UNIVERSITÄT BAYREUTH

Synthese und Derivatisierung der natürlich vorkommenden 3-Acyltetramsäuren Macrocidin Z und Kibdelomycin

Dissertation

zur Erlangung des akademischen Grades einer

Doktorin der Naturwissenschaften (Dr. rer. nat.)

an der Fakultät für Biologie, Chemie, Geowissenschaften

der Universität Bayreuth

vorgelegt von

Laura Treiber

geboren in Erlangen

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Prof. Dr. Stephan Schwarzinger	(Prüfer)

Finis coronat opus. – Das Ende krönt das Werk.

Ovid

INHALTSVERZEICHNIS

ABKÜRZUNGSVERZEICHNIS

In den Formelbildern und im Text werden folgende Abkürzungen verwendet:

9-BBN	9-Borabicyclo(3.3.1)nonan
(+)-L-DET	(+)-L-Diethyltartrat
Ac	Acetyl
Acac	Acetylacetonat
ADDP	1,1'-(Azodicarbonyl)dipiperidin
AgTFA	Silbertrifluoracetat
AISS	Antisense-induzierte Belastungsempfindlichkeit (*antisense-induced strain sensitivity*)
BDMA	Benzaldehyddimethylacetal
BINAP	(2,2'–Bis(diphenylphosphino)–1,1'–binaphthyl)
Bn	Benzyl
Bz	Benzoyl
Boc	*tert*-Butyloxycarbonyl
BOP	Bis(2-oxo-3-oxazolidinyl)phosphinsäure
BTEAC	Benzyltriethylammoniumchlorid
CAM	Cerammoniummolybdat
CD	Circulardichroismus
CHY-b	β-Carotin-Hydroxylase
CHY-e	ε-Carotin-Hydroxylase
Cp	Cyclopentadienyl
CSA	Camphersulfonsäure
DABCO	1,4-Diazabicyclo(2.2.2)octan
DBU	Diazabicycloundecen
DC	Dünnschichtchromatographie
DCC	Dicyclohexylcarbodiimid
DCE	Dichlorethan
DDQ	2,3-Dichlor-5,6-dicyano-1,4-benzochinon
de	Diastereomerenüberschuss (*diastereomeric excess*)
DEAD	Azodicarbonsäurediethylester
$(DHQD)_2PHAL$	Hydrochinidin-1,4-phthalazindiyl-diether
DIBAL	Diisobutylaluminiumhydrid
DIPEA	Diisopropylethylamin
DIPT	Diisopropyltartrat
DMAP	Dimethylaminopyridin
DMAc	Dimethylacetamid

DMF	Dimethylformamid
DMP	Dess-Martin-Periodinan
DMSO	Dimethylsulfoxid
dppf	1,1'-Bis(diphenylphosphino)ferrocen
dr	Diastereomerenverhältnis (*diastereomeric ratio*)
DTBP	Ditertbutylperoxid
DXR	1-Deoxy-D-Xylulose-Reduktoisomerase
DXS	1-Desoxy-D-Xylulose-5-Phosphat-Synthase
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid
EDTA	Ethylendiamintetraessigsäure
ee	Enantimorenüberschuss (*enantiomeric excess*)
FDPP	Pentafluorphenyldiphenylphosphinat
ges.	gesättigt
GGPS	Geranylgeranly-diphosphat-Synthase
HATU	1-[Bis(dimethylamin)methylen]-1*H*-1,2,3-triazol[4,5-b]pyridinium-3-oxid-hexafluorophosphat
HMDS	Hexamethyldisilazan
HMPA	Hexamethylphosphorsäuretriamid
HOAt	1-Hydroxy-7-azabenzotriazol
HOBt	1-Hydroxybenzotriazol
HPLC	Hochleistungsflüssigkeitschromatographie (*high performance liquid chromatography*)
HRMS	Hochauflösende Massenspektrometrie (*high resolution mass spectrometry*)
HWE-Olefinierung	Horner-Wadsworth-Emmons-Olefinierung
*i*Bu	*iso*-Butyl
IBX	2-Iodoxybenzoesäure
IMDA	Intramolekulare Diels-Alder-Reaktion
IPCF	Isopropylchloroformiat
IPI	Isopentenyl-Pyrophosphat-Isomerase
*i*Pr	*iso*-Propyl
kat.	katalytisch
konz.	konzentriert
LC	Flüssigchromatographie (*liquid chromatography*)
LCY-b	Lycopin-β-Cyclase
LCY-e	Lycopin-ε-Cyclase
LDA	Lithiumdiisopropylamin
Lit.	Literatur
Lsg.	Lösung

*m*CPBA	*meta*-Chlorperbenzoesäure
Me	Methyl
MEM	2-Methoxyethoxymethyl
MIC	Minimale Hemm-Konzentration (*minimum inhibitory concentration*)
MoOPH	Oxodiperoxymolybdän-pyridin-hexamethylphosphoramid
Mp	*para*-Methoxyphenyl
MPLC	Mitteldruckflüssigkeitschromatographie (*medium-pressure liquid chromatography*)
MS	Molsieb
Ms	Methansulfonyl (*Mesyl*)
MTBE	Methyl-*tert*-butylether
NaHMDS	Natriumbis(trimethylsilyl)amid
NBS	*N*-Bromsuccinimid
NMO	*N*-Methylmorpholin-*N*-oxid
NMR	Magnetresonanzspektroskopie (*nuclear magnetic resonance*)
NOE	Kern-Overhauser-Effekt (*nuclear overhauser effect*)
NP	Normalphasen
*n*Pent	*n*Pentyl
NXS	Neoaxanthin-Synthase
*o*Nb	*ortho*-Nitrobenzyl
p. a.	*pro analysi*
PAB	*para*-Azidobenzyl
PDC	Pyridiniumdichromat
PDS	Phytoen-Desaturase
Ph	Phenyl
Piv	Pivaloyl
PMB	*para*-Methoxybenzyl
PPTS	Pyridinium-*para*-toluolsulfonat
PSII	Photosystem II
PSY	Phytoen-Synthase
p-TsOH	*para*-Toluolsulfonsäure
RCM	Ringschlussmetathese (*ring-closing-metathesis*)
RP	Umkehrphase (*reversed phase*)
RT	Raumtemperatur
TASF	Trissulfoniumdifluortrimethylsilicat
TBAF	Tetrabutylammoniumfluorid
TBAI	Tetrabutylammoniumiodid
TBS	*tert*-Butyldimethylsilyl

TBDPS	*tert*-Butyldiphenylsilyl
*t*Bu	*tert*-Butyl
TCE	1,1,1-Trichlorethyl
Teoc	2-(Trimethylsilyl)ethoxycarbonyl
TES	Triethylsilyl
Tf	Trifluormethansulfonyl (*Triflyl*)
TFA	Trifluoressigsäure
TFAA	Trifluoressigsäureanhydrid
THF	Tetrahydrofuran
TIPST	Triisopropylsilylthiol
TMS	Trimethylsilyl
Trt	Triphenylmethyl (*Trityl*)
ü. N.	über Nacht
Val	Valin
VED	Violaxanthin-Deepoxidase
ZDS	ζ-Carotin-Desaturase
ZEP	Zeaxanthin-Epoxidase
ZIM	Zentrales Innovationsprogramm Mittelstand
Δ	Erhitzen auf Siedetemperatur

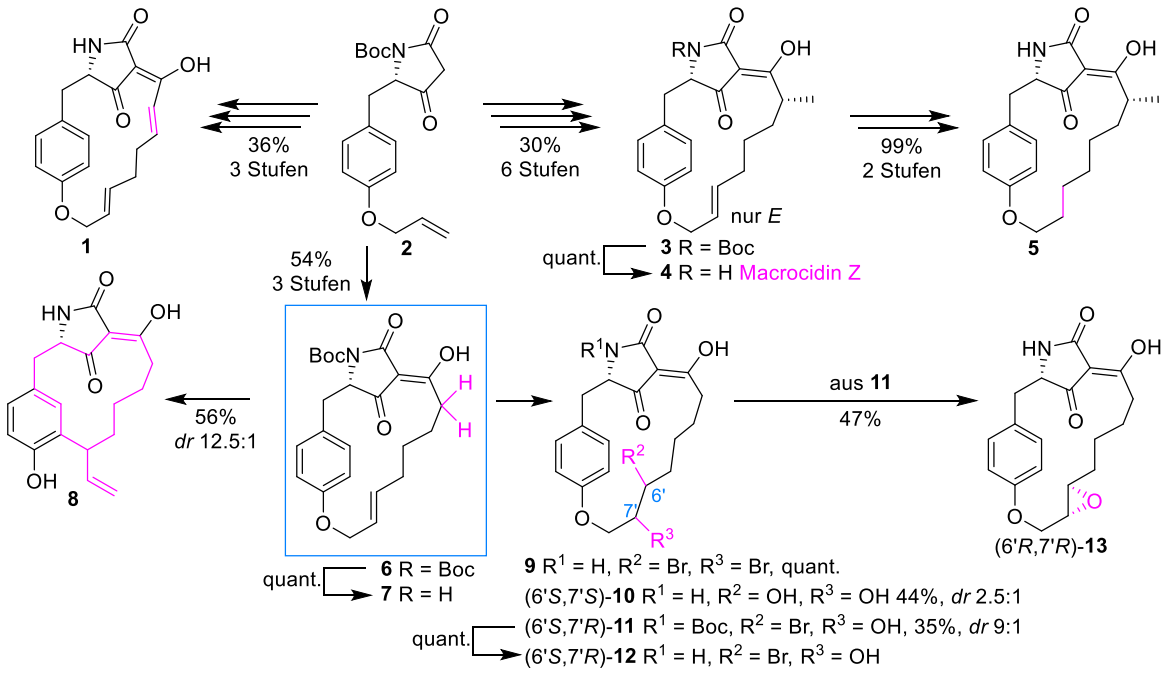
ZUSAMMENFASSUNG

Im Rahmen dieser Dissertation wurden Naturstoffe mit dem zentralen Motiv einer 3-Acyltetramsäure synthetisiert und derivatisiert. Vertreter dieser Klasse sind durch cytotoxische, antibiotische, antivirale oder herbizide Eigenschaften ins Blickfeld der Forschung gerückt. Durch deren Synthesen wird der Zugang zum Wirkstoff an sich, als auch zur Aufstellung von Struktur-Aktivitäts-Beziehungen geebenet. Eine anschließende Derivatisierung ermöglicht die Funktionalisierung oder Simplifizierung der ursprünglichen Struktur unter Wirkerhalt oder sogar Wirksteigerung.

Das erste Projekt beschäftigte sich mit der Darstellung des makrocyclischen Naturstoffs Macrocidin Z (**4**), wobei eine 3-Acyltetramsäure Teil des Ringssystems ist (Schema 1). Die Erstsynthese bestätigte die Strukturannahme und Stereokonfiguration der Substanz, die zusammen mit vier neuen Macrooxazolen aus dem Pilz *Phoma macrostoma* isoliert wurde. Als Startmaterial wurde die von L-Tyrosin abgeleitete 3-H_2-Tetramsäure **2** gewählt. Nach diastereoselektiver Einführung einer Methylgruppe in 6-Heptensäure unter Gebrauch eines Evans-Auxiliars, wurde sie und die Tetramsäure **2** nach dem Yoshii-Yoda-Protokoll in zwei Schritten zur 3-Acyltetramsäure verknüpft. Mittels Ringschlussmetathese unter Verwendung eines Grubbs-Katalysators wurde der Makrocyclus mit hoher *E*-Selektivität geschlossen. Entschützung lieferte schließlich in 30% Gesamtausbeute über sechs Stufen Macrocidin Z (**4**). Neben der bereits bekannten herbiziden Aktivität konnte eine Biofilminhibition und sogar eine Induktion des Biofilmabbaus nachgewiesen werden.

Die Ergebnisse aus den Bioaktivitätsstudien ermutigten zu weiteren Arbeiten am Strukturmotiv der Macrocidine. Es wurden divers funktionalisierte Derivate von Macrocidin Z (**4**) und des ähnlich aufgebauten Naturstoffs Macrocidin A dargestellt und auf verschiedene biologische Aktivitäten getestet. Zunächst gelang durch Hydrierung und Entschützung von Boc-Macrocidin Z **3** die Synthese des Dihydroanalogons **5** in 99% Ausbeute über zwei Stufen. Durch Anwendung der Synthese von 3-Enoyltetramsäuren mittels Ketenylidentriphenylphosphoran und anschließender Ringschlussmetathese sowie Entschützung wurde das doppelt ungesättigte Derivat **1** generiert. Weitere Derivatisierungen sollten ausgehend vom Schlüsselintermediats **6** erfolgen. Dessen Darstellung erfolgte in Analogie zur Synthese von Macrocidin Z (**4**) durch Acylierung der 6-Heptensäure an die Tetramsäure **2**, Umlagerung zur 3-Acyltetramsäure sowie Aufbau des Makrocylcus durch Ringschlussmetathese in 54% über drei Stufen. Anschließend konnte durch Claisen-Umlagerung eine Ringkontraktion in 56% erzielt werden (→ **8**) sowie eine Dibromierung in quantitativer Ausbeute (→ **9**). Außerdem wurde das zentrale Intermediat **6** durch Sharpless-Dihydroxylierung in den Diol (6'*S*,7'*S*)-**10** sowie durch eine regioselektive

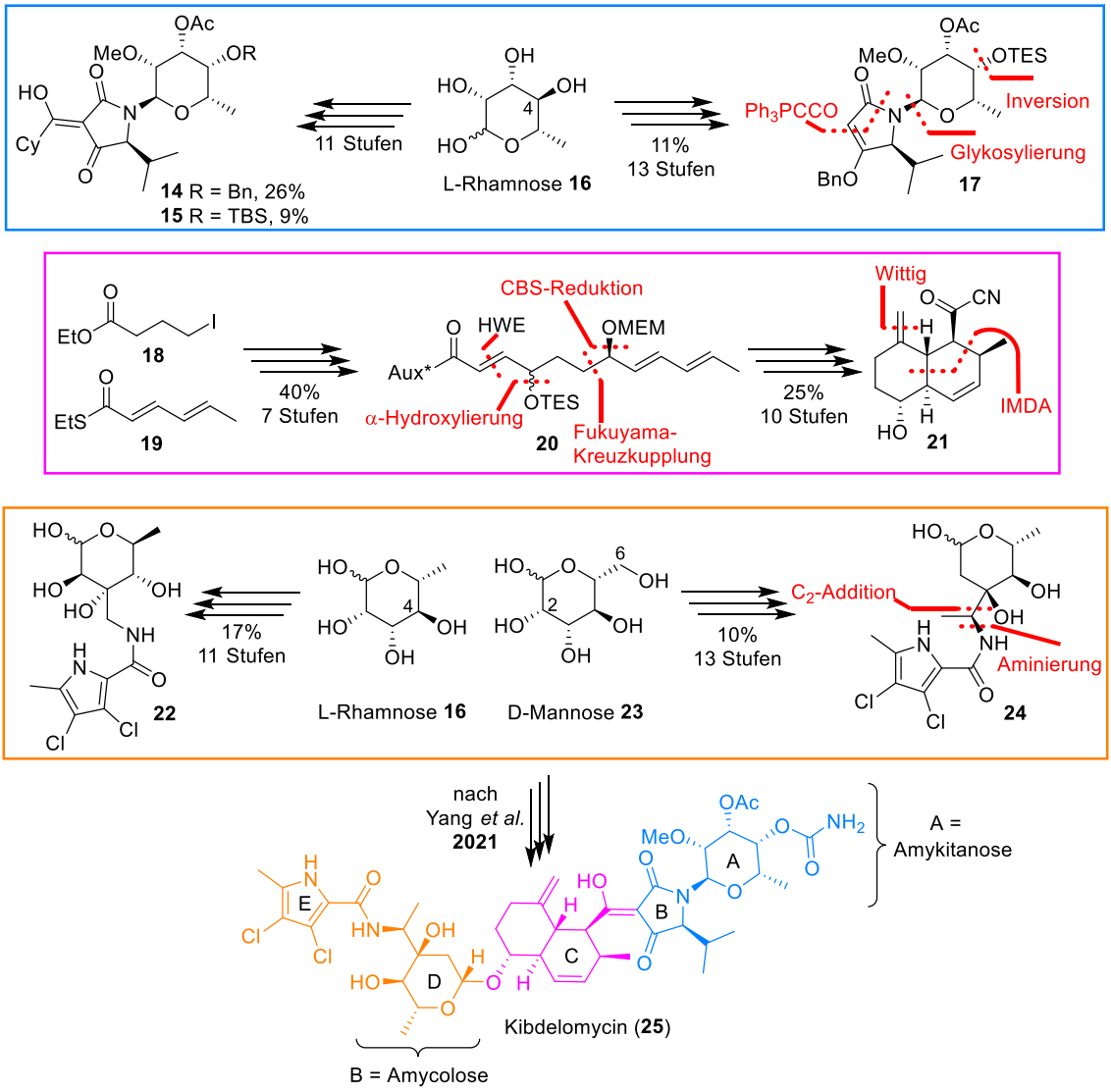
Reaktion mit NBS und H_2O in das *anti*-Bromohydrin (6'*S*,7'*R*)-**11** überführt. Letzteres reagierte unter basischen Bedingungen zum Epoxid (6'*R*,7'*R*)-**13**. Bei allen Reaktionen konnte eine Stereoinduktion durch den Ring beobachtet werden, jedoch konnte nicht für alle Derivate die absolute Stereokonfiguration bestimmt werden.



Schema 1. Synthese des Naturstoffes Macrocidin Z (**4**) sowie dessen Derivatisierung. Pink markiert sind die strukturellen Variationen der Derivate, blau umrandet ist das Schlüsselintermediat.

Im dritten Projekt wurde die formale Totalsynthese von Kibdelomycin (**25**) sowie die Derivatisierung einzelner Fragmente behandelt (Schema 2). Die Darstellung sollte konvergent erfolgen, wobei das Molekül in drei Bausteine unterteilt wurde. Das Zuckerfragment **A**, die Amykitanose, wurde ausgehend von L-Rhamnose (**16**) gebildet. Nach Schützung der anomeren Position und des *syn*-Diols wurde das Stereozentrum an 4-Position durch Oxidation und diastereoselektiver Reduktion invertiert. Es folgten die selektive Einführung der Acetyl- und Methylgruppe. Nach Aktivierung der anomeren Position kann in einer Au-katalysierten Reaktion gemäß Yang *et al*. das Glykosid mit einer 4-*O*-Alkyltetramsäure zum Zielmolekül **17** in einer Gesamtausbeute von 11% über 13 Stufen gekuppelt werden. In Analogie konnten die 3-Acyltetramsäuren **14** und **15** in elf Stufen dargestellt werden. Dabei wurde zum einen die erste direkte *N*-Glykosylierung mit einer 3-Acyltetramsäure entwickelt und zum anderen ein weiterer Zugang zur Amykitanose **A** und Kibdelomycin (**25**) ermöglicht. Das Decalinfragment wurde ausgehend vom Iodid **18** und dem Thioester **19** synthetisiert. Eine Fukuyama-Kupplung ermöglichte deren Verknüpfung zum Keton. CBS-Reduktion, α-Hydroxylierung mit dem Vedejs-Reagenz, eine orthogonale Schutzgruppenstrategie und HWE-Olefinierung lieferten

den acyclischen Vorläufer **20**. Durch eine Auxiliar-gesteuerte IMDA, Wittig-Olefinierung und Formation eines Acylcyanids wurde der Baustein **21** in 10% über 17 Stufen erhalten. Startmaterial für die Synthese der *N*-acylierten Amycolose **24** war D-Mannose (**23**). Schlüsselschritte waren die Desoxygenierung an 2- und 6-Position und die stereoselektive Grignard-Reaktion zur Einführung eine C_2-Einheit. Die Bildung des Amins mit anschließender Kupplung mit dem Pyrrol **E** komplettierten die Synthese der *N*-acylierten Amycolose (**24**) in einer Gesamtausbeute von 10% über 13 Stufen. Die Schlüsselschritte wurden bei der Darstellung eines Derivates **22** ausgehend von L-Rhamnose (**16**) übernommen, wobei zudem eine Strategie zur selektiven Schützung und Integration eines Linkers zum Pyrrol entwickelt wurde. Über diese Route können sämtliche Zucker als Ausgangsmaterial zu Derivatsynthese genutzt werden. Die drei Fragmente **17**, **21** und **24** können in Anlehnung an die Erstsynthese zu Kibdelomycin (**25**) verknüpft werden.



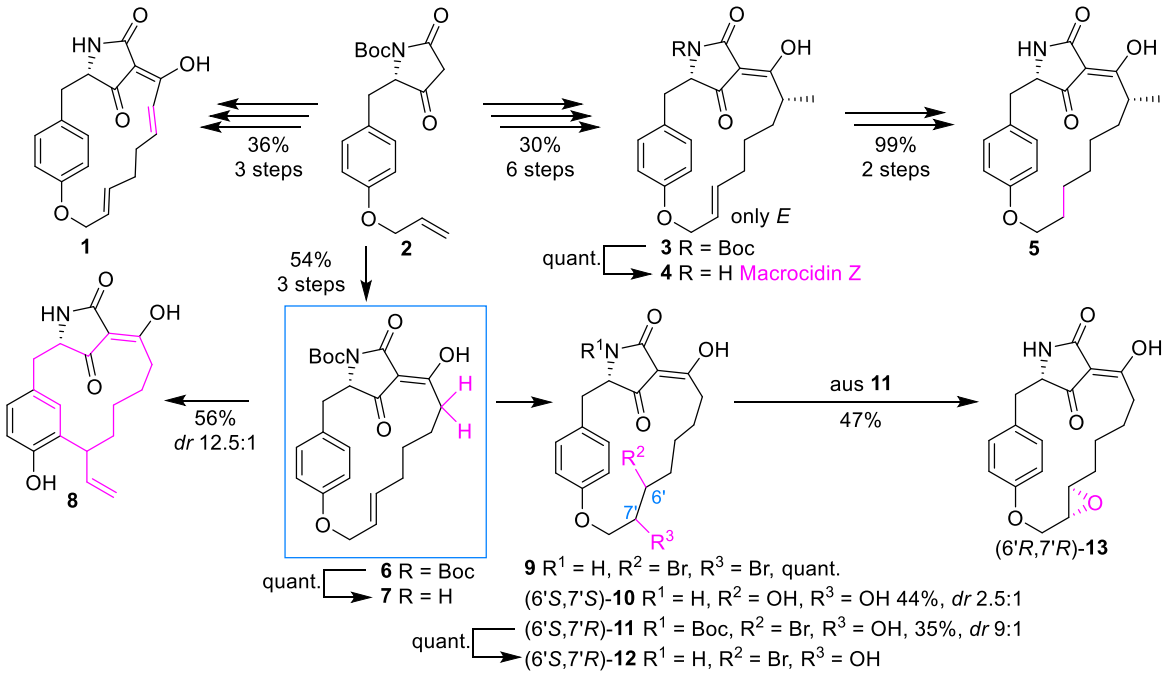
Schema 2. Formale Synthese von Kibdelomycin (**25**) sowie die Derivatisierung von Strukturfragmenten.

SUMMARY

In the context of this dissertation, natural products with the central motif of a 3-acyltetramic acid were synthesised and derivatised. Representatives of this class have become the focus of research because of their cytotoxic, antibiotic, antiviral or herbicidal properties. Through their syntheses, access to the active substance itself as well as to the establishment of structure-activity relationships is provided. Subsequent derivatisation enables the structure to be simplified or functionalised, while maintaining or even increasing its efficacy.

The first project dealt with the first synthesis of the macrocyclic natural product macrocidin Z (**4**), in which a 3-acyltetramic acid is part of the ring system (scheme 3). The synthesis confirmed the proposed structure and stereoconfiguration of the molecule, which was isolated from the fungus *Phoma macrostoma* together with four new macrooxazoles. The starting material used was L-tyrosine derived 3-*H*-tetramic acid **2**. After diastereoselective introduction of a methyl group into 6-heptenoic acid using an Evans auxiliary, it and tetramic acid **2** were linked in two steps according to the Yoshii-Yoda protocol to a 3-acyltetramic acid. The macrocycle was closed with high *E*-selectivity by ring-closing metathesis using a Grubbs catalyst. Finally, deprotection afforded macrocidin Z (**4**) in 30% overall yield over six steps. In addition to the already known herbicidal activity, biofilm inhibition and even induction of biofilm degradation was demonstrated.

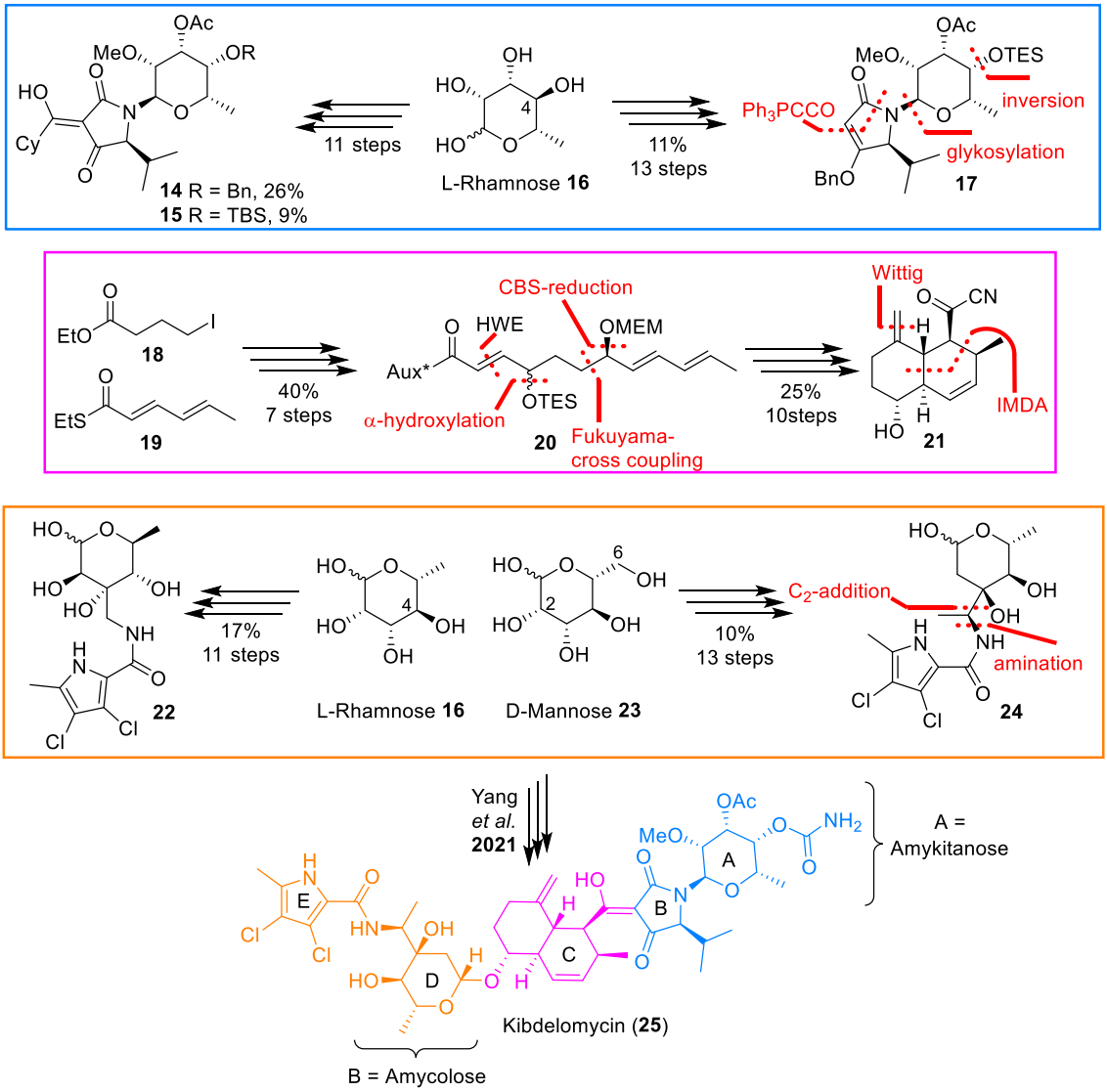
The results from the bioactivity studies encouraged further work on the structural motif of macrocidins. Variously functionalised derivatives of macrocidin Z (**4**) and the structurally related natural product macrocidin A were prepared and tested for a variety of biological activities. First, hydrogenation of Boc-macrocidin Z **3** and deprotection led to the synthesis of the dihydro analogue **5** in 99% yield over two steps. The doubly unsaturated derivative **1** was generated by applying Schobert's synthesis of 3-enoyltetramic acids using ketenylidenetriphenylphosphorane, subsequent ring-closing metathesis and deprotection. Further derivatisation should be done starting from the key intermediate **6**. Its synthesis succeeded in analogy to the first project by acylation of heptenoic acid to tetramic acid **2**, rearrangement to 3-acyltetramic acid and construction of the macrocycle by ring-closing metathesis in 54% over three steps. A ring contraction was achieved by Claisen rearrangement in 56% yield (→ **8**) and a dibromination in quantitative yield (→ **9**). In addition, the key intermediate **6** was converted into diol (6'*S*,7'*S*)-**10** by Sharpless dihydroxylation and into *anti*-bromohydrin (6'*S*,7'*R*)-**11** by regioselective reaction with NBS and H_2O. The latter formed epoxide (6'*R*,7'*R*)-**13** under basic conditions. Stereoinduction by the ring was observed in all reactions, but the absolute stereoconfiguration could not be determined for all derivatives.



Scheme 3. Synthesis of natural product macrocidin Z (**4**) and its derivatisation. Highlighted in pink are the structural variations of the derivatives, highlighted in blue is the key intermediate.

The third project dealt with the formal total synthesis of kibdelomycin (**25**), and the derivatisation of individual fragments (scheme 4). A convergent sequence was chosen, dividing the molecule into three buiding blocks. Fragment **A**, the amykitanose, was synthesised from L-rhamnose (**16**). After protection of the anomeric position and the *syn*-diol, the stereocenter at the 4-position was inverted by oxidation and diastereoselective reduction. This was followed by regioselective introduction of the acetyl and methyl group. After activation of the anomeric position, the sugar could be coupled with a 4-*O*-alkyltetramic acid to the target molecule **17** in an Au-catalyzed reaction according to Yang *et al*. in an overall yield of 11% over thirteen steps. In analogy, the 3-acyltetramic acids **14** and **15** were synthesised in eleven steps, establishing the first direct *N*-glycosylation with a 3-acyltetramic acid. In addition, further access to amykitanose **A** and kibdelomycin (**25**) was enabled. The decalin fragment **21** was synthesised from iodide **18** and thioester **19**. After Fukuyama coupling the corresponding ketone was obtained. CBS reduction, α-hydroxylation with the Vedejs reagent, an orthogonal protecting group strategy, and HWE olefination afforded the acyclic precursor **20**. Auxiliary-directed IMDA, Wittig olefination, and formation of an acyl cyanide led to building block **21** in 10% over 17 steps. Starting material for the synthesis of *N*-acylated amycolose **24** was D-mannose (**23**). Key steps were deoxygenation at the 2- and 6-position and the stereoselective Grignard reaction to introduce a C_2-unit. Formation of the amine followed by coupling with pyrrole **E** completed the synthesis of *N*-acylated amycolose **24** with an overall yield of 10% over 13 steps.

The key steps were adopted in the preparation of a derivative **22** starting from L-rhamnose (**16**), and a strategy for selective protection and integration of a linker to the pyrrole was developed. All sugars can be used as starting material for the synthesis of derivatives via this route. The three fragments **17**, **21** and **24** can be linked based on the first total synthesis to generate kibdelomycin (**25**).

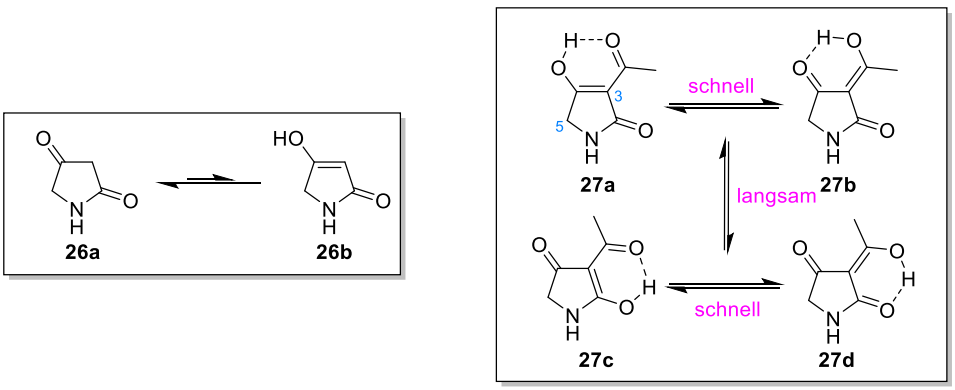


Scheme 4. Formal synthesis of kibdelomycin (**25**) and derivatisation of its fragments.

1 EINLEITUNG

1.1 Tetramsäuren – Eigenschaften und Besonderheiten

Das Motiv der Tetramsäure erlang trotz frühzeitiger Entdeckung erst in den 1960er Jahren Bekanntheit, als es als häufig auftretendes Strukturmuster in Naturstoffen erkannt wurde.[1,2] Diese wiesen meist interessante biologische Aktivitäten auf, welche mit dem Tetramsäure-Motiv in Verbindung gebracht werden konnten und von cytotoxischen über bakterizide, viruzide, herbizide und fungizide Effekte reichen. Tetramsäuren neigen zur Ausbildung tautomerer Strukuren. Sie können sowohl als Pyrrolidin-2,4-dion (**26a**) als auch als in der entsprechenden Enolform **26b** vorliegen (Schema 5), wobei ersteres im Gleichgewicht dominiert.[1,3,4] Die in Naturstoffen häufiger vorkommenden Vertreter, die 3-Acyltetramsäuren verhalten sich ähnlich.[5] Auch sie sind im Gleichgewicht von vier tautomeren Formen **27a-d**, jedoch liegt stets eine der drei Carbonylgruppen aufgrund der Acidität des 3-*H* (pK_s = 3.0-3.5) enolisiert vor.[1] Die vier Tautomere können in die Gruppen der internen Tautomere **27a**/**27b** und **27c**/**27d** und externen Tautomere **27a**/**27c** und **27b**/**27d** eingeteilt werden. Erstere können schnell durch Protonentransfer via Wasserstoffbrückenbindung ineinander überführt werden, wohingegen das Gleichgewicht zwischen Letzteren nur langsam durch Rotation der Acylseitenkette um eine Bindung eingestellt wird.[1,6,7] Welche der vier tautomeren Formen im Gleichgewicht dominiert, hängt unter anderem von den Subsituenten am Stickstoff und an 5-Position sowie dem Lösungsmittel ab.[8–10] Durch ^{13}C-NMR-Experimente und Kristallstruktur-analysen konnte jedoch das *exo*-Enol **27d** in den meisten der untersuchten Fälle als die vorwiegend vorliegende tautomere Form identifiziert werden.[6,11] Die Möglichkeit zur Ausbildung von Wasserstoffbrückenbindungen und die hohe Polarität der Tetramsäuren kann häufig mit der Bioaktivität ihrer Vertreter in Verbindung gebracht werden. Ein Beispiel dafür wäre das Kibdelomycin (**25**), welches in dieser Arbeit noch ausführlich betrachtet wird.



Schema 5. Darstellung der Tautomeren-Gleichgewichte bei 3-H_2-Tetramsäuren und 3-Acyltetramsäuren.[1]

Durch ihre Struktur neigen 3-Acyltetramsäuren zur Ausbildung von Chelatkomplexen mit Metallkationen. Einige bekannte Naturstoffe mit diesem Strukturmotiv konnten sogar ausschließlich als Magnesiumkomplex isoliert werden wie beispielsweise die Tenuazonsäure (**28**) und Magnesidin A (**29**; Abb. 1).[12–14] Das bestätigt auch die Annahme einer erhöhten Stabilität der Verbindungen durch die Komplexbildung.[15] Zudem wird vermutet, dass ein Zusammenhang zwischen der biologischen Wirksamkeit von Tetramsäuren und ihrer Neigung zur Komplexbildung besteht.[12,15–17] Auch die herbizide Wirkung von Macrocidin A (**30**) wird zum Teil durch die Komplexierung von Fe^{2+}-Ionen und einen damit verbundenen Eingriff in die Carotin-Biosynthese der Pflanzen hervorgerufen.[18] Dieser Naturstoff wird ebenfalls in den nachfolgenden Teilen der Arbeit eingehender betrachtet. Des Weiteren führt die Komplexbildung zu einer erhöhten Lipophilie der Naturstoffe, was den Transport durch Zellwände verbessert und somit auch die pharmakologische Wirkung.[16,17]

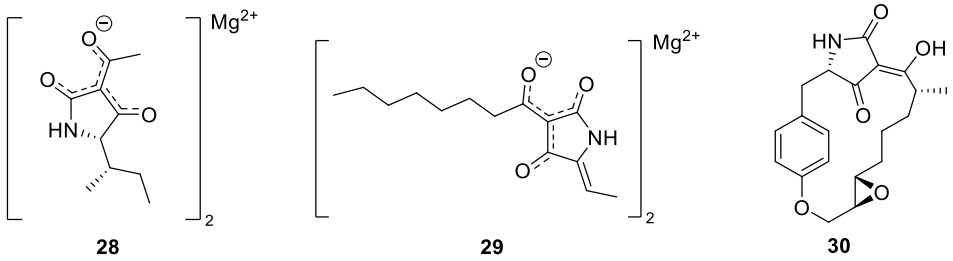


Abb. 1 Strukturformeln des Magnesium-Komplexes der Tenuazonsäure (**28**), Magnesidin A (**29**) und Macrocidin A (**30**).[19]

Die vielfältigen Eigenschaften der Tetramsäuren machen die Synthese von deren Vertretern immer noch erstrebenswert. Es werden weiterhin neue Metabolite mit dem bekannten Pyrrolidin-Motiv isoliert, deren Bioaktivität auch deren Synthese begründet. Dazu zählen auch die Gruppe der Macrocidine und Kibdelomycin, deren synthetische Darstellung in dieser Arbeit behandelt werden.

1.2 Methoden zur Synthese von Tetramsäuren

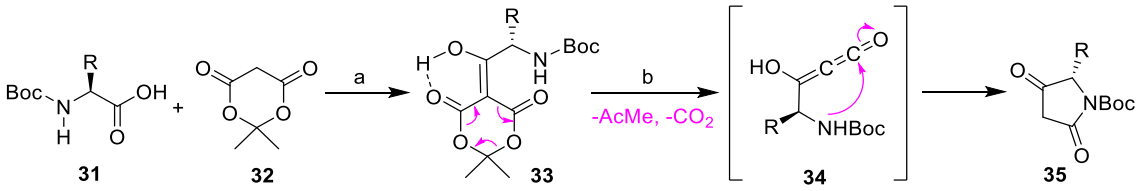
Neben den biologischen Aktivitäten vieler Vertreter der Tetramsäuren, ist auch deren Synthese aus chemischer Sicht weiterhin interessant und herausfordernd. An dem Motiv wird schon seit der ersten Tetramsäuresynthese durch Gabriel *et al*. im Jahr 1913 gearbeitet.[20] Es wurden einige racemische als auch enantioselektive Methoden zur Bildung von 3-H_2-Tetramsäuren als auch zur Bildung von 3-Acyltetramsäuren entwickelt. Letztere lassen sich dabei in zwei grundlegende Gruppen einteilen. Die erste Möglichkeit zur Synthese von 3-Acyltetramsäuren

ist die Acylierung einer 3-H_2-Tetramsäure an 3-Position oder eine 4-*O*-Acylierung mit anschließender Umwandlung in die 3-Acyltetramsäure. Die zweite Gruppe besteht aus Synthesen, die beim Aufbau des Ringsystems direkt eine 3-Acylgruppe mit einführen. Im Folgenden werden sowohl für die Synthese des Tetramsäurerings als auch für die beiden Gruppen der Darstellung von 3-Acyltetramsäuren Beispiele gelistet.

1.2.1 Synthese des Pyrrolidin-2,4-dion-Motivs

1.2.1.1 Meldrumsäuremethode

In Anlehung an die Dieckmann-Cyclisierung eines Aminosäurederivates von Katsuki *et al*., die zu einem racemischen Tetramsäurekerngerüst führte,[21] entwickelten Jouin *et al*. eine bis heute gängige Darstellungsmethode für 3-H_2-Tetramsäuren **35** (Schema 6).[22] Dabei wurden zumeist *N*-Boc-geschützte Aminosäuren **31** nach *in situ* Aktivierung mit IPCF mit Meldrumsäure (**32**) und DMAP umgesetzt. Die Tetramsäuren wurden erhalten durch Erhitzen des Adduktes **33** in EtOAc oder MeCN. Die Reaktion lief unter Erhalt des Stereozentrums ab. Mechanistisch kommt es im ersten Schritt zur intermediären Bildung eines Ketens, worauf ein intramolekularer Angriff des Stickstoffs zum Ringschluss führt.[23]

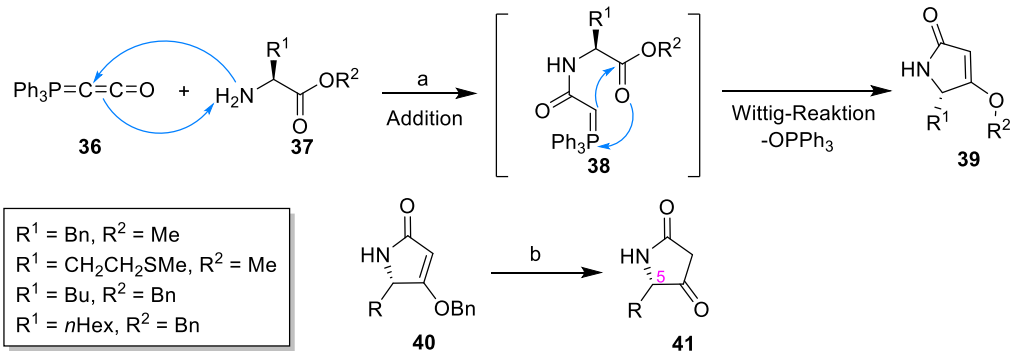


Schema 6. Synthese von Tetramsäuren **35** nach Jouin *et al*.[22]
Reagenzien und Bedingungen: a) DMAP, IPCF, CH_2Cl_2, –5 °C, 2 h; b) Δ, 30 min.

Die Methode wurde durch mehrere Arbeitskreise verbessert. So testeten Jiang *et al*. verschiedene Aktivierungsreagenzien zur Vermeidung des teuren und toxischen IPCF. Die besten Ausbeuten erhielten sie bei Verwendung von BOP-Cl und FDPP.[24] Durch die Anpassung der Aufarbeitung des säurelabilen Meldrumsäureadduktes **33** gelang es Ma *et al.* und später Hosseini *et al.*, die gängigeren Aktivierungsreagenzien DCC oder EDC in der Tetramsäuresynthese einzusetzen.[25,26]

1.2.1.2 Domino-Wittig-Reaktion

Eine weitere Möglichkeit zum Aufbau von Tetramsäuren stammt von Schobert *et al*. und nutzt ebenfalls Aminosäuren aus dem *chiral pool* zum Einbringen der Stereoinformation. Ursprünglich entwickelt zur Synthese von Tetronsäuren,[27] gelang es diese Methode auf die stickstoffhaltigen Analoga zu übertragen.[28,29] Die Aminosäureester **37** wurden dafür mit Ketenylidentriphenylphosphoran (**36**) unter Rückfluss erhitzt (Schema 7). Es erfolgte zunächst die Addition des Stickstoffs an das Reagenz, gefolgt von einer intramolekularen Wittig-Reaktion unter Abspaltung von Triphenylphosphinoxid. Nach Durchführung der Domino-Wittig-Reaktion können die erhaltenen 4-*O*-Benzyltetramsäuren **40** durch hydrogenolytische Spaltung in die Tetramsäuren **41** überführt werden.[30,31] Weniger gute Ausbeute lieferte diese Synthesemethode bei sterisch anspruchsvollen Resten an 5-Position. Durch Zugabe katalytischer Mengen an Benzoesäure wurde der Schritt der Addition beschleunigt, was das Problem löste. Bei säureempfindlichen Resten kann stattdessen *N*-Hydroxysuccinimid genutzt werden.[31,32]



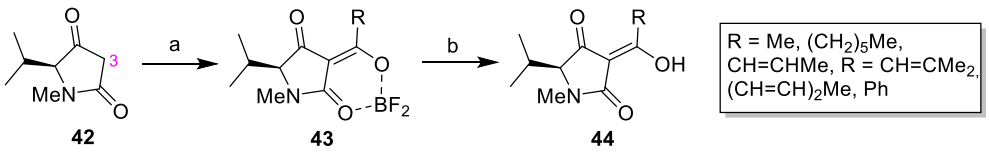
Schema 7. Synthese von Tetramsäuren **41** nach Schobert *et al*. durch eine Domino-Wittig-Reaktion.[22,31] *Reagenzien und Bedingungen*: a) Toluol/Xylol/THF, Δ, 24 h; b) H_2, Pd/C, RT.

Durch den einfachen Zugang zu den Aminosäureestern und dem kumulierten Ylid **36** ist diese Variante besonders interessant. Ketenylidentriphenylphosphoran (**36**) kann in einem Schritt aus Carbomethoxymethylentriphenylphosphoran durch Umsetzung mit NaHMDS gewonnen werden.[33,34] Bei Lagerung unter Argon ist es mehrere Monate bei Raumtemperatur stabil. Jedoch ist in einigen Fällen die Abtrennung des entstehenden Triphenylphosphinoxids problematisch. Durch die Nutzung des polymergebundenen kumulierten Ylids lösten Schobert *et al.* diesen Nachteil.[30,35] Es kann in zwei Schritten aus Polystyrol-gebundenem Triphenylphosphoran dargestellt werden.

1.2.2 Synthese von 3-Acyltetramsäuren durch nachträgliche Acylierung des Pyrrolidin-2,4-dion-Motivs

1.2.2.1 3-Acylierung nach Jones *et al.*

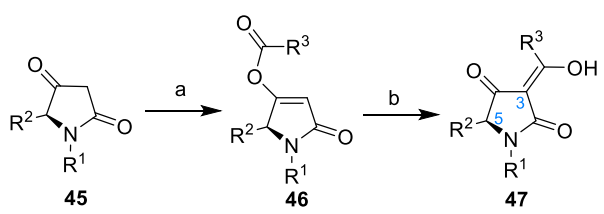
Da in Naturstoffen Tetramsäuren meist an 3-Position acyliert vorliegen, wurden einige Synthesen zur nachträglichen Acylierung von 3-H_2-Tetramsäuren entwickelt. In Anlehnung an die Magnesidin-Synthese nach Bhat *et al*.[36] und die Synthese von 3-Acyltetronsäuren durch Bloomer *et al*.[37] entwickelte die Arbeitsgruppe um Jones eine Lewissäure-vermittelte Reaktion von Carbonsäurechloriden mit dem Tetramsäuregrundgerüst (Schema 8).[38,39] Die 3-H_2-Tetramsäuren **42** wurden mit einem Überschuss verschiedener Säurechloride und diversen Lewissäuren bei erhöhter Temperatur umgesetzt. Nach Anpassung der Aufarbeitung konnten mit $BF_3{\cdot}OEt_2$ die besten Ausbeuten erzielt werden. Jones *et al*. konnten nach wässriger Aufarbeitung die BF_2-Komplexe der *exo*-Enol-Tautomere der 3-Acyltetramsäuren **43** isolieren, welche anschließend durch Erhitzen in Methanol in die 3-Acyltetramsäuren **44** überführt wurden.[39,40] Die Anwendung dieser Methode auf die Synthese von Naturstoffen mit komplizierten oder hoch funktionalisierten Resten an 3-Position kann aufgrund der im hohen Überschuss notwendigen Säurechloride problematisch werden.



Schema 8. 3-Acylierung von 3-Acyltetramsäuren **44** nach Jones *et al*.[38,39]
Reagenzien und Bedingungen: a) RCOCl, $BF_3{\cdot}OEt_2$, 80 °C; b) MeOH, 25-65 °C.

1.2.2.2 Yoshii-Yoda-Acylierung

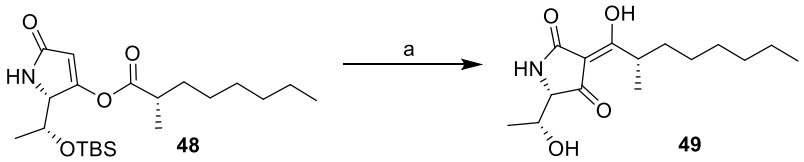
Unter basischen Bedingungen ist eine direkte 3-Acylierung nicht möglich, stattdessen wird meist 4-*O*-acyliert.[40] Nach der ersten Anwendung durch van der Baan *et al.* nutzten Hori *et al*. dies für ihre Methode zur Darstellung von 3-Acyltetramsäuren, die ebenfalls zunächst an 3-Acyltetronsäuren durchgeführt wurde.[41–43] Umsetzung des Pyrrolidingerüsts **45** unter Steglich-Bedingungen mit DCC und einer katalytischen Menge DMAP führte zur Bildung des kinetischen 4-*O*-Acylprodukts **46** (Schema 9).[44] Durch die Zugabe von NEt_3 wurde die langsame Acylmigration zur 3-Acyltetramsäure **47** induziert. Die milden Bedingungen und die Toleranz gegenüber funktionellen Gruppen macht diese Methode für die Synthese von 3-Acyltetramsäuren besonders effektiv.



R^1 = H, R^2 = Bn, R^3 = Bn, *n*Pent, *i*Pr, CHCH=CH_3, $CH_2(CH=CH)_2CH_3$, $(EtO)_2P(O)CH_2$	R^1 = H, R^2 = *i*Pr, R^3 = Bn, *n*Pent, *c*Pr, $CH_2(CH=CH)_2CH_3$, $(EtO)_2P(O)CH_2$
R^1 = CH_3, R^2 = *i*Pr, R^3 = *n*Pent, $(EtO)_2P(O)CH_2$	R^1 = Bn, R^2 = H, R^3 = *n*Pent, CHCH=CH_3, $CH_2(CH=CH)_2CH_3$, $(EtO)_2P(O)CH_2$

Schema 9. 4-*O*-Acylierung mit Umlagerung zur 3-Acyltetramsäure **47** unter milden Bedingungen nach Hori *et al*.[42]
Reagenzien und Bedingungen: a) DCC, DMAP, R^3COOH, CH_2Cl_2, 0 °C→RT; b) NEt_3, CH_2Cl_2, 0°C→RT.

Der Arbeitsgruppe um Yoda gelang zudem eine Optimierung der Bedingungen für die Acylierung sterisch anspruchsvoller oder α-verzweigter Carbonsäuren.[45] Unter den von Hori *et al*. publizierten Bedingungen konnten nur Spuren des von ihnen gewünschten Penicillenol A_1 (**49**) isoliert werden (Schema 10). Entscheidend für die Ausbeutesteigerung war die Anwesenheit von DMAP und $CaCl_2$, wohingegen die Zugabe von NEt_3 keinen Einfluss auf die Reaktion hatte. Es wurde vermutet, dass durch die Komplexierung der Tetramsäure und DMAP die Umlagerung des Acylrestes beschleunigt wird. Im Zuge der Anwendung ihrer Methode an diversen Tetramsäuren konnten sie zudem beweisen, dass die Zugabe des $CaCl_2$ auch die Epimerisierung an 5-Position sowie an α-Position des wandernden Acylrests unterdrückt, die vor allem bei verlängerten Reaktionszeiten mit der Acylmigration konkurrieren.[46]

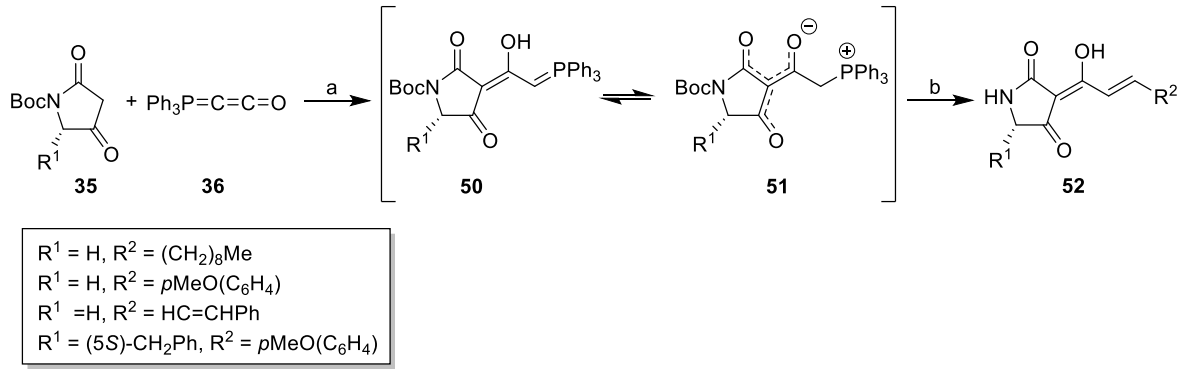


Schema 10. Umlagerung der 4-*O*-Acyltetramsäure **48** zur 3-Acyltetramsäure **49** nach Sengoku *et al*.[27,46]
Reagenzien und Bedingungen: a) 1. DMAP, $CaCl_2$, CH_2Cl_2; 2. NaHMDS, MeI, THF, -40 °C; 3. HCl, MeOH, 0 °C.

1.2.2.3 3-Acylierung nach Schobert

Die Methode von Schlenk *et al*. zur 3-Acylierung von Tetramsäuren hebt sich von den voher beschriebenen Methoden durch ihre Toleranz gegenüber pH-sensitiven Funktionalitäten, ihre Regioselektivität und ihre einfache Durchführung ab.[47] Wie auch in der von der selben Arbeitsgruppe beschriebenen Darstellung von 3-H_2-Tetramsäuren ist das entscheidende Reagenz das kumulierte Ylid **36**.[31] Zunächst erfolgt die Umsetzung der *N*-Boc-geschützten Tetramsäuren **35** mit Ketenylidentriphenylphosphoran (**36**) zu den Yliden **51**, welche im

nächsten Schritt durch KO*t*Bu aktiviert werden (Schema 11). Die entstehenden Kaliumsalze konnten nicht isoliert werden, reagierten jedoch sofort mit Aldeyhden unter Rückfluss zu den 3-Enoyltetramsäuren **52**. Durch katalytische Hydrierung ist zudem die Entfernung der eingeführten konjugierten Doppelbindung möglich, was den Weg zu sämtlichen 3-Acyltetramsäuren ebnet.[47]

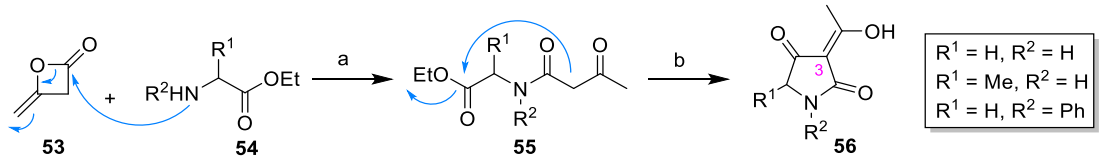


Schema 11. Direkte 3-Ayclierung von Tetramsäuren **35** zu 3-Enoyltetramsäuren **52** nach Schobert.[47]
Reagenzien und Bedingungen: a) THF, Δ; b) 1. KO*t*Bu, THF, Δ; 2. R^2CHO, THF, Δ; 3. TFA, CH_2Cl_2.

1.2.3 Direkte Synthese von 3-Acyltetramsäuren

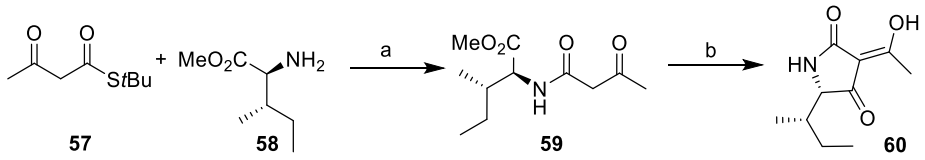
1.2.3.1 Lacey-Dieckmann-Cyclisierung

Die wohl am häufigsten verwendete Methode zum Aufbau von 3-Acyltetramsäuren in der Naturstoffsynthese ist die Lacey-Dieckmann-Cyclisierung. 1954 publizierte Lacey erstmals seine Ergebnisse zur Darstellung von 3-Acetyltetramsäuren **56** in zwei Stufen ausgehend von den entsprechenden Aminosäureethylestern **54** (Schema 12).[48] Diese wurden zunächst mit Diketen (**53**) bei Raumtemperatur zu den β-Ketoamiden **55** umgesetzt, welche nach Deprotonierung mit NaOMe zu den 3-Acetyltetramsäuren **56** im Sinne einer Dieckmann-Cyclisierung reagierten.[48,49] Diese ursprüngliche Variante der Lacey-Dieckmann-Cyclisierung ist jedoch durch die Verwendung des Diketens (**53**) hinsichtlich der Reste an 3-Position beschränkt. Zudem können nur basen- und thermisch stabile Edukte den harschen Bedingungen der Cyclisierung standhalten. Durch das stark basische Milieu der Reaktion können außerdem Stereozentren an aciden Positionen epimerisiert werden.



Schema 12. Lacey-Dieckmann-Cyclisierung der β-Ketoamide **53** zu den 3-Acetyltetramsäuren **56**.[48]
Reagenzien und Bedingungen: a) EtOH, <5°C→RT, 1 h; b) NaOMe, Benzol, Δ, 3h.

Weiter entwickelt wurde die Methode von Ley *et al*. durch die milde Darstellung von β-Ketoamiden aus den entsprechenden β-Ketothioestern und primären oder sekundären Aminen (Schema 13).[50,51] Durch eine silbervermittelte Reaktion unter wasserfreien Bedingungen gelang die Synthese verschiedener β-Ketoamide bei Raumtemperatur. Es kam weder zu Racemisierungen bei Verwendung von Aminosäuren, noch zur Zersetzung funktionalisierter β-Ketoamide.[50,51] Zudem konnten Ley *et al*. durch Modifizierung der Cyclisierungs-Bedingungen die Umsetzung der β-Ketoamide zu den 3-Acyltetramsäuren verbessern. Statt starker Basen wie NaOMe oder KO*t*Bu[52,53] etablierten sie die Verwendung von TBAF als Aktivator für die Cyclisierung. Durch Zugabe von wenigen Äquivalenten cyclisierten die β-Ketoamide in wenigen Minuten bei Raumtemperatur zu den 3-Acyltetramsäuren. Dies ist vor allem vorteilig, da lange Reaktionszeiten und erhöhte Temperaturen zur Racemisierung an 5-Position führen.[54] Als Beispiel für die Reaktionssequenz wurde die Synthese der 3-Acyltetramsäure **60** aus L-Isoleucinmethylester (**58**) abgebildet.



Schema 13. Synthese der 3-Acyltetramsäure **60** ausgehend von L-Isoleucinmethylester (**58**) nach Ley *et al*.[54] *Reagenzien und Bedingungen*: a) Silber(I)-trifluoracetat, THF, RT; b) TBAF, THF, RT, 5 min.

1.3 Macrocidine – Synthesen und Bioaktivität

1.3.1 Macrocidin A (30) und B (61)

Naturstoffe mit 3-Acyltetramsäuremotiv sind vielfältig, einige Vertreter, darunter auch die Macrocidine, sind makrocyclisch. 2003 wurden erstmals zwei Vertreter dieser neuen Naturstoffklasse aus dem Pilz *Phoma macrostoma* Montagne von einer DowAgroSciences Gruppe isoliert.[55] Der Pilz wurde aus Feldisolaten befallener kanadischer Disteln gewonnen. *Phoma macrostoma* Montagne ist als Wundparasit und schwacher Erreger weltweit bekannt, der Chlorose sowie Nekrose bei befallenen Pflanzen auslöst. Graupner *et al*. untersuchten erstmals die Metabolite dieses Pilzes. Kulturextrakte lösten bei breitblättrigen Pflanzen Chlorose auf neu wachsenden Blättern und nach kurzer Zeit sogar Nekrose einiger Pflanzenarten aus. Die Struktur der Hauptverbindung aus den Extrakten – Macrocidin A (**30**) – wurde durch 2D-NMR-Experimente bestimmt, die Stereokonfiguration wurde durch Kristallstrukturanalysen aufgeklärt (Abb. 2).[55] Diese konnte durch die Totalsynthesen aus den Jahren 2010[56] und 2016[57] verifiziert werden. Als weitere Verbindung konnte Macrocidin

B (**61**) isoliert werden. Die Strukturaufklärung gestaltete sich aufgrund der sehr geringen Menge an erhaltenem Metaboliten schwieriger. Graupner *et al*. nahmen an, dass die Konfigurationen an C-5, C-6‘ sowie C-7‘ identisch zu denen in Macrocidin A (**30**) sein müssen, ließen jedoch die verbleibenden Stereozentren unbestimmt. Die erste Totalsynthese des (2’*R*, 3’*S*)-Isomers von Macrocidin B (**61**) nach Weber *et al*. ließ ebenfalls keine genauere Aussage zu den Stereozentren an C-2‘ und C-3‘ zu. Jedoch konnte die (2’*R*, 3’*S*)-Stereokonfiguration aufgrund starker Differenzen bei Vergleich der NMR-Spektren mit denen des Isolats ausgeschlossen werden.[58] Weber *et al*. versuchten zudem, Macrocidin B (**61**) in Isolaten von verschiedenen *Phoma macrostoma* Stämmen zu detektieren und scheiterten. Sie folgerten, dass Macrocidin B (**61**) eventuell kein natürlicher Metabolit des Pilzes ist. Graupner *et al*. publizierten 2006 die Strukturen von vier weiteren Metaboliten, die jedoch nur auf ^{1}H-NMR-Experimenten beruhen und seitdem nicht bestätigt wurden.[59]

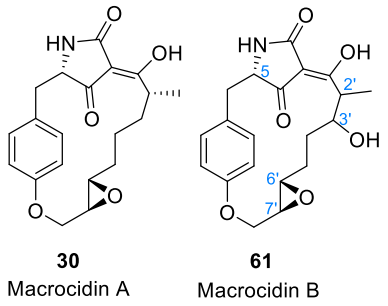


Abb 2. Strukturformeln von Macrocidin A (**30**) und Macrocidin B (**61**).[55]

1.3.2 Biologische Aktivität der Macrocidine

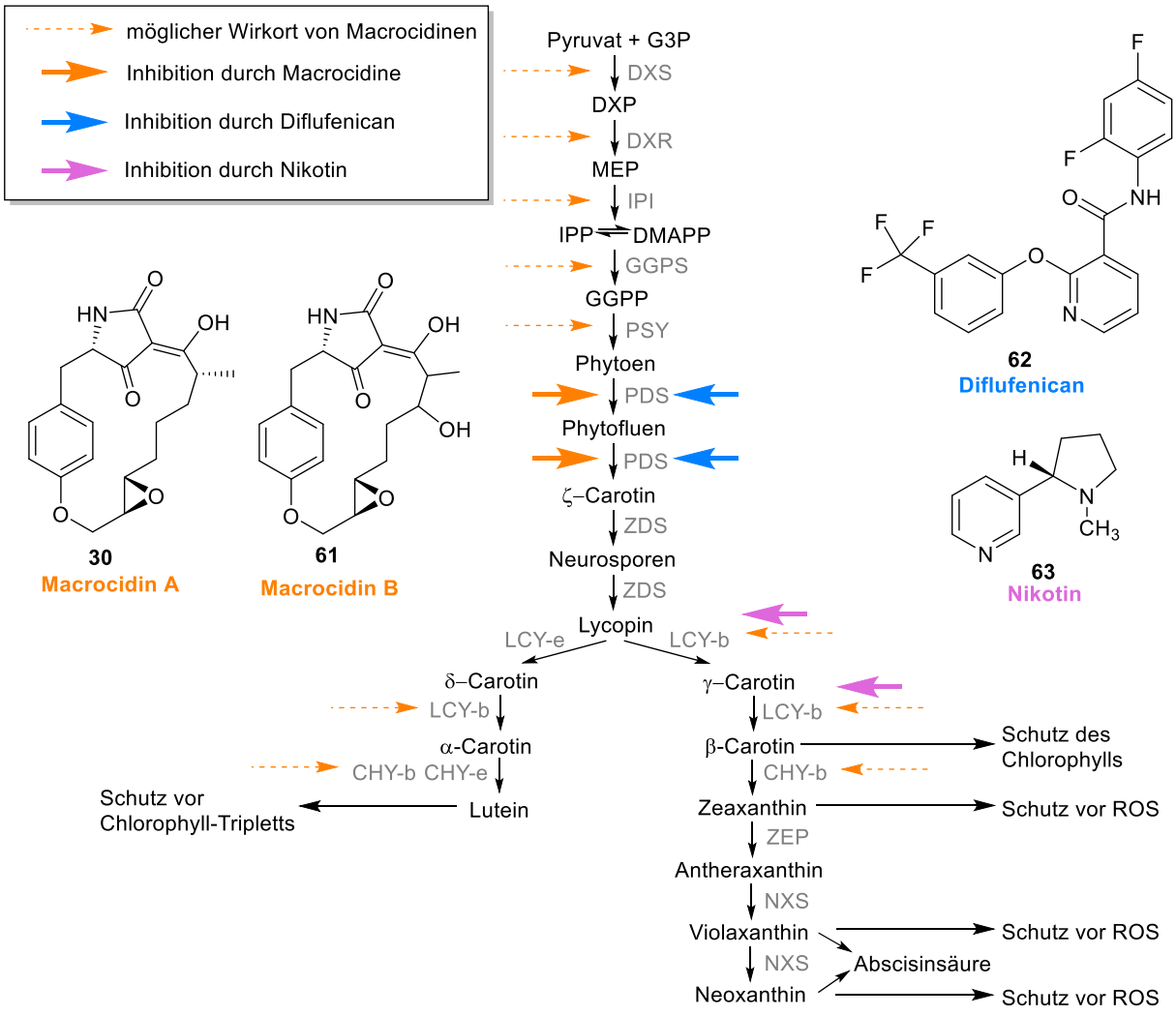
Im Zuge der Isolation von Macrocidin A (**30**) und B (**61**) führten Graupner *et al*. erste biologische Aktivitätstests der Reinsubstanzen durch.[55] Die vorher beobachtete Chlorose und Nekrose bei kanadischen Disteln durch Exktrakte von *Phoma macrostoma* konnten dadurch auf die beiden Metabolite zurückgeführt werden. Chlorosen und Wachstuminhibition konnte vor allem bei breitblättrigen Unkräutern beobachtet werden, wohingegen die Naturstoffe keine Wirkung auf Gräser zeigten.

Durch Aktivitätstest von *Phoma macrostoma* an einigen Nutzpflanzen und Unkräutern legten Bailey *et al*. einen Rahmen für dessen mögliche Anwendung als Bioherbizid fest.[60] Nutzpflanzen aus Familien der Kürbisgewächse (*Cucurbitaceae*; z.B. Wassermelone, Kürbis), Süßgräser (*Poaceae*; z.B. Hafer, Weizen), Leingewächse (*Linaceae*; z.B. Lein) und Nachtschattengewächse (*Solanaceae*; z.B. Tomate, Paprika) waren resistent gegen *Phoma macrostoma*. Dahingegen wurden Pflanzen aus den Familien der Korbblütler (*Asteraceae*; z.B.

Sonnenblume, Blattsalat), Kreuzblütler (*Brassicaceae*; z.B. Raps, Senf), Hülsenfrüchte (*Fabaceae*; z.B. Erbse, Linse) und Rosengewächse (*Rosaceae*; z.B. Erdbeere) durch *P. macrostoma* angegriffen. Bei diesen ist die Anwendung von Bioherbiziden auf Basis von *Phoma macrostoma* folglich ausgeschlossen. Als potenzielles Anwendungsgebiet konnte auch die Gruppe der Zierpflanzen und Gräser gefunden werden, da alle getesteten Familien dieser Gruppe resistent gegen *Phoma macrostoma* waren. Auch die Effekte auf diverse Unkräuter wurden getestet. Wie bei den Nutzpflanzen waren vor allem Spezies der Familien der Korbblütler (*Asteraceae*), Kreuzblütler (*Brassicaceae*) und Hülsenfrüchte (*Fabaceae*) anfällig. Die Arbeitsgruppe um Bailey erarbeitete außerdem erste Ansätze für den Wirkmechanismus der Macrocidine (Schema 14).[61] Die Symptomatik der behandelten Pflanzen zeigte starke Ähnlichkeit zu den Effekten bekannter Inhibitoren der Carotin-Biosynthese wie beispielsweise Diflufenican (**62**). Die Annahme eines vergleichbaren Wirkmechanismus konnte durch intensive Studien an Löwenzähnen und Disteln bestätigt werden. Das in der Carotin-Biosynthese involvierte Enzym Phytoen-Desaturase (PDS) wird sowohl von Diflufenican (**62**) als auch von den Macrocidinen inhibiert, was durch eine erhöhte Phytoenkonzentration und verringerte Konzentrationen von α-, β- und γ-Carotin sowie Violaxanthin in behandelten Pflanzen erkannt wurde. Interessanterweise stieg jedoch der Anteil an Lutein und das β-Carotin/Lutein-Verhältnis sank in den mit Macrocidinen behandelten Pflanzen. Aufgrunddessen schloss die Arbeitsgruppe auf weitere Eingriffe in die Carotin-Biosynthese, die sich von dem Wirkmechanismus von Diflufenican (**62**) unterscheiden. Sie sahen sowohl die Möglichkeit eines Eingriffs vor der PDS als auch in späteren Abschnitten der Carotin-Biosynthese.

In fortführenden Arbeiten gewannen Hubbard *et al*. weitere Erkenntnisse zu anderen Wirkorten der Macrocidine.[18] Zur besseren Einordnung der Ergebnisse wurden als Referenzsubstanzen sowohl Diflufenican (**62**) als auch Nikotin (**63**) als bekannter Inhibitor der Lycopin-β-Cyclase (LCY-b) verwendet. Nikotin (**63**) führt wie die Macrocidine zu einem verringerten β-Carotin/Lutein-Verhältnis in behandelten Pflanzen. Jedoch konnte ein Inhibiton von LCY-b durch die Macrocidine aufgrund von Unterschieden im zeitlichen Einsetzen der Effekte ausgeschlossen werden und stattdessen mit der allgemeinen Anreicherung von Xantophyllen (Lutein und Violaxanthin) nach Behandlung mit Macrocidinen in Verbindung gebracht werden. Der Grund für die Anreicherung der Xantophylle konnte durch Fluoreszenzmessungen gefunden werden. Durch das 3-Acyltetramsäuremotiv der Macrocidine komplexieren diese Fe^{2+}-Ionen, was durch einen komplexen Prozess zur Störung des Photosystems der Pflanzen und zur Beschädigung von β-Carotinen führt. Dies löst schließlich die Anreicherung der

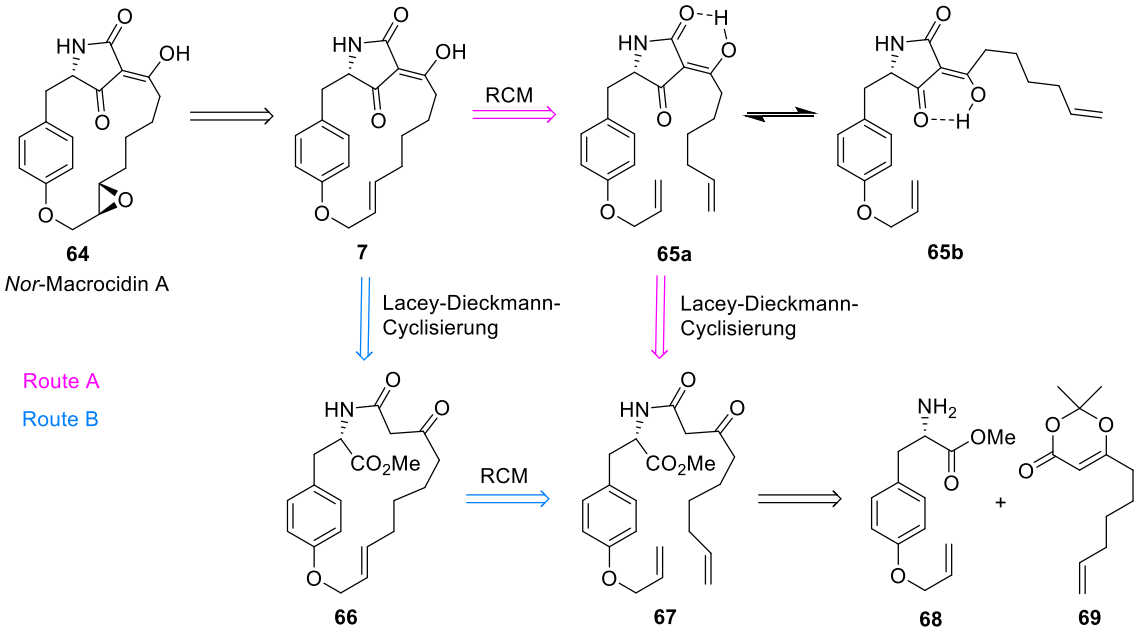
photoprotektiv wirkenden Xantophylle aus. Durch Komplexierung von Mg^{2+}-Ionen inhibieren sie zudem die Enyzme D-Xylulose-Reduktoisomerase (DXR) und Phytoen-Synthase (PSY). Zusammenfassend inhibieren Macrocidine verschiedene Enzyme der Carotin-Biosynthese und stören gleichzeitig das gesamte Photosystem durch Komplexierung von Fe^{2+}- und Mg^{+}-Ionen, was insgesamt zur Abnahme des Chlorophyll-Gehalts der Pflanzen führt.



Schema 14. Carotin-Biosynthese und (potenzielle) Wirkorte von Macrocidinen, Diflufenican (**62**) und Nikotin (**63**). In grau: die an der Biosynthese beteiligten, entscheidenden Enzyme (frei nach Literatur).[61,62]

1.3.3 Synthetische Studien zu Macrocidinen nach Ramana *et al.*

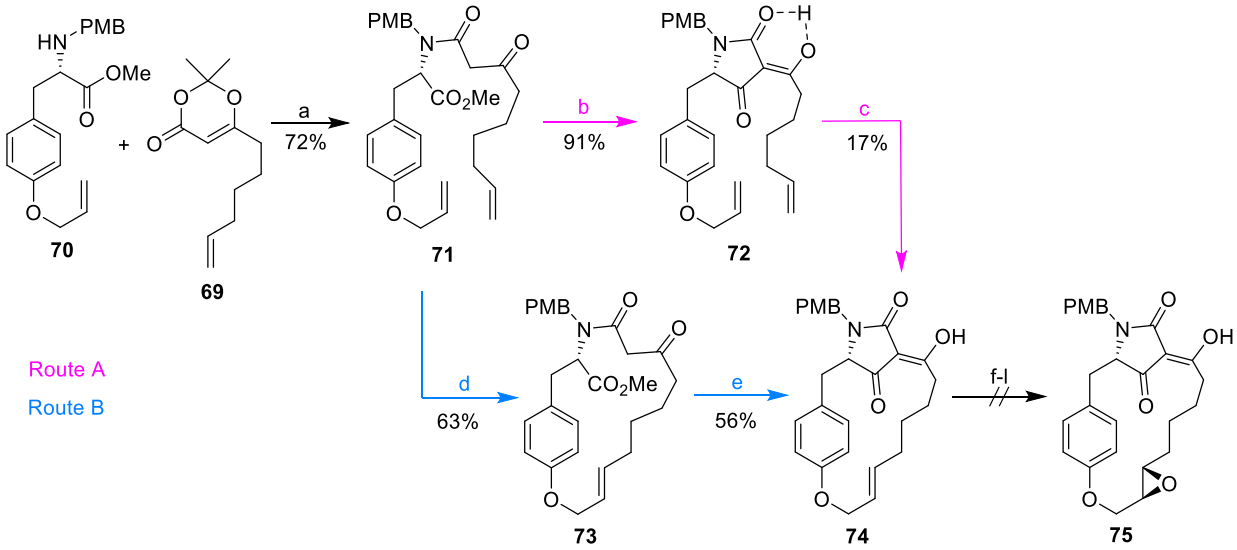
2006 wurden erstmals synthetische Studien zu Macrocidinen von Ramana *et al*. veröffentlicht. Ziel war die Synthese von *Nor*-Macrocidin A (**64**).[63] Die Retrosynthese beinhaltete zwei Routen, die sich in der Reihenfolge der beiden Schlüsselfragmente, der Bildung des Makrocyclus und der Tetramsäure, unterschieden (Schema 15). *Nor*-Macrocidin A (**64**) sollte durch Epoxidierung der *E*-konfigurierten Doppelbindung im Makrocyclus erhalten werden. Das dafür notwendige Zwischenprodukt sollte entweder nach Route A durch Ringschlussmetathese mit vorheriger Lacey-Dieckmann-Cyclisierung zur Tetramsäure **65a** entstehen oder nach Route B durch Lacey-Dieckmann-Cyclisierung nach der Ringschlussmetathese. Als Problem in Route A sahen Ramana *et al*. die mögliche Bildung des Tautomers **65b**, welche die beiden terminalen Doppelbindungen räumlich voneinander entfernt und dadurch die Ringschlussmetathese erschwert. Das für beide Routen notwendige β-Ketoamid **67** sollte aus dem geschützten Tyrosin **68** und dem Dioxin **69** entstehen.



Schema 15. Retrosynthese zu *Nor*-Macrocidin A (**64**) nach Ramana *et al*.[63]

Zuerst wurde der Aminosäureester **70** mit [1,3]-Dioxin-4-on (**69**) unter Rückfluss zum β-Ketoamid **71** umgesetzt (Schema 16).[63] Anschließend gelang die Cyclisierung zur Tetramsäure **72** ausgehend vom β-Ketoamid **71** in 91% Ausbeute. Es stellte sich heraus, dass die Einführung einer Schutzgruppe am Amin für die Bildung der Tetramsäure zwingend erforderlich war. Alle getesteten Reaktionen mit einem freien Amid lieferten nicht das gewünschte Produkt. Die darauffolgende Ringschlussmetathese konnte nur in geringen Ausbeuten realisiert werden (→ **24**). Dies bestätigte die Annahme, dass durch ein im Gleichgewicht auftretendes

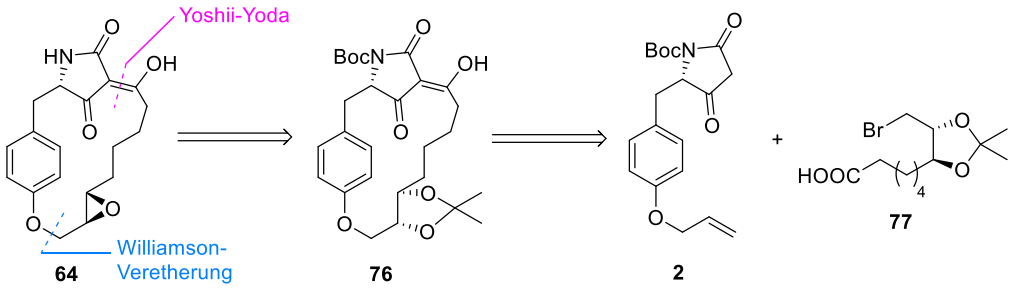
Tautomer der Tetramsäure und die dabei hervorgerufene Entfernung beider terminaler Doppelbindungen die Reaktion verlangsamt. Die Ausbeuten in Route B waren dagegen vielversprechender. Die Makrocyclisierung gelang in 63%, worauf die Lacey-Dieckmann-Cyclisierung zur 3-Acyltetramsäure **74** in 56% Ausbeute folgte. Die Synthese des gewünschten *Nor*-Macrocidin A (**64**) scheiterte schließlich an der Epoxidierung der Doppelbindung. Die üblichen Reagenzien wie *m*CPBA, wässrige Lösungen von H_2O_2, Sharpless-Bedingungen und Oxon führten alle lediglich zur Zersetzung des Edukts **74**. Ramana *et al*. folgerten, dass die 3-Acyltetramsäure **74** unter den getesteten Epoxidierungsbedingungen instabil sein muss.



Schema 16. Schritte innerhalb der versuchten Darstellung von *Nor*-Macrocidin A (**64**) nach Ramana *et al*.[63] *Reagenzien und Bedingungen*: a) PPTS, Toluol, Δ, 7 h; b) KO*t*Bu, *t*BuOH, RT, 30 min; c) Grubbs I (20 Mol-%), CH_2Cl_2, Δ, 12 h; d) Grubbs I (10 Mol-%), CH_2Cl_2, Δ, 36 h; e) KO*t*Bu, *t*BuOH, RT, 30 min; f) *m*CPBA, CH_2Cl_2, –78 °C, 4 h; g) *m*CPBA, CH_2Cl_2, RT; h) Oxon, Aceton, EtOAc, RT; i) H_2O_2, $NaHCO_3$, THF/H_2O; j) H_2O_2, $NaHCO_3$, $CHCl_3$/H_2O; k) H_2O_2, $NaHCO_3$, PhCN/MeOH; l) $Ti(OiPr)_4$, DIPT, *t*BuOOH, CH_2Cl_2, –78 °C.

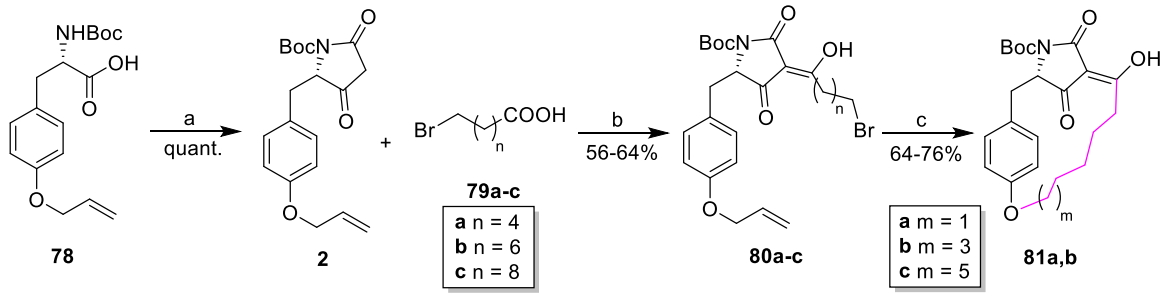
1.3.4 Synthetische Studien zu Macrocidin A (30) nach Barnickel *et al.*

Im Jahr 2010 wurden von der Arbeitsgruppe um Schobert ebenfalls synthetische Studien zu Macrocidinen veröffentlicht.[64] Ziel war, eine Möglichkeit zur Synthese diverser Derivate mit dem Grundgerüst der Macrocidine zu entwickeln. Ähnlich wie Ramana *et al*.[63] wählten sie dafür *Nor*-Macrocidin A (**64**) als Zielmolekül (Schema 17). Das Epoxid sollte auf später Stufe aus einem geschützten Diol entstehen. Davor sollte durch eine Williamson-Ethersynthese in Anlehnung an die Synthese von Rapamycin nach Maddess *et al*.[65] das Ringsystem entstehen. Die Tetramsäure sollte durch eine Yoshii-Yoda-Acylierung der 3-H_2-Tetramsäure **2** mit der Carbonsäure **77** aufgebaut werden.



Schema 17. Retrosynthese von *Nor*-Macrocidin A (**64**) mit retrosynthetischen Schnitten nach Barnickel *et al*.[64]

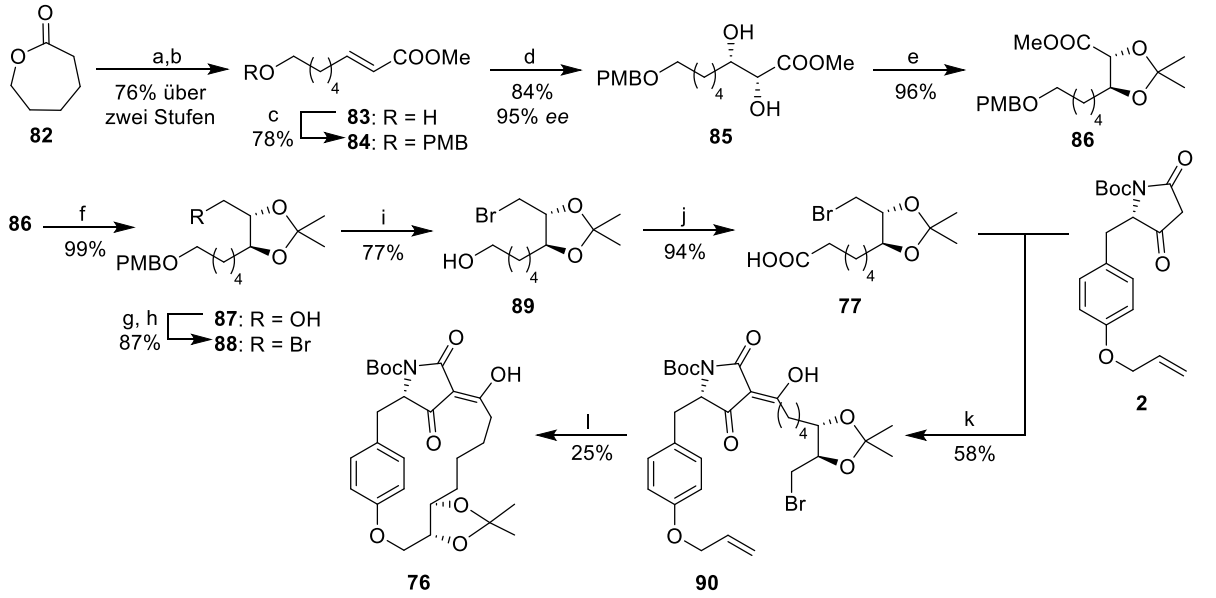
Zum Test der Williamson-Ethersynthese stellten Barnickel *et al*. zunächst drei einfache Derivate **80a-c** der Macrocidine ohne jegliche Funktionalisierung im Linker (pink markiert) dar (Schema 18).[64] Das kommerziell erhältliche geschützte Tyrosin **78** wurde durch Umsetzung mit Meldrumsäure, DCC und DMAP in quantitativer Ausbeute in die Tetramsäure **2** überführt. Diese wurde nach dem Protokoll von Yoshii-Yoda an 3-Position mit den ω-Bromcarbonsäuren **79a-c** acyliert. Darauf folgte die Entfernung der Allyl-Schutzgruppe im schwach Basischen katalysiert durch $Pd(PPh_3)_4$. Bei den gewählten Bedingungen kam es jedoch nicht nur zur Entschützung, sondern sofort zum Ringschluss (→ **81a,b**). Der vergrößerte 20-gliedrige Makrocyclus konnte jedoch nicht isoliert werden.



Schema 18. Synthese vereinfachter Macrocidin-Derivate **81a-b** nach Barnickel *et al*.[64]
Reagenzien und Bedingungen: a) Meldrumsäure, DCC, DMAP, CH_2Cl_2, RT, 2.5 h; b) 1. DMAP, DCC, CH_2Cl_2, 0 °C→RT, 1.5 h; 2. NEt_3, RT→Δ, 22-24 h; c) $Pd(PPh_3)_4$, K_2CO_3, THF/MeOH (5:1), Δ, 27-44 h.

Für die Synthese von *Nor*-Macrocidin A (**64**) musste die passende Carbonsäure **77** synthetisiert werden (Schema 19). Gestartet wurde dafür von ε-Caprolacton (**82**), welches in zwei Schritten zum konjugierten Ester **83** umgesetzt wurde. Nach PMB-Schützung erfolgte eine asymmetrische Sharpless-Dihydroxylierung zum Diol **85** in 84% Ausbeute und 95% *ee*. Der Diol wurde Acetonid-geschützt und der Ester zum Alkohol in 99% Ausbeute reduziert (→ **87**). Nach Mesylierung und Finkelstein-artiger Reaktion entstand das Bromid **88**. Schließlich wurde die PMB-Schutzgruppe entfernt und die primäre Hydroxylfunktion mit PDC zur Carbonsäure **77** in 94% Ausbeute oxidiert. Die anschließende Yoshii-Yoda-Acylierung der Carbonsäure an die Tetramsäure **2** lieferte die 3-Acyltetramsäure **90** in 58% Ausbeute. Darauf sollte die Allylent-

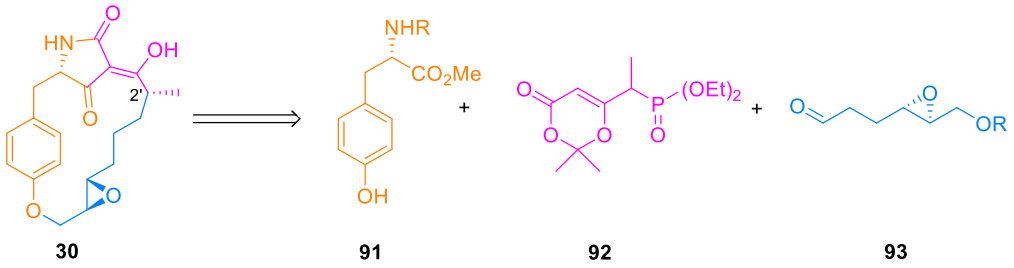
schützung mit Ethersynthese durch Zugabe von K_2CO_3 und $Pd(PPh_3)_4$ erfolgen. Unter den bereits ausgearbeiteten Bedingungen konnte jedoch nur die allylentschützte Tetramsäure erhalten werden. Bei Verwendung von Cs_2CO_3 und $Pd(PPh_3)_4$ wurde in einer Nebenreaktion dehalogeniert statt der eigentlich geplanten Makrocyclisierung. Erfolgreich war schließlich die Verwendung von K_2CO_3 und 18-Krone-6 sowie $Pd(PPh_3)_4$ in *tert*-Butylalkohol. Mit diesem Reagenziengemisch konnte sowohl die Dehalogenierung unterdrückt als auch die Makroveretherung forciert werden. Die komplexierenden Eigenschaften von 3-Acyltetramsäuren führten bei der Aufreinigung des Produktes **76** zu Problemen. Nach Säulenchromatographie über Kieselgel konnte nur eine Mischung aus verschiedenen Metallkomplexen erhalten werden, was in den NMR-Spektren zu Linienverbreiterung führte. Durch RP-Säulenchromatographie mit anschließender wässriger Extraktion mit Na_2EDTA-Lösung konnte die 3-Acyltetramsäure **76** schließlich rein erhalten werden. Die weiteren Schritte zur Synthese von *Nor*-Macrocidin A (**64**) wurden nicht beschrieben.



Schema 19. Synthese der Carbonsäure **77** für die Synthese von *Nor*-Macrocidin A (**64**) und weitere Syntheseschritte zum Schlüsselintermediat **76** nach Barnickel *et al*.[64]
Reagenzien und Bedingungen: a) DIBAL, CH_2Cl_2/THF, –78 °C, 15 min; b) $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, RT, 23 h; c) PMB-Trichloracetimidat, PPTS, CH_2Cl_2, RT, 20 h; d) AD-Mix α, H_2O/*t*BuOH (1:1), RT→0 °C, 3 d; e) $Me_2C(OMe)_2$, *p*TsOH, CH_2Cl_2, RT, 20 min; f) $LiAlH_4$, Et_2O, RT, 19 h; g) MsCl, NEt_3, CH_2Cl_2, 0 °C→RT, 1.5 h; h) LiBr, Aceton, Δ, 2.5 d; i) DDQ, CH_2Cl_2/H_2O (20:1), RT, 1.5 h; j) PDC, DMF, RT, 17 h; k) DCC, DMAP, CH_2Cl_2, 0 °C→RT, 2 h; dann NEt_3, 0 °C→Δ, 19.5 h; l) $Pd(PPh_3)_4$, K_2CO_3, 18-Krone-6, *t*BuOH, Δ, 3 d.

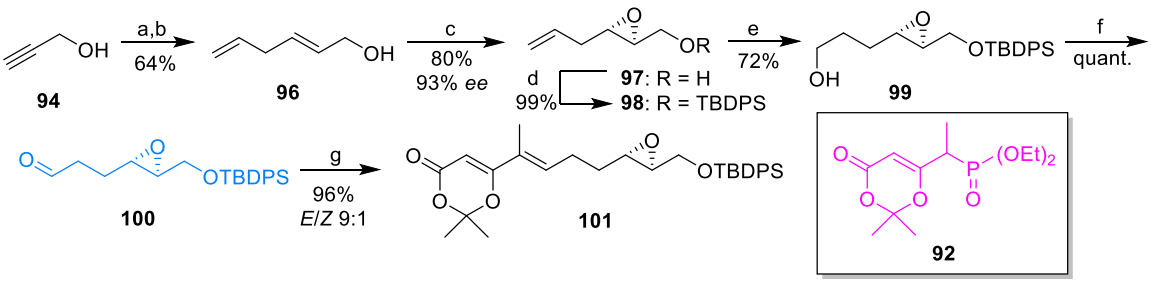
1.3.5 Erstsynthese von Macrocidin A (30) nach Yoshinari *et al.*

Die Erstsynthese von Macrocidin A (**30**) gelang Yoshinari *et al*. 2010.[56] Sie wählten als Schlüsselbausteine das Methyltyrosinat **91**, das Phosphonat **92** und den Aldehyd **93** (Schema 20). Beim Aufbau der Verbindung **93** sahen sie die Einführung des Stereozentrums an C-2‘ als Schlüsselschritt an. Nach Kupplung des Fragmentes **93** mit dem Tyrosin und Makrolactamisierung sollte die Bildung der Tetramsäure durch Lacey-Dieckmann-Cyclisierung die Totalsynthese abschließen.



Schema 20. Retrosynthetische Schnitte im Zielmolekül Macrocidin A (**30**) und die resultierenden Edukte.[56]

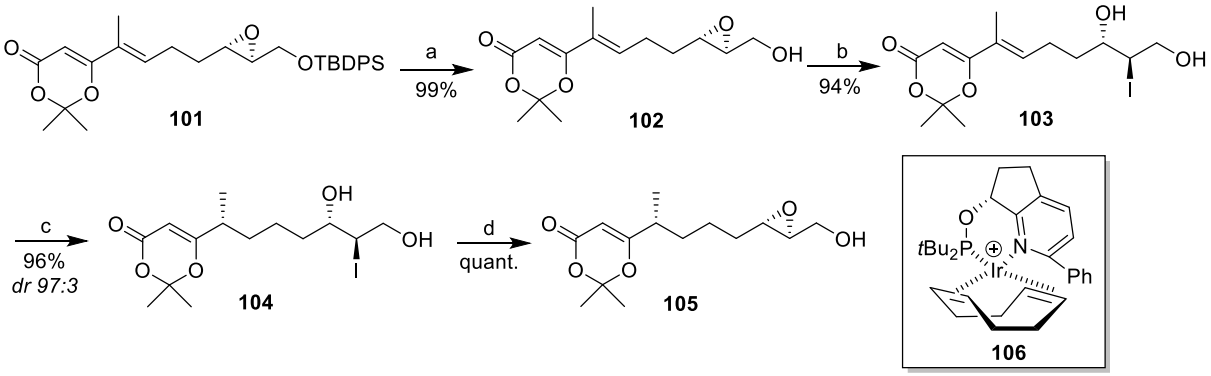
Yoshinari *et al*. starteten mit der Synthese des Aldehyds **100** ausgehend von Propargylalkohol (**94**; Schema 21).[56] Dieser wurde in zwei Schritten durch eine Kupfer-vermittelte Kupplung und anschließende Reduktion in den Allylalkohol **96** überführt. Die Einführung des Epoxids erfolgte durch eine enantioselektive Sharpless-Epoxidierung in 80% Ausbeute und 93% *ee* (→ **97**). Nach Silylschützung der endständigen Hydroxylfunktion folgte eine Rhodium-katalysierte Hydroborierung zum Alkohol **99**. Swern-Oxidation lieferte den ersten Schlüsselbaustein **100** in quantitativer Ausbeute. Der Aldehyd **100** wurde mit dem Phosphonat **92** in einer HWE-Olefinierung verknüpft (→ **101**).



Schema 21. Erste Schritte innerhalb der Synthese von Macrocidin A (**30**) nach Yoshinari *et al*.[56]
Reagenzien und Bedingungen: a) Allylbromid, CuI, NaI, K_2CO_3, AcMe, RT, 5 h; b) $LiAlH_4$, THF, Δ, 2 h; c) $Ti(OiPr)_4$, (+)-L-DET, *t*BuOOH, 4 Å MS, CH_2Cl_2, −20 °C, 24 h; d) TBDPSCl, Imidazol, DMF, RT, 1 h; e) Catecholboran, $[Rh(PPh_3)_3Cl]$, THF, 0 °C, 1.5 h, H_2O_2; f) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C→0 °C, 1.5 h; g) **92**, LDA, HMPA, THF, −78 °C→0 °C.

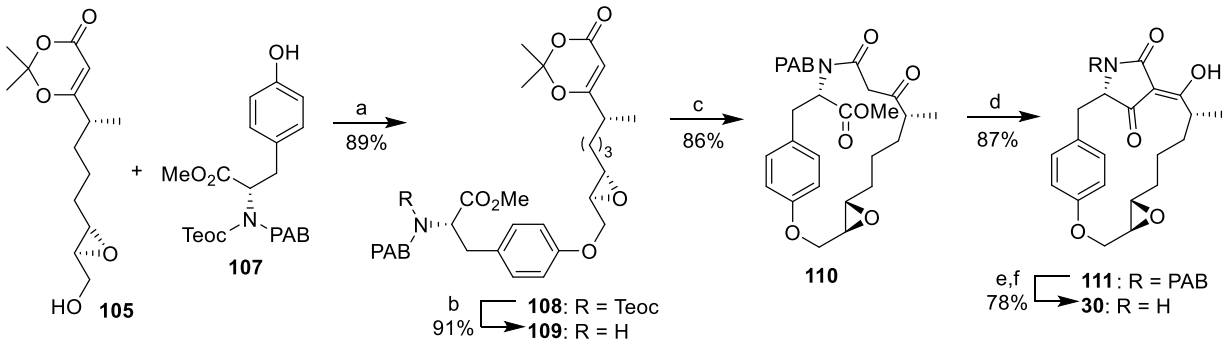
Die enantioselektive Hydrierung des Bausteins **101** war aufgrund der Instabilität des Epoxids unter den notwendigen Bedingungen nur in niedrigen Ausbeuten möglich (Schema 22).[56]

Nach Entfernung der Silylschutzgruppe (→ **102**) und regioselektiver Ringöffnung konnte der Alkohol **103** erhalten werden. Dieser wurde mittels Hydrierung unter Verwendung des Iridium-Katalysators **106** in 96% Ausbeute und einem *dr* von 97:3 zur Verbindung **104** umgesetzt. K_2CO_3 führte anschließend zur regioselektiven Epoxidbildung (→ **105**).



Schema 22. Einführung des Stereozentrums an der Methylgruppe innerhalb der Synthese von Macrocidin A (**30**) nach Yoshinari *et al*.[56]
Reagenzien und Bedingungen: a) TBAF, THF, 0 °C, 1 h; b) NaI, $B(OAc)_3$, AcOH, AcMe, −20 °C→0 °C, 2 h; c) H_2 (10 MPa), **106**, CF_3CH_2OH, 40 °C, 12 h; d) K_2CO_3, MeOH, RT, 1 h.

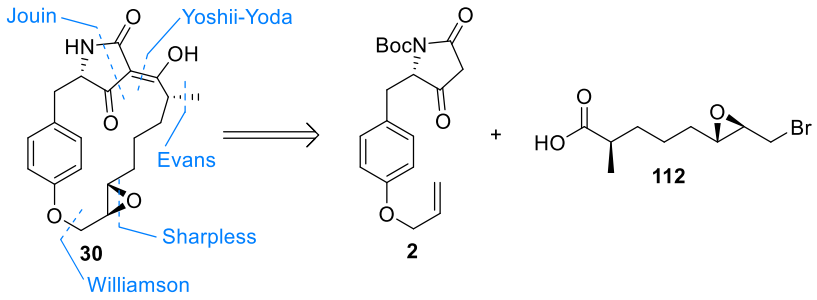
Nach Fertigstellung der Seitenkettensynthese wurden der Alkohol **105** und das geschützte Tyrosin **107** durch eine Williamson-Ethersynthese gekuppelt (Schema 23).[56] Die Schutzgruppen am Amin wurden gewählt, nachdem die Lacey-Dieckmann-Cyclisierung mit dem ursprünglich geplanten freien Amin scheiterte. Nach Entfernung der Teoc-Schutzgruppe folgte die Makrolactamisierung durch Erhitzen in Toluol (→ **110**). Die Lacey-Dieckmann-Cyclisierung induziert durch KO*t*Bu lieferte die Tetramsäure **111** in 87% Ausbeute. Durch Entschützung in zwei Stufen wurde Macrocidin A (**30**) in 78% Ausbeute erhalten.



Schema 23. Letzte Syntheseschritte zu Macrocidin A (**30**) nach Yoshinari *et al*.[56]
Reagenzien und Bedingungen: a) DEAD, PPh_3, PhMe, RT, 3 h; b) TBAF, THF, RT, 4 h; c) PhMe, Δ, 2h; d) KO*t*Bu, *t*BuOH, THF, RT, 30 min; e) H_2, Pd/C (10 Gew.-%), THF/MeOH, RT, 2 h; f) DDQ, H_2O, THF, RT, 0.5 h.

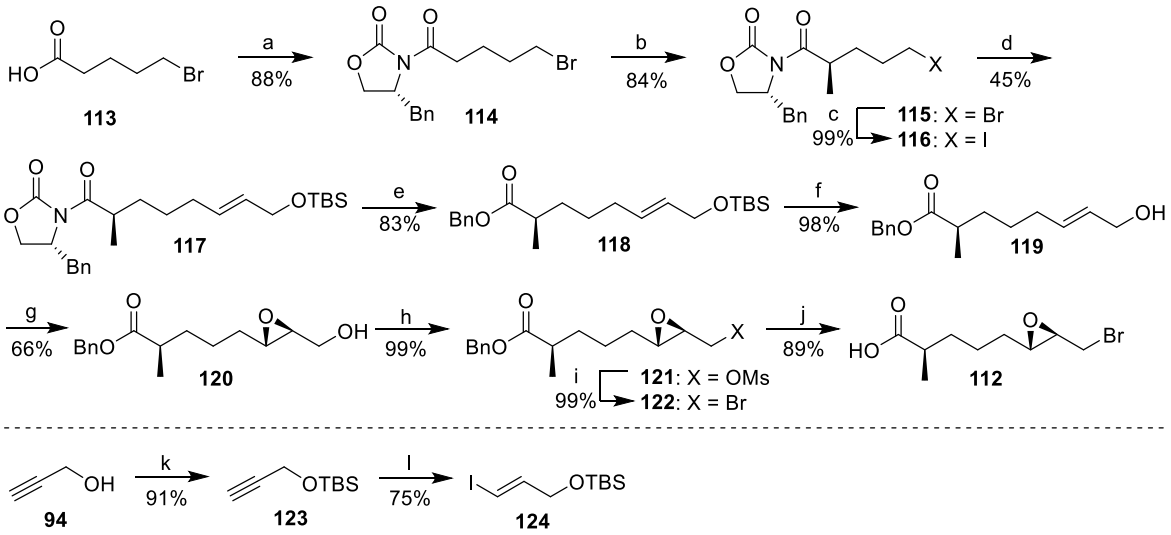
1.3.6 Synthese von Macrocidin A (30) nach Haase *et al.*

Die zweite Totalsynthese von Macrocidin A (**30**) gelang 2016 Haase *et al.*[57] Die retrosynthetischen Schnitte orientierten sich an den Vorversuchen von Barnickel (Schema 24).[64] Der Makrocyclus sollte durch eine Williamson-Makroveretherung entstehen. Vorher sollte die 3-Acyltetramsäure durch 3-Acylierung der 3-H_2-Tetramsäure **2** nach Yoshii-Yoda gebildet werden. Die Carbonsäure **112** sollte bereits alle notwendigen Funktionalisierungen tragen, wobei das Epoxid durch eine Sharpless-Epoxidierung und die Methyl-Gruppe durch eine Evans-Alkylierung eingeführt werden sollte.



Schema 24. Retrosynthetische Schnitte der Totalsynthese von Macrocidin A (**30**) nach Haase *et al.*[57]

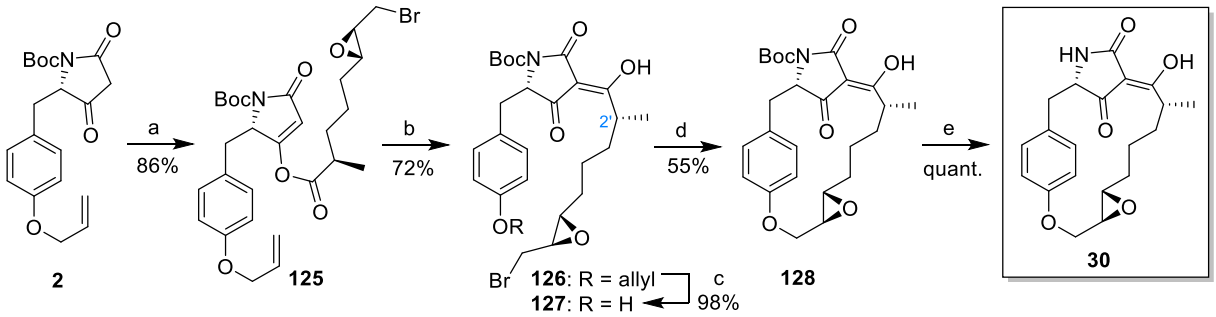
Gestartet wurde mit der Synthese der Carbonsäure **112** ausgehend von ω-Bromvaleriansäure (**113**; Schema 25).[57] An diese wurde im ersten Schritt das Evans-Auxiliar in 88% Ausbeute angebracht. Die diastereoselektive Methylierung lieferte das Bromid **115** mit einem *dr* von 10:1. Durch Säulenchromatographie konnten die Diastereomere getrennt werden. Das benötigte Diastereomer wurde einer Finkelstein-Reaktion unterzogen und das dabei entstandene Iodid **116** wurde in 45% Ausbeute via Negishi-Kupplung mit dem Vinyliodid **124** verknüpft. Letzteres konnte in zwei Schritten aus Propargylalkohol (**94**) gewonnen werden. Der Alkohol **94** musste dafür zunächst TBS-geschützt werden, woraufhin eine Hydrozirkonierung mit dem Schwartz-Reagenz folgte. Durch Zugabe von Iod konnte das entstehende Intermediat in das Iodid **124** überführt werden. Nach der Pd-katalysierten Kreuzkupplung der beiden Iodide folgte die Entfernung des Evans-Auxiliars durch Benzylalkoholyse (→ **118**). Entfernung der TBS-Schutzgruppe mittels HF und eine Sharpless-Epoxidierung lieferten das Epoxid **120** als einzelnes Stereoisomer in 66% Ausbeute. Das Überführen der primären Hydroxygruppe ins Mesylat (→ **121**) und eine finkelsteinartige Reaktion ergaben das Bromid **122** in 99% Ausbeute. Schließlich generierte eine Hydrierung unter hetereogener Pd-Katalyse die Carbonsäure **112** in 89% Ausbeute.



Schema 25. Synthese der Carbonsäure **112** innerhalb der Totalsynthese von Macrocidin A (**30**) nach Haase *et al*.[57]

Reagenzien und Bedingungen: a) 1. PivCl, NEt_3, THF, 0 °C, 20 min; 2. LiCl, (*R*)-4-Benzyl-2-oxazolidinon, THF, 0 °C, 30 min; b) NaHMDS, MeI, THF, –78 °C, 1 h; c) NaI, Aceton, 80 °C, 30 min; d) 1. **116**, Zn, DMAc, 120 °C→80 °C, 3 h, 2. $Pd(dppf)Cl_2$, **124**, DMAc, 0 °C, 2 h; e) BnOLi, THF, 0 °C→RT, 30 min; f) HF (48% in H_2O), MeCN, 0 °C, 15 min; g) $Ti(OiPr)_4$, (+)-L-DET, *t*BuOOH, 4 Å MS, CH_2Cl_2, –25 °C, 5 h; h) MsCl, NEt_3, CH_2Cl_2, RT, 30 min; i) LiBr, Aceton, 80 °C, 1.5 h; j) Pd/C, H_2 (1 atm), MeOH, RT, 1.5 h; k) TBSCl, NEt_3, Imidazol, CH_2Cl_2, RT, 1 h; l) 1. $ZrCp_2Cl_2$, DIBAL, THF, 0 °C→RT, 30 min, 2. I_2, THF, –78 °C, 1 h.

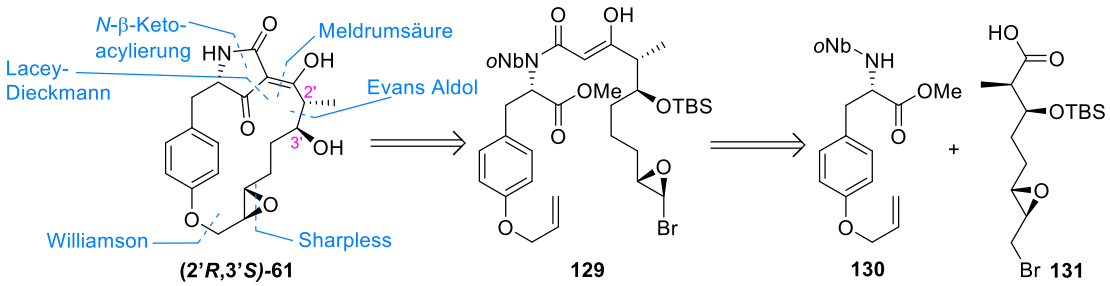
Die Carbonsäure **112** konnte nun mit der Tetramsäure **2** verknüpft werden (Schema 26).[57] Letztere konnte nach dem bekannten Protokoll aus der entsprechenden, geschützten Aminosäure dargestellt werden.[64] Nach Yoshii-Yoda wurde zunächst 4-*O*-acyliert durch Umsetzung mit EDC·HCl und DMAP (→ **125**). Die 4-*O*-Alkyltetramsäure **125** konnte daraufhin mit $CaCl_2$, NEt_3 und DMAP zur 3-Acyltetramsäure **126** in 72% Ausbeute umgelagert werden. Durch die Zugabe von $CaCl_2$ konnte vermutlich aufgrund der damit einhergehenden Komplexbildung die Racemisierung des Stereozentrums an C-2‘ unterdrückt werden. Nach Entfernung der Allyl-Schutzgruppe mit K_2CO_3 und $Pd(PPh_3)_4$ folgte die Williamson-Makroveretherung. Durch Verwendung von katalytische Mengen TBAI wurde *in situ* ein Iodid erzeugt. Durch die Chelatisierung von Kalium-Ionen zwischen dem Carbonyl-Sauerstoff der Amid-Gruppe und der 3-Acyleinheit wird die Konformation fixiert und das Iodid in die Nähe des Phenolats gedrängt. Der Kronenether bindet restliche freie Kalium-Ionen und sorgt somit für ein nacktes Phenolat-Ion. Durch die Gesamtheit dieser Faktoren gelang die Makroveretherung in guten 55% Ausbeute (→ **128**). Die finale Boc-Entschützung mit TFA lieferte Macrocidin A (**30**) in quantitativer Ausbeute. Im Vergleich zur Erstsynthese durch Yoshinari *et al*. könnte die Synthese nach Haase leicht angepasst werden, um sowohl Macrocidin B (**61**) als auch weitere Verbindungen mit Macrocidin-Grundgerüst darzustellen.



Schema 26. Finale Schritte in der Synthese von Macrocidin A (**30**) nach Haase *et al.*[57]
Reagenzien und Bedingungen: a) **112**, EDC·HCl, DMAP, CH_2Cl_2, RT, 3 h; b) $CaCl_2$, DMAP, NEt_3, CH_2Cl_2, RT, 24 h; c) K_2CO_3, $Pd(PPh_3)_4$, MeOH, RT, 2 h; d) K_2CO_3, 18-Krone-6, TBAI, DMF, 100 °C, 24 h; e) TFA, CH_2Cl_2, RT, 5 min.

1.3.7 Synthese eines Isomers von Macrocidin B (61) nach Weber *et al.*

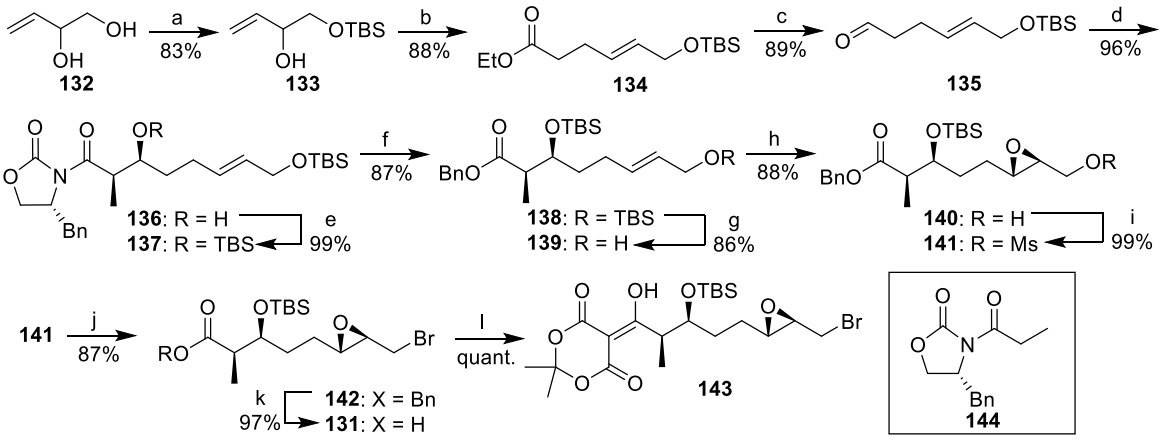
Ebenfalls aus der Arbeitsgruppe um Schobert stammt die erste Synthese eines Isomers von Macrocidin B (**61**).[58] Da die Stereokonfiguration des Naturstoffs bislang ungeklärt ist, wurde nach biosynthetischen Betrachtungen das (2'*R*,3'*S*)-Isomer (2'*R*,3'*S*)-**61** als Zielmolekül gewählt (Schema 27). Die retrosynthetischen Schnitte unterschieden sich vor allem im Aufbau der Tetramsäureeinheit von der vorher publizierten Totalsynthese von Macrocidin A (**30**).[57] Der Tyrosinester **130** sollte zunächst mit der Carbonsäure **131** zu einem β-Ketoamid **129** *N*-acyliert werden, um anschließend einer Lacey-Dieckmann-Cyclisierung unterzogen zu werden. Der Aufbau des Makrocyclus sollte in Anlehnung an die Macrocidin A-Synthese von Haase durch Williamson-Veretherung erfolgen.[57] Die Einführung der Stereozentren im Schlüsselfragment **131** sollte durch Sharpless-Epoxidierung und stereoselektive Evans-Aldol-Reaktion erreicht werden.



Schema 27. Retrosynthese des (2'*R*,3'*S*)-Isomers von Macrocidin B (2'*R*,3'*S*)-**61** nach Weber *et al.*[58]

Gestartet wurde mit der Synthese des Meldrumsäureadduktes **143** (Schema 28). Ausgehend vom Diol **132** wurde zunächst selektiv die primäre Hydroxygruppe TBS-geschützt. Durch eine Johnson-Claisen-Umlagerung nach Umsetzung mit Triethylorthoacetat wurde der Ester **134** in 88% Ausbeute erhalten. Selektive Reduktion mit DIBAL lieferte den Aldehyd **135**. Es folgte eine *syn*-selektive Evans-Aldol-Reaktion mit dem Imid **144** zum Kupplungsprodukt **136** in 96%

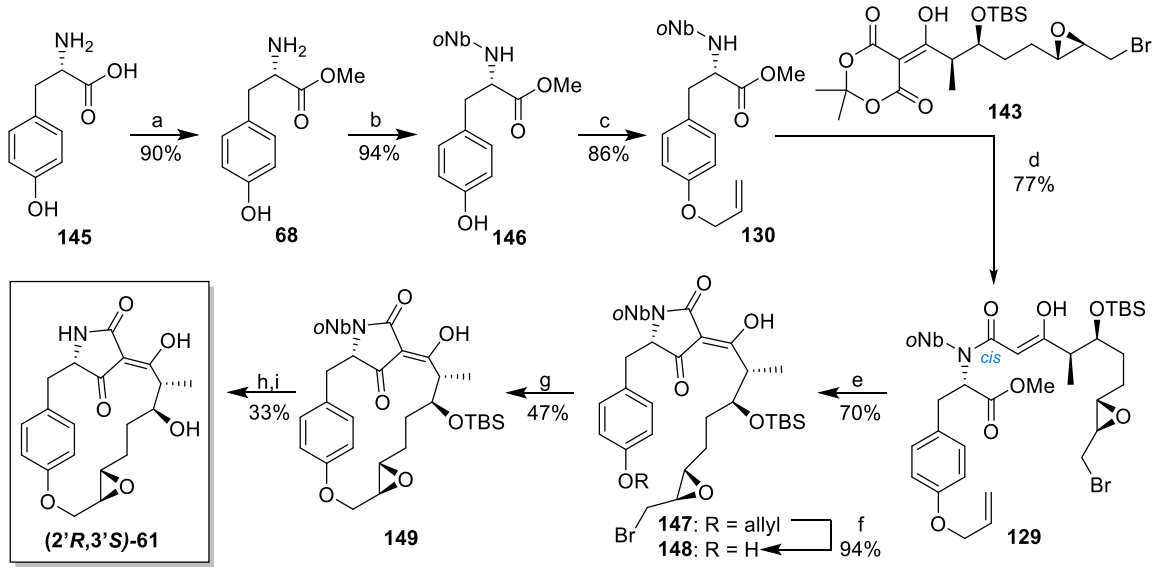
Ausbeute. Nach Silylschützung des sekundären Alkohols wurde das Evans-Auxiliar abgespalten. Der entstandene Ester **138** konnte in 86% Ausbeute regioselektiv an der primären Hydroxyfunktion entschützt werden. Nach Sharpless-Epoxidierung der Doppelbindung, wurde der endständige Alkohol **140** mesyliert und in einer finkelsteinartigen Reaktion mit LiBr in Aceton in das Bromid **142** überführt. Es folgten eine hydrogenolytische Spaltung des Esters **142** in 97% Ausbeute und die Umsetzung mit Meldrumsäure, EDC·HCl und DMAP zum Addukt **143**.



Schema 28. Synthese des Meldrumsäureaddukts **143** innerhalb der Synthese des (2'*R*,3'*S*)-Isomers von Macrocidin B (2'*R*,3'*S*)-**61**.[58]
Reagenzien und Bedingungen: a) TBSCl, NEt_3, DMAP, CH_2Cl_2, RT, 18 h; b) $MeC(OEt)_3$, $EtCO_2H$, DMF, Δ, 3 h; c) DIBAL, CH_2Cl_2, −78 °C, 2 h; d) **144**, Bu_2BOTf, NEt_3, CH_2Cl_2, −78 °C→0 °C, 1 h; e) TBSOTf, 2,6-Lutidin, CH_2Cl_2, RT, 24 h; f) *n*BuLi, BnOH, THF, 0 °C, 6 h; g) H_2O, AcOH, THF, 0 °C, 24 h; h) $Ti(iOPr)_4$, L-DET, *t*BuOOH, 4 Å MS, CH_2Cl_2, −25 °C, 6 h; i) NEt_3, MsCl, CH_2Cl_2, RT, 3 h; j) LiBr, Aceton, 80 °C, 3 h; k) H_2, Pd/C, EtOAc, RT, 24 h; l) EDC·HCl, DMAP, Meldrumsäure, CH_2Cl_2, RT, 24 h.

Es folgte die Darstellung des Fragmentes **130** ausgehend von L-Tyrosin (**145**; Schema 29). Die Aminosäure **145** wurde metyhlverestert und mittels *o*-Nitrobenzaldehyd sowie $NaBH_3CN$ an der Aminofunktion geschützt (→ **146**). Schließlich wurde durch Umsetzung mit K_2CO_3 und Allylbromid in 86% Ausbeute das vollständig geschützte Tyrosinat **130** erhalten. Die Acylierung mit dem Meldrumsäureaddukt **143** konnte durch Erhitzen in 1,4-Dioxan in 77% Rohausbeute realisiert werden (→ **129**). Die durch NaOMe induzierte Lacey-Dieckmann-Cyclisierung lieferte die 3-Acyltetramsäure **147** in 70% Ausbeute. Weber *et al*. postulierten, dass durch die sterisch anspruchsvolle *o*-Nitrobenzylschutzgruppe am Amid die *cis*-Konfiguration der Amid-Bindung induziert wurde und dadurch die Cyclisierung zur Tetramsäure beschleunigt wurde. Der Ringschluss via Williamson-Veretherung in Anlehnung an die Macrocidin A-Synthese lieferte den Makrocyclus **149** in 47% Rohausbeute. Finale Entschützungen der Hydroxyl- und Amidfunktion ergaben das (2'*R*,3'*S*)-Isomer von Macrocidin B (2'*R*,3'*S*)-**61**. Beim Vergleich der NMR-Spektren des synthetisierten Isomers mit denen des

Isolats von Graupner *et al.*[55] zeigten sich deutliche Unterschiede. Die synthetisierte Kombination an Konfigurationen konnte somit für das isolierte Macrocidin B (**61**) ausgeschlossen werden, jedoch bleibt die exakte Struktur unklar.



Schema 29. Zweiter Teil der Synthese des (2'*R*,3'*S*)-Isomers von Macrocidin B (2'*R*,3'*S*)-**61**.[58]
Reagenzien und Bedingungen: a) $SOCl_2$, MeOH, RT, 26 h; b) (*o*-NO_2)C_6H_4CHO, $NaBH_3CN$, 3 Å MS, AcOH in MeOH, RT, 2 h; c) K_2CO_3, Allylbromid, DMF, RT, 24 h; d) 3 Å MS, Dioxan, Δ, 3 h, e) NaOMe, MeOH, RT, 10 min; f) K_2CO_2, $Pd(PPh_3)_4$, MeOH, RT, 27 h; g) K_2CO_3, 18-Krone-6, TBAI, Aceton, 80 °C, 28 h; h) HF, $H_2[SiF_6]$, H_2O, MeCN, RT, 30 min; i) 365 nm, MeCN, H_2O, RT, 48 h.

1.4 Kibdelomycin – Synthesen und Bioaktivität

1.4.1 Isolation und Struktur

Ein weiterer Vertreter der Tetramsäuren ist Kibdelomycin, welches 2011 im Zuge der Suche nach neuen Naturstoffklassen mit antibiotischem Wirkprofil durch Phillipps *et al*. entdeckt wurde.[66] Im Unterschied zu den vorher betrachteten Macrocidinen zeichnet sich Kibdelomycin durch eine starke antibiotische Aktivität aus. Die Arbeitsgruppe um Singh fand durch die Kombination eines neuartigen Screening-Prozesses mit ebenfalls neuen Naturstoff-Produzenten erstmals seit den frühen 1990ern eine neue Klasse von Topoisomerase-Hemmern. Das zuvor entwickelte *target*-basierte Ganzzell-Screening, bei welchem Antisense-RNA den Gen-Knockdown verschiedener Gentargets in *Staphylococcus aureus* auslöst und somit die Sensitivät für Inhibitoren genau dieser *targets* erhöht, führte zum gewünschten Erfolg.[66,67] Zudem wurden Mikroorganismen aus Regionen weltweit und verschiedenen Habitaten *high-throughput*-Fermentationen unterzogen. Eines der getesteten Extrakte aus *Kibdelosporangium* sp. MA 7385, isoliert aus einer Erdprobe in den Wäldern der Zentralafrikanischen Republik, zeigte ein AISS-Profil (*antisense-induced strain sensitivity*) ähnlich zum Antibiotikum Novobiocin. Aus diesem Extrakt wurde Kibdelomycin (**25a**) isoliert (Abb. 3). Durch 2D-NMR-Experimente konnte die Struktur bestimmt werden (→ **25a**). Interessanterweise wurde 2010 ein Naturstoff mit sehr ähnlicher Struktur aus *Amycolatopsis* sp. MK575-fF5 isoliert.[68–70] Die Strukturannahme des Metaboliten Amycolamicin (**25b**) wich nur geringfügig von der Strukturannahme für Kibdelomycin (**25a**) ab. Die Unterschiede lagen lediglich in der Konfiguration der Methylgruppe des *N*-glykosidisch verknüpften Zuckers **A** und der tautomeren Form der 3-Acyltetramsäure **B** sowie der Konfiguration des Stereozentrums im Tetramsäurering **B**. Später wurden die Strukuren beider Naturstoffe revidiert, die überarbeiteten Strukturen von Kibdelomycin (**25**) und Amycolamicin (**25**) waren identisch.[71,72] Durch chemische Spaltung des Naturstoffes in einzelne Fragmente und Kristallstrukturanalysen sowie weiterer NMR-Studien konnte die Konfiguration der Stereozentren bestimmt werden.[71] Diese wurden mittlerweile durch Totalsynthesen aus den Jahren 2021 und 2022 bewiesen.[73–75] Zudem wurde ein Derivat, Kibdelomycin A (**150**) 2012 ebenfalls aus *Kibdelosporangium* sp. von Singh *et al*. isoliert.[76] Strukturell unterschied es sich von Kibdelomycin (**25**) lediglich durch das Fehlen der Methylgruppe am Pyrrolring. Allgemein kann die komplizierte Struktur in fünf Teile gegliedert werden: die Amykitanose **A**, ein hochfunktionalisierter Zucker abgeleitet von L-Talose, die *N*-glykosylierte 3-Acyltetramsäure **B**, das Decalinfragment **C** mit *O*-glykosidischer Verknüpfung zur Amycolose **D** mit einer neuartigen α-Aminoethylverbrückung und

das Pyrrolfragment **E**. Der Zucker **D** und insbesondere die α-Aminoethylverbrückung wurden aufgrund ihrer Seltenheit schon hinsichtlich ihrer Biosynthese untersucht.[68] Außerdem zeigte die Amycolose **D** an sich eine signifikante Unterdrückung des Zellwachstums von Stromazellen.[77] Die einzigartige Struktur macht Kibdelomycin (**25**) nicht nur zu einem interessanten Ziel der organischen Synthese, sondern führt auch zu einer ausgeprägten Aktivität gegen gram-positive Bakterien mit neuartigem Wirkmechanismus.[66] Die Wichtigkeit von Kibdelomycin für die Antibiotika-Forschung wurde durch die Häufung der synthetischen Arbeiten an dem Molekül seit Ende 2021 bestätigt. Auch hervorzuheben ist, dass die erste Totalsynthese von Kibdelomycin (**25**) erst elf Jahre nach der ersten Veröffentlichung[68] der Struktur von Yang *et al*. realisiert wurde,[73] was außerdem die Komplexizität in synthetischer Hinsicht widerspiegelt.

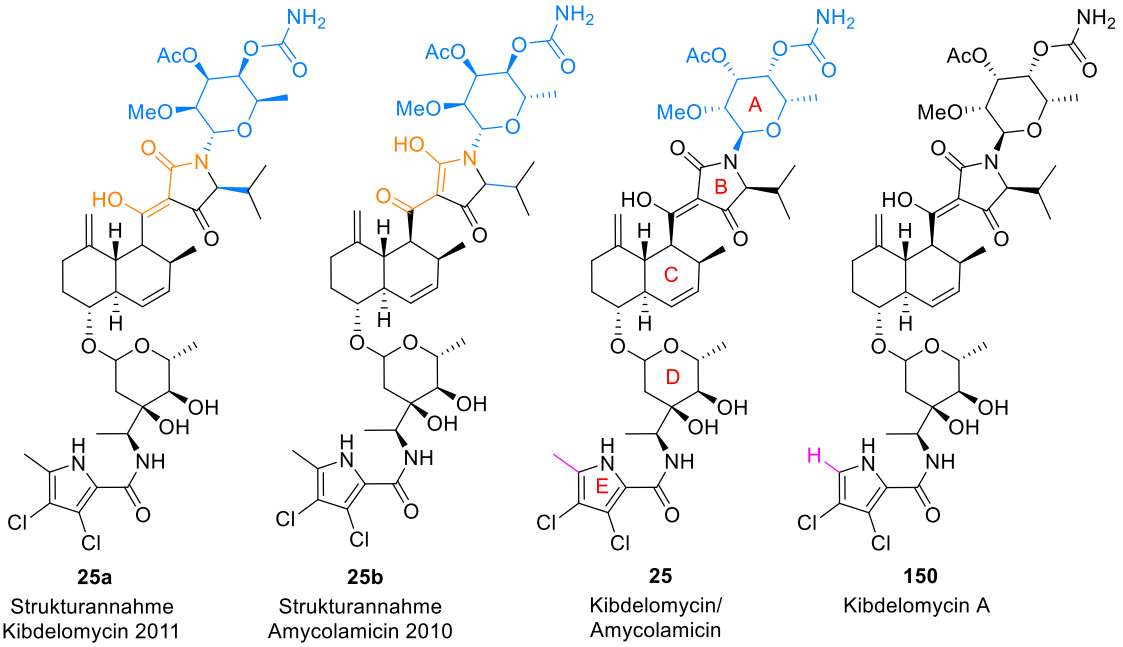


Abb 3. Strukturannahmen der Naturstoffe Kibdelomycin (**25a**) und Amycolamicin (**25b**), überarbeitete Struktur der Naturstoffe **25** sowie Strukturformel von Kibdelomycin A (**150**). In blau/orange: Unterschiede der ursprünglichen Strukturannahmen und der bestätigten Strukturen; in rot: Kennzeichnung der Fragmente; in pink: Struktureller Unterschied zwischen Kibdelomycin (**25**) und Kibdelomycin A (**150**).[66,68,71,72,76]

1.4.2 Bioaktivität von Kibdelomycin (25)

Phillipps *et al*. führten nach Isolation des neuartigen Antibiotikums intensive Studien zur Bioaktivität von Kibdelomycin (**25**) durch.[66] Das AISS-Profil wies deutliche Ähnlichkeit zu dem des Cumarin-Antibiotikums Novobiocin (**151**) auf, welches als Inhibitor der ATPase Aktivität der DNA Gyrase und Topoisomerase IV bekannt ist. Folglich wurde ein ähnlicher Wirkmechanismus für Kibdelomycin (**25**) angenommen und durch einige Assays mit *Staphylococcus aureus* und *Escherichia coli* bestätigt. Die MICs für alle gram-positiven

Bakterienstämme lagen im niedrigen mikromolaren bis nanomolaren Bereich. Es konnte keine signifikante Aktivität gegen den Wildtyp gram-negativer Bakterien mit Ausnahme von *Haemophilus influenzae* festgestellt werden, jedoch eine Inhibition der DNA Gyrase B und Toposisomerase IV z.B. des gram-negativen *Escherichia coli*, was durch eine schlechte Zelldurchdringung und aktiven Efflux erklärt wurde. Durch Anpassungen hinsichtlich dieser Faktoren wäre auch ein Effekt auf gram-negative Bakterien denkbar.[78] Zudem wurde die Aktivität gegen Stämme von *Clostridium difficile* getestet. Diese sind Auslöser von mit Antibiotika-Behandlung verbundener Diarrhoe in Krankenhäusern. Auch hier lagen die MICs im niedrigen mikromolaren Bereich, genauso wie bei den Tests weiterer anaerober, gram-positiver Bakterien bei gleichzeitiger Inaktivität gegen gram-negative Bakterien der Darmflora. Dies könnte eine selektive Behandlung ermöglichen.[79] Außerdem konnten keine Kreuzresistenzen zu den bekannten Topsiomerase II-Inhibitoren Novobiocin (**151**; Cumarin-Antibiotikum) und Ciprofloxacin (**152**; Chinolon-Antibiotikum) festgestellt werden, was auf einen neuartigen Wirkmechanismus schließen ließ. Nennenswert ist zudem die selektive Inhibierung der DNA-Replikation, wobei Effekte auf die RNA-, Protein-, Peptidoglycan- und Phospholipidsynthese ausbleiben.[66,80]

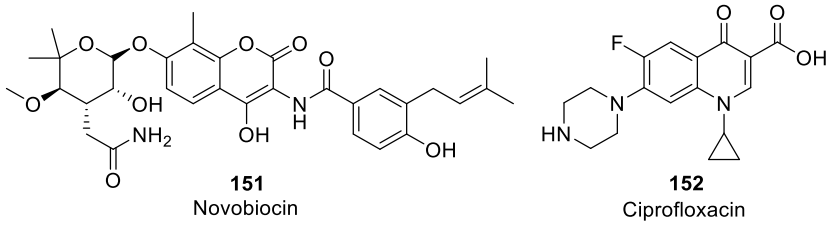
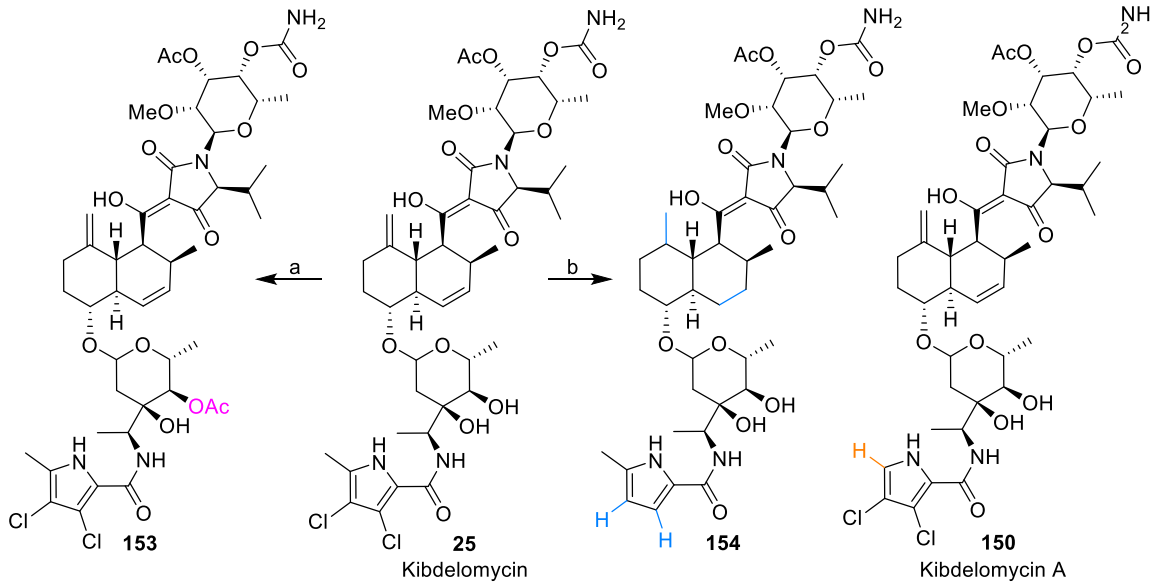


Abb 4. Strukturformeln der bekannten Antibiotika Novobiocin (**151**) und Ciprofloxacin (**152**).

Gleichzeitig mit der Isolation von Kibdelomycin A (**150**) stellten Singh *et al*. zum Aufstellen erster Struktur-Aktivitäts-Beziehungen noch zwei weitere Derivate **153** und **154** dar.[76] Acetylierung mit Essigsäureanhydrid und Pyridin lieferte selektiv das monoacetylierte Derivat **153**. Hydrierung des Naturstoffs mit H_2 und einem hetereogenen Pd-Katalysator lieferte das Tetrahydro-bisdechlor-Analogon **154**. Alle vier Substanzen wurden Bioassays zum Test ihrer Effekte auf das Bakterienwachstum diverser *S. aureus* Stämme unterzogen. Erstaunlicherweise führte bereits das Fehlen der Methylgruppe in Kibdelomycin A (**150**) zum deutlichen Anstieg der MIC-Werte. Auch die Acetylierung der sekundären Hydroxygruppe in der Amycolose führte zu einer verringerten Aktivität. Das hydrierte Derivat **154** zeigte keinerlei Aktivität mehr gegen die getesteten Stämme. Singh *et al.* schlossen darauf, dass es durch die Entfernung der Doppelbindung zu starken Änderungen der Konformation kam.



Schema 30. Synthese des monoacetylierten Derivats **153** und des Tetrahydrobisdechlor-Derivats **154** aus Kibdelomycin (**25**) sowie Kibdelomycin A (**150**). Farblich hervorgehoben sind die strukutrellen Unterschiede.[76]
Reagenzien und Bedingungen: a) Ac_2O, Pyridin; b) H_2, Pd/C (5 Gew.-% Pd).

Auch der Bindungsmodus von Kibdelomycin wurde durch die Arbeitsgruppe um Singh genauer untersucht.[72] Dafür wurden die Kristallstrukturen der Komplexe von Kibdelomycin (**25**) mit Gyrase B (GyrB) sowie Topoisomerase IV (ParE) bestimmt (Abb. 5). Die Bindungsmodi zu beiden Proteinen ähnelten sich stark. Sie zeichneten sich durch eine neuartige zweiarmige, U-förmige Konformation in Domäne I aus. Hier wird beispielhaft die Kokristallstruktur mit ParE genauer betrachtet. Der untere Bindungsarm bestehend aus der Amycolose **D** und dem Pyrrolbaustein **E** ragt in die bekannte ATP-Bindungstasche, wobei sich der obere Bindungsarm bestehend aus der 3-Acyltetramsäure **B** und der Amykitanose **A** weiter in die Domäne I erstreckt. An ParE gebunden wird der untere Bindungsarm durch Wasserstoffbrückenbindungen des Stickstoffs am Pyrrol, des Carbonylsauerstoffs in der Verbrückung von **E** und **D** sowie der sekundären Hydroxygruppe in **D**. Außerdem bilden die Chlorsubsituenten am Pyrrol Van-der-Waals-Wechselwirkungen mit ParE aus. Entscheidend im oberen Bindungsarm sind Wasserstoffbrückenbindungen zwischen der 3-Acyltetramsäure und dem Protein. Der rigide Decalinbaustein **C** bildet mehrere Van-der-Waals-Wechselwirkungen aus. Kibdelomycin wirkt folglich zum einen durch die Blockade der ATP-Bindungstasche, zum anderen behindert der obere Bindungsarm durch seinen sterischen Anspruch die Dimersierung von ParE, was zusätzlich die ATPase-Aktivität inhibiert. Bei dem Vergleich mit anderen Inhibitoren von GyrB oder ParE fällt auf, dass keiner den Bindungsbereich des oberen Arms von Kibdelomycin erreichen kann und es keine identischen Wechselwirkungen mit dem Protein gibt. Somit konnten auch die nicht auftretenden Kreuzresistenzen erklärt werden.

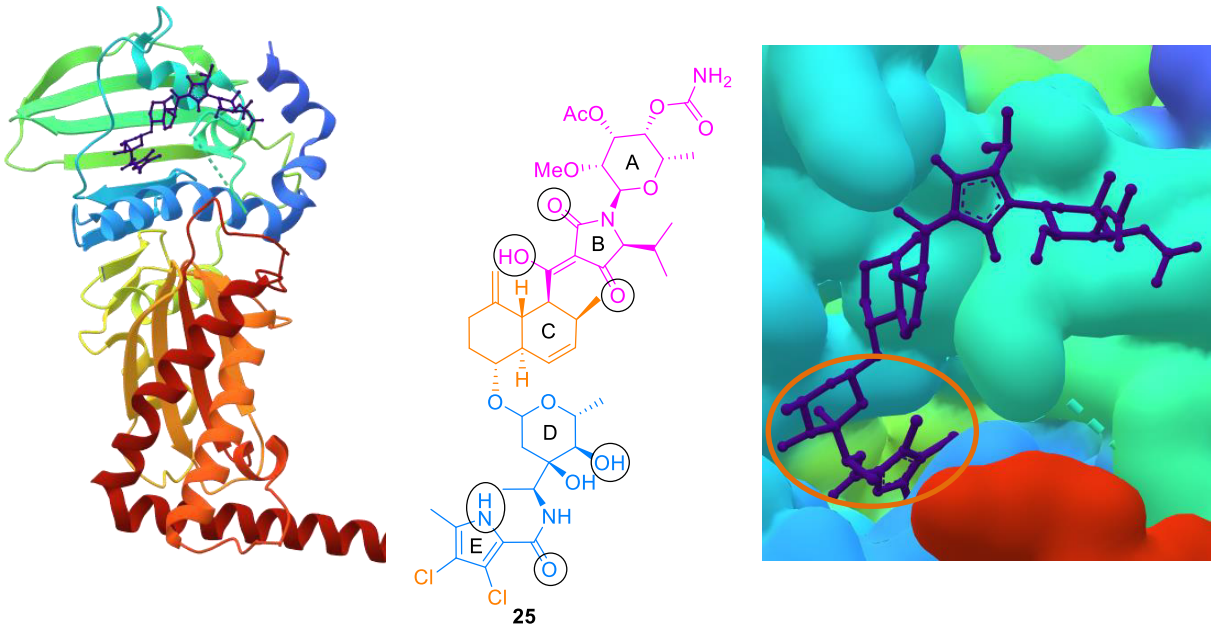
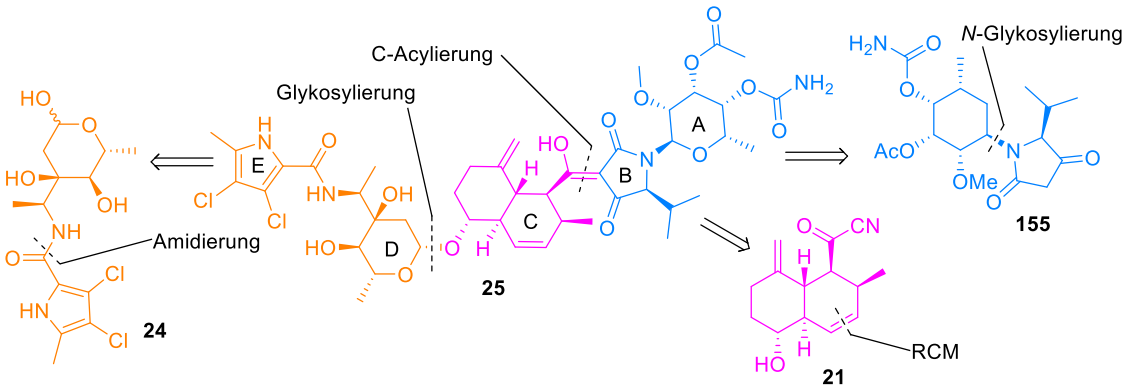


Abb 5. Links/Rechts: Kokristallsstrukuren von Kibdelomycin (**25**) und ParE nach Singh *et al*. Orange umrandet im rechten Bild ist die ATP-Bindungstasche. Mitte: Blau markiert ist der untere Bindungsarm, pink markiert ist der obere Bindungsarm. Positionen, die hydrophobe Wechselwirkungen mit ParE eingehen sind orange markiert. Atome die Wasserstoffbrückenbindungen mit ParE ausbilden sind schwarz umrandet.[72,80,81]

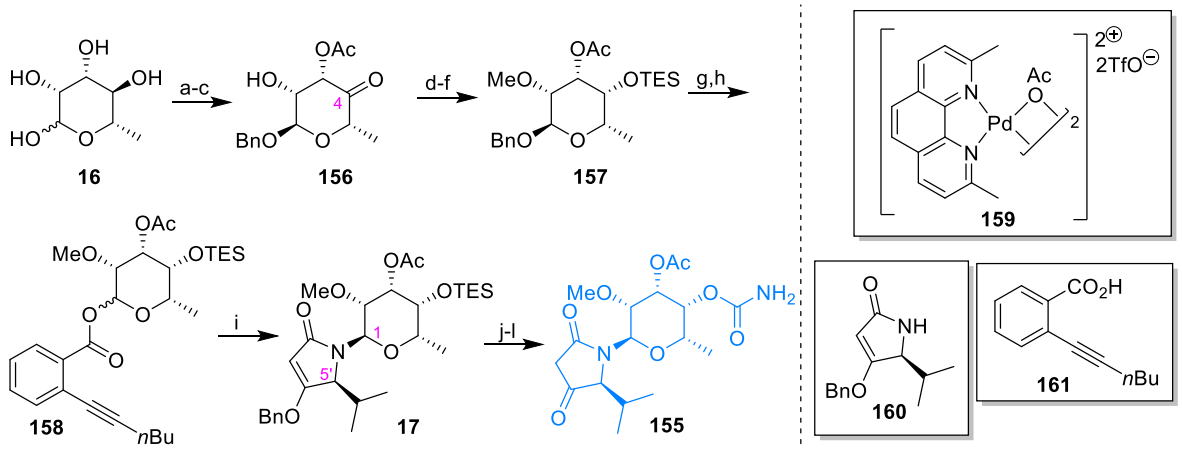
1.4.3 Erstsynthese nach Yang *et al.*

2021 gelang Yang *et al*. die Erstsynthese von Kibdelomycin (**25**). Sie teilten das Molekül in einzelne Fragmente **A**-**E**, um sie dann in einer konvergenten Synthese zusammenzufügen (Schema 31). Die drei Schlüsselbausteine **21**, **24**, **155** sollten in den finalen Schritten durch *C*-Acylierung und *O*-Glykosylierung verknüpft werden. Baustein **155** sollte durch Kupplung der Fragmente **A** und **B** via *N*-Glykosylierung entstehen. Schlüsselschritt beim Aufbau des Decalinfragmentes **21** war die Ringschlussmetathese zum Bicyclus. Die *N*-acylierte Amycolose **24** wiederum sollte durch Kupplung der Fragmente **D** und **E** via Amidierung gebildet werden.



Schema 31. Retrosynthetische Schnitte und Schlüsselbausteine der Erstsynthese von Kibdelomycin (**25**).[73]

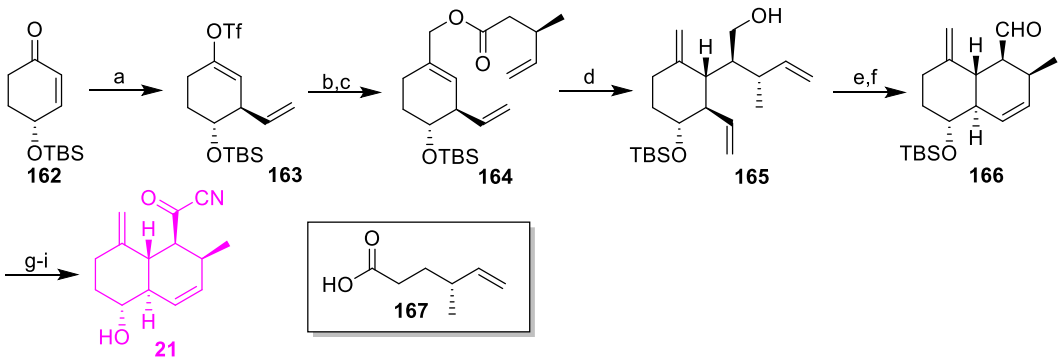
Im Folgenden sind die Synthesen der drei Bausteine dargestellt. Gestartet wurde mit der Synthese des Aminoglykosids **155** ausgehend von L-Rhamnose (**16**; Schema 32). Benzylschützung der anomeren Position in 82% Ausbeute gefolgt von einer selektiven Acetylierung unter Mo-Katalyse und der selektiven Oxidation an 4-Position mit dem Waymouths-Katalysator (**159**) lieferten das Keton **156**. Nach Methylierung der verbleibenden Hydroxyfunktion mit Meerweinsalz konnte diastereoselektiv an 4-Position reduziert werden. Silylschützung der entstehenden Hydroxyfunktion ergab das Glykosid **157**. Dieses konnte durch hydrogenolytische Abspaltung der Benzylgruppe an der anomeren Position und Veresterung mit der Carbonsäure **161** durch Zugabe von DCC und DMAP in das Glykosid **158** überführt werden. Durch Anwendung der von Zhang *et al.* etablierten, Au-katalysierten *N*-Glykosylierung von Nukleotidbasen gelang die α-selektive *N*-Glykosylierung mit der 4-*O*-Alkyltetramsäure **160** in 64% Ausbeute und einem *dr* > 20:1 (→ **17**).[82] Zur Fertigstellung des Bausteins **155** fehlten die Silylentschützung der Hydroxygruppe an 4-Position und deren Funktionalisierung in die Carbamidsäure durch Trichloracetylisocyanat und Umsetzung des entstehenden Intermediats mit NEt_3 in MeOH. Schließlich lieferte die Abspaltung der Benzylgruppe die Tetramsäure **155**.



Schema 32. Synthese des Schlüsselbausteins **155** aus L-Rhamnose (**16**).[73]
Reagenzien und Bedingungen: a) BnOH, NH_2SO_3H, 80 °C, 21 h, 82%, α:β 7.7:1; b) AcCl, $MoO_2(acac)_2$, 2,4,6-Collidin, 1,4-Dioxan, RT, 4 h, 79%; c) **159**, 2,6-Diisopropylphenol, O_2, MeCN, 50 °C, 20 h, 78%; d) Me_3OBF_4, Protonenschwamm, CH_2Cl_2, 0 °C→RT, 12 h, 70%; e) $NaBH_4$, $CeCl_3$, MeOH, −20 °C, 30 min, 76%; f) TESOTf, Pyridin, CH_2Cl_2, 0 °C, 2 h, 95%; g) H_2, Pd/C, EtOAc, RT, 19 h; h) **161**, DCC, DMAP, CH_2Cl_2, 0 °C→RT, 4 h, 65% über zwei Stufen; i) **160**, $Ph_3PAuNTf_2$, Toluol, 40 °C, ü. N., 64%, C-5‘ *dr* 7:1, C-1 *dr* > 20:1; j) $LiBF_4$, MeCN/H_2O, 4 °C, 36 h; k) 1. $Cl_3CCONCO$, CH_2Cl_2, 0 °C→RT, 1 h, 2. NEt_3, MeOH, 0 °C→RT, 2 h, 78% über zwei Stufen; l) H_2, Pd/C, EtOAc, 2 h, 96%.

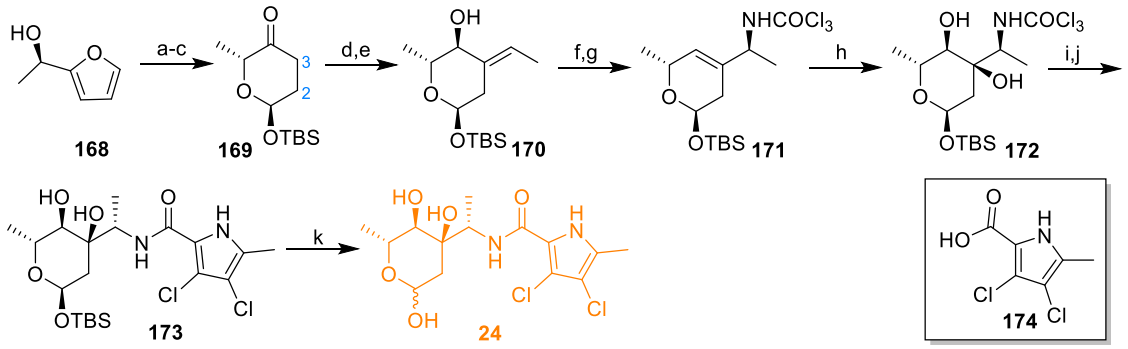
Die Synthese des Decalin-Fragmentes startete vom literaturbekannten Enon **162**,[83] welches in einer diastereoselektiven Addition mit einem Vinylcuprat und Abfangen des Intermediats mit dem Comins-Reagenz ins isomerenreine Triflat **163** überführt wurde (Schema 33).[73] Eine Stille-Kupplung mit (Tributylstannyl)methanol und Veresterung der Carbonsäure **167** mit dem entstandenen Alkohol lieferten den Ester **164**. Eine Ireland-Claisen-Umlagerung sowie sofor-

tige Reduktion mit DIBAL ergaben in einem Schritt in 85% Ausbeute und einem *dr* von 15:1 den Alkohol **165**. Aus der anschließenden Ringschlussmetathese mit dem Grubbs-Katalysator der zweiten Generation und Parikh-Doering-Oxidation der primären Hydroxylgruppe resultierte das Decalin **166**. Durch Cynanosylilierung mit TMSCN, Entfernung der TMS-Gruppe mit NH_4F und Oxidation mit IBX wurde der Aldehyd **166** ins Acylcyanid überführt. Finale TBS-Entschützung setzte den Baustein **21** frei.



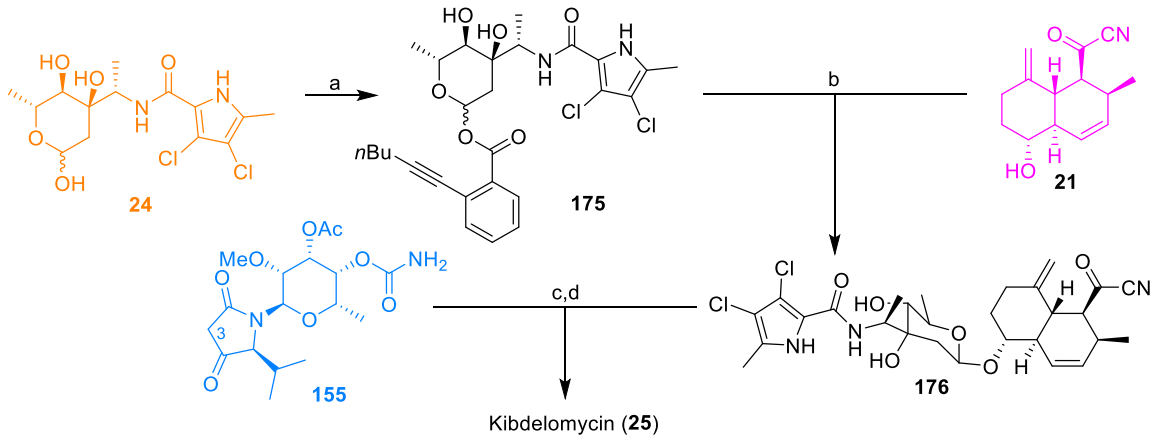
Schema 33. Synthese des Schlüsselbausteins **21** aus dem literaturbekannten Enon **162**.[73]
Reagenzien und Bedingungen: a) 1. CuI, SMe_2, HMPA, VinylMgBr, THF, −78 °C, 3 h, 2. Comins-Reagenz, RT, 18 h, 68%; b) $Pd(PPh_3)_4$, LiCl, $n$$Bu_3SnCH_2OH$, THF, Δ, 3 h, 87%; c) **167**, EDC·HCl, DMAP, NEt_3, CH_2Cl_2, RT, 5 h, 92%; d) 1. TBSOTf, NEt_3, 55 °C, 60 h, 2. DIBAL, 0 °C→RT, 2 h, 85%, *dr* 15:1; e) Grubbs 2. Generation, CH_2Cl_2, Δ, 3 h, 92%; f) SO_3·Pyridin, NEt_3, DMSO, CH_2Cl_2, 0 °C→RT, 2 h, 77%, g) 1. TMSCN, NEt_3, CH_2Cl_2, 0 °C→RT, 12 h, 2. NH_4F, EtOH, 0 °C, 2 h, 84%; h) IBX, EtOAc, 80 °C, 2 h, 83%; i) $LiBF_4$, MeCN/H_2O 10:1, 96%.

Ausgehend von (*R*)-1-(2-Furyl)ethanol (**168**) konnte die *N*-acylierte Amycolose **24** synthetisiert werden (Schema 34).[73] Achmatowicz-Umlagerung, TBS-Schützung und Hydrierung der entstandenen Doppelbindung zwischen C2 und C3 lieferten das Acetal **169** in 70% Ausbeute über drei Stufen Die Einführung einer C_2-Einheit an 3-Position wurde durch die Reaktionssequenz Aldol-Reaktion mit Ethanal, Trifluoracetat-Bildung mit TFAA und Eliminierung realisiert. Eine Luche-Reduktion ergab daraufhin den Alkohol **170** in 84% Ausbeute. Das Hauptisomer wurde in einer Overman-Umlagerung mit CCl_3CN und DBU ins allylische Trichloracetimidat umgesetzt und durch Heizen bei Anwesenheit von K_2CO_3 diastereoselektiv ins Trichloracetamid **171**. Dessen Überführung via Sharpless-Dihydroxylierung in den Diol **172** verlief in 51% Ausbeute über drei Stufen. Reduktion mit DIBAL lieferte das freie Amin, welches mit EDC, HOBt und NEt_3 mit der Carbonsäure **174** gekuppelt wurde (→ **173**). Finale TBS-Entschützung generierte das Glykosid **24** als Anomerengemisch.



Schema 34. Synthese der *N*-acylierten Amycolose **24** aus dem Furan **168**.[73]
Reagenzien und Bedingungen: a) NBS, $NaHCO_3$, NaOAc, THF/H_2O 4:1, 0 °C, 1 h; b) TBSOTf, DIPEA, CH_2Cl_2, −78 °C, 1 h, 70% über zwei Stufen; c) Pd/C, H_2, EtOAc, RT, ü. N., quant., α:β 4:1; d) 1. KHMDS, $ZnBr_2$, CH_3CHO, Toluol, −78 °C, 2 h, 2. TFAA, Pyridin, DBU, 0°C→−20 °C, 1 h, 90%; e) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C, 1 h, 84%; f) CCl_3CN, DBU, CH_2Cl_2, 0 °C, ü.N.; g) K_2CO_3, *p*Xylol, Δ, 8 h; h) $K_2OsO_4 \cdot 2H_2O$, $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, *t*BuOH/H_2O 1:1, RT, 24 h, 51% über drei Stufen, *dr* 6:1; i) DIBAL, Toluol, −78 °C, 1 h; j) **174**, EDC·HCl, HOBt, NEt_3, CH_2Cl_2, 0 °C→RT, ü. N., 57% über zwei Stufen; k) 1 N HCl aq., THF, RT, 3 h, 83%.

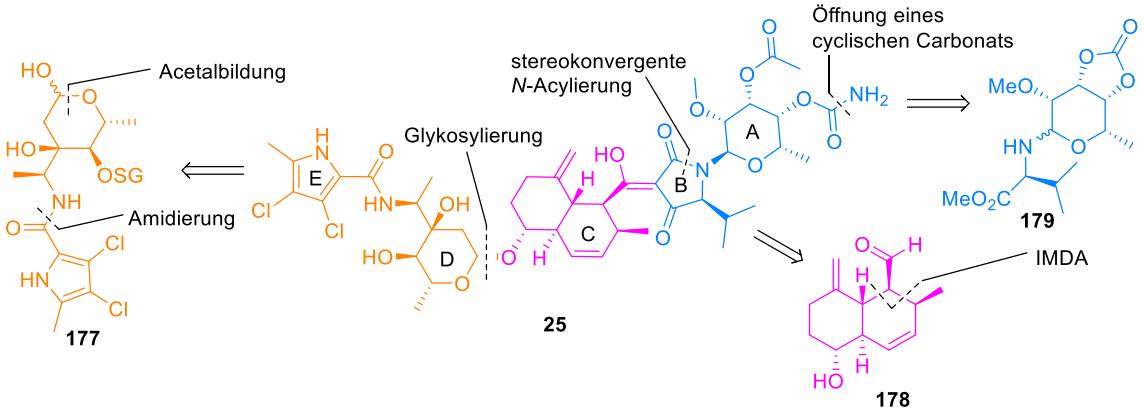
Aufgrund der Tautomerenbildung von 3-Acyltetramsäuren entschieden sich Yang *et al.*, zuerst die Bausteine **24** und **21** zu kuppeln (Schema 35). Dafür wurde zunächst die Carbonsäure **161** mit der *N*-acylierten Amycolose **24** verknüpft (→ **175**). Durch ein leicht abgeändertes Protokoll der bereits vorher verwendeten Bedingungen zur *N*-Glykosylierung konnte das Decalin **21** Au-katalysiert unter Anwesenheit von $Gd(OTf)_3$ in einem α:β-Verhältnis von 1:4 und in 67% Ausbeute *O*-glykosyliert werden (→ **176**). Die Tetramsäure **155** wurde im letzten Schritt direkt 3-acyliert durch Umsetzung mit dem Säurecyanid **176**, HOAt und NEt_3 in 42% Ausbeute. Das erhaltene Triethylamin-Salz von Kibdelomycin konnte unter sauren Bedingungen in den Naturstoff **25** überführt werden.



Schema 35. Finale Schritte in der Totalsynthese von Kibdelomycin (**25**) nach Yang *et al.*[73]
Reagenzien und Bedingungen: a) **161**, EDC·HCl, DMAP, CH_2Cl_2, 0 °C→RT, 3 h, 80%, α:β 1:2; b) PPh_3AuOTf, $Gd(OTf)_3$, 4 Å MS, Toluol/MeCN, −78 °C, 7 h, 67%, α:β 1:4; c) HOAt, NEt_3, CH_2Cl_2, 35 °C, 72 h, 3 d; 42%; d) 0.01 N HCl aq., MeOH.

1.4.4 Totalsynthese nach Meguro *et al.*

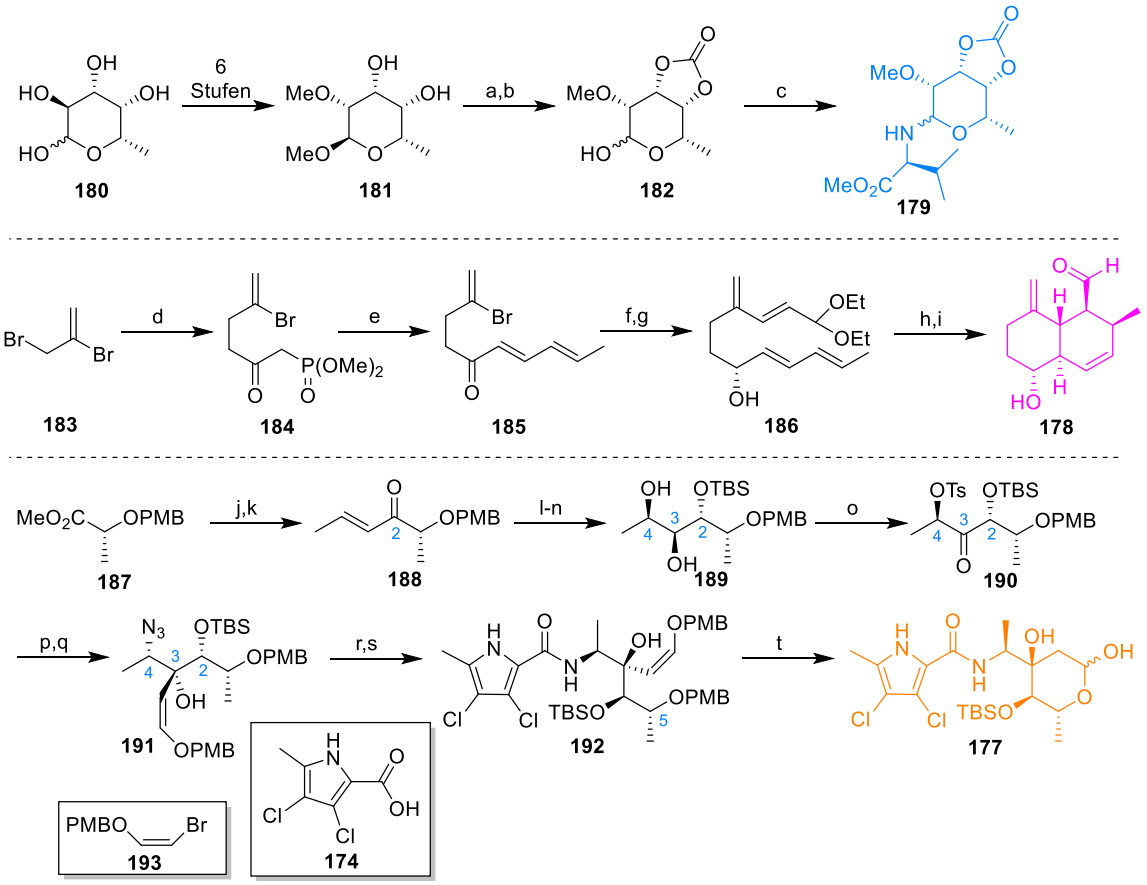
2022 publizierten Meguro *et al*. die zweite Totalsynthese von Kibdelomycin (**25**) und führten damit ihre Vorabeiten zur Synthese der *N*-acylierten Amycolose **24** fort.[74,84] Sie teilten das Molekül in dieselben fünf Fragmente wie zuvor Yang *et al*.[73], wählten jedoch andere Methoden zur Verknüpfung (Schema 36). Durch eine stereokonvergente *N*-Acylierung sollte der Baustein **179** mit dem Decalin verknüpft werden. Dieses sollte davor mit dem Zucker **177** durch eine *O*-Glykosylierung verbunden werden. Schlüsselschritt der Synthese des Decalinfragmentes **178** sollte eine stereoselektive Diels-Alder-Reaktion sein. Die Fragmente **D** und **E** sollten durch eine Amidierung verknüpft werden. Erst im Anschluss sollte der Zucker **D** durch ringschließende Acetalbildung aufgebaut werden.



Schema 36. Retrosynthese von Kibdelomycin (**25**) und Schlüsselbausteine nach Meguro *et al*.[74]

Gestartet wurde mit der Synthese der drei Bausteine **177**, **178** und **179** (Schema 37). Der Zucker **179** wurde ausgehend von L-Fucose (**180**) dargestellt, welche zunächst in sechs Stufen nach einem bekannten Protokoll[71,77,85] in das Methylglykosid **181** überführt wurde. Carbonat-Bildung durch CDI und Hydrolyse des Acetals lieferten das Halbacetal **182**. Dieses konnte durch Umsetzung mit L-Valinmethylester und PPTS in 91% Ausbeute zum Baustein **179** *N*-glykosyliert werden. Die Synthese des Decalin-Fragments **178** startete mit der Darstellung des Phosphonats **184** aus 2,3-Dibrompropen (**183**) und einer HWE-Olefinierung mit Butenal in 51% Ausbeute über zwei Stufen (→ **185**). Es folgte eine Heck-Kupplung mit Acrolein-diethylacetal und eine CBS-Reduktion des Ketons in 95% Ausbeute und 96% *ee* (→ **186**). Nach Hydroylse des Acetals zum Aldehyd wurde das Ringsystem durch eine stereoselektive, intramolekulare Diels-Alder-Reaktion aufgebaut (→ **178**). Der dritte Baustein **177** war bereits Ziel einiger Vorstudien, welche zum Großteil in der Totalsynthese übernommen wurden.[84] Das PMB-geschützte Methyllactat **187** wurde durch Umsetzung ins Phosphonat und anschließende HWE-Olefinierung ins α,β-ungesättigte Keton **188** überführt. Nach dessen diastereo-

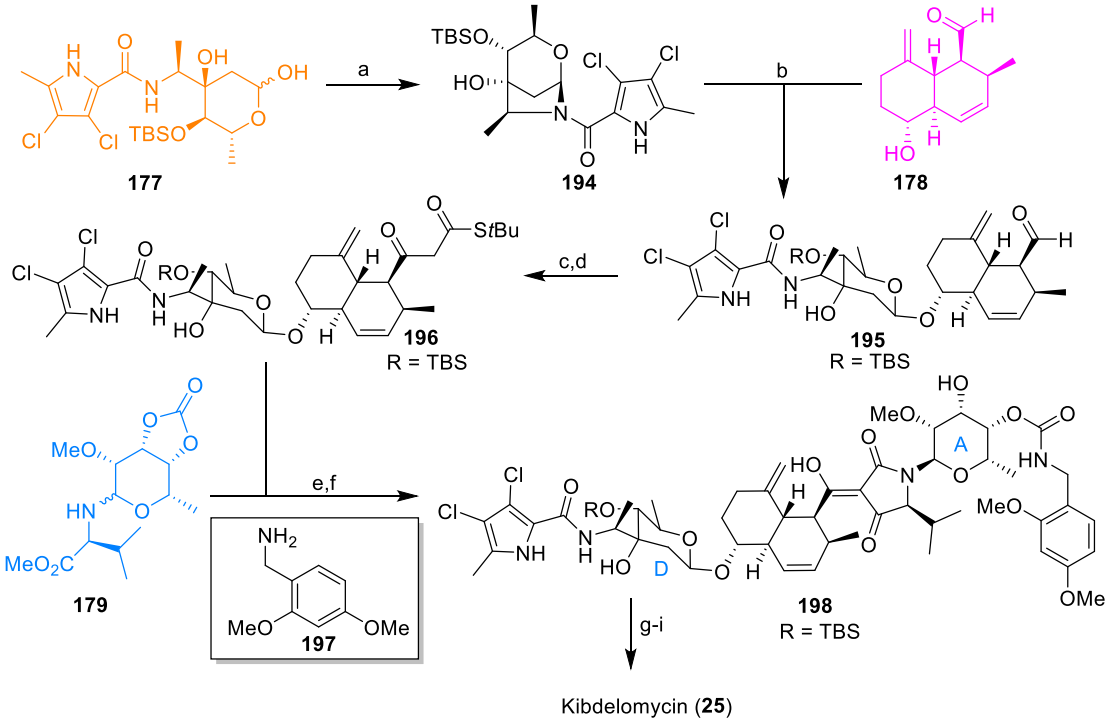
selektiver Reduktion mit $ZnBH_4$, TBS-Schützung und Sharpless-Dihydroxylierung wurde der Diol **189** erhalten. In einer Eintopf-Reaktion wurde regioselektiv an 4-Position tosyliert und mit DMP die Hydroxygruppe an 3-Position in 81% Ausbeute oxidiert (→ **190**). Substitution nach dem S_N2-Mechanismus resultierte im Azid mit invertierter Konfiguration. Durch Umsetzung mit der lithiierten Form des Bromids **193** wurde der tertiäre Alkohol **191** als einzelnes Diastereomer erhalten. Das Azid wurde via Staudinger-Reaktion ins entsprechende Amin überführt, welches mit der Pyrrolcarbonsäure **174** in einer Amidierung verknüpft wurde (→ **192**). Die Zugabe von TFA führte zur PMB-Entschützung und Halbacetalbildung zum Glykosid **177**.



Schema 37. Synthese der Bausteine **177**, **178**, **179** im Zuge der Synthese von Kibdelomycin (**25**) nach Meguro *et al.*[74,84,86]

Reagenzien und Bedingungen: a) CDI, Imidazol, THF, RT, 12 h, 79%; b) $TiBr_4$, CH_2Cl_2/EtOAc, RT, 15 h, 80%, α:β 16:1; c) L-Valinmetyhlester, PPTS, CH_2Cl_2, RT, 48 h, 91%, α:β 1.1:1; d) Dimetyhl(2-oxopropyl)phosphonat, NaH, *n*BuLi, THF, −40 °C, 1 h; e) (*E*)-2-Butenal, LiBr, NEt_3, THF, RT, 6 h, 51% über zwei Stufen, *E*/*Z* 19:1; f) Acroleindietyhlacetal, $Pd(OAc)_2$, K_2CO_3, DMF, 40 °C, 72 h, 76%, *E*/*Z* 16:1; g) (*S*)-Metyl-CBS-Oxazaborolidin, BH_3·THF, THF, −78 °C→−40 °C, 3 h, 95%, 96% *ee*; h) 0.25 M HCl aq., THF, 0 °C, 30 min; i) Et_2AlCl, CH_2Cl_2, −20 °C→0 °C, 9 h, 71% über zwei Stufen, Diastereomerenmischung: 96:2:1:1; j) $(MeO)_2P(O)Me$, *n*BuLi, THF, −78 °C→RT, 24 h, 98%; k) MeCHO, LiCl, *i*Pr_2NEt_2, THF, 0 °C, 24 h, 89%; l) $Zn(BH_4)_2$, THF, −20 °C, 2.5 h, 77%, *dr* >99:1 ; m) TBSCl, DMAP, Imidazol, CH_2Cl_2, 0 °C→RT, 14 h, 95%; n) AD-Mix β, $MeSO_2NH_2$, *t*BuOH/H_2O, 0 °C, 48 h, 94%; o) 1. TsCl, NEt_3, Me_3N·HCl, CH_2Cl_2, 0 °C, 40 min 2. DMP, 0 °C, 1 h, 81%; p) NaN_3, DMF, RT, 30 min, 98%; q) **193**, *t*BuLi, Et_2O, −78 °C, 30 min, 84%, *de* 100%; r) *n*Bu_3P, MeOH, RT, 12 h; s) **174**, EDC·HCl, HOBt, NEt_3, CH_2Cl_2, 0 °C→RT, 2 h, 96% über zwei Stufen; t) TFA, CH_2Cl_2, −20 °C→0 °C, 3 h, 83%, α:β 1:3.

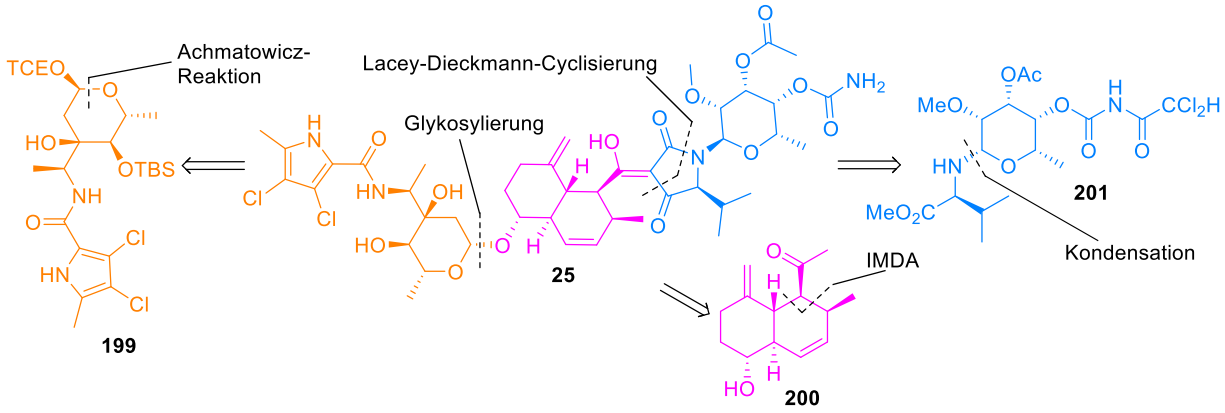
Nach erfolgreicher Synthese der drei Fragmente wurde deren Kupplung erarbeitet (Schema 38).[74] Die Umsetzung des Zuckers **177** mit DBU und Trichloracetonitril lieferte statt dem gewünschten Trichloracetimidat das bicyclische *N*,*O*-Acetal **194**. Der Angriff der Hydroxygruppe des Decalins **178** an den Bicyclus **194** unter saurer Katalyse durch Trifluormethansulfonsäure konnte den Bicyclus öffnen und lieferte das β-glykosidisch verknüpfte Produkt **195**. Der Aldehyd wurde nun via Aldol-Reaktion mit *tert*-Butylthioacetat und Oxidation in den β-Ketothioester **196** überführt. Durch ein Protokoll in Anlehnung an Ley[50] gelang die stereokonvergente *N*-Acylierung. Die *one-pot*-Reaktion aus Lacey-Dieckmann-Cyclisierung des resultierenden β-Ketoamids und Carbonatöffnung mit dem Amin **197** lieferte in 61% Ausbeute die 3-Acyltetramsäure **198**. Die Synthese von Kibdelomycin (**25**) wurde finalisiert durch oxidative Entfernung des benzylischen Restes an der Carbaminsäure im Rest **A** mittels DDQ, Acetylierung der freien, sekundären Hydroxygruppe im Zucker **A** und TBS-Entschützung der Hydroxygruppe im Fragment **D**.



Schema 38. Finale Schritte der Totalsynthese von Kibdelomycin (**25**) nach Meguro *et al.*[74]
Reagenzien und Bedingungen: a) Cl_3CCN, DBU, CH_2Cl_2, RT, 24 h, 86%; b) TfOH, 4 Å MS, CH_2Cl_2, −20 °C→0 C, 14 h, 67%, α:β 1:4.3; c) *S*-*tert*-Butylthioacetat, LiHMDS, THF, −78 °C, 8 h; d) DMP, CH_2Cl_2, RT, 1 h, 95% über zwei Stufen; e) AgTFA, 2,6-Di-*tert*-Butylpyridin, 5 Å MS, THF, 0 °C, 45 min, 72%, 100% α; f) 1. KO*t*Bu, THF, 0 °C, 1.5 h, 2. **197**, Py·HCl, CH_2Cl_2, RT, 3 d, 61%; g) DDQ, 2,6-Di-*tert*-Butylpyridin, CH_2Cl_2/H_2O, 0 °C→RT, 4 h; h) Ac_2O, Li_2CO_3, Pyridin, RT, 24 h, 56% über zwei Stufen; i) TASF, THF/DMF, RT, 4 h, 90%.

1.4.5 Totalsynthese nach He *et al.*

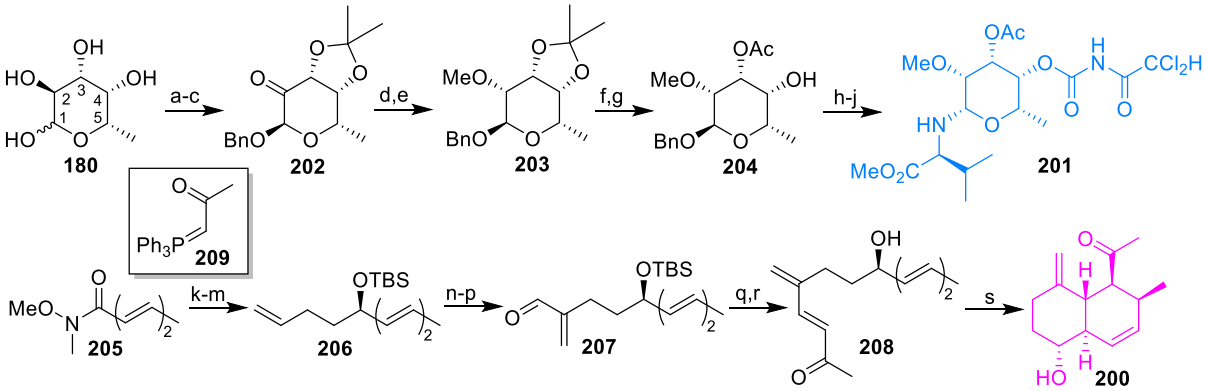
Ebenfalls im Jahr 2022 wurde die dritte Totalsynthese von Kibdelomycin (**25**) von He *et al.* veröffentlicht.[75] Die retrosynthetischen Schnitte aus den ersten beiden Synthesen wurden hier übernommen, jedoch sollten neue Reaktionen zur Verknüpfung der Bausteine genutzt werden (Schema 39). Das Aminoglykosid **201** sollte durch eine Kondensation mit L-Valin zunächst *N*-glykosyliert und letztlich mit dem Decalinfragment **200** über die Zwischenstufe eines β-Ketoamids verknüpft werden, welches via Lacey-Dieckmann-Cyclisierung zur 3-Acyltetramsäure geschlossen werden kann. Vorher sollte die Verknüpfung des Decalins **200** mit dem Zucker **199** mittels Glykosylierung erfolgen, welcher durch eine Achmatowicz-Reaktion aus einer Furan-Vorstufe entstehen sollte. Schlüsselschritt im Aufbau des Decalin-Bausteins **200** sollte eine IMDA werden, ähnlich zur Synthese von Meguro *et al.*[74]



Schema 39. Retrosynthetischer Ansatz zur Synthese von Kibdelomycin (**25**) nach He *et al.*[75]

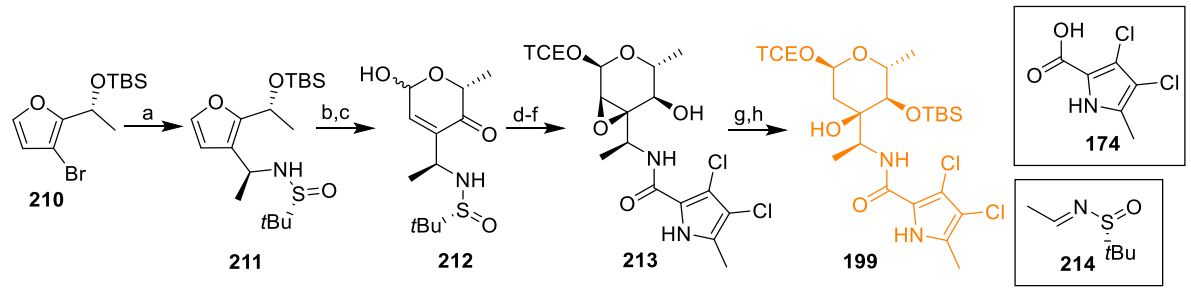
Gestartet wurde mit der Synthese des Aminoglykosids **201** ausgehend von L-Fucose (**180**; Schema 40). Die ersten Schritte waren die Acetalbildung an 1-Position, die Schützung des *syn*-Diols an 3- und 4-Position und die Oxidation der Hydroxygruppe an 2-Position mittels DMP (→ **202**). Durch DIBAL-Reduktion gelang die Inversion des Stereozentrums an dieser Stelle, welche mit MeI und Ag_2O direkt methyliert werden konnte (→ **203**). Anschließend wurde nach Acetalspaltung zunächst selektiv an 3-Position acetyliert (→ **204**) und schließlich mittels Trichloracetylisocyanat ein Carbamat an 4-Position eingeführt. Zeitgleich mit der Entfernung der Benzylgruppe am anomeren C-Atom via hydrogenolytischer Spaltung wurde an der Carbamatfunktion einfach dechloriert, was jedoch unerheblich für die weitere Synthese war. Zuletzt folgte die *N*-Glykosylierung mit Methylvalinat. Es wurde nur das β-Anomer **201** erhalten. Für die Synthese des Naturstoffs ist jedoch das α-Anomer notwendig. Die Arbeiten wurden dennoch fortgesetzt, da die Instabilität dieses Stereozentrums literaturbekannt ist.[71] Das Decalin-Fragment **200** wurde ausgehend vom Weinreb-Amid der Sorbinsäure **205**

dargestellt. Dieses wurde mit Homoallylmagnesiumbromid in einer Grignard-Reaktion zu einem Keton umgesetzt, welches via CBS-Reduktion stereoselektiv zum Alkohol reduziert wurde. Nach dessen TBS-Schützung (→ **206**) erfolgte eine Hydroborierung mit nachfolgender DMP-Oxidation zum Aldehyd. Dieser wurde in einer Mannich-Reaktion mit anschließender Eliminierung des Amins in den Aldehyd **207** mit terminaler Doppelbindung an α-Position überführt. Eine Wittig-Reaktion mit dem Ylen **209** führte zu einer weiteren Verlängerung der Kohlenstoffkette. Für die folgende IMDA musste die TBS-Gruppe entfernt werden (→ **208**), da die Koordination der verwendeten Lewissäure an die freie Hydroxygruppe entscheidend für die Stereokontrolle der Reaktion war. Die Reaktion gelang in 51% Ausbeute und lieferte das Decalin **200**.



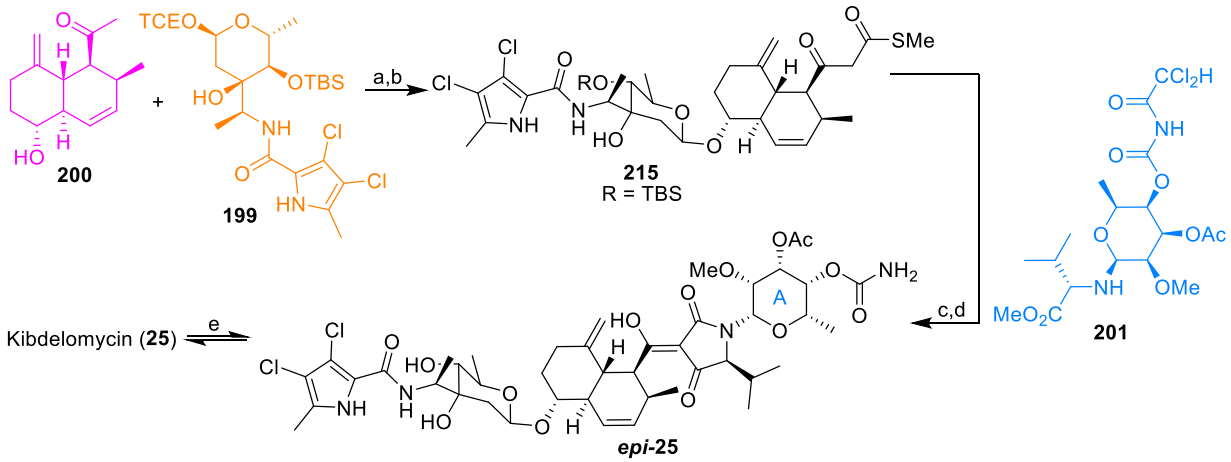
Schema 40. Synthese der Fragmente **200** und **201** im Zuge der Totalsynthese von Kibdelomycin (**25**) nach He *et al.*[75]
Reagenzien und Bedingungen: a) BnOH, *p*TsOH, 80 °C, ü. N.; b) Dimethoxypropan, *p*TsOH, DMF, RT, ü. N., 50% über zwei Stufen; c) DMP, CH_2Cl_2, RT, 2 h; d) DIBAL, THF, −78 °C→RT, ü. N., 87% über zwei Stufen; e) MeI, Ag_2O, CH_2Cl_2, 75 °C, ü. N., 83%; f) AcOH aq., 80 °C, 1 h; g) Ac_2O, NEt_3, DMAP, CH_2Cl_2, RT, ü. N., 80% über zwei Stufen; h) Trichloracetylisocyanat, CH_2Cl_2, 0°C→RT, 1 h, 95%; i) Pd/C, H_2, EtOAc, RT, 3 h; j) L-Val-OMe, PPTS, RT, 6 h, 84% über zwei Stufen; k) Homoallylmagnesiumbromid, THF, 0 °C, 4 h, 93%; l) (*S*)-CBS, BH_3·THF, THF, −78 °C, 5 h, 88%, 99% *ee*; m) TBSCl, Imidazol, DMF, 50 °C, ü. N., 90%; n) 1. 9-BBN, THF, 0 °C→RT, 5h, 2. $NaBO_3$·$4H_2O$, H_2O, 0 °C→RT, ü. N., 96%; o) DMP, CH_2Cl_2, RT, 5 h, 78%; p) 1. L-Prolin, Bn_2NCH_2OMe, DMF, 0 °C→RT, 2 h, 2. SiO_2, CH_2Cl_2, RT, 5 h, 85%; q) **209**, CH_2Cl_2, 45 °C, 24 h, 97%; r) TBAF·$3H_2O$, THF, 0 °C→RT, 2 h, 99%; s) Me_2AlCl, CH_2Cl_2, −20 °C→RT, 18 h, 51%.

Das letzte Fragment wurde ausgehend vom Furan **210** synthetisiert (Schema 41), welches zunächst mit dem chiralen Sulfimin **214** verknüpft wurde (→ **211**). Die TBS-Gruppe wurde entfernt und eine Achmatowicz-Reaktion lieferte das Halbacetal **212**. Die Bildung des Vollacetals mit Trichlorethanol am anomeren C-Atom setzte gleichzeitig das Amin frei, welches mit der Carbonsäure **174** zum Amid gekoppelt wurde. Es folgte eine Luche-Reduktion des Ketons und eine Epoxidierung der Doppelbindung (→ **213**), wobei beide Reaktionen unter vollständiger Stereokontrolle abliefen. Öffnung des Epoxids mit $LiBH_4$ und TBS-Schützung des sekundären Alkohols lieferten das Fragment **199**.



Schema 41. Synthese des Fragmentes **199** im Zuge der Totalsynthese von Kibdelomycin (**25**) nach He *et al.*[75] *Reagenzien und Bedingungen*: a) 1. *n*BuLi, Et_2O, −40 °C, 1 h, 2. **214**, −78 °C→RT, 2 h, 78%, *dr* 5.5:1; b) TBAF·$3H_2O$, THF, RT, 0.5 h, 98%; c) 1.Methylenblau, O_2, CH_2Cl_2, −78 °C, 2.5 h, 2. SMe_2, −78 °C→RT, 2 h, 92%; d) 1. *p*TsOH, TCEOH, RT, 1.5 h, 2. HCl, RT, 1.5 h, 3. **174**, HATU, DIPEA, DMF, RT, 8 h, 44%, *dr* 6:1; e) $NaBH_4$, $CeCl_3·7H_2O$, MeOH, 0 °C, 20 min, 88%; f) CF_3CO_3H, CH_2Cl_2, −40 °C→RT, 2 h, 43%; g) $LiBH_4$, Toluol, 60 °C, 3 h, 53%; h) TBSOTf, NEt_3, DCE, RT→40 °C, 7 h, 56%.

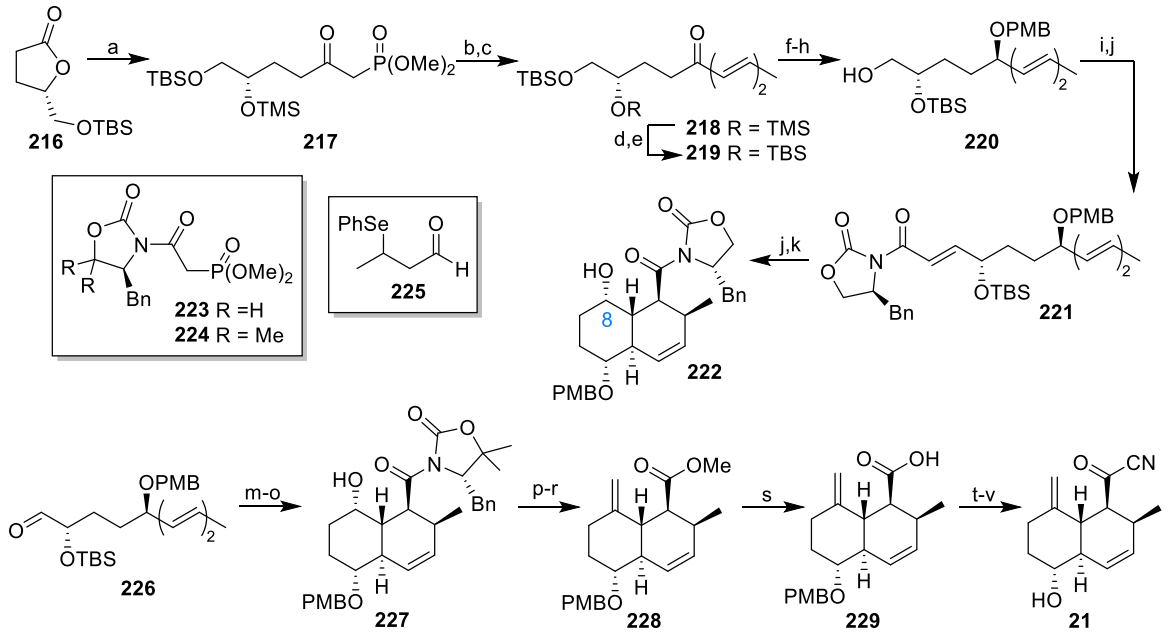
Nun folgten die Kupplungsreaktionen, wobei zuerst durch TfOH in einem Schritt das anomere C-Atom des Zuckers **199** entschützt und mit dem Decalin **200** verknüpft wurde (Schema 42). Das Glykosid wurde als Anomerenmischung isoliert. Nach Abtrennung des α-Anomers erfolgte die Überführung des Ketons am Decalin zum β-Ketothioester **215**. Dieser wurde in einer Ley-Reaktion unter Silberkatalyse mit dem *N*-Glykosid **201** zum β-Ketoamid umgesetzt. Durch NEt_3 wurde das Carbamat im Fragment **A** freigelegt und durch Zugabe von TBAF sowohl die Silyl-Schutzgruppe entfernt als auch die Lacey-Dieckmann-Cyclisierung induziert (→ ***epi*-25**). Im sauren Milieu gelang die teilweise Isomerisierung des Stereozentrums am anomeren C-Atom des Zuckers **A** zu einem 4:3-Gemisch der beiden Epimere.



Schema 42. Finale Schritte der Totalsynthese von Kibdelomycin (**25**) nach He *et al.*[75] *Reagenzien und Bedingungen*: a) TfOH, 4 Å MS, CH_2Cl_2, RT, 2.5 h, 65%, α:β 1:1.6; b) LiHMDS, $CO(SMe)_2$, THF, −78 °C→30 °C, 6.5 h, 78%; c) **201**, AgTFA, 4 Å MS, THF, RT, 2 h; d) 1. NEt_3, MeOH, RT, 10 min, 2. TBAF, THF, RT, 0.5 h, 41% über zwei Stufen; e) 0.1% HCOOH, $MeCN/H_2O$, RT, 24 h, 78% (4:3 ***epi*-25**:**25**).

1.4.6 Weitere Arbeiten am Decalin-Motiv durch Frossard *et al.*

Die Gruppe um Altmann publizierte 2022 ihre Ergebnisse zur Darstellung des Decalin-Fragments von Kibdelomycin (**25**) basierend auf der IMDA-Reaktion eines linearen Vorläufers (Schema 43).[87] Gestartet wurde vom Lacton **216**, welches mit *n*BuLi und einem Methylphosphonat ringgeöffnet und durch Zugabe von TMSCl gleichzeitig die entstehende Hydroxygruppe silyliert wurde (→ **217**). Es folgte eine HWE-Reaktion mit dem abgewandelten Crotonaldehyd **225**. Das enstehende ungesättigte Selenid wurde durch Oxidation und dadurch induzierter Eliminierung in das Keton **218** überführt. Die TMS-Gruppe musste aufgrund ihrer Instabilität in den Folgereaktionen durch eine TBS-Gruppe ersetzt werden (→ **219**). Darauf lieferte eine CBS-Reduktion mit anschließender PMB-Schützung des entstandenen Alkohols und selektive Entschützung der primären Hydroxygruppe den Alkohol **220**. Dieser wurde zum Aldehyd oxidiert und mit dem Phosphonat **223** in einer weiteren HWE-Olefinierung zum Trien **221** umgesetzt, welches in einer Lewissäure-vermittelten, Auxiliar-gesteuerten IMDA zum Decalin reagierte. Daran schloss sich die TBS-Entschützung mittels HF·Pyridin zum Alkohol **222** an. Jegliche Experimente zur Einführung einer Doppelbindung an 8-Position scheiterten, was auf den sterischen Anspruch des Auxiliars zurückgeführt wurde. Auch die Entfernung des Auxiliars durch verschiedene Methoden schlug fehl. Schließlich wurde auf das selektiver entfernbare SuperQuat-Auxiliar zurückgegriffen. Ausgehend vom Aldehyd **226** wurde in Analogie zur vorherigen Route die Synthese des Decalins **227** durch HWE-Olefinierung, IMDA und Silylentschützung realisiert. Tatsächlich gelang daraufhin die Entfernung des Auxiliars durch verschiedene Methoden. Unter anderem konnte durch Umsetzung mit NaOMe der Methylester isoliert werden. Anschließend wurde zum Keton oxidiert und durch eine Wittig-Reaktion die terminale Doppelbindung eingeführt (→ **228**). Die Verseifung des Esters gelang mit dem eher ungewöhnlichen Benzenselenol. Die Carbonsäure **229** reagierte mit Oxalylchlorid zum Säurechlorid, welches durch CuCN und NaI in das Acylcyanid überführt wurde. Nach PMB-Enschützung mittels DDQ konnte das Fragment **21** fertiggestellt werden. Frossard *et al*. gelang damit ein alternativer Zugang zum Baustein, der bereits von Yang *et al*. innerhalb ihrer Totalsynthese von Kibdelomycin (**25**) entwickelt wurde.[73]



Schema 43. Synthese des Decalin-Bausteins **21** nach Frossard *et al*.[87]
Reagenzien und Bedingungen: a) 1. $MePO(OMe)_2$, *n*BuLi, THF, −78 °C, 2 h, 2. LDA, −78 °C→ −20 °C, 30 min, 3. TMSCl, −20 °C→0 °C, ü. N., 89%; b) **225**, NaH, THF, 0 °C, 1 h; c) H_2O_2, CH_2Cl_2, 0 °C, 20 min, 89% über zwei Stufen; d) NaOH, MeOH, RT, 20 min; e) TBSCl, Imidazol, DMAP, DMF, RT, 5 h, 92% über zwei Stufen; f) (*S*)-Me-CBS, $BH_3{\cdot}SMe_2$, THF, −45 °C, 7 h, 96%, *dr* 8:1; g) PMBBr, NaH, TBAI, THF, Δ, 15 h, 92%; h) HF·Pyridin, THF, 0 °C, 39 h, 68%; i) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C→RT, 5 h; j) **223**, LiCl, DIPEA, MeCN, 0 °C→RT, 20 h, 64% über zwei Stufen; k) Me_2AlCl, CH_2Cl_2, −78 °C→0 °C, 17 h, 54%, *dr* 8:1; l) HF·Pyridin, Pyridin, THF, RT, 22 h, 78%; m) **224**, LiCl, DIPEA, MeCN, 0 °C→RT, 20 h, 64% über zwei Stufen; n) Me_2AlCl, CH_2Cl_2, −78 °C→0 °C, 16 h, 34% *dr* 12:1 + 8% *dr* 1.7:1; o) HF·Pyridin, Pyridin, THF, RT, 25 h, 76%; p) NaOMe, CH_2Cl_2, 0 °C, 2 h, 97%; q) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C→RT, 2 h; r) CH_3PPh_3Br, *t*BuOK, THF, 0 °C→RT, 2 h, 87% über zwei Stufen; s) PhSeH, NaH, 18-Krone-6, THF, 80 °C, 5 h, 92%; t) $(COCl)_2$, DMF, CH_2Cl_2, RT, 4 h; u) CuCN, NaI, 4 Å MS, MeCN, 90 °C, 30 min, 64% über zwei Stufen; v) DDQ, CH_2Cl_2/Puffer pH=7, 0 °C→RT, 2 h, 83%.

2 ZIELSETZUNG

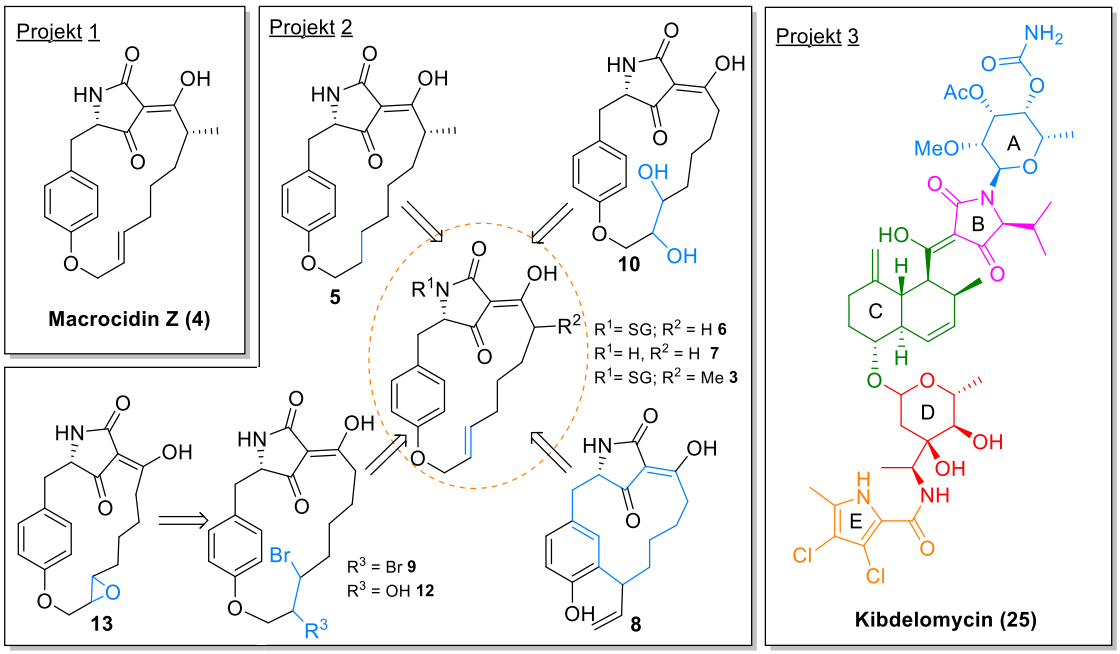
Das Tetramsäuremotiv ist in Naturstoffen weit verbreitet und führt bekannterweise zu ausgeprägten biologischen Aktivitäten.[1] Diese können unterschiedlicher Natur sein. So sind tetramsäureabgeleitete Verbindungen bekannt, die cytotoxisch, antibakteriell, antimykotisch oder herbizid wirken. Ziel dieser Arbeit war die synthetische Betrachtung zweier solcher Naturstoffe mit Tetramsäuremotiv – Macrocidin Z (**4**) und Kibdelomycin (**25**) sowie deren Derivatisierung (Schema 44).

Das erste Teilprojekt entstand aus der bislang nicht bewiesenen Annahme der Existenz eines weiteren Naturstoffes aus *Phoma macrostoma*, der Macrocidin Z (**4**) genannt wurde.[59] In Kooperation mit Blondelle Kemkuignou vom Lehrstuhl *Microbial drugs* des *Helmholtz Centre for Infection Research* war das Ziel der Beweis der Existenz von Macrocidin Z (**4**) sowie die Verifizierung dessen Struktur und Stereokonfiguration. Dafür sollte die stereoselektive Erstsynthese von Macrocidin Z (**4**) erfolgen und anschließend NMR- und CD-Spektren des entsprechenden Isolats und des Syntheseproduktes verglichen werden.

Damit eng verknüpft war das Ziel des zweiten Teilprojekts, die Synthese von Derivaten der Naturstoffe Macrocidin A (**30**) und Z (**4**). Die Erkenntnisse aus der Erstsynthese von Macrocidin Z (**30**) sollten zur Darstellung eines Schlüsselintermediats **6** mit Doppelbindung genutzt werden, welches in weiteren Schritten divers funktionalisiert werden könnte. Die Derivate sollten im Makrocyklus sowohl Halogene als auch Hydroxygruppen oder Doppelbindungen aufweisen oder vereinfacht ohne Funktionalisierung vorliegen. Über die Stufe eines Bromohydrins **12** sollte auch erstmals ein Epoxid am Makrocyclus eingeführt werden (→ **13**), um Macrocidin A und B zu imitieren. Außerdem sollte ein ringverkleinertes Derivat **8** dargestellt werden. Die Funktionalisierungen waren stereoselektiv geplant, wobei im gleichen Zug der Einfluss des Makrocyclus auf die Stereokontrolle betrachtet werden sollte. Alle Produkte könnten schließlich auf ihre herbizidäre Aktivität in Pflanzentests und zudem auf Cytotoxizität, antimikrobielle Wirkung oder biofilminhibierende Wirkung getestet werden. Die daraus gewonnenen Daten sollten zur Aufstellung einer Struktur-Wirkungs-Beziehung genutzt werden.

Ziel des dritten Teilprojektes war die Synthese von Kibdelomycin (**25**), welches sich durch besonders starke antibiotische Effekte mit neuartigem Wirkmechanismus auszeichnet.[76] In Zusammenarbeit mit Manuel Schriefer sollte dabei eine konvergente Synthese ausgearbeitet werden, die die Möglichkeit zur Derivatisierung bietet. Die Struktur wurde dafür aufgeteilt in fünf Fragmente, wobei Ziel dieser Arbeit die Synthese der Amykitanose **A**, des Tetramsäure-

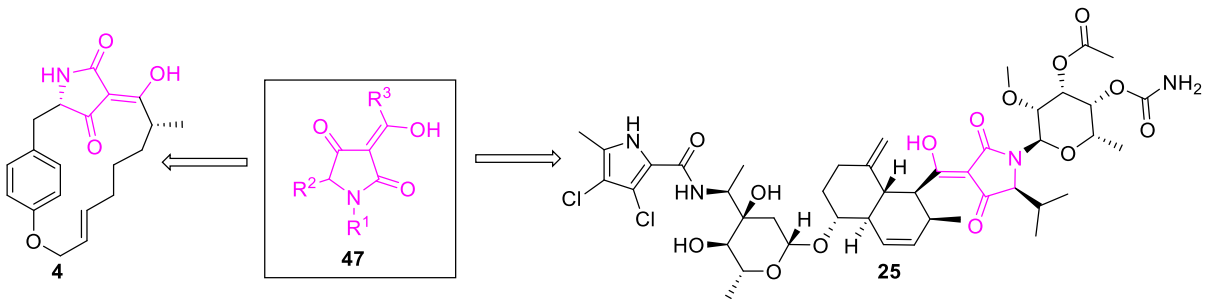
fragmentes **B** und des Decalin-Bausteins **C** sowie deren Verknüpfung war. Außerdem sollten auch für Kibdelomycin erste Versuche zur Derivatisierung unternommen werden und dazu allgemein nutzbare Synthesewege ausgearbeitet werden.



Schema 44. Darstellung der Zielverbindungen der drei Teilprojekte: Macrocidin Z (**4**), Derivate der Macrocidine sowie Kibdelomycin (**25**). Letzteres ist farblich unterteilt in die fünf Fragmente Amykitanose (blau), Tetramsäure (pink), Decalinfragment (grün), Amycolose (rot) und Pyrrolfragment (orange).

3 SYNOPSIS

Zwei Probleme, die den Drang zur Isolation neuartiger Wirkstoffe mit ansprechendem Wirkprofil erhöhen, sind die Herbizid-Resistenzen in Unkräutern[88,89] und Antibiotika-Resistenzen in Mikroorganismen.[90,91] Beide können durch den übermäßigen und inkorrekten Gebrauch der jeweiligen Vertreter und ähnliche Wirkmechanismen verschiedener Wirkstoffklassen erklärt werden. Dies ermöglicht den Pathogenen die rasche Entwicklung von Immunität gegen die eingesetzten Substanzen. Die dadurch anhaltende Suche nach Wirkstoffen jeglicher Art führt zur stetigen Isolierung neuartiger Naturstoffe. Deren Darstellung bietet zum einen die Möglichkeit eines synthetischen Zugangs. Zum anderen kann die Derivatisierung der Naturstoffe zur Aufstellung einer Struktur-Aktivitäts-Beziehung genutzt werden, um dadurch eine Wirksteigerung zu erzielen sowie die Resistenzbildung zu erschweren. Beide oben erwähnten Probleme können durch Naturstoffe mit dem Tetramsäuremotiv angegangen werden. Vertreter dieser Art sind schon seit langem als potentielle Wirkstoffe bekannt und haben unterschiedliche Bioaktivitäten.[92,93] Im Speziellen werden hier die Totalsynthesen von Macrocidin Z (**4**) und Kibdelomycin (**25**) sowie deren Derivatisierung behandelt (Schema 45). Die insbesondere zur Synthese des Tetramsäuremotivs erlangten Erkenntnisse aus der Darstellung des makrocyclischen, strukturell weniger kompliziert aufgebauten Macrocidin Z (**4**) sollen zunächst bei dessen Derivatisierung und schließlich bei der formalen Totalsynthese des lange synthetisch unerreichten Kibdelomycins (**25**) angewandt werden.



Schema 45. Die beiden zur Totalsynthese und Derivatisierung ausgewählten Vertreter von natürlich vorkommenden 3-Acyltetramsäuren– Macrocidin Z (**4**) und Kibdelomycin (**25**).

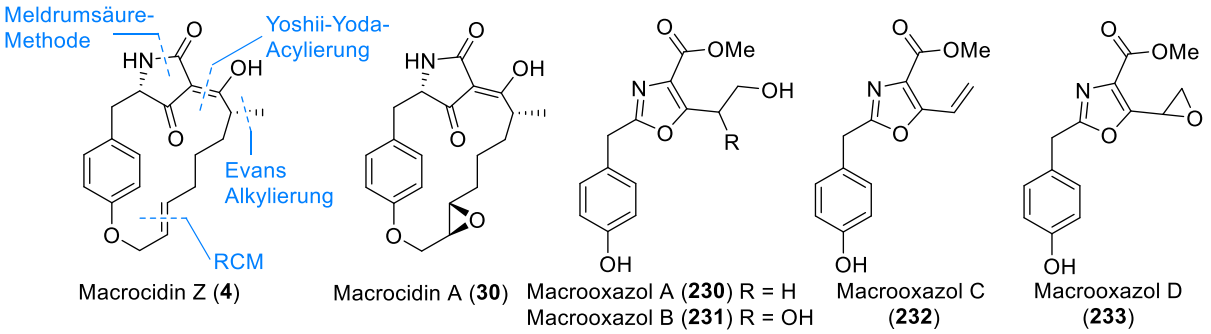
3.1 Macrooxazoles A–D, neue 2,5-disubstituierte Oxazol-4-carboxylsäure-derivate aus dem pflanzenpathogenen Pilz *Phoma macrostoma*

Erster Teil dieser Arbeit war die Synthese des Naturstoffs Macrocidin Z (**4**), welcher zusammen mit den vier neuen Macrooxazolen A-D (**230**-**233**) aus dem Pilz *Phoma macrostoma* isoliert wurde (Schema 46). Die Strukturen der Metabolite wurden mittels intensiver NMR-Studien ermittelt. Im Zuge der ersten Totalsynthese des Naturstoffes gelang auch die Verifikation der Struktur und der absoluten Stereokonfiguration des Moleküls. Alle isolierten Moleküle wurden auf ihre antimikrobiellen und cytotoxischen Eigenschaften untersucht.

Pilz-Metabolite sind spätestens seit der Isolation von Penicillin G aus *Penicillium notatum* von Interesse für die Wirkstoffsuche.[94] Auch aus dem bekannterweise herbizid-wirkenden Pilz *Phoma macrostoma* konnten 2003 erstmals Metabolite isoliert werden, die Macrocidine genannt wurden. Die Bioaktivität der Pilzextrakte konnte auf diese Substanzen zurückgeführt werden. Jedoch wurde die Bildung weiterer, möglicherweise ebenfalls interessanter Naturstoffe durch den Pilz angenommen. Im Rahmen dieser Arbeit wurde durch Fermentation des Pilzes *Phoma macrostoma* diese Annahme bestätigt. Aufreinigung der Extrakte mittels präparativer HPLC führte zur Isolation von vier neuen Metaboliten, den Macrooxazolen A-D (**230-233**) sowie dem bekannten Macrocidin A (**30**) und dem bereits von Graupner 2006 erstmals erwähnten Macrocidin Z (**4**) dessen Strukurannahme jedoch nur auf ^{1}H-Experimenten beruhte.[59] Die Struktur der unbekannten Metabolite wurde durch NMR-Studien bestimmt. Erste Aufschlüsse brachten die massenspektrometrischen Analysen der Isolate nach Trennung durch HPLC. Bei Bestimmung des Drehwertes von Macrooxazol B (**231**) ging der gemessene Wert gegen 0. Dies führte zur Annahme, dass der Naturstoff als racemische Mischung vorliegt. Macrooxazol D (**233**) konnte lediglich als Gemisch mit Macrooxazol B (**231**) isoliert werden. Es konnte nicht gänzlich ausgeschlossen werden, dass letzterer erst bei der Fermentation des Pilzes aus Macrooxazol D (**233**) entsteht.

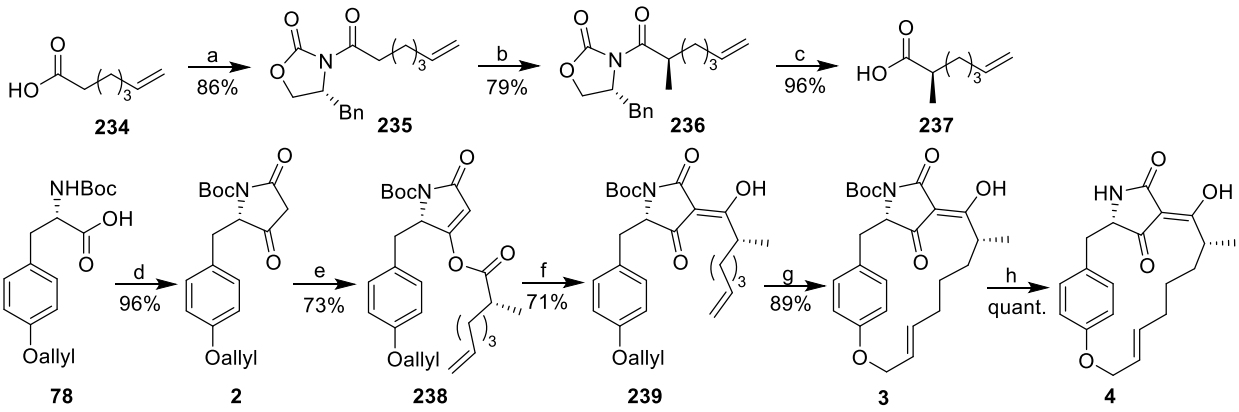
Macrocidin A (**30**) wurde durch Vergleich der NMR- und massenspektrometrischen Daten mit denen aus der Literatur[55] eindeutig erkannt. Zur Bestätigung der Struktur von Macrocidin Z (**4**) wurde im gleichen Zug die Erstsynthese des Naturstoffes angestrebt. Dabei lag die Schwierigkeit in der Identifikation der absoluten Stereokonfiguration und im Aufbau der 3-Acyltetramsäure, die gleichzeitig Teil eines Makrocyclus ist. Ziel war den Ring mittels Metathese zu schließen. Vorher sollte via Yoshii-Yoda-Acylierung eine Carbonsäure mit stereoselektiv eingeführter Methylgruppe in α-Position an eine 3-H_2-Tetramsäure angebracht

werden. Letztere würde vorher durch Anwendung der Meldrumsäure-Methode aus Boc-allyl-geschütztem Tyrosin in Einklang mit der Literatur entstehen.[57]



Schema 46. Abbildung der Strukturen aller Isolate aus dem Pilz *Phoma macrostoma* sowie retrosynthetische Schnitte für die erste Totalsynthese von Macrocidin Z (**4**).

Die Synthese startete mit der Anbringung des Evans-Auxiliars an 6-Heptensäure (**234**) durch Umsetzung mit DCC und DMAP (→ **235**). Anschließend gelang die stereoselektive Einführung einer Methylgruppe in α-Position. Das entstandene Diastereomerengemisch konnte durch Säulenchromatographie aufgetrennt werden, sodass das gewünschte Isomer **236** in 79% Ausbeute isoliert wurde. Mit LiOH und H_2O_2 konnte das Auxiliar in 96% entfernt werden (→ **237**). Daraufhin erfolgte die Synthese der 3-H_2-Tetramsäure **2** aus Boc-allyl-Tyrosin **78** mittels Meldrumsäure-Methode. Sie reagierte mit der Carbonsäure **237** bei Zugabe von EDC·HCl und DMAP zur 4-*O*-Acyltetramsäure **238**, welche dann nach dem Yoshii-Yoda-Protokoll zur 3-Acyltetramsäure **239** umgelagert werden konnte. Schließlich folgte der Schlüsselschritt der Synthese, die Ringschlussmetathese unter Grubbs-Katalyse. Das gewünschte Produkt **3** konnte mit exzellenter *E*-Selektivität von >99% in 89% Ausbeute isoliert werden. Die Kopplungskonstanten von $J = 15.5$ Hz bewiesen eindeutig die *E*-Konfiguration der Doppelbindung. Angenommen wurde, dass die Bildung eines *Z*-Isomers aufgrund der ungünstigen Konformation nicht möglich ist. Letzter Schritt war die Entfernung der Boc-Schutzgruppe durch TFA. Damit gelang die Synthese von Macrocidin Z (**4**) in sehr guten 30% Ausbeute über sieben Stufen. Der Vergleich der ^{1}H- und ^{13}C-Spektren sowie der CD-Spektren des isolierten Metabolits und des synthetischen Produkts bewies sowohl die Strukturannahme als auch die Stereokonfigurationen.



Schema 47. Erstsynthese von Macrocidin Z (**4**) ausgehend von Boc-allyl-Tyrosin **78**.
Reagenzien und Bedingungen: a) (*R*)-Benzyl-2-oxazolidinon, DCC, DMAP, CH_2Cl_2, 0 °C→RT, 23 h; b) 1. NaHMDS, THF, −78 °C, 30 min, 2. MeI, 4.5 h; c) LiOH, H_2O_2, THF/H_2O, 0 °C→RT, 1 d; d) 1. Meldrumsäure, DMAP, EDC·HCl, CH_2Cl_2, RT, 2 h, 2. EtOAc, Rückfluss, 3 h; e) **237**, EDC·HCl, DMAP, CH_2Cl_2, 0 °C→RT, 2 h; f) NEt_3, DMAP, CH_2Cl_2, RT, 24 h; g) Grubbs-Katalysator 2. Generation, CH_2Cl_2, Δ, 15 h; h) TFA, CH_2Cl_2, RT, 15 min.

Alle Isolate wurden gegen diverse Bakterien und Pilze getestet. Nur Macrooxazol C (**232**) der neu isolierten Verbindungen sowie die beiden Macrocidine **4** und **30** zeigten eine Wirkung. Die Mischung der Metabolite **231** und **233** war schwach cytotoxisch gegen HeLa Zellen und Maus Fibroblasten. Ebenfalls getestet wurde die Wirkung der Isolate auf die Bildung von Biofilmen durch *Staphylococcus aureus*. Auch hier fielen vor allem die Macrocidine auf, die nicht nur die Formation der Biofilme inhibierten, sondern auch den Abbau bestehender Biofilme induzierten. Neben ihrer herbiziden Wirkung konnten damit erstmals neue Bioaktivitäten festgestellt werden, die die Macrocidine neben ihrem Potential als Bioherbizide ebenfalls als Zusatzstoffe in Begleitung mit Antibiotika interessant machen. Bei Aufstellung eines Zusammenhangs der beobachteten Effekte mit der Struktur der Metabolite fällt auf, dass das 3-Acyltetramsäuremotiv einen entscheidenen Unterschied macht. Dies machte weitere Arbeiten an den makrocyclischen Tetramsäuren lohnend.

Weitere Details in: Blondelle Matio Kemkuignou, Laura Treiber, Haoxuan Zeng, Hedda Schrey, Rainer Schobert, Marc Stadler

Macrooxazoles A–D, New 2,5-Disubstituted Oxazole-4-Carboxylic Acid Derivatives from the Plant Pathogenic Fungus *Phoma macrostoma*

Molecules **2020**, 25, 5497.

3.2 Duale Agenti: Natürlich vorkommende Macrocidine und synthetische Analoga mit herbiziden und Antibiofilm-Aktivitäten

Im zweiten Teil der Arbeit wurde an den vielversprechenden Biotest-Ergebnissen von Macrocidin Z (**4**) angeschlossen. Die erlangten Erkenntnisse konnten auf die Darstellung eines Schlüsselintermediats angewendet werden, welches den Weg zu divers funktionalisierten Macrocidin-Derivaten ebnete. Diese wurden umfassenden Aktivitätsstudien unterzogen, um schließlich eine Struktur-Aktivitäts-Beziehung zu ermöglichen.

Ziel war es, jeweils ein wichtiges strukturelles Merkmal der Naturstoffe Macrocidin A (**30**) und Z (**4**) wie beispielsweise die Größe des Makrocyclus und die Art der Funktionalität anstelle des Epoxids bzw. der Doppelbindungen zu ändern. Dieser Ansatz führte zur Synthese von acht Derivaten (Abb. 6). Die Synthesen der meisten Derivate sollten ausgehend vom Schlüssel-intermediat **6** erfolgen, das in Analogie zur Synthese von Macrocidin Z (**4**) aufgebaut werden sollte.[95] Es sollte eine Doppelbindung anstatt des Epoxids tragen und keine Methylgruppe in Nachbarschaft zur 3-Acyltetramsäure besitzen.

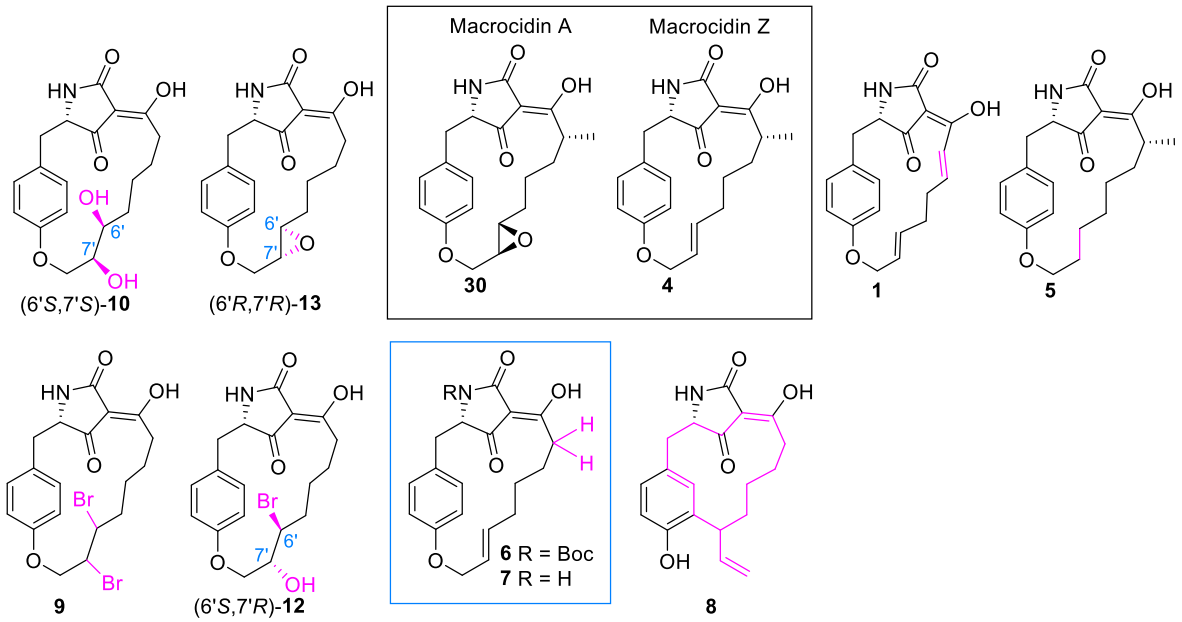
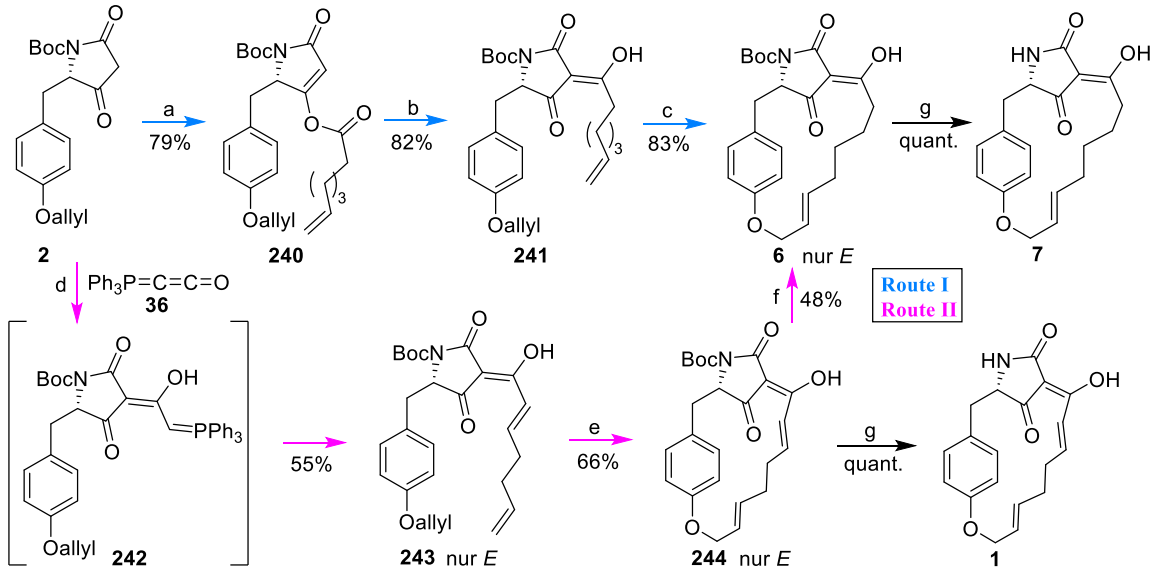


Abb 6. Strukturformeln von Macrocidin A (**30**) und Macrocidin Z (**4**) sowie der acht Derivate **1**, **5**, **7**, **8**, **9**, (6'*S*,7'*S*)-**10**, (6'*S*,7'*R*)-**12**, (6'*R*,7'*R*)-**13**. Pink markiert sind die markanten strukturellen Änderungen im Vergleich zu den Naturstoffen, blau umrandet ist das Schlüsselintermediat der Synthese.

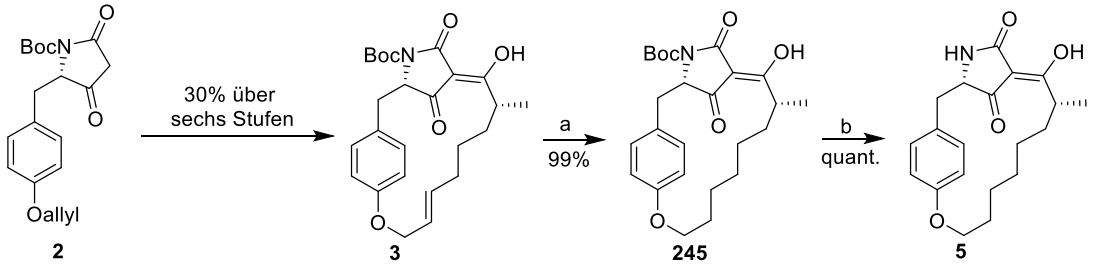
Zunächst wurde die Synthese des Schlüsselintermediats verwirklicht. Dies gelang auf zwei unterschiedliche Routen. Route I wurde in Anlehnung an die Macrocidin Z Synthese gestaltet. Die von Boc-allyl-Tyrosin abgeleitete 3-H_2-Tetramsäure **2** wurde zur 4-*O*-Acyltetramsäure **240** umgesetzt, welche dann mittels NEt_3 und DMAP nach dem Yoshii-Yoda-Protokoll zur 3-Acyl-tetramsäure **241** umgelagert wurde. Dies gelang in 65% Ausbeute über zwei Stufen. Via

Ringschlussmetathese konnte hier der Makrocyclus **6** in guten 83% Ausbeute gebildet werden. Die *E*-Selektivität war hier ebenfalls >99%. Das zentrale Intermediat **6** wurde folglich in 54% Ausbeute über drei Stufen gewonnen. Startmolekül der Route II war erneut die Tetramsäure **2**, welche durch Umsetzung mit Ketenylidentriphenylphosphoran (**36**) und 4-Pentenal direkt in die 3-Acyltetramsäure **243** überführt wurde. Mechanistisch findet zuerst die Bildung des Ylids **242** statt. Durch Zugabe des Aldehyds reagiert dieses in einer Wittig-Reaktion zum gewünschten Produkt **243**, welches als alleiniges Isomer isoliert wurde.[47] Die Ringschluss-metathese der 3-Enoyltetramsäure **243** mit dem Grubbs-Katalysator der zweiten Generation lieferte im nächsten Schritt den Makrocylus **244** in 66% Ausbeute. Die *E*-Selektivität blieb ähnlich hoch, jedoch brach die Ausbeute vermutlich aufgrund der veränderten Sterik durch die zweite Doppelbindung leicht ein. Bekannterweise ist die Entfernung der Doppelbindung von 3-Enoyltetramsäuren mit Hydrierung möglich.[47] Diese Methode konnte hier jedoch aufgrund der zweiten Doppelbindung nicht angewandt werden. Das Schlüsselintermediat **6** wurde schließlich durch Bildung eines nicht isolierten Silylenolethers nach Umsetzung mit dem Wilkinson-Katalysator und Triethylsilan sowie dessen Spaltung mit KF erhalten. Route II ergab eine Gesamtausbeute von 17% über drei Stufen. Die Verbindung **244** und das Intermediat **6** ergaben durch Boc-Entschützung die ersten Derivate **1** und **7**, die sich durch die Anzahl der Doppelbindungen und die Abwesenheit der Methylgruppe in Nachbarschaft zur Tetramsäure von Macrocidin Z (**4**) unterscheiden.



Schema 48. Synthese des Schlüsselintermediats **6** sowie der Derivate **1** und **7**.
Reagenzien und Bedingungen: a) 6-Heptensäure, EDC·HCl, DMAP, CH_2Cl_2, 0 °C→RT, 4 h; b) NEt_3, DMAP, CH_2Cl_2, RT, 24 h; c) Grubbs Katalysator 2. Generation, CH_2Cl_2, Δ, 24 h; d) 1. Ph_3PCCO, THF, Δ, 2 h, 2. KO*t*Bu, THF, Δ, 20 min, 3. 4-Pentenal, THF, Δ→RT, 21 h; e) Grubbs Katalysator 2. Generation, CH_2Cl_2, Δ, 18 h; f) 1. $Rh(PPh_3)_3Cl$, Et_3SiH, CH_2Cl_2, Δ, 19 h, 2. KF, MeOH, −15 °C, 27 h; g) TFA, CH_2Cl_2, RT, 15 min.

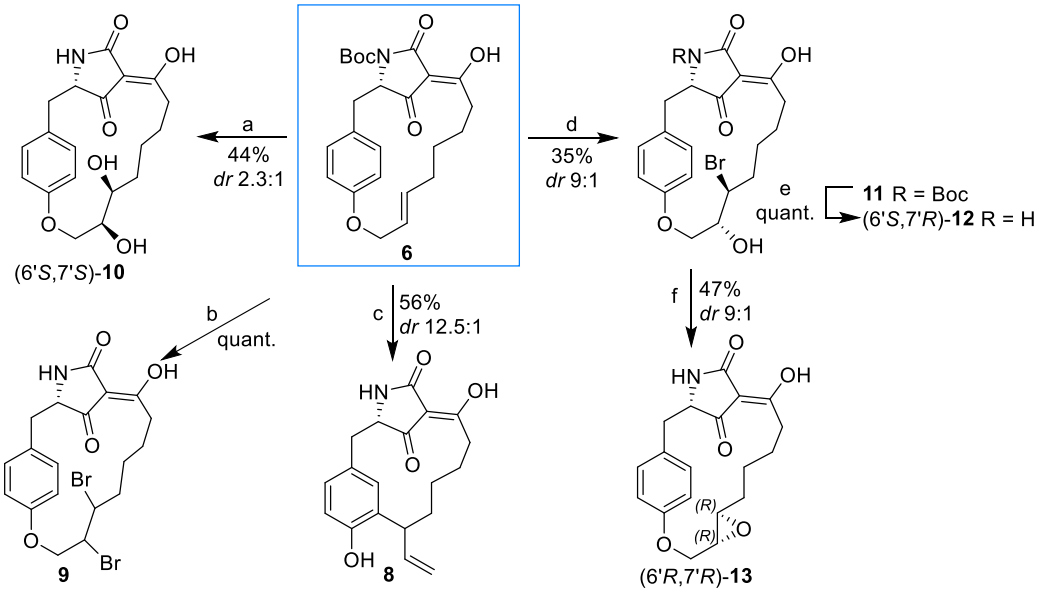
Aus Boc-geschütztem Macrocidin Z (**4**) konnte durch Pd-katalysierte Hydrierung und Entfernung der Schutzgruppe das Dihydroanalogon **5** in 99% über zwei Stufen gewonnen werden (Schema 49).



Schema 49. Synthese des Dihydroanalogon **5** ausgehend von Boc-Macrocidin Z **3**.
Reagenzien und Bedingungen: a) Pd/C, H_2, EtOAc, RT, 31 h; b) TFA, CH_2Cl_2, RT, 15 min.

Anstelle des Epoxids in Macrocidin A (**30**) sollten weitere Funktionalitäten eingefügt werden, wobei allgemein angemerkt werden kann, dass die Polarität der 3-Acyltetramsäuren und die Neigung zur Tautomerie sowohl die Aufreinigung als auch die NMR-spektroskopische Auswertung der Ergebnisse erschwerte. Zunächst wurde nach Umsetzung des Schlüsselintermediats **6** mit AD-Mix α und anschließender Boc-Entschützung der Diol (6'*S*,7'*S*)-**10** erhalten. Die Diastereomere konnten durch RP-Säulenchromatographie nicht getrennt werden. Gemessen an der Literatur zur Sharpless-Dihydroxylierung wäre ein hoher *dr* erwartet worden.[96,97] Der eher geringe *dr* von 2.3:1 weist auf eine Stereoinduktion durch den Makrocyclus hin. Die Reaktion des Schlüsselintermediats **6** mit Brom führte zur Bildung des Dibromids **9**. Es konnte nur als 1:1-Mischung der beiden mechanistisch plausiblen *anti*-Diastereomere isoliert werden. Vermutlich aufgrund der hohen Temperaturen kam es zur gleichzeitigen Entfernung der Boc-Schutzgruppe. Dies konnte ebenfalls bei der Claisen-Umlagerung des Makrocyclus **6** mit einhergehender Ringkontraktion zum Molekül **8** beobachtet werden. Das nun *meta*-verknüpfte Cyclophan **8** wurde als Mischung beider Diastereomere mit einem *dr* von 12.5:1 erhalten. Welche Stereokonfiguration im überwiegend vorliegenden Diastereomer auftritt, konnte nicht geklärt werden. Eine dafür hilfreiche Kristallstrukturanalyse war aufgrund der nicht realisierbaren Kristallzucht nicht möglich. Jedoch bestätigte der hohe *dr* die Annahme der Stereoinduktion durch den Makrocyclus. Dies konnte auch bei der Darstellung des Bromohydrins (6'*S*,7'*R*)-**11** mittels NBS und H_2O beobachtet werden. Nach Bildung eines Bromoniumions greift Wasser regioselektiv an und erzwingt zudem die Entstehung des *anti*-Bromohydrins.[98] Der Boc-geschützte Vorläufer **11** kann mittels Base ins Epoxid (6'*R*,7'*R*)-**13** überführt werden. Hier konnte durch Vergleiche mit

Macrocidin A (**30**) und NMR-Analysen die Stereokonfiguration bestimmt werden, wodurch die Konfiguration des Bromohydrins ebenfalls festgelegt wurde.



Schema 50. Synthese der divers funktionalisierten Derivate **8**, **9**, (6'*S*,7'*S*)-**10**, (6'*S*,7'*R*)-**12**, (6'*R*,7'*R*)-**13** ausgehend vom Schlüsselintermediat **6**.
Reagenzien und Bedingungen: a) 1. AD-Mix α, *t*BuOH/H_2O, 7 °C, 9 d, 2. TFA, CH_2Cl_2, RT, 15 min; b) Br_2, CCl_4, 80 °C, 30 h; c) Diethylanilin, 190 °C, 42 h; d) 1. NBS, DMSO, H_2O, 8 °C→RT, 22 h, 2. TFA, CH_2Cl_2, RT, 15 min; e) TFA, CH_2Cl_2, RT, 15 min; f) KO*t*Bu, THF, 0 °C→RT, 4 d.

Alle Derivate wurden zusammen mit den Naturstoffen Macrocidin A (**30**) und Macrocidin Z (**4**) diversen Biotests unterzogen. Die herbizide Aktivität der Verbindungen wurde im Vergleich zum bekannten Wirkstoff Diflufenican gemessen. Dafür wurden Disteln und Löwenzähne mit Lösungen beziehungsweise Suspensionen der Verbindungen über einen Zeitraum von sechs Wochen behandelt. Die Wirkung auf die Pflanzen wurde an den ausgelösten Nekrosen, Chlorosen und verwelkten Pflanzen gemessen. Alle Macrocidine zeigten eine höhere Aktivität gegen die Disteln. Besonders effektiv waren Macrocidin A (**30**) und Macrocidin Z (**4**). Jegliche Änderungen der Struktur führten zu Aktivitätsverlusten.

Da eine Biofilm-inhibierende Wirkung bereits bei Macrocidinen im Rahmen der Synthese von Macrocidin Z (**4**) festgestellt wurde, wurde nun auch die antibiotische Wirkung auf Bakterienstämme der Art *Staphylococcus aureus, Acinetobacter baumanii* und *Escherichia coli* untersucht. Die antibiotische Aktivität sollte für einen potentiellen biofilminhibierenden Wirkstoff gering ausfallen, um Resistenzbildung zu umgehen.[99] Erfreulicherweise waren nur das Dibromid **9**, Dihydromacrocidin Z **5** und Macrocidin Z (**4**) gegen *Staphylococcus aureus* und *Escherichia coli* sehr schwach antibiotisch aktiv. Keines der Derivate zeigte eine Wirkung gegen *Acinetobacter baumanii*. Ebenfalls günstig war, dass keine cytotoxischen Effekte beobachtet wurden.

Die Inhibition der Biofilm-Formation und die Auflösung bereits existierender Biofilme wurde an *Candida albicans* und *Staphylococcus aureus* getestet. Alle Derivate inhibierten die Bildung mindestens zu 75% im Vergleich zur unbehandelten Kontrolle bei der höchsten getesteten Konzentration. Im subtoxischen Bereich erzielten Macrocidin Z (**4**), Dihydromacrocidin Z **5** und das Dibromid **9** vielversprechende Ergebnisse im Bereich der Positivkontrolle. Diese Substanzen hatten auch deutliche Effekte auf die Auflösung von Biofilmen, die sogar die Wirkung der Positivkontrolle überstiegen. Auffällig war, dass die eher lipophilen und strukturell einfachen Verbindungen die höchsten Aktivitäten zeigten. Einbrüche der Effekte konnten vor allem bei den polaren Verbindungen mit freien Hydroxygruppen (6'*S*,7'*S*)-**10** und (6'*S*,7'*R*)-**12** beobachtetet werden.

Zusammenfassend kann angemerkt werden, dass die Einführung polarer Funktionalitäten im Falle der Derivate (6'*S*,7'*S*)-**10** und (6'*S*,7'*R*)-**12** sowohl zu Verlusten der herbiziden als auch der Antibiofilm-Aktivität führten. Macrocidin Z (**4**) und das sehr ähnliche Dihydroanalogon **5** zeigten sowohl interessante herbizide als auch Antibiofilm-Eigenschaften. Die Aktivität von *Nor*-Macrocidin Z (**7**) hingegen war stets geringer. Die Methylgruppe scheint deshalb einen positiven Effekt auf die Bioaktivität der Verbindungen zu haben. Das Epoxid scheint nicht notwendig für eine herbizide oder biofilminhibierende Wirkung zu sein, anders als in der Literatur oft prognostiziert.

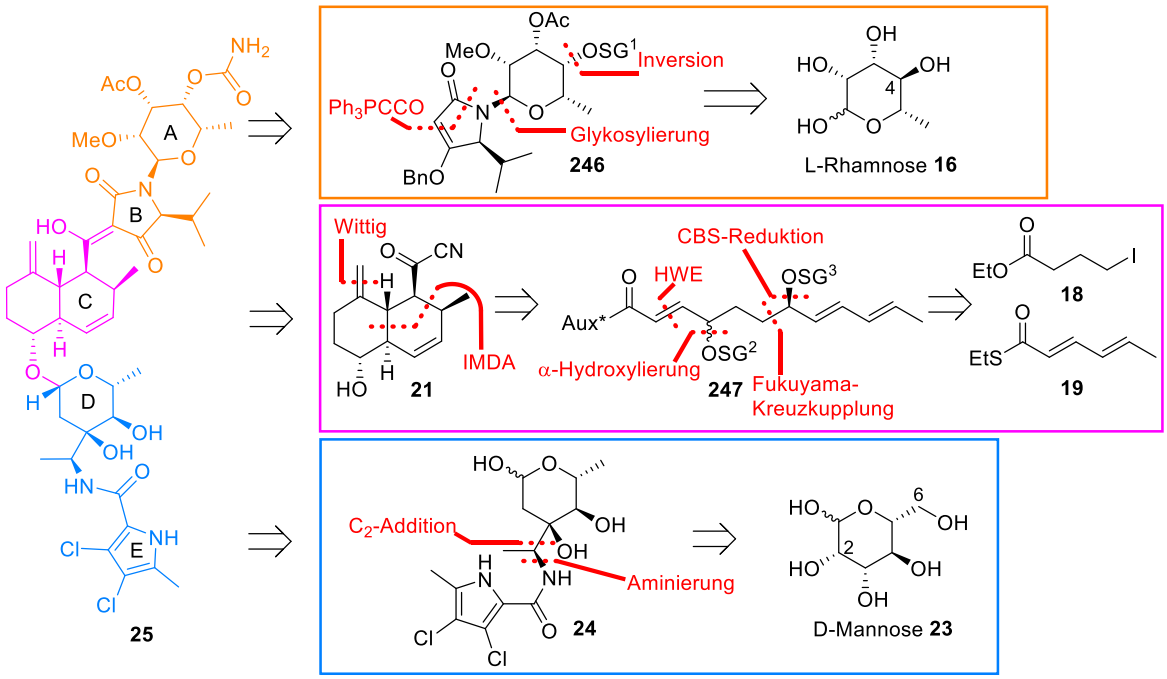
Weitere Details in: Laura Treiber, Christine Pezolt, Haoxuan Zeng, Hedda Schrey, Stefan Jungwirth, Aditya Shekhar, Marc Stadler, Ursula Bilitewski, Maike Erb-Brinkmann, Rainer Schobert

Dual Agents: Fungal Macrocidins and Synthetic Analogues with Herbicidal and Antibiofilm Activities

Antibiotics **2021**, 10, 1022.

3.3 Formale Totalsynthese von Kibdelomycin und Derivatisierung von Amycolose-Glykosiden

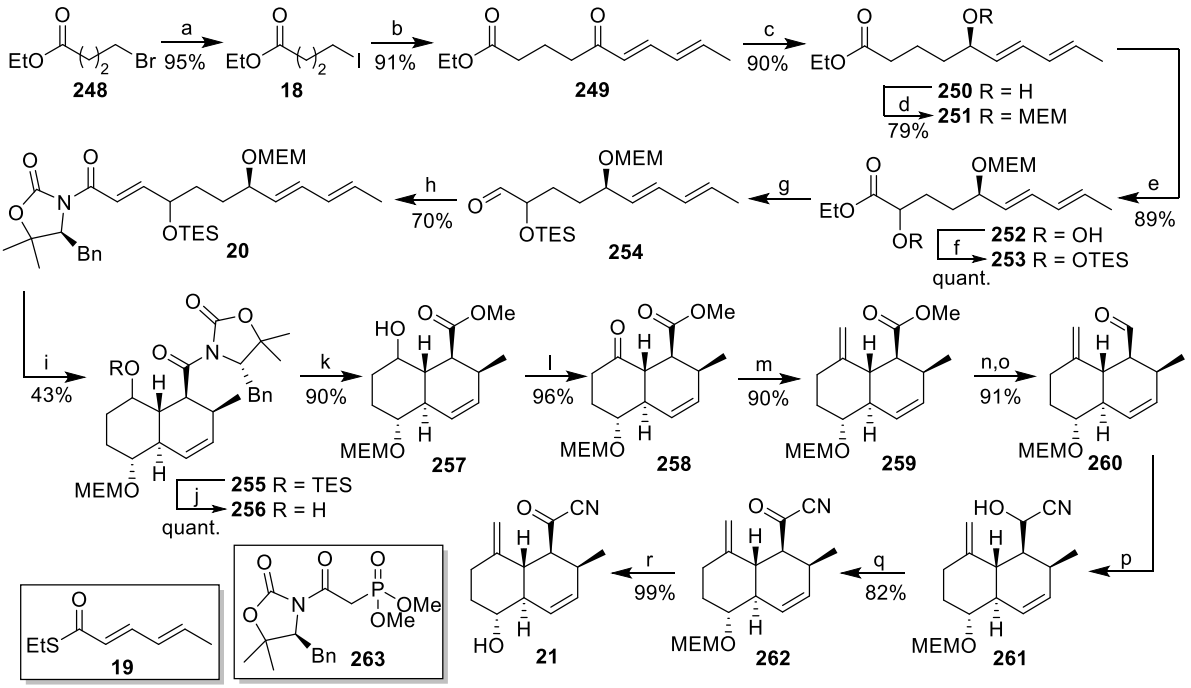
Die in den ersten beiden Teilen gewonnene synthetische Erfahrung zu 3-Acyltetramsäuren und deren Derivatisierung wurden im dritten Teil der Arbeit auf einen strukturell deutlich komplexeren Vertreter dieser Verbindungsklasse – Kibdelomycin (**25**) – angewendet. Kibdelomycin hat starke antibiotische Eigenschaften, vorwiegend auf gram-positive Bakterien mit einem neuen Wirkmechanismus, der sowohl die Bildung von Resistenzen erschwert als auch zur Wirkung auf bereits resistente Keime führt. Die komplexe Struktur wurde für die formale Totalsynthese in drei Fragmente unterteilt (Schema 51): die Amykitanose mit *N*-glykosylierter Tetramsäure **246**, den Decalin-Linker **21** und die *N*-acylierte Amycolose **24**. Dabei sollten die Funktionalitäten der finalen Fragmente identisch zu denen der ersten publizierten Totalsynthese[73] sein, um eine formale Totalsynthese abzuschließen. Der Zucker **246** sollte durch Glykosylierung einer 4-*O*-Alkyltetramsäure entstehen. Letztere sollte in einem Schritt aus der entsprechenden Aminosäure durch Umsetzung mit Ph_3PCCO gebildet werden. Als Startmaterial für das Glykosid **A** wurde L-Rhamnose (**16**) gewählt, wobei zum einen die Stereokonfiguration an 4-Position invertiert werden musste und zum anderen diverse Funktionalisierungen erfolgen mussten. Schlüsselschritt in der Synthese des Decalin-Bausteins **21** war die diastereoselektive IMDA zum Aufbau des Ringsystems. Der lineare Vorläufer **247** sollte nach α-Hydroxylierung, CBS-Reduktion und Fukuyama-Kupplung aus 4-Brombuttersäureethylester (**18**) und dem Sorbinsäurethioester **19** erhalten werden. Die *N*-acylierte Amycolose **24** sollte im letzten Schritt durch Knüpfung der Amidbindung zwischen der Pyrrolcarbonsäure **E** und der Aminofunktion des Glykosids **D** gebildet werden. Davor musste der Zucker **D** ausgehend von D-Mannose (**23**) über diverse Funktionalisierungen unter anderem das stereoselektive Einführen eines C_2-Synthons sowie Desoxygenierungen an 2- und 6-Position dargestellt werden.



Schema 51. Aufteilung von Kibdelomycin (**25**) in drei Fragmente, retrosynthetische Schnitte und Edukte.

Die Synthese des Decalin-Fragementes startete mit einer finkelsteinartigen Reaktion des Bromids **248** zum Iodid **18** (Schema 52). Nach der Pd-katalysierten Fukuyama-Kupplung mit dem Sorbinsäurethioester **19** folgte die CBS-Reduktion des entstandenen Ketons **249**. Der Alkohol **250** wurde in 90% Ausbeute und 91% *ee* gewonnen. Die freie Hydroxygruppe wurde MEM-geschützt und mittels des Vedejs-Reagenzes eine zweite Hydroxygruppe in α-Position zum Ester eingeführt (→ **252**).[91] Nach deren Silyl-Schützung folgte die Reduktion zum Aldehyd **254**, der in einer HWE-Reaktion zum Trien **20** in 70% über zwei Stufen reagierte. Dabei wurde gleichzeitig mit der Bildung der dritten notwendigen Doppelbindung auch das Auxiliar für die nachfolgende diastereoselektive Diels-Alder-Reaktion eingeführt. Zunächst wurde lediglich Benzyloxazolidinon angebracht, was jedoch nach der Diels-Alder-Reaktion nicht mehr entfernt werden konnte. Frossard *et al*. stießen während ihrer Synthese auf ähnliche Probleme und wechselten zum sterisch anspruchsvolleren SuperQuat, das bekanntermaßen selektiver zu entfernen ist.[87,100] Innerhalb der hier dargestellten Synthese konnte dies auch bestätigt werden. Nach erfolgreicher Diels-Alder-Reaktion, die erstmals durch Erhitzen und ohne Verwendung einer Lewissäure mit einem *de* >96% durchgeführt wurde, wurde die Silyl-Gruppe entfernt (→ **256**). Aus sterischen Gründen gelang die Abspaltung des Auxiliars durch Umsetzung mit NaOMe erst mit der freien Hydroxylgruppe (→ **257**). Der entstandene Alkohol **257** wurde schließlich zum Keton **258** oxidiert und dieses in einer Wittig-Reaktion ins terminale Olefin **259** überführt. Der Ester sollte für die Einführung des Acylcyanids zum Aldehyd **260** umgesetzt werden. Dies gelang jedoch nicht wie erwartet in einem Schritt durch Reaktion mit

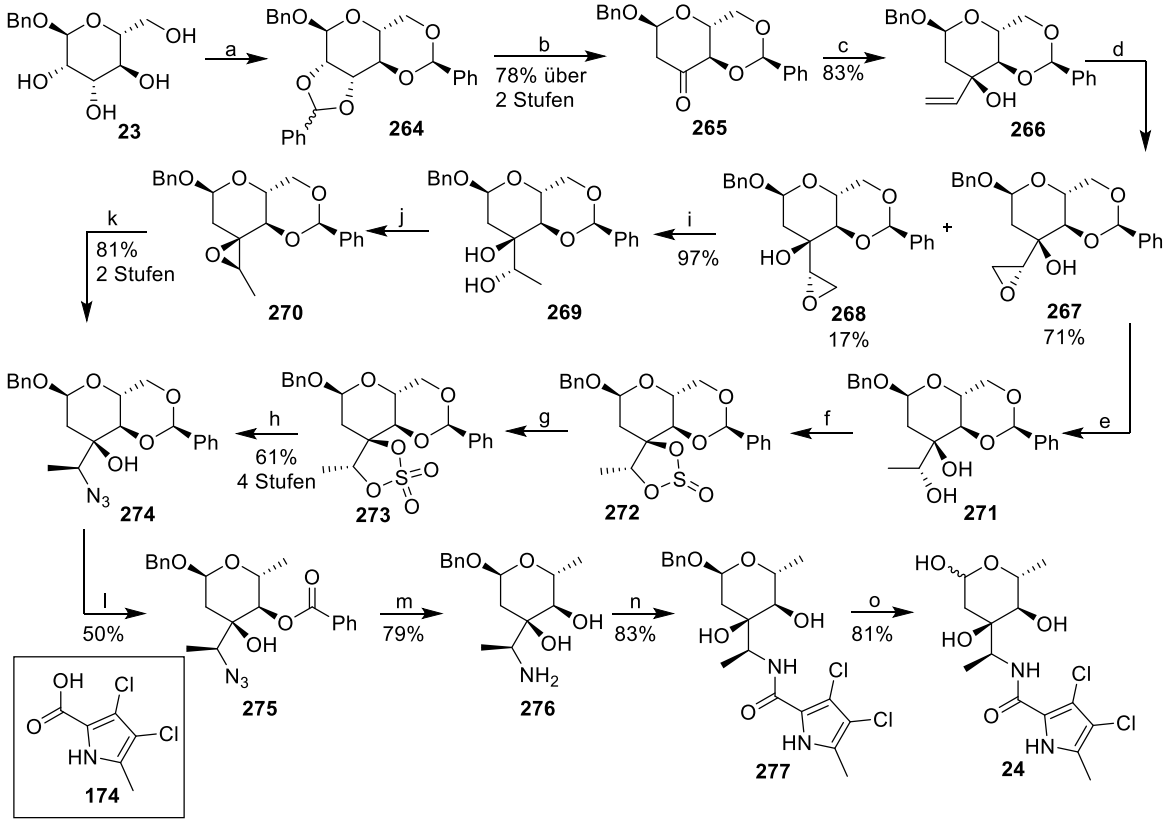
DIBAL. Stattdessen konnte nur der primäre Alkohol isoliert werden, der dann mittels DMP oxidiert wurde. In Anlehnung an die Literatur[73] erfolgte die Reaktion mit TMSCN zum Cyanohydrin **261**, welches erneut mit DMP zum Acylcyanid **262** oxidiert wurde. Zuletzt wurde mittels $LiBF_4$ MEM-entschützt (→ **21**).



Schema 52. Synthese des Decalin-Fragmentes **21** ausgehend von 4-Brombuttersäurethylester (**248**).
Reagenzien und Bedingungen: a) NaI, Aceton, Δ, 21 h; b) 1. **18**, Zn, THF, Rückfluss, 3.5 h, 2. **19**, $Pd(PPh_3)_4$, Toluol, RT, 23 h; c) 1. (*S*)-CBS, BH_3·THF, THF, RT, 1 h, 2. **249**, −35 °C, 3.5 h; d) MEMCl, DIPEA, CH_2Cl_2, 40 °C, 23 h; e) 1. KHMDS, THF, −78 °C, 30 min, 2. MoOPH, −78 °C, 4 h; f) TESCl, Imidazol, DMAP, CH_2Cl_2, 0 °C→40 °C, 4.5 h; g) DIBAL, Toluol, −78 °C, 5 h; h) 1. LiHMDS, **263**, THF, 0 °C, 1 h, 2. **254**, 0 °C→RT, 17 h; i) Toluol, 80 °C, 3 d; j) HF·py, THF, 0 °C, 15 h; k) NaOMe, CH_2Cl_2, 0 °C, 3 h; l) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C→RT, 3 h; m) 1. $MePPh_3Br$, KO*t*Bu, THF, 0 °C, 45 min, 2. **258**, THF, 0 °C→RT, 3 h; n) DIBAL, CH_2Cl_2, 0 °C, 5 h; o) DMP, CH_2Cl_2, 0 °C→RT, 3 h; p) 1. TMSCN, NEt_3, CH_2Cl_2, 0 °C→RT, 4 h, 20 min, 2. NH_4F, EtOH, 0 °C, 2 h; q) DMP, CH_2Cl_2, 0 °C, 1.5 h; r) $LiBF_4$, CH_2Cl_2, 0 °C→RT, 4.5 h.

Die *N*-acylierte Amycolose **24** wurde ausgehend von benzylierter D-Mannose **23** synthetisiert (Schema 53). Diese wurde doppelt Acetal-geschützt und in einer Klemer-Rodemeyer-Fragmentierung in das Keton **265** überführt.[101] Durch eine Grignard-Reaktion mit Vinylmagnesiumbromid wurde der tertiäre Alkohol **266** als einzelnes Diastereomer generiert. Durch Umsetzung mit *m*CPBA entstand eine trennbare Mischung von Epoxiden, wobei beide Diastereomere **267**/**268** durch unterschiedliche Routen zum Zielmolekül führten. Die Stereokonfiguration des Hauptdiastereomers **267** wurde mittels Mosher-Ester-Methode bestimmt. Es wurde durch Angriff eines Hydrids zum sekundären Alkohol **271** ringgeöffnet, dieser ins Sulfit **272** überführt und letzteres zum Sulfat **273** oxidiert. Dieses lieferte durch Reaktion mit NaN_3 das gewünschte Azid **274**. Das zweite Diastereomer **268** wurde ebenfalls

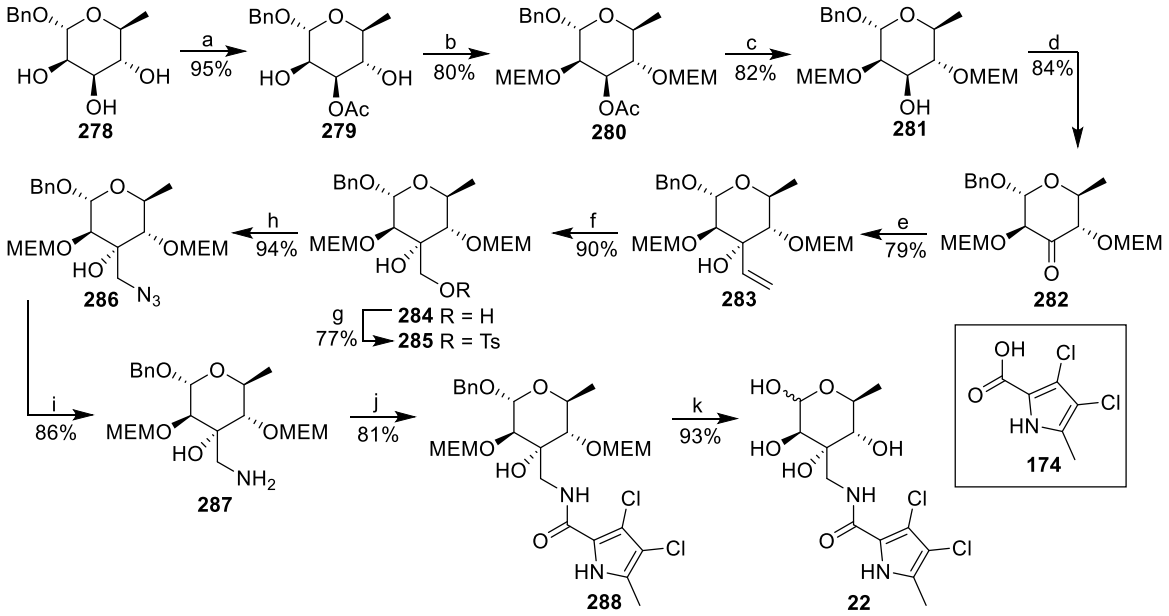
mit $LiAlH_4$ ringgeöffnet und der entstandene Diol **269** anschließend über ein intermediär gebildetes Triflat in das Epoxid **270** überführt. Nach Umsetzung mit NaN_3 konnte auch hier das Azid **274** isoliert werden. Es folgte die Deoxygenierung an 6-Position (→ **275**) und die Entfernung des dabei entstandenen Benzoats unter gleichzeitiger Reduktion des Azids zum Amin **276** mittels $LiAlH_4$.[102] Schließlich wurde mit der Pyrrolcarbonsäure **174** gekuppelt und die Benzylgruppe an der anomeren Position durch BCl_3 entfernt.



Schema 53. Synthese der *N*-acylierten Amycolose **24** ausgehend von benzylierter D-Mannose (**23**).
Reagenzien und Bedingungen: a) BDMA, CSA, $CHCl_3$, 80 °C, 6.5 h; b) *n*BuLi, THF, −78 °C→−35 °C, 3.75 h; c) VinylMgBr, THF, −78 °C, 3 h; d) *m*CPBA, CH_2Cl_2, RT, 22 h; e) $LiAlH_4$, THF, 0 °C→RT, 2.5 h; f) $SOCl_2$, NEt_3, CH_2Cl_2, 0 °C, 3 h, g) $NaIO_4$, $RuCl_3{\cdot}xH_2O$, MeCN, RT, 7 h; h) 1. NaN_3, DMF, 65 °C, 6.75 h, 2. Zitronensäurepuffer, EtOAc, 45 °C, 15 h, 3. Zitronensäure, 3.5 h; i) $LiAlH_4$, THF, 0 °C→RT, 1.75 h; j) Tf_2O, Pyridin, CH_2Cl_2, −78 °C→0 °C, 1.25 h; k) NaN_3, NH_4Cl, MeOH, 80 °C, 12 h; l) TIPST, DTBP, *n*-Octan, 140 °C, 6.75 h; m) $LiAlH_4$, THF, 0 °C→RT, 24 h; n) **174**, HOBt, EDC·HCl, NEt_3, CH_2Cl_2, 0 °C→RT, 16 h; o) BCl_3, CH_2Cl_2, −80 °C, 40 min.

Dass die *N*-acylierte Amycolose **24** an sich als Supressor des Zellwachstums von malignen Zellen in Frage kommt, macht sie zu einem interessanten Ziel für Derivatisierungen. Ausgehend von benzylierter L-Rhamnose **278** wurde erstmals ein solches Amycolose-Derivat synthetisiert, das deutlich vereinfacht und daher auch in einer verkürzten Synthese zu erreichen war (Schema 54). Es sollten alle Hydroxylgruppen inklusive Stereozentren erhalten bleiben und lediglich die Grignard-Reaktion an 3-Position durchgeführt werden, um in weiteren Schritten die Kupplung

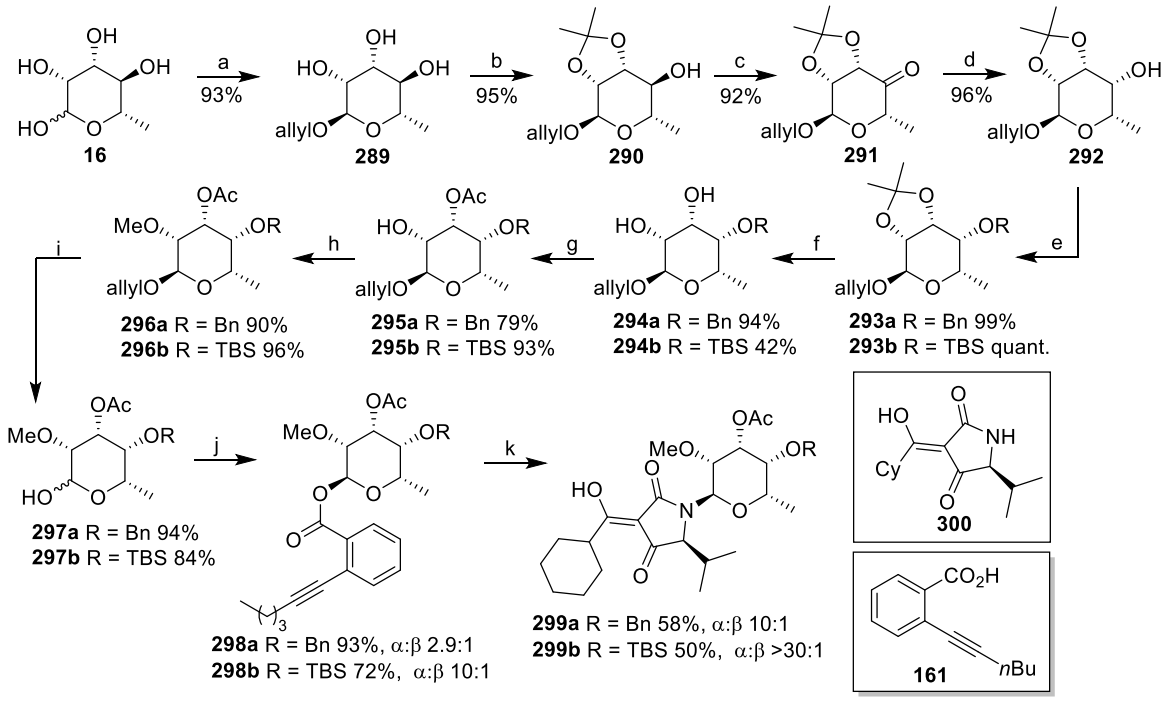
mit der Pyrrolcarbonsäure zu ermöglichen. Die benzylierte Rhamnose **278** wurde selektiv an 3-Position acetyliert und die restlichen Hydroxylgruppen MEM-geschützt (→ **280**). Hier war die Wahl einer schmalen und kleinen Schutzgruppe entscheidend. Nach Acetylentschützung wurde die 3-Position oxidiert (→ **282**). Das resultierende Keton **282** reagierte wie Keton **265** diastereoselektiv mit Vinylmagnesiumbromid zum Alkohol **283**. Die Stereokonfiguration wurde durch NOE-Messungen bestimmt. Nach Ozonolyse und Aktivierung des daraus resultierenden primären Alkohols **284** zum Tosylat **285** konnte dieses zum Azid **286** substituiert werden. Dessen Staudinger-Reaktion lieferte das Amin **287**, welches mit der Pyrrolcarbonsäure **174** acyliert wurde. Zum Schluss wurde das Amid **288** mit BCl_3 global entschützt. Die Synthese des Amycolose-Derivates **22** könnte auf jegliche Pyranosen angewendet werden und ist mit einer Gesamtausbeute von 17% über elf Stufen auch im Grammmaßstab durchführbar.



Schema 54. Synthese eines Derivates der Amycolose **22** ausgehend von benzylierter L-Rhamnose **278**. *Reagenzien und Bedingungen*: a) $MoO_2(acac)_2$, Collidin, AcCl, 1,4-Dioxan, RT, 3 h; b) MEMCl, DIPEA, CH_2Cl_2, 0 °C→40 °C, 1 d; c) DIBAL, Toluol, 0 °C, 3 h; d) DMP, CH_2Cl_2, 0 °C→RT, 5 h; e) VinylMgBr, THF, −78 °C, 5 h; f) 1. O_3, CH_2Cl_2/MeOH, −78 °C, 10 min, 2. $NaBH_4$, RT, 24 h; g) *p*TsCl, DMAP, NEt_3, CH_2Cl_2, RT, 21 h; h) NaN_3, DMF, 65 °C, 17 h; i) 1. PPh_3, THF, RT, 2 d, 2. H_2O, RT, 3 d; j) **174**, EDC·HCl, HOBt, DMAP, CH_2Cl_2, 0 °C→RT, ü. N.; k) BCl_3, CH_2Cl_2, −78 °C, 3.5 h.

Es folgte die Synthese des zweiten Glykosids ausgehend von L-Rhamnose (**16**; Schema 55). Die anomere Position wurde allyl-geschützt (→ **289**) und der *syn*-Diol ins Ketal **290** überführt. Durch Swern-Oxidation und Reduktion wurde die Inversion des Stereozentrums an 4-Position realisiert, wobei ein einzelnes Diastereomer **292** entstand. Nach Benzylschützung der Hydroxylgruppe (→ **293a**) wurde der Diol entschützt (→ **294a**). Durch die nachfolgende selektive Acetylierung und Methylierung wurden die ersten Funktionalitäten eingeführt (→

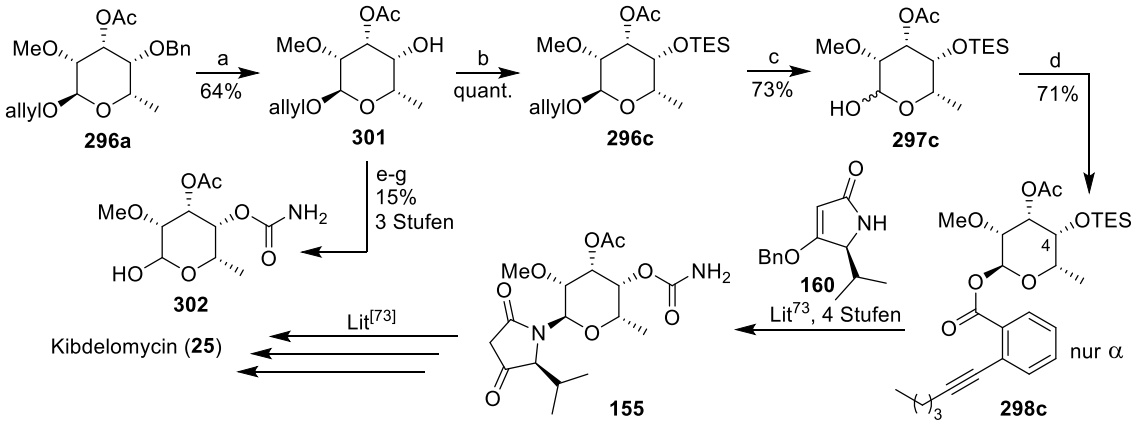
296a). Reaktion mit $Pd(PPh_3)_4$ in AcOH lieferte das Halbacetal **297a**. Einige Versuche einer Kupplung mit der Tetramsäure schlugen fehl. Deshalb wurde, in Anlehnung an die Synthese von Yang[73], der Ester **298a** generiert, der unter Au-Katalyse mit der 3-Acyltetramsäure **300** verknüpft werden konnte (→ **299a**). Der Cyclohexylrest an der 3-Acyltetramsäure sollte das Decalin imitieren. Der Anteil des erwünschten α-Anomers des erhaltenen Glykosids **299a** war deutlich niedriger als in der Literatur[73,82], weswegen ein Zusammenhang mit der Schutzgruppe an 4-Position angenommen wurde. Dies konnte verifiziert werden durch die Synthese des analogen TBS-geschützten Glykosids **299b**, das wie bei Yang *et al*. eine Silylschutzgruppe trug.[73] Das Anomerenverhältnis konnte von α:β=10:1 auf >30:1 gesteigert werden. Die hier etablierte Methode könnte als alternative Kupplung der Fragmente in einer weiteren Totalsynthese und zur Derivatsynthese angewandt werden.



Schema 55. Synthese der Tetramsäuren **299a** und **299b**.
Reagenzien und Bedingungen: a) AcCl, C_3H_5OH, 0 °C→55 °C, 24 h; b) $CuSO_4$, AcMe, RT, 17 h; c) 1. $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 40 min, 2. **290**, 50 min, 3. DIPEA, −78 °C→RT, 16 h; d) $NaBH_4$, EtOH, 0 °C, 1.5 h; e) **293a**: 1. NaH, Imidazol, DMF, 0 °C, 35 min, 2. BnBr, TBAI, RT, 17 h; **293b**: TBSOTf, Pyridin, CH_2Cl_2, 0 °C, 5 h; f) **294a**: AcOH, H_2O, Δ, 1.5 h; **294b**: HCOOH, EtOH, RT, 2.5 h; g) **295a**: 1. Bu_2SnO, Toluol, Δ, 4 h, 2. AcCl, 0 °C, 30 min; **295b**: 1. Bu_2SnO, Toluol, Δ, 3 h, 2. AcCl, RT, 1 h; h) **296a**: $TMSCHN_2$, HBF_4, CH_2Cl_2, 0 °C, 5 h; **296b**: MeO_3BF_4, Protonenschwamm, CH_2Cl_2, 0 °C→40 °C, 21 h; i) **297a**: $Pd(PPh_3)_4$, AcOH, RT, 17 h; **297b**: 1. DABCO, Wilkinson Katalysator, EtOH, Δ, 15 h, 2. I_2, Phosphatpuffer pH=7/H_2O/EtOAc, RT, 10 min; j) **298a**/**298b**: Säure **161**, DCC, DMAP, CH_2Cl_2, RT, 3-3.5 h; k) **299a**/**299b**: Tetramsäure **300**, $AuPPh_3NTf_2$, Toluol, RT→40 °C, 17-20 h.

Zum Abschluss der formalen Totalsynthese musste eine TES-Gruppe an 4-Position von **296a** eingeführt werden (Schema 56). Dies gelang nach Entfernung der Benzylgruppe mit Et_3SiH

und I_2 (→ **301**) und TES-Schützung. Der Zucker **296c** wurde dann analog zur oben beschriebenen Synthese allyl-entschützt und zum Glykosid **297c** umgesetzt. Nach Kupplung mit dem Benzyltetramat **160** und anschließender Einführung des Carbamats an 4-Position (→ **155**) könnte in weiteren Schritten in Anlehnung an die Literatur Kibdelomycin (**25**) gebildet werden.[73] Im Zuge der Synthese des Glykosids **298c** konnte außerdem ausgehend vom Alkohol **301** die Amykitanose (**302**) durch Einführung des Carbamats und Allylentschützung in 15% Ausbeute über drei Stufen dargestellt werden.



Schema 56. Synthese des Glykosids **298c** und finale Schritte analog zur Totalsynthese nach Yang *et al*.[73] *Reagenzien und Bedingungen*: a) 1. I_2, CH_2Cl_2, −65 °C, 35 min, 2. Et_3SiH, −65 °C→−20 °C, 2 h; b) TESOTf, Pyridin, CH_2Cl_2, 0 °C, 2 h; c) 1. DABCO, $Rh(PPh_3)_3Cl$, EtOH, Δ, 5 h, 2. I_2, Phosphatpuffer/H_2O/EtOAc, RT, 25 min; d) DCC, DMAP, CH_2Cl_2, RT, 3 h; e) Trichloracetylisocyanat, CH_2Cl_2, 0°C, 13 min; f) SiO_2, THF/MeOH, 40°C; g) $Pd(PPh_3)_4$, AcOH, RT, 16 h.

In weiteren Arbeiten könnten Derivate von Kibdelomycin (**25**) in Anlehnung an die formale Totalsynthese dargestellt werden. Diese könnten zusammen mit einzelnen Fragmenten Bioaktivitätsstudien unterzogen werden, um weitere Struktur-Aktivitäts-Beziehungen aufzustellen.

Weitere Details in: Manuel G. Schriefer, Laura Treiber, Rainer Schobert

Formal synthesis of kibdelomycin and derivatisation of amycolose glycosides

Chemical Science **2023**, 14, 3562.

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5 PUBLIKATIONEN

5.1 Darstellung des Eigenanteils

Die in dieser Arbeit abgebildeten Publikationen wurden in Zusammenarbeit mit anderen Wissenschaftlern der Universität Bayreuth sowie in Kooperation mit anderen Arbeitsgruppen erarbeitet. Zu Letzteren zählen der Lehrstuhl *Microbial drugs* des *Helmholtz Centre for Infection Research* in Braunschweig unter Leitung von Prof. Dr. Marc Stadler, der Lehrstuhl *Compound Profiling and Screening* des *Helmholtz Centre for Infection Research* in Braunschweig unter Leitung von Prof. Dr. Ursula Bilitewski sowie das Unternehmen PHYTOsolution in Freyburg.

Im Folgenden werden die Eigenanteile der Co-Autoren detailliert aufgelistet.

5.1.1 Eigenanteil Publikation I

Die Arbeiten zu diesem Thema wurden im Journal ***Molecules*** (DOI: 10.3390/molecules25235497) unter dem Titel

„Macrooxazoles A-D, new 2,5-disubstituted oxazole-4-carboxylic acid derivatives from the plant pathogenic fungus phoma macrostoma"

von den Autoren Blondelle Matio Kemkuignou, Laura Treiber, Haoxuan Zeng, Hedda Schrey, Rainer Schobert und Marc Stadler veröffentlicht.

Eigenanteil: Syntheseplanung;
Synthetische Arbeiten und Charakterisierung der neuen Verbindungen;
Beiträge am Verfassen des Manuskriptes.

B. M. Kemkuignou: Fermentierung; Analyse der Extrakte;
Isolation der Verbindungen inklusive Strukturaufklärung;
Bioassays;
Teilnahme am Verfassen des Manuskriptes.

H. Zeng: Antibiofilm-Assay.

H. Schrey: Antibiofilm-Assay; Bearbeitung des Manuskripts.

R. Schobert: Bearbeitung des Manuskripts; Syntheseplanung.

M. Stadler: Bearbeitung und Finalisierung des Manuskriptes; Projektplanung.

5.1.2 Eigenanteil Publikation II

Die Arbeiten zu diesem Thema wurden im Journal ***Antibiotics*** (DOI: 10.3390/antibiotics10081022) unter dem Titel

„Dual agents: fungal macrocidins and synthetic analogues with herbicidal and antibiofilm activities"

von den Autoren Laura Treiber, Christine Pezolt, Haoxuan Zeng, Hedda Schrey, Stefan Jungwirth, Aditya Shekhar, Marc Stadler, Ursula Bilitewski, Maike Erb-Brinkmann und Rainer Schobert veröffentlicht.

Eigenanteil: Projektplanung;
Syntheseplanung;
Synthetische Arbeiten und Charakterisierung der neuen Verbindungen
Verfassen des Manuskriptes.

C. Pezolt: Beitrag zur Synthesearbeit des Schlüsselintermediats.

H. Zeng: Antibiofilm-Assay.

H. Schrey: Antibiofilm-Assay.

S. Jungwirth: Tests zur antimikrobiellen Aktivität.

A. Shekhar: Tests zur antimikrobiellen Aktivität.

M. Stadler: Leitung des Antibiofilm-Assays.

U. Bilitewski: Leitung der Tests zur antimikrobiellen Aktivität.

M. Erb-Brinkmann: Tests der herbiziden Aktivität; Projektplanung.

R. Schobert: Projektplanung und -verwaltung; Verfassen des Manuskriptes.

5.1.3 Eigenanteil Publikation III

Die Arbeiten zu diesem Thema wurden im Journal ***Chemical Science*** (DOI: 10.1039/D3SC00595J) unter dem Titel

„Formal synthesis of kibdelomycin and derivatisation of amycolose glycosides"

von den Autoren Manuel G. Schriefer, Laura Treiber und Rainer Schobert veröffentlicht werden.

Eigenanteil: Projektplanung;
Planung der Synthese der Amykitanose und deren Vorläufer, der 3-Acyltetramsäuren, des Derivates der Amycolose und des Decalin-Fragmentes;
Synthetische Arbeiten und Charakterisierung der neuen Verbindungen im Zuge der Synthese der 3-Acyltetramsäuren, des Derivates der Amycolose und des Decalin-Fragmentes;
Verfassen des Manuskriptes.

M. G. Schriefer: Projektplanung;
Planung der Synthese der Amycolose, dessen Derivats und des Decalin-Fragmentes;
Synthetische Arbeiten und Charakterisierung der neuen Verbindungen innerhalb der Synthese der Amycolose und des Decalin-Fragmentes;
Verfassen des Manuskriptes.

R. Schobert: Projektplanung und -verwaltung; Verfassen des Manuskriptes.

5.2 Publikation I

Macrooxazoles A-D, new 2,5-disubstituted oxazole-4-carboxylic acid derivatives from the plant pathogenic fungus phoma macrostoma

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Macrooxazoles A–D, New 2,5-Disubstituted Oxazole-4-Carboxylic Acid Derivatives from the Plant Pathogenic Fungus *Phoma macrostoma*

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Abstract: In our ongoing search for new bioactive fungal metabolites, four previously undescribed oxazole carboxylic acid derivatives (**1**–**4**) for which we proposed the trivial names macrooxazoles A–D together with two known tetramic acids (**5**–**6**) were isolated from the plant pathogenic fungus *Phoma macrostoma*. Their structures were elucidated based on high-resolution mass spectrometry (HR-MS) and nuclear magnetic resonance (NMR) spectroscopy. The hitherto unclear structure of macrocidin Z (**6**) was also confirmed by its first total synthesis. The isolated compounds were evaluated for their antimicrobial activities against a panel of bacteria and fungi. Cytotoxic and anti-biofilm activities of the isolates are also reported herein. The new compound **3** exhibited weak-to-moderate antimicrobial activity as well as the known macrocidins **5** and **6**. Only the mixture of compounds **2** and **4** (ratio 1:2) showed weak cytotoxic activity against the tested cancer cell lines with an IC_{50} of 23 µg/mL. Moreover, the new compounds **2** and **3**, as well as the known compounds **5** and **6**, interfered with the biofilm formation of *Staphylococcus aureus*, inhibiting 65%, 75%, 79%, and 76% of biofilm at 250 µg/mL, respectively. Compounds **5** and **6** also exhibited moderate activity against *S. aureus* preformed biofilm with the highest inhibition percentage of 75% and 73% at 250 µg/mL, respectively.

Keywords: *Phoma macrostoma*; oxazole derivatives; anti-biofilm; isolation; structure elucidation; macrocidin Z synthesis

1. Introduction

Oxazole and its derivatives are heterocyclic systems which have gained strong interest in recent times due to their increasing importance in the field of medicinal chemistry [1]. They feature a well-known important doubly unsaturated 5-membered ring heterocyclic motif having one oxygen atom at position 1 and a nitrogen at position 3 separated by a carbon in between [2]. Their widespread useful biological activities including antimicrobial [3], anticancer [4], antitubercular [5], anti-inflammatory [6], antidiabetic [7], antiobesity [8] and anthelminthic [9] effects have attracted increasing attention of chemical and pharmacological communities in their search for new lead compounds [1]. Some of

them have shown promising therapeutic potential and have qualified for both preclinical and clinical evaluations [2]. Previous studies reported the isolation of several biologically active substituted oxazole-containing natural products mostly from marine invertebrates and microorganisms [2,10,11]. For instance, hennoxazole A, isolated from a marine sponge *Polyfibrospongia* sp., was reported to possess antiviral activity [12,13] while the phthoxazolins isolated from *Streptomyces* sp. showed selective activity against the oomycete *Phytophthora parasitica* in vitro [14]. As part of our ongoing search of exploring fungi for new biologically active metabolites, we investigated the chemical components of the fermentation extract of the plant pathogenic fungus *Phoma macrostoma* originally isolated from its host, the noxious weed, *Cirsium arvense*. Previous investigations indicated that the liquid culture of the fungus could produce phytotoxic metabolites named macrocidins, which also caused bleaching when applied foliarly to several dicotyledonous species [15,16]. In the present work, four previously undescribed oxazole-4-carboxylic acid derivatives (**1**–**4**) together with two known macrocidins (**5**–**6**) were isolated from the liquid culture of *Phoma macrostoma*. The structures of the isolates were elucidated by means of high resolution electro spray ionization mass spectrometry (HR-ESIMS) data and 1D and 2D nuclear magnetic resonance (NMR) spectroscopic data. The so far contentious structure of macrocidin Z (**6**) was also confirmed by comparison of the isolate with the product of its first total synthesis. All compounds were investigated for antimicrobial and cytotoxic effects. The current paper reports details of their isolation, structural elucidation and biological activities.

2. Results and Discussion

2.1. Structure Elucidation of Compounds **1**–**6**

Liquid fermentation in Q6 $\frac{1}{2}$ medium of *Phoma macrostoma* was carried out as described in the Materials and Methods section. The major metabolites were detected by analytical high performance liquid chromatography (HPLC) as shown in Figure 1 and the preparative HPLC separation to obtain the pure metabolites was guided accordingly. Fractionation of the crude extract using reverse-phase HPLC led to the isolation of four previously undescribed metabolites (**1**–**4**), together with two known compounds, the macrocidins A (**5**) and Z (**6**) (Figure 2). Macrocidin A (**5**) was identified by comparing its NMR and HR-ESIMS data with those reported in the literature [15]. Its absolute configuration was confirmed to be identical to that of the synthetic macrocidin A [17] by comparison of their respective Electronic Circular Dichroism (ECD) spectra (Figure 3). As the structure of macrocidin Z (**6**) was unclear so far [16], we synthesized it for the first time (Scheme 1) and found a perfect match of the ^{1}H and ^{13}C-NMR data, as well as of the ECD spectra of the synthetic and the isolated macrocidin Z samples (see Table 1 and Figure 3). The E-geometry of the Δ^{16-17} double bond in macrocidin Z was determined based on the existence of a coupling constant J = 15.5 Hz between H-16 and H-17.

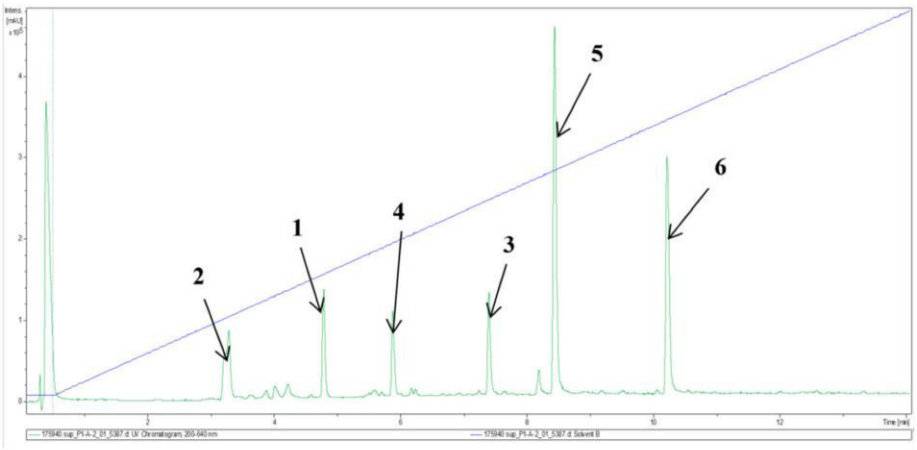


Figure 1. Analytical HPLC–UV/Vis chromatogram of the crude extract from the supernatant of *Phoma macrostoma* (diode array detection at 200–640 nm). Stationary phase: C18 Acquity UPLC BEH column; for gradient and other details on the experimental setup, see the Experimental section; **1**–**6**: Major metabolites detected (chemical structures see Figure 2). % solvent B (acetonitrile (ACN) + 0.1% formic acid 5–100%). Gradient is indicated by the blue line.

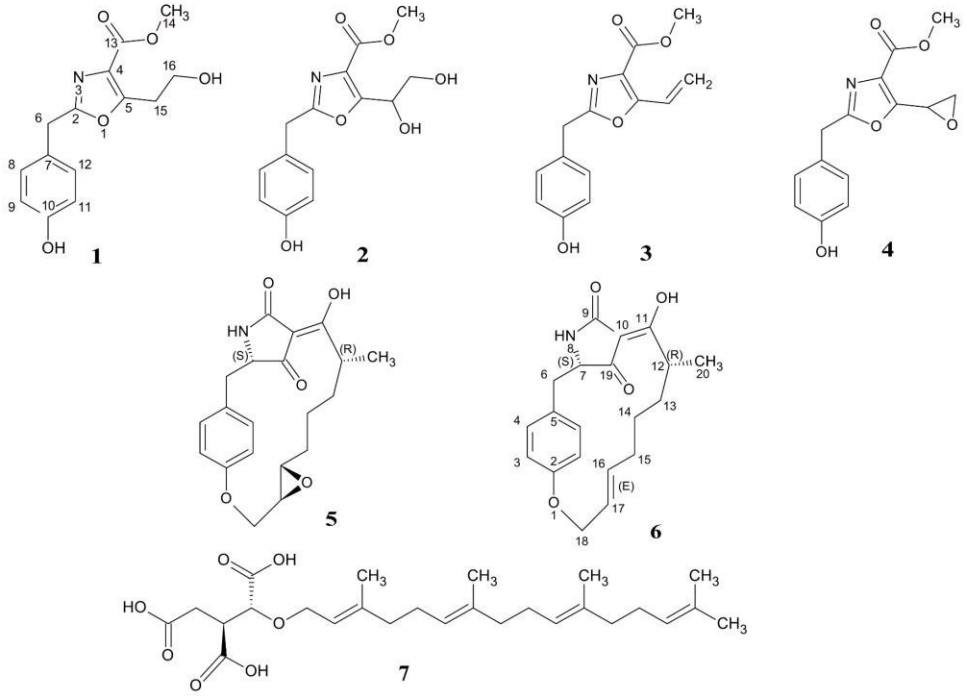


Figure 2. Chemical structures of secondary metabolites isolated from *Phoma macrostoma* (**1**–**6**) and the known biofilm inhibitor microporenic acid A (**7**).

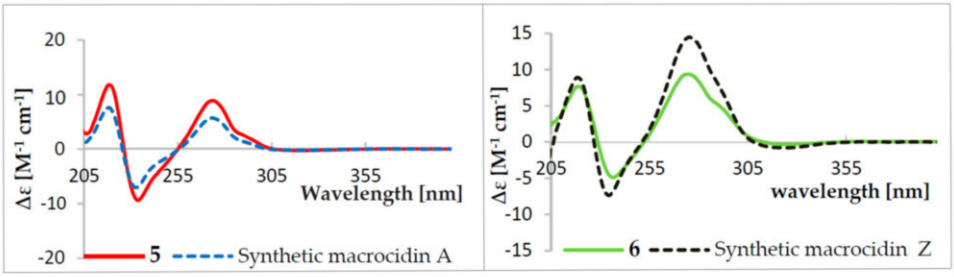


Figure 3. ECD spectra of isolated and synthetic macrocidins A (**5**) and Z (**6**) in MeOH.

Compound **1** was isolated as a yellow oil from both the supernatant and the mycelia. Its molecular formula was established as $C_{14}H_{15}NO_5$ (8 degrees of unsaturation) based on its $[M + H]^+$ ion at *m*/*z* 278.1026 and $[M + Na]^+$ ion at *m*/*z* 300.0837 in the HR-ESIMS.

The ^{1}H-NMR spectroscopic data coupled to the ^{1}H-^{1}H correlation spectroscopy (^{1}H-^{1}H COSY) spectrum revealed two doublets resonating at δ 7.10 (H-8/H-12, d, 9.0) and δ 6.73 (H-9/H-11, d, 9.0) integrating for two aromatic protons each, suggesting a 1,4-disubstituted aromatic ring. One methoxy group singlet resonating at δ 3.87 (H-14, s), a singlet methylene resonating at δ 4.00 (H-6, s), a triplet for methylene protons resonating at δ 3.19 (H-15, t, 6.5), and linked to an oxygenated methylene at δ 3.80 (H-16, t, 6.5) were also recorded.

The ^{13}C-NMR spectrum showed 12 carbon signals instead of 14 as indicated by the molecular formula suggesting the presence of symmetrical carbons and thus confirming the existence of a 1,4-disubstituted aromatic ring. The 12 carbons were further identified as one methoxy, two methylene, one oxymethylene, two aromatic methine carbons and six non-protonated sp^2 carbons from detailed analysis of its ^{1}H-^{13}C heteronuclear single quantum coherence (^{1}H-^{13}C HSQC) spectrum (Table 2). The gross structure of **1** was determined by comprehensive analysis of its 2D NMR including the COSY, HMBC (heteronuclear multiple bond correlation) and NOESY (nuclear overhauser effect spectroscopy) spectra. The chemical shifts of the aromatic carbons as well as the HMBC correlations of H-8/H-12 to C-10 (δ 157.9)/C-6 (δ 34.3), H-9/H-11 to C-10 (δ 157.9)/C-7 (δ 127.0) and H-6 to C-7(δ 127.0)/C-8(δ 131.0) indicated a benzyl group with an oxygen substitution para to the methylene leading to a para-hydroxybenzyl moiety.

Table 1. ^{13}C and ^{1}H-NMR spectroscopic data (^{1}H 500 MH_Z, ^{13}C 125 MH_Z in Methanol-d_4, δ in ppm) for isolated and synthetic compound **6**.

Position	6		Synthetic 6	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
2	157.4, C		157.3, C	
3/21	115.9, CH	6.69, m	115.7, CH	6.71, m
4/22	132.6, CH	6.98, m	132.5, CH	6.97, m
5	127.6, C		127.3, C	
6	36.6, CH_2	3.07, dd (14.1, 3.9) 2.90, dd (14.1, 3.3)	36.5, CH_2	3.07, dd (14.1, 3.9) 2.89, dd (14.1, 3.1)
7	63.8, CH	4.10, t (3.6)	63.8, CH	4.10, m
9	177.3, C		175.5, C	
10	102.3, C		102.1, C	
11	194.0, C		191.8, C	
12	37.2, CH	3.40, sxt (6.8)	36.8, C	3.39, sxt (6.8)
13	35.3, CH_2	1.16, tdd (12.9, 6.4, 4.3) 1.09, m	35.1, CH_2	1.13, m
14	28.3, CH_2	0.83, tddd (12.9, 8.5, 6.5, 4.4) 1.32, m	28.1, CH_2	0.83, m 1.32, m
15	33.6, CH_2	2.06, dq (12.8, 6.2) 1.79, m	33.4, CH_2	2.06, m 1.79, m
16	139.1, CH	5.67, ddd (15.5, 8.8, 5.9)	139.0, CH	5.68, m
17	126.6, CH	5.26, ddd (15.5, 8.9, 3.8)	126.7, CH	5.26, m
18	68.1, CH_2	4.64, dd (13.4, 8.9) 4.53, dd (13.4, 3.8)	67.9, CH_2	4.64, dd (13.4, 9.5) 4.53, m
19	197.3, C		197.1, C	
20	15.4, CH_3	1.05, d (6.8)	15.2, CH_3	

Table 2. ^{13}C and ^{1}H-NMR spectroscopic data (^{1}H 500 MH_Z, ^{13}C 125 MH_Z in Methanol-d_4, δ in ppm) for compounds **1** and **2**.

Position	1		2	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
2	164.4, C	-	165.1, C	-
4	129.0, C	-	129.6, C	-
5	159.1, C	-	159.3, C	-
6	34.3, CH_2	4.00, s	34.3, CH_2	4.04, s
7	127.0, C	-	126.9, C	-
8	131.0, CH	7.10, d (9.0)	131.0, CH	7.12, d (9.0)
9	116.7, CH	6.73, d (9.0)	116.7, CH	6.73, d (9.0)
10	157.9, C	-	158.0, C	-
11	116.7, CH	6.73, d (9.0)	116.7, CH	6.73, d (9.0)
12	131.0, CH	7.10, d (9.0)	131.0, CH	7.12, d (9.0)
13	163.9, C	-	163.6, C	-
14	52.4, CH_3	3.87, s	52.6, CH_3	3.88, s
15	30.8, CH_2	3.19, t (6.5)	67.3, CH	5.33, t (6.5)
16	60.6, CH_2	3.80, t (6.5)	65.1, CH_2	3.73, dd (11, 6.5) 3.78, dd (11, 6.5)

The presence of a methyl-2,5-disubstituted oxazole-4-carboxylate moiety was evidenced by resonance of sp^2 carbon signals at δ 164.4 (C-2), δ 159.1 (C-5), δ 129.0 (C-4), δ 163.9 (C-13), and a methoxy carbon at δ 52.4 (C-14). This was further confirmed by HMBC correlations between H-15 (δ 3.19) and C-5 (δ 159.1)/C-4 (δ 129.0)/C-13 (δ 163.9), H-14 (δ 3.87) and C-13 (δ 163.9)/C-4(δ 129.0), H-6 (δ 4.00) and C-2 (δ 164.4) as well as the ^{15}N-^{1}H HMBC correlation of H-6 (δ 4.00) to N-3 (δ 244.1) (Figure 4). The HMBC correlations of H-6 (δ 4.00) to C-2 (δ 164.4) and N-3 (δ 244.1) revealed the connectivity of the para-hydroxybenzyl moiety to the C-2 carbon of the oxazole moiety confirming unambiguously

the structure of compound **1** as methyl 5-(2-hydroxyethyl)-2-(4-hydroxybenzyl)-oxazole-4-carboxylate, named macrooxazole A.

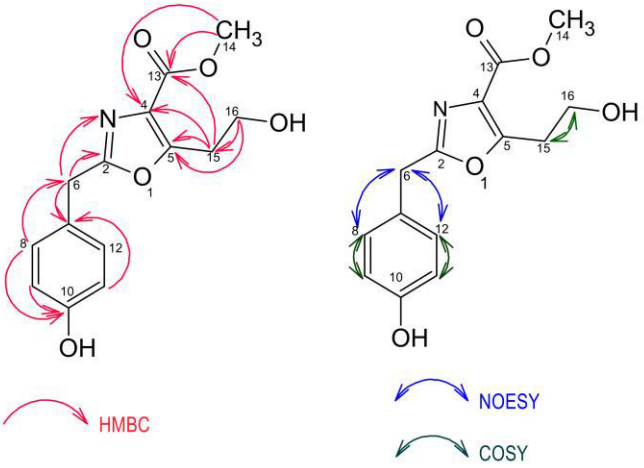


Figure 4. Key HMBC, COSY and NOESY correlations of compound **1**.

Compound **2** was obtained as a yellow oil from both supernatant and mycelia. Its molecular formula $C_{14}H_{15}NO_6$ (8 degrees of unsaturation) was determined by the $[M + H]^+$ ion at *m*/*z* 294.0974, $[M + Na]^+$ ion at *m*/*z* 316.0788 and $[M + H - H_2O]^+$ ion at *m*/*z* 276.0865 from the HR-ESIMS data (positive mode). Its NMR spectroscopic data displayed high similarities to those of compound **1**, suggesting that they are close analogues. The only structural difference was the presence of the hydroxyl group at C-15 of compound **2** which was absent in compound **1**. This was confirmed not only by the ^{1}H-^{1}H COSY coupling of H-15 (δ 5.33) to H-16a (δ 3.73)/H-16b (δ 3.78) but also by the HMBC correlation between H-15 (δ 5.33) and C-16 (δ 65.1). Interestingly, obtaining an optical rotation value approaching zero identified compound **2** to be a racemic mixture. Consequently, compound **2** was determined as a racemic mixture of methyl 5-(1,2-dihydroxyethyl)-2-(4-hydroxybenzyl)-oxazole-4-carboxylate, named macrooxazole B.

The molecular formula of compound **3** isolated from both supernatant and mycelia as a brown oil was established as $C_{14}H_{13}NO_4$ (9 degrees of unsaturation) from the HR-ESIMS which showed an $[M + H]^+$ ion at *m*/*z* 260.0917 and an $[M + Na]^+$ ion at *m*/*z* 282.0737. Analysis of 1D and 2D NMR revealed a similar structure to **1** with the C-16 hydroxyl group missing in compound **3**, but a double bond Δ^{15-16} at δ 123.2 (C-15) and δ 121.0 (C-16) were recorded instead (Table 3). The H-15 (δ 7.14) showed COSY correlations to H-16a (δ 5.58)/H-16b (δ 5.96) and HMBC correlations to C-5 (δ 155.6)/C-16 (δ 121.0) confirming the structure of the previously unreported metabolite **3** as methyl 2-(4-hydroxybenzyl)-vinyloxazole-4-carboxylate, named macrooxazole C.

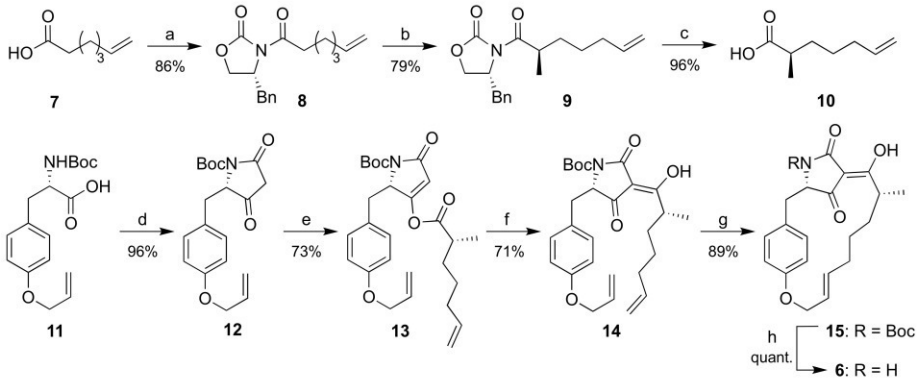
Table 3. ^{13}C and ^{1}H-NMR spectroscopic data (^{1}H 500 MH_Z, ^{13}C 125 MH_Z in Methanol-d_4, δ in ppm) for compounds **3** and **4**.

Position	3		4	
	δ_C, Type	δ_H (*J* in Hz)	δ_C, Type	δ_H (*J* in Hz)
2	164.8, C	-	165.3, C	-
4	128.1, C	-	133.0, C	-
5	155.6, C	-	155.1, C	-
6	34.3, CH_2	4.04, s	34.2, CH_2	4.00, s
7	126.8, C	-	126.6, C	
8	131.0, CH	7.12, d (9.0)	131.0, CH	7.08, d (9.0)
9	116.8, CH	6.75, d (9.0)	116.7, CH	6.73, d (9.0)
10	158.0, C	-	158.0, C	-
11	116.8, CH	6.75, d (9.0)	116.7, CH	6.73, d (9.0)
12	131.0, CH	7.12, d (9.0)	131.0, CH	7.08, d (9.0)
13	163.4, C	-	163.2, C	-
14	52.6, CH_3	3.89, s	52.8, CH_3	3.92, s
15	123.2, CH	7.14, dd (17.5, 11.5)	44.8, CH	4.51, t (3.5)
16	121.0, CH_2	5.58, dd (11.5, 1.1) 5.96, dd (17.5, 1.1)	48.9 *, CH_2	3.22, dd (3.5, 1.6)

* Overlapping with the solvent peak.

Fraction F1 (a mixture of compounds **2** and **4** (ratio 1:2)) was isolated as a yellow oil from both supernatant and mycelial extracts. On the basis of HR-ESIMS and 1D/2D NMR data of this mixture, the structure of compound **4** could be determined independently. HR-ESIMS data revealed the molecular formula of compound **4** as $C_{14}H_{13}NO_5$ (9 degrees of unsaturation) provided by the $[M + H]^+$ ion at *m/z* 276.0866 and $[M + Na]^+$ ion at *m/z* 298.0862. Detailed analysis of its 1D and 2D NMR data showed similar features to those of compound **3**, except that the olefinic bond C-15 (δ 123.2)/C-16 (δ 121.0) was substituted by an epoxide group C-15 (δ 44.8)/C-16 (δ 48.9). The assumption was evidenced from the established molecular formula and was confirmed not only by the COSY correlation of H-15 (δ 4.51) to H-16 (δ 3.22), but also by HMBC correlations of H-16 (δ 3.22) to C-15 (δ 44.8)/C-5 (δ 155.1). Therefore, compound **4** was elucidated unambiguously as methyl 2-(4-hydroxybenzyl)-5-(oxiran-2-yl)-oxazole-4-carboxylate, named macrooxazole D. As can be seen in Figure 1, compounds **2** and **4** are both also present in the crude extract, suggesting they are both genuine natural products and that compound **2** does not only arise from macrooxazole D (**4**) as an isolation artefact during preparative HPLC separation. However, the conversion could already have taken place during fermentation of the fungus.

For an unambiguous confirmation of its structure, macrocidin Z (**6**) was synthesized starting by attaching 6-heptenoic acid (**7**) to the Evans auxiliary (*R*)-benzyl-2-oxazolidinone (Scheme 1) [18]. The resulting imide **8** was deprotonated at the α-position with NaHMDS to give an enolate which was quenched with iodomethane. The resulting 9.8:1 mixture of diastereomers was separated by column chromatography to afford the major isomer **9** in 79% yield. It was converted to the carboxylic acid **10** in 96% yield by adding LiOH and H_2O_2. The tetramic acid **12** was prepared according to a known protocol [17,19,20] by treatment of commercial Boc-Tyr(Allyl)-OH (**11**) with Meldrum's acid. Its acylation with carboxylic acid **10** via the two-step Yoshii-Yoda protocol [21,22] initially afforded 4-*O*-acyltetramate **13**, which was rearranged to the 3-acyltetramic acid **14**. A ring-closing metathesis reaction using Grubbs catalyst gave *N*-Boc-protected macrocidin Z **15** with an *E*-selectivity > 99% in 89% yield. Macrocidin Z (**6**) was obtained quantitatively upon removal of the Boc-protection group with TFA in 30% total yield over seven steps.



Scheme 1. Synthesis of macrocidin Z **(6).** Reagents and conditions: (a) DCC, DMAP, (*R*)-benzyl-2-oxazolidinone, CH_2Cl_2, 23 h; (b) 1. NaHMDS, THF, −78 °C, 30 min, 2. MeI, 4.5 h; (c) LiOH, H_2O_2, THF/H_2O (2:1); (d) Meldrum´s acid, DMAP, EDC·HCl, CH_2Cl_2, rt, 2 h; (e) **10**, DMAP, EDC·HCl, CH_2Cl_2, 0 °C, rt, 2 h; (f) NEt_3, DMAP, CH_2Cl_2, rt, 24 h; (g) Grubbs II catalyst, CH_2Cl_2, Δ, 15 h; (h) TFA, CH_2Cl_2, rt, 15 min. DCC = dicyclohexylcarbodiimide; DMAP = dimethylaminopyridine; NaHMDS = sodium hexamethyldisilazanide; THF = tetrahydrofuran; EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TFA = trifluoroacetic acid.

2.2. Biological Activities

The isolated metabolites were evaluated for their antimicrobial activity against various bacteria and fungi. The Minimum Inhibitory Concentration (MIC) values showed that only the new metabolite **3** as well as the known macrocidins **5** and **6** were active, whereas the remaining compounds were inactive against the organisms tested (See Table S1 in the supporting information). Macrocidin A (**5**) showed the strongest activity against *Bacillus subtilis* with an MIC value of 16.7 µg/mL which is the same value as that of oxytetracyclin used as positive control. The same compound **5** demonstrated weak activity against *Mycobacterium smegmatis* with an MIC value of 33.3 µg/mL. Compound **3** exhibited moderate activity against *Mucor hiemalis* with an MIC value of 66.7 µg/mL equal to that of nystatin used as a positive control. The latter also inhibited the growth of *Bacilus subtilis* at 66.7 µg/mL. Against *Micrococcus luteus*, compound **6** exhibited weak activity with an MIC value of 66.7 µg/mL. Furthermore, the ability of some of the isolated compounds to inhibit the proliferation of two mammalian cell lines including HeLa cells KB3.1 and mouse fibroblasts L929 was examined. Only the mixture of compounds **2** and **4** (ratio 1:2) showed weak cytotoxic activity against HeLa cells KB3.1 and mouse fibroblasts L929 with an IC_{50} value of 23 µg/mL for both cell lines, whereas compound **5** and **6** only showed a slight inhibition of HeLa cells KB3.1 proliferation (See Table S2 in the supporting information).

Moreover, the isolated pure compounds except compound **4** (which was not tested because it was isolated as a mixture) were evaluated for their effectiveness in inhibiting biofilm formation and preformed biofilm of *Staphylococcus aureus* (Table 4). The new compounds **2** and **3** showed moderate-to-weak activity against biofilm formation, with respective inhibition percentages of 65% and 75% at the highest concentration of 250 µg/mL. Compounds **5** and **6** inhibited 61% and 19% of the bacterial biofilm at 15.6 µg/mL, respectively (Figure 5). Interestingly, the test compounds also displayed activity against preformed biofilm of *S. aureus* as represented in Table 4 below. Macrocidins A (**5**) and Z (**6**) exhibited moderate activity against preformed biofilm of *S. aureus* with the highest percentage of inhibition of 75% and 73% at 250 µg/mL, respectively.

Table 4. Inhibition of biofilm and destruction of preformed biofilm in *Staphylococcus aureus* by compounds **1**–**3**, **5**, **6**.

Compounds	Inhibition of Biofilm Formation (%)	Destruction of Preformed Biofilm (%)
1	-	-
2	65 (250 µg/mL) 43 (125 µg/mL)	36 (250 µg/mL) 31 (125 µg/mL)
3	75 (250 µg/mL) 59 (125 µg/mL)	57 (250 µg/mL) 48 (125 µg/mL)
4	n.t	n.t
5	79 (250 µg/mL) 77 (62.5 µg/mL) 61 (15.6 µg/mL)	75 (250 µg/mL) 65 (62.5 µg/mL) 31 (15.6 µg/mL)
6	76 (250 µg/mL) 70 (62.5 µg/mL) 19 (15.6 µg/mL)	73 (250 µg/mL) 59 (62.5 µg/mL) 40 (15.6 µg/mL)
Microporenic acid A	83 (250 µg/mL) 81 (62.5 µg/mL) 48 (15.6 µg/mL)	71 (250 µg/mL) 70 (62.5 µg/mL) 39 (15.6 µg/mL)

n.t: not tested, (-) no activity.

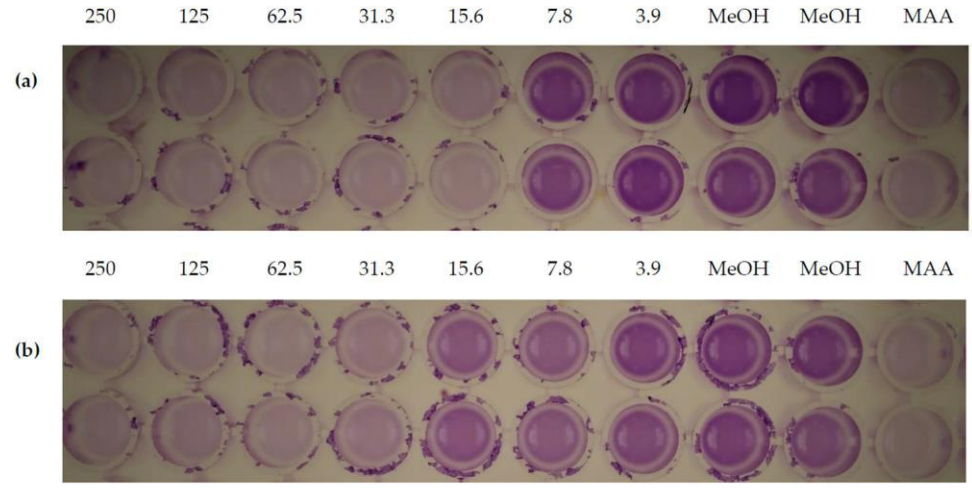


Figure 5. Inhibition of the biofilm formation in *S. aureus* of compounds **5** (**a**) and **6** (**b**). Each column has two replicates in different concentrations of the compounds (250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 µg/mL) and their respective controls. MAA: microporenic acid A (positive control), MeOH: methanol (negative control).

Apart from the strong herbicidal activity of macrocidins [15,16], no other activity has been reported for this class of compounds as far as we know. The current paper therefore constitutes the first extensive evaluation of the biological effects for this class of compounds.

3. Materials and Methods

3.1. General Experimental Procedure

Electrospray mass (ESIMS) spectra were recorded with an UltiMate 3000 Series uHPLC (Thermo Fischer Scientific, Waltman, MA, USA) utilizing a C18 Acquity UPLC BEH column (2.1 × 50 mm, 1.7 µm; Waters, Milford, CT, USA) connected to an amaZon speed ESI-Iontrap-MS (Bruker, Billerica, MA, USA). HPLC parameters were set as follows: solvent A: H_2O + 0.1% formic acid, solvent B: acetonitrile (ACN) + 0.1% formic acid, gradient: 5% B for 0.5 min increasing to 100% B in 19.5 min, then isocratic condition at 100% B for 5 min, a flow rate of 0.6 mL/min, and diode array detection (DAD) in the range of 190–600 nm.

HR-ESIMS (High-resolution electrospray ionization mass spectrometry) spectra were recorded with an Agilent 1200 Infinity Series HPLC–UV system (Agilent Technologies, Santa Clara, CA, USA) (column 2.1 × 50 mm, 1.7 µm, C18 Acquity UPLC BEH (waters), solvent A: H_2O + 0.1% formic acid; solvent B: ACN + 0.1% formic acid, gradient: 5% B for 0.5 min increasing to 100% B in 19.5 min and then maintaining 100% B for 5 min, flow rate 0.6 mL/min, UV/Vis detection 200–640 nm) connected to a MaXis ESI-TOF mass spectrometer (Bruker) (scan range 100–2500 *m*/*z*, capillary voltage 4500 V, dry temperature 200 °C). High-resolution mass spectra of synthetic products were obtained with a UPLC/Orbitrap MS system in ESI mode.

Optical rotations were recorded in methanol (Uvasol, Merck, Darmstadt, Germany) by using an Anton Paar MCP-150 polarimeter (Seelze, Germany) at 25 °C for isolated compounds, and by using a PerkinElmer 241 polarimeter for synthetic products dissolved in $CHCl_3$, MeCN or methanol. UV/Vis spectra were recorded using methanol (Uvasol, Merck, Darmstadt, Germany) with a Shimadzu UV/Vis 2450 spectrophotometer (Kyoto, Japan). ECD spectra were obtained on a J-815 spectropolarimeter (JASCO, Pfungstadt, Germany). Nuclear magnetic resonance (NMR) spectra were recorded with an Avance III 500 spectrometer (Bruker, ^{1}H-NMR: 500 MHz and ^{13}C-NMR: 125 MHz). IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit.

For the purification of synthetic products, chromatography silica gel 60 (40–63 µm) or silica gel RP18 (40–63 µm) were used. Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} pre-coated aluminum-backed plates.

3.2. Fungal Material

The fungal strain *Phoma macrostoma* DAOMC 175,940 was originally isolated from the Canadian thistle *Circium arvense* collected in Quebec, Canada in 1979. It constitutes one of the original producer strains of the macrocidins [15,16,23] and was kindly provided by the CCFC (Canadian Collection of Fungal Cultures, Ottawa, ON, Canada).

3.3. Small-Scale Fermentation and Extraction

The fungus was cultivated in Q6 $\frac{1}{2}$ medium (10 g/mL glycerol, 2.5 g/mL D-glucose, 5 g/mL cotton seed flour and pH = 7.2) [24]. A well-grown culture from a yeast-malt (YM) agar plate (10 g/mL malt extract, 4 g/mL yeast extract, 4 g/mL D-glucose, 1.5% agar and pH = 6.3) was cut into small pieces using a cork borer (7mm), and eight pieces were inoculated into 6 × 500 mL Erlenmeyer flasks, each containing 200 mL of the Q6 $\frac{1}{2}$ medium. The culture was incubated at 23 °C on a rotary shaker (140 rpm). The growth of the fungus was monitored by measuring the amount of free glucose using Diastix Harnzuckerstreifen (Bayer). After glucose depletion, small samples were taken to monitor secondary metabolite production over a period of 14 days (searching for the mass spectra and UV/Vis spectra that were reported to be typical for the macrocidins) and a stagnation of the titres of the putative macrocidin derivatives was observed by HPLC–MS between 8 and 14 days.

Then, the fermentation was terminated and the supernatant and mycelia were separated by filtration. The supernatant was extracted with equal amount of ethyl acetate (200 mL) and filtered through anhydrous sodium sulphate. The resulting ethyl acetate extract was evaporated to dryness by

means of rotary evaporator. The mycelia was extracted with 200 mL of acetone in an ultrasonic bath at 40 °C for 40 min, filtered and the filtrate evaporated. The remaining water phase was suspended in equal amount of distilled water and subjected to same procedure as the supernatant.

3.4. Scale Up of Production in Shake Flask Batches and Extraction

Four well-grown 17-day-old YM agar plates of the mycelial culture were cut into small pieces using a 7 mm cork borer and 8 pieces inoculated in 30 × 500 mL Erlenmeyer flasks containing 200 mL of Q6 $\frac{1}{2}$ medium. The culture was incubated at 23 °C on a rotary shaker (140 rpm) for 13 days. Fermentation was aborted 10 days after the depletion of free glucose.

The mycelia and supernatant from the batch fermentation were separated via filtration. The mycelia was extracted with 4 × 500 mL of acetone in an ultrasonic water bath at 40 °C for 40 min. The extracts were combined and the solvent evaporated by means of a rotary evaporator. The remaining water phase was four times subjected to the same procedure as mycelium in small-scale extraction yielding 949 mg dark brown crude extract. The supernatant (6 L) was extracted with an equal amount of ethyl acetate and filtered through anhydrous sodium sulphate. The resulting ethyl acetate extract was evaporated to dryness by means of rotary evaporator to afford 238 mg of brown crude extract.

3.5. Isolation of Compounds **1**–**6**

The mycelial and the supernatant crude extracts from shake flask batch fermentations (3.4) dissolved in methanol were centrifuged by means of a centrifuge (Hettich Rotofix 32 A, Tuttlingen, Germany) for 10 min at 4000 rpm. The extracts were purified separately using preparative reverse-phase liquid chromatography (PLC 2020; Gilson, Middleton, WI, USA). A VP Nucleodur 100–5 C18ec column (250 × 21 mm, 7 µm: Machery-Nagel, Düren, Germany) was used as the stationary phase. Deionized water (Milli-Q, Millipore, Schwalbach, Germany) with 0.1% formic acid (FA) (solvent A) and acetonitrile (ACN) with 0.1% FA (solvent B) were used as the mobile phase. The elution gradient used was 5–45% solvent B for 20 min, 45–60% B for 15 min, 60–100% B for 10 min and thereafter isocratic condition at 100% solvent B for 5 min. The flow rate was 15 mL/min and the fractions obtained from both the supernatant and mycelial extracts were combined according to UV absorption at 190, 210 and 280 nm as well as concurrent HPLC–MS analyses to yield compound **1** (7.98 mg, t_R: 3.7–3.9 min), **2** (5.20 mg, t_R: 2.3–2.5 min), **3** (8.22 mg, t_R: 6.4–6.6 min), **5** (39.12 mg, t_R: 7.4–7.6 min), **6** (32.10 mg, t_R: 9.3–9.5 min) and fraction F1 (7.20 mg), which was a mixture of compounds **2** (t_R: 2.3–2.5 min) and **4** (t_R: 4.8–5.0 min) with compound **4** as the major component (ratio 1:2).

A total of 5 mg of F1 was further purified by reversed phase HPLC (solvent A (H_2O + 0.1% FA)/solvent B (ACN + 0.1% FA)), elution gradient 20–50% solvent B for 35 min followed by maintaining isocratic condition at 100% solvent B for 5 min with a preparative HPLC column (VP Nucleodur 100–10 C18ec column (250 × 10 mm, 7 µm: Machery-Nagel, Düren, Germany) as stationary phase) and a flow rate of 8 mL/min, to afford only compound **2**. The absence of the peak of compound **4** in the obtained HPLC chromatogram suggests the instability of compound **4** which easily turns into compound **2**.

3.6. Physico-Chemical Characteristics of Compounds **1**–**6**

Methyl 5-(2-Hydroxyethyl)-2-(4-hydroxybenzyl)-oxazole-4-carboxylate (Macrooxazole A (**1**)*):* Yellow oil. UV (MeOH, c = 0.025 mg/mL) λ_{max} (log ε) 202 (4.15), 227 (4.14), 277 (3.36) nm. HR-ESIMS *m/z* 300.0837 $[M + Na]^+$; *m/z* 555.1968 $[2M + H]^+$; *m/z* 278.1026 $[M + H]^+$ (Calcd for $C_{14}H_{16}NO_5$ 278.1023). For NMR data, see Table 2.

Methyl 5-(1,2-Dihydroxyethyl)-2-(4-hydroxybenzyl)-oxazole-4-carboxylate (Macrooxazole B (**2**)*)*: Yellow oil. $[\alpha]^{25}_D = 0\ ^\circ$ (c = 0.002, MeOH); UV (MeOH, c = 0.025 mg/mL) λ_{max} (log ε) 202 (4.15), 227 (4.12), 278 (3.21) nm. HR-ESIMS *m/z* 316.0788 $[M + Na]^+$; *m/z* 276.0865 $[M + H - H_2O]^+$; *m/z* 294.0974 $[M + H]^+$ (Calcd for $C_{14}H_{16}NO_6$ 294.0972). For NMR data, see Table 2.

Methyl 2-(4-Hydroxybenzyl)-vinyloxazole-4-carboxylate (Macrooxazole C (**3**)): Brown oil. UV (MeOH, c = 0.025 mg/mL) λ_{max} (log ε) 201 (4.01), 228 (3.96), 272 (3.89) nm. HR-ESIMS *m/z* 282.0737 $[M + Na]^+$; *m/z* 260.0917 $[M + H]^+$ (Calcd for $C_{14}H_{14}NO_4$ 260.0917). For NMR data, see Table 3.

Methyl 2-(4-Hydroxybenzyl)-5-(oxiran-2-yl)-oxazole-4-carboxylate (macrooxazole D (**4**)): Yellow oil. UV (MeOH, c = 0.025 mg/mL) λ_{max} (log ε) 201 (4.06), 227 (3.96), 274 (3.35) nm. HR-ESIMS *m/z* 298.0682 $[M + Na]^+$; *m/z* 551.1656 $[2M + H]^+$; *m/z* 276.0866 $[M + H]^+$ (Calcd for $C_{14}H_{14}NO_5$ 276.0866). For NMR data, see Table 3.

Macrocidin A (**5**): Beige-yellowish solid. $[\alpha]^{25}_D$ = +45 ° (c = 0.001, MeOH); UV (MeOH, c = 0.025 mg/mL) λ_{max} (log ε) 201 (4.25), 224 (4.16), 281 (4.14) nm; CD (c = 2.8×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 218 (+11.77), 234 (−9.41), 274 (+8.91). HR-ESIMS *m/z* 715.3223 $[2M + H]^+$; *m/z* 340.1547 $[M + H - H_2O]^+$ *m/z* 380.1465 $[M + Na]^+$; *m/z* 358.1655 $[M + H]^+$ (Calcd for $C_{20}H_{24}NO_5$ 358.1649). ^{1}H-NMR (500 MHz, MeOH-*d4*): δ_H 6.82 (2H, d, *J* = 8.7 Hz, H-3/H-21); δ_H 7.04 (2H, br d, *J* = 8.7 Hz, H-4/H-22), δ_H 2.92 (1H, dd, *J* = 14.1, 4.0 Hz, H-6a), δ_H 3.12 (1H, dd, *J* = 14.1, 3.4 Hz, H-6b), δ_H 4.11 (1H, t, *J* = 3.6 Hz, H-7), δ_H 3.59 (1H, m, H-12), δ_H 1.46 (1H, ddd, *J* = 13.1, 11.6, 4.1 Hz, H-13a), δ_H 1.36 (1H, tt, *J* = 13.1, 4.1 Hz, H-13b), δ_H 0.46 (1H, qt, *J* = 12.9, 4.7 Hz, H-14a), δ_H 1.15 (1H, qt, *J* = 13.1, 4.1 Hz, H-14b), δ_H 1.9 (1H, tt, *J* = 12.8, 3.5 Hz, H-15a), δ_H 0.76 (1H, tdd, *J* = 12.9, 9.8, 5.0 Hz, H-15b), δ_H 3.03 (1H, ddd, *J* = 9.9, 3.4, 2.4 Hz, H-16), δ_H 2.58 (1H, dt, *J* = 8.5, 2.0 Hz, H-17), δ_H 4.40 (1H, dd, *J* = 12.7, 1.8 Hz, H-18a), δ_H 3.95 (1H, dd, *J* = 12.7, 8.7 Hz, H-18b), δ_H 1.08 (3H, d, *J* = 6.9 Hz, H-20). (3H, d, *J* = 6.9 Hz, H-20).

Macrocidin Z (**6**): Yellow oil. $[\alpha]^{25}_D$ = +123.5 ° (c = 0.00942, MeOH); UV (MeOH, c = 0.025 mg/mL) λ_{max} (log ε) 201 (4.11), 226 (3.92), 281 (3.90) nm; CD (c = 2.9×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 219 (+7.67), 237 (−4.87), 274 (+9.36). HR-ESIMS *m/z* 683.3324 $[2M + H]^+$; *m/z* 342.1701 $[M + H]^+$ (Calcd for $C_{20}H_{24}NO_4$ 342.1700). For NMR data, see Table 2.

3.7. Synthesis of Macrocidin Z (**6**)

(R)-4-Benzyl-3-(hept-6-enoyl)oxazolidin-2-one (**8**) [25]: 6-Heptenoic acid (**7**; 100 µL, 738 µmol) in dry CH_2Cl_2 (1 mL) at 0 °C was treated with DMAP (dimethylaminopyridine; 8.20 mg, 738 µmol), (R)-benzyl-2-oxazolidinone (119 mg, 671 µmol) and DCC (dicyclohexylcarbodiimide;152 mg, 738 µmol). The mixture was stirred for 23 h at room temperature. The white precipitate was filtered off and washed with CH_2Cl_2. The filtrate was washed with aqueous sat. $NaHCO_3$ solution and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were dried over Na_2SO_4. After removing the solvent under reduced pressure, the crude product was purified by silica gel column chromatography on silica gel 60 using a mobile phase of 12.5% ethyl acetate (EtOAc in hexane, to give imide **8** (166 mg, 86%) as a colorless oil; R_f = 0.68 (25% EtOAc in hexanes); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.49 (quin, *J* = 7.6 Hz, 2H), 1.74 (m, 2H), 2.11 (m, 2H), 2.77 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.95 (m, 2H), 3.30 (dd, *J* = 13.3, 3.3 Hz, 1H), 4.19 (m, 2H), 4.67 (m, 1H), 4.97 (m, 1H), 5.03 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.82 (m, 1H), 7.19–7.36 (m, 5H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 23.8, 28.4, 33.6, 35.5, 38.1, 55.3, 66.3, 114.9, 127.5, 129.1, 129.6, 135.4, 138.6, 153.6, 173.4.

(R,R)-4-Benzyl-3-(2-methylhept-6-enoyl)oxazolidin-2-one (**9**): A solution of imide **8** (1.32 g, 4.60 mmol) in dry THF (11 mL) at –78 °C was treated with 1M NaHMDS in THF (5.29 mL, 5.29 mmol), stirred for 30 min, and then quenched with iodomethane (1.50 mL, 24.0 mmol). The resulting mixture was stirred for 4.5 h, diluted with H_2O (100 mL), and the aqueous phase was extracted with Et_2O (3 × 75 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to give a crude product which was purified by column chromatography (silica gel 60, 10% EtOAc in hexanes) to leave **9** (1.10 g, 79%) as a colorless oil; R_f = 0.72 (25% EtOAc in hexanes); $[\alpha]^{24}_D$–61.6 (c 1.46, $CHCl_3$); IR ν_{max} 3079 (w), 3024 (w), 2974 (w), 2932 (m), 2857 (w), 1779 (s), 1697 (s), 1455 (w), 1385 (m), 1350 (m), 1240 (m), 1211 (m), 1196 (m), 1101 (w), 913 (w), 703 (m) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.23 (d, *J* = 6.88 Hz, 3H), 1.43 (m, 3H), 1.76 (m, 1H), 2.01 (m, 2H), 2.77 (dd, *J* = 13.2, 9.5 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.1 Hz, 1H), 3.72 (m, 1H), 4.19 (m, 2H), 4.67 (m, 1H), 4.95 (m, 1H), 5.01 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.79 (m, 1H), 7.19–7.36 (m, 5H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.5, 26.6, 33.0, 33.8, 37.7,

38.0, 55.5, 66.1, 114.8, 127.5, 129.0, 129.6, 135.4, 138.6, 153.2, 177.3; HRMS (ESI): *m/z* [$C_{18}H_{23}NO_3$ + H^+]: calcd 302.17507, found 302.17438.

(R)-2-Methylhept-6-enoic acid (**10**): A solution of imide **9** (1.20 g, 3.98 mmol) in THF (36 mL) and H_2O (16 mL) was treated with LiOH H_2O (334 mg, 7.96 mmol) and H_2O_2 (30 wt%, 2.03 mL, 19.9 mmol) at 0 °C. After stirring at room temperature for 1 d, sat. aqueous $NaHCO_3$ solution (50 mL) was added, the aqueous layer was extracted with CH_2Cl_2 (50 mL), acidified with 1M HCl to pH = 2, and extracted with Et_2O (3 × 40 mL). The combined organic phases were dried over Na_2SO_4, and concentrated under reduced pressure to give **4** (546 mg, 96%) as a colorless liquid; R_f = 0.37 (25% EtOAc in hexanes); $[\alpha]^{24}_D$ –17.4 (c 0.69, $CHCl_3$); IR ν_{max} 2978 (m), 2936 (m), 2867 (m), 1706 (s), 1466 (w), 1414 (w), 1233 (m), 992 (w), 911 (m) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.19 (d, *J* = 5.9 Hz, 3H), 1.44 (m, 3H), 1.69 (m, 1H), 2.06 (m, 2H), 2.47 (m, 1H), 4.95 (d, *J* = 5.0 Hz, 1H), 5.01 (d, *J* = 5.0 Hz, 1H), 5.79 (m, 1H), 11.5 (br, 1H, OH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.0, 26.5, 33.1, 33.7, 39.3, 114.9, 138.5, 183.1; HRMS (ESI): mboxemphm/z [$C_8H_{14}O_2$ + H^+]: calcd 143.10666, found 143.10656.

(S)-tert-Butyl-2-(4-(allyloxy)benzyl)-3,5-dioxopyrrolidin-1-carboxylate (**12**) [17]: A solution of Boc-Tyr(Allyl)-OH (**11**; 1.28 g, 3.99 mmol) in dry CH_2Cl_2 (13 mL) was treated with Meldrum´s acid (633 mg, 4.39 mmol), DMAP (683 mg, 5.59 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-·HCl (918 mg, 4.79 mmol) at room temperature. The mixture was stirred for 2 h, concentrated under reduced pressure, and the resulting crude product was diluted with EtOAc (100 mL) and washed with 0.5 M H_2SO_4 (3 × 40 mL). The combined aqueous phases were extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with H_2O (100 mL), dried over Na_2SO_4 and refluxed until gas formation ceased. The solvent was removed under reduced pressure to give **12** (1.32 g, 96%) as a yellow foam; R_f = 0.63 (10% MeOH in CH_2Cl_2); IR ν_{max} 2976 (w), 1755 (m), 1711 (m), 1610 (m), 1510 (m), 1364 (m), 1298 (m), 1240 (s), 1149 (s), 1077 (m), 1021 (m), 997 (m), 925 (w), 829 (m), 813 (m), 788 (w), 772 (w), 756 (w), 656 (w) cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ 1.61 (s, 9H), 3.10 (dd, *J* = 14.2, 2.6 Hz, 1H), 3.41 (dd, *J* = 14.2, 5.3 Hz, 1H), 4.47 (dt, *J* = 5.2, 1.6 Hz, 2H), 4.64 (dd, *J* = 5.3, 2.6 Hz, 1H), 5.22 (dq, *J* = 10.5, 1.6 Hz, 1H), 5.37 (dq, *J* = 17.5, 1.6 Hz, 1H), 6.03 (ddt, *J* = 17.5, 10.5, 5.2 Hz, 1H), 6.78 (m, 2H), 6.97 (m, 2H); ^{13}C-NMR (125 MHz, CD_3OD) δ 28.5, 35.0, 62.2, 69.7, 83.9, 115.3, 117.3, 127.5, 131.9, 135.0, 150.9, 159.3, 173.4, 178.2.

(2S)-tert-Butyl-2-(4-(allyloxy)benzyl)-3-(((R)-2-methylhept-6-enoyl)oxy)-5-oxopyrrolidine-1-carboxylate (**13**): A stirred solution of carboxylic acid **10** (397 mg, 2.79 mmol) in dry CH_2Cl_2 (14 mL) at 0 °C was treated with EDC HCl (642 mg, 3.35 mmol) and DMAP (68.2 mg, 558 µmol), allowed to warm to room temperature and stirred for 30 min. The resulting mixture was treated with tetramic acid **12** (1.16 g, 3.35 mmol), stirred for a further 4 h, and then diluted with 0.5M H_2SO_4 (200 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL) and the combined organic phases were washed with brine and dried over Na_2SO_4. After the removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel 60, 10% EtOAc in hexanes → 15% EtOAc in hexanes → 20% EtOAc in hexanes → 25% EtOAc in hexanes → 100% EtOAc) to afford **13** (967mg, 73%) as a yellowish oil; R_f = 0.93 (40% EtOAc in hexanes); $[\alpha]^{24}_D$ 90.3 (*c* 1.15, $CHCl_3$); IR ν_{max} 2971 (w), 2938 (w), 1781 (m), 1742 (s), 1631 (m), 1512 (m), 1368 (m), 1322 (m), 1229 (m), 1217 (m), 1175 (m), 1063 (m), 912 (w), 847 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.25 (t, *J* = 6.9 Hz, 3H), 1.43–1.57 (m, 3H), 1.60 (s, 9H), 1.76 (m, 1H), 2.10 (m, 2H), 2.62 (m, 1H), 3.11 (dd, *J* = 14.2, 2.6 Hz, 1H), 3.36 (dd, *J* = 14.2, 6.0 Hz, 1H), 4.48 (m, 2H), 4.77 (m, 1H), 5.00 (dd, *J* = 10.4, 1H), 5.04 (d, *J* = 17.2, 1H), 5.27 (d, *J* = 10.4, 1H), 5.39 (d, *J* = 17.2, 1H), 5.80 (m, 1H), 6.03 (m, 1H), 6.77 (m, 2H), 6.90 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 16.8, 16.4, 28.3, 32.8, 33.6, 34.8, 40.0, 60.7, 68.9, 83.2, 108.3, 114.8, 115.4, 117.8, 126.0, 130.5, 133.3, 138.0, 149.4, 157.9, 165.1, 168.2, 171.9 [some peaks are doubled due to **13** partially rearranging to **14** on silica gel]; HRMS (ESI): *m/z* [$C_{27}H_{35}NO_6$ + Na^+]: calcd 492.23566, found 492.23499.

(S,Z)-tert-Butyl-2-(4-(allyloxy)benzyl)-4-(1-hydroxy-(2R)-methylhept-6-en-1-ylidene)-3,5-dioxopyrrolidine-1-carboxylate (**14**): A solution of **13** (967 mg, 2.05 mmol) in dry CH_2Cl_2 (20 mL) was treated with NEt_3 (344 µL, 2.46 mmol) and DMAP (125 mg, 1.03 mmol) and stirred at room temperature for 7 h. DMAP (62.6 mg, 0.51 mmol) was added and the stirring continued for 16 h. The reaction was quenched with sat.

aqueous $NaHCO_3$ solution (100 mL), the aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the remainder was purified by column chromatography on reversed phase silica gel (RP18, 40% MeCN in H_2O + 0.01% HCOOH → 60% MeCN in H_2O + 0.01% HCOOH → 80% MeCN in H_2O + 0.01% HCOOH → 100% MeCN + 0.01% HCOOH) to yield **14** (684 mg, 71%) as a yellow oil; R_f = 0.94 (10% MeOH in CH_2Cl_2); $[\alpha]^{24}_D$–26.9 (*c* 2.12, MeOH); IR ν_{max} 3463 (w), 3016 (m), 2970 (m), 2945 (m), 1738 (s), 1599 (w), 1510 (w), 1366 (s), 1229 (s), 1217 (s), 1206 (s), 1156 (w), 907 (m), 787 (w) cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ 1.01 (m, 3H), 1.23 (m, 1H), 1.28–1.42 (m, 2H), 1.63 (m, 1H), 1.63 (s, 9H), 2.04 (q, *J* = 6.7 Hz, 2H), 3.19 (dd, *J* = 14.2, 2.7 Hz, 1H), 3.38 (dd, *J* = 14.2, 5.3 Hz, 1H), 3.56 (m, 1H), 4.47 (m, 2H), 4.58 (br, 1H), 4.95 (m, 1H), 5.01 (dq, *J* = 17.0, 1.7 Hz, 1H), 5.21 (dq, *J* = 10.6, 1.6 Hz, 1H), 5.35 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.78 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H), 6.03 (ddt, *J* = 17.3, 10.6, 5.2 Hz, 1H), 6.76 (m, 2H), 6.90 (m, 2H); ^{13}C-NMR (125 MHz, CD_3OD) δ 17.3, 27.4, 28.4, 33.7, 34.7, 35.8, 37.9, 69.7, 84.9, 115.3, 115.5, 117.4, 127.4, 131.9, 134.8, 139.5, 159.3, 195.1; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.91/1.13 (d, *J* = 6.9 Hz, 3H), 1.15–1.44 (m, 3H), 1.59 (m, 1H), 1.62 (s, 9H), 2.02 (m, 2H), 3.20/3.26 (dd, *J* = 13.8, 2.0 Hz, 1H), 3.34/3.40 (dd, *J* = 13.8, 5.6 Hz, 1H), 3.48/3.65 (m, 1H), 4.38/4.63 (m, 1H), 4.44 (m, 2H), 4.88–5.06 (m, 2H), 5.22–5.41 (m, 2H), 5.75 (m, 1H), 6.01 (m, 1H), 6.73 (m, 2H), 6.91 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.1/17.4, 26.5/26.6, 28.2/28.3, 32.2/33.3, 33.8, 34.9/35.0, 36.3/37.6, 61.8/65.7, 68.7/68.8, 83.4/84.1, 102.1/105.1, 114.7, 114.8/115.1, 117.7/117.8, 126.0/126.4, 130.7/130.9, 133.3, 138.4/138.5, 149.0/150.0, 157.8/157.9, 164.2/173.8, 192.3, 195.6, 197.3, 200.8 [not all quaternary C-atoms are visible in the JMOD in CD_3OD; some of them can be seen via HSQC or HMBC correlations. Some peaks in the ^{1}H-NMR and JMOD in $CDCl_3$ are doubled because of tautomers]; HRMS (ESI): *m*/*z* [$C_{27}H_{35}NO_6$ + Na^+]: calcd 492.23566, found 492.23450.

N-Boc-Macrocidin Z (**15**): A solution of diene **14** (634 mg, 1.35 mmol) in degassed CH_2Cl_2 (270 mL) was treated with 2nd generation Grubbs catalyst (57.3 mg, 67.5 µmol) and heated at reflux for 16 h. The solvent was removed under reduced pressure and the remainder was purified by column chromatography on reversed phase silica gel (RP18, 40% MeCN in H_2O + 0.01% HCOOH → 60% MeCN in H_2O + 0.01% HCOOH → 80% MeCN in H_2O + 0.01% HCOOH) to yield **15** (529 mg, 89%) as a brownish foam; R_f = 0.94 (10% MeOH in CH_2Cl_2); $[\alpha]^{24}_D$ 155.8 (c 0.62, MeOH); IR ν_{max} 3456 (m), 3016 (m), 2970 (s), 2944 (m), 2136 (w), 1740 (s), 1728 (s), 1600 (m), 1436 (m), 1366 (s), 1354 (s), 1299 (m), 1228 (s), 1216 (s), 1296 (m), 1091 (w), 974 (w), 908 (m), 730 (w), 786 (w) cm^{-1};. ^{1}H-NMR (500 MHz, CD_3OD) δ 0.91 (m, 1H), 1.08 (d, *J* = 6.7 Hz, 3H), 1.14 (m, 2H), 1.40 (m, 1H), 1.63 (s, 9H), 1.82 (m, 1H), 2.11 (m, 1H), 3.07 (dd, *J* = 14.3, 3.5 Hz, 1H), 3.36 (dd, *J* = 14.3, 3.1 Hz, 1H), 3.47 (m, 1H), 4.46 (m, 1H), 4.54 (m, 1H), 4.65 (dd, *J* = 13.4, 9.1 Hz, 1H), 5.27 (m, 1H), 5.69 (m, 1H), 6.60–6.97 (m, 4H); ^{13}C-NMR (125 MHz, CD_3OD) δ 15.0, 27.7, 28.3, 33.2, 34.8, 35.6, 37.1, 66.8, 67.9, 85.3, 102.9, 115.6, 119.0, 126.8, 130.8, 132.6, 138.8, 157.6, 174.9, 192.4, 193.8; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.89 (m, 1H), 1.07 (d, *J* = 6.7 Hz, 3H), 1.13 (m, 2H), 1.32 (m, 1H), 1.63 (s, 9H), 1.82 (m, 1H), 2.05 (m, 1H), 3.12 (dd, *J* = 14.3, 3.5 Hz, 1H), 3.37 (dd, *J* = 14.3, 3.1 Hz, 1H), 3.50 (m, 1H), 4.41 (t, *J* = 3.4 Hz, 1H), 4.59 (d, *J* = 6.2 Hz, 2H), 5.26 (dt, *J* = 15.5, 6.2 Hz, 1H), 5.58 (m, 1H), 6.59–7.01 (m, 4H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 13.77/14.97, 26.3/26.6, 28.3/28.4, 32.2/33.3, 33.9, 34.2/34.6/34.9, 35.6/37.1, 61.7/65.7, 67.3/67.7, 84.3, 102.6, 114.5, 118.5, 125.3/125.5, 125.7, 130.0, 131.6, 135.0/137.6, 148.9, 156.0, 174.1, 191.9, 195.4 [not all quaternary C-atoms are visible in the JMOD in CD_3OD, some of them can be seen via HSQC or HMBC correlations. Some peaks in the ^{1}H-NMR and JMOD in $CDCl_3$ are doubled because of tautomers]; HRMS (ESI): *m*/*z* [$C_{25}H_{31}NO_6$ + Na^+]: calcd 464.20436, found 464.20413.

Macrocidin Z (**6**): A solution of Boc-protected macrocidin Z (**15**) (238 mg, 539 µmol) in dry CH_2Cl_2 (10 mL) was treated with TFA (1.00 mL), stirred for 15 min, diluted with toluene (100 mL) and finally concentrated under reduced pressure. This procedure was repeated once to afford macrocidin Z (**6**) (183 mg, quant.) as a pale yellow foam; R_f = 0.60 (10% MeOH in CH_2Cl_2); $[\alpha]^{25}_D$ +126.1 (c 0.85, MeOH); IR ν_{max} 2934 (m), 2864 (w), 1696 (m), 1656 (s), 1607 (s), 1508 (s), 1448 (m), 1338 (m), 1250 (m), 1217 (m), 1177 (w), 976 (m), 843 (w) cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ 0.83 (m, 1H, CH^aHCC=C), 1.05 (d, *J* = 6.8 Hz, 3H, CH_3), 1.13 (m, 2H, CH_2CMe), 1.32 (m, 1H, CHH^bCC=C), 1.79 (m, 1H, CCH^aHC=C), 2.06 (m, 1H,

CCHH^bC=C), 2.89 (dd, J = 14.1, 3.1 Hz, 1H, PhCH^aH), 3.07 (dd, J = 14.1, 3.9 Hz, 1H, PhCHH^b), 3.39 (sex, J = 6.8 Hz, 1H, CHMe), 4.10 (m, 1H, CHN), 4.53 (m, 1H, CH^aHO), 4.64 (dd, J = 13.4, 9.5 Hz, 1H, CHH^bO), 5.26 (m, 1H, OHC=C), 5.68 (m, 1H, C=CHC), 6.71 (m, 2H, H^{ortho}), 6.97 (m, 2H, H^{meta}); ^{13}C-NMR (125 MHz, CD_3OD) δ 15.2 (Me), 28.1 (*C*CC=C), 33.4 (*C*C=C), 35.1 (*C*CMe), 36.5 (PhC), 36.8 (CMe), 63.8 (HCN), 67.9 (OCH_2), 102.1 (NCC=C), 115.7, 118.7 (C^{ortho}), 126.7 (OCH_2C), 127.3 (C^{para}), 131.4, 132.5 (C^{meta}), 139.0 (OCH_2C=*C*), 157.3 (C^{ipso}), 175.5 (NCO), 191.8 (COH), 197.1 (CC(O)C); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.86 (m, 1H), 1.07 (d, J = 6.9 Hz, 3H), 1.09–1.35 (m, 3H), 1.80 (m, 1H), 2.03 (m, 1H), 2.86 (dd, J = 14.4, 2.9 Hz, 1H), 3.20 (dd, J = 14.4, 3.7 Hz, 1H), 3.44 (sex, J = 6.9 Hz, 1H), 4.14 (t, J = 3.7 Hz, 1H), 4.60 (m, 2H), 5.27 (m, 1H), 5.59 (m, 1H), 6.17 (s, 1H), 6.71 (br, 2H), 7.01 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 15.1, 27.0, 32.4, 34.2, 35.5, 36.1, 62.3, 67.4, 100.9, 114.8, 118.1, 125.2, 125.7, 130.3, 131.7, 137.9, 156.0, 175.8, 192.2, 194.3 [not all quaternary C-atoms are visible in the JMOD in CD_3OD, some of them can be seen via HSQC or HMBC correlations]; HRMS (ESI): m/z [$C_{20}H_{23}NO_4$ + H^+]: calcd 342.16998, found 342.16907.

3.8. Antimicrobial Assay

Minimum Inhibitory Concentrations (MIC) of compounds **1–6** were determined in serial dilution assays as described previously [26,27] using different test microorganisms including *Pichia anomala, Schizosaccharomyces pombe, Mucor hiemalis, Candida albicans,* and *Rhodotulas glutinis* for fungal microorganisms; *Micrococcus luteus, Bacillus subtilis, Staphyloccocus aureus* and *Mycobacterium smegmatis* for Gram-positive bacteria; *Chromobacterium violaceum, Escherichia coli* and *Pseudomonas aeruginosa* for Gram-negative bacteria. A detailed protocol can be found in the Supporting Information.

3.9. Cytotoxicity Assay

The in vitro cytotoxicity (IC_{50}) of compounds **1–6** was determined against mammalian cell lines including mouse fibroblast L929 and Hela (KB3.1) cells according to our previously reported procedures [26,27]. A detailed protocol is given in the Supporting Information.

3.10. Biofilm Inhibition Assay

Staphylococcus aureus DSM 1104 from −20 °C stock was incubated in 20 mL CASO (casein-peptone soymeal-peptone) medium at 37 °C on a rotary shaker (100 rpm) overnight. The OD_{600} of the culture solution was measured and adjusted to match the turbidity of a 0.001 McFarland standard. A total of 150 µL of CASO with 4% glucose broth was added together with the serial diluted compounds (250–3.9 µg/mL) and incubated in 96 well microtiter plates (TPP tissue culture ref.no 92196) for 18 h at 37 °C. The biofilm inhibition activity of the test compounds was evaluated by using 0.1% crystal violet staining (Thermo Fisher, Waltham, USA) following previously established protocols [28,29]. In brief, the supernatant was discarded, the biofilm stained at room temperature with 0.1% crystal violet for 15 min and washed three times by using PBS (phosphate-buffered saline) buffer, the dye in the biofilm was extracted with diluted acetic acid (30%), and the absorbance was finally quantified in a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 550 nm. Methanol (5%) was used as a negative control and microporenic acid A [28] (250–7.9 µg/mL) was used as a positive control. Standard deviations (SD) of three repeats with duplicates each were 15% or less. SD values are shown in Table S5 in the Supporting Information.

3.11. Dispersion of Preformed Biofilm

A cell suspension of *Staphylococcus aureus* strain DSM 1104 was adjusted to match the turbidity of a 0.001 McFarland standard and incubated in 96-well tissue microtiter plates for 18 h in CASO with 4% glucose broth. The supernatant was removed from the wells and washed with 150 µL PBS buffer; then, 150 µL of the fresh media (CASO with 4% glucose broth) was added together with the serially diluted compounds (250–3.9 µg/mL) into the wells. The plates were further incubated for 24 h at 37 °C.

Staining of the preformed biofilm and controls were described as for the biofilm inhibition [28,29]. All experiments were made in triplicates with two repetitions.

4. Conclusions

During the course of our search for new biologically active secondary metabolites, four previously undescribed oxazole carboxylic acid derivatives were isolated from the plant pathogenic fungus *Phoma macrostoma*. As far as we know, these metabolites constitute the first series of oxazole derivatives isolated from this genus. Investigation of the antimicrobial activity of the new isolates revealed that only compound **3** displayed moderate activity against *Bacillus subtilis* and *Mucor hiemalis*. Although none of the isolates displayed any antibacterial activity against *S. aureus*, compounds **2** and **3** showed moderate to weak inhibition of biofilm formation and preformed biofilm of the bacteria. Moreover, two known tetramic acids macrocidins, A and Z, were also characterized. The so far unclear structure of macrocidin Z was confirmed in this study by its first total synthesis. Even though the macrocidins are well known for displaying a strong herbicidal activity, their biological effects have also been extensively evaluated in the present work and they turned out to possess an interesting antibiofilm effect against *S. aureus*. Thanks to their ability to inhibit biofilm formation, these are likely to be considered as promising candidates for the development of lead molecules that could function as adjunctive agents in combination therapy with antibiotics.

Supplementary Materials: Tables S1–S5, 1D, 2D NMR, ESIMS and HR-ESIMS spectra of compounds **1**–**6**; NMR spectra of intermediates in the synthesis of **6**. Protocol: Antimicrobial assay; Protocol: Cytotoxicity assay.

Author Contributions: B.M.K. contributed to fermentation, chemical analysis of the extracts, isolation of compounds, structure elucidation, bioactivity assays and manuscript writing. L.T. conceived and carried out the total synthesis of **6** and contributed to manuscript writing. H.Z. contributed to the antibiofilm assay. H.S. contributed to the antibiofilm assay and edited the manuscript. R.S. supervised the synthesis of **6** and co-edited the manuscript. M.S. supervised the project, contributed facilities, experimental guidance, edited and polished the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds are available from the authors.

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Macrooxazoles A-D, new 2,5-disubstituted oxazole-4-carboxylic acid derivatives from the plant pathogenic fungus *Phoma macrostoma*

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Contents

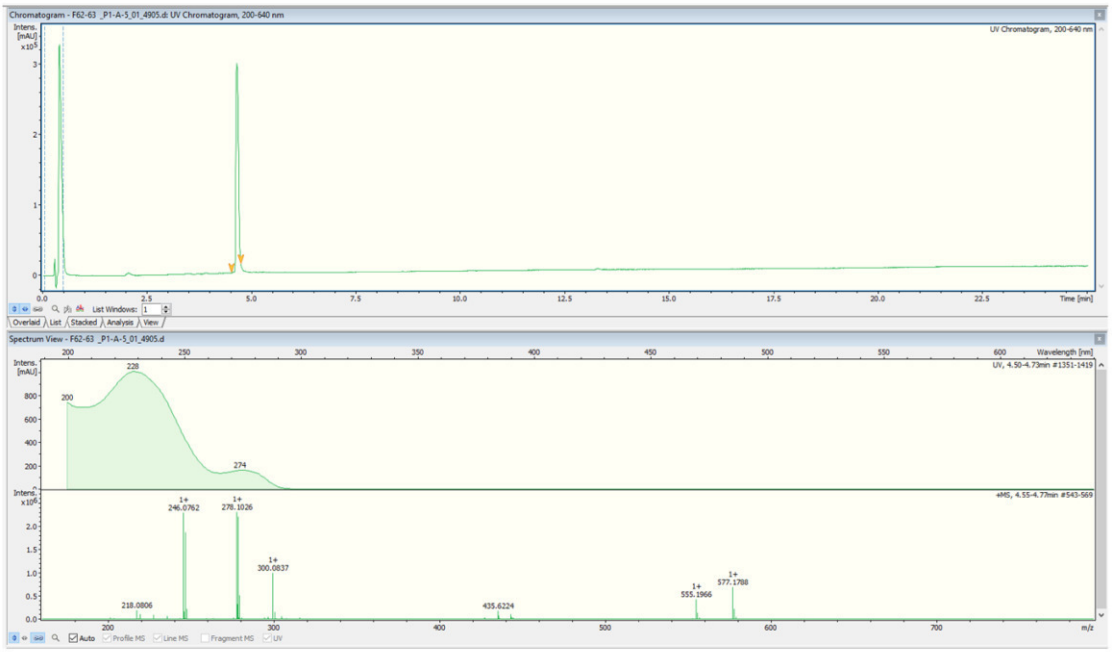

Figure S1: HR-ESIMS data for macrooxazole A (**1**).

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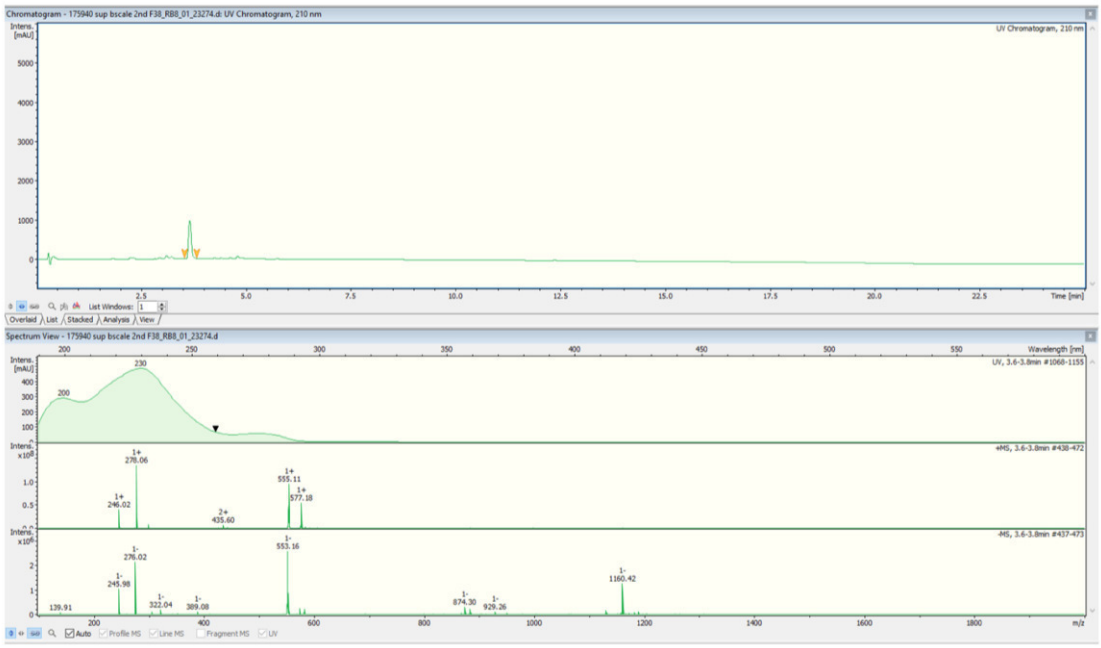

Figure S2: ESIMS data for macrooxazole A (**1**).

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1 D and 2D NMR data for macrooxazole A (1)

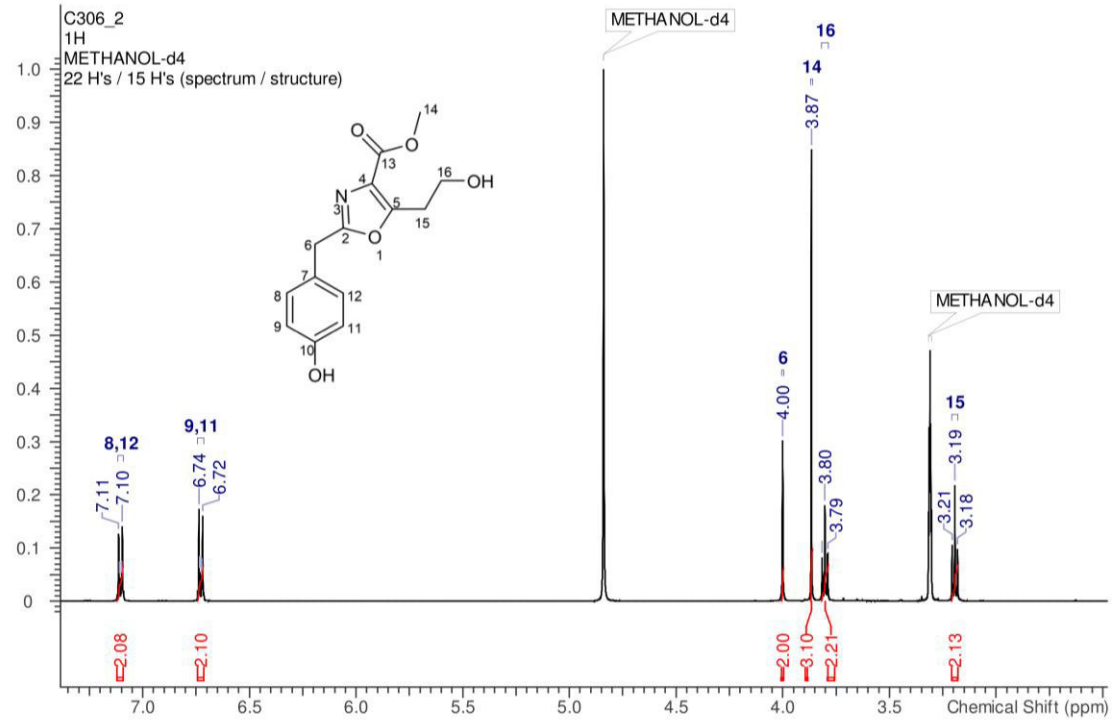

Figure S3: ^{1}H NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole A (**1**).

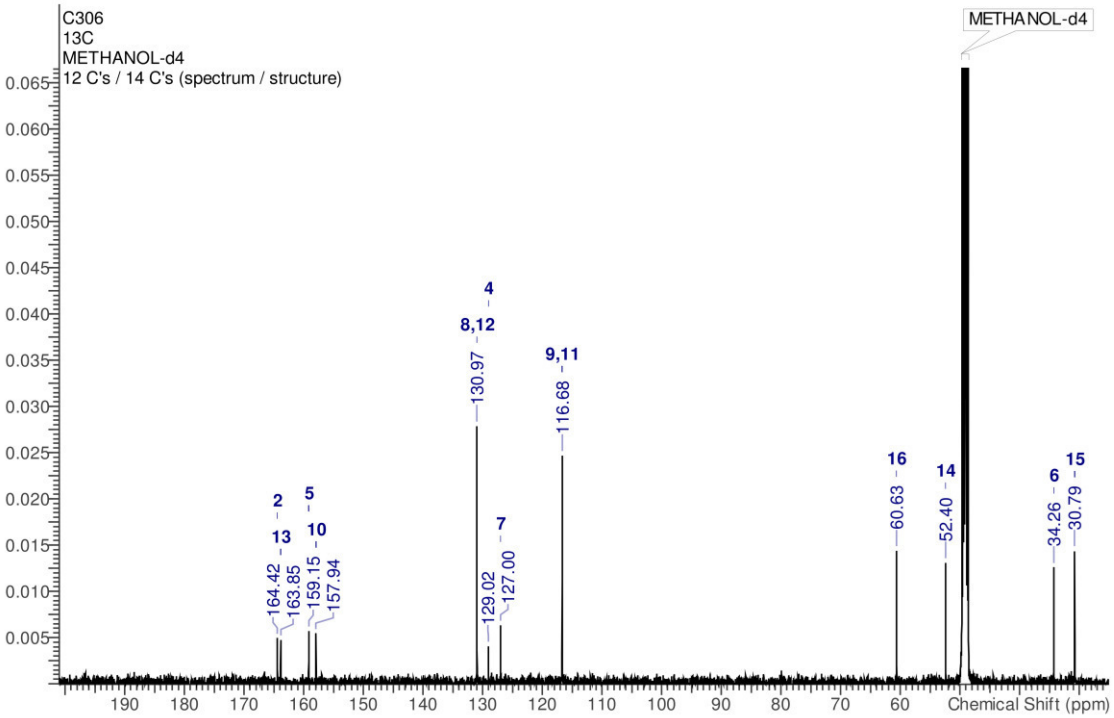

Figure S4: ^{13}C NMR spectrum (MeOH-*d4*, 125 MHz) of macrooxazole A (**1**).

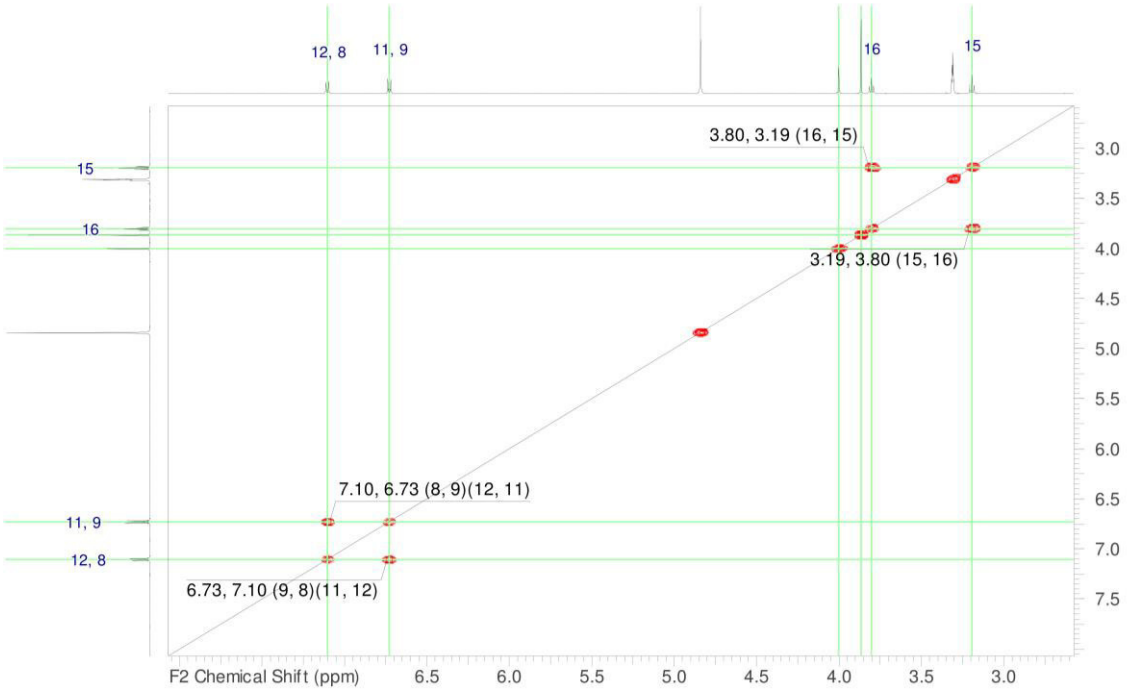

Figure S5: ^{1}H, ^{1}H COSY NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole A (**1**).

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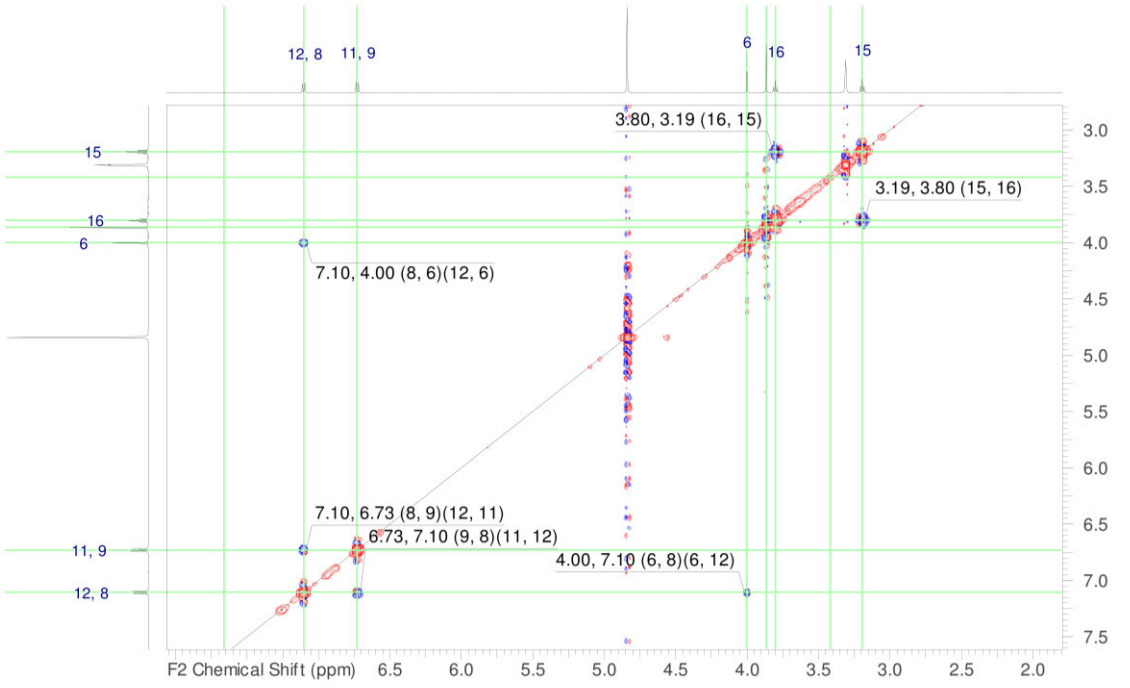

Figure S6: ^{1}H, ^{1}H NOESY NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole A (**1**).

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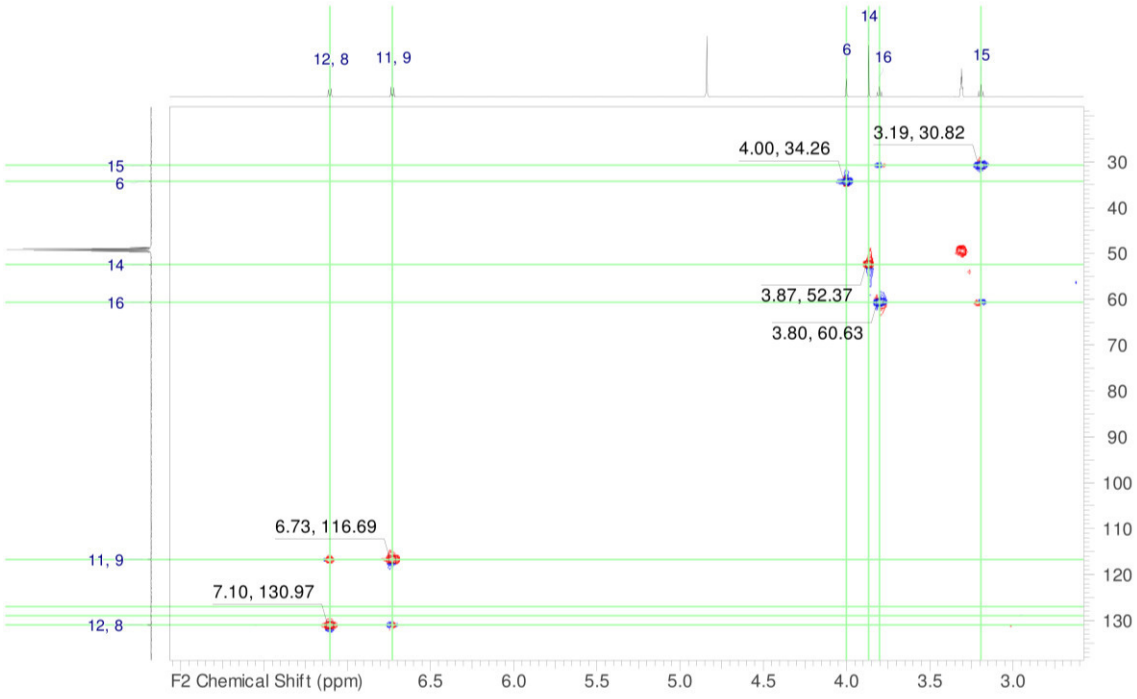

Figure S7: ^{1}H, ^{13}C HSQC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole A (**1**).

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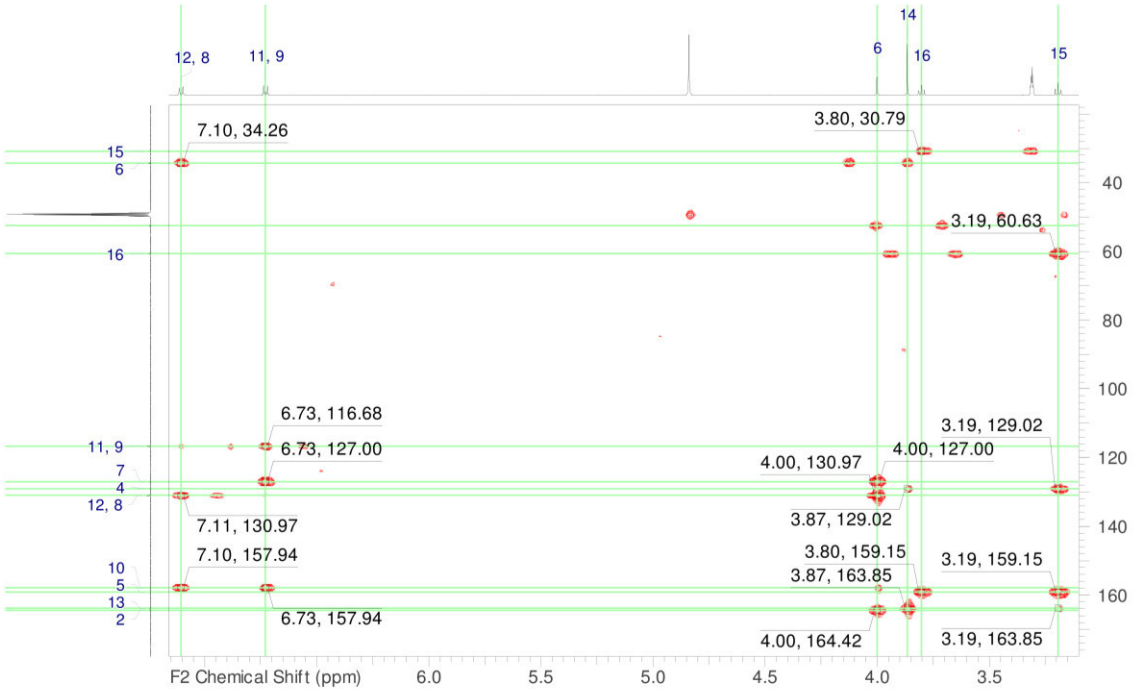

Figure S8: ^{1}H, ^{13}C HMBC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole A (**1**).

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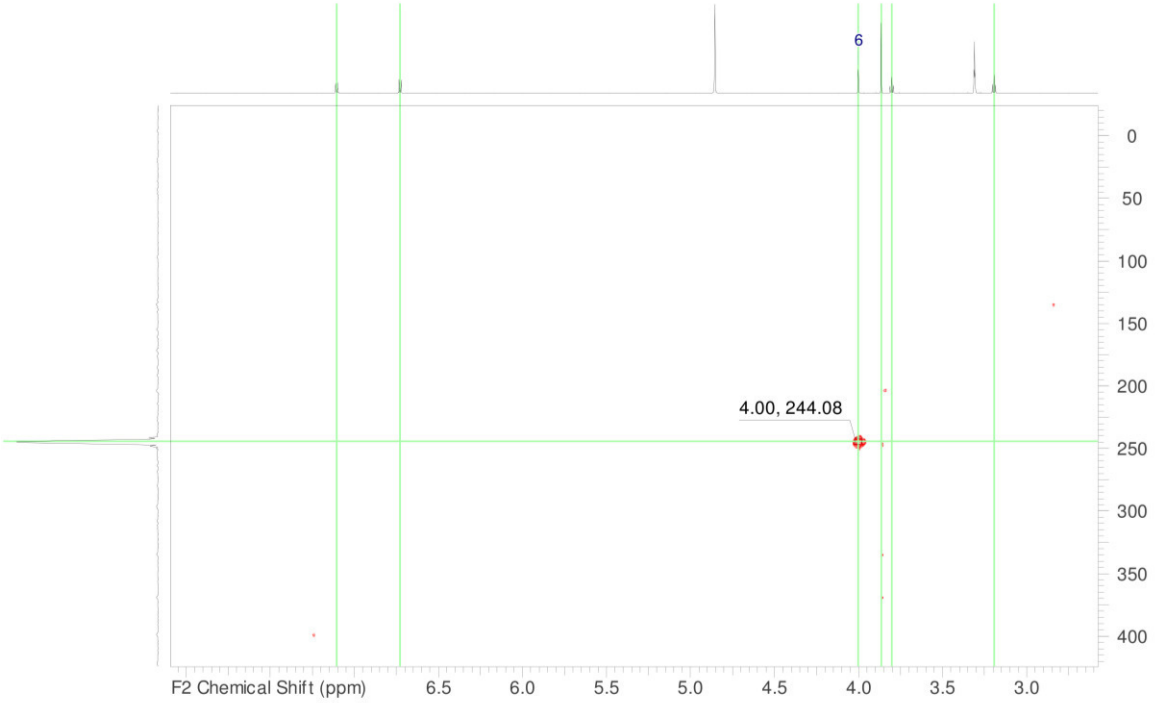

Figure S9: ^{1}H, ^{15}N HMBC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole A (**1**).

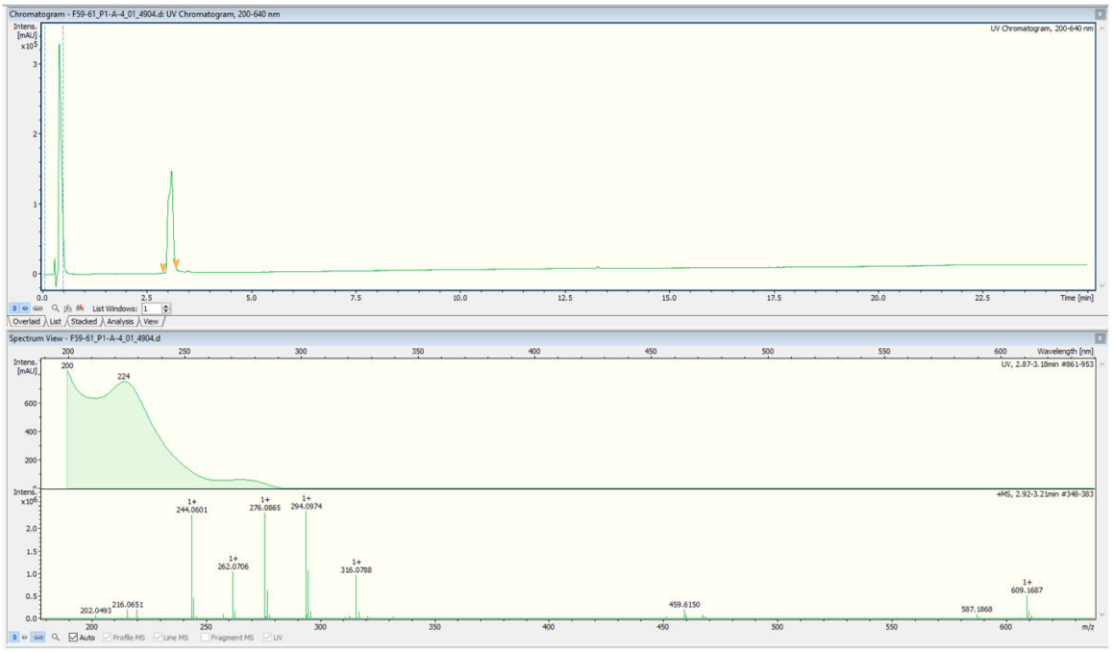

Figure S10: HR-ESIMS data for macrooxazole B (**2**).

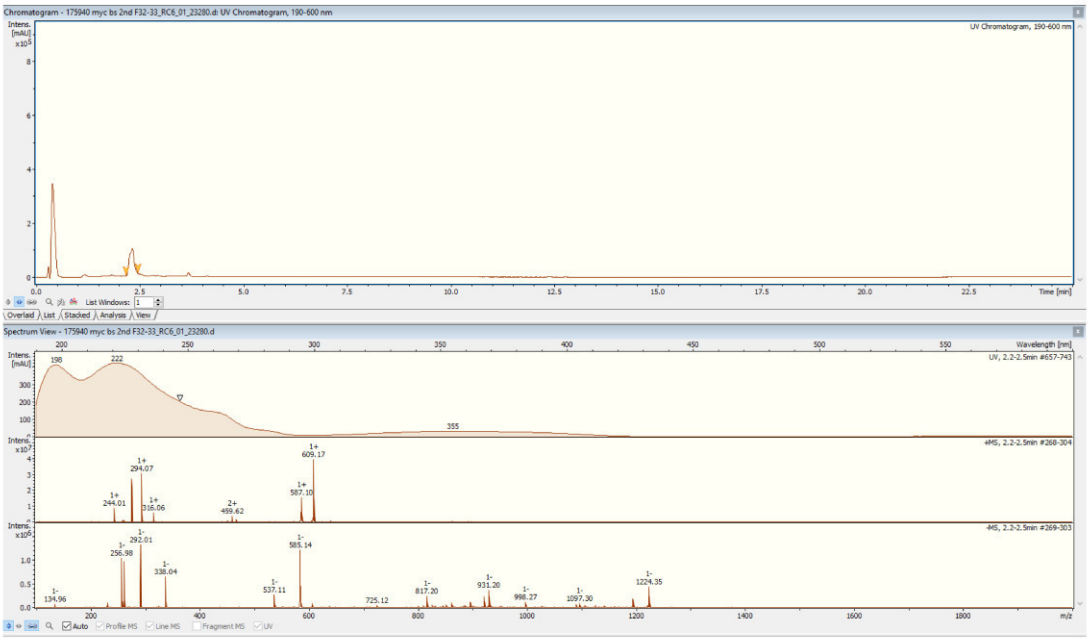

Figure S11: ESIMS data for macrooxazole B (**2**).

1D and 2D NMR data for macrooxazole B (2)

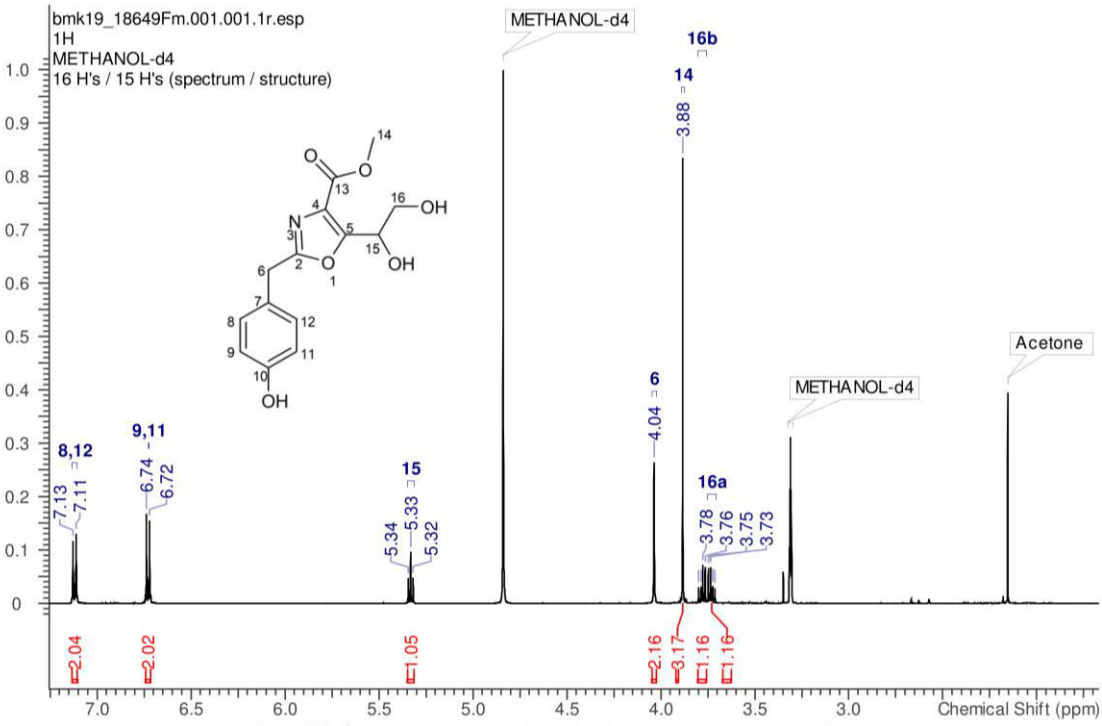

Figure S12: ^{1}H NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole B (**2**).

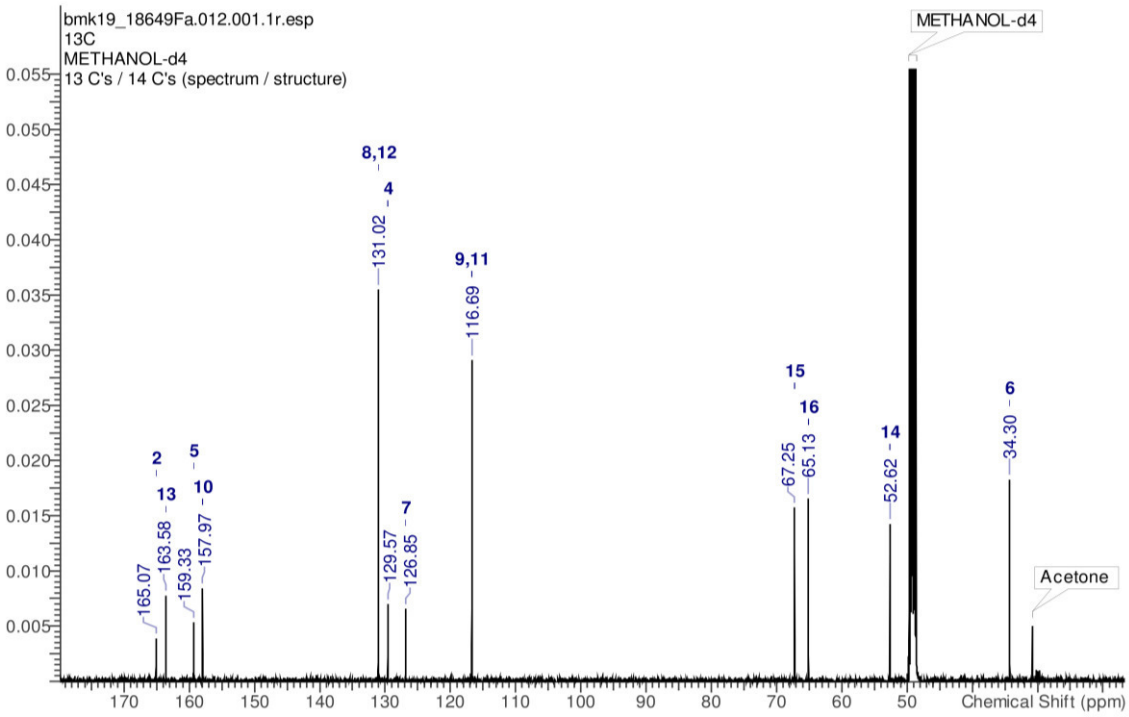

Figure S13: 13C NMR spectrum (MeOH-d4, 125 MHz) of macrooxazole B (**2**).

16

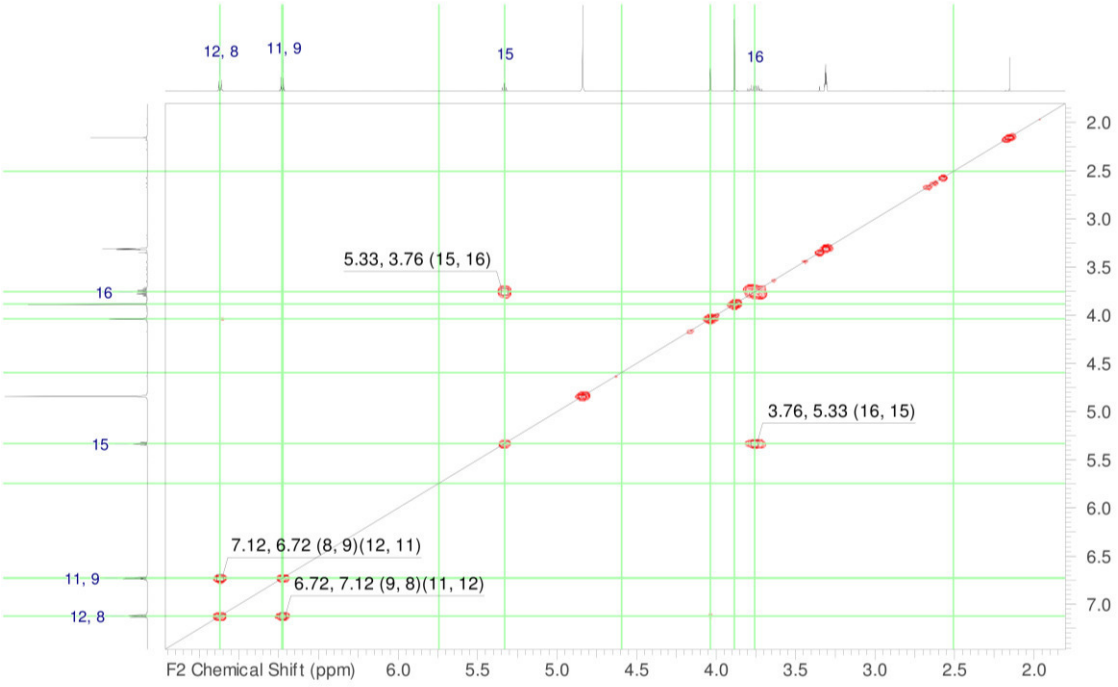

Figure S14: ^{1}H, ^{1}H COSY NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole B (**2**).

17

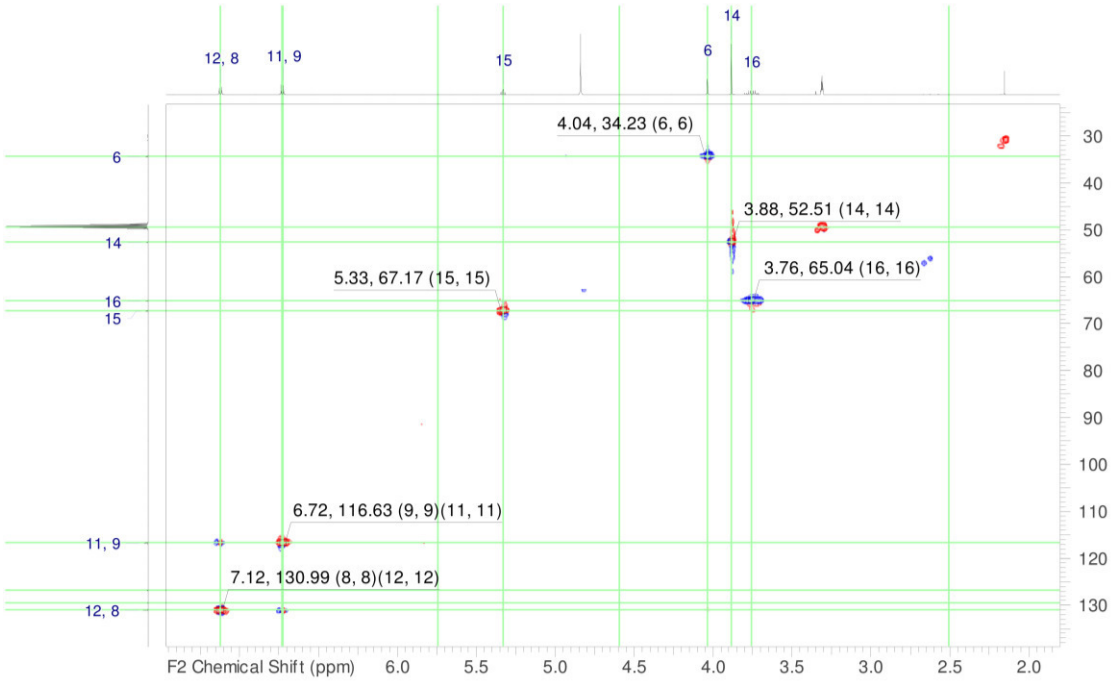

Figure S15: ^{1}H, ^{13}C HSQC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole B (**2**).

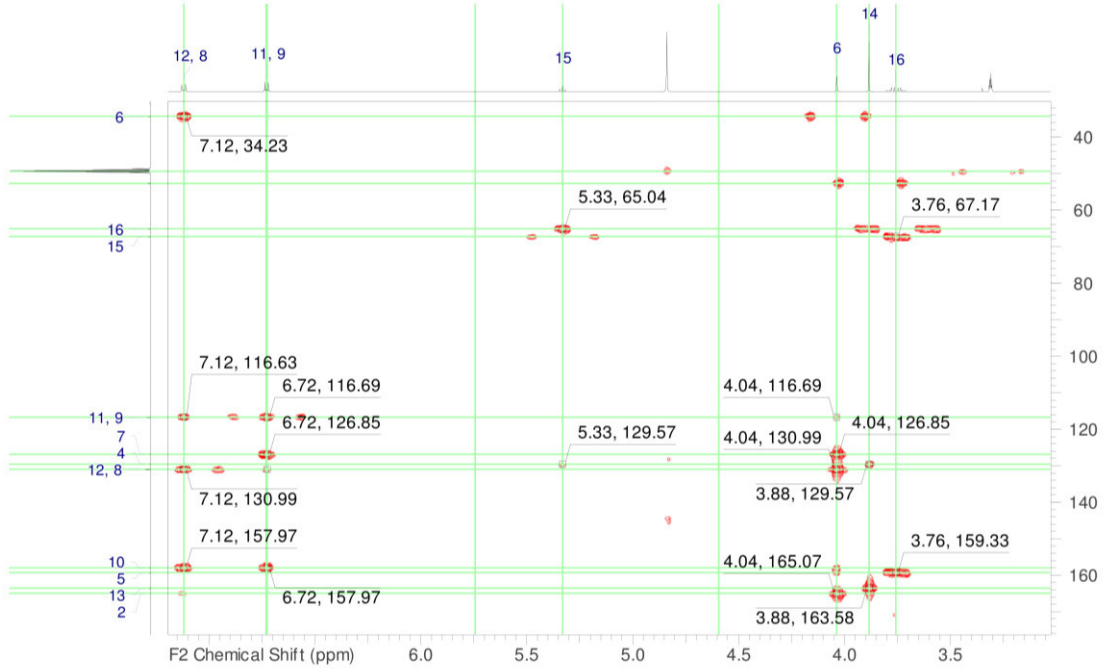

Figure S16: ^{1}H, ^{13}C HMBC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole B (**2**).

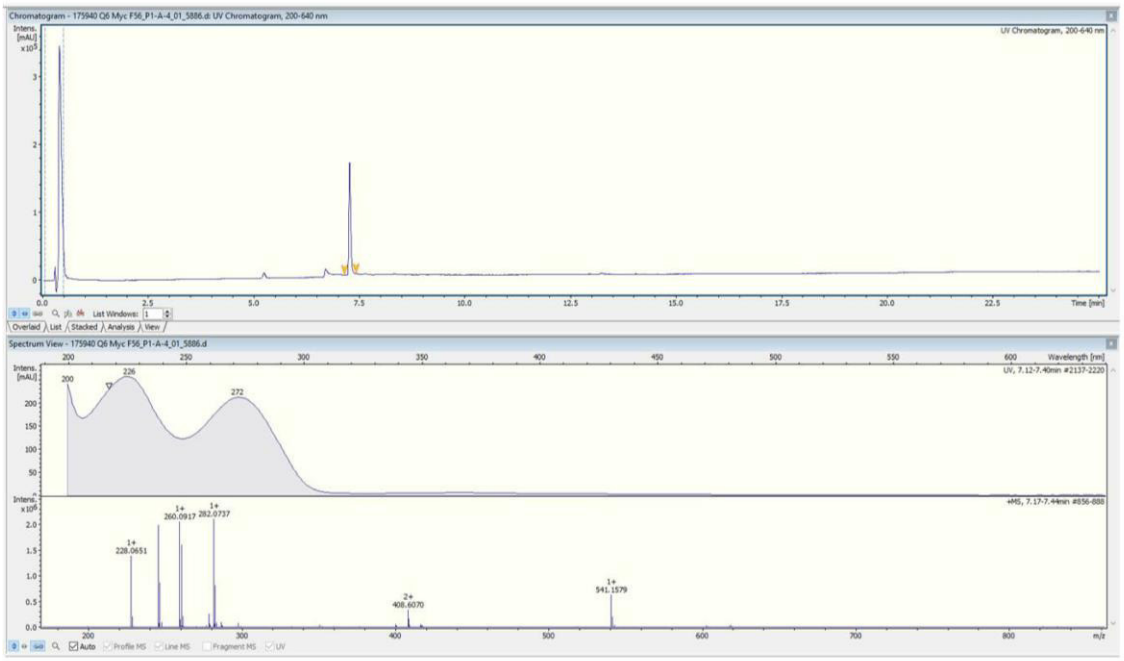

Figure S17: HR-ESIMS data for macrooxazole C (**3**).

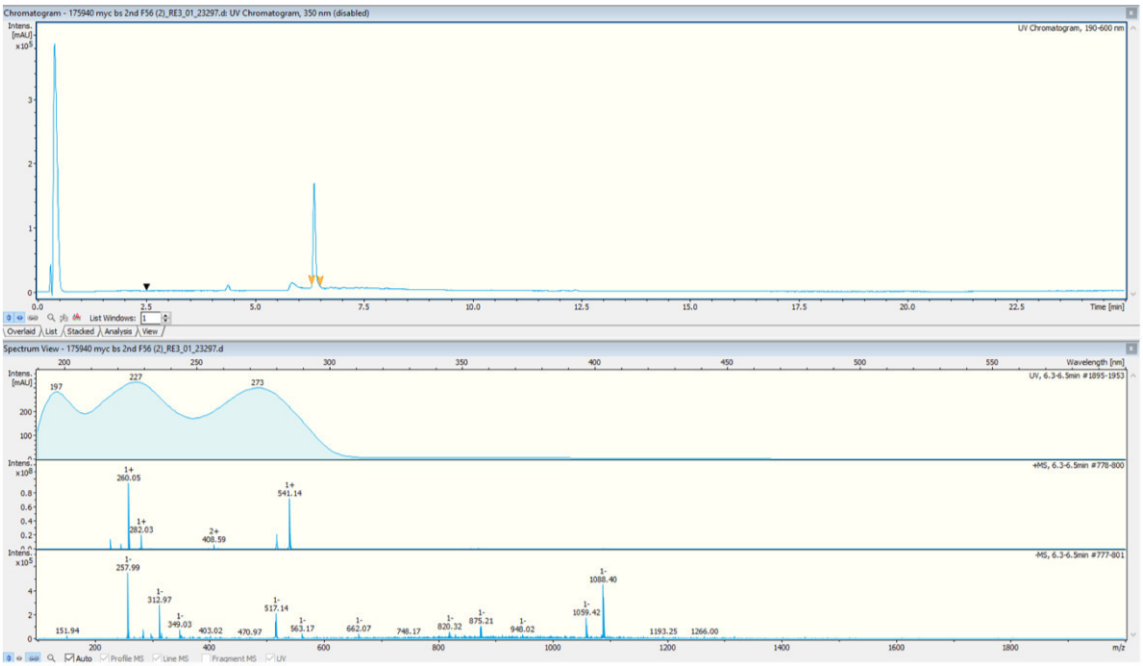

Figure S18: ESIMS data for macrooxazole C (**3**).

1D and 2D NMR data for macrooxazole C (3)

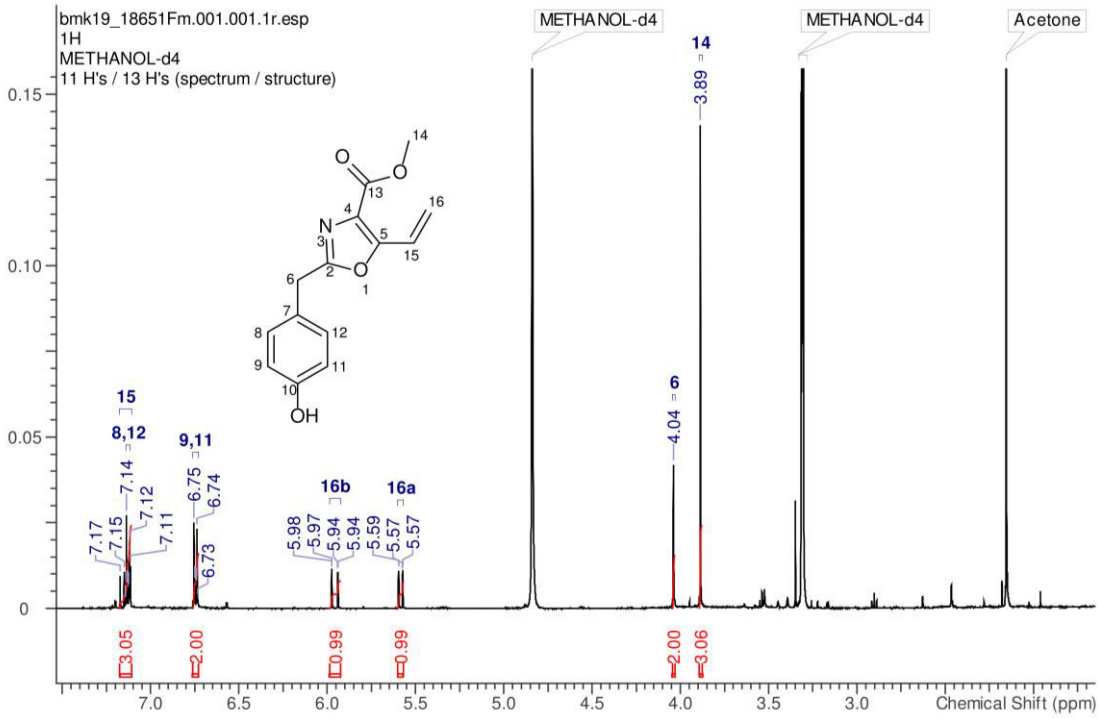

Figure S19: ^{1}H NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole C (**3**).

22

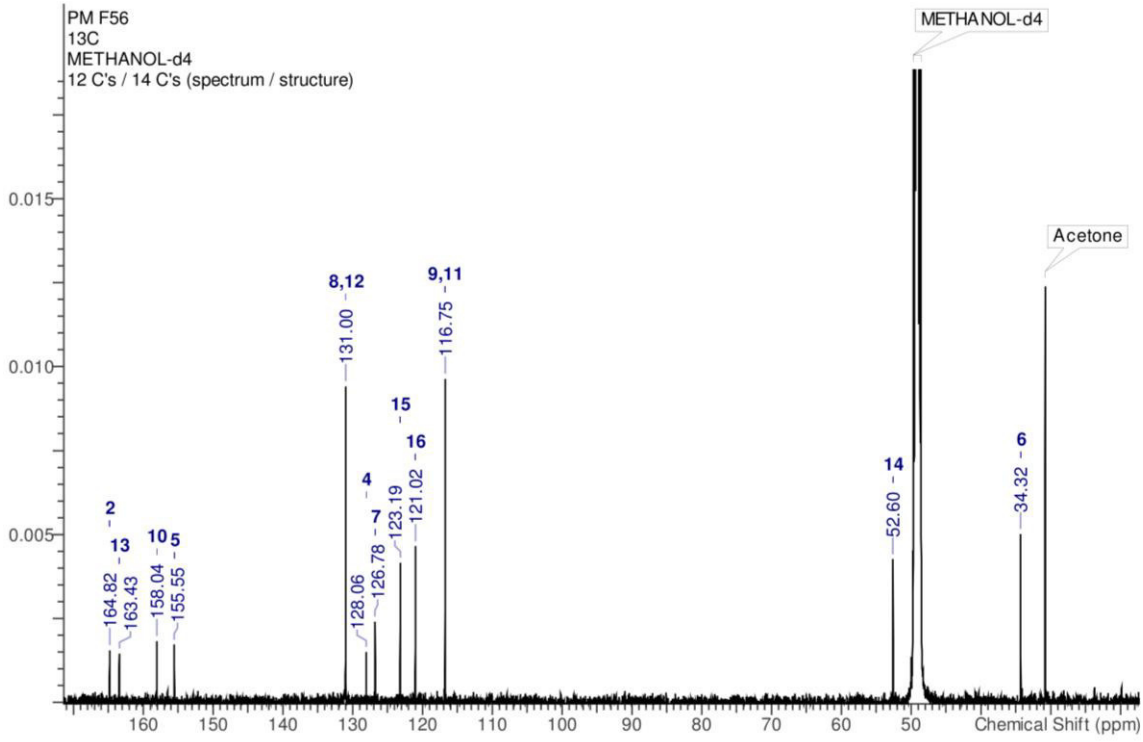

Figure S20: ^{13}C NMR spectrum (MeOH-*d4*, 125 MHz) of macrooxazole C (**3**).

23

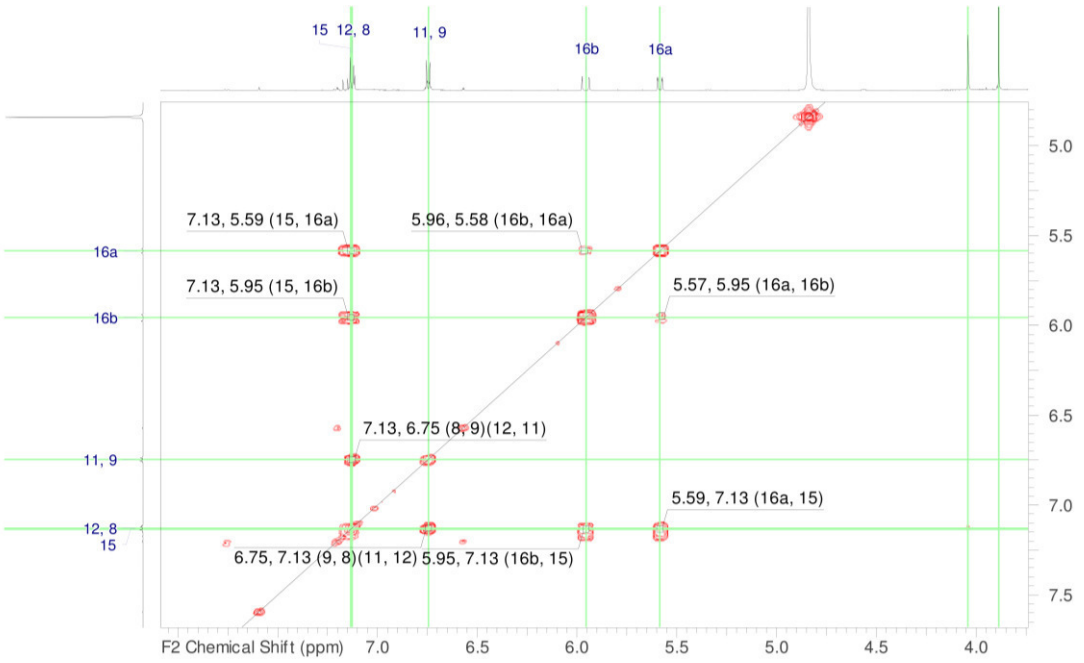

Figure S21: ^{1}H, ^{1}H COSY NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole C (**3**).

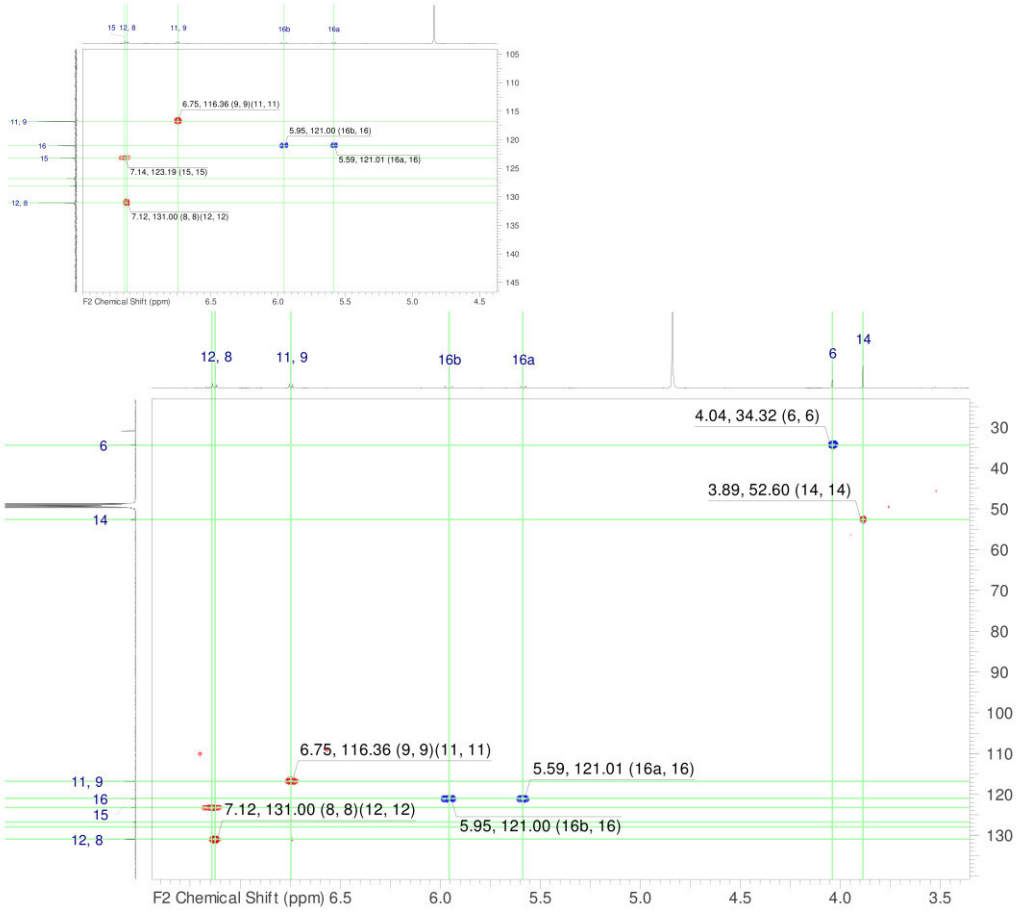

Figure S22: ^{1}H, ^{13}C HSQC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole C (**3**).

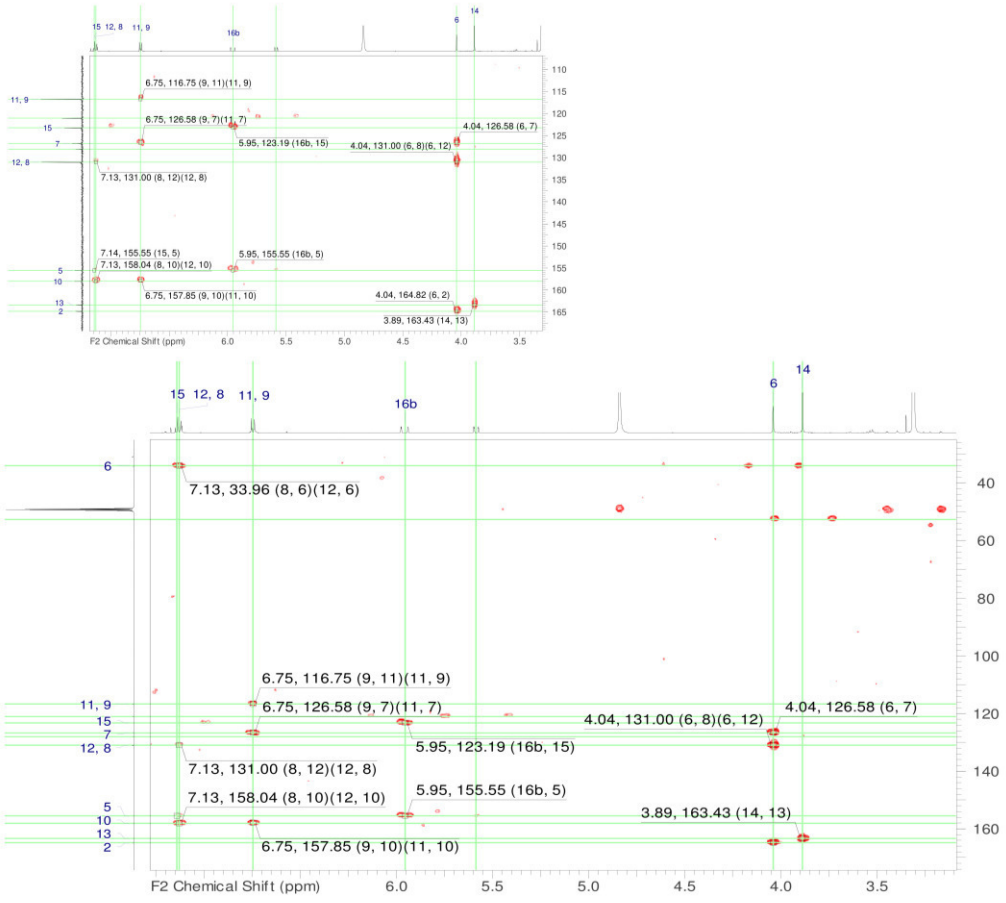

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Figure S23: ^{1}H, ^{13}C HMBC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole C (**3**).

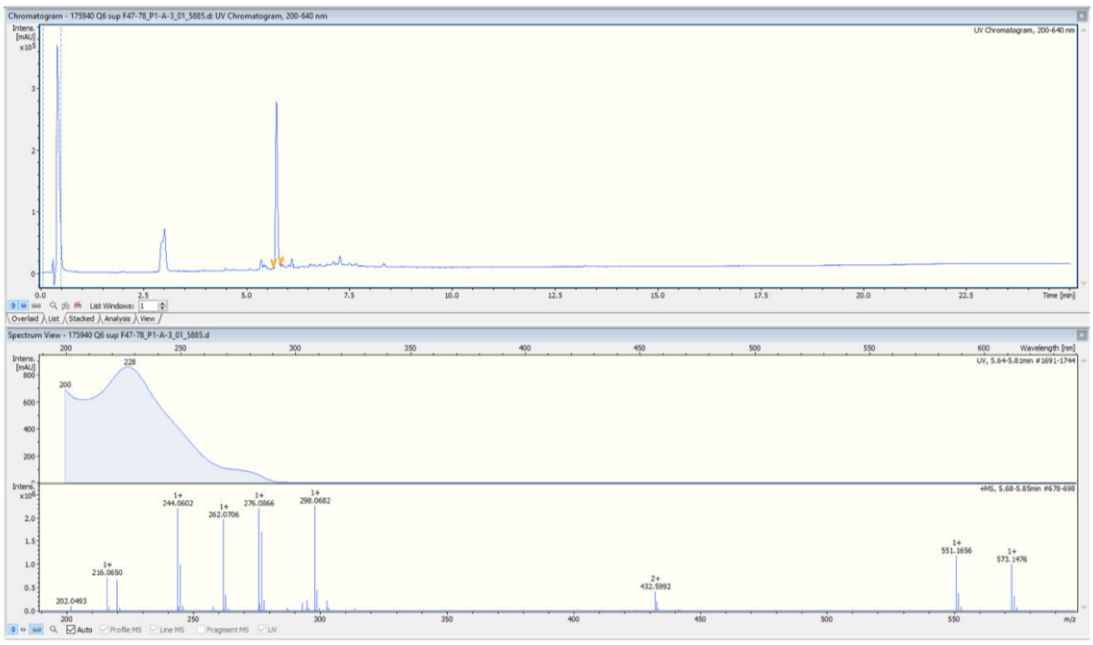

Figure S24: HR-ESIMS data for macrooxazole D (**4**).

27

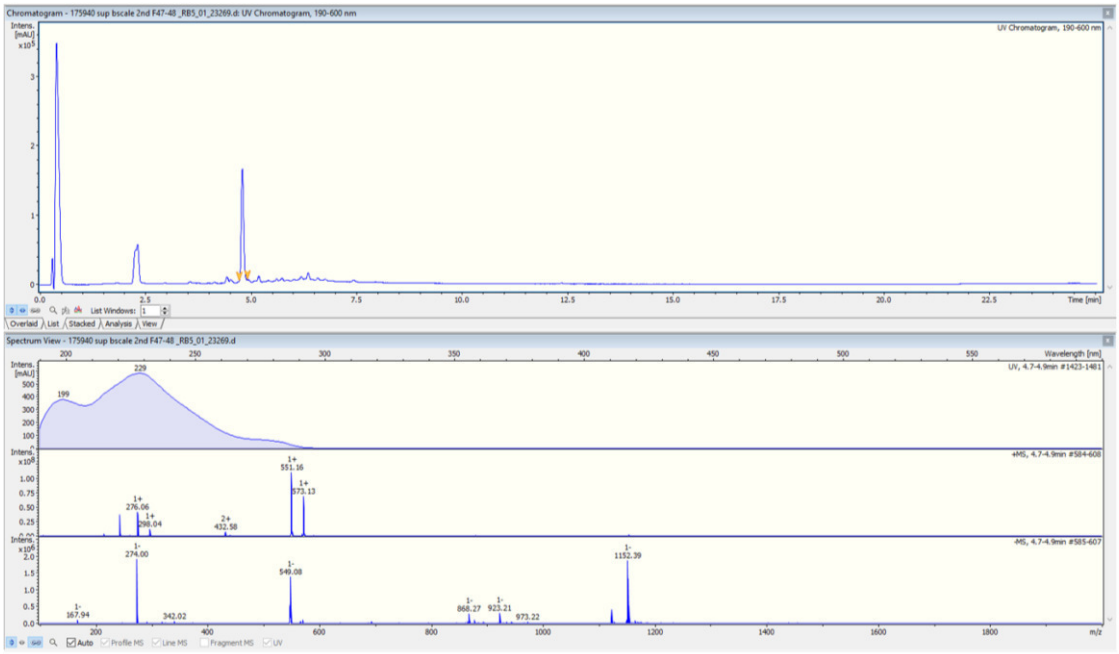

Figure S25: ESIMS data for macrooxazole D (**4**).

1D and 2D NMR data for macrooxazole D (4)

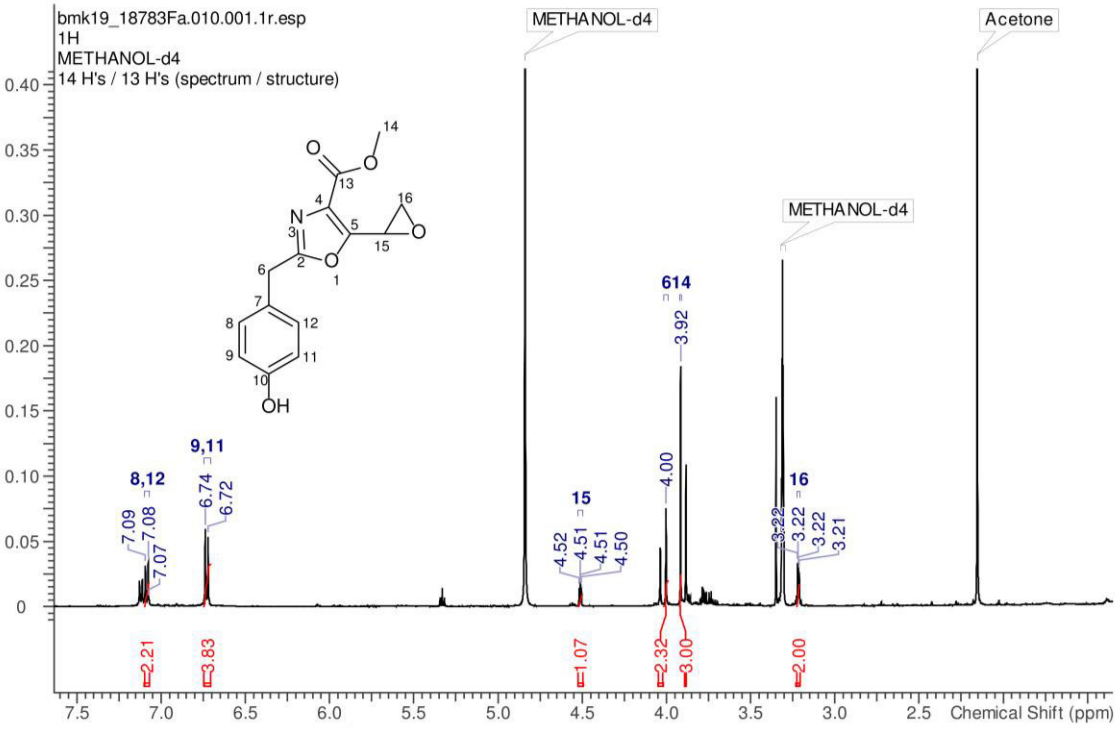

Figure S26: ^{1}H NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole D (**4**).

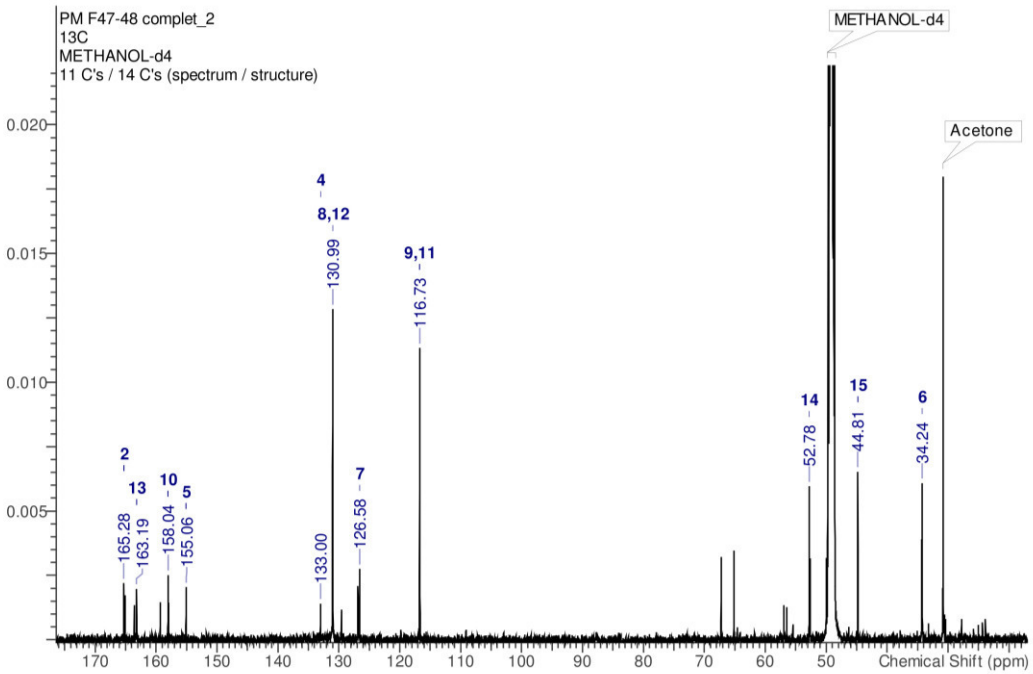

Figure S27: ^{13}C NMR spectrum (MeOH-*d4*, 125 MHz) of macrooxazole D (**4**).

30

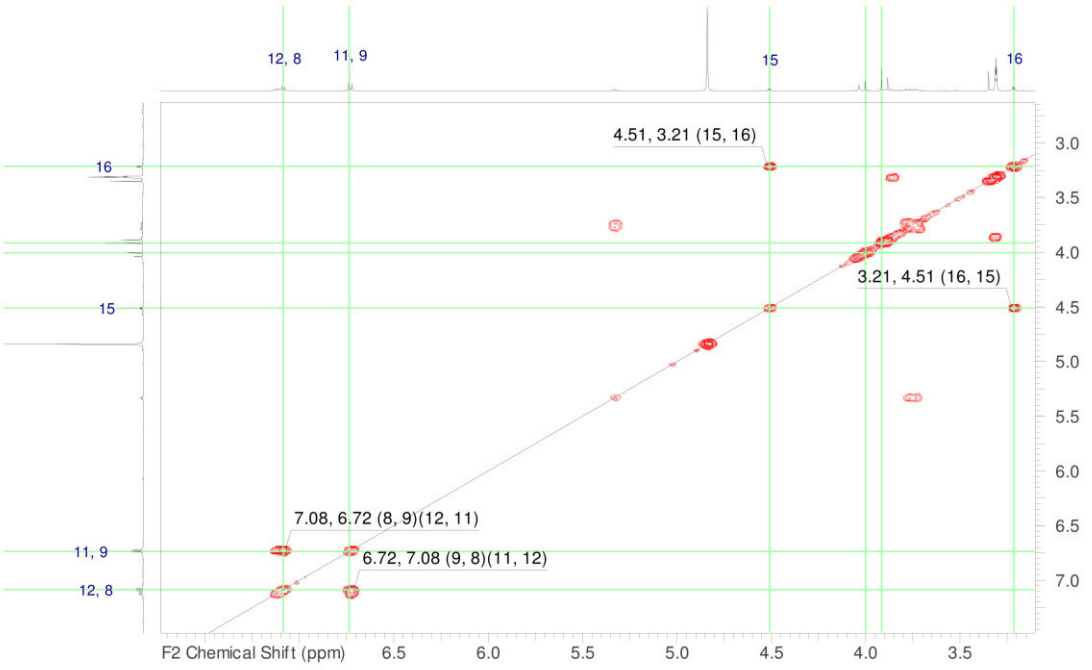

Figure S28: ^{1}H, ^{1}H COSY NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole D (**4**).

31

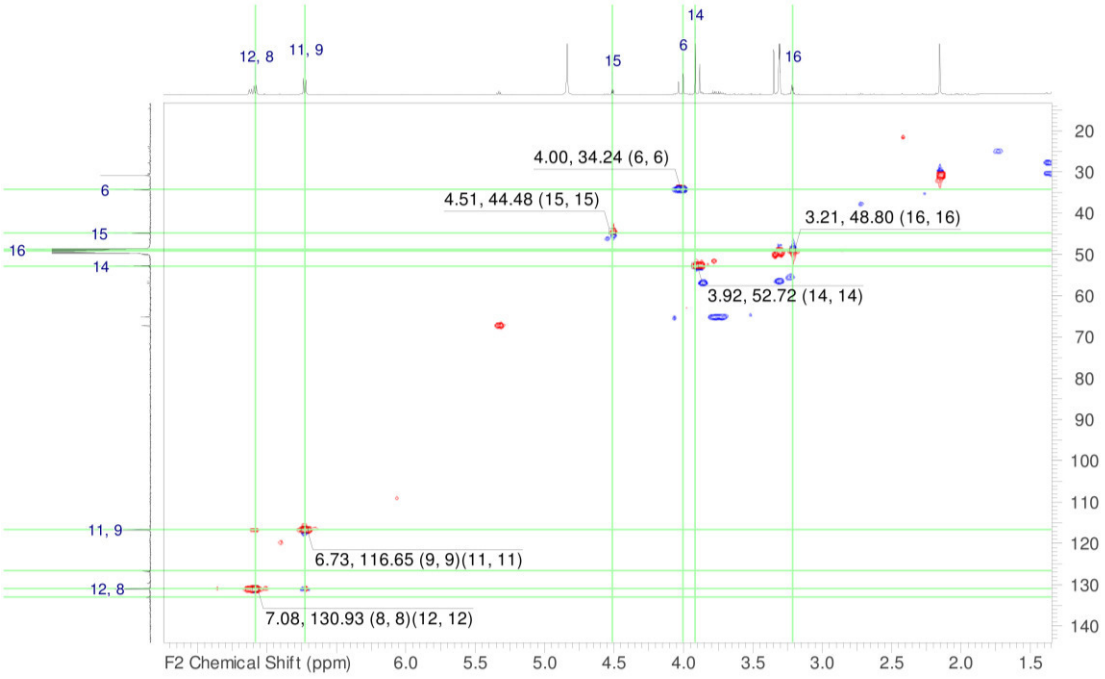

Figure S29: ^{1}H, ^{13}C HSQC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole D (**4**).

32

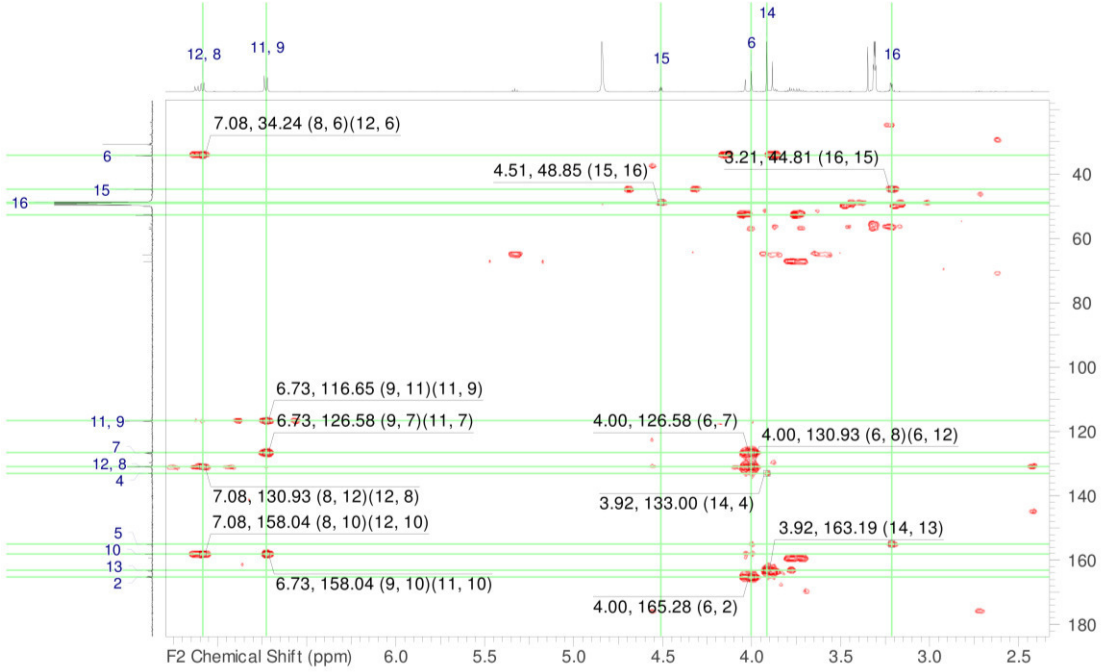

Figure S30: ^{1}H, ^{13}C HMBC NMR spectrum (MeOH-*d4*, 500 MHz) of macrooxazole D (**4**).

33

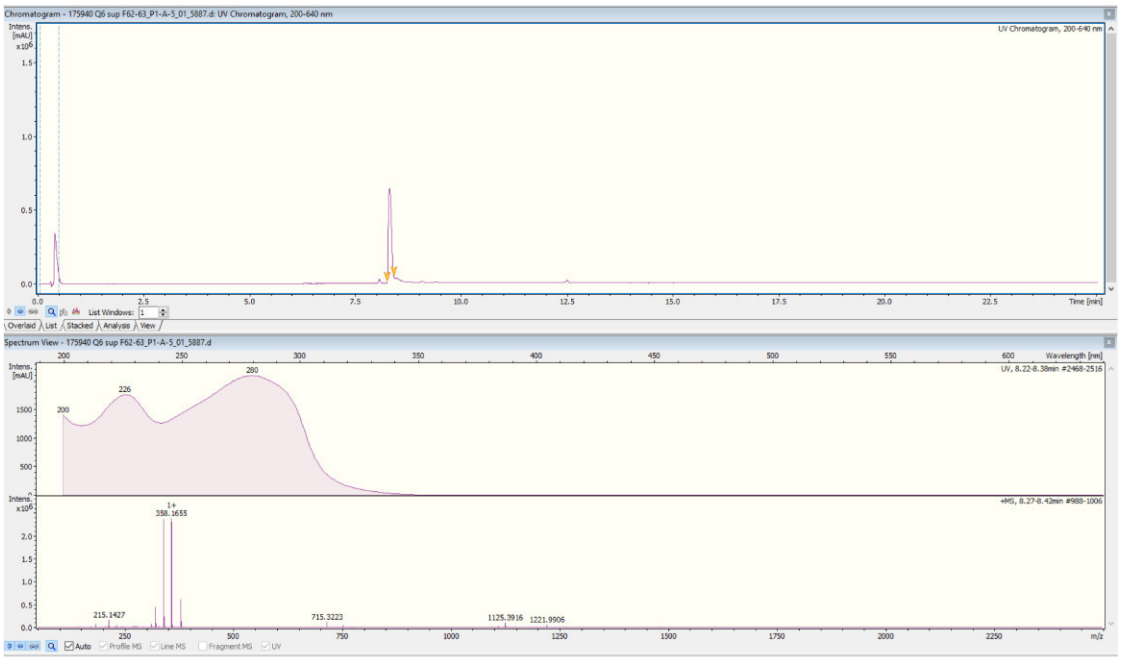

Figure S31: HR-ESIMS data for macrocidin A (**5**).

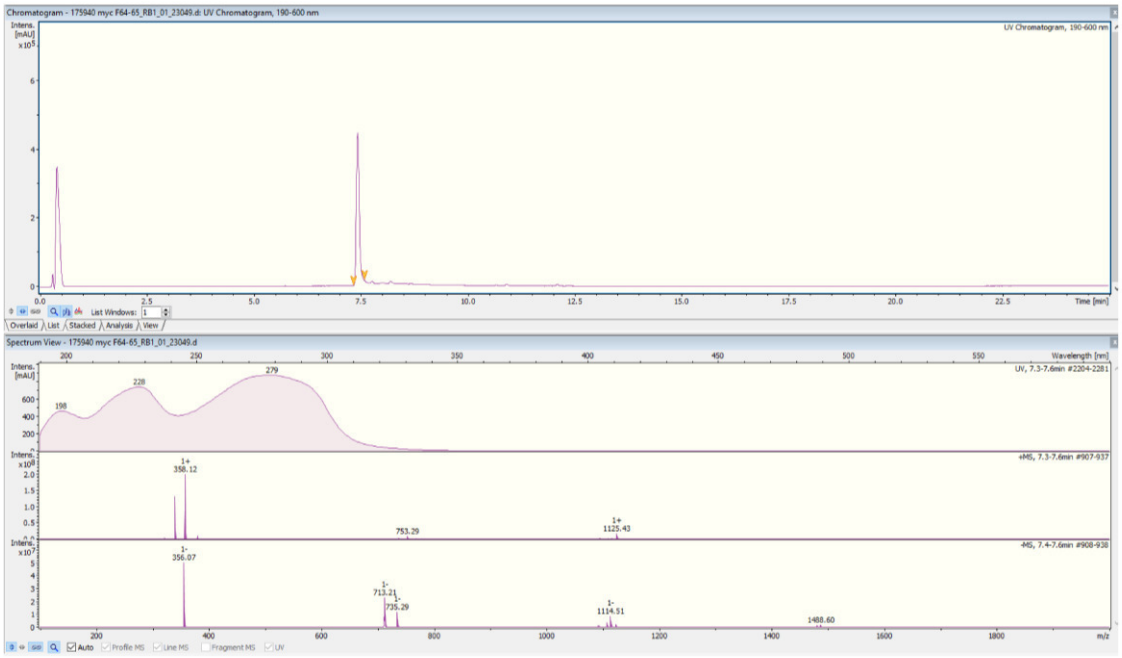

Figure S32: ESIMS data for macrocidin A (**5**).

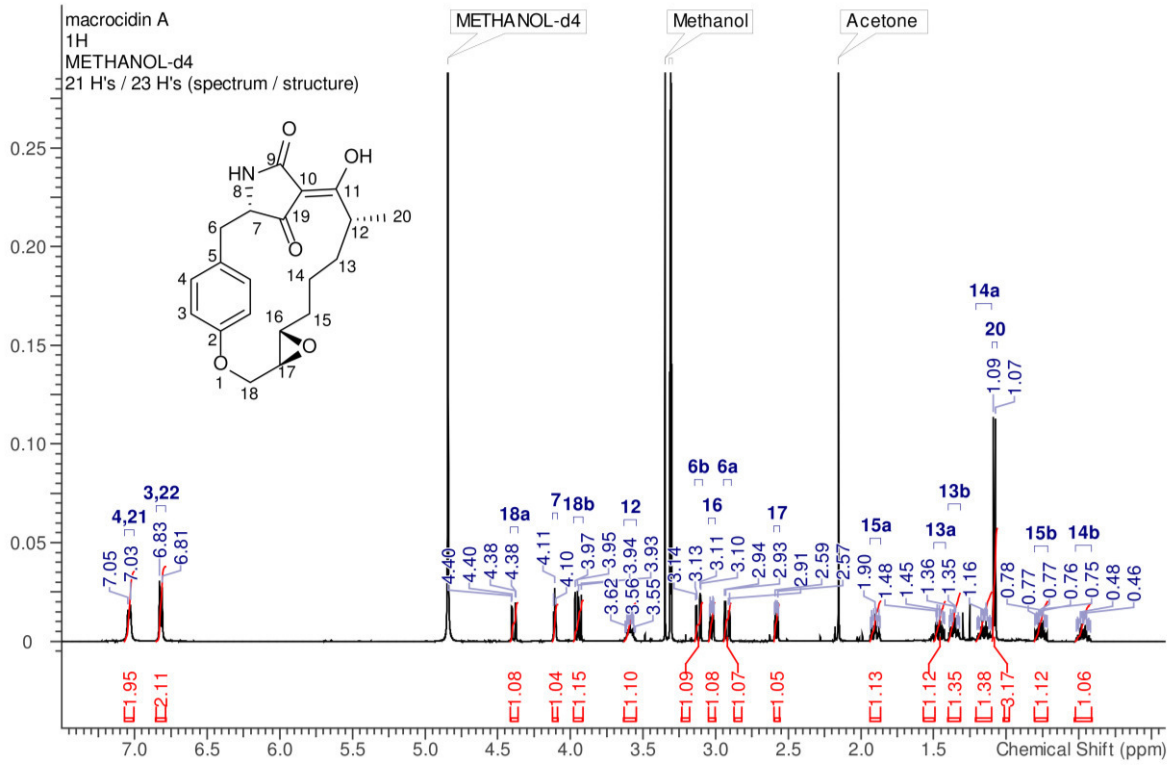

Figure S33: ^{1}H NMR spectrum (MeOH-*d4*, 500 MHz) of macrocidin A (**5**)

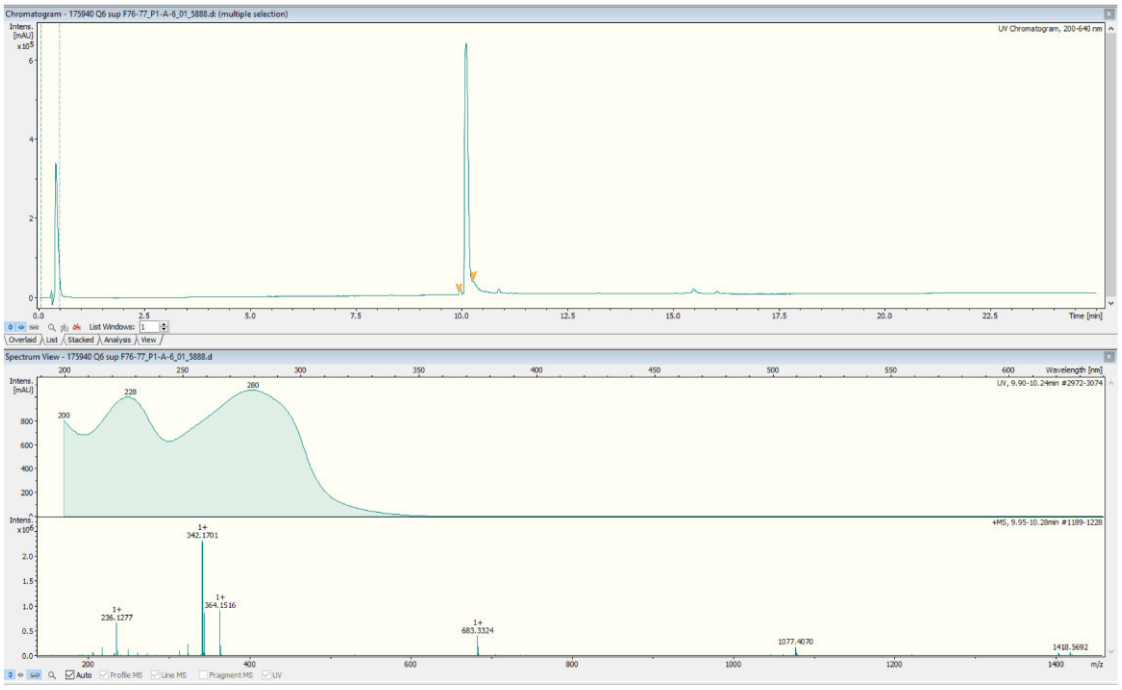

Figure S34: HR-ESIMS data for macrocidin Z (**6**).

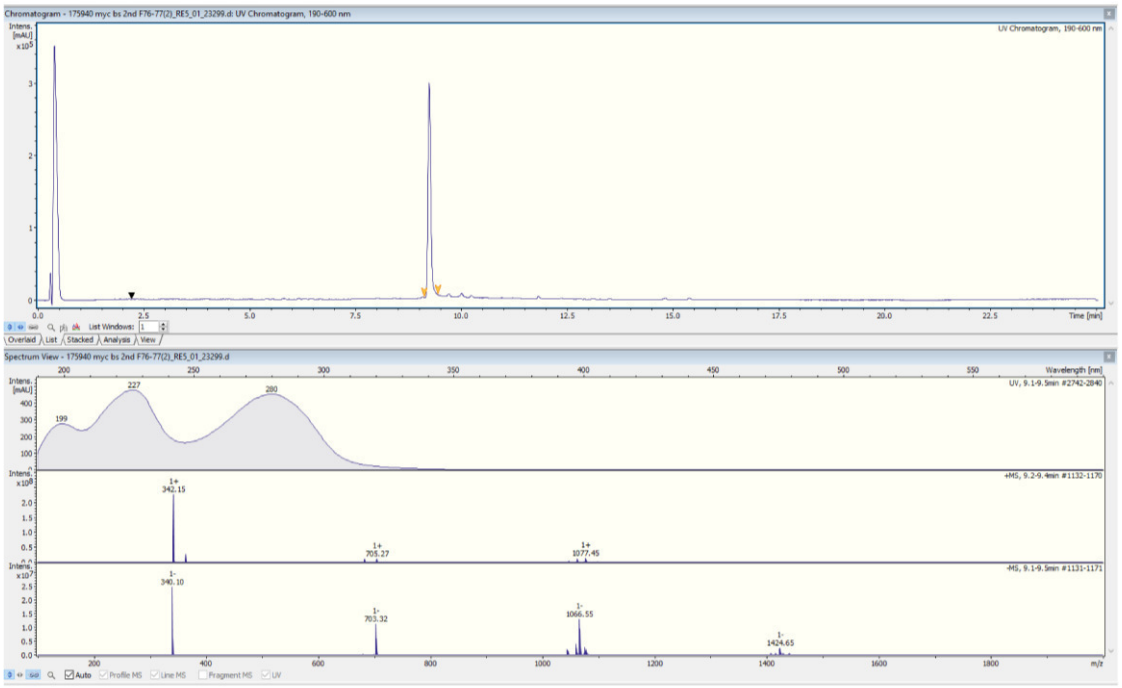

Figure S35: ESIMS data for macrocidin Z (**6**).

1D and 2D NMR data for macrocidin Z (6)

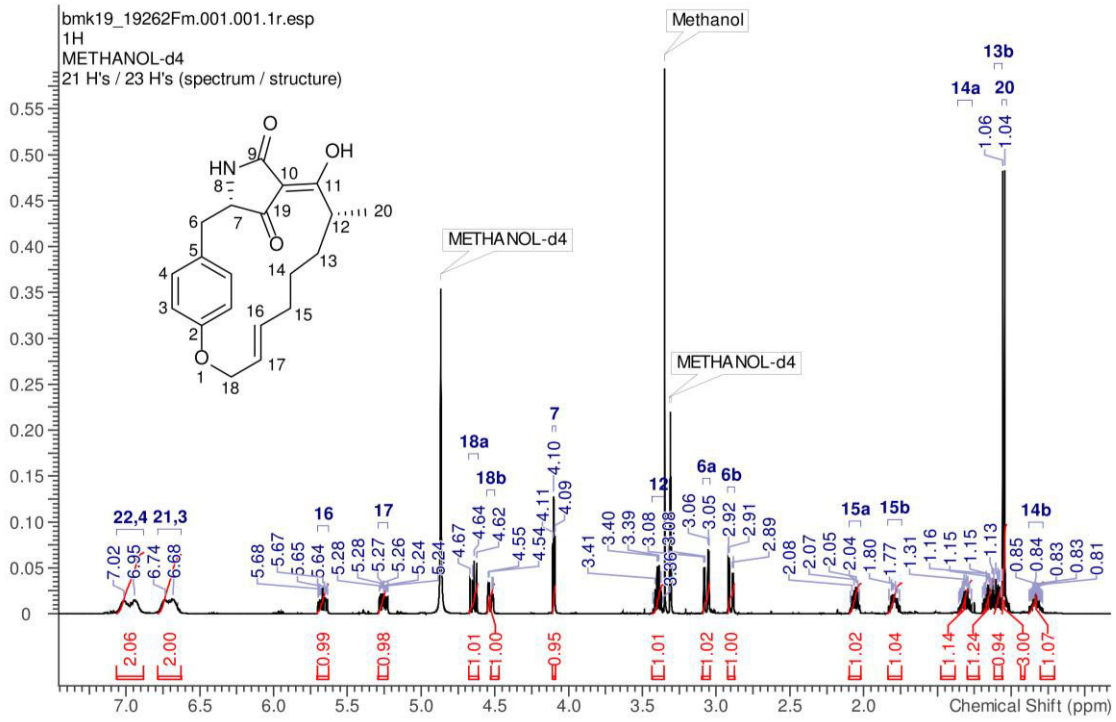

Figure S36: ^{1}H NMR spectrum (MeOH-*d4*, 500 MHz) of macrocidin Z (**6**).

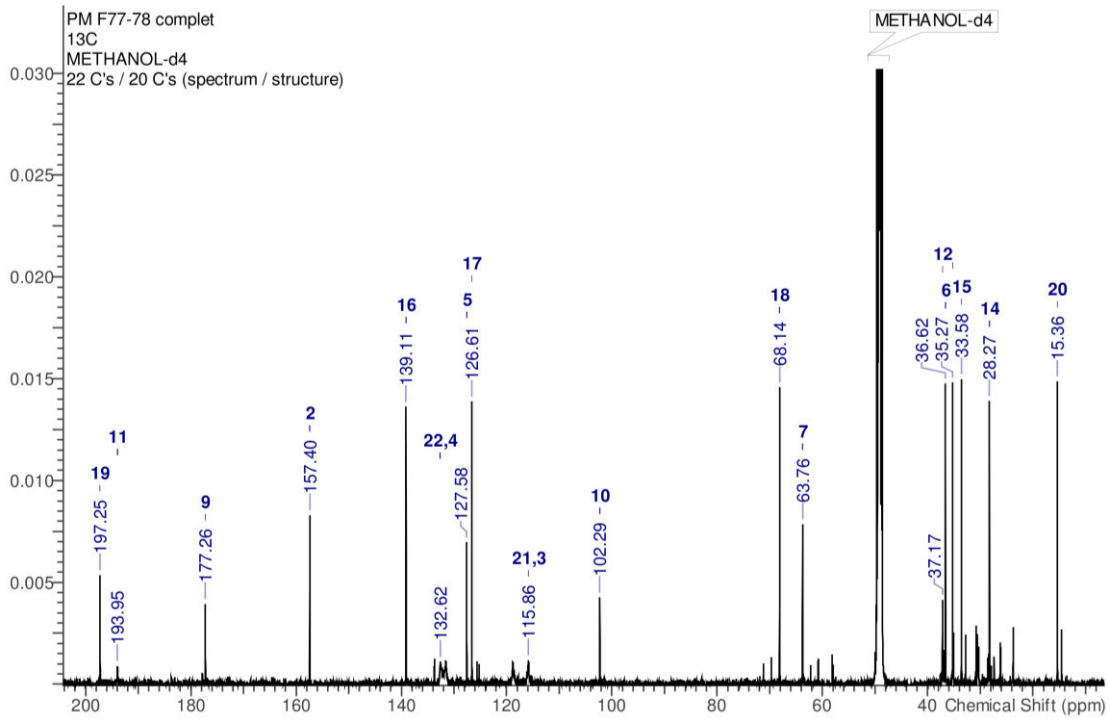

Figure S37: ^{13}C NMR spectrum (MeOH-*d4*, 125 MHz) of macrocidin Z (**6**).

40

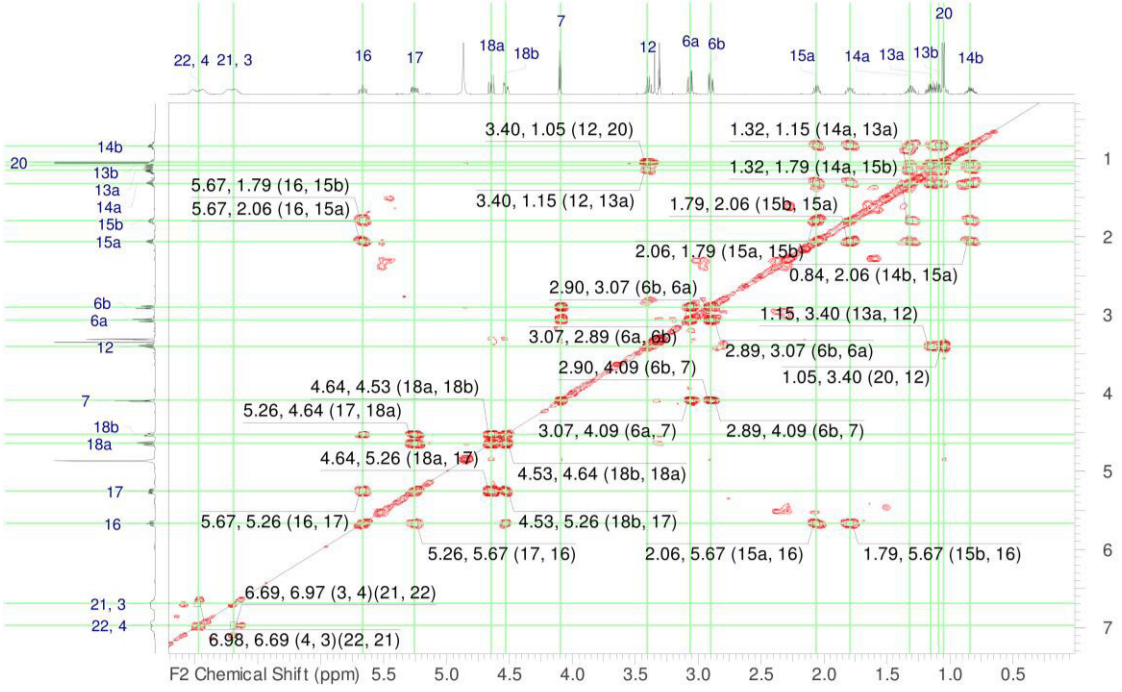

Figure S38: ^{1}H, ^{1}H COSY NMR spectrum (MeOH-*d4*, 500 MHz) of macrocidin Z (**6**).

41

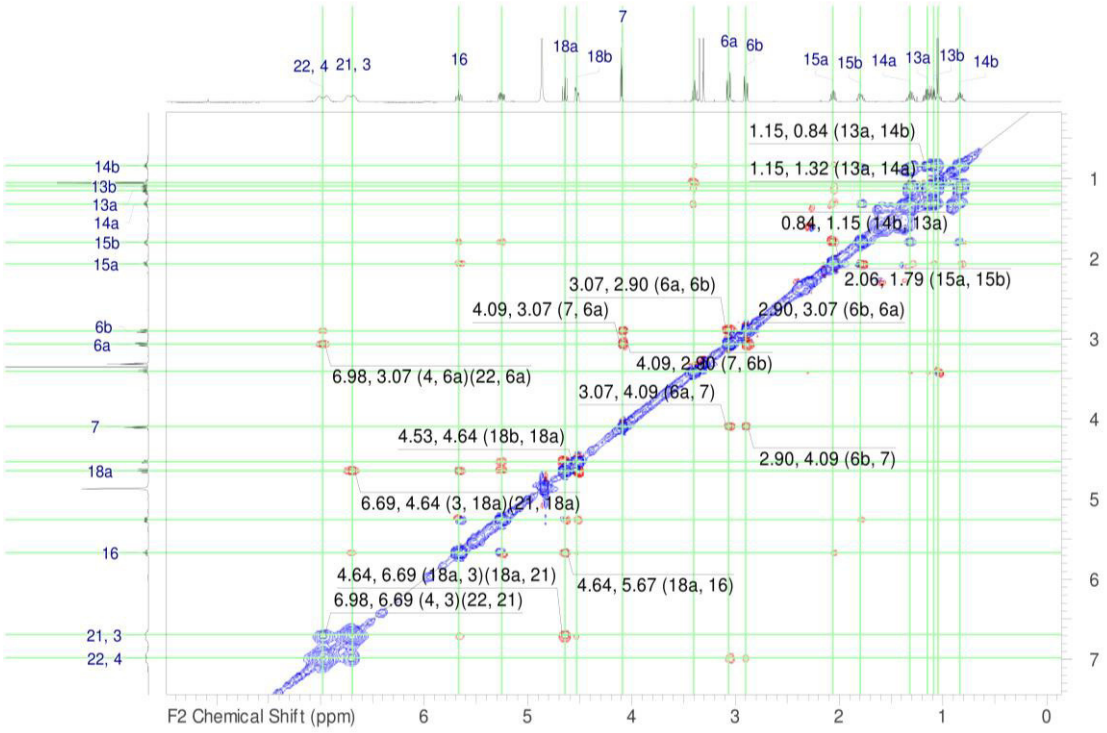

Figure S39: ^{1}H, ^{1}H ROESY NMR spectrum (MeOH-*d4*, 500 MHz) of macrocidin Z (**6**)

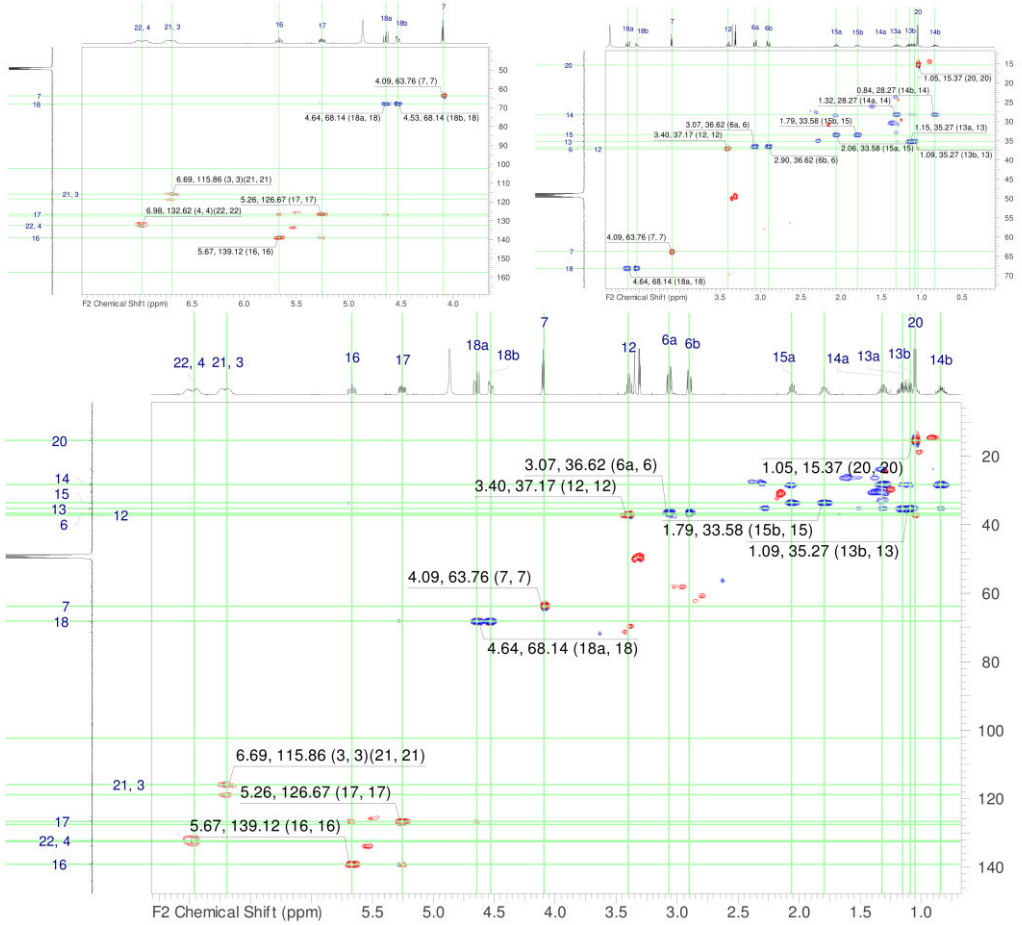

Figure S40: ^{1}H, ^{13}C HSQC NMR spectrum (MeOH-*d4*, 500 MHz) of macrocidin Z (**6**).

Figure S41: ^{1}H, ^{13}C HMBC NMR spectrum (MeOH-*d4*, 500 MHz) of macrocidin Z (**6**)

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Table S1: Minimum Inhibitory concentrations (MIC) of compounds **1-6** against tested microorganisms.

Test organisms	**MIC (µg/mL)**						
	1	**2**	**3**	**2/4** (ratio1:2)	**5**	**6**	**References**
Schizosaccharomyces pombe DSM70572	n.i	n.i	n.i	n.i	n.i	n.i	33.3[n]
Pichia anomala DSM6766	n.i	n.i	n.i	n.i	n.i	n.i	33.3[n]
Mucor hiemalis DSM2656	n.i	n.i	66.7	n.i	n.i	n.i	66.7[n]
Candida albicans DSM1665	n.i	n.i	n.i	n.i	n.i	n.i	66.7[n]
Rhodoturula glutinis DSM10134	n.i	n.i	n.i	n.i	n.i	n.i	8.3[n]
Micrococcus luteus DSM1790	n.i	n.i	n.i	n.i	n.i	66.7	0.4[o]
Escherichia coli DSM1116	n.i	n.i	n.i	n.i	n.i	n.i	1.7[o]
Bacillus subtilis DSM10	n.i	n.i	66.7	n.i	16.7	n.i	16.7[o]
Mycolicibacterium smegmatis ATCC700084	n.i	n.i	n.i	n.i	33.3	n.i	1.7[k]
Staphylococcus aureus DSM346	n.i	n.i	n.i	n.i	n.i	n.i	0.2[o]
Pseudomonas aeruginosa PA14	n.i	n.i	n.i	n.i	n.i	n.i	0.4[g]
Chromobacterium violaceum DSM30191	n.i	n.i	n.i	n.i	n.i	n.i	0.4[o]

n.i.: No inhibition observed under test conditions, g Gentamycin 1 mg/mL, k Kanamycin 1 mg/mL, n Nystatin 1 mg/mL, o Oxytetracyclin 1 mg/mL. Starting concentration for antimicrobial assay were 66.7 µg/mL.

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Table S2: Cytotoxic effect (IC_{50}) of compound **1-6** against two cancer cell lines.

Cell lines	IC_{50} (µg/mL)						
	1	**2**	**3**	**2/4** (ratio 1:2)	**5**	**6**	**Epothilone B**
KB3.1	n.a	n.a	n.a	23	s.i	s.i	0.000016
L929	n.a	n.a	n.a	23	n.a	n.a	0.00026

n.a: Not active; s.i: slight inhibition of cells proliferation; Epothilone B 1mg/mL. Starting concentration for cytotoxicity assay were 37 µg/mL.

NMR spectra of the synthetic intermediates

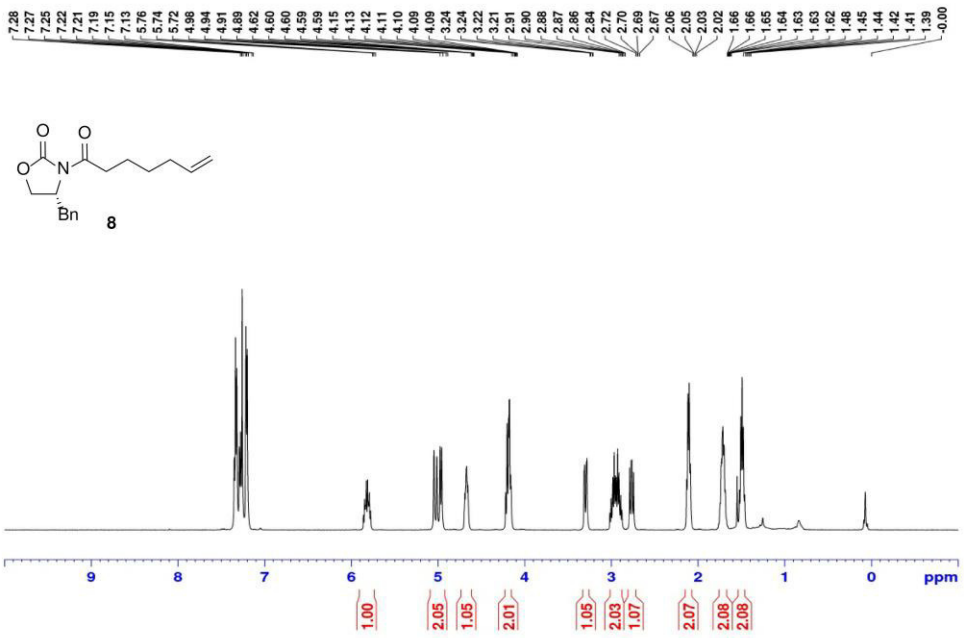

Figure S42: ^{1}H-NMR spectrum of compound **8** in $CDCl_3$.

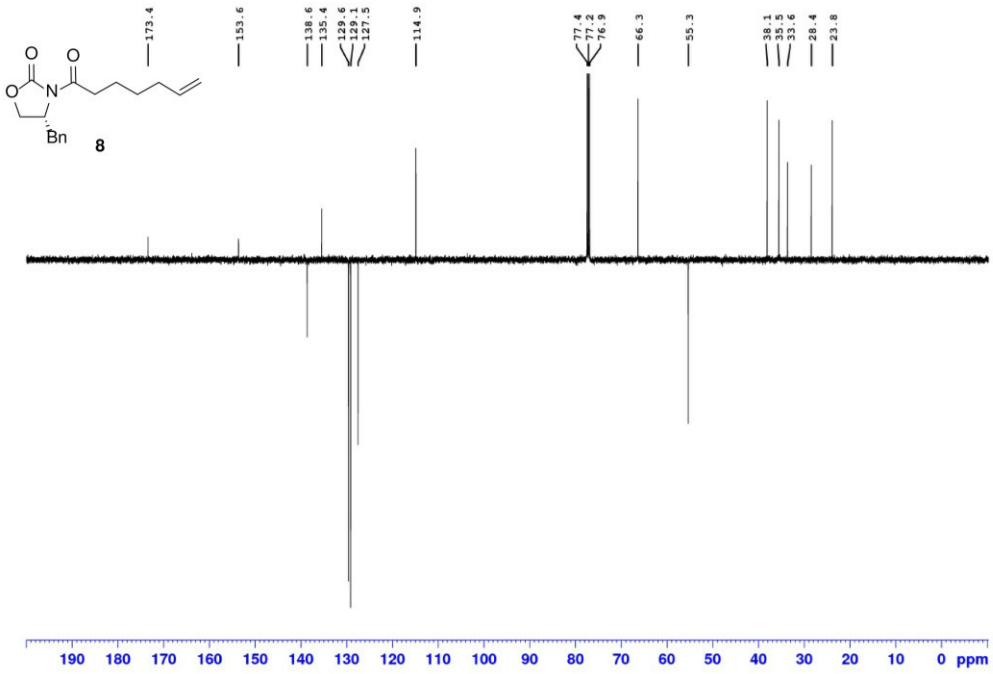

Figure S43: ^{13}C-NMR spectrum of compound **8** in $CDCl_3$.

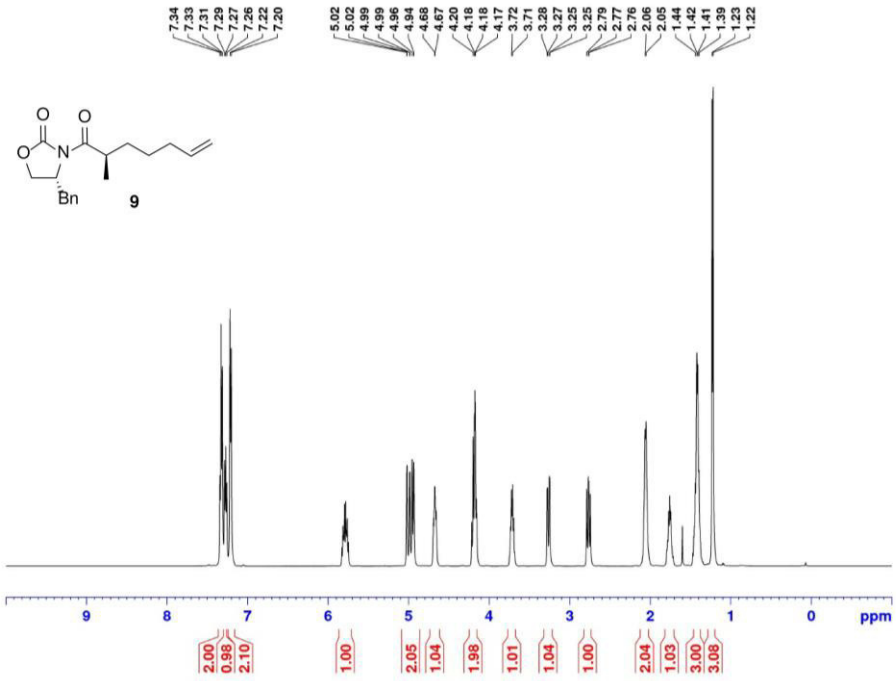

Figure S44: ^{1}H-NMR spectrum of compound **9** in $CDCl_3$.

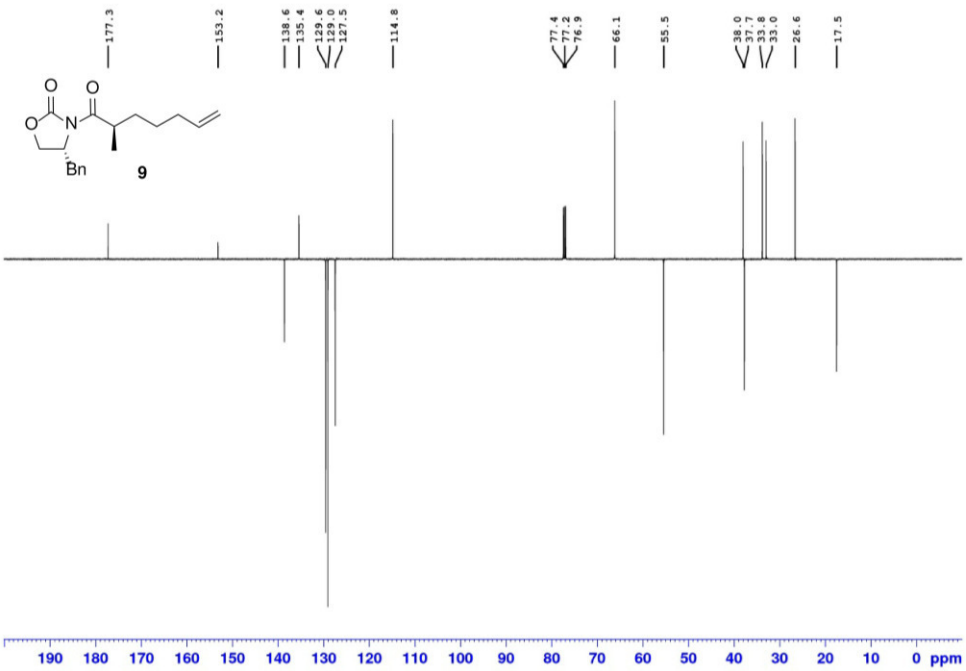

Figure S45: ^{13}C-NMR spectrum of compound **9** in $CDCl_3$.

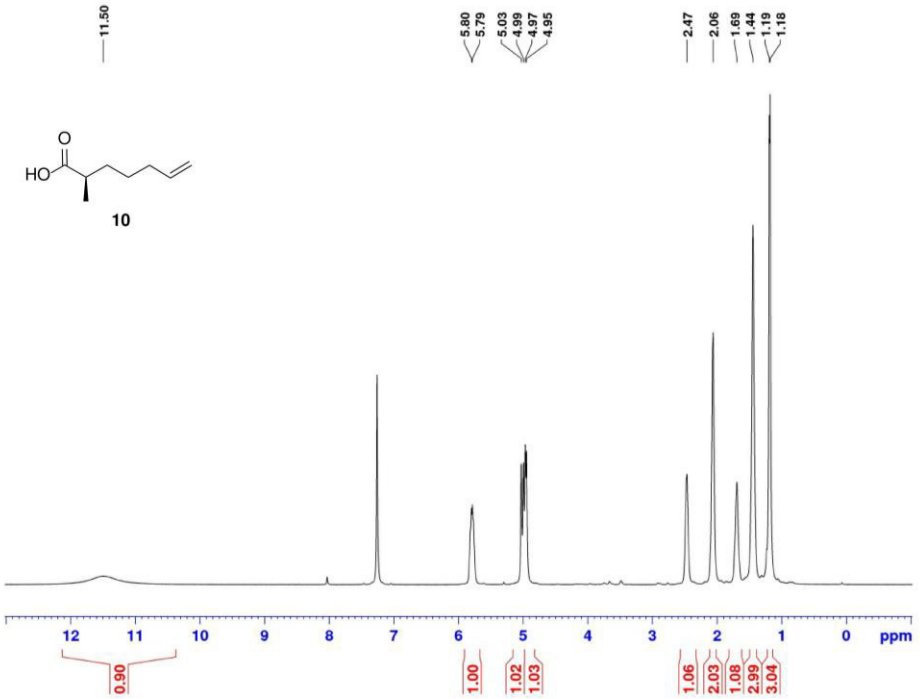

Figure S46: ^{1}H-NMR spectrum of compound **10** in $CDCl_3$.

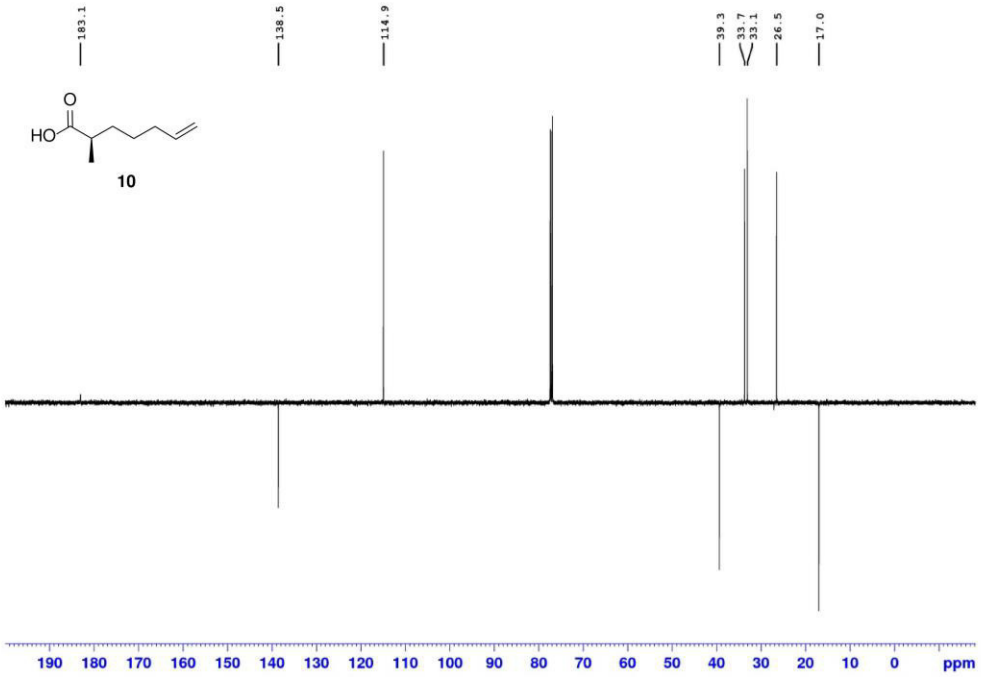

Figure S47: ^{13}C-NMR spectrum of compound **10** in $CDCl_3$.

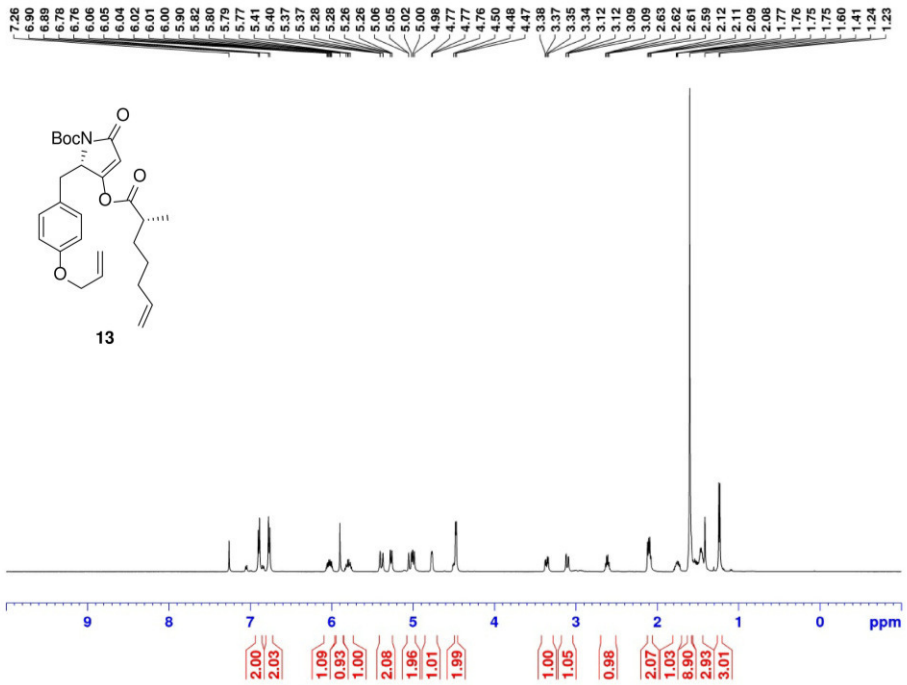

Figure S48: ^{1}H-NMR spectrum of compound **13** in $CDCl_3$.

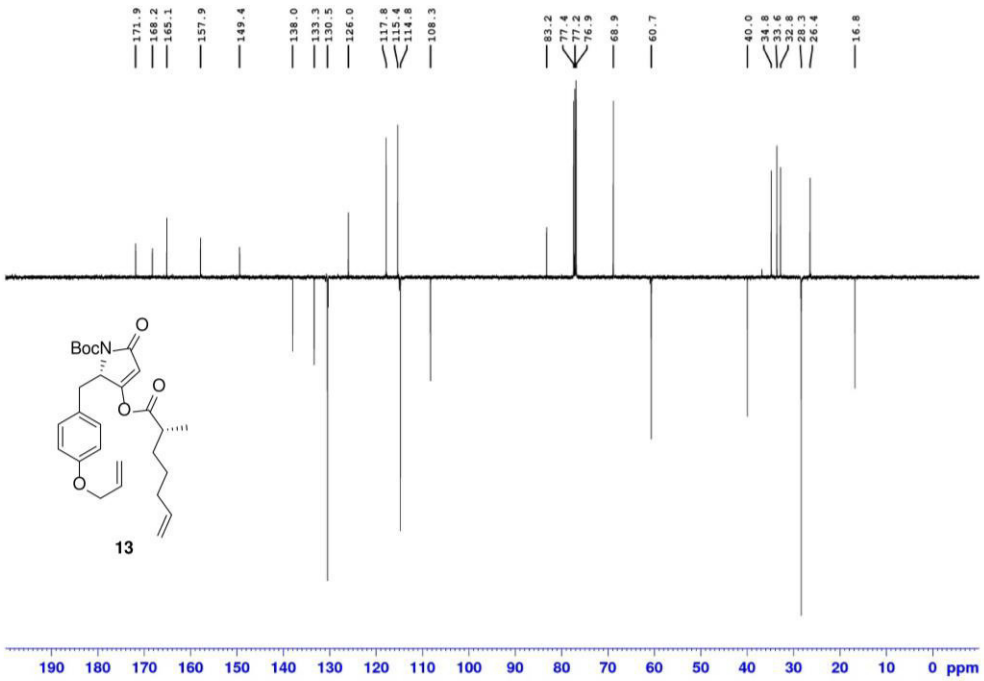

Figure S49: ^{13}C-NMR spectrum of compound **13** in $CDCl_3$.

54

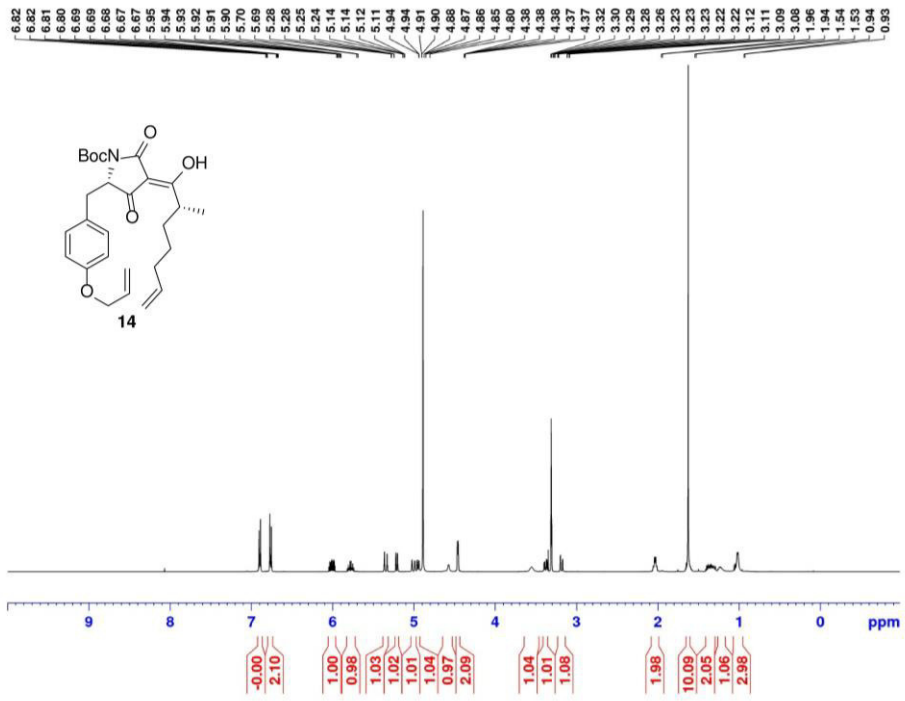

Figure S50: ^{1}H-NMR spectrum of compound **14** in MeOD.

55

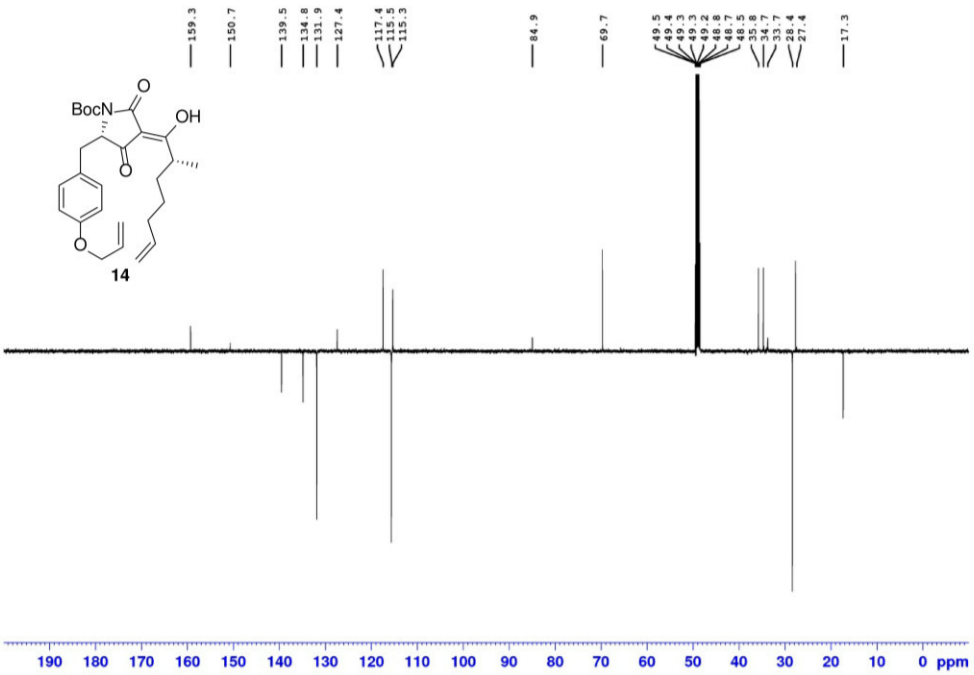

Figure S51: ^{13}C-NMR spectrum of compound **14** in MeOD.

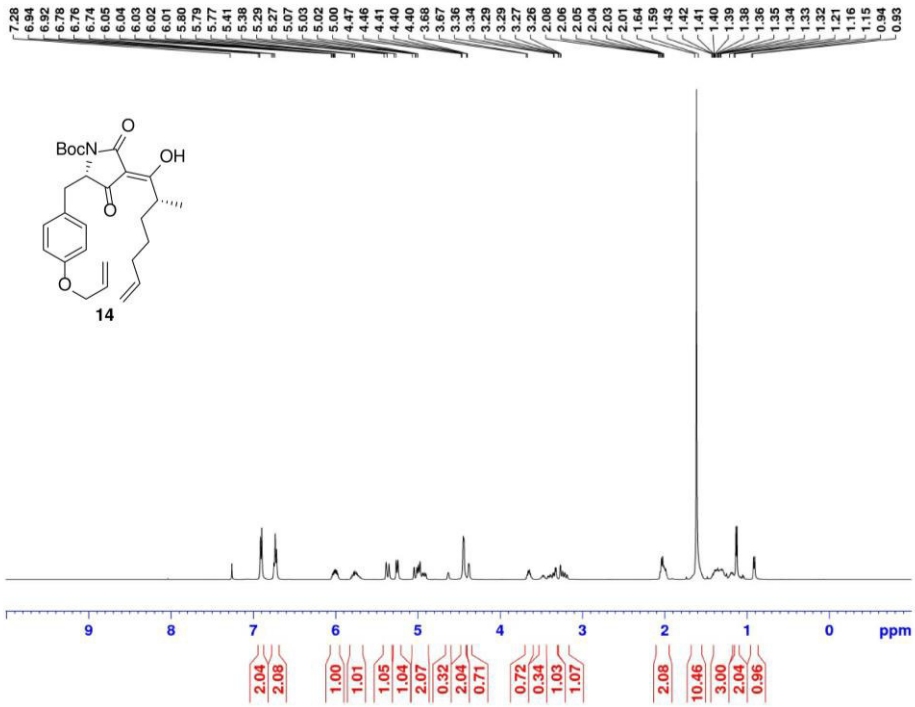

Figure S52: ^{1}H-NMR spectrum of compound **14** in $CDCl_3$.

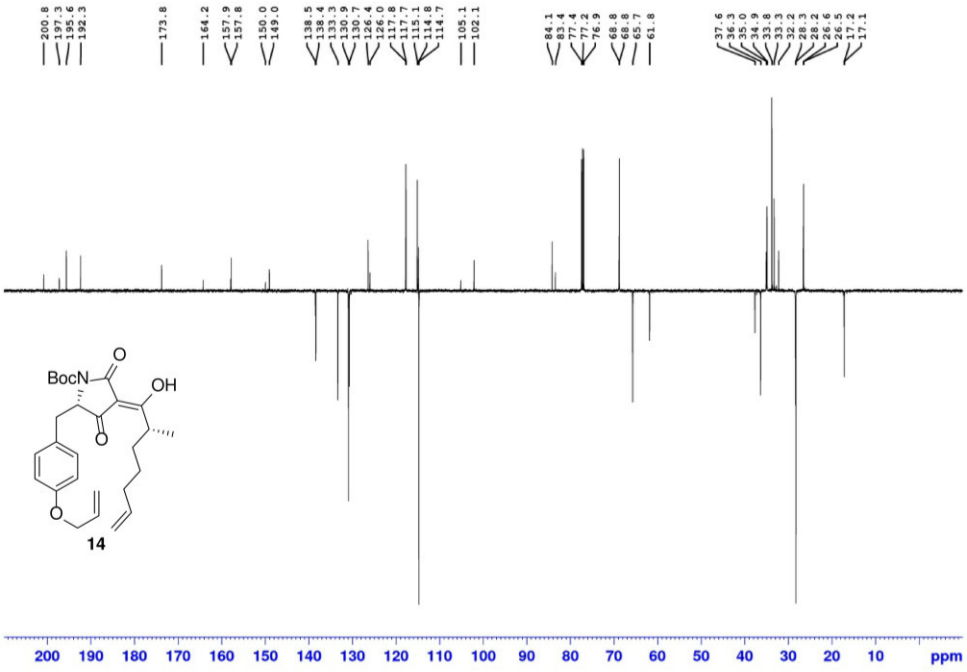

Figure S53: ^{13}C-NMR spectrum of compound **14** in $CDCl_3$.

58

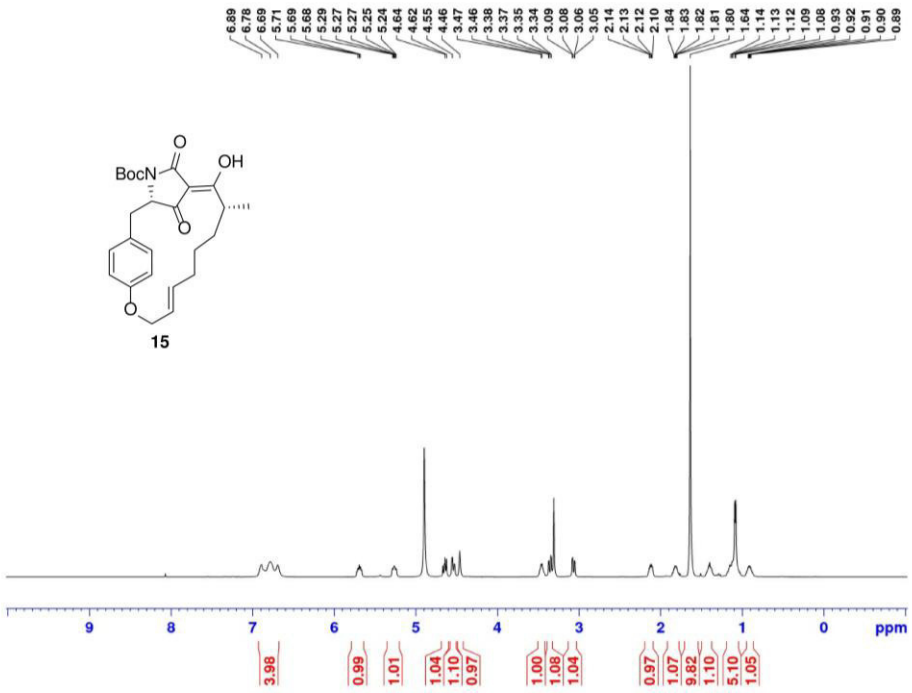

Figure S54: ^{1}H-NMR spectrum of compound **15** in MeOD.

59

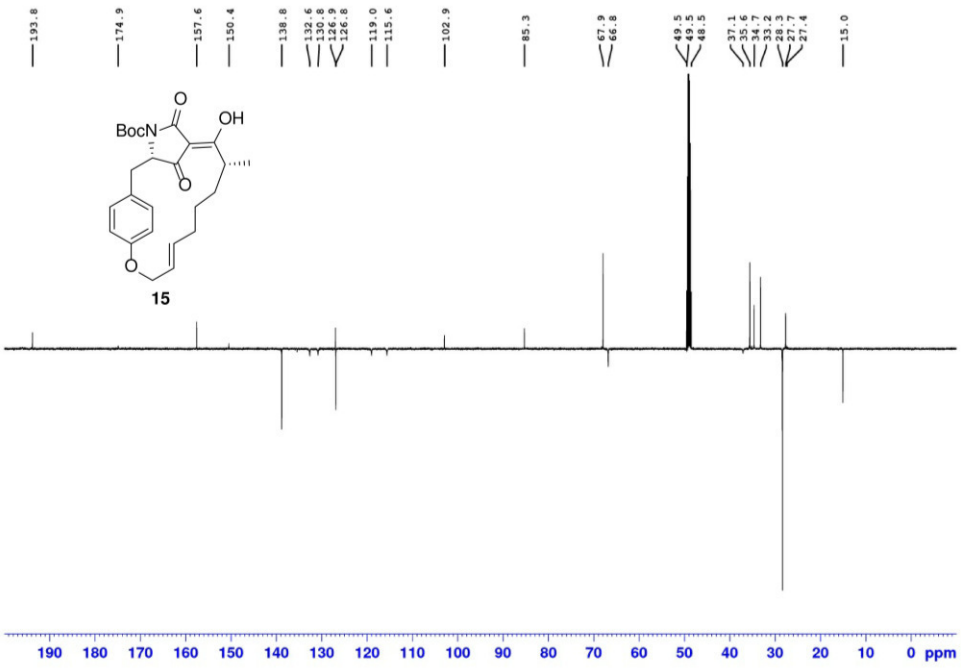

Figure S55: ^{13}C-NMR spectrum of compound **15** in MeOD.

60

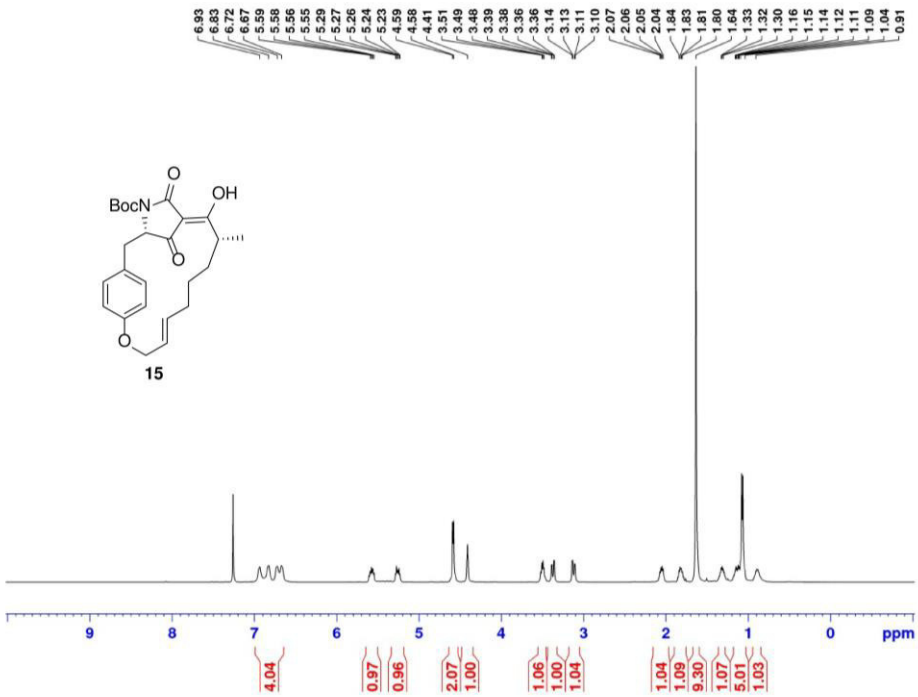

Figure S56: ^{1}H-NMR spectrum of compound **15** in $CDCl_3$.

61

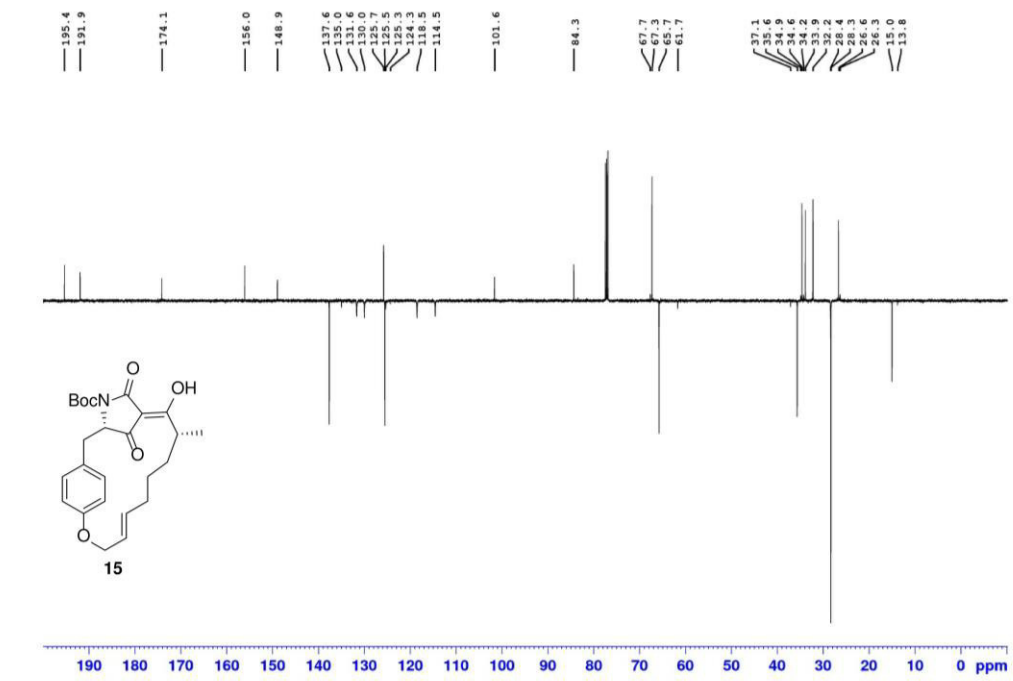

Figure S57: ^{13}C-NMR spectrum of compound **15** in $CDCl_3$.

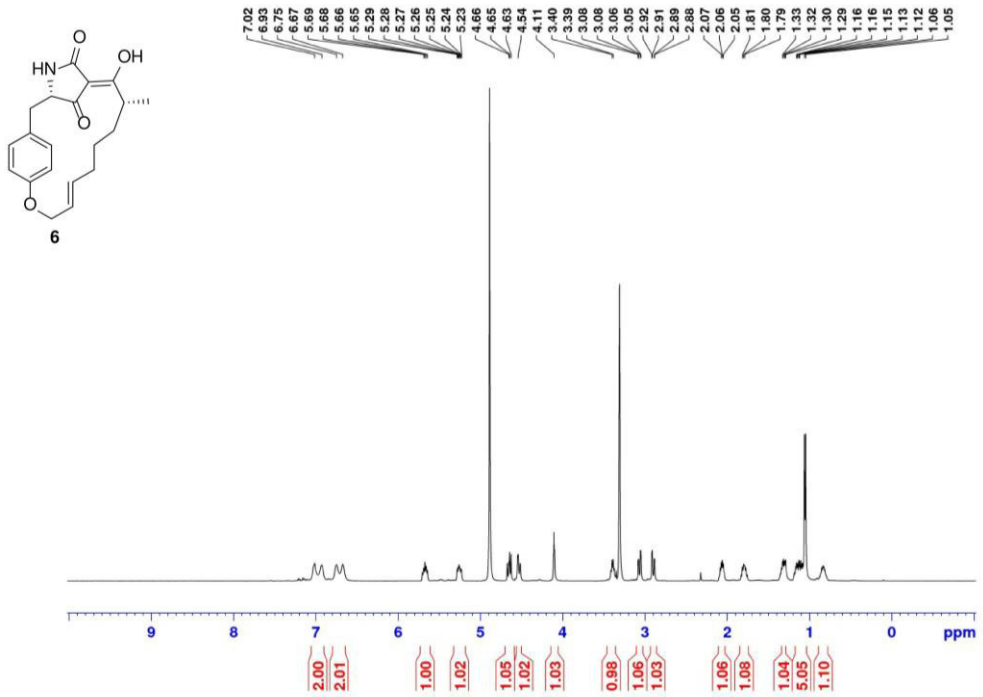

Figure S58: ^{1}H-NMR spectrum of macrocidin Z (**6**) in MeOD.

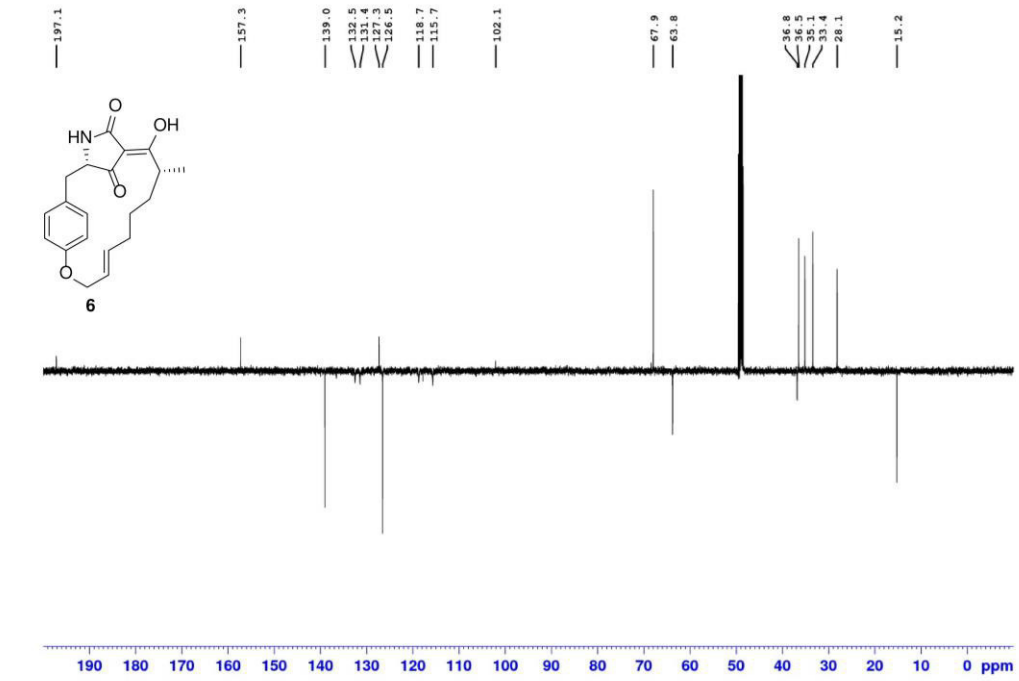

Figure S59: ^{13}C-NMR spectrum of macrocidin Z (**6**) in MeOD.

64

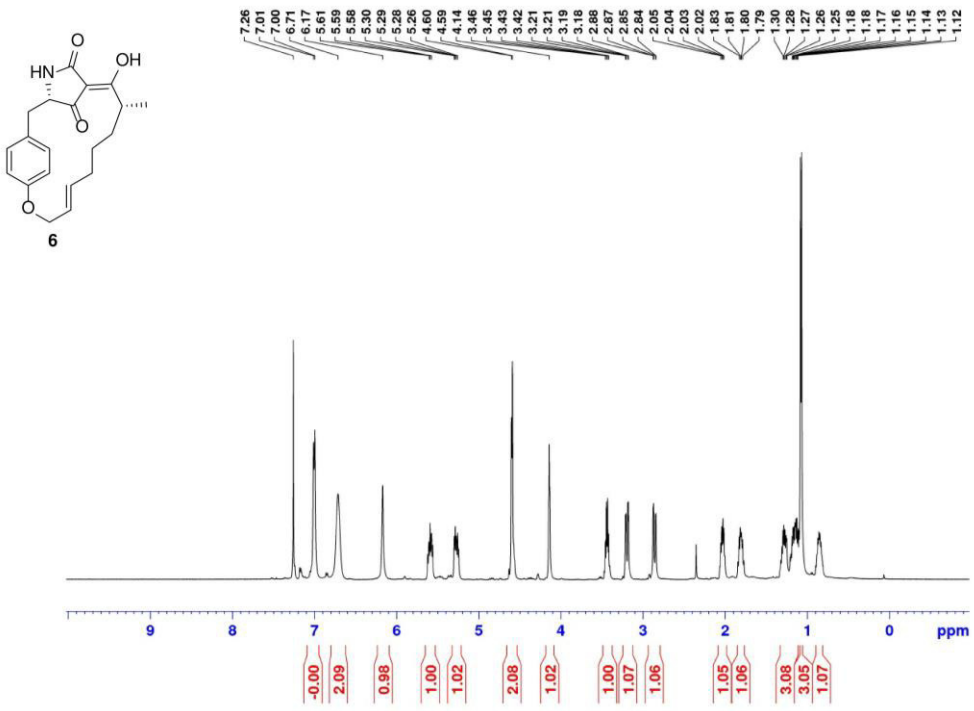

Figure S60: ^{1}H-NMR spectrum of macrocidin Z (**6**) in $CDCl_3$.

65

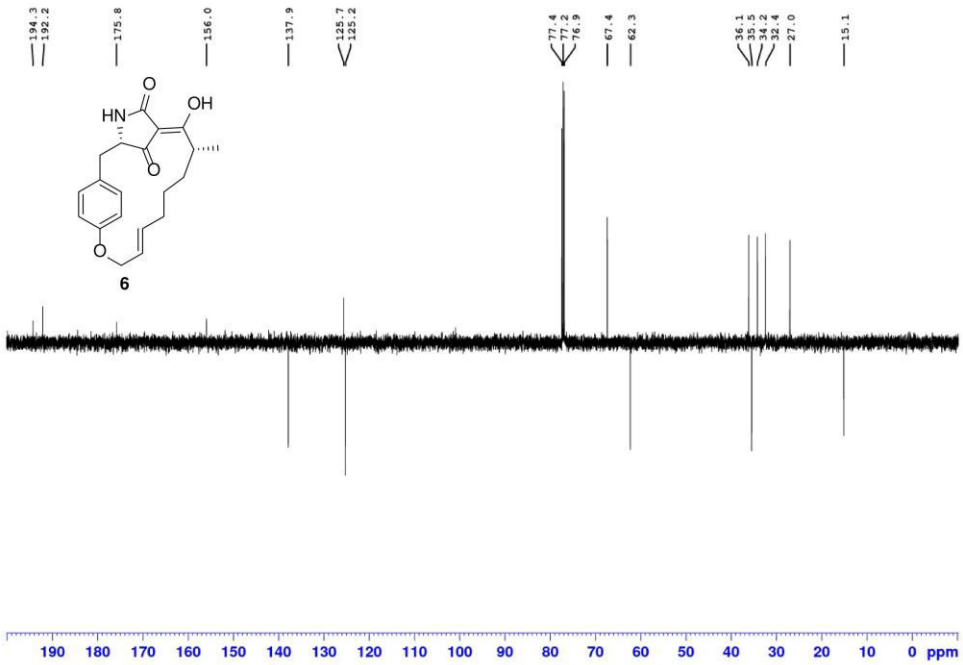

Figure S61: ^{13}C-NMR spectrum of macrocidin Z (**6**) in $CDCl_3$.

Antimicrobial assay

The assay was conducted as a minimum inhibitory concentration (MIC) assay in 96-well roundbottom microtiter plates using the parameters summarized in Table S3 and as already described in [S1].

Stocks of the test organisms were generated by growing the organisms overnight in 50 mL shaking flasks filled with 25 mL of the growth medium at 140 rpm (for media and temperatures see Table S3). If the organisms were well grown the next day, which was checked by occurrence of an optical density (OD)>30 of the suspension ($OD_{600\ nm}$ for bacteria, $OD_{548\ nm}$ for fungi and *M. smegmatis*), aliquots of these were stored in 1.5 mL reaction tubes in a freezer at −80 °C for up to 12 months. Upon use, aliquots were unthawed and the OD of the suspension measured and adjusted by diluting with the respective growth medium. $OD_{600\ nm}$ was adjusted to 0.01 and $OD_{548\ nm}$ to 0.1.

Subsequently, 150 µL of the adjusted suspensions were added to all wells of a 96-well microtiter plate (one test organism per plate). In row A, additional 130 µL of suspensions plus 20 µL of the test compounds (1 mg/mL) and the controls (one compound/column) were added. The test compounds were dissolved in MeOH, MeOH was used as negative control, while different positive controls (references) were used for the test organisms (see Table S3). Then, starting from row A, 150 µL of the suspension were transferred to the next row, the contents thoroughly mixed, and 150 µL transferred to the following row. The remaining 150 µL after row H were discarded. This resulted in a serial dilution of the test compounds, ranging from 66.7 µg/mL in row A to 0.52 µg/mL in row H. The microtiter plates were then incubated overnight on a microplate shaker at 800 rpm at 30 or 37 °C (see Table S3) and were visually evaluated the next day. The MIC is defined as the lowest concentration were no growth of the test organism was observed. A lower MIC thus corresponds to a higher antimicrobial activity of the test compound.

Table S3: MIC assay experiment parameters

Test organisms	Strain No.	Growth medium	Incubation temp. [°C]	Positive controls (references)
Bacillus subtilis	DSM10	MHB [1]	30	oxytetracyclin 1.0 mg/mL
Staphylococcus aureus	DSM346	MHB [1]	30	oxytetracyclin 1.0 mg/mL
Micrococcus luteus	DSM1790	MHB [1]	30	oxytetracyclin 1.0 mg/mL
Chromobacterium violaceum	DSM30191	MHB [1]	30	oxytetracyclin 1.0 mg/mL
Escherichia coli	DSM1116	MHB [1]	37	oxytetracyclin 1.0 mg/mL
Pseudomonas aeruginosa	PA14	MHB [1]	37	gentamicin 0.1 mg/mL
Mycolicibacterium smegmatis	ATCC700084	7H9+ADC [2]	37	kanamycin 0.1 mg/mL
Candida albicans	DSM1665	MYC [3]	30	nystatin 1.0 mg/mL
Schizosaccharomyces pombe	DSM70572	MYC [3]	30	nystatin 1.0 mg/mL
Mucor hiemalis	DSM2656	MYC [3]	30	nystatin 1.0 mg/mL

Pichia anomala	DSM6766	MYC [3]	30	nystatin 1.0 mg/mL
Rhodotorula glutinis	DSM10134	MYC [3]	30	nystatin 1.0 mg/mL

[1] MHB: Müller-Hinton Broth (SN X927.1, Carl Roth GmbH, Karlsruhe, Germany); [2] 7H9+ADC: Middlebrook 7H9 Broth Base + Middlebrook ADC Growth Supplement (SN M0678+M0553, Merck, Darmstadt, Germany); [3] MYC: 1 % w/v, bacto peptone, 1% w/v yeast extract, 2 % w/v glycerol, pH 6.3

[S1]: Becker, K.; Wessel, A. C.; Luangsa-ard, J. J.; Stadler, M. Viridistratins A-C, antimicrobial and cytotoxic benzo[j]fluoranthenes from stromata of Annulohypoxylon viridistratum (Hypoxylaceae, Ascomycota). Biomolecules 2020, 10, 805.

Cytotoxicity assay

The assay was conducted in 96-well flat-bottom microtiter plates using the parameters summarized in Table S4 and as described in [S1, S2].

Cell lines L929 and KB 3.1 were incubated at 37 °C under 10 % CO_2 in Gibco™ DMEM medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10 % FBS. A microtiter plate was filled with 120 µL of this suspension (50,000/mL) in each well.

Separately, another microtiter plate was filled with 100 µL of growth medium in each well. Then, 50 µL of the test compound solutions (1 mg/mL) were given to wells of the first column in two replicates (one compound per row). Cells without additives, MeOH were used as negative control. Starting from the first column, 50 µL of the solutions were gradually transferred to the next column, the contents thoroughly mixed, and 50 µL transferred to the following column. This created a serial dilution of the test compounds ranging from 333 µg/mL to 1.9×10−3 µg/mL. The remaining 50 µL after column twelve were discarded. From this microtiter plate, 60 µL of the solutions from 111 µg/mL to 1.9×10−3µg/mL were given to the first plate containing 120 µL of the cell suspensions (i.e. the highest concentration 333 µg/mL was not used). This resulted in final compound concentrations ranging from 37 µg/mL to 0.6×10−3 µg/mL.

After 5 days of incubation under the aforementioned incubation conditions, the half maximum inhibitory concentrations (IC50) were determined using a colorimetric tetrazolium dye MTT assay [S3]. For this, 20 µL of a 5 mg/mL solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were added to each well and incubated for two hours at 37 °C. Then, the microtiter plate was centrifuged (3,000 rpm, 5 min) and the supernatant removed by holding the plate upside-down and gentle shaking. Afterwards, the wells were washed using 100 µL of phosphate buffered saline (PBS). The plate was again centrifuged and the supernatant removed as described before. Then, 100 µL of an isopropanol:HCl solution (1L isopropanol+4 mL HCl 37 % w/v) were added to the wells. After incubating for 10 min at ambient temperature, the absorption of the wells at 595 nm was measured with an Infinite® 200 Pro microplate reader (TECAN, Männedorf, Schweiz).

The absorption values of the cells without additives were averaged and set to 100 % cell viability. Then, the means of absorption of the two compound replicates were set in relation to the blank media. These percentage values were plotted against the concentration range (37 µg/mL to 0.6×10−3 µg/mL). The IC_{50} value was read from the plot (in µg/mL).

Table S4: Cytotoxicity assay experiment parameters

cell line	type	No.	growth medium
L929	mouse fibroblasts	ACC 2	DMEM [1] + 10 % FBS [2]
KB 3.1	Human endocervical adenocarcinoma (AC)	ACC 158	DMEM [1] + 10 % FBS [2]

[1] DMEM: Dulbecco's Modified Eagle Medium (SN 61965026, Thermo Fisher Scientific, Waltham, MA, USA); [2] FBS: Fetal Bovine Serum (SN 10500064, Thermo Fisher Scientific)

[S2]: Sandargo, B.; Michehl, M.; Praditya, D.; Steinmann, E.; Stadler, M.; Surup, F. Antiviral meroterpenoid rhodatin and sesquiterpenoids rhodocoranes A–E from the Wrinkled Peach Mushroom, *Rhodotus palmatus*. *Organic Letters* **2019**, 21, 3286-3289.

Table S5: *S. aureus* biofilm and preformed biofilm inhibition activity of compounds **1-3**, **5-6**

Compounds	Inhibition of biofilm formation (%)	Inhibition of preformed biofilm (%)
1	-	-
2	65 (250 µg/mL) ± 5 43 (125 µg/mL) ± 9	36 (250 µg/mL) 31 (125 µg/mL)
3	75 (250 µg/mL) ± 3 59 (125 µg/mL) ± 9	57 (250 µg/mL) ± 3 48 (125 µg/mL) ± 15
4	n.t	n.t
5	79 (250 µg/mL) ± 2 77 (62.5 µg/mL) ± 2 61 (15.6 µg/mL) ± 15	75 (250 µg/mL) ± 4 65 (62.5 µg/mL) ± 12 31 (15.6 µg/mL) ± 13

6	76 (250 µg/mL) ± 9 70 (62.5 µg/mL) ± 11 19 (15.6 µg/mL) ± 8	73 (250 µg/mL) ± 7 59 (62.5 µg/mL) ± 10 40 (15.6 µg/mL) ± 15
Microporenic acid A	83 (250 µg/mL) ± 5 81 (62.5 µg/mL) ± 6 48 (15.6 µg/mL) ± 3	71 (250 µg/mL) ± 8 70 (62.5 µg/mL) ± 9 39 (15.6 µg/mL) ± 13

Data are expressed as mean ± SD.

5.3 Publikation II

Dual agents: fungal macrocidins and synthetic analogues with herbicidal and antibiofilm activities

Laura Treiber [1], Christine Pezolt [1], Haoxuan Zeng [2,3], Hedda Schrey [2,3], Stefan Jungwirth [4], Aditya Shekhar [4], Marc Stadler [2,3], Ursula Bilitewski [4], Maike Erb-Brinkmann [5] and Rainer Schobert [1]

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antibiotics

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Article

Dual Agents: Fungal Macrocidins and Synthetic Analogues with Herbicidal and Antibiofilm Activities

Laura Treiber [1], Christine Pezolt [1], Haoxuan Zeng [2,3], Hedda Schrey [2,3], Stefan Jungwirth [4], Aditya Shekhar [4], Marc Stadler [2,3], Ursula Bilitewski [4], Maike Erb-Brinkmann [5] and Rainer Schobert [1,*]

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Abstract: Eight analogues of the bioherbicides macrocidin A (**1**) and Z (**2**) with structural variance in the size of the macrocycle, its *para*- or *meta*-cyclophane character, and its functional groups were synthesized on two modular routes and tested for herbicidal, antibiotic, and antibiofilm activities. Apart from the lead compounds **1** and **2**, the structurally simplified dihydromacrocidin Z (**3**) and normacrocidin Z (**4**) showed high herbicidal activity in either thistles, dandelions or in both. The derivatives **2**, **3**, and dibromide **9** also inhibited the growth of *Staphylococcus aureus* biofilms by ca 70% when applied at subtoxic concentrations as low as ca 20 µM, which are unlikely to induce bacterial resistance. They also led to the dispersion of preformed biofilms of *S. aureus*, exceeding a similar effect by microporenic acid A, a known biofilm inhibitor. Compounds **3** and **9** showed no noticeable cytotoxicity against human cancer and endothelial cells at concentrations below 50 µM, making them conceivable candidates for application as anti-biofilm agents in a medicinal context.

Keywords: macrocidin; polycyclic tetramate macrolactams; 3-acyltetramic acids; antibiotics; biofilms

Citation: Treiber, L.; Pezolt, C.; Zeng, H.; Schrey, H.; Jungwirth, S.; Shekhar, A.; Stadler, M.; Bilitewski, U.; Erb-Brinkmann, M.; Schobert, R. Dual Agents: Fungal Macrocidins and Synthetic Analogues with Herbicidal and Antibiofilm Activities. *Antibiotics* **2021**, *10*, 1022. https://doi.org/10.3390/antibiotics10081022

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1. Introduction

Macrocidins are polycyclic tetramic acid macrolactams (PTMs). Macrocidin A (**1**; Figure 1) was first isolated from the fungus *Phoma macrostoma* Montagne in 2003 by a Dow AgroSciences group headed by Graupner [1]. It was found to induce chlorosis in broadleaf weeds by a unique mode of action implying an interference with the phytoene synthase and desaturase in the chlorophyll and carotenoid biosynthesis [2]. So far, only two total syntheses of macrocidin A (**1**) have been published [3,4]. Macrocidin Z (**2**), which carries an *E*-alkene in lieu of the expoxide, was isolated from *Phoma macrostoma* cultures and synthesised in parallel by us only recently [5]. Although concentrated *Phoma macrostoma* cultures, formulated as broadcast granules, are being used as bioherbicides for environment-friendly weed management in the US and Canada, efforts towards the synthesis of simplified macrocidin derivatives with improved herbicidal properties were sporadically made (e.g., by Graupner's group [6] and Syngenta [7]), albeit without disclosing details. As 3-acyltetramic acids from a broad range of sources were found to have antibiotic or biofilm inhibitory effects [8–12] (e.g., by us in the case of macrocidin Z (**2**) [5]), we now synthesised analogues of the natural macrocidins with variation of the structural key features such as the size of the macrocycle, its *para*- or *meta*-cyclophane character, and its decoration with functional

groups other than epoxide. We tested them for herbicidal, antibacterial, and antibiofilm activities, and for cytotoxicity to human cells.

2. Results

2.1. Chemistry

Figure 1 depicts eight new derivatives **3**–**10** that were prepared on two efficient modular routes. They share the same tetramic acid derived from L-tyrosine, yet differ in their degree of resemblance to the lead macrocidins (**1**) and (**2**). The dihydro derivate **3** of macrocidin Z is the only one retaining the methyl group at an *R*-configured stereocenter. Like macrocidin Z (**2**), the derivatives **4** and **5** feature *E*-alkenes in lieu of the epoxide. Normacrocidin A (**6**) lacks the methyl group while retaining the epoxide, albeit with a non-natural *R*,*R* configuration. In compound **7**, the epoxide is replaced by a vicinal diol, in derivative **8** by a bromohydrin, and in **9** by a vicinal dibromide. Furthest from the natural leads **1** and **2** (in terms of structure) is derivative **10,** which has a 13-membered instead of a 17-membered macrocycle comprising a *meta*- rather than a *para*-cyclophane.

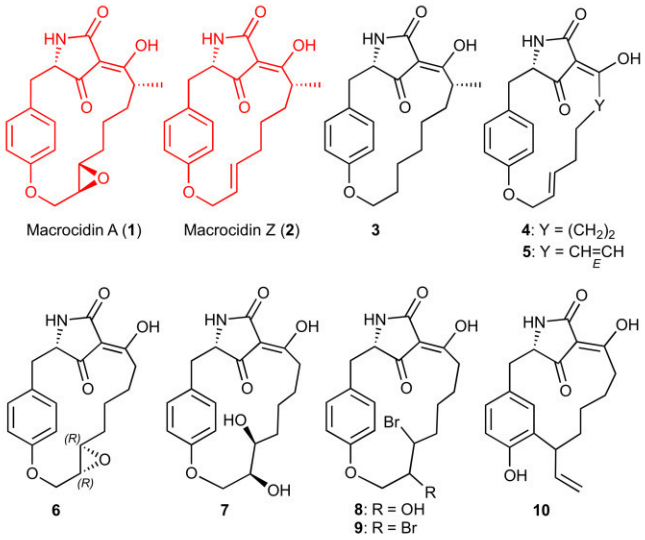
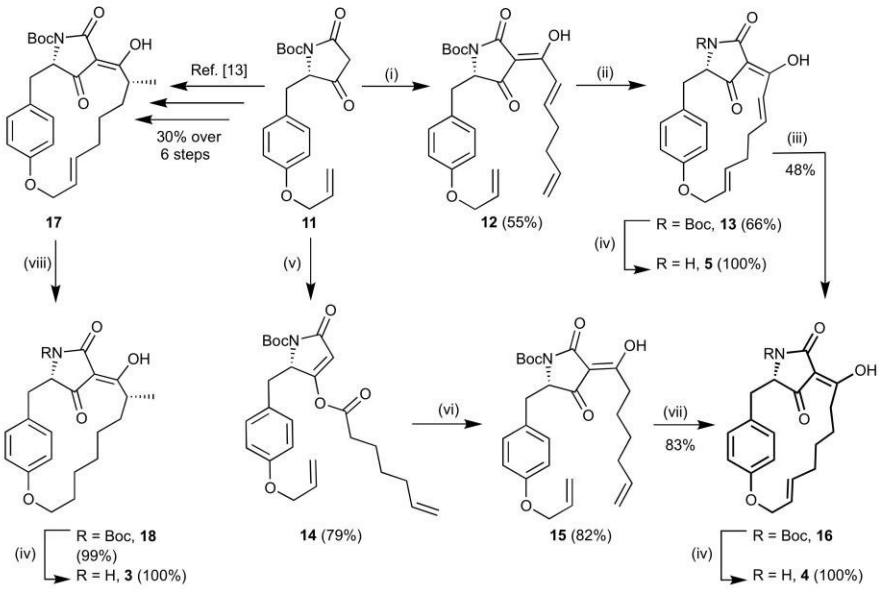


Figure 1. Structures of the natural lead compounds macrocidin A (**1**) and macrocidin Z (**2**) and structural variants **3**–**10**.

In Scheme 1, two alternative synthetic approaches to key intermediate **16**, an *N*-Boc-protected normacrocidin Z, are illustrated. The known L-tyrosine derived tetramic acid **11** [4] was furnished with a 3-(hepta-2,6-dienoyl) residue by a one-pot reaction first with ketenylidentriphenylphosphorane, Ph_3PCCO, to give the corresponding 3-acyl ylide (not shown), followed by a Wittig olefination of the latter with 4-pentenal to leave 3-acyltetramic acid **12** in 55% yield [13]. An *E*-selective ring-closing metathesis reaction with a Grubbs type-II catalyst gave macrocycle **13** in 66% yield as a single stereoisomer. Its reduction with Wilkinson's catalyst and triethylsilane to afford a silyl enol ether (not shown), followed by its cleavage with KF, gave *N*-Boc-protected normacrocidin Z **16** in a 48% yield. Removal of the *N*-Boc-protecting group from compounds **16** and **13** using TFA left test candidates **4** and **5** as pure stereoisomers. For the synthesis of larger quantities of **16**, another route was developed also starting from tetramic acid **11** in analogy to our syntheses of macrocidins A and Z [4,5]. 3-Acyltetramic acid **15** was built up by 4-*O*-acylation of **11** with 6-heptenoic acid and subsequent rearrangement of tetramate **14**. Ring-closing metathesis of **15** with Grubbs type-II catalyst afforded key intermediate **16** in 54% total yield over three steps. Enantiopure dihydromacrocidin Z (**3**) was obtained from *N*-Boc-protected macrocidin Z **17**

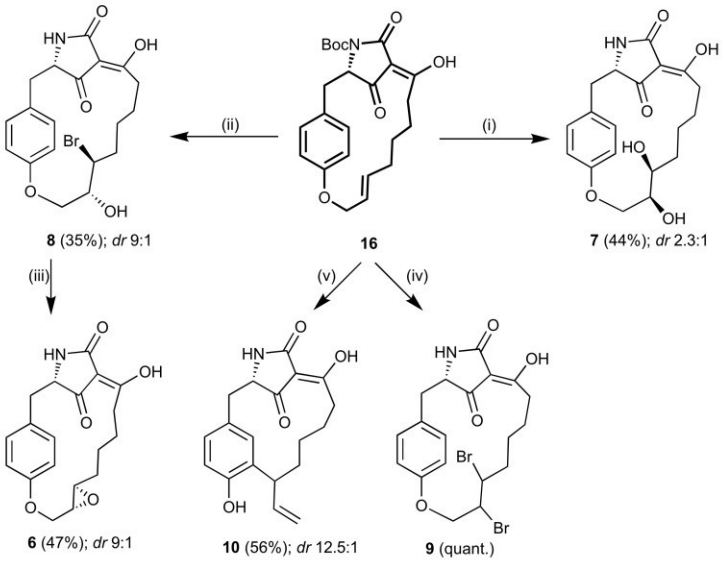
by hydrogenation and removal of the *N*-Boc-protecting group from **18** in 99% yield over two steps [5].



Scheme 1. Syntheses of key intermediate **16** and of macrocidin derivatives **3**, **4**, and **5**. Reagents and conditions: (i) Ph_3PCCO, THF, reflux, 2 h; then KO*t*Bu, THF, reflux, 20 min; then 4-pentenal, THF, reflux → rt, 21 h; (ii) Grubbs II catalyst, CH_2Cl_2, reflux, 18 h; (iii) $Rh(PPh_3)_3Cl$, Et_3SiH, CH_2Cl_2, reflux, 19 h; then KF, MeOH, −15 °C, 27 h; (iv) TFA, CH_2Cl_2, rt, 15 min; (v) EDC HCl, DMAP, 6-heptenoic acid, CH_2Cl_2, 0 °C → rt, 4 h; (vi) NEt_3, DMAP, CH_2Cl_2, rt, 24 h; (vii) Grubbs II catalyst, CH_2Cl_2, reflux, 24 h; (viii) H_2 (1 atm), Pd/C, EtOAc, rt, 31 h.

Key intermediate **16** was also used to introduce further functionalizations, formally replacing the epoxide in macrocidin A (**1**). Its hydroxylation with AD-mix α afforded, after deprotection, diol **7** as an inseparable 2.3:1 mixture of two diastereomers (Scheme 2 shows major diastereomer). Alkene **16** was also converted to an inseparable mixture of two diastereomeric bromohydrins **8** with NBS and H_2O in DMSO [14,15]. A side product (16%) of this reaction, carrying an additional bromo residue next to the enol group, could be separated. Upon treatment with KO*t*Bu, the bromohydrins **8** were converted in 47% yield to a 9:1 mixture of expoxides **6** as indicated by ^{1}H NMR spectra. The configuration of the major isomer of **6** (shown in Scheme 2) was assigned by comparison with a mixture of isomers **6** obtained on a different route. Hence, we assume that the *dr* of precursor bromohydrins **8** was also 9:1 as for the epoxides **6**. After futile attempts by Ramana et al. [16] and our group at direct epoxidation of alkenes such as **4**, **16**, or **17**, this was the first time the epoxide function could be installed in the preformed macrocycle of a macrocidin precursor. Alkene **16** could also be brominated with bromine in CCl_4 to give vicinal dibromide **9** as a mixture of two diastereomers with *trans*-positioned bromo residues as to NMR spectra. Finally, when heated in diethylaniline in a sealed tube, the *para*-cyclophane **16** underwent a Claisen rearrangement to afford *meta*-cyclophane **10** in 56% yield and as a 12.5:1 mixture of diastereomers according to NMR spectra. Like the stereopure derivatives **3**–**5**, the diastereomeric mixtures of derivatives **6**–**10** were tested for bioactivity, although in the case of diol **7** the two diastereomers, present in similar proportions, might dilute or cancel each other out in terms of biological activities. It should be noted, that most derivatives shown in Schemes 1 and 2 were difficult to purify and analyze. Sometimes, only multiple reversed-phase column chromatography runs led to

the desired degree of purity. Their NMR spectra (*cf.* Supporting Information, SI) are rather complex due to the tautomerization of the 3-acyltetramic acid moiety [17].



Scheme 2. Modular synthesis of macrocidin derivatives **6**–**10** starting from key intermediate **16**. Reagents and conditions: (i) AD-mix α, *t*BuOH/H_2O, 7 °C, 9 d; then TFA, CH_2Cl_2, rt, 15 min; (ii) NBS, H_2O, DMSO, 8 °C → rt, 22 h; then TFA, CH_2Cl_2, rt, 15 min; (iii) KO*t*Bu, THF, 0 °C → rt, 4 d; (iv) Br_2, CCl_4, 80 °C, 30 h; (v) diethylaniline, sealed tube, 190 °C, 42 h.

2.2. Herbicidal Activity

Prior to screening them for antimicrobial effects, the new derivatives were tested for herbicidal activity against thistles and dandelions which had been found to be susceptible to the chlorosis-inducing natural macrocidins [2,6]. For both species, they were applied as max. 150 mM solutions to four pots with two plants each, and their bleaching, withering, and necrotizing effects were assessed after two and then after three to six weeks. None of the compounds reached the efficiency of the synthetic commercial herbicide diflufenican, which was used as a positive control. In line with literature, the lead compound macrocidin A (**1**) exhibited the highest maximum herbicidal efficiency of all tested compounds, causing 88% mortality of dandelions and 100% of thistles, three weeks after application of a 100 mM solution in a mixture of isopropanol/water = 1:1 + 0.25% Tween 20 (Figure 2). Interestingly, the epoxide appeared not to be crucial for herbicidal activity, since macrocidin Z (**2**) still displayed a high efficiency of 88% mortality in thistles and of 50% in dandelions after 42 days at 100 mM. Contrary to an earlier assumption by Graupner, Bailey et al. [6], even derivatives with saturated backbones may show herbicidal efficiency, e.g., dihydromacrocidin Z (**3**) (38% mortality in thistles and dandelions). The α-methyl group seemed to be important, apparent from the lower figures for normacrocidin Z (**4**) when compared to **2** (dandelions: 0%, thistles: 63% mortality, 35 days after treatment with 150 mM) and for *S*,*R*,*R*-normacrocidin A (**6**) which was virtually inactive against both plants. The 13-membered macrocyclic *meta*-cyclophane **10** exerted a maximum herbicidal efficiency with 38% mortality in thistles, yet only 13% in dandelions after four weeks at 150 mM. Normacrocidin Z (**4**) and diol **7** also displayed a distinct specificity for thistles over dandelions (for pictures of treated plants *cf.* Figure S68 in the SI).

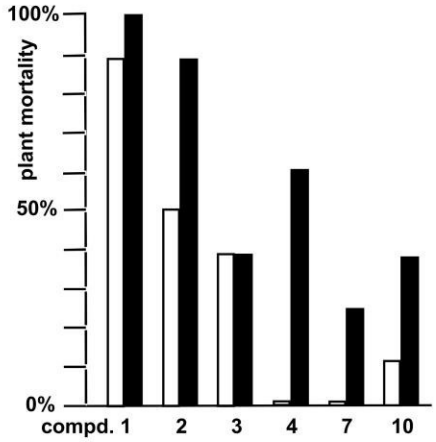


Figure 2. Percentage of final mortality of dandelions (white columns) and thistles (black columns) treated with 0.2 mL/plant of 100–150 mM solutions of active macrocidin derivatives after two to six weeks. Mortality by diflufenican was 100% for either plant species.

2.3. Antimicrobial Activity

As 3-acyltetramic acids were frequently shown to have antimicrobial effects [8,9,12,18–20], the new macrocidin derivatives were tested for activity against three different bacteria, namely the Gram-positive strain *Staphylococcus aureus* (SH1000) and the Gram-negative strains *Acinetobacter baumannii*, *Escherichia coli* with the wild-type strain K12 and the ΔTolC mutant (JW5503), which lacks the AcrAB−TolC efflux system. None of the macrocidin analogues displayed activity against the wild-type strain of *E. coli*. Weak activities against *E. coli* ΔTolC were found only for dibromide **9** (IC_{50} = 75 ± 15 μM), dihydromacrocidin Z (**3**) (IC_{50} = 82 ± 15 μM), and macrocidin Z (**2**) (IC_{50} ca 100 μM). These derivatives were also similarly active against *S. aureus* (*cf.* Table S1 for IC_{50} values and Figures S69–S70 for growth curves in the SI). In comparison, the clinically established antibiotic vancomycin was active with an IC_{50} of ca 12 μM against *S. aureus*, and the antibiotic erythromycin with a nanomolar IC_{50} value. None of the macrocidin derivatives showed a clear antibiotic effect on *A. baumannii*. Even when applied at the highest concentration of 100 μM, the compounds could not prevent the cultures from reaching an OD_{600} of at least 60–70% of the maximum value (*cf.* Figure S71 in the SI). Overall, the toxicity of macrocidin Z (**2**) and its new synthetic analogues **3**–**10** against bacteria is weak.

2.4. Antibiofilm Activity

The macrocidinoids **2**–**10** were tested for inhibitory effects on the formation of biofilms by *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria, as well as for dispersive effects on preformed biofilms of *S. aureus* and the fungal species *Candida albicans* (*cf.* SI for data Table S2). While all compounds **2**–**6** and **8**–**10** inhibited the formation of *S. aureus* biofilms by at least 75% relative to untreated controls (=0%) at the highest tested concentration of 250 μg/mL, only macrocidin Z (**2**), its dihydro derivative **3** and dibromide **9** caused a distinct biofilm inhibition of ca 70% when applied at subtoxic concentrations as low as 7.8 μg/mL (corresponding to 16 μM and 23 μM, respectively). These activities matched or even exceeded that of microporenic acid A (MAA), the known biofilm inhibitor [21] used as a positive control (Figure 3A). A second group of moderately active inhibitors, comprising normacrocidin Z (**4**), diene **5** and phenol **10**, led to an inhibition of biofilm formation of more than 30% when applied at a concentration of 15.6 μg/mL (corresponding to 43–48 μM). The derivatives **7** and **8** had little inhibitory effect at concentrations below 250 μg/mL.

The dispersive effects on preformed biofilms of *S. aureus* were generally slightly less pronounced (Figure 3B). Derivatives **6**–**8** were inactive at all concentrations up to

250 µg/mL. The most distinct effects of at least 35% dispersion over the concentration range from 250 µg/mL down to 15.6 µg/mL were again observed for derivatives **2**, **3,** and **9**, which clearly outperformed MAA. The latter was active only at the highest two concentrations, such as the compounds **4**, **5,** and **10**. In the case of *C. albicans*, compounds **7** and **10** proved inactive, whereas the other compounds, including MAA, showed weak dispersive effects at very high concentrations (250–125 µg/mL) with derivate **5** being the only one active at a lower concentration of 62.5 µg/mL (192 µM) (*cf.* Figure S72 in the SI). None of the tested derivatives inhibited the formation of *P. aeruginosa* biofilms.

This study demonstrated that macrocidin analogues may interfere with the formation and persistence of bacterial and fungal biofilms, depending on their structure and polarity. The strongest effects against *S. aureus* were found for the lipophilic and structurewise "simple" derivatives **2**–**5** and **9**. Interestingly, the activity against biofilms decreased, or even disappeared, when hydroxy groups were introduced into the molecule, as the derivatives **7** and **8** exemplify.

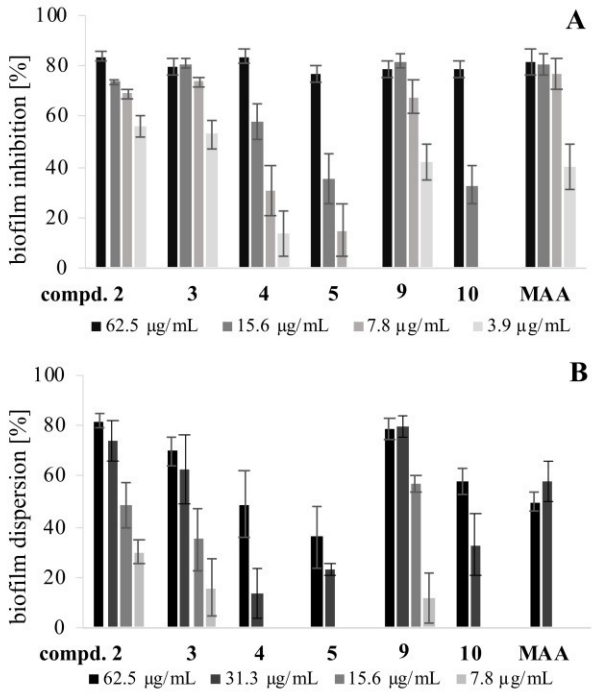


Figure 3. Effects of various concentrations of compounds **2**–**5**, **9**, and **10** on the formation of (**A**) and the dispersion of preformed (**B**) biofilms of *S. aureus*; positive control: microporenic acid A (MAA); error bars indicate SD of two repeats with duplicates.

2.5. Cytotoxicity

The macrocidinoids **2**, **3**, and **6**–**10** were submitted to provisional MTT tests for cytotoxicity/antiproliferative effects against human 518A2 melanoma cells, colon carcinoma cells HCT-116^{wt} and HCT-116$^{p53-/-}$, and KBV cervix carcinoma cells as well as hybrid endothelial EaHy cells. Gratifyingly, none of the compounds but **2** caused signs of toxicity or inhibition of proliferation in the tested cells when applied at concentrations as high as 50 µM (*cf.* Table S3 in the SI). This is far outside of any clinically relevant range and would bode well for a potential future application as biofilm interfering agents in a medicinal context. Even macrocidin Z (**2**) which was antiproliferative with IC_{50} concentrations of ca. 15 to 30 µM in cells of colon carcinoma HCT-116 and cervical carcinoma KBV warrants a more in-depth study of its applicability as an anti-biofilm agent.

3. Discussion

We synthesised eight derivatives of the natural tetramic acids macrocidin A (**1**) and Z (**2**) on two efficient modular routes, which allow the introduction of various functionalities and scaffold modifications on a few key intermediates. The double bonds of intermediates **13** and **16** were converted in good yields to bromides, diols, bromohydrins and saturated bonds using standard reactions. A thermal Claisen rearrangement opened an easy access to a 13-membered macrocycle featuring a *meta*-cyclophane motif. For the first time, we could introduce the epoxide in a macrocidin precursor with a preformed macrocycle. The derivatives and the natural lead compound macrocidin Z (**2**) were tested for herbicidal and antimicrobial activity, as well as for biofilm interference. The incentive for this extended bioscreening were the frequent reports on the high incidence of antimicrobial and antifungal effects by 3-acyltetramic acids in general. Interestingly, the structurally simple compounds macrocidin Z (**2**) and dihydromacrocidin Z (**3**) showed a high herbicidal *and* antibiofilm activity. Normacrocidin Z (**4**) was selectively herbicidal against thistles, and dibromide **9** displayed an *S. aureus* biofilm dispersing effect surpassing even that of the known biofilm inhibitor microporenic acid A. With the exception of diol **7**, which was moderately herbicidal against thistles, hydroxy groups on the alkyl backbone of the macrocycle, appear to be generally detrimental to both herbicidal and antibiofilm effects. Contrary to suppositions in the scant literature on macrocidinoids, the epoxide function is obviously not crucial to either activity. The observed distinct and strain-specific effects of the active macrocidinoids **2**, **3**, and **9** on the biofilms of *S. aureus* are all the more interesting, as their direct antibacterial activities (i.e., toxicities against bacteria) are rather limited. Thus, their application as biofilm inhibitors would probably not induce bacterial resistance. The at best marginal cytotoxicities of compounds **3** and **9** in human cancer and endothelial cells indicate that these compounds would presumably be well tolerated also by higher organisms. Compound **2** might indeed pose a toxicity problem which should be clarified prior to further tests as a biofilm inhibitor.

4. Materials and Methods

4.1. General Information

IR spectra were recorded with a PerkinElmer Spectrum 100 FT-IR spectrophotometer (PerkinElmer, Rodgau, Germany) with ATR sampling unit. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 polarimeter (PerkinElmer, Rodgau, Germany); $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode (ThermoFisher Scientific, Bremen, Germany). NMR spectra were recorded with a Bruker Avance III HD 500 spectrometer (^{1}H NMR: 500 MHz and ^{13}C NMR: 125 MHz) (Bruker, Karlsruhe, Germany). Chemical shifts are given in parts per million, relative to the residual solvent peak as an internal standard, and coupling constants (*J*) are quoted in Hz. Most tetramic acids were measured in $CDCl_3$ and also in CD_3OD where they usually exist as a single (enol) tautomer. Quaternary C-atoms of tetramic acids were sometimes difficult to spot in JMOD or ^{13}C NMR spectra. For these, more signals cropped up in HMBC and/or HSQC correlation spectra and were considered for peak assignment. In $CDCl_3$ solution, signals of virtually all C-atoms of tetramic acids were visible yet split up in multiple, difficult to assign sets for individual tautomers both in ^{1}H and JMOD/^{13}C NMR spectra. In line with literature, we assume the tautomers with exocyclic C–C double bond as drawn for the 3-acyltetramic acids in Figure 1, to be the major tautomer [17]. For the purification of synthetic products, chromatography silica gel 60 (40–63 µm) or silica gel RP18 (40–63 µm) were used. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} pre-coated aluminum-backed plates. Analytical HPLC was performed on a Shimadzu Nexera XR (Shimadzu GmbH, Duisburg, Germany) using a Knauer Eurospher II C18-column (150 × 4 mm) (Knauer GmbH, Berlin, Germany). Chiral HPLC was performed on a Beckmann System Gold Programmable Solvent Modul 126 using a Phenomenex Lux® Amylose-1-HPLC-column (100 × 4.6 mm)

(Phenomenex Ltd., Aschaffenburg, Germany). All air- and water-sensitive reactions were carried out under a dry argon atmosphere.

4.2. Compounds

(*S*,*Z*)-tert-Butyl-2-(4-(allyloxy)benzyl)-4-((*E*)-1-hydroxyhepta-2,6-dien-1-ylidene)-3,5-dioxopyrrolidine-1-carboxylate (12). Tetramic acid **11** [4] (1.90 g, 5.50 mmol, 1.10 eq) in dry THF (305 mL) was treated with ketenylidenetriphenylphosphorane (1.66 g, 5.50 mmol, 1.10 eq) in dry THF (140 mL) over 20 min while refluxing. After stirring for 2 h, KO*t*Bu (0.62 g, 5.50 mmol, 1.10 eq) was added. The solution was stirred for a further 20 min, before 4-pentenal (0.42 g, 5.00 mmol, 1.00 eq) in dry THF (65 mL) was added over a period of 15 min. Stirring at reflux was continued for 4 h and for 17 h at room temperature. The solvent was concentrated under reduced pressure and the crude product was dissolved in CH_2Cl_2 (300 mL). It was washed with sat. NH_4Cl solution (200 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL), the combined organic phases were washed with brine (300 mL) and dried over Na_2SO_4. Removal of the solvent and purification by column chromatography on reversed phase silica gel (RP18, 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 70% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 100% MeCN + 0.1% HCOOH) afforded 3-acyltetramic acid **12** (1.24 g, 2.74 mmol, 55%). R_f = 0.88 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$ −95.0° (c 1.00, MeOH); Major tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 7.29 (dt, *J* = 15.6, 6.9 Hz, 1H), 7.12 (d, *J* = 15.6 Hz, 1H), 6.90 (m, 2H), 6.76 (m, 2H), 6.01 (ddt, *J* = 17.3, 10.7, 5.3 Hz, 1H), 5.83 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H), 5.34 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.20 (dq, *J* = 10.7, 1.6 Hz, 1H), 5.08 (dq, *J* = 17.0, 1.4 Hz, 1H), 5.02 (dq, *J* = 10.3, 1.6 Hz, 1H), 4.53 (m, 1H), 4.47 (dt, *J* = 5.3, 1.6 Hz, 2H), 3.35 (dd, *J* = 14.0, 5.5 Hz, 1H), 3.20 (dd, *J* = 14.0, 2.6 Hz, 1H), 2.45 (q, *J* = 6.9 Hz, 2H), 2.27 (q, *J* = 6.9 Hz, 2H), 1.62 (s, 9H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 5.00 (m, 2H), 4.60 (m, 1H), 4.43 (m, 2H), 3.39 (m, 1H), 3.07 (m, 1H), 2.10 (m, 2H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 159.3, 153.9, 138.2, 134.9, 131.8, 127.6, 122.1, 117.4, 116.2, 115.6, 69.7, 35.9, 35.7, 33.7, 33.0, 28.4 ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.11 (m, 2H), 6.92 (m, 2H), 6.75 (m, 2H), 6.01 (ddt, *J* = 17.2, 10.5, 5.3 Hz, 1H), 5.78 (ddt, *J* = 17.0, 10.3, 6.5 Hz, 1H), 5.37 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.25 (dq, *J* = 10.5, 1.6 Hz, 1H), 5.11–4.91 (m, 2H), 4.46 (dt, *J* = 5.3, 1.6 Hz, 2H), 4.38 (m, 1H), 3.40–3.30 (m, 1H), 3.25 (m, 1H), 2.45 (q, *J* = 6.7 Hz, 2H), 2.27 (q, *J* = 6.7 Hz, 2H), 1.61 (s, 9H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.60 (m, 1H), 3.38 (m, 1H), 3.20 (m, 1H), 2.40 (m, 2H), 2.24 (m, 2H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.5, 176.3, 173.8, 157.7, 152.3, 149.0, 136.8, 133.3, 130.84, 126.5, 121.7, 117.8, 116.0, 114.7, 100.7, 84.1, 68.8, 65.7, 35.1, 32.7, 32.1, 28.2 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.0, 178.4, 164.6, 157.8, 153.2, 150.2, 130.8, 126.4, 121.4, 114.8, 102.9, 83.4, 63.2, 34.9, 32.8, 32.0, 28.3 ppm; IR ν_{max} 2981 (w), 2937 (w), 2367 (w), 1769 (w), 1712 (m), 1642 (m), 1610 (m), 1578 (m), 1511 (m), 1414 (w), 1396 (w), 1370 (m), 1349 (m), 1304 (s), 1248 (m), 1150 (m), 1028 (w), 996 (w) cm^{-1}; HRMS (ESI) *m*/*z* [M + Na]$^+$ calcd. for $C_{26}H_{31}NO_6Na^+$ 476.20436, found 476.20380.

(3*S*,6*Z*,8*E*,12*E*)-4-Aza-*N*-(tert-butoxycarbonyl)-7-hydroxy-15-oxa-5,21-dioxo-tricyclo-[14.2.2.1^{3,6}]henicosa-1(18),6,8,12,16(17),19-hexaene (13). 3-Acyltetramic acid **12** (207 mg, 456 µmol, 1.00 eq) in dry, degassed CH_2Cl_2 (90 mL) was treated with 2nd generation Grubbs catalyst (39 mg, 46 µmol, 10 mol%). The solution was stirred at reflux for 18 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on reversed phase silica gel (RP18, 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 70% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 100% MeCN + 0.1% HCOOH) to afford **13** as pale brown resin (128 mg, 301 µmol, 66%). R_f = 0.75 (5% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$ −19.6° (c 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 6.90 (dt, *J* = 15.6, 7.6 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.74–6.61 (m, 2H), 6.58 (d, *J* = 15.6 Hz, 1H), 5.54 (dt, *J* = 15.3, 7.6 Hz, 1H), 5.41 (dt, *J* = 15.3, 5.7 Hz, 1H), 4.63–4.48 (m, 3H), 3.29 (dd, *J* = 13.8, 3.2 Hz, 1H), 3.04 (dd, *J* = 13.8, 3.2 Hz, 1H), 2.52–2.26 (m, 4H), 1.63 (s, 9H)

ppm; Major tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 174.7 (HMBC correlation), 158.4, 153.1, 134.1, 131.1, 128.3, 126.6, 122.9, 118.0, 117.6, 115.3, 84.9, 67.7, 66.0, 37.0, 33.6, 31.9, 28.4 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 159.9, 132.3, 117.2, 114.9 ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95–6.48 (m, 6H), 5.50 (m, 1H), 5.35 (m, 1H), 4.62–4.39 (m, 3H), 3.27 (dd, *J* = 13.6, 3.6 Hz, 1H), 3.07 (m, 1H), 2.57–2.41 (m, 2H), 2.33–2.12 (m, 2H), 1.63 (s, 9H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.38 (dd, *J* = 13.6, 3.6 Hz, 1H) ppm; C2, C4 and C3 not observed; IR ν_{max} 2973 (w), 2931 (w), 2934 (m), 1764 (m), 1713 (m), 1644 (s), 1608 (w), 1579 (s), 1508 (m), 1394 (w), 1369 (m), 1350 (s), 1306 (s), 1274 (m), 1254 (m), 1222 (m), 1159 (s), 1141 (m), 1109 (w), 978 cm^{-1}; HRMS (ESI) m/z [M + Na]$^+$ calcd. for $C_{24}H_{27}NO_6Na^+$ 448.17306, found 448.17270.

(3*S*,6*Z*,8*E*,12*E*)-4-Aza-7-hydroxy-15-oxa-5,21-dioxo-tri-cyclo [14.2.2.1^{3,6}]henicosa-1 (18),6,8,12,16(17),19-hexaene (5). Tetramic acid **13** (245 mg, 576 µmol, 1.00 eq) in dry CH_2Cl_2 (11 mL) was treated with TFA (1.10 mL) and stirred for 15 min at room temperature. Toluene (75 mL) was added and the solvent was concentrated under reduced pressure. This was repeated once to yield **5** as a pale brown foam (187 mg, 576 µmol, quant.). R_f = 0.63 (10% MeOH in CH_2Cl_2 + 0.1% HCOOH); $[\alpha]_D^{20}$ +92.9° (c 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): Diastereotopic H-atoms indicated as a, b: δ 7.03–6.41 (m, 6H, OC*H*C=C*H*CH_2, CH_{Ar}), 5.57 (m, 1H, OCH_2HC=C*H*), 5.35 (m, 1H, OCH_2*H*C=CH), 4.60/4.48 (m, 2H, $ArOCH_2$), 4.05 (brs, 1H, CHN), 3.01[a] (dd, *J* = 13.6, 3.9 Hz, 1H), 2.85[b] (dd, *J* = 13.6, 2.0 Hz, 1H, ArCH), 2.53–2.13 (m, 4H, OCH_2HC=CH(CH_2)$_2$) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 172.8 (HNCO), 157.9 ($OC_{q,Ar}$), 149.8 (OCHC=*C*HCH_2), 132.8 (OCH_2HC=CH), 131.2 (CH_2CCH_{Ar}), 128.0 (OCH_2HC=*C*H), 126.5 ($CH_2C_{q,Ar}$), 123.2 (OCH*C*=CHCH_2), 117.6 ($OCCH_{Ar}$), 67.4 ($ArOCH_2$), 38.0 ($ArCH_2$), 33.5 (OCHC=*C*HCH_2), 32.5 (OCH_2HC=*C*HCH_2) ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.07–6.42 (m, 6H), 5.57–5.31 (m, 2H), 4.55 (m, 2H), 4.10 (m, 1H), 3.13 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.84 (m, 1H), 2.55–2.11 (m, 4H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.20 (m, 1H), 2.90 (m, 1H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.6, 176.1, 171.8, 156.5, 148.6, 133.5, 132.2, 130.0, 126.8, 125.0, 122.3, 117.0, 111.6, 100.9, 66.5, 62.2, 37.6, 32.6, 31.7 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.7, 172.7, 170.2, 157.1, 149.7, 131.6, 130.8, 127.1, 125.5, 122.0, 115.7, 115.4, 104.0, 67.5, 60.2, 38.1, 32.1, 30.4 ppm; IR ν_{max} 3303 (m), 2927 (w), 2934 (m), 2070 (w), 1643 (s), 1576 (s), 1507 (m), 1428 (m), 1369 (w), 1338 (w), 1254 (m), 1219 (m), 1177 (m), 1115 (m), 975 (s) cm^{-1}; HRMS (ESI) m/z [M + H$^+$] calcd. for $C_{19}H_{20}NO_4^+$ 326.13868, found 326.13785.

(3*S*,6*Z*,12*E*)-4-Aza-*N*-(tert-butoxycarbonyl)-7-hydroxy-15-oxa-5,21-dioxo-tricyclo-henicosa-1(18),6,12, 16(17),19-pentaene (16). Tetramic acid **13** (77.0 mg, 181 µmol, 1.00 eq) and Wilkinson's catalyst (17 mg, 18 µmol, 10 mol%) in dry CH_2Cl_2 (2.5 mL) were treated with Et_3SiH (144 µL, 905 µmol, 5.00 eq). The solution was stirred for 19 h under reflux and the solvent was removed under reduced pressure. The crude product was dissolved in dry MeOH (2.6 mL) and KF (26.3 mg, 453 µmol, 2.50 eq) was added. After stirring for 20 h at −15 °C, more KF (26.3 mg, 453 µmol, 2.50 eq) was added and stirring was continued for a further 7 h at −15 °C. Chilled H_2O (50 mL) and chilled brine (20 mL) were added. The aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic phases were washed with 0.5M H_2SO_4 (40 mL) and dried over Na_2SO_4. Removal of the solvent and purification by column chromatography on reversed phase silica gel (RP18, 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH) gave **16** as a colourless foam (37 mg, 86.6 µmol, 48%). R_f = 0.83 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$ +12.4° (c 1.00, MeOH); Major tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 6.90–6.60 (m, 4H), 5.53 (dt, *J* = 15.4, 7.9 Hz, 1H), 5.40 (dt, *J* = 15.4, 5.3 Hz, 1H), 4.64–4.49 (m, 3H), 3.41 (dd, *J* = 14.1, 4.5 Hz, 1H), 3.10 (dd, *J* = 14.1, 2.9 Hz, 1H), 2.36 (m, 2H), 2.09–1.90 (m, 2H), 1.64 (s, 9H), 1.30 (m, 2H), 1.08 (m, 2H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.29 (dd, *J* = 14.1, 4.5 Hz, 1H), 3.03 (dd, *J* = 14.1, 2.9 Hz, 1H), 2.91 (m, 2H) ppm; Major tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 158.0, 150.8, 135.8, 131.7, 127.1, 127.0, 117.9, 84.7, 68.0, 35.7, 33.6, 33.2,

29.6, 28.4, 28.0 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 158.3, 128.2, 118.5, 67.7, 37.0, 33.4, 29.8, 28.3, 27.7 ppm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02–6.42 (m, 4H), 5.56–5.26 (m, 2H), 4.69–4.37 (m, 3H), 3.49–3.34 (dd, *J* = 14.0, 4.9 Hz, 1H), 3.29 (m, 1H), 3.15–3.03 (m, 1H), 2.48–1.86 (m, 3H), 1.63 (s, 9H), 1.47–0.99 (m, 4H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.4, 196.8, 164.3, 156.6, 149.8, 134.5, 131.9, 125.8, 125.70, 118.4, 102.2, 83.5, 67.6, 62.1, 35.0, 32.7, 32.22, 28.7, 28.3, 26.9 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 192.0, 191.8, 156.1, 136.6, 130.0, 125.75, 114.0, 105.5, 84.4, 67.0, 65.8, 34.7, 34.5, 32.24, 28.4, 27.6, 26.7, 26.0 ppm; IR ν_{max} 2978 (w), 2935 (w), 2863 (w), 1778 (m), 1744 (m), 1712 (s), 1662 (m), 1607 (s), 1509 (s), 1475 (w), 1456 (w), 1440 (m), 1423 (w), 1395 (w), 1366 (m), 1350 (s), 1305 (s), 1272 (m), 1258 (s), 1217 (s), 1150 (s), 1111 (m), 1081 (w), 1017 (w), 971 (m) cm^{-1}; HRMS (ESI) m/z [M + Na$^+$] calcd. for $C_{24}H_{29}NO_6Na^+$ 450.18871, found 450.18776.

(*S*)-tert-Butyl-2-(4-(allyloxy)benzyl)-3-(hept-6-enoyloxy)-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate (14). 6-Heptenoic acid (2.11 mL, 15.6 mmol, 1.00 eq) in dry CH_2Cl_2 (78 mL) was treated with EDC·HCl (3.59 g, 18.7 mmol, 1.20 eq) and DMAP (0.38 g, 3.12 mmol, 0.20 eq) at 0 °C. The solution was stirred for 20 min, before tetramic acid **11** (5.93 g, 17.2 mmol, 1.1 eq) was added at room temperature. After stirring for 4 h, the reaction was quenched with 0.5M H_2SO_4 (250 mL). The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 150 mL). The combined organic phases were washed with brine (200 mL) and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel 60, 10% EtOAc in hexanes → 15% EtOAc in hexanes → 20% EtOAc in hexanes → 25% EtOAc in hexanes) to obtain **14** as an orange resin (5.64 g, 12.4 mmol, 79%). R_f = 0.48 (30% EtOAc in hexanes); $[\alpha]_D^{20}$ +107.5° (c 1.00, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93–6.83 (m, 2H), 6.81–6.71 (m, 2H), 6.03 (m, 1H), 5.88 (s, 1H), 5.79 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H), 5.40 (m, 1H), 5.28 (m, 1H), 5.06–4.94 (m, 2H), 4.77 (dd, *J* = 6.0, 2.8 Hz, 1H), 4.48 (m, 2H), 3.29 (dd, *J* = 14.3, 6.2 Hz, 1H), 3.14 (dd, *J* = 14.3, 2.8 Hz, 1H), 2.48 (td, *J* = 7.4, 1.9 Hz, 2H), 2.09 (m, 2H), 1.74–1.65 (m, 2H), 1.60 (s, 9H), 1.46 (qn, *J* = 7.7 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 168.2, 165.3, 157.9, 149.5, 138.0, 133.3, 130.5, 126.2, 117.9, 115.3, 114.8, 108.3, 83.3, 68.9, 60.7, 35.0, 34.3, 33.4, 28.4, 28.2, 23.9 ppm; IR ν_{max} 3075 (w), 2975 (w), 2939 (w), 2863 (w), 1777 (s), 1744 (s), 1712 (s), 1633 (m), 1611 (w), 1582 (w), 1514 (s), 1478 (w), 1457 (w), 1424 (w), 1392 (w), 1370 (m), 1356 (m), 1320 (s), 1248 (s), 1226 (m), 1172 (s), 1158 (s), 1115 (m), 1064 (s), 1032 (m), 996 (m) cm^{-1}; HRMS (ESI) m/z [M + Na$^+$] calcd. for $C_{26}H_{33}O_6NNa^+$ 478.22001, found 478.21968.

(*S*,*Z*)-tert-Butyl-2-(4-(allyloxy)benzyl)-4-(1-hydroxyhept-6-en-1-ylidene)-3,5-dioxopyrrolidine-1-carboxylate (15). 4-*O*-acyltetramic acid **14** (5.54 g, 12.2 mmol, 1.0 eq) in dry CH_2Cl_2 (122 mL) was treated with dry NEt_3 (2.04 mL, 14.6 mmol, 1.2 eq) at room temperature and stirred for 10 min. DMAP (743 mg, 6.1 mmol, 0.5 eq) was added and the solution was stirred for a further 24 h. $NaHCO_3$ (200 mL) was added and the aqueous phase was extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine (200 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure and purification by column chromatography on reversed phase silica gel (RP18, 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 100% MeCN + 0.1% HCOOH) afforded **15** as an orange resin (4.54 g, 9.97 mmol, 82%). R_f = 0.91 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$ −31.8° (c 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 6.90 (m, 2H), 6.77 (m, 2H), 6.01 (m, 1H), 5.78 (ddt, *J* = 17.1, 10.5, 7.2 Hz, 1H), 5.35 (dd, *J* = 17.1, 1.5 Hz, 1H), 5.21 (m, 1H), 5.01 (m, 1H), 4.94 (m, 1H), 4.58 (s, 1H), 4.46 (d, *J* = 5.2 Hz, 2H), 3.38 (dd, *J* = 14.2, 5.4 Hz, 1H), 3.18 (dd, *J* = 14.2, 2.3 Hz, 1H), 2.75 (t, *J* = 6.4 Hz, 2H), 2.04 (q, *J* = 7.2 Hz, 2H), 1.62 (s, 9H), 1.57–1.45 (m, 2H), 1.34 (m, 2H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 195.2 (HMBC correlation), 159.3, 139.5, 134.9, 131.9, 127.4, 117.4, 115.5, 115.3, 84.8, 69.7, 64.6 (HMBC correlation), 35.8, 34.4, 29.4, 28.4, 26.2 ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (m, 2H), 6.75 (m, 2H), 6.02 (m, 1H), 5.85–5.70 (m, 1H), 5.38 (m, 1H), 5.27 (m, 1H), 5.07–4.91 (m, 2H), 4.39 (dd, *J* = 5.4, 2.7 Hz, 1H), 4.46 (d, *J* = 5.3 Hz, 2H), 3.33 (dd, *J* = 14.1, 5.8 Hz, 1H), 3.24 (dd, *J* = 14.1, 2.6 Hz, 1H),

2.93–2.61 (m, 2H), 2.07 (q, *J* = 7.1 Hz, 2H), 1.61 (s, 9H), 1.60–1.29 (m, 4H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (m, 2H), 6.87 (m, 2H), 4.64 (dd, *J* = 5.4, 2.7 Hz, 1H), 4.52 (d, *J* = 5.3 Hz, 2H), 3.41 (dd, *J* = 14.1, 5.8 Hz, 1H), 3.21 (dd, *J* = 14.1, 2.6 Hz, 1H), 2.01 (q, *J* = 7.1 Hz, 2H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.5, 192.4, 164.4, 157.8, 149.1, 138.3, 133.32, 130.9, 126.4, 117.8, 115.1, 114.7, 102.5, 84.2, 68.82, 65.8, 35.0, 33.4, 32.9, 28.3, 28.2, 25.5 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.9, 150.0, 138.4, 133.30, 130.8, 126.0, 117.9, 115.3, 114.9, 105.6, 83.5, 68.80, 61.9, 34.9, 34.8, 32.7, 28.5, 28.4, 24.7 ppm; IR ν_{max} 3075 (w), 2978 (w), 2932 (w), 2860 (w), 1770 (w), 1744 (m), 1716 (s), 1667 (m), 1640 (m), 1604 (s), 1510 (s), 1457 (w), 1421 (m), 1395 (w), 1366 (m), 1349 (s), 1301 (s), 1241 (s), 1223 (s), 1172 (s), 1151 (s), 1025 (m), 996 (m), 967 (m), 913 (s) cm^{-1}; HRMS (ESI) *m*/*z* [M + Na$^+$] calcd. for $C_{26}H_{33}O_6NNa^+$ 478.22001, found 478.21944.

(3*S*,6*Z*,12*E*)-4-Aza-*N*-(tert-butoxycarbonyl)-7-hydroxy-15-oxa-5,21-dioxo-tricyclohenicosa-1(18),6,12,16(17),19-pentaene (16) from 15. 3-Acyltetramic acid **15** (2.30 g, 5.04 mmol, 1.00 eq) in dry, degassed CH_2Cl_2 (1.00 L) was treated with 2nd generation Grubbs catalyst (428 mg, 504 µmol, 10 mol%). The solution was stirred at reflux for 24 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on reversed phase silica gel (RP18, 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 100% MeCN + 0.1% HCOOH) to yield **16** as a pale brown foam (1.78 g, 4.16 mmol, 83%). For analytical data see above.

(3*S*,6*Z*,12*E*)-4-Aza-7-hydroxy-15-oxa-5,21-dioxo-tricyclo[14.2.2.1^{3,6}]henicosa- 1(18),6,12,16(17),19-pentaene (4). Carbamate **16** (926 mg, 2.17 mmol, 1.00 eq) in dry CH_2Cl_2 (43 mL) was treated with TFA (4.3 mL) and stirred for 15 min at room temperature. Toluene (100 mL) was added and the solvent was concentrated under reduced pressure. This was repeated once to yield **4** as a pale brown foam (709 mg, 2.17 mmol, quant.). R_f = 0.64 (10% MeOH in CH_2Cl_2 + 0.01% HCOOH); $[\alpha]_D^{20}$ +52.2° (c 1.00, MeOH); ^{1}H NMR (500 MHz, CD_3OD): Diastereotopic H-atoms indicated as a, b: δ 7.09–6.68 (m, 4H, CH_{Ar}), 5.58 (dt, *J* = 15.1, 7.8 Hz, 1H, OCH_2HC=CH), 5.33 (m, 1H, OCH_2HC=C*H*), 4.64[a] (dd, *J* = 13.9, 7.7 Hz, 1H, ArOCH), 4.53[b] (m, 1H, ArOCH), 4.15 (t, *J* = 3.3 Hz, 1H, CHN), 3.11–2.67[a] (brs, 1H, OCCH), 3.07[a] (dd, *J* = 14.1, 3.8 Hz, 1H, ArCH), 2.91[b] (dd, *J* = 14.1, 3.5 Hz, 1H, ArCH), 2.42–1.86[b] (brs, 1H, OCCH), 2.05[a] (m, 1H, HC=CHC*H*), 1.94[b] (m, 1H, HC=CHC*H*), 1.34–1.05 (m, 4H, HC=CHCH$_2$(CH_2)$_2$) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 157.5 ($OC_{q,Ar}$), 127.4 (OCH_2*C*H=CH), 126.9 8 ($CH_2C_{q,Ar}$), 118.3 ($OCCH_{Ar}$), 67.8 (ArOCH_2), 36.6 (ArCH_2), 33.6 ($OCCH_2$), 33.4 (HC=CHCH_2), 29.8 (HC=CHCH$_2$(CH_2)$_2$), 28.5 (HC=CHCH_2(CH_2)$_2$) ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10–6.58 (m, 4H), 5.54 (m, 1H), 5.39 (m, 1H), 4.60 (m, 2H), 4.16 (m, 1H), 3.28–3.16 (m, 2H), 2.87 (dd, *J* = 14.1, 1.7 Hz, 1H), 2.12–1.84 (m, 3H), 1.40–1.04 (m, 4H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.49 (m, 1H), 5.32 (m, 1H), 4.29 (s, 1H), 2.87 (m, 1H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.5, 188.6, 175.8, 156.0, 136.8, 132.0, 130.3, 125.7, 125.6, 117.9, 114.1, 101.5, 67.0, 62.4, 36.2, 32.43, 32.41, 28.7, 27.6 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.2 (HMBC correlation), 192.4, 170.3, 156.5, 134.9, 131.2, 118.2, 104.7, 67.6, 59.8, 36.4, 33.1, 32.1, 28.5, 27.1 ppm; IR ν_{max} 3255 (w), 2929 (w), 2857 (w), 1770 (w), 1646 (s), 1608 (s), 1508 (s), 1433 (m), 1367 (w), 1305 (w), 1259 (m), 1214 (s), 1176 (s), 1159 (s), 1113 (m), 1075 (m), 1062 (m), 1015 (m), 973 (s) cm^{-1}. HRMS (ESI) *m*/*z* [M + H$^+$] calcd. for $C_{19}H_{22}NO_4^+$ 328.15433, found 328.15343.

(3*S*,6*Z*,12*S*,13*S*)-4-Aza-7,12,13-trihydroxy-15-oxa-5,21-dioxo-tricyclo[14.2.2.1^{3,6}]henicosa-1(18),6,16(17),19-tetraene (7). AD-mix α (2.86 g, 1.4 g/mmol) in H_2O (10.3 mL) was treated with alkene **16** (874 mg, 2.04 mmol, 1.00 eq) in *t*BuOH (10.3 mL) at 0 °C. The two-phase mixture was stirred at 7 °C for 5 d, before more AD-mix α (1.43 g, 0.7 g/mmol) was added. After stirring for a further 4 d, the mixture was treated with Na_2SO_3 (3.60 g, 28.6 mmol, 14.0 eq) and stirred for 2 h at room temperature. H_2O was added to dissolve the precipitate. The aqueous phase was washed with EtOAc (10 mL) and the organic phase was extracted with H_2O (10 mL). The solvent was removed under reduced pressure

and the crude product was suspended in MeOH. The precipitate was filtered off and washed with MeOH. Concentration of the filtrate and purification of the residue by column chromatography on reversed phase silica gel (RP18, H_2O + 0.1% HCOOH → 20% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH) gave a mixture of Boc-protected and deprotected tetramic acid **7**. This was dissolved in dry CH_2Cl_2 (25 mL) and treated with TFA (1.5 mL). After stirring for 15 min at room temperature, toluene (100 mL) was added. The solvent was concentrated under reduced pressure and more toluene (100 mL) was added. Removal of the solvent under reduced pressure gave diol **7** as a yellowish foam and as a mixture of two diastereomers according to HPLC and NMR spectra. Yield: 323 mg (894 µmol, 44%, *dr* 2.3:1). R_f = 0.63 (10% MeOH in CH_2Cl_2 + 0.1% HCOOH); ^{1}H NMR (500 MHz, CD_3OD): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b: δ 7.13–6.75 (m, 4H, CH_{Ar}), 4.26 (m, 1H, $ArOCH^a$), 4.22 (m, 1H, CHN), 4.07 (dd, J = 11.7, 8.8 Hz, 1H, $ArOCH^{b,B}$), 4.05 (m, 1H, J = 11.8, 8.9 Hz, 1H, $ArOCH^{b,A}$), 3.80 (m, 1H, $ArOCH_2CHOH^A$), 3.60 (m, 2H, $ArOCH_2CHOH^B$, $ArOCH_2CHOHCHOH^B$), 3.46 (dt, J = 9.6, 2.7, 3.9 Hz, 1H, $ArOCH_2CHOHCHOH^A$), 3.10 (dt, J = 14.0, 3.8 Hz, 1H, $ArCH^a$), 2.97 (dt, J = 14.0, 3.2 Hz, 1H, $ArCH^b$), 1.67–1.17 (m, 4H, $(CH_2)_2$), 0.88–0.58 (m, 2H, $OCCH_2CH_2$) ppm; $OCCH_2$ not observed; Major diastereomer: ^{13}C NMR (125 MHz, CD_3OD): δ 189.0 (CHO, HMBC correlation), 157.8 ($OC_{q,Ar}$), 131.1 (CH_2CCH_{Ar}, HMBC correlation), 127.2 ($CH_2C_{q,Ar}$, HMBC correlation), 118.4 ($OCCH_{Ar}$), 113.7 ($OCCH_{Ar}$), 69.9 ($ArOCH_2CHOHCHOH$), 67.5 ($ArOCH_2$), 67.4 ($ArOCH_2CHOH$, HSQC correlation), 36.6 ($ArCH_2$), 34.5 ($ArOCH_2(HCOH)_2CH_2$), 32.9 ($OCCH_2$), 27.3 ($ArOCH_2(HCOH)_2CH_2CH_2$), 25.4 ($OCCH_2CH_2$) ppm; Minor diastereomer ^{13}C NMR (125 MHz, CD_3OD): δ 131.9 (HMBC correlation), 126.5 (HMBC correlation), 119.4, 115.2, 69.7, 68.5, 32.8, 25.9 ppm; IR ν_{max} 3354 (m), 2933 (m), 1646 (s), 1607 (s), 1508 (s), 1433 (w), 1338 (w), 1255 (m), 1216 (m), 1177 (w), 1113 (w), 1016 (w) cm^{-1}; HRMS (ESI) m/z [M + H$^+$] calcd. for $C_{19}H_{24}NO_4^+$ 362.15981, found 362.15894.

(3*S*,6*Z*)-4-Aza-12-bromo-7,13-dihydroxy-15-oxa-5,21-dioxo-tricyclo[14.2.2.1^{3,6}]heni cosa-1(18),6,16(17),19-tetraene (8). Alkene **16** (660 mg, 1.54 mmol, 1.00 eq) in DMSO (8 mL) was treated with H_2O (41.7 µL, 2.32 mmol, 1.50 eq) and NBS (412 mg, 2.23 mmol, 1.50 eq) at 8 °C. After stirring the solution for 22 h at room temperature, sat. $NaHCO_3$ solution (50 mL) was added. The aqueous phase was extracted with EtOAc (3 × 50 mL) and the combined organic phases were dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude mixture of products was purified by column chromatography on reversed phase silica gel (RP18, 20% MeCN in H_2O + 0.1% HCOOH → 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH). Bromohydrins **8** and a side product with an additional bromo substituent were obtained separately and only partially deprotected. The mixture of bromohydrin **8** and its *N*-Boc-protected derivative was dissolved in dry CH_2Cl_2 (9 mL) and treated with TFA (900 µL). The solution was stirred for 15 min at room temperature and toluene (100 mL) was added. The mixture was concentrated under reduced pressure and toluene (50 mL) was added again. Removal of the solvent under reduced pressure gave **8** as a yellowish foam and as a mixture of two inseparable diastereomers of initially unknown *dr* according to ^{13}C and ^{1}H NMR spectra. Yield (**8**): 196 mg (462 µmol, 30%). R_f = 0.41 (10% MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b: δ 7.24–6.75 (m, 4H, CH_{Ar}), 4.67–4.19 (m, 4H, CHN, $ArOCH_2$, CHBr), 3.73 (m, 1H, $CHOH$), 3.14 (m, 2H, $ArCH^a$, $OCCH^{a,A}$), 2.96 (dd, J = 14.0, 3.9 Hz, 1H, $ArCH^b$), 1.60–0.91 (m, 5H, $CBrCH_2CH^aCH_2$), 0.76–0.26 (m, 1H, $CBrCH_2CH^bCH_2$) ppm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20–6.72 (m, 4H), 4.60–4.11 (m, 4H), 3.78 (m, 1H), 3.60–3.20 (m, 2H), 2.94 (m, 1H), 2.20–2.00 (m, 1H), 1.63–1.33 (m, 3H), 1.30–0.96 (m, 2H), 0.66–0.30 (m, 1H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B: δ 194.3^B (CO), 194.2^A (CO), 188.2 (COH), 175.6^B (HNCO),175.5^A

(HNCO), 157.1 ($OC_{q,Ar}$), 131.4^{B} (CH_2CCH_{Ar}), 130.1^{A} (CH_2CCH_{Ar}), 127.6 ($CH_2C_{q,Ar}$), 117.0 ($OCCH_{Ar}$), 101.90^{B} (NCOCCO), 101.88^{A} (NCOCCO), 73.0 (CHOH), 69.6 ($ArOCH_2$), 62.0^{B} (HCNH), 61.9^{A} (HCNH), 55.9 (CHBr), 36.2 ($ArCH_2$), 34.2 ($CHBrCH_2$), 33.1 ($OCCH_2$), 27.4 ($CHBrCH_2$), 26.0 ($OCCH_2CH_2$), 24.6 ($CHBrCH_2CH_2$) ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B: δ 202.6^{B}, 202.5^{A}, 189.03^{B}, 189.00^{A}, 169.1^{B}, 169.0^{A}, 156.3, 133.3^{A}, 132.4^{B}, 127.5, 116.2^{A}, 115.3^{B}, 106.0, 67.7, 59.6^{B}, 59.5^{A}, 53.7, 36.6, 33.4, 31.4, 24.6 ppm; IR ν_{max} 3356 (m), 2936 (m), 1652 (s), 1609 (s), 1508 (s), 1462 (w), 1374 (w), 1254 (m), 1217 (m), 1177 (w), 1114 (w), 1043 (w) cm^{-1}; HRMS (ESI) m/z [M + H$^+$] calcd. for $C_{19}H_{23}NO_5Br^+$ 424.07451, found 424.07521.

(3*S*,6*E*)-4-Aza-13,16-dioxa-5,22-dioxo-tetracyclo[15.2.2.1^{3,6}.0^{12,14}]docosa-1(19),6,17(18),20-tetraene (6). Bromohydrin **8** (248 mg, 473 µmol, 1.00 eq) in dry THF (1.5 mL) was treated with KO*t*Bu (86.7 mg, 709 µmol, 1.50 eq) at 0 °C. The suspension was stirred for 4 d at room temperature and then more KO*t*Bu (58 mg, 473 µmol, 1.00 eq) was added. Stirring was continued for 1 d and H_2O (5 mL) as well as EtOAc (5 mL) were added. The organic phase was separated and extracted with H_2O (5 mL). The combined aqueous phases were concentrated under reduced pressure. The crude product was purified by column chromatography on reversed phase silica gel (RP18, 20% MeCN in H_2O + 0.1% HCOOH → 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH) to yield a virtually pure product. Another column chromatography on reversed phase silica gel (RP18, 0% MeCN in H_2O + 0.1% HCOOH → 20% MeCN in H_2O + 0.1% HCOOH → 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH→ 80% MeCN in H_2O + 0.1% HCOOH) afforded epoxide **6** as a pale brown foam and as a 9:1 mixture of two diastereomers according to ^{13}C and ^{1}H NMR spectra. Yield: 76.0 mg (221 µmol, 47%). R_f = 0.28 (10% MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B, diastereotopic H-atoms indicated as a, b: δ 7.05 (m, 2H, $OC(CH)_2$), 6.77 (m, 2H, $CH_2C(CH)_2$), 4.55^{A,a} (dd, J = 14.0, 3.1 Hz, 1H, ArOCH), 4.40^{B,a} (m, 1H, ArOCH), 4.21 (t, J = 3.2 Hz, 1H, CHN), 4.03^{A,b} (dd, J = 14.0, 3.1 Hz, 1H, ArOCH), 3.95^{B,b} (m, 1H, ArOCH), 3.14^{a} (dd, J = 14.1, 3.5 Hz, 1H, ArCH), 3.22–2.69^{a} (brs, 1H, OCCH), 3.05 (m, 1H, $ArOCH_2CHO$), 2.93^{b} (dd, J = 14.1, 3.5 Hz, 1H, ArCH), 2.65 (m, 1H, $ArOCH_2CHOCH$), 1.71^{a} (m, 1H, $ArOCH_2CHOCHCH$), 1.63–1.15 (m, 3H, OCCH^{b}, $ArOCH_2CHOCHCH_2CH_2$), 1.15–0.41 (m, 3H, $ArOCH_2CHOCHCH^{b}$, $OCCH_2CH_2$) ppm; ^{13}C NMR (125 MHz, CD_3OD): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B: δ 187.5 (HMBC correlation), 157.7 ($OC_{q,Ar}$), 132.7^{A}(CH_2CCH_{Ar}), 132.5^{B} (CH_2CCH_{Ar}), 128.2 ($CH_2C_{q,Ar}$), 116.5^{B} ($OCCH_{Ar}$), 116.0^{A} ($OCCH_{Ar}$), 113.2^{A} ($OCCH_{Ar}$), 66.6^{B} ($ArOCH_2$), 66.4^{A} ($ArOCH_2$), 62.4 (HCNH, HSQC correlation), 58.5 (OCH_2CHOCH), 58.4 (OCH_2CHOCH), 36.3 (CH_2Ar), 33.3 ($ArOCH_2CHOCHCH_2$), 31.9 ($OCCH_2$), 27.3 ($ArOCH_2CHOCHCH_2CH_2$), 24.8^{A} ($OCCH_2CH_2$), 24.7^{B} ($OCCH_2CH_2$) ppm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25–6.43 (m, 5H), 4.53 (t, J = 11.7 Hz, 1H), 4.44–3.83 (m, 2H), 3.40 (m, 1H), 3.24 (m, 1H), 3.12–2.80 (m, 2H), 2.71 (m, 1H), 2.21 (m, 1H), 1.92–1.42 (m, 3H), 1.19–0.35 (m, 3H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B: δ 194.6, 187.6, 175.3, 156.2, 131.5, 126.65, 115.1, 103.5, 65.5, 62.4, 57.2, 57.08, 35.7, 32.1, 30.6, 26.0, 23.5 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B: δ 202.2, 190.0, 169.2, 156.5, 131.8, 126.5, 115.2, 106.3, 66.2, 59.5, 58.0, 57.12, 36.1, 32.4, 31.4, 26.6, 23.4 ppm; IR ν_{max} 3283 (w), 2931 (m), 1706 (s), 1654 (s), 1609 (s), 1509 (s), 1436 (m), 1373 (m), 1306 (w), 1239 (s), 1221 (s), 1180 (m), 1113 (w), 1072 (w), 1044 (m), 915 (m) cm^{-1}; HRMS (ESI) m/z [M + H$^+$] calcd. for $C_{19}H_{22}NO_5^+$ 344.14925, found 344.14904.

(3*S*,6*Z*)-4-Aza-12,13-dibromo-7-hydroxy-15-oxa-5,21-dioxo-tricyclo-[14.2.2.1^{3,6}] henicosa-1(18),6,16(17),19-tetraene (9). A solution of alkene **16** (500 mg, 1.17 mmol, 1.00 eq) in CCl_4 (5.3 mL) was treated with bromine (90.3 µL, 1.75 mmol, 1.50 eq) and stirred in a

sealed tube for 30 h at 80 °C. The solvent was removed under reduced pressure and the remainder was purified by column chromatography on reversed phase silica gel (RP18, 30% MeCN in H_2O + 0.1% HCOOH → 35% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 45% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 100% MeCN in H_2O + 0.1% HCOOH) to afford dibromide **9** as a yellow foam and as a mixture of two diastereomers of unknown *dr*. Yield: 228 mg (468 µmol, 40%). R_f = 0.62 (10% MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67/8.42/8.17 (s, 1H, NH), 7.24–6.72 (m, 4H, CH_{Ar}), 4.82–3.83 (m, 5H, CH_2CHNH, $ArOCH_2$, $(CHBr)_2$), 3.39–2.94 (m, 2H, $ArCH_2$), 2.41–0.75 (m, 8H, $OCCH_2$, $CHBr(CH_2)_3$) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.3 (CO), 193.3 (COH), 168.5 (HNCO), 157.5 ($OC_{q,Ar}$), 133.0 (CH_2CCH_{Ar}), 130.4 (CH_2CCH_{Ar}), 126.8 ($CH_2C_{q,Ar}$), 115.6 ($OCCH_{Ar}$), 101.6 (NCOCCO, HMBC correlation), 70.0 ($ArOCH_2$), 62.5 (HCNH), 59.6 ($(CHBr)_2$), 54.3 ($(CHBr)_2$), 38.2 (CH_2CHBr), 34.9 (CH_2Ar), 27.0, 21.9 ($HOCCH_2$, $CHBrCH_2(CH_2)_2$) ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.7, 132.7, 130.9, 62.4, 61.9, 55.0, 26.4/25.5 ppm; IR ν_{max} 3241 (m), 2929 (m), 1693 (s), 1652 (s), 1605 (s), 1507 (s), 1460 (m), 1431 (m), 1350 (w), 1299 (w), 1250 (m), 1215 (s), 1177 (m), 1112 (w), 1112 (w), 1040 (w), 1016 (m), 925 (w), 906 (m), 858 (m), 811 (w), 769 (w), 733 (s) cm^{-1}; HRMS (ESI) m/z [M + H$^+$] calcd. for $C_{19}H_{22}Br_2NO_4^+$ 487.98896, found 487.98884.

((3*S*,6*Z*)-4-Aza-7,14-dihydroxy-5,18-dioxo-12-vinyl-tricyclo[11.3.1.1^{3,6}]octadeca-1 (17),6,13(14),15-tetraene (10). A solution of alkene **16** (500 mg, 1.17 mmol, 1.00 eq) in degassed diethylaniline (4.7 mL) was stirred in a sealed tube at 190 °C for 42 h. The solution was diluted with EtOAc (50 mL). The organic phase was washed with 2M HCl (2 × 75 mL) and the aqueous phase was extracted with EtOAc (30 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification of the crude product by column chromatography on reversed phase silica gel (RP18, 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 100% MeCN in H_2O + 0.1% HCOOH) afforded **10** as a colourless foam and as an inseparable mixture of two diastereomers. Yield: 218 mg (666 µmol, 57%, *dr* 12.5:1). R_f = 0.28 (10% MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b: δ 6.88–6.72 (m, 2H, $CH_{Ar}CCH_2$), 6.60^A (m, 1H, $CH_{Ar}COH$), 6.56^B (m, 1H, $CH_{Ar}COH$), 6.36^B (m, 1H, C=CH), 5.95^A (ddd, J = 16.0, 10.4, 6.2 Hz, 1H, C=CH), 5.00–4.90 (m, 2H, CH_2=C), 4.00^A (t, J = 3.4 Hz, 1H, CH_2CHNH), 3.96^B (t, J = 3.4 Hz, 1H, CH_2CHNH), 3.71 (m, 1H, CHC=C), 3.58^a (m, 1H, $HOCCH$), 3.14^{A,a} (dd, J = 14.0, 3.5 Hz, ArCH), 3.08^{B,a} (dd, J = 14.0, 3.5 Hz, ArCH), 2.78^b (dd, J = 13.7, 3.5 Hz, 1H, ArCH), 2.13^b (dt, J = 14.0, 4.0 Hz, 1H, $HOCCH$), 1.99^a (m, 1H, $OCCH_2CH$), 1.67^a (m, 1H, $CHCH_2CH$), 1.55^b (m, 1H, $CHCH_2CH$), 1.45–1.26 (m, 2H, $OC(CH_2)_2CH_2$), 0.89^{B,b} (m, 1H, $OCCH_2CH$), 0.44^{A,b} (m, 1H, $OCCH_2CH$) ppm; ^{13}C NMR (125 MHz, CD_3OD): Signals of major diastereomer marked as A, signals of minor diastereomer marked as B: δ 198.9 (CO), 188.8 (COH), 176.2 (HNCO), 155.4 ($OC_{q,Ar}$), 144.1^A (C=CH), 143.1^B (C=CH), 131.1 ($OCCHCH_{Ar}$), 130.9 ($CHC_{q,Ar}$), 130.7^A ($CCH_{Ar}C$), 129.9^B ($CCH_{Ar}C$), 126.2 ($CH_2C_{q,Ar}$), 115.8^B ($OCCH_{Ar}$), 115.2^A ($OCCH_{Ar}$), 113.2^B (C=CH_2), 113.0^A (C=CH_2), 105.4 (NCOCCO), 63.9^A (HCNH), 63.4^B (HCNH), 40.7 (CHC=C), 37.4^A ($ArCH_2$), 37.3^B ($ArCH_2$), 35.4^A ($CHCH_2CH_2$), 32.1^B ($CHCH_2CH_2$), 29.3^A ($HOCCH_2$), 25.9^B ($HOCCH_2$), 24.9^A, 24.8^A ($OC(CH_2)_2CH_2$, $OCCH_2CH_2$), 23.6^B, 23.4^B ($OC(CH_2)_2CH_2$, $OCCH_2CH_2$) ppm; IR ν_{max} 3294 (m), 2931 (m), 2863 (w), 1969 (w), 1656 (s), 1694 (s), 1511 (m), 1435 (m), 1336 (w), 1252 (m), 1169 (w), 1100 (w), 911 (m) cm^{-1}; HRMS (ESI) m/z [M + H$^+$] calcd. for $C_{19}H_{22}NO_4^+$ 328.15433, found 328.15411.

(3*S*,6*Z*,8*R*,12*E*)-4-Aza-*N*-(tert-butoxycarbonyl)-7-hydroxy-8-methyl-15-oxa-5,21-dio xo-tricyclo[14.2.2.1^{3,6}]henicosa-1(18),6,12,16(17),19-pentaene (18). A solution of alkene **17** (263 mg, 596 µmol, 1.00 eq) in EtOAc (6 mL) was treated with Pd on charcoal (26.3 mg, 10 wt%). The resulting suspension was stirred under a H_2-atmosphere for 31 h at room temperature. The solid was filtered off over Celite® and washed with EtOAc. The combined filtrates were concentrated under reduced pressure to give **18** as an orange foam.

Yield: 261 mg (588 µmol, 99%). R_f = 0.93 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$ +38.8° (c 0.75, MeOH); Major tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 7.02–6.57 (m, 4H), 4.50 (m, 1H), 4.26–4.06 (m, 2H), 3.41 (dd, *J* = 14.4, 3.0 Hz, 1H), 3.37 (m, 1H), 3.08 (dd, *J* = 14.8, 3.0 Hz, 1H), 1.63 (s, 9H), 1.60–1.15 (m, 7H), 1.08 (d, *J* = 6.7 Hz, 3H), 1.03 (m, 1H), 0.73–0.42 (m, 2H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 4.68 (m, 1H), 3.54 (m,1H) ppm¸ Major tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 193.7, 174.9, 157.4, 150.6, 131.8, 127.9, 116.1, 104.2, 85.2, 67.8, 66.4, 38.3, 35.4, 34.9, 29.4, 28.4, 27.5, 26.1, 24.7, 17.7 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 63.3, 32.8, 30.4, 28.44, 23.7, 14.3 ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06–6.65 (m, 4H), 4.44 (m, 1H), 4.26–4.06 (m, 2H), 3.42 (m, 1H), 3.41 (dd, *J* = 14.6, 3.0 Hz, 1H), 3.12 (dd, *J* = 14.6, 4.0 Hz, 1H), 1.64 (s, 9H), 1.50–1.13 (m, 7H), 1.06 (m, 3H), 1.04 (m, 1H), 0.73–0.42 (m, 2H) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.62 (m, 1H), 3.55 (m, 2H), 1.60 (s, 9H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.6, 191.6, 174.1, 155.8, 149.0, 132.6, 129.3, 126.6, 117.6, 117.0, 102.8, 84.4, 67.0, 65.5, 36.5, 34.6, 34.1, 28.36, 28.3, 26.1, 24.9, 23.5, 17.6 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 131.1, 130.7, 116.5, 115.4, 62.2, 36.6, 34.9, 33.8, 28.4, 28.0, 26.4, 25.8, 25.4, 23.8, 16.6 ppm; IR ν_{max} 2971 (m), 2935 (m), 2233 (w), 2078 (m), 1740 (s), 1611 (s), 1509 (m), 1440 (m), 1354 (s), 1302 (m), 1256 (w), 1228 (s), 1218 (s), 1156 (m), 1116 (s), 972 (s) cm^{-1}; HRMS (ESI) *m*/*z* [M + Na^+] calcd. for $C_{25}H_{33}NO_6Na^+$ 466.22001, found 466.21899.

(3*S*,6*Z*,8*R*)-4-Aza-7-hydroxy-8-methyl-15-oxa-5,21-dioxo-tricyclo[14.2.2.1^{3,6}] henicosa-1(18),6,16(17),19-tetraene (3). To a solution of tetramic acid **18** (227 mg, 512 µmol, 1.00 eq) in CH_2Cl_2 (9.5 mL) was added TFA (950 µL). After stirring for 15 min at room temperature, toluene (100 mL) was added and the solvent was removed under reduced pressure. Toluene (50 mL) was added again and the solvent was removed, which afforded **3** as an orange foam. Yield: 176 mg (512 µmol, quant.). R_f = 0.51 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$–17.7° (c 0.74, MeOH); Major tautomer: ^{1}H NMR (500 MHz, CD_3OD): diastereotopic H-atoms indicated as a, b: δ 7.14 (m, 1H, CH_{Ar}CCH$_2$), 6.96 (m, 1H, CH_{Ar}CCH$_2$), 6.78 (m, 2H, CH$_{Ar}$CO), 4.23–4.07 (m, 3H, CHN, ArOCH$_2$), 3.33 (m, 1H, CHMe), 3.07[a] (dd, *J* = 14.3, 4.1 Hz, 1H, ArCH), 2.97[b] (dd, *J* = 14.4, 2.4 Hz, 1H, ArCH[b]), 1.52 (m, 2H, OCH$_2$CH_2), 1.44–1.16 (m, 5H, CHMeCH[a], CMeCH$_2$CH_2, O(CH$_2$)$_2$CH_2), 1.08[b] (m, 1H, CHMeCH), 1.05 (d, *J* = 6.9 Hz, 3H, CH$_3$), 0.68–0.40 (m, 2H, CMe(CH$_2$)$_2$CH_2) ppm; Significant signals minor tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 6.64 (m, 2H), 3.53 (m, 1H) ppm; Major tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 198.2 (CO, HMBC correlation), 190.2 (COH), 177.5 (HNCO, HMBC correlation), 157.0 (OC$_{q,Ar}$), 133.3 (CH_{Ar}CCH$_2$), 130.8 (CH_{Ar}CCH$_2$), 128.1 (CH$_2$C$_{q,Ar}$), 118.2 (OCCH$_{Ar}$), 117.4 (OCCH$_{Ar}$), 103.2 (NCOCCO), 67.6 (ArOCH$_2$), 63.6 (CHN), 37.3 (CHMe), 36.4 (ArCH$_2$), 35.2 (CMeCH$_2$), 29.5 (CMeCH$_2$CH$_2$), 27.3 (CMe(CH$_2$)$_2$CH$_2$), 25.9 (OCH$_2$CH$_2$), 24.7 (O(CH$_2$)$_2$CH$_2$), 17.8 (CH$_3$) ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, CD_3OD) δ 131.9, 116.0 ppm; Major tautomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19–7.00 (m, 2H), 6.79 (m, 2H), 4.35–4.09 (m, 3H), 3.38 (m, 1H), 3.28–2.83 (m, 2H), 1.52 (m, 2H), 1.44–1.12 (m, 5H), 1.12 (m, 1H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.67–0.41 (m, 2H) ppm; Significant signal minor tautomer: ^{1}H NMR (500 MHz, CD_3OD) δ 3.63 (m, 1H) ppm; Major tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.3, 192.1, 175.9, 155.7, 132.6, 129.6, 126.4, 117.3, 116.7, 102.0, 66.8, 62.2, 36.0, 34.4, 28.4, 26.0, 24.9, 23.6, 17.6 ppm; Significant signals minor tautomer: ^{13}C NMR (125 MHz, $CDCl_3$) δ 130.5, 116.3, 115.9, 66.9, 63.7, 36.4, 36.1, 29.4, 25.9, 25.1, 23.9, 17.1 ppm; IR ν_{max} 2929 (m), 2853 (m), 1654 (s), 1608 (s), 1509 (s), 1456 (m), 1340 (m), 1259 (m), 1222 (m), 1174 (m) cm^{-1}; HRMS (ESI) *m*/*z* [M + H]$^+$ calcd. for $C_{20}H_{26}NO_4^+$ 344.18563, found 344.18481.

4.3. Experimental Design and Evaluation of Herbicidal Tests

The test compounds were applied as solutions in a mixture of isopropanol/water = 1:1 + 0.25% Tween 20 to arrays of two plant species (dandelions and thistle) that are susceptible to natural macrocidins. Plants were grown in 100 cm^2 pots with two individuals/pot and four replicates/treatment. Within each species there were three treatments: untreated control, the macrocidinoids (100 or 150 mM) and the commercial herbicide diflufenican

(1.2 mM). Spraying was done with 0.4 mL/100 cm^2. Effects were assessed after 14 days and a second time after three to six weeks when mortality was fully developed. A mortality factor, i.e., the percentage of eventually dead plants, was calculated according to the Henderson-Tilton method [22] using the formula:

$$\text{Mortality [\%]} = (1 - T_a \times C_b / T_b \times C_a) \times 100$$

with T_b = number of plants before treatment (=8); T_a = number of vital, treated plants at the end of observation period; C_b = number of vital, untreated control plants at beginning (=8); C_a = number of vital, untreated control plants at the end of observation period.

4.4. Antimicrobial Activity

The antibacterial activities were determined by the so-called broth microdilution method [23]. In brief: all cultivations were done in standard microbiological media such as TSB medium (tryptic soy broth) for *S. aureus* (SH1000) and LB medium (lysogeny broth) for *Escherichia coli* (ATCC25922) and at 37 °C (only *A. baumannii* was cultivated at 30 °C). The overnight cultures of the bacterial test strains were diluted to an OD_{600} of 0.1 and further incubated until an OD_{600} = 0.5 was reached. These cultures were used as working cultures. They were diluted to obtain an OD_{600} of 0.1, determined in 45 µL of the bacterial suspension in each well of a 384-well plate, or in 90 µL of the bacterial suspension in each well of a half-area 96-well plate. Compound solutions were prepared in separate 96-well compound plates starting from 10 mM stock solutions in DMSO. The compound concentrations were adjusted so that the maximum DMSO concentrations in the assay plates were 1%, assuring no interference with growth from the solvent. The respective volumes of the compound solutions were added to the microbial suspensions with the 96-channel semi-automated pipettor CyBio Selma (Analytik Jena). The OD_{600} was determined directly after compound addition and subsequently after 1, 3 and 24 h using the Epoch 2 microplate reader (BioTek Instruments).

4.5. Antibiofilm Activity

Staphylococcus aureus DSM 1104 from a stock kept at −20 °C was precultured in 25 mL CASO (casein-peptone soymeal-peptone) medium in a 250 mL flask at 37 °C and shaken (100 rpm, 20 h. The OD_{600} of the culture solution was adjusted to 0.001 McFarland standard. The solution was incubated in 96-well microtiter plates (TPP tissue culture ref. No. 92196) for 18 h at 37 °C with 150 µL of serially diluted test compounds (250–2 µg/mL) in CASO with 4% glucose broth. Compounds showing high activities (e.g., **2**, **3**, **9**) were diluted in the range of 10–0.3 µg/mL. The inhibition of biofilm formation was evaluated by staining with 150 µL of 0.1% crystal violet (CV; Thermo Fisher, Waltham, MA, USA) following previously established protocols [21,24]. Briefly, the supernatant of the 96-well plate was discarded and the wells were washed once with PBS buffer. The remaining biofilms were stained with 0.1% CV at room temperature for 15 min, washed three times with PBS buffer, and finally dissolved in 150 µL ethanol (95%). The absorbance of the resulting solution at 530 nm was quantified using a plate reader (Synergy 2, BioTek, Santa Clara, CA, USA). Methanol (2.5%) and

Standard deviations (SD) of two repeats with duplicates were 10% or less. Effects on the biofilms and SD values are shown in Table S1 in the Supporting Information (SI).

The precultured bacterial suspension of *S. aureus* strain DSM 1104 was adjusted to 0.001 McFarland standard at OD_{600} and incubated in 96-well tissue microtiter plates for 18 h in 150 µL CASO with 4% glucose broth. The supernatant of the 96-well plate was removed and the remainder was washed with 150 µL PBS buffer. The test compounds were serially diluted in 150 µL of fresh media (CASO with 4% glucose broth) to concentrations of 250–2 µg/mL, and added to the wells. The plates were incubated for a further 24 h at 37 °C. Staining of the preformed biofilm and of the controls was carried out as described above [21,24]. The SD of two repeats with duplicates were 15% or less. SD values are shown in Table S1 in the ESI.

Candida albicans DSM 11225 was taken from a −20 °C stock and precultured in 25 mL YPED (Yeast extract Peptone Dextrose) medium in a 250 mL flask at 30 °C and shaken (100 rpm, 18 h). The OD_{600} of the culture solution was adjusted to 0.05 McFarland standard in RPMI 1640 medium. 150 µL of the solution was added to 96-well non-tissue microtiter plates (Falcon non-tissue plate ref. No. 351172) for 90 min at 37 °C, and shaken with 150 rpm. The supernatant was discarded and the residue washed twice with PBS buffer. The test compounds were serially diluted in 150 µL of fresh medium (RPMI 1640) to concentrations of 250–2 µg/mL and added to the wells. Methanol (2.5%) was used as the negative control. The plates were further incubated at 37 °C and shaken (150 rpm, 24 h). The supernatant of the 96-well plate was discarded, the wells were washed once with PBS buffer, and the biofilms were stained with 150 µL of 0.1% CV at room temperature for 25 min and then washed four times with PBS buffer. The biofilms were dissolved in 150 µL ethanol (95%), and the absorbance of the resulting solution at 610 nm was finally quantified using a plate reader (Synergy 2, BioTek, Santa Clara, CA, USA). SD of two repeats with duplicates each were 10% or less. Dispersal effects on preformed biofilms and SD values are shown in Table S1 (SI).

P. aeruginosa (PA 14) was cultured in 25 mL LB medium (Luria-Bertani Broth) in a 250 mL flask at 37 °C, shaken with 100 rpm, for 18 h. The OD_{600} of the culture solution was adjusted to 0.025 McFarland standard in LB medium. The test compounds were diluted in 100 µL bacterial solution to concentrations of 250–2 µg/mL and the resulting solutions were added to 96-well plates in an MBEC Innovatech incubator (MBEC Assay®, Edmonton, AB, Canada). The plates were incubated at 37 °C at 150 rpm for 24 h. The biofilms were established on the pegs under the growth conditions. The pegs and plates were rinsed once with PBS buffer, the biofilms on pegs were stained with 150 µL 0.1% CV at room temperature for 15 min and then rinsed twice with PBS buffer. The pegs were transferred into a new plate with 150 µL ethanol (95%) and the absorbance at 550 nm was quantified using a plate reader (Synergy 2, BioTek, Santa Clara, CA, USA). Myxovalargin A and methanol (2.5%) were used as positive and negative control.

4.6. Cytotoxicity

The cytotoxic effect upon treatment with macrocidinoids **2**, **3**, **6**–**10** for 72 h was determined by standard MTT assays [25]. The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; abcr) is reduced by viable cells to a violet, water-insoluble formazan. Human 518A2 melanoma cells, colon carcinoma cells HCT-116^{wt} and HCT-$116^{p53-/-}$, and KBV cervix carcinoma cells as well as hybrid endothelial EaHy cells (5×10^4 cells mL^{-1}, 100 µL/well), were seeded in 96-well tissue culture plates and cultured for 24 h at 37 °C, 5% CO_2 and 95% humidity. After treatment with the test compounds (stock solutions 10 mM in DMSO and freshly diluted appropriately with sterile Milli-Q water) incubation of cells was continued for 72 h. Blank and solvent controls were treated identically. After addition of a 5 mg mL^{-1} MTT stock solution in phosphate buffered saline (PBS), microplates were incubated for 2 h at 37 °C, centrifuged at 300 g, 4 °C for 5 min and the supernatant was discarded. The precipitate of formazan crystals was then redissolved in a 10% (*w*/*v*) solution of sodium dodecylsulfate (SDS; Carl Roth) in DMSO containing 0.6% (*v*/*v*) acetic acid. To ensure complete dissolution of the formazan, the microplates were incubated for at least 1 h in the dark. Finally, the absorbance at λ = 570 and 630 nm (background) was measured using a microplate reader (Tecan F200). All experiments were carried out in quadruplicate and the percentage of viable cells was calculated as the mean ± SD with controls set to 100%. The determined IC_{50} (inhibitory concentration) values are shown in Table S2 (*cf.* SI).

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10081022/s1. Figures S1–S67: ^1H and ^{13}C NMR spectra of **3**–**10**, **12**–**16**, **18** and HPLC spectra of **3**–**10**. Figure S68: herbicidal effects on pot plants. Figure S69: growth inhibitory effects of macrocidin derivatives on *E. coli* ΔTolC cultures; Figure S70: growth inhibitory effects of macrocidin derivatives on *Staphylococcus aureus* (SH1000) cultures. Figure S71: growth inhibitory

effects of selected macrocidin derivatives on *Actinetobacter baumannii* cultures. Figure S72: dispersal effects on preformed biofilms of *C. albicans*. Table S1: antibacterial effects of compounds **2**–**10** on *E. coli* ΔTolC and *S. aureus*. Table S2: *S. aureus* and *C. albicans* biofilm growth inhibition and dispersion data. Table S3: IC_{50} values for human cells.

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Abbreviations

DMAP: dimethylaminopyridine; DMSO: dimethylsulfoxide; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; NBS: *N*-bromosuccinimide; NMO: *N*-methylmorpholine-*N*-oxide; PBS: phosphate-buffered saline; TFA: trifluoracetic acid; THF: tetrahydrofuran; sat.: saturated.

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Supporting Information

Dual agents: fungal macrocidins and synthetic analogues with herbicidal and antibiofilm activities

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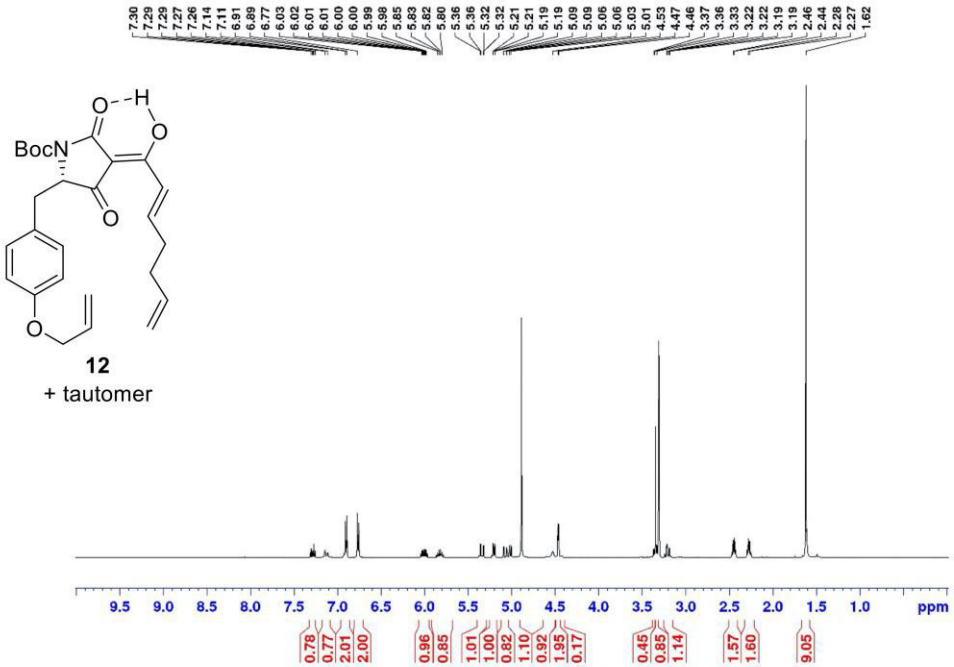

Figure S1. ^{1}H-NMR spectrum of compound **12** in CD_3OD.

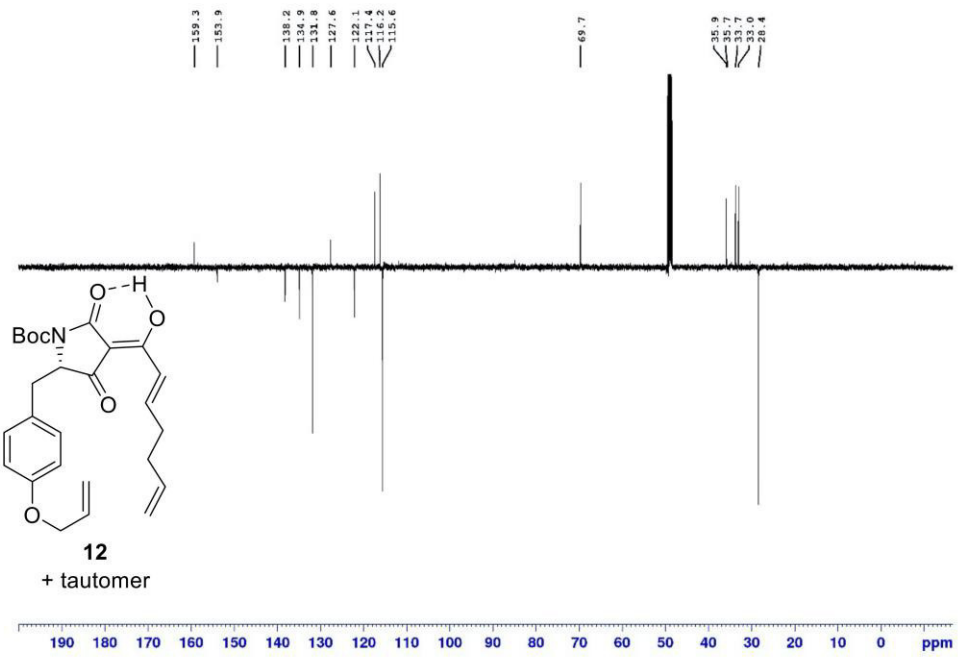

Figure S2. ^{13}C-NMR spectrum of compound **12** in CD_3OD.

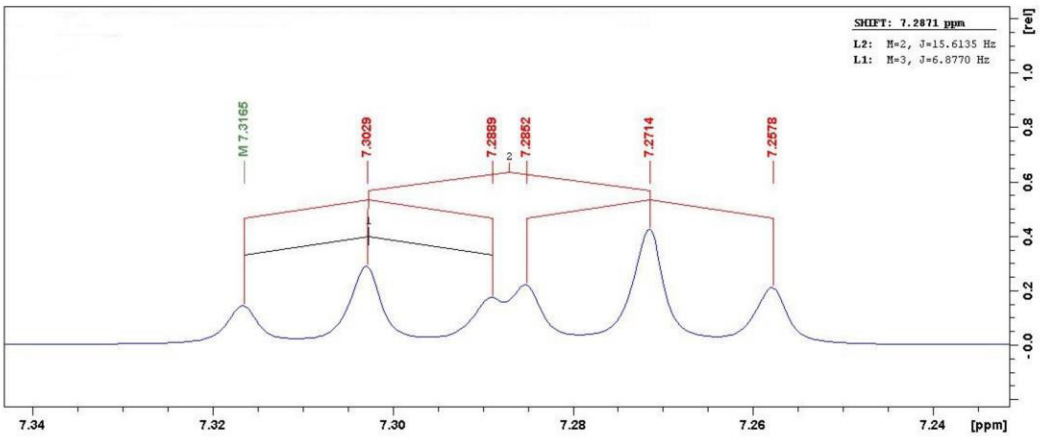

Figure S3. Part of ^{1}H-NMR spectrum of compound **12** in CD_3OD with defined multiplet and coupling constants (J =15.6, 6.9 Hz) that prove the (*E*)-configuration of the resulting double bond.

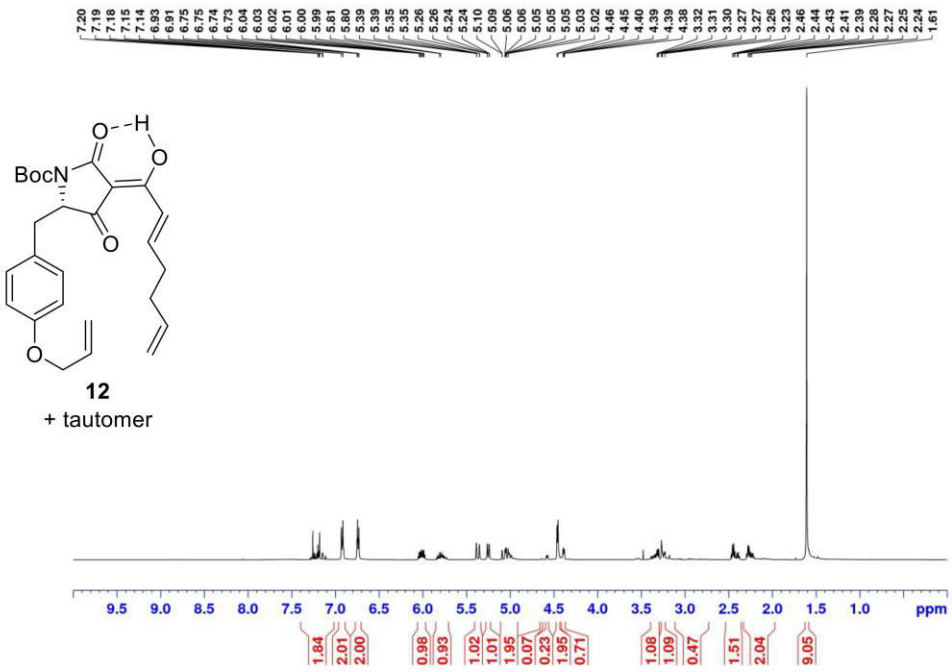

Figure S4. ^{1}H-NMR spectrum of compound **12** in $CDCl_3$.

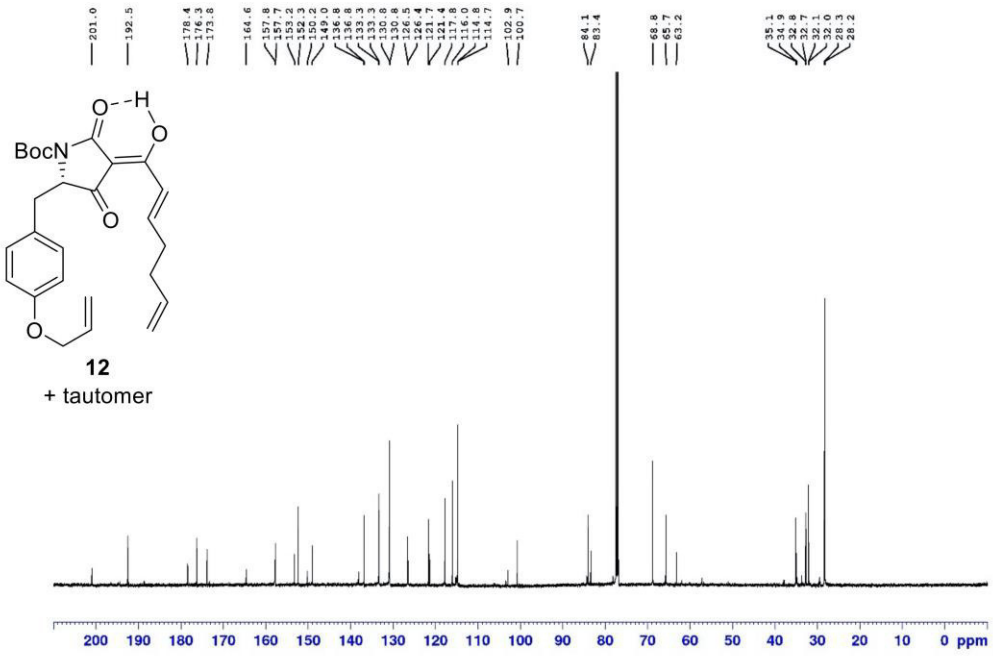

Figure S5. ^{13}C-NMR spectrum of compound **12** in $CDCl_3$.

S3

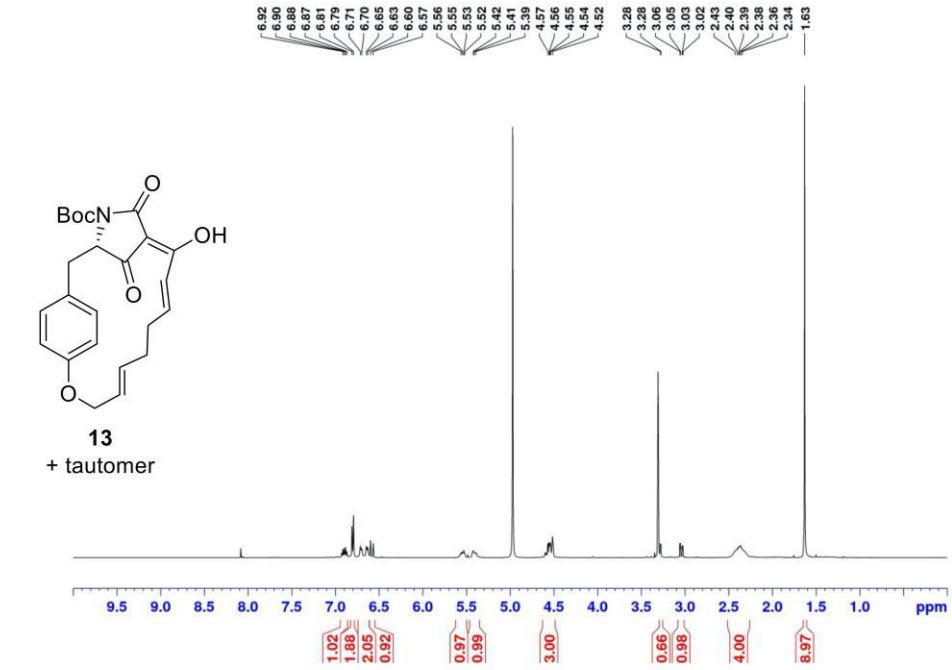

Figure S6. ^{1}H-NMR spectrum of compound **13** in CD_3OD.

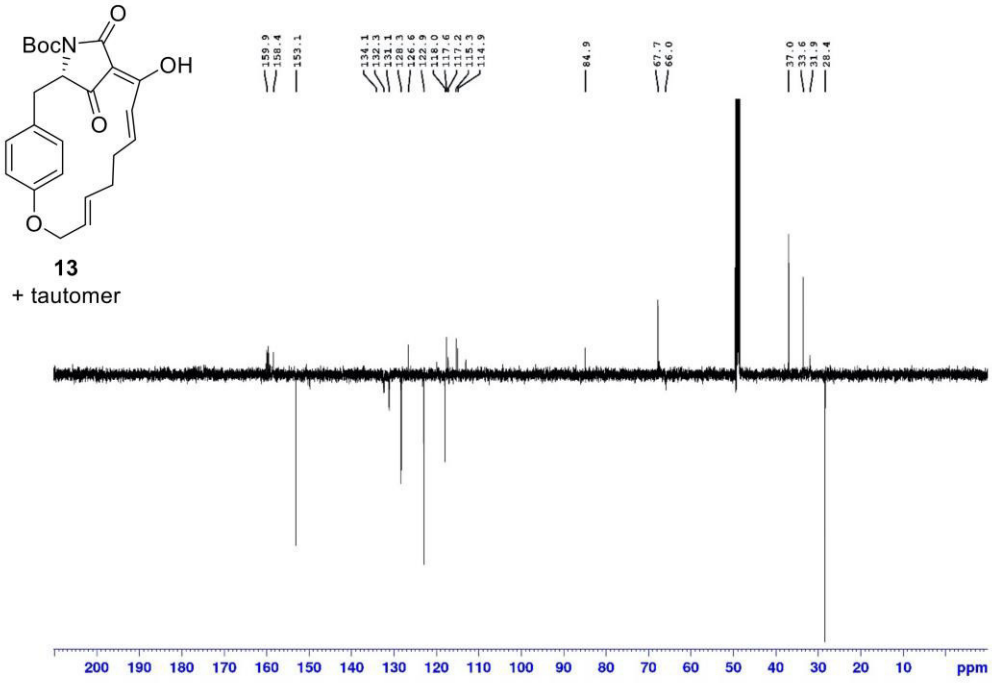

Figure S7. ^{13}C-NMR spectrum of compound **13** in CD_3OD.

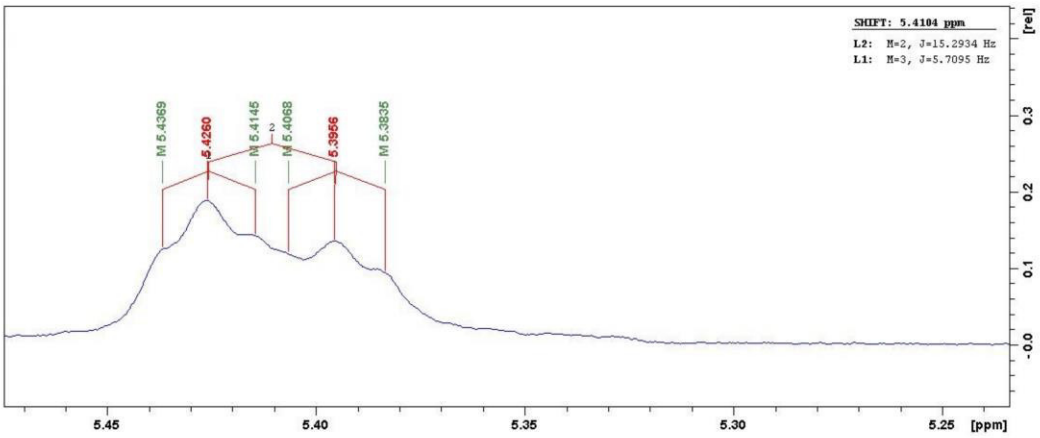

Figure S8. Part of ^{1}H-NMR spectrum of compound **13** in CD_3OD with defined multiplet and coupling constants (J =15.3, 5.7 Hz) that prove the (*E*)-configuration of the resulting isolated double bond.

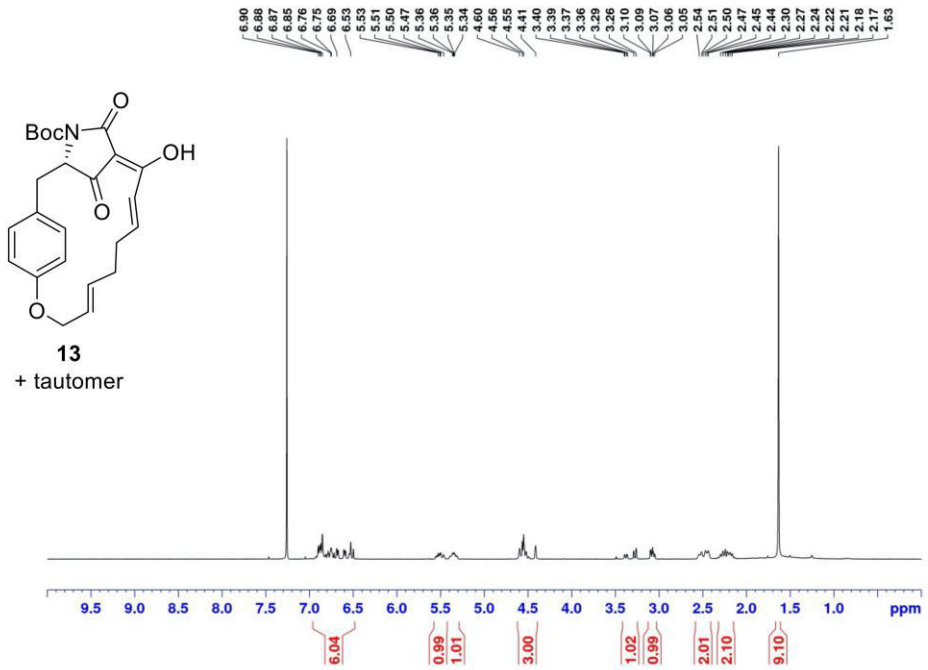

Figure S9. ^{1}H-NMR spectrum of compound **13** in $CDCl_3$.

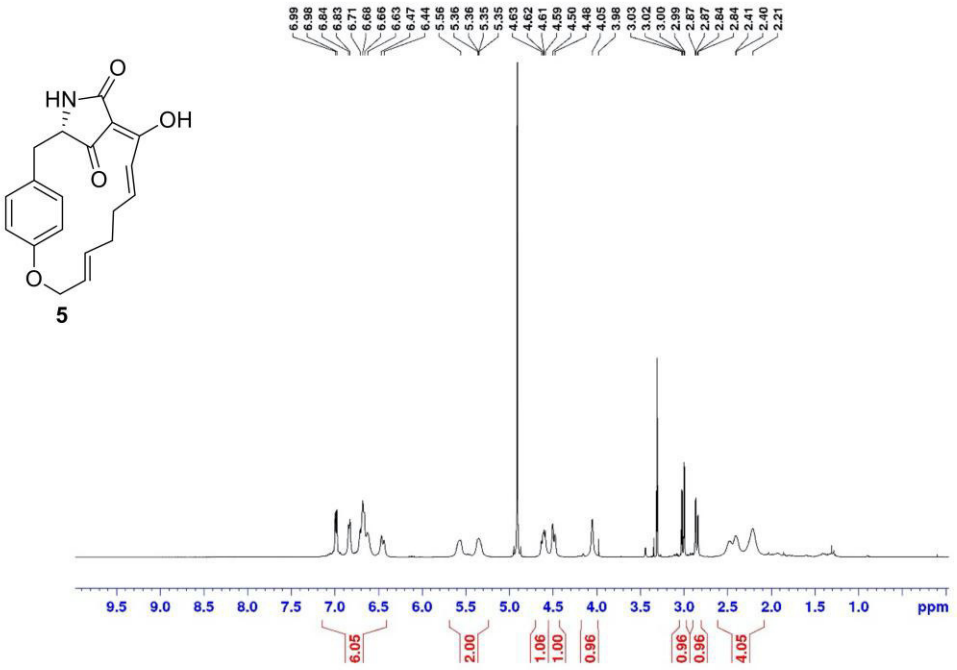

Figure S10. [1]H-NMR spectrum of compound **5** in CD_3OD.

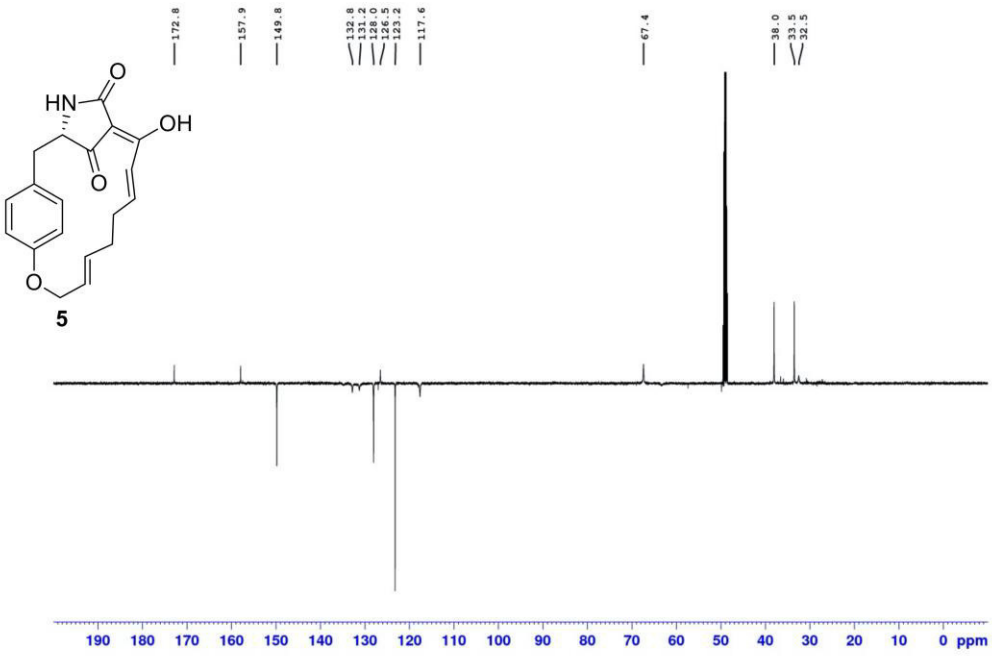

Figure S11. [13]C-NMR spectrum of compound **5** in CD_3OD.

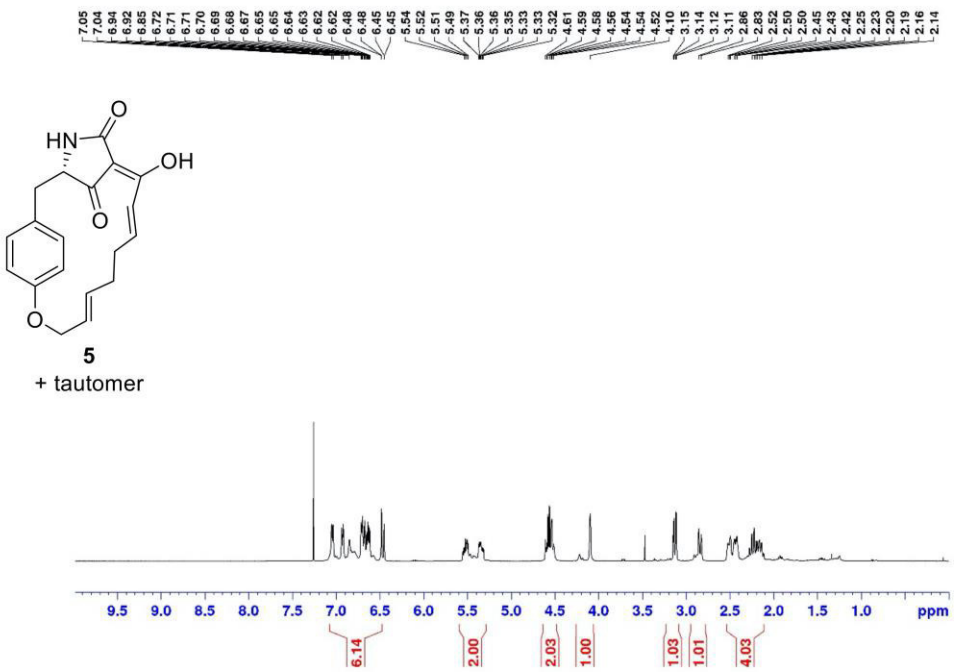

Figure S12. ^{1}H-NMR spectrum of compound **5** in $CDCl_3$.

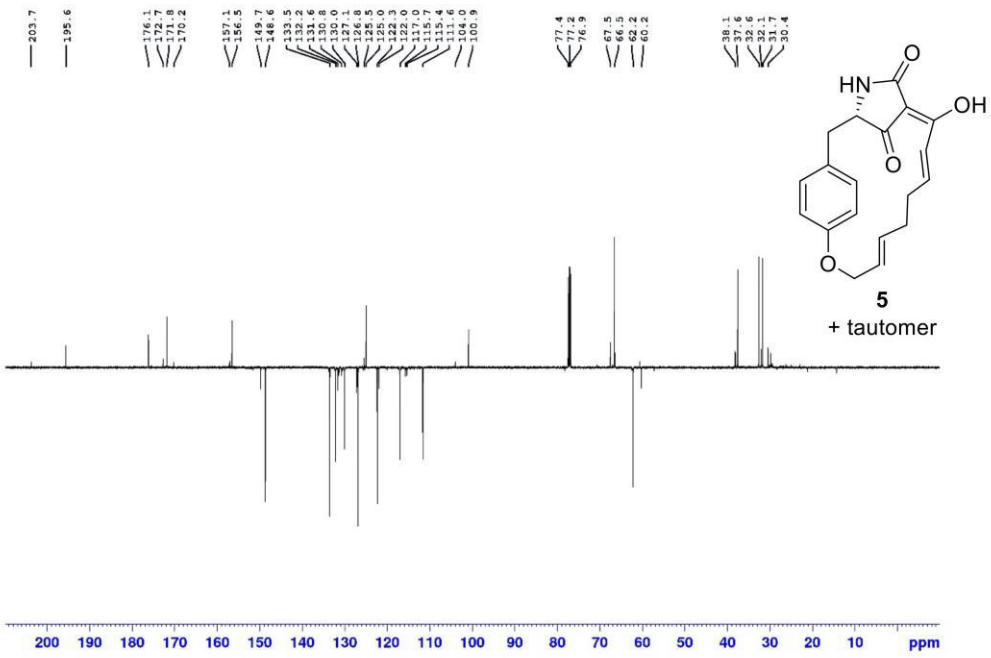

Figure S13. ^{13}C-NMR spectrum of compound **5** in $CDCl_3$.

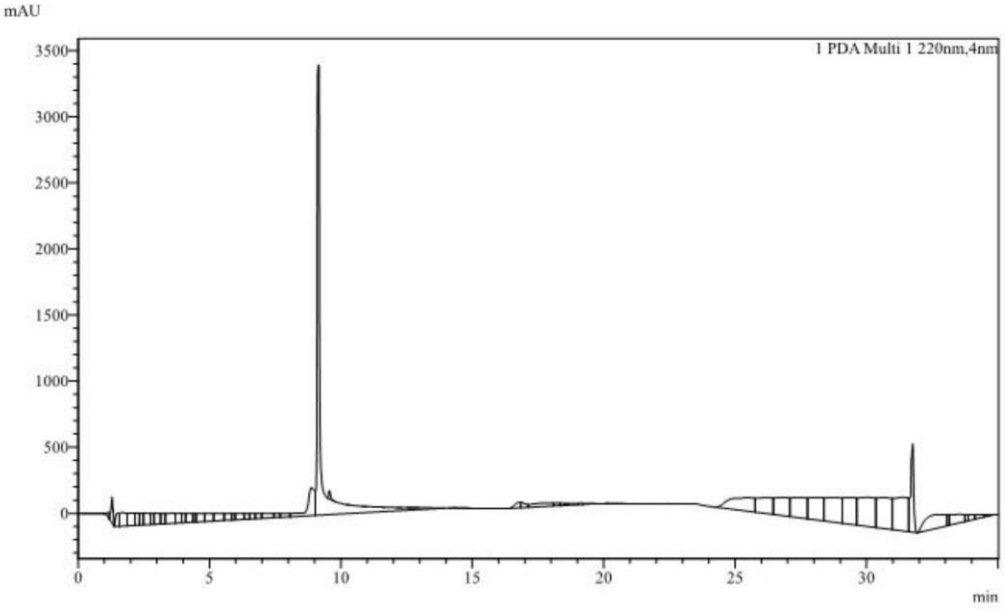

Figure S14. Chromatogram of compound **5**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

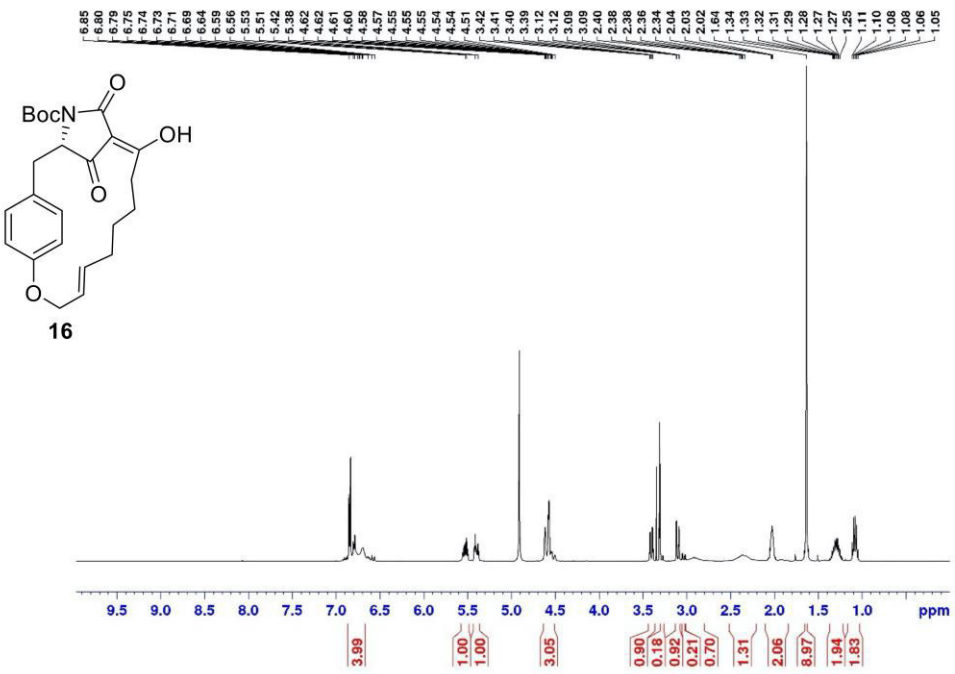

Figure S15. ^{1}H-NMR spectrum of compound **16** in CD_3OD.

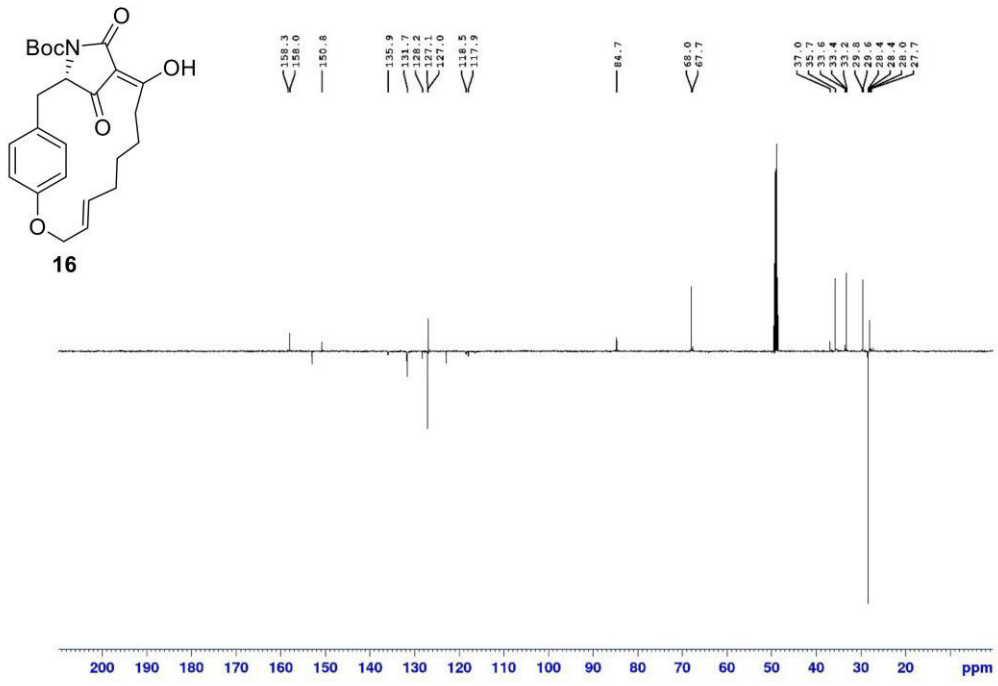

Figure S16. ^{13}C-NMR spectrum of compound **16** in CD_3OD.

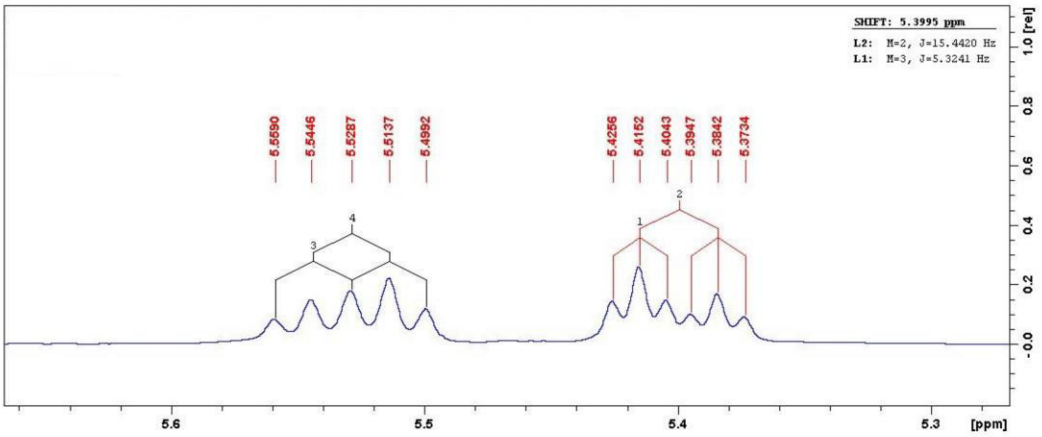

Figure S17. Part of ^{1}H-NMR spectrum of compound **16** in CD_3OD with defined multiplet and coupling constants (J =15.4, 5.3 Hz) that prove the (*E*)-configuration of double bond.

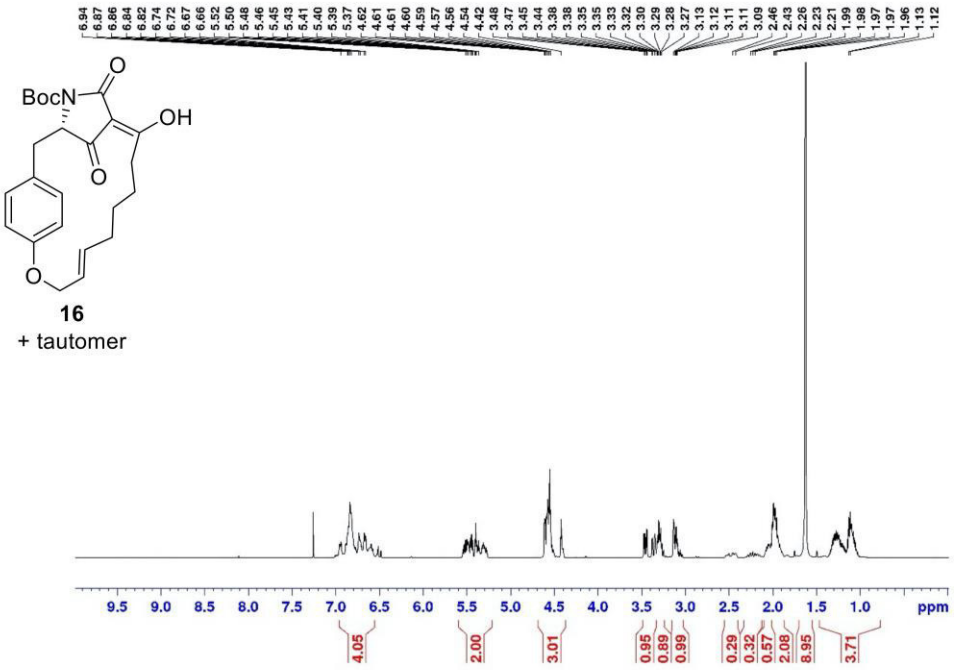

Figure S18. ^{1}H-NMR spectrum of compound **16** in $CDCl_3$.

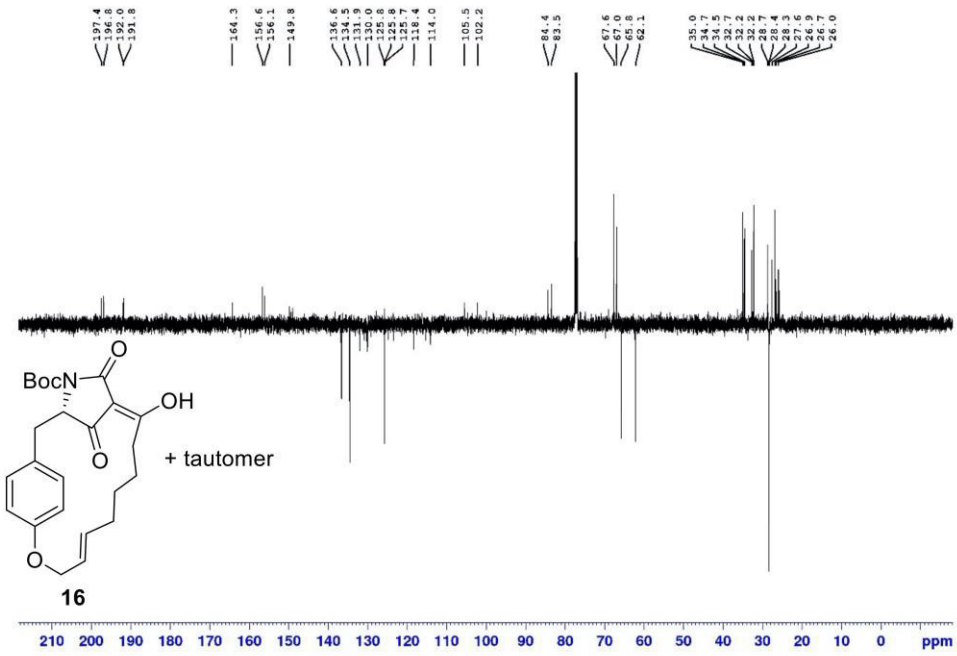

Figure S19. ^{1}H-NMR spectrum of compound **16** in $CDCl_3$.

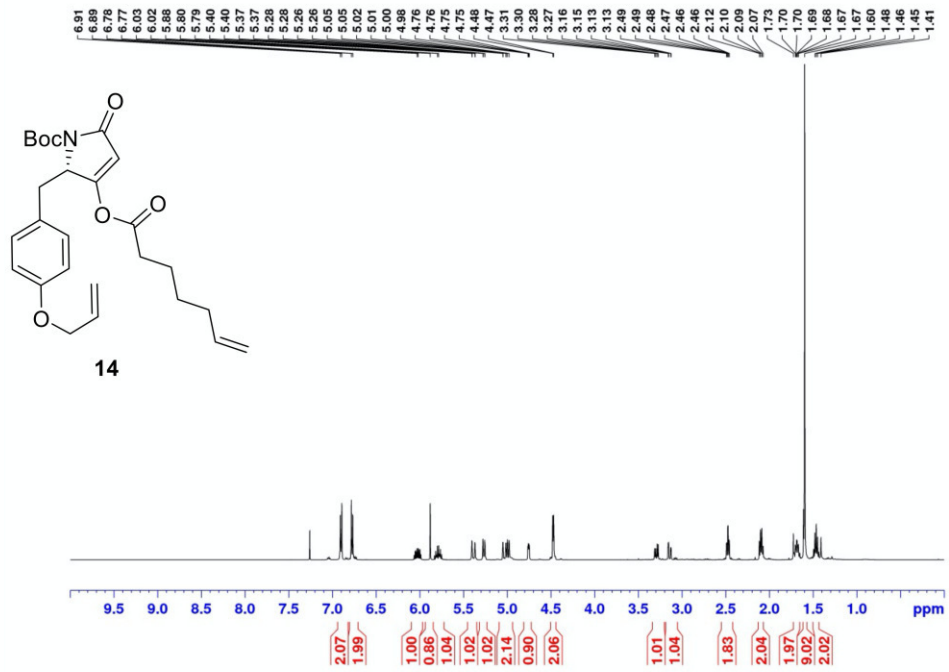

Figure S20. ^{1}H-NMR spectrum of compound **14** in $CDCl_3$.

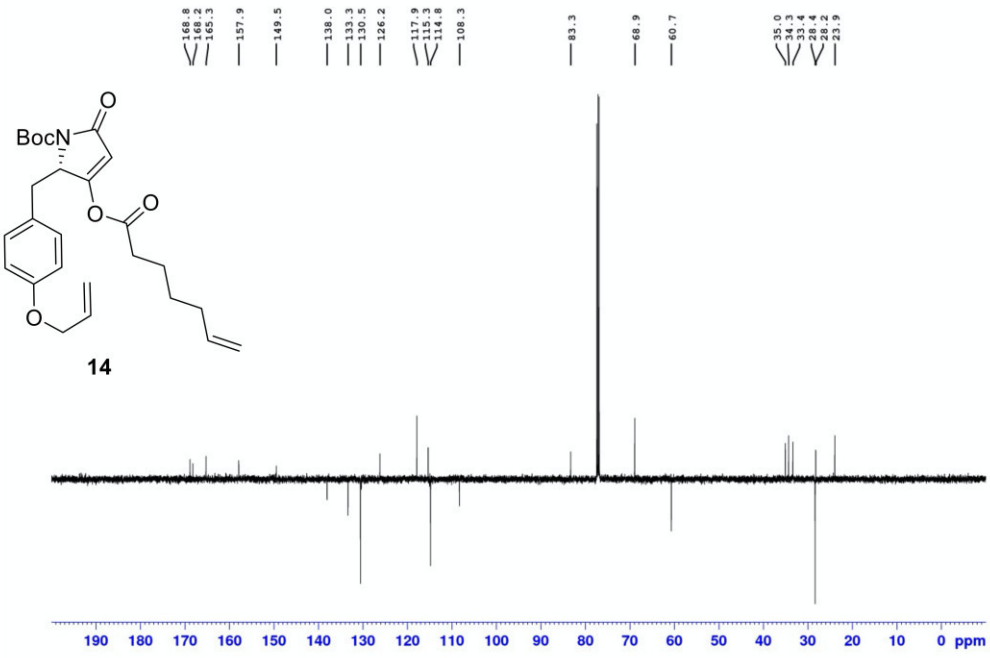

Figure S21. ^{13}C-NMR spectrum of compound **14** in $CDCl_3$.

S11

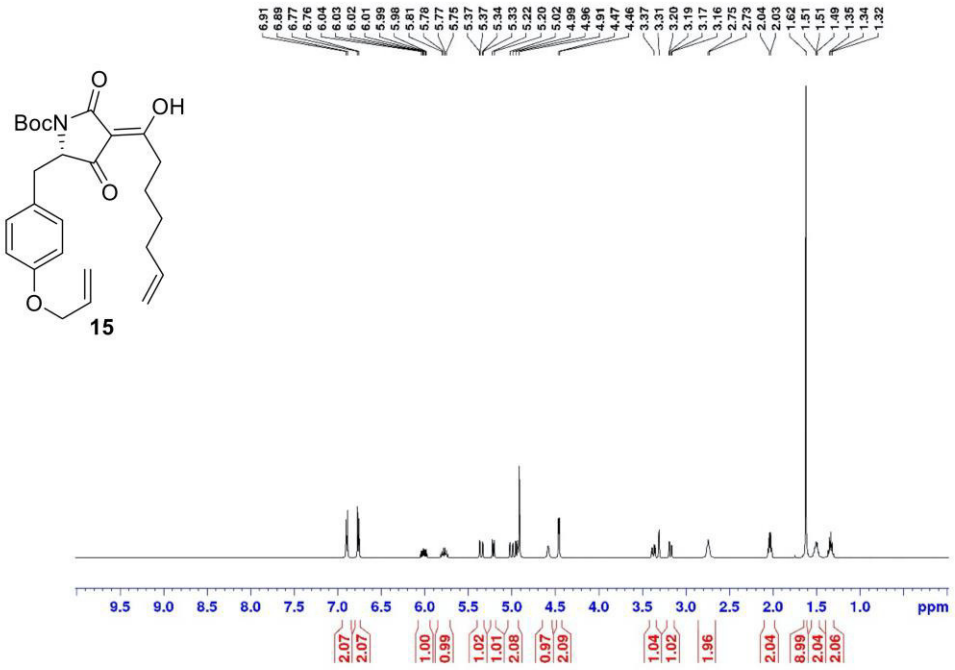

Figure S22. ^{1}H-NMR spectrum of compound **15** in CD_3OD.

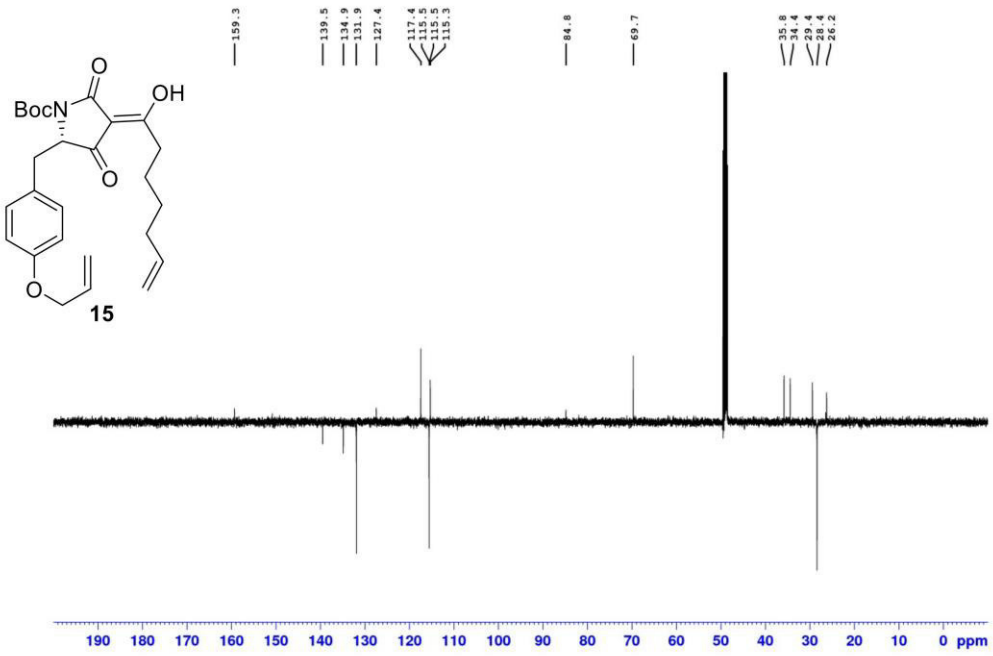

Figure S23. ^{13}C-NMR spectrum of compound **15** in CD_3OD.

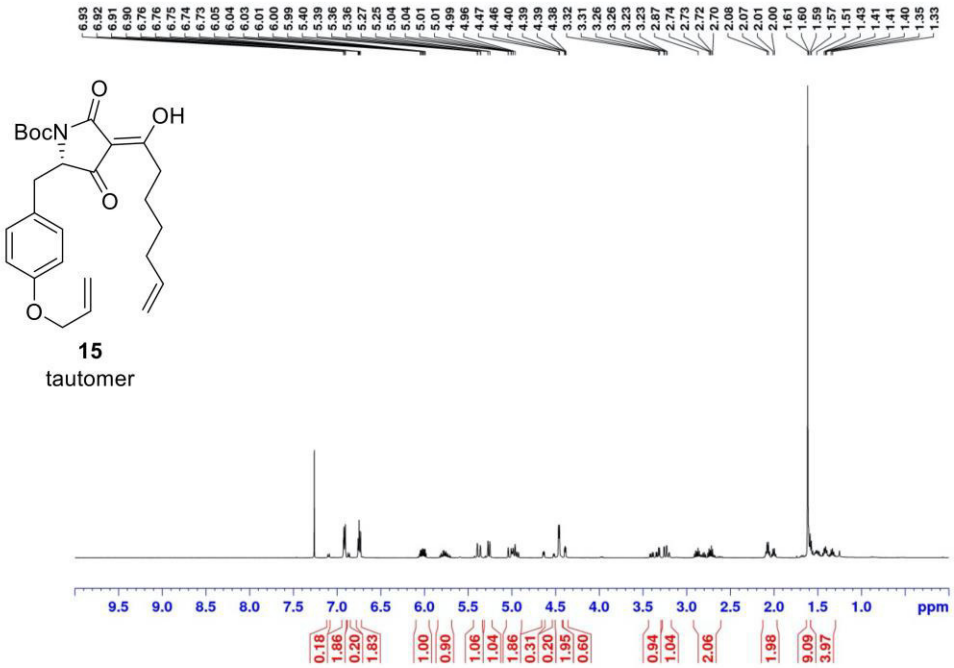

Figure S24. ^{1}H-NMR spectrum of compound **15** in $CDCl_3$.

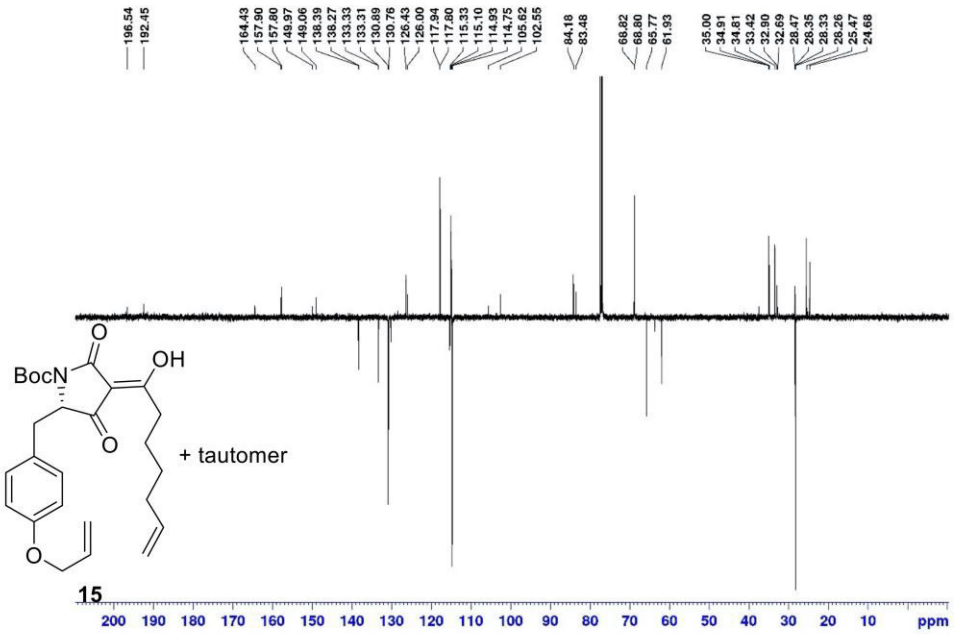

Figure S25. ^{13}C-NMR spectrum of compound **15** in $CDCl_3$.

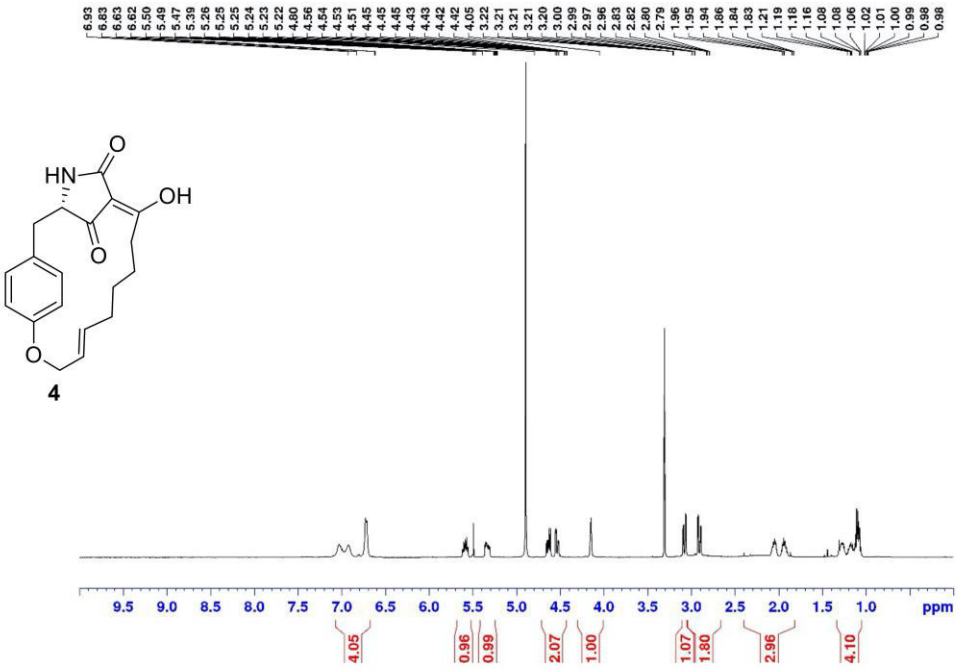

Figure S26. ^{1}H-NMR spectrum of compound **4** in CD_3OD.

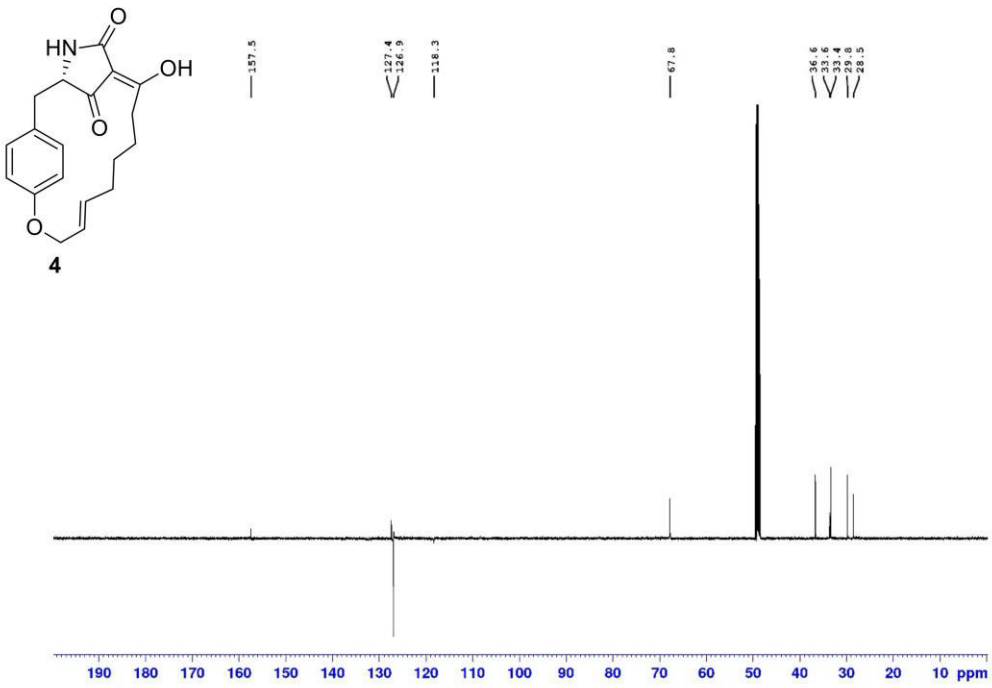

Figure S27. ^{13}C-NMR spectrum of compound **4** in CD_3OD.

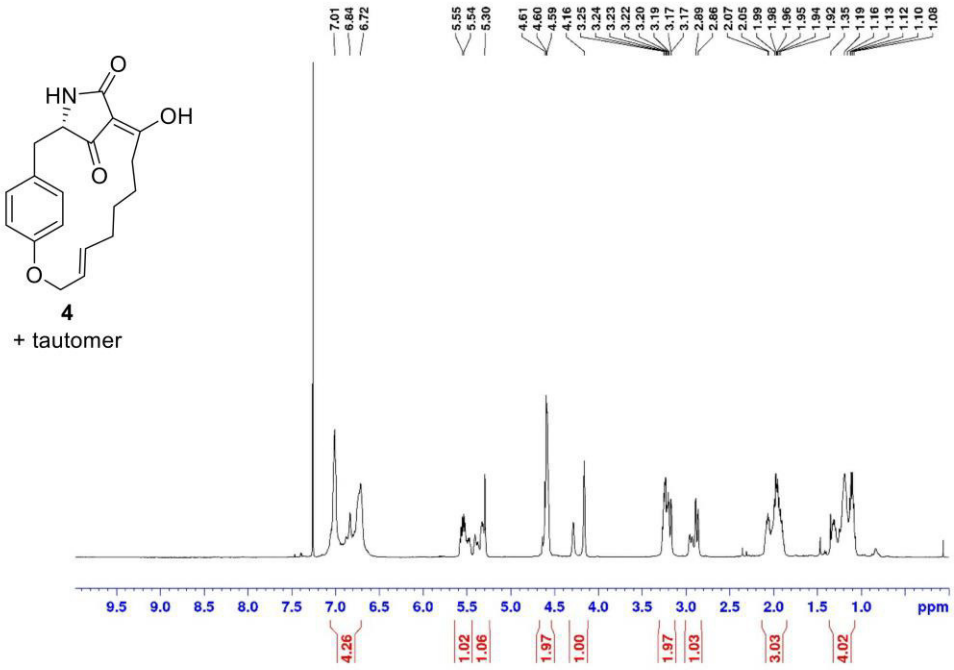

Figure S28. ^{1}H-NMR spectrum of compound **4** in $CDCl_3$.

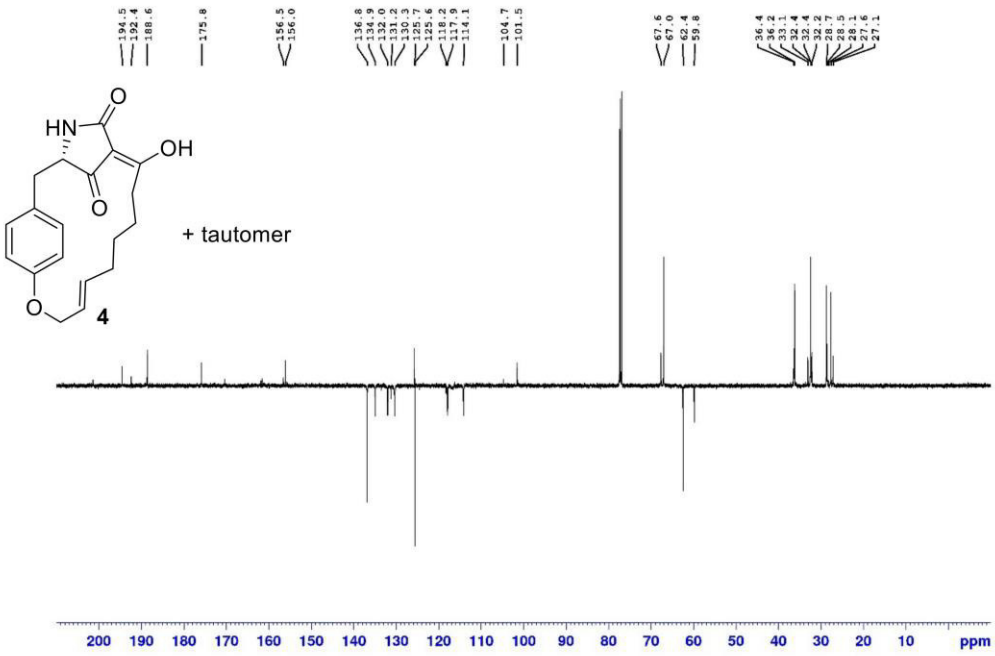

Figure S29. ^{13}C-NMR spectrum of compound **4** in $CDCl_3$.

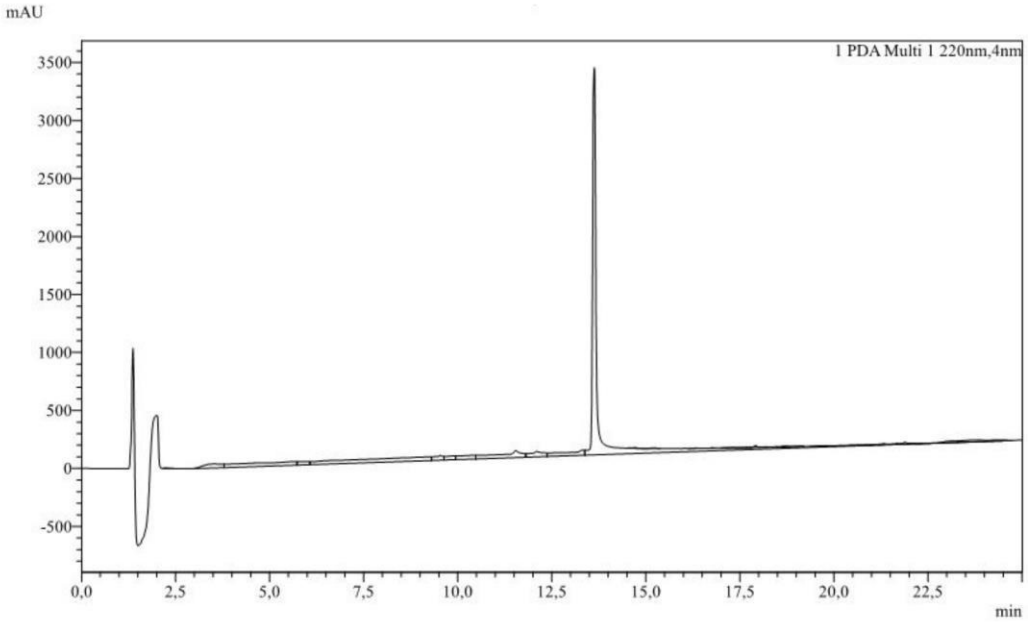

Figure S30. Chromatogram of compound **4**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 10% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

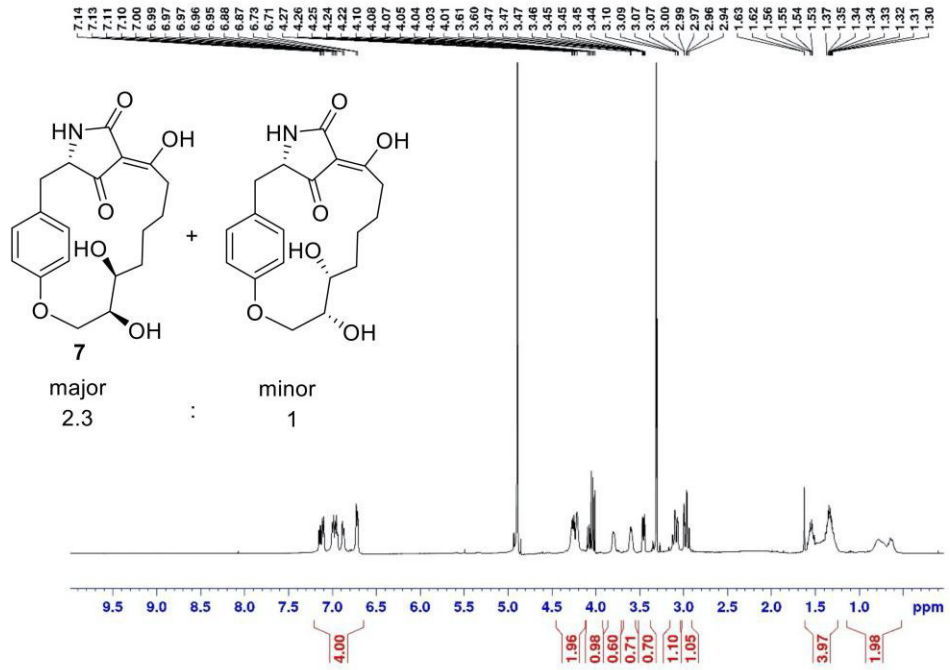

Figure S31. ^{1}H-NMR spectrum of compound **7** in CD_3OD.

S16

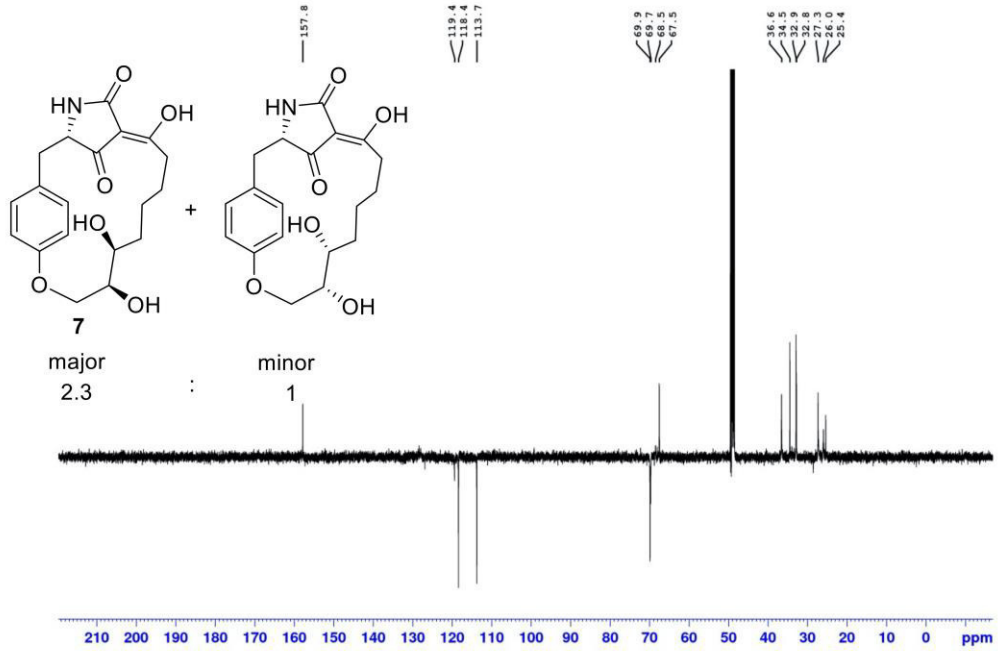

Figure S32. ^{13}C-NMR spectrum of compound **7** in CD_3OD.

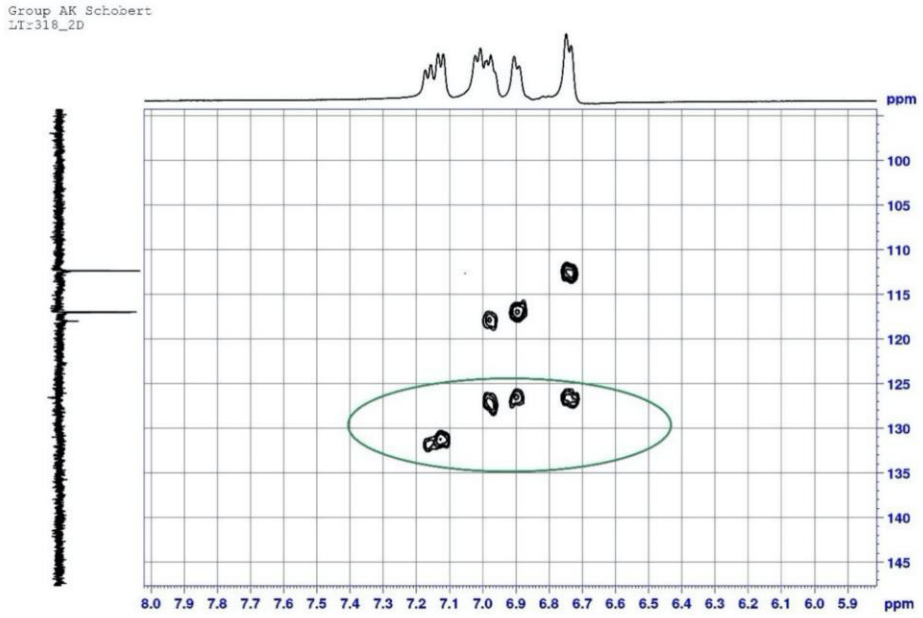

Figure S33. Part of HMBC-2D-NMR spectrum of compound **7** in CD_3OD, which was used for peak assignment.

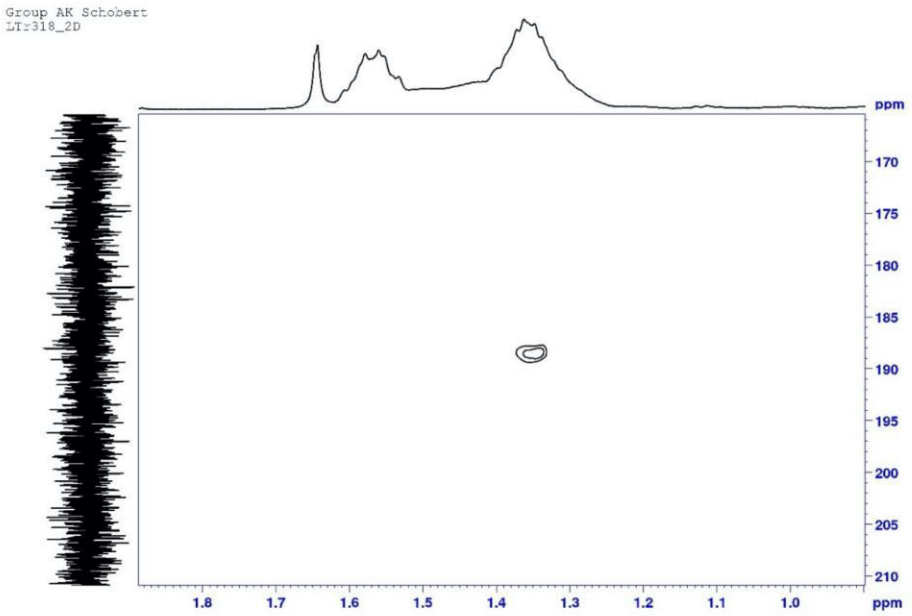

Figure S34. Part of HMBC-2D-NMR spectrum of compound **7** in CD_3OD, which was used for peak assignment.

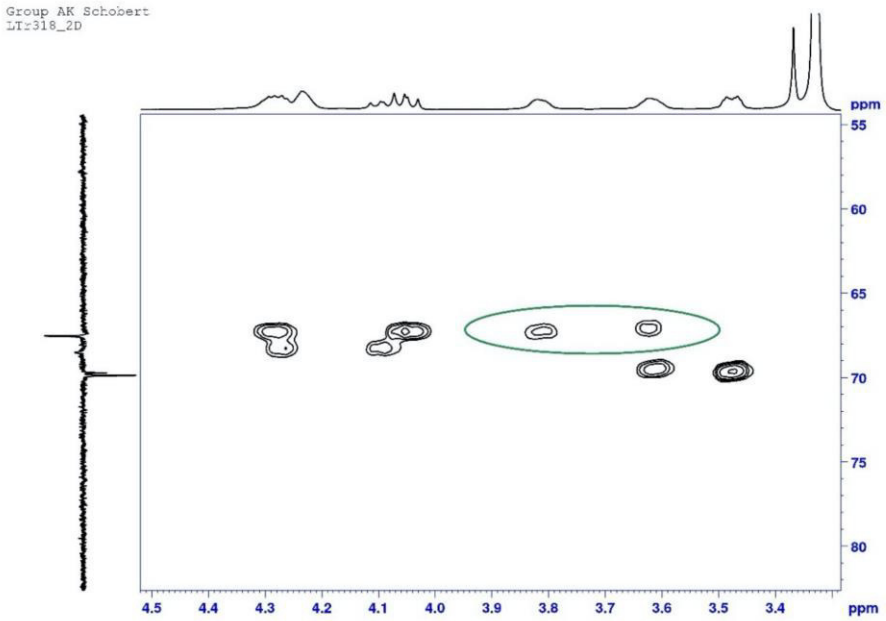

Figure S35. Part of HSQC-2D-NMR spectrum of compound **7** in CD_3OD, which was used for peak assignment.

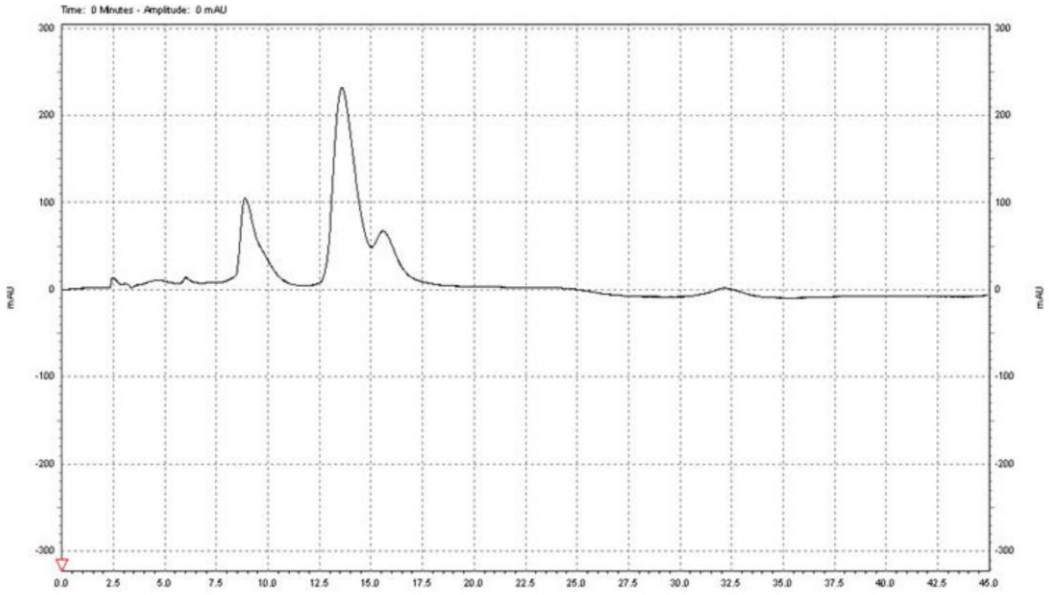

Figure S36. Chromatogram of compound **7**. HPLC: *Beckmann System Gold Programmable Solvent Modul 126,* Beckmann instruments diode array detection module 128, Phenomenex Lux® Amylose-1-HPLC column (100 × 4.6 mm). Method: 30% EtOH in hexanes → 40% EtOH in hexanes → 50% EtOH in hexanes → 60% EtOH in hexanes → 80% EtOH in hexanes, flow: 0.7 mL/min. A mixture of tautomers and diastereomers can be seen

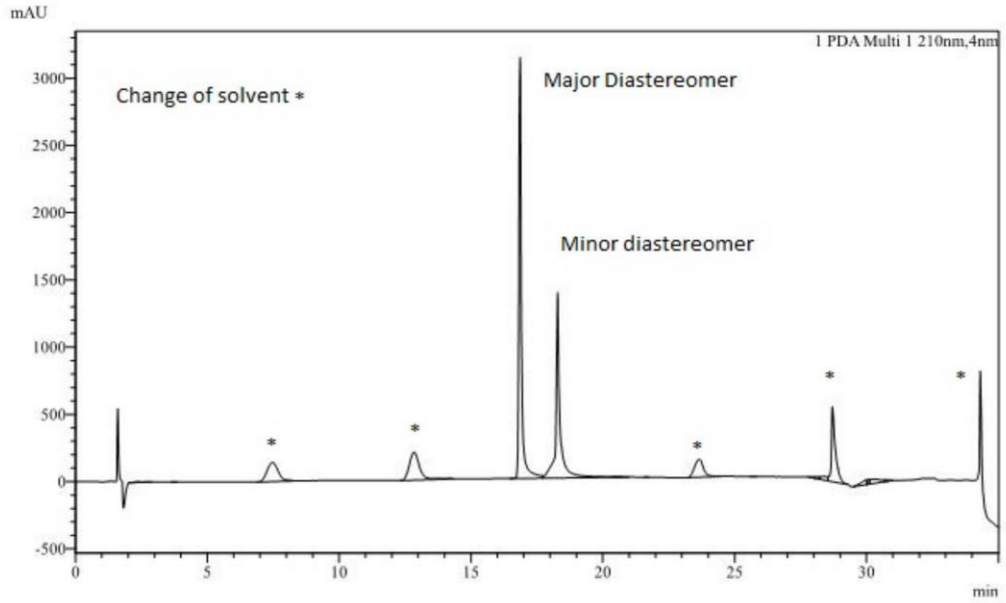

Figure S37. Chromatogram of compound **7**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 5% MeCN in H_2O + 0.1% HCOOH → 10% MeCN in H_2O + 0.1% HCOOH → 20% MeCN in H_2O + 0.1% HCOOH → 30% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH→ 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

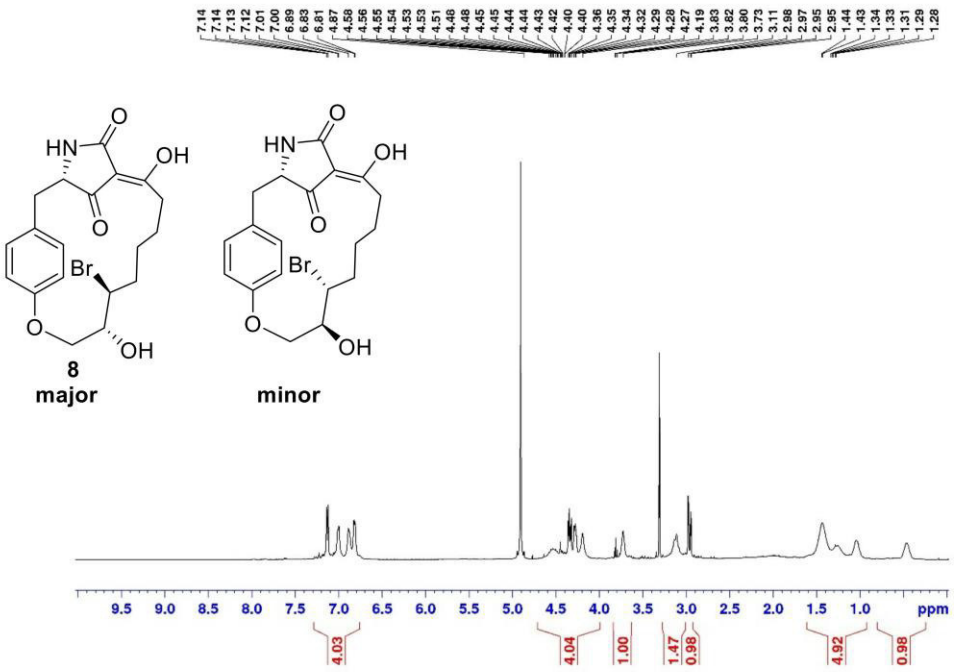

Figure S38. ^{1}H-NMR spectrum of compound **8** in CD_3OD.

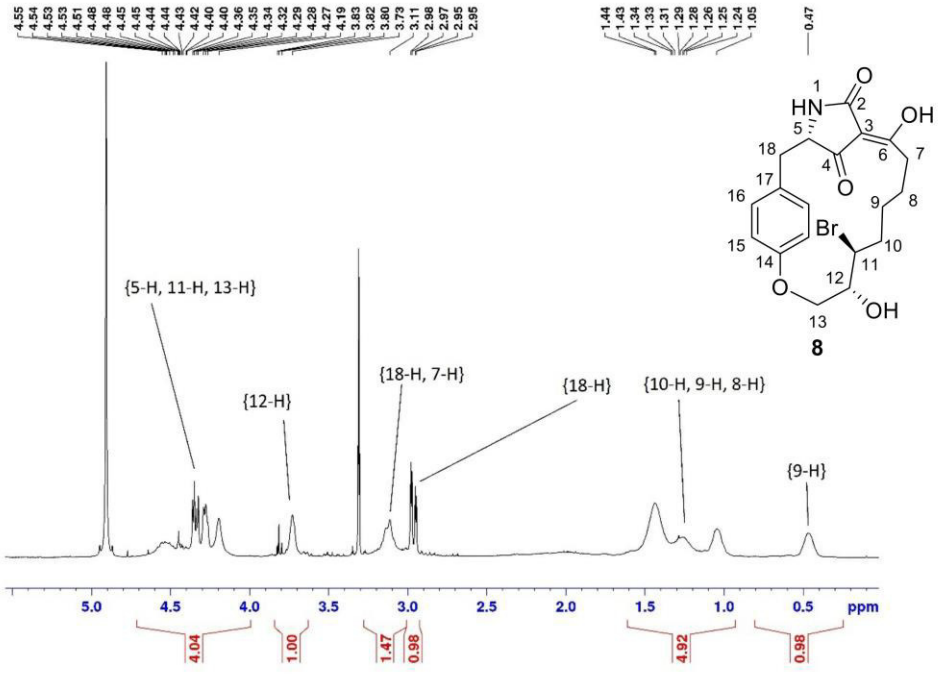

Figure S39. ^{1}H-NMR spectrum of compound **8** between 5.5 ppm and 0.0 ppm in CD_3OD with assignment of signals.

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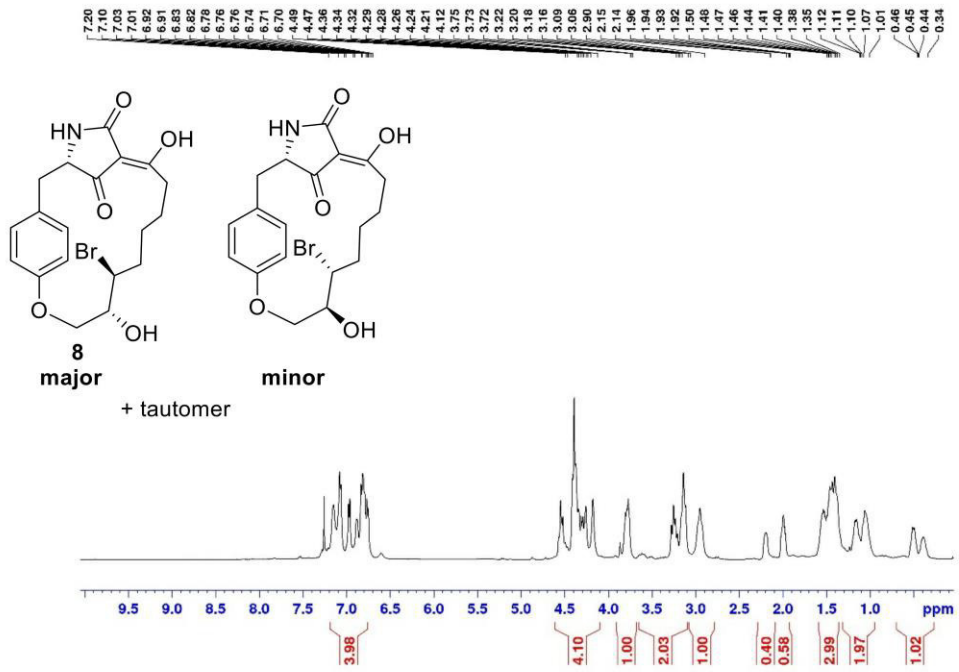

Figure S40. ^{1}H-NMR spectrum of compound **8** in $CDCl_3$.

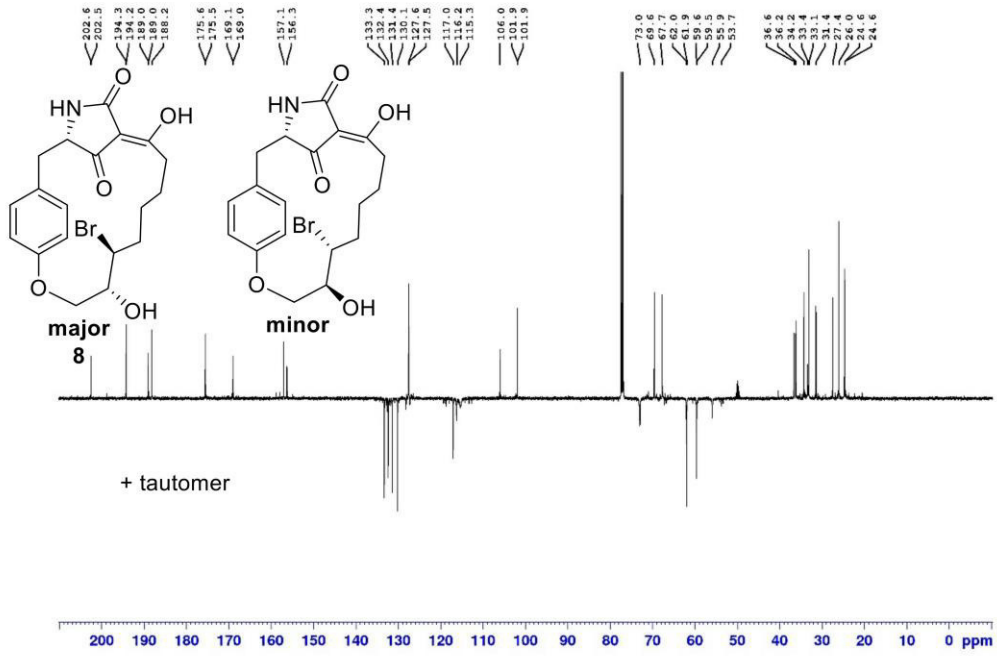

Figure S41. ^{13}C-NMR spectrum of compound **8** in $CDCl_3$.

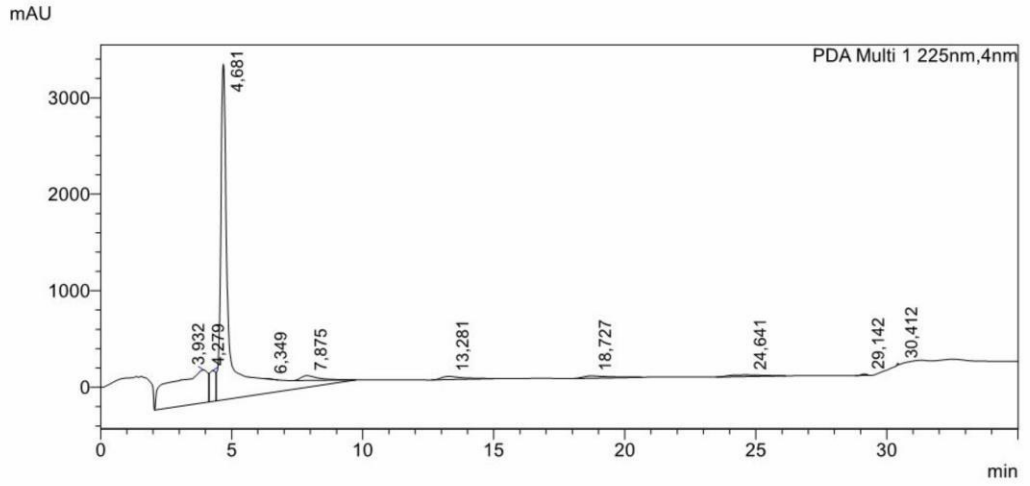

Figure S42. Chromatogram of compound **8**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 30% MeCN in H_2O + 0.1% HCOOH → 35% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 45% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH→ 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

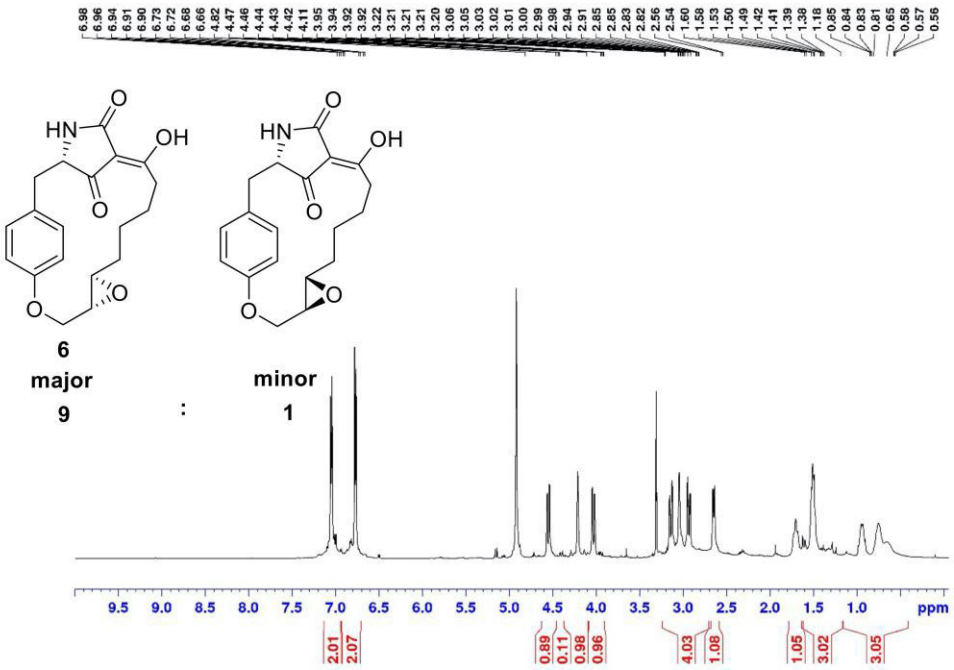

Figure S43. ^{1}H-NMR spectrum of compound **6** in CD_3OD.

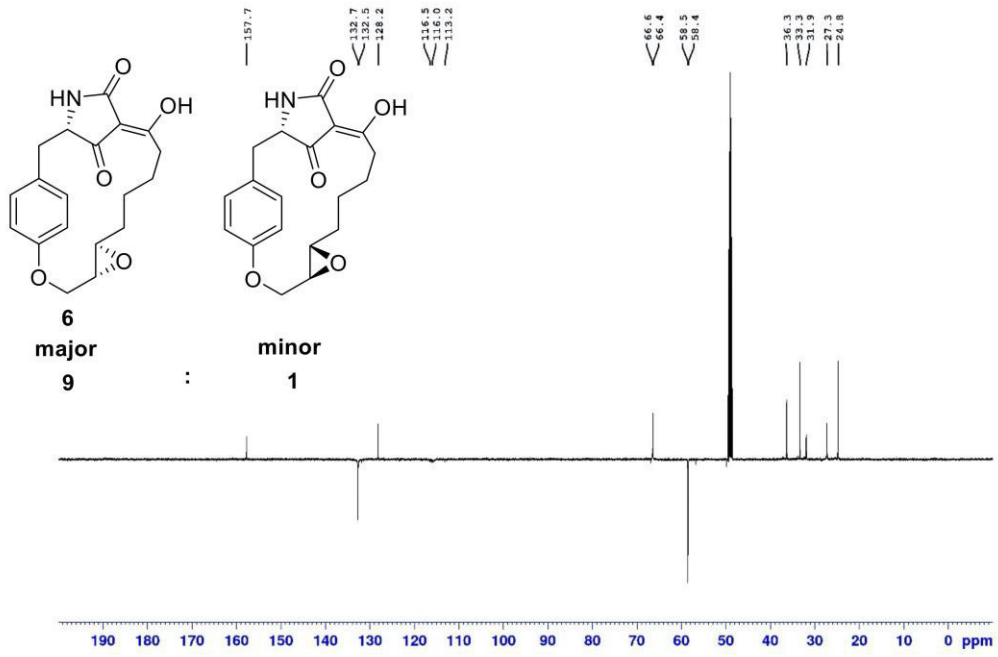

Figure S44. ^{13}C-NMR spectrum of compound **6** in CD_3OD.

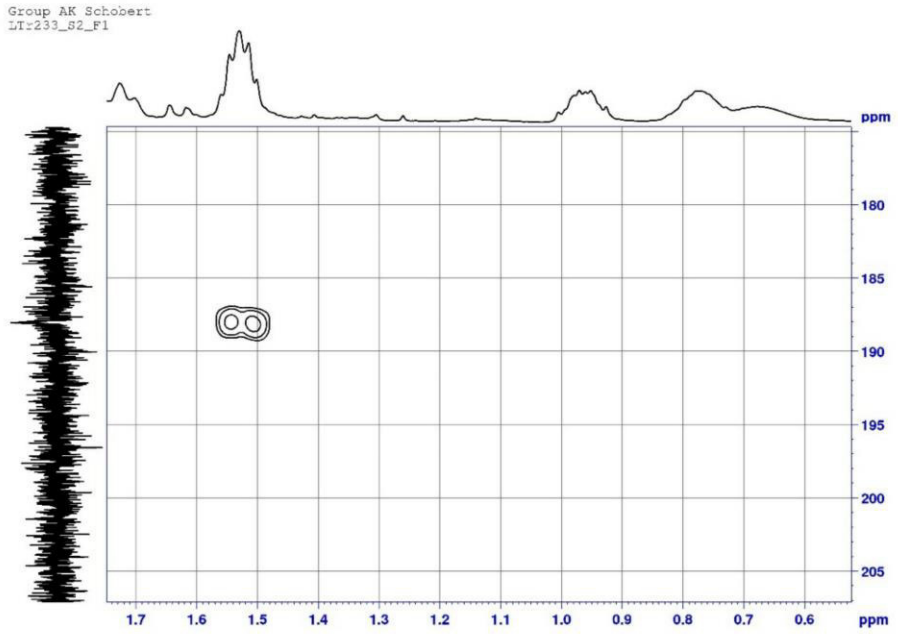

Figure S45. Part of HMBC-2D-NMR spectrum of compound **6** in CD_3OD, which was used for peak assignment.

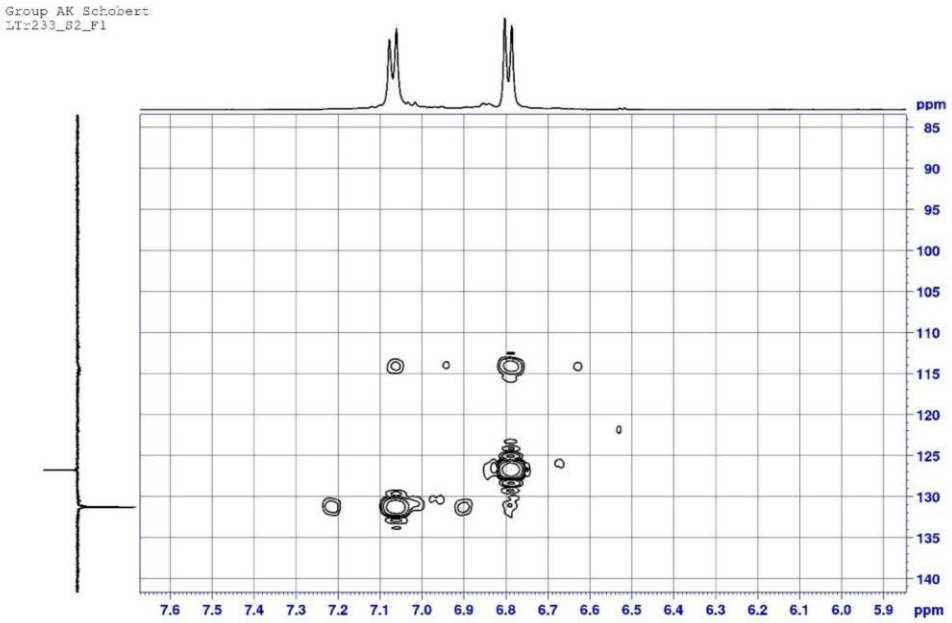

Figure S46. Part of HMBC-2D-NMR spectrum of compound **6** in CD_3OD, which was used for peak assignment.

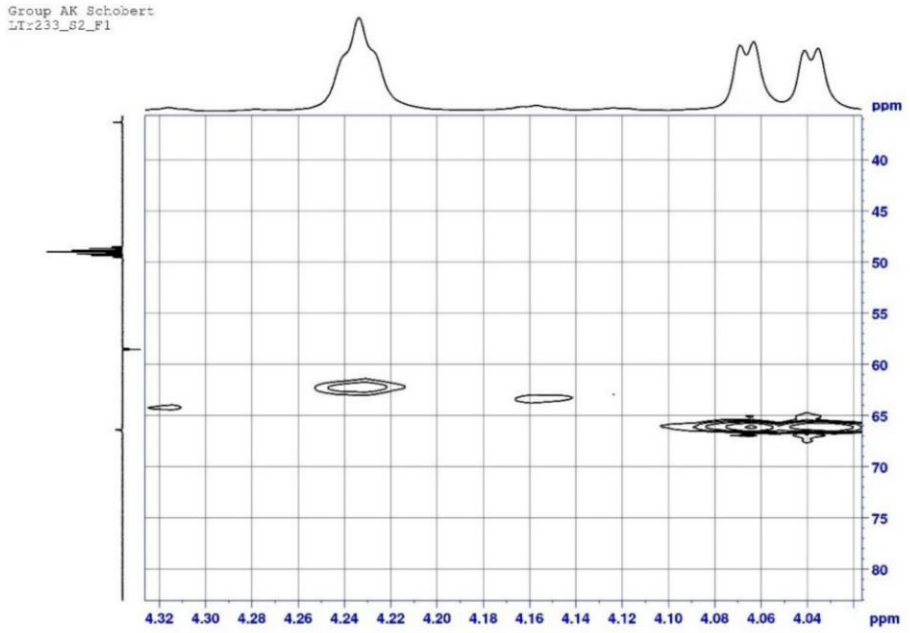

Figure S47. Part of HSQC-2D-NMR spectrum of compound **6** in CD_3OD, which was used for peak assignment.

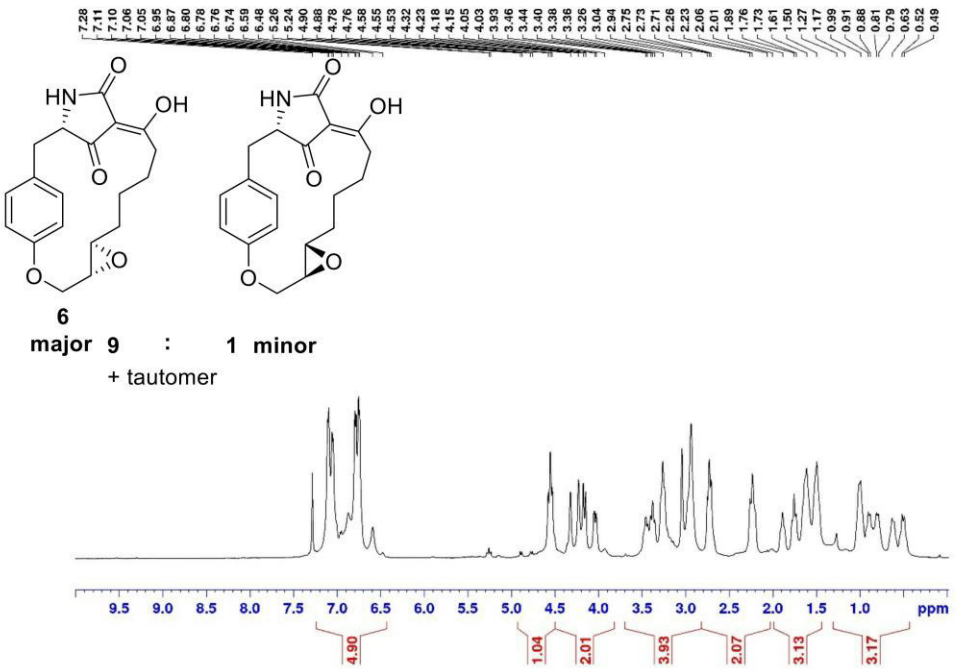

Figure S48. ^{1}H-NMR spectrum of compound **6** in $CDCl_3$.

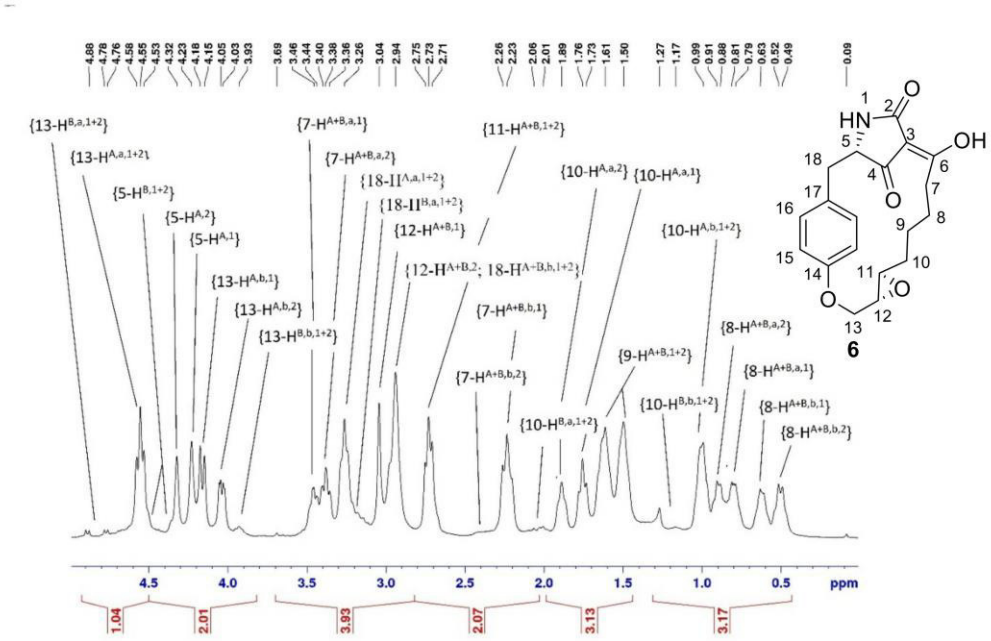

Figure S49. ^{1}H-NMR spectrum of compound **6** between 5.0 ppm and 0.0 ppm in $CDCl_3$ with assignment of signals. Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b; signals of major tautomer marked as 1, signals of minor tautomer marked as 2.

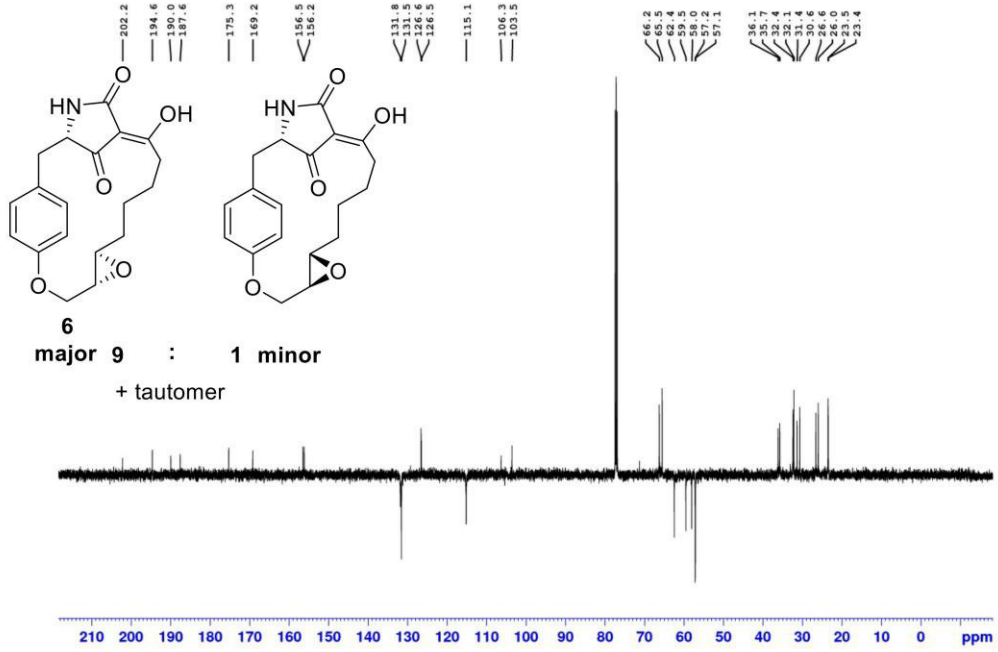

Figure S50. ^{13}C-NMR spectrum of compound **6** in $CDCl_3$.

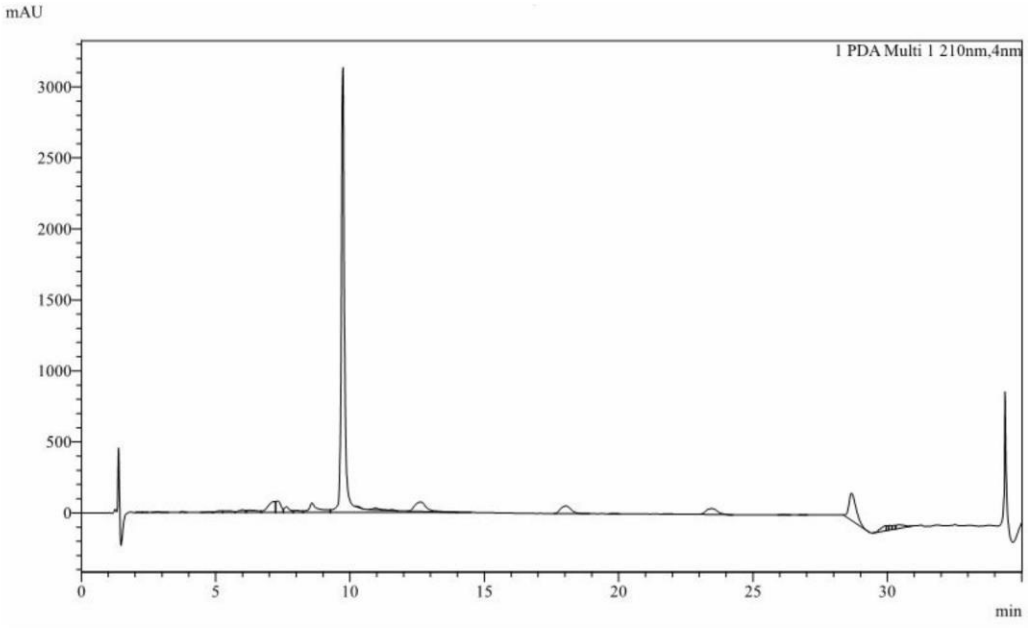

Figure S51. Chromatogram of compound **6**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 30% MeCN in H_2O + 0.1% HCOOH → 35% MeCN in H_2O + 0.1% HCOOH → 40% MeCN in H_2O + 0.1% HCOOH → 45% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH→ 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

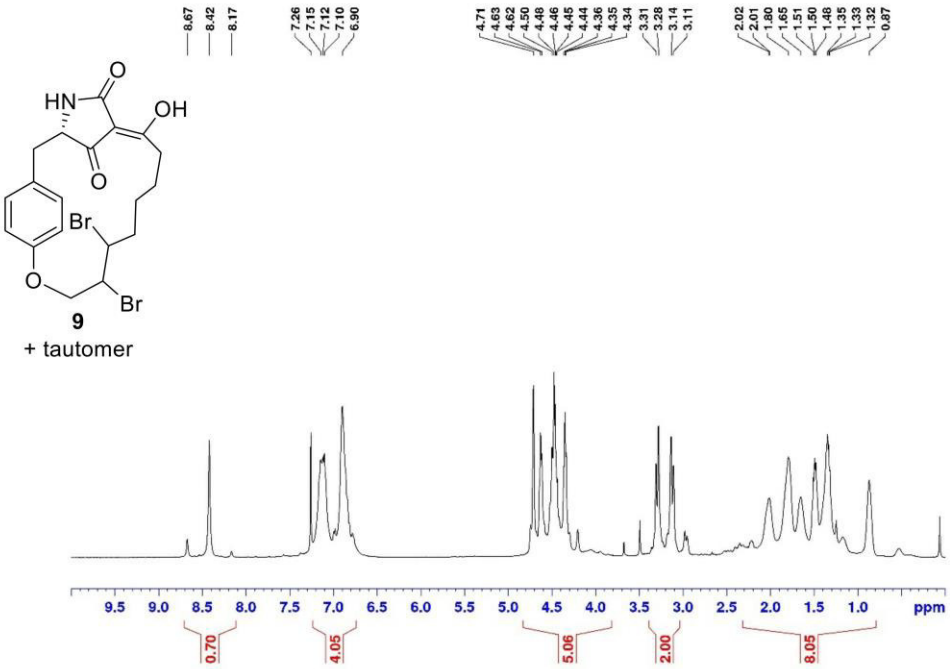

Figure S52. ^{1}H-NMR spectrum of compound **9** in $CDCl_3$.

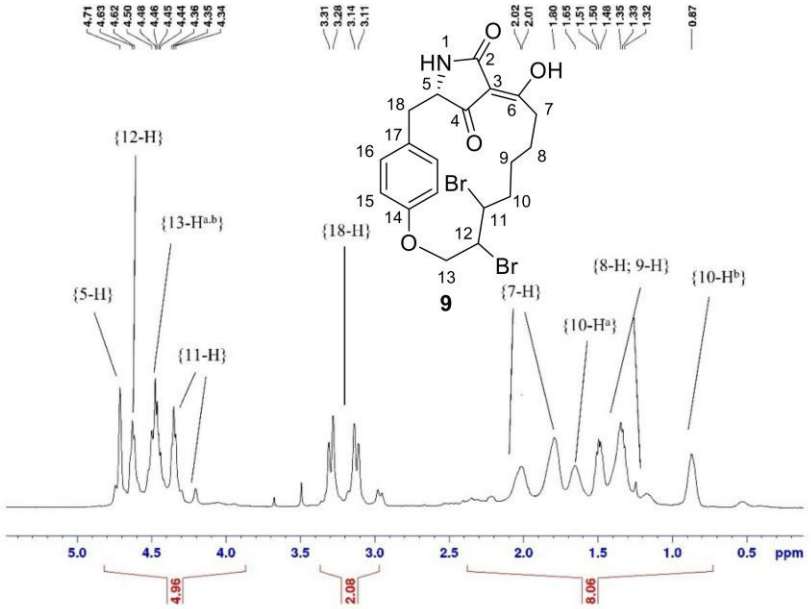

Figure S53. ^{1}H-NMR spectrum of compound **9** between 5.0 ppm and 0.0 ppm in $CDCl_3$ with assignment of signals. Signals of major diastereomer marked as A, signals of minor diastereomer marked as B; diastereotopic H-atoms indicated as a, b; signals of major tautomer marked as 1, signals of minor tautomer marked as 2.

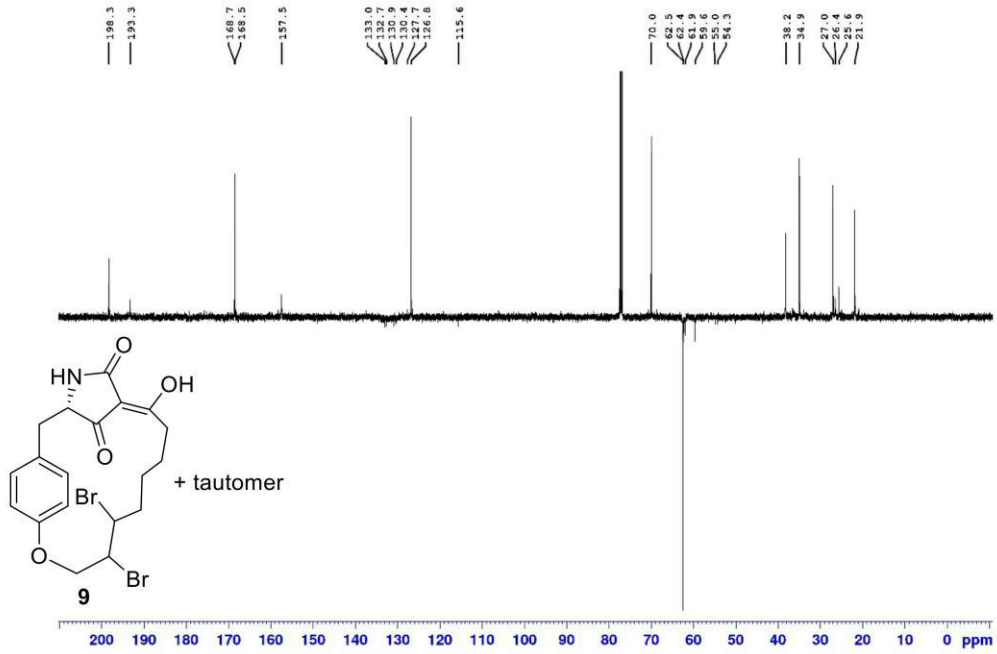

Figure S54. ^{13}C-NMR spectrum of compound **9** in $CDCl_3$.

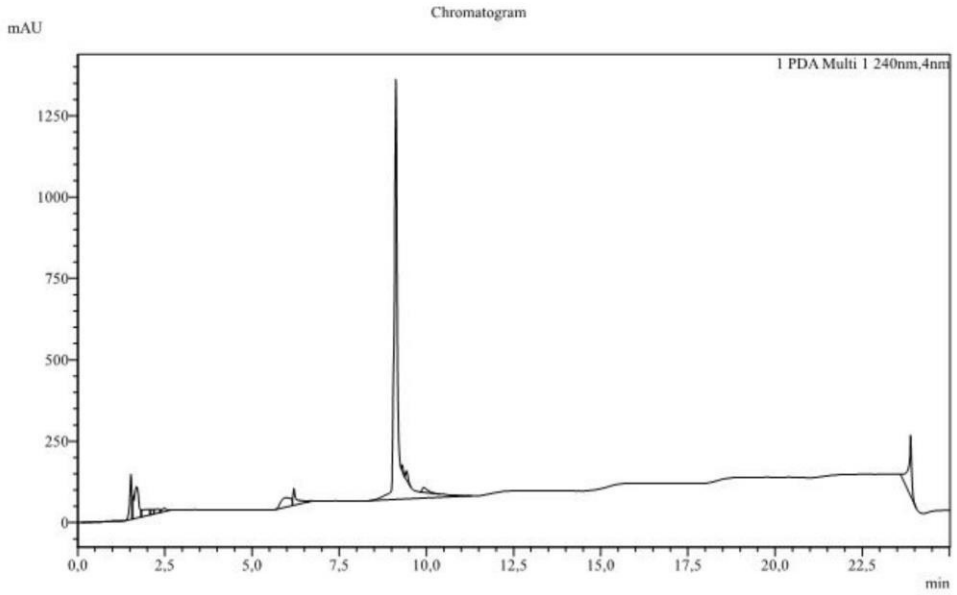

Figure S55. Chromatogram of compound **9**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 70% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 90% MeCN in H_2O + 0.1% HCOOH → 95% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

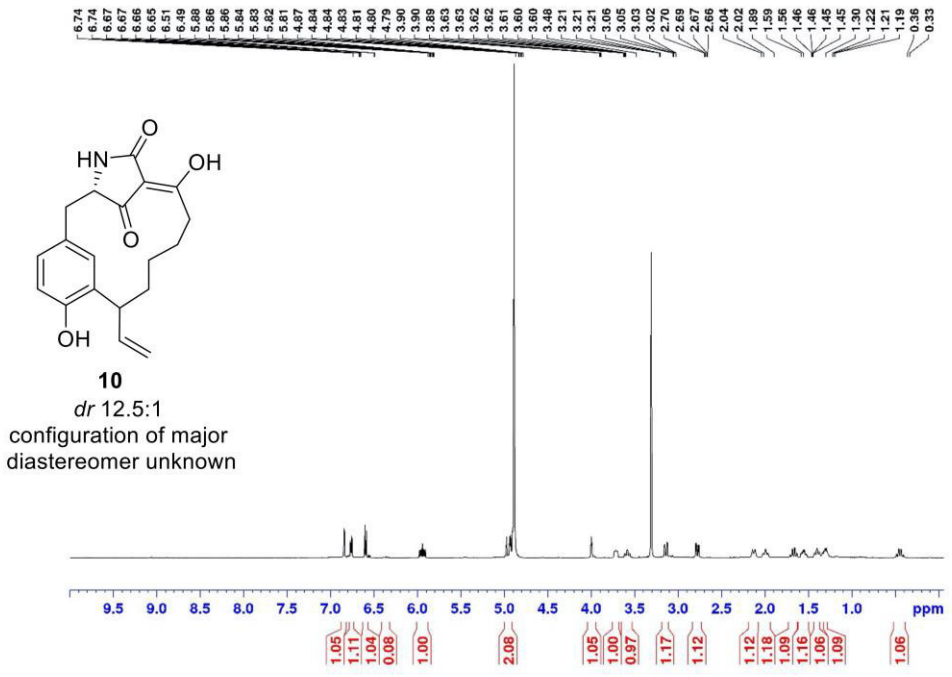

Figure S56. ^{1}H-NMR spectrum of compound **10** in CD_3OD.

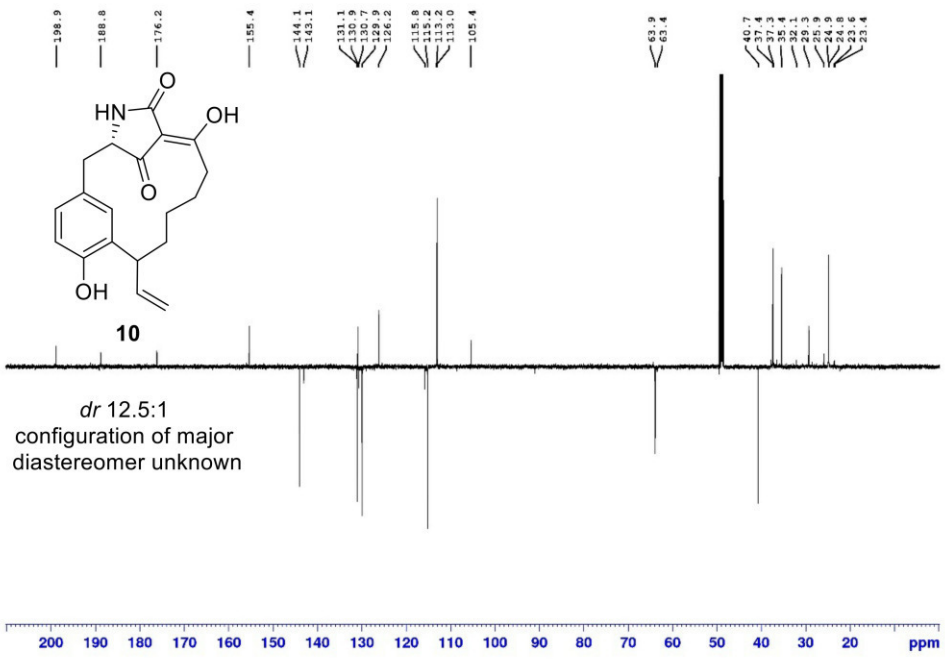

Figure S57. ^{13}C-NMR spectrum of compound **10** in CD_3OD.

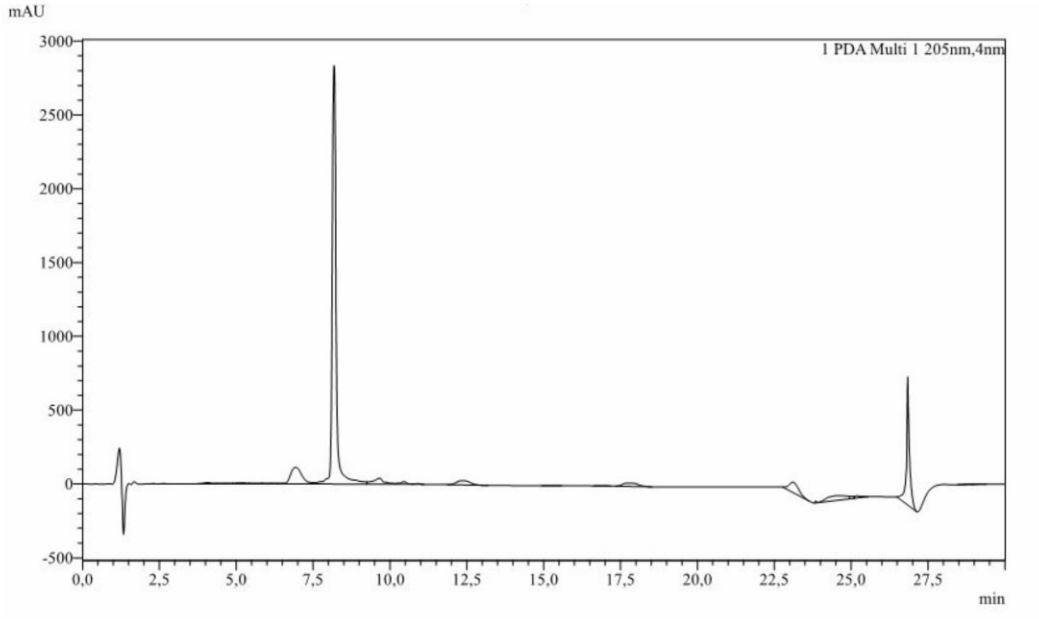

Figure S58. Chromatogram of compound **10**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 50% MeCN in H_2O + 0.1% HCOOH → 55% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

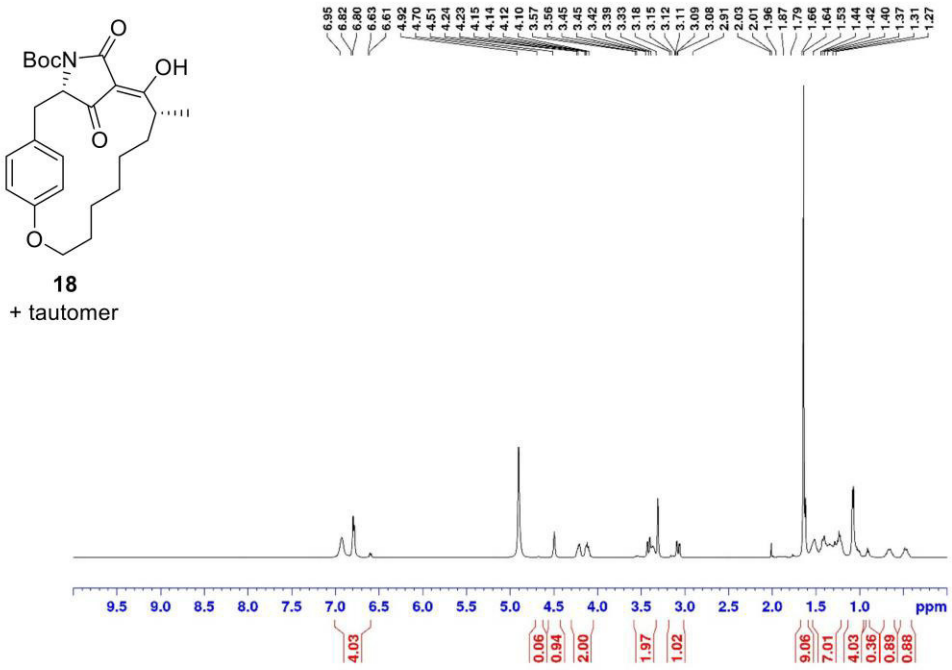

Figure S59. ^{1}H-NMR spectrum of compound **18** in CD_3OD.

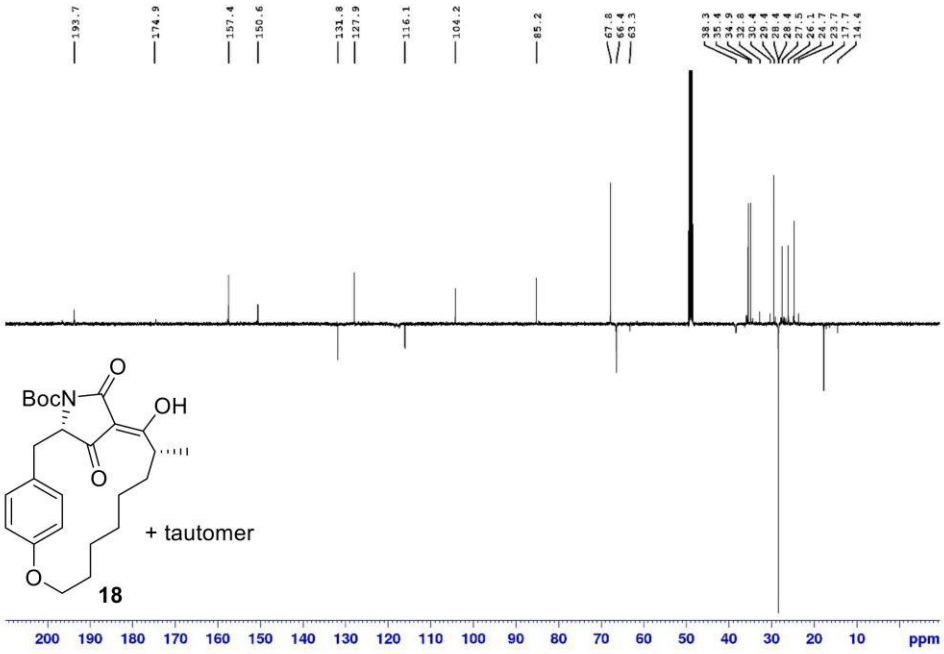

Figure S60. ^{13}C-NMR spectrum of compound **18** in CD_3OD.

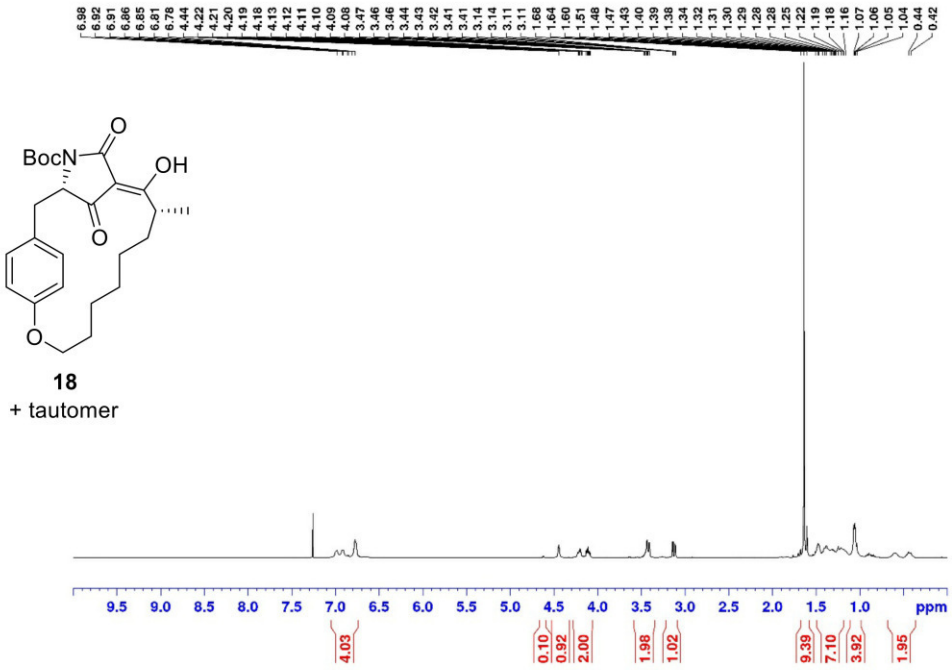

Figure S61. ^{1}H-NMR spectrum of compound **18** in $CDCl_3$.

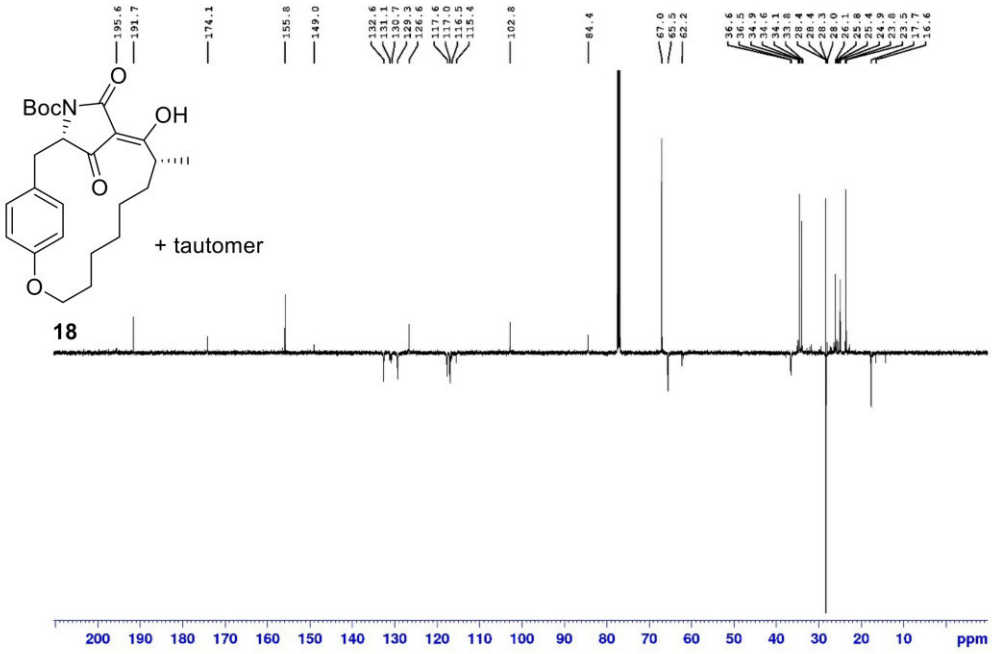

Figure S62. ^{13}C-NMR spectrum of compound **18** in $CDCl_3$.

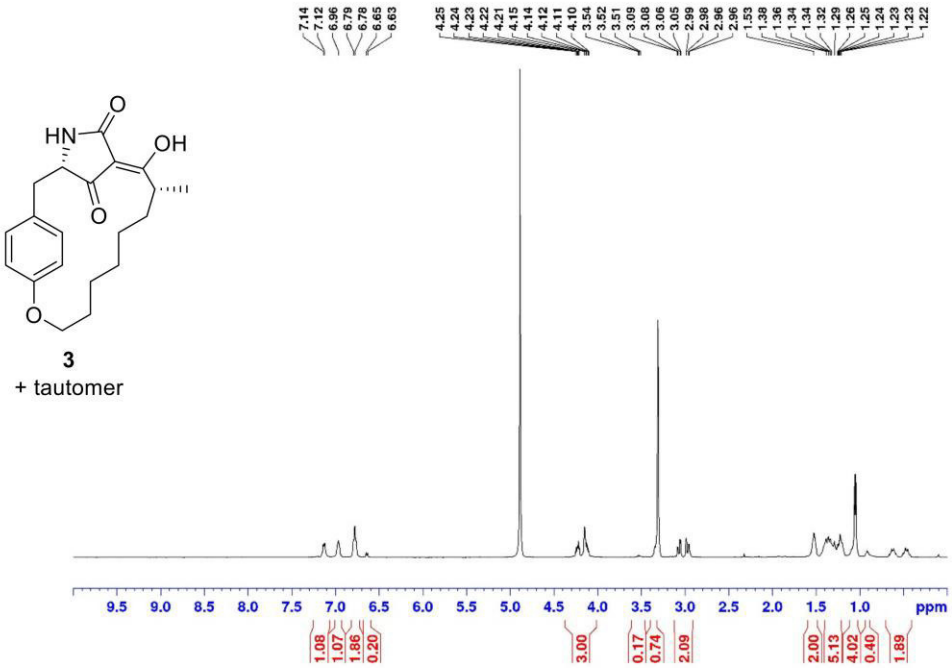

Figure S63. ^{1}H-NMR spectrum of compound **3** in CD_3OD.

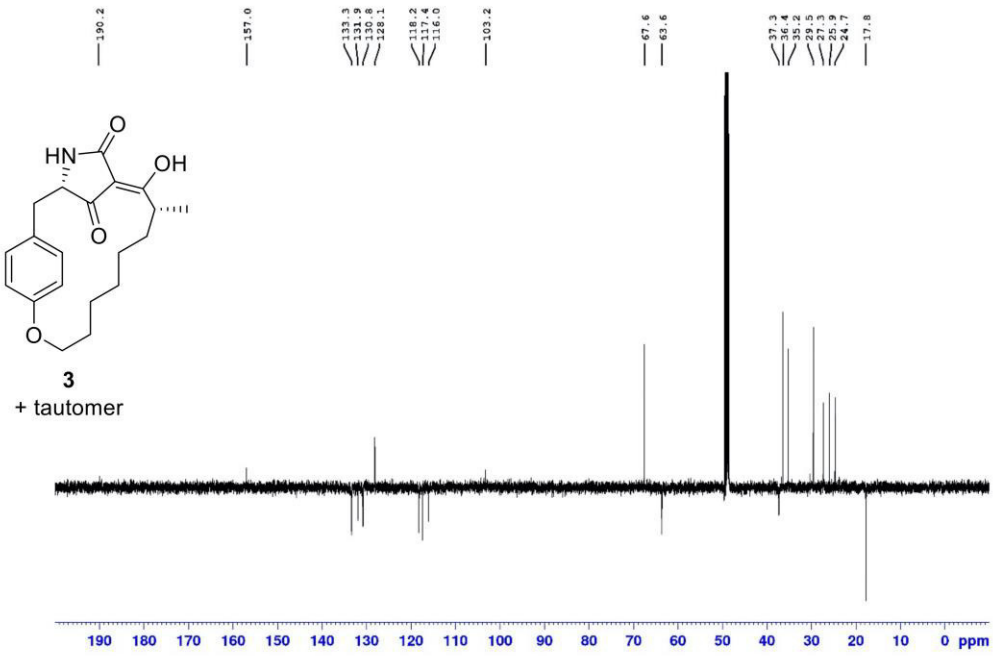

Figure S64. ^{13}C-NMR spectrum of compound **3** in CD_3OD.

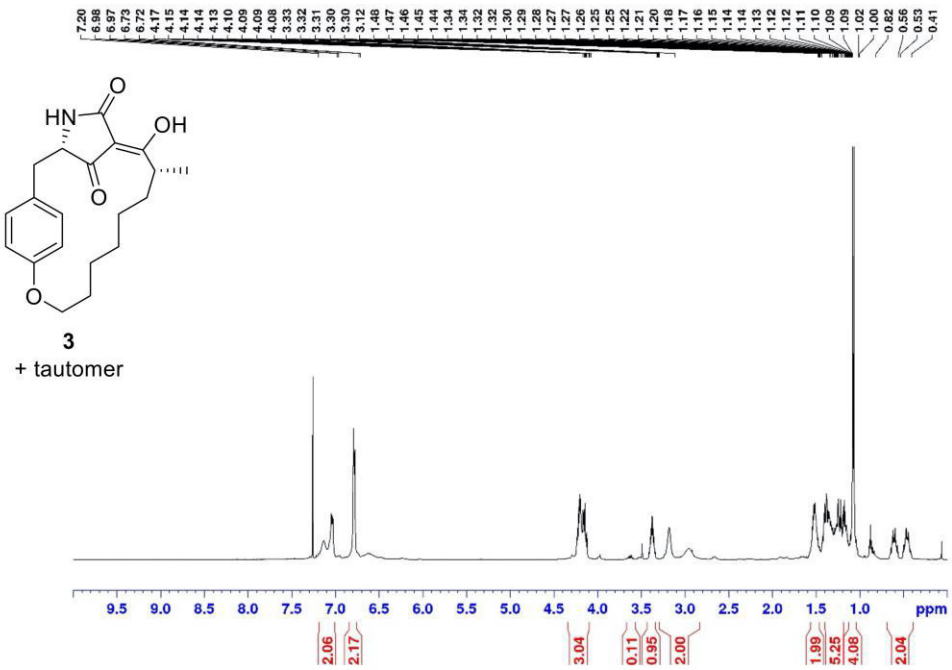

Figure S65. ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

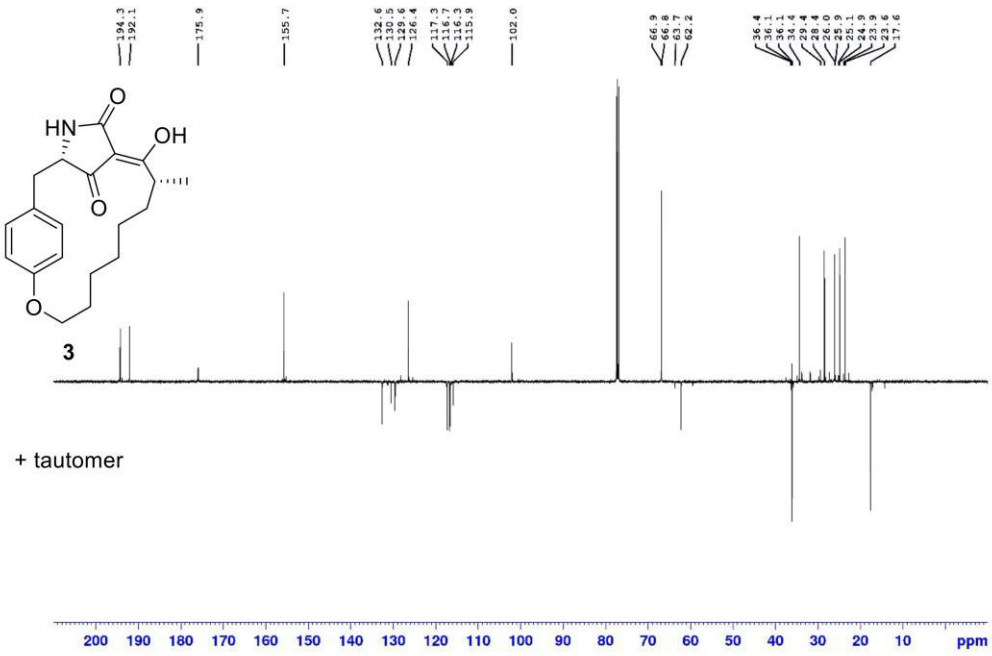

Figure S66. ^{13}C-NMR spectrum of compound **3** in $CDCl_3$.

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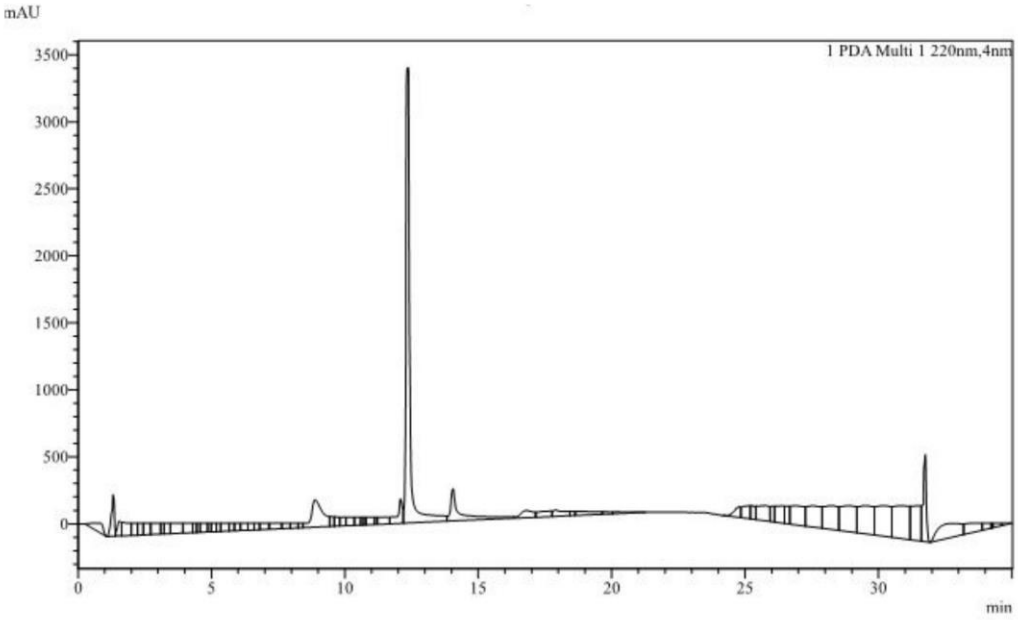

Figure S67. Chromatogram of compound **3**. HPLC: *Shimadzu Nexera XR,* Autosampler *SIL-20A*, diode array detector *SPD-M20A*, C18-column (150 × 4 mm). Method: 40% MeCN in H_2O + 0.1% HCOOH → 60% MeCN in H_2O + 0.1% HCOOH → 80% MeCN in H_2O + 0.1% HCOOH → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

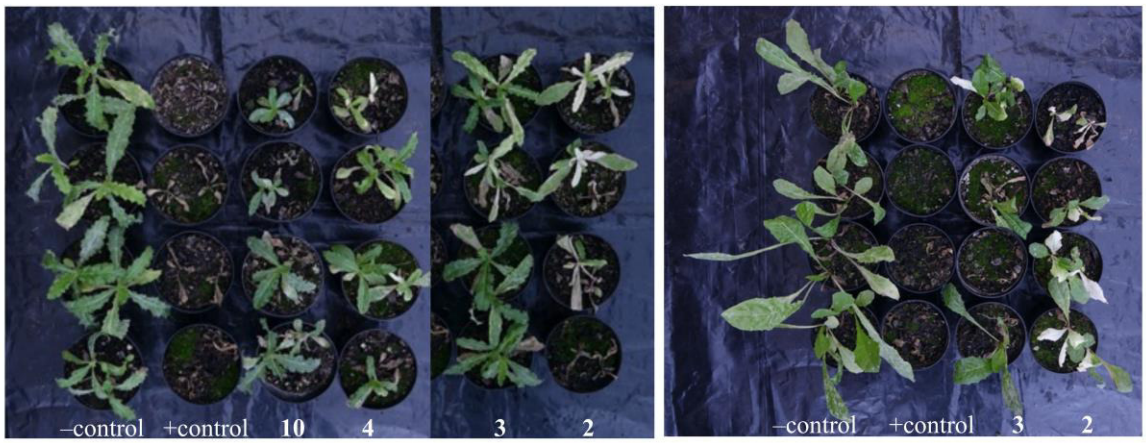

Figure S68: Thistles (left) and dandelions (right), 28 d after treatment with compounds **10** (150 mM), **4** (150 mM), **3** (100 mM) and **2** (100 mM). Negative control: isopropanol:water = 1:1 + 0.25% Tween20; positive control: commercial herbicide diflufenican (1.2 mM).

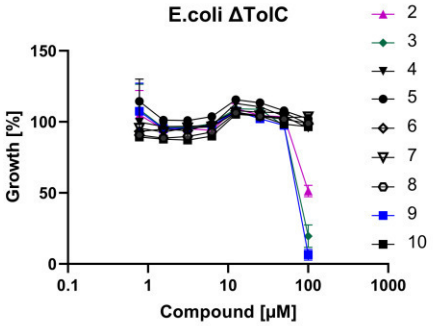

Figure S69. Growth inhibitory effects of various concentrations of macrocidin derivatives on *E. coli ΔTolC* cultures.

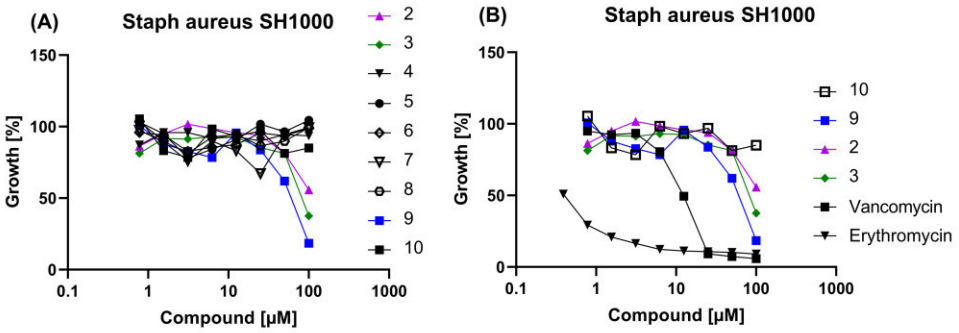

Figure S70. Growth inhibitory effects of various concentrations of (A) all macrocidinoids and of (B) the most active macrocidin derivatives **2**, **3** and **9**, and vancomycin and erythromycin on *S. aureus* (SH1000) cultures.

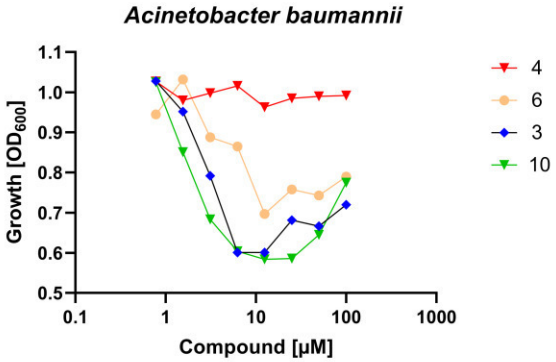

Figure S71. Growth inhibitory effects of various concentrations of selected macrocidin derivatives on *Acinetobacter baumannii* cultures.

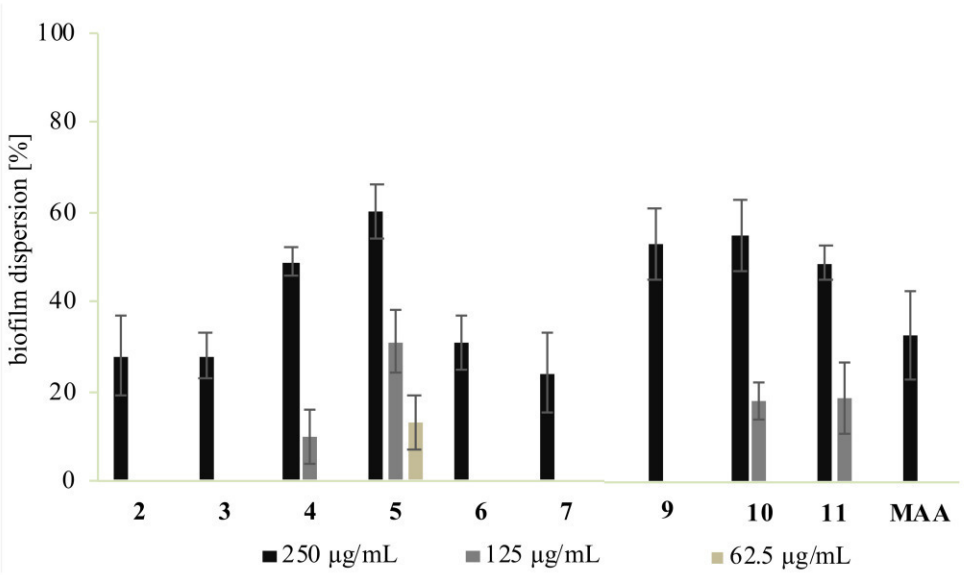

Figure S72. Dispersal effects on preformed biofilms of *C. albicans* by compounds **2**-**7** and **9**-**11** at various concentrations, error bars indicate SD.

Table S1. Antibacterial effects of compounds **2-10** on *E. coli* ΔTolC and *S. aureus*.
Inhibitory concentrations IC_{50} [µM] as determined by the broth microdilution method [ref. 21 and section 4. Materials and methods].

Compound	*Escherichia coli ΔTolC* [µM]	*Staphylococcus aureus SH1000* [µM]
2	100	100 ± 20
3	82 ± 15	83 ± 20
4	inactive	inactive
5	inactive	inactive
6	inactive	inactive
7	inactive	inactive
8	inactive	inactive
9	75 ± 15	57 ± 20
10	inactive	inactive
Vancomycin		12 ± 2

Table S2. Effects of compounds **2-10** on the formation of *S. aureus* biofilms and their dispersive effects on preformed biofilms of *S. aureus* and *C. albicans*, SD values are shown in brackets.

		Biofilm inhibition / dispersion effects [%]								
compounds	**organisms**	**250 µg/mL**	**125 µg/mL**	**62.5 µg/mL**	**31.3 µg/mL**	**15.6 µg/mL**	**7.8 µg/mL**	**3.9 µg/mL**	**2 µg/mL**	**1.3 µg/mL**
	S. aureus	85 (±3)	84 (±3)	84 (±2)	82 (±1)	74 (±1)	69 (±2)	56 (±4)	30 (±5)	30 (±10)
2	**preformed *S. aureus***	82 (±4)	85 (±3)	82 (±3)	74 (±8)	49 (±9)	30 (±5)	/	/	/
	preformed *C. albicans*	28 (±9)	/	/	/	/	/	/	/	/
	S. aureus	83 (±1)	79 (±3)	80 (±3)	80 (±3)	81 (±2)	74 (±2)	53 (±6)	36 (±9)	34 (±9)
3	**preformed S. aureus**	73 (±4)	75 (±3)	70 (±6)	63±14	35 (±12)	16 (±11)	/	/	/
	preformed *C. albicans*	28 (±5)	/	/	/	/	/	/	/	/
	S. aureus	83 (±1)	85 (±1)	84 (±3)	77 (±4)	58 (±7)	31 (±10)	14 (±9)	13 (±7)	/
4	**preformed *S .aureus***	76 (±7)	68 (±12)	49 (±13)	14 (±10)	/	/	/	/	/
	preformed *C. albicans*	49 (±3)	10 (±6)	/	/	/	/	/	/	/
	S. aureus	82 (±3)	81 (±4)	77 (±3)	65 (±10)	35 (±10)	15 (±10)	/	/	/
5	**preformed *S. aureus***	77 (±8)	64 (±13)	36 (±12)	23 (±2)	/	/	/	/	/
	preformed *C. albicans*	60±6	31 (±7)	13 (±6)	/	/	/	/	/	/
	S. aureus	82 (±3)	56 (±3)	17 (±8)	/	/	/	/	/	/
6	**preformed *S. aureus***	25 (±11)	/	/	/	/	/	/	/	/
	preformed *C. albicans*	31 (±6)	/	/	/	/	/	/	/	/
	S. aureus	/	/	/	/	/	/	/	/	/
7	**preformed *S. aureus***	/	/	/	/	/	/	/	/	/
	preformed *C. albicans*	/	/	/	/	/	/	/	/	/
	S. aureus	76 (±10)	28 (±10)	/	/	/	/	/	/	/
8	**preformed *S. aureus***	/	/	/	/	/	/	/	/	/
	preformed *C. albicans*	53 (±8)	/	/	/	/	/	/	/	/
	S. aureus	79 (±4)	81 (±3)	79 (±3)	79 (±6)	82 (±3)	68 (±7)	42 (±7)	35 (±9)	24 (±10)
9	**preformed *S. aureus***	73 (±9)	80 (±4)	79 (±4)	80 (±4)	57 (±3)	12 (±10)	/	/	/
	preformed *C. albicans*	49 (±4)	19 (±8)	/	/	/	/	/	/	/
	S. aureus	82 (±3)	81 (±4)	79 (±3)	53 (±7)	33 (±8)	/	/	/	/
10	**preformed *S. aureus***	83 (±4)	79 (±4)	58 (±15)	33 (±12)	/	/	/	/	/
	preformed *C. albicans*	/	/	/	/	/	/	/	/	/
	S. aureus	83 (±3)	82 (±4)	82 (±5)	80 (±4)	81 (±4)	77 (±6)	40 (±9)	/	/
MAA	**preformed *S. aureus***	68 (±2)	59 (±12)	50 (±4)	58 (±8)	/	/	/	/	/
	preformed *C. albicans*	33 (±10)	/	/	/	/	/	/	/	/
		(/) no activity								

Table S3. Inhibitory concentrations IC_{50} [µM] of compounds **2-10** when applied to KBV cervix carcinoma, 518A2 melanoma, HCT-116, HCT-116$^{p53-/-}$ knockout mutant colon carcinoma and EaHy hybrid endothelial cells. Compounds were tested in a range from 50 nM to 100 µM. Values are the means ± SD determined in four independent experiments and derived from dose-response curves after 72 h incubation using the MTT assay. [n.d. = not determined]

	IC_{50} values [µM]				
	518A2	**HCT-116^{wt}**	**HCT-116^{p53-}**	**EaHy**	**KbV**
2	>50	13.9±0.8	17.2±2	n.d.	31.3±3
3	n.d.	>50	>50	n.d.	>50
6	n.d.	>50	>50	>50	>50
7	>50	>50	>50	>50	>50
8	n.d.	>50	>50	>50	>50
9	n.d.	>50	>50	>50	>50
10	n.d.	>50	>50	>50	>50

5.4 Publikation III

Formal synthesis of kibdelomycin and derivatisation of amycolose glycosides

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Formal synthesis of kibdelomycin and derivatisation of amycolose glycosides†

Manuel G. Schriefer,‡ Laura Treiber‡ and Rainer Schobert *

A convergent total synthesis of bacterial gyrase B/topoisomerase IV inhibitor kibdelomycin (a.k.a. amycolamicin) (**1**) was devised starting from inexpensive D-mannose and L-rhamnose, which were converted in new efficient ways to an *N*-acylated amycolose and an amykitanose derivative as late building blocks. For the former, we developed an expeditious, general method for the introduction of an α-aminoalkyl linkage into sugars *via* 3-Grignardation. The decalin core was built up in seven steps *via* an intramolecular Diels–Alder reaction. These building blocks could be assembled as published previously, making for a formal total synthesis of **1** in 2.8% overall yield. An alternative order of connecting the essential fragments was also made possible by the first protocol for the direct *N*-glycosylation of a 3-acyltetramic acid.

Introduction

Amycolamicin (**1**) (Scheme 1) was first mentioned in 2008/2009 in patents by Igarashi *et al.* who had isolated it from the bacterium *Amycolatopsis* sp. MK575-fF5.[1] In 2010, proposals for its structure and for the biosynthesis of its *N*-acylated amycolose constituent **4**, featuring an unusual α-aminoethyl branched sugar, were put forward.[2] In 2011 Singh and coworkers isolated a compound from *Kibdelsporangium* sp. MA 7385 which they dubbed kibdelomycin and which they assumed to comprise a largely inverted amykitanose moiety when compared to the purported structure of amycolamicin.[3] They recognised its extraordinary efficacy mainly against Gram-positive bacteria, including multidrug resistant pathogens from the ESKAPE panel. In 2012, a Japanese group disclosed a first crystal structure of the β-methyl anomer of amycolose and a revised structure of amycolamicin differing from the earlier one in the configuration of a stereogenic centre in the amykitanose.[4] In 2014, Singh *et al.* settled the dispute over structure and stereochemistry with an X-ray diffraction analysis of crystals of kibdelomycin (**1**) bound to gyrase B/topoisomerase IV.[5] They revised their original structure proposal and so proved that kibdelomycin and amycolamicin are one and the same.

Singh *et al.* also undertook extensive studies of structure activity relationships.[5] Their crystal structure revealed a horseshoe-like conformation in which the dichlorinated pyrrole of amycolose amide **4** penetrates the ATP-binding pocket of gyrase B/topoisomerase IV which is the usual target of known topoisomerase IV inhibitory antibiotics. In contrast to gyrase-inhibiting antibiotics like novobiocin, the decalin, the tetramic acid and the amykitanose fragments of kibdelomycin protrude from the usual binding pocket, a possible explanation for it not showing cross resistance with established gyrase inhibitors.

Regarding its synthesis, kibdelomycin (**1**) can be dissected in three main parts, which are interesting synthetic targets in their own right. There is a decalinoyltetramic acid, a compound class known for its diverse biological activities.[6] The decalin is *O*-glycosidically bound to a 3-α-aminoethyl-3,6-dideoxyhexopyranose. A 6-deoxygenated talose, carrying a methyl ether, an acetate and a carbamic acid, is attached to the tetramic acid by an *N*-glycosidic bond. The first synthetic foray towards kibdelomycin was the preparation of *N*-acyl amycolose **4** by Kuwahara *et al.* in 2019.[7] Then, in quick succession, the groups of Li, Kuwahara and Baran published total syntheses of kibdelomycin within less than one year from December 2021 until 2022.[8,9]

Results and discussion

Our retrosynthetic strategy for kibdelomycin (**1**) took advantage of a convergent route (Scheme 1). Disconnections were set (i) between *N*-acyl amycolose **4** and decalin fragment **5**, requiring a challenging glycosylation of a 2-deoxy sugar in the forward direction, (ii) between decalin fragment **5** and *N*-amykitanosyltetramic acid **2**, to be linked *via* a 3-acylation of the latter, and (iii) between amykitanose (**13**) and 5-isopropyltetramic acid as present in fragment **2**. This strategy would harness our experience with decalinoyl- and *N*-glycosylated tetramic acids.[10] While working on this project the three mentioned total syntheses were released, so that we decided not to frantically avoid a few of

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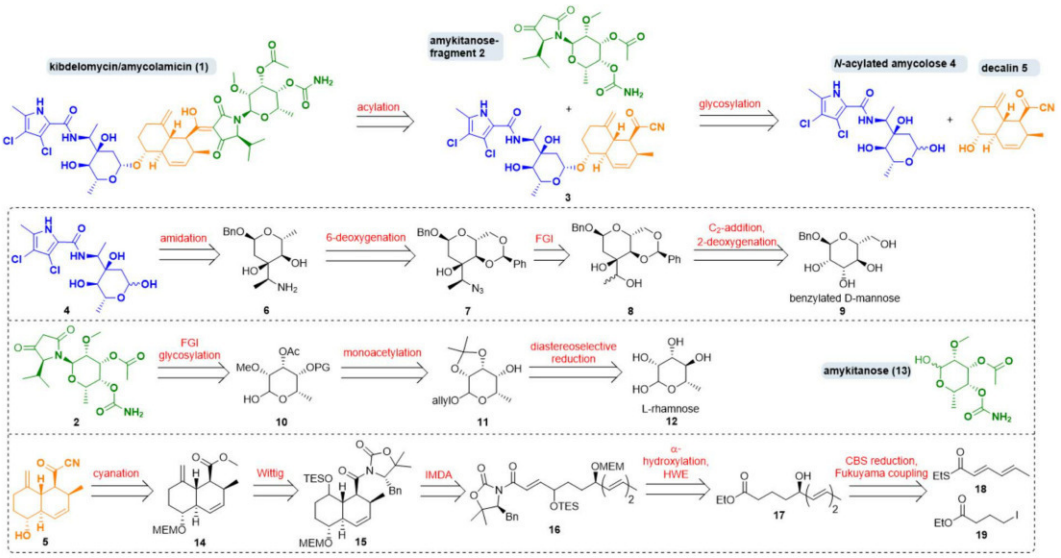
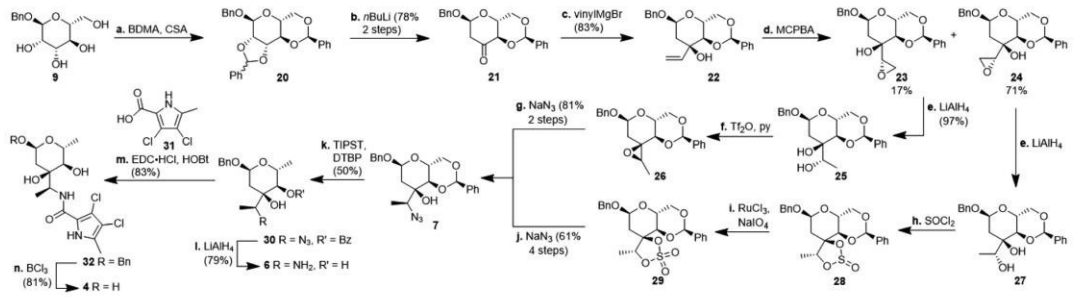


Scheme 1 Retrosynthesis of kibdelomycin (1) and key fragments. FGI: functional group interconversion; IMDA: intramolecular Diels–Alder, HWE: Horner–Wadsworth–Emmons; CBS: Corey–Bakshi–Shibata.

their obvious reaction steps but to concentrate on employing new and more efficient functional group interconversions for the sugar chemistry and to develop an expeditious formal total synthesis of **1**.

The first synthesis by Yang *et al.* resembles ours most because of its convergence and the similarity of some retrosynthetic fragments.[9] However, we chose distinctly different routes to decalin **5**, *N*-acylated amycolose **4** and amykitanose **13**. For the latter two we used a glycal approach with the advantage of not having to build up every single stereogenic centre by means of expensive catalysts and starting materials. For amycolose derivative **4** we decided to start from inexpensive benzylated D-mannose **9**, which first had to be deoxygenated at 2-position, and in which it was necessary to instal an oxidised ethyl group at 3-position. After a second deoxygenation at 6-position and formation of the 3-(α-aminoethyl)sugar **6** the amidation with a dichlorinated pyrrole carboxylic acid should afford **4**. For the synthesis of amykitanose fragment **2** we wanted to start from affordable L-rhamnose (**12**) instead of expensive L-fucose or L-talose. Key steps were the inversion at 4-position, the regioselective monoacetylation at 3-position, the *N*-glycosylation of 5-isopropyltetramic acid, and carbamate formation at C-4. For the synthesis of decalin fragment **5** any reaction other than an intramolecular Diels–Alder (IMDA) cycloaddition was out of the question. In a few steps, starting from thioester **18** and iodide **19**, triene **16** should be accessible *via* Fukuyama coupling, stereoselective reduction of the resulting δ-ketoester to give hydroxyester **17**, α-hydroxylation of the latter, and a chain-lengthening HWE-olefination. The following IMDA should afford mainly the *trans*-decalin scaffold, which had to be olefinated once more and converted to acyl cyanide **5**. Due to the complexity of kibdelomycin (**1**) we had to pursue different synthetic routes to these key fragments. Foundered and abandoned attempts are detailed in the ESI.†

For the synthesis of *N*-acylated amycolose **4**, benzyl protected D-mannose **9** was reacted with benzaldehyde dimethyl acetal (BDMA) and camphorsulfonic acid (CSA) to give bisbenzylidene acetal **20**. This was treated, without prior purification, with *n*BuLi at −78 °C to undergo a Klemer–Rodemeyer fragmentation upon warming to −35 °C, affording ketone **21** in 78% yield over two steps (Scheme 2).[11] It is worthy of note that a *p*-methoxyphenyl (PMP) instead of a methyl, benzyl or propargyl protecting group at the anomeric position was cleaved under these conditions with release of PMPOH. The subsequent Grignard addition of vinyl magnesium bromide occurred exclusively from the side opposite to the neighbouring 4,6-benzylidene acetal. For the introduction of the amino group we intended an initial stereoselective formation of a secondary alcohol at the ethylene group, accessible *via* epoxidation and ensuing ring opening by a metal hydride, and its S_N2-type substitution with sodium azide. The enantio- and diastereoselective Sharpless and $VO(acac)_2$/TBHP epoxidations failed, whereas the Prilezhaev epoxidation gave the epoxides **24** and **23** in 88% yield as a separable 4 : 1 mixture of diastereomers which could both be used for the synthesis of **4**. Epoxide opening by $LiAlH_4$ afforded diols **25** (from **23**) and **27** (from **24**) quantitatively. Applying the Mosher ester method, alcohol **27** was found to be (*S*)-configured (Fig. 1, top).[12] For the retention of its terminal stereogenic centre, diol **25** was submitted to two consecutive S_N2-like reactions. Epoxide formation between the secondary and tertiary alcohol with Tf_2O/pyridine afforded compound **26** which was treated immediately with NaN_3 to furnish azide **7** in 81% over two steps. For the inversion of the terminal stereogenic centre of diol **27**, it was first converted to



Scheme 2 Synthesis of amycolose derivative **4**. BDMA: benzaldehyde dimethyl acetal; CSA: camphorsulfonic acid; MCPBA: 3-chloroperbenzoic acid; Tf_2O: triflic anhydride; TIPST: triisopropylsilanethiol; DTBP: di-*tert*-butylperoxide; EDC: *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide; HOBt: 1-hydroxybenzotriazole.

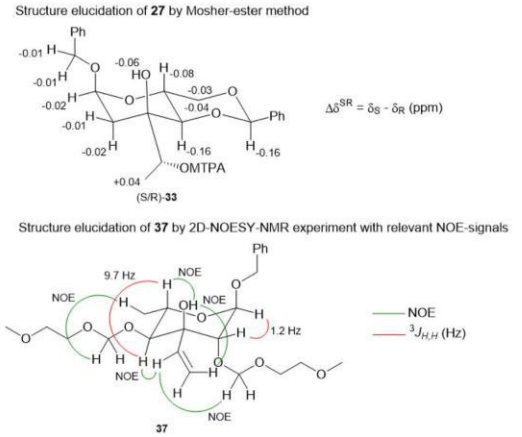
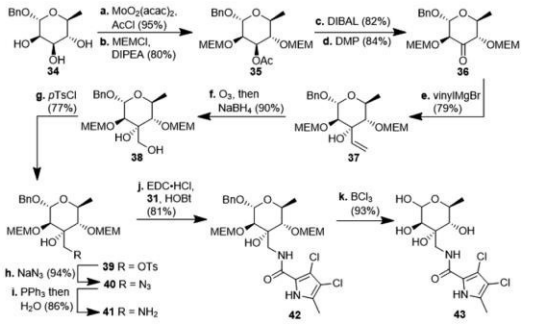


Fig. 1 Structure elucidation of **27** *via* Mosher ester method (top) and significant NOE-signals for the elucidation of the stereoconfiguration of **37** (bottom).

the sulfite **28**. This was oxidised with $RuCl_3/NaIO_4$ to sulfate **29** which was reactive enough to render azide **7** (61% over 4 steps) upon treatment with NaN_3 and subsequent acidic hydrolysis of the intermediate sodium sulfate ester (*cf.* ESI† for details). While on small scale this hydrolysis was possible using aqueous H_2SO_4 (70% yield), at a larger scale aqueous H_2SO_4 led to cleavage of the benzylidene acetal and had to be replaced by a pH 4 citric acid buffer. For the 6-deoxygenation of **7** we followed the protocol of Dang *et al.* and employed a system of DTBP/TIPST for its radical-chain redox rearrangement to give benzoate **30**.[13] After an extensive optimisation this step proceeded with at least 50% yield, which spared us the use of the alternative Hanessian–Hullar reaction with subsequent dehalogenation.[14] Treatment of benzoate **30** with $LiAlH_4$ led to concomitant azide and benzoate reduction with 79% yield. The resulting amine **6** was selectively acylated with carboxylic acid **31** and EDC·HCl/HOBt to give amide **32** in 83% yield. Other amidation reagents such as BOP or HATU were less effective. Because of the potential hydrogenative dechlorination of the pyrrole we used BCl_3 rather than Pd/C and H_2 for the final debenzylation step. We obtained a mixture of α- and β-anomers of **4**, the ratio of which was strongly dependent on the solvent and purification. Next, we checked the applicability of this synthesis to other sugars (Scheme 3). We chose L-rhamnose to test the introduction of an α-aminoalkyl residue. Benzylated L-rhamnose **34** was regioselectively 3-acetylated using a molybdenum catalyst.[15] The hydroxyl groups at 2- and 4-position were MEM-protected (→ **35**, 80%), because the downstream Grignard reaction would not work with bulky (TBS, Bn) or no protecting groups. After removal of the acetyl group by DIBAL (82%) and DMP-oxidation, ketone **36** was obtained with good yield. Its reaction with vinyl magnesium bromide gave the tertiary allyl alcohol **37** in 79% yield and $dr > 30:1$. A 2D-NOESY-experiment proved that the Grignard reagent had attacked from the site opposite to the C4-OMEM group (Fig. 1, bottom). This finding also shows that the group at C4, directing diastereoselective additions, need not be a large 4,6-benzylidene acetal. Next, alkene **37** was converted to primary alcohol **38** by ozonolysis which was tosylated to give **39** that was converted to azide **40**. After Staudinger reaction, the resulting amine **41** was acylated with pyrrole carboxylic acid **31** to give amide **42** in 81% yield. Finally, the benzyl group at the anomeric position as well as both MEM protecting groups of **42** were removed by BCl_3 in

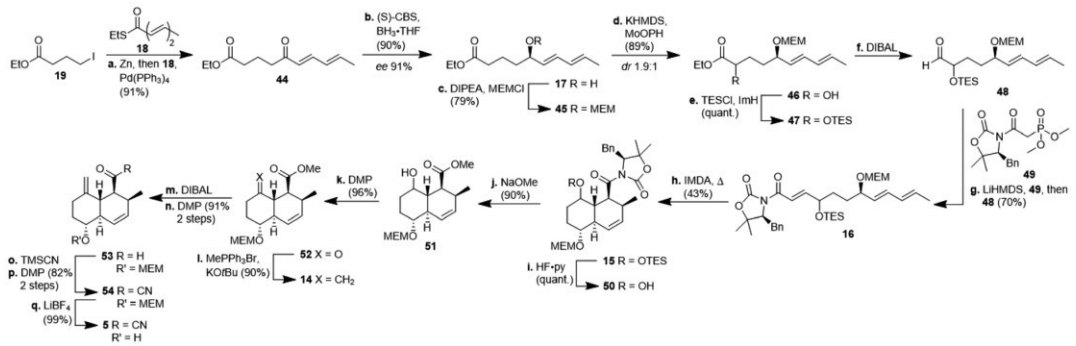


Scheme 3 Synthesis of 3-aminomethyl-6-deoxyhexopyranose derivative **43** starting from benzylated L-rhamnose **34**. MEM: methoxyethoxymethyl; DIPEA: diisopropylethylamine; DMP: Dess–Martin periodinane; Ts: tosyl.

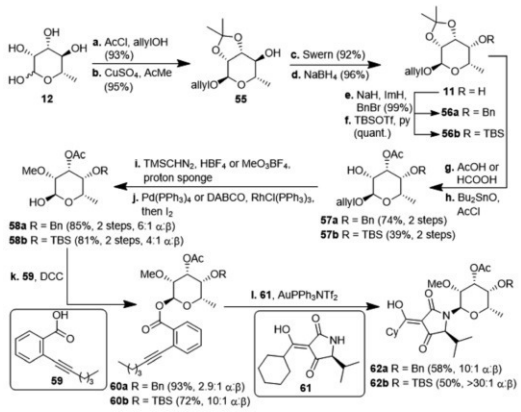
a single step to give the rhamnose derivative **43** in excellent 17% yield over 11 steps. With the synthesis of amycolose and a rhamnose derivative, we demonstrated that this method may be used in general to introduce an α-aminoalkyl linkage in sugars. Moreover, the vinyl group is amenable to a good many other functionalisations (*cf.* ESI†). This aspect might facilitate diversity-oriented syntheses of highly functionalised sugars, including even amycolose, given its known cell growth suppression and possible application as an anticancer medication.[16]

The synthesis of decalin fragment **5** started with a Fukuyama coupling between ethyl 4-iodobutyrate **19** and ethyl (2*E*,4*E*)-hexa-2,4-dienethioate **18** to give δ-ketoester **44** in 91% yield (Scheme 4).[17] The ketone was reduced with BH_3 in the presence of (*S*)-CBS-catalyst affording alcohol **17** with 90% yield and an ee of 91%. This protocol is easier to use on a laboratory scale than a recently published asymmetric Noyori-type hydrogenation of α,β,γ,δ-unsaturated ketones.[18] Unlike other groups who applied a more than quantitative amount of CBS-catalyst, we realised that the reduction proceeded with higher ee when using a merely catalytic amount of CBS-catalyst. After MEM-protection of the alcohol to give ether **45** with 79% yield, a non-trivial α-hydroxylation had to be done at this post-Fukuyama stage, since α-hydroxylated esters from the chiral pool failed to undergo the Fukuyama coupling due to not forming the respective zinc organyl (*cf.* ESI†). After quite a few failed attempts with sulfonyloxaziridines, we identified MoOPH/KHMDS as a viable α-hydroxylating agent affording α-hydroxyester **46** with 89% yield and 1.9 : 1 *dr*. The TES-protected ester **47** was reduced with DIBAL to aldehyde **48** and the latter was submitted to a HWE-olefination with phosphonate **49** to give the triene **16** comprising the SuperQuat auxiliary (70%, two steps). Because HWE-reactions with Evans/Davies auxiliary bearing phosphonates only worked with α-hydroxylated aldehydes but not so with α-methylene substituted aldehydes (*cf.* ESI†) we had to postpone the introduction of the methylene group until after the decalin formation. We opted for Davies' SuperQuat auxiliary for the following Diels–Alder reaction, after many attempts to remove an Evans auxiliary had failed after successful Diels–Alder reaction and in accordance with the results of Frossard *et al.*[19] Unlike most who use $AlMeCl_2$ as a catalyst for the IMDA, we had better results when heating triene **16** in toluene at 80 °C over 3 d which afforded octalin **15** with 43% yield besides some separable undesired *cis*-octalin. Quantitative removal of the TES-protecting group with HF pyridine complex left the alcohol **50** which had its auxiliary cleaved off with sodium methoxide to give hydroxyester **51** with 90% yield. The introduction of the methylene unit was achieved by oxidising alcohol **51** with DMP (96%) and treating the resulting ketone **52** with methylenetriphenylphosphorane. The resulting ester **14** (90%) was reduced to aldehyde **53** in two steps, *i.e.* reduction to the corresponding alcohol with DIBAL and subsequent oxidation with DMP, because of overreduction issues. Reaction of aldehyde **53** with TMSCN led to a cyanohydrin, which was right away oxidised with DMP to acyl cyanide **54**. Cleavage of the MEM-group, liberating decalin **5**, proceeded best using $LiBF_4$ compared to $TiCl_4$ or TFA. This synthesis of the central decalin building block has an edge over those of the previous kibdelomycin syntheses due to its high yielding, simple steps and inexpensive starting materials. Most reactions were performed on a gram scale without yields decreasing.

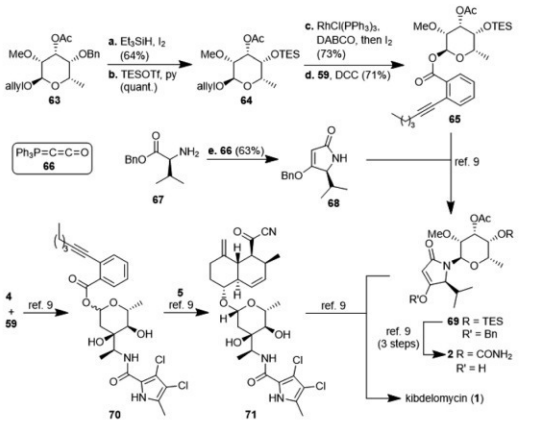
The second, amykitanose-related sugar fragment was synthesised starting from L-rhamnose (**12**) (Scheme 5). It was allylated at the anomeric position in 93% yield and its *syn*-diol was protected as an isopropylidene acetal using anhydrous $CuSO_4$ (95%). The allyl protecting group was chosen since the cleavage of the comparable methyl acetal later on in the synthesis had failed in the presence of other necessary functional groups, *e.g.* because of the instability of the acetyl group. The configuration at the 4-position of the resulting compound **55** was inverted by a sequence of Swern oxidation and ensuing reduction with $NaBH_4$ to give a single diastereomer of **11** in 88% over two steps. Benzylation of the hydroxyl group led to fully protected sugar **56a**. After deprotection of the *syn*-diol, the hydroxyl group at 3-position was acetylated selectively under optimised conditions to afford sugar **57a** in 74% yield over two steps.[20] Methylation at 2-positon was difficult due to the acetyl group getting easily removed under basic conditions, but was eventually achieved



Scheme 4 Synthesis of decalin fragment **5** starting from ethyl 4-iodobutyrate **19**. CBS: Corey–Bakshi–Shibata catalyst; KHMDS: potassium hexamethyldisilazide; MoOPH: oxodiperoxymolybdenum(pyridine)(hexamethylphosphoric triamide); TES: triethylsilyl; ImH: imidazole; LiHMDS: lithium hexamethyldisilazide; TMSCN: trimethylsilyl cyanide.



Scheme 5 Synthesis of *N*-glycosylated 3-cyclohexanoylltetramic acids **62a/b**. TBS: *tert*butyldimethylsilyl; Tf: triflyl; DABCO: 1,4-diazabicyclo[2.2.2]octane; DCC: dicyclohexylcarbodiimide.



Scheme 6 Synthesis of glycoside **65** and tetramate **68** as well as a formal synthesis of kibdelomycin (**1**) according to ref. 9.

using $TMSCHN_2$ and HBF_4. Deprotection of the anomeric position in acidic milieu under Pd-catalysis gave sugar **58a**. All attempts at coupling it with any kind of tetramic acid *via* different customary methods in order to establish analogues of amykitanose fragment **2**, as well as Dieckmann cyclisation based sequences failed (*cf.* ESI†).[21] As a last resort and based on the first total synthesis of kibdelomycin by Li *et al.*,[9] sugar **58a** was esterified with carboxylic acid **59** and the resulting ester **60a** was coupled with 3-cyclohexanoyl-tetramic acid **61** *via* Au-catalysis affording *N*-glycoside **62a** in a decent 58% yield.[22] The cyclohexyl residue was to mimic the octalin moiety. As far as we know, this is the first example of a direct *N*-glycosylation of a 3-acyltetramic acid. The anomeric ratio of 10 : 1 was inferior to the 20 : 1 ratio reported by Li *et al.*[9] for the *N*-glycosylation of 3*H*-5-isopropylpyrrolidin-2,4-dione. The divergent results could only be attributed to the different protecting groups at 4-position of the sugar (Bn *vs.* TES). To verify this assumption, we introduced a silyl protection group as in compound **58b**. The following steps were identical to those for the 4-OBn analogues, albeit with slightly different reaction conditions because of the instability of the TBS-group in an acidic milieu. Even the esterification of **58b** with carboxylic acid **59** showed the influence of the protecting group, since the anomeric ratio of the resulting sugar **60b** increased to 10 : 1 α : β. After coupling with the 3-acyl tetramic acid **61**, the *N*-glycoside **62b** was isolated with an α : β-ratio of >30 : 1. For a strict formal total synthesis, the TES-protected sugar **65** was required (Scheme 6). So, we removed the benzyl group of compound **63**, obtained from methylation of glycoside **57a**, with *in situ* generated HI, and replaced it with a triethylsilyl group to afford compound **64**. Deallylation of the latter and glycosylation with acid **59** gave ester **65** in excellent 15% yield over 12 steps, comparable with the corresponding sequence of the first total synthesis by Li *et al.*[9] Glycoside **65** can then be coupled with 4-*O*-benzyl 5-isopropyltetramate **68** as shown in the first total synthesis of kibdelomycin.[9] Tetramate **68** is readily accessible in one step and 63% yield from reaction of ketenylidenetriphenylphosphorane (**66**) with L-valine benzyl ester (**67**).[23] Removal of the benzyl group in glycoside **63** also opened the door for the synthesis of amykitanose (**13**) in three more steps (*cf.* ESI† for a not yet optimised protocol). The formal synthesis of kibdelomycin (**1**) can be completed by esterification of amycolose derivative **4** with acid **59** to give **70** and subsequent use of the latter for glycosylation of decalin fragment **5** to give compound **71**. Glycoside **65** can be converted to tetramic acid fragment **2** in four steps. Acylation of tetramic acid **2** with ketonitrile **71** using 1-hydroxy-7-azabenzotriazole (HOAt) and triethylamine finally affords kibdelomycin (**1**). For the completion of an alternative total synthesis exploiting the novel *N*-glycosylation of 3-acyltetramic acids *cf.* the ESI.†

Conclusion

In summary we developed an expeditious formal synthesis of kibdelomycin (**1**) starting from inexpensive compounds and employing simple and high-yielding standard protocols, even on a large scale. The stereochemical information stems from the chiral pool or from highly diastereoselective reactions. The longest linear sequence of the factual synthesis of the fragments amounts to a competitive 19 steps. With all fragments in hand, a formal synthesis following the protocol of Yang *et al.* leads to kibdelomycin (**1**, 2.8% overall yield).[9] During our research, we developed a method for introduction of an α-aminoalkyl linkage into sugars *via* Grignard addition to C3 which also opens access to a range of other functionalities. It could be used to synthesise different derivatives of kibdelomycin (**1**) for structure–activity relationship studies or for an optimisation of its applicability and efficacy. As a side benefit, we also report the first *N*-glycosylation of a 3-acyltetramic acid.

Data availability

The datasets and spectra supporting this article have been uploaded as part of the ESI† material.

Author contributions

M. G. S. planned and carried out all reactions concerning amycolose, planned the synthesis of derivatives of amycolose, and wrote parts of the manuscript. L. T. planned and carried out all syntheses concerning amykitanose and rhamnose derivatives and wrote parts of the manuscript. L. T. and M. G. S. planned and realised the synthesis of decalin fragment **5**. R. S. supervised the syntheses and assisted with manuscript preparation.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Formal synthesis of kibdelomycin and derivatisation of amycolose glycosides

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Supporting Information

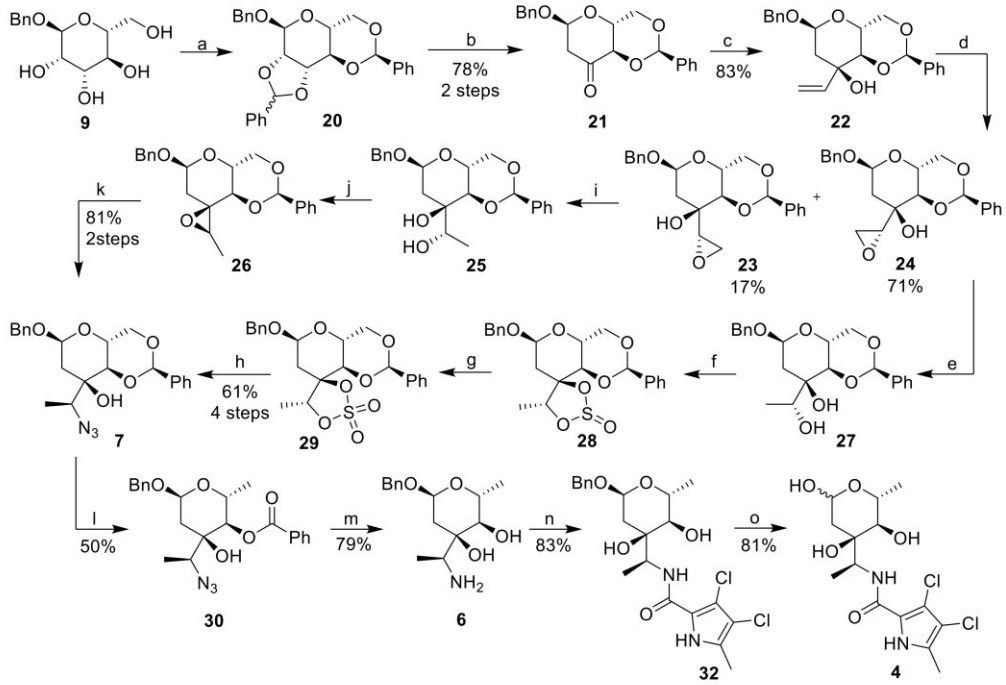
Table of contents

1. General information

Melting points were determined with a Büchi M-565 melting point apparatus and are uncorrected. IR spectra were recorded with a PerkinElmer Spectrum 100 FT-IR spectrophotometer (PerkinElmer, Rodgau, Germany) with ATR sampling unit. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 polarimeter (PerkinElmer, Rodgau, Germany); $[\alpha]_D^{20}$ (c g/100mL, solvent) values are given in 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode (ThermoFisher Scientific, Bremen, Germany). NMR spectra were recorded with a Bruker Avance III HD 500 spectrometer (^{1}H NMR: 500 MHz and ^{13}C NMR: 125 MHz) (Bruker, Karlsruhe, Germany). Chemical shifts are given in parts per million, relative to the residual solvent peak as an internal standard and coupling constants (*J*) are quoted in Hz. Most tetramic acids were measured in $CDCl_3$ and in CD_3OD. In the latter they usually exist as a single (enol) tautomer. Quaternary C-atoms of tetramic acids were sometimes difficult to spot in JMOD or ^{13}C NMR spectra. For these, more signals cropped up in HMBC and/or HSQC correlation spectra and were considered for peak assignment. In $CDCl_3$ solution, signals of virtually all C-atoms of tetramic acids were visible yet split up in multiple, difficult to assign sets for individual tautomers both in ^{1}H and JMOD/^{13}C NMR spectra. In line with literature, we assume the tautomers with exocyclic C–C double bond as drawn for the 3-acyltetramic acids in scheme S10, to be the major tautomer.[1] For the purification of synthetic products, chromatography silica gel 60 (40–63 µm) or silica gel RP18 (40–63 µm) were used. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60 F254 pre-coated aluminum-backed plates. Analytical HPLC was performed on a Shimadzu Nexera XR (Shimadzu GmbH, Duisburg, Germany) using a Knauer Eurospher II C18-column (150 × 4 mm) (Knauer GmbH, Berlin, Germany). Enantiomeric excess was determined by HPLC analysis (Waters Alliance HPLC; Waters 2695 Separation Module, Waters 2487 Dual λ Absorbance Detector) on chiral phase (Daicel Chiralpak OD3). All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran, 1,4-dioxane and toluene which were freshly distilled over sodium/benzophenone, dichloromethane (CH_2Cl_2) which was freshly distilled over CaH_2, dimethylformamide (DMF) which was dried over molecular sieves (3 Å), and methanol (MeOH) which was freshly distilled over Mg. Moisture or air sensitive reactions were routinely carried out in oven-dried glassware under an argon atmosphere using standard Schlenk technique.

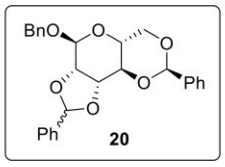
2. Experimental procedure

2.1 Synthesis of amycolose fragment **4**



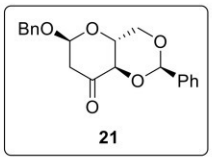
Scheme S1. Synthesis of amycolose derivate **4** starting from benzylated D-mannose **9**.
a) BDMA, CSA, $CHCl_3$, 80 °C, 6.5 h; b) *n*BuLi, THF, −78 °C→−35 °C, 3.75 h; c) VinylMgBr, THF, −78 °C, 3 h; d) *m*CPBA, CH_2Cl_2, rt, 22 h; e) $LiAlH_4$, THF, 0 °C→rt, 2.5 h; f) $SOCl_2$, NEt_3, CH_2Cl_2, 0 °C, 3 h, g) $NaIO_4$, $RuCl_3 \cdot xH_2O$, MeCN, rt, 7 h; h) 1. NaN_3, DMF, 65 °C, 6.75 h, 2. Citric acid buffer, EtOAc, 45 °C, 15 h, 3. Citric acid, 3.5 h; i) $LiAlH_4$, THF, 0 °C→rt, 1.75 h; j) Tf_2O, pyridine, CH_2Cl_2, −78 °C→0 °C, 1.25 h; k) NaN_3, NH_4Cl, MeOH, 80 °C, 12 h; l) TIPST, DTBP, *n*-octane, 140 °C, 6.75 h; m) $LiAlH_4$, THF, 0 °C→rt, 24 h; n) **31**, HOBt, EDC·HCl, NEt_3, CH_2Cl_2, 0 °C→rt, 16 h; o) BCl_3, CH_2Cl_2, −80 °C, 40 min.

(2*R*,4a*R*,6*S*,8a*R*)-6-(Benzyloxy)-2-phenyltetrahydropyrano[3,2-*d*][1,3]dioxin-8(4*H*)-one (21)



Benzylated mannose (**9**, 5.50 g, 20.3 mmol, 1.00 eq.) was solved in $CHCl_3$ (100 mL) and BDMA (7.02 mL, 46.8 mmol, 2.30 eq.) and CSA (709 mg, 3.05 mmol, 0.15 eq.) was added. The solution was heated at 80 °C and the vapor condensed in another flask. The reaction flask was refilled every hour with $CHCl_3$ (ca. 50 mL) and stirred at 80 °C for 6.5 h. The solution was poured into sat. aq. $NaHCO_3$ solution (200 mL) and extracted with CH_2Cl_2 (3×200 mL). The

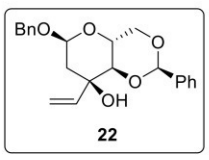
combined organic phases were washed with sat. aq. $NaHCO_3$ solution (3×150 mL) and brine (150 mL), dried over Na_2SO_4 and evaporated. The bis-acetal **20** (7.97 g, quant.) was immediately used without further purification for the next step. It was isolated as a diastereomeric mixture. $\mathbf{R_f}$ = 0.38 (hexanes/EtOAc 6:1); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.56-7.29 (m, 15H), 6.29 (s, 0.60H), 5.96 (s, 0.31H), 5.65 (s, 0.61H), 5.53 (s, 0.32H), 5.28 (s, 0.31H), 5.22 (s, 0.60H), 4.78-4.49 (m, 3H), 4.38-4.19 (m, 2H), 3.94-3.72 (m, 3H) ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{27}H_{27}O_6$ 447.18022, found 447.17924.



21

The raw bis-acetal **20** (7.97 g, 20.3 mmol, 1.00 eq) solved in dry THF (190 mL) at −78 °C under argon atmosphere and was treated with *n*BuLi (2.5M hexanes, 24.4 mL, 2.60 eq.) over 15 minutes. The solution was stirred at −78 °C for 3 h and at −35 °C for 30 min. Sat. aq. NH_4Cl-solution (100 mL) was added and the organic phase was removed by rotary evaporation. The resulting yellow solid was collected by filtration, washed with water (50 mL), crushed, and washed with *n*-pentane (50 mL). The pale yellow solid ketone **21** (5.48 g, 78% over two steps) was dried at the rotary evaporator and was pure enough for the next step. $\mathbf{R_f}$ = 0.47 (hexanes/EtOAc 3:2); **mp** 122 °C (decomposition); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ +81.8° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3069 (w), 3032 (w), 2932 (w), 2869 (w), 1733 (w), 1454 (m), 1379 (m), 1267 (m), 1214 (m), 1129 (s), 1093 (s), 1018 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.51 (m, 2H), 7.39-7.30 (m, 8H), 5.59 (s, 1H), 5.33 (d, 1H, J = 4.8 Hz), 4.72 (d, 1H, J = 12.2 Hz), 4.55 (d, 1H, J = 12.2 Hz), 4.32 (m, 2H), 4.22 (dt, 1H, J = 4.8, 10.0 Hz), 3.91 (t, 1H, J = 10.1 Hz), 2.86 (ddd, 1H, J = 1.2, 4.9, 14.7 Hz), 2.72 (dd, 1H, J = 0.9, 14.7 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 197.7, 136.8, 136.6, 129.5, 128.7, 128.5, 128.2, 128.1, 126.6, 102.3, 98.8, 83.3, 69.6, 69.5, 65.5, 46.5 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{20}H_{20}O_5Na$ 363.12029, found 363.11918.

(2*R*,4a*R*,6*S*,8*R*,8a*R*)-6-(Benzyloxy)-2-phenyl-8-vinylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (22)

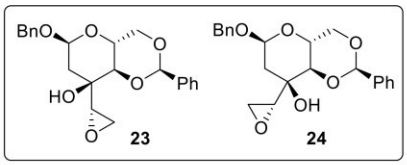


22

Ketone **21** (213 mg, 626 µmol, 1.00 eq.) was solved in dry THF (6.3 mL) under argon atmosphere at −78 °C. VinylMgBr (1M THF, 1.88 mL, 1.88 mmol, 3.00 eq.) was slowly dropped into the solution which was stirred for 3 h at −78 °C. Sat. aq. NH_4Cl solution (30 mL) and H_2O (30 mL) were added, and the aqueous phase was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, and evaporated. The crude allyl alcohol **22** was

purified by column chromatography (SiO_2, pentane/EtOAc 4:1). The alcohol **22** (192 mg, 83%) was obtained as colourless solid. $\mathbf{R_f}$ = 0.82 (hexanes/EtOAc 3:2); **mp** 109.6 °C; $[\alpha]_D^{20}$ +139.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3518 (br. w), 3067 (w), 3033 (w), 2968 (w), 2933 (w), 2863 (w), 1455 (m), 1387 (m), 1116 (s), 1089 (s), 1017 (s), 905 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.48 (m, 2H), 7.40-7.28 (m, 8H), 5.89 (dd, 1H, *J* = 10.8, 17.2 Hz), 5.59 (s, 1H), 5.45 (dd, 1H, *J* = 1.3, 17.2 Hz), 5.21 (dd, 1H, *J* = 1.3, 10.8 Hz), 5.00 (dd, 1H, *J* = 1.2, 3.7 Hz), 4.79 (d, 1H, *J* = 12.0 Hz), 4.56 (d, 1H, *J* = 12.0 Hz), 4.28 (m, 2H), 4.22 (dt, 1H, *J* = 4.8, 10.0 Hz), 3.78 (m, 1H), 3.59 (d, 1H, *J* = 9.3 Hz), 3.56 (s, 1H), 2.05 (dd, 1H, *J* = 1.3, 14.8 Hz), 2.01 (dd, 1H, *J* = 3.8, 14.8 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 140.5, 137.5, 137.0, 129.0, 128.7, 128.3, 128.2, 128.2, 126.3, 115.3, 102.0, 96.4, 82.3, 71.0, 69.7, 69.4, 60.0, 40.4 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{22}H_{24}O_5Na$ 391.15160, found 391.15074.

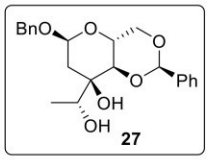
(2*R*,4a*R*,6*S*,8*R*,8a*R*)-6-(Benzyloxy)-8-((*S*)-oxiran-2-yl)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (23) and (2*R*,4a*R*,6*S*,8*R*,8a*R*)-6-(benzyloxy)-8-((*R*)-oxiran-2-yl)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (24)



To a solution of allylalcohol **22** (50 mg, 136 µmol, 1.00 eq.) in CH_2Cl_2 at room temperature was added MCPBA (58.5 mg, 339 µmol, 2.50 eq.). The solution was stirred for 22 h and sat. aq. $Na_2S_2O_3$ solution (2 mL) and sat. aq. $NaHCO_3$ solution (2 mL) was added. The mixture was extracted with EtOAc (3×15 mL), the combined organic phases were washed with 10% K_2CO_3 solution (15 mL) and brine (15 mL), dried over Na_2SO_4 and evaporated. The diastereomeric mixture was separated by SiO_2 column chromatography (pentane/EtOAc 5:1 to 2:1). The optical pure epoxides **24** (37 mg, 71%) and **23** (9 mg, 17%) were isolated as colourless crystalline solids. **24**: $\mathbf{R_f}$ = 0.39 (hexanes/EtOAc 2:1); **mp** 113.9 °C; $[\alpha]_D^{20}$ +99.0° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3507 (br. w), 3067 (w), 3035 (w), 2934 (w), 2864 (w), 1455 (m), 1388 (m), 1099 (s), 1018 (s), 905 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.49 (m, 2H), 7.39-7.28 (m, 8H), 5.65 (s, 1H), 5.06 (d, 1H, *J* = 3.5 Hz), 4.76 (d, 1H, *J* = 11.9 Hz), 4.54 (d, 1H, *J* = 11.9 Hz), 4.33 (dd, 1H, *J* = 5.1, 10.2 Hz), 4.23 (dt, 1H, *J* = 5.1, 10.0 Hz), 3.82 (t, 1H, *J* = 10.0 Hz), 3.69 (d, 1H, *J* = 9.6 Hz), 3.63 (s, 1H), 3.16 (dd, 1H, *J* = 2.7, 4.1 Hz), 2.90 (dd, 1H, *J* = 2.7, 5.0 Hz), 2.78 (dd, 1H, *J* = 4.1, 5.0 Hz), 1.99 (dd, 1H, *J* = 1.3, 14.7 Hz), 1.91 (dd, 1H, *J* = 4.0, 14.7 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.4, 136.7, 129.1, 128.7, 128.3, 128.3, 128.2, 126.3, 102.0, 96.6, 80.6, 69.8, 69.4, 68.9, 59.6, 54.3, 43.8, 35.8 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{22}H_{24}O_6Na$ 407.14651,

found 407.14562. **23**: $\mathbf{R_f}$ = 0.32 (hexanes/EtOAc 2:1); **mp** 120.6 °C; $[\boldsymbol{\alpha}]_D^{20}$ +58.7° (c 0.6 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3506 (br. w), 3067 (w), 3035 (w), 2975 (w), 2931 (w), 2864 (w), 1455 (m), 1386 (w), 1119 (s), 1096 (s), 1025 (s) 911 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.49 (m, 2H), 7.40-7.16 (m, 8H), 5.63 (s, 1H), 4.99 (d, 1H, *J* = 4.4 Hz), 4.77 (d, 1H, *J* = 12.2 Hz), 4.58 (d, 1H, *J* = 12.2 Hz), 4.28 (m, 2H), 3.77 (m, 1H), 3.62 (m, 1H), 3.23 (s, 1H), 3.02 (dd, 1H, *J* = 2.7, 4.1 Hz), 2.90 (dd, 1H, *J* = 2.7, 5.2 Hz), 2.69 (dd, 1H, *J* = 4.1, 5.2 Hz), 2.04 (dd, 1H, *J* = 1.1, 14.9 Hz), 1.97 (dd, 1H, *J* = 0.8, 14.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.3, 137.2, 129.1, 128.7, 128.4, 128.3, 128.1, 126.2, 101.7, 95.8, 80.5, 69.6, 69.3, 68.5, 59.2, 55.9, 43.7, 37.2 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{22}H_{24}O_6Na$ 407.14651, found 407.14557.

(2*R*,4a*R*,6*S*,8*R*,8a*R*)-8-((*S*)-1-Azidoethyl)-6-(benzyloxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (7)



27

$LiAlH_4$ (128 mg, 3.38 mmol, 2.00 eq.) was suspended in dry THF (14 mL) at 0 °C under argon atmosphere and epoxide **24** (649 mg, 1.69 mmol, 1.00 eq) in dry THF (20 mL) was added dropwise. The solution was stirred at 0 °C for 30 min and at room temperature for 2 h. AcMe (1.7 mL) was added, the solution stirred for 5 min, poured into a mixture of EtOAc (20 mL) and sat. aq. Na,K-tartrate solution (300 mL) and stirred for 2 h. The aqueous phase was separated and extracted with EtOAc (3×100 mL). The organic phases were washed with brine (100 mL), dried over Na_2SO_4 and the solvent removed in vacuo. Alcohol **27** (669 mg, quant.) was obtained as colourless resin and used without further purification in the next step. $\mathbf{R_f}$ = 0.55 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_D^{20}$ +81.5° (c 0.9 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3500 (br. w), 3067 (w), 3032 (w), 2971 (w), 2934 (w), 2873 (w), 1455 (m), 1397 (m), 1095 (s), 1078 (s), 1014 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.49 (m, 2H), 7.40-7.12 (m, 8H), 5.62 (s, 1H), 5.06 (t, 1H, *J* = 2.7 Hz), 4.77 (d, 1H, *J* = 12.0 Hz), 4.55 (d, 1H, *J* = 12.0 Hz), 4.31 (dd, 1H, *J* = 5.1, 10.0 Hz), 4.24 (dt, 1H, *J* = 5.1, 9.8 Hz), 3.94 (qn, 1H, *J* = 6.4 Hz), 3.87 (d, 1H, *J* = 9.6 Hz), 3.79 (t, 1H, *J* = 10.1 Hz), 3.64 (s, 1H), 1.98 (m, 2H), 1.78 (m, 1H), 1.25 (d, 3H, *J* = 6.5 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.5, 137.0, 129.2, 128.7, 128.4, 128.3, 128.2, 126.3, 101.9, 97.0, 79.0, 72.3, 69.7, 69.5, 69.0, 59.6, 34.1, 17.5 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{22}H_{26}O_6Na$ 409.16216, found 409.16121.

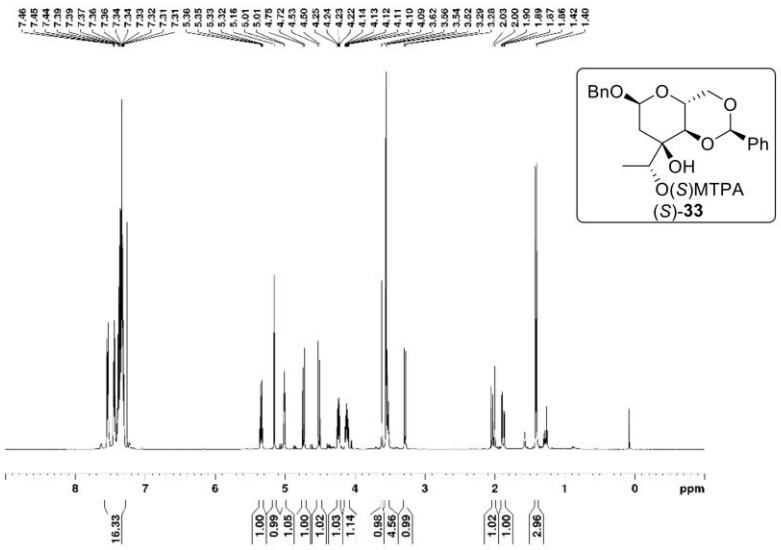


Fig. S1. ^{1}H-NMR-spectrum of (*S*)-**33**. (*S*)-Mosher ester of **27**.

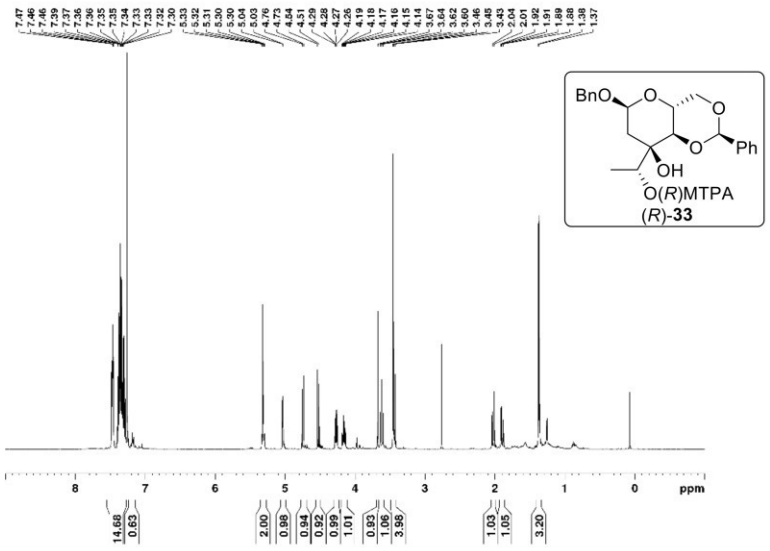
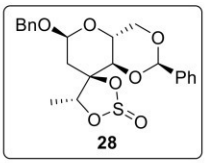


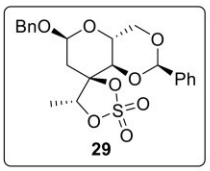
Fig. S2. ^{1}H-NMR-spectrum of (*R*)-**33**. (*R*)-Mosher ester of **27**.

The stereogenic centre of the secondary alcohol in **27** was determined by Mosher ester method. Comparison of the ^{1}H-NMR-spectra of (*S*)-**33** (fig. S1) and (*R*)-**33** (fig. S1) indicated the secondary alcohol to be (*R*)-configurated. Exact $\Delta\delta^{SR} = \delta^{S} - \delta^{R}$–values are shown in Figure 1 (main manuscript). The stereogenic determination was made by standard procedure.



28

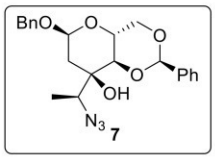
To a solution of diol **27** (654 mg, 1.69 mmol, 1.00 eq.) and dest. dry NEt_3 (1.06 mL, 7.61 mmol, 4.50 eq.) in dry CH_2Cl_2 (16.9 mL) under argon atmosphere was added $SOCl_2$ (307 µL, 4.23 mmol, 2.50 eq.) at 0 °C. The solution was stirred at 0 °C for 3 h and sat aq. NH_4Cl solution (25 mL) was mixed by. The aqueous phase was extracted with EtOAc (4×25 mL) and the combined organic phases were washed with sat. aq. NH_4Cl solution (2×20 mL), sat. aq. $NaHCO_3$ solution (20 mL) and brine (20 mL). The solution was dried over Na_2SO_4, evaporated and the raw sulfite **28** (774 mg, quant.) used without purification. $\mathbf{R_f}$ = 0.50 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −10.8° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3065 (w), 3030 (w), 2980 (w), 2932 (w), 2870 (w), 1455 (m), 1386 (m), 1207 (s) 1101 (s), 1026 (s), 911 (s), 878 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.65-7.27 (m, 10H), 5.64 (s, 0.28H), 5.58 (s, 0.72H), 4.98 (m, 1H), 4.78 (m, 1H), 4.69 (q, 0.75H, J = 6.5 Hz) 4.56 (m, 1H), 4.37-4.27 (m, 1.30H), 4.23 (m, 1H), 3.80-3.66 (m, 1.58H), 3.58 (d, 0.73H, J = 9.4 Hz), 2.29 (d, 0.73H, J = 14.8 Hz), 2.10 (m, 1H), 1.95 (dd, 0.29H, J = 4.7, 14.8 Hz), 1.61 (d, 0.81H, J = 6.6 Hz), 1.55 (d, 2.13H, J = 6.5 Hz) ppm; major diastereomer: **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.5, 137.0, 129.3, 128.6, 128.4, 127.8, 127.8, 126.1, 101.1, 94.8, 87.5, 80.9, 77.0, 69.4, 69.2, 58.7, 37.3, 13.7 ppm; minor diastereomer: **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.4, 137.1, 129.1, 128.6, 128.4, 128.0, 128.0, 126.7, 101.9, 94.9, 85.0, 85.0, 77.1, 69.5, 69.3, 59.6, 39.4, 16.1 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{22}H_{24}O_7SNa$ 455.11349, found 455.11272.



29

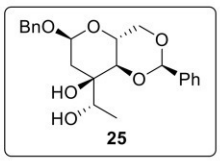
The sulfite **28** (724 mg, 1.58 mmol, 1.00 eq.) was solved in MeCN (9 mL)/H_2O (4.5 mL) at room temperature and $NaIO_4$ (355 mg, 1.66 mmol, 1.05 eq.) and $RuCl_3 \cdot xH_2O$ (16 mg, 79.0 µmol, 5 mol%) were added. The mixture was stirred at room temperature for 7 h, sat. aq. $Na_2S_2O_3$ solution (40 mL) was added and extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, and evaporated. The crude sulfate **29** (678 mg, 96%) was pure enough for the next step without purification. $\mathbf{R_f}$ = 0.34 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ +74.1° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3069 (w), 3033 (w), 2926 (w), 2871 (w), 1455 (m), 1380 (s), 1208 (s), 1130 (m), 1105 (s), 1026 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.55 (m, 2H), 7.41-7.27 (m, 8H), 5.62 (s, 1H), 4.97 (d, 1H, J = 4.7 Hz), 4.75 (d, 1H, J = 12.4 Hz), 4.71 (q, 1H, J = 6.5 Hz), 4.54 (d, 1H, J = 12.4 Hz), 4.30 (dt, 1H, J = 5.2, 9.9 Hz), 4.23 (dd, 1H, J = 5.2, 10.4 Hz), 3.74 (t, 1H, J = 10.4 Hz), 3.71 (d, 1H, J = 9.9 Hz), 2.33 (d, 1H, J = 15.1 Hz), 1.98 (dd, 1H, J = 4.7, 15.1 Hz), 1.25 (d, 3H, J = 6.5 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.2, 136.7, 129.4, 128.6, 128.5, 128.0, 127.9, 126.4, 101.7, 94.5, 88.6, 83.7,

77.0, 69.5, 69.4, 69.1, 58.8, 37.5, 13.6 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{22}H_{25}O_8S$ 449.12646, found 449.12551.



A solution of sulfate **29** (640 mg, 1.43 mmol, 1.00 eq.) in dry DMF (7.1 mL) under argon atmosphere was treated with NaN_3 (464 mg, 7.14 mmol, 5.00 eq.) and stirred at 65 °C for 6.75 h. The resulting sodium sulfate was hydrolyzed by adding pH 4.5 citrate-buffer (50 mL) and EtOAc (20 mL) and stirring at 45 °C for 15 h. Further citric acid (5 g) was added and stirring at 45 °C was continued for 3.5 h. The mixture was extracted with EtOAc (4×50 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ solution (50 mL), H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated. Column chromatography (SiO_2, pentane/EtOAc 6:1) led to azide **7** (370 mg, 63%; 61% over 4 steps) as colourless solid. $\mathbf{R_f}$ = 0.38 (hexanes/EtOAc 4:1); **mp** 86.3 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ +106.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3504 (br. m), 3069 (w), 3037 (w), 2980 (w), 2934 (w), 2872 (w), 2092 (br. s), 1455 (m), 1402 (m), 1264 (m), 1117 (s), 1096 (s), 1019 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.51 (m, 2H), 7.41-7.18 (m, 8H), 5.59 (s, 1H), 5.09 (d, 1H, *J* = 3.8 Hz), 4.78 (d, 1H, *J* = 11.9 Hz), 4.56 (d, 1H, *J* = 11.9 Hz), 4.34 (dd, 1H, *J* = 5.1, 10.2 Hz), 4.22 (dt, 1H, *J* = 5.1, 9.8 Hz), 4.08 (s, 1H), 3.84 (q, 1H, *J* = 6.9 Hz), 3.80 (t, 1H, *J* = 10.2 Hz), 3.64 (d, 1H, *J* = 9.5 Hz), 2.06 (d, 1H, *J* = 14.8 Hz), 1.94 (dd, 1H, *J* = 4.0, 14.8 Hz), 1.27 (d, 3H, *J* = 6.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.3, 136.6, 129.2, 128.8, 128.4 (2 signals), 128.3, 126.3, 101.9, 97.0, 79.9, 73.8, 69.9, 69.4, 62.4, 59.7, 35.0, 15.0 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{22}H_{25}O_5N_3Na$ 434.16864, found 434.16775.

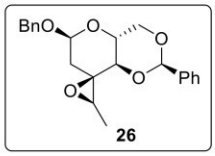
(2*R*,4a*R*,6*S*,8*R*,8a*R*)-6-(Benzyloxy)-8-((*S*)-1-hydroxyethyl)-2-phenylhexahydropyrano-[3,2-*d*][1,3]dioxin-8-ol (25)



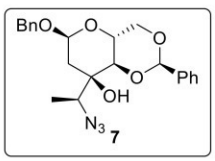
Epoxide **23** (475 mg, 1.24 mmol, 1.00 eq.) in dry THF (5 mL) was added to a suspension of $LiAlH_4$ (93.7 mg, 2.47 mmol, 2.00 eq.) in dry THF (20 mL) under argon atmosphere at 0 °C. The solution was stirred at 0 °C for 5 min and at room temperature for 1.75 h. EtOAc (15 mL) was added, the mixture stirred for 5 min and poured into Na,K-tartrate solution (150 mL). After stirring for 40 min the mixture was extracted with EtOAc (3×75 mL). The combined organic phases were washed with brine (75 mL), dried over Na_2SO_4 and evaporated. After column chromatography (SiO_2, pentane/EtOAc 4:1) the diol **25** (462 mg, 97%) was obtained as colourless resin. $\mathbf{R_f}$ =

0.46 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ +121.8° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3499 (br. m), 3033 (w), 2975 (w), 2934 (w), 2871 (w), 1455 (m), 1397 (m), 1101 (s), 1018 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.47 (m, 2H), 7.39-7.16 (m, 8H), 5.59 (s, 1H), 5.07 (d, 1H, J = 3.8 Hz), 4.78 (d, 1H, J = 12.0 Hz), 4.56 (d, 1H, J = 12.0 Hz), 4.31 (dd, 1H, J = 5.1, 10.2 Hz), 4.24 (dt, 1H, J = 5.1, 9.8 Hz), 4.06 (q, 1H, J = 6.5 Hz), 3.92 (s, 1H), 3.78 (t, 1H, J = 10.1 Hz), 3.64 (d, 1H, J = 9.4 Hz), 2.74 (s, 1H), 2.08 (dd, 1H, J = 1.0, 14.7 Hz), 1.82 (dd, 1H, J = 4.2, 14.7 Hz), 1.25 (d, 3H, J = 6.5 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.2, 136.8, 129.3, 128.7, 128.4, 128.3 (2 signals), 126.3, 102.0, 96.9, 81.3, 72.9, 70.2, 69.8, 69.5, 59.6, 34.0, 16.0 ppm; **HRMS** ESI m/z [M + Na$^+$] calcd. for $C_{22}H_{26}O_6Na$ 409.16216, found 409.16120.

(2R,2'R,3R,4a'R,6'S,8a'R)-6'-(Benzyloxy)-3-methyl-2'-phenyltetrahydro-4'H-spiro-[oxirane-2,8'-pyrano[3,2-d][1,3]dioxine] (26)



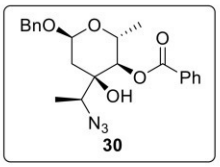
To a solution of diol **25** (100 mg, 259 µmol, 1.00 eq.) in dry CH_2Cl_2 (2 mL) and pyridine (200 µL) under argon atmosphere at −78 °C was added Tf_2O (87.1 µL, 518 µmol, 2.00 eq.). The solution was stirred at 0 °C for 1.25 h. Sat. aq. $NaHCO_3$ solution(20 mL) and $NaHCO_3$ (solid, 1 g) was mixed by and stirred for 30 min at room temperature. The emulsion was extracted with CH_2Cl_2 (3×20 mL). After washing the combined organic phases with H_2O (20 mL) and brine (20 mL), they were dried over Na_2SO_4 and solvent was removed in vacuo. The pinkish white solid (105 mg, quant.) was used without further purification. **R_f** = 0.85 (hexanes/EtOAc 1:1); **mp** 142 °C; $[\alpha]_D^{20}$ +96.0° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3067 (w), 3032 (w), 2968 (w), 2927 (w), 2864 (w), 1454 (m), 1384 (m), 1126 (s), 1095 (s), 1022 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.47-7.26 (m, 10H), 5.58 (s, 1H), 4.98 (d, 1H, J = 4.2 Hz), 4.78 (d, 1H, J = 12.3 Hz), 4.57 (d, 1H, J = 12.3 Hz), 4.30 (dt, 1H, J = 5.0, 9.9 Hz), 4.24 (d, 1H, J = 5.0, 10.3 Hz), 4.05 (d, 1H, J = 9.5 Hz), 3.77 (t, 1H, J = 10.3 Hz), 2.86 (q, 1H, J = 5.7 Hz), 2.37 (dd, 1H, J = 4.2, 14.8 Hz), 1.60 (dd, 1H, J = 0.7, 14.8 Hz), 1.54 (d, 3H, J = 5.7 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.6, 137.4, 129.0, 128.5, 128.3, 128.1, 127.8, 126.3, 101.7, 96.0, 69.8, 69.2, 61.8, 58.8, 58.3, 38.7, 14.1 ppm; **HRMS** ESI m/z [M + K$^+$] calcd. for $C_{22}H_{24}O_5K$ 407.12553, found 407.12479.



Half of the crude epoxide **26** (52.5 mg, 129 µmol, 1.00 eq.) was suspended in MeOH (1.2 mL)/H_2O (300 µL) and treated with NaN_3 (33.5 mg, 516 µmol, 4.00 eq.) and NH_4Cl (13.8 mg, 258 µmol, 2.00 eq.). The mixture was heated at 80 °C for 12 h. The volatile components were

removed by rotary evaporation and the remainder dissolved in EtOAc (15 mL)/H_2O (15 mL). The aqueous phase was separated and extracted with EtOAc (2×10 mL). The combined organic phases were washed with brine (15 mL), dried over Na_2SO_4 and evaporated. The crude azide **7** was chromatographed (SiO_2, pentane/EtOAc 3:1) and the pure compound (43 mg, 81%) was obtained as colourless solid. For analytical data see prior performed synthesis of azide **7**.

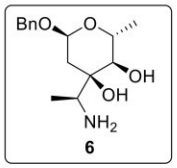
(2*R*,3*R*,4*R*,6*S*)-4-((*S*)-1-Azidoethyl)-6-(benzyloxy)-4-hydroxy-2-methyltetrahydro-2*H*-pyran-3-yl benzoate (30)



30

Azide **7** (360 mg, 875 µmol, 1.00 eq.) was placed in a sealed vessel with TIPST (187 µL, 875 µmol, 1.00 eq), DTBP (81.1 µL, 438 µmol, 0.50 eq.) and degassed *n*-octane (18 mL) under argon atmosphere. The solution was heated at 140 °C for 6.75 h, the solvent was removed in vacuo and the remainder was chromatographed (SiO_2, pentane/EtOAc 15:1 to 12:1). The ester **30** (179 mg, 50%) was obtained as colourless solid. $\mathbf{R_f}$ = 0.59 (hexanes/EtOAc 4:1); **mp** 90.1 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ +111.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3492 (br. m), 2981 (w), 2937 (w), 2912 (w), 2093 (s), 1721 (s), 1453 (m), 1267 (s), 1113 (s), 1027 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 8.11 (m, 2H), 7.60 (tt, 1H, J = 1.3, 7.4 Hz), 7.47 (m, 2H), 7.42-7.30 (m, 5H), 5.13 (d, 1H, J = 3.8 Hz), 5.01 (d, 1H, J = 9.7 Hz), 4.78 (d, 1H, J = 11.9 Hz), 4.57 (d, 1H, J = 11.9 Hz), 4.40 (s, 1H), 4.24 (dq, 1H, J = 6.4, 9.7 Hz), 3.60 (q, 1H, J = 6.9 Hz), 2.16 (dd, 1H, J = 1.0, 14.6 Hz), 1.87 (dd, 1H, J = 4.0, 14.6 Hz), 1.22 (d, 3H, J = 6.3 Hz), 1.15 (d, 3H, J = 6.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 166.1, 136.7, 133.7, 130.1, 129.5, 128.8, 128.7, 128.4, 128.3, 96.7, 75.7, 74.4, 69.9, 63.5, 62.1, 34.0, 17.5, 15.0 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{22}H_{25}O_6N_3Na$ 434.16864, found 434.16795.

(2*R*,3*R*,4*R*,6*S*)-4-((*S*)-1-Aminoethyl)-6-(benzyloxy)-2-methyltetrahydro-2*H*-pyran-3,4-diol (6)

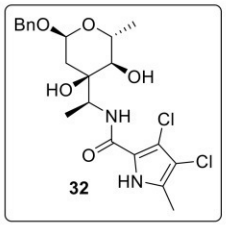


6

To a suspension of $LiAlH_4$ (22 mg, 583 µmol, 3.00 eq.) in dry THF (4 mL) under argon atmosphere at 0 °C was added dropwise ester **30** (80 mg, 194 µmol, 1.00 eq.). The solution was stirred at 0 °C for 7 h and further 17 h at room temperature. EtOAc (1 mL) was mixed by, stirred for 5 min and poured into sat. aq. Na,K-tartrate solution (10 mL). The suspension was stirred further 2 h and extracted with EtOAc (3×40 mL). The combined organic phases were washed with brine, dried

over Na_2SO_4, and evaporated. After column chromatography (SiO_2, CH_2Cl_2/MeOH+0.5% NEt_3 30:1 to 4:1) amine **6** (43 mg, 79%) was obtained as colourless resin. $\mathbf{R_f}$ = 0.11 (CH_2Cl_2/MeOH 4:1); $[\alpha]_D^{20}$ +108.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3500-2500 (m), 3031 (m), 2970 (m), 2931 (m), 1735 (w), 1586 (m), 1455 (m), 1379 (m), 1258 (m), 1126 (s), 1064 (s), 1019 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.36-7.26 (m, 5H), 4.97 (d, 1H, J = 3.8 Hz), 4.71 (d, 1H, J = 11.8 Hz), 4.46 (d, 1H, J = 11.9 Hz), 4.09 (br. s, 4H), 3.85 (dq, 1H, J = 6.2, 9.4 Hz), 3.32 (d, 1H, J = 9.5 Hz), 3.05 (q, 1H, J = 6.5 Hz), 1.96 (dd, 1H, J = 0.8, 14.5 Hz), 1.57 (dd, 1H, J = 4.0, 14.5 Hz), 1.31 (d, 3H, J = 6.2 Hz), 1.12 (d, 3H, J = 6.5 Hz) ppm; **^{1}H-NMR** (500 MHz, CD_3OD) δ 7.42-7.26 (m, 5H), 5.03 (d, 1H, J = 3.8 Hz), 4.71 (d, 1H, J = 11.8 Hz), 4.51 (d, 1H, J = 11.9 Hz), 3.88 (dq, 1H, J = 6.3, 9.5 Hz), 3.23 (d, 1H, J = 9.5 Hz), 3.18 (q, 1H, J = 6.7 Hz), 1.93 (dd, 1H, J = 1.1, 14.5 Hz), 1.70 (dd, 1H, J = 4.0, 14.5 Hz), 1.27 (d, 3H, J = 6.3 Hz), 1.08 (d, 3H, J = 6.8 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.0, 128.6, 128.2, 128.1, 96.4, 77.6, 71.7, 69.3, 65.0, 54.3, 36.0, 18.3, 17.8 ppm; **^{13}C-NMR** (125 MHz, CD_3OD) δ 138.7, 129.5, 129.2, 129.0, 98.0, 75.9, 74.5, 70.4, 66.1, 52.6, 34.4, 18.2, 16.4 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{15}H_{24}O_4N$ 282.16998, found 282.16969.

_N_-((_S_)-1-((2_R_,3_R_,4_R_,6_S_)-6-(Benzyloxy)-3,4-dihydroxy-2-methyltetrahydro-2_H_-pyran-4-yl)ethyl)-3,4-dichloro-5-methyl-1_H_-pyrrole-2-carboxamide (32)



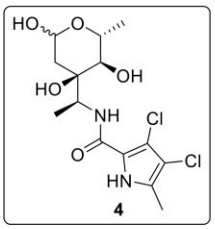
32

A solution of amine **6** (45 mg, 160 µmol, 1.00 eq.), carboxylic acid **31** (38.8 mg, 200 µmol, 1.25 eq.), HOBt (30.6 mg, 200 µmol, 1.25 eq.) and dry NEt_3 (55.8 µL, 400 µmol, 2.50 eq.) in dry CH_2Cl_2 (2 mL) was treated with EDC·HCl (61.3 mg, 320 µmol, 2.00 eq.) at 0 °C under argon atmosphere. The solution was slowly warmed to room temperature over 3 h and stirred further 13 h at room temperature. The reaction was quenched with 1M HCl (2 mL) and poured into a mixture of EtOAc (40 mL) and 1M HCl (40 mL). The organic phase was separated, and the aqueous phase extracted with EtOAc (2×40 mL). The combined organic phases were washed with 1M HCl (40 mL), sat. aq. $NaHCO_3$ solution (2×40 mL) and brine (40 mL). After drying over Na_2SO_4, the organic phase was evaporated and chromatographed (SiO_2, CH_2Cl_2/MeOH 100:1 to 40:1). The amide **32** (61 mg, 83%) was obtained as a reddish solid foam. $\mathbf{R_f}$ = 0.74 (CH_2Cl_2/MeOH 9:1); **mp** 68.6 °C; $[\alpha]_D^{20}$ +90.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3412 (br. m), 3208 (br. m), 2976 (w), 2933 (m), 1629 (s), 1532 (s), 1455 (m), 1413 (m), 1272 (m), 1126 (m), 1047 (s), 1023 (m), 759 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 11.00, (s, 1H), 7.40-7.28 (m, 5H), 6.93 (d, 1H, J = 8.8 Hz), 5.04 (d, 1H, J = 3.4 Hz), 4.72 (d, 1H, J =

11.8 Hz), 4.50 (d, 1H, *J* = 11.9 Hz), 4.46 (m, 1H), 4.16 (s, 1H), 3.76 (dq, 1H, *J* = 6.2, 9.4 Hz), 3.27 (d, 1H, *J* = 9.3 Hz), 2.47 (br. s, 1H), 2.25 (s, 3H), 2.02 (d, 1H, *J* = 14.4 Hz), 1.86 (dd, 1H, *J* = 3.9, 14.4 Hz), 1.34 (d, 3H, *J* = 6.2 Hz), 1.26 (d, 3H, *J* = 6.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 159.6, 136.7, 128.7, 128.5, 128.3, 128.2, 118.5, 111.0, 110.1, 96.3, 74.3, 73.5, 69.6, 65.7, 50.5, 35.2, 18.0, 16.4, 11.2 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{21}H_{26}O_5N_2Cl_2Na$ 479.11110, found 479.11029.

3,4-Dichloro-5-methyl-*N*-((1*S*)-1-((2*R*,3*R*,4*R*)-3,4,6-trihydroxy-2-methyltetrahydro-2*H*-pyran-4-yl)ethyl)-1*H*-pyrrole-2-carboxamide (4)

To a solution of amide **32** (20 mg, 43.7 µmol, 1.00 eq.) in dry CH_2Cl_2 (2 mL) under argon atmosphere was added BCl_3 (1M CH_2Cl_2, 219 µL, 219 µmol, 5.00 eq.) at −80 °C. The solution was stirred at −80 °C for 40 min and a few drops of H_2O were added. The emulsion was evaporated to dryness and chromatographed (SiO_2, CH_2Cl_2/MeOH 40:1 to 15:1). The anomeric mixture of amycolose derivative **4** (13 mg, 81%) was obtained as colourless resin. **R_f** = 0.35, 0.42 (CH_2Cl_2/MeOH 9:1); **IR** ν_{max}/cm^{-1} 3668-3028 (br. m), 2976 (w), 2932 (m), 1758 (w), 1706 (m), 1627 (s), 1536 (s), 1416 (m), 1377 (m), 1269 (m), 1067 (s), 1001 (m), 803 (w), 764 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.62 (s, 0.83H), 9.56 (s, 0.19H), 6.65 (d, 1H, *J* = 6.5 Hz), 6.21 (br. s, 0.72H), 5.64 (br. s, 0.64H), 5.23 (d, 0.81H, *J* = 3.5 Hz), 5.15 (dd, 0.19H, *J* = 2.1, 9.3 Hz), 4.41 (qn, 1H, *J* = 6.8 Hz), 4.00 (dq, 0.82H, *J* = 6.2, 9.3 Hz), 3.69 (dq, 0.19H, *J* = 6.3, 9.2 Hz), 3.19 (d, 0.74H, *J* = 9.3 Hz), 3.17 (d, 0.26H, *J* = 9.1 Hz), 2.94-1.53 (m, 6.78H), 2.29 (s, 2.23H), 2.28 (s, 0.78H), 1.99 (dd, 0.24H, *J* = 2.3, 13.3 Hz), 1.95 (d, 0.95H, *J* = 1.0, 13.9 Hz), 1.70 (dd, 0.87H, *J* = 3.9, 13.9 Hz), 1.46 (dd, 0.26H, *J* = 9.3, 13.0 Hz), 1.34 (d, 3H, *J* = 6.2 Hz), 1.31 (d, 3H, *J* = 7.0 Hz) ppm; **^{1}H-NMR** (500 MHz, CD_3OD) δ 5.21 (m, 0.75H), 5.05 (d, 0.31H, *J* = 2.1, 9.5 Hz), 4.37 (m, 1H), 4.05 (dq, 0.68H, *J* = 6.2, 9.4 Hz), 3.73 (dq, 0.30H, *J* = 6.2, 9.2 Hz), 3.21 (d, 0.73H, *J* = 9.4 Hz), 3.17 (d, 0.33H, *J* = 9.3 Hz), 2.23 (s, 3H), 1.90 (dd, 0.73H, *J* = 1.4, 14.1 Hz), 1.88 (dd, 0.27H, *J* = 2.1, 13.3 Hz), 1.80 (dd, 0.73H, *J* = 3.9, 14.1 Hz), 1.53 (dd, 0.31H, *J* = 9.5, 13.3 Hz), 1.26 (m, 6H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 161.7, 161.5, 129.3, 129.1, 117.4, 117.3, 112.6, 112.4, 111.2, 111.1, 92.9, 92.2, 77.2, 76.3, 74.1, 74.0, 70.8, 64.7, 52.6, 52.5, 37.5, 33.6, 18.2, 18.1, 16.3, 11.5, 11.5 ppm; **^{13}C-NMR** (125 MHz, CD_3OD) δ 161.7, 161.6, 129.4, 129.4, 120.0, 120.0, 112.3, 112.2, 110.6, 110.6, 93.5, 92.9, 76.3, 76.1, 75.0, 74.7, 71.6, 65.8, 52.3, 52.0, 39.2, 35.5, 18.6, 18.5, 16.2, 10.8 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{14}H_{20}O_5N_2Cl_2Na$ 389.06415, found 389.06320.



4

S13

α-/β-Anomeric ratio and signal form of O*H*-groups in ^{1}H-NMR-spectra depends on the purification method as well as solvent and pH.

Spectroscopic data corresponded to those reported in the literature.[2]

Trace impurities in the NMR-spectra of the compounds in the amycolose-sequence can result from the formation of different α-/β-anomers best observed in the ^{1}H-NMR at the anomeric and benzylic position as shown below (fig. S3). The amount of the wrong anomer in the synthesis sequence depends on the purity of the benzyl α-D-mannopyranoside (**9**) as starting material but has no influence on the (diastereoselective) reactions.

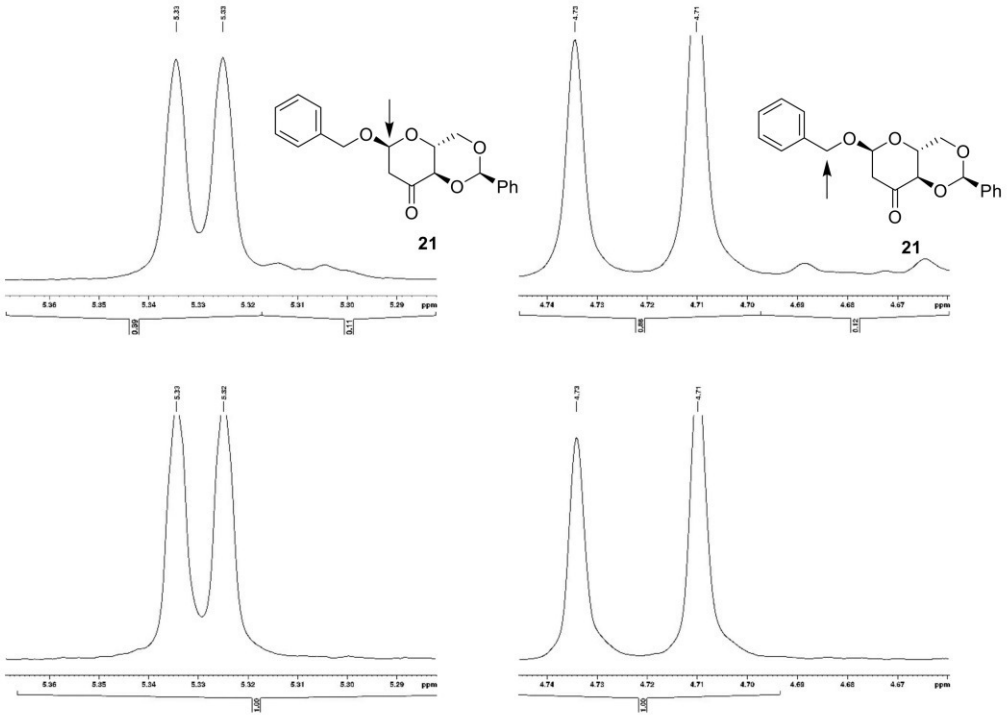
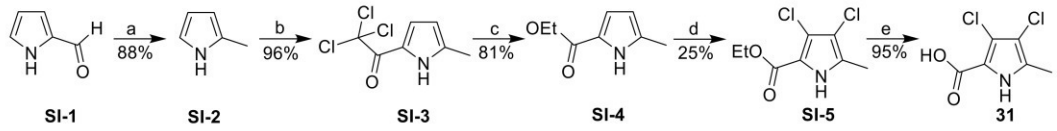


Fig. S3. Comparison of the anomeric (left) and benzylic (right) position of ketone **21** in the ^{1}H-NMR-spectra with different pure starting materials. The upper spectra show a α/β-ratio of ca. 9:1, while the others show 100% α.

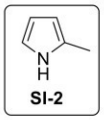
2.2 Synthesis of pyrrole carboxylic acid **31**



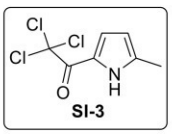
Scheme S2. Synthesis of pyrrole carbonic acid **31**.
Reagents and conditions: a) NaOH, ethylene glycol, $N_2H_4 \cdot xH_2O$, 210 °C, 2.5 h; b) trichloroacetyl chloride, THF, 0 °C, 16 h; c) Na, EtOH, rt, 35 min; d) SO_2Cl_2, CH_2Cl_2, 0 °C, 3.5 h; e) NaOH, H_2O/MeOH, rt, 22 h.

The route is also possible with a methyl ester (Methyl esterification by K_2CO_3/MeOH, 79%).

2,2,2-Trichloro-1-(5-methyl-1*H*-pyrrol-2-yl)ethan-1-one (SI-3)



Pyrrole-2-carbaldehyde (**SI-1**, 5.71 g, 60.0 mmol, 1.00 eq.) and NaOH (12.5 g, 312 mmol, 5.20 eq.) were solved in ethylene glycol (80 mL) under argon atmosphere and hydrazine hydrate (18.1 mL, 372 mmol, 6.20 eq.) was added. The flask was equipped with a Dean-Stark apparatus and heated at 210 °C for 2.5 h. An azeotrope of glycol and 2-methyl pyrrole (**SI-2**) was condensed at the reflux condenser and collected in the Dean-Stark trap as biphasic mixture which was added to Et_2O (200 mL). The organic phase was washed with H_2O (100 mL, 2×50 mL), dried over Na_2SO_4 and evaporated. The raw methyl pyrrole (**SI-2**, 4.28 g, 88%) was used without further purification. **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.88 (br. s, 1H), 6.67 (q, 1H, J = 2.2 Hz), 6.15 (q, 1H, J = 2.8 Hz), 5.93 (m, 1H), 2.30 (s, 3H) ppm.
Spectroscopic data corresponded to those reported in the literature.[3]

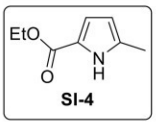


To a solution of trichloro acetylchloride (2.47 mL, 22.0 mmol, 1.10 eq.) in dry THF (10 mL) was slowly added 2-methyl pyrrole (**SI-2**, 1.72 mL, 20.0 mmol, 1.00 eq.) under argon atmosphere at 0 °C. The red solution was stirred at room temperature for 16 h and sat. aq. $NaHCO_3$ solution (100 mL) and 10% aq. K_2CO_3 solution (50 mL) were added. The mixture was extracted with EtOAc (4×50 mL) and the combined organic phases were washed with 10% aq. K_2CO_3 solution (50 mL) as well as brine (50 mL), dried over $NaSO_4$ and evaporated. The pyrrole **SI-3** (4.35 g, 96%) was obtained as

shiny black solid and was pure enough for the next step. **R_f** = 0.85 (hexanes/EtOAc 1:1); **IR** ν_{max}/cm^{-1}3315 (s), 3141 (w), 3102 (w), 2957 (w), 2920 (w), 1764 (w), 1636 (s), 1493 (m), 1399 (m), 1365 (s), 1262 (s), 1218 (s), 1054 (s), 842 (s), 808 (s), 784 (s), 743 (s), 726 (s), 681 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.47 (br. s, 1H), 7.32 (dd, 1H, J = 2.6, 3.7 Hz), 6.11 (t, 1H, J = 3.7 Hz), 2.40 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 172.7, 139.5, 122.8, 122.0, 111.3, 68.7, 13.6 ppm.

Spectroscopic data corresponded to those reported in the literature.[4]

Ethyl 5-methyl-1*H*-pyrrole-2-carboxylate (SI-4)

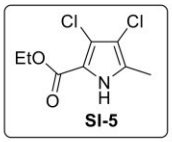


SI-4

Sodium (924 mg, 40.2 mmol, 1.30 eq.) was added to absolute EtOH (33 mL) and stirred until full dilution. Trichloro acetate **SI-3** (7.00 g, 30.9 mmol, 1.00 eq.) was added at room temperature and the solution was stirred for 35 min. It was concentrated at the rotary evaporator and 3M HCl (25 mL) was added. The solution was extracted with Et_2O (3×50 mL) and the organic phases were washed with sat. aq. $NaHCO_3$ solution (50 mL) and brine (50 mL). After drying over Na_2SO_4, the solvent was removed by rotary evaporation. The pale brown pyrrole **SI-4** (3.81 g, 81%) was used without purification. **mp** 97.2 °C, **R_f** = 0.87 (hexanes/EtOAc 2:1); **IR** ν_{max}/cm^{-1} 3288 (s), 3143 (w), 2987 (w), 2913 (w), 1667 (s), 1494 (m), 1321 (s), 1220 (s), 1152 (s), 1025 (s), 801 (s), 774 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 8.97 (s, 1H), 6.81 (m, 1H), 5.95 (m, 1H), 4.30 (q, 2H, J = 7.1 Hz), 2.31 (s, 3H), 1.34 (t, 3H, J = 7.1 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 161.3, 133.7, 121.6, 116.1, 109.0, 60.2, 14.6, 13.3 ppm; **HRMS** ESI m/z $[M + H]^+$ calcd. for $C_8H_{12}NO_2$ 154.08626 found 154.08601.

Spectroscopic data corresponded to those reported in the literature.[4]

Ethyl 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate (SI-5)

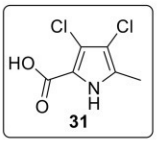


SI-5

Ester **SI-4** (2.06 g, 13.4 mmol, 1.00 eq.) was solved in CH_2Cl_2 (67 mL) at 0 °C and SO_2Cl_2 (2.17 mL, 26.9 mmol, 2.00 eq.) was slowly added. The solution was stirred for 3.5 h at 0 °C and sat. aq. $Na_2S_2O_3$ solution (80 mL) and sat. aq. $NaHCO_3$ solution (100 mL) were added. The mixture was extracted with EtOAc (2×100 mL), the combined organic phases were washed with brine (100 mL), dried over Na_2SO_4 and evaporated. The crude product was chromatographed (SiO_2, pentane/EtOAc 7:1 to 5:1). Pyrrole **SI-5** (741 mg, 25%) was obtained as colourless needles. **R_f** = 0.59

(hexanes/EtOAc 2:1); **IR** ν_{max}/cm^{-1} 3315 (s), 3141 (w), 3102 (w), 2957 (w), 2920 (w), 1764 (m), 1636 (s), 1558 (m), 1493 (m), 1399 (m), 1365 (s), 1262 (s), 1218 (s), 1054 (s), 943 (w), 880 (w), 842 (s), 808 (s), 784 (s), 743 (s), 726 (s), 681 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.02 (s, 1H), 4.35 (q, 2H, J = 7.1 Hz), 2.29 (s, 3H), 1.38 (t, 3H, J = 7.1 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 160.0, 129.1, 117.6, 116.2, 111.9, 61.1, 14.5, 11.7 ppm (quaternary C-atoms indicated by HMBC-correlations); **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_8H_{10}Cl_2NO_2$ 222.00831, found 222.00833.

3,4-Dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (31)

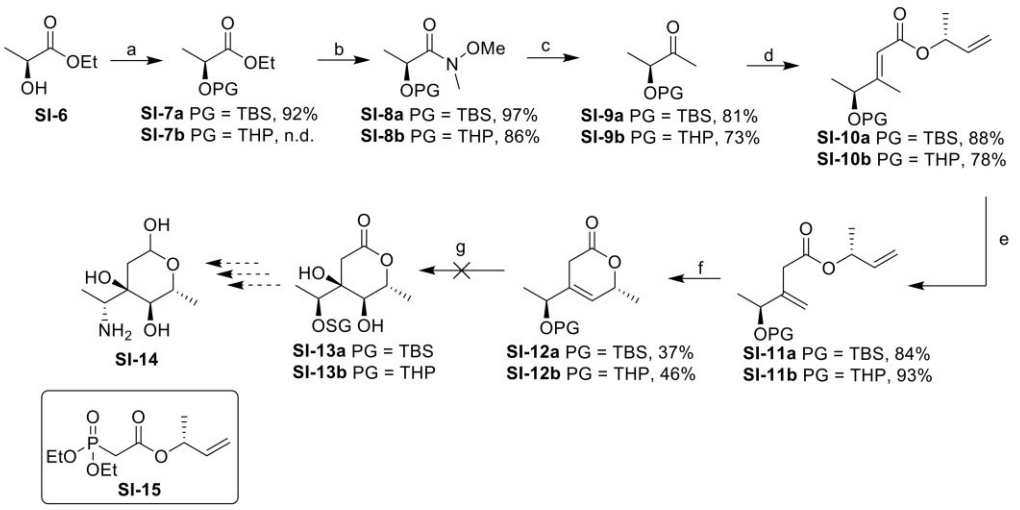


31

Ester **SI-5** (732 mg, 3.30 mmol, 1.00 eq.) was suspended in MeOH (33 mL) and H_2O (8.8 mL) at room temperature and 3M NaOH (4.40 mL, 13.2 mmol, 4.00 eq.) was added. The mixture was stirred for 22 h and further 3M NaOH (20 mL) was added. The mixture was extracted once with EtOAc (20 mL) and the aqueous phase was acidified to pH 1-2 with 1M HCl. The aqueous phase was extracted with EtOAc (3×50 mL). These organic phases were dried over Na_2SO_4 and evaporated. The carboxylic acid **31** (608 mg, 95%) was obtained as red solid. **mp** 102 °C (decomposition).**R_f** = 0.49 (hexanes/EtOAc 2:1); **IR** ν_{max}/cm^{-1} 3113 (s), 2924 (s), 2590 (m), 2325 (s),1646 (s), 1544 (m), 1572 (m), 1498 (s), 1466 (m), 1360 (m), 1326 (m), 1283 (m), 1249 (m), 1102 (m), 1036 (m), 763 (m), 711 (m); **^{1}H-NMR** (500 MHz, CD_3OD) δ 2.23 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, CD_3OD) δ 162.2, 130.6, 117.9, 117.2, 111.6, 10.9 ppm; **HRMS** ESI *m/z* $[M - H]^-$ calcd. for $C_6H_4Cl_2NO_2$ 191.96246, found 191.96179.

2.3 Failed routes amycolose derivative **4**

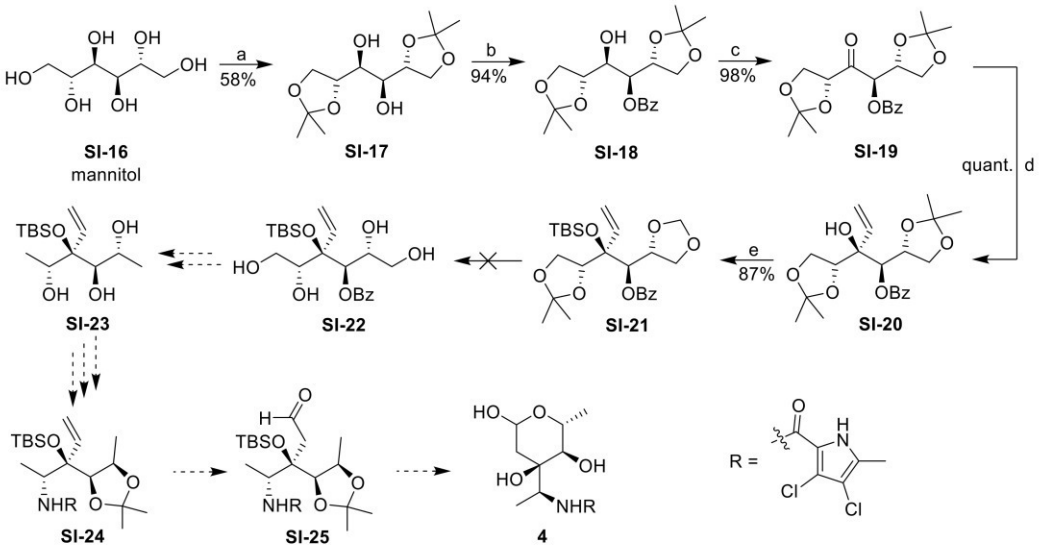
Our first try to build up amycolose derivative **4** was starting from lactic acid ester **SI-6** and perform a *de novo* synthesis of the sugar scaffold. Formation of ketones **SI-9a/b** was accomplished using Weinreb amide method. α,β-unsaturated esters **SI-10a/b** were synthesised in a HWE-olefination of ketones **SI-9a/b** with phosphonate **SI-15** which was itself synthesised by semihydrogenation under Lindlar-conditions of the corresponding alkyne. A base mediated deconjugation formed terminal dienes **SI-11a/b** which led to only low yields in the following Grubbs metathesis reaction. The Sharpless dihydroxylation to diols **SI-13a/b** was not observed. The following steps towards amycolose derived carbohydrate **4** should have been introduction of an amine and reduction of the lactone.



Scheme S3. Attempts to synthesise amycolose derivative **4** starting from lactic acid ester **SI-6**. Reagents and conditions: a) **SI-7a**: TBSCl, imidazole, DMAP, CH_2Cl_2, rt, 19 h; **SI-7b**: DHP, PPTS, CH_2Cl_2, rt; b) MeONHMe·HCl, *i*PrMgCl, LiCl/BuLi, THF, 0 °C, 19 h; c) MeMgBr/MeLi, THF; d) BuLi, LiHMDS, **SI-15**, THF; e) LDA/LiHMDS, HMPT, THF, −78 °C, then AcOH, Et_2O; f) Grubbs catalyst 2nd generation, Ti(O*i*Pr)$_4$, CH_2Cl_2, reflux, 21 h; g) AD-mix.

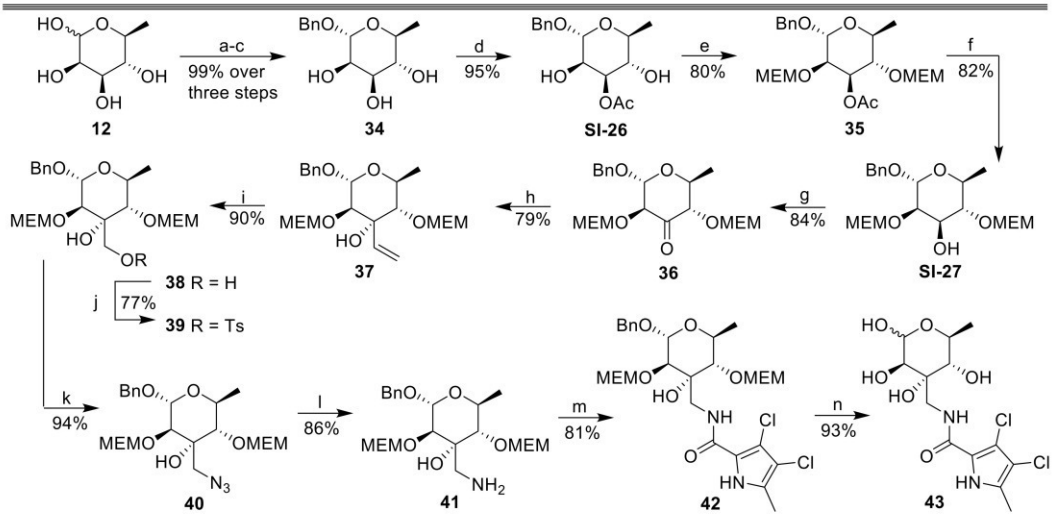
Another idea synthesising amycolose derivative **4** was starting from sugar based mannitol (**SI-16**) using a fully diastereoselective approach. After acetonide protection of both terminal diols a monobenzoylation was carried out (→**SI-18**). The free hydroxyl group was oxidised and ketone **SI-19** was treated with vinylMgBr. After protection of alcohol **SI-20**, the following acetonide deprotection was not possible. The next steps should have been the deoxygenation of

the primary position, protection of the vicinal hydroxy groups as well as amine and aldehyde formation and ultimate deprotection to carbohydrate **4**.



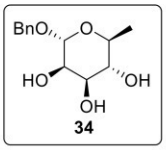
Scheme S4. Attempts to synthesise amycolose starting from mannitol (**SI-16**).
Reagents and conditions: a) $ZnCl_2$, acetone, rt, 15 h; b) Cu(bipy), DIPEA, BzCl, $CH_2Cl_2/CHCl_3$, 0 °C→rt, 5 h; c) DMP, $NaHCO_3$, CH_2Cl_2, rt, 3 h; d) vinylMgBr, THF, −78 °C, 40 min; e) 1. KH, THF, 0 °C, 10 min, 2. TBSCl, rt, 2 h.

2.4 Synthesis of sugar **43** – derivatization of amycolose



Scheme S5. Synthesis of amycolose derived carbohydrate **43**.
Reagents and conditions: a) Ac_2O, pyridine, rt, 22 h; b) BnOH, $BF_3{\cdot}OEt$, 4 Å MS, CH_2Cl_2, 0 °C→rt, on; c) NaOMe, MeOH, rt, 4 d; d) $MoO_2(acac)_2$, collidine, AcCl, 1,4-dioxane, RT, 3 h; e) MEMCl, DIPEA, CH_2Cl_2, 0 °C→40 °C, 1 d; f) DIBAL, toluene, 0 °C, 3 h; g) DMP, CH_2Cl_2, 0 °C→rt, 5 h; h) vinylMgBr, THF, −78 °C, 5 h; i) 1. O_3, CH_2Cl_2/MeOH, −78 °C, 10 min; 2. $NaBH_4$, rt, 24 h; j) *p*TsCl, DMAP, NEt_3, CH_2Cl_2, rt, 21 h; k) NaN_3, DMF, 65 °C, 17 h; l) 1. PPh_3, THF, rt, 2 d; 2. H_2O, rt, 3 d; m) **31**, EDC·HCl, HOBt, DMAP, CH_2Cl_2, 0 °C→rt, on; n) BCl_3, CH_2Cl_2, −78 °C, 3.5 h.

(3*R*,4*R*,5*R*,6*S*)-2-(Benzyloxy)-6-methyltetrahydro-2*H*-pyran-3,4,5-triol (34)



L-Rhamnose (**12**, 10.0 g, 54.9 mmol, 1.00 eq.) was dissolved in Ac_2O (57.0 mL) and pyridine (57.0 mL) at room temperature. The solution was stirred for 22 h and the volatiles were removed under reduced pressure. The crude product was diluted with CH_2Cl_2 and a sat. aq. Cu_2SO_4 solution. The aqueous phase was extracted thrice with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and the solvents were removed under reduced pressure. After purification by column chromatography (SiO_2, pentane/EtOAc 5:1→3:1→2:1) the product (18.2 g, 54.9 mmol) was isolated in quantitative yield.

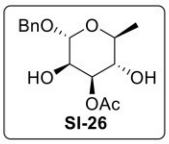
The peracetylated rhamnose (18.0 g, 54.3 mmol, 1.00 eq.) in dry CH_2Cl_2 (147 mL) was treated with BnOH (28.2 mL, 271 mmol, 5.00 eq.) and 4 Å molecular sieve (12 g) at room temperature. After stirring for 30 min $BF_3{\cdot}OEt_2$ (55.0 mL, 434 mmol, 8.00 eq.) was added at 0 °C over a period of 45 min. The mixture was allowed to warm to room temperature overnight. After TLC showed complete conversion of the starting material, the reaction was quenched by slow

addition of H_2O. The mixture was diluted with CH_2Cl_2. The aqueous phase was extracted four times with CH_2Cl_2, and the combined organic phases were dried over Na_2SO_4. The volatiles were removed under reduced pressure and the crude product was used without further purification.

Fully protected rhamnose (20.7 g, 54.3 mmol, 1.00 eq.) was dissolved in dry MeOH (180 mL) and treated with NaOMe (25wt%, 3.72 mL, 16.3 mmol, 0.30 eq.) at room temperature. After 18 h of stirring, another portion of NaOMe (25wt%, 3.72 mL, 16.3 mmol, 0.30 eq.) was added. Stirring was continued for 3 d. The mixture was neutralised by addition of DOWEX. The solid was filtered off over celite® and the solvents were removed under reduced pressure. Purification by column chromatography (SiO_2, pentane/EtOAc 1:1→0:1) gave the product **34** (13.7 g, 99%, α:β >10:1) as a light yellow resin, minor impurities occurred due to β-anomer. $\mathbf{R_f}$ = 0.40 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −8.52° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3392 (m), 2991 (w), 2906 (w), 1455 (w), 1276 (m), 1261 (m), 1131 (m), 1049 (m), 980 (m), 911 (w), 810 (w), 764 (s), 750 (s), 698 (m); **^{1}H-NMR** (500 MHz, CD_3OD) δ 7.37-7.22 (m, 5H), 4.75 (d, 1H, J = 1.6 Hz), 4.69 (d, 1H, J = 11.9 Hz), 4.51 (d, 1H, J = 11.9 Hz), 3.82 (dd, 1H, J = 1.6, 3.4 Hz), 3.68 (dd, 1H, J = 3.4, 9.5 Hz), 3.62 (dq, 1H, J = 6.2, 9.5 Hz), 3.39 (t, 1H, J = 9.5 Hz), 1.27 (d, 3H, J = 6.2 Hz) ppm; **^{13}C-NMR** (125 MHz, CD_3OD) δ 139.1, 129.4, 129.1, 128.8, 100.8, 74.0, 72.4, 72.3, 70.01, 70.00, 18.0 ppm.

Spectroscopic data corresponded to those reported in the literature.[5]

(3*R*,4*R*,5*S*,6*S*)-2-(Benzyloxy)-3,5-dihydroxy-6-methyltetrahydro-2*H*-pyran-4-yl acetate (SI-26)

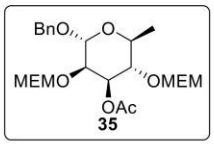


SI-26

Benzylated rhamnose **34** (900 mg, 3.54 mmol, 1.00 eq.) in dry 1,4-dioxane (29 mL) was treated with $MoO_2(acac)_2$ (57.7 mg, 177 µmol, 0.05 eq.), collidine (937 µL, 7.08 mmol, 2.00 eq.) and AcCl (379 µL, 5.31 mmol, 1.50 eq.) at room temperature. The mixture was stirred for 3 h and diluted with H_2O and CH_2Cl_2. The aqueous phase was extracted twice with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4. The volatiles were removed under reduced pressure. Purification by column chromatography (SiO_2, pentane/EtOAc 2:1→1:1) afforded the product **SI-26** (994 mg, 95%) as a colourless resin. The product was isolated as major isomer of a mixture of different regiosiomers (100:10:7). $\mathbf{R_f}$ = 0.64 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −74.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3439 (m), 2980 (w), 2933 (w), 1717 (m), 1497 (w), 1455 (w), 1372

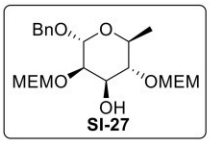
(m), 1275 (m), 1260 (s), 1128 (m), 1049 (s), 983 (m), 886 (w), 842 (w), 805 (w), 764 (s), 750 (s), 699 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.38-7.27 (m, 5H), 5.08 (dd, 1H, J = 3.3, 9.8 Hz), 4.83 (d, 1H, J = 1.7 Hz), 4.72 (d, 1H, J = 12.0 Hz), 4.52 (d, 1H, J = 12.0 Hz), 4.05 (dd, 1H, J = 1.7, 3.3 Hz), 3.78 (dq, 1H, J = 6.2, 9.5 Hz), 3.64 (t, 1H, J = 9.8 Hz), 2.45 (br. s, 2H), 2.14 (s, 3H), 1.35 (d, 3H, J = 6.2 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 171.9, 137.0, 128.6, 128.2, 128.1, 98.5, 75.1, 71.7, 70.0, 69.3, 68.9, 21.3, 17.7 ppm; HRMS ESI *m/z* $[M + Na]^+$ calcd. for $C_{15}H_{20}O_6Na$ 315.11521 found 315.11417.

(3*R*,4*R*,5*S*,6*S*)-2-(Benzyloxy)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyltetrahydro-2*H*-pyran-4-yl acetate (35)



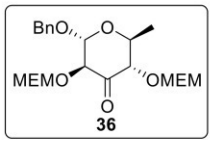
Carbohydrate **SI-26** (11.4 g, 38.3 mmol, 1.00 eq.) in dry CH_2Cl_2 (58 mL) was treated with DIPEA (20.0 mL, 115 mmol, 3.00 eq.) and MEMCl (13.1 mL, 115 mmol, 3.00 eq.) at 0 °C. After 30 min at 0 °C, the solution was allowed to warm to room temperature. DIPEA (6.67 mL, 38.3 mmol, 1.00 eq.) and MEMCl (4.37 mL, 38.3 mmol, 1.00 eq.) were added after 7 h at 0 °C. The solution was stirred at room temperature overnight and for 6 h at 40 °C. As soon as TLC showed complete conversion, the mixture was allowed to come to room temperature and EtOAc as well as sat. aq. K_2CO_3 solution were added. The organic phase was separated and washed with 1M HCl. The combined aqueous phases were extracted thrice with EtOAc. All organic phases were washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure and purification of the crude product by column chromatography (SiO_2, pentane/EtOAc 4:1→3:1→2:1→1:1) gave the product **35** (14.4 g, 80%) as a colourless resin and as a mixture of regioisomers. $\mathbf{R_f}$ = 0.38 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ −79.9° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2935 (m), 2888 (m), 2816 (w), 1743 (m), 1456 (m), 1367 (m), 1237 (s), 1111 (m), 1035 (s), 750 (m) 700 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.36-7.26 (m, 5H), 5.23 (dd, 1H, J = 3.3, 9.5 Hz), 4.86 (d, 1H, J = 6.7 Hz), 4.85 (d, 1H, J = 2.0 Hz), 4.73 (d, 1H, J = 6.7 Hz), 4.73 (d, 1H, J = 6.7 Hz), 4.72 (d, 1H, J = 6.7 Hz), 4.70 (d, 1H, J = 12.0 Hz), 4.51 (d, 1H, J = 12.0 Hz), 4.05 (dd, 1H, J = 2.0, 3.2 Hz), 3.80-3.63 (m, 6H), 3.53 (m, 2H), 3.45 (m, 2H), 3.38 (s, 3H), 3.35 (s, 3H), 2.08 (s, 3H), 1.31 (d, 3H, J = 6.2 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.2, 137.3, 128.5, 128.0, 127.9, 97.7, 96.9, 95.8, 77.7, 75.0, 73.2, 71.8, 71.6, 69.2, 68.0, 67.8, 67.2, 59.22, 59.17, 21.3, 18.1 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{23}H_{36}O_{10}$ 495.21925 found 495.22007.

(3*R*,4*R*,5*R*,6*S*)-2-(Benzyloxy)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyltetrahydro-2*H*-pyran-4-ol (SI-27)



The fully protected sugar **35** (859 mg, 1.82 mmol, 1.00 eq.) in dry toluene (25.0 mL) was treated with DIBAL (3.49 mL, 3.49 mmol, 1.90 eq.) at 0 °C. After stirring for 3 h at this temperature, sat. aq. Na,K-tartrate solution, Na,K-tartrate and acetone were added. The mixture was stirred for 40 min at room temperature. The organic phase was separated, and the aqueous phase was extracted thrice with CH_2Cl_2. The combined organic phases were washed with brine and dried over Na_2SO_4. Removal of the solvents and purification by column chromatography (SiO_2, pentane/EtOAc 2:1→1:1→0:1) afforded product **SI-27** (640 mg, 82%) as a colourless oil and as a mixture of regioisomers. $\mathbf{R_f}$ = 0.71 (CH_2Cl_2/MeOH 95:5); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −61.9° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3463 (m), 2980 (m), 2924 (m), 2889 (m), 2826 (w), 1455 (m), 1366 (w), 1276 (m), 1261 (m), 1112 (m), 1024 (s), 984 (m), 845 (m), 800 (w), 764 (s), 750 (s), 700 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.37-7.26 (m, 5H), 4.93 (d, 1H, J = 6.8 Hz), 4.91 (d, 1H, J = 1.5 Hz), 4.91 (d, 1H, J = 6.8 Hz), 4.80 (d, 1H, J = 7.1 Hz), 4.78 (d, 1H, J = 7.1 Hz), 4.70 (d, 1H, J = 11.9 Hz), 4.48 (d, 1H, J = 11.9 Hz), 3.96 (m, 1H), 3.89 (m, 2H), 3.78 (m, 2H), 3.70 (m, 3H), 3.56 (m, 2H), 3.50 (m, 2H), 3.41 (t, 1H, J = 8.5 Hz), 3.38 (s, 3H), 3.36 (s, 3H), 1.29 (d, 3H, J = 6.3 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.6, 128.6, 127.90, 127.89, 98.1, 97.1, 96.6, 83.0, 77.9, 71.8, 71.7, 70.2, 69.2, 67.8, 67.4, 67.3, 59.2, 59.1, 17.9 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{21}H_{34}O_9Na$ 453.20890 found 453.20950.

(3*S*,5*S*,6*S*)-2-(Benzyloxy)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyltetrahydro-4*H*-pyran-4-one (36)

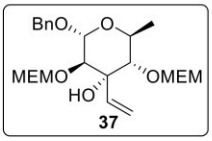


Partially protected rhamnose **SI-27** (5.53 g, 12.8 mmol, 1.00 eq.) was dissolved in CH_2Cl_2 *p.a.* (51.0 mL) and DMP (6.53 g, 15.4 mmol, 1.20 eq.) was added at 0 °C. The suspension was allowed to warm to room temperature after 30 min. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and sat. aq. $NaHCO_3$ solution after 5 h. The aqueous phase was extracted thrice with EtOAc, combined organic phases were washed with sat. aq. $Na_2S_2O_3$ solution, sat. aq. $NaHCO_3$ solution, brine and dried over Na_2SO_4. Solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 2:1) to give a mixture of product and residues of DMP. It was diluted in EtOAc and washed

twice with sat. aq. $Na_2S_2O_3$ solution and sat. aq. $NaHCO_3$ solution alternately. The product **36** (4.58 g, 84%) was obtained as a colourless oil and as a mixture of regioisomers. $\mathbf{R_f}$ = 0.67 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ −143.9° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2938 (m), 2896 (m), 2826 (w), 1745 (m), 1137 (s), 1123 (s), 1052 (s), 997 (m), 751 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.35-7.23 (m, 5H), 5.06 (d, 1H, *J* = 1.6 Hz), 4.82 (d, 1H, *J* = 7.1 Hz), 4.76 (d, 1H, *J* = 7.1 Hz), 4.74 (s, 2H), 4.68 (d, 1H, *J* = 12.2 Hz), 4.51 (d, 1H, *J* = 12.2 Hz), 4.40 (d, 1H, *J* = 9.4 Hz), 4.02 (d, 1H, *J* = 1.6 Hz), 3.98 (dq, 1H, *J* = 6.1, 9.4 Hz), 3.76 (m, 2H), 3.71-3.61 (m, 2H), 3.52 (m, 2H), 3.46 (m, 2H), 3.36 (s, 3H), 3.32 (s, 3H), 1.40 (d, 3H, *J* = 6.2 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 202.2, 136.6, 128.5, 128.0, 127.9, 99.7, 95.5, 95.2, 81.2, 80.0, 71.7, 71.6, 70.5, 69.1, 67.8, 67.6, 59.1, 59.0, 18.7 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{21}H_{32}O_9Na$ 451.19321 found 451.19385.

(3*R*,5*S*,6*S*)-2-(Benzyloxy)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyl-4-vinyltetrahydro-2*H*-pyran-4-ol (37)

Ketone **36** (4.45 g, 10.4 mmol, 1.00 eq.) in dry THF (100 mL) was treated slowly with vinylMgBr solution (1M in THF, 30.1 mL, 30.1 mmol, 3.00 eq., 1.00 mL per minute) at −78 °C. After 5 h at this temperature, the reaction was quenched by addition of sat. aq. NH_4Cl solution. The organic phase was separated, and the aqueous phase was extracted thrice with EtOAc, combined organic phases were washed with brine and dried over Na_2SO_4. Removal of the solvent under vacuum and purification by column chromatography (SiO_2, pentane/EtOAc 3:1→2:1→1:1) gave the product **37** (3.73 g, 79%, dr >30:1 determined by NMR) as a colourless oil and as a mixture of regioisomers. $\mathbf{R_f}$ = 0.52 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ −90.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3498 (m), 2942 (m), 2891 (m), 1455 (w), 1362 (w), 1200 (w), 1173 (m), 1135 (m), 1112 (m), 1024 (s), 958 (m), 847 (w), 739 (w), 700 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.30-7.07 (m, 5H), 6.07 (ddd, 1H, *J* = 1.2, 10.7, 17.2 Hz), 5.61 (dd, 1H *J* = 2.0, 17.2 Hz), 5.22 (dd, 1H, *J* = 2.0, 10.7 Hz), 4.91 (d, 1H, *J* = 0.9 Hz), 4.77 (d, 1H, *J* = 11.7 Hz), 4.74 (s, 2H), 4.70 (s, 2H), 4.55 (d, 1H, *J* = 11.7 Hz), 4.09 (d, 1H, *J* = 1.2 Hz), 4.00 (dq, 1H, *J* = 6.3, 9.7 Hz), 3.72 (m, 2H), 3.66 (m, 1H), 3.60 (m, 2H), 3.53 (d, 1H, *J* = 9.7 Hz), 3.51 (m, 2H), 3.48-3.39 (m, 2H), 3.38 (s, 3H), 3.35 (s, 3H), 1.33 (d, 3H, *J* = 6.3 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 139.5, 136.7, 128.7, 128.32, 128.27, 116.3, 98.0, 97.1, 96.1, 79.71, 79.69, 74.4, 71.8, 71.6, 69.8, 67.9, 67.5, 64.4, 59.21, 59.15, 18.1 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{23}H_{36}O_9Na$ 479.22483 found 479.22515.



37

S24

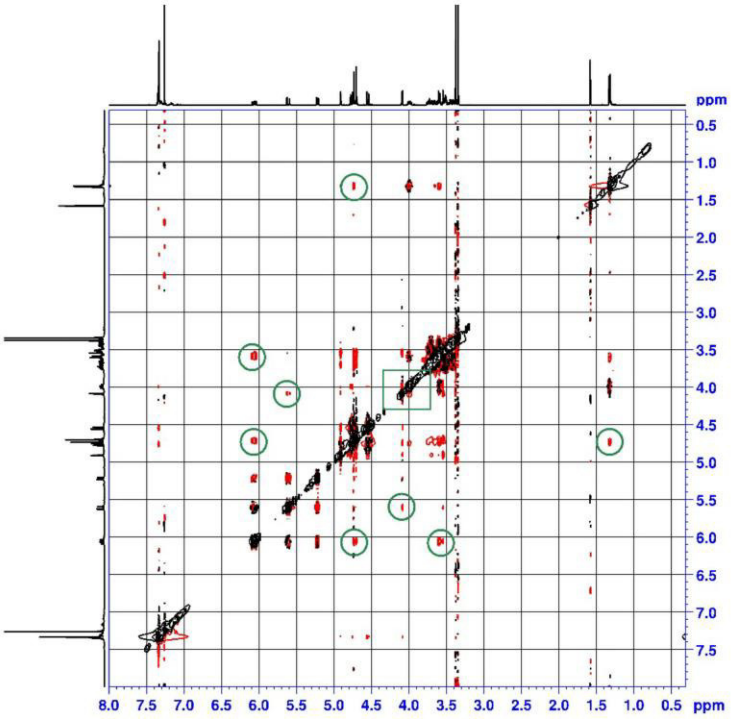


Fig. S4. Relevant NOE-signals for elucidation of stereoconfiguration of glycoside **37.**

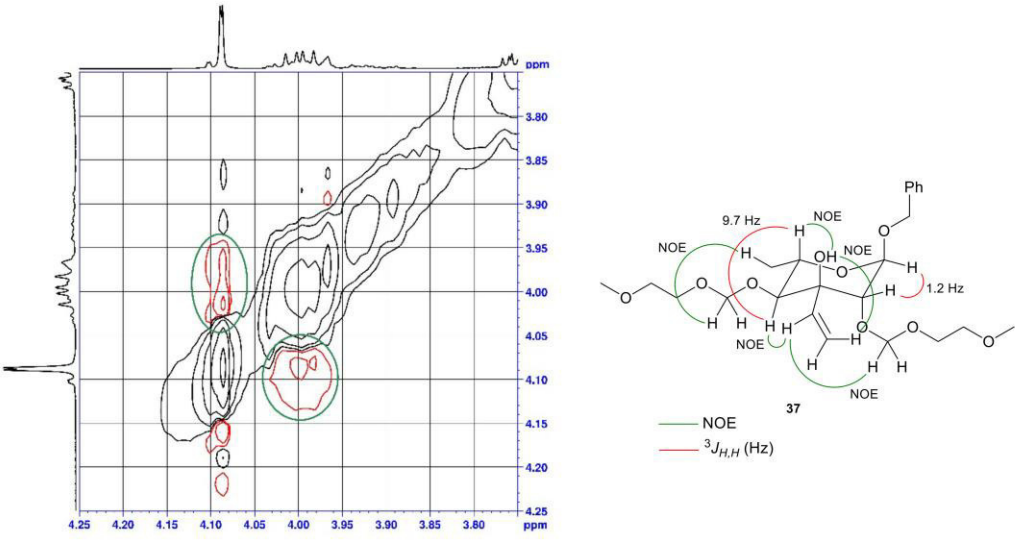
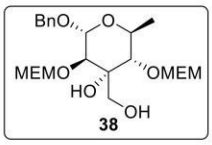


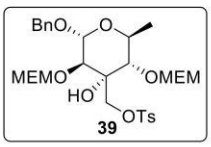
Fig. S5. Relevant NOE-signals for elucidation of stereoconfiguration of glycoside **37.**

(3*R*,5*S*,6*S*)-2-(Benzyloxy)-4-(hydroxymethyl)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyltetrahydro-2*H*-pyran-4-ol (38)



Carbohydrate **37** (3.61 g, 7.90 mmol, 1.00 eq.) was dissolved in MeOH *p.a.* (120 mL) and CH_2Cl_2 *p.a.* (120 mL) and cooled to −78 °C. O_3/O_2 was bubbled through the solution until it turned blue. This was followed by passing oxygen through the solution up to the blue colour disappeared. $NaBH_4$ (724 mg, 19.1 mmol, 2.40 eq.) was added and the solution was slowly allowed to come to room temperature. After stirring for 24 h, the residues were filtered off over celite® and the volatiles were removed under reduced pressure. Purification of the crude product by column chromatography (SiO_2, CH_2Cl_2/MeOH 19:1) gave the product **38** (3.28 g, 90%) as a colourless oil and as a mixture of regioisomers. $\mathbf{R_f}$ = 0.25 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_D^{20}$ −52.4° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3486 (m), 2977 (m), 2935 (m), 2886 (m), 2819 (w), 1456 (m), 1363 (w), 1276 (m), 1261 (m), 1112 (m), 1024 (s), 847 (w), 764 (s), 750 (s), 701 (w); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.36-7.26 (m, 5H), 4.99 (s, 1H), 4.84 (d, 1H, J = 7.0 Hz), 4.80 (d, 1H, J = 7.0 Hz), 4.76 (d, 1H, J = 7.0 Hz), 4.75 (d, 1H, J = 11.5 Hz), 4.73 (d, 1H, J = 7.0 Hz), 4.54 (d, 1H, J = 11.5 Hz), 4.11 (d, 1H, J = 1.2 Hz), 3.98 (dq, 1H, J = 6.3, 9.7 Hz), 3.86 (d, 1H, J = 1.0 Hz), 3.79 (ddd, 1H, J = 3.8, 5.3, 9.1 Hz), 3.76-3.63 (m, 5H), 3.56-3.42 (m, 5H), 3.38 (s, 3H), 3.36 (s, 3H), 2.47 (dd, 1H, J = 3.8, 9.8 Hz), 1.31 (d, 3H, J = 6.3 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 136.5, 128.7, 128.32, 128.26, 98.1, 97.8, 96.0, 78.8, 75.11, 75.06, 71.7, 71.6, 69.9, 68.4, 67.6, 64.1, 63.9, 59.2, 59.1, 18.0 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{22}H_{36}O_{10}Na$ 483.21957 found 483.22007.

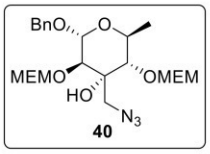
((3*R*,5*S*,6*S*)-2-(Benzyloxy)-4-hydroxy-3,5-bis((2-methoxyethoxy)methoxy)-6-methyl-tetrahydro-2*H*-pyran-4-yl)methyl-4-methylbenzenesulfonate (39)



Carbohydrate **38** (36.0 mg, 78.2 µmol, 1.00 eq.) was dissolved in dry CH_2Cl_2 (550 µL) and treated with *p*TsCl (22.4 mg, 117 µmol, 1.50 eq.), dry NEt_3 (16.3 µL, 117 µmol, 1.50 eq.) and DMAP (478 µg, 3.91 µmol, 0.05 eq.) at room temperature. The solution was stirred for 21 h and H_2O was added. The organic phase was separated, and the aqueous phase was extracted thrice with EtOAc. The combined organic phases were washed with 1M HCl, H_2O as well as brine and dried over Na_2SO_4. The solvents were removed under vacuum and the crude product was purified by column chromatography (SiO_2, pentane/EtOAc 2:1). The tosylated sugar **39**

(37.1 mg, 77%) was isolated as a colourless oil. It was pure enough for next step. $\mathbf{R_f}$ = 0.41 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −58.8° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3482 (m), 2931 (m), 2890 (m), 1600 (w), 1456 (m), 1362 (m), 1177 (s), 1114 (m), 1033 (s), 972 (m), 841 (m), 752 (w), 700 (m), 663 (w); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.79 (d, 2H, J = 8.3 Hz), 7.36-7.26 (m, 7H), 5.06 (s, 1H), 4.80 (d, 1H, J = 7.3 Hz), 4.73 (d, 1H, J = 7.2 Hz), 4.71 (d, 1H, J = 11.5 Hz), 4.69 (d, 1H, J = 7.2 Hz), 4.69 (d, 1H, J = 7.3 Hz), 4.51 (d, 1H, J = 11.5 Hz), 4.27 (dd, 1H, J = 2.2, 9.8 Hz), 4.10 (d, 1H, J = 9.8 Hz), 3.93 (dq, 1H, J = 6.1, 9.6 Hz), 3.78 (d, 1H, J = 1.2 Hz), 3.76 (ddd, 1H, J = 2.8, 6.3, 9.3 Hz), 3.69 (m, 1H), 3.61-3.47 (m, 5H), 3.44-3.39 (m, 2H), 3.40 (s, 3H), 3.36 (s, 3H), 2.41 (s, 3H), 1.31 (d, 3H, J = 6.1 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 144.8, 136.4, 133.1, 129.9, 128.7, 128.6, 128.4, 128.33, 128.27, 128.1, 98.0, 97.6, 96.8, 78.9, 75.2, 74.2, 71.6, 70.1, 68.6, 67.6, 63.8, 59.3, 59.1, 21.8, 17.7 ppm; **HRMS** ESI m/z $[M + Na]^+$ calcd. for $C_{29}H_{42}O_{12}SNa$ 637.22820 found 637.22892.

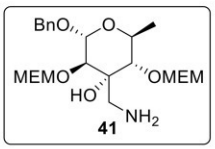
(3*R*,5*S*,6*S*)-4-(Azidomethyl)-2-(benzyloxy)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyl-tetra-hydro-2*H*-pyran-4-ol (40)



Tosylated sugar **39** (2.40 g, 3.90 mmol, 1.00 eq.) in dry DMF (15 mL) was treated with NaN_3 (760 mg, 11.7 mmol, 3.00 eq.) at room temperature. The mixture was stirred at 65 °C for 17 h and NaN_3 (760 mg, 11.7 mmol, 3.00 eq.) was added again. After stirring for a further 35 h at 70 °C, it was allowed to come to room temperature and H_2O was added. The aqueous phase was extracted thrice with EtOAc and the combined organic phases were washed with H_2O, brine and dried over Na_2SO_4. After removal of the solvents under vacuum, the crude product was purified by column chromatography (SiO_2, pentane/EtOAc 2:1→1.5:1) to give azide **40** (1.78 g, 94%) as a colourless oil, minor impurities occur due to regioisomers. $\mathbf{R_f}$ = 0.53 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −41.1° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3484 (m), 2928 (m), 2880 (m), 2826 (w), 2099 (s), 1455 (m), 1364 (w), 1276 (m), 1261 (m), 1200 (w), 1134 (m), 1111 (s), 1022 (s), 977 (m), 919 (m), 847 (m), 764 (m), 750 (s), 700 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.38-7.26 (m, 5H), 5.05 (d, 1H, J = 0.9 Hz), 4.85 (d, 1H, J = 7.1 Hz), 4.80 (d, 1H, J = 7.1 Hz), 4.77 (d, 1H, J = 7.1 Hz), 4.75 (d, 1H, J = 11.5 Hz), 4.73 (d, 1H, J = 7.1 Hz), 4.55 (d, 1H, J = 11.5 Hz), 4.18 (d, 1H, J = 2.2 Hz), 3.95 (dq, 1H, J = 6.5, 9.9 Hz), 3.86 (d, 1H, J = 1.4 Hz), 3.85 (ddd, 1H, J = 4.1, 4.9, 10.8 Hz), 3.75 (ddd, 1H, J = 2.9, 6.2, 10.8 Hz), 3.69 (ddd, 1H, J = 4.1, 4.9, 10.8 Hz), 3.65 (d, 1H, J = 12.6 Hz), 3.59 (ddd, 1H, J = 2.9, 6.4, 10.8 Hz), 3.55 (m, 2H), 3.49 (ddd, 1H, J = 2.9, 6.2, 10.8 Hz), 3.42 (ddd, 1H, J = 2.9, 6.4, 10.8 Hz), 3.39 (s, 3H), 3.36

(s, 3H), 3.37 (m, 1H), 3.23 (dd, 1H, *J* = 2.4, 12.5 Hz), 1.29 (d, 3H, *J* = 6.5 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 136.4, 128.8, 128.4 (2 signals), 98.2, 97.9, 96.4, 80.0, 75.9, 75.3, 71.8, 71.6, 70.1, 68.6, 67.6, 64.1, 59.22, 59.16, 54.5, 17.9 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{22}H_{35}N_3O_9Na$ 508.22636 found 508.22655.

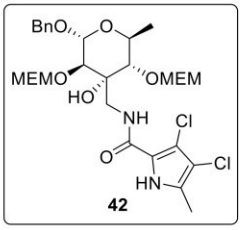
(2*R*,3*R*,4*S*,5*S*,6*S*)-4-(Aminomethyl)-2-(benzyloxy)-3,5-bis((2-methoxyethoxy)methoxy)-6-methyltetrahydro-2*H*-pyran-4-ol (41)



Azide **40** (952 mg, 1.96 mmol, 1.00 eq.) in dry THF (20 mL) was treated with PPh_3 (1.29 g, 4.90 mmol, 2.50 eq.) and stirred until TLC showed full consumption of starting material. H_2O (384 µL, 19.6 mmol, 10.0 eq.) was added and stirring was continued for 3 days. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, 15% MeOH in CH_2Cl_2 + 0.5% NEt_3→10 % MeOH in CH_2Cl_2+0.5% NEt_3). Amin **41** (772 mg, 86%) was isolated as a colourless oil, minor impurities occur due to regioisomers. $\mathbf{R_f}$ = 0.24 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ −59.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3495 (m), 2926 (m), 2882 (m), 1456 (m), 1363 (w), 1276 (m), 1261 (m), 1201 (w), 1111 (m), 1021 (s), 846 (m), 765 (s), 750 (s), 846 (m), 765 (s), 750 (s), 700 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 4.97 (d, 1H, *J* = 0.9 Hz), 4.82 (d, 1H, *J* = 6.9 Hz), 4.81 (d, 1H, *J* = 7.0 Hz), 4.78 (d, 1H, *J* = 6.9 Hz), 4.76 (d, 1H, *J* = 11.8 Hz), 4.73 (d, 1H, *J* = 7.0 Hz), 4.55 (d, 1H, *J* = 11.8 Hz), 3.98 (dq, 1H, *J* = 6.4, 9.8 Hz), 3.82 (d, 1H, *J* = 1.4 Hz), 3.80 (ddd, 1H, *J* = 3.4, 5.6, 10.9 Hz), 3.76-3.69 (m, 3H), 3.59-3.46 (m, 4H), 3.41 (d, 1H, *J* = 9.8 Hz), 3.39 (s, 3H), 3.37 (s, 3H), 2.97 (d, 1H, *J* = 13.3 Hz), 2.82 (d, 1H, *J* = 13.3 Hz), 1.89 (br. s, 3H), 1.31 (d, 3H, *J* = 6.4 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 136.5, 128.8, 128.4, 128.3, 98.0, 97.9, 95.8, 80.3, 75.0, 74.6, 71.8, 71.7, 69.9, 68.5, 68.0, 64.3, 59.24, 59.21, 44.9, 18.1 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{22}H_{38}NO_9$ 460.25411 found 460.25302.

***N*-(((2*R*,3*R*,4*S*,5*S*,6*S*)-2-(Benzyloxy)-4-hydroxy-3,5-bis((2-methoxyethoxy)methoxy)-6-methyltetrahydro-2*H*-pyran-4-yl)methyl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (42)**

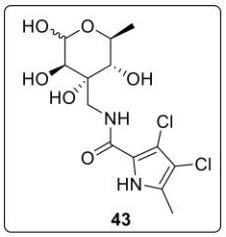
To a solution of amin **41** (42.0 mg, 91.4 µmol, 1.00 eq.) and carbonic acid **31** (21.3 mg, 110 µmol, 1.20 eq.) in dry CH_2Cl_2 (1 mL) was added dry NEt_3 (31.8 µL, 228 µmol, 2.50 eq.), EDC·HCl (26.3 mg, 137 µmol, 1.50 eq.) and HOBt (16.8 mg, 110 µmol, 1.20 eq.) at 0 °C. The



42

mixture was allowed to warm to room temperature overnight. Reaction was quenched by addition of sat. aq. $NaHCO_3$ solution. Aqueous phase was extracted with EtOAc thrice and combined organic phases were dried over Na_2SO_4. Removal of solvents under reduced pressure and purification by column chromatography (SiO_2, pentane/EtOAc 1:1→CH_2Cl_2/MeOH 50:1) gave amide **42** (47.2 mg, 81%) as a light red oil. Minor impurities occur due to regioisomers. $\mathbf{R_f}$ = 0.35 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_D^{20}$ −42.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3407 (m), 3208 (m), 2924 (m), 2882 (m), 1629 (m), 1533 (m), 1455 (m), 1417 (w), 1379 (w), 1276 (m), 1262 (m), 1113 (m), 1024 (s9, 847 (m), 764 (s), 750 (s), 700 (m); major regioisomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.41 (m, 1H), 7.38-7.27 (5H, m), 7.23 (m, 1H), 5.02 (d, 1H, J = 1.3 Hz), 4.83 (d, 1H, J = 7.0 Hz), 4.78 (d, 1H, J = 7.0 Hz), 4.75 (d, 1H, J = 11.8 Hz), 4.71 (d, 1H, J = 7.3 Hz), 4.64 (d, 1H, J = 7.3 Hz), 4.56 (d, 1H, J = 11.8 Hz), 4.34 (d, 1H, J = 1.6 Hz), 4.00 (m, 2H), 3.75 (t, 2H, J = 4.7 Hz), 3.71 (ddd, 1H, J = 2.9, 5.8, 10.9 Hz), 3.68 (m, 1H), 3.55 (m, 3H), 3.47 (d, 1H, J = 9.5 Hz), 3.46-3.36 (m, 2H), 3.34 (s, 3H), 3.32 (m, 1H), 3.29 (s, 3H), 2.29 (s, 3H), 1.58 (m, 1H), 1.33 (d, 3H, J = 6.3 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 159.8, 136.5, 128.7, 128.31, 128.27, 128.2, 118.6, 111.3, 110.2, 109.2, 98.4, 98.0, 96.8, 79.8, 76.3, 74.4, 71.7, 71.5, 70.0, 68.5, 67.4, 64.4, 59.2, 59.0, 41.9, 29.8, 18.1, 11.3 ppm; **HRMS** ESI *m/z* [M + H]$^+$ calcd. for $C_{28}H_{41}Cl_2N_2O_{10}$ 635.21328 found 635.21334.

3,4-Dichloro-5-methyl-*N*-(((2*R*,3*R*,4*S*,5*S*,6*S*)-2,3,4,5-tetrahydroxy-6-methyltetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-pyrrole-2-carboxamide (43)

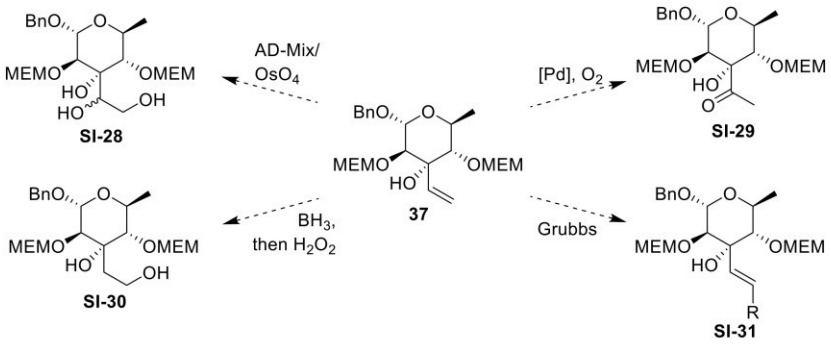


43

Carbohydrate **42** (24.3 mg, 37.8 µmol, 1.00 eq.) in dry CH_2Cl_2 (1 mL) was treated dropwise with BCl_3 (1M CH_2Cl_2, 453 µL, 12.0 eq.) at −78 °C. The solution was stirred at this temperature for 2 h, before BCl_3 (113 µL, 4.00 eq.) was added again. Stirring was continued for 1.5 h and H_2O was added to stop the reaction. All volatiles were removed at the rotary evaporator and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2 19:1→10:1 MeOH in CH_2Cl_2). This yielded the product **43** (13.3 mg, 93%, α:β 1.7:1) as a colourless foam. $\mathbf{R_f}$ = 0.37 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_D^{20}$+7.17° (c 1.0 in $CHCl_3$); IR ν_{max}/cm^{-1} 3310 (s), 2925 (s), 2530 (m), 1606 (s), 1499 (s), 1450 (s), 1323 (m), 1272 (m), 1164 (m), 1071 (s), 761 (m); major regioisomer, α-anomer **^{1}H-NMR** (500 MHz, CD_3OD) δ 5.06 (d, 1H, J = 1.2 Hz), 3.87 (m, 1H), 3.71 (dq, 1H, J = 6.2, 9.5 Hz), 3.52 (m, 1H), 3.46 (d, 1H, J = 1.0 Hz),

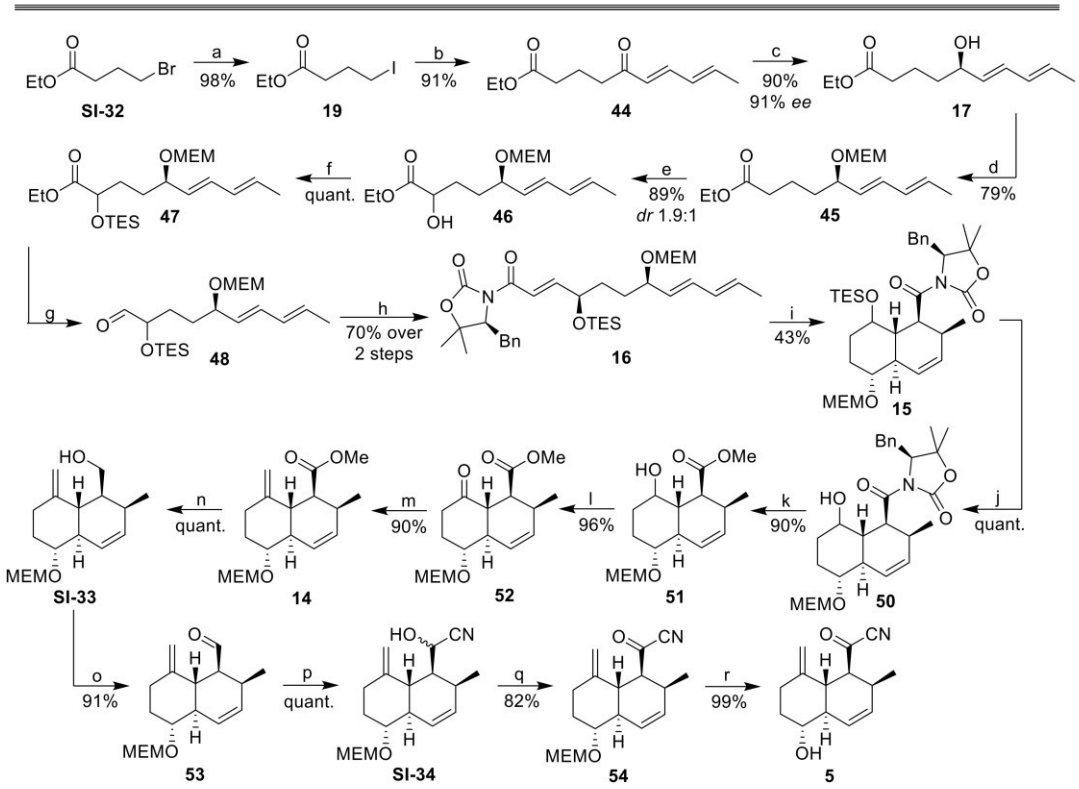
3.34 (d, 1H, *J* = 9.5 Hz), 2.23 (s, 3H), 1.27 (d, 3H, *J* = 6.2 Hz) ppm; major regioisomer, β-anomer **^{1}H-NMR** (500 MHz, CD_3OD) δ 4.96 (d, 1H, *J* = 1.2 Hz), 4.02 (dq, 1H, *J* = 6.2, 9.7 Hz), 3.89 (m, 1H), 3.57 (d, *J* = 1.5 Hz), 3.55 (m, 1H), 3.40 (d, 1H, *J* = 9.7 Hz), 2.23 (s, 3H), 1.29 (d, 3H, *J* = 6.2 Hz) ppm; major regioisomer **^{13}C-NMR** (125 MHz, CD_3OD) δ 162.3, 129.7, 119.6, 112.4, 96.3, 75.7, 72.5, 70.6, 65.6, 44.4, 18.2, 10.8 ppm; minor regioisomer **^{13}C-NMR** (125 MHz, CD_3OD) δ 162.4, 129.6, 119.6, 110.7, 93.6, 75.5, 73.2, 72.6, 71.3, 45.2, 18.3, 14.5 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{13}H_{19}Cl_2N_2O_6$ 369.06147 found 369.06091.

The vinyl group in **37** is amenable to a good many other functionalisations, e.g., dihydroxylations affording vicinal diols such as **SI-28**, Wacker-type oxidations leading to methyl ketones such as **SI-29**, hydroborations to give primary alcohols like **SI-30**, or Grubbs-catalysed metathesis to non-terminal alkenes like **SI-31** (Scheme S6).



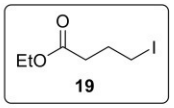
Scheme S6. Possible transformations of olefin **37** as a common intermediate

2.5 Synthesis of decalin fragment **5**



Scheme S7. Synthesis of decalin core **5**.
Reagents and conditions: a) NaI, acetone, reflux, 21 h; b) 1. **19**, Zn, THF, reflux, 3.5 h, 2. Thioester **18**, $Pd(PPh_3)_4$, toluene, rt, 23 h; c) 1. (*S*)-CBS-catalyst, BH_3·THF, rt, 1 h, 2. **44**, −35 °C, 3.5 h; d) MEMCl, DIPEA, CH_2Cl_2, 40 °C, 23 h; e) 1. KHMDS, THF, −78 °C, 30 min, 2. MoOPH, −78 °C, 4 h; f) TESCl, imidazole, DMAP, CH_2Cl_2, 0 °C→40 °C, 4.5 h; g) DIBAL, toluene, −78 °C, 5 h; h) 1. LiHMDS, phosphonate **49**, THF, 0 °C, 1 h, 2. **48**, 0 °C→rt, 17 h; i) toluene, 80 °C, 3 d; j) HF·py, THF, 0 °C, 15 h; k) NaOMe, CH_2Cl_2, 0 °C, 3 h; l) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C→rt, 3 h; m) 1. $MePPh_3Br$, KO*t*Bu, THF, 0 °C, 45 min, 2. **52**, THF, 0 °C→rt, 3 h; n) DIBAL, CH_2Cl_2, 0 °C, 5 h; o) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C→rt, 3 h; p) 1. TMSCN, NEt_3, CH_2Cl_2, 0 °C→rt, 4 h 20 min, 2. NH_4F, EtOH, 0 °C, 2 h; q) DMP, CH_2Cl_2, 0 °C, 1.5 h; r) $LiBF_4$, MeCN/H_2O, rt→55 °C, 4.5 h.

Ethyl 4-iodobutanoate (19)

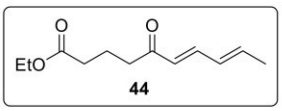


Bromo-butyric acid ester **SI-32** (20.0 mL, 133 mmol, 1.00 eq.) dissolved in acetone *p.a.* (1.3 L) was treated with NaI (100 g, 667 mmol, 5.00 eq.) at room temperature. The mixture was stirred under reflux for 21 h. The suspension was filtered off over celite® and washed with Et_2O. The filtrate was washed with H_2O. The aqueous phase was reextracted with Et_2O thrice and dried over Na_2SO_4. Removal of the solvent and purification by column chromatography (SiO_2, pentane→pentane/EtOAc 30:1) furnished

iodide **19** (31.6 g, 98%) as a yellow liquid. $\mathbf{R_f}$ = 0.61 (hexanes/EtOAc 98:2); **IR** ν_{max}/cm^{-1} 2981 (m), 2936 (w), 2908 (w), 1732 (s), 1444 (m), 1374 (m), 1352 (w), 1308 (w), 1226 (m), 1192 (s), 1163 (m), 1121 (m), 1097 (w), 1032 (m), 857 (w), 769 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 4.13 (q, 2H, J = 7.1 Hz), 3.24 (t, 2H, J = 6.7 Hz), 2.44 (t, 2H, J = 7.1 Hz), 2.13 (qn, 2H, J = 7.0 Hz), 1.26 (t, 3H, J = 7.1 Hz) ppm.

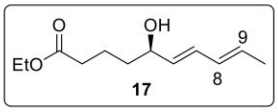
Spectroscopic data corresponded to those reported in the literature.[6]

Ethyl (6*E*,8*E*)-5-oxodeca-6,8-dienoate (44)



A solution of iodide **19** (26.7 g, 109 mmol, 3.00 eq.) in dry THF (120 mL) was treated with Zn (14.1 g, 215 mmol, 5.90 eq.) and stirred under reflux for 3.5 h. This mixture was added to a solution of thioester **18** (5.99 g, 36.3 mmol, 1.00 eq.) in dry. toluene (125 mL) at room temperature. The mixture was treated with $Pd(PPh_3)_4$ (2.10 g, 1.82 mmol, 0.05 eq.) and stirred for 23 h at room temperature. The solids were filtered off over celite® and the organic phases were washed with 1M HCl, sat. aq. $NaHCO_3$ solution as well as brine and dried over Na_2SO_4. The solvents were removed under vacuum and the crude product was purified by column chromatography (SiO_2, pentane/EtOAc 9:1→8:1) to give product **44** (6.93 g, 91%) as a light-yellow oil. $\mathbf{R_f}$ = 0.68 (hexanes/EtOAc 8:1); IR ν_{max}/cm^{-1} 2979 (m), 2940 (m), 1732 (s), 1687 (m), 1664 (m), 1639 (m), 1596 (m), 1447 (w), 1418 (w), 1376 (m), 1323 (w), 1197 (m), 1100 (m), 1028 (m), 1000 (m), 949 (w), 858 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.13 (m, 1H), 6.19 (m, 2H), 6.05 (d, 1H, J = 15.4 Hz), 4.12 (q, 2H, J = 7.2 Hz), 2.62 (t, 2H, J = 7.2 Hz), 2.35 (t, 2H, J = 7.2 Hz), 1.94 (qn, 2H, J = 7.3 Hz), 1.86 (d, 3H, J = 4.9 Hz), 1.25 (t, 3H, J = 7.3 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 200.1, 173.4, 143.2, 140.6, 130.4, 127.7, 60.5, 39.4, 33.6, 19.6, 19.0, 14.4 ppm; **HRMS** ESI m/z $[M + H]^+$ calcd. for $C_{12}H_{19}O_3$ 211.13287 found 211.13260.

Ethyl (*R*,6*E*,8*E*)-5-hydroxydeca-6,8-dienoate (17)



A solution of (*S*)-CBS-catalyst (3.95 g, 14.3 mmol, 1.50 eq.) in dry THF (90 mL) was treated with BH_3·THF (10.5 mL, 10.5 mmol, 1.10 eq.) at room temperature. After stirring for 1 h, ketone **44** (2.00 g, 9.51 mmol, 1.00 eq.) was added dissolved in dry THF (22 mL) at -35 °C over 1.5 h. The reaction was stirred for a further 2h and quenched with sat. aq. NH_4Cl solution. The phases

were separated, and the organic phase was washed with sat. aq. NH_4Cl solution again. The combined aqueous phases were reextracted with Et_2O twice, the combined organic phases were washed with brine and dried over Na_2SO_4. The volatiles were removed under reduced pressure. Column chromatography (SiO_2, pentane/EtOAc 8:1→6:1→5:1→4:1→3:1) gave product **17** (1.82 g, 90%, 91% *ee*, E/Z 11:1) as a light-yellow liquid. E/Z isomerization occurred at double bond between position 8 and 9. $\mathbf{R_f}$ = 0.30 (hexanes/EtOAc 4:1); $[\boldsymbol{\alpha}]_D^{20}$ −6.97° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3439 (m), 2985 (m), 2935 (m), 2875 (w), 1732 (s), 1448 (m), 1374 (m), 1276 (s), 1261 (s), 1163 (m), 1099 (m), 1030 (m), 990 (m), 860 (w), 765 (s), 750 (s); *E,E*-isomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.18 (dd, 1H, J = 10.5, 15.2 Hz), 6.03 (ddq, 1H, J = 1.4, 10.5, 15.0 Hz), 5.71 (dq, 1H, J = 6.7, 15.0 Hz), 5.55 (dd, 1H, J = 7.1, 15.2 Hz), 4.12 (q, 2H, J = 7.2 Hz), 4.12 (m, 1H), 2.33 (t, 2H, J = 7.3 Hz), 1.75 (dd, 3H, J = 1.4, 6.7 Hz), 1.74-1.52 (m, 4H), 1.25 (t, 3H, J = 7.1 Hz) ppm; significant signals *E,Z*-isomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.53 (ddt, 1H, J = 0.9, 11.1, 15.2 Hz), 6.00 (m, 1H), 5.66 (m, 1H), 5.52 (m, 1H), 4.19 (m, 1H), 1.25 (t, 3H, J = 7.1 Hz) ppm; *E,E*-isomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 173.7, 132.9, 131.2, 130.7, 130.2, 72.4, 60.3, 36.6, 34.1, 20.9, 18.1, 14.3 ppm; significant signals *E,Z*-isomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 135.2, 128.5, 127.2, 125.9, 72.5 ppm; **HRMS** ESI *m/z* [M − OH]$^+$ calcd. for $C_{12}H_{19}O_2$ 195.13796 found 195.13789.

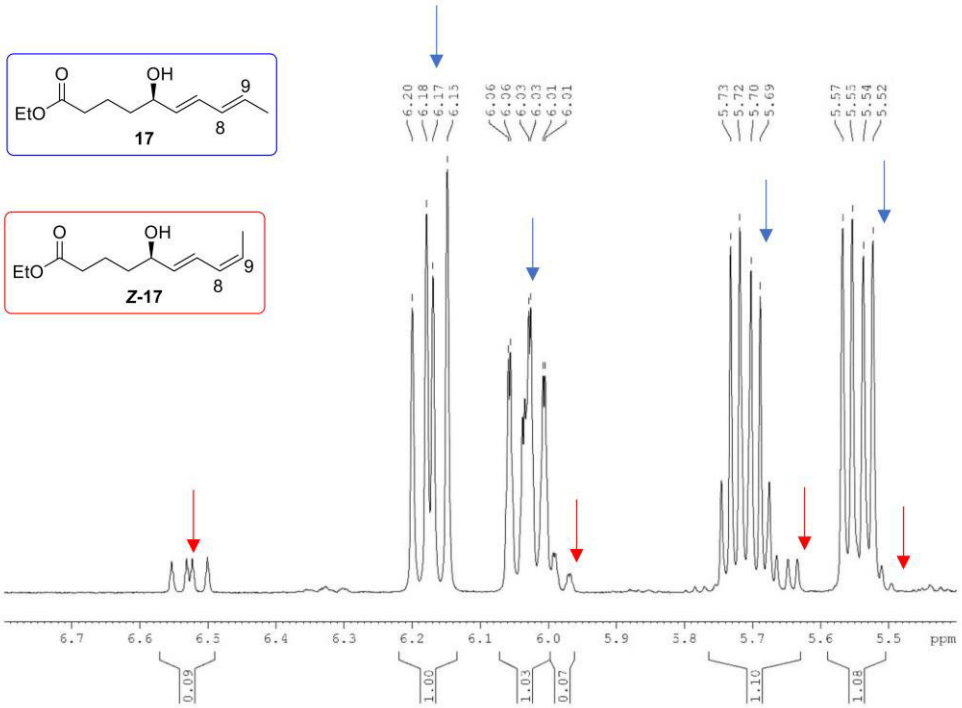
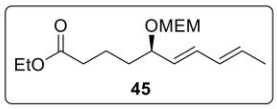


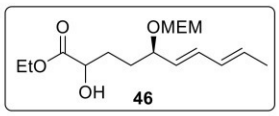
Fig. S6. Differentiation of **17** and *Z*-**17** in ^{1}H-NMR-spectrum.

Ethyl (*R*,6*E*,8*E*)-5-((2-methoxyethoxy)methoxy)deca-6,8-dienoate (45)



Alcohol **17** (2.28 g, 10.7 mmol, 1.00 eq.) in dry CH_2Cl_2 (100 mL) was treated with MEMCl (2.46 mL, 21.5 mmol, 2.00 eq.) and DIPEA (5.48 mL, 32.2 mmol, 3.00 eq.) at room temperature. The solution was stirred for 23 h at 40 °C. 0.5M HCl was added, and the aqueous phase was extracted with EtOAc thrice. The combined organic phases were washed with brine and dried over Na_2SO_4. Removal of the solvent under vacuum and purification by column chromatography (SiO_2, pentane/EtOAc 7:1→5:1) gave MEM-protected alcohol **45** (2.55 g, 79%) as a colourless liquid in 79% yield. $\mathbf{R_f}$ = 0.43 (hexanes/EtOAc 4:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −96.0° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2977 (m), 2931 (m), 2879 (m), 1733 (s), 1451 (m), 1372 (m), 1276 (m), 1260 (m), 1178 (m), 1135 (m), 1089 (m), 1023 (s), 990 (s), 931 (w), 852 (m), 765 (s), 750 (s); *E,E*-isomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.15 (dd, 1H, *J* = 10.5, 15.3 Hz), 6.02 (ddq, 1H, *J* = 1.3, 10.5, 15.1 Hz), 5.70 (dq, 1H, *J* = 6.8, 15.1 Hz), 5.33 (dd, 1H, *J* = 8.2, 15.3 Hz), 4.76 (d, 1H, *J* = 7.1 Hz), 4.61 (d, 1H, *J* = 7.1 Hz), 4.11 (q, 2H, *J* = 7.1 Hz), 4.04 (m, 1H), 3.79 (ddd, 1H, *J* = 2.9, 4.9, 10.3 Hz), 3.60 (m, 1H), 3.55 (m, 2H), 3.39 (s, 3H), 2.30 (t, 2H, *J* = 7.4 Hz), 1.74 (dd, 3H, *J* = 1.3, 6.8 Hz), 1.73-1.48 (m, 4H), 1.24 (t, 3H, *J* = 7.1 Hz) ppm; significant signals *E,Z*-isomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.49 (ddt, 1H, *J* = 0.9, 11.1, 15.3 Hz), 5.98 (m, 1H), 5.51 (dqu, 1H, *J* = 7.0, 10.7 Hz), 5.33 (dd, 1H, *J* = 8.0, 15.3 Hz), 4.79 (d, 1H, *J* = 7.1 Hz), 4.63 (d, 1H, *J* = 7.1 Hz), 4.12 (q, 1H, 7.1 Hz), 4.11 (m, 1H), 3.82 (m, 1H), 3.65 (m, 1H), 3.57 (m, 2H), 3.39 (s, 3H), 2.31 (m, 2H), 1.25 (t, 3H, *J* = 7.1 Hz) ppm; *E,E*-isomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 173.7, 133.7, 130.8, 130.5, 130.0, 92.6, 76.2, 71.9, 67.0, 60.4, 59.2, 35.2, 34.3, 21.1, 18.3, 14.4 ppm; significant signals *E,Z*-isomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 132.4, 128.6, 128.4, 127.4, 92.7, 76.4, 67.1 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{16}H_{28}O_5Na$ 323.18290 found 323.18275.

Ethyl (5*R*,6*E*,8*E*)-2-hydroxy-5-((2-methoxyethoxy)methoxy)deca-6,8-dienoate (46)



Ester **45** (2.50 g, 8.32 mmol, 1.00 eq.) was dissolved in dry THF (83 mL) and treated with KHMDS (12.5 mL, 12.5 mmol, 1.50 eq.) at −78 °C. The solution was stirred for 30 min, before MoOPH (4.04 g, 12.5 mmol, 1.50 eq.) was added. Another portion of MoOPH (1.35 g, 4.16 mmol, 0.5 eq.) was added after 2.5 h of stirring at −78 °C. Stirring was continued for 1.5 h and the reaction was quenched with sat. aq. NH_4Cl solution and sat. aq. $Na_2S_2O_3$ solution. The aqueous

phase was extracted thrice with EtOAc, organic phases were washed with H_2O, brine and dried over Na_2SO_4. Crude product was purified by column chromatography (SiO_2, pentane/EtOAc 4:1→3:1) to yield α-hydroxylated ester **46** (2.34 g, 89%, *dr* 1.6:1) as a colourless liquid. $\mathbf{R_f}$ = 0.24 (hexanes/EtOAc 4:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −93.9° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3462 (w), 2980 (m), 2933 (m), 2884 (m), 1735 (m), 1449 (w), 1368 (w), 1261 (m), 1276 (m), 1199 (m), 1103 (m), 1024 (m), 991 (m), 853 (w), 764 (s), 750 (s); *E,E*-isomer major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.16 (dd, 1H, J = 10.5, 15.2 Hz), 6.02 (dd, 1H, J = 10.4, 15.0 Hz), 5.72 (dq, 1H, J = 6.8, 15.0 Hz), 5.34 (m, 1H), 4.76 (d, 1H, J = 6.9 Hz), 4.62 (d, 1H, J = 6.9 Hz), 4.23 (m, 2H), 4.18 (m, 1H), 4.09 (m, 1H), 3.80 (m, 1H), 3.60 (m, 1H), 3.55 (m, 2H), 3.39 (s, 3H), 2.93 (m, 1H), 1.88 (m, 1H), 1.75 (d, 3H, J = 6.8 Hz), 1.78-1.58 (m, 3H), 1.29 (t, 3H, J = 7.1 Hz) ppm; significant signals *E,E*-isomer minor diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 4.62 (d, 1H, J = 7.0 Hz), 3.39 (s, 3H), 2.89 (m, 1H), 1.29 (t, 3H, J = 7.1 Hz) ppm; significant signals *E,Z*-isomer major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.49 (dd, 1H, J = 11.0, 15.2 Hz), 5.98 (m, 1H), 5.52 (dq, 1H, J = 7.1, 10.6 Hz), 5.34 (m, 1H), 4.78 (d, 1H, J = 7.1 Hz), 4.64 (d, 1H, J = 7.1 Hz), 3.40 (s, 3H) ppm; *E,E*-isomer major diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 175.1, 133.6, 130.6, 130.4, 129.6, 92.5, 76.2, 71.8, 70.4, 67.0, 61.6, 59.1, 31.1, 30.4, 18.1, 14.2 ppm; significant signals *E,E*-isomer minor diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 175.1, 133.6, 130.6, 130.4, 129.6, 92.5, 76.0, 71.8, 70.1, 67.0, 61.7, 59.1, 30.7, 30.2 ppm; significant signals *E,Z*-isomer major diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 133.1, 128.5, 128.3, 127.3, 92.6, 76.4 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{16}H_{28}O_6Na$ 339.17781 found 339.17700.

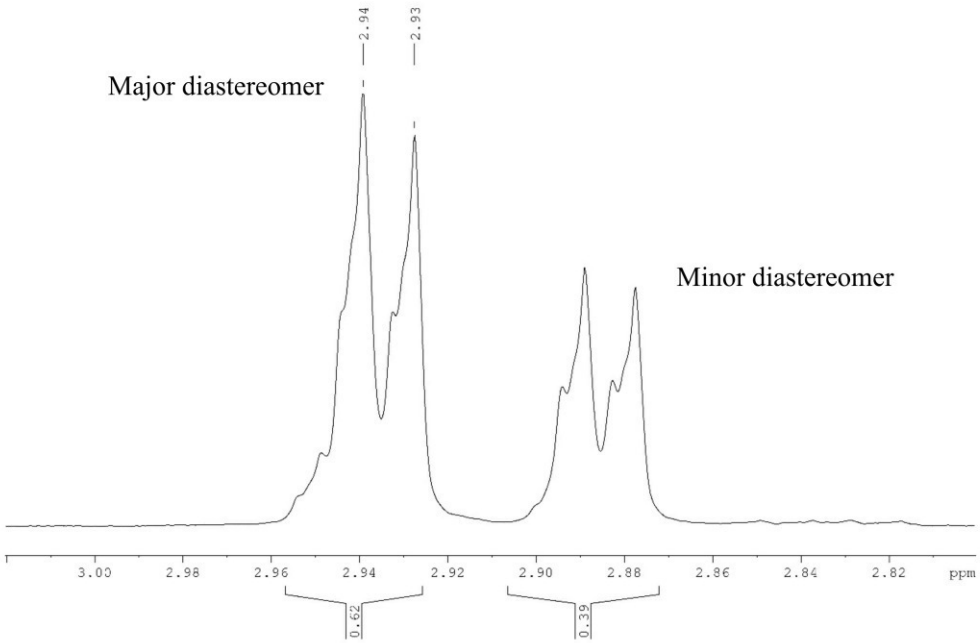
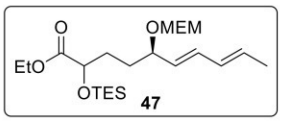


Fig. S7. Significant signals in ^{1}H-NMR-spectrum of ester **46**.

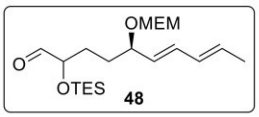
Ethyl (5*R*,6*E*,8*E*)-5-((2-methoxyethoxy)methoxy)-2-((triethylsilyl)oxy)deca-6,8-dienoate (47)



To a solution of α-hydroxylated ester **46** (2.29 g, 7.22 mmol, 1.00 eq.) in dry CH_2Cl_2 (72 mL) TESCl (2.42 mL, 14.4 mmol, 2.00 eq.), imidazole (1.47 g, 21.7 mmol, 3.00 eq.) and DMAP (88.2 mg, 722 µmol, 0.10 eq.) were added at 0 °C. The suspension was stirred at 40 °C for 4.5 h. Sat. aq. NH_4Cl solution was added. The aqueous phase was extracted with CH_2Cl_2 thrice and organic phases were dried over Na_2SO_4. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 8:1) to give TES-protected α-hydroxylated ester **47** (3.27 g, quant.) as a colourless liquid. $\mathbf{R_f}$ = 0.24 (hexanes/EtOAc 4:1); $[\boldsymbol{\alpha}]_D^{20}$ −61.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2956 (m), 2914 (m), 2878 (m), 1752 (m), 1726 (m), 1458 (m), 1276 (m), 1261 (m), 1134 (m), 1023 (m), 990 (m), 764 (s), 750 (s); *E,E*-isomer major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.14 (dd, 1H, J = 10.5, 15.2 Hz), 6.02 (ddq, 1H, J = 1.4, 10.4, 15.0 Hz), 5.69 (dq, 1H, J = 6.8, 15.0 Hz), 5.33 (dd, 1H, J = 8.2, 15.2 Hz), 4.76 (d, 1H, J = 6.9 Hz), 4.61 (d, 1H, J = 6.9 Hz), 4.17 (m, 3H), 4.04 (m, 1H), 3.77 (m, 1H), 3.59 (m, 1H), 3.55

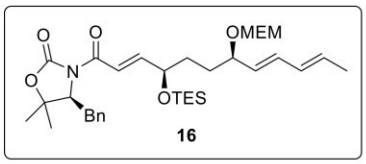
(m, 2H), 3.38 (s, 3H), 1.89-1.76 (m, 1H), 1.75 (d, 3H, *J* = 6.5 Hz), 1.73-1.58 (m, 3H), 1.27 (t, 3H, *J* = 7.1 Hz), 0.95 (t, 9H, *J* = 8.0 Hz), 0.61 (q, 6H, *J* = 8.0 Hz) ppm; significant signals *E,E*-isomer minor diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 4.76 (d, 1H, *J* = 6.9 Hz), 3.39 (s, 3H), 1.75 (d, 3H, *J* = 6.7 Hz), 1.27 (t, 3H, *J* = 7.1 Hz) ppm; significant signals *E,Z*-isomer major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.48 (dd, 1H, *J* = 11.0, 15.3 Hz), 5.98 (m, 1H), 5.51 (dq, 1H, *J* = 7.2, 10.8 Hz), 5.44 (dd, 1H, *J* = 8.2, 15.2), 4.77 (d, 1H, *J* = 7.1 Hz), 4.63 (d, 1H, *J* = 7.1 Hz), 4.10 (m, 1H), 3.39 (s, 3H) ppm; *E,E*-isomer major diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 173.8, 133.7, 130.8, 130.4, 129.9, 92.6, 76.6, 72.2, 71.9, 67.0, 60.9, 59.2, 31.5, 31.4, 18.3, 14.4, 6.86, 4.71 ppm; significant signals *E,E*-isomer minor diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 130.8, 130.4, 129.9, 92.5, 76.1, 71.8, 31.2, 31.1 ppm; significant signals *E,Z*-isomer major diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 132.34, 132.29, 128.7, 128.4 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{22}H_{42}O_6SiNa$ 453.26429 found 453.26346.

(S)-4-Benzyl-3-((2*E*,4*R*,7*R*,8*E*,10*E*)-7-((2-methoxyethoxy)methoxy)-4-((triethylsilyl)oxy)-dodeca-2,8,10-trienoyl)-5,5-dimethyloxazolidin-2-one (16)



48

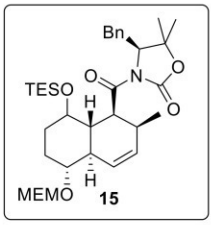
Ester **47** (1.20 g, 2.79 mmol, 1.00 eq.) in dry toluene (28 mL) was treated dropwise with DIBAL (4.18 mL, 4.18 mmol, 1.50 eq.) at −78 °C. The reaction was stirred at this temperature for 5 h, before it was stopped by addition of acetone (1 mL) and sat. aq. Na,K-tartrate solution. The two-phase mixture was stirred vigorously at room temperature for 2 h. The organic phase was separated, and the aqueous phase was extracted with EtOAc four times. The combined organic phases were washed with H_2O and dried over Na_2SO_4. Aldehyde **48** was used without further purification. **R_f** = 0.24 (hexanes/EtOAc 4:1); **IR** ν_{max}/cm^{-1} 3435 (w), 2954 (m), 2933 (m), 2908 (m), 2877 (m), 1731 (m), 1696 (w), 1457 (m), 1414 (m), 1367 (m), 1240 (m), 1199 (w), 1104 (s), 1042 (s), 1018 (s), 975 (s), 849 (m), 809 (m), 741 (s); *E,E*-isomer major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.58 (t, 1H, *J* = 1.6 Hz), 6.14 (dd, 1H, *J* = 10.4, 15.8 Hz), 6.02 (ddq, 1H, *J* = 1.4, 10.5, 15.0 Hz), 5.70 (dq, 1H, *J* = 6.8, 15.0 Hz), 5.32 (dd, 1H, *J* = 8.1, 15.2 Hz), 4.76 (d, 1H, *J* = 7.0 Hz), 4.61 (d, 1H, *J* = 7.0 Hz), 4.03 (m, 1H), 3.97 (m, 1H), 3.77 (m, 1H), 3.59 (m, 1H), 3.55 (m, 2H), 3.38 (s, 3H), 1.80-1.57 (m, 4H), 1.75 (d, 3H, *J* = 6.5 Hz), 0.95 (t, 9H, *J* = 7.9 Hz), 0.61 (q, 6H, *J* = 7.9 Hz) ppm; significant signals *E,Z*-isomer major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.59 (t, 1H, *J* = 1.4 Hz), 6.48 (dd, 1H, *J* = 11.2, 15.2 Hz), 5.99 (m, 1H), 5.52 (dq, 1H, *J* = 7.1, 10.8 Hz), 5.44 (dd, 1H, *J* = 8.2, 15.2 Hz), 4.10 (m, 1H), 3.40 (s, 3H) ppm.



16

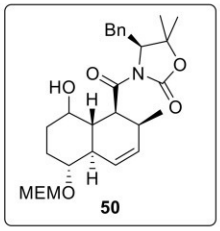
A solution of phosphonate **49** (1.24 g, 3.49 mmol, 1.25 eq.) in dry THF (7 mL) was treated with LiHMDS (3.35 mL, 3.35 mmol, 1.20 eq.) at 0 °C. After stirring for 1 h, crude aldehyde **48** (1.08 g, 2.79 mmol, 1.00 eq.) dissolved in dry THF (3 mL) was added dropwise. The mixture was allowed to warm to room temperature overnight. Sat. aq. NH_4Cl solution stopped the reaction after 17 h of stirring. The aqueous phase was extracted with EtOAc thrice, combined organic phases were washed with H_2O, brine and dried over Na_2SO_4. Removal of the solvent under vacuum and purification by column chromatography (SiO_2, pentane/EtOAc 8:1→6:1→4:1→2:1) furnished trien **16** (1.18 g, 70% over two steps) as a colourless oil. $\mathbf{R_f}$ = 0.38 (hexanes/EtOAc 4:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+27.4° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2952 (m), 2936 (m), 2877 (m), 1778 (s), 1687 (m), 1640 (w), 1497 (w), 1456 (w), 1354 (m), 1329 (w), 1274 (w), 1242 (w), 1207 (w), 1180 (w), 1159 (w), 1100 (s), 1040 (s), 821 (w), 729 (m), 702 (w); *E,E*-isomer major diastereomer ^{1}**H-NMR** (500 MHz, $CDCl_3$) δ 7.39 (dt, 1H, J = 1.3, 15.3 Hz), 7.32-7.20 (m, 5H), 7.04 (ddd, 1H, J = 2.3, 5.2, 15.3 Hz), 6.14 (dd, 1H, J = 10.6, 15.2 Hz), 6.03 (dd, 1H, J = 10.6, 14.8 Hz), 5.69 (dq, 1H, J = 6.8, 14.8 Hz), 5.32 (dd, 1H, J = 8.3, 15.2 Hz), 4.76 (d, 1H, J = 7.0 Hz), 4.61 (d, 1H, J = 7.0 Hz), 4.55 (dt, 1H, J = 3.6, 9.6 Hz), 4.38 (m, 1H), 4.02 (m, 1H), 3.79 (m, 1H), 3.60 (m, 1H), 3.55 (m, 2H), 3.38 (s, 3H), 3.21 (m, 1H), 2.89 (tt, 1H, J = 6.0, 9.7 Hz), 1.75 (d, 3H, J = 6,7 Hz), 1.71-1.53 (m, 4H), 1.38 (s, 3H), 1.35 (s, 3H), 0.95 (t, 9H, J = 8.0 Hz), 0.61 (q, 6H, J = 8.0 Hz) ppm; significant signals *E,E*-isomer minor diastereomer ^{1}**H-NMR** (500 MHz, $CDCl_3$) δ 4.76 (d, 1H, J = 6.9 Hz), 3.39 (s, 3H), 1.37 (s, 3H), 1.36 (s, 3H), 0.96 (t, 3H, J = 7.9 Hz) ppm; significant signals *E,Z*-isomer major diastereomer ^{1}**H-NMR** (500 MHz, $CDCl_3$) δ 7.40 (dt, 1H, J = 1.5, 15.3 Hz), 6.48 (dd, 1H, J = 10.7, 15.3 Hz), 5.98 (m, 1H), 5.51 (dq, 1H, J = 7.1, 10.4 Hz), 5.44 (dd, 1H, J = 8.3, 15.3 Hz), 4.09 (m, 1H), 3.39 (s, 3H) ppm; *E,E*-isomer major diastereomer ^{13}**C-NMR** (125 MHz, $CDCl_3$) δ 165.4, 152.8, 152.6, 137.2, 133.7, 130.8, 130.4, 130.0, 129.2, 128.8, 126.9, 119.6, 92.5, 82.2, 76.7, 72.0, 71.9, 67.0, 63.9, 59.2, 35.4, 33.5, 31.2, 28.8, 22.5, 18.3, 6.99, 4.95 ppm; significant signals *E,E*-isomer minor diastereomer ^{13}**C-NMR** (125 MHz, $CDCl_3$) δ 165.3, 152.7, 137.3, 130.4, 130.0, 119.5, 82.2, 76.4, 71.8, 63.9, 33.4, 31.0, 28.7, 22.5 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{34}H_{53}NO_7SiNa$ 638.34835 found 638.34784.

(4*S*)-4-Benzyl-3-((1*S*,2*S*,4a*R*,5*R*,8a*S*)-5-((2-methoxyethoxy)methoxy)-2-methyl-8-((triethylsilyl)oxy)-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carbonyl)-5,5-dimethyl-oxazolidin-2-one (15)



Trien **16** (513 mg, 833 µmol, 1.00 eq.) was dissolved in dry toluene (28 mL) and heated at 80 °C for 2 days. Temperature was raised to 100°C and stirring was continued for 1 d. The solvent was removed at the rotary evaporator. Crude product was purified by column chromatography (SiO_2, pentane/EtOAc 6:1→8:1) to give Diels-Alder-product **15** (219 mg, 43%, *de* >96%) as a colourless resin. $\mathbf{R_f}$ = 0.50 (hexanes/EtOAc 4:1); $[\alpha]_D^{20}$+63.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3030 (w), 2934 (m), 2876 (m), 1776 (s), 1690 (m), 1497 (w), 1456 (m), 1393 (w), 1374 (m), 1352 (m), 1301 (w), 1273 (m), 1242 (m), 1207 (w), 1221 (w), 1180 (w); 1159 (w), 1129 (w), 1101 (s), 1086 (s), 1039 (s), 1005 (s), 984 (m), 919 (m), 882 (w), 839 (w), 821 (m), 805 (w), 764 (w), 727 (s), 702 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.32 (d, 4H, *J* = 4.4 Hz), 7.24 (sex, 1H, *J* = 4.4 Hz), 5.88 (d, 1H, *J* = 10.0 Hz), 5.61 (ddd, 1H, *J* = 2.6, 4.8, 10.0 Hz), 4.89 (d, 1H, *J* = 7.1 Hz), 4.75 (d, 1H, *J* = 7.1 Hz), 4.57 (dd, 1H, *J* = 2.3, 11.0 Hz), 4.30 (s, 1H), 4.05 (dd, 1H, *J* = 5.9, 11.2 Hz), 3.78 (dt, 1H, *J* = 4.6, 11.1 Hz), 3.73 (dt, 1H, *J* = 4.6, 11.1 Hz), 3.58 (t, 2H, *J* = 4.6 Hz), 3.39 (s, 3H), 3.33 (dd, 1H, *J* = 2.1, 14.3 Hz), 3.24 (dt, 1H, *J* = 4.4, 10.7 Hz), 2.84 (m, 1H), 2.79 (dd, 1H, *J* = 11.2, 14.3 Hz), 2.53 (tq, 1H, *J* = 2.0, 10.7 Hz), 1.93 (m, 1H), 1.81 (dq, 1H, *J* = 3.2, 14.0 Hz), 1.77-1.67 (m, 2H), 1.55 (m, 1H), 1.33 (d, 6H, *J* = 6.9 Hz), 0.93 (t, 9H, *J* = 7.9 Hz), 0.85 (d, 3H, *J* = 7.1 Hz), 0.60-0.46 (m, 6H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 174.6, 151.8, 137.3, 131.1, 129.0, 128.9, 126.9, 126.2, 94.8, 81.5, 79.6, 71.9, 67.2, 66.0, 64.0, 59.2, 43.7, 39.0, 38.8, 35.2, 31.9, 31.0, 29.3, 27.0, 23.2, 17.7, 7.16, 5.43 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{34}H_{53}NO_7SiNa$ 638.34835 found 638.34778.

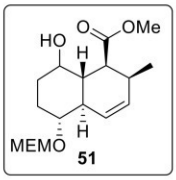
(4*S*)-4-Benzyl-3-((1*S*,2*S*,4a*R*,5*R*,8a*S*)-8-hydroxy-5-((2-methoxyethoxy)methoxy)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carbonyl)-5,5-dimethyloxazolidin-2-one (50)



Diels-Alder product **15** (198 mg, 321 µmol, 1.00 eq.) was dissolved in THF *p.a.* (3.2 mL) and treated with HF·pyridine (459 µL, 17.7 mmol, 55.0 eq.) at 0 °C. The solution was stirred 15 h at this temperature and quenched with sat. aq. $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc four times, combined organic phases were washed

with brine and dried over Na_2SO_4. The deprotected alcohol **50** (161 mg, quant.) was used without further purification. $\mathbf{R_f}$ = 0.42 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$+69.5° (c 1.0 in MeOH); **IR** ν_{max}/cm^{-1} 3485 (w), 2927 (m), 2880 (m), 1775 (s), 1692 (m), 1497 (w), 1455 (m), 1394 (m), 1373 (m), 1353 (m), 1297 (m), 1276 (m), 1230 (m), 1207 (m), 1176 (m), 1159 (m), 1101 (s), 1087 (s), 1036 (s), 956 (m), 921 (w), 844 (w), 822 (w), 766 (w), 730 (s), 700 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.32-7.27 (m, 4H), 7.23 (m, 1H), 5.88 (d, 1H, *J* = 10.0 Hz), 5.63 (ddd, 1H, *J* = 2.6, 4.6, 10.0 Hz), 4.88 (d, 1H, *J* = 7.1 Hz), 4.74 (d, 1H, *J* = 7.1 Hz), 4.56 (dd, 1H, *J* = 4.0, 9.7 Hz), 4.10 (m, 1H), 4.07 (dd, 1H, *J* = 5.8, 11.2 Hz), 3.77 (dt, 1H, *J* = 4.6, 11.1 Hz), 3.71 (dt, 1H, *J* = 4.6, 11.1 Hz), 3.56 (t, 2H, *J* = 4.6 Hz), 3.39 (s, 3H), 3.24 (m, 1H), 3.14 (dd, 1H, *J* = 4.0, 14.3 Hz), 2.88 (dd, 1H, *J* = 9.7, 14.3 Hz), 2.78 (m, 1H), 2.31 (tq, 1H, *J* = 2.6, 11.2 Hz), 1.98 (m, 1H), 1.84 (m, 1H), 1.74 (dt, 1H, *J* = 2.2, 11.2 Hz), 1.55 (m, 2H), 1.35 (d, 6H, *J* = 7.7 Hz), 1.27 (d, 1H, *J* = 5.3 Hz), 0.80 (d, 3H, *J* = 7.1 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 174.0, 152.7, 137.0, 131.8, 129.2, 128.8, 127.0, 125.8, 94.7, 82.5, 79.0, 71.9, 67.2, 65.3, 63.8, 59.2, 43.9, 39.9, 38.4, 35.6, 31.4, 31.3, 28.3, 26.7, 22.3, 17.4 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{28}H_{39}NO_7Na$ 524.26187 found 524.26081.

Methyl(1*S*,2*S*,4a*R*,5*R*,8a*S*)-8-hydroxy-5-((2-methoxyethoxy)methoxy)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (51)

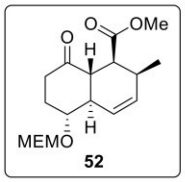


51

Alcohol **50** (554 mg, 1.10 mmol, 1.00 eq.) in dry CH_2Cl_2 (11 mL) was treated with NaOMe (50 wt%, 505 µL, 2.21 mmol, 2.00 eq.) at 0 °C. After stirring for 3 h, sat. aq. NH_4Cl solution was added, and the aqueous phase was extracted wit EtOAc four times. The combined organic phases were washed with sat. aq. $NaHCO_3$ solution as well as brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure and purification by column chromatography (SiO_2, pentane/EtOAc 3:1→2:1→2:3→1:2) gave methylester **51** (325 mg, 90%) in 90% yield as a colourless oil. $\mathbf{R_f}$ = 0.35 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$+82.8° (c 1.0 in MeOH); **IR** ν_{max}/cm^{-1} 3484 (w), 3024 (w), 2932 (m), 2877 (m), 1732 (s), 1453 (w), 1436 (w), 1366 (w), 1296 (w), 1243 (w), 1199 (m), 1172 (m), 1127 (s), 1107 (s), 1032 (s), 1019 (s), 956 (m), 937 (m), 871 (m), 849 (w), 775 (w), 750 (m), 730 (m), 676 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.89 (d, 1H, *J* = 10.0 Hz), 5.61 (ddd, 1H, *J* = 2.6, 4.4, 10.0 Hz), 4.88 (d, 1H, *J* = 7.1 Hz), 4.74 (d, 1H, *J* = 7.1 Hz), 4.21 (s, 1H), 3.77 (dt, 1H, *J* = 4.6, 11.1 Hz), 3.71 (dt, 1H, *J* = 4.6, 11.1 Hz), 3.69 (s, 3H), 3.56 (t, 2H, *J* = 4.6 Hz), 3.39 (s, 3H), 3.23 (dt, 1H, *J* = 3.9, 10.6 Hz), 2.90 (dd, 1H, *J* = 6.0, 11.6 Hz), 2.59 (m, 1H), 2.34 (tq, 1H, *J* = 2.6, 10.6 Hz), 1.99 (m, 1H), 1.84 (m, 1H), 1.72-1.56

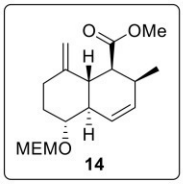
(m, 3H), 1.28 (m, 1H), 0.90 (d, 3H, J = 7.1 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 174.2, 131.5, 126.2, 94.7, 79.2, 71.9, 67.2, 65.4, 59.2, 51.5, 45.1, 39.6, 38.1, 32.2, 31.5, 26.7, 17.6 ppm; **HRMS** ESI m/z $[M + Na]^+$ calcd. for $C_{17}H_{28}NO_6Na$ 351.17781 found 351.17722.

Methyl(1*S*,2*S*,4a*R*,5*R*,8a*S*)-5-((2-methoxyethoxy)methoxy)-2-methyl-8-methylene-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylate (14)



52

To a solution of alcohol **51** (305 mg, 928 µmol, 1.00 eq.) in CH_2Cl_2 *p.a.* (9.3 mL) was added DMP (590 mg, 1.39 mmol, 1.50 eq.) and $NaHCO_3$ (390 mg, 4.64 mmol, 5.00 eq.) at 0 °C. The suspension was allowed to warm to room temperature and stirred for 3 h. After addition of sat. aq. $Na_2S_2O_3$ solution and sat. aq. $NaHCO_3$ solution, the aqueous phase was extracted with EtOAc four times. The combined organic phases were washed with sat. aq. $NaHCO_3$ solution, sat. aq. $Na_2S_2O_3$ solution as well as brine and dried over Na_2SO_4. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 3:1→3:2→1:1) to give ketone **52** (290 mg, 96%) as a colourless resin in 96% yield. The product wasn´t further purified, but directly used in the next reaction. **R_f** = 0.53 (hexanes/EtOAc 1:1); **$[\alpha]_D^{20}$**+111.1° (c 1.0 in MeOH); **IR** ν_{max}/cm^{-1} 3035 (w), 2958 (m), 2928 (m), 2877 (m), 1737 (s), 1720 (s), 1455 (m), 1436 (m), 1375 (w), 1326 (w), 1255 (m), 1197 (m), 1174 (m), 1145 (m), 1097 (s), 1034 (s), 927 (w), 854 (w), 814 (w), 742 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.84 (d, 1H, J = 10.0 Hz), 5.70 (ddd, 1H, J = 2.6, 4.4, 10.0 Hz), 4.89 (d, 1H, J = 7.1 Hz), 4.79 (d, 1H, J = 7.1 Hz), 3.77 (dt, 1H, J = 4.7, 10.9 Hz), 3.71 (m, 1H), 3.69 (s, 3H), 3.57 (t, 2H, J = 4.6 Hz), 3.40 (s, 3H), 2.84 (dd, 1H, J = 6.4, 11.5 Hz), 2.71 (t, 1H, J = 12.0 Hz), 2.66-2.47 (m, 4H), 2.39 (m, 1H), 2.17 (m, 1H), 1.71 (dq, 1H, J = 5.7, 13.4 Hz), 0.86 (d, 3H, J = 7.2 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 209.6, 174.2, 132.7, 124.7, 95.2, 77.7, 71.8, 67.5, 59.2, 51.7, 46.8, 45.1, 42.6, 38.8, 32.9, 31.0, 17.8 ppm; **HRMS** ESI m/z $[M + Na]^+$ calcd. for $C_{17}H_{26}O_6Na$ 349.16216 found 349.16156.

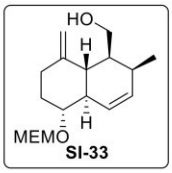


14

Methylphosphoniumbromide (2.14 g, 6.00 mmol, 1.20 eq.) in dry THF (10 mL) was treated with KO*t*Bu (561 mg, 5.00 mmol, 1.00 eq.) at 0 °C. The suspension was stirred for 45 min. A solution of ketone **52** (268 mg, 821 µmol, 1.00 eq.) in dry THF (4.3 mL) was treated with the suspension of ylide (0.5M, 3.28 mL, 1.64 mmol, 2.00 eq.) at 0 °C and stirred for 3 h at room temperature. Sat. aq. NH_4Cl solution was added, and the aqueous phase was extracted with EtOAc four times. The combined organic phases were dried over Na_2SO_4 and the solvents were removed *in vacuo*. Purification of the crude product by column chromatography (SiO_2, pentane/EtOAc 5:1)

delivered decalin **14** (240 mg, 90%) as a colourless liquid in 90% yield. $\mathbf{R_f}$ = 0.74 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$+101.6° (c 1.0 in MeOH); **IR** ν_{max}/cm^{-1} 2934 (m), 2877 (m), 1742 (s), 1653 (w), 1455 (m), 1436 (m), 1365 (w), 1325 (m), 1300 (w), 1256 (m), 1192 (m), 1132 (s), 1109 (s), 1058 (m), 1032 (s), 931 (m), 892 (m), 852 (m), 818 (w), 775 (w), 745 (m), 730 (m), 670 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.84 (dt, 1H, J = 1.4, 10.0 Hz), 5.68 (ddd, 1H, J = 2.6, 4.6, 10.0 Hz), 4.87 (d, 1H, J = 7.1 Hz), 4.75 (d, 1H, J = 7.1 Hz), 4.73 (s, 1H), 4.39 (s, 1H), 3.76 (dt, 1H, J = 4.7, 11.0 Hz), 3.70 (dt, 1H, J = 4.7, 11.0 Hz), 3.67 (s, 3H), 3.57 (t, 2H, J = 4.6 Hz), 3.40 (dt, 1H, J = 4.6, 10.8 Hz), 3.40 (s, 3H), 2.92 (dd, 1H, J = 6.3, 11.9 Hz), 2.62 (m, 1H), 2.38-2.28 (m, 2H), 2.23-2.13 (m, 2H), 1.87 (tq, 1H, J = 2.2, 10.8 Hz), 1.46-1.35 (m, 1H), 0.89 (d, 3H, J = 7.2 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 174.7, 150.3, 131.7, 125.4, 104.7, 95.0, 79.5, 71.9, 67.2, 59.2, 51.5, 48.5, 45.4, 38.5, 34.9, 34.7, 31.7, 18.3 ppm; **HRMS** ESI m/z $[M + H]^+$ calcd. for $C_{18}H_{29}O_5$ 325.20095 found 325.19994.

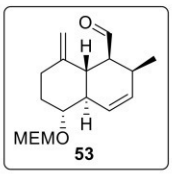
(1*S*,2*S*,4a*R*,5*R*,8a*S*)-5-((2-Methoxyethoxy)methoxy)-2-methyl-8-methylene-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carbaldehyde (53)



SI-33

Ester **14** (220 mg, 678 µmol, 1.00 eq.) dissolved in dry CH_2Cl_2 (6.8 mL) was treated with DIBAL (2.03 mL, 2.03 mmol, 3.00 eq.) at 0 °C. After stirring at this temperature for 4 h another portion of DIBAL (339 µL, 339 µmol, 0.50 eq.) was added. As soon as TLC showed complete conversion of the starting material, sat. aq. Na,K-tartrate solution was added and the two-phase mixture was stirred vigorously at room temperature for 45 min. The aqueous phase was extracted with EtOAc thrice, combined organic phases were washed with brine and dried over Na_2SO_4. Solvents were removed at the rotary evaporator. Crude product **SI-33** (211 mg, quant.) was used without further purification. $\mathbf{R_f}$ = 0.53 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$+76.5° (c 0.9 in MeOH); **IR** ν_{max}/cm^{-1} 3424 (w), 3027 (w), 2930 (m), 2875 (m), 1649 (m), 1454 (m), 1394 (w), 1366 (m), 1296 (w), 1242 (w), 1200 (w), 1178 (w), 1155 (w), 1109 (m), 1086 (m), 1052 (s), 1037 (s), 1014 (s), 982 (m), 923 (m), 896 (m), 849 (w), 830 (w), 749 (m), 739 (m), 720 (w), 677 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.80 (d, 1H, J = 10.1 Hz), 5.76 (ddd, 1H, J = 1.9, 4.4, 10.1 Hz), 4.88 (s, 1H), 4.85 (d, 1H, J = 7.1 Hz), 4.76 (s, 1H), 4.74 (d, 1H, J = 7.1 Hz), 4.15 (dt, 1H, J = 4.4, 11.3 Hz), 3.76 (dt, 1H, J = 4.7, 10.9 Hz), 3.70 (dt, 1H, J = 4.7, 10.9 Hz), 3.56 (t, 2H, J = 4.7 Hz), 3.55 (m, 1H), 3.40 (s, 3H), 3.37 (dt, 1H, J = 4.5, 10.7 Hz), 2.50 (m, 1H), 2.31 (m, 2H), 2.21 (m, 1H), 2.05 (dt, 1H, J = 4.6, 13.0 Hz), 1.93 (tq, 1H, J = 1.9, 10.7 Hz), 1.75 (t, 1H, J = 10.7 Hz), 1.43 (m, 1H), 1.20 (t, 1H, J = 5.3 Hz), 0.99 (d, 3H, J = 7.1 Hz) ppm; **^{13}C-NMR**

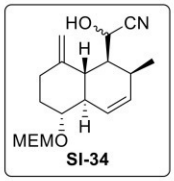
(125 MHz, $CDCl_3$) δ 150.3, 133.4, 125.2, 106.3, 95.0, 80.1, 71.9, 67.2, 62.1, 59.2, 50.6, 39.2, 38.5, 35.8, 35.6, 30.5, 16.4 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{17}H_{29}O_4$ 297.20604 found 297.20509.



53

A solution of alcohol **SI-33** (180 mg, 607 µmol, 1.00 eq.) in CH_2Cl_2 *p.a.* (6 mL) was treated with $NaHCO_3$ (255 mg, 3.04 mmol, 5.00 eq.) and DMP (386 mg, 911 µmol, 1.50 eq.) at 0 °C. The suspension was stirred at this temperature for 1 h and at room temperature for 2 h. Sat. aq. $NaHCO_3$ solution and sat. aq. $Na_2S_2O_3$ solution were added. The aqueous phase was extracted with EtOAc thrice, the combined organic phases were washed with sat. aq. $NaHCO_3$ solution, sat. $Na_2S_2O_3$ aq. solution as well as brine and dried over Na_2SO_4. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 6:1→5:1) to give aldehyde **53** (163 mg, 91%) as a colourless liquid. $\mathbf{R_f}$ = 0.71 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+43.0° (c 0.4 in MeOH); **IR** ν_{max}/cm^{-1} 2929 (m), 2878 (m), 1720 (m), 1652 (w), 1455 (m), 1366 (w), 1261 (m), 1199 (w), 1166 (w), 1102 (s), 1094 (s), 1032 (s), 895 (m), 849 (w), 803 (m), 741 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 9.64 (d, 1H, *J* = 4.3 Hz), 5.87 (dt, 1H, *J* = 1.5, 10.1 Hz), 5.67 (ddd, 1H, *J* = 2.6, 4.5, 10.1 Hz), 4.88 (d, 1H, *J* = 7.1 Hz), 4.82 (s, 1H), 4.76 (d, 1H, *J* = 7.1 Hz), 4.38 (s, 1H), 3.77 (dt, 1H, *J* = 4.8, 10.8 Hz), 3.71 (dt, 1H, *J* = 4.8, 10.8 Hz), 3.57 (t, 2H, *J* = 4.8 Hz), 3.44 (dt, 1H, *J* = 4.6, 10.7 Hz), 3.40 (s, 3H), 2.74-2.62 (m, 2H), 2.42-2.29 (m, 3H), 2.18 (dt, 1H, *J* = 4.6, 13.5 Hz), 1.92 (tq, 1H, *J* = 2.1, 10.7 Hz), 1.44 (m, 1H), 1.01 (d, 3H, *J* = 6.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 207.5, 148.8, 132.0, 125.8, 107.5, 94.9, 79.3, 71.9, 67.3, 59.2, 50.3, 48.5, 37.4, 34.74, 34.67, 32.4, 16.9 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{17}H_{27}O_4$ 295.19039 found 295.18976.

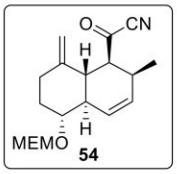
(1*S*,2*S*,4a*R*,5*R*,8a*S*)-5-((2-Methoxyethoxy)methoxy)-2-methyl-8-methylene-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carbonyl cyanide (54)



SI-34

Aldehyde **53** (86.2 mg, 292 µmol, 1.00 eq.) in dry CH_2Cl_2 (3 mL) was treated with TMSCN (110 µL, 876 µmol, 3.00 eq.) and dry NEt_3 (121 µL, 876 µmol, 3.00 eq.) at 0 °C. The solution was stirred at this temperature for 20 min and at room temperature for 4 h. The volatiles were removed at the rotary evaporator. Crude product was dissolved in EtOH *p.a.* and NH_4F (48.7 mg, 1.31 mmol, 4.50 eq.) was added at 0 °C. After 2 h of stirring TLC showed complete conversion of the starting material. H_2O was added and the aqueous phase was extracted with EtOAc thrice. Combined organic phases were washed with brine and dried over Na_2SO_4. The solvents were

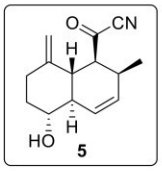
removed under reduced pressure and the oily, colourless product **SI-34** (92.3 mg, quant., *dr* 1.1:1) was used without further purification. $\mathbf{R_f}$ = 0.64 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+61.1° (c 0.5 in MeOH); **IR** ν_{max}/cm^{-1} 3385 (m), 3076 (w), 3030 (w), 2933 (m), 2881 (m), 1651 (m), 1455 (m), 1395 (w), 1366 (w), 1296 (w), 1244 (w), 1170 (m), 1098 (s), 1036 (s), 894 (m), 848 (m), 754 (m), 737 (w), 677 (w); major diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.83 (d, 1H, *J* = 10.0 Hz), 5.74 (ddd, 1H, *J* = 2.5, 5.1, 10.0 Hz), 5.30 (m, 1H), 4.93 (s, 1H), 4.85 (d, 1H, *J* = 7.1 Hz), 4.74 (d, 1H, *J* = 7.1 Hz), 4.68 (s, 1H), 3.75 (dt, 1H, *J* = 4.7, 10.9 Hz), 3.69 (dt, 1H, *J* = 4.7, 10.9 Hz), 3.56 (t, 2H, *J* = 4.7 Hz), 3.43 (m, 1H), 3.39 (s, 3H), 2.73 (m, 1H), 2.55 (m, 1H), 2.40-2.25 (m, 3H), 2.22-1.95 (m, 3H), 1.44 (m, 1H), 1.19 (d, 3H, *J* = 7.1 Hz) ppm; minor diastereomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.81 (d, 1H, *J* = 10.0 Hz), 5.72 (ddd, 1H, *J* = 2.5, 5.1, 10.0 Hz), 5.30 (m, 1H), 4.94 (s, 1H), 4.85 (d, 1H, *J* = 7.1 Hz), 4.74 (d, 1H, *J* = 7.1 Hz), 4.65 (s, 1H), 3.75 (dt, 1H, *J* = 4.7, 10.9 Hz), 3.69 (dt, 1H, *J* = 4.7, 10.9 Hz), 3.56 (t, 2H, *J* = 4.7 Hz), 3.43 (m, 1H), 3.40 (s, 3H), 2.63 (m, 1H), 2.35 (m, 3H), 2.22-1.95 (m, 4H), 1.44 (m, 1H), 1.21 (d, 3H, *J* = 7.1 Hz) ppm; major diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 150.7, 132.7, 125.4, 119.4, 106.2, 95.0, 79.8, 71.9, 67.2, 61.1, 59.2, 50.5, 40.0, 38.0, 35.8, 35.7, 31.7, 18.2 ppm; minor diastereomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 150.1, 132.7, 125.4, 119.4, 105.7, 95.0, 79.9, 71.9, 67.2, 60.9, 59.2, 50.7, 40.0, 39.5, 35.65, 35.61, 29.3, 17.1 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{18}H_{28}NO_4$ 322.20128 found 322.20044.



54

A solution of cyanohydrin **SI-34** (87.0 mg, 271 µmol, 1.00 eq.) in dry CH_2Cl_2 (2.7 mL) was treated with DMP (138 mg, 325 µmol, 1.20 eq.) at 0 °C. The suspension was stirred for 1.5 h at this temperature, before it was filtered off over celite®. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (SiO_2, pentane/EtOAc 5:1) to give the acylcyanide **54** (71.3 mg, 82%) as a colourless liquid. $\mathbf{R_f}$ = 0.77 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+111.3° (c 1.0 in MeOH); **IR** ν_{max}/cm^{-1} 2934 (m), 2879 (m), 2217 (w), 1708 (m), 1653 (w), 1455 (w), 1177 (m), 1096 (s), 1054 (s), 1026 (s), 897 (m), 744 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.91 (dt, 1H, *J* = 1.5, 10.1 Hz), 5.70 (ddd, 1H, *J* = 2.6, 4.6, 10.1 Hz), 4.87 (s, 1H), 4.87 (d, 1H, *J* = 7.1 Hz), 4.75 (d, 1H, *J* = 7.1 Hz), 4.29 (s, 1H), 3.76 (dt, 1H, *J* = 4.8, 10.9 Hz), 3.70 (dt, 1H, *J* = 4.8, 10.9 Hz), 3.56 (t, 2H, *J* = 4.8 Hz), 3.45 (dt, 1H, *J* = 4.6, 10.7 Hz), 3.39 (s, 3H), 3.18 (dd, 1H, *J* = 6.3, 12.1 Hz), 2.82 (m, 1H), 2.45-2.33 (m, 3H), 2.22 (dt, 1H, *J* = 4.7, 13.5), 1.94 (tq, 1H, *J* = 2.1, 10.7 Hz), 1.43 (m, 1H), 1.04 (d, 3H, *J* = 7.1 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 180.6, 148.8, 130.8, 125.9, 113.6, 107.2, 94.9, 79.0, 71.8, 67.4, 59.2, 52.7, 48.4, 37.8, 34.7, 34.3, 32.0, 17.4 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{18}H_{25}NO_4Na$ 342.16758 found 342.16726.

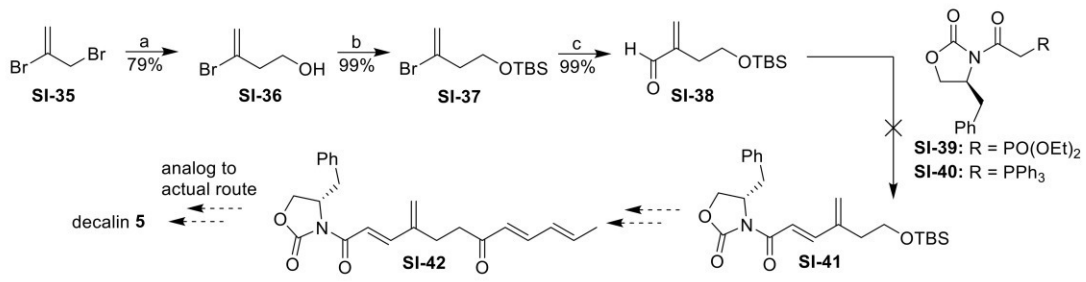
(1*S*,2*S*,4a*R*,5*R*,8a*S*)-5-Hydroxy-2-methyl-8-methylene-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carbonyl cyanide (5)



MEM-ether **54** (25.1 mg, 78.3 µmol, 1.00 eq.) in MeCN *p.a.* (1.5 mL) and H_2O (0.1 mL) was treated with $LiBF_4$ (183 mg, 1.96 mmol, 25.0 eq.) at room temperature. The mixture was stirred at 55 °C for 4.5 h. H_2O was added at 0 °C and the aqueous phase was extracted with EtOAc thrice. The combined organic phases were washed with brine and dried over Na_2SO_4. Purification of the crude product by column chromatography (SiO_2, pentane/EtOAc 4:1→3:1) gave product **5** (18.2 mg, 99%) as a colourless resin. $\mathbf{R_f}$ = 0.30 (hexanes/EtOAc 3:1); $[\alpha]_D^{20}$+157.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3375 (m), 3081 (w), 3032 (w), 2965 (w), 2939 (m), 2877 (m), 2217 (m), 1708 (s), 1652 (m), 1454 (m), 1377 (w), 1328 (w), 1260 (w), 1163 (m), 1062 (s), 1029 (s), 999 (w), 896 (m), 868 (w), 838 (w), 741 (s), 674 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 6.00 (dt, 1H, J = 1.4, 10.1 Hz), 5.73 (ddd, 1H, J = 2.6, 4.6, 10.1 Hz), 4.89 (s, 1H), 4.30 (s, 1H), 3.52 (dt, 1H, J = 4.6, 10.5 Hz), 3.18 (dd, 1H, J = 6.3, 12.2 Hz), 2.83 (m, 1H), 2.43 (m, 1H), 2.36 (t, 1H, J = 11.6 Hz), 2.30-2.22 (m, 2H), 1.84 (tq, 1H, J = 2.2, 10.6 Hz), 1.65 (br. s, 1H), 1.52-1.43 (m, 1H), 1.05 (d, 3H, J = 7.2 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 180.7, 148.8, 130.9, 125.8, 113.6, 107.4, 73.3, 52.6, 49.9, 37.8, 37.5, 34.5, 32.1, 17.4 ppm.

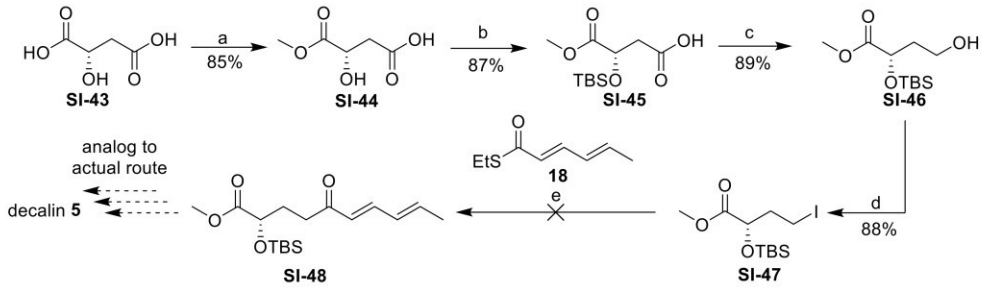
2.6 Failed routes to the decalin

One promising and short route started from dibromide **SI-35** which was elongated by a tin mediated reaction to alcohol **SI-36**. After TBS-protection and formylation an HWE- or Wittig olefination with an auxiliary based phosphonate **SI-39** or ylide **SI-40** was not possible. The following steps should have been performed analogously to the actual route.



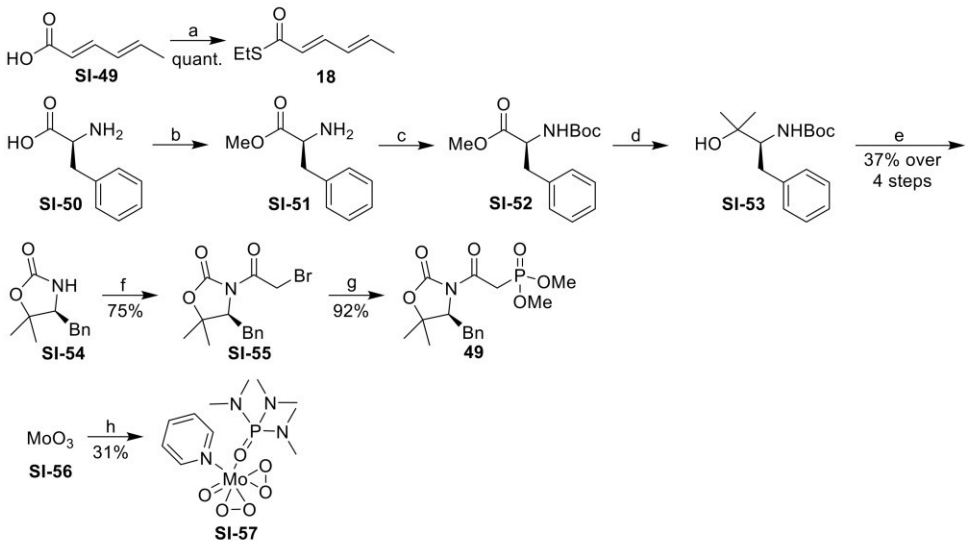
Scheme S8. Attempt to synthesise triene **SI-42** starting from vinylbromide **SI-35**.
Reagents and conditions: a) Sn, CH_2O, cat. HBr, Et_2O/H_2O, rt, 19 h; b) TBSCl, imidazole, DMAP, CH_2Cl_2, rt, 21 h, c) 1. *t*BuLi, Et_2O, −78 °C, 30 min, 2. DMF, 3.5 h.

After failure of the olefination of an α,β-unsaturated aldehyde we had the plan to introduce the terminal alkene after the olefination reaction. Starting with malic acid (**SI-43**) it was first chemoselectively esterified and TBS-protected (→ **SI-45**). The carboxyl group was reduced to alcohol **SI-46** which was iodinated in an Appel-reaction (→**SI-47**). The following Fukuyama coupling was not successful due to low formation of the zinc organyl.



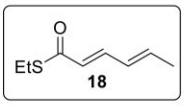
Scheme S9. Tested route to α-hydroxylated ester **SI-48**.
Reagents and conditions: a) 1. $(TFA)_2$, rt, 3 h, 2. MeOH, rt, 22.5 h, b) 1. TBSCl, imidazole, DMAP, CH_2Cl_2, rt, 23 h, 2. K_2CO_3, H_2O, MeOH, rt, 2.5 h, c) 1. EtOCOCl, NMM, THF, −10 °C, 1.2 h, 2. $NaBH_4$, H_2O, 1 h; d) PPh_3, imidazole, I_2, THF, 0 °C, 1 h.

2.7 Synthesis of reagents for the decalin fragment



Scheme S10. Synthesis of thioester **18**, phosphonate **49** and molybdenum reagent **SI-57** needed for formation of decalin **5**.
Reagents and conditions: a) DCC, DMAP, EtSH, CH_2Cl_2, 0 °C→rt, 21 h; b) $SOCl_2$, MeOH, 0 °C→reflux, 20 h; c) Boc_2O, NEt_3, imidazole, CH_2Cl_2, 17 h; d) MeMgBr, THF, 0 °C→rt, 2 d; e) KO*t*Bu, THF, 0 °C, 30 min; f) 1. *n*BuLi, THF, −80 °C, 10 min, 2. Bromoacetylbromide, −80 °C→rt, 13.5 h; g) $P(OMe)_3$, 20.5 h, rt→60 °C; h) 1. H_2O_2, 40 °C, 4.25 h, 2. HMPA, rt, 5 min, 3. Pyridine, THF, rt, 15 min.

(*S*)-Ethyl (2*E*,4*E*)-hexa-2,4-dienethioate (18)



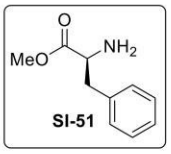
Sorbic acid (**SI-49**) (5.00 g, 44.6mmol, 1.00 eq.) was dissolved in dry CH_2Cl_2 (203 mL). DCC (9.66 g, 46.8 mmol, 1.05 eq.), DMAP (545 mg, 4.46 mmol, 0.10 eq.) were added at room temperature. At 0 °C EtSH (4.29 mL, 58.0 mmol, 1.30 eq.) was dripped to the mixture and it was stirred for 21 h at room temperature. The reaction mixture was filtered off over celite® and the solvents were partially removed. The organic phase was washed with sat. aq. $NaHCO_3$ solution and H_2O. The combined aqueous phases were reextracted with CH_2Cl_2 once and the organic phases were washed with brine. It was dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 30:1→20:1) to give thioester **18** (6.97 g, quant.) as a light-yellow liquid. $\mathbf{R_f}$ = 0.92 (CH_2Cl_2/MeOH 25:1); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.17 (dd, 1H, J = 10.2, 15.2 Hz), 6.25-6.11 (m, 2H), 6.06 (d, 1H, J =

15.2 Hz), 2.95 (q, 2H, *J* = 7.4 Hz), 1.86 (d, 3H, *J* = 6.1 Hz), 1.28 (t, 3H, *J* = 7.4 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 190.3, 141.0, 140.8, 129.8, 126.3, 23.3, 19.0, 15.0 ppm.

Spectroscopic data corresponded to those reported in the literature.[7]

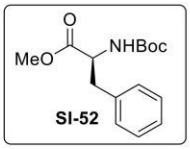
(*S*)-4-Benzyl-5,5-dimethyloxazolidin-2-one (SI-54)

L-Phenylalanin (**SI-50**, 19.8 g, 120 mmol, 1.00 eq.) in MeOH *p.a.* (300 mL) was treated with $SOCl_2$ (26.1 mL, 360 mmol, 3.00 eq.) at 0 °C. The mixture was stirred at reflux for 20 h. The volatiles were removed under reduced pressure. The crude product was dissolved in MeOH *p.a.* and solvents were removed. This procedure was carried out multiple times. Methylesterhydrochlorid **SI-51** (25.7 g, quant.) was isolated as a colourless solid and used without further purification.



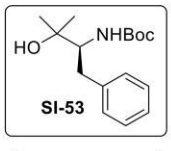
SI-51

Methylester **SI-51** (25.7 g, 119 mmol, 1.00 eq.) in dry CH_2Cl_2 (300 mL) was treated with dry NEt_3 (18.3 mL) and Boc_2O (27.3 g, 125 mmol, 1.05 eq.) in dry CH_2Cl_2 (100 mL) at 0 °C. The suspension was stirred at this temperature for 20 min, dry NEt_3 (4.15 mL, 29.8 mmol, 0.25 eq.) was added again and stirring was continued at room temperature for 16 h. Imidazole (810 mg, 11.9 mmol, 0.10 eq.) was added and stirring was continued for 30 min. The mixture was poured into citric acid solution (1M). Organic phase was separated and washed with citric acid solution (1M) twice, with 1 vol% HCl twice and with brine once. They were dried over Na_2SO_4, and solvents were removed at the rotary evaporator. The Boc-protected phenylalanineester **SI-52** (33.1 g, 92%) was isolated as a clear brownish resin and was used without further purification.



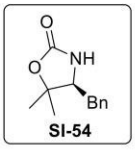
SI-52

Ester **SI-52** (27.9 g, 100 mmol, 1.00 eq.) in dry THF (200 mL) was treated with MeMgBr (3M in Et_2O, 133 mL, 400 mmol) at 0 °C over 45 min. Solution was stirred at room temperature for 2 d. MeOH and H_2O was added, and the suspension was filtered off over celite®. The solvent was removed under reduced pressure and the crude product was suspended in Et_2O, filtered off over celite® and the solvent was again removed at the rotary evaporator. This procedure was repeated once. Alcohol **SI-53** (25.4 g, 91%) was isolated as a pale brown resin.



SI-53

Alcohol **SI-53** (25.4 g, 90.9 mmol, 1.00 eq.) in dry THF (364 mL) was treated with KO*t*Bu (12.2 g, 109 mmol, 1.20 eq.) at 0 °C. After stirring for 30 min, sat. aq. NH_4Cl solution and EtOAc were added, and the aqueous phase was extracted with EtOAc twice. Combined organic phases were washed with brine and dried

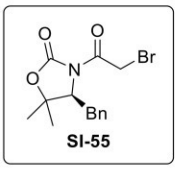


SI-54

over Na_2SO_4. After removal of the volatiles under reduced pressure, the crude product was recrystallised with pentane/Et_2O twice. Oxazolidinone **SI-54** (8.29 g, 44%) was obtained as colourless needles. $\mathbf{R_f}$ = 0.26 (hexanes/EtOAc 2:1); **mp** 66.5 °C; Lit.[8] **mp** 66-67 °C; **IR** ν_{max}/cm^{-1} 3263 (m), 3030 (w), 2980 (m), 2933 (w), 1739 (s), 1604 (w), 1496 (m), 1455 (m), 1374 (m), 1298 (m), 1271 (m), 1241 (w), 1218 (w), 1189 (w), 1143 (w), 1085 (m), 995 (m), 967 (w), 940 (w), 914 (w), 884 (w), 771 (m), 744 (m), 700 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.34 (m, 2H), 7.27 (m, 1H), 7.18 (m, 2H), 4.87 (br. s , 1H), 3.69 (ddd, 1H, J = 0.6, 3.7, 10.8 Hz), 2.84 (dd, 1H, J = 3.7, 13.3 Hz), 2.67 (dd, 1H, J = 10.8, 13.3 Hz), 1.48 (s, 3H), 1.46 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 158.0, 137.0, 129.2, 129.0, 127.4, 127.1, 83.3, 63.2, 37.2, 27.7, 22.1 ppm.

Spectroscopic data corresponded to those reported in the literature.[8]

***(S)*-4-Benzyl-3-(2-bromoacetyl)-5,5-dimethyloxazolidin-2-one (SI-55)**

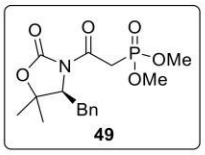


SI-55

A solution of oxazolidinone **SI-54** (6.00 g, 29.2 mmol, 1.00 eq.) in dry THF (73 mL) was treated with *n*BuLi (12.3 mL, 30.7 mmol, 1.05 eq.) at −80 °C. After 10 min, bromoacetylbromide (2.67 mL, 32.7 mmol, 1.12 eq.) was added at −80 °C and stirring was continued for 13.5 h at room temperature. Sat. aq. NH_4Cl solution and EtOAc were added and the aqueous phase was extracted with EtOAc thrice. Combined organic phases were washed with sat. aq. $NaHCO_3$ solution as well as brine and dried over Na_2SO_4. Crude product was purified by column chromatography (SiO_2, pentane/EtOAc 5:1) to yield bromide **SI-55** (7.14 g, 75%) as a light-yellow oil. $\mathbf{R_f}$ = 0.76 (hexanes/EtOAc 4:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$−26.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3060 (w), 3028 (w), 2983 (w), 2940 (w), 1773 (s), 1698 (s), 1605 (w), 1497 (w), 1455 (w), 1415 (w), 1393 (m), 1358 (s), 1327 (m), 1276 (s), 1234 (m), 1207 (m), 1184 (m), 1161 (m), 1142 (m), 1094 (s), 1024 (w), 962 (m), 920 (w), 902 (w), 849 (w), 761 (m), 731 (m), 700 (m), 653 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.35-7.21 (m, 5H), 4.58 (d, 1H, J = 12.4 Hz), 4.51 (dd, 1H, J = 3.8, 9.7 Hz), 4.44 (d, 1H, J = 12.4 Hz), 3.19 (dd, 1H, J = 3.8, 14.6 Hz), 2.90 (dd, 1H, J = 9.7, 14.6 Hz), 1.42 (s, 3H), 1.41 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 166.4, 152.3, 136.6, 129.2, 128.9, 127.1, 83.4, 64.2, 35.1, 28.8, 28.5, 22.4 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{14}H_{17}NO_3Br$ 326.03863 found 326.03800.

Spectroscopic data corresponded to those reported in the literature.[9]

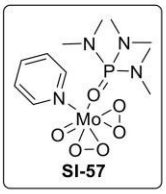
Dimethyl-(*S*)-(2-(4-benzyl-5,5-dimethyl-2-oxooxazolidin-3-yl)-2-oxoethyl)phosphonate (49)



49

Bromide **SI-55** (5.17 g, 15.8 mmol, 1.00 eq.) was treated with $P(OMe)_3$ (9.36 mL, 79.2 mmol, 5.00 eq.) at room temperature. The mixture was stirred for 17 h at room temperature and for 3.5 h at 60 °C. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, EtOAc) to give phosphonate **49** (5.15 g, 92%) as a colourless resin. $\mathbf{R_f}$ = 0.59 (EtOAc); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −12.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3011 (w), 2957 (w), 2854 (w), 1771 (s), 1695 (s), 1605 (w), 1498 (w), 1456 (w), 1396 (m), 1357 (s), 1322 (m), 1265 (s), 1211 (m), 1185 (m), 1160 (m), 1094 (m), 1056 (m), 1020 (s), 964 (m), 926 (w), 901 (w), 882 (m), 846 (m), 806 (m), 764 (m), 731 (s), 700 (m), 677 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.33-7.21 (m, 5H), 4.53 (dd, 1H, J = 3.7, 9.8 Hz), 4.06 (dd, 1H, J = 14.1, 22.0 Hz), 3.82 (d, 3H, J = 4.9 Hz), 3.80 (d, 3H, J = 4.9 Hz), 3.56 (dd, 1H, J = 14.1, 22.2 Hz), 3.18 (dd, 1H, J = 3.7, 14.6 Hz), 2.89 (dd, 1H, J = 9.8, 14.6 Hz), 1.40 (s, 3H), 1.37 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 165.0 (d, J = 7.0 Hz), 152.8, 136.9, 129.2, 128.8, 127.0, 82.8, 64.1, 53.4 (d, J = 5.8 Hz), 53.3 (d, J = 5.8 Hz), 35.3, 34.4, 33.3, 28.5, 22.4 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{17}H_{22}NO_6P$ 356.12575 found 356.12491.

Oxodiperoxymolybdenum(pyridine) (hexamethylphosphoric triamide) (SI-57)

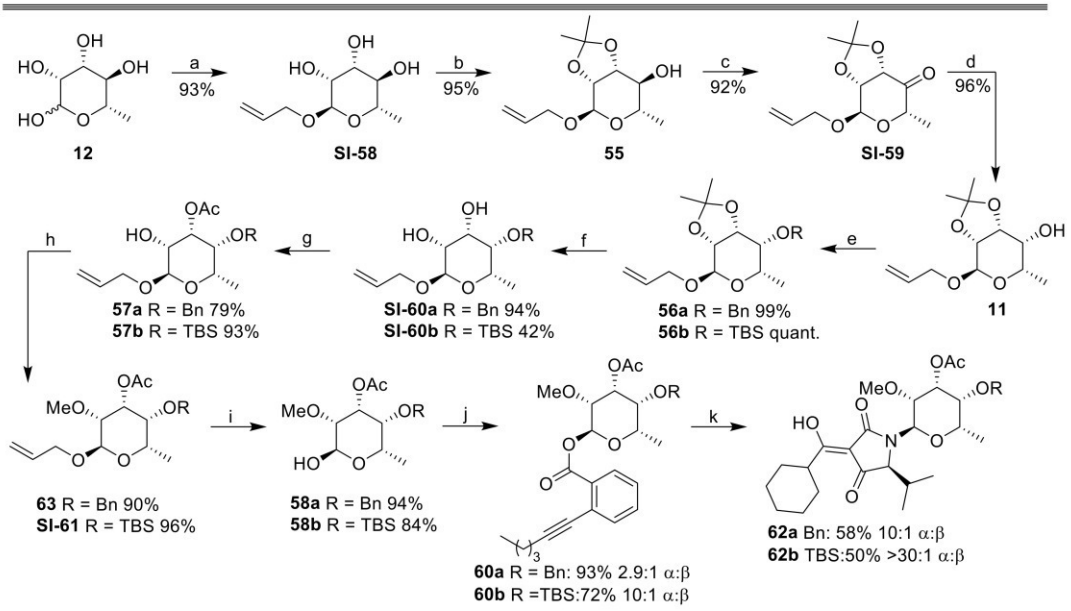


SI-57

MoO_3 (**SI-56**, 30.0 g, 208 mmol, 1.00 eq.) was dissolved in H_2O_2 (30 wt%, 150 mL) and stirred at 40 °C. Temperature was strictly kept at max. 40 °C, while stirring for 4.25 h. The suspension was filtered off over celite® and the mother liquor was treated with HMPA (36.2 mL, 208 mmol, 1.00 eq.) and stirred vigorously for 5 min. It was again filtered off and the solid was recrystallized in MeOH. The solid (27.6 g, 77.4 mmol, 1.00 eq.) was dried in the desiccator and dissolved in dry THF (115 mL). Pyridine (6.26 mL, 77.4 mmol, 1.00 eq.) was added at room temperature and the mixture was stirred for 15 min. The solid was filtered off, washed with dry THF as well as dry Et_2O and dried in a desiccator filled with P_2O_5. The Vedejs-reagent (**SI-57**, 27.8 g, 31%) was isolated as yellow crystals.

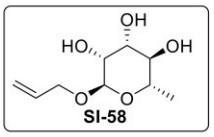
There is no convenient analytical method for characterization of this compound.[10]

2.8 Synthesis of glycosides **62a** and **62b**



Scheme S11. Synthesis of glycosides **62a/b**.
Reagents and conditions: a) AcCl, allylOH, 0 °C→55 °C, 24 h; b) $CuSO_4$, AcMe, rt, 17 h; c) 1. $(ClCO)_2$, DMSO, −78 °C, 40 min, 2. **55**, 50 min, 3. DIPEA, −78 °C→rt, 16 h; d) $NaBH_4$, 0 °C, 1.5 h; e) 1. **56a**: NaH, imidazole, DMF, 0 °C, 35 min, 2. BnBr, TBAI, rt, 17 h; **56b**: TBSOTf, pyridine, CH_2Cl_2, 0 °C, 5 h; f) **SI-60a**: AcOH, H_2O, reflux, 1.5 h; **SI-60b**: HCOOH, EtOH, rt, 2.5 h; g) **57a**: 1. Bu_2SnO, toluene, reflux, 4 h, 2. AcCl, 0 °C, 30 min; **57b**: 1. Bu_2SnO, toluene, reflux, 3 h, 2. AcCl, rt, 1 h; h) **63**: $TMSCHN_2$, HBF_4, CH_2Cl_2, 0 °C, 5 h; **SI-61** MeO_3BF_4, proton sponge, CH_2Cl_2, 0 °C→40 °C, 21 h; i) **58a**: $Pd(PPh_3)_4$, AcOH, rt, 17 h; **58b**: 1. DABCO, Wilkinson´s catalyst, EtOH, Δ, 15 h, 2. I_2, phosphate buffer pH=7/H_2O/EtOAc, rt, 10 min; j) **60a/b**: acid **59**, DCC, DMAP, CH_2Cl_2, rt, 3-3.5 h; k) **62a/b**: tetramic acid **61**, $AuPPh_3NTf_2$, rt→40 °C, 17-20 h.

(3*R*,4*R*,5*R*,6*S*)-2-(Allyloxy)-6-methyltetrahydro-2*H*-pyran-3,4,5-triol (SI-58)

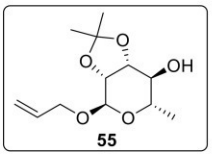


L-Rhamnose (**12**; 10.0 g, 54.9 mmol, 1.00 eq.) was added to a solution of AcCl (10.1 mL, 141 mmol, 1.10 eq.) and allylic alcohol (100 mL) at 0 °C. The mixture was stirred at 55 °C for 24 h. The reaction was quenched with $NaHCO_3$ and the solid was filtered off. The volatiles were removed in vacuo, toluene was added, and the solvent was concentrated under reduced pressure. This procedure was repeated twice. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 10:1→9:1→8:1) to yield the allylated carbohydrate **SI-58** (10.3 g, 93%, α:β 9:1) as a colourless resin. $\mathbf{R_f}$ = 0.74 (CH_2Cl_2/MeOH 4:1); $[\boldsymbol{\alpha}]_D^{20}$ −85.6° (c 1.0, $CHCl_3$); **IR** ν_{max}/cm^{-1} 3376 (s), 2978 (m), 2919 (m), 1451 (w), 1423 (w), 1384 (w), 1265 (w), 1130 (m), 1050 (s), 985 (m), 927 (w), 810 (w); α-anomer: **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.89 (dddd, 1H, J = 5.1, 6.0, 10.7, 16.9 Hz), 5.29 (dq, 1H, J = 1.5, 16.9 Hz), 5.20 (dq, 1H, J = 1.5, 10.7 Hz),

4.83 (d, 1H, *J* = 1.0 Hz), 4.18 (ddt, 1H, *J* = 1.3, 5.1, 13.0 Hz), 3.99 (ddt, 1H, *J* = 1.3, 6.0, 13.0 Hz), 3.96 (m, 1H), 3.79 (m, 1H), 3.69 (m, 1H), 3.49 (d, 1H, *J* = 5.5 Hz), 3.46 (dt, 1H, *J* = 3.5, 9.4 Hz), 3.04-2.86 (br. s, 1H), 2.78-2.56 (br. s, 2H), 1.32 (d, 3H, *J* = 6.3 Hz) ppm; β-anomer: **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.93 (m, 1H), 5.30 (m, 1H), 5.23 (m, 1H), 4.51 (s, 1H), 4.40 (ddt, 1H, *J* = 1.3, 5.2, 12.8 Hz), 4.13 (ddt, 1H, *J* = 1.3, 6.6, 12.8 Hz), 3.99 (m, 2H), 3.79 (m, 1H), 3.69 (m, 1H), 3.27 (m, 1H), 2.93 (br. s, 1H), 1.64 (br. s, 1H), 1.37 (d, 3H, *J* = 6.2 Hz), 0.99 (m, 1H) ppm. α-anomer: **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 133.8, 117.7, 99.0, 73.1, 71.9, 71.1, 68.3, 68.1, 17.7 ppm; β-anomer: **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 133.6, 118.6, 98.6, 74.2, 72.9, 72.2, 71.2, 70.1, 17.7 ppm.

Spectroscopic data corresponded to those reported in the literature.[11]

(3a*R*,6*S*,7*S*,7a*R*)-4-(Allyloxy)-2,2,6-trimethyltetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-7-ol (55)

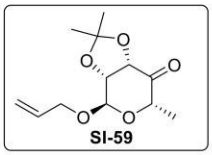


55

A solution of glycoside **SI-58** (7.74 g, 37.9 mmol, 1.00 eq.) in acetone (1.60 L) was treated with $CuSO_4$ (96.8 g, 606 mmol, 16.0 eq.) and stirred for 17 h at room temperature. The solid was removed by filtration over celite®. Removing of the solvent under reduced pressure gave the product **55** (8.68 g, 94%, α:β 16:1) as a colourless resin. $\mathbf{R_f}$ = 0.75 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ −26.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3470 (m), 2985 (m), 2937 (m), 2905 (m), 1456 (w), 1383 (m), 1244 (m), 1220 (m), 1141 (m), 1077 (s), 1053 (s), 1023 (s), 997 (m), 922 (w), 860 (m), 818 (w); α-Anomer: **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.91 (dddd, 1H, *J* = 5.3, 6.2, 10.3, 17.0 Hz), 5.31 (dq, 1H, *J* = 1.4, 17.0 Hz), 5.22 (dq, 1H, *J* = 1.4, 10.3 Hz), 5.01 (s, 1H), 4.20 (ddt, 1H, *J* = 1.4, 2.8, 5.3 Hz), 4.17 (d, 1H, *J* = 5.8 Hz), 4.10 (dd, 1H, *J* = 5.8, 7.1 Hz), 4.01 (ddt, 1H, *J* = 1.4, 6.2, 12.8 Hz), 3.70 (dq, 1H, *J* = 6.3, 9.1 Hz), 3.42 (ddd, 1H, *J* = 4.6, 7.1, 9.1 Hz), 2.19 (m, 1H), 1.53 (s, 3H), 1.36 (s, 3H), 1.30 (d, 3H, *J* = 6.3 Hz) ppm; β-Anomer: **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.96 (m, 1H), 5.31 (m, 1H), 5.23 (m, 1H), 4.78 (d, 1H, *J* = 2.2 Hz), 4.43 (ddt, 1H, *J* = 1.5, 4.9, 13.0 Hz), 4.25 (dd, 1H, *J* = 2.2, 5.7 Hz), 4.19 (m, 1H), 4.10 (m, 1H), 3.54 (m, 1H), 3.30 (m, 1H), 2.11 (m, 1H), 1.57 (s, 3H), 1.39 (s, 3H), 1.35 (m, 3H) ppm. α-Anomer: **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 133.7, 118.0, 109.6, 96.4, 78.5, 75.9, 74.6, 68.1, 66.1, 28.1, 26.3, 17.6 ppm; β-Anomer: **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 133.9, 118.5, 111.0, 97.0, 80.3, 75.1, 75.0, 71.1, 70.3, 28.2, 26.4, 17.9 ppm.

Spectroscopic data corresponded to those reported in the literature.[12]

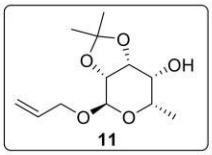
(3a*R*,6*S*,7a*S*)-4-(Allyloxy)-2,2,6-trimethyldihydro-4*H*-[1,3]dioxolo[4,5-c]pyran-7(6*H*)-one (SI-59)



Oxalyl chloride (7.90 mL, 92.1 mmol, 2.00 eq.) was dissolved in dry CH_2Cl_2 (38 mL) and treated with dry DMSO (13.1 mL, 184 mmol, 4.00 eq.) at -78 °C. After stirring for 40 min, glycoside **55** (11.3 g, 46.1 mmol, 1.00 eq.) was added. Stirring was continued for 50 min at −78 °C and DIPEA (31.5 mL, 184 mmol, 4.00 eq.) was dropped into the mixture. The solution was allowed to warm to room temperature and stirred for a further 16 h. Sat. aq. $Na_2S_2O_3$ solution was added, and the aqueous phase was extracted thrice with CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography (SiO_2, pentane/EtOAc 8:1) afforded ketone **SI-59** (9.52 g, 92%, only α) as a colourless oil. $\mathbf{R_f}$ = 0.79 (hexanes/EtOAc 7:3); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −125.1° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2989 (m), 2938 (m), 2922 (m), 2876 (w), 1742 (s), 1456 (w), 1375 (m), 1228 (m), 1162 (m), 1107 (s), 1979 (s), 1012 (s), 932 (m), 857 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.89 (m, 1H), 5.31 (m, 1H), 5.24 (m, 1H), 5.00 (s, 1H), 4.45 (q, 2H, J = 5.7 Hz), 4.28 (q, 1H, J = 6.8 Hz), 4.24 (m, 1H), 4.08 (m, 1H), 1.49 (s, 3H), 1.39 (d, 3H, J = 6.8 Hz), 1.36 (s, 3H), ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 204.8, 133.1, 118.5, 111.5, 96.1, 78.9, 76.1, 70.2, 68.9, 26.9, 25.6, 16.0 ppm.

Spectroscopic data corresponded to those reported in the literature.[13]

(3a*R*,6*S*,7*R*,7a*R*)-4-(Allyloxy)-2,2,6-trimethyltetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-7-ol (11)

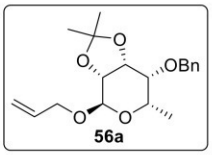


A solution of ketone **SI-59** (9.52 g, 39.3 mmol, 1.00 eq.) in EtOH *p.a.* (157 mL) was treated with $NaBH_4$ (1.64 g, 43.2 mmol, 1.10 eq.) at 0 °C. The suspension was stirred for 1.5h and the solid was filtered off over celite®. The solvent was removed under reduced pressure. Column chromatography (SiO_2, pentane/EtOAc, 7:1→6:1→4:1) gave alcohol **11** (9.19 g, 96%, only α) as a colourless liquid. $\mathbf{R_f}$ = 0.53 (hexanes/EtOAc 3:2); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −38.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3528 (m), 2984 (m), 2936 (m), 1381 (m), 1255 (m), 1215 (m), 1152 (m), 1073 (s), 1019 (m), 991 (s), 852 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.92 (m, 1H), 5.31 (d, 1H, J = 17.0 Hz), 5.22 (d, 1H, J = 10.3 Hz), 5.08 (s, 1H), 4.22 (q, 1H, J = 5.9 Hz), 4.20 (m, 1H), 4.07 (d, 1H, J = 6.2 Hz), 4.03 (dd, 1H, J = 6.2, 12.8 Hz), 3.89 (q, 1H, J = 6.7 Hz), 3.55 (t, 1H, J =

5.9 Hz), 2.18 (d, 1H, *J* = 6.7 Hz), 1.59 (s, 3H), 1.38 (s, 3H), 1.32 (d, 3H, *J* = 6.7 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 133.8, 118.0, 109.4, 96.8, 73.4, 73.1, 68.4, 67.0, 64.5, 26.0, 25.4, 16.8 ppm.

Spectroscopic data corresponded to those reported in the literature.[13]

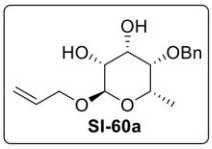
(3a*R*,6*S*,7*R*,7a*R*)-4-(Allyloxy)-7-(benzyloxy)-2,2,6-trimethyltetrahydro-4*H*-[1,3]dioxolo-[4,5-c]pyran (56a)



A solution of alcohol **11** (8.98 g, 36.8 mmol, 1.00 eq.) in dry DMF (142 mL) was treated with NaH (2.82 g, 118 mmol, 3.20 eq.) and imidazole (225 mg, 3.31 mmol, 0.09 eq.) at 0 °C. The solution was stirred for 35 min, BnBr (6.33 mL, 53.3 mmol, 1.45 eq.) and TBAI (1.36 g, 3.68 g, 0.10 eq.) were added and stirring was continued for 17 h at room temperature. H_2O and EtOAc were added, the phases were separated, and the aqueous phase was extracted thrice with EtOAc. The combined organic phases were washed with H_2O and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography (SiO_2, pentane/EtOAc 7:1) afforded benzylated glycoside **56a** (12.2 g, quant., only α) as a colourless solid. $\mathbf{R_f}$ = 0.76 (hexanes/EtOAc 3:2); **mp** 27 °C; $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −12.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2984 (m), 2933 (m), 2910 (m), 1455 (m), 1380 (m), 1369 (m), 1252 (m), 1214 (m), 1161 (m), 1144 (m), 1055 (s), 1025 (s), 924 (w), 858 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.40-7.26 (m, 5H), 5.89 (dddd, 1H, *J* = 5.6, 6.2, 10.5, 17.1 Hz), 5.27 (dq, 1H, *J* = 1.6, 17.1 Hz), 5.18 (d, 1H, *J* = 1.6, 10.5 Hz), 4.98 (d, 1H, *J* = 1.5 Hz), 4.85 (d, 1H, *J* = 12.0 Hz), 4.56 (d, 1H, *J* = 12.0 Hz), 4.40 (dd, 1H, *J* = 4.6, 6.6 Hz), 4.18 (ddt, 1H, *J* = 1.5, 5.1, 12.7 Hz), 4.07 (dd, 1H, *J* = 1.7, 6.7 Hz), 4.01 (ddt, 1H, *J* = 1.5, 6.3, 12.8 Hz), 3.88 (dq, 1H, *J* = 3.3, 6.7 Hz), 3.59 (dd, 1H, *J* = 3.3, 4.3 Hz), 1.56 (s, 3H), 1.37 (s, 3H), 1.20 (d, 3H, *J* = 6.7 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 138.1, 134.0, 128.7, 128.4, 127.9, 117.7, 110.1, 97.0, 74.5, 74.3, 73.8, 72.8, 68.6, 65.8, 26.4, 25.6, 16.9 ppm. **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{26}O_5Na$ 357.16685, found 357.16725.

(3*R*,4*S*,5*S*,6*S*)-2-(Allyloxy)-5-(benzyloxy)-6-methyltetrahydro-2*H*-pyran-3,4-diol (SI-60a)

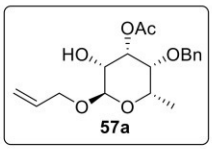
Carbohydrate **56a** (12.2 g, 36.4 mmol, 1.00 eq.) was dissolved in H_2O (7 mL) and AcOH (64 mL). The solution was stirred at 110 °C for 1.5 h. Toluene was added and the volatiles were



SI-60a

removed under reduced pressure. This procedure was repeated twice. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 4:1→2:1) to give deprotected carbohydrate **SI-60a** (9.94 g, 93%, only α) as a colourless oil in 93% yield. $\mathbf{R_f}$ = 0.65 (hexanes/EtOAc 3:2); $[\alpha]_D^{20}$ −103.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3475 (m), 2932 (m), 1736 (w), 1455 (w), 1383 (w), 1360 (w), 1103 (s), 1052 (s), 1008 (s), 928 (w), 813 (m), 737 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.38-7.29 (m, 5H), 5.89 (m, 1H), 5.28 (dq, 1H, J = 1.4, 17.2 Hz), 5.19 (dq, 1H, J = 1.4, 10.4 Hz), 4.90 (d, 1H, J = 1.1 Hz), 4.78 (d, 1H, J = 11.0 Hz), 4.70 (d, 1H, J = 11.0 Hz), 4.15 (ddt, 1H, J = 1.4, 5.1, 13.0 Hz), 3.99 (ddt, 1H, J = 1.4, 6.0, 13.0 Hz), 3.92 (q, 1H, J = 6.6 Hz), 3.88 (dt, 1H, J = 3.4, 10.3 Hz), 3.69 (m, 1H), 3.64 (m, 1H), 3.39 (m, 1H), 2.79 (m, 1H), 1.27 (d, 3H, J = 6.6 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 137.6, 133.9, 128.7, 128.3, 128.2, 117.4, 100.2, 81.5, 76.8, 70.9, 68.3, 66.9, 66.1, 17.1 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{16}H_{22}O_5Na$ 317.13568, found 317.13594.

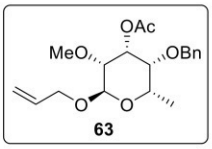
(3*R*,4*S*,5*R*,6*S*)-2-(Allyloxy)-5-(benzyloxy)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-4-yl acetate (57a)



57a

A solution of diol **SI-60a** (6.12 g, 21.0 mmol, 1.00 eq.) in toluene *p.a.* (1.00 L) was treated with Bu_2SnO (6.27 g, 25.2 mmol, 1.20 eq.) and stirred for 4 h under reflux with a water separator. AcCl (1.60 mL, 22.1 mmol, 1.05 eq.) was added at 0 °C and stirred for a further 30 min. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, pentane/EtOAc 5:1) to give product **57a** (5.56 g, 79%) as a colourless oil. $\mathbf{R_f}$ = 0.56 (hexanes/EtOAc 3:2); $[\alpha]_D^{20}$ −128.0° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3487 (m), 2937 (w), 1740 (s), 1432 (w), 1455 (w), 1362 (m), 1229 (s), 1150 (m), 1116 (s), 1045 (s), 1011 (s), 919 (m), 752 (m), 731 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H), 5.88 (m, 1H), 5.28 (dq, 1H, J = 1.4, 17.2 Hz), 5.19 (d, 1H, J = 1.4, 10.4 Hz), 5.08 (t, 1H, J = 3.1 Hz), 4.90 (d, 1H, J = 1.5 Hz), 4.77 (d, 1H, J = 11.3 Hz), 4.61 (d, 1H, J = 11.3 Hz), 4.16 (ddt, 1H, J = 1.3, 5.3, 13.0 Hz), 4.11, (d, 1H, J = 11.1 Hz), 4.00 (m, 2H), 3.83 (m, 1H), 3.77 (m, 1H), 2.11 (s, 3H), 1.25 (d, 3H, J = 6.5 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.6, 137.3, 133.9, 128.7, 128.4, 128.4, 117.6, 100.7, 79.0, 76.1, 70.1, 69.3, 68.4, 66.4, 21.3, 16.9 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{18}H_{24}O_6Na$ 359.14651, found 359.14602.

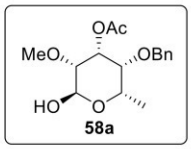
(3*R*,4*R*,5*R*,6*S*)-2-(Allyloxy)-5-(benzyloxy)-3-methoxy-6-methyltetrahydro-2*H*-pyran-4-yl acetate (63)



63

A solution of carbohydrate **57a** (2.75 g, 8.20 mmol, 1.00 eq.) in dry CH_2Cl_2 (33 mL) was treated with $TMSCHN_2$ (1.8-2.4M in hexanes, 20.5 mL, 40.9 mmol, 5.00 eq.) and HBF_4 (50 wt% in H_2O, 2.00 mL, 16.4 mmol, 2.00 eq.) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C, $TMSCHN_2$ (1.8-2.4M in hexanes, 20.5 mL, 40.9 mmol, 5.00 eq.) and HBF_4 (50 wt% in H_2O, 2.00 mL, 16.4 mmol, 2.00 eq.) were added again and stirring was continued for 1 h. This was repeated a second time. The reaction was quenched by addition of sat. aq. $NaHCO_3$ solution. The aqueous phase was extracted thrice with CH_2Cl_2, the combined organic phases were washed with brine and dried over Na_2SO_4. The volatiles were removed under reduced pressure. Purification by column chromatography (SiO_2, pentane/EtOAc 4:1→2:1) gave product **63** (2.58 g, 90%) as a colourless resin. $\mathbf{R_f}$ = 0.61 (hexanes/EtOAc 3:2); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −79.1° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3004 (w), 2989 (w), 1744 (w), 1276 (m), 1261 (m), 1092 (w), 1051 (w), 764 (s), 750 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.39-7.25 (m, 5H), 5.89 (m, 1H), 5.28 (dq, 1H, J = 11.6, 7.2 Hz), 5.18 (d, 1H, J = 1.4, 10.4 Hz), 5.17 (t, 1H, J = 3.5 Hz), 4.95 (d, 1H, J = 2.1 Hz), 4.71 (d, 1H, J = 12.2 Hz), 4.65 (d, 1H, J = 12.2 Hz), 4.17 (ddt, 1H, J = 1.5, 5.1, 13.0 Hz), 4.02-3.94, (m, 2H), 3.61 (m, 1H), 3.51 (s, 3H), 3.43 (m, 1H), 2.03 (s, 3H), 1.24 (d, 3H, J = 6.7 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 138.6, 134.0, 128.4, 128.3, 127.7, 127.6, 117.4, 97.5, 77.5, 76.1, 74.7, 71.3, 68.2, 67.0, 59.9, 21.3, 16.6 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{26}O_6Na$ 373.16216, found 373.16129.

(2*S*,3*R*,4*R*,5*R*)-3-(Benzyloxy)-6-hydroxy-5-methoxy-2-methyltetrahydro-2*H*-pyran-4-yl acetate (58a)

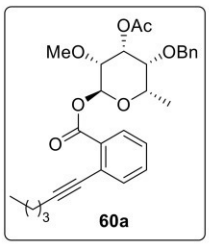


58a

Glycoside **63** (1.00 g, 2.85 mmol, 1.00 eq.) was dissolved in AcOH (29 mL) and $Pd(PPh_3)_4$ (989 mg, 856 µmol, 0.30 eq.) was added at room temperature. The mixture was stirred for 17 h and quenched with sat. aq. $NaHCO_3$ solution as well as solid $NaHCO_3$. The aqueous phase was extracted thrice with EtOAc, the combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and crude product was purified by column chromatography (SiO_2, pentane/EtOAc 1.5:1→1:1) to afford hemi-acetal **58a** (784 mg, 89%, α:β 6:1) as a light yellow resin. $\mathbf{R_f}$ = 0.52 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −41.1° (c

1.0 in $CHCl_3$); IR ν_{max}/cm^{-1} 3438 (m), 2977 (w), 2934 (m), 2896 (m), 2837 (w), 1739 (s), 1497 (w), 1455 (m), 1372 (m), 1236 (s), 1157 (m), 1132 (m), 1096 (s), 1044 (s), 968 (m), 913 (m), 817 (w), 750 (s), 699 (m), 677 (m); α-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 5.31 (t, 2H, J = 3.2 Hz), 4.71 (d, 1H, J = 12.1 Hz), 4.64 (d, 1H, J = 12.1 Hz), 4.22 (dq, 1H, J = 2.6, 6.6 Hz), 3.63 (t, 1H, J = 2.8 Hz), 3.51 (s, 3H), 3.87 (t, 1H, J = 3.2 Hz), 2.70 (d, 1H, J = 3.7 Hz), 2.06 (s, 3H), 1.28 (d, 3H, J = 6.7 Hz) ppm; β-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 4.85 (t, 1H, J = 3.3 Hz), 4.75 (d, 1H, J = 12.3 Hz), 4.68 (dd, 1H, J = 1.8, 12.8 Hz), 4.63 (d, 1H, J = 12.3 Hz), 4.04 (d, 1H, J = 12.8 Hz), 3.67 (s, 3H), 3.56 (m, 2H), 3.53 (m, 1H), 1.99 (s, 3H), 1.30 (m, 3H) ppm; α-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 138.4, 128.4, 128.3, 127.8, 92.7, 77.9, 75.7, 74.2, 70.3, 67.8, 59.7, 21.3, 16.4, ppm; β-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.3, 138.3, 128.8, 128.4, 128.0, 93.8, 77.9, 75.6, 75.4, 74.4, 71.4, 61.6, 21.1, 16.9 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{16}H_{22}O_6Na$ 333.13033, found 333.213086.

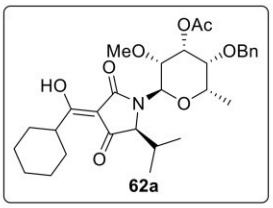
(3*R*,4*R*,5*R*,6*S*)-4-Acetoxy-5-(benzyloxy)-3-methoxy-6-methyltetrahydro-2*H*-pyran-2-yl-2-(hex-1-yn-1-yl)benzoate (60a)



A solution of hemi-acetal **58a** (784 mg, 2.53 mmol, 1.00 eq.) in dry CH_2Cl_2 (3.6 mL) was treated with acid **59** (656 mg, 3.03 mmol, 1.20 eq.), DCC (782 mg, 3.79 mmol, 1.50 eq.) and DMAP (463 mg, 3.79 mmol, 1.50 eq.) at room temperature. After stirring for 3 h, the solids were filtered off over celite®. The organic phase was washed with sat. aq. $NaHCO_3$ solution and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, and solvents were removed under reduced pressure. Purification by column chromatography (SiO_2, pentane/EtOAc 6:1→4:1→2:1) gave product **60a** (1.16 g, 93% mmol, α:β 2.9:1) as a colourless resin. $\mathbf{R_f}$ = 0.71 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ −9.6° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2934 (w), 2872 (w), 2229 (w), 1737 (s), 1596 (w), 1567 (w), 1484 (w), 1456 (w), 1366 (m), 1275 (m), 1233 (s), 1131 (m), 1069 (s), 1042 (s), 989 (m), 946 (m), 916 (m), 751 (s), 698 (m); α-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.89 (dd, 1H, J = 1.2, 8.0 Hz), 7.53 (dd, 1H, J = 1.2, 8.0 Hz), 7.45 (dq, 1H, J = 2.3, 7.6 Hz), 7.40-7.27 (m, 6H), 6.51 (d, 1H, J = 2.0 Hz), 5.23 (t, 1H, J = 3.5 Hz), 4.75 (d, 1H, J = 12.2 Hz), 4.69 (d, 1H, J = 12.2 Hz), 4.24 (dq, 1H, J = 1.8, 6.5 Hz), 3.73 (m, 1H), 3.60 (s, 3H), 3.59 (s, 1H), 2.47 (m, 2H), 2.06 (s, 3H), 1.61 (m, 2H), 1.48 (m, 2H), 1.28 (d, 3H, J = 6.6 Hz), 0.94 (t, 3H, J = 7.4 Hz) ppm; β-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 8.09 (dd, 1H, J = 1.2,

8.0 Hz), 7.53 (dd, 1H, *J* = 1.2, 8.0 Hz), 7.45 (dq, 1H, *J* = 2.3, 7.6 Hz), 7.40-7.27 (m, 6H), 6.07 (d, 1H, *J* = 2.2 Hz), 5.24 (t, 1H, *J* = 3.5 Hz), 4.72 (d, 1H, *J* = 12.2 Hz), 4.68 (d, 1H, *J* = 12.2 Hz), 3.89 (dq, 1H, *J* = 2.9, 6.7 Hz), 3.69 (dd, 1H, *J* = 1.8, 3.5 Hz), 3.65 (t, 1H, *J* = 3.5 Hz), 3.57 (s, 3H), 2.45 (m, 2H), 2.09 (s, 3H), 1.61 (m, 2H), 1.48 (m, 2H), 1.37 (d, 3H, *J* = 6.7 Hz), 0.94 (t, 3H, *J* = 7.3 Hz) ppm; α-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 164.4, 138.3, 135.0, 132.1, 130.7, 130.6, 128.4, 128.3, 127.8, 125.0, 96.6, 93.2, 79.6, 76.3, 75.5, 74.8, 70.9, 70.0, 60.1, 30.8, 22.2, 21.2, 19.6, 16.8, 13.8 ppm; significant signals β-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 164.0, 138.2, 134.6, 130.75, 130.73, 128.34, 128.27, 127.3, 127.0, 125.7, 97.1, 92.2, 79.1, 76.4, 74.2, 73.7, 72.2, 60.7, 30.8, 22.2, 21.1, 19.7, 17.0 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{29}H_{34}O_7Na$ 517.21967, found 517.21924.

(2*S*,3*R*,4*R*,5*R*)-3-(Benzyloxy)-6-((*S*,*Z*)-3-(cyclohexyl(hydroxy)methylene)-5-isopropyl-2,4-dioxopyrrolidin-1-yl)-5-methoxy-2-methyltetrahydro-2*H*-pyran-4-yl acetate (62a)



62a

Ester **60a** (200 mg, 404 µmol, 1.00 eq.) and 3-acyl tetramic acid **61** (152 mg, 607 µmol, 1.50 eq.) were dissolved in dry toluene (1.00 mL). $AuPPh_3NTf_2$ (59.8 mg, 80.9 µmol, 0.20 eq.) was added and the mixture was stirred at 40 °C for 17 h. All volatiles were removed in vacuo. The crude product was purified by column chromatography (SiO_2 C-18, 40% MeCN in H_2O + 0.1% HCO_2H→60% MeCN in H_2O + 0.1% HCO_2H→80% MeCN in H_2O + 0.1% HCO_2H→100% MeCN in H_2O + 0.1% HCO_2H) to give product **62a** as a light-yellow resin (127 mg, 58%, α:β 10:1). Anomers were separated by HPLC. Minor impurities occurred due to third tautomer $\mathbf{R_f}$ = 0.49 (hexanes/EtOAc 1:1); $[\boldsymbol{\alpha}]_D^{20}$ −8.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2932 (m), 2857 (w), 1744 (s), 1796 (s), 1647 (s), 1607 (s), 1453 (m), 1364 (w), 1312 (w), 1232 (s), 1089 (s), 1027 (w), 752 (m), 698 (w); α-anomer **^{1}H-NMR** (500 MHz, CD_3OD) δ 7.40-7.26 (m, 5H), 6.00 (t, 1H, *J* = 3.0 Hz), 5.09 (br. s, 1H), 4.67 (d, 1H, *J* = 11.6 Hz), 4.51 (d, 1H, *J* = 11.6 Hz), 4.26 (m, 2H), 3.84 (br. s, 1H), 3.83 (dd, 1H, *J* = 3.2, 6.5 Hz), 3.43 (tt, 1H, *J* = 3.3, 11.5 Hz), 3.32 (s, 3H), 2.24 (m, 1H), 2.13 (s, 3H), 1.86-1.70 (m, 5H), 1.51 (m, 2H), 1.43 (d, 3H, *J* = 7.1 Hz), 1.39 (m, 2H), 1.27 (m, 1H), 1.17 (d, 3H, *J* = 7.1 Hz), 0.89 (d, 3H, *J* = 7.1 Hz) ppm; α-anomer major tautomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 193.9, 192.6, 175.9, 170.4, 137.8, 128.6, 128.0, 127.7, 101.2, 75.6, 74.2, 73.2, 71.9, 71.0, 66.4, 57.1, 41.0, 30.4, 29.0, 28.5, 25.8, 25.71, 25.70, 25.6, 21.4, 18.1, 16.1, 13.6 ppm; significant signals α-anomer minor tautomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 199.7, 197.6, 170.5, 168.4, 137.8, 128.6, 128.2, 127.7, 104.8, 73.1, 71.7, 70.9, 66.6, 57.0, 41.8, 30.2,

29.1, 28.4, 25.8, 25.5, 21.4, 18.2, 15.7, 13.6 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{30}H_{42}NO_8$ 544.29049, found 544.28949.

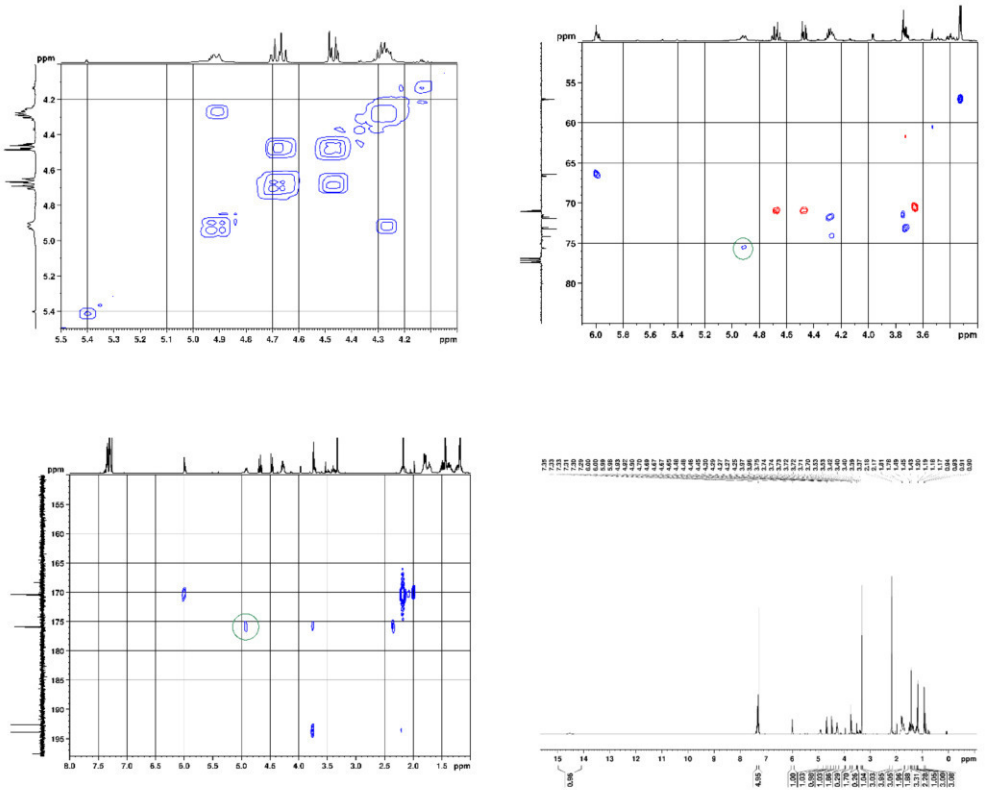


Fig. S8. 2D-NMR-spectra [^{1}H-^{1}H-COSY (top, left), ^{1}H-^{13}C-HSQC (top, right), ^{1}H-^{13}C-HMBC (bottom, left)] of **62a** for elucidation of *N*,*O*-acetal formation. ^{1}H-NMR-spectrum ($CDCl_3$) of **62a** (bottom, right).

2D-NMR-spectra (COSY, HSQC, HMBC) as well as 1D-NMR-spectra (^{1}H and ^{13}C, $CDCl_3$) clearly showed the exclusive formation of an *N*,O-acetal. An *O*-glycosylation with tautomers of 3-acyl-tetramic acids could is conceivable, yet was not observed.[1] Via COSY and HSQC the signal at 4.92 ppm was assigned to the anomeric proton (Fig. S8, top). The chemical shift of the anomeric C-atom (δ = 75.6 ppm) had a distinct high-field shift compared to an *O*,*O*-acetal (δ ≈ 95 ppm). The chemical shifts of the anomeric position are in full accordance with the results of Yang *et al*.[2] As known from the literature the enolization of the amide is highly unfavoured and therefore an *O*-glycosylation with enolized amide is unlikely.[1] HMBC indicated a coupling of the anomeric proton of talose-derivative with amide-C-atom (Fig. S8, bottom left, green circle) confirming the spatial proximity to the amide-C. In the ^{1}H-NMR-spectrum a signal for an enolic

proton was found at 14.5 ppm while no signal for NH was observed. In an additional experiment for *N*-glycosylation of tetramic acid derivatives, the *O*-glycosylation took place (for synthesis see Scheme S19). For proof of *N*-glycosylation the spectra can be compared with those of the accidently formed *O*,*O*-acetal **SI-62**. In the ^{1}H-spectrum of **SI-62** (Fig. S9, top) a signal for an amide proton (no HSQC-correlation, Fig. S9 bottom) instead of enolic proton signal was indicated at 5.84 ppm. The anomeric H-atom (δ = 5.52 ppm) and the anomeric C-atom (δ = 98.3 ppm) of **SI-62** were shifted downfield compared to the *N*,*O*-acetal **62a**.

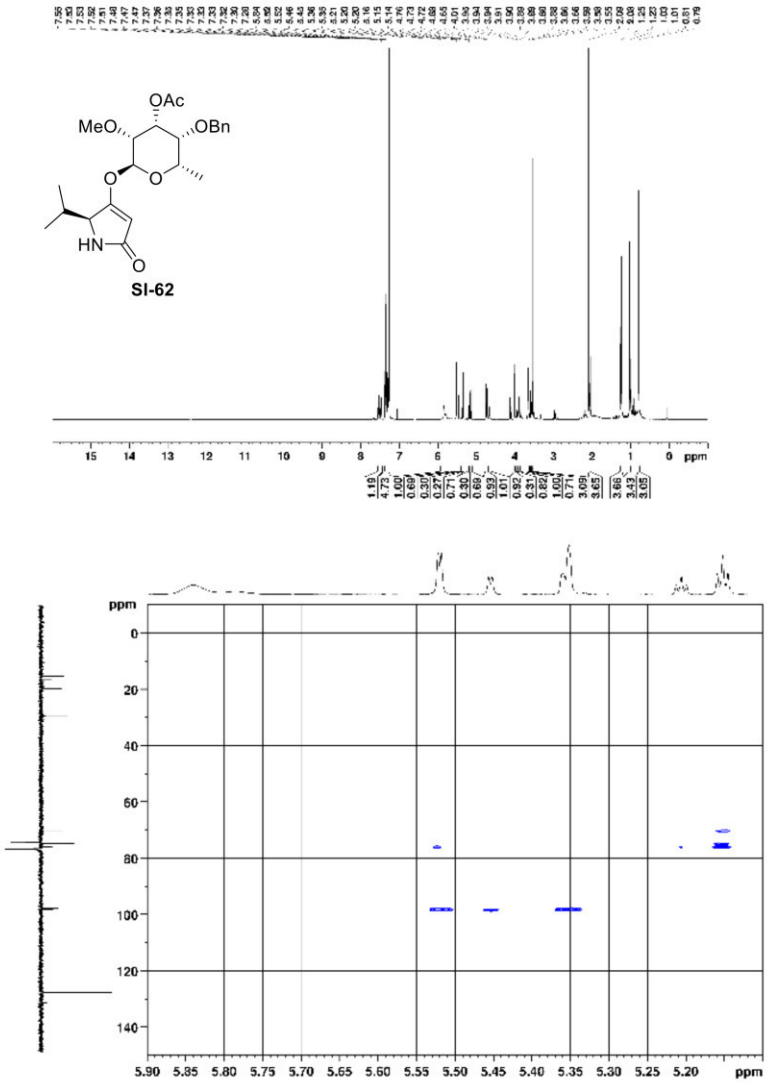
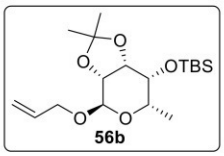


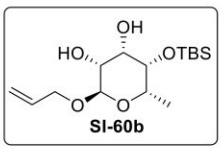
Fig. S9. ^{1}H-NMR-spectrum (top) and ^{1}H-^{13}C-HSQC-spectrum (bottom) of **SI-62** for comparison with spectra of the *N*,*O*-acetal.

(((3a*R*,6*S*,7*R*,7a*S*)-4-(Allyloxy)-2,2,6-trimethyltetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-7-yl)oxy)(*tert*-butyl)dimethylsilane (56b)



Alcohol **11** (772 mg, 3.16 mmol, 1.00 eq.) in dry CH_2Cl_2 (55 mL) was treated with pyridine (2.55 mL, 31.6 mmol, 10.0 eq.) and TBSOTf (2.18 mL, 9.48 mmol, 3.00 eq.) at 0 °C. The solution was stirred for 5 h and the reaction was quenched by addition of sat. aq. $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc thrice and the combined organic phases were washed with brine as well as dried over Na_2SO_4. After removal of the solvent *in vacuo* the crude product was purified by column chromatography (SiO_2, pentane/EtOAc 6:1) to give TBS-ether **56b** (1.16 g, quant.) as a colourless liquid. $\mathbf{R_f}$ = 0.88 (hexanes/EtOAc 3:2); $[\alpha]_D^{20}$ −58.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2988 (m), 2933 (m), 2889 (m), 2865 (m), 1473 (w), 1381 (w), 1276 (s), 1260 (s), 1979 (m), 1056 (m), 838 (m), 764 (s), 750 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.92 (dddd, 1H, J = 5.2, 6.3, 10.7, 17.2 Hz), 5.30 (dq, 1H, J = 1.5, 17.2 Hz), 5.19 (dq, 1H, J = 1.5, 10.7 Hz), 4.85 (d, 1H, J = 4.1 Hz), 4.29 (dd, 1H, J = 3.6, 7.5 Hz), 4.26 (ddt, 1H, J = 1.3, 5.2, 12.8 Hz), 4.13 (dd, 1H, J = 3.6, 4.1 Hz), 4.08 (ddt, J = 1.3, 6.3, n.d. Hz), 4.07 (m, 1H), 3.95 (m, 1H), 1.52 (s, 3H), 1.33 (s, 3H), 1.33 (d, 3H, J = 6.5 Hz), 0.92 (s, 9H), 0.11 (s, 3H), 0.07 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 134.2, 117.6, 110.2, 97.2, 76.2, 75.5, 69.8, 69.2, 67.5, 26.7, 26.2, 24.8, 18.5, 17.2, −4.01, −4.58 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{18}H_{34}O_5SiNa$ 381.20677, found 381.20547.

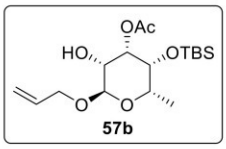
(3*R*,4*S*,5*S*,6*S*)-2-(Allyloxy)-5-((*tert*-butyldimethylsilyl)oxy)-6-methyltetrahydro-2*H*-pyran-3,4-diol (SI-60b)



Fully protected carbohydrate **56b** (310 mg, 865 µmol, 1.00 eq.) was dissolved in EtOH *p.a.* (1.3 mL) and formic acid (1.3 mL). The solution was stirred at room temperature for 2.5 h. After addition of sat. aq. $NaHCO_3$ solution, the aqueous phase was extracted with EtOAc thrice. The combined organic phases were dried over Na_2SO_4, and the volatiles were removed *in vacuo*. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 9:1) to yield deprotected diol **SI-60b** (116 mg, 42%) as a colourless solid. $\mathbf{R_f}$ = 0.58 (hexanes/EtOAc 3:1); **mp** 69 °C; $[\alpha]_D^{20}$ −91.6° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3405 (m), 3359 (m), 2945 (m), 2929 (m), 2882 (w), 2858 (m), 1471 (w), 1425 (w), 1351 (w), 1276 (m), 1260 (m), 1167 (w), 1143 (w), 1104 (m), 1067 (m), 1044 (w), 1014 (m), 996 (m), 916 (w), 837 (m), 765 (s), 749 (s),

678 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.89 (dddd, 1H, J = 5.2, 6.2, 10.7, 17.0 Hz), 5.28 (dq, 1H, J = 1.6, 17.0 Hz), 5.19 (dq, 1H, J = 1.6, 10.7 Hz), 4.92 (d, 1H, J = 1.3 Hz), 4.16 (ddt, 1H, J = 1.4, 5.2, 13.0 Hz), 4.00 (ddt, J = 1.4, 6.0, 13.0 Hz), 3.89 (q, 1H, J = 6.6 Hz), 3.80 (m, 1H), 3.75 (dt, 1H, J = 3.1, 10.7 Hz), 3.68 (m, 1H), 3.43 (d, 1H, J = 12.0 Hz), 2.61 (d, 1H, J = 10.7 Hz), 1.23 (d, 3H, J = 6.6 Hz), 0.95 (s, 9H), 0.19 (s, 3H), 0.12 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 134.0, 117.5, 100.2, 75.1, 71.4, 68.3, 66.6, 66.4, 26.1, 18.4, 17.6, −3.88, −4.50 ppm; **HRMS** ESI m/z $[M + Na]^+$ calcd. for $C_{15}H_{30}O_5SiNa$ 341.17547, found 341.17505.

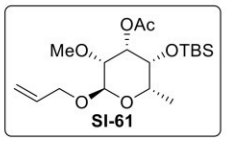
(3*R*,4*S*,5*R*,6*S*)-2-(Allyloxy)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-4-yl acetate (57b)



57b

To a solution of diol **SI-60b** (210 mg, 659 µmol, 1.00 eq.) in dry toluene (33 mL) was added Bu_2SnO (197 mg, 791 µmol, 1.20 eq.). The suspension was stirred under reflux for 3 h. AcCl (49.4 µL, 692 µmol, 1.05 eq.) was added at room temperature and stirring was continued for 1 h. All volatiles were removed under reduced pressure. Purification of the crude product (SiO_2, pentane/EtOAc 9:1) resulted in acetylated carbohydrate **57b** (220 mg, 93%) as a colourless liquid. **R_f** = 0.35 (hexanes/EtOAc 5:1); $[\alpha]_D^{20}$ −93.8° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3504 (m), 2956 (m), 2932 (m), 2900 (m), 2860 (m), 1741 (m), 1473 (w), 1432 (w), 1374 (w), 1276 (m), 1260 (s), 1235 (m), 1180 (w), 1118 (m), 1070 (m), 1001 (s), 938 (w), 839 (m), 765 (s), 750 (s), 680 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.89 (dddd, 1H, J = 5.1, 6.2, 10.5, 17.1 Hz), 5.29 (dq, 1H, J = 1.6, 17.1 Hz), 5.19 (dq, 1H, J = 1.6, 10.5 Hz), 5.00 (t, 1H, J = 2.9 Hz), 4.90 (d, 1H, J = 1.5 Hz), 4.17 (ddt, 1H, J = 1.5, 5.1, 13.0 Hz), 4.11 (d, 1H, J = 11.1 Hz), 4.01 (ddt, J = 1.5, 6.2, 13.0 Hz), 3.99 (q, 1H, J = 6.6 Hz), 3.92 (m, 1H), 3.80 (m, 1H), 2.15 (s, 3H), 1.23 (d, 3H, J = 6.6 Hz), 0.96 (s, 9H), 0.15 (s, 3H), 0.10 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.6, 134.0, 117.6, 110.7, 73.2, 69.66, 69.65, 68.4, 66.8, 26.0, 21.5, 18.3, 17.5, −4.24, −4.41 ppm; **HRMS** ESI m/z $[M + Na]^+$ calcd. for $C_{17}H_{32}O_6SiNa$ 383.18604, found 383.18468.

(3*R*,4*S*,5*R*,6*S*)-2-(Allyloxy)-5-((*tert*-butyldimethylsilyl)oxy)-3-methoxy-6-methyltetrahydro-2*H*-pyran-4-yl acetate (SI-61)

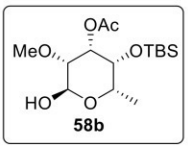


SI-61

Alcohol **57b** (40 mg, 111 µmol, 1.00 eq.) in dry CH_2Cl_2 (1.10 mL) was treated with Me_3OBF_4 (65.6 mg, 444 µmol, 4.00 eq.) and proton sponge (95.1 mg, 444 µmol, 4.00 eq.) at 0 °C and stirred at 40 °C for 21 h. The

reaction was quenched by addition of sat. aq. NH_4Cl solution. The aqueous phase was extracted with EtOAc thrice. Combined organic phases were washed with sat. aq. citric acid solution as well as brine and dried over Na_2SO_4. After removal of the solvent *in vacuo* and purification of the crude product by column chromatography (SiO_2, pentane/EtOAc 9:1) product **SI-61** (40 mg, 96%) was isolated as a colourless liquid. $\mathbf{R_f}$ = 0.63 (hexanes/EtOAc 4:1); $[\alpha]_D^{20}$ −30.0° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2930 (m), 2900 (m), 2857 (m), 1745 (s), 1463 (w), 1374 (w), 1237 (s), 1197 (w), 1130 (m), 1091 (m), 1053 (m), 1004 (m), 859 (m), 838 (m), 765 (s), 750 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.91 (dddd, 1H, *J* = 5.2, 6.1, 10.5, 17.1 Hz), 5.29 (dq, 1H, *J* = 1.6, 17.1 Hz), 5.18 (dq, 1H, *J* = 1.6, 10.5 Hz), 5.16 (t, 1H, *J* = 3.4 Hz), 4.93 (d, 1H, *J* = 2.9 Hz), 4.18 (ddt, 1H, *J* = 1.4, 5.1, 13.0 Hz), 4.01 (ddt, *J* = 1.4, 6.1, 13.0 Hz), 3.96 (dq, 1H, *J* = 2.6, 6.6 Hz), 3.80 (t, 1H, *J* = 2.8 Hz), 3.43 (s, 3H), 3.36 (m, 1H), 2.13 (s, 3H), 1.25 (d, 3H, *J* = 6.6 Hz), 0.93 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 134.1, 117.5, 97.1, 77.5, 71.3, 70.4, 70.1, 68.4, 68.3, 59.6, 26.0, 21.5, 18.5, 16.6, −4.40, −4.48 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{18}H_{34}O_6SiNa$ 397.20169, found 397.20114.

(2*S*,3*R*,4*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-6-hydroxy-5-methoxy-2-methyltetrahydro-2*H*-pyran-4-yl acetate (58b)

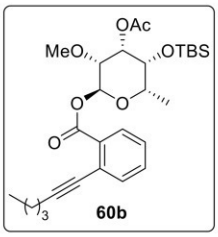


58b

Glycoside **SI-61** (820 mg, 2.19 mmol, 1.00 eq.) dissolved in EtOH *p.a.* (15 mL) was treated with DABCO (128 mg, 1.09 mmol, 0.50 eq.) and Wilkinson catalyst (101 mg, 109 µmol, 0.05 eq.). The reaction mixture was stirred at reflux for 15 h. After cooling down to room temperature, the suspension was filtered off over celite® and solvents were removed under reduced pressure. The crude product was dissolved in EtOAc *p.a.* (226 mL), H_2O (226 mL) and phosphate buffer (22.6 mL). A solution of I_2 (1.67 g, 6.57 mmol, 3.00 eq.) in EtOAc *p.a.* (92 mL) was added dropwise at room temperature. The mixture was stirred vigorously for 10 min. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution. The aqueous phase was extracted with EtOAc thrice. Combined organic phases were washed with sat. aq. $Na_2S_2O_3$ solution as well as sat. aq. $NaHCO_3$ solution and dried over Na_2SO_4. After removal of the solvents under reduced pressure, purification of the crude product by column chromatography (SiO_2, pentane/EtOAc 2:1) gave semi-acetal **58b** (618 mg, 84%, α:β 4:1) as a colourless liquid. $\mathbf{R_f}$ = 0.86 (hexanes/EtOAc 4:1); $[\alpha]_D^{20}$ −45.9° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3402 (m), 2949 (m), 2931 (m), 2886 (w), 2858 (m), 1746 (m), 1464 (w), 1373 (m), 1276 (s), 1260 (s), 1198 (m), 1139 (m), 1090 (m), 1047 (m), 962 (w), 858 (m), 837 (m), 765 (s), 750 (s); α-anomer **^{1}H-NMR**

(500 MHz, $CDCl_3$) δ 5.32 (t, 1H, J = 3.3 Hz), 5.25 (t, 1H, J = 4.3 Hz), 4.15 (m, 1H), 3.85 (t, 1H, J = 3.4 Hz), 3.44 (s, 3H), 3.28 (m, 1H), 2.57 (d, 1H, J = 4.1 Hz), 2.12 (s, 3H), 1.30 (d, 3H, J = 6.8 Hz), 0.92 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H) ppm; β-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 4.84 (t, 1H, J = 3.3 Hz), 4.70 (dd, 1H, J = 1.9, 12.5 Hz), 3.99 (d, 1H, J = 12.5 Hz), 3.72 (dt, 1H, J = 1.3, 3.3 Hz), 3.56 (dd, 1H, J = 1.6, 6.6 Hz), 3.54 (s, 3H), 3.49 (m, 1H), 2.17 (s, 3H), 1.28 (d, 3H, J = 6.6 Hz), 0.96 (s, 9H), 0.10 (s, 3H), 0.06 (s, 3H) ppm; α-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.4, 91.7, 78.3, 70.5, 69.9, 69.6, 59.1, 26.0, 21.4, 18.4, 15.9, −4.54, −4.60 ppm; significant signals β-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 93.5, 77.9, 74.1, 71.7, 69.5, 61.5, 26.1, 21.5, 17.5, −4.28 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{15}H_{30}O_6SiNa$ 357.17039, found 357.17020.

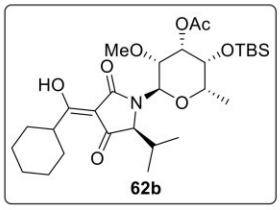
(3*R*,4*S*,5*R*,6*S*)-4-Acetoxy-5-((*tert*-butyldimethylsilyl)oxy)-3-methoxy-6-methyltetra-hydro-2*H*-pyran-2-yl 2-(2,2-dimethyl-2l6-but-1-yn-1-yl)benzoate (60b)



Semi-acetal **58b** (52.8 mg, 158 µmol, 1.00 eq.) in dry CH_2Cl_2 (1.2 mL) was treated with acid **59** (41.0 mg, 190 µmol, 1.20 eq.), DMAP (28.9 mg, 237 µmol, 1.50 eq.) and DCC (48.9 mg, 237 µmol, 1.50 eq.) at room temperature. The reaction mixture was stirred for 3.5 h and quenched by addition of sat. aq. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 thrice and combined organic phases were dried over Na_2SO_4. After removal of all volatiles and purification by column chromatography (SiO_2, pentane/EtOAc 11:1) glycoside **60b** (59.1 mg, 72%, α:β 10:1) was isolated as a light-yellow oil. $\mathbf{R_f}$ = 0.80 (hexanes/EtOAc 3:1); $[\boldsymbol{\alpha}]_D^{20}$ −60.9° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2931 (m), 2854 (m), 1744 (s), 1276 (s), 1260 (s), 1136 (m), 1081 (m), 838 (m), 762 (s), 750 (s); α-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.90 (dd, 1H, J = 1.3, 8.1 Hz), 7.53 (d, 1H, J = 7.8 Hz), 7.44 (dt, 1H, J = 1.3, 7.5 Hz), 7.32 (dt, 1H, J = 1.3 Hz, 7.5 Hz), 6.47 (d, 1H, J = 2.7 Hz), 5.23 (t, 1H, J = 3.5 Hz), 4.20 (dq, 1H, J = 2.2, 6.7 Hz), 3.89 (t, 1H, J = 2.7 Hz), 3.54 (t, 1H, J = 3.2 Hz), 3.45 (s, 3H), 2.46 (dt, 2H, J = 3.3, 7.2 Hz), 2.15 (s, 3H), 1.62 (m, 2H), 1.50 (m, 2H), 1.30 (d, 3H, J = 6.7 Hz), 0.95 (s, 9H), 0.95 (t, 3H, J = 7.0 Hz), 0.10 (s, 3H), 0.06 (s, 3H) ppm; β-anomer significant signals **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 8.23 (d, 1H, J =7.8 Hz), 7.67 (dt, 1H, J = 1.5, 7.8 Hz), 7.45 (m, 1H), 7.34 (m, 1H), 6.23 (s, 1H), 2.55-2.39 (m, 2H), 1.72-1.37 (m, 4H) ppm ; α-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 164.5, 135.0, 132.1, 130.9, 130.6, 127.3, 125.1, 96.7, 92.9, 79.6, 76.3, 70.9, 70.8, 69.9, 59.7, 30.9, 26.0, 22.2, 21.5, 19.7, 18.6, 16.9, 13.8,

−4.34, −4.48 ppm; **HRMS** ESI *m/z* [M + Na]$^+$ calcd. for $C_{28}H_{42}O_7SiNa$ 541.25868, found 541.25920.

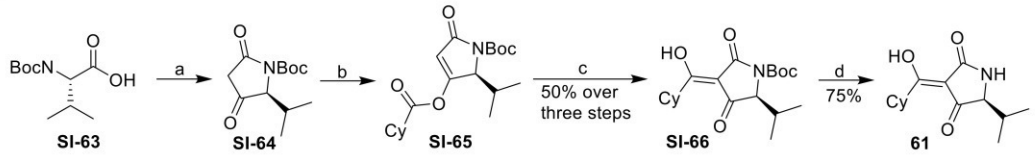
(2*S*,3*R*,4*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-6-((*S*,*Z*)-3-(cyclohexyl(hydroxy)-methylene)-5-isopropyl-2,4-dioxopyrrolidin-1-yl)-5-methoxy-2-methyltetrahydro-2*H*-pyran-4-yl acetate (62b)



62b

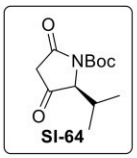
Glycoside **60b** (200 mg, 386 µmol, 1.00 eq.) and tetramic acid **61** (145 mg, 578 µmol, 1.50 eq.) were dissolved in toluene and the solvent was removed on a rotary evaporator. This procedure was repeated twice. The substances were dissolved in dry toluene (1 mL) and treated with $AuPPh_3NTf_2$ (57.0 mg, 77.1 µmol, 0.20 eq.) at room temperature. After stirring for 20 h at 40 °C the volatiles were removed *in vacuo* and crude product was purified by column chromatography (SiO_2 C-18, 40% MeCN in H_2O + 0.1% HCOOH→60% MeCN in H_2O + 0.1% HCOOH→80% MeCN in H_2O + 0.1% HCOOH→90% MeCN in H_2O + 0.1% HCOOH). The product **62b** (110 mg, 50%, α:β >30:1) was isolated as a light-yellow solid. $\mathbf{R_f}$ = 0.40 (hexanes/EtOAc 3:1); **mp** 88 °C; $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −44.6° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2991 (w), 2931 (m), 2858 (m), 1748 (m), 1705 (m), 1652 (m), 1607 (m), 1452 (m), 1361 (w), 1276 (m), 1260 (m), 1231 (m), 1106 (m), 1987 (m), 1007 (w), 963 (m), 863 (m), 838 (m), 764 (s), 751 (s); α-anomer **^{1}H-NMR** (500 MHz, CD_3OD) δ 5.72 (t, 1H, J = 3.3 Hz), 5.06 (br. s, 1H), 4.26 (br. s, 1H), 4.10 (m, 2H), 3.85 (br. s, 1H), 3.45 (m, 1H), 3.30 (s, 3H, under solvent signal), 2.23 (m, 1H), 2.12 (s, 3H), 1.86-1.69 (m, 5H), 1.50 (m, 2H), 1.41 (d, 3H, J = 6.8 Hz), 1.44-1.23 (m, 3H), 1.17 (d, 3H, J = 6.9 Hz), 0.89 (s, 9H), 0.89 (d, 3H, J = 6.9 Hz), 0.13 (s, 3H), 0.11 (s, 3H) ppm; α-anomer major tautomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 193.9, 192.6, 175.9, 170.2, 104.9, 101.3, 75.4, 74.0, 73.7, 71.5, 69.8, 67.7, 57.1, 41.0, 30.3, 29.0, 28.5, 25.8, 25.7, 25.6, 18.1, 18.0, 16.0, 13.1, −4.89, −4.98 ppm; significant signals α-anomer minor tautomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 199.7, 197.6, 170.2, 75.9, 73.5, 73.0, 71.3, 70.1, 67.8, 57.0, 41.8, 30.2, 29.1, 28.4, 25.8, 25.7, 21.4, 18.2, 15.7, 13.1, −4.90, −4.98 ppm; **HRMS** ESI *m/z* [M + H]$^+$ calcd. for $C_{29}H_{50}NO_8Si$ 568.33002, found 568.32990.

2.9 Synthesis of 3-acyltetramic acid **61**

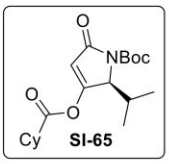


Scheme S12. Synthesis of 3-acyltetramic acid **61**.
Reagents and conditions: a) 1. Meldrum´s acid, DMAP, EDC·HCl, CH_2Cl_2, rt, 3 h, 2. EtOAc, Δ, 2 h; b) 1. cyclohexylcarbonic acid, EDC·HCl, DMAP, CH_2Cl_2, 0 °C, 50 min, 2. tetramic acid **SI-64**, rt, 2.5 h; c) NEt_3, DMAP, CH_2Cl_2, rt, 2 d; d) TFA, CH_2Cl_2, rt, 20 min.

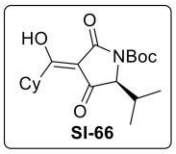
tert-Butyl(*S*,*Z*)-3-(cyclohexyl(hydroxy)methylene)-5-isopropyl-2,4-dioxopyrrolidine-1-carboxylate (SI-66)



Amino acid **SI-63** (5.00 g, 23.0 mmol, 1.00 eq.) in dry CH_2Cl_2 (74 mL) was treated with Meldrum´s acid (3.65 g, 25.3 mmol, 1.10 eq.), DMAP (3.93 g, 32.2 mmol, 1.40 eq.) and EDC·HCl (5.29 g, 27.6 mmol, 1.20 eq.). at room temperature. The reaction mixture was stirred for 3 h. 0.5M H_2SO_4 and EtOAc were added. The organic phase was separated, and the aqueous phase was extracted thrice with EtOAc. Combined organic phases were washed with H_2O and dried over Na_2SO_4. After filtration, organic phase was stirred under reflux for 2 h. The solvent was removed under reduced pressure. The product **SI-64** was used without further purification.



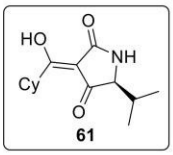
Cyclohexylcarbonic acid (2.58 mL, 20.9 mmol, 1.00 eq.) in dry CH_2Cl_2 (70 mL) was treated with EDC·HCl (4.79 g, 25.0 mmol, 1.20 eq.) and DMAP (511 mg, 4.18 mmol, 0.20 eq.) at 0 °C. After 50 min at room temperature, tetramic acid **SI-64** (5.55 g, 23.0 mmol, 1.10 eq.) in dry CH_2Cl_2 (55 mL) was added. Stirring was continued for 2.5 h. Addition of CH_2Cl_2 and 0.5M H_2SO_4 was followed by separation of organic phase. The aqueous phase was extracted thrice with CH_2Cl_2, combined organic phases were washed with brine, dried over Na_2SO_4 and volatiles were removed under reduced pressure. Purification over a short SiO_2-plug (SiO_2, pentane/EtOAc 20:1 →10:1→7:1→5:1) led to 4-*O*-acyl tetramic acid **SI-65** (6.65 g). It was pure enough for the next step. $\mathbf{R_f}$ = 0.92 (hexanes/EtOAc 3:1); **^{1}H-NMR** (500 MHz, CD_3OD) δ 6.10 (d, 1H, J = 0.7 Hz), 4.49 (dd, 1H, J = 0.7, 2.4 Hz), 2.49 (m, 2H), 1.99 (m, 2H), 1.79 (m, 2H), 1.67 (m, 1H), 1.54 (s, 9H), 1.51 (m, 1H), 1.32 (m, 4H), 1.12 (d, 3H, J = 6.8 Hz), 0.82 (d, 3H, J = 6.8Hz) ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{29}NO_5Na$ 374.19375, found 374.19308.



SI-66

4-*O*-Acyltetramic acid **SI-65** (6.65 g, 18.8 mmol, 1.00 eq.) in dry CH_2Cl_2 (190 mL) was treated with dry NEt_3 (3.20 mL, 22.6 mmol, 1.20 eq.) and DMAP (1.15 g, 9.40 mmol, 0.50 eq.) at room temperature. After stirring for 22 h DMAP (575 mg, 4.70 mmol, 0.25 eq.) was added again and stirring was continued for 24 h. Sat. aq. $NaHCO_3$ solution and CH_2Cl_2 were added. The aqueous phase was extracted thrice with CH_2Cl_2, combined organic phases were washed with brine and dried over Na_2SO_4. Removal of all volatiles under reduced pressure and purification by column chromatography (SiO_2 C-18, 40% MeCN in H_2O + 0.1% HCO_2H→60% MeCN in H_2O + 0.1% HCO_2H→80% MeCN in H_2O + 0.1% HCO_2H→100% MeCN in H_2O + 0.1% HCO_2H) gave 3-acyl tetramic acid **SI-66** as an orange resin (4.04 g, 50% over three steps). $\mathbf{R_f}$ = 0.72 (CH_2Cl_2/MeOH 9:1); $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ +37.2° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2970 (m), 2933 (m), 2857 (m), 1771 (m), 1744 (m), 1713 (s), 1652 (m), 1599 (s), 1452 (m), 1393 (m), 1228 (m), 1308 (s), 1277 (s), 1259 (s), 1154 (s), 1022 (w), 931 (m), 913 (m), 857 (w), 764 (s), 751 (s); **^{1}H-NMR** (500 MHz, CD_3OD) δ 4.33 (s, 1H), 3.46 (tt, 1H, J = 3.0, 11.5 Hz), 2.45 (dqn, 1H, J = 3.0, 7.1 Hz), 1.84 (m, 4H), 1.75 (m, 1H), 1.55 (s, 9H), 1.48 (dt, 2H, J = 2.9, 12.1 Hz), 1.40 (m, 2H), 1.28 (m, 1H), 1.17 (d, 3H, J = 7.1Hz), 0.82 (d, 3H, J = 7.1 Hz); mixture of three tautomers **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 201.2, 197.7, 195.4, 192.4, 174.5, 165.8, 165.0, 163.3, 149.7, 149.0, 117.3, 104.5, 101.4, 84.0, 83.5, 83.3, 69.1, 65.6, 61.8, 42.7, 41.3, 30.8, 30.3, 29.2, 28.8, 28.6, 28.4, 28.3, 28.1, 26.0, 25.8, 25.7, 25.6, 25.5, 19.0, 18.6, 18.5, 16.2, 15.7, 15.1 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{29}NO_5Na$ 374.19379, found 374.19296.

(*S*,*Z*)-3-(Cyclohexyl(hydroxy)methylene)-5-isopropylpyrrolidine-2,4-dione (61)

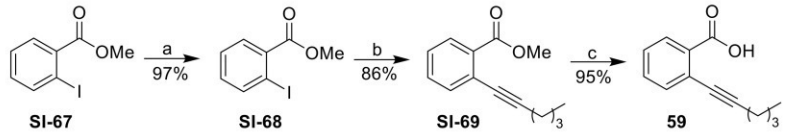


61

Tetramic acid **SI-66** (606 mg, 1.71 mmol, 1.00 eq.) was dissolved in dry CH_2Cl_2 (32 mL) and treated with TFA (3.20 mL, 10 vol% CH_2Cl_2) at room temperature. The solution was stirred for 20 min. All volatiles were removed at the rotary evaporator. The crude product was purified by column chromatography (SiO_2 C-18, 40% MeCN in H_2O + 0.1% HCO_2H→50% MeCN in H_2O + 0.1% HCO_2H→60% MeCN in H_2O + 0.1% HCO_2H→80% MeCN in H_2O + 0.1% HCO_2H→100% MeCN in H_2O + 0.1% HCO_2H) to afford product **61** as a light orange solid (323 mg, 75%). $\mathbf{R_f}$ = 0.68 (CH_2Cl_2/MeOH 9:1); **mp** 109 °C; $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ −109.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3219 (m), 2931 (m), 2856 (m), 1653 (s), 1606 (s), 1448 (m), 1352 (m), 1308 (m), 1276 (m), 1261 (m), 1227 (m), 1137 (w), 1024 (w), 920 (m), 817 (m), 765 (s), 750 (s); **^{1}H-NMR** (500 MHz, CD_3OD) δ 3.75 (br. s, 1H), 3.40 (br. s, 1H), 2.17 (m, 1H), 1.86-1.70 (m, 4H), 1.56-1.20 (m,

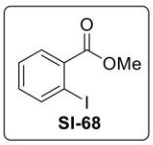
6H), 1.03 (d, 3H, J = 7.1 Hz), 0.82 (d, 3H, J = 7.1 Hz) ppm; major tautomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 196.6, 192.6, 176.7, 100.4, 67.3, 41.0, 30.26, 28.9, 28.6, 25.74, 25.67, 25.61, 19.6, 16.0 ppm; minor tautomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 201.5, 194.9, 169.5, 103.8, 64.0, 41.6, 30.30, 28.9, 28.8, 25.8, 25.61, 25.56, 19.3, 16.3 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{14}H_{22}NO_3$ 252.15942, found 252.15883.

2.10 Synthesis of acid **59**



Scheme S13. Synthesis of acid **59**.
Reagents and conditions: a) $SOCl_2$, MeOH, −10 °C→40 °C, 17 h; b) 1. $PdCl_2(PPh_3)_2$, PPh_3, CuI, iPr$_2$NH, rt, 1 h, 2. 1-hexyne, 0 °C→rt, 18.5 h; c) NaOH, THF, 50 °C, 19 h.

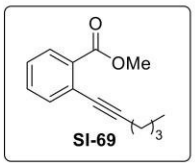
Methyl 2-iodobenzoate (SI-68)



2-Iodobenzoic acid **SI-67** (5.00 g, 20.2 mmol, 1.00 eq.) was dissolved in dry MeOH (35.0 mL) and $SOCl_2$ (2.20 mL, 30.2 mmol, 1.20 eq.) was slowly added at −10 °C. After 15 min the solution was heated to 40 °C and stirred for a further 17 h. The reaction was quenched by addition of sat. aq. $NaHCO_3$ solution and EtOAc. The organic phase was separated, and the aqueous phase was extracted with EtOAc thrice, combined organic phases were washed with H_2O twice and dried over Na_2SO_4. The solvents were removed *in vacuo*. Purification by column chromatography (SiO_2, pentane/EtOAc 6:1) afforded product **SI-68** (5.11 g, 97%) as a colourless liquid. **R_f** = 0.70 (hexanes/EtOAc 4:1); **IR** ν_{max}/cm^{-1} 2950 (m), 1727 (s), 1583 (m), 1562 (w), 1465 (m), 1432 (s), 1289 (s), 1251 (s), 1191 (m),1131 (s), 1104 (s), 1043 (m), 1016 (s), 963 (m), 826 (w), 739 (s), 688 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.99 (d, 1H, J = 7.9 Hz), 7.80 (dd, 1H, J = 1.5, 7.9 Hz), 7.40 (t, 1H, J = 7.7 Hz), 7.15 (t, 1H, J = 7.7 Hz), 3.93 (s, 3H) ppm.

Spectroscopic data corresponded to those reported in the literature.[14]

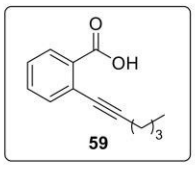
Methyl 2-(hex-1-yn-1-yl)benzoate (SI-69)



Methyl 2-iodobenzoate (**SI-68**, 100 mg, 382 µmol, 1.00 eq.) was dissolved in *i*Pr_2NH (1.00 mL) and treated with $PdCl_2(PPh_3)_2$ (13.4 mg, 19.1 µmol, 5 mol%), PPh_3 (10.0 mg, 38.2 µmol, 10 mol%) and CuI (3.63 mg, 19.1 µmol, 5 mol%). The mixture was stirred at room temperature for 1h. At 0 °C, hexyne (65.7 µL, 572 µmol, 1.50 eq.) was added, stirring was continued for a further 18.5 h and the mixture was allowed to warm to room temperature. Addition of sat. aq. NH_4Cl solution stopped the reaction. Pentane was added and the organic phase was separated. The aqueous phase was extracted with pentane/EtOAc 100:1 and the combined organic phases were washed with H_2O and brine. They were dried over Na_2SO_4 and all volatiles were removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, pentane/EtOAc 30:1→20:1) to give alkyne **SI-69** as a colourless liquid (76.0 mg, 86%). $\mathbf{R_f}$ = 0.79 (hexanes/EtOAc 9:1); **IR** ν_{max}/cm^{-1} 2956 (m), 2934 (m), 2873 (m), 1733 (s), 1718 (s), 1597 (w), 1577 (w), 1485 (m), 1447 (m), 1433 (m), 1294 (s), 1276 (s), 1249 (s), 1190 (w), 1129 (m), 1083 (s), 1043 (w), 966 (w), 757 (s), 702 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.88 (dd, 1H, J = 1.1, 7.9 Hz), 7.51 (dd, 1H, J = 1.1, 7.9 Hz), 7.42 (dt, 1H, J = 1.4, 7.6 Hz), 7.31 (t, 1H, J = 1.4, 7.6 Hz), 3.91 (s, 3H), 2.48 (t, 2H, J = 7.1 Hz), 1.62 (m, 2H), 1.51 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz) ppm.

Spectroscopic data corresponded to those reported in the literature.[15]

2-(Hex-1-yn-1-yl)benzoic acid (59)

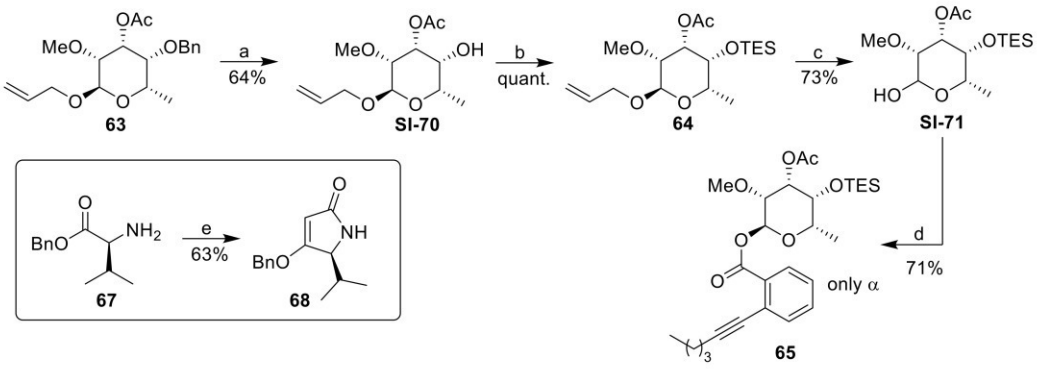


Ester **SI-69** (76.0 mg, 330 µmol, 1.00 eq.) in THF *p.a.* (1.40 mL) and 1M NaOH (1.40 mL) was stirred at 50 °C for 19 h. The solution was treated with conc. HCl until pH value reached 1. The aqueous phase was extracted five times with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, and the volatiles were removed under reduced pressure. Product **59** (68.2 mg, 95%) was isolated as a colourless resin and used without further purification. $\mathbf{R_f}$ = 0.23 (hexanes/EtOAc 9:1); **IR** ν_{max}/cm^{-1} 3077 (m), 2958 (m), 2932 (m), 2873 (m), 2656 (m), 2229 (w), 1693 (s), 1600 (w), 1568 (w), 1487 (w), 1455 (w), 1409 (m), 1379 (w), 1297 (m), 1274 (m), 1141 (w),1086 (w), 922 (w), 756 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 8.11 (d, 1H, 7.7 Hz), 7.51 (dd, 1H, J = 1.2, 7.7 Hz), 7.42 (dt, 1H, J = 1.2, 7.7 Hz), 7.31 (t, 1H, J = 1.2, 7.7 Hz), 2.48

(t, 2H, *J* = 7.1 Hz), 1.62 (m, 2H), 1.51 (m, 2H), 0.96 (t, 3H, *J* = 7.3 Hz) ppm. COO*H* not detectable.

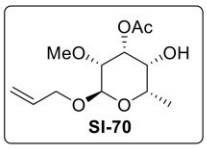
Spectroscopic data corresponded to those reported in the literature.[15]

2.11 Synthesis of glycoside **65** for formal synthesis



Scheme S14. Synthesis of glycoside **65** for formal synthesis.
Reagents and conditions: a) 1. I_2, CH_2Cl_2, −65 °C, 35 min, 2. Et_3SiH, −65 °C→−20 °C, 2 h; b) TESOTf, pyridine, CH_2Cl_2, 0 °C, 2 h; c) 1. DABCO, Wilkinson´s catalyst, EtOH, Δ, 5 h, 2. I_2, phosphate buffer/H_2O/EtOAc, rt, 25 min; d) DCC, DMAP, CH_2Cl_2, rt, 3 h; e) Ph_3PCCO (**66**), benzoic acid, THF, 60 °C, 22 h.

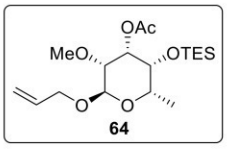
(3*R*,4*R*,5*R*,6*S*)-2-(Allyloxy)-5-hydroxy-3-methoxy-6-methyltetrahydro-2*H*-pyran-4-yl acetate (SI-70)



Glycoside **63** (141 mg, 402 µmol, 1.00 eq.) in dry CH_2Cl_2 (10.9 mL) was treated with I_2 (153 mg, 604 µmol, 1.50 eq.) at −65 °C. The mixture was stirred for 35 min and Et_3SiH (96.4 µL, 604 µmol, 1.50 eq.) was added. After 40 min at −65 °C, the solution was allowed to warm to −20 °C. Stirring was continued for 1 h 30 min. Allylic alcohol (136 µL, 2.01 mmol, 5.00 eq.) and $NaHCO_3$ (169 mg, 2.01 mmol, 5.00 eq.) were added. After stirring for 10 min, the mixture was treated with sat. aq. $Na_2S_2O_3$ solution and CH_2Cl_2. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 twice. The combined organic phases were washed with brine and dried over Na_2SO_4. After removal of the volatiles and purification by column chromatography (SiO_2, pentane/EtOAc 3:1) product **SI-70** (67.0 mg, 64%) was obtained as a colourless liquid. $\mathbf{R_f}$ = 0.63 (hexanes/EtOAc 4:1); $[\alpha]_D^{20}$ −110.3° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 3510 (m), 2987 (m), 2938 (m), 1744 (m), 1429 (m), 1375 (m), 127 (m), 1237 (s), 1178 (w), 1114 (s), 1984 (m), 1045 (s), 981 (m), 933 (w), 764 (s), 750 (s),687 (w); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.90 (dddd, 1H, J = 5.2, 6.1, 10.4, 17.3 Hz), 5.30 (dq, 1H, J = 1.5, 17.3 Hz), 5.21 (dq, 1H, J = 1.5, 10.4 Hz), 5.07 (t, 1H, J = 3.3 Hz), 4.97 (d, 1H, J = 0.8 Hz), 4.20 (ddt, 1H, J = 1.4, 5.2, 12.8 Hz), 4.01 (ddt, 1H, J = 1.4, 6.1, 12.8 Hz), 3.94 (d, 1H, J = 6.6 Hz), 3.70 (m, 1H), 3.55

(m, 1H), 3.52 (s, 3H), 3.38 (d, 1H, *J* = 11.0 Hz), 2.13 (s, 3H), 1.29 (d, 3H, *J* = 6.6 Hz) ppm; **^{13}CNMR** (125 MHz, $CDCl_3$) δ 170.5, 133.7, 117.8, 96.7, 78.7, 71.0, 69.9, 68.3, 67.5, 59.8, 21.3, 16.4 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{12}H_{20}O_6Na$ 283.11521, found 283.11435.

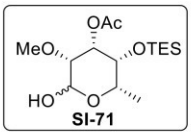
(3*R*,4*S*,5*R*,6*S*)-2-(Allyloxy)-3-methoxy-6-methyl-5-((triethylsilyl)oxy)tetrahydro-2*H*-pyran-4-yl acetate (64)



64

Carbohydrate **SI-70** (30.0 mg, 115 µmol, 1.00 eq.) in dry CH_2Cl_2 (2.30 mL) was treated with pyridine (576 µL, 231 µL, 5.00 eq.) and TESOTf (52.1 µL, 231 µL, 2.00 eq.) at 0 °C. After stirring at this temperature for 2 h, sat. aq. $NaHCO_3$ solution and CH_2Cl_2 were added. The aqueous phase was extracted with CH_2Cl_2 thrice and the combined organic phases were dried over Na_2SO_4. After removal of the volatiles under reduced pressure and purification by column chromatography (SiO_2, pentane/EtOAc 4:1) product **64** (43.1 mg, quant.) was isolated as a colourless liquid. $\mathbf{R_f}$ = 0.59 (hexanes/EtOAc 3:1); $[\boldsymbol{\alpha}]_D^{20}$ −77.5° (c 1.0 in $CHCl_3$); **I**R ν_{max}/cm^{-1} 2854 (m), 2878 (m), 1744 (s), 1459 (m), 1413 (w), 1374 (m), 1235 (s), 1197 (m), 1128 (m), 1090 (s), 1052 (s), 1031 (s), 1003 (s), 962 (m), 848 (m), 747 (s), 724 (s), 677 (m); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.90 (dddd, 1H, *J* = 5.3, 6.0, 10.5, 16.9 Hz), 5.29 (dq, 1H, *J* = 1.6, 16.9 Hz), 5.18 (dq, 1H, *J* = 1.6, 10.5 Hz), 5.10 (t, 1H, *J* = 3.5 Hz), 4.94 (d, 1H, *J* = 2.5 Hz), 4.17 (ddt, 1H, *J* = 1.5, 5.3, 12.9 Hz), 4.00 (ddt, *J* = 1.5, 6.0, 12.9 Hz), 3.95 (dq, 1H, *J* = 2.1, 6.6 Hz), 3.80 (m, 1H), 3.42 (s, 3H), 3.39 (m, 1H), 2.14 (s, 3H), 1.26 (d, 3H, *J* = 6.6 Hz), 0.98 (t, 9H, *J* = 7.9 Hz), 0.65 (q, 6H, *J* = 7.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 134.1, 117.4, 97.3, 77.5, 71.2, 70.3, 68.3, 67.9, 59.8, 21.4, 16.5, 7.07, 5.14 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{18}H_{35}O_6Si$ 375.21930, found 375.21974.

(3*R*,4*S*,5*R*,6*S*)-2-Hydroxy-3-methoxy-6-methyl-5-((triethylsilyl)oxy)tetrahydro-2*H*-pyran-4-yl acetate (SI-71)

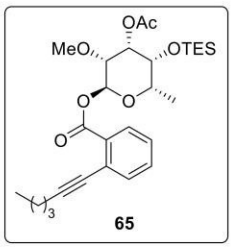


SI-71

Glycoside **64** (168 mg, 449 µmol, 1.00 eq.) was dissolved in EtOH *p.a.* (3.00 mL) and treated with Wilkinson catalyst (4.15 mg, 44.9 µmol, 1 mol%) as well as DABCO (7.55 mg, 67.3 µmol, 15 mol%). The suspension was stirred at 95 °C for 24 h. Rhodium-catalyst (4.15 mg, 44.9 µmol, 1 mol%) and DABCO (7.55 mg, 67.3 µmol, 15 mol%) were added again at room temperature. Stirring was continued for 24 h at 95 °C. A third portion of Wilkinson catalyst (4.15 mg, 44.9 µmol,

1 mol%) and DABCO (7.55 mg, 67.3 µmol, 15 mol%) was added. After stirring for a further 3 days the mixture was filtered off over celite® and the volatiles were removed under reduced pressure. The crude product was dissolved in EtOAc (48 mL) and H_2O (48 mL). A buffer (pH=7, 4.8 mL) was added. The mixture was treated dropwise with a solution of iodine (342 mg, 1.35 mmol, 3.00 eq.) in EtOAc (19 mL). After 25 min, sat. aq. $Na_2S_2O_3$ solution was added. The aqueous phase was extracted with EtOAc thrice, combined organic phases were washed with sat. aq. $NaHCO_3$ solution and dried over Na_2SO_4. Removal of the volatiles *in vacuo* and purification by column chromatography (SiO_2, pentane/EtOAc 2:1→1:1) afforded product **SI-71** (109 mg, 73%) as a colourless resin. $\mathbf{R_f}$ = 0.33 (hexanes/EtOAc 2:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ −64.5° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2930 (m), 2900 (m), 2857 (m), 1745 (s), 1463 (w), 1374 (m), 1238 (s), 1130 (m), 1091 (s), 1053 (s), 1004 (m), 940 (w), 859 (m), 838 (m), 765 (s), 750 (s); α-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 5.27 (t, 1H, J = 2.6 Hz), 5.22 (t, 1H, J = 3.4 Hz), 4.16 (dq, 1H, J = 3.0, 6.7 Hz), 3.82 (t, 1H, J = 3.0 Hz), 3.43 (s, 3H), 3.32 (dt, 1H, J = 0.6, 3.5 Hz), 3.03 (br. s, 1H), 2.13 (s, 3H), 1.69 (br. s, 1H), 1.29 (d, 3H, J = 6.7 Hz), 0.97 (t, 9H, J = 7.9 Hz), 0.66 (q, 6H, J = 7.9 Hz) ppm; β-anomer **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 4.81 (t, 1H, J = 3.2 Hz), 4.67 (dd, 1H, J = 1.6, 12.5 Hz), 4.09 (d, 1H, J = 12.6 Hz), 3.72 (dt, 1H, J = 1.1, 3.2 Hz), 3.55 (s, 3H), 3.55 (dq, 1H, J = 1.4, 6.7 Hz), 3.50 (m, 1H), 2.18 (s, 3H), 1.29 (d, 3H, J = 6.7 Hz), 0.99 (t, 9H, J = 7.9 Hz), 0.66 (q, 6H, J = 7.9 Hz) ppm; α-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.4, 92.2, 78.1, 70.6, 69.9, 69.1, 59.5, 21.3, 16.0, 7.01, 5.05 ppm; β-anomer **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.3, 93.8, 73.9, 71.9, 69.7, 61.7, 21.3 17.1, 7.12, 5.22 ppm; **HRMS** ESI m/z $[M + Na]^+$ calcd. for $C_{15}H_{30}O_6SiNa$ 357.17039 found 357.16962.

(2*S*,3*R*,4*S*,5*R*,6*S*)-4-Acetoxy-3-methoxy-6-methyl-5-((triethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl 2-(hex-1-yn-1-yl)benzoate (65)

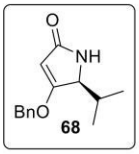


Semi-acetal **SI-71** (110 mg, 329 µmol, 1.00 eq.) and acid **59** (85.4 mg, 395 µmol, 1.20 eq.) were dissolved in dry CH_2Cl_2 (1.5 mL) and treated with DCC (102 mg, 493 µmol, 1.50 eq.) as well as DMAP (60.3 mg, 493 µmol, 1.50 eq.) at room temperature. The suspension was stirred for 3 h, before sat. aq. $NaHCO_3$ solution was added. The aqueous phase was extracted with CH_2Cl_2 thrice and the combined organic phases were dried over Na_2SO_4. Removal of the solvent at the rotary evaporator and purification by column chromatography (SiO_2, pentane/EtOAc 6:1→4:1) as well as a second column chromatography (SiO_2, pentane/EtOAc 9:1→8:1) furnished glycoside **65** (122 mg, 71%, single diastereomer) as

a colourless oil. $\mathbf{R_f}$ = 0.80 (hexanes/EtOAc 3:1); $[\alpha]_D^{20}$ −61.7° (c 1.0 in $CHCl_3$); **IR** ν_{max}/cm^{-1} 2956 (m), 2938 (m), 2877 (m), 1744 (m), 1458 (w), 1375 (w), 1276 (s), 1261 (s), 1236 (m), 1136 (m), 1081 (m), 1031 (w), 921 (w), 853 (w), 764 (s), 750 (s); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.90 (dd, 1H, *J* = 1.0, 7.9 Hz), 7.53 (dd, 1H, *J* = 1.0, 7.9 Hz), 7.44 (dt, 1H, 7.8 Hz), 7.32 (dt, 1.2, 7.5 Hz), 6.49 (d, 1H, *J* = 2.3 Hz), 5.18 (t, 1H, *J* = 3.5 Hz), 4.22 (dq, 1H, *J* = 1.7, 6.5 Hz), 3.89 (m, 1H), 3.56 (ddd, 1H, *J* = 0.9, 2.3, 3.5 Hz), 3.50 (s, 3H), 2.46 (dt, 2H, *J* = 3.2, 7.2 Hz), 2.17 (s, 3H), 1.61 (m, 2H), 1.49 (m, 2H), 1.31 (d, 3H, *J* = 6.5 Hz), 1.00 (t, 9H, *J* = 7.9 Hz), 0.95 (t, 3H, *J* = 7.3 Hz), 0.68 (q, 6H, *J* = 7.9 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 170.5, 164.5, 135.0, 132.1, 130.9, 130.7, 127.3, 125.1, 96.7, 93.1, 79.7, 76.3, 70.7, 70.1, 60.0, 30.9, 22.3, 21.4, 19.7, 16.8, 13.8, 7.05, 5.15 ppm; **HRMS** ESI *m/z* $[M + Na]^+$ calcd. for $C_{28}H_{42}O_7SiNa$ 541.25920, found 541.25885.

Spectroscopic data corresponded to those reported in the literature.[2]

(*S*)-4-(Benzyloxy)-5-isopropyl-1,5-dihydro-2*H*-pyrrol-2-one (68)



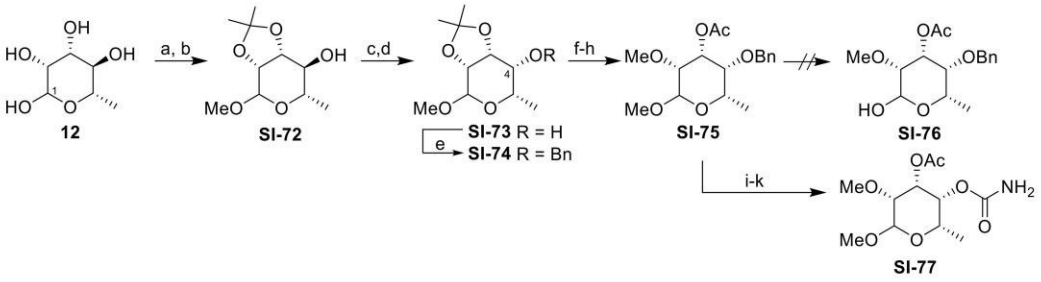
68

Amino acid **67** (500 mg, 2.41 mmol, 1.00 eq.) in dry THF (8.00 mL) was treated with Ph_3PCCO (**66**, 802 mg, 2.65 mmol, 1.10 eq.) and benzoic acid (58.9 mg, 482 µmol, 0.20 eq.) at room temperature. The mixture was heated to 60 °C and stirred for 22 h. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, acetone/CH_2Cl_2 19:1→6:1→4:1→3:1 →2:1→1.5:1) to furnish 4-*O*-alkyl tetramic acid **68** (351 mg, 1.52 mmol) as a colourless solid. $\mathbf{R_f}$ = 0.59 (CH_2Cl_2/MeOH 9:1); **mp** 129 °C; **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.37 (m, 5H), 6.76 (br. s, 1H), 5.10 (d, 1H, *J* = 1.5 Hz), 4.99 (d, 1H, *J* = 11.6 Hz), 4.94 (d, 1H, *J* = 11.6 Hz), 4.04 (d, 1H, *J* = 3.3 Hz), 2.14 (dqn, 1H, *J* = 3.3, 7.0 Hz), 1.03 (d, 3H, *J* = 7.0 Hz), 0.80 (d, 3H, *J* = 7.0 Hz) ppm; **^{13}C-NMR** (125 MHz, $CDCl_3$) 176.3, 175.2, 135.0, 128.82, 128.78, 127.9, 95.4, 73.2, 63.0, 29.4, 19.6, 15.2 ppm; **HRMS** ESI *m/z* $[M + H]^+$ calcd. for $C_{14}H_{18}NO_2$ 232.13321, found 232.13260.

Spectroscopic data corresponded to those reported in the literature.[2]

2.12 Failed routes to amykitanose

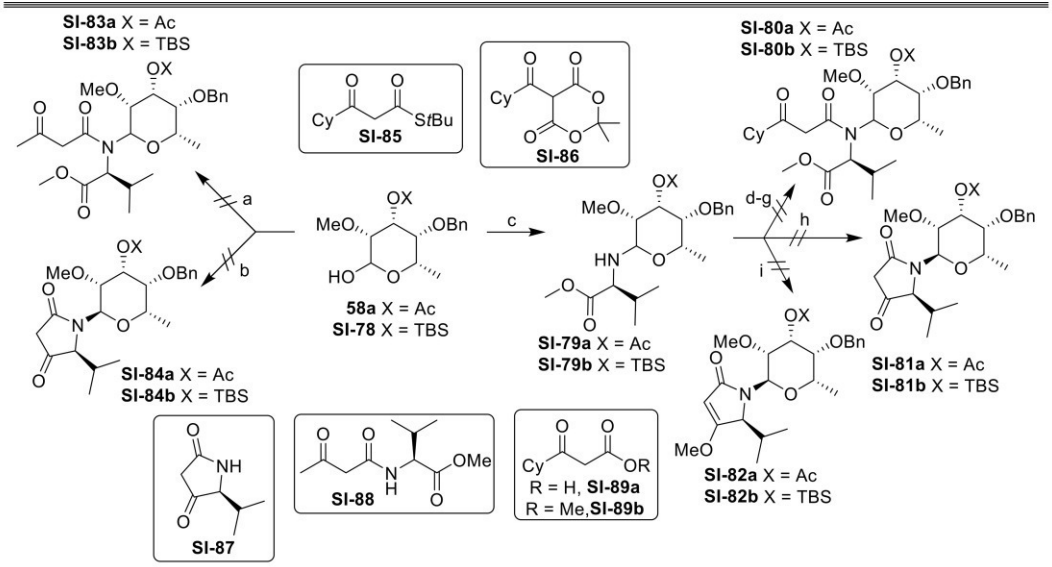
Before the synthesis with an allyl function at the anomeric position was completed, we tried to use a methyl acetal at 1-position. It was introduced with sulfuric acid in MeOH in quantitative yield. Protection of the *syn*-diol furnished carbohydrate **SI-72** in 93% yield. Swern-oxidation in 77% yield and consequent reduction with $NaBH_4$ in 99% yield gave alcohol **SI-73** with inverted stereoconfiguration at 4-position as a single diastereomer. The remaining hydroxyl group was benzylated in 99%. Removal of the acetal with $BiCl_3$ provided a diol, which was regioselectively acetylated at 3-position. This was followed by methylation with $TMSCHN_2$ and HBF_4 (→ **SI-75**). Different acidic conditions were used to cleave the acetal at the anomeric position. However, either the acetyl group was removed too, or no reaction was observed. Therefore, it was switched to the allyl group at the anomeric position. It was also tried, to introduce the carbamate at 4-position. The benzyl group was removed via hydrogenation. The resulting hydroxyl group reacted quickly with trichloroacetylisocyanate to an intermediate, which was converted to carbamate **SI-77** by stirring with SiO_2 in a THF/MeOH mixture.



Scheme S15. Performed steps towards methyl-acetal of L-rhamnose **SI-76**.
Reagents and conditions: a) H_2SO_4, MeOH, RT, on, quant.; b) $CuSO_4$, acetone, rt, 21 h, 93%; c) 1. $(ClOC)_2$, DMSO, CH_2Cl_2, −78 °C, 30 min, 2. **SI-72**, 30 min, 3. DIPEA, rt, 18 h, 77%; d) $NaBH_4$, EtOH, 0 C, 21 h, 99%, single diastereomer; e) 1. NaH, imidazole, DMF, 0 °C→rt, 35 min, 2. BnBr, TBAI, rt, 18 h, 99%; f) $BiCl_3$, MeCN/H_2O, rt, 1 d, 99%; g) 1. Bu_2SnO, toluene, reflux, 2 h, 2. AcCl, rt, 3 h, 85%; h) $TMSCHN_2$, HBF_4, CH_2Cl_2, 0 °C, 5 h, 77%; i) Pd/C, H_2, MeOH, 20 h, quant.; j) trichloroacetylisocyanate, CH_2Cl_2, 0 °C, 10 min; k) SiO_2, THF/MeOH, 40 °C, 16 h, 65% over two steps.

The main problem of the synthesis of the upper part of kibdelomycin was the coupling of the sugar and tetramic acid. Our first concept was to build *N*-glycosides **SI-79a/b** with L-valine, which we achieved in excellent 99% yield and α:β-ratio of 2:1 by simply adding the amino acid in EtOH or MeOH. However, it was not possible to convert the aminoglycosides **SI-79a/b** into the corresponding β-ketoamides **SI-80a/b**, tetramic acids **SI-81a/b** or 4-*O*-alkyl tetramic acids

SI-82a/b. All of them could be converted to 3-acyltetramic acid in well studied reactions and therefore could have been possible intermediates. For building β-ketoamides **SI-80a/b**, we focused on Ley´s acylation with β-ketothioester **SI-85**. This method was successfully used for acylation of a aminoglycoside by our group in 2016.[16] Different equivalents, reaction time, temperature, different silver salts and additional reagents were tested (Table S1). Most of the times the acetyl group or valine was removed, sometimes complete decomposition was observed or educt was reisolated. Also, an attempt to introduce a β-ketoamide by conversion with adduct **SI-86** under reflux only led to removal of the acetyl group. Likewise, the *in situ* formation of the acid chloride of carboxylic acid **SI-89a** and conversion with aminoglycoside **SI-79a** under basic conditions gave decomposition of starting materials. After multiple attempts, the acetyl group turned out to be instable under different conditions. So instead of the acetyl group, a TBS protecting group was introduced to try some of the reactions already carried out again. Each of them also lead to decomposition or removal of acetyl group or no transformation. Further attempts to convert the aminoglycosides **SI-79a/b** into a tetramic acid via Meldrum´s acid method led to elimination of valine. Also, the conversion with ketenylidentriphenylphosphorane to give 4-*O*-alkyltetramic acids **SI-82a/b** wasn´t successful, only decomposition products were isolated. After trials to convert the aminoglycoside, the β-ketoamide or tetramic acid should be introduced directly. Therefore, a Mitsunobu reaction with β-ketoamide **SI-88** was carried out, but only educt was reisolated. Conversion of semi-acetal **58a** with tetramic acid **SI-87** and *p*TsOH led to decomposition. The experiments with TBS-group instead of acetyl group led to similar results.



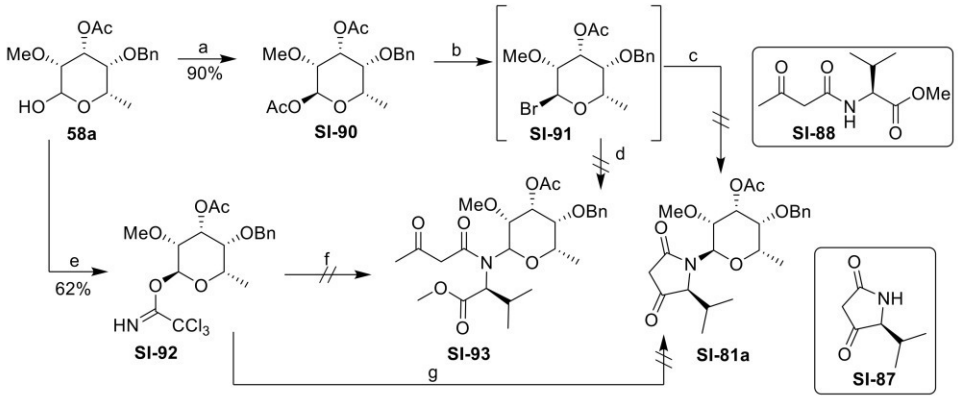
Scheme S16. Failed attempts to attach a tetramic acid or β-ketoamide at the glycoside or aminoglycoside.
Reagents and conditions: a) PPh_3, DIAD, β-ketoamide **SI-88**, THF, −78 °C; b) **SI-87**, *p*TsOH, CH_2Cl_2, reflux, 2 d; c) X = Ac L-valine methyl ester, EtOH/MeOH, rt, 3 d, 99%; X = TBS 86%; d) Table S1 e) X = Ac adduct **SI-86**, toluene, 120 °C, 2 h; f) X = Ac 1. oxalyl chloride, acid **SI-89a**, DMF, 0 °C, 2 h, 2. **SI-79a**, 0 °C, 21 h; g) X = Ac β-ketoester **SI-89b**, toluene, reflux, 22 h; h) X = Ac/TBS 1. Meldrum´s acid, DMAP, EDC·HCl, CH_2Cl_2, rt, 3 h, 2. EtOAc, reflux, 3 h, i) X = Ac/TBS Ph_3PCCO, THF, reflux, 19 h.

Table S1. Reaction conditions for Ley-acylation of aminoglycosides **SI-79a/b**.

Entry	X	Reagents and conditions	Temperature[°C]	Time	Result
1	Ac	Educt (1.00 eq.), **SI-85** (1.25 eq.), AgO_2CCF_3 (1.60 eq.), 4 Å MS, THF, aq. Work-up	0	3 h	Removal of Ac
2	Ac	Educt (1.00 eq.), **SI-85** (1.25 eq.), AgO_2CCF_3 (1.60 eq.), 4 Å MS, THF, without aq. work-up	0	3 h	Removal of Ac/valine
3	Ac	Educt (1.20 eq.), **SI-85** (1.00 eq.), AgO_2CCF_3 (1.20 eq.), NEt_3, THF	0	3 h	Removal of valine
4	Ac	Educt (1.00 eq.), **SI-85** (1.20 eq.), AgO_2CCF_3 (1.50 eq.), NEt_3, THF	0	3 h	Removal of Ac/valine

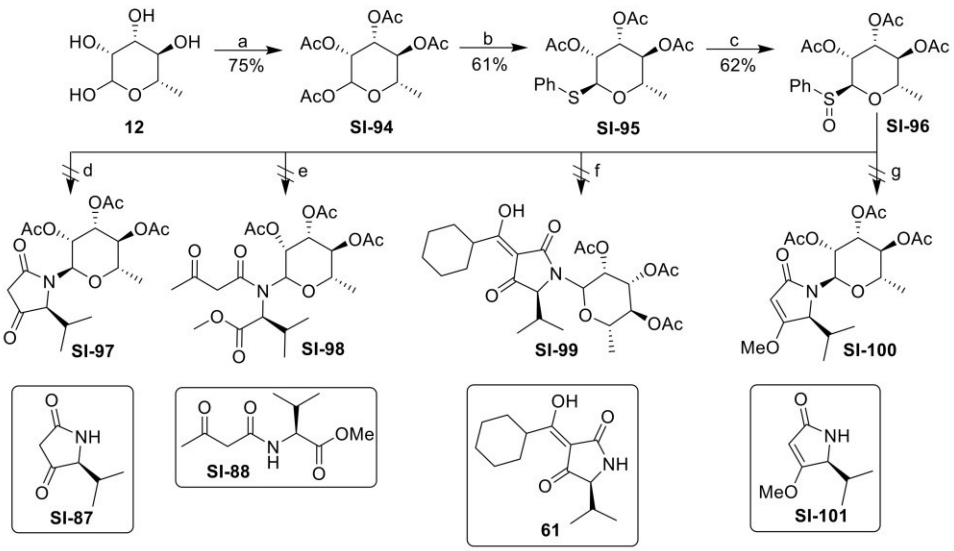
6	Ac	Educt (1.00 eq.), **SI-85** (1.20 eq.), AgO_2CCF_3 (1.25 eq.), Na_2KHPO_4, THF	0	6 h	Removal of valine
7	Ac	Educt (1.00 eq.), **SI-85** (1.25 eq.), AgO_2CCF_3 (1.25 eq.), 4 Å MS, THF	−78	1 h	Removal of Ac
8	Ac	Educt (1.00 eq.), **SI-85** (1.20 eq.), AgO_2CCF_3 (1.25 eq.), Na_2KHPO_4, THF	−78	1.5 h	Removal of Ac
9	Ac	Educt (1.00 eq.), **SI-85** (1.50 eq.), AgO_3SCF_3 (2.00 eq.), NEt_3, THF	0	6 h	educt
10	Ac	Educt (1.00 eq.), **SI-85** (1.25 eq.), AgO_3SCF_3 (1.60 eq.), 4 Å MS, THF	0	22 h	Removal of Ac/valine
11	TBS	Educt (1.00 eq.), **SI-85** (1.25 eq.), AgO_2CCF_3 (1.25 eq.), 4 Å MS, THF	−78	4 h	Decompo-sition
12	TBS	Educt (1.00 eq.), **SI-85** (1.25 eq. + 1.25 eq.), AgO_2CCF_3 (1.25 eq. + 1.25 eq.), Na_2KHPO_4, THF	−78→rt	1 d	Decompo-sition
13	TBS	Educt (1.20 eq.), **SI-85** (1.00 eq.), AgO_2CCF_3 (1.20 eq.), NEt_3, THF	0→rt	2 d	educt

Some reactions were carried out with activated forms of carbohydrate **58a**. Therefore, it was first acetylated at the anomeric position in 90% yield. The bromide **SI-91** was formed by addition of TMSBr and had to be used directly in the next step because of its instability. On the one hand it was reacted with tetramic acid **SI-87** and KO*t*Bu and on the other hand it was converted with β-ketoamide **SI-88** and KO*t*Bu. Both reactions led to decomposition of starting material. The trichloroacetimidate **SI-92** was easily built by conversion of sugar **58a** with trichloroacetonitrile in 62% yield. Though, the attempts to couple it with tetramic acid **SI-87** or β-ketoamide **SI-88** weren´t successful and led to reisolation of starting material and decomposition, respectively.



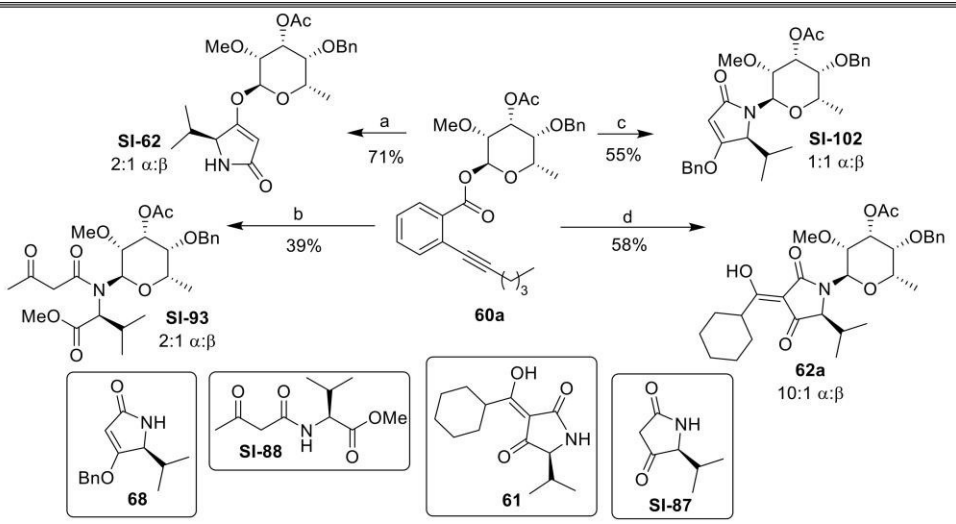
Scheme S17. Failed attempts to attach a tetramic acid or β-ketoamide at activated glycosides **SI-92** and **SI-91**.
Reagents and conditions: a) Ac_2O, pyridine, rt, 2 h; b) TMSBr, CH_2Cl_2, 0 °C, 2 h; c) tetramic acid **SI-87**, KO*t*Bu, THF, 0 °C, 20 h; d) β-ketoamide **SI-88**, KO*t*Bu, THF, 0 °C, 20 h; e) DBU, Cl_3CCN, CH_2Cl_2, 0 °C→rt, 1 d, f) β-ketoamide **SI-88**, TMSOTf, 4 Å MS, CH_3NO_2, rt, 4 d; g) tetramic acid **SI-87**, TMSOTf, 4 Å MS, CH_3NO_2, 0 °C, 1 d.

On the basis of the work of Beretta *et al.*[17] we synthesized the sulfoxide donor **SI-96** in three steps out of L-Rhamnose (**12**) by complete acetylation, *S*-glycosylation and oxidation to the sulfoxide with *m*CPBA. This sugar was used instead of the ready functionalised sugar to try the coupling reactions. Sulfoxide **SI-96** was reacted with tetramic acid **SI-87**, β-ketoamide **SI-88**, 3-acyltetramic acid **61** and 4-*O*-alkyltetramic acid **SI-101**. Before, they were activated by conversion with BSA, which should silylate the nitrogen. Second step is the addition of sugar **SI-96** and a lewis-acid, for which we choose TMSOTf. All the experiments led to decomposition of the starting material.



Scheme S18. Failed attempts to attach a tetramic acid or β-ketoamide to sulfoxide **SI-96**.
Reagents and conditions: a) Ac_2O, pyridine, rt, 22 h; b) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 22 h; c) *m*CBPA, CH_2Cl_2, −78 °C→0 °C, 7 h; d) 1. tetramic acid **SI-87**, BSA, dichloroethane, 90 °C, 2 h, 2. **SI-96**, TMSOTf, rt, 23 h; e) 1. β-ketoamide **SI-88**, BSA, dichloroethane, 90 °C, 2 h, 2. **SI-96**, TMSOTf, rt, 19 h; f) 1. 3-acyl tetramic acid **61**, BSA, dichloroethane, 90 °C, 1 h, 2. **SI-96**, TMSOTf, rt, 22 h; g) 1. 4-*O*-alkyltetramic acid **SI-101**, BSA, dichloroethane, 90 °C, 2 h, 2. **SI-96**, TMSOTf, rt, 20 h.

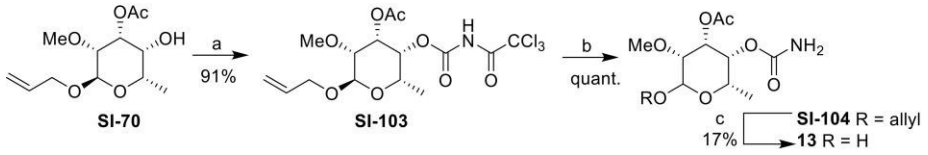
Finally, we decided to use the established method of the first total synthesis.[2] Ester **60a** was treated with gold-catalyst and all of the coupling products used before. Conversion with tetramic acid **SI-87** led to a defined product. 2D-NMR-experiments indicated that tetramic acid is bound to the sugar via a *O*-glycosidic linkage. This is possible because of the tautomeric character of tetramic acid **SI-87**. Reaction with β-ketoamide **SI-88** led to a product mixture. Here *O*-, *C*- or *N*-glycosidic linkages are possible. The different products couldn´t be separated. The glycosylation with 4-*O*-alkyltetramic acid **68** as well as 3-acyltetramic acid **61** gave the desired products but with a α:β ratio of 1:1 and 10:1, respectively.



Scheme S19. Investigations on attaching different forms of tetramic acids to a glycoside via an Au-catalysed reaction.
Reagents and conditions: a) tetramic acid **SI-87**, $AuPPh_3NTf_2$, toluene, 40 °C, 20 h; b) β-ketoamide **SI-88**, $AuPPh_3NTf_2$, toluene, 40 °C, 20 h; c) 4-*O*-alkyltetramic acid **68**, $AuPPh_3NTf_2$, toluene, 40 °C, 20 h; d) 3-acyltetramic acid **61**, $AuPPh_3NTf_2$, toluene, 40 °C, 20 h.

2.13 Synthesis of amykitanose (**13**)

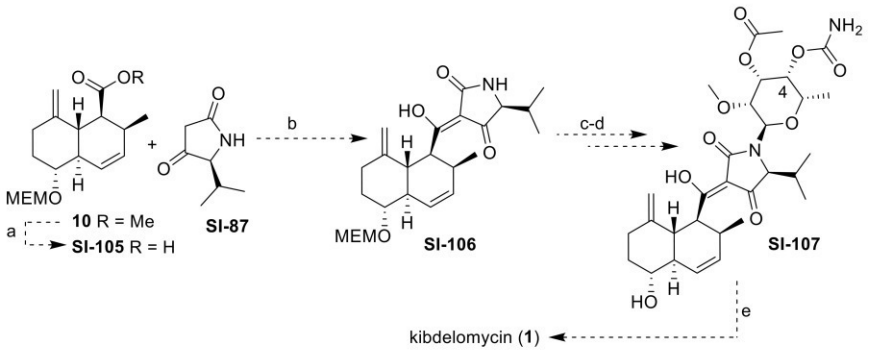
Glycoside **SI-70** was reacted with trichloroacetylisocyanate to give product **SI-103**, which gave the carbamate **SI-104** after stirring with SiO_2 in 91% yield over two steps. Deprotection at the anomeric position in 17% yield gave amykitanose (**13**). The synthesis wasn´t optimised yet but can easily be used to introduce the carbamate function.



Schema S20. Synthesis of amykitanose (**13**).
Reagents and conditions: a) trichloroacetylisocyanate, CH_2Cl_2, 0°C, 13 min; b) SiO_2, THF/MeOH, 40°C; c) $Pd(PPh_3)_4$, AcOH, rt, 16 h.

2.14 Alternative formal synthesis of kibdelomycin (**1**)

For the completion of an alternative total synthesis exploiting the novel *N*-glycosylation of 3-acyltetramic acids, tetramic acid **SI-87** would have to be attached to the decalin fragment **SI-105** via an established Yoshii-Yoda acylation (Scheme S21).[18] The resulting 3-acyltetramic acid **SI-106** would then be *N*-glycosylated with the sugar fragments **60a/b** via the known Au-catalysed reaction and the 4-position be converted into a carbamic acid to give **SI-107** (analogue to the synthesis of amykitanose (**13**) *cf.* Scheme S20). Finally, building block **SI-107** would be *O*-glycosylated with the amycolose derivative **4** to afford kibdelomycin (**1**).



Schema S21. Synthetic plan for an alternative synthesis of kibdelomycin (**1**).
Reagents and conditions: a) LiOH; b) EDC·HCl, DMAP, then NEt_3, DMAP, $CaCl_2$; c) **60a/b**, $AuPPh_3NTf_2$; d) deprotection 4-position, then $Cl_3CCONCO$, then SiO_2, then MEM-deprotection; e) **4**, TfOH.

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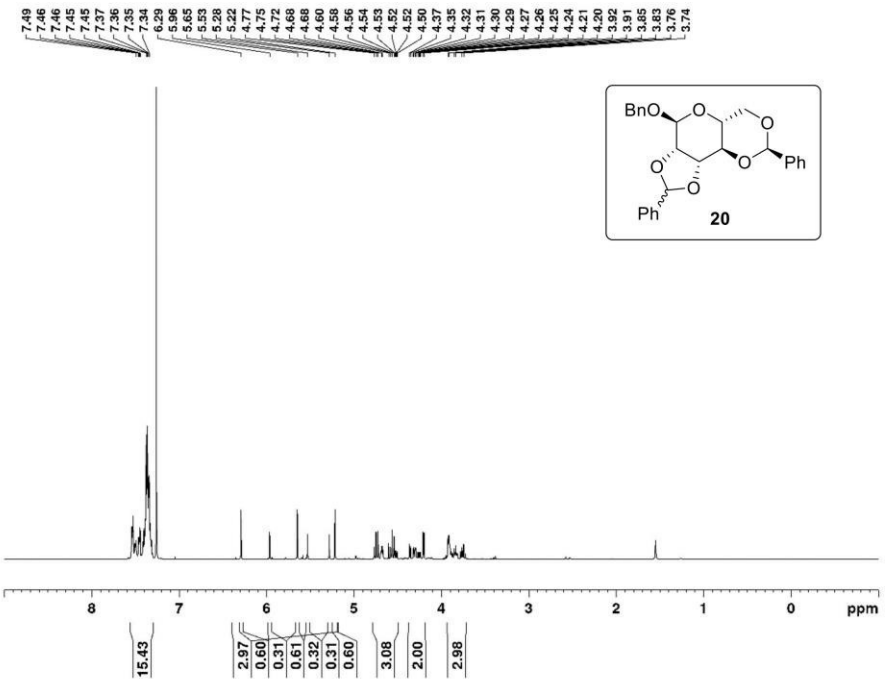
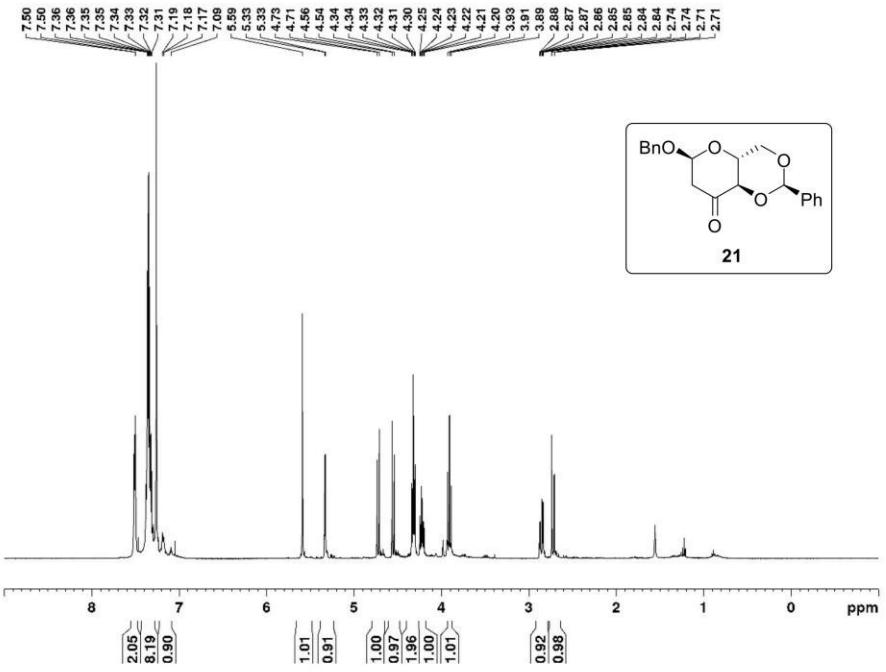
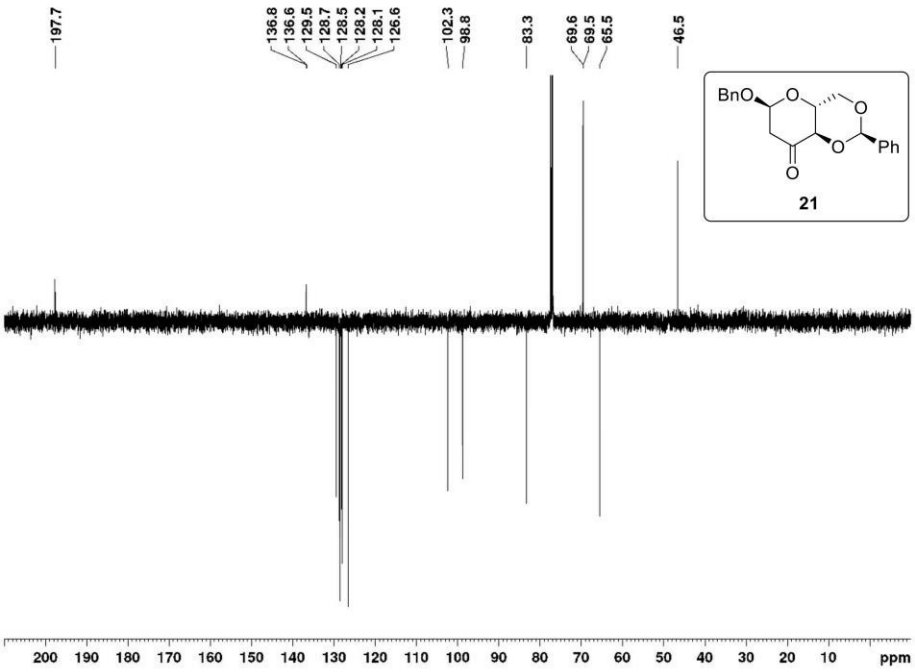
4. NMR-Spectra



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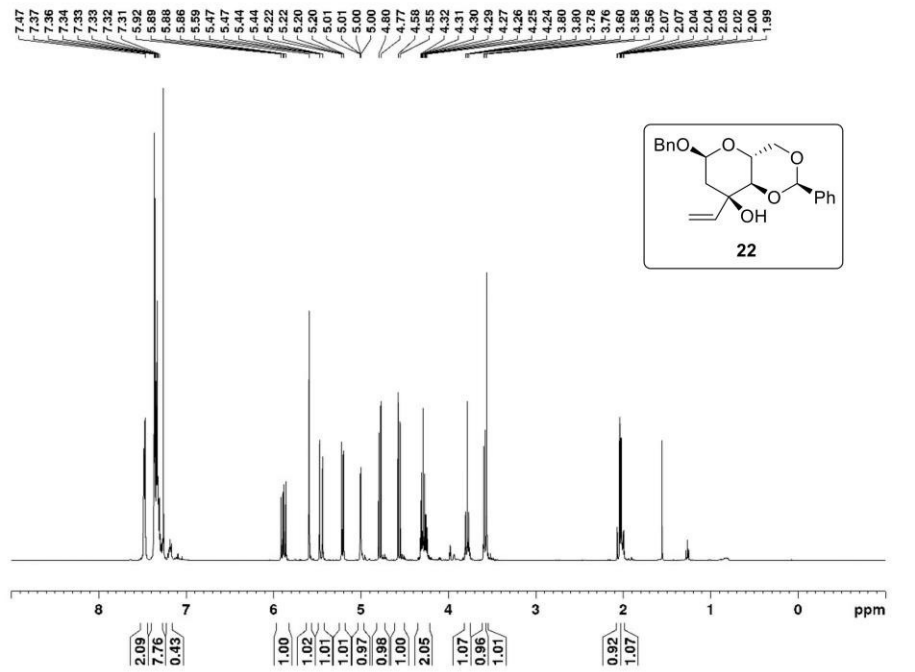
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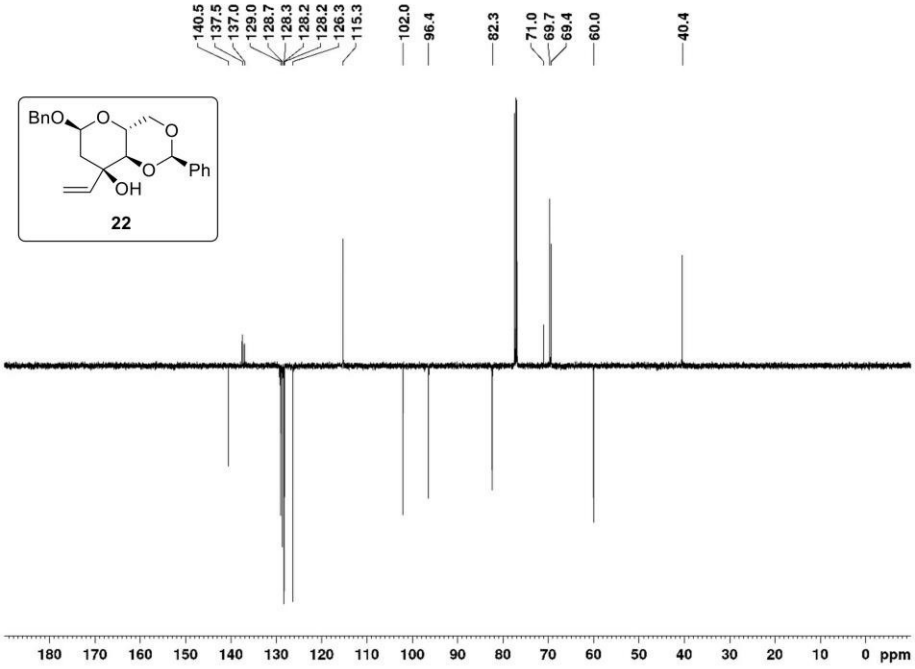
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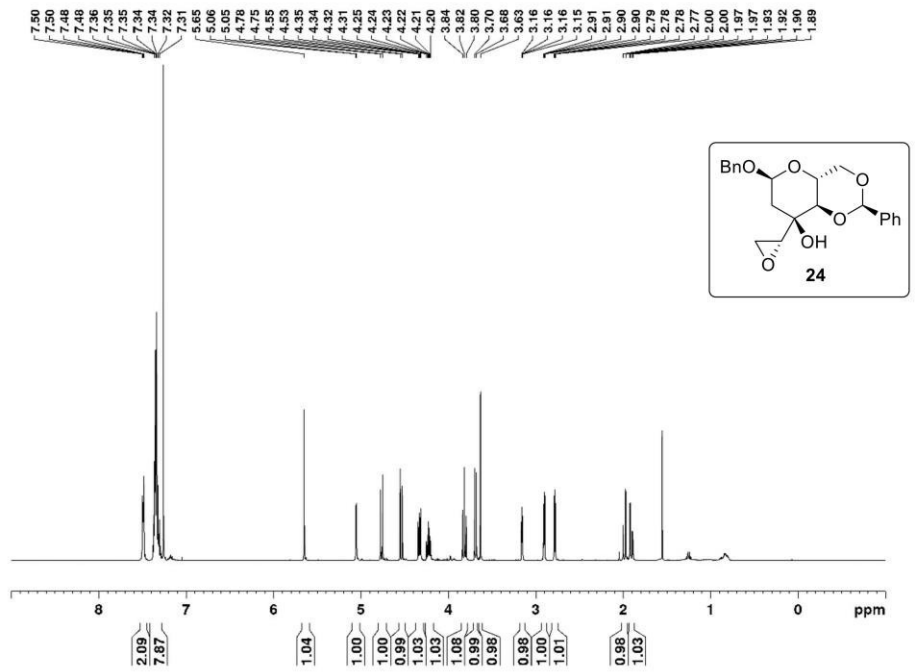
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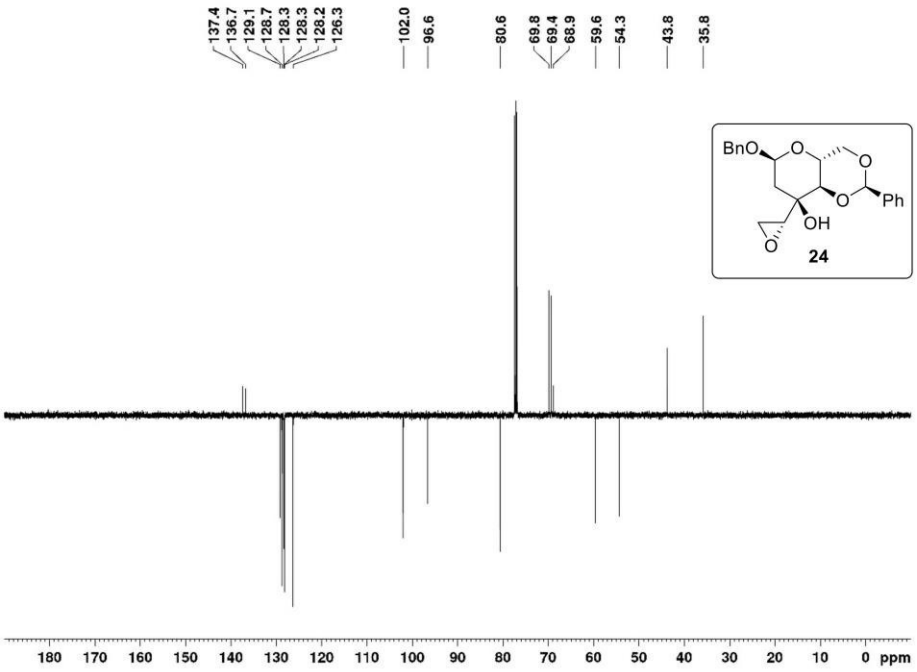
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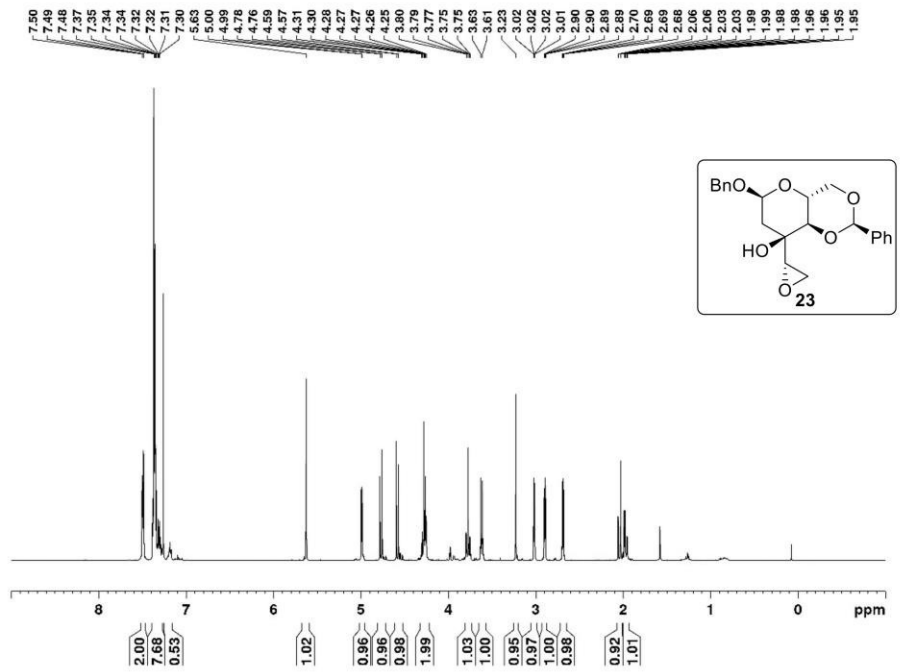
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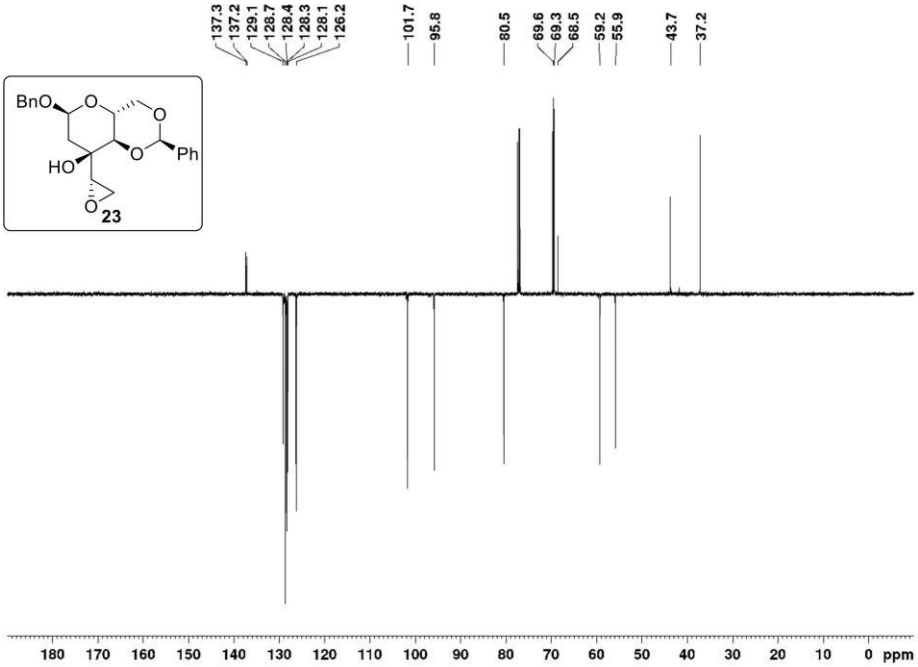
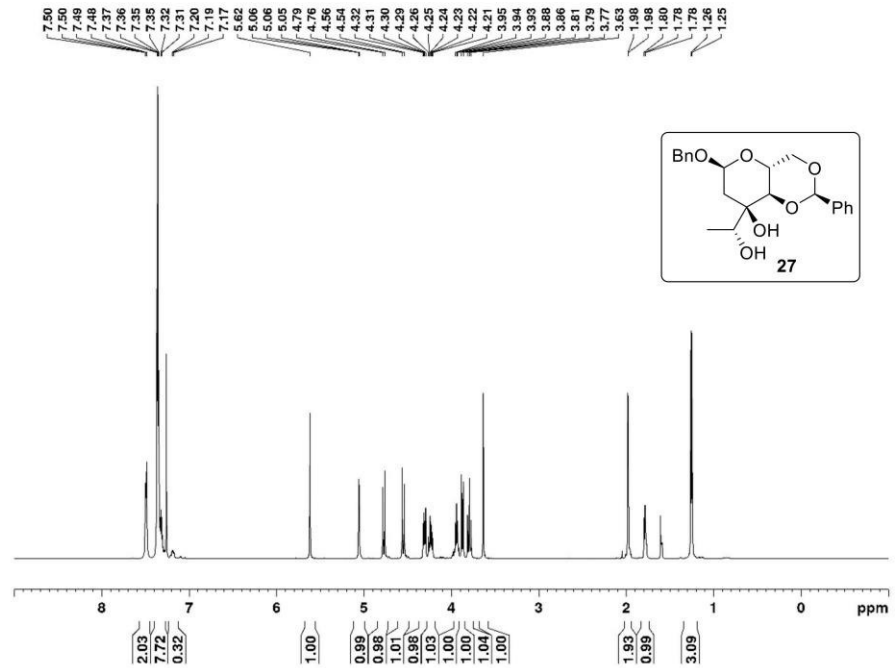
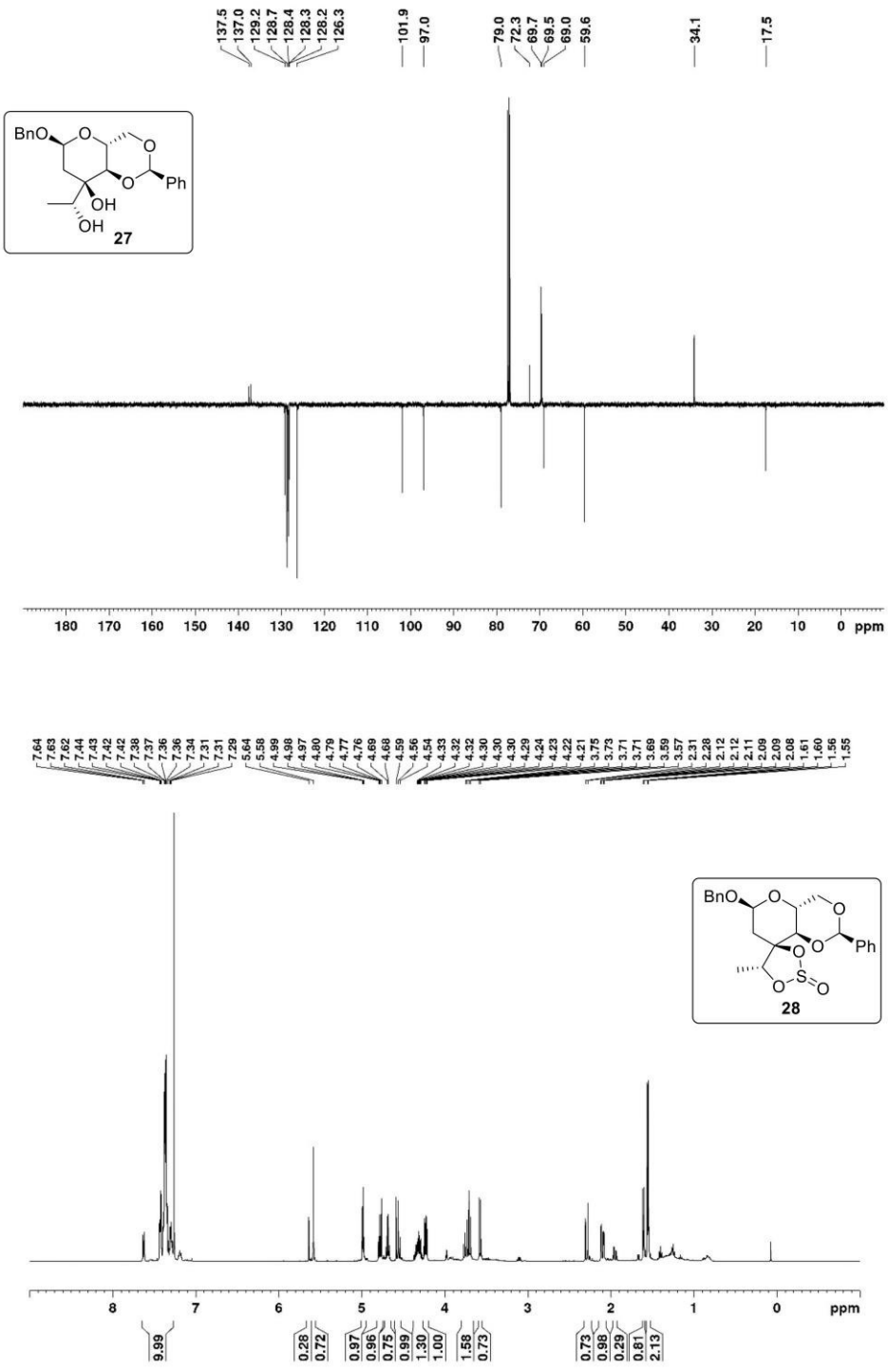
2.06
2.06
2.03
2.03
1.99
1.99
1.98
1.98
1.96
1.96
1.95
1.95
BnO
O
O
O
Ph
HO
O
23
8 7 6 5 4 3 2 1 0 ppm
2.00
7.68
0.53
1.02
0.96
0.96
0.98
1.99
1.03
1.00
0.95
0.97
1.00
0.98
0.92
1.01

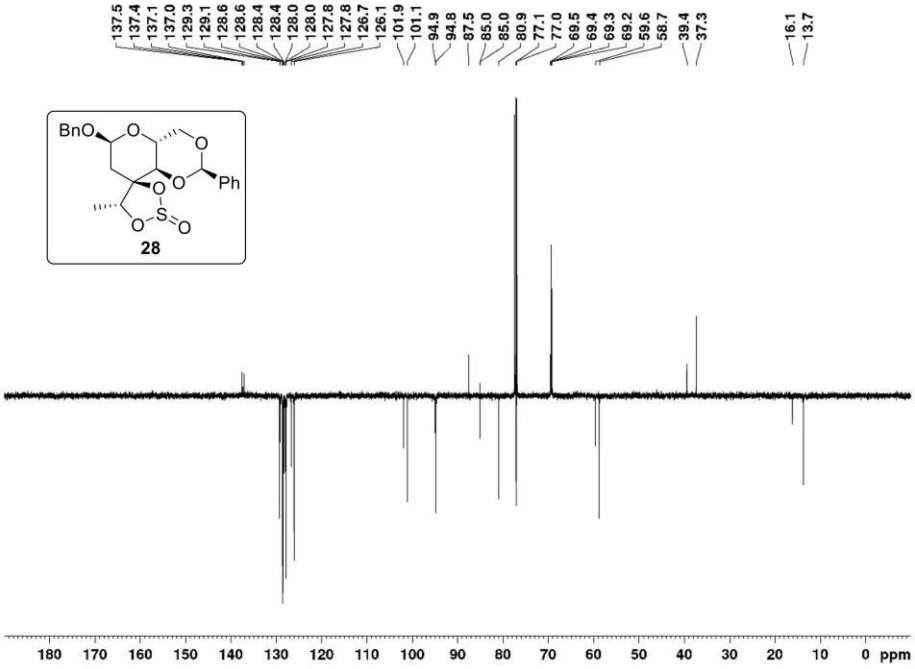

137.3
137.2
129.1
128.7
128.4
128.3
128.1
126.2
101.7
95.8
80.5
69.6
69.3
68.5
59.2
55.9
43.7
37.2
BnO
HO
Ph
23
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

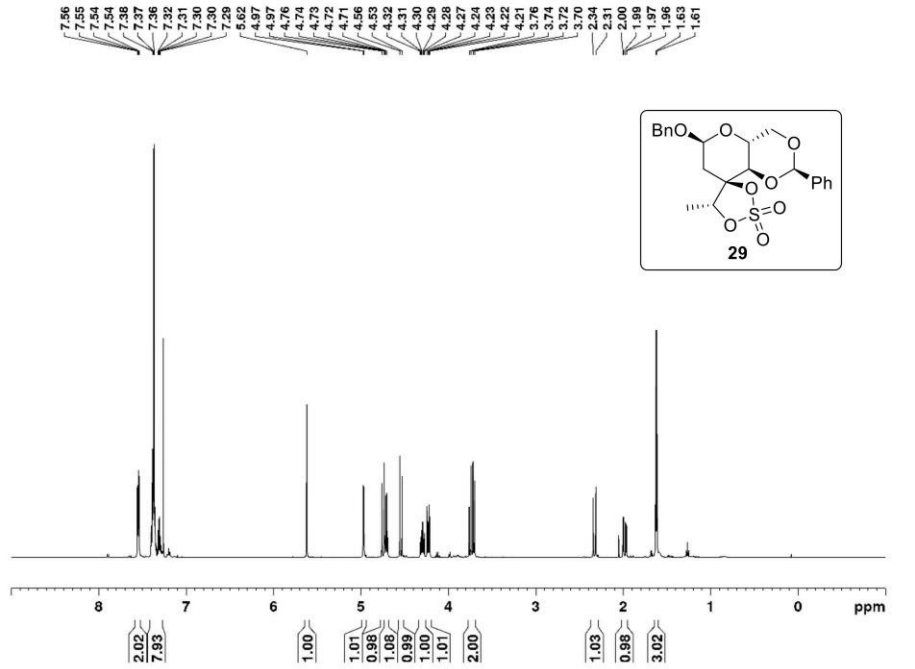

BnO
OH
OH
Ph
27
8 7 6 5 4 3 2 1 0 ppm
2.03
7.72
0.32
1.00
0.99
0.98
1.01
0.98
1.03
1.00
1.00
1.04
1.00
1.93
0.99
3.09

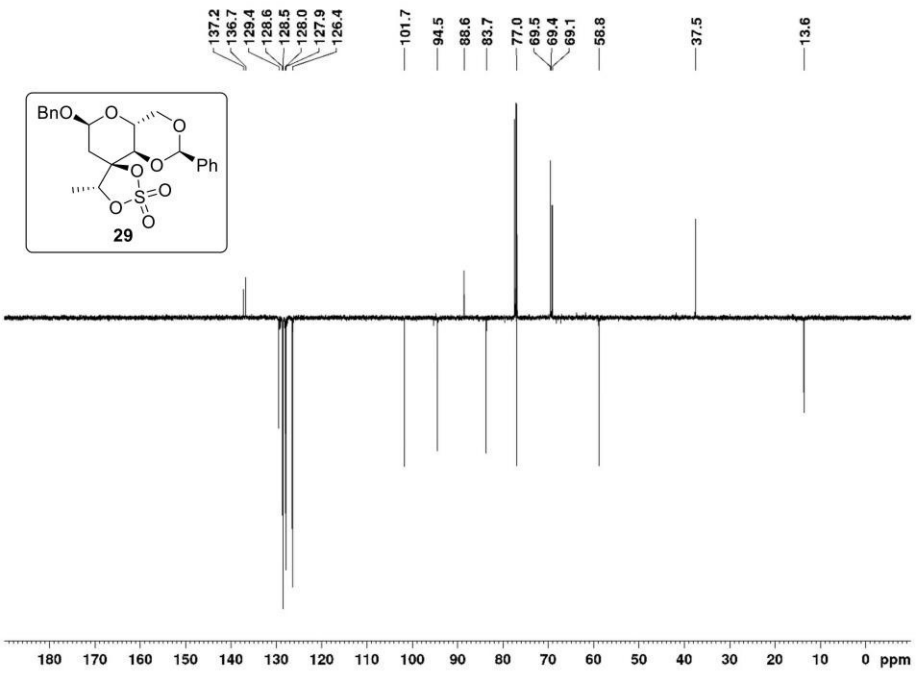
S88

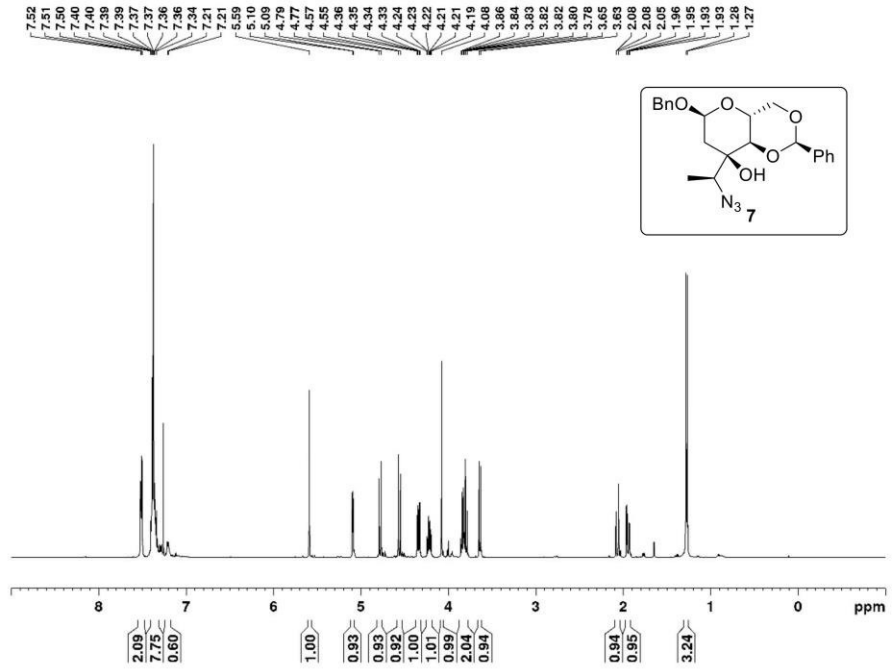

137.5
137.0
129.2
128.7
128.4
128.3
128.2
126.3
101.9
97.0
79.0
72.3
69.7
69.5
69.0
59.6
34.1
17.5
BnO
O
O
O
Ph
OH
OH
27
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
7.64
7.63
7.62
7.44
7.43
7.42
7.42
7.38
7.37
7.36
7.36
7.34
7.31
7.31
7.29
5.64
5.58
4.99
4.98
4.97
4.80
4.79
4.77
4.76
4.69
4.68
4.59
4.56
4.54
4.33
4.32
4.32
4.30
4.30
4.30
4.29
4.24
4.23
4.22
4.21
3.75
3.73
3.71
3.71
3.69
3.59
3.57
2.31
2.28
2.12
2.12
2.11
2.09
2.09
2.08
1.61
1.60
1.56
1.55
BnO
O
O
O
Ph
O
S
O
O
28
8 7 6 5 4 3 2 1 0 ppm
9.99
0.28
0.72
0.97
0.96
0.75
0.99
1.30
1.00
1.58
0.73
0.73
0.98
0.29
0.81
2.13

S89

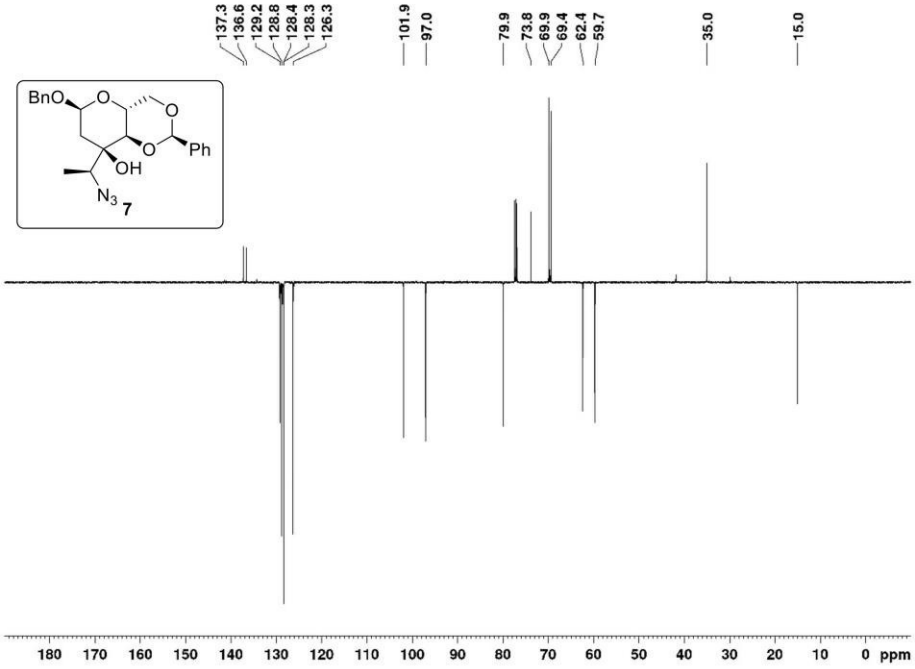

137.5
137.4
137.1
137.0
129.3
129.1
128.6
128.6
128.4
128.4
128.0
128.0
127.8
127.8
126.7
126.1
101.9
101.1
94.9
94.8
87.5
85.0
85.0
80.9
77.1
77.0
69.5
69.4
69.3
69.2
59.6
58.7
39.4
37.3
16.1
13.7
BnO
Ph
28
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

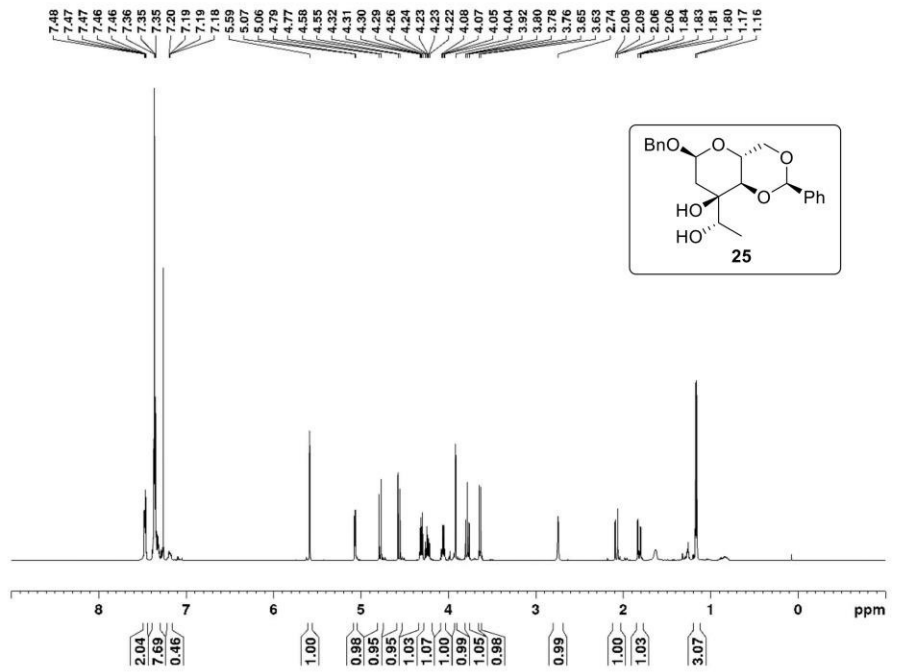

7.56
7.55
7.54
7.54
7.38
7.37
7.36
7.32
7.31
7.30
7.30
7.29
5.62
4.97
4.97
4.76
4.74
4.73
4.72
4.71
4.56
4.53
4.32
4.31
4.30
4.29
4.28
4.27
4.24
4.23
4.22
4.21
3.76
3.74
3.72
3.70
2.34
2.31
2.00
1.99
1.97
1.96
1.63
1.61
BnO
Ph
29
8 7 6 5 4 3 2 1 0 ppm
2.02
7.93
1.00
1.01
0.98
1.08
0.99
1.00
1.01
2.00
1.03
0.98
3.02


137.2
136.7
129.4
128.6
128.5
128.0
127.9
126.4
101.7
94.5
88.6
83.7
77.0
69.5
69.4
69.1
58.8
37.5
13.6
BnO
O
O
Ph
O
O
S
O
O
29
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

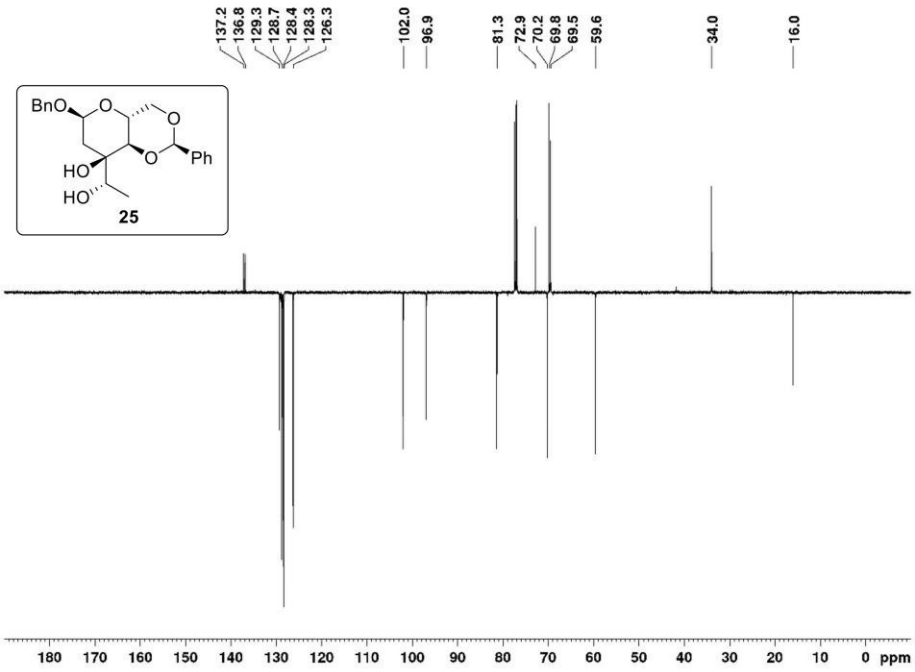

7.52
7.51
7.50
7.40
7.40
7.39
7.39
7.37
7.37
7.36
7.36
7.34
7.21
7.21
5.59
5.10
5.09
4.79
4.77
4.57
4.55
4.36
4.35
4.34
4.33
4.24
4.23
4.22
4.21
4.21
4.19
4.08
3.86
3.84
3.83
3.82
3.82
3.80
3.78
3.65
3.63
2.08
2.08
2.05
1.96
1.95
1.93
1.93
1.28
1.27
BnO
O
O
Ph
O
OH
N3
7
8 7 6 5 4 3 2 1 0 ppm
2.09
7.75
0.60
1.00
0.93
0.93
0.92
1.00
1.01
0.99
2.04
0.94
0.94
0.95
3.24

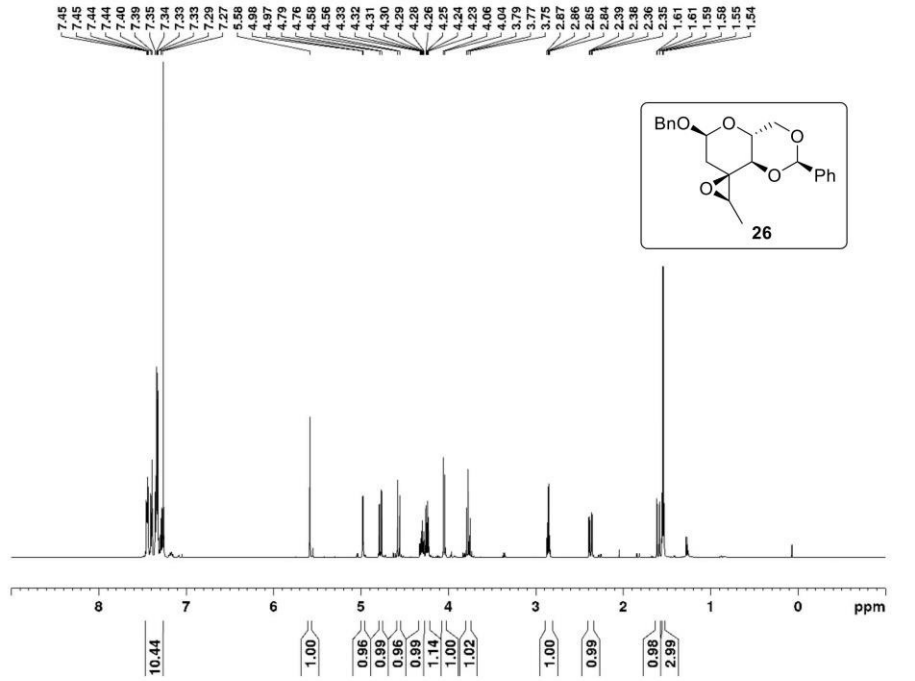
S91


137.3
136.6
129.2
128.8
128.4
128.3
126.3
101.9
97.0
79.9
73.8
69.9
69.4
62.4
59.7
35.0
15.0
BnO
O
O
O
Ph
OH
N3
7
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

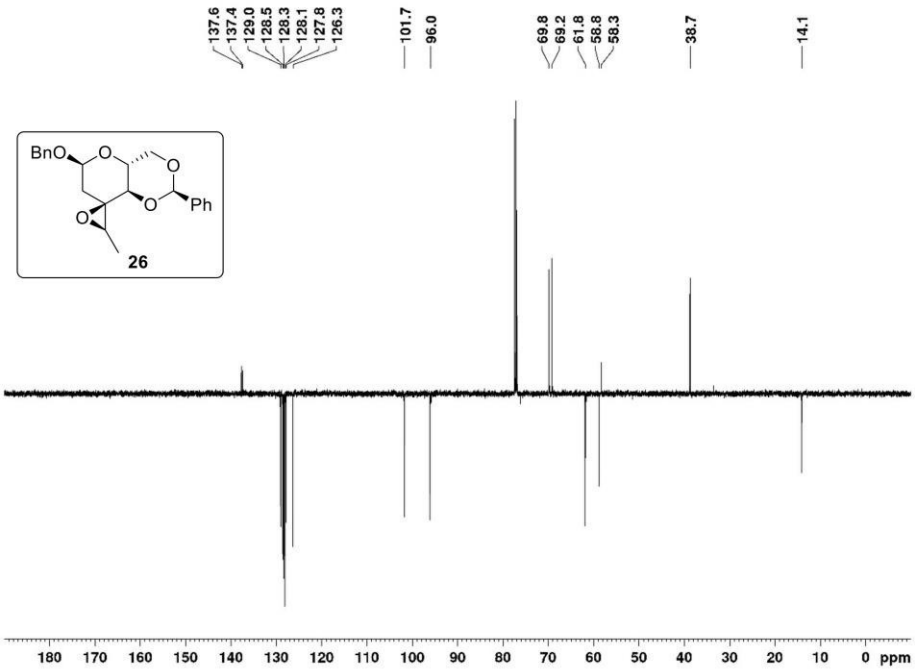

7.48
7.47
7.47
7.46
7.46
7.36
7.35
7.35
7.20
7.19
7.19
7.18
5.59
5.07
5.06
4.79
4.77
4.58
4.55
4.32
4.31
4.30
4.29
4.26
4.24
4.23
4.23
4.22
4.08
4.07
4.05
4.04
3.92
3.80
3.78
3.76
3.65
3.63
2.74
2.09
2.09
2.06
2.06
1.84
1.83
1.81
1.80
1.17
1.16
BnO
O
O
O
Ph
HO
HO
25
8 7 6 5 4 3 2 1 0 ppm
2.04
7.69
0.46
1.00
0.98
0.95
0.95
1.03
1.07
1.00
0.99
1.05
0.98
0.99
1.00
1.03
3.07

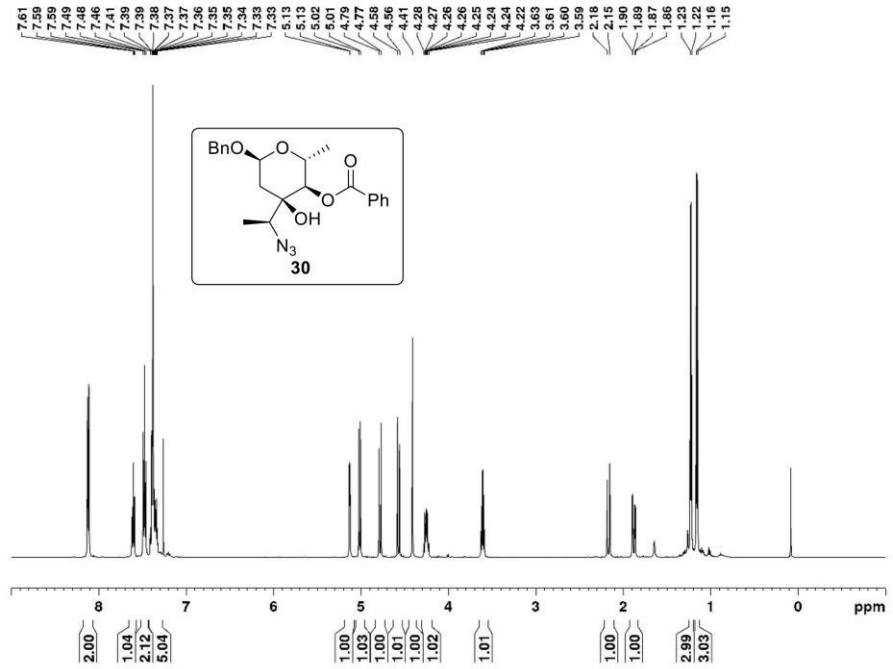
S92


137.2
136.8
129.3
128.7
128.4
128.3
126.3
102.0
96.9
81.3
72.9
70.2
69.8
69.5
59.6
34.0
16.0
BnO
O
O
O
Ph
HO
HO
25
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

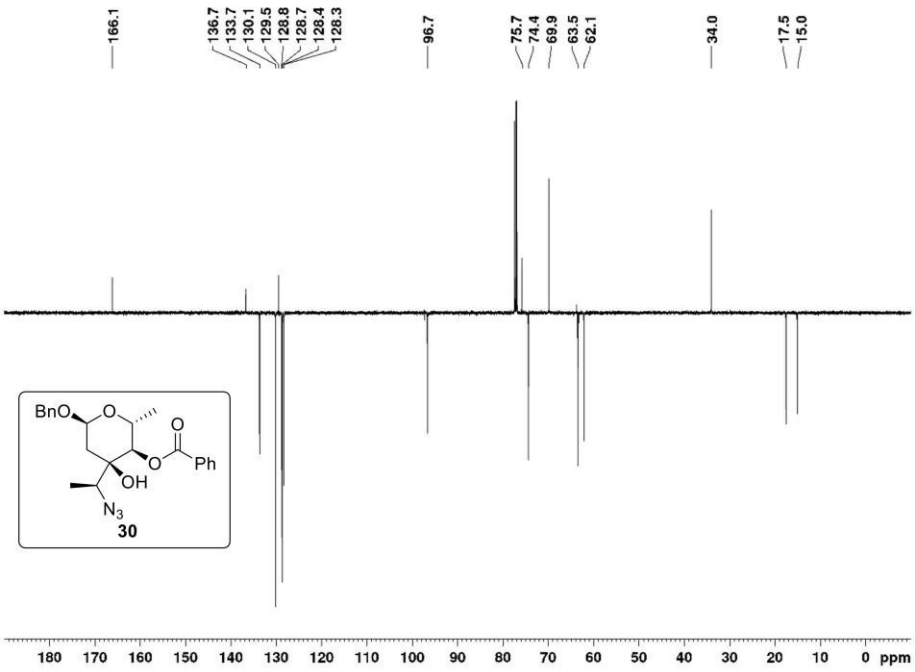

7.45
7.45
7.44
7.44
7.40
7.39
7.35
7.34
7.33
7.33
7.29
7.27
5.58
4.98
4.97
4.79
4.76
4.58
4.56
4.33
4.32
4.31
4.30
4.29
4.28
4.26
4.25
4.24
4.23
4.06
4.04
3.79
3.77
3.75
2.87
2.86
2.85
2.84
2.39
2.38
2.36
2.35
1.61
1.61
1.59
1.58
1.55
1.54
BnO
O
O
O
Ph
O
26
8 7 6 5 4 3 2 1 0 ppm
10.44
1.00
0.96
0.99
0.96
0.99
1.14
1.00
1.02
1.00
0.99
0.98
2.99

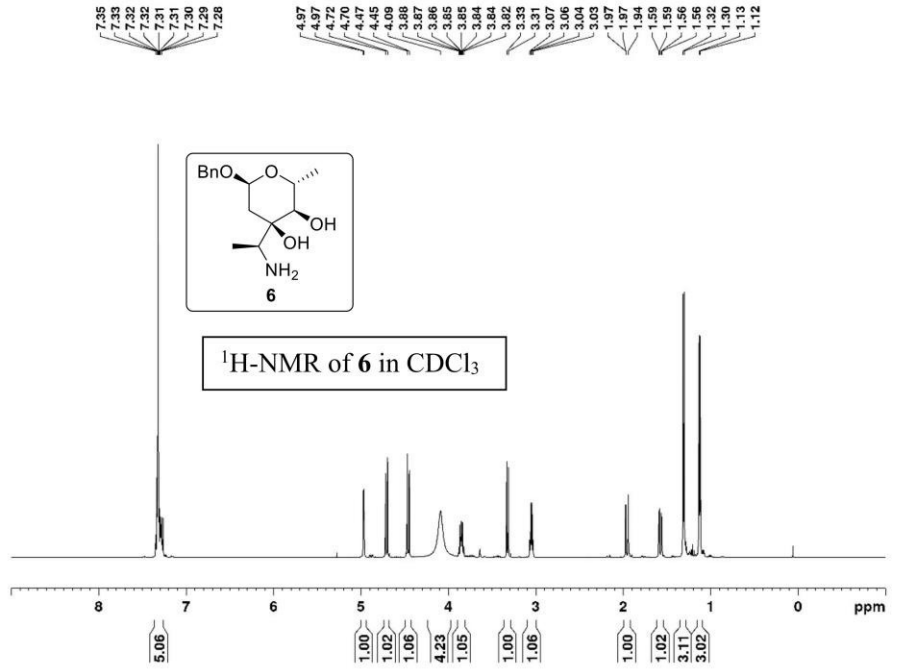
S93

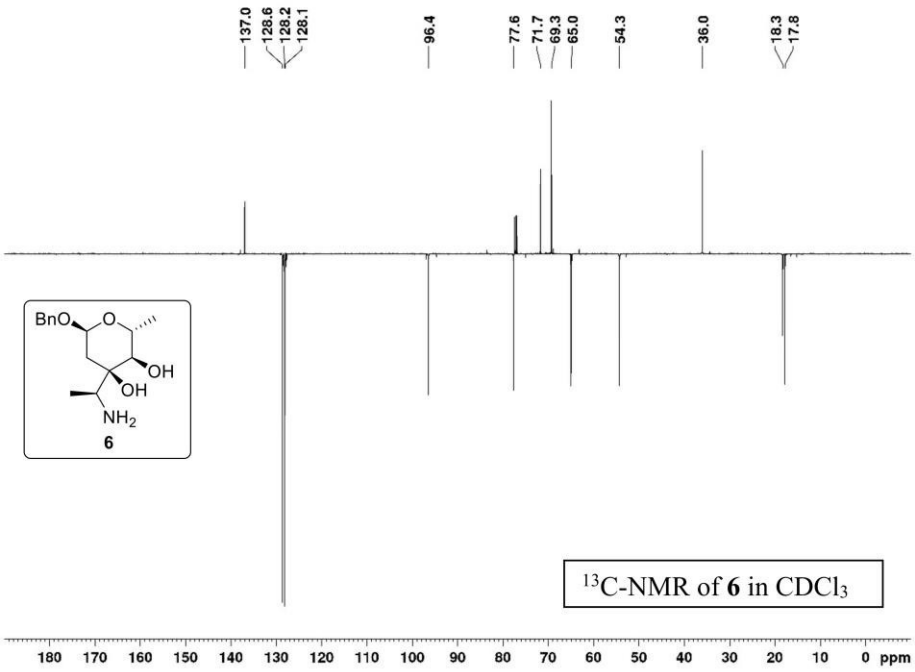

137.6
137.4
129.0
128.5
128.3
128.1
127.8
126.3
101.7
96.0
69.8
69.2
61.8
58.8
58.3
38.7
14.1
BnO
O
O
O
O
Ph
26
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

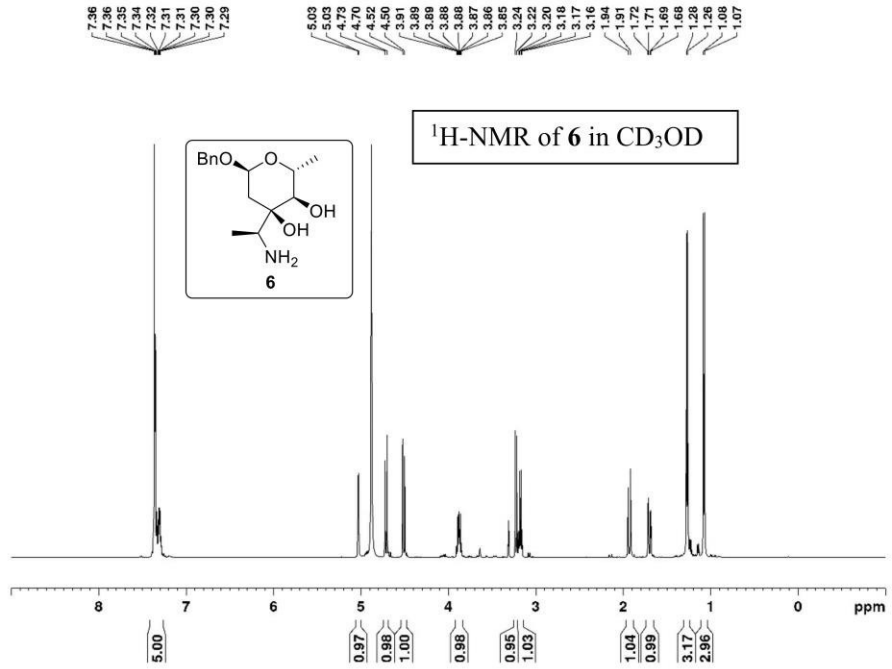

7.61
7.59
7.59
7.49
7.48
7.46
7.41
7.39
7.39
7.38
7.37
7.37
7.36
7.35
7.35
7.34
7.33
7.33
5.13
5.13
5.02
5.01
4.79
4.77
4.58
4.56
4.41
4.28
4.27
4.26
4.26
4.25
4.24
4.24
4.22
3.63
3.61
3.60
3.59
2.18
2.15
1.90
1.89
1.87
1.86
1.23
1.22
1.16
1.15
BnO
O
O
O
Ph
OH
N3
30
8 7 6 5 4 3 2 1 0 ppm
2.00
1.04
2.12
5.04
1.00
1.03
1.00
1.01
1.00
1.02
1.01
1.00
1.00
2.99
3.03

S94

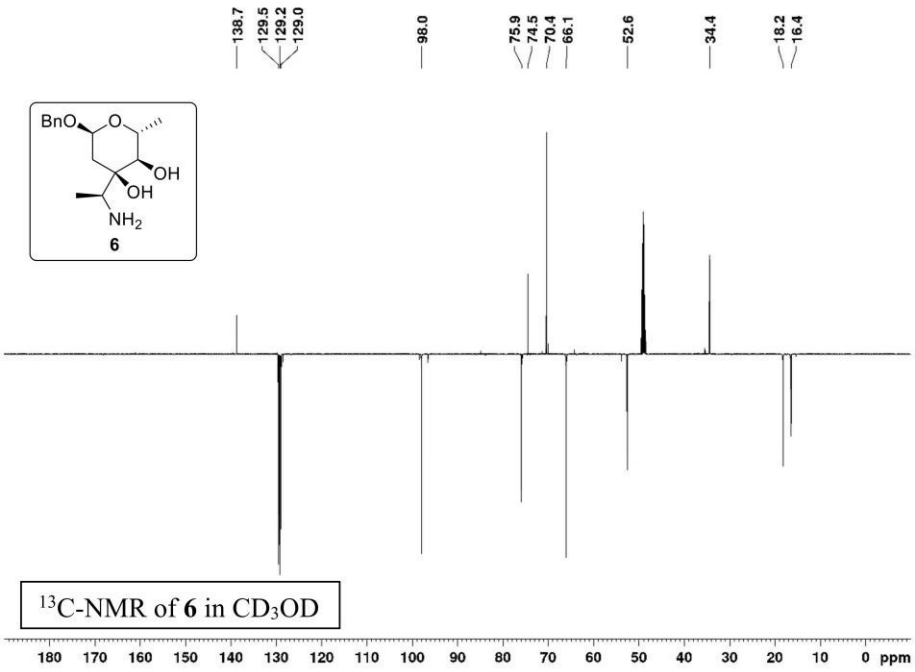

166.1
136.7
133.7
130.1
129.5
128.8
128.7
128.4
128.3
96.7
75.7
74.4
69.9
63.5
62.1
34.0
17.5
15.0
BnO
O
O
Ph
OH
N3
30
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


7.35
7.33
7.32
7.32
7.31
7.31
7.30
7.29
7.28
4.97
4.97
4.72
4.70
4.47
4.45
4.09
3.88
3.87
3.86
3.85
3.85
3.84
3.84
3.82
3.33
3.31
3.07
3.06
3.04
3.03
1.97
1.97
1.94
1.59
1.59
1.56
1.56
1.32
1.30
1.13
1.12
BnO
OH
OH
NH2
6
1H-NMR of 6 in CDCl3
8 7 6 5 4 3 2 1 0 ppm
5.06
1.00
1.02
1.06
4.23
1.05
1.00
1.06
1.00
1.02
3.11
3.02

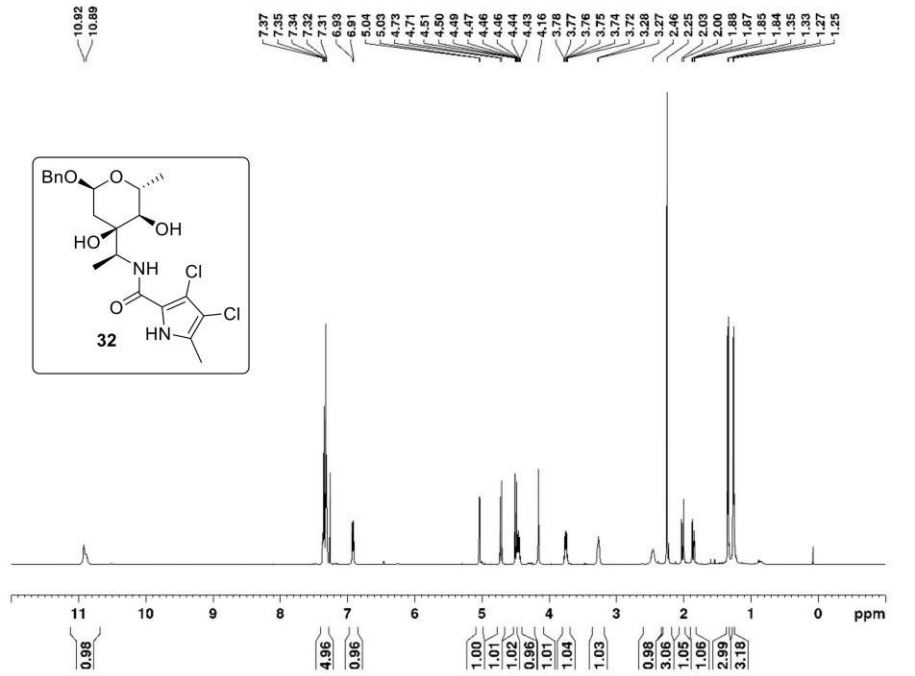

137.0
128.6
128.2
128.1
96.4
77.6
71.7
69.3
65.0
54.3
36.0
18.3
17.8
BnO
O
OH
OH
NH2
6
13C-NMR of 6 in CDCl3
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

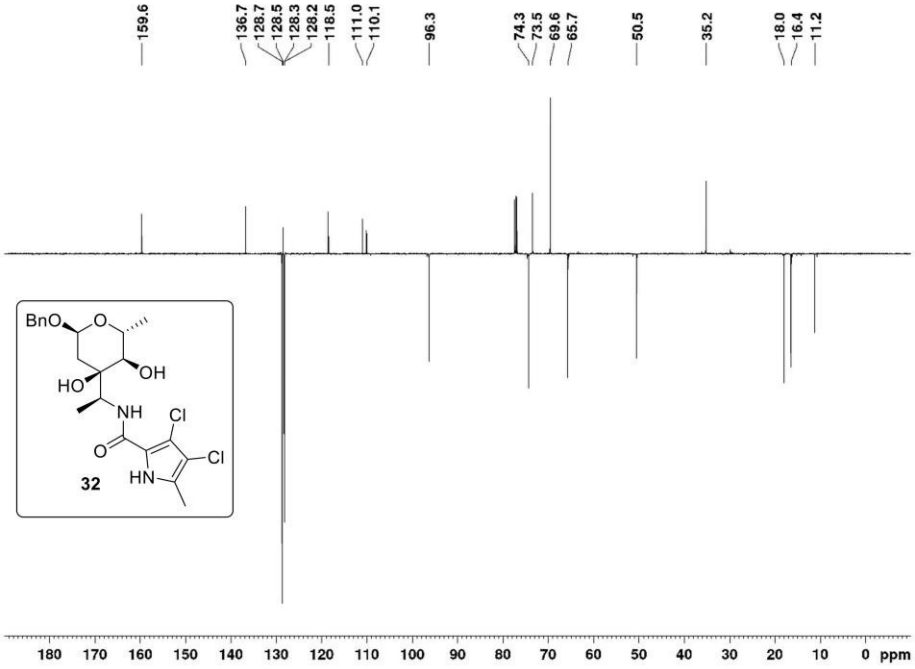

7.36
7.36
7.35
7.34
7.32
7.31
7.31
7.30
7.30
7.29
5.03
5.03
4.73
4.70
4.52
4.50
3.91
3.89
3.89
3.88
3.88
3.87
3.86
3.85
3.24
3.22
3.20
3.18
3.17
3.16
1.94
1.91
1.72
1.71
1.69
1.68
1.28
1.26
1.08
1.07
1H-NMR of 6 in CD3OD
BnO
O
OH
OH
NH2
6
8 7 6 5 4 3 2 1 0 ppm
5.00
0.97
0.98
1.00
0.98
0.95
1.03
1.04
0.99
3.17
2.96

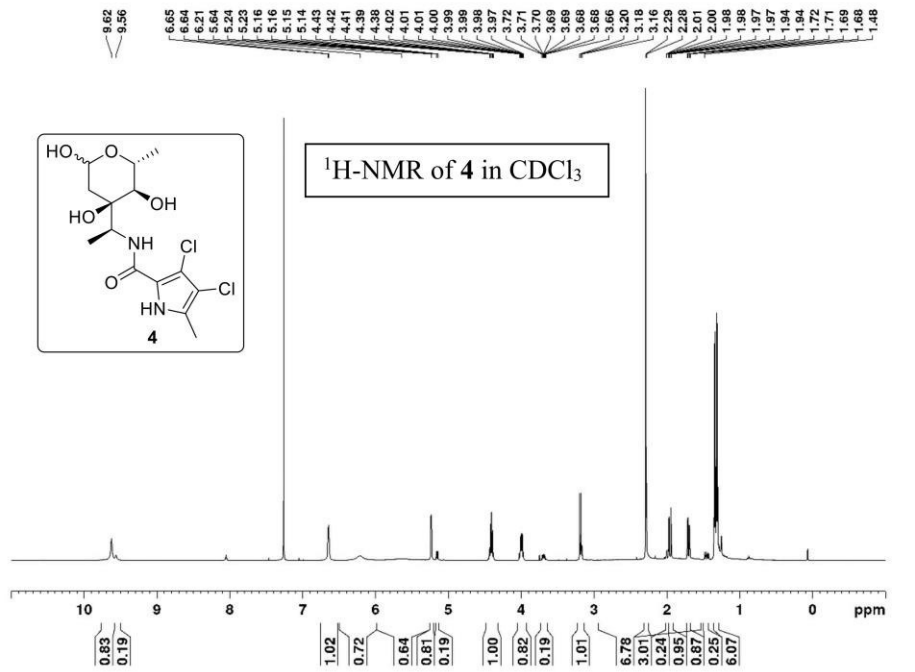
S96

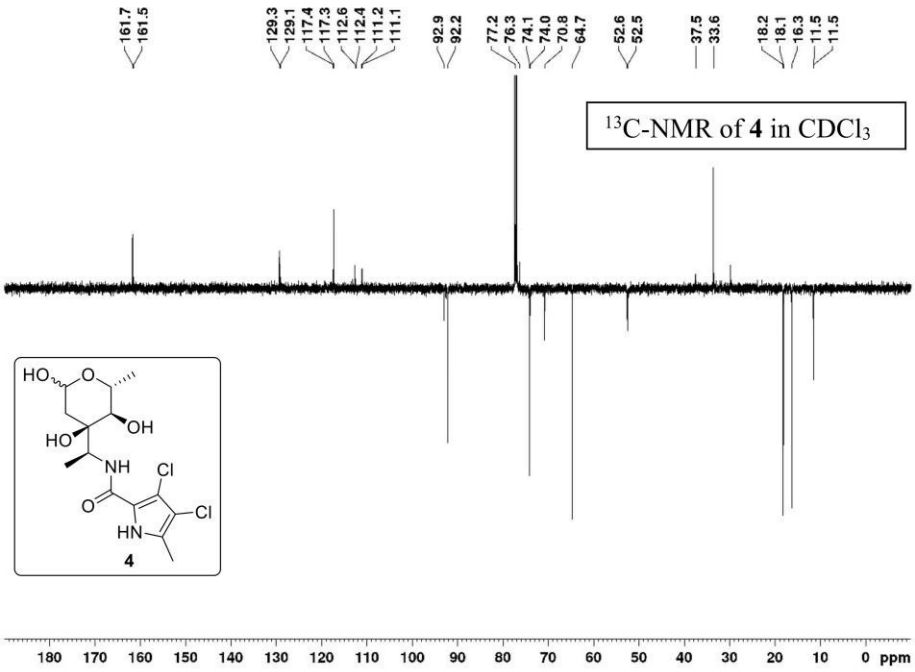


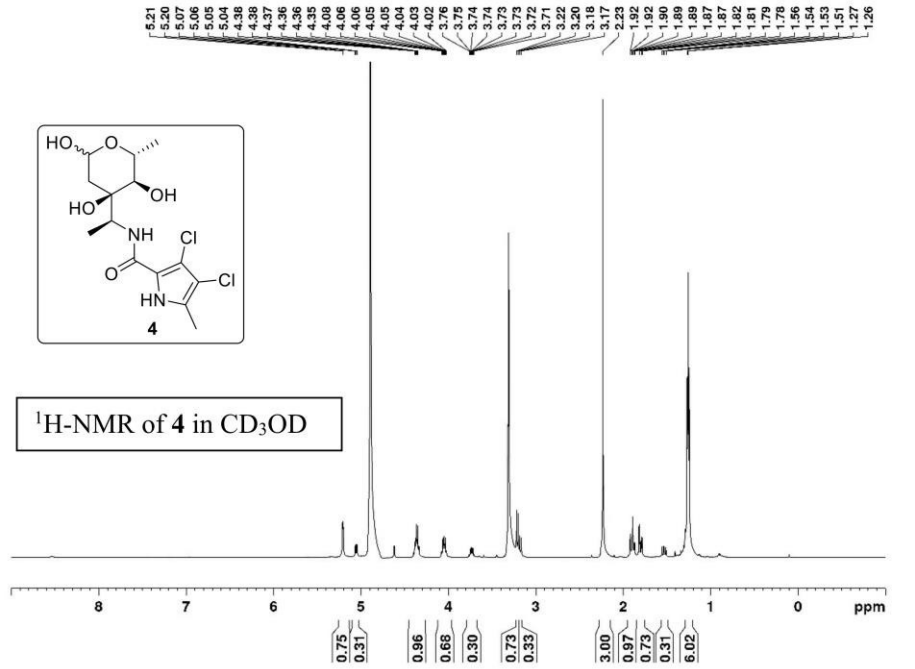
^{13}C-NMR of **6** in CD_3OD



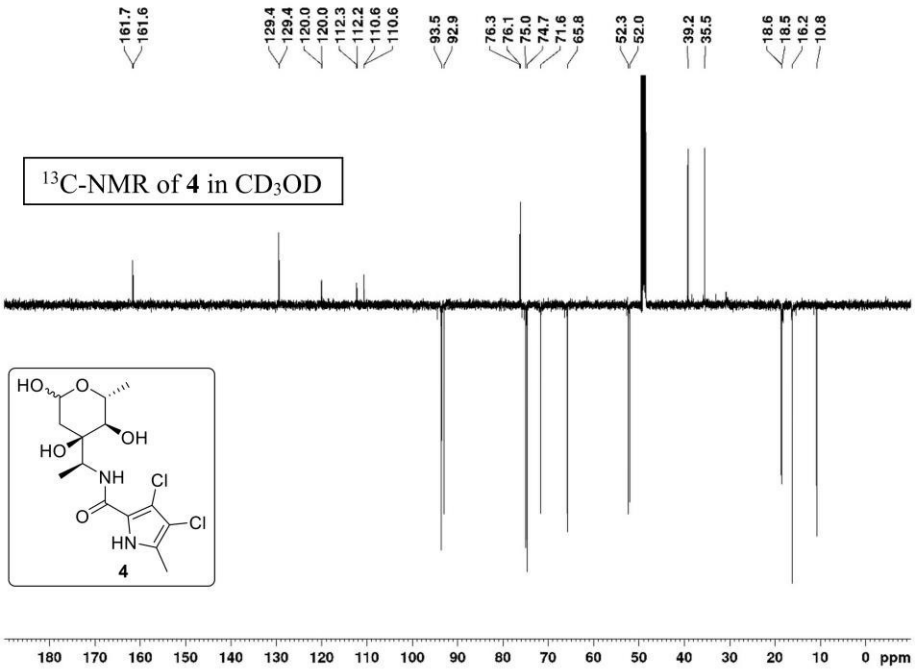

159.6
136.7
128.7
128.5
128.3
128.2
118.5
111.0
110.1
96.3
74.3
73.5
69.6
65.7
50.5
35.2
18.0
16.4
11.2
BnO
HO
OH
NH
Cl
Cl
O
HN
32
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

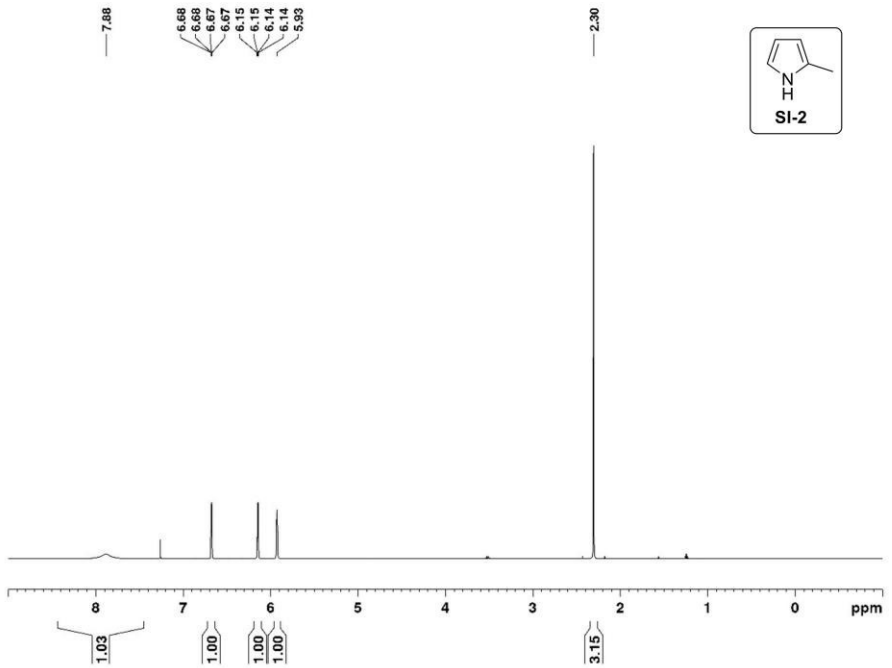

9.62
9.56
6.65
6.64
6.21
5.64
5.24
5.23
5.16
5.16
5.15
5.14
4.43
4.42
4.41
4.39
4.38
4.02
4.01
4.01
4.00
3.99
3.99
3.98
3.97
3.72
3.71
3.70
3.69
3.69
3.68
3.68
3.66
3.20
3.18
3.16
2.29
2.28
2.01
2.00
1.98
1.98
1.97
1.97
1.94
1.94
1.72
1.71
1.69
1.68
1.48
HO
OH
HO
NH
Cl
Cl
O
HN
4
^{1}H-NMR of 4 in CDCl$_3$
10 9 8 7 6 5 4 3 2 1 0 ppm
0.83
0.19
1.02
0.72
0.64
0.81
0.19
1.00
0.82
0.19
1.01
6.78
3.01
0.24
0.95
0.87
0.25
6.07

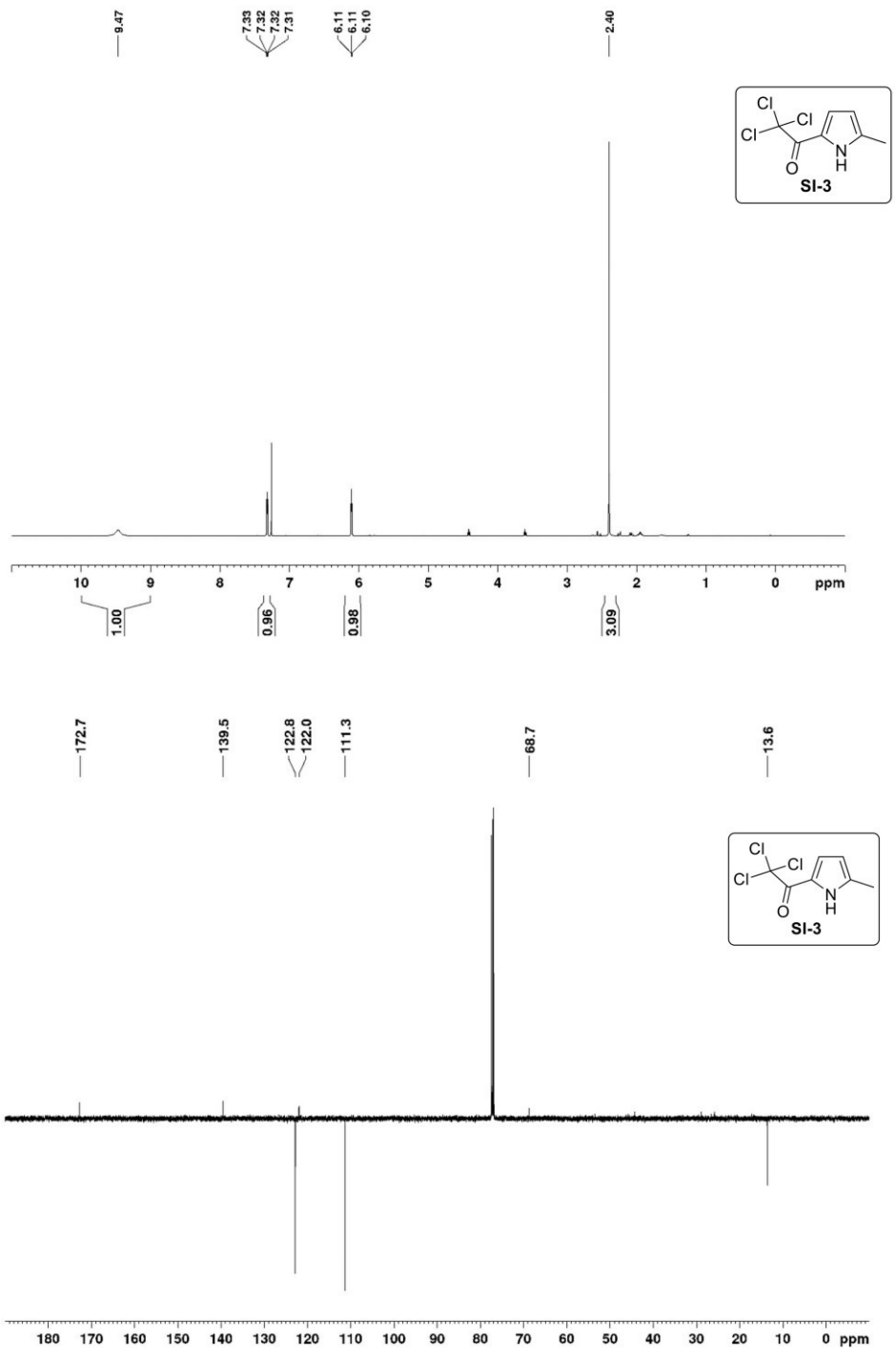

161.7
161.5
129.3
129.1
117.4
117.3
112.6
112.4
111.2
111.1
92.9
92.2
77.2
76.3
74.1
74.0
70.8
64.7
52.6
52.5
37.5
33.6
18.2
18.1
16.3
11.5
11.5
^{13}C-NMR of **4** in $CDCl_3$
HO
O
OH
HO
NH
Cl
Cl
O
HN
4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


5.21
5.20
5.07
5.06
5.05
5.04
4.38
4.37
4.36
4.36
4.35
4.08
4.06
4.06
4.05
4.05
4.04
4.03
4.02
3.76
3.75
3.74
3.74
3.73
3.73
3.72
3.71
3.22
3.20
3.18
3.17
2.23
1.92
1.92
1.90
1.89
1.89
1.87
1.87
1.82
1.81
1.79
1.78
1.56
1.54
1.53
1.51
1.27
1.26
HO
O
OH
HO
NH
Cl
Cl
O
HN
4
^{1}H-NMR of **4** in CD_3OD
8 7 6 5 4 3 2 1 0 ppm
0.75
0.31
0.96
0.68
0.30
0.73
0.33
3.00
0.97
0.73
0.31
6.02

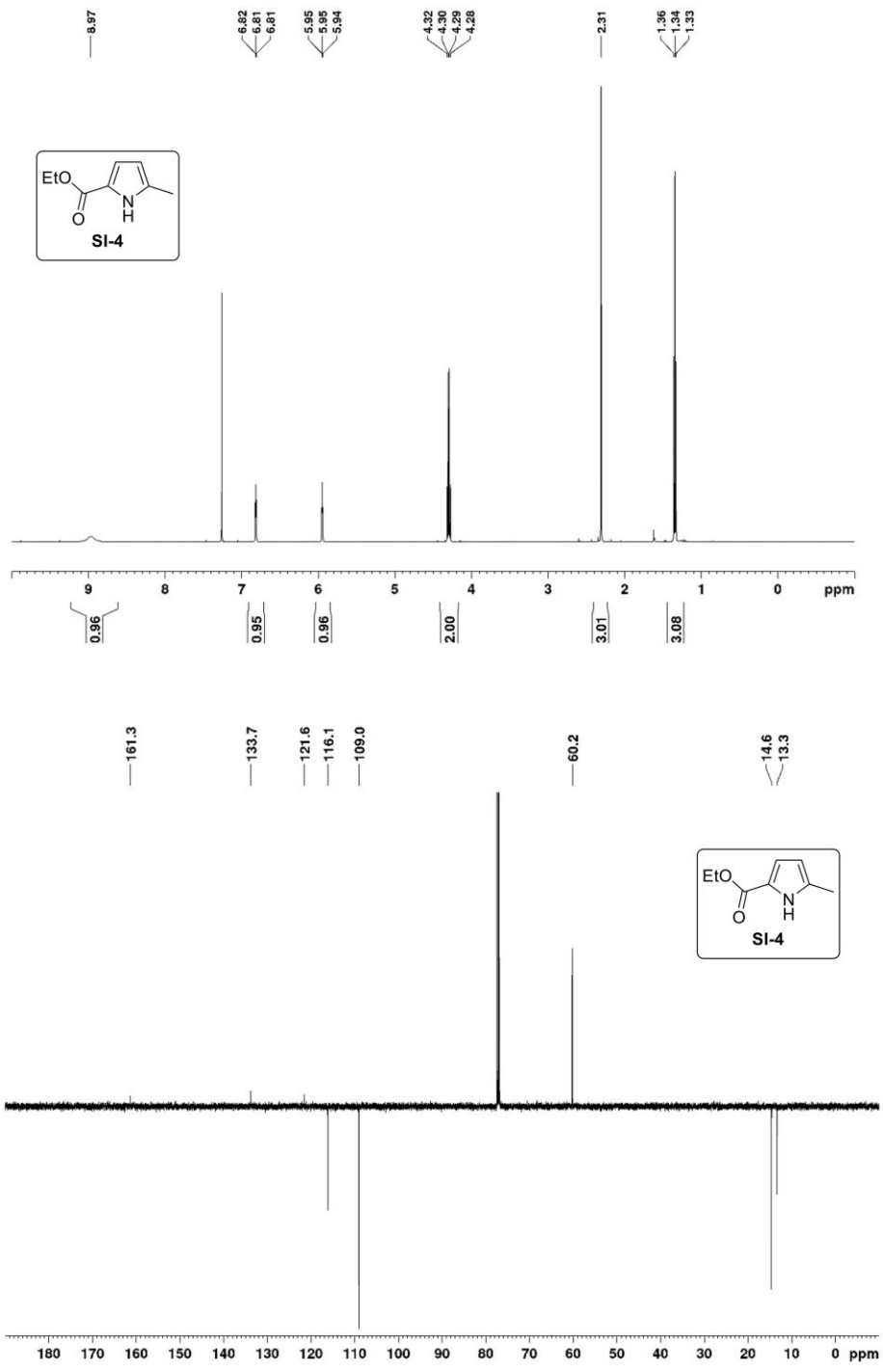
S99


161.7
161.6
129.4
129.4
120.0
120.0
112.3
112.2
110.6
110.6
93.5
92.9
76.3
76.1
75.0
74.7
71.6
65.8
52.3
52.0
39.2
35.5
18.6
18.5
16.2
10.8
^{13}C-NMR of **4** in CD_3OD
HO
O
OH
HO
NH
Cl
O
Cl
HN
4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

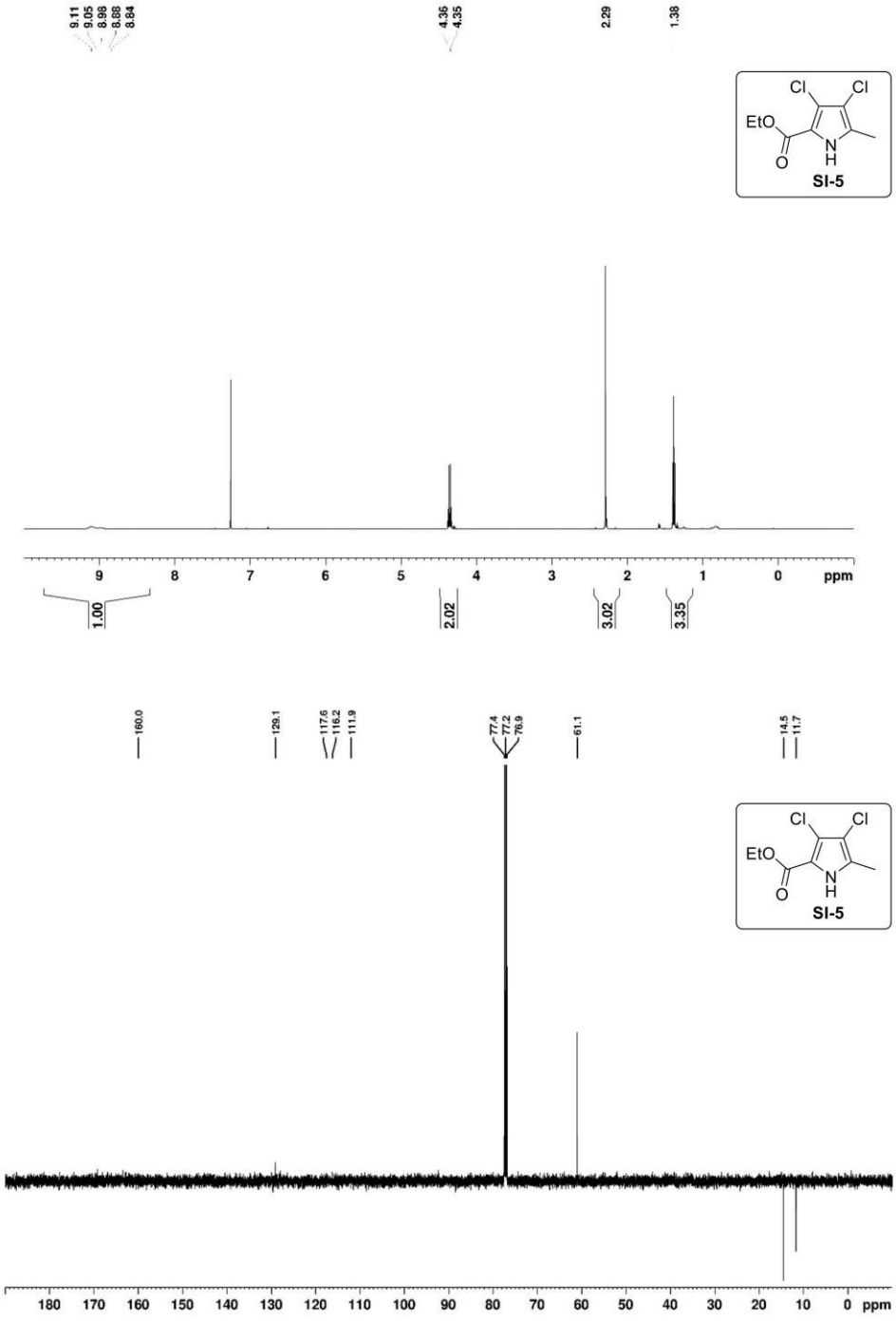

7.68
6.68
6.68
6.67
6.67
6.15
6.15
6.14
6.14
5.93
2.30
N
H
SI-2
8 7 6 5 4 3 2 1 0 ppm
1.03
1.00
1.00
1.00
3.15


8.47
7.33
7.32
7.32
7.31
6.11
6.11
6.10
2.40
Cl
Cl
Cl
O
N
H
SI-3
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.96
0.98
3.09
172.7
139.5
122.8
122.0
111.3
68.7
13.6
Cl
Cl
Cl
O
N
H
SI-3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

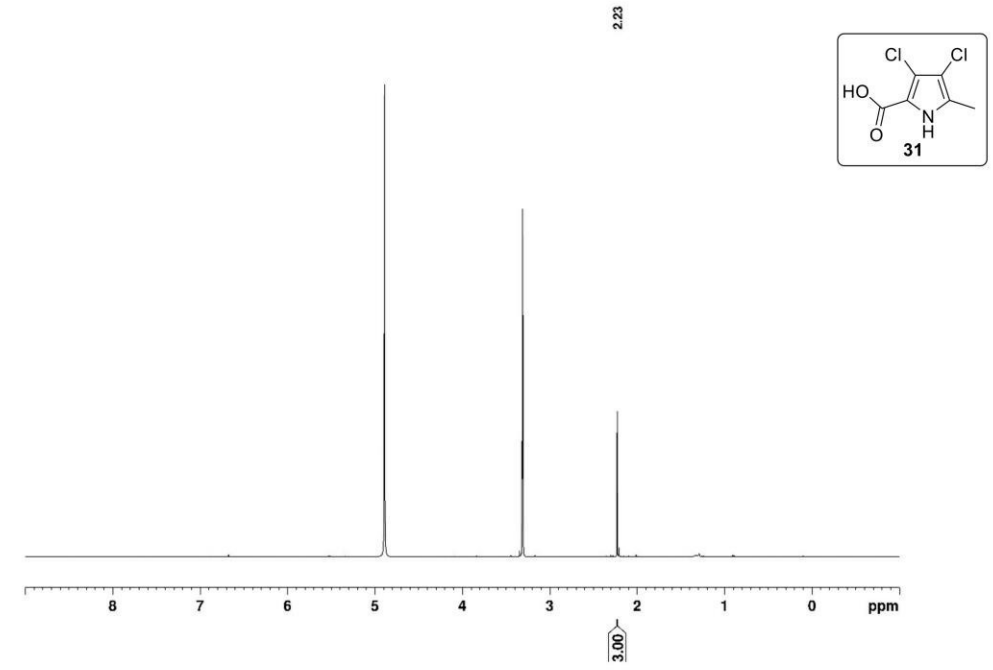
S101

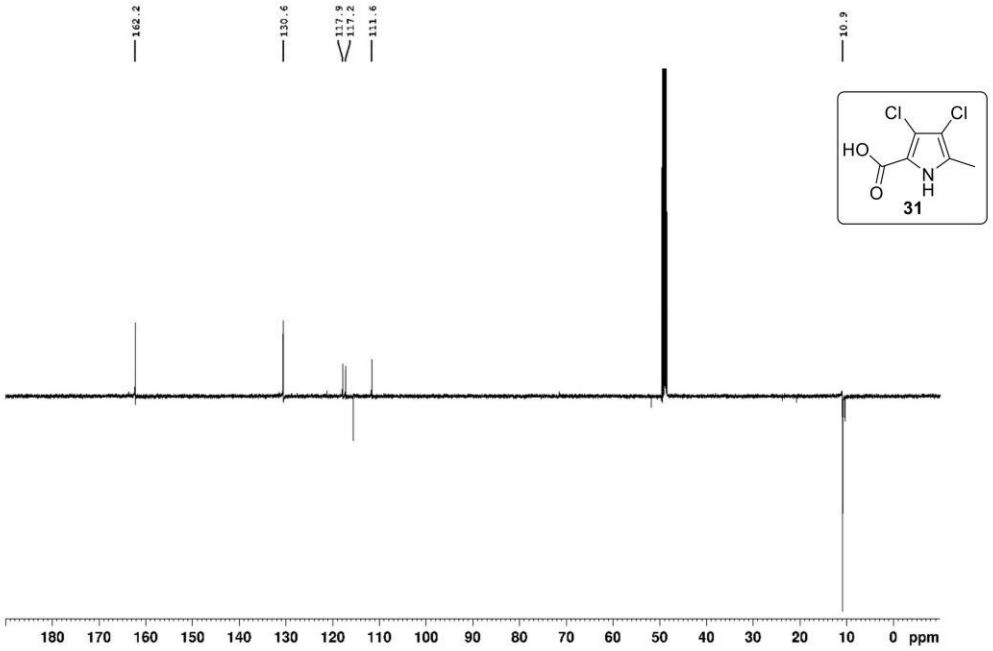

8.97
6.82
6.81
6.81
5.95
5.95
5.94
4.32
4.30
4.29
4.28
2.31
1.36
1.34
1.33
EtO
O
N
H
SI-4
9
8
7
6
5
4
3
2
1
0
ppm
0.96
0.95
0.96
2.00
3.01
3.08
161.3
133.7
121.6
116.1
109.0
60.2
14.6
13.3
EtO
O
N
H
SI-4
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

S102

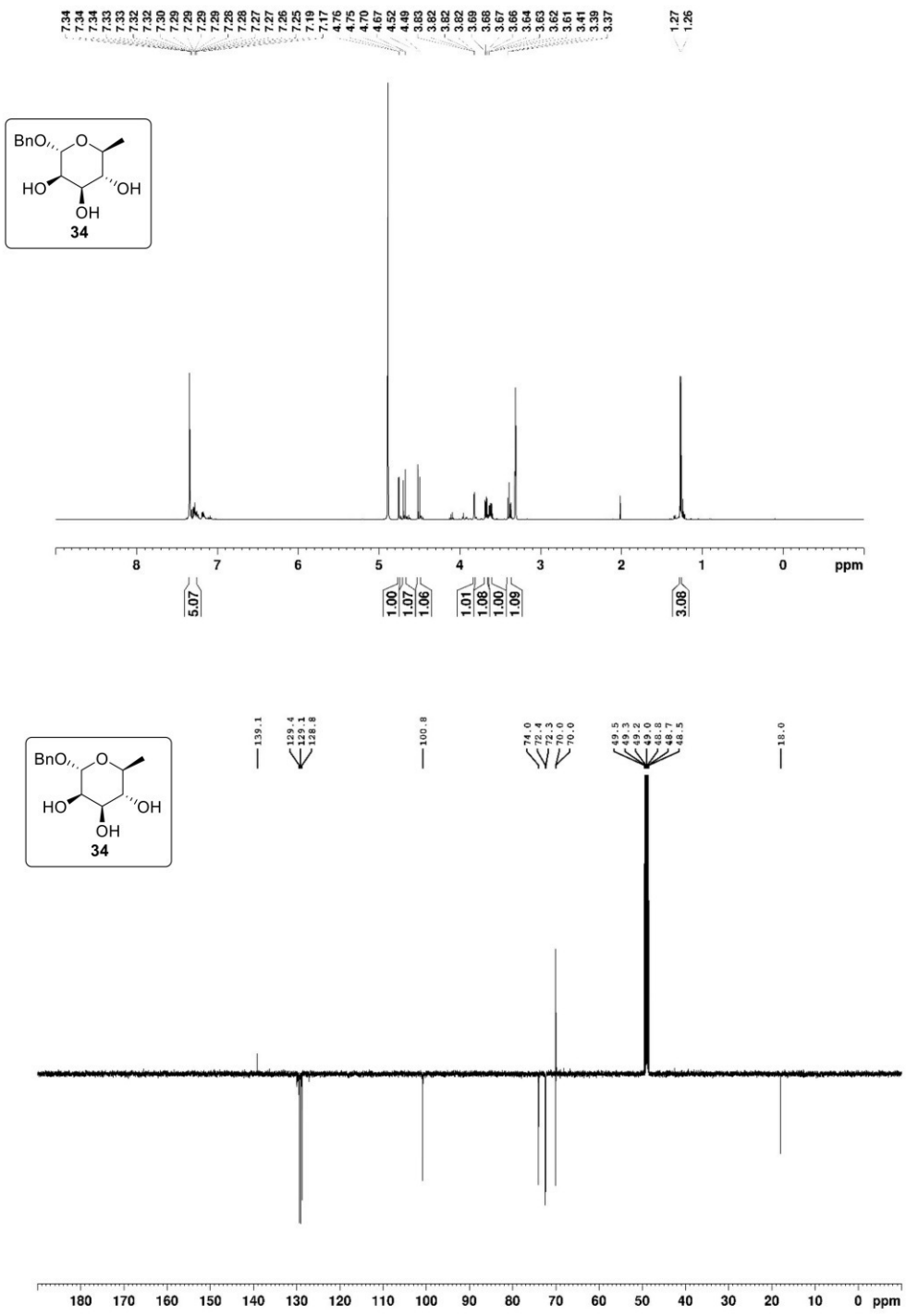

9.11
9.05
8.98
8.88
8.84
4.36
4.35
2.29
1.38
Cl
Cl
EtO
O
N
H
SI-5
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.02
3.02
3.35
160.0
129.1
117.6
116.2
111.9
77.4
77.2
76.9
61.1
14.5
11.7
Cl
Cl
EtO
O
N
H
SI-5
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

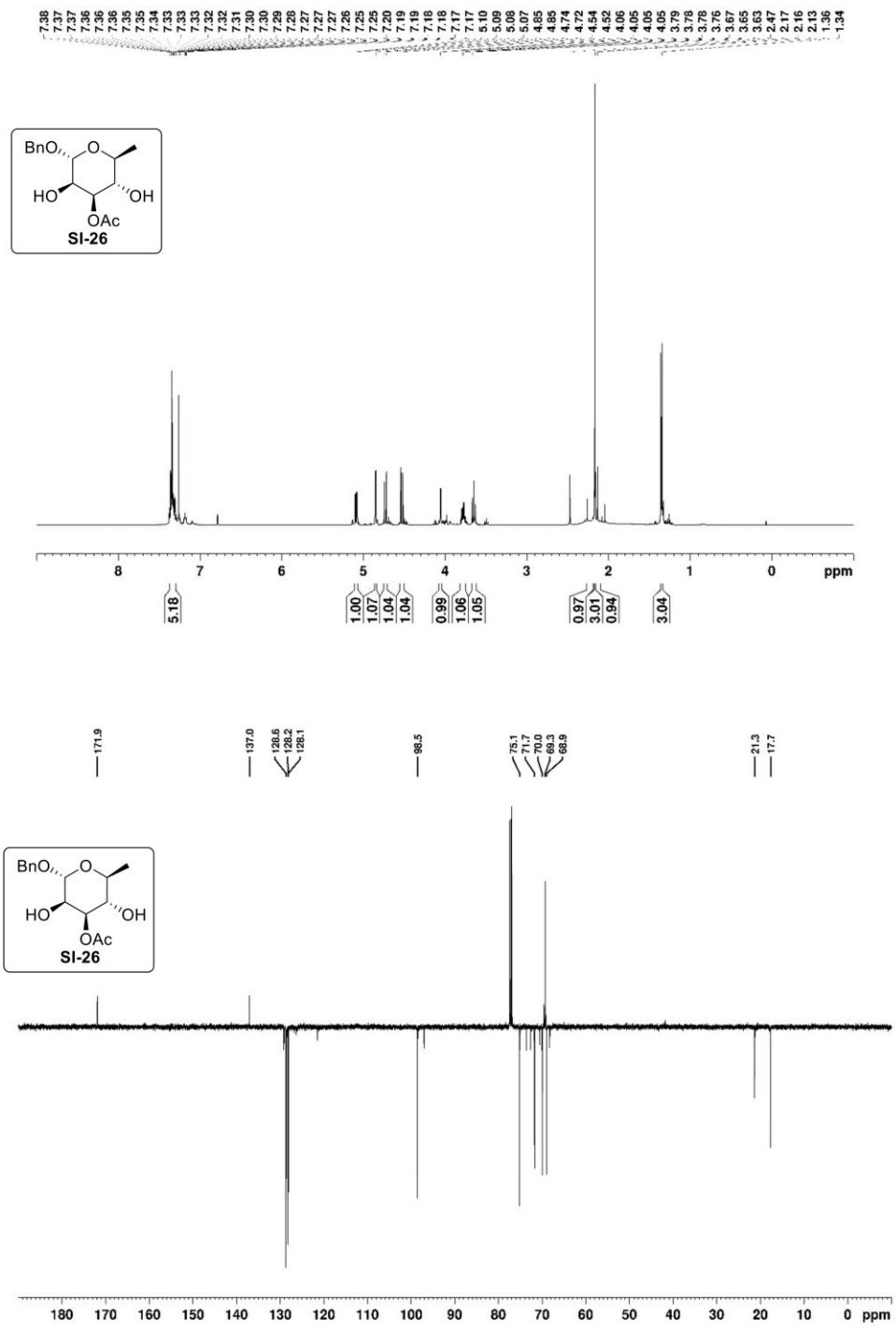
S103

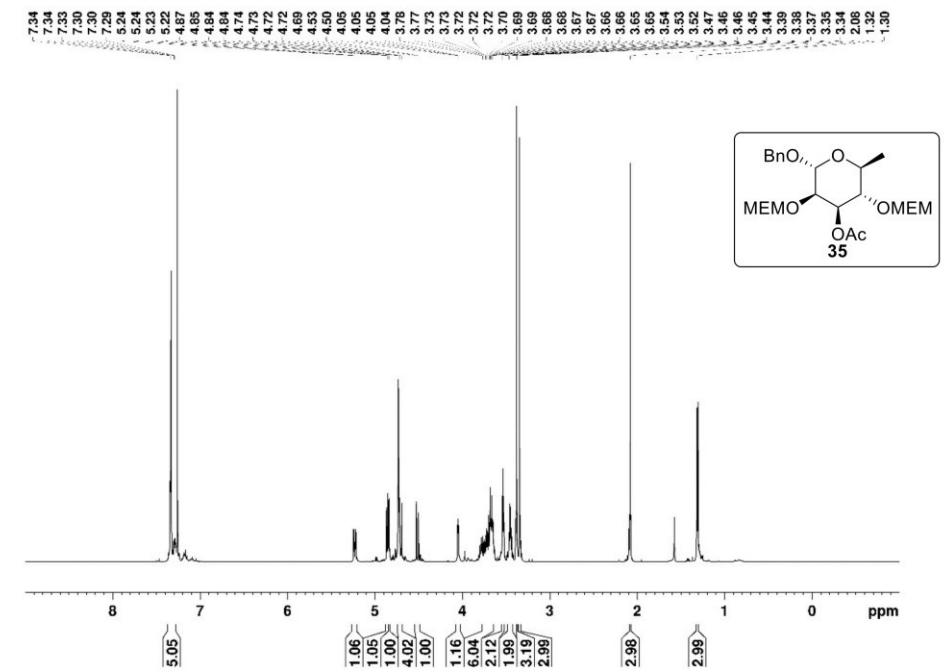

2.23
Cl
Cl
HO
O
N
H
31
8
7
6
5
4
3
2
1
0
ppm
3.00

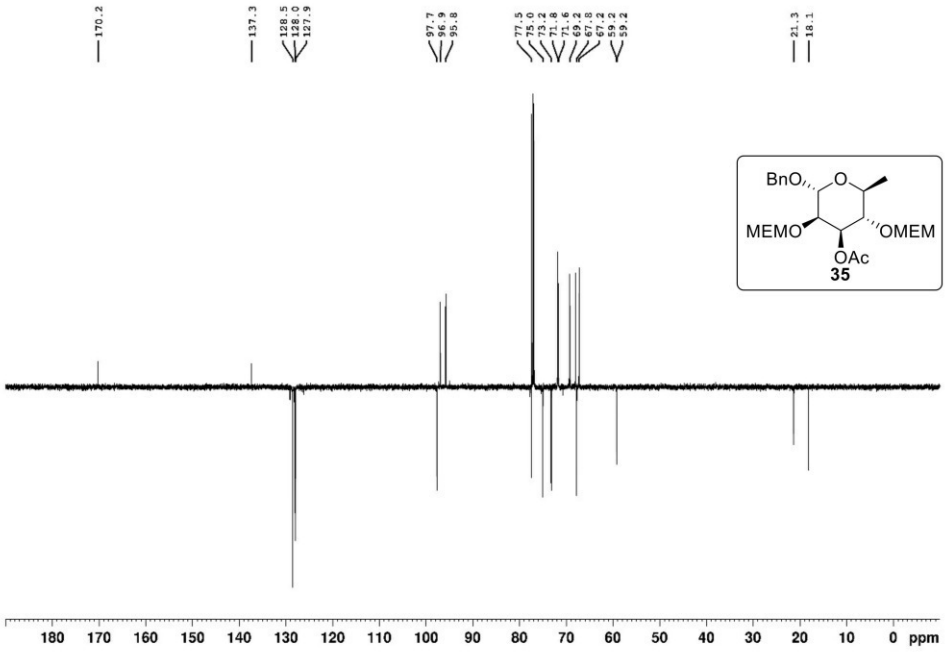

162.2
130.6
117.9
117.2
111.6
10.9
Cl
Cl
HO
O
N
H
31
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

S104

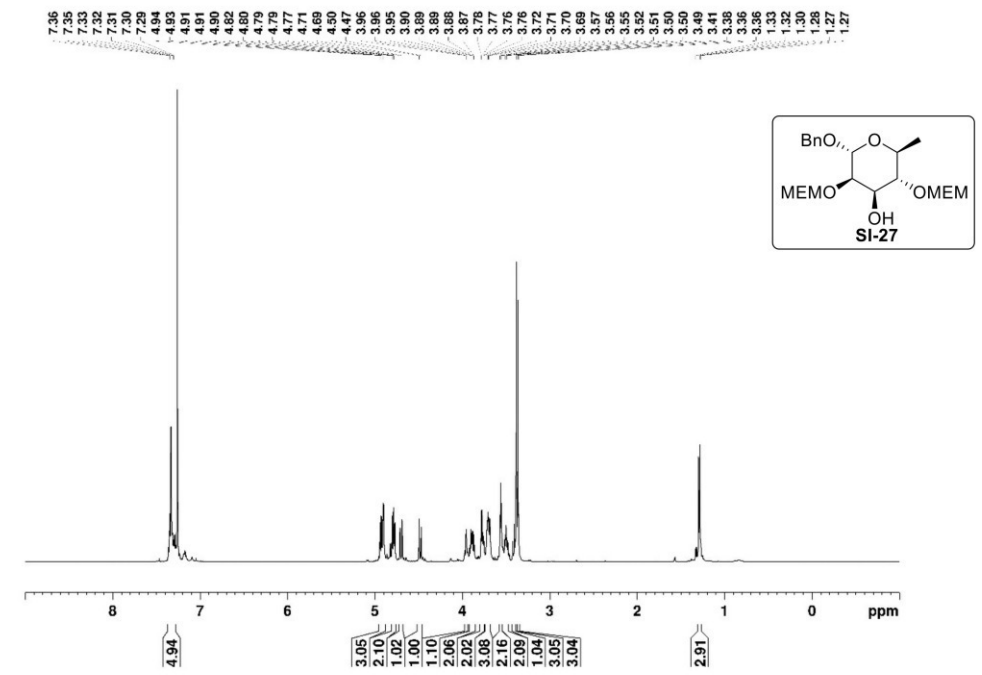

BnO
HO
OH
OH
34
ppm
5.07
1.00
1.07
1.06
1.01
1.08
1.00
1.09
3.08
BnO
HO
OH
OH
34
ppm

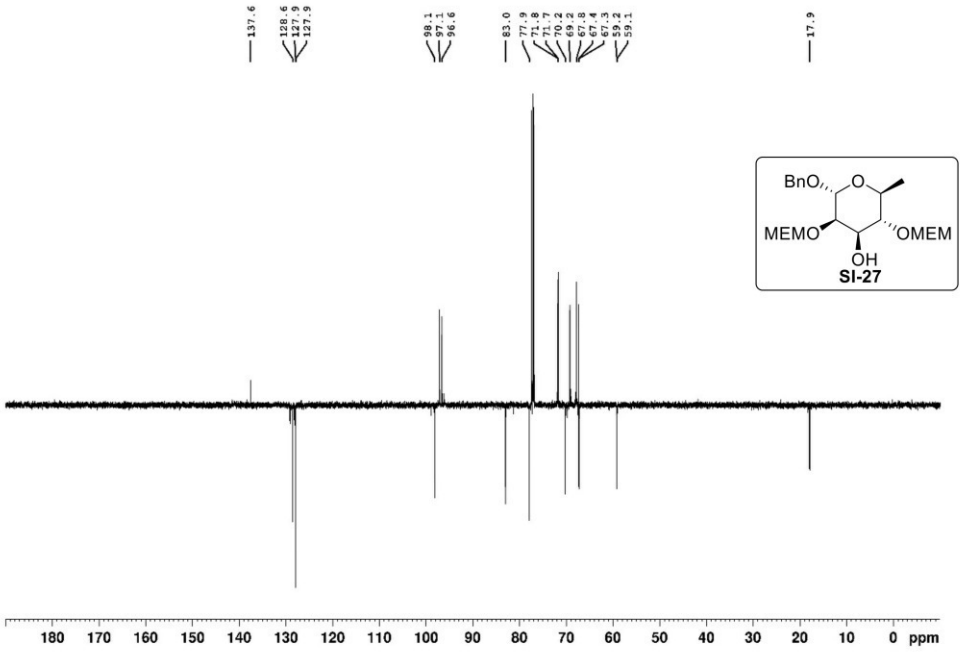

BnO
O
HO
OH
OAc
SI-26
ppm
5.18
1.00
1.07
1.04
1.04
0.99
1.06
1.05
0.97
3.01
0.94
3.04
171.9
137.0
128.6
128.2
128.1
98.5
75.1
71.7
70.0
69.3
68.9
21.3
17.7

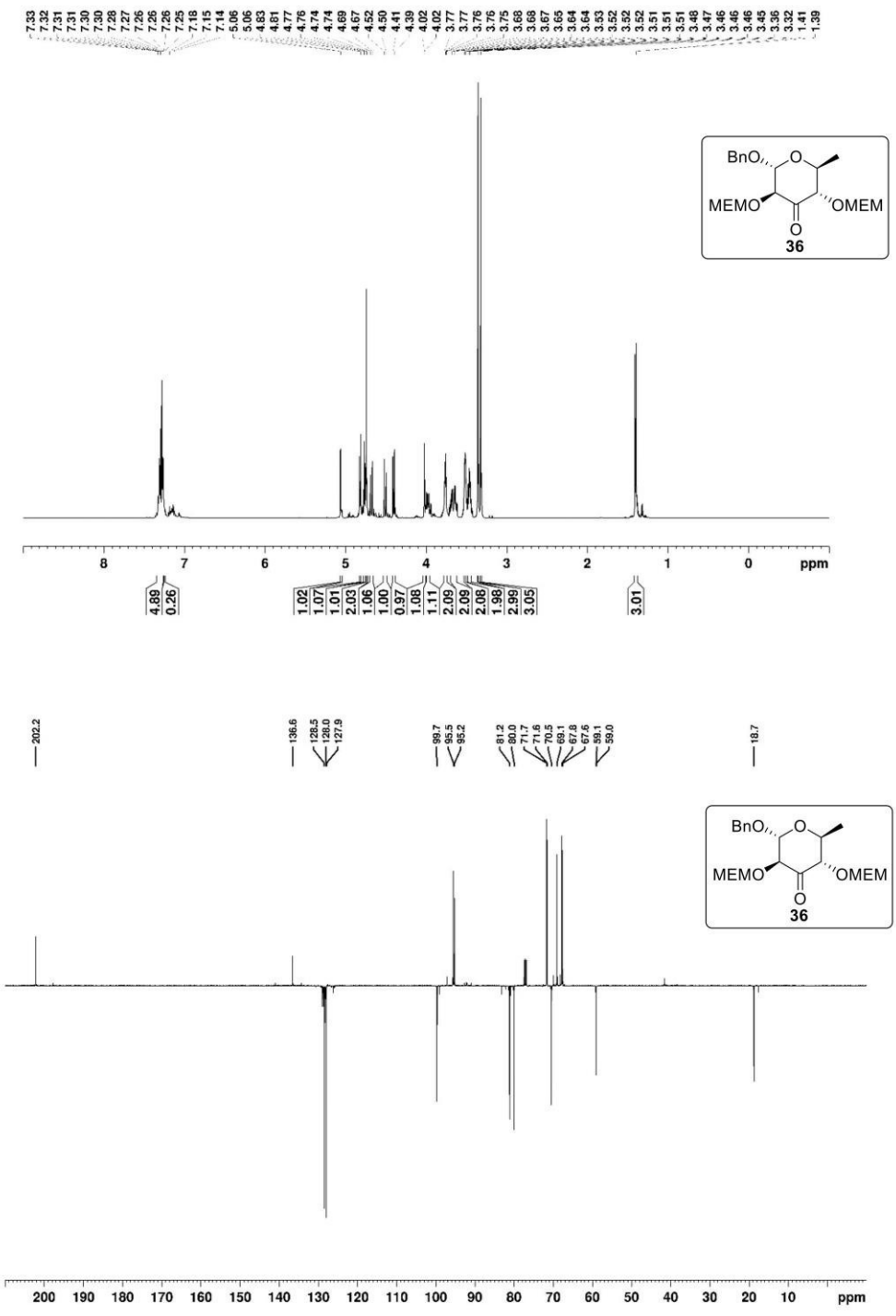




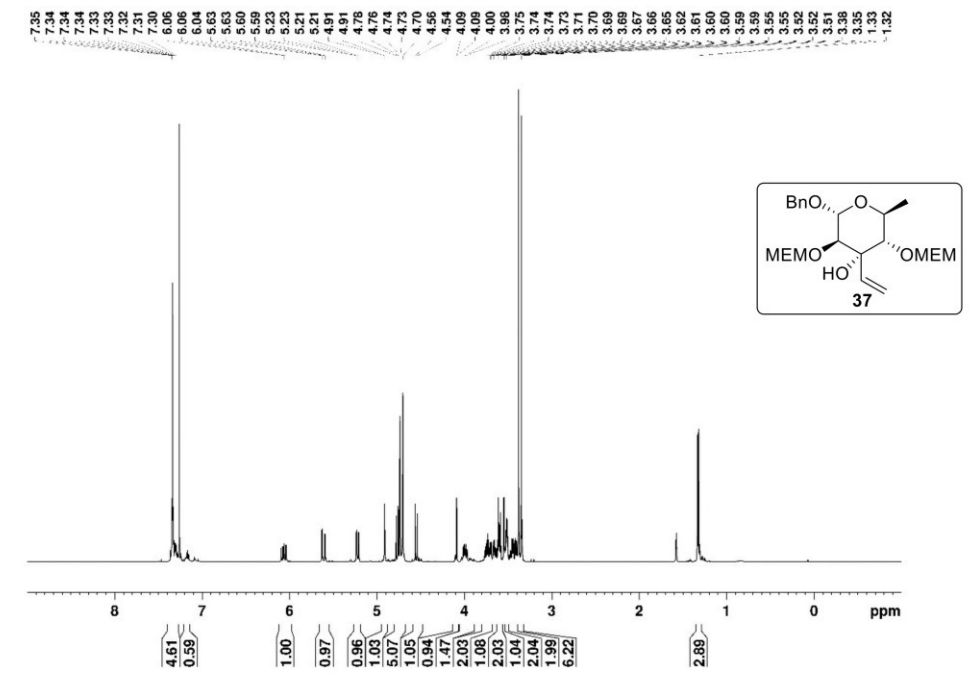
S107

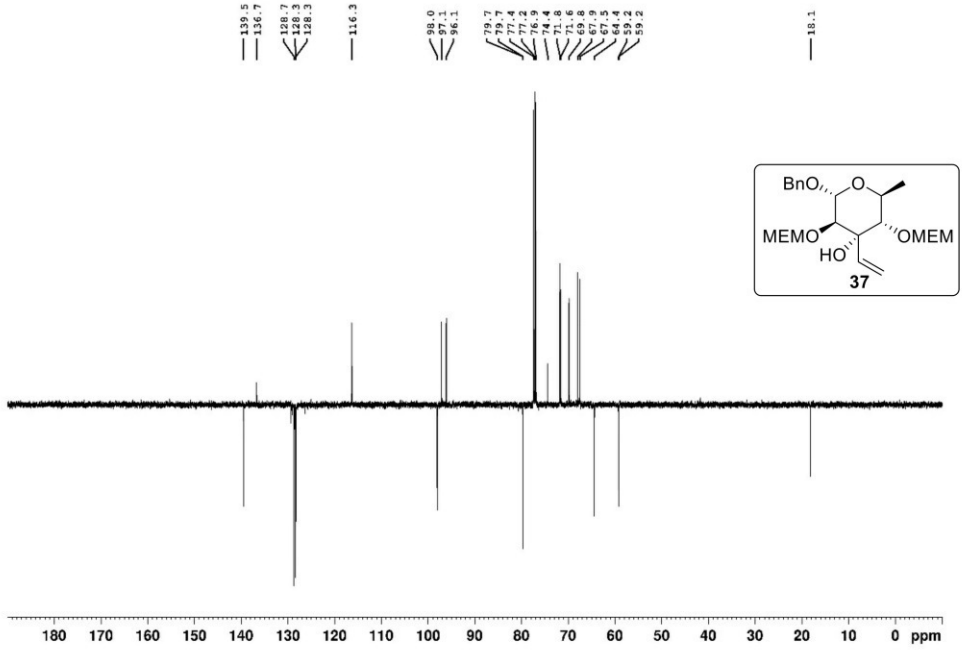

BnO
O
MEMO
OMEM
OH
SI-27
ppm


BnO
O
MEMO
OMEM
OH
SI-27
ppm

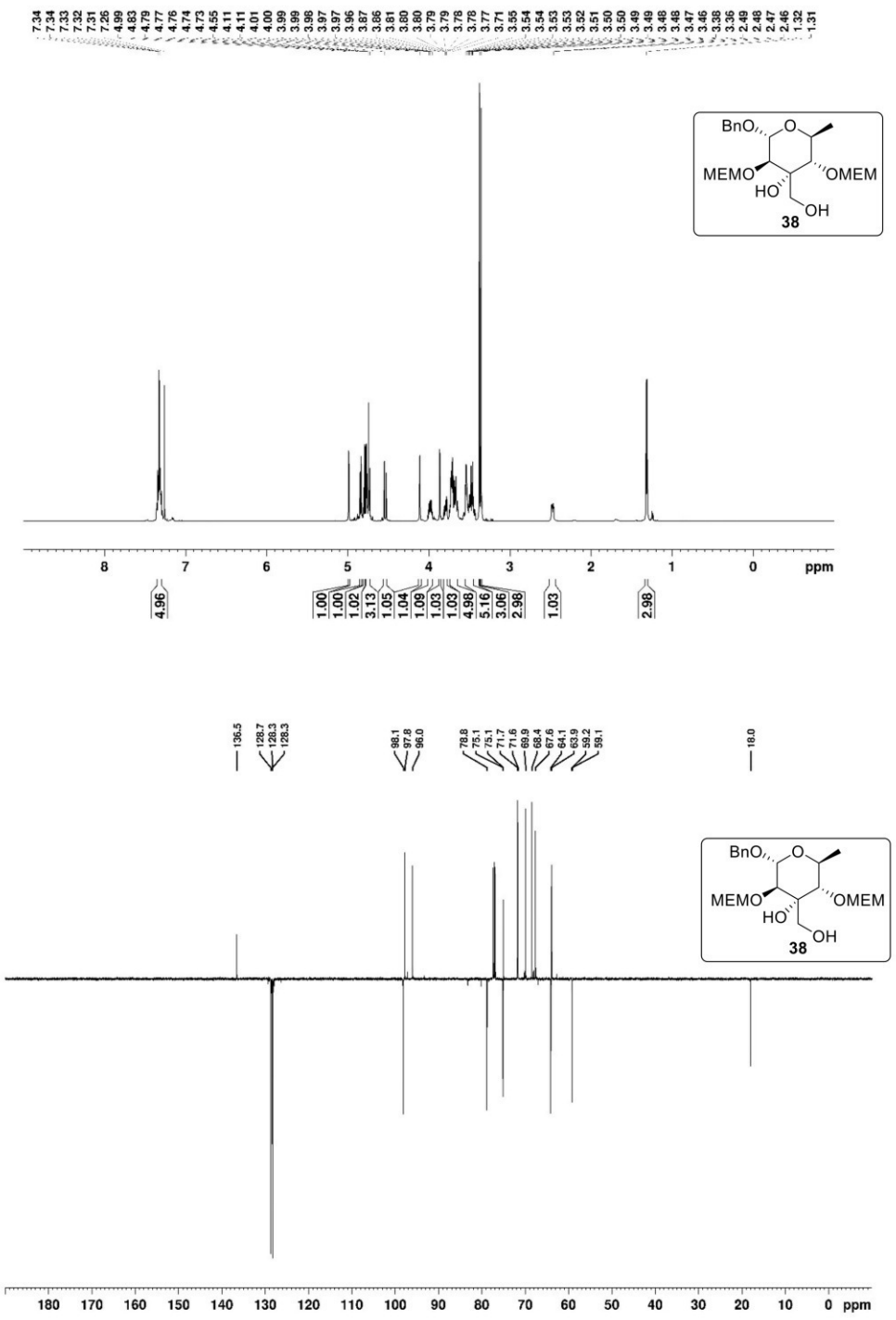

BnO
MEMO
OMEM
O
36
ppm
202.2
136.6
128.5
128.0
127.9
99.7
95.5
95.2
81.2
80.0
71.7
71.6
70.5
69.1
67.8
67.6
59.1
59.0
18.7

S109

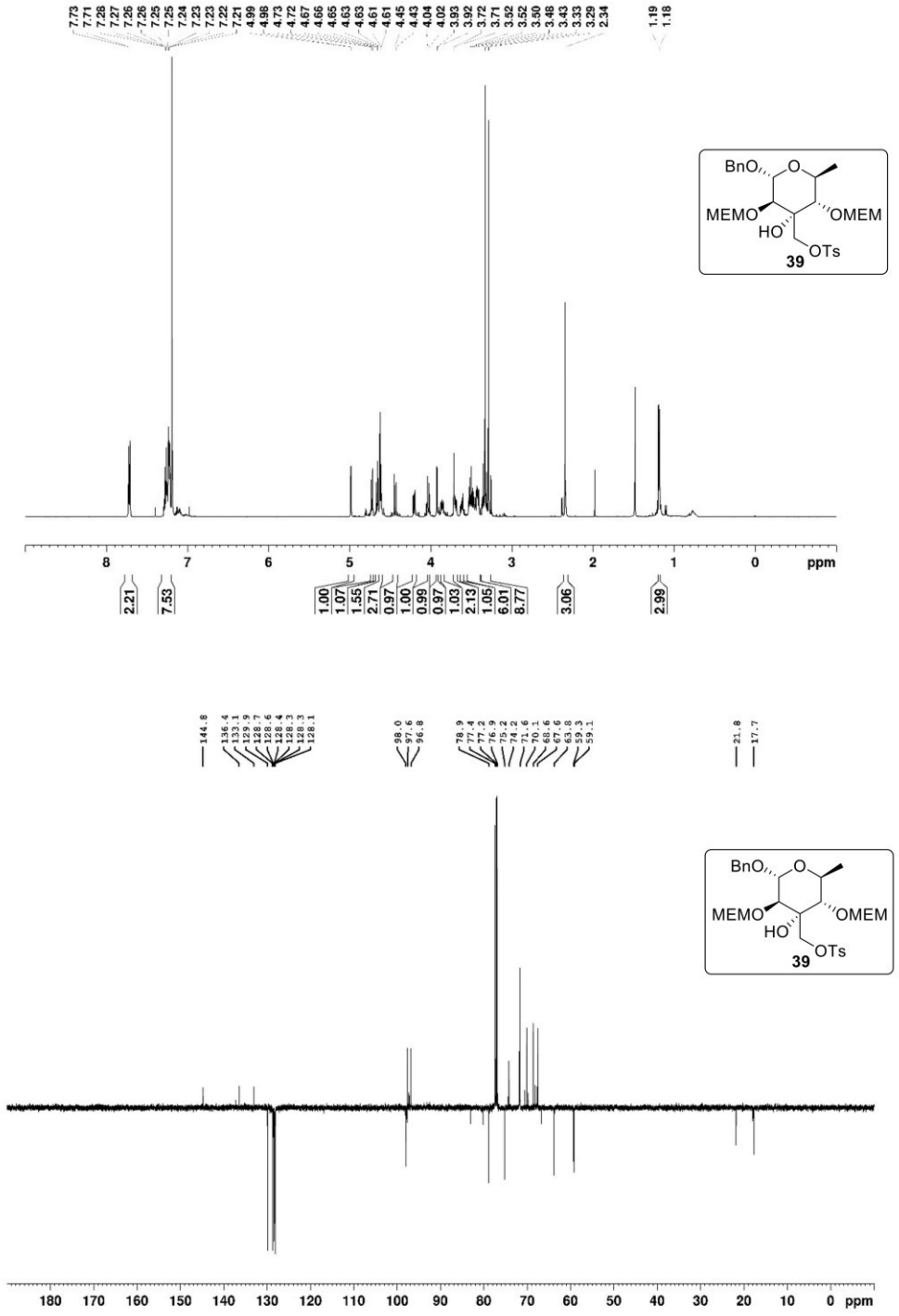


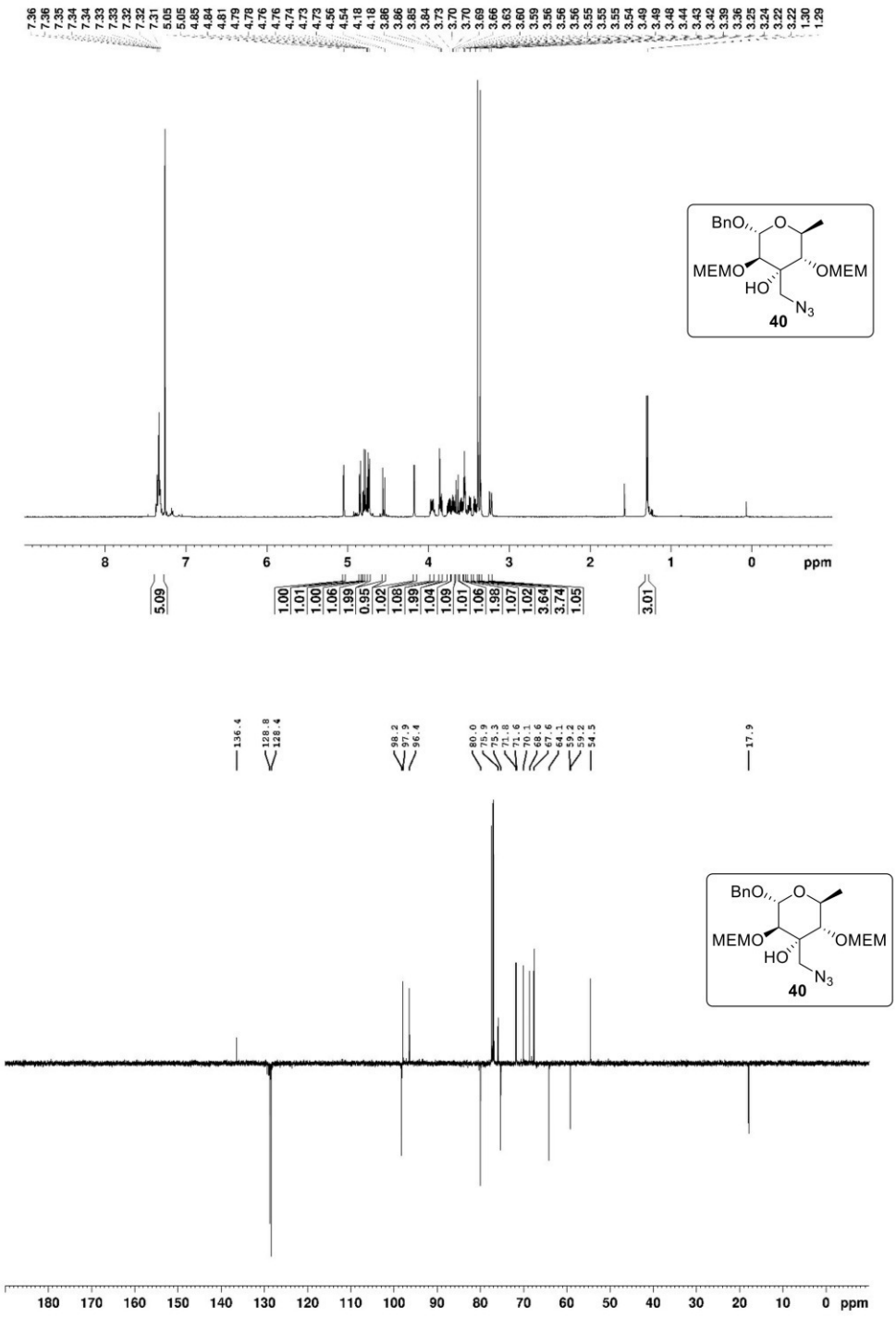


S110

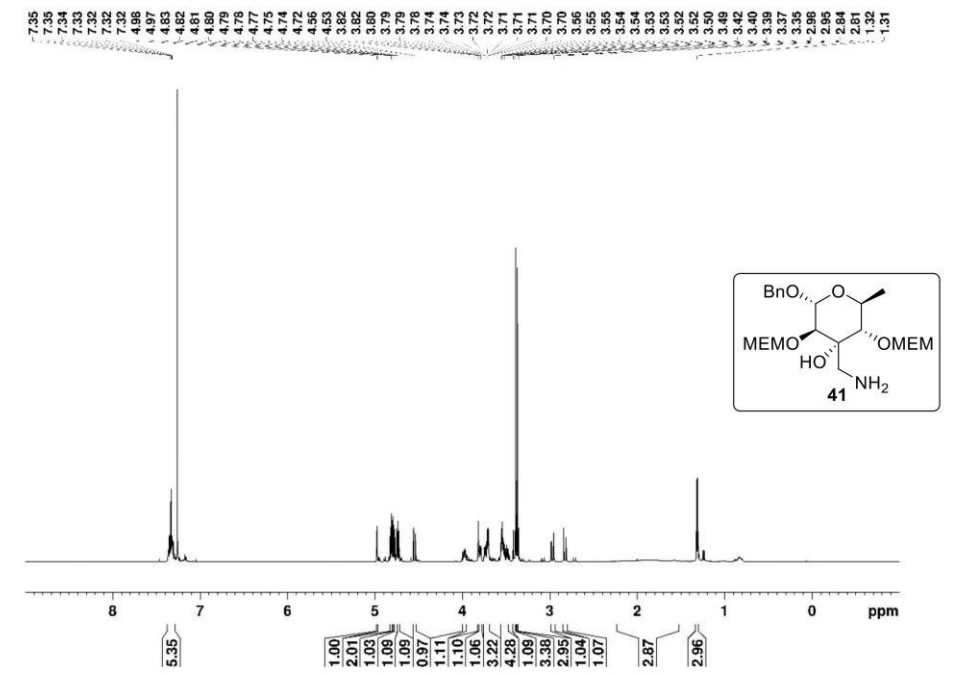

BnO
O
MEMO
OMEM
HO
OH
38
ppm
4.96
1.00
1.00
1.02
3.13
1.05
1.04
1.09
1.03
1.03
4.98
5.16
3.06
2.98
1.03
2.98
136.5
128.7
128.3
128.3
98.1
97.8
96.0
78.8
75.1
75.1
71.7
71.6
69.9
68.4
67.6
64.1
63.9
59.2
59.1
18.0

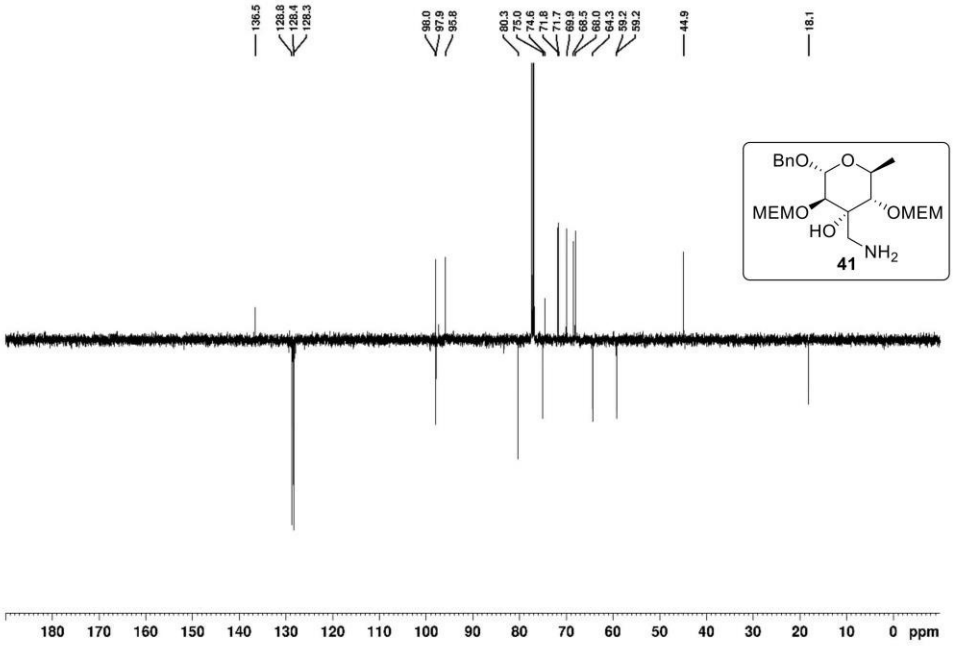
S111

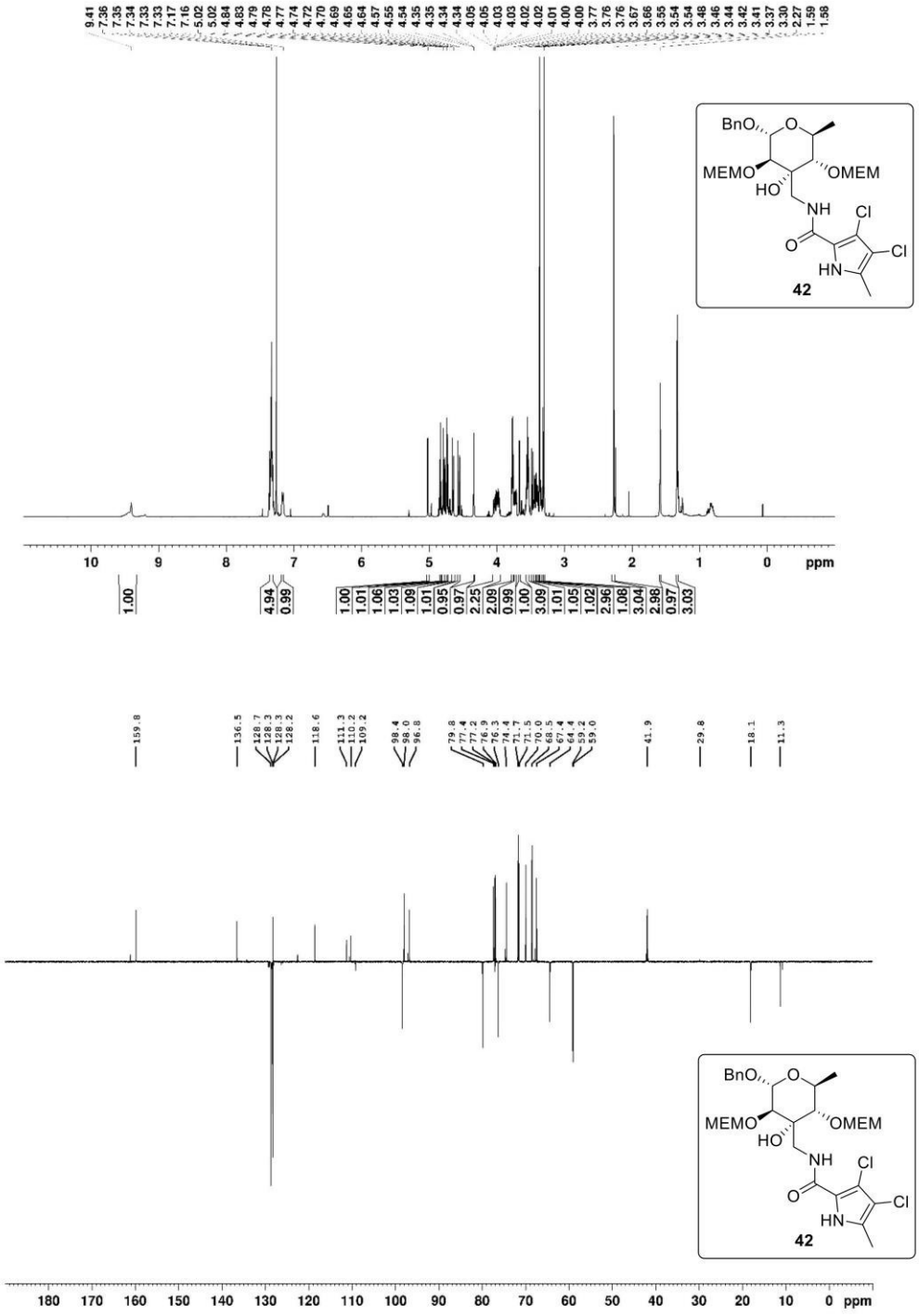

BnO
MEMO
HO
OMEM
OTs
39
ppm

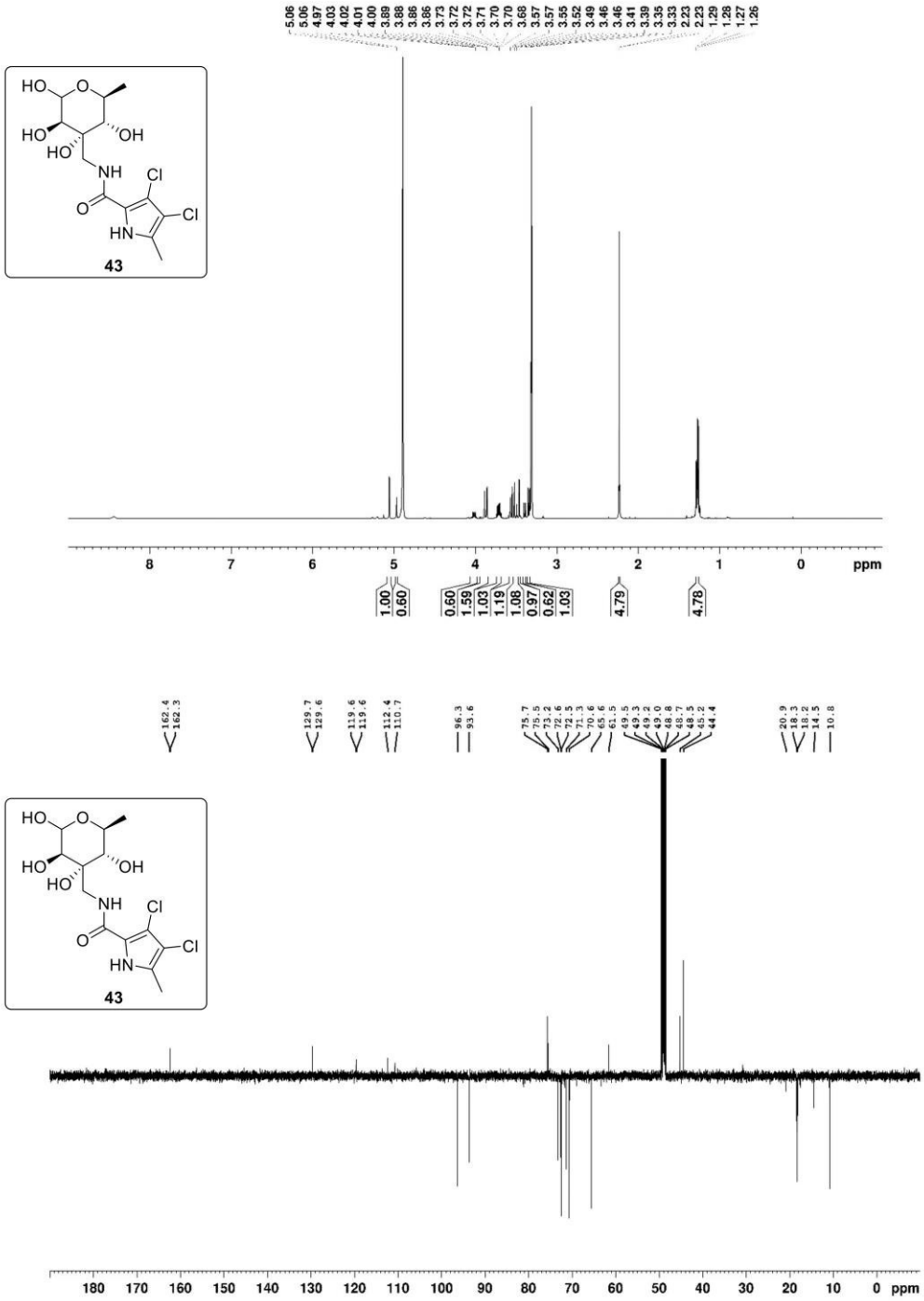


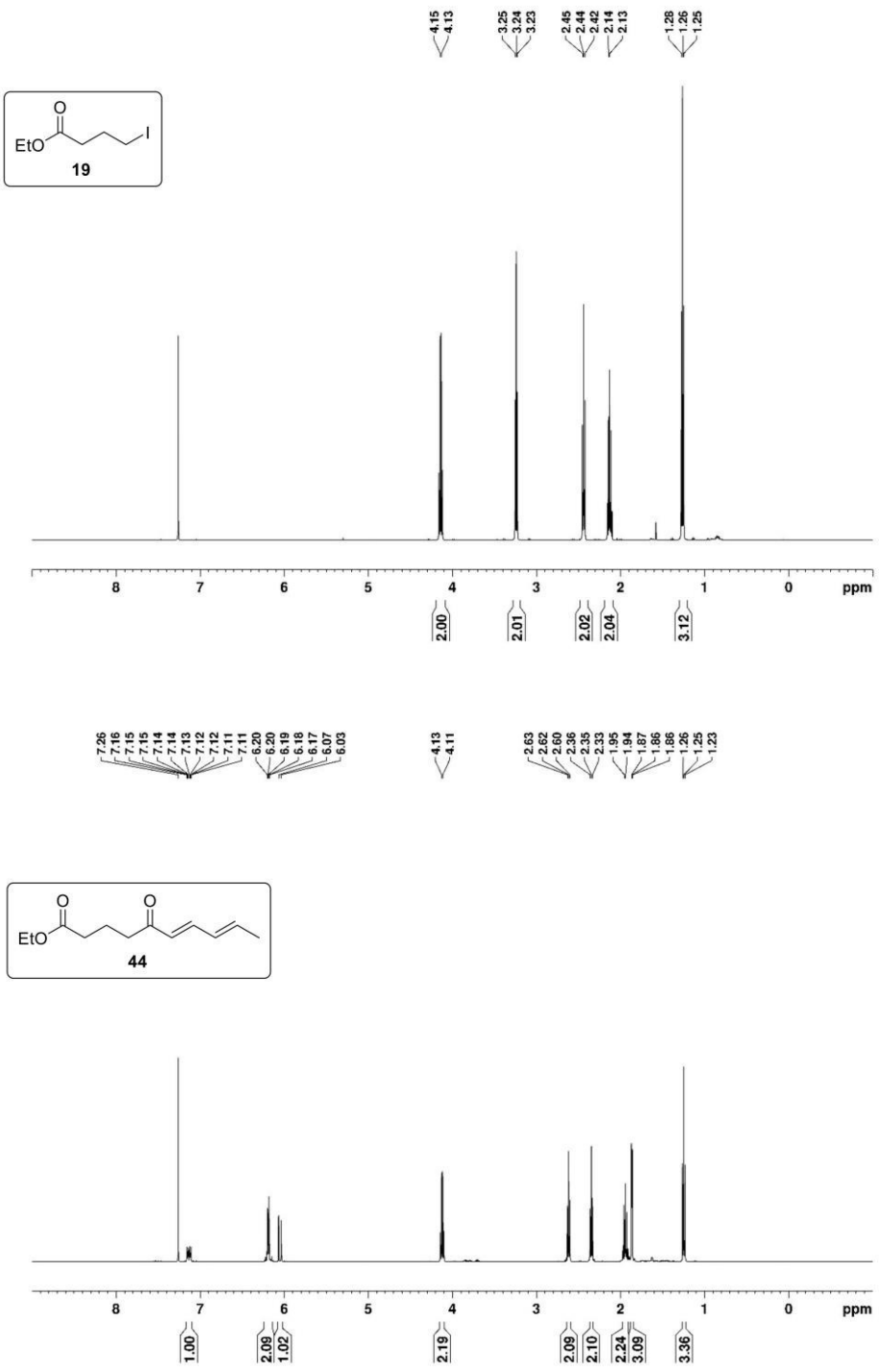
S113


BnO
MEMO
OMEM
HO
NH2
41
ppm

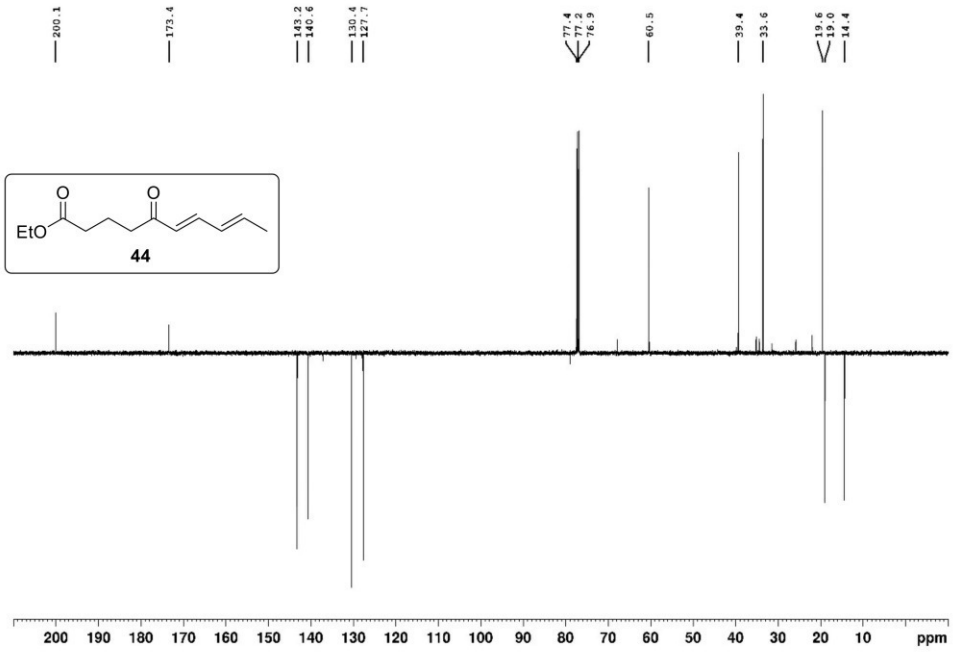

BnO
MEMO
OMEM
HO
NH2
41
ppm

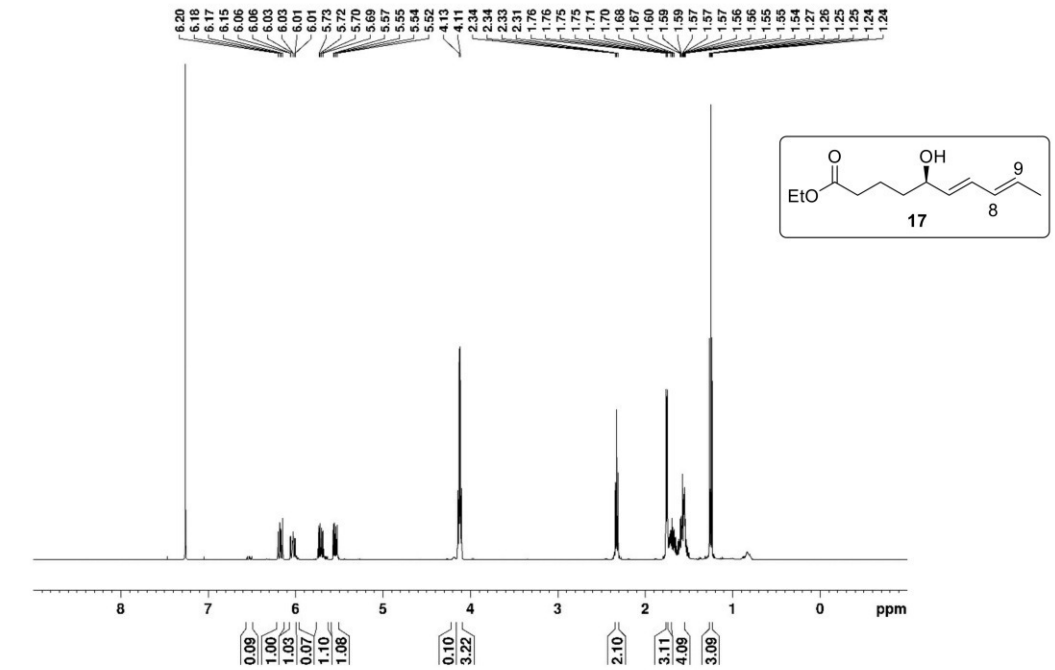

BnO
O
MEMO
OMEM
HO
NH
Cl
O
Cl
HN
42
ppm
159.8
136.5
128.7
128.3
128.3
128.2
118.6
111.3
110.2
109.2
98.4
98.0
96.8
79.8
77.4
77.2
76.9
76.3
74.4
71.7
71.5
70.0
68.5
67.4
64.4
59.2
59.0
41.9
29.8
18.1
11.3
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


43
ppm
43
ppm

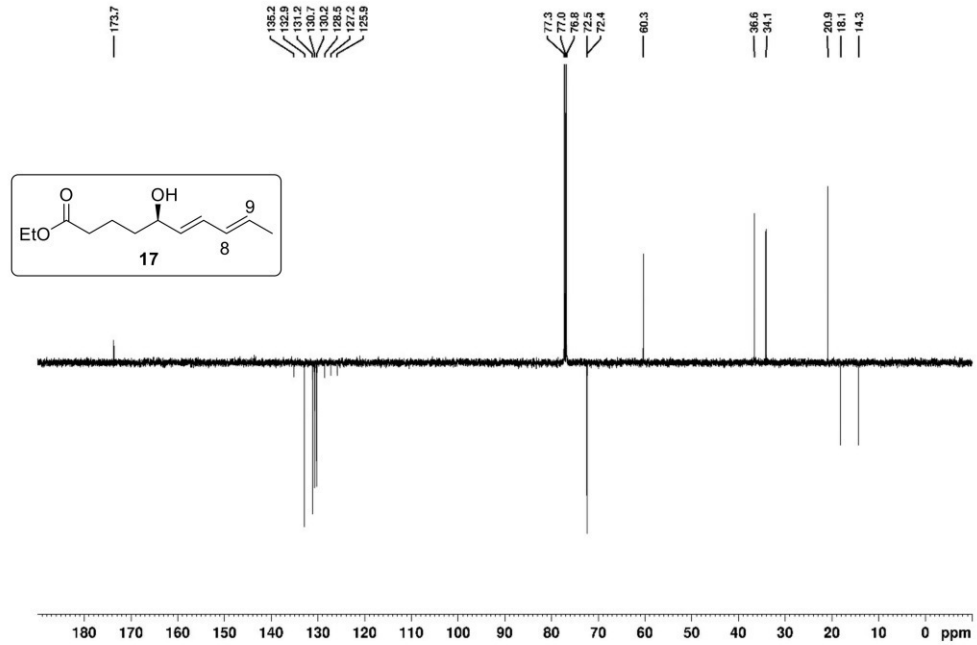

4.15
4.13
3.25
3.24
3.23
2.45
2.44
2.42
2.14
2.13
1.28
1.26
1.25
EtO
O
I
19
8
7
6
5
4
3
2
1
0
ppm
2.00
2.01
2.02
2.04
3.12
7.26
7.16
7.15
7.15
7.14
7.14
7.13
7.12
7.12
7.11
7.11
6.20
6.20
6.19
6.18
6.17
6.07
6.03
4.13
4.11
2.63
2.62
2.60
2.36
2.35
2.33
1.95
1.94
1.87
1.86
1.86
1.26
1.25
1.23
EtO
O
O
44
1.00
2.09
1.02
2.19
2.09
2.10
2.24
3.09
3.36

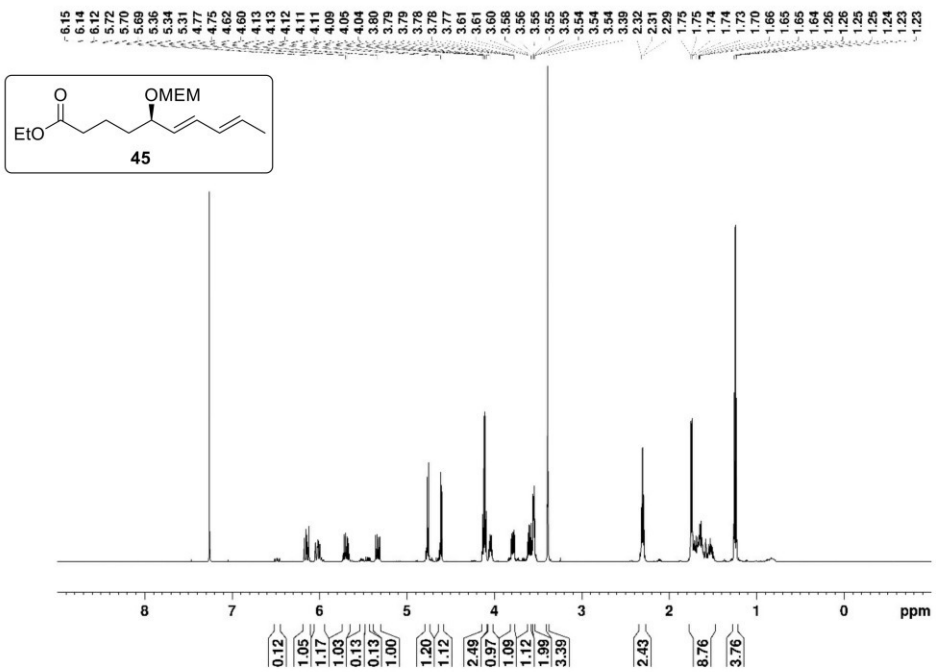
S117


200.1
173.4
143.2
140.6
130.4
127.7
77.4
77.2
76.9
60.5
39.4
33.6
19.6
19.0
14.4
EtO
44
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

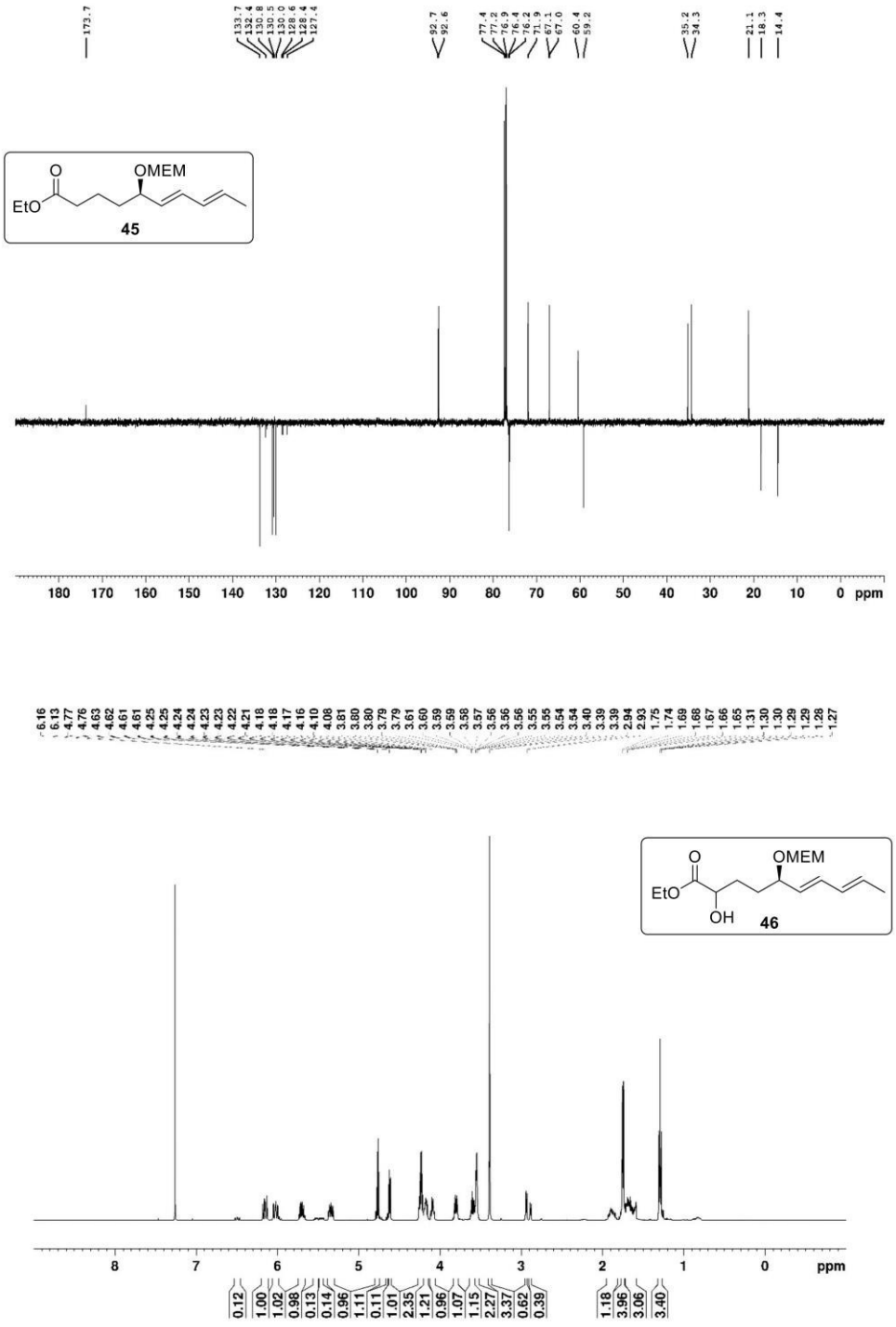

6.20
6.18
6.17
6.15
6.06
6.06
6.03
6.03
6.01
6.01
5.73
5.72
5.70
5.69
5.57
5.55
5.54
5.52
4.13
4.11
2.34
2.34
2.33
2.31
1.76
1.76
1.75
1.75
1.71
1.70
1.68
1.67
1.60
1.59
1.59
1.57
1.57
1.57
1.56
1.56
1.55
1.55
1.54
1.27
1.26
1.25
1.25
1.24
1.24
EtO
OH
9
8
17
8 7 6 5 4 3 2 1 0 ppm
0.09
1.00
1.03
0.07
1.10
1.08
0.10
3.22
2.10
3.11
4.09
3.09

S118

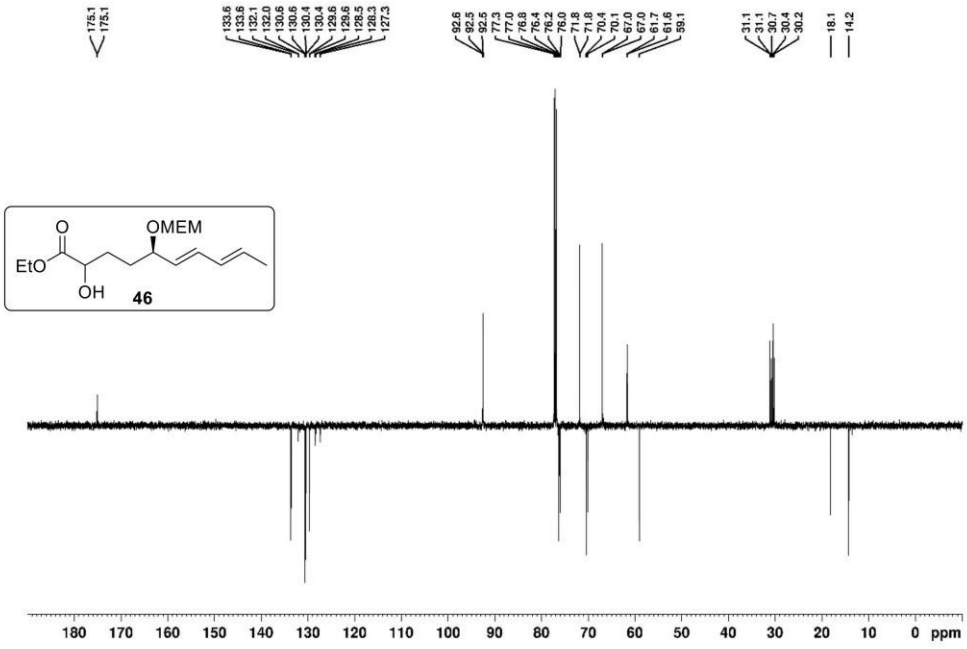

173.7
135.2
132.9
131.2
130.7
130.2
128.5
127.2
125.9
77.3
77.0
76.8
72.5
72.4
60.3
36.6
34.1
20.9
18.1
14.3
O
OH
EtO
9
8
17
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

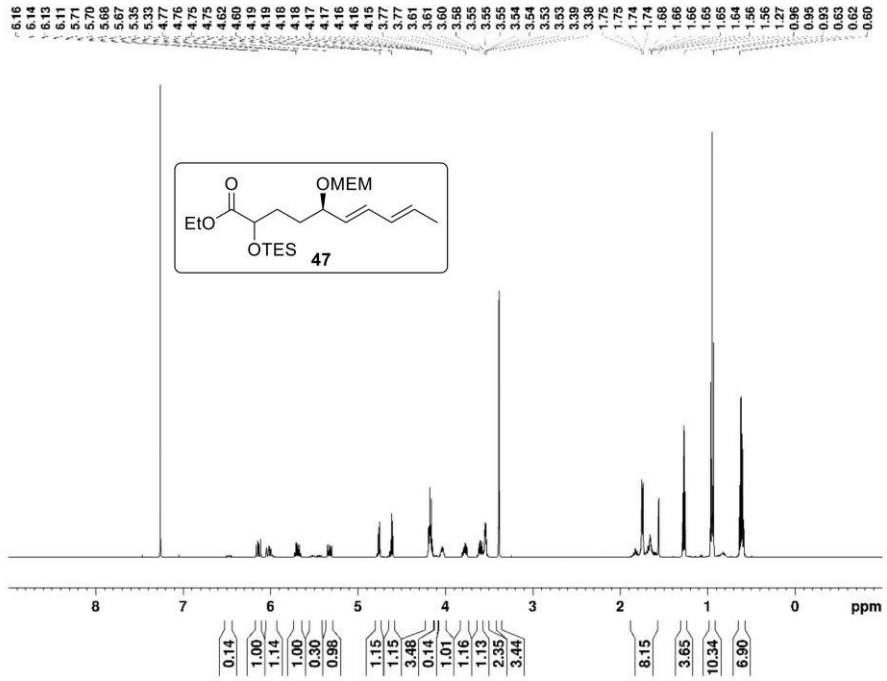

O
OMEM
EtO
45
8 7 6 5 4 3 2 1 0 ppm
0.12
1.05
1.17
1.03
0.13
1.00
1.20
1.12
2.49
0.97
1.09
1.12
1.99
3.39
2.43
8.76
3.76

S119

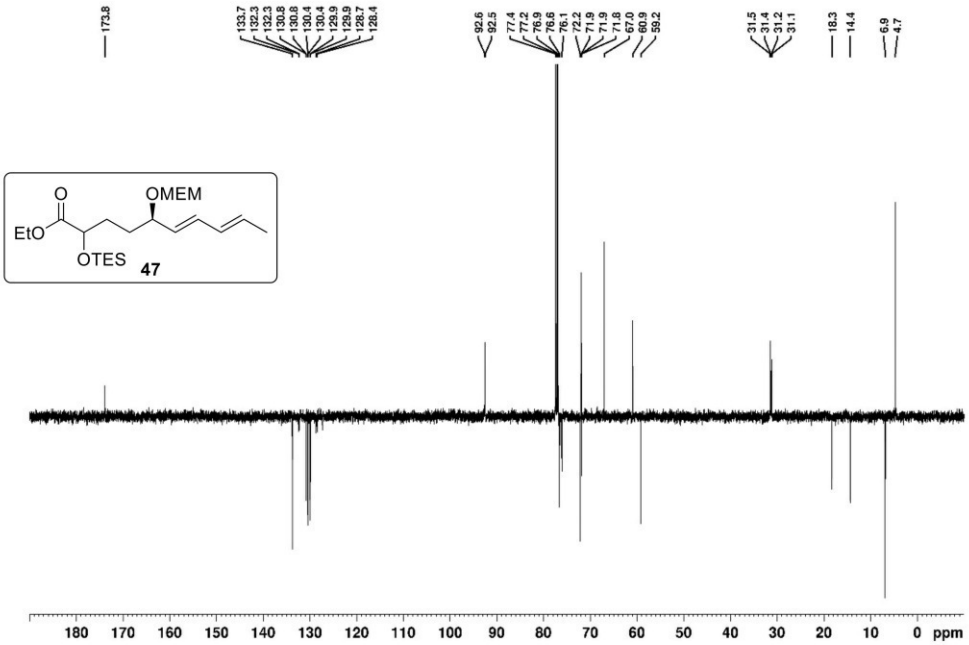


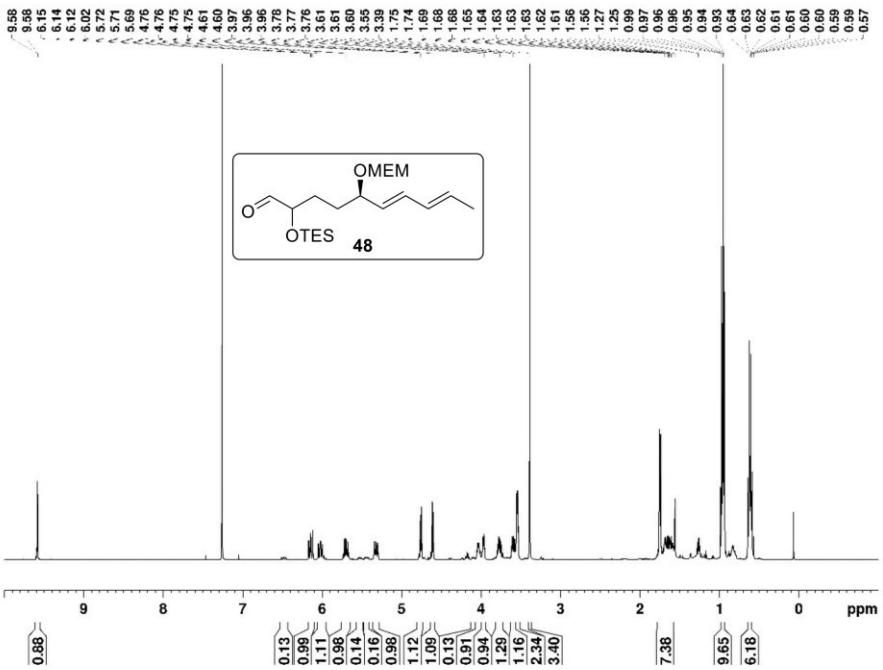
S120


175.1
175.1
133.6
133.6
132.1
132.0
130.6
130.6
130.4
130.4
129.6
129.6
128.5
128.3
127.3
92.6
92.5
92.5
77.3
77.0
76.8
76.4
76.2
76.0
71.8
71.8
70.4
70.1
67.0
67.0
61.7
61.6
59.1
31.1
31.1
30.7
30.4
30.2
18.1
14.2
O
OMEM
EtO
OH
46
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

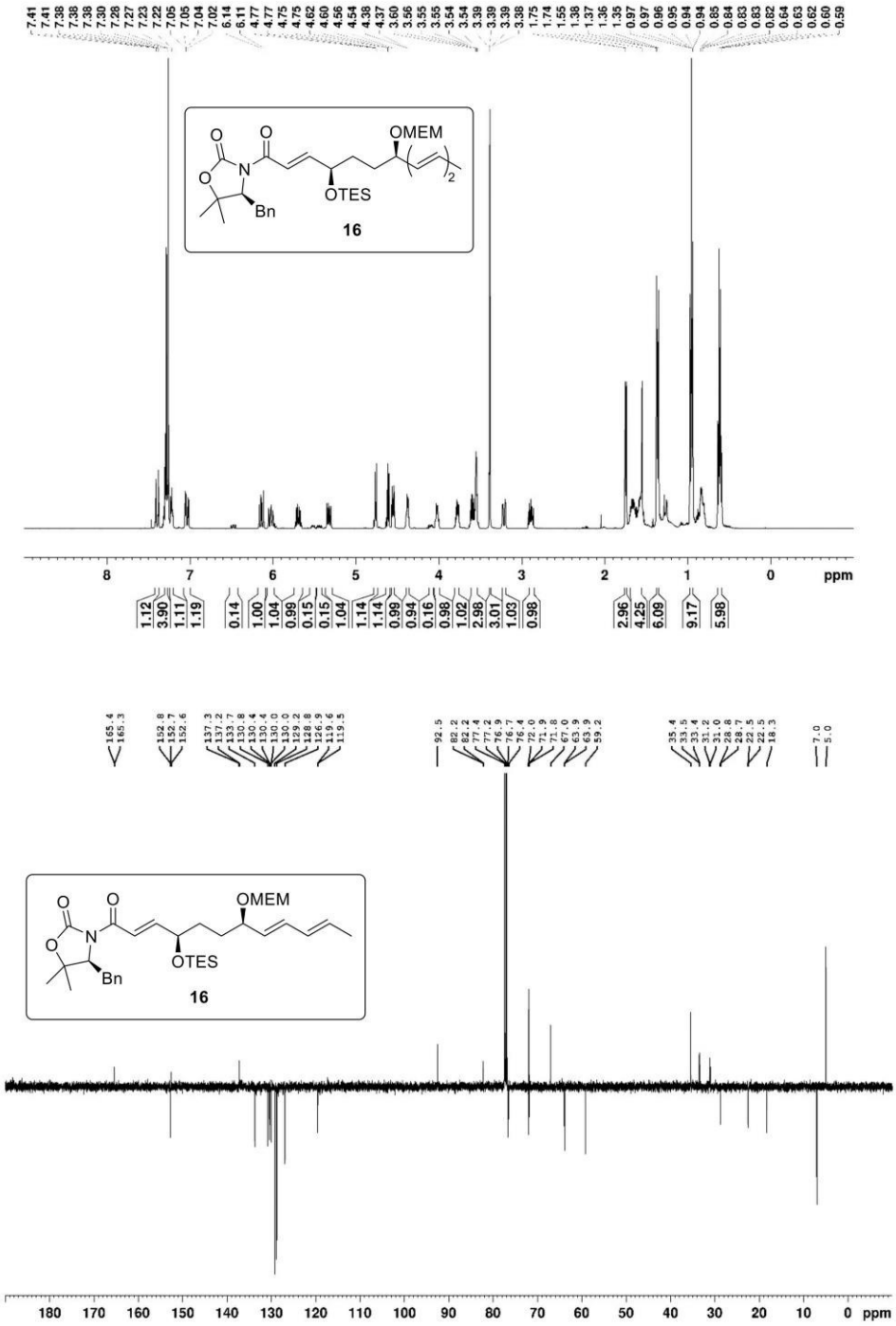

6.16
6.14
6.13
6.11
5.71
5.70
5.68
5.67
5.35
5.33
4.77
4.76
4.75
4.75
4.62
4.60
4.19
4.19
4.18
4.18
4.17
4.17
4.16
4.16
4.15
3.77
3.77
3.61
3.61
3.60
3.58
3.55
3.55
3.55
3.54
3.54
3.53
3.53
3.39
3.38
1.75
1.75
1.74
1.74
1.68
1.66
1.66
1.65
1.65
1.64
1.56
1.56
1.27
0.96
0.95
0.93
0.63
0.62
0.60
O
OMEM
EtO
OTES
47
8 7 6 5 4 3 2 1 0 ppm
0.14
1.00
1.14
1.00
0.30
0.98
1.15
1.15
3.48
0.14
1.01
1.16
1.13
2.35
3.44
8.15
3.65
10.34
6.90

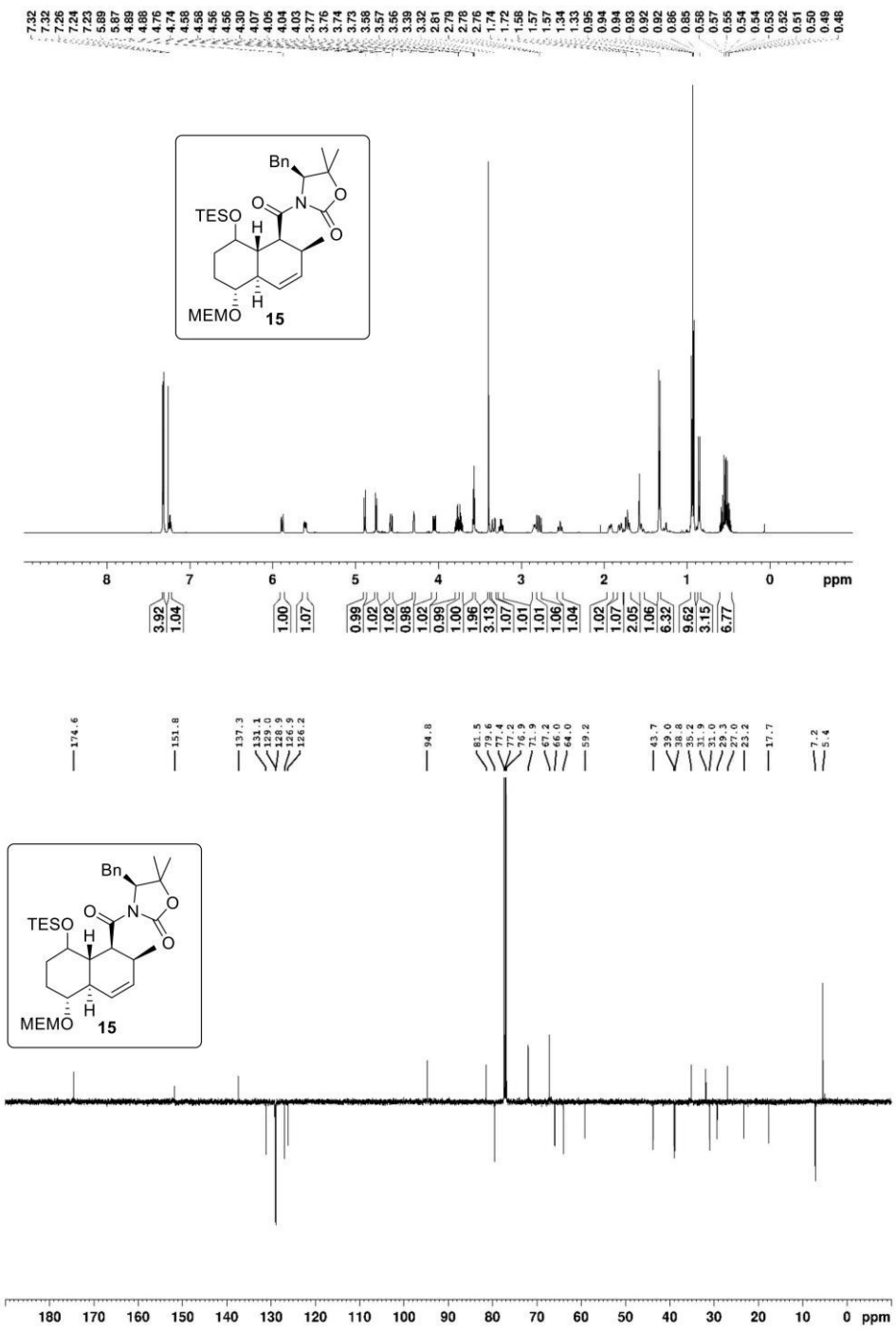
S121

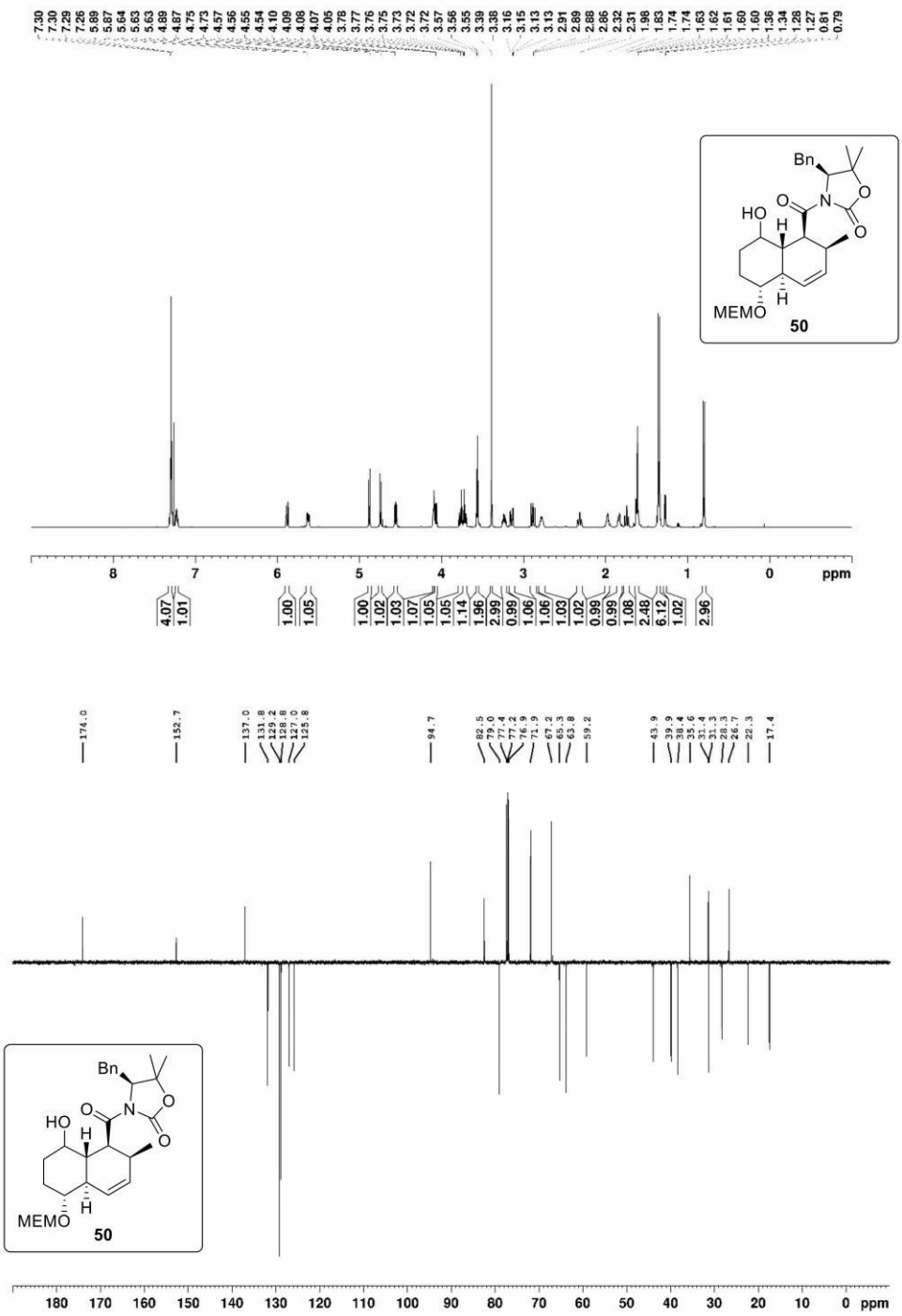

173.8
133.7
132.3
132.3
130.8
130.8
130.4
130.4
129.9
129.9
128.7
128.4
92.6
92.5
77.4
77.2
76.9
76.8
76.1
72.2
71.9
71.9
71.8
67.0
60.9
59.2
31.5
31.4
31.2
31.1
18.3
14.4
6.9
4.7
O
OMEM
EtO
OTES
47
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

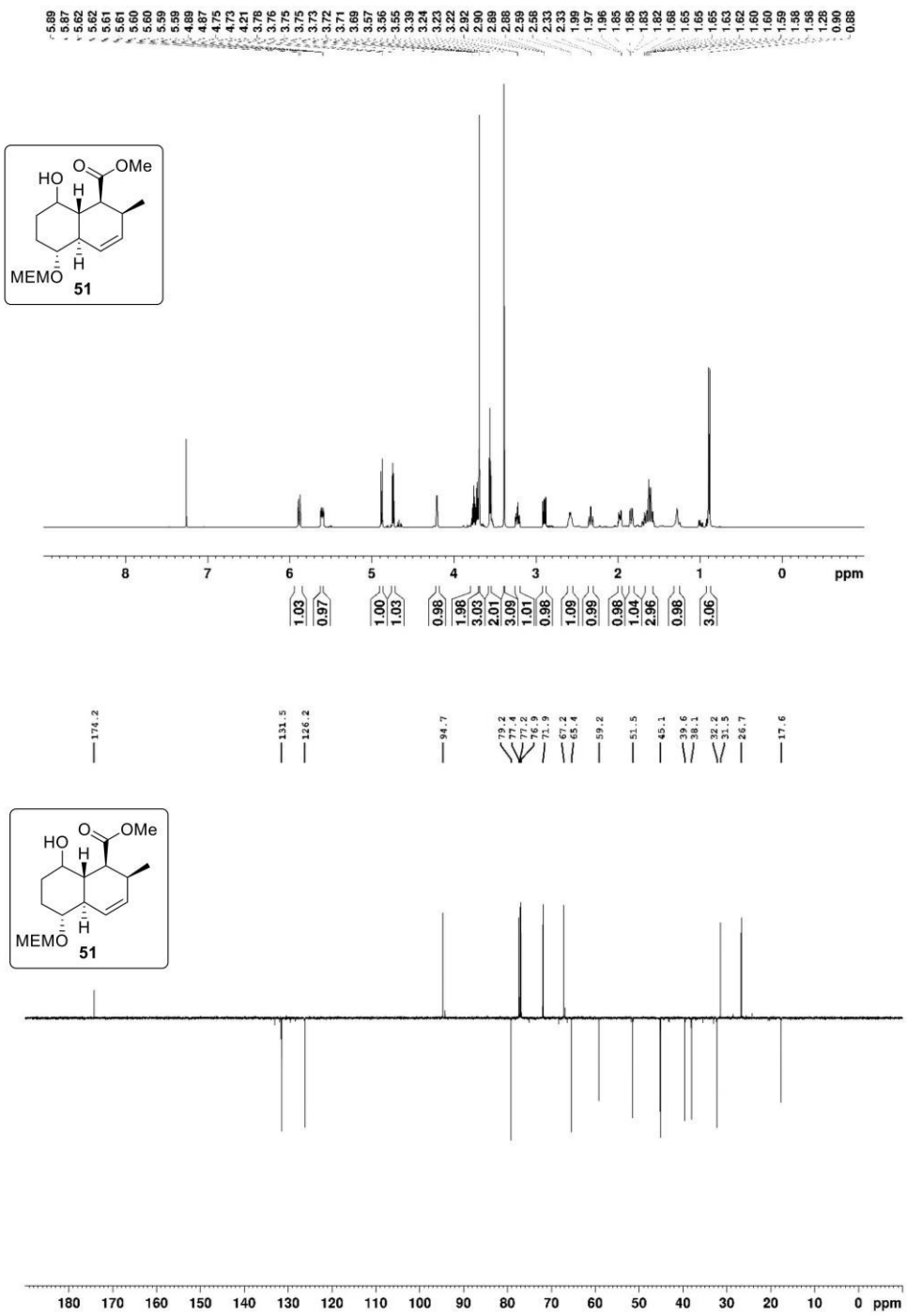

9.58
9.58
6.15
6.14
6.12
6.02
5.72
5.71
5.69
4.76
4.76
4.76
4.75
4.75
4.61
4.60
3.97
3.96
3.96
3.78
3.77
3.76
3.61
3.61
3.60
3.55
3.39
1.75
1.74
1.69
1.69
1.68
1.68
1.65
1.64
1.63
1.63
1.63
1.62
1.61
1.56
1.56
1.27
1.25
0.99
0.97
0.96
0.96
0.95
0.94
0.93
0.64
0.63
0.62
0.61
0.61
0.60
0.60
0.59
0.59
0.57
OMEM
O
OTES
48
9 8 7 6 5 4 3 2 1 0 ppm
0.88
0.13
0.99
1.11
0.98
0.14
0.16
0.98
1.12
1.09
0.13
0.91
0.94
1.29
1.16
2.34
3.40
7.38
9.65
6.18

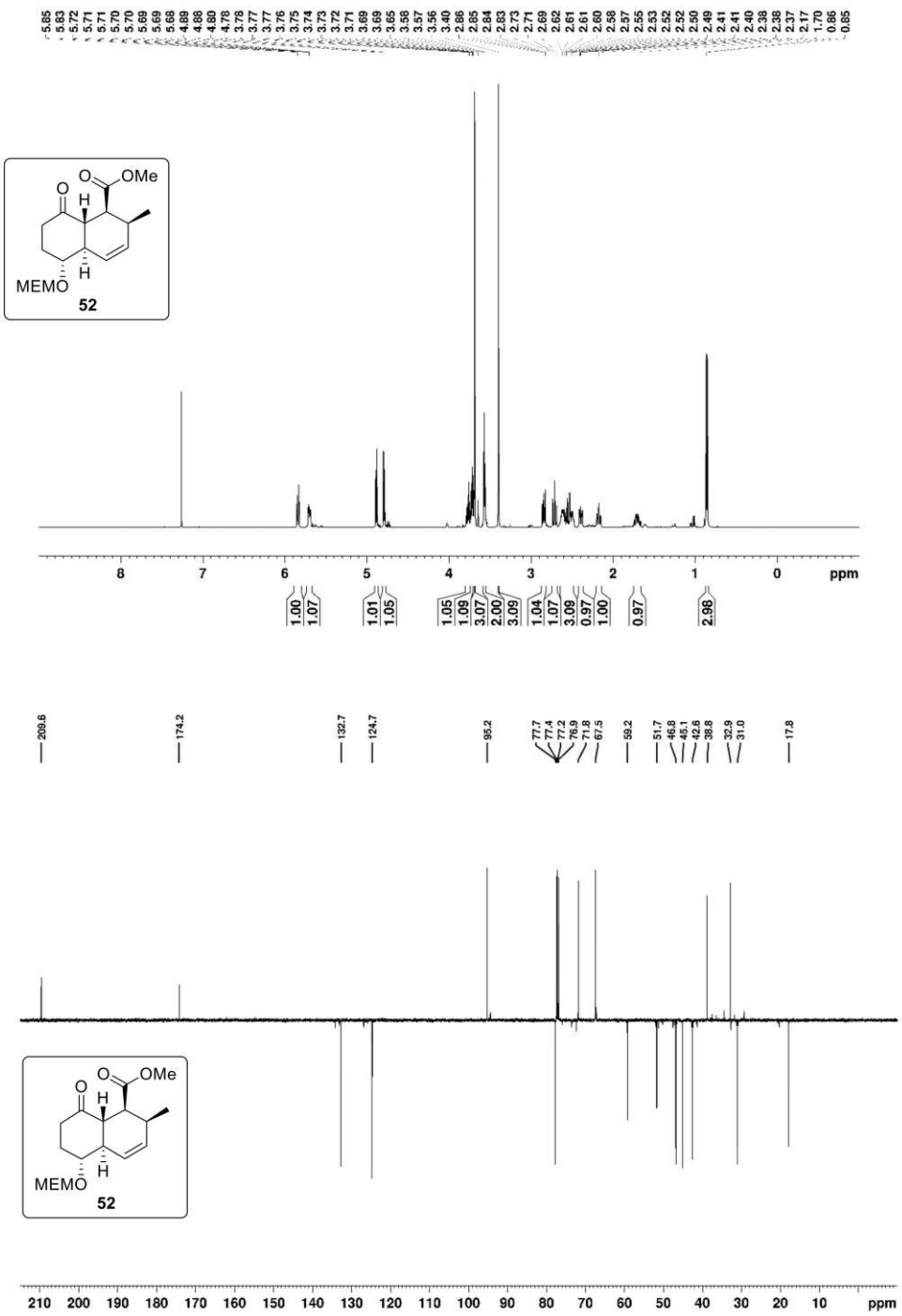
S122


OMEM
OTES
Bn
16
ppm
OMEM
OTES
Bn
16
ppm

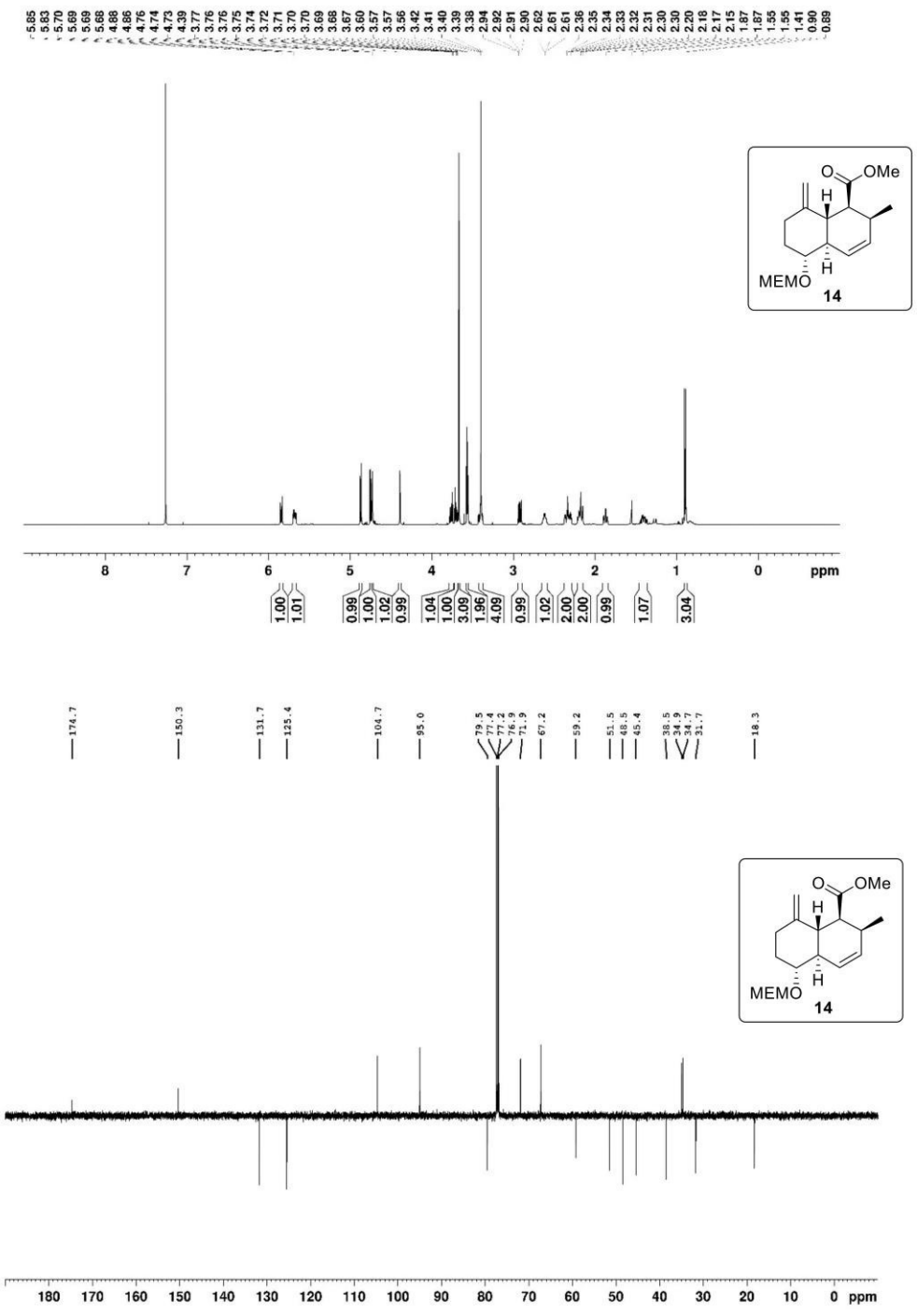

Bn
TESO
MEMO
15
ppm
Bn
TESO
MEMO
15
ppm

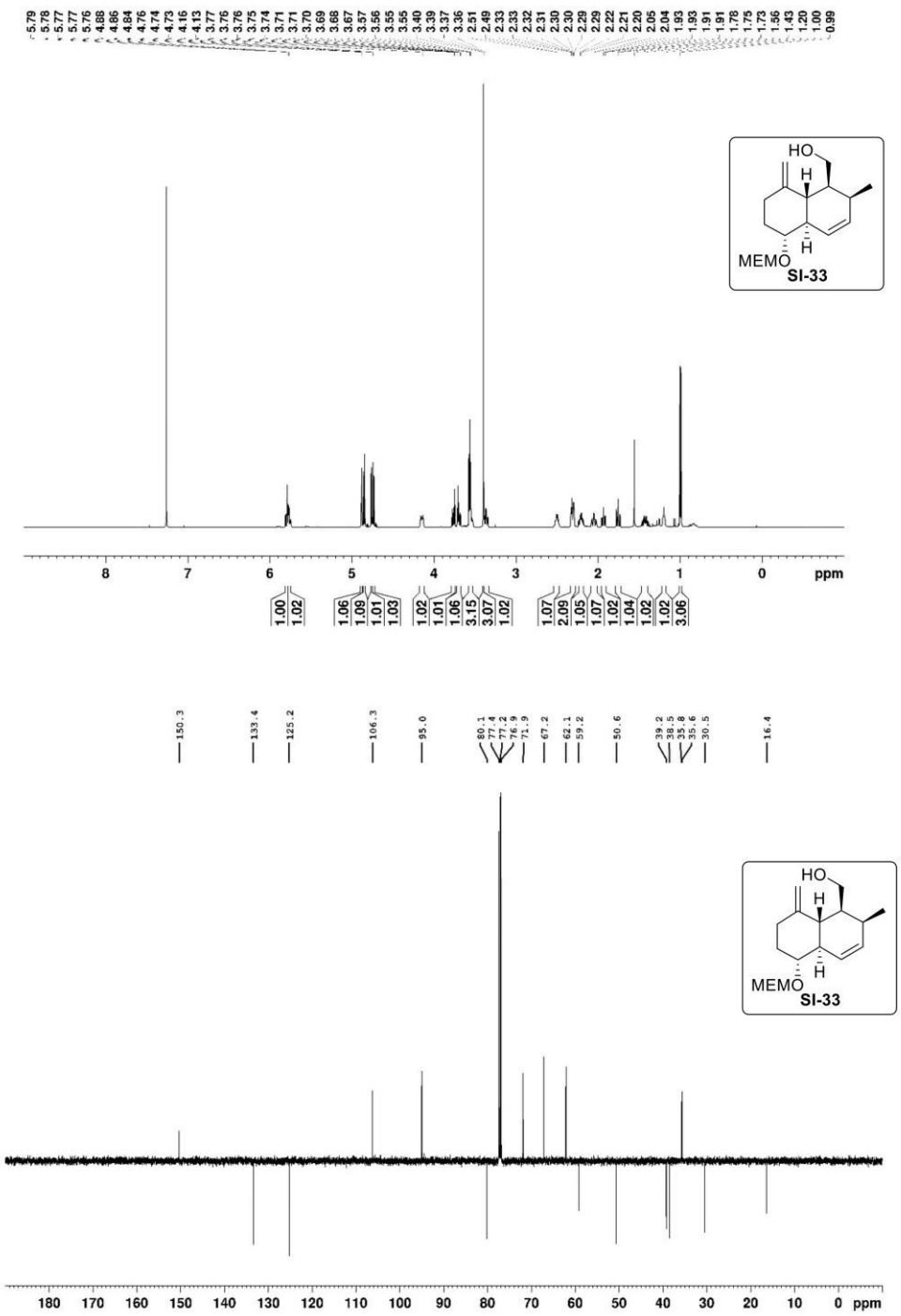

Bn
HO
MEMO
50
ppm
Bn
HO
MEMO
50
ppm

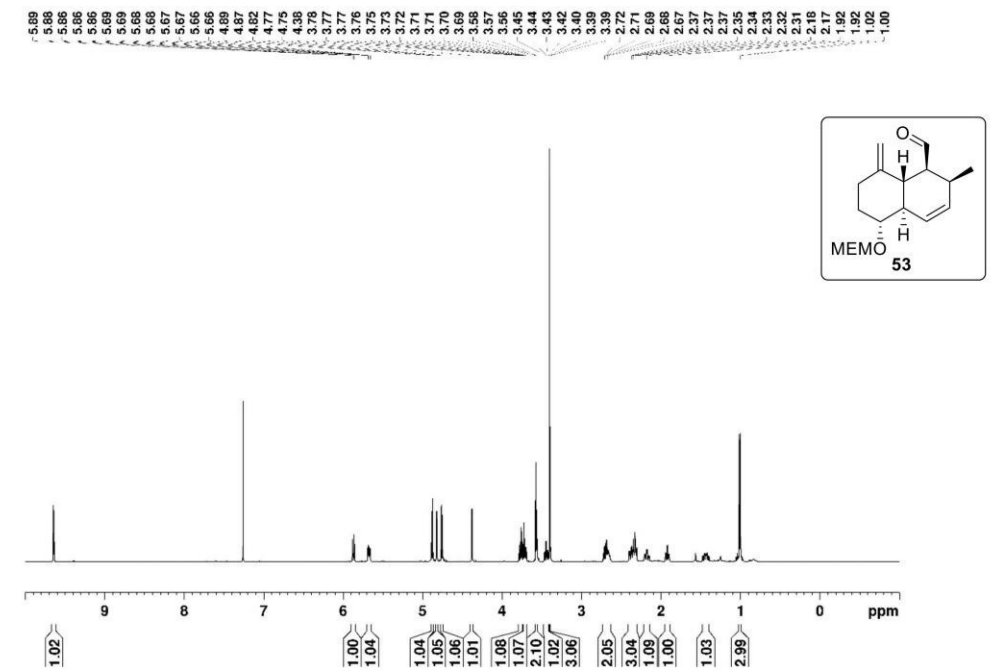

HO
O
OMe
H
MEMO
H
51
ppm
174.2
131.5
126.2
94.7
79.2
77.4
77.2
76.9
71.9
67.2
65.4
59.2
51.5
45.1
39.6
38.1
32.2
31.5
26.7
17.6
ppm

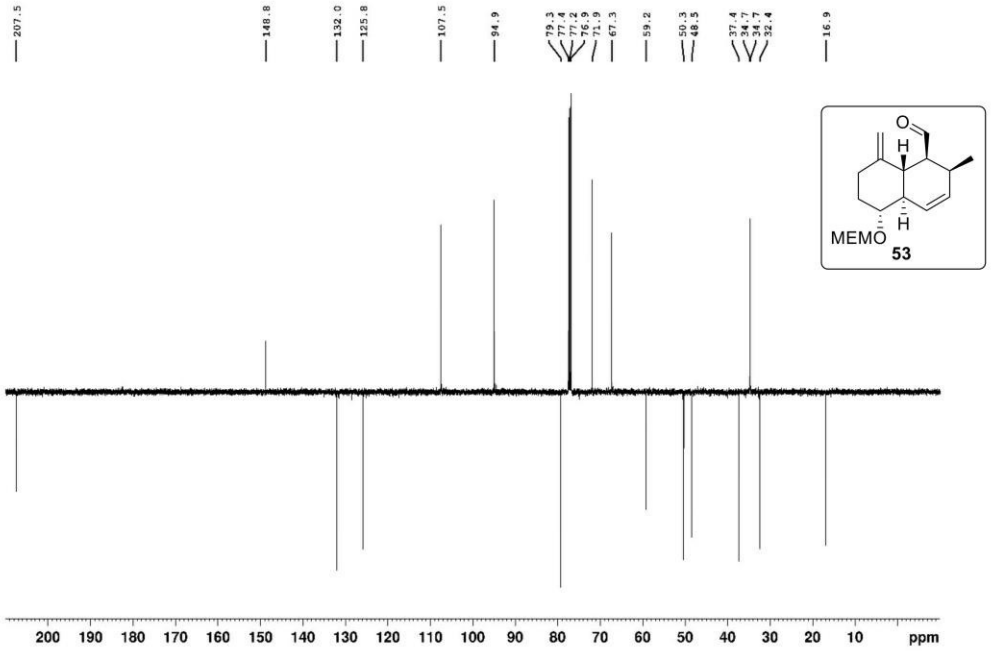

OMe
O
H
MEMO
52
ppm
209.6
174.2
132.7
124.7
95.2
77.7
77.4
77.2
76.9
71.8
67.5
59.2
51.7
46.8
45.1
42.6
38.8
32.9
31.0
17.8

S127

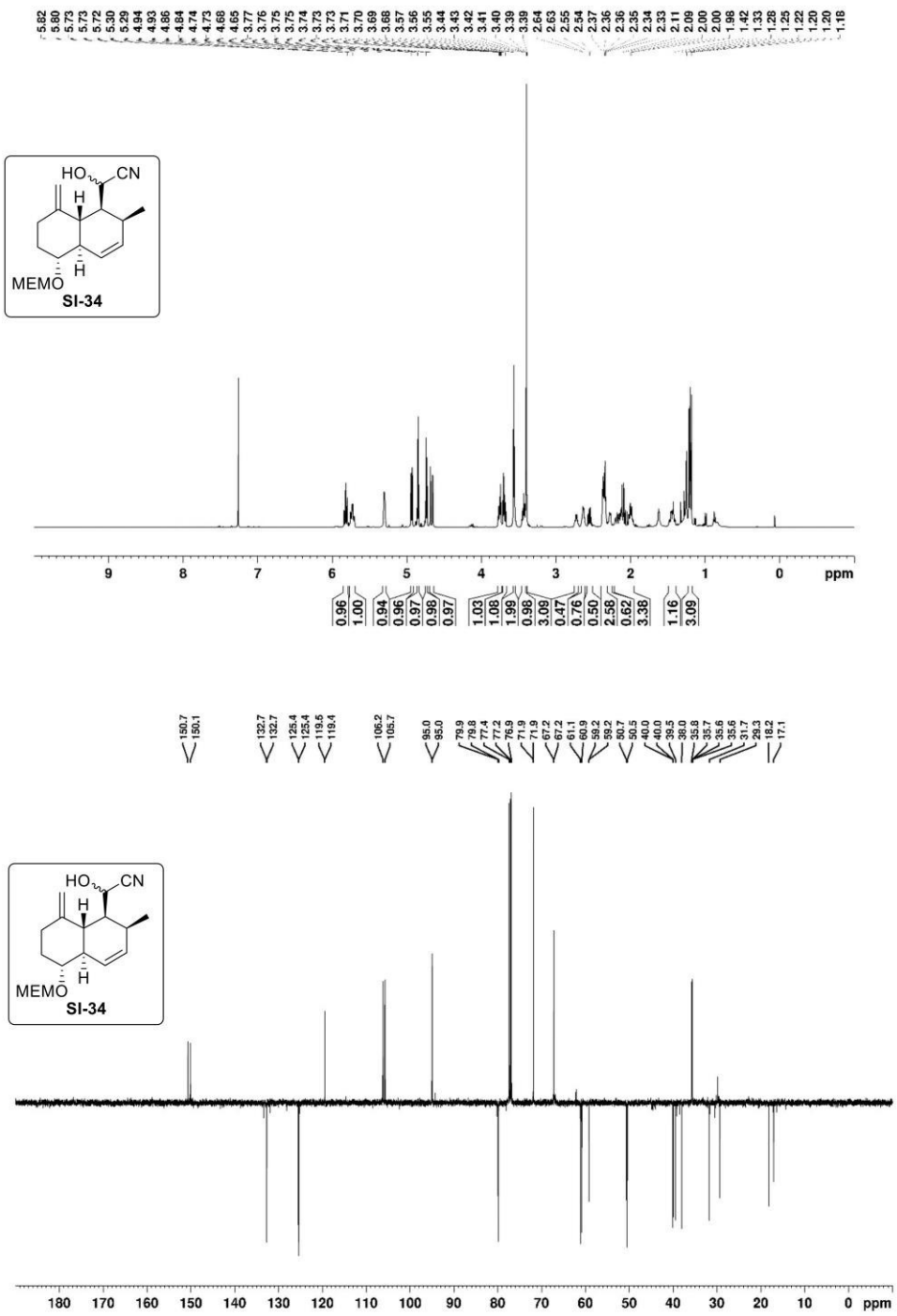

5.85 5.83 5.70 5.69 5.69 5.68 4.88 4.86 4.76 4.74 4.73 4.39 3.77 3.76 3.76 3.75 3.74 3.72 3.71 3.70 3.70 3.69 3.68 3.67 3.60 3.57 3.57 3.56 3.42 3.41 3.40 3.39 3.38 2.94 2.92 2.91 2.90 2.62 2.61 2.61 2.36 2.35 2.34 2.33 2.32 2.31 2.30 2.30 2.20 2.18 2.17 2.15 1.87 1.87 1.55 1.55 1.41 0.90 0.89
O
OMe
H
H
MEMO
14
8 7 6 5 4 3 2 1 0 ppm
1.00 1.01 0.99 1.00 1.02 0.99 1.04 1.00 3.09 1.96 4.09 0.99 1.02 2.00 2.00 0.99 1.07 3.04
174.7 150.3 131.7 125.4 104.7 95.0 79.5 77.4 77.2 76.9 71.9 67.2 59.2 51.5 48.5 45.4 38.5 34.9 34.7 31.7 18.3
O
OMe
H
H
MEMO
14
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

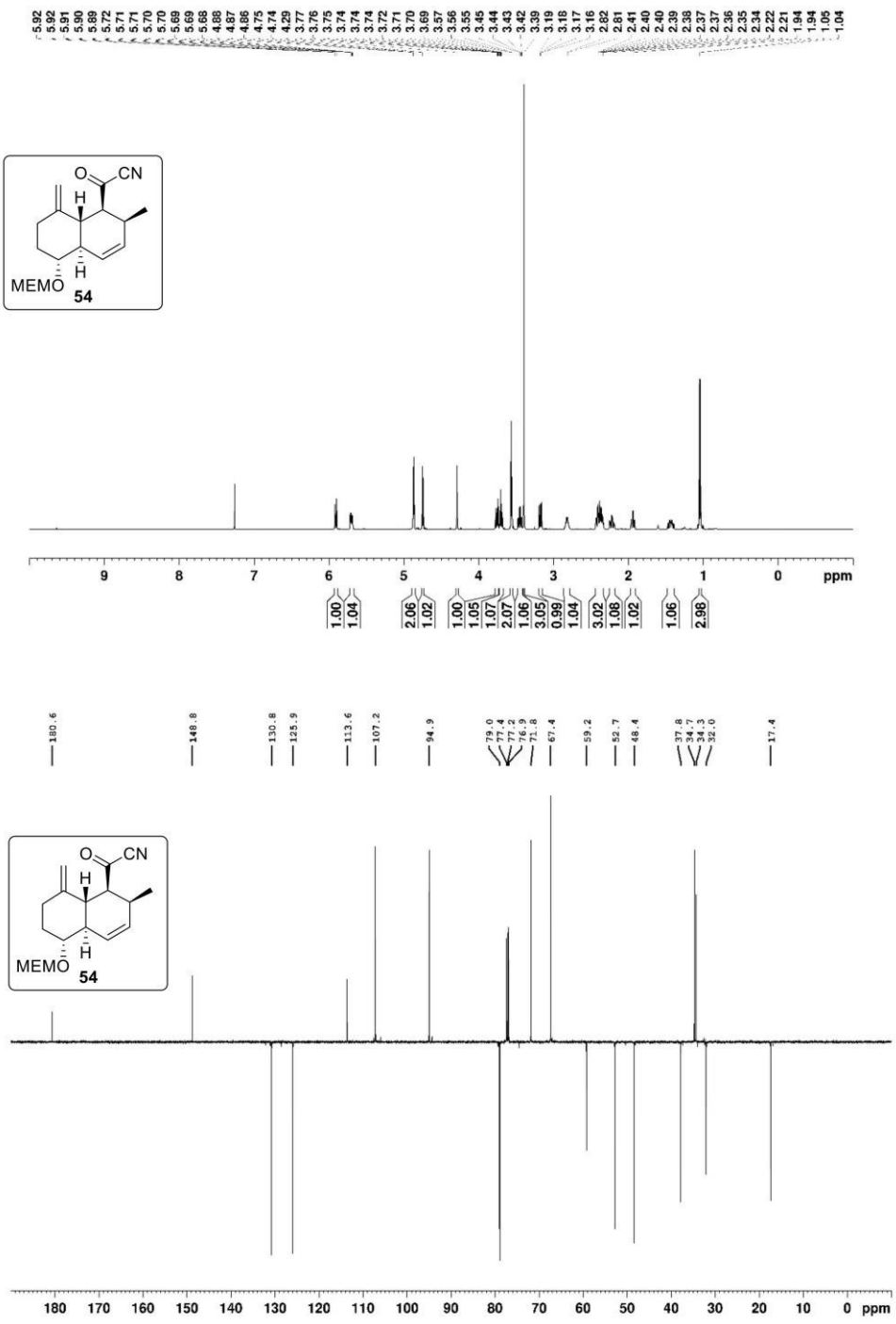

HO
H
H
MEMO
SI-33
ppm
HO
H
H
MEMO
SI-33
ppm

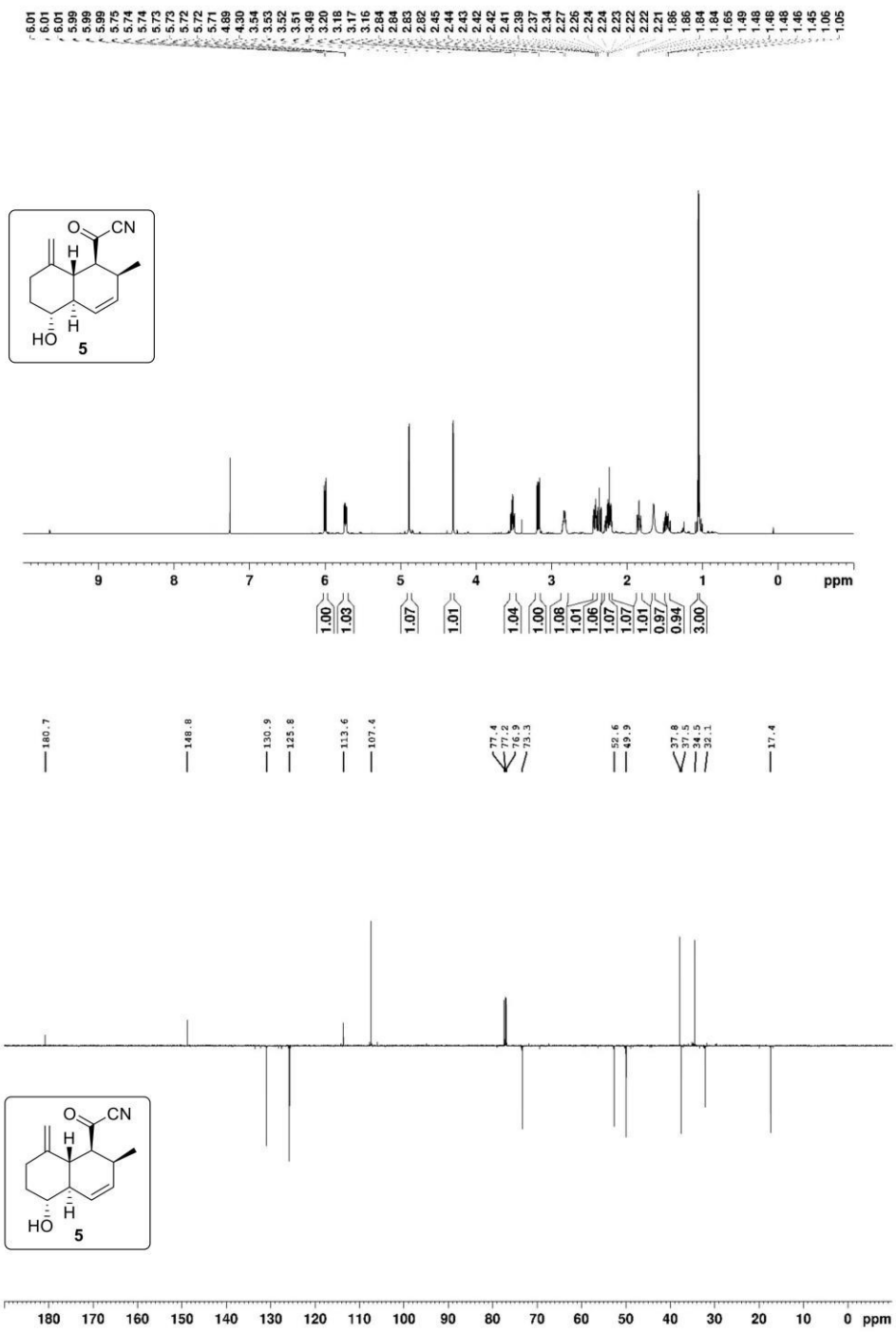


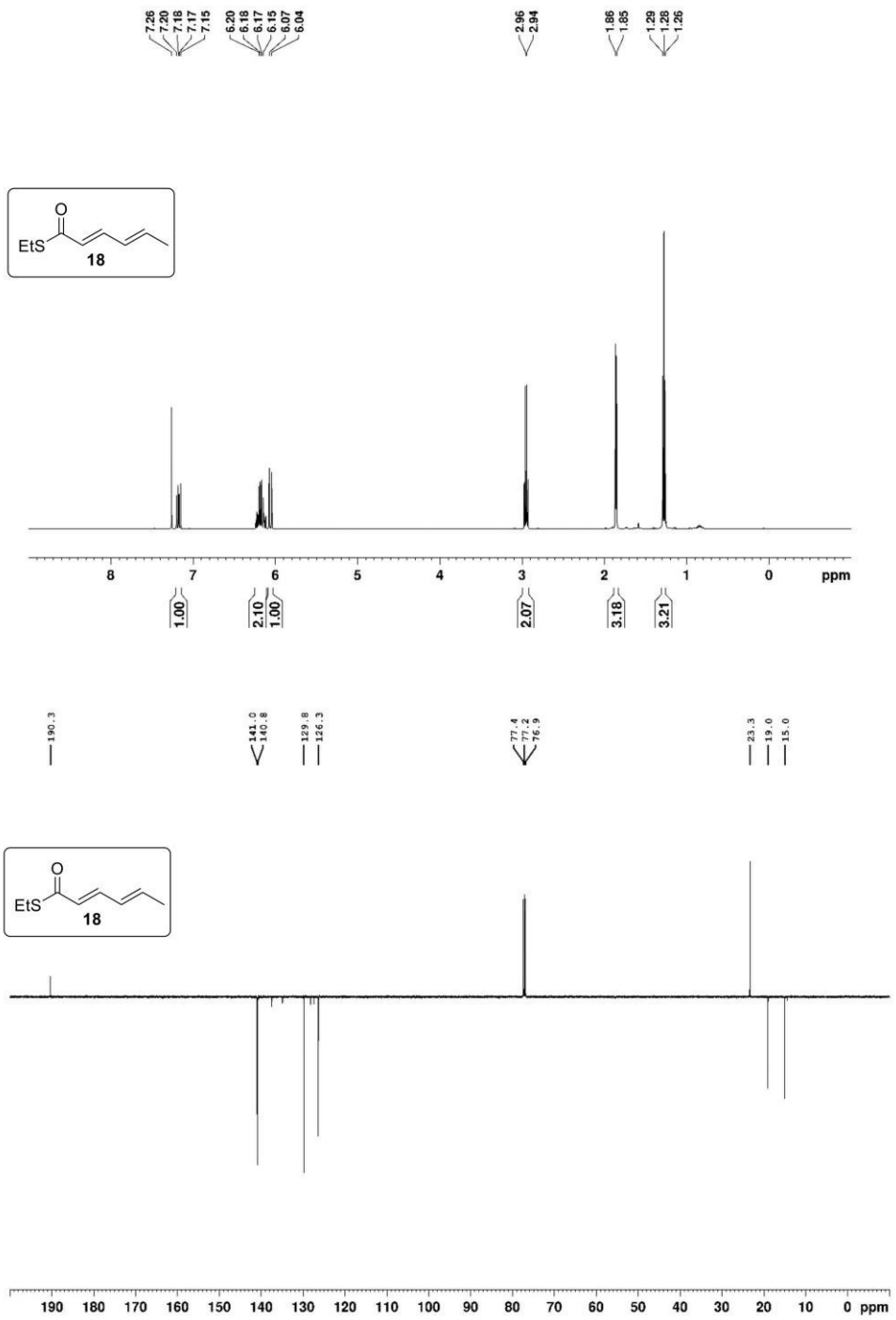


S130

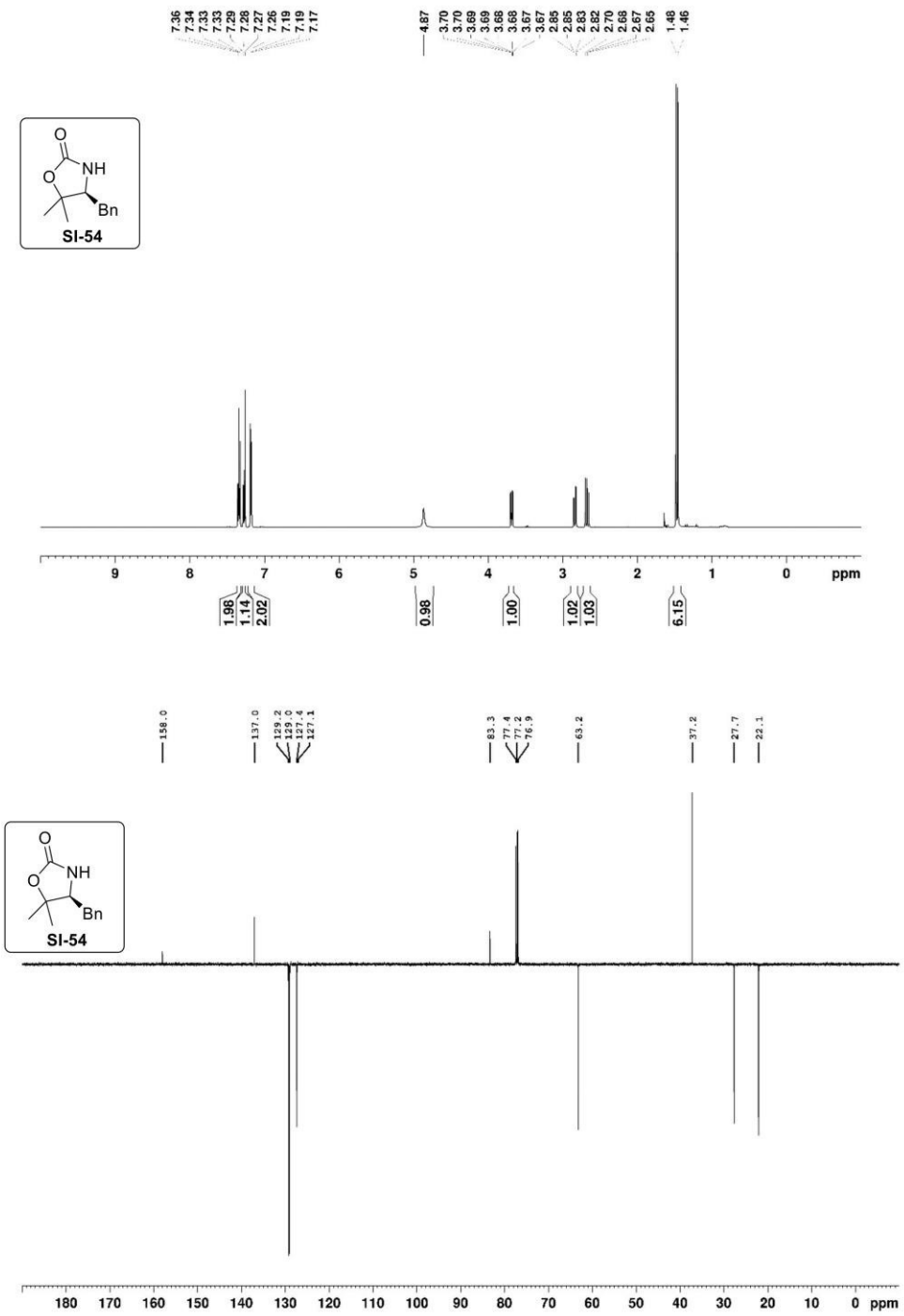

HO
CN
H
H
MEMO
SI-34
ppm
HO
CN
H
H
MEMO
SI-34
ppm


5.92 5.92 5.91 5.90 5.89 5.72 5.71 5.71 5.70 5.70 5.69 5.69 5.68 5.68 4.87 4.86 4.75 4.74 4.29 3.77 3.76 3.75 3.74 3.74 3.74 3.72 3.71 3.70 3.69 3.57 3.56 3.55 3.45 3.44 3.43 3.42 3.39 3.19 3.18 3.17 3.16 2.82 2.81 2.41 2.40 2.40 2.39 2.38 2.37 2.37 2.36 2.35 2.34 2.22 2.21 1.94 1.94 1.05 1.04
O
CN
H
H
MEMO
54
9 8 7 6 5 4 3 2 1 0 ppm
1.00 1.04 2.06 1.02 1.00 1.05 1.07 2.07 1.06 3.05 0.99 1.04 3.02 1.08 1.02 1.06 2.98
180.6 148.8 130.8 125.9 113.6 107.2 94.9 79.0 77.4 77.2 76.9 71.8 67.4 59.2 52.7 48.4 37.8 34.7 34.3 32.0 17.4
O
CN
H
H
MEMO
54
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

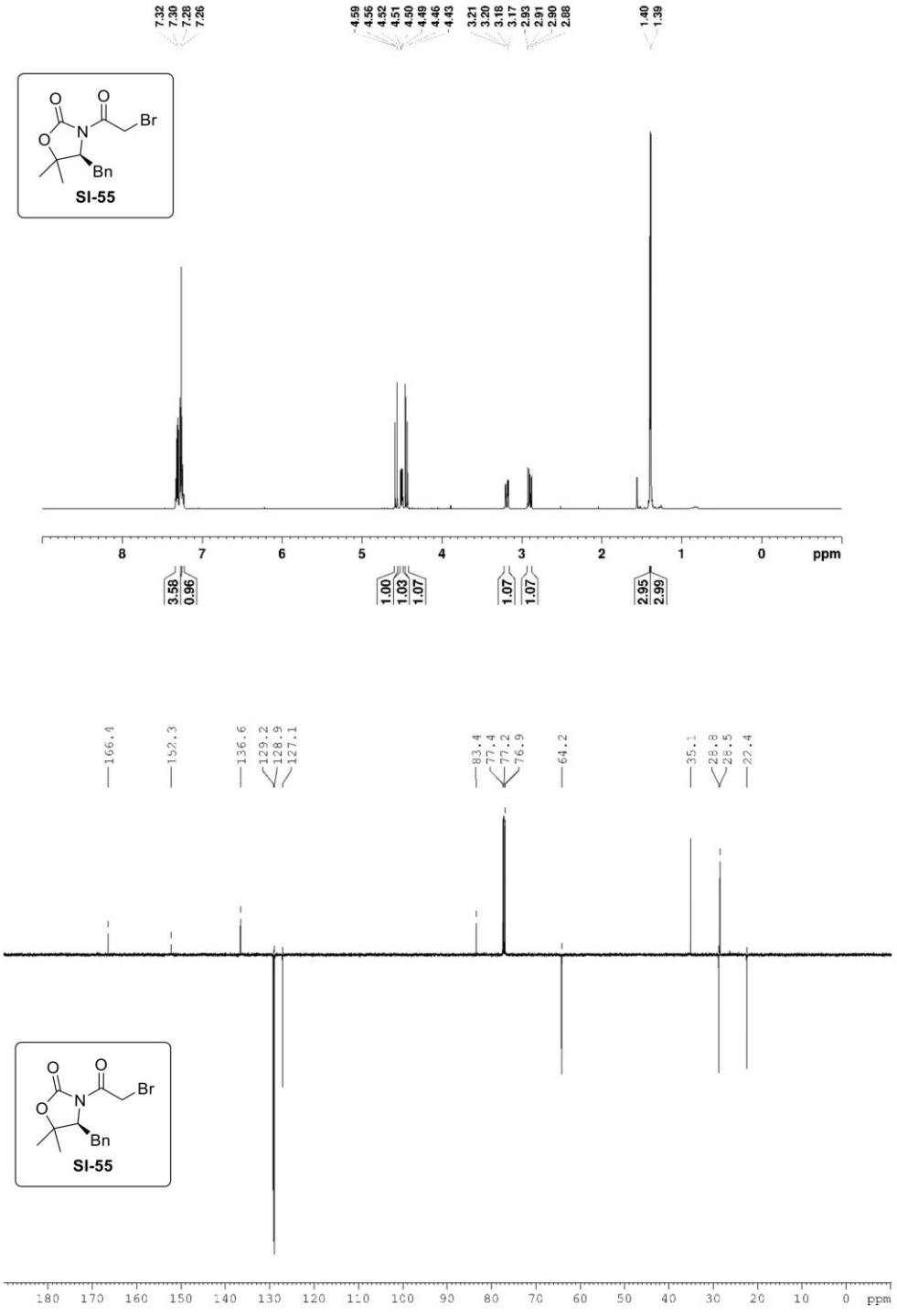

6.01 6.01 6.01 5.99 5.99 5.99 5.99 5.75 5.74 5.74 5.73 5.73 5.72 5.72 5.71 4.89 4.30 3.54 3.53 3.52 3.51 3.49 3.20 3.18 3.17 3.16 2.84 2.84 2.83 2.82 2.45 2.44 2.43 2.42 2.42 2.41 2.39 2.37 2.34 2.27 2.26 2.24 2.24 2.23 2.22 2.22 2.21 1.86 1.86 1.84 1.84 1.65 1.49 1.48 1.48 1.46 1.45 1.06 1.05
O
CN
H
HO
H
5
9 8 7 6 5 4 3 2 1 0 ppm
1.00 1.03 1.07 1.01 1.04 1.00 1.08 1.01 1.06 1.07 1.07 1.01 0.97 0.94 3.00
180.7 148.8 130.9 125.8 113.6 107.4 77.4 77.2 76.9 73.3 52.6 49.9 37.8 37.5 34.5 32.1 17.4
O
CN
H
HO
H
5
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

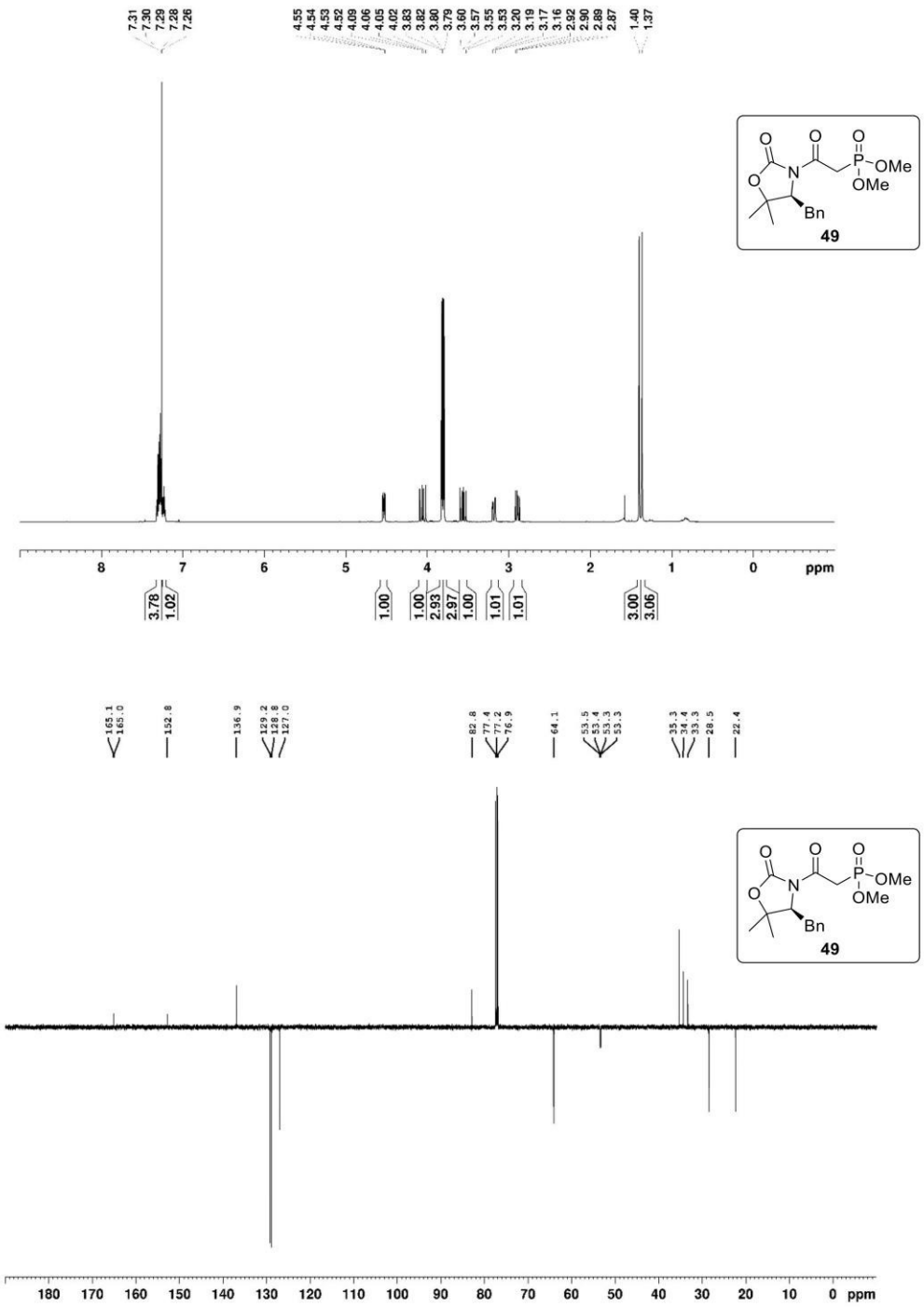

7.26
7.20
7.18
7.17
7.15
6.20
6.18
6.17
6.15
6.07
6.04
2.96
2.94
1.86
1.85
1.29
1.28
1.26
EtS
O
18
8
7
6
5
4
3
2
1
0
ppm
1.00
2.10
1.00
2.07
3.18
3.21
190.3
141.0
140.8
129.8
126.3
77.4
77.2
76.9
23.3
19.0
15.0
EtS
O
18
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

S134

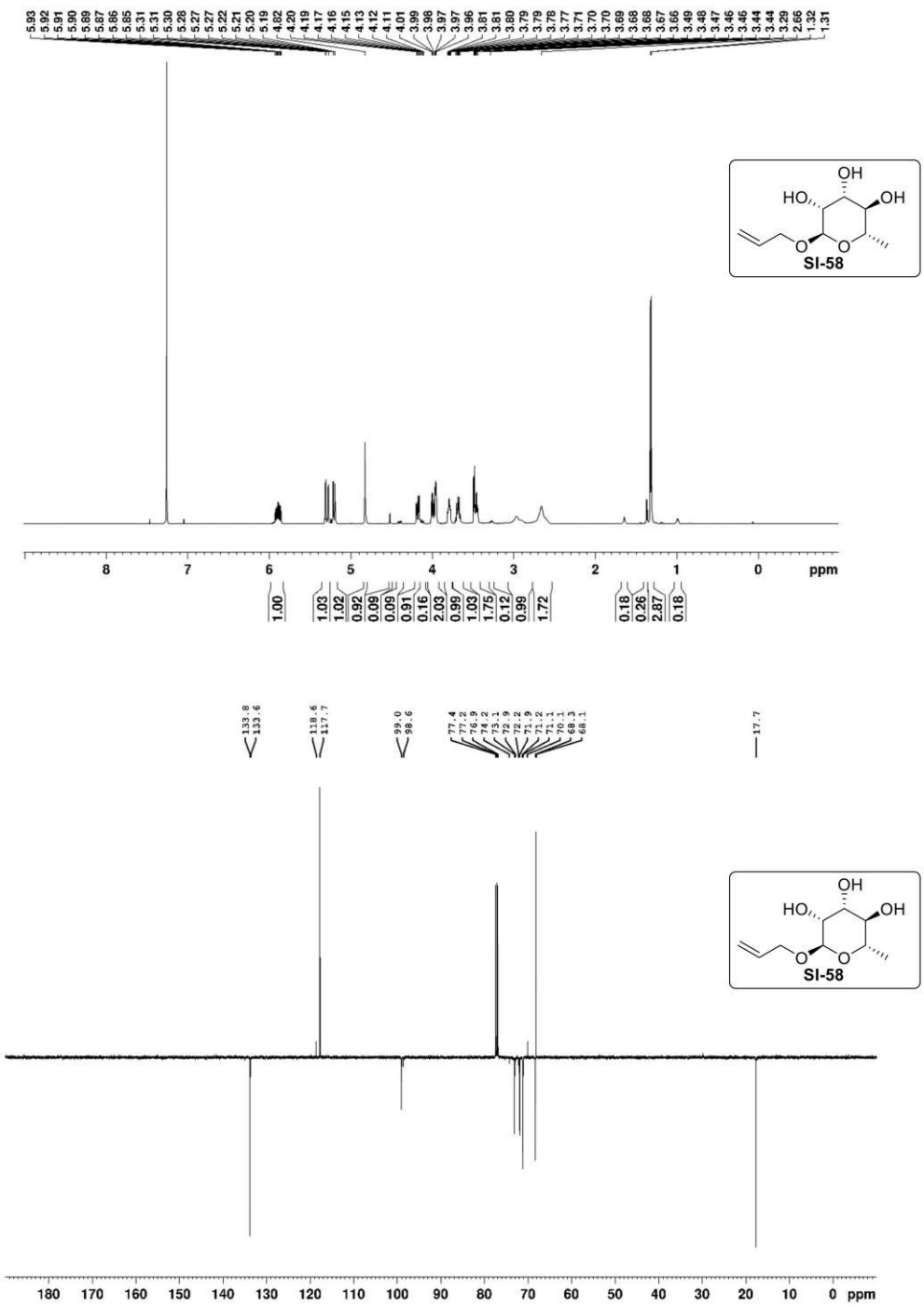

7.36
7.34
7.33
7.33
7.29
7.28
7.27
7.26
7.19
7.19
7.17
4.87
3.70
3.70
3.69
3.69
3.68
3.68
3.67
3.67
2.85
2.85
2.83
2.82
2.70
2.68
2.67
2.65
1.48
1.46
O
NH
O
Bn
SI-54
9
8
7
6
5
4
3
2
1
0
ppm
1.96
1.14
2.02
0.98
1.00
1.02
1.03
6.15
158.0
137.0
129.2
129.0
127.4
127.1
83.3
77.4
77.2
76.9
63.2
37.2
27.7
22.1
O
NH
O
Bn
SI-54
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

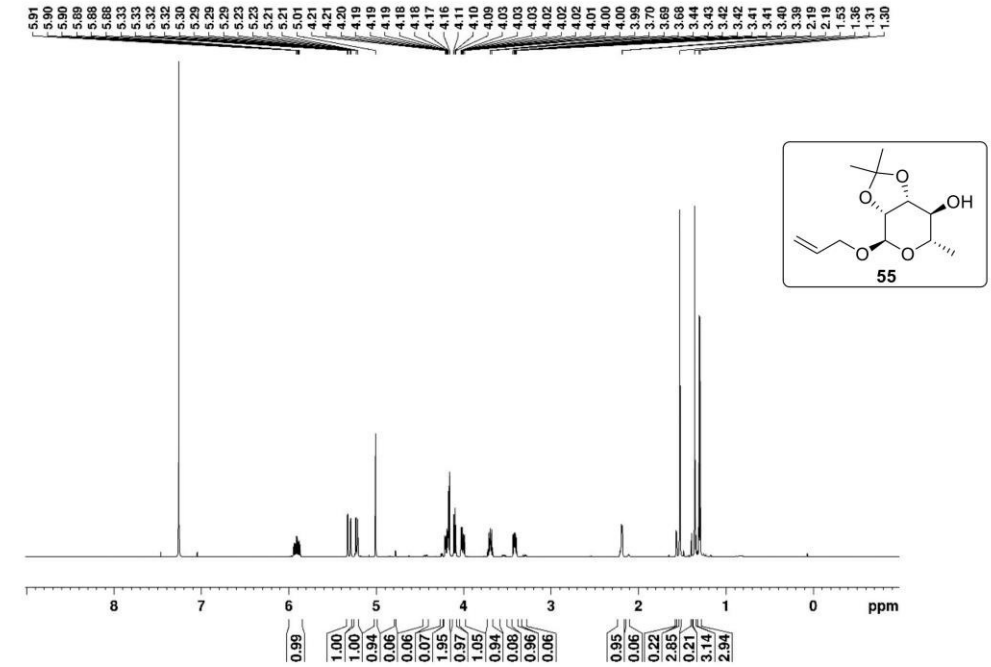
S135

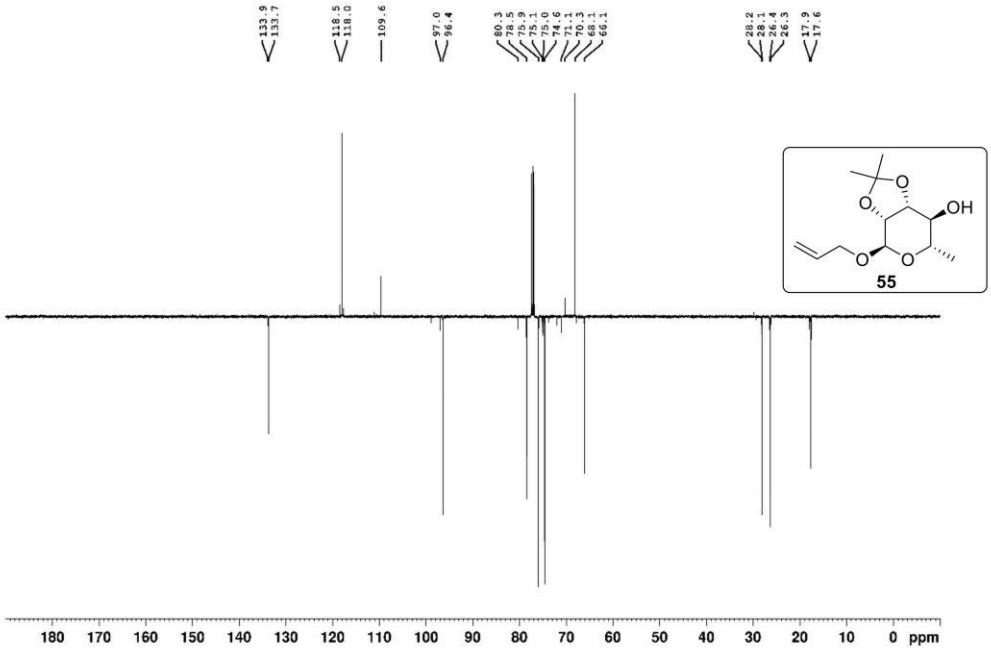

7.32
7.30
7.28
7.26
4.59
4.56
4.52
4.51
4.50
4.49
4.46
4.43
3.21
3.20
3.18
3.17
2.93
2.91
2.90
2.88
1.40
1.39
Br
Bn
SI-55
8
7
6
5
4
3
2
1
0
ppm
3.58
0.96
1.00
1.03
1.07
1.07
1.07
2.95
2.99
166.4
152.3
136.6
129.2
128.9
127.1
83.4
77.4
77.2
76.9
64.2
35.1
28.8
28.5
22.4
Br
Bn
SI-55
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

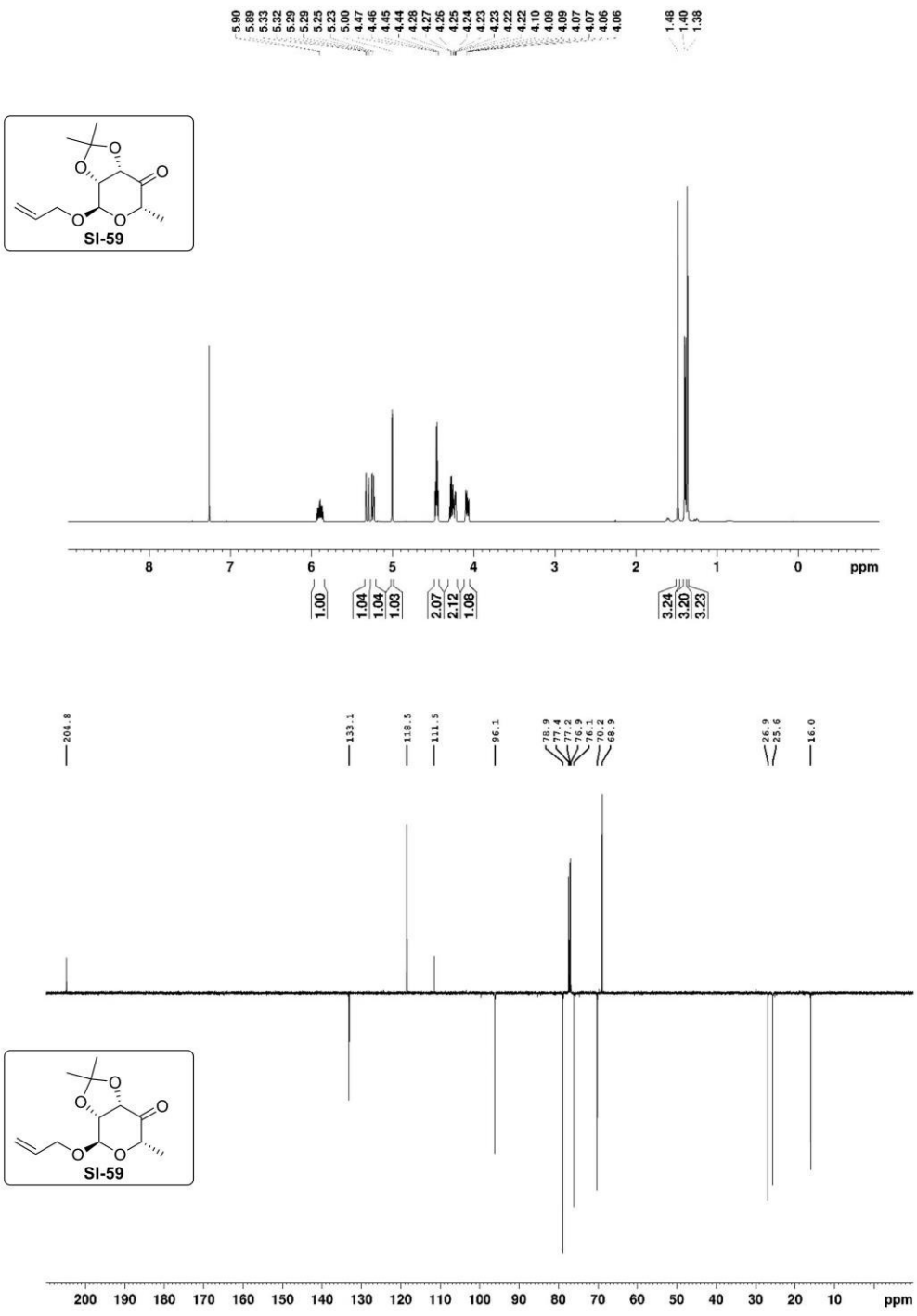

7.31
7.30
7.29
7.28
7.26
4.55
4.54
4.53
4.52
4.08
4.06
4.05
4.02
3.83
3.82
3.80
3.79
3.60
3.57
3.55
3.53
3.20
3.19
3.17
3.16
2.92
2.90
2.89
2.87
1.40
1.37
OMe
OMe
Bn
49
ppm
3.78
1.02
1.00
1.00
2.93
2.97
1.00
1.01
1.01
3.00
3.06
165.1
165.0
152.8
136.9
129.2
128.8
127.0
82.8
77.4
77.2
76.9
64.1
53.5
53.4
53.3
53.3
35.3
34.4
33.3
28.5
22.4
OMe
OMe
Bn
49
ppm

S137

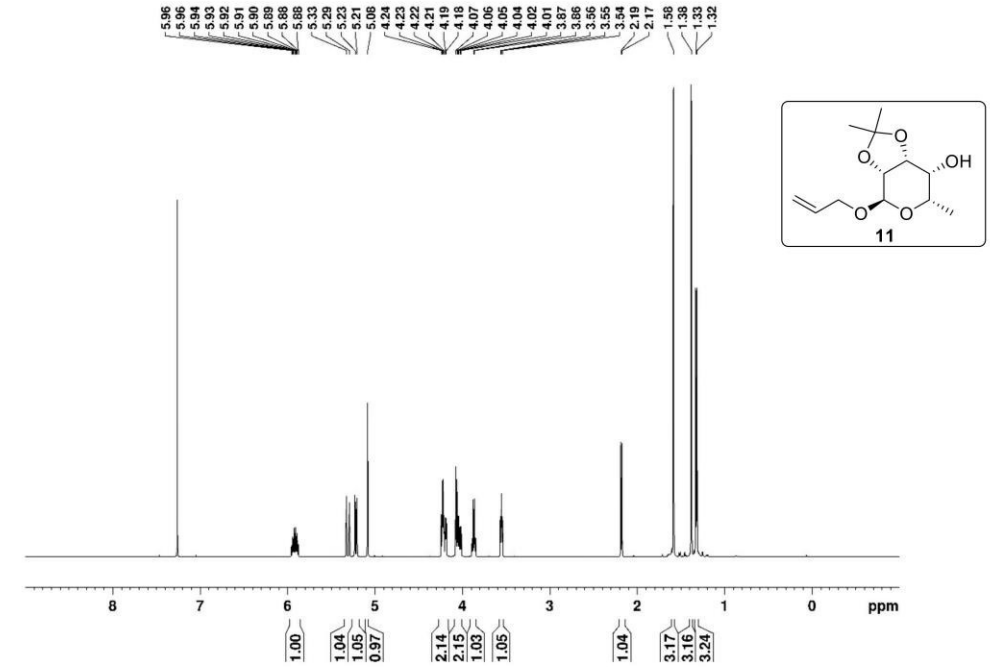

OH
HO
OH
O
O
SI-58
ppm
OH
HO
OH
O
O
SI-58
ppm

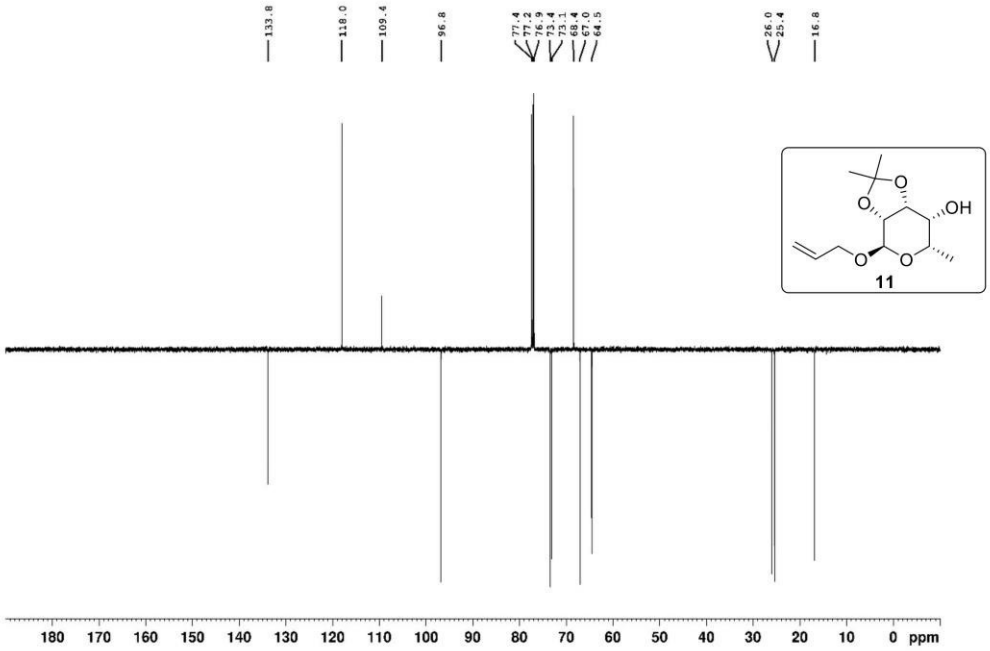

OH
55
ppm


OH
55
ppm

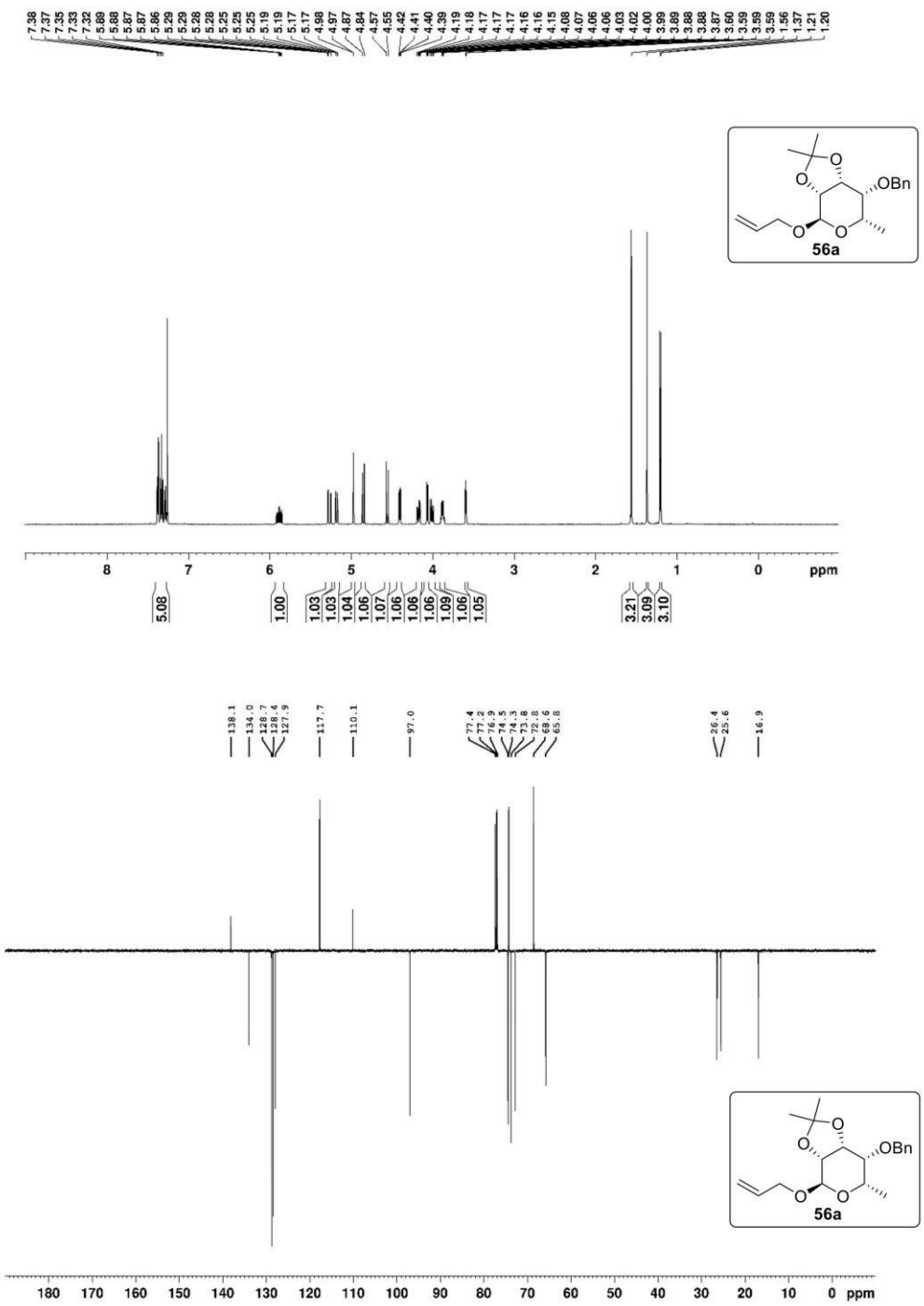

5.90
5.89
5.33
5.32
5.29
5.29
5.25
5.23
5.00
4.47
4.46
4.45
4.44
4.28
4.27
4.26
4.25
4.24
4.23
4.23
4.22
4.22
4.10
4.09
4.09
4.07
4.07
4.06
4.06
1.48
1.40
1.38
SI-59
8
7
6
5
4
3
2
1
0
ppm
1.00
1.04
1.04
1.03
2.07
2.12
1.08
3.24
3.20
3.23
204.8
133.1
118.5
111.5
96.1
78.9
77.4
77.2
76.9
76.1
70.2
68.9
26.9
25.6
16.0
SI-59
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

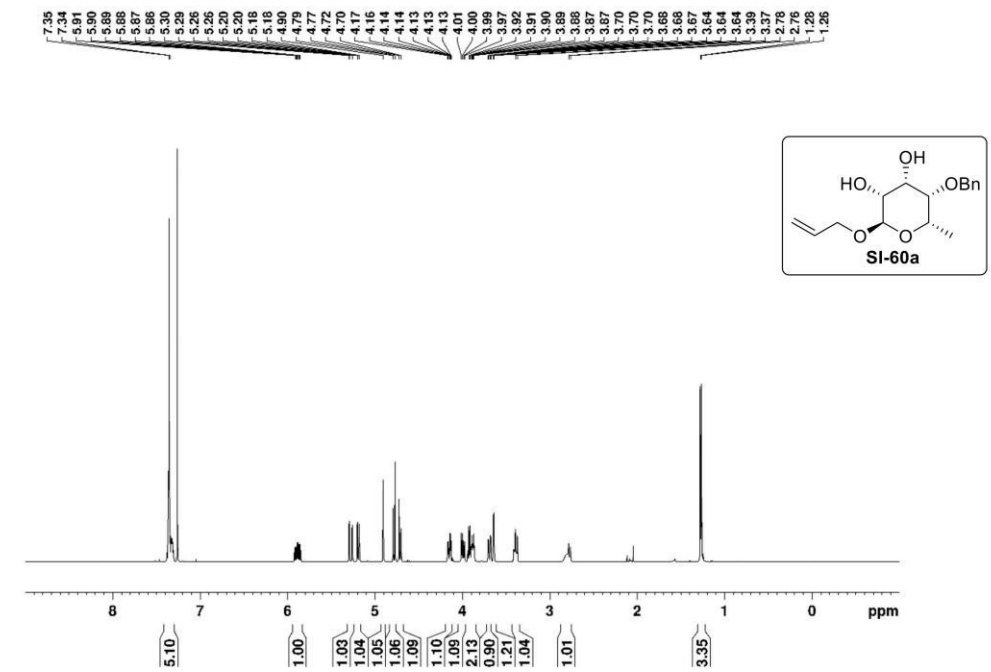
S140

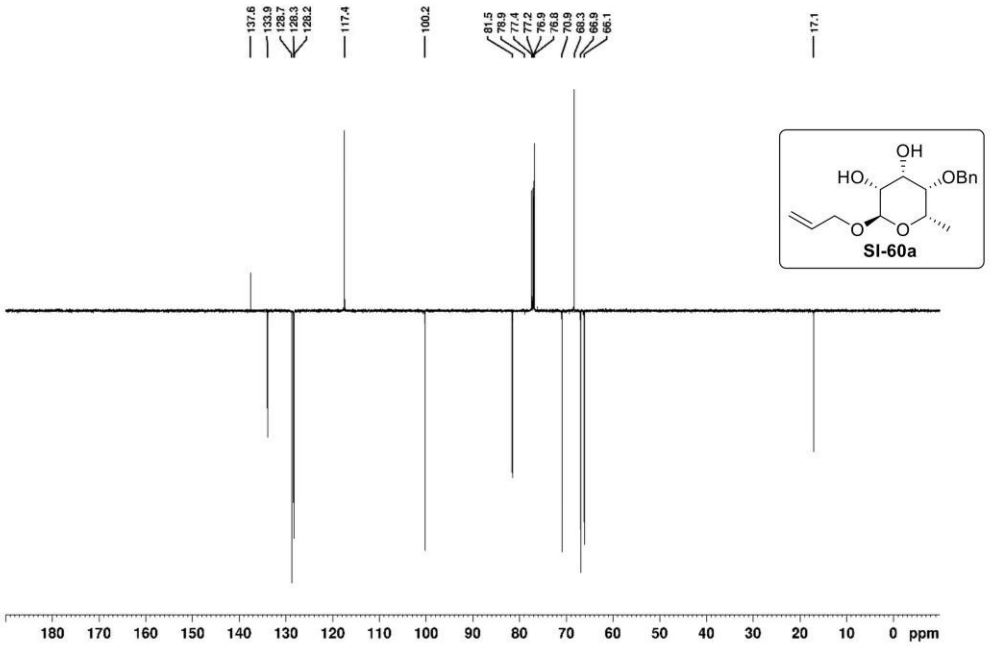

5.96
5.96
5.94
5.94
5.93
5.92
5.91
5.90
5.89
5.88
5.88
5.33
5.29
5.23
5.21
5.06
4.24
4.23
4.22
4.21
4.19
4.18
4.07
4.06
4.05
4.04
4.02
4.01
3.87
3.86
3.56
3.55
3.54
2.19
2.17
1.58
1.38
1.33
1.32
OH
O
O
O
O
11
8
7
6
5
4
3
2
1
0
ppm
1.00
1.04
1.05
0.97
2.14
2.15
1.03
1.05
1.04
3.17
3.16
3.24


133.8
118.0
109.4
96.8
77.4
77.2
76.9
73.4
73.1
68.4
67.0
64.5
26.0
25.4
16.8
OH
O
O
O
O
11
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

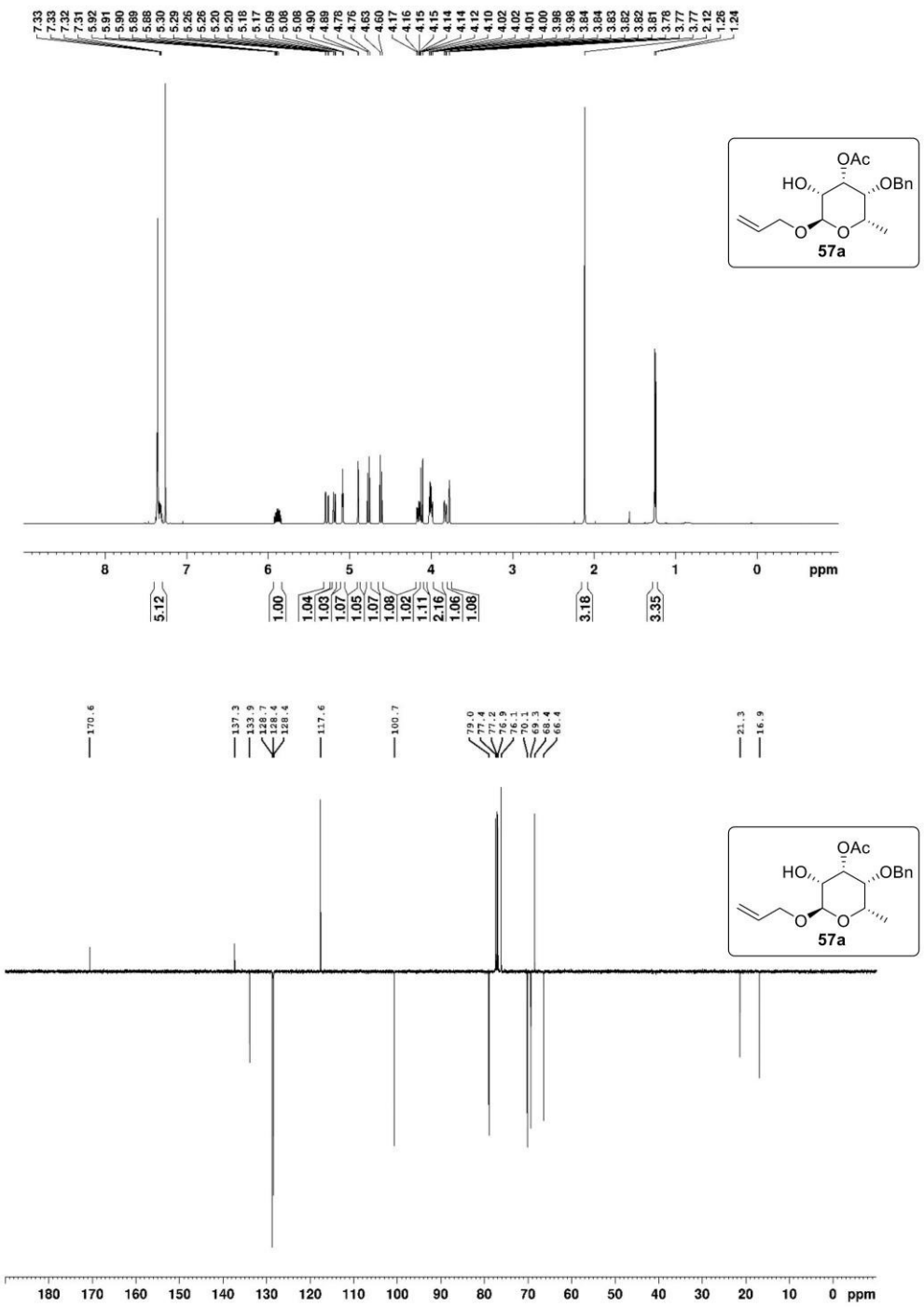
S141

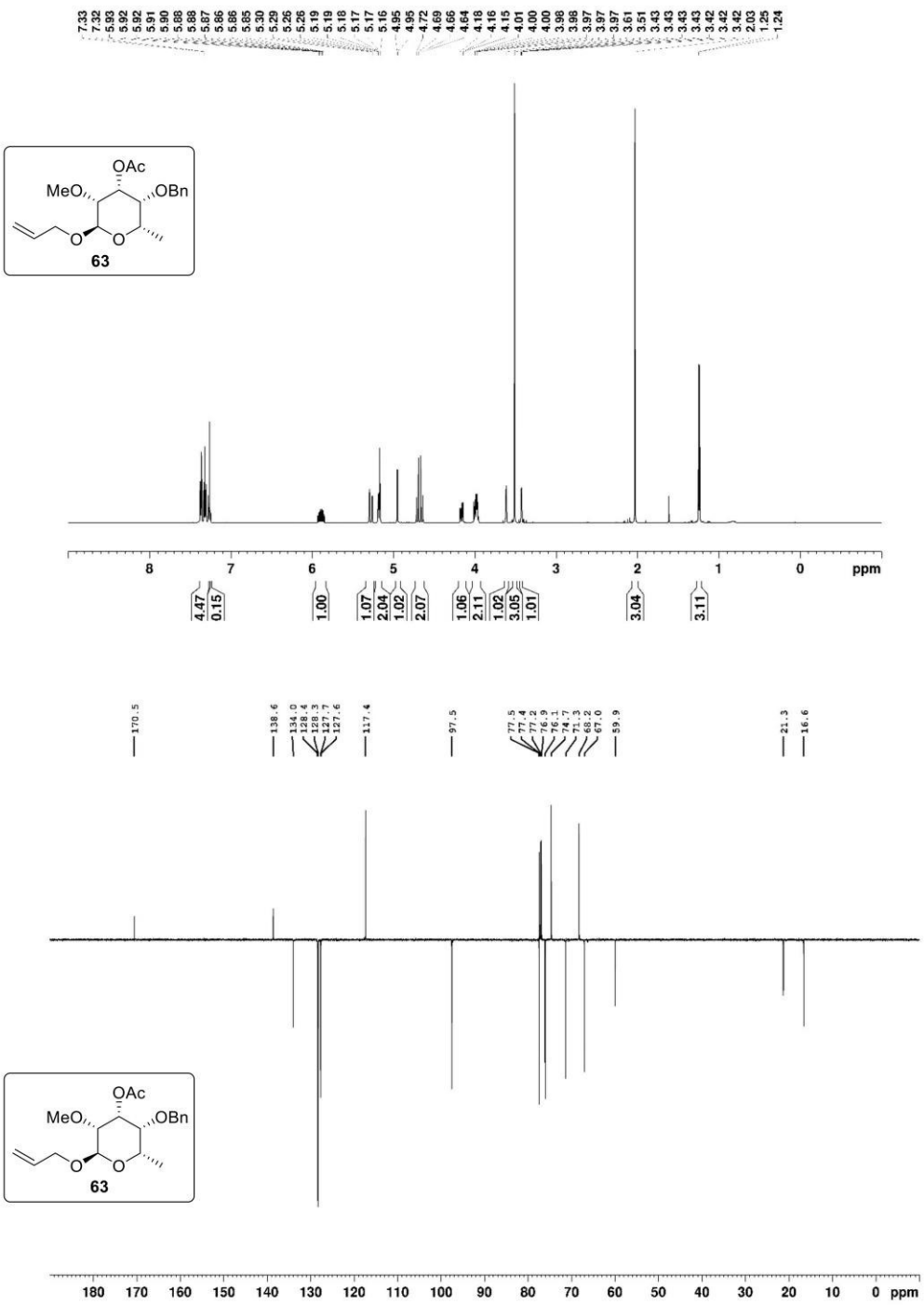

OBn
56a
ppm
OBn
56a
ppm

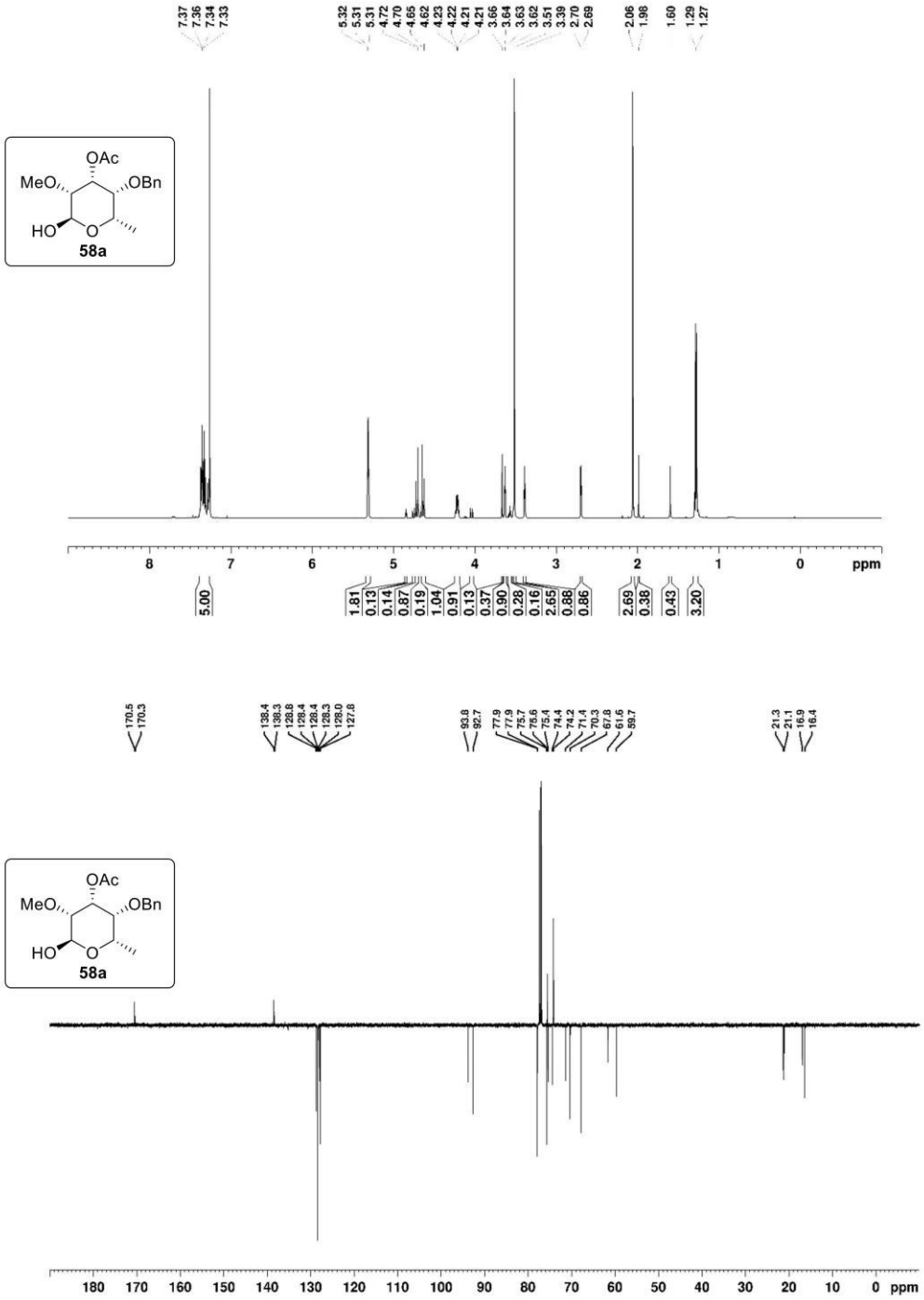

7.35
7.34
5.91
5.90
5.89
5.88
5.87
5.86
5.30
5.29
5.26
5.26
5.20
5.20
5.18
5.16
4.90
4.79
4.77
4.72
4.70
4.17
4.16
4.14
4.14
4.13
4.13
4.13
4.01
4.00
3.99
3.97
3.92
3.91
3.90
3.89
3.88
3.87
3.87
3.70
3.70
3.70
3.68
3.68
3.67
3.64
3.64
3.64
3.39
3.37
2.78
2.76
1.28
1.26
OH
HO
OBn
O
O
SI-60a
8
7
6
5
4
3
2
1
0
ppm
5.10
1.00
1.03
1.04
1.05
1.06
1.09
1.10
1.09
2.13
0.90
1.21
1.04
1.01
3.35

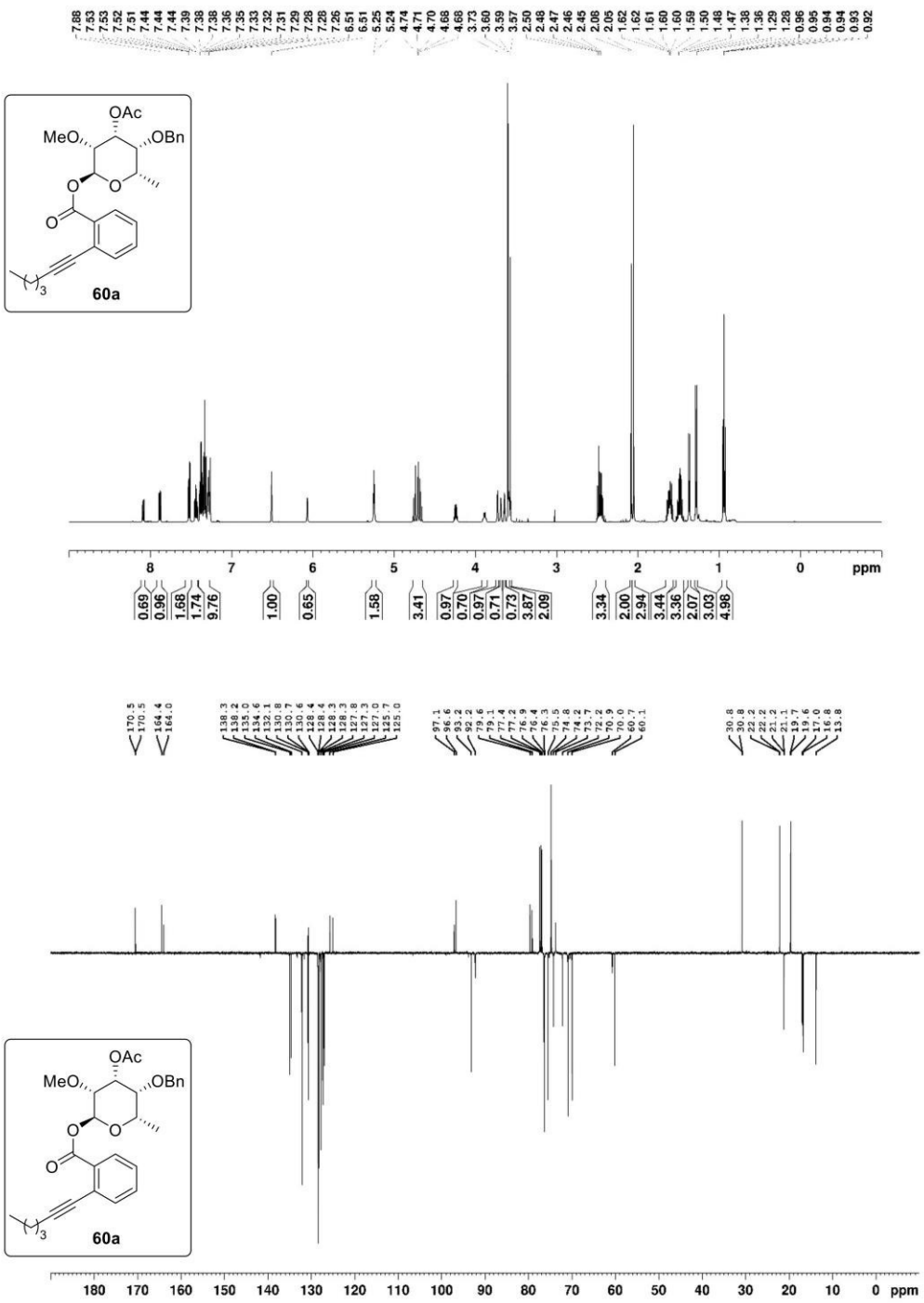

137.6
133.9
128.7
128.3
128.2
117.4
100.2
81.5
78.9
77.4
77.2
76.9
76.8
70.9
68.3
66.9
66.1
17.1
OH
HO
OBn
O
O
SI-60a
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

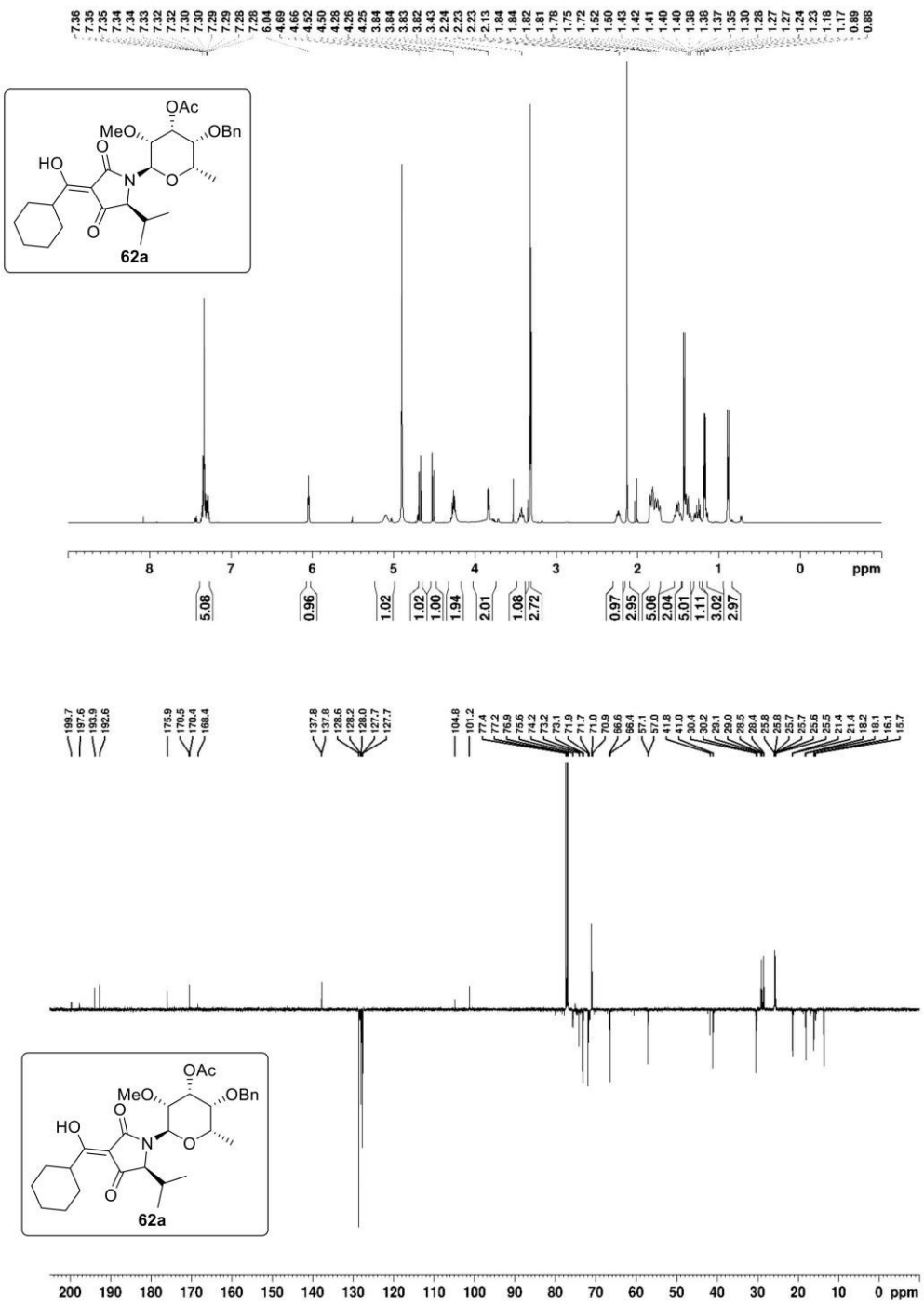
S143

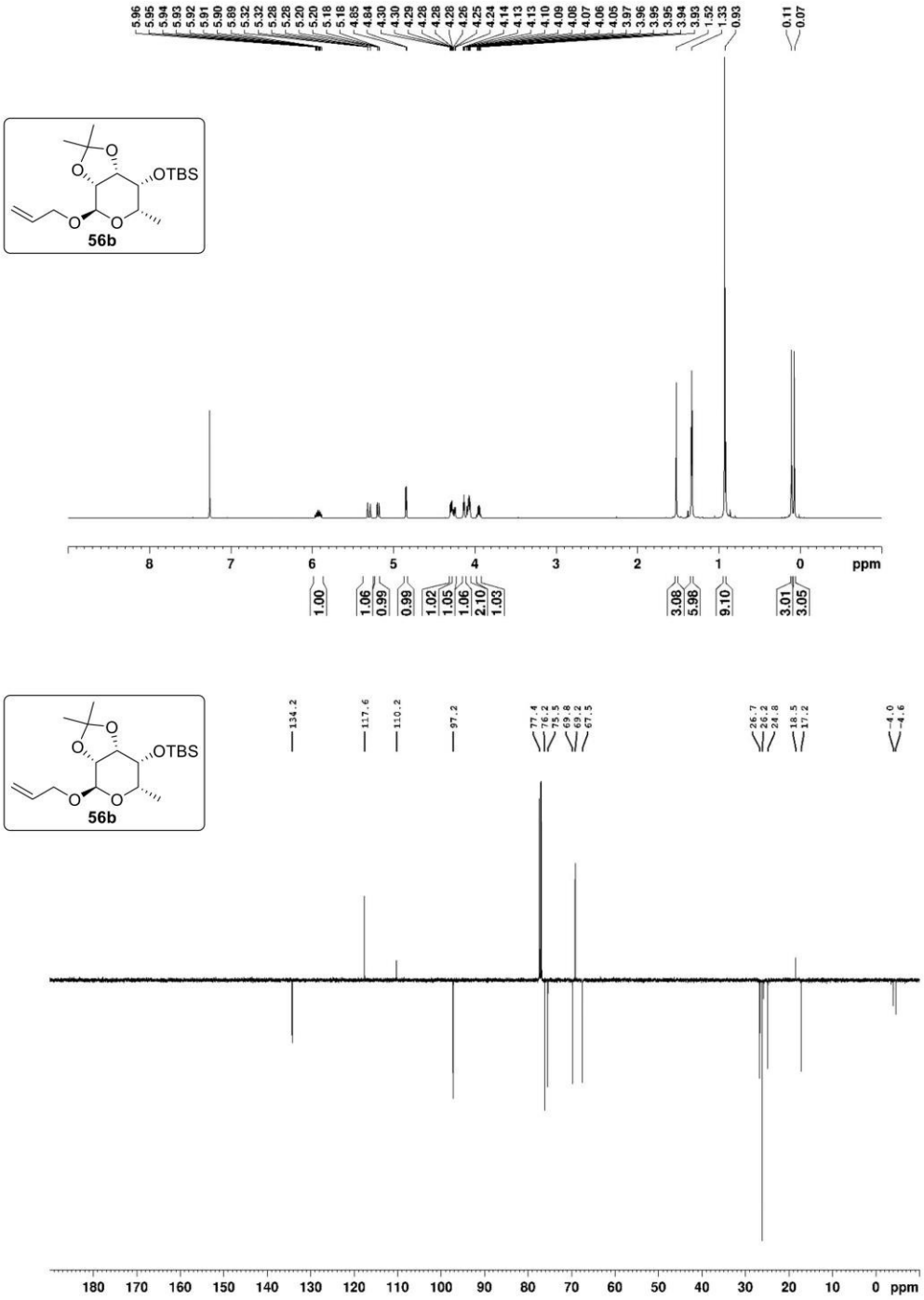

OAc
HO
OBn
O
O
57a
ppm
OAc
HO
OBn
O
O
57a
ppm

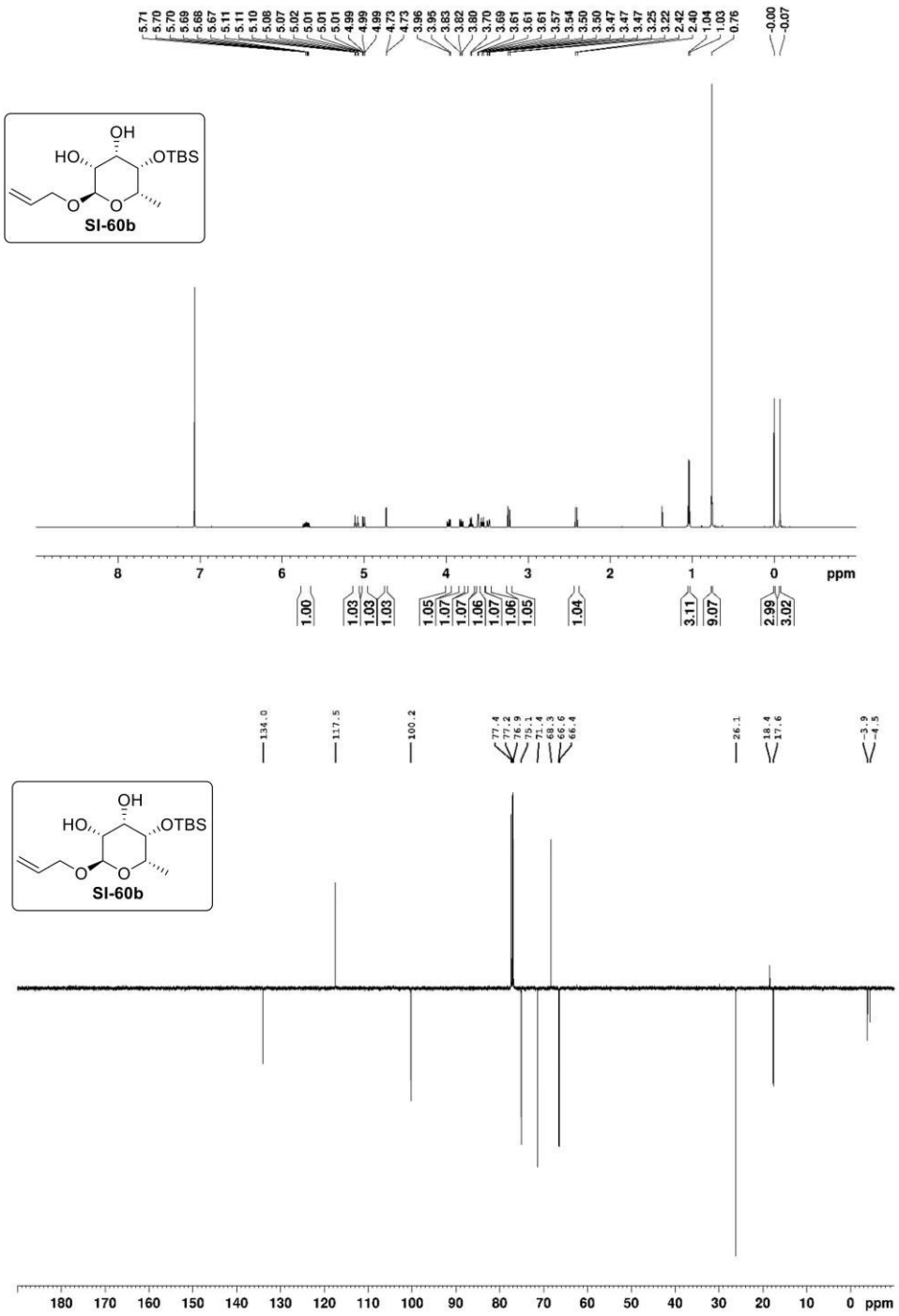

OAc
MeO
OBn
O
O
63
ppm
OAc
MeO
OBn
O
O
63
ppm

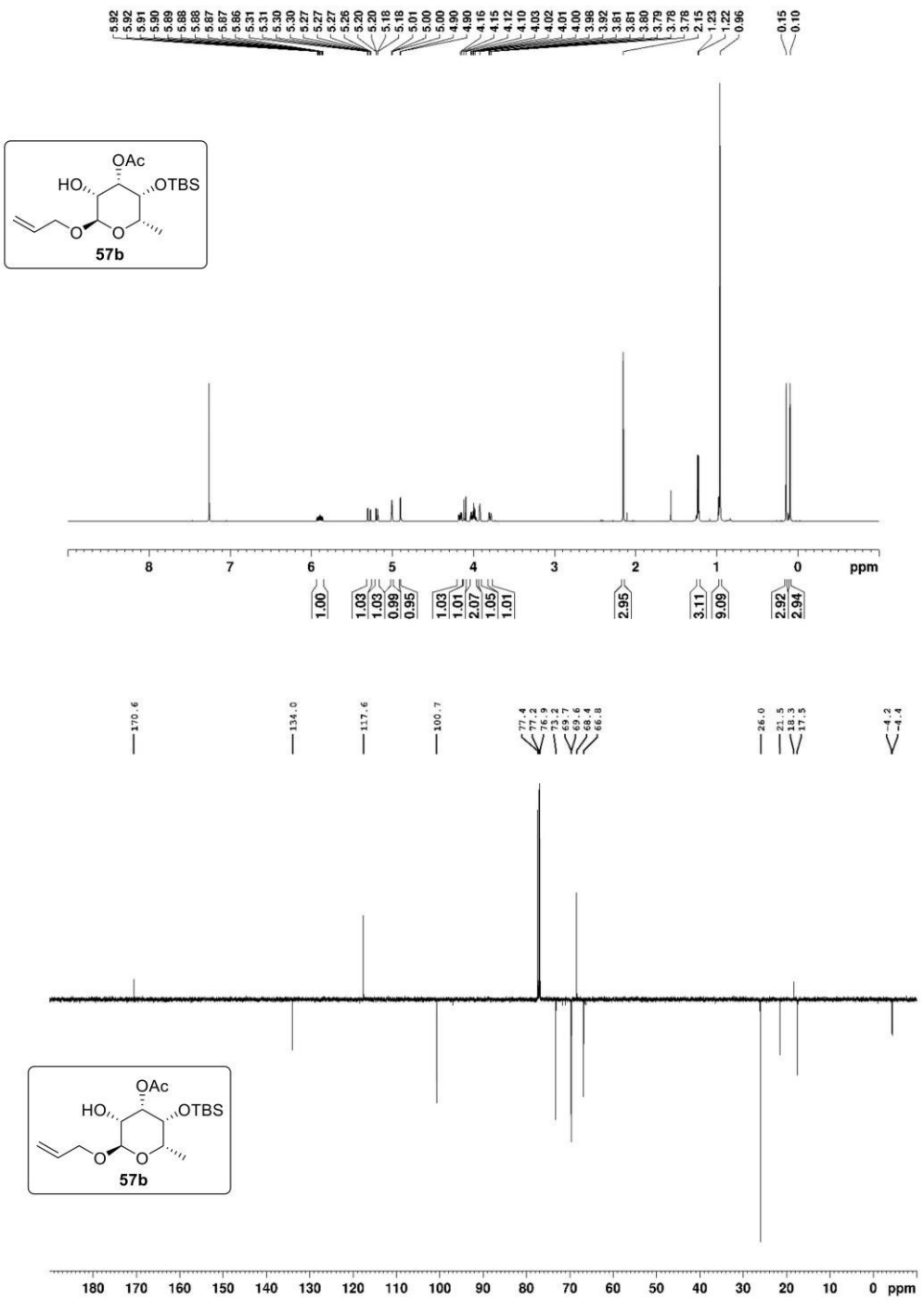

OAc
MeO
OBn
HO
O
58a
ppm
OAc
MeO
OBn
HO
O
58a
ppm

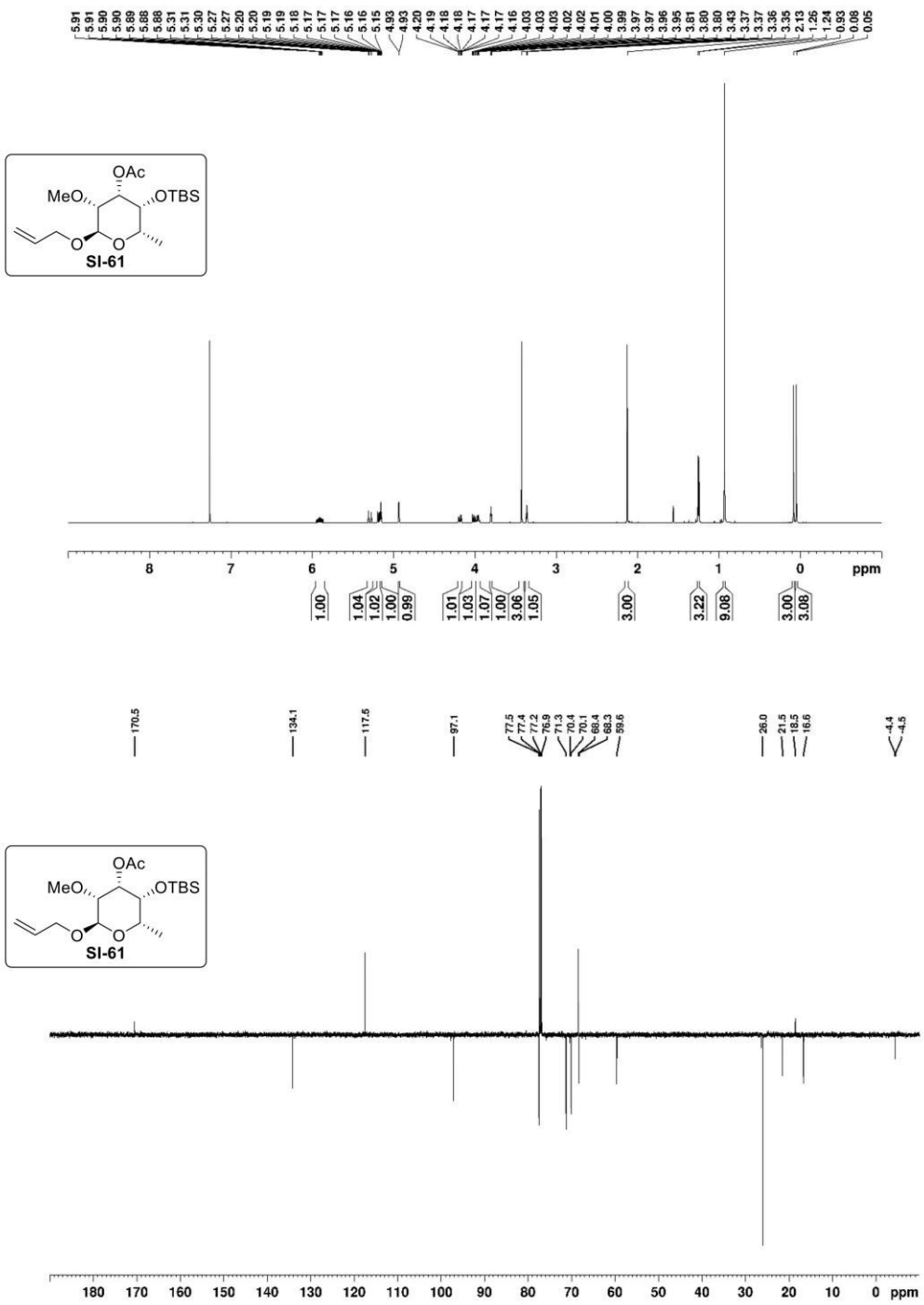

OAc
MeO
OBn
O
O
O
3
60a
ppm
OAc
MeO
OBn
O
O
O
3
60a
ppm

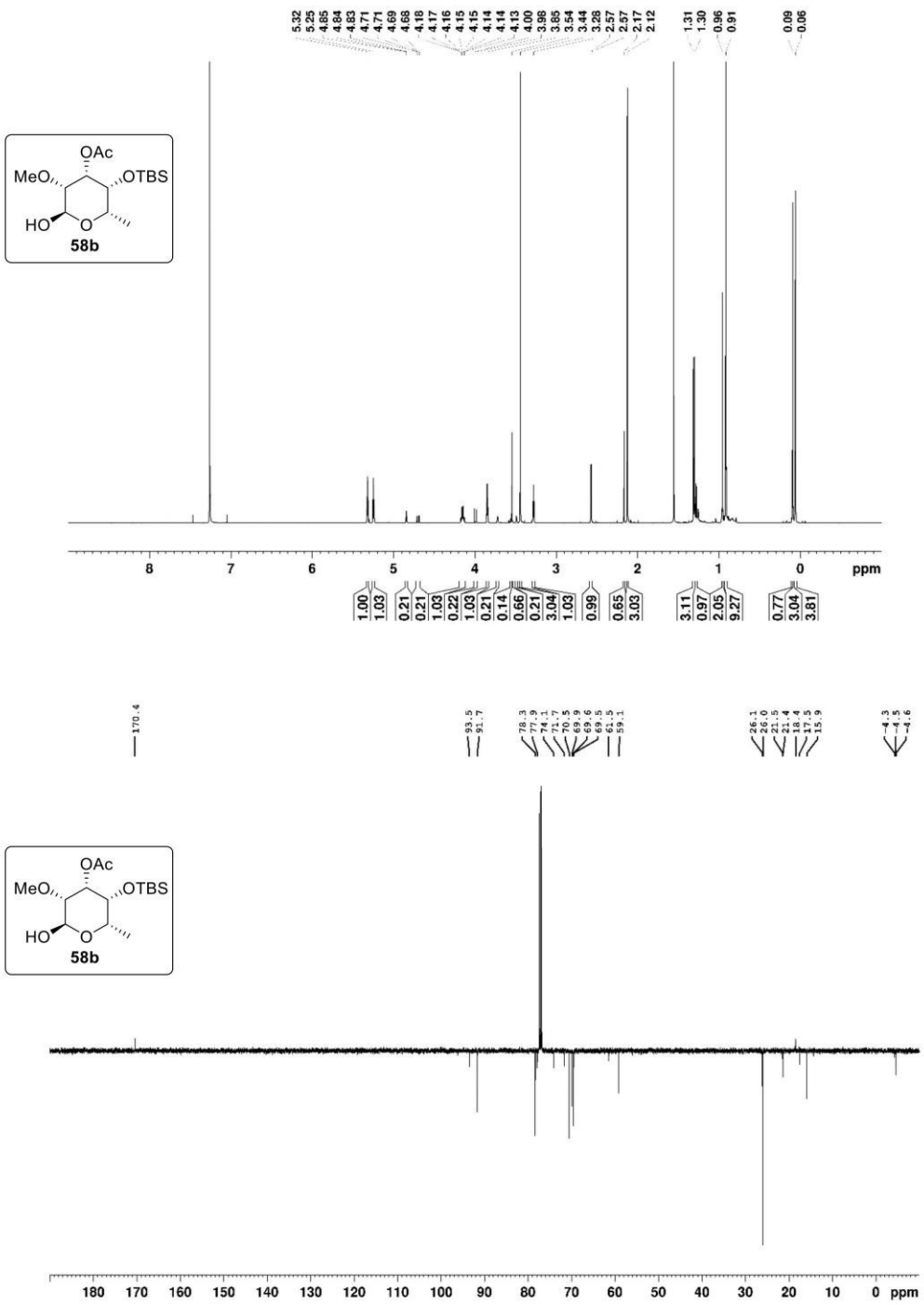

OAc
MeO
OBn
HO
O
N
O
O
62a
ppm
OAc
MeO
OBn
HO
O
N
O
O
62a
ppm

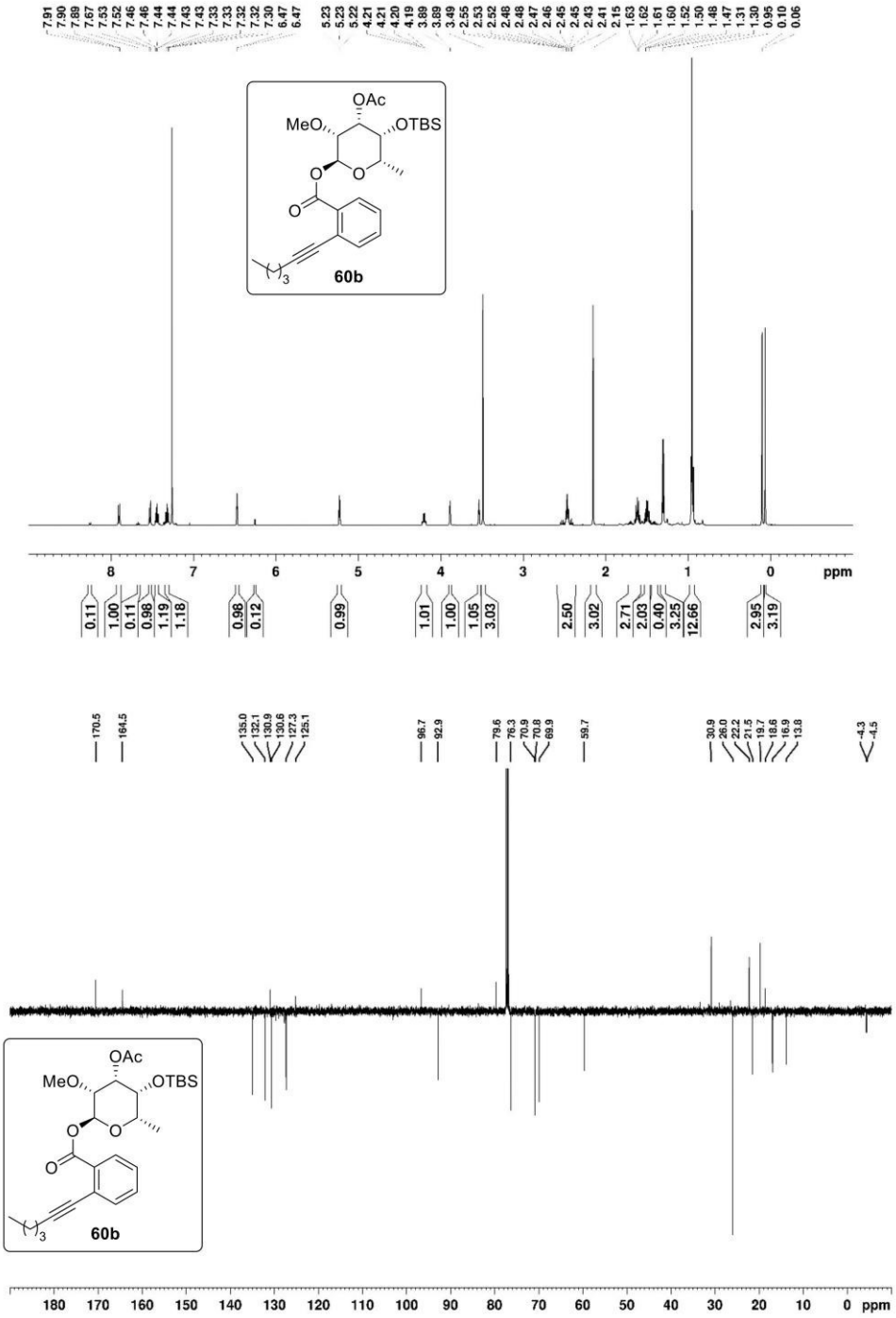

OTBS
56b
ppm
OTBS
56b
ppm

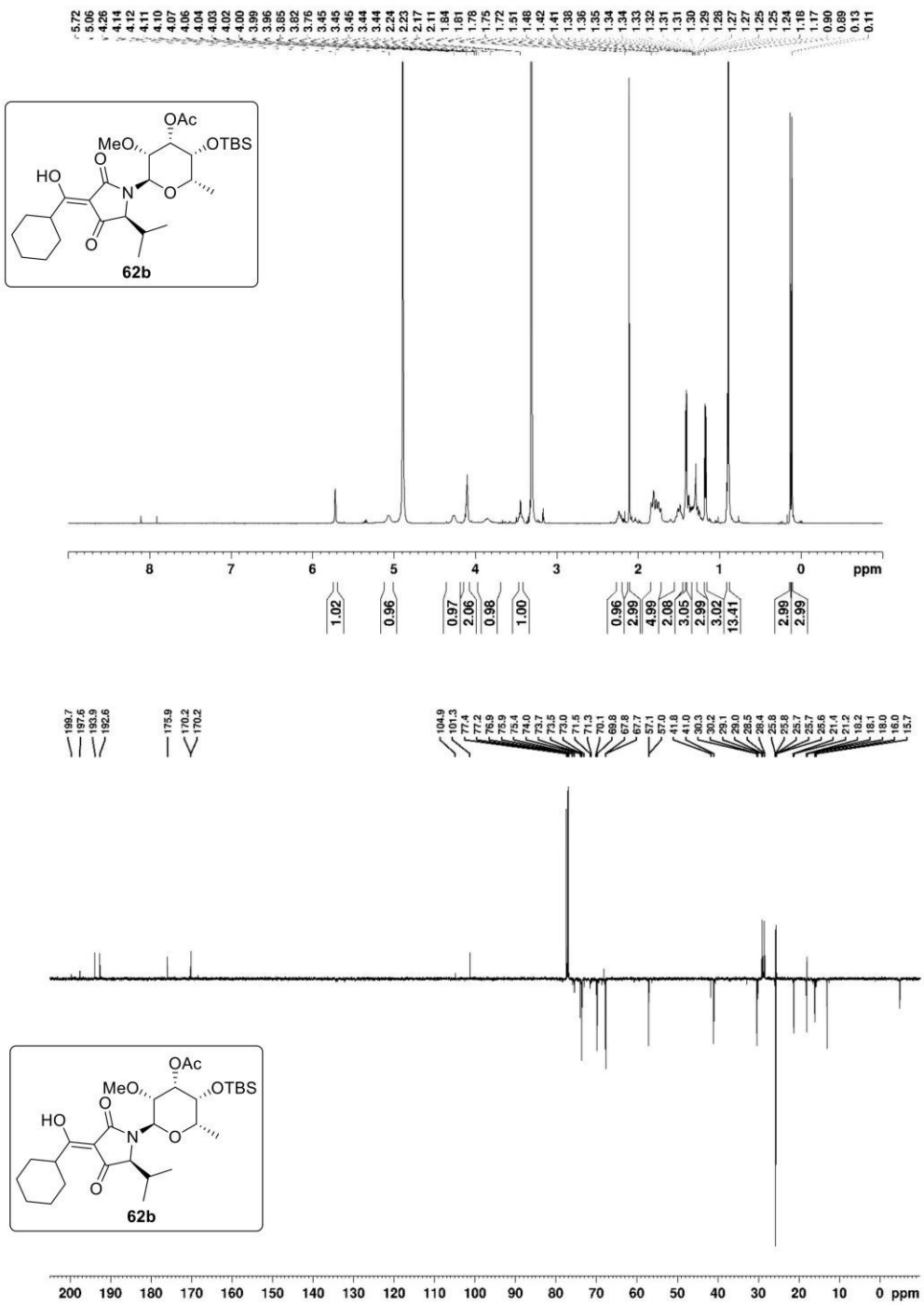

OH
HO
OTBS
O
O
SI-60b
ppm
OH
HO
OTBS
O
O
SI-60b
ppm

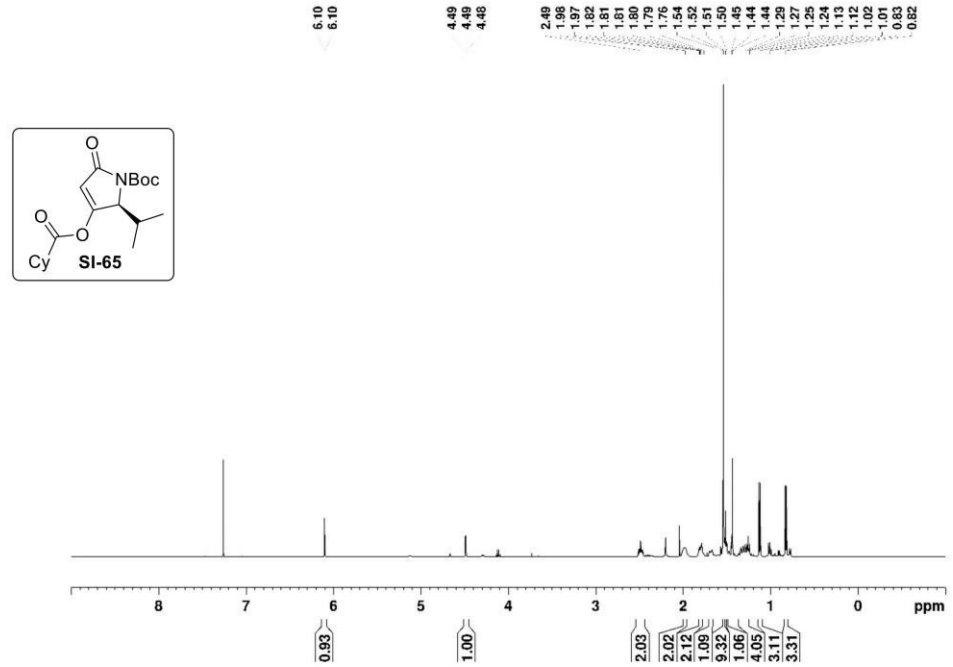

OAc
HO
OTBS
O
O
57b
ppm
OAc
HO
OTBS
O
O
57b
ppm

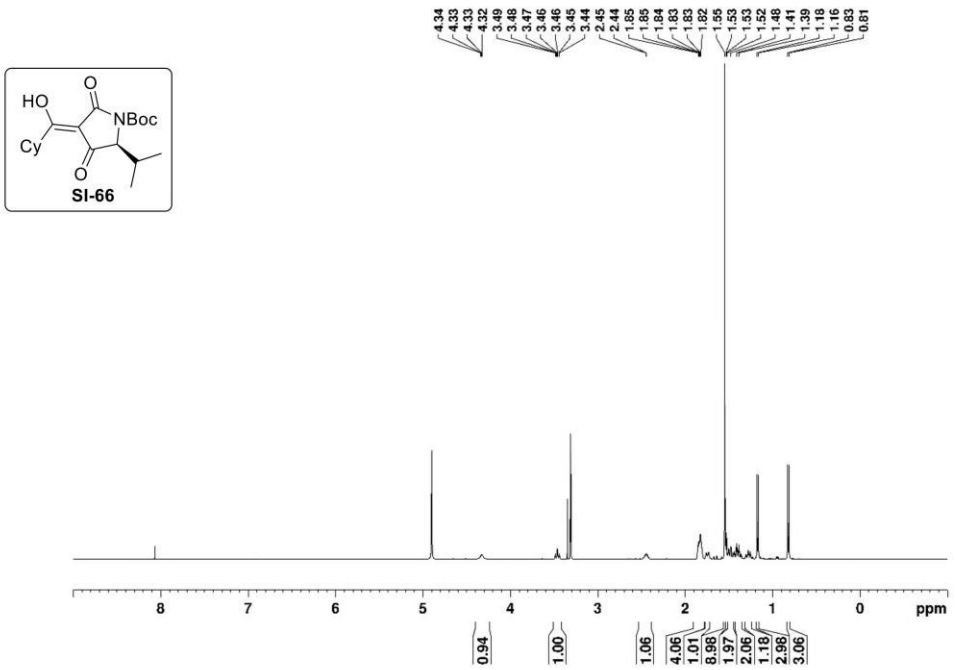

OAc
MeO
OTBS
SI-61
ppm
OAc
MeO
OTBS
SI-61
ppm


OAc
MeO
OTBS
HO
O
58b
ppm
OAc
MeO
OTBS
HO
O
58b
ppm

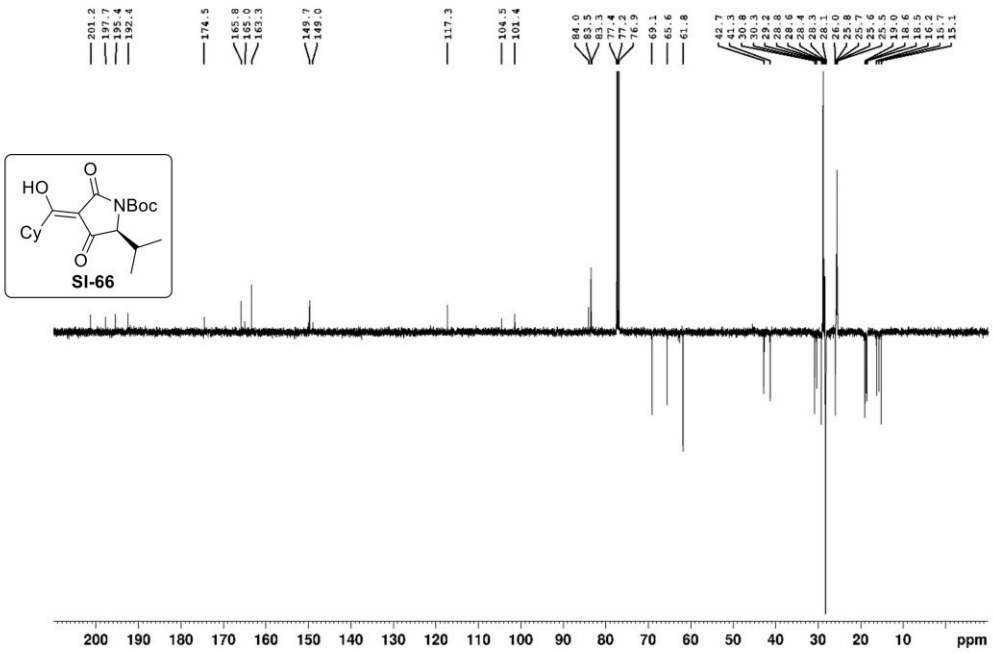

OAc
MeO
OTBS
O
O
O
60b
ppm
OAc
MeO
OTBS
O
O
O
60b
ppm

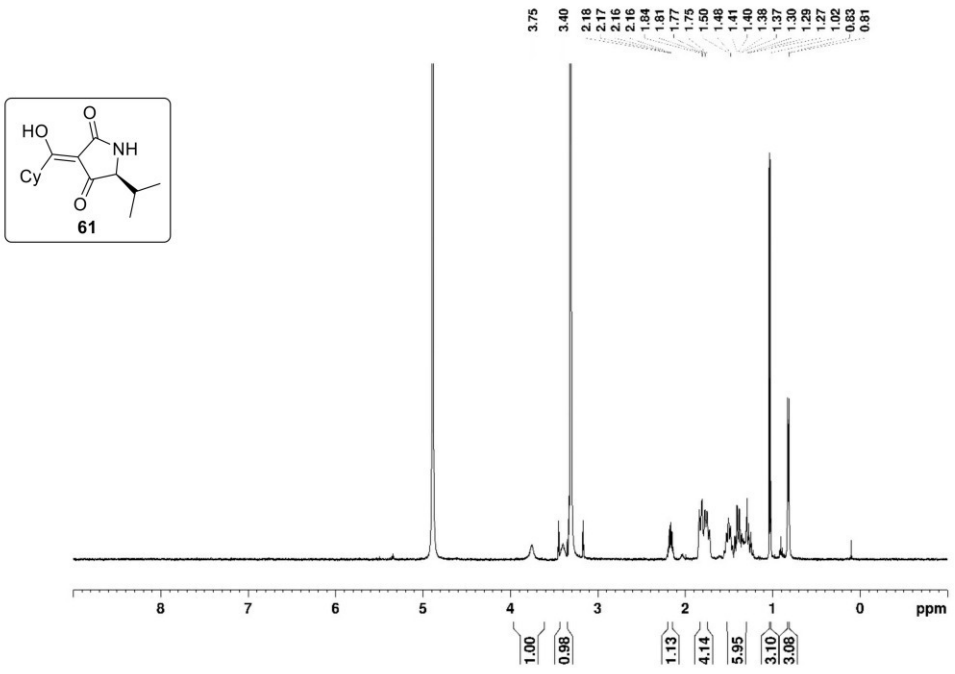

OAc
MeO
OTBS
HO
O
N
O
O
62b
ppm
1.02
0.96
0.97
2.06
0.98
1.00
0.96
2.99
4.99
2.06
3.05
2.99
3.02
13.41
2.99
2.99
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


6.10
6.10
4.49
4.49
4.48
2.49
1.98
1.97
1.82
1.81
1.81
1.80
1.79
1.76
1.54
1.52
1.51
1.50
1.45
1.44
1.44
1.29
1.27
1.25
1.24
1.13
1.12
1.02
1.01
0.83
0.82
O
NBoc
O
O
Cy
SI-65
8
7
6
5
4
3
2
1
0
ppm
0.93
1.00
2.03
2.02
2.12
1.09
9.32
1.06
4.05
3.11
3.31

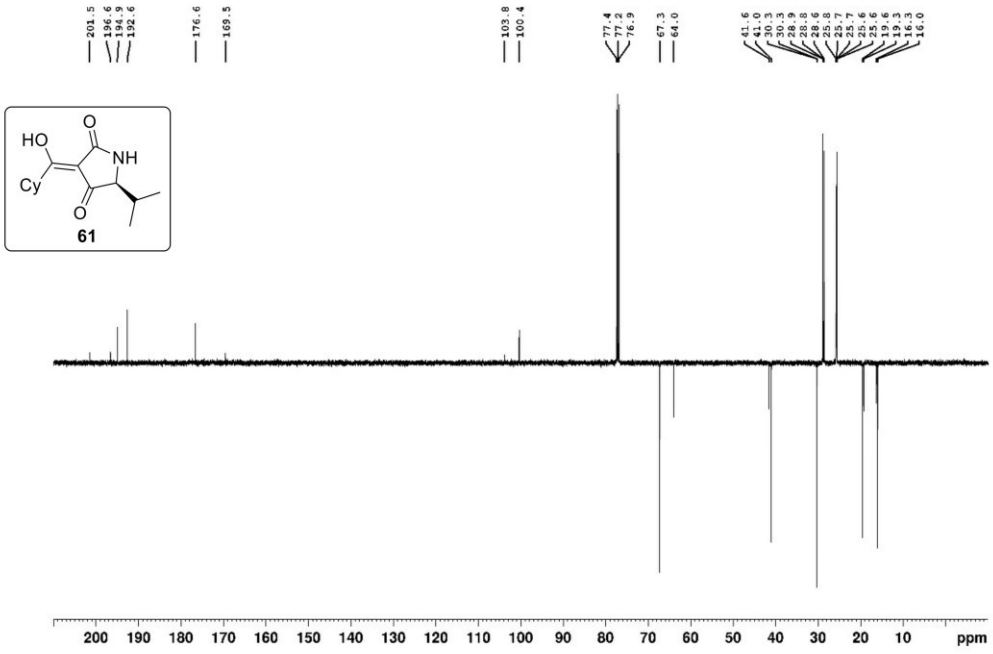

4.34
4.33
4.33
4.32
3.49
3.48
3.47
3.46
3.46
3.45
3.44
2.45
2.44
1.85
1.85
1.84
1.83
1.83
1.82
1.55
1.53
1.53
1.52
1.46
1.41
1.39
1.18
1.16
0.83
0.81
HO
O
NBoc
Cy
O
SI-66
8
7
6
5
4
3
2
1
0
ppm
0.94
1.00
1.06
4.06
1.01
8.98
1.97
2.06
1.18
2.96
3.06

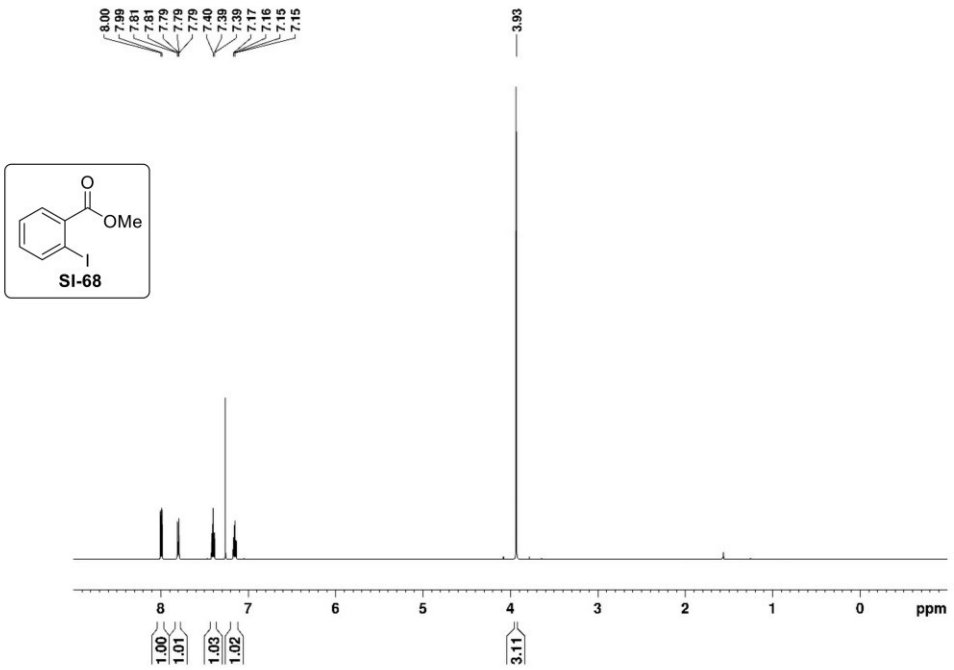
S156

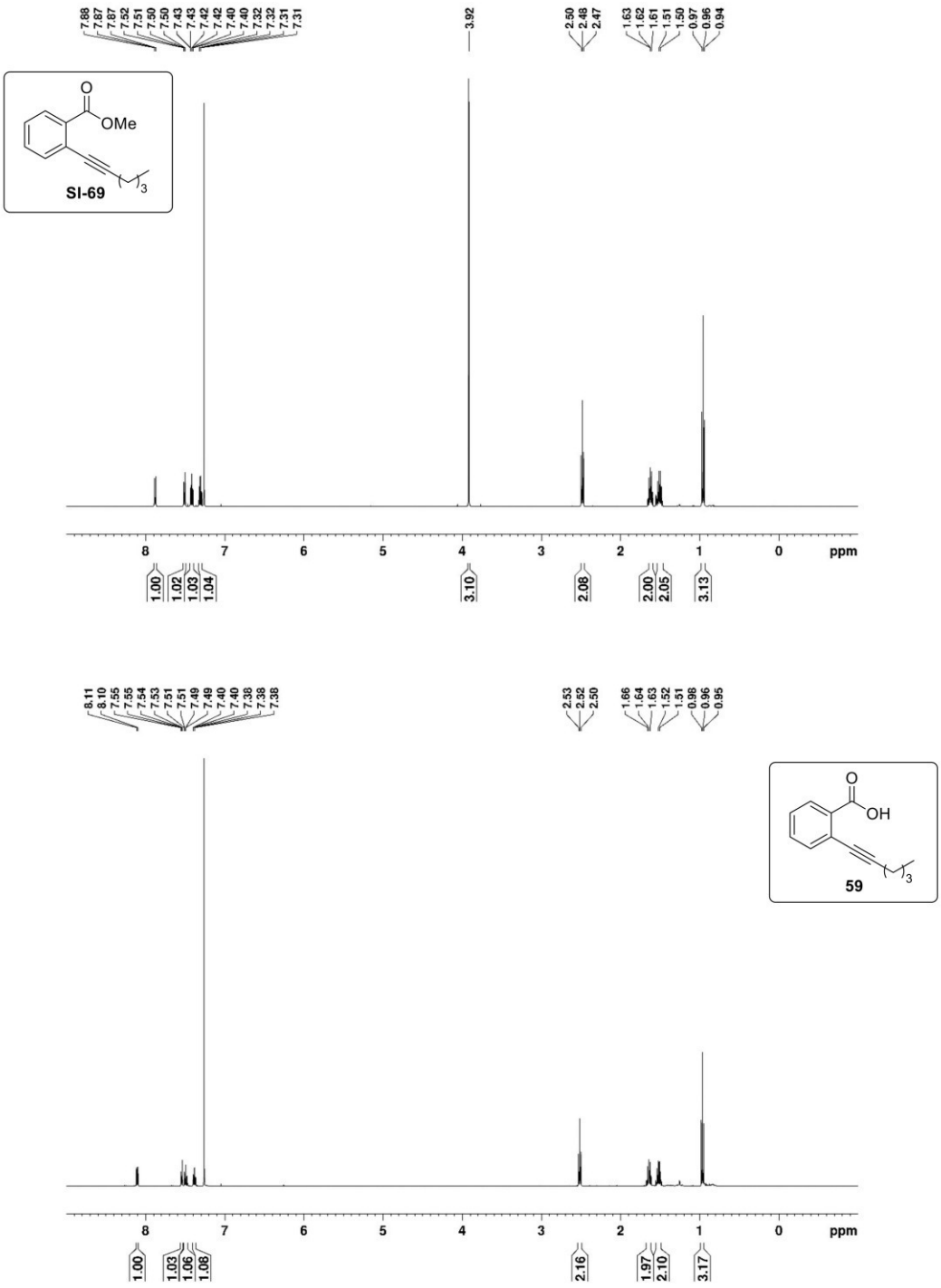

HO
O
NBoc
Cy
O
SI-66
201.2
197.7
195.4
192.4
174.5
165.8
165.0
163.3
149.7
149.0
117.3
104.5
101.4
84.0
83.5
83.3
77.4
77.2
76.9
69.1
65.6
61.8
42.7
41.3
30.8
30.3
29.2
28.8
28.6
28.4
28.3
28.1
26.0
25.8
25.7
25.6
25.5
19.0
18.6
18.5
16.2
15.7
15.1
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

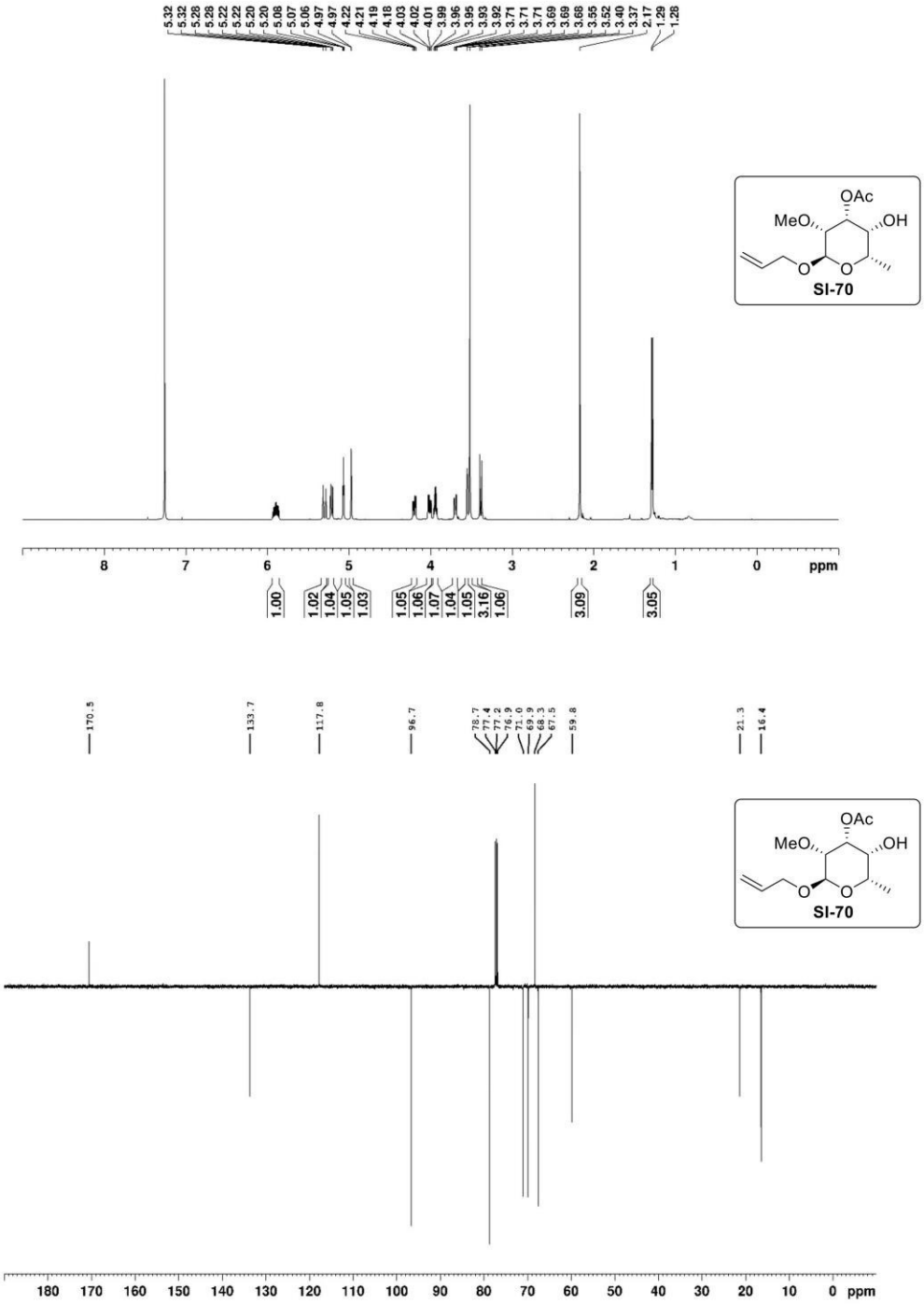

HO
O
NH
Cy
O
61
3.75
3.40
2.18
2.17
2.16
2.16
1.84
1.81
1.77
1.75
1.50
1.48
1.41
1.40
1.38
1.37
1.30
1.29
1.27
1.02
0.83
0.81
8 7 6 5 4 3 2 1 0 ppm
1.00
0.98
1.13
4.14
5.95
3.10
3.08

S157


201.5
196.6
194.9
192.6
176.6
169.5
103.8
100.4
77.4
77.2
76.9
67.3
64.0
41.6
41.0
30.3
30.3
28.9
28.8
28.6
25.8
25.7
25.7
25.6
25.5
19.6
19.3
16.3
16.0
HO
O
NH
Cy
O
61
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


8.00
7.99
7.81
7.81
7.79
7.79
7.79
7.40
7.39
7.39
7.17
7.16
7.15
7.15
3.93
O
OMe
I
SI-68
8 7 6 5 4 3 2 1 0 ppm
1.00
1.01
1.03
1.02
3.11


7.88
7.87
7.87
7.52
7.51
7.50
7.50
7.43
7.43
7.42
7.42
7.40
7.40
7.32
7.32
7.31
7.31
3.92
2.50
2.48
2.47
1.63
1.62
1.61
1.51
1.50
0.97
0.96
0.94
O
OMe
SI-69
3
8
7
6
5
4
3
2
1
0
ppm
1.00
1.02
1.03
1.04
3.10
2.08
2.00
2.05
3.13
8.11
8.10
7.55
7.55
7.54
7.53
7.51
7.51
7.49
7.49
7.40
7.40
7.38
7.38
7.38
2.53
2.52
2.50
1.66
1.64
1.63
1.52
1.51
0.98
0.96
0.95
O
OH
59
3
8
7
6
5
4
3
2
1
0
ppm
1.00
1.03
1.06
1.08
2.16
1.97
2.10
3.17


5.32
5.32
5.28
5.28
5.22
5.22
5.20
5.20
5.08
5.07
5.06
4.97
4.97
4.22
4.21
4.19
4.18
4.03
4.02
4.01
3.99
3.96
3.95
3.93
3.92
3.71
3.71
3.71
3.71
3.69
3.69
3.68
3.55
3.52
3.40
3.37
2.17
1.29
1.28
OAc
MeO
OH
O
O
SI-70
8
7
6
5
4
3
2
1
0
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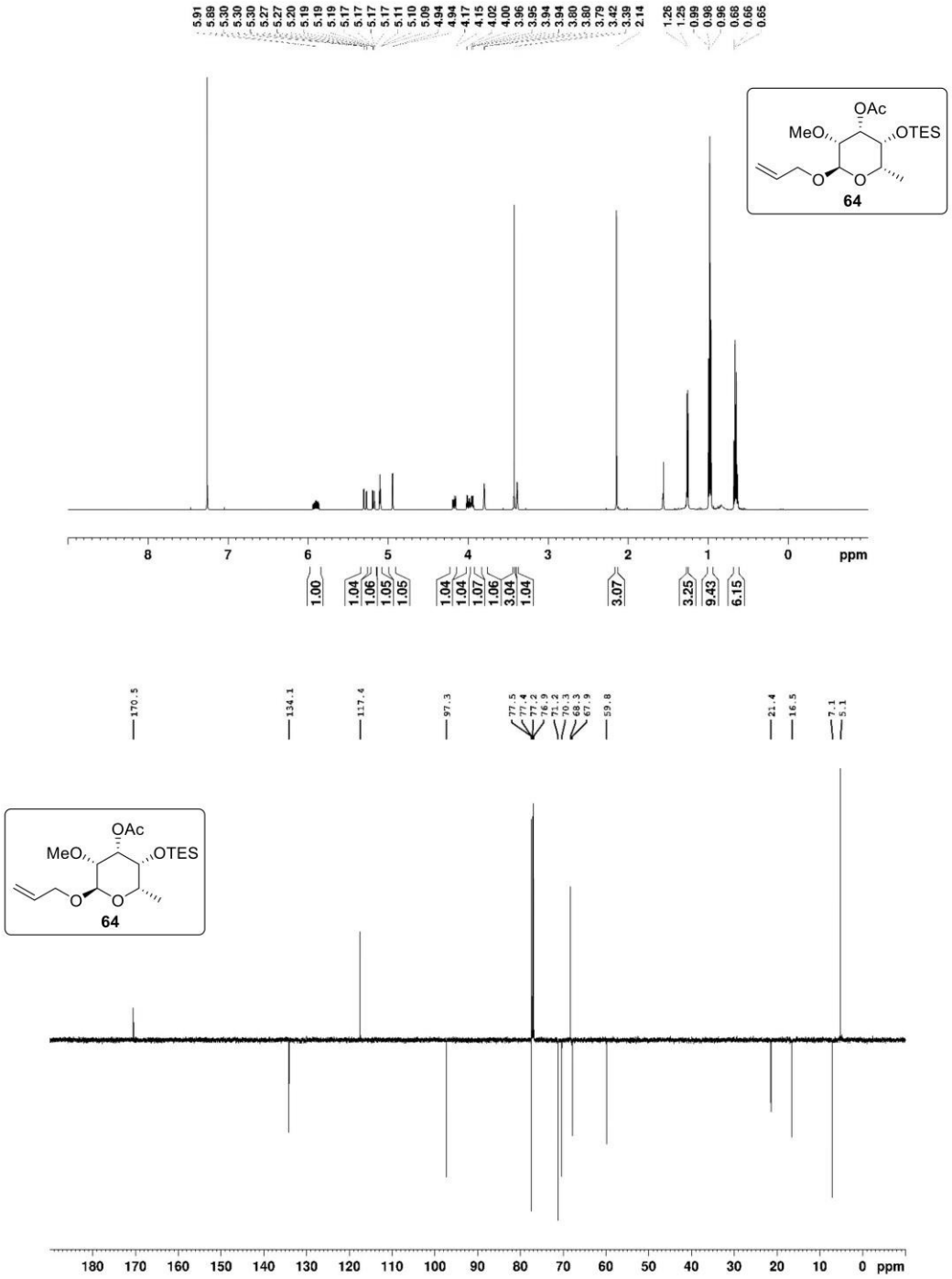
S160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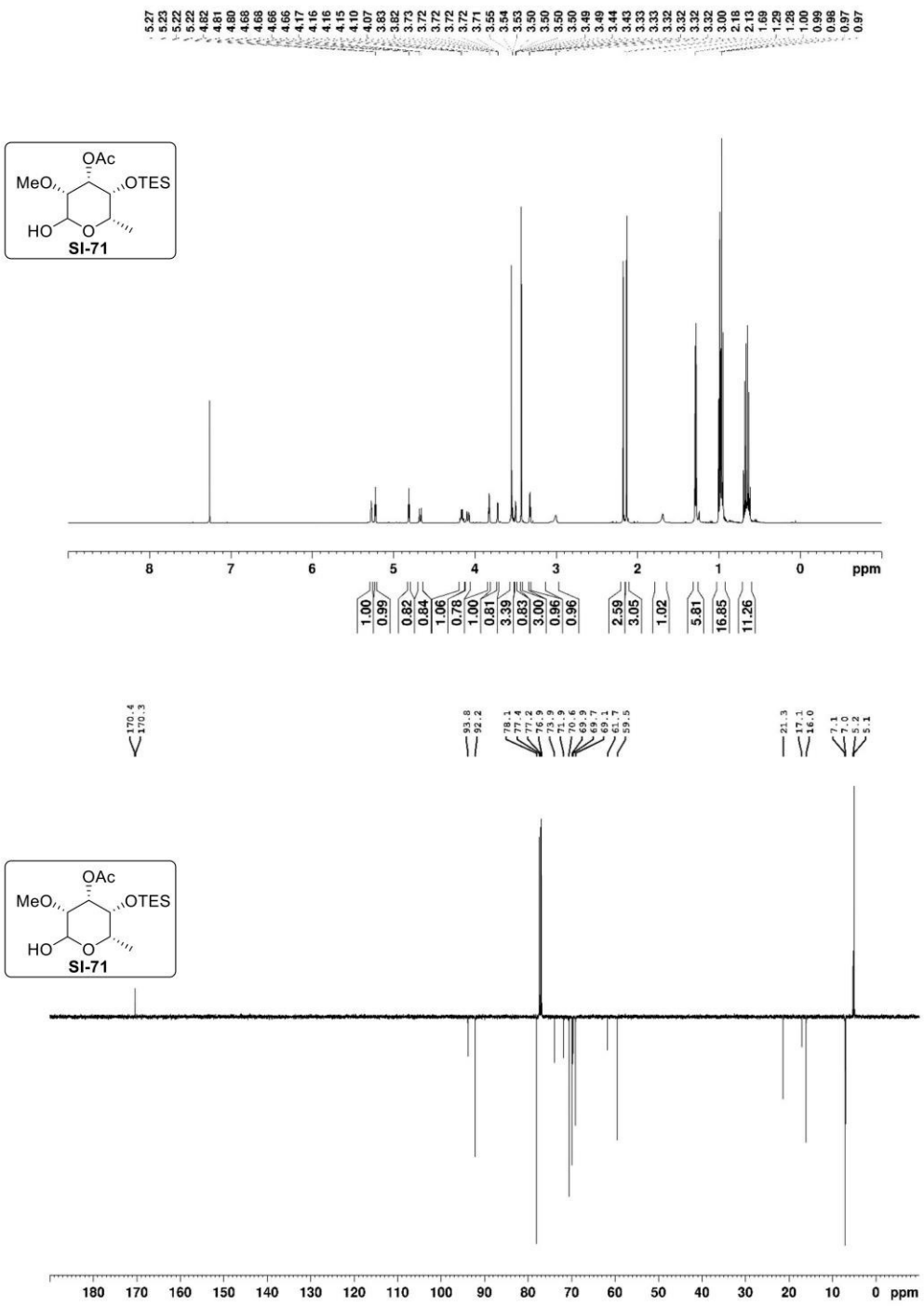
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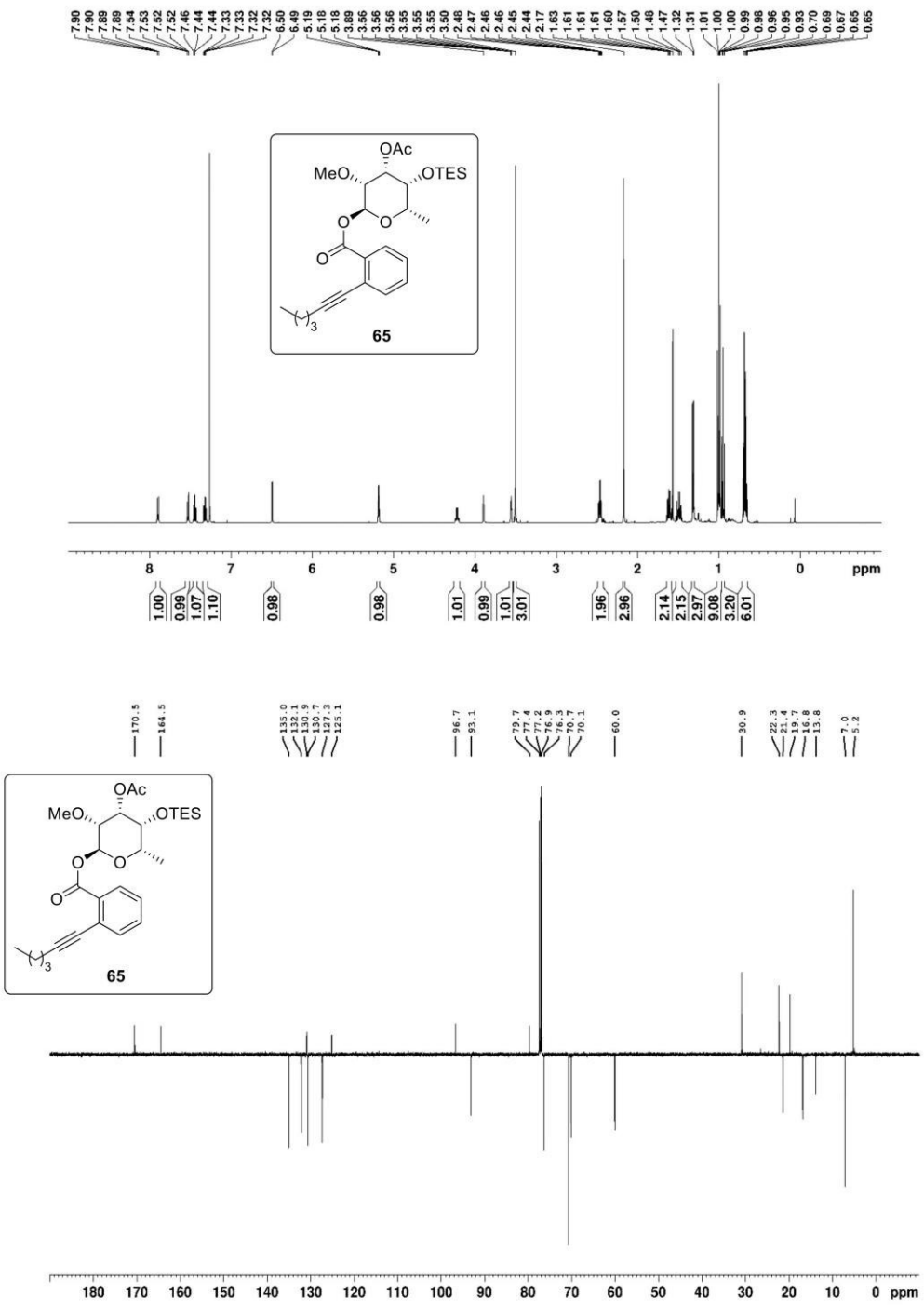
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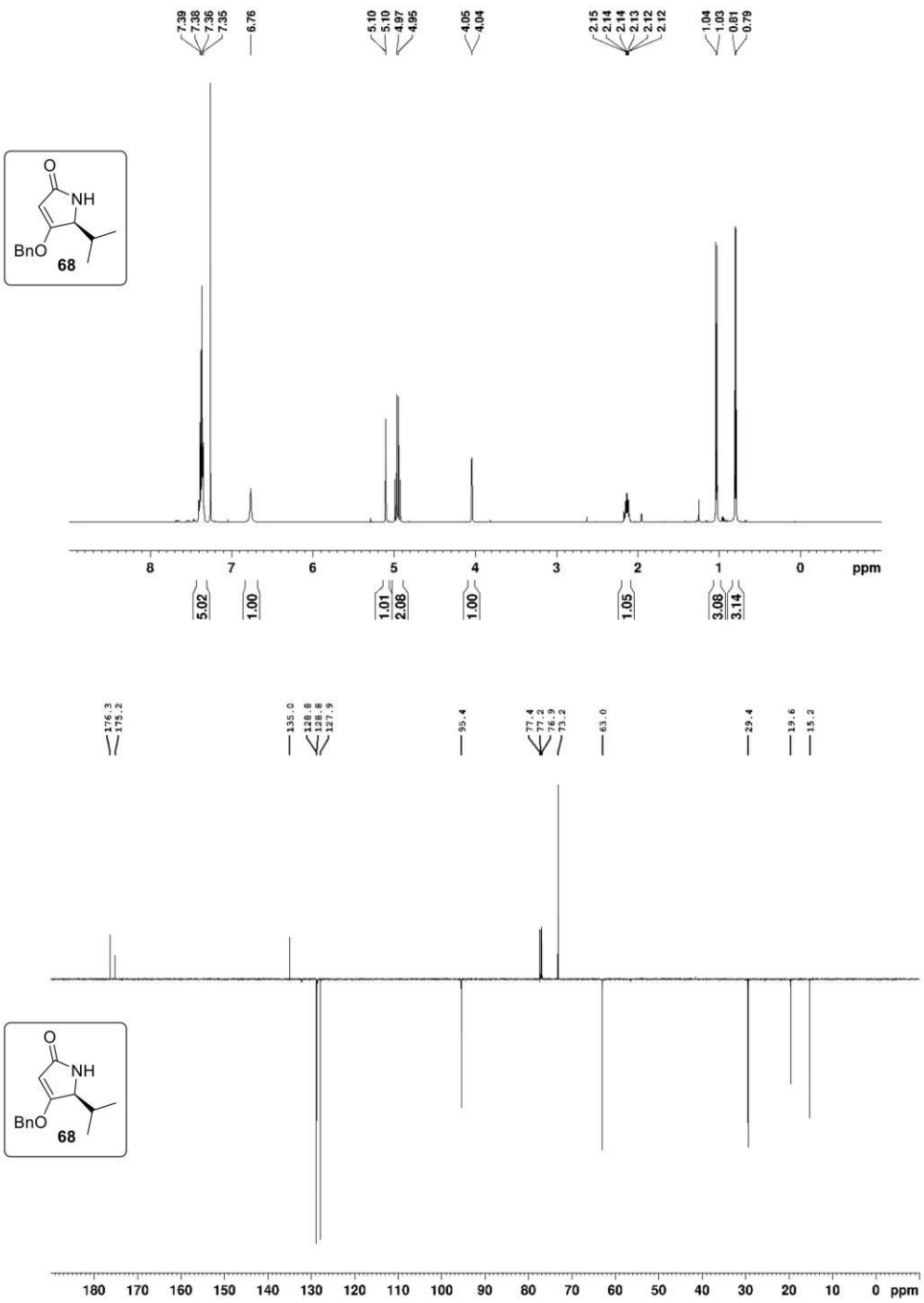
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BnO
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1.01
2.08
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1.05
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128.8
127.9
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77.2
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73.2
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15.2
O
NH
BnO
68
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

S164

Publikationsliste

1. *„Macrooxazoles A-D, new 2,5-disubstituted oxazole-4-carboxylic acid derivatives from the plant pathogenic fungus phoma macrostoma"* von Blondelle Matio Kemkuignou, Laura Treiber, Haoxuan Zeng, Hedda Schrey, Rainer Schobert und Marc Stadler, ***Molecules*** (DOI: 10.3390/molecules25235497); Molecules **2020**, 25, 5497.
2. „*Dual agents: fungal macrocidins and synthetic analogues with herbicidal and antibiofilm activities*" von Laura Treiber, Christine Pezolt, Haoxuan Zeng, Hedda Schrey, Stefan Jungwirth, Aditya Shekhar, Marc Stadler, Ursula Bilitewski, Maike Erb-Brinkmann und Rainer Schobert, ***Antibiotics*** (DOI: 10.3390/antibiotics10081022); Antibiotics **2021**, 10, 1022.
3. „*Formal synthesis of kibdelomycin and derivatisation of amycolose glycosides*" von Manuel G. Schriefer, Laura Treiber und Rainer Schobert, ***Chemical Science*** (DOI: 10.1039/D3SC00595J); Chemical Science **2023**, 14, 3562.

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Ort, Datum, Unterschrift