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BAYREUTH

*Entwicklung flexibler Synthesestrategien  
für bioaktive Sphingolipide*

DISSERTATION

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**Abkürzungsverzeichnis**

Ac	Acetyl
ATPase	Adenosintriphosphatase
Bn	Benzyl
Boc	<i>tert</i> -Butyloxycarbonyl
Bu	Butyl
bzw.	beziehungsweise
C1PP	Ceramid-1-Phosphat-Phosphatase
CD	Circulardichroismus
CD95	<i>cluster of differentiation 95</i>
CDase	Ceramidase
Cer	Ceramid
CerS	Ceramidsynthase
CERT	<i>ceramide transfer protein</i>
CGT	Ceramid-Galactosyltransferase
CK	Ceramidkinase
CoA	Coenzym A
CuTc	Kupfer-(I)-Thiophencarboxylat
dba	Dibenzalacetone
DBU	Diazabicycloundecen
de	Diastereomenüberschuss
DEAD	Azodicarbonsäurediethylester
DH-Des	Dihydroceramid-Desaturase
DIPEA	Diisopropylethylamin
DMAP	4-(Dimethylamino)-pyridin
(DHQD) <sub>2</sub> -PHAL	Hydrochinidin-1,4-phthalazindiyl-diether
DMF	Dimethylformamid
DMMP	Dimethylmethylphosphonat
DMSO	Dimethylsulfoxid
DPPA	Diphenylazidophosphat
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	Enantiomenüberschuss
Et	Ethyl
EGF	<i>epidermal growth factor</i>
ER	Endoplasmatisches Retikulum
FG	<i>functional group</i>
GalC	Galactosylceramidase
GCAs	Glucosylceramidase
GCS	Glucosylceramid-Synthase
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphat
HMDS	1,1,1,3,3,3-Hexamethyldisilazan
HMPT	Hexamethylphosphorsäuretriimid

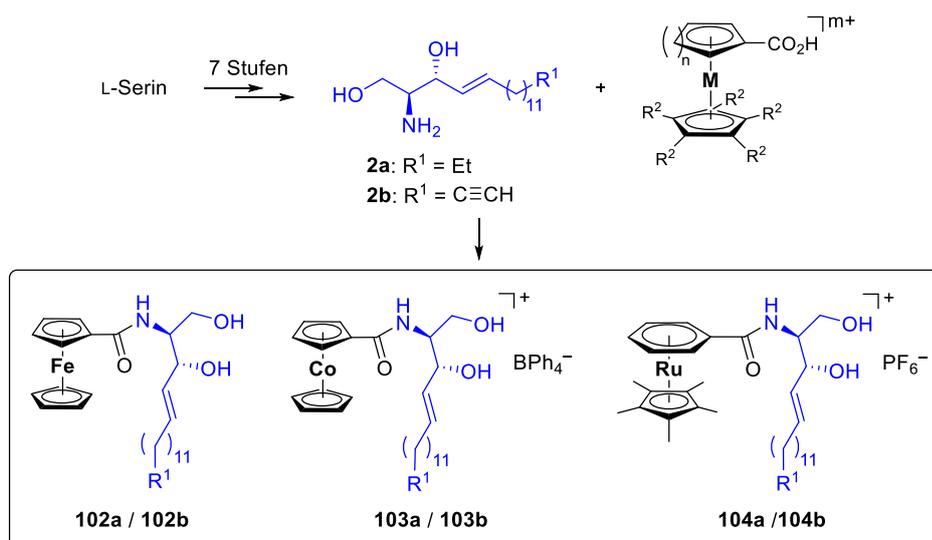
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HPLC	<i>high performance liquid chromatography</i>
HSN1	<i>hereditary sensory neuropathy type 1</i>
HWE	Horner-Wadsworth-Emmons
IL-1	Interleukin-1
KHMDS	Kaliumhexamethyldisilazid
KSR	Ketosphinganin-Reduktase
Me	Methyl
Ms	Methansulfonyl
NOE	<i>N</i> -Oleylethanolamin
NGF	<i>nerve growth factor</i>
NMR	<i>nuclear magnetic resonance</i>
NKT-Zellen	natürliche <i>killer</i> T-Zellen
PDGF	<i>platelet-derived growth factor</i>
Ph	Phenyl
PPTS	Pyridinium <i>para</i> -Toluolsulfonat
Pr	Propyl
pTsOH	<i>para</i> -Toluolsulfonsäure
rac.	racemisch
rf	unter Rückfluss
ROS	<i>reactive oxygen species</i>
rt	Raumtemperatur
S1P	Sphingosin-1-Phosphat
S1PL	Sphingosin-1-Phosphaat-Lyase
S1PP	Sphingosin-1-Phosphat-Phosphatase
SAR	<i>structure activity relationship</i>
SK	Sphingosinkinase
SMase	Sphingomyelinase
SMS	Sphingomyelinsynthase
SPT	Serin-Palmitoyltransferase
TBAB	Tetrabutylammoniumbromid
TBS	<i>tert</i> -Butyldimethylsilyl
Tf	Trifluoromethansulfonyl
TFA	Trifluoressigsäure
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TNF	Tumornekrosefaktor
Tr	Trityl
TRAF2	<i>TNF receptor associated factor 2</i>
TRAIL	<i>TNF-related apoptosis-inducing ligand</i>
VEGF	<i>vascular endothelial growth factor</i>
vgl.	vergleiche

## Zusammenfassung

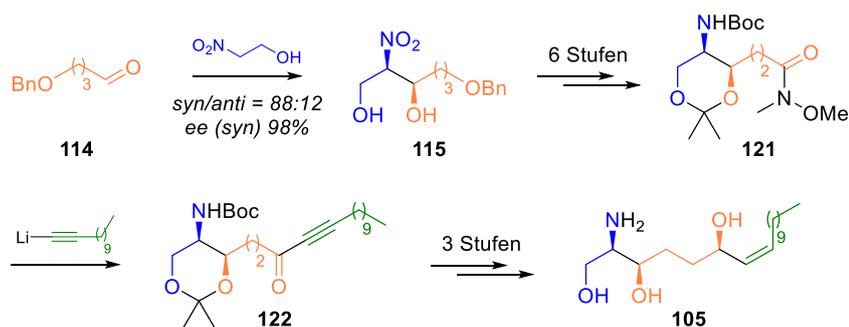
Sphingolipide sind aufgrund ihrer strukturellen Vielfalt und ihrer biologischen Bedeutung beliebte synthetische Ziele. Sie kommen ubiquitär in Eukaryoten als Bestandteile zellulärer Membranen vor. Bestimmte Sphingolipid-Metabolite, insbesondere Ceramid und Sphingosin-1-Phosphat, sind als Signalmoleküle an grundlegenden zellbiologischen Prozessen wie der Proliferation, der Zellerhaltung und der Zellbeweglichkeit, aber auch der Apoptose beteiligt. Die Regulierung des metabolischen Gleichgewichts zwischen diesen Signal-Lipiden ist von entscheidender Bedeutung für die Zellhomöostase. Anomalien der Sphingolipid-Biosynthese werden mit der Entstehung verschiedener schwerwiegender Krankheiten in Verbindung gebracht, darunter Sphingolipidosen, Alzheimer, Diabetes und Krebs. Bestimmte bioaktive Sphingolipide, sowie Enzyme der Sphingolipid-Biosynthese, stellen folglich Leitstrukturen bzw. *targets* für die Entwicklung neuartiger Wirkstoffe und Therapien dar. Ziel dieser Dissertation war es, möglichst flexible Synthesestrategien zur Darstellung bioaktiver Sphingolipide zu entwickeln, um deren biologische Eigenschaften zu untersuchen. Die Zielverbindungen sind sowohl natürlich-vorkommende Sphingolipide als auch synthetische Derivate, welche antiproliferative Aktivität gegenüber Tumorzellen besitzen.

Im ersten Teilprojekt wurde eine Reihe metallorganischer Ceramid-Derivate (**102 - 104**, Schema 1) zur Verwendung als Substrat-analoge Inhibitoren des Enzyms saure Ceramidase synthetisiert und ihre antiproliferativen Eigenschaften gegenüber verschiedenen Krebszelllinien untersucht. Hierzu wurden zuerst die natürliche Sphingoidbase *D-erythro*-Sphingosin (**2a**) und das Alkin-markierte Sphingosin-Derivat **2b** innerhalb von 7 Stufen ausgehend von L-Serin dargestellt. Verknüpfung der Sphingoidbasen mit verschiedenen Metallkomplexen ergab eine Bibliothek metallorganischer Ceramid-Derivate (**102 - 104**). Alle Komplexe inhibierten rekombinant gewonnene saure Ceramidase, vor allem der kationische Ru-Komplex **104a**. Anhand der Alkin-markierten Derivate konnte die Akkumulation der Komplexe innerhalb der Lysosomen von Krebszellen gezeigt werden, wo sich das *target* saure Ceramidase hauptsächlich befindet. Alle Komplexe wiesen zytotoxische Aktivität im niedrigen mikromolaren Bereich gegenüber verschiedenen Krebszelllinien, sowie weitere antiproliferative Effekte wie die Aktivierung der Caspasen -3/-7 auf.



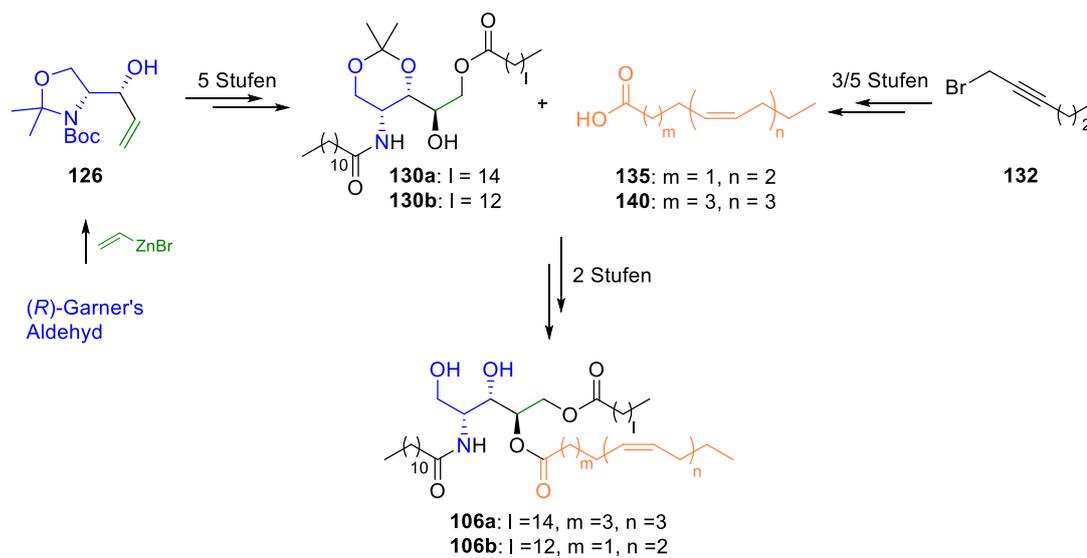
**Schema 1:** Synthese einer Bibliothek metallorganischer Ceramidase Inhibitoren (detaillierter in Kap. 2.2 und 4.2).

Das zweite Teilprojekt befasste sich mit der Totalsynthese der aus dem Meeresschwamm *Haliclona Reniera* isolierten Sphingoidbase Halisphingosin A (**105**), welche zytotoxische Aktivität gegenüber verschiedenen Tumorzelllinien im einstellig-mikromolaren Bereich aufweist (Schema 2). Die *threo*-konfigurierte Kopfgruppe wurde durch eine *syn*- und enantioselektive Henry-Reaktion zwischen Aldehyd **114** und 2-Nitroethanol aufgebaut. Der dabei entstandene Nitro-Alkohol **115** konnte anschließend über 6 Stufen zu dem Weinreb-Amid **121** umgesetzt werden. Kupplung von **121** mit Dodecynyl-Lithium ergab das Alkinon **122**. Der *Z*-allylische Alkohol wurde anschließend durch stereoselektive Transfer-Hydrierung der Keto-Gruppe und *Z*-selektive partielle Hydrierung des Alkins erhalten. Auf diese Art und Weise wurde (*2R,3R,6R,7Z*)-Halisphingosin A enantioselektiv über 11 Stufen in einer Gesamtausbeute von 29% dargestellt. Das synthetische Halisphingosin A wies zytotoxische Aktivität gegenüber verschiedenen Krebszelllinien im niedrigen mikromolaren Bereich auf, was der Aktivität des natürlichen Isolats entspricht.



**Schema 2:** Übersicht der Totalsynthese von Halisphingosin A (**105**, detaillierter in Kap 2.3 und 4.3).

Im dritten Teilprojekt wurde eine kurze und flexible Syntheseroute zur Darstellung der (2*R*,3*S*,4*R*)-Isomere der Bathymodiolamide A und B (**106a** und **106b**, Schema 3) entwickelt. Beide Naturstoffe weisen zytotoxische Aktivität gegenüber bestimmten Krebszelllinien im nanomolaren Bereich auf. Eine potenzielle Verwendung von **106a** und **106b** als Chemotherapeutika wurde kürzlich patentiert. Das Aminotetraol-Grundgerüst wurde durch *syn*-selektive Vinylierung von (*R*)-Garner's Aldehyd und späterer Dihydroxylierung des Alkens aufgebaut. Die mehrfach ungesättigten Carbonsäuren **135** und **140** können ausgehend von 1-Bromhex-2-in (**132**) durch Kupfer-vermittelte Halogen-Alkin-Kupplungen und *Z*-selektiver partieller Hydrierung der entsprechenden Alkine erhalten werden. Veresterung der sekundäre Alkohole **130a** und **130b** mit den ungesättigten Carbonsäuren **135** und **140** gefolgt von Acetonid-Entschützung ergab **106a** und **106b** in Gesamtausbeuten von 38 bzw. 39%. Abweichungen der chemischen Verschiebungen der <sup>1</sup>H und <sup>13</sup>C NMR-Spektren im Vergleich zu den Literaturdaten legen eine inkorrekte Zuordnung der absoluten Konfiguration der isolierten Naturstoffe nahe. Zudem zeigten die hier dargestellten Verbindungen nahezu keine zytotoxische Aktivität. Aufgrund ihrer flexiblen und modularen Natur, ermöglicht die hier beschriebene Syntheseroute jedoch auch den synthetischen Zugang zu den anderen Stereoisomeren der Bathymodiolamide A und B, sowie zu weiteren Derivaten.

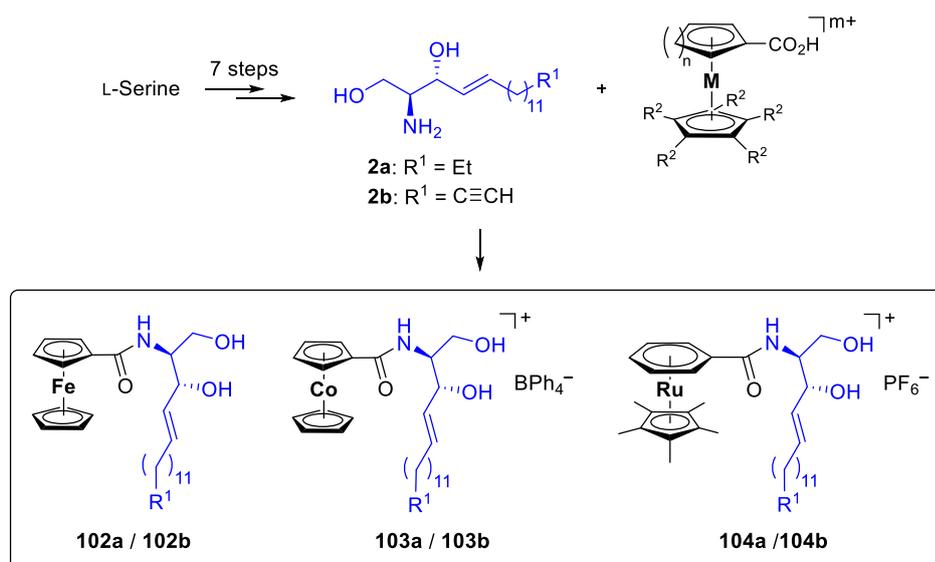


**Schema 3:** Übersicht der Synthese der (2R,3S,4R)-Isomere von Bathymodiolamid A und B (**106a** und **106b**, detaillierter in Kap. 2.4 und 4.4).

## Summary

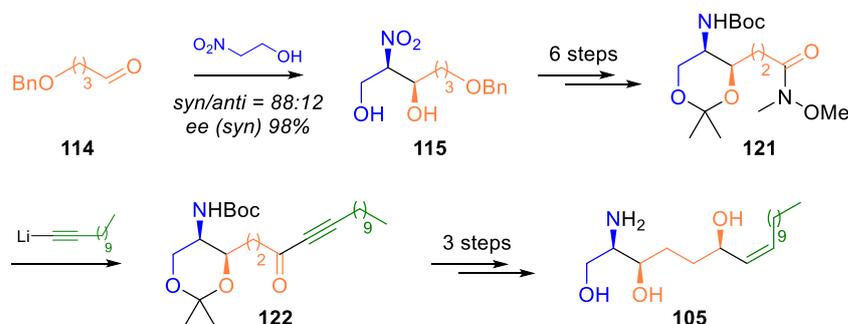
Due to their structural diversity and biological activities, Sphingolipids are interesting synthetic targets. They are essential constituents of plasma membranes in eukaryotic organisms. Besides their structural role as cell membrane components, some metabolites, in particular ceramide and sphingosine-1-phosphate, have been recognized as signaling molecules and are involved in the regulation of fundamental cellular processes like cell growth, cell survival, cell motility and apoptosis. Regulation of the metabolic balance between these signaling lipids is of fundamental importance for cell homeostasis. There is increasing evidence that alterations of this balance contribute to the onset and/or progression of diseases including sphingolipidoses, Alzheimer's disease, diabetes, and cancer. Thus, utilization of bioactive sphingolipids or targeting specific enzymes of sphingolipid metabolism are different approaches for the discovery of novel drugs or therapies. The focus of this thesis was the development of flexible synthetic routes towards bioactive Sphingolipids, in order to evaluate their biological activities. The target compounds include natural sphingolipids as well as synthetic derivatives, displaying cytotoxic activities against cancer cells.

In the first sub-project of this, thesis novel *N*-Metalloenoylsphingosines were synthesized as targeted ceramidase inhibitors. For this purpose, the natural sphingoid base *D*-erythro-sphingosine (**2a**) and an alkyne-tagged derivative (**2b**) were prepared over 7 steps starting from L-Serine (Scheme 1). Both sphingoid bases were then linked to different metalorganic carboxylic acids to give a library of metalorganic ceramide analogues (**102** - **104**). All of the prepared complexes inhibited recombinant acid ceramidase, most strongly the cationic Ru-complex **104a**. The complexes accumulated preferentially in the lysosomes of cancer cells like their target acid ceramidase. They displayed cytotoxicity with mostly single-digit micromolecular IC<sub>50</sub> values against different cancer cell lines as well as additional antiproliferative effects like caspase-3/-7 activation.



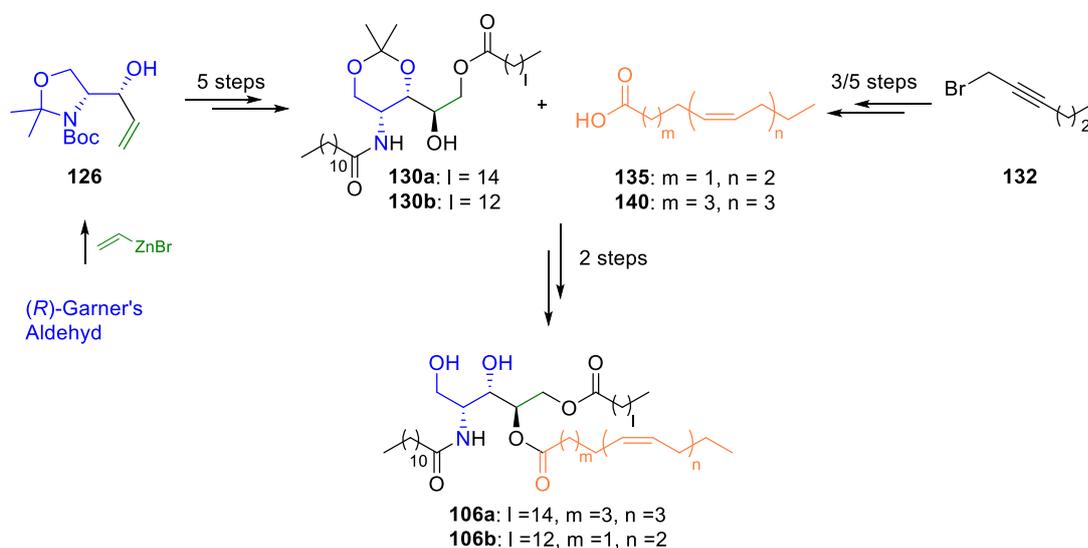
**Scheme 1:** Synthesis of metalorganic Ceramide-analogues **102** – **104** (for more details see chapters 2.1 and 4.2).

The second part of this thesis was the enantioselective total synthesis of the marine sponge metabolite halisphingosine A (**105**, Scheme 2), which showed moderate cytotoxic activity against different tumor cell lines. The *threo*-configured head group of **105** was established employing a *syn*- and enantioselective Henry-reaction of aldehyde **114** with 2-nitroethanol. The resulting nitro-diol **115** was converted into the Weinreb-amide **121** over six steps. Coupling of **121** with dodecynyl-Lithium yielded alkynone **122**. The *Z*-allylic alcohol moiety was established by stereoselective transfer hydrogenation of the keto group and *Z*-selective partial hydrogenation of the alkyne. Overall (2*R*,3*R*,6*R*,7*Z*)-halisphingosine A (**105**) was prepared in 29% yield over eleven steps. It showed antiproliferative activities against six different tumor cell lines at clinically relevant concentrations, comparable to the activity of the isolated natural product.



**Scheme 2:** Overview of the Synthesis of Halisphingosine A (**105**, for more details see chapters 2.2 and 4.3).

In the third part of this thesis, a straightforward and modular synthetic route for the proposed *2R,3S,4R*-isomers of bathymodiolamides A and B (**106a** and **106b**, Scheme 3) was developed. Both deep-sea mussel metabolites showed nanomolar cytotoxic activities in cancer cells and were recently patented for potential use in cancer chemotherapy. The aminotetraol moiety was established by *syn*-selective Vinylation of (*R*)-Garner's aldehyde and substrate-controlled dihydroxylation of the resulting alkene at a later stage. The required dienoic and trienoic acids **135** and **140** were prepared from 1-bromohex-2-yne by copper mediated coupling of corresponding bromides and alkynes followed by *Z*-selective partial hydrogenation of the resulting skipped polyynes. Esterification of the secondary alcohols **130a** and **130b** with carboxylic acids **135** and **140**, followed by acetone deprotection afforded **106a** and **106b** in 38 and 39% yield over eight steps. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra deviate distinctly from those reported, suggesting an incorrect stereochemical assignment of the natural isolates. Furthermore, **106a** and **106b** did not show any cytotoxic activity. However, the here reported synthetic route is facile and sufficiently flexible to allow the synthesis of the other stereoisomers of **106** as well as other derivatives.



**Scheme 3:** Overview of the synthesis of the *2R,3S,4R*-Isomers of Bathymodiolamide A and B (**106a** and **106b**, for more details see chapters 2.3 and 4.4).

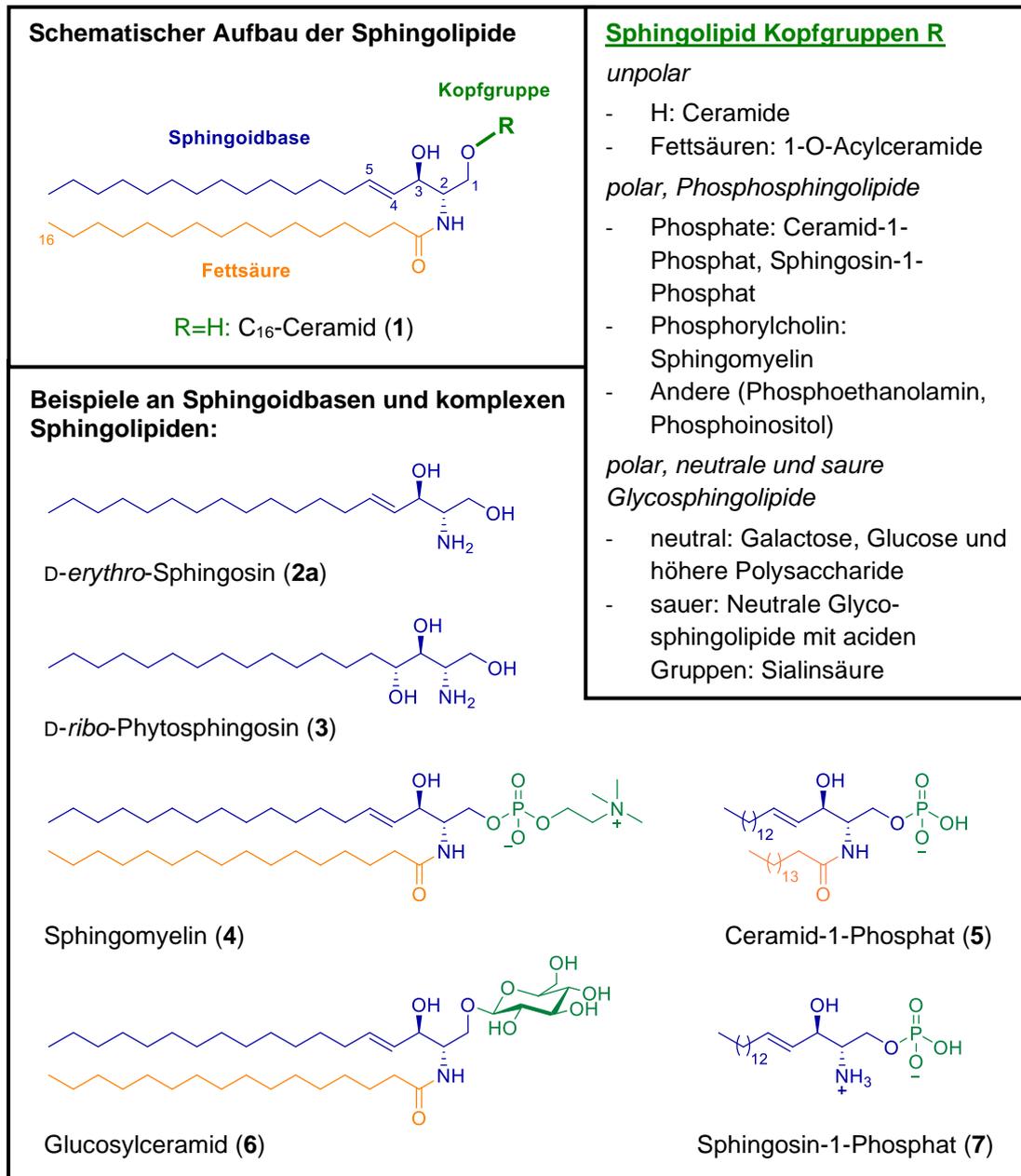
# 1 Kenntnisstand

## 1.1 Sphingolipide: Vorkommen, Struktur und Eigenschaften

### 1.1.1 Einleitung

Sphingolipide sind eine strukturell diverse Klasse der Lipide und kommen ubiquitär in eukaryotischen Organismen, sowie in einigen Bakterien und Viren vor.<sup>[1]</sup> Sie sind hauptsächlich innerhalb zellulärer Plasmamembranen und in Zellorganellen wie dem endoplasmatischen Retikulum, dem Golgi Apparat, den Lysosomen und den Mitochondrien vorhanden. Erste Vertreter dieser Stoffklasse wurden 1884 von Johann Thudichum aus menschlichen Gehirnzellen isoliert.<sup>[2]</sup> Entgegen Thudichums Erwartungen war das Hydrolyseprodukt dieser neuartigen Lipide nicht Glycerol und zeigte zudem basische Eigenschaften. In Anlehnung an die mythische Kreatur Sphinx nannte er diese Verbindung aufgrund ihrer damals rätselhaften Natur „Sphingosin“. Carter *et al.* bestimmten 1947 die Struktur von Sphingosin und führten den Begriff „Sphingolipide“ als Bezeichnung für Lipide, die sich von Sphingosin ableiten lassen, ein.<sup>[3]</sup> Seitdem wurden hunderte weitere einfache und komplexe Sphingolipide isoliert.

Sphingolipide zeichnen sich durch ihren modularen Aufbau aus. Sie besitzen als Grundgerüst eine sogenannte Sphingoidbase, was sie von anderen Lipiden wie den Glycerolipiden unterscheidet. In Säugetieren ist die am häufigsten vorkommende Sphingoidbase *D-erythro*-Sphingosin (**2a**, Abbildung 1). In Pflanzen, Pilzen, Protozoen und Hefen hingegen, werden vor allem *D-ribo*-Phytosphingosin (**3**) und entsprechende Stereoisomere von **3** gebildet.<sup>[4]</sup> Neben Sphingosin und Phytosphingosin gibt es eine Vielzahl weiterer Sphingoidbasen, die sich in Kettenlänge, Stereochemie, Anzahl an Doppelbindungen und Hydroxygruppen, sowie dem Verzweigungsgrad unterscheiden. Freie Sphingoidbasen sind normalerweise nur in sehr geringen Mengen vorhanden, da sie meist mit einer langkettigen Fettsäure verknüpft als Amide vorliegen, welche pauschal als Ceramide bezeichnet werden. Die Fettsäure-Reste sind für gewöhnlich gesättigt und besitzen eine Kettenlänge von 16 bis 26 Kohlenstoffatomen, wobei *N*-Palmitoyl-Sphingosin im Allgemeinen Ceramid (**1**), oder C<sub>16</sub>-Ceramid, genannt wird. Ceramide der Haut, der Epithelzellen und von Pflanzen besitzen oft  $\alpha$ -Hydroxy-Fettsäuren als Seitenkette.<sup>[5]</sup>



**Abbildung 1:** Grundstruktur der Sphingolipide und Beispiele an Sphingoidbasen und komplexen Sphingolipiden.

Durch Modifikation der primären Hydroxygruppe mit verschiedenen Kopfgruppen werden unterschiedliche Klassen an komplexen Sphingolipiden aufgebaut. Hierbei wird zwischen Phospho- und Glycosphingolipiden unterschieden. Wichtige Phosphosphingolipide in Säugetieren sind das Membranlipid Sphingomyelin (4) und Ceramid-1-Phosphat (5). In Pflanzen und Pilzen werden stattdessen hauptsächlich Phosphoinositole produziert.<sup>[6]</sup> Glycosphingolipide besitzen Mono- oder Oligosaccharide mit bis zu 20 Zuckerbausteinen als Kopfgruppe. Bisher wurden über 500 verschiedene Kohlenhydrat-Strukturen beschrieben.<sup>[7]</sup> Je nach Art der Zucker und anderer funktioneller Gruppen werden Glycosphingolipide in weitere Unterkategorien

eingeteilt. Die einfachsten Vertreter sind Glucosyl-Ceramid (**6**) und Galctosyl-Ceramid, aus denen höhere Glycosphingolipide wie Ganglioside oder Sulfatide aufgebaut werden. In geringen Mengen werden auch die entsprechenden „Lyso-Formen“ komplexer Sphingolipide wie Sphingosin-1-Phosphat (S1P, **7**) gebildet. Durch Modifikation der verschiedenen Einzelbausteine entsteht in der Natur eine immense Anzahl strukturell einzigartiger Sphingolipide, welche meist hochspezifische Funktionen besitzen.

Innerhalb der zweiten Hälfte des letzten Jahrhunderts konnte die Biosynthese der Sphingolipide in Säugetieren nahezu vollständig aufgeklärt werden. Heute sind alle beteiligten Enzyme und die zugrundeliegenden biochemischen Reaktionswege bekannt. Ursprünglich wurde angenommen, dass die grundlegende Funktion des Sphingolipid-Metabolismus die Bereitstellung von Lipid-Bausteinen für den Aufbau von Zellmembranen ist.<sup>[8]</sup> So sind Sphingomyelin und in geringerem Maße Glycosphingolipide die am häufigsten vorkommenden Sphingolipide und machen neben Glycerophospholipiden und Cholesterol einen Anteil von bis zu 30% der Lipide innerhalb zellulärer Membranen aus.<sup>[9]</sup> Weiterhin bildet Sphingomyelin zusammen mit Cholesterol und anderen Sphingolipiden Mikrodomänen innerhalb der Lipiddoppelschicht zellulärer Membranen, welche auch *lipid rafts* genannt werden.<sup>[10]</sup> Sphingolipide bestimmen so die biophysikalischen Eigenschaften von Membranen, und können mit Membranproteinen, der extrazellulären Matrix und anderen Zellen wechselwirken.

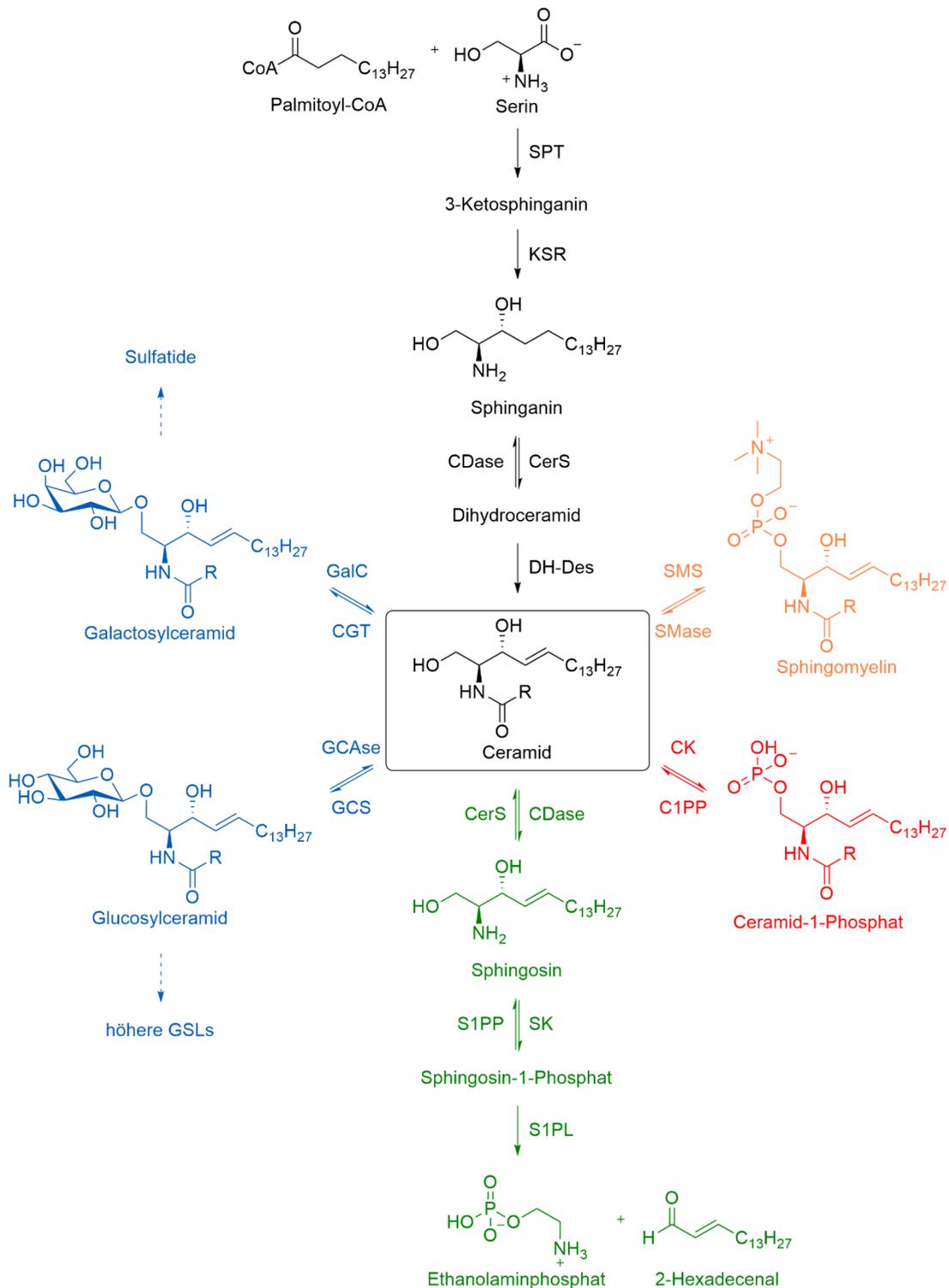
1986 zeigten Hannun *et al.*, dass Sphingosin die Aktivität von Proteinkinase C inhibiert, einem Enzym von zentraler Bedeutung für die zelluläre Signalübertragung.<sup>[11]</sup> Es wurde erkannt, dass bestimmte Sphingolipid-Metabolite als *second messenger* regulatorische Funktionen innerhalb diverser Signaltransduktionswege besitzen. Wenige Zeit später wurden auch molekulare *targets* von Ceramid<sup>[12]</sup>, Sphingosin-1-Phosphat<sup>[13]</sup> und Ceramid-1-Phosphat<sup>[14]</sup> identifiziert. Diese Sphingolipid-Metabolite werden als Reaktion auf spezifische Stimuli gebildet und regulieren entsprechende nachgeordnete *targets* und Effektoren. Sie sind an nahezu allen Prozessen der Zellbiologie beteiligt, darunter dem Zellwachstum und der Zellerhaltung, der Zellmotilität, der Zellseneszenz, dem Stillstand des Zellzyklus und verschiedenen Mechanismen des Zelltods (Apoptose, Anoikis, Autophagie, Nekrose).<sup>[15]</sup> Der Sphingolipid-Stoffwechsel ist daher entscheidend für die Zell-Homöostase, Anomalien können zur Entstehung

schwerwiegender Krankheiten führen. So sind Sphingolipidosen, eine Reihe genetisch vererbbarer lysosomaler Speicherkrankheiten, wobei Defekte oder Defizite Sphingolipid-abbauender Enzyme die toxische Akkumulation bestimmter Sphingolipid-Spezies verursachen.<sup>[16]</sup> Weiterhin trägt die Dysregulation von Enzymen des Sphingolipid-Metabolismus zur Pathogenese vieler humaner Krankheiten bei, dazu zählen Krebs,<sup>[17]</sup> Diabetes,<sup>[18]</sup> Alzheimer,<sup>[19]</sup> und Herz-Kreislauf-Erkrankungen.<sup>[20]</sup>

Es ist somit kaum verwunderlich, dass Sphingolipide attraktive synthetische Ziele sind. Zum einen weisen natürliche Sphingolipide ein breites Spektrum an biologischer Aktivität auf und viele dieser Verbindungen bzw. Derivate stellen potenzielle Leitstrukturen dar. Zum anderen sind bestimmte Enzyme des Sphingolipid-Stoffwechsels aufgrund ihrer Rolle bei der Entstehung diverser Erkrankungen vielversprechende *targets* für die Entwicklung neuer Wirkstoffe und Therapien. Synthetische Sphingolipid-Analoga sind zudem nützliche Werkzeuge zur Aufklärung der biologischen Funktionen und der Stoffwechselwege bestimmter Sphingolipide. Es besteht daher nach wie vor ein großes Interesse an der Entwicklung einfacher und flexibler Synthesestrategien, die den Zugang zu neuartigen Sphingolipiden gewährleisten und deren Modifizierung bzw. Derivatisierung ermöglichen.

### 1.1.2 Die Sphingolipid-Biosynthese

Eine Übersicht der Sphingolipid-Biosynthese in Säugetieren ist in Schema 4 dargestellt. Die *de novo* Biosynthese beginnt im endoplasmatischen Retikulum (ER) mit der Kondensation von Serin und Palmitoyl-CoA zu 3-Ketosphinganin durch das Enzym Serin-Palmitoyltransferase (SPT).<sup>[21]</sup> Dies ist zugleich der geschwindigkeitsbestimmende Schritt der Sphingolipid-Biosynthese. Palmitoyl-CoA wird dabei als Substrat bevorzugt, weshalb Sphingoidbasen in der Regel eine Kettenlänge von achtzehn Kohlenstoffen besitzen. Mutationen von SPT sind die Ursache der autosomal-dominanten Erbkrankheit HSN1 (*hereditary sensory neuropathy type 1*). Durch die veränderte Substratspezifität des Enzyms, werden Alanin und Glycin anstelle von Serin bei der *de novo* Biosynthese verwendet, was zu der Akkumulation toxischer 1-Desoxysphingolipide führt.<sup>[22]</sup> Das von SPT gebildete 3-Ketosphinganin wird im nächsten Schritt stereoselektiv zu Sphinganin reduziert. Ceramidsynthasen katalysieren die *N*-Acylierung von Sphinganin zu Dihydroceramid, welches anschließend *trans*-selektiv zur Bildung der Ceramide desaturiert wird.



**Schema 4:** Übersicht der Sphingolipid-Biosynthese; *de novo* Biosynthese (schwarz), Sphingomyelin Kreislauf (orange), Glycosphingolipid-Synthese (blau), Phosphorylierung von Ceramid (rot) und metabolischer Abbau über S1P (grün).

Ceramide sind die Vorstufe aller höheren Sphingolipide und stellen einen zentralen Knotenpunkt des Sphingolipid Metabolismus dar. Aufgrund ihrer hydrophoben Natur werden Ceramide nach ihrer Synthese im ER entweder vesikulär, oder durch das *ceramide transfer protein* (CERT) in den Golgi-Apparat transportiert, wo sie zu den

komplexen Sphingolipiden weiter funktionalisiert werden.<sup>[23]</sup> Das Enzym Sphingomyelinsynthase (SMS) überträgt die Phosphocholin-Kopfgruppe auf die primäre Hydroxygruppe von Ceramid zur Bildung von Sphingomyelin. Die Glykosylierung von Ceramid wird von Glucosyl- und Galactosyl-Transferasen katalysiert. Aus Glucosyl- und Galactosyl-Ceramid werden durch sukzessive Addition weiterer Zucker und anderer Bausteine höhere Glycosphingolipide aufgebaut. Nach ihrer Biosynthese gelangen Sphingomyelin und die verschiedenen Glycosphingolipide durch vesikulären Transport zur Plasmamembran.<sup>[24]</sup> Weiterhin kann Ceramid von Ceramidkinase zu Ceramid-1-Phosphat phosphoryliert werden.<sup>[25]</sup>

Neben der *de novo* Biosynthese innerhalb des ER, kann Ceramid durch die Hydrolyse von Sphingomyelin innerhalb der Plasmamembran,<sup>[26]</sup> sowie über den Abbau komplexer Sphingolipide in den Endosomen und Lysosomen gebildet werden.<sup>[24]</sup> Dort gebildetes Ceramid wird wiederum von saurer Ceramidase zu Sphingosin hydrolysiert, welches im Gegensatz zu Ceramid die Lysosomen verlassen kann. Sphingosin kann anschließend durch Re-Acylierung zur Synthese von Ceramiden und komplexen Sphingolipiden wiederverwendet werden. Derartiges Recycling von Sphingoidbasen wird auch als „*salvage pathway*“ bezeichnet und macht zwischen 50% und 90% der Sphingolipid-Biosynthese aus.<sup>[27]</sup> Alternativ kann Sphingosin von Sphingosinkinasen zu S1P phosphoryliert werden. Durch S1P-Lyase wird S1P zu Ethanolaminphosphat und 2-Hexadecenal irreversibel abgebaut, was den einzigen Austrittspunkt aus dem Sphingolipid Metabolismus darstellt.<sup>[21]</sup>

### 1.1.3 Natürlich vorkommende Sphingolipide

Sphingolipide zeichnen sich vor allem durch ihre bemerkenswerte strukturelle Diversität und durch ihr breites Spektrum an biologischer Aktivität aus. Insbesondere Pilze und Meeresschwämme produzieren Sphingolipid-Derivate mit interessanten biologischen Eigenschaften, darunter Lipide mit ungewöhnlichen „*backbones*“, wie 1-Desoxy- und  $\alpha,\omega$ -bifunktionelle Sphingoidbasen, von Sphingoidbasen abgeleitete Heterozyklen, sowie eine Vielfalt an einzigartigen Glycosphingolipiden. Eine kleine Auswahl dieser Verbindungen ist in Abbildung 2 dargestellt.

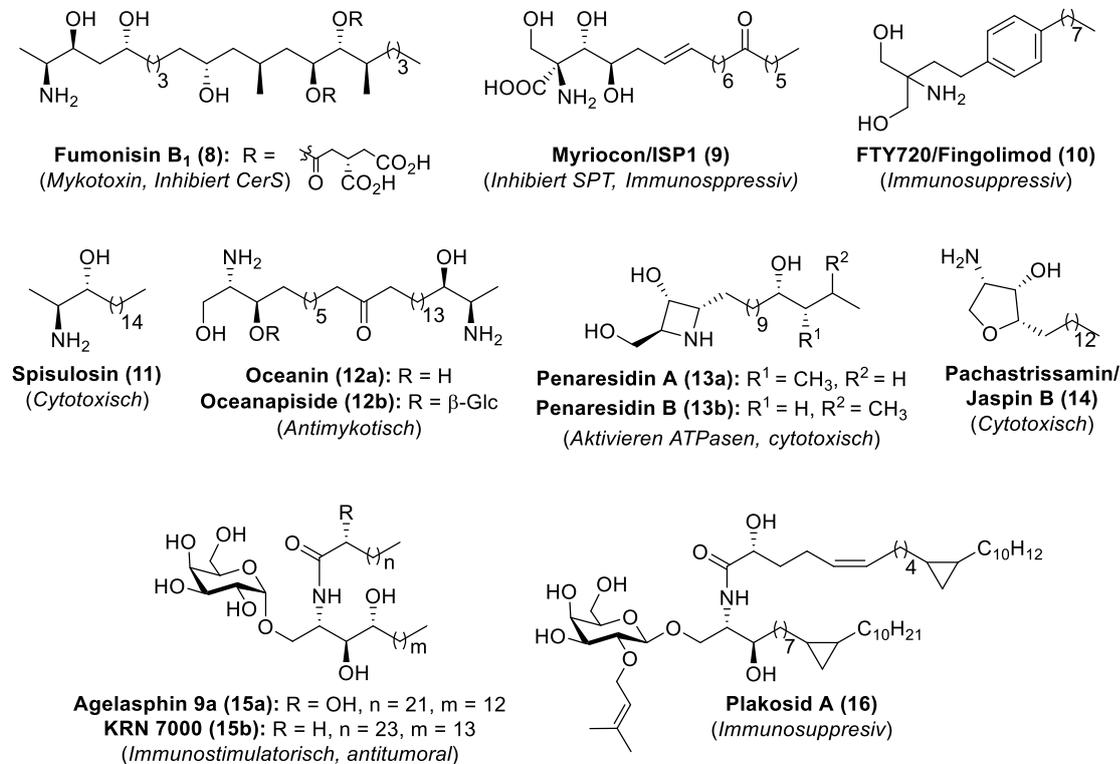
Bestimmte sphingolipidartige Naturstoffe sind vor allem aufgrund ihrer Toxizität bekannt. So sind Fumonisine wie Fumonisin B<sub>1</sub> (**8**) Mykotoxine, die von Pilzen wie *Fusarium* spp. und *Aspergillus* spp. produziert werden.<sup>[28]</sup> Sie sind für das Verderben von Getreide und daraus hergestellten Lebensmittel verantwortlich und stehen unter

dem Verdacht, kanzerogen zu sein. Ihre Toxizität beruht unter anderem auf ihrer Eigenschaft das Enzym Ceramid-Synthase (CerS) zu inhibieren.<sup>[29]</sup> Als CerS-Inhibitor wurde **8** in zahlreichen Studien zur Aufklärung des Sphingolipid-Metabolismus und der Funktion von Ceramiden und komplexen Sphingolipiden verwendet.<sup>[30]</sup> Ein weiterer Pilz-Metabolit, welcher ebenfalls als Werkzeug zur Aufklärung der Sphingolipid-Biosynthese verwendet wurde, ist Myriocin oder ISP1 (**9**). **9** ist ein spezifischer Inhibitor des Enzyms SPT und besitzt zudem immunosuppressive Eigenschaften.<sup>[31]</sup> SAR-Studien führten zu der Entwicklung des Wirkstoffes FTY720 oder Fingolimod (**10**), welcher zur Behandlung von multipler Sklerose eingesetzt wird.<sup>[32]</sup>

Die simple 1-Desoxy-Sphingoidbase Spisulosin (**11**), auch als E-285 bezeichnet, ist ein Metabolit der Muschel *Spisula polynyma* und weist zytotoxische Aktivitäten gegenüber verschiedenen Krebszelllinien mit IC<sub>50</sub>-Werten im niedrig-mikromolaren bis in den nanomolaren Bereich auf.<sup>[33]</sup> Oceanin (**12a**) hingegen, das Aglycon von Oceanapiside (**12b**), ist ein Beispiel für eine  $\alpha,\omega$ -bifunktionelle Sphingoidbase. **12b** wurde aus dem Meeresschwamm *Oceanapia phillipensis* isoliert.<sup>[34]</sup> Sowohl **12a**, als auch **12b** besitzen antimykotische Aktivität gegenüber der pathogenen und Fluconazol-resistenten Hefe *Candida Galbarata*. Die von Sphingosin abgeleiteten Azetidine Penaresidin A und B (**13a**, **13b**) wurden aus der Schwamm-Gattung *Penares* isoliert; beide Verbindungen aktivieren Actomyosin-ATPasen.<sup>[35]</sup> Eine Reihe synthetischer Analoga wies sowohl zytotoxische Aktivität gegenüber verschiedenen Krebszelllinien, als auch antibakterielle Aktivität gegenüber gram-positiven Bakterien auf.<sup>[36]</sup> Das Anhydrophytosphingosin Pachastrissamin bzw. Jaspin B (**14**), ein Metabolit der Schwämmen *Pachastrissa sp.*<sup>[37]</sup> und *Jaspis sp.*<sup>[38]</sup>, besitzt ebenfalls zytotoxische Eigenschaften im klinisch relevanten Bereich. **14** ist ein beliebtes synthetisches Ziel und es existieren mehrere Totalsynthesen, die den Zugang zu **14** und dessen Stereoisomeren ermöglichen.<sup>[39]</sup>

Die Algelasphine sind eine Familie von  $\alpha$ -Galactosyl-Ceramiden, welche 1993 von Natori *et al.* aus dem Meeresschwamm *Agelas mauritianus* isoliert wurden, darunter auch Algelasphin 9a (**15a**).<sup>[40]</sup> Sie hemmen das Wachstum von Tumorzellen, obwohl sie nur im geringen Maße zytotoxisch sind, indem sie das Immunsystem durch Aktivierung von NKT-Zellen (*natural killer T cells*) stimulieren.<sup>[41]</sup> Das synthetische Derivat KRN7000 (**15b**) ist ein vielversprechender Wirkstoff für die Krebstherapie und befindet sich in verschiedenen klinischen Studien der Phasen I und II.<sup>[42]</sup> Im

Gegensatz zu den immunostimulierenden Algelasphinen, weist das aus dem Schwamm *Plakortis simplex* isolierte  $\beta$ -Galactosyl-Ceramid Plakosid A (16) immunosuppressive Eigenschaften auf.<sup>[43]</sup>



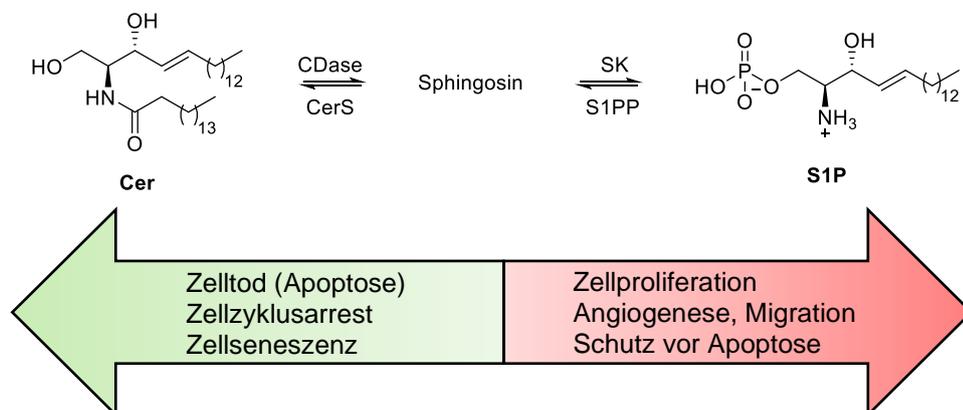
**Abbildung 2:** Beispiele an natürlich vorkommenden, biologisch aktiven Sphingolipiden und davon abgeleiteten synthetischen Derivaten.

### 1.1.4 Das Sphingolipid Rheostat

Bestimmte Sphingolipide, vor allem Ceramid (Cer) und Sphingosin-1-Phosphat (S1P), stellen aufgrund ihrer essentiellen biologischen Funktionen als Signalmoleküle die wohl am besten untersuchten Sphingolipide dar.<sup>[15]</sup> Im Allgemeinen wird Cer als Reaktion auf zellulären Stress gebildet und spielt eine entscheidende Rolle bei Signalkaskaden zur Induktion von Zelltodmechanismen, vor allem der Apoptose.<sup>[44]</sup> Eine große Zahl an Stimuli, darunter Hitze, und ionisierende Strahlung, oxidativer Stress, Hypoxie, bestimmte Cytokine (TNF, Fas-Liganden) und Chemotherapeutika (z. B. Daunorubicin, Camptothecin), regen die Bildung von endogenem Cer über die *de novo* Synthese oder die Hydrolyse von Sphingomyelin an.<sup>[45]</sup> Zu den molekularen *targets* von Cer zählen die Phosphoprotein Phosphatasen 1 und 2A, Proteinkinase C- $\zeta$ , und Cathepsin D.<sup>[46]</sup> Weiterhin führen erhöhte Mengen an Cer innerhalb von Zellmembranen zur Bildung Ceramid-reicher Makrodomänen.<sup>[47]</sup> Diese dienen als

„Signal-Plattformen“, da in ihnen (Todes)-Rezeptoren (z.B. TNF-Rezeptoren, TRAIL-Rezeptoren, CD95) und Signalmoleküle aggregieren, wodurch deren Funktion der Signalübertragung verstärkt wird.<sup>[48]</sup> Zudem ist Cer in der Lage, Kanäle in der Mitochondrienmembran zu bilden, was Apoptose durch Freisetzung pro-apoptotischer Proteine auslösen kann.<sup>[49]</sup>

Durch Hydrolyse von Cer und nachfolgende Phosphorylierung des dabei entstehenden Sphingosins wird S1P gebildet. Viele Verbindungen können das Enzym Sphingosin-Kinase I aktivieren, oder die Expression des entsprechenden Gens chronisch erhöhen. Dazu zählen verschiedene Wachstumsfaktoren (EGF, PDGF, VEGF, NGF), Cytokine (TNF- $\alpha$ , IL-1) und bestimmte Hormone. S1P wird größtenteils aus der Zelle sezerniert und bindet an bestimmte G-Protein-gekoppelte Rezeptoren (S1P-Rezeptoren 1-5).<sup>[50]</sup> Dadurch werden Signale vermittelt, welche vor allem erhöhte Proliferation und Zellmotilität zur Folge haben. Intrazellulär kann S1P mit bestimmten nachgeordneten *targets* wechselwirken, beispielsweise TRAF2, dessen antiapoptotische Wirkung S1P verstärkt,<sup>[51]</sup> oder Histondeacetylasen 1 und 2, deren Aktivität S1P inhibiert.<sup>[52]</sup> Über intrazelluläre oder Rezeptor-vermittelte Mechanismen spielt S1P eine wichtige Rolle bei der Regulation von Zellwachstum und Zellüberleben,<sup>[53]</sup> der Angiogenese und Zellmigration,<sup>[54]</sup> dem Egress von Lymphozyten und Entzündungs-Reaktionen.<sup>[55]</sup> Cer und S1P besitzen somit gegensätzliche Eigenschaften, auf der einen Seite vermittelt Cer anti-proliferative und pro-apoptotische Signale, auf der anderen Seite fördert S1P das Zellwachstum, erhöhte Zellmotilität und Resistenzen gegenüber Zelltodmechanismen. Das metabolische Gleichgewicht zwischen beiden Lipiden ist von entscheidender Bedeutung für die Zell-Homöostase und kann in einem vereinfachten Modell als „Sphingolipid-Rheostat“ beschrieben werden (Abbildung 3).<sup>[56]</sup>



**Abbildung 3:** Vereinfachtes Modell des Sphingolipid-Rheostats.

## 1.2 Sphingolipide in der Krebstherapie

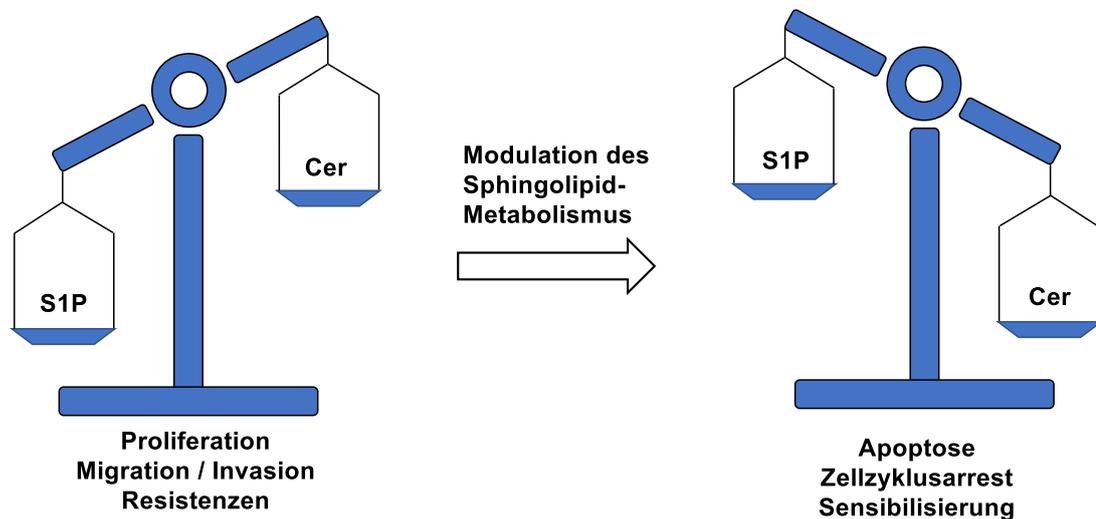
### 1.2.1 Der Ceramid-Stoffwechsel in Tumorzellen

Innerhalb der letzten Jahrzehnte häuften sich die Erkenntnisse, dass Anomalien des Sphingolipid-Stoffwechsels mit der Entstehung und Progression von Krebserkrankungen in Zusammenhang stehen.<sup>[57]</sup> Im Allgemeinen wird Cer auch als „*tumor-suppressor lipid*“ bezeichnet und sowohl endogene, als auch exogene Ceramide, tragen aufgrund ihrer Rolle bei der Induktion von Zelltodmechanismen dazu bei, die Tumor-Progression zu verlangsamen, oder zu unterdrücken.<sup>[58]</sup> Bereits 1993 zeigten Hannun *et al.*, dass durch Behandlung mit exogenen Ceramiden Apoptose in HL60 Zellen induziert werden kann.<sup>[59]</sup> Krebszellen weisen jedoch typischerweise erhebliche Veränderungen in der Expression und Aktivität der Enzyme des Sphingolipid-Stoffwechsels auf. In vielen Tumorarten sind Ceramid-abbauende Enzyme wie saure Ceramidase (aCDase), Ceramidkinase (CK), Glucosylceramid-Synthase (GCS) und Sphingomyelin-Synthase (SMS) überexprimiert.<sup>[60]</sup> Durch die gesteigerte Verstoffwechslung von Cer werden zelluläre Ceramid-Konzentrationen reduziert und die Akkumulation von Ceramiden verhindert, was zu der Resistenz von Krebszellen gegenüber Ceramid-vermittelten Zelltodmechanismen führt (vgl. Abbildung 4). Auf der anderen Seite wurde eine erhöhte Expression von Sphingosin-Kinase I in diversen Tumorarten beobachtet und die gesteigerte Bildung von mitogenen S1P korreliert mit abnormalem Zellwachstum, der Tumor-Angiogenese, der Migration und Adhäsion von Tumorzellen, sowie der Resistenz gegenüber Apoptose.<sup>[61]</sup> Weiterhin werden erhöhte zelluläre Konzentrationen an S1P und insbesondere die Akkumulation von Glycosphingolipiden mit der Entstehung von (multiplen) Wirkstoff-Resistenzen in Verbindung gebracht. So ist die erhöhte Expression bzw. Aktivität von GCS eine häufig vorkommende Eigenschaft resistenter Krebszelllinien.<sup>[62]</sup>

Sowohl die Behandlung mit chemotherapeutischen Wirkstoffen als auch mit ionisierender Strahlung beeinflusst den Cer-Stoffwechsel von Krebszellen. So wurden erhöhte Cer-Konzentrationen bei der Behandlung von Krebszellen mit verschiedenen klinisch-relevanten Chemotherapeutika, darunter Anthracycline, Doxorubicin, Daunorubicin, Etoposide, Irinotecan, Vincristin und Paclitaxel nachgewiesen.<sup>[63]</sup> Viele dieser Verbindungen verursachen zellulären Stress, was zu der Bildung endogener Ceramide führt und es wird angenommen, dass die Induktion von Apoptose durch

Ceramid zu der Wirkung dieser Verbindungen beiträgt. Auf der anderen Seite verringert die Inhibition von Enzymen der *de novo* Synthese von Cer die Wirkung bestimmter Chemotherapeutika, wie zum Beispiel Daunorubicin.<sup>[64]</sup> Auch die Behandlung von Krebszellen mit ionisierender Strahlung führt in vielen Tumor-Modellen zur Akkumulation endogener Ceramide, in diesem Fall vor allem durch die Hydrolyse von Sphingomyelin innerhalb der Plasmamembran.<sup>[65]</sup>

Die gezielte Modulation des Sphingolipid-Stoffwechsels bzw. der zellulären Konzentrationen bestimmter Sphingolipide, stellt eine interessante Strategie zur Entwicklung neuartiger Therapien oder zur Sensibilisierung resistenter Krebszelllinien gegenüber herkömmlichen Chemotherapeutika dar (vgl. Abbildung 4). Die anti-proliferativen Eigenschaften von Cer legen eine Erhöhung der zellulären Cer-Konzentration in Tumorzellen nahe. Auf der anderen Seite birgt die Hemmung der Biosynthese bzw. der Aktivität von S1P therapeutischen Nutzen. Zu den verschiedenen Herangehensweisen, die innerhalb der letzten Jahrzehnte untersucht wurden, zählen die Entwicklung von (1) synthetischen Cer-Mimetika, (2) niedermolekularen Inhibitoren Cer-abbauender Enzyme (CDase, GCS, SMS, CK), (3) SK-Inhibitoren, (4) S1P-Rezeptor-Antagonisten und (5) CERT-Inhibitoren.



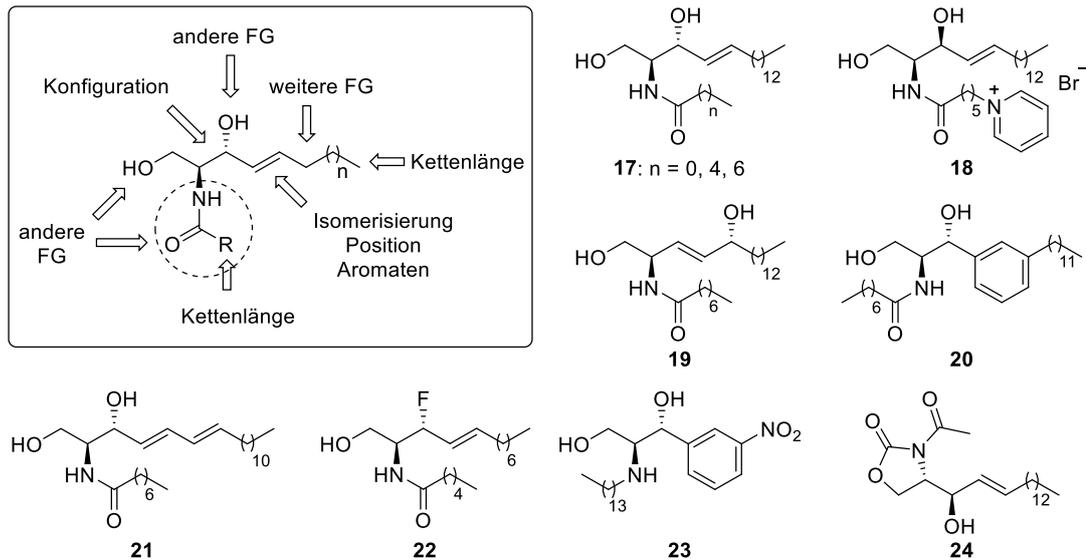
**Abbildung 4:** Schematische Darstellung des Sphingolipid-Rheostats in Tumorzellen und therapeutischer Ansatz zur Modulation des Sphingolipid-Metabolismus.

### 1.2.2 Synthetische Ceramid-Mimetika

Einer der ersten Ansätze, um neuartige Therapien basierend auf der Wirkung bioaktiver Sphingolipide zu entwickeln, war es, durch Verwendung exogener Cer-Analoga Apoptose in Krebszellen auszulösen. Da natürliche Ceramide in wässrigen Medien nahezu unlöslich sind, wurden anfangs die langkettigen *N*-Acyl-Reste durch kürzere substituiert. Kurzkettigen C<sub>2</sub>-, C<sub>6</sub>- und C<sub>8</sub>-Ceramide (**17**, Abbildung 5) wurden ursprünglich zur Aufklärung der biologischen Funktionen von Cer verwendet.<sup>[59, 66]</sup>

Derartige „Zell-permeable“ Ceramide zeigten innerhalb verschiedener *in vitro* Studien ähnliche Aktivitäten wie natürliche vorkommende Ceramide und sind in der Lage Apoptose und Zellzyklusarrest in Krebszellen auszulösen.<sup>[67-70]</sup> Entscheidende Strukturelemente für die biologische Aktivität sind die Doppelbindung zwischen C<sub>4</sub> und C<sub>5</sub>, eine ausreichende Kettenlänge der Sphingoidbase mit einem Optimum bei 18-C-Atomen, sowie ein hydrophobes Strukturelement in der Nähe der Kopfgruppe.

Auf der Suche nach neuen antiproliferativen Wirkstoffen wurde eine Vielzahl an Cer-Derivaten synthetisiert, eine Übersicht der häufigsten strukturellen Modifikationen ist in Abbildung 5 dargestellt. Um die schlechte Wasserlöslichkeit der lipophilen Ceramide zu erhöhen, wurde eine Reihe kationischer Pyridinium-Ceramide wie Verbindung **18** dargestellt und ihre Aktivität gegenüber verschiedenen Tumorarten evaluiert. Im Allgemeinen weisen Pyridinium-Ceramide höhere Zytotoxizitäten und eine höhere zelluläre Aufnahme auf als die entsprechenden ungeladenen Derivate. Aufgrund ihrer positiven Ladung und Lipophilie akkumulieren sie in den Mitochondrien, was zur Verringerung des mitochondrialen Membranpotentials, Freisetzung von mitochondrialem Cytochrom C, Aktivierung von Caspasen 3 und 9 und apoptotischem Zelltod führt.<sup>[71-74]</sup> Weitere strukturelle Modifikationen der Cer-Grundstruktur sind die Veränderung der Position der Doppelbindung und deren Substitution durch Aromaten (**19**, **20**),<sup>[75]</sup> die Einführung und Substitution weiterer funktioneller Gruppen (**21**<sup>[70]</sup>, **22**<sup>[76]</sup>), sowie die Synthese neuartiger Struktur motive, darunter „Ceramine“ wie Verbindung **23**,<sup>[77]</sup> oder heterozyklische Cer-Derivate wie das Carbamat **24**.<sup>[78]</sup> Die verschiedenen Derivate **19-24** zeigen zytotoxische Aktivitäten gegenüber Tumorzellen und weisen zum Teil bessere pharmakologische Eigenschaften als die entsprechenden Vorgänger-Substanzen auf.



**Abbildung 5:** Modifikationsmöglichkeiten der Cer-Grundstruktur und Beispiele zytotoxischer Cer-Derivate.

Um die zelluläre Aufnahme exogener Cer-Analoga zu verbessern, wurden verschiedene *drug delivery* Systeme auf der Basis von Nanopartikeln entwickelt. Durch Verkapselung von C<sub>6</sub>-Ceramid innerhalb künstlicher Nanoliposomen konnte die wachstumshemmende Wirkung gegenüber humanen Brustkrebs-Zellen im Vergleich zu freiem C<sub>6</sub>-Ceramid erheblich gesteigert werden.<sup>[79]</sup> Die sogenannten *Ceramid nanoliposomes* (CNLs) erwiesen sich in verschiedenen *in vivo* Studien als wirksam und befinden sich momentan in klinischen Studien der Phase I.<sup>[80]</sup>

Neben ihrer geringen Wasserlöslichkeit und Zellpermeabilität besteht jedoch auch das Problem, dass Ceramid-Analoga mit verschiedenen zellulären und biochemischen *targets* innerhalb der Sphingolipid-Biosynthese wechselwirken können. Zur Entwicklung neuer Antikrebsmittel, die den Sphingolipid-Stoffwechsel beeinflussen, wird daher hauptsächlich in Richtung spezifischer Enzym-Inhibitoren geforscht.

### 1.2.3 Ceramidase Inhibitoren

CDasen sind eine Familie bestehend aus fünf unterschiedlichen Enzymen, die die Hydrolyse von Cer zu Sphingosin katalysieren. Sie werden je nach pH-Optimum ihrer Aktivität in saure Ceramidase (aCDase), neutrale Ceramidase und drei basische Ceramidasen eingeteilt. Sie unterscheiden sich zudem in ihrer Lokalisation und Substratspezifität. So kommt aCDase hauptsächlich in den Lysosomen vor<sup>[81]</sup> und zeigt höhere Spezifität gegenüber ungesättigten Ceramiden mittlerer Kettenlänge.<sup>[82]</sup> Aufgrund der Rolle bei der lysosomalen Speicherkrankheit Farber-Syndrom, die durch

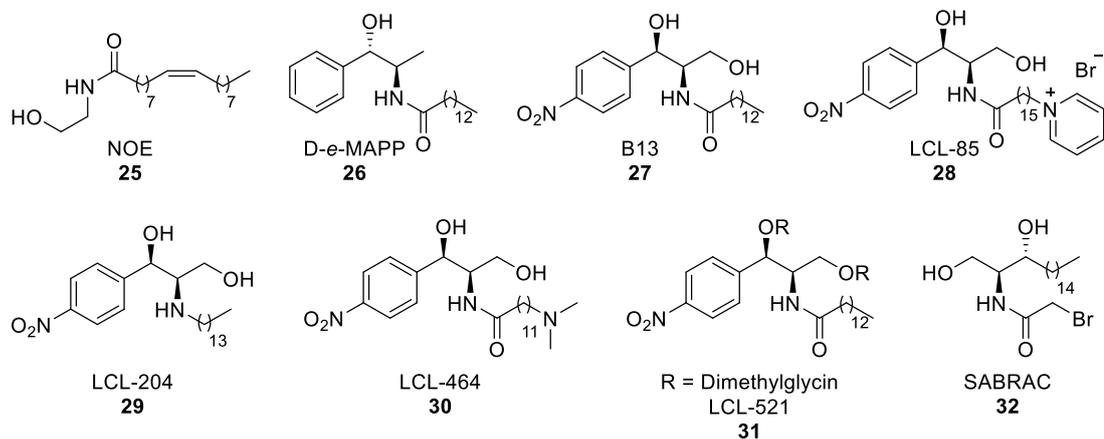
einen genetisch bedingten Aktivitätsmangel an aCDase verursacht wird, ist aCDase das wohl am besten untersuchte Enzym dieser Familie. aCDase ist eines der Schlüsselenzyme des Sphingolipid-Rheostats und ist in verschiedenen Tumorarten überexprimiert, was erhöhte zelluläre Konzentrationen an S1P und somit dauerhafte Proliferation und gesteigerte Überlebensraten zur Folge hat.<sup>[60]</sup>

Innerhalb der letzten beiden Jahrzehnte wurden verschiedene aCDase-Inhibitoren entwickelt, die sich strukturell von Ceramid ableiten lassen (sogenannte aCDase-Inhibitoren der ersten Generation, Abbildung 6). Der erste Inhibitor dieser Art war *N*-Oleoyl-ethanolamid (**25**, NOE).<sup>[83]</sup> NOE wurde als aCDase-Inhibitor in verschiedenen grundlegenden Studien verwendet, erwies sich jedoch aufgrund seiner geringen Wirksamkeit und Selektivität für therapeutische Zwecke ungeeignet.<sup>[84]</sup> 1992 berichteten Bielawska *et al.* von einer Gruppe aromatischer Ceramid-Derivate, darunter *D*-*e*-MAPP (**26**), welches antiproliferative Aktivität gegenüber HL-60 Zellen analog zu C<sub>2</sub>-Ceramid aufweist.<sup>[85]</sup> **26** inhibiert basische CDase (IC<sub>50</sub> ~ 1-5 μM) aus HL-60-Zellextrakten und führt in HL-60 Zellen zur Akkumulation endogener Ceramide, sowie zu Zellzyklusarrest in der G<sub>0</sub>/G<sub>1</sub>-Phase.<sup>[86]</sup> Nach einer widersprüchlichen Studie weist **26** geringe inhibitorische Aktivität gegenüber aCDase (IC<sub>50</sub> ~ 500 μM) in Melanom- und HaCat-Zelllinien auf, nicht jedoch gegenüber basischer CDase.<sup>[87]</sup> In selbiger Studie stellte sich die Verbindung B13 (**27**), als selektiver aCDase-Inhibitor heraus (IC<sub>50</sub> ~ 10 μM). Weiterführende Untersuchungen ergaben, dass B13 die endogenen Cer-Konzentrationen in verschiedenen Krebszelllinien erhöht und in der Lage ist, Apoptose zu induzieren.<sup>[87-89]</sup> Es ist jedoch fraglich, ob die antiproliferative Aktivität von B13 auf die Inhibition von aCDase zurückzuführen ist, da B13 als lipophiles und ungeladenes Molekül nur in geringem Maße in die Lysosomen gelangen kann, wo sich das *target* aCDase befindet.

Durch Modifikation mit kationischen oder basischen funktionellen Gruppen, wurde versucht die zelluläre Aufnahme neuer B13-Derivate zu verbessern und deren Ansammlung in Zellkompartimenten wie den Mitochondrien bzw. den Lysosomen zu erreichen. Beispiele hierfür sind die kationische Verbindung LCL-85 (**28**) und das Amin LCL-204 (**29**).<sup>[90-91]</sup> Im Allgemeinen weisen derartige B13-Derivate eine verbesserte zelluläre Aufnahme und höhere Zytotoxizität gegenüber MCF-7-Zellen als B13 auf. Insbesondere Amine wie **29** akkumulieren in den aciden Zellkompartimenten, erhöhen endogene Cer-Konzentrationen und verringern zelluläre Konzentrationen an Sphingosin. **29** verursacht jedoch auch die Permeabilisierung der

Lysosomen und Degradation von aCDase, was zu der Entwicklung weiterer Strukturanaloga führte. Eine Reihe an  $\omega$ -*N*-Aminoacyl-Derivaten, darunter LCL-464 (**30**), weisen ähnliche Eigenschaften wie **29** auf, ohne derartige unerwünschte Nebeneffekte auszulösen. **30** besitzt eine deutlich höhere inhibitorische Aktivität gegenüber zellulärer aCDase in MCF-7-Zellen als B13, jedoch nicht gegenüber aCDase aus Zelllysaten.<sup>[92]</sup> Neuere Serien weiterer strukturverwandter Verbindungen beinhalten Sulfonamid-Analoga,<sup>[93]</sup> sowie B13-dimethylglycinester wie LCL-521 (**31**).<sup>[94]</sup> Der Wirkmechanismus der B13-Derivate wurde bisher noch nicht aufgeklärt.

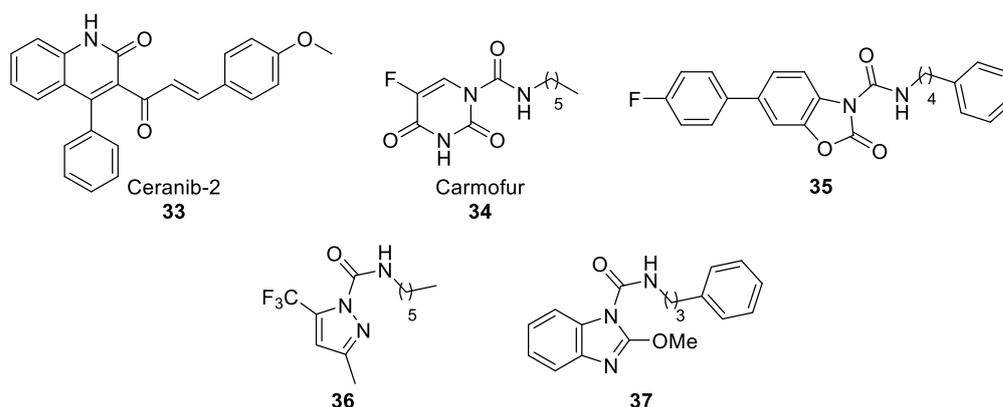
Shtraizent *et al.* schlugen 2008 einen Mechanismus für die Aktivierung von aCDase vor, wonach aCDase als Proenzym vorliegt, und durch eine autokatalytische Spaltung der  $\alpha$ - und  $\beta$ -Untereinheit des Enzyms aktiviert wird, wobei ein *N*-terminales Cystein als aktives Zentrum freigelegt wird.<sup>[95]</sup> Camacho *et al.* untersuchten deshalb eine Reihe von Cer-Derivaten mit Thiol-reaktiven Strukturmotiven, um das aktive Zentrum des Enzyms zu blockieren. Dabei stellte sich SABRAC (**32**) als aktivste Verbindung heraus und inhibierte aCDase in FDX10-Zellen und in Zelllysaten ( $IC_{50} \sim 52$  nM). In PC-3/Mc-Zellen inhibierte **32** das Zellwachstum signifikant, und führte zu der Ansammlung endogener Ceramide.<sup>[96]</sup>



**Abbildung 6:** Auswahl an Ceramidase-Inhibitoren der ersten Generation.

In der Regel weisen Cer-Analoga jedoch aufgrund ihrer hohen Hydrophobizität schlechte pharmakologische Eigenschaften auf und werden generell nicht als „drug-like“ eingestuft. Auf der Suche nach neuen aCDase-Inhibitoren, die nicht strukturverwandt mit Cer sind (sogenannte Ceramidase-Inhibitoren der zweiten Generation, Abbildung 7), identifizierten Draper *et al.* bei dem *screening* einer 50000

Verbindungen umfassenden Bibliothek eine Reihe an Quinolinen, darunter Ceranib-2 (**33**). **33** inhibiert die Aktivität von aCDase in Zell-basierten Assays ( $IC_{50} \sim 28 \mu M$ ) und inhibiert das Zellwachstum bestimmter Krebszelllinien.<sup>[97]</sup> Bei einem weiteren *screening* einer kommerziellen Bibliothek zeigten Realini *et al.*, dass der antineoplastische Wirkstoff Carmofur (**34**) rekombinante aCDase inhibiert ( $IC_{50} \sim 29 nM$ ), was zu der Zytotoxizität von **34** beiträgt.<sup>[98]</sup> Weiterführende Studien derselben Arbeitsgruppe ergaben, dass eine Reihe an Benzoxalon-Carboxamiden, wie Verbindung **35**, aCDase sowohl in bestimmten Zelllinien als auch *in vivo* in Modellmäusen inhibiert. Dabei entsteht eine kovalente Bindung zwischen dem Cystein im aktiven Zentrum von aCDase und der Carboxamid-Seitenkette.<sup>[99]</sup> Obwohl derartige Verbindungen eine hohe Wirksamkeit aufweisen, zeichnen sie sich dennoch durch geringe Wasserlöslichkeit und moderate chemische und metabolische Stabilitäten aus. Neuere Klassen derartiger Verbindungen sind Pyrazol-Carboxamide wie **36**, und Benzimidazol-Carboxamide, wie **37**.<sup>[100]</sup>



**Abbildung 7:** Auswahl an Ceramidase-Inhibitoren der zweiten Generation.

Vor kurzem konnte die Struktur menschlicher aCDase mittels Röntgenkristallographie von Gebai *et al.* aufgeklärt werden, was zukünftig das rationale Design neuer Generationen an aCDase-Inhibitoren ermöglicht.<sup>[101]</sup>

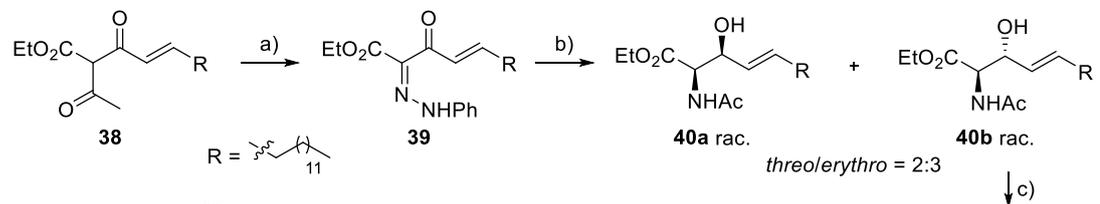
### 1.3 Synthetische Darstellung von Sphingoidbasen

Aufgrund ihrer biologischen Bedeutung und ihrer modularen Struktur sind Sphingolipide seit den 1950er Jahren beliebte Syntheseeziele. Im Allgemeinen können Ceramide und Glycosphingolipide durch *N*-Acylierung bzw. Glycosylierung entsprechend modifizierter Sphingoidbasen dargestellt werden. Insbesondere für die

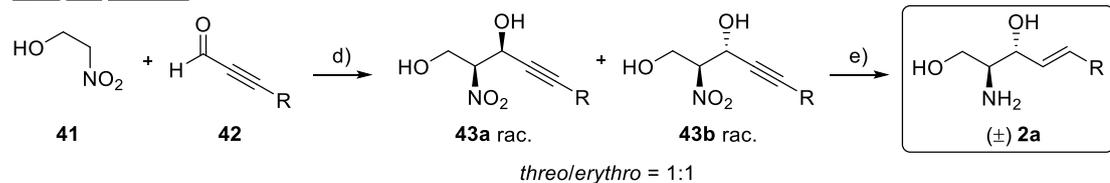
drei großen Gruppen der Sphingosine, Phytosphingosine und Sphinganine wurden zahlreiche Synthesemethoden entwickelt.

Die erste racemische Synthese von Sphingosin wurde 1954 von Shapiro und Segal veröffentlicht (Schema 5).<sup>[102]</sup> Eine Japp-Klingemann-Reaktion von  $\beta$ -Ketoester **38** mit Phenylhydrazoniumchlorid und nachfolgende Reduktion des dabei entstehenden Phenylhydrazons mit Zn/AcOH und NaBH<sub>4</sub> ergab die Alkohole **40a** und **40b** als Mischung aus Stereoisomeren mit einem *erythro*/*threo*-Verhältnis von 3:2. Durch Trennung der Diastereomere mittels Kristallisation und anschließender Entschützung des *erythro*-Diastereomers **40b**, wurde racemisches Sphingosin erhalten. Kurze Zeit später verfolgten Grob und Gadiant einen alternativen Ansatz, basierend auf der Kondensation zwischen 2-Nitroethanol (**41**) und Hexadecinal (**42**).<sup>[103]</sup> Die *erythro*- und *threo*-Isomere des Nitrodiols **43** wurden wiederum durch Kristallisation voneinander getrennt und Reduktion von **43b** lieferte die Zielverbindung ( $\pm$ )-**2a** als Racemat. Seitdem wurden diverse Strategien zur Darstellung von Sphingoidbasen beschrieben. Detaillierte Zusammenstellungen können der Literatur entnommen werden.<sup>[104-106]</sup>

Shapiro und Segal:<sup>[102]</sup>



Grob und Gadiant:<sup>[103]</sup>



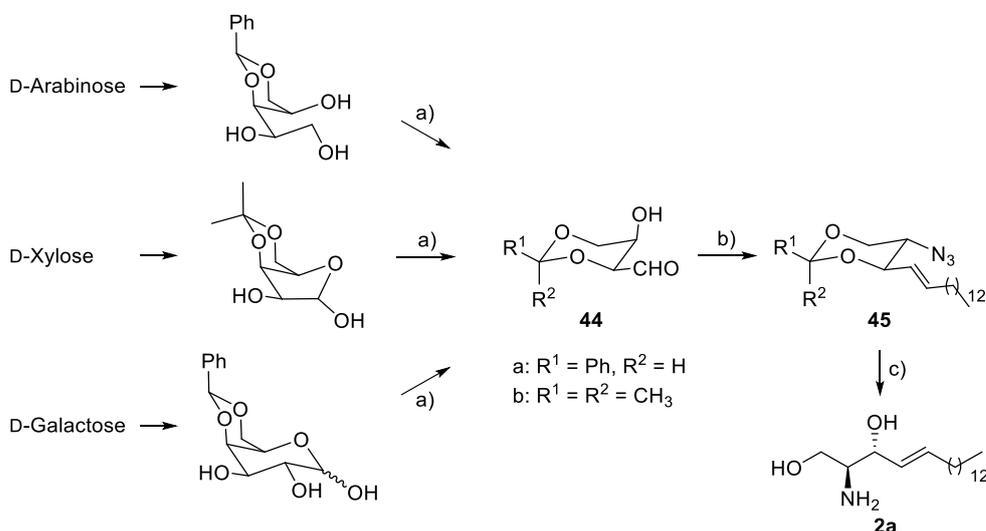
**Schema 5:** Racemische Synthesen von Sphingosin. Reagenzien und Bedingungen: a) PhN<sub>2</sub>Cl, NH<sub>4</sub>Cl; b) 1. Zn/AcOH, Ac<sub>2</sub>O; 2. NaBH<sub>4</sub>, Kristallisation; c) 1. HCl; 2. LiAlH<sub>4</sub>; d) K<sub>2</sub>CO<sub>3</sub>, MeOH, 0 °C, 15 min, Kristallisation; e) 1. Zn/HCl, EtOH, 0 °C, 30 min; 2. LiAlH<sub>4</sub>, THF, rf, 4 h.

### 1.3.1 Ausgehend von Kohlenhydraten

Eine der größten Herausforderungen bei der Synthese von Sphingoidbasen ist der stereoselektive Aufbau der Kopfgruppe. Einer der ersten Ansätze zur Synthese von Sphingoidbasen war die Verwendung von Kohlenhydraten als Quelle der stereochemischen Information. Vorteil dieser Strategie ist die große Auswahl an geschützten Ausgangsmaterialien bekannter Konfiguration. Häufig wird hierbei ein

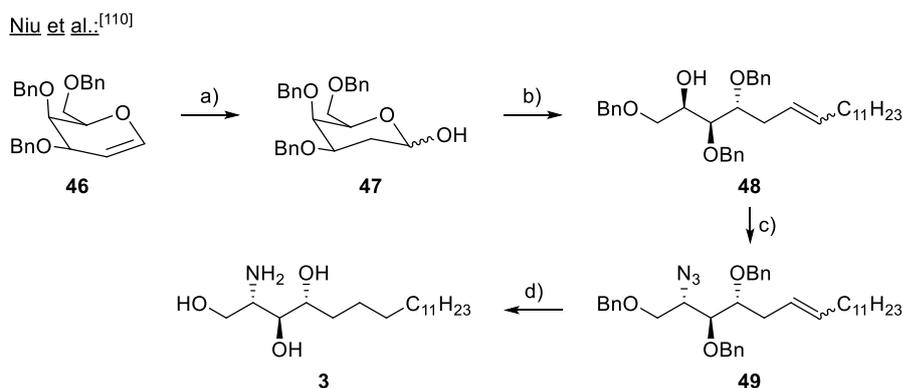
Zucker-Baustein in einen entsprechenden Aldehyd überführt, der in einer Wittig-Reaktion zu dem Grundgerüst der Sphingoidbase umgesetzt wird. So verwendeten Schmidt, Zimmermann und Kiso *et al.* 2,4-*O*-geschützte Threosen wie **44a** und **44b**, die in wenigen Stufen aus D-Galaktose, D-Xylose oder D-Arabinose erhalten werden können (Schema 6).<sup>[107-109]</sup> Eine Schlosser-Variante der Wittig Reaktion gefolgt von chromatographischer Trennung der entstehenden *E/Z*-Isomere und Substitution der freien Hydroxy-Gruppe ergab die Azide **45a** und **45b**. Acetal-Entschützung gefolgt von der Reduktion der Azid-Gruppe lieferte Sphingosin in der gewünschten *D-erythro*-Konfiguration.

Schmidt, Zimmermann und Kiso *et al.*:<sup>[107-109]</sup>



**Schema 6:** Darstellung von *D-erythro*-Sphingosin (**2a**) ausgehend von verschiedenen Zuckerbausteinen. Reagenzien und Bedingungen: a)  $\text{NaIO}_4$ , MeOH, rt, 3 h; b) 1. Tetradecyl-Triphenylphosphoniumbromid, PhLi, THF,  $-30^\circ\text{C}$ , 1 h; 2. MsCl, Pyridin, rt, 6 h; oder  $\text{Tf}_2\text{O}$ , Pyridin,  $-15^\circ\text{C}$ , 15 min; dann  $\text{NaN}_3$ , DMF,  $110^\circ\text{C}$ , 18 h; c) 1. AcOH,  $\text{H}_2\text{O}$ ,  $45^\circ\text{C}$ ; oder *p*-TsOH,  $\text{CH}_2\text{Cl}_2$ , rt, 12 h; 2.  $\text{NaBH}_4$ , 2-Propanol, rf, 48 h; oder  $\text{H}_2\text{S}$ , Pyridin,  $\text{H}_2\text{O}$ , rt, 48 h.

Auch für die Stereoisomere von Phytosphingosin existieren verschiedene Synthesemethoden, die auf der Verwendung von Zucker-Bausteinen basieren. Beispielsweise veröffentlichten Niu *et al.* 2008 eine kurze und effiziente Syntheseroute für *D-ribo*-Phytosphingosin ausgehend von kommerziell erhältlichen 3,4,6-*O*-benzylidene-D-Galactal (**46**) (Schema 7).<sup>[110]</sup> **46** wurde in den 2-Desoxy-Zucker **47** überführt und nachfolgende Wittig Olefinierung von **47** mit Dodecyl-Triphenylphosphoniumbromid und *n*-BuLi ergab Alkohol **48**. Mittels Mitsunobu-Reaktion wurde die Azid-Gruppe von **49** eingeführt und anschließende Hydrierung ergab die Zielverbindung **3** mit einer Gesamtausbeute von 74% über 5 Stufen.



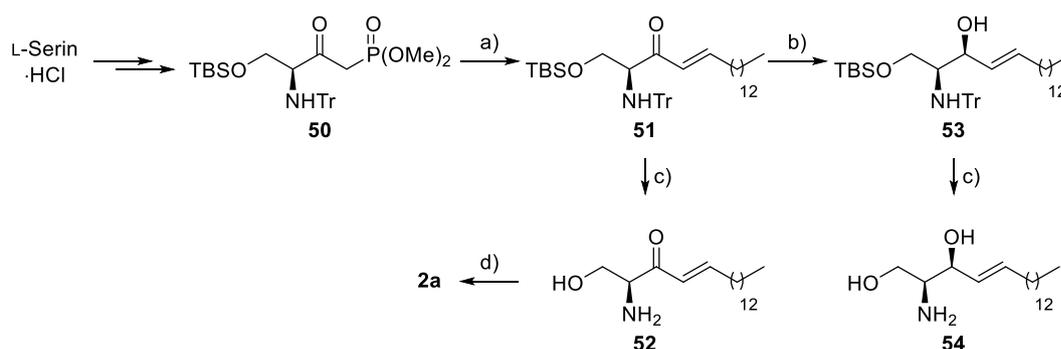
**Schema 7:** Synthese von *D-ribo*-Phytosphingosin (**3**) ausgehend von 3,4,6-*O*-benzyl-*D*-Galactal (**46**). Reagenzien und Bedingungen: a) HBr, PPh<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 24 h; b) Dodecyl-Triphenylphosphoniumbromid, *n*-BuLi, THF, -10 °C, 2 h; c) DPPA, PPh<sub>3</sub>, DEAD, rt, 24 h; d) H<sub>2</sub>, Pd/C, MeOH/AcOEt/AcOH, rt, 4 d.

### 1.3.2 Ausgehend von Serin

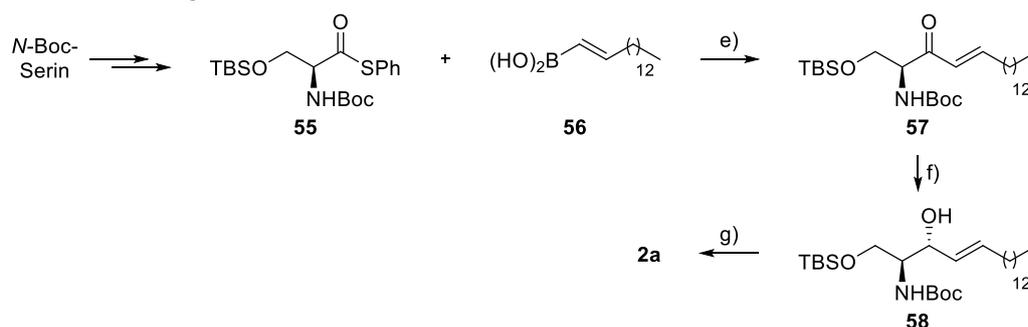
Der Nachteil bei der Verwendung von Kohlenhydraten als Ausgangsverbindungen ist die zum Teil aufwendige Darstellung der benötigten Zucker-Bausteine, weshalb bei der Synthese von Sphingoidbasen vor allem leicht zugängliche Derivate der natürlichen Aminosäure Serin genutzt werden. Bei diesen Synthesemethoden wird meist mittels C-C-Verknüpfung eine 3-Keto-Sphingoidbase dargestellt, welche anschließend diastereoselektiv reduziert wird, um die Stereoinformation an Position 3 einzuführen. Chung *et al.* veröffentlichten 2002 eine kurze Syntheseroute die Zugang zu allen 4 Stereoisomeren von Sphingosin ermöglicht (Schema 8).<sup>[111]</sup> Hierfür wurde L-Serin-Hydrochlorid innerhalb von 3 Stufen in das  $\beta$ -Ketophosphonat **50** überführt. HWEOlefinierung mit Myristylaldehyd lieferte das 3-Ketosphingosin **51**. *syn*-Selektive Reduktion von **51** zu **53** mittels NaBH<sub>4</sub> und nachfolgende saure Entschützung ergab *L-threo*-Sphingosin **54**. Durch saure Entschützung von **51** und anschließende *anti*-selektive Reduktion von **52** wurde *D-erythro*-Sphingosin (**2a**) erhalten. Analog dazu wurden ausgehend von *D*-Serin-Hydrochlorid die entsprechenden *D-threo*- und *L-erythro*-Isomere dargestellt. Libeskind und Yang entwickelten eine Methode zur Palladium-katalysierten Kreuzkupplung zwischen geschützten Thioestern und Vinyl-Borsäure-Verbindungen.<sup>[112]</sup> Durch Kupplung des *L*-Serin-Thioesters **55** mit der Vinyl-Borsäure **56**, anschließender *anti*-selektiver Reduktion des Enons **57** mit LiAlH(O*t*Bu)<sub>3</sub> und Entschützung mit TFA wurde **2a** in einer Gesamtausbeute von 71% über 6 Stufen erhalten. Katsumura *et al.* verwendeten ebenfalls LiAlH(O*t*Bu)<sub>3</sub> um das Vinylketon **60** *anti*-selektiv zu reduzieren.<sup>[113]</sup> **60** wurde durch Addition von Vinylmagnesiumbromid an Weinreb-Amid **59** erhalten. Je nach Zielverbindung,

wurden die Amino- und die primäre Hydroxy-Gruppe des Allylalkohols **61** modifiziert. Anschließend wurde die Seitenkette der Sphingoidbase mittels Olefinmetathese eingeführt. Nach dieser Methode wurden verschiedene Sphingolipide synthetisiert, darunter Sphingosin, Cer, SIP, Sphingomyelin und einige Derivate mit Fluoreszenz-markierten funktionellen Gruppen.

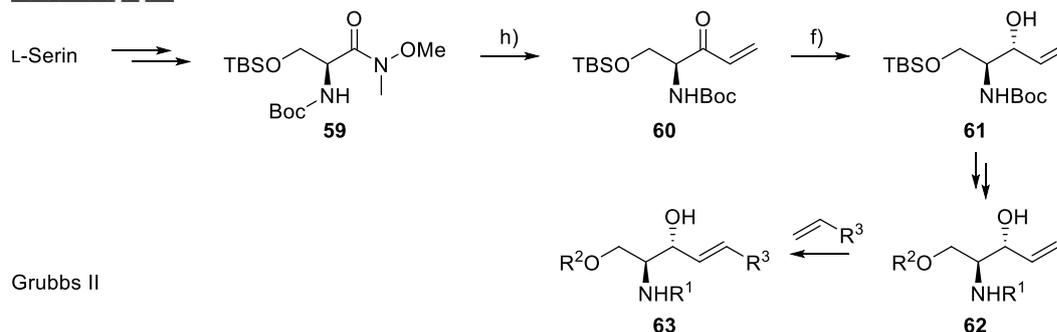
Chung et al.<sup>[111]</sup>



Libeskind und Yang.<sup>[112]</sup>



Katsumura et al.<sup>[113]</sup>



**Schema 8:** Synthese von Sphingosin ausgehend von Serin. Reagenzien und Bedingungen: a) Myristylaldehyd, DBU, LiCl, THF, rt, 5 h; b) NaBH<sub>4</sub>, CeCl<sub>3</sub>·7H<sub>2</sub>O, MeOH, 0 °C, 3 h; c) 2N HCl, THF/MeOH, 40 °C, 5 h; d) Zn(BH<sub>4</sub>)<sub>2</sub>, THF, rt, 5 h; e) Pd<sub>2</sub>(dba)<sub>3</sub>, CuTC, P(OEt)<sub>3</sub>, THF, rt, 5 h; f) LiAlH(O*t*Bu)<sub>3</sub> EtOH, -78 °C, 2 h; g) TFA, MeOH, 0 °C, 30 min; h) VinylMgBr, THF, rt, 10 min.

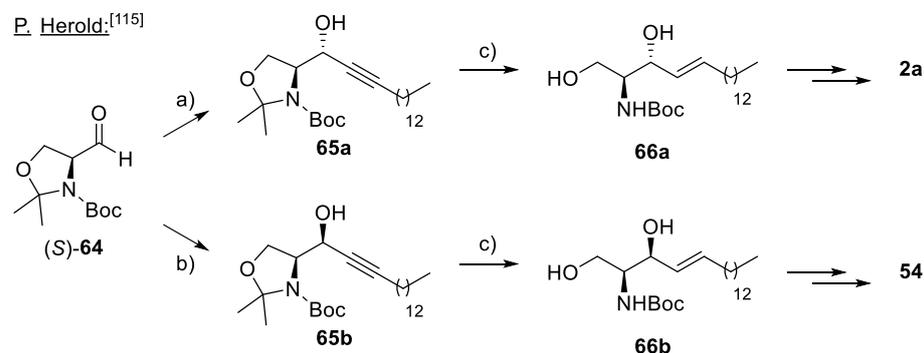
### 1.3.3 Ausgehend von Garner's Aldehyd

Unter den Serin-Derivaten wird vor allem Garner's Aldehyd (**64**) häufig zur Synthese von Sphingoidbasen verwendet (Schema 9). Die Addition von Metallorganen an **64** stellt einen einfachen Zugang zu 2-Amino-1,3-dihydroxypropyl-Strukturmotiven dar. Je nach Metallorganyl und Reaktionsbedingungen kann die Addition an **64** sowohl *anti*- als auch *syn*-selektiv verlaufen.<sup>[114]</sup> Im Allgemeinen entstehen hohe *anti*-Selektivitäten durch einen Angriff auf der sterisch ungehinderten Seite des Aldehyds, hohe *syn*-Selektivitäten werden durch Chelat-Kontrolle erreicht.

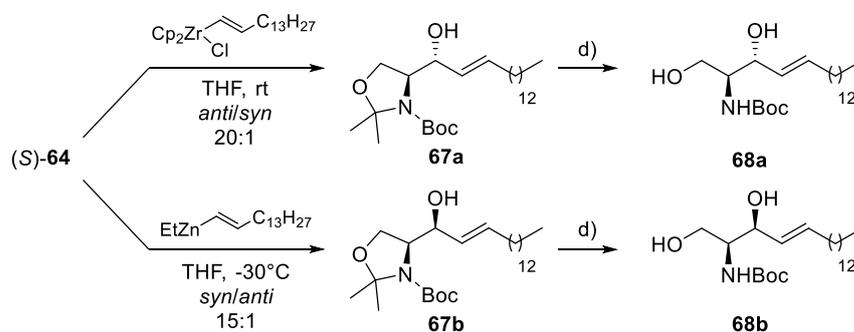
P. Herold stellte durch *anti*-selektive Addition von Pentadecynyllithium an (*S*)-**64** den propargylischen Alkohol **65a** mit einem *de* von 95% dar.<sup>[115]</sup> Addition der entsprechenden Alkynyl-Zink-Verbindung, durch Transmetallierung von Pentadecynyl-Lithium auf ZnBr<sub>2</sub> vor der Zugabe des Aldehyds, ergab das *syn*-Produkt **65b** (*de* = 95%). Acetonid-Entschützung und partielle Reduktion des Alkins mit Red-Al lieferte die Diole **66a** und **66b**, die anschließend durch Boc-Entschützung in die *erythro*- und *threo*-Isomere von Sphingosin (**2a**, **54**) überführt wurden. Murakami und Furusawa verwendeten Alk-1-enyl-Nucleophile, um direkt die beiden Alkohole **67a** und **67b** zu synthetisieren.<sup>[116]</sup> Addition von Pentadecenyl-Zirconoceniumchlorid ergab das *erythro*-Isomer **67a** in einem *anti/syn*-Verhältnis von 20:1. Die Addition von Pentadecenyl-Ethyl-Zink hingegen, ergab das entsprechende *threo*-Isomer **67a** in einem *syn/anti*-Verhältnis von 15:1. Nachfolgende Acetonid-Entschützung lieferte die *N*-Boc-Sphingosine **68a** und **68b**. Arenz *et al.* stellten verschiedene Sphingosin-Derivate ausgehend von (*S*)-**64** durch Addition von Vinylmagnesiumbromid dar.<sup>[117]</sup> Mittels Olefinmetathese können anschließend verschiedene Seitenketten eingeführt werden. So wurde der Allylalkohol **69** mit 1-Pentadecen zu **67a** umgesetzt und nachfolgende Entschützung lieferte *D-erythro*-Sphingosin (**2a**).

Eine alternative Synthesemethode zur Darstellung von Phytosphingosin-Derivaten ausgehend von Garner's Aldehyd wurde von Kim *et al.* entwickelt.<sup>[118]</sup> Dabei wurde (*S*)-**64** mit Pentadecyl-Triphenylphosphoniumbromid und KHMDS in einer Wittig-Olefinierung zu Verbindung **70** umgesetzt. Durch Modifikation der Schutzgruppen wurden die *Z*-Allylamide **71a** und **71b** erhalten. Eine OsO<sub>4</sub>-katalysierte Dihydroxylierung diente zum Aufbau der Stereozentren an Position 3 und 4, wobei die Dihydroxylierung gefolgt von Acetylierung der dabei entstehenden Hydroxy-Gruppen von **71a** das *syn*-Isomer **72a** ergab, ein geschütztes *L-arabino*-Phytosphingosin-

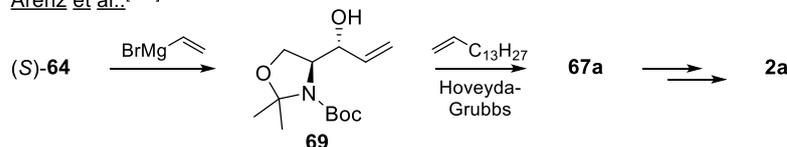
Derivat. Dihydroxylierung der *N,N*-DiBoc-geschützten Verbindung **71b** ergab das entsprechende *anti*- bzw. *D-ribo*-Phytosphingosin-Derivat **72b**.



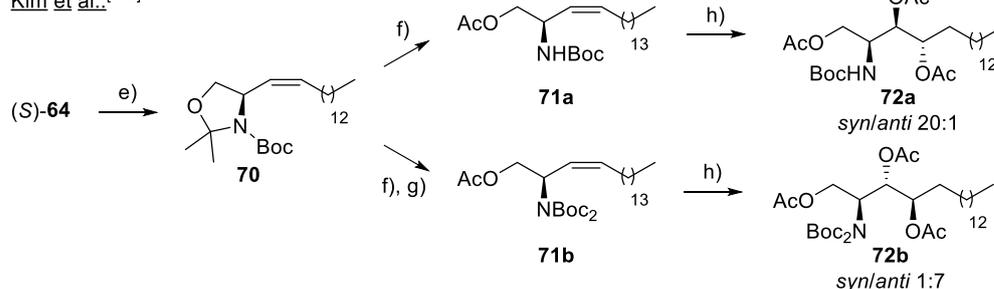
Murakami und Furusawa:<sup>[116]</sup>



Arenz et al.:<sup>[117]</sup>



Kim et al.:<sup>[118]</sup>

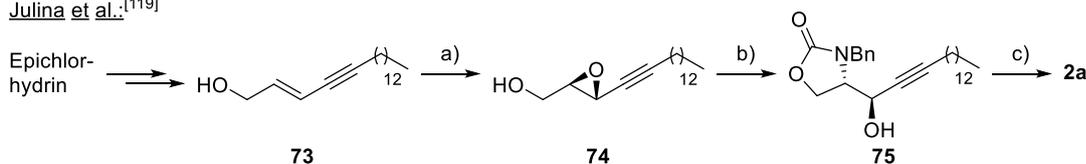
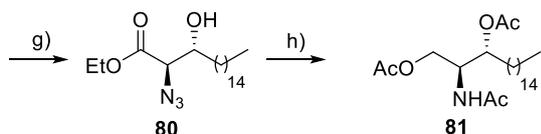
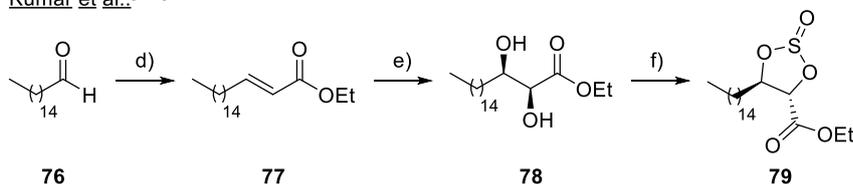
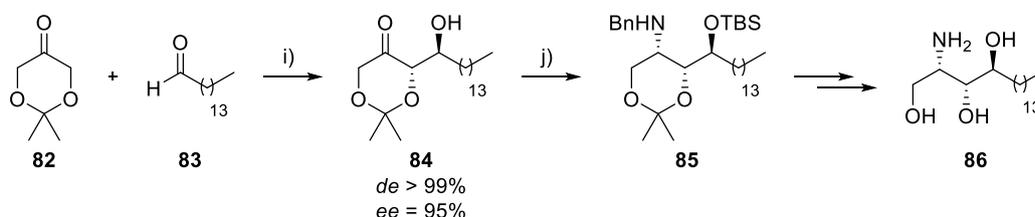
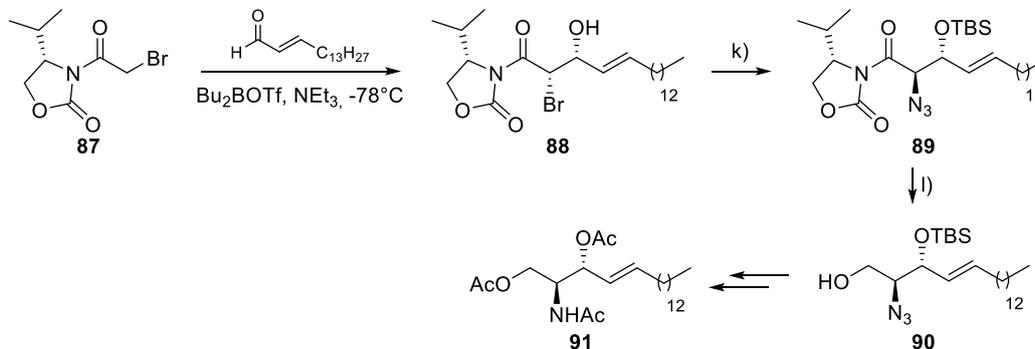


**Schema 9:** Synthese von Sphingolipiden ausgehend von Garner's Aldehyd (**64**). Reagenzien und Bedingungen: a) Pentdecin, *n*-BuLi, HMPT, THF, -78 °C, 2 h, de = 95%; b) Pentdecin, *n*-BuLi, ZnBr<sub>2</sub>, THF, -78 °C  $\rightarrow$  rt, 24 h, de = 95%; c) 1. Amberlyst 15, MeOH, rt, 41 h; 2. Red-Al, Et<sub>2</sub>O, rt, 24 h; d) AcOH, 50 °C, 5 h; e) Pentadecyl-Triphenylphosphoniumbromid, KHMDS, THF, -78 °C; f) 1. Dowex 50Wx4-100, MeOH, rt; 2. Ac<sub>2</sub>O, NEt<sub>3</sub>, DMAP; g) Boc<sub>2</sub>O, NEt<sub>3</sub>, DMAP; h) 1. OsO<sub>4</sub>, NMO; 2. Ac<sub>2</sub>O, NEt<sub>3</sub>, DMAP.

### 1.3.4 Asymmetrische Reaktionen und chirale Auxiliare

Neben Synthesen, die auf Ausgangsmaterialien aus dem chiralen Pool basieren, wurden verschiedene Strategien entwickelt, um die Stereoinformation mithilfe von asymmetrischen Reaktionen oder chiralen Auxiliaren zu erzeugen, wobei vor allem Sharpless-Dihydroxylierungen und -Epoxidierungen, sowie asymmetrische Aldol-Reaktionen verwendet wurden. Einige Beispiele sind in Schema 10 dargestellt. So stellten Julina *et al.* Allylalkohol **73** in wenigen Stufen ausgehend von Epichlorhydrin dar.<sup>[119]</sup> Die Sharpless-Epoxidierung von **73** ergab das Oxiran **74** mit einem ee von 98%, welches durch regioselektive Ringöffnung mit Benzylisocyanat in das Oxazolidinon **75** überführt wurde. Nachfolgende Reduktion des Alkins gefolgt von basischer Ringöffnung lieferte die Zielverbindung *D-erythro*-Sphingosin **2a**. Kumar *et al.* hingegen verwendeten eine Sharpless-Dihydroxylierung, um *D-erythro*-Sphinganin-Triacetat (**81**) herzustellen.<sup>[120]</sup> Hierzu wurde Hexadecanal (**76**) in einer Wittig-Reaktion zu dem Olefin **77** umgesetzt. Eine Sharpless-Dihydroxylierung ergab den Diol **78** mit einem ee von 99%, welcher anschließend mit Thionylchlorid in das zyklische Sulfit **79** überführt wurde. Regioselektive nucleophile Ringöffnung des Sulfits mit LiN<sub>3</sub> gefolgt ergab den Azidoalkohol **80**. Durch Reduktion mittels LiAlH<sub>4</sub> und anschließender Acetylierung wurde die Zielverbindung **81** erhalten.

Enders *et al.* optimierten die (*S*)-Prolin-katalysierte Aldolreaktion zwischen Dioxanon **82** und Pentadecanal (**83**).<sup>[121]</sup> Auf diese Art und Weise wurde das Aldolprodukt **84** mit einem de von >99% und einem ee (*anti*) von 95% erhalten. Nach TBS-Schätzung der freien Hydroxygruppe, wurde das Keton durch eine substratgesteuerte reduktive Aminierung zu Verbindung **85** mit einem de von 99% umgesetzt. Nachfolgende Entschätzungen ergaben *D-arabino*-Phytosphingosin (**86**). Nicolaou *et al.* verwendeten ein chirales Oxazolidinon als Auxiliar zur Darstellung von *D-erythro*-Sphingosin-Derivaten.<sup>[122]</sup> Dabei ergab die Aldolreaktion zwischen dem Bor-Enolat von *N*-acyl-Oxazolidinon **87** und Hexadec-2-en-1-al ausschließlich das *syn*-Isomer **88**. Substitution des Bromides durch NaN<sub>3</sub> und TBS-Schätzung der freien Hydroxy-Gruppe lieferte Verbindung **89**. Durch Abspaltung des Auxiliars wurde Azid **90** erhalten, das eine geeignete Vorstufe für die Synthese von Glycosphingolipiden ist. Weiterhin wurde **90** zu *D-erythro*-Sphingosin-Triacetat (**91**) umgesetzt.

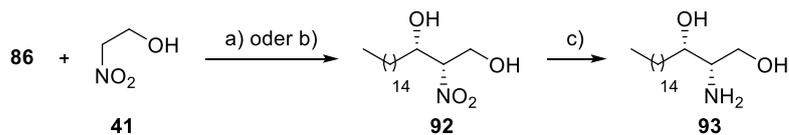
Julina et al.:<sup>[119]</sup>Kumar et al.:<sup>[120]</sup>Enders et al.:<sup>[121]</sup>Nicolaou et al.:<sup>[122]</sup>**Schema 10:** Synthese von Sphingolipiden mithilfe von Auxiliaren und asymmetrischen Reaktionen.

Reagenzien und Bedingungen: a) (-)-Diethyltartrat,  $\text{Ti}(\text{O}t\text{Bu})_4$ ,  $t\text{BuOOH}$ ,  $\text{CH}_2\text{Cl}_2$ , 5 h,  $-30^\circ\text{C}$ , *ee* = 98%; b)  $\text{BnNCO}$ ,  $\text{NaH}$ , THF,  $60^\circ\text{C}$ , 3 h; c) 1.  $\text{Li}$ ,  $\text{EtNH}_2$ ,  $t\text{BuOH}$ ;  $-78^\circ\text{C}$ , 2 h; 2.  $\text{NaOH}$ ,  $\text{EtOH}$ ,  $80^\circ\text{C}$ , 2,5 h; d)  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$ , THF, rf, 18 h; e)  $(\text{DHQD})_2\text{-PHAL}$ ,  $\text{K}_3\text{Fe}(\text{CN})_6$ ,  $\text{OsO}_4$ ,  $\text{MeSO}_2\text{NH}_2$ ,  $t\text{BuOH}/\text{H}_2\text{O}$ ,  $0^\circ\text{C}$ , 24 h, *ee* = 99%; f)  $\text{SOCl}_2$ ,  $\text{CCl}_4$ , rf, 1,5 h; g) 1.  $\text{LiN}_3$ , DMF,  $100^\circ\text{C}$ , 18 h; 2.  $\text{H}_2\text{SO}_4/\text{Et}_2\text{O}$ , rt, 12 h; h) 1.  $\text{LiAlH}_4$ ,  $\text{Et}_2\text{O}$ , rt, 24 h; 2.  $\text{Ac}_2\text{O}$ , Pyridin, rt, 18 h; i) (*S*)-Prolin,  $\text{CHCl}_3$ , rt, 4 d, *de* > 99%, *ee* (*anti*) = 95%; j) 1. TBSOTf, 2,6-Lutidin,  $\text{CH}_2\text{Cl}_2$ ,  $-20^\circ\text{C}$ ; 2.  $\text{BnNH}_2$ ,  $\text{NaBH}(\text{OAc})_3$ ,  $\text{AcOH}$ ,  $\text{CH}_2\text{Cl}_2$ , 2 C *de* > 99%; k) 1.  $\text{NaN}_3$ , DMSO, rt, 2 h; 2. TBSOTf, 2,6-Lutidin,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ , 12 h; l)  $\text{LiBH}_4$ , THF,  $0^\circ\text{C}$ , 3 h.

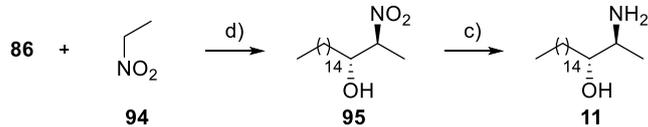
Die Henry-Reaktion stellt eine weitere interessante Methode zum Aufbau des 2-Amino-1,3-dihydroxypropyl-Strukturmotivs ausgehend von prochiralen Aldehyden und Nitroalkanen dar. Einfache Sphingoidbasen können so in nur zwei Stufen (Henry-Reaktion gefolgt von der Reduktion der Nitro-Gruppe) synthetisiert werden. Bisher sind jedoch nur wenige diastereo- und enantioselektive Beispiele bekannt, die zudem zur Darstellung von Sphingolipiden verwendet wurden. Shibasaki *et al.* entwickelten Lanthan-Lithium-BINOL-Komplexe wie **99** (Schema 11), mit denen hohe Diastereo- und Enantioselektivitäten erreicht werden können.<sup>[123]</sup> Sie erprobten ihre Methode bei der Kondensation von Hexadecanal (**86**) mit Nitroethanol (**41**), wobei unter Verwendung von **99** der entsprechende Nitrodiol **92** in einem *syn/anti*-Verhältnis von 91:9 mit einem ee (*syn*) von 97% erhalten wurde. Nachfolgende Hydrierung der Nitro-Gruppe ergab *L-threo*-Sphingamin (**93**), ein Proteinkinase C-Inhibitor, der auch als Safingol bekannt ist. Ein alternatives Protokoll zur Durchführung von *syn*- und enantioselektiven Henry-Reaktionen wurde von Chen *et al.* veröffentlicht, welche ebenfalls **93** mit ihrer Methode synthetisierten.<sup>[124]</sup> So wurde bei der Kondensation von **86** mit **41** in der Anwesenheit von Cu(OAc)<sub>2</sub> und Ligand **100** ein *syn/anti*-Verhältnis von 16,5:1 und ein ee (*syn*) von 98% erreicht.

Eine Methode für Kupfer-katalysierte, *anti*-selektive Henry-Reaktionen wurde von Wang *et al.* entwickelt und bei der Synthese der 1-Desox-Sphingoidbase Spisulosin (**11**) benutzt.<sup>[125]</sup> Die Nitroaldolreaktion zwischen **86** und Nitroethan (**94**) unter Verwendung von CuBr<sub>2</sub> und Ligand **101** lieferte den entsprechenden Nitroalkohol **95** mit einem *anti/sny*-Verhältnis von 10,6:1 und einem ee (*anti*) von 95%. Anschließende Hydrierung ergab Spisulosin (**11**). Selbige Methode wurde von Filippova *et al.* bei der Totalsynthese des Naturstoffs Obscruaminol A (**98**) ausgehend von Aldehyd **96** verwendet.<sup>[126]</sup> Da die Reduktion des entstehenden Nitroalkohols **97** mit SmI<sub>2</sub> in geringem Maße Epimerisierung auftrat, waren weitere Schritte nötig, um die Zielverbindung **98** enantiomerenrein zu erhalten.

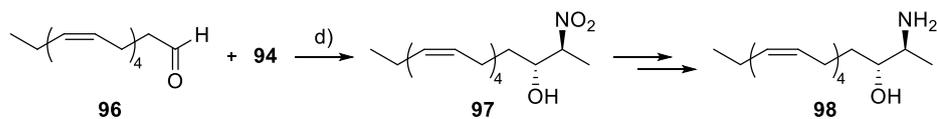
Shibasaki et al.<sup>[123]</sup> und Chen et al.<sup>[124]</sup>



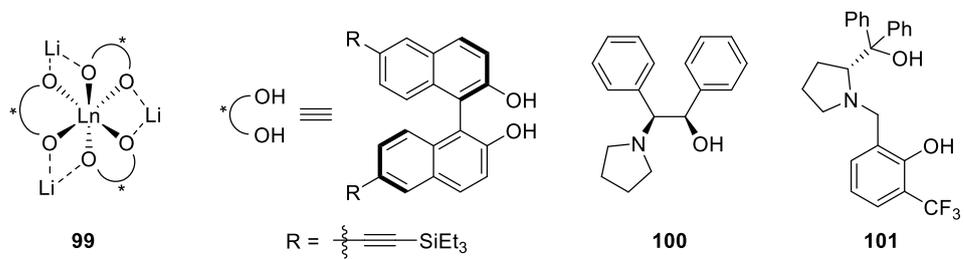
Wang et al.<sup>[125]</sup>



Filippova et al.<sup>[126]</sup>



Katalysatoren und Liganden:



**Schema 11:** Synthese von Sphingoidbasen mithilfe von diastereo- und enantioselektiven Henry-Reaktionen: Reagenzien und Bedingungen: a) **99**, THF,  $-40\text{ }^\circ\text{C}$ , 163 h, *syn/anti* = 91:9, ee (*syn*) = 97%; b) **100**,  $\text{Cu}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ , THF,  $15\text{ }^\circ\text{C}$ , 48 h, *syn/anti* = 16,5:1, ee (*syn*) = 98%; c)  $\text{H}_2$ , Pd/C, MeOH, rt, 2 h; d) **101**,  $\text{CuBr}_2$ ,  $\text{Cs}_2\text{CO}_3$ , THF,  $0\text{ }^\circ\text{C}$ , 73 h, *anti/sny* = 10,6:1, ee (*anti*) = 95%.

## 2 Synopsis

### 2.1 Zielsetzung und Überblick über die Teilprojekte

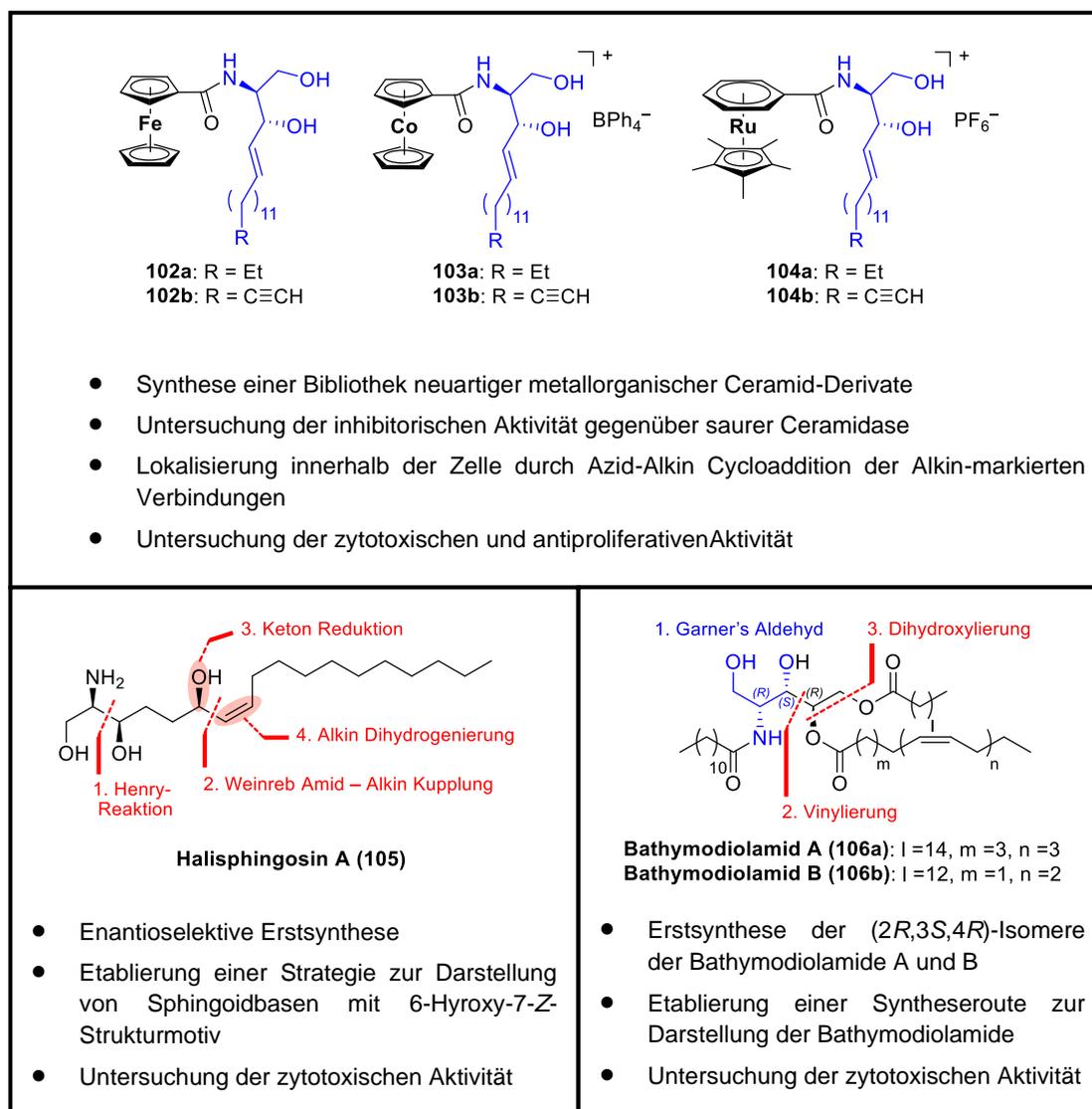
Die vorliegende kumulative Dissertationsschrift umfasst drei Publikationen, welche in Kapitel 4 abgedruckt sind. Ziel dieser Arbeiten war es, einen möglichst modularen Zugang zu natürlichen Sphingolipiden, sowie zu synthetischen Derivaten zu schaffen, um deren biologische Eigenschaften untersuchen zu können. Eine Übersicht der Zielverbindungen und Ergebnisse der vorliegenden Dissertation ist in Abbildung 8 dargestellt.

Das erste Teilprojekt dieser Arbeit befasste sich mit der Synthese metallorganischer Cer-Analoga zur Verwendung als neuartige aCDase-Inhibitoren. Hierzu sollte basierend auf modifizierten Literaturmethoden eine modulare Syntheseroute zur Darstellung der von Sphingosin-Derivaten etabliert werden. Die Sphingoidbasen wurden anschließend mit verschiedenen Metallkomplexen verknüpft, um eine Bibliothek metallorganischer Ceramide (**102-104**) zu erhalten. Durch die Substitution der Fettsäure von Cer mit metallorganischen Fragmenten sollte eine verbesserte Löslichkeit und zelluläre Aufnahme gegenüber natürlichen Ceramiden, sowie die Akkumulation der Komplexe in den Ceramidase-haltigen Zellkompartimenten erreicht werden. Das Einbringen eines terminalen Alkins in der Seitenkette der Sphingoidbase, ermöglicht eine spätere Lokalisation der Zielverbindungen innerhalb der Zellen durch Azid-Alkin Cycloaddition mit Azidocoumarin.<sup>[127]</sup> Weiterhin sollte die inhibitorische Aktivität der dargestellten Verbindungen gegenüber rekombinant gewonnener aCDase, sowie ihre antiproliferative Aktivität untersucht werden.

Im zweiten Teilprojekt dieser Arbeit sollte eine enantioselektive Syntheseroute zur Darstellung der neuartigen Sphingoidbase Halisphingosin A (**105**) entwickelt werden, um die biologische Aktivität von **105** zu untersuchen. **105** wurde erstmals aus Extrakten des Meeresschwamms *Haliclona Reniera* von Mansoor et al. isoliert.<sup>[128]</sup> Später isolierten Molinski *et al.* Halisphingosin A in Form des *N*-Boc-Derivats und bestätigten die ursprüngliche Zuordnung der (2*R*,3*R*,6*R*)-Konfiguration anhand von CD-Methoden.<sup>[129]</sup> Neben der *L-threo*-Konfiguration der Kopfgruppe, weist **105** ein für Sphingoidbasen ungewöhnliches *Z*-Allylalkohol-Strukturmotiv auf. In *in vitro* Studien zeigte **105** zytotoxische Aktivität gegenüber verschiedenen Tumorzelllinien

im einstellig mikromolaren Bereich, und inhibierte die Produktion von Peroxyl-Radikalen sowie die Blutgerinnung.<sup>[130]</sup>

Innerhalb des dritten Teilprojekts sollte eine kurze und flexible Syntheseroute zur Darstellung der Bathymodiolamide A und B (**106a** und **106b**) entwickelt werden, die die Synthese weiterer Isomere und Derivate für die Aufklärung von Struktur-Wirkungs-Beziehungen ermöglicht. **106a** und **106b** sind mit Cer verwandte Lipid-Metabolite der Tiefseemuschel *Bathymodiolus Thermophilus*, welche an hydrothermalen Quellen vorkommt.<sup>[131]</sup> Sie wurden neben einer Reihe weiterer Derivate isoliert, die sich in der Struktur ihrer Fettsäureester unterscheiden. **106a** und **106b** weisen eine zytotoxische Aktivität gegenüber verschiedenen Krebszelllinien im nanomolaren Bereich auf. Die Verwendung der Bathymodiolamide als Chemotherapeutika wurde vor kurzem patentiert.<sup>[132]</sup>



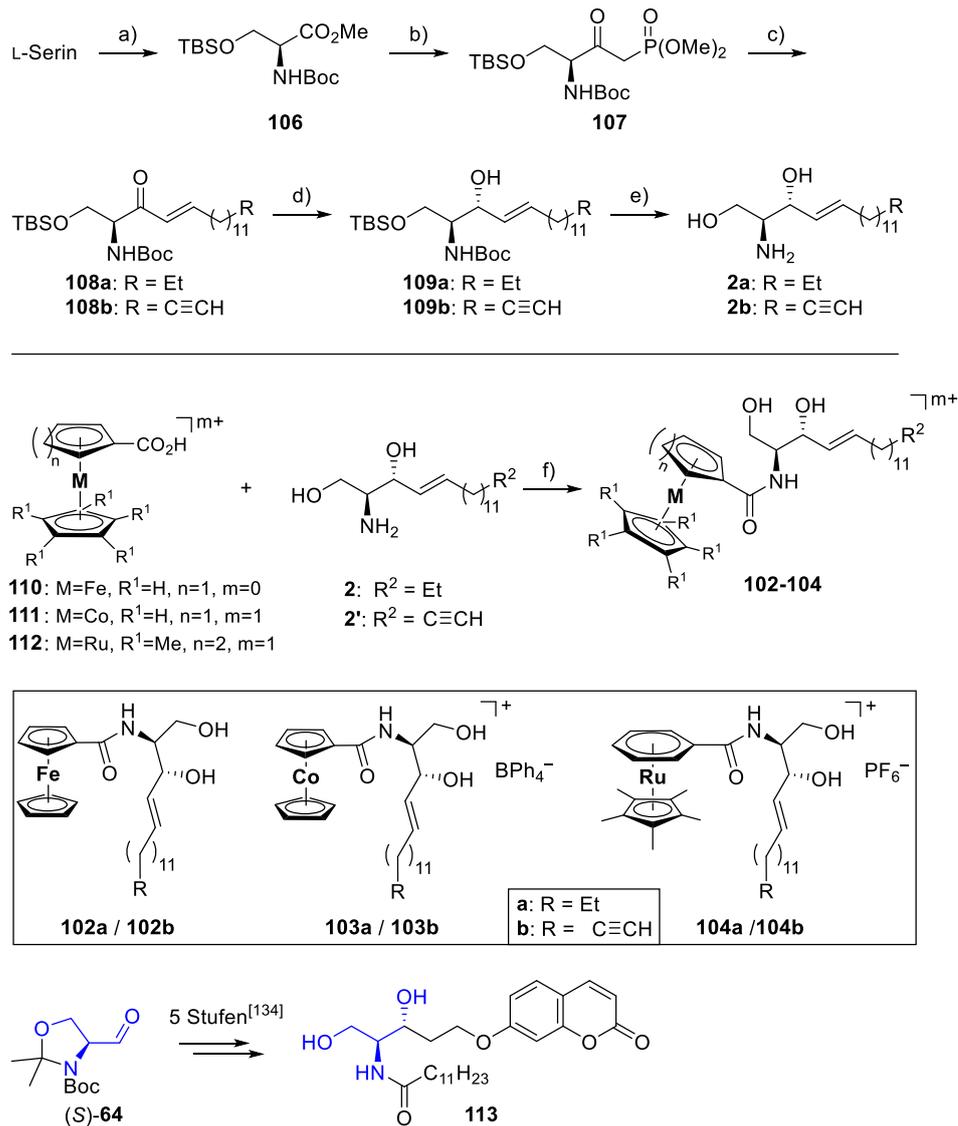
**Abbildung 8:** Übersicht über Zielverbindungen und Ergebnisse der vorliegenden Dissertation.

## 2.2 Die Synthese von *N*-Metallocenoyl-Sphingosin-Komplexen

Erster Teil dieser Arbeit war die Synthese einer Bibliothek metallorganischer Ceramide als Substrat-analoge Inhibitoren menschlicher aCDase (Schema 12). Hierzu wurde L-Serin in sein Methylester-Hydrochlorid überführt, sowie *N*-Boc und *O*-TBS geschützt. Der geschützte Aminosäureester **106** wurde anschließend, analog zu der Sphingosin-Synthese von Chung *et al.*,<sup>[111]</sup> in das  $\beta$ -Ketophosphonat **107** überführt. Eine HWE-Reaktion mit Tetradecanal ergab das 3-Ketosphingosin **108a**. Diastereoselektive Reduktion des Enons nach der Methode von Hoffman *et al.*<sup>[133]</sup> mittels  $\text{LiAlH}(\text{O}t\text{Bu})_3$ , gefolgt von saurer Abspaltung der TBS- und Boc-Schutzgruppen lieferte D-erythro-Sphingosin (**2a**) in einer Gesamtausbeute von 65% über 7 Stufen. Die Verwendung anderer Aldehyde bei der HWE-Reaktion erlaubt den Aufbau weiterer Sphingolipid-Derivate. So wurde das Alkin-markierte Sphingosin-Derivat **2b** unter Verwendung von Tetradec-13-inal mit einer Gesamtausbeute von 62% dargestellt.

Mithilfe von HBTU und DIPEA wurden beide Sphingoidbasen mit Carboxyferrocen (**110**), Carboxycobalocenium-hexafluorophosphat (**111**) und dem Ruthenium-Aren Komplex **112** verknüpft. Nach Aufreinigung wurden die entsprechenden Amide in guten Ausbeuten erhalten. Um die inhibitorische Aktivität der Komplexe **102** - **104** gegenüber aCDase bestimmen zu können, wurde für die Durchführung eines Fluoreszenz-Assays das Ceramidase-Substrat Rbm 14-12 (**113**) analog zur Literatur<sup>[134]</sup> innerhalb von 5 Stufen in einer Gesamtausbeute von 22% dargestellt.

Die inhibitorische Aktivität der Komplexe **102** - **104** gegenüber rekombinant gewonnener aCDase wurde anschließend untersucht. Es wurde gezeigt, dass die hier dargestellten Komplexe aCDase inhibieren, insbesondere der kationische Ruthenium-Komplex **104a** ( $\text{IC}_{50} = 23,5 \pm 0,5 \mu\text{M}$ ). Durch bioorthogonale Azid-Alkin Cycloaddition der Alkin-markierten Verbindungen und Cumarinazid konnte die Akkumulation der Komplexe (vor allem der kationischen Komplexe **103b** und **104b**) innerhalb der Lysosomen nachgewiesen werden. Alle Komplexe zeigten cytotoxische Aktivität gegenüber verschiedenen Krebszelllinien. **102** - **104** wiesen zudem weitere antiproliferative Eigenschaften auf, die nicht mit der Inhibition von aCDase korrelieren, darunter die Erhöhung der ROS Konzentration und Induktion von Caspasen 3 und 7, Apoptose vermittelnden Enzymen.



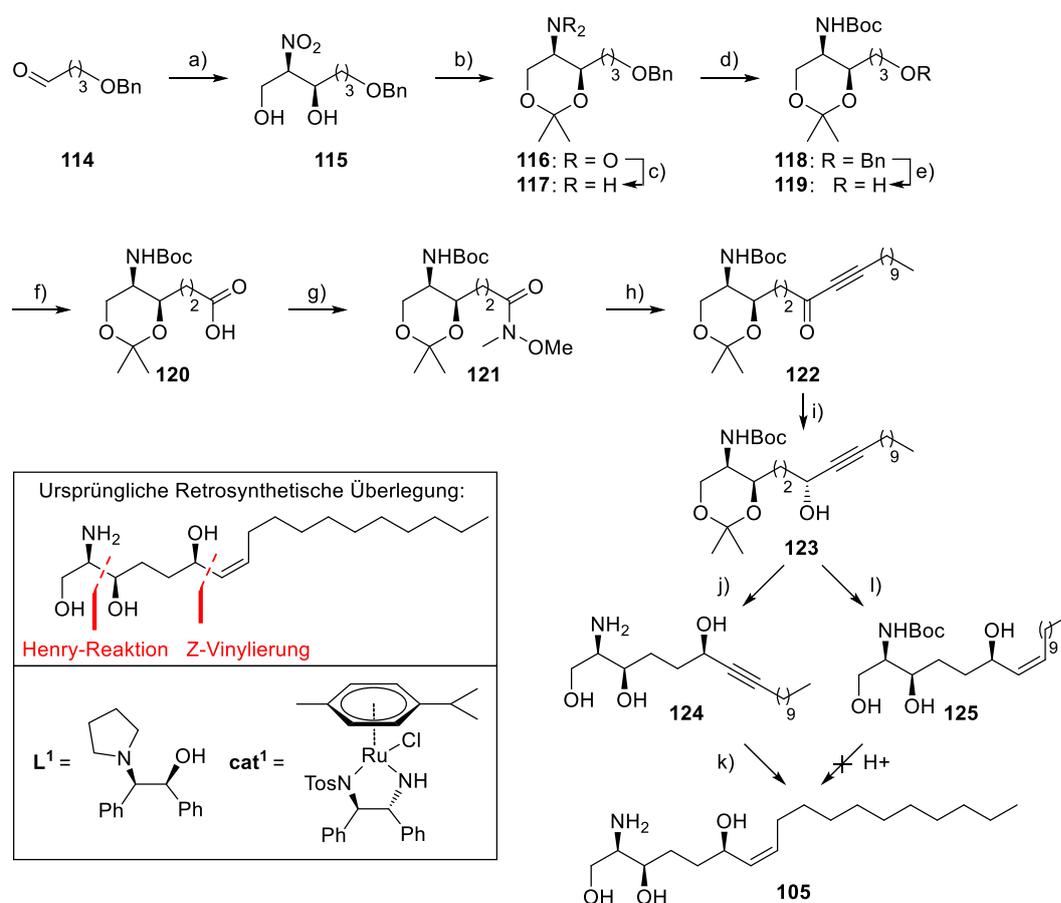
**Schema 12:** Synthese einer Bibliothek metallorganischer Ceramidase-Inhibitoren und von Rbm1 4-12. Reagenzien und Bedingungen: a) 1. SOCl<sub>2</sub>, Et<sub>2</sub>O, rf, 18 h; 2. Boc<sub>2</sub>O, Net<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 18 h; 3. TBSCl, Imidazol, DMF, rt, 18 h, 97%; b) *n*-BuLi, DMMP, THF, -78 °C, 2 h, 99%; c) Tetradecanal oder Tetradec-13-inal, Net<sub>3</sub>, LiCl, THF, rt, 24 h, 91% **108a**; 94% **108b**; d) LiAlH(O*t*Bu)<sub>3</sub>, EtOH, -78 °C, 1,5 h; **109a** 75%, **109b** 77%; e) TFA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h, **2a** 99%, **2b** 94%; f) HBTU, DIPEA, DMF, rt, 1 h, 51%-91%.

### 2.3 Synthese und antiproliferative Aktivität des Meeresschwamm-Metaboliten Halisphingosin A

Ziel des zweiten Teilprojekts war es, anhand einer enantioselektiven Erstsynthese den Zugang zu der neuartigen Sphingoidbase Halisphingosin A zu etablieren. Die geplante Syntheseroute basierte auf zwei Schlüsselreaktionen, einer *syn*- und enantioselektiven Henry-Reaktion nach der Methode von Chen *et al.*<sup>[124]</sup>, um beide Stereozentren der Aminoalkohol-Kopfgruppe innerhalb einer Stufe einzuführen, sowie der stereoselektiven Addition von *Z*-vinyl-Zinkorganylen an Aldehyde nach Walsh *et al.*<sup>[135]</sup> für den direkten Aufbau des *Z*-Allylalkohols (Schema 13). Bei ersten Modellversuchen erwies sich die katalytische Henry-Reaktion als vielversprechend, die direkte *Z*-Vinylierung gelang jedoch nur mit moderaten Enantioselektivitäten. Letzteres ist auf den geringen sterischen Anspruch des Alkyl-Restes des benötigten Aldehyds zurückzuführen. Aus diesem Grund wurde der Allylalkohol durch zweifache Reduktion eines entsprechenden Alkinons aufgebaut. Hierfür wurden der Noyori Katalysator **cat**<sup>1</sup> zur stereoselektiven Transferhydrierung des Ketons,<sup>[136-137]</sup> sowie die Nickelborid-Methode nach Brown *et al.* zur *Z*-selektiven Hydrierung des Alkins verwendet.<sup>[138]</sup>

Die Synthese von Halisphingosin A (**105**) erfolgte ausgehend von Aldehyd **114**. Die katalytische Henry-Reaktion von **114** mit Nitroethanol (**41**) unter Verwendung von Cu(OAc)<sub>2</sub> und Ligand **L**<sup>1</sup> ergab Nitrodiol **115** als Diastereomerengemisch mit einem *syn/anti*-Verhältnis von 88:12 sowie einen ee (*syn*) von 98%. Anschließend wurde der instabile Nitrodiol **115** in das Acetonid **116** überführt, was eine Trennung der Diastereomere mittels Säulenchromatographie ermöglichte. Reduktion der Nitrogruppe gefolgt von der Boc-Schützung des rohen Amins **117** und der Hydrierung der Benzyl-Schutzgruppe von **118** lieferte den Alkohol **119**. Oxidation der primären Hydroxygruppe mit RuCl<sub>3</sub>/NaIO<sub>4</sub> ergab die Carbonsäure **120**, welche zu dem Weinreb Amid **121** umgesetzt wurde. Durch Reaktion von **121** mit 1-Dodecynyl-Lithium wurde das Inon **122** dargestellt. Die Reduktion von **122** unter Verwendung von Katalysator **cat**<sup>1</sup> unter Phasentransfer-Bedingungen<sup>[139]</sup> gelang in exzellenten Ausbeuten und ergab den sekundären Alkohol **123** mit einem ee von 95%, welcher anhand der <sup>19</sup>F NMR-Spektren der entsprechenden Mosher-Ester<sup>[140]</sup> bestimmt wurde. Weiterhin wurde die absolute Konfiguration aller Stereozentren anhand der <sup>1</sup>H-NMR-Spektren der jeweiligen Mosher-Ester und -Amide bestätigt (vgl. Kapitel 4.3).<sup>[141]</sup>

*N*-Boc-Halisphingosin A (**125**) wurde durch Acetonid-Entschützung von **123** mit PPTS und nachfolgender partieller Hydrierung des Rohprodukts gemäß der Methode von Brown *et al.*<sup>[138]</sup> dargestellt. Saure Entschützung der Boc-Schutzgruppe ergab die Zielverbindung **105** jedoch nur in Form von Mischungen verschiedener Isomere. Durch komplette Entschützung von **123** mit AcCl in MeOH und nachfolgender partieller Hydrierung des Aminotriols **124** konnte Halisphingosin A (**105**) ohne die Bildung von Nebenprodukten in einer Gesamtausbeute von 29% über 11 Stufen erhalten werden.



**Schema 13:** Enantioselective Erstsynthese von (2*R*,3*R*,6*R*,7*Z*)-Halisphingosin A. Reagenzien und Bedingungen: a) Nitroethanol, Cu(OAc)<sub>2</sub>,  $L^1$ , THF, -5 °C, 48 h, 93%, *syn/anti* = 88:12, ee (*syn*) 98%; b) Me<sub>2</sub>C(OMe)<sub>2</sub>, BF<sub>3</sub>·Et<sub>2</sub>O, rt, 2 h, 85%; c) NaBH<sub>4</sub>, NiCl<sub>2</sub>, MeOH, rt, 1 h, 99%; d) Boc<sub>2</sub>O, Net<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 18 h, 96%; e) H<sub>2</sub>, Pd/C, EtOAc, rt, 1 h, 99%; f) RuCl<sub>3</sub>, NaIO<sub>4</sub>, MeCN/EtOAc/H<sub>2</sub>O, rt, 1 h, 85%; g) MeHNOMe·HCl, EDC, DMAP, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 18 h, 91%; h) Dodecin, *n*-BuLi, THF -78 °C, 1 h; dann **121**, -78 °C, 2 h, 76%; i)  $cat^1$ , NaHCO<sub>2</sub>, TBAB, H<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>, rt, 24 h, 98%, de 95%; j) AcCl, MeOH, 0 °C, 1 h, 73%; k) H<sub>2</sub>, Ni(OAc)<sub>2</sub>, NaBH<sub>4</sub>, (NH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>, EtOH, rt, 3 h, 94%; l) 1. PPTS, MeOH, rt, 18 h; 2. H<sub>2</sub>, Ni(OAc)<sub>2</sub>, NaBH<sub>4</sub>, (NH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>, EtOH, rt, 3 h, 81%.

Der spezifische Drehwert von **105** entsprach dem des natürlichen Isolats, die chemischen Verschiebungen der Atome der Kopfgruppe der <sup>1</sup>H- und <sup>13</sup>C-NMR-Spektren wichen jedoch geringfügig von den durch Mansoor *et al.* beschriebenen

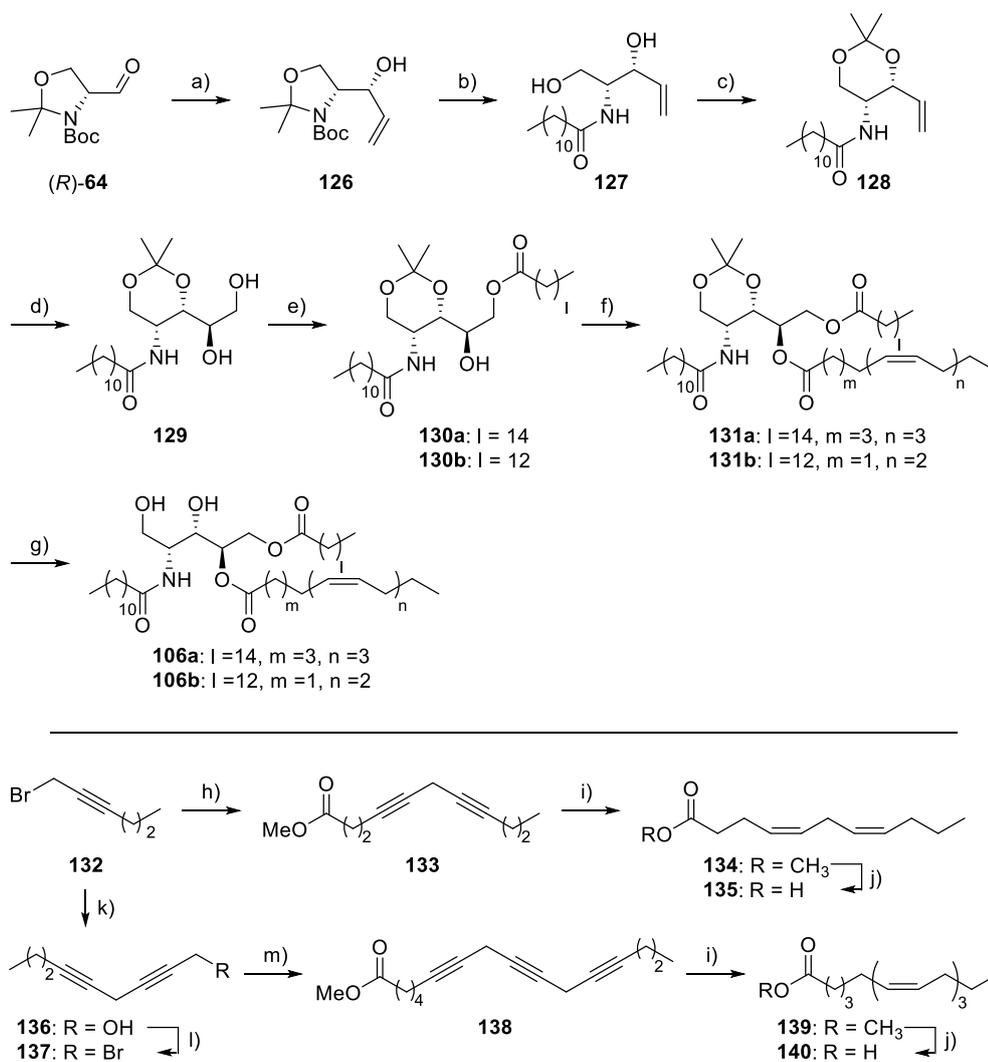
Werten ab.<sup>[128]</sup> Die chemischen Verschiebungen der Atome an C-4 und C-5 des *N*-Boc-Derivates **125** wiesen ebenfalls Abweichungen zu den von Molinski *et al.*<sup>[129]</sup> beschriebenen Werten auf. Ein möglicher Grund hierfür könnten unterschiedliche Mengen an H<sub>2</sub>O in dem NMR-Lösungsmittel Methanol-d<sub>4</sub> und damit zusammenhängende Effekte durch Aggregation bzw. Wasserstoffbrücken-Bindungen aufgrund der amphiphilen Natur von **105** sein. Eine inkorrekte Zuordnung der Stereochemie des natürlichen Isolats kann jedoch nicht gänzlich ausgeschlossen werden. Andererseits zeigte das synthetische Halisphingosin A jedoch zytotoxische Aktivität gegenüber verschiedenen Krebszelllinien im niedrigen mikromolaren Bereich, was der Aktivität des natürlichen Isolats entspricht.<sup>[128]</sup>

## 2.4 Synthese der 2*R*,3*S*,4*R*-Isomere der Tiefseemuschel-Metabolite Bathymodiolamid A und B

Ziel dieses Teilprojekts war es, eine kurze und flexible Syntheseroute zur Darstellung der stark zytotoxischen Ceramid-Analoga Bathymodiolamid A und B zu etablieren. Anhand der NMR- und Massenspektren, sowie des chemischen Abbaus der isolierten Verbindungen, wurde eine absolute Konfiguration von 2*R*,3*S*,4*R* analog zur verwandten Sphingoidbase *L-arabino*-Phytosphingosin postuliert.<sup>[131]</sup> Das Stereozentrum 2*R*, sowie die C-Atome C1-C3 sollten dem chiralen Pool in Form von *R*-Garners Aldehyd entnommen werden. Durch *syn*-selektive Vinylierung von (*R*)-**64** und diastereoselektive Dihydroxylierung der resultierenden Doppelbindung sollte das Aminotetraol-Grundgerüst und somit die beiden Stereozentren 3*S* und 4*R* aufgebaut werden. Die benötigten ungesättigten Fettsäuren sollten durch konsekutive Kupplung entsprechender Alkine und Halogenide mithilfe von Kupfersalzen, sowie *Z*-selektive Hydrierung der Alkine mittels Nickelborid nach Brown *et al.*<sup>[138]</sup> aufgebaut werden. Vinylierung von (*R*)-**64** mit Vinyl-Zinkbromid gemäß einer modifizierten Literaturmethode<sup>[142]</sup> ergab Allyl-Alkohol **126** in einem *syn/anti* Verhältnis von 5:1 (Schema 14). Da bei späterer Boc-Entschützung nach der Veresterung mit den ungesättigten Fettsäuren Probleme auftraten, wurde bereits auf der nächsten Stufe das Fettsäure-Amid eingeführt. Hierzu wurde **126** sauer entschützt und das rohe Amin mit Lauroylchlorid *N*-acyliert. Der dabei entstandene Diol **127** wurde anschließend Acetonid-geschützt. Dihydroxylierung der Doppelbindung von **128** unter Substratkontrolle mittels Kaliumosmat und NMO lieferte den Diol **129** in einem *syn/anti* Verhältnis von 1:5. Die entstandenen Diastereomere wurden mittels Säulenchromatographie voneinander getrennt und die absolute Konfiguration des neuen Stereozentrums wurde anhand der <sup>1</sup>H-NMR-Spektren der entsprechenden Mosher-Ester<sup>[141]</sup> als 4*R* bestätigt (vgl. Kapitel 4.4). Konsekutive Veresterung des primären Alkohols mit Palmitin- bzw. Myristinsäure und des sekundären Alkohols mit den entsprechenden ungesättigten Carbonsäuren **135** und **140**, gefolgt von der Entschützung des Acetonids ergab die Zielverbindungen **106a** und **106b**.

Beide Seitenketten wurden ausgehend von 1-Bromohex-2-in (**132**) dargestellt. Die Kupplung von **132** mit dem Methylester von 4-Pentinsäure ergab den Ester **133**. Partielle Hydrierung der beiden Dreifachbindungen von **133** und nachfolgende Verseifung des Methylesters von **134** lieferte die für die Synthese von Bathymodiolamid B benötigte ungesättigte Säure **135**. Die dreifachungesättigte Säure

**140** für die Synthese von Bathymodiol A wurde auf analoge Art und Weise dargestellt. Hierzu wurde **132** mit Propargylalkohol gekuppelt und die Bromierung des entstandenen Alkohols **136** ergab das Bromid **137**, welches anschließend mit dem 6-Heptinsäuremethylester gekuppelt wurde. Partielle Hydrierung der Dreifachbindungen von **138**, gefolgt der Verseifung des Methylrestes, lieferte die ungesättigte Säure **140**.



**Schema 14:** Synthese der (2*R*,3*S*,4*R*)-Isomere der Bathymodiolamide A und B. Reagenzien und Bedingungen: a) 1. Tetravinylstannan, MeLi, Et<sub>2</sub>O, 0 °C, 15 min; dann ZnBr<sub>2</sub>, Et<sub>2</sub>O, -78 °C zu rt, 2 h; 92 %, *syn/anti* 5:1; b) 1. AcCl, MeOH, rt, 30 min; 2. Laurylchlorid, Net<sub>3</sub>, THF, rt, 18 h, 99%; c) Me<sub>2</sub>C(OMe)<sub>2</sub>, BF<sub>3</sub>·Et<sub>2</sub>O, rt, 2 h, 94%; d) K<sub>2</sub>OsO<sub>4</sub>, NMO, THF/H<sub>2</sub>O, rt, 18 h, 77%, *syn/anti* 1:5; e) Palmitin- oder Myristinsäure, EDC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt, 18 h, **131a** 82%, **131b** 69%; f) **136** oder **141**, EDC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt, 18 h, **132a** 74%, **132b** 88%; g) pTsOH, MeOH, rt, 2,5 h, **106a** 94%, **106b** 97%; h) Pent-4-insäure Methylester, CuI, NaI, Cs<sub>2</sub>CO<sub>3</sub>, DMF, rt, 18 h, 68%; i) H<sub>2</sub>, Ni(OAc)<sub>2</sub>, NaBH<sub>4</sub>, (NH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>, EtOH, rt, 3 h, 95-98%; j) LiOH, THF/H<sub>2</sub>O, rt, 18 h, 88-98%; k) Propargylalkohol, CuI, NaI, Cs<sub>2</sub>CO<sub>3</sub>, DMF, rt, 18 h, 89%; l) PBr<sub>3</sub>, Pyridin, Et<sub>2</sub>O, rt, 2 h, 98%; m) Hept-6-insäure Methylester, CuI, NaI, Cs<sub>2</sub>CO<sub>3</sub>, DMF, rt, 18 h, 76%.

Neben geringen Abweichungen der spezifischen Drehwerte, weisen die NMR-spektroskopischen Daten erhebliche Unterschiede gegenüber den Literaturdaten<sup>[131]</sup> auf, wobei vor allem die chemischen Verschiebungen der Atome der Aminotetraol-Einheit von denen der natürlichen Isolate abweichen. Dies legt eine inkorrekte Zuordnung der absoluten Konfiguration der Naturstoffe nahe, die auf dem Vergleich mit analytischen Daten literaturbekannter Verbindungen basierte.<sup>[131]</sup> Weiterhin zeigten **106a** und **106b**, im Gegensatz zu den natürlichen Isolaten, nahezu keine zytotoxische Aktivität in MTT-Assays an verschiedenen Krebszelllinien. Zusammenfassend wurden die (2*R*,3*S*,4*R*)-Isomere der Bathymodiolamide A und B in Gesamtausbeuten von 38% bzw. 39% über 8 Stufen dargestellt. Die hier vorgestellte Syntheseroute ermöglicht sowohl die Synthese der anderen Stereoisomere zur Aufklärung der absoluten Konfiguration beider Naturstoffe als auch die Darstellung geeigneter Derivate, um Struktur-Wirkungs-Beziehungen zu erarbeiten.

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## 4 Darstellung des Eigenanteils und Publikationen

### 4.1 Darstellung des Eigenanteils

Die in dieser Dissertation vorgestellten Publikationen sind in Kooperation mit anderen Wissenschaftlern entstanden. Der Eigenanteil an den jeweiligen Veröffentlichungen soll im Folgenden beschrieben werden.

#### **zu Publikation I (Kapitel 4.2):**

Diese Arbeit wurde publiziert in *Bioorganic Chemistry* (*Bioorg. Chem.* **2020**, 97, 103703) unter dem Titel

#### ***N*-Metalloenoylsphingosines as targeted ceramidase inhibitors: Syntheses and antitumoral effects**

von den Autoren *Matthias Rothemund, Alexander Bär, Leonhard Köhler, Felix Klatt, Sascha Weidler, Carlo Unverzagt, Claus Kuhn und Rainer Schobert*

Diese Arbeit wurde in Kooperation mit den Lehrstühlen Bioorganische Chemie und Biochemie VI der Universität Bayreuth durchgeführt. Die Synthese der Zielverbindungen wurde in Zusammenarbeit mit Prof. Rainer Schobert geplant. Die synthetischen Arbeiten und die Charakterisierung der neuen Verbindungen wurden von mir durchgeführt. Die Experimente zur Stabilität der Verbindungen, Inhibition von saurer Ceramidase, intrazellulären Lokalisation, Zytotoxizität (MTT-Tests), Caspaseaktivierung und zu reaktiven Sauerstoffspezies, sowie die Zellzyklusanalysen und Zytoskelettfärbungen wurden von Matthias Rothemund geplant und durchgeführt. Leonhard Köhler war an der Bestimmung der Inhibition von saurer Ceramidase beteiligt. Die rekombinante Expression ASAH1 und Reinigung des Proteins wurde von Matthias Rothemund in Zusammenarbeit mit Sascha Weidler und Felix Klatt durchgeführt. Die Publikation wurde von mir in Zusammenarbeit mit Matthias Rothemund und Prof. Dr. Rainer Schobert verfasst.

**zu Publikation II (Kapitel 4.3):**

Diese Arbeit wurde publiziert in *Organic and Biomolecular Chemistry (Org. Biomol. Chem.* **2020**, *18*, 7565) unter dem Titel

**Synthesis and Anticancer Activity of the Marine Sponge Metabolite  
Halisphingosine A**

von den Autoren *Alexander Bär, Sofia I. Bär und Rainer Schobert*

Die Synthesestrategie wurde von mir erarbeitet. Die synthetischen Arbeiten, sowie die Charakterisierung der neuen Verbindungen wurden von mir durchgeführt. Die Experimente zur Bestimmung der Zytotoxizität (MTT-Tests) wurden von Sofia Bär durchgeführt. Die Publikation wurde von mir in Zusammenarbeit mit Prof. Dr. Rainer Schobert verfasst.

**zu Publikation III (Kapitel 4.4):**

Diese Arbeit wurde publiziert in *The Journal of Organic Chemistry (J. Org. Chem.* **2021**, *86*, 1868-1873) unter dem Titel

**Synthesis of the Proposed Isomers of the Deep-sea Mussel Metabolites  
Bathymodiolamides A and B**

von den Autoren *Alexander Bär, Sofia I. Bär, Moritz Röder und Rainer Schobert*

Die Synthesestrategie wurde von mir erarbeitet. Die synthetischen Arbeiten, sowie die Charakterisierung der neuen Verbindungen wurden von mir durchgeführt. Die Experimente zur Bestimmung der Zytotoxizität (MTT-Tests) wurden von Sofia Bär durchgeführt. Moritz Röder war an der Synthese der ungesättigten Fettsäuren beteiligt. Die Publikation wurde von mir in Zusammenarbeit mit Prof. Dr. Rainer Schobert verfasst.

## 4.2 Publikation I

### N-Metallocenoyl sphingosines as targeted ceramidase inhibitors: Syntheses and antitumoral effects

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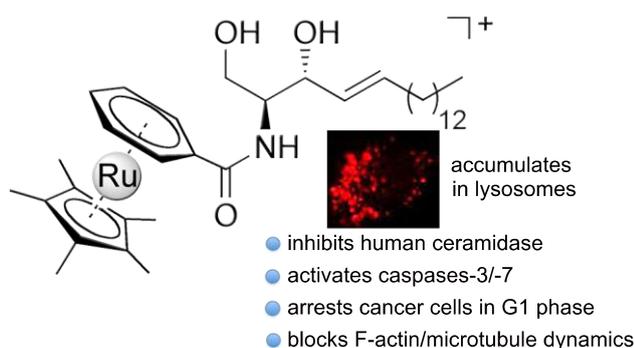
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## N-Metalloceoylsphingosines as targeted ceramidase inhibitors: Syntheses and antitumoral effects

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### ABSTRACT

Three N-metalloceoylsphingosines with variance in the central metal (Fe, Co, Ru), the charge (neutral or cationic), and the arene ligands (Cp<sub>2</sub>, Cp\*Ph) were synthesized from serine and metallocene carboxylic acids as substrate-analogous inhibitors of human acid ceramidase (AC). Their inhibitory potential was examined using the recombinant full length ASAHI enzyme, expressed and secreted from High Five insect cells, and the fluorescent substrate Rbm14-12. All complexes inhibited AC, most strongly so ruthenium(II) complex **13a**. Some antitumoral effects of the complexes, such as the interference with the microtubular and F-actin cytoskeleton of cancer cells, were correlated to their AC-inhibition, whereas others, e.g. their cytotoxicity and their induction of caspase-3/-7 activity in cancer cells, were not. All complexes accumulated preferentially in the lysosomes of cancer cells like their target AC, arrested the cells in G1 phase of the cell cycle, and displayed cytotoxicity with mostly single-digit micromolar IC<sub>50</sub> values while inducing cancer cell apoptosis.

### 1. Introduction

The sphingolipid rheostat is a delicately balanced network of sphingosine derivatives and pertinent enzymes, regulating growth, differentiation, motility, survival and apoptosis of endothelial and cancer cells [1–4]. Key players of this rheostat are the cell death promoting ceramides (**1**, cer) and sphingosine (**2a**, sph), as well as sphingosine-1-phosphate (**3**, s1p) which promotes cell survival and proliferation (Fig. 1) [5–8]. Increased levels of cer and sph are associated with greater permeability of lysosomal and mitochondrial membranes, and thus with an activation of the lysosomal and the intrinsic apoptotic pathway [9–11]. Both metabolites were reported to impair cell cycle progression. Imbalancing the rheostat led to Golgi fragmentation and loss of cell-cell contacts, and eventually to anoikis and cell starvation [12,13]. The anti-apoptotic s1p can be generated only by conversion of cer to sph by ceramidases and a subsequent phosphorylation of sph by sphingosine kinases (SK) [14,15]. In human cells three types of

ceramidases are known, called alkaline, neutral and acid ceramidase according to their pH optimum [1,2]. Due to its role in Farber's disease, a deficiency in lysosomal N-acylsphingosine-amidohydrolase activity, acid ceramidase (AC) probably is the most thoroughly investigated and most important ceramidase [16]. Ceramidases are often upregulated in cancer cells, resulting in an increase of s1p and consequently in higher survival rates and sustained cell proliferation [17–20]. Thus, the cer/s1p rheostat was recognized as a promising target for chemotherapeutic intervention in the treatment of cancer [6]. The inhibition of ceramidases is expected to lead to an accumulation of pro-apoptotic cer and to a depletion of anti-apoptotic s1p.

Early ceramidase inhibitors were structurally modelled on the natural ceramides, yet showed low activities and poor selectivities in *in vitro* assays [21–24]. Only a few derivatives showed a significant activity, e.g. analogues of N-oleylethanolamine (NOE) and the ceramide analogue (1S,2R)-D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (D-e-MAPP), whose antitumoral and ceramidase inhibitory effects were

**Abbreviations:** AC, acid ceramidase; ASAHI, N-acylsphingosine amidohydrolase 1; cer, ceramide; cpm, cells per mL; DIPEA, diisopropylethylamide; DMF, dimethylformamide; HBTU, (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); JACoP, Just another colocalization plugin; LICQ, Li's Intensity Correlation Quotient; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBT, nitroblue tetrazolium; PC, Pearson correlation coefficient; P-gp1, p-glycoprotein 1; sph, sphingosine; SK, sphingosine kinase; s1p, sphingosine-1-phosphate; vbl, vinblastine; vpm, verapamil

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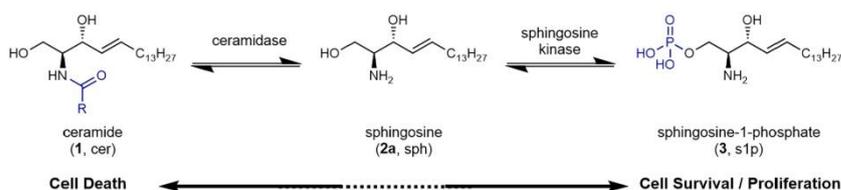


Fig. 1. Ceramide/sphingosine-1-phosphate rheostat: sphingosine-1-phosphate (3, s1p), promoting cell survival and proliferation, can be synthesized via hydrolyzation of cell death promoting ceramides (1, cer) by ceramidase to give sphingosine (2a, sph) and its downstream phosphorylation by sphingosine kinases (SK).

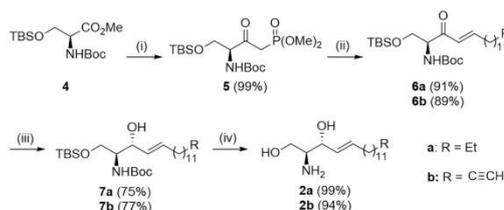
studied in detail [21,25,26]. More potent inhibitors, structurally unrelated to ceramide, were later identified by Draper et al. (e.g. ceranib-2) [27], Reaini et al. (carmofur) [28] and others [21]. A major problem with developing new ceramidase inhibitors is the low comparability of literature data. Multiple methods for determining the efficacy of ceramidase inhibitors are in use, that differ in the conditions of the cellular assays, the purity of the enzymes, and the nature of the reporter substrates. More often than not, potential inhibitors were tested not on purified enzymes but on crude cell extracts of unknown enzyme concentration.

Following our successful strategy of covalently combining natural metabolites with bioactive metal complex fragments and metallocenes in particular [29], we developed new sphingosine–metallocene conjugates and investigated their antitumoral modes of action, as well as their AC-inhibitory activity on a purified human ceramidase enzyme.

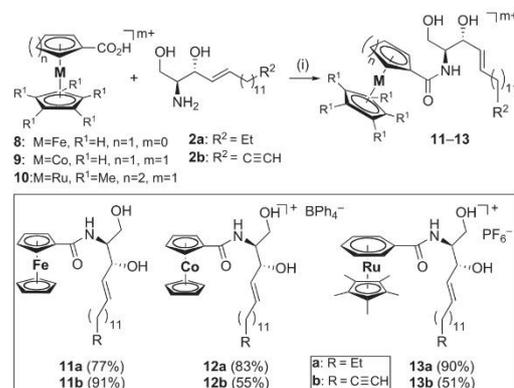
## 2. Results and discussion

### 2.1. Chemistry

A series of novel organometallic AC-inhibitor candidates was prepared by *N*-acylation of sph (2a) and alkyne-labeled sph (2b) with different metallocene carboxylic acids. The two sphingoid bases 2 were synthesized from doubly protected serine ester 4 which was treated with excess lithium dimethyl methylphosphonate to be converted to the  $\beta$ -ketophosphonate 5 in excellent yield (Scheme 1). Its HWE reactions with tetradecanal and tetradec-13-ynal using  $\text{NEt}_3$  as a base in the presence of anhydrous LiCl afforded the corresponding 3-keto-sphingosines 6a and 6b [30]. The diastereoselective reduction of the enones was achieved using  $\text{LiAlH}(\text{O}t\text{Bu})_3$  in analogy to a literature protocol [31]. Gratifyingly, only the desired *anti*-configured alcohols 7 were obtained in 75% and 77% yield, respectively. Their deprotection with TFA afforded the free sphingoid bases 2 in total yields of 67% and 64% over four steps. The sphingoid bases 2 were then linked to carboxyferrocene (8), carboxycobaltocenium hexafluorophosphate (9) and  $[(\eta^5\text{-Cp}^*)(\eta^6\text{-C}_6\text{H}_5\text{CO}_2\text{H})\text{Ru}]\text{PF}_6$  (10) using HBTU as a coupling reagent in the presence of excess DIPEA (Scheme 2). Purification by column chromatography over silica gel (for 11 and 13), or precipitation with  $\text{NaBPh}_4$  in MeOH (for 12) afforded the corresponding amides 11–13 in



Scheme 1. Synthesis of sphingoid bases 2a and 2b. Reagents and conditions: (i) *n*-BuLi, dimethyl methylphosphonate, THF,  $-78^\circ\text{C}$ , 2 h; (ii) tetradecanal or tetradec-13-ynal,  $\text{NEt}_3$ , LiCl, THF, r.t., 24 h; (iii)  $\text{LiAlH}(\text{O}t\text{Bu})_3$ , EtOH,  $-78^\circ\text{C}$ , 1.5 h; (iv) TFA,  $\text{CH}_2\text{Cl}_2$ , r.t., 1 h.



Scheme 2. Synthesis of organometallic ceramide analogues 11–13. Reagents and conditions: (i) HBTU, DIPEA, DMF, 1 h, r.t.

good yields.

To estimate the stability of the *N*-metallocenoylsphingosines 11a, 12a and 13a under biological conditions, UV–vis spectra of their solutions in a water–DMF (99:1) mixture were recorded over the course of 48 h (ESI Figure S1). They showed distinct spectra with a maximum at  $\lambda = 240\text{--}260\text{ nm}$  (11a, 13a), or two absorption maxima in the same range (12a). While the shape of the spectral curves remained largely the same, their height (= absorbance) diminished over time, most rapidly so for the cobalt complex 12a. This observation can be rationalized by a decreasing concentration of the amphiphilic complexes due to partial precipitation or micelle formation. However, all complexes appear to be sufficiently long-lived to be taken up by cancer cells and to reach their biological targets [32].

### 2.2. Inhibition of purified acid ceramidase

The *N*-metallocenoylsphingosines 11a, 12a, and 13a were tested for their ability to inhibit human AC (ASAH1) in vitro. Using a fluorescence based assay developed by Bedia et al. [16] we measured the  $\text{IC}_{50}$  values for the inhibition of AC by complexes 11a, 12a and 13a (Fig. 2). In contrast to the original work by Bedia et al. we expressed human acid ceramidase recombinantly in High Five insect cells and purified the enzyme by affinity chromatography and gel filtration, instead of using a crude cell extract (cf Supporting Information). In this way we were able to control the exact amount of enzyme used in our assays, rather than relying on an approximation for its quantification. By a modified variant of Bedia's fluorescence assay, we measured a  $K_M$  value of  $14\ \mu\text{M}$  for the affinity of our purified enzyme for the Rbm14-12 substrate, compared to  $26\ \mu\text{M}$  for the affinity of Bedia's enzyme sample. Using this measured  $K_M$  value as a basis for the Rbm14-12 substrate concentration, we found ruthenium complex 13a ( $23.5 \pm 0.5\ \mu\text{M}$ ) to be the best AC inhibitor, while complexes 11a ( $142.6 \pm 7.6\ \mu\text{M}$ ) and 12a

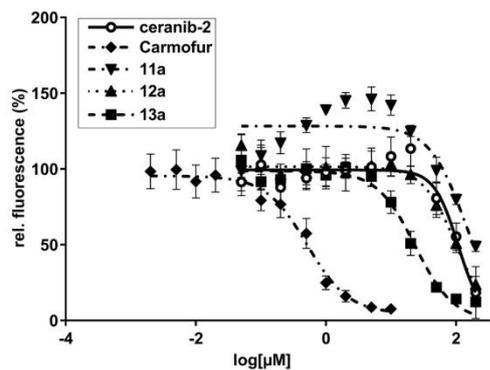


Fig. 2. Inhibition of AC (0.05  $\mu\text{g}$ ) after 30 min of incubation with ceranib-2, 11a, 12a or 13a at 37  $^{\circ}\text{C}$  at a final concentration range of 200–0.05  $\mu\text{M}$ , or 10  $\mu\text{M}$ –2 nM of carmofur. Means  $\pm$  SD from four independent values [16].

(98.1  $\pm$  14.0  $\mu\text{M}$ ) were approximately five to seven times less active. The  $\text{IC}_{50}$  value of ca. 110  $\mu\text{M}$  obtained for ceranib-2 under these assay conditions is not reliable due to its limited solubility. In earlier inhibition assays with cellular ceramidase enzymes under less well-defined conditions ceranib-2 was active with an  $\text{IC}_{50}$  = 28  $\mu\text{M}$  [27]. For the better soluble, known AC-inhibitor carmofur [33] we measured, as expected, a superior activity with  $\text{IC}_{50}$  = 0.55  $\pm$  0.09  $\mu\text{M}$ .

### 2.3. Intracellular localization

Acid ceramidases are predominantly found in lysosomes or nuclei of mammalian cells [34–36]. To test if complexes 11a, 12a or 13a not only inhibit AC but actually accumulate in these organelles we treated 518A2 melanoma cells with the alkyne-tagged *N*-metallocoenylsphingosines 11b, 12b, and 13b (30  $\mu\text{M}$ , 0.5 h). Due to the bioorthogonality of the acetylene group, these derivatives behave just like their saturated *a*-analogues. We tracked their intracellular localization by “clicking” them with a mixture of 3-azido-7-hydroxycoumarin, sodium ascorbate and copper sulphate to form a fluorescent triazole which was visualized by confocal fluorescence microscopy (Fig. 3) [37–41]. We found all three complexes to colocalize with lysosomes. The neutral ferrocenyl complex 11b was also present in other cell compartments, resulting in a lower Pearson correlation coefficient (PC = 1 for a complete match; PC = 0.56 for 11b). Due to overlapping fluorescence spectra of the triazole products and stained nuclei, their co-staining was not possible. In contrast to 11b, the positively charged cobalt and ruthenium complexes 12b and 13b were found mainly in the lysosomes, resulting in high values for PC and Li’s Intensity Correlation Coefficient (12b: PC = 0.92, LICQ = 0.47; 13b: PC = 0.95, LICQ = 0.42).

### 2.4. Inhibition of cancer cell growth

Having shown that the *N*-metallocoenylsphingosines inhibit AC and accumulate mainly in AC-rich lysosomes of cancer cells, we studied them for other antitumoral effects and their correlation with AC-inhibition. The cytotoxicities of complexes 11a, 12a, 13a and their alkyne derivatives 11b, 12b, 13b, of sph, and of the known ceramidase inhibitor ceranib-2 were determined in MTT-assays against nine human cancer cell lines of four entities, including the HCT116 p53 knock out mutant HCT116<sup>-/-</sup> and the P-gp1 overexpressing multi-drug resistant (mdr) cell line KB-V1<sup>Vbl</sup>. The resulting  $\text{IC}_{50}$  values are listed in table 1. All complexes were active with  $\text{IC}_{50}$  values in the low micromolar range. Except for the HCT116 cells, ferrocene 11a was the most active metal complex. None of the three *a*-type complexes appears to be a

substrate of efflux transporter P-gp1 since each showed similar activities against the mdr KB-V1<sup>Vbl</sup> cervix carcinoma cells, both in the presence and absence of the competitive P-gp1 inhibitor verapamil. In the HCT116 wildtype cell line (HCT116<sup>wt</sup>) 12a was the most active complex, whereas in the p53 knock-out mutant (HCT116<sup>-/-</sup>) Ru complex 13a was most active, again with low single-digit micromolar  $\text{IC}_{50}$  values. None of the complexes was as cytotoxic as ceranib-2.

### 2.5. Cell cycle progression

The effect of 11a, 12a, 13a, sph and ceranib-2 on the cell cycle of 518A2 melanoma cells was investigated by flow cytometry (Fig. 4). Upon treatment for 24 h with varying concentrations, all complexes arrested the melanoma cells in G1-phase. While ferrocene complex 11a showed effects only at 10  $\mu\text{M}$ , the cobaltocenium and ruthenium arene complexes 12a and 13a already had a noticeable impact on the cell cycle at a concentration of 2  $\mu\text{M}$ . This is at variance with the results of the MTT assays. A higher concentration of 11a was also necessary to cause distinct effects in other biological assays, as discussed below. Compounds 12a and 13a seem to arrest the melanoma cells during the G1 phase without causing cell death over the course of 24 h. Sph had a negligible effect on the cell cycle, increasing the cell population in G1-phase only slightly. Ceranib-2 showed the greatest effect, arresting cells at submicromolar concentrations in S- and G2-phase which hints at a different mode of action. Neither sph nor ceranib-2 induced cell death at these concentrations.

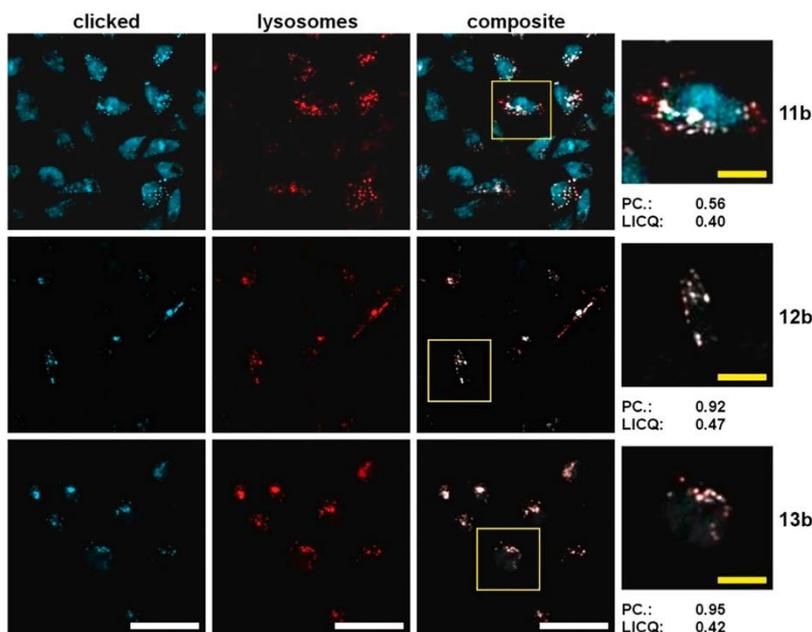
### 2.6. Reactive oxygen species (ROS)

Ceramides can have an indirect impact on the function of membrane associated proteins. Moreover, ceramide accumulation is associated with elevated ROS levels, due to deregulated mitochondrial functions [11]. We monitored ROS levels in 518A2 melanoma cells treated with complexes 11a, 12a and 13a using the nitroblue tetrazolium (NBT) assay. In comparison to the solvent control all complexes caused an increase in ROS levels after 24 h of incubation, accompanied by a reduced cell vitality. Complexes 11a and 13a doubled the ROS levels at the highest concentration of 10  $\mu\text{M}$ , which is less than their  $\text{IC}_{50}$  (24 h) values. In contrast, the cobalt complex 12a nearly quadrupled the ROS per vital cell at this concentration, which is, however, about twice its  $\text{IC}_{50}$  (24 h) value (Fig. 5).

### 2.7. Interference with cytoskeleton

The microtubules and F-actin components of the cytoskeleton of 518A2 melanoma cells, previously treated with complexes 11a, 12a, or 13a at concentrations close to their  $\text{IC}_{50}$  values, were visualized by immunofluorescence staining and the results were documented in fluorescence images (Fig. 6). While the microtubules were evenly distributed in control cells, they seemed to be focussed near cell nuclei and to radiate toward peripheral cell compartments in cells treated with the metal complexes. The microtubules of cells treated with ruthenium complex 13a appeared to have lost the regular filamentous structure visible in control cells. All complexes strongly influenced the organization of the actin cytoskeleton, causing stress fiber formation and depolymerization of actin filaments throughout the cells.

As mentioned in the introduction, increased levels of ceramides are frequently associated with Golgi fragmentation. Thus, we examined 518A2 melanoma cells treated with 11a, 12a or 13a for such effects by staining their nuclei with DAPI (blue) and by staining  $\alpha$ -*N*-acetylglucosamine residues in their Golgi apparatus with Alexa Fluor 647 HPA lectin antibody (Supporting Information, Figure S2). In comparison to the control, treated cells indeed revealed a Golgi apparatus that was more diffuse around the nucleus, hinting at its fragmentation.



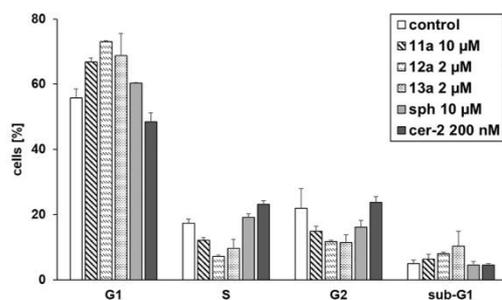
**Fig. 3.** Colocalization of alkyne-labeled complexes **11b**, **12b**, **13b** (30  $\mu\text{M}$ , 0.5 h) and lysosomes (red) in 518A2 melanoma cells after “click” reaction with 3-azido-7-hydroxycoumarin to give triazoles (cyan). Yellow box: cell that was analyzed for PC and LICQ values using ImageJ (JaCOPI) [42–44]; PC, Pearson Coefficient; LICQ, Li’s Intensity Correlation Quotient. Scales: white: 50  $\mu\text{m}$ ; yellow 15  $\mu\text{m}$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 2.8. Induction of caspase activity

Caspase-3 and -7 are often activated upon deregulation of the cer/s1p rheostat, especially by elevated levels of cer or sph. By means of Apo-One® Homogeneous Caspase-3/-7 Assays (Promega) we ascertained that all complexes **11a**, **12a** and **13a** caused caspase-3/-7 activation in 518A2 melanoma cells, most strongly so the cobaltocenium complex **12a**, which was, however, still inferior to the established apoptosis inducer staurosporine (Fig. 7)

### 3. Conclusions

The replacement of the fatty acyl residue in ceramides by metallo-acyl residues led to metallo-drugs **11–13** with properties distinctly different from those of ceramides. Our main objective, to find new substrate-like, targeted inhibitors of ceramidase was realized with ruthenium complex **13a**. It inhibited purified human AC with an  $\text{IC}_{50}$  of



**Fig. 4.** Cell cycle analysis of 518A2 melanoma cells, treated with **11a** (10  $\mu\text{M}$ ), **12a** (2  $\mu\text{M}$ ), **13a** (2  $\mu\text{M}$ ), sph (10  $\mu\text{M}$ ), cer2=ceranib-2 (200 nM) or the solvent as control for 24 h.

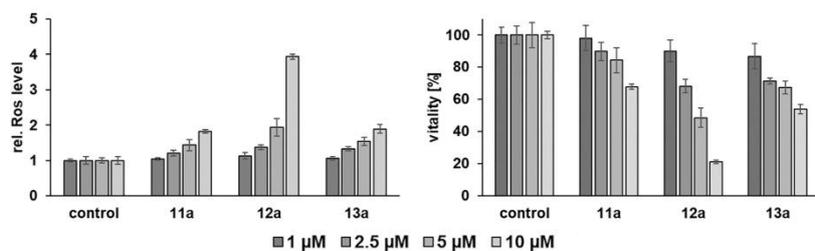
**Table 1**

Means  $\pm$  SD of  $\text{IC}_{50}$  values [ $\mu\text{M}$ ] of compounds **11–13**, sph, and ceranib-2 in MTT assays with human cell lines<sup>a</sup> after 72 h of incubation as calculated from four independent measurements.

	Cell lines									
	518A2	HT-29	HCT116 <sup>wt</sup>	HCT116 <sup>-/-</sup>	DLD-1	EaHy.926	U87	Hela	Kb-V1 <sup>Vbl b</sup>	Kb-V1 <sup>Vbl + vpm b</sup>
<b>11a</b>	5.0 $\pm$ 0.1	1.4 $\pm$ 0.3	6.6 $\pm$ 0.7	11.9 $\pm$ 0.2	7.3 $\pm$ 0.2	4.7 $\pm$ 0.2	3.3 $\pm$ 0.7	4.2 $\pm$ 0.3	7.5 $\pm$ 0.4	4.4 $\pm$ 0.5
<b>11b</b>	3.6 $\pm$ 0.5	4.1 $\pm$ 0.2	10.0 $\pm$ 0.1	11.8 $\pm$ 0.5	13.0 $\pm$ 0.6	6.5 $\pm$ 0.3	4.5 $\pm$ 0.1	5.7 $\pm$ 0.2	7.6 $\pm$ 0.9	4.9 $\pm$ 0.5
<b>12a</b>	5.8 $\pm$ 0.2	4.3 $\pm$ 0.5	2.4 $\pm$ 0.2	6.3 $\pm$ 0.3	10.0 $\pm$ 0.4	7.3 $\pm$ 0.5	5.7 $\pm$ 0.5	10.3 $\pm$ 0.8	20.4 $\pm$ 0.7	12.0 $\pm$ 0.6
<b>12b</b>	5.7 $\pm$ 0.8	6.3 $\pm$ 0.3	8.9 $\pm$ 0.4	3.2 $\pm$ 0.7	17.4 $\pm$ 1.0	6.6 $\pm$ 0.3	8.4 $\pm$ 1.0	11.4 $\pm$ 1.0	31.2 $\pm$ 1.3	24.5 $\pm$ 0.4
<b>13a</b>	7.6 $\pm$ 0.1	10.6 $\pm$ 0.2	9.4 $\pm$ 0.3	2.8 $\pm$ 0.1	12.8 $\pm$ 0.8	5.2 $\pm$ 0.6	7.6 $\pm$ 1.0	5.6 $\pm$ 0.2	27.4 $\pm$ 0.4	32.9 $\pm$ 1.7
<b>13b</b>	5.7 $\pm$ 0.9	27.3 $\pm$ 0.5	27.8 $\pm$ 0.3	16.9 $\pm$ 0.5	20.9 $\pm$ 2.2	27.4 $\pm$ 0.3	21.5 $\pm$ 0.7	28.8 $\pm$ 0.4	31.7 $\pm$ 3.0	44.2 $\pm$ 2.8
sph	11.9 $\pm$ 0.9	6.4 $\pm$ 0.4	12.7 $\pm$ 0.7	15.6 $\pm$ 0.7	16.6 $\pm$ 1.9	21.8 $\pm$ 0.9	19.1 $\pm$ 1.5	31.4 $\pm$ 0.6	9.5 $\pm$ 1.8	4.7 $\pm$ 0.4
ceranib-2	0.70 $\pm$ 0.03	0.59 $\pm$ 0.04	1.2 $\pm$ 0.0	0.78 $\pm$ 0.05	0.69 $\pm$ 0.08	0.20 $\pm$ 0.05	3.1 $\pm$ 0.2	0.63 $\pm$ 0.11	0.79 $\pm$ 0.11	0.69 $\pm$ 0.02

<sup>a</sup> 518A2 – melanoma, HT-29 – colon adenocarcinoma, HCT116<sup>wt</sup> – colon carcinoma (wildtype), HCT116<sup>-/-</sup> – colon carcinoma (p53 knock-out mutant), DLD-1 – Dukes type C colorectal adenocarcinoma, Ea.Hy926 – endothelial hybrid, U87-MG – likely glioblastoma, Hela – cervix carcinoma, Kb-V1<sup>Vbl</sup> – cervix carcinoma.

<sup>b</sup> Vbl – vinblastine, vpm – verapamil.



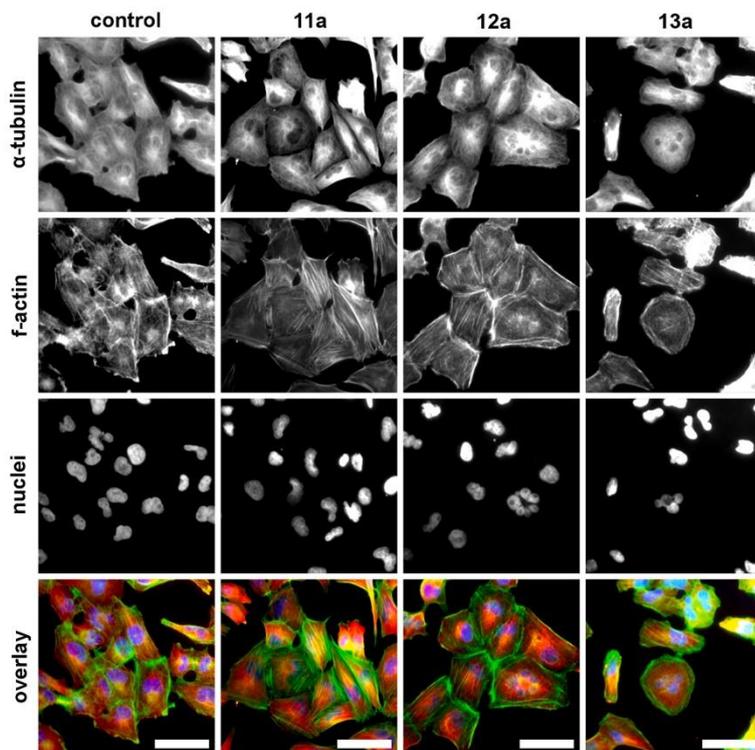
**Fig. 5.** Left: Relative levels of reactive oxygen species (ROS) in 518A2 melanoma cells, after 24 h incubation with 1, 2.5, 5 or 10  $\mu\text{M}$  of **11a**, **12a** or **13a** or the solvent as control, calculated in relation to the percentage of vital cells. Right: Vitality of 518A2 melanoma cells from analogously conducted MTT proliferation assays. Means  $\pm$  SD from three independent values.

ca. 23  $\mu\text{M}$ , a value which, though inferior by a factor of 10 to that of dedicated AC-inhibitors such as carmofur, is pharmacologically meaningful. The distinctly weaker ceramidase inhibition by the neutral ferrocene conjugate **11a** and the “cationic” cobaltocenium derivative **12a** indicates that the steric demand of the arene ligands might be more decisive for their interference with the target enzyme than the complex charge and the nature of the central metal. The complex charge, however, seems to be crucial for the intracellular accumulation: the cationic cobalt and ruthenium complexes readily accumulated in lysosomes, whereas the neutral ferrocene complex **11a** was also found in the cytosol. Thus, our data suggest that the next generation of AC-inhibitors of this type should carry bulky ligands and a positive charge in order to accumulate at sites rich in the target enzyme.

Concerning the antiproliferative activity, the less effective AC-

inhibitors **11a** and **12a** were on average slightly more antiproliferative against our cancer cell lines than complex **13a**. This indicates that ceramidase inhibition is only one aspect among several antitumoral factors. All three metallocenoylsphingosines **11–13** showed additional antitumoral effects, whose intensities are also not stringently correlated with their AC-inhibitory potential. For instance, the best AC-inhibitor **13a** also had the most pronounced impact on the cytoskeleton of melanoma cells, while the cobalt complex was the strongest inducer of caspase-3/-7 activity and ROS production in these cells.

The results of this study warrant a broader screening of a library of (cationic) metallocenoylsphingosines with variance in the arene ligands to establish a structure–activity relation and to identify those secondary effects that are most closely associated with AC-inhibition.



**Fig. 6.** Immunofluorescence staining of microtubules (red), actin filaments (green) and nuclei (blue) of 518A2 melanoma cells after 24 h of incubation with **11a** (10  $\mu\text{M}$ ), **12a** (5  $\mu\text{M}$ ), **13a** (5  $\mu\text{M}$ ), or the solvent as control. Scale: 50  $\mu\text{m}$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

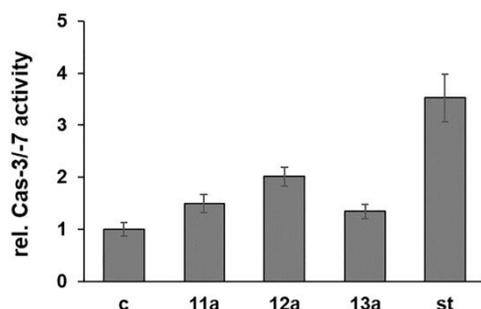


Fig. 7. Activation of caspase-3 and -7 after 4 h of incubation with 11a (10  $\mu$ M), 12a (5  $\mu$ M), 13a (5  $\mu$ M); negative control: DMF; positive control: staurosporine (st, 2  $\mu$ M). Means  $\pm$  SD from three independent experiments.

#### 4. Experimental

##### 4.1. Chemistry

Melting points are uncorrected; IR spectra were recorded on an FT-IR spectrometer with ATR sampling unit; NMR spectra were run on a 500 MHz spectrometer; chemical shifts are given in ppm ( $\delta$ ) downfield from tetramethylsilane as internal standard; Mass spectra: direct inlet, EI, 70 eV; HRMS: UPLC/Orbitrap MS system in ESI mode; Microanalyses: Elementar Unicube analyzer. Specific optical rotations were measured at 589 nm (Na-D-line) and are given in  $\text{deg cm}^3 \text{g}^{-1} \text{dm}^{-1}$ . Absorption and fluorescence measurements were obtained using a plate reader (Tecan Infinite F200). Cell cycle analysis was done using a flow cytometer (Beckman Coulter Cytomics FC500). Fluorescence images were obtained using an AxioCAM MRM (Zeiss, AxioPlan2) or a Leica TCS SP5 confocal microscope. Serine ester **4** [45], tetradecanal [46], tetradec-13-ynal [47], **9** [48], **10** [49], and Rbm14-12 [50] were prepared according to literature procedures. All tested compounds were  $> 95\%$  pure by elemental analysis and/or UPLC-HRMS.

##### 4.1.1. *tert*-Butyl (*S*)-1-((*tert*-Butyldimethylsilyloxy)-4-(dimethoxyphosphoryl)-3-oxobutan-2-yl)carbamate (**5**)

A solution of dimethyl methylphosphonate (5.34 mL, 49.5 mmol, 3.3 equiv) in THF (150 mL) at  $-78^\circ\text{C}$  was treated with *n*-BuLi (2.5 M in hexane, 19.2 mL, 48.0 mmol, 3.2 equiv). After stirring for 30 min, a solution of **4** (5.0 g, 15.0 mmol, 1.0 equiv) in THF (50 mL) was added dropwise. The mixture was stirred for 1 h at  $-78^\circ\text{C}$ . Then sat. aq.  $\text{NH}_4\text{Cl}$  (200 mL) was added, the phases were separated, and the aqueous phase was extracted with EtOAc ( $2 \times 100$  mL). The combined organic phases were washed with brine (300 mL), dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, *n*-hexane/ethyl acetate 1:1) to give **5** as a colorless oil. Yield: 6.31 g (14.8 mmol, 99%).  $[\alpha]_D^{25} = +62.3$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 5.53$  (d,  $J = 7.6$  Hz, 1H), 4.41–4.49 (m, 1H), 4.07 (dd,  $J = 10.4$ , 3.7 Hz, 1H), 3.84 (dd,  $J = 10.4$ , 4.0 Hz, 1H), 3.80 (d,  $J = 2.9$  Hz, 3H), 3.78 (d,  $J = 2.9$  Hz, 3H), 3.41 (dd,  $J = 22.0$ , 14.6 Hz, 1H), 3.14 (dd,  $J = 22.0$ , 14.6 Hz, 1H), 1.45 (s, 9H), 0.86 (s, 9H), 0.04 (s, 6H).  $^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.7$ , 155.2, 79.9, 63.0, 61.9, 60.4, 53.1, 38.5 (d,  $^1J(\text{C,P}) = 131.7$  Hz), 28.3, 25.7, 18.2,  $-5.6$ . HRMS (ESI):  $m/z$  calculated for  $\text{C}_{17}\text{H}_{30}\text{O}_7\text{NPSi} + \text{Na}^+$  [ $\text{M} + \text{Na}^+$ ]: 448.1891. Found: 448.1890.

##### 4.1.2. *tert*-Butyl (*S,E*)-1-((*tert*-Butyldimethylsilyloxy)-3-oxooctadec-4-en-17-yn-2-yl)carbamate (**6b**)

$\text{NEt}_3$  (3.1 mL, 22.44 mmol, 3.0 equiv) was added to a solution of **5** (3.18 g, 7.46 mmol, 1.0 equiv), LiCl (0.95 g, 22.44 mmol, 3.0 equiv) and tetradec-13-ynal (3.12 g, 15.0 mmol, 2.0 equiv) in THF (150 mL)

and the resulting mixture was stirred at room temperature for 18 h. Then 1 M citric acid (150 mL) was added, the phases were separated and the aqueous phase was extracted with EtOAc ( $2 \times 100$  mL). The combined organic phases were washed with brine (300 mL), dried over  $\text{MgSO}_4$ , and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, *n*-hexane/ethyl acetate 30:1) to leave **6b** as a colorless oil. Yield: 3.38 g (6.66 mmol, 89%).  $[\alpha]_D^{25} = +43.4$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 6.95$  (dt,  $J = 15.8$ , 7.0 Hz, 1H), 6.26 (d,  $J = 15.8$  Hz, 1H), 4.53 (dt,  $J = 7.4$ , 4.0 Hz, 1H), 3.96 (dd,  $J = 10.4$ , 4.0 Hz, 1H), 3.82 (dd,  $J = 10.4$ , 4.0 Hz, 1H), 2.21 (q,  $J = 7.0$  Hz, 2H), 2.17 (td,  $J = 7.1$ , 2.6 Hz, 2H), 1.92 (t,  $J = 2.6$  Hz, 1H), 1.47–1.55 (m, 2H), 1.43 (m, 11H), 1.34–1.40 (m, 2H), 1.22–1.32 (m, 12H), 0.81–0.85 (m, 9H),  $-0.01$  (m, 6H).  $^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ ):  $\delta = 196.4$ , 155.3, 149.2, 126.9, 79.6, 68.0, 63.6, 59.4, 32.6, 29.5, 29.4, 29.35, 29.2, 29.1, 28.7, 28.35, 28.3, 28.0, 25.7, 18.4, 18.2. HRMS (ESI):  $m/z$  calculated for  $\text{C}_{29}\text{H}_{55}\text{O}_4\text{NSi} + \text{Na}^+$  [ $\text{M} + \text{Na}^+$ ]: 530.3636. Found: 530.3627.

##### 4.1.3. *tert*-Butyl ((2*S,E*)-1-((*tert*-Butyldimethylsilyloxy)-3-hydroxyoctadec-4-en-17-yn-2-yl)carbamate (**7b**)

**7b** was prepared according to a literature procedure [31] from **6b** (1.33 g, 2.60 mmol, 1.0 equiv) and  $\text{LiAlH}(\text{O}^t\text{Bu})_3$  (1.32 g, 5.20 mmol, 2.0 equiv) as a colorless oil. Yield: 1.02 g (2.00 mmol, 77%).  $[\alpha]_D^{25} = +16.6$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 5.75$  (dtd,  $J = 15.3$ , 6.9, 0.9 Hz, 1H), 5.50 (dd,  $J = 15.3$  Hz, 5.9, 2H), 5.24 (d,  $J = 8.2$  Hz, 1H), 4.16–4.22 (m, 1H), 3.93 (dd,  $J = 10.2$ , 3.1 Hz, 1H), 3.75 (dd,  $J = 10.2$ , 2.4 Hz, 1H), 3.53–3.60 (m, 1H), 3.33 (d,  $J = 8.24$  Hz, 1H), 2.17 (dt,  $J = 7.2$ , 2.7 Hz, 2H), 2.03–2.07 (m, 2H), 1.93 (t,  $J = 2.7$  Hz, 1H), 1.48–1.55 (m, 2H), 1.44 (s, 9H), 1.33–1.41 (m, 4H), 1.26 (br. s., 12H), 0.89 (s, 9H), 0.06 (d,  $J = 1.53$  Hz, 6H).  $^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ ):  $\delta = 155.8$ , 133.0, 129.4, 84.8, 79.4, 74.66, 68.0, 63.5, 54.4, 32.3, 29.6, 29.55, 29.5, 29.2, 29.1, 28.7, 28.5, 28.4, 25.8, 18.4, 18.1. HRMS (ESI):  $m/z$  calculated for  $\text{C}_{29}\text{H}_{55}\text{O}_4\text{NSi} + \text{Na}^+$  [ $\text{M} + \text{Na}^+$ ]: 532.3793. Found: 532.3784.

##### 4.1.4. (2*S,E*)-2-Aminoctadec-4-en-17-yn-1,3-diol (**2b**)

TFA (20 mL) was added to a solution of **7b** (1.89 g, 3.73 mmol, 1.0 equiv) in  $\text{CH}_2\text{Cl}_2$  (20 mL) at  $0^\circ\text{C}$ . The mixture was stirred at room temperature for 1.5 h. Volatiles were removed *in vacuo*. To remove excess TFA the residue was dissolved in MeOH (10 mL) and concentrated at reduced pressure. This was repeated twice. The remaining residue was dissolved in 1 M NaOH (10 mL) and extracted with EtOAc ( $3 \times 10$  mL). The combined organic phases were dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel,  $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$  95:5:1) to give **2b** as a white solid. Yield: 1.04 g (3.50 mmol, 94%). mp =  $69^\circ\text{C}$ .  $[\alpha]_D^{25} = +8.9$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 5.76$  (dtd,  $J = 15.5$ , 6.8, 0.6 Hz, 1H), 5.47 (ddt,  $J = 15.5$ , 6.8, 1.2 Hz, 1H), 4.05 (t,  $J = 6.8$  Hz, 1H), 3.66 (qd,  $J = 10.8$ , 5.3 Hz, 2H), 2.86 (q,  $J = 5.3$  Hz, 1H), 2.18 (td,  $J = 7.2$ , 2.7 Hz, 2H), 2.06 (q,  $J = 6.8$  Hz, 2H), 1.94 (t,  $J = 2.7$  Hz, 1H), 1.48–1.58 (m, 2H), 1.34–1.43 (m, 4H), 1.24–1.33 ppm (m, 12H).  $^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ ):  $\delta = 134.7$ , 129.3, 84.8, 75.5, 68.0, 64.1, 56.1, 32.3, 29.55, 29.5, 29.4, 29.2, 29.1, 28.7, 28.6, 18.4. HRMS (ESI):  $m/z$  calculated for  $\text{C}_{18}\text{H}_{34}\text{O}_2\text{N} [\text{M} + \text{H}^+]$ : 296.2576. Found: 296.2584.

##### 4.1.5. General procedure for the synthesis of complexes 11–13 from 2

To a solution of the respective organometallic carboxylic acid **8–10** (1.0 equiv) in dry dimethylformamide (0.05 M), diisopropylethylamine (2.0 equiv), HBTU (1.1 equiv) and the corresponding sphingoid base **2** (1.1 equiv) were added at  $0^\circ\text{C}$ . The mixture was stirred for 1 h while it was allowed to reach room temperature. Volatiles were removed under reduced pressure and the remainder was dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL), washed with 1 M NaOH (10 mL), 1 M aqueous HCl (10 mL), and water (10 mL). The organic phase was dried ( $\text{MgSO}_4$ ) and evaporated to dryness.

#### 4.1.6. *N*-((2*S*,3*R*,*E*)-1,3-Dihydroxyoctadec-4-en-2-yl)ferrocenecarboxamide (**11a**)

**11a** was prepared according to the general procedure from **8** (50 mg, 0.22 mmol), DIPEA (74  $\mu$ L, 0.43 mmol), HBTU (91 mg, 0.24 mmol) and **2a** (71 mg, 0.24 mmol). The crude product was purified by flash chromatography (silica gel, *n*-hexane/acetone 2:1) to give an orange solid. Yield: 86 mg (0.17 mmol, 77%). mp = 81 °C.  $[\alpha]_D^{25} = +7.7$  (c = 0.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 6.58 (d, *J* = 7.6 Hz, 1H), 5.83 (dt, *J* = 15.5, 7.0 Hz, 1H), 5.57 (dd, *J* = 15.5, 6.4 Hz, 1H), 4.71 (d, *J* = 8.9 Hz, 2H), 4.39–4.37 (m, 1H), 4.35 (s, 2H), 4.20–4.23 (m, 5H), 4.01–4.06 (m, 1H), 3.96 (dd, *J* = 11.3, 4.0 Hz, 1H), 3.76 (dd, *J* = 11.3, 2.7 Hz, 1H), 3.56 (br. s., 2H), 2.06 (q, *J* = 7.0 Hz, 2H), 1.33–1.41 (m, 2H), 1.20–1.32 (m, 20H), 0.87 ppm (t, *J* = 6.9 Hz, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 171.6, 134.2, 128.8, 75.3, 74.1, 70.7, 69.9, 68.4, 68.3, 62.4, 54.9, 32.3, 31.9, 29.7, 29.6, 29.6, 29.5, 29.3, 29.2, 29.2, 22.7, 14.1. MS (ESI<sup>+</sup>): *m/z* 512.4 [M+H<sup>+</sup>]. Elemental analysis calculated (%) for C<sub>29</sub>H<sub>45</sub>FeNO<sub>3</sub>: C 68.09, H 8.87, N 2.74. Found: C 68.33, H 8.60, N 3.01.

#### 4.1.7. *N*-((2*S*,3*R*,*E*)-1,3-Dihydroxyoctadec-4-en-17-yn-2-yl)ferrocenecarboxamide (**11b**)

**11b** was prepared according to the general procedure from **8** (50 mg, 0.22 mmol), DIPEA (74  $\mu$ L, 0.43 mmol), HBTU (91 mg, 0.24 mmol) and **2b** (71 mg, 0.24 mmol). The crude product was purified by flash chromatography (silica gel, *n*-hexane/acetone 2:1) to leave an orange solid. Yield: 100 mg (0.20 mmol; 91%). mp = 67 °C.  $[\alpha]_D^{25} = +12.5$  (c = 0.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 6.51 (d, *J* = 7.0 Hz, 1H), 5.86 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.61 (dd, *J* = 15.6, 6.4 Hz, 1H), 4.70–4.73 (m, 2H), 4.42–4.47 (m, 1H), 4.36–4.39 (m, 2H), 4.24 (s, 5H), 4.01–4.08 (m, 2H), 3.78–3.84 (m, 1H), 2.78–2.83 (m, 2H), 2.19 (td, *J* = 7.1, 2.6 Hz, 2H), 2.10 (q, *J* = 7.0 Hz, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.49–1.57 (m, 2H), 1.36–1.44 (m, 4H), 1.24–1.33 ppm (m, 12H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 171.3, 134.4, 128.9, 74.7, 70.6, 69.8, 68.3, 68.2, 68.0, 62.6, 54.7, 32.3, 29.6, 29.5, 29.4, 29.2, 29.1, 28.7, 28.5, 18.4. MS (ESI<sup>+</sup>): *m/z* 508.4 [M+H<sup>+</sup>]. Elemental analysis calculated (%) for C<sub>29</sub>H<sub>41</sub>FeNO<sub>3</sub>: C 68.63, H 8.14, N 2.76. Found: C 68.89, H 8.46, N 2.88.

#### 4.1.8. *N*-((2*S*,3*R*,*E*)-1,3-Dihydroxyoctadec-4-en-2-yl)cobaltoceniumcarboxamide Tetraphenylborate (**12a**)

**12a** was prepared according to the general procedure from **9** (100 mg, 0.26 mmol), DIPEA (90  $\mu$ L, 0.53 mmol), HBTU (109 mg, 0.29 mmol) and **2a** (110 mg, 0.29 mmol). The crude product was dissolved in 3 mL MeOH followed by the addition of 90 mg of NaBPh<sub>4</sub> (0.26 mmol, 1.0 equiv) in 2 mL MeOH. A yellow precipitate formed, which was isolated by filtration, washed with MeOH (5 mL) and dried *in vacuo* to give a yellow solid. Yield: 184 mg (83%). mp = 109 °C.  $[\alpha]_D^{25} = +25.2$  (c = 0.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>):  $\delta$  = 7.74 (d, *J* = 8.9 Hz, 1H), 7.35 (m, 8H), 6.94 (t, *J* = 7.5 Hz, 8H), 6.76–6.82 (m, 4H), 6.35–6.37 (m, 1H), 6.32 (m, 1H), 5.83 (s, 5H), 5.80 (t, *J* = 2.1 Hz, 2H), 5.74 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.60 (dd, *J* = 15.6, 6.1 Hz, 1H), 4.24–4.30 (m, 1H), 4.11–4.18 (m, 1H), 3.98 (t, *J* = 5.5 Hz, 1H), 3.77–3.88 (m, 2H), 2.08–2.03 (m, 2H), 1.33–1.40 (m, 2H), 1.22–1.32 (m, 20H), 0.88 ppm (t, *J* = 7.0 Hz, 3H). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>):  $\delta$  = 165.9, 165.5, 165.1, 164.7, 162.6, 137.4, 137.4, 133.9, 131.8, 126.4, 122.7, 95.5, 87.6, 87.4, 87.3, 85.6, 85.2, 73.5, 61.8, 57.9, 33.4, 33.0, 30.8, 30.7, 30.6, 30.4, 30.3, 30.1, 30.0, 29.8, 23.7, 14.8. MS (ESI<sup>+</sup>): *m/z* 514.3 [M - BPh<sub>4</sub><sup>-</sup>]. MS (ESI<sup>-</sup>): *m/z* 319.1 [BPh<sub>4</sub><sup>-</sup>]. Elemental analysis calculated (%) for C<sub>53</sub>H<sub>65</sub>BCoNO<sub>3</sub>: C 76.34, H 7.86, N 1.68. Found: C 76.44, H 7.54, N 2.08.

#### 4.1.9. *N*-((2*S*,3*R*,*E*)-1,3-Dihydroxyoctadec-4-en-17-yn-2-yl)Cobaltoceniumcarboxamide tetraphenylborate (**12b**)

**12b** was prepared according to the general procedure from **9** (50 mg, 0.13 mmol), DIPEA (45  $\mu$ L, 0.26 mmol), HBTU (55 mg, 0.14 mmol) and **2b** (42 mg, 0.14 mmol). The crude product was

dissolved in 3 mL MeOH followed by the addition of 45 mg of NaBPh<sub>4</sub> (0.13 mmol, 1.0 equiv) in 2 mL MeOH. A yellow precipitate formed, which was isolated by filtration, washed with MeOH (5 mL) and dried *in vacuo* to leave a yellow solid. Yield: 59 mg (55%). mp = 97.5 °C.  $[\alpha]_D^{25} = +27.5$  (c = 0.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>):  $\delta$  = 7.70–7.80 (m, 1H), 7.32–7.39 (m, 8H), 6.94 (t, *J* = 7.5 Hz, 8H), 6.77–6.82 (m, 6H), 6.34–6.38 (m, 1H), 6.30–6.33 (m, 1H), 5.81–5.84 (m, 5H), 5.80 (t, *J* = 2.1 Hz, 2H), 5.71–5.78 (m, 1H), 5.60 (dd, *J* = 15.3, 6.1 Hz, 1H), 4.24–4.29 (m, 1H), 4.11–4.19 (m, 1H), 3.97 (t, *J* = 5.3 Hz, 1H), 3.76–3.89 (m, 2H), 2.31 (t, *J* = 2.7 Hz, 1H), 2.16 (td, *J* = 7.0, 2.7 Hz, 2H), 2.01–2.09 (m, 2H), 1.45–1.53 (m, 2H), 1.22–1.44 ppm (m, 16H). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>):  $\delta$  = 165.9, 165.5, 165.2, 164.8, 162.6, 137.5, 133.9, 131.9, 126.5, 122.8, 87.6, 87.4, 87.3, 85.6, 85.2, 73.5, 70.3, 61.9, 57.9, 33.4, 30.8, 30.7, 30.6, 30.5, 30.3, 30.1, 30.0, 29.9, 29.8, 19.1. MS (ESI<sup>+</sup>): *m/z* 510.3 [M - BPh<sub>4</sub><sup>-</sup>]. MS (ESI<sup>-</sup>): *m/z* 319.1 [BPh<sub>4</sub><sup>-</sup>]. Elemental analysis calculated (%) for C<sub>53</sub>H<sub>61</sub>BCoNO<sub>3</sub>: C 76.71, H 7.41, N 1.67. Found: C 76.77, H 7.85, N 2.03.

#### 4.1.10. $\{\eta^6$ -[*N*-((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-17-yn-2-yl)benzamide)]( $\eta^5$ -1,2,3,4,5-pentamethylcyclopentadienyl)-ruthenium (**13a**)

**13a** was prepared according to the general procedure from **10** (50 mg, 0.10 mmol), DIPEA (34  $\mu$ L, 0.20 mmol), HBTU (42 mg, 0.11 mmol) and **2a** (33 mg, 0.11 mmol). The crude product was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) to give a white solid. Yield: 70 mg (0.05 mmol, 90%). mp = 129 °C.  $[\alpha]_D^{25} = +7.5$  (c = 0.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.42 (d, *J* = 7.9 Hz, 1H), 6.36–6.45 (m, 2H), 5.79–5.97 (m, 4H), 5.54 (dd, *J* = 15.6, 6.1 Hz, 1H), 4.39–4.44 (m, 1H), 4.00–4.09 (m, 2H), 3.81 (d, *J* = 8.2 Hz, 1H), 2.00–2.08 (m, 2H), 1.87–1.96 (m, 15H), 1.32–1.39 (m, 2H), 1.18–1.31 (m, 26H), 0.87 (t, *J* = 7.3 Hz, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 163.3, 134.0, 128.5, 97.8, 92.3, 88.3, 87.2, 87.1, 85.6, 85.4, 74.0, 61.7, 55.9, 32.3, 31.9, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.2, 29.1, 22.7, 14.1, 10.1. MS (ESI<sup>+</sup>): *m/z* 640.4 [M - PF<sub>6</sub><sup>-</sup>]. MS (ESI<sup>-</sup>): *m/z* 144.9 [PF<sub>6</sub><sup>-</sup>]. Elemental analysis calculated (%) for C<sub>35</sub>H<sub>56</sub>F<sub>6</sub>NO<sub>3</sub>PRu: C 53.56, H 7.19, N 1.78. Found: C 53.28, H 7.28, N 2.07.

#### 4.1.11. $\{\eta^6$ -[*N*-((2*S*,3*R*,*E*)-1,3-Dihydroxyoctadec-4-en-17-yn-2-yl)benzamide)]( $\eta^5$ -1,2,3,4,5-pentamethylcyclopentadienyl)ruthenium (**13b**)

**13b** was prepared according to the general procedure from **10** (50 mg, 0.10 mmol), DIPEA (34  $\mu$ L, 0.20 mmol), HBTU (42 mg, 0.11 mmol) and **2b** (32 mg, 0.11 mmol). The crude product was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 98:2) to leave a white solid. Yield: 40 mg (0.05 mmol, 51%). mp = 118 °C.  $[\alpha]_D^{25} = +6.5$  (c = 0.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.35 (d, *J* = 7.6 Hz, 1H), 6.41 (dd, *J* = 14.5, 6.0 Hz, 2H), 5.91–5.96 (m, 1H), 5.80–5.91 (m, 3H), 5.55 (dd, *J* = 15.4, 6.0 Hz, 1H), 4.44 (s, 1H), 4.01–4.10 (m, 2H), 3.80–3.88 (m, 1H), 3.40 (br. s., 1H), 3.12–3.24 (br. m, 1H), 2.18 (td, *J* = 7.1, 2.6 Hz, 2H), 2.02–2.10 (m, 2H), 1.95 (s, 16H), 1.53 (quin, *J* = 7.3 Hz, 2H), 1.33–1.43 (m, 4H), 1.22–1.32 ppm (m, 12H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 163.2, 134.1, 128.4, 97.8, 92.3, 88.2, 87.1, 85.6, 85.4, 84.8, 74.0, 68.0, 61.7, 55.8, 32.3, 29.6, 29.5, 29.2, 29.1, 28.7, 28.5, 18.4, 10.1. MS (ESI<sup>+</sup>): *m/z* 636.4 [M - PF<sub>6</sub><sup>-</sup>]. MS (ESI<sup>-</sup>): *m/z* 144.9 [PF<sub>6</sub><sup>-</sup>]. Elemental analysis calculated (%) for C<sub>35</sub>H<sub>52</sub>F<sub>6</sub>NO<sub>3</sub>PRu: C 53.84, H 6.71, N 1.79. Found: C 53.55, H 6.85, N 2.13.

## 4.2. Biological evaluation

### 4.2.1. Recombinant expression of ASAH1 in High Five insect cells

Recombinant full length ASAH1 was expressed and secreted from High Five insect cells. The used vector containing the genetic information for ASAH1 was obtained from Genescript. The first 21 amino acids were exchanged for the melittin signal sequence (MKFLVNVALLVFMVYISYIYA) [51]. At the C-terminus a TEV site, followed by a His-

tag (ENLYFQGGGGHHHHHH) was added. 48 h after infection with the respective baculovirus, the cells (1.2 mio cells/mL) were centrifuged (4500 rpm, 4 °C, 5 min), and the supernatant containing the secreted ASAH1 was dialysed against Ni<sup>2+</sup>-IMAC binding buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, pH 7.8, 4 × 1 L, 4 °C). After dialysis, Ni<sup>2+</sup>-IMAC was performed (washing buffer 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 20 mM imidazol, pH 7.8; elution buffer 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 125 mM imidazole, pH 7.8). The protein was concentrated (amicon stirred cell, NADIR/UP020P) and the protein was further purified via gel filtration (flow 0.6 mL/min, Hiloalad 16/600 Superdex 200 pg, buffer 50 mM Tris/HCl, 200 mM NaCl, pH 7.6). The purity of the protein was determined via SDS-page (cf. Supporting Information). The protein concentration was determined at A280 using the extinction coefficient  $\epsilon = 79215 \text{ M}^{-1} \text{ cm}^{-1}$ . From 320 mL of medium we obtained 2.4 mg of pure enzyme.

#### 4.2.2. Determination of $K_M$ of the fluorescence substrate Rbm14-12

The assay for the determination of the  $K_M$  and  $IC_{50}$  values is based on the fluorescence assay of Bedia et al. [16]. In 1.5 mL Eppendorf reaction tubes 750  $\mu\text{L}$  of reaction mix containing 645  $\mu\text{L}$  NaOAc buffer (25 mM, pH 4.5), 5  $\mu\text{L}$  of Rbm14-12 predilutions in EtOH (final concentrations 0, 2.5, 5, 7.5, 10, 20, 40, 60  $\mu\text{M}$ , 0.5% EtOH) were prepared. The reactions were started with 250  $\mu\text{L}$  of ASAH1 solution in NaOAc buffer (final concentration 500 ng/mL). The reaction tubes were incubated at 37 °C. After 0, 5, 10, 15, 20, 30, 40, and 50 min, 100  $\mu\text{L}$  samples were withdrawn from each tube and added to 50  $\mu\text{L}$  of MeOH in the wells of a 96 black well plate to stop the enzyme reaction. 100  $\mu\text{L}$  of a 2.5 mg/mL NaIO<sub>4</sub> solution (100 mM glycine/NaOH, pH 10.6) were added to each well and the plate was incubated at room temperature in the dark for 2 h. For background measurements reaction tubes containing blanks were treated analogously. The fluorescence of the resulting umbelliferone was measured at  $\lambda_{\text{ex}} = 340$  and  $\lambda_{\text{em}} = 465$  nm. The  $K_M$  value (cf. Supporting Information) was determined using GraphPad Prism.

#### 4.2.3. Determination of ceramidase inhibition ( $IC_{50}$ values)

In a 96 black well plate 75  $\mu\text{L}$  of reaction mix, containing 64.5  $\mu\text{L}$  NaOAc buffer (25 mM, pH 4.5), 0.5  $\mu\text{L}$  of fluorescence substrate Rbm14-12 (final concentration 14  $\mu\text{M}$ ), and 10  $\mu\text{L}$  of a predilution of **11a**, **12a**, **13a**, carmofer, ceranib-2 or the solvent in NaOAc buffer (final concentrations 200  $\mu\text{M}$  to 50 nM, or 10  $\mu\text{M}$  to 2 nM in the case of carmofer) were prepared. The enzyme reaction was started by addition of 25  $\mu\text{L}$  of NaOAc containing 0.05  $\mu\text{g}$  ASAH1, resp. 25  $\mu\text{L}$  of pure NaOAc for background fluorescence determination, into each well. The reaction was stopped after 30 min (37 °C) by addition of 50  $\mu\text{L}$  of MeOH to each well. Then 100  $\mu\text{L}$  of a 2.5 mg/mL NaIO<sub>4</sub> solution (100 mM glycine/NaOH, pH 10.6) were added into each well and the plate was incubated at room temperature in the dark for 2 h. The fluorescence of the product umbelliferone was measured at 340 nm<sub>ex</sub> / 465 nm<sub>em</sub>.  $IC_{50}$  values (means  $\pm$  SD) were calculated from four independent values using GraphPad Prism. We checked that the metal complexes do not themselves interfere with the fluorescence intensity of umbelliferone (cf. Figure S5).

#### 4.2.4. Cell culture conditions

The cell lines used for the biological evaluation were cultivated in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS and 1% Antibiotic-Antimycotic. The cells were cultivated at 37 °C, 5% CO<sub>2</sub> and 95% humidity.

#### 4.2.5. Intracellular colocalization

518A2 cells were seeded at a density of  $0.03 \times 10^6$  cells per mL (cpm) onto glass cover slips ( $\emptyset$  12 mm) inside the wells of a 24 well plate (0.5 mL/well). After 24 h of incubation under cell culture conditions the medium was exchanged for 500  $\mu\text{L}$  of complete DMEM containing 6  $\mu\text{L}$  of Cell light Lysosomes-RFP, BacMam (Thermo Fisher)

and the plate was again incubated for 24 h. The cells were treated with 30  $\mu\text{M}$  of **11b**, **12b** or **13b** for 30 min at 37 °C. The old medium was discarded and the cells were washed three times with 1 mL of PBS. After fixation (4% formaldehyde in PBS) for 20 min and permeabilization (0.5% Triton X-100, 1% BSA in PBS), 200  $\mu\text{L}$  of click working solution (2 mM CuSO<sub>4</sub>, 5 mM sodium ascorbate, 0.1 mM 3-azido-7-hydroxycoumarin, 1% BSA in PBS) were added into each well. The cells were incubated at room temperature in the dark for 30 min before the solution was discarded once more. The cells were washed twice with PBS (1% BSA) before the coverslips were embedded in mowiol. Fluorescence images were taken using a Leica TCS SP5 confocal microscope (pinhole at 1 Airy). Colocalization parameters were calculated for one cell using the ImageJ plugin JaCOP [42–44].

#### 4.2.6. $IC_{50}$ determination using the MTT assay

The cytotoxicity of **11–13**, sph and ceranib-2 was evaluated via MTT based proliferation assays. Cells were seeded at  $0.05 \times 10^6$  cpm or  $0.1 \times 10^6$  cpm (DLD-1) into the wells of 96 well plates (100  $\mu\text{L}$ /well) and incubated for 24 h to establish confluency. Appropriate predilutions in H<sub>2</sub>O made from fresh stock solutions (10 mM) in DMF or DMSO (ceranib-2) or EtOH (sph) were added into the wells to reach final concentrations ranging from 25 nM to 100  $\mu\text{M}$ . After 72 h of incubation at 37 °C the plates were centrifuged for 5 min (300 g, 4 °C) and the medium was discarded by swiftly turning the plates onto fresh cell tissue paper. 50  $\mu\text{L}$  of an MTT solution (0.05% in PBS) was added into each well and the plates were further incubated at 37 °C for 2 h. The MTT solution was removed as before, and the cells and the formazan were lysed by addition of 25  $\mu\text{L}$  of SDS/DMSO (1%, 0.6% acetic acid). For complete solution of the formazan the plates were incubated for at least another hour at 37 °C before the absorptions of the formazan and the background were measured at 570 nm, respectively at 630 nm. The vitality of the cells treated with the solvent was set to 100% viable cells for each concentration and the vitality of the cells inside the wells treated with **11**, **12**, **13** was calculated accordingly. The  $IC_{50}$  values were finally calculated via GraphPad Prism. Means  $\pm$  SDs were calculated from four independent values.

#### 4.2.7. Cell cycle analysis

The effects of **11–13**, sph, and ceranib-2 on the progression of the cell cycle of 518A2 melanoma cells were analyzed via propidium iodide (PI) staining and flow cytometry. Cells were seeded at  $0.05 \times 10^6$  cpm into the wells of 6-well plates (3 mL/well) and the plates were incubated under standard cell culture conditions for 24 h. Dilutions of the test complexes in H<sub>2</sub>O were added to the wells to reach final concentrations of 10  $\mu\text{M}$  (**11a**, sph), 2  $\mu\text{M}$  (**12a**, **13a**), 200 nM (ceranib-2) or equal amounts of the respective solvent. The cells were incubated for another 24 h before the medium of each well (3 wells per concentration) was transferred into an ice cooled centrifugation tube, the cells were washed with 1 mL of PBS which was transferred into the respective tube as well. The cells were harvested via trypsinization and thorough washing of the wells with PBS, and were transferred into the respective tube. The cells were centrifuged at 300 g (5 min, 4 °C) and the supernatant was discarded. The resulting pellet was resuspended in 1 mL of ice cold EtOH (70%) and kept on ice for at least 1 h. For PI staining the cells were centrifuged at 400 g (5 min, 23 °C) and the supernatant was discarded. The cells were layered with 1 mL PBS and incubated for 5 min at 23 °C. After centrifugation (5 min, 400 g, 4 °C) the PBS was discarded and the pellet was resuspended with 200  $\mu\text{L}$  of PI staining buffer (50  $\mu\text{g}/\text{mL}$  propidium iodide, 0.1% sodium citrate, 50  $\mu\text{g}/\text{mL}$  RNase I) and the cells were incubated for 30 min at 37 °C. Thereafter, flow cytometry measurements were done with a Cytomics™ FC 500.

#### 4.2.8. (Immuno-)fluorescence staining

518A2 cells were seeded at  $0.05 \times 10^6$  cpm analogously to the preparation of the colocalization. The cells were incubated at standard

cell culture conditions for 24 h and then treated with **11a** (10  $\mu$ M), **12a** (5  $\mu$ M), **13a** (5  $\mu$ M) for another 24 h. The medium was removed and the cells were washed thrice with PBS. After fixation with 3.7% formaldehyde in PBS for 20 min at room temperature, the cells were again washed three times with PBS.

**Microtubules.** For staining of the microtubules the cells were incubated for 30 min (room temperature) with blocking and permeabilization buffer (1% BSA, 0.1% Triton X-100 in PBS). The buffer was replaced with the primary mouse anti-tubulin antibody (1:500 in 1% BSA in PBS) and the plates were incubated for 1 h at 37 °C. The buffer was discarded and the cells were washed three times with PBS, before the secondary goat anti-mouse phalloidin 488 antibody (1:500 in 1% BSA in PBS) was added to each well. After another hour of incubation at room temperature and in the dark, the buffer was discarded again and the cells were washed twice with PBS before the coverslips were layered with 500  $\mu$ L of sterile water. The coverslips were carefully embedded into mowiol mounting buffer (2.5% DABCO, 1 mg/mL DAPI) and stored at 4 °C.

**Actin filaments.** Staining of the actin filaments was done using Actin-stain™ 488 phalloidin (Cytoskeleton) and following the protocol provided by the manufacturer. After staining the cells were washed and the coverslips embedded as described above.

**Golgi apparatus.** Staining of the Golgi apparatus was done similar to microtubule staining. After treatment with the complexes, the cells were fixed and permeabilized as before. The Golgi apparatus was stained using Lectin HPA Alexa Fluor 647 conjugate (20  $\mu$ g/mL in PBS) for 30 min (room temperature). The cells were embedded as before.

#### 4.2.9. ROS level determination

The generation of reactive oxygen species was monitored by means of Nitro blue tetrazolium chloride (NBT) assays in 518A2 melanoma cells after 24 h of incubation with **11a**, **12a**, **13a**. NBT is reduced to a formazan by reactive oxygen species. The cells were seeded at  $0.1 \times 10^6$  cells/mL into the wells of a 96-well plate (100  $\mu$ L/well) and incubated for 24 h at 37 °C. Appropriate dilutions in sterile H<sub>2</sub>O were added into the wells (end concentrations 1, 2.5, 5, 10  $\mu$ M) and the cells were again incubated for another 24 h. The plates were centrifuged (300 g, 5 min, 4 °C) and the supernatant was removed as described for the MTT assay. 50  $\mu$ L of NBT in PBS (0.1%) was added into each well. After another 4 h of incubation at 37 °C the plates were centrifuged as before and the NBT solution was replaced with 50  $\mu$ L of a 2 M KOH solution, and subsequently, 65  $\mu$ L of DMSO was added into each well. After 30 min of incubation at 37 °C the absorbance of the formazan was measured at 405 nm and 630 nm. Analogously, a second 96-well plate was prepared and an MTT assay was performed as described.

#### 4.2.10. Caspase-3/-7 activation

The activation of caspases 3 and 7 was monitored using the ApoOne® Homogeneous Caspase-3/7 Assay (Promega), largely following the given protocol. 518A2 cells were seeded at  $0.1 \times 10^6$  cpm into the wells of a 96-black well plate (67.5  $\mu$ L/well) and the plate was incubated for 24 h under standard cell culture conditions. For each concentration, wells containing just DMEM without cells were prepared for background measurements. The cells were treated with **11a** (10  $\mu$ M), **12a** and **13a** (each 5  $\mu$ M), staurosporine (st) as a positive control (2  $\mu$ M), and an analogous amount of the solvent as a negative control for 4 h at 37 °C. The provided substrate and buffer were prepared accordingly (1:100 dilution) and added to the wells (75  $\mu$ L/well). After 2 h of incubation at room temperature in the dark, the fluorescence of the converted substrate was measured at 499 nm<sub>ex</sub> / 527 nm<sub>em</sub>. Analogously, a second plate for MTT assays was prepared and measured.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgement

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#### Appendix A. Supplementary material

Experimental data of **2a**, **6a**, and **7a**; NMR spectra of **2-13**; UV-vis stability studies of **2-8**; Fluorescence staining of Golgi apparatus in 518A2 melanoma cells; SEC chromatogram and SDS-page of the expressed ASAHI; Michaelis Menten curve of Rbm14-12 at doi:xxxx. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2020.103703>.

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## Supporting Information

# *N*-Metalloenoylsphingosines as targeted ceramidase inhibitors: syntheses and antitumoral effects

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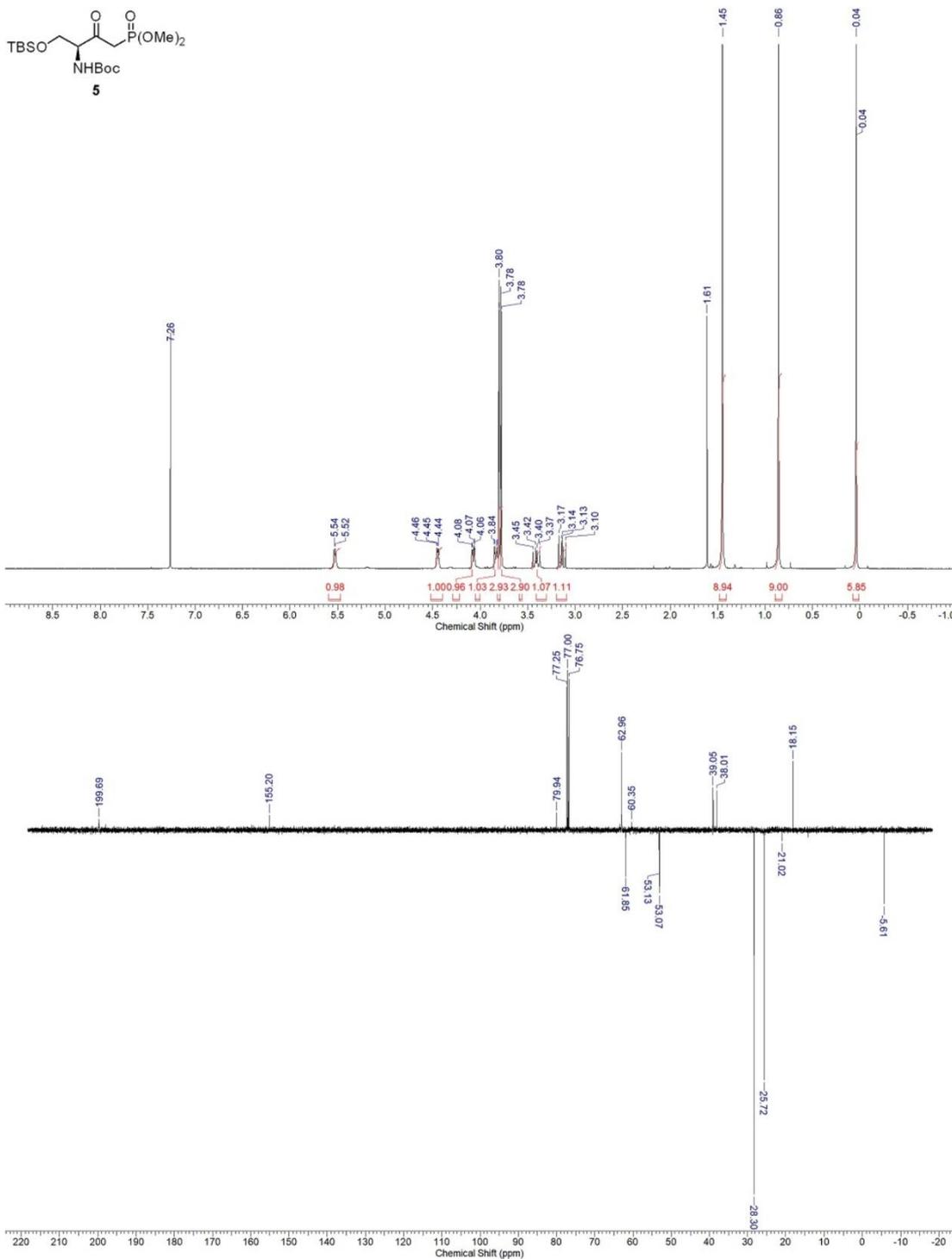
Experimental procedures for compounds <b>2a</b> , <b>6a</b> and <b>7a</b>	S2-3
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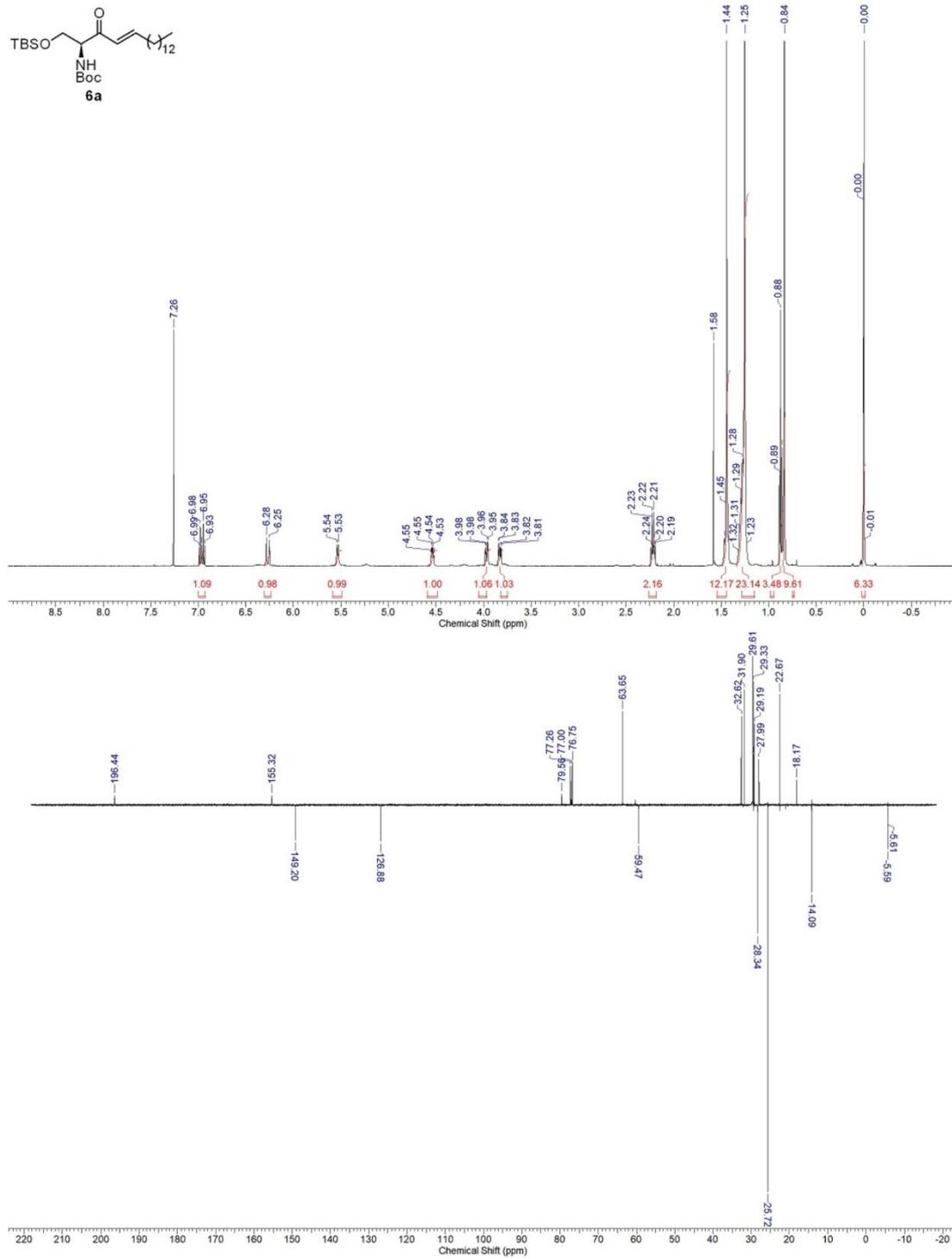
**(-)-D-erythro-Sphingosine (2a).** TFA (10 mL) was added to a solution of **7a** (453 mg, 0.88 mmol, 1.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at 0 °C. The mixture was stirred at room temperature for 1.5 h. Volatiles were removed *in vacuo*. To remove excess TFA the residue was dissolved in MeOH (10 mL) and concentrated at reduced pressure. This was repeated twice. The remaining residue was dissolved in 1 M NaOH (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, CHCl<sub>3</sub>/MeOH/NH<sub>4</sub>OH 95:5:1) to give **2a** as a white solid. Yield: 264 mg (0.88 mmol, 99%).  $[\alpha]_D^{23} = -1.5$  (c = 1.5; CHCl<sub>3</sub>). Mp = 82 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ = 5.75 (dt, *J* = 15.6, 6.4 Hz, 1H), 5.45 (dd, *J* = 15.6, 7.0 Hz, 1H), 4.06 (t, *J* = 6.4 Hz, 1H), 3.61 - 3.71 (m, 2H), 2.85 - 2.88 (m, 1H), 2.65 (br. s., 4H), 2.05 (q, *J* = 7.0 Hz, 2H), 1.33 - 1.41 (m, 2H), 1.19 - 1.32 (m, 20H), 0.87 ppm (t, *J* = 6.7 Hz, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ = 134.6, 129.1, 75.1, 63.7, 56.2, 32.4, 29.75, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.2, 22.7, 14.2. HRMS (ESI): *m/z* calculated for C<sub>18</sub>H<sub>38</sub>O<sub>2</sub>N+Na<sup>+</sup> [M + Na<sup>+</sup>]: 300.2897. Found: 300.2890. Analytical data agree with those reported.<sup>1</sup>

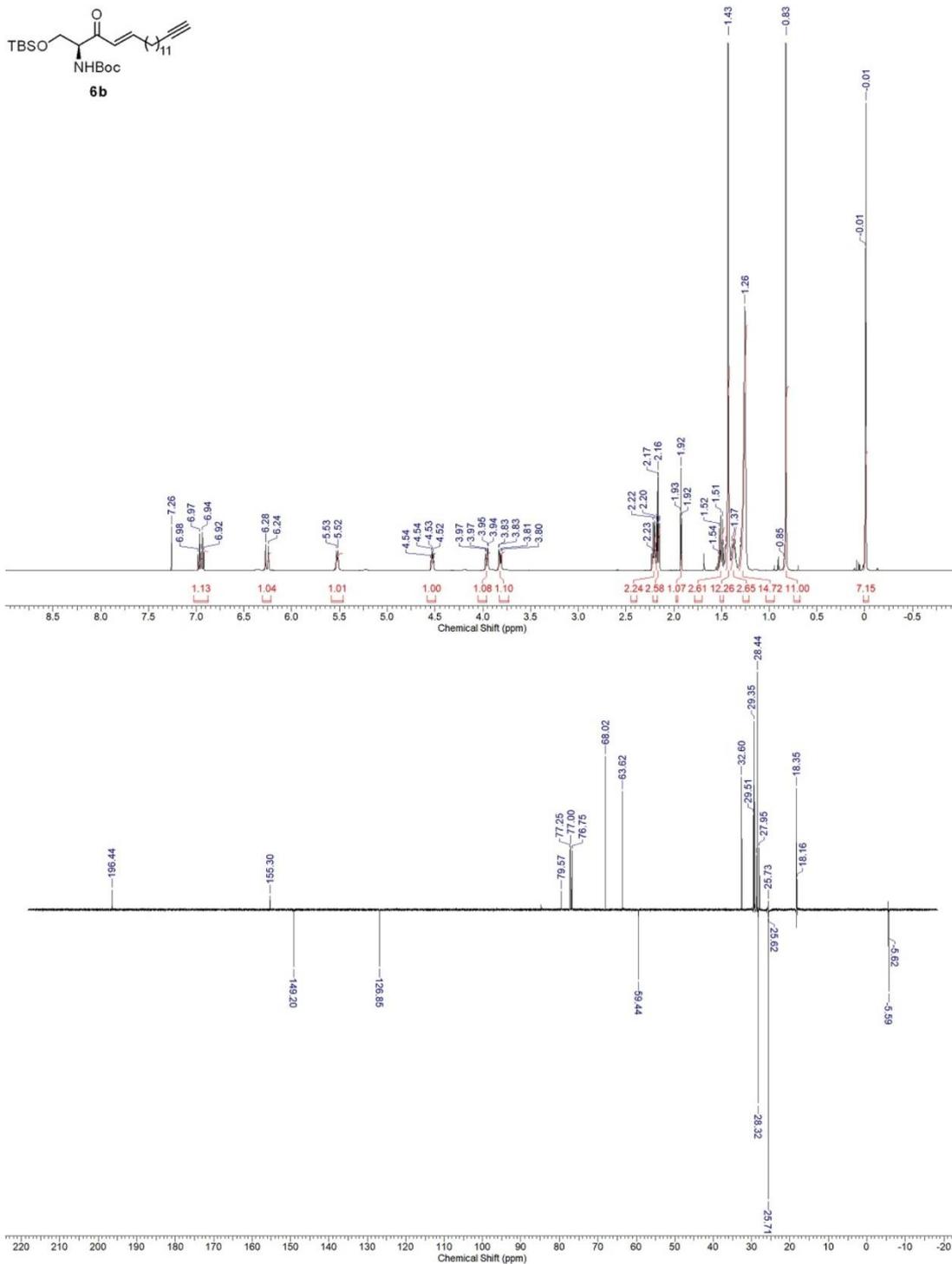
**tert-Butyl (S,E)-(1-((tert-Butyldimethylsilyloxy)-3-oxooctadec-4-en-2-yl)carbamate (6a).** NEt<sub>3</sub> (4.6 mL, 32.92 mmol, 3.0 equiv) was added to a solution of **5** (4.67 g, 10.97 mmol, 1.0 equiv), LiCl (1.40 g, 32.92 mmol, 3.0 equiv) and tetradecanal (4.66 g, 21.94 mmol, 2.0 equiv) in THF (150 mL) and the resulting mixture was stirred at room temperature for 18 h. Then 1 M citric acid (150 mL) was added, the phases were separated and the aqueous phase was extracted with EtOAc (2 x 100 mL). The combined organic phases were washed with brine (300 mL), dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, *n*-hexane/ethyl acetate 30:1) to give **6a** as a colorless oil. Yield: 5.13 g (10.0 mmol, 91%).  $[\alpha]_D^{23} = + 35.5$  (c = 1.1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ =

6.96 (dt,  $J = 15.7, 7.1$  Hz, 1H), 6.27 (d,  $J = 15.7$  Hz, 1H), 5.53 (d,  $J = 7.6$  Hz, 1H), 4.48 - 4.60 (m, 1H), 3.97 (dd,  $J = 10.3, 3.5$  Hz, 1H), 3.83 (dd,  $J = 10.3, 4.6$  Hz, 1H), 2.22 (qd,  $J = 7.1, 1.7$  Hz, 2H), 1.40 - 1.49 (m, 11H), 1.22 - 1.34 (m, 20H), 0.85 - 0.90 (m, 3H), 0.84 (s, 9H), -0.01 - 0.02 ppm (m, 6H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta = 196.4, 155.3, 149.2, 126.9, 79.6, 63.7, 59.5, 32.62, 31.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.3, 29.2, 28.3, 25.7, 22.7, 18.2, 14.1, 5.6$ . HRMS (ESI):  $m/z$  calculated for  $\text{C}_{29}\text{H}_{57}\text{O}_4\text{NSi} + \text{Na}^+ [\text{M} + \text{Na}^+]$ : 534.3947. Found: 534.3949. Analytical data agree with those reported.<sup>1</sup>

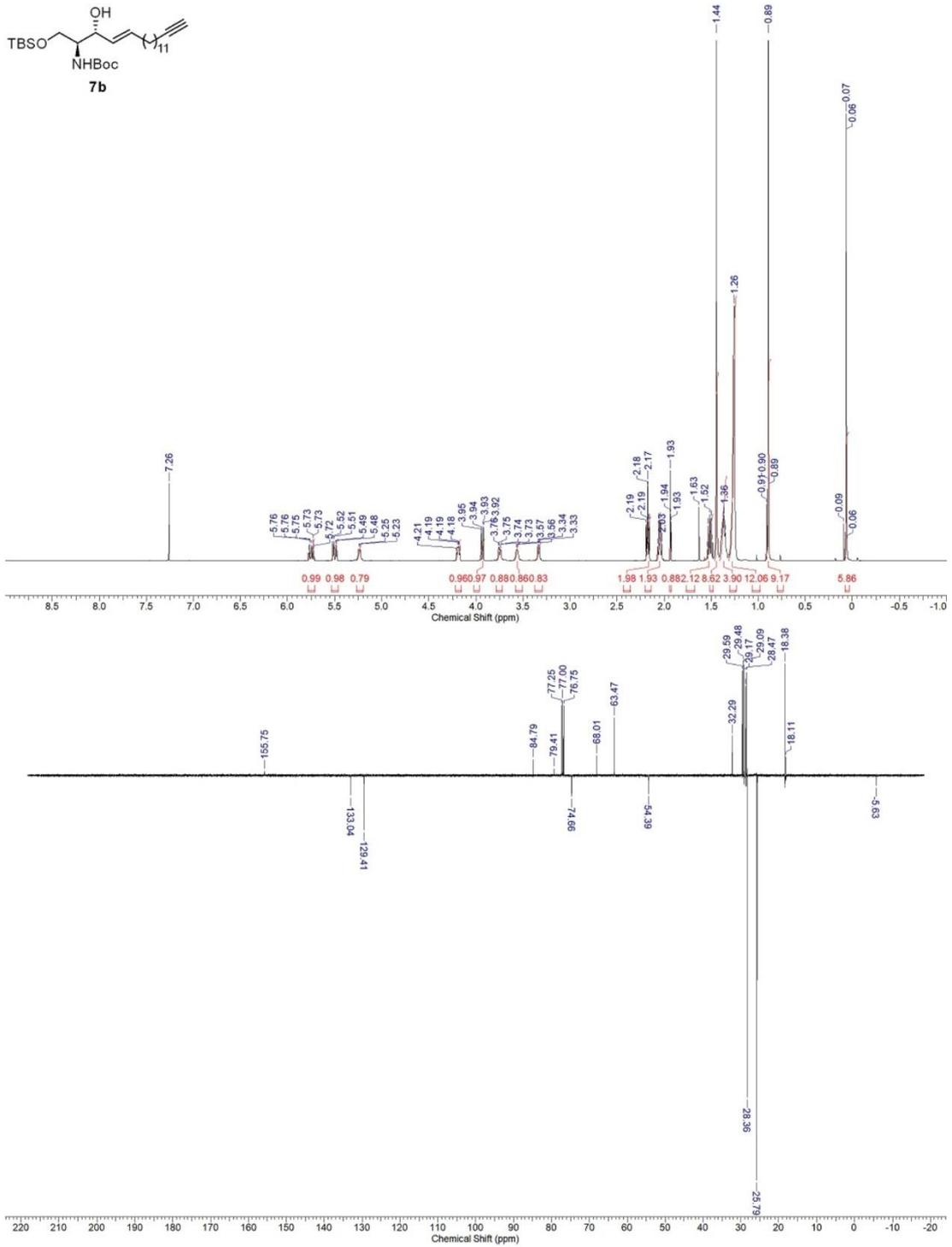
***tert*-Butyl ((2*S*,3*R*,*E*)-1-((*tert*-Butyldimethylsilyloxy)-3-hydroxy-octadec-4-en-2-yl)carbamate (7a).** **7a** was prepared according to a literature procedure(1) from **6a** (1.55 g, 3.04 mmol, 1.0 equiv) and  $\text{LiAlH}(\text{O}t\text{Bu})_3$  (1.54 g, 6.07 mmol, 2.0 equiv) as a white solid. Yield: 1.17 g (2.28 mmol, 75%); mp = 63-65 °C.  $[\alpha]_{\text{D}}^{23} = +10.8$  (c = 1.0,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 5.73$  (dt,  $J = 15.5, 6.7$  Hz, 1H), 5.49 (dd,  $J = 15.5, 5.8$  Hz, 1H), 4.14 - 4.21 (m, 1H), 3.91 (dd,  $J = 10.4, 3.1$  Hz, 1H), 3.73 (d,  $J = 10.4$  Hz, 1H), 3.52 - 3.58 (m, 1H), 3.35 (d,  $J = 8.9$  Hz, 1H), 1.99 - 2.08 (m, 2H), 1.41 - 1.45 (m, 9H), 1.32 - 1.38 (m, 2H), 1.20 - 1.30 (m, 20H), 0.87 - 0.90 (m, 9H), 0.04 - 0.06 ppm (m, 6H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta = 155.8, 130.1, 129.4, 79.5, 74.7, 63.5, 54.4, 32.3, 32.0, 29.75, 29.7, 29.5, 29.4, 29.2, 28.4, 25.8, 22.7, 14.2, -5.6, -5.65$ . HRMS (ESI):  $m/z$  calculated for  $\text{C}_{29}\text{H}_{59}\text{O}_4\text{NSi} + \text{Na}^+ [\text{M} + \text{Na}^+]$ : 536.4105. Found: 536.4105. Analytical data agree with those reported.<sup>1</sup>

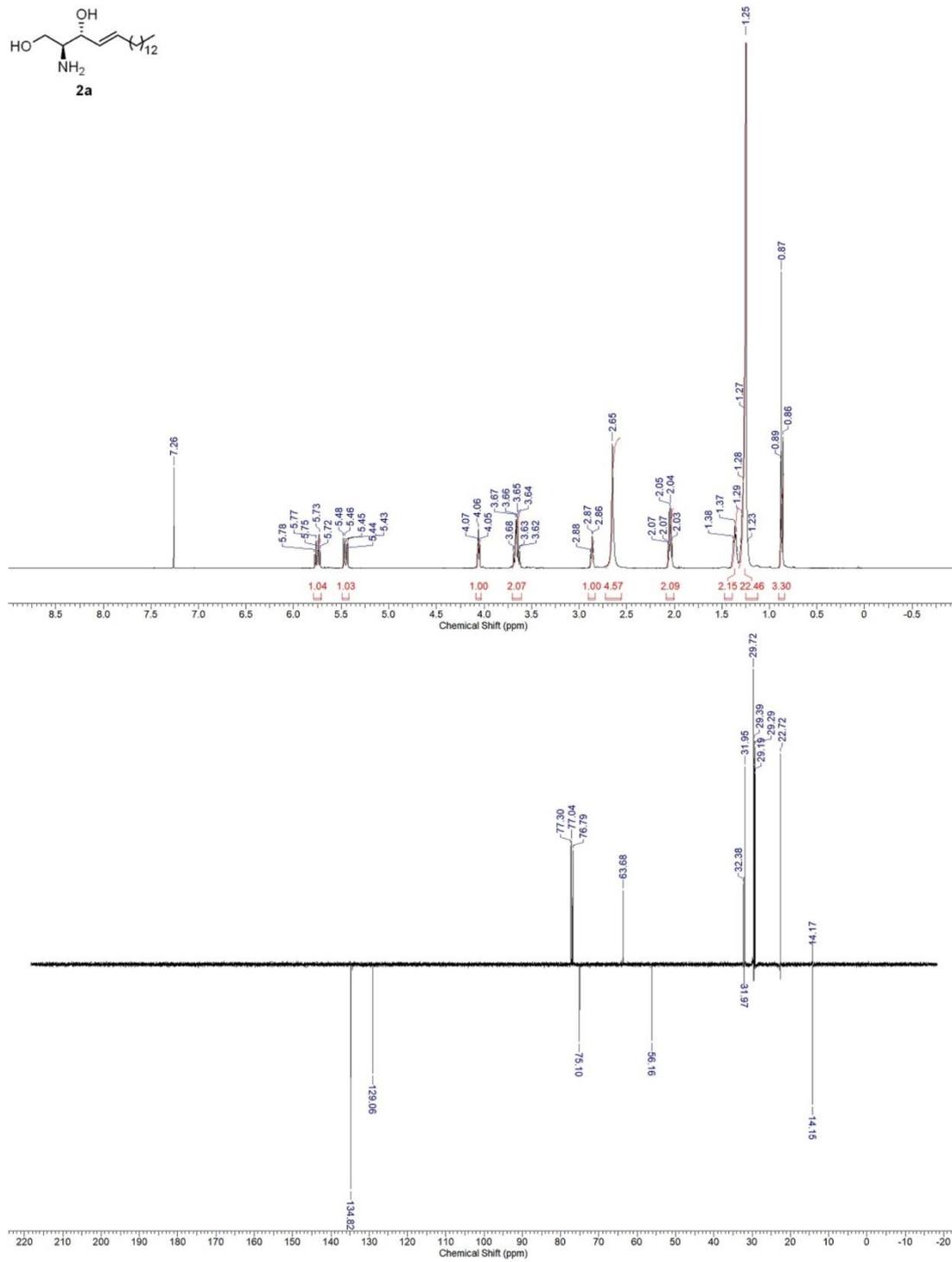
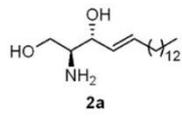


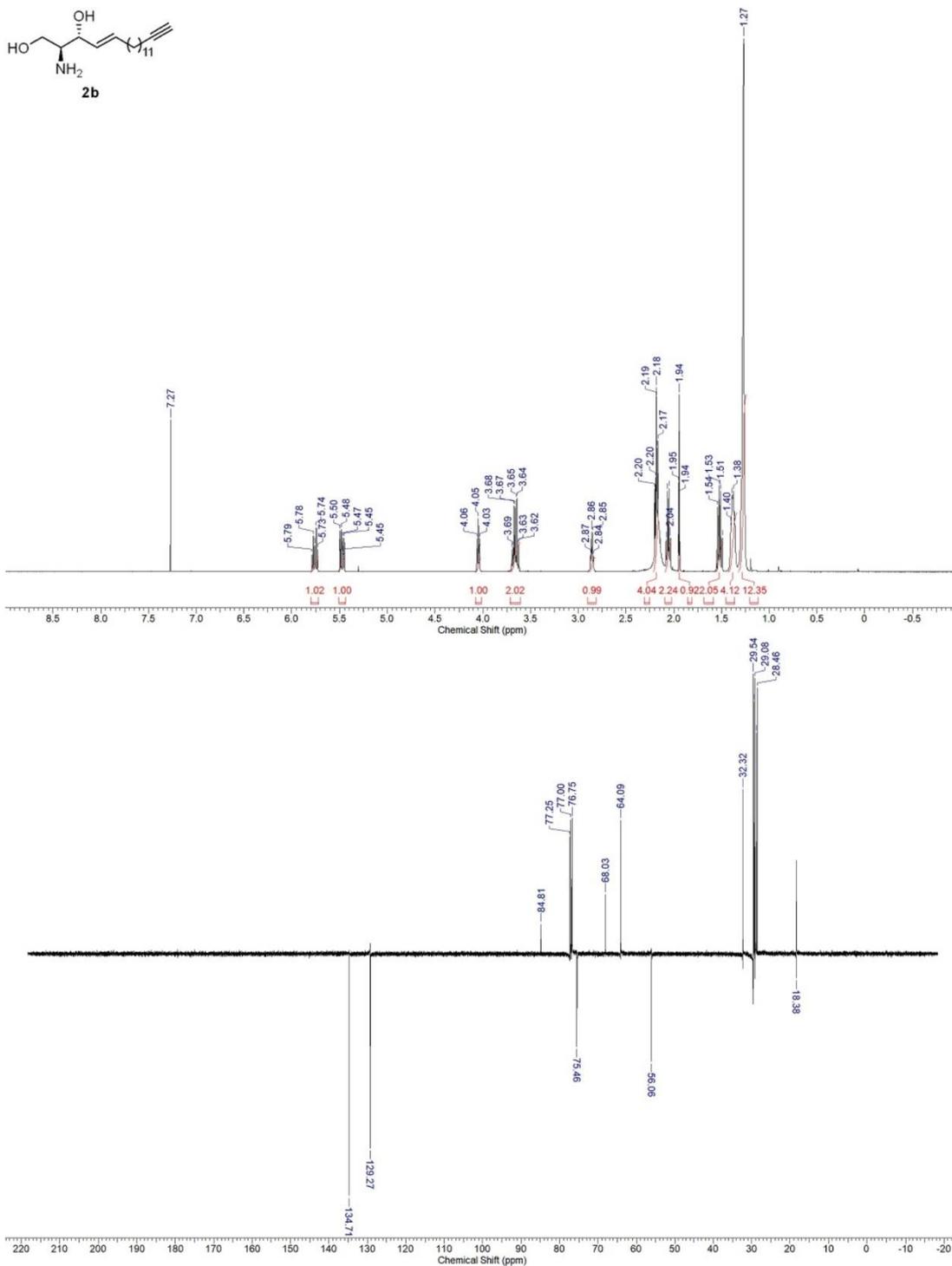


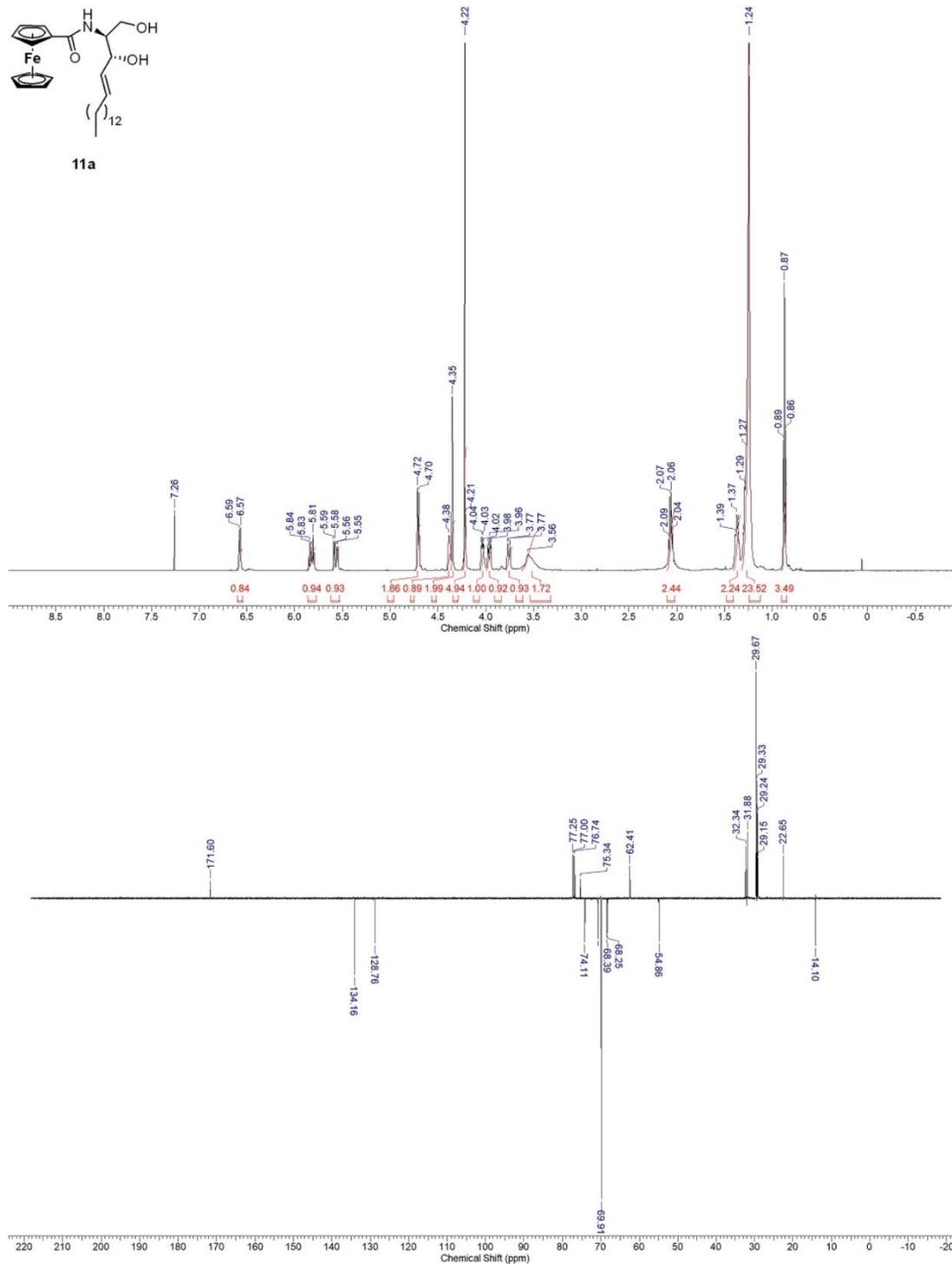
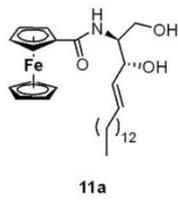


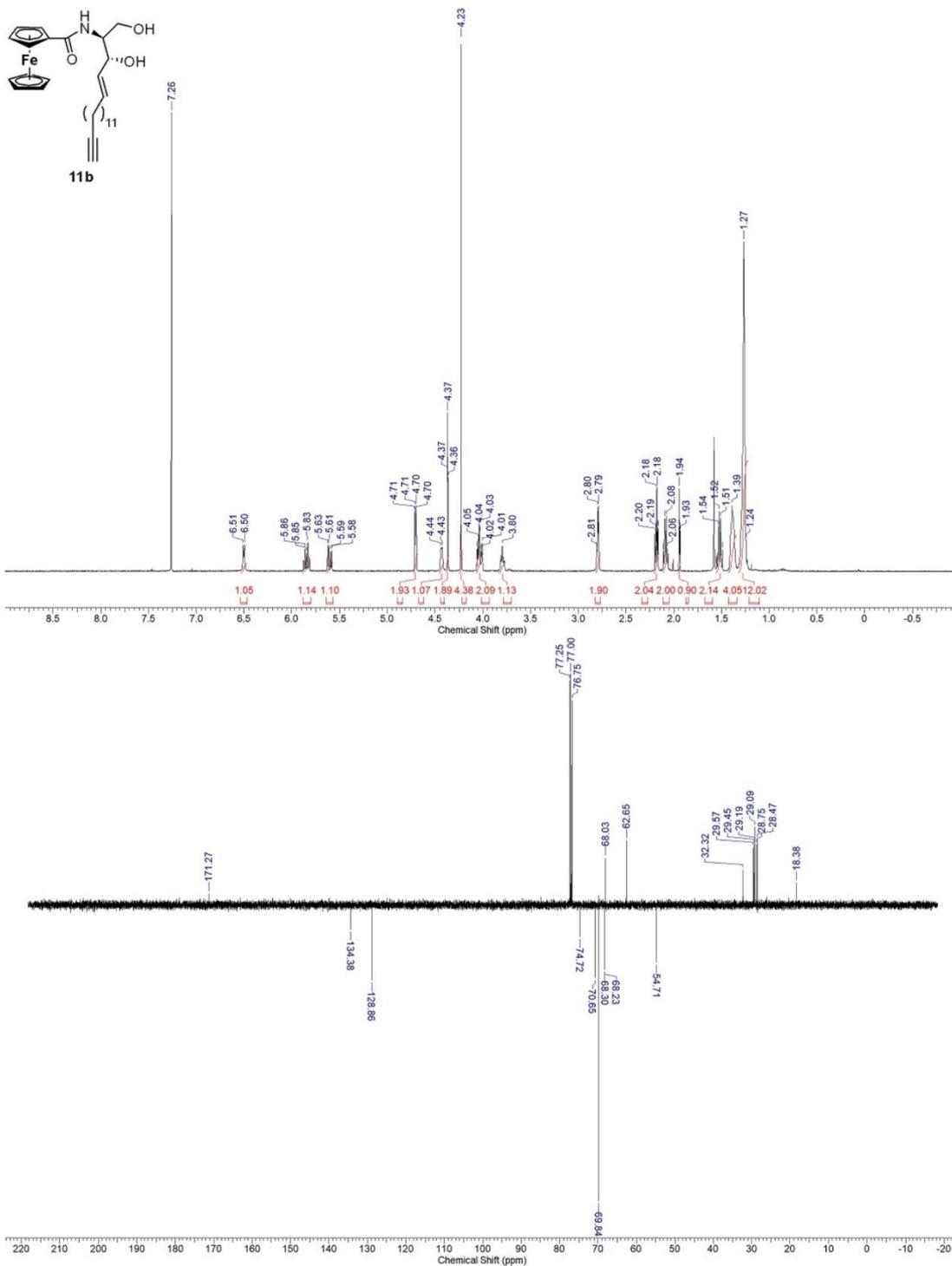


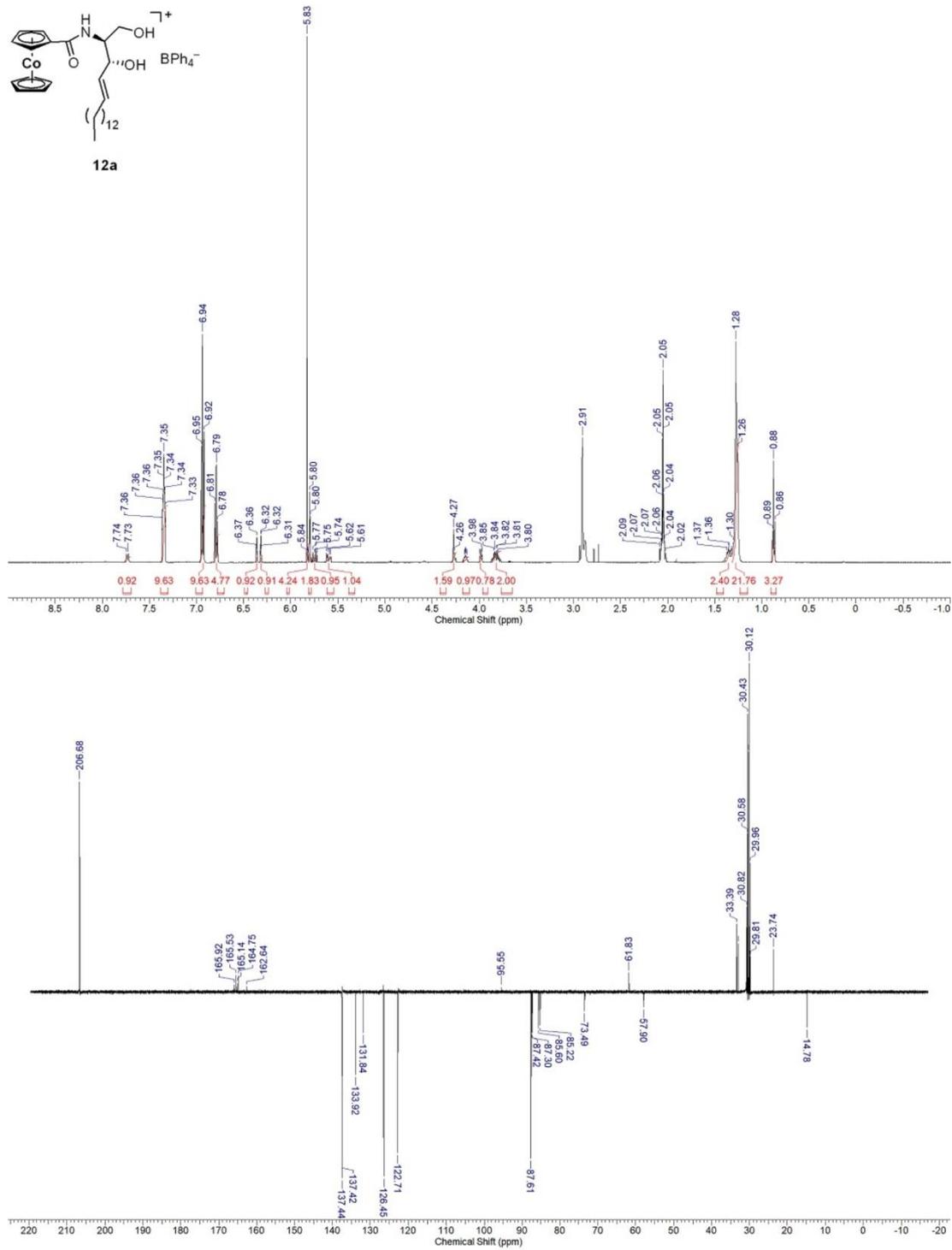


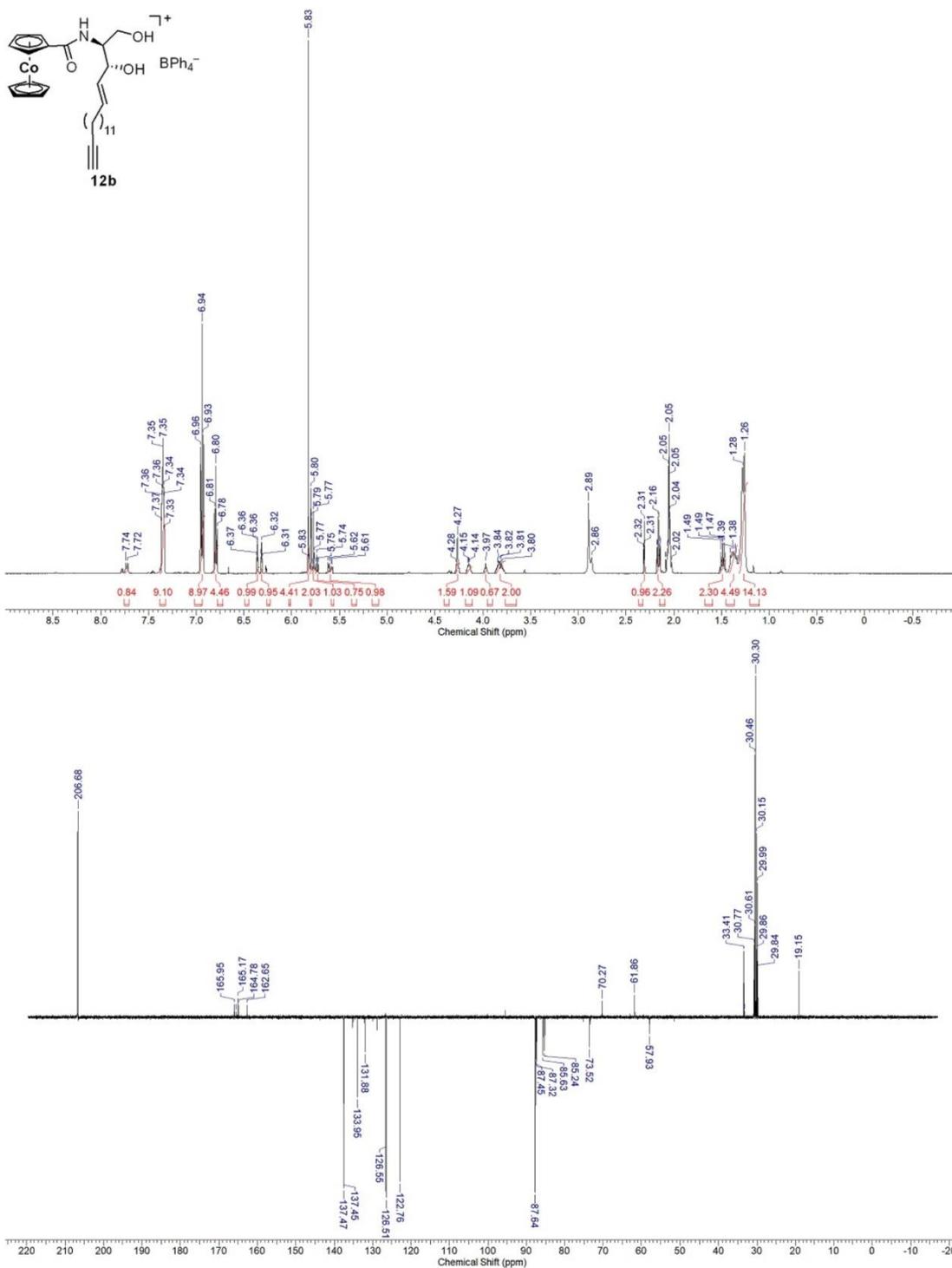


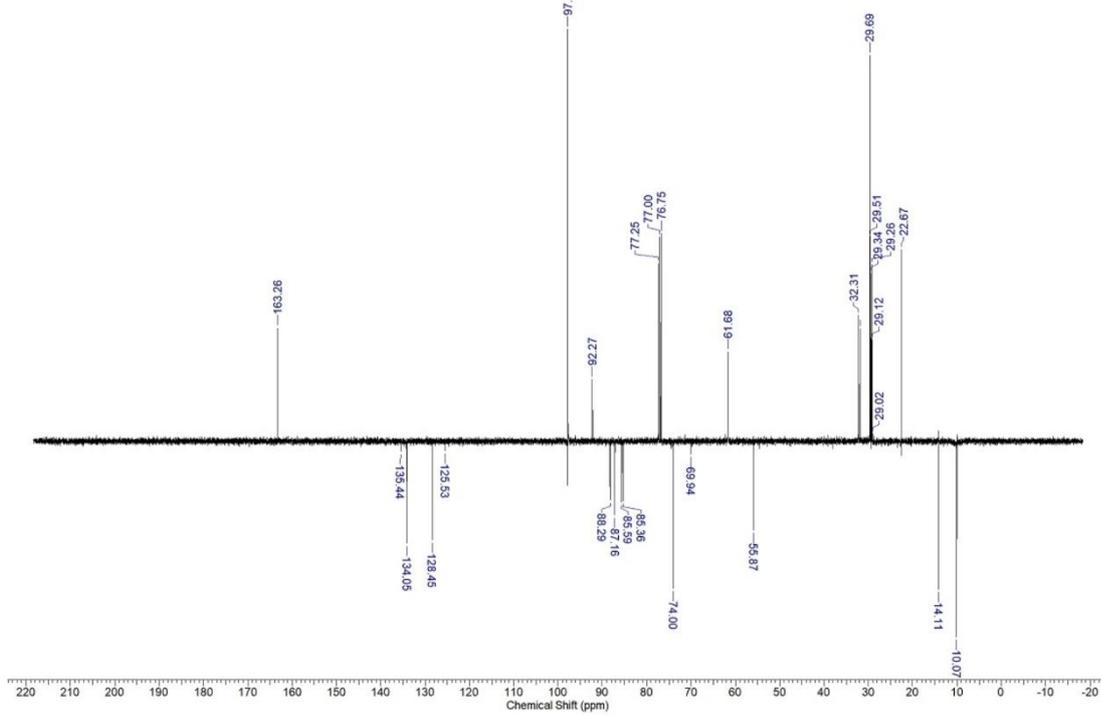
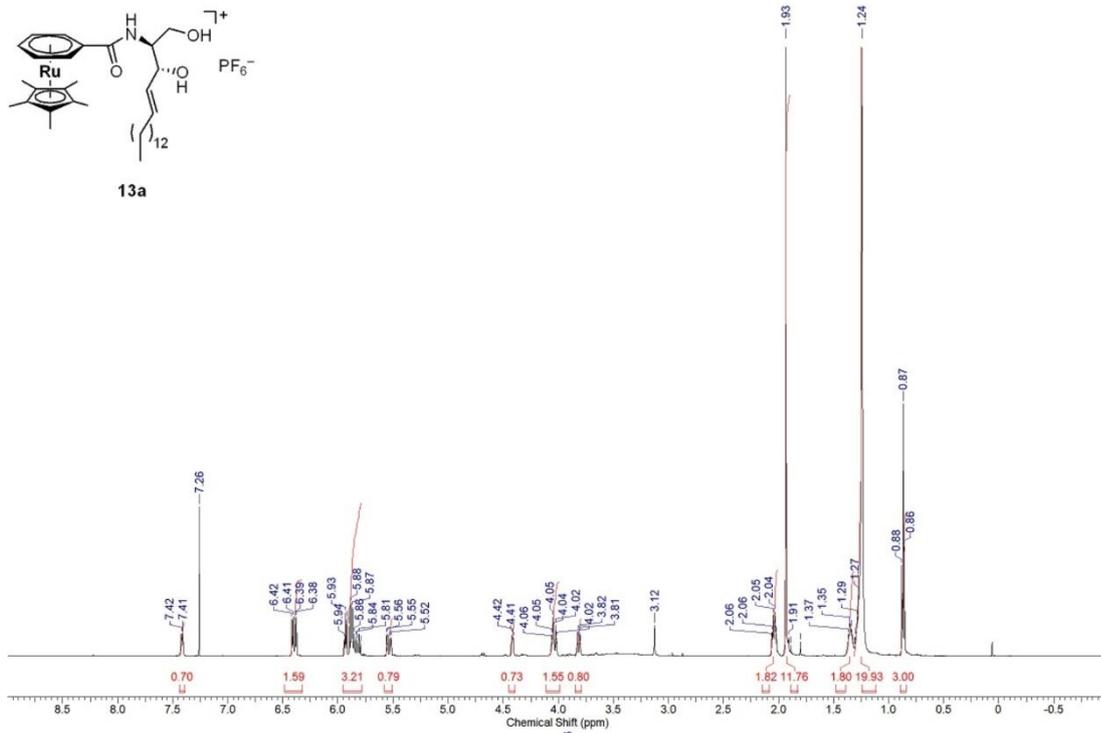
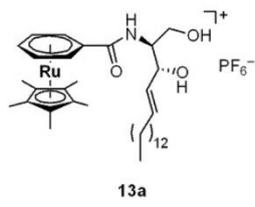


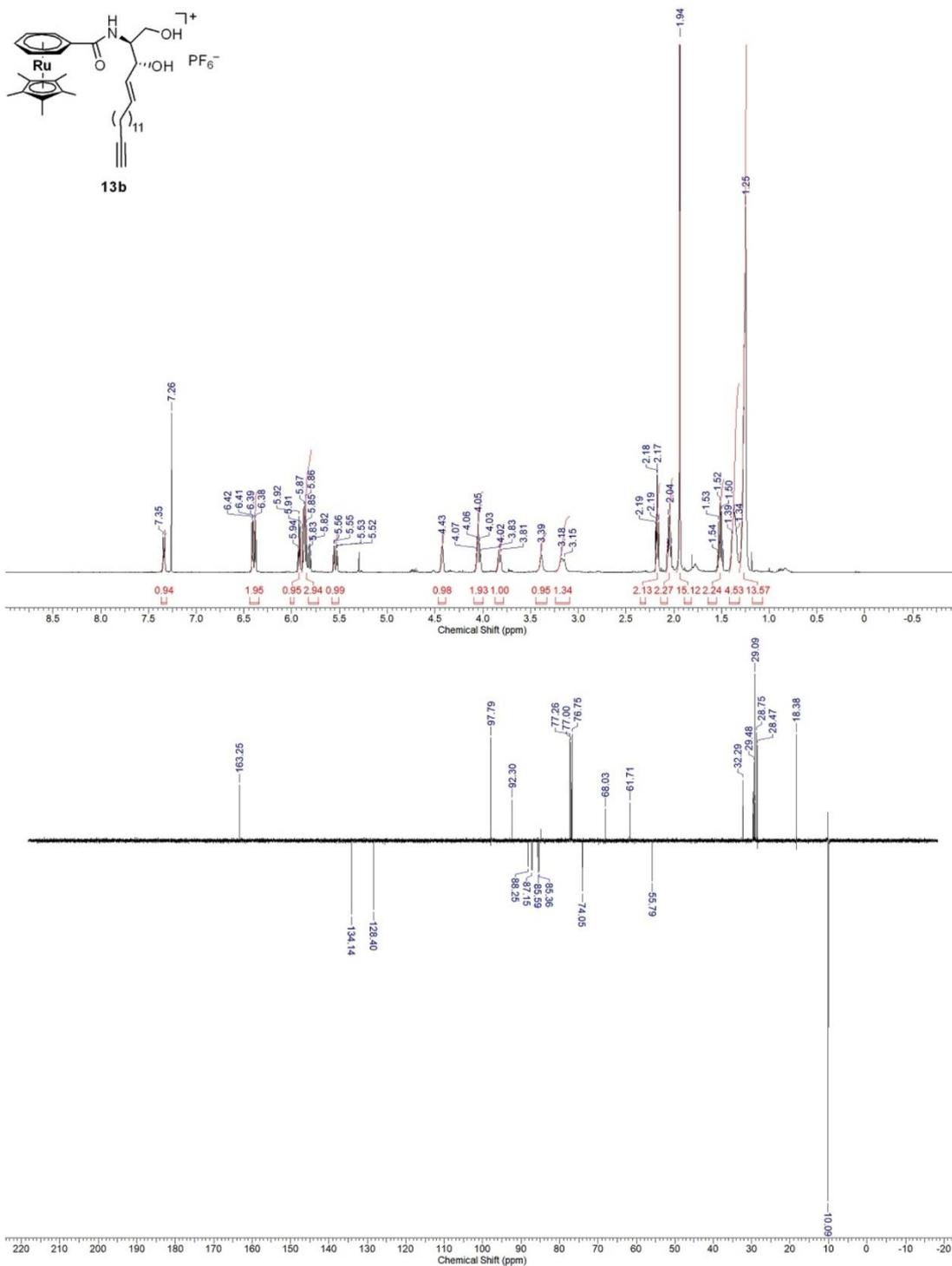




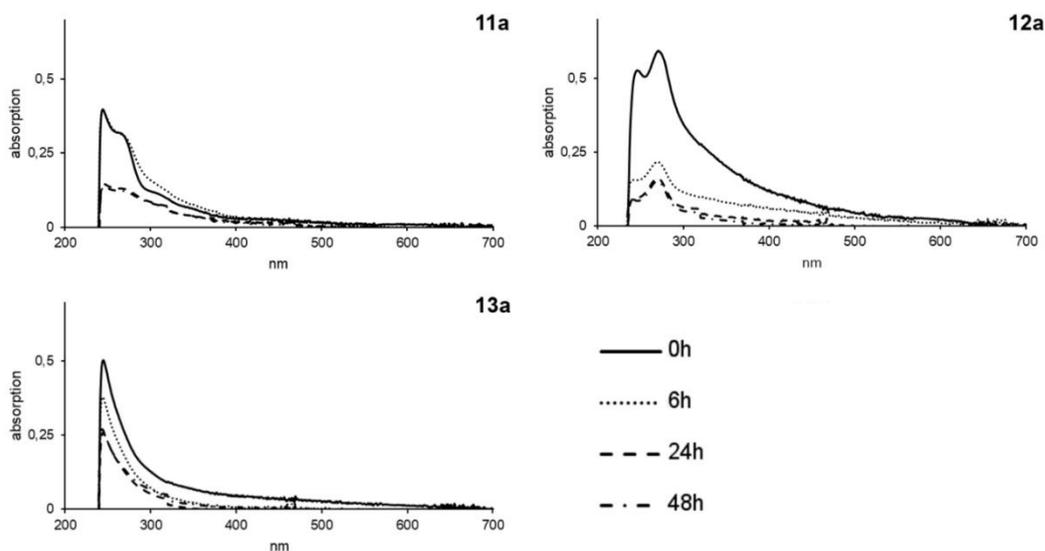






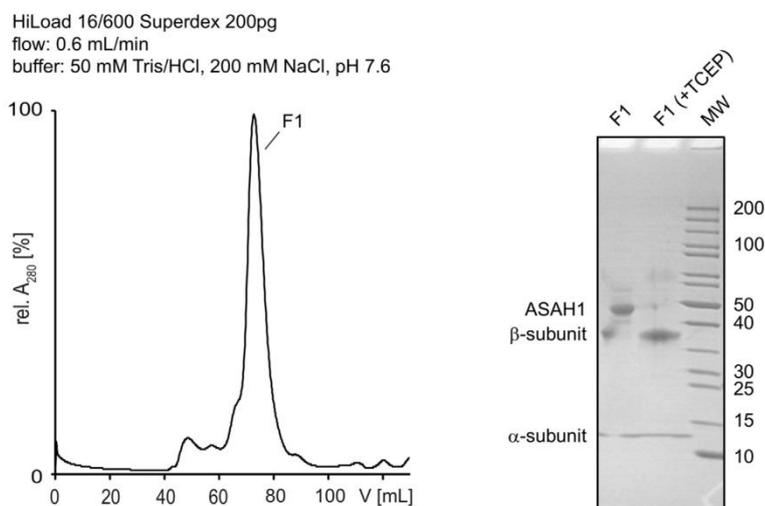


## UV-Vis stability studies of 11a, 12a and 13a

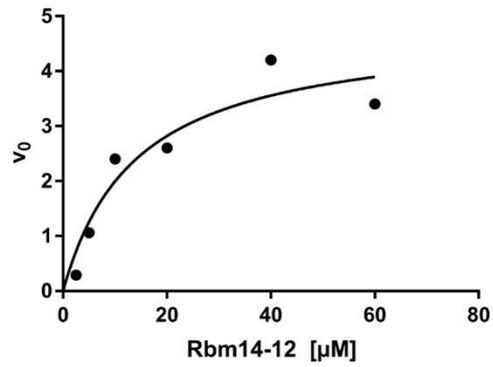


**Figure S1** UV-Vis stability studies of **11a** ( $100 \mu\text{M}$ ), **12a** ( $25 \mu\text{M}$ ) and **13a** ( $100 \mu\text{M}$ ) in Water/DMF (99:1). Stem solutions were kept at  $37^\circ\text{C}$  and absorption spectra of aliquots were measured after 0 h, 6 h, 24 h and 48 h using an Agilent Cary 60 UV-Vis spectrophotometer.

## SEC chromatogram and SDS-page of the expressed ASAH1

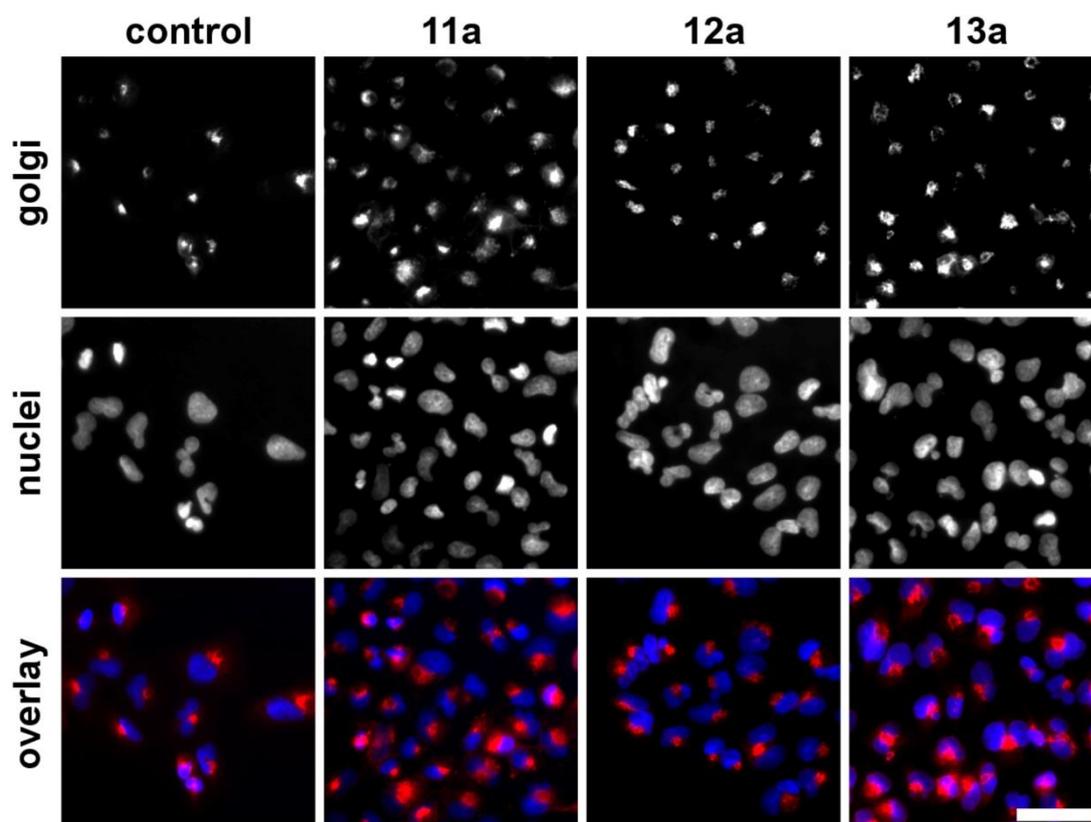


**Figure S2** Purification of the recombinantly expressed ASAH1 (F1) via SEC and SDS-page of the collected F1 fraction and a reduced (F1+TCEP) sample.

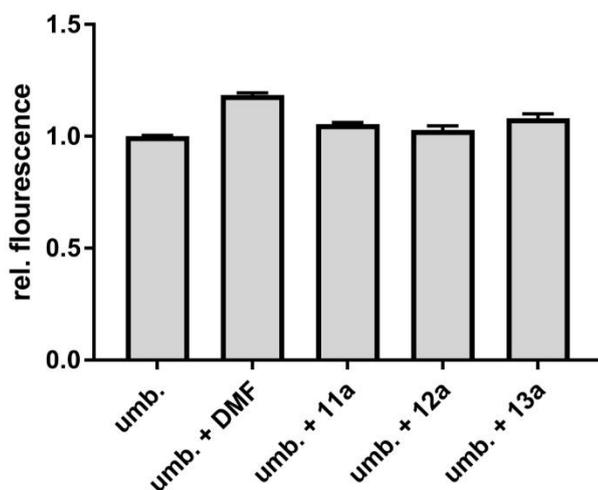
**Michaelis Menten curve of Rbm14-12**

**Figure S3** Michaelis Menten curve for  $K_M$  determination of the Rbm14-12 substrate and the purified ASAHI enzyme.

## Fluorescence staining of Golgi apparatus in 518A2 melanoma cells



**Figure S4** Immunofluorescence staining of Golgi apparatus and nuclei of 518A2 cells after 24 h of incubation with **11a** (10  $\mu\text{M}$ ), **12a** (5  $\mu\text{M}$ ), **13a** (5  $\mu\text{M}$ ), or DMF as control; Golgi apparatus - red, DAPI - blue. Scale bar: 50  $\mu\text{m}$ .

**Proof of non-interference of the title complexes with umbelliferone fluorescence**

**Figure S5** Fluorescence intensities of solutions of umbelliferone ( $14 \mu\text{M}$ ) or umbelliferone ( $14 \mu\text{M}$ ) plus DMF or plus any of the title complexes **11a**, **12a**, or **13a** ( $100 \mu\text{M}$ ) in assay buffer.

**References**

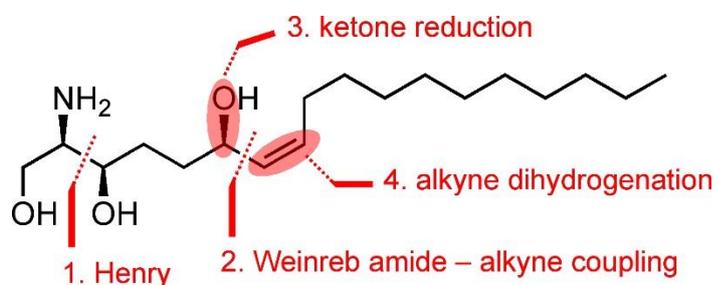
- (1) Yang, H.; Liebeskind, L. S. A concise and scalable synthesis of high enantiopurity (-)-D-erythro-sphingosine using peptidyl thiol ester-boronic acid cross-coupling. *Org. Lett.* **2007**, *9*, 2993-2995.

### 4.3 Publikation II

## Synthesis and antiproliferative effect of the proposed stereoisomer of the marine sponge metabolite halisphingosine A

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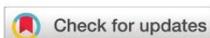


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## PAPER



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## Synthesis and antiproliferative effect of the proposed stereoisomer of the marine sponge metabolite halisphingosine A†

Alexander Bär, Sofia I. Bär and Rainer Schobert \*

The first synthesis of the proposed natural stereoisomer of halisphingosine A, a metabolite of the marine sponge *Haliclona tubifera*, was accomplished in 11 steps including an enantioselective Henry reaction, a Weinreb amide – acetylide coupling, and stereoselective reductions of the resulting ynone to afford the *R,Z*-configured allyl alcohol moiety. The synthetic product differed from the natural isolate in some <sup>13</sup>C-NMR data. It showed antiproliferative activity at clinically relevant concentrations against six tumour cell lines including such lacking functional tumor suppressor gene p53.

### Introduction

The halisphingosines A (1) and B (2) are C<sub>18</sub> sphingoid bases that were isolated from various species of the marine sponge genus *Haliclona*,<sup>1,2</sup> which had earlier been identified as a rich source of other bioactive metabolites such as macrolides<sup>3</sup> and alkaloids.<sup>4</sup> Their configuration was determined as *2R,3R,6R* (Fig. 1).<sup>2</sup> Extracts of the sponge *Haliclona tubifera*, rich in sphingoid bases, proved moderately cytotoxic against two brain tumour cell lines with IC<sub>50</sub> values <5 μM and were shown to inhibit the production of peroxy radicals and blood coagulation.<sup>5</sup> We now report on a stereoselective synthesis of *2R,3R,6R,7Z*-halisphingosine A (1) in quantities allowing a thorough assessment of its biological activities.

### Results and discussion

Our retrosynthetic approach is outlined in Scheme 1. The *Z*-allyl alcohol moiety was to be established by stereoselective reductions of ynone 3, since explorative attempts to obtain it by addition of *Z*-vinyl zinc reagents to aldehydes, as described by Walsh *et al.*,<sup>6</sup> were unsatisfactory in terms of yields and ee. The allyl alcohol was to be introduced by a twofold reduction of ynone 3, employing first Noyori's transfer hydrogenation catalyst<sup>7,8</sup> for the enantioselective reduction of the ketone, and

then Brown's P-2 Ni protocol<sup>9</sup> for the *Z*-selective alkyne dihydrogenation. Ynone 3 should be accessible by addition of 1-dodecynyllithium (5) to Weinreb amide 4, which in turn was to be prepared by oxidation of alcohol 6 to the corresponding carboxylic acid and its coupling with *N,O*-dimethylhydroxylamine. The two stereogenic centers of 6 were planned to be installed in one step by a *syn*- and enantioselective Henry reaction of aldehyde 8 with 2-nitroethanol (9) according to the method reported by Chen *et al.*,<sup>10</sup> followed by acetamide protection of the resulting nitrodiol to give 7, the nitro group of which was to be hydrogenated and *N*-Boc protected.

The catalytic Henry reaction of readily available aldehyde 8<sup>11</sup> with 2-nitroethanol (9) in the presence of Cu(OAc)<sub>2</sub> and ligand L<sup>1</sup> at –5 °C, as reported by Chen *et al.*,<sup>10</sup> gave nitrodiol 10 as a mixture of diastereomers with a *syn/anti* ratio of 88 : 12 and an ee (*syn*) of 98% in 93% overall yield (Scheme 2). After conversion of the delicate nitrodiol 10 into acetamide 7, the diastereomers were separated *via* column chromatography furnishing the *syn* diastereomer in 85% yield. Reduction of 7 with NiCl<sub>2</sub>/NaBH<sub>4</sub> in methanol and Boc-protection of the crude amine 11 afforded the fully protected amino alcohol 12 in 95% yield (over two steps). Hydrogenation of the benzyl protecting

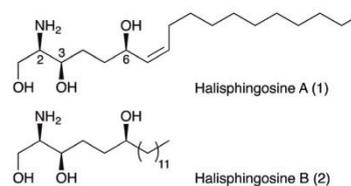
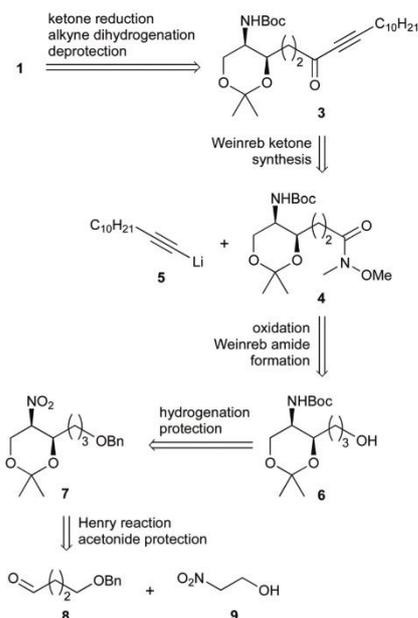


Fig. 1 Proposed structures of halisphingosines A (1) and B (2).

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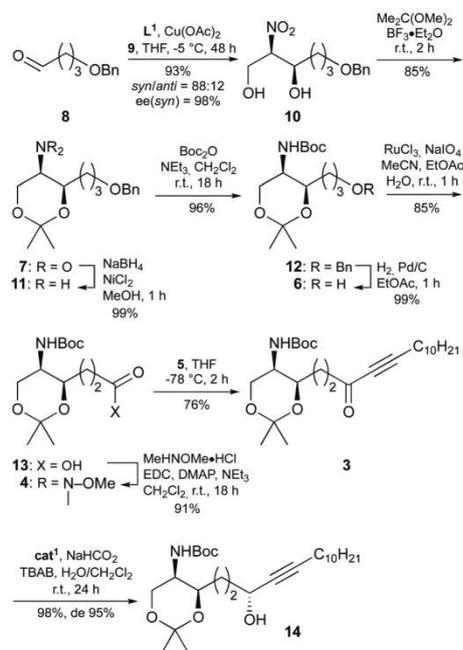
† Electronic supplementary information (ESI) available: Synthesis and analysis of Mosher amides and esters; comparison of NMR data of synthetic and isolated 1; NMR spectra and chromatogrammes; cell culture conditions and MTT-assay; <sup>1</sup>H and <sup>13</sup>C NMR spectra of new compounds. See DOI: 10.1039/d0ob01786h



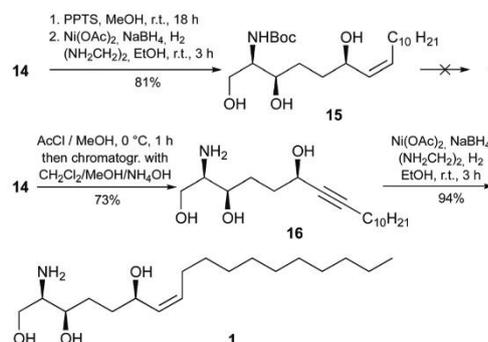
Scheme 1 Retrosynthesis of halisphingosine A (1).

group and oxidation of the resulting alcohol **6** using  $\text{RuCl}_3/\text{NaIO}_4$  gave carboxylic acid **13**, which was converted into Weinreb amide **4**. Ynone **3** was obtained by reaction of **4** with *in situ* generated 1-dodecynyllithium (**5**) at  $-78^\circ\text{C}$  in THF. The last of the three stereogenic centres was introduced by an enantioselective transfer hydrogenation using sodium formate and Noyori's  $\text{cat}^1$  under phase-transfer conditions.<sup>12</sup> The de of propargyl alcohol **14** was determined to be 95% according to the  $^{19}\text{F}$  NMR spectra of the corresponding Mosher esters.<sup>13</sup> The absolute configuration of all three stereogenic centres was ascertained to be *R* by analysis of the  $^1\text{H}$  NMR spectra of the corresponding Mosher esters and amides (*cf.* ESI<sup>†</sup> for details).<sup>14</sup>

Acetonide deprotection and *Z*-selective alkyne hydrogenation of **14** using nickel boride and ethylene diamine, as described by Brown *et al.*,<sup>9</sup> gave *N*-Boc-protected halisphingosine A (**15**, Scheme 3). *N*-Boc-cleavage of **15** under acidic conditions afforded **1**, albeit only in inseparable mixtures of different isomers, presumably resulting from intramolecular condensation and loss of configurational integrity at the allyl alcohol. However, when alkyne **14** was first deprotected by addition of  $\text{AcCl}$  to a solution in MeOH, the aminotriol **16**, obtained upon chromatography with  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ , was *Z*-selectively dihydrogenated under identical conditions to afford the target compound **1** as the sole product. After purification it showed a specific optical rotation of  $[\alpha]_{\text{D}}^{23} = +11.6$  ( $c = 0.1$ , MeOH), fairly agreeing with  $[\alpha]_{\text{D}}^{22} = +10.0$  ( $c = 0.1$ , MeOH)<sup>1</sup> as reported for the natural isolate. However, the chemical



Scheme 2 Establishment of the three stereogenic centres.

Scheme 3 *Z*-alkene formation and deprotection.

shifts of atoms C-4 and C-5 in  $^{13}\text{C}$  NMR spectra of our synthetic product **1** deviated conspicuously ( $\Delta\delta = 1.9$  ppm/3.9 ppm) from those reported by Mansoor *et al.*<sup>1</sup> for the natural isolate. Interestingly, the chemical shifts of the same

**Table 1** IC<sub>50</sub> values [μM] of 2*R*,3*R*,6*R*,7*Z*-halisphingosine A (**1**)

Cell line <sup>a</sup>	HCT-116 <sup>-/-</sup>	HCT-116 <sup>wt</sup>	518A2	U87	Ea-Hy 926	HeLa
IC <sub>50</sub> <sup>b</sup>	8.5 ± 1.1	14.1 ± 1.5	4.9 ± 1.4	5.3 ± 0.1	11.8 ± 1.6	11.9 ± 1.8

<sup>a</sup> HCT-116<sup>-/-</sup> (colon carcinoma, p53 knock-out mutant); HCT-116<sup>wt</sup> (colon carcinoma, wildtype); 518A2 (melanoma); U87 (glioblastoma); Ea-Hy926 (endothelial hybrid); HeLa (cervix carcinoma). <sup>b</sup> Values are the means ± SD determined in four independent experiments and derived from dose–response curves after 72 h incubation using the MTT assay.

atoms C-4 and C-5 of our synthetic *N*-Boc-protected halisphingosine A **15** and those reported by Molinski *et al.*<sup>2</sup> for the corresponding *N*-Boc-protected natural isolate also disagreed significantly ( $\Delta\delta = 1.6$  ppm/3.4 ppm; *cf.* ESI†). Thus we cannot exclude an incorrect assignment of the stereochemistry of the natural isolate, which was done by means of CD spectra.<sup>2</sup>

Pure synthetic **1** was finally tested for antiproliferative activity against six different human tumour cell lines (Table 1). It showed cytotoxic activity with IC<sub>50</sub> concentrations in a clinically relevant low micromolar range, comparable to the activity of the isolated natural product<sup>1</sup> and of extracts of *Haliclona tubifera*.<sup>5</sup> It is worthy of note, that its activity is independent of functional p53, the tumor suppressor protein whose gene is mutated in many tumours.

## Conclusions

In summary, we accomplished the first synthesis of 2*R*,3*R*,6*R*,7*Z*-halisphingosine A (**1**) in an overall yield of 29% over 11 steps. The stereogenic centres were build up using a *syn*- and enantioselective Henry reaction and enantioselective reductions. An NMR analysis cast some doubt on the validity of the stereochemical assignment of the natural isolate and further stereoisomers will now be synthesised to clarify this point. Our synthetic halisphingosine A inhibited the growth of various tumour cell lines at clinically relevant low micromolar IC<sub>50</sub> concentrations and in a p53-independent manner.

## Experimental section

### General information

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were obtained using a Bruker DRX 500 spectrometer. Chemical shifts are given in parts per million using the residual solvent peak as an internal standard, *i.e.* 7.26 ppm (proton) and 77.00 ppm (carbon) for CDCl<sub>3</sub> and 3.31 ppm (proton) and 49.15 ppm (carbon) for CD<sub>3</sub>OD. Coupling constants (*J*) are quoted in Hz. Multiplicity abbreviations used: s singlet, d doublet, t triplet, q quartet and m multiplet. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. IR spectra were recorded with a PerkinElmer Spectrum 100 FT-IR spectrophotometer with ATR sampling unit. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 Polarimeter. Melting points were obtained by a Büchi Melting

Point M-565 and are uncorrected. Analytical HPLC measurements were performed on a Beckman System Gold Programmable Solvent Module 125 using a Phenomenex Lux® Amylose-1-HPLC column, length 100 × 4.6 mm, pore size 100 Å, particle size 5 μm. Detection by a Beckman Instruments Diode Array Detection Module 168. All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran and dichloromethane which were freshly distilled according to standard procedures. Reactions were routinely carried out under an argon atmosphere unless stated otherwise. All glassware was flame-dried before use. Analytical thin layer chromatography was carried out using Merck Kieselgel 60 GF254 pre-coated aluminum-backed plates and/or Merck 60 RP-18 F254S foil plates. The compounds were visualized with UV light (254 nm and/or 360 nm) and/or potassium permanganate. Flash chromatography was performed at medium pressure using Machery-Nagel silica gel 60, pore size 40–63 μm with the eluent specified. Ligand **L**<sup>1</sup> and aldehyde **8** were prepared according to literature procedures.<sup>11,15</sup>

(2*R*,3*R*)-6-Benzoyloxy-2-nitrohexane-1,3-diol (**10**). **10** was prepared analogously to a literature procedure.<sup>10</sup> A stirred solution of Cu(OAc)<sub>2</sub>·H<sub>2</sub>O (1.02 g, 5.1 mmol, 0.1 equiv.) and ligand **L**<sup>1</sup> (1.37 g, 5.1 mmol, 0.1 equiv.) in dry THF (125 mL) was treated with 2-nitroethanol (14.11 g, 153.9 mmol, 3.0 equiv.) and aldehyde **8** (9.14 g, 51.3 mmol, 1.0 equiv.). The mixture was stirred at –5 °C for 48 h, and then concentrated under reduced pressure. The residue was redissolved in Et<sub>2</sub>O (100 mL). The organic phase was washed with 1 M HCl (2 × 100 mL) and brine (100 mL), dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 7 : 3) to give **10** as a colourless oil (12.79 g, 47.5 mmol, 93%). *R*<sub>f</sub> = 0.17 (*n*-hexane/ethyl acetate 7 : 3);  $[\alpha]_D^{25} = +10.3$  (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.53–1.64 (1 H, m, 4-H<sup>a</sup>), 1.70–1.84 (3 H, m, 4-H<sup>b</sup>, 5-H), 2.42–2.55 (1 H, br m, OH), 3.48–3.57 (2 H, m, CH<sub>2</sub>OBn), 3.62–3.68 (1 H, m, OH), 3.95–4.03 (1 H, m, 3-H), 4.06–4.16 (2 H, m, 1-H), 4.48–4.55 (2 H, m, OCH<sub>2</sub>Ph), 4.55–4.60 (1 H, m, CHNO<sub>2</sub>), 7.28–7.33 (3 H, m, Ph), 7.34–7.39 (2 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  25.8 (C-5), 31.2 (C-4), 61.3 (CH<sub>2</sub>OH), 69.8 (CH<sub>2</sub>OBn), 69.9 (C-3), 73.3 (OCH<sub>2</sub>Ph), 92.5 (CHNO<sub>2</sub>), 127.9 (Ph), 128.5 (Ph), 137.5 (Ph); IR ( $\nu_{\text{max}}$ /cm<sup>-1</sup>) 3371, 2939, 2870, 1549, 14454, 1362, 1074, 1027, 867, 740, 699; HRMS (ESI) *m/z* [M]<sup>+</sup> calcd for C<sub>13</sub>H<sub>20</sub>O<sub>5</sub>N<sup>+</sup> 270.1336, found 270.1329; diastereomeric ratios (dr) and enantiomeric excess (ee) were determined by HPLC (Phenomenex Lux® Amylose-1, 95 : 5 Hex/IPA,

1.0 mL min<sup>-1</sup>): *anti* isomer: tr (major) = 24.93 min, tr (minor) = 19.85 min, 89% ee; *syn* isomer: tr (major) = 30.15 min, tr (minor) = 22.17 min, 98% ee; dr (*syn/anti*) = 88 : 12.

**(4*R*,5*R*)-4-(3-(Benzyloxy)propyl)-2,2-dimethyl-5-nitro-1,3-dioxane (7).** A solution of **10** (3.47 g, 12.9 mmol, 1.0 equiv.) in acetone (90 mL) was treated with 2,2-dimethoxypropane (31.6 mL, 257.7 mmol, 20.0 equiv.) and BF<sub>3</sub>·Et<sub>2</sub>O (185 mg, 1.3 mmol, 0.1 equiv.). The mixture was stirred at room temperature for 2 h. NEt<sub>3</sub> (0.5 mL) was added, and volatiles were removed under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate 5 : 1) to give **7** as a colourless oil (3.39 g, 11.0 mmol, 85%). *R*<sub>f</sub> = 0.23 (*n*-hexane/ethyl acetate 4 : 1); [α]<sub>D</sub><sup>23</sup> = -1.8 (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.39 (3 H, s, CH<sub>3</sub>), 1.46 (3 H, s, CH<sub>3</sub>), 1.61–1.85 (4 H, m, CH<sub>2</sub>COBn, CH<sub>2</sub>CCOBn), 3.45–3.53 (2 H, m, CH<sub>2</sub>OBn), 4.08–4.15 (2 H, m, 6-H<sup>a</sup>, 4-H), 4.33 (1 H, dd, *J* = 13.3, 2.9 Hz, 6-H<sup>b</sup>), 4.44–4.53 (3 H, m, CH<sub>2</sub>Ph, CHNO<sub>2</sub>), 7.26–7.37 (5 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 19.5 (CH<sub>3</sub>), 25.5 (CCOBn), 27.9 (CH<sub>3</sub>), 28.4 (CCCOBn), 60.8 (C-6), 68.4 (C-4), 69.5 (CH<sub>2</sub>OBn), 73.0 (CH<sub>2</sub>Ph), 81.2 (C-5), 99.7 (C-2), 127.67 (Ph), 128.4 (Ph), 138.3 (Ph); IR (ν<sub>max</sub>/cm<sup>-1</sup>) 2994, 2942, 2864, 1546, 1454, 1374, 1359, 1272, 1197, 1088, 957, 876, 738, 698; HRMS (ESI) *m/z* [M + Na]<sup>+</sup> calcd for C<sub>16</sub>H<sub>23</sub>O<sub>5</sub>NNa<sup>+</sup> 332.1468, found 332.1461; HPLC (Phenomenex Lux® Amylose-1, 95 : 5 Hex/IPA, 1.0 mL min<sup>-1</sup>): *syn* isomer: tr (major) = 11.73 min, tr (minor) = 5.80 min.

**(4*R*,5*R*)-5-Amino-4-(3-benzyloxypropyl)-2,2-dimethyl-1,3-dioxane (11).** NaBH<sub>4</sub> (2.05 g, 54.3 mmol, 1.0 equiv.) was slowly added to **7** (2.40 g, 7.8 mmol, 1.0 equiv.) and NiCl<sub>2</sub>·6H<sub>2</sub>O (2.77 g, 11.6 mmol, 1.0 equiv.) in MeOH (100 mL). The mixture was stirred for 30 min at room temperature. The suspension was filtered through Celite and volatiles were evaporated under reduced pressure. The residue was taken up in 1 M NaOH (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to dryness to give **11** as a colourless oil (2.15 g, 7.7 mmol, 99%). *R*<sub>f</sub> = 0.57 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95 : 5); [α]<sub>D</sub><sup>23</sup> = +6.3 (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.38 (3 H, s, CH<sub>3</sub>), 1.40 (3 H, s, CH<sub>3</sub>), 1.50–1.77 (4 H, m, CH<sub>2</sub>COBn, CH<sub>2</sub>CCOBn), 2.42–2.44 (1 H, m, 5-H), 3.44–3.53 (2 H, m, CH<sub>2</sub>OBn), 3.70 (1 H, dd, *J* = 11.6, 1.5 Hz, 6-H<sup>a</sup>), 3.82–3.85 (1 H, m, 4-H), 4.06 (1 H, dd, *J* = 11.6, 2.0 Hz, 6-H<sup>b</sup>), 4.45–4.54 (2 H, m, CH<sub>2</sub>Ph), 7.26–7.30 (1 H, m, Ph), 7.31–7.36 (4 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 18.6 (CH<sub>3</sub>), 25.4 (CCOBn), 28.6 (CCCOBn), 29.7 (CH<sub>3</sub>), 47.7 (C-5), 67.4 (C-6), 70.0 (CH<sub>2</sub>OBn), 71.7 (C-4), 72.9 (CH<sub>2</sub>Ph), 98.7 (C-2), 127.5 (Ph), 127.6 (Ph), 128.3 (Ph), 138.4 (Ph); IR (ν<sub>max</sub>/cm<sup>-1</sup>) 2991, 2860, 1455, 1379, 1271, 1196, 1096, 1071, 980, 844, 736, 698; HRMS (ESI) *m/z* [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>26</sub>O<sub>3</sub>N<sup>+</sup> 280.1907, found 280.1902.

**(4*R*,5*R*)-*tert*-Butyl 4-(3-benzyloxypropyl)-2,2-dimethyl-1,3-dioxan-5-yl carbamate (12).** **11** (1.68 g, 6.0 mmol, 1.0 equiv.) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (60 mL) and treated with Boc<sub>2</sub>O (1.44 g, 6.6 mmol, 1.1 equiv.) and NEt<sub>3</sub> (1.7 mL, 12.0 mmol, 2.0 equiv.). The mixture was stirred for 18 h at room temperature. 60 mL 1 M HCl were added and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL). The combined organic

phases were washed with brine (50 mL), dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 5 : 1) to give **12** as a colourless oil (2.20 g, 5.8 mmol, 96%). *R*<sub>f</sub> = 0.25 (*n*-hexane/ethyl acetate 5 : 1); [α]<sub>D</sub><sup>23</sup> = -8.7 (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.38 (3 H, s, CH<sub>3</sub>), 1.40 (3 H, s, CH<sub>3</sub>), 1.44 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.49–1.73 (4 H, m, CH<sub>2</sub>COBn, CH<sub>2</sub>CCOBn), 3.42–3.52 (3 H, m, CH<sub>2</sub>OBn, CHNH), 3.73 (1 H, dd, *J* = 11.9, 1.5 Hz, 6-H<sup>a</sup>), 3.89–3.94 (1 H, m, 4-H), 4.01 (1 H, dd, *J* = 11.9, 1.8 Hz, 6-H<sup>b</sup>), 4.44–4.52 (2 H, m, CH<sub>2</sub>Ph), 5.29 (1 H, d, *J* = 9.8 Hz, NH), 7.24–7.36 (5 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 18.5 (CH<sub>3</sub>), 25.1 (CCOBn), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 28.5 (CCCOBn), 29.7 (CH<sub>3</sub>), 46.8 (C-5), 65.3 (C-6), 69.9 (CH<sub>2</sub>OBn), 71.1 (C-4), 72.8 (CH<sub>2</sub>Ph), 79.3 (C(CH<sub>3</sub>)<sub>3</sub>), 98.9 (C-2), 127.5 (Ph), 127.6 (Ph), 128.3 (Ph), 138.5 (Ph), 155.7 (NCO); IR (ν<sub>max</sub>/cm<sup>-1</sup>) 2978, 2867, 1710, 1496, 1456, 1381, 1365, 1272, 1244, 1197, 1164, 1084, 1028, 984, 952, 850, 736, 698; HRMS (ESI) *m/z* [M + Na]<sup>+</sup> calcd for C<sub>21</sub>H<sub>33</sub>O<sub>5</sub>NNa<sup>+</sup> 402.2251, found 402.2245.

**(4*R*,5*R*)-*tert*-Butyl 4-(3-hydroxypropyl)-2,2-dimethyl-1,3-dioxan-5-yl carbamate (6).** To a solution of **12** (1.49 g, 4.0 mmol, 1.0 equiv.) in EtOAc (30 mL) was added 5% Pd/C (150 mg, 10 wt-%) and the mixture was stirred under a hydrogen atmosphere at ambient temperature for 1 h. The suspension was filtered over Celite and the resulting filtrate was concentrated *in vacuo* to give **6** as a colourless oil (1.13 g, 3.9 mmol, 99%). [α]<sub>D</sub><sup>23</sup> = -2.0 (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.39 (3 H, s, CH<sub>3</sub>), 1.42–1.45 (9 H, m, C(CH<sub>3</sub>)<sub>3</sub>), 1.45 (3 H, s, CH<sub>3</sub>), 1.49–1.70 (4 H, m, CH<sub>2</sub>COH, CH<sub>2</sub>CCOH), 3.51 (1 H, dt, *J* = 9.8, 1.6 Hz, CHNH), 3.61 (2 H, t, *J* = 5.5 Hz, CH<sub>2</sub>OH), 3.71–3.76 (1 H, m, 6-H<sup>a</sup>), 3.93 (1 H, t, *J* = 6.3 Hz, 4-H), 4.06 (1 H, d, *J* = 11.9 Hz, 6-H<sup>b</sup>), 5.32 (1 H, d, *J* = 9.8 Hz, NH); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 18.5 (CH<sub>3</sub>), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 28.5 (CH<sub>2</sub>COH), 28.6 (CH<sub>2</sub>CCOH), 29.6 (CH<sub>3</sub>), 47.0 (CHNH), 62.5 (CH<sub>2</sub>OH), 65.2 (C-6), 71.7 (C-4), 79.5 (C(CH<sub>3</sub>)<sub>3</sub>), 99.1 (C-2), 155.9 (NCO); IR (ν<sub>max</sub>/cm<sup>-1</sup>) 3448, 2939, 2873, 1694, 1499, 1366, 1244, 1164, 1083, 1057, 985, 840; HRMS (ESI) *m/z* [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>28</sub>O<sub>5</sub>N<sup>+</sup> 290.1962, found 290.1957.

**(4*R*,5*R*)-3-(5-(*tert*-Butoxycarbonylamino)-2,2-dimethyl-1,3-dioxan-4-yl)propanoic acid (13).** To a solution of **6** (150 mg, 0.52 mmol, 1.0 equiv.) in MeCN/EtOAc 1 : 1 (4 mL) at 0 °C was added a solution of NaIO<sub>4</sub> (455 mg, 2.13 mmol, 4.1 equiv.) and RuCl<sub>3</sub>·H<sub>2</sub>O (3 mg, 0.02 mmol, 0.03 equiv.) in H<sub>2</sub>O (3 mL). The mixture was stirred at ambient temperature for 2 h, filtered over Celite and diluted with H<sub>2</sub>O (20 mL). The aqueous phase was extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (50 mL), dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1 : 1) to give **13** as a colourless oil (127 mg, 0.44 mmol, 85%). *R*<sub>f</sub> = 0.29 (*n*-hexane/ethyl acetate 1 : 1); [α]<sub>D</sub><sup>23</sup> = -5.5 (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.39 (3 H, s, CH<sub>3</sub>), 1.44 (3 H, s, CH<sub>3</sub>), 1.44–1.46 (9 H, m, C(CH<sub>3</sub>)<sub>3</sub>), 1.71–1.87 (2 H, m, CH<sub>2</sub>CCO<sub>2</sub>), 2.44 (2 H, td, *J* = 7.2, 1.7 Hz, CH<sub>2</sub>CO<sub>2</sub>), 3.53 (1 H, dd, *J* = 9.8, 1.9 Hz, CHNH), 3.74 (1 H, dd, *J* = 11.9, 1.9 Hz, 6-H<sup>a</sup>), 4.00 (1 H, ddd, *J* = 8.5, 5.1, 1.9 Hz, 4-H), 4.06 (1 H, dd, *J* = 11.9, 1.9 Hz, 6-H<sup>b</sup>), 5.31 (1 H, d, *J* = 9.8 Hz, NH); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)

$\delta$  18.5 (CH<sub>3</sub>), 26.7 (CH<sub>2</sub>CCO<sub>2</sub>), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 29.4 (CH<sub>2</sub>CO<sub>2</sub>), 29.5 (CH<sub>3</sub>), 46.8 (CHNH), 65.2 (C-6), 70.3 (C-4), 79.7 (C(CH<sub>3</sub>)<sub>3</sub>), 99.2 (C-2), 155.8 (NCO), 178.4 (COOH); IR ( $\nu_{\max}/\text{cm}^{-1}$ ) 2977, 1708, 1501, 1367, 1243, 1162, 1083, 982, 849, 733; HRMS (ESI)  $m/z$  [M + Na]<sup>+</sup> calcd for C<sub>14</sub>H<sub>25</sub>O<sub>6</sub>NNa<sup>+</sup> 326.1574, found 326.1565.

**(4R,5R)-tert-Butyl 4-(3-(methoxymethylamino)-3-oxopropyl)-2,2-dimethyl-1,3-dioxan-5-yl carbamate (4).** A mixture of **13** (160 mg, 0.53 mmol, 1.0 equiv.), *N,O*-dimethylhydroxylamine hydrochloride (67 mg, 0.69 mmol, 1.3 equiv.), DMAP (13 mg, 0.11 mmol, 0.2 equiv.) and dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at 0 °C was treated with NEt<sub>3</sub> (220  $\mu$ L, 1.58 mmol, 3.0 equiv.) and EDC·HCl (131 mg, 0.69 mmol, 1.3 equiv.). The resulting mixture was stirred at room temperature for 18 h and then diluted with CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The organic phase was washed with 1 M HCl (25 mL), aq. NaHCO<sub>3</sub> (25 mL) and brine (25 mL), dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1 : 1) to give **4** as a colourless oil (167 mg, 0.48 mmol, 91%).  $R_f$  = 0.27 (*n*-hexane/ethyl acetate 1 : 1); [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -11.8 (c 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.36–1.41 (3 H, m, CH<sub>3</sub>), 1.42–1.47 (12 H, m, CH<sub>3</sub>, C(CH<sub>3</sub>)<sub>3</sub>), 1.73–1.86 (2 H, m, CH<sub>2</sub>CCON), 2.41–2.57 (2 H, m, CH<sub>2</sub>CON), 3.17 (3 H, s, NCH<sub>3</sub>), 3.50–3.55 (1 H, m, CHNH), 3.66–3.69 (3 H, m, OCH<sub>3</sub>), 3.71–3.76 (1 H, m, 6-H<sup>a</sup>), 4.01–4.08 (2 H, m, 6-H<sup>b</sup>, 4-H), 5.29 (1 H, d,  $J$  = 9.8, NH); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  18.6 (CH<sub>3</sub>), 26.6 (CH<sub>2</sub>CCON), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 29.7 (CH<sub>3</sub>), 46.8 (CHNH), 61.2 (OCH<sub>3</sub>), 65.3 (C-6), 70.3 (C-4), 79.4 (C(CH<sub>3</sub>)<sub>3</sub>), 99.0 (C-2), 155.7 (NCO), 174.0 (NCO); IR ( $\nu_{\max}/\text{cm}^{-1}$ ) 2977, 2938, 1707, 1662, 1496, 1381, 1365, 1245, 1163, 1082, 983, 851; HRMS (ESI)  $m/z$  [M + Na]<sup>+</sup> calcd for C<sub>16</sub>H<sub>30</sub>O<sub>6</sub>N<sub>2</sub>Na<sup>+</sup> 369.1996, found 369.1986.

**(4R,5R)-tert-Butyl 2,2-dimethyl-4-(3-oxopentadec-4-yn-1-yl)-1,3-dioxan-5-yl carbamate (3).** 1-Dodecyne (110  $\mu$ L, 0.50 mmol, 2.2 equiv.) in dry THF (2.5 mL) was treated with 2.2 M *n*-BuLi in hexanes (210  $\mu$ L, 0.46 mmol, 2.0 equiv.) at -78 °C. The mixture was stirred for 1 h at -78 °C. A solution of amide **4** (80 mg, 0.23 mmol, 1.0 equiv.) in dry THF (2 mL) was added, the resulting mixture was warmed to room temperature and stirred for 2 h. Saturated aqueous ammonium chloride (10 mL) was added and the aqueous phase was extracted with Et<sub>2</sub>O (3  $\times$  10 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 5 : 1) to give **3** as a colourless oil (79 mg, 0.17 mmol, 76%).  $R_f$  = 0.29 (*n*-hexane/ethyl acetate 5 : 1); [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -6.5 (c 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.85–0.90 (3 H, m, CH<sub>3</sub>), 1.24–1.32 (12 H, m, (CH<sub>2</sub>)<sub>6</sub>), 1.36–1.40 (5 H, m, CH<sub>3</sub>, CH<sub>2</sub>), 1.42 (3 H, s, CH<sub>3</sub>), 1.43–1.45 (9 H, m, C(CH<sub>3</sub>)<sub>3</sub>), 1.56 (2 H, quin,  $J$  = 7.2 Hz, CH<sub>2</sub>CC $\equiv$ C), 1.76–1.83 (2 H, m, CH<sub>2</sub>CCO), 2.34 (2 H, t,  $J$  = 7.2 Hz, CH<sub>2</sub>C $\equiv$ C), 2.61 (2 H, t,  $J$  = 7.3 Hz, CH<sub>2</sub>CO), 3.50 (1 H, dd,  $J$  = 9.8, 1.8 Hz, CHNH), 3.73 (1 H, dd,  $J$  = 12.0, 1.8 Hz, 6-H<sup>a</sup>), 3.94 (1 H, ddd,  $J$  = 7.7, 5.9, 1.8 Hz, 4-H), 4.03 (1 H, dd,  $J$  = 12.0, 1.8 Hz, 6-H<sup>b</sup>), 5.27 (1 H, d,  $J$  = 9.8 Hz, NH); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  14.1 (CH<sub>3</sub>), 18.5 (CH<sub>3</sub>), 18.9 (CH<sub>2</sub>), 22.6 (CH<sub>2</sub>), 26.1 (CH<sub>2</sub>), 27.7 (CH<sub>2</sub>), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 28.9 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 29.6 (CH<sub>3</sub>), 31.8 (CH<sub>2</sub>), 40.6

(CH<sub>2</sub>), 46.8 (CHNH), 65.2 (C-6), 70.1 (C-4), 79.5 (C(CH<sub>3</sub>)<sub>3</sub>), 80.8 (C $\equiv$ CCO), 94.5 (C $\equiv$ CCO), 99.1 (C(CH<sub>3</sub>)<sub>2</sub>), 155.7 (NCO), 187.4 (CO); IR ( $\nu_{\max}/\text{cm}^{-1}$ ) 2926, 2856, 2213, 1713, 1674, 1496, 1365, 1243, 1164, 1084, 982, 851; HRMS (ESI)  $m/z$  [M + Na]<sup>+</sup> calcd for C<sub>26</sub>H<sub>45</sub>O<sub>5</sub>NNa<sup>+</sup> 474.3189, found 474.3181.

**(4R,5R,3'R)-tert-Butyl 4-(3'-hydroxypentadec-4'-yn-1'-yl)-2,2-dimethyl-1,3-dioxan-5-yl carbamate (14).** A mixture of **3** (60 mg, 0.13 mmol, 1.0 equiv.), TBAB (13 mg, 0.04 mmol, 0.3 equiv.), NaHCO<sub>2</sub> (90 mg, 1.30 mmol, 10.0 equiv.) and CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O 1 : 1 (4 mL) was treated with RuCl(*p*-cymene)[(R,R)-TsDPEN] (1 mg, 1.3  $\mu$ mol, 0.01 equiv.) and stirred at ambient temperature for 24 h. H<sub>2</sub>O (5 mL) was added and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  5 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 3 : 1) to give **14** as a colourless oil (59 mg, 0.13 mmol, 98%).  $R_f$  = 0.20 (*n*-hexane/ethyl acetate 3 : 1); [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -5.4 (c 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.85–0.90 (3 H, m, CH<sub>3</sub>), 1.22–1.31 (12 H, m, (CH<sub>2</sub>)<sub>6</sub>), 1.31–1.38 (4 H, m, (CH<sub>2</sub>)<sub>2</sub>), 1.40 (3 H, s, CH<sub>3</sub>), 1.44–1.48 (12 H, m, C(CH<sub>3</sub>)<sub>3</sub>, CH<sub>3</sub>), 1.48–1.53 (2 H, m, CH<sub>2</sub>), 1.60–1.67 (2 H, m, CH<sub>2</sub>), 1.73–1.80 (2 H, m, CH<sub>2</sub>), 2.18 (2 H, td,  $J$  = 7.2, 2.0 Hz, CH<sub>2</sub>C $\equiv$ C), 2.32–2.35 (1 H, br m, OH), 3.52 (1 H, dd,  $J$  = 9.8, 1.8 Hz, CHNH), 3.75 (1 H, dd,  $J$  = 11.9, 1.8 Hz, 6-H<sup>a</sup>), 3.96 (1 H, ddd,  $J$  = 7.4, 5.7, 1.8 Hz, 4-H), 4.06 (1 H, dd,  $J$  = 11.9, 1.8 Hz, 6-H<sup>b</sup>), 4.33–4.39 (1 H, m, 3'-H), 5.30 (1 H, d,  $J$  = 9.8 Hz, NH); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  14.1 (CH<sub>3</sub>), 18.6 (CH<sub>3</sub>), 18.7 (CH<sub>2</sub>), 22.7 (CH<sub>2</sub>), 27.8 (CH<sub>2</sub>), 28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 28.7 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 29.6 (CH<sub>3</sub>), 34.1 (CH<sub>2</sub>), 47.0 (CHNH), 62.5 (C-3'), 65.3 (C-6), 71.5 (C-4), 79.5 (C(CH<sub>3</sub>)<sub>3</sub>), 80.8 (CH<sub>2</sub>C $\equiv$ C), 85.5 (CH<sub>2</sub>C $\equiv$ C), 99.2 (C(CH<sub>3</sub>)<sub>2</sub>), 155.9 (NCO); IR ( $\nu_{\max}/\text{cm}^{-1}$ ) 3454, 2928, 2856, 1715, 1501, 1366, 1245, 1167, 1086, 850; HRMS (ESI)  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>48</sub>O<sub>5</sub>N<sup>+</sup> 454.3527, found 454.3522.

**(2R,3R,6R,7Z)-N-Boc-Halispingosine A (15).** A solution of carbamate **14** (50 mg, 0.11 mmol, 1.0 equiv.) in MeOH (5 mL) was treated with PPTS and stirred at ambient temperature for 18 h. Volatiles were removed *in vacuo*. The residue was taken up in EtOAc and filtered over a plug of silica. The filtrate was concentrated under reduced pressure. The crude product was added to a mixture of Ni(OAc)<sub>2</sub>·4H<sub>2</sub>O (27 mg, 0.11 mmol, 1.0 equiv.), NaBH<sub>4</sub> (4 mg, 0.11 mmol, 1.0 equiv.) and ethylene diamine (29  $\mu$ L, 0.44 mmol, 4.0 equiv.) in EtOH (5 mL). The mixture was stirred under hydrogen atmosphere at ambient temperature for 3 h. The suspension was filtered over Celite and the resulting filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1 : 2) to give **15** as a colourless oil (37 mg, 89  $\mu$ mol, 81%).  $R_f$  = 0.23 (*n*-hexane/ethyl acetate 1 : 2); [ $\alpha$ ]<sub>D</sub><sup>23</sup> = +9.8 (c 0.2 in CHCl<sub>3</sub>) {lit.<sup>2</sup> [ $\alpha$ ]<sub>D</sub><sup>23</sup> = +12.7 (c 0.2 in MeOH)}; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.90 (3 H, t,  $J$  = 7.0 Hz, CH<sub>3</sub>), 1.24–1.41 (16 H, m, (CH<sub>2</sub>)<sub>8</sub>), 1.42–1.46 (9 H, m, C(CH<sub>3</sub>)<sub>3</sub>), 1.47–1.57 (3 H, m, 4-H<sup>a</sup>, 5-H), 1.64–1.73 (1 H, m, 4-H<sup>b</sup>), 2.03–2.15 (2 H, m, =CHCH<sub>2</sub>), 3.51–3.58 (2 H, m, CH<sub>2</sub>OH), 3.58–3.64 (1 H, m, CHNH), 3.72–3.79 (1 H, m, 3-H), 4.37 (1 H, dt,  $J$  = 8.5, 6.6 Hz, 6-H), 5.33 (1 H, dd,  $J$  = 11.0, 8.5 Hz, 7-H), 5.44 (1 H, dt,  $J$  = 11.0,

7.6 Hz, 8-H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  14.5 ( $\text{CH}_3$ ), 23.8 ( $\text{CH}_2$ ), 28.7 ( $\text{CH}_2$ ), 28.8 ( $\text{C}(\text{CH}_3)_3$ ), 30.5 ( $\text{CH}_2$ ), 30.6 ( $\text{CH}_2$ ), 30.8 ( $\text{CH}_2$ ), 30.9 ( $\text{CH}_2$ ), 31.2 ( $\text{CH}_2$ ), 33.1 ( $\text{CH}_2$ ), 35.1 ( $\text{CH}_2$ ), 56.8 ( $\text{CHNH}$ ), 63.2 ( $\text{CH}_2\text{OH}$ ), 68.4 (C-6), 71.2 (C-3), 80.1 ( $\text{C}(\text{CH}_3)_3$ ), 132.4 (C-8), 133.9 (C-7), 158.4 (NCO); IR ( $\nu_{\text{max}}/\text{cm}^{-1}$ ) 3358, 2923, 2854, 1688, 1502, 1420, 1366, 1169, 1048, 722; HRMS (ESI)  $m/z$   $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{23}\text{H}_{45}\text{O}_5\text{NNa}^+$  438.3190, found 438.3207.

**(2R,3R,6R)-2-Amino-octadec-7-yne-1,3,6-triol (16).** **14** (40 mg, 96  $\mu\text{mol}$ ) in MeOH (3 mL), was treated with AcCl (0.5 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h. Volatiles were evaporated and the crude product was purified by flash chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$  89:10:1) to give **16** as a white solid (22 mg, 70  $\mu\text{mol}$ , 73%) of mp 85 °C;  $R_f$  = 0.09 ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$  89:10:1);  $[\alpha]_{\text{D}}^{23}$  = +20.5 (c 1.0 in  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.90 (3 H, t,  $J$  = 7.0 Hz,  $\text{CH}_3$ ), 1.24–1.37 (14 H, m, ( $\text{CH}_2$ )), 1.37–1.45 (2 H, m,  $\text{CH}_2$ ), 1.46–1.53 (2 H, m,  $\text{CH}_2$ ), 1.55–1.64 (1 H, m, 4-H<sup>a</sup>), 1.65–1.75 (2 H, m,  $\text{CH}_2$ , 5-H), 1.81–1.91 (1 H, m, 4-H<sup>b</sup>), 2.20 (2 H, td,  $J$  = 7.0, 1.8 Hz, 9-H), 2.77 (1 H, dt,  $J$  = 6.7, 5.2 Hz,  $\text{CHNH}_2$ ), 3.53 (1 H, dd,  $J$  = 11.0, 6.7 Hz, 1-H<sup>a</sup>), 3.60 (1 H, ddd,  $J$  = 8.9, 5.2, 3.7 Hz, 3-H), 3.66 (1 H, dd,  $J$  = 11.0, 4.6 Hz, 1-H<sup>b</sup>), 4.31–4.35 (1 H, m, 6-H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  14.6 ( $\text{CH}_3$ ), 19.5 ( $\text{CH}_2$ ), 23.9 ( $\text{CH}_2$ ), 30.1 ( $\text{CH}_2$ ), 30.4 ( $\text{CH}_2$ ), 30.5 ( $\text{CH}_2$ ), 30.8 ( $\text{CH}_2$ ), 30.9 ( $\text{CH}_2$ ), 33.2 ( $\text{CH}_2$ ), 35.7 ( $\text{CH}_2$ ), 58.4 ( $\text{CHNH}_2$ ), 62.9 (C-6), 63.8 ( $\text{CH}_2\text{OH}$ ), 71.5 (C-3), 82.6 (C-8), 85.6 (C-7); IR ( $\nu_{\text{max}}/\text{cm}^{-1}$ ) 3336, 2923, 2854, 1674, 1466, 1202, 1137, 1048, 722; HRMS (ESI)  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{18}\text{H}_{36}\text{O}_3\text{N}^+$  314.2689, found 314.2685.

**(2R,3R,6R,7Z)-Halisphingosine A (1).** Ni(OAc) $_2$ ·4H $_2$ O (32 mg, 0.13 mmol, 1.0 equiv.) in EtOH (2 mL) was treated with NaBH $_4$  (5 mg, 0.13 mmol, 1.0 equiv.) at 0 °C. The mixture was stirred under a hydrogen atmosphere at ambient temperature for 30 min. Ethylene diamine (35  $\mu\text{L}$ , 0.51 mmol, 4.0 equiv.) and **16** (40 mg, 0.13 mmol, 1.0 equiv.) were added. The mixture was stirred under a hydrogen atmosphere at ambient temperature for a further 3 h. The resulting suspension was filtered over Celite and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$  80:19:1) to give **1** as a colourless oil (38 mg, 0.12 mmol, 94%).  $R_f$  = 0.19 ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$  80:19:1);  $[\alpha]_{\text{D}}^{23}$  = +11.6 (c 0.1 in MeOH) {lit.<sup>1</sup>  $[\alpha]_{\text{D}}^{23}$  = +10.0 (c 0.1 in MeOH)};  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (3 H, t,  $J$  = 7.6 Hz,  $\text{CH}_3$ ), 1.32–1.41 (16 H, m, ( $\text{CH}_2$ ) $_8$ ), 1.43–1.64 (3 H, m, 4-H<sup>a</sup>, 5-H), 1.67–1.77 (1 H, m, 4-H<sup>b</sup>), 2.03–2.16 (2 H, m, 9-H), 2.77 (1 H, dt,  $J$  = 6.7, 5.3 Hz,  $\text{CHNH}_2$ ), 3.52 (1 H, dd,  $J$  = 11.0, 6.7 Hz, 1-H<sup>a</sup>), 3.55–3.60 (1 H, m, 3-H), 3.65 (1 H, dd,  $J$  = 11.0, 4.6 Hz, 1-H<sup>b</sup>), 4.41, (1 H, td,  $J$  = 7.8, 5.8 Hz, 6-H), 5.36 (1 H, dd,  $J$  = 11.0, 9.0 Hz, 7-H), 5.45 (1 H, dt,  $J$  = 11.0, 7.3 Hz, 8-H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  14.5 ( $\text{CH}_3$ ), 23.7 ( $\text{CH}_2$ ), 28.7 ( $\text{CH}_2$ ), 30.5

( $\text{CH}_2$ ), 30.7 ( $\text{CH}_2$ ), 30.8 ( $\text{CH}_2$ ), 30.9 ( $\text{CH}_2$ ), 33.1 ( $\text{CH}_2$ ), 34.8 ( $\text{CH}_2$ ), 58.4 ( $\text{CHNH}_2$ ), 63.4 ( $\text{CH}_2\text{OH}$ ), 68.1 (C-6), 71.4 (C-3), 132.4 (C-8), 133.8 (C-7); IR ( $\nu_{\text{max}}/\text{cm}^{-1}$ ) 3337, 2923, 2854, 1674, 1457, 1202, 1138, 1048, 721; HRMS (ESI)  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{18}\text{H}_{38}\text{O}_3\text{N}^+$  316.2846, found 316.2843.

## Conflicts of interest

There are no conflicts to declare.

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## Supporting Information

### Synthesis and antiproliferative effect of the proposed stereoisomer of the marine sponge metabolite halisphingosine A

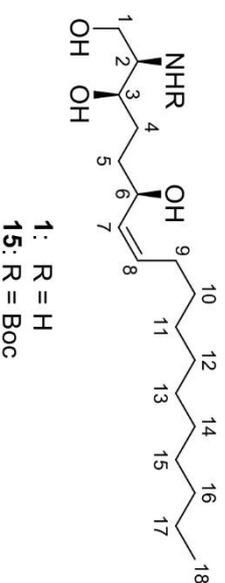
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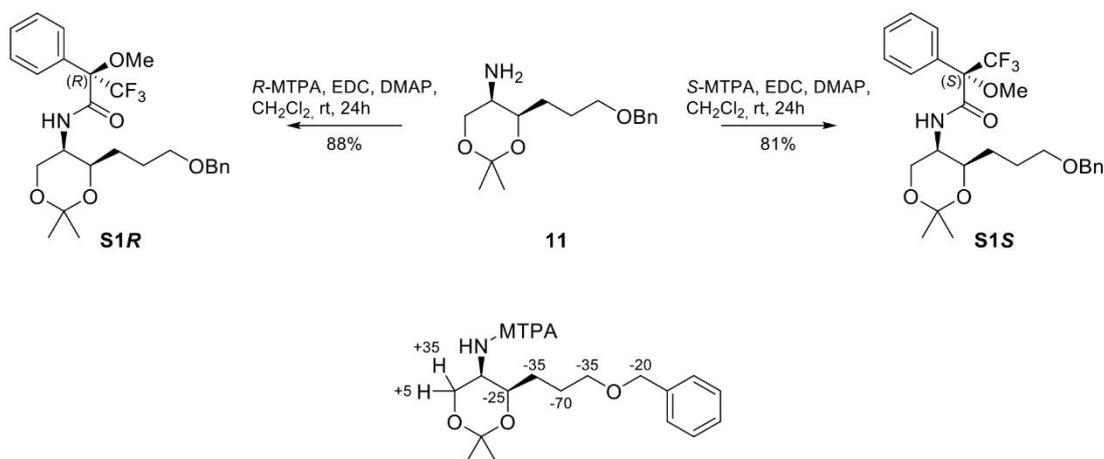
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Table 1. Comparison of  $^{13}\text{C}$  and  $^1\text{H}$  NMR shifts of isolated and synthetic Halisphingosine A (**1**) and *N*-Boc-Halisphingosine A (**15**)

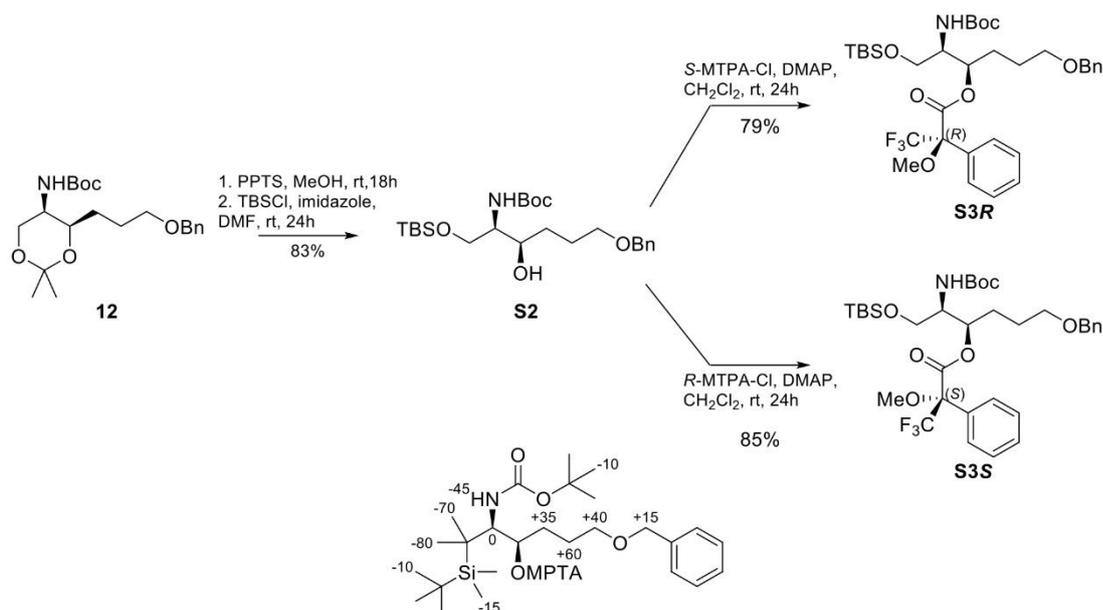
	isolated <b>1</b> (lit. <sup>1</sup> ) in CD <sub>3</sub> OD		synthetic <b>1</b> in CD <sub>3</sub> OD		<b>15</b> prepared from isolated <b>1</b> (lit. <sup>2</sup> ) in CD <sub>3</sub> OD		our fully synthetic <b>15</b> in CD <sub>3</sub> OD	
Pos.	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> [Hz])	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> [Hz])	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> [Hz])	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> [Hz])
1	60.9	3.74 (dd, 11.5, 4.5)	63.4	3.65 (dd, 11.0, 4.6)	62.8	3.60 (m)	63.2	3.60 (m)
		3.62 (dd, 11.5, 7.0)		3.52 (dd, 11.0, 6.7)		3.55 (m)		3.56 (m)
2	59.0	2.99 (dt, 7.0, 4.5)	58.4	2.77 (dt, 6.7, 5.2)	56.5	3.54 (m)	56.8	3.53 (m)
3	69.3	3.64 (m)	71.4	3.58 (m)	70.7	3.75 (m)	71.2	3.76 (m)
4	35.0	1.52 (m)	33.08	1.58 (m)	34.7	1.45 (m)	33.1	1.48 (m)
		1.42 (m)		1.52 (m)				
5	38.7	1.55 (m)	34.8	1.72 (m)	38.5	1.56 (m)	35.1	1.69 (m)
		1.35 (m)		1.48 (m)		1.38 (m)		1.53 (m)
6	68.3	4.35 (m)	68.1	4.41 (td, 7.8, 5.8)	67.9	4.37 (dt, 9.0, 5.7)	68.4	4.37 (dt, 8.5, 6.7)
7	134.0	5.31 (dd, 11.0, 9.0)	133.8	5.36 (dd, 11.0, 9.0)	133.7	5.31 (ddd, 9.3, 7.8, 1.5)	133.9	5.33 (dd, 11.0, 8.5)
8	132.2	5.44 (dt, 11.0, 7.5)	132.4	5.45 (dt, 11.0, 7.3)	131.8	5.45 (dt, 9.3, 6.4)	132.4	5.44 (dt, 11.0, 7.6)
9	28.7	2.08 (m)	28.7	2.08 (m)	28.4	2.09 (m)	28.7	2.09 (m)
10-17	23.7 - 32.9	1.28 - 1.37 (m)	23.7 - 30.9	1.24 - 1.40 (m)	23.5 - 32.5	1.29-1.41 (m)	23.8 - 31.2	1.28 - 1.40 (m)
18	14.1	0.90 (t, 7.0)	14.5	0.90 (t, 7.0)	14.1	0.91 (t, 6.7)	14.5	0.90 (t, 7.0)
Boc-CH <sub>3</sub>	-	-	-	-	28.5	1.45 (s)	28.8	1.45 (s)
Boc-C=O	-	-	-	-	156.4	-	158.4	-
Boc-CCH <sub>3</sub>	-	-	-	-	79.6	-	80.1	-

Synthesis and analysis of Mosher amides and Mosher esters **S1**-**S4**<sup>3,4</sup>

Scheme S1. Synthesis and  $\Delta\delta_{SR} = \delta_S - \delta_R$  values in Hz of the  $^1\text{H}$  NMR spectra of Mosher-amides **S1R** and **S1S**.

**(R)- and (S)-N-((4R,5R)-4-(3-(benzyloxy)propyl)-2,2-dimethyl-1,3-dioxan-5-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide (S1R, S1S)**

A solution of amine **11** (25 mg, 89  $\mu\text{mol}$ , 1.0 equiv.) in dry  $\text{CH}_2\text{Cl}_2$  (1 mL) was treated with  $\text{NEt}_3$  (25  $\mu\text{L}$ , 179  $\mu\text{mol}$ , 2.0 equiv.), EDC-HCl (21 mg, 107  $\mu\text{mol}$ , 1.2 equiv.) and (*S*)- or (*R*)-MTPA (25 mg, 107  $\mu\text{mol}$ , 1.2 equiv.). The mixture was stirred at ambient temperature for 18 h and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 2:1) to give **S1R** and **S1S** as colorless oils;  $R_f = 0.57$  (*n*-hexane/ethyl acetate 2:1); IR ( $\nu_{\text{max}}/\text{cm}^{-1}$ ) 3427, 2951, 1698, 1508, 1271, 1166, 1103, 714, 698; HRMS (ESI)  $m/z$  [ $\text{M} + \text{H}$ ] $^+$  calcd for  $\text{C}_{26}\text{H}_{33}\text{O}_5\text{NF}_3$  496.2305, found 496.2288, [ $\text{M} + \text{Na}$ ] $^+$  calcd for  $\text{C}_{26}\text{H}_{32}\text{O}_5\text{NF}_3\text{Na}^+$  518.2125, found 518.2105; **S1R** (43 mg, 72  $\mu\text{mol}$ , 81%):  $[\alpha]^{23}_{\text{D}} = -33.8$  (*c* 1.0 in  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.37-1.41 (3 H, s,  $\text{CH}_3$ ), 1.42-1.46 (3 H, s,  $\text{CH}_3$ ), 1.48-1.57 (1 H, m,  $\text{CHHCCOBn}$ ), 1.58-1.77 (3 H, m,  $\text{CHHCH}_2\text{COBn}$ ), 3.39 (3 H, s,  $\text{OCH}_3$ ), 3.43-3.52 (2 H, m,  $\text{CH}_2\text{OBn}$ ), 3.70, (1 H, dd,  $J = 12.1, 2.0$  Hz, 6- $\text{H}^{\text{a}}$ ), 3.90 (1 H, ddd,  $J = 9.5, 1.7, 1.7$  Hz,  $\text{CHNH}$ ), 4.00-4.04 (1 H, m, 4- $\text{H}$ ), 1.06 (1 H, dd,  $J = 12.1, 2.0$  Hz, 6- $\text{H}^{\text{b}}$ ), 4.44-4.54 (2 H, m,  $\text{CH}_2\text{Ph}$ ), 7.26-7.37 (4 H, m, Ph), 7.39-7.45 (3 H, m, Ph), 7.53-7.63 (3 H, m, Ph);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  18.5 ( $\text{CH}_3$ ), 25.0 ( $\text{CCOBn}$ ), 28.6 ( $\text{CCCOBn}$ ), 29.7 ( $\text{CH}_3$ ), 45.7 (C-5), 54.9 ( $\text{OCH}_3$ ), 64.6 (C-6), 69.8 ( $\text{CH}_2\text{OBn}$ ), 70.8 (C-4), 72.9 ( $\text{CH}_2\text{Ph}$ ), 84.0 ( $^2J_{\text{FC}} = 26.3$  Hz,  $\text{CCF}_3$ ), 99.1 (C-2), 123.9 ( $^1J_{\text{FC}} = 290.6$  Hz,  $\text{CF}_3$ ), 123.9, 127.5, 127.6, 127.7, 128.3, 128.6, 129.4, 132.2, 138.5, 166.0 (NCO); **S1S** (47 mg, 79  $\mu\text{mol}$ , 88%):  $[\alpha]^{23}_{\text{D}} = -5.8$  (*c* 1.0 in  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22-1.37 (2 H, m,  $\text{CH}_2\text{CCOBn}$ ), 1.39 (3 H, s,  $\text{CH}_3$ ), 1.42 (3 H, s,  $\text{CH}_3$ ), 1.48-1.65 (2 H, m,  $\text{CH}_2\text{COBn}$ ), 3.28-3.40 (2 H, m,  $\text{CH}_2\text{OBn}$ ), 3.50 (3 H, s,  $\text{OCH}_3$ ), 3.77 (1 H, dd,  $J = 11.9, 1.8$  Hz, 6- $\text{H}^{\text{a}}$ ), 3.90 (1 H, ddd,  $J = 9.7, 1.8, 1.8$  Hz,  $\text{CHNH}$ ), 3.98 (1 H, td,  $J = 6.9, 1.8$  Hz, 4- $\text{H}$ ), 4.07 (1 H, dd,  $J = 11.9, 1.8$  Hz, 6- $\text{H}^{\text{b}}$ ), 4.45 (2 H, q,  $J = 12.1$  Hz,  $\text{CH}_2\text{Ph}$ ), 7.26-7.40 (8 H, m, Ph), 7.52-7.62 (2 H, m, Ph);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  18.5 ( $\text{CH}_3$ ), 24.9 ( $\text{CCOBn}$ ), 28.6 ( $\text{CCCOBn}$ ), 29.7 ( $\text{CH}_3$ ), 45.5 (C-5), 55.1 ( $\text{OCH}_3$ ), 64.8 (C-6), 69.7 ( $\text{CH}_2\text{OBn}$ ), 70.8 (C-4), 72.9 ( $\text{CH}_2\text{Ph}$ ), 84.0 ( $^2J_{\text{FC}} = 25.4$  Hz,  $\text{CCF}_3$ ), 99.2 (C-2), ( $^1J_{\text{FC}} = 289.7$  Hz,  $\text{CF}_3$ ) 127.4, 127.6, 127.7, 128.3, 128.4, 129.4, 132.9, 128.4, 166.1 (NCO); The absolute configuration of amine **S1** was confirmed to be *R*.



Scheme S2. Synthesis of alcohol **S2**; synthesis and  $\Delta\delta_{SR} = \delta_S - \delta_R$  values in Hz of the <sup>1</sup>H NMR spectra of Mosher esters **S3R** and **S3S**.

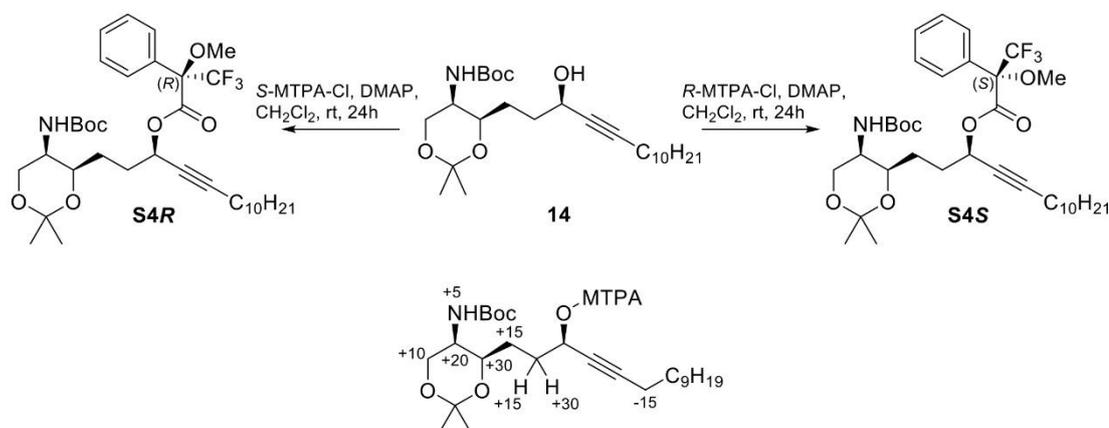
**tert-Butyl ((2R,3R)-6-(benzyloxy)-1-((tert-butyldimethylsilyl)oxy)-3-hydroxyhexan-2-yl)carbamate (S2)**

A solution of carbamate **12** (100 mg, 264  $\mu$ mol, 1.0 equiv.) in MeOH (2 mL), was treated with PPTS (28 mg, 111  $\mu$ mol, 0.5 equiv.) and stirred at ambient temperature for 18 h. Volatiles were removed *in vacuo*. The residue was taken up in EtOAc (10 mL) and filtered through a plug of silica. The filtrate was concentrated to dryness. The crude diol was dissolved in DMF (2 mL) and imidazole (30 mg, 441  $\mu$ mol, 2.0 equiv.) and TBSCl (33 mg, 218  $\mu$ mol, 1.0 equiv.) were added. The mixture was stirred at ambient temperature for 18 h. Saturated aqueous NH<sub>4</sub>Cl (10 mL) was added and the aqueous phase was extracted with Et<sub>2</sub>O (3 x 10 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 6:1) to give alcohol **S2** as a colorless oil (70 mg, 135  $\mu$ mol, 51%).  $R_f = 0.35$  (*n*-hexane/ethyl acetate 6:1);  $[\alpha]^{23}_D = -16.7$  (*c* 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.02-0.09 (6 H, m, Si(CH<sub>3</sub>)<sub>2</sub>), 0.84-0.92 (9 H, m, SiC(CH<sub>3</sub>)<sub>3</sub>), 1.44 (9 H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.51-1.82 (4 H, m, 4-H, 5-H), 3.47-3.55 (3 H, m, CH<sub>2</sub>OBn, CHNH), 3.77 (1 H, dd,  $J = 10.3, 2.9$  Hz, 1-H<sup>a</sup>), 3.87 (1 H, dd,  $J = 10.3, 4.0$  Hz, 1-H<sup>b</sup>), 3.93-3.99 (1 H, m, 3-H), 4.46 (2 H, m, CH<sub>2</sub>Ph), 5.16 (1 H, d,  $J = 8.9$  Hz, NH), 7.26-7.30 (1 H, m, Ph), 7.31-7.36 (4 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  -5.5 (Si(CH<sub>3</sub>)<sub>2</sub>), 18.1 (SiC(CH<sub>3</sub>)<sub>3</sub>); 25.8 (SiC(CH<sub>3</sub>)<sub>3</sub>), 26.0 (C-5), 28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 31.0 (C-4), 53.8 (C-2), 66.2 (C-6), 70.2 (C-1), 72.8 (C-3), 72.9 (CH<sub>2</sub>Ph), 79.2 (C(CH<sub>3</sub>)<sub>3</sub>), 127.5, 127.6, 128.3, 138.4, 156.0 (NCO); IR ( $\nu_{max}/cm^{-1}$ ) 3449, 2928, 2857, 1715, 1692, 1496, 1365, 1252, 1168, 1096, 835, 776, 734, 696; HRMS (ESI)  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>24</sub>H<sub>44</sub>O<sub>5</sub>NSi<sup>+</sup> 454.2983, found 454.2970, [M+Na]<sup>+</sup> calcd for C<sub>24</sub>H<sub>43</sub>O<sub>5</sub>NNaSi<sup>+</sup> 476.2803, found 476.2790.

**(2R,3R)-6-(benzyloxy)-2-((tert-butoxycarbonyl)amino)-1-((tert-butyldimethylsilyl)oxy)hexan-3-yl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (S3R, S3S)**

A solution of alcohol **S2** (25 mg, 55  $\mu$ mol, 1.0 equiv.) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was treated with DMAP (14 mg, 110  $\mu$ mol, 2.0 equiv.), and (*S*)- or (*R*)-MTPA-Cl (12  $\mu$ L, 66  $\mu$ mol, 1.2 equiv.). The mixture was stirred at ambient temperature for 18 h and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 8:1) to give the esters **S3R** and **S3S** as colorless oils.  $R_f = 0.43$  (*n*-hexane/ethyl acetate 8:1); IR ( $\nu_{max}/cm^{-1}$ ) 2953, 2930, 2857, 1746, 1717, 1497,

1253, 1166, 1103, 1016, 835, 778, 697; HRMS (ESI)  $m/z$   $[M + H]^+$  calcd for  $C_{34}H_{51}O_7NF_3Si^+$  670.3381, found 670.3360,  $[M + Na]^+$  calcd for  $C_{34}H_{50}O_7NF_3NaSi^+$  692.3201, found 692.3178; **S3R** (28 mg, 42  $\mu$ mol, 77%):  $[\alpha]_D^{23} = +7.8$  (c 1.0 in  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  0.05 (6 H, s,  $Si(CH_3)_2$ ), 0.90 (9 H, s,  $SiC(CH_3)_3$ ), 1.39-1.46 (9 H, m,  $C(CH_3)_3$ ), 1.49-1.61 (2 H, m, 4-H), 1.76 (2 H, q,  $J = 7.4$  Hz, 5-H), 3.40 (2 H, t,  $J = 6.1$  Hz,  $CH_2OBn$ ), 3.47 (1 H, dd,  $J = 10.2$ , 6.6 Hz, 1-H<sup>a</sup>), 3.53 (3 H, s,  $OCH_3$ ), 3.62 (1 H, dd,  $J = 10.2$ , 4.4 Hz, 1-H<sup>b</sup>), 3.84-3.93 (1 H, m,  $CHNH$ ), 4.45 (2 H, s,  $CH_2Ph$ ), 4.66 (1 H, d,  $J = 9.8$  Hz, NH), 5.39 (1 H, q,  $J = 6.1$  Hz, 3-H), 7.26-7.36 (5 H, m, Ph), 7.36-7.43 (3 H, m, Ph), 7.49-7.58 (2 H, m, Ph);  $^{13}C$  NMR (126 MHz,  $CDCl_3$ )  $\delta$  -5.5 ( $Si(CH_3)_2$ ), 18.1 ( $SiC(CH_3)_3$ ), 25.1 (C-5), 25.7 ( $SiC(CH_3)_3$ ), 27.5 (C-4), 28.3 ( $C(CH_3)_3$ ), 53.7 (C-2), 55.3 ( $OCH_3$ ), 62.0 (C-1), 69.4 ( $CH_2OBn$ ), 72.7 ( $CH_2Ph$ ), 74.9 (C-3), 79.6 ( $C(CH_3)_3$ ), 84.6 ( $^2J_{FC} = 27.7$  Hz,  $CCF_3$ ), 123.3 ( $^1J_{FC} = 288.8$  Hz;  $CF_3$ ), 127.4, 128.4, 129.6, 132.0, 138.4, 155.4 (NCO), 166.1 (COO); **S3S** (31 mg, 46  $\mu$ mol, 85%):  $[\alpha]_D^{23} = -16.6$  (c 1.0 in  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  0.02 (6 H, s,  $Si(CH_3)_2$ ), 0.88 (9 H, s,  $SiC(CH_3)_3$ ), 1.38-1.47 (9 H, m,  $C(CH_3)_3$ ), 1.57-1.76 (2 H, m, 4-H), 1.78-1.68 (2 H, m, 5-H), 3.31 (1 H, dd,  $J = 9.9$ , 7.5 Hz, 1-H<sup>a</sup>), 3.45-3.51 (3 H, m, 1-H<sup>b</sup>,  $CH_2OBn$ ), 3.53 (3 H, s,  $OCH_3$ ), 3.85-3.93 (1 H, m,  $CHNH$ ), 4.48 (2 H, s,  $CH_2Ph$ ), 4.52 (1 H, d,  $J = 9.8$  Hz, NH), 5.42 (1 H, td,  $J = 6.4$ , 3.7 Hz, 3-H), 7.27-7.36 (5 H, m, Ph), 7.37-7.44 (3 H, m, Ph), 7.53-7.55 (2 H, m, Ph);  $^{13}C$  NMR (126 MHz,  $CDCl_3$ )  $\delta$  -5.5 ( $Si(CH_3)_2$ ), 18.1 ( $SiC(CH_3)_3$ ), 25.3 (C-5), 25.7 ( $SiC(CH_3)_3$ ), 27.7 (C-4), 28.3 ( $C(CH_3)_3$ ), 55.3 (C-2), 55.4 ( $OCH_3$ ), 61.7 (C-1), 69.4 ( $CH_2OBn$ ), 72.7 ( $CH_2Ph$ ), 74.8 (C-3), 79.6 ( $C(CH_3)_3$ ), 123.4 ( $^1J_{FC} = 288.7$  Hz;  $CF_3$ ), 127.5, 127.6, 128.3, 128.4, 129.7, 132.1, 138.4, 155.4 (NCO), 166.1 (COO); The absolute configuration of alcohol **S2** was confirmed to be *R*.



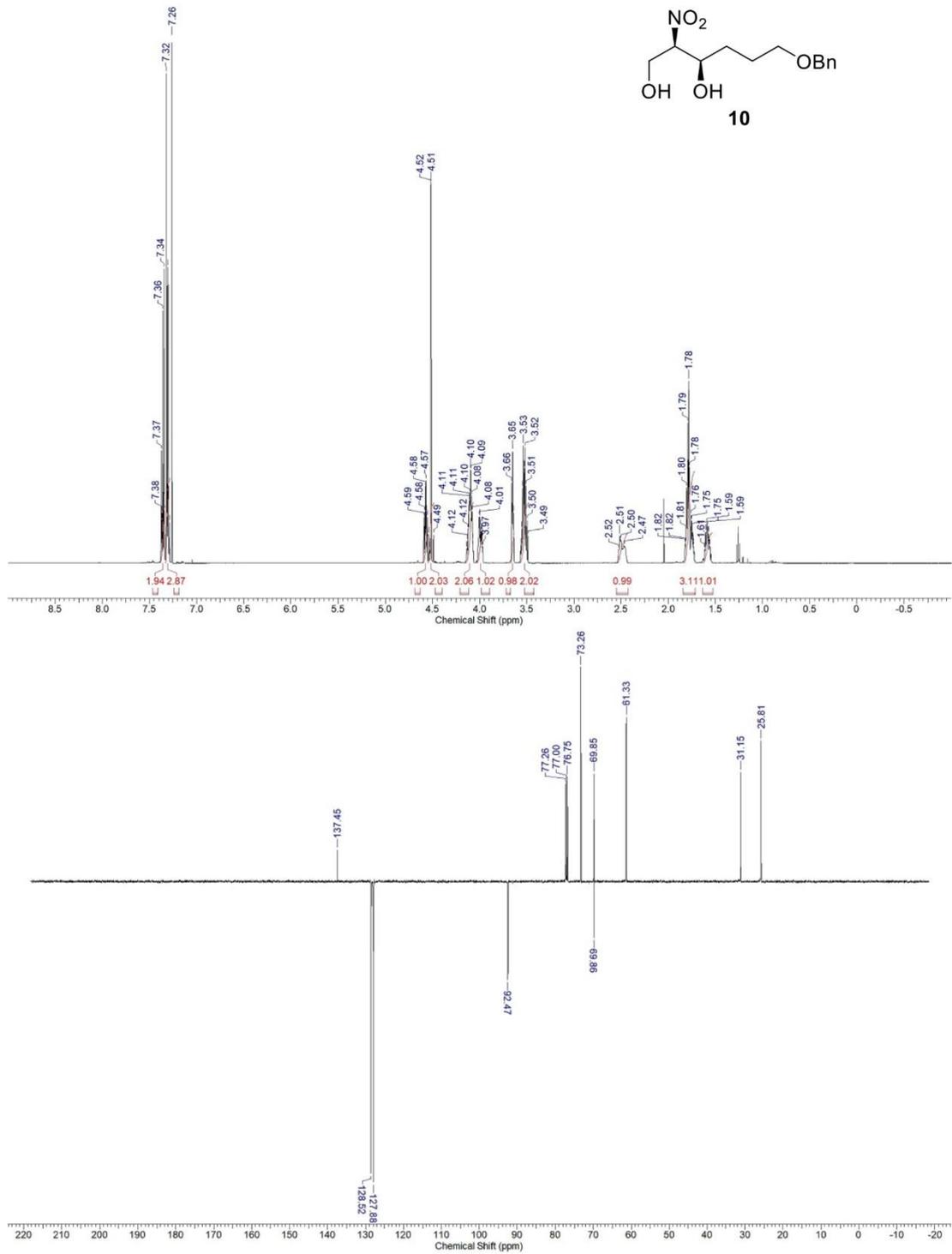
Scheme S3. Synthesis and  $\Delta\delta_{SR} = \delta_S - \delta_R$  values in Hz of the  $^1H$  NMR spectra of Mosher-esters **S4R** and **S4S**

**(R)-1-((4''*R*,5''*R*)-5''-((tert-butoxycarbonyl)amino)-2'',2''-dimethyl-1'',3''-dioxan-4''-yl)pentadec-4'-yn-3'-yl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (**S4R**, **S4S**)**

A solution of alcohol **14** (25 mg, 55  $\mu$ mol, 1.0 equiv.) in dry  $CH_2Cl_2$  (1 mL) was treated with DMAP (14 mg, 110  $\mu$ mol, 2.0 equiv.), and (*S*)- or (*R*)-MTPA-CL (12  $\mu$ L, 66  $\mu$ mol, 1.2 equiv.). The mixture was stirred at ambient temperature for 18 h and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to give **S4R** and **S4S** as colorless oils.  $R_f = 0.35$  (*n*-hexane/ethyl acetate 9:1); IR ( $\nu_{max}/cm^{-1}$ ) 2962, 2856, 1750, 1713, 1496, 1244, 1164, 1119, 1081, 986, 718; HRMS (ESI)  $m/z$   $[M + H]^+$  calcd for  $C_{36}H_{55}O_7NF_3^+$  670.3925, found 670.3904,  $[M + Na]^+$  calcd for  $C_{36}H_{54}O_7NF_3Na^+$  692.3745, found 692.3721; **S4R** (32 mg, 50  $\mu$ mol, 90%):  $[\alpha]_D^{23} = +17.1$  (c 1.0 in  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  0.78-0.83 (3 H, m,  $CH_3$ ), 1.15-1.24 (12 H, m,  $(CH_2)_6$ ), 1.28-1.31 (3 H, m,  $CH_3$ ), 1.32-1.35 (3 H, m,  $CH_3$ ), 1.35-1.40 (9 H, m,  $C(CH_3)_3$ ), 1.40-1.55 (4 H, m, 4-H,  $CH_2$ , 1'-H), 1.67-

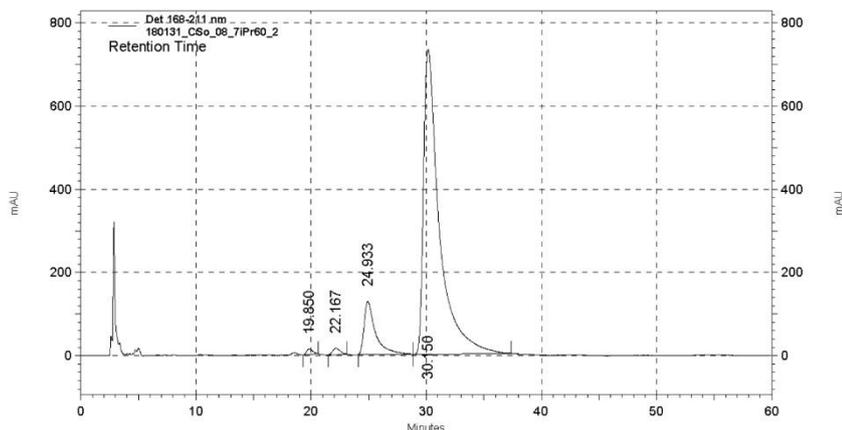
1.84 (2 H, m, 2'-H), 2.13 (2 H, td,  $J = 7.2, 2.1$  Hz, 6'-H), 3.37 (1 H, dd,  $J = 9.8, 1.8$  Hz, CHNH), 3.51 (3 H, s, OCH<sub>3</sub>), 3.62-3.69 (1 H, m, 6''-H<sup>a</sup>), 3.79 (1 H, ddd,  $J = 8.5, 5.1, 1.8$  Hz, 4''-H), 3.94 (1 H, dd,  $J = 12.1, 2.0$  Hz, 6''-H<sup>b</sup>), 5.17 (1 H, d,  $J = 9.8$  Hz, NH), 5.45 (1 H, tt,  $J = 6.7, 1.9$  Hz, 3'-H), 7.30-7.38 (3 H, m, Ph), 7.46-7.52 (2 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  14.1 (CH<sub>3</sub>), 18.5 (CH<sub>3</sub>), 18.6 (CH<sub>2</sub>), 22.7 (CH<sub>2</sub>), 27.1 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 28.8 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 29.6 (CH<sub>3</sub>), 30.4 (CH<sub>2</sub>), 31.9 (CH<sub>2</sub>), 46.9 (C-5''), 55.4 (OCH<sub>3</sub>), 65.2 (C-6''), 66.4 (C-3'), 70.6 (C-4''), 76.2 (C-5'), 79.4 (C(CH<sub>3</sub>)<sub>3</sub>), 87.9 (C-4'), 99.1 (C-2''), 123.3 (<sup>1</sup>J<sub>FC</sub> = 288.8 Hz, CF<sub>3</sub>), 126.6, 128.3, 129.6, 129.9, 132.4, 155.7 (NCO), 165.7 (COO); <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>)  $\delta$  -71.6 (major), -71.9 (minor); **S4S** (30 mg, 47  $\mu$ mol, 86%): [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -14.1 (c 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): 0.85-0.90 (3 H, m, CH<sub>3</sub>), 1.20-1.35 (12 H, m, (CH<sub>2</sub>)<sub>6</sub>), 1.37-1.40 (3 H, m, CH<sub>3</sub>), 1.41-1.49 (12 H, m, CH<sub>3</sub>, C(CH<sub>3</sub>)<sub>3</sub>), 1.52-1.66 (2 H, m, 1'-H), 1.76-1.97 (2 H, m, 2'-H), 2.16 (2 H, td,  $J = 7.2, 1.8$  Hz, 6'-H), 3.47 (1 H, dd,  $J = 9.9, 1.9$  Hz, CHNH), 3.55 (3 H, s, OCH<sub>3</sub>), 3.73 (1 H, dd,  $J = 12.0, 1.8$  Hz, 6''-H<sup>a</sup>), 3.92 (1 H, ddd,  $J = 8.2, 5.2, 1.8$  Hz, 4''-H), 4.02 (1 H, dd,  $J = 12.0, 2.0$  Hz, 6''-H<sup>b</sup>), 5.25 (1 H, d,  $J = 10.1$  Hz, NH), 5.45 (1 H, tt,  $J = 6.7, 1.8$  Hz, 3'-H), 7.38-7.42 (3 H, m, Ph), 7.52-7.55 (2 H, m, Ph); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  14.1 (CH<sub>3</sub>), 18.5 (CH<sub>3</sub>), 18.6 (CH<sub>2</sub>), 22.7 (CH<sub>2</sub>), 27.4 (CH<sub>2</sub>), 28.4 (C(CH<sub>3</sub>)<sub>3</sub>), 28.8 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 29.6 (CH<sub>3</sub>), 30.3 (CH<sub>2</sub>), 31.9 (CH<sub>2</sub>), 46.9 (C-5''), 55.5 (OCH<sub>3</sub>), 65.2 (C-6''), 66.8 (C-3'), 70.8 (C-4''), 76.0 (C-5'), 79.5 (C(CH<sub>3</sub>)<sub>3</sub>), 87.7 (C-4'), 99.1 (C-2''), 123.2 (<sup>1</sup>J<sub>FC</sub> = 287.9 Hz, CF<sub>3</sub>), 127.5, 128.3, 129.6, 132.0, 155.7 (NCO), 165.6 (COO); <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>)  $\delta$  -71.6 (minor), -71.9 (major); The absolute configuration of alcohol **14** was confirmed to be *R*.

## NMR spectra and chromatograms



## Custom Report

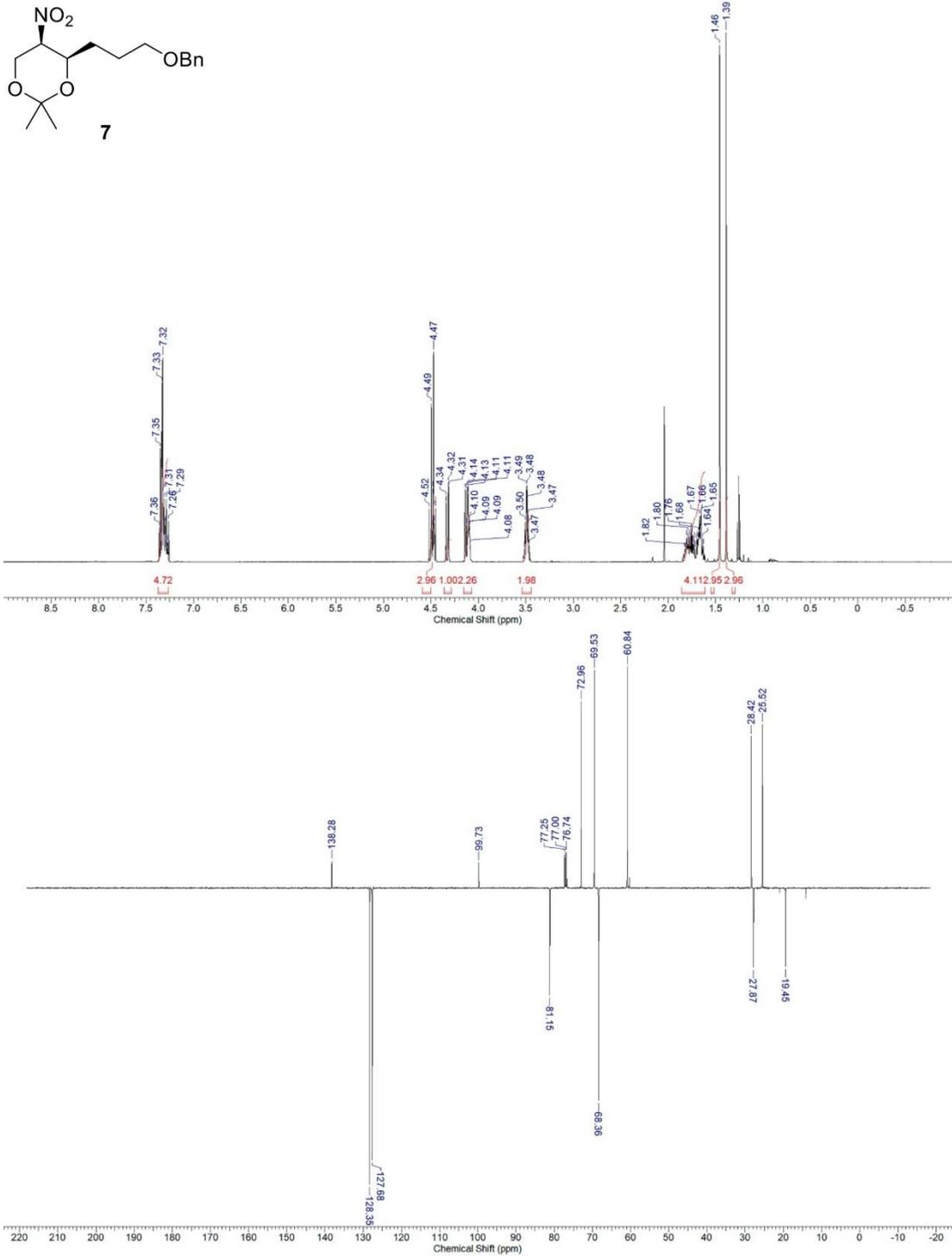
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 Instrument Name: HPLC2 (Offline)  
 Injection Volume: 20  $\mu$ L  
 Concentration: 0.9 mg/mL in 10% *i*-PrOH in *n*-Hexane  
 Analyst: Admin  
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 Analyzed: 4/12/2018 12:58:38 PM  
 Printed: 4/12/2018 12:59:15 PM



Det 168-211 nm  
 Results

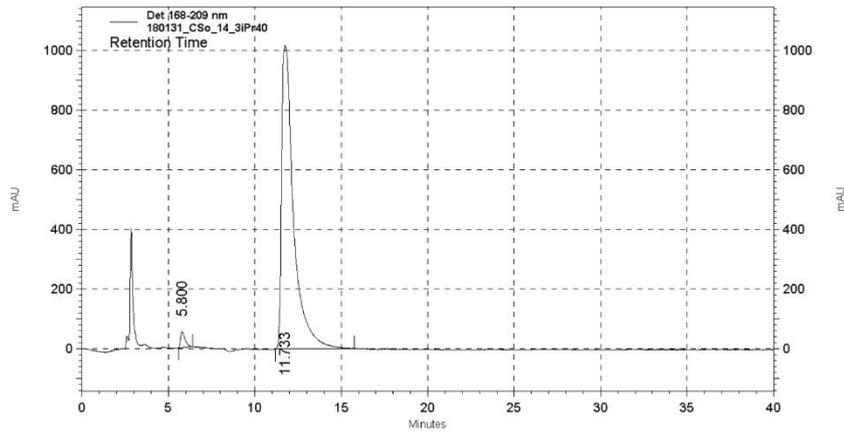
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3	24.933	8758190	127522	10.981
4	30.150	69848641	732468	87.576
<b>Totals</b>		79757741	888541	100.000

HPLC trace of **10**; Phenomenex Lux® Amylose-1; 95:5 Hex/IPA; 1.0 mL/min; *anti* isomer: *tr* (major) = 24.93 min, *tr* (minor) = 19.85 min, 89% *ee*; *syn* isomer: *tr* (major) = 30.15 min, *tr* (minor) = 22.17 min, 98% *ee*; *dr* (*syn/anti*) = 88:12.



## Custom Report

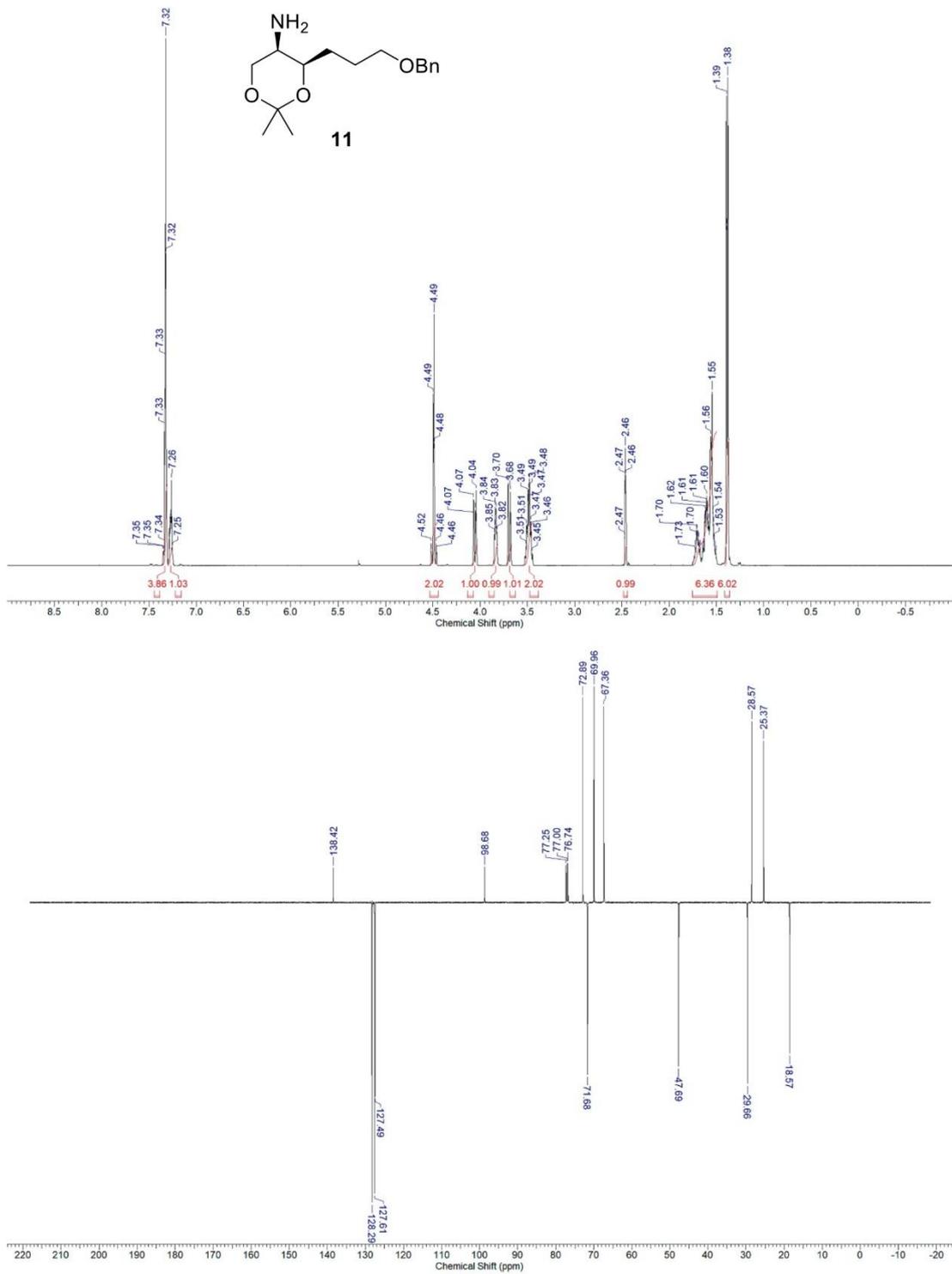
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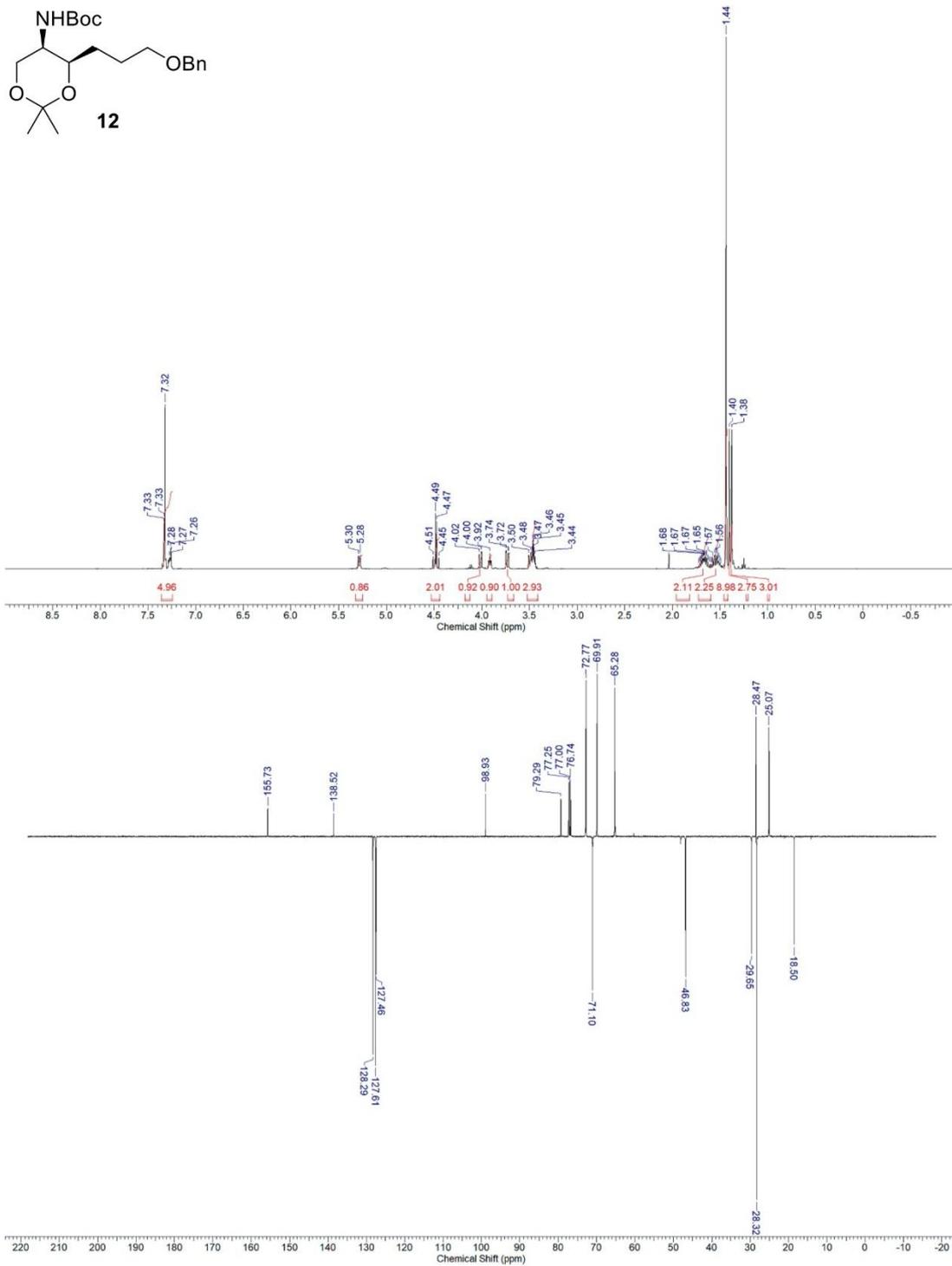


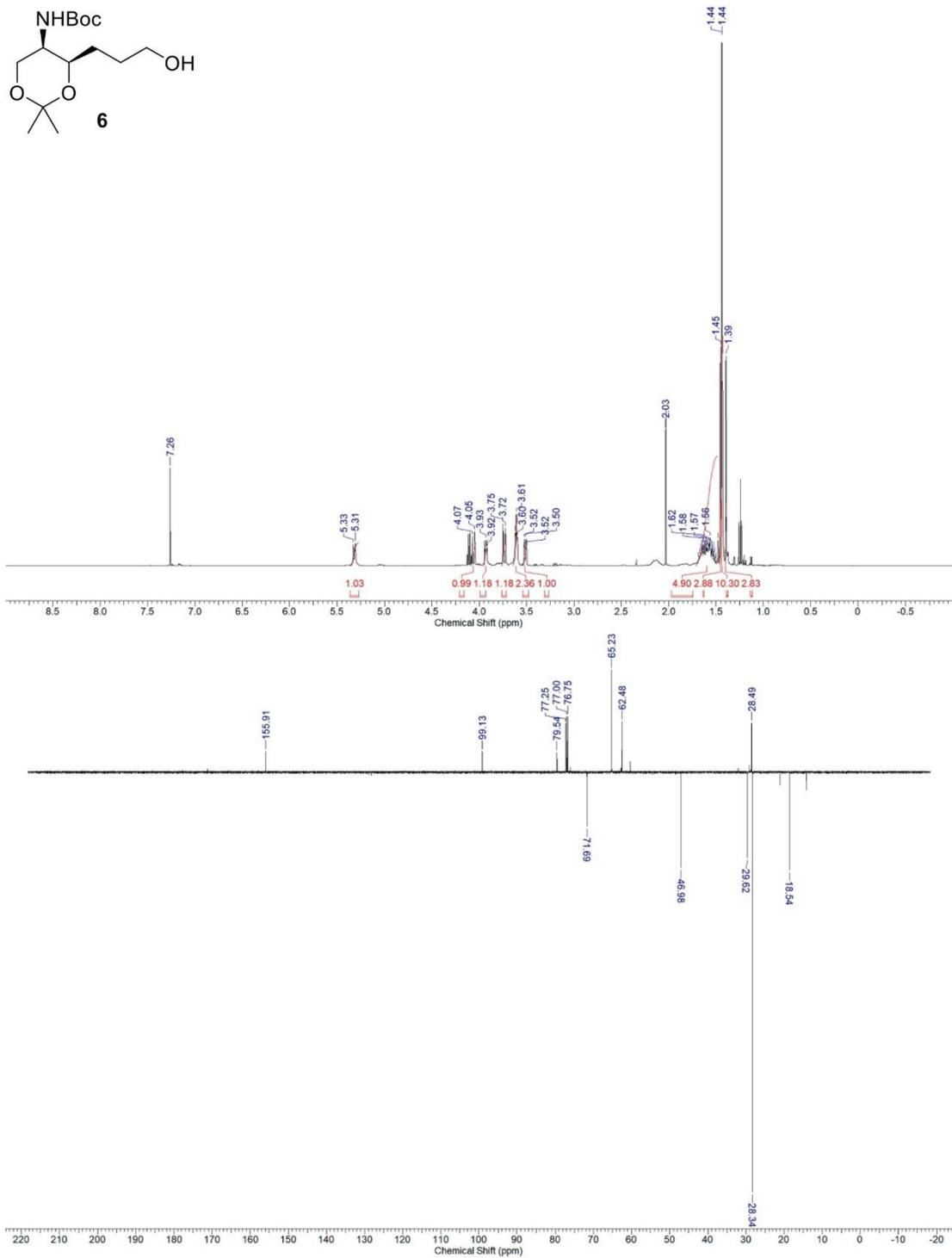
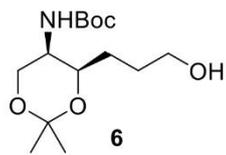
## Det 168-209 nm Results

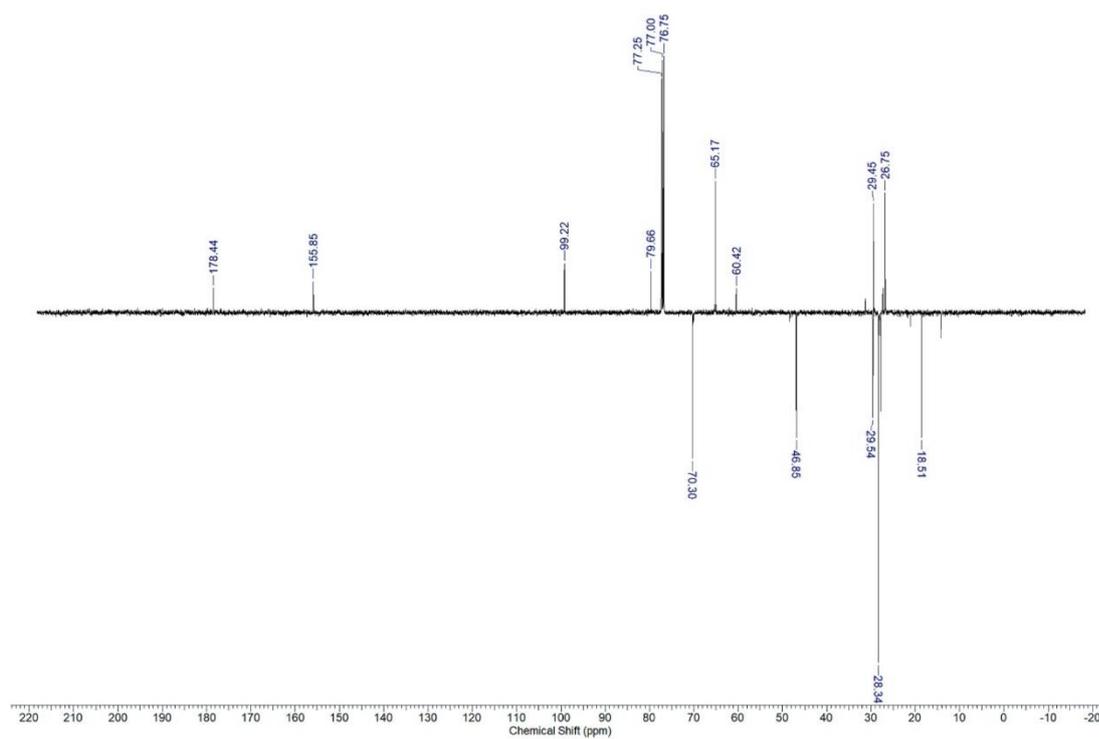
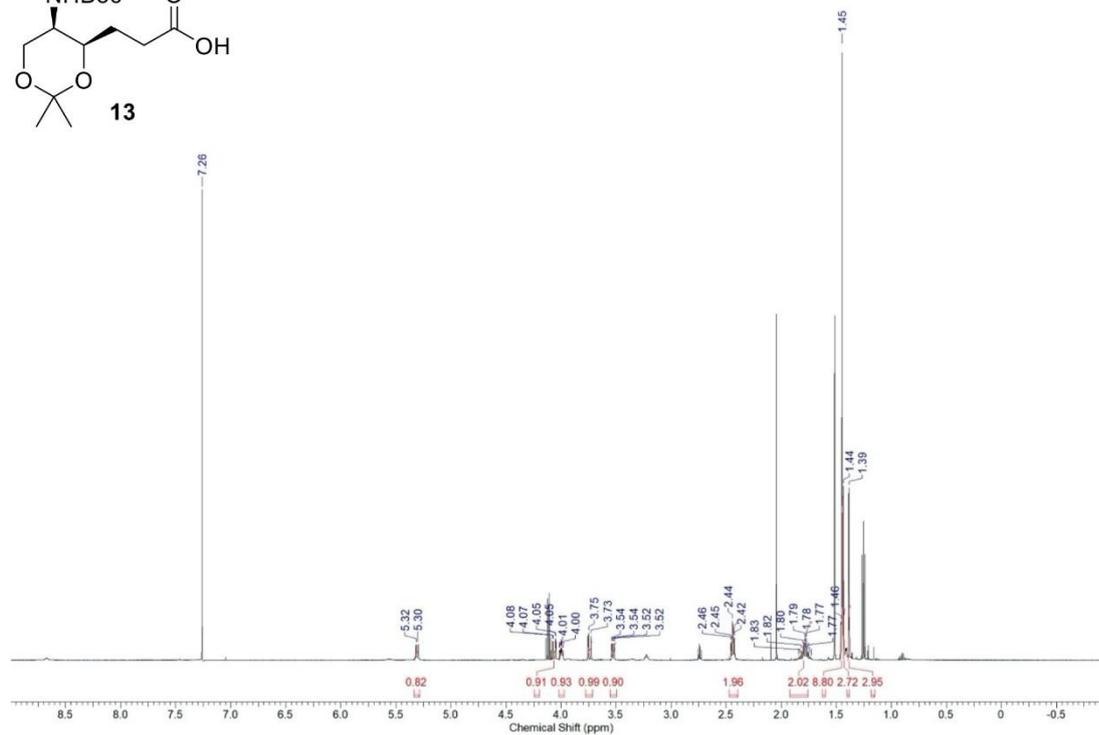
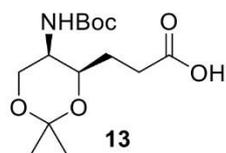
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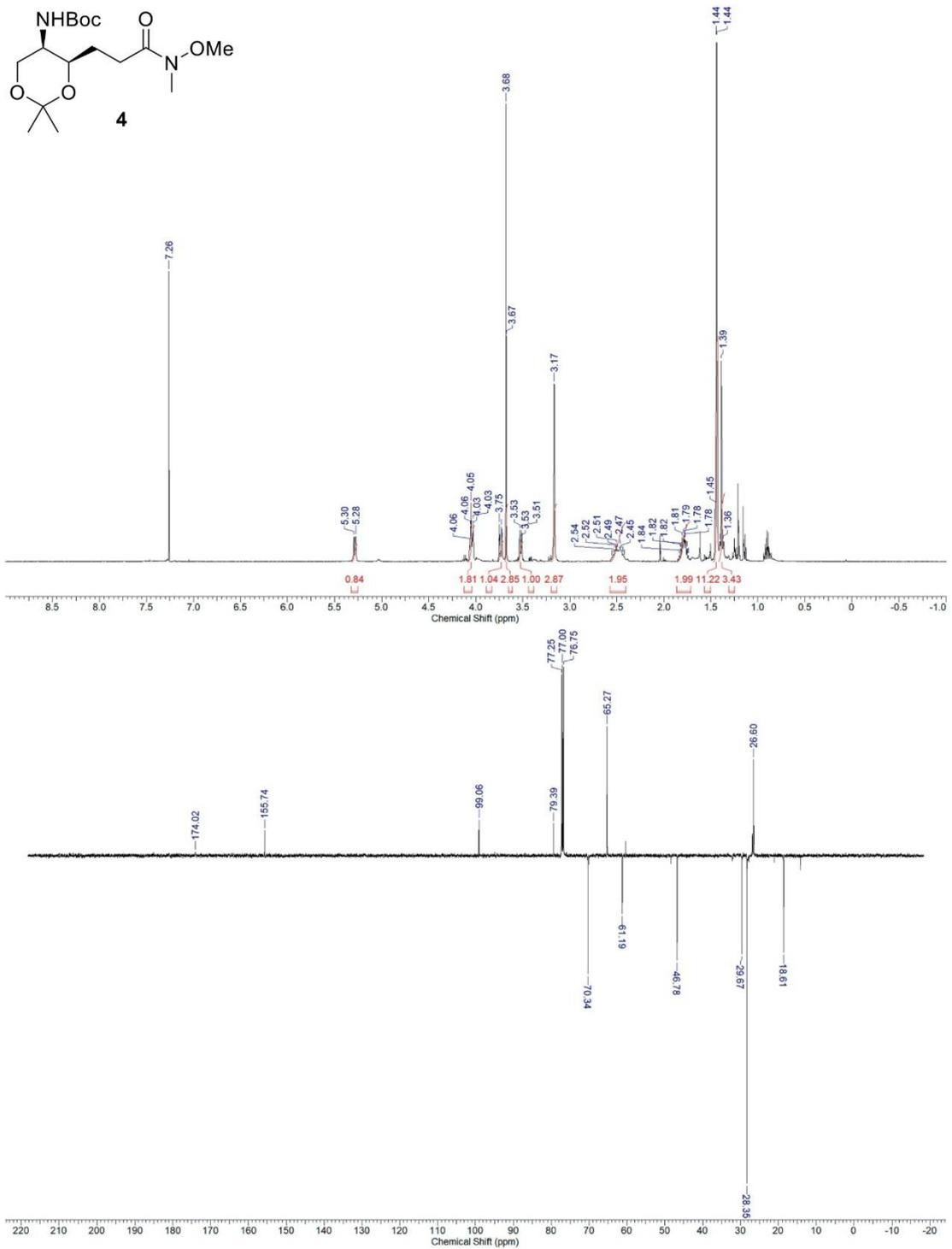
HPLC trace of **7**; Phenomenex Lux<sup>®</sup> Amylose-1; 95:5 Hex/IPA; 1.0 mL/min; *syn* isomer: *tr* (major) = 11.73 min, *tr* (minor) = 5.80 min

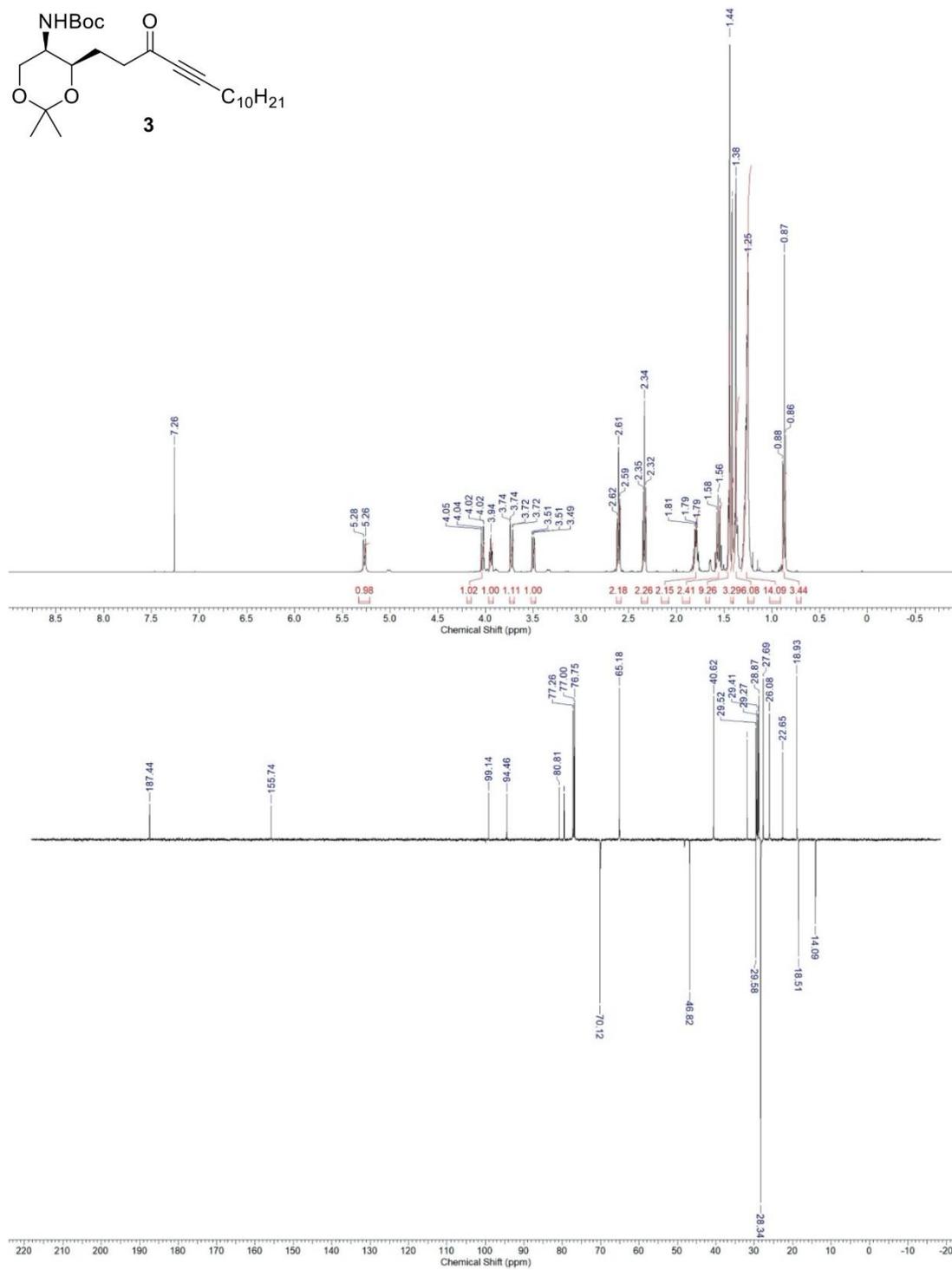
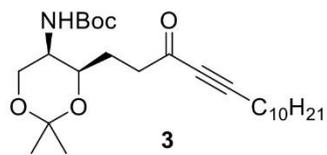


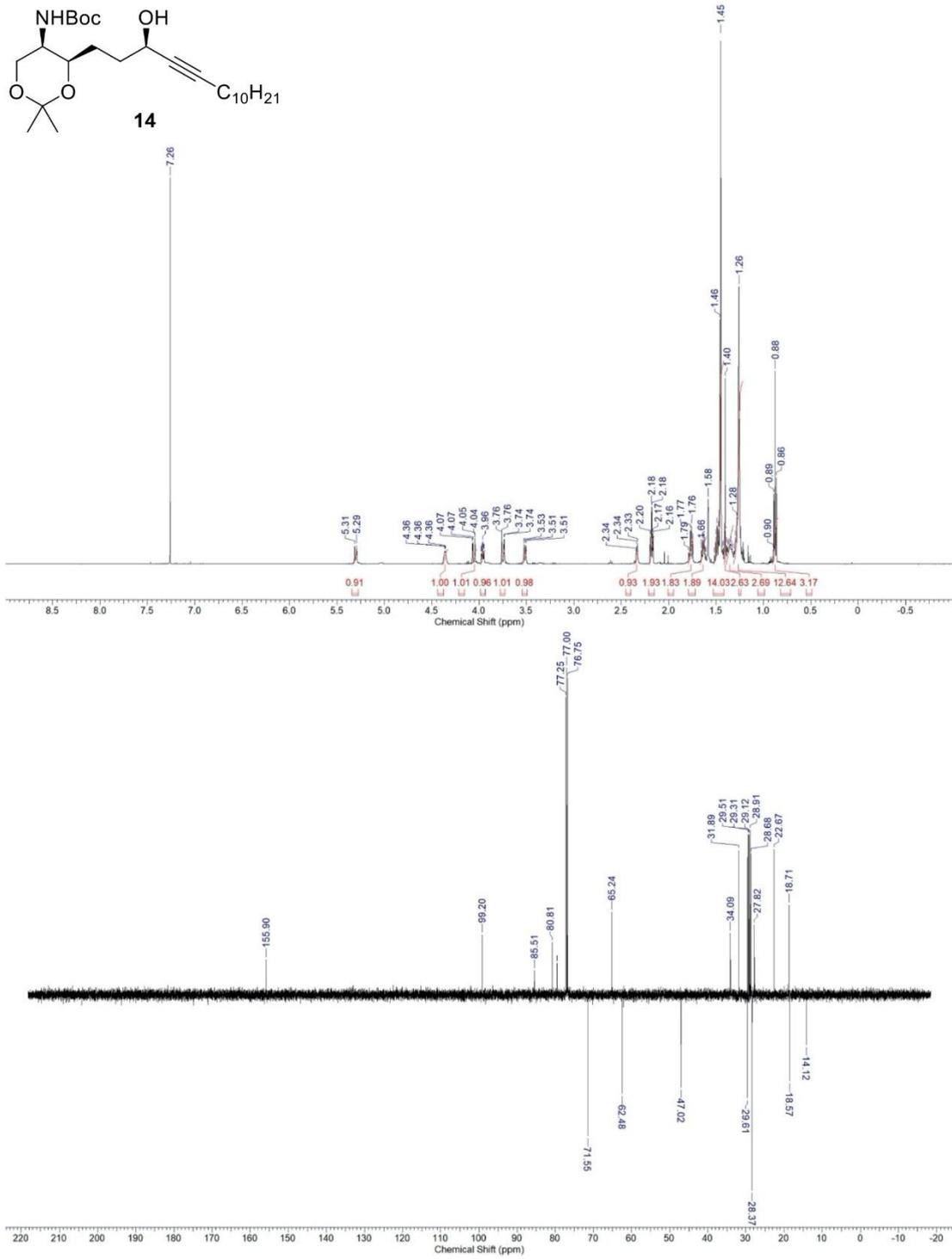


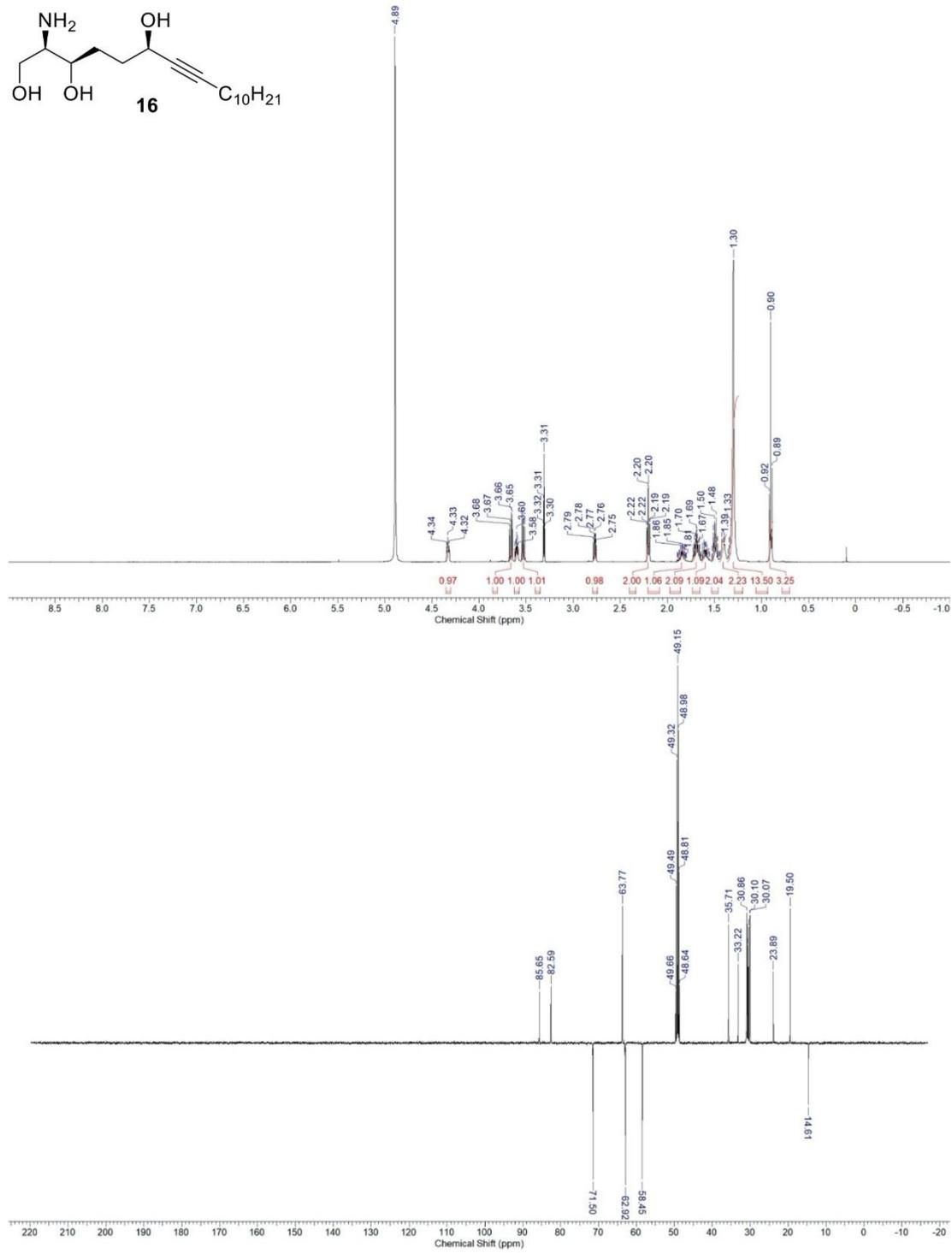
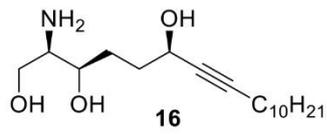


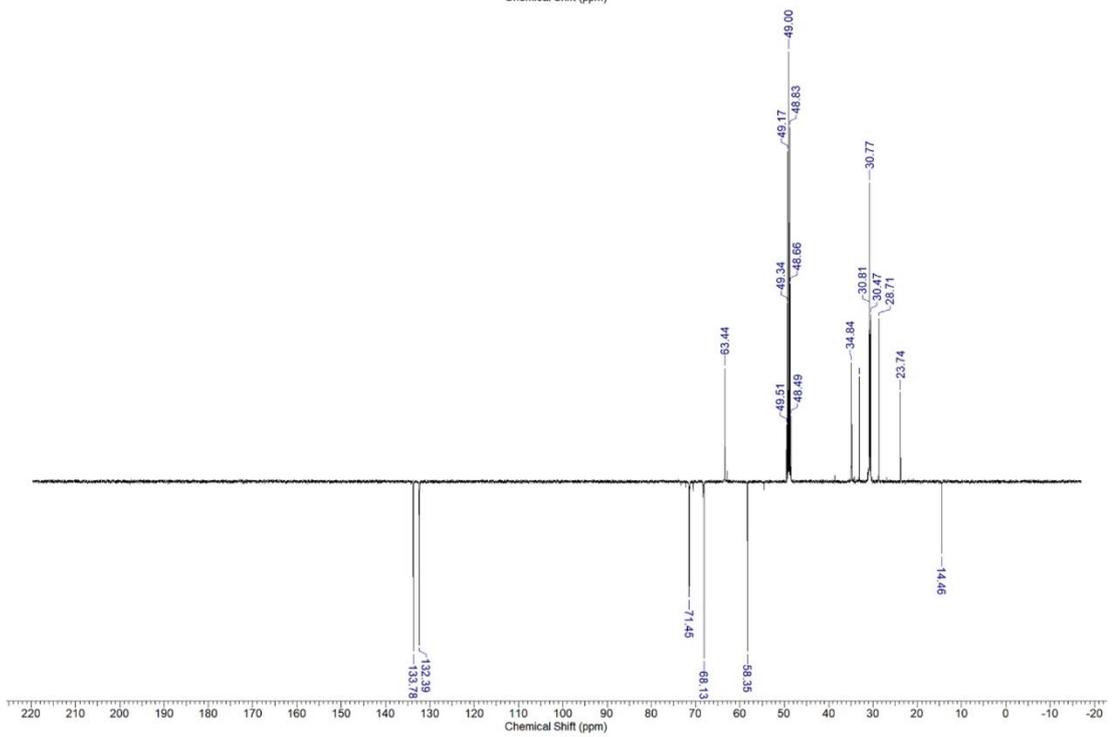
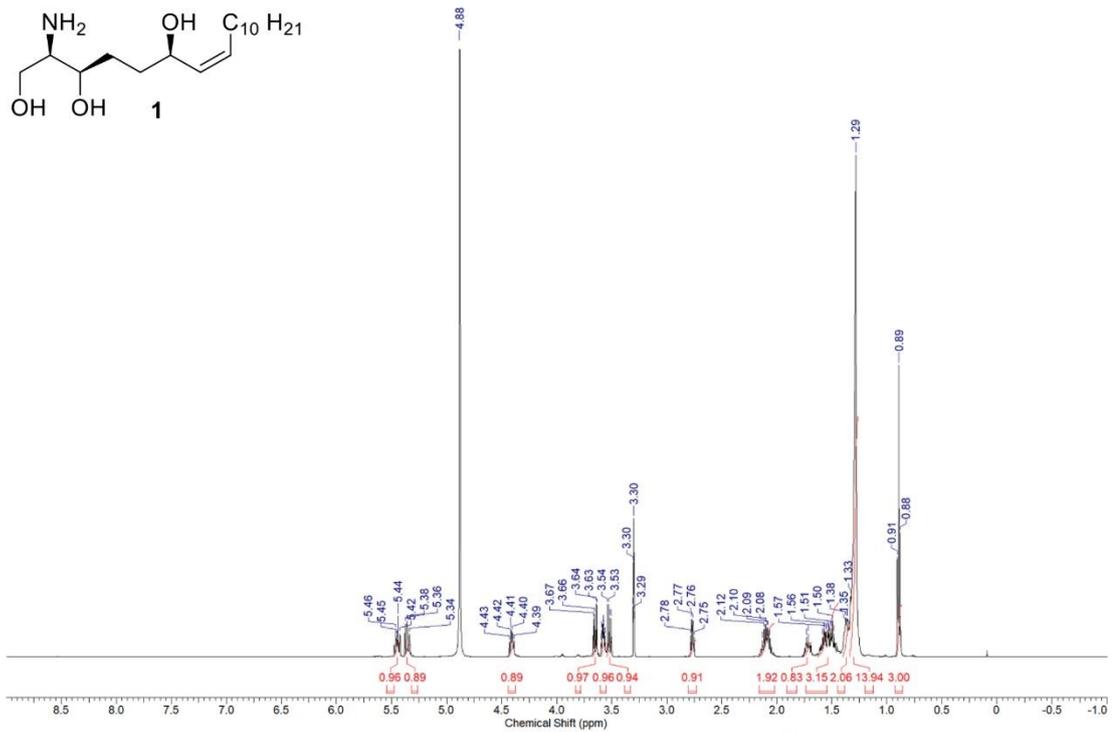
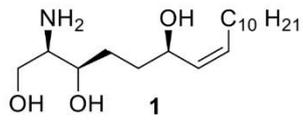


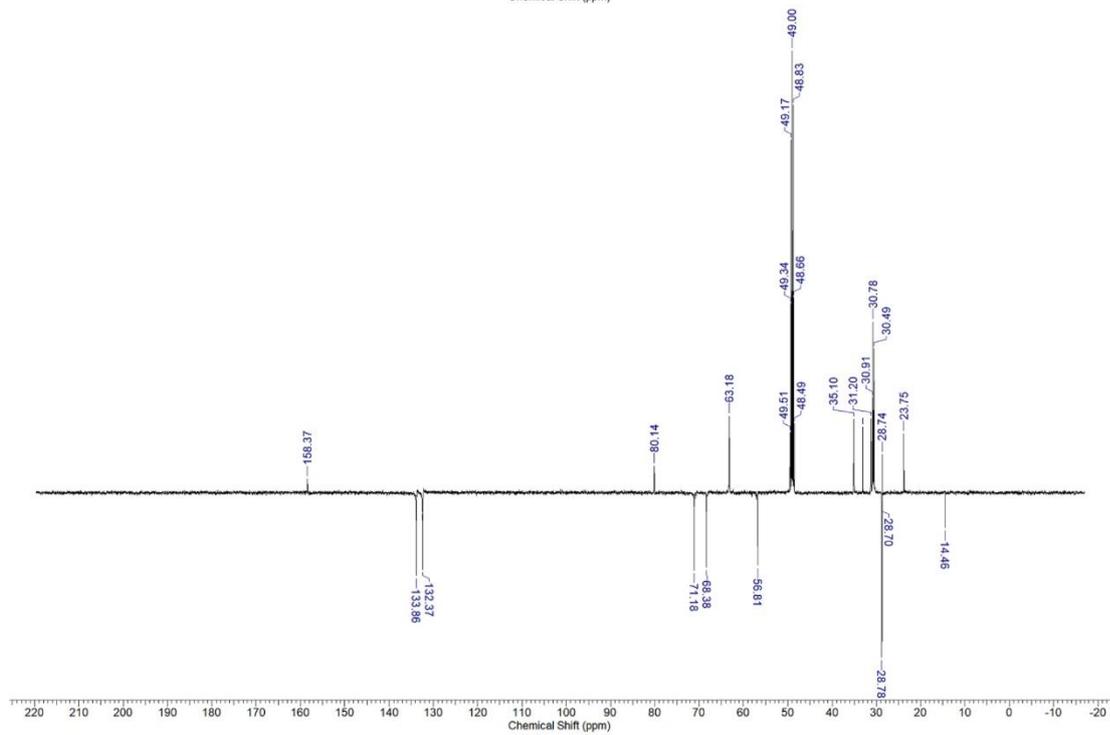
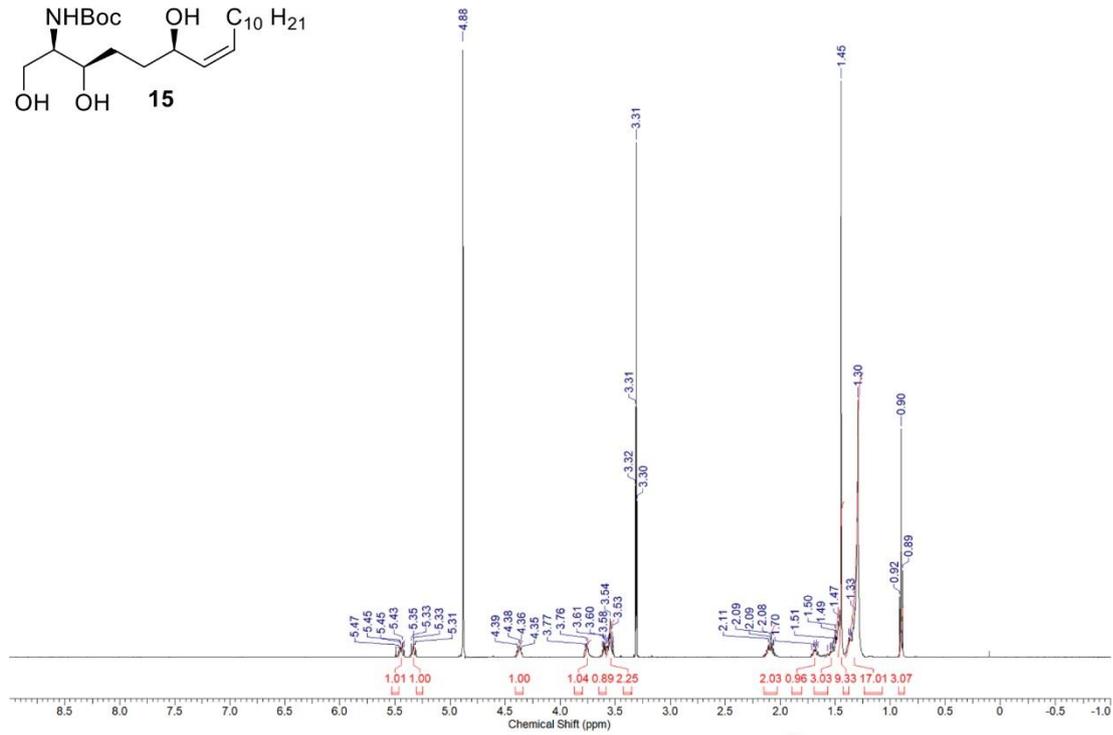
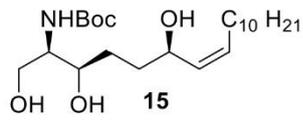


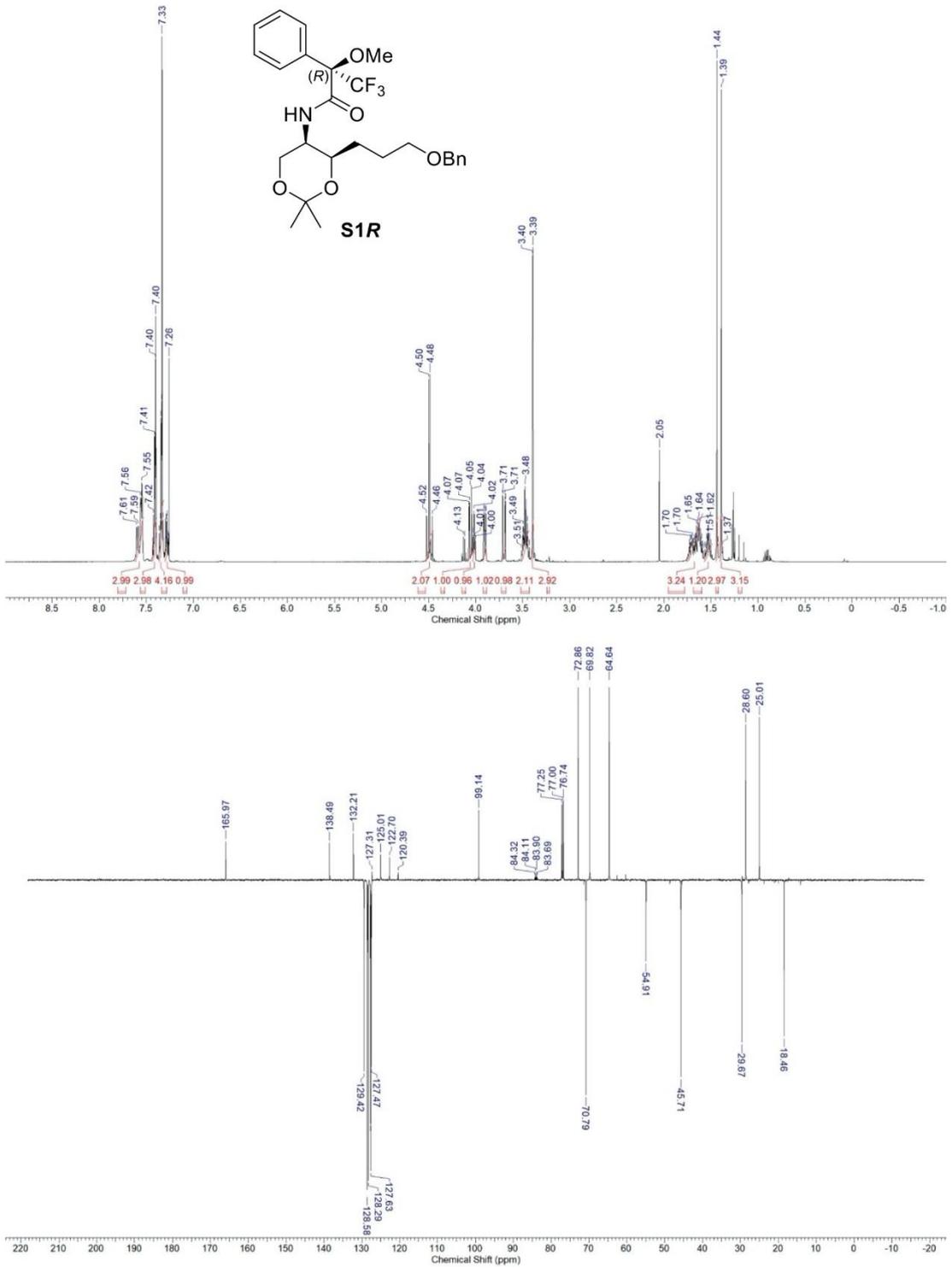


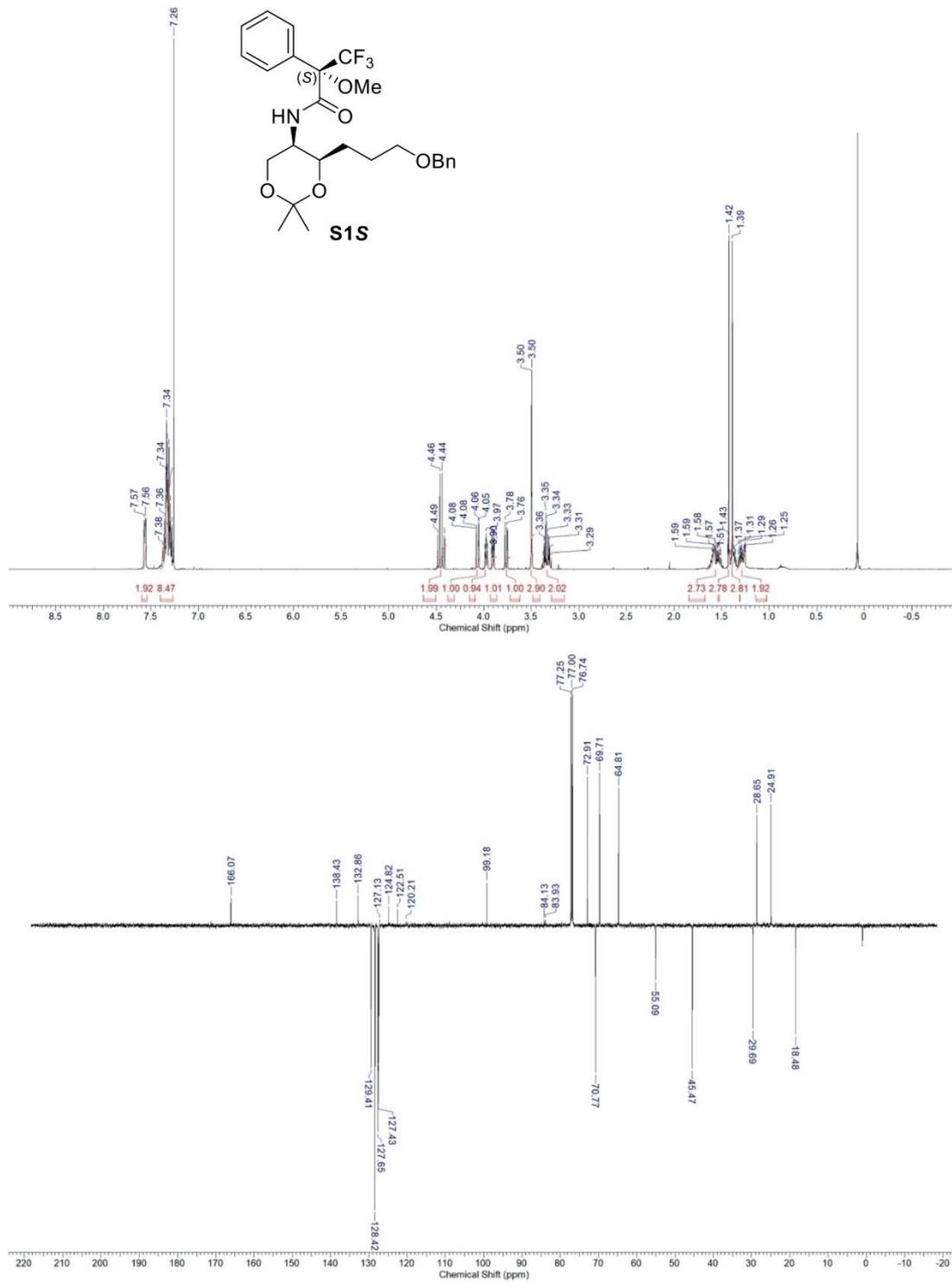


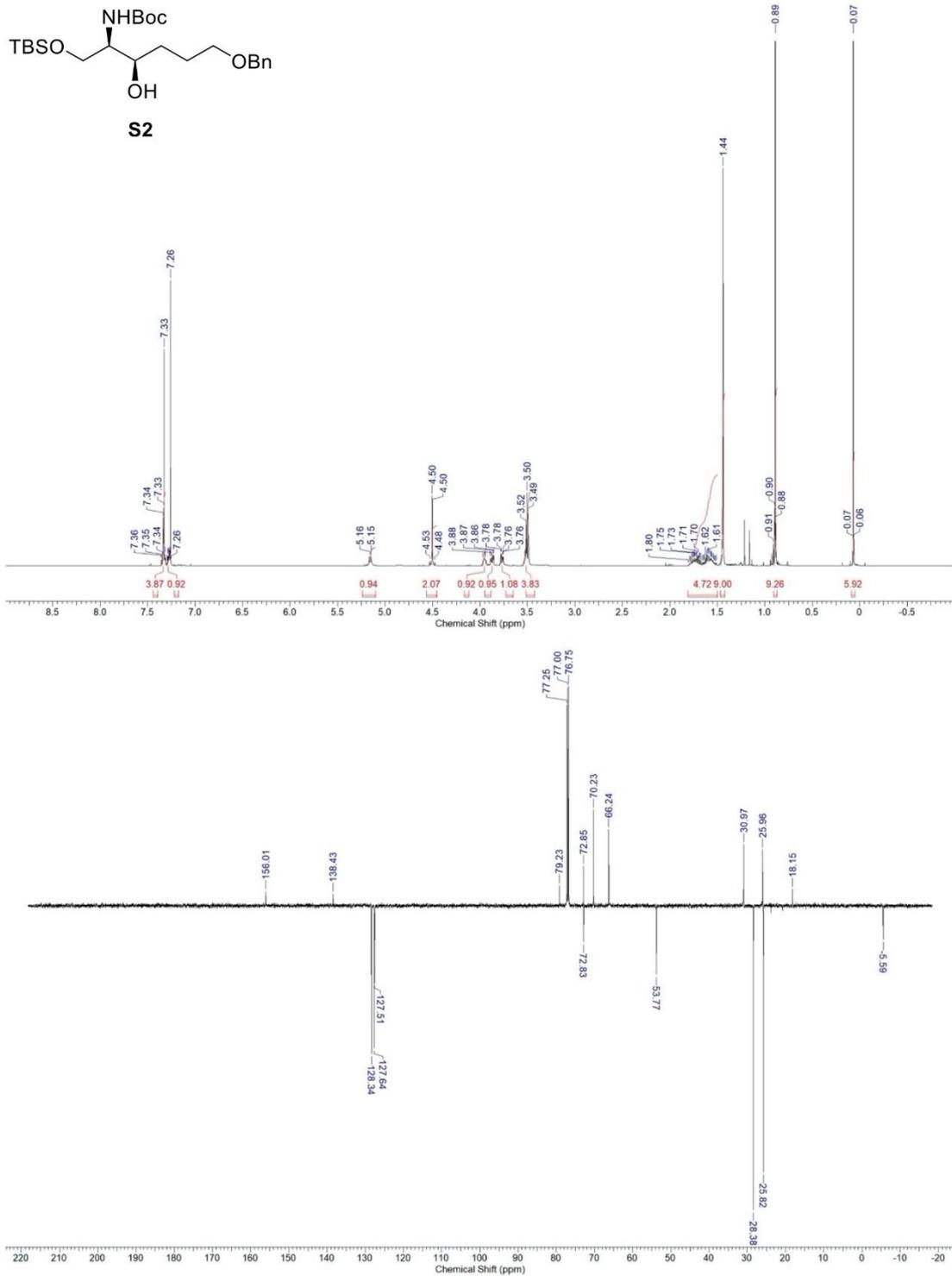


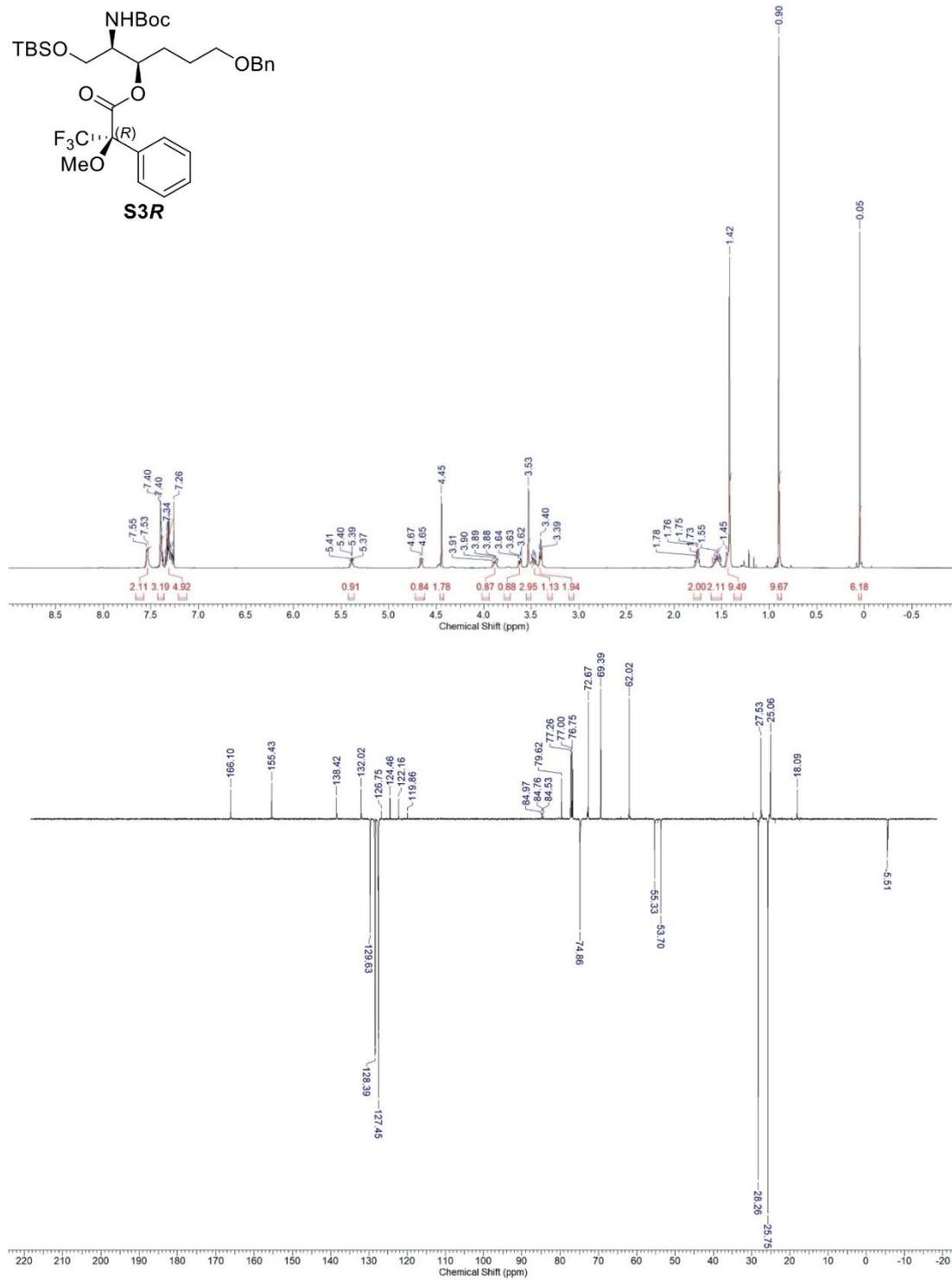


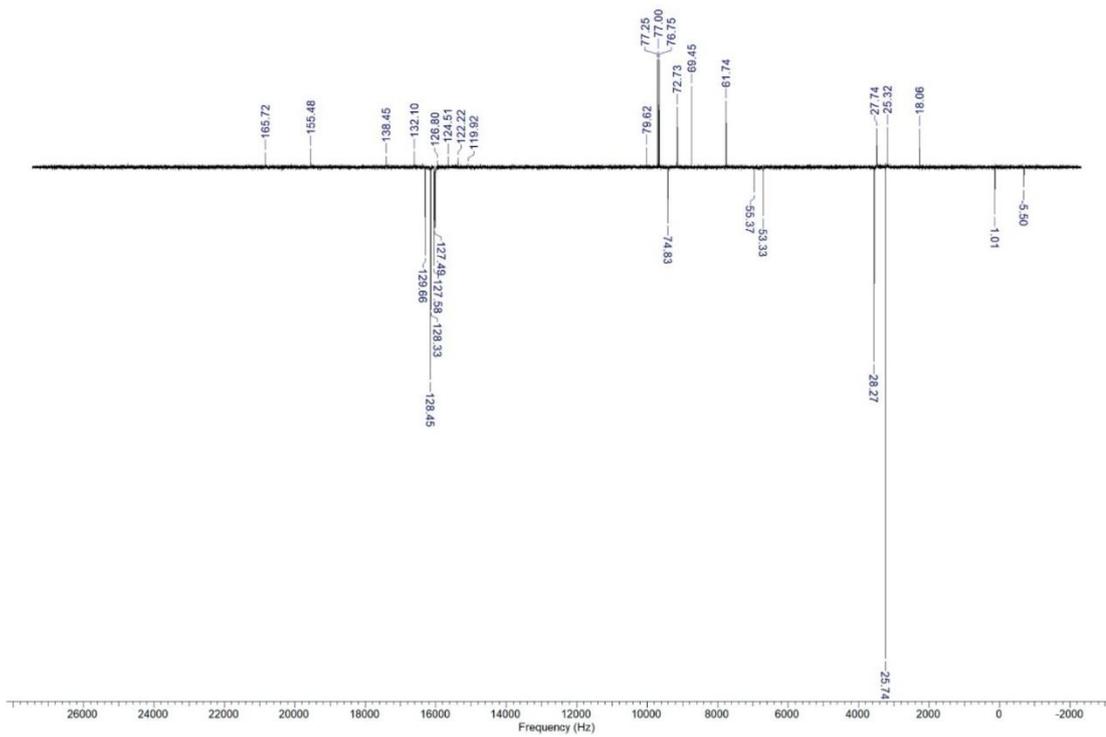
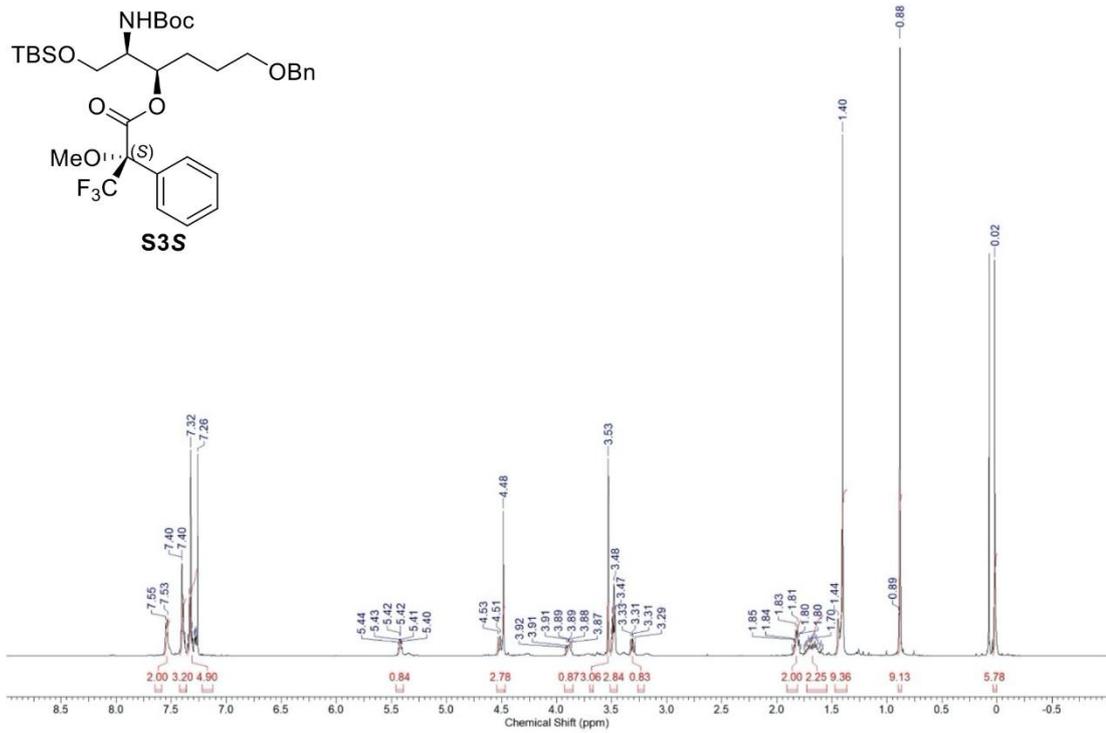
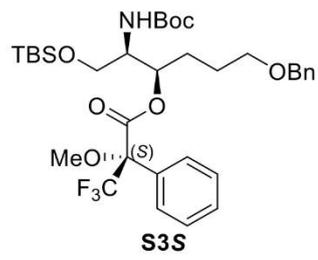


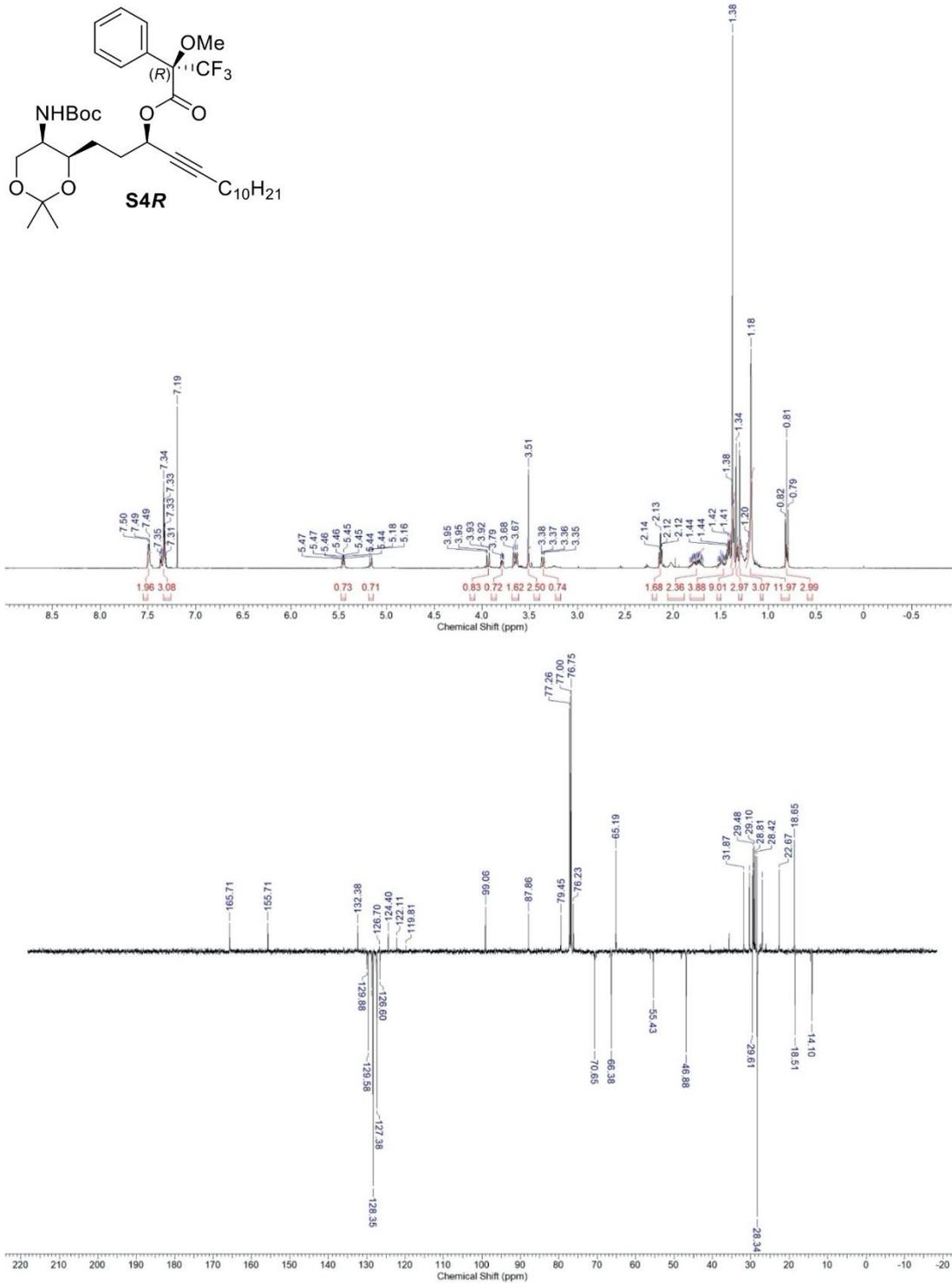


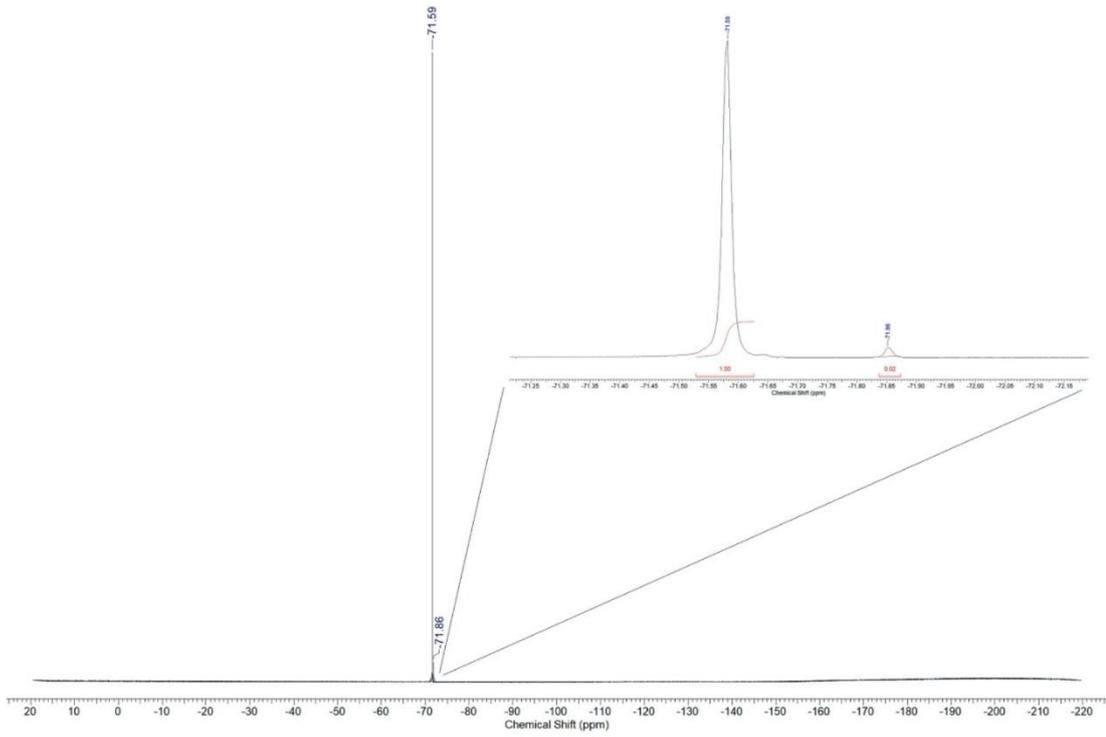


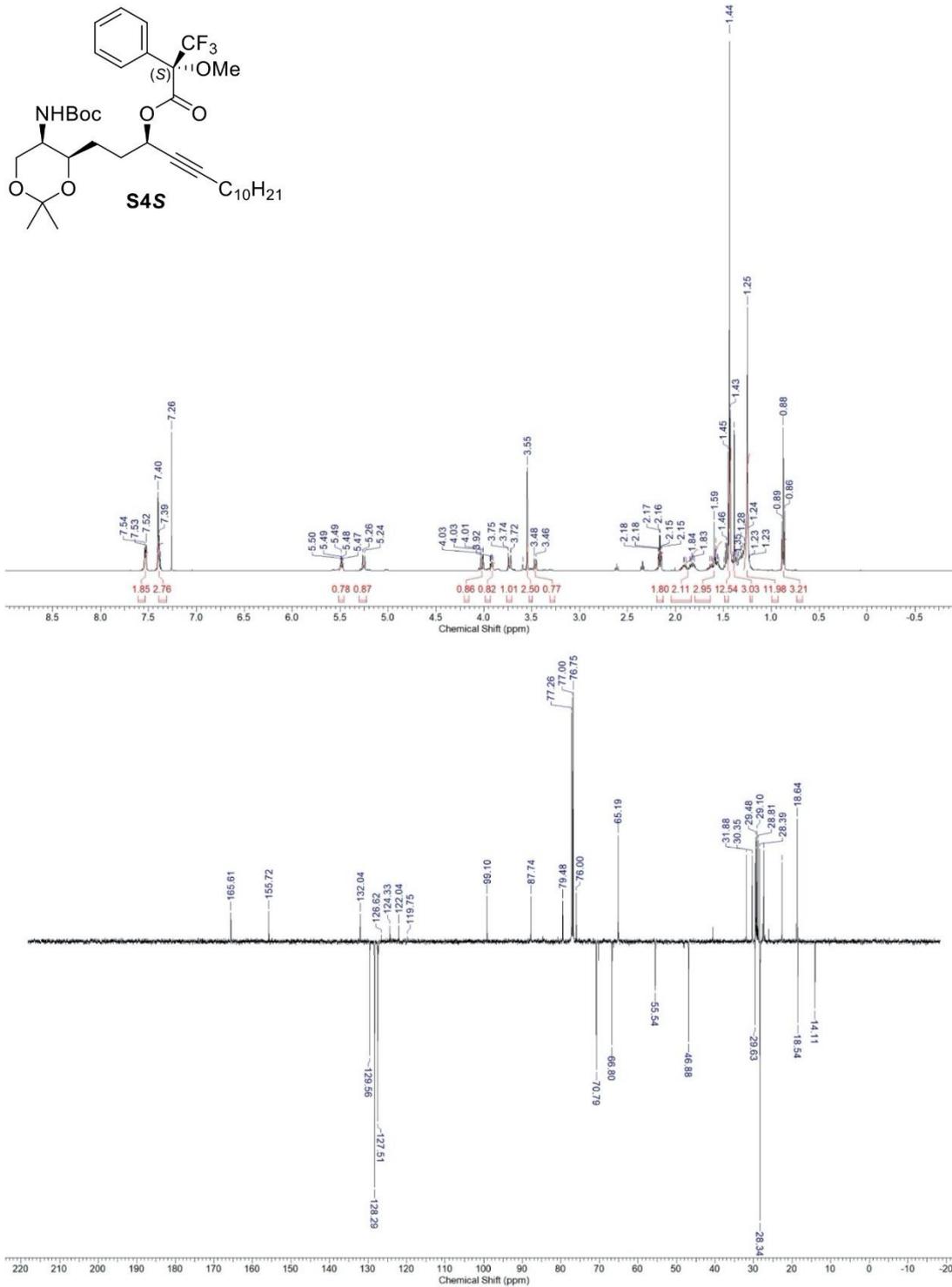


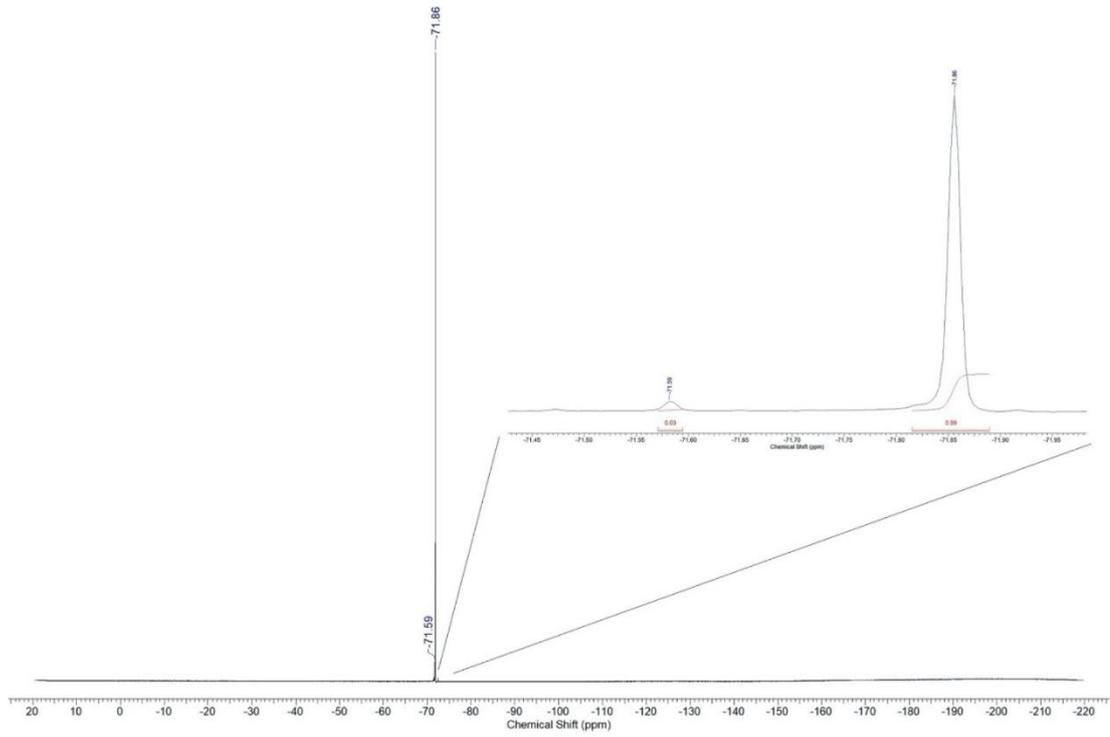












## Cell culture conditions and MTT-assay

### *Cell culture conditions and stock solutions*

The HCT-116<sup>wt</sup> (DSMZ ACC-581) as well as its knockout mutant HCT-116<sup>p53<sup>-/-</sup></sup> colon carcinoma cells, the 518A2 (Department of Radiotherapy & Radiobiology, University Hospital Vienna, Austria) melanoma cells, the U87 glioblastoma cells, the HeLa (DSMZ ACC-57) cervix carcinoma cells and the Ea.Hy926 (ATCC CRL-2922) endothelial cells were cultivated in Dulbeccos Modified Eagle Medium (Gibco, ThermoFisher), supplemented with 10% fetal bovine serum (Biochrom) and 1% Antibiotic-Antimycotic (Gibco, ThermoFisher) at 37 °C, 95% humidity and 5% CO<sub>2</sub>. If not indicated otherwise all incubation steps of the assay were conducted under these cell culture conditions. Halisphingosine A (**1**) was dissolved in DMSO (10 mM) and freshly diluted appropriately with sterile Milli-Q water.

### *Anti-proliferative activity (MTT-assay)<sup>5</sup>*

**1** was investigated for its anti-proliferative effect on various human carcinoma cell lines via the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Glentham life sciences) based proliferation assay. Cells were seeded at  $0.05 \times 10^6$  cells per mL (cpm) into the wells of 96-well microtiter plates (100 µL/well) and incubated for 24 h. Appropriate dilutions in H<sub>2</sub>O of halisphingosine A (**1**) or equal amounts of the solvent were added into the wells (final concentrations 100 µM–5 nM) and the cells were further incubated for 72 h. Before staining of the viable cells the plates were centrifuged (300 g, 5 min, 4 °C) and the back medium was discarded. 50 µL of a 0.05% MTT solution (PBS) were added to each well. After another 2 h of incubation the plates were centrifuged as before and the MTT solution was discarded again. To dissolve the cells and the formed violet water-insoluble formazan, 25 µL of an SDS/DMSO solution (10%, 0.6% AcOH) were added to each well and the plates were further incubated for at least 1 h. The absorbances at 570 nm (formazan) and at 630 nm (background) were measured. The absorbance of formazan is directly linked to the amount of metabolically active (viable) cells in the wells. The absorbance of the wells treated with the solvent was set to 100% viable cells, and the percentage of viable cells in the wells treated with halisphingosine A was calculated accordingly. IC<sub>50</sub> values were determined using Graphpad Prism, means and SD were calculated from four independent experiments.

**References**

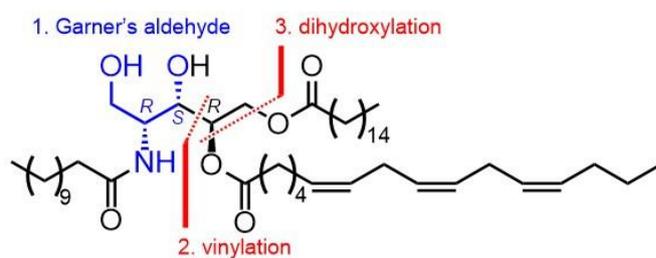
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## 4.4 Publikation III

### Synthesis of the Proposed Isomers of the Deep-Sea Mussel Metabolites Bathymodiolamides A and B

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## Synthesis of the Proposed Isomers of the Deep-Sea Mussel Metabolites Bathymodiolamides A and B

Alexander Bär, Sofia I. Bär, Moritz Röder, and Rainer Schober<sup>\*†</sup>

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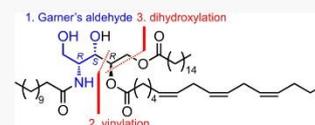
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**ABSTRACT:** The first total synthesis of bathymodiolamides A and B, ceramide-like metabolites of the deep-sea hydrothermal vent mussel *Bathymodiolus thermophilus*, was accomplished in eight linear steps starting from Garner's aldehyde and three carboxylic acids. A sequence of vinylation of Garner's aldehyde, N-acylation with lauric acid, dihydroxylation of the terminal alkene, and stepwise Steglich–Hassner esterifications of the resulting vicinal diol with the respective saturated and unsaturated carboxylic acids, which had to be prepared separately, afforded the target products in 38 and 39% yield. We found distinct discrepancies between their NMR data and antiproliferative activities and those reported for the natural isolates.



### INTRODUCTION

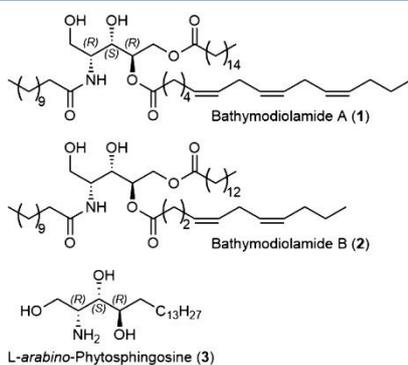
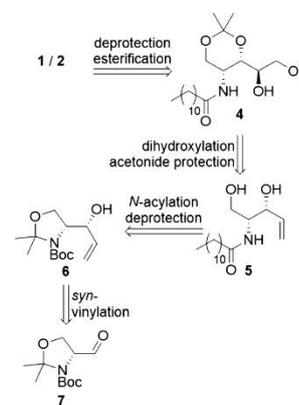
The bathymodiolamides A (**1**) and B (**2**) are ceramide-related lipid metabolites that were isolated from the deep-sea hydrothermal vent mussel *Bathymodiolus thermophilus*.<sup>1</sup> Their absolute configurations were deduced based on NMR and mass spectra, chemical degradation, and on a comparison of specific optical rotations of related known compounds. These data suggested the absolute configurations of the stereotriad of **1** and **2** to be identical to those of the known congener *L*-arabino phytosphingosine (**3**),<sup>2</sup> that is, 2*R*,3*S*,4*R* (Figure 1). Because of their distinct apoptosis induction and cytotoxic effect in cancer cells, they and further structurally related metabolites from the same source organism were recently patented for potential use in cancer chemotherapy.<sup>3</sup> We now report a short flexible synthetic route to the proposed (2*R*,3*S*,4*R*)-isomers of bathymodiolamides A and B, and a

comparison of their physical data and anticancer activities with those reported for the natural isolates.

### RESULTS AND DISCUSSION

Our retrosynthetic approach is outlined in Scheme 1. Both target compounds **1** and **2** were finished by consecutive attachment of the corresponding saturated and unsaturated

#### Scheme 1. Retrosynthesis of Bathymodiolamides A (**1**) and B (**2**)



**Figure 1.** Structures of bathymodiolamides A (**1**) and B (**2**) and *L*-arabino phytosphingosine (**3**).

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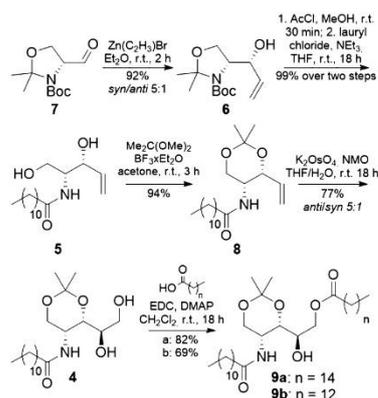
Published: January 4, 2021



carboxylic acids to diol **4** followed by acetonide deprotection. Acetonide protection of amide **5** and asymmetric dihydroxylation of the alkene under substrate control should predominantly furnish diol **4**, thus installing stereogenic center **4R**. Amide **5** was accessible by deprotection and N-acylation of allylic alcohol **6** which contributes stereogenic center **3S**, established by *syn*-selective vinylation of R-Garner's aldehyde (**7**). The latter is readily available from D-serine<sup>4</sup> and served as a source of carbon atoms C1–C3 and of stereogenic center **2R**.

Vinylation of **7** with vinyl zinc bromide according to modified literature procedures<sup>5</sup> and purification by column chromatography afforded the *syn*-configured allylic alcohol **6** in 92% yield (Scheme 2). It was treated with a mixture of acetyl

**Scheme 2. Synthesis of Diol 4 and Its Mono Esters 9a and 9b**

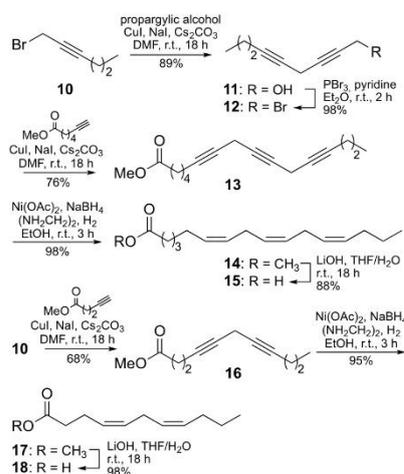


chloride and methanol to cleave the boc and the acinal groups resulting in an amine which was acylated right away with commercially available lauryl chloride to give amide **5** in 99% yield. Acetonide protection of its hydroxy groups left alkene **8** which was dihydroxylated under substrate control with potassium osmate/NMO furnishing predominantly diol **4** with a *syn*/*anti* ratio of 1:5. Gratifyingly, **4** was easy to purify *via* column chromatography and the absolute configuration of the secondary alcohol was ascertained to be *R* by analysis of the <sup>1</sup>H NMR spectra of the corresponding Mosher esters (*cf.* Supporting Information for details).<sup>6</sup> The primary hydroxy group of **4** was then esterified with palmitic acid or myristic acid under Steglich–Hassner conditions affording the hydroxyesters **9a** and **9b** in 82 and 69% yield.

The required skipped trienoic and dienoic acids **15** and **18** were both prepared starting from readily available 1-bromohex-2-yne (**10**)<sup>7</sup> (Scheme 3). The copper-mediated coupling of **10** with propargylic alcohol afforded alcohol **11**, which in turn was converted into propargylic bromide **12**. Coupling of **12** with 6-heptynoic acid methyl ester<sup>8</sup> gave triyne **13**. Z-selective reduction of **13** according to Brown's P-2 Ni protocol<sup>9</sup> and saponification of resulting ester **14** led to hexadeca-6*Z*,9*Z*,12*Z*-trienoic acid (**15**) in 57% overall yield. Undeca-4*Z*,7*Z*-dienoic acid (**18**) was prepared analogously in 63% overall yield by coupling **10** with 4-pentynoic acid methyl ester<sup>10</sup> followed by Z-selective reduction of diyne **16** and saponification of ester **17**.

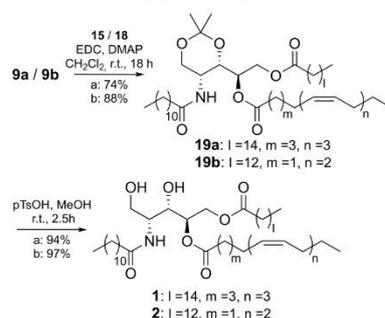
To complete the synthesis, hydroxy esters **9a** and **9b** were esterified with the respective oligoenoic acid **15** or **18**, again

**Scheme 3. Synthesis of Skipped Polyunsaturated Fatty Acids 15 and 18**



under Steglich–Hassner conditions, to afford the diester amides **19a** and **19b** which were eventually deprotected with pTsOH in methanol to give the target compounds **1** and **2** in 38 and 39% overall yield (Scheme 4). Their specific optical

**Scheme 4. Final Steps Affording (2*R*,3*S*,4*R*)-Bathymodiolamides A (**1**) and B (**2**)**



rotations of  $[\alpha]_D^{23} +14.8$  ( $c$  0.08 in MeOH) for **1** and  $[\alpha]_D^{23} +6.7$  ( $c$  0.08 in MeOH) for **2** deviated somewhat from  $[\alpha]_D^{23} +10.8$  ( $c$  0.08 in MeOH)<sup>1</sup> as reported for both natural isolates. Even more conspicuous are the differences in the <sup>1</sup>H and <sup>13</sup>C NMR spectra of our synthetic products when compared with those reported by Andrianasolo *et al.*,<sup>1</sup> for the natural isolates, in particular, the marked differences in the chemical shifts of the atoms of the aminotetraol moiety and in some coupling constants in the <sup>1</sup>H NMR spectra (*cf.* Table 1, Supporting Information). This casts some doubt on the stereochemical assignment of the aminotetraol moiety of the isolates.

The isolating group also reported the cytotoxicities of **1** and **2** as measured via a modified MTT assay. They found an  $IC_{50} = 0.4 \mu\text{M}$  for **1** and  $0.5 \mu\text{M}$  for **2** against HeLa cervical cancer cells and an  $IC_{50} = 0.1 \mu\text{M}$  for **1** and  $0.2 \mu\text{M}$  for **2** against MCF7 breast carcinoma cells.<sup>1</sup> We tested our synthetic products **1** and **2** for antiproliferative effects in MTT assays against cells of HeLa, MCF7, as well as HCT-116 and its p53

mutant HCT-116<sup>p53-/-</sup> colon carcinoma, 518A2 human melanoma, and U87 human glioblastoma. However, both were virtually inactive with IC<sub>50</sub> > 50 μM in all tested cancer cell lines (cf. Table 2, Supporting Information). This inactivity suggests a possibly incorrect structural assignment for both natural compounds.

## CONCLUSIONS

In summary, we developed efficient eight-step syntheses for the (2*R*,3*S*,4*R*)-isomers purported for the natural bathymodiolamides A and B. However, discrepancies in the NMR data and *in vitro* anticancer activities of our synthetic products and those reported for the natural isolates suggest a re-evaluation of the latter. As our synthetic route is straightforward and sufficiently flexible to allow the synthesis of other stereoisomers of 1 and 2, it could be used to clarify the configurations of the natural bathymodiolamides A and B and to establish reliable structure–activity relationships.

## EXPERIMENTAL SECTION

**General Remarks.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained using a Bruker DRX 500 spectrometer. Chemical shifts are given in parts per million using the residual solvent peak as an internal standard 7.26 ppm (proton) and 77.00 ppm (carbon) for CDCl<sub>3</sub> and 3.31 ppm (proton) and 49.15 ppm (carbon) for MeOD. Coupling constants (*J*) are quoted in Hz. Multiplicity abbreviation used: s singlet, d doublet, t triplet, q quartet, quin quintet, sxt sextet, and m multiplet. High-resolution mass spectra were obtained with a UPLC/Orbitrap MS system in electrospray ionization (ESI) mode. IR spectra were recorded with a Perkin-Elmer Spectrum 100 FT-IR spectrophotometer with an ATR sampling unit. Optical rotations were measured at 589 nm (Na D line) on a PerkinElmer 241 polarimeter. Melting points were obtained by a Büchi melting point M-565 and are uncorrected. All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were used as supplied, except tetrahydrofuran and dichloromethane which were freshly distilled according to standard procedures. Reactions were routinely carried out under an argon atmosphere unless stated otherwise. All glassware was flame-dried before use. Analytical thin layer chromatography was carried out using Merck Kieselgel 60GF254 pre-coated aluminum-backed plates. The compounds were visualized with UV light (25 and/or 360 nm) and/or potassium permanganate. Flash chromatography was performed at medium pressure using Macherey-Nagel silica gel 60, pore size 40–63 μm with the eluent specified. (*R*)-Garner's aldehyde (7),<sup>8</sup> 1-bromohex-2-yne (10),<sup>7</sup> 6-heptynoic acid methyl ester,<sup>8</sup> and 4-pentynoic acid methyl ester<sup>10</sup> were prepared according to literature procedures.

*tert*-Butyl (*R*)-4-((*R*)-1-hydroxyallyl)-2,2-dimethylloxazolidine-3-carboxylate (6) was prepared according to a modified literature procedure.<sup>5a</sup> Tetravinyltin (2.0 mL, 10.9 mmol) in dry Et<sub>2</sub>O (50 mL) was treated with MeLi (27.3 mL, 1.6M in Et<sub>2</sub>O, 43.6 mmol) at 0 °C. The mixture was stirred for 15 min at 0 °C before ZnBr<sub>2</sub> (9.8 g, 43.6 mmol) was added. After stirring for 1 h at ambient temperature, the mixture was added to a solution of (*R*)-Garner's aldehyde (7) (2.5 g, 10.9 mmol) and ZnBr<sub>2</sub> (2.5 g, 10.9 mmol) in dry Et<sub>2</sub>O (50 mL) at –35 °C. The mixture was allowed to warm to ambient temperature and stirred for 3 h. Saturated aqueous NH<sub>4</sub>Cl (100 mL) was added, and the phases were separated. The aqueous phase was extracted with Et<sub>2</sub>O (2 × 100 mL). The combined organic phases were washed with brine (200 mL), dried over MgSO<sub>4</sub>, and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 5:1) to give 6 (2.58 g, 10.0 mmol, 92%) as a white solid; analytical data agreed with those reported.<sup>5a</sup>

*N*-((2*R*,3*R*)-1,3-Dihydroxypent-4-en-2-yl)dodecanamide (5). A solution of 6 (1.5 g, 5.8 mmol) in MeOH (30 mL) was treated with 2 mL AcCl at 0 °C. The mixture was stirred for 30 min at room

temperature and volatiles were evaporated. The residue was taken up in THF (30 mL), and NEt<sub>3</sub> (2.4 mL, 17.49 mmol) and lauroyl chloride (1.5 mL, 6.41 mmol) were added. The mixture was stirred for 18 h at room temperature, then diluted with EtOAc (50 mL), and the organic phase was washed with 1 M HCl (75 mL), aqueous NaHCO<sub>3</sub> (75 mL), and brine (75 mL), dried over MgSO<sub>4</sub>, and evaporated to dryness. The crude product was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) to give 5 (1.73 g, 5.78 mmol, 99%) as a white solid of mp 70–71 °C; *R*<sub>f</sub> = 0.49 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5); [α]<sub>D</sub><sup>23</sup> +20.8 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): δ 5.86 (ddd, *J* = 5.5, 10.5, 17.2 Hz, 1H), 5.30 (td, *J* = 1.6, 17.2 Hz, 1H), 5.14 (td, *J* = 1.6, 10.5 Hz, 1H), 4.34 (tdd, *J* = 1.4, 3.4, 5.5 Hz, 1H), 3.98–3.92 (m, 1H), 3.68 (dd, *J* = 6.4, 10.9 Hz, 1H), 3.55 (dd, *J* = 6.4, 10.9 Hz, 1H), 2.21 (dt, *J* = 1.5, 7.5 Hz, 2H), 1.65–1.55 (m, 2H), 1.35–1.25 (m, 16H), 0.90 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>3</sub>OD, 126 MHz): δ 176.7, 139.9, 116.0, 72.0, 62.4, 56.4, 37.3, 33.2, 30.9, 30.8, 30.7, 30.5, 27.3, 23.9, 14.6; IR *ν*<sub>max</sub> 3364, 2923, 2853, 2415, 1622, 1456, 1054, 991, 922 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>34</sub>O<sub>3</sub>N<sup>+</sup>, 300.2533; found 300.2523.

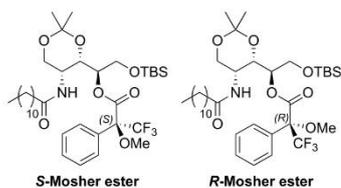
*N*-((4*R*,5*R*)-2,2-Dimethyl-4-vinyl-1,3-dioxan-5-yl)dodecanamide (8). A solution of 5 (1.29 g, 4.32 mmol) in acetone (20 mL) was treated with 2,2-dimethoxypropane (5.3 mL, 43.24 mmol) and BF<sub>3</sub>·Et<sub>2</sub>O (55 μL, 0.43 mmol). The mixture was stirred for 3 h at ambient temperature. NEt<sub>3</sub> (0.5 mL) was added, and volatiles were removed under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate 2:1) to give 8 (1.37 g, 4.05 mmol, 94%) as colorless oil; *R*<sub>f</sub> = 0.28 (*n*-hexane/ethyl acetate 3:1); [α]<sub>D</sub><sup>23</sup> +5.9 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 6.11 (d, *J* = 9.2 Hz, 1H), 5.71 (ddd, *J* = 4.4, 10.7, 17.3 Hz, 1H), 5.31 (td, *J* = 1.7, 17.3 Hz, 1H), 5.19 (td, *J* = 1.7, 10.7 Hz, 1H), 4.56 (dd, *J* = 1.7, 4.3 Hz, 1H), 4.12 (dd, *J* = 1.8, 12.0 Hz, 1H), 3.97 (dd, *J* = 1.8, 9.2 Hz, 1H), 3.75 (dd, *J* = 1.8, 12.0 Hz, 1H), 2.20 (dt, *J* = 3.1, 7.6 Hz, 2H), 1.65–1.57 (m, 2H), 1.50 (s, 3H), 1.46 (s, 3H), 1.33–1.21 (m, 16H), 0.87 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 172.9, 134.7, 116.6, 99.2, 71.3, 64.6, 45.5, 36.8, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 25.7, 22.7, 18.5, 14.1; IR *ν*<sub>max</sub> 3322, 2924, 2854, 1651, 1505, 1380, 1269, 1237, 1199, 1087, 985, 856 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>38</sub>O<sub>3</sub>N<sup>+</sup>, 340.2846; found 340.2838.

*N*-((4*S*,5*R*)-4-((*R*)-1,2-Dihydroxyethyl)-2,2-dimethyl-1,3-dioxan-5-yl)dodecanamide (4). Alkene 8 (1.32 g, 3.9 mmol) was dissolved in THF (20 mL), and K<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O (75 mg, 0.19 mmol) and NMO (50% in H<sub>2</sub>O, 2.5 mL, 11.7 mmol) were added. The mixture was stirred for 18 h at room temperature. Water (50 mL) was added, and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (150 mL), dried over MgSO<sub>4</sub>, and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:4) to give diol 4 (1.12 g, 3.0 mmol, 77%) as a colorless oil; *R*<sub>f</sub> = 0.30 (*n*-hexane/ethyl acetate 1:2); [α]<sub>D</sub><sup>23</sup> +40.6 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 6.35 (d, *J* = 7.9 Hz, 1H), 5.04 (d, *J* = 3.7 Hz, 1H), 4.21 (dd, *J* = 2.1, 12.2 Hz, 1H), 3.97 (dd, *J* = 1.5, 8.5 Hz, 1H), 3.88 (dd, *J* = 1.2, 9.2 Hz, 1H), 3.80–3.73 (m, 2H), 3.57 (td, *J* = 5.6, 11.3 Hz, 1H), 3.34–3.27 (m, 1H), 2.30 (dt, *J* = 1.4, 7.7 Hz, 2H); 2.24–2.16 (m, 1H), 1.70–1.62 (m, 2H), 1.47 (s, 3H), 1.40 (s, 3H), 1.37–1.22 (m, 16H), 0.87 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 175.3, 99.3, 71.7, 69.4, 64.3, 63.1, 44.0, 36.5, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 25.7, 22.7, 18.5, 14.1; IR *ν*<sub>max</sub> 3316, 2923, 2853, 1640, 1528, 1460, 1382, 1271, 1199, 1087, 849, 662 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>40</sub>O<sub>5</sub>N<sup>+</sup>, 374.2901; found, 374.2894. The *anti/syn* ratio of 5:1 was determined by analysis of <sup>1</sup>H NMR spectrum of the crude product.

**Mosher Esters of Diol 4.** *N*-((4*S*,5*R*)-4-((*R*)-2'-((*tert*-Butyldimethylsilyloxy)-1'-hydroxyethyl)-2,2-dimethyl-1,3-dioxan-5-yl)dodecanamide. A solution of diol 4 (100 mg, 0.27 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was treated with imidazole (36 mg, 0.54 mmol) and TBSCl (44 mg, 0.29 mmol) and was stirred at ambient temperature for 18 h. Saturated aqueous NH<sub>4</sub>Cl (10 mL) was added, and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 10 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-

hexane/ethyl acetate 5:1) to give the primary TBS-ether of diol **4** as colorless oil (121 mg, 0.25 mmol, 93%);  $R_f = 0.50$  (*n*-hexane/ethyl acetate 2:1);  $[\alpha]_D^{23} +50.2$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 6.28 (d, *J* = 8.9 Hz, 1H), 4.40 (s, 1H), 4.18 (dd, *J* = 1.8, 12.2 Hz, 1H), 4.04 (dd, *J* = 1.5, 8.9 Hz, 1H), 3.99 (dd, *J* = 1.5, 9.2 Hz, 1H), 3.78 (dd, *J* = 2.1, 10.7 Hz, 1H), 3.76 (dd, *J* = 1.8, 12.2 Hz, 1H), 3.68 (dd, *J* = 4.3, 10.7 Hz, 1H), 3.26 (ddd, *J* = 2.1, 4.3, 9.2 Hz, 1H), 2.27 (t, *J* = 7.6 Hz, 2H), 1.65 (quin, *J* = 7.6 Hz, 2H), 1.46 (s, 3H), 1.39 (s, 3H), 1.36–1.18 (m, 16H), 0.90 (s, 9H), 0.87 (t, *J* = 7.0 Hz, 3H), 0.07 (s, 3H), 0.06 (s, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 174.6, 99.2, 70.3, 70.2, 64.7, 63.2, 43.8, 36.6, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 26.0, 25.8, 22.7, 18.6, 18.5, 14.1, –5.4, –5.3; IR  $\nu_{max}$  3318, 2957, 2925, 2855, 1642, 1524, 1462, 1391, 1252, 1199, 1121, 1090, 834, 777 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>26</sub>H<sub>54</sub>O<sub>3</sub>NSi<sup>+</sup>, 488.3766; found, 488.3767.

(*R*)-2'-((*tert*-Butyldimethylsilyloxy)-1'-((4*S*,5*R*)-5-dodecanamido-2,2-dimethyl-1,3-dioxan-4-yl) ethyl (5*S*)-(*R*)-3',3'',3'''-trifluoro-2'-methoxy-2'-phenylpropanoate (*S*- and *R*-Mosher esters).



A solution of the primary TBS-ether of diol **4** (35 mg, 72 μmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was treated with 4-dimethylaminopyridine (DMAP) (18 mg, 140 μmol) and (*R*)- or (*S*)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetyl chloride (MTPA-Cl; 16 μL, 86 μmol). The mixture was stirred at ambient temperature for 18 h, and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 5:1) to give *S*-Mosher ester (40 mg, 79%) or *R*-Mosher ester (24 mg, 48%) as colorless oils;  $R_f = 0.34$  (*n*-hexane/ethyl acetate 5:1); IR  $\nu_{max}$  3440, 2927, 2656, 1755, 1678, 1500, 1463, 1383, 1254, 1169, 1108, 1017, 977, 833, 776, 719 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>36</sub>H<sub>61</sub>O<sub>7</sub>NF<sub>3</sub>Si<sup>+</sup>, 704.4163; found, 704.4161; *S*-Mosher ester:  $[\alpha]_D^{23} -35.4$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.61–7.56 (m, 2H), 7.42–7.37 (m, 3H), 6.00 (d, *J* = 9.2 Hz, 1H), 5.20 (td, *J* = 3.8, 7.9 Hz, 1H), 4.32 (dd, *J* = 1.8, 7.9 Hz, 1H), 3.97 (dd, *J* = 1.8, 12.1 Hz, 1H), 3.88 (qd, *J* = 1.8, 9.2 Hz, 1H), 3.82 (t, *J* = 3.8 Hz, 2H), 3.73 (dd, *J* = 1.8, 12.1 Hz, 1H), 3.62–3.58 (m, 3H), 2.07 (dt, *J* = 1.7, 7.5 Hz, 2H), 1.57 (quin, *J* = 7.5 Hz, 2H), 1.46 (s, 3H), 1.40 (s, 3H), 1.32–1.21 (m, 16 H), 0.89 (s, 9H), 0.87 (t, *J* = 7.3 Hz, 3H), 0.07 (s, 3H), 0.06 (s, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 172.6, 165.5, 132.7, 129.54, 128.4, 127.4, 123.3 (<sup>1</sup>*J*<sub>FC</sub> = 288.8 Hz), 99.6, 84.5 (<sup>2</sup>*J*<sub>FC</sub> = 28.2 Hz), 74.1, 67.8, 64.8, 60.1, 55.6, 42.9, 36.6, 31.9, 29.6, 29.5, 29.4, 29.3, 25.7, 25.6, 22.7, 18.5, 18.1, 14.1, –5.6, –5.7; *R*-Mosher ester:  $[\alpha]_D^{23} -15.6$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.59–7.53 (m, 2H), 7.43–7.37 (m, 3H), 6.13 (d, *J* = 8.9 Hz, 1H), 5.14 (td, *J* = 3.8, 7.8 Hz, 1H), 4.39 (dd, *J* = 1.8, 7.8 Hz, 1H), 4.01 (dd, *J* = 1.8, 12.0 Hz, 1H), 3.95 (qd, *J* = 1.8, 8.9 Hz, 1 H), 3.80 (dd, *J* = 1.8, 12.0 Hz, 1 H), 3.73 (ddd, *J* = 3.8, 11.0, 17.1 Hz, 2 H), 3.56–3.53 (m, 3 H), 2.08 (t, *J* = 7.9 Hz, 2 H), 1.61–1.54 (m, 2 H), 1.47 (s, 3 H), 1.43 (s, 3 H), 1.32–1.22 (m, 17 H), 0.87 (t, *J* = 7.0 Hz, 4 H), 0.83 (s, 9 H), –0.01 (s, 3 H), –0.03 (s, 3 H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 172.9, 165.9, 132.0, 129.6, 128.5, 127.7, 123.2 (<sup>1</sup>*J*<sub>FC</sub> = 288.8 Hz), 99.6, 84.9 (<sup>2</sup>*J*<sub>FC</sub> = 28.2 Hz), 74.3, 67.5, 64.7, 59.7, 55.5, 43.1, 36.6, 31.9, 29.6, 29.5, 29.4, 29.3, 25.7, 25.6, 22.7, 18.5, 18.1, 14.1, –5.6, –5.7. For an analysis<sup>6</sup>

confirming the absolute configuration of diol **4** cf. the Supporting Information.

(*R*)-2'-((4*S*,5*R*)-5-Dodecanamido-2,2-dimethyl-1,3-dioxan-4-yl)-2-hydroxyethyl palmitate (**9a**). A solution of **4** (780 mg, 2.01 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was treated with DMAP (26 mg, 0.20 mmol), EDC·HCl (400 mg, 2.01 mmol), and palmitic acid (477 mg, 2.01 mmol) and stirred for 18 h at ambient temperature. The organic phase was washed with 1M HCl (20 mL), aqueous NaHCO<sub>3</sub> (20 mL), and brine (20 mL), dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 2:1) to give **9a** (806 mg, 1.32 mmol, 82%) as a white solid; mp 52–53 °C;  $R_f = 0.49$  (*n*-hexane/ethyl acetate 2:1);  $[\alpha]_D^{23} +38.7$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 6.34 (d, *J* = 8.5 Hz, 1H), 4.99 (s, 1H), 4.25 (dd, *J* = 2.3, 11.5 Hz, 1H), 4.20 (dd, *J* = 1.7, 12.3 Hz, 1H), 4.12 (dd, *J* = 5.7, 11.5 Hz, 1H), 4.01 (dd, *J* = 1.7, 8.5 Hz, 1H), 3.88 (dd, *J* = 1.2, 9.2 Hz, 1H), 3.77 (dd, *J* = 1.7, 12.3 Hz, 1H), 3.45 (ddd, *J* = 2.3, 5.7, 9.2 Hz, 1H), 2.33 (t, *J* = 7.6 Hz, 2H), 2.30 (dt, *J* = 3.1, 7.3 Hz, 2H), 1.69–1.56 (m, 4H), 1.43 (s, 3H), 1.38 (s, 3H), 1.35–1.19 (m, 40H), 0.87 (t, *J* = 7.3 Hz, 6H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 175.3, 173.9, 99.4, 71.1, 67.8, 64.4, 64.3, 43.8, 36.4, 34.2, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 25.7, 24.9, 22.6, 18.4, 14.1; IR  $\nu_{max}$  3322, 2922, 2853, 1736, 1644, 1526, 1457, 1381, 1269, 1234, 1199, 1175, 1090, 850 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>36</sub>H<sub>70</sub>O<sub>6</sub>N<sup>+</sup>, 612.5197; found, 612.5189.

(*R*)-2'-((4*S*,5*R*)-5-Dodecanamido-2,2-dimethyl-1,3-dioxan-4-yl)-2-hydroxyethyl tetradecanoate (**9b**). Analogous to **9a**, ester **9b** (647 mg, 1.11 mmol, 69%) was prepared as a white solid from alcohol **4** (600 mg, 1.61 mmol), DMAP (20 mg, 0.16 mmol), EDC·HCl (308 mg, 1.61 mmol), and myristic acid (367 mg, 1.61 mmol); mp 40–41 °C;  $R_f = 0.37$  (*n*-hexane/ethyl acetate 2:1);  $[\alpha]_D^{23} +38.3$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 6.34 (d, *J* = 8.5 Hz, 1H), 4.97 (s, 1H), 4.23 (dd, *J* = 2.1, 11.5 Hz, 1H), 4.18 (dd, *J* = 1.5, 12.3 Hz, 1H), 4.11 (dd, *J* = 5.6, 11.5 Hz, 1H), 3.99 (dd, *J* = 1.5, 8.5 Hz, 1H), 3.86 (dd, *J* = 1.2, 9.2 Hz, 1H), 3.76 (dd, *J* = 1.5, 12.3 Hz, 1H), 3.43 (ddd, *J* = 2.1, 5.6, 9.2 Hz, 1H), 2.34–2.26 (m, 4H), 1.68–1.56 (m, 4H), 1.41 (s, 3H), 1.37 (s, 3H), 1.34–1.17 (m, 36H), 0.85 (t, *J* = 7.0 Hz, 6H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 175.2, 173.9, 99.3, 71.0, 67.7, 43.8, 36.4, 34.1, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 25.6, 24.9, 22.6, 18.3, 14.0; IR (cm<sup>-1</sup>, neat)  $\nu_{max}$  3344, 2922, 2853, 1737, 1645, 1526, 1461, 1381, 1200, 1090, 850 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>34</sub>H<sub>66</sub>O<sub>6</sub>N<sup>+</sup>, 584.4884; found, 584.4879.

*Nona*-2,5-diyne-1-ol (**11**). Cul (4.60 g, 24.1 mmol), NaI (3.62 g, 24.1 mmol), Cs<sub>2</sub>CO<sub>3</sub> (7.86 g, 24.1 mmol), and propargylic alcohol (1.39 mL, 24.1 mmol) were added to a solution of **10** (3.0 g, 18.6 mmol) in dry DMF (25 mL). The mixture was stirred for 18 h at ambient temperature. Saturated aqueous NH<sub>4</sub>Cl (50 mL) was added, and the mixture was filtered over celite. The filtrate was extracted with Et<sub>2</sub>O (3 × 50 mL), and the combined organic phases were washed with brine (2 × 100 mL), dried over MgSO<sub>4</sub>, and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 5:1) to give **11** (2.25 g, 16.5 mmol, 89%) as colorless oil;  $R_f = 0.12$  (*n*-hexane/ethyl acetate 5:1); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 4.29–4.23 (m, 2H), 3.22–3.16 (m, 2H), 2.16–2.10 (m, 2H), 1.63 (s, 1H), 1.51 (sxt, *J* = 7.3 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 81.0, 80.9, 79.3, 73.4, 51.3, 22.1, 20.6, 13.5, 9.8; IR  $\nu_{max}$  3348, 2964, 2934, 2874, 1729, 1414, 1313, 1113, 1010 cm<sup>-1</sup>; HRMS (ESI) *m/z*: [M + H]<sup>+</sup> calcd for C<sub>6</sub>H<sub>13</sub>O<sup>+</sup>, 137.0961; found, 137.0960.

1-Bromonona-2,5-diyne (**12**). PBr<sub>3</sub> (0.63 mL, 6.6 mmol) and 0.1 mL pyridine were added to a solution of **11** (2.25 g, 16.5 mmol) in Et<sub>2</sub>O (100 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C and brine (100 mL) was added. The layers were separated, and the aqueous phase was extracted with Et<sub>2</sub>O (2 × 50 mL). The combined organic phases were dried over MgSO<sub>4</sub> and evaporated to dryness. The crude product was purified by flash chromatography (*n*-pentane/Et<sub>2</sub>O 20:1) to give **12** (3.22 g, 16.2 mmol, 98%) as colorless oil;  $R_f = 0.50$  (*n*-pentane/Et<sub>2</sub>O 20:1); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 3.91 (t, *J* = 2.3 Hz, 2H), 3.21 (quin, *J* = 2.3 Hz, 2H), 2.13 (tt, *J* = 2.3, 7.2 Hz, 2H), 1.51 (sxt, *J* = 7.2 Hz, 2H), 0.96 (t, *J* = 7.2 Hz, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 82.1, 81.2, 75.2, 72.9, 22.1, 20.6, 14.9,

13.5, 10.1; IR  $\nu_{\text{max}}$  2962, 2934, 2872, 1718, 1463, 1411, 1313, 1209, 1123  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_9H_{12}Br^+$ , 199.0117; found, 199.0117.

**Methyl hexadeca-6,9,12-trienoate (13).** Analogous to **11**, ester **13** (937 mg, 3.63 mmol, 76%) was prepared as colorless oil from **12** (950 mg, 4.77 mmol), 6-heptynoic acid methyl ester (870 mg, 6.20 mmol), CuI (1.18 g, 6.20 mmol), NaI (930 mg, 6.20 mmol), and  $Cs_2CO_3$  (2.02 g, 6.20 mmol);  $R_f = 0.27$  (*n*-hexane/ethyl acetate 30:1);  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  3.69 (s, 3H), 3.16–3.14 (m, 4H), 2.34 (t,  $J = 7.5$  Hz, 2H), 2.20 (tt,  $J = 2.2, 7.1$  Hz, 2H), 2.15 (tt,  $J = 2.2, 7.1$  Hz, 2H), 1.77–1.69 (m, 2H), 1.58–1.50 (m, 4H), 0.98 (t,  $J = 7.5$  Hz, 3H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  173.9, 80.7, 80.1, 74.9, 74.7, 74.2, 73.8, 51.5, 33.6, 28.1, 24.1, 22.1, 20.7, 18.4, 13.5, 9.8; IR  $\nu_{\text{max}}$  2936, 1735, 1435, 1317, 1198, 1172, 1147  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{17}H_{23}O_2^+$  259.1693; found 259.1693.

**Methyl (6Z,9Z,12Z)-hexadeca-6,9,12-trienoate (14).** A suspension of  $Ni(OAc)_2 \cdot 4H_2O$  (566 mg, 2.28 mmol) in EtOH (10 mL) was treated with  $NaBH_4$  (86 mg, 2.28 mmol) at 0 °C. The mixture was stirred under a hydrogen atmosphere at ambient temperature for 30 min. Ethylene diamine (610  $\mu$ L, 9.13 mmol) and **13** (590 mg, 2.28 mmol) were added, and stirring under a hydrogen atmosphere at ambient temperature was continued for 3 h. The resulting suspension was filtered over celite, and saturated aqueous  $NH_4Cl$  (50 mL) was added. The aqueous phase was extracted with  $Et_2O$  ( $3 \times 50$  mL). The combined organic phases were dried over  $MgSO_4$  and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 30:1) to give **14** (561 mg, 2.12 mmol, 98%) as colorless oil;  $R_f = 0.27$  (*n*-hexane/ethyl acetate 30:1);  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  5.44–5.31 (m, 6H), 2.80 (t,  $J = 5.8$  Hz, 2H), 2.31 (t,  $J = 7.6$  Hz, 2H), 2.11–2.01 (m, 4H), 1.69–1.60 (m, 2H), 1.44–1.33 (m, 4H), 0.91 (t,  $J = 7.0$  Hz, 3H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  174.2, 130.2, 129.6, 128.4, 128.2, 128.1, 127.8, 51.5, 34.0, 29.3, 29.1, 26.9, 25.7, 25.6, 24.6, 22.8, 13.8; IR  $\nu_{\text{max}}$  3011, 2929, 2858, 1741, 1436, 1198, 1171, 713  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{17}H_{29}O_2^+$ , 265.2162; found, 265.2162.

**(6Z,9Z,12Z)-Hexadeca-6,9,12-trienoic acid (15).** Ester **14** (550 mg, 2.08 mmol) in THF/ $H_2O$  1:1 (10 mL) was treated with  $LiOH \cdot H_2O$  (175 mg, 4.16 mmol), and the mixture was stirred for 18 h at ambient temperature. 1 M HCl was added (20 mL), and the aqueous phase was extracted with EtOAc ( $3 \times 20$  mL). The combined organic phases were dried over  $MgSO_4$  and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 3:1) to give **15** (458 mg, 1.83 mmol, 88%) as colorless oil;  $R_f = 0.4$  (*n*-hexane/ethyl acetate 3:1);  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  11.51 (s, 1H), 5.50–5.28 (m, 6H), 2.81 (t,  $J = 6.0$  Hz, 4H), 2.36 (t,  $J = 7.5$  Hz, 2H), 2.13–2.00 (m, 4H), 1.71–1.61 (m, 2H), 1.47–1.33 (m, 4H), 0.91 (t,  $J = 7.5$  Hz, 3H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  179.9, 130.2, 129.5, 128.4, 128.3, 128.0, 127.8, 33.9, 29.3, 29.0, 26.8, 25.6, 24.3, 22.8, 13.8; IR  $\nu_{\text{max}}$  3011, 2928, 2859, 1707, 1413, 1287, 1233, 928, 711  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{16}H_{27}O_2^+$ , 251.2006; found, 251.2009.

**Methyl undeca-4,7-dienoate (16).** Analogous to **13**, ester **16** (1.21 mg, 6.30 mmol, 68%) was prepared as colorless oil from **10** (1.50 g, 9.3 mmol), 4-pentynoic acid methyl ester (1.35 g, 12.0 mmol), CuI (2.29 g, 12.0 mmol), NaI (1.80 g, 12.0 mmol), and  $Cs_2CO_3$  (3.92 g, 12.0 mmol);  $R_f = 0.3$  (*n*-hexane/ethyl acetate 30:1);  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  3.67 (s, 3H), 3.09 (quin,  $J = 2.3$  Hz, 2H), 2.53–2.43 (m, 4H), 2.11 (tt,  $J = 2.3, 7.1$  Hz, 2H), 1.49 (sxt,  $J = 7.4$  Hz, 2H), 0.94 (t,  $J = 7.4$  Hz, 3H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  172.4, 80.5, 78.2, 75.4, 74.2, 51.7, 33.3, 22.1, 20.6, 14.6, 13.4, 9.6; IR  $\nu_{\text{max}}$  2962, 2935, 2875, 1737, 1437, 1366, 1314, 1198, 1166, 1038  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{12}H_{17}O_2^+$ , 193.1223; found, 193.1223.

**Methyl (4Z,7Z)-undeca-4,7-dienoate (17).** Analogous to **14**, ester **17** (1.46 g, 7.42 mmol, 95%) was prepared as colorless oil from **16** (1.50 g, 7.81 mmol),  $Ni(OAc)_2 \cdot 4H_2O$  (1.94 g, 7.81 mmol),  $NaBH_4$  (295 mg, 7.81 mmol), and ethylene diamine (2.1 mL, 31.22 mmol);  $R_f = 0.3$  (*n*-hexane/ethyl acetate 30:1);  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  5.44–5.29 (m, 4H), 3.66 (s, 3H), 2.79 (t,  $J = 7.0$  Hz, 2H), 2.42–2.34 (m, 4H), 2.03 (q,  $J = 7.3$  Hz, 2H), 1.37 (sxt,  $J = 7.3$  Hz, 2H),

0.90 (t,  $J = 7.3$  Hz, 3H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  173.6, 130.2, 129.7, 127.6, 127.5, 51.5, 34.0, 29.3, 25.5, 22.8, 13.8; IR  $\nu_{\text{max}}$  3011, 2957, 2872, 1740, 1436, 1360, 1196, 1160, 717  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{12}H_{21}O_2^+$  197.1536; found 197.1538.

**(4Z,7Z)-Undeca-4,7-dienoic acid (18).** Analogous to **15**, carboxylic acid **18** (700 mg, 3.84 mmol, 98%) was prepared as colorless oil from **17** (770 mg, 3.92 mmol) and  $LiOH \cdot H_2O$  (330 mg, 7.85 mmol);  $R_f = 0.35$  (*n*-hexane/ethyl acetate 3:1);  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  5.46–5.30 (m, 4H), 2.80 (t,  $J = 7.0$  Hz, 2H), 2.43–2.36 (m, 4H), 2.03 (q,  $J = 7.3$  Hz, 2H), 1.38 (sxt,  $J = 7.3$  Hz, 2H), 0.90 (t,  $J = 7.3$  Hz, 3H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  179.1, 130.3, 129.9, 127.5, 127.2, 34.0, 29.3, 25.6, 22.7, 22.5, 13.8; IR  $\nu_{\text{max}}$  3011, 2958, 2929, 2873, 1708, 1412, 1279, 1250, 1211, 931, 712  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{11}H_{19}O_2^+$ , 183.1379; found, 183.1380.

**(R)-1-((4S,5R)-5-Dodecanamido-2,2-dimethyl-1,3-dioxan-4-yl)-2-(palmitoyloxy)ethyl (6Z,9Z,12Z)-hexadeca-6,9,12-trienoate (19a).** A solution of **9a** (244 mg, 0.40 mmol) in dry  $CH_2Cl_2$  (10 mL) was treated with DMAP (98 mg, 0.80 mmol), EDC-HCl (92 mg, 0.48 mmol), and **15** (100 mg, 0.4 mmol) and stirred for 18 h at ambient temperature. The organic phase was washed with 1M HCl (20 mL), aqueous  $NaHCO_3$  (20 mL), and brine (20 mL), dried over  $MgSO_4$ , and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 5:1) to give **19a** (250 mg, 0.30 mmol, 74%) as colorless oil;  $R_f = 0.4$  (*n*-hexane/ethyl acetate 5:1);  $[\alpha]_D^{25} +9.9$  (c 1.0,  $CHCl_3$ );  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  6.07 (d,  $J = 10.1$  Hz, 1H), 5.43–5.30 (m, 6H), 4.93 (ddd,  $J = 2.4, 4.6, 9.7$  Hz, 1H), 4.38 (dd,  $J = 2.0, 12.2$  Hz, 1H), 4.19 (dd,  $J = 2.0, 9.7$  Hz, 1H), 4.14–4.06 (m, 3H), 3.71 (dd,  $J = 1.5, 12.2$  Hz, 1H), 2.84–2.76 (m, 4H), 2.39–2.23 (m, 4H), 2.22–2.13 (m, 2H), 2.10–1.97 (m, 4H), 1.65–1.54 (m, 6H), 1.46 (s, 3H), 1.41 (s, 3H), 1.41–1.35 (m, 4H), 1.29–1.21 (m, 40H) 0.91 (t,  $J = 7.5$  Hz, 3H), 0.88 (t,  $J = 6.9$  Hz, 6H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  173.4, 172.6, 172.3, 130.1, 129.7, 128.4, 128.1, 128.0, 127.8, 99.5, 68.8, 68.3, 65.1, 62.1, 41.9, 36.7, 34.1, 33.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.9, 25.6, 25.5, 24.9, 24.2, 22.8, 22.7, 18.3, 14.1, 13.8; IR  $\nu_{\text{max}}$  2922, 2853, 1744, 1679, 1501, 1462, 1381, 1236, 1199, 1142, 1093, 843, 721  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{52}H_{94}O_7N^+$ , 844.7025; found, 844.7018.

**(R)-2-((4S,5R)-5-Dodecanamido-2,2-dimethyl-1,3-dioxan-4-yl)-2-(((4Z,7Z)-undeca-4,7-dienoyl)oxy)ethyl tetradecanoate (19b).** Analogous to **19a**, ester **19b** (658 mg, 0.88 mmol, 88%) was prepared as colorless oil from alcohol **9b** (584 mg, 1.00 mmol), DMAP (245 mg, 2.00 mmol), EDC-HCl (230 mg, 1.20 mmol), and acid **18** (182 mg, 1.00 mmol);  $R_f = 0.37$  (*n*-hexane/ethyl acetate 2:1);  $[\alpha]_D^{25} +10.7$  (c 1.0,  $CHCl_3$ );  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  6.09 (d,  $J = 9.8$  Hz, 1H), 5.45–5.29 (m, 4H), 4.94 (ddd,  $J = 2.1, 4.3, 9.8$  Hz, 1H), 4.37 (dd,  $J = 2.1, 12.2$  Hz, 1H), 4.20 (dd,  $J = 2.0, 9.8$  Hz, 1H), 4.14–4.07 (m, 3H), 3.71 (dd,  $J = 1.8, 12.2$  Hz, 1H), 2.80 (t,  $J = 6.0$  Hz, 2H), 2.45–2.32 (m, 4H), 2.30 (dt,  $J = 1.5, 7.5$  Hz, 2H), 2.23–2.11 (m, 2H), 2.06–2.00 (m, 2H), 1.64–1.55 (m, 4H), 1.46 (s, 3H), 1.41 (s, 3H), 1.40–1.34 (m, 2H), 1.32–1.22 (m, 36H) 0.90 (t,  $J = 7.3$  Hz, 3H), 0.87 (t,  $J = 7.0$  Hz, 6H);  $^{13}C\{^1H\}$  NMR ( $CDCl_3$ , 126 MHz):  $\delta$  173.4, 172.6, 171.9, 130.2, 129.4, 127.8, 99.5, 68.8, 68.4, 65.1, 62.1, 41.9, 36.7, 34.11, 33.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 25.6, 25.5, 24.9, 22.8, 22.7, 22.4, 18.3, 14.1, 13.8; IR  $\nu_{\text{max}}$  2923, 2853, 1744, 1677, 1505, 1464, 1380, 1236, 1199, 1147, 1086, 843, 721  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[M + H]^+$  calcd for  $C_{45}H_{82}O_7N^+$ , 748.6086; found, 748.6088.

**Bathymodiolamide A (1).** A solution of **19a** (250 mg, 0.30 mmol) in 5 mL MeOH was treated with pTsOH (25 mg, 0.15 mmol) and stirred for 2.5 h at ambient temperature. Volatiles were removed under reduced pressure, and the crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1) to give **1** (223 mg, 0.28 mmol, 94%) as a white solid; mp 46–47 °C;  $R_f = 0.39$  (*n*-hexane/ethyl acetate 1:1);  $[\alpha]_D^{25} +14.8$  (c 0.08, MeOH)  $[\text{lit}^1: [\alpha]_D^{25} +10.8$  (c 0.08, MeOH)];  $^1H$  NMR ( $CD_3OD$ , 500 MHz):  $\delta$  5.43–5.31 (m, 6H), 4.92–4.89 (m, 1H), 4.62 (dd,  $J = 2.3, 12.1$  Hz, 1H), 4.12 (dd,  $J = 5.8, 12.1$  Hz, 1H), 4.12–4.09 (m, 1H), 4.04 (dd,  $J = 1.5, 9.5$  Hz, 1H), 3.61 (dd,  $J = 8.2, 10.7$  Hz, 1H), 3.53 (dd,  $J = 5.8, 10.7$  Hz, 1H),

2.84–2.82 (m, 4H), 2.38–2.27 (m, 4H), 2.22–2.15 (m, 2H), 2.14–2.02 (m, 4H), 1.66–1.55 (m, 6H), 1.45–1.36 (m, 4H), 1.36–1.24 (m, 40H), 0.93 (t,  $J = 7.3$  Hz, 3H), 0.90 (t,  $J = 7.3$  Hz, 6H);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CD}_3\text{OD}$ , 126 MHz):  $\delta$  176.5, 175.3, 174.1, 131.1, 130.9, 129.5, 129.4, 129.3, 129.2, 71.8, 68.2, 64.3, 62.5, 51.9, 37.3, 35.2, 33.3, 31.0, 30.8, 30.7, 30.6, 30.5, 30.4, 28.2, 27.2, 26.8, 26.2, 25.4, 24.0, 23.9, 14.7, 14.4; IR  $\nu_{\text{max}}$  3320, 2922, 2853, 1744, 1633, 1457, 1377, 1173, 1057, 720  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{49}\text{H}_{90}\text{O}_7\text{N}^+$ , 804.6712; found, 804.6703.

(2*R*,3*S*,4*R*)-Bathymodiolamide B (2). Analogous to 1, bathymodiolamide B (184 mg, 0.26 mmol, 97%) was prepared as a white solid from acetonide 19b (200 mg, 0.27 mmol) and pTsOH (23 mg, 0.14 mmol); mp 43–44 °C;  $R_f = 0.3$  (*n*-hexane/ethyl acetate 1:1);  $[\alpha]_{\text{D}}^{23} +6.7$  (c 0.08, MeOH) {lit<sup>1</sup>:  $[\alpha]_{\text{D}}^{23} +10.8$  (c 0.08, MeOH)};  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz):  $\delta$  5.41–5.32 (m, 4H), 4.93–4.89 (m, 1H), 4.60 (dd,  $J = 2.4, 12.2$  Hz, 1H), 4.14 (dd,  $J = 5.8, 12.2$  Hz, 1H), 4.12–4.09 (m, 1H), 4.05 (dd,  $J = 1.5, 9.5$  Hz, 1H), 3.61 (dd,  $J = 8.5, 10.7$  Hz, 1H), 3.53 (dd,  $J = 5.8, 10.7$  Hz, 1H), 2.84–2.42 (m, 2H), 2.42–2.32 (m, 4H), 2.30 (t,  $J = 7.3$  Hz, 2H), 2.24–2.12 (m, 2H), 2.07 (q,  $J = 6.7$  Hz, 2H), 1.61–1.57 (m, 4H), 1.44–1.36 (m, 2H), 1.36–1.24 (m, 36H), 0.93 (t,  $J = 7.3$  Hz, 3H), 0.90 (t,  $J = 7.3$  Hz, 9H);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CD}_3\text{OD}$ , 126 MHz):  $\delta$  176.5, 175.3, 173.6, 131.1, 130.5, 129.2, 71.9, 68.2, 62.5, 51.9, 37.3, 35.3, 35.2, 33.3, 31.0, 30.8, 30.7, 30.6, 30.5, 30.4, 27.2, 26.7, 26.2, 24.1, 23.9, 23.8, 14.7, 14.4; IR  $\nu_{\text{max}}$  3363, 2920, 2853, 1745, 1640, 1457, 1377, 1152, 1061, 722  $\text{cm}^{-1}$ ; HRMS (ESI)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{42}\text{H}_{78}\text{O}_7\text{N}^+$ , 708.5772; found, 708.5765.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.joc.0c02726>.

Comparison of synthetic and isolated bathymodiolamides A and B; analysis of the Mosher esters of alcohol 4; NMR spectra of all compounds; and cell culture conditions and inhibition of cell growth (PDF)

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### Notes

The authors declare no competing financial interest.

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**Supporting information****Synthesis of the proposed isomers of the deep-sea mussel metabolites bathymodiolamides A and B**

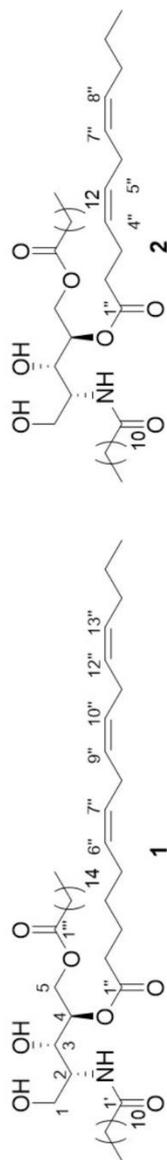
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**Comparison of synthetic and isolated bathymodiolamides A (1) and B (2)**

**Table 1.** Comparison of  $^{13}\text{C}\{^1\text{H}\}$  and  $^1\text{H}$  NMR shifts of isolated and synthetic Bathymodiolamides A (1) and B (2)

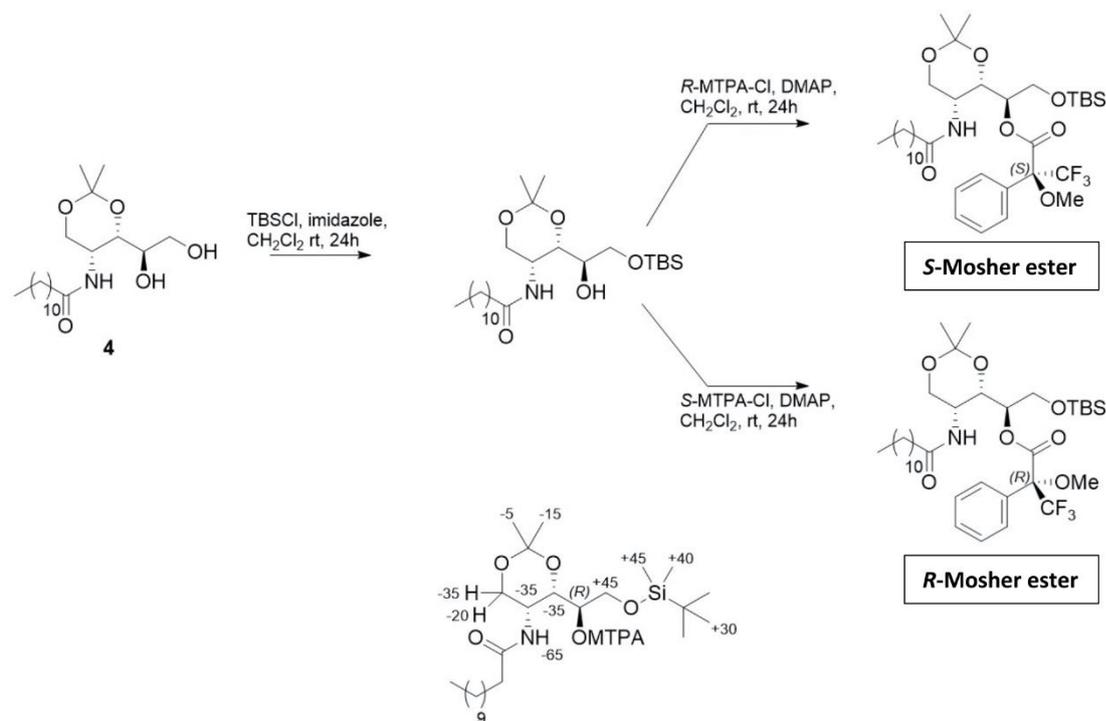


Pos.	natural 1 in $\text{CD}_3\text{OD}^1$		synthetic 1 in $\text{CD}_3\text{OD}$		natural 2 in $\text{CD}_3\text{OD}^1$		synthetic 2 in $\text{CD}_3\text{OD}$	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., J [Hz])						
1a	63.7	3.64 (d, 4.5)	62.5	3.61 (dd, 10.7, 8.2)	63.7	3.65 (d, 4.5)	62.5	3.61 (dd, 10.7, 8.5)
1b		3.65 (d, 4.5)		3.53 (dd, 10.7, 5.8)		3.65 (d, 4.5)		3.53 (dd, 10.7, 5.8)
2	53.3	4.29 (dd, 4.5, 2)	51.9	4.12-4.09 (m)	53.5	4.29 (dd, 4.5, 2)	51.9	4.12-4.09 (m)
3	62.5	4.01 (dd, 6.5, 2)	68.2	4.04 (dd (9.5, 1.5)	62.5	4.01 (dd, 6.5, 2)	68.2	4.05 (dd (9.5, 1.5)
4	66.1	5.25 (dt, 6.5, 3)	71.8	4.92-4.89 (m)	66.1	5.25 (dt, 6.5, 3)	71.9	4.93-4.89 (m)
5a	59.9	4.20 (dd, 12, 3)	64.2	4.12 (dd (12.1, 5.8)	59.9	4.20 (dd, 12, 3)	64.2	4.14 (dd, 12.2, 5.8)
5b		4.45 (dd, 12, 3)		4.62 (dd, 12.1, 2.3)		4.45 (dd, 12, 3)		4.60 (dd, 12.2, 2.4)
1'	173.2		176.5		173.2		176.5	
2'	30.0	2.31 (t, 7.5)	33.3	2.22-2.15 (m)	30.0	2.31 (t, 7.5)	37.3	2.24-2.13 (m)
3'	29.9	1.29 (brs)	31.0	1.68-1.55 (m)	29.9	1.29 (brs)	26.2	1.61-1.57 (m)
4'	29.8	1.29 (brs)	31.0	1.36-1.25 (m)	29.8	1.29 (brs)	30.8	1.35-1.26 (m)
5'	29.8	1.29 (brs)	31.0	1.36-1.25 (m)	29.8	1.29 (brs)	30.8	1.35-1.26 (m)
6'	29.8	1.29 (brs)	31.0	1.36-1.25 (m)	29.8	1.29 (brs)	30.8	1.35-1.26 (m)
7'	29.7	1.29 (brs)	30.8	1.36-1.25 (m)	29.7	1.29 (brs)	30.7	1.35-1.26 (m)
8'	29.7	1.29 (brs)	30.8	1.36-1.25 (m)	29.7	1.29 (brs)	30.6	1.35-1.26 (m)
9'	29.5	1.29 (brs)	30.5	1.36-1.25 (m)	29.5	1.29 (brs)	30.6	1.35-1.26 (m)
10'	29.5	1.29 (brs)	30.5	1.36-1.25 (m)	29.5	1.29 (brs)	30.5	1.35-1.26 (m)
11'	28.9	1.29 (brs)	23.9	1.36-1.25 (m)	28.9	1.29 (brs)	30.4	1.35-1.26 (m)
12'	13.2	0.89 (t, 7.0)	14.7	0.90 (t, 7.3)	13.2	0.89 (t, 7.0)	14.7	0.90 (t, 7.3)
1''	173.4		174.1		173.4		173.6	
2''	30.1	2.35 (t, 7.2)	35.2	2.38-2.37 (m)	30.1	2.35 (t, 7.2)	35.3	2.42-2.32 (m)

3''	22.5	1.68 (m)	30.4	1.68-1.55 (m)	24.5	2.08 (m)	23.8	2.42-2.32 (m)
4''	27.5	1.33 (m)	28.2	1.45-1.36 (m)	127.5	5.38 (m)	131.1	5.41-5.32 (m)
5''	24.5	2.08 (m)	26.2	2.09-2.02 (m)	128.1	5.36 (m)	131.1	5.41-5.32 (m)
6''	129.5	5.38 (m)	131.1	5.42-5.32 (m)	26.5	2.81 (m)	26.7	2.84-2.82 (m)
7''	127.8	5.36 (m)	129.3	5.42-5.32 (m)	127.9	5.36 (m)	130.5	5.41-5.32 (m)
8''	26.5	2.81 (m)	27.1	2.84-2.82 (m)	128.1	5.36 (m)	129.2	5.41-5.32 (m)
9''	127.9	5.36 (m)	129.5	5.42-5.32 (m)	26.5	2.01 (m)	30.4	2.07 (q, 6.7)
10''	128.1	5.36 (m)	129.4	5.42-5.32 (m)	22.5	1.40 (m)	23.9	1.44-1.36 (m)
11''	26.5	2.81 (m)	26.8	2.84-2.82 (m)	13.1	0.91 (t, 7.5)	14.4	0.93 (t, 7.3)
12''	128.0	5.36 (m)	129.2	5.42-5.32 (m)				
13''	128.4	5.36 (m)	130.9	5.42-5.32 (m)				
14''	25.5	2.01 (m)	25.6	2.09-2.02 (m)				
15''	22.5	1.40 (m)	24.1	1.45-1.36 (m)				
16''	13.1	0.91 (m)	14.4	0.93 (t, 7.3)				
1'''	173.8		175.3		173.8		175.3	
2'''	30.2	2.36 (t, 7.5)	37.3	2.38-2.37 (m)	30.2	2.36 (t, 7.5)	35.3	2.30 (t, 7.3)
3'''	29.9	1.29 (brs)	31.0	1.68-1.55 (m)	29.9	1.29 (brs)	27.2	1.61-1.57 (m)
4'''	29.8	1.29 (brs)	31.0	1.36-1.25 (m)	29.8	1.29 (brs)	30.8	1.35-1.26 (m)
5'''	29.8	1.29 (brs)	31.0	1.36-1.25 (m)	29.8	1.29 (brs)	30.8	1.35-1.26 (m)
6'''	29.8	1.29 (brs)	30.8	1.36-1.25 (m)	29.8	1.29 (brs)	30.8	1.35-1.26 (m)
7'''	29.7	1.29 (brs)	30.8	1.36-1.25 (m)	29.7	1.29 (brs)	30.8	1.35-1.26 (m)
8'''	29.7	1.29 (brs)	30.7	1.36-1.25 (m)	29.7	1.29 (brs)	30.7	1.35-1.26 (m)
9'''	29.6	1.29 (brs)	30.6	1.36-1.25 (m)	29.6	1.29 (brs)	30.7	1.35-1.26 (m)
10'''	29.6	1.29 (brs)	30.6	1.36-1.25 (m)	29.6	1.29 (brs)	30.6	1.35-1.26 (m)
11'''	29.5	1.29 (brs)	30.5	1.36-1.25 (m)	29.5	1.29 (brs)	30.5	1.35-1.26 (m)
12'''	29.5	1.29 (brs)	30.5	1.36-1.25 (m)	29.5	1.29 (brs)	30.4	1.35-1.26 (m)
13'''	29.5	1.29 (brs)	30.5	1.36-1.25 (m)	28.4	1.29 (brs)	30.4	1.35-1.26 (m)
14'''	29.4	1.29 (brs)	30.5	1.36-1.25 (m)	13.0	0.90 (t, 7.5)	14.7	0.90 (t, 7.3)
15'''	28.9	1.29 (brs)	23.9	1.36-1.25 (m)				
16'''	13.0	0.90 (t, 7.5)	14.7	0.90 (t, 7.3)				

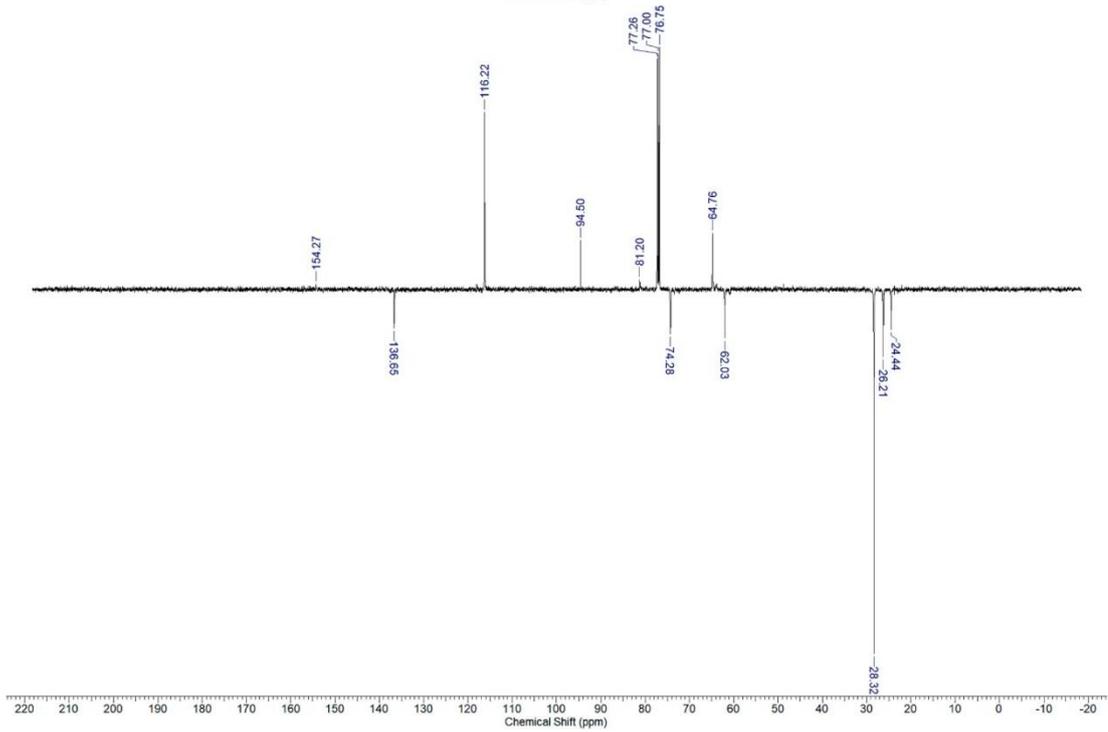
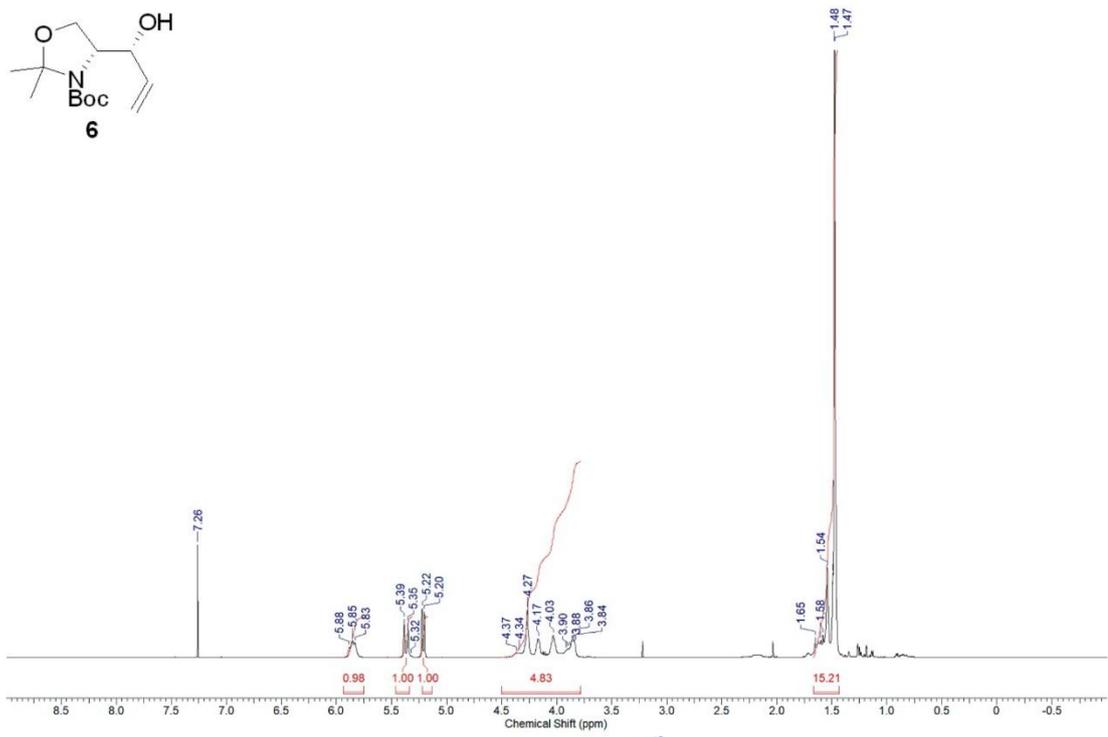
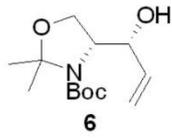
**Analysis of the Mosher esters of alcohol 4 (according to literature<sup>2</sup>)**

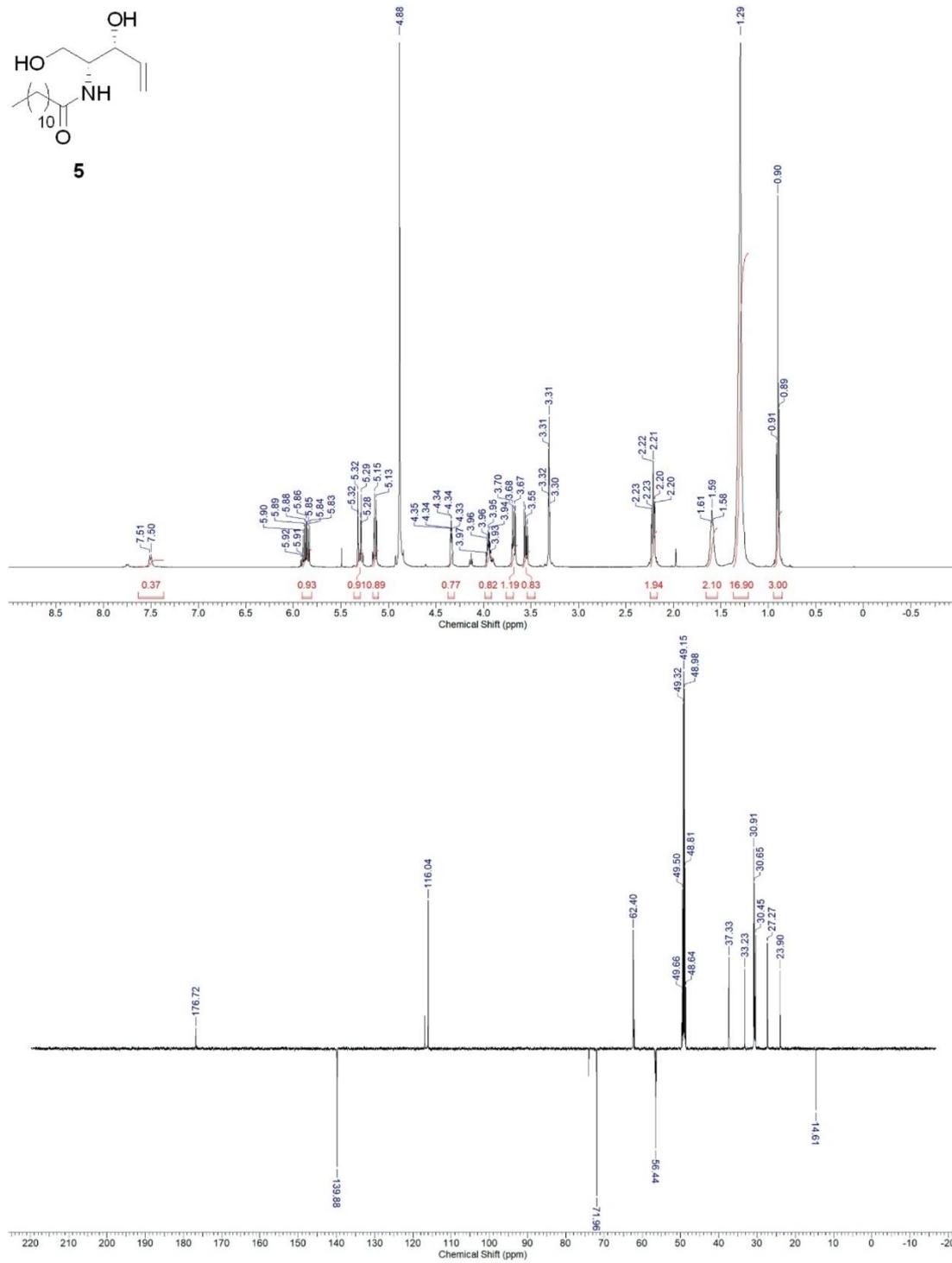
**Scheme 1.** Synthesis of the TBS-ether of alcohol 4 and of the *S*- and *R*-Mosher esters as well as  $\Delta\delta_{SR} = \delta_S - \delta_R$  values in Hz calculated from their <sup>1</sup>H NMR spectra.

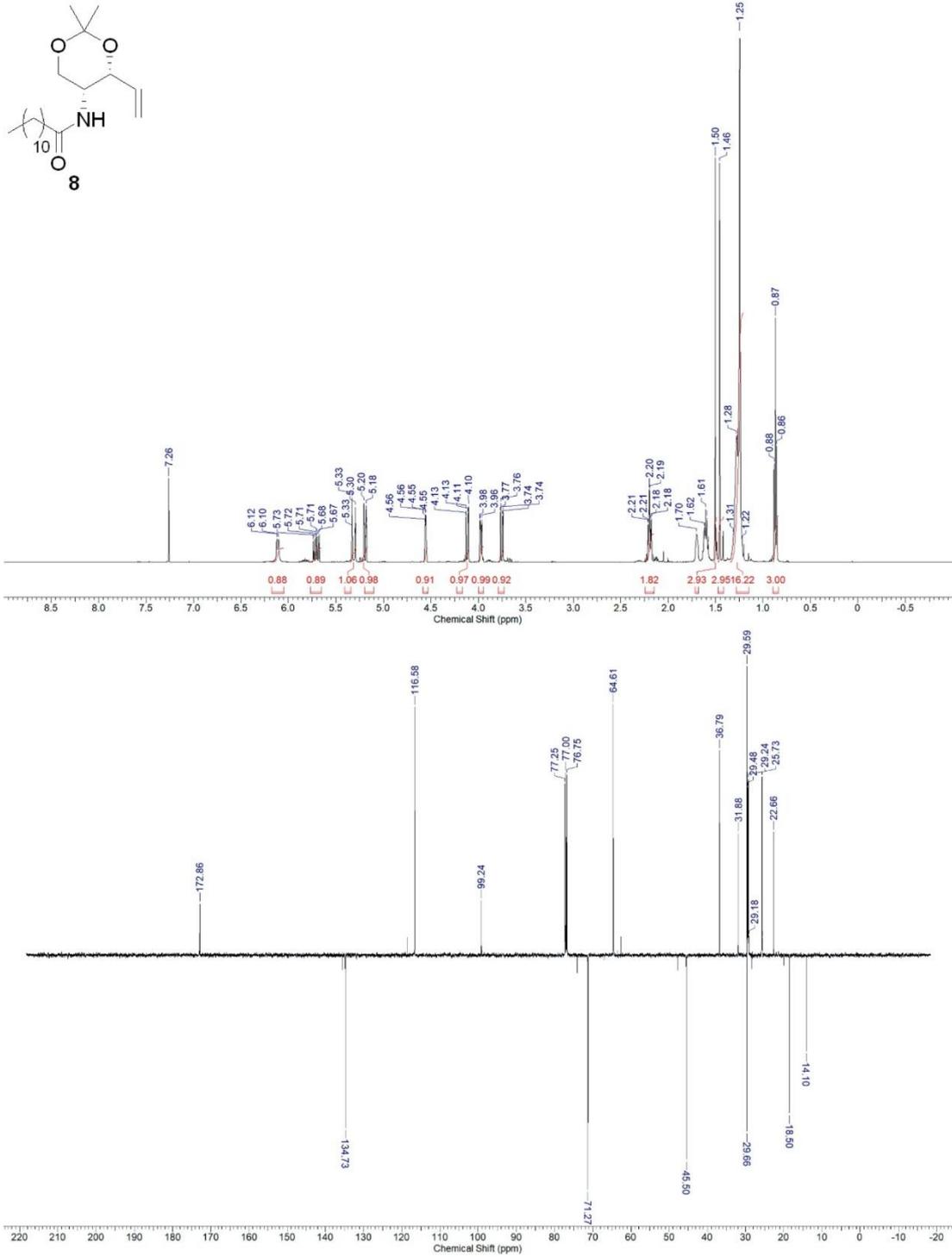


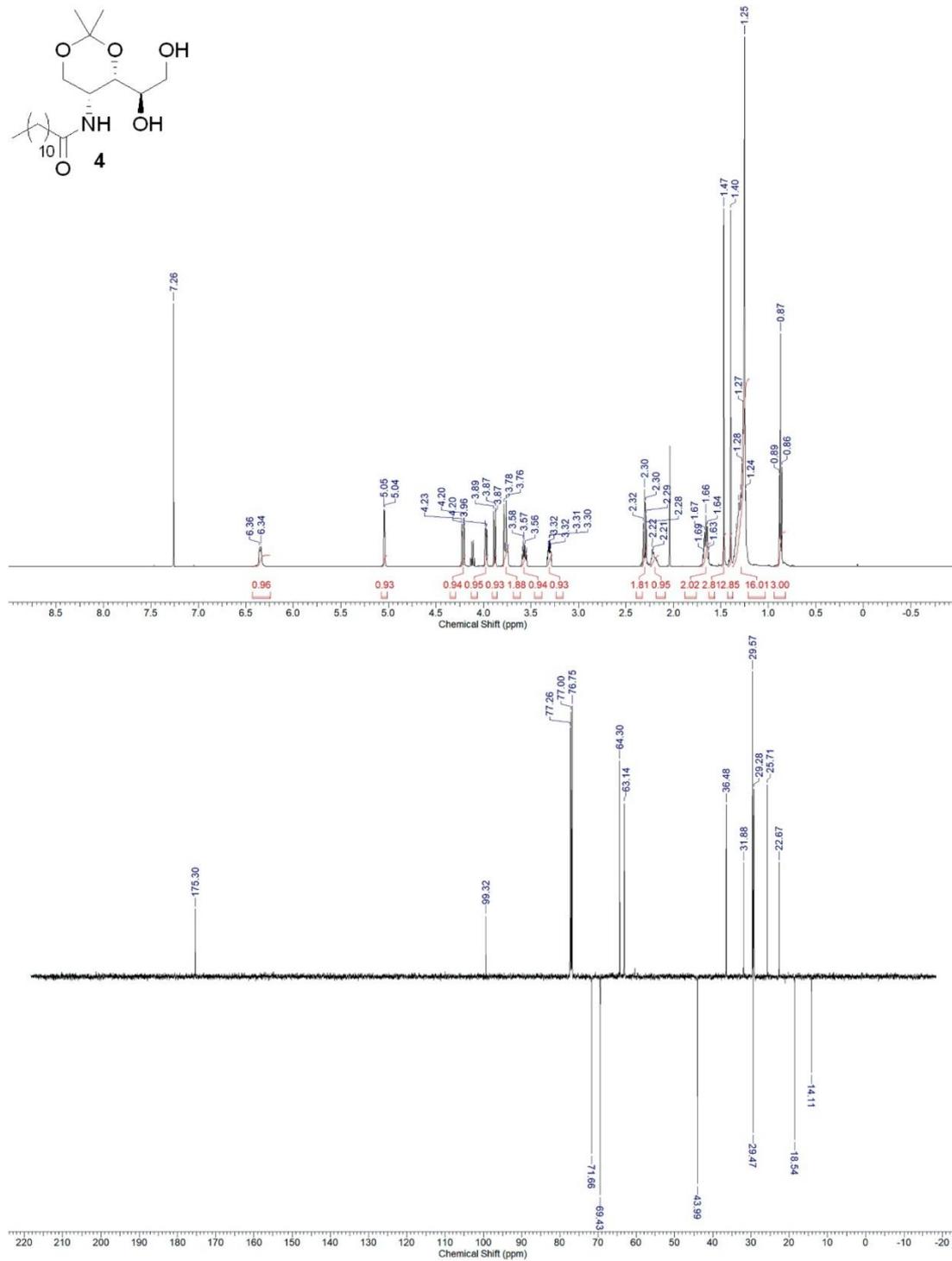
position	$\delta_S$ (in ppm)	$\delta_R$ (in ppm)	$\Delta\delta_{SR}$ (in Hz)
4	4.32	4.39	-35
5	3.88	3.95	-35
6a	3.97	4.01	-20
6b	3.73	3.80	-35
2-CH <sub>3a</sub>	1.46	1.47	-5
2-CH <sub>3b</sub>	1.40	1.43	-15
NH	6.00	6.13	-65
1'	5.20	5.14	
2'	3.82	3.73	+45
Si-CH <sub>3a</sub>	0.07	-0.01	+40
SiCH <sub>3b</sub>	0.06	-0.03	+45
SiC(CH <sub>3</sub> ) <sub>3</sub>	0.89	0.83	+30

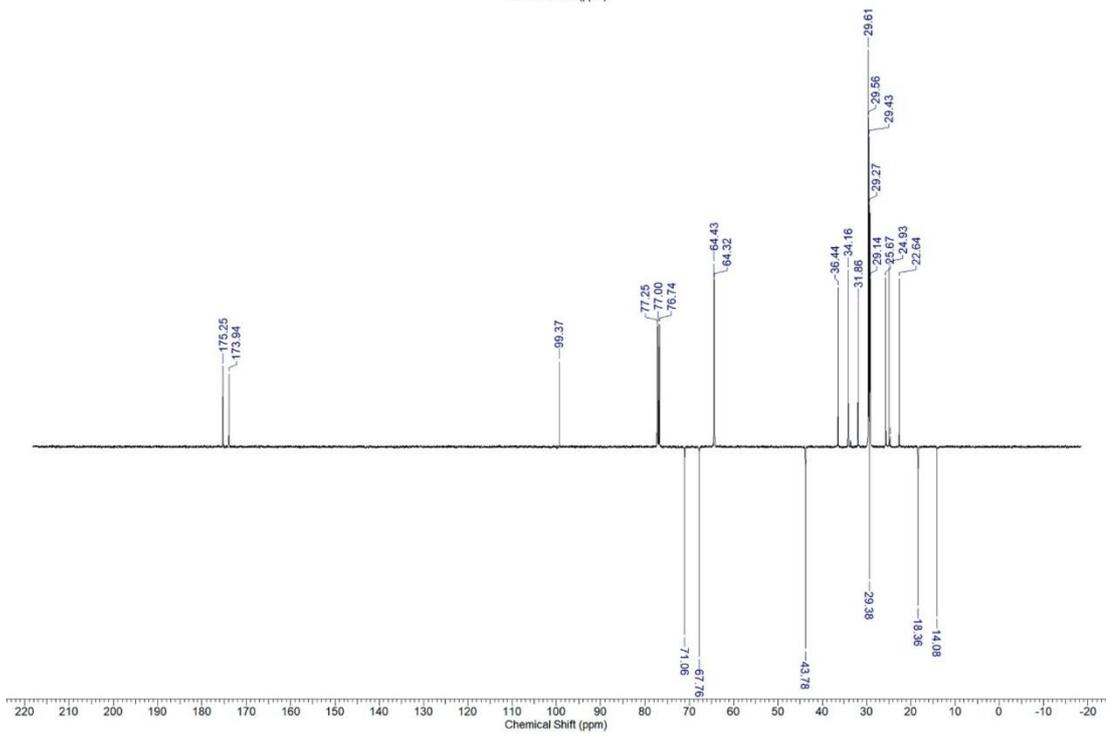
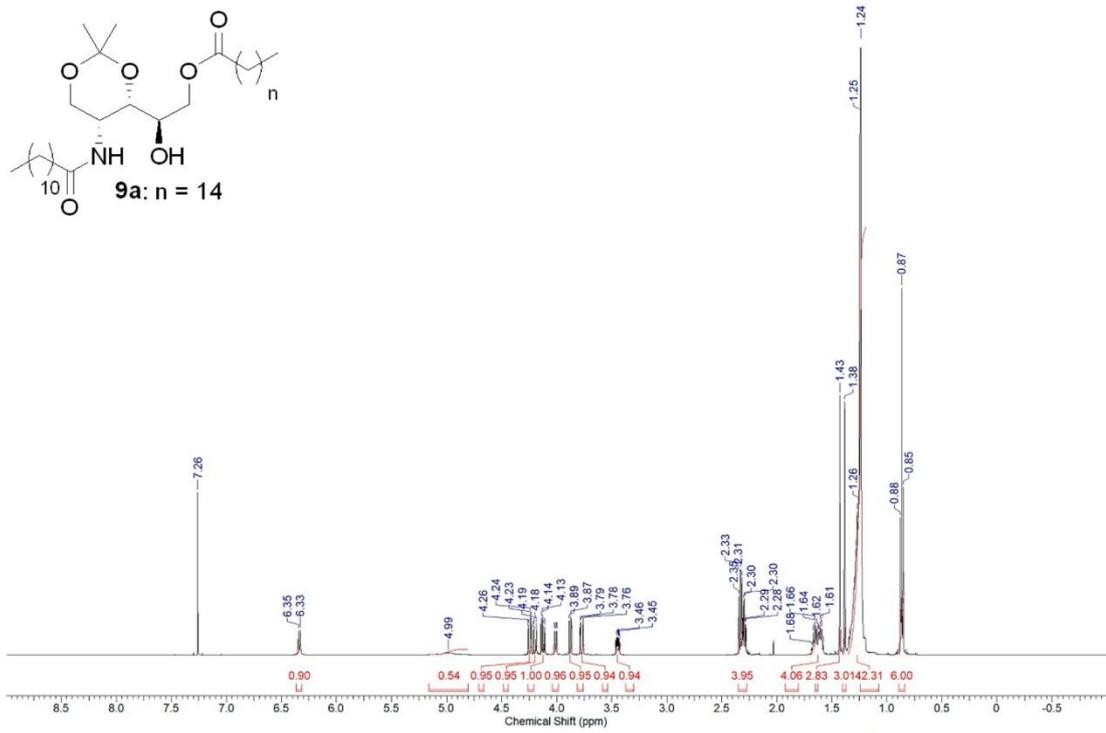
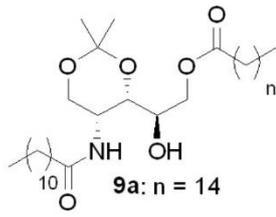
$^1\text{H}$  and  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra

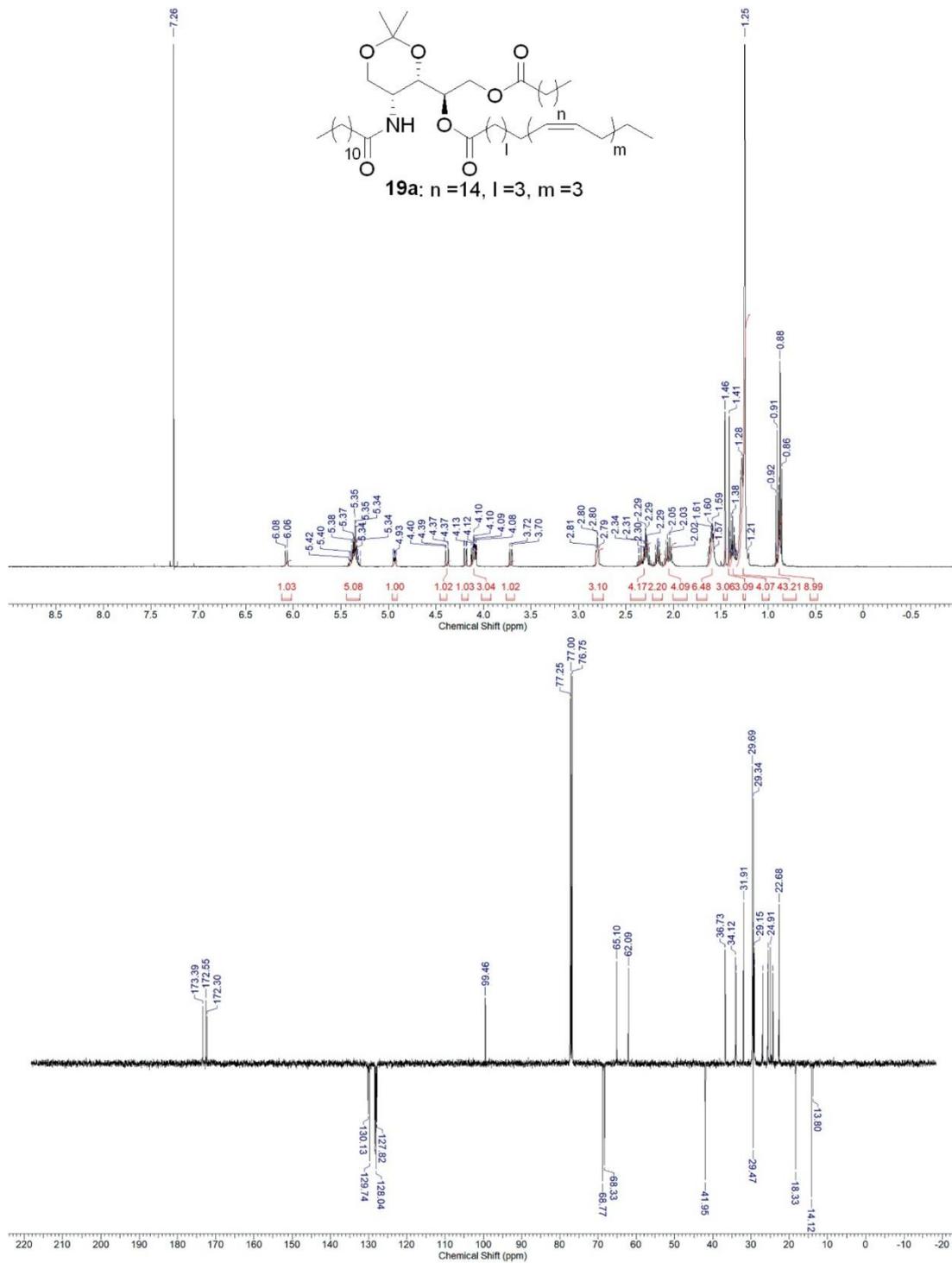


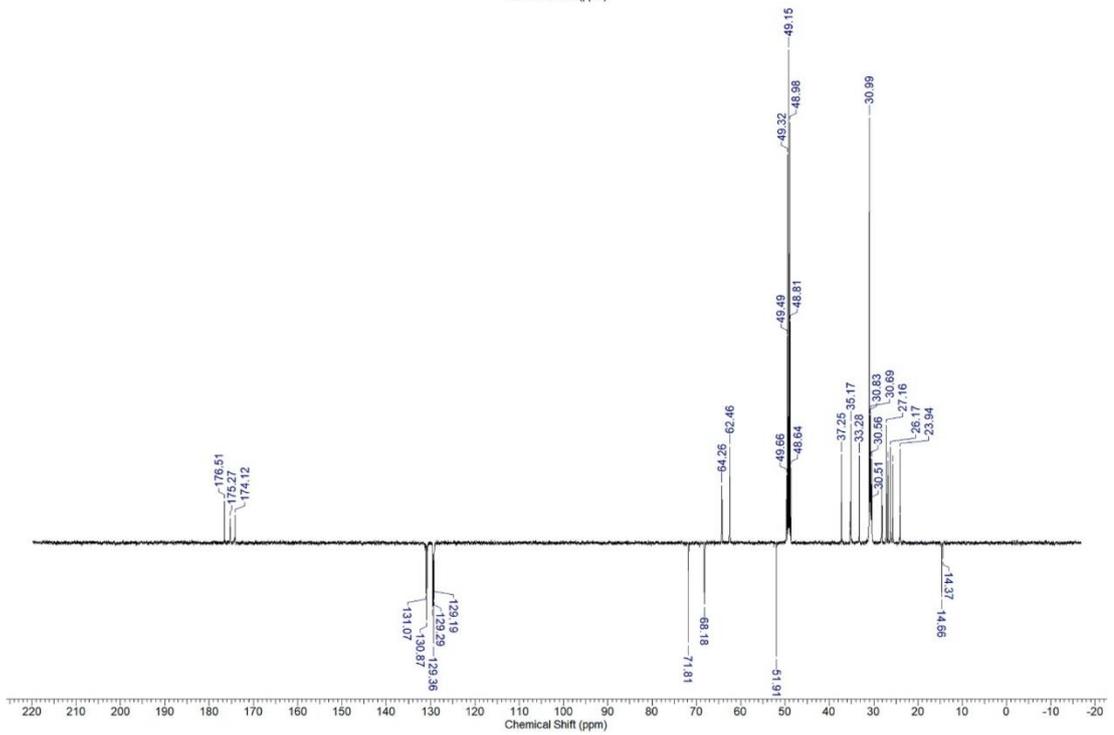
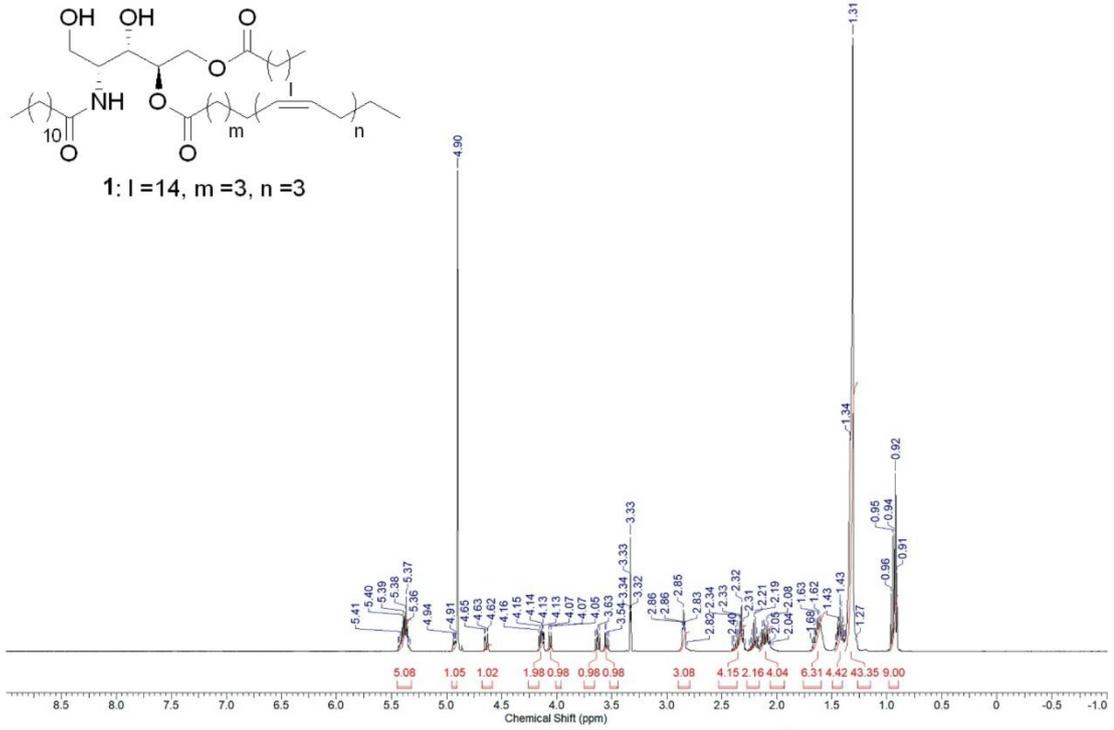
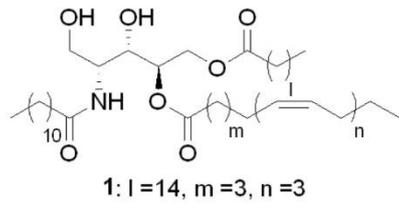


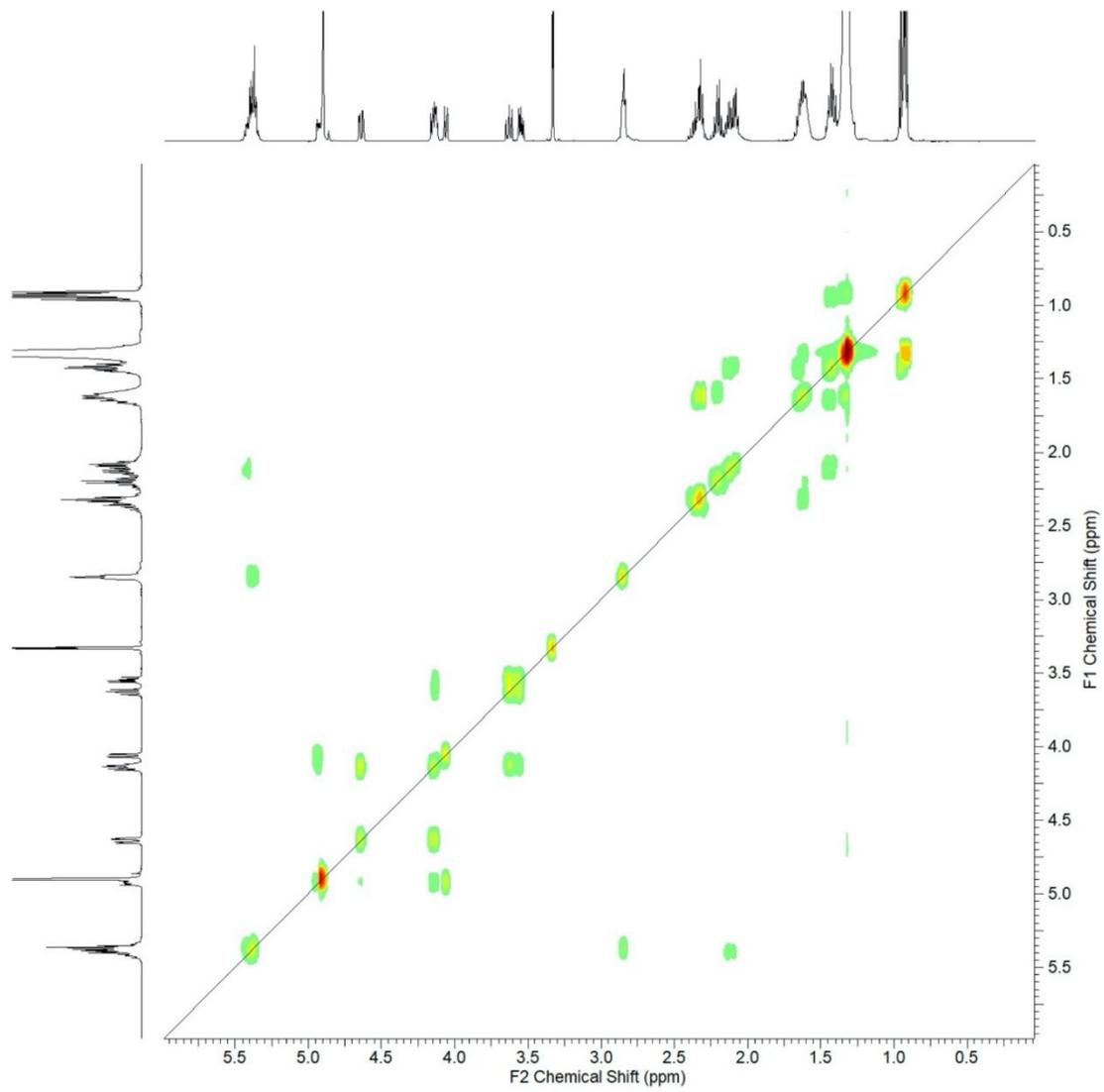




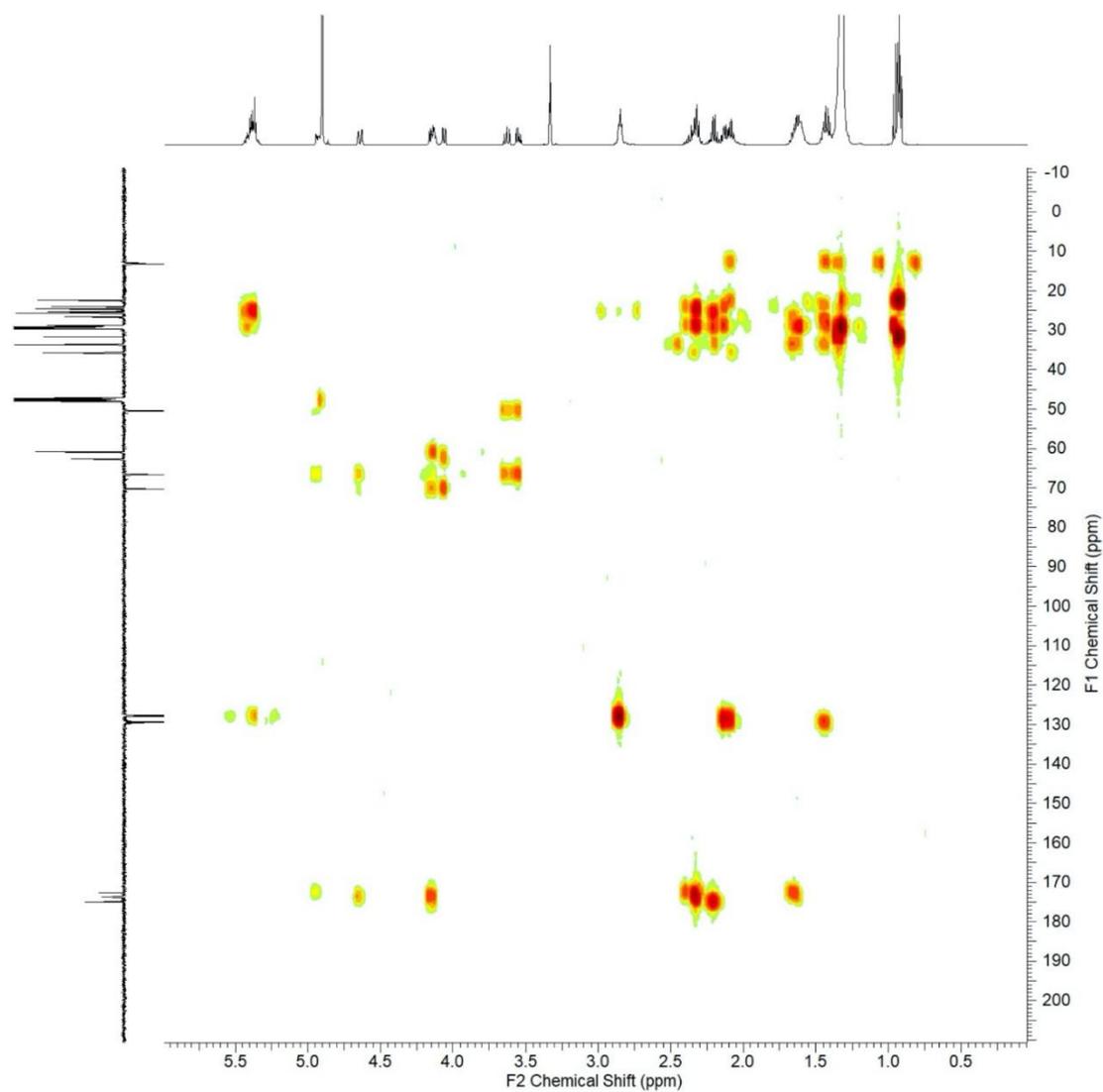




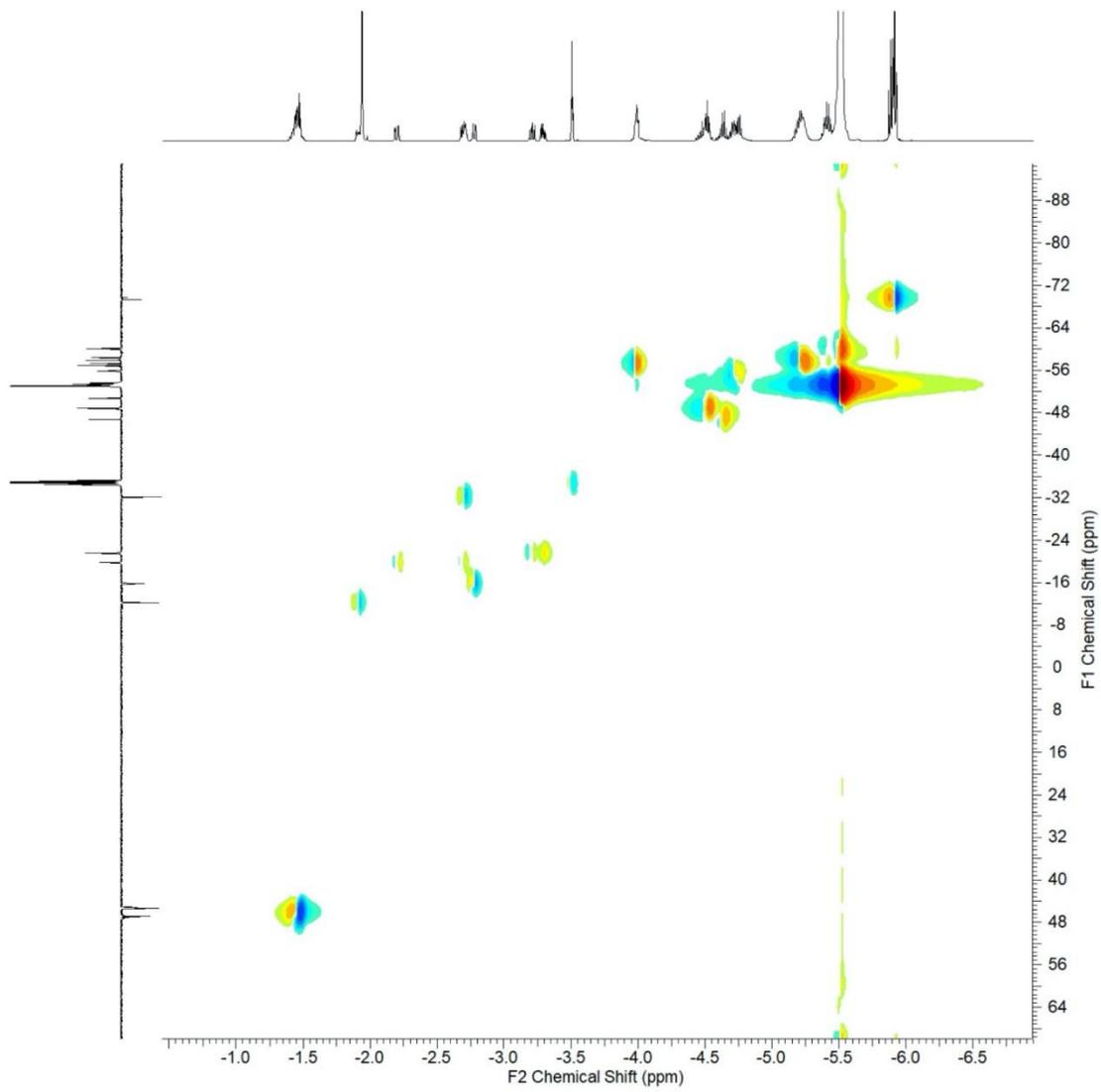


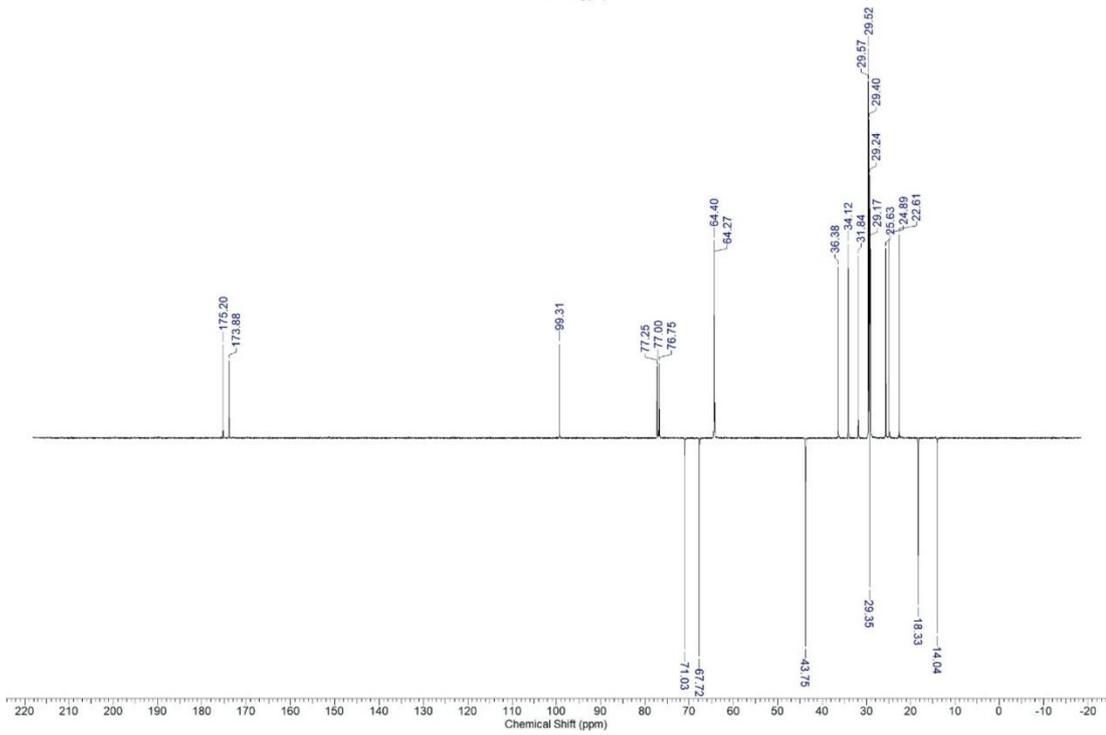
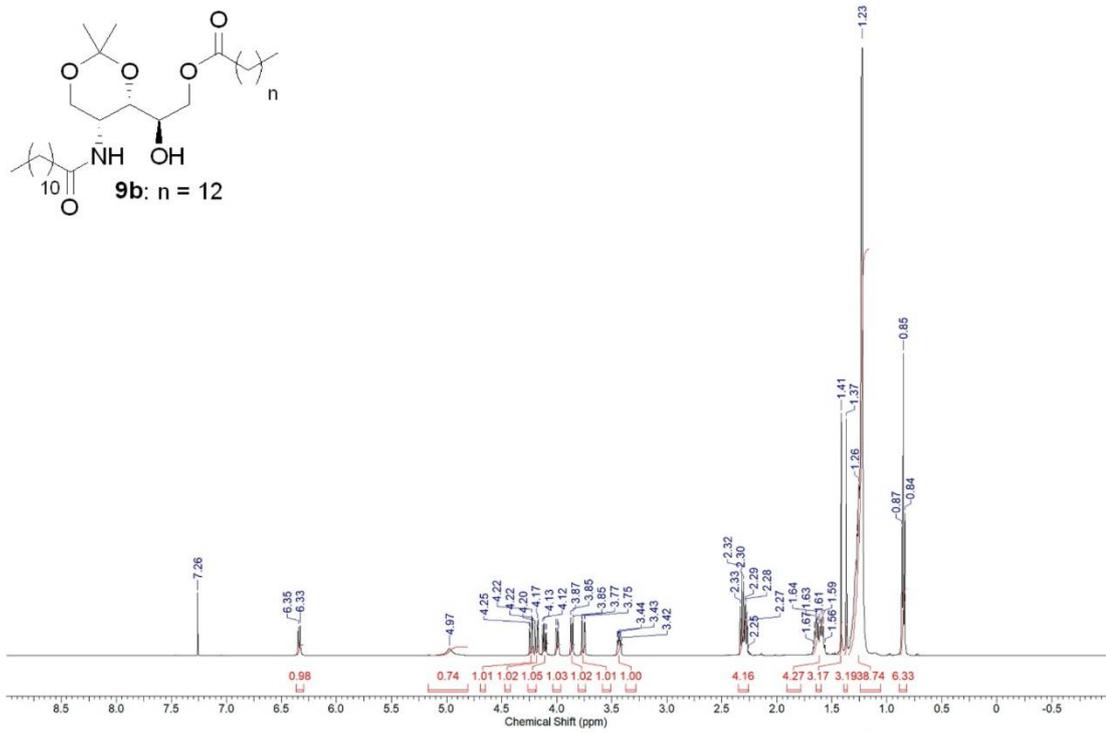
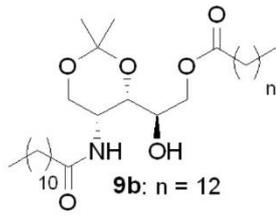
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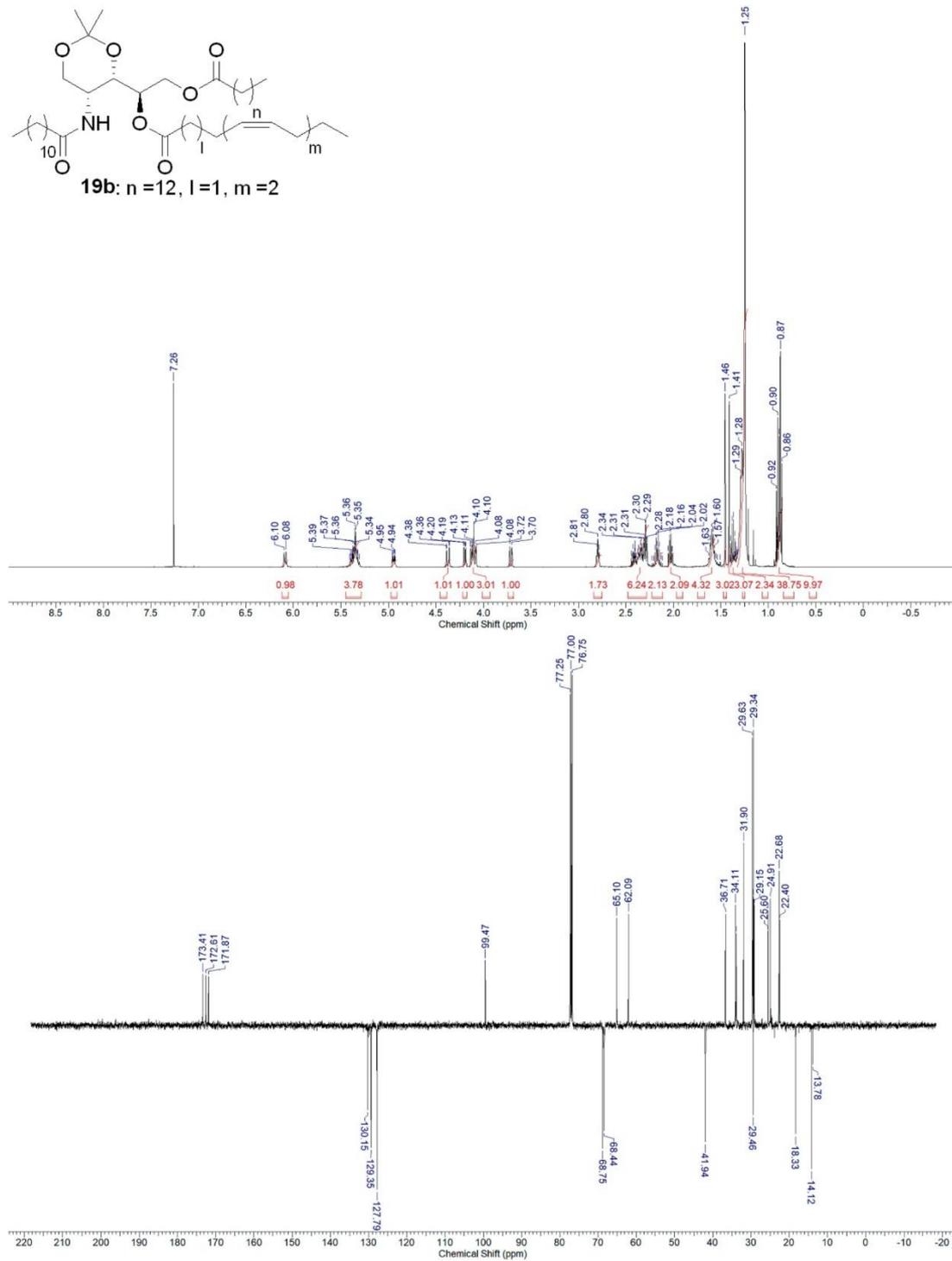
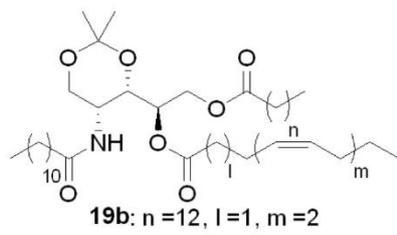
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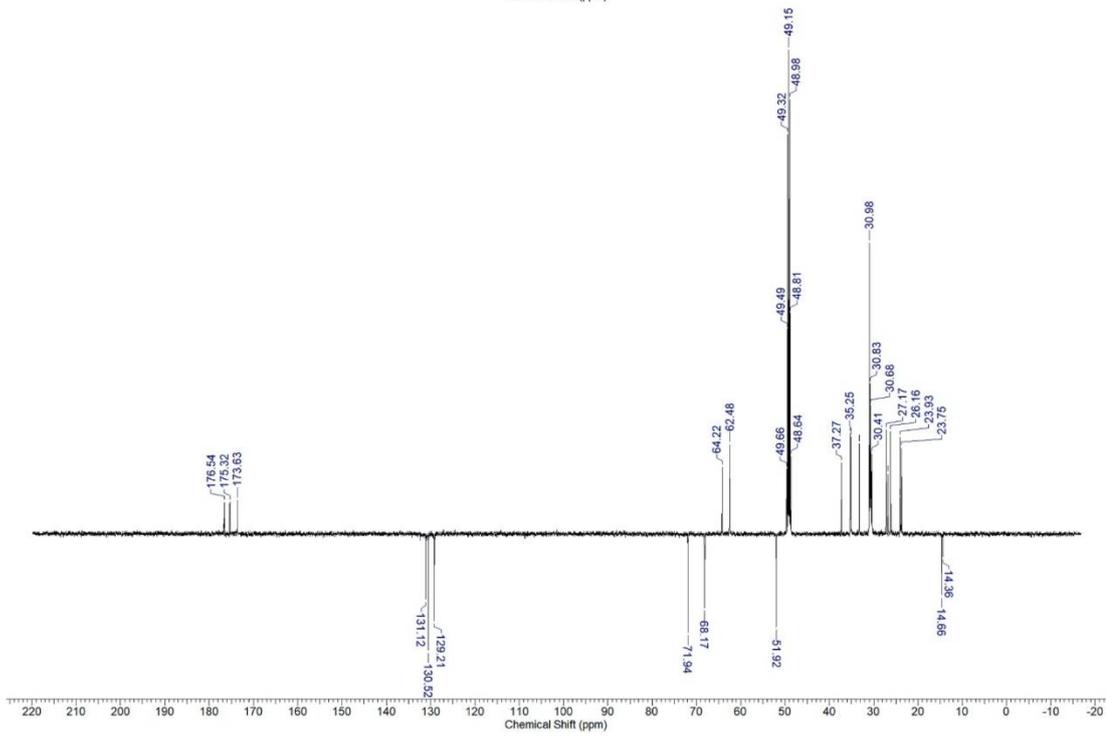
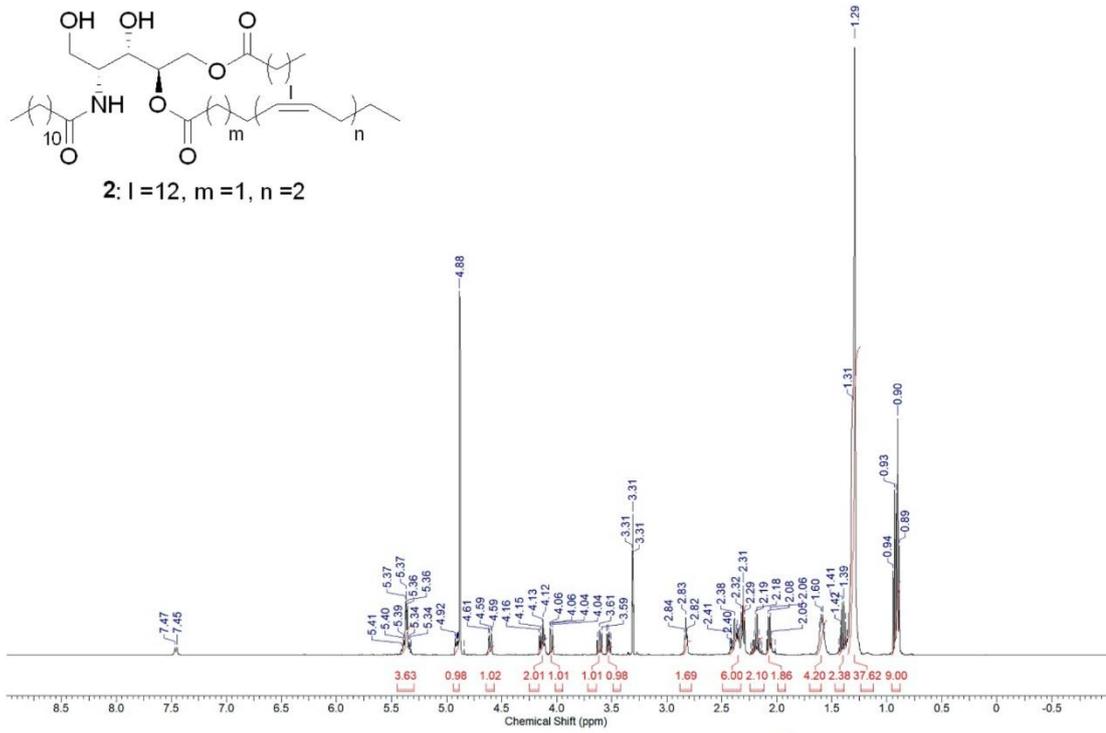
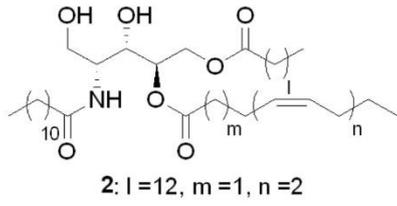


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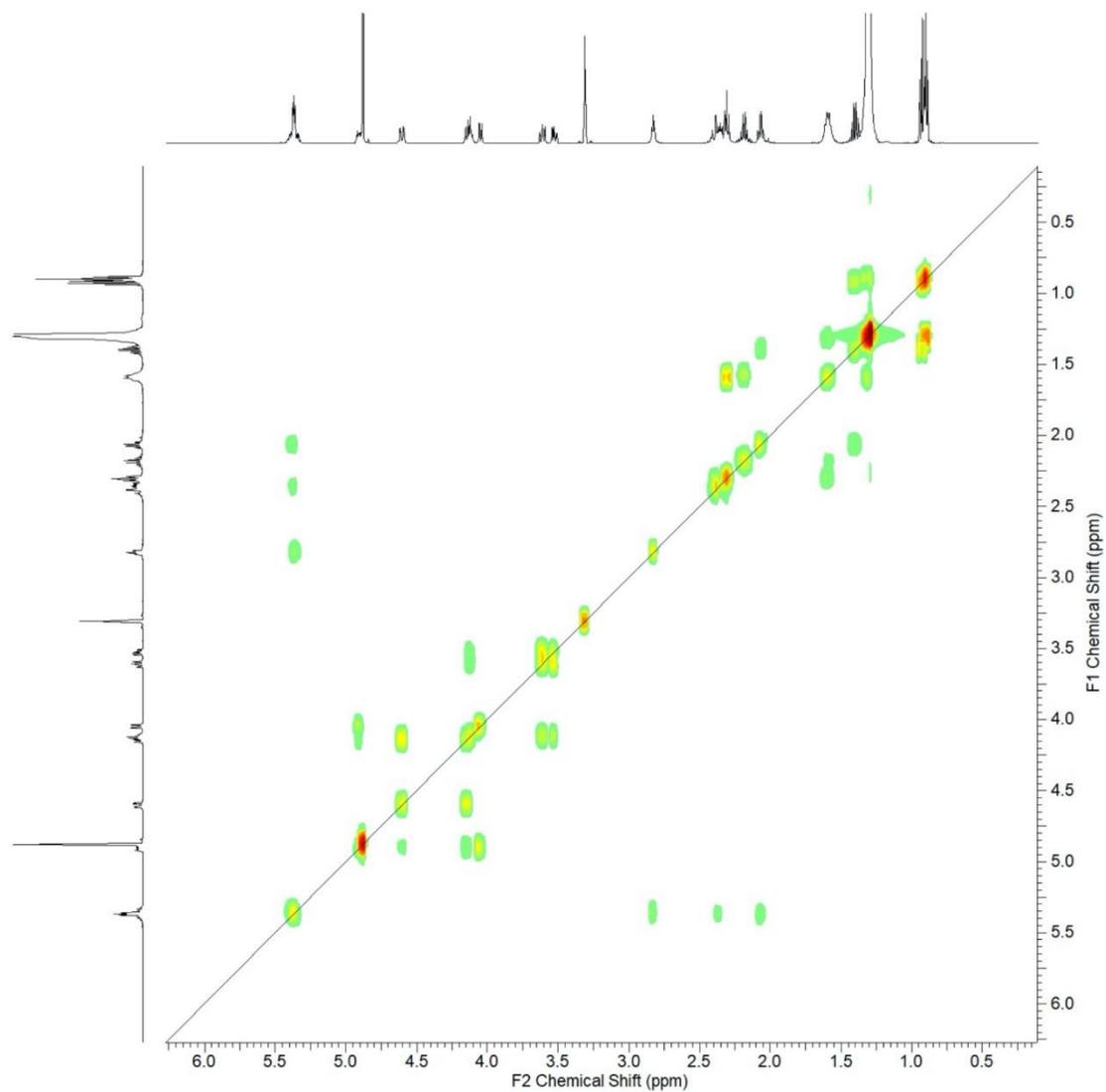




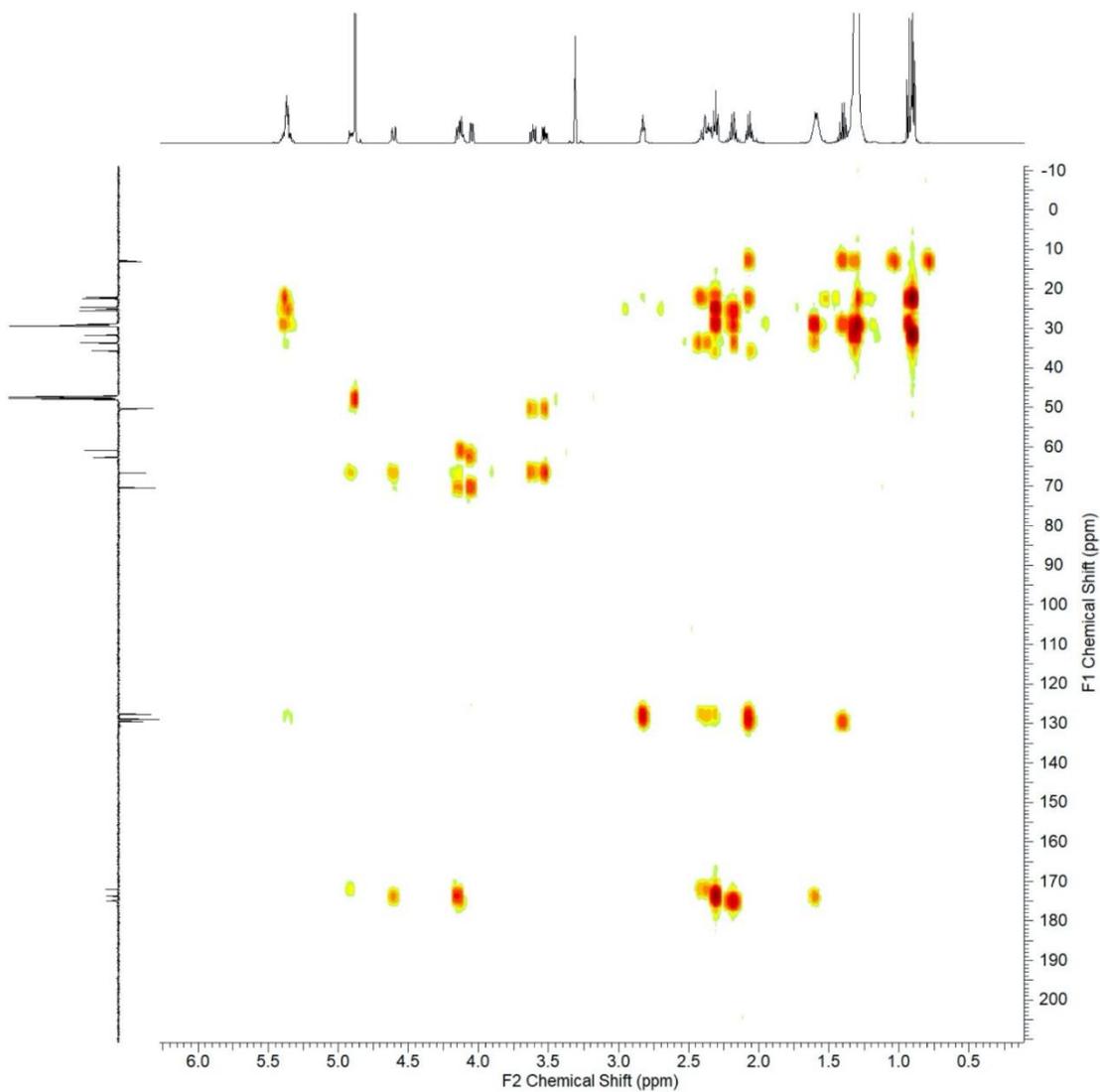




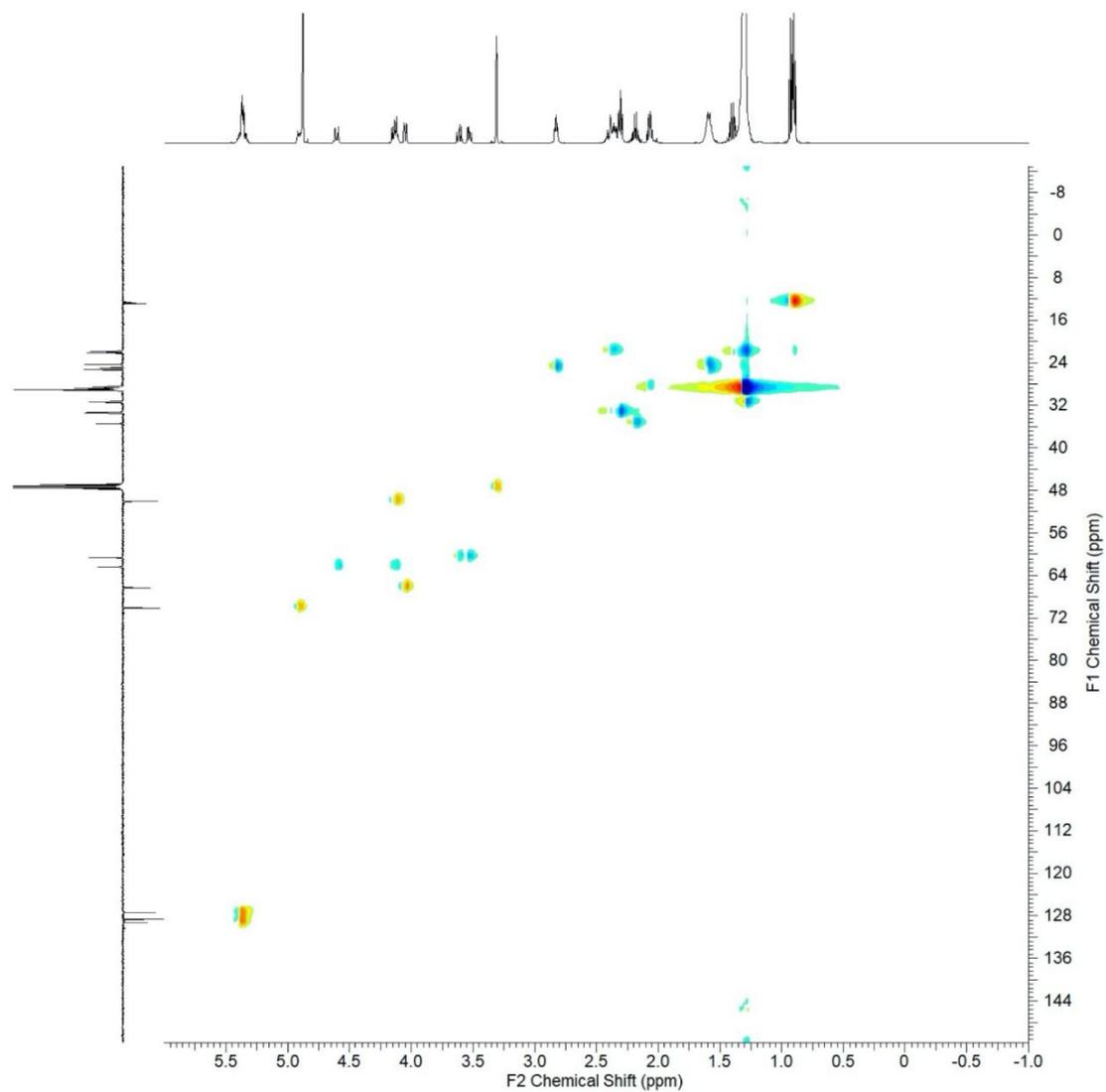
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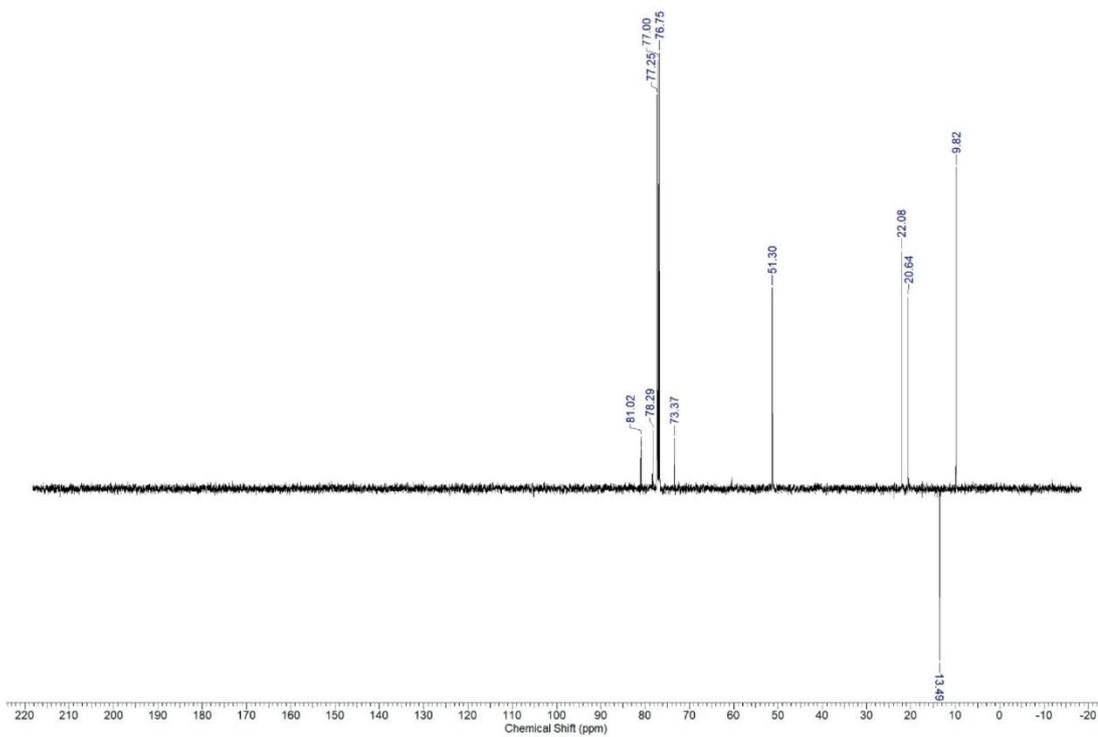
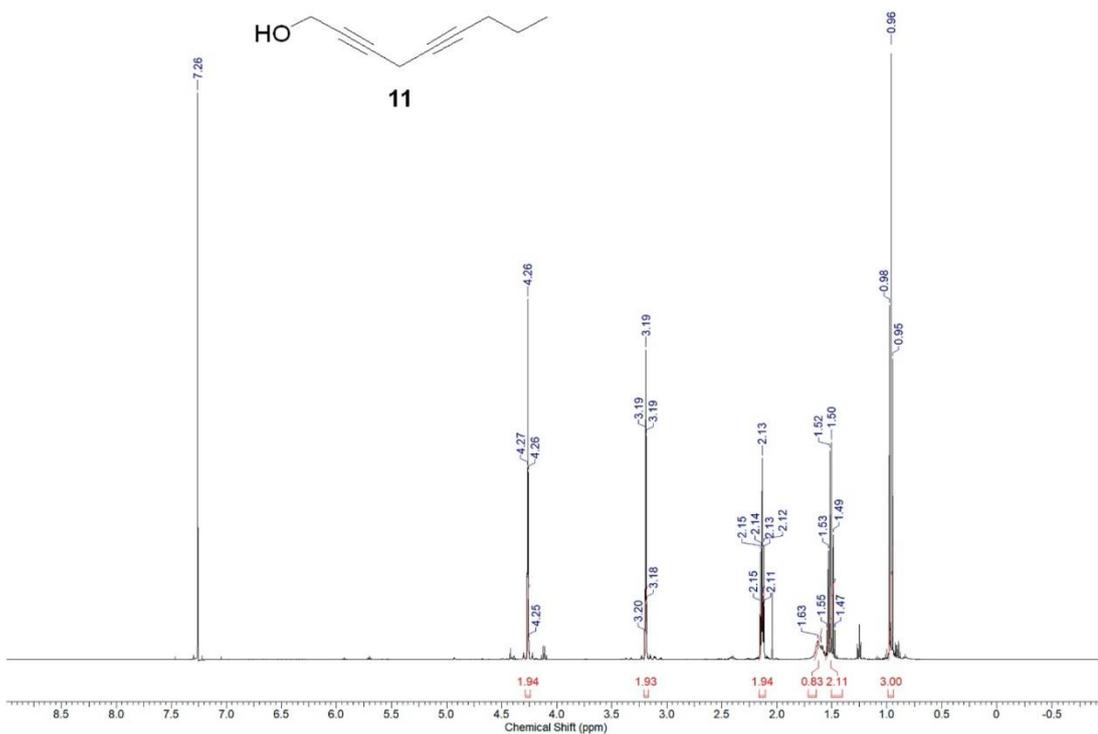


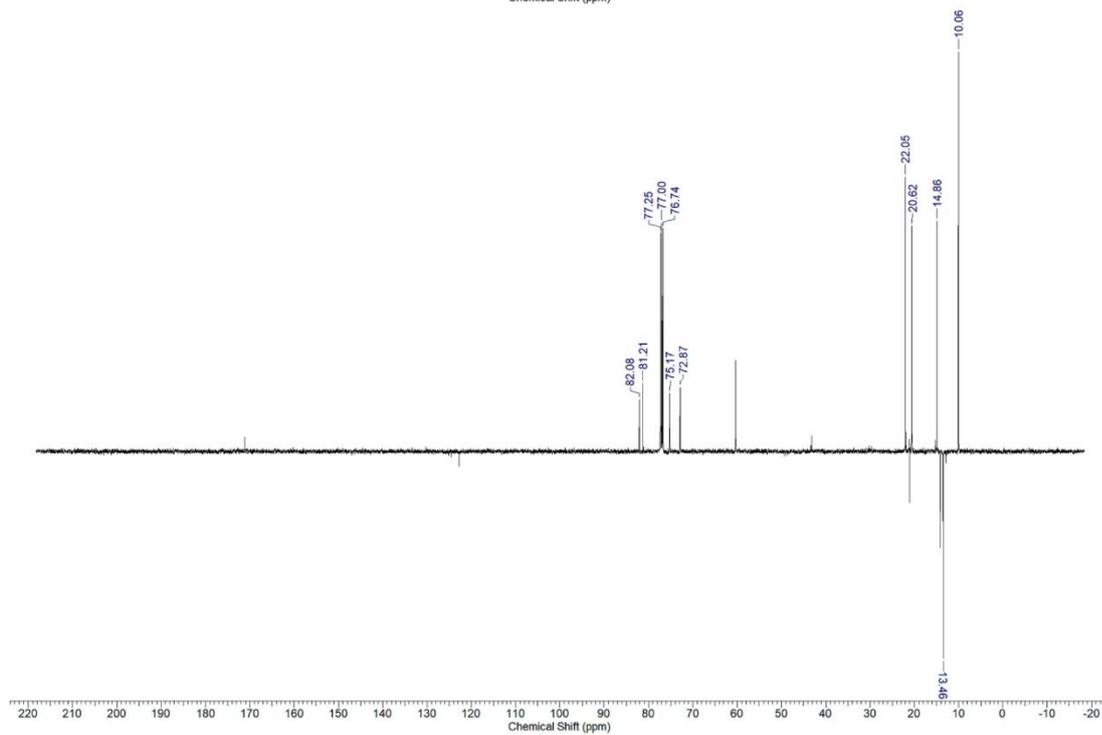
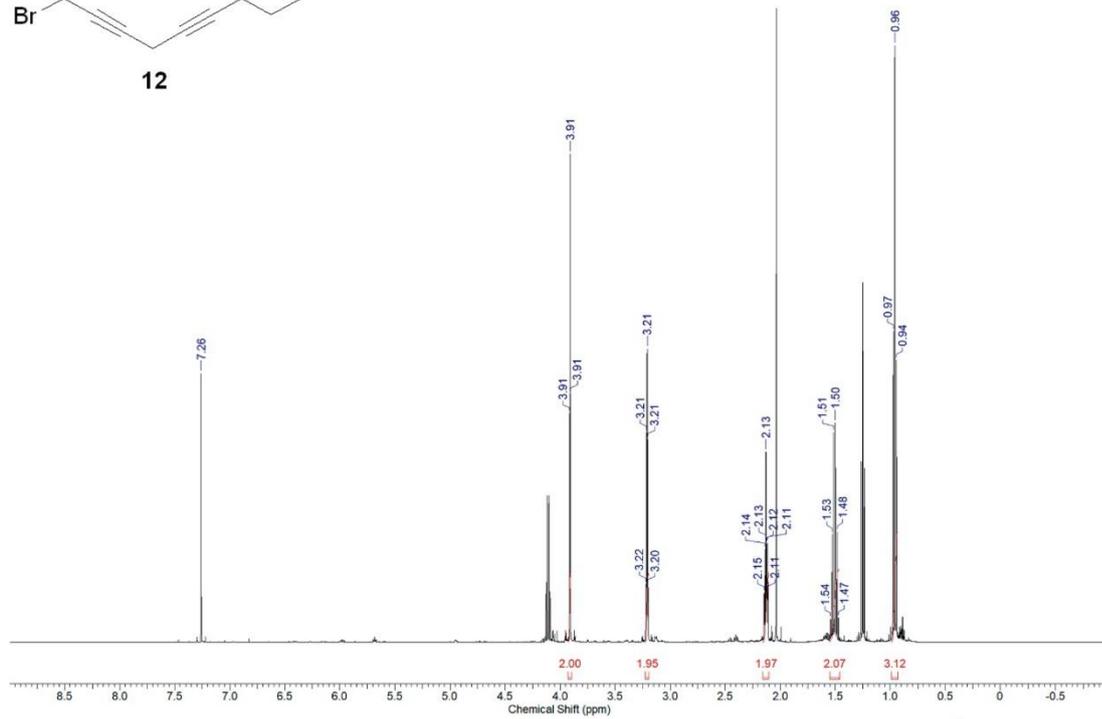
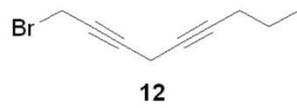
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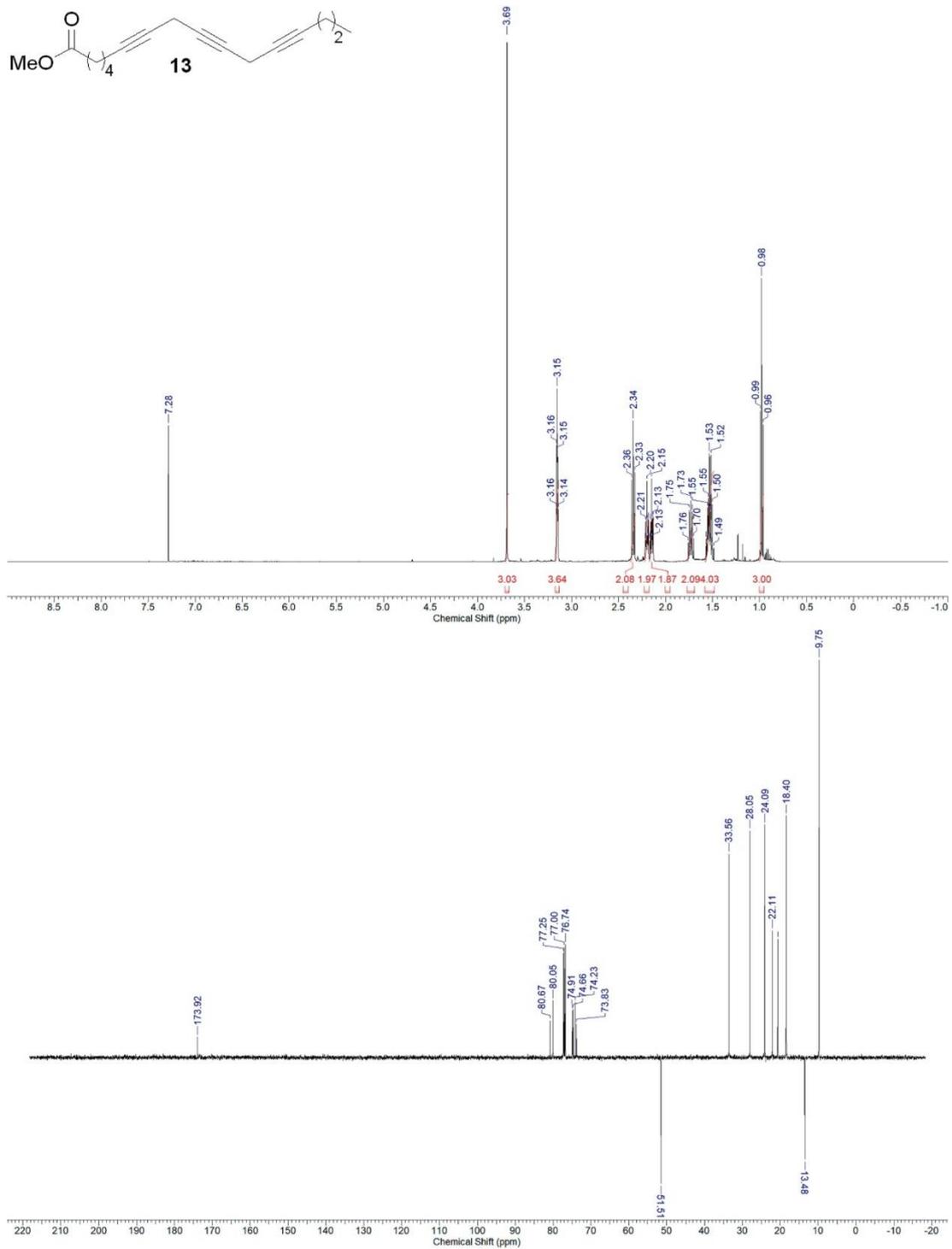


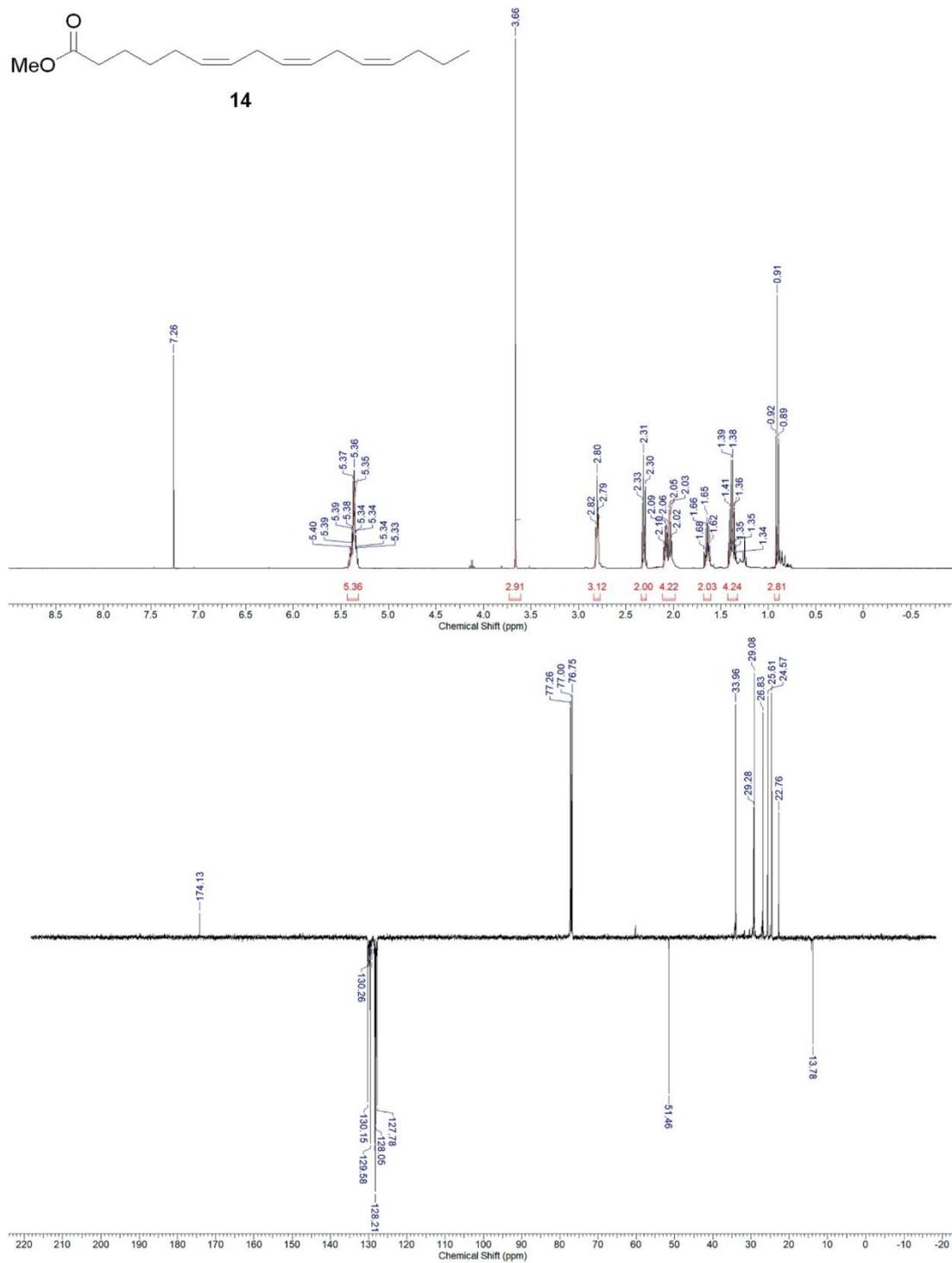
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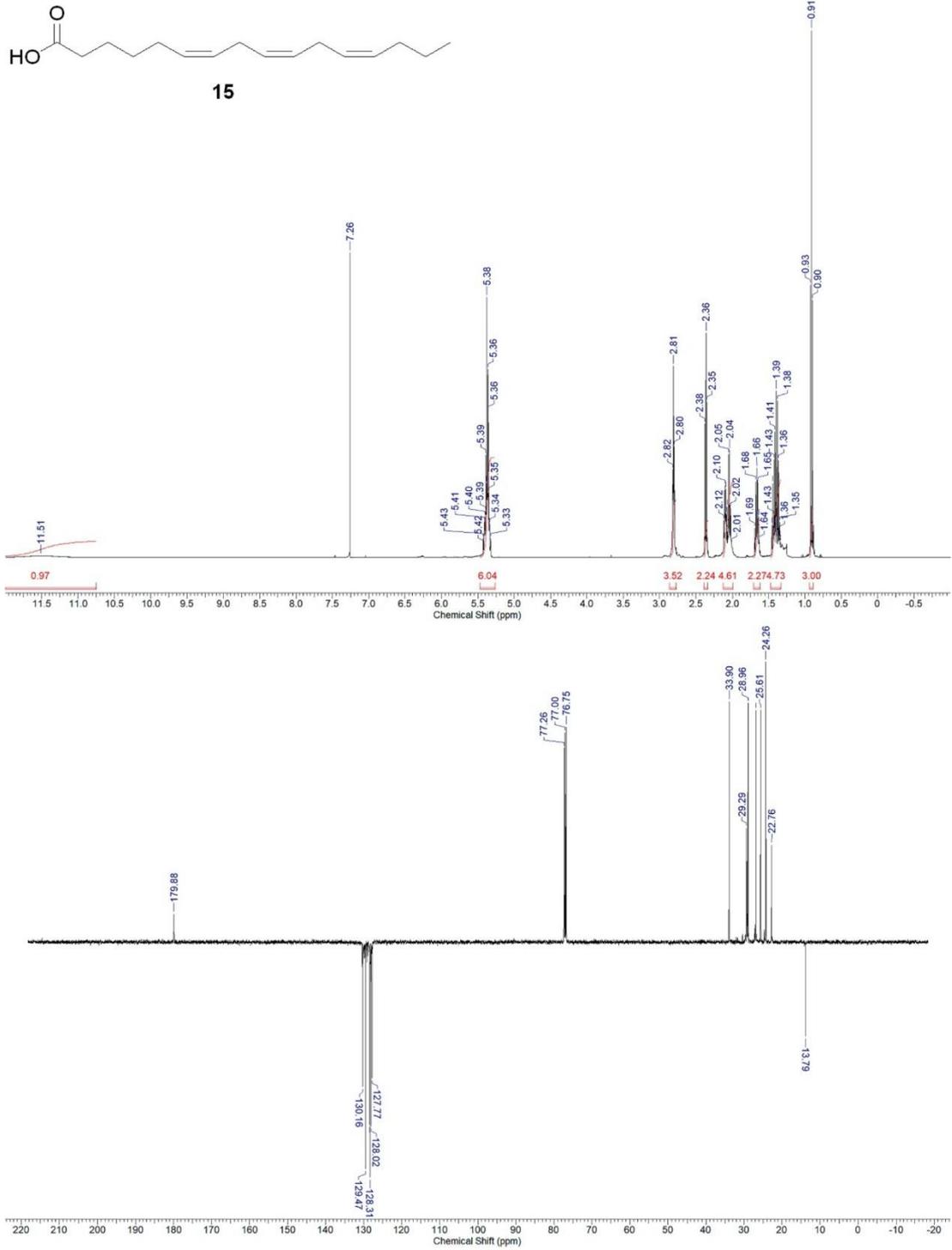


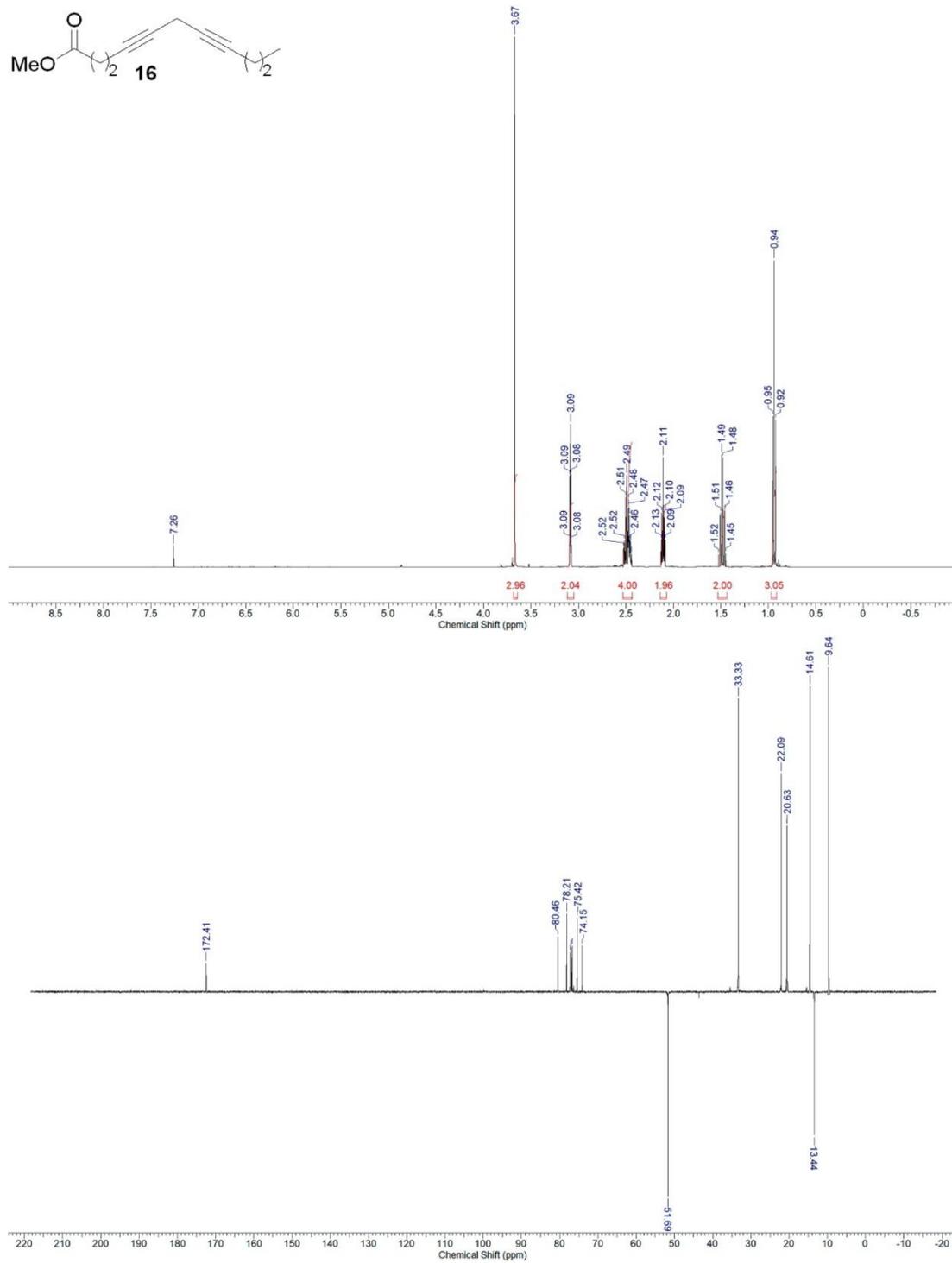


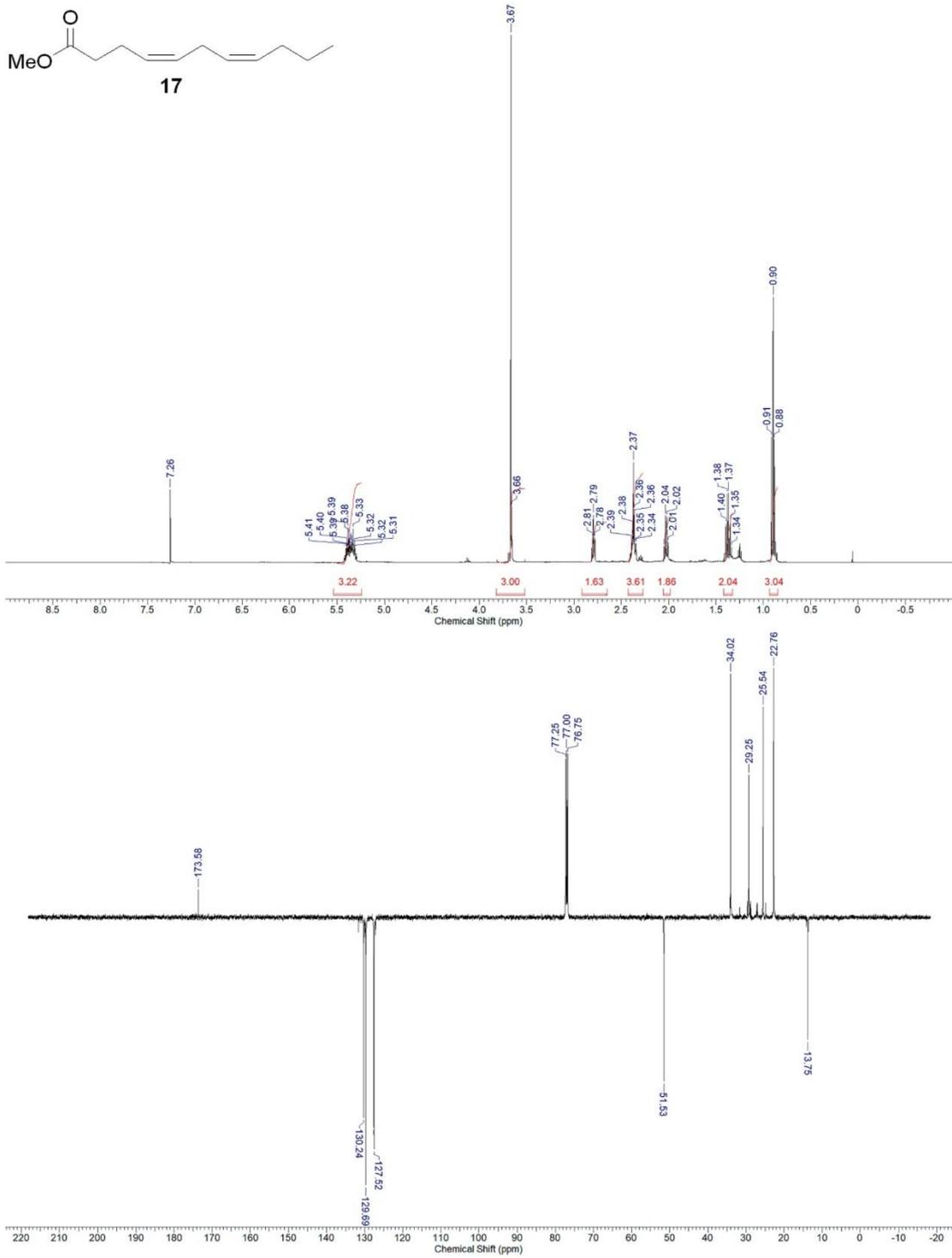


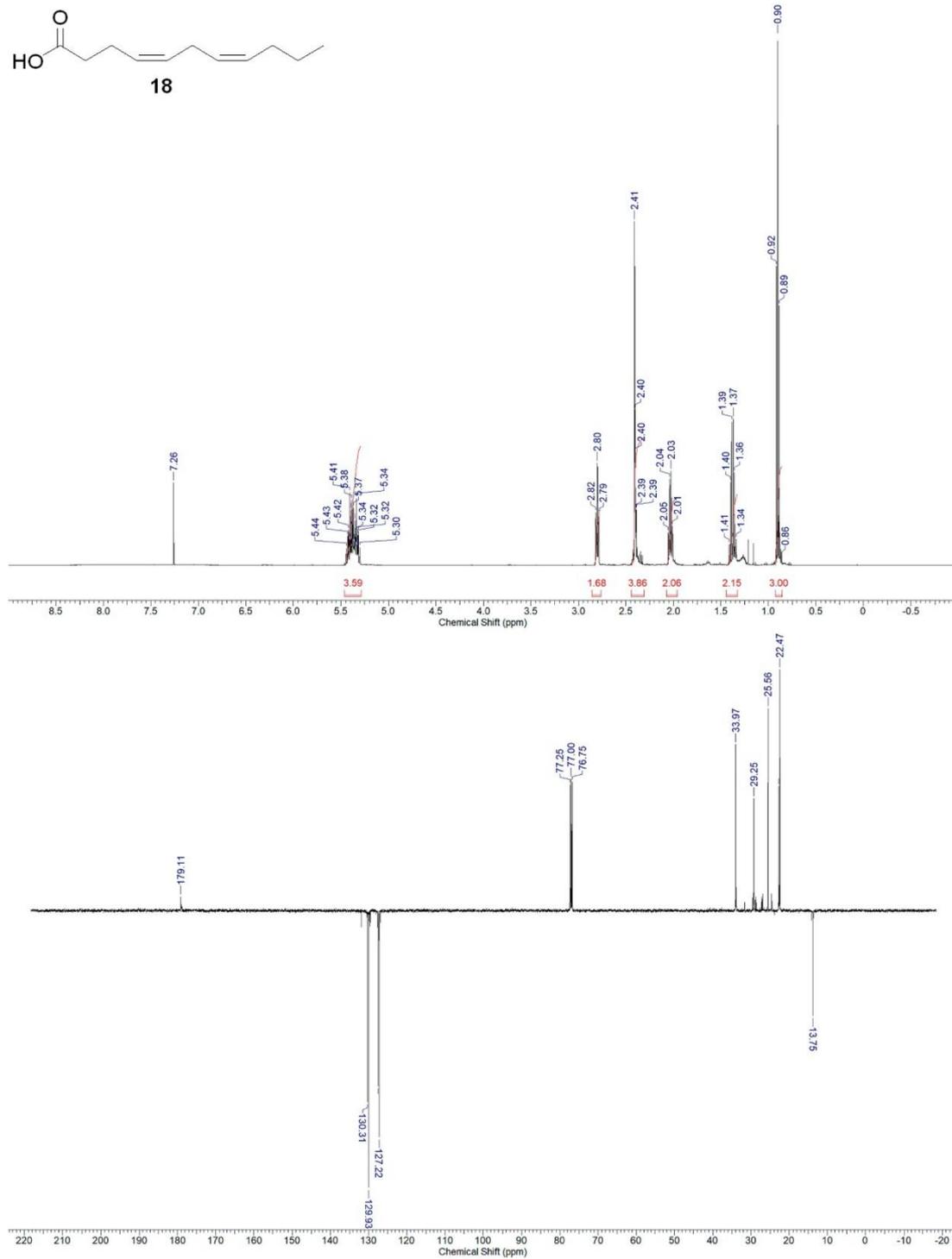


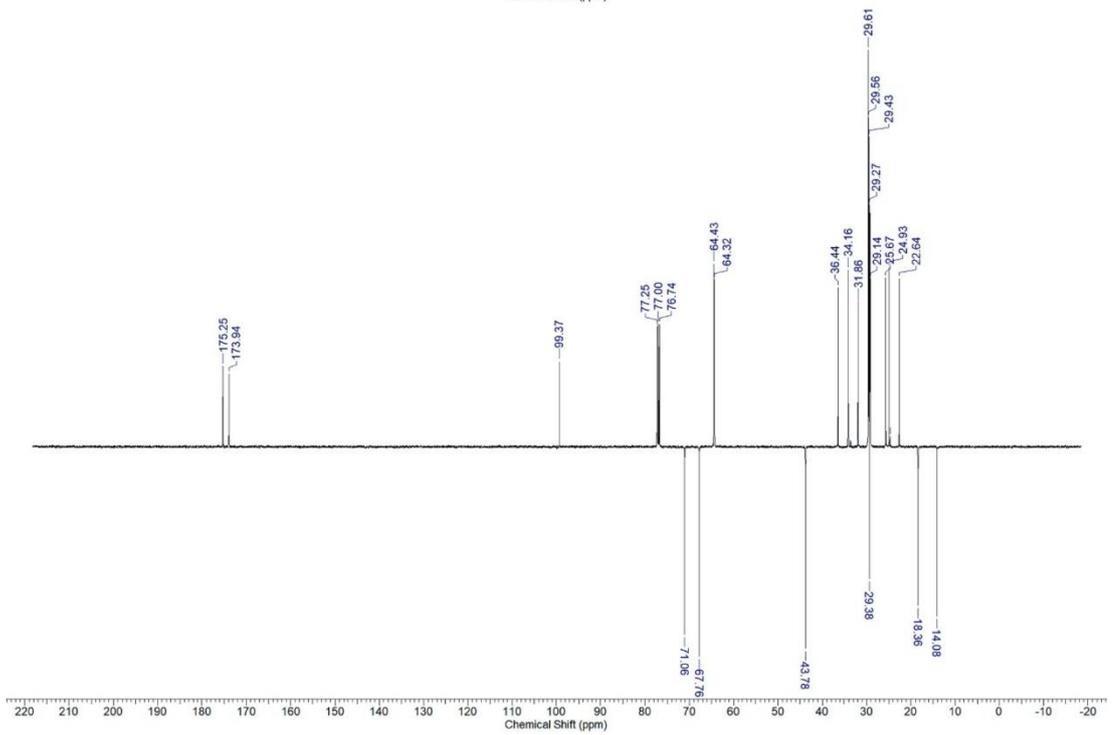
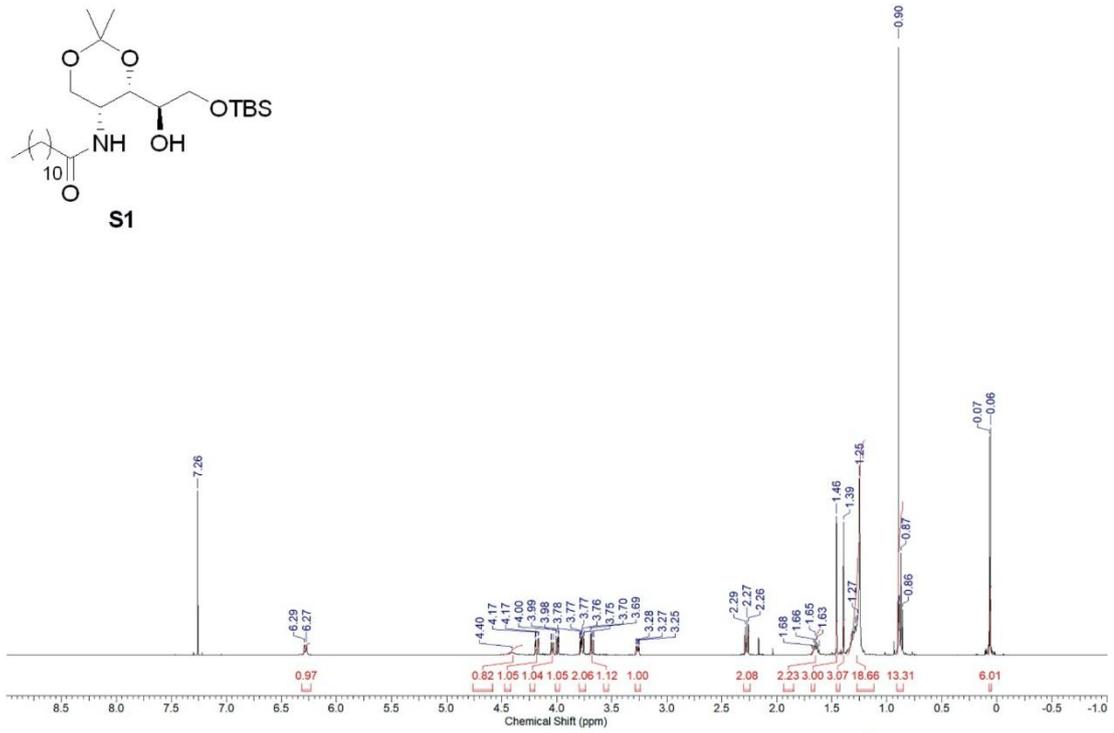
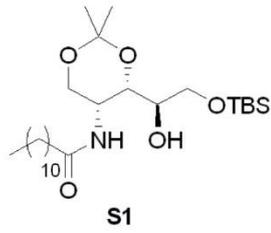


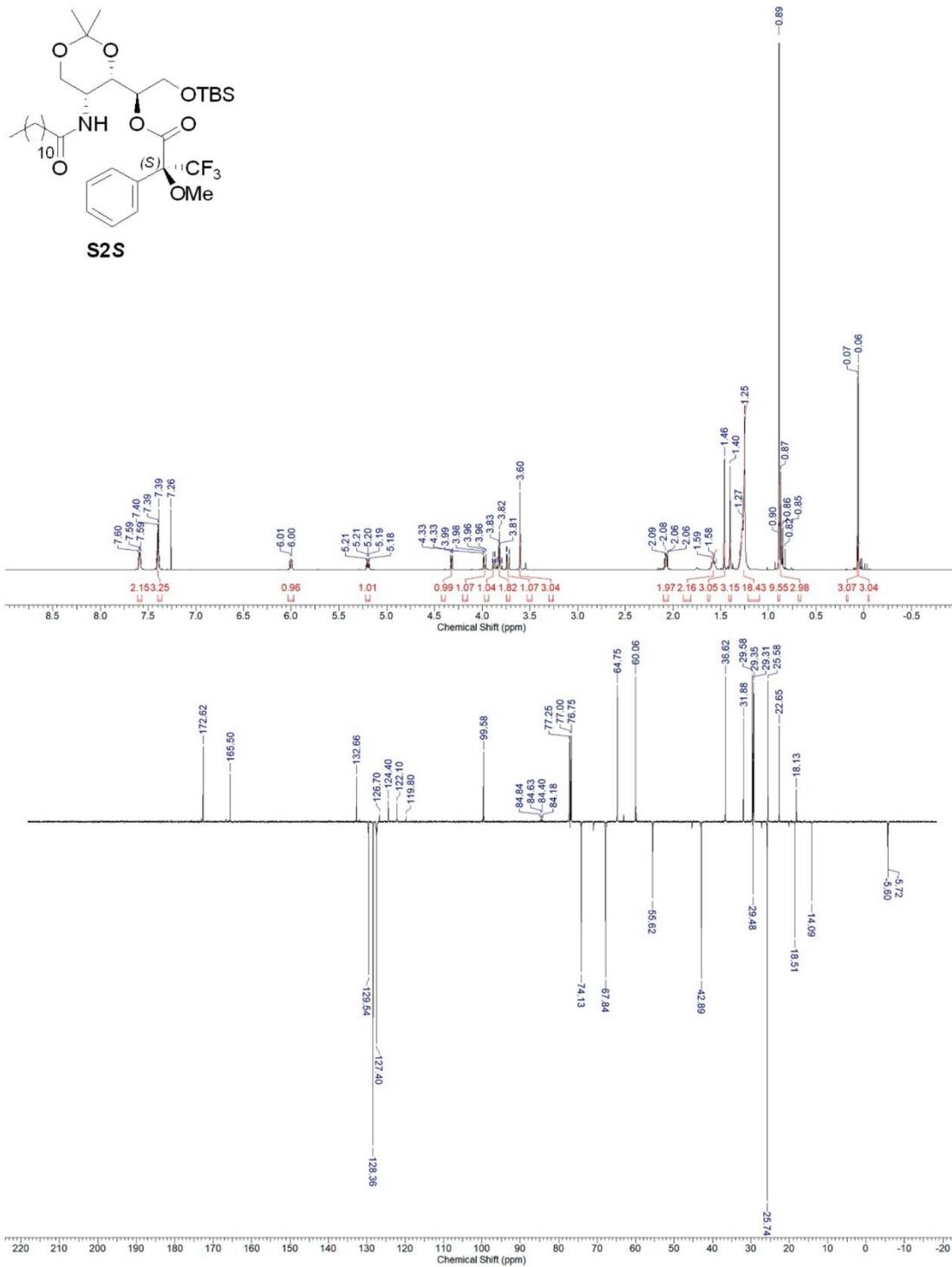


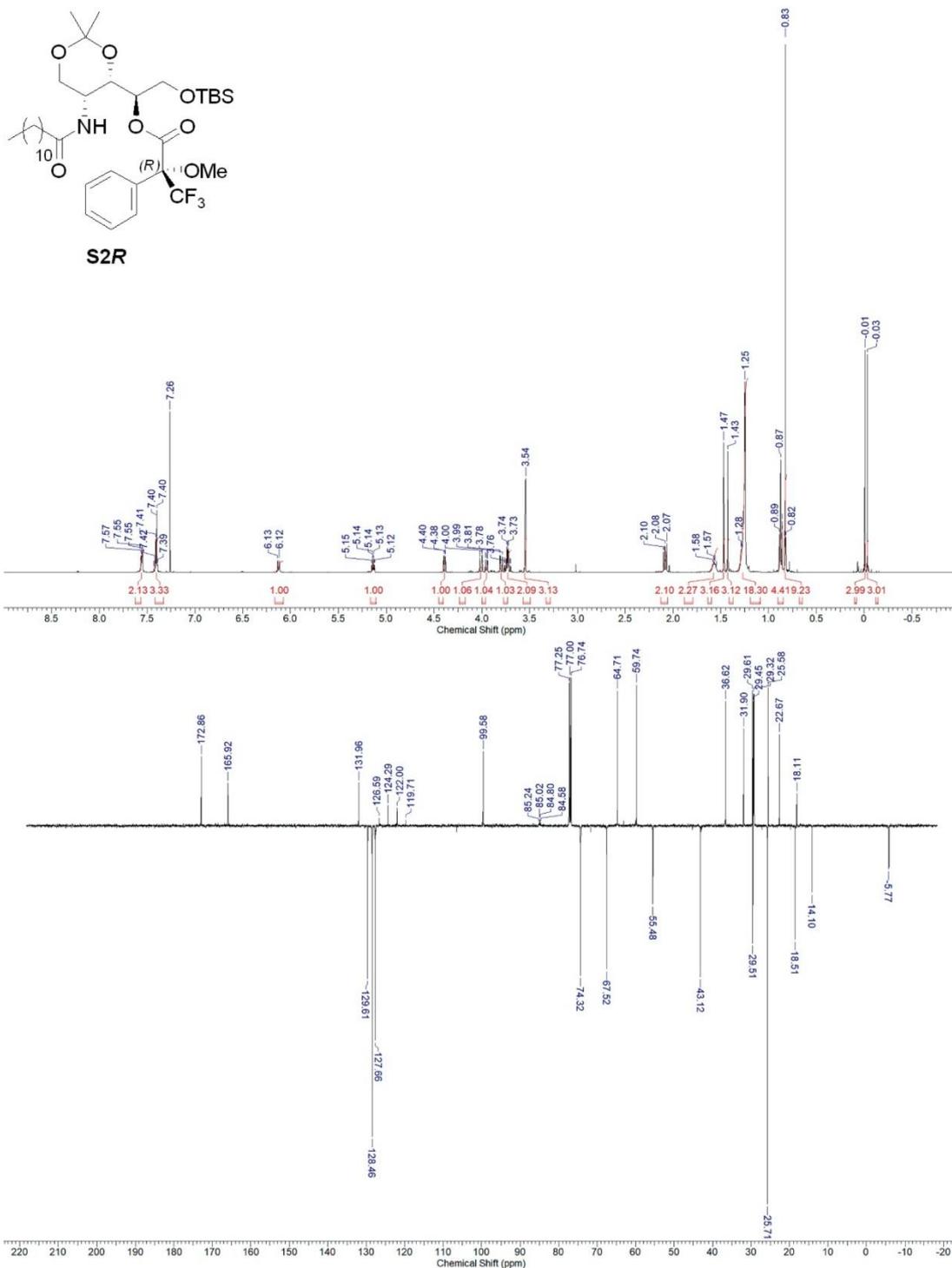












## Cell culture conditions and inhibition of cell growth

### *Cell culture conditions and stock solutions*

518A2 (Department of Radiotherapy and Radiobiology, University Hospital Vienna) human melanoma cells, HCT-116 (ACC-581) human colon carcinoma cells, as well as its p53 knockout mutant cell line HCT-116<sup>p53<sup>-/-</sup></sup>, HeLa human cervix carcinoma, U87 human glioblastoma and MCF-7 human breast carcinoma cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Biochrom) supplemented with 10% (v/v) fetal bovine serum (FBS; Biochrom) and 1% (v/v) Antibiotic-Antimycotic solution (Gibco). The cells were incubated at 37 °C, 5% CO<sub>2</sub>, 95% humidified atmosphere and were serially passaged following trypsinisation by using 0.05% trypsin/0.02% EDTA (w/v; Biochrom GmbH, Berlin, Germany). Mycoplasma contamination was frequently monitored, and only mycoplasma-free cultures were used.

### *Inhibition of cell growth (MTT-assay)<sup>3</sup>*

The cytotoxic effect upon treatment with bathymodiolamides A (**1**) and B (**2**) for 72 h was determined by standard MTT assays. The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; abcr) is reduced by viable cells to a violet, water-insoluble formazan. 518A2 melanoma cells, colon carcinoma cells HCT-116 and HCT-116<sup>p53<sup>-/-</sup></sup> and HeLa cervix carcinoma cells ( $5 \times 10^4$  cells mL<sup>-1</sup>, 100 µL/well), as well as U87 and MCF-7 cells ( $10 \times 10^4$  cells mL<sup>-1</sup>, 100 µL/well) were seeded in 96-well tissue culture plates and cultured for 24 h at 37 °C, 5% CO<sub>2</sub> and 95% humidity. After treatment with the test compounds **1** and **2** (stock solutions 10 mM in DMSO and freshly diluted appropriately with sterile Milli-Q water) incubation of cells was continued for 72 h. Blank and solvent controls were treated identically. After addition of a 5 mg mL<sup>-1</sup> MTT stock solution in phosphate buffered saline (PBS), microplates were incubated for 2 h at 37 °C, centrifuged at 300 g, 4 °C for 5 min and the supernatant was discarded. The precipitate of formazan crystals was then redissolved in a 10% (w/v) solution of sodium dodecylsulfate (SDS; Carl Roth) in DMSO containing 0.6% (v/v) acetic acid. To ensure complete dissolution of the formazan, the microplates were incubated for at least 1 h in the dark. Finally, the absorbance at  $\lambda = 570$  and 630 nm (background) was measured using a microplate reader (Tecan F200). All experiments were carried out in quadruplicate and the percentage of viable cells was calculated as the mean  $\pm$  SD with controls set to 100%. The determined IC<sub>50</sub> (inhibitory concentration) values are shown in table 1.

**Table 2.** Inhibitory concentrations IC<sub>50</sub> [µM] of (2R,3S,4R)-bathymodiolamides A **1** and B **2** when applied to HeLa cervix carcinoma, MCF7 breast carcinoma, 518A2 melanoma, HCT-116, HCT-116<sup>p53<sup>-/-</sup></sup> knockout mutant colon carcinoma and U87 glioblastoma cells. Values are the means  $\pm$  SD determined in four independent experiments and derived from dose-response curves after 72 h incubation using the MTT assay.

	IC <sub>50</sub> (µM)					
	HeLa	MCF7	518A2	HCT-116	HCT-116 <sup>p53<sup>-/-</sup></sup>	U87
<b>1</b>	>50	>50	>50	>50	>50	>50
<b>2</b>	>50	>50	>50	>50	>50	>50

Neither of the two synthetic bathymodiolamides **1** and **2** showed a cytotoxic effect on the six human cell lines in the concentration range up to 50 µM

**References**

- (1) Andrianasolo, E. H.; Haramaty, L.; McPhail, K. L.; White, E.; Vetriani, C.; Falkowski, P.; Lutz, R. Bathymodiolamides A and B, Ceramide Derivatives from a Deep-Sea Hydrothermal Vent Invertebrate Mussel, *Bathymodiolus thermophilus*. *J. Nat. Prod.* **2011**, *74*, 842–846.
- (2) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. Absolute configurations of marine diterpenes possessing a xenicane skeleton. An application of an advanced Mosher's method. *Tetrahedron Lett.* **1989**, *30*, 3147–3150.
- (3) Mosmann, T. J. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **1983**, *65*, 55-63

## 4.5 Auflistung aller Publikationen

### 1. N-Metallocenoylsphingosines as targeted ceramidase inhibitors: Syntheses and antitumoral effects

Matthias Rothmund, Alexander Bär, Felix Klatt, Sascha Weidler, Leonhard Köhler, Carlo Unverzagt, Claus-D. Kuhn und Rainer Schobert  
*Bioorg. Chem.* **2020**, *97*, 103703

### 2. Synthesis and antiproliferative effect of the proposed stereoisomer of the marine sponge metabolite halisphingosine A

Alexander Bär, Sofia I. Bär und Rainer Schobert  
*Org. Biomol. Chem.* **2020**, *18*, 7565

### 3. Synthesis of the Proposed Isomers of the Deep-Sea Mussel Metabolites Bathymodiolamides A and B

Alexander Bär, Sofia I. Bär, Moritz Röder, und Rainer Schobert  
*J. Org. Chem.* **2021**, *86*, 1868

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