UNIVERSITÄT BAYREUTH

Neue flexible Synthesestrategien für bioaktive Dihydro-3(2*H*)-furanone der Ophiofuranon-, Thiocarboxyl- und Gregatin-Familien

DISSERTATION

zur Erlangung des akademischen Grades eines

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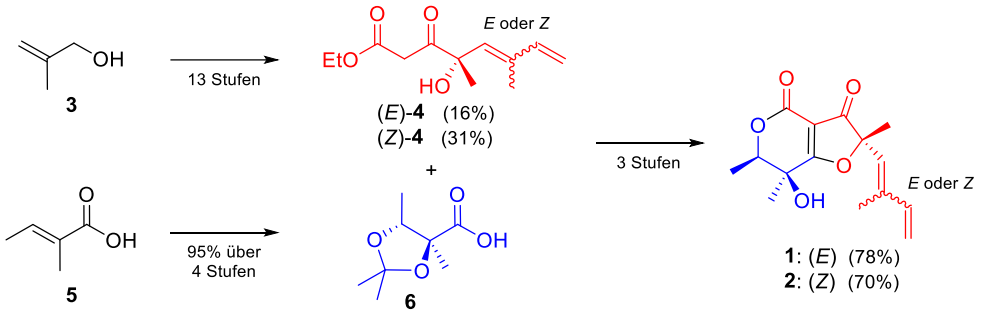
INHALTSVERZEICHNIS

ZUSAMMENFASSUNG

Ziel dieser Arbeit war die Entwicklung flexibler, enantioselektiver Syntheserouten für natürliche, bioaktive Dihydro-3(2*H*)-furanone. Die Vertreter dieser Stoffklasse wurden aus endophytischen Pilzen isoliert und weisen eine große Bandbreite an biologischen Aktivitäten auf, was sie zu interessanten Leitstrukturen in der Wirkstoffforschung macht.

Im ersten Teil dieser Arbeit ging es um die Entwicklung einer Totalsynthese für die Naturstoffe Ophiofuranon A (**1**) und B (**2**), um sie das erste Mal auf ihre biologischen Aktivitäten zu untersuchen (Schema 1). Die für ihre Synthese benötigten Bausteine (*E*)-**4** und (*Z*)-**4** wurden ausgehend von β-Methallylalkohol (**3**) in 13 Stufen hergestellt. Das Stereozentrum wurde via Sharpless-Epoxidierung und die konjugierten Doppelbindungen mittels zweier Wittig-Reaktionen (→(*E*)-**4**) bzw. einer Wittig- und einer Ando-modifizierten HWE-Reaktion (→(*Z*)-**4**) aufgebaut. Der zweite benötigte Baustein **6** wurde ausgehend von Tiglinsäure (**5**) in 4 Stufen dargestellt und die Stereozentren mittels Sharpless-Dihydroxylierung eingeführt. Ophiofuranon A (**1**) und B (**2**) konnten durch drei aufeinander folgende Kondensationsreaktionen (Yamaguchi-Veresterung, Knoevenagel-ähnliche Cyclisierung und Lactonisierung) zum ersten Mal totalsynthetisch dargestellt werden. Die Gesamtausbeute betrug über die längste lineare Synthese mit 16 Stufen 12% (**1**) bzw. 22% (**2**).



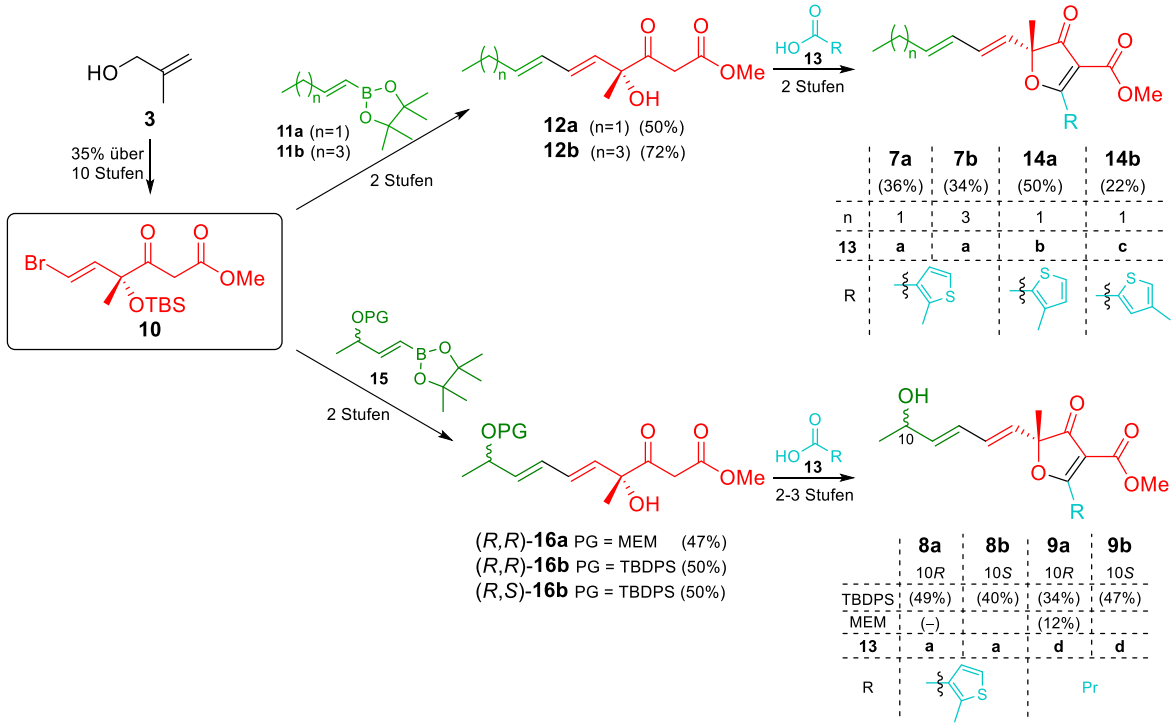
Schema 1: Totalsynthese von Ophiofuranon A (**1**) und B (**2**) ausgehend von β-Methallylalkohol (**3**) und Tiglinsäure (**5**).

Die synthetischen Naturstoffe **1** und **2** wurden auf ihre antimikrobielle, zytotoxische und Antibiofilm-Aktivität gegen verschiedene Bakterien, Pilze und Zelllinien in Kooperation mit dem Helmholtz-Zentrum für Infektionsforschung in Braunschweig getestet. Ophiofuranon A (**1**) und B (**2**) wiesen dabei nur eine schwache Aktivität gegen den Pilz *Mucor hiemalis* mit MICs von 66.6 µg/mL auf und zeigten keine zytotoxische Aktivität gegen menschliche Tumorzelllinien und nicht bösartige Fibroblasten. Die Dispersion eines *Candida albicans*-Biofilms wurde von **1** zu 24% bei einer Konzentration von 250 µg/mL gehemmt.

Im zweiten Projekt sollten die Thiocarboxyle A (**7a**), C_1 (**8a**) und C_2 (**8b**) und die Gregatine G_1 (**9a**) und G_2 (**9b**) totalsynthetisch dargestellt werden, da die Isolate gegen *Escherichia coli*, *Staphylococcus aureus* und *Candida albicans* gute antimikrobielle Wirkungen mit MICs im Bereich von 1.7-7.0 µg/mL

aufwiesen. Dazu wurde eine flexible Synthese für Dihydro-3(2*H*)-furanone entwickelt, bei der sowohl die Seitenkette (grün) als auch der Rest am Furanon (blau) auf einer späten Stufe variiert werden konnte (Schema 2). Dadurch war sowohl die erste Totalsynthese der Naturstoffe **7a**, **8** und **9** wie auch die Darstellung der Derivate **7b** und **14** möglich.

Die Synthesestrategie wurde an Thiocarboxyl A (**7a**) bzw. seinem um 2 C-Atome längeren Derivat **7b** erarbeitet (Schema 2, oben). Vinylbromid **10**, das über 10 Stufen ausgehend von β-Methallylalkohol (**3**) synthetisiert wurde, stellte ein Schlüsselbaustein der flexiblen Synthese dar. Ausgehend von **10** wurden die γ-Hydroxy-β-ketoester **12a** und **12b** via Suzuki-Kupplung mit den Boronsäureestern **11a** oder **11b** und TBS-Entschützung dargestellt. Diese wurden mittels Yamaguchi-Veresterung mit 3-Methyl-2-thiophencarbonsäure (**13a**) und Knoevenagel-ähnlicher Cyclisierung zu den Furanonen **7a** und **7b** umgesetzt. Die chemischen Verschiebungen der vollständig substituierten C-Atome der Thiopheneinheit der ^{13}C-NMR Spektren des synthetischen und isolierten Thiocarboxyls A (**7a**) wiesen geringe Abweichungen auf. Daraufhin wurden die Analoga **14a** und **14b** mit unterschiedlich substituierten Thiophenresten in jeweils 2 Stufen ausgehend von **12a** synthetisiert, um die tatsächliche Struktur von **7a** aufzuklären. Am Ende wurde jedoch festgestellt, dass die publizierte Struktur von **7a** korrekt ist. Thiocarboxyl A (**7a**) wurde somit über 14 Stufen ausgehend von **3** mit einer Gesamtausbeute von 6% synthetisiert.



Schema 2: Synthese der Dihydro-3(2*H*)-furanone Thiocarboxyl A (**7a**) und dessen Derivate **7b**, **14a** und **14b** sowie der Epimerenpaare Thiocarboxyl $C_{1,2}$ (**8a,b**) und Gregatin $G_{1,2}$ (**9a,b**).

Die etablierte Synthese wurde im Folgenden zur Darstellung der zwei Epimerenpaare Thiocarboxyl C_1 (**8a**) und C_2 (**8b**) und Gregatin G_1 (**9a**) und G_2 (**9b**) weiterentwickelt, deren Seitenketten mit einer

zusätzlichen Hydroxygruppe substituiert sind (Schema 2, unten). Dafür wurde eine geeignete, orthogonale Schutzgruppenstrategie erarbeitet, wobei sich TBDPS als geeignete Schutzgruppe des sekundären Alkohols der Seitenkette erwies.

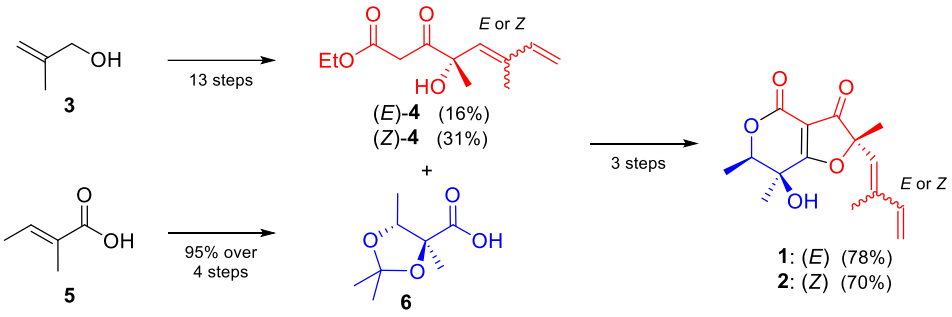
Allgemein wurde Vinylbromid **10** mittels Suzuki-Kupplung mit dem geschützten Boronat **15** und TBS-Entschützung in die γ-Hydroxy-β-ketoester **16** überführt und diese jeweils mit 3-Methyl-2-thiophencarbonsäure (**13a**) bzw. Buttersäure (**13d**) verestert. Knoevenagel-ähnliche Kondensation und finale Entschützung führte zu den Thiocarboxylen C_1 (**8a**) und C_2 (**8b**) und Gregatinen G_1 (**9a**) und G_2 (**9b**). Die Gesamtausbeuten über die 5 Stufen ausgehend von Vinylbromid **10** betrugen 17%-24%.

Da gute antimikrobielle Aktivitäten der isolierten Naturstoffe **7a**, **8** und **9** gegen *E. coli* and *S. aureus* publiziert wurden, wurden die synthetischen Thiocarboxyle A (**7a**) und $C_{1,2}$ (**8a,b**), Gregatine $G_{1,2}$ (**9a,b**) und die drei hergestellten Derivate **7b**, **14a** und **14b** gegen diese Bakterienstämme getestet. Überraschenderweise waren dabei alle acht Testverbindungen inaktiv. Nur gegen die empfindlichere *E. coli* ΔTolC-Mutante ohne ArcAB-TolC-Effluxsystem war **7a** mit einem IC_{50} von 44.3 µM minimal wachstumshemmend. Ebenfalls zeigte keine der acht Verbindungen eine zytotoxische Aktivität gegen die Zelllinien A549, L929 und Huh7.

SUMMARY

The aim of this work was to develop flexible, enantioselective synthetic routes for natural occurring, bioactive dihydro-3(2*H*)-furanones. The members of this substance class have been isolated from endophytic fungi and show a wide range of biological activities, which makes them interesting lead structures in drug discovery.

The first part of this work focused on the development of a total synthesis for the natural products ophiofuranone A (**1**) and B (**2**) to study their biological activities (Scheme 1). The building blocks (*E*)-**4** and (*Z*)-**4** were prepared in 13 steps starting from β-methallyl alcohol (**3**). The stereogenic center was built via Sharpless epoxidation and the conjugated double bonds via two Wittig reactions (→(*E*)-**4**) respectively one Wittig and one Ando-modified HWE reaction (→(*Z*)-**4**). The second required building block **6** was prepared starting from tiglic acid (**5**) in 4 steps and the stereogenic centres were introduced by Sharpless dihydroxylation. Ophiofuranone A (**1**) and B (**2**) were synthesized for the first time by three consecutive condensation steps (Yamaguchi esterification, Knoevenagel-type cyclization, and lactonization). The overall yields over the longest linear synthesis with 16 steps were 12% (**1**) and 22% (**2**).



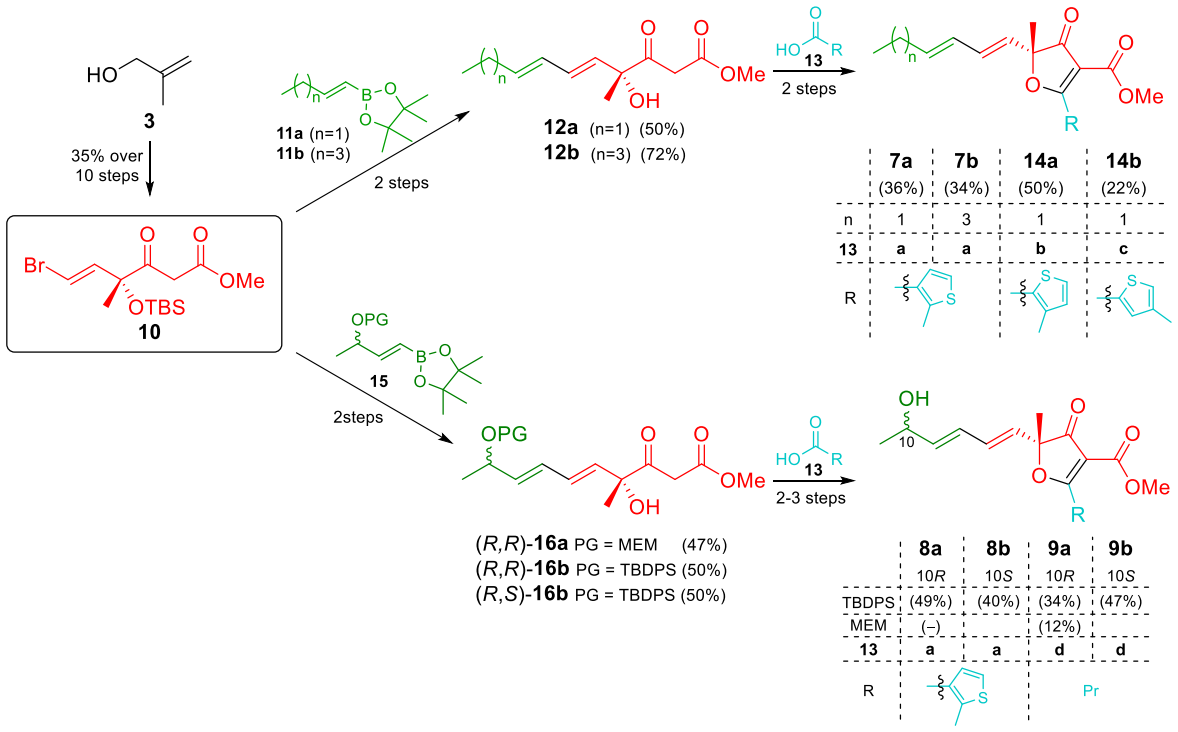
Scheme 1: Total synthesis of ophiofuranone A (**1**) and B (**2**) starting from β-methallyl alcohol (**3**) and tiglic acid (**5**).

The synthetic natural products **1** and **2** were tested for their antimicrobial, cytotoxic and antibiofilm activity against a number of bacteria, fungi and cell lines in cooperation with the Helmholtz Centre for Infection Research in Braunschweig. Thereby, ophiofuranone A (**1**) and B (**2**) had only weak activity against the fungus *Mucor hiemalis* with MICs of 66.6 µg/mL and showed no cytotoxicity against human tumor cell lines and nonmalignant fibroblasts. Dispersion of *Candida albicans* biofilm was inhibited from **1** to 24% at a concentration of 250 µg/mL.

In the second project, thiocarboxylic A (**7a**), C_1 (**8a**), and C_2 (**8a**) and gregatins G_1 (**9a**) and G_2 (**9b**) should be synthesized because the isolates showed good antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* with MICs between 1.7-7.0 µg/mL. For this a flexible synthesis for dihydro-3(2*H*)-furanones was developed, in which both the side chain (green) and the residue of the furanone (blue) could be varied at a late stage (Scheme 2). This allowed the first

total synthesis of the natural products **7a**, **8** and **9** as well as the preparation of the derivatives **7b** and **14**.

The synthetic strategy was elaborated on thiocarboxylic A (**7a**) and its derivative **7b**, featuring a 2 C-atoms longer side chain (Scheme 2, top). Vinyl bromide **10** synthesized via 10 steps of β-methallyl alcohol (**3**) represented a key building block of the flexible synthesis. Compounds **12a** and **12b** were synthesized via Suzuki coupling of **10** with boronates **11a** or **11b** and TBS deprotection. These were converted to furanones **7a** and **7b** by Yamaguchi esterification with 3-methyl-2-thiophenecarboxylic acid (**13a**) and Knoevenagel-type cyclization. The chemical shifts oft the non-hydrogenated C-atoms oft he thiophene residue of the ^{13}C-NMR spectra of the synthetic and isolated thiocarboxylic A (**7a**) showed minor derivations. Subsequently, analogues **14a** and **14b** were synthesized with differently substituted thiophene residues in 2 steps starting from **12a** to elucidate the actual structure of **7a**. However, the published structure of **7a** was determined to be correct. Thiocarboxylic A (**7a**) was therefore synthesized over 14 steps with a total yield of 6%.



Scheme 2 Flexible synthesis of thiocarboxylic A (**7a**) and its derivatives **7b**, **14a**, and **14b** and the pairs of epimers thiocarboxylic $C_{1,2}$ (**8a,b**) and gregatin $G_{1,2}$ (**9a,b**).

The established synthesis was developed to synthesize the two pairs of epimers thiocarboxylic $C_{1,2}$ (**8a,b**) and gregatin $G_{1,2}$ (**9a,b**), whose side chains are substituted with an additional hydroxy group (Scheme 2, bottom). For this reason, a orthogonal protecting group strategy was elaborated, and TBDPS was found to be a appropriate protecting group of the secondary alcohol of the side chain.

In general, building block **10** was converted to the γ-hydroxy-β-keto esters **16** by Suzuki coupling with a protected boronate **15** and TBS deprotection. Compounds **16** were esterified with 3-methyl-2-

thiophenecarboxylic acid (**13a**) or butyric acid (**13d**). Knoevenagel-type condensation and deprotection resulted in thiocarboxylics C_1 (**8a**) and C_2 (**8b**) and gregatins G_1 (**9a**) and G_2 (**9b**). The overall yields over the 5 steps starting from vinyl bromide **10** were 17%-24%.

Because good antimicrobial activities of the isolated natural products **7a**, **8** and **9** against *E. coli* and *S. aureus* have been published, the synthetic thiocarboxylics A (**7a**) and $C_{1,2}$ (**8a,b**), gregatins $G_{1,2}$ (**9a,b**) and the three prepared derivatives **7b**, **14a** and **14b** were tested against those bacterial strains. Surprisingly, all eight test compounds were inactive. Only against the more sensitive *E. coli* ΔTolC mutant lacking the ArcAB-TolC efflux system **7a** was minimally growth inhibitory, with an IC_{50} of 44.3 µM. Also none of the eight compounds showed cytotoxicity against the cell lines A549, L929 and Huh7.

ABKÜRZUNGSVERZEICHNIS

Elementbezeichnungen und Summenformeln sowie SI- und sich daraus ableitende Einheiten werden im Abkürzungsverzeichnis nicht aufgeführt. In den Formelbildern und im Text werden nachfolgende Abkürzungen sowie Kombinationen daraus verwendet.

Ac	Acetyl
ACP	Acyl-Carrier-Protein
AD	Asymmetrische Dihydroxylierung
AT	Acyltransferase
Bn	Benzyl
Bu	Butyl
CAN	Ammoniumcer(IV)-nitrat
CoA	Coenzym A
CSA	Camphersulfonsäure
dba	Dibenzylidenaceton
DH	Dehydratase
DIBAL-H	Diisobutylaluminiumhydrid
DIPT	Diisopropyltartrat
DMAP	4-(Dimethylamino)pyridin
DMF	*N,N*-Dimethylformamid
DMSO	Dimethylsulfoxid
DMP	Dess-Martin-Periodinan
DNA	Desoxyribonukleinsäure
DDQ	Dichlor-5,6-dicyano-1,4-benzochinon
EAC	Ehrlich Aszitеskarzinom
ECD	*electronic circular dichroism*
ee	*enantiomeric excess*
ER	Enoylreduktase
ESI	*electron spray ionization*
Et	Ethyl
et al.	*et alii*
HRMS	*high resolution mass spectrometry*
HWE	Horner-Wadsworth-Emmons
i	*iso*
IC_{50}	*half maximal inhibitory concentration*
IR	Infrarot
J	Kopplungskonstante

konz.	konzentriert
KR	Ketoreduktase
KS	Ketosynthase
LDA	Lithiumdiisopropylamid
LPS	Lipopolysaccharid
MCoA	Malonyl-Coenzym A
Me	Methyl
MEM	Methoxyethoxymethyl
MIC	minimale Hemm-Konzentration
MT	Methyltransferase
n	*normal*
n.b.	nicht bestimmt
NBS	*N*-Bromsuccinimid
NMO	*N*-Methylmorpholin-*N*-oxid
NMP	*N*-Methyl-2-pyrrolidon
NMR	*nuclear magnetic resonance*
p	*para*
PG	*protecting group*
PKS	Polyketidsynthase
PMB	*para*-Methoxybenzyl
Ph	Phenyl
PPTS	Pyridinium-*para*-toluolsulfonat
Pr	Propyl
py	Pyridin
quant.	quantitativ
R	Rest
RF	Erhitzen unter Rückfluss
RT	Raumtemperatur
SAM	*S*-Adenosylmethionin
t	*tert*
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
TC	2-Thiophencarboxylat
TE	Thioesterase
Tf	Trifluormethansulfonyl
Tfa	Triflouroacetat
TFA	Trifluoressigsäure

TGF	Transformierender Wachstumsfaktor
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TMS	Trimethylsilyl
Tol	Tolyl
TPAP	Tetrapropylammoniumperruthenat
Ts	Toluolsulfonyl
vgl.	vergleiche

1 EINLEITUNG

1.1 Naturstoffsynthese

Sekundärmetabolite, sogenannte Naturstoffe, sind die wichtigste Quelle für potenzielle Arzneimittel und deren Leitstrukturen.[1–3] Sie bieten mit ihrer einzigartigen strukturellen Vielfalt die Möglichkeiten für die Entdeckung neuartiger Wirkstoffe und Pharmakophore, da erst weniger als 10% der weltweiten Biodiversität auf ihre potenzielle biologische Aktivität hin untersucht wurde.[2] Wenn man die Arzneimittelentwicklung vor dem Aufkommen des Hochdurchsatz-Screenings betrachtet, waren mehr als 80% der Arzneimittel Naturstoffe oder von einem Naturstoff abgeleitet.[3,4] Ein Vergleich der Informationen über die Quellen neuer Arzneimittel von 1981 bis 2014 zeigt, dass fast die Hälfte der seit 1994 zugelassenen Wirkstoffe auf Naturstoffen basieren.[5] Von 2005 bis 2007 wurden dreizehn Medikamente auf der Basis von Naturstoffen zugelassen[6] und 2008 befanden sich mehr als 100 Verbindungen in der klinischen Entwicklung, insbesondere als Zytostatika und Antiinfektiva.[3]

Da Naturstoffe aus natürlichen Ressourcen zunächst nur selten ausreichend zur Verfügung stehen, müssen die meist komplexen Verbindungen aus einfachen Bausteinen enantioselektiv synthetisiert werden, um ausreichend Material für biologische Aktivitätsstudien zu erhalten.[7,8] Der Bereich der Naturstoffsynthese dient zusätzlich dazu die Strukturen der isolierten Naturstoffen zu bestätigen[7] und zur Identifikation und Modifikation von Leitstrukturen und Pharmakophoren. Durch die Derivatisierung dieser Leitmotive können eine verbesserte therapeutische Wirkung oder eine Verringerung der Nebenwirkungen erzielt werden.[9,10] Ebenso steigt die Zahl der Resistenzen gegenüber zytotoxischer und antibiotischer Arzneimitteln kontinuierlich, weswegen die Suche nach neuen Leitstrukturen und Wirkstoffen, um diese Resistenzen zu umgehen, immer noch von großer Bedeutung ist.

Sekundärmetabolite werden von den verschiedensten Organismen produziert, dazu zählen Bakterien, Pflanzen und niedere Tiere, wie zum Beispiel Algen, Korallen und Schwämme.[11] Darüber hinaus dienen (endophytische) Pilze als Quelle neuer Wirkstoffe. Die aus ihnen isolierten Verbindungen werden über verschiedenste Biosynthesewege dargestellt und zählen unter anderem zu den Isoprenoiden und Polyketiden oder sind Aminosäurederivate.[12]

1.2 Natürlich vorkommende Dihydro-3(2*H*)-furanone

Eine interessante Stoffklasse, die bisher ausschließlich aus endophytischen Pilzen isoliert wurde und zu den Polyketiden zählt, besitzt ein Dihydro-3(2*H*)-furanon als Leitmotiv. Sie besteht mittlerweile aus fast 40 Vertretern, die unter anderem aus Pilzen der Gattungen *Aspergillus*,[13–16] *Cephalosporium*,[17,18], und *Penicillium*[19–23] isoliert wurden. Die Vertreter der Stoffklasse sind strukturell divers, haben aber alle ein Dihydro-3(2*H*)-furanon als Kernstruktur (Abbildung 1, blau). Gemeinsam haben auch alle

einen Methylsubstituenten am C-2 (rot), sowie eine ungesättigte Seitenkette am quartären Stereozentrum des Furanonrings, die meist (*E*,*E*)- in seltenen Fällen auch (*E*,*Z*)-konfiguriert oder wie bei Huaspenon B (**21**)[16] nur einfach ungesättigt ist. An C-4 findet sich bei fast allen Naturstoffen dieser Klasse eine Acylgruppe, die beinahe immer als Methylester oder bei wenigen Vertretern als freie Säure vorliegt (orange). Eine Ausnahme hierzu ist das 1988 isolierte Cyclogregatin (**18**),[14] da es neben dem Dihydro-3(2*H*)-furanon noch ein α-Pyron-Motiv enthält, wodurch sich eine bicylische Furopyran-3,4-dion-Grundstruktur ergibt, die bis heute nur in zwei weiteren Naturstoffen gefunden wurde (siehe Kapitel 1.2.5).[24] Der größte Unterschied liegt bei der Strukturklasse im Substituenten an C-5 des Furanons (schwarz), der von kurzen gesättigten, ungesättigten oder hydroxylierten Kohlenstoffketten wie bei Gregatin A (**17**),[17] Huaspenon B (**21**)[16] und Aspertetronin B (**22**)[13] bis hin zu hochsubstituierten Dihydronaphtyl-Resten wie der von Penicilfuranon A (**19**)[21] oder ganzen Steroid-Resten wie in Isopenicin A (**20**)[22] reicht. 2015 wurden die ersten “dimeren“ Vertreter der Dihydro-3(2*H*)-furanone, z.B. das Miniolin A (**23**), isoliert.[20]

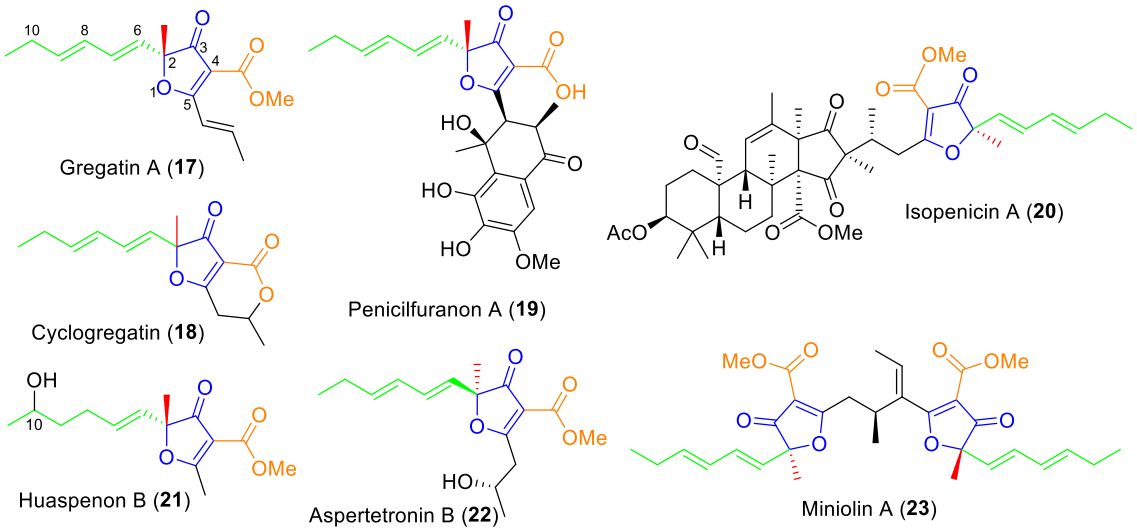


Abbildung 1: Einige Beispiele für natürlich vorkommende Dihydro-3(2*H*)-furanone.

Fast alle natürlich vorkommenden Dihydro-3(2*H*)-furanone weisen eine große Bandbreite an biologischen Aktivitäten auf. Sie wirken unter anderem phytotoxisch,[17,18,25] antibiotisch,[14,15,17,23,25–28] antimykotisch [14,17,25,27] und auch zytotoxisch,[14,20,22,29] was sie zu interessanten Leitstrukturen in der Wirkstoffforschung macht.

1.2.1 Stukturrevision des Leitmotivs

Bis die Leitstruktur der Stoffklasse der Dihydro-3(2*H*)-furanone vollständig aufgeklärt war sind nach der Isolierung der ersten Vertreter 1969[13] über 40 Jahre vergangen.[30–39] So wurde die Kernstruktur der 1969 isolierten Aspertetronine ursprünglich als 3-Acyl-4-methoxyfuran-2(5*H*)-on **24** veröffentlicht (Abbildung 2; 1. Generation).[13] Durch die Totalsynthese des racemischen Gregatins B von Clemo und Pattenden[31] im Jahr 1982 wurde die Leitstruktur zu 4-Acyl-5-methoxyfuran-3(2*H*)-on **25** revidiert

und durch weitere Synthesearbeiten anderer Gruppen[32–34] im selben Jahr bestätigt. Durch Extrapolation wurde die gleiche Art von Strukturrevision für alle bis dahin isolierten Vertreter der Naturstoffklasse vorgeschlagen,[31] was in der 2. Generation der Strukturklasse resultierte. Burghart-Stoll und Brückner[38] synthetisieren 2011 Gregatin B der 2. Generation, was durch Zufall zur zweiten Strukturrevision der Naturstoffklasse führte. Durch Analyse eines Nebenproduktes stellten sie fest, dass der Furanonring des Naturstoffs identisch zur 2. Generation ist, aber die Substituenten des Rings anders angeordnet sind, wodurch sich 4-(Methoxycarbonyl)furan-3(2*H*)-on **26** als neue und bis heute gültige Leitstruktur für die Strukturklasse ergab (3. Generation).

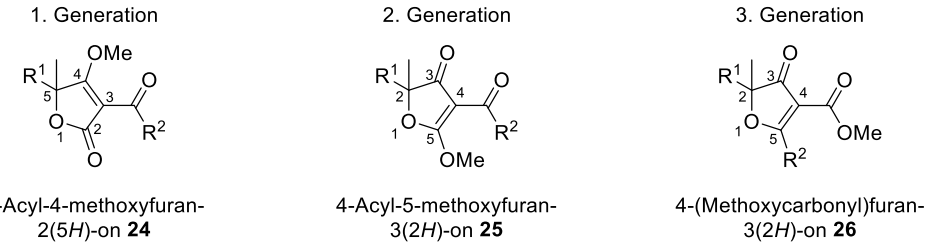


Abbildung 2: Ursprünglich publizierte Struktur **24** für die natürlich vorkommenden Furanone (1. Generation)[13] und die durch jeweils eine Strukturrevision publizierte 2. Generation[31] **25** und 3. Generation[38] **26**.

1.2.2 Vertreter und ihre biologischen Aktivitäten

Zu den bekanntesten, natürlich vorkommenden Vertretern der Dihydro-3(2*H*)-furanone gehören die 1975 erstmals aus dem Pilz *Cephalosporium gregatum* isolierten Gregatine A-E (**17**, **27**-**30**),[17,25,40] die in Abbildung 3 dargestellt sind. Es wurde festgestellt, das Gregatin A (**17**), C (**28**) und D (**29**) das Welken und Absterben der Blätter sowie Gefäßbräune von Stecklingen der Adzukibohne und der Mungobohne verursachten. Damit lag nahe, dass diese Verbindungen an der Pathogenese der braunen Stängelfäule beteiligt sind, die zu dieser Zeit ein großes Problem beim Anbau der Bohnen darstellte. Gregatin B (**27**) und E (**30**) hatten die gleiche Auswirkung auf die Blätter der Hülsenfrüchte, führten jedoch nicht zur Gefäßbräune.[17]

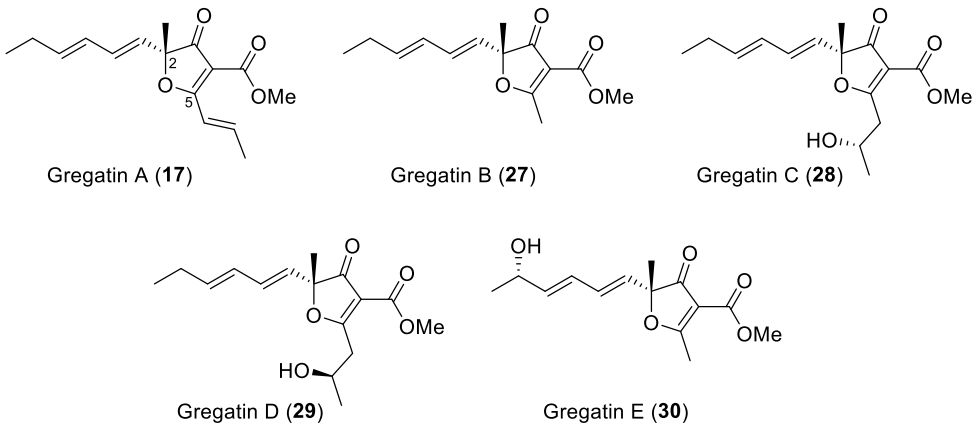


Abbildung 3: Strukturen der 1975 isolierten phytotoxischen Gregatine A (**17**), B (**27**), C (**28**), D (**29**) und E (**30**).[17]

Neben ihren phytotoxischen Eigenschaften wurden die Vertreter der Gregatin-Familie zusätzlich auf ihre antibiotischen und antimykotischen Aktivitäten untersucht.[25] Die Keimung von Sporen einiger Pilze (*Mucor hiemalis*, *Rhizopus* sp. und *Aspergillus flavus*) wurde durch Gregatin A (**17**) mit einer Konzentration von 50 µg/mL vollständig gehemmt. Eine wesentlich höhere Konzentration wurde von Gregatin C (**28**) und D (**29**) benötigt, um den gleichen Effekt zu erzielen. Das Myzelwachstum einiger Pilze wie *Aphanomyces cochlioides* und *Rhizoaonia solani* wurde durch Gregatin A (**17**), C (**28**) und D (**29**) bei Konzentrationen unter 25 µg/mL vollständig inhibiert. Die gleichen Verbindungen führten auch zu Wachstumshemmungen der Hefe *Rhodotorula glutinis* und grampositiver Bakterien, insbesondere *Bacillus subtilis* und *Staphylococcus aureus*. Sie waren dagegen jedoch unwirksam gegen gramnegative Bakterien. Gregatin B (**27**) und E (**30**) waren weitgehend inaktiv gegen die getesteten Mikroorganismen.[25]

Gregatin A (**17**), B (**27**) und D (**29**) wurden zum zweiten Mal 1980 von Schwab und Achenbach mit drei weiteren Metaboliten aus dem Pilz *Aspergillus panamensis* isoliert und auf ihre biologische Aktivität untersucht. Hierbei lag die minimale Hemm-Konzentration von Gregatin A (**17**) sowohl gegen gramnegative Bakterien wie *Acinetobacter calcoaceticus* als auch gegen grampositive Bakterien wie *Bacillus brevis* oder *Staphylococcus aureus* bei 1 µg/mL.[15] Gregatin A (**17**) wurde ebenfalls gegen 25 unterschiedliche Pilzstämme getestet und führte bei fast allen bei der niedrigeren Konzentration von 10 µg/disc zu Wachstumshemmungen. Schwab und Achenbach vermuteten, dass die Doppelbindung der Crotonyl-Seitenkette von Gregatin A (**17**) eine wichtige Rolle bei der biologischen Aktivität spielt, da es bei allen anderen Gregatinen, die diese Doppelbindung nicht besitzen, zu einer enormen Aktivitätsabnahme kommt.[15] So wurde beispielsweise bei Gregatin D (**29**) eine wesentlich geringere Phytotoxizität[25] und auch 100-fach höhere MICs gegen Bakterien festgestellt.[15] Ein ähnlicher Effekt des Aktivitätsverlust durch Entfernung der Doppelbindung einer Crotonyl-Gruppe wurde 1979 für Pyrenocin A, einem phytotoxischen Metabolit von *Pyrenochaeta terrestris* berichtet.[41] Dies lässt vermuten, dass die Doppelbindung der Crotonyl-Seitenkette an C-5 von großer Bedeutung für die biologische Aktivität von Gregatin A (**17**) ist.[15]

Im Jahr 1977 wurde ebenfalls aus einem Pilz der Gattung *Cephalosporium* ein weiteres Phytotoxin isoliert[18] und 1979 auf seine biologischen Eigenschaften untersucht.[27] Das sogenannte Graminin A (**31**, Abbildung 4), ebenfalls mit ungesättigter Seitenkette an C-5, führte bei niedrigen Konzentrationen spezifisch zu Chlorose sowie Braunfärbung der Blätter und des Gefäßgewebes von Weizenstecklingen und löste somit die gleichen Symptome bei Weizen aus wie Gregatin A (**17**) bei Adzukibohnen. Graminin A (**31**) zeigte gegen einige Pilze und drei grampositive Bakterienarten MICs von unter 25 µg/mL und besitzt zusätzlich antimykotische und antibakterielle Eigenschaften. Das namentlich verwandte Graminin B (**32**, Abbildung 4) wurde 2014 aus dem Pilz *Paraconiothyrium* sp. isoliert.[26] Es unterscheidet sich lediglich durch die fehlende Doppelbindung des Substituenten am C-5 des Furanons (R^2) und der Geometrie der Δ^8-Doppelbindung. Graminin B (**32**) war der erste isolierte

Vertreter der Dihydro-3(2*H*)-furanone mit einer (*Z*)-konfigurierten Doppelbindung der Hexadienyl-Seitenkette. Es wurde in einer Reihe von antimikrobiellen Assays getestet und zeigte dabei lediglich eine moderate Wirkung gegen *E. Coli* und gegen Methicillin-resistentes *S. aureus*. Eine antimykotische oder zytostatische Wirkung von **32** konnte nicht festgestellt werden.[26] 2015 folgte mit der Isolation von Graminin C (**33**, Abbildung 4), neben Graminin B (**32**) und *cis*-Gregatin B, aus dem Pilz *Cupressus arizonica* der dritte Vertreter dieser Familie.[28] Durch Untersuchung der antibakteriellen Aktivität von Graminin B (**32**) und C (**33**) wurde festgestellt, dass sich die zusätzliche Doppelbindung von R^2 positiv auf ihre Wirkung ausübt. Es wurde daher vermutet, dass die antibiotische Aktivität dieser Verbindungen von der Anzahl der in Konjugation mit den Carbonyl-Gruppen stehenden Doppelbindungen abhängt.[28]

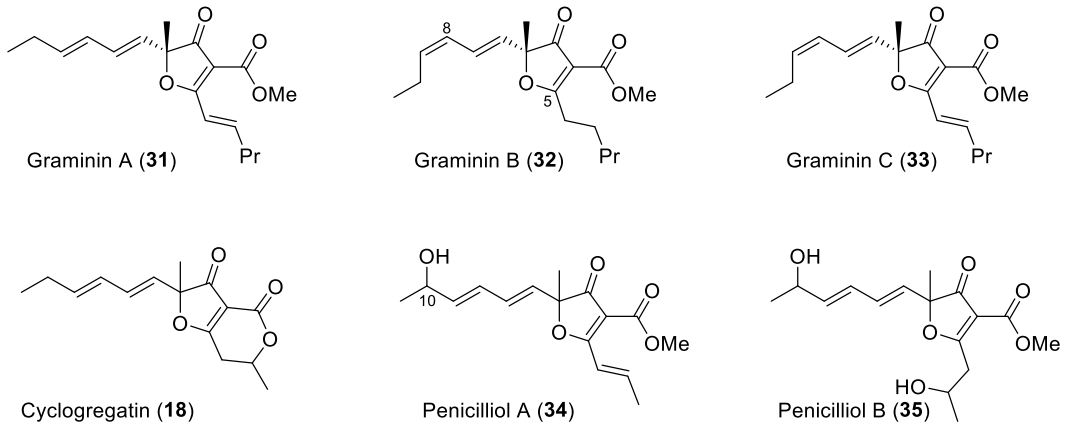


Abbildung 4: Strukturen der Graminine A (**31**),[18] B (**32**)[26] und C (**33**),[28] des bicyclischen Cyclogregatins (**18**)[14] und der Penicilliole A (**34**) und B (**35**).[19]

Das im Verlauf der Isolierung von Gregatin A (**17**) aus *Aspergillus panamensis* von Steglich *et al.* 1988 entdeckte bicyclische Cyclogregatin (**18**, Abbildung 4) wurde im Vergleich mit **17** auf seine biologischen Eigenschaften untersucht.[14] Die meisten Bakterien-, Hefe- und Pilzstämme gegen die Gregatin A (**17**) sensitiv waren, wiesen ebenfalls eine Sensitivität gegen Cyclogregatin (**18**) auf, jedoch mit 5-10-fach höheren MICs. Die beiden Naturstoffe wurden zusätzlich auf ihre zytotoxische Aktivität gegen EAC-Zellen getestet. Gregatin A (**17**) wies auch hier eine bessere Aktivität mit einem IC_{50}-Wert von 2 µg/mL gegenüber Cyclogregatin (**18**) mit einem Wert von 10 µg/mL auf.[14]

Im Jahr 2009 wurden zwei weitere Vertreter der Dihydro-3(2*H*)-furanone aus *Penicillium daleae* isoliert, die Penicilliole A (**34**) und B (**35**) (Abbildung 4).[19] Metabolit **34** hat ebenfalls wie Gregatin A (**17**) den Crotonyl-Substituenten am C-5 des Furanons, während seine Dienyl-Seitenkette an Position 10 zusätzlich hydroxyliert ist. Penicilliol B (**35**) enthält zwei einfach hydroxylierte Seitenketten. Beide Verbindungen hemmen selektiv die Aktivität von eukaryotischen DNA-Polymerasen der Y-Familie, wobei **34** ein stärkerer Inhibitor als **35** ist. Auch hier wurde vermutet, dass die Doppelbindung der Crotonyl-Gruppe eine wichtige Rolle bei der biologischen Aktivität spielt. Die Inhibition von Polymerasen der A-, B-, und X-Familie und einigen Enzymen des DNA-Metabolismus wurden von

den Penicilliolen A (**34**) und B (**35**) nicht inhibiert. Die Polymerasen der Y-Familie gehören zu den DNA-Polymerasen, welche die Fähigkeit besitzen auch über beschädigte Stellen DNA zu replizieren, was zu erhöhter Mutagenese führt. Eine gezielte Hemmung der Y-Polymerasen kann somit für die Entwicklung neuer Chemotherapeutika interessant sein.[19]

Koseki *et al.* haben 2012 gleich acht Dihydro-3(2*H*)-furanone aus *Microdiplodia* sp. KS 75-1 isoliert.[42] Darunter die in Abbildung 5 gezeigten Metabolite **36-41**, welche besonders macht, dass sie statt des Methylesters in Position 4 des Furanonrings eine freie Säure (**36-38**) oder sogar gar keinen Acylrest (**39-41**) tragen. Alle sechs Verbindungen wiesen antimikrobielle Wirkungen gegen *Candida albicans* und *Staphylococcus aureus* auf.[42]

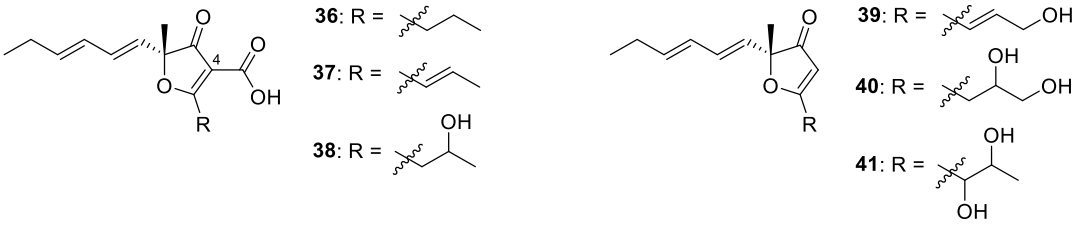


Abbildung 5: Strukturen der Dihydro-3(2*H*)-furanone mit freier Säure am Furanon **36-38** und ohne Acylgruppe an Position 4 **39-41**, die aus *Microdiplodia* sp. KS 75-1 isoliert wurden.[42]

Die 1969 bis 2014 isolierten Dihydrofuranone sind strukturell recht einfache Vertreter dieser Naturstoffklasse. Sie unterscheiden sich oft nur durch die Konfiguration eines Stereozentrums oder einer Doppelbindung, einer Hydroxygruppe oder der Acylgruppe am Furanon. Ab 2015 wurden auch strukturell wesentlich komplexere Vertreter dieser Klasse isoliert, die zum Teil als "Dimere" der gleichen oder auch aus unterschiedlichen Stoffklassen vorliegen.

Die Sekundärmetabolite Minioline A (**23**), B (**42**) und C (**43**) wurden 2015 von Gao *et al*. aus dem Pilz *Penicillium minioluteum* isoliert und sind "Dimere" des 1969 als ersten Vertreter dieser Strukturklasse isolierten Aspertetronins A (**44**)[13] (Abbildung 6).[20] Dieses bildet das Enantiomer zu Gregatin A (**17**) und wurde bis dahin nicht auf seine biologische Aktivität untersucht. Gao *et al*. testeten sowohl die Minioline A (**23**), B (**42**) und C (**43**) als auch Aspertetronin A (**44**) auf ihre zytotoxischen Eigenschaften gegen verschiedene Hela-Zelllinien.

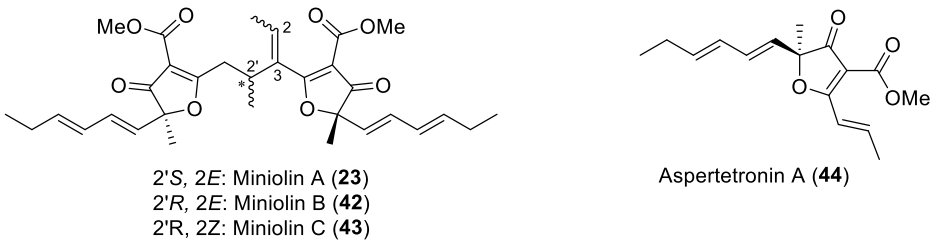


Abbildung 6: Strukturen der Minioline A (**23**), B (**42**) und C (**43**),[20] die ersten isolierten "dimeren" Dihydrofuranone, und deren vermuteter, biosynthetischer Vorläufer Aspertetronin A (**44**).[13]

Die Dimere **23**, **42** und **43** zeigten dabei jedoch lediglich eine moderate Wirkung mit IC_{50}-Werten im mittleren bis unteren zweistelligen µM-Bereich, wohingegen Aspertetronin A (**22**) gar keine Wirkung aufwies.[20]

Einige der strukturell verschiedensten Naturstoffe der Klasse der Dihydro-3(2*H*)-furanone sind in Abbildung 7 gezeigt. Das hoch oxygenierte Penicilfuranon A (**19**) wurde 2016 mit dessen biosynthetischem Vorläufer Gregatin A (**17**) aus dem Pilz *Penicillium* sp. sh18 isoliert.[21] Es zeigt eine signifikante antifibrotische Wirkung in aktiven hepatischen Sternzellen durch negative Regulierung des TGFβ/Smad-Signalwegs. Es wird angenommen, dass die Aktivierung der hepatischen Sternzellen und ihre Transdifferenzierung in Myofibroblasten ein Schlüsselprozess bei Leberschäden und Fibrogenese ist. Daher stellt die Hemmung der Aktivierung und Funktion von Sternzellen ein primäres therapeutisches Ziel bei Leberfibrose dar, was Penicilfuranon A (**19**) zu einer interessanten Leitstruktur dafür macht.[21]

Die 2017 von Yin *et al*. aus dem Pilz *Aspergillus* sp. isoliert Citrifurane A-D (**47-50**, Abbildung 7) sind ungewöhnliche "Dimere" aus einem Dihydrofuranon und aromatischen Polyketiden.[43] Sie sind die ersten Heterodimere aus Azaphilon- und Furanonderivaten. Es wird angenommen, dass die neue C-C-Bindung zwischen den zwei Polyketideinheiten durch eine Michael-Addition geknüpft wurde, wobei zwei unterschiedliche Verknüpfungsmuster, je nach Michael-Donator des Furanonderivats, entstehen können. Die Citrifurane A-C (**47-50**) zeigten eine moderate Inhibition der LPS-induzierten NO-Produktion in RAW 264.7 Makrophagen mit IC_{50}-Werten von 18.3, 22.6, bzw. 25.3 µM, was sie zu potentiellen entzündungshemmenden Wirkstoffen macht.[44]

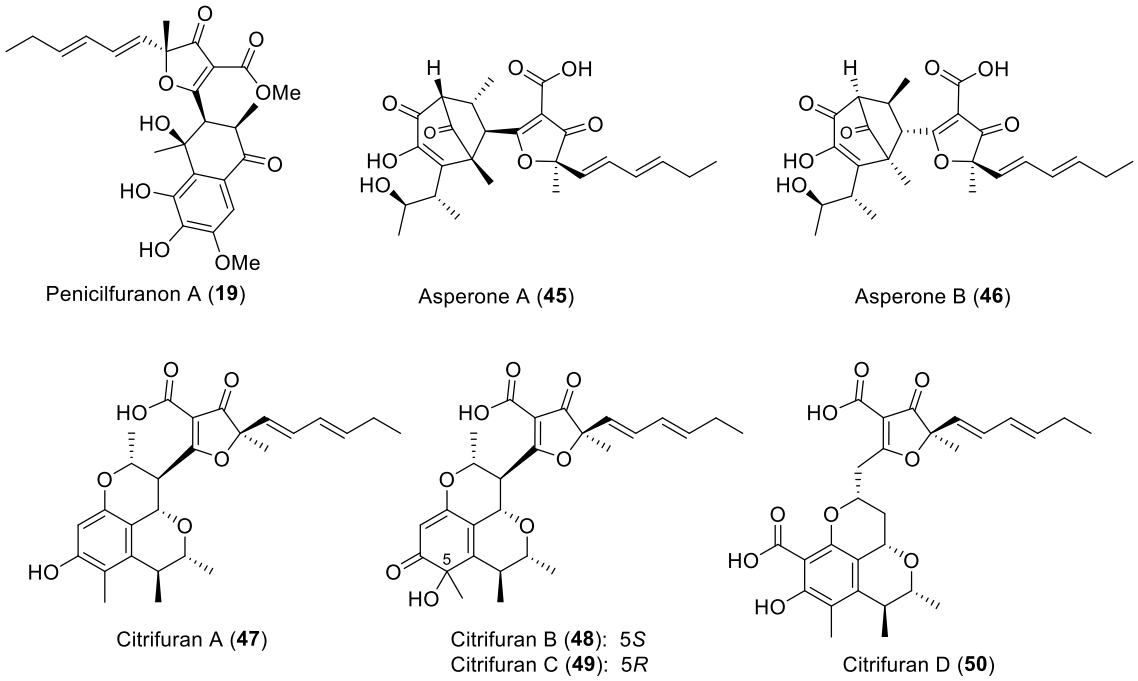


Abbildung 7: Strukturen der strukturell komplexen Dihydro-3(2*H*)-furanone Penicilfuranon A (**19**),[21] Asperon A (**45**) und B (**46**)[44] und Citrifuran A-D (**47-50**).[43]

Yin *et al*.[44] isolierten ein Jahr später ebenfalls aus dem Pilz *Aspergillus* sp. erneut "dimere" Dihydro-3(2*H*)-furanone, die Asperone A (**45**) und B (**46**) (Abbildung 7). Auch hierbei handelt es sich um Metabolite aus einer Furanoneinheit und einem aromatischen Polyketid, welche vermutlich über eine [3+2]-Cycloaddition verknüpft wurden. Asperon A (**45**) und B (**46**) wurden ebenfalls auf ihre hemmende Wirkung auf die LPS-induzierte NO-Produktion sowie auf ihre Zytotoxizität in RAW 264.7-Makrophagenzellen getestet. Sie zeigten beide keine Zytotoxizität bei einer Konzentration von 50 µM. Asperon B (**46**) hemmte jedoch die LPS-induzierte NO-Produktion mit einem IC_{50} von 16.0 µM. Zusätzlich wurden **45** und **46** auf ihre antibiotischen, antimykotischen und antitumoralen Aktivitäten untersucht, wobei Asperon B (**46**) eine schwache antibiotische Wirkung gegen *Pseudomonas aeruginosa* mit einer minimalen Hemm-Konzentration von 71.6 µM aufwies.[44]

Aus dem gleichen Pilz wie Penicilfuranon A (**19**) wurden 2019 die Isopenicine A (**20**) und B (**51**) isoliert (Abbildung 8).[22] Diese Meroterpenoide besitzen ein neuartiges Terpenoid-Polyketid-Hybridgerüst, da sie sowohl zur Gruppe der Dihydro-3(2*H*)-furanone als auch zu den Steroiden zählen. Sie unterscheiden sich lediglich in der Konfiguration des Stereozentrums in Position 15. Es wurde festgestellt, dass Isopenicin A (**20**) ein potenter Inhibitor des Wnt-Signalwegs von Darmkrebszellen ist. Eine abnorme Aktivierung dieses Signalwegs ist an der Entwicklung und dem Fortschreiten verschiedener Krebsarten beteiligt, weshalb sich Antagonisten des Wnt-Signalwegs als wirksame antitumorale Wirkstoffe erweisen können.

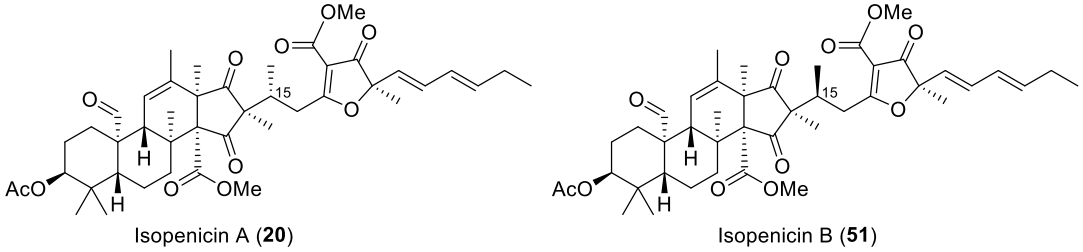
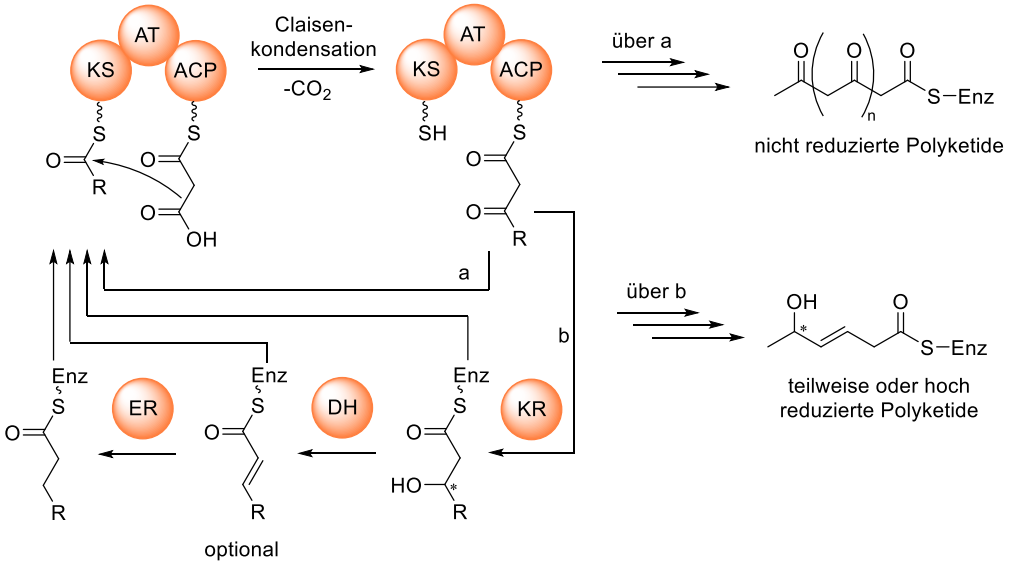


Abbildung 8: Strukturen der 2019 isolierten Isopenicine A (**20**) und B (**51**).[22]

Wie in diesem Kapitel beschrieben, weisen die Vertreter der Dihydo-3(2*H*)-furanone einige interessante biologische Aktivitäten auf, was sie zu einer potenziellen Leitstruktur für neue Wirkstoffe macht.

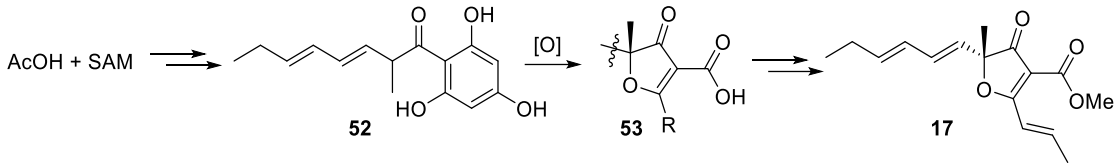
1.2.3 Biosynthese

Die Biosynthese der Dihydro-3(2*H*)-furanone erfolgt über den Polyketidweg durch multimodulare Polyketidsynthasen (PKS) vom Typ I, wobei die Verlängerung der wachsenden Polyketidkette durch decarboxylierende Claisen-Thioester-Kondensation einer aktivierten Startereinheit mit einem Malonsäure-Halbthioester katalysiert wird (Schema 3). Jedes Modul besteht aus verschiedenen Domänen und enthält immer ein Acyl-Carrier-Protein (ACP), eine Ketosynthase (KS) und eine Acyltransferase (AT), die in linearer Sequenz ein Zwischenprodukt um zwei Kohlenstoffatome verlängert. Die AT belädt das ACP mit einem Coenzym A-aktivierten Baustein (meist Acetyl-CoA oder Malonyl-CoA) und die KS katalysiert die Bildung einer Kohlenstoff-Kohlenstoff-Bindung zwischen dem Zwischenprodukt aus dem vorgeschalteten Modul und dem Acyl-ACP. Darüber hinaus können die Module auch Domänen enthalten, die die β-Oxo-Funktion nach dem Verlängerungsschritt nacheinander in eine Hydroxygruppe (Ketoreduktase (KR)), eine Doppelbindung (Dehydratase (DH)) oder eine Einfachbindung (Enoylreduktase (ER)) reduzieren.[45,46] Je nachdem welche Domänen ein Modul enthält wird die Reduktion auf verschiedenen Stufen beendet, was ein komplexes Funktionalisierungsmuster zur Folge hat.[46] Die ACP verwendet einen Phosphopantethein-Arm und eine Thioesterbindung, um Zwischenprodukte und Bausteine zu binden und sie zur Beladung, Verlängerung oder Modifizierung auf die entsprechenden katalytischen Domänen zu übertragen. Anschließend transferiert das ACP das Zwischenprodukt auf die KS des nachfolgenden Moduls zur weiteren Verlängerung oder auf die Thioesterase (TE) des letzten Moduls zur Freisetzung des Polyketidrückgrats. Die Ablösung von der PKS kann durch Hydrolyse, Lactonisierung, reduktiv oder durch andere nukleophile Angriffe erfolgen, wobei es zur Cyclisierung des Grundgerüsts kommen kann.[45,46] Die Primärprodukte können im Anschluss durch zusätzliche Cyclisierungen, Bindungsspaltungen oder Umlagerungen noch weiter modifiziert werden.[46]



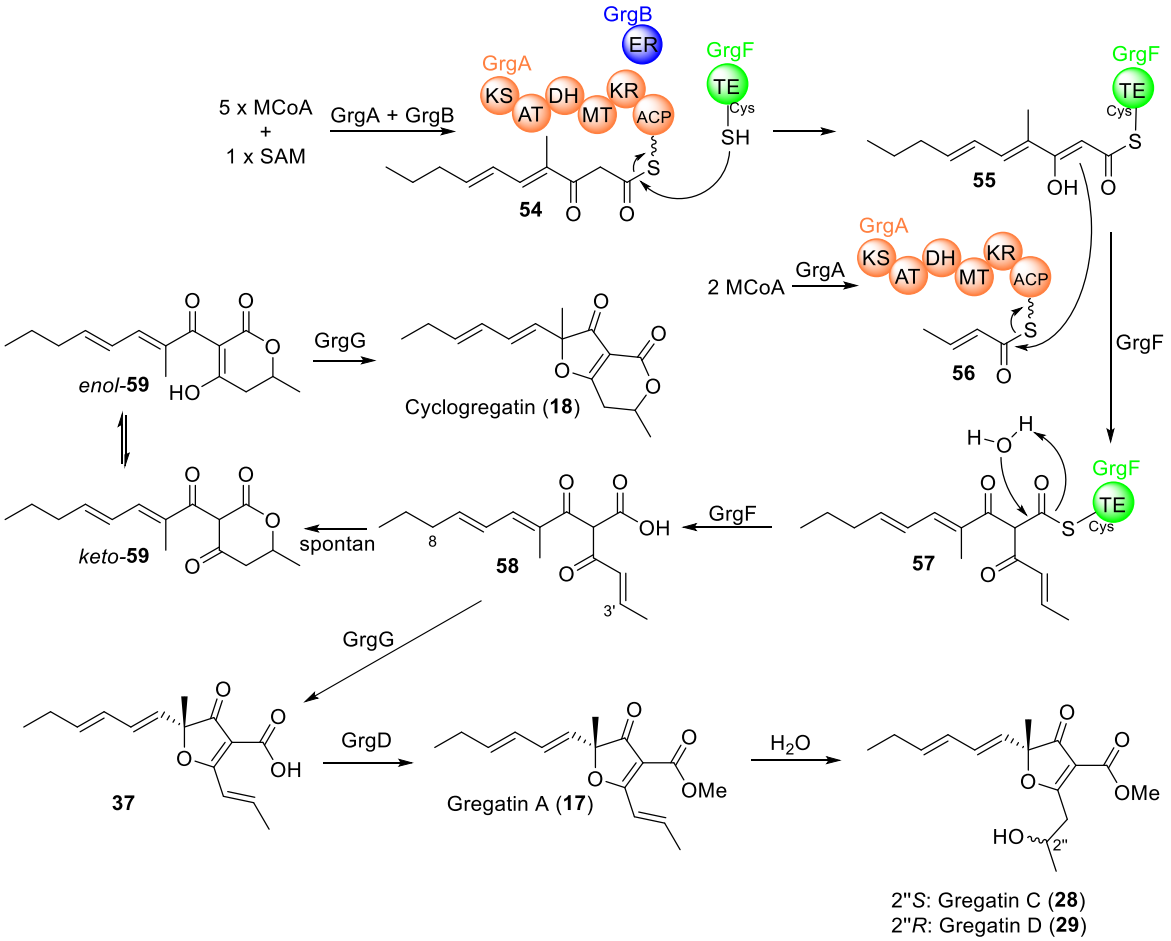
Schema 3: Grundmechanismus der Polyketid-Biosynthese: a) Verlängerung ohne Reduktion; b) Verlängerung mit optionalen Reduktionsschritten des Polyketidrückgrats durch Ketoreduktase (KR), Dehydratase (DH) und Enoylreduktase (ER).[46]

Über die Biosynthese der Dihydro-3(2*H*)-furanone war lange Zeit nichts bekannt. Die ersten Vermutungen wurden 2017 angestellt und gingen davon aus, dass Vorläufer **52** durch eine Polyketidsynthase aus Essigsäure und *S*-Adenosylmethionin (SAM) biosynthetisiert wird (Schema 4).[43] Die oxidative Spaltung des Aromaten von **52** würde zum Grundgerüst der Dihydro-3(2*H*)-furanone **53** führen, welches durch weitere Modifikationen in den jeweiligen Naturstoff[43,44] wie zum Beispiel Gregatin A (**17**), umgewandelt werden soll.[47] Es wurden jedoch keine experimentellen Untersuchungen durchgeführt, um diese Hypothese zu unterstützen.[47]



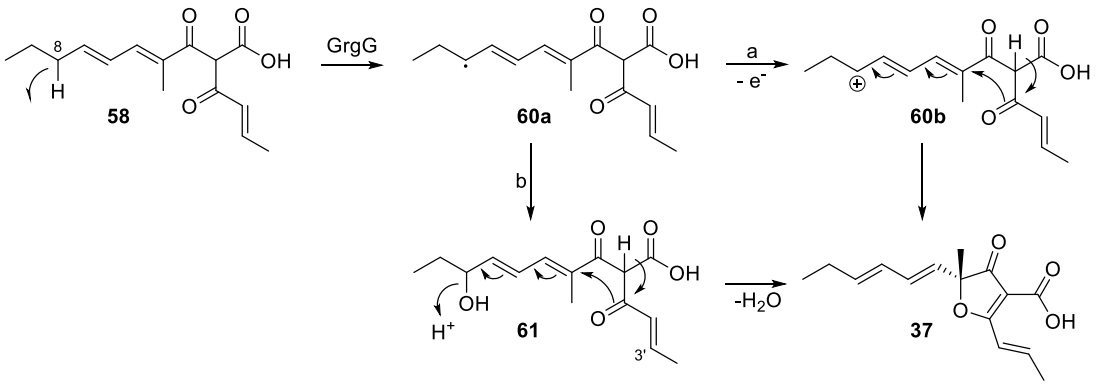
Schema 4: Vorgeschlagene Biosynthese zur Bildung der Furanon-Grundstruktur von Gregatin A (**17**).[43,47]

Im Jahr 2020 untersuchten Matsuda *et al*. das erste Mal die Biosynthese der Dihydro-3(2*H*)-furanone, insbesondere die von Gregatin A (**17**, Schema 5).[47]



Schema 5: Von Matsuda *et al*. untersuchte Biosynthese von Gregatin A (**17**) und vorgeschlagene Biosynthese für Gregatin C (**28**) und D (**29**) und Cyclogregatin (**18**).[47,48]

Sie identifizierten dabei das biosynthetische Gencluster von Gregatin A (**17**) in *Penicillium* sp. sh18 und rekonstruierten seine Biosynthese in dem heterogenen Pilz *Aspergillus oryzae*. Isotopenmarkierungsexperimente zeigten, dass **17** durch Fusion zweier Kohlenstoffketten, die von einem einzigen PKS GrgA dargestellt werden, mit Hilfe einer *trans*-wirkenden Enoylreduktase GrgB biosynthetisiert wird. Aufgrund ihrer experimentellen Daten schlugen sie den in Schema 5 dargestellten Biosyntheseweg für Gregatin A (**17**) vor. Demnach synthetisiert die PKS GregA die C_{11}-Polyketidkette **54** in Gegenwart von *trans*-ER GrgB aus fünf Malonyl-CoA (MCoA) und *S*-Adenosylmethionin und die C_4-Kette **56** in Abwesenheit von GrgB aus zwei MCoA-Einheiten.[47] Die Thioesterase GrgF katalysiert anschließend die Fusion der beiden Polyketidketten durch eine Claisen-Kondensation (→**57**) gefolgt durch eine hydrolytische Freisetzung des linearen Dimers **58**.[47,48] Verbindung **58** wird vermutlich durch den Angriff von C-3' des konjugierten Olefins an die Carboxygruppe (→*keto*-**59**), gefolgt von einer Keto-Enol-Tautomerie spontan in *enol*-**59** umgesetzt. Bevor dies geschieht, nimmt GrgG **58** als Substrat auf und führt die oxidative Cyclisierung durch, um das Furanongerüst **37** zu bilden, das bereits 2012 von Koseki *et al.* aus *Microdiplodia* sp. KS 75-1 isoliert wurde (Abschnitt 1.2.2).[42] Hierbei würde GrgG erst ein Wasserstoffatom des C-8 von **58** abspalten, was zur Isomerisierung der Doppelbindungen und zur Heterocyclisierung zu Desmethylgregatin A (**37**) führen würde. Letztlich methyliert die *O*-Methyltransferase (MT) von GrgD die Carboxylgruppe von **37**, um Gregatin A (**17**) zu erhalten. Der vorgeschlagene Mechanismus der oxidativen Cyclisierung von **58** zu Furanon **37** ist in Schema 6 gezeigt. Nach Aufnahme von **58** als Substrat von GrgG soll zuerst ein Wasserstoffatom von C-8 homolytisch abgespalten werden, wodurch Radikal **60a** entsteht. Von **60a** kann nun ein Elektron auf das Eisen-Hem-Zentrum von GrgG übertragen werden, um die carbokationische Spezies **60b** zu erhalten, die durch Heterocyclisierung und Isomerisierung zu Furanon **37** reagiert (Pfad a). Alternativ kann von GrgG aus **60a** das hydroxylierte Intermediat **61** geliefert werden, welches durch Dehydratisierung Furanon **37** bildet (Pfad b).[47]



Schema 6: Zwei vorgeschlagene Mechanismen der oxidativen Heterocyclisierung von **58** zu Desmethylgregatin (**37**).[47]

Des Weiteren stellten sie Vermutungen zur Biosynthese von Cyclogregatin (**18**) und Gregatin C (**28**) und D (**29**) an, da sie diese im Laufe ihrer Studien ebenfalls isolieren konnten (Schema 5).

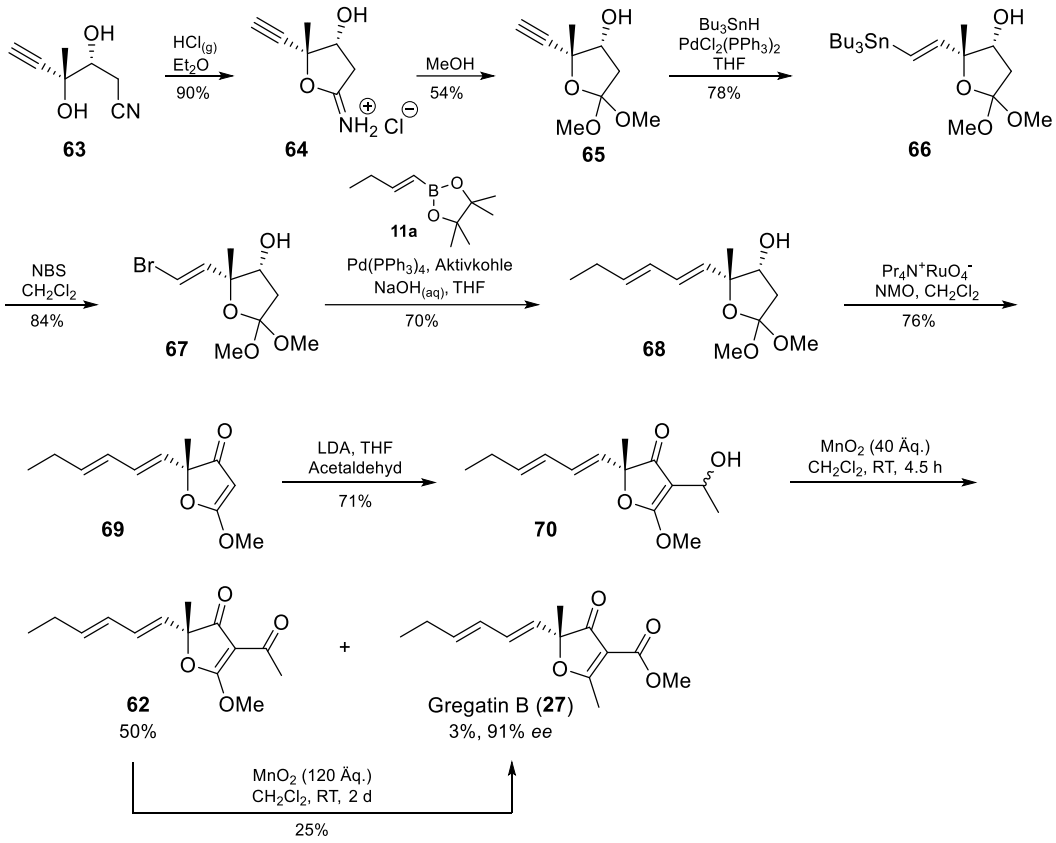
Cyclogregatin (**18**) soll nach einem ähnlichen Mechanismus aus Intermediat *enol*-**59** entstehen, wie Furanon **37** aus Vorläufer **58**. Gregatin C (**28**) und D (**29**) wurden als nicht-enzymatische, von Gregatin A (**17**) abgeleitete Metabolite, isoliert, weshalb Matsuda *et al*. vermuteten, dass sie durch einen stereoselektiven Michael-Angriff von Wasser an das C-2" von Gregatin A (**17**) entstehen.[47]

1.2.4 Totalsynthesen

Burghart-Stoll und Brückner[38] lieferten 2011 zufällig die erste Totalsynthese von Gregatin B (**27**) und damit die 2. Strukturrevision der Strukturklasse. Sie wollten ursprünglich Gregatin B der 2. Generation **62** darstellen (Abbildung 9), stellten aber fest, dass die analytischen Daten von **62** nicht mit denen des isolierten Naturstoffs übereinstimmten. Durch Analyse eines Nebenproduktes der letzten Stufe stellten sie fest, dass es sich bei der Leitstruktur der Naturstoffklasse nicht wie bisher angenommen um ein 4-Acyl-5-methoxyfuran-3(2*H*)-on sondern um ein 4-(Methoxycarbonyl)furan-3(2*H*)-on handelt. Ebenfalls revidierten sie die absolute Konfiguration des Stereozentrums durch Darstellung beider Enantiomere und den Vergleich ihrer spezifischen Drehwinkel mit denen des Naturprodukts von (*S*) zu (*R*).[38]

Abbildung 9: Struktur von Gregatin B der 2. Generation **62** und der 3. Generation **27**.

Im Folgenden wird die erste Totalsynthese von Gregatin B (**27**) der 3. Generation beschrieben (Schema 7). Sie beginnt mit der Darstellung von Imidolacton-Hydrochlorid **64** via Pinner-Cyclisierung durch Umsetzung von Dihydroxynitril **63** mit HCl. Methanolyse von **64** lieferte Ortholacton **65**, welches Pd(II)-katalysiert mit Tributylzinnhydrid in Stannan **66** überführt wurde. Stannan **66** lag als 93:7-Gemisch mit seinem Regioisomer vor und wurde mit *N*-Bromsuccinimid in Bromoolefin **67** überführt, welches mit Boronsäureester **11a** in einer Suzuki-Kreuzkupplung umgesetzt wurde (→**68**), um die Hexadienyl-Seitenkette einzuführen. Ley-Griffith-Oxidation von **68** mit TPAP und NMO lieferte unter Eliminierung von Methanol Furanon **69**. Die Acetylierung von **69** erfolgte in zwei Stufen durch sukzessive Behandlung mit LDA und Acetaldehyd (→**70**) und Oxidation mit aktiviertem Mangandioxid. Hauptprodukt **62**, was bisher für die richtige Struktur von Gregatin B gehalten wurde, wurde mit 50% Ausbeute erhalten. Die spektroskopischen Daten unterschieden sich jedoch von denen des Naturstoffs. Überraschenderweise lieferte die Oxidation von **70** mit 3% Ausbeute ein Nebenprodukt, bei dem es sich laut NMR-Daten um Gregatin B (**27**) handelte. Durch weitere Experimente wurde festgestellt, dass sich **62** durch Umsetzung mit 120 Äquivalenten Mangandioxid bei Raumtemperatur nach zwei Tagen mit 25% Ausbeute in Gregatin B (**27**) isomerisieren ließ. Wurde *para*-Toluolsulfonsäure zur Isomerisierung verwendet kam es zur Racemisierung des Stereozentrums von Gregatin B (**27**).[38]

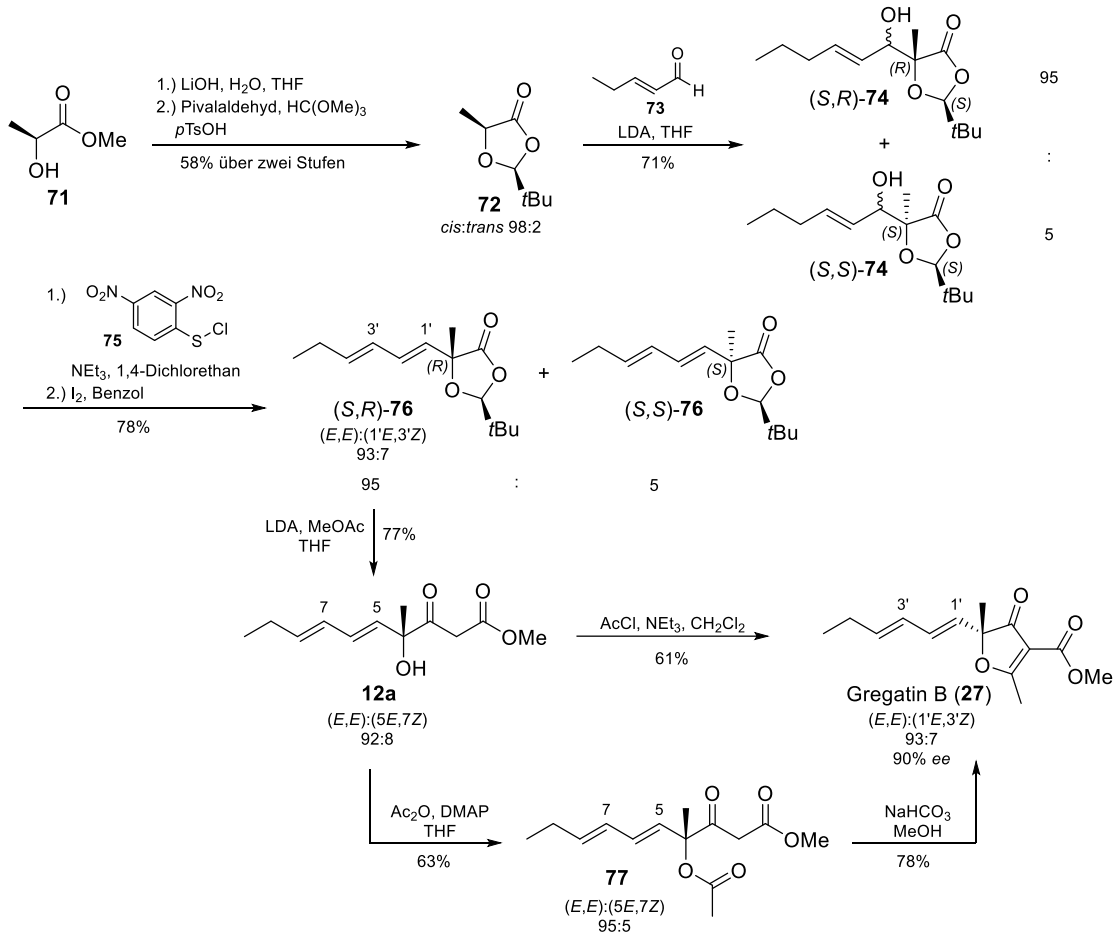


Schema 7: Erste Totalsynthese von Gregatin B (**27**) von Burghart-Stoll und Brückner.[38]

Durch Vergleich der spektroskopischen Daten des synthetischen Gregatins B (**27**) und denen der anderen bis dahin isolierten Vertreter der Strukturklasse wie die Gregatine, Aspertetronine, Graminine und Penicilliole wurden deren Strukturen ebenfalls revidiert.[38]

Da die Synthese von Gregatin B (**27**) von Burghart-Stoll und Brückner nur zufällig war, publizierten sie nur ein Jahr später einen gezielten Zugang zu der Strukturklasse durch die Synthese der Gregatine A (**17**), B (**27**), D (**29**) und Aspertetronin A (**44**).[39]

Gregatin B (**27**) wurde nun ausgehend von L-Milchsäuremethylester (**71**) in 7 bzw. 8 Stufen dargestellt (Schema 8). Dazu wurde **71** zuerst mit Lithiumhydroxid verseift und mit Pivalaldehyd acetalisiert, wobei Acetal **72** als *cis*:*trans*-Gemisch mit einem Verhältnis von 98:2 erhalten wurde. Die Hydroxyalkylierung mittels Aldol-Reaktion über das korrespondierende Lithiumenolat von **72** mit (*E*)-Hex-2-enal (**73**) führte zu einer Mischung der Diastereomere (*S*,*R*)-**74** und (*S*,*S*)-**74** in einem Verhältnis von 95:5, welche nicht voneinander getrennt werden konnten. Das Gemisch wurde anschließend mit 2,4-Dinitrobenzolsulfonylchlorid (**75**) und Triethylamin sulfoniert, wobei die entstandenen Intermediate *in situ* durch eine 2,3-Umlagerung und β-Eliminierung des Sulfoxides zu den voneinander trennbaren Dienen (*S*,*R*)-**76** und (*S*,*S*)-**76** umgesetzt wurden.[39]

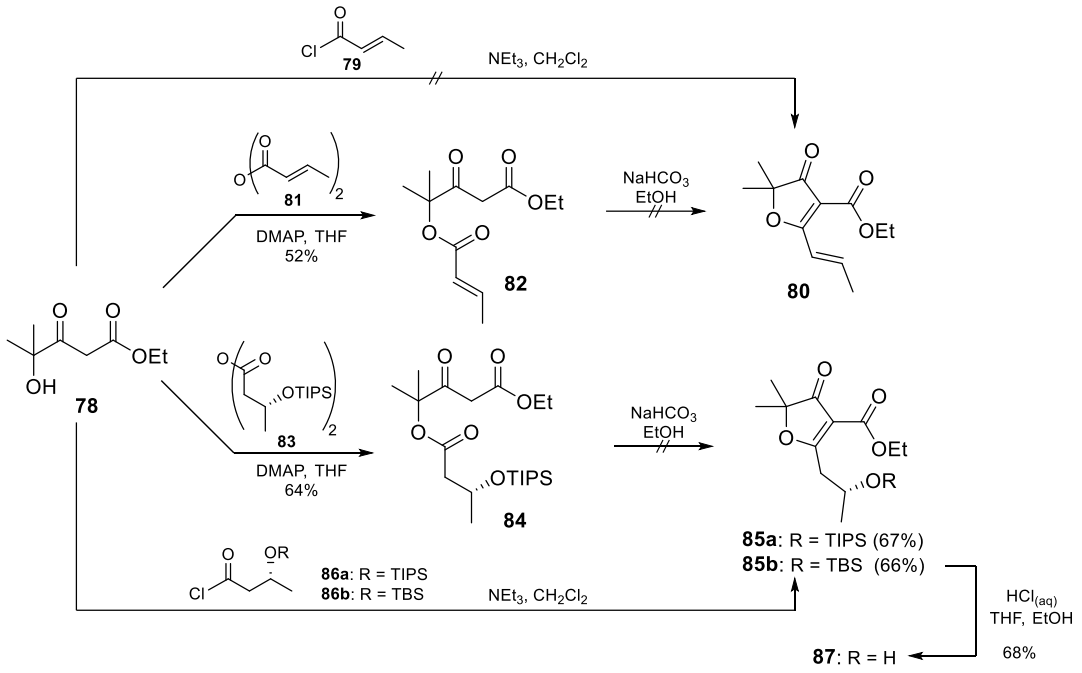


Schema 8: Zweite Totalsynthese von Gregatin B (**27**) von Burghart-Stoll und Brückner durch Hydroxyalkylierung, Dehydrierung, Acylierung und Knoevenagel-ähnlicher Kondensation.[39]

Dien (*S*,*R*)-**76** lag nach zusätzlicher Iod-katalysierter Isomerisierung als (*E*,*E*):(1'*E*,3'*Z*)-Gemisch im Verhältnis 93:7 vor. γ-Hydroxy-β-ketoester **12a** wurde durch Claisen-Kondensationen zwischen dem Lithiumenolat von Methylacetat und Acetal (*S*,*R*)-**76** dargestellt. Verbindung **12a** wurde mit Essigsäurechlorid und Triethylamin zuerst acetyliert und anschließend *in situ* zu Gregatin B (**27**) cyclisiert. Alternativ wurde **12a** mit Essigsäureanhydrid und DMAP zu Ester **77** überführt, welcher in einer baseninduzierten Knoevenagel-ähnlichen Reaktion zu Furanon **27** cyclisiert wurde. Das Problem dieser Synthese war, dass Gregatin B (**27**) nicht isomerenrein erhalten werden konnte. Es enthielt aufgrund der mangelnden Stereokontrolle bei der Dehydratisierung des Aldol-Additionsproduktes **74** 5% seines (*E*,*E*)-Enantiomers und 7 rel-% seines (1'*E*,3'*Z*)-Diastereomers. Nichtsdestotrotz wurde Gregatin B (**27**) ausgehend von L-Milchsäuremethylester (**71**) mit einer guten Gesamtausbeute von 15% über 7 Stufen bzw. mit 12% über 8 Stufen erhalten.

Diesen neuen Zugang zu der Strukturklasse der Dihydro-3(2*H*)-furanone wollten Burghart-Stoll und Brückner auf die Synthese von Gregatin A (**17**) und D (**29**) anwenden, indem sie γ-Hydroxy-β-ketoester **12a** mit aktivierter Crotonsäure oder einer aktivierten, geschützten β-Hydroxybuttersäure acylierten und das Zwischenprodukt cyclisierten. Um die Reaktionsbedingungen zu testen,

verwendeten sie den vereinfachten γ-Hydroxy-β-ketoester **78** als Modellverbindung (Schema 9), die zuerst mit den Säureanhydriden **81** und **83** in die entsprechenden Ester **82** und **84** überführt wurde. Beide Ester **82** und **84** konnten überraschenderweise jedoch nicht mittels baseninduzierter Cyclokondensation zu den gewünschten Furanonen **80** und **85a** cyclisiert werden. Ester **82** dimerisierte durch eine intermolekulare Michael-Addition seines Enolats an die Doppelbindung des Crotonylrests. Ester **84** war dahingegen nahezu inert unter den gewählten Reaktionsbedingungen, was Burghart-Stoll und Brückner auf die strukturell anspruchsvolle TIPS-Gruppe zurückführten.[39]

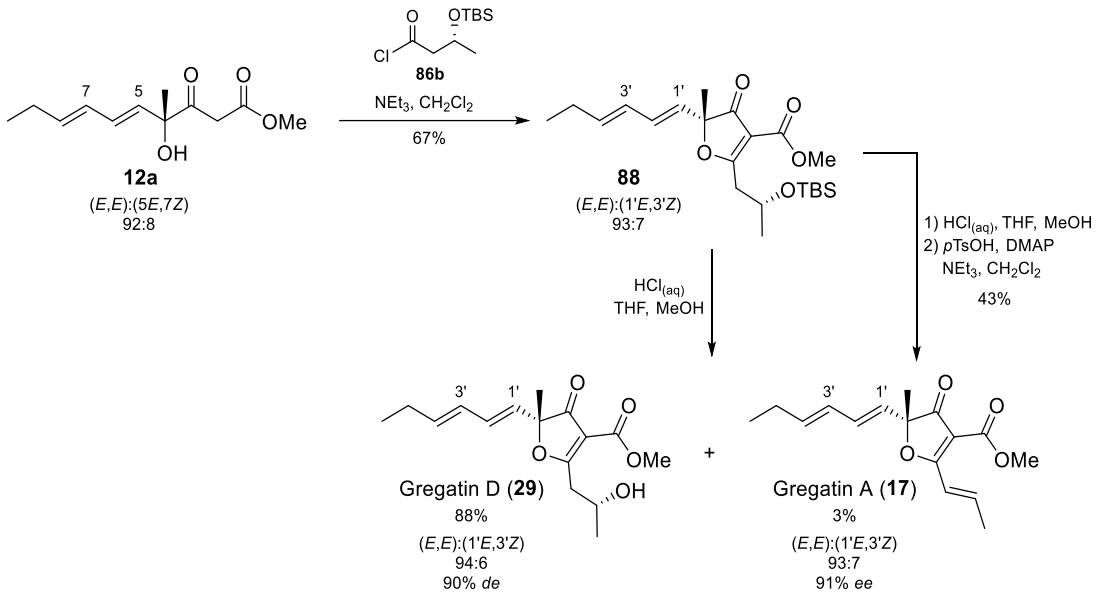


Schema 9: Modellversuche zur Darstellung des Furanons mit Crotonylrest **80** und des Furanons mit hydroxyliertem Rest **87**.[39]

Sie versuchten daher Modelverbindung **78** unter Verwendung eines Säurechlorids direkt ins Furanon zu überführen. Dazu wurde **78** mit Crotonsäurechlorid (**79**) in Gegenwart von Triethylamin umgesetzt, was jedoch lediglich zu einer Vielzahl an nicht identifizierten Nebenprodukten führte. Die Umsetzung von **78** mit den Silyl-geschützen Säurechloriden **86a** und **86b** lieferten jedoch die gewünschten Furanone **85a** bzw. **85b**. Furanon **85b** wurde mit HCl in heißem, wässrigem Ethanol TBS-entschützt, wodurch das hydroxylierte Furanon **87** erhalten wurde. Zusätzlich entstanden bei der Reaktion 4% des entsprechenden β-Eliminierungsprodukts **80**, welches nicht gezielt synthetisiert werden konnte. Aufgrund dieser Beobachtung sollte der Crotonylrest von Gregatin A (**17**) durch β-Eliminierung der OH-Gruppe eines β-Hydroxypropylsubstituenten eingeführt werden.

Die letzten Stufen der Synthese von Gregatin A (**17**) und D (**29**) sind in Schema 10 dargestellt. Hydroxy-β-ketoester **12a** wurde dafür mit dem TBS-geschützten Säurechlorid **86b** in Furanon **88** überführt. Dieses wurde anschließend mit HCl in wässrigem Methanol zu Gregatin D (**29**) entschützt, wobei ebenfalls 3% von Gregatin A (**17**) isoliert werden konnte. Dieses wurde gezielt synthetisiert

indem **88** erst mit HCl entschützt, das Rohprodukt tosyliert und im gleichen Schritt die Abgangsgruppe eliminiert wurde. Ausgehend von L-Milchsäuremethylester (**71**) wurde Gregatin D (**29**) somit in 8 Stufen mit einer Gesamtausbeute von 15% und Gregatin A (**17**) in 9 Stufen mit 7% dargestellt.



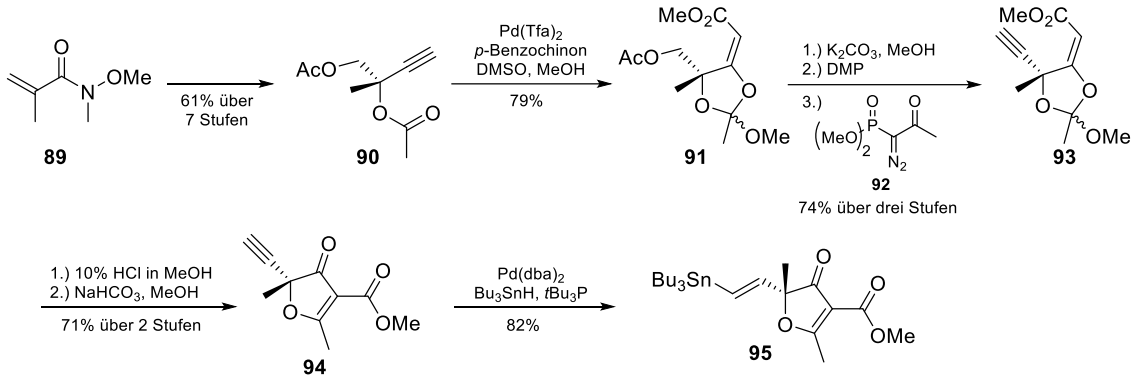
Schema 10: Erste Totalsynthese von Gregatin A (**17**) und D (**29**) ausgehend von γ-Hydroxy-β-ketoester **12a**.[39]

Analog zur Syntheseroute für Gregatin A (**17**) haben Burghart-Stoll und Brückner zusätzlich Aspertetronin A (**44**) ausgehend von D-Milchsäuremethylester mit einer Gesamtausbeute von 15% in 9 Stufen dargestellt. Ebenfalls war es ihnen möglich, die Enantiomere von Gregatin B (**27**) und Gregatin C (**28**) herzustellen.[39]

Zusammenfassend konnten Burghart-Stoll und Brückner zum zweiten Mal Gregatin B (**27**) und zum ersten Mal Gregatin A (**17**) und D (**29**) sowie Aspertetronin A (**44**) durch einen modularen Zugang zur Strukturklasse der Dihydro-3(2*H*)-furanone mit (*E*,*E*)-Hexadienyl-Seitenkette synthetisieren. So konnten zwar verschiedene Reste am C-3 des Furanons eingebracht werden, die Seitenkette war jedoch nicht variabel.

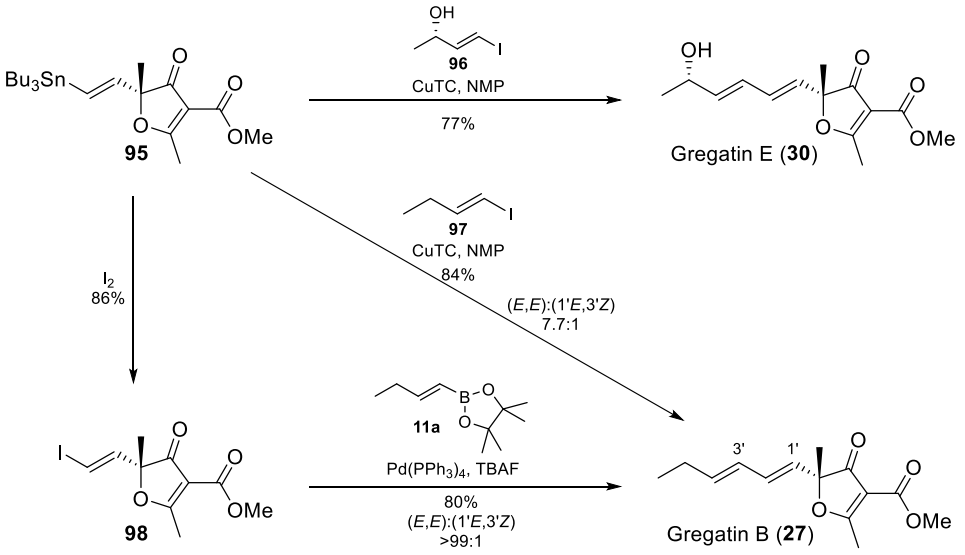
Im Jahr 2014 veröffentlichte Kato *et al*. eine weitere Synthese von Gregatin B (**27**) und die erste Totalsynthese von Gregatin E (**30**).[49] Im Gegensatz zu der vorherigen Synthese von Burghart-Stoll und Brückner[39] wurde hierbei die Furanon-Leitstruktur vor den jeweiligen Seitenketten etabliert, da sich Gregatin E (**30**) nur durch eine zusätzliche Hydroxyfunktion in Position 10 der Seitenkette von **27** unterscheidet.[49] Die Synthese begann mit Herstellung des Propargylacetats **90** in 7 Stufen ausgehend von Weinrebamid **89** (Schema 11). Eine Pd(II)-katalysierte, oxidative, cyclisierende Methoxycarbonylierung von Propargylacetat **90** in DMSO und Methanol ergab den cyclischen Orthoester **91** als einziges Produkt. Bei einer Durchführung der Reaktion ohne DMSO als Lösungsmittel wurde die Dimerisierung von Orthoester **90** beobachtet. Die Acetoxymethylgruppe von **91** wurde anschließend in einer dreistufigen Sequenz in das endständige Alkin **93** überführt. Dazu wurde **91** erst hydrolysiert,

der erhaltene Alkohol zum Aldehyd oxidiert und dieser mit dem Bestmann-Ohira-Reagenz (**92**) zu dem terminalen Alkin **93** umgesetzt. Durch Säurebehandlung von Verbindung **93** gefolgt von einer Knoevenagel-ähnlichen Kondensation des offenen Esters wurde Furanon **94** in zwei Stufen erhalten. Regioselektive Pd(II)-katalysierte Hydrostannylierung von **94** führte zu Vinylstannan **95**.[49]



Schema 11: Synthese des Vinylstannans **95** ausgehend von Weinrebamid **89** in 14 Stufen zur Synthese von Gregatin B (**27**) und E (**30**) von Kato *et al.*[49]

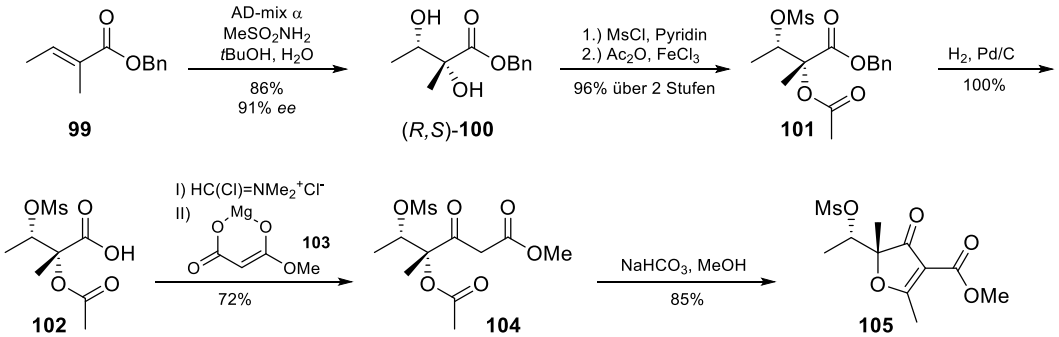
Ausgehend von Vinylstannan **95** sollten die fehlenden Reste der Seitenketten von Gregatin B (**27**) und E (**30**) mittels Stille-Kreuzkupplung mit einem Vinyliodid eingeführt werden (Schema 12). Die beste Ausbeute mit 77% lieferte die Kupfer-katalysierte Kreuzkupplung mit dem ungeschützten Vinyliodid **96**. Versuche mit einem TBS-geschützten Vinyliodid zeigten, dass die getesteten Fluorid-vermittelten Desilylierungen nicht zielführend waren, da sich der Naturstoff unter den gewählten Entschützungsbedingungen als instabil erwies. Die Stille-Kupplung von Stannan **95** und Vinyliodid **97** lieferte Gregatin B (**27**) mit einer guten Ausbeute von 84%. Es kam jedoch zur Isomerisierung der eingeführten Doppelbindung, wodurch **27** nur mit einem (*E*:*Z*)-Verhältnis von 7.7:1 erhalten wurde.



Schema 12: Dritte Totalsynthese von Gregatin B (**27**) und erste Totalsynthese von Gregatin E (**30**) mittels Kreuzkupplung von Kato *et al.*[49]

Um zu testen, ob die Stille-Kupplung mit inversen Funktionalitäten ohne Isomerisierung abläuft, wurde Stannan **95** in Iodid **98** überführt. Die Stille-Kupplung von **98** mit dem entsprechenden Stannan als Kupplungsreagenz führte tatsächlich ohne Isomerisierung zu Gregatin B (**27**), aber lediglich mit einer moderaten Ausbeute von 60%. Daher wurde eine Pd(II)-katalysierte Suzuki-Kreuzkupplung von Vinyliodid **98** mit Boronsäureester **11a** durchgeführt, wodurch Gregatin B (**27**) mit einer Ausbeute von 80% ohne Isomerisierung dargestellt werden konnte.
Kato *et al*. lieferten somit einen interessanten Ansatz für die Synthese von Dihydro-3(2*H*)-furanonen, bei dem erst das Furanon aufgebaut und anschließend durch Kreuzkupplung die Seitenkette eingeführt wurde. Diese Synthese ist jedoch in der Variabilität des Restes am Furanonring begrenzt.

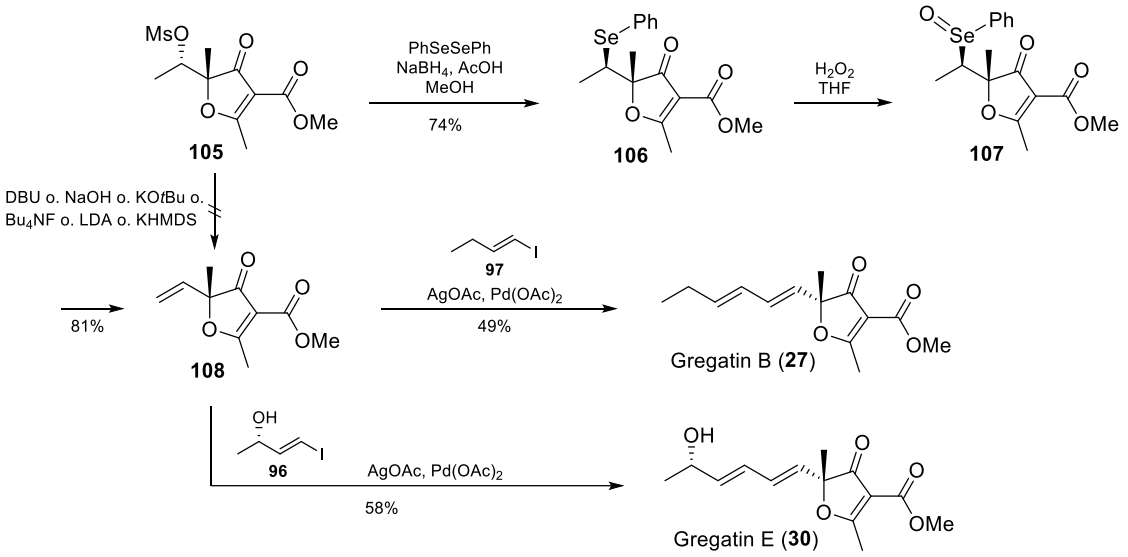
Im Jahr 2014 wurde eine weitere Synthese von Gregatin B (**27**) und E (**30**) von Weber und Brückner publiziert, die einen ähnlichen Ansatz verfolgten.[50] Es wurde auch hier zuerst der Furanonring aufgebaut und anschließend die entsprechenden Seitenketten durch Kreuzkupplung eingeführt (Schema 13). Die Synthese ist für beide Naturstoffe mit jeweils 9 Stufen wesentlich kürzer als die von Kato *et al*.,[49] die für Gregatin E (**30**) 15 Stufen und für Gregatin B (**27**) 16 Stufen lang ist.
Weber und Brückner begannen ihre Synthese mit einer asymmetrischen Sharpless-Dihydroxylierung von Benzyltiglat (**99**) zu Diol (*R,S*)-**100** mit 91% *ee* (Schema 13). Die sekundäre Hydroxygruppe wurde mesyliert und die tertiäre mit Essigsäureanhydrid und $FeCl_3$ als Katalysator acetyliert (→**101**). Nach Hydrierung des Benzylesters **101** wurde die freie Säure **102** in β-Ketoester **104** überführt, wobei sich die Erhaltung des Mesylats als schwierig erwies. Die gängigen Methoden zur Aktivierung der Carbonsäure [$(COCl)_2$, $(COCl)_2$ + kat. DMF, CDI und Meldrumsäure/Pyridin] lieferten hier nicht das gewünschte Produkt. Durch Aktivierung von **102** mit dem Vilsmeier-Reagenz konnte das gewünschte Carbonsäurechlorid letztlich erhalten werden, welches ohne Aufreinigung direkt mit dem Dianion von Monomethylmalonat **103**, aus Monomethylmalonat und 2 Äquivalenten *i*PrMgCl in THF, zu β-Ketoester **104** umgesetzt wurde. Die anschließende Knoevenagel-ähnliche Kondensation erfolgte unter milden basischen Bedingungen und führte zu Furanon **105**.



Schema 13: Synthese des Furanons **105** als Vorläufer für Gregatin B (**27**) und E (**30**) nach Weber und Brückner.[50]

Da die Seitenketten mittels Heck-Kupplung eingeführt werden sollten, musste das sekundäre Mesylat in **105** durch β-Eliminierung in eine terminale Doppelbindung überführt werden (Schema 14). Der

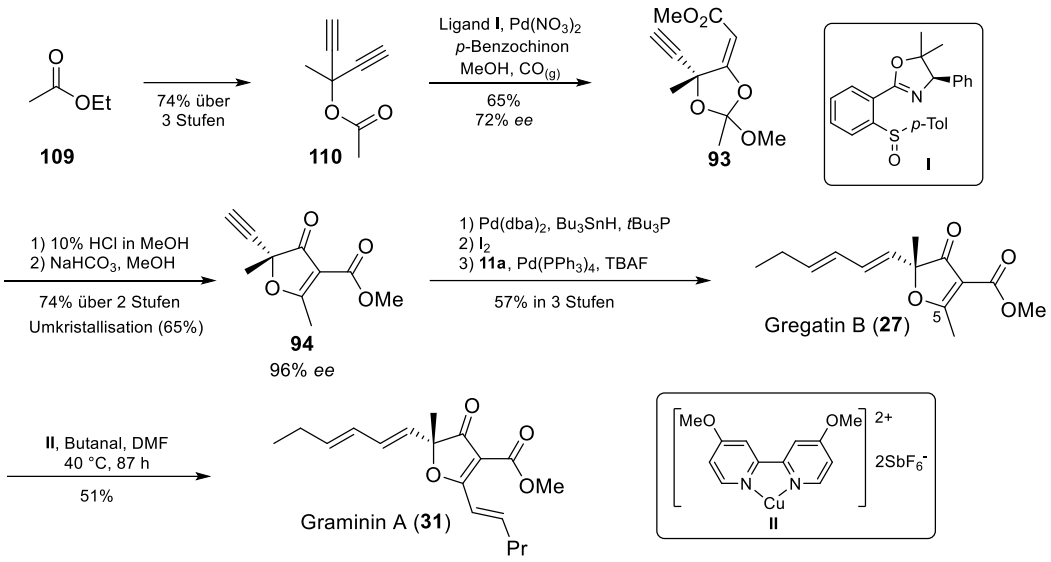
direkte Weg führte auch unter Verwendung verschiedener Basen/Lösungsmittel-Kombinationen nicht zum gewünschten Produkt **108**, weshalb das benötigte Furanon **108** über einen Umweg dargestellt werden musste. Dazu wurde die Mesylat-Gruppe von **105** mit gepuffertem Diphenyldiselenid substituiert (→**106**), anschließend mit H_2O_2 in Amin-freiem THF zu **107** oxidiert und dieses unter den gleichen Bedingungen zum Vinyl-substituierten Furanon **108** eliminiert. Durch Heck-Kupplung von Vinylfuranon **108** mit (*E*)-1-Iodobut-1-en (**97**) oder mit (*S*,*E*)-3-Hydroxy-1-iodobut-1-en (**96**) wurden Gregatin B (**27**) und Gregatin E (**30**) synthetisiert.[50] Durch diesen 9-stufigen Syntheseansatz von Weber und Brückner war es möglich Gregatin B (**27**) im Gramm-Maßstab und 15% Gesamtausbeute darzustellen.



Schema 14: Vierte Totalsynthese von Gregatin B (**27**) und zweite von Gregatin E (**30**) nach Weber und Brückner.[50]

Kato *et al*. veröffentlichten im Jahr 2019 eine verbesserte Synthese von Gregatin B (**27**), die auf ihrer bisherigen Synthese[49] beruhte, und entwickelten sie durch die erste Totalsynthese von Graminin A (**31**) weiter.[51] Der benötigte Orthoester **93** wurde bisher in insgesamt 11 Stufen ausgehend von Weinrebamid **89** und einer Ausbeute von 48% dargestellt (siehe Schema 11).[49] Nun verkürzten sie die Route auf nur 4 Stufen (Schema 15).[51] Ausgehend von Essigsäureethylester (**109**) wurde das racemische Propargylacetat **110** durch Zugabe von TMS-Acetylid, Desilylierung und Acetylierung mit 74% über 3 Stufen hergestellt. Die Pd(II)-katalysierte cyclisierende Carbonylierung von Propargylacetat **110** musste in diesem Fall asymmetrisch durchgeführt werden, wofür ein- bis dreizahnige Sulfoxid-Oxazolin-Liganden untersucht wurden. Dabei spielte die Stereochemie des Oxazolins eine wichtige Rolle bei der Stereoinduktion. Ebenfalls beeinflusste das Gegenion des Palladiums sowohl die Ausbeute als auch die Enantioselektivität der Reaktion. So führte die Verwendung von Palladium(II)-nitrat statt Palladium(II)-trifluoroacetat zu einer Verbesserung von beidem. Letztlich konnte mit Ligand **I** und $Pd(NO_3)_2$ Orthoester **93** mit 65% und einem *ee* von 72% erhalten werden. Verbindung **93** wurde anschließend analog zur bereits 2013 publizierten Synthese in

Furanon **94** überführt.[49,51] Die optische Reinheit von **94** wurde durch Umkristallisation aus Dichlormethan und Hexan erhöht und der *ee* auf 96% verbessert.[51] Regioselektive Pd-katalysierte Hydrostannylierung, Iodierung und Suzuki-Kupplung führten zu Gregatin B (**27**). Graminin A (**31**) konnte durch Aldol-Reaktion von Gregatin B (**27**) mit Butanal in 51% Ausbeute dargestellt werden. Die Aldol-Reaktion wurde weder unter sauren noch unter basischen Bedingungen durchgeführt, da dies meist zu komplexen Nebenverbindungen führte, sondern unter Zugabe des Bipyridin-Komplexes **II**. Der Kupfer-Komplex **II** aktivierte den β-Ketoester von **27**, wodurch die Methylgruppe am C-5 des Furanons acide wurde.[51]



Schema 15: Überarbeitete Totalsynthese von Gregatin B (**27**) und Weiterentwicklung durch die erste Totalsynthese von Graminin A (**31**) mittels Aldolreaktion nach Kato *et al*.[51]

Durch Weiterentwicklung ihrer ersten Totalsynthese für Dihydro-3(2*H*)-furanone[49] konnte Gregatin B (**27**) diesmal über nur 10 Stufen und mit einer Gesamtausbeute von 13% dargestellt werden. Ebenfalls lieferten sie die erste Totalsynthese von Graminin A (**31**) mit 11 Stufen und einer Gesamtausbeute von 7%.

Zusammenfassend wurden somit bisher 5 verschiedene, synthetische Zugänge zu den Dihydro-3(2*H*)-furanonen von den Arbeitsgruppen Brückner[38,39,50] und Kato[49,51] veröffentlicht, wodurch Gregatin A (**17**),[39] B (**27**),[38,39,49,50] D (**29**),[39] E (**30**),[39,49,50] Aspertetronin A (**44**)[39] und Graminin A (**31**)[51] totalsynthetisch dargestellt wurden. Die gewählten synthetischen Ansätze waren jedoch alle entweder bei der Einführung variabler Reste der Seitenkette oder am Furanonring eingeschränkt oder lieferten die Naturstoffe mit mäßigen Enantioselektivitäten oder als Isomerengemische.

1.2.5 Ophiofuranone

Lou *et al*.[24] isolierten 2019 aus dem Pilz *Ophiosphaerella korrae*, der in der Flechte *Physciaceae physcia* aus der chinesischen Provinz Xinjiang gefunden wurde, sechs neue Metabolite, darunter die zwei Ophiofuranone A (**1**) und B (**2**) mit einem Dihydro-3(2*H*)-furanon-Motiv (Abbildung 10). Der Pilz *O. korrae* gilt als einer der drei Erreger, der für die sogenannte *spring dead spot*-Krankheit von Bermudagras, die im Frühjahr gut definierte, kreisförmige, tote Flecken im Gras hervorruft.[52] Die Ethylacetat-Rohextrakte von *O. korrae* ergaben drei zytotoxische Fraktionen, aus denen die neuen Naturstoffe isoliert und deren Struktur mittels NMR-, ESI-HRMS- und IR-Analysen aufgeklärt wurden. Dabei wurde festgestellt, dass die Ophiofuranone A (**1**) und B (**2**) ein seltenes 4*H*-Furo[3,2-*c*]pyran-3,4(2*H*)-dion-Gerüst besitzen, welches bisher nur bei dem 1988 aus *A. panamensis* isolierten Cyclogregatin (**18**)[14] beobachtet wurde. Ihre 2-Methylbutadien-Seitenkette unterscheidet sich ebenfalls von denen der anderen Dihydro-3(2*H*)-furanone, da sie eine Methylverzweigung in Position 2' aufweist und der typische Ethylrest fehlt. Zusätzlich besitzen sie in Position **7** ein quartäres Stereozentrum im Gegensatz zu Cyclogregatin (**2**), dessen C-7 nicht substituiert ist. Die absolute Konfiguration der Stereozentren der Ophiofuranone wurde durch Vergleiche der gemessenen ECD-Spektren der Isolate mit berechneten ECD-Spektren für verschiedene Konfigurationen bestimmt. Dadurch ergab sich für Ophiofuranon A (**1**) ebenso wie für Ophiofuranon B (**2**) eine absolute Konfiguration von (2*S*,6*R*,7*S*).[24]

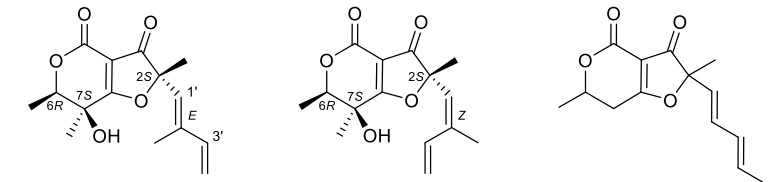
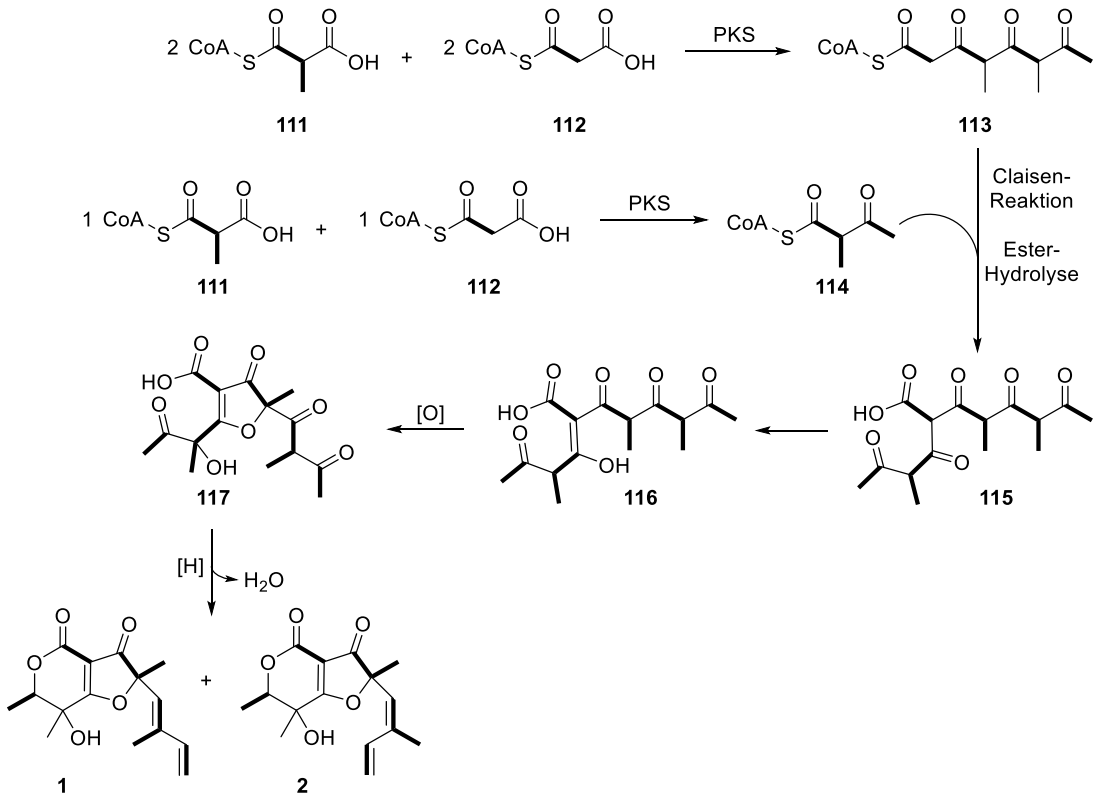


Abbildung 10: Strukturen der 2019 von Lou *et al.* isolierten Ophiofuranone A (**1**) und B (**2**)[24] und des strukturell verwandten Cyclogregatins (**18**).[14]

Ebenfalls wurde von Lou *et al.* eine mögliche Biosynthese der Ophiofuranone postuliert (Schema 16).[24] Sie gehen davon aus, dass mittels Polyketidsynthase das Polyketon **113** aus zwei Malonyl- (**111**) und zwei Methylmalonyl-CoA-Einheiten (**112**) hergestellt werden. Aus jeweils einer Einheit **111** und **112** wird eine zweite, kürzere Kette **114** gebildet, beide über eine Claisen-Kondensation verknüpft und mittels Esterhydrolyse von der PKS freigesetzt. Die entstandene Polyketonsäure **115** tautomerisiert zu Enol **116**, welches oxidativ zu Dihydro-3(2*H*)-furanon **117** cyclisiert. Durch Lactonisierung und selektive Reduktion der Ketone der Seitenkette sollen Ophiofuranon A (**1**) und B (**2**) letztlich biosynthetisch dargestellt werden.[24] Die vorgeschlagene Biosynthese von Lou *et al*.[24] für die Ophiofuranone unterscheidet sich in einigen Punkten von den Ergebnissen der Studie zur Biosynthese von Gregatin A (**17**) und die vorgeschlagene Biosynthese des strukturverwandten Cyclogregatins (**18**) von Matsuda *et al*. (siehe Kapitel 1.2.3).[47] Gemeinsam haben jedoch beide Ansätze, dass das

Grundgerüst durch Fusion zweier separat aufgebauter Polyketidketten gebildet und das Furanon durch oxidative Cyclisierung geschlossen wird. Matsuda *et al*. äußerten ebenfalls eine Vermutung zur Biosynthese von Cyclogregatin (**18**), in welcher der 6-Ring vor dem 5-Ring geschlossen werden soll. Wie die Biosynthese der Ophiofuranone A (**1**) und B (**2**) genau abläuft, muss jedoch in weiterführenden Studien eindeutig untersucht werden.



Schema 16: Von Lou *et al*. vorgeschlagene Biosynthese von Ophiofuranon A (**1**) und B (**2**).[24]

Die isomeren Dihydro-3(2*H*)-furanone Ophiofuranon A (**1**) und B (**2**) weisen mit ihrem 4*H*-Furo[3,2-*c*]pyran-3,4(2*H*)-dion-Gerüst ein interessantes Strukturmerkmal auf,[24] das bis heute nur in einem einzigen weiteren Naturstoff, dem Cyclogregatin (**18**) gefunden wurde.[14] In der Literatur finden sich bisher keine Untersuchungen zu den biologischen Eigenschaften von **1** und **2**.

1.2.6 Thiocarboxyle und neue Vertreter der Gregatin-Familie

Im Jahr 2020 wurden zehn neue und sieben bekannte Vertreter der Dihydro-3(2*H*)-furanone aus dem Pilz *Penicillium* sp. sb62 von Ruan *et al*. isoliert.[23] Sechs der neuen Verbindungen wurden von ihnen „Thiocarboxylic" A (**7a**), B (**118**), C (**8**) und D (**119**) genannt. Da sich dies schwer ins Deutsche übertragen lässt, werden die Verbindungen im weiteren Verlauf dieser Arbeit daher „Thiocarboxyle" A-D genannt.

Die Thiocarboxyle A-D weisen als Rest am Furanon eine Thiopheneinheit auf, was für Dihydrofuranone neuartig ist. Thiocarboxyl B (**118**) ist zusätzlich am Thiophen carboxyliert. Außerdem wurden mit Gregatin F (**120**) und G (**9**) zwei weitere Vertreter der Gregatin-Familie isoliert,

die einen Propylsubstituenten am Furanon tragen. Das quartäre Stereozentrum ist bei allen Metaboliten (*R*)-konfiguriert. Da acht der neuen Naturstoffe in Position 10 der Seitenkette hydroxyliert sind, ergeben sich insgesamt vier Epimerenpaare, darunter Thiocarboxyl $C_{1,2}$ (**8a,b**) und $D_{1,2}$ (**119a,b**), Gregatin $F_{1,2}$ (**120a,b**) und $G_{1,2}$ (**9a,b**).[23]

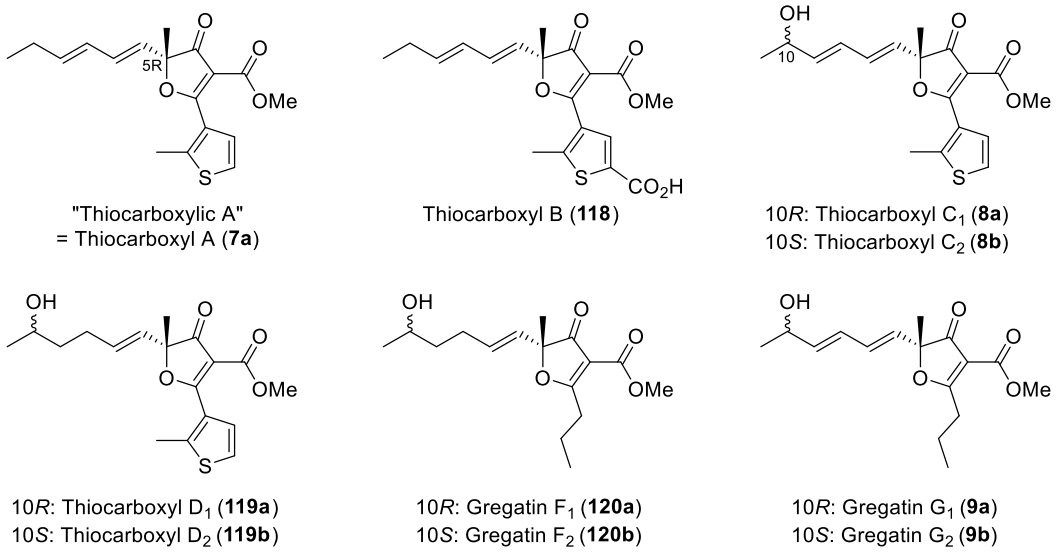
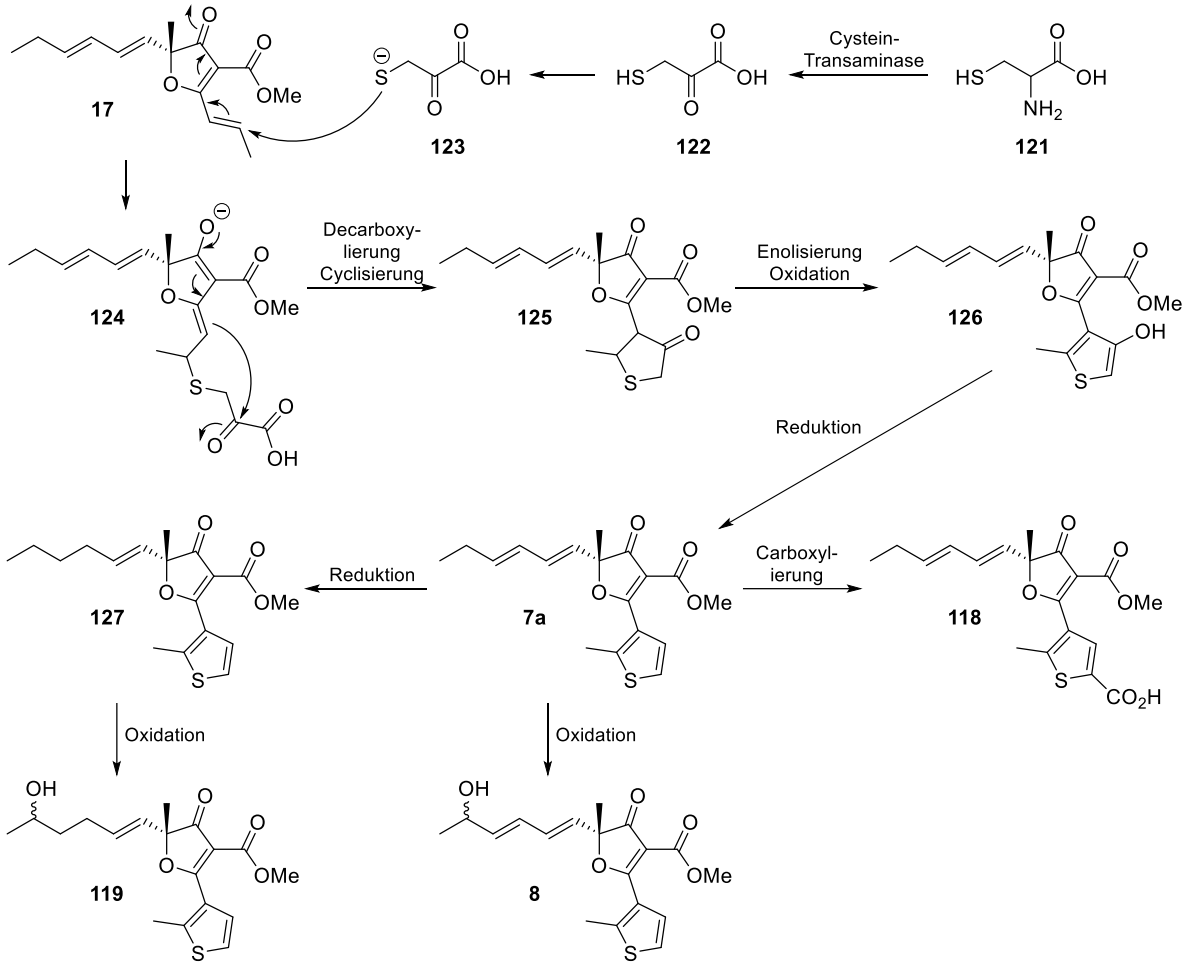


Abbildung 11: Strukturen der zehn neuen von Ruan *et al.* isolierten Dihydro-3(2*H*)-furanone: Thiocarboxyl A (**7a**), B (**118**), C (**8**) und D (**119**) und Gregatin F (**120**) und G (**9**).[23]

Ihre Strukturen wurden mittels ESI-HRMS und NMR-Daten zugeordnet und ihre absoluten Konfigurationen durch ECD-Kalkulationen und im Falle von **8**, **9**, **119** und **120** zusätzlich durch Vergleiche der spezifischen optischen Rotationswerte und der modifizierten Mosher-Methode[53] bestimmt.[23]

Ruan *et al.* vermuten, dass Gregatin A (**17**), welches ebenfalls isoliert wurde, und Cystein **121** die biosynthetischen Vorläufer der Thiocarboxyle sind (Schema 17). Cystein **121** soll demnach mittels Transaminase in α-Ketosäure **122** überführt werden. Nach Deprotonierung von **122** soll Mercaptopyruvat **123** an das verlängerte Michael-System von Gregatin A (**17**) angreifen, wodurch Intermediat **124** entsteht. Durch decarboxylierende Cyclisierung (→**125**), Enolisierung, Oxidation (→**126**), und Reduktion soll das Thiophen-Motiv aufgebaut und somit Thiocarboxyl A (**7a**) hergestellt werden. Metabolit **7a** könnte daraufhin carboxyliert (→**118**) oder nicht stereoselektiv hydroxyliert werden, um das Epimerenpaar **8a,b** zu erhalten. Eine Doppelbindung von Thiocarboxyl A (**7a**) könnte auch erst reduziert (→**127**) und anschließend oxidiert werden, wodurch die Epimere **119a** und **119b** dargestellt werden würden.[23]



Schema 17: Vorgeschlagene Biosynthese von Ruan *et al.* für die Thiocarboxyl-Familie mit Thiophen-Motiv ausgehend von Gregatin A (**17**) und Cystein **121**.[23]

Die neuen Verbindungen **7a**, **8**, **9** und **118-120** wurden *in vitro* auf ihre antimikrobiellen Eigenschaften gegen die gramnegativen Bakterien *Escherichia coli*, die grampositiven Bakterien *Staphylococcus aureus* und den Pilz *Candida albicans* untersucht (Tabelle 1). Sie zeigten unterschiedlich starke Aktivitäten mit minimalen Hemm-Konzentrationen zwischen 0.9 und 7.0 µg/mL. Die Thiocarboxyle A (**7a**) und B (**118**) wiesen die höchsten antimikrobiellen Aktivitäten auf, was darauf hindeutet, dass ein zusätzlicher Hydroxysubstituent oder eine fehlende Doppelbindung der Seitenkette zu einem Aktivitätsverlust führt. Die Verbindungen **7a** und **118** zeigten mit MICs von 0.9 und 1.7 µg/mL gegen *E. coli* eine ausgezeichnete Aktivität, die besser war als die der Referenz Streptomycin. Die Konfiguration der Hydroxygruppe der Seitenkette hatte keinen Einfluss auf die Wirkung, da die vier Epimerenpaare **8a,b**, **9a,b**, **119a,b** und **120a,b** jeweils die gleiche Aktivität zeigten.[23]

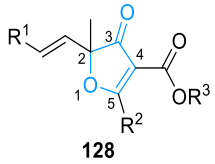
Tabelle 1: Antimikrobielle Aktivität der Verbindungen **7a**, **8**, **9**, **118**-**120** gegen *E. coli*, *S. aureus* und *C. albicans* und der jeweiligen Referenzen Streptomycin und Amphotericin B in µg/mL.[23]

Verbindung	MIC (µg/mL)		
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
7a	1.7	1.7	3.3
118	0.9	1.9	3.8
8a	7.0	3.5	7.0
8b	7.0	3.5	7.0
119a	3.5	3.5	7.0
119b	3.5	3.5	7.0
120a	3.0	3.0	5.9
120b	3.0	3.0	5.9
9a	2.9	2.9	4.4
9b	2.9	2.9	4.4
Streptomycin	2.3	0.1	n.b.
Amphotericin B	n.b.	n.b.	0.1

MIC: Minimale Hemm-Konzentration; n.b.: nicht bestimmt.

2 ZIELSETZUNG

Eine interessante Stoffklasse, die fast ausschließlich aus endophytischen Pilzen isoliert wurde, besitzt ein Dihydro-3(2*H*)-furanon als Leitmotiv (**128**, Abbildung 12). Sie weisen eine große Bandbreite an biologischen Aktivitäten auf. Sie wirken phytotoxisch,[17,18] antibiotisch,[14,15,18,25,26,28,42] antimykotisch,[14,15,18,25,28,42] zytotoxisch,[14,20,22] antifibrotisch,[21] und entzündungshemmend,[43,44] oder haben die Eigenschaft selektiv die Aktivität von eukaryotischen DNA-Polymerasen der Y-Familie zu inhibieren.[19] Viele der natürlichen Dihydro-3(2*H*)-furanone wurden bisher gar nicht oder nur teilweise auf ihre biologischen Aktivitäten untersucht.



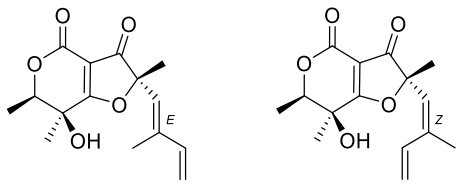
128

Abbildung 12: Allgemeine Struktur der Dihydro-3(2*H*)-furanone **128**.

Bislang wurden fünf verschiedene synthetische Zugänge zur Stoffklasse der Dihydro-3(2*H*)-furanone von den Arbeitsgruppen Brückner *et al*.[38,39,50] und Kato *et al*.[49,51] veröffentlicht. Diese Synthesen sind jedoch entweder in der Variabilität des Restes der Seitenkette R^1 oder des Restes am Furanonring R^2 eingeschränkt oder lieferten die Naturstoffe mit mäßigen Enantioselektivitäten oder als Isomerengemische.

Das Ziel dieser Arbeit war es daher flexible Syntheserouten zur Strukturklasse der Dihydro-3(2*H*)-furanone zu entwickeln, durch die es möglich ist sowohl R^1 als auch R^2, optimalerweise auf einer späten Stufe, zu variieren. Durch diese synthetischen Zugänge sollten natürliche Dihydro-3(2*H*)-furanone dargestellt werden, um sie anschließend auf ihre biologischen Eigenschaften zu untersuchen.

Bei zwei der Zielverbindungen handelte es sich um die 2019 isolierten bicyclischen Ophiofuranone A (**1**) und B (**2**),[24] da es bisher keine Totalsynthese zu Naturstoffen mit dieser interessanten Furopyran-3,4-dion-Grundstruktur gibt (Abbildung 13). Zusätzlich sollten die Ophiofuranone A (**1**) und B (**2**), die bisher nicht auf ihre biologischen Aktivitäten untersucht wurden, und zwei ihrer synthetischen Vorläufer ebenfalls mit Dihydro-3(2*H*)-furanon-Motiv auf ihre antimikrobiellen, zytotoxischen und Anti-Biofilm-Aktivitäten getestet werden.

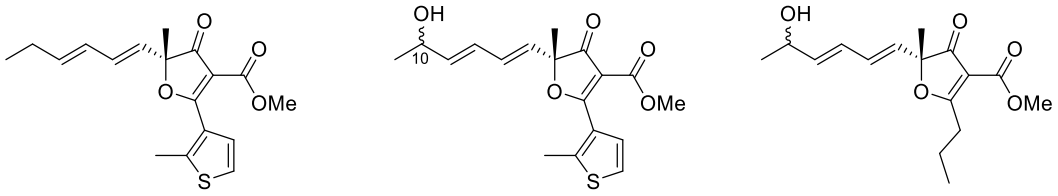


Ophiofuranon A (**1**) Ophiofuranon B (**2**)

Abbildung 13: Zwei der Zielverbindungen dieser Arbeit: Ophiofuranon A (**1**) und B (**2**).

Im zweiten Projekt sollten die Thiocarboxyle A (**7a**), C_1 (**8a**) und C_2 (**8a**) und Gregatine G_1 (**9a**) und G_2 (**9b**) totalsynthetisch dargestellt und auf ihre biologischen Aktivitäten untersucht werden, da die Isolate gegen *Escherichia coli*, *Staphylococcus aureus* und *Candida albicans* gute antimikrobielle Wirkungen mit minimalen Hemm-Konzentrationen im Bereich von 1.7-7.0 µg/mL aufwiesen (Abbildung 14).[23]

Zuerst sollte eine Synthese von Thiocarboxyl A (**7a**) etabliert und im Folgenden auf die Darstellung der Epimerenpaare Thiocarboxyl C_1 (**8a**) und C_2 (**8b**) und Gregatin G_1 (**9a**) und G_2 (**9b**) mit geeigneter Schutzgruppenstrategie angewendet werden.



Thiocarboxyl A (**7a**)

10*R*: Thiocarboxyl C_1 (**8a**)
10*S*: Thiocarboxyl C_2 (**8b**)

10*R*: Gregatin G_1 (**9a**)
10*S*: Gregatin G_2 (**9b**)

Abbildung 14: Weitere Zielverbindungen der vorliegenden Arbeit.

3 SYNOPSIS

Die vorliegende kumulative Dissertation umfasst zwei Publikationen und ein eingereichtes Manuskript, welche in Kapitel 5 zu finden sind. Die Arbeit handelt von der Entwicklung modularer Totalsynthesen natürlicher Dihydro-3(2*H*)-furanone **128**, um deren biologische Eigenschaften in Kooperation mit dem Helmholtz-Zentrum für Infektionsforschung in Braunschweig untersuchen zu können und ist in zwei Projekte unterteilt.

Im ersten Projekt wurde die erste bekannte Totalsynthese für die isomeren Ophiofuranone A (**1**) und B (**2**) mit Furopyran-3,4-dion-Grundstruktur entwickelt (Abbildung 15, oben) und die Naturstoffe **1** und **2** auf ihre antimikrobiellen, zytotoxischen und Anti-Biofilm-Aktivitäten untersucht. Die durch die Synthese der Ophiofuranone gewonnenen Erkenntnisse wurden im zweiten Projekt zur Etablierung eines modularen Zugangs zu weiteren Dihydro-3(2*H*)-furanonen genutzt, bei dem sowohl der Rest R^1 der Seitenkette als auch der Rest R^2 am Furanonring auf einer späten Stufe variabel eingeführt werden konnte. Mit dieser flexiblen Synthese wurden sowohl natürliche Dihydro-3(2*H*)-furanone als auch synthetische Derivate der Strukturklasse dargestellt (Abbildung 15, unten). Die Synthese wurde anhand von Derivat **7b** entwickelt, dessen Seitenkette um 2 C-Atome länger als die von Thiocarboxyl A (**7a**) ist.

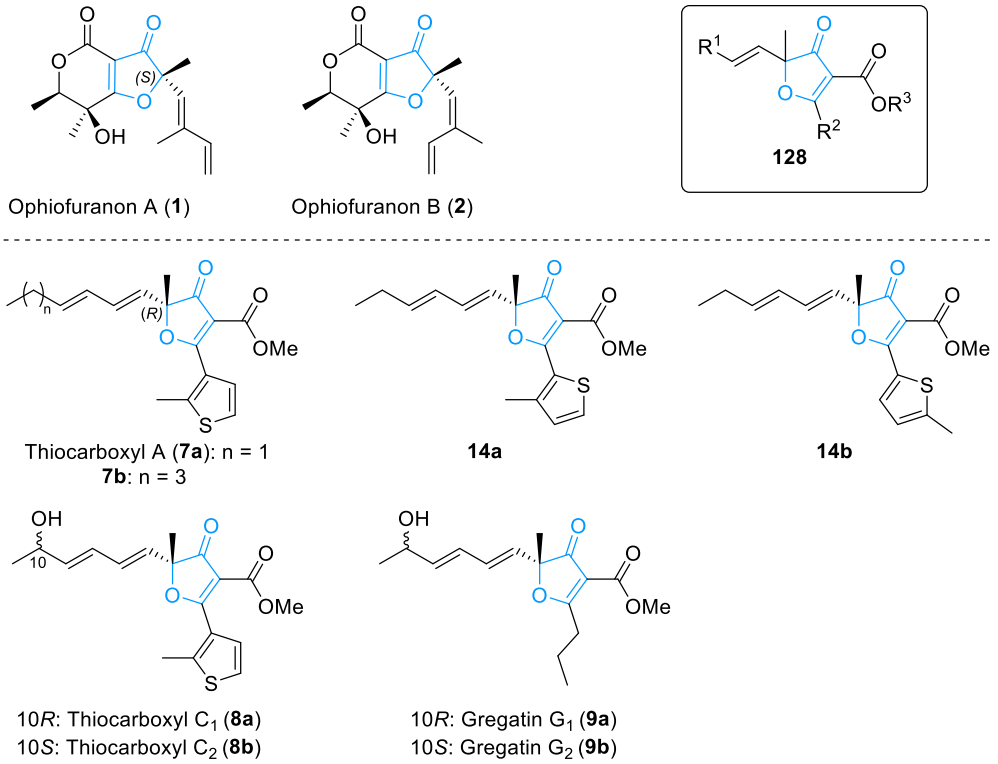
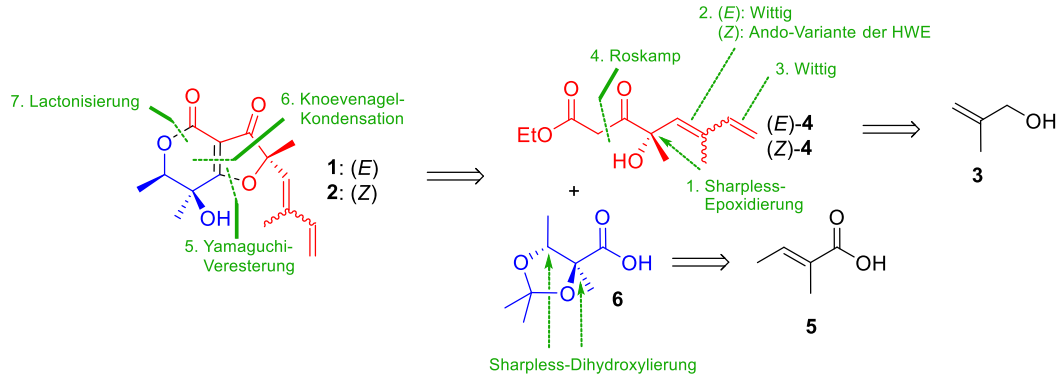


Abbildung 15: Übersicht aller natürlichen und synthetischen Dihydro-3(2*H*)-furanone, die in der vorliegenden Arbeit dargestellt wurden.

Anschließend wurden die eigentliche Zielverbindung Thiocarboxyl A (**7a**) und die zwei Analoga **14a** und **14b** hergestellt, da zuerst fraglich war, ob die Substituenten des Thiophens des Naturstoffs **7a** von Ruan *et al*.[23] richtig zugeordnet worden sind. Thiocarboxyl A (**7a**) und dessen drei Derivate **7b**, **14a** und **14b** wurden auf ihre antimikrobiellen und zytotoxischen Aktivitäten getestet. Im Folgenden wurden mit dem flexiblen Ansatz und Erarbeitung einer geeigneten, orthogonalen Schutzgruppenstrategie zusätzlich die beiden Epimerenpaare Thiocarboxyl $C_{1,2}$ (**8a,b**) und Gregatin $G_{1,2}$ (**9a,b**) synthetisiert und ebenfalls auf ihre antimikrobiellen und zytotoxischen Eigenschaften untersucht.

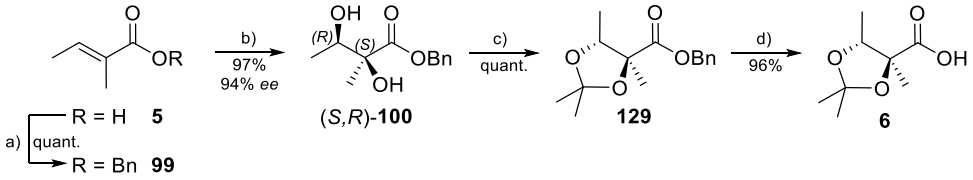
3.1 Synthese und Bioaktivität von Ophiofuranon A und B

Im ersten Teil dieser Arbeit wurde eine Totalsynthese der 2019 isolierten Ophiofuranone A (**1**) und B (**2**) (vgl. Kapitel 1.2.5) etabliert. Es folgte daraufhin die erste Untersuchung auf ihre biologischen Aktivitäten in Kooperation mit dem Helmholtz-Zentrum für Infektionsforschung in Braunschweig.
Die Ophiofuranone A (**1**) und B (**2**) konnten retrosynthetisch auf zwei Bausteine, den γ-Hydroxy-β-ketoester **4** und die von Tiglinsäure (**5**) abgeleitete Carbonsäure **6**, zurückgeführt werden (Schema 18). Baustein **4**, dessen eine Doppelbindung entweder (*E*)- oder (*Z*)-konfiguriert ist, wurde ausgehend von β-Methallylalkohol (**3**) aufgebaut.



Schema 18: Retrosynthetische Analyse von Ophiofuranon A (**1**) und B (**2**).

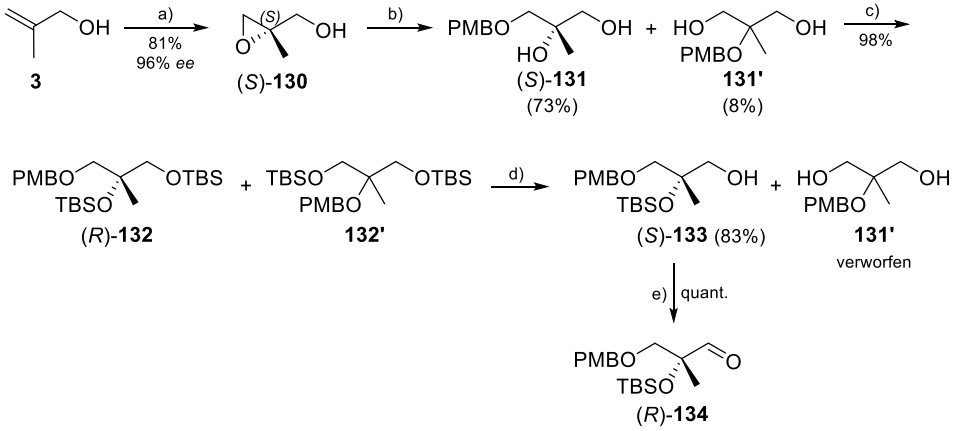
Zur Darstellung der Acetonid-geschützten Säure **6** wurde zu Beginn Tiglinsäure (**5**) nach Hamada *et al*. quantitativ in den Benzylester **99** überführt und dieser mittels asymmetrischer Sharpless-Dihydroxylierung[54] zu Diol (*S*,*R*)-**100** mit 97% Ausbeute und 95% *ee* umgesetzt (Schema 19). Nach quantitativer Acetonid-Schützung[55] des Diols (*S*,*R*)-**100** und Hydrierung mit H_2 und Pd/C wurde die freie Säure **6** mit 96% Ausbeute erhalten.



Schema 19: Synthese der Säure **6** ausgehend von Tiglinsäure (**5**) in 4 Stufen.
Reagenzien und Bedingungen: a) BnBr, Cs_2CO_3, DMF, RT, 16 h; b) AD-mix β, $MeSO_2NH_2$, *t*BuOH, H_2O, 0 °C, 5 d; c) $Me_2C(OMe)_2$, (+)-CSA, Aceton, RT, 3.5 h; d) H_2, Pd/C, EtOAc, RT, 2 h.

Für die Synthese der γ-Hydroxy-β-ketoester **4** wurde zuerst Aldehyd (*R*)-**134** ausgehend von β-Methallylalkohol (**3**) dargestellt (Schema 20). Zur Einführung des dritten, (*S*)-konfigurierten Stereozentrums von Ophiofuranon A (**1**) und B (**2**) wurde Allylalkohol **3** in 81% mit 96% *ee* nach Sharpless[56,57] epoxidiert (→(*S*)-**130**). Öffnung des Epoxids (*S*)-**130** mit *para*-Methoxybenzylalkohol und frisch hergestelltem $Ti(OPMB)_4$ in Anlehnung an Literatur[58,59] lieferte eine untrennbare

Mischung der Regioisomere (*S*)-**131** und **131'** im Verhältnis 8.6:1 mit insgesamt 81% Ausbeute. TBS-Schützung beider Hydroxygruppen der Diole (*S*)-**131** und **131'** führte zu den Bis(Silylether)n (*R*)-**132** und **132'** ebenfalls im Verhältnis 8.6:1. Nach Desilylierung der primären Alkohole mit Essigsäure wurde eine leicht trennbare Mischung des Monools (*S*)-**133** und des Diols **131'** erhalten. Alkohol (*S*)-**133** wurde mit Dess-Martin-Periodinan[60] quantitativ zu Aldehyd (*R*)-**134** oxidiert.

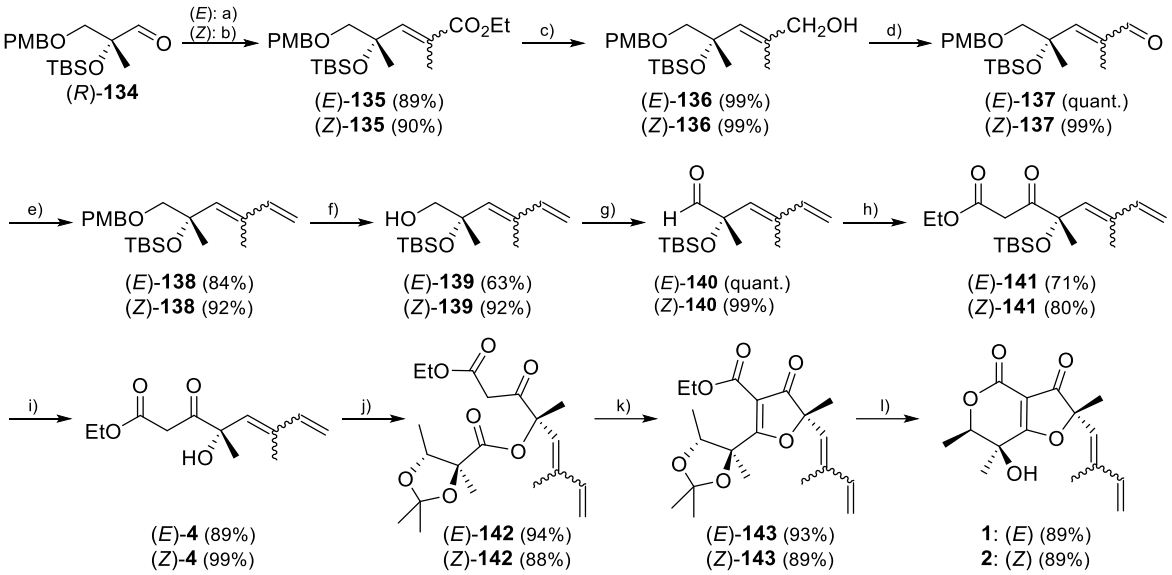


Schema 20: Synthese des chiralen Aldehyds (*R*)-**134** ausgehend von Allylalkohol **3** in 5 Stufen.
Reagenzien und Bedingungen: a) L-(+)-DIPT, Ti(O*i*Pr)$_4$, Cumolhydroperoxid, CH_2Cl_2, –35 °C auf –20 °C, 44 h; b) PMB-OH, Ti(OPMB)$_4$, Benzol, RT, 22 h; c) TBSOTf, 2,6-Lutidin, CH_2Cl_2, RT, 15 h; d) AcOH, H_2O, THF, RT, 43 h; e) DMP, CH_2Cl_2, RT, 3.5 h.

Die beiden Doppelbindungen der Seitenketten wurden in Anlehnung der Synthesesequenz von Sabitha *et al.*[61] eingeführt. Dazu wurde Aldehyd (*R*)-**134** entweder mit dem stabilisierten Ylid [1-(Ethoxycarbonyl)-ethylen]triphenylphosphoran[62,63] in einer Wittig-Reaktion zu (*E*)-Alken (*E*)-**135** oder mit Phosphonat $(PhO)_2P(O)CH(Me)CO_2Et$[64,65] in einer Ando-modifizierten HWE-Reaktion[65] zu (*Z*)-Alken (*Z*)-**135** umgesetzt (Schema 21). Beide Alkene **135** wurden mit ca. 90% Ausbeute erhalten. Nahezu quantitative Reduktion der Estergruppen mit DIBAL-H lieferte die Allylalkohole (*E*)-**136** und (*Z*)-**136**, welche mit DMP quantitativ in die Aldehyde (*E*)-**137** und (*Z*)-**137** überführt wurden. Durch Wittig-Reaktion mit Methylentriphenylphosphoran konnten die konjugierten Diene (*E*)-**138** und (*Z*)-**138** mit guten Ausbeuten erhalten werden. Die oxidative Entschützung der PMB-Gruppe von (*E*)-Alken (*E*)-**138** mit DDQ[66] lieferte Alkohol (*E*)-**139** lediglich mit 63%. Solche geringen Ausbeuten bei der PMB-Entschützung mit DDQ in Gegenwart von konjugierten Dienen wurden in der Literatur bereits häufiger beschrieben.[67–70] Andere Entschützungsmethoden (Hydrogenolyse, Reduktion mit Lewis-Säure oder CAN, TFA)[68,71] waren aufgrund der Funktionalitäten von (*E*)-**138** ungeeignet oder noch weniger erfolgreich. Dahingegen konnte das Alken (*Z*)-**138** mit einer sehr guten Ausbeute von 92% mit DDQ zu Alkohol (*Z*)-**139** entschützt werden. Die Alkohole (*E*)-**139** und (*Z*)-**139** wurden anschließend mit DMP quantitativ oxidiert und die erhaltenen Aldehyde (*E*)-**140** und (*Z*)-**140** mittels Roskamp-Reaktion[72] mit Ethyldiazoacetat und $SnCl_2$ in β-Ketoester (*E*)-**141** und (*Z*)-**141** mit 71% bzw. 80% Ausbeute überführt. Im Gegensatz zur Originalliteratur musste $SnCl_2$ äquimolar statt nur

katalytisch eingesetzt und die Reaktion wesentlich länger gerührt werden, um einen vollständigen Umsatz der Aldehyde (*E*)-**140** und (*Z*)-**140** zu erreichen. Durch TBS-Entschützung mit TBAF konnten die Bausteine (*E*)-**4** und (*Z*)-**4** mit 89% bzw 99% synthetisiert werden.

Die Synthese der Ophiofuranone A (**1**) und B (**2**) wurde durch jeweils drei aufeinanderfolgende Kondensationsschritte beendet. Yamaguchi-Veresterung[73,74] zwischen der Carbonsäure **6** und den γ-Hydroxy-β-ketoestern (*E*)-**4** und (*Z*)-**4** führte zu den entsprechenden Estern (*E*)-**142** und (*Z*)-**142** mit ca. 90% Ausbeute, welche mittels baseninduzierter Knoevenagel-ähnlicher Kondensation[39,75] zu den Dihydro-3(2*H*)-furanonen (*E*)-**143** und (*Z*)-**143** in ähnlich guten Ausbeuten cyclisiert wurden. Die Umsetzung von (*E*)-**143** und (*Z*)-**143** mit *para*-Toluolsulfonsäure in Methanol[76] führte zur Abspaltung der jeweiligen Acetonid-Gruppen und zur Lactonisierung der entstandenen Diole, wodurch die Zielverbindungen Ophiofuranon A (**1**) und B (**2**) mit jeweils 89% dargestellt werden konnten. Ausgehend von β-Methallylalkohol (**3**) konnten sie somit in 16 Stufen und 12% (**1**) bzw. 22% (**2**) Gesamtausbeute synthetisiert werden.



Schema 21: Synthese der Ophiofuranone A (**1**) und B (**2**) ausgehend von Aldehyd (*R*)-**134**.

Reagenzien und Bedingungen: a) $Ph_3PC(Me)CO_2Et$, Toluol, RF, 18 h; b) $(PhO)_2P(O)CH(Me)CO_2Et$, NaH, THF, –78 °C auf RT, 20 h; c) DIBAL-H, CH_2Cl_2, –78 °C, 2.5 h; d) DMP, CH_2Cl_2, RT, 3 h; e) Ph_3PCH_3Br, *n*BuLi, THF, RT, 17-19 h; f) DDQ, Puffer (pH = 7), CH_2Cl_2, 0 °C, 1 h; g) DMP, CH_2Cl_2, RT, 3 h; h) N_2CHCO_2Et, $SnCl_2$, CH_2Cl_2, RT, 18 h; i) TBAF, THF, 0 °C, 2.5-3 h; j) **6**, $(C_6Cl_3H_2)COCl$, NEt_3, DMAP, Toluol, RT, 60-80 min; k) Piperidin, Benzol, RF, 18-23 h; l) *p*TsOH, MeOH, RT, 16-23 h.

Die NMR-Daten der synthetischen Ophiofuranone A (**1**) und B (**2**) entsprachen den für die natürlichen Isolate publizierten Daten, die spezifischen Drehwerte, bis auf die Vorzeichen, jedoch nicht.[24] Da die gemessenen ECD-Spektren der synthetischen Produkte **1** und **2** jedoch sehr gut mit denen der Isolate übereinstimmten, nehme ich an, dass die von Lou *et al*.[24] vorgeschlagenen absoluten Konfigurationen für die Naturstoffe korrekt sind, jedoch die Drehwerte aufgrund der geringen Menge der isolierten Verbindungen und geringer Reinheit derer falsch bestimmt wurden.

Die synthetischen Ophiofuranone A (**1**) und B (**2**) sowie die Vorstufen (*E*)-**143** und (*Z*)-**143** mit Dihydro-3(2*H*)-furanon-Motiv wurden auf ihre antimikrobiellen, zytotoxischen und Antibiofilm-Aktivitäten gegen verschiedene Bakterien, Pilze und Zelllinien in Kooperation mit dem Helmholtz-Zentrum für Infektionsforschung in Braunschweig getestet. Die Ophiofuranone A (**1**) und B (**2**) wiesen mit MICs von 66.6 µg/mL nur eine schwache Aktivität gegen den Pilz *Mucor hiemalis* auf. Keine der getesteten Verbindungen **1**, **2**, (*E*)-**143** und (*Z*)-**143** zeigten eine zytotoxische Aktivität gegen menschliche Tumorzelllinien und nicht bösartige Mausfibroblasten. Nur Verbindung (*Z*)-**143** hemmte zu 52% die Bildung eines Biofilms durch *Staphylococcus aureus* bei einer Konzentration von 250 µg/mL. Eine Dispersion von *Candida albicans*-Biofilmen wurde für **1**, (*E*)-**143** und (*Z*)-**143** zu 16%-51% beobachtet.

3.2 Entwicklung einer modularen Synthese für Dihydro-3(2*H*)-furanone

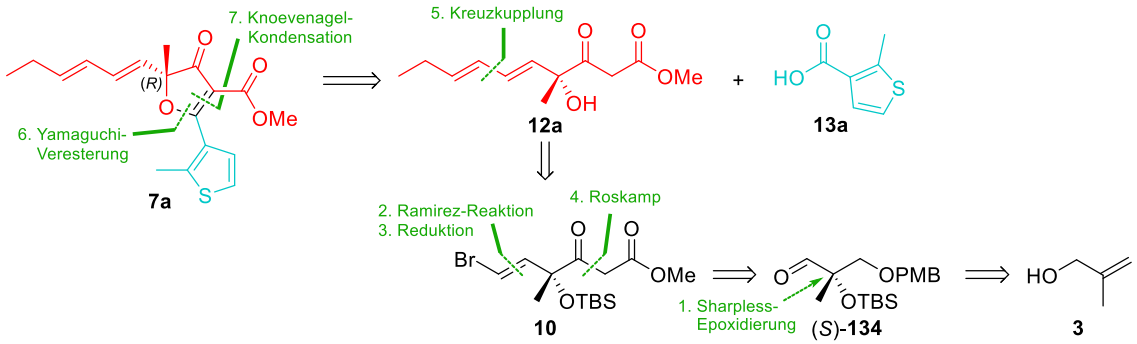
Im zweiten Projekt dieser Arbeit wurde eine flexible Syntheseroute für Dihydro-3(2*H*)-furanone aufbauend auf den Erkenntnissen der Synthese der Ophiofuranone entwickelt. Bei dieser Synthese war es möglich sowohl die Seitenkette als auch den Rest am Furanonring auf einer späten Stufe einzuführen und somit variieren zu können, was sie von den fünf bisherig veröffentlichten Synthesen zur Strukturklasse der Dihydro-3(2*H*)-furanone[38,39,49–51] abhebt.

Die erarbeitete Synthese wurde zur Darstellung einiger 2020 von Ruan *et al.*[23] isolierter Dihydro-3(2*H*)-furanone (siehe Kapitel 1.2.6) angewendet, da deren Isolate interessante biologische Aktivitäten aufwiesen. Die synthetischen Naturstoffe wurden in Kooperation mit dem Helmholtz-Zentrum für Infektionsforschung in Braunschweig auf ihre biologischen Aktivitäten untersucht.

3.2.1 Synthese und Bioaktivität von Thiocarboxyl A und dessen Derivaten

Ein großes Interesse lag in der Synthese von Thiocarboxyl A (**7a**) aufgrund des neuartigen Thiophen-Motivs und der guten antimikrobiellen Aktivitäten gegen *Escherichia coli*, *Staphylococcus aureus* und *Candida albicans* mit minimalen Hemmkonzentrationen von 1.7 bis 3.0 µg/mL.[23]

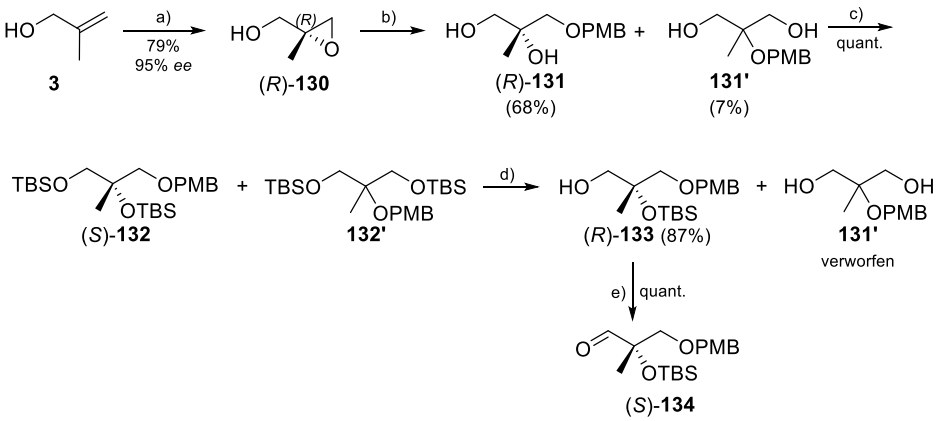
Retrosynthetisch konnte Thiocarboxyl A (**7a**) von dem γ-Hydroxy-β-ketoester **12a** und der käuflichen 3-Methyl-2-thiophencarbonsäure (**13a**) abgeleitet werden (Schema 22). Der Butenylrest der Seitenkette von **12a** wurde über eine Kreuzkupplung von Vinylbromid **10** eingeführt. Je nach Wahl der Säure **13** oder des Reagenzes für die Kreuzkupplung war es möglich den Rest der Seitenkette oder den Rest am Furanon zu variieren.



Schema 22: Retrosynthetische Analyse von Thiocarboxyl A (**7a**).

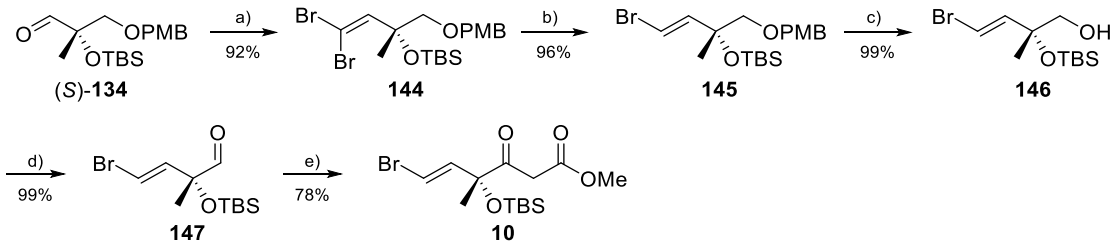
Baustein **10** wurde auf Aldehyd (*S*)-**134** zurückgeführt, der bereits für die Synthese der Ophiofuranone, jedoch mit (*R*)-konfiguriertem Stereozentrum, verwendet wurde (vgl. Kapitel 3.1). Aldehyd (*S*)-**134** wurde deshalb analog zu seinem Enantiomer (*R*)-**134** ausgehend von β-Methallylalkohol (**3**) aufgebaut (Schema 23). Für die Sharpless-Epoxidierung wurde diesmal entsprechend D-(−)-Diisopropyltartrat eingesetzt, um (*R*)-**130** mit 95% *ee* zu synthetisieren. Das Regioisomerenverhältnis von (*R*)-**131** und

131' nach der Epoxidöffnung von (*R*)-**130** betrug hier 10:1. Nach folgender Schützung, Desilylierung und Oxidation wurde Aldehyd (*S*)-**134** in fünf Stufen ausgehend von **3** mit 41% Ausbeute erhalten.



Schema 23: Synthese des chiralen Aldehyds (*S*)-**134** ausgehend von Allylalkohol **3** in 5 Stufen.
Reagenzien und Bedingungen: a) D-(–)-DIPT, Ti(O*i*Pr)$_4$, Cumolhydroperoxid, CH_2Cl_2, –35 °C auf –20 °C, 46 h; b) PMB-OH, Ti(OPMB)$_4$, Benzol, RT, 18 h; c) TBSOTf, 2,6-Lutidin, CH_2Cl_2, RT, 19 h; d) AcOH, H_2O, THF, RT, 48 h; e) DMP, CH_2Cl_2, RT, 3 h.

Um Schlüsselbaustein **10** zu erhalten, wurde Aldehyd (*S*)-**134** nach dem Ramirez-Protokoll[77] in *gem*-Dibromoolefin **144** überführt, welches mit Diethylphosphit und Triethylamin zu dem (*E*)-konfigurierten Vinylbromid **145** reduziert wurde (Schema 24).[78] Die Einführung des β-Ketoesters wurde ebenfalls analog der Ophiofuranonsynthese (vgl. Kapitel 3.1) durchgeführt. Dazu wurde zuerst der primäre Alkohol von **145** mit DDQ[66] zum Alkohol **146** entschützt. Aufgrund einer fehlenden zweiten konjugierten Doppelbindung in **145** verlief die Entschützung hier annähernd quantitativ. Nach Oxidation von Alkohol **146** mit DMP[60] zu Aldehyd **147** und dessen Roskamp-Reaktion[72] mit frisch hergestelltem Methyldiazoacetat und $SnCl_2$ wurde β-Ketoester **10** erhalten. Die Ausbeute über die 5 Stufen betrug 68%.



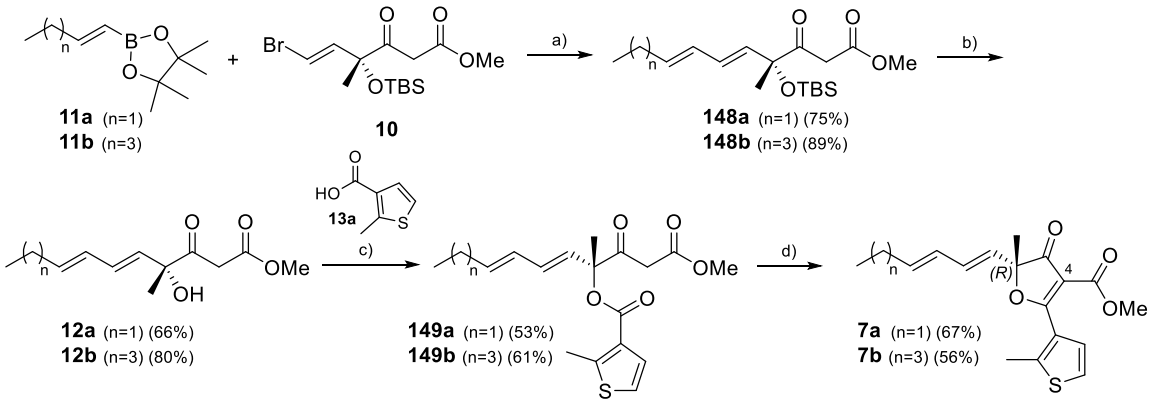
Schema 24: Synthese des für die Kreuzkupplung benötigten Vinylbromids **10** ausgehend von Aldehyd (*S*)-**134**.
Reagenzien und Bedingungen: a) CBr_4, PPh_3, CH_2Cl_2, RT, 4 h; b) Diethylphosphit, NEt_3, DMF, RT, 25 h; c) DDQ, Puffer (pH = 7), CH_2Cl_2, RT, 1 h; d) DMP, CH_2Cl_2, RT, 3.5 h; e) N_2CHCO_2Me, $SnCl_2$, CH_2Cl_2, RT, 19 h.

Zur Untersuchung der optimalen Bedingungen für die folgende Kreuzkupplung, wurde zuerst 1-Hexin als Vorläufer für das Kupplungsreagenz **11** verwendet, da es besser verfügbar und einfacher

handzuhaben war als das gasförmige 1-Butin. Aufgrund dessen wurde vor Thiocarboxyl A (**7a**) das nicht natürliche Derivat **7b** hergestellt (Schema 25).

Da es bisher kaum Literatur zu Kreuzkupplungen in Gegenwart von β-Ketoestern gibt, waren einige Versuche dazu nötig. Die getesteten Negishi-Kupplungen[79–81] von **10** führten dabei nicht zum gewünschten Kupplungsprodukt **148b**. Im Anschluss wurden unterschiedliche Bedingungen für eine Suzuki-Kupplung[49,51,82–84] mit Boronsäureester **11b**[85] (n=3) getestet, wobei die Verwendung von 8 mol% Tetrakis(triphenylphosphan)palladium(0) in Gegenwart von Kaliumcarbonat in einem Wasser/1,4-Dioxan-Gemisch und unter Sauerstoffauschluss nach Guiry *et al.*[86] mit einer guten Ausbeute von 89% (*E,E*)-Dien **148b** lieferte. Die Kreuzkupplung von **10** mit Boronsäureester **11a**[38] (n=1) führte mit 75% zu Dien **148a**. Beide Kupplungsprodukte **148a** und **148b** wurden ohne das jeweilige (*Z*)-Isomer erhalten. Durch Abspaltung der TBS-Gruppen von **148a** und **148b** mit TBAF und Essigsäure konnten die γ-Hydroxy-β-ketoester **12a** und **12b** dargestellt werden. Baustein **12a** wurde bereits 2012 von Burghart-Stoll und Brückner bei der Synthese von Gregatin A-D und Aspertetronin A hergestellt (vgl. Kapitel 1.2.4).[39] Ihre Synthese von **12a** ist zwar kürzer, jedoch ist sie nicht flexibel in R^1 und lieferte **12a** mit einem *ee* von nur 90% und als untrennbares Gemisch von (*E,Z*)-Isomeren, welche dadurch ebenfalls in den hergestellten Naturstoffen enthalten waren.

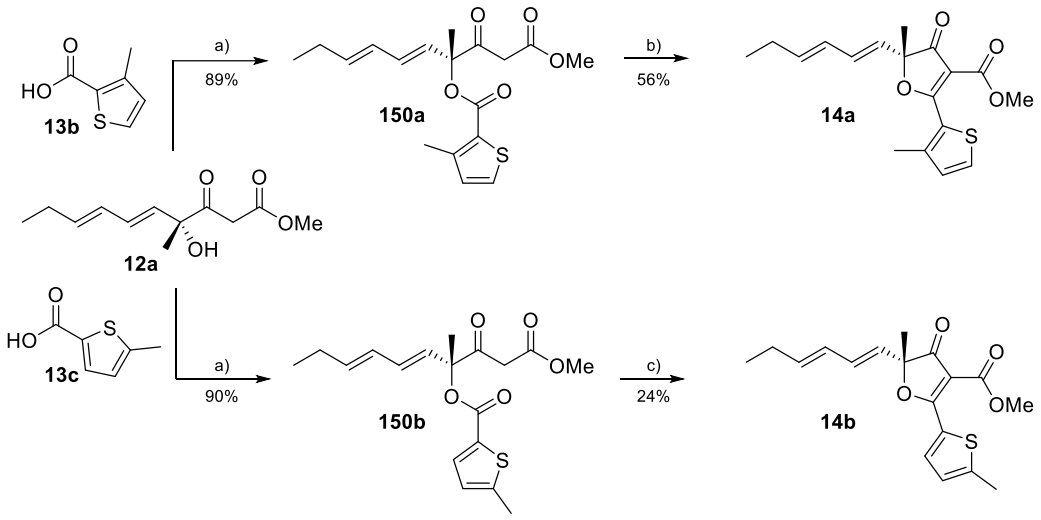
Eine Yamaguchi-Veresterung[73,74] von **12a** und **12b** mit 3-Methyl-2-thiophencarbonsäure (**13a**) führte zu den entsprechenden Estern **149a** und **149b**, welche mit einer baseninduzierten Knoevenagel-ähnlichen Kondensation in Thiocarboxyl A (**7a**) und in dessen um 2 C-Atome längeres Derivat **7b** überführt werden konnten.



Schema 25: Synthese von Thiocarboxyl A (**7a**) und dessen um 2 C-Atome längerem Derivat **7b**.
Reagenzien und Bedingungen: a) $Pd(PPh_3)_4$, K_2CO_3, H_2O, 1,4-Dioxan, 50 °C, 17-23 h; b) TBAF, AcOH, THF, 0 °C, 6-7 h; c) $(C_6Cl_3H_2)COCl$, NEt_3, DMAP, Toluol, RT, 1-1.5 h; d) Piperidin, Benzol, RF, 16-24 h.

Das ^{1}H-NMR-Spektrum des synthetisch dargestellten Thiocarboxyls A (**7a**) stimmte sehr gut mit dem des Isolats[23] überein. Das ^{13}C-NMR-Spektrum entsprach ebenfalls dem der Literatur, mit Ausnahme der chemischen Verschiebungen der vollständig substituierten C-Atome der Thiophen-Einheit und des C-Atoms der Position 4. Da die spezifischen Drehwerte außer im Vorzeichen ebenfalls nicht übereinstimmten, wurde vermutet, dass Ruan *et al.*[23] die Substituenten des Thiophen-Rests falsch

zugeordnet haben könnten. Es wurden daher zwei weitere Derivate **14a** und **14b** hergestellt, um sie mit den analytischen Daten des Isolats zu vergleichen. Aufgrund der Multiplizität der Doppelbindung des Thiophen-Rests (d, J = 5.4 Hz) des Isolats war nur eine Synthese dieser beiden Konstitutionsisomere **14a** und **14b** sinnvoll. Sie konnten aufgrund der flexiblen Route in jeweils zwei Stufen ausgehend von **12a** hergestellt werden (Schema 26). Baustein **12a** wurde dazu mit den Thiophensäuren **13b** und **13c** verestert und die resultierenden Ester **150a** und **150b** mit Piperidin in Benzol zu den Thiocarboxyl A-Analoga **14a** und **14b** cyclisiert.



Schema 26: Synthese der Thiocarboxyl A-Analoga **14a** und **14b** ausgehend von γ-Hydroxy-β-ketoester **12a** in jeweils 2 Stufen.

Reagenzien und Bedingungen: a) $(C_6Cl_3H_2)COCl$, NEt_3, DMAP, Toluol, RT, 1 h; b) Piperidin, Benzol, 50 °C, 23 h.

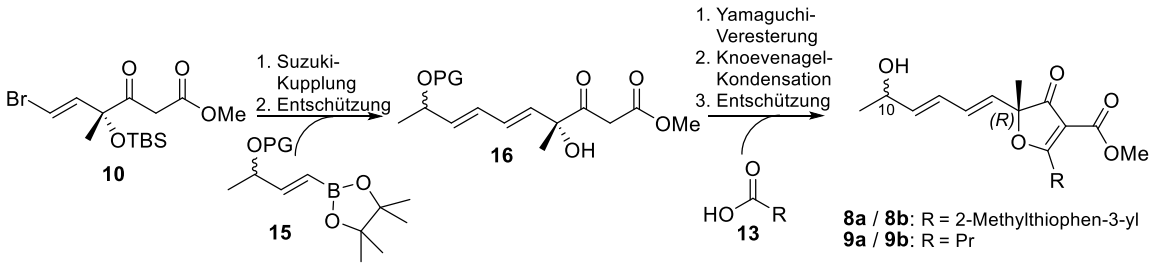
Nach Vergleich der erhaltenen ^{1}H-NMR-Spektren von **14a** und **14b** mit dem des isolierten Naturstoffs war klar, dass es sich bei keiner der beiden Verbindungen um die korrekte Struktur des Naturstoffs handelt. Eine genauere Betrachtung des ^{13}C-NMR-Spektrums des isolierten Naturstoffs zeigte, dass die Signale der fraglichen C-Atome im von Ruan *et al.*[23] abgebildeten ^{13}C-NMR-Spektrum nicht sichtbar waren. Es wurde daher angenommen, dass die angegebenen Verschiebungen durch automatische Signalspitzenauswahl ohne weitere Überprüfung der 2D-Korrelationen zugewiesen wurden. Aufgrund dessen ist davon auszugehen, dass es sich bei dem synthetischen Thiocarboxyl A (**7a**) um die korrekte Struktur des Naturstoffs handelt, da alle Verschiebungen des ^{1}H- und die anderen Verschiebungen des ^{13}C-NMR-Spektrums mit denen des Isolats übereinstimmen.

Da von Ruan *et al.*[23] ebenfalls eine gute antimikrobielle Aktivität des Isolats **7a** gegen *E. coli* and *S. aureus* berichtet wurde, wurden sowohl Thiocarboxyl A (**7a**) als auch die drei hergestellten Derivate **7b**, **14a** und **14b** gegen diese Bakterienstämme getestet. Überraschenderweise waren alle vier Testverbindungen inaktiv. Nur gegen die empfindlichere *E. coli* ΔTolC-Mutante ohne ArcAB-TolC-Effluxsystem war **7a** mit einem IC_{50} von 44.3 µM minimal wachstumshemmend. Ebenfalls zeigte keine der vier Verbindungen eine zytotoxische Aktivität gegen die Zelllinien A549, L929 und Huh7.

3.2.2 Synthese und antibiotische Aktivitäten der Seitenketten-Epimere Gregatin G und Thiocarboxyl C

Die in Kapitel 3.2.1 entwickelte flexible Synthese zur Darstellung von Thiocarboxyl A (**7a**) und dessen Derivaten **7b**, **14a** und **14b** wurde nun zur Synthese weiterer natürlicher Dihydro-3(2*H*)-furanone genutzt. Es wurden die 2020 von Ruan *et al*.[23] isolierten, strukturell anspruchsvolleren Epimerenpaare Thiocarboxyl $C_{1,2}$ (**8a,b**) und Gregatin $G_{1,2}$ (**9a,b**) synthetisiert. Es musste jedoch wegen der zusätzlichen Hydroxygruppe der Seitenkette zuerst eine geeignete Schutzgruppenstrategie entwickelt werden.

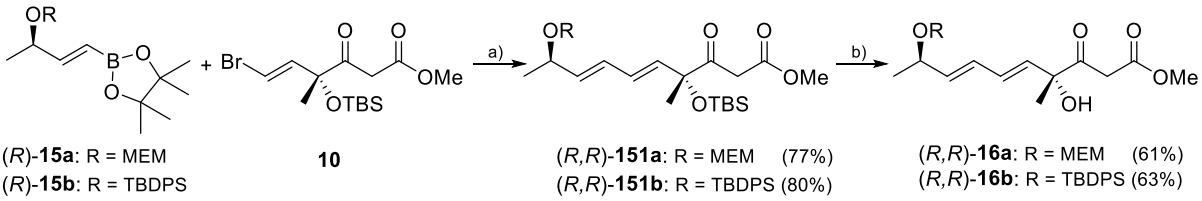
Die Synthese ging von dem bereits in 10 Stufen hergestellten Vinylbromid mit β-Ketoesterfunktion **10** aus (vgl. Kapitel 3.2.1), das in einer Suzuki-Kupplung mit einem Boronsäureester **15** mit geschützter Hydroxygruppe umgesetzt wurde (Schema 27). Das nach TBS-Entschützung erhaltene Kupplungsprodukt **16** wurde durch Yamaguchi-Veresterung mit Carbonsäure **13** und Knoevenagel-ähnlicher Cyclisierung in die entsprechenden Dihydro-3(2*H*)-furanone überführt, welche zu den Zielverbindungen **8a**, **8b**, **9a** und **9b** entschützt wurden.



Schema 27: Erweiterung der flexiblen Synthese für Dihydro-3(2*H*)-furanone zur Darstellung der Epimerenpaare Thiocarboxyl C (**8**) und Gregatin G (**9**) mit orthogonaler Schutzgruppenstrategie.

Aufgrund der TBS-Gruppe in Vinylbromid **10** und der enthaltenen Funktionalitäten der Naturstoffe war die Auswahl einer geeigneten Schutzgruppe für den sekundären Alkohol begrenzt. Keto *et al*.[49] beschrieben 2013 bei ihren Synthesearbeiten für Gregatin B und E, dass die Fluorid-vermittelte finale Desilylierung vom racemischen, TBS-geschützten Gregatin E aufgrund der Instabilität des Naturstoffs nur mit 25% gelang. Nichtsdestotrotz entschied ich mich für die sterisch noch anspruchsvollere Silylschutzgruppe TBDPS und zusätzlich für MEM als orthogonale Schutzgruppen zu TBS, um die finale Entschützung sowohl im Sauren als auch im Basischen bzw. Fluorid-vermittelt durchführen zu können.

Zuerst wurden Thiocarboxyl C_1 (**8a**) und Gregatin G_1 (**9a**) synthetisiert, um eine geeignete Schutzgruppenstrategie zu erarbeiten, welche anschließend zur Darstellung ihrer Epimere **8b** und **9b** angewendet werden sollte. Suzuki-Kupplung von Vinylbromid **10** mit den unterschiedlich geschützten Boronsäureestern (*R*)-**15a** und (*R*)-**15b** unter den bereits erarbeiteten Bedingungen (Kapitel 3.2.1) führte zu den Dienen (*R,R*)-**151a** und (*R,R*)-**151b**. Durch Entschützung ihrer jeweiligen TBS-Gruppen wurden die γ-Hydroxy-β-ketoester (*R,R*)-**16a** und (*R,R*)-**16b** erhalten (Schema 28).

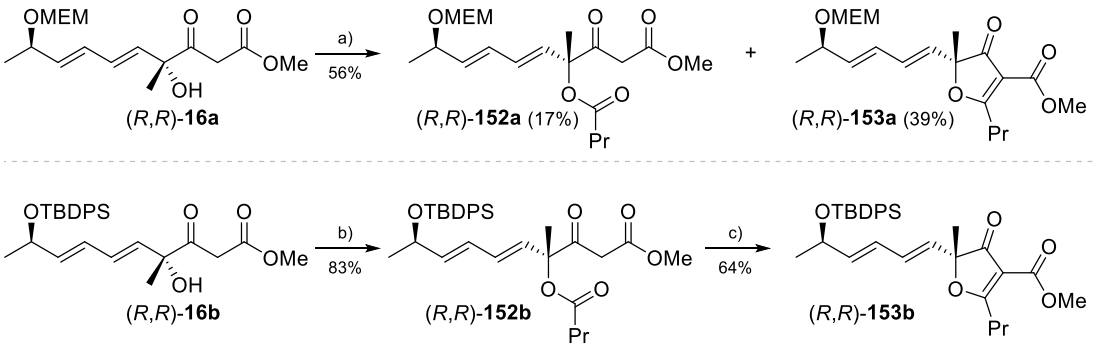


Schema 28: Synthese der unterschiedlich geschützten γ-Hydroxy-β-ketoester (*R*,*R*)-**16a** und (*R*,*R*)-**16b**.

Reagenzien und Bedingungen: a) $Pd(PPh_3)_4$, K_2CO_3, H_2O, 1,4-Dioxan, 50 °C, 18 h; b) TBAF, AcOH, THF, 0 °C, 4-4.5 h.

Im Gegensatz zur bisherigen Synthesestrategie wurde Alkohol (*R*,*R*)-**16a** in Anlehnung an Burghart-Stoll und Brückner[39] in einer Stufe mit Buttersäurechlorid und Triethylamin in Dihydro-3(2*H*)-furanon (*R*,*R*)-**153a** überführt (Schema 29, oben). Problem hierbei war jedoch, dass der *in situ* gebildete Diester (*R*,*R*)-**152a** nie vollständig zu Furanon (*R*,*R*)-**153a** cyclisierte und beide Verbindungen durch mehrfache Säulenchromatographie nicht vollständig getrennt werden konnten. Somit wurden nur 39% sauberes Furanon (*R*,*R*)-**153a** und 17% vom Diester (*R*,*R*)-**152a** erhalten.

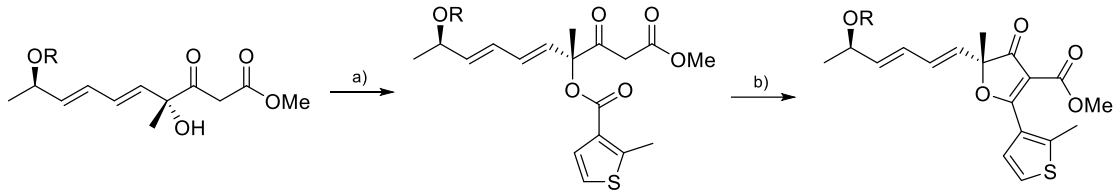
Dihydro-3(2*H*)-furanon (*R*,*R*)-**153b** wurde darum wieder in zwei Stufen mittels Yamaguchi-Veresterung mit Buttersäure (**13d**) und baseninduzierter Knoevenagel-ähnlicher Kondensation hergestellt, wobei die Ausbeute über beide Stufen 53% betrug (Schema 29, unten).



Schema 29: Synthesen der 3-Furanone (*R*,*R*)-**153a** und (*R*,*R*)-**153b** mit MEM- (oben) oder TBDPS-geschütztem (unten) Alkohol.

Reagenzien und Bedingungen: a) Buttersäurechlorid, NEt_3, CH_2Cl_2, 50 °C, 17 h; b) Buttersäure (**13d**), $(C_6Cl_3H_2)COCl$, NEt_3, DMAP, Toluol, RT, 1 h; c) $NaHCO_3$, MeOH, RT, 23 h.

Die unterschiedlich geschützten Vorstufen für Thiocarboxyl C_1 (*R*,*R*)-**155a** und (*R*,*R*)-**155b** wurden ebenfalls in zwei Stufen ausgehend von den γ-Hydroxy-β-ketoestern (*R*,*R*)-**16a** und (*R*,*R*)-**16b** synthetisiert (Schema 30). Die Alkohole (*R*,*R*)-**16a** und (*R*,*R*)-**16b** wurden dazu jeweils mit 3-Methyl-2-thiophencarbonsäure (**13a**) verestert (→(*R*,*R*)-**154a** und (*R*,*R*)-**154b**) und der Furanonring mittels Knoevenagel-ähnlicher Kondensation geschlossen.



(*R*,*R*)-**16a**: R = MEM
(*R*,*R*)-**16b**: R = TBDPS

(*R*,*R*)-**154a**: R = MEM (70%)
(*R*,*R*)-**154b**: R = TBDPS (77%)

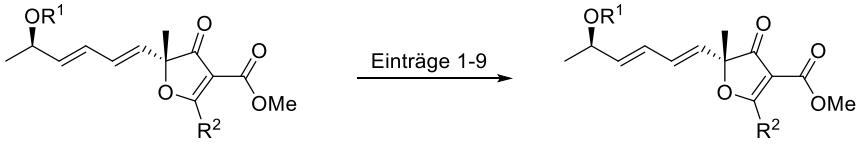
(*R*,*R*)-**155a**: R = MEM (56%)
(*R*,*R*)-**155b**: R = TBDPS (66%)

Schema 30: Synthesen der 3-Furanone (*R*,*R*)-**155a** und (*R*,*R*)-**155b** mit MEM oder TBDPS-geschütztem Alkohol und Thiophenrest am Furanon.

Reagenzien und Bedingungen: a) **13a**, $(C_6Cl_3H_2)COCl$, NEt_3, DMAP, Toluol, RT, 1 h; b) Piperidin, Benzol, RF, 17-20 h.

Die Ergebnisse aller Entschützungsversuche der MEM- bzw. TBDPS-Gruppe der Verbindungen (*R*,*R*)-**153** und (*R*,*R*)-**155** sind in Tabelle 2 gezeigt. Gregatin G_1 (**9a**) wurde durch MEM-Entschützung von (*R*,*R*)-**153a** unter (Lewis)[87]-sauren[88,89] Bedingungen aufgrund der Instabilität des Furanonrings unter den gewählten Bedingungen nur mit Ausbeuten von 11-32% erhalten (Einträge 1-3). So öffnete sich das Furanon von (*R*,*R*)-**153a** zum Teil durch die Verwendung von $ZnBr_2$ wieder zum Diester (*R*,*R*)-**152a** (Eintrag 1). Die Entschützung von (*R*,*R*)-**155a** unter den gleichen Bedingungen führte nicht zu Thiocarboxyl C_1 (**8a**), da der elektronenreiche Heteroaromat das Furanon vermutlich noch mehr destabilisierte (Eintrag 4). Die Verwendung von TFA in Anlehnung an Literatur[90] führte zur vollständigen Zersetzung von (*R*,*R*)-**155a** (Eintrag 5), wobei die Behandlung mit konzentrierter HCl[89] mit 22% Ausbeute zu Produkt **8a** führte (Eintrag 6).

Tabelle 2: Bedingungen und Ausbeuten der versuchten Entschützungen der MEM- und TBDPS-Gruppen der Verbindungen (*R*,*R*)-**153** und (*R*,*R*)-**155** zur Darstellung von Gregatin G_1 (**9a**) und Thiocarboxyl C_1 (**8a**).



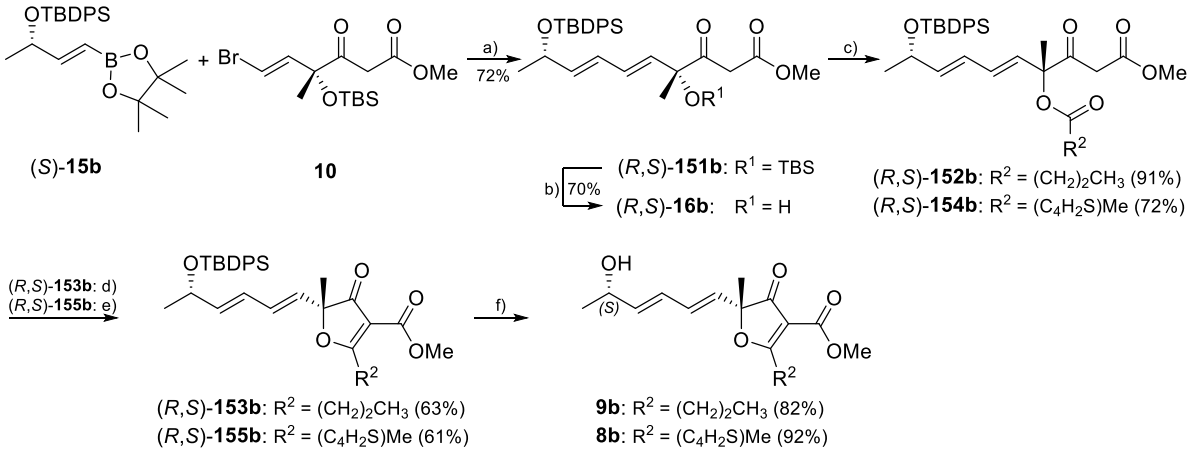
(*R*,*R*)-**153a**: R^1 = MEM; R^2 = $(CH_2)_2CH_3$
(*R*,*R*)-**153b**: R^1 = TBDPS; R^2 = $(CH_2)_2CH_3$
(*R*,*R*)-**155a**: R^1 = MEM; R^2 = $(C_4H_2S)Me$
(*R*,*R*)-**155b**: R^1 = TBDPS; R^2 = $(C_4H_2S)Me$

9a: R^1 = H; R^2 = $(CH_2)_2CH_3$
8a: R^1 = H; R^2 = $(C_4H_2S)Me$

Eintrag	Edukt	Bedingungen	Produkt	Ausbeute [%]
1[87]	(*R*,*R*)-**153a**	$ZnBr_2$, CH_2Cl_2, RT, 22 h	**9a**	23
2[88]	(*R*,*R*)-**153a**	PPTS, *t*BuOH, RF, 24 h	**9a**	11
3[89]	(*R*,*R*)-**153a**	konz. HCl, MeOH, RT, 24 h	**9a**	32
4[87]	(*R*,*R*)-**155a**	$ZnBr_2$, CH_2Cl_2, RT, 22 h	**8a**	–
5[90]	(*R*,*R*)-**155a**	TFA, CH_2Cl_2, RT, 1 h	**8a**	–
6[89]	(*R*,*R*)-**155a**	konz. HCl, MeOH, RT, 24 h	**8a**	22
7[91]	(*R*,*R*)-**153b**	TBAF, THF, 45 °C, 7 h	**9a**	64
8[91]	(*R*,*R*)-**155b**	TBAF, THF, 45 °C, 7 h	**8a**	–
9[92]	(*R*,*R*)-**155b**	HF-py, THF, RT, 23 h	**8a**	96

Die Desilylierung von TBDPS-Ether (*R,R*)-**153b** mit TBAF bei 45 °C nach Literatur[91] lieferte Gregatin G_1 (**9a**) mit akzeptablen 64% (Eintrag 7). Entschützung von (*R,R*)-**155b** unter den gleichen Bedingungen führte aufgrund der Thiophen-Einheit jedoch zur vollständigen Zersetzung (Eintrag 8). Thiocarboxyl C_1 (**8a**) wurde letztlich mit einer sehr guten Ausbeute von 96% durch die Umsetzung von (*R,R*)-**155b** mit HF-Pyridin erhalten (Eintrag 9).
Bezogen auf Vinylbromid **10** konnte Thiocarboxyl C_1 (**8a**) mit einer Ausbeute von 25% mit TBDPS als Schutzgruppe und mit 4% unter Verwendung von MEM dargestellt werden. Gregatin G_2 (**9a**) wurde mit 6% (MEM) bzw. 17% (TBDPS) synthetisiert.

Da für beide dargestellten Naturstoffe **8a** und **9a** die Gesamtausbeute mit TBDPS als Schutzgruppe besser war als bei der Verwendung von MEM, wurden Thiocarboxyl C_2 (**8b**) und Gregatin G_2 (**9b**) ausgehend von Vinylbromid **10** und dem TBDPS-geschützten, (*S*)-konfigurierten Boronsäureester (*S*)-**15b** jeweils in 5 Stufen hergestellt (Schema 31). Die Gesamtausbeute von Thiocarboxyl C_2 (**8b**) betrug dabei 29% und die von Gregatin G_2 (**9b**) 23%.



Schema 31: Synthese von Gregatin G_2 (**9b**) und Thiocarboxyl C_2 (**8b**) in jeweils 5 Stufen ausgehend von Vinylbromid **10**. *Reagenzien und Bedingungen:* a) $Pd(PPh_3)_4$, K_2CO_3, H_2O, 1,4-Dioxan, 50 °C, 22 h; b) TBAF, AcOH, THF, 0 °C, 3.5 h; c) **13d** (→(*R,S*)-**152b**) oder **13a** (→(*R,S*)-**154b**), $(C_6Cl_3H_2)COCl$, NEt_3, DMAP, Toluol, RT, 1 h; d) $NaHCO_3$, MeOH, RT, 22 h; e) Piperidin. Benzol, RF, 17 h; f) HF-py, THF, RT, 23-25 h.

Die NMR-Daten aller synthetisierten Naturstoffe **8a,b** und **9a,b** stimmten sehr gut mit denen der isolierten Verbindungen überein, die spezifischen Drehwerte jedoch bis auf die Vorzeichen wieder nicht. Trotz allem ist davon auszugehen, dass die Strukturen für Thiocarboxyl $C_{1,2}$ (**8a,b**) und Gregatin $G_{1,2}$ (**9a,b**) von Ruan *et al*.[23] korrekt bestimmt wurden.

Wie Thiocarboxyl A (**7a**) wies keine der synthetischen Dihydro-3(2*H*)-furanone **8a,b** und **9a,b** antibiotische Wirkung gegen *E. Coli* oder *S. aureus* auf, trotz der guten publizierten antimikrobiellen Aktivitäten. Eine Zytotoxizität gegen die Krebszelllinien A549, L929 und Huh7.konnte ebenfalls bei keiner der vier Substanzen festgestellt werden.

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5 PUBLIKATIONEN

5.1 Darstellung des Eigenanteils

Die in dieser Dissertation vorgestellten Publikationen und dem Manuskript wurden in Kooperation mit anderen Wissenschaftlern der Universität Bayreuth, sowie mit Arbeitsgruppen anderer Institutionen erarbeitet. Hierzu zählen das *Department of Microbial Drugs* und das *Department of Compound Profiling and Screening* des Helmholtz-Zentrums für Infektionsforschung in Braunschweig. Der Eigenanteil an den jeweiligen Veröffentlichungen soll im Folgenden detailliert dargestellt werden.

5.1.1 Eigenanteil: Publikation I

Die Ergebnisse zu dieser Arbeit wurden im Journal *The Journal of Organic Chemistry* (*J. Org. Chem.* **2022**, *87*, 6520−6523; DOI: 10.1021/acs.joc.2c00521) unter folgendem Titel veröffentlicht:

„Synthesis and Bioactivity of Ophiofuranones A and B“

von den Autoren

Franziska Gillsch, Haoxuan Zeng, Sofia I. Bär, Hedda Schrey und Rainer Schobert.

Die Arbeit wurde in Kooperation mit dem *Department of Microbial Drugs* des Helmholtz-Zentrums für Infektionsforschung in Braunschweig durchgeführt.

Die Synthesestrategie wurde von mir erarbeitet. Probleme während der Synthese wurden in wissenschaftlichen Diskussionen zwischen Prof. Dr. Rainer Schobert und mir erörtert und gelöst. Die synthetischen Arbeiten, die anfallende Analytik der Präparate und deren Auswertung wurde von mir durchgeführt. Die HPLC- und ECD-Messungen wurden ebenfalls von mir durchgeführt. Die biologischen Untersuchungen der Verbindungen wurden von Haoxuan Zeng, Sofia I. Bär und Hedda Schrey durchgeführt.

Prof. Dr. Rainer Schobert und ich waren an der Abfassung, der Diskussion sowie der Korrektur und der Revision des Manuskripts beteiligt. Haoxuan Zeng und Dr. Hedda Schrey waren zusätzlich an der Korrektur des Manuskripts beteiligt.

Geschätzter Eigenanteil: 70%

5.1.2 Eigenanteil: Publikation II

Die Ergebnisse zu dieser Arbeit wurden im Journal *Journal of Natural Products* (*J. Nat. Prod*. **2022**, *85*, 2828−2835; DOI: 10.1021/acs.jnatprod.2c00870) unter folgendem Titel veröffentlicht:

„Synthesis and Bioactivity of Thiocarboxylic A and Derivatives“

von den Autoren

Franziska Gillsch, Fredrick Mbui, Ursula Bilitewski und Rainer Schobert.

Die Arbeit wurde in Kooperation mit dem *Department of Compound Profiling and Screening* des Helmholtz-Zentrums für Infektionsforschung in Braunschweig durchgeführt.

Die Synthesestrategie wurde von mir erarbeitet. Probleme während der Synthese wurden in wissenschaftlichen Diskussionen zwischen Prof. Dr. Rainer Schobert und mir erörtert und gelöst. Die synthetischen Arbeiten, die anfallende Analytik der Präparate und deren Auswertung wurde von mir durchgeführt. Die HPLC-Messungen wurden ebenfalls von mir durchgeführt. Die biologischen Untersuchungen der Verbindungen wurden von Dr. Fredrick Mbui durchgeführt.

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Geschätzter Eigenanteil: 80%

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Die Ergebnisse zu dieser Arbeit wurden im Journal *Chemistry & Biodiversity* (*Chem. Biodiversity* **2023**, *20*, e202300181; DOI: 10.1002/cbdv.202300181) unter folgendem Titel veröffentlicht:

„Syntheses and Antibacterial Evaluation of New Penicillium Metabolites Gregatins G and Thiocarboxylics C“

von den Autoren

Franziska Gillsch, Fredrick Mbui, Ursula Bilitewski und Rainer Schobert.

Die Arbeit wurde in Kooperation mit dem *Department of Compound Profiling and Screening* des Helmholtz-Zentrums für Infektionsforschung in Braunschweig durchgeführt.

Die Synthesestrategie wurde von mir erarbeitet und Probleme während der Synthese von mir gelöst. Die synthetischen Arbeiten, die anfallende Analytik der Präparate und deren Auswertung wurde von mir durchgeführt. Die HPLC-Messungen wurden ebenfalls von mir durchgeführt. Die biologischen Untersuchungen der Verbindungen wurden von Dr. Fredrick Mbui durchgeführt.

Dieses Manuskript wurde von mir verfasst. Prof. Dr. Rainer Schobert und ich waren an der Diskussion sowie der Korrektur des Manuskripts beteiligt. Prof. Dr. Ursula Bilitewski und Dr. Fredrick Mbui waren an der Korrektur beteiligt.

Geschätzter Eigenanteil: 85%

5.2 Publikation I

Synthesis and Bioactivity of Ophiofuranones A and B

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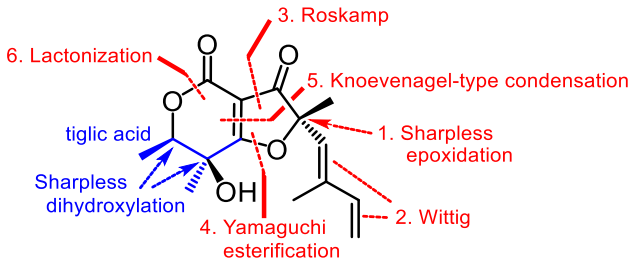

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Synthesis and Bioactivity of Ophiofuranones A and B

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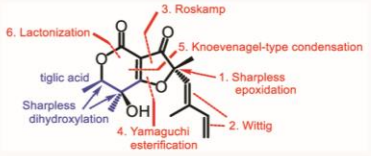
ABSTRACT: Ophiofuranones A and B, metabolites of the fungus *Ophiosphaerella korrae*, were synthesized in 16 steps and 12%/22% yield. The stereogenic centers were established by Sharpless dihydroxylations and epoxidation, the 1,3-dienes via Wittig or HWE olefinations. The rings were closed through Knoevenagel-type condensation and lactonization. The ophiofuranones proved nontoxic at relevant concentrations against tumor cells, fibroblasts, and various bacteria and fungi. Ophiofuranone A and the monocyclic precursors **4** were weakly active against microbial biofilms.



The ophiofuranones A (**1**) and B (**2**) were isolated in 2019 by Lou et al.[1] from the endolichenic fungus *Ophiosphaerella korrae*, and structurally assigned mainly by means of ECD and NMR spectra (Figure 1). They were shown to feature a

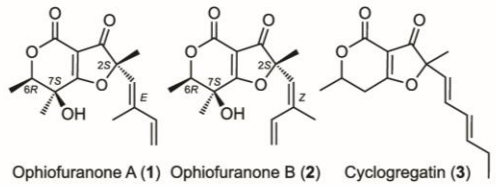
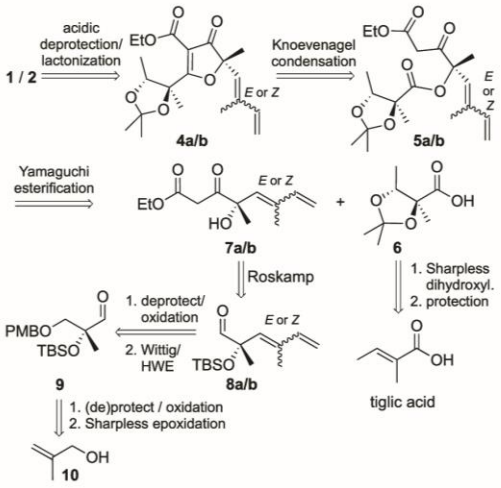


Figure 1. Structures of ophiofuranones A (**1**) and B (**2**) and the related cyclogregatin (**3**).

rare 4*H*-furo[3,2-*c*]pyran-3,4(2*H*)-dione, reminiscent of cyclogregatin (**3**), which had been isolated from *Aspergillus panamensis* by Steglich et al.[2] and which had its structure later revised by Brückner et al.[3] Lou et al. reported metabolites **1** and **2** to have no particular bioactivities, unlike most of the other low-molecular compounds isolated from this source fungus, and in contrast to the weakly antimicrobial cyclogregatin (**3**). Herein, we present the first synthesis of these structurewise interesting compounds and report on some bioactivities.

Our retrosynthetic approach to both ophiofuranones A (**1**) and B (**2**) is depicted in Scheme 1. We intended to close the six-membered ring in the final step via lactonization by removing acid-labile protecting groups from the alcohols of a fully fitted precursor **4**. Its five-membered ring was to be closed by a base-induced Knoevenagel-type condensation of the respective *β*-ketoacyl esters **5**.[4] These in turn should be accessible by a Yamaguchi esterification of the *β*-keto-*γ*-hydroxyocta-5,7-dienoates **7** with carboxylic acid **6**, derived from tiglic acid and bearing two stereogenic centers introduced by an asymmetric Sharpless dihydroxylation.[5] *β*-Ketoesters **7** were to be prepared by a Roskamp reaction[6] of aldehydes **8** with ethyl diazoacetate (EDA) in the presence of $SnCl_2$. The

Scheme 1. Retrosynthesis of Ophiofuranones A (1) and B (2)



aldehydes **8** should be accessible from aldehyde **9** via a series of two Wittig (for the *E*-isomer **8a**) or an Ando's modification of HWE[7] and a Wittig (for the *Z*-isomer **8b**) olefinations with additional oxidation/reduction steps. The chiral aldehyde **9** was to be prepared by a Sharpless epoxidation of 2-methylallyl alcohol,[8] followed by ring opening of the resulting epoxide with *para*-methoxybenzylate, protection of the resulting secondary alcohol, and oxidation of the primary one.

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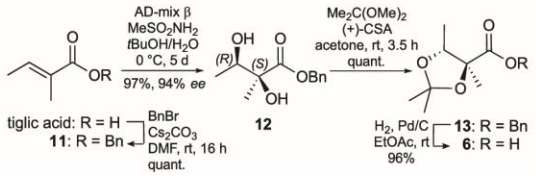
J. Org. Chem. 2022, 87, 6520–6523

Carboxylic acid **6** was prepared from tiglic acid (Scheme 2). Asymmetric hydroxylation of the quantitatively accessible

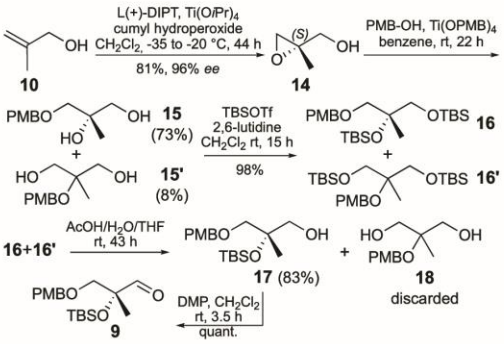
Scheme 2. Synthesis of Carboxylic Acid 6



benzyl ester **11** with AD-mix β under conditions as reported by Hamada et al.[9] afforded diol **12** in 97% yield with 94% *ee*. Protection furnished acetonide **13**, the benzyl ester of which was cleaved with H_2, Pd/C to leave carboxylic acid **6** in 93% overall yield, relative to tiglic acid.

The synthesis of β-ketoesters **7** started by establishing the third, *S*-configured stereogenic center of ophiofuranones A (**1**) and B (**2**) by an asymmetric Sharpless epoxidation of 2-methylallyl alcohol[8] **10** that afforded (*S*)-1-hydroxymethyl-2-methyloxirane **14** in 81% yield and with 96% *ee* (Scheme 3).

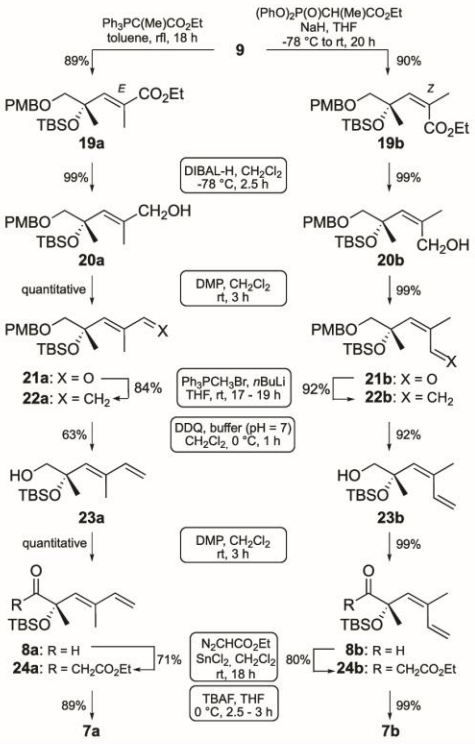
Scheme 3. Synthesis of Aldehyde 9



Opening of the expoxide with *p*-methoxybenzyl alcohol (PMB−OH) in the presence of $Ti(OPMB)_4$ gave an inseparable 8.6:1-mixture of regioisomeric diols **15** and **15′** in 81% overall yield. Protection of both hydroxy groups of these diols gave an 8.6:1-mixture of the bis(silyl ether)s **16** and **16′** which had their primary TBS protecting groups removed with acetic acid to afford an easily separable mixture of the desired monoprotected alcohol **17** and the diol **18**. Alcohol **17** was quantitively oxidized with Dess-Martin periodinane (DMP)[10] to afford pivotal aldehyde **9**.

The latter was olefinated in parallel with the stabilized ylide [1-(ethoxycarbonyl)ethylene]triphenylphosphorane[11] to give *E*-alkene **19a** and with the anion of phosphonate $(PhO)_2P(O)CH(Me)CO_2Et$[7,12] to afford *Z*-alkene **19b** in ca. 90% yield (Scheme 4). Reduction of the ester groups with DIBAL-H at −78 °C left the respective allyl alcohol **20a** or **20b** in near quantitative yield. Their oxidation with DMP[10] furnished the corresponding enals **21a** and **21b** in quantitative yield. Wittig olefination of aldehydes **21a** and **21b** with methylenetriphenylphosphorane afforded the conjugated dienes **22a** and **22b** in 84% (*E*) and 92% (*Z*). Oxidative cleavage of the PMB protecting groups with DDQ[13] liberated the primary alcohols **23a** and **23b**. Alcohol **23a** was obtained in a merely moderate

Scheme 4. Synthesis of β-Keto-γ-hydroxyesters 7



yield of 63%. Low yields of the deprotection of methoxybenzyl groups in the presence of conjugated dienes due to decomposition were repeatedly reported in the literature.[14] Alternative deprotection methods (hydrogenolysis, reduction with Lewis acids or CAN, TFA)[14a,15] were even less successful or were unsuitable for compound **22a**. Surprisingly, the *Z*-isomer **23b** was obtained in a yield of 92%. The alcohols **23a** and **23b** were quantitatively oxidized with DMP[10] to the respective aldehydes **8a** and **8b**. These were converted to β-ketoesters **24a** and **24b** via a Roskamp extension reaction[6] with EDA/$SnCl_2$ in 71% (*E*) and 80% (*Z*). In contrast to the original literature, tin(II) chloride had to be used equimolarly rather than catalytically and the reaction mixture had to be stirred much longer to get a complete conversion of **8a** and **8b**. Cleavage of the TBS groups with TBAF in good yields eventually afforded the building blocks **7a** and **7b**.

The syntheses of ophiofuranones A (**1**) and B (**2**) were finished in three further condensation steps (Scheme 5). Yamaguchi esterification[16] between carboxylic acid **6** and either of the two β-keto-γ-hydroxyesters **7** gave the corresponding esters **5a** and **5b** in ca. 90% yield. Base-induced Knoevenagel-type cyclization of the latter afforded the respective 3-furanones **4a** and **4b** in similarly good yields.[3,4] Their treatment with pTsOH in methanol led to the cleavage of the acetonide group and a subsequent lactonization of the so-formed diol to give the target compounds **1** and **2** in 89% yield. Related to allyl alcohol **10** (longest linear sequence), they were prepared in 16 steps and 12% (**1**) and 22% (**2**) yield.

The NMR data of our synthetic ophiofuranones A (**1**) and B (**2**) agreed well with those of the literature.[1] This was not so for the specific optical rotations. While synthetic product **1**

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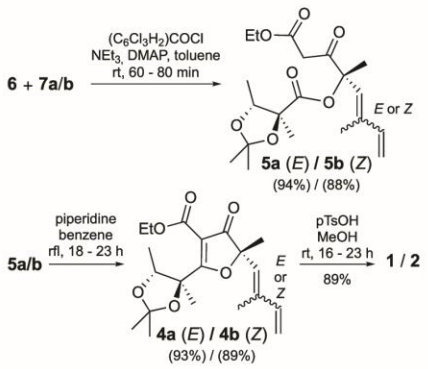
J. Org. Chem. 2022, 87, 6520−6523

Scheme 5. Final Condensation Steps Affording 1 and 2



showed an $[\alpha]_D^{24} = -272.4$ (c 0.10 in MeOH) and synthetic compound **2** an $[\alpha]_D^{24} = -248.8$ (c 0.10 in MeOH), a value of $[\alpha]_D^{20} = -38.7$ (c 0.1 in MeOH) was reported for isolate **1** and of $[\alpha]_D^{20} = -36.2$ (c 0.1 in MeOH) for isolate **2**. These deviations might partly be due to impurities of the isolates as visible in their ^{1}H and ^{13}C NMR spectra. Their scale, however, is more indicative of an error in measurement. The ECD spectra of our synthetic samples, though, are a close match (compare with the Supporting Information, SI) for those reported for the natural isolates.[1] Hence, we assume that the absolute configurations proposed by Lou et al. for the natural products are correct.

The synthetic ophiofuranones A (**1**), B (**2**) as well as their precursors **4a** and **4b** were tested for antimicrobial, cytotoxic, and antibiofilm activities against various bacteria, fungi, and cell lines. Concerning their minimum inhibitory concentration (MIC),[17,18] ophiofuranones A (**1**), B (**2**), and **4a** showed weak activities only against *Mucor hiemalis* (66.6 μg/mL) (compare with SI Table S3). No cytotoxic effects against human tumor cell lines and nonmalignant fibroblasts were observed for the synthetic compounds **1**, **2**, **4a**, and **4b** when applied in concentrations up to 37 μg/mL (133 μM for **1**/**2**; 101 μM for **4a**/**4b**).

As the isolated ophiofuranones had not been evaluated for antibiofilm effects, we tested our synthetic products **1** and **2** and their precursors **4a** and **4b** for inhibition of biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as for dispersal effects on preformed biofilms of *S. aureus* and *Candida albicans* (Table 1).[19−21] Only **4b** exhibited a 52% inhibition of the formation of biofilms by *S. aureus* when applied at 250 μg/mL. Dispersion of *C. albicans* biofilms to an extent between 16% and 51% was observed for **1**, **4a**, and **4b** when applied at 250 μg/mL or 125 μg/mL.

Table 1. Inhibition [%] of biofilm formation by *S. aureus* and dispersion [%] of preformed biofilms of *C. albicans* by ophiofuranones A (1), B (2), and precursors 4a and 4b[a]

compounds	biofilm inhibition (*S. aureus*) [% ± SD]	biofilm dispersion (*C. albicans*) [% ± SD]
1	-	24 ± 6 (250 μg/mL)[c]
2	-	-
4a	-	51 ± 4 (250 μg/mL)[c]
	-	43 ± 8 (125 μg/mL)[c]
4b	52 ± 7 (250 μg/mL)[b]	48 ± 10 (250 μg/mL)[c]
		16 ± 9 (125 μg/mL)[c]

[a]References (%). [b]Microporenic acid A (MAA): 82 (250 μg/mL), 84 (7.8 μg/mL), 34 (3.9 μg/mL). [c]Farnesol: 78 (250 μg/mL), 70 (31.3 μg/mL), 42 (15.6 μg/mL); SD: standard deviation; -: not active.

In summary, we synthesized ophiofuranones A and B in 12% (A) and 22% (B) yield starting from cheap tiglic acid and 2-methylallyl alcohol. Their four stereogenic elements were installed via stereoselective Sharpless and Wittig/HWE reactions. Matching NMR and ECD spectra corroborated the structural assignment of the isolated natural compounds. Their lack of cytotoxic effects on human cells and bacteria could prove beneficial as synthetic ophiofuranone A and monocyclic precursors were found weakly effective against biofilms of *S. aureus* and *C. albicans*. Our flexible synthesis should allow further structural optimization toward derivatives potentially suitable for the control of infections on implanted medical devices and mucosal surfaces without eliciting microbial resistance.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.2c00521.

Experimental details of chemical syntheses; characterization of new compounds; NMR spectra of all new compounds; HPLC chromatograms of ophiofuranones A and B; NMR and ECD comparison of isolated and synthetic ophiofuranones A and B; and descriptions of assays for antimicrobial, cytotoxic, biofilm inhibitory, and biofilm dispersal activities (PDF)

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Author Contributions

The manuscript was written through substantial contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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Supporting Information

Synthesis and Bioactivity of Ophiofuranones A and B

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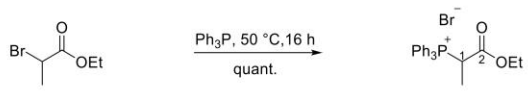
Table of Contents

General information

All reagents were purchased from commercial sources (ABCR, Acros, Alfa Aeser, Carbolution, chemPur, Fluorochem, Merck, Sigma Aldrich, TCI) and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran and toluene which were freshly distilled over sodium/benzophenone, and benzene, dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and dichloromethane which were dried over molecular sieve (3 Å). Moisture or air sensitive reactions were routinely carried out in oven-dried glassware under an argon atmosphere using standard Schlenk technique. Reactions that required heating were set up in an oil bath. Melting points were determined with a Büchi M-565 melting point apparatus and are uncorrected. IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. ^{1}H-NMR, ^{13}C-NMR (APT) and ^{31}P-NMR spectra were obtained using a Bruker Avance III HD 500 spectrometer. Chemical shifts of NMR signals are given in parts per million (δ) using the residual solvent peak as an internal standard, i.e., 7.26 ppm (proton) and 77.16 ppm (carbon) for $CDCl_3$, 3.31 ppm (proton) and 49.0 ppm (carbon) for MeOD and 2.50 ppm (proton) and 39.52 ppm (carbon) for DMSO-d_6. Coupling constants (*J*) are quoted in Hz. Multiplicity abbreviations used: s singlet, d doublet, t triplet, q quartet, br broad and m multiplet. Proton and carbon resonances were assigned by standard 2D experiments (for all substances HSQC and HMBC and for some additionally H,H-COSY or NOESY). These 2D NMR spectra are available from the corresponding author upon reasonable request. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 Polarimeter using solutions in chloroform or methanol. Electronic circular dichroism spectra were recorded on a Jasco Spectropolarimeter J-710 using a Peltier device to control the temperature (PTC-348 WI). ECD spectra were measured with a substance concentration of 0.05 and 0.1 mg/mL in MeOH in the wavelength range 200-400 nm at 25 °C with a 1 nm bandwidth in a 1 mm cuvette. Data were normalized by subtraction of MeOH spectrum. For chromatography silica gel 60 (230–400 mesh) was used. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60GF_{254} pre-coated aluminum-backed plates. The compounds were visualized with UV light (254 nm) or the Hanessian's stain or potassium permanganate. Flash chromatography was performed at medium pressure using dry packed Macherey-Nagel silica gel 60, pore size 40–63 μm with the eluent specified in the respective experimental procedures. The determination of the enantiomeric excess (*ee*) of chiral compounds was performed on a gas chromatograph GC-FID 2010 equipped with an autosampler AOC 20i, a Lipotex A column (length: 25 m; inside diameter: 0.25 mm; bonded) and a flame ionization detector (FID) 2010 or on a HPLC Waters Alliance HPLC equipped with a Waters 2695 Separation Module, a Waters 2487 Dual λ Absorbance Detector and a Daicel Chiralpak AD-H column. Analytical HPLC measurements were carried out on a Shimadzu Nexera XR with autosampler SIL-20A using a Knauer Eurospher II C-18 column (150 × 4 mm), pore size 100 Å, particle size 3 μm. Detection was executed by a diode array detector SPD-M20A.

Experimental procedures

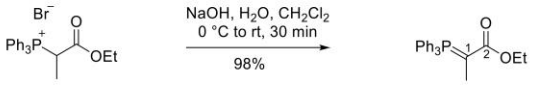
(1-Ethoxycarbonylethyl)triphenylphosphonium bromide



(1-Ethoxycarbonylethyl)triphenylphosphonium bromide was prepared according to literature.[1] Triphenylphosphine (26.2 g, 100 mmol, 1.00 eq.) and ethyl 2-bromopropionate (15.6 mL, 120 mmol, 1.20 eq.) were heated without solvent for 16 h at 50 °C. The resulting solid was grained in a mortar into fine powder which was washed with *n*-hexane (4 × 60 mL). The volatiles were evaporated under reduced pressure and (1-ethoxycarbonylethyl)triphenylphosphonium bromide was obtained as a colorless solid (44.3 g, 100 mmol, quantitative).

^{1}H-NMR ($CDCl_3$, 500 MHz): 8.01-7.93 (m, 6 H, H-Ar), 7.78-7.72 (m, 3 H, H-Ar), 7.69-7.63 (m, 6 H, H-Ar), 6.94-6.83 (m, 1 H, H-1), 3.98 (dtq, *J* = 18.6, 11.0, 7.2 Hz, 2 H, OCH_2CH_3), 1.66 (dd, *J* = 18.5, 7.2 Hz, 3 H, Me), 0.98 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3). — **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz) 168.0 (C-2), 135.0 (d, *J* = 3.6 Hz, C-Ar), 134.3 (d, *J* = 10.0 Hz, C-Ar), 130.3 (d, *J* = 12.7 Hz, C-Ar), 117.8 (d, *J* = 86.7 Hz, C-Ar_q), 62.9 (OCH_2CH_3), 36.7 (d, *J* = 51.0 Hz, C-1), 13.7 (OCH_2CH_3), 13.0 (d, *J* = 2.7 Hz, Me). — **^{31}P-NMR** ($CDCl_3$, 202 MHz): 28.5.
The preceding data is consistent with those reported in literature.[1,2]

Ethyl 2-(triphenylphosphoranylidene)propionate[1]



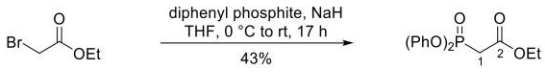
Ethyl 2-(triphenylphosphoranylidene)propionate was prepared according to literature.[1] A solution of NaOH (4.40 g, 110 mmol, 1.10 eq.) in H_2O (80 mL) was treated slowly with (1-ethoxycarbonylethyl)triphenylphosphonium bromide (44.3 g, 100 mmol, 1.00 eq.) in CH_2Cl_2 (90 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for 30 min. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were washed with brine (3 × 150 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Before all CH_2Cl_2 was removed, *n*-hexane (100 mL) was added to the residue to precipitate ethyl 2-(triphenylphosphoranylidene)propionate. After removing the volatiles in vacuo ethyl 2-(triphenylphosphoranylidene)propionate was obtained as a yellow hygroscopic solid (35.5 g, 98.0 mmol, 98%). The product was obtained as a mixture of a *major* and a *minor* rotamer with a ratio of 2.6:1 after dynamic equilibrium was established in $CDCl_3$ (determined via ^{1}H NMR).

^{1}H-NMR *major* ($CDCl_3$, 500 MHz): 7.64-7.57 (m, 6 H, H-Ar), 7.56-7.49 (m, 3 H, H-Ar), 7.48-7.41 (m, 6 H, H-Ar), 3.71 (q, *J* = 7.0 Hz, 2 H, OCH_2CH_3), 1.62 (d, *J* = 13.7 Hz, 3 H, Me), 0.45 (t, *J* = 7.1 Hz, 3

H, OCH_2CH_3). — **$^{13}C\{^1H\}$-NMR** *major* ($CDCl_3$, 125 MHz): 170.8 (C-2), 133.7 (d, *J* = 9.9 Hz, C-Ar), 131.7 (d, *J* = 3.5 Hz, C-Ar), 128.5 (d, *J* = 11.8 Hz, C-Ar), 128.5 (d, *J* = 90.6 Hz, C-Ar_q), 57.5 (OCH_2CH_3), 31.9 (d, *J* = 120.5 Hz, C-1), 14.3 (OCH_2CH_3), 13.0 (d, *J* = 12.7 Hz, Me). — **^{31}P-NMR** *major* ($CDCl_3$, 202 MHz): 22.6. — **^1H-NMR** *minor* ($CDCl_3$, 500 MHz): 7.64-7.57 (m, 6 H, H-Ar), 7.56-7.49 (m, 3 H, H-Ar), 7.48-7.41 (m, 6 H, H-Ar), 4.05 (q, *J* = 7.0 Hz, 2 H, OCH_2CH_3), 1.60 (d, *J* = 14.3 Hz, 3 H, Me), 1.24 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3). — **$^{13}C\{^1H\}$-NMR** *minor* ($CDCl_3$, 125 MHz): 170.7 (C-2), 133.8 (d, *J* = 9.1 Hz, C-Ar), 132.2 (d, *J* = 10.0 Hz, C-Ar), 128.6 (d, *J* = 12.7 Hz, C-Ar), 128.0 (d, *J* = 89.8 Hz, C-Ar_q), 58.2 (OCH_2CH_3), 32.9 (d, *J* = 126.6 Hz, C-1), 15.5 (OCH_2CH_3), 12.3 (d, *J* = 11.8 Hz, Me). — **^{31}P-NMR** *minor* ($CDCl_3$, 202 MHz): 22.5.

The preceding data is consistent with those reported in literature.[1,2]

Ethyl 2-(diphenoxyphosphoryl)acetate

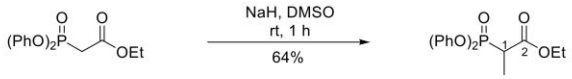


According to literature[3] diphenyl phosphite (10.0 mL, 50.0 mmol, 1.00 eq.) was slowly added to NaH (60 wt% in mineral oil, 2.00 g, 50.0 mmol, 1.00 eq.) in dry THF (40 mL) at 0 °C. After 1 h at 0 °C ethyl bromoacetate (5.52 mL, 50.0 mmol, 1.00 eq.) was slowly added at 0 °C and the reaction mixture was allowed to warm up to ambient temperature. The reaction mixture was stirred for 17 h at room temperature. Sat. aqueous NH_4Cl (20 mL) and H_2O (20 mL) were added to the mixture and the phases were separated. The aqueous layer was extracted with Et_2O (3 × 50 mL) and the combined organic phases were dried over $MgSO_4$. The volatiles were removed in vacuo and the remainder was purified by column chromatography (15% to 40% EtOAc in *n*-pentane). Ethyl 2-(diphenoxyphosphoryl)acetate was obtained as a colorless hygroscopic oil (6.92 g, 21.6 mmol, 43%).

^1H-NMR ($CDCl_3$, 500 MHz): 7.38-7.29 (m, 4 H, H-Ar), 7.25-7.16 (m, 6 H, H-Ar), 4.23 (q, *J* = 7.2 Hz, 2 H, OCH_2CH_3), 3.27 (d, *J* = 21.6 Hz, 2 H, H-1), 1.28 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3). — **$^{13}C\{^1H\}$-NMR** ($CDCl_3$, 125 MHz): 164.9 (d, *J* = 6.4 Hz, C-2), 150.1 (d, J = 8.4 Hz, C-Ar_q), 130.0 (C-Ar), 125.7 (d, *J* = 1.7 Hz, C-Ar), 120.8 (d, *J* = 4.6 Hz, C-Ar), 62.2 (OCH_2CH_3), 34.2 (d, *J* = 137.0 Hz, C-1), 14.2 (OCH_2CH_3). — **^{31}P-NMR** ($CDCl_3$, 202 MHz): 12.8.

The preceding data is consistent with those reported in literature.[3]

Ethyl 2-(diphenoxyphosphoryl)propanoate



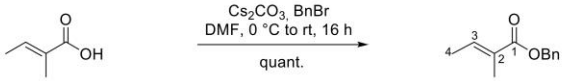
Ethyl 2-(diphenoxyphosphoryl)propanoate was prepared according to literature.[4] To a stirred solution of ethyl 2-(diphenoxyphosphoryl)acetate (4.61 g, 14.4 mmol, 1.00 eq.) in dry DMSO (17 mL) was added

NaH (60 wt% in mineral oil, 576 mg, 14.4 mmol, 1.00 eq.) at 15 °C. After 20 min at room temperature MeI (975 µL, 15.8 mmol, 1.10 eq.) was added and the reaction mixture was stirred for 1 h. The reaction was quenched with sat. aqueous NH_4Cl (30 mL) and the resulting mixture was extracted with EtOAc (3 × 40 mL). The combined organic phases were washed with H_2O (2 × 100 mL) and brine (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The remainder was purified by column chromatography (10% to 30% EtOAc in *n*-pentane) to afford ethyl 2-(diphenoxyphosphoryl)-propanoate as a colorless oil (3.09 g, 9.24 mmol, 64%) along with ethyl 2-(diphenoxyphosphoryl)acetate (967 mg, 3.02 mmol, 21%) and the dialkylation product (547 mg, 1.57 mmol, 11%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 7.35-7.29 (m, 4 H, H-Ar), 7.23-7.14 (m, 6 H, H-Ar), 4.27-4.18 (m, 2 H, OCH_2CH_3), 3.37 (dq, *J* = 23.6, 7.3 Hz, 1 H, H-1), 1.64 (dd, *J* = 19.4, 7.3 Hz, 3 H, Me), 1.27 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3). — **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 168.9 (d, *J* = 4.5 Hz, C-2), 150.44 (d, *J* = 5.4 Hz, C-Ar_q), 150.37 (d, *J* = 5.4 Hz, C-Ar_q), 129.9 (C-Ar), 125.5 (d, *J* = 2.5 Hz, H-Ar), 120.74 (d, *J* = 4.6 Hz, H-Ar), 120.65 (d, *J* = 3.6 Hz, H-Ar), 62.0 (OCH_2CH_3), 39.7 (d, *J* = 135.4 Hz, C-1), 14.2 (OCH_2CH_3), 11.9 (d, *J* = 6.4 Hz, Me). — **^{31}P-NMR** ($CDCl_3$, 202 MHz): 16.7.

The preceding data is consistent with those reported in literature.[4]

Benzyl (*E*)-2-methylbut-2-enoate (11)



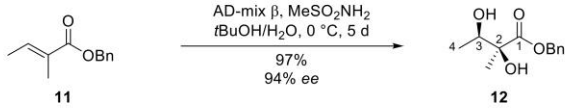
ticlic acid **11**

A stirred solution of tiglic acid (3.50 g, 35.0 mmol, 1.00 eq.) in dry DMF (70 mL) was treated with Cs_2CO_3 (11.4 g, 35.0 mmol, 1.00 eq.) at room temperature and BnBr (4.57 mL, 38.5 mmol, 1.10 eq.) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and was allowed to warm up to room temperature. After the reaction mixture was stirred for 16 h at this temperature, it was quenched with H_2O (100 mL) and the phases were separated. The aqueous phase was extracted with Et_2O (3 × 50 mL) and the combined organic phases were washed with brine (2 × 150 mL) and dried over $MgSO_4$. The volatiles were evaporated under reduced pressure and the remainder was purified by column chromatography (*n*-pentane to 1.5% EtOAc in *n*-pentane). Benzyl ester **11** was obtained as a hygroscopic colorless oil (6.65 g, 35.0 mmol, quantitative).

R_f = 0.68 (10% EtOAc in *n*-hexane). — **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.41-7.29 (m, 5 H, H-Ar), 6.92 (qq, *J* = 7.1, 1.3 Hz, 1 H, H-3), 5.19 (s, 2 H, Ar-CH_2), 1.87 (s, 3 H, Me), 1.80 (dq, *J* = 7.2, 0.8 Hz, 3 H, H-4). — **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 168.0 (C-1), 137.8 (C-3), 136.6 (C-Ar_q), 128.6 (C-Ar), 128.2 (C-2), 128.1 (C-Ar), 128.0 (C-Ar), 66.3 (Ar-CH_2), 14.5 (C-4), 12.2 (Me). — **IR** (cm^{-1}) $\tilde{\nu}$ = 2946, 1711, 1651, 1258, 1127, 731, 699. — **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{12}H_{15}O_2^+$ 191.1067; found 191.1064.

The preceding data is consistent with those reported in literature.[5]

Benzyl (2*S*,3*R*)-2,3-dihydroxy-2-methylbutanoate (12)



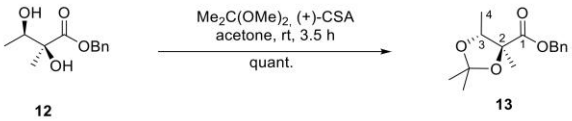
Water (58 mL) and *t*BuOH (58 mL) were treated with AD-mix β (16.0 g), $MeSO_2NH_2$ (1.09 g, 11.5 mmol, 1.00 eq.) and benzyl ester **11** (2.18 g, 11.5 mmol, 1.00 eq.) at 0 °C. The reaction mixture stirred at 0 °C for 5 d. Solid sodium sulfite (10 g) was added at 0 °C. The mixture was allowed to warm up to ambient temperature and was stirred for 1 h. The aqueous phase was extracted with EtOAc (5 × 150 mL), the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The remainder was purified by column chromatography (30% EtOAc in *n*-pentane) to afford product **12** as a colorless oil that turned solid at –20 °C (2.49 g, 11.1 mmol, 97%, 94% *ee*).

Analytical HPLC: Chiralpak AD-H, *n*-hexane:*i*PrOH = 90:10, 215 nm, t_R = 20.58 min (2*S*,3*R*), t_R = 25.27 min (2*R*,3*S*).

R_f = 0.19 (30% EtOAc in *n*-hexane). **—** $[\alpha]_D^{24}$: +1.5 (*c* 1.00 in $CHCl_3$) [Lit.[6] value for the enantiomer of **12**: $[\alpha]_D^{20}$: –1.15 (*c* 4.68 in $CHCl_3$)]. **— ^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.41-7.32 (m, 5 H, H-Ar), 5.25 (s, 2 H, Ar-CH_2), 3.98 (dq, *J* = 9.0, 6.4 Hz, 1 H, H-3), 3.37 (s, 1 H, OH^2), 2.00 (d, *J* = 9.0 Hz, 1 H, OH^3), 1.33 (s, 3 H, Me), 1.22 (d, *J* = 6.4 Hz, 3 H, H-4). **— ^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 176.3 (C-1), 135.3 (C-Ar_q), 128.8 (C-Ar), 128.7 (C-Ar), 128.2 (C-Ar), 77.4 (C-2), 71.8 (C-3), 67.9 (Ar-*C*H_2), 21.9 (Me), 16.8 (C-4). **— IR** (cm^{-1}) $\tilde{\nu}$ = 3447 (br.), 2983, 1729, 1127, 696. **— HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{12}H_{16}O_4Na^+$ 247.0941; found 247.0937.

The preceding data is consistent with those reported in literature.[7]

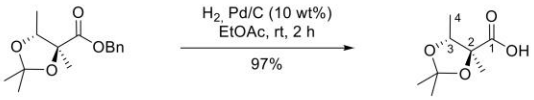
Benzyl (4*S*,5*R*)-2,2,4,5-tetramethyl-1,3-dioxolane-4-carboxylate (13)



Compound **12** (600 mg, 2.68 mmol, 1.00 eq.) was dissolved in acetone (50 mL) and treated with 2,2-dimethoxypropane (328 µL, 2.68 mmol, 1.00 eq.) and (+)-CSA (311 mg, 1.34 mmol, 0.50 eq.). After 3.5 h at room temperature the solvent was concentrated in vacuo. The residue was treated with CH_2Cl_2 (30 mL) and sat. aqueous $NaHCO_3$ (30 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic phases were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure and acetonide **13** was obtained as a colorless oil without further purification (705 mg, 2.68 mmol, quantitative).

R_f = 0.38 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{24}$: –15.5 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.39-7.29 (m, 5 H, H-Ar), 5.22 (d, *J* = 12.4 Hz, 1 H, Ar-C*H*^{a}H^b), 5.18 (d, *J* = 12.4 Hz, 1 H, Ar-CH^a*H*^{b}), 4.34 (q, *J* = 6.3 Hz, 1 H, H-3), 1.47 (s, 3 H, acetonide-Me), 1.37 (s, 3 H, acetonide-Me), 1.34 (s, 3 H, Me), 1.30 (d, *J* = 6.3 Hz, 3 H, H-4). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 173.2 (C-1), 135.7 (C-Ar$_q$), 128.7 (C-Ar), 128.5 (C-Ar), 128.2 (C-Ar), 108.9 (acetonide-C$_q$), 82.9 (C-2), 75.9 (C-3), 67.1 (Ar-*C*H$_2$), 28.3 (acetonide-Me), 25.6 (acetonide-Me), 19.8 (Me), 14.9 (C-4). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1730, 1259, 1107, 696. ─ **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{15}H_{21}O_4^+$ 265.1434; found 265.1432.

(4*S*,5*R*)-2,2,4,5-Tetramethyl-1,3-dioxolane-4-carboxylic acid (6)

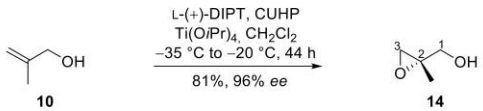


13 **6**

To a solution of benzyl ester **13** (2.18 g, 8.24 mmol, 1.00 eq.) in EtOAc (82 mL) under an argon atmosphere was added Pd/C (10 wt%, 327 mg). The reaction flask and the suspension were purged with H_2 and the reaction mixture was stirred at room temperature for 2 h under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over a plug of Celite® and the filtrate was washed with EtOAc (300 mL). The volatiles were evaporated under reduced pressure and carboxylic acid **6** was obtained as a colorless oil without further purification (1.39 g, 8.00 mmol, 97%.).

R_f = 0.10 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{25}$: –42.4 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 9.62 (br. s, 1 H, CO_2*H*), 4.30 (q, *J* = 6.3 Hz, 1 H, H-3), 1.49 (s, 3 H, acetonide-Me), 1.44 (s, 3 H, acetonide-Me), 1.38 (s, 3 H, Me), 1.36 (d, *J* = 6.3 Hz, 3 H, H-4). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 175.9 (C-1), 109.4 (acetonide-C$_q$), 82.9 (C-2), 75.9 (C-3), 28.2 (acetonide-Me), 25.7 (acetonide-Me), 19.2 (Me), 14.5 (C-4). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3501 (br.), 1723, 1104. ─ **HRMS** (ESI) *m/z* $[M-H]^-$: calculated for $C_8H_{13}O_4^-$ 173.0808; found 173.0809.

(*S*)-(2-Methyloxiran-2-yl)methanol (14)



10 **14**

According to literature[8] a mixture of crushed 4 Å activated molecular sieves (4.00 g) in dry CH_2Cl_2 (90 mL) was cooled to –35 °C. Ti(O*i*Pr)$_4$ (887 µL, 3.00 mmol, 0.10 eq.) and L-(+)-DIPT (770 µL, 4.49 mmol, 0.15 eq.) were added by syringe. After the mixture was stirred at –35 °C for 30 min, 2-methyl-prop-2-en-1-ol (**10**) (2.52 mL, 30.0 mmol, 1.00 eq.) in dry CH_2Cl_2 (10 mL) was added, followed by cumene hydroperoxide (8.38 mL, 44.9 mmol, 1.50 eq.). The reaction mixture was stirred at –35 °C for 1 h and then at –20 °C for 44 h. Aqueous saturated Na_2SO_4 (3 mL) was added at –20 °C and

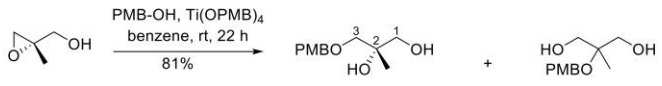
the mixture was diluted with Et_2O (30 mL). After the mixture was stirred at ambient temperature for 2 h, the resulting slurry was filtered through a pad of Celite® and the resulting solution was concentrated under reduced pressure. The residue was purified by column chromatography (50% Et_2O in *n*-pentane) to yield epoxide **14** as a colorless oil (2.14 g, 24.3 mmol, 81%, 96% *ee*).

Analytical GC: Lipotex A, 50 °C, t_R = 6.85 min (*S*), t_R = 10.60 min (*R*).

R_f = 0.33 (75% Et_2O in *n*-pentane) ─ $[\alpha]_D^{25}$: –8.9 (*c* 1.00 in $CHCl_3$) [Lit.[8] $[\alpha]_D^{25}$: –9.7 (*c* 2.10 in $CHCl_3$)]. ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 3.72 (d, *J* = 12.3 Hz, 1 H, H-1^a), 3.61 (d, *J* = 12.3 Hz, 1 H, H-1^b), 2.91 (d, *J* = 4.7 Hz, 1 H, H-3^a), 2.65 (d, *J* = 4.9 Hz, 1 H, H-3^b), 1.98 (br. s., 1 H, OH), 1.36 (s, 3 H, Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 64.3 (C-3), 57.3 (C-2), 51.1 (C-1), 18.2 (Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3413 (br.), 1042, 799. ─ **HRMS** (ESI) *m/z* [M+H$^+$] calculated for $C_4H_9O_2^+$ 89.0597; found 89.0600.

Analytical data agree with those reported.[8]

(*S*)-3-((4-Methoxybenzyl)oxy)-2-methylpropane-1,2-diol (15)



14 **15** **15'**

regioisomeric ratio 8.6:1

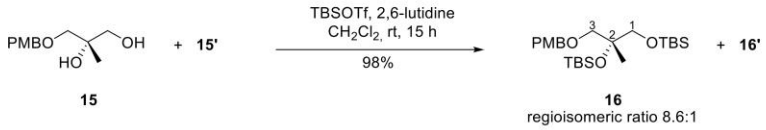
A mixture of $Ti(OiPr)_4$ (1.01 mL, 3.40 mmol, 1.50 eq.) and 4-methoxybenzyl alcohol (4.66 mL) was stirred under reduced pressure (8 mbar) for 60 min to remove the 2-propanol. The stirred solution was treated with epoxide **14** (200 mg, 2.27 mmol, 1.00 eq.) in dry benzene (12 mL) and the resulting mixture was stirred at ambient temperature for 22 h. The solution was diluted with Et_2O (20 mL) and aqueous sulfuric acid (0.5M, 15 mL) was added. The two-phase mixture was stirred vigorously until two clear phases formed. The layers were separated and the aqueous one was extracted with Et_2O (3 × 15 mL). The combined organic phases were dried over $MgSO_4$ and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (30% EtOAc in *n*-pentane) afforded regioisomeric mixture **15** and **15'** as a colorless oil that turned solid at –20 °C (414 mg, 1.83 mmol, 81%). The regioisomeric ratio of the epoxide opening amounted to 1:8.6 (determined via ^{1}H-NMR). A small amount of the regioisomeric mixture of **15** and **15'** was purified additionally by column chromatography (10% to 30% EtOAc in *n*-pentane) to get pure diol **15** for analytics.

R_f = 0.41 (EtOAc). ─ $[\alpha]_D^{24}$: +8.1 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.25-7.22 (m, 2 H, H-Ar), 6.91-6.86 (m, 2 H, H-Ar), 4.50 (d, *J* = 11.6 Hz, 1 H, Ar-C*H*^{a}H^b), 4.47 (d, *J* = 11.6 Hz, 1 H, Ar-CH^a*H*^{b}), 3.81 (s, 3 H, OMe), 3.62 (d, *J* = 11.1, 4.6 Hz, 1 H, H-1^a), 3.48 (d, *J* = 9.2 Hz, 1 H, H-3^a), 3.43 (dd, *J* = 11.0, 5.5 Hz, 1 H, H-1^b), 3.39 (d, *J* = 9.3 Hz, 1 H, H-3^b), 2.80 (s, 1 H, OH^2), 2.38 (br. s, 1 H, OH^1), 1.12 (s, 3 H, Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.5 (C-Ar$_q$), 129.9 (C-Ar$_q$), 129.5 (C-Ar), 111.0 (C-Ar), 76.2 (C-3), 73.5 (Ar-*C*H$_2$), 71.9 (C-2), 68.9 (C-1), 55.4 (OMe), 21.5 (Me). ─ **IR**

(cm^{-1}) $\tilde{\nu}$ =3322 (broad), 1512, 1237, 1035. ─ **mp** 44-45 °C ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{12}H_{18}O_4Na^+$ 249.1097; found 249.1096.

Analytical data agree with those reported.[9]

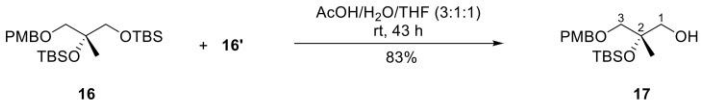
(*R*)-1,2-Bis(*tert*-butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)-2-methylpropane (16)



To a solution of regioisomeric mixture **15** and **15'** (8.16 g, 36.1 mmol, 1.00 eq.) in dry CH_2Cl_2 (360 mL), 2,6-lutidine (33.5 mL, 289 mmol, 8.00 eq.) and TBSOTf (50.1 mL, 216 mmol, 6.00 eq.) were added at 0 °C. The reaction mixture was stirred for 15 h at ambient temperature. The reaction was quenched with sat. aqueous NH_4Cl (350 mL) at 0 °C. The aqueous phase was extracted with Et_2O (3 × 250 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The remainder was purified by column chromatography (*n*-pentane to 5% EtOAc in *n*-pentane), to afford bis(silyl ether)s **16** and **16'** as a colorless oil (16.1 g, 35.4 mmol, 98%). The regioisomeric ratio was still 1:8.6 (determined via ^{1}H-NMR). A small amount of the regioisomeric mixture of **16** and **16'** was purified additionally by column chromatography (*n*-pentane to 3% EtOAc in *n*-pentane) to get pure silyl ether **16** for analytics.

$\mathbf{R_f}$ = 0.39 (4% EtOAc in *n*-hexane). ─ $[\alpha]_D^{25}$: +3.4 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.26-7.23 (m, 2 H, H-Ar), 6.89-6.84 (m, 2 H, H-Ar), 4.45 (d, *J* = 11.6 Hz, 1 H, Ar-H^aH^b), 4.42 (d, *J* = 11.6 Hz, 1 H, Ar-H^aH^b), 3.81 (s, 3 H, OMe), 3.52 (d, *J* = 9.5 Hz, 1 H, H-1^a), 3.362 (d, *J* = 9.6 Hz, 1 H, H-1^b), 3.360 (d, J = 9.2 Hz, H-3^a), 3.28 (d, *J* = 9.3 Hz, 1 H, H-3^b), 1.15 (s, 3 H, Me), 0.88 (s, 9 H, TBS-*t*Bu), 0.84 (s, 9 H, TBS-*t*Bu), 0.06 (s, 3 H, TBS-Me), 0.05 (s, 3 H, TBS-Me), 0.030 (s, 3 H, TBS-Me), 0.028 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.1 (C-Ar_q), 131.1 (C-Ar_q), 129.2 (C-Ar), 113.7 (C-Ar), 76.1 (C-2), 79.0 (C-3), 73.1 (Ar-CH_2), 68.1 (C-1), 55.4 (OMe), 26.1 (TBS-*t*Bu), 26.0 (TBS-*t*Bu), 22.6 (Me), 18.43 (TBS-*t*Bu_q), 18.35 (TBS-*t*Bu_q), –2.21 (TBS-Me), –2.24 (TBS-Me), –5.3 (TBS-Me), –5.4 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 2929, 1247, 1095, 831, 773. ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{24}H_{46}O_4Si_2Na^+$ 477.2827; found 477.2818.

(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)-2-methylpropan-1-ol (17)

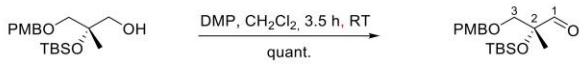


A solution of the mixture of silyl ethers **16** and **16'** (220 mg, 0.484 mmol, 1.00 eq.) in dry THF (1.8 mL) was treated with H_2O (1.8 mL) und AcOH (5.4 mL) at 0 °C and the resulting mixture was stirred at

ambient temperature for 43 h. The reaction was slowly quenched with aqueous NaOH (4.75M, 20 mL) at 0 °C. The aqueous phase was extracted with CH_2Cl_2 (4 × 20 mL) and the combined organic phases were washed with sat. aqueous $NaHCO_3$ (60 mL). Drying the organic layer over Na_2SO_4, removing the volatiles in vacuo and purification of the residue by column chromatography (10% EtOAc in *n*-pentane) yielded alcohol **17** as a colorless oil (137 mg, 0.402 mmol, 83%) together with residual **16** (13.0 mg, 28.6 µmol, 6%).

R$_f$ = 0.22 (10% EtOAc in *n*-hexane). — $[\alpha]_D^{22}$: +3.8 (*c* 1.00 in $CHCl_3$). — **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.25-7.22 (m, 2 H, H-Ar), 6.90-6.85 (m, 2 H, H-Ar), 4.47 (d, *J* = 11.6 Hz, 1 H, Ar-C*H*^{a}H^b), 4.44 (d, *J* = 11.6 Hz, 1 H, Ar-CH^a*H*^{b}), 3.81 (s, 3 H, OMe), 3.51 (dd, *J* = 10.8, 8.2 Hz, 1 H, H-1^a), 3.44 (d, *J* = 8.9 Hz, 1 H, H-3^a), 3.42 (dd, *J* = 10.8, 4.7 Hz, 1 H, H-1^b), 3.31 (d, *J* = 8.9 Hz, 1 H, H-3^b), 2.17 (dd, *J* = 8.1, 4.7 Hz, 1 H, OH), 1.23 (s, 3 H, Me), 0.86 (s, 9 H, TBS-*t*Bu), 0.10 (s, 3 H, TBS-Me), 0.08 (s, 3 H, TBS-Me). — **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.3 (C-Ar$_q$), 130.4 (C-Ar$_q$), 129.3 (C-Ar), 113.9 (C-Ar), 75.6 (C-2), 75.4 (C-3), 73.3 (Ar-*C*H$_2$), 69.2 (C-1), 55.4 (OMe), 25.9 (TBS-*t*Bu), 22.6 (Me), 18.3 (TBS-*t*Bu$_q$), –2.16 (TBS-Me), –2.17 (TBS-Me). — **IR** (cm^{-1}) $\tilde{\nu}$ = 3687 (br.), 1514, 1246, 1094, 833, 774. — **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{18}H_{32}O_4SiNa^+$ 363.1962; found 363.1955.

(*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)-2-methylpropanal (9)

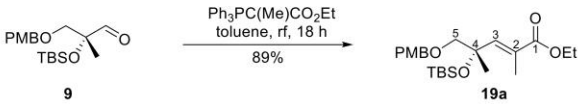


17 **9**

To a stirred solution of alcohol **17** (1.00 g, 2.94 mmol, 1.00 eq.) in dry CH_2Cl_2 (30 mL) was added DMP (1.37 g, 3.23 mmol, 1.10 eq.) at 0 °C. The reaction mixture was allowed to warm up to room temperature. After 3.5 h sat. aqueous $NaHCO_3$ (30 mL) and sat. aqueous $Na_2S_2O_3$ (30 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 15 mL). The combined organic phases were washed with sat. aqueous $Na_2S_2O_3$ (2 × 150 mL) and sat. aqueous $NaHCO_3$ (2 × 150 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure and aldehyde **9** was obtained without further purification as a slightly yellowish oil (995 mg, 2.94 mmol, quantitative).

R$_f$ = 0.61 (10% EtOAc in *n*-hexane). — $[\alpha]_D^{22}$: +3.2 (*c* 1.00 in $CHCl_3$). — **^{1}H-NMR** ($CDCl_3$, 500 MHz): 9.60 (s, 1 H, H-1), 7.24-7.18 (m, 2 H, H-Ar), 6.89-6.85 (m, 2 H, H-Ar), 4.45 (d, *J* = 11.7 Hz, 1 H, Ar-C*H*^{a}H^b), 4.41 (d, *J* = 11.7 Hz, 1 H, Ar-CH^a*H*^{b}), 3.81 (s, 3 H, OMe), 3.50 (d, *J* = 9.7 Hz, 1 H, H-3^a), 3.45 (d, *J* = 9.7 Hz, 1 H, H-3^b), 1.25 (s, 3 H, Me), 0.89 (s, 9 H, TBS-*t*Bu), 0.094 (s, 3 H, TBS-Me), 0.087 (s, 3 H, TBS-Me). — **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 204.1 (C-1), 159.2 (C-Ar$_q$), 129.9 (C-Ar$_q$), 129.3 (C-Ar), 113.8 (C-Ar), 80.3 (C-2), 74.8 (C-3), 73.2 (Ar-*C*H$_2$), 55.3 (OMe), 25.8 (TBS-*t*Bu), 20.7 (Me), 18.3 (TBS-*t*Bu$_q$), –2.56 (TBS-Me), –2.60 (TBS-Me). — **IR** (cm^{-1}) $\tilde{\nu}$ = 1739, 1514, 1247, 1036, 833, 776. — **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{18}H_{31}O_4Si^+$ 339.1986; found 339.1989.

Ethyl (*S*,*E*)-4-((*tert*-butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2,4-dimethylpent-2-enoate (19a)

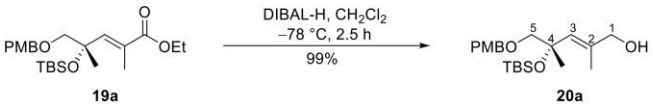


9 **19a**

Aldehyde **9** (990 mg, 2.92 mmol, 1.00 eq.) was dissolved in dry toluene (30 mL) and stabilized ylide [1-(ethoxycarbonyl)ethylene]triphenylphosphorane (4.79 g 13.2 mmol, 4.50 eq.) was added to the solution at ambient temperature. The reaction mixture was heated at reflux for 18 h. The volatiles were removed in vacuo and the remainder was purified by column chromatography (2% to 3% EtOAc in *n*-pentane). Product **19a** was obtained as a colorless oil (1.11 g, 2.63 mmol, 89%). In addition, 6% of the *Z*-isomer (79.0 mg, 0.187 mmol) was obtained, which was completely removed by column chromatography.

R$_f$ = 0.56 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: –7.7 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.26-7.23 (m, 2 H, H-Ar), 6.89-6.84 (m, 2 H, H-Ar), 6.80 (q, *J* = 1.4 Hz, 1 H, H-3), 4.49 (d, *J* = 11.7 Hz, 1 H, Ar-C*H*^{a}H^b), 4.46 (d, *J* = 11.7 Hz, 1 H, Ar-CH^a*H*^{b}), 4.18 (qd, *J* = 7.1, 1.1 Hz, 2 H, OC*H*$_2$CH$_3$), 3.81 (s, 3 H, OMe), 3.41 (d, *J* = 9.3 Hz, 1 H, H-5^a), 3.38 (d, *J* = 9.3 Hz, 1 H, H-5^b), 1.99 (d, *J* = 1.4 Hz, 3 H, Me^2), 1.43 (s, 3 H, Me^4), 1.30 (t, *J* = 7.1 Hz, 3 H, OCH$_2$C*H*$_3$), 0.87 (s, 9 H, TBS-*t*Bu), 0.08 (s, 3 H, TBS-Me), 0.07 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 169.0 (C-1), 159.2 (C-Ar$_q$), 145.4 (C-3), 130.4 (C-Ar$_q$), 129.39 (C-2), 129.35 (C-Ar), 113.8 (C-Ar), 77.3 (C-5), 75.6 (C-4), 73.2 (Ar-*C*H$_2$), 60.8 (O*C*H$_2$CH$_3$), 55.4 (OMe), 26.4 (Me^4), 26.1 (TBS-*t*Bu), 18.5 (TBS-*t*Bu$_q$), 14.4 (OCH$_2$*C*H$_3$), 13.9 (Me^2), –2.08 (TBS-Me), –2.13 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1710, 1514, 1246, 1033, 833, 774. ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{23}H_{38}O_5SiNa^+$ 445.2381; found 445.2377.

(*S*,*E*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2,4-dimethylpent-2-en-1-ol (20a)

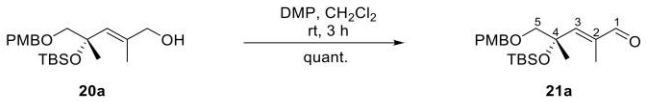


19a **20a**

A stirred solution of ester **19a** (295 mg, 0.698 mmol, 1.00 eq.) in dry CH_2Cl_2 (7 mL) was treated with DIBAL-H (1M in *n*-hexane, 1.74 mL, 1.74 mmol, 2.50 eq.) at –78 °C and stirred for 2.5 h. Acetone (250 µL) and sat. aqueous potassium sodium tartrate (20 mL) were added at –78 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for a further 2 h. The phases were separated and the aqueous phase was extracted with Et_2O (4 × 20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated to give alcohol **20a** as a colorless oil without further purification (263 mg, 0.691 mmol, 99%).

R_f = 0.27 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{25}$: –8.8 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.26-7.23 (m, 2 H, H-Ar), 6.90-6.84 (m, 2 H, H-Ar), 5.52 (q, *J* = 1.4 Hz, 1 H, H-3), 4.51 (d, *J* = 11.8 Hz, 1 H, Ar-C*H*^{a}H^b), 4.46 (d, *J* = 11.8 Hz, 1 H, Ar-CH^a*H*^{b}), 3.95 (d, *J* = 5.6 Hz, 2 H, H-1), 3.81 (s, 3 H, OMe), 3.38 (d, *J* = 9.2 Hz, 1 H, H-5^a), 3.35 (d, *J* = 9.2 Hz, 1 H, H-5^b), 1.80 (d, *J* = 1.1 Hz, 3 H, Me^2), 1.40 (s, 3 H, Me^4), 0.86 (s, 9 H, TBS-*t*Bu), 0.06 (s, 6 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.2 (C-Ar$_q$), 137.3 (C-2), 130.7 (C-Ar$_q$), 130.5 (C-3), 129.3 (C-Ar), 113.8 (C-Ar), 78.0 (C-5), 75.3 (C-4), 73.2 (Ar-*C*H$_2$), 69.7 (C-1), 55.4 (OMe), 27.2 (Me^4), 26.2 (TBS-*t*Bu), 18.5 (TBS-*t*Bu$_q$), 15.2 (Me^2), –1.96 (TBS-Me), –2.02 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3440 (br.), 1513, 1246, 832, 773. ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{21}H_{36}O_4SiNa^+$ 403.2275; found 403.2271.

(*S*,*E*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2,4-dimethylpent-2-enal (21a)

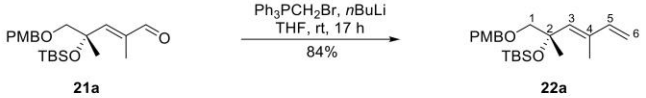


20a **21a**

To a stirred solution of alcohol **20a** (480 mg, 1.26 mmol, 1.00 eq.) in dry CH_2Cl_2 (13 mL) was added DMP (592 mg, 1.39 mmol, 1.10 eq.) at 0 °C. The reaction mixture was allowed to warm up to room temperature. After 3 h sat. aqueous $NaHCO_3$ (15 mL) and sat. aqueous $Na_2S_2O_3$ (15 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic phases were washed with sat. aqueous $Na_2S_2O_3$ (2 × 100 mL) and sat. aqueous $NaHCO_3$ (2 × 100 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure and aldehyde **21a** was obtained without further purification as a colorless oil (476 mg, 1.26 mmol, quantitative).

R_f = 0.69 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{25}$: –17.1 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 9.35 (s, 1 H, H-1), 7.24-7.20 (m, 2 H, H-Ar), 6.89-6.85 (m, 2 H, H-Ar), 6.46 (q, *J* = 1.2 Hz, 1 H, H-3), 4.47 (s, 2 H, Ar-C*H*$_2$), 3.81 (s, 3 H, OMe), 3.46 (d, *J* = 9.3 Hz, 1 H, H-5^a), 3.43 (d, *J* = 9.3 Hz, 1 H, H-5^b), 1.87 (d, *J* = 1.2 Hz, 3 H, Me^2), 1.48 (s, 3 H, Me^4), 0.87 (s, 9 H, TBS-*t*Bu), 0.09 (s, 3 H, TBS-Me), 0.08 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 196.4 (C-1), 159.4 (C-Ar$_q$), 158.5 (C-3), 139.3 (C-2), 130.1 (C-Ar$_q$), 129.4 (C-Ar), 113.9 (C-Ar), 77.1 (C-5), 76.0 (C-4), 73.3 (Ar-*C*H$_2$), 55.4 (OMe), 26.05 (Me^4), 26.04 (TBS-*t*Bu), 18.4 (TBS-*t*Bu$_q$), 10.4.20 (Me^2), –2.0 (TBS-Me), –2.1 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1689, 1513, 1247, 1031, 827, 774. ─ **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{21}H_{35}O_4Si^+$ 379.2299; found 379.2294.

(*S*,*E*)-2-((*tert*-Butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)oxy)-2,4-dimethylhexa-3,5-diene (22a)

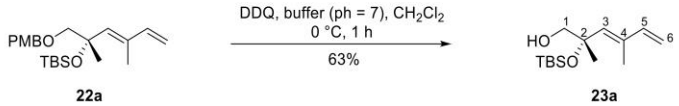


21a **22a**

Ph_3PCH_2Br (734 mg, 2.06 mmol, 2.00 eq.) in dry THF (5 mL) was treated with *n*BuLi (2.5M in *n*-hexane, 742 µL, 1.85 mmol, 1.80 eq.) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature. Aldehyde **21a** (389 mg, 1.03 mmol, 1.00 eq.) in dry THF (5 mL) was added and the reaction mixture was stirred for 17 h at room temperature. The reaction was quenched with H_2O (10 mL), the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (2% Et_2O in *n*-pentane) afforded product **22a** as a colorless oil (327 mg, 0.868 mmol, 84%).

R_f = 0.46 (5% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: –13.1 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.26-7.23 (m, 2 H, H-Ar), 6.90-6.84 (m, 2 H, H-Ar), 6.32 (dd, *J* = 17.4, 10.7 Hz, 1 H, H-5), 5.56 (s, 1 H, H-3), 5.15 (d, *J* = 17.4 Hz, 1 H, H-6^a), 4.97 (d, *J* = 10.7 Hz, 1 H, H-6^b), 4.51 (d, *J* = 11.7 Hz, 1 H, Ar-C*H*^{a}H^b), 4.45 (d, *J* = 11.7 Hz, 1 H, Ar-CH^a*H*^{b}), 3.81 (s, 3 H, OMe), 3.40 (d, *J* = 9.3 Hz, 1 H, H-1^a), 3.38 (d, *J* = 9.3 Hz, 1 H, H-1^b), 1.90 (d, *J* = 1.1 Hz, 3 H, Me^4), 1.43 (s, 3 H, Me^2), 0.86 (s, 9 H, TBS-*t*Bu), 0.06 (s, 3 H, TBS-Me), 0.05 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.2 (C-Ar$_q$), 142.6 (C-5), 137.6 (C-3), 136.2 (C-4), 130.8 (C-Ar$_q$), 129.3 (C-Ar), 113.8 (C-Ar), 111.7 (C-6), 78.0 (C-1), 75.4 (C-2), 73.1 (Ar-*C*H$_2$), 55.4 (OMe), 27.4 (Me^2), 26.2 (TBS-*t*Bu), 18.5 (TBS-*t*Bu$_q$), 13.3 (Me^4), –2.0 (TBS-Me), –2.2 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1513, 1246, 1035, 833, 773. ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{22}H_{36}O_3SiNa^+$ 399.2326; found 399.2319.

(*S*,*E*)-2-((*tert*-Butyldimethylsilyl)oxy)-2,4-dimethylhexa-3,5-dien-1-ol (23a)



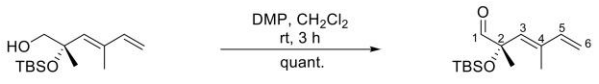
22a **23a**

To a stirred solution of compound **22a** (100 mg, 0.297 mmol, 1.00 eq.) in dry CH_2Cl_2 (3 mL) phosphate buffer (pH = 7, 300 µL) was added at room temperature and DDQ (81.0 mg, 0.356 mmol, 1.20 eq.) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and thereafter it was quenched with sat. aqueous $NaHCO_3$ (5 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were washed with sat. aqueous $NaHCO_3$ (15 mL) and dried over Na_2SO_4. After removing the volatiles in vacuo and purification of the residue by column chromatography (6% Et_2O in *n*-pentane) alcohol **23a** was obtained as a colorless oil (48.0 mg, 0.187 mmol, 63%). In addition, 5% of educt **22a** (5.0 mg, 14.9 µmol) was isolated.

R_f = 0.35 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: +49.9 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.32 (ddd, *J* = 17.4, 10.7, 0.6 Hz, 1 H, H-5), 5.53 (s, 1 H, H-3), 5.19 (d, *J* = 17.4 Hz, 1 H, H-6^a), 5.01 (d, *J* = 10.7 Hz, 1 H, H-6^b), 3.60 (dd, *J* = 10.8, 4.8 Hz, 1 H, H-1^a), 3.38 (dd, *J* = 10.8, 8.2 Hz, 1 H, H-1^b), 2.00 (dd, *J* = 8.3, 4.8 Hz, 1 H, OH), 1.91 (d, *J* = 1.2 Hz, 3 H, Me^4), 1.45 (s, 3 H, Me^2), 0.89 (s, 9 H, TBS-*t*Bu), 0.11 (s, 3 H, TBS-Me), 0.08 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 142.2 (C-5),

137.0 (C-4), 136.6 (C-3), 112.5 (C-6), 76.5 (C-2), 70.8 (C-1), 26.6 (Me^2), 26.2 (TBS-*t*Bu), 18.4 (TBS-*t*Bu_q), 13.5 (Me^4), –1.9 (TBS-Me), –2.5 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3440 (br.), 1003, 834, 773. No HRMS of **23a** could be determined because of decomposition of compound **23a** probably into a conjugated triene during the measurement by elimination of TBSOH. We assume this based on the following determination: HRMS (ESI) *m/z* $[M–TBSOH+H]^+$: calculated for $C_8H_{13}O^+$ 125.0961; found 125.0961.

(*S*,*E*)-2-((*tert*-Butyldimethylsilyl)oxy)-2,4-dimethylhexa-3,5-dienal (8a)

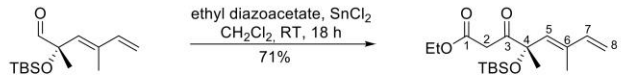


23a **8a**

To a stirred solution of alcohol **23a** (163 mg, 0.636 mmol, 1.00 eq.) in dry CH_2Cl_2 (6 mL) was added DMP (297 mg, 0.699 mmol, 1.10 eq.) at 0 °C. The reaction mixture was allowed to warm up to room temperature. After 3 h sat. aqueous $NaHCO_3$ (8 mL) and sat. aqueous $Na_2S_2O_3$ (8 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 8 mL). The combined organic phases were washed with sat. aqueous $Na_2S_2O_3$ (2 × 50 mL) and sat. aqueous $NaHCO_3$ (2 × 50 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure and aldehyde **8a** was obtained without further purification as a colorless oil (162 mg, 0.636 mmol, quantitative).

$\mathbf{R}_f$ = 0.87 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{27}$: –25.3 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 9.58 (s, 1 H, H-1), 6.34 (dd, *J* = 17.3, 10.6 Hz, 1 H, H-5), 5.49 (s, 1 H, H-3), 5.24 (d, *J* = 17.4 Hz, 1 H, H-6^a), 5.08 (d, *J* = 10.7 Hz, 1 H, H-6^b), 1.80 (d, *J* = 0.8 Hz, 3 H, Me^4), 1.45 (s, 3 H, Me^2), 0.91 (s, 9 H, TBS-*t*Bu), 0.11 (s, 3 H, TBS-Me), 0.06 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 200.9 (C-1), 140.9 (C-5), 139.7 (C-4), 133.2 (C-3), 114.1 (C-6), 79.6 (C-2), 25.9 (TBS-*t*Bu), 25.2 (Me^2), 18.4 (TBS-*t*Bu_q), 14.1 (Me^4), –2.2 (TBS-Me), –2.5 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1738, 1115, 1004, 834, 775. ─ **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{14}H_{27}O_2Si^+$ 255.1775; found 255.1773.

Ethyl (*S*,*E*)-4-((*tert*-butyldimethylsilyl)oxy)-4,6-dimethyl-3-oxoocta-5,7-dienoate (24a)



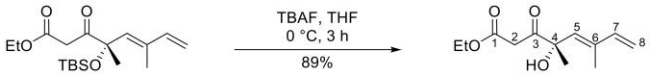
8a **24a**

Aldehyde **8a** (119 mg, 0.468 mmol, 1.00 eq.) was dissolved in dry CH_2Cl_2 (5 mL) and ethyl diazoacetate (solution with 13 wt% CH_2Cl_2, 167 µL, 1.40 mmol, 3.00 eq.) and $SnCl_2$ (87.0 mg, 0.468 mmol, 1.00 eq.) were added at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for 18 h at room temperature. The reaction was quenched with H_2O (5 mL) and diluted with Et_2O (10 mL). The phases were separated and the aqueous phase was extracted five times with CH_2Cl_2 (5 × 5 mL) because of poor phase separation. The combined organic phases were dried over Na_2SO_4 and

the solvent was evaporated. The residue was purified by column chromatography (2% to 4% Et_2O in *n*-pentane) to yield β-ketoester **24a** as a colorless oil (113 mg, 0.332 mmol, 71%).

R_f = 0.25 (4% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: +20.5 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.31 (dd, *J* = 17.2, 10.7 Hz, 1 H, H-7), 5.56 (s, 1 H, H-5), 5.22 (d, *J* = 17.2 Hz, 1 H, H-8^a), 5.07 (d, *J* = 10.7 Hz, 1 H, H-8^b), 4.18 (q, *J* = 7.1 Hz, 2 H, OCH_2CH$_3$), 3.75 (d, *J* = 16.5 Hz, 1 H, H-2^a), 3.68 (d, *J* = 16.5 Hz, 1 H, H-2^b), 1.69 (d, *J* = 0.9 Hz, 3 H, Me^6), 1.54 (s, 3 H, Me^4), 1.26 (t, *J* = 7.1 Hz, 3 H, OCH$_2$CH_3), 0.91 (s, 9 H, TBS-*t*Bu), 0.13 (s, 3 H, TBS-Me), 0.07 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 205.0 (C-3), 168.1 (C-1), 140.9 (C-7), 138.6 (C-6), 135.3 (C-5), 114.0 (C-8), 81.2 (C-4), 61.3 (OCH$_2$CH$_3$), 44.8 (C-2), 27.0 (Me^4), 26.0 (TBS-*t*Bu), 18.3 (TBS-*t*Bu$_q$), 14.3 (OCH$_2$CH$_3$),13.5 (Me^6), –2.3 (TBS-Me), –2.5 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1749, 1723, 1254, 828, 776. ─ **HRMS** (ESI) *m/z* [M+Na]$^+$: calculated for $C_{18}H_{32}O_4SiNa^+$ 363.1962; found 363.1955.

Ethyl (*S*,*E*)-4-hydroxy-4,6-dimethyl-3-oxoocta-5,7-dienoate (7a)

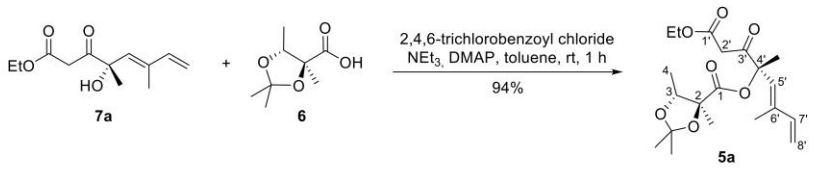


8a **7a**

Silyl ether **8a** (83.0 mg, 0.244 mmol, 1.00 eq.) was dissolved in dry THF (2.5 mL) and TBAF (1M in THF, 487 µL, 0.487 mmol, 2.00 eq.) was added to the solution at 0 °C. After 3 h at 0 °C sat. aqueous NH_4Cl (3 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (10% EtOAc in *n*-pentane) to yield γ-hydroxy-β-ketoester **7a** as a colorless oil (49.0 mg, 0.217 mmol, 89%).

R_f = 0.25 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: –9.4 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.33 (ddd, *J* = 17.3, 10.8, 0.5 Hz, 1 H, H-7), 5.58 (s, 1 H, H-5), 5.27 (d, *J* = 17.2 Hz, 1 H, H-8^a), 5.10 (d, *J* = 10.7 Hz, 1 H, H-8^b), 4.17 (qd, *J* = 7.1, 1.4 Hz, 2 H, OCH_2CH$_3$), 3.75 (s, 1 H, OH), 3.58 (s, 2 H, H-2), 1.76 (d, *J* = 1.1 Hz, 3 H, Me^6), 1.52 (s, 3 H, Me^4), 1.26 (t, *J* = 7.2 Hz, 3 H, OCH$_2$CH_3). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 204.9 (C-3), 167.0 (C-1), 141.1 (C-6), 140.7 (C7), 131.0 (C-5), 114.6 (C-8), 78.5 (C-4), 61.8 (OCH$_2$CH$_3$), 44.1 (C-2), 27.5 (Me^4), 14.2 (OCH$_2$CH$_3$), 12.5 (Me^6). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3471 (br.), 1743, 1711, 1312, 1260, 1022. ─ **HRMS** (ESI) *m/z* [M+H]$^+$: calculated for $C_{12}H_{19}O_4^+$ 227.1278; found 227.1274.

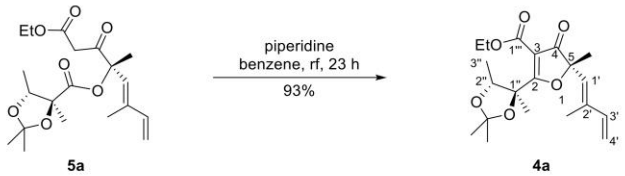
(*S*,*E*)-1-Ethoxy-4,6-dimethyl-1,3-dioxoocta-5,7-dien-4-yl (4*S*,5*R*)-2,2,4,5-tetramethyl-1,3-dioxolane-4-carboxylate (5a)



To a stirred solution of carboxylic acid **6** (40.0 mg, 0.227 mmol, 1.00 eq.) and alcohol **7a** (59.0 mg, 0.261 mmol, 1.15 eq.) in dry toluene (5 mL) were added NEt_3 (94.0 µL, 0.681 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (56.0 µL, 0.359 mmol, 1.58 eq.) and DMAP (42.0 mg, 0.341 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 1 h before being quenched with sat. aqueous $NaHCO_3$ (5 mL). After phase separation the aqueous phase was extracted with Et_2O (3 × 5 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10% EtOAc in *n*-pentane) afforded ester **5a** as a colorless oil (82.0 mg, 0.214 mmol, 94%).

R$_f$ = 0.46 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{24}$: –46.3 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.34 (dd, *J* = 17.2, 10.7 Hz, 1 H, H-7'), 5.79 (s, 1 H, H-5'), 5.26 (d, *J* = 17.4 Hz, 1 H, H-8'^{a}), 5.11 (d, *J* = 10.7 Hz, 1 H, H-8'^{b}), 4.41 (q, *J* = 6.3 Hz, 1 H, H-3), 4.16 (q, *J* = 7.2 Hz, 2 H, OC*H*$_2$CH$_3$), 3.53-3.42 (m, 2 H, H-2'), 1.83 (d, *J* = 0.9 Hz, 3 H, Me$^{6'}$), 1.74 (s, 3 H, Me$^{4'}$), 1.47 (s, 3 H, acetonide-Me), 1.40 (s, 3 H, acetonide-Me), 1.35 (s, 3 H, Me^{2}), 1.32 (d, *J* = 6.3 Hz, 3 H, H-4), 1.24 (t, *J* = 7.2 Hz, 3 H, OCH$_2$C*H*$_3$). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 197.3 (C-3'), 172.1 (C-1), 166.8 (C-1'), 140.9 (C-7'), 139.4 (C-6'), 128.2 (C-5'), 114.6 (C-8'), 109.1 (acetonide-C$_q$), 86.1 (C-4'), 82.7 (C-2), 75.8 (C-3), 61.6 (O*C*H$_2$CH$_3$), 43.6 (C-2'), 28.3 (acetonide-Me), 25.6 (acetonide-Me), 22.2 (Me$^{4'}$), 19.7 (Me^{2}), 14.8 (C-4), 14.2 (OCH$_2$*C*H$_3$), 12.9 (Me$^{6'}$). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1750, 1727, 1257, 1109, 102. ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{20}H_{30}O_7Na^+$ 405.1884; found 405.1871.

Ethyl (*S*)-5-methyl-5-((*E*)-2-methylbuta-1,3-dien-1-yl)-4-oxo-2-((4*S*,5*R*)-2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-4,5-dihydrofuran-3-carboxylate (4a)

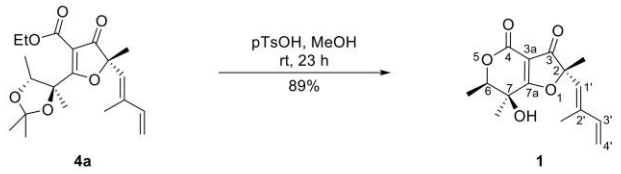


Compound **5a** (105 mg, 0.275 mmol, 1.00 eq.) was dissolved in dry benzene (2.8 mL) and piperidine (109 µL, 1.10 mmol, 4.00 eq.) was added to the solution at room temperature. The reaction mixture was heated at reflux for 23 h before it was allowed to cool to room temperature. The volatiles were removed

in vacuo and the remainder was purified by column chromatography (5% EtOAc in *n*-pentane). Product **4a** was obtained as a colorless oil that turned solid at –20 °C (93.0 mg, 0.255 mmol, 93%).

R$_f$ = 0.49 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{24}$: –199.7 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.33 (dd, *J* = 17.3, 10.8 Hz, 1 H, H-3'), 5.53 (s, 1 H, H-1'), 5.28 (d, *J* = 17.4 Hz, 1 H, H-4$^{'a}$), 5.13 (d, *J* = 10.7 Hz, 1 H, H-4$^{'b}$), 4.36 (q, *J* = 6.3 Hz, 1 H, H-2''), 4.33-4.19 (m, 2 H, O*C*H_2CH$_3$), 1.92 (d, *J* = 1.1 Hz, 3 H, Me$^{2'}$), 1.58 (s, 3 H, Me^5), 1.49 (s, 3 H, Me$^{1''}$), 1.46 (s, 3 H, acetonide-Me), 1.39 (d, *J* = 6.3 Hz, 3 H, C-3''), 1.33 (t, *J* = 7.1 Hz, 3 H, OCH$_2$C*H*$_3$), 1.29 (s, 3 H, acetonide-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 200.1 (C-4), 191.0 (C-2), 162.8 (C-1'''), 140.5 (C-3'), 140.3 (C-2'), 127.1 (C-1'), 114.9 (C-4'), 109.1 (acetonide-C$_q$), 109.0 (C-3), 90.7 (C-5), 81.9 (C-1''), 76.4 (C-2''), 61.4 (O*C*H$_2$CH$_3$), 28.6 (acetonide-Me), 25.6 (acetonide-Me), 24.4 (Me^5), 20.5 (Me$^{1''}$), 15.5 (C-3''), 14.3 (OCH$_2$*C*H$_3$), 13.6 (Me$^{2'}$). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1735, 1703, 1375, 1270, 1104. ─ **mp** 48-49 °C ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{20}H_{28}O_6Na^+$ 387.1778; found 387.1766.

Ophiofuranone A (1)



4a **1**

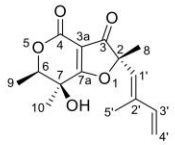
To a stirred solution of compound **4a** (139 mg, 0.381 mmol, 1.00 eq.) in MeOH (4 mL) was added *para*-toluenesulfonic acid monohydrate (218 mg, 1.14 mmol, 3.00 eq.). The reaction mixture was stirred at ambient temperature for 23 h. The reaction was quenched with sat. aqueous $NaHCO_3$ (5 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (10% to 30% EtOAc in *n*-pentane) to yield ophiofuranone A (**1**) as a colorless solid (94.0 mg, 0.338 mmol, 89%).

R$_f$ = 0.13 (30% EtOAc in *n*-hexane). ─ $[\alpha]_D^{24}$: –272.4 (*c* 0.10 in MeOH) [Lit.[10] $[\alpha]_D^{20}$: –38.7 (*c* 0.1 in MeOH)]. ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.32 (dd, *J* = 17.4, 10.7 Hz, 1 H, H-3'), 5.52 (s, 1 H, H-1'), 5.31 (d, *J* = 17.2 Hz, 1 H, H-4$^{'a}$), 5.15 (d, *J* = 10.7 Hz, 1 H, H-4$^{'b}$), 4.43 (q, *J* = 6.5 Hz, 1 H, H-6), 3.79-3.65 (m, 1 H, OH), 1.91 (d, *J* = 1.1 Hz, 3 H, Me$^{2'}$), 1.71 (s, 3 H, Me^2), 1.51 (d, *J* = 6.4 Hz, 3 H, Me^6), 1.49 (s, 3 H, Me^7). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 196.6 (C-3), 194.5 (C-7a), 160.5 (C-4), 141.0 (C-2'), 140.3 (C-3'), 126.0 (C-1'), 115.4 (C-4'), 102.7 (C-3a), 95.2 (C-2), 81.3 (C-6), 68.1 (C-7), 23.8 (Me^2), 18.5 (Me^7), 13.4 (Me$^{2'}$), 13.0 (Me^6). **^{1}H-NMR** (DMSO-d_6, 500 MHz): 6.41 (s, 1 H, OH), 6.41 (dd, *J* = 17.3, 10.9 Hz, 1 H, H-3'), 5.57 (s, 1 H, H-1'), 5.35 (d, *J* = 17.3 Hz, 1 H, H-4$^{'a}$), 5.17 (d, *J* = 10.8 Hz, 1 H, H-4$^{'b}$), 4.61 (q, *J* = 6.5 Hz, 1 H, H-6), 1.82 (d, *J* = 0.9 Hz, 3 H, Me$^{2'}$), 1.56 (s, 3 H, Me^2), 1.35 (s, 3 H, Me^7), 1.32 (d, *J* = 6.6 Hz, 3 H, Me^6). ─ **^{13}C{^{1}H}-NMR** (DMSO-d_6, 125 MHz): 196.6 (C-3), 195.3 (C-7a), 159.1 (C-4), 140.24 (C-3'), 140.21 (C-2'), 126.5 (C-1'), 115.6 (C-4'), 102.2 (C-3a), 93.3

(C-2), 80.1 (C-6), 66.9 (C-7), 23.5 (Me^{2}), 18.4 (Me^{7}), 12.9 ($Me^{2'}$), 12.8 (Me^{6}). **— IR** (cm^{-1}) $\tilde{\nu}$ = 3419, 1756, 1683, 1328, 902, 724. **— mp** 142-143 °C **— HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{15}H_{18}O_5Na^+$ 301.1046; found 301.1038.

The preceding data is consistent with those reported in literature.[10] The NMR data of the synthetic ophiofuranone A (**1**) agreed very well with those of the literature (Table S1). Though our synthetic product showed an optical rotation of $[\alpha]_D^{24}$ –272.4 (*c* 0.10 in MeOH) in contrast to $[\alpha]_D^{20}$ –38.7 (*c* 0.1 in MeOH) reported for the natural isolate.

Table S1. Comparison of NMR spectra of isolated and synthetic ophiofuranone A (**1**) in DMSO-*d*6.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C{^{1}H} δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			93.2	93.3
3			196.5	196.6
3a			102.1	102.2
4			159.0	159.1
6	4.61 q (6.4)	4.61 q (6.5)	80.0	80.1
7			66.8	66.9
7a			195.2	195.3
8	1.56 s	1.56 s	23.4	23.5
9	1.32 d (6.4)	1.32 d (6.6)	12.8	12.8
10	1.36 s	1.35 s	18.4	18.4
1'	5.58 s	5.57 s	126.4	126.5
2'			140.1	140.21
3'	6.41 dd (17.2, 10.8)	6.41 dd (17.3, 10.9)	140.2	140.24
4'[a]	5.35 d (17.2)	5.35 d (17.3)	115.5	115.6
4'[b]	5.16 d (10.8)	5.17 d (10.8)		
5'	1.82 s	1.82 d (0.9)	12.9	12.9
OH	6.40 s	6.41 s		

[a] ^{1}H and ^{13}C{^{1}H} NMR data recorded at 400 MHz and 100 MHz. [b] ^{1}H and ^{13}C{^{1}H} NMR data recorded at 500 MHz and 125 MHz.

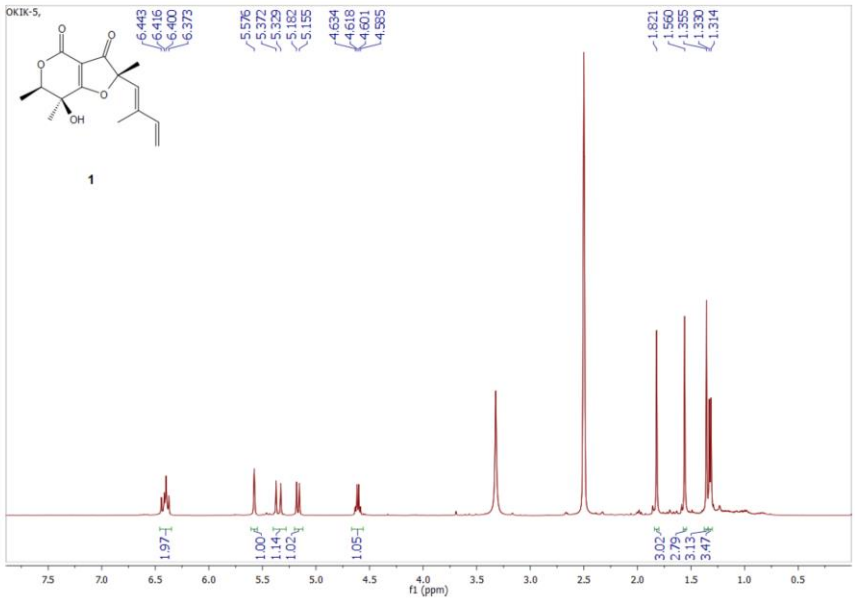


Figure S1i: ^{1}H-NMR spectrum of isolated ophiofuranone A (**1**). [10]

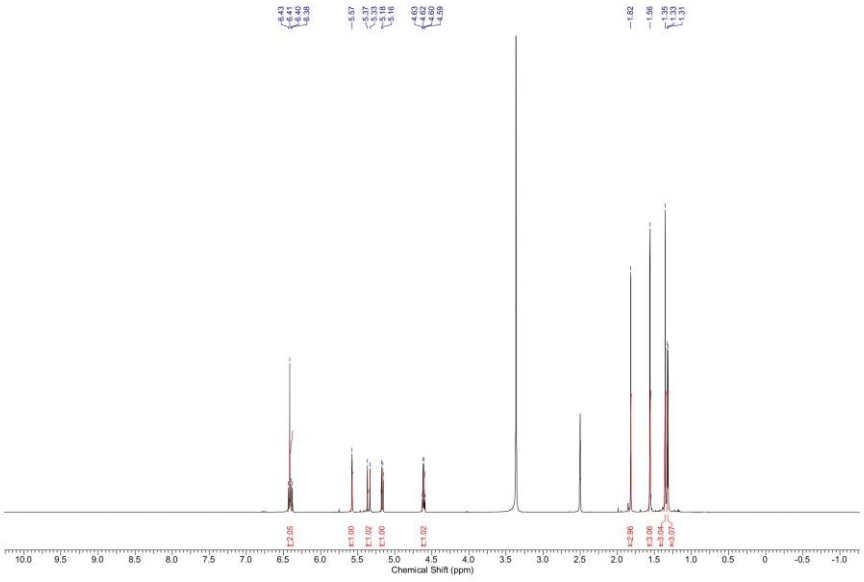


Figure S2s: ^{1}H-NMR spectrum of synthetic ophiofuranone A (**1**) in DMSO-*d*6 (500 MHz).

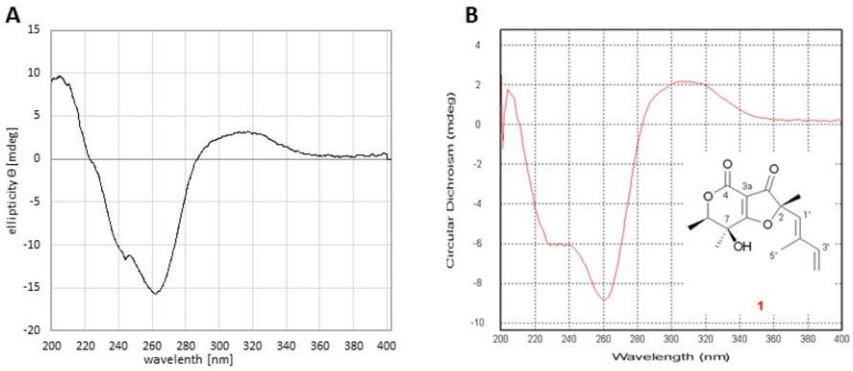
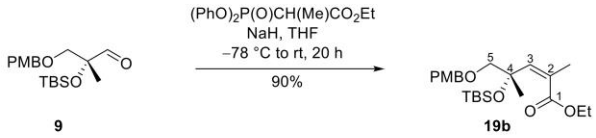


Figure S3. A: CD spectrum of synthetic ophiofuranone A (**1**) (0.05 mg/mL in MeOH); **B**: CD spectrum of isolated ophiofuranone A (**1**).[10]

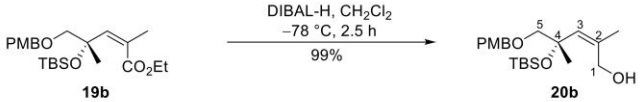
Ethyl (*S*,*Z*)-4-((*tert*-butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2,4-dimethylpent-2-enoate (19b)



NaH (60 wt% in mineral oil, 238 mg, 5.95 mmol, 2.90 eq.) in dry THF (3 mL) was treated with phosphonate $(PhO)_2P(O)CH(Me)CO_2Et$ (2.06 g, 6.16 mmol, 3.00 eq.) in dry THF (9 mL) at 0 °C. The solution was stirred for 20 min at 0°C and was then cooled to –78 °C. Aldehyde **9** (692 mg, 2.04 mmol, 1.00 eq.) in dry THF (9 mL) was added slowly and the reaction mixture was stirred at –78 °C for 1 h. The reaction mixture was allowed to warm up to ambient temperature and was stirred at this temperature for another 20 h. The reaction was quenched with H_2O (30 mL), the phases were separated and the aqueous phase was extracted with EtOAc (3 × 10mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (3% EtOAc in *n*-pentane) afforded product **19b** as a colorless oil (778 mg, 1.84 mmol, 90%). In addition, 2% of the *E*-isomer (18.0 mg, 42.6 µmol) was isolated, which was completely removed by column chromatography.

R_f = 0.52 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{24}$: +6.4 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.27-7.23 (m, 2 H, H-Ar), 6.89-6.84 (m, 2 H, H-Ar), 5.64 (q, *J* = 1.5 Hz, 1 H, H-3), 4.49 (d, *J* = 12.2 Hz, 1 H, Ar-C*H*^{a}H^b), 4.46 (d, *J* = 12.2 Hz, 1 H, Ar-CH^a*H*^{b}), 4.17-4.09 (m, 2 H, OC*H*$_2$CH$_3$), 3.80 (s, 3 H, OMe), 3.48 (d, *J* = 9.5 Hz, 1 H, H-5^a), 3.43 (d, *J* = 9.5 Hz, 1 H, H-5^b), 1.91 (d, *J* = 1.5 Hz, 3 H, Me^2), 1.39 (s, 3 H, Me^4), 1.25 (t, *J* = 7.1 Hz, 3 H, OCH$_2$C*H*$_3$), 0.85 (s, 9 H, TBS-*t*Bu), 0.07 (s, 3 H, TBS-Me), 0.06 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 169.9 (C-1), 159.1 (C-Ar$_q$), 139.0 (C-3), 130.9 (C-Ar$_q$), 129.2 (C-Ar), 128.2 (C-2), 113.7 (C-Ar), 77.7 (C-5), 75.9 (C-4), 73.2 (Ar-*C*H$_2$), 60.6 (O*C*H$_2$CH$_3$), 55.4 (OMe), 26.0 (TBS-*t*Bu), 25.0 (Me^4), 22.6 (Me^2), 18.4 (TBS-*t*Bu$_q$), 14.1 (OCH$_2$*C*H$_3$), –2.20 (TBS-Me), –2.23 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1727, 1246, 1035, 833, 773. ─ **HRMS** (ESI) *m/z* [M+Na]$^+$: calculated for $C_{23}H_{38}O_5SiNa^+$ 445.2381; found 445.2372.

(*S*,*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2,4-dimethylpent-2-en-1-ol (20b)

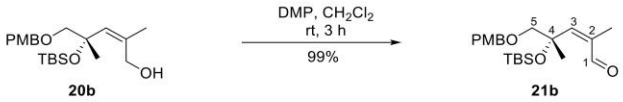


A stirred solution of ester **19b** (1.98 g, 4.69 mmol, 1.00 eq.) in dry CH_2Cl_2 (47 mL) was treated with DIBAL-H (1M in *n*-hexane, 11.7 mL, 11.7 mmol, 2.50 eq.) at –78 °C and stirred for 2.5 h. Acetone (500 µL) and sat. aqueous potassium sodium tartrate (40 mL) were added at –78 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for a further 2 h. The phases were

separated and the aqueous phase was extracted with Et_2O (5 × 50 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated to give alcohol **20b** as a colorless oil without further purification (1.77 g, 4.64 mmol, 99%).

R_f = 0.38 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: –0.8 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.27-7.22 (m, 2 H, H-Ar), 6.90-6.85 (m, 2 H, H-Ar), 5.28 (s, 1 H, H-3), 4.47 (s, 2 H, Ar-CH_2), 4.29 (dd, *J* = 12.4, 5.3 Hz, 1 H, H-1^a), 4.04 (dd, *J* = 12.2, 6.9 Hz, 1 H, H-1^b), 3.80 (s, 3 H, OMe), 3.37 (d, *J* = 9.3 Hz, 1 H, H-5^a), 3.35 (d, *J* = 9.3 Hz, 1 H, H-5^b), 2.46 (br. s, 1 H, OH), 1.79 (d, *J* = 1.2 Hz, 3 H, Me^2), 1.39 (s, 3 H, Me^4), 0.88 (s, 9 H, TBS-*t*Bu), 0.092 (s, 3 H, TBS-Me), 0.086 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.4 (C-Ar$_q$), 137.9 (C-2), 132.3 (C-3), 130.1 (C-Ar$_q$), 129.6 (C-Ar), 113.9 (C-Ar), 78.0 (C-5), 75.9 (C-4), 73.3 (Ar-*C*H$_2$), 62.2 (C-1), 55.4 (OMe), 27.9 (Me^4), 26.1 (TBS-*t*Bu), 23.6, (Me^2), 18.4 (TBS-*t*Bu$_q$), –1.9 (TBS-Me), –2.0 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3403 (br.), 1514, 1246, 1033, 1003, 833, 773. ─ **HRMS** (ESI) *m/z* [M+Na]$^+$: calculated for $C_{21}H_{36}O_4SiNa^+$ 403.2275; found 403.2271.

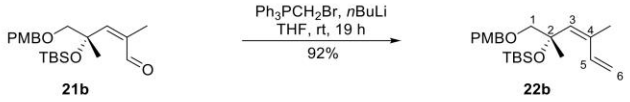
(*S*,*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2,4-dimethylpent-2-enal (21b)



To a stirred solution of alcohol **20b** (1.76 g, 4.62 mmol, 1.00 eq.) in dry CH_2Cl_2 (46 mL) was added DMP (2.16 g, 5.08 mmol, 1.10 eq.) at 0 °C. The reaction mixture was allowed to warm up to room temperature. After 3 h sat. aqueous $NaHCO_3$ (100 mL) and sat. aqueous $Na_2S_2O_3$ (100 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 30 mL). The combined organic phases were washed with sat. aqueous $Na_2S_2O_3$ (2 × 1 L) and sat. aqueous $NaHCO_3$ (2 × 1 L) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure and aldehyde **21b** was obtained without further purification as a colorless oil (1.73 g, 4.57 mmol, 99%).

R_f = 0.77 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{25}$: –52.0 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 10.6 (s, 1 H, H-1), 7.25-7.19 (m, 2 H, H-Ar), 6.90-6.85 (m, 2 H, H-Ar), 6.46 (q, *J* = 1.2 Hz, 1 H, H-3), 4.48 (s, 2 H, Ar-CH_2), 3.81 (s, 3 H, OMe), 3.44 (d, *J* = 9.2 Hz, 1 H, H-5^a), 3.39 (d, *J* = 9.2 Hz, 1 H, H-5^b), 1.77 (d, *J* = 1.2 Hz, 3 H, Me^2), 1.49 (s, 3 H, Me^4), 0.85 (s, 9 H, TBS-*t*Bu), 0.09 (s, 3 H, TBS-Me), 0.08 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 194.9 (C-1), 159.4 (C-Ar$_q$), 150.7 (C-3), 137.0 (C-2), 130.0 (C-Ar$_q$), 129.4 (C-Ar), 113.9 (C-Ar), 78.1 (C-5), 76.5 (C-4), 73.2 (Ar-*C*H$_2$), 55.4 (OMe), 28.5 (Me^4), 26.1 (TBS-*t*Bu), 18.4 (TBS-*t*Bu$_q$), 17.2 (Me^2), –2.18 (TBS-Me), –2.21 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1676, 1514, 1247, 1032, 834, 774. ─ **HRMS** (ESI) *m/z* [M+Na]$^+$: calculated for $C_{21}H_{34}O_4SiNa^+$ 401.2119; found 401.2119.

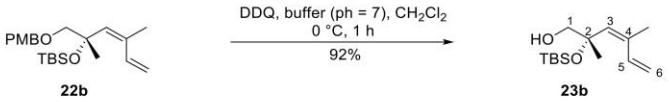
(*S*,*Z*)-2-((*tert*-Butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)oxy)-2,4-dimethylhexa-3,5-diene (22b)



Ph_3PCH_2Br (3.30 g, 9.24 mmol, 2.03 eq.) in dry THF (23 mL) was treated with *n*BuLi (2.5M in *n*-hexane, 3.33 mL, 8.32 mmol, 1.82 eq.) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature. Aldehyde **21b** (1.73 g, 4.56 mmol, 1.00 eq.) in dry THF (23 mL) was added and the mixture was stirred for 19 h at room temperature. The reaction was quenched with H_2O (50 mL), the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (0.5% to 1% Et_2O in *n*-pentane) afforded product **22b** as a colorless oil (1.58 g, 4.20 mmol, 92%).

R_f = 0.73 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: –10.5 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.28 (dd, *J* = 17.6, 10.9 Hz, 1 H, H-5), 7.27-7.22 (m, 2 H, H-Ar), 6.89-6.85 (m, 2 H, H-Ar), 5.46 (s, 1 H, H-3), 5.16 (dd, *J* = 17.6, 1.0 Hz, 1 H, H-6^a), 5.05 (ddd, *J* = 11.0, 1.4, 1.4 Hz, 1 H, H-6^b), 4.52 (d, *J* = 11.8 Hz, 1 H, Ar-C*H*^{a}H^b), 4.46 (d, *J* = 11.8 Hz, 1 H, Ar-CH^a*H*^{b}), 3.81 (s, 3 H, OMe), 3.40 (d, *J* = 9.5 Hz, 1 H, H-1^a), 3.38 (d, *J* = 9.5 Hz, 1 H, H-1^b), 1.82 (d, *J* = 1.1 Hz, 3 H, Me^4), 1.42 (s, 3 H, Me^2), 0.87 (s, 9 H, TBS-*t*Bu), 0.06 (s, 3 H, TBS-Me), 0.05 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 159.2 (C-Ar$_q$), 135.9 (C-5), 134.9 (C-3), 134.6 (C-4), 130.8 (C-Ar$_q$), 129.3 (C-Ar), 113.81 (C-6), 113.79 (C-Ar), 78.6 (C-1), 75.6 (C-2), 73.2 (Ar-*C*H$_2$), 55.4 (OMe), 28.2 (Me^2), 26.2 (TBS-*t*Bu), 20.6 (Me^4), 18.6 (TBS-*t*Bu$_q$),–2.0 (TBS-Me), –2.1 (TBS-Me). ─ **^{1}H-NMR** (MeOD, 500 MHz): 7.32 (ddd, *J* = 17.5, 11.0, 0.4 Hz, 1 H, H-5), 7.26-7.21 (m, 2 H, H-Ar), 6.90-6.86 (m, 2 H, H-Ar), 5.47 (s, 1 H, H-3), 5.18 (dd, *J* = 17.6, 1.0 Hz, 1 H, H-6^a), 5.03 (ddd, *J* = 11.0, 1.5, 1.5 Hz, 1 H, H-6^b), 4.48 (d, *J* = 11.6 Hz, 1 H, Ar-C*H*^{a}H^b), 4.43 (d, *J* = 11.6 Hz, 1 H, Ar-CH^a*H*^{b}), 3.78 (s, 3 H, OMe), 3.40 (d, *J* = 9.3 Hz, 1 H, H-1^a), 3.37 (d, *J* = 9.3 Hz, 1 H, H-1^b), 1.81 (d, *J* = 1.2 Hz, 3 H, Me^4), 1.40 (s, 3 H, Me^2), 0.87 (s, 9 H, TBS-*t*Bu), 0.06 (s, 3 H, TBS-Me), 0.05 (s, 3 H, TBS-Me). ─ **^{13}C-NMR** (MeOD, 125 MHz): 160.8 (C-Ar$_q$), 137.1 (C-5), 135.8 (C-3), 135.7 (C-4), 131.7 (C-Ar$_q$), 130.5 (C-Ar), 114.7 (C-6), 114.1 (C-Ar), 79.4 (C-1), 76.8 (C-2), 74.1 (Ar-*C*H$_2$), 55.7 (OMe), 28.5 (Me^2), 26.7 (TBS-*t*Bu), 20.6 (Me^4), 19.4 (TBS-*t*Bu$_q$),–1.87 (TBS-Me), –1.93 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1513, 1246, 1035, 831, 773. ─ **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{22}H_{36}O_3SiNa^+$ 399.2326; found 399.2322.

(*S*,*Z*)-2-((*tert*-Butyldimethylsilyl)oxy)-2,4-dimethylhexa-3,5-dien-1-ol (23b)

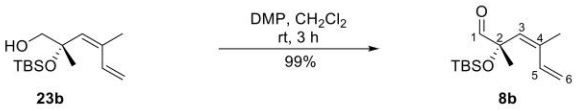


To a stirred solution of compound **22b** (1.56 g, 4.14 mmol, 1.00 eq.) in dry CH_2Cl_2 (40 mL) was added phosphate buffer (pH = 7, 4.1 mL) at room temperature and DDQ (1.13 g, 4.97 mmol, 1.20 eq.) at 0 °C.

The reaction mixture was stirred at 0 °C for 1 h and thereafter it was quenched with sat. aqueous $NaHCO_3$ (50 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with sat. aqueous $NaHCO_3$ (150 mL) and dried over Na_2SO_4. After removing the volatiles in vacuo and purification of the residue by column chromatography (5% Et_2O in *n*-pentane) alcohol **23b** was obtained as a colorless oil (972 mg, 3.79 mmol, 92%).

R_f = 0.49 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{22}$: +78.1 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.14 (dd, *J* = 17.5, 11.0 Hz, 1 H, H-5), 5.41 (s, 1 H, H-3), 5.21 (dd, *J* = 17.5, 0.8 Hz, 1 H, H-6^a), 5.11 (ddd, *J* = 10.9, 1.4, 1.4 Hz, 1 H, H-6^b), 3.61 (dd, *J* = 10.8, 4.4 Hz, 1 H, H-1^a), 3.36 (dd, *J* = 10.8, 8.7 Hz, 1 H, H-1^b), 2.00 (dd, *J* = 8.7, 4.4 Hz, 1 H, OH), 1.84 (d, *J* = 0.8 Hz, 3 H, Me^4), 1.43 (s, 3 H, Me^2), 0.89 (s, 9 H, TBS-*t*Bu), 0.11 (s, 3 H, TBS-Me), 0.07 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 135.8 (C-4), 135.2 (C-5), 133.8 (C-3), 115.0 (C-6), 76.5 (C-2), 71.5 (C-1), 27.4 (Me^2), 26.2 (TBS-*t*Bu), 20.7 (Me^4), 18.5 (TBS-*t*Bu$_q$),–1.8 (TBS-Me), –2.4 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 3414 (br.), 1003, 834, 773. No HRMS of **23b** could be determined because of decomposition of compound **23b** during the measurement probably into a conjugated triene by elimination of TBSOH. We assume this based on the following determination: **HRMS** (ESI) *m/z* $[M–TBSOH+H]^+$: calculated for $C_8H_{13}O^+$ 125.0961; found 125.0963.

(*S,Z*)-2-((*tert*-Butyldimethylsilyl)oxy)-2,4-dimethylhexa-3,5-dienal (8b)

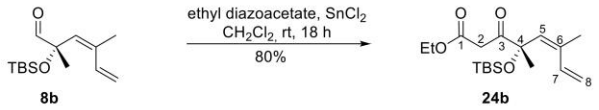


23b **8b**

To a stirred solution of alcohol **23b** (750 mg, 2.92 mmol, 1.00 eq.) in dry CH_2Cl_2 (29 mL) was added DMP (1.49 g, 3.51 mmol, 1.20 eq.) at 0 °C. The reaction mixture was allowed to warm up to room temperature. After 3 h sat. aqueous $NaHCO_3$ (50 mL) and sat. aqueous $Na_2S_2O_3$ (50 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 30 mL). The combined organic phases were washed with sat. aqueous $Na_2S_2O_3$ (2 × 700 mL) and sat. aqueous $NaHCO_3$ (2 × 700 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure and aldehyde **8b** was obtained without further purification as a colorless oil (739 mg, 2.90 mmol, 99%).

R_f = 0.83 (10% EtOAc in *n*-hexane). ─ $[\alpha]_D^{25}$: –55.2 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 9.57 (s, 1 H, H-1), 6.87 (dd, *J* = 17.4, 10.8 Hz, 1 H, H-5), 5.48 (s, 1 H, H-3), 5.23 (d, *J* = 17.2 Hz, 1 H, H-6^a), 5.17 (ddd, *J* = 10.7, 1.3, 1.3 Hz, 1 H, H-6^b), 1.85 (d, *J* = 1.1 Hz, 3 H, Me^4), 1.44 (s, 3 H, Me^2), 0.91 (s, 9 H, TBS-*t*Bu), 0.10 (s, 3 H, TBS-Me), 0.05 (s, 3 H, TBS-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 200.8 (C-1), 138.3 (C-4), 135.0 (C-5), 130.6 (C-3), 116.5 (C-6), 79.7 (C-2), 26.0 (TBS-*t*Bu), 25.6 (Me^2), 20.1 (Me^4), 18.4 (TBS-*t*Bu$_q$), –2.2 (TBS-Me), –2.4 (TBS-Me). ─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1737, 1003, 835, 775. ─ **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{14}H_{27}O_2Si^+$ 255.1775; found 255.1775.

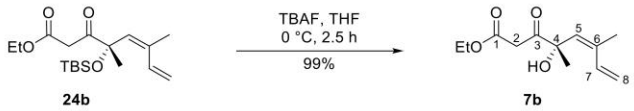
Ethyl (*S*,*Z*)-4-((*tert*-butyldimethylsilyl)oxy)-4,6-dimethyl-3-oxoocta-5,7-dienoate (24b)



Aldehyde **8b** (731 mg, 2.87 mmol, 1.00 eq.) was dissolved in dry CH_2Cl_2 (29 mL) and ethyl diazoacetate (solution with 13 wt% CH_2Cl_2, 1.27 mL, 8.61 mmol, 3.00 eq.) and $SnCl_2$ (545 mg, 2.87 mmol, 1.00 eq.) were added to the solution at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for 18 h at this temperature. The reaction was quenched with H_2O (35 mL). The phases were separated and the aqueous phase was extracted with Et_2O (4 × 50 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (2% to 10% Et_2O in *n*-pentane) to yield β-ketoester **24b** as a colorless oil (784 mg, 2.30 mmol, 80%).

R_f =0.49 (4% EtOAc in *n*-hexane). **—** $[\alpha]_D^{24}$: +29.6 (*c* 1.00 in $CHCl_3$). **— ^{1}H-NMR** ($CDCl_3$, 125 MHz): 6.60 (dd, *J* = 17.2, 10.8 Hz, 1 H, H-7), 5.45 (s, 1 H, H-5), 5.26 (d, *J* = 17.1 Hz, 1 H, H-8^a), 5.16 (ddd, *J* = 10.7, 1.2, 1.2 Hz, 1 H, H-8^b), 4.16 (q, *J* = 7.2 Hz, 4 H, OC*H*$_2$CH$_3$), 3.75 (d, *J* = 16.3 Hz, 1 H, H-2^a), 3.66 (d, *J* = 16.3 Hz, 1 H, H-2^b), 1.84 (d, *J* = 1.2 Hz, 3 H, Me^6), 1.54 (s, 3 H, Me^4), 1.25 (t, *J* = 7.2 Hz, 3 H, OCH$_2$C*H*$_3$), 0.91 (s, 9 H, TBS-*t*Bu), 0.12 (s, 3 H, TBS-Me), 0.07 (s, 3 H, TBS-Me). **— ^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 205.0 (C-3), 168.0 (C-1), 136.8 (C-6), 134.0 (C-7), 132.8 (C-5), 116.8 (C-8), 81.2 (C-4), 61.3 (O*C*H$_2$CH$_3$), 44.7 (C-2), 27.6 (Me^4), 26.0 (TBS-*t*Bu), 20.2 (Me^6), 18.4 (TBS-*t*Bu$_q$), 14.3 (OCH$_2$*C*H$_3$),–2.2 (TBS-Me), –2.4 (TBS-Me). **— IR** (cm^{-1}) $\tilde{\nu}$ = 1749, 1723, 1253, 835, 776. **— HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{18}H_{32}O_4SiNa^+$ 363.1962; found 363.1955.

Ethyl (*S*,*Z*)-4-hydroxy-4,6-dimethyl-3-oxoocta-5,7-dienoate (7b)

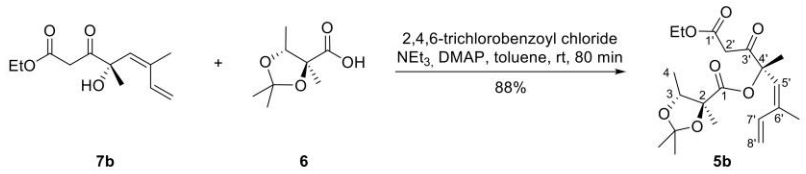


Silyl ether **24b** (44.0 mg, 0.129 mmol, 1.00 eq.) was dissolved in dry THF (1.3 mL) and TBAF (1M in THF, 258 µL, 0.258 mmol, 2.00 eq.) was added to the solution at 0 °C. After 2.5 h at 0 °C sat. aqueous NH_4Cl (2 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (10% to 18% EtOAc in *n*-pentane) to yield γ-hydroxy-β-ketoester **7b** as a colorless oil (29.0 mg, 0.128 mmol, 99%).

R_f = 0.24 (10% EtOAc in *n*-hexane). **—** $[\alpha]_D^{24}$: –44.3 (*c* 1.00 in $CHCl_3$). **— ^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.76 (ddd, *J* = 17.3, 10.9, 0.6 Hz, 1 H, H-7), 5.49 (s, 1 H, H-5), 5.31 (dd, *J* = 17.2, 0.5 Hz, 1 H, H-8^a), 5.20 (ddd, *J* = 11.0, 1.3, 1.3 Hz, 1 H, H-8^b), 4.17 (qd, *J* = 7.1, 1.6 Hz, 2 H, OC*H*$_2$CH$_3$), 3.70 (s, 1 H,

OH), 3.60 (d, *J* = 15.7 Hz, 1 H, H-2[a]), 3.56 (d, *J* = 15.7 Hz, 1 H, H-2[b]), 1.87 (d, *J* = 1.1 Hz, 3 H, Me[6]), 1.50 (s, 3 H, Me[4]), 1.25 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3). — **$^{13}C\{^1H\}$-NMR** ($CDCl_3$, 125 MHz): 205.4 (C-3), 167.1 (C-1), 139.2 (C-6), 133.2 (C-7), 128.6 (C-5), 117.7 (C-8), 78.6 (C-4), 61.7 (OCH_2CH_3), 44.2 (C-2), 27.9 (Me[4]), 20.4 (Me[6]), 14.2 (OCH_2CH_3). — **IR** (cm^{-1}) $\tilde{\nu}$ = 3484 (br.), 1742, 1712, 1312, 1022. — **HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{12}H_{19}O_4^+$ 227.1278; found 227.1275.

(*S*,*Z*)-1-Ethoxy-4,6-dimethyl-1,3-dioxooct-5,7-dien-4-yl (4*S*,5*R*)-2,2,4,5-tetramethyl-1,3-dioxolane-4-carboxylate (5a)

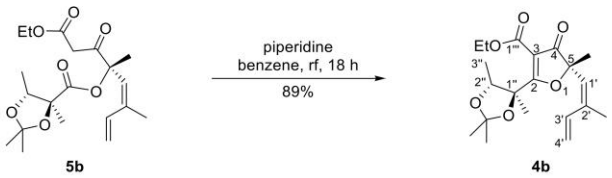


7b **6** **5b**

To a stirred solution of carboxylic acid **6** (50.0 mg, 0.285 mmol, 1.00 eq.) and alcohol **7b** (74.0 mg, 0.327 mmol, 1.15 eq.) in dry toluene (6 mL) were added NEt_3 (119 µL, 0.855 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (70.4 µL, 0.450 mmol, 1.58 eq.) and DMAP (52.0 mg, 0.428 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 80 min before being quenched with sat. aqueous $NaHCO_3$ (7 mL). After phase separation the aqueous phase was extracted with Et_2O (3 × 7 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10% EtOAc in *n*-pentane) afforded ester **5b** as a volatile colorless oil (96.0 mg, 0.251 mmol, 88%).

$\mathbf{R}_f$ = 0.54 (20% EtOAc in *n*-hexane). — $[\alpha]_D^{23}$: –50.5 (*c* 1.00 in $CHCl_3$). — **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.83 (dd, *J* = 17.2, 10.9 Hz, 1 H, H-7'), 5.77 (s, 1 H, H-5'), 5.23 (d, *J* = 17.2 Hz, 1 H, H-8[ra]), 5.11 (ddd, *J* = 10.8, 1.4, 1.4 Hz, 1 H, H-8[rb]), 4.39 (q, *J* = 6.3 Hz, 1 H, H-3), 4.16 (q, *J* = 7.2 Hz, 2 H, OCH_2CH_3), 3.51 (d, *J* = 15.6 Hz, 1 H, H-2[ra]), 3.44 (d, *J* = 15.6 Hz, 1 H, H-2[rb]), 1.87 (d, *J* = 1.2 Hz, 3 H, Me[6']), 1.74 (s, 3 H, Me[4']), 1.47 (s, 3 H, acetonide-Me), 1.39 (s, 3 H, acetonide-Me), 1.33 (s, 3 H, Me[2]), 1.32 (d, *J* = 6.4 Hz, 3 H, H-4), 1.25 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3). — **$^{13}C\{^1H\}$-NMR** ($CDCl_3$, 125 MHz): 197.9 (C-3'), 172.0 (C-1), 166.8 (C-1'), 137.5 (C-6'), 133.3 (C-7'), 125.6 (C-5'), 117.8 (C-8'), 109.0 (acetonide-C_q), 85.9 (C-4'), 82.7 (C-2), 75.7 (C-3), 61.6 (OCH_2CH_3), 43.3 (C-2'), 28.3 (acetonide-Me), 25.6 (acetonide-Me), 23.2 (Me[4']), 20.5 (Me[6']), 19.8 (Me[2]), 14.8 (C-4), 14.2 (OCH_2CH_3). — **IR** (cm^{-1}) $\tilde{\nu}$ = 1749, 1728, 1258, 1108, 1022. — **HRMS** (ESI) *m/z* $[M+Na]^+$: calculated for $C_{20}H_{30}O_7Na^+$ 405.1884; found 405.1868.

Ethyl (*S*)-5-methyl-5-((*Z*)-2-methylbuta-1,3-dien-1-yl)-4-oxo-2-((4*S*,5*R*)-2,2,4,5-tetramethyl-1,3-dioxolan-4-yl)-4,5-dihydrofuran-3-carboxylate (4b)

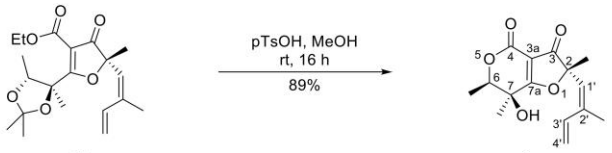


5b **4b**

Compound **5b** (203 mg, 0.531 mmol, 1.00 eq.) was dissolved in dry benzene (5.3 mL) and piperidine (210 µL, 2.12 mmol, 4.00 eq.) was added to the solution at room temperature. The reaction mixture was heated at reflux for 18 h before it was allowed to cool to room temperature. The volatiles were removed in vacuo and the remainder was purified by column chromatography (5% EtOAc in *n*-pentane). Product **4b** was obtained as a volatile colorless oil (172 mg, 0.472 mmol, 89%).

$\mathbf{R}_f$ = 0.65 (20% EtOAc in *n*-hexane). ─ $[\alpha]_D^{24}$: –208.3 (*c* 1.00 in $CHCl_3$). ─ **^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.89 (dd, *J* = 17.4, 10.8 Hz, 1 H, H-3'), 5.45 (s, 1 H, H-1'), 5.34 (d, *J* = 17.2 Hz, 1 H, H-4ta), 5.24 (ddd, *J* = 10.8, 1.2, 1.2 Hz, 1 H, H-4^{tb}), 4.35-4.27 (m, 2 H, OCH_2CH$_3$), 4.26-4.18 (m, 1 H, H-2''), 1.87 (d, *J* = 1.2 Hz, 3 H, Me$^{2'}$), 1.58 (s, 3 H, Me^5), 1.48 (s, 3 H, Me$^{1''}$), 1.45 (s, 3 H, acetonide-Me), 1.36 (d, *J* = 6.3 Hz, 3 H, H-3''), 1.32 (t, *J* = 7.2 Hz, 3 H, OCH$_2$CH_3), 1.28 (s, 3 H, acetonide-Me). ─ **^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 200.1 (C-4), 190.6 (C-2), 162.8 (C-1'''), 138.7 (C-2'), 134.0 (C-3'), 125.0 (C-1'), 117.4 (C-4'), 109.1 (acetonide-C$_q$), 108.9 (C-3), 90.8 (C-5), 81.7 (C-1''), 76.4 (C-2''), 61.4 (OCH$_2$CH$_3$), 28.5 (acetonide-Me), 25.5 (acetonide-Me), 24.9 (Me^5), 20.4 (Me$^{1''}$), 20.2 (Me$^{2'}$), 15.2 (C-3''), 12.3 (OCH$_2$$CH_3$).─ **IR** (cm^{-1}) $\tilde{\nu}$ = 1742, 1713, 1373, 1105, 1030. ─ **HRMS** (ESI) *m/z* [M+Na]$^+$: calculated for $C_{20}H_{28}O_6Na^+$ 387.1778; found 387.1762.

Ophiofuranone B (2)



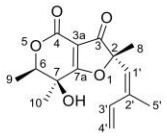
4b **2**

To a stirred solution of compound **4b** (31.0 mg, 85.1 µmol, 1.00 eq.) in MeOH (1 mL) was added *para*-toluenesulfonic acid monohydrate (49.0 mg, 0.255 mmol, 3.00 eq.). The reaction mixture was stirred at ambient temperature for 16 h. The reaction was quenched with sat. aqueous $NaHCO_3$ (2 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 2 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (10% EtOAc in *n*-pentane) to yield ophiofuranone B (**2**) as a colorless solid (21.0 mg, 75.5 µmol, 89%).

R_f = 0.13 (30% EtOAc in *n*-hexane). **—** $[\alpha]_D^{24}$: –248.8 (*c* 0.10 in MeOH) [Lit.[10] $[\alpha]_D^{20}$: –36.2 (*c* 0.1 in MeOH)]. **— ^{1}H-NMR** ($CDCl_3$, 500 MHz): 6.92 (ddd, *J* = 17.2, 10.8, 0.6 Hz, 1 H, H-3'), 5.42 (s, 1 H, H-1'), 5.40 (d, *J* = 17.2 Hz, 1 H, H-4'^{a}), 5.33 (ddd, *J* = 10.9, 1.1 Hz, 1 H, H-4'^{b}), 4.42 (q, *J* = 6.5 Hz, 1 H, H-6), 3.85 (br. s., 1 H, OH), 1.87 (d, *J* = 1.2 Hz, 3 H, $Me^{2'}$), 1.70 (s, 3 H, Me^2), 1.50 (d, *J* = 6.6 Hz, 3 H, Me^6), 1.48 (s, 3 H, Me^7). **— ^{13}C{^{1}H}-NMR** ($CDCl_3$, 125 MHz): 196.6 (C-3), 194.4 (C-7a), 160.7 (C-4), 139.5 (C-2'), 133.7 (C-3'), 123.7 (C-1'), 118.0 (C-4'), 102.5 (C-3a), 95.3 (C-2), 81.4 (C-6), 68.1 (C-7), 24.3 (Me^2), 20.4 ($Me^{2'}$), 18.6 (Me^7), 13.0 (Me^6). **— ^{1}H-NMR** (DMSO-*d*6, 500 MHz): 6.76 (dd, *J* = 17.2, 10.8 Hz, 1 H, H-3'), 6.41 (s, 1 H, OH), 5.46 (s, 1 H, H-1'), 5.40 (dd, *J* = 17.2, 0.6 Hz, 1 H, H-4'^{a}), 5.33 (ddd, *J* = 10.9, 1.4, 1.4 Hz, 1 H, H-4'^{b}), 4.62 (q, *J* = 6.4 Hz, 1 H, H-6), 1.85 (d, *J* = 1.2 Hz, 3 H, $Me^{2'}$), 1.55 (s, 3 H, Me^2), 1.32 (s, 3 H, Me^7), 1.31 (d, *J* = 6.5 Hz, 3 H, Me^6). **— ^{13}C{^{1}H}-NMR** (DMSO-*d*6, 125 MHz): 196.5 (C-3), 195.1 (C-7a), 159.1 (C-4), 138.8 (C-2'), 133.3 (C-3'), 124.1 (C-1'), 118.6 (C-4'), 102.0 (C-3a), 93.2 (C-2), 80.0 (C-6), 66.9 (C-7), 24.2 (Me^2), 19.9 ($Me^{2'}$), 18.5 (Me^7), 12.8 (Me^6) **— IR** (cm^{-1}) $\tilde{\nu}$ = 3435, 1754, 1685, 1596, 1432. **— mp** 169-170 °C **— HRMS** (ESI) *m/z* $[M+H]^+$: calculated for $C_{15}H_{19}O_5^+$ 279.1227; found 279.1220.

The preceding data is consistent with those reported in literature.[10] The NMR data of the synthetic ophiofuranone B (**2**) agreed very well with those of the literature (Table S2). Though our synthetic product showed an optical rotation of $[\alpha]_D^{24}$: –248.8 (*c* 0.10 in MeOH) in contrast to $[\alpha]_D^{20}$: –36.2 (*c* 0.1 in MeOH) reported for the natural isolate. This deviation might be due to impurities visible in the ^{1}H spectra and the small amount of the isolate (Figure S3), but this cannot normally explain such a large variance. It is also very unlikely that the isolate is a non-racemic mixture of enantiomers, since circular dichroism spectra of the natural isolate and the synthetic sample match very well (Figure S4).[10]

Table S2. Comparison of NMR spectra of isolated and synthetic ophiofuranone B (**2**) in DMSO-*d*6.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C{^{1}H} δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			93.2	93.2
3			196.4	196.5
3a			102.0	102.0
4			159.1	159.1
6	4.62 q (6.6)	4.62 q (6.4)	80.0	80.0
7			66.8	66.9
7a			195.0	195.1
8	1.55 s	1.55 s	24.1	24.2
9	1.32 d (6.6)	1.31 d (6.5)	12.8	12.8
10	1.32 s	1.32 s	18.5	18.5
1'	5.46 s	5.46 s	124.1	124.1
2'			133.3[c]	138.8[c]
3'	6.77 dd (17.4, 10.8)	6.76 dd (17.2, 10.8)	138.7[c]	133.3[c]
4'[a]	5.40 d (17.2)	5.40 dd (17.2, 0.6)	118.5	118.6
4'[b]	5.33 dt (10.8, 1.2)	5.33 ddd (10.8, 1.4, 1.4)		
5'	1.82 s	1.85 d (1.2)	19.5	19.9
OH	6.40 s	6.41 s		

[a] ^{1}H and ^{13}C{^{1}H} NMR data recorded at 600 MHz and 150 MHz. [b] ^{1}H and ^{13}C{^{1}H} NMR data recorded at 500 MHz and 125 MHz. [c] For the ^{13}C NMR of the isolated ophiofuranone B (**2**) the signal by 133.3 ppm was assigned for position 2' and 138.7 ppm for position 3'.[10] However, it could be clearly proved by jmod and 2D NMR experiments of the synthetic one that the assignment is exactly the other way round.

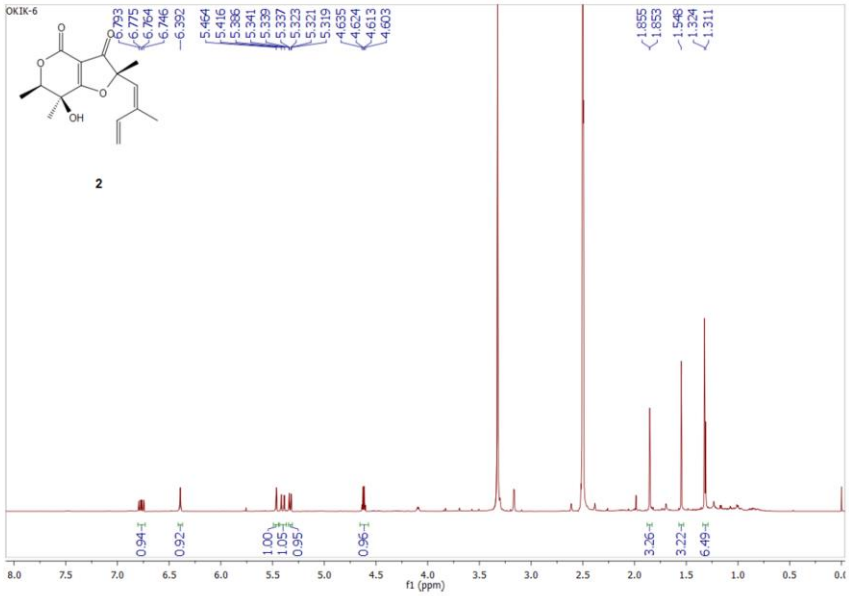


Figure S4i: ^{1}H-NMR spectra of isolated ophiofuranone B (**2**).[10]

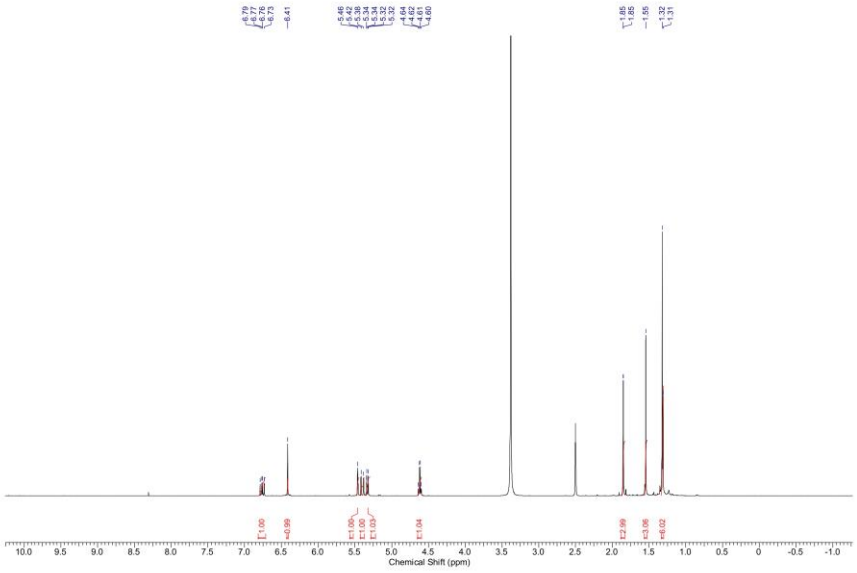


Figure S5s: ^{1}H-NMR spectra of synthetic ophiofuranone B (**2**) in $CDCl_3$ (500 MHz).

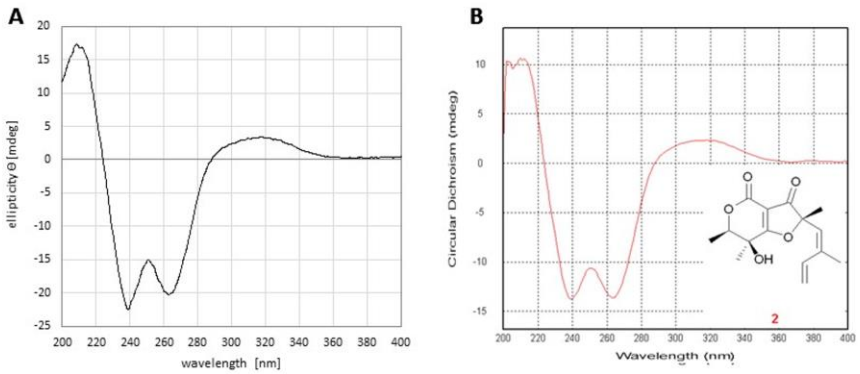


Figure S6. A: CD spectrum of synthetic ophiofuranone B (**2**) (0.1 mg/mL in MeOH); **B**: CD spectrum of isolated ophiofuranone B (**2**).[10]

Antimicrobial assay

Minimum Inhibitory Concentrations (MIC) of ophiofuranones A (**1**), B (**2**), **4a**, and **4b** were quantified by serial dilution assays as described previously[11,12] using the following organisms: *Pichia anomala, Schizosaccharomyces pombe, Mucor hiemalis, Candida albicans, Candida auris,* and *Rhodotulas glutinis* for fungal microorganisms; *Bacillus subtilis, Staphyloccocus aureus* and *Mycobacterium smegmatis* for Gram-positive bacteria, *Acinetobacter baumannii*, *Chromobacterium violaceum, Escherichia coli* and *Pseudomonas aeruginosa* for Gram-negative bacteria.

Table S3. Antimicrobial activity of synthetic ophiofuranones A (**1**), B (**2**), **4a**, and **4b**.

		MIC [µg/mL]				
Tested organisms	**Strain No.**	**1**	**2**	**4a**	**4b**	**Reference**
Bacteria						
Bacillus subtilis	DSM 10	–	–	–	–	8.3[a]
Staphylococcus aureus	DSM 346	–	–	–	–	1.7[a]
Mycobacterium smegmatis	ATCC 700084	–	–	–	–	1.7[b]
Acinetobacter baumannii	DSM 30008	–	–	–	–	0.5[c]
Chromobacterium violaceum	DSM 30191	–	–	–	–	0.4[a]
Escherichia coli	DSM 1116	–	–	–	–	1.7[a]
Pseudomonas aeruginosa	PA14	–	–	–	–	0.4[d]
Fungi						
Mucor hiemalis	DSM 2656	66.6	66.6	66.6	–	4.2[e]
Pichia anomala	DSM 6766	–	–	–	–	4.2[e]
Rhodoturula glutinis	DSM 10134	–	–	–	–	2.1[e]
Candida albicans	DSM 1665	–	–	–	–	8.3[e]
Candida auris	DSM 21095	–	–	–	–	31.2[e]
Schizosaccharomyces pombe	DSM 70572	–	–	–	–	4.2[e]

References: [a] oxytetracycline; [b] kanamycin; [c] ciprobay; [d] gentamicin; [e] nystatin; – : not active.

Biofilm inhibition assay

The stock of *Staphylococcus aureus* DSM 1104 from –20 °C was incubated in 25 mL CASO (casein-peptone soymeal-peptone) medium at 37 °C on a rotary shaker (100 rpm) for 18 h. The OD_{600} of the culture solution was examinated and adjusted to match the turbidity of a 0.001 McFarland standard. 150 µL of CASO with 4% glucose broth was added together with the serial diluted compounds (250-2 µg/mL) and incubated in 96 well microtiter plates (TPP tissue culture ref.no 92196) for 18 h at 37 °C. The anti-biofilm activity was evaluated by using 0.1% crystal violet staining (Thermo Fisher, Waltham, USA) following previously established protocols.[13,14] In brief, the supernatant was discarded, the biofilm was stained at room temperature for 15 min and subsequently washed three times by PBS (phosphate-buffered saline) buffer, the dye combined with biofilm was dissolved in acetic acid (30%),

and the absorbance was finally quantified by using a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 530 nm. Methanol (2.5%) was used as negative control and microporenic acid A[13] (250-0.2 µg/mL) was used as positive control.

P. aeruginosa (PA 14) DSM 19882 was precultured in 25 mL LB medium (Luria-Bertani Broth) with a 250 mL flask at 37 °C with shaking 100 rpm overnight. The OD_{600} of the suspended solution was measured and adjusted to 0.1 McFarland standard in M63 medium, which is supplemented with magnesium sulfate, glucose and casamino acids as previously described.[15] The compounds were added into 150 µL bacterial solution at the concentration (250–2 µg/mL) then the solution was added in U-bottom 96 well plate (Falcon non-tissue plate with U-Bottom ref.no 351177). The plates were incubated at 37 °C at 150 rpm for 24 h and biofilms were established at the air liquid interface. The plates were rinsed once by using PBS buffer, the biofilms were stained by 150 µL 0.1% CV at room temperature for 15 min and then rinsed two times by using PBS buffer. The absorbance was quantified with the plate reader (Synergy 2, BioTek, Santa Clara, USA) at 550 nm using ethanol (95%). Methanol (2.5 %) and Myxovalargin A (250–2 µg/mL) were used as negative control and positive control, respectively.[14]

Biofilm dispersion assay

A cell suspension of *Staphylococcus aureus* strain DSM 1104 was adjusted to match 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 (250-2 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.[14]

Candida albicans DSM 11225 grew in 25 mL YPED (Yeast extract Peptone Dextrose) medium in a 250 mL flask at 30 °C in a shaker (100 rpm) for 18 h. The OD_{600} of the bacterial suspension was measured and adjusted to 0.05 McFarland standard in RPMI 1640 medium. The 150 µL bacterial solution was added in 96 well non-tissue microtiter plates (Falcon non-tissue plate ref.no 351172) at 37 °C (150 rpm). After 90 min incubation, the supernatant was discarded and rinsed two times by using PBS buffer. Serially compounds were diluted in 150 µL of the fresh media (RPMI 1640) at the concentration (250–2 µg/mL) and added into the wells of 96 wells plate. Methanol (2.5 %) and farnesol (250–2 µg/mL) were separately used as a negative control and positive control, respectively. The plates were further incubated at 37 °C at 150 rpm for 24 h. At last, the supernatant was removed and washed with PBS buffer. Biofilms were stained by 150 µL 0.1% CV for 25 min at room temperature. Afterwards, the plates were washed four times with PBS buffer and 150 µL ethanol (95%) was added to the wells to dissolve the biofilms. The absorbance was finally quantified using a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 610 nm. SD of two repeats with duplicates each were 10% or less.[14]

Cytotoxicity assay

518A2 melanoma cells (university hospital vienna), $HCT116^{wt}$ and $HCT116^{p53-/-}$ (ACC 581) colon carcinoma, HeLa (ACC 57) and KB-3-1 (ACC 158) cervix carcinoma as well as L929 (ACC 2) mouse fibroblasts were cultured under standard cell culture conditions (37 °C, 5% CO_2, 95% humidified atmosphere). The cells were maintained in high glucose DMEM containing 10% foetal bovine serum and 100 U/mL penicillin and streptomycin (all from Gibco, Thermo Fisher Scientific). Cell lines were serially passaged after trypsinisation, using 0.05% trypsin / 0.02% EDTA solution. The cultures were routinely monitored for potential contamination, and only mycoplasma free cultures were used. The test compounds were investigated for their anti-proliferative effect on cell lines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based cell viability assay.[11,16] Cells were seeded in 96 well microtiter plates (Sarstedt) with a cell density of 0.05×10^6 cells per mL and 100 µL per well and were incubated for 24 h. A dilution series of test compounds was added to the wells, ranging in eleven steps from 100 µM – 5nM (37 µg/mL – 0.63 ng/mL), and equal amounts of DMSO or methanol were added as negative controls. Treated cells were incubated for a further 72 h. Then, 12.5 µL per well of MTT solution (0.05% in PBS) were added, followed by another 2 h of incubation. The plates were centrifuged, the medium was discarded and 25 µL per well of SDS solution in DMSO (10% SDS, 0.6% AcOH in DMSO) were added to dissolve the formazan. The plates were incubated for another hour. Absorbances at 570 nm and 630 nm were measured with a plate reader (Tecan). Background absorbance (630 nm) was subtracted from the formazan signal (570 nm). The resulting absorbance is directly proportional to the amount of viable cells. The control was normalized to 100% viable cells, and the viability of cells treated with the test compounds was calculated accordingly. IC_{50} values were calculated based on a sigmoidal fit model using GraphPad Prism. Means and SD were calculated from at least four independent experiments.

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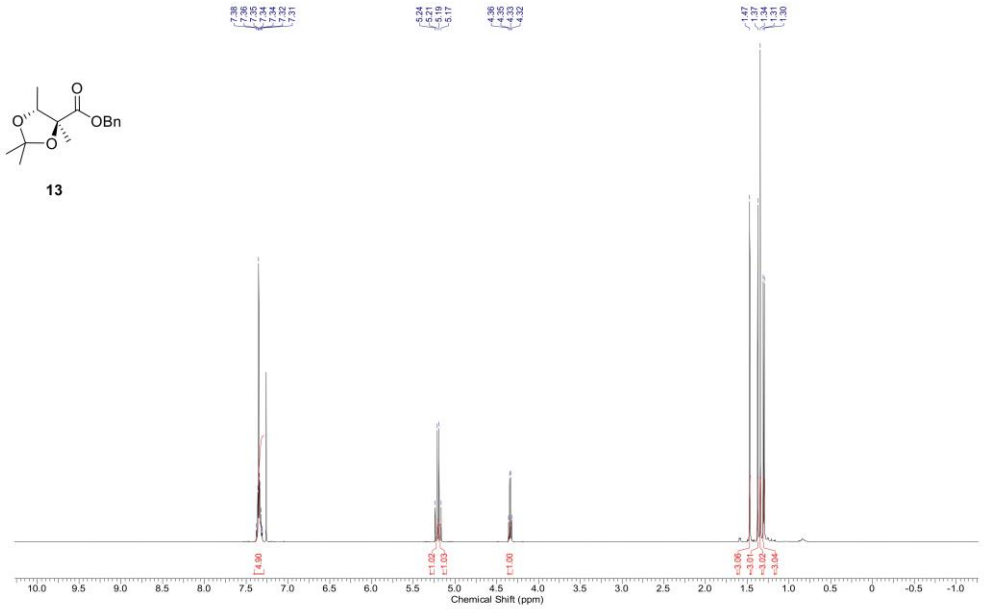
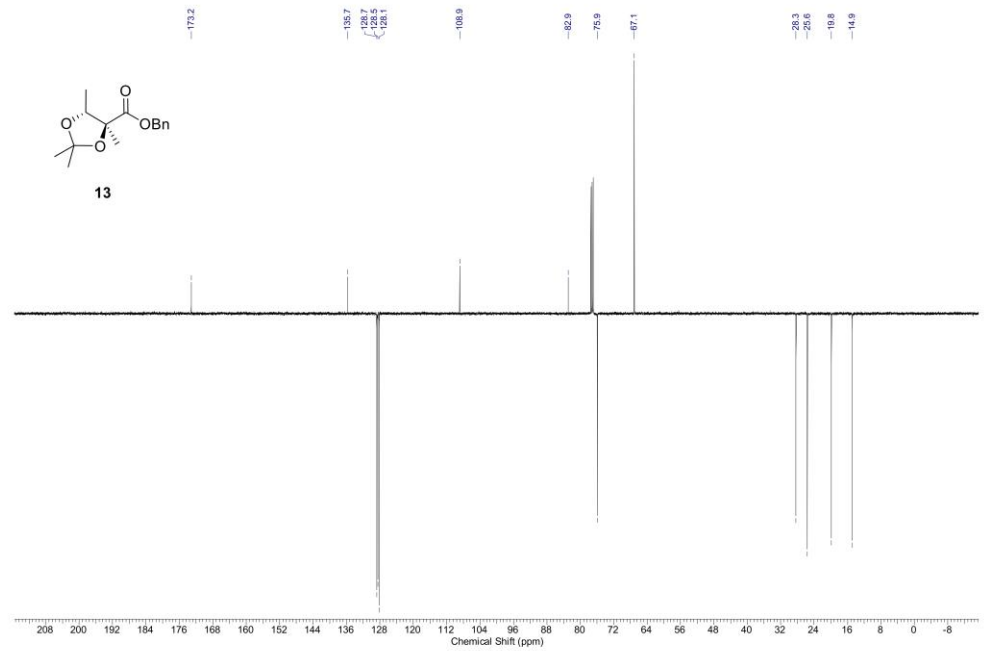
NMR spectra of all new compounds



^{1}H-NMR spectrum of compound **13** in $CDCl_3$ (500 MHz).



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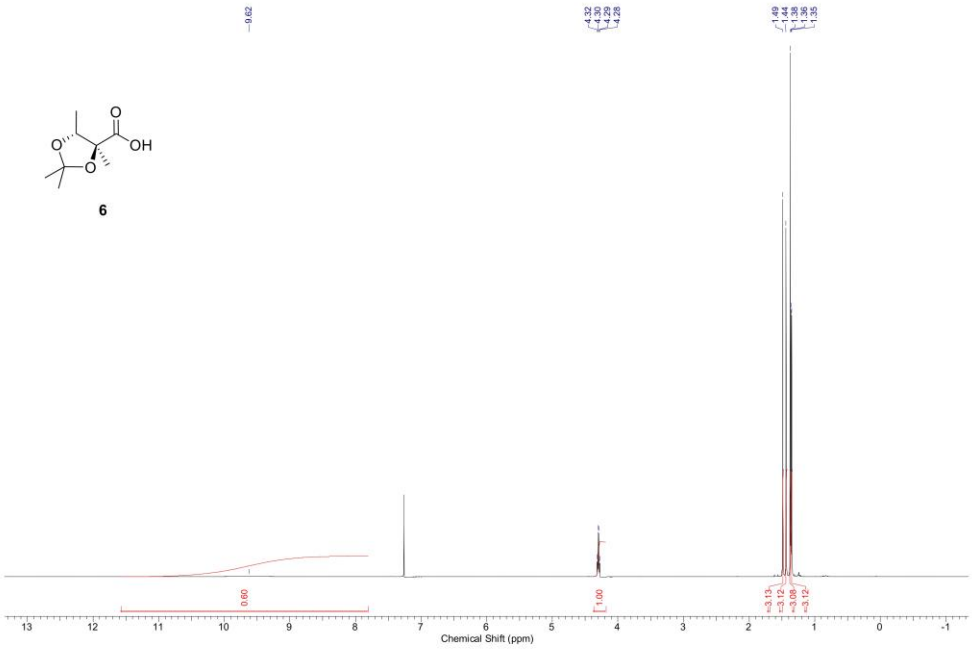
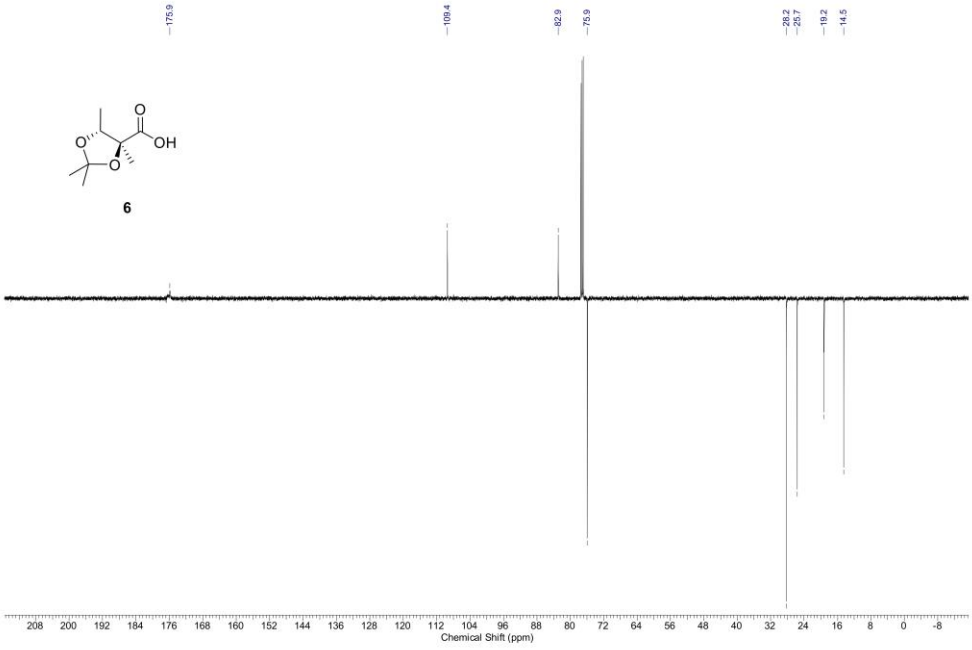


^{1}H-NMR spectrum of compound **6** in $CDCl_3$ (500 MHz).

S40



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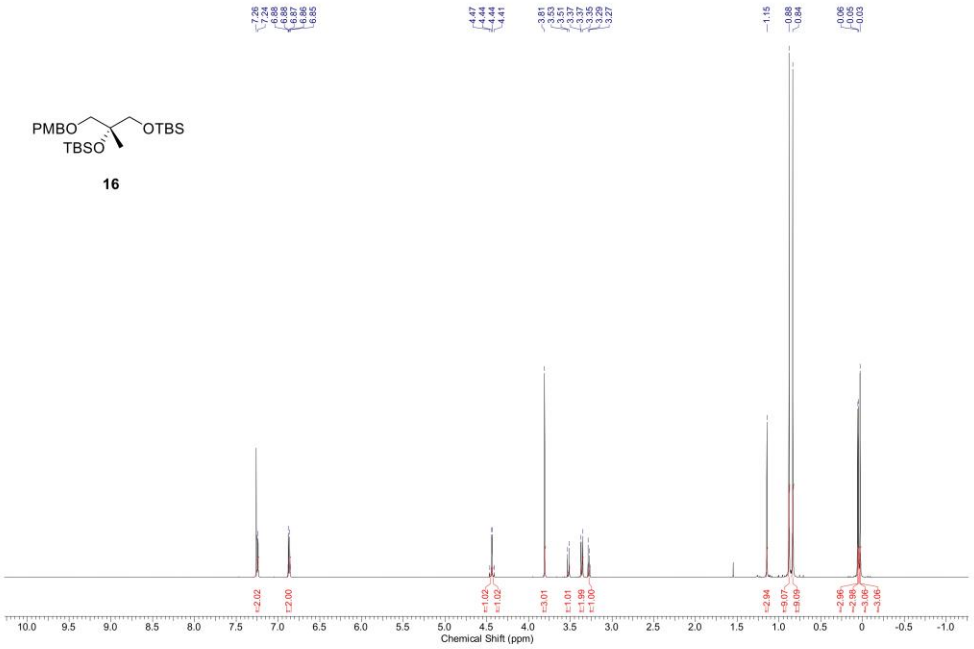
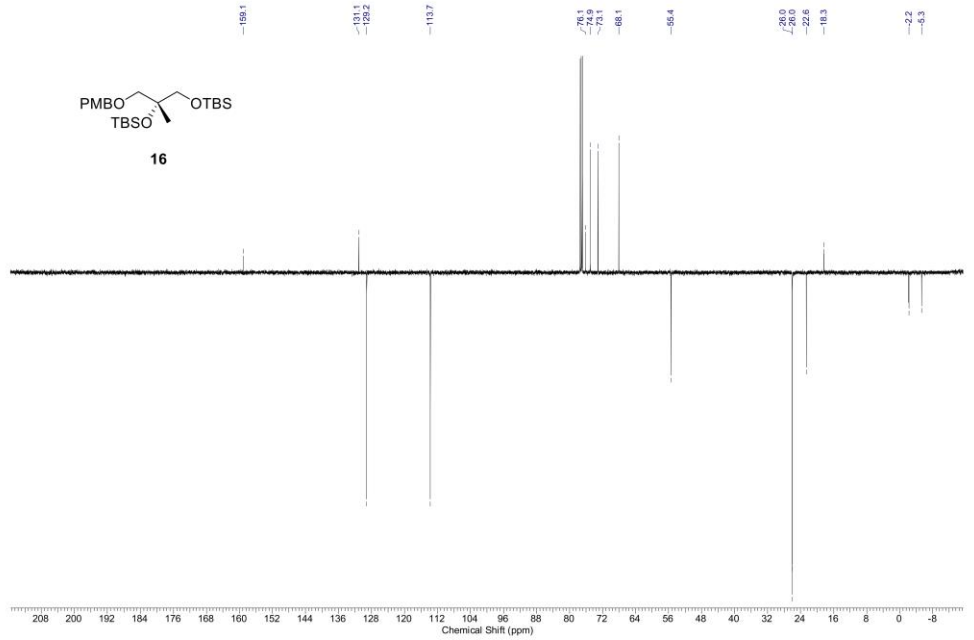
S41



^{1}H-NMR spectrum of compound **16** in $CDCl_3$ (500 MHz).



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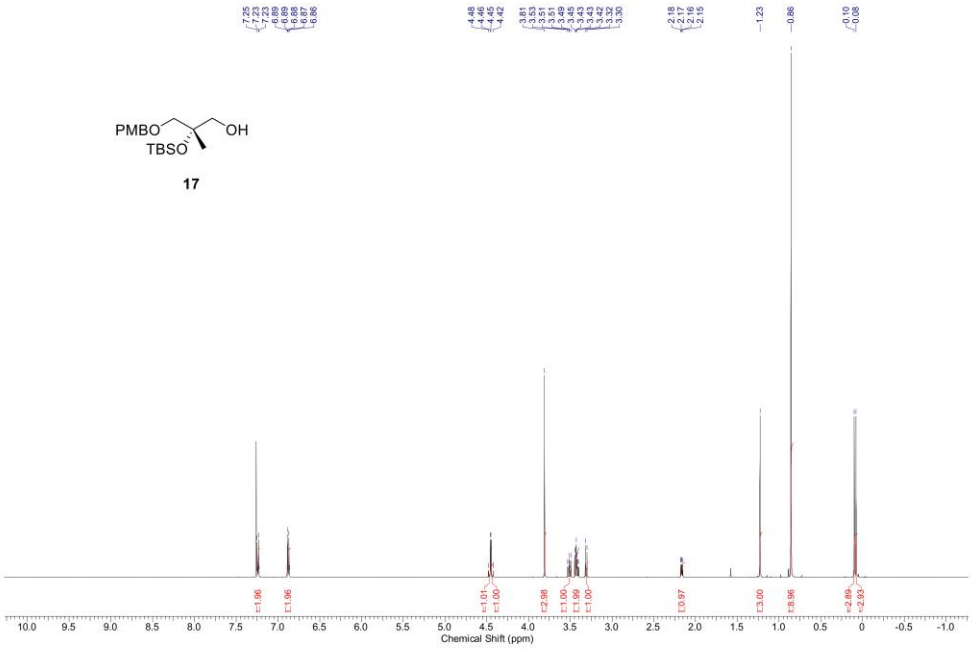
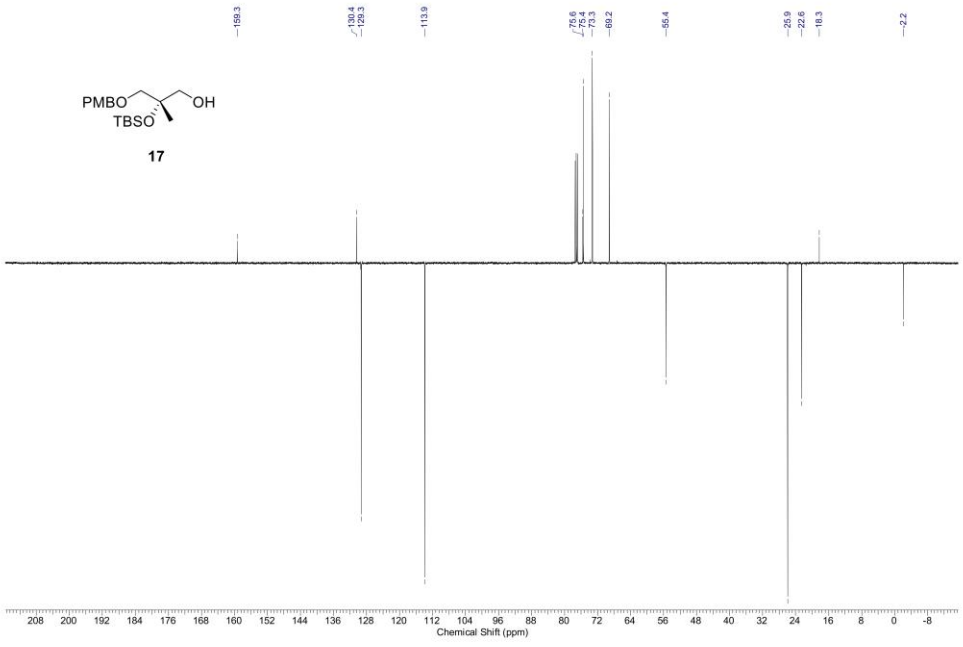


^{1}H-NMR spectrum of compound **17** in $CDCl_3$ (500 MHz).



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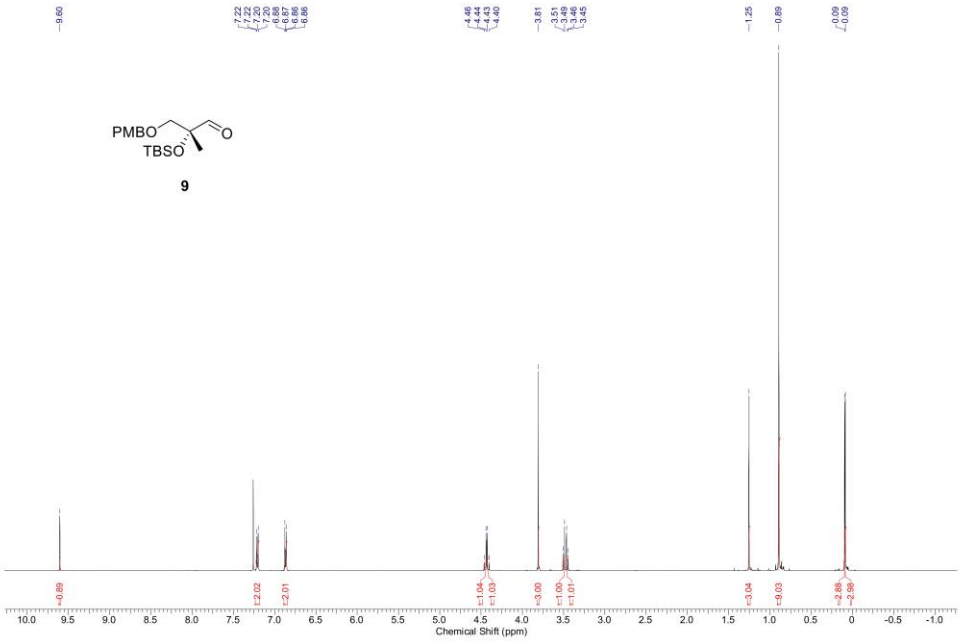

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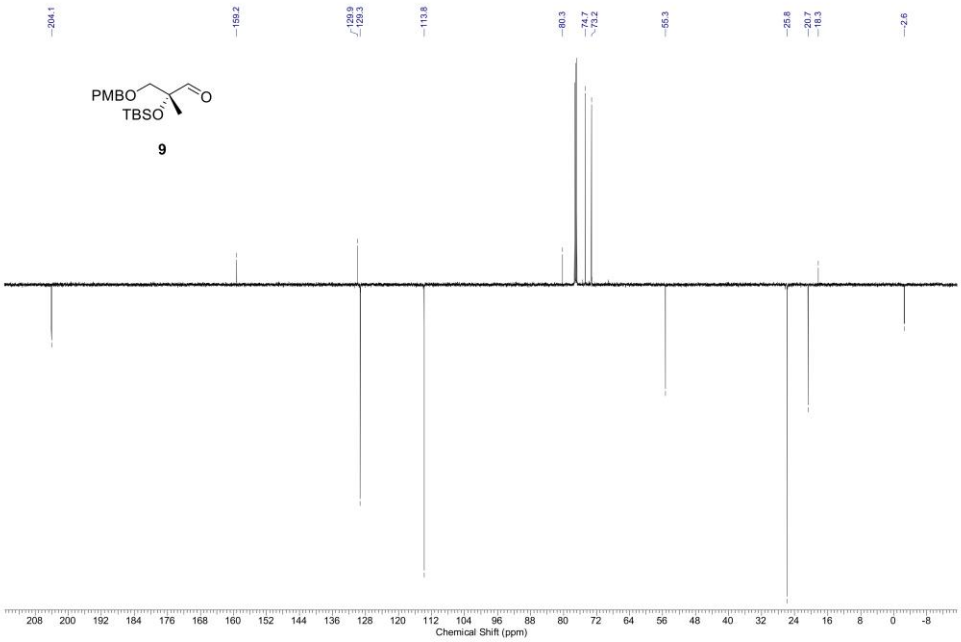

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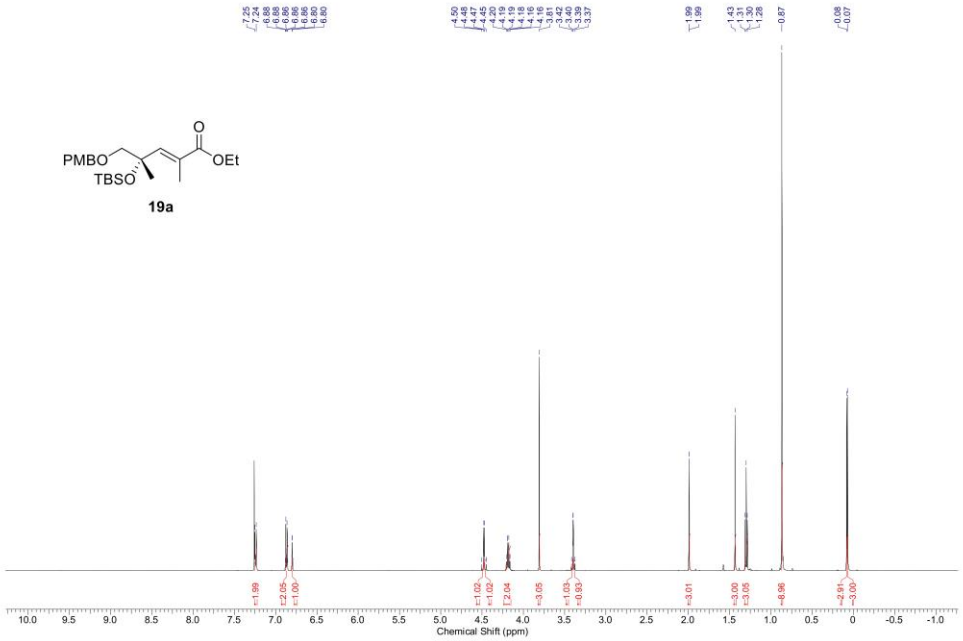
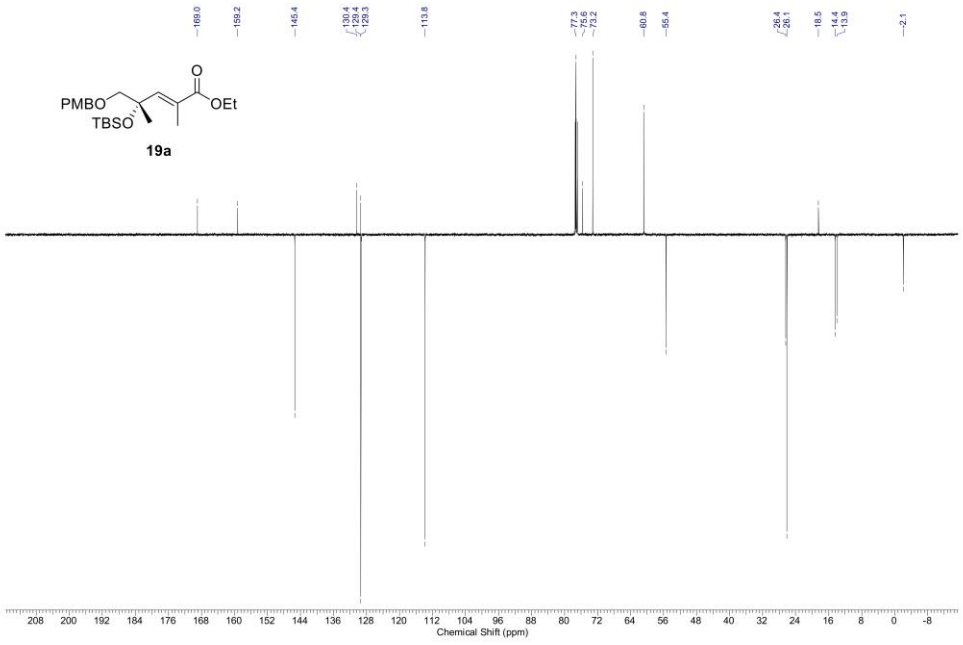


^{1}H-NMR spectrum of compound **19a** in $CDCl_3$ (500 MHz).



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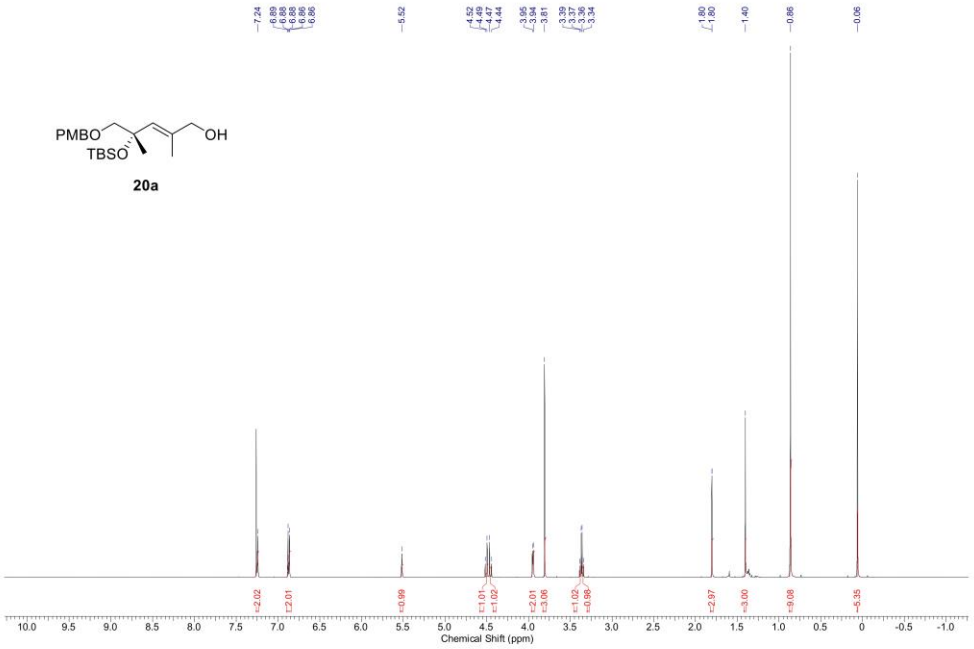

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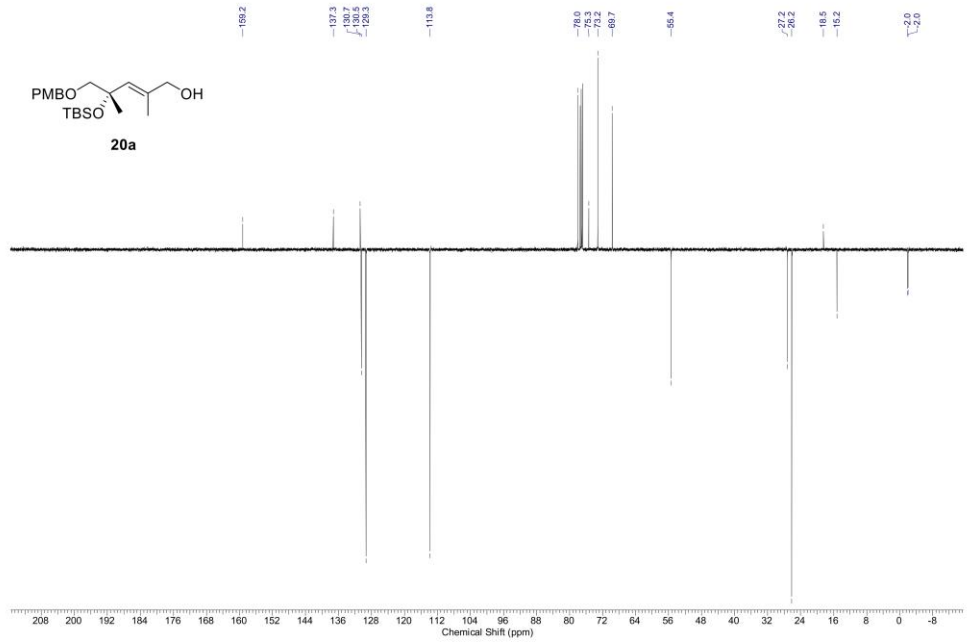

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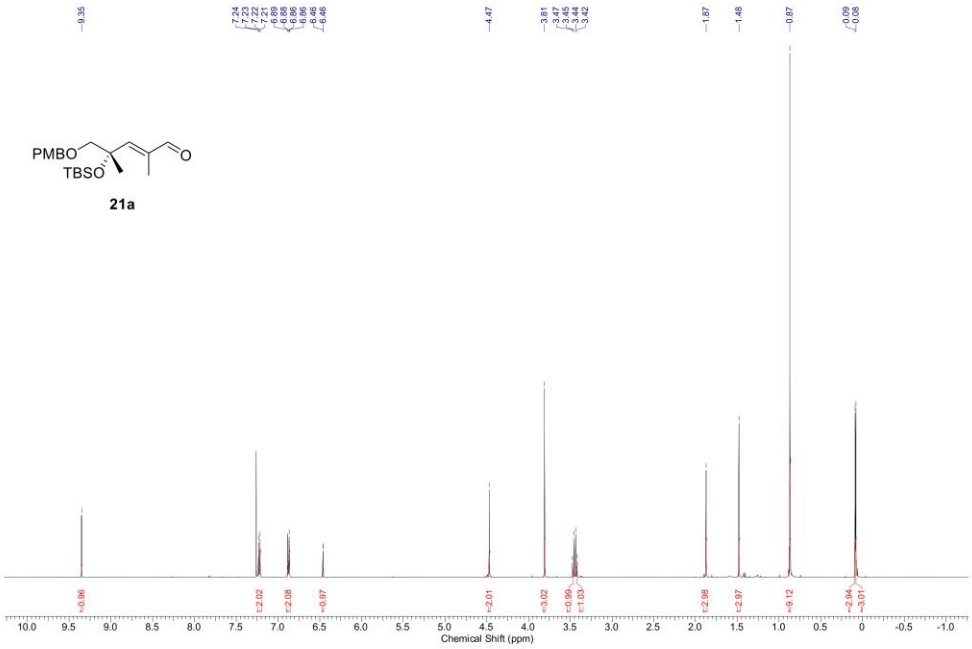
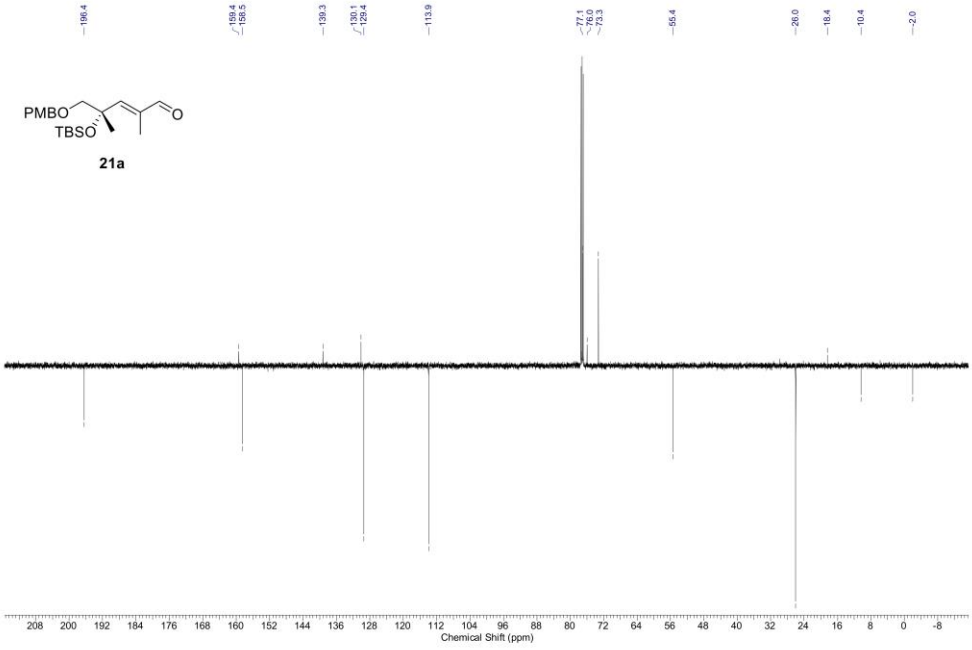


^{1}H-NMR spectrum of compound **21a** in $CDCl_3$ (500 MHz).



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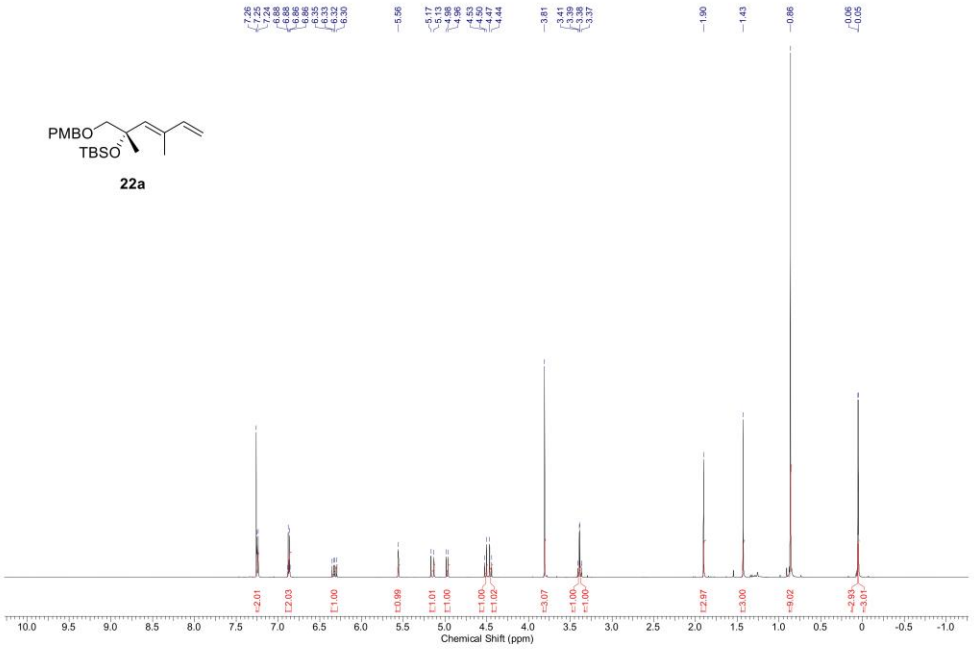
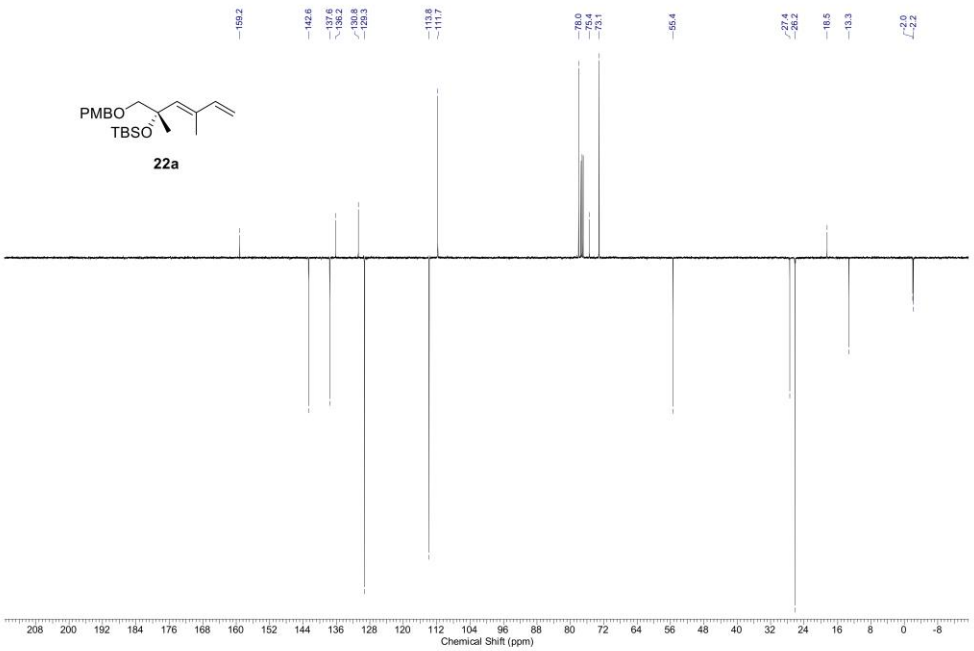


^{1}H-NMR spectrum of compound **22a** in $CDCl_3$ (500 MHz).



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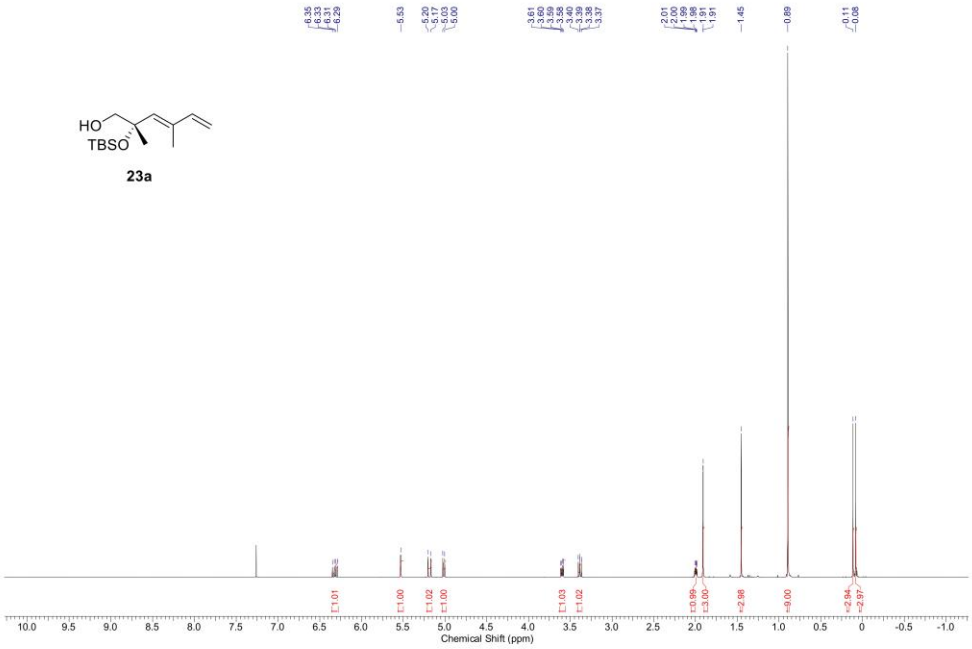

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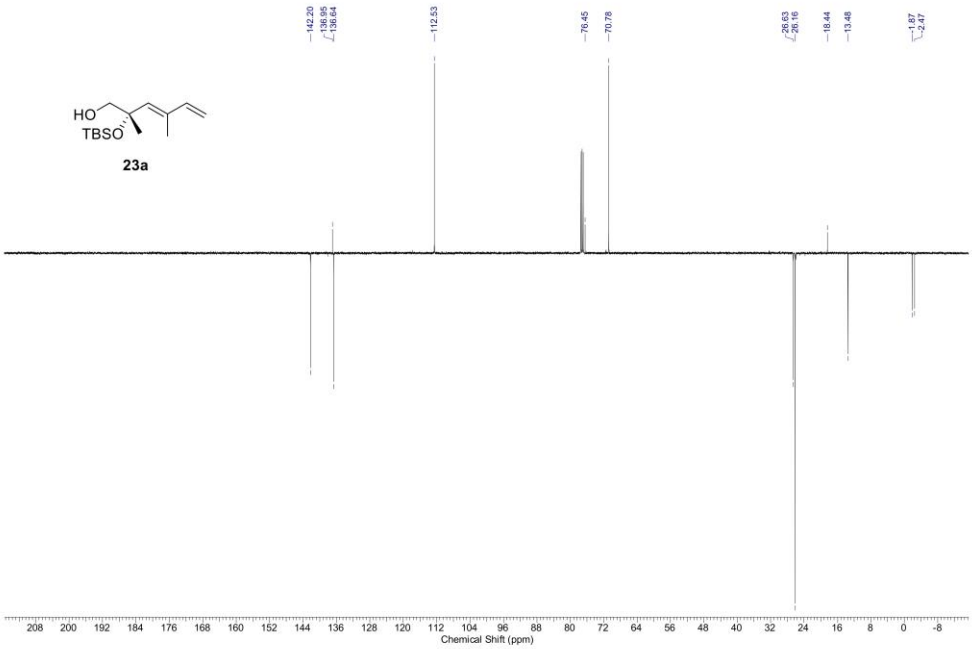

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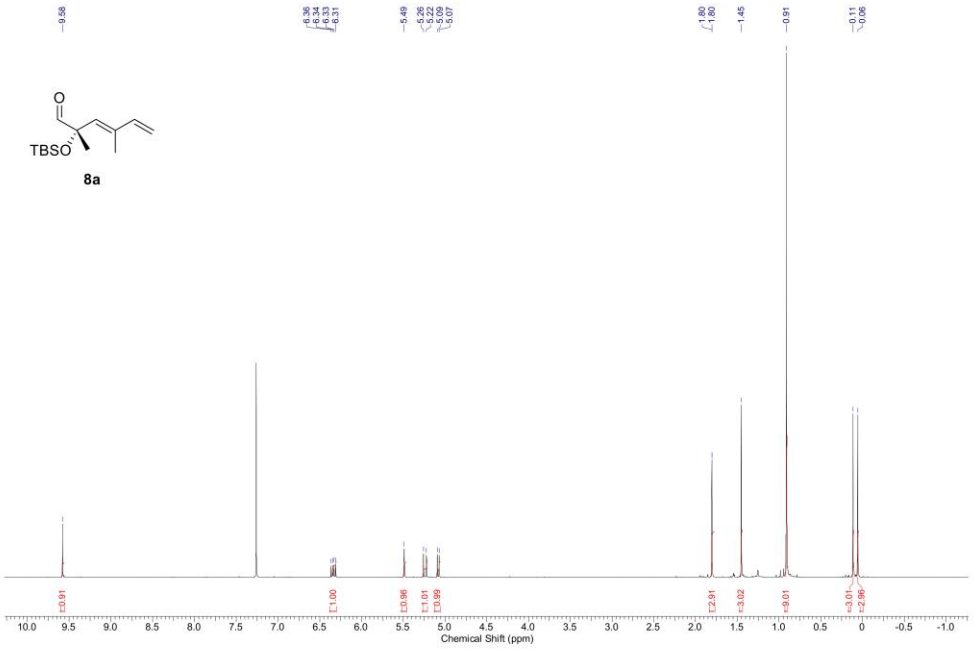

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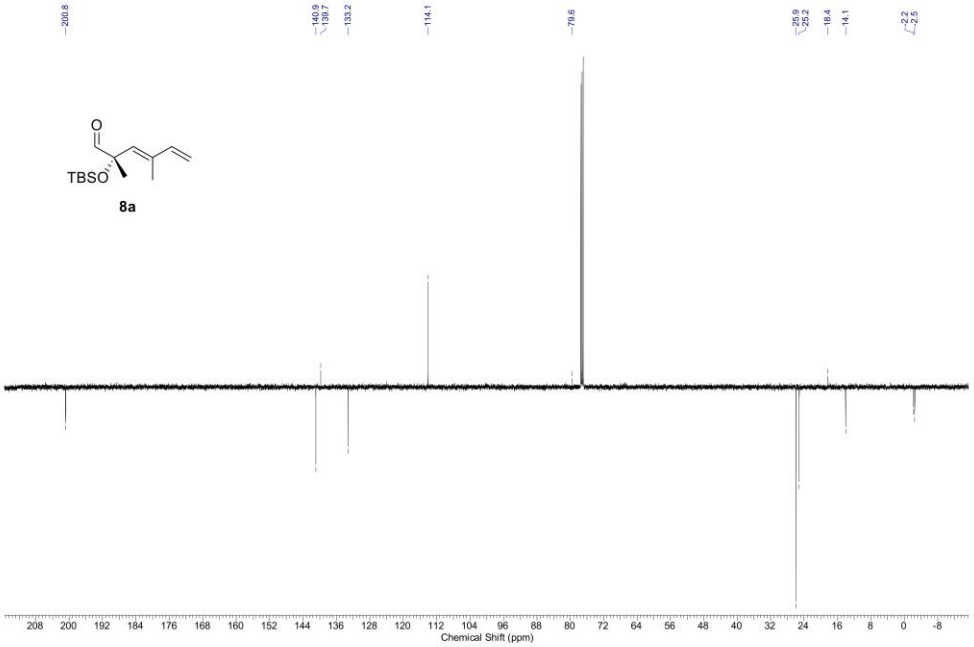

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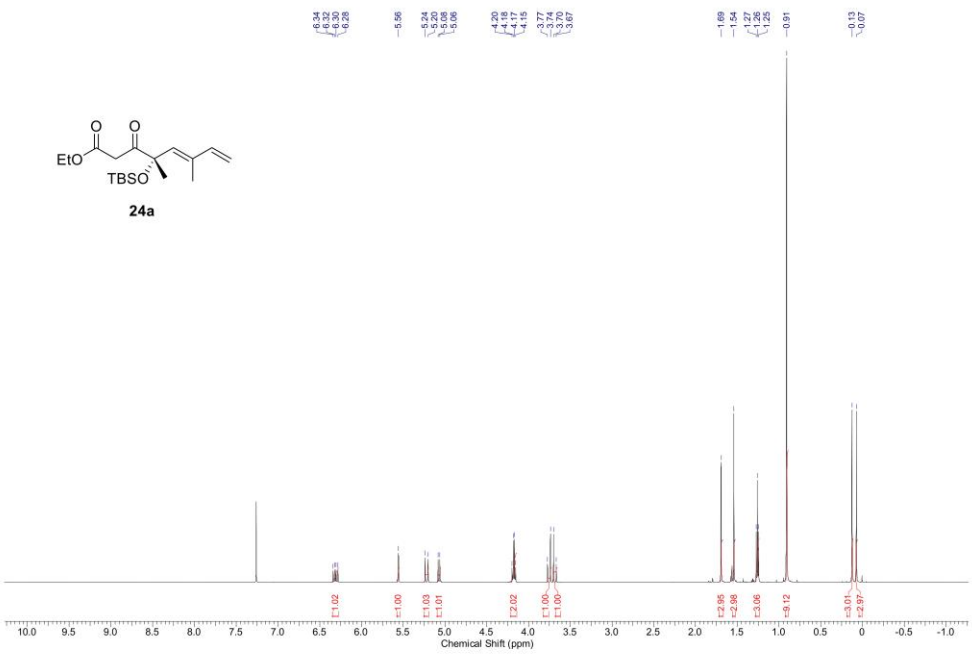
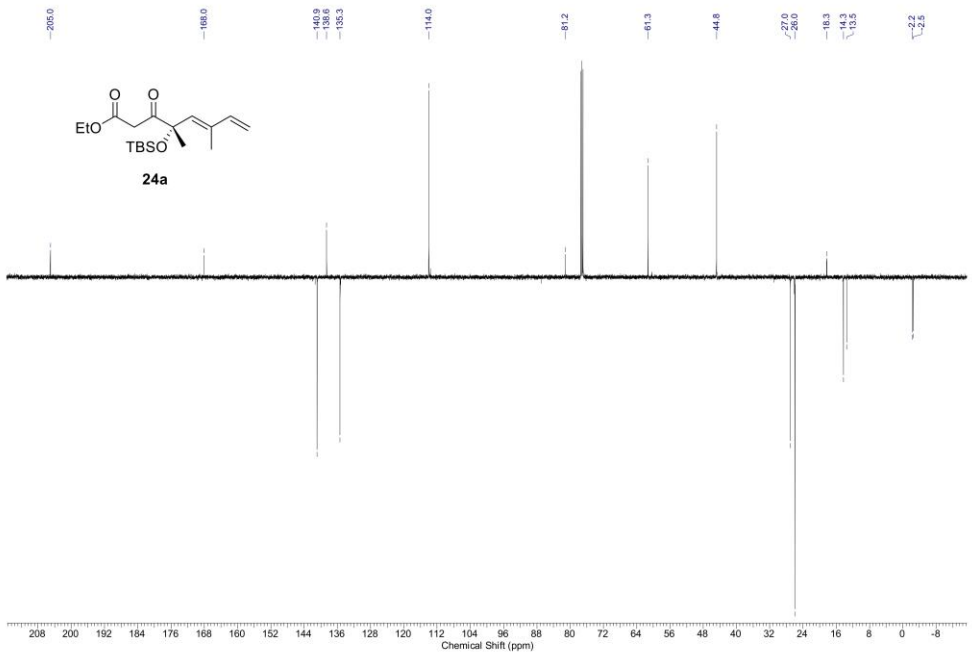


^{1}H-NMR spectrum of compound **24a** in $CDCl_3$ (500 MHz).

S60



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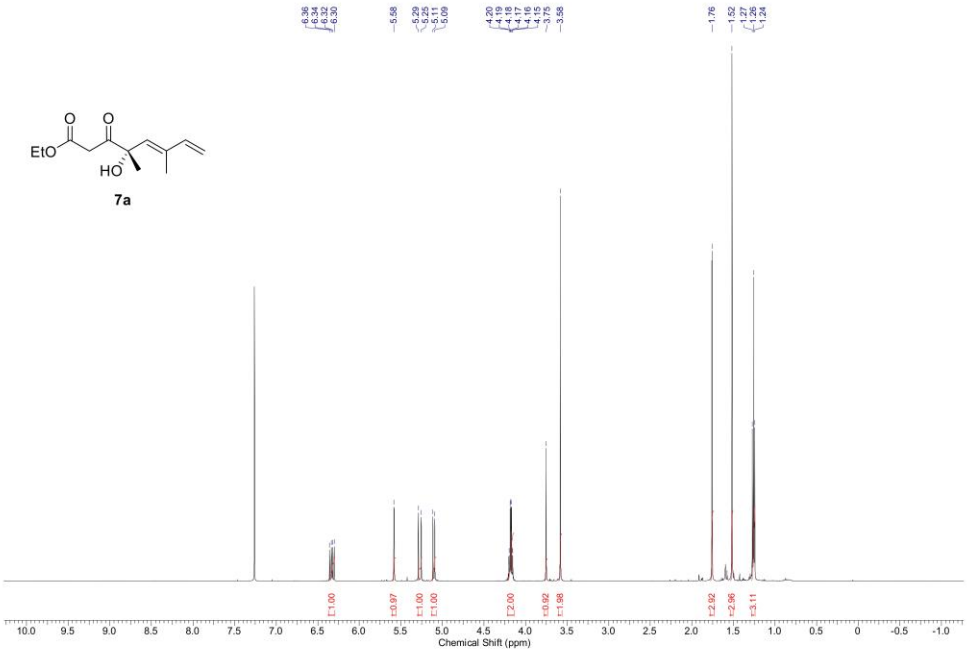
S61



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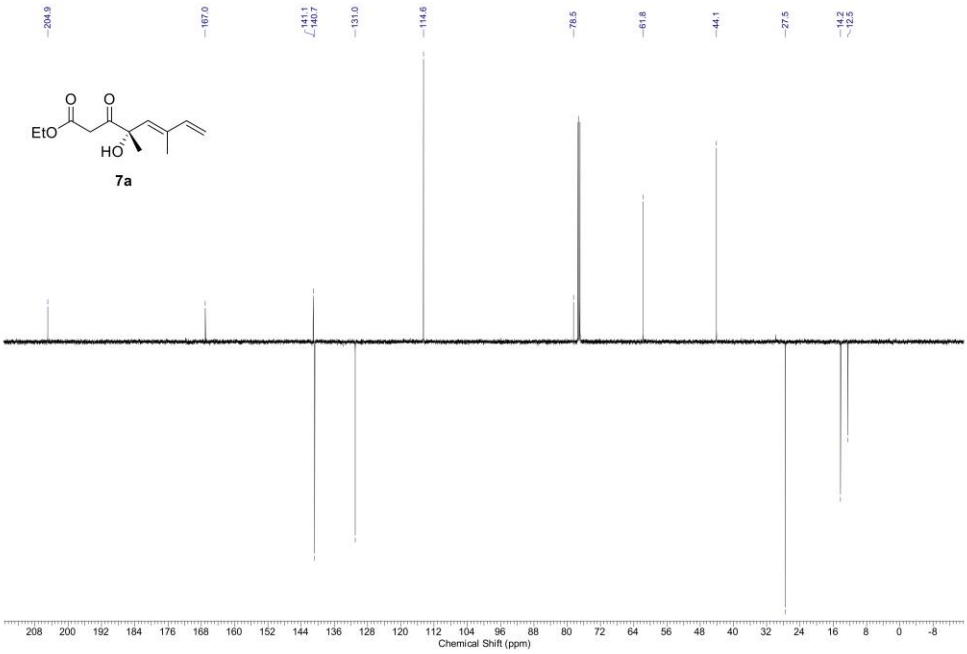

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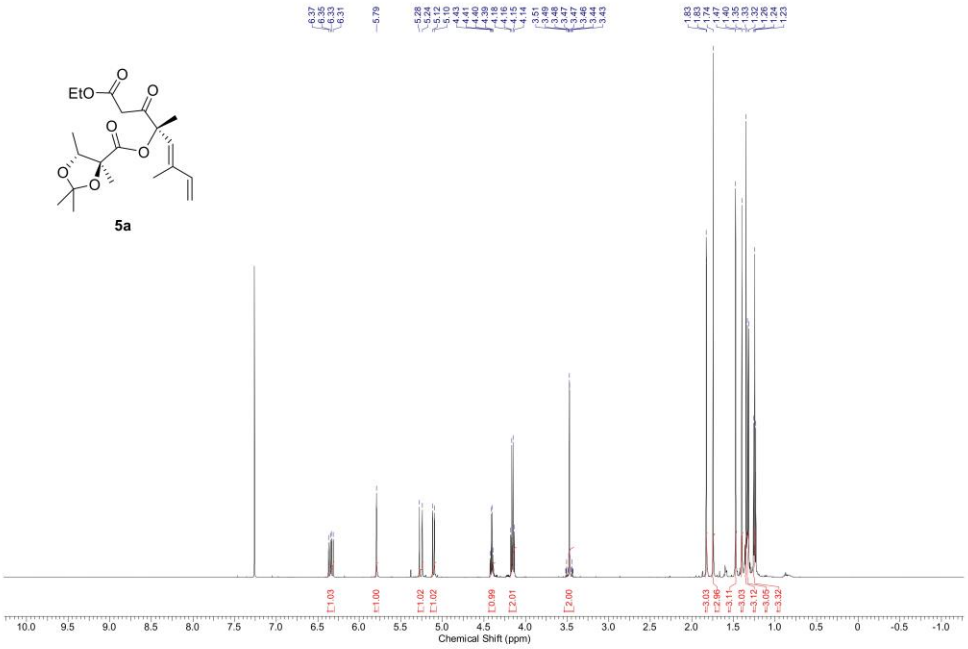

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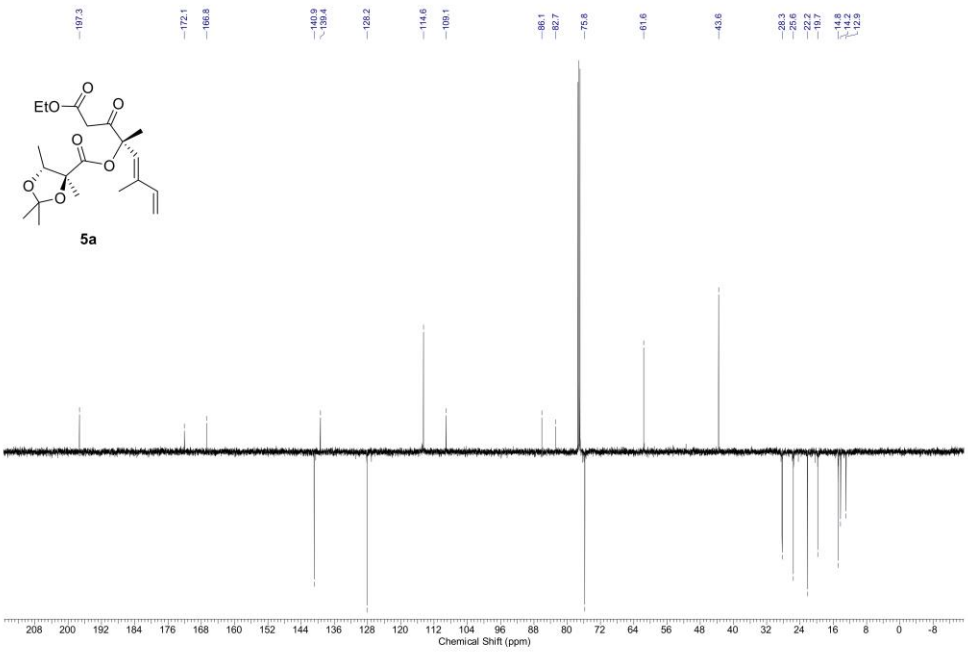
S64



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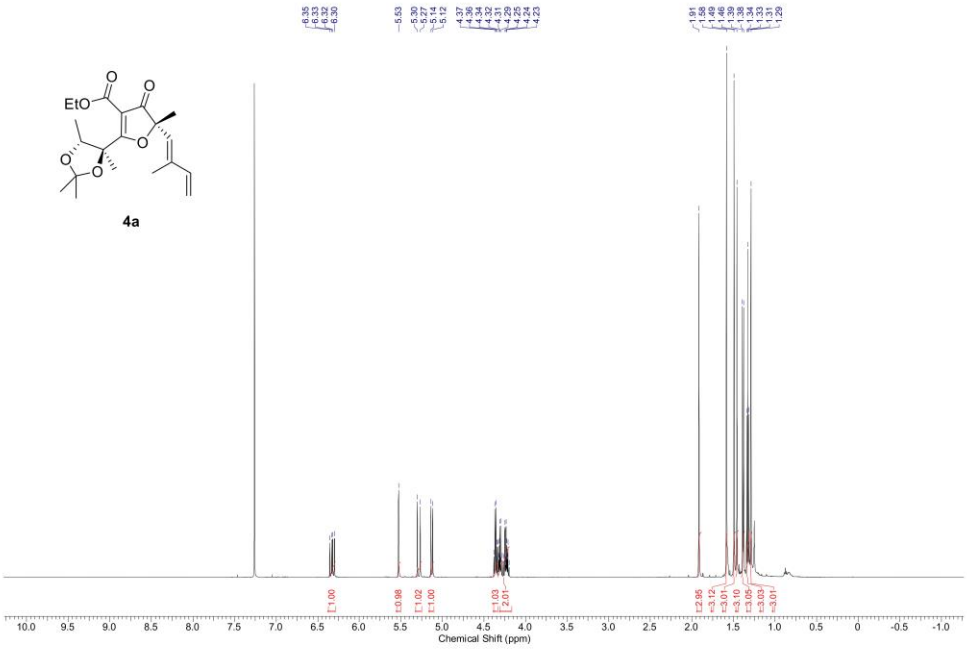
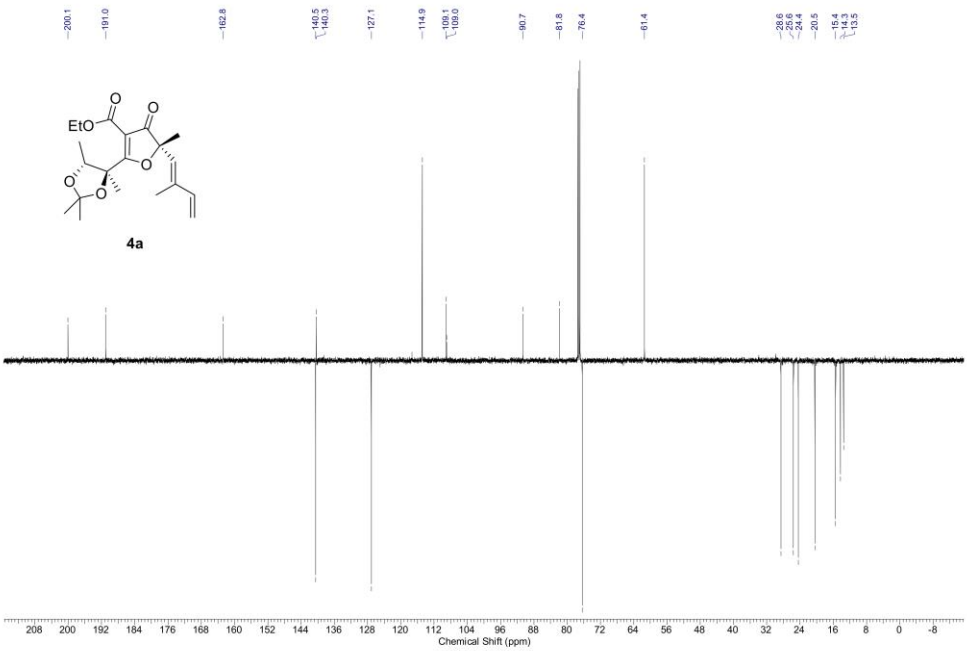
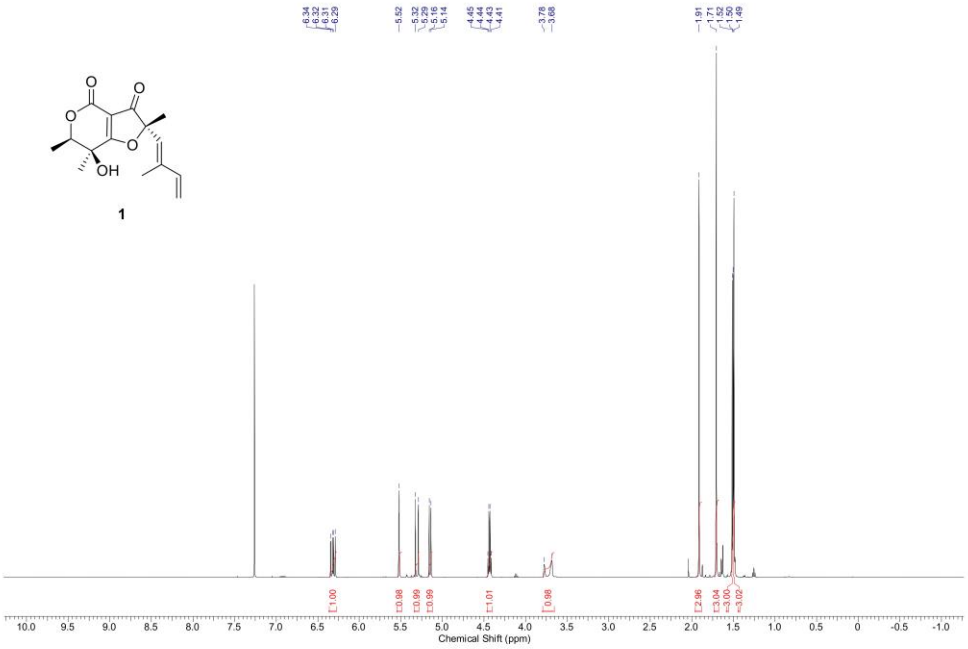
S65



^{1}H-NMR spectrum of compound **4a** in $CDCl_3$ (500 MHz).



^{13}C{^{1}H}-NMR (APT) spectrum of compound **4a** in $CDCl_3$ (125 MHz).



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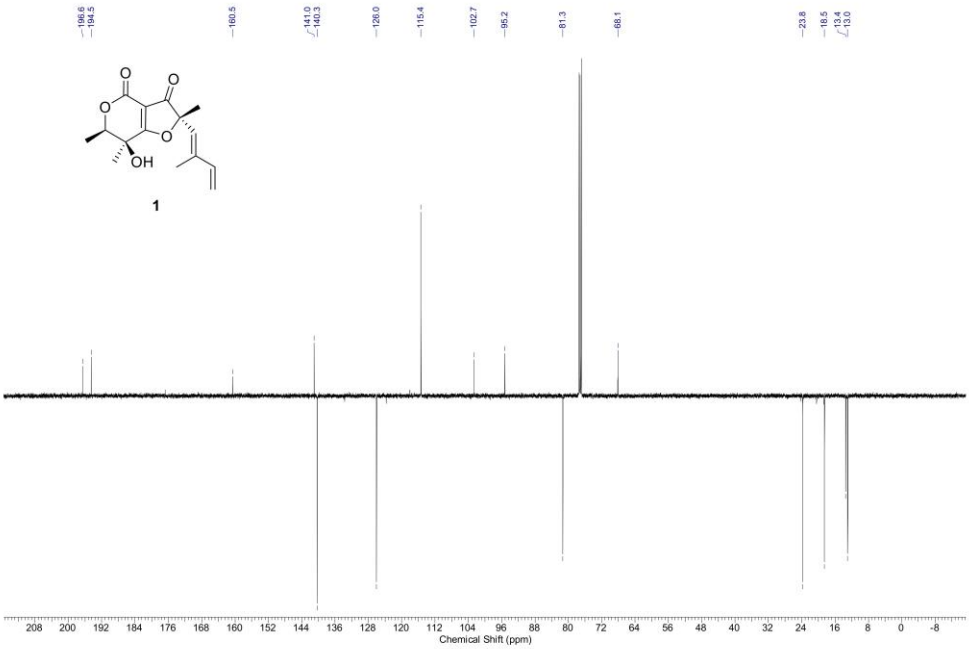
^{1}H-NMR spectrum of compound **1** in $CDCl_3$ (500 MHz).

S68



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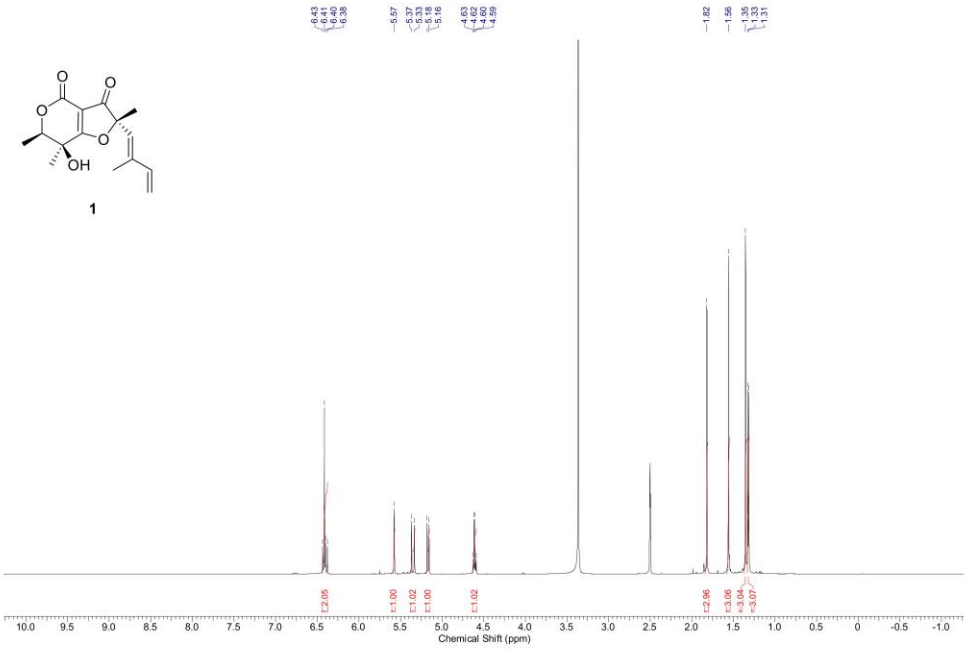
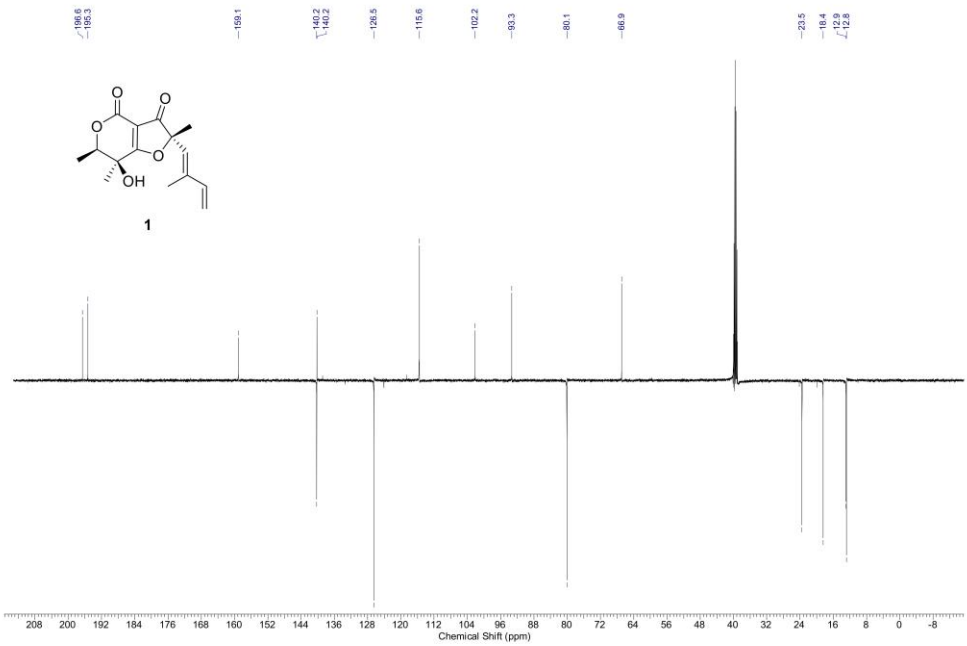
^{13}C{^{1}H}-NMR (APT) spectrum of compound **1** in $CDCl_3$ (125 MHz).

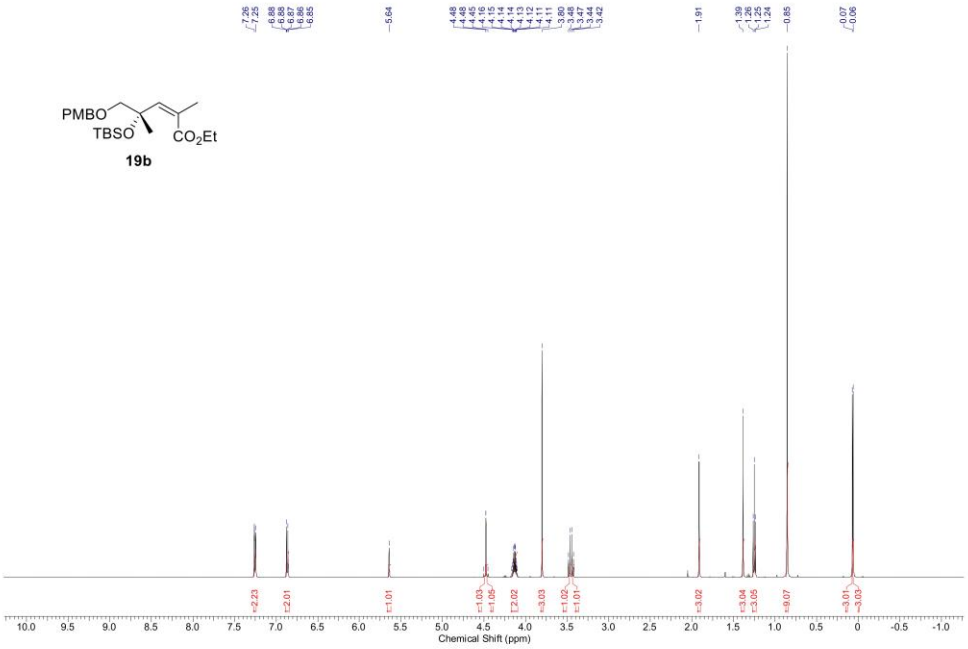
S69



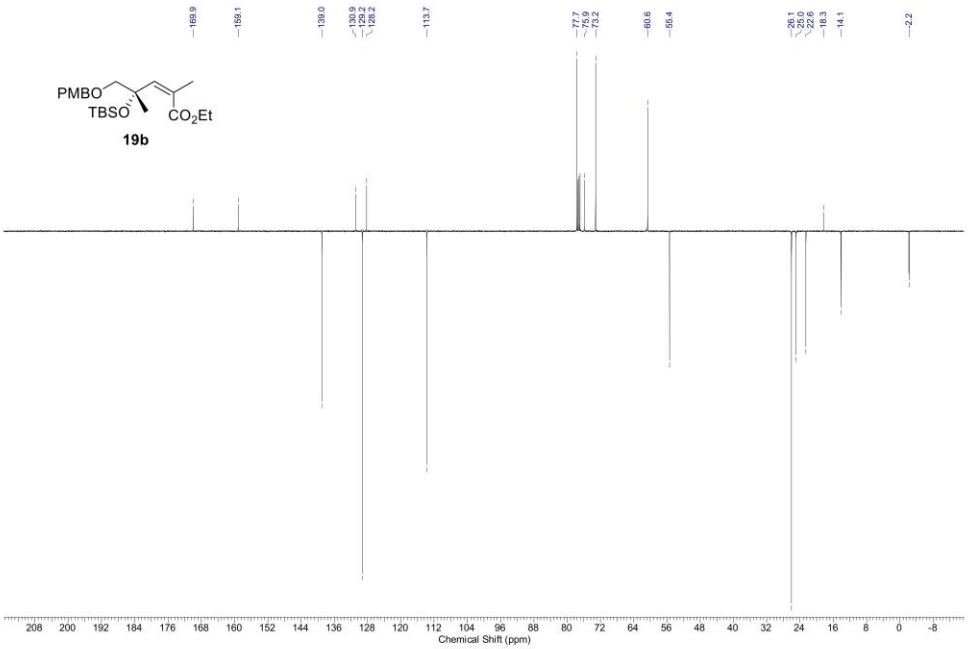
^{1}H-NMR spectrum of compound **1** in DMSO-*d*6 (500 MHz).



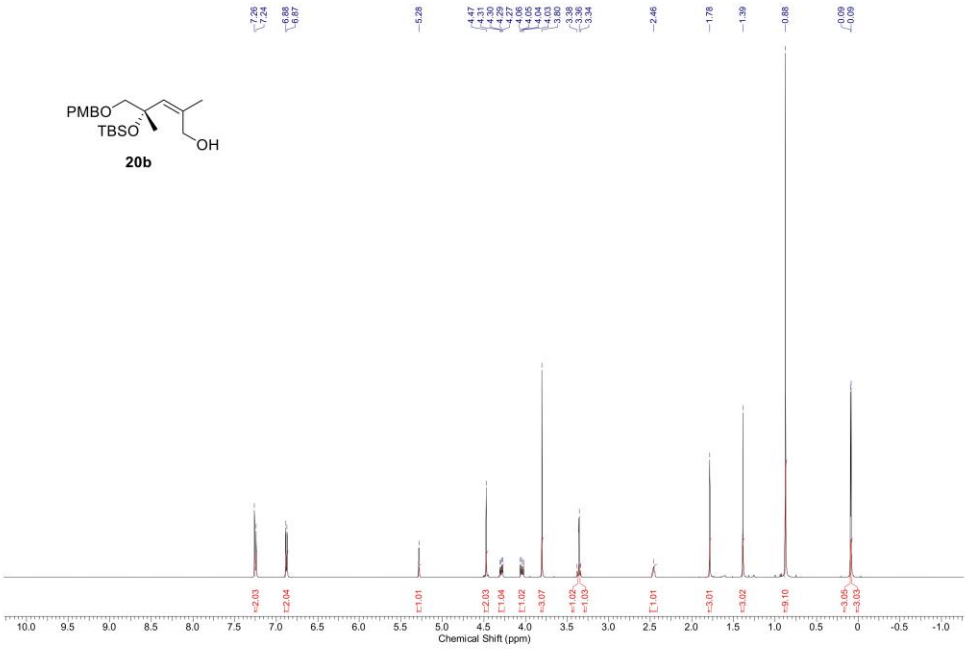
^{13}C{^{1}H}-NMR (APT) spectrum of compound **1** in DMSO-*d*6 (125 MHz).



^{1}H-NMR spectrum of compound **19b** in $CDCl_3$ (500 MHz).

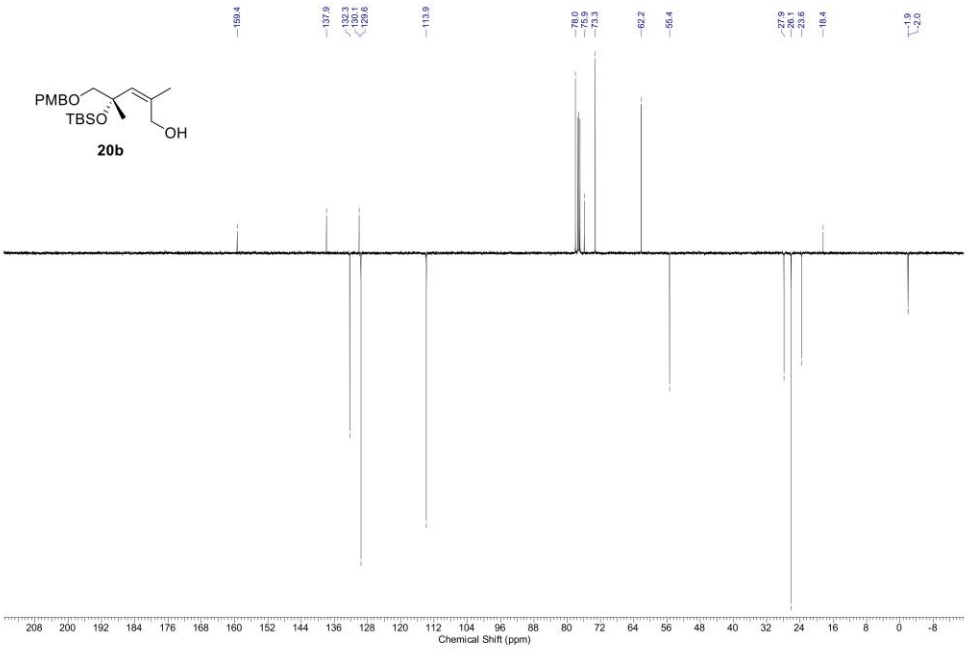


^{13}C{^{1}H}-NMR (APT) spectrum of compound **19b** in $CDCl_3$ (125 MHz).



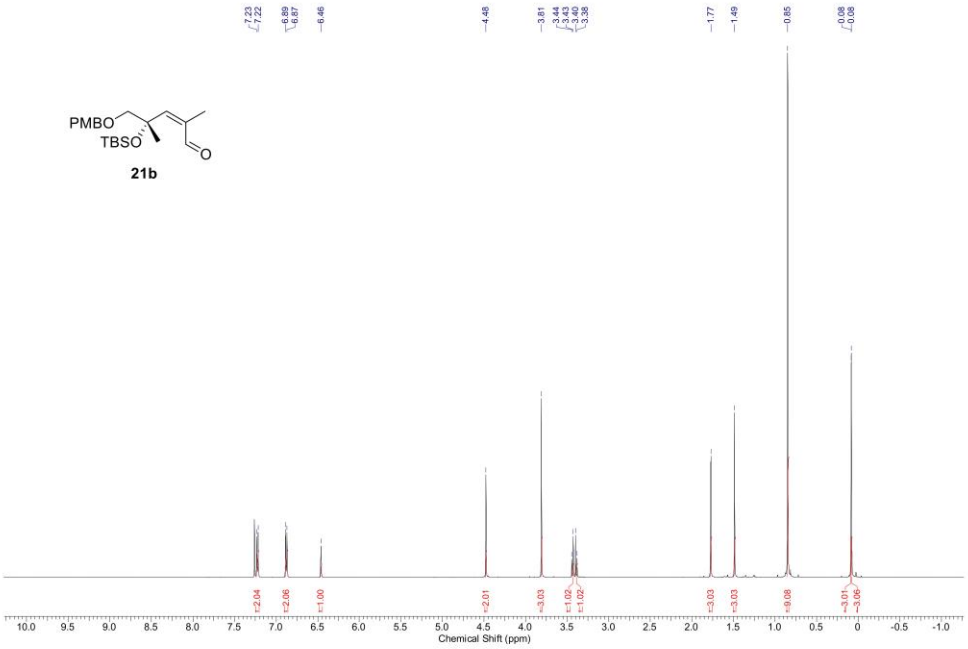
^{1}H-NMR spectrum of compound **20b** in $CDCl_3$ (500 MHz).

S74



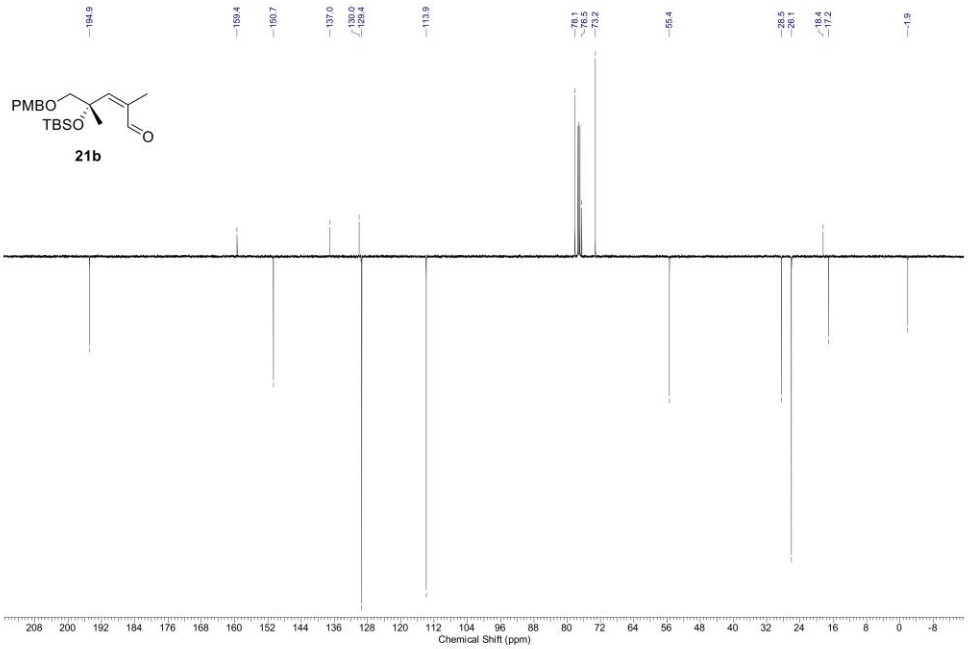
$^{13}C\{^{1}H\}$-NMR (APT) spectrum of compound **20b** in $CDCl_3$ (125 MHz).

S75



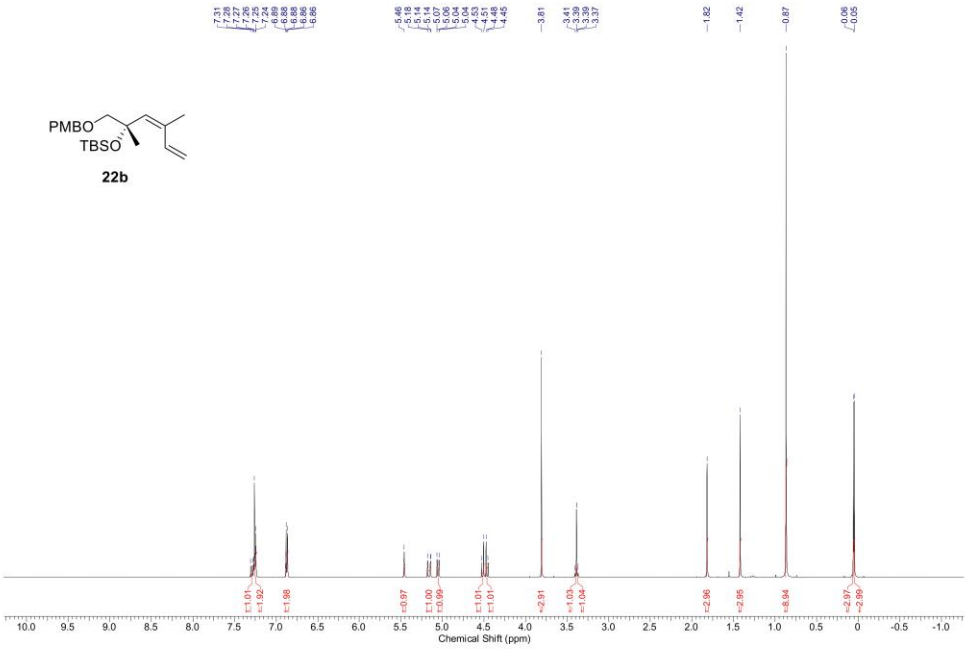
^{1}H-NMR spectrum of compound **21b** in $CDCl_3$ (500 MHz).

S76

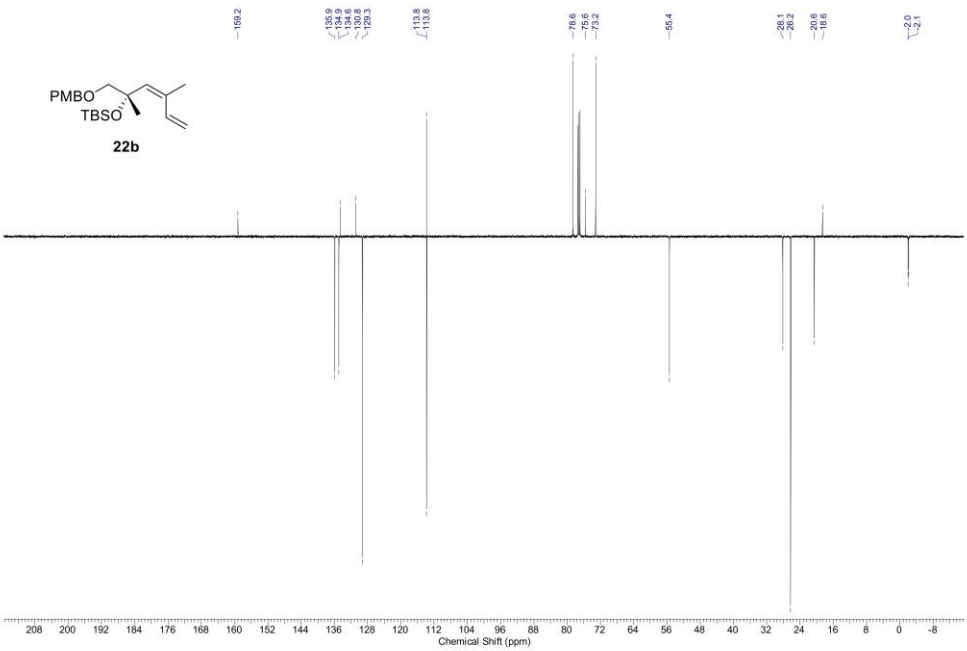


^{13}C{^{1}H}-NMR (APT) spectrum of compound **21b** in $CDCl_3$ (125 MHz).

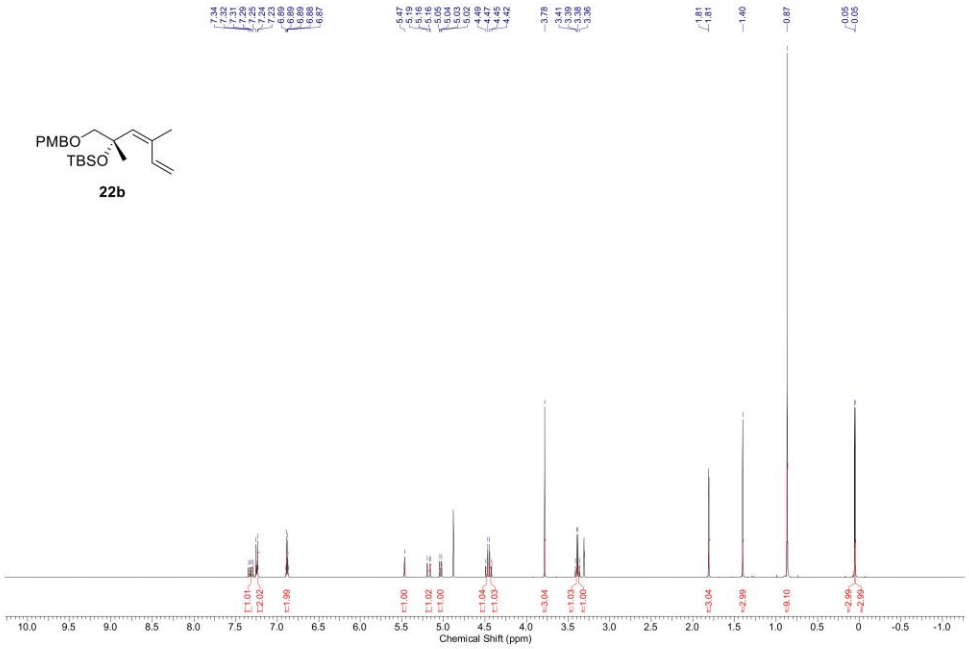
S77



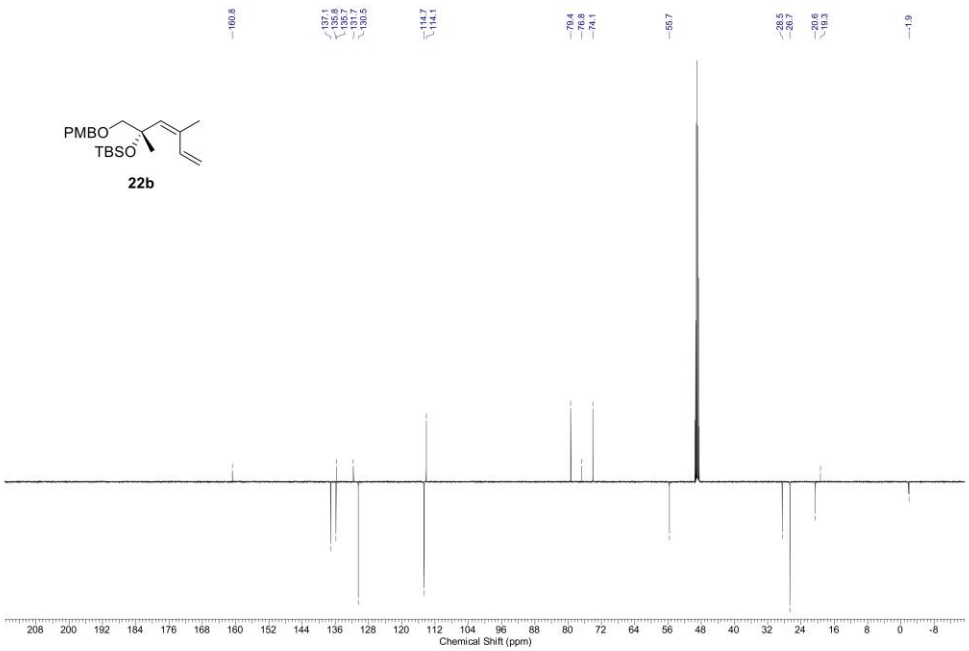
^{1}H-NMR spectrum of compound **22b** in $CDCl_3$ (500 MHz).



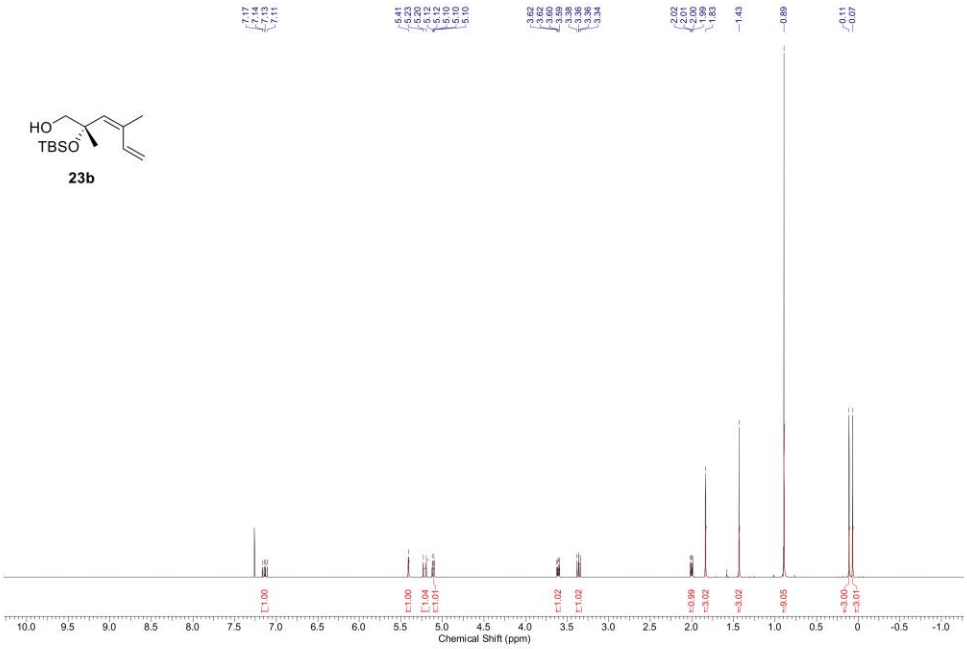
^{13}C{^{1}H}-NMR (APT) spectrum of compound **22b** in $CDCl_3$ (125 MHz).



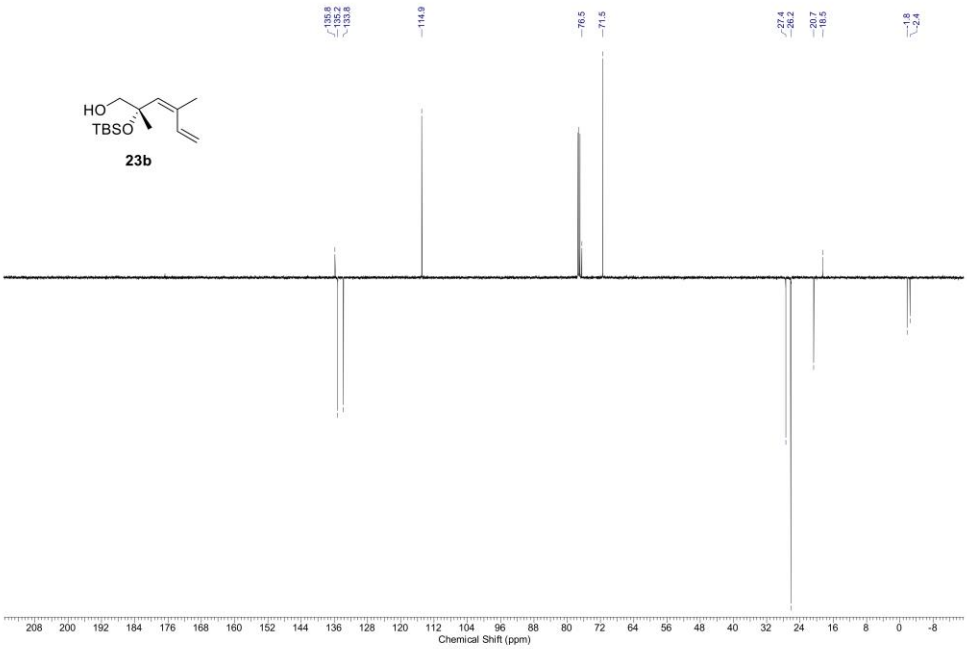
^{1}H-NMR spectrum of compound **22b** in MeOD (500 MHz).



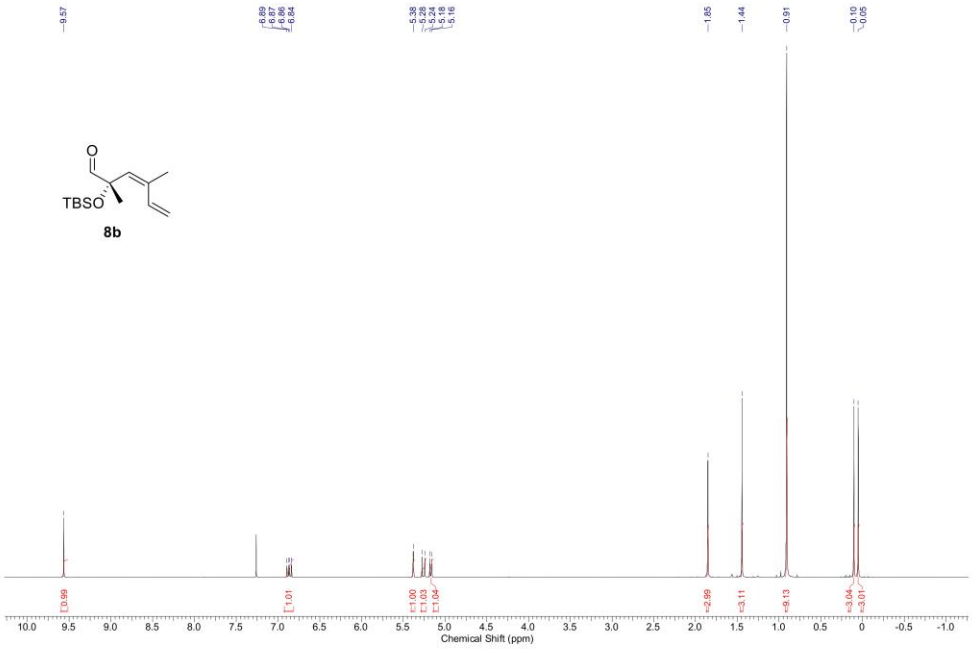
^{13}C{^{1}H}-NMR (APT) spectrum of compound **22b** in MeOD (125 MHz).



^{1}H-NMR spectrum of compound **23b** in $CDCl_3$ (500 MHz).

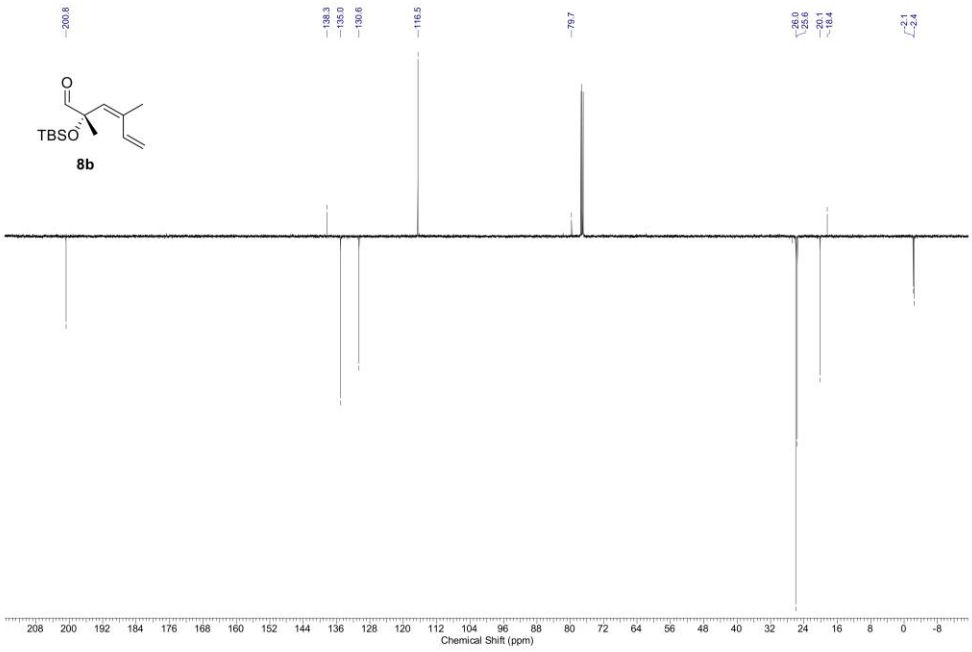


^{13}C{^{1}H}-NMR (APT) spectrum of compound **23b** in $CDCl_3$ (125 MHz).



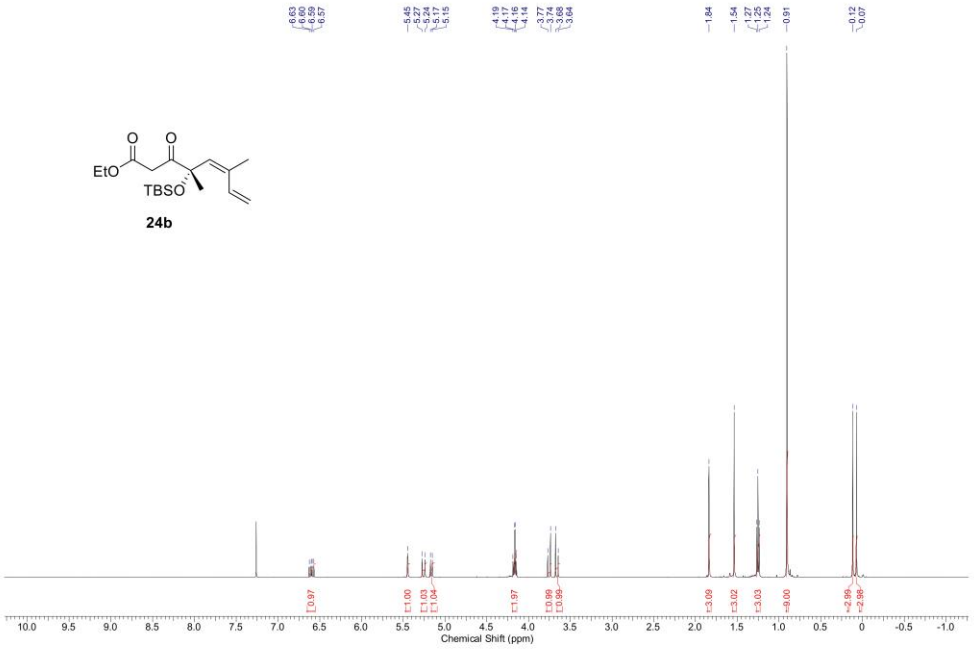
^{1}H-NMR spectrum of compound **8b** in $CDCl_3$ (500 MHz).

S84

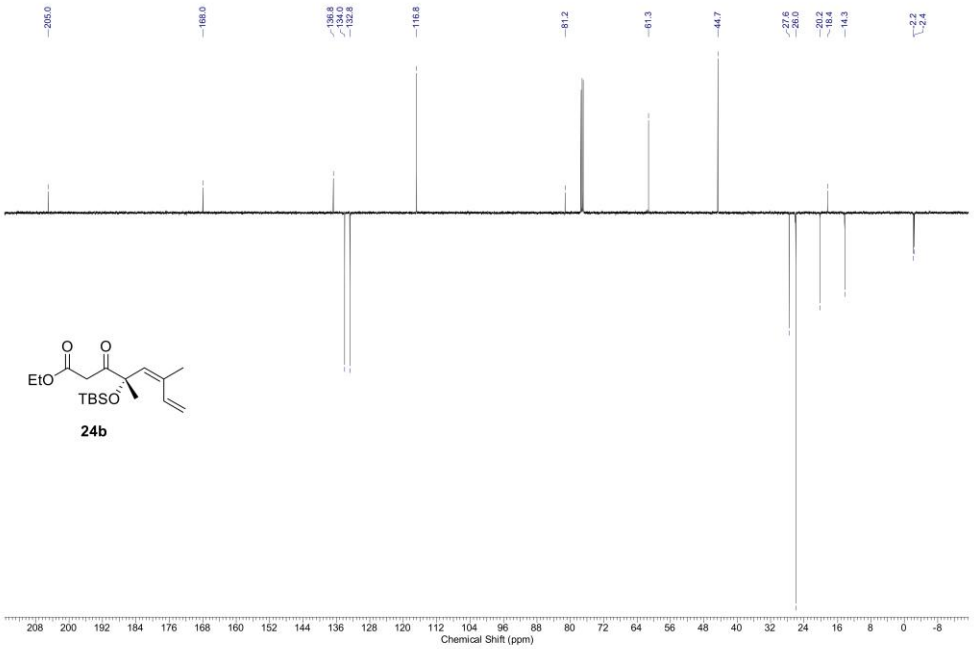


^{13}C{^{1}H}-NMR (APT) spectrum of compound **8b** in $CDCl_3$ (125 MHz).

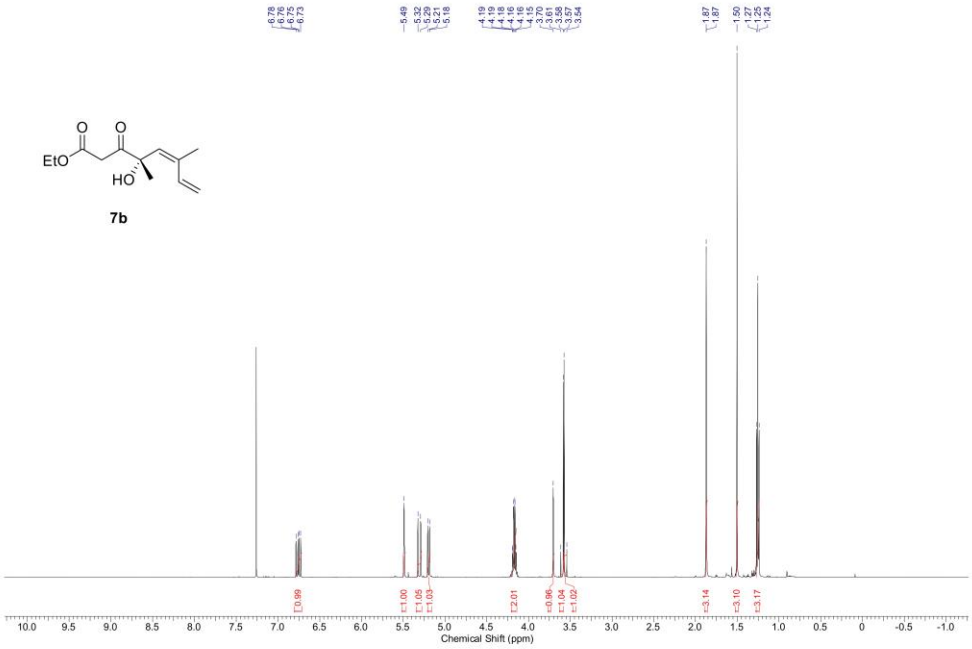
S85



^{1}H-NMR spectrum of compound **24b** in $CDCl_3$ (500 MHz).

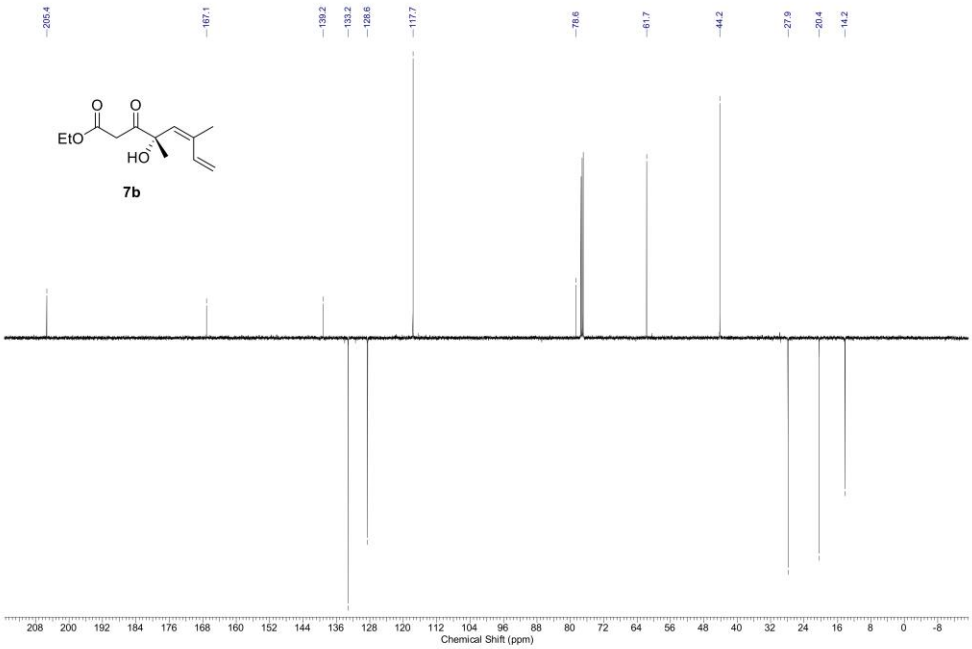


^{13}C{^{1}H}-NMR (APT) spectrum of compound **24b** in $CDCl_3$ (125 MHz).



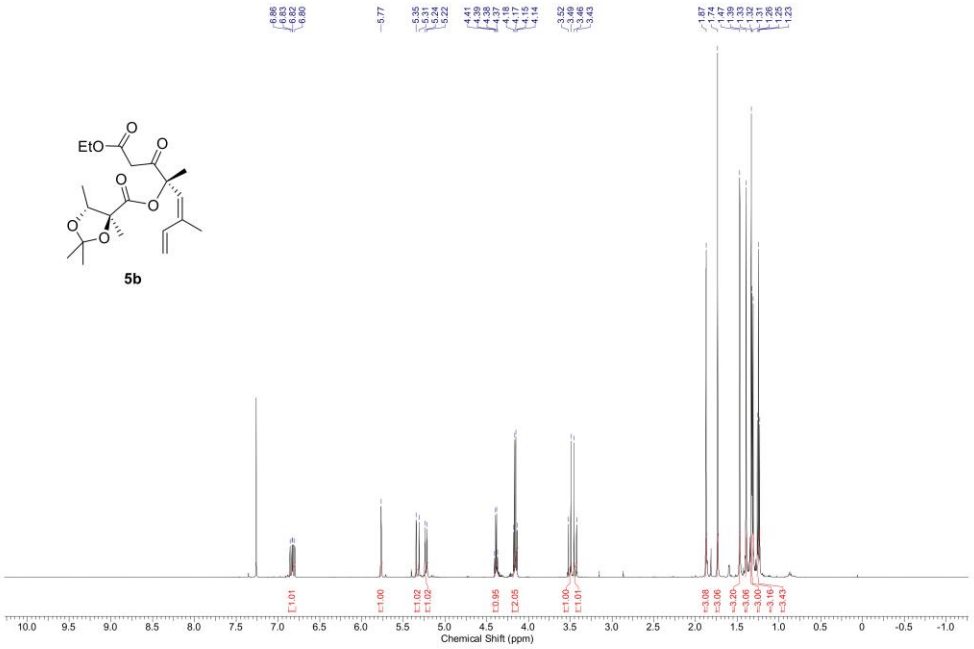
^{1}H-NMR spectrum of compound **7b** in $CDCl_3$ (500 MHz).

S88

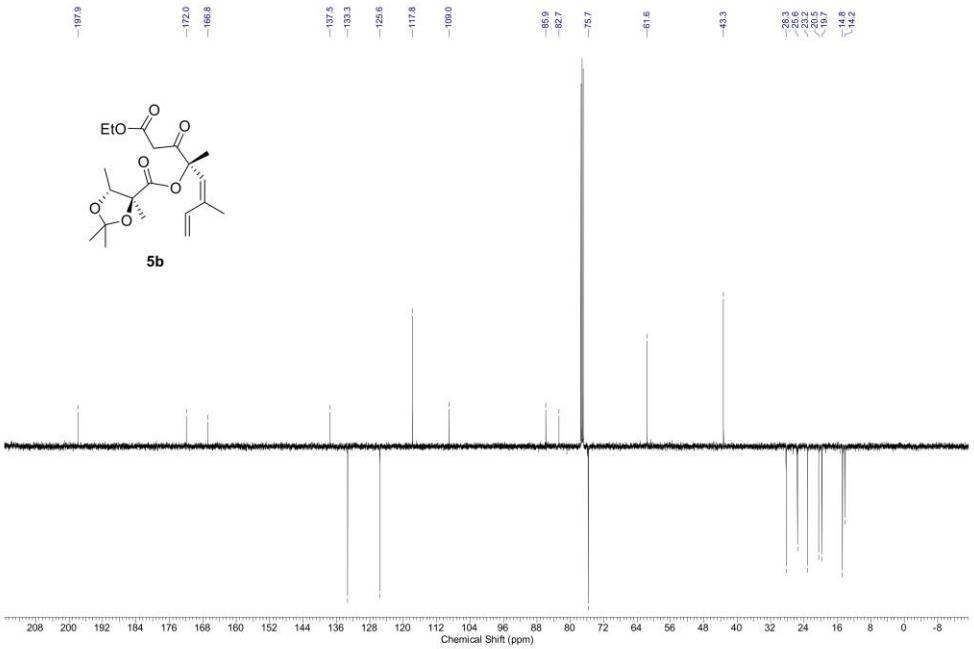


$^{13}C\{^{1}H\}$-NMR (APT) spectrum of compound **7b** in $CDCl_3$ (125 MHz).

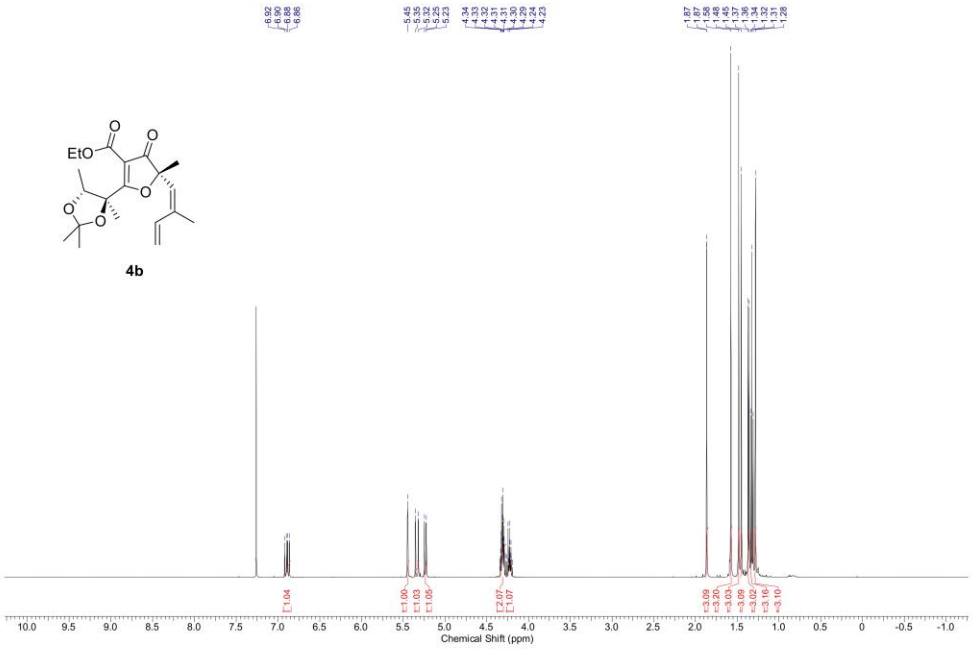
S89



^{1}H-NMR spectrum of compound **5b** in $CDCl_3$ (500 MHz).

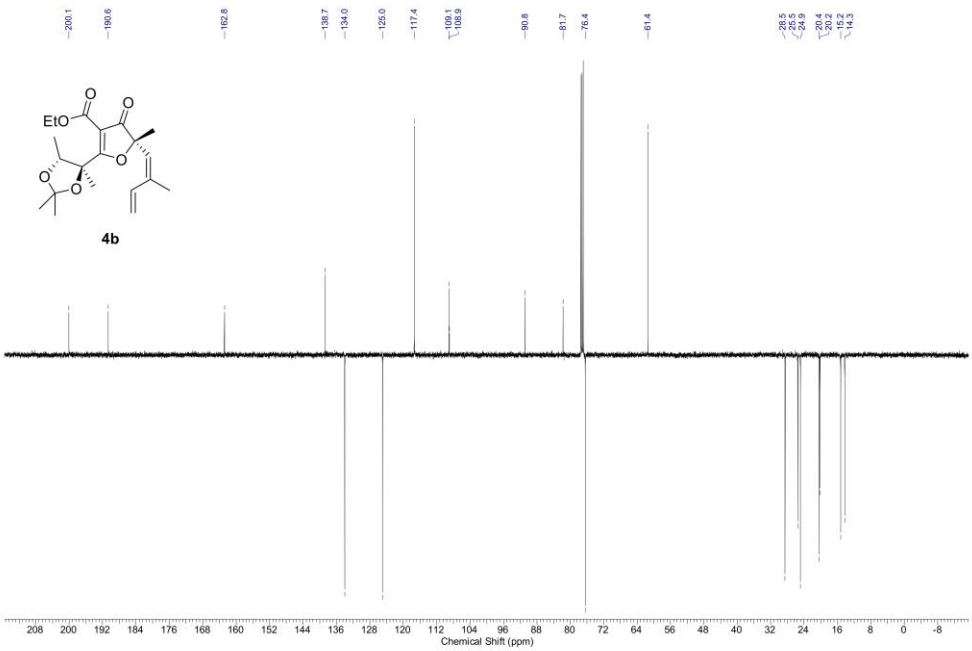


^{13}C{^{1}H}-NMR (APT) spectrum of compound **5b** in $CDCl_3$ (125 MHz).



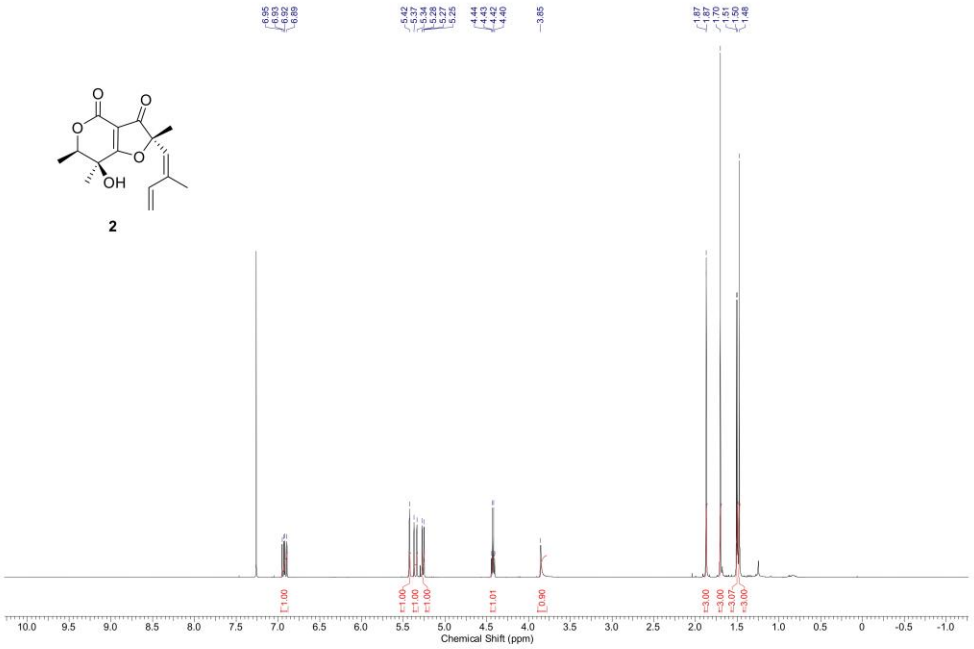
^{1}H-NMR spectrum of compound **4b** in $CDCl_3$ (500 MHz).

S92

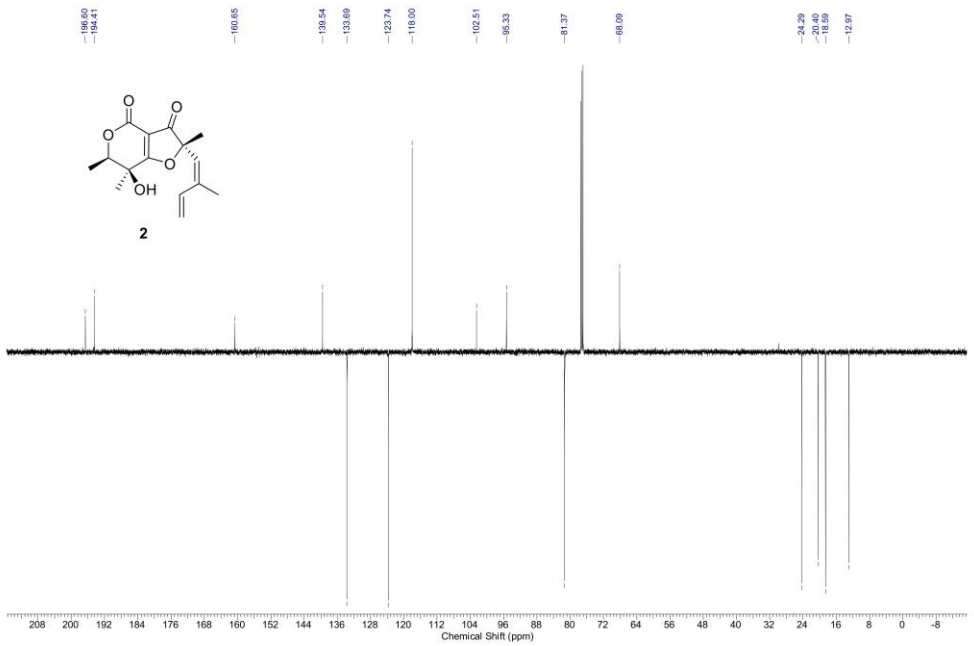


$^{13}C\{^{1}H\}$-NMR (APT) spectrum of compound **4b** in $CDCl_3$ (125 MHz).

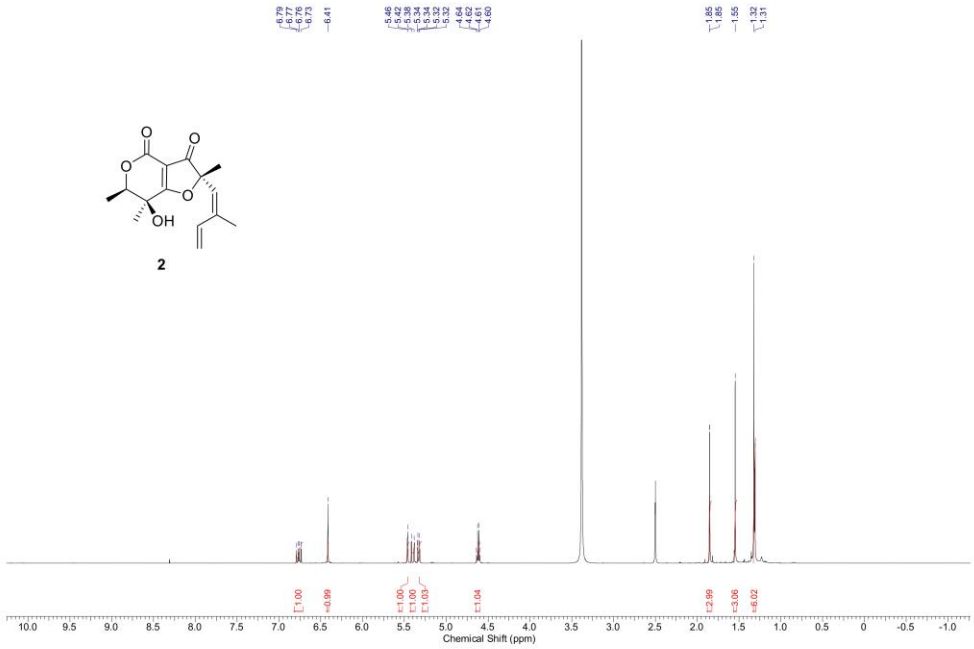
S93



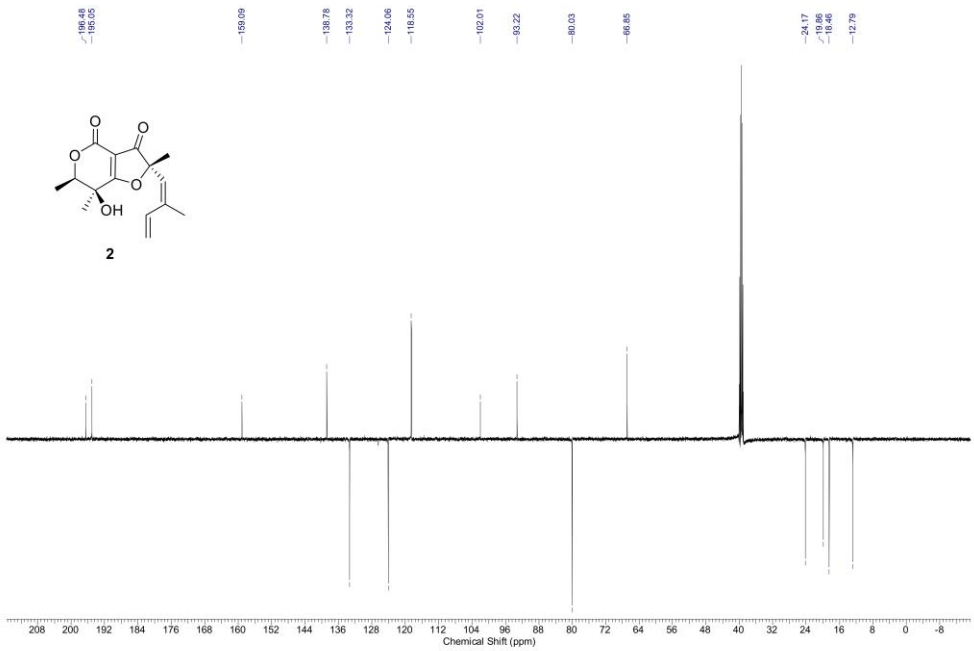
^{1}H-NMR spectrum of compound **2** in $CDCl_3$ (500 MHz).



^{13}C{^{1}H}-NMR (APT) spectrum of compound **2** in $CDCl_3$ (125 MHz).



^{1}H-NMR spectrum of compound **2** in DMSO-*d*6 (500 MHz).



^{13}C{^{1}H}-NMR (APT) spectrum of compound **2** in DMSO-*d*6 (125 MHz).

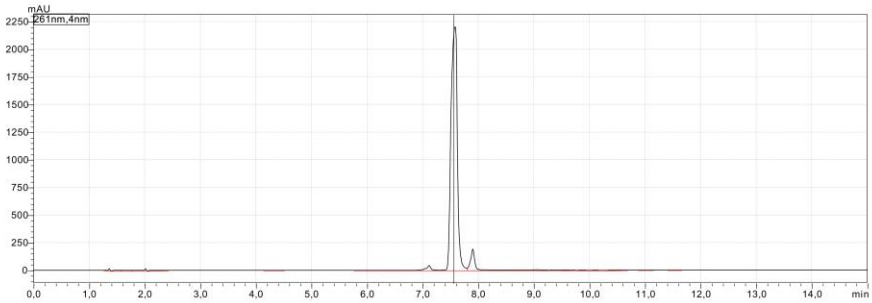


Figure S5: HPLC chromatogram of ophiofuranone A (**1**). HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 10% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

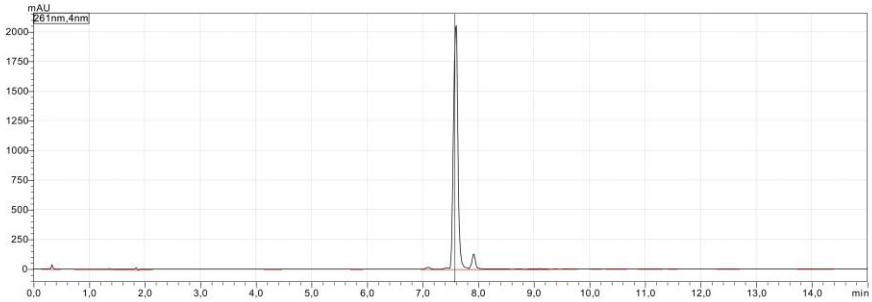


Figure S6: HPLC chromatogram of ophiofuranone B (**2**). HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 10% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

5.3 Publikation II

Synthesis and Bioactivity of Thiocarboxylic A and Derivatives

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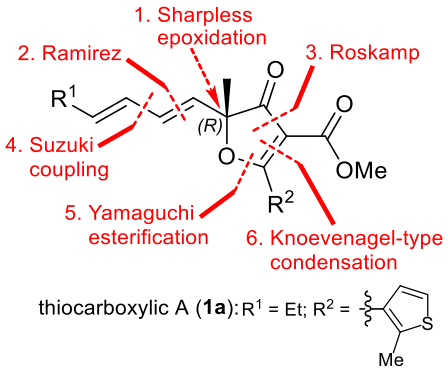

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Synthesis and Bioactivity of Thiocarboxylic A and Derivatives

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Cite This: *J. Nat. Prod.* 2022, 85, 2828−2835 Read Online

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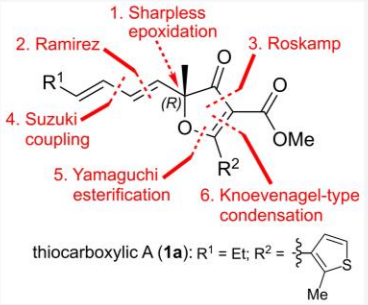
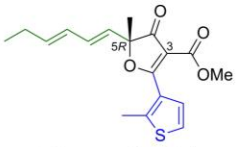
ABSTRACT: The *Penicillium* metabolite thiocarboxylic A (**1a**) and three close analogues were synthesized in 14 steps. The stereogenic elements were installed via stereoselective Sharpless epoxidation, (*E*)-selective reduction of a dibromide, and a Suzuki cross coupling. Thiocarboxylic A (**1a**) was obtained in 6% overall yield. The synthetic product and the natural isolate differed markedly in their specific rotations and antibiotic activities against *Escherichia coli* and *Staphylococcus aureus*. This modular synthetic route should be flexible enough to allow the synthesis of other natural and non-natural 3-methoxycarbonyldihydrofuran-4-ones for biological studies.



Thiocarboxylic A (**1a**) was isolated in 2020 by Ruan et al.[1] from the fungus *Penicillium* sp. Sb62 together with 16 other 3-methoxycarbonyl-dihydrofuran-4-ones. The structure was assigned mainly by means of NMR and ECD spectra. Four of the compounds featured a thiophene moiety, which is quite uncommon for this class of natural products. Thiocarboxylic A (**1a**) showed greater antimicrobial activity than analogues lacking this thienyl residue against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* with MIC values ranging from 1.7 to 3.0 μg/mL.



Thiocarboxylic A (**1a**)

To date, five different synthetic approaches to related dihydrofuranones were reported by the groups of Brückner et al.[2] and Kato et al.,[3] exemplified by the total syntheses of gregatins A,[2b] B,[2,3a] C,[2b] D,[2b] and E,[2c,3a] aspertetronin A,[2b] and graminin A.[3b] While their introduction of the side chain via a cross coupling reaction[2a,c,3] and their ring closure of the dihydrofuranone by a Knoevenagel-type reaction[2b,c,3] are similar to our synthesis, these syntheses are limited as to either the residue of the side chain (R^1, green) or the residue on the 4-furanone (R^2, blue). We present a new approach to the natural product class of 3-methoxycarbonyl-dihydrofuran-4-ones which allows a flexible introduction of both residues at a late stage, rendering the majority of the natural products of this class easily accessible. Herein, we focus on a synthesis of the novel thiocarboxylic A (**1a**). As it has already shown antimicrobial activity, we also investigated its bioactivities in more detail.

RESULTS AND DISCUSSION

The retrosynthetic approach to thiocarboxylic A (**1a**) as depicted in Scheme 1 is based on key steps applied in our recently published synthesis of the structurally related ophiofuranones A and B.[4] We intended to close the dihydrofuranone ring by a final base-induced Knoevenagel-type condensation of β-ketoacyl ester **2a**.[4,5] The latter should be accessible by a Yamaguchi esterification of methyl β-keto-γ-hydroxydeca-5,7-dienoate **3a** with 2-methylthiophene-3-carboxylic acid (**4**).[4,6] For the sake of flexibility in R^1 the conjugated (*E*,*E*)-diene **3a** should be built up by a Pd-mediated cross coupling of vinyl bromide **5** and the required, in principle structurally flexible, coupling reagent **6a**.[7] β-Ketoester **5** was to be prepared by a Roskamp reaction[4,8] of aldehyde **7** with methyl diazoacetate in the presence of $SnCl_2$. Aldehyde **7** was assumed accessible from aldehyde **8** via a sequence comprised of a Ramirez olefination[9] with Ph_3P/CBr_4, followed by reduction of the formed 1,1-dibromoalkene to afford the corresponding (*E*)-1-bromoalkene,[9,10] the primary alcohol of which was to be deprotected and finally oxidized. The chiral aldehyde **8** should be prepared by Sharpless epoxidation of 2-methylallyl alcohol **9**,[4,11] followed by epoxide

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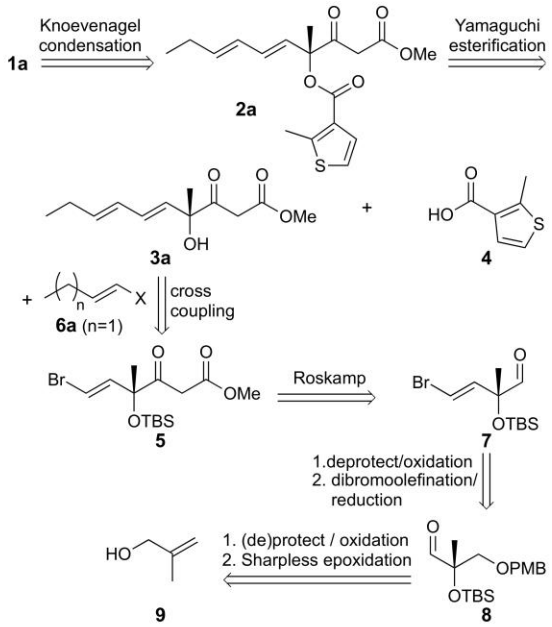
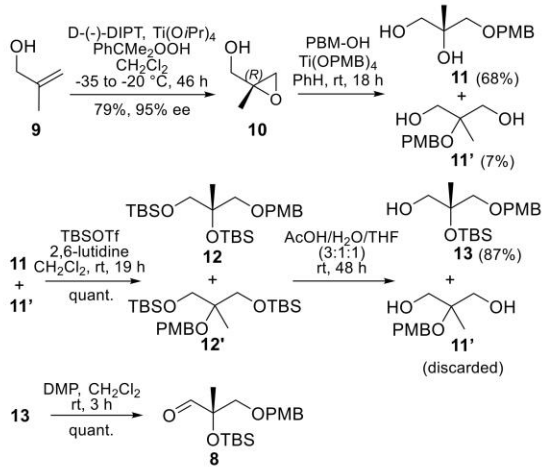
Scheme 1. Retrosynthesis of Thiocarboxylic A (1a)



opening with *para*-methoxybenzylate, protection of the resulting secondary alcohol, and oxidation of the primary one.[4]

Aldehyde **8** was prepared analogously to its enantiomer[4] in five steps starting with 2-methylallyl alcohol **9** (Scheme 2). Its

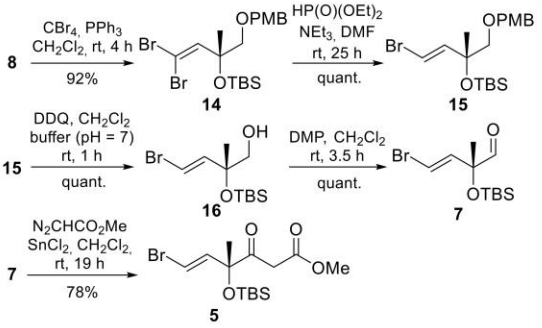
Scheme 2. Synthesis of Aldehyde 8



asymmetric Sharpless epoxidation afforded (*R*)-1-hydroxymethyl-1-methyloxirane (**10**) in 79% yield and with 95% ee. Opening of the epoxide **10** with *p*-methoxybenzyl alcohol (PMB-OH) in the presence of $Ti(OPMB)_4$ gave an inseparable 10:1 mixture of regioisomeric diols **11** and **11′** in 75% overall yield. Quantitative protection of both hydroxy groups of these diols gave a 10:1 mixture of the bis(silyl ether)s **12** and **12′** which had their primary TBS protecting groups removed with acetic acid to afford an easily separable mixture of the desired monoprotected alcohol **13** in 87% yield and the diol **11′**. Alcohol **13** was quantitatively oxidized with Dess-Martin periodinane (DMP)[12] to afford aldehyde **8**.

Aldehyde **8** was olefinated according to the Ramirez protocol[9] to give *gem*-dibromoolefin **14** in 92% yield (Scheme 3). Reduction of the dibromide with diethylphosphite and

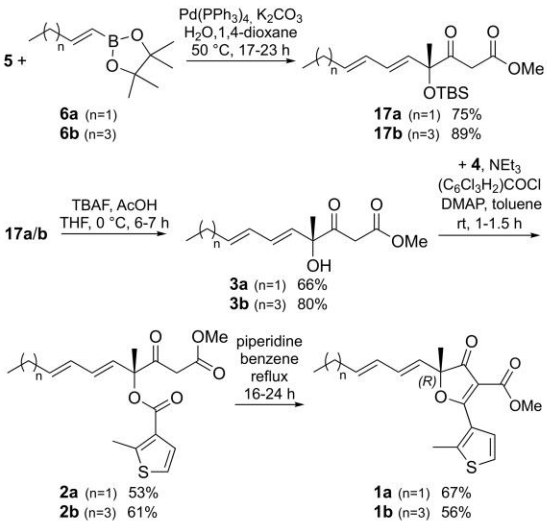
Scheme 3. Synthesis of Vinyl Bromide 5



NEt_3 led to (*E*)-configured vinyl bromide **15** in 96% yield.[10] Oxidative cleavage of the PMB protecting group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[4,13] liberated the primary alcohol **16** in near quantitative yield. Oxidation with DMP[12] yielded aldehyde **7** in 99% yield, which was converted to β-ketoester **5** via a Roskamp extension reaction[4,8] with methyl diazoacetate[14]/$SnCl_2$ in 78% yield.

In order to explore possible cross coupling reactions of vinyl bromide **5**, and because 1-hexyne is more readily available and easier to handle as a precursor of coupling reagent **6** when compared with 1-butyne, the non-natural derivative **1b** was synthesized prior to thiocarboxylic A (**1a**) (Scheme 4). As literature is rather scant on cross coupling reactions in the

Scheme 4. Synthesis of Thiocarboxylic A (1a) and Derivative 1b



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J. Nat. Prod. 2022, 85, 2828−2835

presence of β-ketoesters, some experimentation was necessary. Negishi couplings[15] did not afford product **17b**. After trying Suzuki couplings with boronate **6b** (n = 3) under different conditions,[3,16] we found that the coupling worked efficiently with 8 mol % of $Pd(PPh_3)_4$ and in the presence of K_2CO_3 as reported by Guiry et al.[17] (E,E)-Dienes **17a** and **17b** were eventually obtained with 75% and 89% yield, respectively, and void of (Z)-isomers. As boronates with other alkenyl residues are readily available[18] from the corresponding 1-alkynes, Cp_2TiClH, and pinacolborane, the residue R^1 in analogues of **1a** is flexibly selectable. With alkylboronates, (E)-monoenes should be accessible.[19]

Cleavage of the TBS groups of **17a** or **17b** with tetra-*n*-butylammonium fluoride (TBAF) and AcOH afforded β-keto-γ-hydroxyester **3a** or **3b**. Building block **3a** had already been synthesized by Burghart-Stoll and Brückner in 2012 during their synthesis of gregatins A−D and aspertetronin A.[2b] While their route is shorter than ours, it lacks the flexibility in R^1 and afforded the product with an ee of merely 90% and as an inseparable mixture of (E,Z)-isomers as occurring also in the synthetic natural products. Yamaguchi esterification[4,6] between 2-methylthiophene-3-carboxylic acid (**4**) and either of the two β-keto-γ-hydroxyesters **3** gave the corresponding esters **2a** and **2b** in moderate yields. By using other carboxylic acids, it should be possible to vary the residue R^2 on the 4-furanone ring. Base-induced Knoevenagel-type cyclization[4] of the esters **2** afforded thiocarboxylic A (**1a**) and the two C atoms longer derivative **1b** with 67% and 53% yield, respectively.

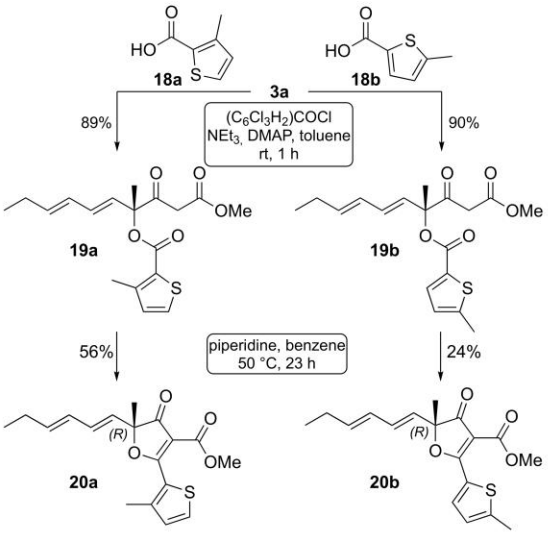
The ^{1}H NMR spectrum of our synthetic thiocarboxylic A (**1a**) agreed very well with those in the literature.[1] The ^{13}C NMR spectrum also was a good match, except for the chemical shifts of the non-hydrogenated C atoms of the thiophene and the C atom in position 3 (Table S1, Supporting Information). The specific rotation of $[\alpha]_D^{21}$ −78 (c 0.2, $CHCl_3$) for synthetic compound **1a** also deviated from $[\alpha]_D^{20}$ −43 (c 0.2, $CHCl_3$)[1] as reported for the natural isolate. Because of these discrepancies, we suspected that the thiophene residue might have been incorrectly assigned by Ruan et al.[1] We therefore prepared further derivatives with other thiophene-carboxylic acids to compare their analytical data with those of the isolate. Due to the multiplicity of the ^{1}H NMR signals of the thiophene double bond (d, J = 5.4 Hz) in the isolate, there were only two possible constitutional isomers left.

Thus, building block **3a** was esterified with the carboxylic acids **18a** and **18b** in good yields (Scheme 5). Cyclization of the resulting β-ketoesters **19a** and **19b** with piperidine in benzene led to the thiocarboxylic A analogues **20a** and **20b**.

Even a cursory comparison of the ^{1}H and ^{13}C NMR spectra of **20a** and **20b** with those reported for the isolate revealed that they are quite different. Neither of the derivatives **20** represents the structure of the natural isolate. A closer scrutiny of the ^{13}C NMR spectrum of the isolated natural product showed that the signals of the non-hydrogenated C atoms in question were not really visible. Their shifts were possibly assigned by peak picking without a careful check of the 2D NMR correlations. At this point we thus assumed that the structure of thiocarboxylic A (**1a**) was assigned correctly by Ruan et al.[1]

As this group had reported antimicrobial activity of their isolated thiocarboxylic A (**1a**) with an MIC of 5.1 μM against both *E. coli* and *S. aureus*,[1] we also tested our synthetic products **1a**, **1b**, **20a**, and **20b** for their activities against these bacteria (Supporting Information). Somewhat surprisingly, all

Scheme 5. Synthesis of Analogues 20a and 20b



four test compounds were virtually inactive with IC_{50}, let alone MIC, values beyond the highest tested concentration of 100 μM. Only against the more sensitive *E. coli* ΔTolC mutant, which lacks the ArcAB−TolC efflux system, was synthetic **1a** growth inhibitory with an IC_{50} of 44 μM. The divergent bioactivities and specific rotations of isolated and synthetic thiocarboxylic A might be due to minor impurities of the isolate as visible in the ^{1}H NMR spectrum.[1]

The thiophene regioisomers **20a** and **20b** were less active against *E. coli* ΔTolC, while the analogue **1b**, featuring a longer octadienyl side chain, was virtually inactive. The four synthetic end products **1a**,**b** and **20a**,**b** proved also not to be cytotoxic in concentrations up to 100 μM against cell lines A549 (human lung carcinoma), L929 (mouse fibroblasts), and Huh7 (human hepatocellular carcinoma).

■ CONCLUSIONS

In summary, we developed a flexible 14-step-long route to thiocarboxylic A starting from inexpensive 2-methylallyl alcohol. The stereogenic elements were installed via a stereoselective Sharpless epoxidation, (E)-selective reduction of a dibromide, and a Suzuki cross coupling. Thiocarboxylic A was obtained in 6% overall yield. The cause for the divergent specific rotations and antibiotic activities of isolated natural and our synthetic thiocarboxylic A is unclear, with slight impurities being a conceivable explanation. Work is in progress to apply our flexible modular approach to the synthesis of other natural and non-natural 3-methoxycarbonyldihydrofuran-4-ones.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with a Büchi M-565 melting point apparatus and are uncorrected. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 polarimeter using solutions in $CHCl_3$. IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance III HD 500 spectrometer. Chemical shifts of NMR signals are given using the residual solvent peak as an internal

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J. Nat. Prod. 2022, 85, 2828−2835

standard, i.e., 7.26 ppm (proton) and 77.16 ppm (carbon) for $CDCl_3$. High-resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. Elemental analyses were measured with a PerkinElmer 2400 CHN elemental analyzer. The determination of the enantiomeric excess (ee) of chiral compounds was performed on a gas chromatograph GC-FID 2010 equipped with an autosampler AOC 20i, a Lipotex A column (length: 25 m; inside diameter: 0.25 mm; bonded), and a flame ionization detector (FID) 2010. Analytical HPLC measurements were carried out on a Shimadzu Nexera XR with a SIL-20A autosampler using a Knauer Eurospher II C-18 column (150 × 4 mm), pore size 100 Å, particle size 3 μm. Detection was executed by an SPD-M20A diode array detector.

Chemicals. All reagents were purchased from commercial sources and were used without further purification. Boronates **6a**[2a] and **6b**[18b] were prepared according to literature methods. All anhydrous solvents were used as supplied, except tetrahydrofuran (THF), toluene, and 1,4-dioxane, which were freshly distilled over sodium/benzophenone, and benzene, dimethylformamide (DMF), and CH_2Cl_2, which were dried over molecular sieves (3 Å). Moisture- or air-sensitive reactions were routinely carried out in oven-dried glassware under an argon atmosphere using standard Schlenk techniques.

Chromatography. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel $60GF_{254}$ precoated aluminum-backed plates. The compounds were visualized with UV light (254 nm) and/or ceric ammonium molybdate (CAM). Column chromatography was performed at medium pressure using dry packed Macherey-Nagel silica gel 60, pore size 40−63 μm, with the eluent specified.

(*R*)-(2-Methyloxiran-2-yl)methanol (10). A mixture of crushed 4 Å activated molecular sieves (8.00 g) in dry CH_2Cl_2 (200 mL) was cooled to −35 °C. $Ti(OiPr)_4$ (1.78 mL, 6.00 mmol, 0.10 equiv) and diisopropyl D-(−)-tartrate (1.88 mL, 9.00 mmol, 0.15 equiv) were added by syringe. After the mixture was stirred at −35 °C for 30 min, 2-methyl-prop-2-en-1-ol (**9**) (5.10 mL, 60.0 mmol, 1.00 equiv) in dry CH_2Cl_2 (20 mL) was added, followed by cumene hydroperoxide (16.8 mL, 90.0 mmol, 1.50 equiv). The reaction mixture was stirred at −35 °C for 1 h and then at −20 °C for 46 h. Aqueous saturated Na_2SO_4 (6 mL) was added at −20 °C, and the mixture was diluted with Et_2O (60 mL). After the mixture was stirred at ambient temperature for 1 h, the resulting slurry was filtered through a pad of Celite and the resulting solution was concentrated under reduced pressure. The residue was purified by column chromatography (50% Et_2O in *n*-pentane) to yield epoxide **10** as a colorless oil (4.16 g, 47.2 mmol, 79%, 95% ee): R_f = 0.36 (80% Et_2O in *n*-pentane); $[\alpha]_D^{22}$ +10.6 (*c* 1.00, $CHCl_3$) [lit.[20] $[\alpha]_D^{25}$ +11.1 (*c* 0.9, $CHCl_3$)]; IR (neat) ν_{max} 3386 (broad), 2925, 1391, 1069, 1041, 883, 800, 704, 686 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 3.72 (dd, *J* = 12.2, 4.4 Hz, 1 H), 3.60 (dd, *J* = 12.3, 8.6 Hz, 1 H), 2.91 (d, *J* = 4.7 Hz, 1 H), 2.65 (d, *J* = 4.7 Hz, 1 H), 1.87 (dd, *J* = 8.5, 4.6 Hz, 1 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 64.3, 57.3, 51.1, 18.2; HRESIMS *m*/*z* 89.0600 $[M + H^+]$ (calcd for $C_4H_9O_2^+$, 89.0597).

(*R*)-3-((4-Methoxybenzyl)oxy)-2-methylpropane-1,2-diol (11). A mixture of $Ti(OiPr)_4$ (14.1 mL, 47.7 mmol, 1.50 equiv) and 4-methoxybenzyl alcohol (62 mL) was stirred under reduced pressure (8 mbar) for 4 h to remove the 2-propanol. The stirred solution was treated with epoxide **10** (2.80 g, 31.8 mmol, 1.00 equiv) in dry benzene (280 mL), and the resulting mixture was stirred at ambient temperature for 18 h. The solution was diluted with Et_2O (300 mL), and aqueous sulfuric acid (0.5 M, 300 mL) was added. The two-phase mixture was stirred vigorously until two clear phases formed. The layers were separated, and the aqueous one was extracted with Et_2O (3 × 200 mL). The combined organic phases were dried over Na_2SO_4, and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (30% EtOAc in *n*-pentane to EtOAc) afforded a regioisomeric mixture of **11** and **11′** as a colorless oil that turned solid at −20 °C (5.38 g, 23.8 mmol, 75%). The regioisomeric ratio of the epoxide opening amounted to 10:1 (determined via ^1H NMR). A small amount of the regioisomeric mixture of **11** and **11′** was purified additionally by column chromatography (10% to 30% EtOAc in *n*-pentane) to get pure diol **11** for analytics: R_f = 0.48 (EtOAc); mp 43−44 °C (lit.[4] value for the enantiomer of **11**: 44−45 °C); $[\alpha]_D^{22}$ −8.7 (*c* 1.00, $CHCl_3$) [lit.[4] value for the enantiomer of **11**: $[\alpha]_D^{24}$ +8.1 (*c* 1.00, $CHCl_3$)]; IR (neat) ν_{max} 3337 (broad), 2911, 1611, 1512, 1255, 1237, 1078, 1048, 1035, 817, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.25−7.22 (m, 2 H), 6.91−6.87 (m, 2 H), 4.50 (d, *J* = 11.5 Hz, 1 H), 4.47 (d, *J* = 11.5 Hz, 1 H), 3.81 (s, 3 H), 3.63 (dd, *J* = 11.1, 4.6 Hz, 1 H), 3.48 (d, *J* = 9.1 Hz, 1 H), 3.43 (dd, *J* = 10.9, 8.2 Hz, 1 H), 3.39 (d, *J* = 9.3 Hz, 1 H), 2.82−8.78 (m, 1 H), 2.41−2.33 (m, 1 H), 1.13 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 159.5, 129.8, 129.5 (2 C atoms), 114.0 (2 C atoms), 76.2, 73.5, 71.9, 68.9, 55.4, 21.5; HRESIMS *m*/*z* 249.1095 $[M + Na^+]$ (calcd for $C_{12}H_{18}O_4Na^+$, 249.1097).

(*S*)-1,2-Bis(*tert*-butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)-2-methylpropane (12). To a solution of regioisomeric mixture **11** and **11′** (11.2 g, 49.5 mmol, 1.00 equiv) in dry CH_2Cl_2 (500 mL) were added 2,6-lutidine (40.2 mL, 347 mmol, 7.00 equiv) and TBSOTf (57.0 mL, 247 mmol, 5.00 equiv) at 0 °C. The reaction mixture was stirred for 19 h at ambient temperature. The reaction was quenched with saturated aqueous NH_4Cl (400 mL) at 0 °C. The aqueous phase was extracted with Et_2O (3 × 300 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The remainder was purified by column chromatography (*n*-pentane to 6% EtOAc in *n*-pentane), to afford bis(silyl ether)s **12** and **12′** as a colorless oil (22.5 g, 49.4 mmol, 100%). The regioisomeric ratio was still 10:1 (determined via ^1H NMR). A small amount of the regioisomeric mixture of **12** and **12′** was purified additionally by column chromatography (*n*-pentane to 5% EtOAc in *n*-pentane) to get pure silyl ether **12** for analytics: R_f = 0.29 (2% EtOAc in *n*-hexane); $[\alpha]_D^{22}$ −3.8 (*c* 1.00, $CHCl_3$) [lit.[4] value for the enantiomer of **12**: $[\alpha]_D^{25}$ +3.4 (*c* 1.00, $CHCl_3$)]; IR (neat) ν_{max} 2929, 1514, 1247, 1095, 1040, 831, 805, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.26−7.23 (m, 2 H), 6.89−6.85 (m, 2 H), 4.45 (d, *J* = 11.6 Hz, 1 H), 4.43 (d, *J* = 11.6 Hz, 1 H), 3.81 (s, 3 H), 3.52 (d, *J* = 9.5 Hz, 1 H), 3.363 (d, *J* = 9.6 Hz, 1 H), 3.361 (d, *J* = 9.3 Hz, 1 H), 3.28 (d, *J* = 9.2 Hz, 1 H), 1.15 (s, 3 H), 0.88 (s, 9 H), 0.84 (s, 9 H), 0.06 (s, 3 H), 0.05 (s, 3 H), 0.031 (s, 3 H), 0.029 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 159.1, 131.1, 129.2 (2 C atoms), 113.7 (2C atoms), 76.1, 74.9, 73.1, 68.1, 55.4, 26.1 (3 C atoms), 26.0 (3C atoms), 22.6, 18.44, 18.35, −2.21, −2.24, −5.32, −5.34; HRESIMS *m*/*z* 477.2823 $[M + Na^+]$ (calcd for $C_{24}H_{46}O_4Si_2Na^+$, 477.2827).

(*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)-oxy)-2-methylpropan-1-ol (13). A solution of the mixture of silyl ethers **12** and **12′** (4.01 g, 8.82 mmol, 1.00 equiv) in dry THF (36 mL) was treated with H_2O (36 mL) and AcOH (108 mL) at 0 °C, and the resulting mixture was stirred at ambient temperature for 48 h. The reaction was slowly quenched with aqueous NaOH (0.95 M, 200 mL) at 0 °C. The aqueous phase was extracted with CH_2Cl_2 (4 × 150 mL), and the combined organic phases were washed with saturated aqueous $NaHCO_3$ (500 mL). Drying the organic layer over Na_2SO_4, removing the volatiles *in vacuo*, and purification of the residue by column chromatography (5% EtOAc in *n*-pentane) yielded alcohol **13** as a colorless oil (2.61 g, 7.66 mmol, 87%): R_f = 0.53 (20% EtOAc in *n*-hexane); $[\alpha]_D^{22}$ −3.2 (*c* 1.00, $CHCl_3$) [lit.[4] value for the enantiomer of **13**: $[\alpha]_D^{22}$ +3.8 (*c* 1.00, $CHCl_3$)]; IR (neat) ν_{max} 3453 (broad), 2929, 1613, 1514, 1246, 1092, 1036, 833, 808, 774 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.25−7.22 (m, 2 H), 6.89−6.86 (m, 2 H), 4.47 (d, *J* = 11.6 Hz, 1 H), 4.44 (d, *J* = 11.6 Hz, 1 H), 3.81 (s, 3 H), 3.51 (dd, *J* = 10.8, 8.2 Hz, 1 H), 3.44 (d, *J* = 9.0 Hz, 1 H), 3.42 (dd, *J* = 10.8, 4.6 Hz, 1 H), 3.31 (d, *J* = 8.9 Hz, 1 H), 2.17 (dd, *J* = 8.2, 4.7 Hz, 1 H), 1.23 (s, 3 H), 0.86 (s, 9 H), 0.10 (s, 3 H), 0.08 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 159.3, 130.4, 129.3 (2 C atoms), 113.9 (2 C atoms), 75.6, 75.4, 73.3, 69.2, 55.4, 25.9 (3 C atoms), 22.6, 18.3, −2.15, −2.16; HRESIMS *m*/*z* 363.1956 $[M + Na^+]$ (calcd for $C_{18}H_{32}O_4SiNa^+$, 363.1962).

(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)-oxy)-2-methylpropanal (8). To a stirred solution of alcohol **13** (3.00 g, 8.81 mmol, 1.00 equiv) in dry CH_2Cl_2 (90 mL) was added DMP (4.48 g, 10.6 mmol, 1.20 equiv) at 0 °C. The reaction mixture was allowed to warm up to rt. After 3 h saturated aqueous $NaHCO_3$ (90 mL) and saturated aqueous $Na_2S_2O_3$ (90 mL) were added, and

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J. Nat. Prod. 2022, 85, 2828−2835

the layers were separated. The aqueous layer was extracted with Et_2O (3 × 40 mL). The combined organic phases were washed with saturated aqueous $Na_2S_2O_3$ (2 × 500 mL) and saturated aqueous $NaHCO_3$ (2 × 500 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure, and aldehyde **8** was obtained without further purification as a colorless oil (2.98 g, 8.80 mmol, 100%): R_f = 0.64 (10% EtOAc in *n*-hexane); $[\alpha]_D^{22}$ −6.4 (*c* 1.00, $CHCl_3$) [lit.[4] value for the enantiomer of **8**: $[\alpha]_D^{22}$ +3.2 (*c* 1.00, $CHCl_3$)]; IR (neat) ν_{max} 2930, 1739, 1514, 1247, 1097, 1036, 833, 810, 776 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 9.60 (s, 1 H), 7.23−7.19 (m, 2 H), 6.89−6.85 (m, 2 H), 4.45 (d, *J* = 11.7 Hz, 1 H), 4.41 (d, *J* = 11.7 Hz, 1 H), 3.81 (s, 3 H), 3.50 (d, *J* = 9.7 Hz, 1 H), 3.45 (d, *J* = 9.7 Hz, 1 H), 1.25 (s, 3 H), 0.89 (s, 9 H), 0.093 (s, 3 H), 0.087 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 204.2, 159.3, 130.0, 129.4 (2 C atoms), 113.9 (2 C atoms), 80.4, 74.8, 73.3, 55.4, 25.9 (3 C atoms), 20.8, 18.4, −2.47, −2.50; HRESIMS *m*/*z* 339.1989 [M + H^+] (calcd for $C_{18}H_{31}O_4Si^+$, 339.1986).

(*R*)-*tert*-Butyl((4,4-dibromo-1-((4-methoxybenzyl)oxy)-2-methylbut-3-en-2-yl)oxy)dimethylsilane (14). A solution of aldehyde **8** (1.04 g, 3.06 mmol, 1.00 equiv) in dry CH_2Cl_2 (30 mL) was treated with CBr_4 (1.52 g, 4.59 mmol, 1.50 equiv) in one portion and PPh_3 (2.41 g, 9.18 mmol, 3.00 equiv) in portions at 0 °C. The dark red reaction mixture was stirred for 4 h at ambient temperature before it was diluted with *n*-pentane (100 mL) to precipitate the remaining PPh_3 and triphenylphosphine oxide. The mixture was filtered through a pad of Celite, and the resulting solution was concentrated under reduced pressure. The residue was purified by column chromatography (*n*-pentane to 2% EtOAc in *n*-pentane) to afford dibromide **14** as a colorless oil (1.39 g, 2.81 mmol, 92%): R_f = 0.63 (4% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +2.2 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2929, 1612, 1513, 1247, 1095, 1035, 833, 824, 808, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.28−7.24 (m, 2 H) (signal is located under the signal of $CDCl_3$), 6.90−6.87 (m, 2 H), 6.71 (s, 1 H), 4.48 (s, 2 H), 3.81 (s, 3 H), 3.47 (d, *J* = 9.4 Hz, 1 H), 3.42 (d, *J* = 9.3 Hz, 1 H), 1.46 (s, 3 H), 0.87 (s, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 159.3, 143.5, 130.4, 129.4 (2 C atoms), 113.9 (2 C atoms), 87.8, 77.0, 76.2, 73.2, 55.4, 26.1 (3 C atoms), 25.1, 18.4, −2.2, −2.3; anal. calcd for $C_{19}H_{30}Br_2O_3Si$ C, 46.16; H, 6.12, found C, 45.98; H, 5.92.

(*R,E*)-((4-Bromo-1-((4-methoxybenzyl)oxy)-2-methylbut-3-en-2-yl)oxy)(*tert*-butyl)dimethylsilane (15). A solution of dibromide **14** (11.2 g, 22.7 mmol, 1.00 equiv) in dry DMF (230 mL) was treated with NEt_3 (31.5 mL, 227 mmol, 10.0 equiv) and diethylphosphite (29.2 mL, 227 mmol, 10.0 equiv) at 0 °C. The reaction mixture was allowed to warm up to rt. After 25 h H_2O (200 mL) was added and the layers were separated. The aqueous one was extracted with Et_2O (3 × 200 mL). The combined organic phases were washed with aqueous HCl (1 M, 500 mL) and brine (500 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure, and the remainder was purified by column chromatography (*n*-pentane to 6% EtOAc in *n*-pentane) to obtain vinyl bromide **15** as a colorless oil (9.04 g, 21.8 mmol, 96%): R_f = 0.63 (4% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +2.2 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2930, 1614, 1513, 1247, 1095, 1036, 834, 825, 807, 774 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.26−7.22 (m, 2 H), 6.90−6.86 (m, 2 H), 6.30 (s, 2 H), 4.46 (s, 2 H), 3.81 (s, 3 H), 3.29 (d, *J* = 9.2 Hz, 1 H), 3.27 (d, *J* = 9.2 Hz, 1 H), 1.32 (s, 3 H), 0.86 (s, 9 H), 0.072 (s, 3 H), 0.066 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 159.3, 143.0, 130.5, 129.3 (2 C atoms), 113.9 (2 C atoms), 106.1, 77.7, 76.6, 73.2, 55.4, 26.0 (3 C atoms), 25.1, 18.4, −2.12, −2.13; anal. calcd for $C_{19}H_{31}BrO_3Si$ C, 54.93; H, 7.52, found C, 55.43; H, 7.38.

(*R,E*)-4-Bromo-2-((*tert*-butyldimethylsilyl)oxy)-2-methylbut-3-en-1-ol (16). To a stirred solution of compound **15** (464 mg, 1.12 mmol, 1.00 equiv) in dry CH_2Cl_2 (11 mL) were added phosphate buffer (pH = 7, 1.1 mL) and DDQ (380 mg, 1.68 mmol, 1.50 equiv) at 0 °C. The reaction mixture was stirred at ambient temperature for 1 h. Thereafter it was quenched with saturated aqueous $NaHCO_3$ (15 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were dried over Na_2SO_4. After removing the volatiles *in vacuo* and purification of the residue by column chromatography (10% Et_2O in *n*-pentane) alcohol **16** was obtained as a colorless oil (328 mg, 1.11 mmol, 99%): R_f = 0.37 (10% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ −36.9 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 3426 (br), 2930, 1617, 1463, 1253, 1115, 1050, 1003, 834, 774 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 6.33 (d, *J* = 13.8 Hz, 1 H), 6.28 (d, *J* = 13.8 Hz, 1 H), 3.41 (dd, *J* = 10.7, 7.0 Hz, 1 H), 3.37 (dd, *J* = 10.7, 6.3 Hz, 1 H), 1.92 (dd, *J* = 6.9, 6.3 Hz, 1 H), 1.33 (s, 3 H), 0.89 (s, 9 H), 0.12 (s, 3 H), 0.11 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 141.8, 107.2, 77.0, 70.9, 26.0 (3 C atoms) 23.6, 18.3, −2.1, −2.3; anal. calcd for $C_{11}H_{23}BrO_2Si$ C, 44.74; H, 7.85, found C, 45.26; H, 7.68.

(*R,E*)-4-Bromo-2-((*tert*-butyldimethylsilyl)oxy)-2-methylbut-3-enal (7). To a stirred solution of alcohol **16** (3.03 g, 10.3 mmol, 1.00 equiv) in dry CH_2Cl_2 (100 mL) was added DMP (5.22 g, 12.3 mmol, 1.20 equiv) at 0 °C. The reaction mixture was allowed to warm up to rt. After 3.5 h saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous $Na_2S_2O_3$ (100 mL) were added, and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic phases were washed with saturated aqueous $Na_2S_2O_3$ (2 × 600 mL) and saturated aqueous $NaHCO_3$ (2 × 600 mL) and were dried over Na_2SO_4. The volatiles were evaporated under reduced pressure, and aldehyde **7** was obtained without further purification as a colorless oil (2.99 g, 10.2 mmol, 99%): R_f = 0.85 (10% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +60.1 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} = 2931, 1737, 1255, 1199, 1128, 1004, 936, 834, 774 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 9.37 (s, 1 H), 6.49 (d, *J* = 13.6 Hz, 1 H), 6.08 (d, *J* = 13.4 Hz, 1 H), 1.42 (s, 3 H), 0.93 (s, 9 H), 0.13 (s, 3 H), 0.11 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 199.3, 137.3, 109.7, 81.8, 25.9 (3 C atoms), 22.8, 18.4, −2.25, −2.34; HRESIMS *m*/*z* 293.0557 [M + H^+] (calcd for $C_{11}H_{22}BrO_2Si^+$, 293.0567).

Methyl (*R,E*)-6-Bromo-4-((*tert*-butyldimethylsilyl)oxy)-4-methyl-3-oxohex-5-enoate (5). To a solution of glycine methyl ester hydrochloride (18.7 g, 149 mmol, 15.0 equiv) in dry CH_2Cl_2 (40 mL) was added a solution of $NaNO_2$ (12.4 g, 180 mmol, 18.2 equiv) in H_2O (20 mL). The biphasic mixture was stirred for 1.5 h at ambient temperature. Saturated aqueous $NaHCO_3$ (2.8 mL) was added, the phases were separated, and the organic phase was dried over $MgSO_4$. The freshly prepared solution of methyl diazoacetate in CH_2Cl_2 was immediately added to a solution of aldehyde **7** (2.90 g, 9.90 mmol, 1.00 equiv) in dry CH_2Cl_2 (65 mL) at 0 °C. Anhydrous $SnCl_2$ (1.91 g, 10.4 mmol, 1.05 equiv) was also added at 0 °C. The reaction mixture was allowed to warm up to rt and was stirred for 19 h at rt. The reaction was quenched with brine (100 mL) and diluted with Et_2O (200 mL). The phases were separated, and the aqueous phase was extracted five times with Et_2O (5 × 150 mL) because of poor phase separation. The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (2% to 4% EtOAc in *n*-pentane) to yield *β*-ketoester **5** as an orange oil (2.82 g, 7.71 mmol, 78%). The keto−enol ratio of **5** was 10:1 (determined via ^1H NMR): R_f = 0.31 (4% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +60.2 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2955, 1752, 1724, 1255, 1207, 1124, 992, 938, 836, 777 cm^{-1}; ^1H NMR *keto* ($CDCl_3$, 500 MHz) 6.46 (d, *J* = 13.6 Hz, 1 H), 6.18 (d, *J* = 13.7 Hz, 1 H), 3.73, (s, 3 H), 3.70 (d, *J* = 16.5 Hz, 1 H), 3.65 (d, *J* = 16.5 Hz, 1 H), 1.50 (s, 3 H), 0.93 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H); ^{13}C NMR *keto* ($CDCl_3$, 125 MHz) 203.8, 168.1, 139.1, 109.1, 83.3, 52.5, 43.7, 25.9 (3 C atoms), 24.2, 18.4, −2.2, −2.3; anal. calcd for $C_{14}H_{25}BrO_4Si$: C, 46.03; H, 6.90, found C, 46.11; H, 6.71.

Methyl (*R,5E,7E*)-4-((*tert*-Butyldimethylsilyl)oxy)-4-methyl-3-oxodeca-5,7-dienoate (17a). The Suzuki coupling was carried out under an argon atmosphere using standard Schlenk techniques for oxygen exclusion. Vinyl bromide **5** (201 mg, 0.550 mmol, 1.00 equiv) in dry 1,4-dioxane (5 mL) was treated with $Pd(PPh_3)_4$ (51.0 mg, 44.0 *μ*mol, 8 mol %). The reaction mixture was stirred for 5 min at ambient temperature. Boronic ester **6a** (150 mg, 0.825 mmol, 1.50 equiv) in 1,4-dioxane (7 mL) and K_2CO_3 (152 mg, 1.10 mmol, 2.00 equiv) in H_2O (1.2 mL) were added. The reaction mixture was heated to 50 °C and stirred for 17 h at this temperature. After cooling to rt, the reaction mixture was diluted with CH_2Cl_2 (15 mL), and the

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J. Nat. Prod. 2022, 85, 2828−2835

phases were separated. The organic phase was washed with saturated aqueous NH_4Cl (15 mL), H_2O (15 mL), and brine (15 mL) and was dried over Na_2SO_4. The volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (*n*-pentane to 3% EtOAc in *n*-pentane) afforded coupling product **17a** as a colorless oil (141 mg, 0.414 mmol, 75%): R_f = 0.52 (10% EtOAc in *n*-hexane); $[\alpha]_D^{26}$ +137 (*c* 1.00 in $CHCl_3$); IR (neat) ν_{max} 2956, 1751, 1724, 1366, 1254, 1228, 1217, 1203, 990, 835, 776 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 6.30 (dd, *J* = 15.4, 10.4 Hz, 1 H), 6.00 (ddt, *J* = 15.2, 10.4, 1.3 Hz, 1 H), 5.78 (dt, *J* = 15.1, 6.6 Hz, 1 H), 5.48 (d, *J* = 15.4 Hz, 1 H), 3.74 (d, *J* = 16.5 Hz, 1 H), 3.71 (s, 3 H), 3.62 (d, *J* = 16.5 Hz, 1 H), 2.11 (qdd, 7.5, 6.6, 1.4 Hz, 2 H), 1.50 (s, 3 H), 1.01 (t, *J* = 7.5 Hz, 3 H), 0.94 (s, 9 H), 0.12 (s, 3 H), 0.10 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 205.0, 168.6, 138.6, 132.0, 131.8, 128.2, 82.6, 52.3, 43.7, 26.0 (3 C atoms), 25.8, 24.4, 18.4, 13.5, −2.1, −2.2; HRESIMS *m*/*z* 363.1956 $[M + H^+]$ (calcd for $C_{18}H_{32}O_4SiNa^+$, 363.1962).

Methyl (*R*,5*E*,7*E*)-4-((*tert*-Butyldimethylsilyl)oxy)-4-methyl-3-oxododeca-5,7-dienoate (17b). The Suzuki coupling was carried out under an argon atmosphere using standard Schlenk techniques for oxygen exclusion. A stirred solution of vinyl bromide **5** (70.0 mg, 0.192 mmol, 1.00 equiv) in dry 1,4-dioxane (1.5 mL) was treated with $Pd(PPh_3)_4$ (17.7 mg, 15.4 μmol, 8 mol %), and the reaction mixture was stirred for 5 min at ambient temperature. Boronic ester **6b** (52.0 mg, 0.247 mmol, 1.29 equiv) in 1,4-dioxane (2.5 mL) and K_2CO_3 (53.0 mg, 0.384 mmol, 2.00 equiv) in H_2O (1 mL) were added. The reaction mixture was heated to 50 °C and stirred for 23 h at this temperature. After cooling to rt, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and the phases were separated. The organic phase was washed with saturated aqueous NH_4Cl (5 mL) and was dried over Na_2SO_4. The volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (*n*-pentane to 5% EtOAc in *n*-pentane) afforded coupling product **17b** as a colorless oil (63.0 mg, 0.171 mmol, 89%): R_f = 0.58 (10% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +129 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2930, 1753, 1723, 1254, 990, 835, 776 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 6.29 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.99 (ddt, *J* = 15.2, 10.5, 1.2 Hz, 1 H), 5.73 (dt, *J* = 15.2, 7.0 Hz, 1 H), 5.47 (d, *J* = 15.4 Hz, 1 H), 3.75 (d, *J* = 16.5 Hz, 1 H), 3.71 (s, 3 H), 3.62 (d, *J* = 16.5 Hz, 1 H), 2.08 (tdd, 7.0, 6.8, 1.2 Hz, 2 H), 1.50 (s, 3 H), 1.40−1.28 (m, 4 H), 0.94 (s, 9 H), 0.89 (t, *J* = 7.2 Hz, 3 H), 0.12 (s, 3 H), 0.10 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 205.0, 168.6, 137.2, 132.0, 131.7, 129.1, 82.6, 52.3, 43.7, 32.5, 31.4, 26.0 (3 C atoms), 24.4, 22.4, 18.4, 14.1, −2.1, −2.2; HRESIMS *m*/*z* 391.2268 $[M + Na^+]$ (calcd for $C_{20}H_{36}O_4SiNa^+$, 391.2275).

Methyl (*R*,5*E*,7*E*)-4-Hydroxy-4-methyl-3-oxodeca-5,7-dienoate (3a). Silyl ether **17a** (670 mg, 1.97 mmol, 1.00 equiv) was dissolved in dry THF (20 mL). AcOH (225 μL, 3.93 mmol, 2.0 equiv) and TBAF (1 M in THF, 3.93 mL, 3.93 mmol, 2.00 equiv) were added to the solution at 0 °C. After 6 h at 0 °C saturated aqueous NH_4Cl (15 mL) and saturated aqueous $NaHCO_3$ (15 mL) were added, and the layers were separated. The aqueous one was extracted with Et_2O (3 × 20 mL), the combined organic phases were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (30% Et_2O in *n*-pentane) to yield γ-hydroxy-β-ketoester **3a** as a colorless oil (294 mg, 1.30 mmol, 66%): R_f = 0.26 (20% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +37.6 (*c* 0.30, $CHCl_3$) [lit.[2b] $[\alpha]_D^{20}$ +29.6 (*c* 1.1, $CHCl_3$)]; IR (neat) ν_{max} 3491 (broad), 2964, 1748, 1715, 1438, 1319, 1268, 993 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 6.37 (dd, *J* = 15.3, 10.5 Hz, 1 H), 6.03 (ddt, *J* = 14.9, 10.5, 1.2 Hz, 1 H), 5.82 (dt, *J* = 15.1, 6.6 Hz, 1 H), 5.56 (d, *J* = 15.4 Hz, 1 H), 3.74 (s, 3 H), 3.69 (d, *J* = 15.7 Hz, 1 H), 3.58 (d, *J* = 15.7 Hz, 1 H), 3.50 (br s, 1 H), 2.11 (qdd, *J* = 7.4, 6.4, 1.2 Hz, 2 H), 1.49 (s, 3 H), 1.01 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 204.5, 168.0, 139.1, 132.3, 130.2, 128.0, 79.7, 52.7, 43.4, 25.8, 25.0, 13.4; HRESIMS *m*/*z* 209.1170 $[M - H_2O + H^+]$ (calcd for $C_{12}H_{17}O_3^+$, 209.1172).

Methyl (*R*,5*E*,7*E*)-4-Hydroxy-4-methyl-3-oxododeca-5,7-dienoate (3b). Silyl ether **17b** (20.0 mg, 54.3 μmol, 1.00 equiv) was dissolved in dry THF (550 μL), and AcOH (36.2 μL, 0.109 mmol, 2.0 equiv) and TBAF (1 M in THF, 109 μL, 0.109 mmol, 2.00 equiv) were added to the solution at 0 °C. After 7 h at 0 °C saturated aqueous NH_4Cl (1 mL) and saturated aqueous $NaHCO_3$ (1 mL) were added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL), the combined organic phases were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (30% to 40% Et_2O in *n*-pentane) to yield γ-hydroxy-β-ketoester **3b** as a colorless oil (11.0 mg, 43.3 μmol, 80%): R_f = 0.32 (20% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ −7.1 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 3479 (broad), 2929, 1751, 1724, 1438, 1316, 1265, 1136, 990 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 6.37 (dd, *J* = 15.3, 10.5 Hz, 1 H), 6.02 (ddt, *J* = 15.0, 10.6, 1.3 Hz, 1 H), 5.78 (dt, *J* = 15.2, 7.0 Hz, 1 H), 5.54 (d, *J* = 15.4 Hz, 1 H), 3.73 (s, 3 H), 3.69 (d, *J* = 15.8 Hz, 1 H), 3.58 (d, *J* = 15.8 Hz, 1 H), 3.51 (br s, 1 H), 2.09 (tdd, 7.3, 6.9, 1.1 Hz, 2 H), 1.48 (s, 3 H), 1.40−1.28 (m, 4 H), 0.89 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 204.5, 168.0, 137.8, 132.3, 130.1, 128.9, 79.7, 52.7, 43.4, 32.5, 31.4, 25.0, 22.4, 14.1; HRESIMS *m*/*z* 237.1478 $[M - H_2O + H^+]$ (calcd for $C_{14}H_{21}O_3^+$, 237.1485).

(*R*,5*E*,7*E*)-1-Methoxy-4-methyl-1,3-dioxodeca-5,7-dien-4-yl-2-methylthiophene-3-carboxylate (2a). A stirred solution of 2-methylthiophene-3-carboxylic acid (**4**) (72.7 mg, 0.511 mmol, 1.00 equiv) and alcohol **3a** (133 mg, 0.588 mmol, 1.15 equiv) in dry toluene (10 mL) was treated with NEt_3 (212 μL, 1.53 mmol, 3.00 equiv), 2,4,6-trichlorobenzoyl chloride (126 μL, 0.807 mmol, 1.58 equiv), and DMAP (94.0 mg, 0.767 mmol, 1.50 equiv). The reaction mixture was stirred at rt for 1 h before being quenched with saturated aqueous $NaHCO_3$ (10 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10% EtOAc in *n*-pentane) afforded ester **2a** as a colorless oil (95.0 mg, 0.271 mmol, 53%): R_f = 0.53 (20% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ +185 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2964, 1750, 1719, 1709, 1532, 1436, 1370, 1267, 1093, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.43 (d, *J* = 5.5 Hz, 1 H), 7.03 (d, *J* = 5.3 Hz, 1 H), 6.42 (dd, *J* = 15.6, 10.4 Hz, 1 H), 6.06 (ddt, *J* = 15.3, 10.4, 1.2 Hz, 1 H), 5.85 (dt, *J* = 15.2, 6.5 Hz, 1 H), 5.74 (d, *J* = 15.6 Hz, 1 H), 3.69 (s, 3 H), 3.60 (d, *J* = 15.6 Hz, 1 H), 3.51 (d, *J* = 15.6 Hz, 1 H), 3.75 (s, 3 H), 2.13 (qdd, *J* = 7.6, 6.2, 1.3 Hz, 2 H), 1.72 (s, 3 H), 1.02 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.4, 167.6, 162.6, 151.5, 139.6, 132.9, 129.2, 128.1, 127.23, 127.16, 121.5, 85.8, 52.5, 43.1, 25.8, 21.6, 15.8, 13.4; HRESIMS *m*/*z* 373.1073 $[M + Na^+]$ (calcd for $C_{18}H_{22}O_5SNa^+$, 373.1080).

(*R*,5*E*,7*E*)-1-Methoxy-4-methyl-1,3-dioxododeca-5,7-dien-4-yl-2-methylthiophene-3-carboxylate (2b). To a stirred solution of 2-methylthiophene-3-carboxylic acid (**4**) (9.70 mg, 68.4 μmol, 1.00 equiv) and alcohol **3b** (20.0 mg, 78.6 μmol, 1.15 equiv) in dry toluene (1.5 mL) were added NEt_3 (28.4 μL, 0.205 mmol, 3.00 equiv), 2,4,6-trichlorobenzoyl chloride (16.9 μL, 0.108 mmol, 1.58 equiv), and DMAP (12.5 mg, 0.103 mmol, 1.50 equiv). The reaction mixture was stirred at rt for 1.5 h before being quenched with saturated aqueous $NaHCO_3$ (2 mL). After phase separation the aqueous one was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic phases were dried over Na_2SO_4, and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10% EtOAc in *n*-pentane) afforded ester **2b** as a colorless oil (15.8 mg, 41.7 μmol, 61%): R_f = 0.60 (20% EtOAc in *n*-hexane); $[\alpha]_D^{26}$ +84.5 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2971, 1739, 1727, 1713, 1367, 1268, 1229, 1217, 1204, 1093 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.43 (d, *J* = 5.5 Hz, 1 H), 7.03 (d, *J* = 5.3 Hz, 1 H), 6.41 (dd, *J* = 15.6, 10.4 Hz, 1 H), 6.05 (ddt, *J* = 15.3, 10.4, 1.2 Hz, 1 H), 5.81 (dt, *J* = 15.3, 7.0 Hz, 1 H), 5.72 (d, *J* = 15.6 Hz, 1 H), 3.69 (s, 3 H), 3.60 (d, *J* = 15.6 Hz, 1 H), 3.51 (d, *J* = 15.6 Hz, 1 H), 3.75 (s, 3 H), 2.10 (tdd, *J* = 7.2, 6.9, 1.2 Hz, 2 H), 1.72 (s, 3 H), 1.41−1.29 (m, 4 H), 0.90 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.4, 167.6, 162.6, 151.5, 138.2, 132.9, 129.2, 129.0, 127.2, 127.1, 121.5, 85.8, 52.5, 43.1, 32.5, 31.3, 22.4, 21.6, 15.8, 14.0; HRESIMS *m*/*z* 401.1385 $[M + Na^+]$ (calcd for $C_{20}H_{26}O_5SNa^+$, 401.1393).

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Thiocarboxylic A (1a). Compound **2a** (18.0 mg, 51.4 μmol, 1.00 equiv) was dissolved in dry benzene (1 mL), and piperidine (25.4 μL, 0.257 mmol, 5.00 equiv) was added to the solution at rt. The reaction mixture was heated at reflux for 16 h before it was allowed to cool to rt. The volatiles were removed *in vacuo*, and the remainder was purified by column chromatography (20% to 30% Et_2O in *n*-pentane). Thiocarboxylic A (**1a**) was obtained as a colorless oil (11.4 mg, 34.2 μmol, 67%): R_f = 0.53 (20% EtOAc in *n*-hexane); $[\alpha]_D^{21}$ −78 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 2963, 1742, 1712, 1580, 1439, 1415, 1337, 1103, 1055, 992 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.36 (d, *J* = 5.5 Hz, 1 H), 7.12 (d, *J* = 5.3 Hz, 1 H), 6.34 (dd, *J* = 15.5, 10.5 Hz, 1 H), 6.00 (ddt, *J* = 15.2, 10.5, 1.2 Hz, 1 H), 5.82 (dt, *J* = 15.3, 6.6 Hz, 1 H), 5.62 (d, *J* = 15.6 Hz, 1 H), 3.82 (s, 3 H), 2.64 (s, 3 H), 2.10 (qdd, *J* = 7.5, 6.6, 1.2 Hz, 2 H), 1.62 (s, 3 H), 0.99 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.8, 184.4, 163.3, 147.8, 139.7, 132.2, 129.5, 127.8, 126.7, 125.9, 122.2, 106.2, 90.8, 52.0, 25.9, 23.0, 15.9, 13.4; HRESIMS *m/z* 333.1147 $[M + H^+]$ (calcd for $C_{18}H_{21}O_4S^+$, 333.1155).

Methyl (*R*)-5-Methyl-2-(2-methylthiophen-3-yl)-5-((1*E*,3*E*)-octa-1,3-dien-1-yl)-4-oxo-4,5-dihydrofuran-3-carboxylate (1b). Compound **2b** (17.0 mg, 44.9 μmol, 1.00 equiv) was dissolved in dry benzene (1 mL), and piperidine (22.2 μL, 0.225 mmol, 5.00 equiv) was added to the solution at rt. The reaction mixture was heated at reflux for 24 h before it was allowed to cool to rt. The volatiles were removed *in vacuo*, and the remainder was purified by column chromatography (5% to 20% EtOAc in *n*-pentane). Product **1b** was obtained as a colorless oil (9.00 mg, 25.0 μmol, 56%): R_f = 0.35 (20% EtOAc in *n*-hexane); $[\alpha]_D^{24}$ −66.4 (*c* 0.80, $CHCl_3$); IR (neat) ν_{max} 2927, 1741, 1713, 1580, 1439, 1415, 1337, 1103 1054, 991 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.36 (d, *J* = 5.5 Hz, 1 H), 7.12 (d, *J* = 5.5 Hz, 1 H), 6.33 (dd, *J* = 15.5, 10.5 Hz, 1 H), 5.99 (ddt, *J* = 15.2, 10.5, 1.2 Hz, 1 H), 5.77 (dt, *J* = 15.3, 7.0 Hz, 1 H), 5.61 (d, *J* = 15.4 Hz, 1 H), 3.82 (s, 3 H), 2.64 (s, 3 H), 2.08 (tdd, *J* = 7.1, 7.0, 1.2 Hz, 2 H), 1.62 (s, 3 H), 1.38−1.27 (m, 4 H), 0.88 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.8, 184.4, 163.3, 147.8, 138.4, 132.2, 129.5, 128.8, 126.7, 125.8, 122.2, 106.2, 90.8, 52.0, 32.5, 31.3, 23.0, 22.3, 15.9, 14.0; HRESIMS *m/z* 361.1458 $[M + H^+]$ (calcd for $C_{20}H_{25}O_4S^+$, 361.1468).

(*R*,5*E*,7*E*)-1-Methoxy-4-methyl-1,3-dioxodeca-5,7-dien-4-yl-3-methylthiophene-2-carboxylate (19a). 3-Methyl-2-thiophenecarboxylic acid (**18a**) (42.1 mg, 0.296 mmol, 1.00 equiv) and alcohol **3a** (77.0 mg, 0.340 mmol, 1.15 equiv) in dry toluene (6 mL) were treated with NEt_3 (123 μL, 0.888 mmol, 3.00 equiv), 2,4,6-trichlorobenzoyl chloride (73.1 μL, 0.467 mmol, 1.58 equiv), and DMAP (54.2 mg, 0.444 mmol, 1.50 equiv). The reaction mixture was stirred at rt for 1 h before being quenched with saturated aqueous $NaHCO_3$ (5 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10% EtOAc in *n*-pentane) afforded ester **19a** as a colorless oil (87.0 mg, 0.248 mmol, 84%): R_f = 0.54 (20% EtOAc in *n*-hexane); $[\alpha]_D^{25}$ +123 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} 2962, 1753, 1723, 1708, 1410, 1267, 1070, 993, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.46 (d, *J* = 4.9 Hz, 1 H), 6.95 (d, *J* = 5.0 Hz, 1 H), 6.45 (dd, *J* = 15.6, 10.4 Hz, 1 H), 6.06 (ddt, *J* = 15.0, 10.6, 1.3 Hz, 1 H), 5.87 (dt, *J* = 15.2, 6.5 Hz, 1 H), 5.70 (d, *J* = 15.6 Hz, 1 H), 3.70 (s, 3 H), 3.62 (d, *J* = 15.8 Hz, 1 H), 3.53 (d, *J* = 15.9 Hz, 1 H), 3.56 (s, 3 H), 2.13 (qdd, *J* = 7.6, 6.2, 1.2 Hz, 2 H), 1.73 (s, 3 H), 1.02 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.4, 167.6, 161.7, 148.1, 139.7, 133.0, 132.2, 131.3, 128.1, 127.0, 125.5, 86.3, 52.5, 43.1, 25.9, 21.6, 16.2, 14.4; HRESIMS *m/z* 373.1070 $[M + Na^+]$ (calcd for $C_{18}H_{22}O_5SNa^+$, 373.1080).

(*R*,5*E*,7*E*)-1-Methoxy-4-methyl-1,3-dioxodeca-5,7-dien-4-yl-5-methylthiophene-2-carboxylate (19b). 5-Methyl-2-thiophenecarboxylic acid (**18b**) (42.1 mg, 0.296 mmol, 1.00 equiv) and alcohol **3a** (77.0 mg, 0.340 mmol, 1.15 equiv) in dry toluene (6 mL) were treated with NEt_3 (123 μL, 0.888 mmol, 3.00 equiv), 2,4,6-trichlorobenzoyl chloride (73.1 μL, 0.467 mmol, 1.58 equiv), and DMAP (54.2 mg, 0.444 mmol, 1.50 equiv). The reaction mixture was stirred at rt for 1 h before being quenched with saturated aqueous $NaHCO_3$ (5 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10% EtOAc in *n*-pentane) afforded ester **19b** as a colorless oil (72.0 mg, 0.205 mmol, 69%): R_f = 0.51 (20% EtOAc in *n*-hexane); $[\alpha]_D^{25}$ +93 (*c* 0.80, $CHCl_3$); IR (neat) ν_{max} 2962, 1753, 1724, 1706, 1458, 1292, 1267, 1085, 993, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.68 (d, *J* = 3.7 Hz, 1 H), 6.81 (dq, *J* = 3.7, 1.0 Hz, 1 H), 6.34 (dd, *J* = 15.4, 10.4 Hz, 1 H), 6.05 (ddt, *J* = 15.2, 10.5, 1.3 Hz, 1 H), 5.87 (dt, *J* = 15.1, 6.6 Hz, 1 H), 5.63 (d, *J* = 15.6 Hz, 1 H), 3.69 (s, 3 H), 3.62 (d, *J* = 15.7 Hz, 1 H), 3.52 (d, *J* = 15.7 Hz, 1 H), 2.55 (d, *J* = 0.6 Hz, 3 H), 2.13 (qdd, *J* = 7.2, 6.2, 1.2 Hz, 2 H), 1.72 (s, 3 H), 1.02 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.4, 167.6, 161.2, 149.5, 139.7, 135.2, 132.9, 130.1, 128.1, 127.0, 126.9, 86.4, 52.5, 43.1, 25.9, 21.5, 16.0, 13.4; HRESIMS *m/z* 373.1071 $[M + Na^+]$ (calcd for $C_{18}H_{22}O_5SNa^+$, 373.1080).

Methyl (*R*)-5-((1*E*,3*E*)-Hexa-1,3-dien-1-yl)-5-methyl-2-(3-methylthiophen-2-yl)-4-oxo-4,5-dihydrofuran-3-carboxylate (20a). Compound **19a** (54.0 mg, 0.154 mmol, 1.00 equiv) was dissolved in dry benzene (2 mL), and piperidine (76.3 μL, 0.770 mmol, 5.00 equiv) was added to the solution at rt. The reaction mixture was heated to 50 °C for 23 h before it was allowed to cool to rt. The volatiles were removed *in vacuo*, and the remainder was purified by column chromatography (20% Et_2O in *n*-pentane). Product **20a** was obtained as a colorless oil (29.0 mg, 87.2 μmol, 56%): R_f = 0.27 (20% EtOAc in *n*-hexane); $[\alpha]_D^{25}$ −120 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2925, 1739, 1705, 1574, 1547, 1436, 1407, 1368, 1261, 1198, 1103, 1089, 1022, 989, 798 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.64 (d, *J* = 5.0 Hz, 1 H), 7.00 (d, *J* = 5.0 Hz, 1 H), 6.34 (dd, *J* = 15.6, 10.4 Hz, 1 H), 5.99 (ddt, *J* = 15.2, 10.5, 1.3 Hz, 1 H), 5.82 (dt, *J* = 15.1, 6.6 Hz, 1 H), 5.63 (d, *J* = 15.4 Hz, 1 H), 3.84 (s, 3 H), 2.46 (s, 3 H), 2.10 (qdd, *J* = 7.5, 6.6, 1.3 Hz, 2 H), 1.62 (s, 3 H), 0.99 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.5, 182.0, 163.3, 146.5, 139.7, 133.0, 132.0, 131.8, 127.9, 126.1, 124.8, 105.4, 90.6, 52.1, 25.8, 23.0, 17.7, 13.4; HRESIMS *m/z* 333.1146 $[M + H^+]$ (calcd for $C_{18}H_{21}O_4S^+$, 333.1155).

Methyl (*R*)-5-((1*E*,3*E*)-Hexa-1,3-dien-1-yl)-5-methyl-2-(5-methylthiophen-2-yl)-4-oxo-4,5-dihydrofuran-3-carboxylate (20b). Compound **19b** (61.0 mg, 0.174 mmol, 1.00 equiv) was dissolved in dry benzene (2 mL), and piperidine (86.2 μL, 0.870 mmol, 5.00 equiv) was added to the solution at rt. The reaction mixture was heated to 50 °C for 23 h before it was allowed to cool to rt. The volatiles were removed *in vacuo*, and the remainder was purified by column chromatography (20% Et_2O in *n*-pentane). Product **19b** was obtained as a colorless oil (14.0 mg, 42.1 μmol, 24%): R_f = 0.10 (20% EtOAc in *n*-hexane); $[\alpha]_D^{25}$ −240 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2933, 1740, 1704, 1560, 1448, 1377, 1261, 1219, 1103, 1056, 991, 798 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 8.38 (d, *J* = 4.0 Hz, 1 H), 6.94 (dq, *J* = 4.0, 0.9 Hz, 1 H), 6.31 (dd, *J* = 15.4, 10.4 Hz, 1 H), 5.98 (ddt, *J* = 15.1, 10.5, 1.3 Hz, 1 H), 5.80 (dt, *J* = 15.1, 6.6 Hz, 1 H), 5.62 (d, *J* = 15.4 Hz, 1 H), 3.89 (s, 3 H), 2.61 (s, 3 H), 2.09 (qdd, *J* = 7.6, 6.5, 1.3 Hz, 2 H), 1.60 (s, 3 H), 0.98 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) 198.5, 180.2, 164.0, 153.0, 139.4, 137.7, 131.5, 128.4, 127.9, 127.4, 126.5, 102.3, 90.0, 52.0, 25.8, 22.8, 16.0, 13.5; HRESIMS *m/z* 355.0962 $[M + Na^+]$ (calcd for $C_{18}H_{20}O_4SNa^+$, 355.0975).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.2c00870.

NMR spectra of all new compounds; HPLC chromatograms of thiocarboxylic A and three derivatives; NMR comparison of isolated and synthetic thiocarboxylic A; descriptions of and tables and diagrams from assays for antimicrobial and cytotoxic activities (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting Information

Synthesis and Bioactivity of Thiocarboxylic A and Derivatives

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Table of Contents

Antimicrobial assay

Staphylococcus aureus and *Escherichia coli* bacteria strains were grown in Trypton-Soja-Bouillon (TSB) media or Luria-Bertani (LB) media respectively. TSB media (4 mL) was inoculated with *S. aureus* wild-type strain - USA 300 while LB media (4 mL) was inoculated with *E. coli* wild-type strain or an *E. coli* strain lacking the outer membrane protein TolC - *E.coli* TolC.[1] The bacteria strains were incubated under aerobic conditions at 37 °C with shaking at 150 rpm and grown overnight. Bacterial density was determined by measuring the optical density (OD) at 600 nm followed by the establishment of a working culture by diluting the overnight culture to OD 600 nm = 0.1 in fresh media in an Erlenmeyer flask. Bacteria cultures were grown until OD 600 nm ~ 0.5 and then diluted to OD 600 nm = 0.01 in fresh media. The diluted bacteria suspension was seeded in transparent 96 well half-area plates. 1µL of serially diluted (1.5-fold) compounds was added per well using the liquid handler CyBioSelma 96/60 (Analytik Jena) resulting in final concentrations ranging from 100 µM to 1.73 µM. The OD 600 nm of the seeded bacteria was measured, and the plates were incubated at 37 °C, 5% CO_2 for 24 h. Bacteria growth was determined by subtracting the OD 600 nm measured at time 0 h from OD 600 nm at time 24 h of incubation.

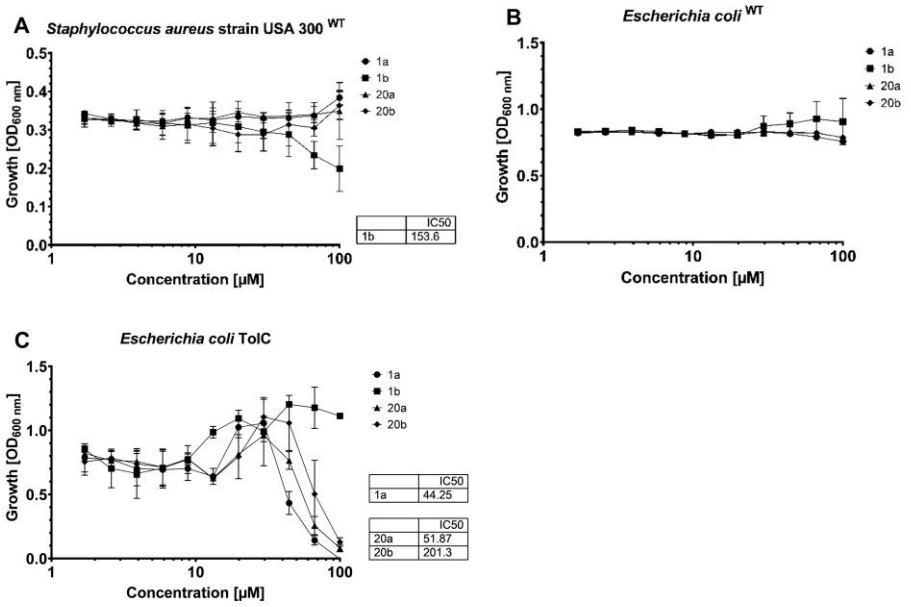


Figure S1. Antimicrobial Activity of thiocarboxylic A (**1a**) and derivatives **1b**, **20a**, and **20b**. The graphs represent the average growth of bacteria strains *Staphylococcus aureus* (A), *Escherichia coli* wild type (B), and *Escherichia coli* TolC mutant (C). Each symbol represents the average bacteria growth of at least duplicate measurements, and the error bars represent the standard deviation of the mean.

Cytotoxicity assay

L929 cells (mouse fibroblasts cell line), A549 cells (lung epithelial cell line) and HuH7.5 cells (human hepatoma cell line) were cultured and maintained in Dulbecco's modified Eagle's medium high glucose, GlutaMAX™ Supplement (Life Technologies, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) in a 37 °C incubator with a humidified atmosphere and 5% CO_2. Confluent cells (90–100%) were split and seeded in a 96-well plate at a seeding density of 2×10^4 cells/99 µL/well. Cells were incubated for 24 h to allow adherence before treatment with 1µL of serially diluted (1.5-fold) compounds, resulting in final concentrations ranging from 100 µM to 1.73 µM. Cells were incubated for 72 h before determining their viability by a resazurin reduction method (alamarBlue assay) according to manufacturer recommendation.[2] Cell viability was calculated and expressed as a percentage relative to the untreated control (cells in DMSO).

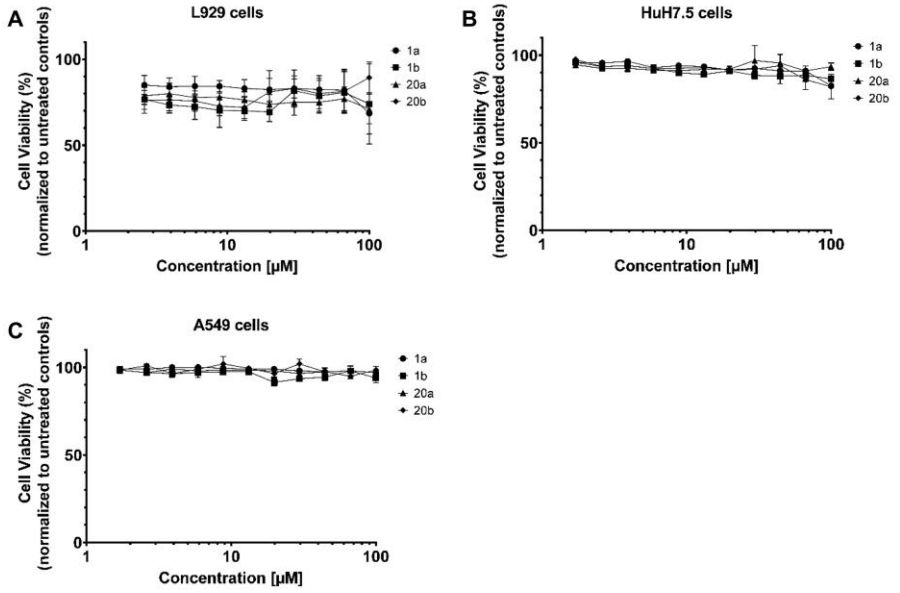
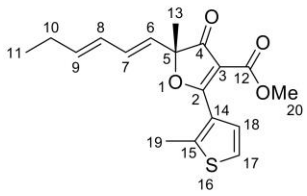


Figure S2. Cytotoxicity of thiocarboxylic A (**1a**) and derivatives **1b**, **20a**, and **20b**. The graphs represent the percent viability of L929 cells (A), HuH7.5 cells (B), and A549 cells (C). Each symbol represents the average cell viability of at least duplicate measurements, and the error bars represent the standard deviation of the mean

Table S1. Comparison of NMR spectra of isolated[3] and synthetic thiocarboxylic A (**1a**) in $CDCl_3$.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C{^{1}H} δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			184.4	184.4
3			111.2	106.2
4			198.5	198.8
5			90.8	90.8
6	5.62 d (15.5)	5.62 d (15.6)	125.9	125.9
7	6.34 dd (15.5, 10.4)	6.34 dd (15.5, 10.5)	132.2	132.2
8	6.00 dd (15.3, 10.4)	6.00 ddt (15.2, 10.5, 1.2)	127.9	127.8
9	5.83 dt (15.2, 7.4)	5.82 dt (15.3, 6.6)	139.7	139.7
10	2.09 m	2.10 qdd (7.5, 6.6, 1.2)	25.8	25.9
11	0.99 t (7.4)	0.99 t (7.4)	13.4	13.4
12			163.2	163.3
13	1.62 s	1.62 s	23.0	23.0
14			128.6	126.7
15			141.9	147.8
17	7.12 d (5.4)	7.12 d (5.3)	122.2	122.2
18	7.36 d (5.4)	7.36 d (5.5)	129.5	129.5
19	2.64 s	2.64 s	15.8	15.9
20	3.82 s	3.82 s	52.0	52.0

[a] ^{1}H and ^{13}C NMR data recorded at 400 MHz and 100 MHz. [b] ^{1}H and ^{13}C NMR data recorded at 500 MHz and 125 MHz.

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NMR spectra of all new compounds

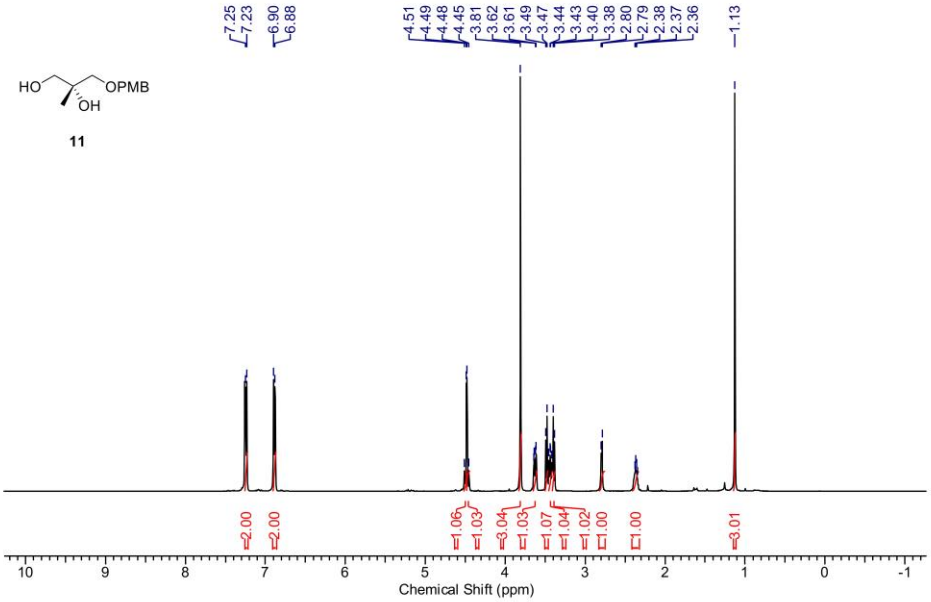

Figure S3. ^{1}H NMR spectrum of compound **11** in $CDCl_3$ (500 MHz).

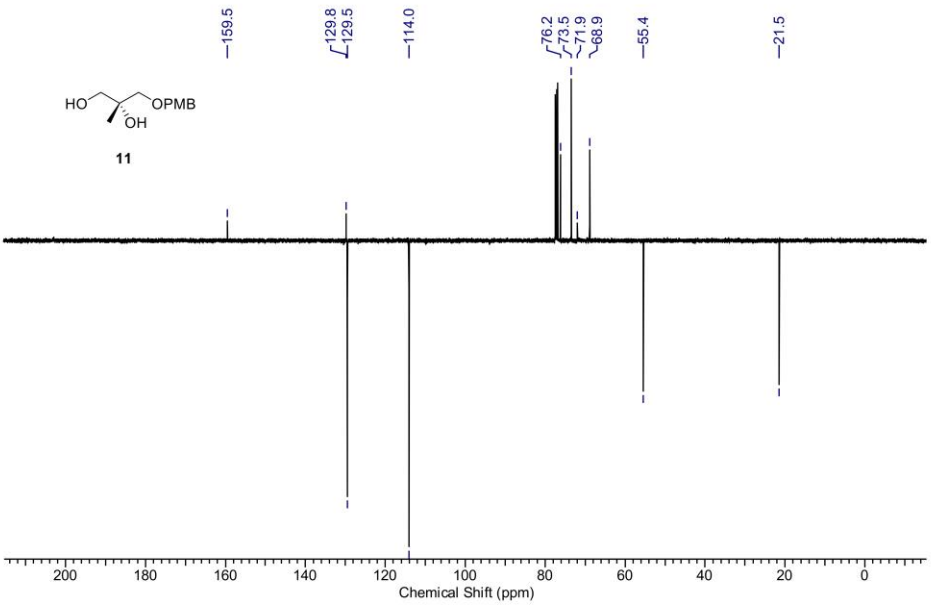

Figure S4. ^{13}C NMR (APT) spectrum of compound **11** in $CDCl_3$ (125 MHz).

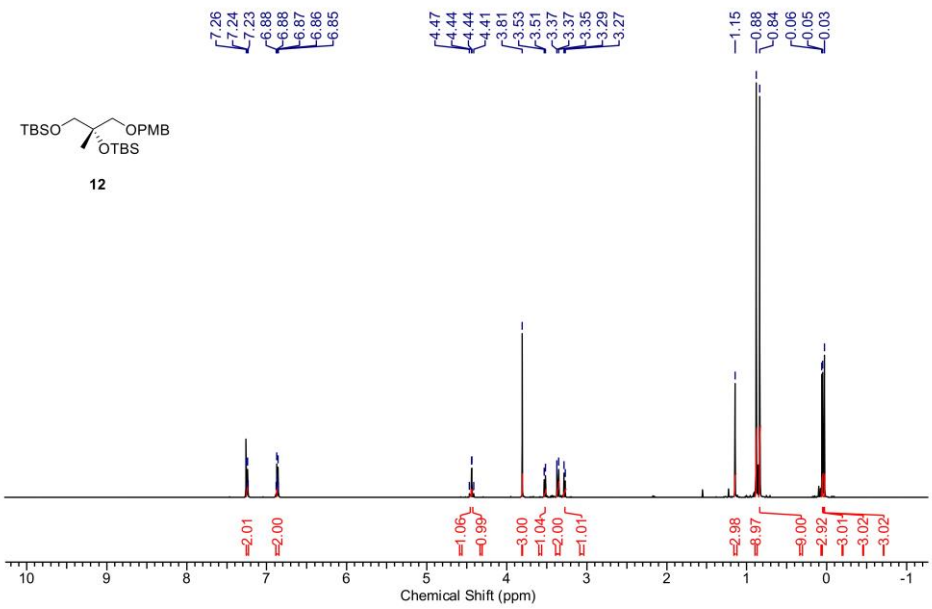

Figure S5. ^{1}H NMR spectrum of compound **12** in $CDCl_3$ (500 MHz).

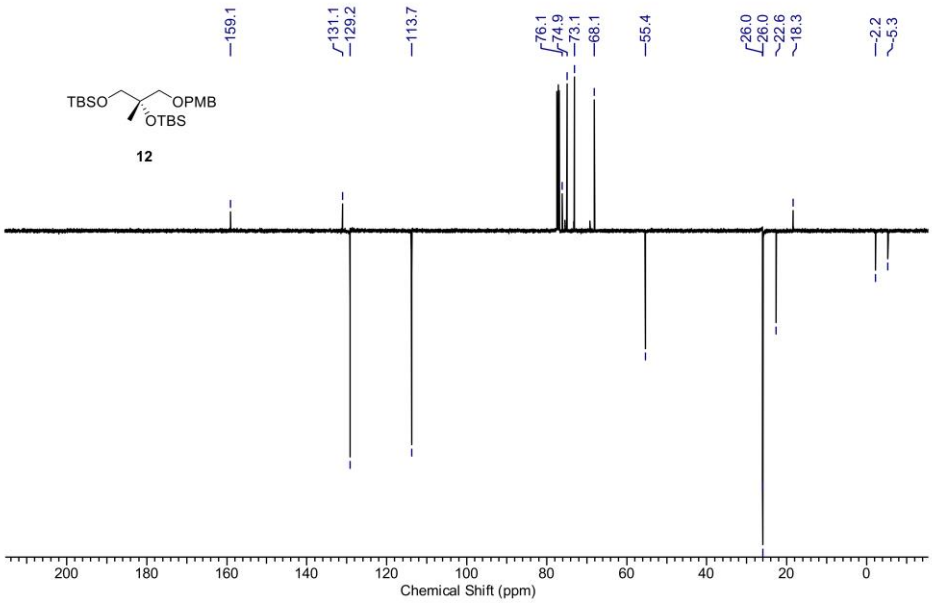

Figure S6. ^{13}C NMR (APT) spectrum of compound **12** in $CDCl_3$ (125 MHz).

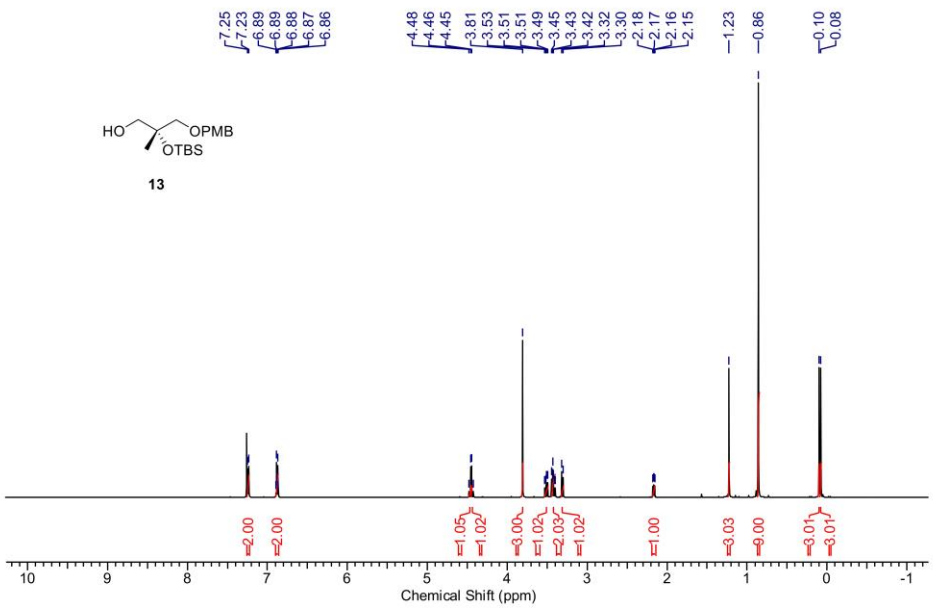

Figure S7. ^{1}H NMR spectrum of compound **13** in $CDCl_3$ (500 MHz).

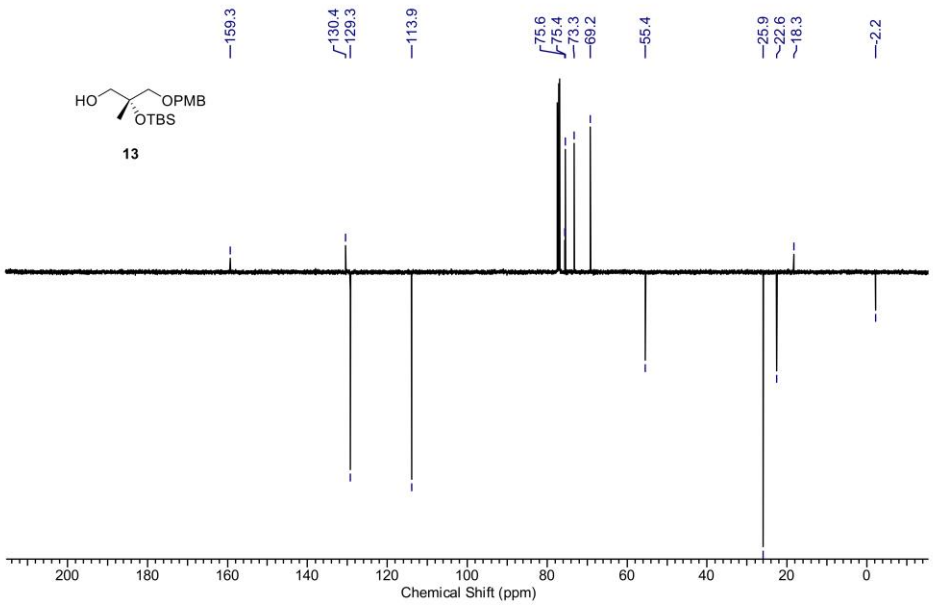

Figure S8. ^{13}C NMR (APT) spectrum of compound **13** in $CDCl_3$ (125 MHz).

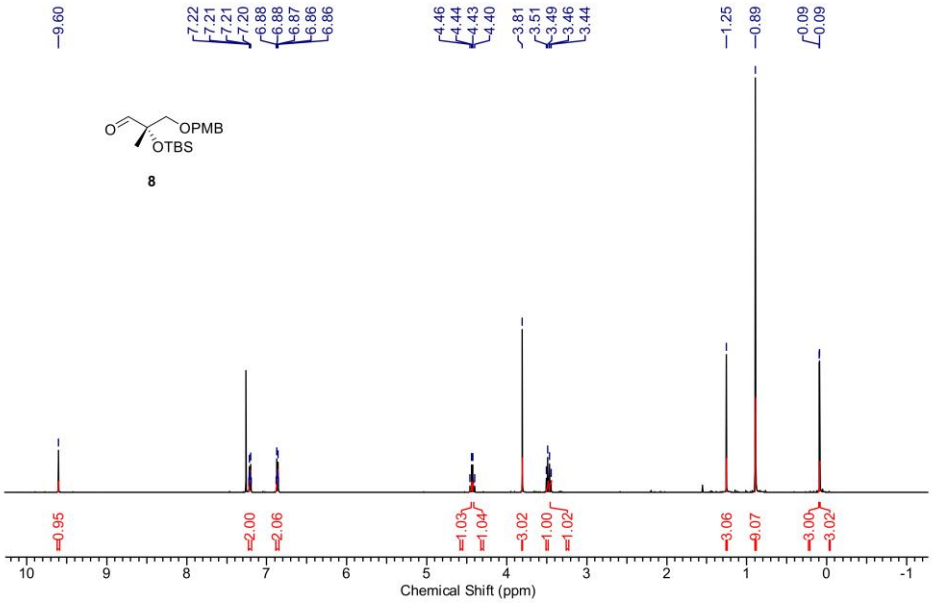

Figure S9. ^{1}H NMR spectrum of compound **8** in $CDCl_3$ (500 MHz).

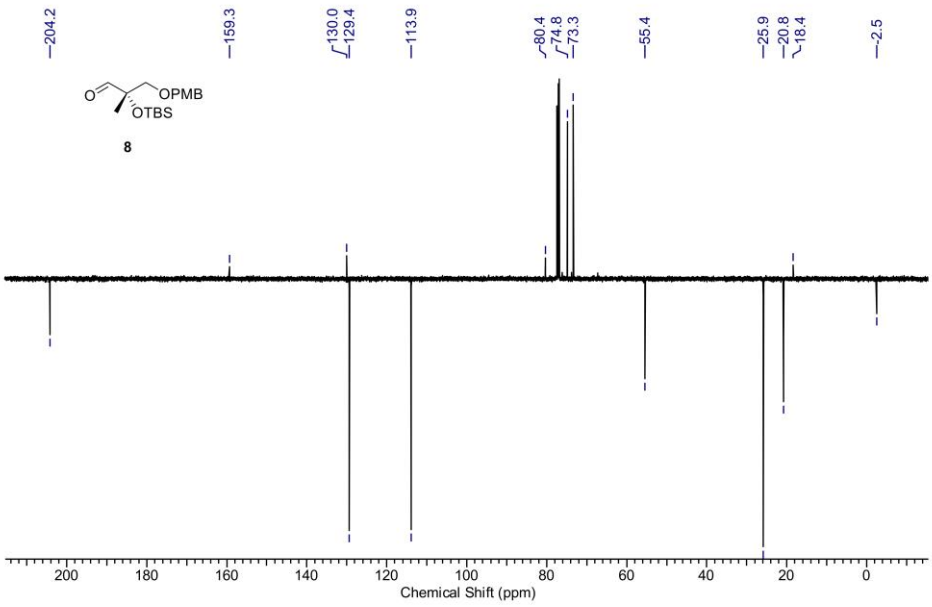

Figure S10. ^{13}C NMR (APT) spectrum of compound **8** in $CDCl_3$ (125 MHz).

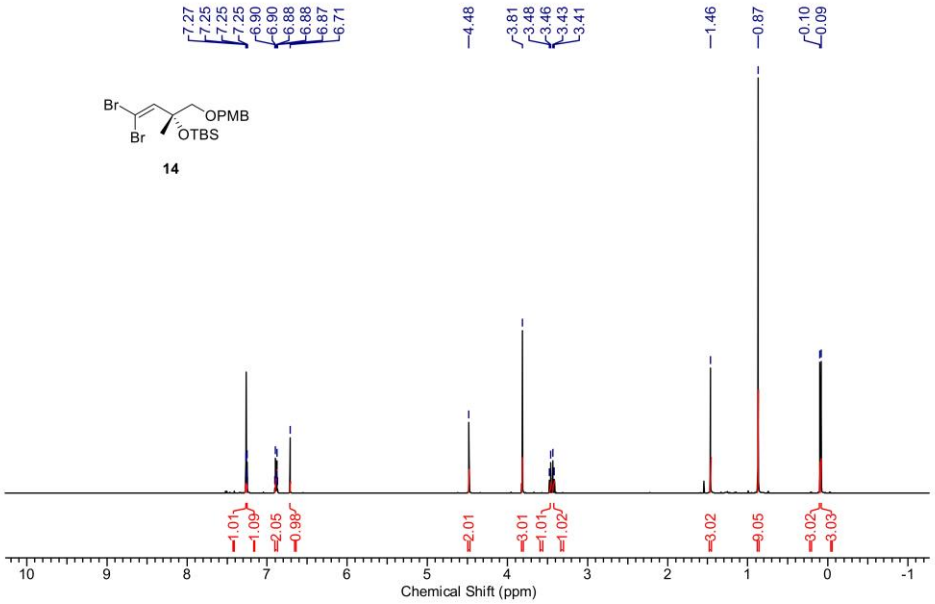

Figure S11. ^{1}H NMR spectrum of compound **14** in $CDCl_3$ (500 MHz).

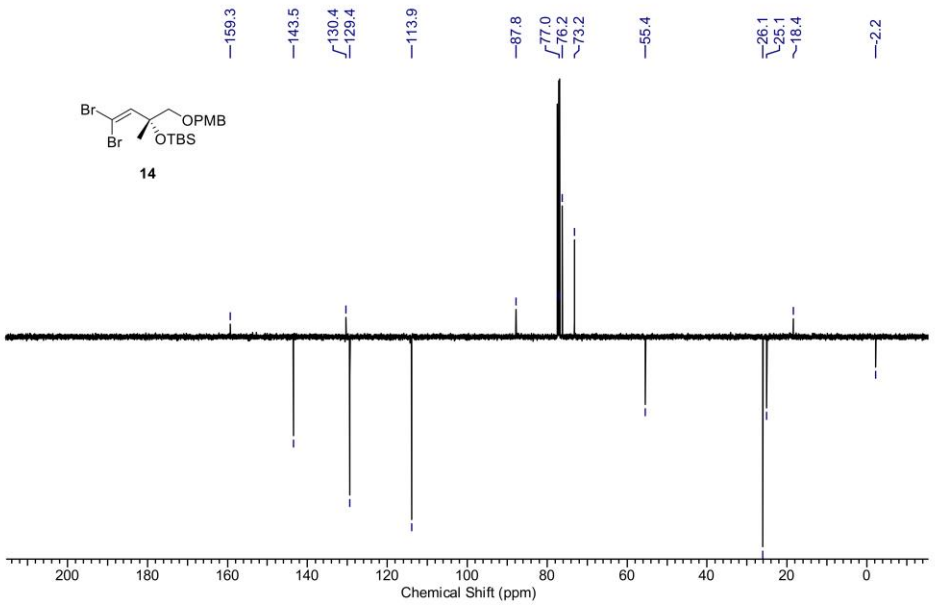

Figure S12. ^{13}C NMR (APT) spectrum of compound **14** in $CDCl_3$ (125 MHz).

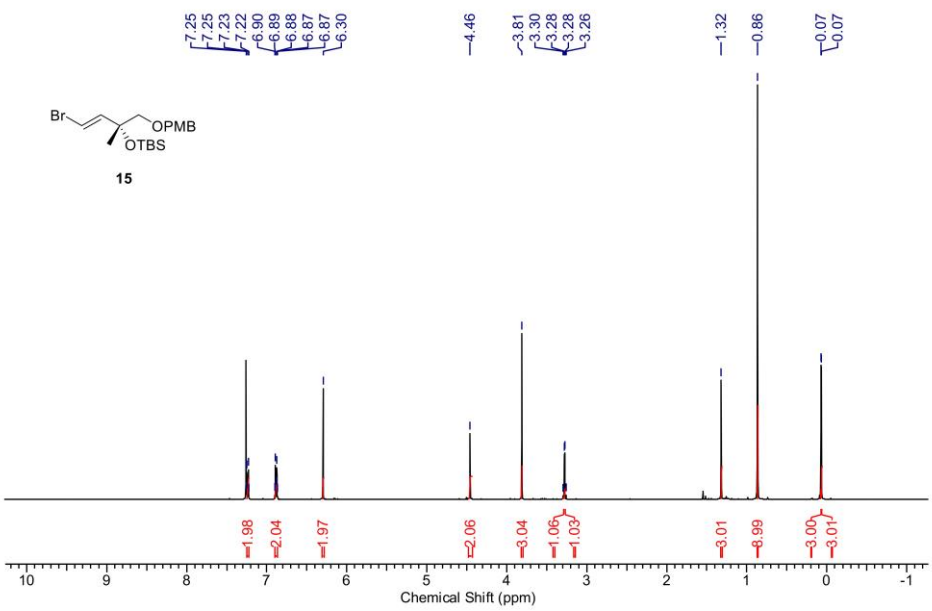

Figure S13. ^{1}H NMR spectrum of compound **15** in $CDCl_3$ (500 MHz).

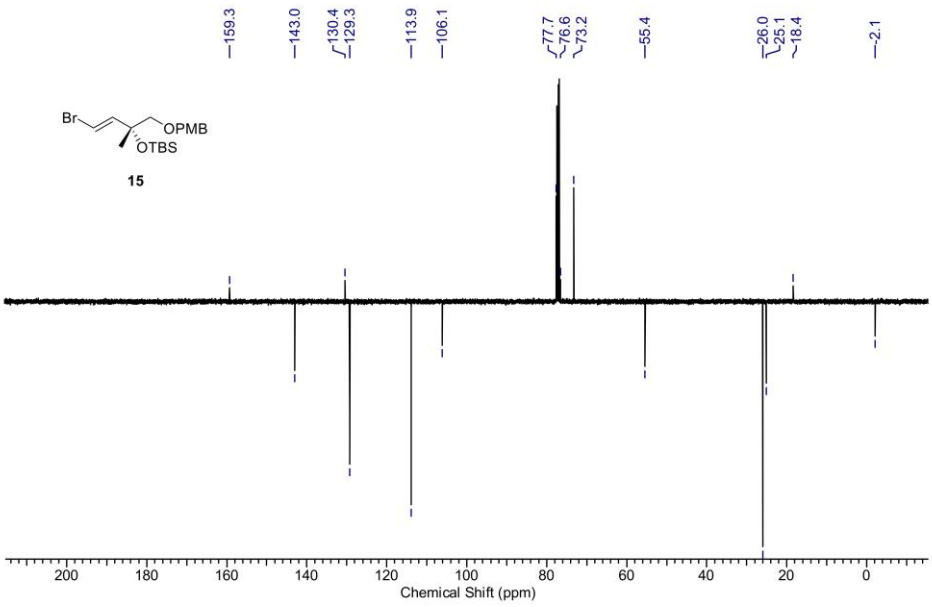

Figure S14. ^{13}C NMR (APT) spectrum of compound **15** in $CDCl_3$ (125 MHz).

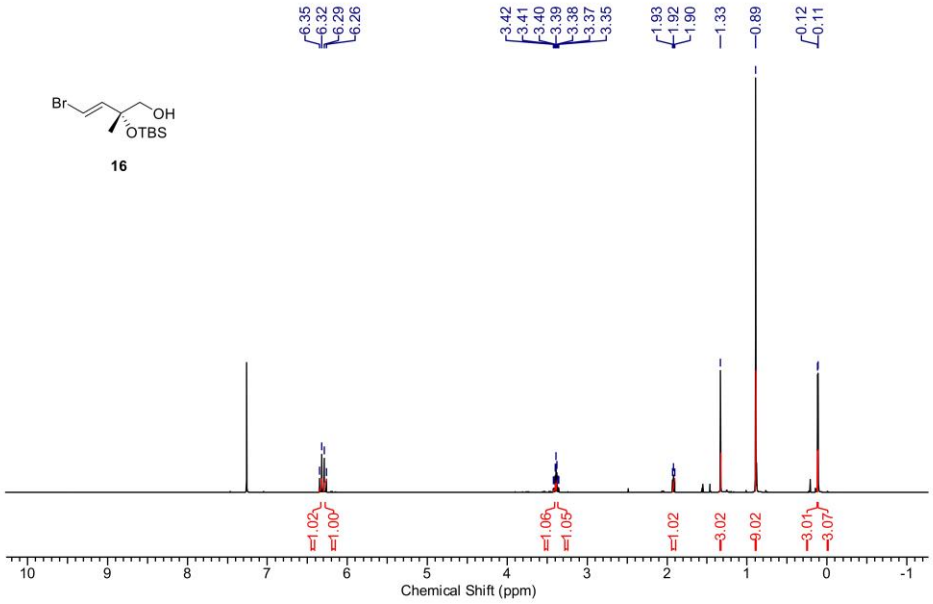

Figure S15. ^{1}H NMR spectrum of compound **16** in $CDCl_3$ (500 MHz).

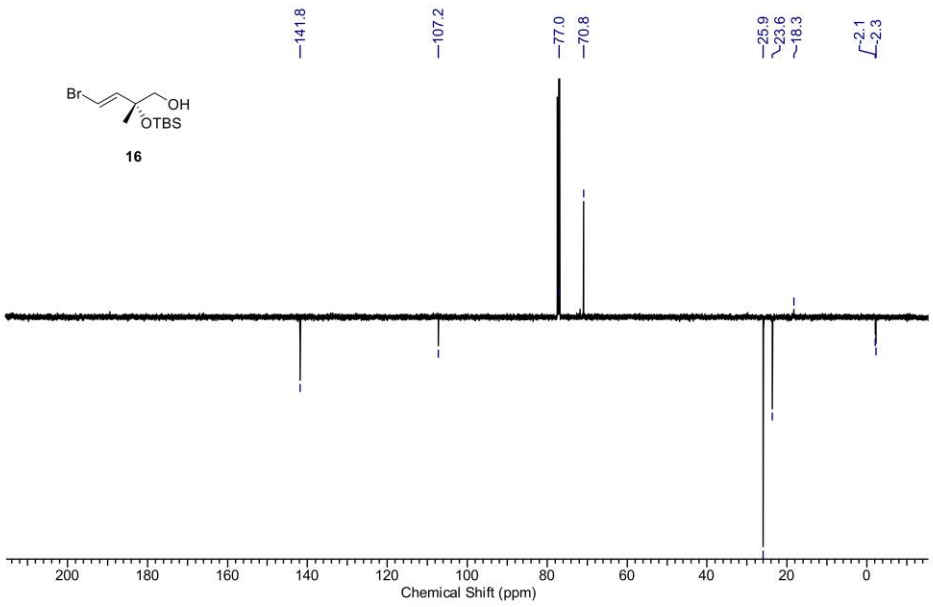

Figure S16. ^{13}C NMR (APT) spectrum of compound **16** in $CDCl_3$ (125 MHz).

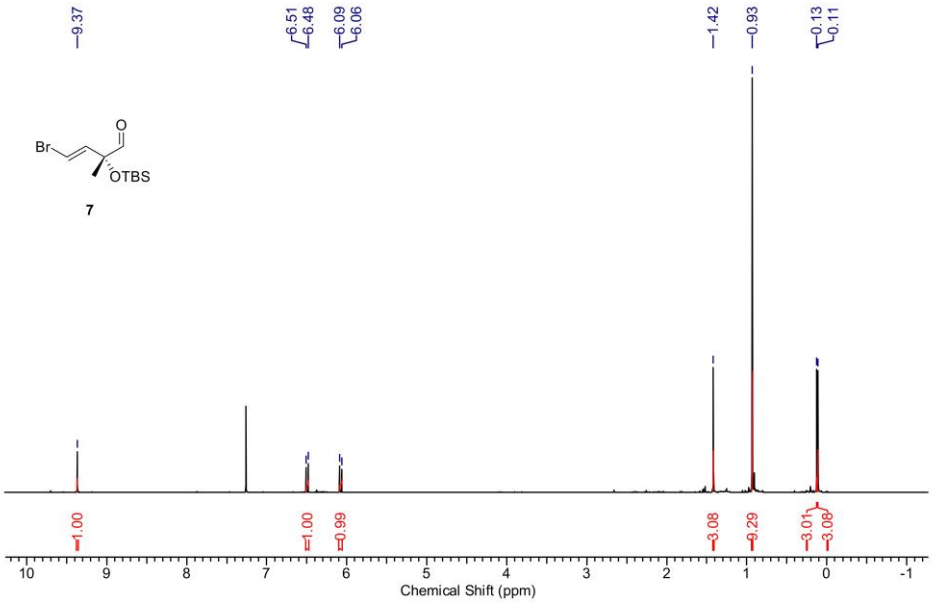

Figure S17. ^{1}H NMR spectrum of compound **7** in $CDCl_3$ (500 MHz).

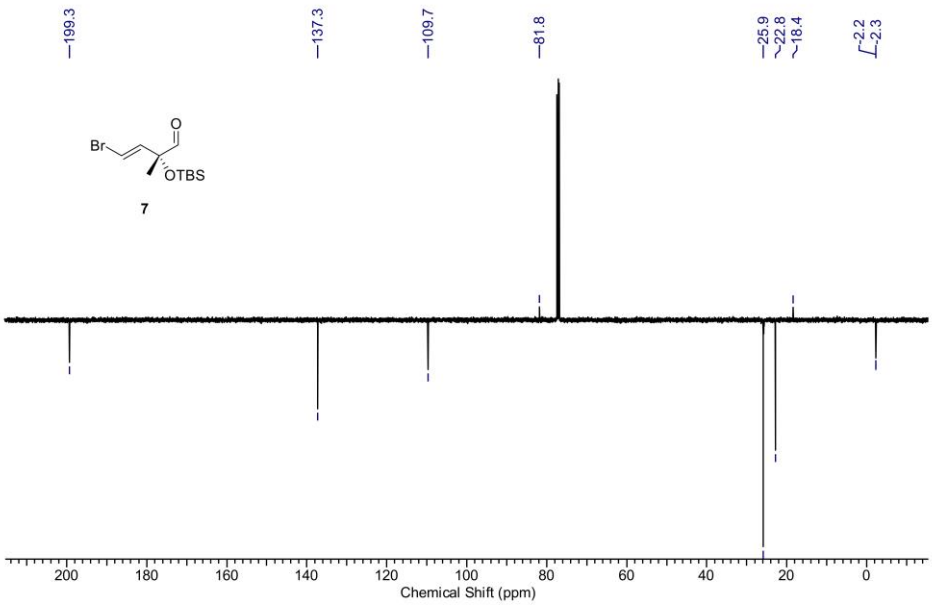

Figure S18. ^{13}C NMR (APT) spectrum of compound **7** in $CDCl_3$ (125 MHz).

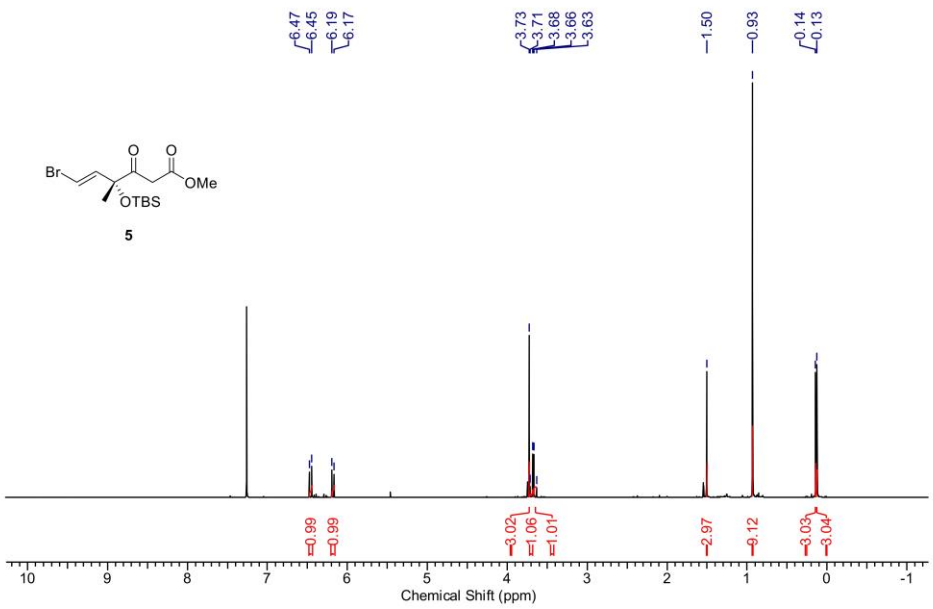

Figure S19. ^{1}H NMR spectrum of compound **5** in $CDCl_3$ (500 MHz).

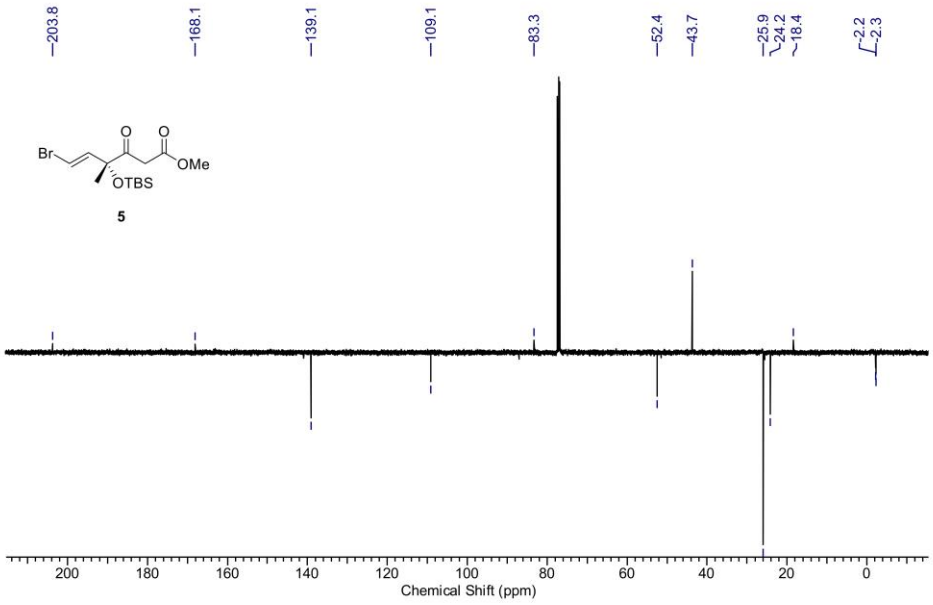

Figure S20. ^{13}C NMR (APT) spectrum of compound **5** in $CDCl_3$ (125 MHz).

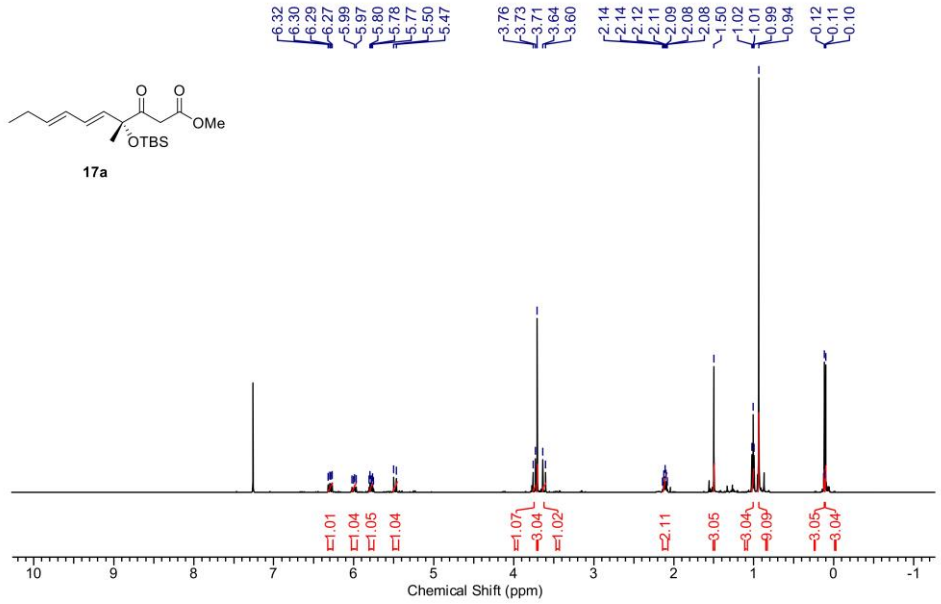

Figure S21. ^{1}H NMR spectrum of compound **17a** in $CDCl_3$ (500 MHz).

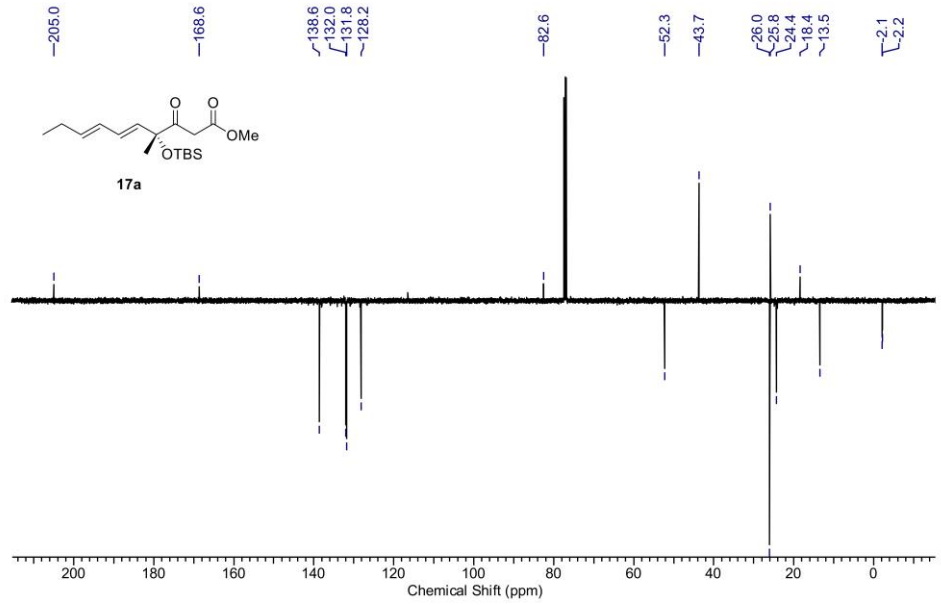

Figure S22. ^{13}C NMR (APT) spectrum of compound **17a** in $CDCl_3$ (125 MHz).

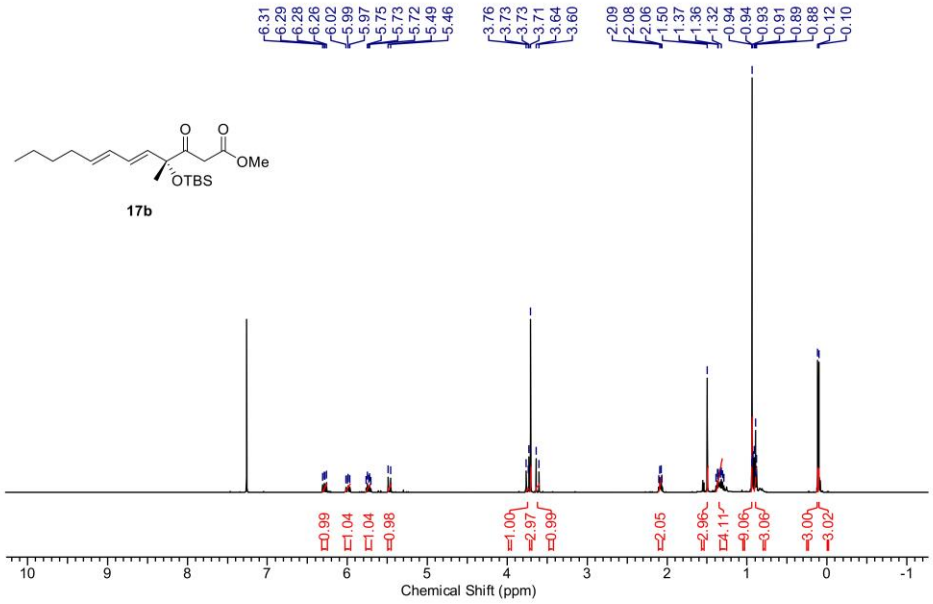

Figure S23. ^{1}H NMR spectrum of compound **17b** in $CDCl_3$ (500 MHz).

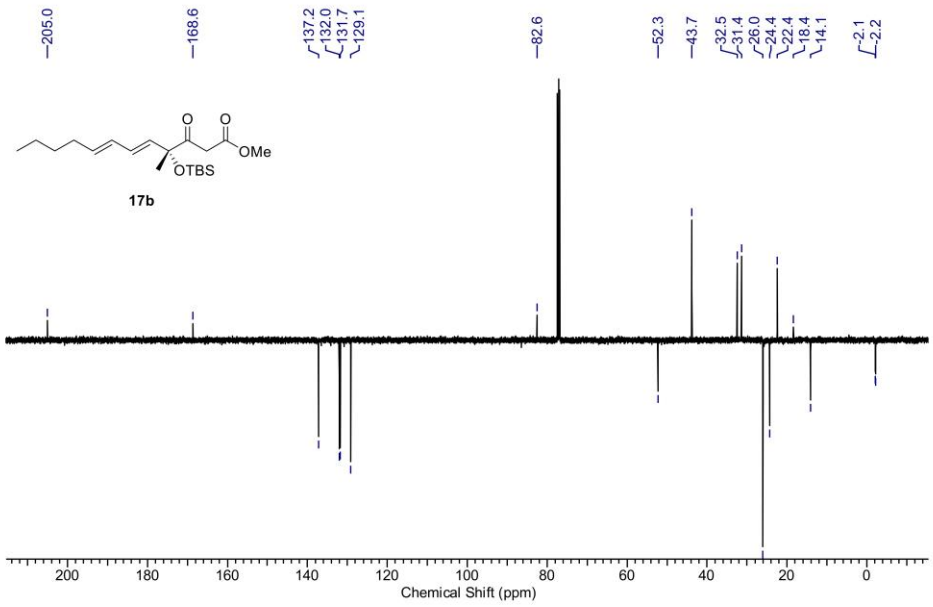

Figure S24. ^{13}C NMR (APT) spectrum of compound **17b** in $CDCl_3$ (125 MHz).

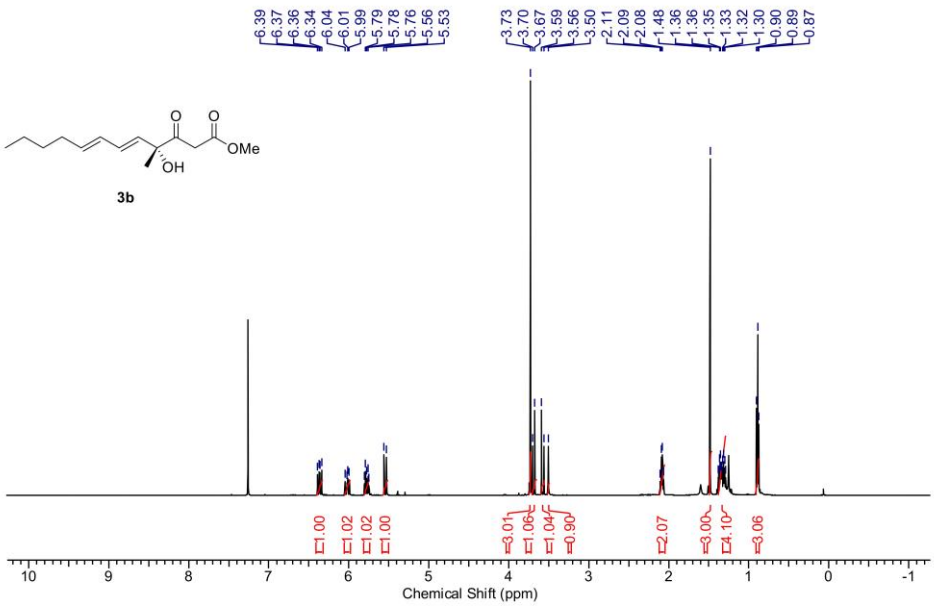

Figure S25. ^{1}H NMR spectrum of compound **3b** in $CDCl_3$ (500 MHz).

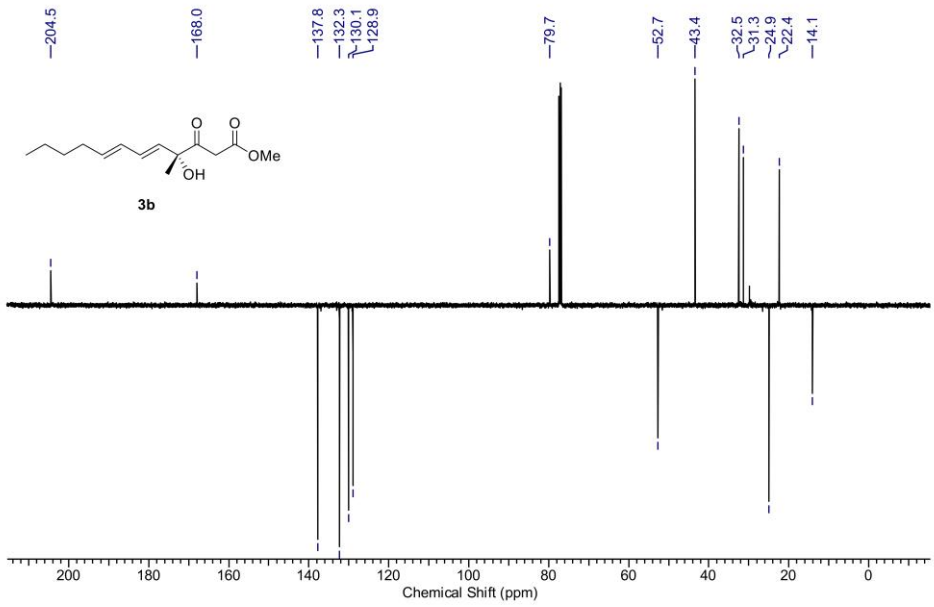

Figure S26. ^{13}C NMR (APT) spectrum of compound **3b** in $CDCl_3$ (125 MHz).

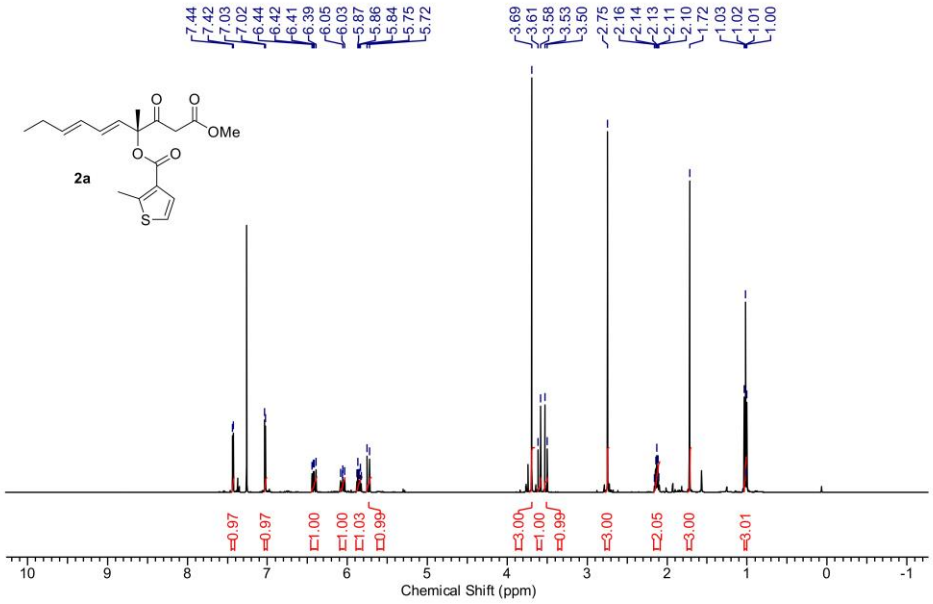

Figure S27. ^{1}H NMR spectrum of compound **2a** in $CDCl_3$ (500 MHz).

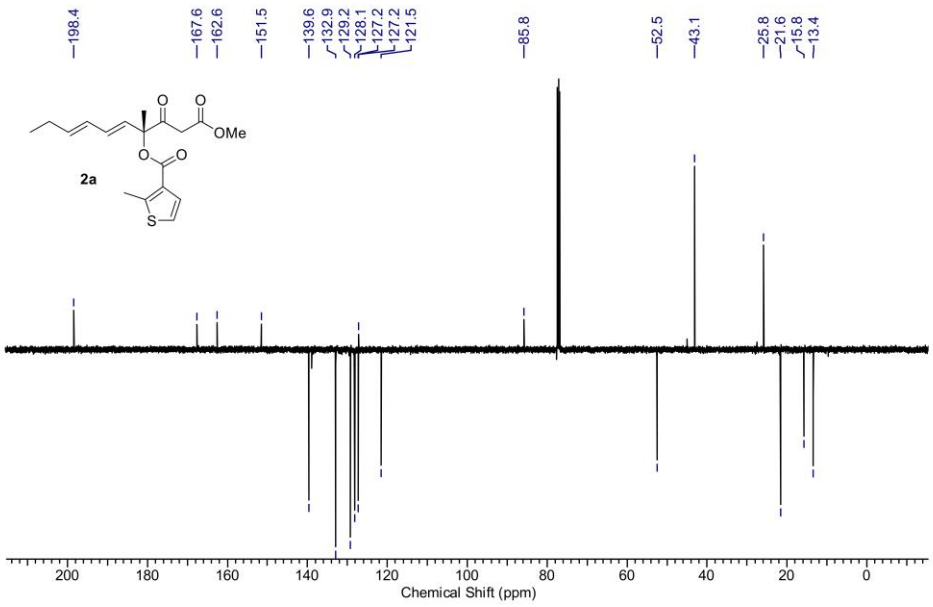

Figure S28. ^{13}C NMR (APT) spectrum of compound **2a** in $CDCl_3$ (125 MHz).

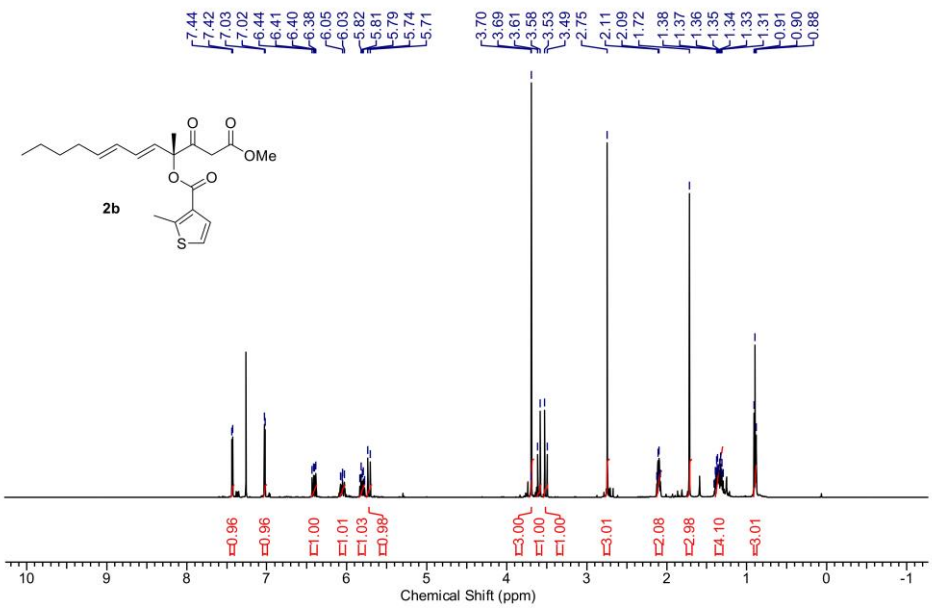

Figure S29. ^{1}H NMR spectrum of compound **2b** in $CDCl_3$ (500 MHz).

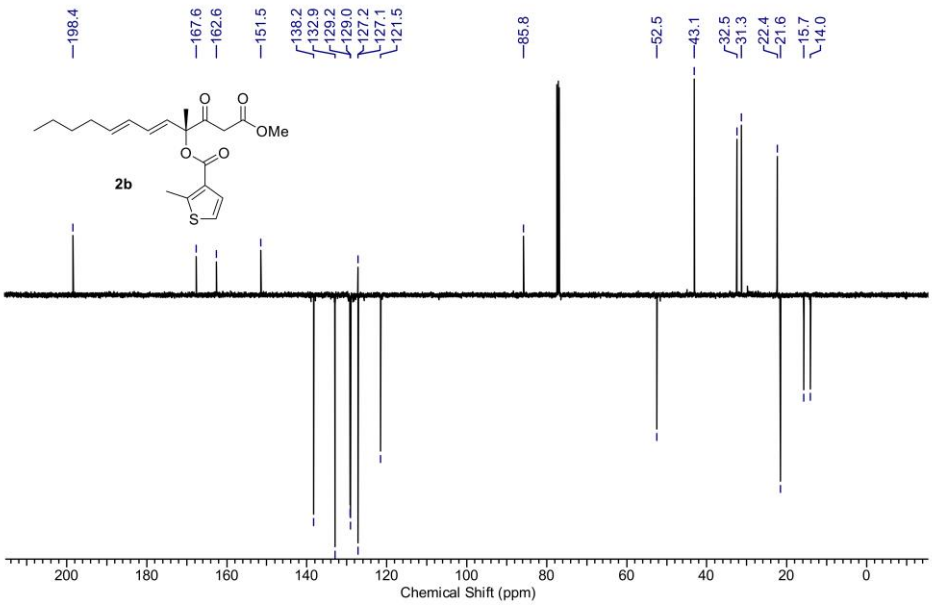

Figure S30. ^{13}C NMR (APT) spectrum of compound **2b** in $CDCl_3$ (125 MHz).

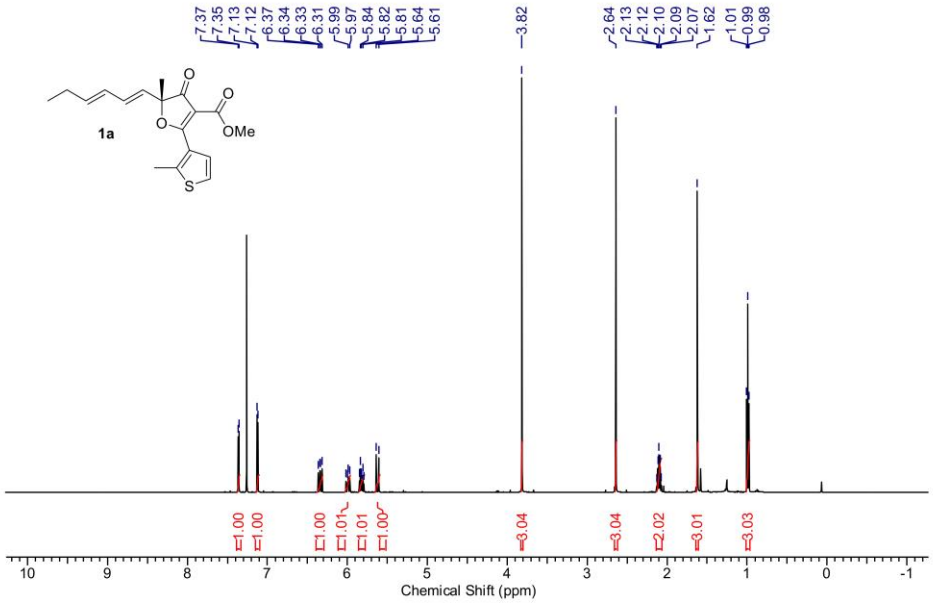

Figure S31. ^{1}H NMR spectrum of compound **1a** in $CDCl_3$ (500 MHz).

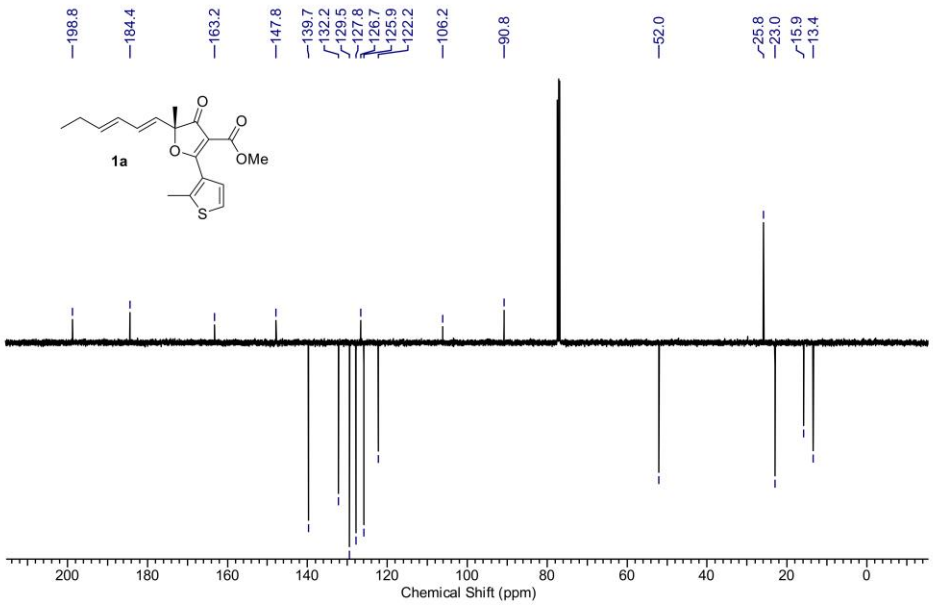

Figure S32. ^{13}C NMR (APT) spectrum of compound **1a** in $CDCl_3$ (125 MHz).

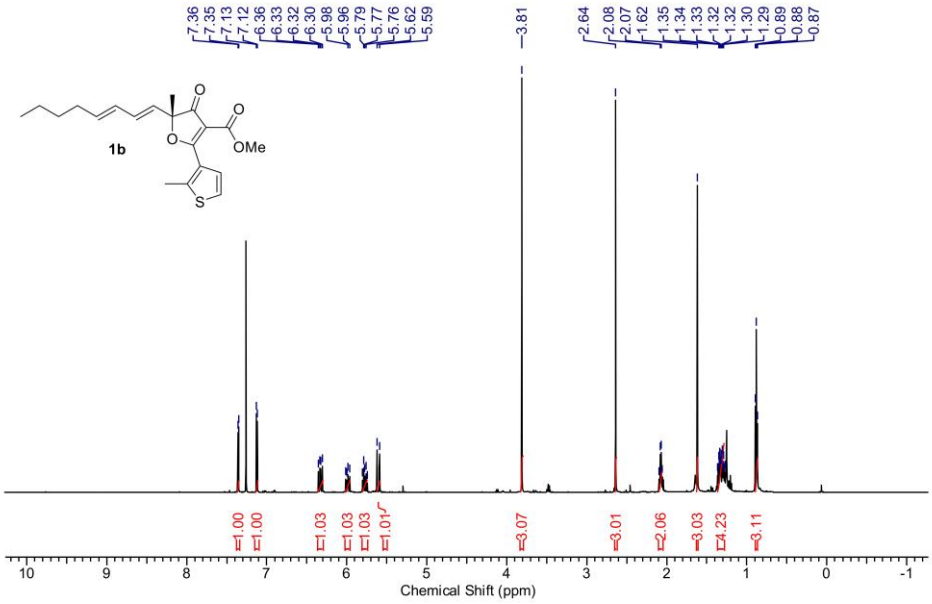

Figure S33. ^{1}H NMR spectrum of compound **1b** in $CDCl_3$ (500 MHz).

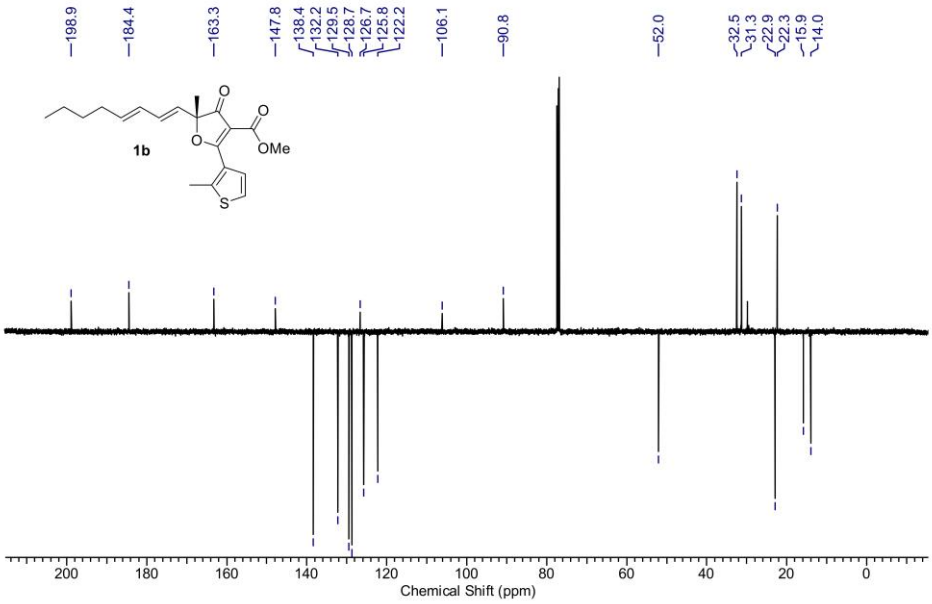

Figure S34. ^{13}C NMR (APT) spectrum of compound **1b** in $CDCl_3$ (125 MHz).

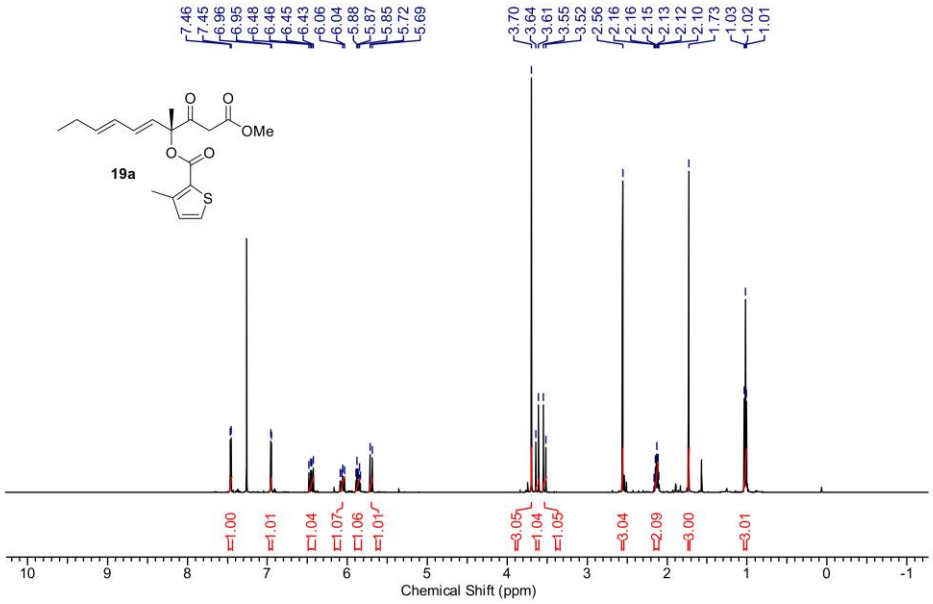

Figure S35. ^{1}H NMR spectrum of compound **19a** in $CDCl_3$ (500 MHz).

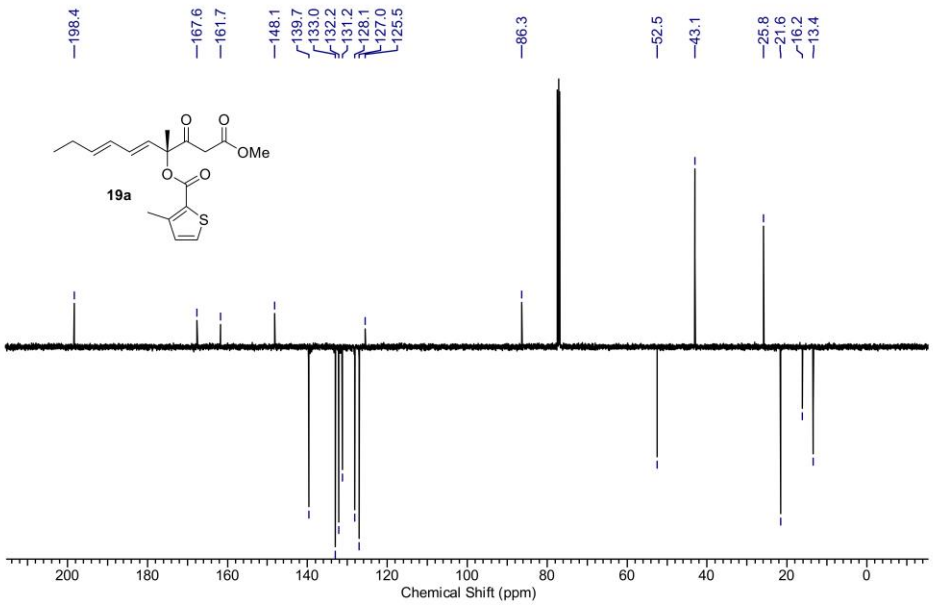

Figure S36. ^{13}C NMR (APT) spectrum of compound **19a** in $CDCl_3$ (125 MHz).

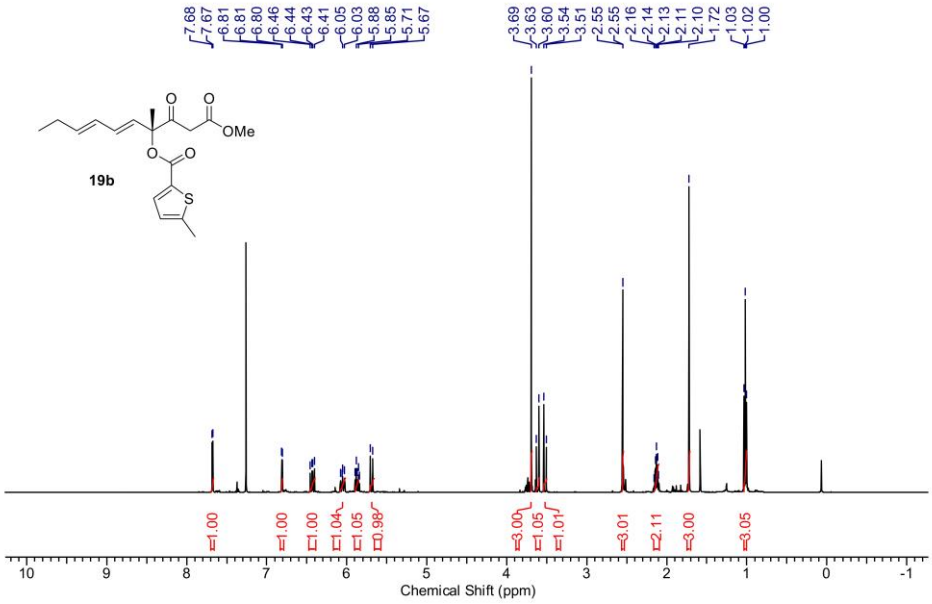

Figure S37. ^{1}H NMR spectrum of compound **19b** in $CDCl_3$ (500 MHz).

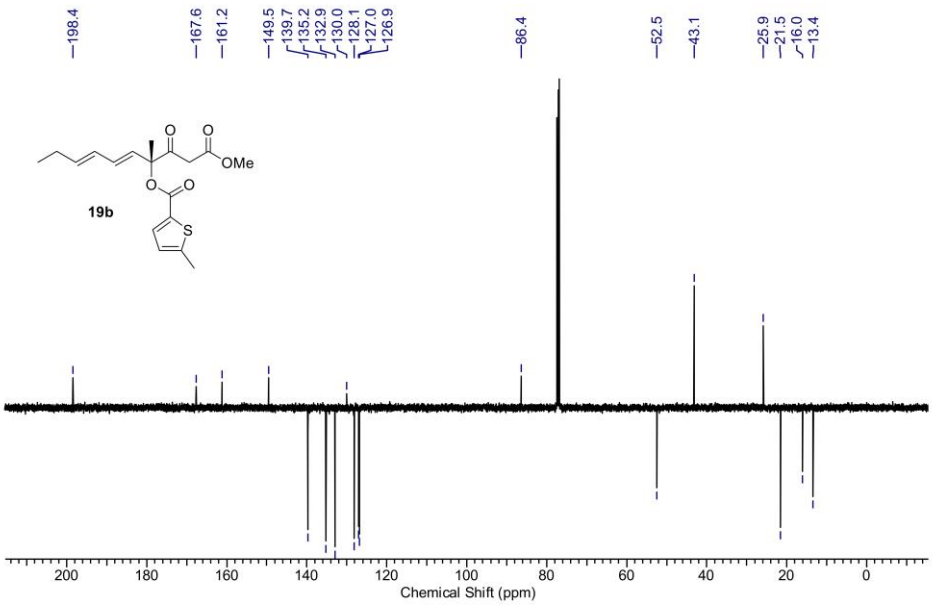

Figure S38. ^{13}C NMR (APT) spectrum of compound **19b** in $CDCl_3$ (125 MHz).

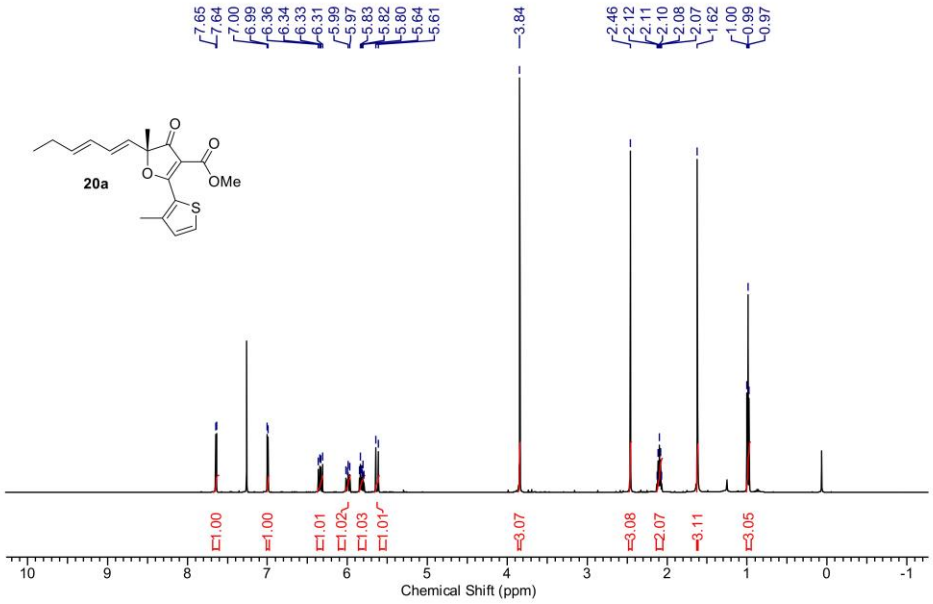

Figure S39. ^{1}H NMR spectrum of compound **20a** in $CDCl_3$ (500 MHz).

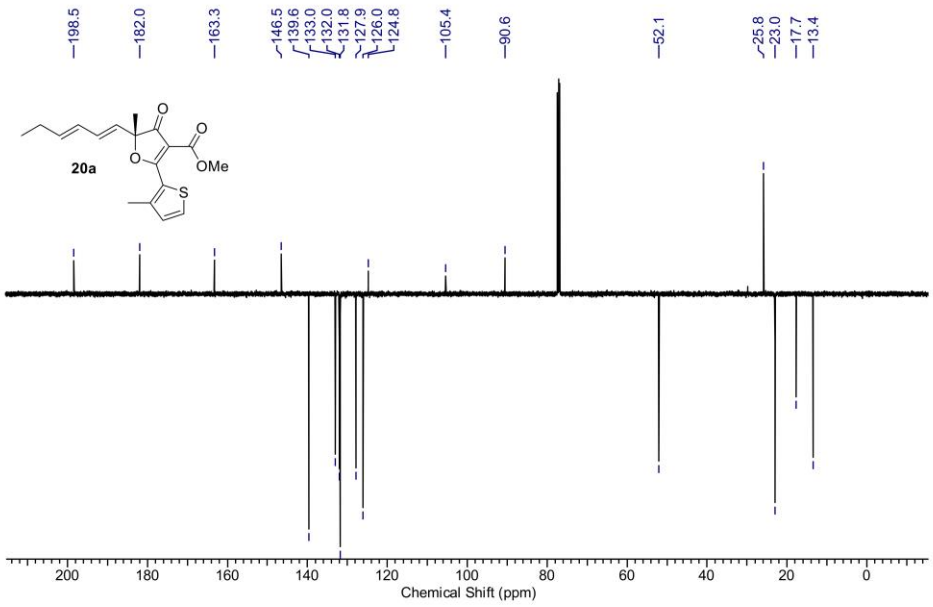

Figure S40. ^{13}C NMR (APT) spectrum of compound **20a** in $CDCl_3$ (125 MHz).

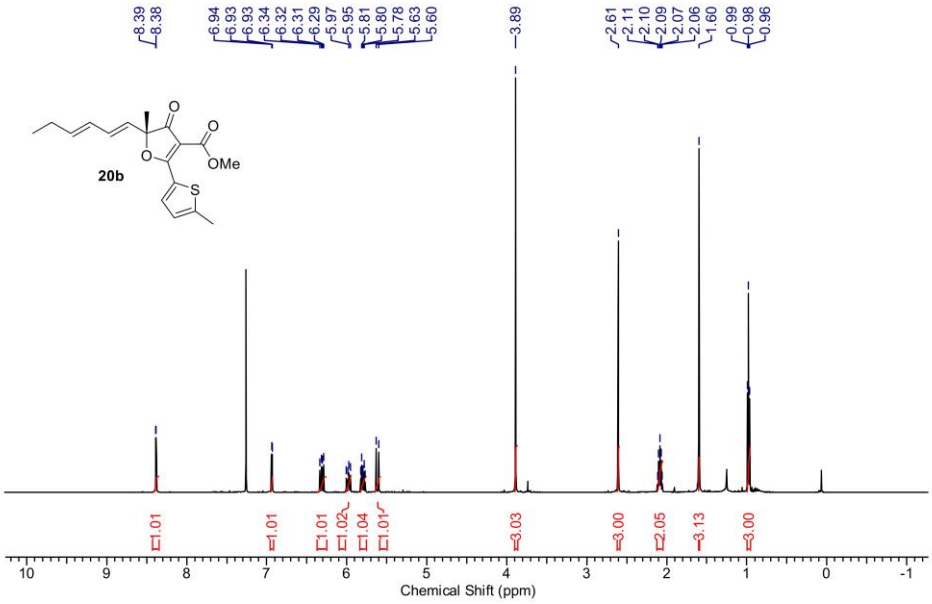

Figure S41. ^{1}H NMR spectrum of compound **20b** in $CDCl_3$ (500 MHz).

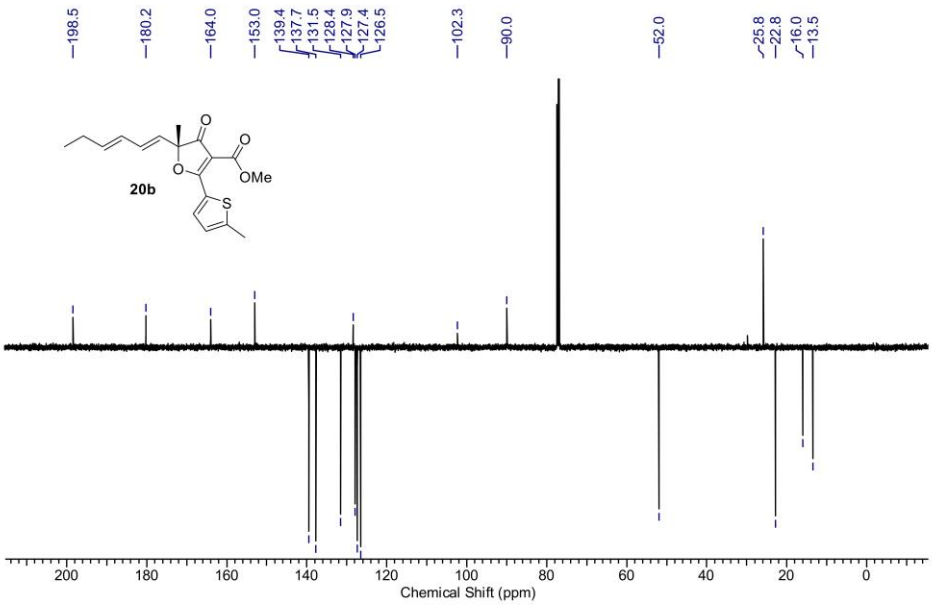

Figure S42. ^{13}C NMR (APT) spectrum of compound **20b** in $CDCl_3$ (125 MHz).

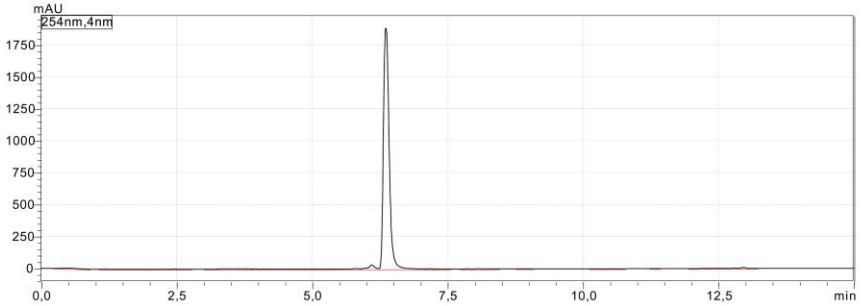

Figure S43: HPLC chromatogram of compound **1b**. HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

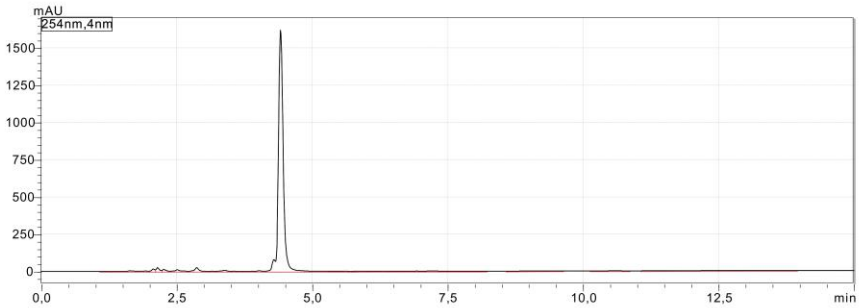

Figure S44: HPLC chromatogram of thiocarboxylic A (**1a**). HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

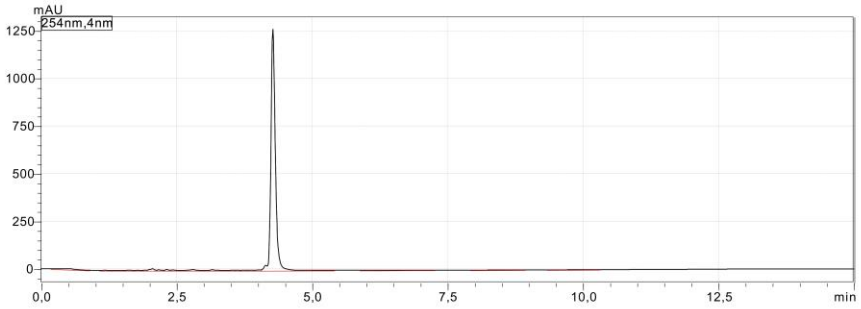

Figure S45: HPLC chromatogram of derivative **20a**. HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

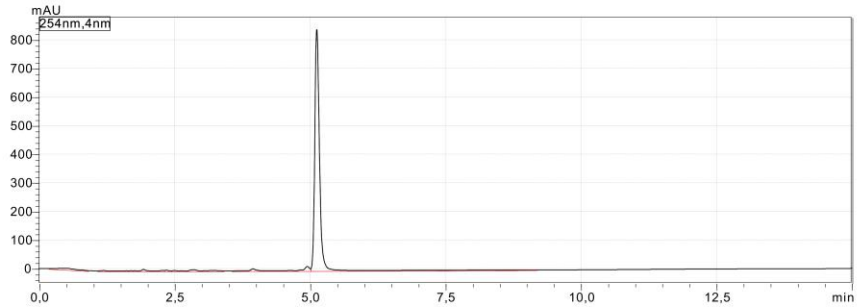

Figure S46: HPLC chromatogram of derivative **20b**. HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

5.4 Publikation III

Syntheses and Antibacterial Evaluation of New *Penicillium* Metabolites Gregatins G and Thiocarboxylics C

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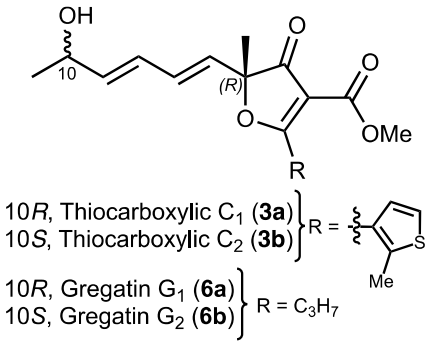

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doi.org/10.1002/cbdv.202300181 **RESEARCH ARTICLE** CHEMISTRY & BIODIVERSITY

Syntheses and Antibacterial Evaluation of New *Penicillium* Metabolites Gregatins G and Thiocarboxylics C

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Two pairs of side-chain epimeric 3-methoxycarbonyl-dihydrofuran-4-ones with structures purported for thiocarboxylics $C_{1/2}$ and gregatins $G_{1/2}$, isolated from *Penicillium* sp. Sb62, were synthesised for the first time in five steps and 17–25% yield. Key steps were a Suzuki cross-coupling, a Yamaguchi esterification, and a base-induced Knoevenagel-type condensation. The optimum protecting group for the 10-OH group in the dienyl side-chain, orthogonal to necessary protecting groups on O-10 of the furanone, was found to be *t*-butyldiphenylsilyl (TBDPS). The specific rotations of our synthetic products deviated markedly from those reported for the natural isolates. In contrast to the isolates, the synthetic products were not active against *Escherichia coli* and *Staphylococcus aureus* bacteria.

Keywords: 3-furanone, bioactivity, gregatin, penicillium, thiocarboxylic.

Introduction

The 3-methoxycarbonyl-dihydrofuran-4-one motif is rare in natural products with gregatins A–E,[1–4] aspertetronins A and B,[5,6] graminins A and B[7] and huspenones A and B[6] having been studied in more detail. In 2020 seven known and ten novel (**1**–**6**) methyl 4-oxo-dihydrofuran-3-carboxylates, including four pairs of epimers, were isolated by *Ruan et al.*[8] from the fungus *Penicillium* sp. Sb62. They comprise gregatin F (**5**) and G (**6**), and the thiophenyl substituted thiocarboxylics A–D (**1**–**4**) (*Figure 1*).

Eight of the new natural products **3**–**6** feature a 10-hydroxylated side-chain giving rise to four pairs of epimers since C-5 is *R*-configured throughout. The side-chains carry either one (**4** and **5**) or two alkenes (**1**–**3** and **6**). All new compounds **1**–**6** showed

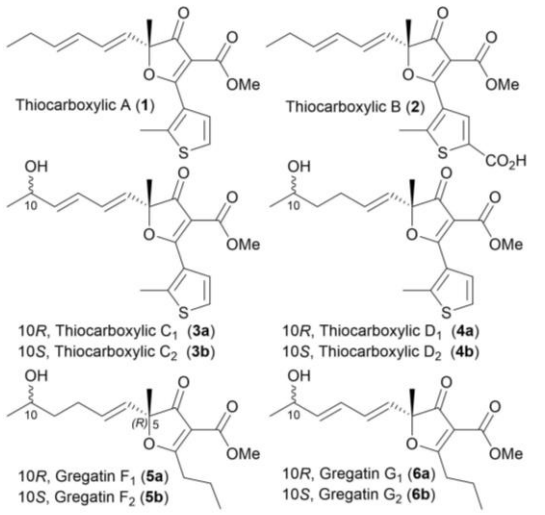
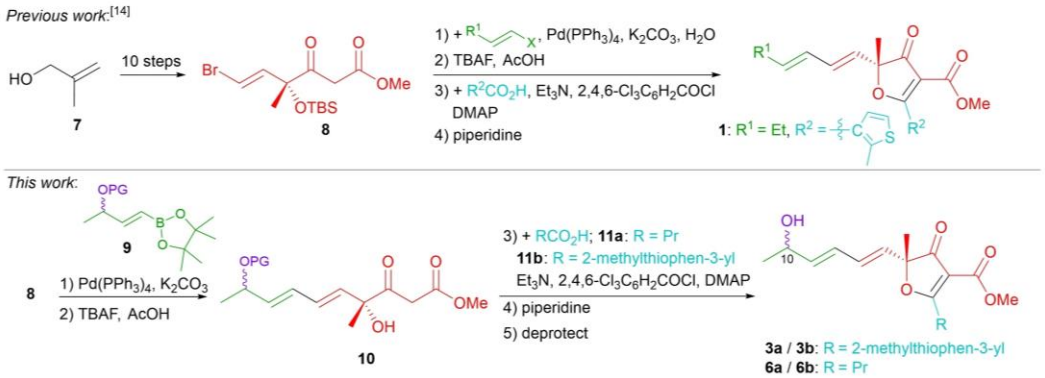


Figure 1. Structures of the thiocarboxylics A–D (**1**–**4**) and gregatins F (**5**) and G (**6**).

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202300181

Chem. Biodiversity **2023**, *20*, e202300181



Scheme 1. *Previous work*: synthesis of methyl 4-oxo-dihydrofuran-3-carboxylates such as thiocarboxylic A (**1**) with flexibility as to the 2-aryl and 5-alkadienyl residues.[14] *This work*: extension to analogs thiocarboxylic C (**3**) and gregatin G (**6**), carrying additional stereogenic alcohols, using suitable protecting groups.

antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* with MIC values ranging from 0.9 to 7.0 μg/mL.

Brückner et al.[9–11] and *Kato et al.*[12,13] published five different syntheses of related dihydrofuranones, e.g., of gregatins A,[10] B,[9–12] C,[10] D,[10] E,[11,12] aspertetronin A[10] and graminin A.[13] Previously, our group reported a new approach to the natural product class of 3-methoxycarbonyl-dihydrofuran-4-ones which allows a more flexible introduction of both residues at C-2 and C-5 of the furanones at a late stage, rendering the majority of the natural products of this class easily accessible (*Scheme 1*, *previous work*).[14] R^1 of the alkenyl side-chain was introduced via a Suzuki cross-coupling of vinyl bromide **8**, synthesized in 10 steps from 2-methylallyl alcohol **7**, and R^2 via a Yamaguchi esterification of the deprotected coupling product alcohol and a subsequent ring-closing Knoevenagel-type condensation. In this way, we synthesised thiocarboxylic A (**1**) for the first time and three derivatives of it with variance in R^1 and R^2.[14]

Herein we report an extension of this synthetic route, accommodating OH-groups in the side-chain at C-10 by identifying protecting groups that are compatible with the OTBS group in precursor **8**. The resulting product epimers gregatin G_1 (**6a**) and G_2 (**6b**), and thiocarboxylic C_1 (**3a**) and C_2 (**3b**) were also evaluated for their antibiotic activities in comparison to the natural isolates.

Results and Discussion

We intended to synthesise thiocarboxylic C (**3**) and gregatin G (**6**) by first reacting vinyl bromide **8**[14] with boronate **9**, carrying an aptly protected hydroxy group at a stereogenic center, in a Suzuki cross-coupling (*Scheme 1*, *this work*). After cleavage of the TBS group, tertiary alcohol **10** should be converted to the corresponding β-ketoacyl esters by a Yamaguchi esterification with carboxylic acids **11**, introducing R. The five-membered ring was to be closed by a base-induced Knoevenagel-type condensation to afford the respective *O*-protected dihydrofuranones. Deprotection of the secondary alcohol would eventually furnish thiocarboxylics C_1 (**3a**) and C_2 (**3b**), or gregatins G_1 (**6a**) and G_2 (**6b**).

Due to the TBS group in vinyl bromide **8** and the functionalities of the natural products, the choice of protecting groups for boronates **9** is limited. *Kato et al.* described that the fluoride-mediated desilylation of a TBS group at the same position in racemic gregatin E only led to a maximum yield of 25% because of the instability of the product under these conditions.[12] Since we were not sure whether the natural products would be more stable under basic or acidic deprotection conditions, we tried two different orthogonal protecting group strategies, employing TBDPS and 2-methoxyethoxymethyl (MEM) as protecting groups for the secondary alcohol. We started with the *R*-configured boronates **9a**[1] and **9b**[1] to get gregatin G_1

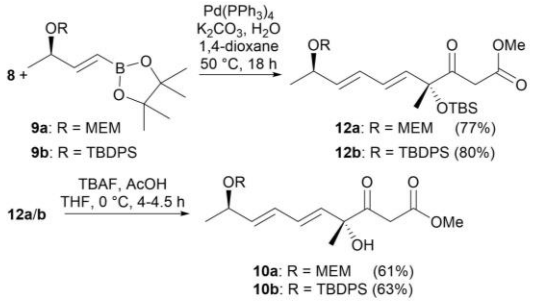
[1]The synthesis of the boronates **9a**–**c** can be found in detail in the *Supporting Information*.

Chem. Biodiversity **2023**, *20*, e202300181

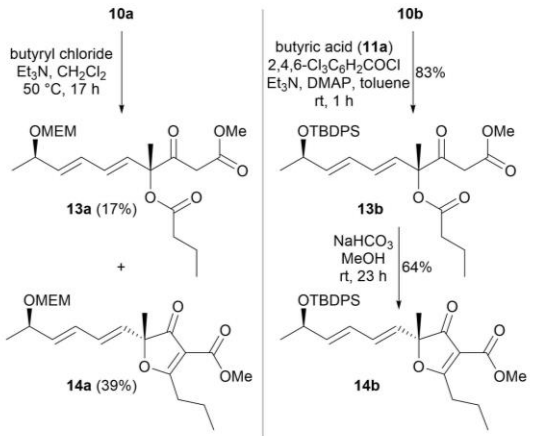
(**6a**) and thiocarboxylic C_1 (**3a**) before synthesising their epimers **3b** and **6b**.

The Suzuki cross-couplings of vinyl bromide **8** and the boronates **9a** or **9b** were run under the conditions already elaborated in our earlier work, using 8 mol% $Pd(PPh_3)_4$ in the presence of K_2CO_3 (*Scheme 2*).[14] (*E,E*)-Dienes **12a** and **12b** were obtained with good yields and void of (*Z*)-isomers. Cleavage of the TBS group of **12a** or **12b** with tetra-*n*-butylammonium fluoride (TBAF) and AcOH afforded β-keto-γ-hydroxyesters **10a** or **10b** in moderate yields, irrespective of the second protecting group.

Contrary to our original plan we tried to convert alcohol **10a** in one step into furanone **14a** with butyryl chloride and Et_3N according to the procedure of *Burghart-Stoll* and *Brückner*[10] (*Scheme 3*, *left*). The problem was that ester **13a**, which formed in situ, did not react completely to furanone **14a** and that the two compounds could not be separated fully by column chromatography due to virtually equal polarity, as to TLC. After three purification cycles by column chromatography, we obtained only 39% of pure dihydrofuranone **14a** and 17% of diester **13a**. Next, we prepared furanone **14b** in two steps. Yamaguchi esterification[14] of alcohol **10b** with butyric acid (**11a**) gave pure ester **13b** which was submitted to a Knoevenagel-type cyclisation with $NaHCO_3$ (*Scheme 3*, *right*).[10,15] The 53% yield of **14b** over these two steps was slightly better than that of the single-step approach. In addition, compounds **13b** and **14b** could be easily purified by a single column chromatography.



Scheme 2. Synthesis of tertiary alcohols **10** with MEM (**10a**) or TBDPS (**10b**) protected secondary alcohols.

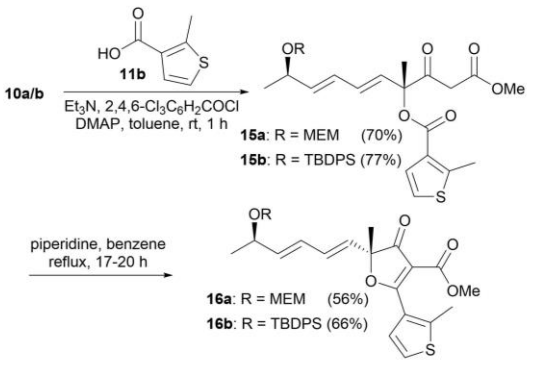


Scheme 3. *Left*: One-step synthesis of MEM-protected furanone **14a** starting from alcohol **10a**; *right*: synthesis of TBDPS-protected furanone **14b** in two condensation steps starting from alcohol **10b**.

Based on these results, we decided to stick to the original plan to synthesise the differently protected precursors for thiocarboxylic C_1 **16a** and **16b** in two steps (*Scheme 4*).

Starting from alcohols **10a**, or **10b** we obtained **16a** and **16b** with yields of 70% and 77% for the esterification with 2-methylthiophene-3-carboxylic acid (**11b**), and 56% and 66% for the ring-closure. The results of the final cleavage of the MEM and TBDPS groups, respectively, are shown in *Table 1*. Gregatin G_1 (**6a**) was obtained by deprotection of **14a** under (Lewis)[16] acidic[17,18] conditions, yet only in yields of 11–32% because of the instability of **6a** under these conditions (entries 1–3). For example, using $ZnBr_2$,[16] the dihydrofuranone ring of **14a** partly opened to give back the acyclic diester **13a** (entry 1).

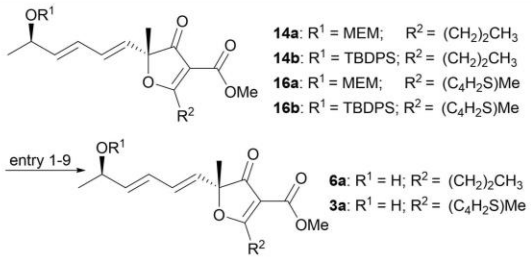
Deprotection of **16a** under the same conditions failed to give thiocarboxylic C_1 (**3a**) because the electron-rich heteroarene destabilises the furanone even more (entry 4). With trifluoroacetic acid (TFA),[19]



Scheme 4. Two-step synthesis of furanones **16a** and **16b**, carrying a thiophene moiety, starting from alcohols **10a** or **10b**.

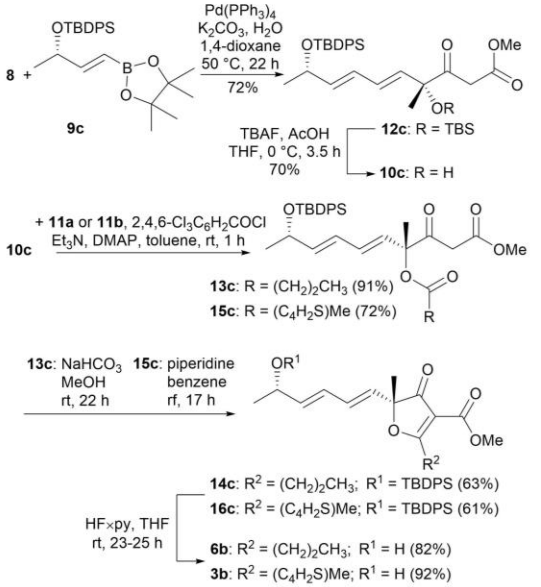
Chem. Biodiversity **2023**, *20*, e202300181

Table 1. Cleavage of the MEM and TBDPS groups of compounds **14** and **16** to afford gregatin G_1 (**6a**) and thiocarboxylic C_1 (**3a**); conditions and yields.



entry	educt	conditions	product	yield [%]
1[16]	**14a**	(A)	**6a**	23
2[17]	**14a**	(B)	**6a**	11
3[18]	**14a**	(C)	**6a**	32
4[16]	**16a**	(A)	**3a**	–
5[19]	**16a**	(D)	**3a**	–
6[18]	**16a**	(C)	**3a**	22
7[20]	**14b**	(E)	**6a**	64
8[20]	**16b**	(E)	**3a**	–
9[21]	**16b**	(F)	**3a**	96

Conditions: (A) $ZnBr_2$, CH_2Cl_2, r.t., 22; (B) PPTS, *t*BuOH, refl., 24; (C) conc. HCl, MeOH, r.t., 24; (D) TFA, CH_2Cl_2, r.t., 1; (E) TBAF, THF, 45 °C, 7; (F) HF·py, THF, r.t., 23.



Scheme 5. Synthesis of thiocarboxylic C_2 (**3b**) and gregatin G_2 (**6b**) in five steps starting from vinyl bromide **8**.

16a was even completely decomposed (entry 5), while when treated with conc. HCl[18] gave product **3a** with 22% yield (entry 6). Desilylation of TBDPS ether **14b** with TBAF[20] at 45 °C afforded gregatin G_1 (**6a**) with a yield of 64% (entry 7), which was acceptable, given the low yields of the attempts to cleave off the MEM group. Though we had already suspected that the desilylation of **16b** with TBAF might proceed less well than that of **14b** due to the thiophene moiety, the complete decomposition occurring under these conditions came as a surprise (entry 8). However, thiocarboxylic C_1 (**3a**) was obtained with an excellent yield of 96% upon treating silyl ether **16b** with 80 equivalents of HF-pyridine (entry 9).[21] Related to starting vinyl bromide **8**, thiocarboxylic C_1 (**3a**) was obtained with an overall yield of 25% when using a TBDPS protecting group, and with a mere 4% yield when using a MEM group. Gregatin G_1 (**6a**) was synthesised with a total yield of 6% (MEM) and 17% (TBDPS).

Since for both natural products the yields of all steps, and especially those of the final deprotection, were better when using TBDPS as the protecting group of the secondary alcohol, we decided to prepare gregatin G_2 (**6b**) and thiocarboxylic C_2 (**3b**) also with this protecting group strategy (*Scheme 5*). Hence, we performed the Suzuki cross-coupling of vinyl bromide **8** with the TBDPS protected, *S*-configured boronate **9c**[1] and obtained the coupling product **12c** with 72% yield. The latter was desilylated with TBAF and AcOH to give alcohol **10c**, which was esterified with butyric acid (**11a**) or 2-methylthiophene-3-carboxylic acid (**11b**) to give the esters **13c** or **15c** with 91% and 72% yield, respectively. The Knoevenagel-type reaction of **13c** was induced by $NaHCO_3$ and that of **15c** by piperidine, and afforded the dihydrofuranones **14c** and **16c** with yields slightly above 60%. The final cleavage of the TBDPS group using HF-pyridine gave thiocarboxylic C_2 (**3b**) and gregatin G_2 (**6b**) in good yields. Related to **8**, the overall yields amounted to 24% (**6b**) and 20% (**3b**).

The NMR data of our synthetic thiocarboxylic C_1 (**3a**), C_2 (**3b**), gregatin G_1 (**6a**) and G_2 (**6b**) agreed well with those reported in the literature[8] for the isolates (*cf. Supporting Information*). Not so the specific rotations. While synthetic thiocarboxylic C_1 (**3a**) showed an $[\alpha]_D^{25} = -99$ ($c = 0.25$, $CHCl_3$) and synthetic compound **3b** an $[\alpha]_D^{27} = -108$ ($c = 1.0$, $CHCl_3$), a value of $[\alpha]_D^{20} = -44$ ($c = 0.1$, $CHCl_3$)[8] was reported for isolate **3a** and $[\alpha]_D^{20} = -40$ ($c = 0.1$, $CHCl_3$)[8] for isolate **3b**. The values for gregatin G_1 (**6a**) and G_2 (**6b**) also differed from those of the isolate. The values of the isolates with $[\alpha]_D^{20} = +177$ ($c = 0.1$, $CHCl_3$)[8] for **6a** and $[\alpha]_D^{20} =$

Chem. Biodiversity **2023**, *20*, e202300181

+171 ($c=0.1$, $CHCl_3$)[8] for **6b** are higher than the values of our synthetic compounds with $[\alpha]_D^{25}=+96$ ($c=0.50$, $CHCl_3$) for **6a** and $[\alpha]_D^{25}=+104$ ($c=1.00$, $CHCl_3$) for **6b**. These deviations might be due to impurities of the isolated compounds as visible in their ^{1}H-NMR spectra.[8]

As *Ruan et al.* had reported antimicrobial activity of their isolated thiocarboxylic $C_{1/2}$ (**3a**,**b**) with MIC of 10.5 and 21 μM and of gregatin $G_{1/2}$ (**6a**,**b**) with MIC of 8.7 μM against *E. coli* and *S. aureus*,[8] we also tested our synthetic products **3a**, **3b**, **6a** and **6b** for their activities against these bacteria (*cf. Supporting Information*). However, they were all virtually inactive with IC_{50} values beyond the highest tested concentration of 100 μM. They proved also not cytotoxic in concentrations up to 100 μM against cell lines A549 (human lung carcinoma), L929 (mouse fibroblasts) and Huh7 (human hepatocellular carcinoma). A plausible assumption for the activities of the natural isolates observed by Ruan *et al.* would be the presence of other, unidentified active metabolites.

Conclusions

Based on our recently established synthetic approach to natural products with 3-methoxycarbonyl-dihydrofuran-4-one structures,[14] we now synthesised two pairs of epimers of more complex furanones, bearing an additional stereogenic center in the 5-alkadienyl side-chain. Compounds supposed to have these structures were isolated by *Ruan et al.* in 2020.[8] We tested two different orthogonal protecting group strategies, and identified TBDPS as the protecting group of choice for the secondary alcohol of the side-chain. Starting from a TBS-protected vinyl bromide with β-ketoester functionality, we built up gregatin G_1 and G_2 as well as thiocarboxylic C_1 and C_2 by a sequence of Suzuki cross-coupling, Yamaguchi esterification, base-induced Knoevenagel-type condensation, and two desilylation steps. The total yields over these five steps ranged from 17% to 25%. As was the case with thiocarboxylic A,[14] our synthetic gregatins $G_{1/2}$ and thiocarboxylics $C_{1/2}$ exhibited specific rotations and antibiotic activities strikingly at variance with those reported of the natural isolates. In contrast to the isolates, our synthetic products showed no antibiotic activity against *Escherichia coli* and *Staphylococcus aureus*. Given the matching NMR spectra, an erroneous structure assignment of the natural isolates appears rather unlikely, whereas the entrainment of trace amounts of other active *Penicillium* metabolites remains conceivable.

Experimental Section

General Information

Melting points were determined with a Büchi M-565 melting point apparatus and are uncorrected. Optical rotations were measured at 589 nm (Na–D line) on a PerkinElmer 241 Polarimeter using solutions in $CHCl_3$. IR spectra were recorded with a FT-IR spectrophotometer equipped with an ATR unit. ^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Bruker Avance III HD 500 spectrometer. Chemical shifts of NMR signals are given using the residual solvent peak as an internal standard, i.e., 7.26 ppm (proton) and 77.16 ppm (carbon) for $CDCl_3$. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. Analytical HPLC measurements were carried out on a Shimadzu Nexera XR with autosampler SIL-20 A using a Knauer Eurospher II C-18 column (150×4 mm), pore size 100 Å, particle size 3 μm. Detection was executed by a diode array detector SPD–M20 A.

Chemicals. All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran (THF), toluene and 1,4-dioxane which were freshly distilled over sodium/benzophenone, and benzene, MeOH and CH_2Cl_2 which were dried over molecular sieves (3 Å). Moisture or air sensitive reactions were routinely carried out in oven-dried glassware under an argon atmosphere using standard Schlenk technique.

Chromatography. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel $60GF_{254}$ pre-coated aluminum-backed plates. The compounds were visualized with UV light (254 nm) and/or ceric ammonium molybdate (CAM). Column chromatography was performed at medium pressure using dry packed Macherey-Nagel silica gel 60, pore size 40–63 μm with the eluent specified.

General Procedures

*Suzuki Cross-Coupling of Vinyl Bromide **8** with Boronates **9a–c**[1] Into (E,E)-Dienes **12a–c***

The Suzuki coupling was carried out under an argon atmosphere using standard Schlenk technique for

Chem. Biodiversity **2023**, *20*, e202300181

oxygen exclusion. To a stirred solution of vinyl bromide **8** (1.00 equiv.) in dry 1,4-dioxane was added $Pd(PPh_3)_4$ (8 mol%) and the reaction mixture was stirred for 5 min at ambient temperature. Boronic ester **9a**, **9b** or **9c** (1.40–1.50 equiv.) in 1,4-dioxane and K_2CO_3 (2.00 equiv.) in H_2O were added. The reaction mixture was heated to 50 °C and stirred for 18–22 at this temperature. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 and the phases were separated. The organic phase was washed with sat. aqueous NH_4Cl, H_2O and brine and was dried over Na_2SO_4. The volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (**12a**: 5% to 10% AcOEt in *n*-pentane; **12b** and **12c**: 2% AcOEt in *n*-pentane) afforded coupling product **12**.

Desilylation of the Coupling Products **12a–c** *Into Alcohols* **10a–c**

Silyl ether **12** (1.00 equiv.) was dissolved in dry THF and AcOH (2.0 equiv.) and TBAF (1M in THF, 2.00 equiv.) were added to the solution at 0 °C. After 3.5–4.5 at 0 °C sat. aqueous NH_4Cl and sat. aqueous $NaHCO_3$ were added and the layers were separated. The aqueous layer was extracted three times with CH_2Cl_2, the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (**10a**: 50% AcOEt in *n*-pentane; **10b** and **10c**: 20% AcOEt in *n*-pentane) to yield γ-hydroxy-β-ketoester **10**.

Yamaguchi Esterification of γ-hydroxy-β-ketoesters **10a–c** *Into β-ketoacyl Esters* **13b–c** *and* **15a–c**

A stirred solution of alcohol **10** (1.15 equiv.) and 2-methylthiophene-3-carboxylic acid (**11b**, 1.00 equiv.) or butyric acid (**11a**, 1.00 equiv.) in dry toluene was treated with Et_3N (3.00 equiv.), 2,4,6-trichlorobenzoyl chloride (1.58 equiv.) and DMAP (1.50 equiv.). The reaction mixture was stirred at room temperature for 1 before being quenched with sat. aqueous $NaHCO_3$. After phase separation the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (**13b**, **13c**, **15b** and **15c**: 10% AcOEt in *n*-pentane; **15a**: 20% AcOEt in *n*-pentane) afforded esters **13b–c** or **15**.

Knoevenagel-Type Condensation of **15a–c** *Into 3-furanones* **16a–c**

Ester **15** (1.00 equiv.) was dissolved in dry benzene and piperidine (5.00 equiv.) was added to the solution at room temperature. The reaction mixture was heated at reflux for 17–20 before it was allowed to cool to room temperature. The volatiles were removed in vacuo and the remainder was purified by column chromatography (**16a**: 20% AcOEt in *n*-pentane, **16b**: 10% AcOEt in *n*-pentane; **16c**: 10% to 20% AcOEt in *n*-pentane) to obtain product **16**.

Knoevenagel-Type Condensation of **13b–c** *Into 3-furanone* **14b–c**

Compound **13b** or **13c** (1.00 equiv.) was dissolved in dry MeOH and $NaHCO_3$ (2.50 equiv.) was added to the solution at room temperature. The reaction mixture was stirred for 22–23 at this temperature before being quenched with H_2O. After phase separation the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (10% AcOEt in *n*-pentane) afforded product **14b–c**.

Conversion of Alcohol **10a** *Into Ester* **13a** *and 3-furanone* **14a**

A stirred solution of alcohol **10a** (1.00 equiv.) in dry CH_2Cl_2 was treated with butyryl chloride (3.00 equiv.) and Et_3N (6.00 equiv.). The reaction mixture was heated at reflux for 17 before being quenched with H_2O. The phases were separated and the aqueous phase was extracted three times with Et_2O. The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by three column chromatography runs (20% AcOEt in *n*-pentane) afforded product **14a** together with ester **13a**.

Synthesis of Gregatin G_1 (**6a**)

[2]Silyl ether **14b** (1.00 equiv.) was dissolved in dry THF and TBAF (1M in THF, 4.00 equiv.) was added to the solution at 0 °C. After 6.5 h at 0 °C sat. aqueous NH_4Cl was added and the layers were separated. The

[2]Only the TBDPS deprotection is mentioned. The MEM deprotection can be found in the *Supporting Information.*

Chem. Biodiversity **2023**, *20*, e202300181

aqueous layer was extracted three times with CH_2Cl_2, the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (30% to 40% AcOEt in *n*-pentane) to yield gregatin G_1 (**6a**).

Desilylation of the TBDPS-Group to Obtain Thiocarboxylic $C_{1,2}$ (**3a**,[2]**b**) *and Gregatin G_2* (**6b**)

Silyl ether **14c**, **16b** or **16c** (1.00 equiv.) was dissolved in dry THF and 70% HF-pyridine (80.0 equiv.) was added to the solution at 0 °C. After 23–25 at room temperature sat. aqueous K_2CO_3 and H_2O were added and the layers were separated. The aqueous layer was extracted three times with Et_2O, the combined organic phases were washed with sat aqueous $CuSO_4$ and were dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (**3a** and **6b**: 30% to 50% AcOEt in *n*-pentane; **3b**: 30% to 40% AcOEt in *n*-pentane) to yield the natural products **3a**, **3b** or **6b**.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contribution Statement

The manuscript was written through substantial contributions of all authors (FG, chemical synthesis and analytics; FM and UB, biotests and data evaluation; RS, supervision, manuscript drafting). All authors have given approval to the final version of the manuscript.

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Supporting Information

Syntheses and Antibacterial Evaluation of New *Penicillium* Metabolites Gregatins G and Thiocarboxylics C

Franziska Gillsch, Fredrick Mbui, Ursula Bilitewski, and Rainer Schobert*

S1

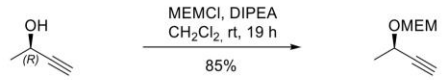
Table of Contents

General information

All reagents were purchased from commercial sources (ABCR, Acros, Alfa Aeser, Carbolution, chemPur, Fluorochem, Merck, Sigma Aldrich, TCI) and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran (THF), toluene and 1,4-dioxane which were freshly distilled over sodium/benzophenone, and benzene, methanol and dichloromethane which were dried over molecular sieve (3 Å). Moisture or air-sensitive reactions were routinely carried out in oven-dried glassware under an argon atmosphere using standard Schlenk technique. Reactions that required heating were set up in an oil bath. Melting points were determined with a Büchi M-565 melting point apparatus and are uncorrected. IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. ^{1}H-NMR, ^{13}C-NMR (APT) were obtained using a Bruker Avance III HD 500 spectrometer. Chemical shifts of NMR signals are given in parts per million (δ) using the residual solvent peak as an internal standard, i.e., 7.26 ppm (proton) and 77.16 ppm (carbon) for $CDCl_3$. Coupling constants (*J*) are quoted in Hz. Multiplicity abbreviations used: s singlet, d doublet, t triplet, q quartet, br broad and m multiplet. High-resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 Polarimeter using solutions in chloroform. For chromatography silica gel 60 (230–400 mesh) was used. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60GF_{254} pre-coated aluminum-backed plates. The compounds were visualized with UV light (254 nm) and/or ceric ammonium molybdate (CAM). Column chromatography was performed at medium pressure using dry packed Macherey-Nagel silica gel 60, pore size 40–63 µm with the eluent specified in the respective experimental procedures. Analytical HPLC measurements were carried out on a Shimadzu Nexera XR with autosampler SIL-20A using a Knauer Eurospher II C-18 column (150 × 4 mm), pore size 100 Å, particle size 3 µm. Detection was executed by a diode array detector SPD-M20A.

Experimental procedures

(*R*)-3-((2-Methoxyethoxy)methoxy)but-1-ynediphenylsilane (S2a)

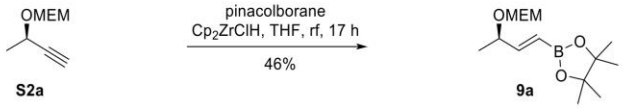


S1a **S2a**

To a solution of (*R*)-3-butyn-2-ol (**S1a**, 1.00 mL, 12.7 mmol, 1.00 eq.) in dry CH_2Cl_2 (25 mL) were added MEMCl (4.35 mL, 38.1 mmol, 3.00 eq.) and DIPEA (6.48 mL, 38.1 mmol, 3.00 eq.) at 0 °C. The reaction mixture was allowed to warm up to ambient temperature and was stirred for 19 h. The reaction was quenched with sat. aqueous $NaHCO_3$ (30 mL) and the phases were separated. The aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were washed with sat. aqueous NH_4Cl (50 mL), dried over $MgSO_4$ and concentrated in vacuo. The remainder was purified by column chromatography (20% Et_2O in *n*-pentane), to afford MEM-ether **S2a** as a colorless oil (1.70 g, 10.7 mmol, 85%).

R_f = 0.50 (20% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +217.9 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 3260 (br.), 2890, 1737, 1119, 1086, 1035, 1016, 880, 849 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 4.98 (d, *J* = 7.0 Hz, 1 H), 4.72 (d, *J* = 7.2 Hz, 1 H), 4.48 (qd, *J* = 6.7, 2.1 Hz, 1 H), 3.79–3.74 (m, 1 H), 3.68–3.63 (m, 1 H), 3.57–3.54 (m, 2 H), 3.39 (s, 3 H), 2.39 (d, *J* = 2.0 Hz, 1 H), 1.45 (d, *J* = 6.7 Hz, 3 H. ^{13}C-NMR (125 MHz, $CDCl_3$): 93.2, 83.5, 72.9, 71.9, 67.3, 61.4, 59.2, 22.1. HR-MS: 181.0832 ($[M+Na]^+$, $C_8H_{14}O_3Na^+$; calc. 181.0835).

(*R*,*E*)-2-(3-((2-Methoxyethoxy)methoxy)but-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (9a)

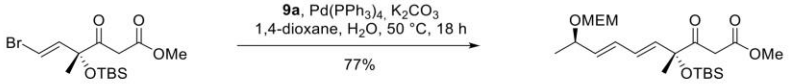


S2a **9a**

Zirconocene dichloride (924 mg, 3.16 mmol, 0.50 eq.) in dry THF (3 mL) was treated with DIBAL-H (1M in *n*-hexane, 3.22 mL, 3.22 mmol, 0.51 eq.) at 0 °C. The reaction mixture was stirred 60 min at 0 °C. Thereafter alkyne **S2a** (1.00 g, 6.32 mmol, 1.0 eq.) in dry THF (8 mL) and pinacolborane (1.38 mL, 9.48 mmol, 1.50 eq.) were added to the solution of Schwartz' Reagent at 0 °C. The reaction mixture was allowed to warm up to room temperature and was heated at reflux for 17 h. H_2O (10 mL) was added carefully to quench the reaction. The phases were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The combined organic phases were dried over $MgSO_4$ and volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (30% Et_2O in *n*-pentane) to yield boronic ester **9a** as a colorless oil (828 mg, 2.89 mmol, 46%).

R_f = 0.30 (20% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +47.0 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2977, 1362, 1323, 1144, 1105, 1095, 1032, 998, 936, 850 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.50 (dd, *J* = 18.2, 6.0 Hz, 1 H), 5.60 (dd, *J* = 18.2, 1.2 Hz, 1 H), 4.72 (d, *J* = 7.0 Hz, 1 H), 4.70 (d, *J* = 7.2 Hz, 1 H), 4.27 (dqd, *J* = 6.5, 6.0, 1.2 Hz, 1 H), 3.79–3.73 (m, 1 H), 3.67–3.61 (m, 1 H), 3.55 (dd, *J* = 4.8, 4.6 Hz, 2 H), 3.39 (s, 3 H), 1.26 (s, 12 H), 1.24 (d, *J* = 5.8 Hz, 3 H. ^{13}C-NMR (125 MHz, $CDCl_3$): 153.8, 93.4, 83.4 (2 C-atoms), 73.9, 71.9, 67.0, 59.2, 24.94 (2 C-atoms), 24.90 (2 C-atoms), 20.8 (The other alkenyl C-atom could not be observed. This special occurrence was already reported in the literature[1]). HR-MS: 309.1842 ($[M+Na]^+$, $C_{14}H_{27}O_5BNa^+$; calc. 309.1844).

Methyl (4*R*,5*E*,7*E*,9*R*)-4-((*tert*-butyldimethylsilyl)oxy)-9-((2-methoxyethoxy)methoxy)-4-methyl-3-oxodeca-5,7-dienoate (12a)

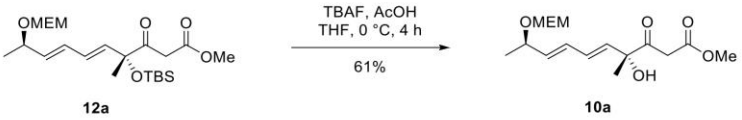


8 **12a**

The Suzuki coupling was carried out under an argon atmosphere using standard Schlenk technique for oxygen exclusion. To a stirred solution of vinyl bromide **8** (500 mg, 1.37 mmol, 1.00 eq.) in dry 1,4-dioxane (20 mL) was added $Pd(PPh_3)_4$ (127 mg, 0.110 mmol, 8 mol%) and the reaction mixture was stirred for 5 min at ambient temperature. Boronic ester **9a** (514 mg, 1.92 mmol, 1.40 eq.) in 1,4-dioxane (10 mL) and K_2CO_3 (379 mg, 2.74 mmol, 2.00 eq.) in H_2O (3 mL) were added. The reaction mixture was heated to 50 °C and stirred for 18 h at this temperature. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and the phases were separated. The organic phase was washed with sat. aqueous NH_4Cl (20 mL), H_2O (20 mL) and brine (20 mL) and was dried over Na_2SO_4. The volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (5% to 10 % EtOAc in *n*-pentane) afforded coupling product **12a** as a light yellowish oil (469 mg, 1.05 mmol, 77%).

R_f = 0.25 (20% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +173.5 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2931, 1752, 1723, 1257, 1136, 1108, 1088, 1036, 1024, 994, 837, 778 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.31 (dd, *J* = 15.2, 10.6 Hz, 1 H), 6.16 (dd, *J* = 15.2, 10.6 Hz, 1 H), 5.61 (dd, *J* = 15.2, 7.4 Hz, 1 H), 5.60 (d, *J* = 15.3 Hz, 1 H), 4.74 (d, *J* = 7.1 Hz, 1 H), 4.67 (d, *J* = 7.0 Hz, 1 H), 4.26 (dq, *J* = 7.4, 6.4 Hz, 1 H), 3.79–3.73 (m, 1 H), 3.74 (d, *J* = 16.6 Hz, 1 H), 3.71 (s, 3 H), 3.66–3.61 (m, 1 H), 3.61 (d, *J* = 16.6 Hz, 1 H), 3.57–3.53 (m, 2 H), 3.39 (s, 3 H), 1.50 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H), 0.94 (s, 9 H), 0.11 (s, 3 H), 0.10 (s, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 204.8, 168.5, 136.3, 135.0, 130.7, 130.4, 93.0, 82.6, 72.1, 71.9, 67.0, 59.2, 52.3, 43.7, 26.0 (3 C-atoms), 24.4, 21.3, 18.4, –2.1, –2.2. HR-MS: 467.2426 ($[M+Na]^+$, $C_{22}H_{40}O_7SiNa^+$; calc. 467.2436).

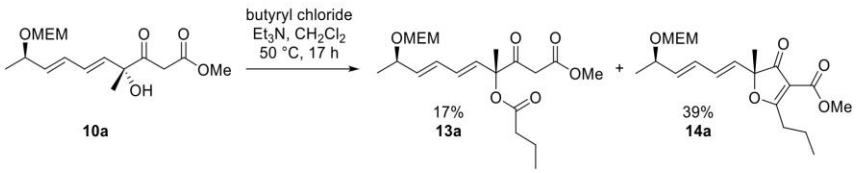
Methyl (4*R*,5*E*,7*E*,9*R*)-4-hydroxy-9-((2-methoxyethoxy)methoxy)-4-methyl-3-oxodeca-5,7-dienoate (10a)



Silyl ether **12a** (403 mg, 0.906 mmol, 1.00 eq.) was dissolved in dry THF (10 mL) and AcOH (104 µL, 1.81 mmol, 2.0 eq.) and TBAF (1M in THF, 1.81 mL, 1.81 mmol, 2.00 eq.) were added to the solution at 0 °C. After 4 h at 0 °C sat. aqueous NH_4Cl (5 mL) and sat. aqueous $NaHCO_3$ (5 mL) were added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (50% EtOAc in *n*-pentane) to yield γ-hydroxy-β-ketoester **10a** as a colorless oil (184 mg, 0.557 mmol, 61%).

R_f = 0.33 (50% EtOAc in *n*-hexane). $[\alpha]_D^{26}$ = +31.4 (*c* = 0.30, $CHCl_3$). IR (neat) ν_{max} = 3452 (br.), 2926, 1748, 1717, 1438, 1318, 1134, 1085, 1032, 1019, 995, 845 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.40 (dd, *J* = 15.3, 10.7 Hz, 1 H), 6.16 (dd, *J* = 15.3, 10.6 Hz, 1 H), 5.68 (d, *J* = 15.1 Hz, 1 H), 5.66 (dd, *J* = 15.4, 7.3 Hz, 1 H), 4.73 (d, *J* = 7.0 Hz, 1 H), 4.67 (d, *J* = 6.9 Hz, 1 H), 4.26 (dq, *J* = 7.3, 6.4 Hz, 1 H), 3.79–3.74 (m, 1 H), 3.73 (s, 3 H), 3.69 (d, *J* = 15.8 Hz, 1 H), 3.66–3.61 (m, 1 H), 3.58 (d, *J* = 16.0 Hz, 1 H), 3.57–3.54 (m, 2 H), 3.52 (br. s, 1 H), 3.39, (s, 3 H), 1.49 (s, 3 H), 1.26 (d, *J* = 6.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 204.3, 167.9, 136.8, 133.1, 131.0, 130.2, 92.9, 79.8, 72.1, 71.9, 66.9, 59.2, 52.8, 43.5, 25.1, 21.4. HR-MS: 353.1568 ($[M+Na]^+$, $C_{16}H_{26}O_7Na^+$; calc. 353.1571).

Methyl (4*R*,5*E*,7*E*,9*R*)-9-((*tert*-butyldiphenylsilyl)oxy)-4-(butyryloxy)-4-methyl-3-oxodeca-5,7-dienoate (13a) and Methyl (*R*)-5-((*R*,1*E*,3*E*)-5-((2-methoxyethoxy)methoxy)hexa-1,3-dien-1-yl)-5-methyl-4-oxo-2-propyl-4,5-dihydrofuran-3-carboxylate (14a)

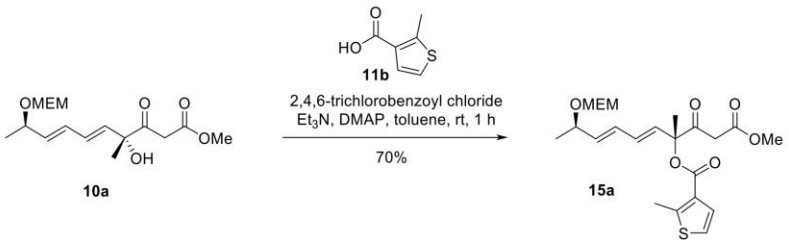


A stirred solution of alcohol **10a** (62.0 mg, 0.188 mmol, 1.00 eq.) in dry CH_2Cl_2 (2 mL) was treated with butyryl chloride (58.2 µL, 0.563 mmol, 3.00 eq.) and Et_3N (156 µL, 1.13 mmol, 6.00 eq.). The reaction mixture was heated at reflux for 17 h before being quenched with H_2O (3 mL). The phases were separated and the aqueous phase was extracted with Et_2O (3 × 3 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by three column chromatography runs (20% EtOAc in *n*-pentane) afforded product **14a** as a colorless oil (28.0 mg, 73.3 µmol, 39%) together with ester **13a** (13.0 mg, 32.0 µmol, 17%).

13a: R_f = 0.59 (50% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +187 (*c* = 0.60, $CHCl_3$). IR (neat) ν_{max} = 2931, 1751, 1728, 1438, 1261, 1137, 1088, 1021, 994, 802 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.35 (dd, *J* = 15.5, 10.6 Hz, 1 H), 6.18 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.75 (d, *J* = 15.4 Hz, 1 H), 5.68 (dd, *J* = 15.3, 7.2 Hz, 1 H), 4.73 (d, *J* = 7.1 Hz, 1 H), 4.67 (d, *J* = 7.0 Hz, 1 H), 4.27 (dq, *J* = 7.2, 6.4 Hz, 1 H), 3.79–3.73 (m, 1 H), 3.70 (s, 3 H), 3.66–3.60 (m, 1 H), 3.57–3.54 (m, 2 H), 3.51 (d, *J* = 15.6 Hz, 1 H), 3.44 (d, *J* = 15.6 Hz, 1 H), 3.39 (s, 3 H), 2.36 (t, *J* = 7.4 Hz, 2 H), 1.68 (qt, *J* = 7.5, 7.4 Hz, 2 H), 1.61 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H), 0.98 (t, *J* = 7.5 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.3, 172.9, 167.4, 137.3, 131.6, 130.1, 130.0, 93.0, 85.4, 72.0, 71.9, 67.0, 59.2, 52.5, 43.2, 36.3, 21.4, 21.3, 18.4, 13.8. HR-MS: 423.1977 ($[M+Na]^+$, $C_{20}H_{32}O_8Na^+$; calc. 423.1989).

14a: R_f = 0.55 (50% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +175 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2931, 1748, 1707, 1584, 1739, 1387, 1198, 1129, 1089, 1038, 1022, 993, 798, 780 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.25 (dd, *J* = 15.3, 10.6 Hz, 1 H), 6.12 (dd, *J* = 15.2, 10.8 Hz, 1 H), 5.64 (d, *J* = 15.3 Hz, 1 H), 5.63 (dd, *J* = 15.3, 7.4 Hz, 1 H), 4.70 (d, *J* = 7.0 Hz, 1 H), 4.64 (d, *J* = 7.0 Hz, 1 H), 4.23 (dq, *J* = 7.4, 6.6 Hz, 1 H), 3.81 (s, 3 H), 3.76–3.71 (m, 1 H), 3.63–3.58 (m, 1 H), 3.55–3.51 (m, 2 H), 3.37 (s, 3 H), 3.06–2.96 (m, 2 H), 1.77 (qt, *J* = 7.4, 7.4 Hz, 2 H), 1.51 (s, 3 H), 1.23 (d, *J* = 6.4 Hz, 3 H), 1.02 (t, *J* = 7.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 199.1, 198.2, 163.4, 137.4, 130.5, 129.8, 128.6, 106.4, 92.9, 91.0, 72.0, 71.9, 67.0, 59.2, 51.8, 32.8, 22.7, 21.4, 20.1, 14.0. HR-MS: 405.1874 ($[M+Na]^+$, $C_{20}H_{30}O_7Na^+$; calc. 405.1884).

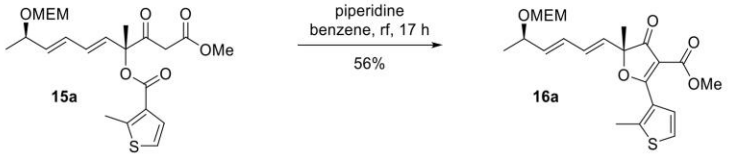
(8*R*,9*E*,11*E*,13*R*)-8,13-Dimethyl-14,16-dioxo-2,5,7,17-tetraoxaoctadeca-9,11-dien-13-yl 2-methylthiophene-3-carboxylate (15a)



A stirred solution of alcohol **10a** (90.0 mg, 0.272 mmol, 1.15 eq.) and 2-methylthiophene-3-carboxylic acid (**11b**, 33.7 mg, 0.237 mmol, 1.00 eq.) in dry toluene (3 mL) was treated with Et_3N (98.6 µL, 0.711 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (58.6 µL, 0.374 mmol, 1.58 eq.) and DMAP (43.4 mg, 0.356 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 1 h before being quenched with sat. aqueous $NaHCO_3$ (4 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 4 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (20% EtOAc in *n*-pentane) afforded ester **15a** as a colorless oil (75.0 mg, 0.165 mmol, 70%).

R_f = 0.61 (50% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +172.4 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2931, 1751, 1722, 1709, 1266, 1091, 1023, 993, 844, 711 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.43 (d, *J* = 5.3 Hz, 1 H), 7.04 (d, *J* = 5.3 Hz, 1 H), 6.42 (dd, *J* = 15.4, 10.5 Hz, 1 H), 6.22 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.87 (d, *J* = 15.6 Hz, 1 H), 5.69 (dd, *J* = 15.3, 7.2 Hz, 1 H), 4.74 (d, *J* = 7.1 Hz, 1 H), 4.68 (d, *J* = 7.0 Hz, 1 H), 4.28 (dq, *J* = 7.2, 6.4 Hz, 1 H), 3.79–3.74 (m, 1 H), 3.69 (s, 3 H), 3.66–3.61 (m, 1 H), 3.58 (d, *J* = 15.7 Hz, 1 H), 3.57–3.55 (m, 2 H), 3.52 (d, *J* = 15.7 Hz, 1 H), 3.39 (s, 3 H), 2.75 (s, 3 H), 1.72 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.3, 167.5, 162.5, 151,7, 137.3, 131.6, 130.3, 130.1, 129.2, 127.0, 121.6, 93.0, 85.6, 72.0, 71.9, 67.0, 59.2, 52.5, 43.1, 21.8, 21.3, 15.8. HR-MS: 477.1542 ($[M+Na]^+$, $C_{22}H_{30}O_8SNa^+$; calc. 477.1554).

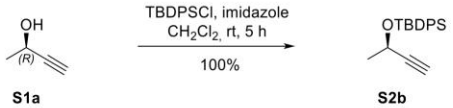
Methyl (*R*)-5-((*R*,1*E*,3*E*)-5-((2-methoxyethoxy)methoxy)hexa-1,3-dien-1-yl)-5-methyl-2-(2-methylthiophen-3-yl)-4-oxo-4,5-dihydrofuran-3-carboxylate (16a)



Ester **15a** (54.0 mg, 0.119 mmol, 1.00 eq.) was dissolved in dry benzene (1.2 mL) and piperidine (58.8 µL, 0.594 mmol, 5.00 eq.) was added to the solution at room temperature. The reaction mixture was heated at reflux for 17 h before it was allowed to cool to room temperature. The volatiles were removed in vacuo and the remainder was purified by column chromatography (20% EtOAc in *n*-pentane). Product **16a** was obtained as a colorless oil (29.0 mg, 66.4 µmol, 56%).

R_f = 0.57 (50% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = –20.0 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2928, 1741, 1709, 1579, 1439, 1337, 1102, 1087, 1045, 1022, 992, 800 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.37 (d, *J* = 5.5 Hz, 1 H), 7.13 (d, *J* = 5.5 Hz, 1 H), 6.34 (dd, *J* = 15.4, 10.5 Hz, 1 H), 6.15 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.74 (d, *J* = 15.4 Hz, 1 H), 5.66 (dd, *J* = 15.3, 7.2 Hz, 1 H), 4.72 (d, *J* = 7.0 Hz, 1 H), 4.66 (d, *J* = 7.0 Hz, 1 H), 4.26 (dq, *J* = 7.2, 6.4 Hz, 1 H), 3.82 (s, 3 H), 3.78–3.73 (m, 1 H), 3.65–3.59 (m, 1 H), 3.56–3.53 (m, 2 H), 3.39 (s, 3 H), 2.65 (s, 3 H), 1.63 (s, 3 H), 1.25 (d, *J* = 6.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.5, 184.4, 163.2, 148.0, 137.5, 130.8, 129.8, 129.5, 128.8, 126.6, 122.3, 106.1, 93.0, 90.5, 72.0, 71.9, 67.0, 59.2, 52.1, 23.0, 21.4, 15.9. HR-MS: 459.1436 ($[M+Na]^+$, $C_{22}H_{28}O_7SNa^+$; calc. 459.1448).

(*R*)-(But-3-yn-2-yloxy)(*tert*-butyl)diphenylsilane (S2b)



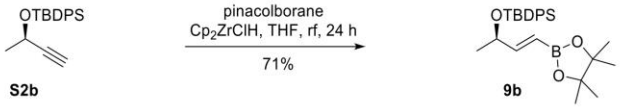
S1a **S2b**

To a solution of (*R*)-3-butyn-2-ol (**S1a**, 1.00 mL, 12.7 mmol, 1.00 eq.) in dry CH_2Cl_2 (120 mL) were added TBDPSCl (3.63 mL, 14.0 mmol, 1.10 eq.) and imidazole (1.30 g, 19.1 mmol, 1.50 eq.) at 0 °C. The reaction mixture was allowed to warm up to ambient temperature and stirred at this temperature for 5 h. The reaction was quenched with sat. aqueous NH_4Cl (100 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The remainder was purified by column chromatography (*n*-pentane to 2% EtOAc in *n*-pentane), to afford silyl ether **S2b** as a colorless oil (3.90 g, 12.6 mmol, 100%).

R_f = 0.81 (4% EtOAc in *n*-hexane). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.77–7.73 (m, 2 H), 7.71–7.67 (m, 2 H), 7.46–7.41 (m, 2 H), 7.41–7.35 (m, 4 H), 4.45 (qd, *J* = 6.5, 2.1 Hz, 1 H), 2.34 (d, *J* = 2.1 Hz, 1 H), 1.39 (d, *J* = 6.4 Hz, 3 H), 1.08 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 136.1 (2 C-atoms), 135.9 (2 C-atoms), 133.8, 133.5, 129.9, 129.8, 127.8 (2 C-atoms), 127.6 (2 C-atoms), 86.2, 71.6, 59.9, 27.0 (3 C-atoms), 25.3, 19.3.

Analytical data agree with those reported.[2]

(*R*,*E*)-*tert*-Butyldiphenyl((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-en-2-yl)oxy)silane (9b)



S2b **9b**

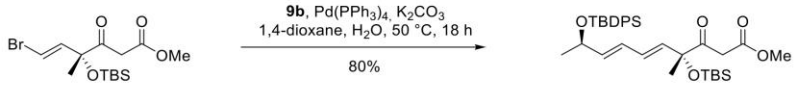
Zirconocene dichloride (189 mg, 0.648 mmol, 0.10 eq.) in dry THF (1 mL) was treated with DIBAL-H (1M in *n*-hexane, 680 µL, 0.680 mmol, 0.105 eq.) at 0 °C. The reaction mixture was stirred 30 min at 0 °C. Thereafter alkyne **S2b** (2.00 g, 6.48 mmol, 1.0 eq.) in dry THF (10 mL) and pinacolborane (1.08 mL, 7.46 mmol, 1.15 eq.) were added to the solution of Schwartz' Reagent at 0 °C. The reaction mixture was allowed to warm up to room temperature and was heated at reflux for 24 h. H_2O (15 mL) was added carefully to quench the reaction. The phases were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The combined organic phases were dried over $MgSO_4$ and volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (2% EtOAc in *n*-pentane) to yield boronic ester **9b** as a colorless oil (2.01 g, 4.61 mmol, 71%).

R_f = 0.23 (4% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +35.6 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2977, 1642, 1362, 1335, 1320, 1142, 1105, 1089, 998, 850, 822, 740, 700 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.70–7.67 (m, 2 H), 7.66–7.62 (m, 2 H), 7.44–7.31 (m, 6 H), 6.59 (dd, *J* = 18.0, 4.3 Hz, 1 H), 5.65 (dd, *J* = 17.9,

1.6 Hz, 1 H), 4.36 (qdd, *J* = 6.4, 4.7, 1.6 Hz, 1 H), 1.27 (s, 12 H), 1.07 (d, *J* = 6.3 Hz, 3 H), 1.06 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 156.8, 136.0 (4 C-atoms), 134.7, 134.0, 129.7, 129.6, 127.6 (4 C-atoms), 83.3 (2 C-atoms), 70.9, 27.2 (3 C-atoms), 24.9 (4 C-atoms), 23.7, 19.4 (The other alkenyl C-atom could not be observed. This special occurrence was already reported in the literature[1]). HR-MS: 437.2672 ($[M+H]^+$, $C_{26}H_{38}O_3BSi^+$; calc. 437.2678).

Analytical data agree with those reported.[3]

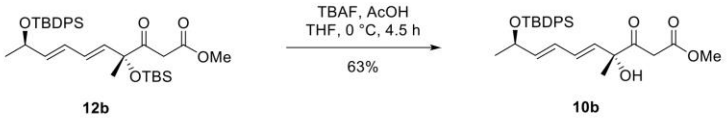
Methyl (4*R*,5*E*,7*E*,9*R*)-4-((*tert*-butyldimethylsilyl)oxy)-9-((*tert*-butyldiphenylsilyl)oxy)-4-methyl-3-oxodeca-5,7-dienoate (12b)



The Suzuki coupling was carried out under an argon atmosphere using standard Schlenk technique for oxygen exclusion. To a stirred solution of vinyl bromide **8** (500 mg, 1.37 mmol, 1.00 eq.) in dry 1,4-dioxane (10 mL) was added $Pd(PPh_3)_4$ (127 mg, 0.110 mmol, 8 mol%) and the reaction mixture was stirred for 5 min at ambient temperature. Boronic ester **9b** (896 mg, 2.05 mmol, 1.50 eq.) in 1,4-dioxane (20 mL) and K_2CO_3 (379 mg, 2.74 mmol, 2.00 eq.) in H_2O (3 mL) were added. The reaction mixture was heated to 50 °C and stirred for 18 h at this temperature. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and the phases were separated. The organic phase was washed with sat. aqueous NH_4Cl (20 mL), H_2O (20 mL) and brine (20 mL) and was dried over Na_2SO_4. The volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (2% EtOAc in *n*-pentane) afforded coupling product **12b** as a colorless oil (652 mg, 1.10 mmol, 80%).

R_f = 0.45 (10% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +80.8 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2931, 1754, 1724, 1258, 1140, 1111, 992, 837, 778, 740, 702 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.70–7.65 (m, 2 H), 7.65–7.62 (m, 2 H), 7.43–7.31 (m, 6 H), 6.24 (dd, *J* = 15.3, 10.6 Hz, 1 H), 5.94 (dd, *J* = 15.3, 10.6 Hz, 1 H), 5.70 (dd, *J* = 15.3, 5.8 Hz, 1 H), 5.46 (d, *J* = 15.3 Hz, 1 H), 4.32 (qd, *J* = 6.4, 5.8 Hz, 1 H), 3.75 (d, *J* = 16.5 Hz, 1 H), 3.71 (s, 3 H), 3.60 (d, *J* = 16.6 Hz, 1 H), 1.50 (s, 3 H), 1.16 (d, *J* = 6.3 Hz, 3 H), 1.06 (s, 9 H), 0.94 (s, 9 H), 0.102 (s, 3 H), 0.097 (s, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 204.9, 168.5, 139.5, 136.02 (2 C-atoms), 136.00 (2 C-atoms), 134.5, 134.1, 133.8, 131.2, 129.72, 129.69, 127.64 (2 C-atoms), 127.60 (2 C-atoms), 127.5, 82.5, 69.8, 52.3, 43.7, 27.1 (3 C-atoms), 26.0 (3 C-atoms), 24.4, 24.2, 19.4, 18.4, –2.1, –2.2. HR-MS: 617.3078 ($[M+Na]^+$, $C_{34}H_{50}O_5Si_2Na^+$; calc. 617.3089).

Methyl (4*R*,5*E*,7*E*,9*R*)-9-((*tert*-butyldiphenylsilyl)oxy)-4-hydroxy-4-methyl-3-oxodeca-5,7-dienoate (10b)

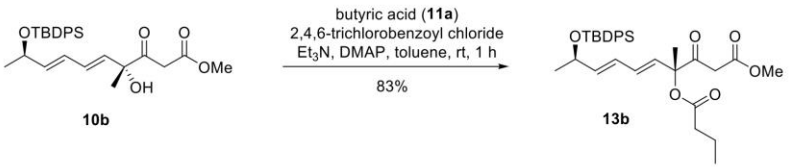


12b **10b**

Silyl ether **12b** (596 mg, 1.00 mmol, 1.00 eq.) was dissolved in dry THF (10 mL) and AcOH (114 µL, 2.00 mmol, 2.0 eq.) and TBAF (1M in THF, 2.00 mL, 2.00 mmol, 2.00 eq.) were added to the solution at 0 °C. After 4.5 h at 0 °C sat. aqueous NH_4Cl (5 mL) and sat. aqueous $NaHCO_3$ (5 mL) were added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (20% EtOAc in *n*-pentane) to yield γ-hydroxy-β-ketoester **10b** as a colorless oil (302 mg, 0.628 mmol, 63%).

R_f = 0.27 (10% EtOAc in *n*-hexane). $[\alpha]_D^{26}$ = +62.5 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 3483 (br.), 2930, 1749, 1717, 1428, 1319, 1140, 1111, 1084, 993, 740, 702 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.70–7.65 (m, 2 H), 7.65–7.62 (m, 2 H), 7.43–7.32 (m, 6 H), 6.33 (dd, *J* = 15.3, 10.6 Hz, 1 H), 6.01 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.76 (dd, *J* = 15.3, 5.6 Hz, 1 H), 5.55 (d, *J* = 15.3 Hz, 1 H), 4.34 (qd, *J* = 6.4, 5.6 Hz, 1 H), 3.74 (s, 3 H), 3.68 (d, *J* = 15.7 Hz, 1 H), 3.57 (d, *J* = 15.7 Hz, 1 H), 3.51 (s, 1 H), 1.49 (s, 3 H), 1.13 (d, *J* = 6.4 Hz, 3 H), 1.06 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 204.4, 167.9, 140.1, 136.01 (2 C-atoms), 135.99 (2 C-atoms), 134.5, 134.1, 132.0, 131.6, 129.74, 129.70, 127.7 (2 C-atoms), 127.6 (2 C-atoms), 127.1, 79.8, 69.7, 52.7, 43.4, 27.1 (3 C-atoms), 24.9, 24.2, 19.4. HR-MS: 503.2216 ($[M+Na]^+$, $C_{28}H_{36}O_5SiNa^+$; calc. 503.2224).

Methyl (4*R*,5*E*,7*E*,9*R*)-9-((*tert*-butyldiphenylsilyl)oxy)-4-(butyryloxy)-4-methyl-3-oxodeca-5,7-dienoate (13b)



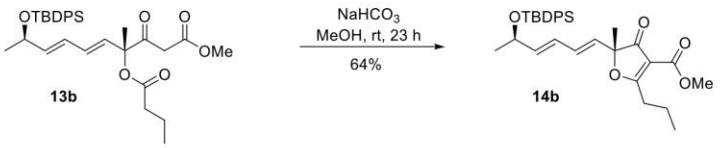
10b **13b**

A stirred solution of butyric acid (**11a**, 22.7 µL, 0.246 mmol, 1.00 eq.) and alcohol **10b** (136 mg, 0.283 mmol, 1.15 eq.) in dry toluene (3 mL) was treated with Et_3N (102 µL, 0.738 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (60.8 µL, 0.389 mmol, 1.58 eq.) and DMAP (45.0 mg, 0.369 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 1 h before being quenched with sat. aqueous $NaHCO_3$ (5 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced

pressure. Purification of the residue by column chromatography (10% EtOAc in *n*-pentane) afforded ester **13b** as a colorless oil (116 mg, 0.205 mmol, 83%).

R_f = 0.58 (20% EtOAc in *n*-hexane). $[\alpha]_D^{25}$ = +182 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2964, 1750, 1732, 1729, 1428, 1262, 1140, 1105, 1082, 992, 823, 740, 702 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.68–7.66 (m, 2 H), 7.65–7.62 (m, 2 H), 7.43–7.33 (m, 6 H), 6.29 (dd, *J* = 15.4, 10.5 Hz, 1 H), 5.99 (dd, *J* = 15.4, 10.5 Hz, 1 H), 5.76 (dd, *J* = 15.3, 5.6 Hz, 1 H), 5.62 (d, *J* = 15.6 Hz, 1 H), 4.35 (qd, *J* = 6.4, 5.6 Hz, 1 H), 3.69 (s, 3 H), 3.51 (d, *J* = 15.7 Hz, 1 H), 3.44 (d, *J* = 15.7 Hz, 1 H), 2.37 (t, *J* = 7.4 Hz, 2 H), 1.69 (qt, *J* = 7.4, 7.3 Hz, 2 H), 1.62 (s, 3 H), 1.14 (d, *J* = 6.3 Hz, 3 H), 1.06 (s, 9 H), 0.98 (t, *J* = 7.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.4, 172.9, 167.4, 140.6, 136.01 (2 C-atoms), 135.99 (2 C-atoms), 134.4, 134.1, 132.0, 129.8, 129.7, 128.9, 127.7 (2 C-atoms), 127.6 (2 C-atoms), 127.2, 85.5, 69.7, 52.5, 43.3, 36.3, 27.1 (3 C-atoms), 24.2, 21.4, 19.4, 18.4, 13.8. HR-MS: 573.2628 ($[M+Na]^+$, $C_{32}H_{42}O_6SiNa^+$; calc. 573.2643).

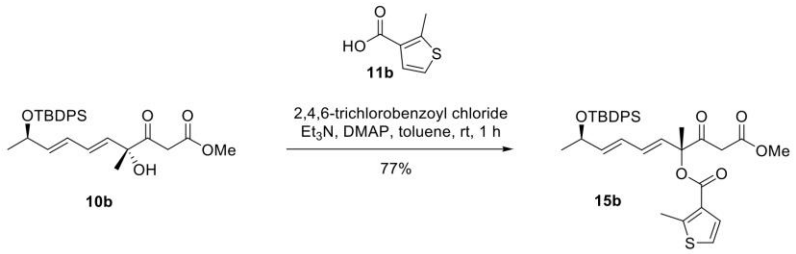
Methyl (*R*)-5-((*R*,1*E*,3*E*)-5-((*tert*-butyldiphenylsilyl)oxy)hexa-1,3-dien-1-yl)-5-methyl-4-oxo-2-propyl-4,5-dihydrofuran-3-carboxylate (14b)



Compound **13b** (68.0 mg, 0.120 mmol, 1.00 eq.) was dissolved in dry MeOH (1.5 mL) and $NaHCO_3$ (25.2 mg, 0.300 mmol, 2.50 eq.) was added to the solution at room temperature. The reaction mixture was stirred for 23 h at this temperature before being quenched with H_2O (2 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (10% EtOAc in *n*-pentane) afforded product **14b** as a colorless oil (41.0 mg, 77.0 µmol, 64%).

R_f = 0.43 (20% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +129 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2964, 1749, 1710, 1587, 1439, 1389, 1112, 1047, 992, 703 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.69–7.64 (m, 2 H), 7.63–7.60 (m, 2 H), 7.44–7.32 (m, 6 H), 6.21 (dd, *J* = 15.4, 10.7 Hz, 1 H), 5.99 (dd, *J* = 15.5, 10.6 Hz, 1 H), 5.74 (dd, *J* = 15.1, 5.5 Hz, 1 H), 5.54 (d, *J* = 15.4 Hz, 1 H), 4.33 (qd, *J* = 6.3, 5.6 Hz, 1 H), 3.83 (s, 3 H), 3.03 (dd, *J* = 7.8, 7.2 Hz, 2 H), 1.79 (dqd, *J* = 7.8, 7.6, 7.2 Hz, 2 H), 1.53 (s, 3 H), 1.10 (d, *J* = 6.3 Hz, 3 H), 1.05 (s, 9 H), 1.04 (t, *J* = 7.6 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 199.0, 198.4, 163.4, 140.6, 135.99 (2 C-atoms), 135.97 (2 C-atoms), 134.5, 134.0, 131.0, 129.7 (2 C-atoms), 127.6 (4 C-atoms), 127.5, 126.8, 106.3, 91.2, 69.6, 51.7, 32.9, 27.1 (3 C-atoms), 24.2, 22.7, 20.1, 19.4, 14.0. HR-MS: 533.2706 ($[M+H]^+$, $C_{32}H_{41}O_5Si^+$; calc. 533.2718).

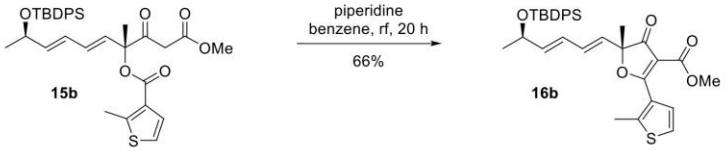
(4*R*,5*E*,7*E*,9*R*)-9-((*tert*-Butyldiphenylsilyl)oxy)-1-methoxy-4-methyl-1,3-dioxodeca-5,7-dien-4-yl 2-methylthiophene-3-carboxylate (15b)



A stirred solution of 2-methylthiophene-3-carboxylic acid (**11b**, 35.0 mg, 0.246 mmol, 1.00 eq.) and alcohol **10b** (136 mg, 0.283 mmol, 1.15 eq.) in dry toluene (3 mL) was treated with Et_3N (102 µL, 0.738 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (60.8 µL, 0.389 mmol, 1.58 eq.) and DMAP (45.0 mg, 0.369 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 1 h before being quenched with sat. aqueous $NaHCO_3$ (5 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (10% EtOAc in *n*-pentane) afforded ester **15b** as a colorless oil (114 mg, 0.188 mmol, 77%).

R_f = 0.52 (20% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = +127 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2932, 1752, 1723, 1713, 1428, 1267, 1141, 1091, 991, 823, 741, 702 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.70–7.66 (m, 2 H), 7.65–7.62 (m, 2 H), 7.44 (d, *J* = 5.3 Hz, 1 H), 7.43–7.32 (m, 6 H), 7.04 (d, *J* = 5.3 Hz, 1 H), 6.35 (dd, *J* = 15.6, 10.5 Hz, 1 H), 6.02 (dd, *J* = 15.3, 10.7 Hz, 1 H), 5.77 (dd, *J* = 15.2, 5.7 Hz, 1 H), 5.72 (d, *J* = 15.6 Hz, 1 H), 4.36 (qd, *J* = 6.3, 5.7 Hz, 1 H), 3.68 (s, 3 H), 3.58 (d, *J* = 15.7 Hz, 1 H), 3.51 (d, *J* = 15.7 Hz, 1 H), 2.75 (s, 3 H), 1.72 (s, 3 H), 1.14 (d, *J* = 6.3 Hz, 3 H), 1.07 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198,4, 167.5, 162.5, 151.6, 140.5, 136.02 (2 C-atoms), 136.00 (2 C-atoms), 134.4, 134.1, 132.1, 129.8, 129.7, 129.2, 129.1, 127.7 (2 C-atoms), 127.6 (2 C-atoms), 127.3, 127.1, 121.6, 85.7, 69.7, 52.5, 43.1, 27.1 (3 C-atoms), 24.2, 21.6, 19.4, 15.8. HR-MS: 627.2195 ([M+Na]$^+$, $C_{34}H_{40}O_6SiSNa^+$; calc. 627.2207).

Methyl (*R*)-5-((*R*,1*E*,3*E*)-5-((*tert*-butyldiphenylsilyl)oxy)hexa-1,3-dien-1-yl)-5-methyl-2-(2-methylthiophen-3-yl)-4-oxo-4,5-dihydrofuran-3-carboxylate (16b)



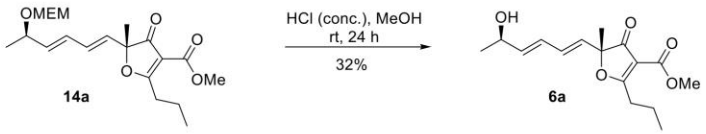
Compound **15b** (69.0 mg, 0.114 mmol, 1.00 eq.) was dissolved in dry benzene (1.2 mL) and piperidine (56.5 µL, 0.570 mmol, 5.00 eq.) was added to the solution at room temperature. The reaction mixture

was heated at reflux for 20 h before it was allowed to cool to room temperature. The volatiles were removed in vacuo and the remainder was purified by column chromatography (10% EtOAc in *n*-pentane). Product **16b** was obtained as a colorless oil (44.3 mg, 75.5 µmol, 66%).

R_f = 0.36 (20% EtOAc in *n*-hexane). $[\alpha]_D^{23}$ = –49.8 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2932, 1743, 1713, 1583, 1439, 1337, 1106, 1056, 992, 822, 740, 703, 690 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.68–7.65 (m, 2 H), 7.64–7.61 (m, 2 H), 7.44–7.32 (m, 7 H), 7.13 (d, *J* = 5.5 Hz, 1 H), 6.29 (dd, *J* = 15.4, 10.5 Hz, 1 H), 6.03 (ddd, *J* = 15.3, 10.5, 0.6 Hz, 1 H), 5.76 (dd, *J* = 15.3, 5.5 Hz, 1 H), 5.63 (d, *J* = 15.4 Hz, 1 H), 4.34 (qd, *J* = 6.3, 5.5 Hz, 1 H), 3.83 (s, 3 H), 2.65 (s, 3 H), 1.63 (s, 3 H), 1.10 (d, *J* = 6.4 Hz, 3 H), 1.06 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198,6, 184.4, 163.2, 147.9, 140.7, 136.0 (4 C-atoms), 134.5, 134.0, 131.3, 129.7, 129.4, 129.1, 127.7, 127.6 (4 C-atoms), 126.8, 126.6, 122.3, 106.1, 90.7, 69.6, 52.0, 27.1 (3 C-atoms), 24.2, 23.0, 19.4, 15.9. HR-MS: 587.2270 ($[M+H]^+$, $C_{34}H_{39}O_5SiS^+$; calc. 587.2282).

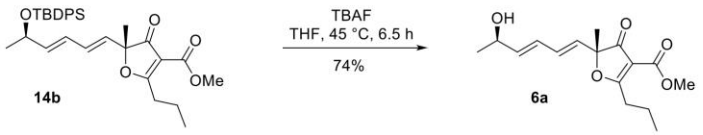
Gregatin G_1 (6a)

Method A:



MEM-ether **14a** (12.0 mg, 31.4 µmol, 1.00 eq.) was dissolved in MeOH (300 µL) and conc. HCl (6 µL) was added to the solution at room temperature. The reaction mixture was stirred for 24 h at this temperature before being quenched with sat. aqueous $NaHCO_3$ (500 µL). After phase separation the aqueous phase was extracted with Et_2O (3 × 1 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (30% EtOAc in *n*-pentane) to yield gregatin G_1 (**6a**) as a colorless oil (3.00 mg, 10.2 µmol, 32%).

Method B:

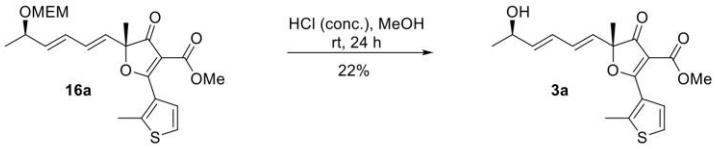


Silyl ether **14b** (22.0 mg, 41.3 µmol, 1.00 eq.) was dissolved in dry THF (1 mL) and TBAF (1M in THF, 165 µL, 0.165 mmol, 4.00 eq.) was added to the solution at 0 °C. After 6.5 h at 0 °C sat. aqueous NH_4Cl (2 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (30% to 40% EtOAc in *n*-pentane) to yield gregatin G_1 (**6a**) as a colorless oil (9.0 mg, 30.6 µmol, 74%).

R_f = 0.39 (50% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +95.8 (*c* = 0.50, $CHCl_3$). IR (neat) ν_{max} = 3484 (br.), 2968, 1741, 1707, 1439, 1390, 1260, 1198, 1127, 1044, 1025, 991, 798, 753 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.28 (dd, *J* = 15.3, 10.6 Hz, 1 H), 6.15 (ddd, *J* = 15.3, 10.6, 0.8 Hz, 1 H), 5.81 (dd, *J* = 15.2, 6.2 Hz, 1 H), 5.66 (d, *J* = 15.4 Hz, 1 H), 4.35 (qd, *J* = 6.4, 6.2 Hz, 1 H), 3.82 (s, 3 H), 3.08–2.97 (m, 2 H), 1.78 (qt, *J* = 7.5, 7.4 Hz, 2 H), 1.53 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H), 1.03 (t, *J* = 7.5 Hz, 3 H) (OH was not observed). ^{13}C-NMR (125 MHz, $CDCl_3$): 199.1, 198.2, 163.4, 140.0, 130.6, 128.5, 127.8, 106.4, 91.0, 68.3, 51.8, 32.8, 23.3, 22.7, 20.1, 13.9. HR-MS: 317.1351 ($[M+Na]^+$, $C_{16}H_{22}O_5Na^+$; calc. 317.1359).

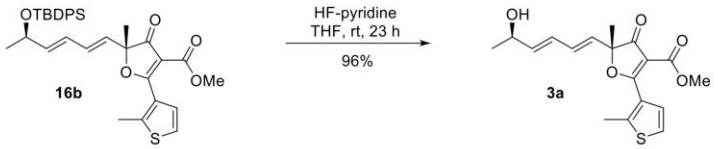
Thiocarboxylic C_1 (3a)

Method A:



MEM-ether **16a** (14.0 mg, 32.4 µmol, 1.00 eq.) was dissolved in MeOH (300 µL) and conc. HCl (6 µL) was added to the solution at room temperature. The reaction mixture was stirred for 24 h at this temperature before being quenched with sat. aqueous $NaHCO_3$ (500 µL). After phase separation the aqueous phase was extracted with Et_2O (3 × 1 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (30% to 40% EtOAc in *n*-pentane) to yield thiocarboxylic C_1 (**3a**) as a colorless oil (2.50 mg, 7.18 µmol, 22%).

Method B:

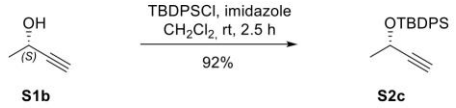


Silyl ether **16b** (7.00 mg, 11.9 µmol, 1.00 eq.) was dissolved in dry THF (120 µL) and 70% HF-pyridine (24.8 µL, 0.954 mmol, 80.0 eq.) was added to the solution at 0 °C. After 23 h at room temperature sat. aqueous K_2CO_3 (0.5 mL) and H_2O (0.5 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 1.5 mL), the combined organic phases were washed with sat aqueous $CuSO_4$ (4 mL) and were dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (30% to 50% EtOAc in *n*-pentane) to yield thiocarboxylic C_1 (**3a**) as a colorless oil (4.00 mg, 11.5 µmol, 96%).

R_f = 0.18 (40% EtOAc in *n*-hexane). $[\alpha]_D^{25}$ = –99.4 (*c* = 0.25, $CHCl_3$). IR (neat) ν_{max} = 3463 (br.), 2927, 1733, 1708, 1575, 1440, 1338, 1104, 1052, 991, 909, 799, 729 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.36

(d, *J* = 5.3 Hz, 1 H), 7.13 (d, *J* = 5.5 Hz, 1 H), 6.36 (dd, *J* = 15.4, 10.7 Hz, 1 H), 6.18 (ddd, *J* = 15.2, 10.6, 0.5 Hz, 1 H), 5.82 (dd, *J* = 15.3, 6.3 Hz, 1 H), 5.75 (d, *J* = 15.4 Hz, 1 H), 4.36 (qd, *J* = 6.4, 6.3 Hz, 1 H), 3.82 (s, 3 H), 2.64 (s, 3 H), 1.63 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H) (OH was not observed). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.5, 184.4, 163.2, 148.0, 140.1, 131.0, 129.4, 128.7, 127.8, 126.6, 122.3 106.1, 90.5, 68.4, 52.1, 23.4, 23.0, 15.9. HR-MS: 371.0918 ($[M+Na]^+$, $C_{18}H_{20}O_5SNa^+$; calc. 371.0924).

(*S*)-(But-3-yn-2-yloxy)(*tert*-butyl)diphenylsilane (S2c)



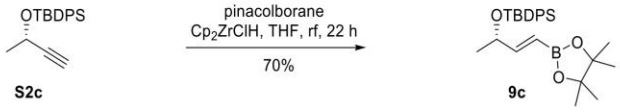
S1b **S2c**

To a solution of (*S*)-3-butyn-2-ol (**S1b**, 1.12 mL, 14.3 mmol, 1.00 eq.) in dry CH_2Cl_2 (140 mL) were added TBDPSCl (4.08 mL, 15.7 mmol, 1.10 eq.) and imidazole (1.46 g, 21.5 mmol, 1.50 eq.) at 0 °C. The reaction mixture was allowed to warm up to ambient temperature and stirred at this temperature for 2.5 h. The reaction was quenched with sat. aqueous NH_4Cl (140 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 120 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The remainder was purified by column chromatography (*n*-pentane to 2% EtOAc in *n*-pentane), to afford silyl ether **S2c** as a colorless oil (4.06 g, 13.2 mmol, 92%).

R_f = 0.82 (4% EtOAc in *n*-hexane). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.78–7.73 (m, 2 H), 7.71–7.67 (m, 2 H), 7.46–7.41 (m, 2 H), 7.41–7.36 (m, 4 H), 4.46 (qd, *J* = 6.5, 2.1 Hz, 1 H), 2.34 (d, *J* = 2.0 Hz, 1 H), 1.40 (d, *J* = 6.4 Hz, 3 H), 1.08 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 136.1 (2 C-atoms), 135.9 (2 C-atoms), 133.8, 133.5, 129.9, 129.8, 127.8 (2 C-atoms), 127.6 (2 C-atoms), 86.2, 71.6, 59.9, 27.0 (3 C-atoms), 25.3, 19.3.

Analytical data agree with those reported.[4]

(S,*E*)-*tert*-Butyldiphenyl((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-en-2-yl)oxy)silane (9c)



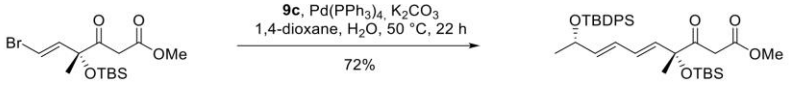
S2c **9c**

Zirconocene dichloride (568 mg, 1.94 mmol, 0.20 eq.) in dry THF (3 mL) was treated with DIBAL-H (1M in *n*-hexane, 2.04 mL, 2.04 mmol, 0.21 eq.) at 0 °C. The reaction mixture was stirred 1 h at 0 °C. Thereafter alkyne **S2c** (3.00 g, 9.72 mmol, 1.0 eq.) in dry THF (15 mL) and pinacolborane (1.83 mL, 12.6 mmol, 1.3 eq.) were added to the solution of Schwartz' Reagent at 0 °C. The reaction mixture was allowed to warm up to room temperature and was heated at reflux for 22 h. H_2O (20 mL) was added carefully to quench the reaction. The phases were separated and the aqueous phase was extracted with

Et_2O (3 × 15 mL). The combined organic phases were dried over $MgSO_4$ and volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (2% EtOAc in *n*-pentane) to yield boronic ester **9c** as a colorless oil (2.96 g, 6.77 mmol, 70%).

R_f = 0.24 (4% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = –36.3 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2977, 1642, 1362, 1335, 1320, 1142, 1105, 1089, 1054, 998, 850, 822, 740, 700 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.72–7.68 (m, 2 H), 7.67–7.63 (m, 2 H), 7.45–7.32 (m, 6 H), 6.61 (dd, *J* = 17.9, 4.3 Hz, 1 H), 5.67 (dd, *J* = 18.0, 1.5 Hz, 1 H), 4.38 (qdd, *J* = 6.4, 4.7, 1.5 Hz, 1 H), 1.29 (s, 12 H), 1.09 (d, *J* = 6.4 Hz, 3 H), 1.08 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 156.8, 136.0 (4 C-atoms), 134.7, 134.0, 129.7, 129.6, 127.6 (4 C-atoms), 83.2 (2 C-atoms), 70.9, 27.2 (3 C-atoms), 24.9 (4 C-atoms), 23.7, 19.4 (The other alkenyl C-atom could not be observed. This special occurrence was already reported in the literature[1]). HR-MS: 437.2667 ($[M+H]^+$, $C_{26}H_{38}O_3BSi^+$; calc. 437.2678).

Methyl (4*R*,5*E*,7*E*,9*S*)-4-((*tert*-butyldimethylsilyl)oxy)-9-((*tert*-butyldiphenylsilyl)oxy)-4-methyl-3-oxodeca-5,7-dienoate (12c)



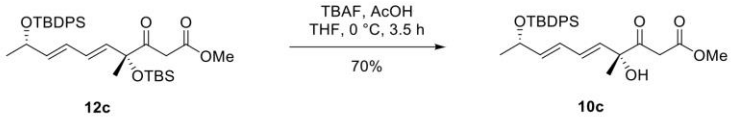
8 **12c**

The Suzuki coupling was carried out under an argon atmosphere using standard Schlenk technique for oxygen exclusion. To a stirred solution of vinyl bromide **8** (1.00 g, 2.74 mmol, 1.00 eq.) in dry 1,4-dioxane (20 mL) was added $Pd(PPh_3)_4$ (253 mg, 0.219 mmol, 8 mol%) and the reaction mixture was stirred for 5 min at ambient temperature. Boronic ester **9c** (1.80 g, 4.11 mmol, 1.50 eq.) in 1,4-dioxane (40 mL) and K_2CO_3 (757 mg, 5.48 mmol, 2.00 eq.) in H_2O (6 mL) were added. The reaction mixture was heated to 50 °C and stirred for 22 h at this temperature. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (40 mL) and the phases were separated. The organic phase was washed with sat. aqueous NH_4Cl (40 mL), H_2O (40 mL) and brine (40 mL) and was dried over Na_2SO_4. The volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (2% EtOAc in *n*-pentane) afforded coupling product **12c** as a colorless oil (1.17 g, 1.96 mmol, 72%).

R_f = 0.26 (4% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +38.0 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2931, 1753, 1723, 1258, 1139, 1106, 989, 836, 822, 776, 739, 700 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.69–7.66 (m, 2 H), 7.66–7.62 (m, 2 H), 7.43–7.32 (m, 6 H), 6.23 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.92 (dd, *J* = 15.3, 10.6 Hz, 1 H), 5.70 (dd, *J* = 15.2, 6.0 Hz, 1 H), 5.46 (d, *J* = 15.3 Hz, 1 H), 4.33 (qd, *J* = 6.3, 6.0 Hz, 1 H), 3.74 (d, *J* = 16.6 Hz, 1 H), 3.71 (s, 3 H), 3.60 (d, *J* = 16.6 Hz, 1 H), 1.49 (s, 3 H), 1.16 (d, *J* = 6.3 Hz, 3 H), 1.06 (s, 9 H), 0.94 (s, 9 H), 0.110 (s, 3 H), 0.108 (s, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 205.0, 168.5, 139.6, 136.03 (2 C-atoms), 136.00 (2 C-atoms), 134.5, 134.2, 133.7, 131.2, 129.72, 129.68, 127.64 (2

C-atoms), 127.58 (2 C-atoms), 127.5, 82.6, 69.9, 52.3, 43.8, 27.1 (3 C-atoms), 26.0 (3 C-atoms), 24.3, 24.2, 19.4, 18.4, –2.0, –2.2. HR-MS: 617.3082 ([M+Na]$^+$, $C_{34}H_{50}O_5Si_2Na^+$; calc. 617.3089).

Methyl (4*R*,5*E*,7*E*,9*S*)-9-((*tert*-butyldiphenylsilyl)oxy)-4-hydroxy-4-methyl-3-oxodeca-5,7-dienoate (10c)

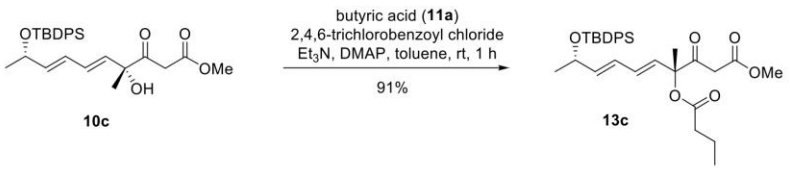


12c **10c**

Silyl ether **12c** (894 mg, 1.50 mmol, 1.00 eq.) was dissolved in dry THF (15 mL) and AcOH (172 µL, 3.00 mmol, 2.0 eq.) and TBAF (1M in THF, 3.00 mL, 3.00 mmol, 2.00 eq.) were added to the solution at 0 °C. After 3.5 h at 0 °C sat. aqueous NH_4Cl (8 mL) and sat. aqueous $NaHCO_3$ (8 mL) were added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (20% EtOAc in *n*-pentane) to yield γ-hydroxy-β-ketoester **10c** as a colorless oil (506 mg, 1.05 mmol, 70%).

R_f = 0.26 (10% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = –63.2 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 3483 (br.), 2932, 1747, 1716, 1428, 1318, 1139, 1105, 1085, 991, 739, 700 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.69–7.66 (m, 2 H), 7.65–7.62 (m, 2 H), 7.43–7.33 (m, 6 H), 6.33 (dd, *J* = 15.3, 10.6 Hz, 1 H), 6.01 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.75 (dd, *J* = 15.3, 5.6 Hz, 1 H), 5.55 (d, *J* = 15.3 Hz, 1 H), 4.35 (qd, *J* = 6.4, 5.6 Hz, 1 H), 3.74 (s, 3 H), 3.69 (d, *J* = 15.6 Hz, 1 H), 3.58 (d, *J* = 15.7 Hz, 1 H), 3.50 (s, 1 H), 1.48 (s, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H), 1.06 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 204.4, 167.9, 140.1, 136.01 (2 C-atoms), 135.99 (2 C-atoms), 134.5, 134.1, 132.0, 131.6, 129.73, 129.70, 127.7 (2 C-atoms), 127.6 (2 C-atoms), 127.1, 79.8, 69.7, 52.7, 43.5, 27.1 (3 C-atoms), 25.0, 24.2, 19.4. HR-MS: 503.2212 ([M+Na]$^+$, $C_{28}H_{36}O_5SiNa^+$; calc. 503.2224).

Methyl (4*R*,5*E*,7*E*,9*S*)-9-((*tert*-butyldiphenylsilyl)oxy)-4-(butyryloxy)-4-methyl-3-oxodeca-5,7-dienoate (13c)



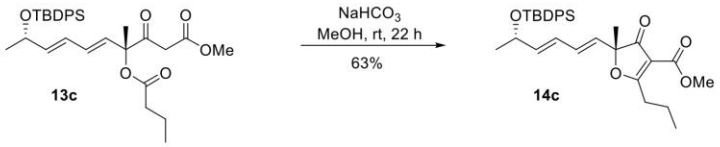
10c **13c**

A stirred solution of butyric acid (**11a**, 26.0 µL, 0.280 mmol, 1.00 eq.) and alcohol **10c** (155 mg, 0.322 mmol, 1.15 eq.) in dry toluene (6 mL) was treated with Et_3N (116 µL, 0.840 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (69.2 µL, 0.442 mmol, 1.58 eq.) and DMAP (51.3 mg, 0.420 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 1 h before being quenched with sat.

aqueous $NaHCO_3$ (6 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 6 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (10% EtOAc in *n*-pentane) afforded ester **13c** as a colorless oil (144 mg, 0.255 mmol, 91%).

R_f = 0.52 (20% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +43.1 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2966, 1752, 1735, 1731, 1428, 1262, 1139, 1105, 1085, 990, 822, 740, 701 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.69–7.66 (m, 2 H), 7.65–7.62 (m, 2 H), 7.44–7.33 (m, 6 H), 6.29 (dd, *J* = 15.5, 10.6 Hz, 1 H), 5.99 (dd, *J* = 15.6, 10.6 Hz, 1 H), 5.77 (dd, *J* = 15.3, 5.6 Hz, 1 H), 5.62 (d, *J* = 15.6 Hz, 1 H), 4.35 (qd, *J* = 6.4, 5.6 Hz, 1 H), 3.70 (s, 3 H), 3.51 (d, *J* = 15.6 Hz, 1 H), 3.44 (d, *J* = 15.7 Hz, 1 H), 2.37 (t, *J* = 7.4 Hz, 2 H), 1.69 (qt, *J* = 7.4, 7.4 Hz, 2 H), 1.61 (s, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H), 1.06 (s, 9 H), 0.99 (t, *J* = 7.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.4, 172.9, 167.4, 140.6, 136.00 (2 C-atoms), 135.99 (2 C-atoms), 134.4, 134.1, 132.2, 129.8, 129.7, 128.9, 127.7 (2 C-atoms), 127.6 (2 C-atoms), 127.2, 85.5, 69.7, 52.5, 43.3, 36.3, 27.1 (3 C-atoms), 24.2, 21.4, 19.4, 18.4, 13.8. HR-MS: 573.2636 ($[M+Na]^+$, $C_{32}H_{42}O_6SiNa^+$; calc. 573.2643).

Methyl (*R*)-5-((*S*,1*E*,3*E*)-5-((*tert*-butyldiphenylsilyl)oxy)hexa-1,3-dien-1-yl)-5-methyl-4-oxo-2-propyl-4,5-dihydrofuran-3-carboxylate (14c)

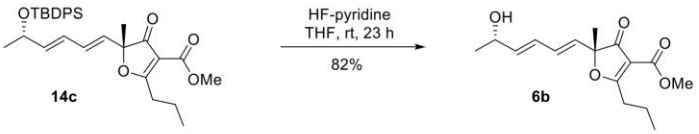


Compound **13c** (136 mg, 0.240 mmol, 1.00 eq.) was dissolved in dry MeOH (2.5 mL) and $NaHCO_3$ (50.0 mg, 0.601 mmol, 2.50 eq.) was added to the solution at room temperature. The reaction mixture was stirred for 22 h at this temperature before being quenched with H_2O (3 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (10% EtOAc in *n*-pentane) afforded product **14c** as a colorless and highly viscous oil (81.0 mg, 0.152 mmol, 63%).

R_f = 0.43 (20% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +79.3 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2964, 1747, 1709, 1584, 1439, 1389, 1105, 1044, 989, 800, 740, 701 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.68–7.64 (m, 2 H), 7.63–7.60 (m, 2 H), 7.44–7.32 (m, 6 H), 6.22 (dd, *J* = 15.4, 10.5 Hz, 1 H), 5.97 (dd, *J* = 15.3, 10.5 Hz, 1 H), 5.74 (dd, *J* = 15.3, 5.6 Hz, 1 H), 5.53 (d, *J* = 15.4 Hz, 1 H), 4.33 (qd, *J* = 6.3, 5.6 Hz, 1 H), 3.84 (s, 3 H), 3.03 (dd, *J* = 7.8, 7.2 Hz, 2 H), 1.79 (dqd, *J* = 7.8, 7.4, 7.2 Hz, 2 H), 1.52 (s, 3 H), 1.11 (d, *J* = 6.3 Hz, 3 H), 1.05 (s, 9 H), 1.04 (t, *J* = 7.4 Hz, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 199.0, 198.3, 163.5, 140.6, 135.98 (2 C-atoms), 135.96 (2 C-atoms), 134.5, 134.0, 131.1, 129.7 (2 C-atoms), 127.6 (4

C-atoms), 127.5, 126.9, 106.4, 91.2, 69.6, 51.7, 32.9, 27.1 (3 C-atoms), 24.2, 22.6, 20.0, 19.4, 13.9. HR-MS: 555.2516 ([M+Na]$^+$, $C_{32}H_{40}O_5SiNa^+$; calc. 555.2537).

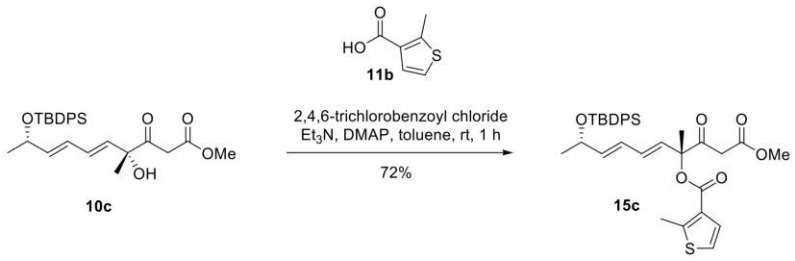
Gregatin G_2 (6b)



Silyl ether **14c** (22.0 mg, 41.3 µmol, 1.00 eq.) was dissolved in dry THF (400 µL) and 70% HF-pyridine (85.8 µL, 3.30 mmol, 80.0 eq.) was added to the solution at 0 °C. After 23 h at room temperature sat. aqueous K_2CO_3 (1 mL) was added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 2 mL), the combined organic phases were washed with sat. aqueous $CuSO_4$ (5 mL) and were dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (30% to 50% EtOAc in *n*-pentane) to yield gregatin G_2 (**6b**) as a colorless oil (10.0 mg, 34.0 µmol, 82%).

R_f = 0.18 (40% EtOAc in *n*-hexane). $[\alpha]_D^{25}$ = +104 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 3473 (br.), 2967, 1740, 1706, 1439, 1390, 1260, 1197, 1128, 1043, 1023, 991, 767, 732 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.28 (dd, *J* = 15.3, 10.6 Hz, 1 H), 6.14 (ddd, *J* = 15.3, 10.6, 0.6 Hz, 1 H), 5.81 (dd, *J* = 15.3, 6.1 Hz, 1 H), 5.65 (d, *J* = 15.4 Hz, 1 H), 4.35 (qd, *J* = 6.4, 6.1 Hz, 1 H), 3.82 (s, 3 H), 3.08–2.95 (m, 2 H), 1.78 (qt, *J* = 7.4, 7.4 Hz, 2 H), 1.52 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H), 1.03 (t, *J* = 7.4 Hz, 3 H) (OH was not observed). ^{13}C-NMR (125 MHz, $CDCl_3$): 199.1, 198.2, 163.4, 140.0, 130.7, 128.5, 127.8, 106.4, 91.0, 68.3, 51.7, 32.8, 23.3, 22.6, 20.1, 13.9. HR-MS: 317.1353 ([M+Na]$^+$, $C_{16}H_{22}O_5Na^+$; calc. 317.1359).

(4*R*,5*E*,7*E*,9*S*)-9-((*tert*-Butyldiphenylsilyl)oxy)-1-methoxy-4-methyl-1,3-dioxodeca-5,7-dien-4-yl 2-methylthiophene-3-carboxylate (15c)

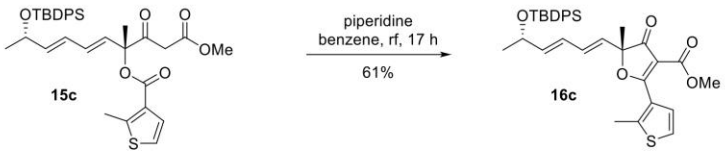


A stirred solution of 2-methylthiophene-3-carboxylic acid (**11b**, 82.6 mg, 0.581 mmol, 1.00 eq.) and alcohol **10c** (321 mg, 0.668 mmol, 1.15 eq.) in dry toluene (12 mL) was treated with Et_3N (242 µL, 1.74 mmol, 3.00 eq.), 2,4,6-trichlorobenzoyl chloride (144 µL, 0.918 mmol, 1.58 eq.) and DMAP (106 mg, 0.872 mmol, 1.50 eq.). The reaction mixture was stirred at room temperature for 1 h before

being quenched with sat. aqueous $NaHCO_3$ (10 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were evaporated under reduced pressure. Purification of the residue by column chromatography (10% EtOAc in *n*-pentane) afforded ester **15c** as a colorless oil (253 mg, 0.428 mmol, 72%).

R_f = 0.51 (20% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = +116 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2931, 1753, 1721, 1711, 1428, 1268, 1141, 1091, 991, 823, 740, 702 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.69–7.66 (m, 2 H), 7.65–7.62 (m, 2 H), 7.44 (d, *J* = 5.3 Hz, 1 H), 7.43–7.32 (m, 6 H), 7.04 (d, *J* = 5.3 Hz, 1 H), 6.36 (dd, *J* = 15.6, 10.5 Hz, 1 H), 6.02 (dd, *J* = 15.5, 10.6 Hz, 1 H), 5.77 (dd, *J* = 15.3, 5.6 Hz, 1 H), 5.73 (d, *J* = 15.6 Hz, 1 H), 4.36 (qd, *J* = 6.4, 5.6 Hz, 1 H), 3.69 (s, 3 H), 3.58 (d, *J* = 15.7 Hz, 1 H), 3.52 (d, *J* = 15.7 Hz, 1 H), 2.76 (s, 3 H), 1.71 (s, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H), 1.06 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.4, 167.5, 162.5, 151.6, 140.6, 136.01 (2 C-atoms), 135.99 (2 C-atoms), 134.4, 134.1, 132.1, 129.8, 129.7, 129.2, 129.1, 127.7 (2 C-atoms), 127.6 (2 C-atoms), 127.2, 127.1, 121.6, 85.7, 69.7, 52.5, 43.1, 27.1 (3 C-atoms), 24.2, 21.6, 19.4, 15.8. HR-MS: 627.2204 ($[M+Na]^+$, $C_{34}H_{40}O_6SiSNa^+$; calc. 627.2207).

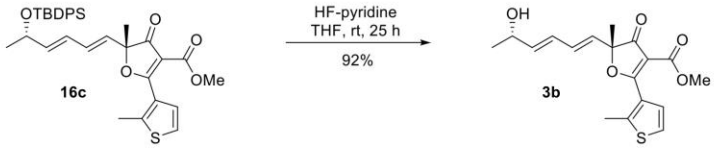
Methyl (*R*)-5-((*S*,1*E*,3*E*)-5-((*tert*-butyldiphenylsilyl)oxy)hexa-1,3-dien-1-yl)-5-methyl-2-(2-methylthiophen-3-yl)-4-oxo-4,5-dihydrofuran-3-carboxylate (16c)



Compound **15c** (141 mg, 0.233 mmol, 1.00 eq.) was dissolved in dry benzene (2.5 mL) and piperidine (115 µL, 1.17 mmol, 5.00 eq.) was added to the solution at room temperature. The reaction mixture was heated at reflux for 17 h before it was allowed to cool to room temperature. The volatiles were removed in vacuo and the remainder was purified by column chromatography (10% to 20 % EtOAc in *n*-pentane). Product **16c** was obtained as a colorless oil (84.0 mg, 0.143 mmol, 61%).

R_f = 0.32 (20% EtOAc in *n*-hexane). $[\alpha]_D^{27}$ = –108 (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 2930, 1742, 1710, 1579, 1439, 1335, 1104, 1053, 988, 822, 737, 700, 688 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.68–7.65 (m, 2 H), 7.64–7.60 (m, 2 H), 7.44–7.32 (m, 7 H), 7.14 (d, *J* = 5.3 Hz, 1 H), 6.29 (dd, *J* = 15.4, 10.5 Hz, 1 H), 6.01 (ddd, *J* = 15.2, 10.5, 0.5 Hz, 1 H), 5.75 (dd, *J* = 15.2, 5.6 Hz, 1 H), 5.62 (d, *J* = 15.4 Hz, 1 H), 4.34 (qd, *J* = 6.3, 5.6 Hz, 1 H), 3.83 (s, 3 H), 2.65 (s, 3 H), 1.63 (s, 3 H), 1.11 (d, *J* = 6.4 Hz, 3 H), 1.05 (s, 9 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 198.6, 184.4, 163.2, 147.9, 140.7, 135.99 (2 C-atoms), 135.97 (2 C-atoms), 134.5, 134.0, 131.4, 129.7, 129.5, 129.1, 127.7, 127.6 (4 C-atoms), 126.9, 126.6, 122.3, 106.2, 90.7, 69.6, 52.1, 27.1 (3 C-atoms), 24.2, 22.9, 19.4, 15.9. HR-MS: 609.2093 ($[M+Na]^+$, $C_{34}H_{38}O_5SiSNa^+$; calc. 609.2101).

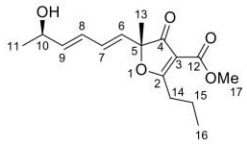
Thiocarboxylic C_2 (3b)



Silyl ether **16c** (33.0 mg, 56.2 µmol, 1.00 eq.) was dissolved in dry THF (500 µL) and 70% HF-pyridine (146 µL, 5.62 mmol, 100 eq.) was added to the solution at 0 °C. After 25 h at room temperature sat. aqueous K_2CO_3 (1 mL) and H_2O (1 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 2 mL), the combined organic phases were washed with sat aqueous $CuSO_4$ (6 mL) and were dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (30% to 40% EtOAc in *n*-pentane) to yield thiocarboxylic C_2 (**3b**) as a colorless oil (18.0 mg, 51.7 µmol, 92%).

R_f = 0.39 (50% EtOAc in *n*-hexane). $[\alpha]_D^{25} = -108$ (*c* = 1.00, $CHCl_3$). IR (neat) ν_{max} = 3482 (br.), 2926, 1735, 1709, 1575, 1440, 1338, 1104, 1052, 991, 910, 799, 731 cm^{-1}.f ^{1}H-NMR (500 MHz, $CDCl_3$): 7.36 (d, *J* = 5.5 Hz, 1 H), 7.12 (d, *J* = 5.5 Hz, 1 H), 6.35 (dd, *J* = 15.5, 10.6 Hz, 1 H), 6.17 (dd, *J* = 15.5, 10.5 Hz, 1 H), 5.82 (dd, *J* = 15.3, 6.3 Hz, 1 H), 5.74 (d, *J* = 15.4 Hz, 1 H), 4.35 (qd, *J* = 6.4, 6.3 Hz, 1 H), 3.81 (s, 3 H), 2.64 (s, 3 H), 1.62 (s, 3 H), 1.27 (d, *J* = 6.4 Hz, 3 H) (OH was not observed). ^{13}C-NMR f90.5, 68.3, 52.0, 23.3, 22.9, 15.9. HR-MS: 371.0915 ($[M+Na]^+$, $C_{18}H_{20}O_5SNa^+$; calc. 371.0924).

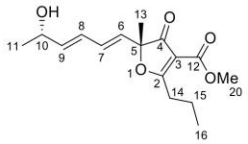
Table S1. Comparison of NMR spectra of isolated[5] and synthetic gregatin G_1 (**6a**) in $CDCl_3$.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			199.0	199.1
3			106.3	106.4
4			198.2	198.2
5			91.0	91.0
6	5.64 d (15.3)	5.66 d (15.3)	128.4	128.5
7	6.27 dd (15.4, 10.5)	6.28 dd (15.3, 10.6)	130.7	130.6
8	6.14 dd (15.4, 10.5)	6.15 ddd (15.3, 10.6, 0.8)	127.7	127.8
9	5.80 dd (15.0, 6.2)	5.81 dd (15.2, 6.2)	140.0	140.0
10	4.34 q (6.3)	4.35 qd (6.4, 6.2)	68.3	68.3
11	1.26 d (6.4)	1.27 d (6.4)	23.3	23.3
12			163.3	163.4
13	1.51 s	1.53 s	22.6	22.7
14	3.01 td (7.0, 6.4, 4.2)	2.97–3.08 m	32.8	32.8
15	1.76 q (7.5)	1.78 qt (7.5, 7.4)	20.0	20.1
16	1.02 t (7.3)	1.03 t (7.5)	13.9[c]	13.9
17	3.81 s	3.82 s	51.7	51.8

[a] ^{1}H- and ^{13}C-NMR data recorded at 400 MHz and 100 MHz. [b] ^{1}H- and ^{13}C-NMR data recorded at 500 MHz and 125 MHz. [c] In the NMR assignment in the table of the isolation the signal was indicated as 23.3 ppm. But in the ^{13}C-NMR spectrum the signal appears at 13.9 ppm.[5]

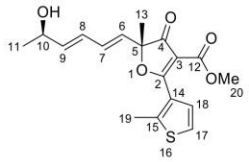
Table S2. Comparison of NMR spectra of isolated[5] and synthetic gregatin G_2 (**6b**) in $CDCl_3$.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			199.0	199.1
3			106.3	106.4
4			198.2	198.2
5			91.0	91.0
6	5.66 d (15.3)	5.65 d (15.4)	128.4	128.5
7	6.29 dd (15.4, 10.4)	6.28 dd (15.3, 10.6)	130.7	130.7
8	6.15 dd (15.1, 10.4)	6.14 ddd (15.3, 10.6, 0.6)	127.7	127.8
9	5.81 dd (15.0, 6.1)	5.81 dd (15.3, 6.1)	140.0	140.0
10	4.35 q (6.3)	4.35 qd (6.4, 6.1)	68.3	68.3
11	1.28 d (6.4)	1.27 d (6.4)	23.3	23.3
12			163.3	163.4
13	1.53 s	1.52 s	22.6	22.6
14	3.02 td (7.0, 6.4, 4.2)	2.95–3.08 m	32.8	32.8
15	1.75 q (7.4)	1.78 qt (7.4, 7.4)	20.0	20.1
16	1.04 t (7.3)	1.03 t (7.4)	13.9[c]	13.9
17	3.83 s	3.83 s	51.7	51.7

[a] ^{1}H- and ^{13}C-NMR data recorded at 400 MHz and 100 MHz. [b] ^{1}H- and ^{13}C-NMR data recorded at 500 MHz and 125 MHz. [c] In the NMR assignment in the table of the isolation the signal was indicated as 23.3 ppm. But in the ^{13}C-NMR spectrum the signal appears at 13.9 ppm.[5]

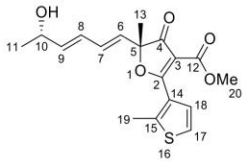
Table S3. Comparison of NMR spectra of isolated[5] and synthetic thiocarboxylic C_1 (**3a**) in $CDCl_3$.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			184.4	184.4
3			106.2	106.1
4			198.5	198.5
5			90.5	90.5
6	5.75 d (15.4)	5.75 d (15.4)	128.7	128.7
7	6.36 dd (15.4, 10.5)	6.36 dd (15.4, 10.7)	131.0	131.0
8	6.18 dd (15.3, 10.5)	6.18 ddd (15.2, 10.6, 0.5)	127.8	127.8
9	5.83 dd (15.3, 6.1)	5.82 dd (15.3, 6.3)	140.1	140.1
10	4.36 m	4.36 qd (6.4, 6.3)	68.3	68.4
11	1.28 d (6.4)	1.27 d (6.4)	23.4	23.4
12			163.1	163.2
13	1.63 s	1.63 s	23.0	23.0
14			126.6	126.6
15			147.9	148.0
17	7.13 d (5.5)	7.13 d (5.5)	122.3	122.3
18	7.36 d (5.4)	7.36 d (5.3)	129.4	129.4
19	2.64 s	2.64 s	15.9	15.9
20	3.82 s	3.82 s	52.0	52.1

[a] ^{1}H- and ^{13}C-NMR data recorded at 400 MHz and 100 MHz. [b] ^{1}H- and ^{13}C-NMR data recorded at 500 MHz and 125 MHz.

Table S4. Comparison of NMR spectra of isolated[5] and synthetic thiocarboxylic C_2 (**3b**) in $CDCl_3$.



Position	^{1}H-Signals δ (ppm), multipl., *J* (Hz)		^{13}C δ (ppm)	
	Isolation[a]	Synthetic[b]	Isolation[a]	Synthetic[b]
2			184.4	184.4
3			106.2	106.1
4			198.5	198.5
5			90.5	90.5
6	5.75 d (15.4)	5.74 d (15.4)	128.7	128.6
7	6.36 dd (15.4, 10.5)	6.35 dd (15.5, 10.6)	131.0	131.0
8	6.18 dd (15.3, 10.5)	6.17 dd (15.5, 10.5)	127.8	127.8
9	5.83 dd (15.3, 6.1)	5.82 dd (15.3, 6.3)	140.1	140.1
10	4.36 m	4.35 qd (6.4, 6.3)	68.3	68.3
11	1.28 d (6.4)	1.27 d (6.4)	23.4	23.3
12			163.1	163.1
13	1.63 s	1.62 s	23.0	22.9
14			126.6	126.5
15			147.9	147.9
17	7.13 d (5.5)	7.12 d (5.5)	122.3	122.3
18	7.36 d (5.4)	7.36 d (5.5)	129.4	129.4
19	2.64 s	2.64 s	15.9	15.9
20	3.82 s	3.81 s	52.0	52.0

[a] ^{1}H- and ^{13}C-NMR data recorded at 400 MHz and 100 MHz. [b] ^{1}H- and ^{13}C-NMR data recorded at 500 MHz and 125 MHz.

Antimicrobial assay

Staphylococcus aureus and *Escherichia coli* bacteria strains were grown in Trypton-Soja-Bouillon (TSB) media or Luria-Bertani (LB) media respectively. TSB media (4 mL) was inoculated with *Staphylococcus aureus* wild-type strain - USA 300 while LB media (4 mL) was inoculated with *Escherichia coli* wild-type strain or an *E. coli* strain lacking the outer membrane protein TolC - *E.coli* TolC.[6] The bacteria strains were incubated under aerobic conditions at 37 °C with shaking at 150 rpm and grown overnight. Bacterial density was determined by measuring the optical density (OD) at 600 nm followed by the establishment of a working culture by diluting the overnight culture to OD 600 nm = 0.1 in fresh media in an Erlenmeyer flask. Bacteria cultures were grown until OD 600 nm ~ 0.5 and then diluted to OD 600 nm = 0.01 in fresh media. The diluted bacteria suspension was seeded in transparent 96 well half-area plates. 1µL of serially diluted (1.5-fold) compounds was added per well using the liquid handler CyBioSelma 96/60 (Analytik Jena) resulting in final concentrations ranging from 100 µM to 1.73 µM. The OD 600 nm of the seeded bacteria was measured, and the plates were incubated at 37 °C, 5% CO_2 for 24 h. Bacteria growth was determined by subtracting the OD 600 nm measured at time 0 h from OD 600 nm at time 24 h of incubation.

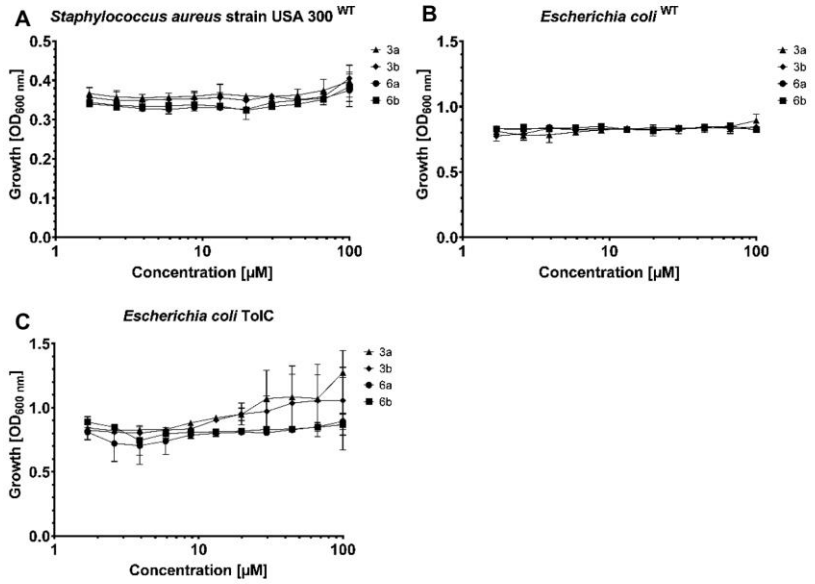


Figure S1. Antimicrobial activity of thiocarboxylic C_1 (**3a**), thiocarboxylic C_2 (**3b**), gregatin G_1 (**6a**) and gregatin G_2 (**6b**). The graphs represent the average growth of bacteria strains *Staphylococcus aureus* (A), *Escherichia coli* wild type (B), and *Escherichia coli* TolC mutant (C). Each symbol represents the average bacteria growth of at least duplicate measurements, and the error bars represent the standard deviation of the mean.

Cytotoxicity assay

L929 cells (mouse fibroblasts cell line), A549 cells (lung epithelial cell line) and HuH7.5 cells (human hepatoma cell line) were cultured and maintained in Dulbecco's modified Eagle's medium high glucose, GlutaMAX™ Supplement (Life Technologies, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) in a 37 °C incubator with a humidified atmosphere and 5% CO_2. Confluent cells (90–100%) were split and seeded in a 96-well plate at a seeding density of 2×10^4 cells/99 µL/well. Cells were incubated for 24 h to allow adherence before treatment with 1µL of serially diluted (1.5-fold) compounds, resulting in final concentrations ranging from 100 µM to 1.73 µM. Cells were incubated for 72 h before determining their viability by a resazurin reduction method (alamarBlue assay) according to manufacturer recommendation.[7] Cell viability was calculated and expressed as a percentage relative to the untreated control (cells in DMSO).

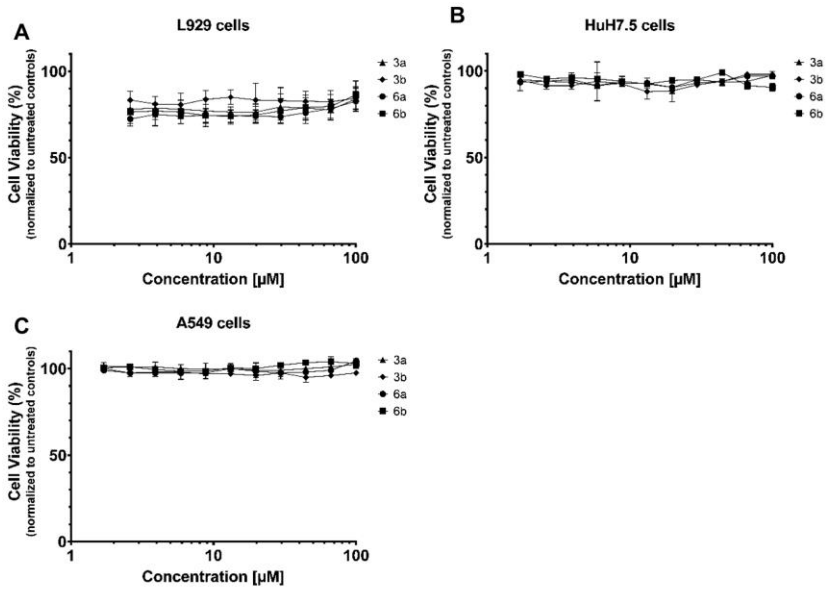


Figure S2. Cytotoxicity of thiocarboxylic C_1 (**3a**), thiocarboxylic C_2 (**3b**), gregatin G_1 (**6a**) and gregatin G_2 (**6b**). The graphs represent the percent viability of L929 cells (A), HuH7.5 cells (B), and A549 cells (C). Each symbol represents the average cell viability of at least duplicate measurements, and the error bars represent the standard deviation of the mean

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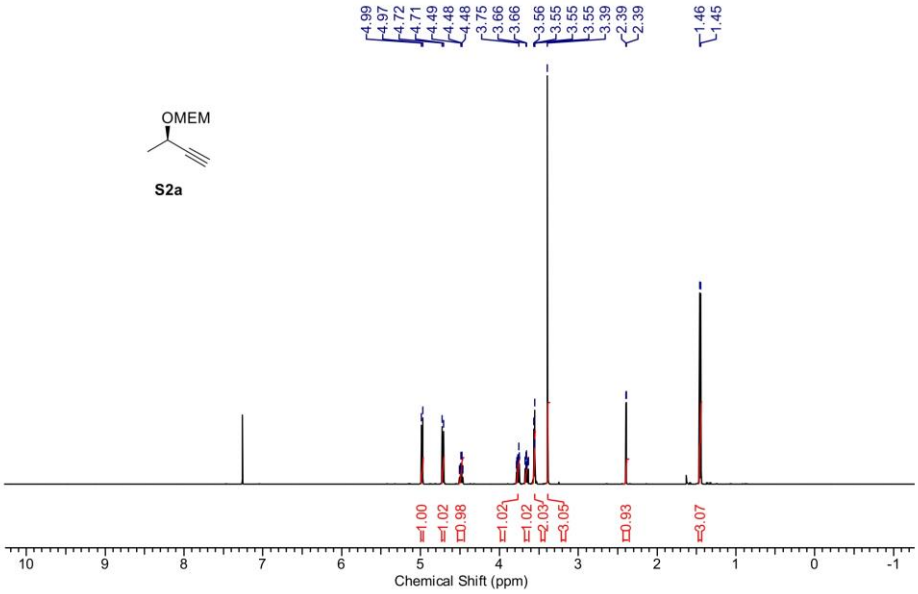
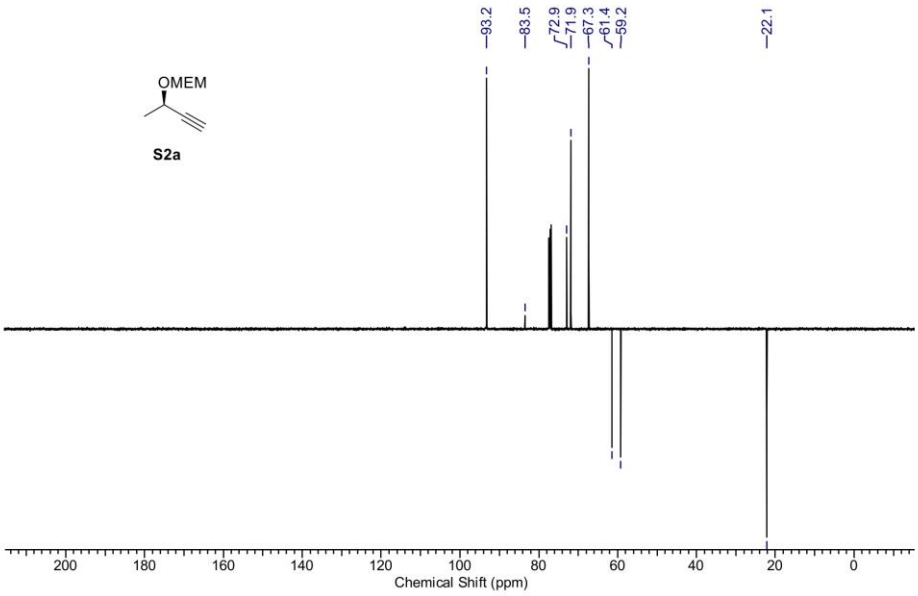
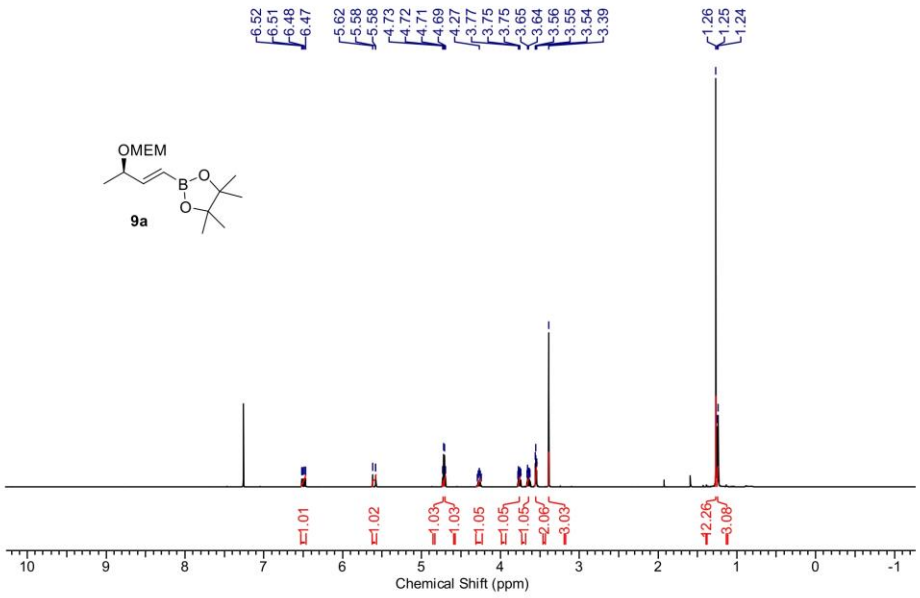
NMR spectra of all new compounds



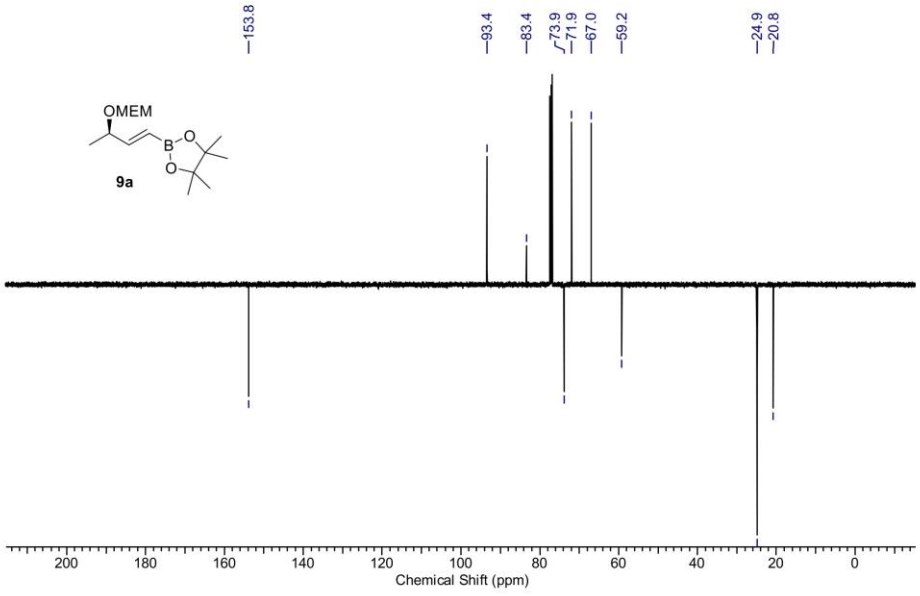
^{1}H-NMR spectrum of compound **S2a** in $CDCl_3$ (500 MHz).



^{13}C-NMR (APT) spectrum of compound **S2a** in $CDCl_3$ (125 MHz).



^{1}H-NMR spectrum of compound **9a** in $CDCl_3$ (500 MHz).



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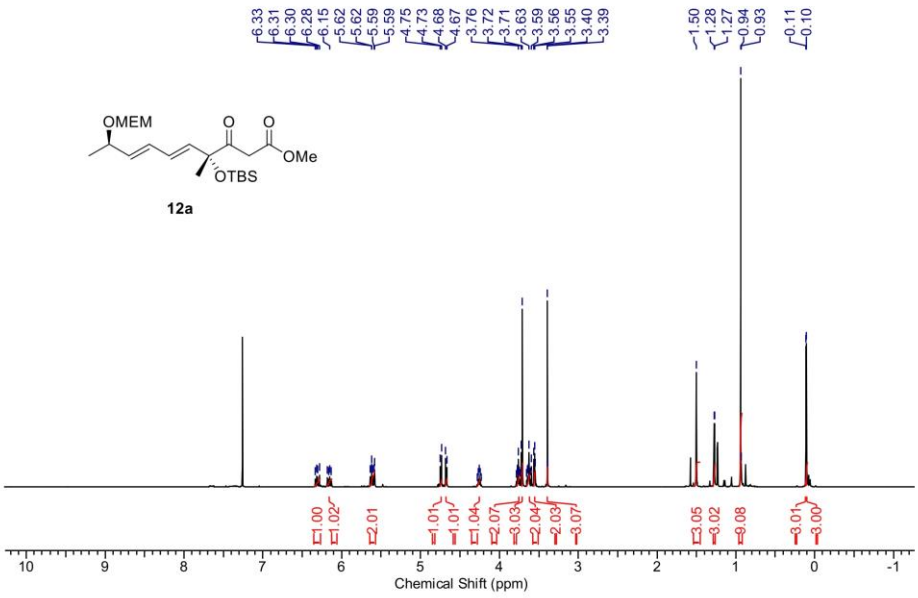
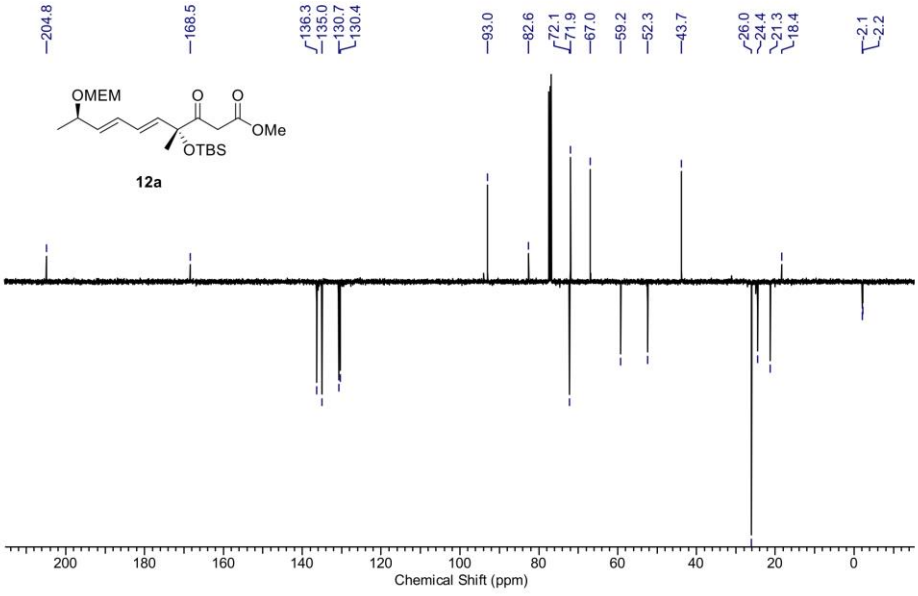


^{1}H-NMR spectrum of compound **12a** in $CDCl_3$ (500 MHz).



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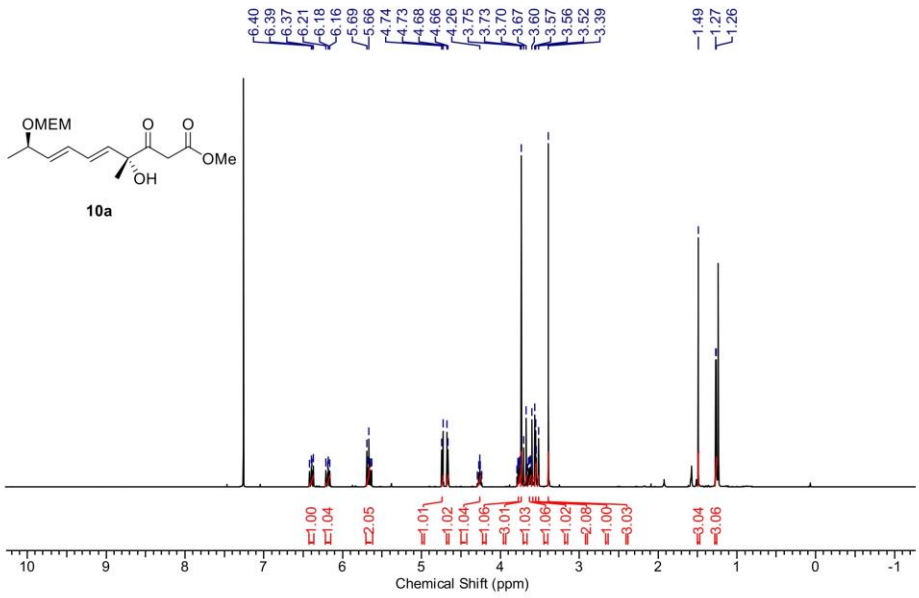
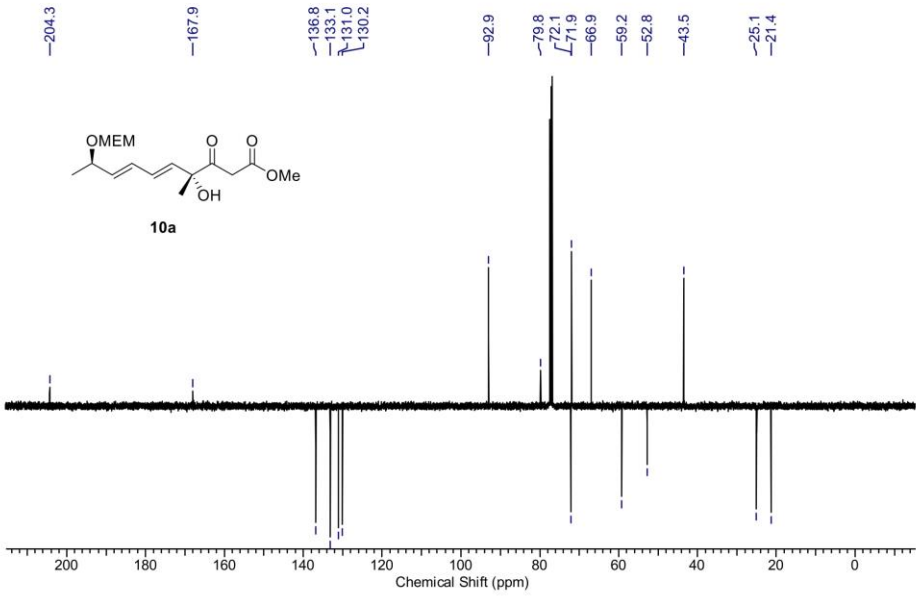
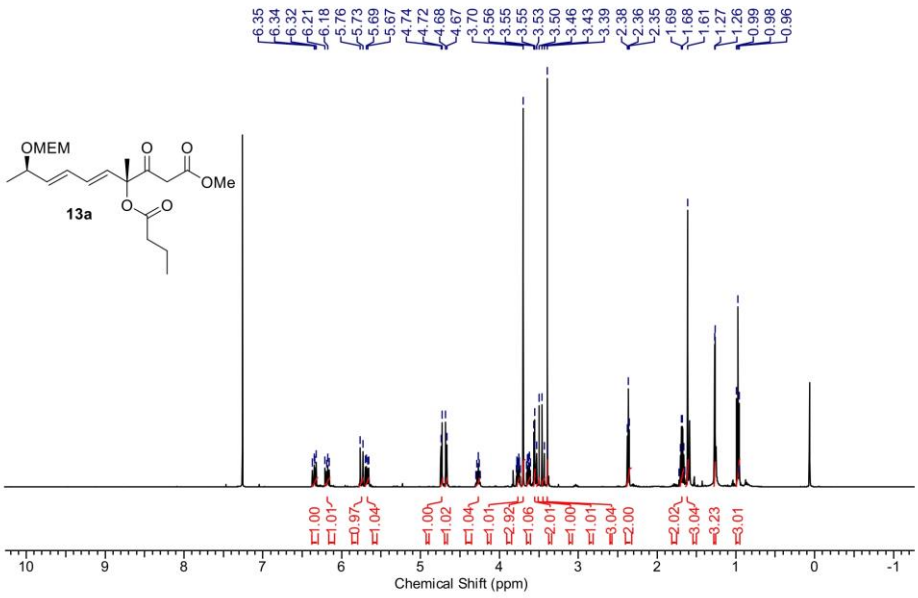


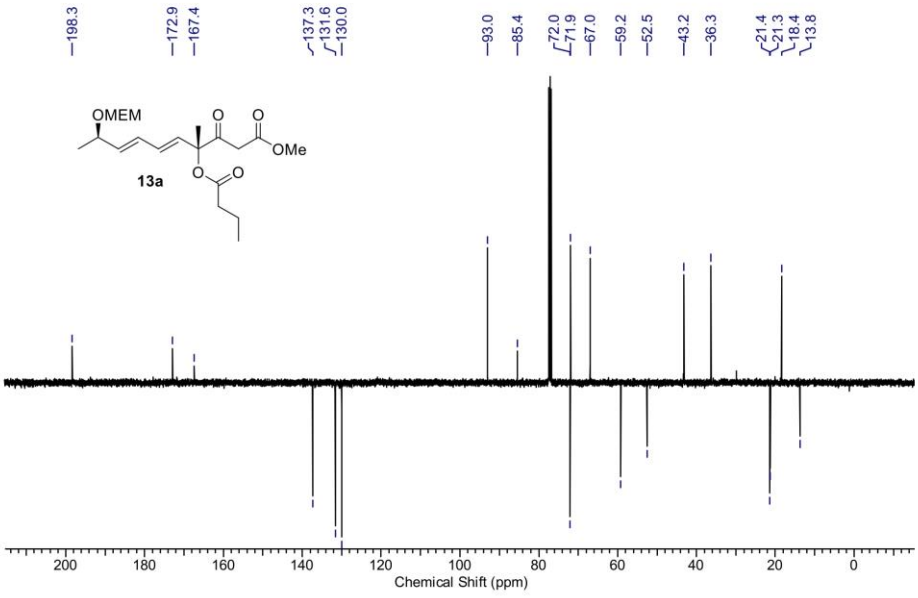
^{1}H-NMR spectrum of compound **10a** in $CDCl_3$ (500 MHz).



^{13}C-NMR (APT) spectrum of compound **10a** in $CDCl_3$ (125 MHz).



^{1}H-NMR spectrum of compound **13a** in $CDCl_3$ (500 MHz).



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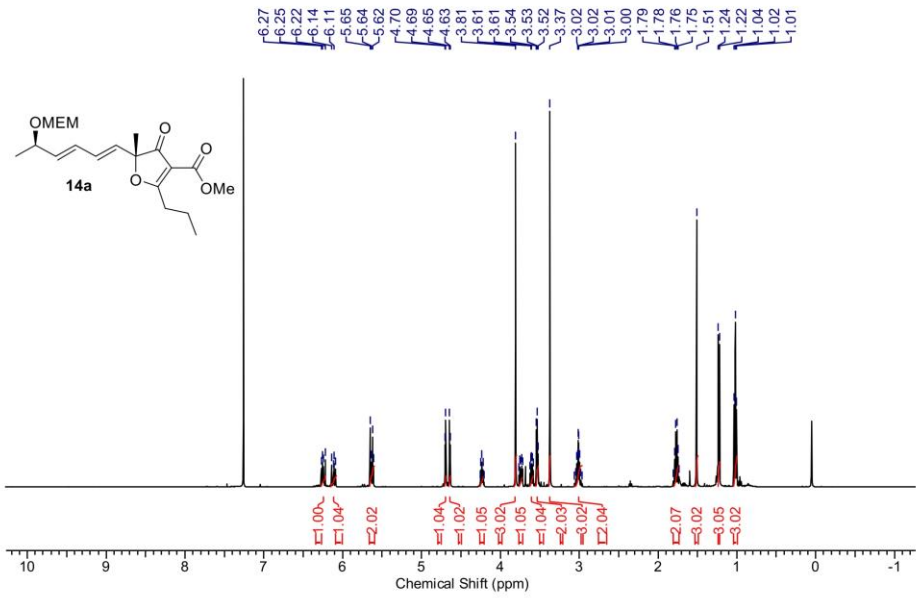

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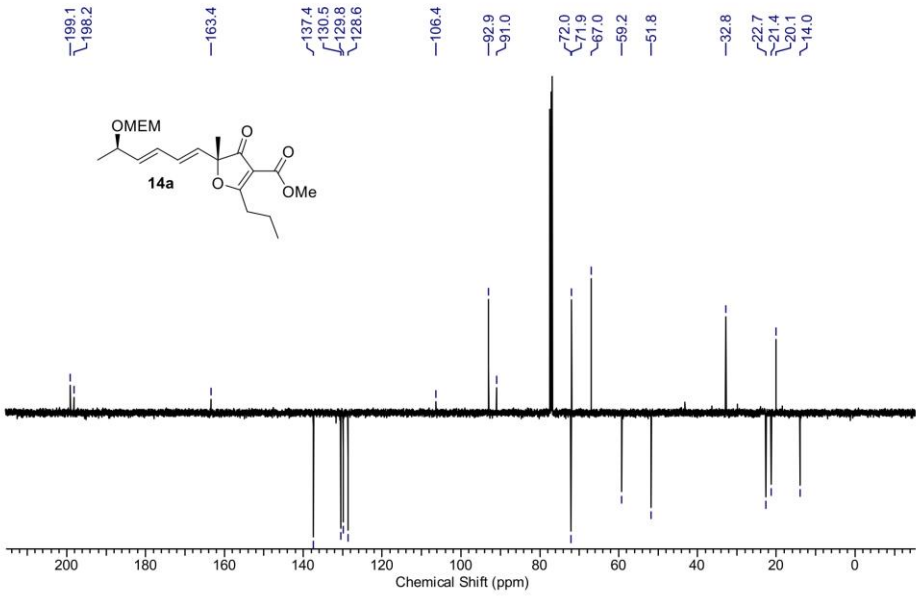

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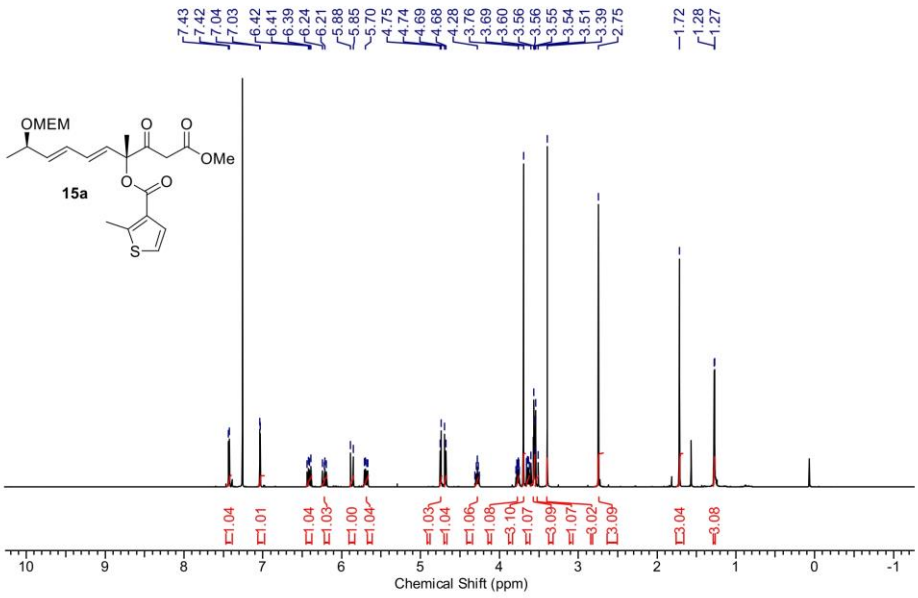

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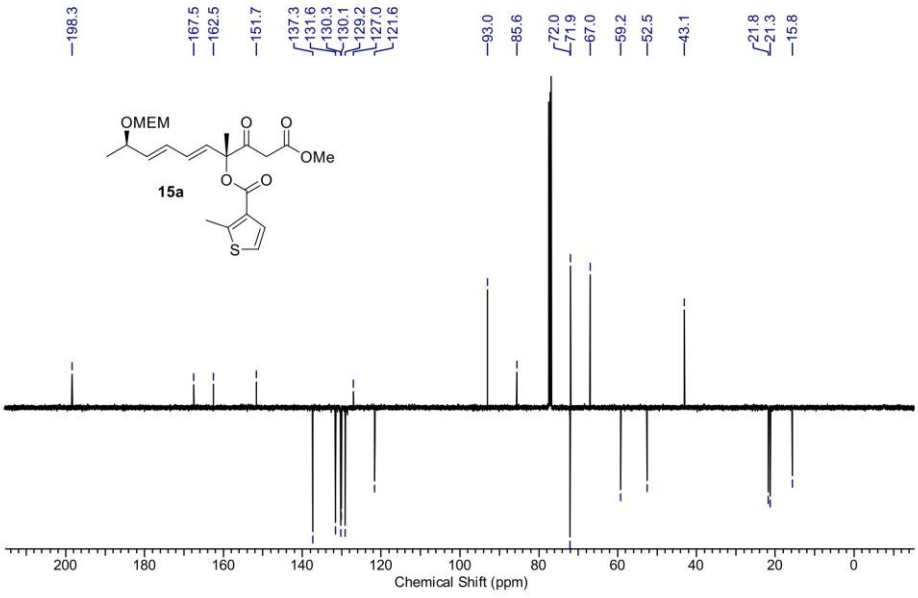

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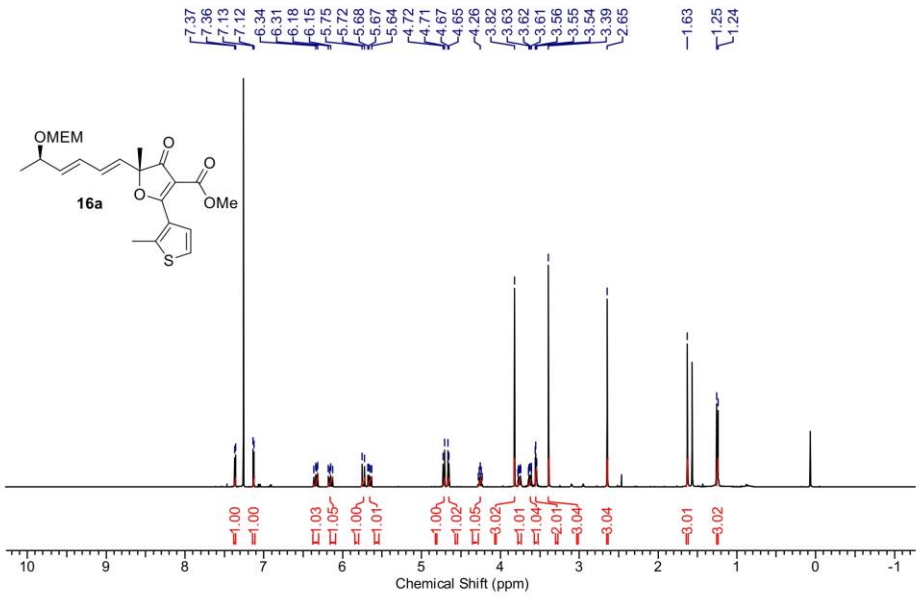

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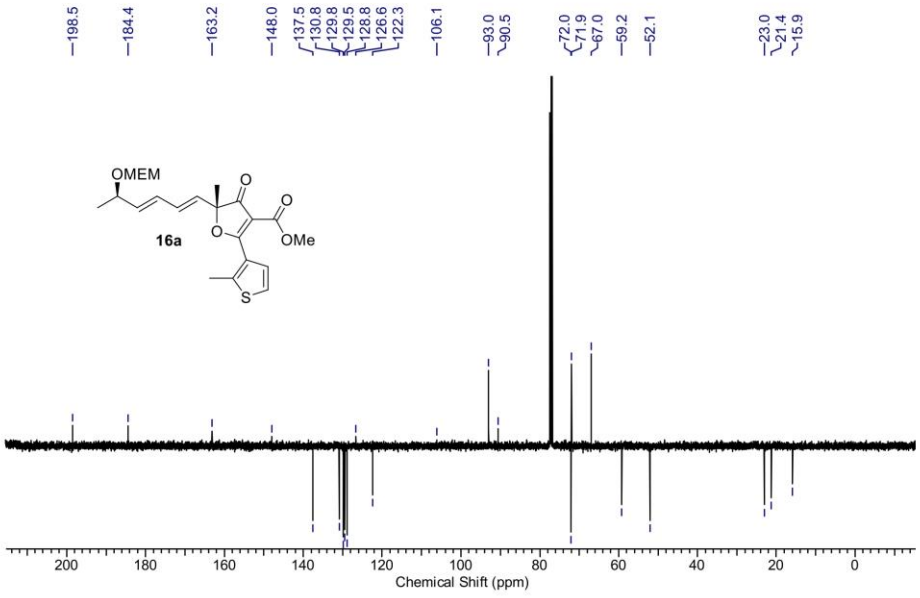

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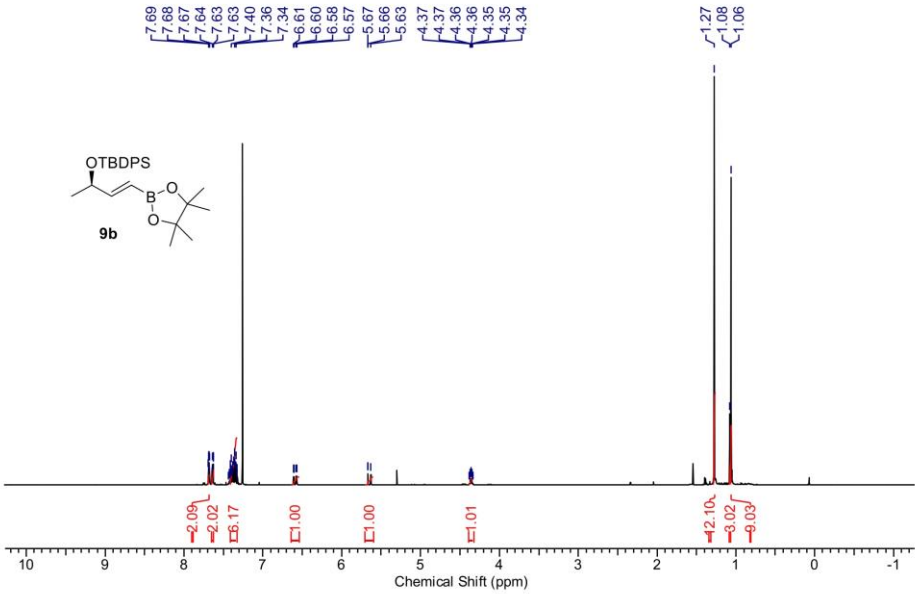

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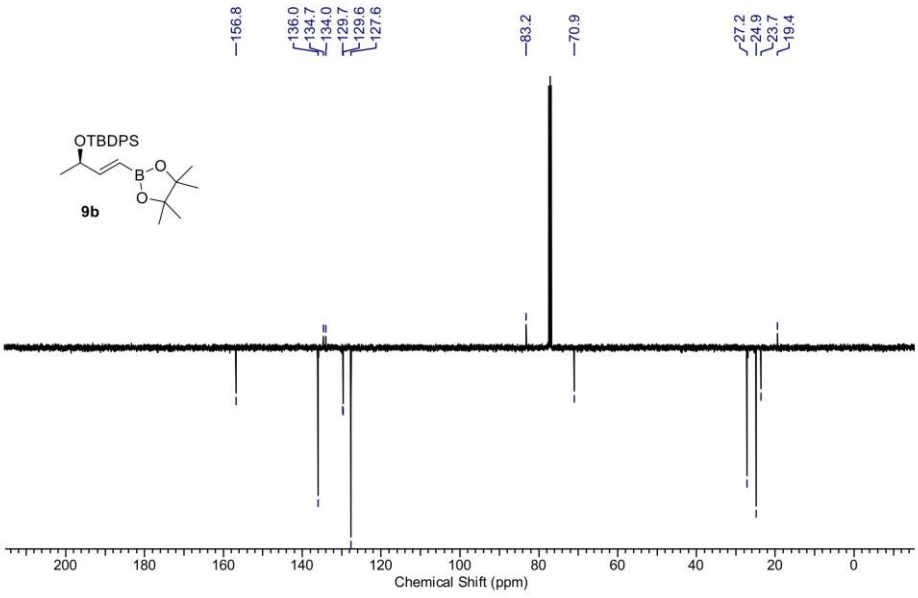

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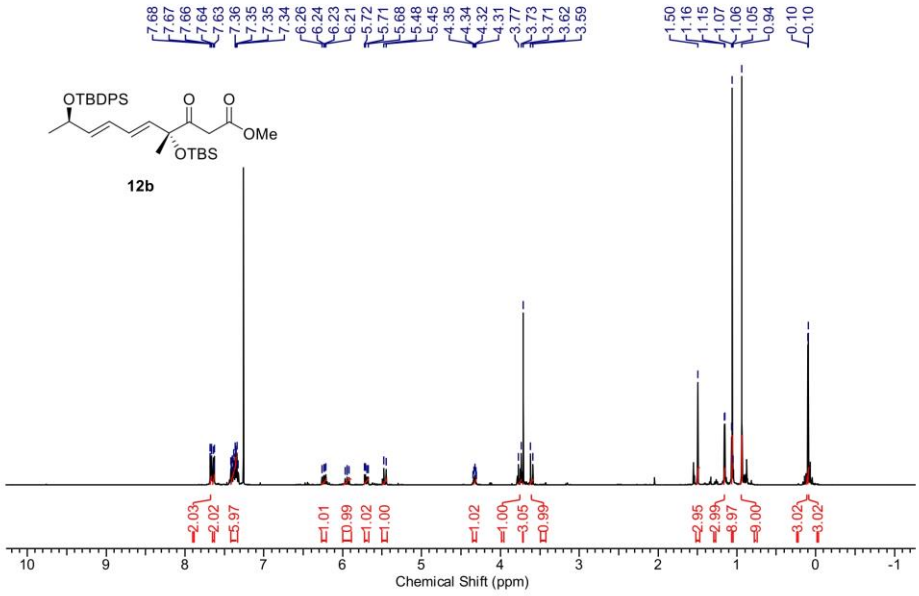

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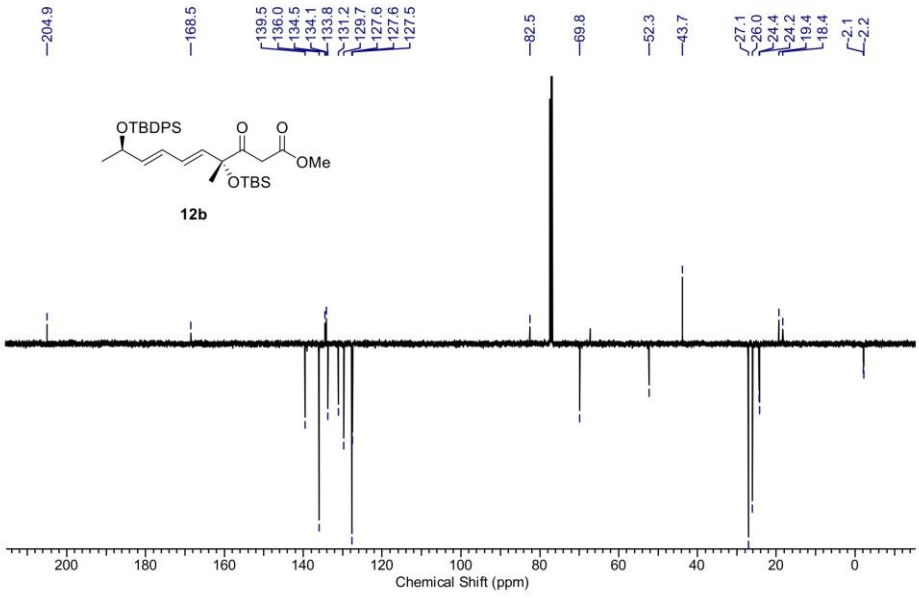

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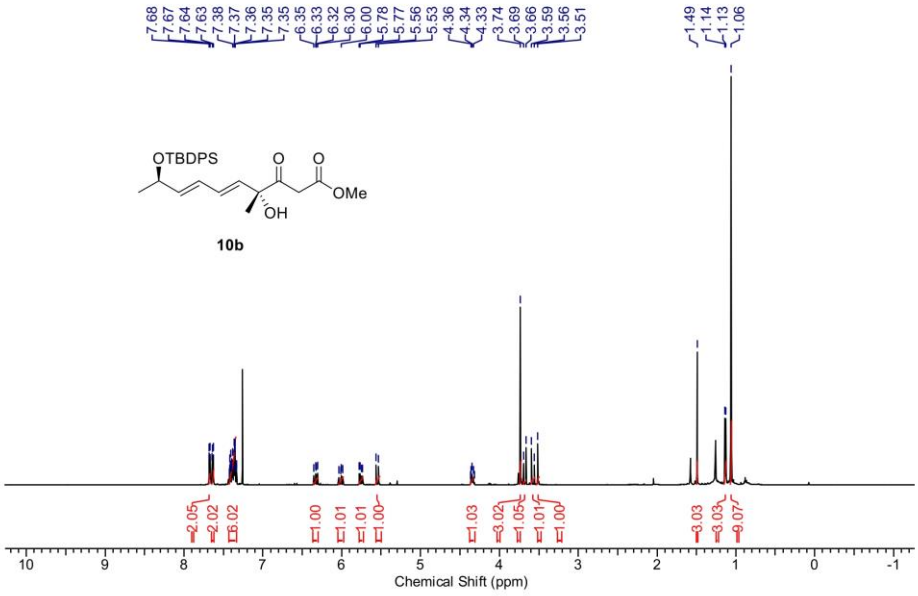

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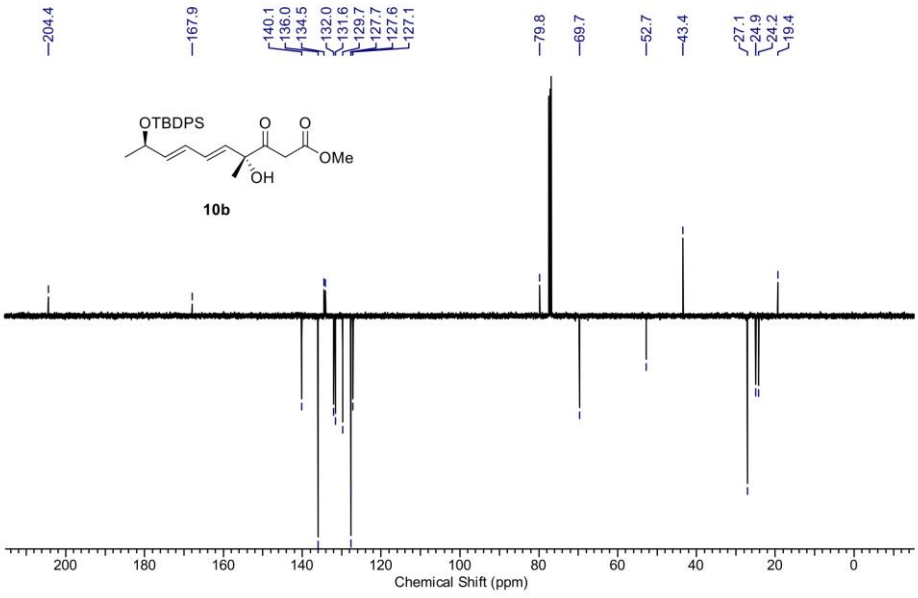

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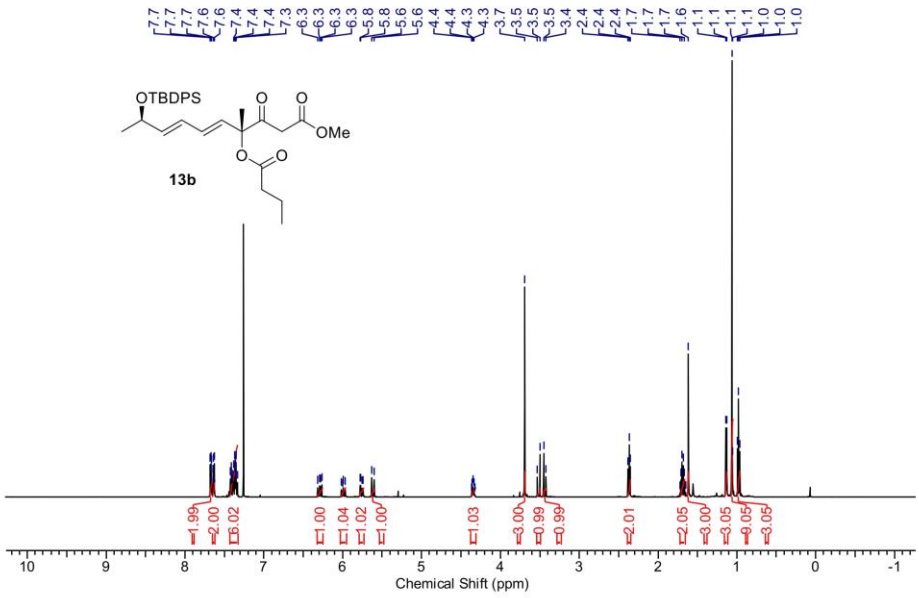

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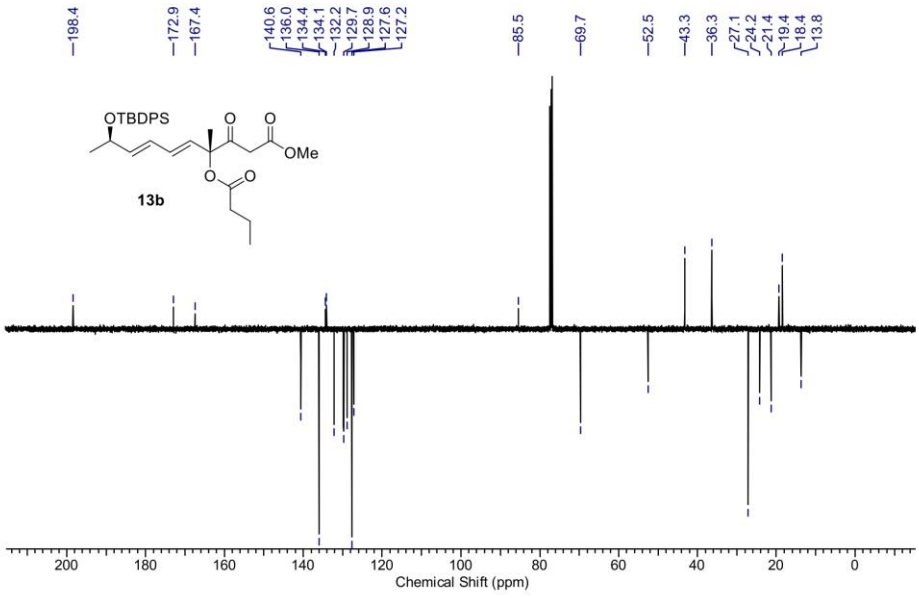

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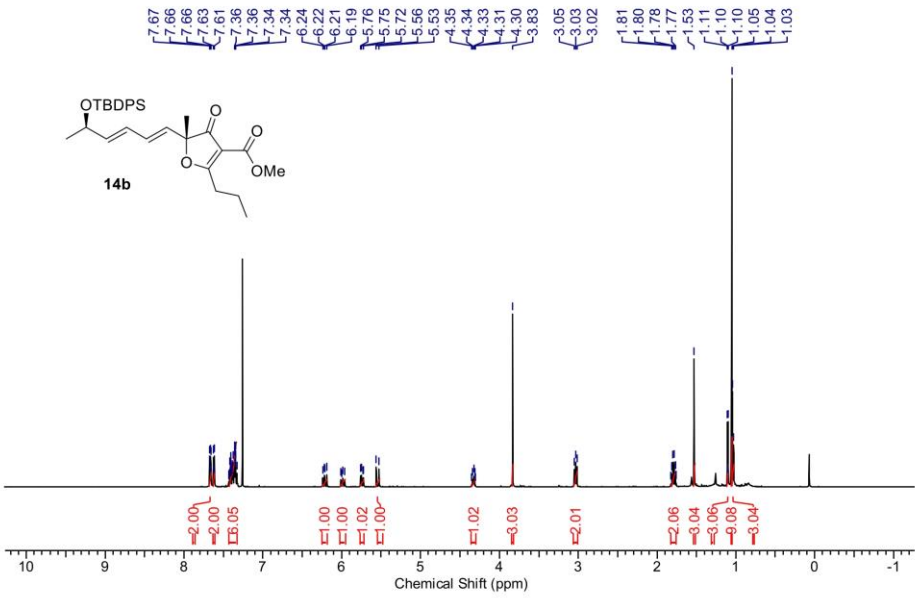

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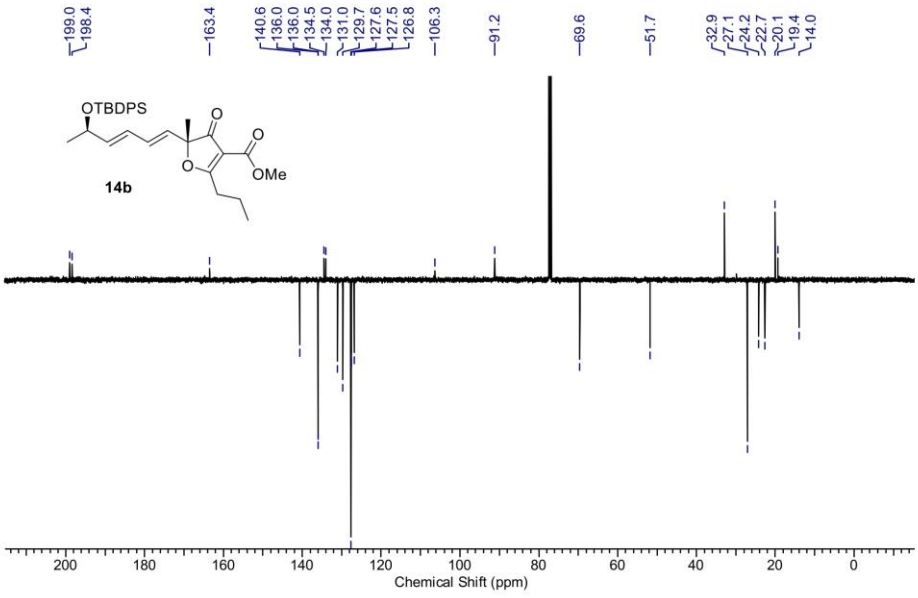

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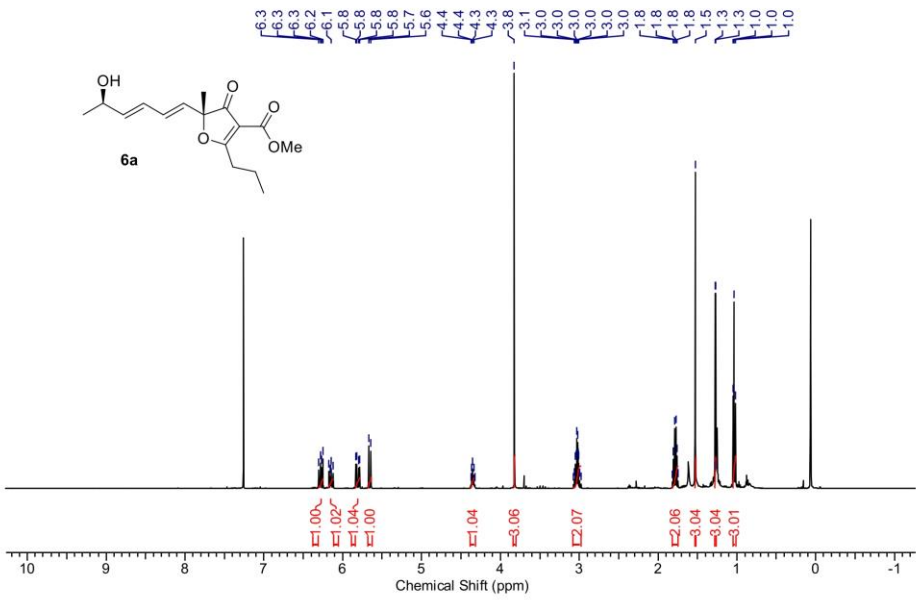

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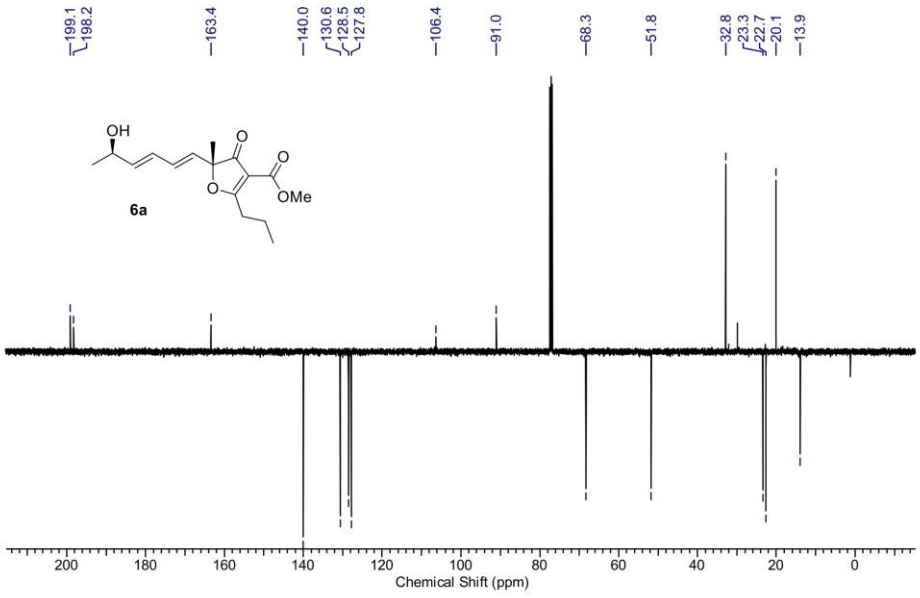

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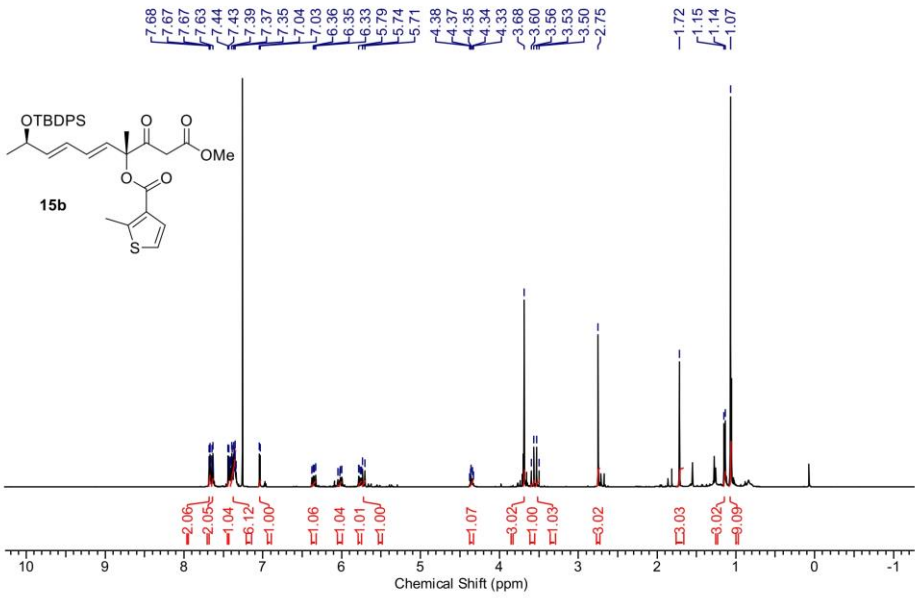

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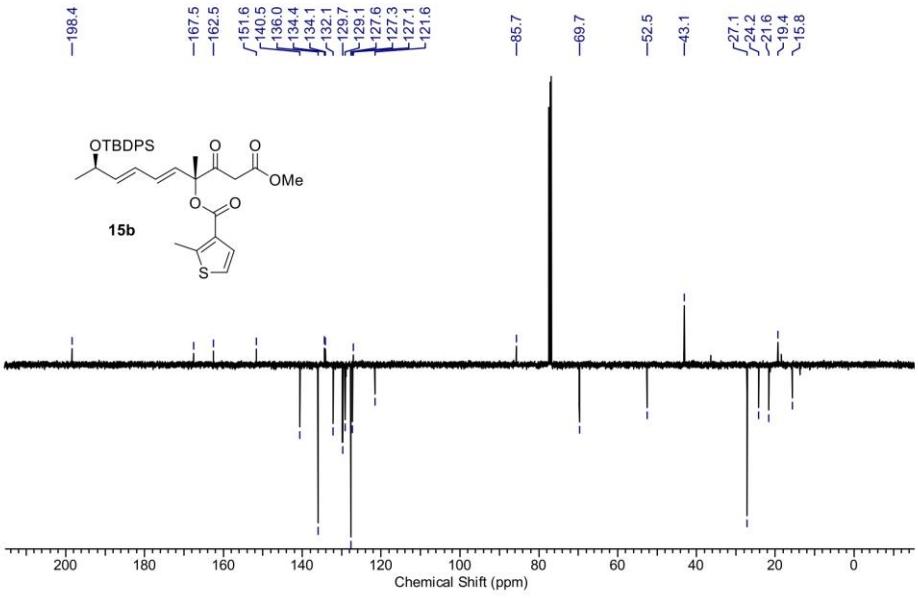

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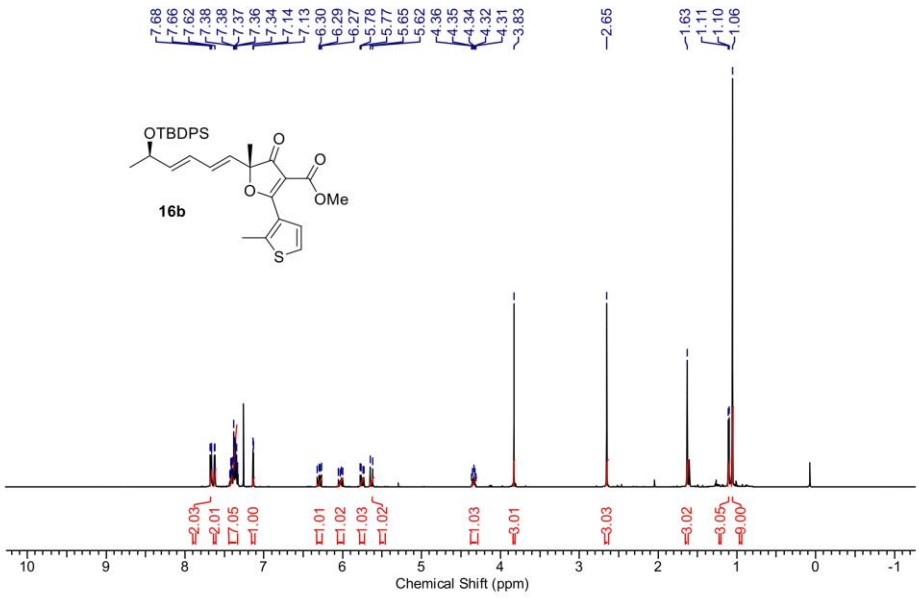

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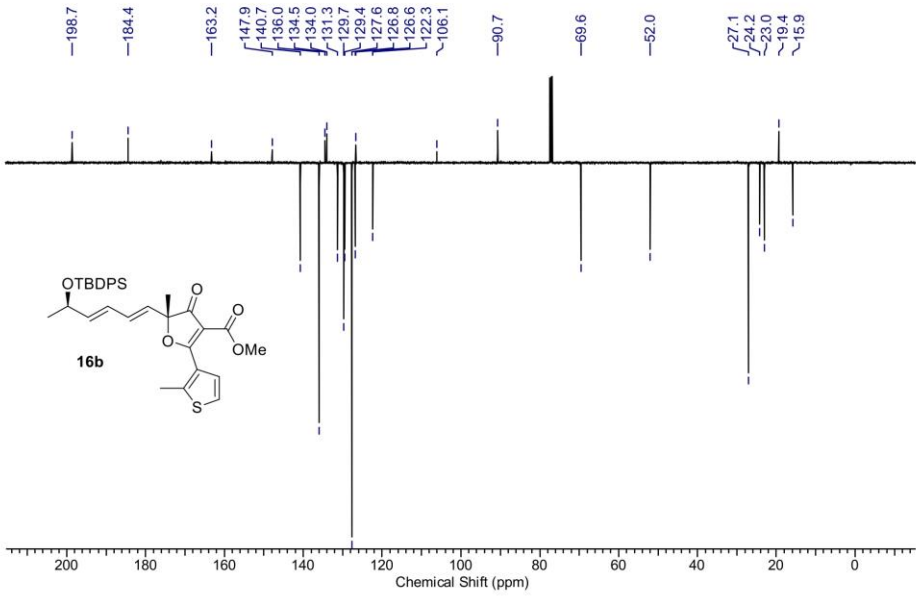

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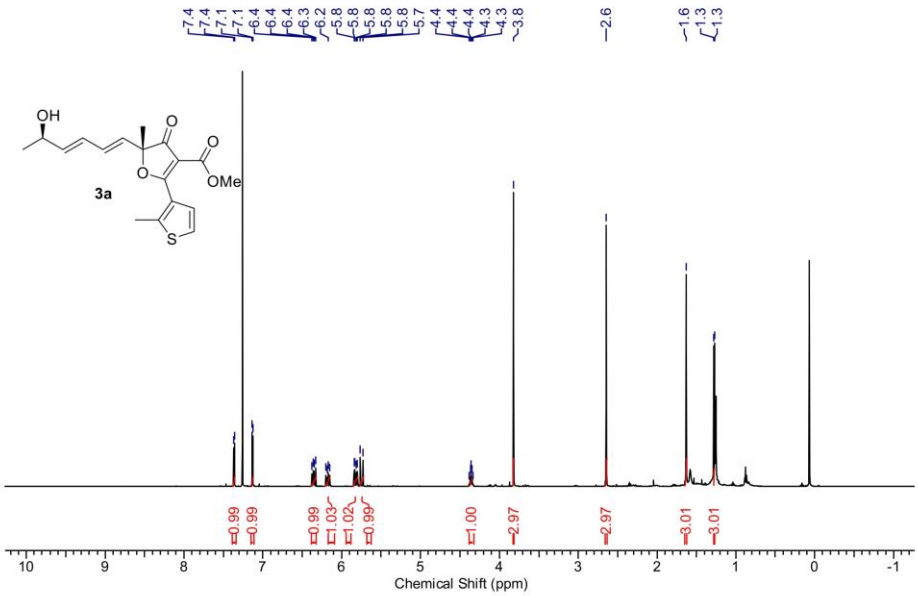

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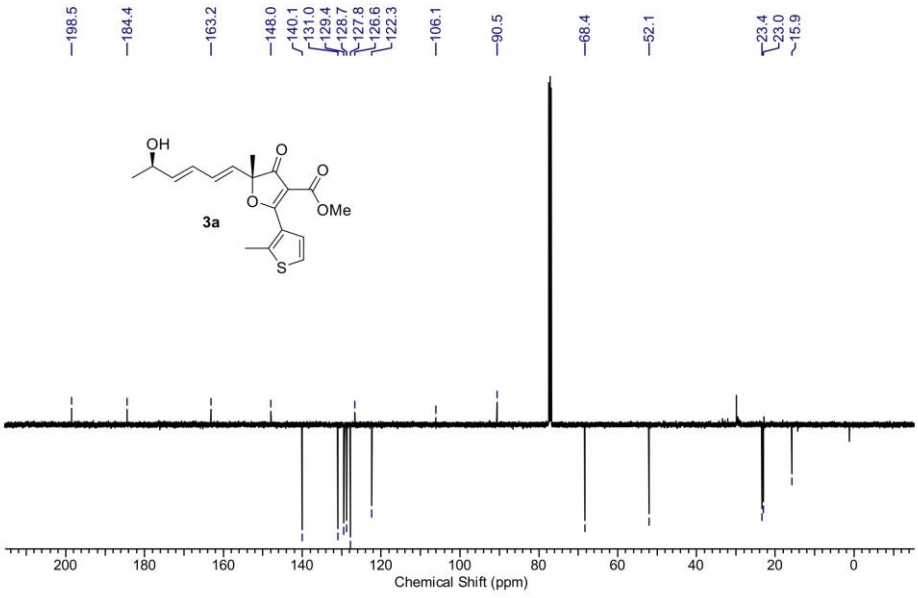

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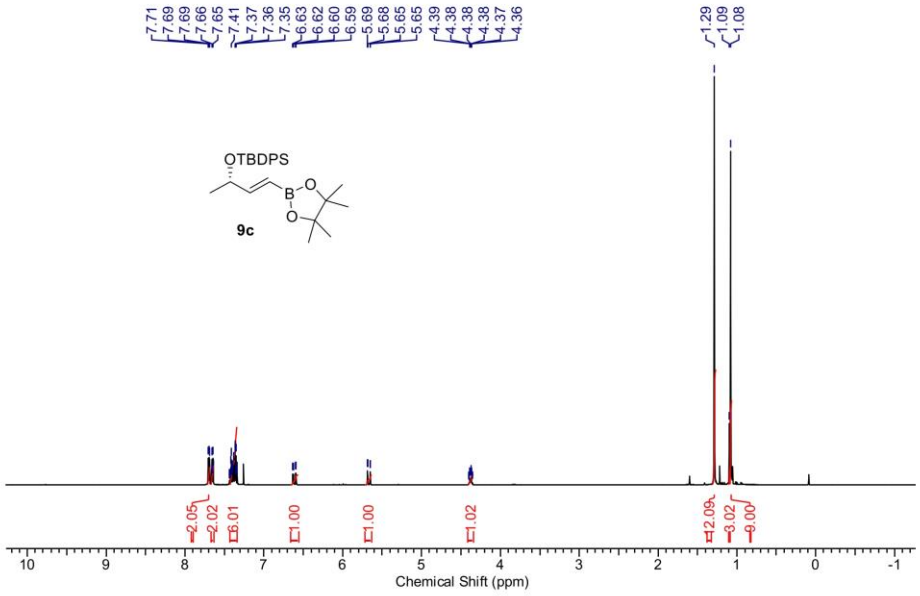

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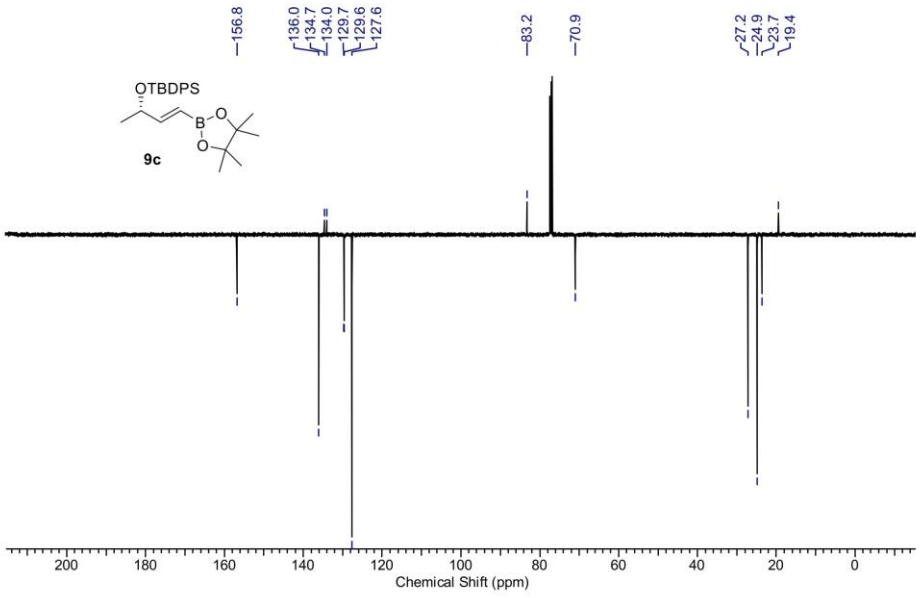

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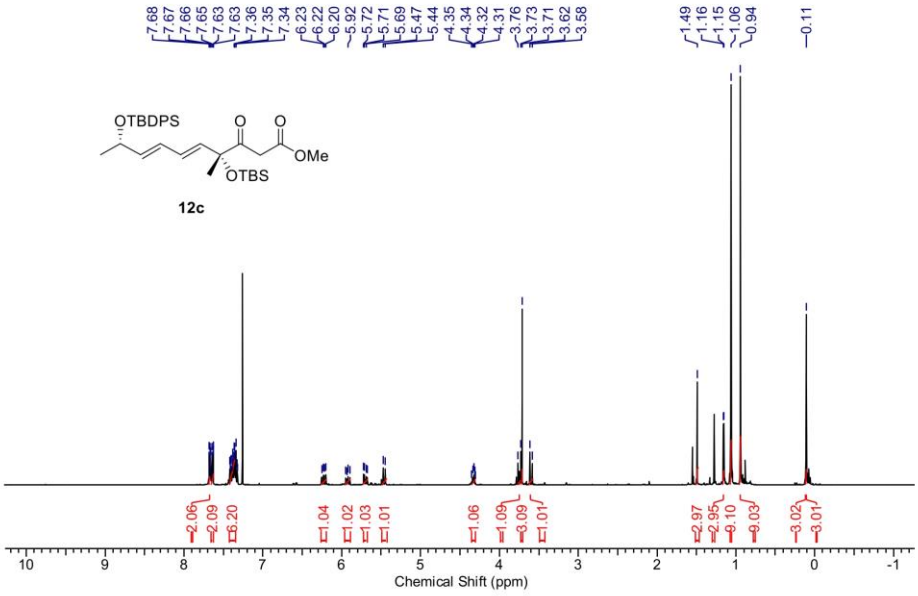

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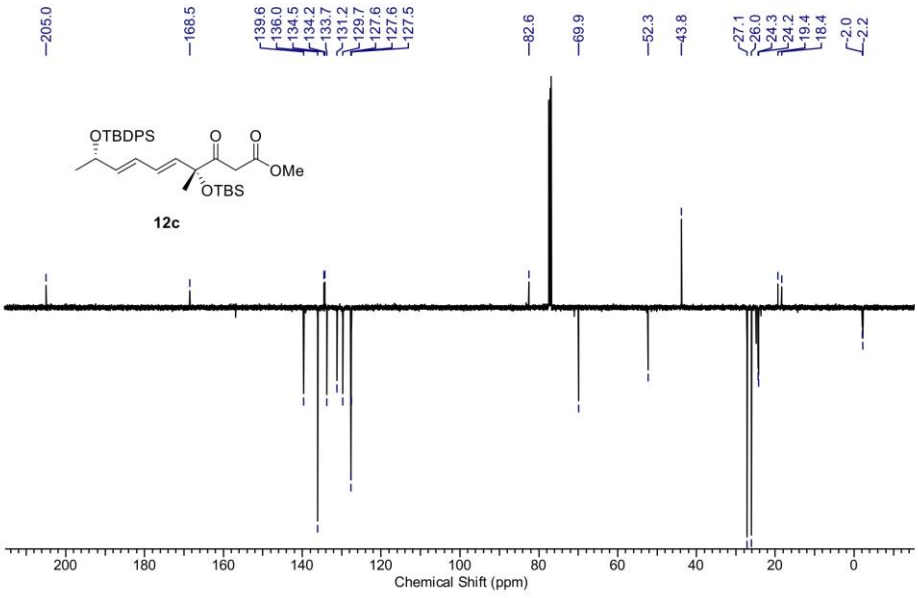
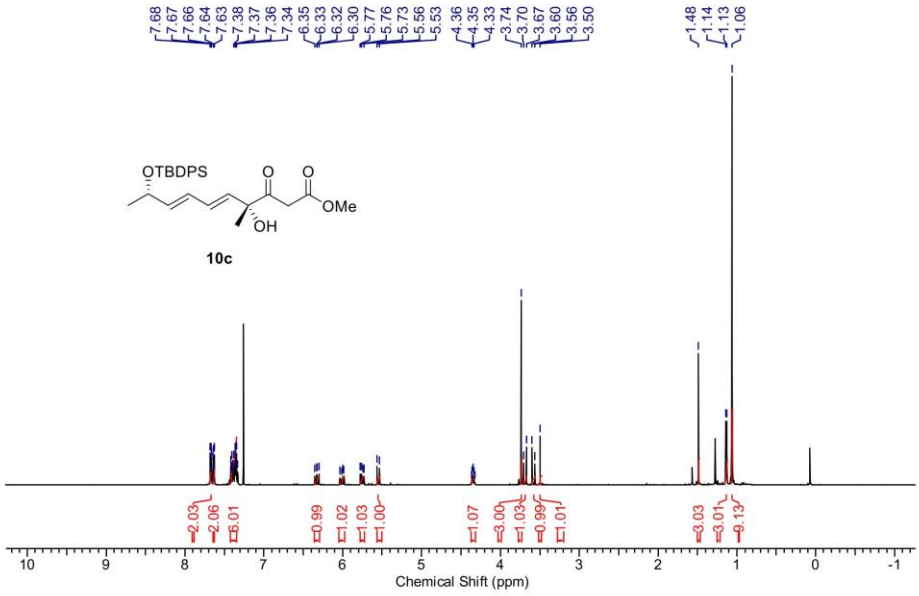


^{13}C-NMR (APT) spectrum of compound **12c** in $CDCl_3$ (125 MHz).

S48



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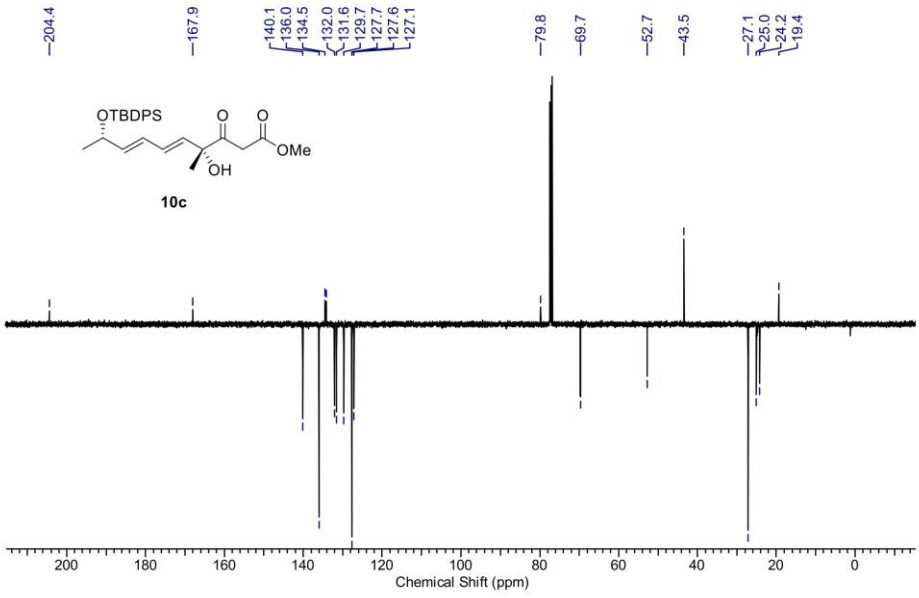

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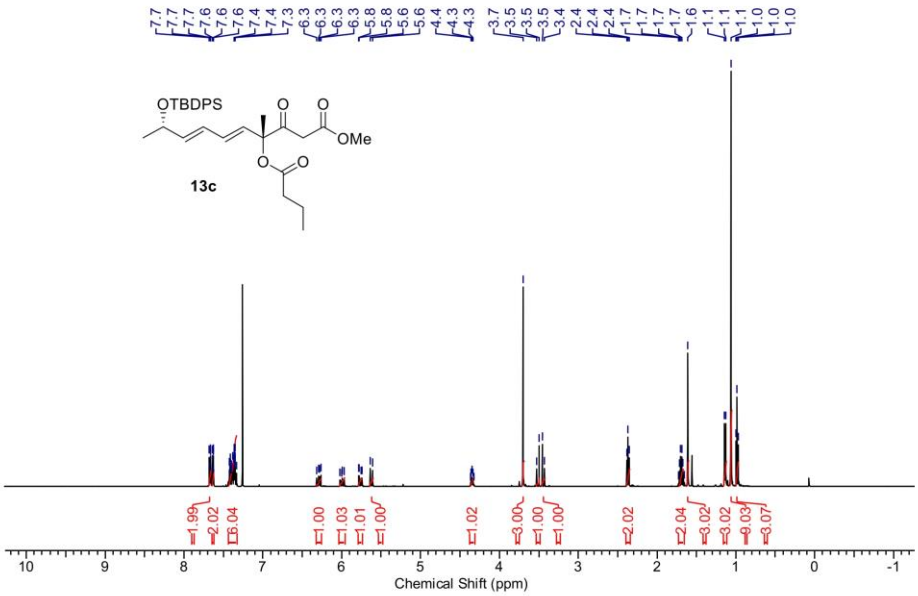

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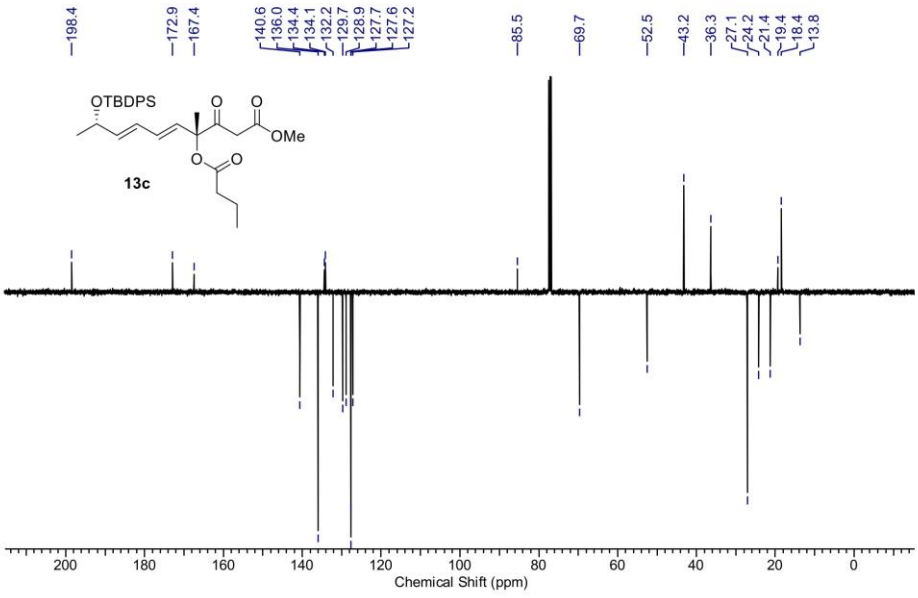
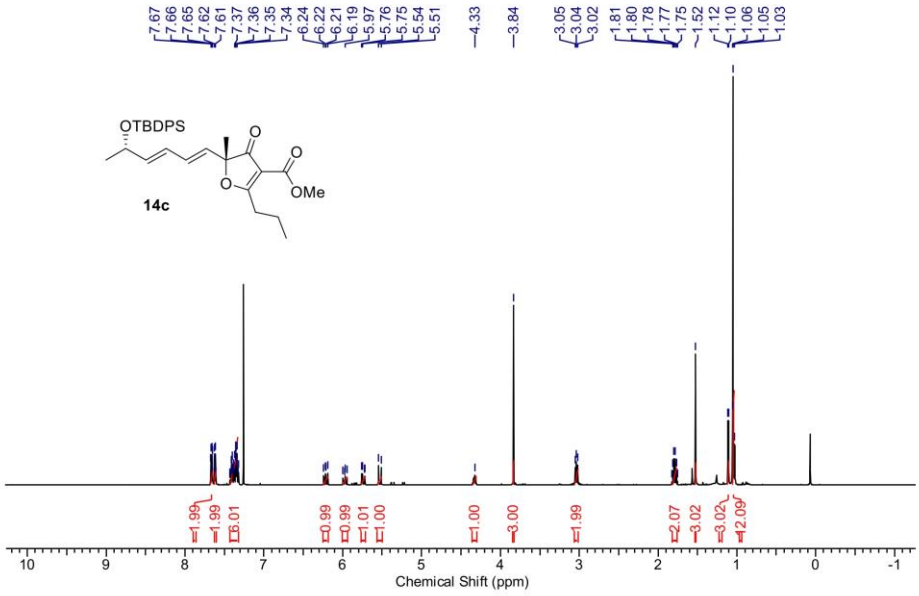
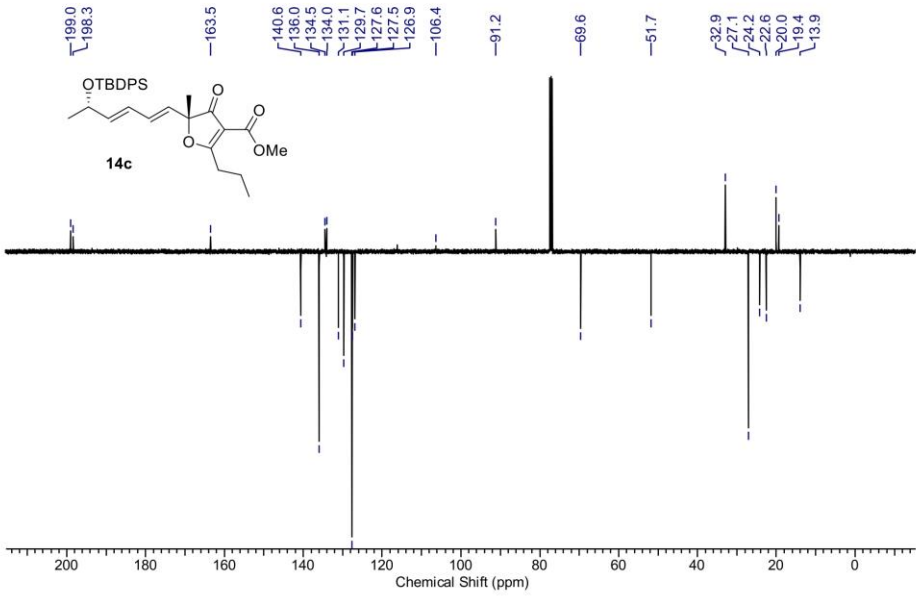


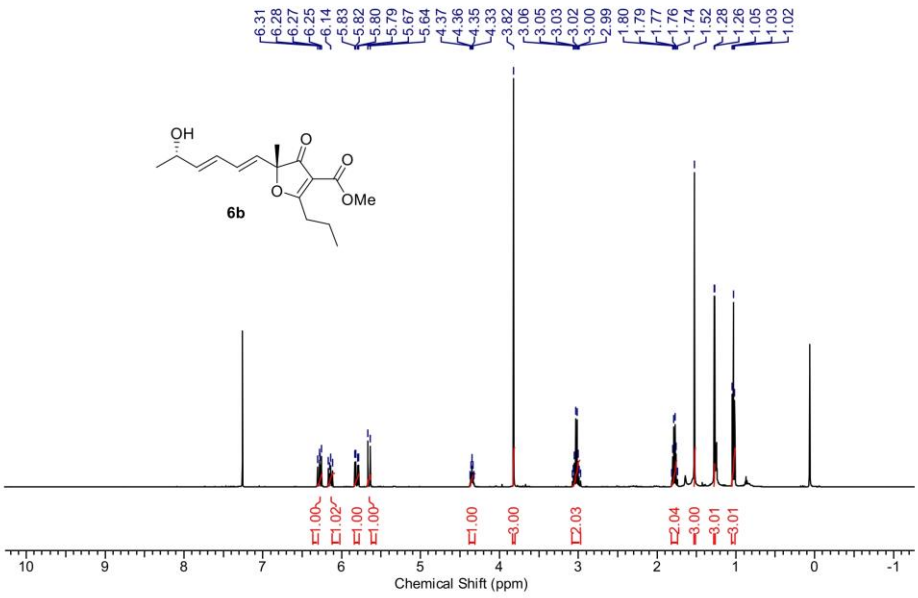
^{13}C-NMR (APT) spectrum of compound **13c** in $CDCl_3$ (125 MHz).



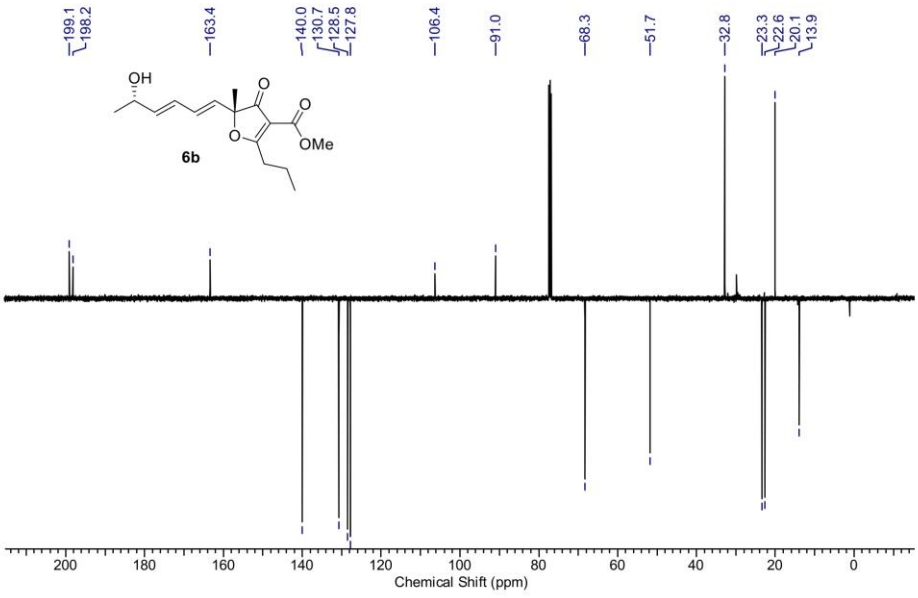
^{1}H-NMR spectrum of compound **14c** in $CDCl_3$ (500 MHz).



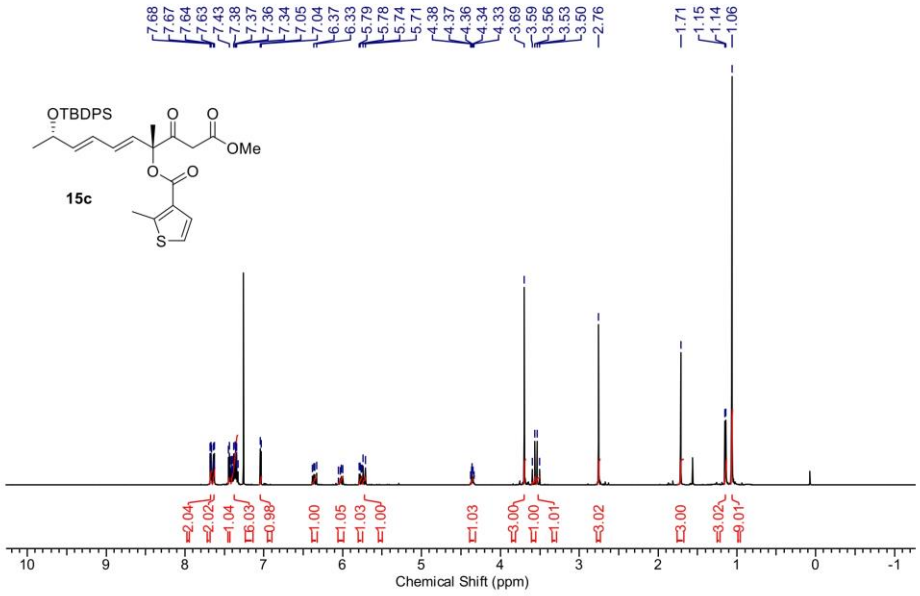
^{13}C-NMR (APT) spectrum of compound **14c** in $CDCl_3$ (125 MHz).



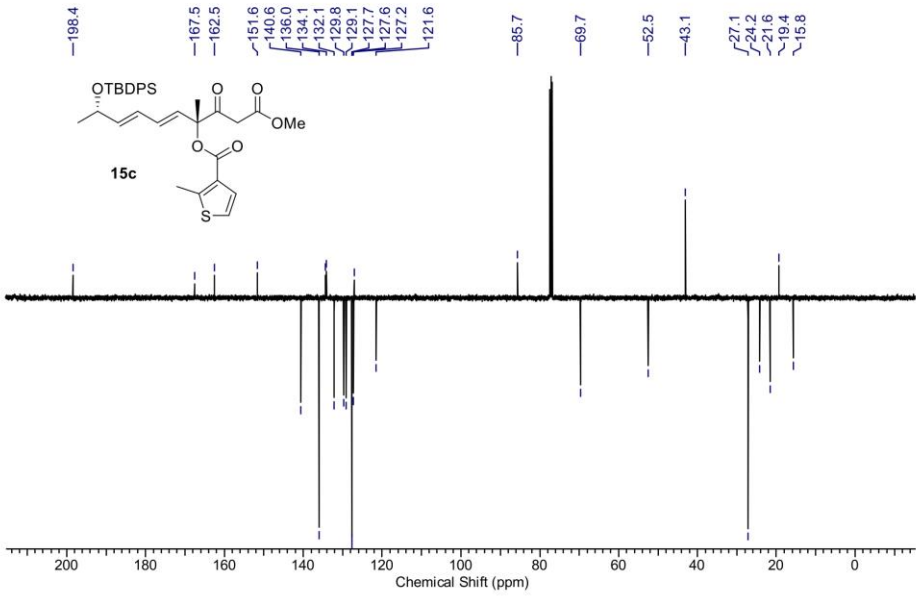
^{1}H-NMR spectrum of compound **6b** in $CDCl_3$ (500 MHz).



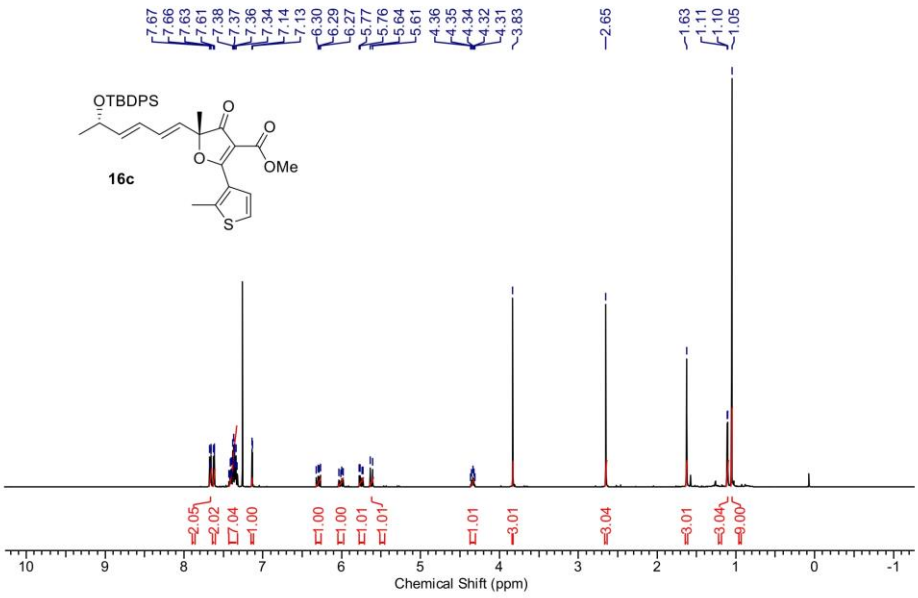
^{13}C-NMR (APT) spectrum of compound **6b** in $CDCl_3$ (125 MHz).



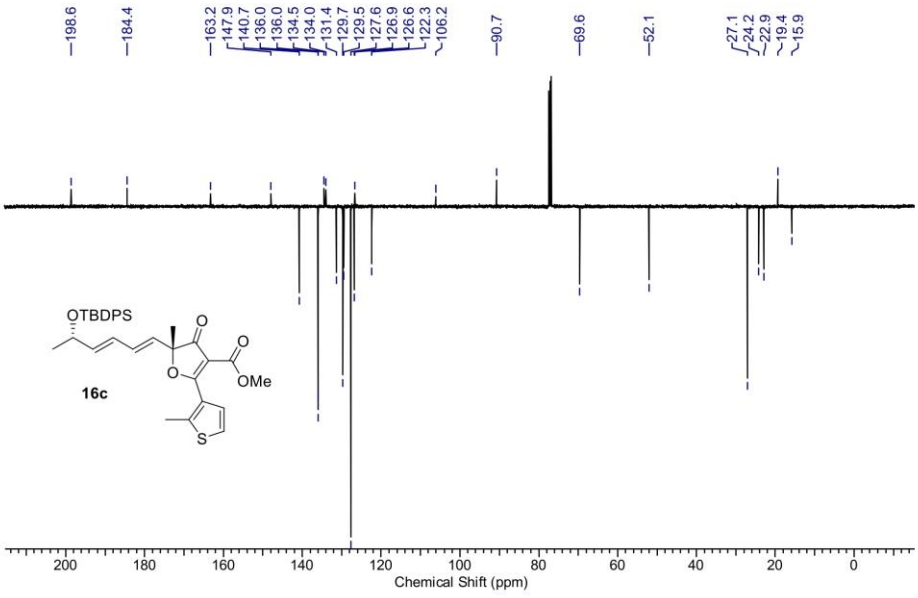
^{1}H-NMR spectrum of compound **15c** in $CDCl_3$ (500 MHz).



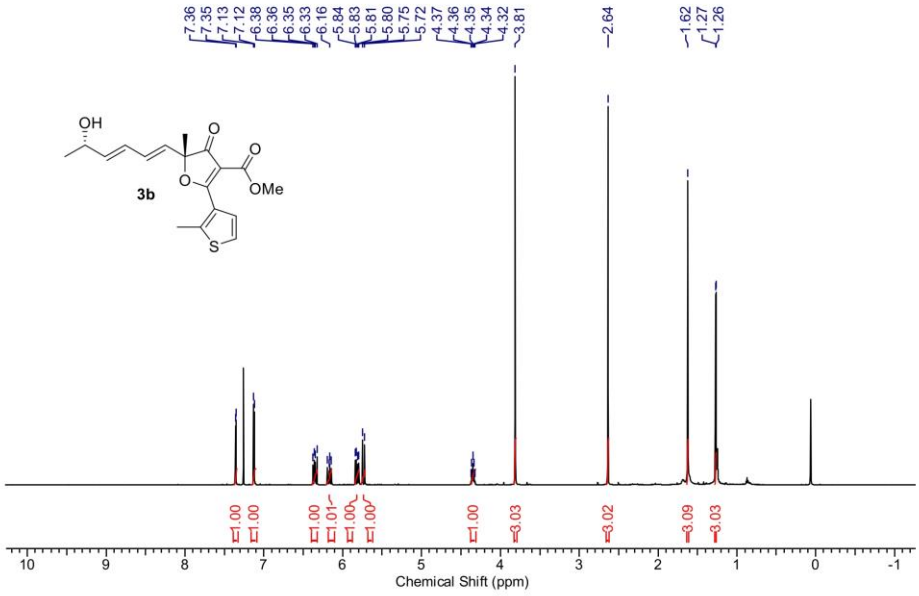
^{13}C-NMR (APT) spectrum of compound **15c** in $CDCl_3$ (125 MHz).



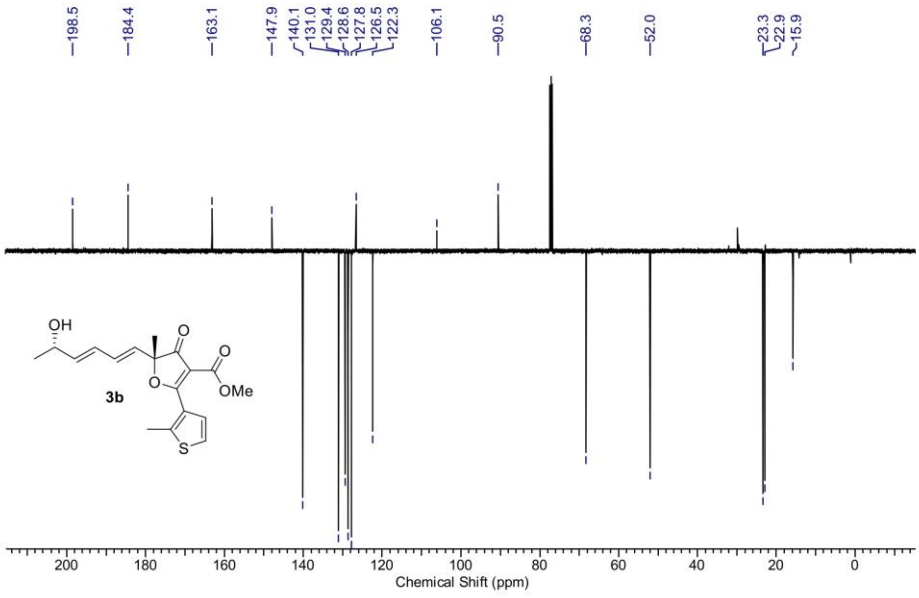
^{1}H-NMR spectrum of compound **16c** in $CDCl_3$ (500 MHz).



^{13}C-NMR (APT) spectrum of compound **16c** in $CDCl_3$ (125 MHz).



^{1}H-NMR spectrum of compound **3b** in $CDCl_3$ (500 MHz).



^{13}C-NMR (APT) spectrum of compound **3b** in $CDCl_3$ (125 MHz).

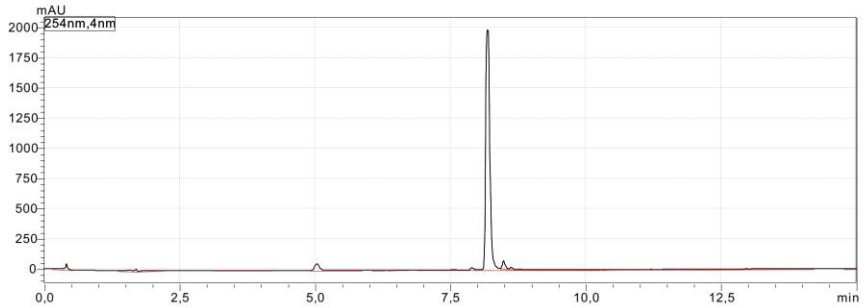


Figure S3. HPLC chromatogram of gregatin G_1 (**6a**). HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

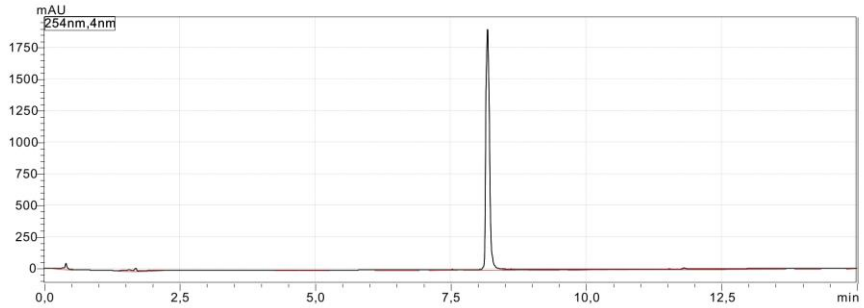


Figure S4. HPLC chromatogram of gregatin G_2 (**6b**). HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

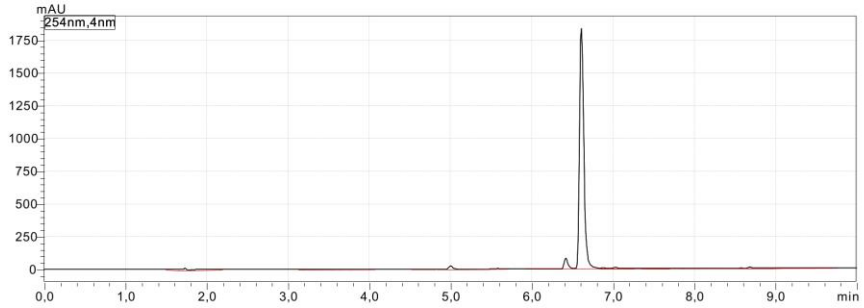


Figure S5. HPLC chromatogram of thiocarboxylic C_1 (**3a**) HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

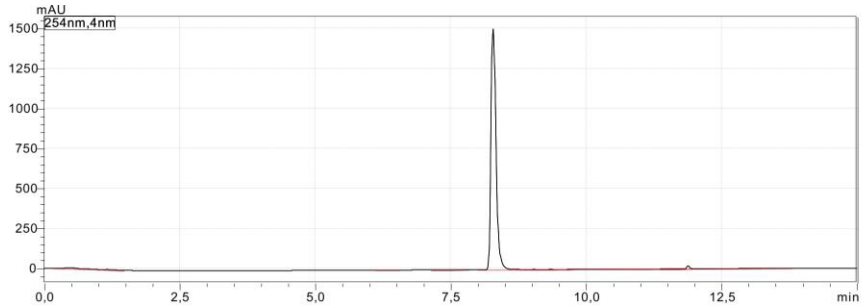


Figure S6. HPLC chromatogram of thiocarboxylic C_2 (**3b**). HPLC: Shimadzu Nexera XR, Autosampler SIL-20A, diode array detector SPD-M20A, C18-column (150 × 4 mm). Method: 70% → 97% MeCN in H_2O + 0.1% HCOOH, flow: 1.0 mL/min.

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EIDESSTATTLICHE VERSICHERUNG UND ERKLÄRUNG DES VERFASSERS

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