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Hiyun Kim

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ALLENE-BASED APPROACH TO THE SYNTHESIS OF DE NOVO ERYTHROMYCINOIDS

by

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Dr. Lawrence J. Williams Ph.D

and approved by

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New Brunswick, New Jersey

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ABSTRACT OF THE DISSERTATION

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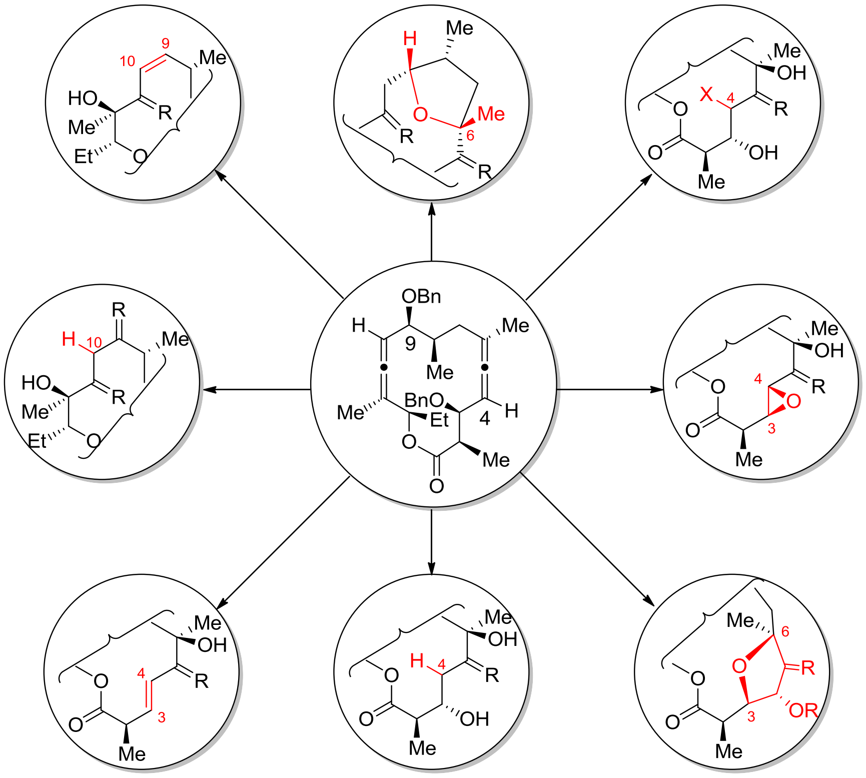
By HIYUN KIM

Dissertation Director:

Dr. Lawrence J. Williams Ph.D



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We prepared an advanced synthetic module (bis[allene] macrolactone at center) equipped with two allenes embedded in a macrolactone scaffold. The plan was to effect heterogeneous derivatization of the allenes, in tandem or separately. In addition to diversity, this approach is maximally concise and economic, especially in terms of steps. Moreover, immediate derivatives of the macrocyclic bis[allene] can be taken into further steps, thus providing canonical build-up of erythromycin analogs.

To date, our bis[allene] macrolactone has been converted to over 30 novel macrolides. These *de novo* analogs serve to validate the strategy and lay the ground work for further work. Taken together, the allene-based reactions/transformations employed in this study, such as DMDO oxidation/nucleophile addition, allene osmylation/electrophile addition, bromination, allene oxide rearrangement, spirodiepoxide rearrangement, benzylic migration/elimination, mono- and bis-oxidation of bis[allene], chelation-controlled reduction and oxime formation, demonstrate that 8 of the 11 modifiable carbons in this antibiotic can be modified. It is especially noteworthy that each congener was made in less than three steps from the bis[allene] macrolactone.

Acknowledgements

“The mind of man plans his way, But the LORD directs his steps (Proverbs 16:9)”

I would like to note my sincere appreciation to my advisor Prof. Lawrence J. Williams. He has been an excellent teacher, researcher, and writer who possesses “naturally-born” scientific insight and care for the students at the same time. He gave me the second opportunity to pursue a Ph.D. degree at Rutgers. From the first day in his group, everything was new. However, I quickly realized that the novelty which I observed was the extension of creativity, enthusiasm, scientific contribution, and pride in his research. I worked on several very challenging projects, including quite a few different interdisciplinary projects, which were scientifically and experimentally challenging, but fully rewarding at the end. In his group, I have had my eyes opened to science.

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Dedication

To My Lord, Jesus Christ, and Orphans in North Korea

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List of Abbreviations

°C	degrees Celsius
Å	Angstroms
Ac	acetyl
Ag_2O	silver (I) oxide
Akt	protein kinase B
AZM	azithromycin
Bn	benzyl
Bu_2BOTf	dibutylboron trifluoromethanesulfonate
CAM	clarithromycin
$ClTi(O^iPr)_3$	chlorotriisopropoxytitanium (IV)
DABCO	diazabicyclo[2.2.2]octane
DCC	*N*,*N'*-dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	diisopropyl azodicarboxylate
DIC	*N*,*N'*-dicycloisopropylcarbodiimide
DMAP	4-(N,N-dimethylamino)pyridine
DMDO	dimethyldioxirane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DTT	dithiothreitol

eGFP	enhanced green fluorescence protein
EGFRvIII	epidermal growth factor receptor variant III
equiv.	equivalents
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
FCC	flash column chromatography
GBM	glioblastoma multifome
Hex	hexane
HFL	heterofunctional linkers
HIV	human imminodeficiency virus
HMBC	heteronuclear multiple-bond correlation spectroscopy
i-Bu	*iso*-butyl
imid	imidazole
i-Pr	*iso*-propyl
^{i}-Pr_2NEt	diisopropylethyamine
LDA	lithium diisopropylamide
LiCCH-EDA	lithium acetylide-ethylenediamine
m-	meta-
M.S.	molecular sieves
Me	methyl
MLS_B	macrolide-lincosamide-*streptogramin* B
MsCl	methanesulfonyl chloride

mTOR	mammalian target of Rapamycin
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
nOe	nuclear overhauser effect
o-	ortho-
OsO_4	osmium (VIII) tetroxide
p-	para-
PBS	phosphate buffered saline
PEG	polyethylene glycol
Ph	phenyl
PhH	benzene
PhMe	toluene
PI13K	phosphatidylinositol 3-kinase
PKS	polyketide synthase
PTEN	protein tyrosine phosphatase
pyr	pyridine
QD	quantum dot
RGD	arginine-glycine-aspartate peptide
RISC	RNA-induced silencing complex
rt	room temperature
RuO_4	ruthenium (VIII) tetroxide
SAR	structure activity relationship

SDE	spirodiepoxide
siRNA	small interfering RNA
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TEA	triethyl amine
TESCl	triethylsilyl chloride
TFA	trifluoroacetic acid
TOPO	tri-octylphosphine oxide
wt%	weight percent

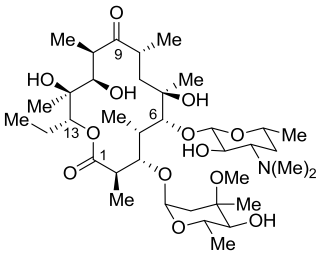
Chapter 1

Erythromycin and Its Derivatives

1.1. Introduction

Erythromycin **1.1**, an archetypal 14-membered macrolide, originally exhibited unique and superior biological properties such as its activity, safety, and mode of action in inhibiting bacterial protein synthesis.[1] Recently, Rubin et al. reported other biological functions such as the selective uptake by macrophages, extracellular kinase activity, and anti-asthmatic functions.[2]

Figure 1.1-1: Erythromycin



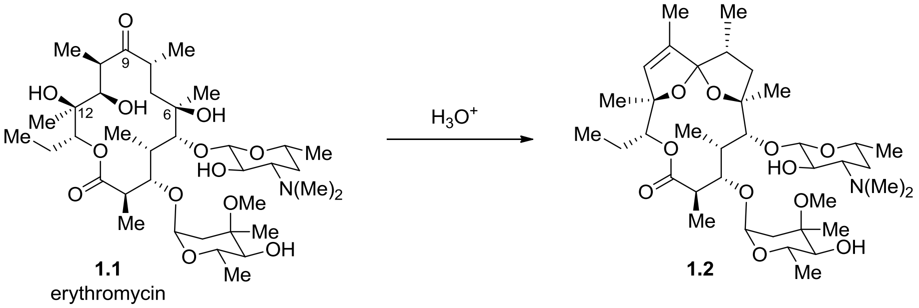
1.1
erythromycin

Even though its profuse utility for clinical use in human medicine since 1950, it showed several disadvantages, such as weak activity against gram-negative bacteria, induction of macrolide resistance, a bitter taste, and a tendency to produce gastrointestinal cramp as a consequence of the instability in the acidic medium of the stomach. This acid-instability caused a low and inconsistent bioavailability. These were the initial major problems which directed the tremendous synthetic efforts to modify erythromycin.[1, 3, 4]

1.2. Erythromycin Derivatives

It had been recognized that the degradation of erythromycin proceeds via interaction of the 6- and 12-hydroxy, 9-carbonyl and 8-hydrogen groups through intramolecular ketalization (Scheme 1.2-1).[5] The resulting compound mimics the activity of motilin which causes stomach cramps.[4] Consequently, significant research was devoted to the modification of these specific positions. The 2nd generation macrolides, clarithromycin **1.3**, azithromycin **1.4**, roxithromycin **1.5**, flurithromycin **1.6** and dirithromycin **1.7** were developed in the 1980s, and eventually commercialized in the 1990s (Figure 1.2-1).

Scheme 1.2-1: Acid Induced Transformation of Erythromycin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OMe
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H_3O^+
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OH
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Me
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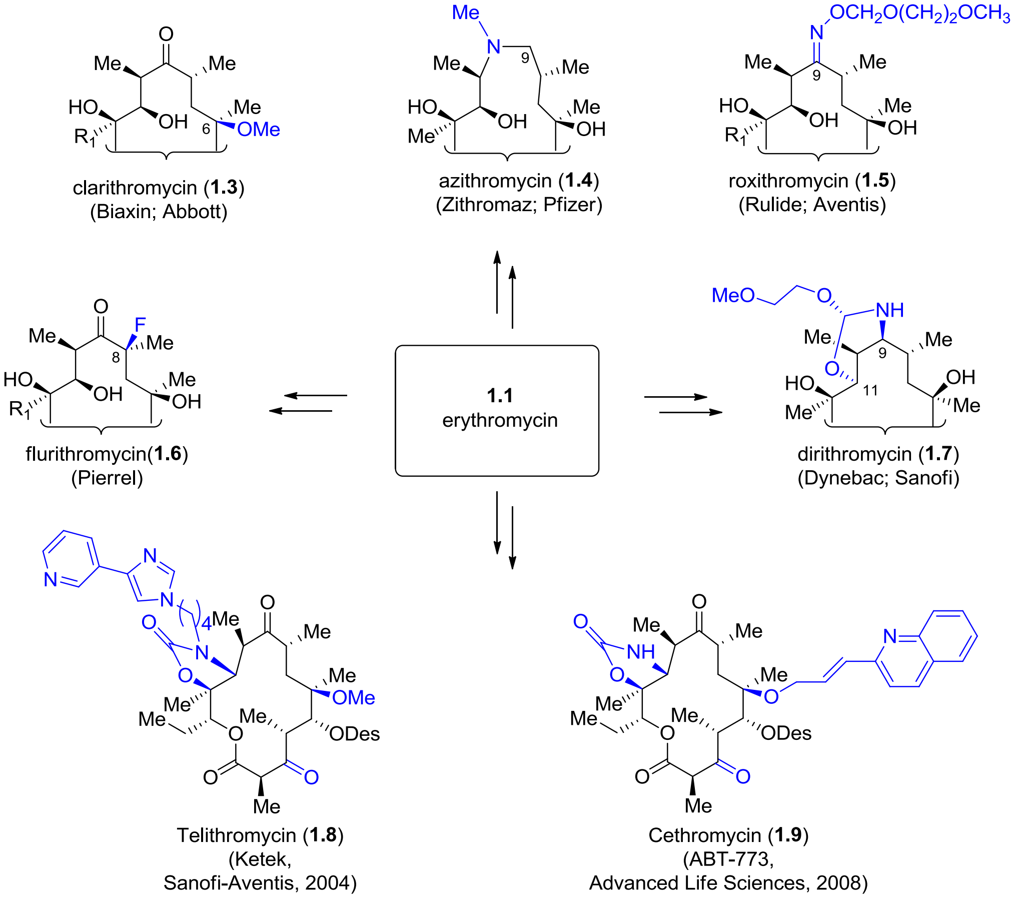
Figure 1.2-1: Commercialized Erythromycin Derivatives



clarithromycin (**1.3**)
(Biaxin; Abbott)

azithromycin (**1.4**)
(Zithromaz; Pfizer)

roxithromycin (**1.5**)
(Rulide; Aventis)

flurithromycin(**1.6**)
(Pierrel)

1.1
erythromycin

dirithromycin (**1.7**)
(Dynebac; Sanofi)

Telithromycin (**1.8**)
(Ketek,
Sanofi-Aventis, 2004)

Cethromycin (**1.9**)
(ABT-773,
Advanced Life Sciences, 2008)

All these drugs, however, present several drawbacks. They are inactive against macrolide, lincosamide, type B streptogramin (MLS_B)-resistant *streptococci* and *Streptococcus pneumoniae* and, with exception of azithromicin, weakly active against *Haemophilus influenzae*.[4] Furthermore, the resistance of *S. pneumonia* to erythromycin has increased significantly in recent years.[6]

A 2005 report indicated that ~30% of the *S. pnuemoniae* strains in the United States were resistant to macrolides (erythromycin, azithromycin and clarithromycin). Although resistance among *S. pnuemoniae* to single agents is problematic, more troublesome is the emergence and dissemination of multidrug-resistant (MDR) strains: MDR phenotypes cross several drugs, including macrolides, β-lactams, sulphonamides and tetracyclines. This emerging drug resistance clearly suggested an exigency for the 3rd generation macrolide and this quest resulted in the development of ketolides, which exhibit increased affinity to the ribosome as well as apparent lower sensitivity to known mechanisms of resistance. These 14-membered ring drugs are characterized by the keto group at the C3 position of the lactone ring, rather than the cladinose sugar found in the macrolides. Among many ketolides, two compounds were commercialized, telithromycin **1.8** and cethromycin **1.9**. Both feature an 11,12-carbamate group (Figure 1.2-1). In addition, telithromycin has an extended alkyl-aryl extending from the 11,12-carbamate group, whereas cethromycin has a quinolylallyl side chain at the C6 position of the lactone ring.

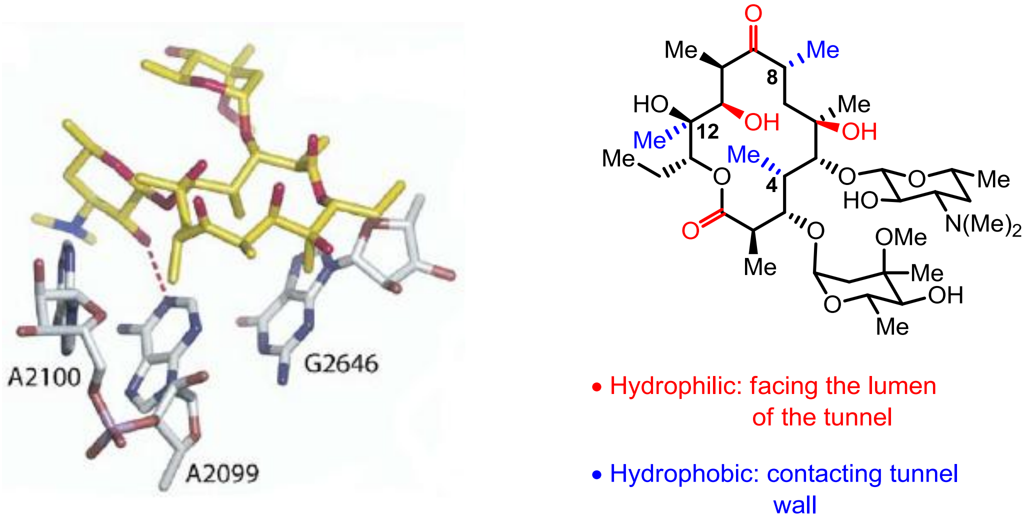
1.3. Binding Site and Modes of Action

The details of the molecular interactions of the various classes of macrolides with the ribosome were determined from X-ray crystallographic structures of the archaeal (*H. marismortui*) and bacterial (*D. radiocurans*) large ribosomal subunits in the laboratories of Tom Steitz[7, 8] and Ada Yonath[9, 10] respectively. The X-ray crystallographic structures revealed that the macrolide binding site is located on the large ribosomal subunit inside the nascent peptide exit tunnel near the peptidyl transferase center. Basically, this binding blocks the exit tunnel of the ribosome to prevent the departure of the nascent polypeptide, thereby halting protein synthesis. A number of nucleotide residues in domain V of 23S rRNA are involved in bonding with the macrolide molecule. Important contacts are found between the C5 mono- or disaccharide side chains of 14-, 15-, and 16-membered ring macrolides and rRNA. The desosamine sugar of erythromycin and other related 14-membered ring macrolides forms hydrogen bonding with the nitrogen bases of the nucleotide residues A2058, A2059 (*E. coli* numbering). The nucleotide, occupying position 2057, may also be involved in hydrogen bonding with the C5 desosamine of macrolide, or it may be establish hydrophobic interactions with the lactone ring. In addition, the desosamine sugar can potentially interact with the backbone phosphate oxygen of G2505. Hence, certain resistant microorganisms with mutational changes in components of this subunit of the ribosome fail to bind the drug.

Ketolides displayed increased binding to the ribosome as compared to the macrolides of previous generations. Their binding site overlaps the macrolide-binding site of domain V of 23S rRNA. In addition, ketolides bind to domain II. For example, telithromycin[11] binds to a specific adenine (A752) on domain II of 23S rRNA via the alkyl-aryl extension from the 11,12-carbamate bridge, whereas the quinolallyl side chain at the C6 position in cethromycin[12] makes a contact with U790 of domain II of 23S rRNA.

Binding affinity is affected not only by hydrogen bonding, but also by the hydrophobic interactions of the lactone ring with the ribosomal subunit. Steitz[13] illustrated that the side of the lactone ring facing the lumen of the tunnel is hydrophilic, including two axially oriented hydroxyl (C6 & C11) and two axially oriented carbonyl oxygens (C1 & C9). In contrast, the face of the lactone ring that contacts the tunnel wall is hydrophobic. It contains no hydrogen bond donors or acceptors, only consisting of three methyl groups (C4, C8 &C12) that reside in the hydrophobic pocket formed by the base of A2100 (A2059Ec), the hydrophobic C2 edge of A2099 (A2058Ec), and the base and sugar of G2546 (C2611Ec).

Figure 1.3-1: Macrolides in Hydrophobic Pocket (adapted with permission from ref.[14])

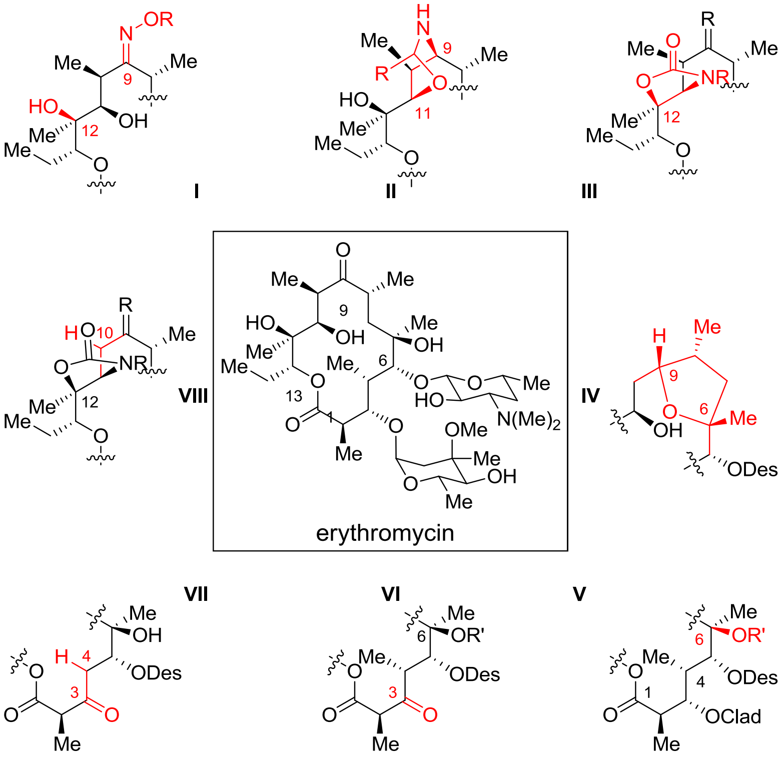

A2100
G2646
A2099
O
Me
Me
8
HO
OH
Me
OH
Me
12
Me
Me
4
O
O
HO
Me
N(Me)2
O
OMe
O
Me
Me
O
OH
Me
• Hydrophilic: facing the lumen of the tunnel
• Hydrophobic: contacting tunnel wall

Regardless of the structures, the nucleotide at position 2058Ec was shown to play a major role in the binding of macrolides to the ribosome. As a consequence, it also plays a main role in the resistance to macrolides, often acquired by steric hindrance caused by its methylation or A-to-G mutation.[15, 16]

1.4. Previous SAR Studies

The combination of low structural processability and high structural complexity of the erythromycins pose serious obstacles to addressing the problem of bacterial resistance. The known structure/activity profile (Figure 1.4-1) for erythromycin represents a tremendous effort.[17-19] Briefly, a *priori* study has shown that: *(a)* portions of the glycans, especially desosamine, are critical and both the hydrophilic character of the β-face and hydrophobic character of the α-face dictate binding (erythromycin);[20] *(b)* C9 amine or oxime/ether functionality improves pharmacokinetic profiles (**I**);[17, 19] *(c)* C9-C11 or C11-C12 heteroannulation can improve binding and appears to provide opportunities to overcome MLS_B resistance (**II, III**);[21, 22] *(d)* ring formation between C6 and C9 can be beneficial,[1] leading to interesting bioactivities such as gastrointestinal motor stimulant albeit with non-antibiotic function (**IV**);[23] *(e)* retention of the C6 and C12 heteroatom connectivity is desirable and ether formation at C6 may improve antibiotic activity and suppress other activity (**I, V**); [24, 25] *(f)* C3 ketone derivatives can improve efflux resistance (**VI**);[22, 26] and *(g)* alterations to the hydrophobic face of the macrocycle (e.g. at C4, C8, and C10, **VII** and **VIII**) may overcome resistance.[14, 27]

Figure 1.4-1: A Priori SAR Study of Erythromycin (adapted with permission from ref.[28])



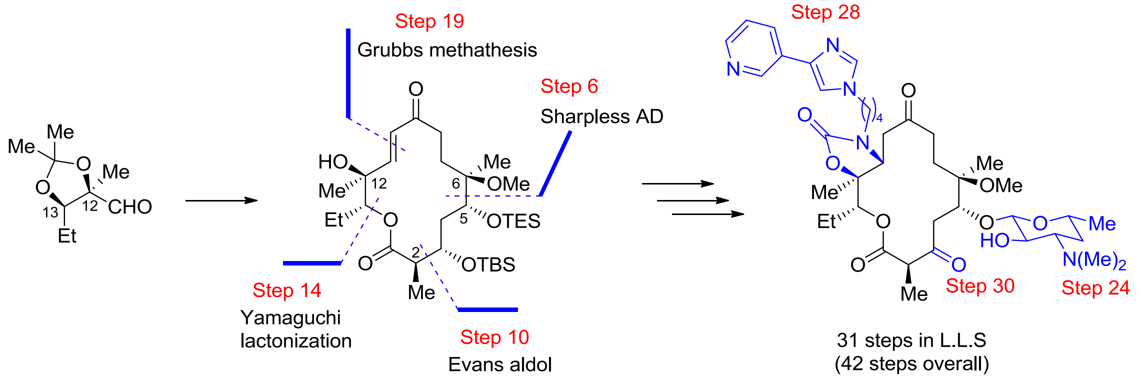
Hence, modification on the C3-C6 and C9-C12 regions offer opportunities to improve drug properties and avoid bacterial resistance.[14] We therefore aimed to directly access diverse modifiable erythromycinoid variants to thereby expedite lead optimization studies.

Conventional medicinal chemistry approaches rely on erythromycin as a starting material (*See* Figure 1.1-2). However, total synthesis represents the only means by which to gain unrestricted access to this structure space. Indeed, *de novo* synthesis of the erythromycins has a rich history, and the knowledge gained from efforts to synthesize these targets is considerable.[29]

1.5. Total Synthetic Approach to Erythromycin Derivatives

In 2011, Andrade et al. reported the total synthesis of 4,8,10-tridesmethyl telithromycin.[14] As described in Section 1.2, telithromycin is a 3rd generation semisynthetic drug derived from erythromycin and characterized by the 11,12-carbamate side chain and 3-keto group. Their desmethylation strategy is grounded in structural data by Steitz and co-workers.[13] Bacterial strains resistant to macrolide, lincosamide, type B streptogramin, and ketolide (MLS_BK) antibiotics commonly possess either an N-methyl transferase that methylates the exocyclic N6 amine in A2058Ec in 23S rRNA or a mutated 23S rRNA, often A2058G.[16] Steitz and coworkers postulated the cause of resistance might due to a steric clash of the amino group of guanine 2058 with the C4 methyl of the drug.[13]

Scheme 1.5-1 Andrades' Synthesis of 4,8,10-Tridesmethyl Telithromycin


Step 19
Grubbs methathesis
Step 6
Sharpless AD
Step 14
Yamaguchi lactonization
Step 10
Evans aldol
Step 28
Step 30
Step 24
Me
Me
O
O
Me
13
12
CHO
Et
HO
Me
Et
OMe
OTES
OTBS
N(Me)2
HO
31 steps in L.L.S
(42 steps overall)

1.10 **1.11** **1.12**

The synthesis commenced with the known aldehyde **1.10** for the construction of C11-C13 unit. Sharpless asymmetric dihydroxylation established the requisite stereochemistry at C5 and C6 at *step 6*. Construction of C2 and C3 bond was accomplished via an Evans aldol reaction at *step 10*. After ring-closing methathesis (RCM) at *step 19*, the glycosylation (*step 24*), the elaboration with C11,C12-carbamate (*step 28*) and the oxidation at C3 (*step 30*) were applied. From macrocylic structure **1.11**, it took additional 12 steps to finish the synthesis. The synthesis was very lengthy (42 steps overall, 31 steps in the longest linear sequence) though **1.12** exhibited antibacterial activity.

Andrades' synthesis demonstrated the advantage of total synthesis over semi-synthesis, namely the ability to modify the carbon skeleton selectively. However, the access to the structural/biological space of erythromycin analogs is far from the reality with single target-oriented synthesis.

1.6. Conclusion

Despite the development of new synthetic methods and strategies toward the erythromycin family, structural complexity and low structural processability are serious obstacles to addressing the problem of bacterial resistance through semi- and/or total synthesis. It is clear that comprehensive knowledge of structure/function space is essential prerequisite for the development of better drugs. Hence, the development of a *de novo* strategy that grants efficient access to diverse structures is imperative in order to conduct a comprehensive SAR study for erythromycin-based antibiotics.

This dissertation focuses on our advances to the efficient generation of erythromycinoids.

Chapter 2: Strategy for the Synthesis of *De Novo* Erythromycinoids

Chapter 3: Synthesis of the Bis[Allene] Macrolactone

Chapter 4: Synthesis of Erythromycinoids

Chapter 5: Allene Osmylation and Multicomponent Coupling

Additionally, a multidisciplinary collaborative project with Prof. Ki-Bum Lee (Department of Chemistry & Chemical Biology, Rutgers) is described in Chapter 6: The Ligand Design and Synthesis for SiRNA Delivery.

1.7. References

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Chapter 2

Strategy for the Synthesis of De Novo Erythromycinoids: Bis[Allene] Macrolactone

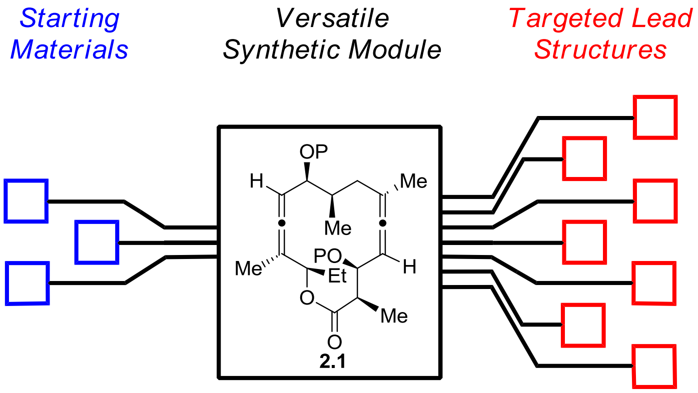
2.1. Introduction

Ideally, unrestricted access to diverse erythromycin analogs, including skeletal and stereochemical modifications, would require a minimal number of manipulations and no dramatic change in the synthetic route. Considering the model study and our extensive experience with and knowledge of allene transformations, we designated the fully functionalized bis[allene] macrolactone as a modular starting point for the construction of new analog scaffolds. Herein, I disclose our bis[allene] macrolactone strategy for the synthesis of *de novo* erythromycinoids.

2.2. Efficient Access to Diverse in Erythromycin Congeners

Our aim is to enable access to the diverse erythromycinoid structure space through efficient synthesis. The synthetic strategy considers: a) the structural space contains skeletal and stereochemical diversity; b) the versatile synthetic module leads to new sets of expandable intermediates, which ultimately constitute sets of targeted lead structure; and c) synthetic maneuvers should be minimal: the routes should be short.

Figure 2.2-1: Synthetic Strategy: Bis[Allene] Macrolactone (adapted with permission from ref.[25])


Starting Materials
Versatile Synthetic Module
Targeted Lead Structures
OP
H
Me
Me
Me
PO
Et
H
O
Me
O
2.1

2.3. *Synthetic Strategy: Bis[Allene] Macrolactone*

To achieve the aim, we developed a bis[allene] macrolactone strategy, which is characterized by the following key points:

- Total synthetic approach
- Late-stage functionalization: allene oxidation
- Macrocyclic stereocontrol
- Convergent synthesis of bis[allene] macrolactone
- Divergent synthesis of erythromycinoids

The most common method for erythromycin analog synthesis has been the derivatization of erythromycin itself. This semi-synthetic method has its own benefit from bountiful supply of erythromycin. However, the lack of synthetic flexibility not only requires lengthy synthetic manipulations of erythromycin but also limits the accessibility of potentially useful chemical and biological space.

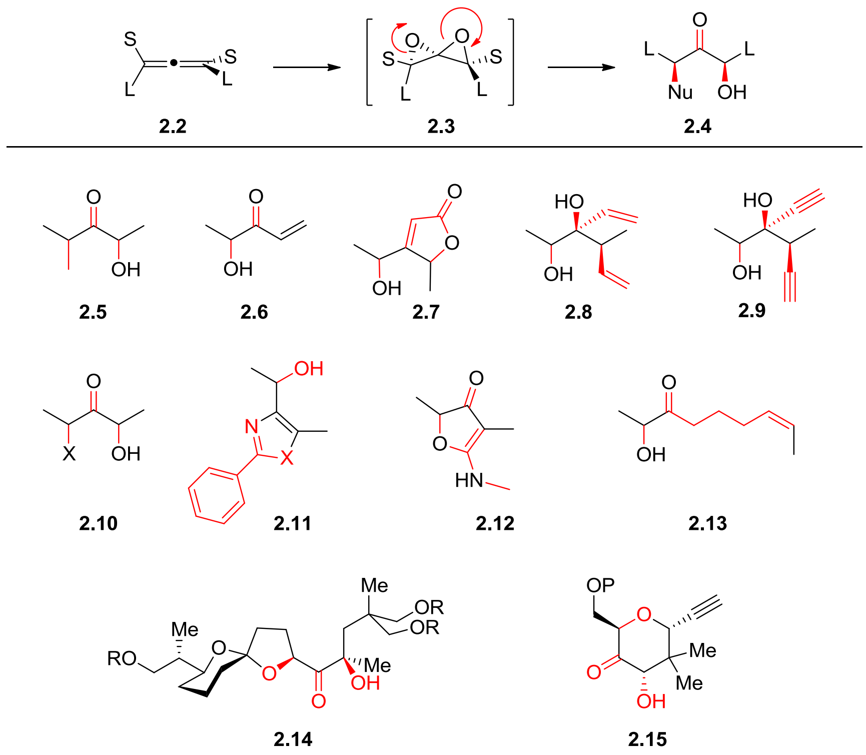
Total synthesis might be a very lengthy way to generate analogs, but in the context of diversity-oriented synthesis,[1, 2] it provides an excellent means of modification, especially on the carbon backbone, which is intractable when using the semi-synthetic method. Late-stage introduction of heterofunctionality[3] is not considered an the ideal strategy for target-oriented synthesis due to the risk of failure at a late stage. However, when it is combined with the versatile synthetic module, it can be greatly appreciated in terms of efficiency and diversity.

2.4. Allene Oxidation: Spirodiepoxide (SDE) Chmistry

As a result of their inherent chirality and high reactivity in such diverse transformations as addition, cyclization/cycloaddition, cycloisomerization, and cross-coupling reactions,[4, 5] functionalized allenes have been employed in recent years as highly versatile precursors to natural products and other target molecules. Despite their demonstrated utility, allenes are still considered to be an “under-developed” functional group. Prof. Williams’ group has studied the synthesis[6] and reactivity[7] of allenes, developing many novel transformations to access diverse motifs (*See* Figure 2.4.-1).

Crandall’s pioneering work[8-16] demonstrated that upon DMDO oxidation of allenes, the corresponding spirodiepoxides (SDEs) formed and various nucleophiles could be added. However, the synthetic utility of allenes in complex molecule synthesis was realized through the work in the Williams group.

Figure 2.4-1: Allene-Based Chemical Transformation from the Williams Group

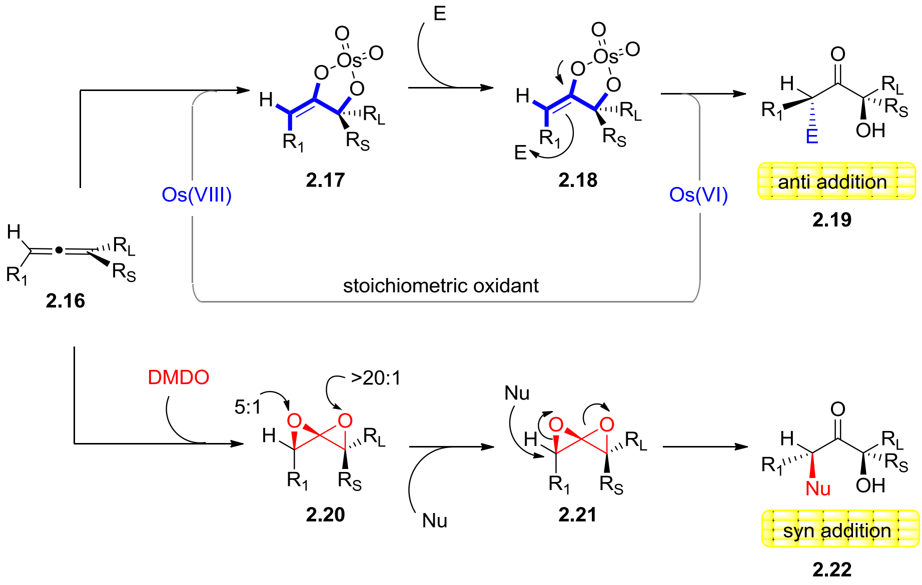

S
L
S
L
2.2
O
O
S
S
L
L
2.3
O
L
L
Nu
OH
2.4
O
OH
2.5
O
OH
2.6
O
O
OH
2.7
HO
OH
2.8
HO
OH
2.9
O
X
OH
2.10
OH
N
X
2.11
O
O
HN
2.12
O
OH
2.13
Me
OR
OR
Me
RO
O
O
O
Me
OH
2.14
OP
O
Me
O
Me
OH
2.15

Various heteronucleophiles and carbon nucleophiles as well as ambiphilic reagents such as benzamide, thiobenzamide, benamidine, azide, intramolecular oxygen, and organometallic carbon[17] are compatible with SDE chemistry and result in a complex motif such as a vicinal triad composed of hydroxyl, ketone, and syn-substituted nucleophiles,[18] haloketones, α'-hydroxy enone,[19] heterocycles (oxazoles, thiazoles, and imidazoles),[20] dihydrofuranone,[7] butenolides, diendiols, diyndiol, γ-lactones, spiroketals,[21] and pyrans[22] with high regio- and stereoselectivity in a single step. The utility of SDEs was extended in synthetic studies of complex natural products such as epoxomicin,[18] psymberin,[22] pectenotoxin 4,[23] and erythromycin.[24, 25]

2.5. Allene Oxidation: Osmylation

We have recently developed a method of allene osmylation. Osmium tetroxide (OsO_4) readily oxidized simple and complex linear allenes and the hydrolysis of the intermediate osmate ester gave α-hydroxy ketones both under catalytic and stoichiometric conditions (*See* Chapter 5 for details). When an electrophile is present upon the oxidation, it not only accelerated the hydrolysis of osmate ester **2.18** but also formed a multi-component adduct, giving α-hydroxy-α'-electrophile ketones (**2.16 → 2.19**). For now, this OsO_4-catalyzed multicoupling reaction of allenes is not fully appreciated in complex settings nor has the scope of electrophiles been fully explored. However, studies are ongoing. So far we have found compatible electrophiles are N-bromosuccinimide (NBS), N-chlorosuccinimide (NCS), Selectfluor, Eshenmoser's salt (dimethylmethylidene ammonium chloride), and chloramines-T (N-chloro tosylamide).

Scheme 2.5-1: Allene Oxidation

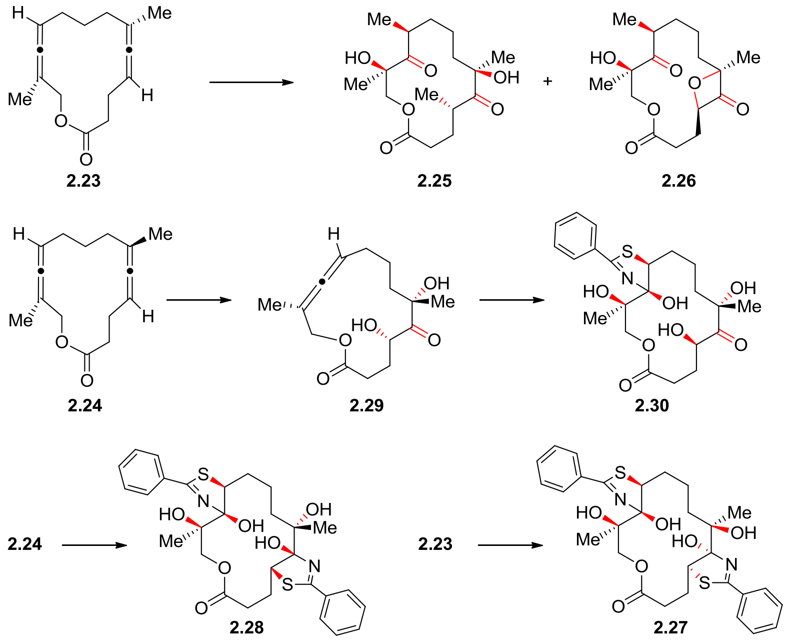

E
Os(VIII)
2.17
2.18
Os(VI)
anti addition
2.19
2.16
stoichiometric oxidant
DMDO
5:1
>20:1
Nu
2.20
2.21
Nu
syn addition
2.22

When applied on chiral allenes, the stereochemical outcome of the reaction complements our allene spirodiepoxidation/nucleophile capture methology (**2.16** → **2.22**). Therefore, when applied on the macrocyclic bis[allene], it provides access to stereoisomers, and their unique SAR profiles, without invoking a new asymmetric route. Furthermore, the stereoselectivity is enhanced due the macrocyclic stereocontrol. The use of protecting groups was minimized via late-stage macrocyclic stereocontrol; furthermore, highly stereoselective reactions were achieved due to the conformational preferences of the macrolide intermediates.

2.6. Model Study: Bis[allene] Macrolactone

Dr. Ghosh and co-workers in Prof. Williams' group demonstrated the macrocyclic bis[allene] strategy[24] on a simplified racemic macrocyclic bis[allene] (Figure 2.6-1). The study demonstrated that: *(a)* heterogeneous derivatization of the bis[allene] by stepwise oxidation; *(b)* successful addition of an organocuprate (MeCuCNLi) to the SDE, which represented the feasibility of SDE chemistry for polyketide-containing natural product synthesis; *(c)* complete macrocyclic stereocontrol; and *(d)* compatibility of SDE/nucleophile addition chemistry in a macrocylic platform.

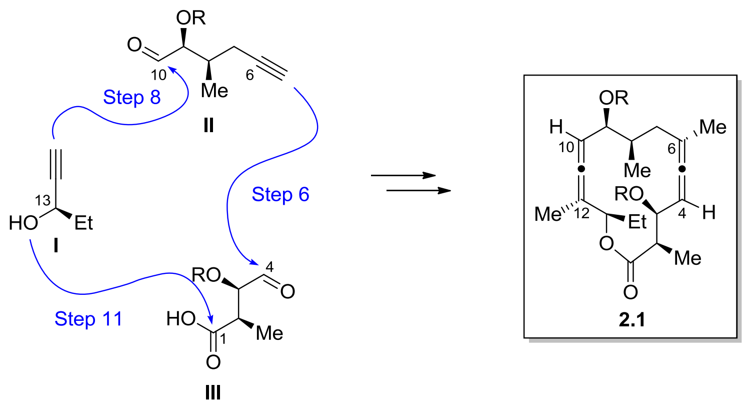
Figure 2.6-1: Previous Model Study of the Bis [Allene] Macrolactone Strategy



2.7. Convergent Synthesis of Bis[Allene] Macrolactone

Single-flask allene preparation,[7] a method recently developed in Prof. Williams' group, helped us envision the synthesis of both allene (C4 to C6, C10 to C12) moieties in **2.1** from propargyl alcohols. The convergent, recursive alkynylation sequence (*step 6* and *step 8*), coordinated allene installation (*step 9*), and lactone formation (*step 11*) produce bis[allene] macrolactone (**2.1**).

Figure 2.7-1: Convergent & Recursive Assembly of a Macrocyclic Bis[Allene] (adapted with permission from ref.[25])



The synthesis of three building blocks, an alkynol (**I**), an alkynal (**II**), and an aldehyde (**III**), and subsequent coupling of these for the synthesis of (**2.1)** will be discussed in detail in the following chapter.

2.8. Conclusion

The bis[allene] macrolactone strategy is designed to confer the efficiency and diversity on the structure/function space of erythromycinoids. Diversity will be achieved by late-stage oxidation of the bis[allene], which will undergo selective independent or concurrent modification. We have shown that allene oxidation, depending on the oxidant, can enable multi-component coupling with various nucleophiles and electrophiles in a highly stereoselective manner. This coordinated synthetic maneuver will provide the stereochemical and skeletal diversities, which can easily be expanded into functional group and appendage diversities. The route is designed so as to enable modification on the carbon skeleton where precedent methodologies cannot access.

Hence, the unexplored structure/function space of erythromycin antibiotics can be realistically reached.

In the following chapter, I discuss the synthesis of the bis[allene] macrolactone.

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Chapter 3

Synthesis of the Bis[Allene] Macrolactone

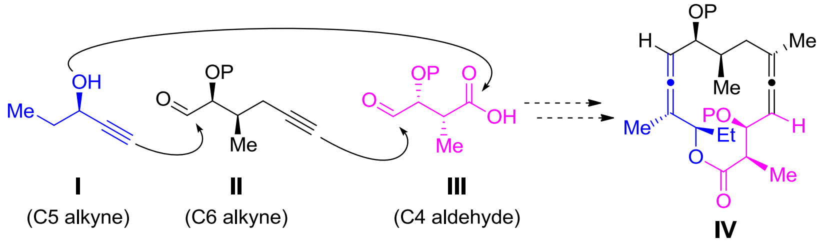
3.1. Introduction

We have focused on developing a single short route for the chemical synthesis of diverse erythromycinoids. The bis[allene] macrolactone was envisioned as a versatile synthetic module. The first generation of the synthesis was carried out by our former colleague, Dr. Partha Ghosh.[1] Kai Liu and I have since made considerable improvements, especially in terms of reproducibility. This chapter focuses on the experimental details of the synthetic route for the bis[allene] macrolactone.

3.2. Synthesis of Coupling Units

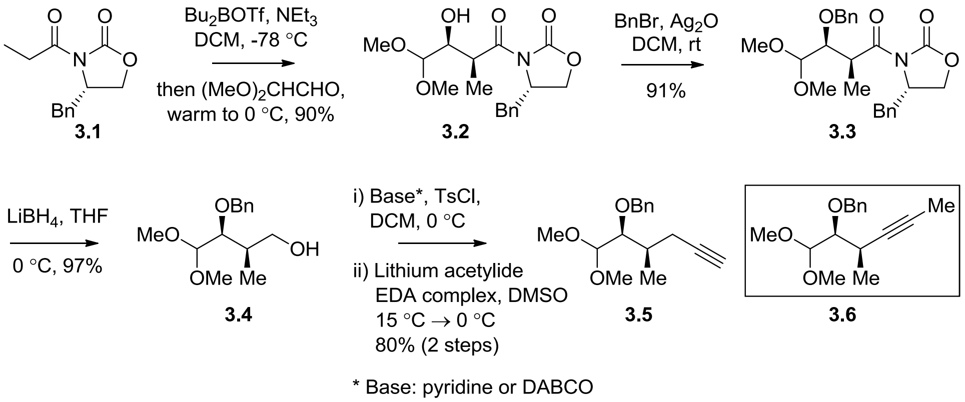
Three readily available fragments (**I**, **II**, and **III**) were prepared and combined to provide the bis[allene] macrolactone **IV** (Figure 3.2-1). For the sake of convenience, they are referred to here as the C5 alkyne, C6 alkyne, and C4 aldehyde for **I**, **II**, and **III**, respectively. Two versions of the bis[allene] macrolactone were prepared, one with benzylic ethers and the other with *meta*-fluorobenzyl ethers, masking the hydroxyl groups at C3 and C9.

Figure 3.2-1: Convergent Synthesis



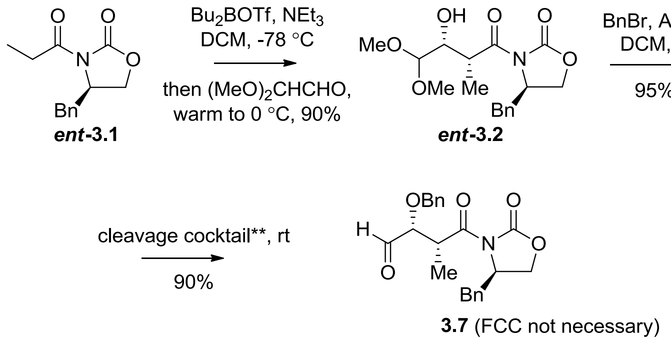
The synthesis of C6 alkyne **3.5** commenced with an Evans asymmetric aldol reaction (Scheme 3.2-1).[2] The addition of the enolate, derived from oxazolidinone **3.1**, to commercially available dimethoxyacetalaldehyde afforded the expected aldol product **3.2** as a single isomer (90%). 1,1-dimethoxyacetaldehyde is commercially available as a solution in H_2O. The anhydrous form of the aldehyde was obtained by extraction with dichloromethane (DCM) and drying over 4Å molecular sieves. Subsequent benzyl etherification was accomplished using benzyl bromide, Ag_2O, and 4Å molecular sieves in anhydrous DCM **3.3** (95%).[3] Though the reaction was done cleanly, it was quite slow (2 days). Our former colleague Dr. Ghosh reported that when using conventional NaH and benzyl bromide, the benzylation gave several by-products. He also noted that this is the first example of an Ag_2O mediated benzylation of an Evans aldol product.

Scheme 3.2-1: Synthesis of the C6 Alkyne Coupling Partner


Bu2BOTf, NEt3
DCM, -78 °C
then (MeO)2CHCHO,
warm to 0 °C, 90%
BnBr, Ag2O
DCM, rt
91%
LiBH4, THF
0 °C, 97%
i) Base*, TsCl,
DCM, 0 °C
ii) Lithium acetylide
EDA complex, DMSO
15 °C → 0 °C
80% (2 steps)
* Base: pyridine or DABCO
3.1
3.2
3.3
3.4
3.5
3.6

The hydride reduction[4] provided the desired alcohol **3.4** (97%). It was observed that the yield was reproducible when the temperature was kept at 0 °C, rather than increasing the temperature from 0 °C to room temperature. The tosylation was done within 1 hour when employing 1,4-diazabicyclo[2.2.2]octane (DABCO) as the base.[5] Dr. Ghosh reported that the tosylation took about 12 hours with pyridine. The primary tosylate was not purified; rather, the crude material was subjected to lithium acetylide to give alkyne **3.5** (80% over two steps).[6] Upon quenching with saturated aqueous solution of NH_4Cl, the side-product **3.6** formed considerably and was very difficult to separate from **3.5**. Side-product formation was prevented by cooling to 0 °C before quenching.

Scheme 3.2-2: Synthesis of the C5 Aldehyde Coupling Partner

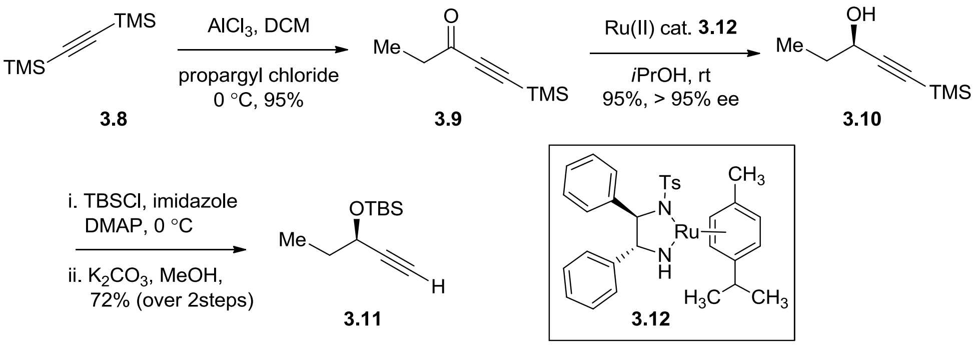


ent-3.1
Bu2BOTf, NEt3
DCM, -78 °C
then (MeO)2CHCHO,
warm to 0 °C, 90%
ent-3.2
BnBr, Ag2O
DCM, rt
95%
ent-3.3
cleavage cocktail**, rt
90%
3.7 (FCC not necessary)

** HOAc:TFA: H_2O (4:1:1), 3hrs 30mins
HOAc:H_2O (4:1), 12hrs

The antipode of **3.3** was prepared from ***ent*-3.1** using identical procedures (Scheme 3.2-2). For the coupling with alkyne **3.5**, ***ent*-3.3** was hydrolyzed under acidic conditions. Dr. Ghosh reported that the hydrolysis took 12 hours in 80% aqueous acetic acid, but when trifluoroacetic acid was added into the cleavage cocktail, the hydrolysis went remarkably faster (3.5 hours). It was found that purification of **3.7** by flash column chromatography (FCC) was not necessary for the subsequent alkynylation step. Notably, significant amount of **3.7** decomposed on silica gel.

Scheme 3.2-3: Synthesis of the C5 Alkyne Coupling Partner



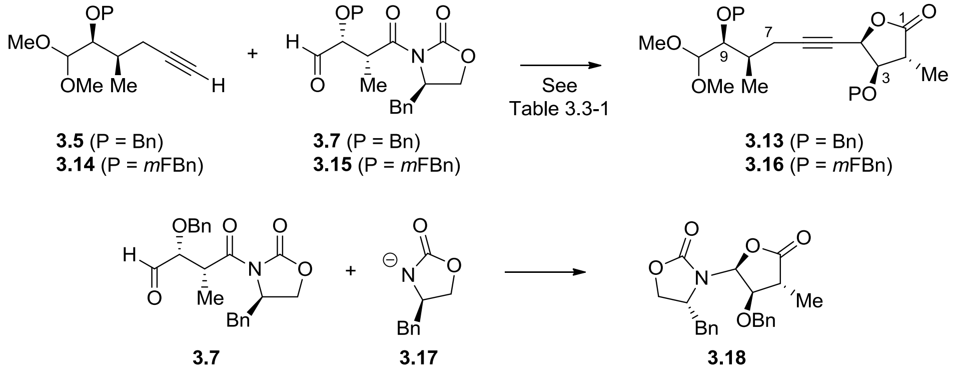
3.8
AlCl3, DCM
propargyl chloride
0 °C, 95%
3.9
Ru(II) cat. 3.12
iPrOH, rt
95%, > 95% ee
3.10
i. TBSCl, imidazole
DMAP, 0 °C
ii. K2CO3, MeOH,
72% (over 2steps)
3.11
3.12

Propargyl alcohol **3.11** was prepared from commercially available bis-TMS acetylene **3.8**, adopting a procedure developed by our former colleague, Dr. Stephen D. Lotesta (Scheme 3.2-3).[7] $AlCl_3$-promoted Friedel Crafts-type acylation gave the alkynone **3.9** (95%), which was followed by a Noyori asymmetric reduction of the ketone. The propargyl alcohol was obtained in 90% yield with >95% ee (Mosher ester analysis).[8] The alcohol was protected as the *tert*-butyldimethylsilyl (TBS) ether, then the trimethylsilyl (TMS) group was cleaved in the same pot (72% yield over two steps).

3.3. Optimization of the 1st Coupling (Step 6): C1 to C9

Two coupling units, aldehyde **3.7** and alkyne **3.5**, were chosen for a chelation-controlled addition to generate propargyl lactone **3.13** (Scheme 3.3-1). Dr. Ghosh reported that the chelation-controlled addition of the zinc alkynylide of alkyne **3.5** to aldehyde **3.7**, followed by spontaneous *in situ* lactonization gave **3.13** in a modest 64% yield as a 8:1 mixture of diastereomers (entry 1, Table 3.3-1). Using the *meta*-fluorobenzyl ether, he also reported **3.16** in a 35 % yield as a 6:1 mixture of diastereomers (entry 4, Table 3.3-1). However, my colleague Kai Liu and I realized that the reaction yield is not reproducible using Dr. Ghoshs' condition for both benzylic and *meta*-fluorobenzylic ethers.

Scheme 3.3-1: The 1st Coupling: Chelation-Controlled Alkynylation



In his thesis[1], he argued that the propargylation competes with a side reaction in which the starting aldehyde **3.7** undergoes rearrangement to **3.18**, promoted by the auxiliary anion **3.17**. The amount of anion **3.17** increases as the propargylation proceeds. Consequently, the relative rate of the side reaction increase, relative to the rate of formation of the desired product.[9] Minimizing this side reaction by adjusting temperature proved difficult as the propargylation only takes place within a narrow temperature range. The reaction is sluggish below -10 °C, but proceeds rapidly at 0 °C.

Table 3.3-1: Optimization of 1st Coupling Reaction

Entry	Equivalents			Addition temp. (°C)		Addition Rate	Yield
	Alkyne	Aldehyde	Base (metal) [b]	$ZnBr_2$	Aldehyde		
1[a] (P=Bn)	1	1	1	- 40 → -10	-10	1 hr	57% (8:1)
2 (P=Bn)	2.3	1	2.3	- 40 → -10	10	1 hr	58% (6:1)
3 (P=Bn)	2.3	1	2.3 (3.3)	- 40 → -10	10	1 hr	63% (8:1)
4[a] (P=*m*FBn)	1.3	1	1.3	-78	-10	2 hrs	35% (6:1)

5 (P=*m*FBn)	3.5	1	3.5	-78	-10	30min	30% (5:1)
6 (P=*m*FBn)	2.5	1	2	-78 (Et_2AlCl)	-10	30min	63% (1:2)

a) Reaction done by Dr. Ghosh and reported in his Ph.D thesis and not reproducible.[1] b) Indicated when the equivalence of metal is different from that of base.

This reaction was optimized by altering several variables such as the equivalents of alkyne and base, the equivalents of chelation metal, the addition temperature of aldehyde, the chelation metal, and the rate of aldehyde addition (Table 3.3-1).

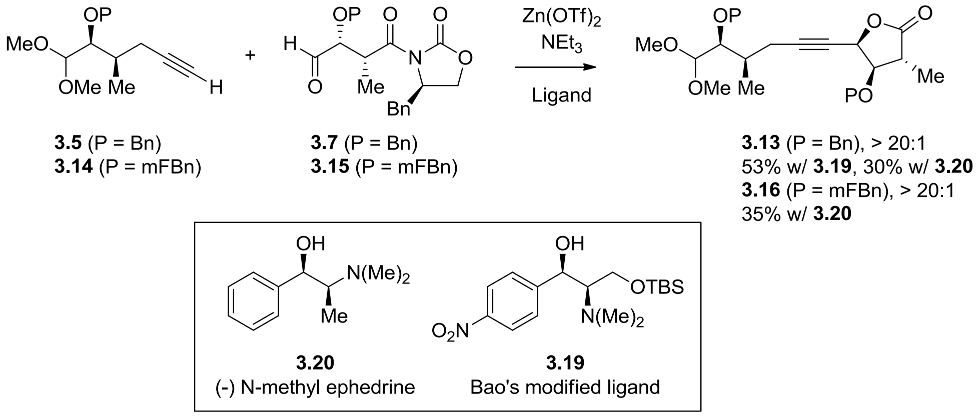
For the benzylic ether, the reaction yield and selectivity were reproducible when using a large excess of alkyne (entry 2), and the diastereoselectivity was better when the equivalents of chelation metal was the sum of the equivalents of alkyne and aldehyde (entry 3). To avoid the side reaction, the temperature was kept at 10 °C for 8 hours after the addition of aldehyde.

After thorough optimization, reproducible conditions were found and the reaction was scaled up to a maximum of 3.5 g (C4 aldehyde). The highest obtained yield of **3.13** was 63% (entry 3). Based on recovered alkyne **3.5**, however, the yield is an excellent 95%.

Optimization studies using the *meta*-fluorobenzylic ether resulted in a highest yield of 35%. From all the data that was collected, it was concluded that zinc may not be an effective metal in the case of the *meta*-fluorobenzylic ether. The electron-withdrawing nature of the *meta*-fluorobenzyl group may be reducing the ability of the aldehyde oxygen to chelate with the metal. Therefore, we explored alternative metals. Using diethylaluminum chloride as a Lewis acid, we achieved a higher combined yield of the product, but the selectivity was poor. The undesired diastereomer was preferred under these conditions, giving a 2:1 ratio of undesired/desired diastereomers.

Chelation-controlled addition of zinc alkynilides to α-alkoxy aldehydes is known to proceed with high *syn* selectivity.[10] Although the expected stereochemistry was confirmed later by X-ray crystallography data of erythromycinoids (*See* Chapter 4), it was not clear at this moment if the desired diastereoselection was achieved. The *syn* selectivity in the major diastereomer of **3.13** was rationalized a number of ways such that: *a)* the coupling constant of the propargylic proton in ^{1}H NMR appeared (1H, td, 2.0 Hz, 6.5 Hz) in the major isomer and (1H, td, 2.0 Hz, 5.0 Hz) in the minor isomer. Although this difference is small, the trend is the same as that for similar substrates reported in the literature;[11] *b)* Carreira's asymmetric alkynylation gave the desired product **3.13** as the sole product.[12] When (-)-*N*-methylephedrine **3.20** was used, the reaction yield was 30% with complete diastereoselection. Jiang et al. reported the modified ligand **3.19** for the Carreira reaction.[13]

Scheme 3.3-2: The 1st Coupling: Carreira Asymmetric Alkynylation



3.5 (P = Bn)
3.14 (P = mFBn)

3.7 (P = Bn)
3.15 (P = mFBn)

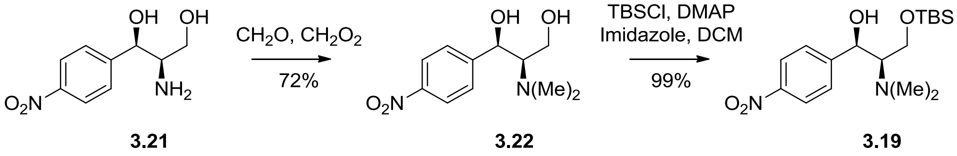
3.13 (P = Bn), > 20:1
53% w/ **3.19**, 30% w/ **3.20**
3.16 (P = mFBn), > 20:1
35% w/ **3.20**

3.20
(-) N-methyl ephedrine

3.19
Bao's modified ligand

3.19 was synthesized in two steps according to Jiang et al. (Scheme 3.3-3).[14] For both protecting groups, it gave complete diastereoselection with moderate yields, but a large excess of alkyne was required (4 equivalents). Since among the three coupling units the synthesis of alkyne **3.5** is the longest linear sequence, it makes $ZnBr_2$-mediated chelation-controlled alkynylation the most practical approach to pushing material forward.

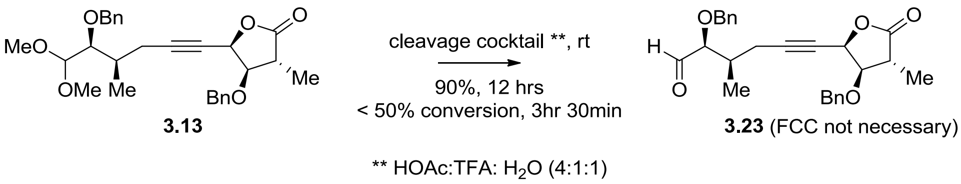
Scheme 3.3-3: Synthesis of Jiang's Modified Carreira Ligand[14]


OH
OH
NH2
O2N
3.21
CH2O, CH2O2
72%
OH
OH
N(Me)2
O2N
3.22
TBSCl, DMAP
Imidazole, DCM
99%
OH
OTBS
N(Me)2
O2N
3.19

3.4. Optimization of the 2nd Coupling: C1 to C14

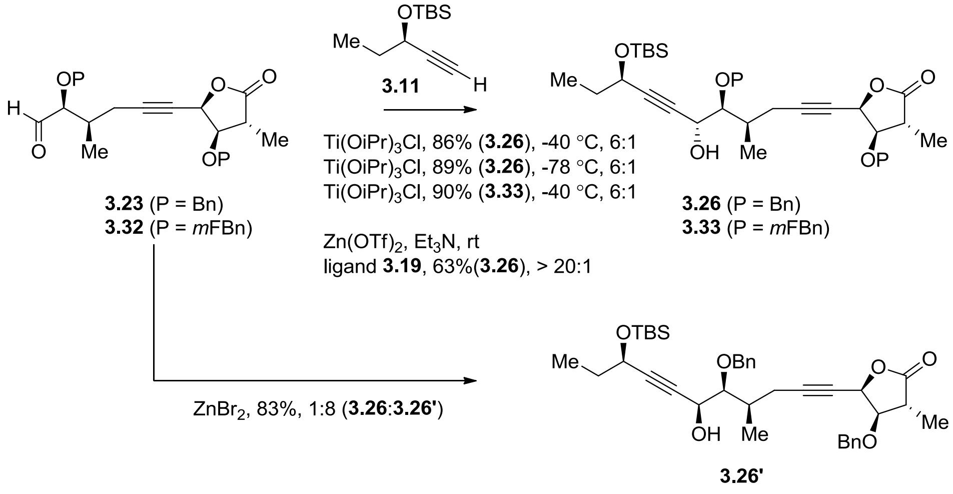
When propargyl lactone **3.13** was subjected to the cleavage cocktail (HOAc:TFA:H_2O = 4:1:1), less than 50% was converted to the desired aldehyde **3.23** in 3.5 hours. This was in sharp contrast to the conversion of ***ent*-3.3** → **3.7** in 90% yield under the same conditions (Scheme 3.4-1).

Scheme 3.4-1: Hydrolysis of the Acetal (Step 7)


OBn
MeO
OMe Me
BnO
Me
O
O
3.13
cleavage cocktail **, rt
90%, 12 hrs
< 50% conversion, 3hr 30min
** HOAc:TFA: H2O (4:1:1)
H
O
OBn
Me
BnO
Me
O
O
3.23 (FCC not necessary)

Nonetheless, subjecting the acetal **3.13** to a 4:1:1 mixture of HOAc, TFA and H_2O afforded the aldehyde **3.23** in 90% yield after 12 hours of stirring at room temperature. The aldehyde **3.23** turned out to be somewhat unstable to silica gel chromatography. Therefore, it was quickly advanced to the next reaction without chromatography.

Scheme 3.4-2: The Second Coupling (Step 8)

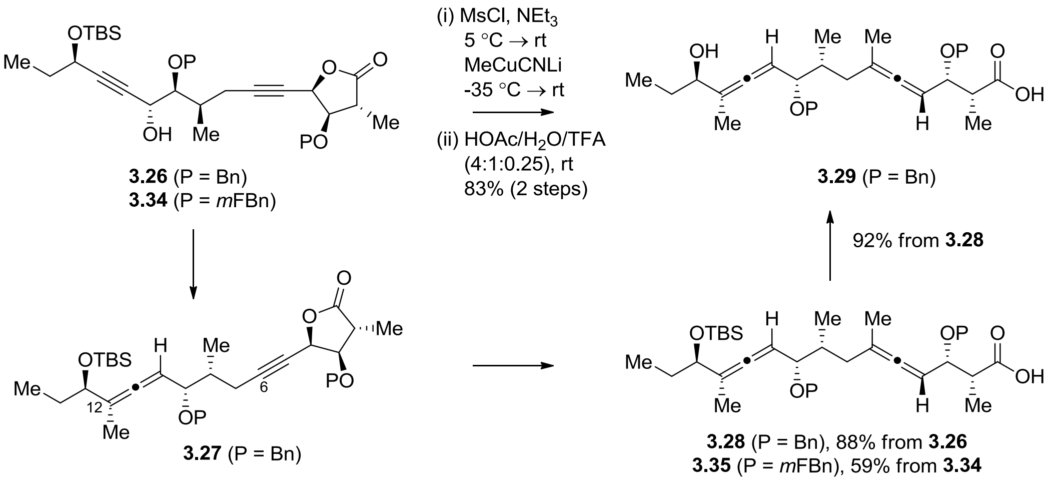

OTBS
Me
3.11
H
OP
H
O
Me
O
O
OP
Me
3.23 (P = Bn)
3.32 (P = mFBn)
Ti(OiPr)3Cl, 86% (3.26), -40 °C, 6:1
Ti(OiPr)3Cl, 89% (3.26), -78 °C, 6:1
Ti(OiPr)3Cl, 90% (3.33), -40 °C, 6:1
Zn(OTf)2, Et3N, rt
ligand 3.19, 63%(3.26), > 20:1
OTBS
Me
OP
OH
Me
O
O
OP
Me
3.26 (P = Bn)
3.33 (P = mFBn)
ZnBr2, 83%, 1:8 (3.26:3.26')
OTBS
Me
OBn
OH
Me
O
O
BnO
Me
3.26'

Non-chelation controlled additions of titanium triisopropoxy alkynilides to α-alkoxy aldehydes are known to give products with high *anti* selectivity.[15] The alkynylide derived from **3.11** was combined with **3.23** in the presence of titanium triisopropoxide chloride. These addition products were also readily separable and the major product (**3.26**) was taken forward (89%, dr 6:1). The stereochemistry was postulated at this stage of the synthesis by the comparison of a major product from chelation controlled zinc alkynilide, using $ZnBr_2$ instead of $Ti(OiPr)_3Cl$ and Carreira asymmetric alkynylation. Using $ZnBr_2$ as the chelation metal, Dr. Ghosh reported that the product **3.27** was obtained in 83% yield, which strongly supported the structural assignment. Carreira's asymmetric alkynylation with the modified ligand **3.19** gave the desired product with complete diastereoselection in 63% yield.

3.5. Synthesis of the Bis[allene] Seco-acid

A single-flask conversion of the hydroxyl unit of **3.26** to bis[allene] **3.28** was accomplished by converting **3.26** to the mesylate followed by addition of excess lower order methyl cyanocuprate (Scheme 3.5-1). Deprotection of the TBS group under acidic conditions gave the bis[allene] seco-acid **3.29** in 92% yield. It was found that crude **3.28** could also be converted to **3.29** without flash column chromatography (83% over two steps).

Scheme 3.5-1: Synthesis of the Bis[Allene] Seco-Acid (Step 10)


OTBS
Me
OP
O
O
OH
Me
PO
Me
3.26 (P = Bn)
3.34 (P = mFBn)
(i) MsCl, NEt3
5 °C → rt
MeCuCNLi
-35 °C → rt
(ii) HOAc/H2O/TFA
(4:1:0.25), rt
83% (2 steps)
OH
H
Me
Me
OP
O
OH
Me
OP
H
Me
3.29 (P = Bn)
92% from 3.28
O
O
Me
OTBS
H
Me
Me
12
6
PO
OP
Me
3.27 (P = Bn)
OTBS
H
Me
Me
OP
O
OH
Me
OP
H
Me
3.28 (P = Bn), 88% from 3.26
3.35 (P = mFBn), 59% from 3.34

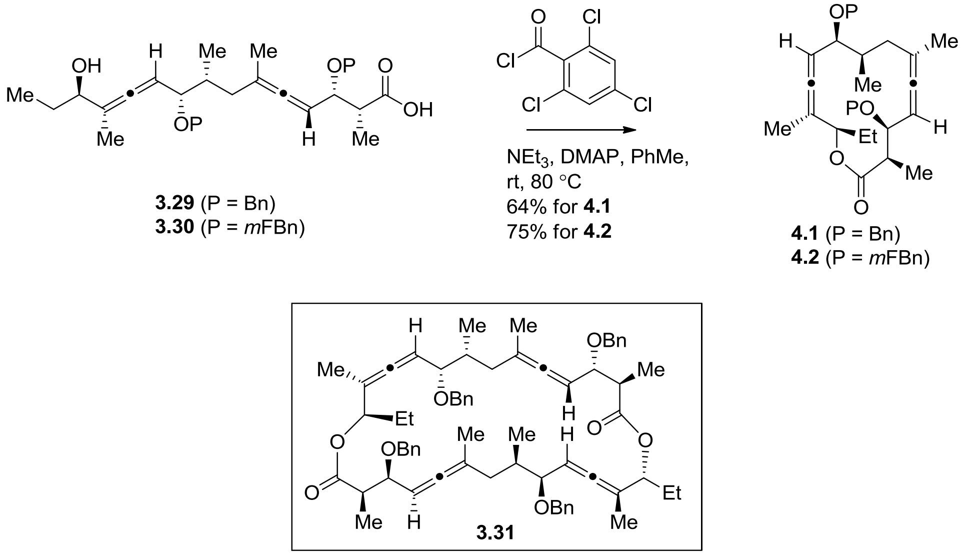
When the temperature was kept below -20 °C after the addition of lower order methyl cyanocuprate to *in situ* mesylate, the major product was mono[allene] **3.27**. Although the single-flask conversion to the bis[allene] is very convenient, mono[allene]

3.27 is a useful intermediate that offers the flexibility to generate heterogeneous functionalities on C6 and C12.

3.6. Macrolactonization

Yamaguchi macrolactonization of seco-acids **3.29** and **3.30** provided bis[allene] macrolactones **4.1** and **4.2** in 64% and 75% yield, respectively. It is well precedented that the addition of sp^2 centers on the periphery of the ring decreases the transannular ring strain, thereby it facilitates the ring closure.[16] The major byproduct of these lactonizations is the dimer **3.31** derived from the seco-acids. Four methyl groups on allenes as doublet of doublet in 1H NMR confirm the dimer formation.

Scheme 3.6-1: Synthesis of Bis[Allene] Macrolactone (Step 11)


OH
H
Me
Me
OP
O
Me
OH
Me
OP
H
Me
O
Cl
Cl
Cl
Cl
OP
H
Me
Me
PO
Me
Et
H
O
Me
O
H
Me
Me
OBn
Me
Me
OBn
H
Et
O
Me
Me
H
O
O
OBn
O
Et
Me
H
OBn
Me

3.29 (P = Bn)
3.30 (P = *m*FBn)

NEt$_3$, DMAP, PhMe,
rt, 80 °C
64% for **4.1**
75% for **4.2**

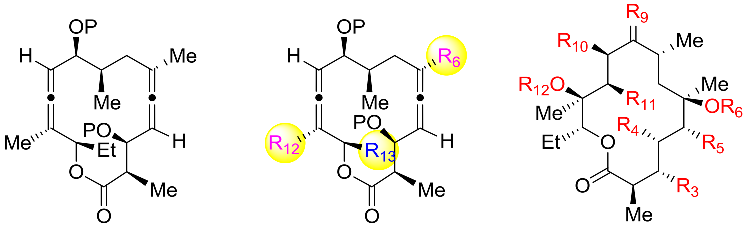
4.1 (P = Bn)
4.2 (P = *m*FBn)

3.31

3.7. Conclusion: Flexibility of the Synthetic Route

The bis[allene]macrolactone was prepared in a longest linear sequence of 11 steps (overall yield ~ 18%) by two consecutive alkynations (*steps 6 & 8*), single-flask bis[allene] synthesis (*step 9*) and macrocyclization (*step 11*). Considering the relatively late-stage elaboration of the carbon backbone (C6, C12, and C13) and adjustable methodologies, the flexibility of the current synthetic route can be easily envisioned for incorporation of hetero-functionalities at C6, C12, and C13 (**V**, Figure 3.7-1).

Figure 3.7-1: Flexibility of the Synthetic Route


OP
H
Me
Me
PO
Et
H
O
Me
O
OP
H
R_6
Me
PO
R_{12}
R_{13}
H
O
Me
O
R_9
R_{10}
Me
$R_{12}O$
R_{11}
Me
OR_6
Me
Et
O
R_4
R_5
O
R_3
Me

IV **V** **VI**

In Chapter 4, I will discuss the synthesis of erythromycinoids (**IV** → **VI**).

3.8. References

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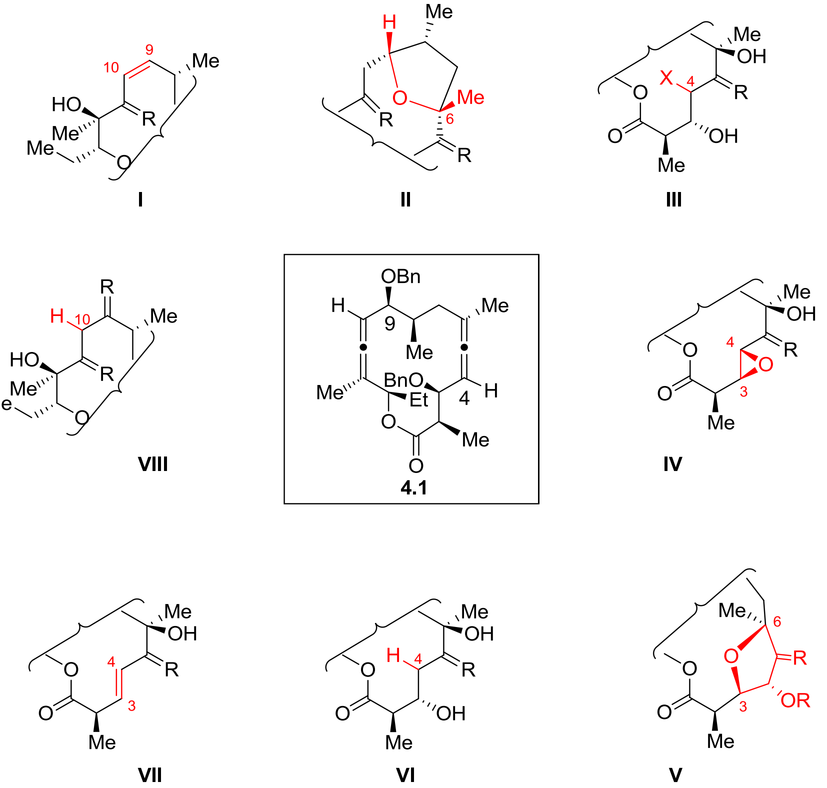
Chapter 4

The Synthesis of De Novo Erythromycinoids

4.1. Introduction

To date, bis[allene] macrolactone **4.1** has been converted to over 30 novel macrolides (Figure 4.1-1). These de novo analogs serve to validate the strategy and lay the foundation for further work. Taken together, the allene-based transformations employed in this study, such as DMDO oxidation/nucleophile addition, allene osmylation/electrophile addition, halohydrin formation, allene oxide rearrangement, spirodiepoxide (SDE) rearrangement, benzylic migration/elimination, mono- and bis-oxidation of bis[allene], chelation-controlled reduction, and oxime formation, demonstrate that 9 of 11 carbons in ring core of erythromycin can be modified. It is especially noteworthy that each congener was made in less than three steps from a bis[allene] macrolactone of type **4.1**. The congeners contain a variety of functional groups including enone, epoxy ketone, furanone, α'-halo ketone (fluoro-, chloro-, bromo-), vinyl bromide, and furan. These variations of the macrolactone core skeleton are novel, particularly in comparison to conventional analogs, inaccessible from the natural product, and, most importantly, represent further opportunities to create new derivatives. Herein is presented the detailed reaction conditions, mechanistic rationales, and synthetic significance of each compound synthesized to date.

Figure 4.1-1: Synthesis of Erythromycinoids



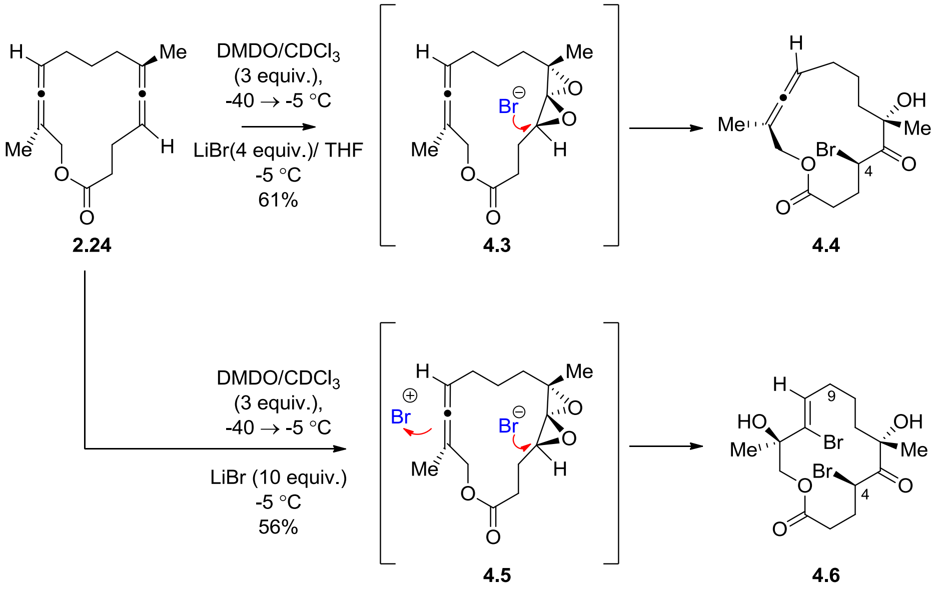
4.2. *DMDO Oxidation and Nucleophile Addition*

A recent disclosure[1] from our group demonstrated that lithium halide salts are better nucleophiles than tetraalkylammonium salts for addition reactions to SDEs. The former reacts rapidly and reliably to give haloketones in excellent yield upon addition to SDEs, derived from simple trisubstituted allenes.

The model macrolactone, **2.24**, was treated with DMDO (3 equiv.). Scheme 4.2-1 outlines the epoxidation/bromide addition reactions of model macrolactone **2.24**. Addition of LiBr to crude SDE **4.3** gave *α*-bromo ketone **4.4**. X-ray crystallographic analysis (Figure 4.2-1) confirmed the bromide addition at C4 which is anti to the hydroxy at C6 as expected.[2]

However, the addition of large excess LiBr (10 equiv.) to the SDE, without removing excess DMDO, resulted in bromine addition to the central carbon (C11) of the unreacted allene (**4.5** → **4.6**). Bromine isotope effect (^{79}Br and ^{81}Br) in HRMS confirmed the presence of two bromines ([M+]:[M+2]:[M+4] = 51:100:47) in **4.6**.

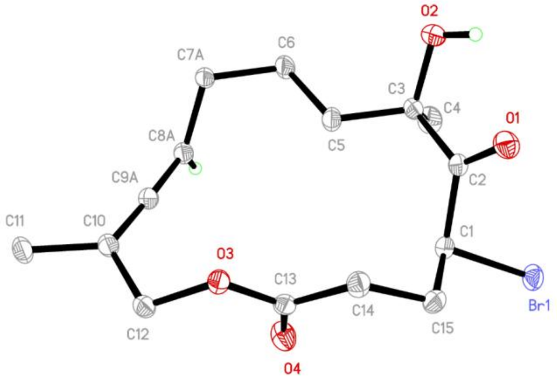
Scheme 4.2-1: Nucleophile Addition to SDE



DMDO/CDCl$_3$
(3 equiv.),
-40 → -5 °C
LiBr(4 equiv.)/ THF
-5 °C
61%
DMDO/CDCl$_3$
(3 equiv.),
-40 → -5 °C
LiBr (10 equiv.)
-5 °C
56%
2.24
4.3
4.4
4.5
4.6
H
Me
O
OH
Br
4
9
HO

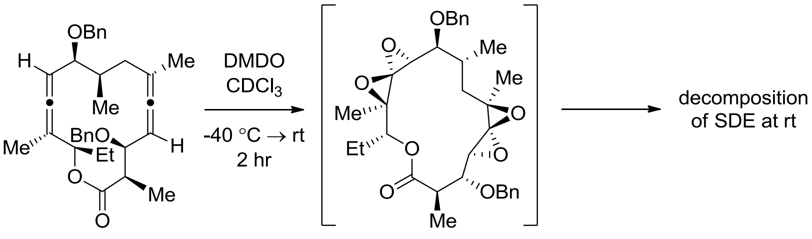
^{1}H NMR spectrum of **4.6** revealed the presence of a single vinyl proton resonance (6.03 ppm, CDCl$_3$). The vinyl proton resonance was coupled to both methylene protons of C9 (2.37 ppm and 2.31 ppm, CDCl$_3$) as determined by HMBC spectroscopy. A strong nOe was observed between the vinyl proton and the methyl protons at C12, confirming Z-vinyl bromide/β-C12 alcohol in **4.6**.

Figure 4.2-1: X-ray Crystal Structure of 4.4


O2
C7A
C6
C3
C4
O1
C8A
C5
C2
C9A
C1
C11
C10
O3
C13
C14
Br1
C12
C15
O4

The same strategy was applied to fully functionalized bis[allene] macrolactone **4.1**. Treatment of **4.1** with DMDO (6 equiv.) in $CDCl_3$ gave bis[SDE] **4.7** (Scheme 4.2-2). Similar to the model study,[3] one allene underwent oxidation to SDE below 0 °C while the other allene persisted. After 15 minutes at room temperature, the unreacted allene was completely oxidized to give the second SDE. However, at room temperature, significant cleavage of the benzyl ether was observed (~ 25% by ^{1}H NMR).[4] Use of $CHCl_3$ as solvent instead of $CDCl_3$ resulted in the recovery of more benzyl cleaved product.

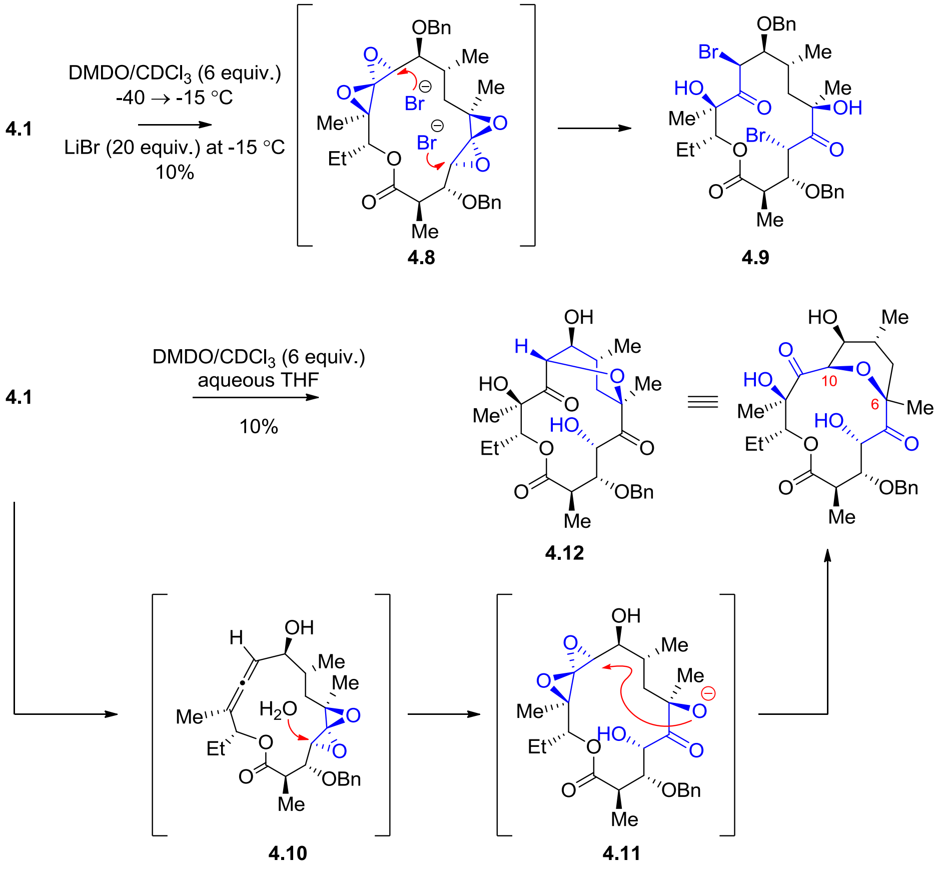
Scheme 4.2-2: Dr. Ghosh's Attempt to Add Nucleophile to Bis[SDE][4]


OBn
H
Me
Me
BnO
Et
Me
H
O
O
Me
DMDO
CDCl3
-40 °C → rt
2 hr
OBn
O
Me
Me
O
Me
O
Et
O
O
O
OBn
Me
decomposition
of SDE at rt

4.1 **4.7**

Due to the instability of SDEs, LiBr was added at -15 °C to bis[SDE]s (**4.1** → **4.8**, Scheme 4.2-3). Albeit in low yield (10%), we isolated the first nucleophile adduct **4.9** to the corresponding SDEs from fully functionalized macrocyclic bis[allene] **4.1**. Remarkably, the reaction installed two ketones, two halides, and four stereocenters in a single flask transformation. The well-known versatility of haloketones[5] also gives more importance on the utility of SDE chemistry.

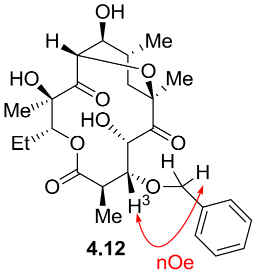
Scheme 4.2-3: Nucleophile Addition to SDE


4.1
DMDO/CDCl$_3$ (6 equiv.)
-40 → -15 °C
LiBr (20 equiv.) at -15 °C
10%
4.8
4.9
4.1
DMDO/CDCl$_3$ (6 equiv.)
aqueous THF
10%
4.12
4.10
4.11
OBn
Me
Br
HO
OH
Et
H$_2$O
10
6

Crandall et al. reported the formation of α,α’-dihydroxy ketones upon the oxidation of acyclic trisubstituted allenes by DMDO in aqueous THF.[6] Under the same conditions, we have also observed hydroxyl addition to SDEs **4.12**, however the tertiary C6 alkoxide subsequently added into the C10 SDE terminus **4.11**. It was not a surprising outcome when considering the reactivity of the hydroxyl at C6 to form 6,9-hemiketal in erythromycin.[7] Excess DMDO was not removed in either reaction in light of the instability of the macrocyclic SDE and its tendency to give complex mixtures upon DMDO removal at room temperature.

As per the previous observation of Dr. Partha Ghosh,[4] there was benzylic ether cleavage. This type of oxidative cleavage is known and presumed to proceed via insertion of oxygen from DMDO into the benzylic C-H bond to give a hemiacetal which collapses into the alcohol and benzaldehyde.[8] HRMS confirmed the cleavage of one benzylic group and nOe between the benzylic protons and H3 confirmed the location of cleavage is at C3 (*See* Figure 4.2-2).

Figure 4.2-2: Key nOe Analysis for Benzylic Ether Location in 4.12



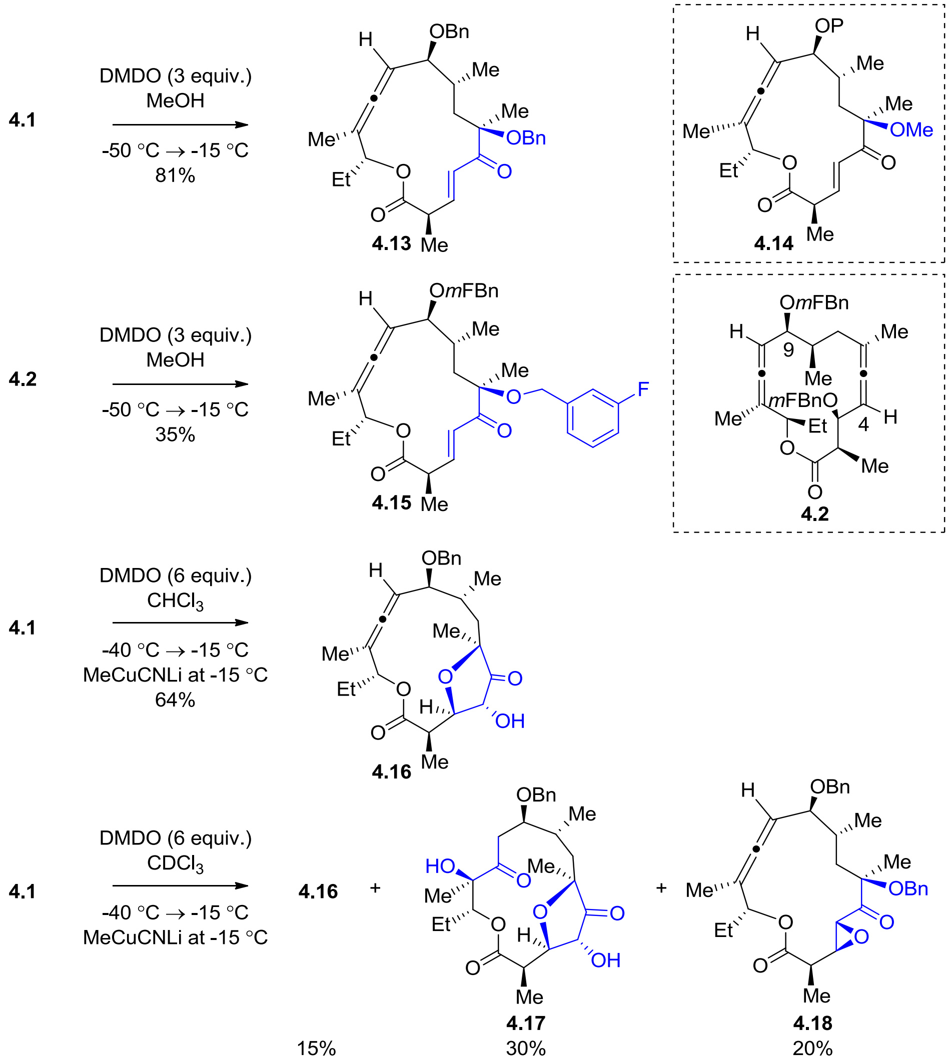
4.12

4.3. *DMDO Oxidation and Rearrangement*

Scheme 4.3-1 shows products derived from epoxidation of **4.1** and **4.2** by DMDO. Upon the exposure of **4.1** to DMDO in methanol, the reaction smoothly delivered αbenzyloxyenone **4.13**. However, epoxidation with DMDO in chloroform, followed by treatment with Lewis acid,[1] delivered dihydrofuranone **4.16**. Remarkably, lower order lithium methyl-cyanocuprate efficiently promoted this reaction giving **4.16** in 64% yield.

Benzylic migration product **4.13** is presumably due to hydrogen-bond donation[9, 10] by methanol to the highly reactive allene oxide intermediate leading to opening of the strained heterocycle faster than a 2nd epoxidation event.

Scheme 4.3-1: DMDO Oxidation and Rearrangement

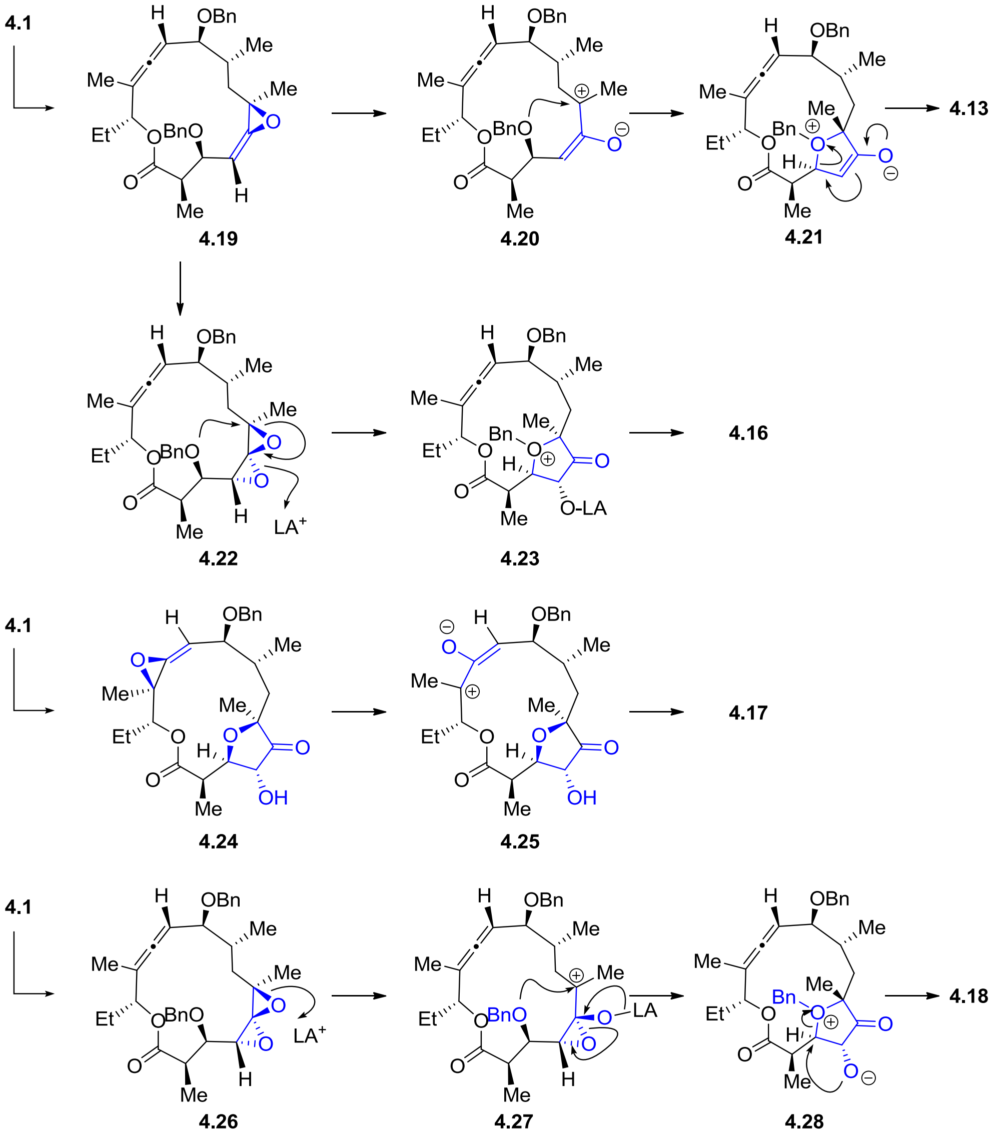

4.1
DMDO (3 equiv.)
MeOH
-50 °C → -15 °C
81%
4.13
4.14
4.2
DMDO (3 equiv.)
MeOH
-50 °C → -15 °C
35%
4.15
4.2
4.1
DMDO (6 equiv.)
CHCl3
-40 °C → -15 °C
MeCuCNLi at -15 °C
64%
4.16
4.1
DMDO (6 equiv.)
CDCl3
-40 °C → -15 °C
MeCuCNLi at -15 °C
4.16
+
4.17
+
4.18
15%
30%
20%

When the less electron-rich *meta*-fluorobenzyl group is used for the protection of the C3 and C9 hydroxyls (**4.2**), I observed the same pattern of migration from C3 → C6 as shown in **4.13** giving **4.15**. This suggests that it might be a good methodology for the installation of methoxy group at C6 (**4.14**), which is essential to avoid 8,9-anhydro-6,9-hemi-ketal formation from erythromycin.[7] Crandall et al. reported the addition of alcohols (e.g., *n*-PrOH, *i*-PrOH) to SDEs in the presence of base (i.e., K_2CO_3, NaH),[6] however no methanol addition product was observed in reaction of **4.1** and **4.2**.

Upon the oxidation of allene **4.1** using DMDO, SDE formation occurred readily at the C4-C6 allene at low temperature (-20 °C). The nearby electron withdrawing ester functionality retarded oxidation at the C10 to C12 allene. When 6 equivalents of DMDO were used, the mixture of products was obtained including mono-oxidized product **4.16**, bis-oxidized product **4.17**, containing dihydrofuranone and 10-desmethyl α-hydroxy ketone functionalities, and epoxy ketone **4.18**.

Interestingly in the cases where benzylic migration occurred (**4.13** and **4.18**), the stereochemistry at C6 is retained. However, when the benzylic ether is lost (**4.16** and **4.17**), an inversion of configuration at C6 is observed. We postulate that formation of **4.13**, **4.16**, **4.17**, and **4.18** are closely related and involve either allene oxide opening (**4.19** → **4.20**) or SDE opening (**4.22** → **4.23**) at C6 and subsequent capture of the C3 benzyloxy group (Scheme 4.3-2). The retained stereochemistry at C6 in **4.13** may reflect the comparatively high stability of oxyallyl zwitterions **4.20**, which could explain benzyloxy capture with overall retention of configuration. In the case of **4.16**, two factors such as the comparatively low stability of a cation derived from SDE and the proximity of the C3 benzyl ether to C6 could lead to **4.23** directly with inversion of configuration at C6. This mechanistic framework is also consistent with the reaction conditions used for these transformations though further studies are needed to evaluate the hypothesis.

Scheme 4.3-2: Mechanistic Rationale for 4.13, 4.16, 4.17, and 4.18


4.1
4.19
4.20
4.21
4.13
4.22
LA+
4.23
O-LA
4.16
4.1
4.24
4.25
4.17
4.1
4.26
LA+
4.27
LA
4.28
4.18
H
OBn
Me
Et
BnO
O
OH

Analogous to **4.20**, subsequent bis-oxidized product **4.17** may involve the formation of oxyallyl zwitterion **4.25** and subsequent water addition from the more accessible top face accounts for retention of configuration at C12 (Scheme 4.3-2). The instability of SDEs in the presence of Lewis acid could form a transient carbocation **4.26**. If the carbocation was captured by C3 benzyloxy group and concurrent (or subsequent) second epoxide opening **4.27** could lead to oxonium **4.28**. The cyclic oxonium ion can then be opened up by closure of the oxygen anion onto C3 furnishing benzyloxy migrated epoxy ketone **4.18**.

4.4. Structural Analysis of 4.13 and 4.18

Analysis of the spectral evidence (^{1}H NMR, ^{13}C NMR IR) presented below established the structure of compounds **4.13** and **4.18**.

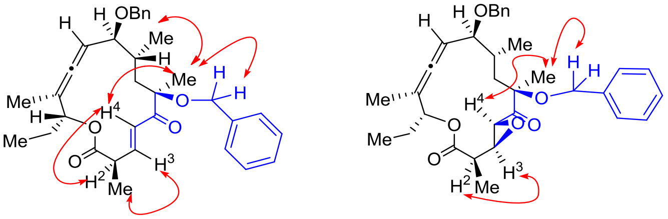
The NMR-spectra of **4.13** revealed the presence of olefin protons (6.73 and 7.05 ppm, $CDCl_3$). A *trans* configuration was established by the large vicinal couplings constants (ca. 15.7 Hz) observed (Figure 4.4-1). A coupling constant of 1.1 Hz corresponding to allylic coupling of H4 with H2 was observed as well as vicinal coupling of H2 with H3 (8.2 Hz). This firmly established the structure of this portion of the molecule.

The NMR-spectra of **4.18** revealed the presence of epoxide protons (3.37 and 3.97 ppm, $CDCl_3$) as well as the corresponding ^{13}C NMR signals (59.4 and 52.4 ppm, $CDCl_3$). The epoxide protons were split by vicinal couplings (ca. 1.8 Hz) and the magnitude of these couplings established the epoxide geometry as *trans*, which was expected as per our mechanistic rationale.

Benzylic migration from C3 to C6 is unambiguously shown by nOes observed between the benzylic protons and the C6 methyl protons in **4.13** and **4.18**. Extensive 2D nOe analysis revealed that the stereochemistry at C6 of **4.13** and **4.18** is retained in comparison to **4.1** (*See* Figure 4.4-1).

Observation of a signal at 1653cm^{-1} in the infrared spectrum, characteristic of the carbonyl at enones, confirmed the presence of an enone moiety in **4.13**.

Figure 4.4-1: Key nOe Analysis of 4.13 and 4.18



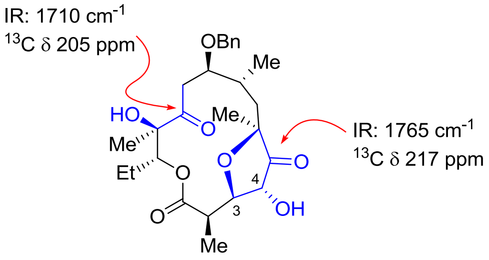
4.13

4.18

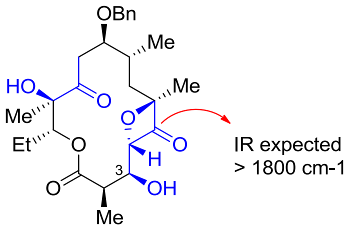
4.5. Structural Analysis of 4.16 and 4.17

Due to their structural complexity, it is very hard to elucidate the structural assignment of **4.16** and **4.17** unambiguously. For compounds **4.16** & **4.17**, the initial concern was whether the newly formed ring was dihydrofuranone or oxetanone. It was initially considered to be an oxetanone (**4.29**, Figure 4.5-1) because the ^{13}C NMR signal at 217 ppm was in close agreement with literature values for other compounds containing oxetanone functionality ($\delta \approx 215$ ppm). Additionally, previous studies on model macrocyclic bis[allene] in which oxetanone was formed encouraged us to favor structure **4.29**.[3] However, FTIR analysis showed a carbonyl absorption frequency at 1765 cm^{-1}, which is consistent with furanone.[11] Literature precedent for oxetanone containing molecules showed that an IR signal at 1802-1800 cm^{-1} corresponded to the oxetanone carbonyl.[12]

Figure 4.5-1: Structural Analysis of 4.17


IR: 1710 cm-1
13C δ 205 ppm
OBn
Me
HO
Me
Me
Et
O
O
O
O
O
3
4
OH
Me
IR: 1765 cm-1
13C δ 217 ppm

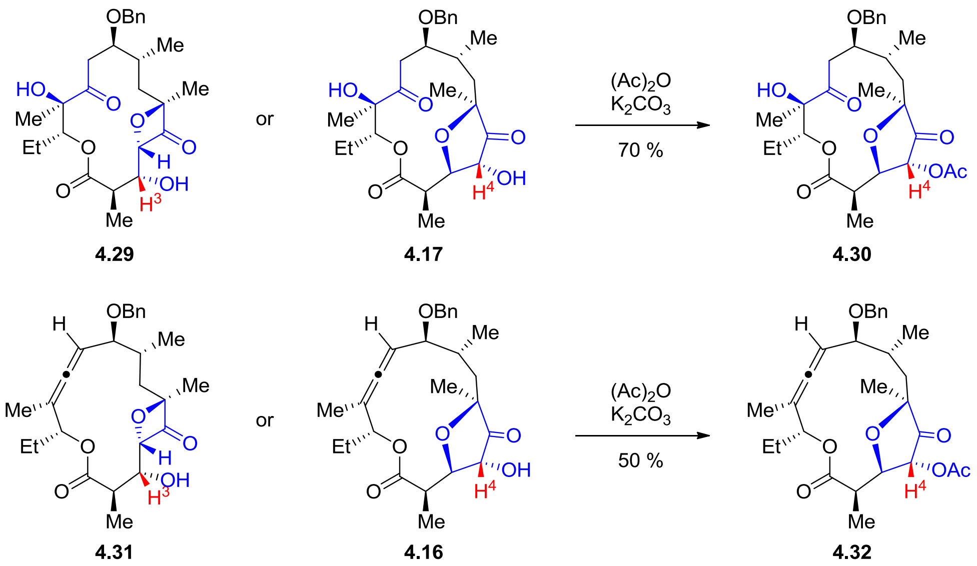
4.17


OBn
Me
Me
HO
Me
Et
O
O
O
O
O
H
3
OH
Me
IR expected
> 1800 cm-1

4.29

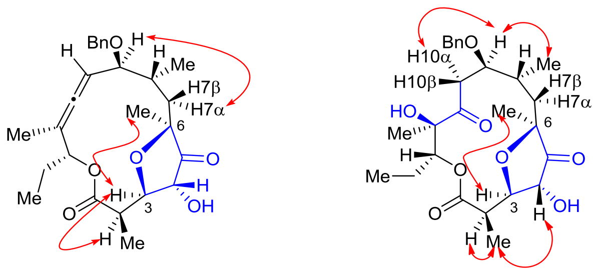
To further verify ring size and, by extension, the location of the hydroxyl (C4 in **4.17**, C3 in **4.29**), the material was acylated using acetic anhydride (Scheme 4.5-1). Upon acylation, the H4 proton in **4.30** shifted 1 ppm downfield in the 1H NMR spectrum, but the H3 proton did not show the shift. The same acylation strategy was used for mono-oxidized compound and returned similar data confirming the structure of **4.32**.

Scheme 4.5-1: Acylation of 4.13 & 4.14 for Structural Proof of Furanone


4.29
or
4.17
(Ac)2O
K2CO3
70 %
4.30
4.31
or
4.16
(Ac)2O
K2CO3
50 %
4.32

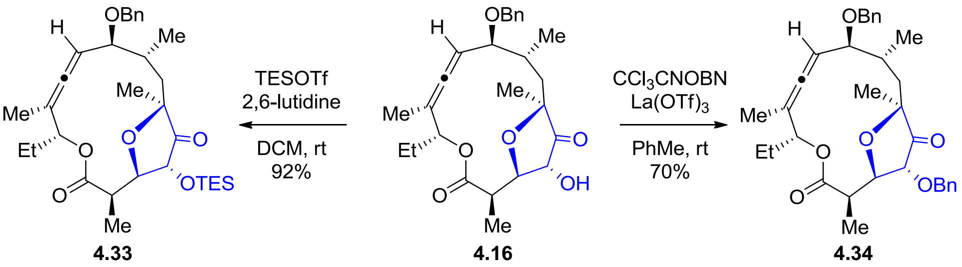
Strong nOe between the C6 methyl protons and the H3 proton revealed that the stereochemistry at C6 of **4.16** and **4.17** is reversed in comparison to **4.1** (Figure 4.5-2).

Figure 4.5-2: Key nOe Analysis of 4.16 and 4.17



Mono-oxidation products such as **4.13**, **4.16**, and **4.18** provide the unique opportunities to enhance the heterogeneity among the erythromycinoids. The reactive secondary hydroxyl in **4.16** was protected either with TES or benzylic ether for further derivatization of the second allene.

Scheme: 4.5-2: Hydroxyl Protection in 4.16

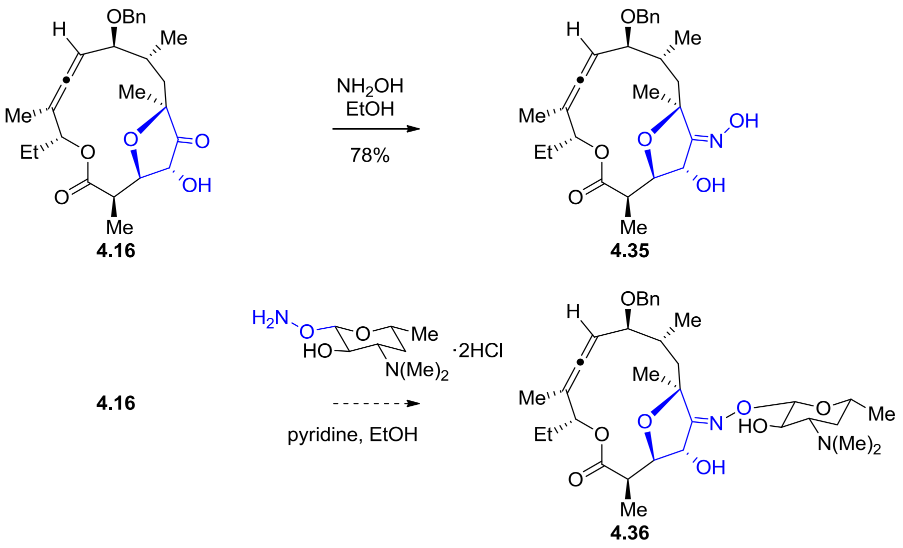


TESOTf
2,6-lutidine
DCM, rt
92%
CCl3CNOBN
La(OTf)3
PhMe, rt
70%
OBn
Me
H
Et
O
OTES
OH
OBn
4.33
4.16
4.34

4.6. *De Novo Glycosylation Strategy*

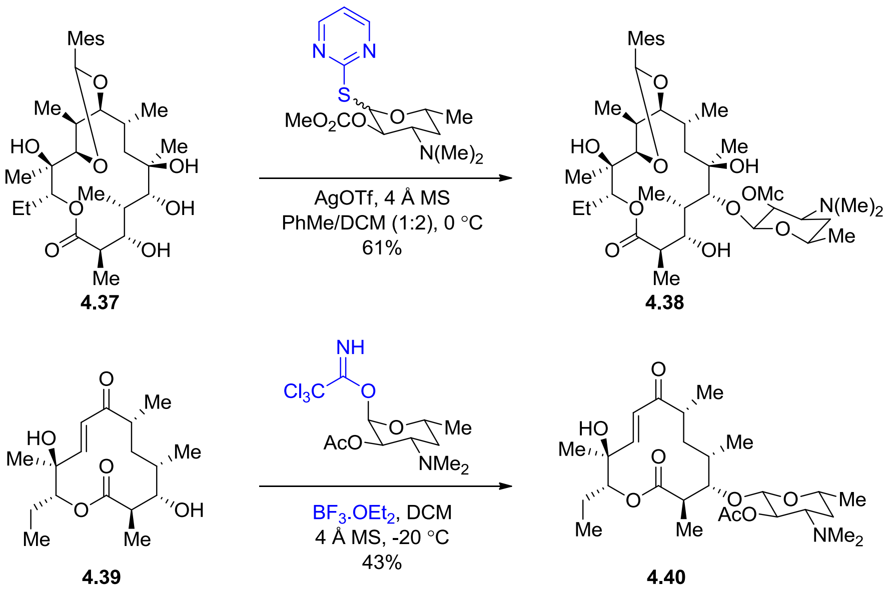
The first reported oxime formation[13] at C9 in the erythromycin macrolide family lead to invention of 2nd and 3rd generation erythromycins. When **4.16** was treated with hydroxylamine, oxime formation at C5 proceeded smoothly to give **4.35** in 78% yield (Scheme 4.6-1). This clean oxime formation suggested the possibility of adding the C5 sugar as a nucleophile (**4.16** → **4.36**) in contrast to the conventional methodologies.

Scheme 4.6-1: Oxime Formation


OBn
H
Me
Me
Me
Et
O
O
O
OH
Me
4.16
NH2OH
EtOH
78%
N
OH
4.35
H2N
O
HO
N(Me)2
·2HCl
pyridine, EtOH
4.16
4.36

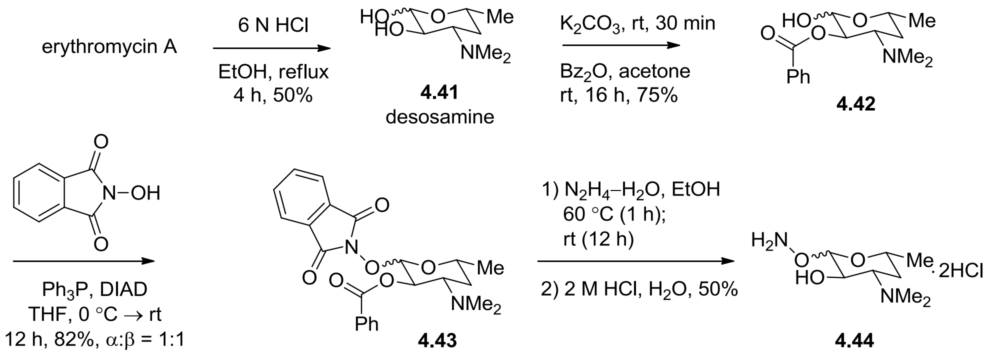
For example, in Martin’s synthesis of erythromycin B, a sugar bearing a pyrimidyl thioglycoside at the anomeric position can be added via the intermediate oxocarbenium ion (Section 4.6-2).[14] Woodward and Toshima employed similar methods to glycosylate erythromycin A.[15,16] These strategies require the 2'-OH on the desosamine sugar to be masked. An alternative method for glycosylation with desosamine is to prepare the corresponding acetimidate as in the synthesis of Methymycin by Kang et al. [17] Though the reaction proceeded regio- and stereoselectively to afford the desired C5-glycosylated β-glycoside as the sole anomer, the reaction requires rigorously anhydrous conditions and low yields are not uncommon.

Scheme 4.6-2: Glycosylation


Mes
Me
Me
HO
Me
OH
Me
OH
Et
O
OH
O
Me
4.37
N
N
S
MeO2CO
O
Me
N(Me)2
AgOTf, 4 Å MS
PhMe/DCM (1:2), 0 °C
61%
OMc
N(Me)2
Me
4.38
O
Me
HO
Me
Me
OH
Me
Me
4.39
NH
Cl3C
O
AcO
NMe2
BF3.OEt2, DCM
4 Å MS, -20 °C
43%
AcO
NMe2
4.40

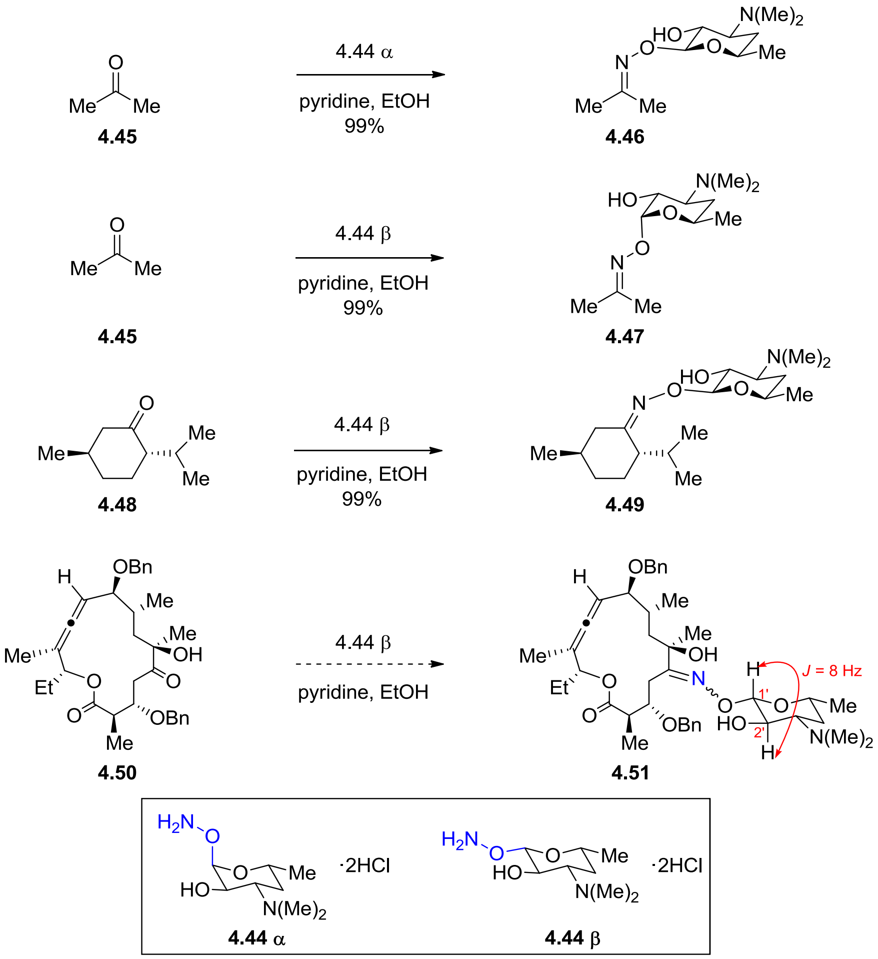
In contrast, the aminooxydesosamine hydrochloride salt will react in aqueous conditions. The aminooxydesosamine hydrochloride salt[18] was prepared in 4 steps (Scheme 4.6-3) by my colleague, Libing Yu, and the hypothesis was tested on simple ketones and macrolide **4.50** (Scheme 4.6-4). The desosamine was isolated from the degradation of erythromycin. Benzoyl protection of the 2'-OH gave **4.42**. Mitsunobo reaction of the anomeric alcohol with *N*-hydroxyphthalimide provided **4.43** as a 1:1 mixture of s α:β anomers. Oxyphthalimide **4.43** was then reduced by hydrazine followed by the removal of benzoyl ether to give hydrochloride salt **4.44** in a single flask transformation.

Scheme 4.6-3: Modified Synthesis of Desosamine Hydrochloric Salt


erythromycin A
6 N HCl
EtOH, reflux
4 h, 50%
HO
HO
O
Me
NMe2
4.41
desosamine
K2CO3, rt, 30 min
Bz2O, acetone
rt, 16 h, 75%
HO
O
O
Me
NMe2
Ph
4.42
O
N–OH
O
Ph3P, DIAD
THF, 0 °C → rt
12 h, 82%, α:β = 1:1
O
N
O
O
O
O
Me
NMe2
Ph
4.43
1) N2H4–H2O, EtOH
60 °C (1 h);
rt (12 h)
2) 2 M HCl, H2O, 50%
H2N
O
O
Me
· 2HCl
HO
NMe2
4.44

Yu coupled both α and β glycosidic aminohydroxyl desosamine salts with simple ketones (i.e., acetone and menthone) to obtain oxime in 99% yield in all cases (Scheme 4.6-4). When macrolactone **4.50** was treated with **4.44β**, ^{13}C NMR data showed the appearance of the characteristic oxime signal (δ = 168 ppm in **4.51**) and the absence of the ketone signal of **4.50** (δ = 212 ppm). The stereochemistry of the glycoside bond was confirmed by ^{1}H NMR coupling constant analysis ($J_{1',2'}$ = 8.0 Hz).

Scheme 4.6-4: Addition of Hydroxyl Amine Desosamine


4.45
4.44 α
pyridine, EtOH
99%
4.46
4.45
4.44 β
pyridine, EtOH
99%
4.47
4.48
4.44 β
pyridine, EtOH
99%
4.49
4.50
4.44 β
pyridine, EtOH
4.51
J = 8 Hz
1'
2'
·2HCl
4.44 α
·2HCl
4.44 β

4.7. OsO₄ Oxidation/Addition/Rearrangement /Application

According to the outcome of osmylation on linear allenes (*See* Chapter 5 for more detail), we hypothesized that the osmylation of macrocyclic bis[allene] **4.1** in the absence of an electrophile would give 4,10-desmethyl erythromycinoid **4.52** (Scheme 4.7-1). Desmethyl erythromycinoid **4.52** was targeted because Steitz and coworkers demonstrated the cause of resistance might be due to a steric clash of the amino group of guanine 2058 of the large ribosomal subunit with C4 methyl of the drug.[19]

Upon oxidation of **4.1** with 2 equivalents of OsO_4, Kai Liu isolated (9*S*)-dihydro-6,9-anhydro derivative **4.53** (46%). Initially, we thought that the bis-oxidized product was **4.52**. However, long-range coupling between the proton at C9 and the methyl carbon at C6 suggested the formation of 6,9-furan. Furthermore, the tertiary hydroxyl at C12 was protected with a TES group and the product was recrystallized to unambiguously determine the structure to be **4.54** by X-ray crystallographic analysis (Figure 4.7-1). It is well precedented that 6,9-linked analogs, so called “motilide”, display biological activity as motilin acceptors, thus promoting gastrointestinal prokinetic activities.[20, 21]

Scheme 4.7-1: Desmethyl Erythromycinoids

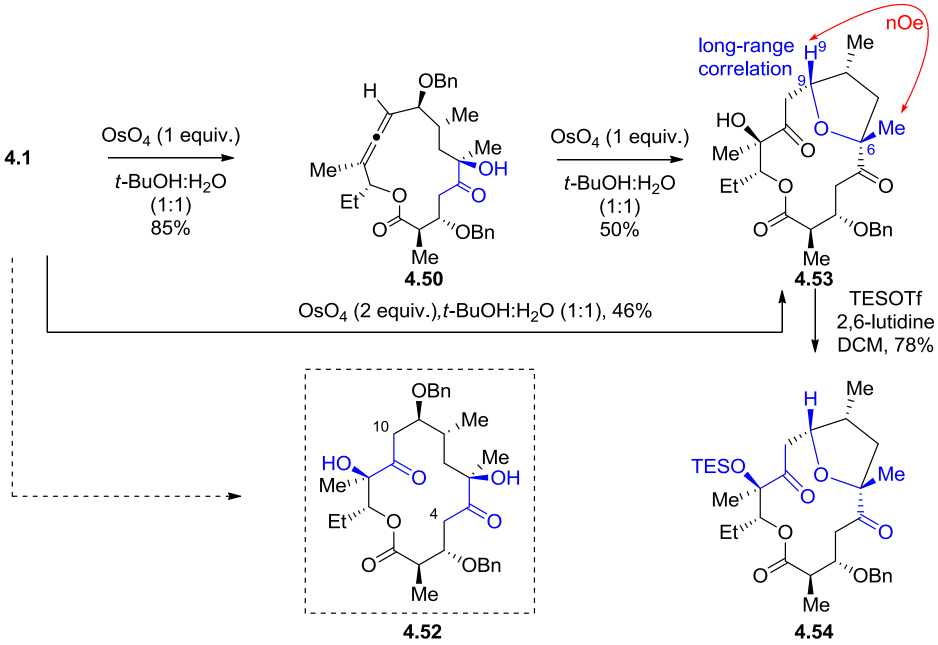
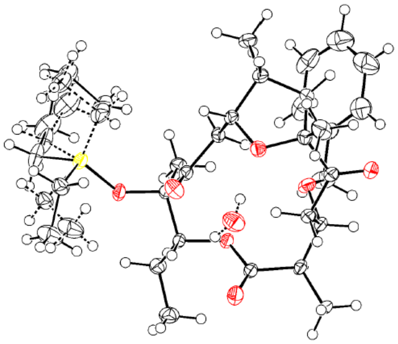

4.1
OsO4 (1 equiv.)
t-BuOH:H2O (1:1) 85%
4.50
OsO4 (1 equiv.)
t-BuOH:H2O (1:1) 50%
4.53
long-range correlation
nOe
H9
9
6
OsO4 (2 equiv.), t-BuOH:H2O (1:1), 46%
TESOTf
2,6-lutidine
DCM, 78%
4.52
4.54
OBn
Me
OH
HO
Et
TESO
10
4

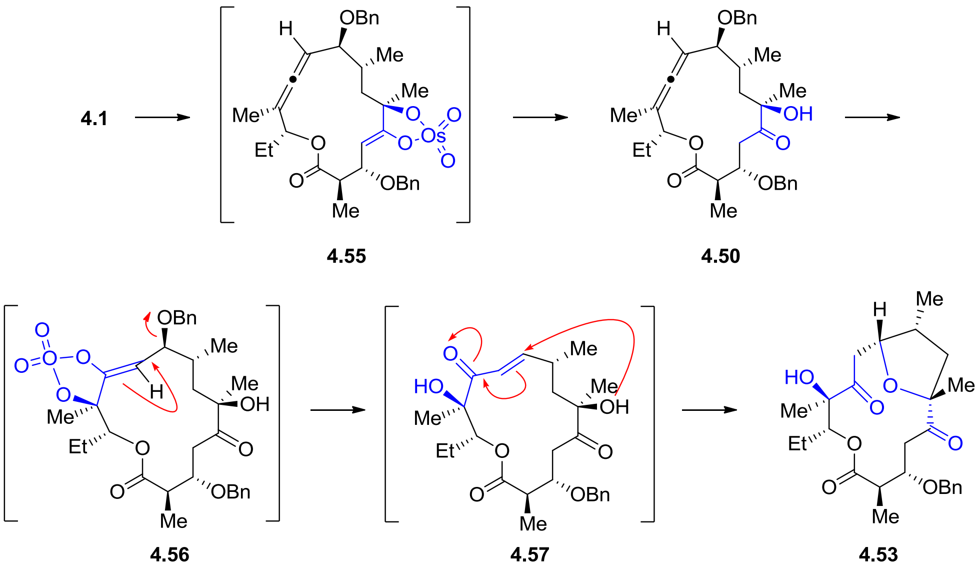
Figure 4.7-1: X-ray Crystal Structure of 4.54



In considering the different reactivity of the two allenes in macrocyclic bis[allene] **4.1**, it was realized that we could oxidize them in a stepwise manner. Thereby, the 2nd allene can be modified heterogeneously. In an attempt to affect stepwise oxidation, macrocyclic bis[allene] **4.1** was treated with 1 equivalent of OsO_4 to give mono-oxidized 4-desmethyl macrolide **4.50** (85%). Addition of a 2nd equivalent of OsO_4 oxidized the second allene to **4.53** (50%).

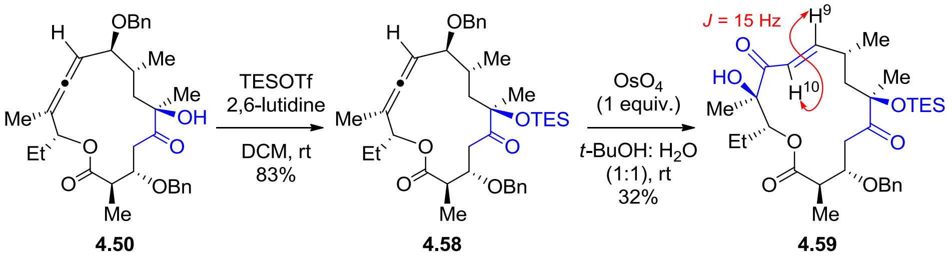
Although the precise structures of the intermediates are a matter of speculation, the osmium adducts formed and were subsequently hydrolyzed (i.e., **4.55** → **4.50**, **4.56** → **4.57**, Scheme 4.7-2). Considering that these intermediates are also enolates,[22] unlike simple alkene-derived osmate esters, β-elimination (**4.56** → **4.57**) and subsequent intramolecular conjugate addition (**4.57** → **4.53**) is reasonable and suggestive of other possible transformations. Interestingly, the C3 benzyloxy group is retained whereas the C9 group is eliminated, a phenomenon most likely traceable to the orientation of this group relative to the osmate ester.

Scheme 4.7-2: Mechanistic Rationale for 6,9-Bicyclolide 4.53



C9-C12 α-hydroxyenone **4.59** was isolated when hydroxyl ketone **4.50** was protected with a TES group (**4.51** → **4.58**), followed by a second osmyation (**4.58** → **4.59**, Scheme 4.7-3). α-Hydroxyenone **4.59** adopts a *trans* configuration, as confirmed by vicinal coupling constants ($^{3}J_{\mathrm{H9,\ H10}}$ = 15.5 Hz). Considering the synthetic versatility of enones, this functionality in **4.59** and **4.13** provides enormous opportunity to derivatize C3, C4, C6, C9, C10 and C11 to include diverse functional arrays.

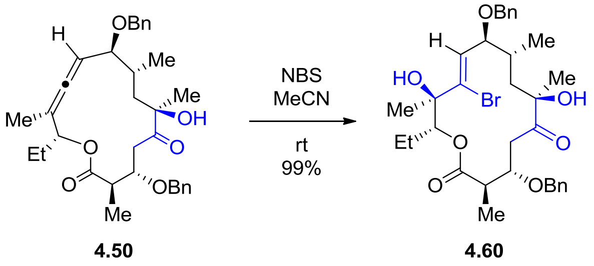
Scheme 4.7-3: Formation of C9-C12 α-Hydroxyenone, 4.59


TESOTf
2,6-lutidine
DCM, rt
83%
OsO4
(1 equiv.)
t-BuOH: H2O
(1:1), rt
32%
J = 15 Hz
H9
H10
4.50
4.58
4.59

4.8. *Allene Halohydration*

Ionic addition to simple acyclic allenes is known,[23] but has never been applied in asymmetric synthesis. We hypothesized that an NBS addition/hydrolysis reaction will be highly stereoselective due to macrocyclic stereocontrol in allene **4.50**. Herein, I described the combination of allene osmylation and electrophilic bromination (Scheme 4.8-1).

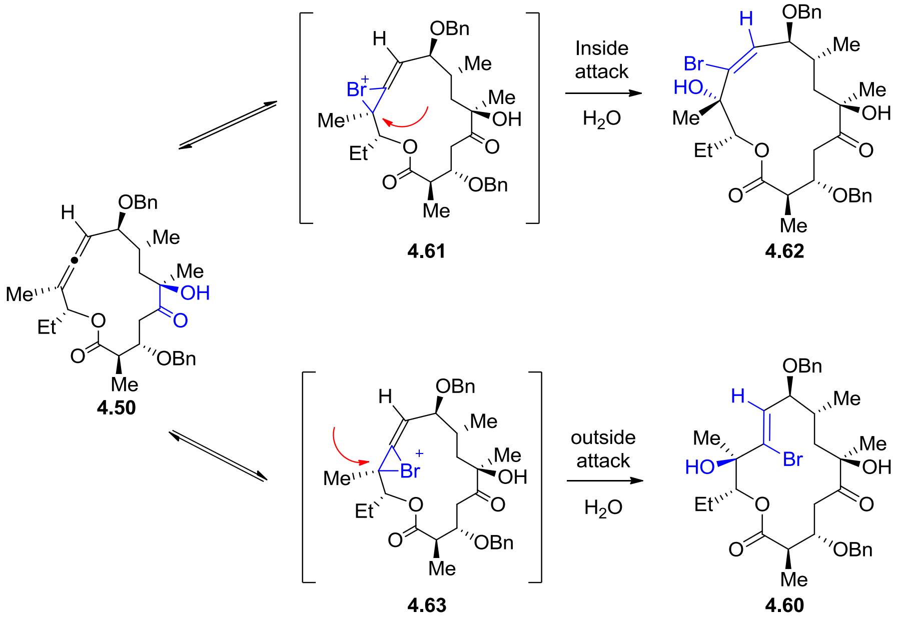
Scheme 4.8-1: Electrophilic Bromination


NBS
MeCN
rt
99%
4.50
4.60

Following osmylation of **4.1**, the resultant ketoalcohol **4.50** was treated with NBS in acetonitrile.[24] The C10-C11 *Z*-vinyl bromide/β-C12 hydroxyl **4.60** was isolated in excellent yield.

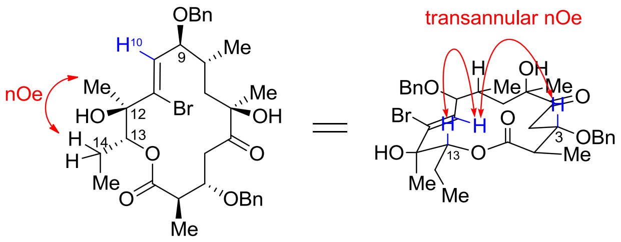
Although there are 2 possible faces of approach for the electrophilic bromination ion to the allene of **4.50** (**4.61** and **4.63**, Scheme 4.8-2), approach of the nucleophile is expected to occur from outside of the macrocycle. This would lead to Z-bromide **4.60** at a significantly faster rate than **4.62**.

Scheme 4.8-2: Mechanistic Postulation for 4.60



The NMR-spectra revealed the presence of a single olefin proton (6.14 ppm in **4.60**). The olefin proton was split by vicinal coupling ($^3J_{H10, H9}$ = 8.4 Hz in **4.60**). The transannular nOe of H10 with H13 and H3 unambiguosly set *Z*-vinylbromide configuration of **4.60** (*See* Figure 4.8-1). The absence of an nOe for H10 with the C12 methyl group set the stereochemistry of the C12 hydroxyl as β as well as the nOe between the C12 methyl with the C14 methylene protons in **4.60**.

Figure 4.8-1: Key nOe Analysis of 4.60

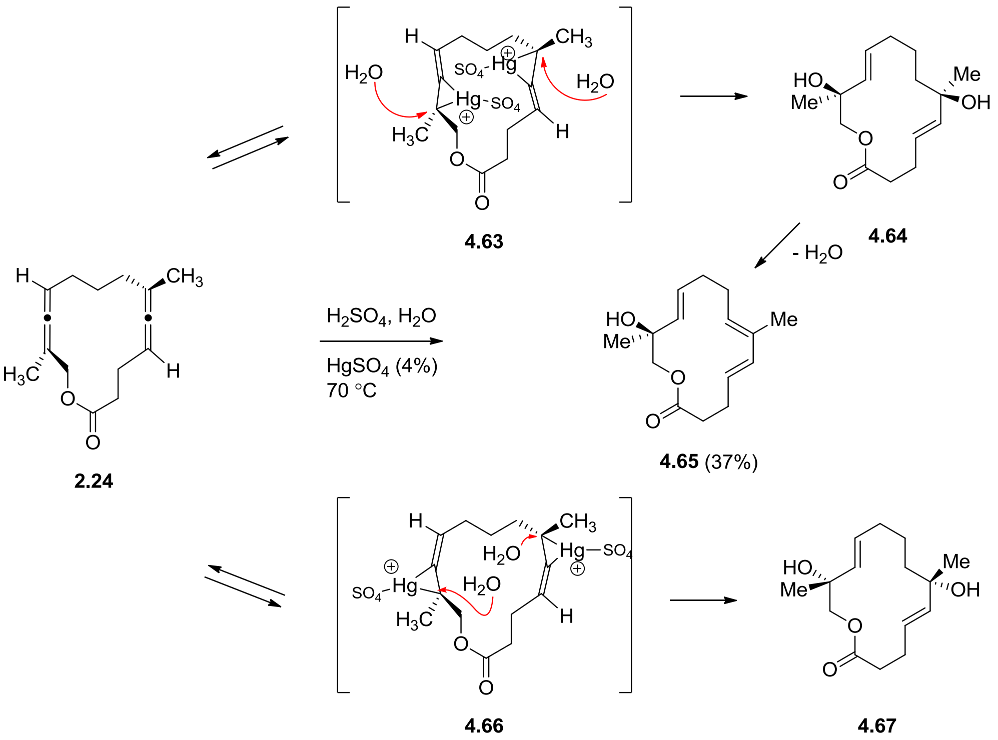

OBn
H10
9
Me
nOe
Me
HO
12
Br
Me
OH
H
14
13
H
O
O
Me
O
OBn
Me
transannular nOe
H
OH
BnO
Me
Me
O
Br
H
H
O
3
OBn
HO
13
O
Me
Me
Me

4.60

4.9. Alternative to DMDO Oxidation

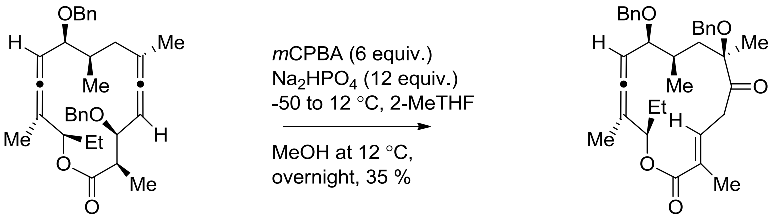
Oxymercuration/demercuration[25, 26] of allenes has been well studied, however it has never been applied in the context of macrocyclic allene systems. The stereochemical outcome of product formation in oxymercuration/demercuration is analogous to that of halohydration. Upon exposure of the allene to mercuric sulfate, the formation of the purported mercurium bridge inside the ring would allow for facile attack by a nucleophile from outside the macrocycle (**2.24** → **4.63**) in comparison to formation of the mercurium bridge outside the ring and attack of the nucleophile from inside the macrocycle (**2.24** → **4.66**). The former would lead to *trans* allylic alcohol **4.64**. However, the demercuration step (reflux at 70 °C in sulfuric acid and water) lead to the elimination product **4.65**.

Scheme 4.9-1: Oxymercuration/Demercuration



Oxidative cleavage[8, 27, 28] of benzylic ethers and arenes is known and accounts for the instability of SDEs prepared from macrocycle **4.1** upon reaching ambient temperature. In contrast to DMDO oxidation, exposure of arene-containing allenes to *m*CPBA seems to give SDEs.[29] Hence, bis[allene] macrolactone **4.1** was oxidized with *m*CPBA (6 equiv.) in methanol. Instead of the methanol addition adduct, **4.68** was isolated containing a C2 olefin forms. This product was similar to **4.13**, though differed by the position of the olefin.

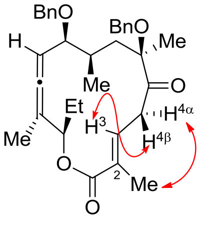
Scheme 4.9-2: *m*CPBA Oxidation of 4.1


OBn
H
Me
Me
BnO
Et
H
Me
O
O
*m*CPBA (6 equiv.)
Na$_2$HPO$_4$ (12 equiv.)
-50 to 12 °C, 2-MeTHF
MeOH at 12 °C,
overnight, 35 %
BnO
BnO
H
Me
Me
O
Et
H
Me
O
Me
O

4.1 **4.68**

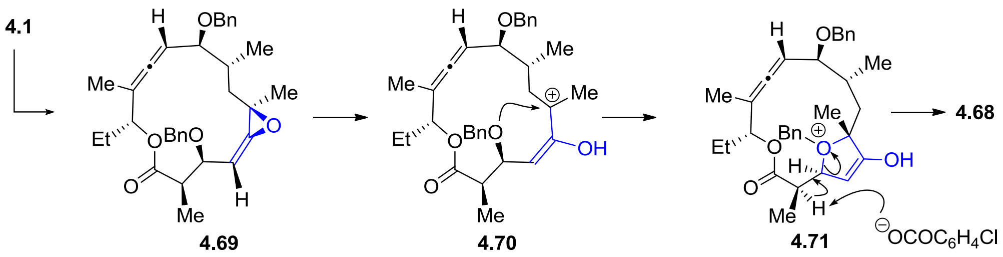
A *trans* configuration of the olefin at C2-C3 in **4.68** was confirmed by nOe analysis. The C3 proton shows an nOe with H4β only, however in contrast, the C2-methyl protons shows an nOe with H4α only (Figure 4.9-1). In the *cis* olefin configuration, an nOe between H4α and the C2-methyl protons is unlikely. The mechanism is analogous to the formation of **4.13**. The highly reactive allene oxide intermediate **4.69** opens up before the second epoxidation event due to hydrogen bond donation. The nearby benzyloxy added to the carbocation of intermediate **4.70** followed by removal of a proton by base promoted by the benzyloxonium ion in **4.71**(Scheme 4.9-3).

Figure 4.9-1: Key nOe Analysis of 4.68


BnO
BnO
H
Me
Me
O
Et
H^3
H$^{4\alpha}$
H$^{4\beta}$
Me
O
2
Me
O

4.68

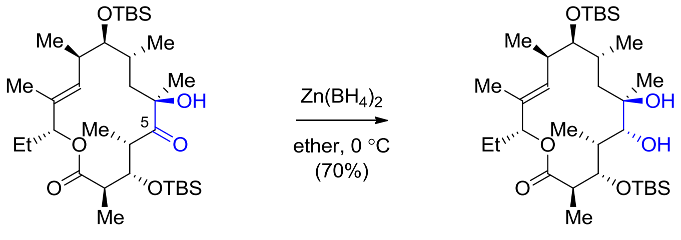
Scheme 4.9-3: Mechanistic Rationale for 4.68


4.1
4.69
4.70
4.71
4.68
OBn
Me
OH
$^{\ominus}OCOC_6H_4Cl$

4.10. Chelation-Controlled Reduction

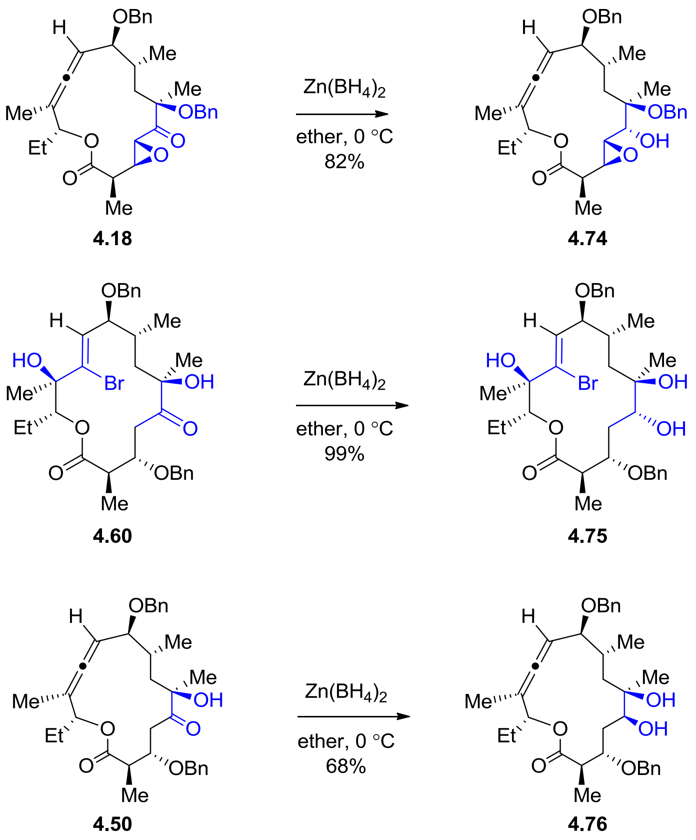
$Zn(BH_4)_2$ reduction of optically active α,β-epoxy ketones[30] and α-hydroxy ketones[31] in linear systems was reported to afford optically active *erythro* isomers with high selectivity. However, for macrocyclic systems, the stereochemical outcome is different from what is expected in linear systems. It depends on the topolographical bias of the macrocycle. Contrary to the stereochemical prediction for linear systems, Paterson showed *threo* reduction at C5 by $Zn(BH_4)_2$ (**4.72** → **4.73**, Scheme 4.10-1).[32]

Scheme 4.10-1: $Zn(BH_4)_2$ Reduction on α-Hydroxy Ketone by Paterson[32]


OTBS
Me
OH
5
Zn(BH4)2
ether, 0 °C
(70%)

4.72 **4.73**

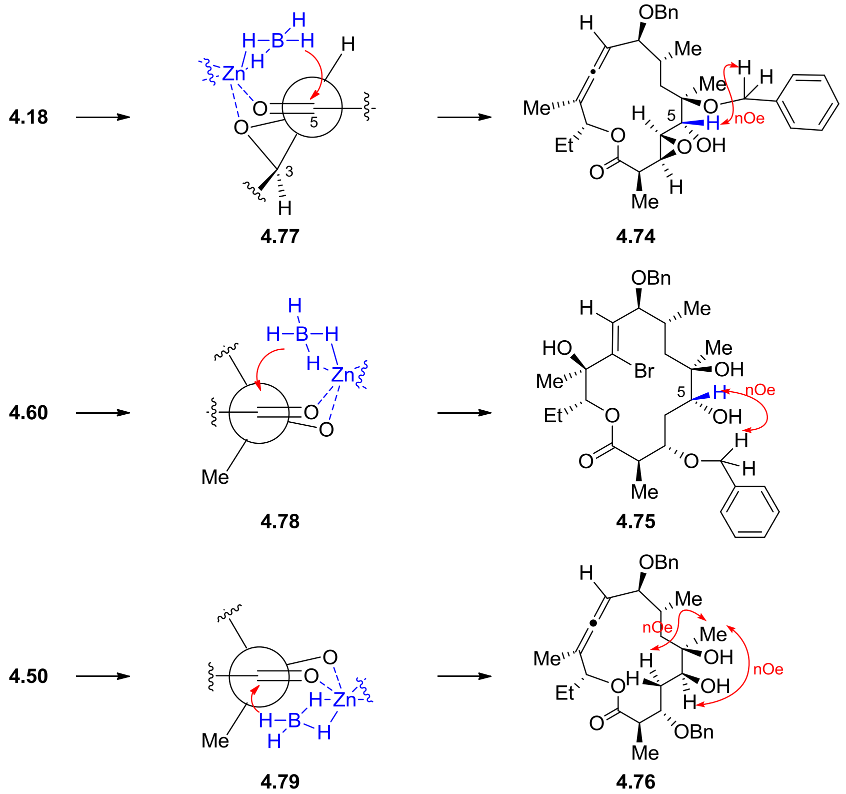
Scheme 4.10-2: $Zn(BH_4)_2$ Reduction


4.18
$Zn(BH_4)_2$
ether, 0 °C
82%
4.74
4.60
$Zn(BH_4)_2$
ether, 0 °C
99%
4.75
4.50
$Zn(BH_4)_2$
ether, 0 °C
68%
4.76

Upon reduction with $Zn(BH_4)_2$, the macrolactones with epoxy ketone **4.18**, keto alcohol with vinyl bromide **4.60**, and keto alcohol **4.50** show the complete diastereoselection with moderate to good yield (Scheme 4.10-2). The careful and extensive NMR analysis established the structure of the reduction products.

In case of **4.18**, the hydride attack would be more accessible from the opposite side of the epoxide **4.77**, which account for α-hydroxy at C5 in **4.74**. The stereochemistry was confirmed by nOe between H5 and benzylic protons at C6 in **4.74**.

Scheme 4.10-3: Key nOe Analysis of Alcohols at C5

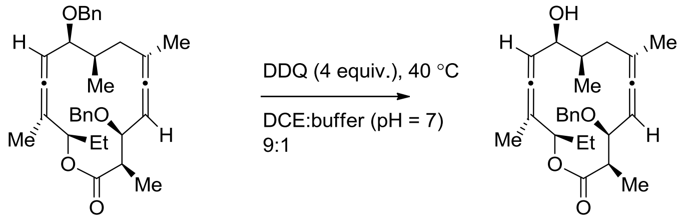


For keto alcohols **4.60** and **4.50**, hydride attack on the ketone at C5 from the top face was predicted to be unlikely due to steric hindrance. **4.75** showed a very strong transannular nOe among H10, H3, and H13 and this led us to assume that the benzylic protons at C3 were positioned at the top face of the compound. The same benzylic protons showed an nOe with H5. This confirmed hydride attack at C5 from the top face as shown **4.78**. However $Zn(BH_4)_2$ reduction of **4.50** gave β-hydroxy formation at C5 as a single diastereomer (**4.76**). The nOe between the C6-methyl and C5-α-hydroxy confirmed the assigned stereochemistry in **4.76**.

4.11. Benzylic Ether Cleavage by DDQ

As shown in this chapter, the presence of benzylic ether resulted in a very interesting rearrangement due to its reactivity under the employed oxidation conditions. However, more stable protecting groups such as silyl ethers were also advised.

Scheme 4.11-1: Benzylic Ether Cleavage by DDQ

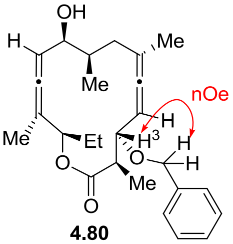

OBn
H
Me
Me
BnO
Me
Et
H
O
Me
O
DDQ (4 equiv.), 40 °C
DCE:buffer (pH = 7)
9:1
OH
H
Me
Me
BnO
Me
Et
H
O
Me
O

4.1

4.80 (60%)

Instead of incorporating the different protecting group at the beginning of the synthesis, if we can manipulate the protecting groups at a later stage of the synthesis, this will ease the synthetic maneuver. Often times, benzylic ethers are cleaved by palladium catalyzed hydrogenolysis, however due to allene reactivity, we affected the cleavage with DDQ. The cleavage occurred stepwise leading to cleavage of the C9 benzyl ether before the C3 benzyl ether.

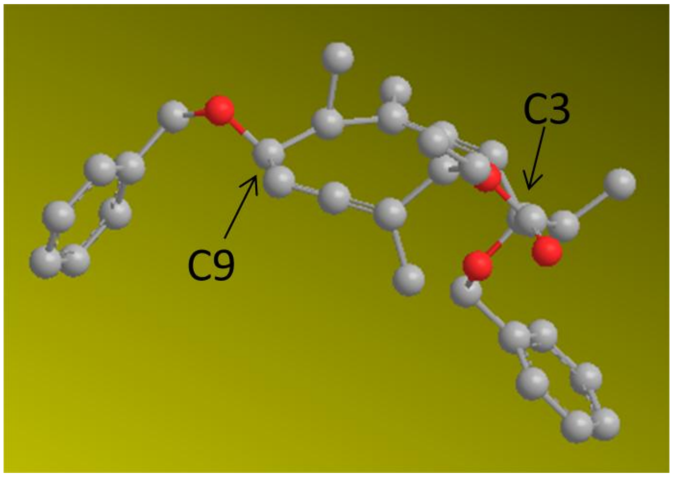
Figure 4.11-1: Key nOe Analysis for Benzylic Ether Location in 4.80


OH
H
Me
Me
nOe
H
Me
Et
H3
H
O
H
O
Me
O

4.80

Computational analysis performed at the MM2 level suggests that the ground state of the bis[allene] macrolactone **4.1** populates a favorable conformation in which the benzylic ether at C9 is more open in space whereas the benzylic ether at C3 is tucked into the ring. This conformation is also consistent with nOe analysis.

Figure 4.11-2: Ground State Minimum Energy Conformation of 4.1



4.12. Conclusion

In summary, the four sites of unsaturation in a bis[allene] macrolactone were transformed with apparently complete selectively. The observed order of reactivity is C5-C6 > C4-C5 > C11-C12 and then C10-C11. In all of the reactions, the products were isolated as single stereoisomers. The observed stereochemical outcomes reflect the cooperative effects of intrinsic allene stereoselectivity, macrocyclic stereocontrol,[33] and (for the intramolecular transformations) proximity of the reacting partners. Bis[allene] macrolactone has proven to be a processable intermediate and all compounds were prepared from this species in 3 steps or less. 9 out of 11 modifiable carbons were synthetically manipulated and the new functional moieties included enone, epoxy ketone, furanone, α'-halo ketone (fluoro-, chloro-, bromo-), halohydrin, and furan. Most importantly, this new class of erythromycinoids would allow us to systematically vary the point of interest on the macrolactone carbon backbone to shed light on the complete survey of structure/function space and the mechanisms by which these compounds work.

Taken all together, the bis[allene] macrolactone is a structurally processable intermediate that integrates the routes to targets that intersect with underexplored erythromycinoid structure space. As part of our program to develop *de novo* strategies for the synthesis of erythromycinoids, we have been interested in improving methods to oxidize an allene to synthesize erythromycinoids. Allene osmylation and multicomponent coupling is discussed in more detail in chapter 5.

4.13. References

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Chapter 5

Allene Osmylation and Multi-component Coupling

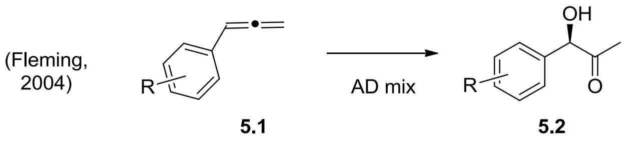
5.1. Introduction

A mechanistic rationale that reconciles earlier methodological shortcomings of allene osmylation is advanced and tested. These insights provide the basis for new efficient methods of catalytic allene osmylation and stereoselective multi-component allene hydroxylation/electrophile capture reactions. These methods constitute a powerful complement to allene spirodiepoxidation/nucleophile capture methods. Simple and complex substrates are used to demonstrate the generality of these new methods.

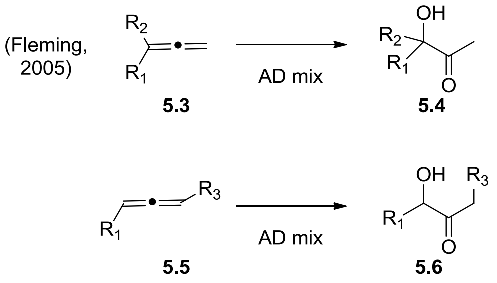
5.2. History of Allene Osmylation

In contrast to the extensive studies of alkene osmylation in the literature, we have found few studies on allene osmylation. These reports detail allene osmylation as sluggish and low yielding reactions with significant byproduct formation that is extremely limited in substrate scope. Fleming reported the asymmetric dihydroxylation of mono-substituted aryl allenes of type **5.1** using AD-mix (Scheme 5.2-1).[1] The reaction gave chiral α-hydroxy ketones with complete regioselectivity and a high degree of stereoselectivity, however the yields were only moderate (45 – 63%) with the exception of the *para*-methoxy substituted allene (79%). In the case of non-aryl 1,1-disubstituted allenes of type **5.3**, the yield and enentioselectivity were poor.[2] Crabbe disclosed the stoichiometric osmium tetroxide (OsO_4)-mediated oxidation of steroid **5.7** gave dihydroxy-keto steroid **5.8** (Scheme 5.2-2).[3]

Scheme 5.2-1: Asymmetric Dihydroxylation on Allene by Fleming

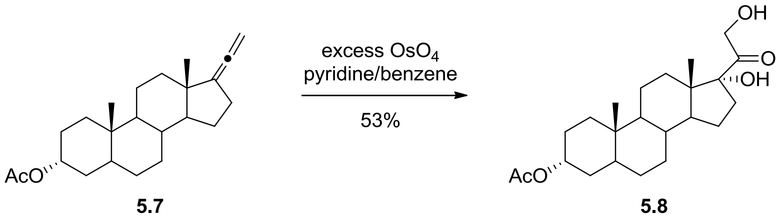

(Fleming, 2004)
R
5.1
AD mix
OH
R
O
5.2

Ar	Hydroxy ketone	% ee
Ph-	45%	88 (R)
4-MePh-	49%	89 (R)
4-MeOPh-	79%	92 (R)
4-ClPh-	58%	82 (R)
2-MePh-	52%	88 (R)
2-Naph-	63%	92 (R)


(Fleming, 2005)
R2
R1
5.3
AD mix
OH
R2
R1
O
5.4
R3
R1
5.5
AD mix
OH R3
R1
O
5.6

Allene	R1	R2	Hydroxy ketone	% ee	Product
5.3a	Hex	Me	29%	50 (R)	5.4a
5.3b	Pen	Et	28%	<5 (R)	5.4b
5.3c	Ph	Me	72%	86 (R)	5.4c
5.5a	Ph	Hex	29%	79 (R)	5.6a
5.5b	Naph	Hex	40%	89 (R)	5.6b
5.5c	Ph	*t*-Bu	40%	64 (R)	5.6c

Scheme 5.2-2: Stoichiometric Allene Osmylation on a Steroidal Substrate

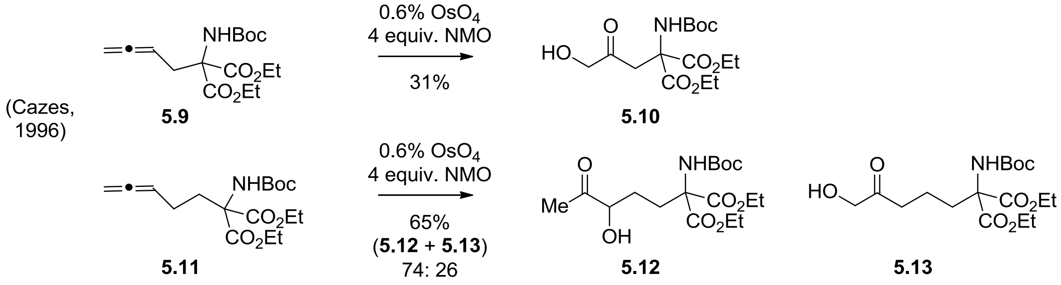

AcO
5.7
excess OsO_4
pyridine/benzene
53%
OH
O
OH
AcO
5.8

(Crabbe, 1971)

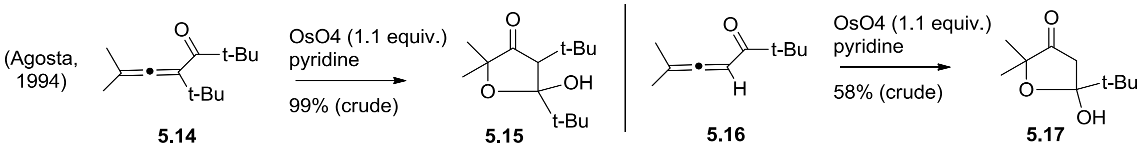
Previous allene osmylations showed that oxidation occurs at the more electron-rich terminus. However Cazes demonstrated a steric effect, that led unexpectedly to unique α-hydroxyketone **5.10** from *N*-Boc aminomalonate **5.9** while osmylation of homolog **5.11** resulted in reversed regioselectivity giving regiomers **5.12** and **5.13** in a

74:26 ratio (Scheme 5.2-3).[4] OsO_4 was also shown by Agosta to oxidize allenic ketones to cyclic hemiketals (**5.14** → **5.15**, **5.16** → **5.17**, Scheme 5.2-4).[5]

Scheme 5.2-3: Effect of Sterics on Regioselectivity


(Cazes, 1996)
NHBoc
CO2Et
CO2Et
5.9
0.6% OsO4
4 equiv. NMO
31%
HO
O
NHBoc
CO2Et
CO2Et
5.10
NHBoc
CO2Et
CO2Et
5.11
0.6% OsO4
4 equiv. NMO
65%
(5.12 + 5.13)
74: 26
O
Me
OH
NHBoc
CO2Et
CO2Et
5.12
HO
O
NHBoc
CO2Et
CO2Et
5.13

Scheme 5.2-4: Osmylation on Allenic Ketones

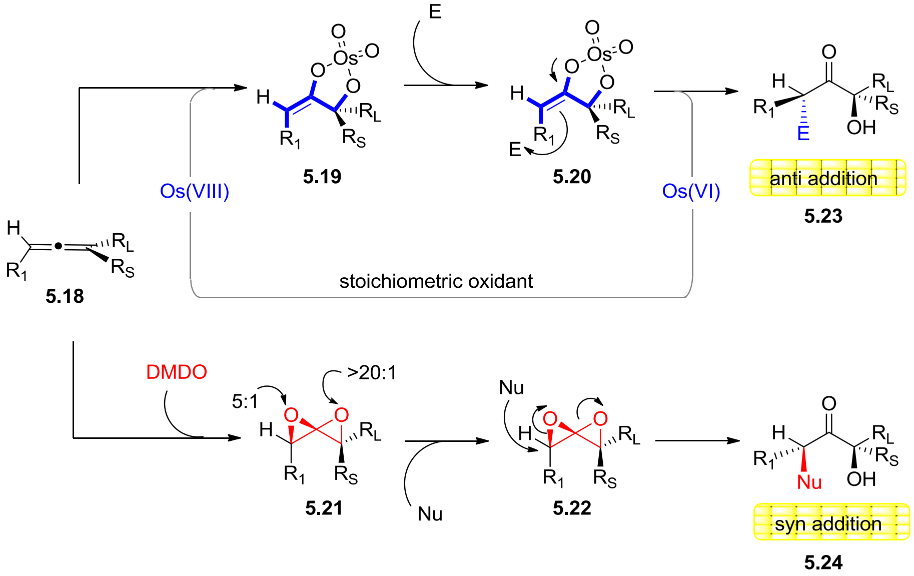

(Agosta, 1994)
O
t-Bu
t-Bu
5.14
OsO4 (1.1 equiv.)
pyridine
99% (crude)
O
t-Bu
O
OH
t-Bu
5.15
O
t-Bu
H
5.16
OsO4 (1.1 equiv.)
pyridine
58% (crude)
O
O
t-Bu
OH
5.17

5.3. Mechanistic Rationale and Stereochemical Outcome

The Sharpless group has reported their findings on "ligand-accelerated catalyst" systems in which the addition of a specific ligand increases the reaction rate of an already existing catalytic transformation .[6, 7] The nature of the ligands bound to a metal center or complex always affects the selectivity and rate of the organic transformations catalyzed by such a species. If the reaction rate is increased upon ligand binding, the stereoselective pathway can dominate over the nonselective one.

Along similar lines, we hypothesized that the addition of an electrophile to an allene osmylation reaction would accelerate decomposition of the relatively stable osmate enol ester intermediate (**5.20** → **5.23**, Figure 5.3-1), thereby increasing reaction rate and selectivity. Allenes are oxidized at the most electron-rich terminus and subsequent decomposition of the osmate ester captures the electrophile syn-facial to the smaller substituent (R_s, **5.20**). This provides regio- and stereoselective allene hydroxylation/electrophile addition anti-adduct. The stereochemical outcome of the reaction is complementary to allene spirodiepoxidation/nucleophile addition syn-adduct **5.24**.

Figure 5.3-1: Allene Oxidation


E
Os(VIII)
Os(VI)
5.19
5.20
anti addition
5.23
5.18
stoichiometric oxidant
DMDO
5:1
>20:1
Nu
5.21
5.22
syn addition
5.24

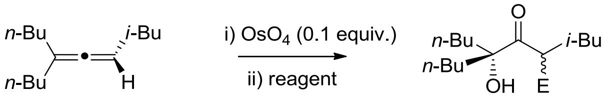
In the DMDO oxidation of acyclic allenes, the first epoxidation will also occur at the most electron-rich terminus with excellent stereoselectivity (> 20:1), favoring the less sterically hindered side (H vs. R_1 on **5.21**). The second epoxidation is less selective, but still favors the smaller substituent (R_S). Nucleophile addition then occurs at the least sterically hindered SDE terminus on the face of the larger substituent (R_L), providing the syn-addition product (**5.24**).

5.4. Allene osmylation and multi-component coupling

These hypotheses were tested on achiral tri-substituted allene **5.25** as a model system (Table 5.4-1). All reactions were carried out under catalytic conditions (0.1 equiv. OsO_4) with a specific stoichiometric oxidant. The typical experimental procedure is as follows: to the allene in a *t*-BuOH:pH 7.4 phosphate buffer (1:1) solution was added an electrophile (Selectfluor, NCS, or NBS at room temperature). The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.1 equiv.) was added slowly followed by stoichiometric oxidant. After the complete disappearance of allene by TLC observation, the reaction mixture was quenched with a saturated sodium sulfite solution. Purification by flash column chromatography on silica gel following aqueous workup afforded the corresponding α-hydroxy-α'-halo ketone. Under anhydrous conditions (entry 5), a solution of allene in dichloromethane (DCM) was added to a solution of OsO_4 (0.1 equiv.), NMO (1.2 equiv.), acetic acid (4 equiv.) and phenylboronic acid (1.2 equiv.) in DCM. The workup and purification process was identical to that of the above aqueous conditions and afforded the corresponding α-hydroxy ketone.

In all cases, the reaction proceeds with complete regioselectivity. As expected, allene **5.25** is oxidized at the more substituted (electron-rich) terminus and decomposition of the osmate enol ester captures the electrophile at the less substituted terminus. In the presence of a halide electrophile, the reaction provides an α-hydroxy-α’-halo ketone and the rate of the reaction increases (cf. entries 1, 9, 12, and 13) with NMO as the stoichiometric oxidant in a *t*-BuOH:pH 7.4 phosphate buffer (1:1) solution.

Table 5.4-1: Catalytic Allene Oxidation



5.25

5.26 (E = H), **5.27** (E = F)
5.28 (E = Cl), **5.29** (E = Br)

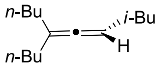
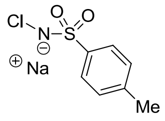
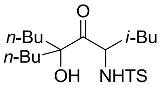
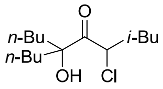
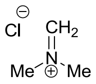
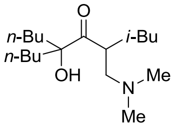
Entry	Stoichiometric oxidant (equiv.)	Additive (equiv)	E	Solvent	Time	Yield
1	NMO (2)	-	-	*t*-BuOH: H_2O (1:1)	48 hr	trace
2	AD-mix (1.4g/mmol)	$MeSO_2NH_2$(0.2)	-	*t*-BuOH: H_2O (1:1)	48 hr	56 %
3	$K_3Fe(CN)_6$ (3)	K_2CO_3(0.2)	-	*t*-BuOH: H_2O (1:1)	12 hr	65 %
4	NMO (2)	DABCO (0.1)	-	*t*-BuOH: H_2O (1:1)	12 hr	68 %
5	NMO (1.2)	AcOH w/ $PhB(OH)_2$	-	DCM	2 hr	65 %
6	NMO (2)	AcOH	-	*t*-BuOH: H_2O (1:1)	4 hr	70 %
7	NMO (2)		NCS	*t*-BuOH: H_2O (1:1)	12 hr	10 %
8	NMO (2)		NCS	acetone: H_2O (1:1)	3 hr	34 %
9	NMO (2)		NCS	*t*-BuOH: buffer (1:1)	24 hr	67 %
10	NMO (2)		NCS	acetone: buffer (1:1)	24 hr	68 %
11	NMO (2)		NBS	acetone: buffer (1:1)	2 d	31 %
12	NMO (2)		NBS	*t*-BuOH: buffer (1:1)	5 hr	68 %
13	NMO (2)		Selectfluor	*t*-BuOH: buffer (1:1)	12 hr	40 %

AD-mix with methanesulfonamide also accelerates the rate of the reaction (entry 2). Sharpless et al.[8] demonstrated that hydrolysis of the osmium (VI) glycolate product can be accelerated considerably by methanesulfonamide.[4] Potassium cyanoferrate ($K_3Fe(CN)_6$) in the presence of K_2CO_3, was also evaluated and found to react smoothly in the osmylation of **5.25** (entry 3). Sharpless argued that using $K_3Fe(CN)_6$, instead of NMO, eliminates the second catalytic cycle because there is no oxidant other than OsO_4

in the organic layer due to biphasic conditions with $K_3Fe(CN)_6$.[5] Thereby, the reported ee under $K_3Fe(CN)_6/K_2CO_3$ is better than ones using NMO. The tertiary amine, DABCO, is also capable of accelerating the reaction (entry 4).

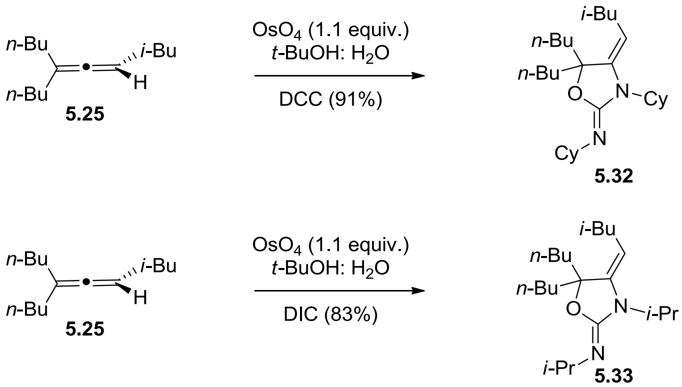
The reaction can be conducted in organic solvent alone (entry 5). For the dihydroxylation of olefins, aqueous conditions are usually employed to hydrolyze the osmate ester. However, this not only makes it difficult to isolate water soluble polyol derivatives but many electrophiles also react fast with water. Iwasawa, Kato, and Narasaka reported that the use of phenylboronic acid as a diol captor prevents over-oxidation and also enables the reaction to be performed in organic solvent alone.[9]

Table 5.4-2: Allene Oxidation

allene	electrophile	solvent	products
 **5.25**		*t*-BuOH: H_2O	 **5.30** (10%)  **5.28** (50%)
5.25		DCM	 **5.31** (63%)

Other than halide electrophiles, we also tried C-N and C-C bond forming reactions. The addition of Chloramine-T was performed under stoichiometric conditions (1.1 equiv. OsO_4) to give **5.30** (10% yield, Table 5.4-2). The majority of the product was chlorine addition **5.28** (50% yield). The addition of Eshenmoser salt (dimethylmethylideneammonium chloride) was carried out under catalytic conditions with NMO as the co-oxidant in anhydrous DCM giving **5.31** in good yield (63% yield). When DCC or DIC were added, heterocycles **5.32** and **5.33** were isolated in good yield (Scheme 5.4-1).

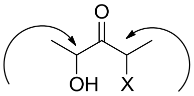
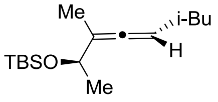
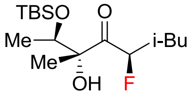
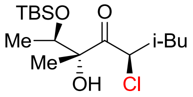
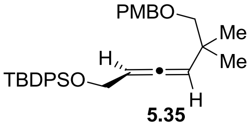
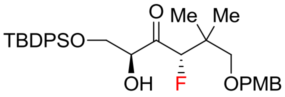
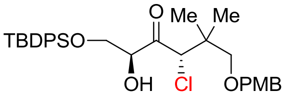
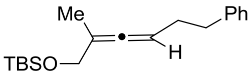
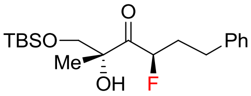
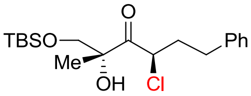
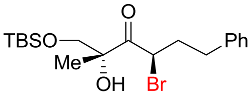
Scheme 5.4-1: DCC and DIC Addition


n-Bu
n-Bu
i-Bu
H
5.25
OsO4 (1.1 equiv.)
t-BuOH: H2O
DCC (91%)
i-Bu
n-Bu
n-Bu
O
N
Cy
N
Cy
5.32
n-Bu
n-Bu
i-Bu
H
5.25
OsO4 (1.1 equiv.)
t-BuOH: H2O
DIC (83%)
i-Bu
n-Bu
n-Bu
O
N
i-Pr
N
i-Pr
5.33

5.5. Chiral allene: osmylation and multi-component coupling

The osmylation reaction was also performed on chiral di- and tri-substituted allenes (Table 5.5-1). Optically active allenes **5.34** and **5.35** were prepared according to known procedures,[10, 11] however the racemate of **5.36** was used. Three electrophilic halide sources (Selectfluor, NCS, and NBS) were tested for both the catalytic and stoichiometric conditions. The reaction conditions for the catalytic and stoichiometric processes were the same as described in section 5.4.

Table 5.5-1: Chiral Allene Osmylation and Halide Addition

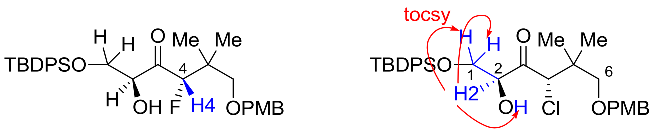
entry^{A}	allene	product^{B}	selectivity^{C}		yield^{D}(%)
			 r_1	r_2	
1	 **5.34**	 **5.37**	> 20:1	5.9 : 1	68
2	**5.34**	 **5.38**	> 20:1	5.3 : 1	78
3	 **5.35**	 **5.39**	> 20:1 (> 20:1)	> 20:1 (> 20:1)	64 (62)
4	**5.35**	 **5.40**	> 20:1 (> 20:1)	3.2 : 1 (1.5:1)	68 (79)
5	 **5.36**	 **5.41**	(> 20:1)	(7: 1)	74
6	**5.36**	 **5.42**	(> 20:1)	(2: 1)	50
7	**5.36**	 **5.43**	(> 20:1)	(1.7: 1)	60

A) stoichiometric reactions employed 1.1 equiv. of OsO$_4$ w/ electrophile (2 or 5 equiv.) at rt. catalytic reactions employed 0.1 equiv of OsO$_4$ w/ NMO (2 equiv.) & electrophile (2 or 5 equiv.) at rt. B) only anti product is shown: C) black = selectiviy in stoichiometric condition, blue = selectiviy in catalytic condition: D) combined yield of anti and syn products

In all cases, we observed complete regioselectivity. In tri-substitued allenes (entries 1, 2, 5, 6, and 7), the osmate enol ester formed at the more substituted terminus as expected, leading to carbon-halide bond formation at the less substituted terminus. In

1,3-disubstituted allenes (entries 3 and 4), the steric effect, induced by gem-dimethyl substituents, overrides the electronic effect of the electron withdrawing TBDPS group. The site of fluorine addition (**5.35** → **5.39**) was confirmed by observation of H4 geminal splitting by fluorine which appeared as a doublet with a characteristic strong splitting constant (Figure 5.5-1, *J* = 47.2 Hz) while the regioselectivity of **5.40** was confirmed by TOCSY and vicinal splitting of H2, which appeared as a triplet of doublets (Figure 5.5-1).

Figure 5.5-1: Regiochemistry of 5.39 and 5.40



^{2}J (H4, F) = 47.2 Hz, d

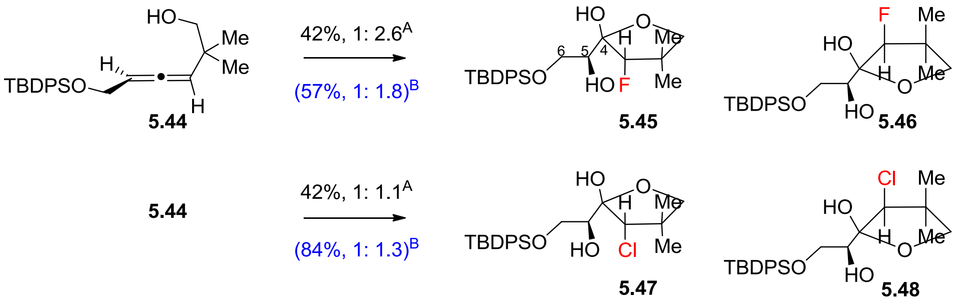
5.39

^{3}J (H2) = 1.7, 5.4 Hz, td

5.40

The stereoselectivity is particularly good with bulky Selectfluor in comparison to NCS and NBS. It is likely that the asymmetric bias is enhanced due to the bulkiness of SelectFlour.[12] In all cases, the stoichiometric reaction gave better selectivity than catalytic reactions. For the addition of bromine, NBS had to be pre-solvated in *t*-BuOH:buffer mixture, then added to the mixture of OsO_4, NMO, and allene slowly over at least 1 hour. If these requirements are not met, the allene will react with NBS faster than osmylation can occur.

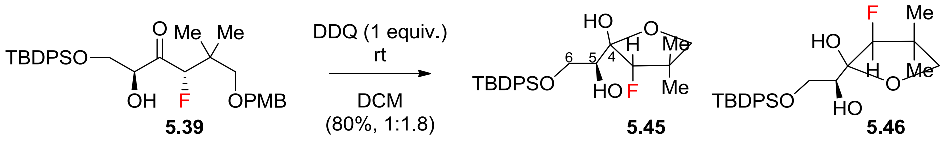
Scheme 5.5-1: Halogenated Furanose


HO
Me
Me
H
TBDPSO
H
5.44
42%, 1: 2.6^A
(57%, 1: 1.8)^B
HO
O
H
Me
6
5
4
TBDPSO
HO
F
Me
5.45
F
Me
HO
H
O
Me
TBDPSO
HO
5.46
5.44
42%, 1: 1.1^A
(84%, 1: 1.3)^B
HO
O
H
Me
TBDPSO
HO
Cl
Me
5.47
Cl
Me
HO
H
O
Me
TBDPSO
HO
5.48

A) Reactions employed 1.1 equiv. of OsO_4 w/ electrophile (2 or 5 equiv.) at rt.
B) Reactions employed 0.1 equiv. of OsO_4 w/ NMO (2 equiv.) & electrophile (2 or 5 equiv.) at rt.

When the substrate without the para-methoxy benzyl (PMB) group **5.44** is oxidized in the presence of electrophiles, the osmylation reaction gave halo-substituted furanose (Scheme 5.5-1). Unlike the osmylation on the substrate with PMB group **5.35**, I found the mixture of two diastereomers for fluorine adducts (Scheme 5.5-1). Upon the cleavage of PMB group in **5.39** by DDQ (Scheme 5.5-2), the reaction generated the same products **5.45** and **5.46** in the same ratio (1:1.8). This experiment indicated that the diastereoselectivity on fluorine addition is >20:1.

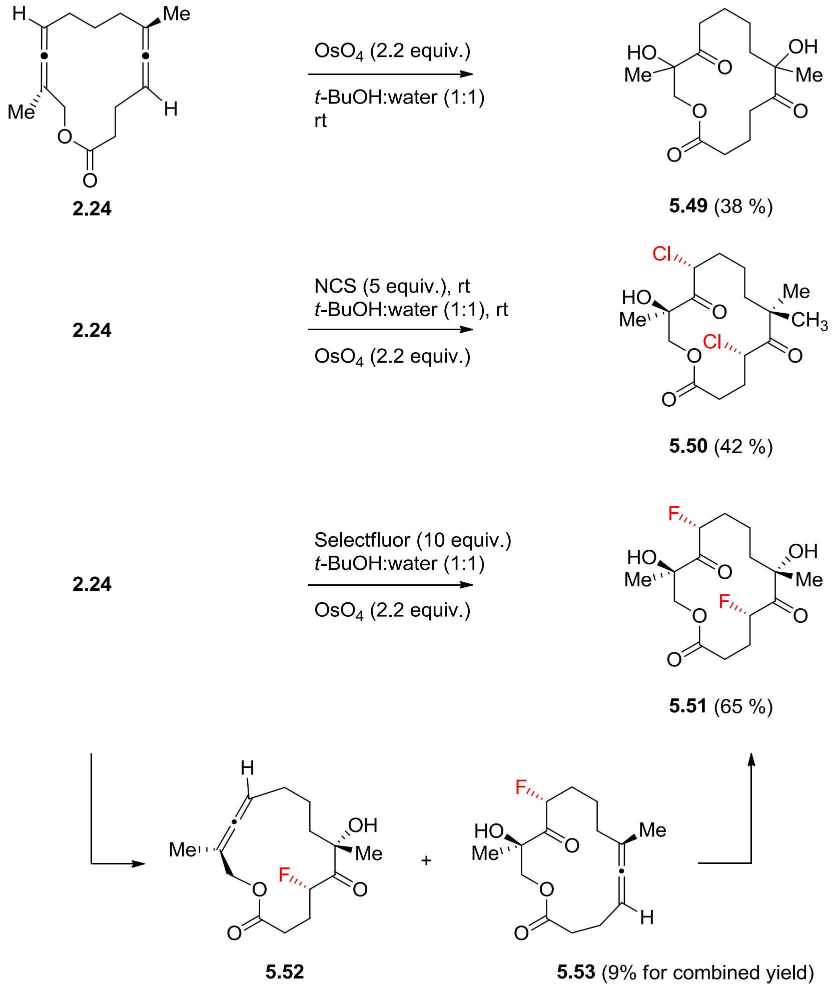
Scheme 5.5-2: In-Situ Cleavage and Cyclization of 5.39


TBDPSO
O
Me
Me
OH
F
OPMB
5.39
DDQ (1 equiv.)
rt
DCM
(80%, 1:1.8)
HO
O
H
Me
6
5
4
TBDPSO
HO
F
Me
5.45
F
Me
HO
H
O
Me
TBDPSO
HO
5.46

5.6. Cyclic Allene Osmylation: Model Study

Macroyclic bis[allene] **2.24** and compounds of this type provide a unique platform for diversity oriented synthesis due to the reactivity of allenes in various chemical transformations, the heterogeneous manipulation of bis[allene] and enhanced macrocyclic stereocontrol.[13, 14] Since acyclic allenes show complete regioselectivity and moderate stereoselectivity, the successful application of allene osmylation/electrophile addition methodology for cyclic bis[allene]s of type **2.24** will further prove the utility of the methodology.

Scheme 5.6-1: Cyclic Chiral Allene Osmylation

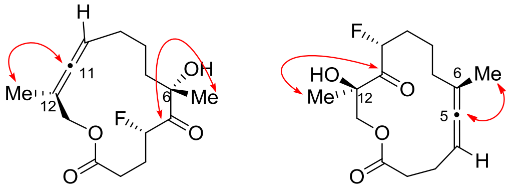

2.24
OsO4 (2.2 equiv.)
t-BuOH:water (1:1)
rt
5.49 (38 %)
2.24
NCS (5 equiv.), rt
t-BuOH:water (1:1), rt
OsO4 (2.2 equiv.)
5.50 (42 %)
2.24
Selectfluor (10 equiv.)
t-BuOH:water (1:1)
OsO4 (2.2 equiv.)
5.51 (65 %)
+
5.52
5.53 (9% for combined yield)

All reactions (Scheme 5.6-1) were carried out under stoichiometric conditions (2.2 equiv. OsO_4). The typical experimental procedure is as follows: to the allene in a *t*-BuOH:water (1:1) solution was added an electrophile (SelectFlour, NCS) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution was added dropwise. After the complete disappearance of allene by TLC observation, the reaction mixture was quenched with a saturated sodium sulfite solution.

In the absence of an electrophile, bis[keto] alcohol **5.49** was isolated as expected. Both NCS and Selectfluor proved to be the compatible electrophiles for macrocyclic bis[allene] **2.24** under the above reaction conditions giving the desired products (**2.24** → **5.50**, **2.24** → **5.51**) in moderate yields. Under the reaction conditions, the conversion from mono-oxidized products of types **5.52** and **5.53** to bis-oxidized product **5.51** is easily monitored by TLC over 1 or 2 hours. Interestingly, the oxidation rate of the two allenes of the macyclic lactone (**2.24**) did not show much difference, which could be confirmed by the isolation of a 1:1 mixture of **5.52** and **5.53**. This observation is contrary to the bis[allene] macrolactone oxidation of **2.24** by DMDO (*See* Chapter 4).

HMBC spectroscopy shows that the central allenic carbon resonances (C11 in **5.52**, C5 in **5.53**) was coupled to methyl protons at C12 in **5.52** and C6 in **5.53** while the tertiary methyl proton resonances at C6 in **5.52** and C12 in **5.53** was coupled carbonyl carbon resonances (C5 in **5.52**, C11 in **5.53**).

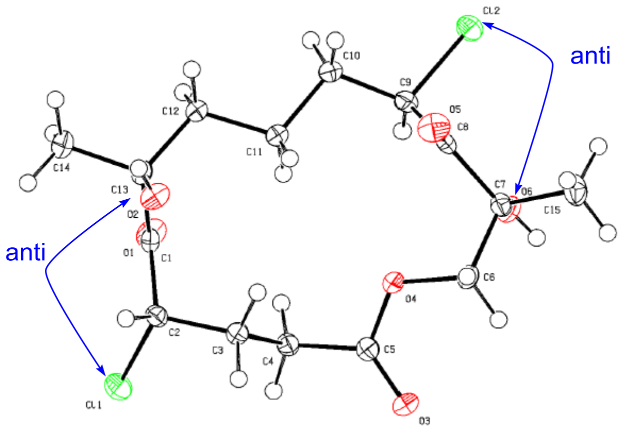
Figure 5.6-1: HMBC Confirmation of Two Central Allenic Carbons



5.52 **5.53**

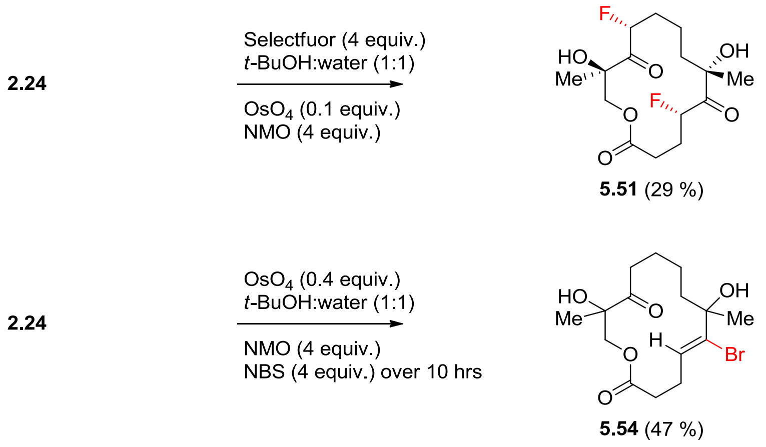
The bis[allene] hydroxylation/electrophile addition showed complete diastereoselection in the products. Kai Liu successfully recrystallized chlorine addition product **5.50** and was therefore able to establish the stereochemistry at C4 and C10 unambiguously (Figure 5.6-1).

Figure 5.6-2: X-ray Crystallography Data of Chlorine Addition, 5.50


CL2
anti
C10
C9
O5
C8
C12
C11
C14
C13
O2
O1
C1
C7
O6
C15
anti
C6
O4
C2
C3
C4
C5
CL1
O3

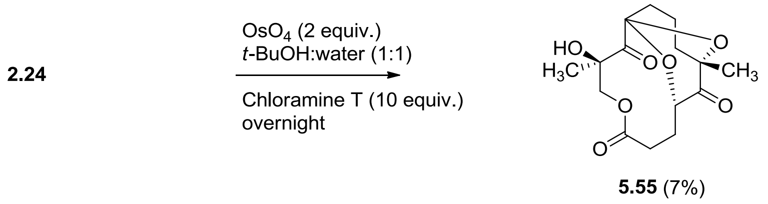
Catalytic conditions with NMO as co-oxidant were also tried for fluorine and bromine additions (Scheme 5.6-2). With Selectfluor, bis-fluorine addition product **5.51** was isolated in 29% yield. Since NBS is able to react with allenes independently, NBS was premixed in *t*-BuOH:water and added over 10 hours into the mixture of allene, OsO_4, and NMO in *t*-BuOH:water. However, the isolated halohydrin (**5.54**) showed NBS addition to the central carbon.

Scheme 5.6-2: Catalytic Osmylation on Macrocyclic Bis[Allene] 2.24


2.24
Selectfuor (4 equiv.)
t-BuOH:water (1:1)
OsO4 (0.1 equiv.)
NMO (4 equiv.)
F
HO
Me
O
OH
Me
O
F
O
O
5.51 (29 %)
2.24
OsO4 (0.4 equiv.)
t-BuOH:water (1:1)
NMO (4 equiv.)
NBS (4 equiv.) over 10 hrs
HO
Me
O
OH
Me
H
Br
O
O
5.54 (47 %)

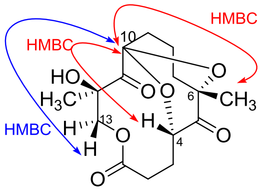
Stoichiometric conditions with OsO_4 in the presence of Chloramine-T led to the formation of polycyclic ether **5.55**. The detailed mechanism for the formation of **5.55** was not clear, but one can presume that the over-oxidation product must be involved as an intermediate.

Scheme 5.6-3: Polycyclic Ether Formation


2.24
OsO4 (2 equiv.)
t-BuOH:water (1:1)
Chloramine T (10 equiv.)
overnight
HO
H3C
O
O
O
CH3
O
O
O
5.55 (7%)

Long-range correlations through oxygens between the C6 methyl protons and C10 carbon (C6-CH_3 ↔ C10) as well as the C4 protons and C10 carbon (C4-H ↔ C10) confirmed the formation of cyclic ethers. The position of C10 was confirmed by long-range correlation between the C13 protons and C10 carbon (C13-H ↔ C10).

Figure 5.6-3: Long-Range Correlations Through Oxygens in 5.55

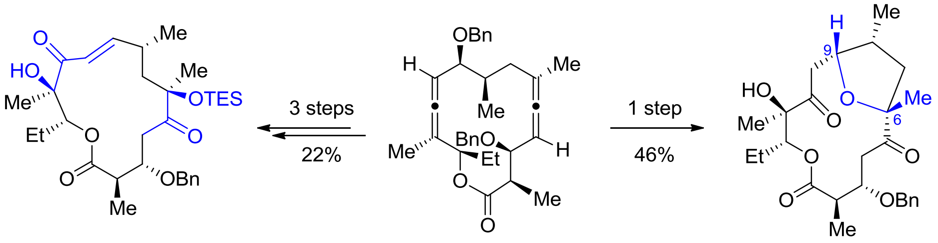


5.55

5.7. *Cyclic Allene Osmylation*

In chapter 4, we showed that treatment of macrocyclic bis[allene] **4.1** with OsO_4 can give 6,9-bicyclomacrolide **4.53** or C9-C12 hydroxyenone **4.59** if the osmylation was done stepwise and the hydroxyl group at C6 was masked before subsequent oxidation.

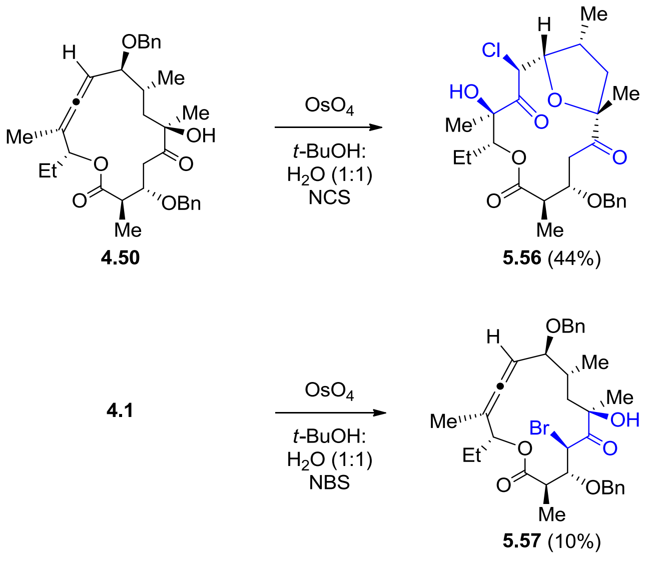
Scheme 5.7-1: Osmylation of 4.1



4.59 **4.1** **4.53**

Model macrolactone of type **2.24** demonstrated that hydrolysis of the osmate ester in the presence of an electrophile will lead to the electrophile-captured adduct even in bis[allene] macocyclic platform. We can easily envision the synthetic utility of halogenated macrolide. Hence, Kai Liu reacted mono-osmylated adduct **4.50** with OsO_4 (1.1 equiv.) in *t*-BuOH:H_2O. NCS in *t*-BuOH and H_2O solution was slowly added to the osmate enol ester in solution for 3 hours. The product showed chlorine addition **5.56** at C10 (Scheme 5.7-2). In the analogous way, NBS in *t*-BuOH and H_2O solution was added to the osmate enol ester from bis[allene] macrolactone **4.1**. Due to the reactivity of NBS with allenes, the complex mixture of products was observed on TLC and they were very hard to separate from flash column chromatography. After exhaustive chromatography, we could isolate mono bromine adduct **5.57** adduct in 10% yield.

Scheme 5.7-2: Chlorine Addition to Macrolactone


OBn
H
Me
Me
OH
Me
Et
O
O
O
OBn
Me
OsO4
t-BuOH:
H2O (1:1)
NCS
H
Me
Cl
HO
O
Me
Me
O
Et
O
O
O
OBn
Me
OsO4
t-BuOH:
H2O (1:1)
NBS
OBn
H
Me
Me
OH
Me
Br
O
Et
O
O
OBn
Me

4.50

5.56 (44%)

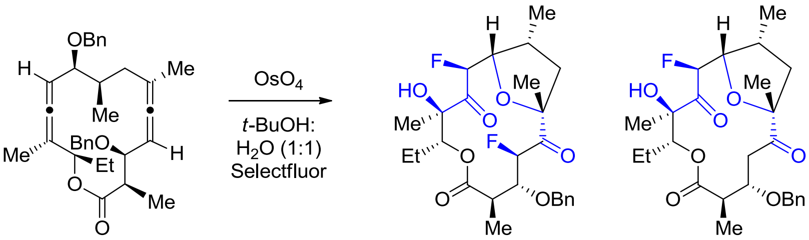
4.1

5.57 (10%)

Since chlorine and bromine atoms possess similar Van der Waals radii to a methyl group (Cl = 1.80 Å, Br = 1.95 Å, Me = 2.00 Å), they could be possible candidates for methyl group bioisostere in erythromycin analogs.[15] Especially, the chlorine atom is viewed not only to be isosteric but also isolipophillic with the methyl group and its replacement showed the alternation in drug metabolism.[15]

In modern medicinal chemistry, fluorinated compounds are synthesized on a routine basis.[16] Owing to its chemical stability and small size, fluorine is often regarded as a hydrogen bioisostere and has become an important tool in modern drug discovery to fine-tune biological effects and for studies of binding affinity in protein-ligand complexes.[17]

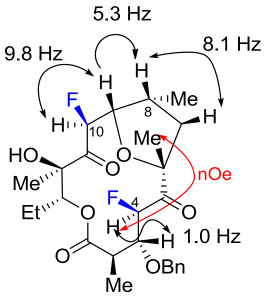
Scheme 5.7-3: Fluorine and Bromine Addition to Macrolactone



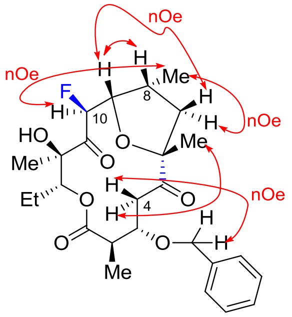
4.1 **5.58** (10%) **5.59** (21%)

To incorporate fluorine, bis[allene] macrolactone **4.1** was treated with OsO_4 followed by a solution of Selectfluor in *t*-BuOH:H_2O (1:1) (Scheme 5.7-3). This reaction was not optimized. The isolated products (**5.58** and **5.59**) showed fluorine addition. The syn addition of fluorine at C10 was confirmed by observation of a strong nOe between H10 and the C8 methyl group. Contrary to this, fluorine addition at C4 showed anti addition as expected.

Figure 5.7-1: Coupling Constants and nOe Analysis of Cyclic Ether Formation


5.3 Hz
9.8 Hz
8.1 Hz
F
H
H
Me
H
10
8
Me
HO
O
Me
O
nOe
Et
O
F
4
O
H
H
1.0 Hz
O
OBn
Me

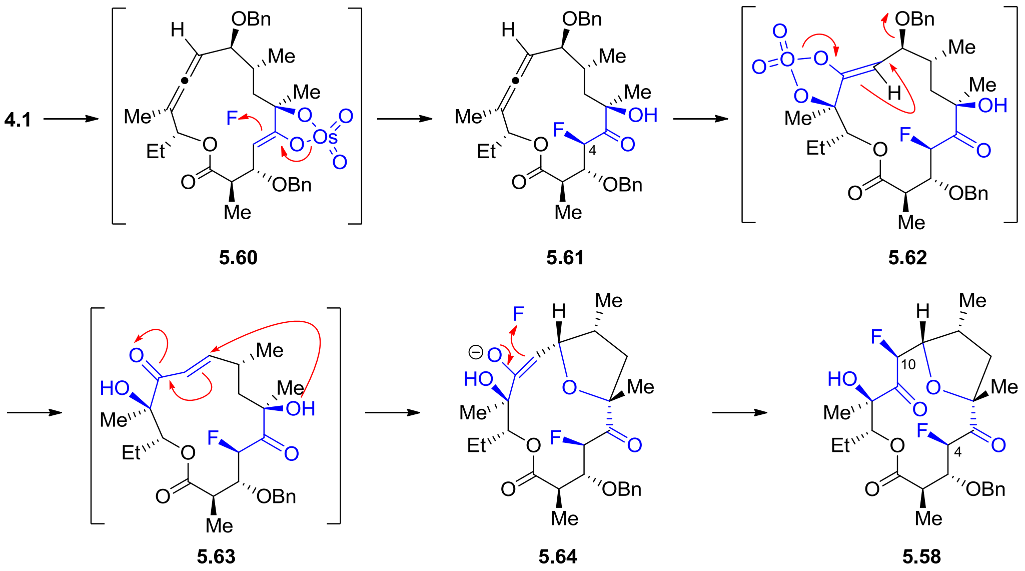
5.58


nOe
nOe
H
H
Me
F
H
10
8
H
H
nOe
HO
O
Me
Me
O
H
Et
O
4
O
nOe
H
H
O
O
H
Me

5.59

Although the precise structures of the intermediates are a matter of speculation, the osmate enol ester intermediate **5.60** formed and subsequent decomposition of the osmate enol ester captured the electrophile at C4 syn-facial to the smaller substituent (methyl vs. osmate ester at C6). However, the osmylation at the C10-C12 allene resulted in β-elimination (**5.62** → **5.63**) and subsequent intramolecular conjugate addition formed enolate (**5.63** → **5.64**). Considering the relative ground state minimum energies, z-enolate is preferred and the sterics in z-enolate preferred the top face access of electrophile. The same mechanistic rationale could be applied for the stereochemistry of chlorine and bromine adducts (**5.56** and **5.57**).

Scheme 5.7-4: Mechanistic Rationale for Stereochemistry of Bis[Fluorine] Addition



5.8. Conclusion

We discovered that *a)* the catalytic amount of OsO_4 with co-oxidants can be utilized to oxidize the chiral allenes with high regio- and stereoselective manner; *b)* the presence of electrophiles not only accelerates the rate of the reaction but also provides the multicomponent allene hydroxylation/electrophile capture adduct; *c)* when applied on the cyclic allene system, the reaction showed the complete stereoselection; *d)* the compatible electrophiles are Selectfluor, NCS, NBS, Chloramine-T, and Eshenmoser’s salt; *e)* taken together these advances have led to one general procedure which is applicable to a wide range of allenic substrates.

5.9. References

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Chapter 6

The Ligand Design and Synthesis for siRNA Delivery

6.1. Introduction

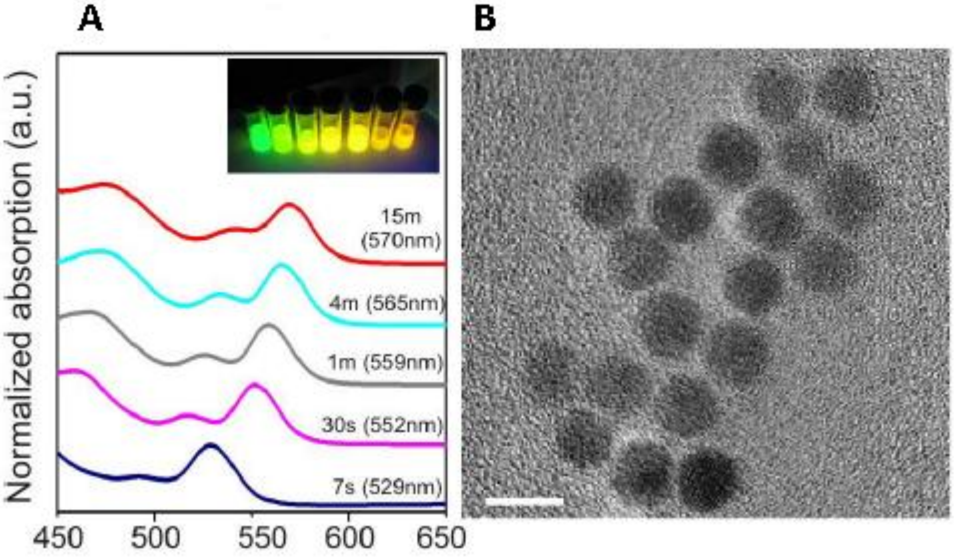
Nanoscience refers to the study of manipulating matter on an atomic and molecular scale of approximately 1~1000 nm and their applications in various fields such as solar cells, electronic devices, drug/gene delivery, regenerative medicine, tissue engineering, and biological diagnosis.[1, 2] This field has been rapidly growing in the past two decades, and its biological application has been especially widely demonstrated as a proof-of-principle. However, a practical application to clinical medicine is required to solve many key problems concerning cancer biology for therapy as well as regenerative medicine using stem cells.

In this study, I focused on the design and synthesis of target specific quantum dot-based platforms to induce the apoptosis of brain cancer cells and to show their capacity to be extended to in vivo systems for the further clinical purpose of using them as theragnostics which combines therapy and diagnosis.[3] This work in chapter 6 was all done in collaboration with Prof. Ki-Bum Lee’s group at Rutgers.

6.2. Quantom Dots (QDs)

QDs have been regarded as a representative nanosystem because it shows novel quantum physical properties such as quantum confinement effect and quantum size effect beyond classical mechanics.[4, 5] According to their sizes, QDs emit photons with different wavelengths due to different band gap energies between the conduction and valence bands: smaller sizes emit in the short wavelength range and larger size emits longer wavelength photons, which means the emission wavelength is readily tunable.

Figure 6.2-1: UV-Vis Absorption Spectra and TEM Image of Quantum Dots (adapted with permission from ref. [3])



(A) Normalized absorption spectra of CdS core while these were growing; (B) TEM image of DHLA-PEG8-amine coated CdSe/CdS/ZnS coreshell QDs (scale bar = 10 nm)

Moreover, they have broad absorption ranges and narrow emission peaks, which enable multicolor detection with a single light source. Most of all, high quantum yields and extinction coefficients generate about fifty times higher brightness than conventional organic dyes. Their enhanced photostability makes them ideal candidates for the substitution of organic dyes in the fields of single molecule biophysics and cell biology. In addition, QDs are especially suitable in biological applications since they provide high surface area which is ideal for a delivery agent and high quantum yield makes them the ideal tool for tracking agent.

However, despite of its advantages as described above, several drawbacks exist and need to be addressed for practical application, especially on cell biology. Its toxicity to cells is a major issue. Often times, the coating of quantum dots is done with mercaptoacetic acid, which is cytotoxic.[6] Even though the coating of the quantum dots is compromised, the metallic core (i.e., cadnium or selenium ions) or the dissolution of the core can be toxic. Quantum dot metabolism and degradation within the body is still unknown and several studies have shown the accumulation of the quantums dots in the kidney, spleen, and liver.[7] In addition, the bioconjugation with different molecules will increase the size of the dots making delivery into cells more difficult.

6.3. Glioblastoma Multiforme (GBM) and the Mutant Epidermal Growth Factors Receptor (EGFRvIII)

Glioblastoma multiforme (GBM) originating from glial cells is known to be the most malignant, invasive, fast-progressive, and difficult-to-treat brain tumor with just 10-12 months of the mean survival rate after cancer treatment.[8-10] Therefore, it is necessary to develop novel early diagnostic tools and therapeutic strategies and nanotechnology can be a promising tool.

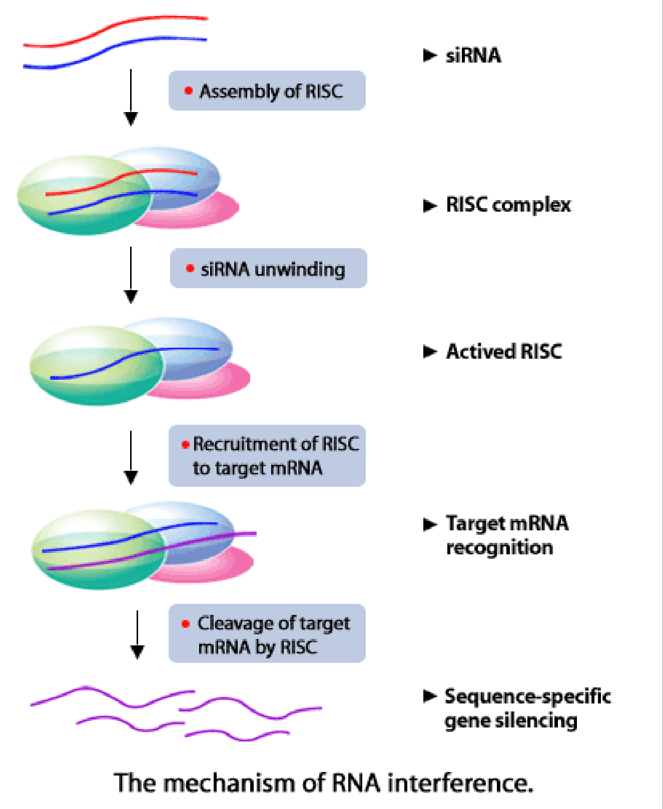
About 40% of glioblastoma show overexpression and amplification of the epidermal growth factor receptor (EGFR) gene locus and about half of these tumors express a mutant receptor (EGFRvIII) which lacks amino acids from 6 to 273 in the extracellular domain and is always activated without ligand binding.[11]

We targeted EGFRvIII, since it is known that knockdown of this gene is one of the most effective ways to down-regulate the PI3K/Akt signaling pathway.[12, 13] The PI3K/Akt signaling pathway plays a critical role in cell proliferation and apoptosis. Therefore, several drug molecules were developed to inhibit the function of the proteins in this pathway (i.e., Erlotinib/Gefinib for EGFR, and Rapamycin for mTOR). EGFR forms a dimeric complex upon the ligand binding on the extracellular domain. The kinase domain in cytoplasm region of each monomer is close together and subsequently phosphorylates the tyrosine residue on the long and flexible tail. This complex sends the proliferation triggering signal to following proteins in the downstream such as Akt, mTOR, and S6.

6.4. siRNA

RNA interference (RNAi) is a system within living cells that is responsible for controlling which genes are active and how active they are. Two types of small RNA molecules; microRNA (miRNA) and small interfering RNA (siRNA), are central to RNA interference pathway. RNAs are the direct products of genes. These small RNAs can bind to target messenger RNA (mRNA) and either increase or decrease their activity. This usually results in translational repression or target degradation and gene silencing. RNAi-based approach can sequence-specifically inhibit the expression of targeted oncogenes. Therefore, it enables modulation of signaling pathways and regulates the behavior of malignant tumor cells.[14, 15]

Figure 6.4-1: Mechanism of RNA interference[16]


siRNA
Assembly of RISC
RISC complex
siRNA unwinding
Actived RISC
Recruitment of RISC to target mRNA
Target mRNA recognition
Cleavage of target mRNA by RISC
Sequence-specific gene silencing
The mechanism of RNA interference.

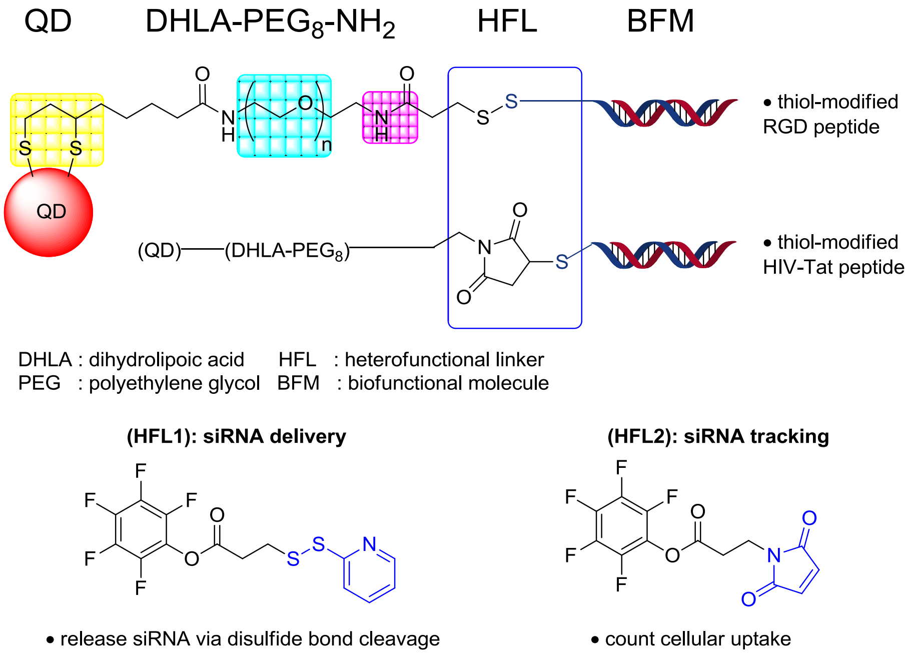
However, in order to harness the full potential of this approach, the prime requirements are to deliver the siRNA molecules with high selectivity and efficiency into tumor cells and to monitor both siRNA delivery and the resulting knock-down effects at the single cell level.

Herein, we describe the synthesis and target-specific delivery of multifunctional siRNA-QD constructs for selectively inhibiting the expression of EGFRvIII in target human U87 glioblastoma cells, and subsequently monitor the resulting down-regulated signaling pathway with high efficiency.

6.5. Design and Synthesis of the multifunctional QD platform for delivery and tracking of siRNA

We synthesized two types of siRNA-QD conjugates, one for siRNA delivery and the other for siRNA tracking (Figure 6.5-1). My former colleague Dr. Jongjin Jung prepared CdSe/CdS/ZnS core-shell QDs[17, 18] with a diameter of 7 nm and coated with either trioctylphosphine oxide (TOPO) or hexadecylamine (HDA). In order to make the QD constructs water-soluble and suitable for conjugating with siRNA, we replaced these hydrophobic ligands with a dihydrolipoic acid (DHLA) derivatized with an amine-terminated polyethylene glycol (PEG) spacers.

Figure 6.5-1: General Diagram of Multifunctional QD Conjugates (adapted with permission from ref. [3])


QD
DHLA-PEG8-NH2
HFL
BFM
• thiol-modified RGD peptide
• thiol-modified HIV-Tat peptide
(QD)—(DHLA-PEG8)
DHLA : dihydrolipoic acid
PEG : polyethylene glycol
HFL : heterofunctional linker
BFM : biofunctional molecule
(HFL1): siRNA delivery
(HFL2): siRNA tracking
• release siRNA via disulfide bond cleavage
• count cellular uptake

We hypothesized that since the dithiol moiety would provide strong coordination to the QD surface and increase stability in aqueous media, the PEG space would increase water solubility and reduce nonspecific binding, and the amine group would enable conjugation to the siRNA element.

Two heterofunctional linkers (HFL) were synthesized and evaluated for siRNA conjugation. The linker **HFL1** was designed to release siRNA upon entering the cell by cleavage of the disulfide linkage, through enzymatic reduction or ligand exchange (e.g. glutathione).[19] The second linker **HFL2** was designed to be more robust, thereby enabling evaluation of cellular uptake and localization of the siRNA construct within the cellular compartments.[20]

The final design component was the combination of three biofunctional molecules, siRNA, thiol-modified RGD (Arginine-Glycine-Aspartic Acid) peptide and thiol-modified HIV-Tat derived peptide.

Since brain tumor cells (U87 and U87-EGFRvIII) overexpress the integrin receptor protein $\alpha_v\beta_3$, they strongly bind to the RGD binding domain.[21] Thereby, RGD-functionalized siRNA-QDs selectively accumulate in brain tumor cells in vitro, and can be tracked by fluorescence microscopy. In addition, the HIV-Tat peptide promotes efficient transfection of siRNA-QDs in cells when it is directly attached to the QD surface.[22]

6.6. Transfection efficiency & target –specific delivery of siRNA-QDs

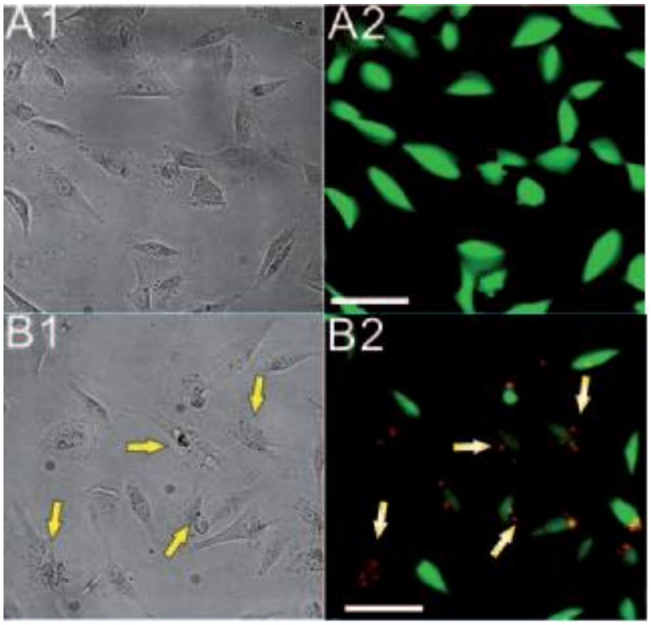
Prior to targeting EGFRvIII, the transfection efficiency was evaluated with EGFP (Enhanced Green Fluorescent Protein). Three different **siRNA-QD** constructs were prepared as follows.

- Construct modified with the RGD peptide only (**siRNA-QD-RDG**)
- Construct with HIV-Tat peptide only (**siRNA-QD-HIV-Tat**)
- Construct with both HIV-Tat and RGD peptide (**siRNA-QD-RGD-HIV-Tat**)

Among three, **siRNA-QD-RGD-HIV-Tat**, (the ratio of siRNA/RGD/HIV-Tat being 1:1:10 per QD) showed the maximum internalization within U87-EGFP cells. Therefore, this optimal condition was used for subsequent experiments.

The U87-EGFP cell line was then treated with siRNA-QD, modified with HIV-Tat and RGD, then simultaneously imaged by fluorescent microscopy.

Figure 6.6-1: Transfection and Knockdown Effect by siRNA-QD Conjugates (adapted with permission from ref. [3])



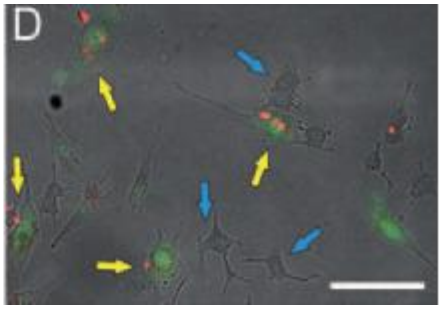
(A) Control U87 cells without siRNA-QDs; (B) U87 cells with siRNA-QDs:
A1, B1 = phase-contrast image, A2, B2 = fluorescence image. Scale bar: 100 μm.

In comparison to control U87-EGFP cells without siRNA-QD conjugates (A1 and A2, Figure 6.6-1), U87-EGFP cells with siRNA-QD conjugates (B1 and B2, Figure 6.6-1) clearly indicate the significant internalization of siRNA-QDs into the cells, which were easily distinguishable from the control cells owing to the bright fluorescence (red dots) of the QDs. Yellow arrows (B1 and B2, Figure 6.6-1) also indicate knockdown of EGFP in U87 cells as expected.

6.7. Target–specific delivery of siRNA-QDs

To further demonstrate the target-specific delivery of the siRNA-QDs, we incubated the siRNA-QDs, modified with HIV-Tat and RGD peptides, in co-cultures of the U87-EGFP cell line with PC-12 cell lines which are less-tumorigenic and have less integrin receptors. We hypothesized that the malignant U87 cells have higher cellular uptake of siRNA-QDs than the less tumorigenic PC-12 because the presence of RGD peptide on the surface of siRNA-QD led to specific binding with integrin receptors which over-expressed in the malignant U87 cells. As expected, the U87-EGFP cells showed selective accumulation of the QDs demonstrating the target-specific delivery of the siRNA-QDs (Figure 6.7-1).

Figure 6.7-1: Co-cultures of U87-EGFP cells and PC-12 (adapted with permission from ref. [3])

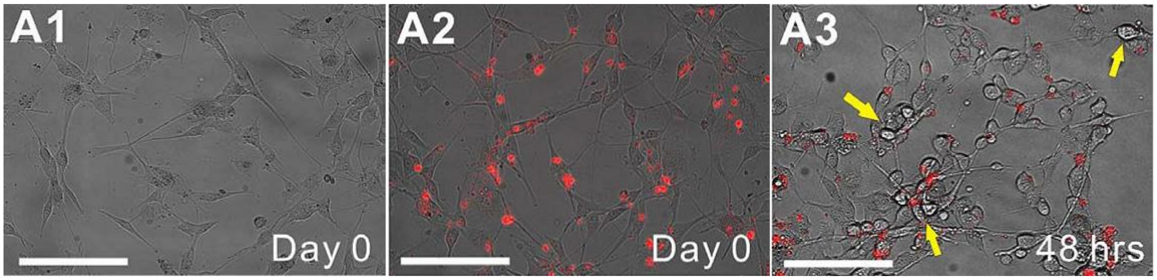


EGFP cells (yellow arrow) and PC12 (blue arrow), Scale bar: 100 μm

6.8. Knockdown of EGFRvIII as a chemotherapeutic target by siRNA-QDs

The successful demonstration of the selective manipulation of the U87-EGFP cell line led us to the examination of siRNA-QD constructs with EGFRvIII. U87-EGFRvIII cells were genetically modified to overexpress EGFRvIII, which only expressed within cancer cells. This cell type was incubated with our siRNA-QDs, modified with Tat and RGD peptides. The cells were simultaneously imaged for the internalization of siRNA-QDs using fluorescence microscopy. Relative to the control (U87-EGFRvIII without siRNA-QDs), significant cell death was observed in the wells loaded with siRNA-QDs against EGFRvIII after 48 hours (A3, Figure 6.8-1). After 48 hours, the cells have clearly shrunk and appear to have collapsed (yellow arrow, A3, Figure 6.8-1).

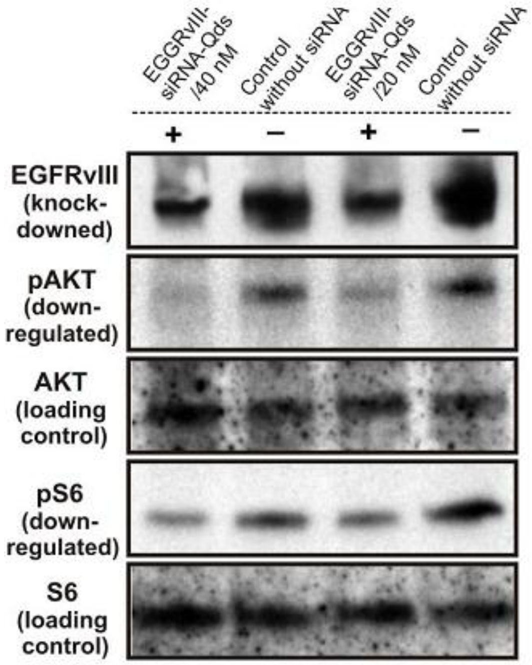
Figure 6.8-1: Knockdown of EGFRvIII in U87-EGFvIII (adapted with permission from ref. [3])



Morphology of (a) U87-EGFRvIII cells before incubation with siRNA-QDs on Day 0; (b) U87-EGFRvIII cells after incubation with siRNA-QDs on Day 0; (c) U87-EGFRvIII cells 48 hrs after incubation with siRNA-QDs, Scale Bar: 100μm.

Western immunoblotting (Figure 6.8-2) showed the considerable decrease in the expression of EGFRvIII, and down-regulation of phosphor-Akt and phopho-S6 relative to control.

Figure 6.8-2: Silencing Effect of EGFRvIII Gene (adapted with permission from ref. [3])


EGGRvIII-siRNA-Qds /40 nM
Control without siRNA
EGGRvIII-siRNA-Qds /20 nM
Control without siRNA
+
–
+
–
EGFRvIII (knock-downed)
pAKT (down-regulated)
AKT (loading control)
pS6 (down-regulated)
S6 (loading control)

Taken all together, these results demonstrate the specificity of the siRNA against EGFRvIII, the inherent noncytotoxicity of the QDs, and the facile evaluation and manipulation of cancer cell proliferation with multifunctional QD constructs.

6.9. Conclusion

The small interfering RNA (siRNA) enables manipulation of key oncogenes that modulate signaling pathways and thereby regulate the behavior of malignant tumor cells. In collaboration with Prof. Lee's group at Rutgers, we demonstrated as follows; *(a)* the synthesis and target-specific delivery of multifunctional siRNA-QD constructs; *(b)* the selective inhibition of the expression of epidermal growth factor receptor variant III

(EGFRvIII) in target human U87 glioblastoma cells; *(c)* the subsequent down-regulated signaling pathway with high efficiency was monitored.

6.10. References

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Chapter 7

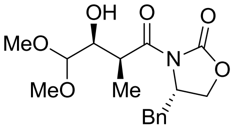
Experimental

7.1. General

All the commercial available starting materials, reagents and solvents were purchased from the suppliers (Aldrich–Sigma, Acros, Strem, TCI America, Fischer Scientific and Ochem.) and used without purification unless otherwise stated. Anhydrous solvent such as tetrahydrofurn (THF), diethyl ether (Et_2O), dichloromethane (DCM), toluene (PhMe) were purchased from Sigma–Aldrich, and then passed over a solvent purification system containing alumina based columns and dried by activated 4 Å molecular sieves for 1 hour before use. Anhydrous THF was further distilled from calcium hydride before use. All the glassware used for anhydrous reaction was flame–dried under vacuum. All the reactions were conducted under the atmosphere of high purity argon gas. The progress of all reactions were monitored by silica gel thin layer chromatography (TLC, Dynamic Absorbents Inc.), visualized under UV and/or stained using different stains such as anisaldehyde, vanillin, cerium ammonium molybdate, $KMnO_4$. Crude products were purified by flash column chromatography (FCC) on 120–400 mesh silica gel if necessary. Proton nuclear magnetic resonance spectra (^1H NMR) were obtained from either a Varian–300 instrument (300 MHz), Varian–400 instrument (400 MHz), Varian–500 instrument (500 MHz) or Inova–600 instrument (600 MHz). Chemical shifts are reported in ppm with tetramethylsilane (TMS) being the internal standard. Data is reported as follows: chemical shift (multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet, coupling constants in Hz and integration).

Carbon nuclear magnetic resonance spectra (^{13}C NMR) were obtained from either a Varian–400 instrument (100 MHz), Varian–500 instrument (125 MHz) or Inova–600 instrument (150 MHz). Chemical shifts are reported in ppm with tetramethylsilane (TMS) being the internal standard. Optical rotations were recorded at 25 °C using the sodium D line (589 nm), using Perkin–Elmer 241 polarimeter. Mass spectra were recorded on a Finnigan LCQ–DUO mass spectrometer (ESIMS) or Finnigan high resolution mass spectrometer (HRMS). Infrared (FTIR) spectra were recorded on ATI Mattson Genesis Series FT–Infrared spectrophotometer.

7.2. Chapter 3

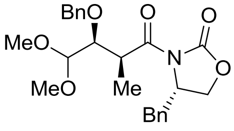


3.2

The 4(S)–benzyl *N*–propionyl oxazolidinone **3.1** (20.3 g, 87.0 mmol) was dissolved in anhydrous DCM (607 mL). To this solution was added di–*n*butylboron triflate (96.0 mL, 96.0 mmol) and TEA (10.6 g, 104 mmol) dropwise at –78 °C. The reaction mixture was then warmed to 0 °C and stirred for 1 hour at 0 °C, and then cooled back to –78 °C. Anhydrous DCM solution of 1.1–dimethoxy acetaldehyde (87.0 mL, 148 mmol, 0.18 g/mL) was added to the reaction mixture slowly at –78 °C, then the resulting solution was slowly warmed to 0 °C over 1 hour and stirred for 1 hour at 0 °C. The reaction was quenched with 100 mL solution of methanol and phosphate buffer (pH = 7.4) in 1:3 ratio at 0 °C, followed by the addition of 100 mL solution of 30% H_2O_2 and methanol (1:2 ratio). The reaction was stirred for 10 minutes at 0 °C, diluted with 200 mL DCM, washed with cold water (2 x 100 mL). Organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*. Silica gel column chromatography using 40% EtOAc in hexane gave aldol product **3.2** as white solid (26.4 g, 78.3 mmol, 90% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.25 (m, 3H), 7.21 (d, *J* = 7.0 Hz, 2H), 4.72 – 4.65 (m, 1H), 4.33 (d, *J* = 6.0 Hz, 1H), 4.23 – 4.15 (m, 2H), 4.05 – 3.96 (m, 1H), 3.42 (s, 3H), 3.38 (s, 3H), 3.26 (dd, *J* = 13.5, 3.5 Hz, 1H), 2.78 (dd, *J* = 13.5, 10 Hz, 1H), 2.68 – 2.60 (bs, 1H), 1.32 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 153.2, 135.4, 129.6 (2), 129.1 (2), 127.5, 104.9, 71.4, 66.3, 55.4, 54.9, 54.4, 39.2, 38.1, 12.8; *m/z*

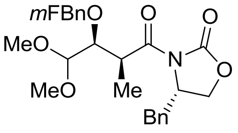
(ESIMS) calculated for $[C_{17}H_{23}NO_6+Na]^+$: 360.2, found: 360.2; $[\alpha]^{25}_D$ = 51.5 (*c* = 0.01, $CHCl_3$); IR ν_{max}(neat)/cm^{-1} 3485, 2937, 1778, 1696, 1386.



3.3

Aldol product **3.2** (17.6 g, 52.2 mmol), 4 Å powdered molecular sieve (20.0 g) and Ag_2O (33.0 g, 142 mmol) were mixed in anhydrous DCM (332 mL). Stirred for 10 minutes at room temperature, and then BnBr (24.4 g, 143 mmol) was added. The reaction mixture was then stirred thoroughly for 2 days under argon in the darkness at room temperature, and then filtered over a short column of celite. The solid residue was rinsed with DCM (3 x 100 mL). The solvent was removed *in vacuo*, silica gel column chromatography using 20% EtOAc in hexane gave compound **3.3** as colorless oil (20.3 g, 47.5 mmol, 91% yield).

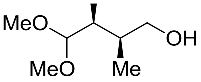
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 8H), 7.19 (d, *J* = 7.0 Hz, 2H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.34 (d, *J* = 6.0 Hz, 1H), 4.14 – 4.03 (m, 3H), 3.84 (dd, *J* = 7.5, 6.5 Hz, 1H), 3.43 (s, 3H), 3.34 (s, 3H), 3.24 (dd, *J* = 13.5, 3.5 Hz, 1H), 2.75 (dd, *J* = 13.5, 9.5 Hz, 1H), 1.31 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.2, 153.3, 138.6, 135.5, 129.6 (2), 129.0 (2), 128.4 (2), 128.2 (2), 127.8, 127.4, 107.0, 79.8, 74.4, 66.1, 55.5, 55.4, 55.2, 39.5, 38.1, 13.8; *m/z* (ESIMS) calculated for $[C_{24}H_{29}NO_6Na]^+$: 450.2, found: 450.2; $[\alpha]^{25}_D$ = 22.0 (*c* = 0.01, $CHCl_3$); IR ν_{max}(neat)/cm^{-1} 2934, 1778, 1698, 1383, 1107.



3.32

Aldol product **3.2** (8.25 g, 24.5 mmol), Ag_2O (17.0 g, 73.4 mmol) and 4 Å powdered molecular sieve (12.0 g) were mixed together and to that dry DCM (156 mL) was added. After stirring for 10 minutes at room temperature, 3–flurobenzyl bromide (6.00 mL, 48.9 mmol) was added to the reaction. The reaction was then stirred thoroughly for 5 days under nitrogen at room temperature. The reaction was then filtered through celite and the solid residue was rinsed with DCM (3 x 100 mL). Evaporation of the organic filtrate gave the crude product, which upon further purification by silica gel column chromatography using 20% EtOAc in hexane gave **3.32** as colorless oil (10.3 g, 23.1 mmol, 94% yield).

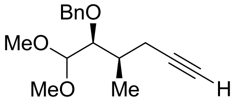
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.27 (m, 4H), 7.23 – 7.19 (m, 2H), 7.14 – 7.08 (m, 2H), 6.99 – 6.93 (m, 1H), 4.80 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.64 – 4.60 (m, 1H), 4.35 (d, *J* = 6.4 Hz, 1H), 4.15 – 4.08 (m, 2H), 4.10 (d, *J* = 7.1 Hz, 1H), 3.82 (dd, *J* = 7.6, 6.1 Hz, 1H), 3.43 (s, 3H), 3.35 (s, 3H), 3.42 (dd, *J* = 13.5, 3.3 Hz, 1H), 2.76 (dd, *J* = 13.5, 2.8 Hz, 1H), 1.30 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 163.8, 153.2, 141.1, 135.3, 129.8, 129.4, 128.9, 127.3, 123.2, 114.7, 114.4, 114.3, 106.7, 79.9, 73.4, 66.0, 55.3, 55.2, 55.0, 39.3, 38.0, 15.3.



3.4

Compound **3.3** (17.1 g, 40.0 mmol) was dissolved in Et_2O (370 mL), and then 11.0 mL methanol was added. The reaction mixture was cooled to 0 °C and then added a solution of $LiBH_4$ (35.2 mL, 77.4 mmol, 2.20 M solution in THF) slowly. Stirred at 0 °C for 2 hours, and then quenched with aqueous NH_4Cl (50 mL), extracted with EtOAc (3 x 200 mL). Organic layer combined, dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*. Silica gel column chromatography using 15% EtOAc in hexane gave **3.4** as colorless oil (9.90 g, 38.9 mmol, 97% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 4H), 7.30 – 7.25 (m, 2H), 4.82(d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.39 (d, *J* = 6.5 Hz, 1H), 3.59 (dd, *J* = 6.0, 3.5 Hz, 1H), 3.59 – 3.46 (m, 2H), 3.49 (s, 3H), 3.41 (s, 3H), 2.02 – 1.96 (m, 1H), 1.90 (bs, 1H), 0.94 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 128.5 (2), 128.2 (2), 127.8, 106.5, 79.6, 74.1, 65.8, 56.2, 54.6, 36.9, 11.4; *m/z* (ESIMS) calculated for $[C_{14}H_{22}O_4Na]^+$: 277.2, found: 277.2; IR ν_{max}(neat)/cm^{-1} 3431, 2934, 1454, 1071.

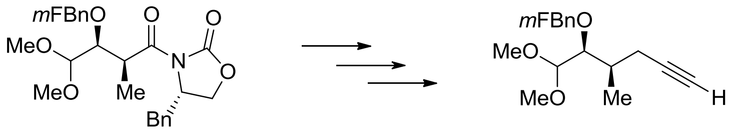


3.5

Primary alcohol **3.4** (6.00 g, 23.7 mmol) was dissolved in anhydrous DCM (184 mL), and then added diazabicyclo[2.2.2]octane (DABCO) (3.66 g, 32.6 mmol). The reaction mixture was cooled down to 0 °C and tosyl chloride (4.97 g, 26.1 mmol) was added. The reaction mixture was warmed up to room temperature and stirred for 1 hour. The reaction was then diluted with 150 mL of DCM, washed with aqueous NH_4Cl (3 x50 mL), water (50 mL). Organic layer combined and dried over anhydrous Na_2SO_4, and then concentrated down to dryness to give the crude tosylate, which could be used for the next step immediately without further purification.

The crude tosylate was azeotroped in toluene, and then dissolved in anhydrous DMSO (50 mL). To this, lithium acetylide–ethylenediamine (4.37 g, 47.4 mmol) solution in 100 mL DMSO was added at 0 °C slowly for 10 minutes. The reaction was stirred for 4 hours at room temperature. Upon the complete consumption of the tosylate, the reacton mixture was cooled down to 0 °C, and then quenched carefully with aqueous NH_4Cl (50 mL). The organic layer was extracted with EtOAc (300 mL), washed with water (3 x 100 mL), and then dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*. Silica gel column chromatography using 5% EtOAc in hexane gave **3.5** as colorless oil (4.90 g, 18.7 mmol, 80% yield).

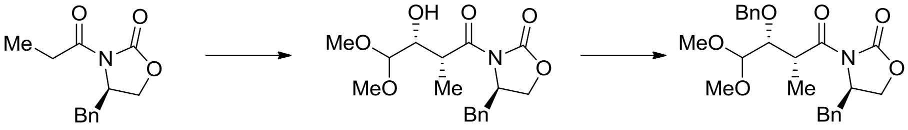
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 4H), 7.28 – 7.22 (m, 1H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.56 (d, *J* = 11.5 Hz, 1H), 4.36 (d, *J* = 7.0 Hz, 1H), 3.65 (dd, *J* = 7.0, 3.0 Hz, 1H), 3.47 (s, 3H), 3.38 (s, 3H), 2.23 – 2.12 (m, 2H), 2.10 – 2.00 (m, 1H), 1.96 (t, *J* = 7.5 Hz, 1H), 0.98 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.3, 128.4 (2), 127.9 (2), 127.6, 106.4, 83.7, 80.3, 74.8, 69.6, 56.0, 53.8, 34.4, 23.5, 14.0; *m/z* (ESIMS) calculated for $[C_{16}H_{22}O_3Na]^+$: 285.1, found: 285.2; IR ν_{max}(neat)/cm^{-1} 3295, 2935, 2116, 1096.



3.32 **3.14**

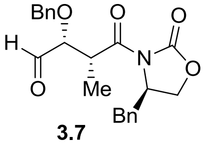
The alkyne **3.14** was prepared from **3.32** in 3 steps using same procedure used for the synthesis of alkyne **3.5**.

^{1}H NMR (500 MHz, $CDCl_3$) δ (7.34 – 7.23 (1H, m), 7.14 – 7.03 (2H, m), 6.97 – 6.91 (1H, m), 4.86 (1H, d, *J* = 12.0 Hz), 4.56 (1H, d, *J* = 12.0 Hz), 4.37 (1H, d, *J* = 7.0 Hz), 3.66 (1H, dd, *J* = 7.0, 2.5 Hz), 3.47 (3H, s), 3.39 (3H, s), 2.32 – 2.12 (2H, m), 2.10 – 2.00 (1H, m), 1.97 (1H, t, *J* = 2.5 Hz), 0.99 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.0, 162.1, 142.0 (d, J = 69 Hz), 129.9 (d, J = 66 Hz), 123.1 (d, J = 29 Hz), 114.6, 114.4, 114.4, 114.2, 106.3, 83.5, 80.4, 73.9 (d, J = 7.5 Hz), 69.7, 56.0, 53.8, 34.4, 23.4, 14.0; *m/z* (ESIMS) calculated for $[C_{16}H_{21}FO_3Na]^+$: 303.2, found: 303.2; IR ν_{max}(neat)/cm^{-1} 3306, 2936, 2117, 1789, 1592.



***ent*-3.1** ***ent*-3.2** ***ent*-3.3**

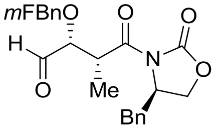
Compound ***ent*–3.2** and ***ent*–3.3** could be synthesized by using the same procedure used for the synthesis of compound **3.2** and **3.3,** respectively. The values for the observed optical rotation ($[\alpha]^{25}_D$) are –50.0 (*c* = 0.01, $CHCl_3$) for the compound ***ent*–3.2** and –21.0 (*c* = 0.01, $CHCl_3$) for the compound ***ent*–3.3**.



3.7

Benzyl protected aldol product ***ent*–3.3** (3.90 g, 9.22 mmol) was dissolved in 46 mL of water: acetic acid: trifluoroacetic acid = 1:4:1 mixed solution at room temperature for 3hrs 30 minutes. The acidic solution was azeotroped with toluene (5 x 20 mL) and resulting crude product was taken into the next step immediately without further purification. It could also be further purified by silica gel column chromatography using 15% EtOAc in hexane gave **3.7** as colorless viscous oil (3.16 g, 8.30 mmol, 90% yield).

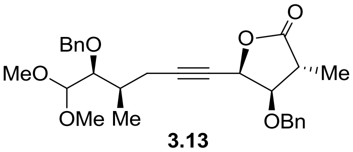
^{1}H NMR (500 MHz, CDCl$_3$) δ 9.81 (s, 1H), 7.38 – 7.22 (m, 8H), 7.17 (d, *J* = 7.0 Hz, 2H), 4.75 (d, *J* = 12.5 Hz, 1H), 4.64 – 4.54 (m, 1H), 4.59 (d, *J* = 12.5 Hz, 1H), 4.32 – 4.24 (m, 1H), 4.16 – 4.06 (m, 2H), 3.92 (d, *J* = 6.0 Hz, 1H), 3.20 (dd, *J* = 13.5, 3.5 Hz, 1H), 2.76 (dd, *J* = 13.5, 10 Hz, 1H), 1.33 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125MHz, CDCl$_3$) δ 202.0, 173.8, 153.1, 137.2, 135.1, 129.6 (2), 129.1 (2), 128.7 (2), 128.4, 128.3 (2), 127.5, 83.3, 73.1, 66.4, 55.4, 41.5, 37.8, 13.4; *m/z* (ESIMS) calculated for $[C_{22}H_{23}NO_5Na]^+$: 404.2, found: 404.2; IR ν_{max}(neat)/cm^{-1} 1778, 1730, 1693, 1390, 1212.



3.15

3–flurobenzyl protected aldol product ***ent*–3.32** (1.23 g, 2.77 mmol) was dissolved in water: acetic acid: trifluoroacetic acid = 1:4:1 mixed solution (13.7 mL) and stirred for 2 hours at room temperature. The mixture was azeotroped with toluene (3 x 50 mL) and resulting crude product upon further purification by silica gel column chromatography using 15% EtOAc in hexane gave **3.15** as colorless viscous oil (1.05 g, 2.63 mmol, 95% yield).

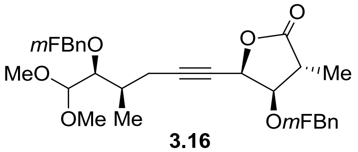
^{1}H NMR (500 MHz, CDCl$_3$) δ 9.82 (s, 1H), 7.34 – 7.24 (m, 4H), 7.19 – 6.95 (m, 4H), 4.73 (d, *J* = 12.5 Hz, 1H), 4.71 – 4.63 (m, 2H), 4.57 (d, *J* = 12.5 Hz, 1H), 4.34 – 4.26 (m, 1H), 4.16 – 4.13 (m, 1H), 4.12 (quartet, *J* = 7.0, 1H), 3.92 (dd, *J* = 6.5, 1.2 Hz, 1H), 3.24 (dd, *J* = 13.3, 3.1 Hz, 1H), 2.78 (dd, *J* = 13.3, 9.4 Hz, 1H), 1.33 (d, *J* = 7.4 Hz, 3H), 1.25 (d, *J* = 7.0 Hz, 3H).



3.13

Alkyne **3.5** (3.44 g, 13.1 mmol) was dissolve in Et_2O (130 mL) then cooled to –78 °C. To that mixture was added *n*–BuLi (8.2 mL, 13 mmol) slowly. The reaction mixture was stirred at –78 °C for 1 hour and added a solution of $ZnBr_2$ (4.13 g, 18.4 mmol) in Et_2O (35.9 mL). The reaction flask was moved to 0 °C bath. At 10 °C, the solution of aldehyde **3.7** (2.00 g, 5.24 mmol) in Et_2O (12.5 mL) was added dropwise over 1 hour via syringe pump. The reaction was stirred overnight. The reaction was quenched with aqueous NH_4Cl (50 mL) at 0 °C, and then extracted with EtOAc (200 mL). The organic layer washed with water (2 x 50 mL), and then dried over Na_2SO_4. The organic layer was concentrated down to dryness to give the crude product (8:1 ratio by ^{1}H NMR). Silica gel column chromatography using 10% EtOAc in hexane gave major isomer **3.13** as colorless oil (1.53 g, 3.29 mmol, 63% yield).

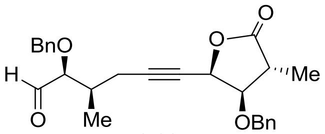
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 10H), 5.12 (td, *J* = 2.0 Hz, 6.5 Hz, 1H), 4.82 (d, *J* =11.5 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 3.88 (dd, *J* = 9.5, 6.5 Hz, 1H), 3.57 (dd, *J* = 10.0, 7.0 Hz, 1H), 3.47 (s, 3H), 3.37 (s, 3H), 2.86 – 2.76 (m, 1H), 2.38 – 2.20 (m, 2H), 2.10 – 2.00 (m, 1H), 1.25 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 139.1, 137.1, 128.8 (2), 128.5 (2), 128.4, 128.1 (2), 127.9 (2), 127.7, 106.4, 90.5, 81.0, 80.5, 74.7, 73.9, 72.4, 70.5, 56.1, 54.1, 39.4, 34.4, 24.0, 14.0, 12.7; *m/z* (ESIMS) calculated for $[C_{28}H_{34}O_6Na]^+$: 489.3, found: 489.2; IR ν_{max}(neat)/cm^{-1} 2935, 2238, 1786, 1454.



3.16

Alkyne **3.14** (54.9 mg, 196 μmol) was dissolve in Et_2O (2 mL) then cooled to –78 °C. To that mixture was added *n*–BuLi (78 μL, 0.20 mmol) slowly. The reaction mixture was stirred at –78 °C for 1 hour and added a solution of $ZnBr_2$ (44.1 mg, 196 μmol) in Et_2O (0.4 mL). The reaction flask was moved to 0 °C bath. At 10 °C, the solution of aldehyde **3.15** (60.2 mg, 151 μmol) in Et_2O (0.4 mL) was added dropwise over 1 hour via syringe pump. The reaction was stirred overnight. The reaction was quenched with aqueous NH_4Cl (50 mL) at 0 °C, and then extracted with EtOAc (20 mL). The organic layer washed with water (2 x 5 mL), and then dried over Na_2SO_4. The organic layer was concentrated down to dryness to give the crude product (8:1 ratio by ^{1}H NMR). Silica gel column chromatography using 10% EtOAc in hexane gave major isomer **3.16** as colorless oil (26.6 mg, 52.9 μmol, 35% yield).

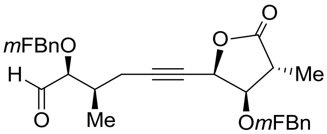
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 3H), 7.09 – 6.93 (m, 5H), 4.88 – 4.82 (m, 2H), 4.75 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.36 (d, *J* = 6.8 Hz, 1H), 3.89 (dd, *J* = 7.1, 5.6 Hz, 1H), 3.54 (dd, *J* = 7.0, 2.9 Hz, 1H), 3.48 (s, 3H), 3.39 (s, 3H), 2.68 (quartet, *J* = 7.3 Hz, 1H), 2.37 – 2.19 (m, 2H), 2.13 – 2.01 (m, 1H), 1.36 (d, *J* = 7.3 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H).



3.23

Acetal **3.13** (1.90 g, 4.10 mmol) was dissolved in 42.5 mL mixed solution of acetic acid, trifluoroacetic acid and water (4:1:1) at room temperature and stirred for 12 hours. The solvent was azeotroped with toluene (5 x 100 mL) to give a crude product which could be used for the next step without further purification. It could also be further purified by silica gel column chromatography using 12% EtOAc in hexane gave **3.23** as colorless viscous oil (1.50 g, 3.57 mmol, 90% yield).

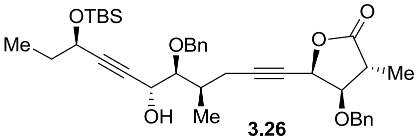
^{1}H NMR (500 MHz, CDCl$_3$) δ 9.65 (s, 1H), 7.44 – 7.25 (m, 10H), 5.11 (d, *J* = 6.0 Hz, 1H), 4.67 (d, *J* = 13.0 Hz, 2H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 3.95 – 3.85 (m, 2H), 2.85 – 2.75 (m, 1H), 2.48 – 2.26 (m, 2H), 2.26 – 2.16 (m, 1H), 1.27 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 204.3, 175.9, 137.6, 137.1, 128.8 (2), 128.7 (2), 128.5, 128.3, 128.2 (2), 128.0 (2), 89.2, 85.1, 81.0, 74.9, 73.4, 72.4, 70.4, 39.4, 35.2, 23.1, 14.5, 12.7; *m/z* (ESIMS) calculated for $[C_{26}H_{28}O_5Na]^+$: 443.2, found: 443.2; IR ν_{max}(neat)/cm^{-1} 2935, 2240, 1786, 1730, 1455.



3.32

Acetal **3.16** (36.3 mg, 72.0 μmol) was dissolved in 0.75 mL mixed solution of acetic acid, trifluoroacetic acid and water (4:1:1) at room temperature and stirred for 12 hours. The solvent was azeotroped with toluene (5 x 100 mL) to give a crude product which could be used for the next step without further purification. It could also be further purified by silica gel column chromatography using 12% EtOAc in hexane gave **3.32** as colorless viscous oil (31.3 mg, 69.0 μmol, 95% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.67 (s, 1H), 7.44 – 6.92 (m, 8H), 5.21– 5.11 (m, 1H), 4.71 (d, *J* = 12.0 Hz, 2H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 3.96 – 3.84 (m, 2H), 2.90 – 2.78 (m, 1H), 2.50 – 2.19 (m, 2H), 1.29 (d, *J* = 7.3 Hz, 3H), 1.01 (d, *J* = 6.7 Hz, 3H).

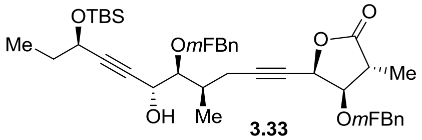


3.26

A solution of alkyne **3.11** (1.38 g, 7.00 mmol) in THF (33 mL) was cooled to –78 °C. To that was added MeLi (3.70 mL, 5.87 mmol) slowly. The reaction mixture was stirred for 30 minutes and to that mixture was added the hexane solution of chlorotriisopropoxyltitanium (IV) (5.87 mL, 5.87 mmol). The reaction was stirred for 1 hour and to that a solution of aldehyde **3.23** (1.50 g, 3.70 mmol) in THF (15 mL) was added slowly at –78 °C. The reaction was then warmed slowly to –30 °C over 1 hour. The reaction was quenched with aqueous NH_4Cl, extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4. . Evaporation of the organic filtrate gave the crude product (6:1 ratio by ^{1}H NMR) which upon further purification by silica gel column chromatography using 10% EtOAc in hexane gave major isomer of **3.26** as colorless oil (2.00 g, 3.23 mmol, 89% combined yield for both diastereomers).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 – 7.44 (m, 10H), 5.11 (td, *J* = 2.1 Hz, 6.0 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.58 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.43 – 4.54 (m, 1H), 4.32 (t, *J* = 6.6 Hz, 1H), 3.87 (dd, *J* = 9.6, 6.6

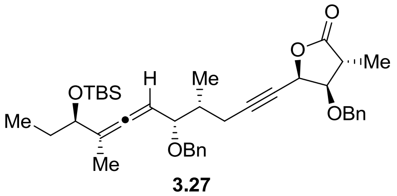
Hz, 1H), 3.58 (dd, *J* = 5.4, 5.4 Hz, 1H), 2.88 – 2.76 (m, 1H), 2.46 – 2.13 (m, 1H), 1.75 – 1.60 (m, 1H), 1.24 (d, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 6.9 Hz, 3H), 0.87 (s, 9H), 0.08 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 138.4, 137.0, 128.8, 128.6, 128.5, 128.0, 127.9, 127.9, 89.7, 88.4, 83.5, 82.6, 80.9, 77.4, 77.2, 76.9, 74.6, 74.3, 72.3, 70.4, 64.4, 63.5, 39.4, 34.6, 31.9, 26.0, 23.8, 18.4, 14.9, 12.6, 9.8, 0.2, –4.2, –4.8; *m/z* (ESIMS) calculated for $[C_{37}H_{50}O_6SiNa]^+$: 641.3, found: 641.3.



3.33

A solution of alkyne **3.11** (26.8 mg, 135 μmol) in THF (640 μL) was cooled to –78 °C. To that was added MeLi (75 μL, 0.12 mmol) slowly. The reaction mixture was stirred for 30 minutes and to that mixture was added the hexane solution of chlorotriisopropoxyltitanium (IV) (120 μL, 120 μmol). The reaction was stirred for 1 hour and to that a solution of aldehyde **3.32** (36.3 mg, 80.0 μmol) in THF (320 μL) was added slowly at –78 °C. The reaction was then warmed slowly to –30 °C over 1 hour. The reaction was quenched with aqueous NH_4Cl, extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4. . Evaporation of the organic filtrate gave the crude product (6:1 ratio by ^{1}H NMR) which upon further purification by silica gel column chromatography using 10% EtOAc in hexane gave major isomer of **3.33** as colorless oil (46.9 mg, 72.0 mmol, 90% combined yield for both diastereomers).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.24 (m, 3H), 7.15 – 6.93 (m, 3H), 5.18 (td, *J* = 2.1 Hz, 6.2 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.58 (d, *J* = 5.3 Hz, 1H), 4.54 (d, *J* = 5.3 Hz, 1H), 4.50 (dd, *J* = 11.7, 5.6 Hz, 1H), 4.32 (td, *J* = 6.5, 1.5 Hz, 1H), 3.90 (dd, *J* = 9.4, 6.2 Hz, 1H), 3.58 (dd, *J* = 5.6, 4.4 Hz, 1H), 2.89 – 2.79 (m, 1H), 2.43 – 2.16 (m, 4H), 1.71 – 1.61 (m, 2H), 1.30 (d, *J* = 7.3 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H)0.89 (s, 9H), 0.14 (s, 3H), 0.07 (s, 3H).

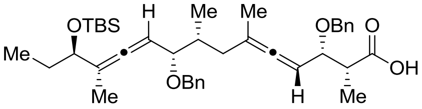


3.27

To a solution of **3.26** (0.25 g, 0.40 mmol) in 8 mL of Et_2O was added TEA (60.7 mg, 0.600 mmol) and methanesulfonyl chloride (68.7 mg, 0.606 mmol) respectively at 5 °C. The reaction mixture was warmed to room temperature and stirred for 1 hr 20 minutes at room temperature. The mesylate solution was added a solution of methyl cyanocuprate, prepared from CuCN (0.21 g, 2.4 mmol) and MeLi (1.5 mL, 2.4 mmol) in 12 mL of Et_2O at –35 °C. The temperature was kept below –20 °C for 1 hour. The reaction was then quenched with aqueous NH_4Cl (5 mL), and then extracted in Et_2O (3 x 10 mL). The organic layer was washed with water (10 mL) and dried over anhydrous $MgSO_4$. After removing the solvent under reduced pressure, the crude material was subject to silica gel column chromatography giving **3.27** (0.12g, 0.020 mmol) in 50% yield.

Data of **3.27**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 – 7.26 (m, 10H), 5.17 – 5.12 (m, 1H), 5.00 – 4.93 (m, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* =12.2 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.30 (d, *J* = 12.0 Hz, 1H), 4.01 (t, *J* = 6.1 Hz, 1H), 3.89 (t, *J* = 6.4 Hz, 1H), 3.71 (dd, *J* = 8.8, 5.4 Hz, 1H), 2.86 – 2.80 (m, 1H), 2.68 – 2.62 (m, 1H), 2.19 (dd, *J* = 9.5, 2.0 Hz, 1H), 1.73 (d, *J* = 2.9 Hz, 3H), 1.64 (d, *J* = 3.0 Hz, 1H), 1.63 – 1.37 (m, 2H), 1.27

(d, *J* = 7.1 Hz, 3H), 1.10 (d, *J* = 6.6 Hz, 3H), 0.91 (s, 9H), 0.88 (t, *J* = 7.3 Hz, 3H), 0.04 (s, 3H), 0.03 (s, 3H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.5, 175.7, 138.5, 136.9, 128.5 (2), 128.2 (2), 127.8(4), 127.5, 127.4, 102.7, 90.3, 89.4, 80.8, 80.7, 75.3, 73.4, 72.0, 70.3, 69.9, 39.1, 38.2, 29.2(3), 25.8, 18.2, 15.1, 13.8, 12.4, 10.0, –4.6, –5.0.

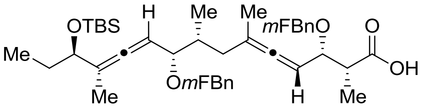


3.28

To a solution of **3.26** (1.24 g, 2.00 mmol) in 40 mL of Et_2O was added TEA (0.30 g, 3.0 mmol) and methanesulfonyl chloride (0.34 g, 3.0 mmol) respectively at 5 °C. The reaction mixture was warmed to room temperature and stirred for 1 hr 20 minutes at room temperature. The mesylate solution was added a solution of methyl cyanocuprate, prepared from CuCN (1.07 g, 12.0 mmol) and MeLi (7.49 mL, 12.0 mmol) in 60 mL of Et_2O at – 35 °C. The reaction mixture was then warmed to room temperature and stirred for 1h. The reaction was then quenched with aqueous NH_4Cl (50 mL) and extracted in Et_2O (3 x 100 mL). The organic layer was washed with water (100 mL), and then dried over anhydrous $MgSO_4$.. Evaporation of solvent gave the crude product **3.28** (1.27 g) and it is subjected to the next step without silica gel column chromatography.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.26 (m, 10H), 5.05 – 4.90 (m, 2H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.69 (d, *J* =11.5 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.39 (d, *J* = 12.0 Hz, 1H), 4.03 (dd, *J* = 8.0 Hz, 6.0 Hz, 1H), 4.00 (t, *J* = 6.0 Hz, 1H), 3.66 (dd, *J* = 9.5, 5.5 Hz, 1H), 2.84 – 2.72 (m, 1H), 2.42 – 2.32 (m, 1H), 1.95 – 1.85 (m, 1H), 1.72 (d, *J* = 3.0 Hz, 3H), 1.65 (d, *J* = 3.0 Hz, 3H), 1.60 – 1.50 (m, 2H), 1.24 (d, *J* = 7.0 Hz, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.88 (s, 9H), 0.85 (t, *J* = 7.5 Hz, 3H), 0.02 (s, 3H), 0.00 (s, 3H) ; ^{13}C

NMR (125 MHz, $CDCl_3$) δ 203.9, 202.9, 177.0, 138.9, 137.8, 128.6 (2), 128.5 (2), 128.0 (4), 127.8, 127.6, 102.5, 100.0, 89.7, 87.7, 82.2, 79.8, 75.8, 70.7, 70.2, 37.7, 36.7, 29.5, 26.1 (3), 19.0, 18.4, 15.7, 13.8, 12.9, 10.3, –4.3, –4.8; *m/z* (ESIMS) calculated for $[C_{39}H_{56}O_5SiNa]^+$: 655.4, found: 655.4; $[\alpha]^{25}_{D}$ = 47 (*c* = 0.01, $CHCl_3$); IR ν_{max}(neat)/cm^{-1} 2929, 1965, 1710, 1456, 1066.

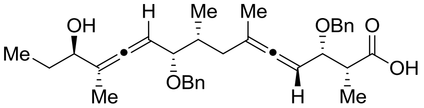


3.35

To a solution of **3.33** (10.5 mg, 16.0 μmol) in 320 μL of Et_2O was added TEA (4.9 mg, 48 μmol) and methanesulfonyl chloride (5.5 mg, 48 μmol) respectively at 5 °C. The reaction mixture was warmed to room temperature and stirred for 1 hr 20 minutes at room temperature. The mesylate solution was added a solution of methyl cyanocuprate, prepared from CuCN (14.4 mg, 160 μmol) and MeLi (100 μL, 160 μmol) in 800 μL of Et_2O at – 35 °C. The reaction mixture was then warmed to room temperature and stirred for 1h. The reaction was then quenched with aqueous NH_4Cl (5 mL) and extracted in Et_2O (3 x 10 mL). The organic layer was washed with water (10 mL), and then dried over anhydrous $MgSO_4$.. Evaporation of solvent gave the crude product is subjected to silica gel column chromatography, giving **3.35** (9.2 mg, 14 μmol) in 86% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 – 7.23 (m, 3H), 7.12 – 6.89 (m, 5H), 6.13 – 6.00 (m, 1H), 5.63 – 5.52 (m, 1H), 5.63 – 5.52 (m, 1H), 4.64 (d, *J* = 12.3 Hz, 1H), 4.36 (d, *J* = 12.6 Hz, 1H), 3.99 (t, *J* = 6.2 Hz, 1H), 3.73 (dd, *J* = 8.5 Hz, 5.3 Hz, 1H), 2.59 – 2.49 (m, 1H), 2.25 – 2.15 (m, 2H), 1.96 – 1.86 (m, 2H), 1.70 (d, *J* = 2.6 Hz, 3H), 1.65 –

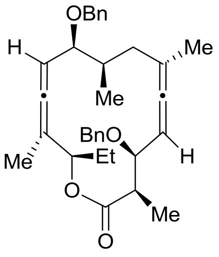
1.49 (m, 2H), 1.29 (d, *J* = 6.8 Hz, 3H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.92 (s, 9H), 0.79 (t, *J* = 7.5 Hz, 3H), 0.02 (s, 3H), 0.00 (s, 3H).



3.29

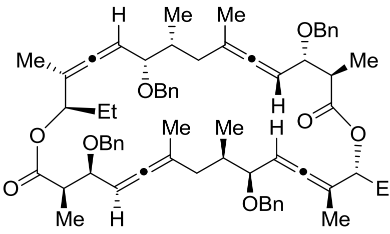
The crude Bis[allene] **3.28** was dissolved in 60 mL of acetic acid, water, and trifluoroacetic acid mixture (4:1:0.25 ratio) and stirred for 1 hour at rt. The solvent was azeotroped with toluene (3 x 50 mL) and resulting crude product upon further purification by silica gel column chromatography using 30% EtOAc in hexane gave **3.29** as colorless oil (860 mg, 1.66 mmol, 83% yield over 2 steps, **3.26** → **3.29**).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.27 (m, 10H), 5.18 – 5.10 (m, 1H), 5.08 – 5.02 (m, 1H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.45 (d, *J* = 11.5 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.04 (t, *J* = 6.4, 1H), 3.99 (t, *J* = 6.8 Hz, 1H), 3.69 (dd, *J* = 8.5, 5 Hz, 1H), 2.84 – 2.76 (m, 1H), 2.38 – 2.32 (m, 1H), 1.94 – 1.84 (m, 1H), 1.75 (d, *J* = 3 Hz, 3H), 1.67 (d, *J* = 2.5 Hz, 3H), 1.66 – 1.50 (m, 2H), 1.23 (d, *J* = 7 Hz, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.90 (t, *J* = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.6, 201.7, 175.7, 138.9, 137.7, 128.7 (2), 128.5 (2), 128.1, 128.1 (2), 127.8 (2), 127.7, 103.4, 100.6, 92.5, 88.0, 82.1, 79.3, 74.2, 70.7, 70.4, 44.4, 37.6, 37.0, 27.9, 19.4, 15.4, 15.1, 13.3, 9.8; *m/z* (ESIMS) calculated for $[C_{33}H_{42}O_5Na]^+$: 541.3, found: 541.3; $[\alpha]^{25}_D$ = 45.0 (*c* = 0.01, $CHCl_3$); IR ν_{max}(neat)/cm^{-1} 3388, 2933, 1965, 1710, 1454.



4.1

+

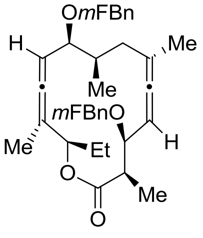


3.31

The seco acid **3.29** (195 mg, 0.380 mmol) was azeotroped with toluene (3 x 10 mL) and then dissolved in 20 mL toluene. TEA (0.19 mg, 1.9 mmol) and 2,4,6–trichlorobenzoyl chloride (0.46 g, 1.9 mmol) was added to this solution slowly at room temperature, and then stirred for 6 hours at room temperature. The resulting active ester was then added dropwise over 2 hours to a solution of 4–(*N*, *N*–dimethylamino)pyridine (DMAP) (0.46 g, 3.5 mmol) in toluene (163 mL) at 80 °C. The mixture was then cooled back to room temperature and quenched with aqueous NH_4Cl (100 mL). The organic layer was washed with water (2 x 100 mL) and dried over Na_2SO_4. The organic layer was concentrated down to dryness under reduced pressure. Silica gel column chromatography using 5% EtOAc in hexanes gave the bis[allene] macrolactone **4.1** (121 mg, 0.240 mmol, 64% yield) and **3.31** (19 mg, 19 μmol, 5% yield) as a colorless oil.

For detailed NMR analysis of **4.1**, see page 204; *m/z* (ESIMS) calculated for $[C_{33}H_{40}O_4Na]^+$: 523.3, found: 523.3; $[\alpha]^{25}_D = 50.0$ ($c = 0.01$, $CHCl_3$); IR ν_{max}(neat)/cm^{-1} 2969, 2934, 1960, 1731, 1454, 1369, 1248, 1065.

Data of **3.31**; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.16 (m, 20H), 5.12 – 5.02 (m, 4H), 4.98 – 4.90 (m, 2H), 4.65 – 4.58 (m, 4H), 4.37 – 4.28 (m, 4H), 3.97 – 3.94 (t, J = 7.8 Hz, 2H), 3.71 – 3.67 (m, 2H), 2.73 – 2.60 (m, 3H), 2.36 – 2.28 (m, 2H), 1.95 – 1.78 (m, 5H), 1.74 – 1.60 (m, 12H), 1.24 – 1.23 (m, 8H), 1.03 – 0.97 (m, 6H), 0.89 – 0.81 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.6, 202.6, 173.3, 138.9, 138.6, 128.2, 128.1, 127.5, 127.3, 99.1, 98.8, 92.2, 88.2, 81.3, 79.9, 76.1, 70.4, 70.3, 45.9, 38.5, 36.6, 31.9, 29.7, 29.6, 29.4, 25.8, 22.7, 18.3, 14.7, 14.6, 14.1, 13.6, 15.1, 9.7; *m/z* (ESIMS) calculated for $[C_{66}H_{80}O_8Na]^+$: 1023.3, found: 1023.3; IR ν_{max}(neat)/cm^{-1} 2922, 1968, 1735, 1453, 1377, 1068.



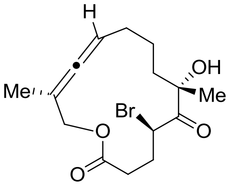
4.2

The seco acid **3.30** (280 mg, 0.510 mmol) was azeotroped with toluene (3 x 10 mL) and then dissolved in 15 mL toluene. TEA (260 mg, 2.52 mmol) and 2,4,6–trichlorobenzoyl chloride (617 mg, 2.52 mmol) was added to this solution slowly at room temperature, and then stirred for 6 hours at room temperature. The resulting active ester was then added dropwise over 2 hours to a solution of 4–(*N*, *N*–dimethylamino)pyridine (DMAP) (610 mg, 5.00 mmol) in toluene (250 mL) at 80 °C. The mixture was then cooled back to room temperature and quenched with aqueous NH_4Cl (100 mL). The organic layer was washed with water (2 x 100 mL) and dried over Na_2SO_4. The organic layer was concentrated down to dryness under reduced pressure. Silica gel column chromatography using 5% EtOAc in hexanes gave the bis[allene] macrolactone **4.2** (202 mg, 0.380 mmol, 75% yield) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 – 7.22 (2H, m), 7.11 – 7.01 (4H, m), 6.97 – 6.89 (2H, m), 5.38 – 5.30 (1H, m), 5.28 (1H, t, *J* = 6.5 Hz), 5.16 – 5.08 (1H, m), 4.64 (1H, d, *J* = 12.5 Hz), 4.52 (1H, d, *J* = 12.5 Hz), 4.48 (1H, d, *J* = 12.5 Hz), 4.31 (1H, d, *J* =

12.0 Hz), 3.96 (1H, dd, *J* = 8.0, 4.0 Hz), 3.77 (1H, dd, *J* = 7.0, 7.0 Hz), 2.86 – 2.76 (1H, m), 2.20 – 2.14 (1H, m), 2.04 – 1.94 (1H, m), 1.71 (3H, d, *J* = 3.0 Hz), 1.70 (3H, d, *J* = 3.0 Hz), 1.72 – 1.62 (2H, m), 1.26 (3H, d, *J* = 7.0 Hz), 1.05 (3H, d, *J* = 7.0 Hz), 0.90 (3H, t, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.8, 201.3, 173.8, 164.1 (d, *J* = 84 Hz), 162.1 (d, *J* = 89 Hz), 142.1(d, *J* = 75 Hz), 141.6 (d, *J* = 69 Hz), 129.9 (d, *J* = 46 Hz), 129.8 (d, *J* = 79 Hz), 123.1 (d, *J* = 27 Hz), 122.9 (d, *J* = 27 Hz), 114.6 – 114.3 (4C, m), 102.6, 99.3, 91.6, 90.5, 81.7, 77.9, 75.8, 70.0 (d, *J* = 17 Hz), 68.1 (d, *J* = 19 Hz), 44.9, 37.7, 35.9, 25.1, 20.5, 17.5, 15.3, 14.0, 9.9; $[\alpha]^{25}_{D}$ = –3.0 (*c* = 0.01, $CHCl_3$); *m/z* (ESIMS) calculated for $[C_{33}H_{38}F_2O_4Na]^+$: 559.3, found: 559.3; IR ν_{max}(neat)/cm^{-1} 2971, 2935, 1968, 1732, 1617, 1592, 1174.

7.3. Chapter 4

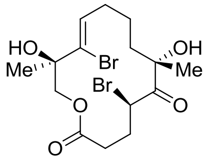


4.4

To a solution of bis[allene] macrolactone **2.24** (26.6 mg, 115 μmol) in $CHCl_3$ (1 mL) was added a solution of DMDO in $CDCl_3$ (1.1 mL, 0.30 mmol) dropwise at –40 °C. The reaction was stirred under nitrogen and let it to warm to –5 °C over 1 hr 30 minutes. After the consumption of **2.24**, excess DMDO was removed under reduced pressure and the crude mixture was azeotroped with toluene. To the crude mixture was added a solution of LiBr (39.8 mg, 0.450 mmol) in THF (1 mL) via syringe pump for 1 hour at –5 °C After the consumption of SDE, the reaction mixture was diluted with water and then extracted with DCM. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave **4.4** (24.2 mg, 69.9 μmol, 61%) of as white solid. The product was recrystallized in 20% EtOAc in hexane.

For detailed X–ray crystallography, see page 214; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.27 – 5.17 (m, 1H), 4.94 (dd, *J* = 12.4, 2.9 Hz, 1H), 4.70 (t, *J* = 7.0 Hz, 1H), 4.11 (dd, *J* = 12.5, 1.8 Hz, 1H), 3.72 (s, 1H), 2.69 – 2.54 (m, 1H), 2.52 – 2.30 (m, 3H), 2.06 – 1.97 (m, 2H), 1.73 (d, J = 2.93 Hz, 3H), 1.59 – 1.57 (m, 2H), 1.55 (s, 3H), 1.39 – 1.24 (m, 1H), 0.96 – 0.80 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.9, 203.2, 171.7, 95.8, 92.0, 79.1, 65.8, 41.7, 37.9, 31.8, 29.7, 28.4, 26.8, 22.7, 17.6; *m/z* (HRMS) calculated for

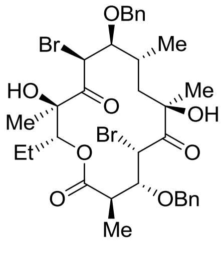
$[C_{15}H_{21}BrO_4Na]^+$: 369.1, found: 369.1; IR ν_{max} (neat)/cm^{-1} 3502, 2954, 2924, 2854, 1969, 1736, 1712, 1454, 1376, 1236, 1200, 1154.



4.6

To a solution of bis[allene] macrolactone **2.24** (47.0 mg, 202 μmol) in $CDCl_3$ (1 mL) was added a solution of DMDO in $CDCl_3$ (2.0 mL, 0.60 mmol) dropwise at –40 °C. The reaction was stirred under nitrogen and let to warm to –5 °C over 1 hr 30 minutes. To the reaction mixture was added LiBr (52.8 mg, 0.610 mmol) at –5 °C. TLC showed the unreacted SDE. To the reaction mixture was added LiBr (123 mg, 1.42 mmol). After the complete consumption of SDE, the reaction was then diluted with water and extracted with DCM. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 20% EtOAc in hexanes gave **4.6** as colorless oil (50.0 mg, 113 μmol, 56% yield).

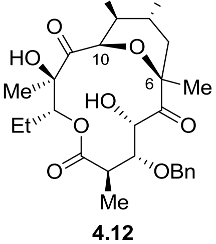
For detailed NMR analysis, see page 222; *m/z* (HRMS) calculated for $[C_{15}H_{21}Br_2O_5H]^+$: 441.9, found: 422.9, 424.9, 426.9 $[C_{15}H_{21}Br_2O_5H{-}H_2O]$; IR ν_{max} (neat)/cm^{-1} 3495, 2920, 2860, 1740, 1721, 1454, 1372, 1241, 1143, 1002.



4.9

To a solution of bis[allene] macrolactone **4.1** (15.0 mg, 30.0 μmol) in $CDCl_3$ (1 mL) was added a solution of DMDO in $CDCl_3$ (0.4 mL, 0.2 mmol) dropwise at –40 °C. The reaction was stirred under nitrogen and let to warm to –15 °C over 30 minutes. To the reaction mixture was added LiBr (53.2 mg, 0.610 mmol) at –15 °C. After the complete consumption of SDE, the reaction was diluted with water and extracted with DCM. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave **4.9** as colorless oil (2.0 mg, 3.1 μmol, 11% yield).

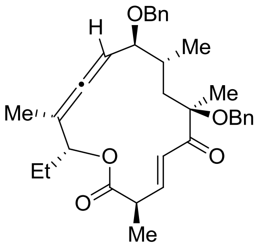
For detailed NMR analysis, see page 235; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.33 (m, 10H), 6.00 (d, *J* = 8.51 Hz, 1H), 5.17 (dd, *J* = 2.64, 10.56 Hz, 2H), 4.55 (d, *J* = 7.92 Hz, 1H), 3.86 (d, *J* = 9.39 Hz, 1H), 3.83 (dd, *J* = 3.82, 9.69 Hz, 1H), 3.17 (t, *J* = 6.45 Hz, 1H), 1.50 (s, 3H), 1.36 (d, *J* = 7.34, 3H), 1.21 (s, 3H), 1.05 (d, *J* = 6.75, 3H), 0.99 – 0.91 (m, 3H); IR ν_{max} (neat)/cm^{-1} 3435, 2955, 2922, 2851, 1726, 1460, 1377, 1161, 1072.



4.12

The solution of freshly prepared DMDO in $CHCl_3$ (0.5 mL, 0.2 M, 0.1 mmol) was added to bis[allene] macrolactone **4.1** (18.0 mg, 36.0 μmol), dissolved in THF/water (1:1, total 1 mL) dropwise at 5 °C. Upon the consumption of allene, the reaction mixture was removed under reduced pressure. The crude was chromatographed with 20% EtOAc in hexane through silica gel and the product was obtained in **4.12** (1.3 mg, 2.6 μmol, 7%).

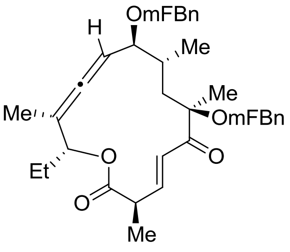
For detailed NMR analysis, see page 237; m/z (HRMS) calculated for $[C_{26}H_{36}O_9H]^+$: 493.3, found 493.3.



4.13

To a solution of bis[allene] macrolactone **4.1** (12.0 mg, 23.9 μmol) in methanol (3 mL) was added a solution of DMDO (0.38 mL, 0.14 mmol) dropwise at –50 °C. The reaction was stirred under nitrogen and let to warm to –15 °C over 1 hr 30minutes. Evaporation of solvent and silica gel column chromatography using 5% EtOAc in hexanes gave **4.13** (10.0 mg, 19.4 μmol, 80%) as colorless oil.

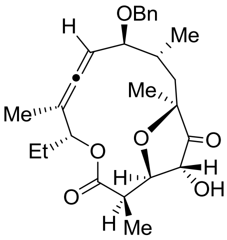
For detailed NMR analysis, see page 247; *m/z* (HRMS) calculated for $[C_{33}H_{40}O_5Na]^+$: 539.3, found: 539.3; $[\alpha]25^D$ = 3.3° (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3065, 2919, 2849, 1966, 1723, 1653, 1455, 1376, 1175, 1066.



4.15

To a solution of bis[allene] macrolactone **4.2** (32.9 mg, 65.5 μmol) in methanol (2 mL) was added a solution of DMDO (1.15 mL, 180 μmol) dropwise at –20 °C. The temperature was kept at –20 °C for 5 hours. Evaporation of solvent and silica gel column chromatography using 5% EtOAc in hexanes gave **4.15** (10.0 mg, 18.1 μmol, 30%) of as colorless oil.

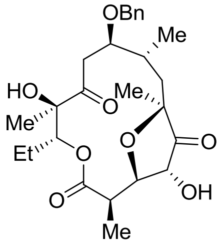
For detailed NMR analysis, see page 272; *m/z* (HRMS) calculated for $[C_{33}H_{38}F_2O_5Na]^+$: 575.3, found: 575.3; $[\alpha]25^D$ = –85.9° (c = 0.005, CHCl$_3$); IR ν_{max} (neat)/cm^{-1} 3066, 2972, 2933, 2876, 1964, 1738, 1696, 1624, 1450, 1379, 1177, 1086.



4.16

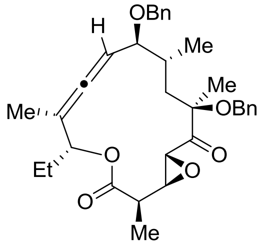
To a solution of macrolactone **4.1** (17.7 mg, 35.4 μmol) in CDCl$_3$ (0.5 mL) was added a solution of DMDO (0.56 mL, 0.21 mmol, 0.38 M in CDCl$_3$) dropwise at −40 °C, warmed up to −15 °C over 30 minutes, then lower order methyl cyanocuprate (MeCuCNLi, 0.71 mmol) was added, prepared by addition of MeLi (0.44 mL, 0.71 mmol) to a slurry of CuCN (63.3 mg, 0.710 mmol) in 2–methyl THF (6 mL) at −78 °C and then warming to –15 °C. The mixture was warmed to –2 °C over 1 hr 30minutes, quenched with saturated aqueous solution of NH_4OH and NH_4Cl (1:4 ratio) and then extracted with Et_2O. The combined organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure to give the crude product, which was purified by silica gel column chromatography using 15% EtOAc in hexane to afford **4.16** (10.0 mg, 22.6 μmol, 64% yield) as a colorless oil.

For detailed NMR analysis, see page 287; *m/z* (HRMS) calculated for $[C_{26}H_{34}O_6Na]^+$: 465.2, found: 465.5; $[\alpha]^{25}D = 5.9°$ (c = 0.005, $CHCl_3$); IR νmax (neat)/cm^{-1} 3434, 2968, 2925, 1959, 1764, 1725, 1452, 1370, 1155.



4.17

+

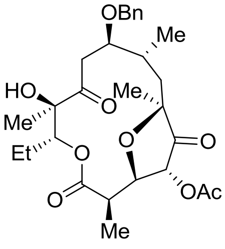


4.18

To the neat bis[allene] macrolactone **4.1** (25.3 mg, 50.6 μmol) was added a solution of DMDO (800 μL, 303 μmol, 0.380 M in $CDCl_3$) dropwise at –40 °C. The temperature was increased to –15 °C over 1 hr. In another flask, lower order methyl cyanocuprate was prepared by addition of MeLi (632 μL, 1.01 mmol) to a slurry of CuCN (91.0 mg, 1.01 mmol) in Et_2O (10 mL) at –78 °C and then warming to –15 °C. SDE solution was added to the cuprate solution at a –15 °C. After the complete consumption of SDE, the reaction was then quenched with water and extracted with Et_2O. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave a mixture of **4.16** (3.3 mg, 7.5 μmol, 15% yield), **4.17** (7.3 mg, 15 μmol, 30% yield) and **4.18** (5.4 mg, 10 μmol, 20% yield) as colorless oil.

Data of **4.17**: For detailed NMR analysis, see page 311; *m/z* (HRMS) calculated for $[C_{26}H_{36}O_8H]^+$: 477.5, found: 477.2; IR ν_{max} (neat)/cm^{-1} 3427, 3063, 2968, 2934, 2878, 1764, 1734, 1454, 1375, 1164, 1068.

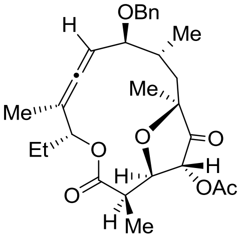
Data of **4.18**: For detailed NMR analysis, see page 329; *m/z* (HRMS) calculated for $[C_{33}H_{40}O_6Na]^+$: 555.3, found: 555.3; $[\alpha]25^D$ = 11.1° (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3436, 3063, 3031, 2969, 2933, 2876, 1966, 1729, 1454, 1376, 1270, 1179, 1065.



4.30

To a solution of alcohol **4.17** (7.4 mg, 16 μmol) in acetone (500 μl) was added grinded K_2CO_3 (4.3 mg, 31 μmol) and stirred for 30 minutes at room temperature. To the mixture was added acetic anhydride (6 μl, 0.07 mmol) at room temperature and stirred for overnight. The reaction mixture was diluted in DCM and the organic layer was washed with water. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 7% EtOAc in hexanes gave **4.30** (5.6 mg, 11 μmol, 70% yield) as colorless oil.

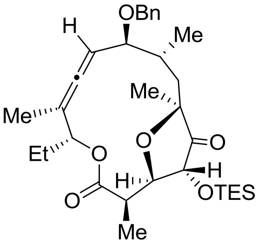
For detailed NMR analysis of **4.30**, see page 345; *m/z* (HRMS) found: 541.2, calculated for $[C_{28}H_{38}O_9Na]^+$: 541.2.



4.32

To a solution of alcohol **4.16** (3.1 mg, 7.0 μmol) in acetone (500 μl) was added grinded K_2CO_3 (9.6 mg, 70 μmol) and stirred for 30 minutes at room temperature. To the mixture was added acetic anhydride (6 μl, 0.07 mmol) at room temperature and stirred for overnight. The reaction mixture was diluted in DCM and the organic layer was washed with water. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 7% EtOAc in hexanes gave **4.32** (1.7 mg, 3.5 μmol, 50% yield) as colorless oil.

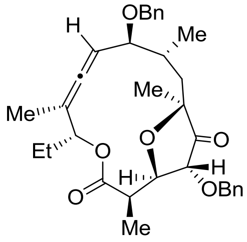
For detailed NMR analysis of **4.32**, see page 347; *m/z* (HRMS) calculated for $[C_{28}H_{36}O_7Na]^+$: 507.2, found: 507.2.



4.33

To the solution of **4.16** (6.2 mg, 14 μmol) in 1 mL of anhydrous DCM was added imidazole (2.9 mg, 40 μmol) and DMAP (0.5 mg, 4 μmol). After stirring for 5 minutes at room temperature, triethylsilyl chloride (12.6 mg, 83.6 μmol) was added to the mixture at room temperature. Upon the completion of the reaction, the mixture was quenched with aqueous NH_4Cl and the organic layer was extracted with DCM, dried over Na_2SO_4. The crude was chromatographed with 3% EtOAc in hexane through silica gel, giving **4.33** (7.2 mg, 13 μmol, 92% yield).

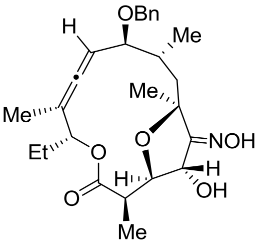
For detailed NMR analysis of **4.33**, see page 353; *m/z* (HRMS) calculated for $[C_{32}H_{48}O_6SiH]^+$: 557.3, found: 557.3; IR ν_{max} (neat)/cm^{-1} 3439, 2957, 2931, 2987, 2897, 2858, 1723, 1471, 1376, 1195, 1103, 837.



4.34

To a stirred solution of macrolactone **4.16** (10.0 mg, 22.6 μmol) in toluene (155 μL) was added benzyl trichloroacetimidate (8.0 mg, 32 μmol) and lanthanium triflate (1.2 mg, 2.2 μmol). Then the mixture was stirred at room temperature for 30 minutes. The reaction mixture was concentrated under vacuum and the residue was subjected to purification by silica gel column chromatography yielding **4.34** (8.4 mg, 16 μmol, 70%).

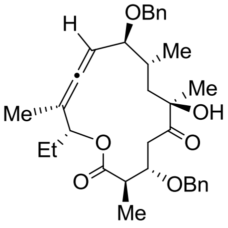
For detailed NMR analysis, see page 367; *m/z* (HRMS) calculated for $[C_{33}H_{40}O_6Na]^+$: 557.3, found: 555.3; IR ν_{max} (neat)/cm^{-1} 3400, 2965, 2923, 2877, 2851, 1900, 1759, 1725, 1453, 1374, 1186, 1079.



4.35

To a solution of **4.16** (3.4 mg, 7.7 µmol) in ethanol (1 mL) was added hydroxylamine hydrochloride (16.0 mg, 0.230 mmol). The reaction mixture was stirred for 2hrs, then KOH (12.9 mg, 0.230 mmol) was added at room temperature. The reaction was diluted with water and extracted with Et_2O. The combined organic phase was dried over anhydrous Na_2SO_4. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave **4.35** (2.7 mg, 6.0 µmol, 78% yield) as colorless oil.

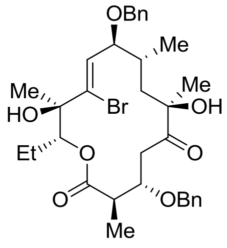
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.31 (m, 5H), 5.83 (d, *J* = 6.60 Hz, 1H), 5.57 (dd, *J* = 8.80, 5.58 Hz, 1H), 5.07 – 5.03 (m, 1H), 4.61 (d, *J* = 11.74 Hz, 1H), 4.34 (d, *J* = 11.99 Hz, 1H), 3.93 (dd, *J* = 6.36 Hz, 4.40, 1H), 3.79(dd, *J* = 9.05 Hz, 1.46, 1H), 3.09 (s, 1H), 2.98 – 2.91(m, 1H), 2.03 – 1.96 (m, 1H), 1.76 (d, *J* = 2.69 Hz, 3H), 1.73 – 1.60 (m, 4H), 1.31(d, *J* = 8.56 Hz, 3H), 0.93 (d, *J* = 7.33 Hz, 3H), 0.91 (s, 3H), 0.90 – 0.84 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 174.3, 167.1, 139.2, 128.2, 127.5, 127.2, 98.6, 90.4, 83.2, 82.3, 76.7, 75.4, 70.5, 67.8, 42.5, 39.9, 34.1, 24.2, 22.7, 14.8, 14.0, 13.6, 10.1; *m/z* (HRMS) calculated for $[C_{26}H_{35}NO_6Na]^+$: 480.2, found: 480.2; $[\alpha]^{25}_D$ = 6.3° (c = 0.005, $CHCl_3$); IR ν_{max}(neat)/cm^{-1} 3309, 2917, 2849, 1959, 1729, 1709, 1458, 1375, 1156, 1070.



4.50

The macrolactone **4.1** (26.0 mg, 52.0 μmol) was dissolved in the mixture of *t*–BuOH and water (1:1 ratio, total 2 mL). To the solution was added 4% aqueous OsO_4 solution (495 μL, 62.2 μmol) at room temperature, and then stirred for 45 minutes. After the complete consumption of **4.1**, the reaction was quenched by 20 mL saturated Na_2SO_3 solution, and then extracted with Et_2O (2 x 20 mL). The solvent was removed *in vacuo*. Silica gel column chromatography using 10% EtOAc in hexane gave the macrolactone **4.50** as light yellowish oil (23.0 mg, 43.0 μmol, 83% yield).

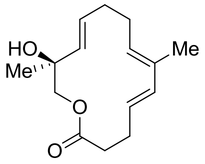
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.17 (m, 8H), 5.15 (t, *J* = 6.0 Hz, 2H), 4.94 (dd, *J* = 6.8, 3.1 Hz, 1H), 4.73 (dd, *J* = 13.4, 8.4 Hz, 1H), 4.58 (dd, *J* = 21.0, 11.6 Hz, 2H), 4.35 (d, *J* = 12.2 Hz, 1H), 4.15 (ddd, *J* = 8.7, 6.9, 4.0 Hz, 1H), 3.83 (s, 1H), 3.44 (dd, *J* = 8.0, 6.9 Hz, 1H), 2.95 (qd, *J* = 17.8, 5.4 Hz, 2H), 2.58 (dt, *J* = 15.5, 6.8 Hz, 1H), 1.90 (dd, *J* = 15.0, 3.1 Hz, 1H), 1.79 (d, *J* = 2.9 Hz, 2H), 1.76 – 1.64 (m, 2H), 1.57 – 1.46 (m, 2H), 1.42 – 1.28 (m, 4H), 1.28 – 1.15 (m, 2H), 1.07 (t, *J* = 13.0 Hz, 2H), 0.99 – 0.69 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.5, 208.4, 178.2, 143.0, 142.8, 133.2, 132.9, 132.9, 132.5, 132.5, 132.3, 132.3, 132.1, 131.6, 104.1, 96.1, 86.7, 83.3, 81.9, 81.6, 81.4, 80.8, 80.4, 78.2, 74.4, 50.0, 47.3, 47.0, 38.6, 34.3, 31.8, 29.6, 23.0, 19.9, 18.1, 14.0; *m/z* (HRMS) calculated for $[C_{33}H_{42}O_6Na]^+$: 557.3, found: 557.3; IR ν_{max}(neat)/cm^{-1} 3475, 3065, 2930, 2919, 2872, 2849, 1963, 1727, 1453, 1375, 1172, 1069.



4.60

To a stirred solution of macrolactone **4.50** (7.8 mg, 15 μmol) in 1 mL of acetonitrile was added *N*–Bromosuccinimide (34.0 mg, 191 μmol) at room temperature. Starting material was consumed right away. The reaction mixture was quenched with 1 mL of saturated aqueous solution of $Na_2S_2O_3$, and then extracted with Et_2O. The solvent was removed *in vacuo*, and the residue was subjected to purification by silica gel column chromatography (14% EtOAc in hexane) yielding **4.60** (9.1 mg, 15 μmol, 99% yield).

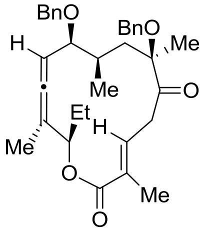
For detailed NMR analysis, see page 372; *m/z* (ESIMS) calculated for $[C_{33}H_{43}BrO_7Na]^+$: 653.3, 655.3, found: 653.2, 655.2; $[\alpha]^{25}_D = 7.6°$ (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3442, 3062, 2956, 2922, 2850, 1728, 1711, 1454, 1376, 1165, 1070.



4.65

To a solution of bis[allene] macrolactone **2.24** (36.6 mg, 158 μmol) in THF (2 mL) was added sulfuric acid (15.5 mg, 0.160 mmol), water (13 μL, 0.71 mmol), mercuric sulfate (1.9 mg, 6.3 μmol) sequentially. The reaction mixture was heated under reflux at 70 °C for 5 hours. After the complete consumption of bis[allene] macrolactone, the reaction was cooled down to room temperature and diluted with water. The organic phase was extracted with EtOAc and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and silica gel column chromatography using 20% EtOAc in hexanes gave **4.65** (15.9 mg, 63.6 μmol, 40%) as colorless oil.

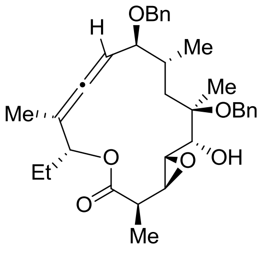
For detailed NMR analysis, see page 393; *m/z* (HRMS) calculated for $[C_{15}H_{22}O_3Na]^+$: 273.1, found: 273.1.



4.68

To a solution of bis[allene] macrolactone **4.1** (15.0 mg, 30.0 μmol) in 2–methyl THF (3 mL) was added Na_2HPO_4(51.0 mg, 0.360 mmol) and *m*CPBA (31.0 mg, 0.180 mmol) sequentially at –50 °C. The temperature was slowly increased to 12 °C over 5 hr 30 minute. To the reaction mixture was methanol (4 mL) and stirred overnight. The reaction was quenched with aqueous $NaHCO_3$ and extracted with EtOAc. The combined organic phase was dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and silica gel column chromatography using 15% EtOAc in hexanes gave **4.68** as colorless oil (7.0 mg, 14 μmol, 45% yield).

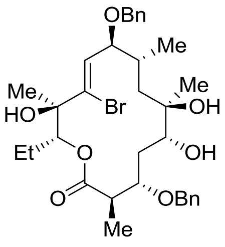
For detailed NMR analysis, see page 419; *m/z* (ESIMS) calculated for $[C_{33}H_{40}O_5+Na]^+$: 539.6, found: 539.1.



4.74

To epoxy ketone **4.18** (1.7 mg, 3.2 µmol) in Et_2O (1 mL) was added $Zn(BH_4)_2$ (46 µl, 14 µmol, 0.30 M in Et_2O) dropwise at 0 °C, then $Zn(BH_4)_2$ (115 µl, 35.0 µmol, 0.300 M in Et_2O) twice at room temperature. After the complete consumption of **4.18**, the reaction was quenched with aqueous NH_4Cl and extracted with Et_2O. The combined organic phase was dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and silica gel column chromatography using 15% EtOAc in hexanes gave **4.74** (1.4 mg, 2.6 µmol, 82% yield) as colorless oil.

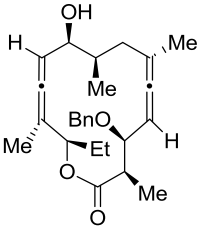
For detailed NMR analysis, see page 442; *m/z* (HRMS) calculated for $[C_{33}H_{42}O_6H]^+$: 535.3, found: 535.3.



4.75

To a stirred solution of macrolactone **4.60** (5.0 mg, 7.9 µmol) in 1 mL of anhydrous Et_2O was $Zn(BH_4)_2$ solution in Et_2O (91 µl, 12 µmol) at 0 °C. The mixture was stirred for 30 minutes at 0 °C. The reaction mixture was quenched with 1 mL of aqueous NH_4Cl, and then extracted with Et_2O. The crude was subjected to purification by silica gel column chromatography (20% EtOAc in hexane) yielding **4.75** (4.9 mg, 7.9 μmol, 99% yield).

For detailed NMR analysis, see page 463; *m/z* (HRMS) calculated for $[C_{33}H_{45}BrO_7Na]^+$: 655.2, 657.2, found: 655.2, 657.2; $[\alpha]^{25}_D$ = 5.8° (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3433, 2925, 2851, 1729, 1454, 1375, 1164, 1068.

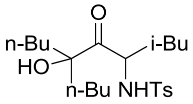


4.80

To a solution of bis[allene] macrolactone **4.1** (21.5 mg, 43.0 μmol) in 1,2–dichloroethane (3.6 mL) and phosphate buffer (0.4 mL, pH = 7) was added DDQ (39.0 mg, 172 μmol) and stirred for 15 minutes at room temperature. Then, raise the temperature to 40 °C until the complete disappearance of **4.1**. The aqueous $NaHCO_3$ (3 mL) was added and the resulting dark solution was stirred vigorously for 1 hr. The crude mixture was extracted with DCM and the excess solvent was removed under reduced pressure. The crude mixture was chromatographed, yielding **4.80** (10.5 mg, 25.6 μmol, 60%) as colorless oil.

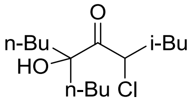
For detailed NMR analysis, see page 500; *m/z* (HRMS) calculated for $[C_{26}H_{34}O_4Na]^+$: 433.2, found: 433.2; $[\alpha]^{25}_D$ = 15.1° (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3435, 2968, 2917, 2848, 1963, 1729, 1453, 1376, 1177, 1069.

7.4. Chapter 5



5.30

+

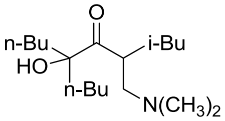


5.28

To the solution of allene (31.3 mg, 0.150 mmol) **5.25** in *t*–BuOH (2 mL) and water (0.7 mL) was added Chloramine T–trihydrate (73.8 mg, 0.300 mmol) at room temperature. The mixture was stirred for 1 minute, and then 4% aqueous OsO_4 solution (1.29 mL, 165 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.30** (6.2 mg, 10% yield) and **5.28** (20.8 mg, 50% yield).

Data of **5.30**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.68 (2H, m), 7.30 – 7.23 (2H, m), 5.31 (1H, d, *J* = 9.5 Hz), 4.51 (1H, dd, *J* = 2.7, 9.5 Hz), 2.39 (3H, s), 1.98 (3H, s), 1.91 – 1.82 (1H, m), 1.66 – 1.00 (14H, m), 0.99 – 0.80 (12H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.9, 143.4, 137.3, 129.6, 82.3, 55.6, 41.6, 38.1, 37.4, 22.5, 24.7, 24.4, 23.5, 22.9, 22.8, 20.8, 13.8, 13.8; IR ν_{max} (neat)/cm^{-1} 3516, 3282, 3028, 2956, 2931, 2870, 1712, 1598, 1466, 1332, 1159.

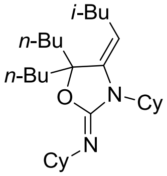
Data of **5.28**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.79 (1H, dd, *J* = 10.3, 3.91 Hz), 3.02 (1H, s), 1.92 – 1.03 (15H, m), 0.99 – 0.98 (3H, d, *J* = 6.60 Hz), 0.95 – 0.91 (3H, d, *J* = 6.60 Hz), 0.91 – 0.87 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.8, 82.7, 54.2, 41.9, 38.6, 38.5, 25.6, 25.5, 24.7, 23.1, 22.9 (2), 21.1, 13.9, 13.8; IR ν_{max} (neat) /cm^{-1} 3513, 2958, 2932, 2872, 1720, 1467; *m/z* (HRMS) calculated for $[C_{15}H_{29}ClO_2H]^+$: 277.2, found: 277.2.



5.31

To the solution of allene (30.0 mg, 0.140 mmol) **5.25** in anhydrous DCM (3 mL), dimethylmethylideneammonium chloride (70.0 mg, 0.700 mmol), and OsO_4 solution (3.0 mL, 0.15 mmol, 0.050 M in DCM), and NMO (20.0 mg, 0.150 mmol) were added at room temperature. The mixture was stirred for 1 hour, and then quenched by saturated solution of Na_2SO_3. The organic layer was extracted in DCM. The crude was purified by silica gel column chromatography, yielding **5.31** (27.3 mg, 63% yield).

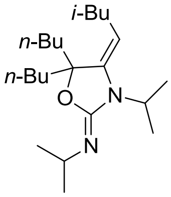
Data of **5.31**: ^{1}H NMR (500 MHz, $CDCl_3$) 3.92 (1H, s), 3.50 – 3.38 (1H, m), 2.46 (2H, t, *J* = 7.6), 2.30 (6H, s), 1.76 – 1.00 (15H, m), 0.96 – 0.84 (12H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 219.7, 81.2, 64.4, 45.6, 42.3, 39.0, 38.7, 37.4, 26.3, 25.6, 25.5, 23.2, 23.1, 23.0, 22.7, 14.1, 13.9; *m/z* (HRMS) calculated for $[C_{18}H_{37}NO_2H]^+$: 300.3, found: 300.3.



5.32

To the solution of allene (10.0 mg, 48.0 μmol) **5.25** in *t*–BuOH (900 μL) and water (150 μL) was added *N,N'*–dicyclohexylcarbodiimide (76.5 mg, 371 μmol) at room temperature. The mixture was stirred for 20 minutes, and then 4% aqueous OsO_4 solution (415 μL, 53.0 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in DCM. The crude was purified by silica gel column chromatography, yielding **5.32** (16.4 mg, 79% yield).

For detailed NMR analysis, see page 528; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.24 (1H, t, *J* = 7.8 Hz), 4.21 – 4.30 (2H, tt, *J* = 4.1 Hz), 2.04 – 2.08 (1H, m), 1.87 – 1.91 (2H, m), 1.7 (1H, m), 0.96 (3H, s), 0.95 (3H, s), 1.15 – 2.20 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.6, 166.8, 100.5, 94.6, 59.8, 59.2, 41.3, 39.8, 33.9, 32.2, 32.0, 31.9, 31.8, 29.5, 25.9, 25.8, 25.7, 25.6(2), 25.5(2), 23.1, 22.6, 22.5, 22.5, 13.9(2)

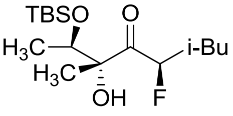


5.33

To the solution of allene (9.4 mg, 45 μmol) **5.25** in *t*–BuOH (900 μL) and water (150 μL) was added *N,N'*–diisopropylcarbodiimide (114 mg, 902 μmol) at room temperature. The mixture was stirred for 20 minutes, and then 4% aqueous OsO_4 solution (390 μL, 50.0 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in DCM. The crude was purified by silica gel column chromatography, yielding **5.33** (8.8 mg, 56% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.68 (2H, m), 7.30 – 7.23 (2H, m), 5.31 (1H, d, *J* = 9.5 Hz), 4.51 (1H, dd, *J* = 2.7, 9.5 Hz), 2.39 (3H, s), 1.98 (3H, s), 1.91 – 1.82 (1H, m), 1.66 – 1.00 (14H, m), 0.99 – 0.80 (12H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ

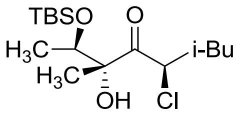
212.9, 143.4, 137.3, 129.6, 82.3, 55.6, 41.6, 38.1, 37.4, 22.5, 24.7, 24.4, 23.5, 22.9, 22.8, 20.8, 13.8, 13.8.



5.37

To the solution of allene (17.5 mg, 65.2 μmol) **5.34** in *t*–BuOH (1 mL), phosphate buffer (0.50 mL, pH= 7.4), and acetone (0.5 mL) was added Selectfluor (115 mg, 326 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.51 mL, 65 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture of **5.37** (13.4 mg, 41.8 μmol, 64% combined yield for anti and syn in 5.9:1 ratio)

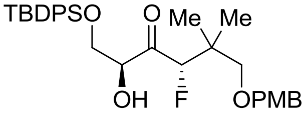
Data of **5.37** (anti): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.48 (1H, dddd, *J* = 2.20, 10.27, 51.35 Hz), 4.05 (1H, q, *J* = 6.11 Hz), 2.83 (1H, s), 1.96 – 1.88 (1H, m), 1.81 – 1.68 (2H, m), 1.35 (3H, s), 1.08 (3H, d, *J* = 6.36 Hz), 1.01 (3H, d, *J* = 6.85 Hz), 0.98 (6H, d, *J* = 6.26 Hz), 0.90 (9H, s), 0.86 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.4 (J_{CF} = 16.75), 92.2, 82.1, 72.4, 39.7, 25.8 (3), 25.7, 24.7, 23.3, 21.2, 18.6, 18.0, –4.3, –4.9; *m/z* (HRMS) calculated for $[C_{16}H_{33}FO_3SiNa]^+$: 343.2, found: 343.2; $[\alpha]^{25}_D$ = –46.5° (c = 0.01, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3546, 2958, 2931, 2858, 1733, 1464, 1374, 1256, 1159, 1120, 1037, 982, 836.



5.38

To the solution of allene (17.5 mg, 65.2 μmol) **5.34** in *t*–BuOH (1 mL) and water (0.5 mL) was added NCS (43.5 mg, 326 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.51 mL, 65 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.38** (17.1 mg, 50.9 μmol, 78% combined yield for anti and syn in 5.3:1 ratio).

Data of **5.38** (anti): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.02 (1H, dd, *J* = 4.7, 10.2 Hz), 3.97 (1H, q, *J* = 6.3 Hz), 2.77 (1H, s), 1.90 – 1.82 (1H, m), 1.71 – 1.66 (2H, m), 1.42 (3H, s), 1.04 (3H, d, *J* = 6.3 Hz), 0.97 (3H, d, *J* = 6.7 Hz), 0.93 (3H, d, *J* = 6.3 Hz), 0.91 (3H, s), 0.90 (9H, s), 0.88 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.2, 82.1, 73.3, 55.7, 40.9, 25.8 (3), 25.7, 24.8, 23.1, 21.1, 18.4, 18.0, –4.26, –4.81; *m/z* (HRMS) calculated for $[C_{16}H_{33}ClO_3SiNa]^+$: 359.2, found: 359.2; $[\alpha]^{25}_D$ = 24.8° (c = 0.01, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3544, 2958, 2931, 2858, 1723, 1464, 1375, 1257, 1169, 1104, 1029, 1008, 837.



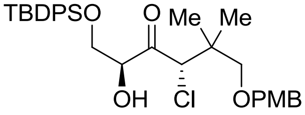
5.39

Stoichiometric condition is as follows; to the solution of allene (24.6 mg, 49.2 μmol) **5.35** in *t*–BuOH (1 mL), phosphate buffer (0.60 mL, pH = 7.4), and acetone (0.5

mL) was added Selectfluor (87.0 mg, 246 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.39 mL, 49 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.39** (17.4 mg, 31.5 μmol, 64% yield, single diastereomer).

Catalytic condition is as follows; to the solution of allene (35.0 mg, 70.0 μmol) **5.35**, and Selectfluor (124 mg, 349 μmol) in *t*–BuOH (1 mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (1 mL) was added 4% aqueous OsO_4 solution (55 μL, 7.0 μmol) was added slowly. Then, NMO (16.4 mg, 140 μmol) was added. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.39** (23.8 mg, 43.1 μmol, 62% yield, single diastereomer).

Data of **5.39** (anti): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 – 7.63 (4H, m), 7.45 – 7.36 (6H, m), 7.21 – 7.18 (2H, m), 6.87 – 6.84 (2H, m), 5.16 (1H, d, *J* = 47.2 Hz), 4.49 – 4.46 (1H, m), 4.37 (2H, s), 3.96 (1H, ddd, *J* = 1.0, 3.4, 10.8 Hz), 3.89 (1H, ddd, *J* = 1.0, 4.4, 10.8 Hz), 3.80 (3H, s), 3.53 (1H, dd, *J* = 6.9 Hz), 3.19 (1H, dd, *J* = 9.3 Hz), 1.02 (9H, s), 1.00 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.0, 207.8, 159.2, 135.7, 135.6, 132.9, 132.6, 129.8, 129.1, 127.7, 113.7, 97.0, 95.5, 76.5, 75.0, 72.8, 65.2, 55.2, 40.2, 40.1, 26.7, 20.9, 19.2; *m/z* (HRMS) calculated for $[C_{32}H_{41}FO_5SiNa]^+$: 575.3, found: 575.3; $[\alpha]^{25}_D$ = 18.4° (c = 0.01, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3071, 3048, 2957, 2931, 2857, 1725, 1427, 1362, 1247, 1173, 1112, 1035, 1008, 736, 702.

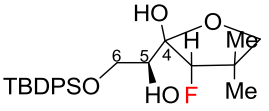


5.40

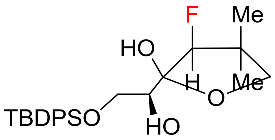
Stoichiometric condition is as follows; to the solution of allene (24.6 mg, 49.2 μmol) **5.35** in *t*–BuOH (1 mL), phosphate buffer (0.60 mL, pH = 7.4), acetone (0.5 mL) was added NCS (32.8 mg, 246 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.39 mL, 49 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture of **5.40** (19.2 mg, 33.8 μmol, 69% yield for anti and syn in 3.2:1 ratio).

Catalytic condition is as follows; to the solution of allene (18.2 mg, 36.4 μmol) **5.35** in *t*–BuOH (1 mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (0.5 mL) were added *N*–Methylmorpholine *N*–oxide (NMO) (8.5 mg, 73 μmol) and 4% aqueous OsO_4 solution (28 μL, 3.6 μmol). Then, NCS (24.3 mg, 182 μmol), dissolved in *t*–BuOH (0.5 mL), phosphate buffer (0.50 mL, pH = 7.4), and acetone (0.5 mL), was added dropwise over 3 hours via syringe pump at room temperature After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture of **5.40** (16.3 mg, 28.2 μmol, 77% yield for anti and syn in 1.5:1 ratio).

Data of **5.40** (anti): ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.69 – 7.66 (4H, m), 7.44 – 7.36 (6H, m), 7.20 – 7.19 (2H, m), 6.87 – 6.83 (2H, m), 4.87 (1H, s), 4.39 (2H, s), 4.35 (1H, dt, *J* = 1.7, 5.4 Hz), 3.92 (1H, dd, *J* = 3.7, 10.8 Hz), 3.82 (1H, dd, *J* = 5.4, 10.8 Hz), 3.79 (3H, s), 3.44 (1H, d, *J* = 7.1 Hz), 3.32 (1H, d, *J* = 9.3 Hz), 3.12 (1H, d, *J* = 9.23 Hz), 1.09 (6H, s), 1.04 (9H, s); ^{13}C NMR (125 MHz, CDCl$_3$) δ 206.0, 159.2, 135.7, 132.8, 132.6, 129.9, 129.8, 129.8, 129.2, 127.8, 113.8, 77.9, 76.1, 73.0, 65.2, 62.4, 55.2, 26.7, 22.2, 20.8, 19.2; *m/z* (HRMS) calculated for $[C_{32}H_{41}ClO_5SiNa]^+$: 591.2, found: 591.2; $[\alpha]^{25}_D$ = 25.6° (c = 0.01, CHCl$_3$); IR ν_{max} (neat)/cm^{-1} 3071, 3049, 2955, 2927, 2855, 1728, 1427, 1364, 1248, 1173, 1112, 1036, 1008, 737, 702.



5.45

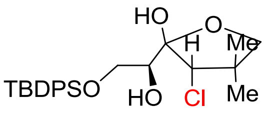


5.46

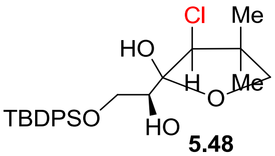
Stoichiometric condition is as follows; to the solution of allene (29.6 mg, 77.8 μmol) **5.44** in *t*–BuOH (1 mL), phosphate buffer (0.50 mL, pH = 7.4), acetone (0.5 mL) was added Selectfluor at room temperature The mixture was stirred for 20 minutes, then 4% aqueous OsO$_4$ solution (0.61 mL, 78 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (14.2 mg, 32.9 μmol, 42% yield for **5.45** and **5.46** in 1: 2.6 ratio).

Catalytic condition is as follows; to the solution of allene (30.0 mg, 78.9 μmol) **5.44**, Selectfluor (140 mg, 394 μmol), and NMO (18.5 mg, 158 μmol) in *t*–BuOH (1 mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (1 mL) was added 4% aqueous OsO_4 solution (62 μL, 7.9 μmol) slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (19.3 mg, 44.7 μmol, 57% yield for **5.45** and **5.46** in 1: 1.8 ratio).

For detailed NMR analysis, see page 549; *m/z* (HRMS) calculated for $[C_{24}H_{33}FO_4SiNa]^+$: 455.2; IR ν_{max} (neat)/cm^{-1} 3427, 3071, 3049, 2957, 2929, 2890, 2856, 1471, 1427, 1392, 1113, 907, 731, 702.



5.47

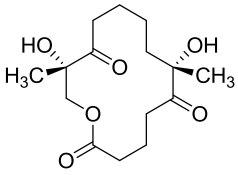


5.48

Stoichiometric condition is as follows; to the solution of allene (30.2 mg, 79.4 μmol) **5.44** in *t*–BuOH (1 mL), phosphate buffer (0.37 mL, pH = 7.4), and acetone (0.5 mL) was added NCS (53.0 mg, 397 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.62 mL, 79 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (17.4 mg, 38.8 μmol, 49% yield for **5.47** and **5.48** in 1: 1.1 ratio).

Catalytic condition is as follows; to the solution of allene (30.0 mg, 78.9 μmol) **5.44**, NCS (52.6 mg, 394 μmol), and NMO (18.5 mg, 158 μmol) in *t*–BuOH (1 mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (1 mL) was added 4% aqueous OsO_4 solution (62 μL, 7.9 μmol) slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (29.7 mg, 84% yield for **5.47** and **5.48** in 1:1.3 ratio).

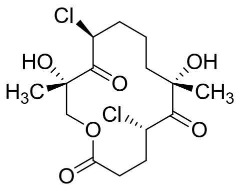
For detailed NMR analysis, see page 556; *m/z* (HRMS) calculated for $[C_{24}H_{33}ClO_4SiNa]^+$: 471.2, 473.2 found: 471.2, 473.2; IR ν_{max} (neat)/cm^{-1} 3421, 3071, 3049, 2957, 2928, 2856, 1428, 1417, 1374, 1112, 1070, 916, 737, 702.



5.49

To the solution of allene (9.4 mg, 40 μmol) **2.24** in *t*–BuOH (400 μL) and water (50 μL) was added 4% aqueous OsO_4 solution (0.64 mL, 81 μmol) slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.49** (4.6 mg, 38% yield).

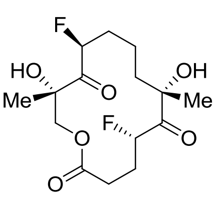
For detailed NMR analysis of **5.49**, see page 573; *m/z* (HRMS) calculated for $[C_{15}H_{24}O_6Na]^+$: 323.2, found: 323.2; IR ν_{max} (neat)/cm^{-1} 3479, 2923, 2851, 1713, 1458, 1374, 1133.



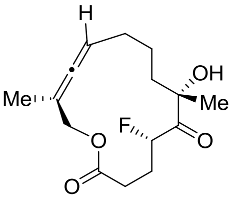
5.50

To the solution of allene (10.5 mg, 45.0 μmol) **2.24** in *t*–BuOH (400 μL) and water (50 μL) was added NCS (30.2 mg, 226 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO_4 solution (0.70 mL, 90 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.50** (10.0 mg, 65% yield).

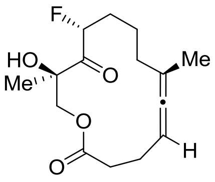
For detailed NMR analysis of **5.50**, see page 585; *m/z* (HRMS) calculated for $[C_{15}H_{22}Cl_2O_6H]^+$: 367.1, 369.1, 372.1, found: 367.1, 369.1, 372.1; IR ν_{max} (neat)/cm^{-1} 3433, 2920, 2850, 1739, 1462, 1375, 1241, 1157.



5.51



5.52



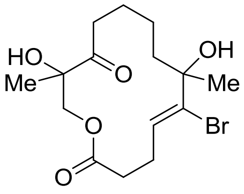
5.53

To the solution of allene (10.4 mg, 45.0 μmol) **2.24** in *t*–BuOH (2 mL) and water (1.3 mL) was added Selectfluor (109 mg, 448 μmol) at room temperature. The mixture was stirred for 7 minutes, then 4% aqueous OsO_4 solution (0.70 mL, 90 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc.

The crude was purified by silica gel column chromatography, yielding **5.51** (6.3 mg, 42% yield) and the mixture of **5.52** and **5.53** (1.2 mg, 9% combined yield).

For detailed NMR analysis of **5.51**, see page 603; *m/z* (HRMS) calculated for $[C_{15}H_{22}F_2O_6Na]^+$: 359.1, found: 359.1; IR ν_{max} (neat)/cm^{-1} 3479, 2927, 2853, 1727, 1455, 1372, 1162.

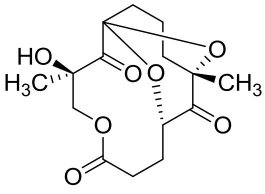
For detailed NMR analysis of **5.52** and **5.53**, see page 618; *m/z* (HRMS) calculated for $[C_{15}H_{21}FO_4Na]^+$: 307.1, found: 307.1; IR ν_{max} (neat)/cm^{-1} 3492, 2929, 1723, 1461, 1371, 1155.



5.54

To a solution of bis[allene] macrolactone **2.24** (10.0 mg, 43.0 μmol) in *t*–BuOH and water mixture (1:1, total 2 mL) was added NMO (20.2 mg, 0.170 mmol) and 4% aqueous OsO4 solution (135 μL, 17.0 μmol) sequentially. NBS (23.0 mg, 129 μmol) in in *t*–BuOH (0.5 mL), phosphate buffer (0.5 mL, pH = 7) and acetone (0.5 mL) was added slowly over 10 hours. After the complete disappearance of SM, the reaction was quenched saturated solution of Na_2SO_3 and the organic layer was extracted with EtOAc. The organic phase was dried with Na_2SO_4 and the excess solvent was removed under reduced pressure. The crude material was chromatographed in silica gel, yielding **5.54** (6.0 mg, 47% yield).

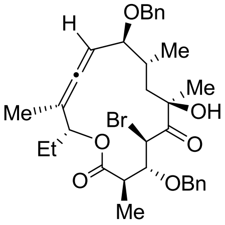
For detailed NMR analysis, see page 631; IR ν_{max} (neat)/cm^{-1} 3459, 2923, 2850, 1735, 1712, 1459, 1370, 1163.



5.55

To the solution of allene (24.5 mg, 0.110 mmol) **2.24** in *t*–BuOH (2 mL) and water (1.3 mL) was added Chloramine T–trihydrate (259 mg, 1.05 mmol) at room temperature. The mixture was stirred for 1 minute, then 4% aqueous OsO_4 solution (1.66 mL, 0.210 mmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.55** (2.3 mg, 7% yield).

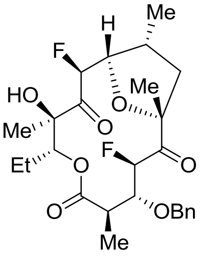
For detailed NMR analysis, see page 638; *m/z* (HRMS) calculated for $[C_{15}H_{20}O_7Na]^+$: 335.1, found: 335.1; IR ν_{max} (neat)/cm^{-1} 3498, 2921, 2850, 1730, 1446, 1357.



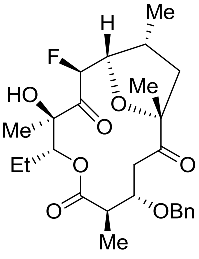
5.57

To the solution of allene (20.7 mg, 41.0 μmol) **4.1** in *t*–BuOH (1 mL), phosphate buffer (0.29 mL, pH = 7.4), and acetone (0.50 mL) was added 4% aqueous OsO_4 solution (0.71 mL, 91 μmol) slowly. To the mixture was added NBS (14.7 mg, 83.0 μmol), dissolved in *t*–BuOH (0.5 mL), phosphate buffer (0.50 mL, pH = 7.4), and acetone (0.5 mL). After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The organic phase was dried with Na_2SO_4 and the excess solvent was removed under reduced pressure. The crude was purified by silica gel column chromatography, yielding **5.57** (2.5 mg, 10% yield).

For detailed NMR analysis of **5.57**, see page 651; *m/z* (HRMS) calculated for $[C_{33}H_{41}BrO_6Na]^+$: 635.2, 637.2, found: 635.2, 637.2; IR ν_{max} (neat)/cm^{-1} 3452, 3030, 2924, 2852, 1959, 1736, 1454, 1371, 1179, 1068, 697.



5.58



5.59

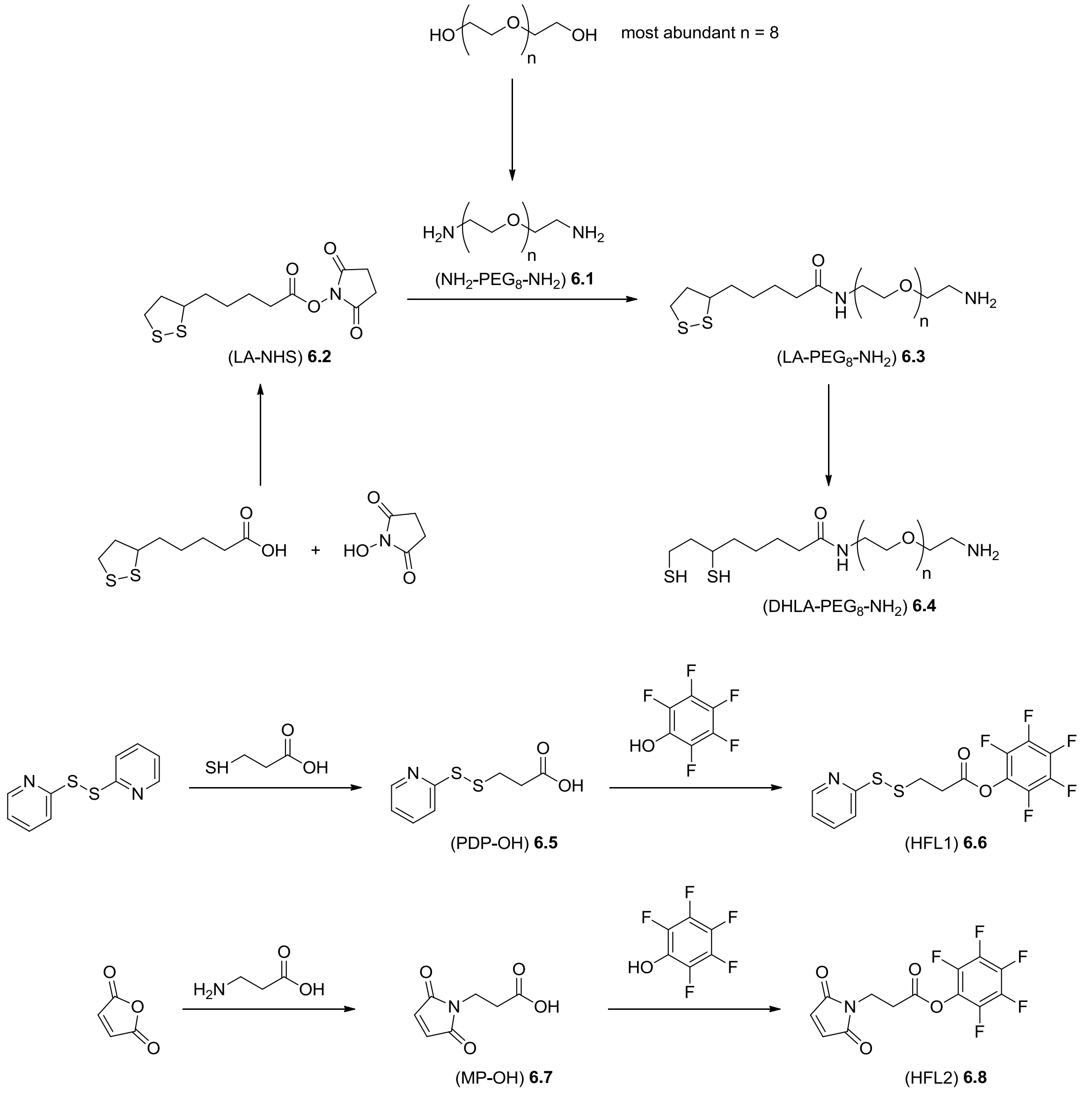
To the solution of allene (25.0 mg, 45.0 μmol) **4.1** in *t*–BuOH (1 mL), water (0.27 mL), and acetone (2 mL) was added Selectfluor (177 mg, 499 μmol) at room temperature. The mixture was stirred for 5 minutes, then 4% aqueous OsO_4 solution (1.18 mL, 90.0 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na_2SO_3 and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.58** (2.6 mg, 10% yield) and **5.59** (5.0 mg, 21% yield).

For detailed NMR analysis of **5.58**, see page 653; *m/z* (HRMS) calculated for $[C_{26}H_{34}F_2O_7Na]^+$: 519.2, found: 519.2; $[\alpha]^{25}_D = 7.3°$ (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3452, 2952, 2922, 2851, 1737, 1456,1377, 1174, 1068.

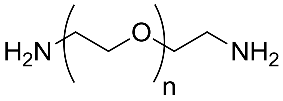
For detailed NMR analysis of **5.59**, see page 671; *m/z* (HRMS) calculated for $[C_{26}H_{35}FO_7Na]^+$: 501.2, found: 501.2; $[\alpha]^{25}_D = 1.3°$ (c = 0.005, $CHCl_3$); IR ν_{max} (neat)/cm^{-1} 3500, 2918, 2849, 1736, 1455, 1375, 1169, 1069, 1011.

7.5. Chapter 6

Synthetic Routes to DHLA–PEG–NH_2 and Heterofunctional Linkers: (adapted with permission from ref.[11])


most abundant n = 8
(NH2-PEG8-NH2) 6.1
(LA-NHS) 6.2
(LA-PEG8-NH2) 6.3
(DHLA-PEG8-NH2) 6.4
(PDP-OH) 6.5
(HFL1) 6.6
(MP-OH) 6.7
(HFL2) 6.8

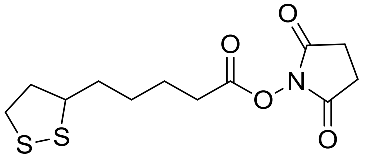
Synthesis of the Core–Shell Quantum Dots: CdSe/CdS/ZnS core–shell QDs were synthesized using well established protocols.[1, 2] The QDs were then characterized for size and its distribution using a transmission electron microscope (TEM); See Figure 6.2-1. The spectroscopic properties of the QDs were obtained using a fluorometer (FluoroMax–3, HORIBA Scientific).



Diamino–PEG (NH_2–PEG_8–NH_2) (**6.1**)

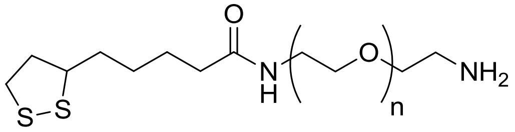
This procedure from the literature[3] was modified. Neat PEG_8 (20.0 g, 50.0 mmol, average MW 400 g/mol) was dried at 100 °C for 4 hours with stirring to remove all traces of water. The flask was cooled on an ice bath before thionyl chloride (10.5 mL, 145 mmol) was added dropwise. The solution was warmed to room temperature and stirred for 22 hours. After 22 hours, TLC indicated complete conversion to the bis–chloride; TLC (R_f 0.75, 90% DCM/MeOH, plate dried under vacuum prior to running) and ESI–MS (*m/z* 517.4 $[M+H]^+$). The crude product was diluted with DMF (20 mL) and the solvent removed under reduced pressure. After repeating this three times, the flask was left under high vacuum overnight to remove all traces of thionyl chloride. To the crude dark yellow product was added a solution of sodium azide (9.40 g, 145 mmol) in 250 mL of DMF. The resulting dark-orange solution was stirred for 16 hours at 85 °C, indicating complete conversion to the diazide by TLC (R_f 0.6, 90% DCM/MeOH) and ESI–MS (*m/z* 531.4 $[M+H]^+$). The solvent was removed under reduced pressure, and diluted with 200 mL of DCM. The precipitate was removed by vacuum filtration through Celite® and the solvent removed under high vacuum overnight to yield the intermediate diazide as brown oil. The sample was dissolved in 300 mL of THF, and triphenylphosphine (27.8 g, 106 mmol) was added to the mixture. The dark orange solution was stirred at room temperature for 24 hours, showing complete consumption of the diazide by ESI–MS and formation of the iminophosphorane intermediate by TLC (R_f 0.4, 90% DCM/MeOH). To the crude mixture, 4 mL of water was added and the dark brown mixture was stirred for 24 hours, showing complete reduction to the bis–amine **6.1** by TLC (R_f 0.1, 90% DCM/MeOH) and ESI–MS (*m/z* 457.4 $[M+H]^+$). THF was removed *in vacuo*, yielding a crude orange solid. 100 mL of water was added, and the precipitate was removed by vacuum filtration through a B*ü*chner funnel. The filtrate was washed with toluene (3 x 50 mL). The water was removed *in vacuo* to give a dark orange oil. The crude product was purified by alumina column chromatography (DCM/MeOH, 95:5), giving **6.1** as a light yellow oil (19.8 g, 99%). Spectroscopic data matched those previously reported.[3] Traces of triphenylphosphine oxide were detected by ^{1}H NMR.

The previously reported protocols[3, 4] for the synthesis of **6.1** required multiple steps; therefore, we applied a Mitsunobu reaction[5] to acquire the bis–amine in a single step: PEG_8 (10.0 g, 25.0 mmol), previously dried over 3Å molecular sieves, triphenylphosphine (13.1 g, 2.0 equiv.), and phthalimide (7.4 g, 2.0 equiv.) were mixed in 150 mL of DCM and cooled to 0 °C under nitrogen. To this solution was added 95% diisopropylazodicarboxylate (DIAD, 10.4 mL, 2.0 equiv.) dropwise. The temperature being kept at or below 20 °C. After the addition was complete, the reaction was allowed to warm to room temperature and was stirred for the additional 1 hour. To the mixture was then added 55% hydrazine (3.6 mL, 2.5 equiv.) and the reaction was refluxed overnight under nitrogen. It was then cooled to room temperature and filtered. The filtrate was stirred with 15 g of Amberlyst® 15 cation exchange resin for 5 hours. The resin was then filtered and washed with DCM (3 x 50 mL). It was then added to a solution of 2.9 g of NaOH in 100 mL of anhydrous EtOH and mechanically stirred overnight. The resin was filtered and washed with 95% EtOH (3 x 50 mL). The filtrate was concentrated *in vacuo* to give **6.1** as an amber oil (9.2 g, 93%). Spectroscopic data matched those previously reported.[3]



Lipoic Acid *N*–Hydroxysuccinimide–Ester (LA–NHS) (**6.2**)

The literature procedure[3] was modified. To a solution of lipoic acid (5.00 g, 24.2 mmol) and *N*–hydroxysuccinimide (3.35 g, 29.1 mmol) in 150 mL of THF at 4 °C was added slowly a solution of dicyclohexylcarbodiimide (DCC, 6.00 g, 29.1 mmol) in 10 mL of THF. The mixture was warmed to room temperature and stirred for 8 hours, then showing complete conversion to the coupling product **6.2** by TLC (R_f 0.7, 90% DCM/MeOH). The precipitate was removed by vacuum filtration through Celite® and the solvent was evaporated *in vacuo*, giving a yellow solid. The crude product was redissolved in EtOAc and filtered again. This filtrate was concentrated *in vacuo*, giving pale–yellow oil. Recrystallization from a solution of hot ethyl acetate/hexane (1:1 v/v) afforded pure dithiane **6.2** a pale–yellow solid (3.47 g, 47%). The mother liquor was concentrated down and saved for future recrystallizations. Spectroscopic data matched those previously reported.[3]

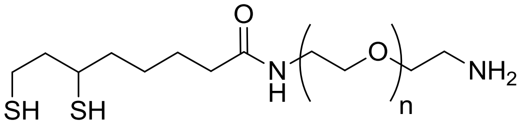


Lipoic Acid–PEG–Amine (LA–PEG$_8$–NH$_2$) (**6.3**)

This procedure from the literature[3] was modified. To a yellow solution of **6.1** (13.0 g, 32.8 mmol) and sodium bicarbonate (2.75 g, 32.8 mmol) in DMF/water (100 mL, 50:50 v/v) at 4 °C was added dropwise a solution of recrystallized pure **6.2** (1.80 g, 5.90 mmol) in 10 mL of DMF over 1 hour, turning the reaction mixture a lighter yellow. The solution was warmed to room temperature and stirred for additional 16 hours, showing complete conversion to the amide **6.3** by TLC (R_f 0.15, 90% DCM/MeOH) and ESI–MS (*m/z* 601.3 $[M+H]^+$, see page 686, adapted with permission from ref.[11]). The crude product mixture was extracted with chloroform (3 x 30 mL). The combined organic extracts were washed with water (3 x 30 mL), dried over Na_2SO_4 and filtered. After removing the solvent under reduced pressure, the crude product was purified by alumina column (DCM/MeOH, 95:5), yielding **6.3** as a yellow oil (1.93 g, 58%). The remainder of the product was contaminated with traces of **6.1**. This material was recycled along with excess **6.1** which recovered from the aqueous layer. Spectroscopic data matched those previously reported.[3]

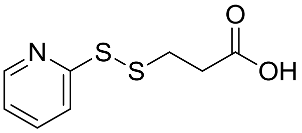
The published procedure[3] for the synthesis of **6.3** required excessive extractions from the DMF/water layer; therefore, we developed the following procedure: **6.1** (7.8 g, 5.0 equiv.) was dissolved in 50 mL of DCM. To this solution was added a solution of **6.2** in 50 mL of DCM, dropwise with stirring, over 1 hour. After another 30 minutes, the solvent was removed *in vacuo* and the residue was subject to silica gel chromatograph

(1% NH_4OH/10% MeOH/DCM). The compound **6.3** was obtained as an amber oil (2.6 g, 96% yield). Spectroscopic data matched those previously reported.[3]



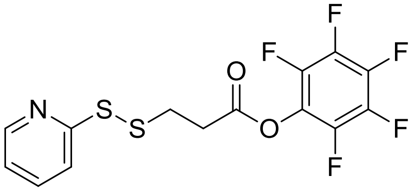
Dihydrolipoic Acid–PEG–Amine (DHLA–PEG_8–NH_2) (**6.4**)

This procedure from the literature[3] was modified. To a yellow solution of **6.1** (0.20 g, 0.33 mmol) in 4:1 water/ethanol (3 mL) at 4 °C was slowly added 4 equiv. of sodium borohydride (50.0 mg, 1.33 mmol) over 30 minutes. The bubbling solution was stirred for 4 hours at 4 °C, showing only 67% conversion to the reduction product **6.4** by ESI–MS (*m/z* 603.3 $[M+H]^+$, see page 688, adapted with permission from ref.[11]). The product and starting material were not distinguishable due to overlapping TLC spots (R_f 0.15, 90% DCM/MeOH). The reaction mixture was left stirring overnight at room temperature. No further conversion was observed, so a total of 8 more equiv. of sodium borohydride was added over 48 hours, driving the reaction to completion in 72 hours. The crude reaction mixture was acidified to pH 2 with 1 M HCl, and extracted with chloroform (3 x 15 mL). The combined organic layer was dried over magnesium sulfate and filtered. The solvent was removed *in vacuo* to furnish the dithiol **6.4** as yellow oil (0.196 g, 98% yield). Spectroscopic data matched those previously reported.[3] Traces of triphenylphosphine oxide were detected by ^1H NMR. A stability study was conducted on the dithiol. A 6 mg of **6.4** in $CDCl_3$ was kept under argon in a capped NMR tube for 48 hours, and no change was observed in the ^1H NMR spectrum. The sample was then exposed to the atmosphere in an uncapped NMR tube for 48 hours, again showing no change by ^{1}H NMR. This product appeared to be air–stable.



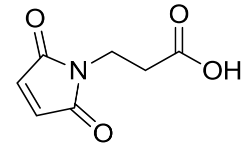
3–(2–Pyridyl)–dithiopropionic acid (PDP–OH) (**6.5**)

This procedure from the literature[6] was modified. Pyridyl disulfide (3.11 g, 14.1 mmol) was dissolved in 30 mL of anhydrous EtOH and cooled to –10 °C. Mercaptopropionic acid (MPA, 1.0 g, 9.4 mmol) in 20 mL of anhydrous EtOH was added dropwise over 30 minutes at –10 °C. The reaction mixture was allowed to warm to room temperature. After 24 hours, the solvent was removed *in vacuo* and the residue was chromatographed (15% EtOAc/hexane). The compound **6.5** was isolated as a pale yellow solid (1.3 g, 64%). Spectroscopic data matched those previously reported.[6]



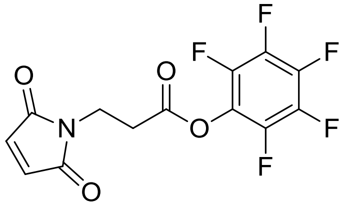
3–(2–Pyridyl)–dithiopropionic acid pentafluorophenyl ester (HFL1) (**6.6**)

This procedure was modified from the literature.[7] **6.5** (1.3 g, 6.0 mmol), pentafluorophenol (PFP, 1.1 g, 1 equiv.), and DCC (1.25 g, 1 equiv.) were dissolved in 30 mL of DCM and the reaction mixture was stirred at room temperature under nitrogen. After 48 hours, another 100 mg of both PFP and DCC were added to drive the coupling to completion within 3 hours. The solvent was removed *in vacuo*, and the residue was purified by silica gel column chromatography (5% EtOAc/hexane). **6.6** was obtained as a yellow oil (1.88 g, 82% yield). Spectroscopic data matched those previously reported.[8] Traces of DCC were detected by ^{1}H NMR.



3–Maleimidopropionic acid (MP–OH) (**6.7**)

According to the literature,[9] MP-OH was synthesized. **6.7** was obtained as a white solid (43% yield). Spectroscopic data matched those previously reported.[9]



3–Maleimidopropionic acid pentafluorophenyl ester (HFL2) **(6.8)**

HFL2 was synthesized according to the literature.[7] **6.8** was acquired as a white solid (74% yield). ^{1}H–NMR (CDCl$_3$) δ: 6.72 (2H, s, maleimide), 3.95 (2H, t, $–CH_2^{\alpha}–$), 3.04 (2H, t, $–CH_2^{\beta}–$). Our spectrum agreed with that of the reported hexanoic analogue.[7]

Synthesis of Quantum Dot Conjugates with siRNA: The siRNA molecules for eGFP and EGFRvIII containing 5’ thiol group were purchased from Dharmacon, Invitrogen. The siRNA for eGFP was designed as follows: sense sequence was 5’–thiol–GGCUACGUCCAGGAGCGCACC and antisense sequence was 5’–phosphate–

UGCGCUCCUGGACGUAGCCUU. For the knockdown of EGFRvIII, sense sequence was 5'–thiol–GAAAGGUAAUUAUGUGGUGdTdT and antisense sequence was 5'–phosphate–CACCACAUAAUUACCUUUCdTdT. QDs in the PBS were mixed with a 1000–fold excess of the cross linker, **6.6** and **6.8**, for 1 hour. After removing the unreacted linkers by ultracentrifugation, siRNA molecules were coupled to the QDs by mixing them together for 1 hour. After the conjugation, free siRNA was removed using ultracentrifugation.

To quantify the ratio of siRNA molecules per QD, the siRNA linked to QDs via **6.6** (disulfide bond) were treated with DTT for 1 hour. After centrifugation, the supernatant was analyzed under UV at 260 nm to quantify the amount of siRNA detached from QDs. By determining the concentration of QDs in the same volume at the first absorption peak,[10] the number of siRNA molecules per QD was estimated. For the conjugation of HIV–Tat(CYGRKKRRQRRR) and RGD(RGDC) to siRNA–QD, the same method as that used to conjugate siRNA to QDs was used to determine the ratio of 1:10:10 (siRNA:RGD:HIV–Tat).

7.6. References

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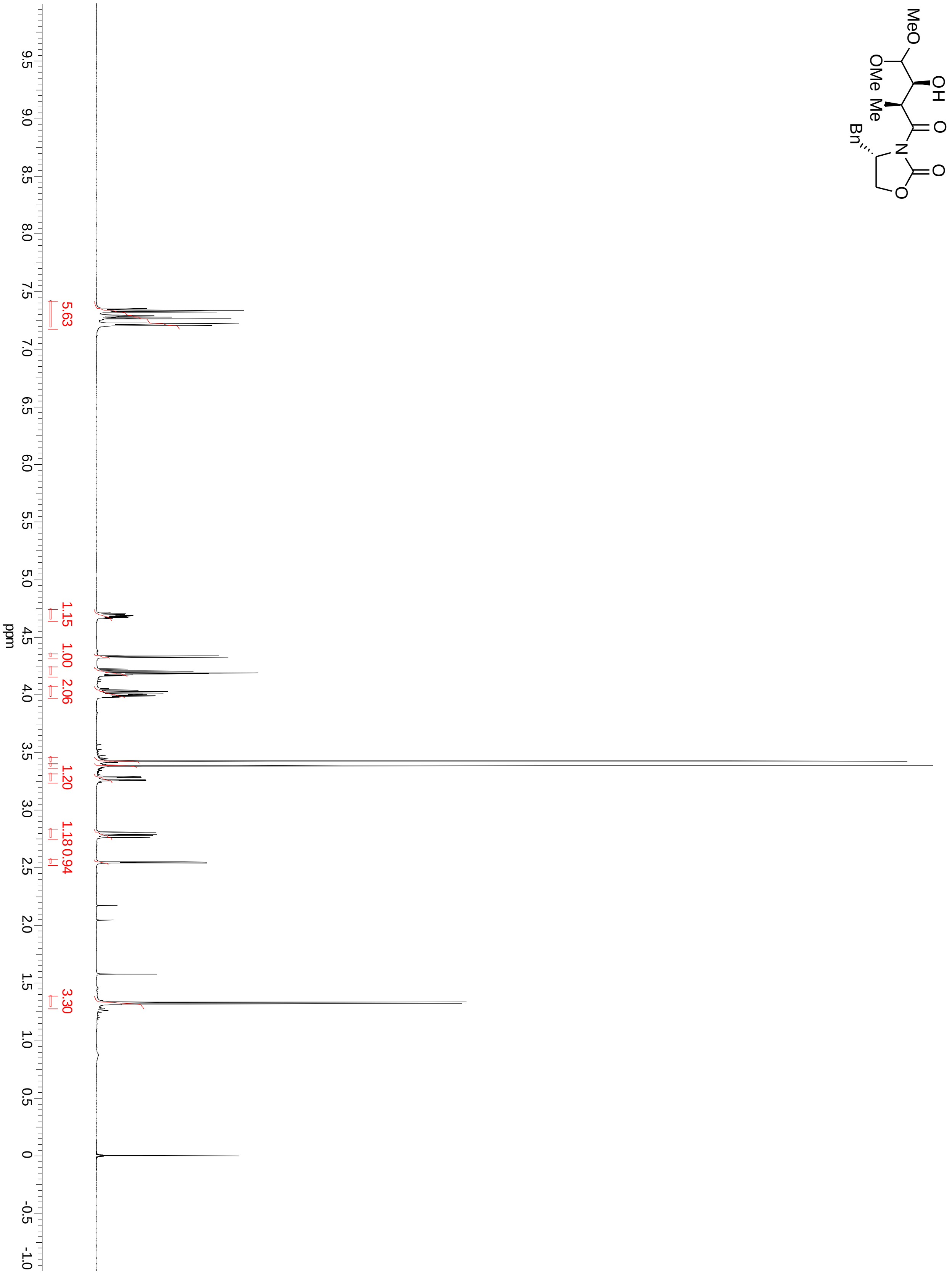
Appendix: Spectral Data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4.0
3.5
3.0
2.5
2.0
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1.0
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1.00
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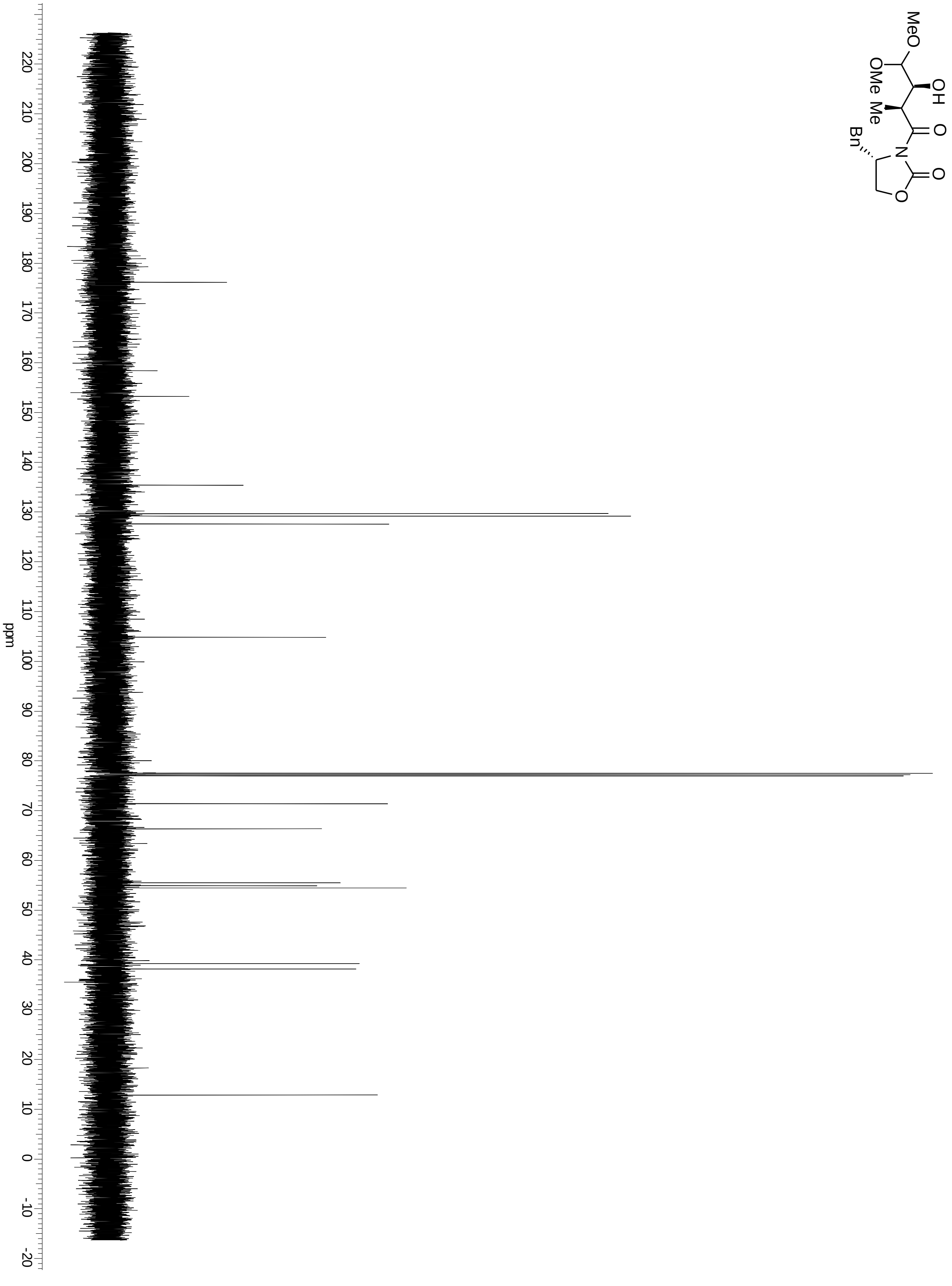
3.30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40
30
20
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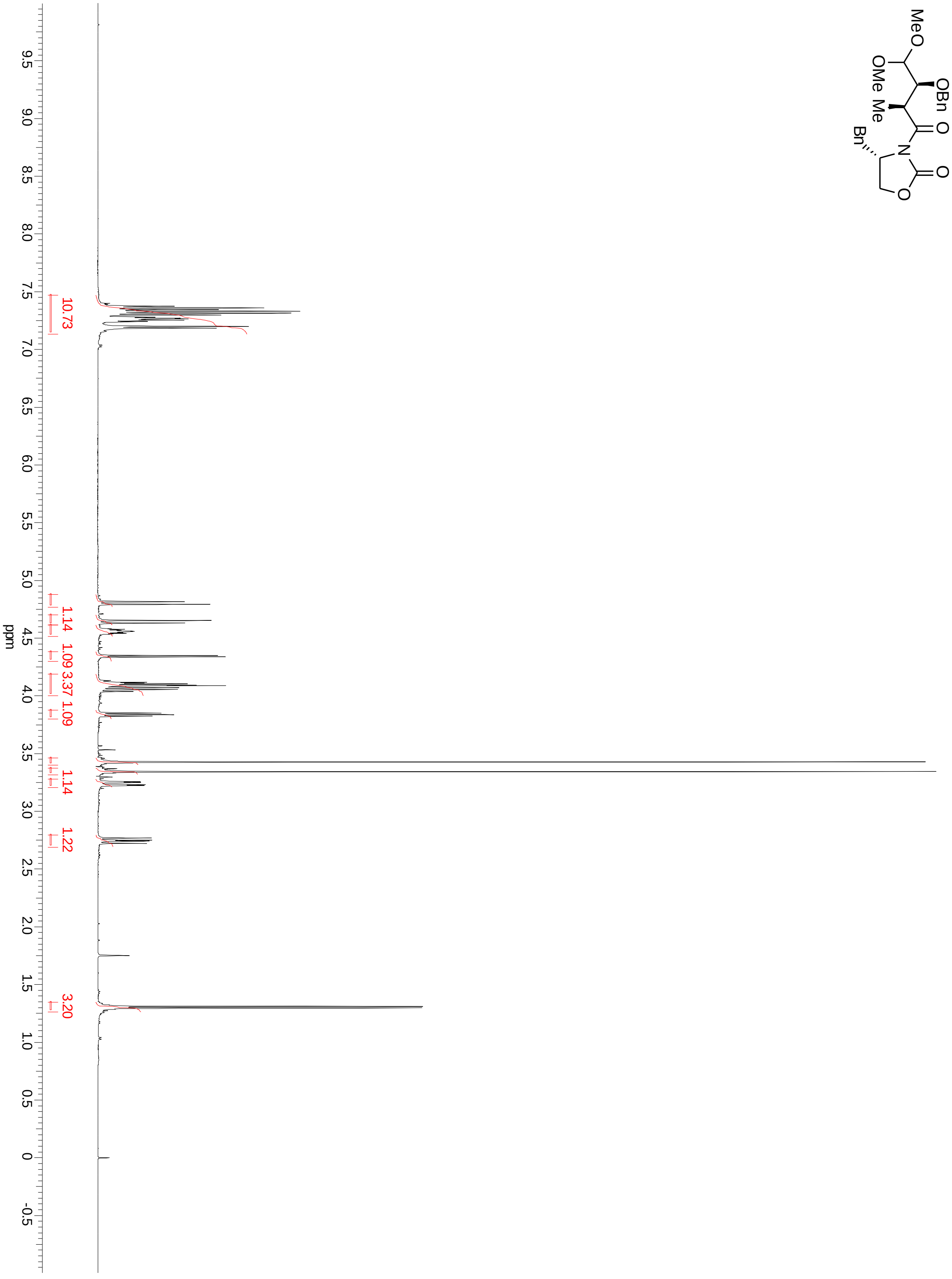
0
-10
-20
ppm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1.09
1.14
1.22
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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2.0
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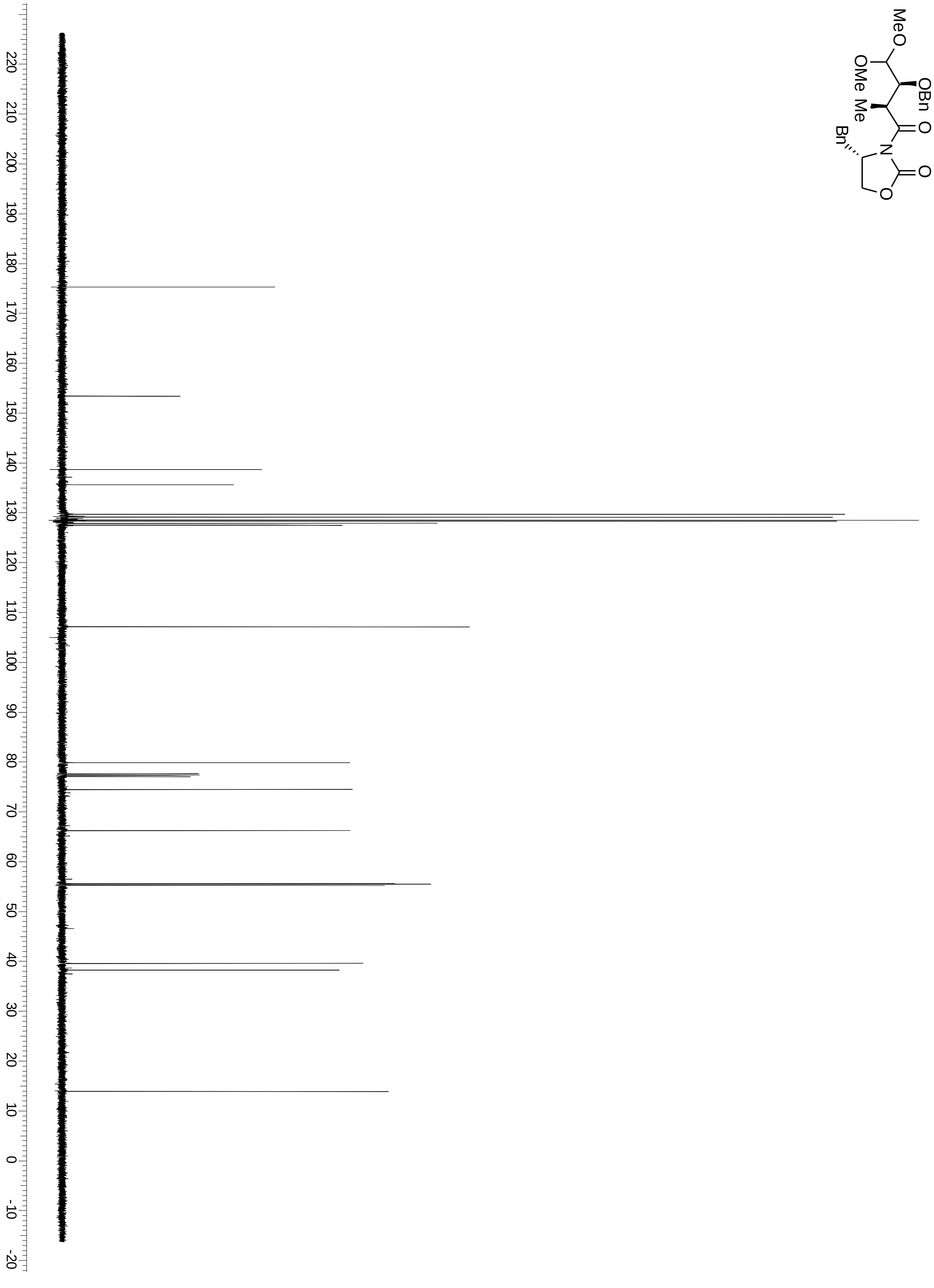
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O
Bn
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100
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40
30
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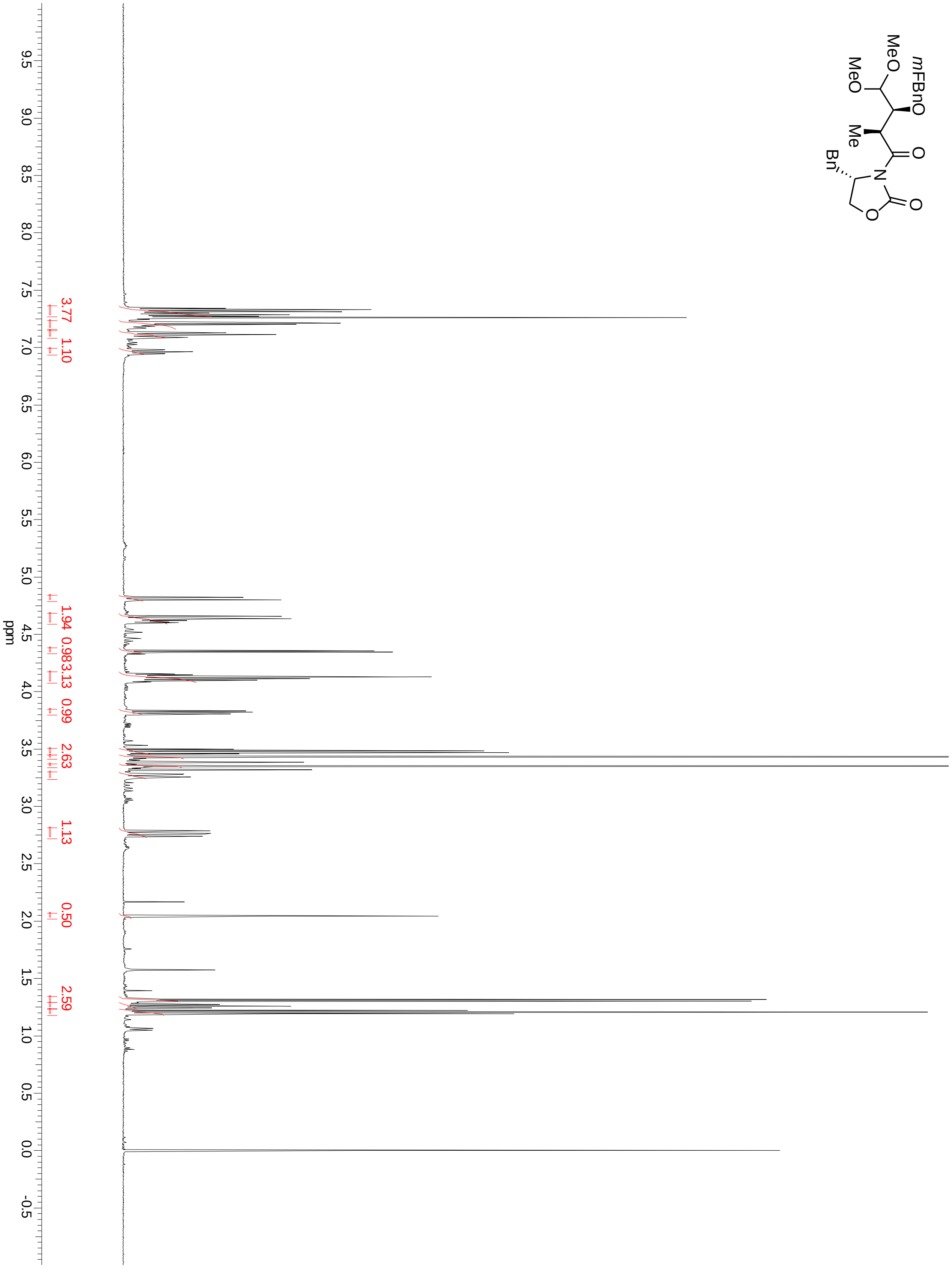
10
0
-10
-20
ppm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4.0
3.5
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2.0
1.5
1.0
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0.0
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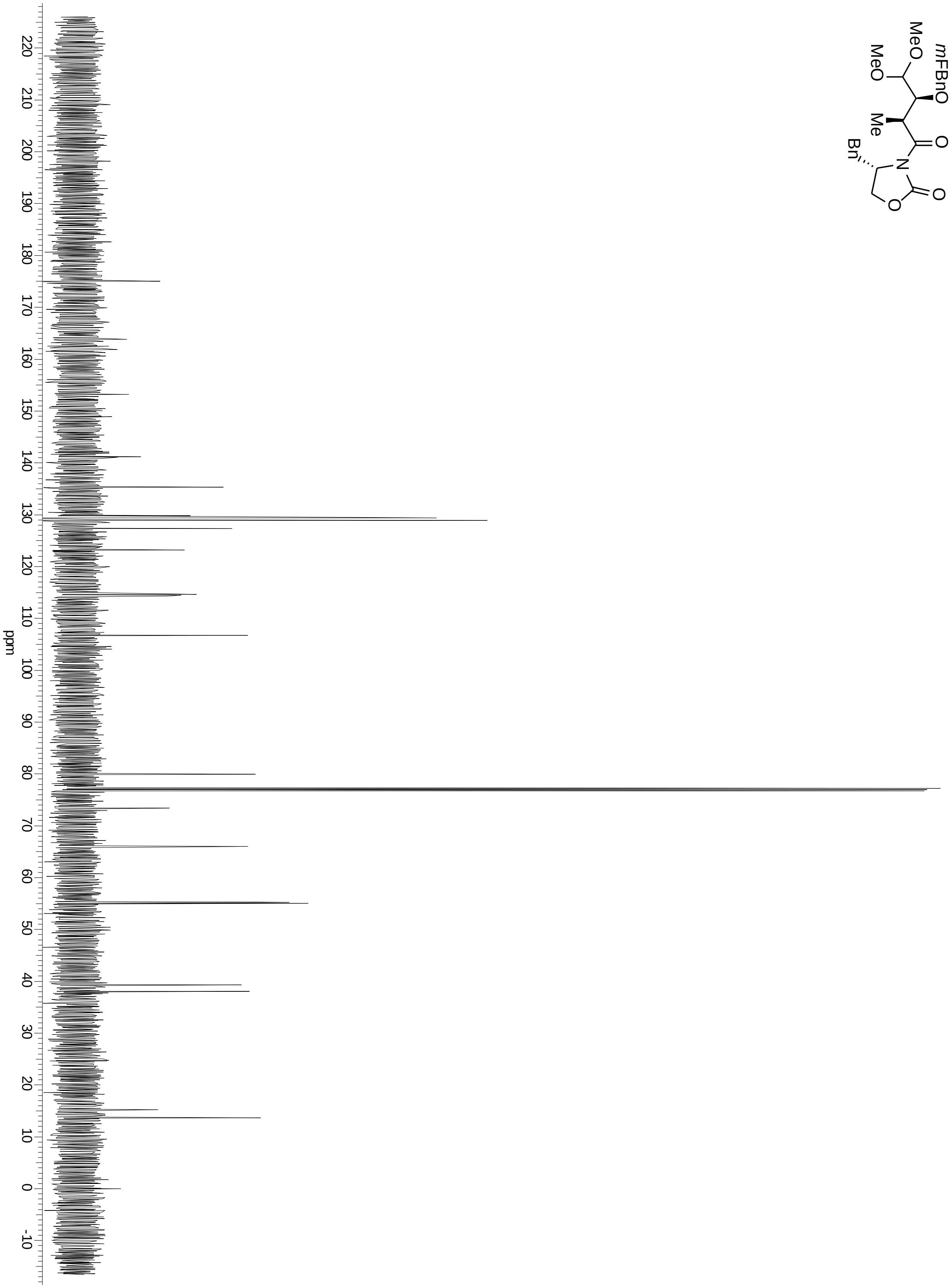
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O
O
Bn
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210
200
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180
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130
120
110
100
90
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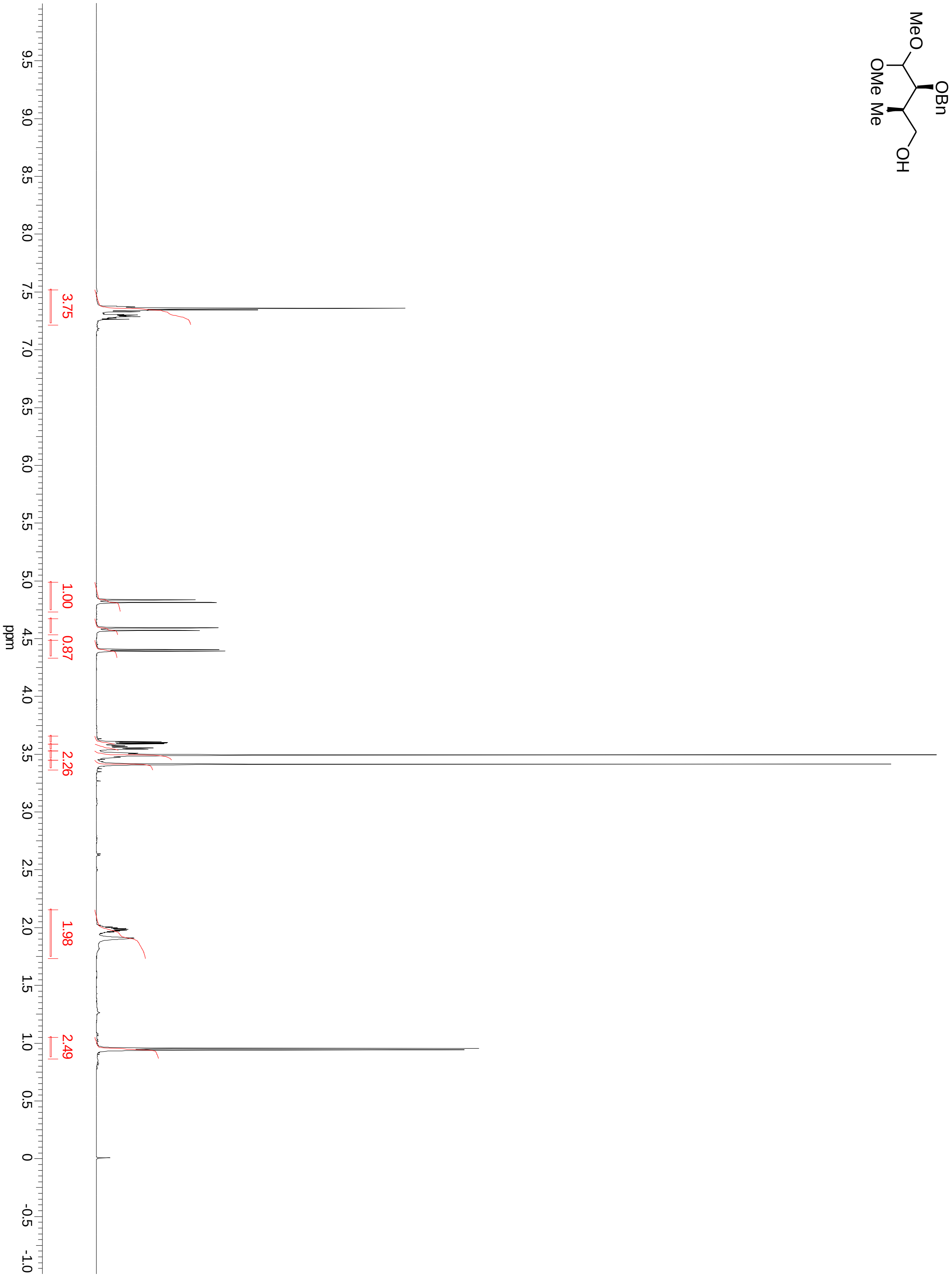
50
40
30
20
10
0
-10
ppm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5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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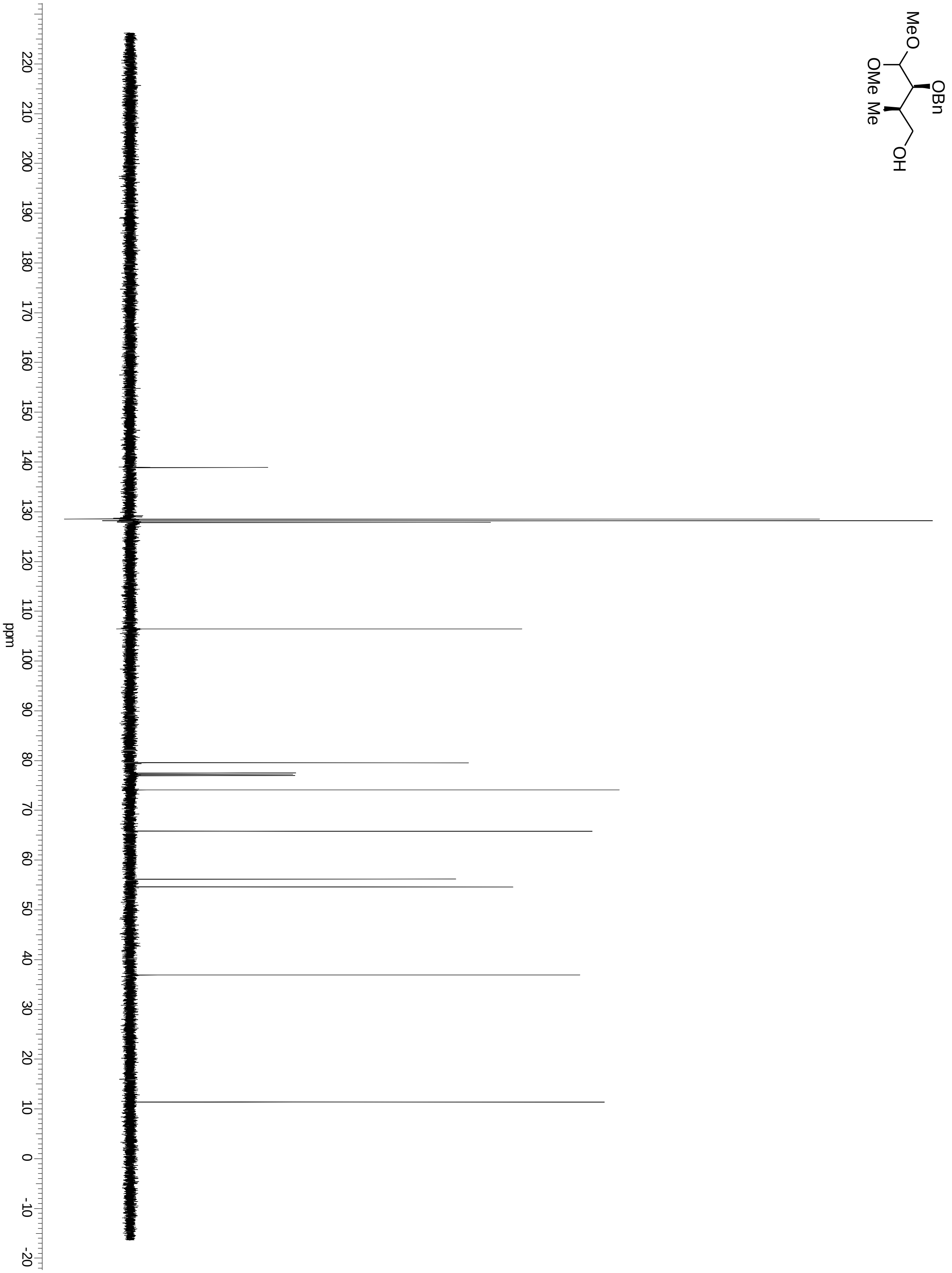
1.98
2.49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OMe Me
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180
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40
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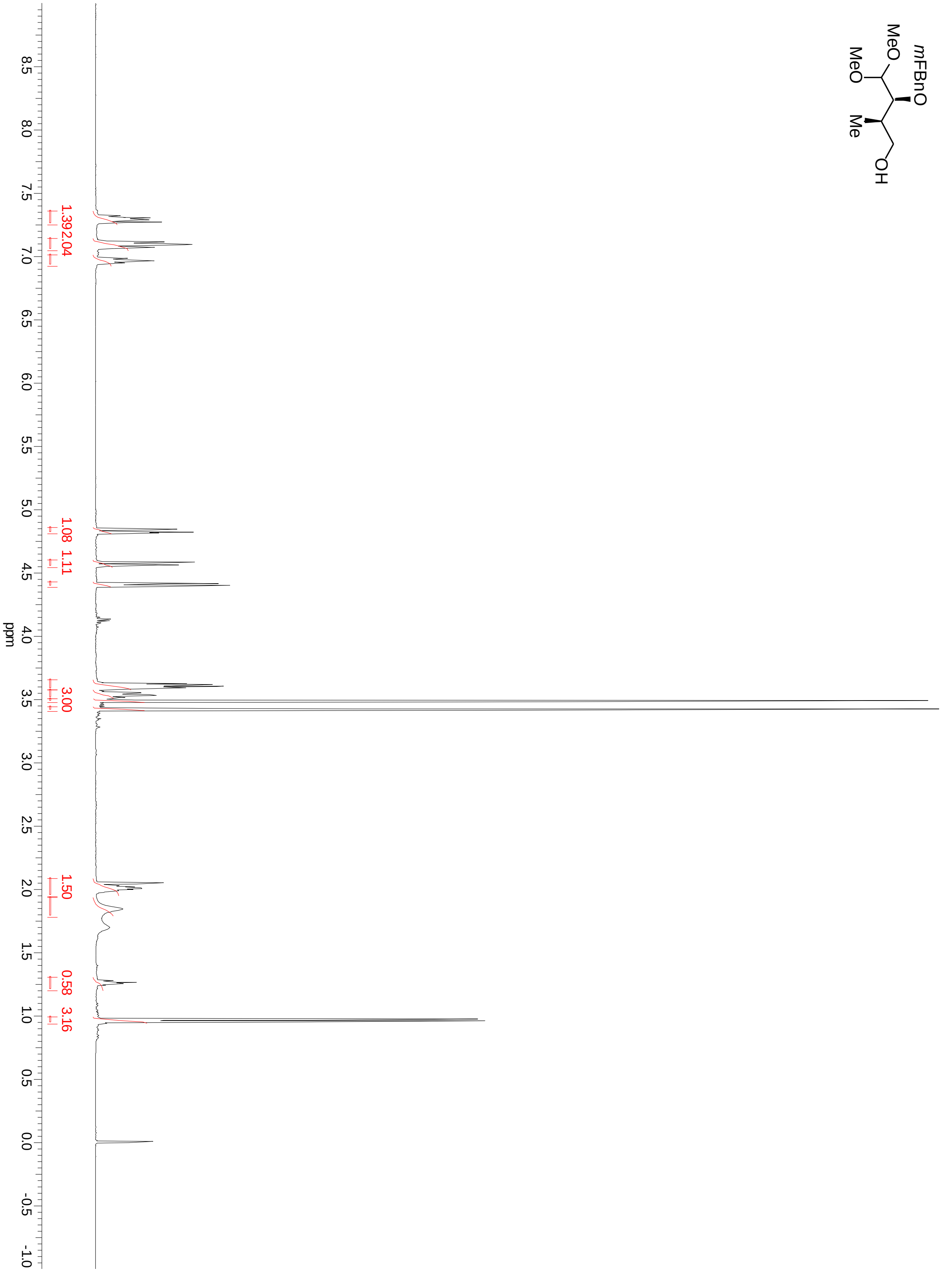
-10
-20
ppm



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MeO
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8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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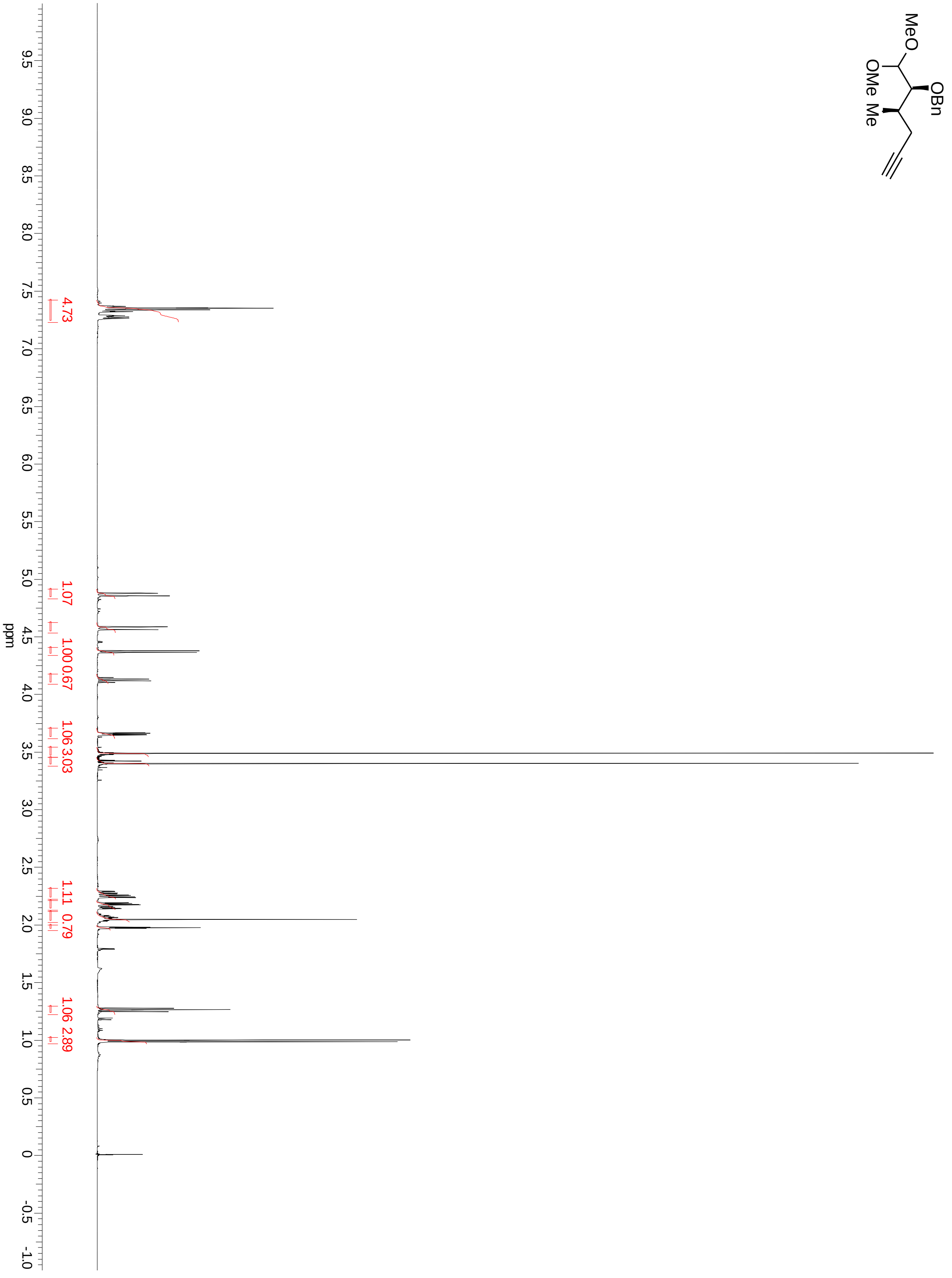
-0.5
-1.0
ppm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6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
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1.06
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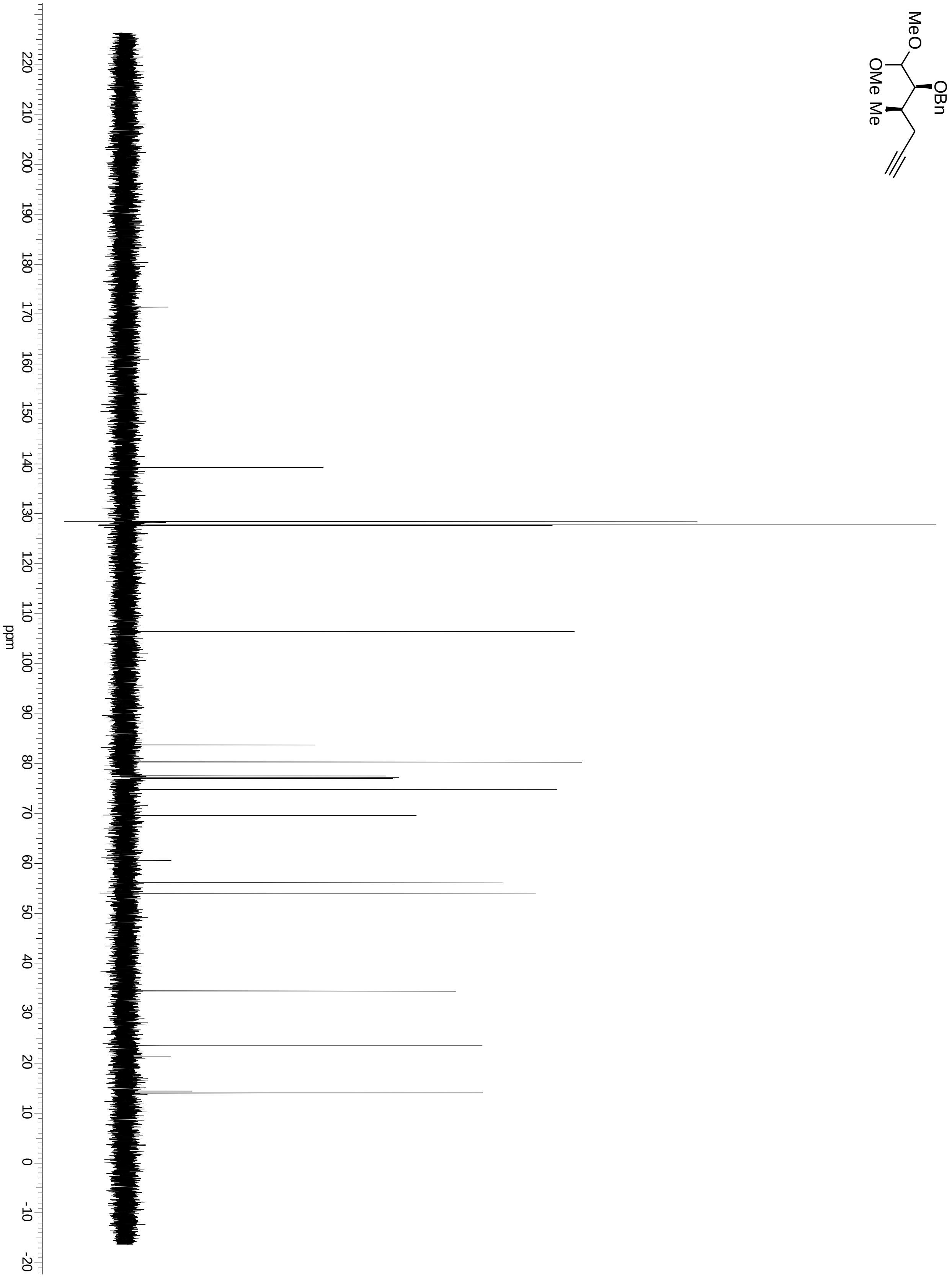
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OBn
OMe Me
220
210
200
190
180
170
160
150
140
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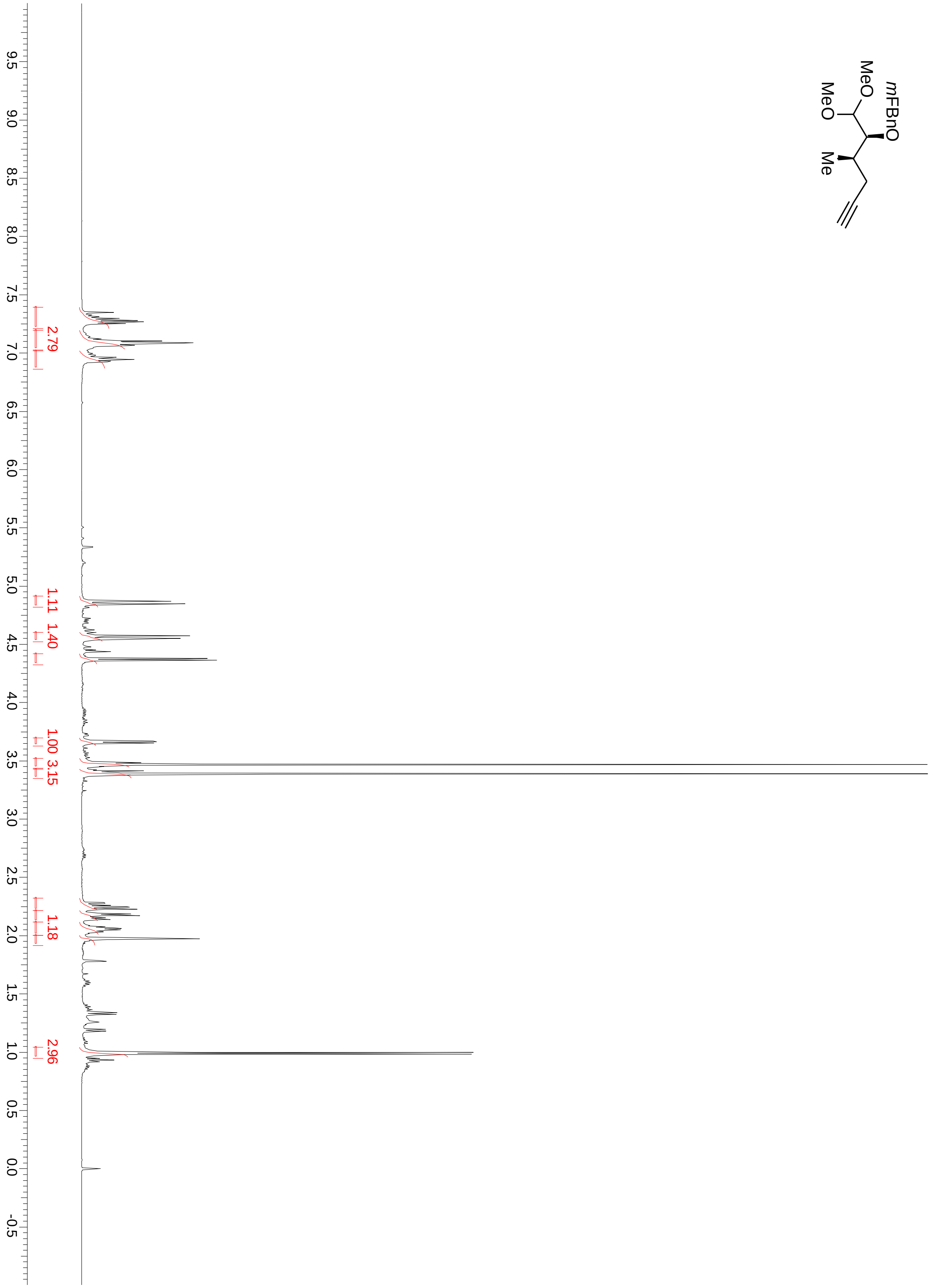
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm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4.0
3.5
3.0
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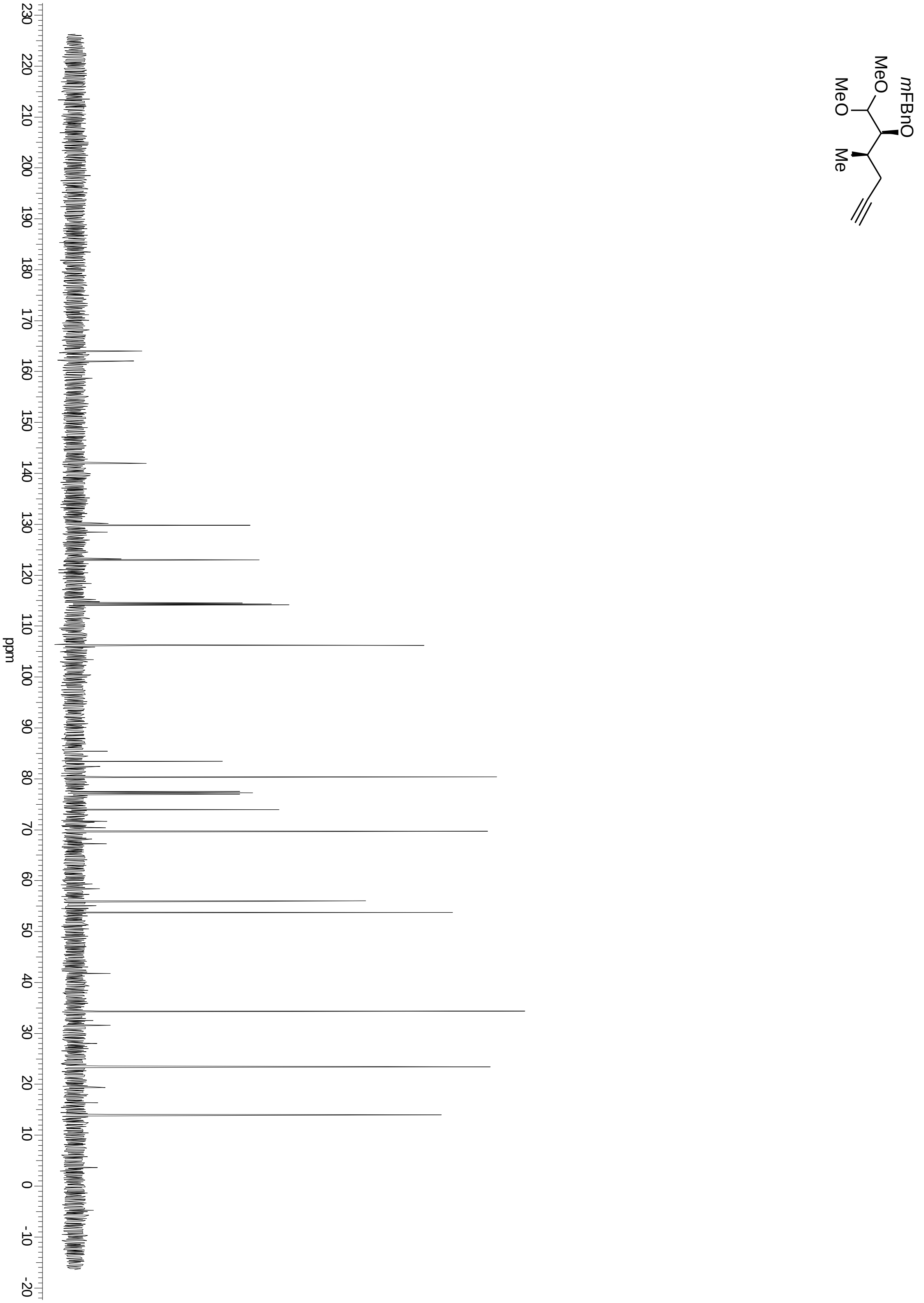
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10
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-20
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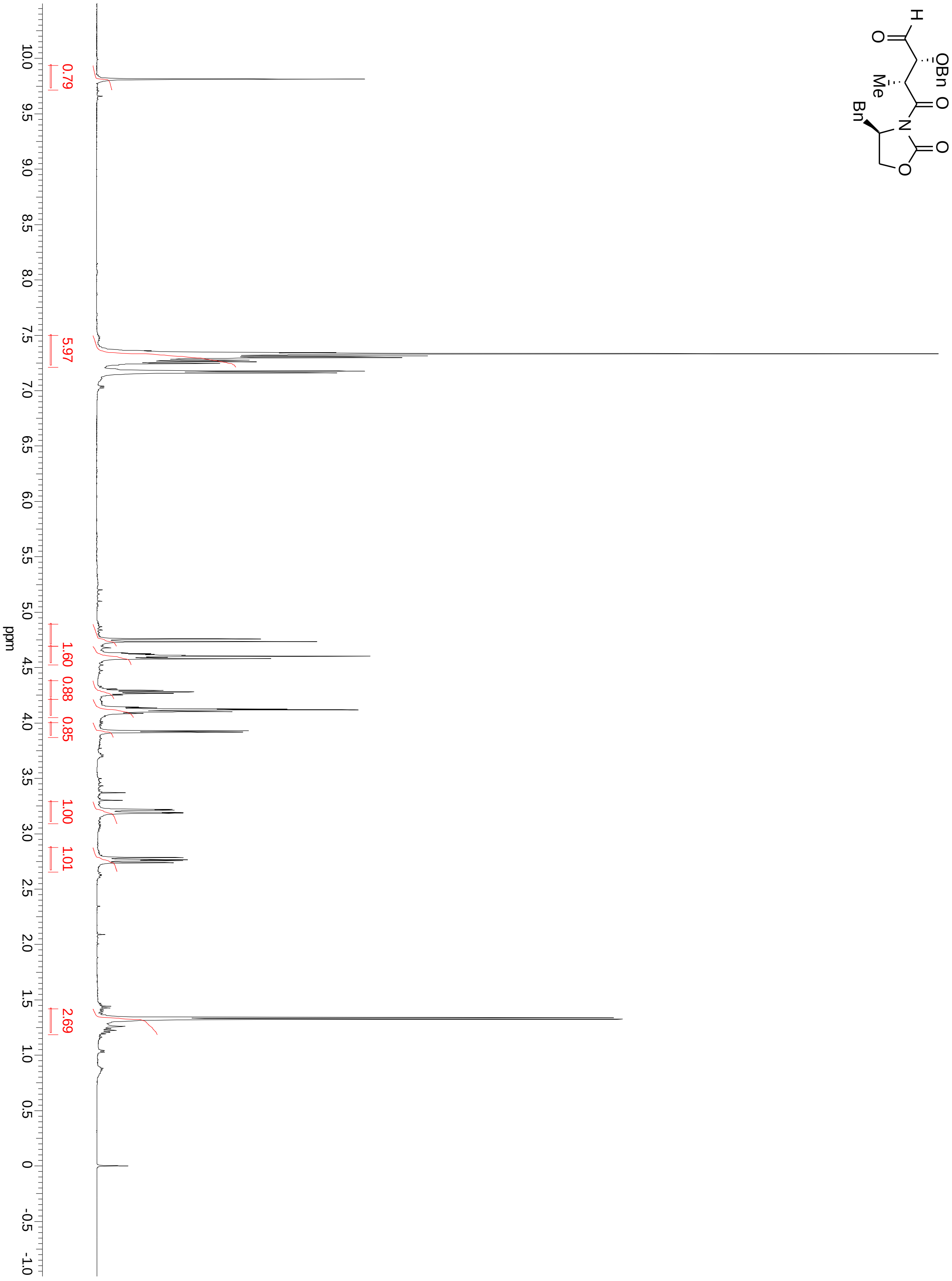
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O
Me
Bn
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6.0
5.5
5.0
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2.0
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0.5
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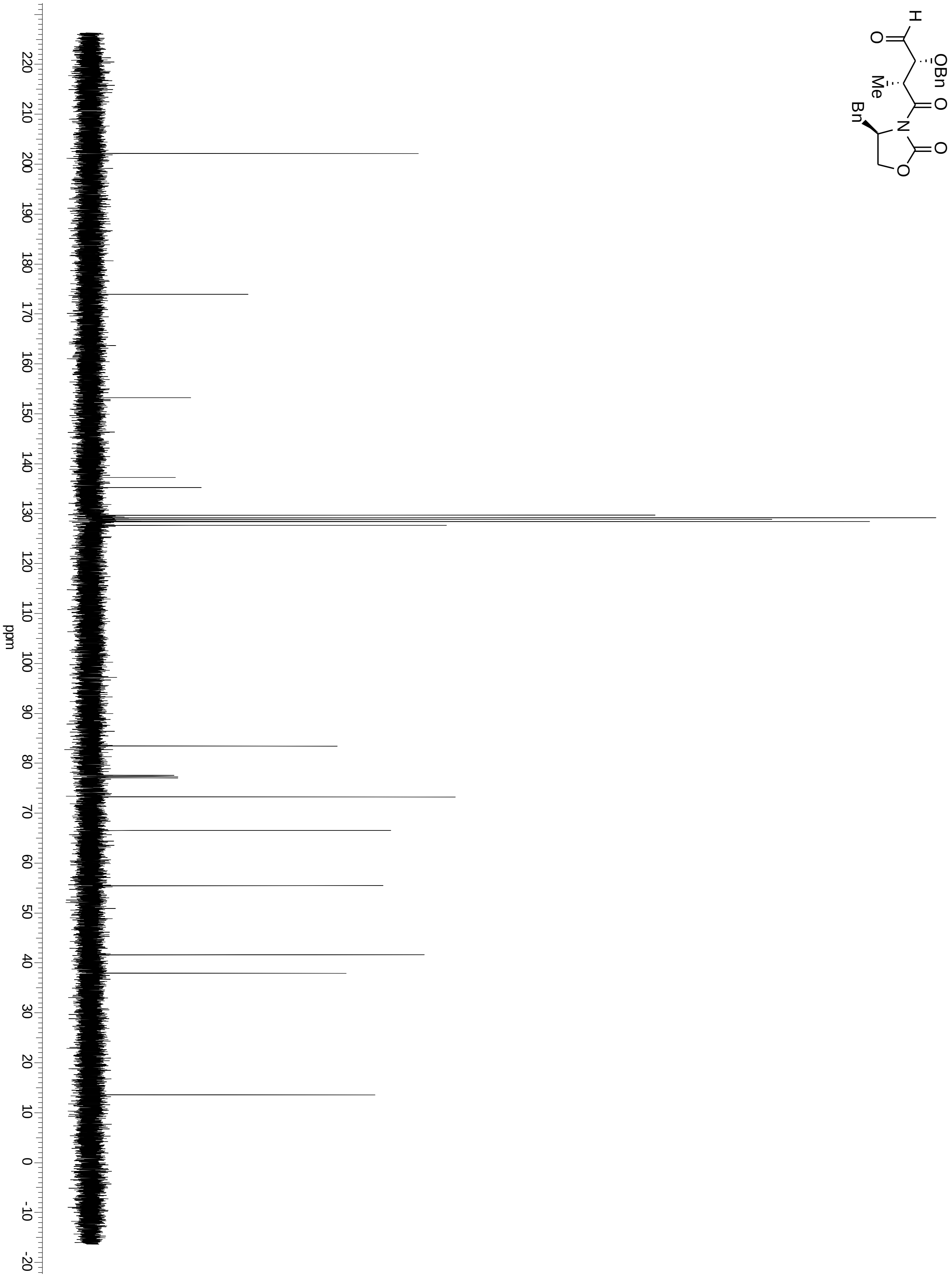
-0.5
-1.0
ppm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O
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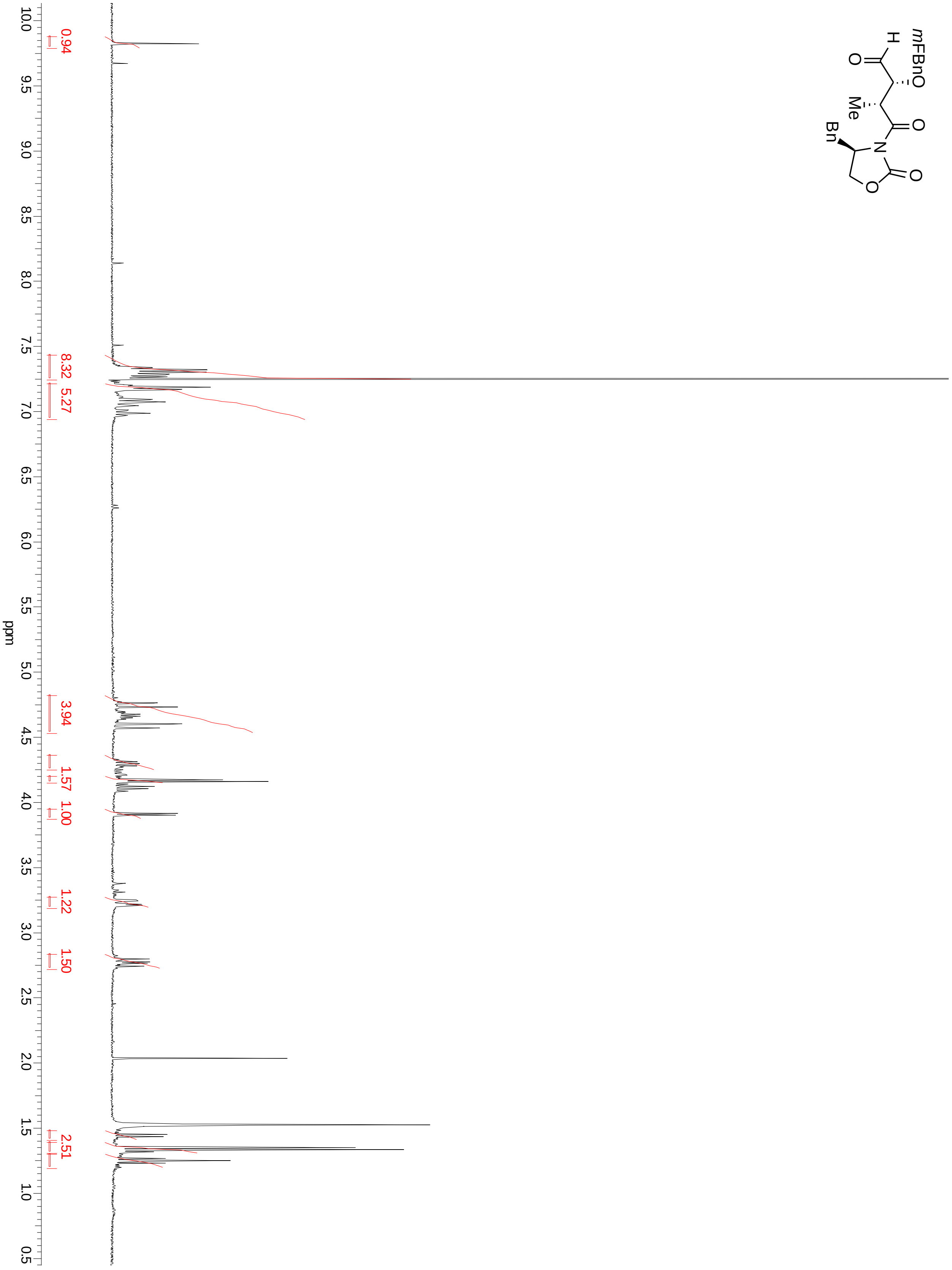
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O
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8.5
8.0
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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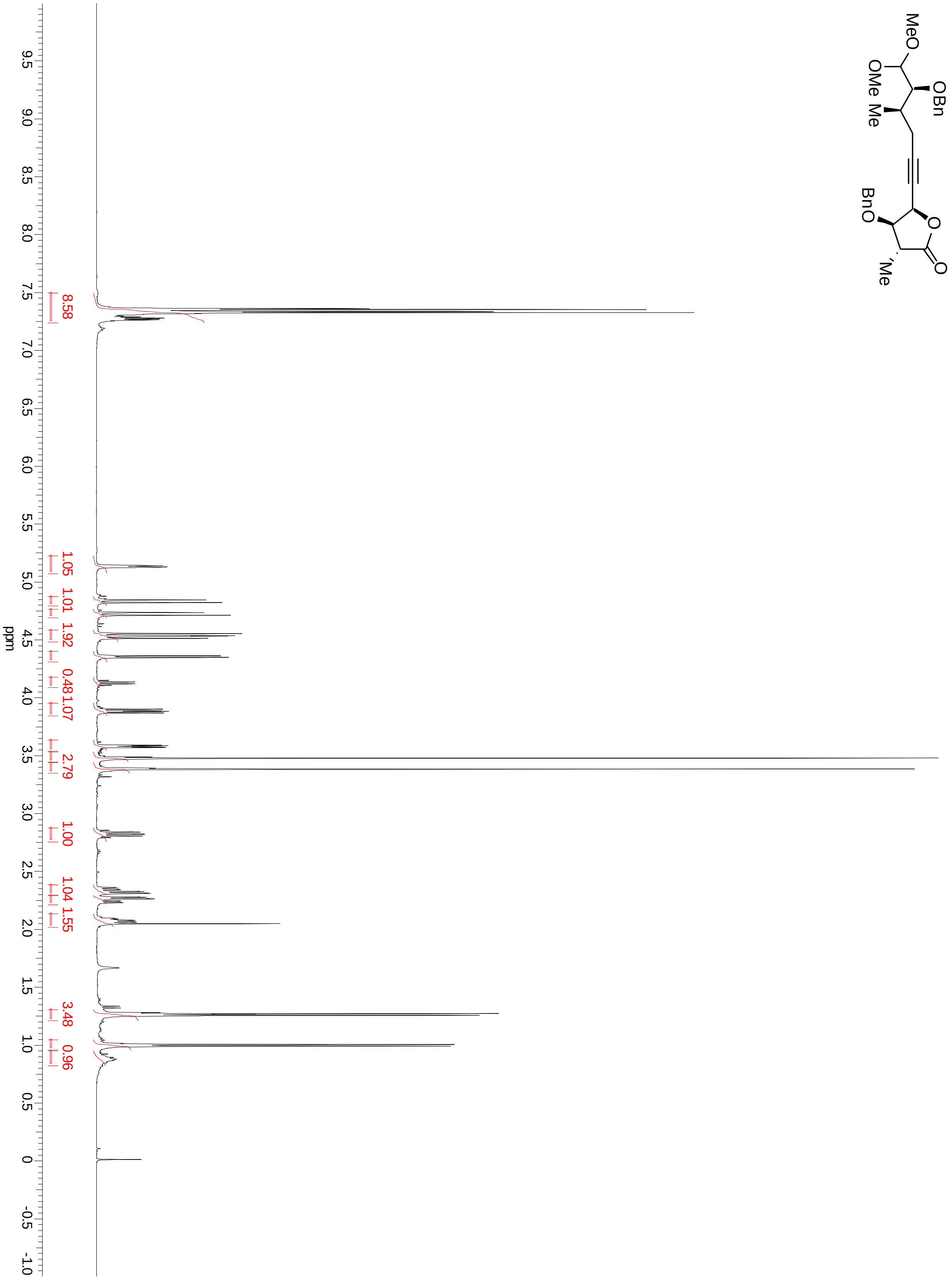
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7.0
6.5
6.0
5.5
5.0
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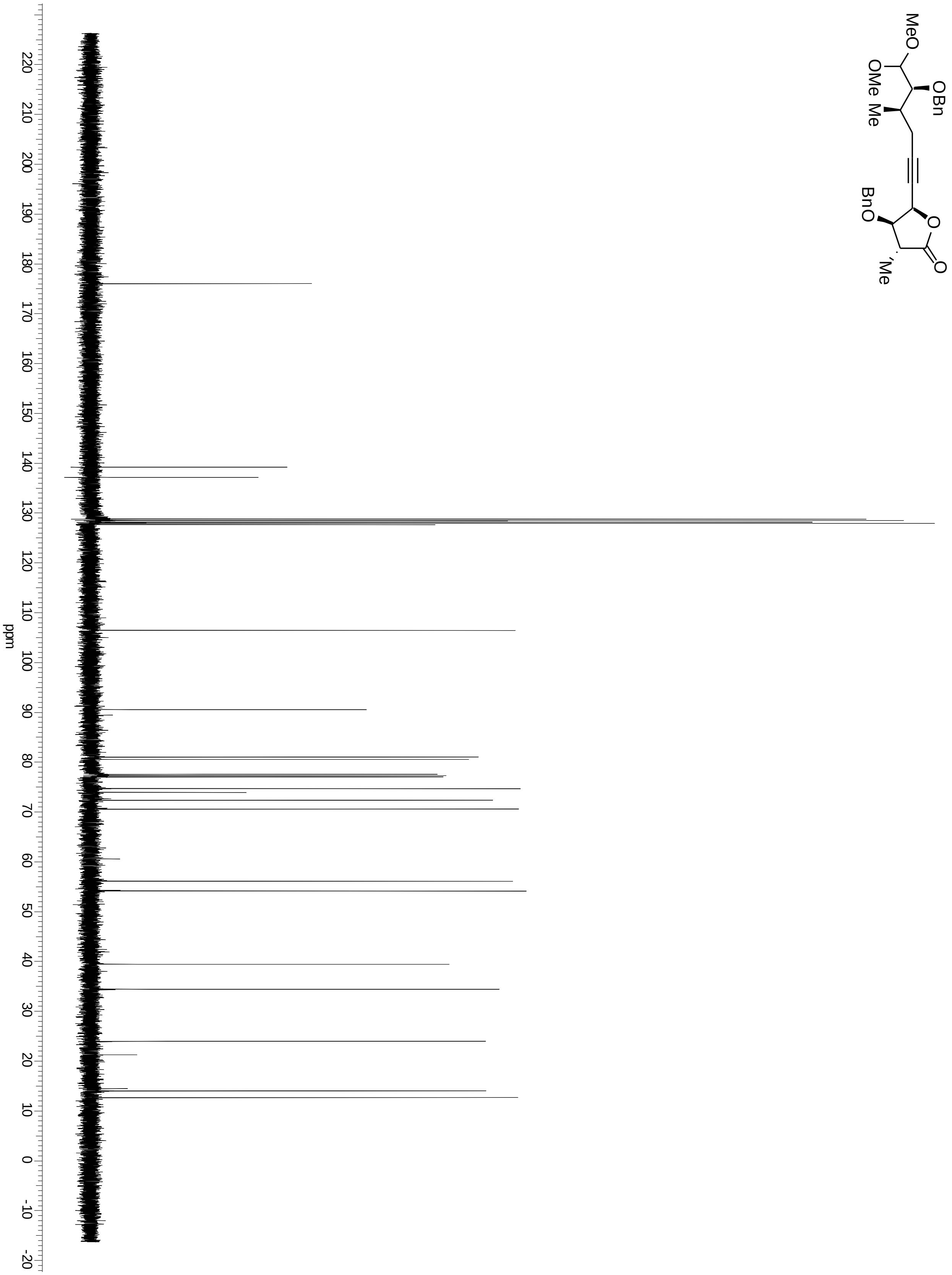
-1.0
ppm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OBn
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O
Me
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200
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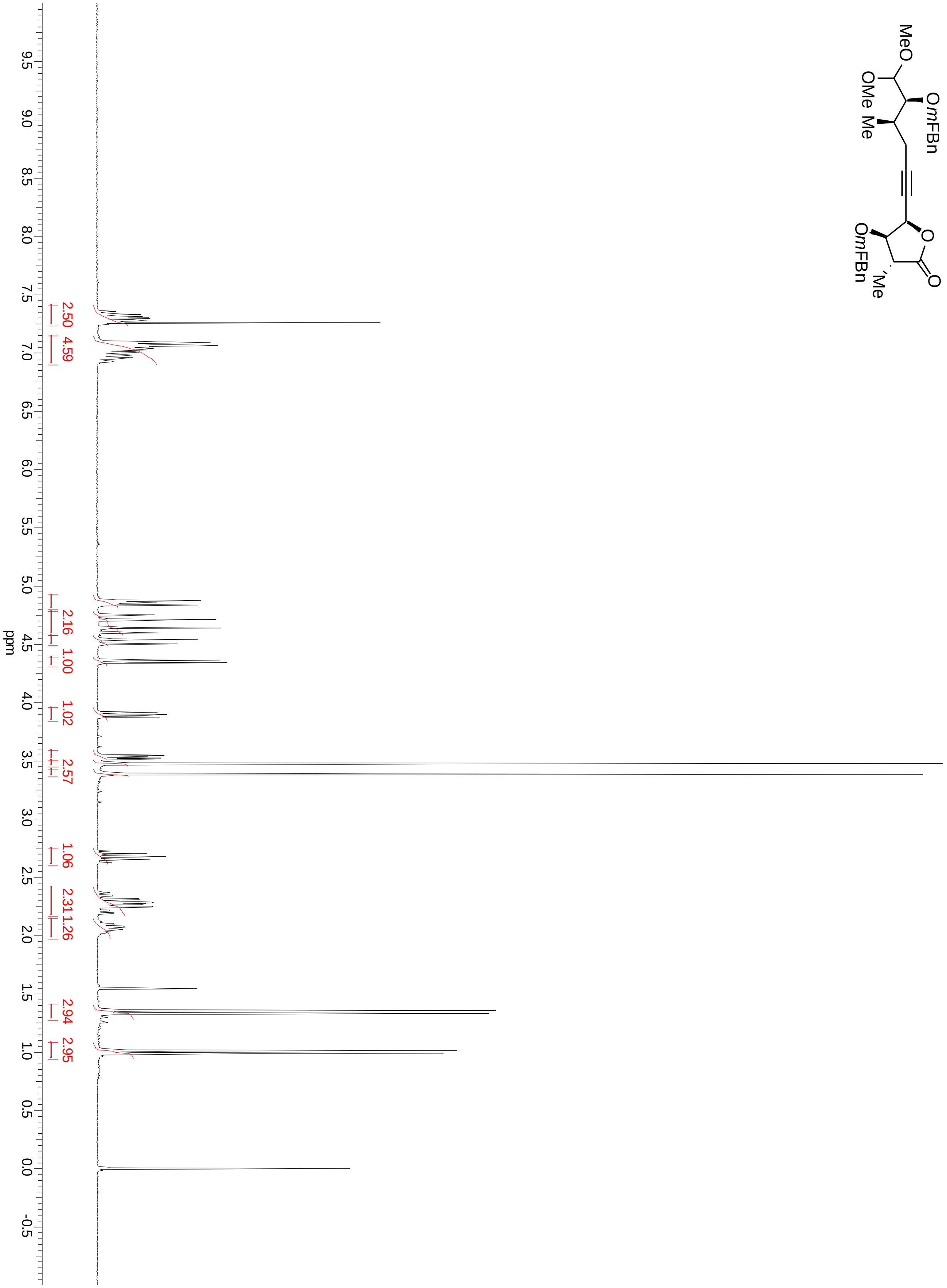
ppm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6.0
5.5
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3.0
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2.0
1.5
1.0
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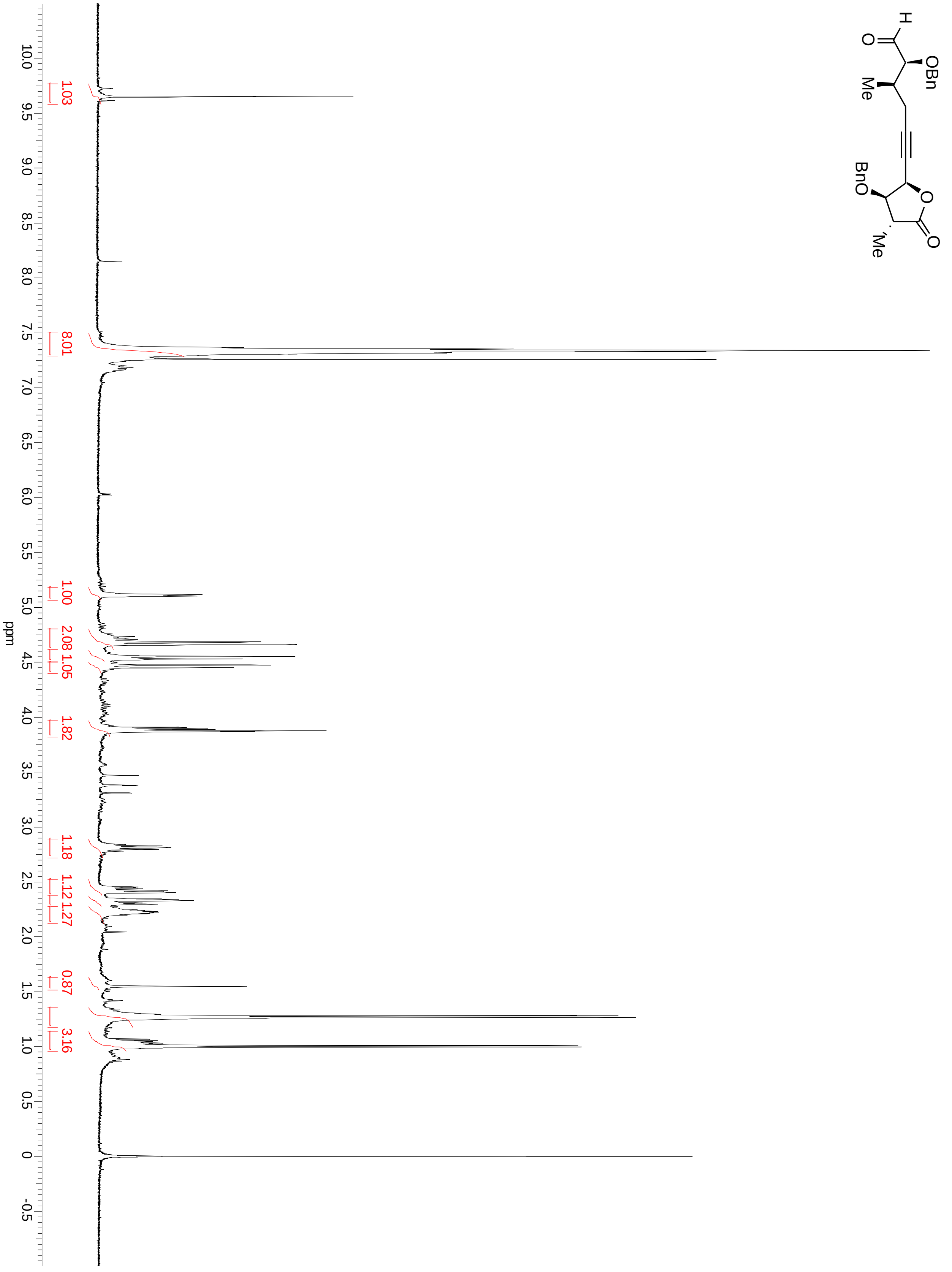
2.95



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6.5
6.0
5.5
5.0
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4.0
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3.0
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0.5
0
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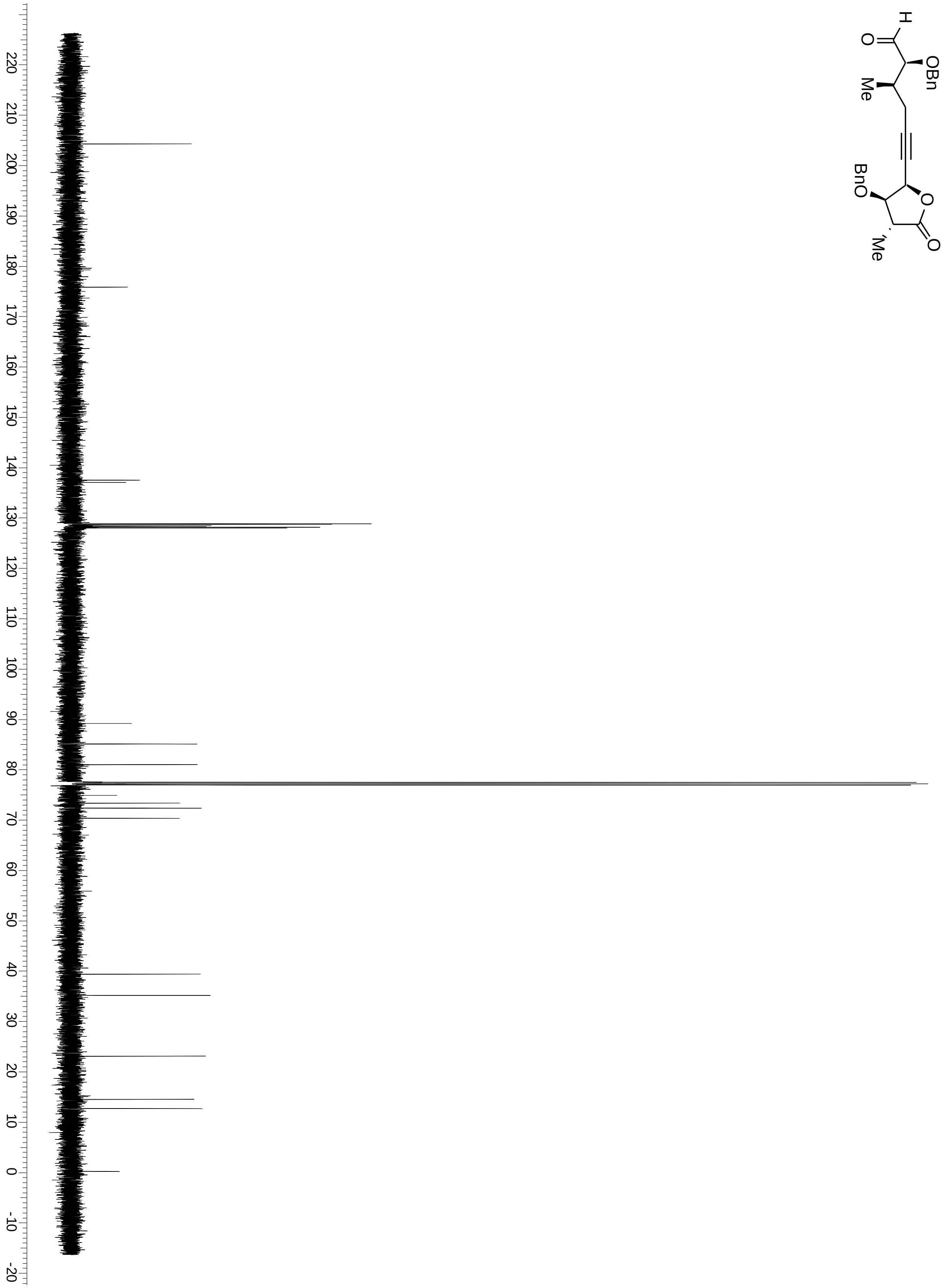
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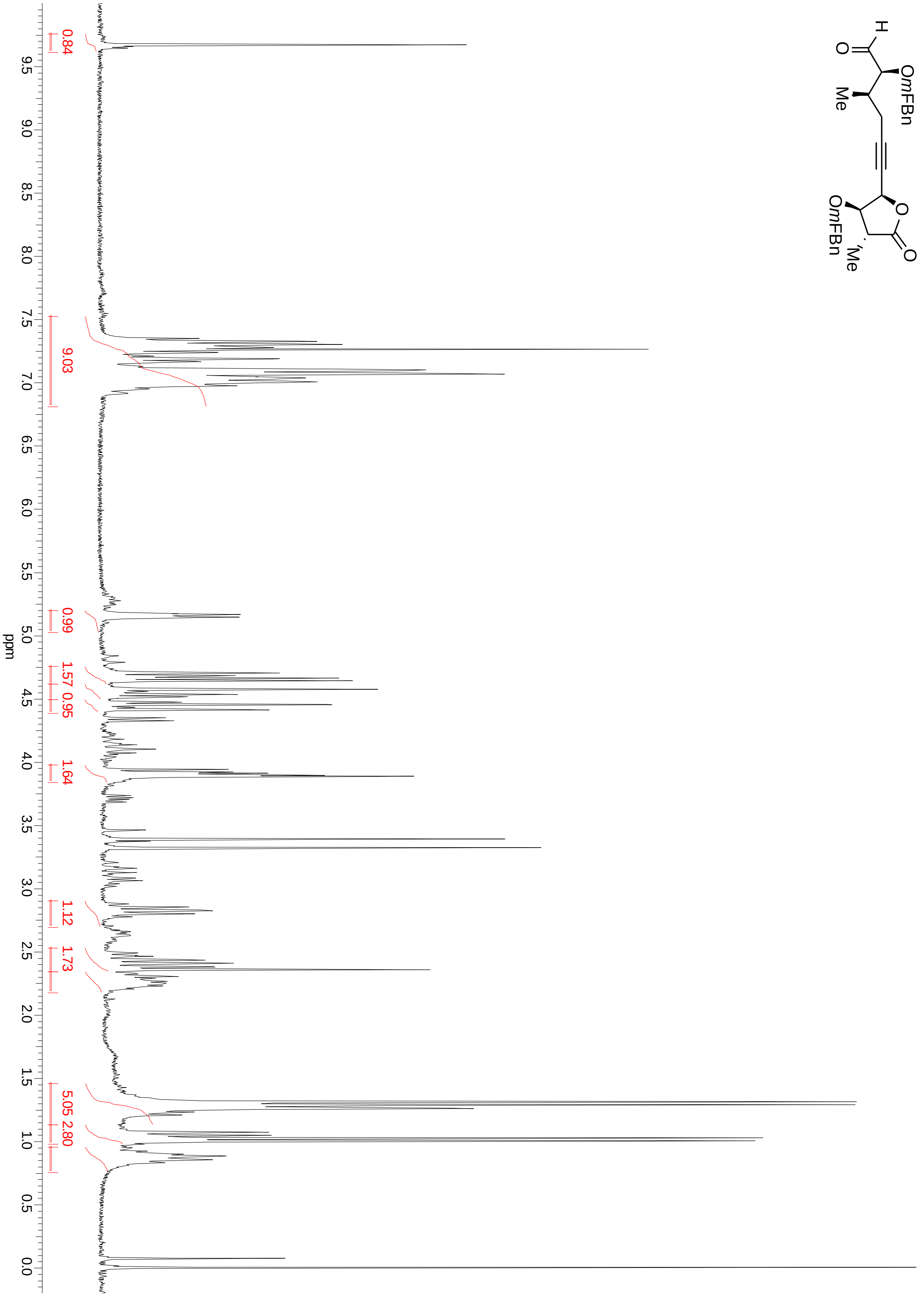
-20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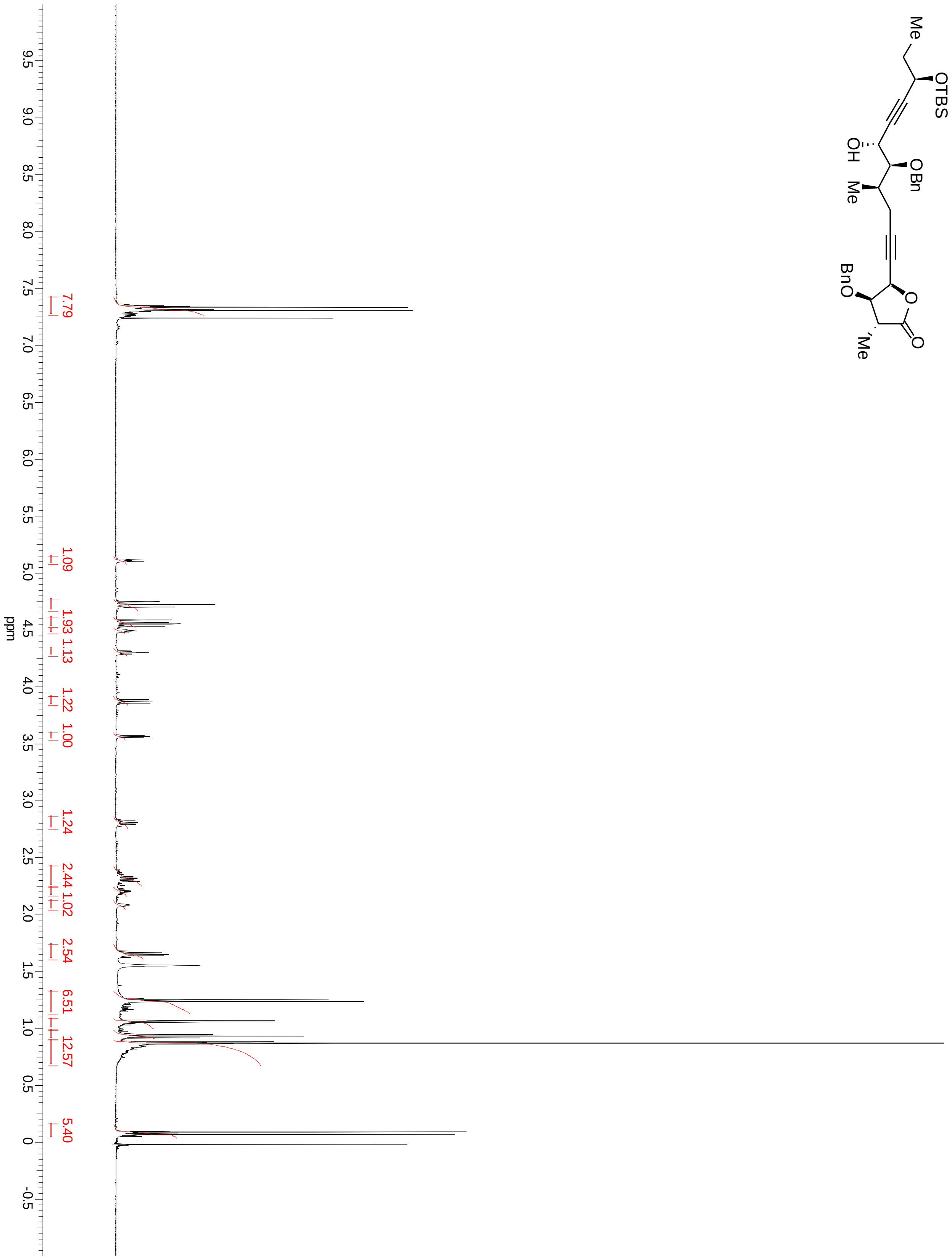
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2.54
6.51
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7.0
6.5
6.0
5.5
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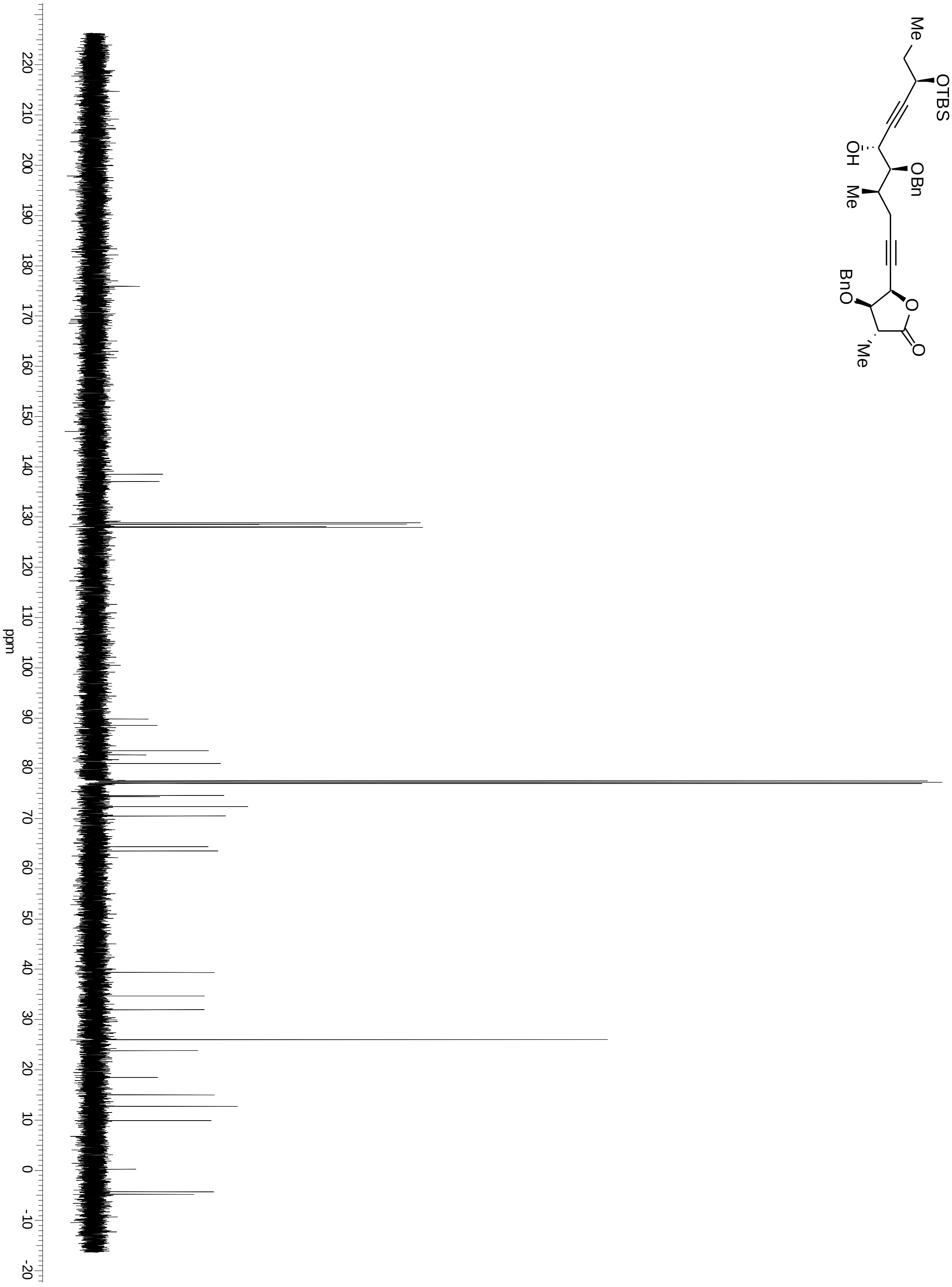
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-20
ppm
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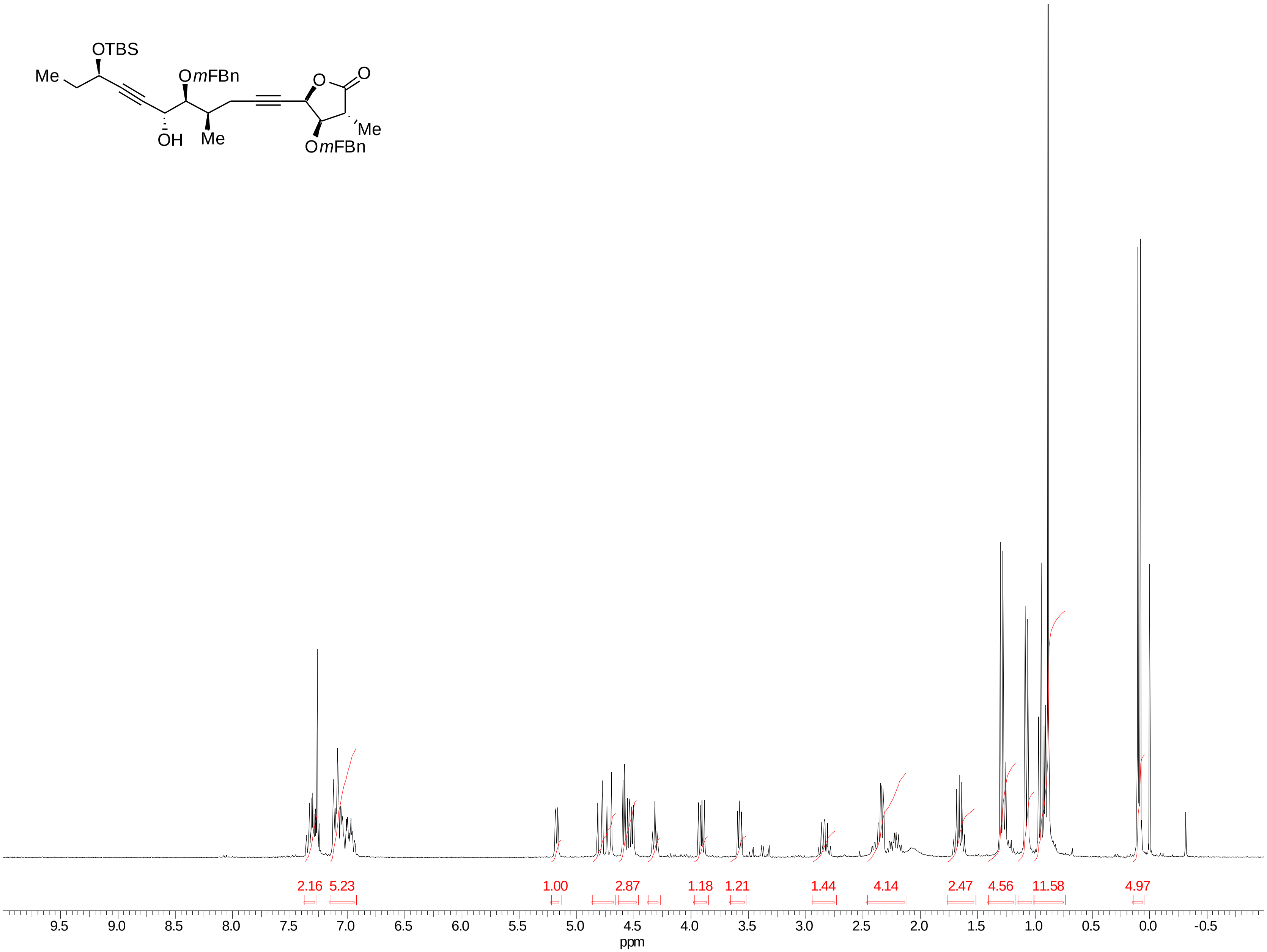
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11.58
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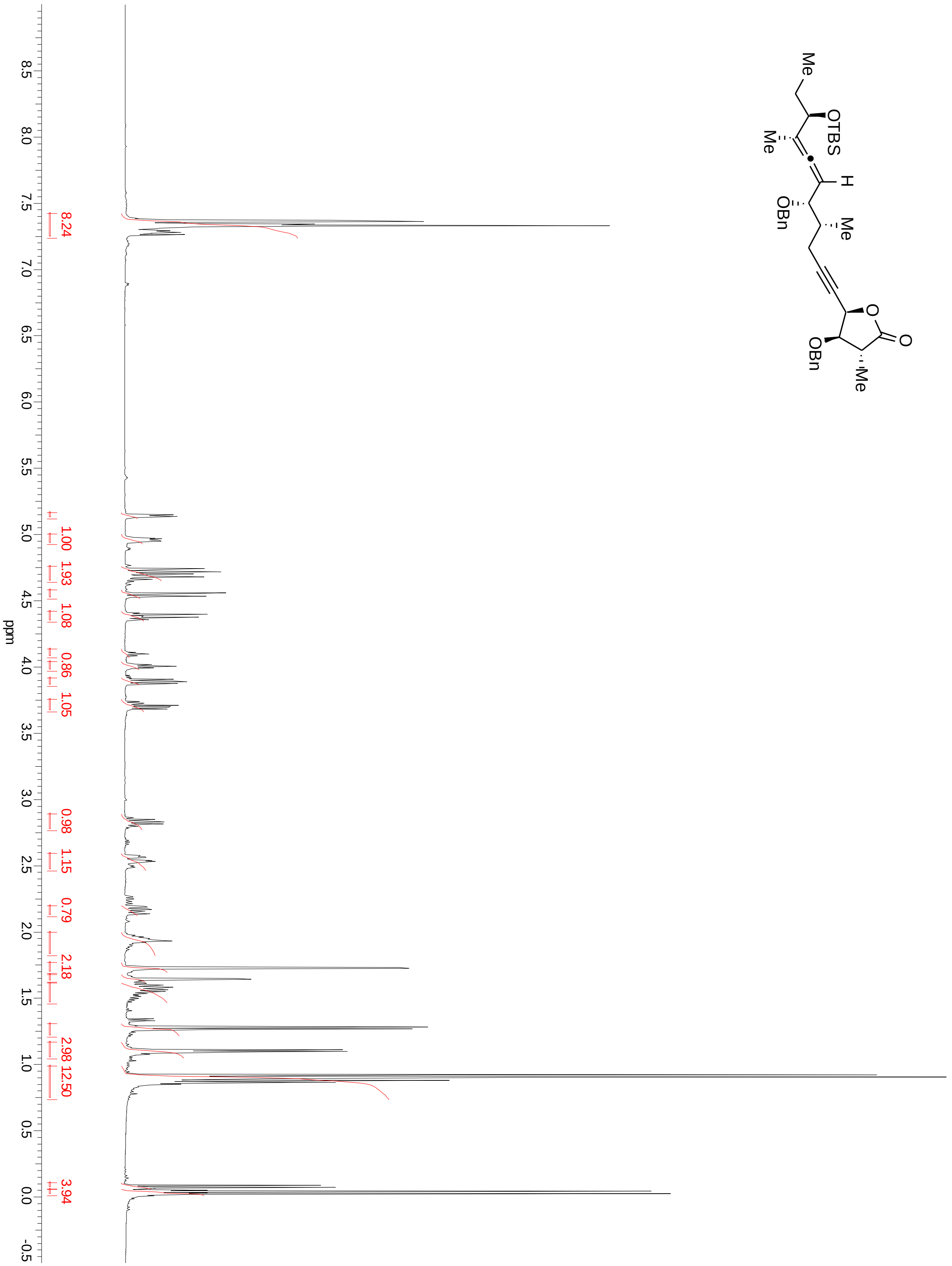
1.0
0.5
0.0
-0.5
ppm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8.5
8.0
7.5
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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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2.0
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0.0
-0.5
ppm
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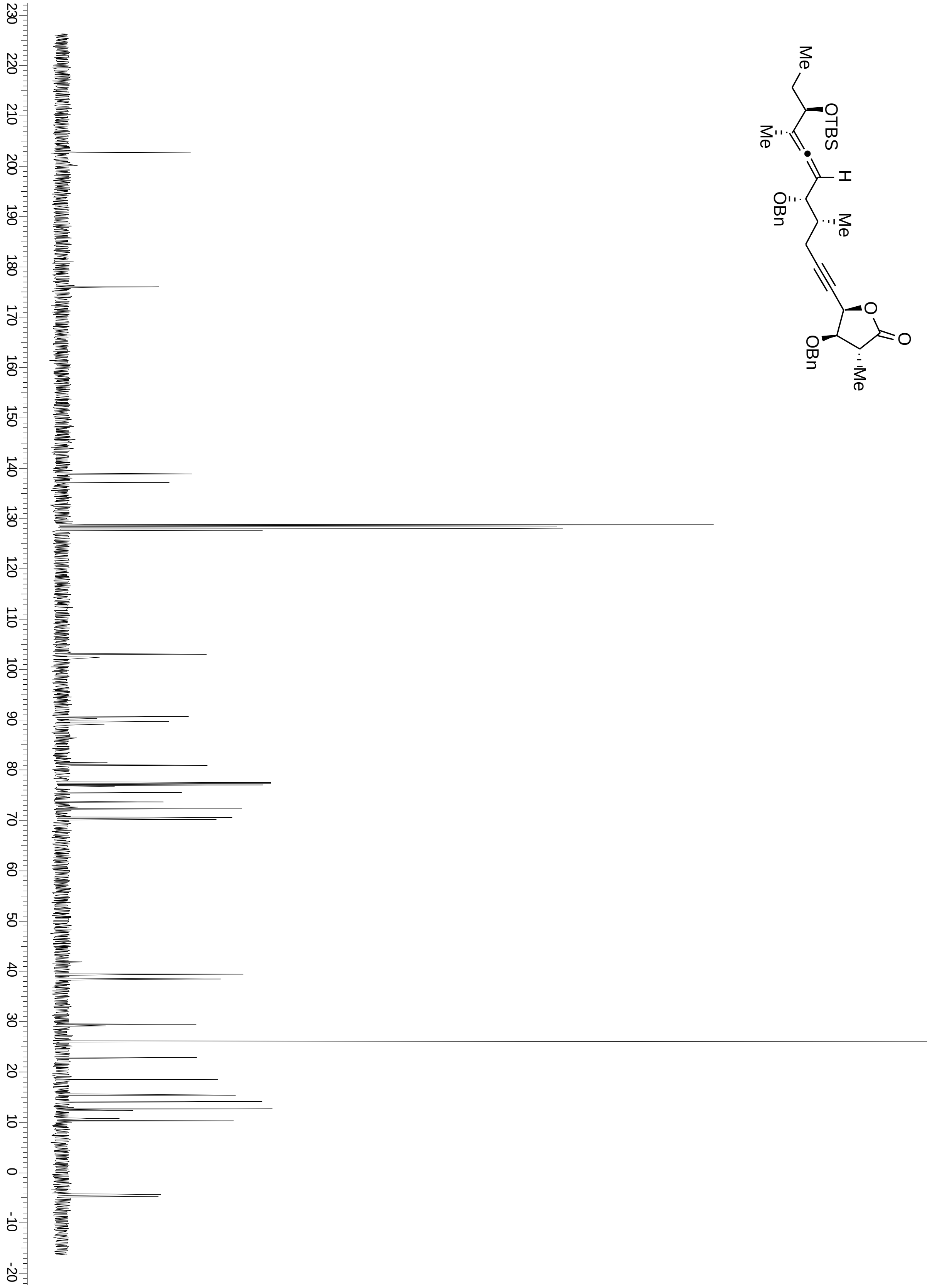
3.94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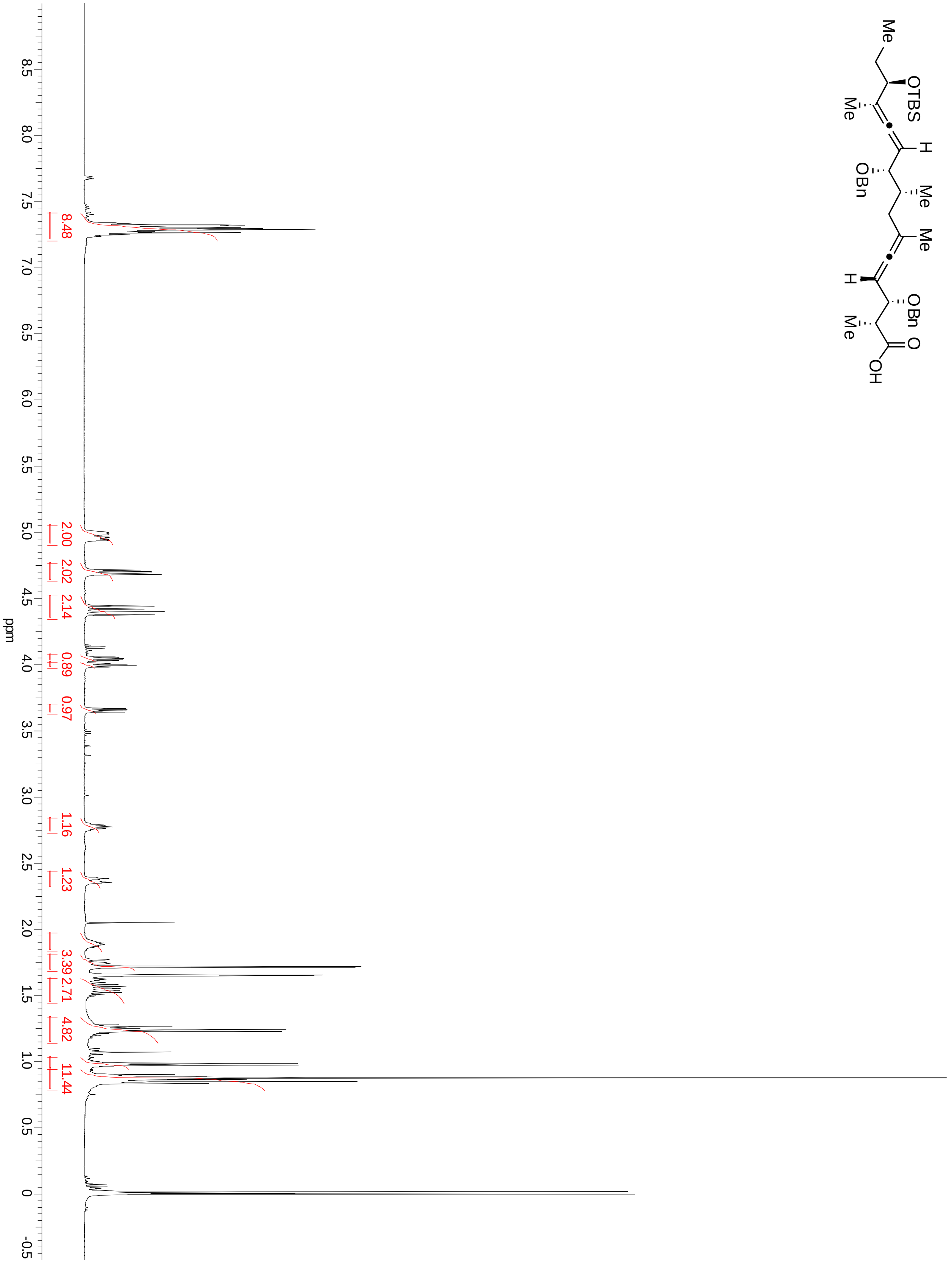
-20
ppm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OTBS
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H
OBn
Me
O
OH
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
ppm
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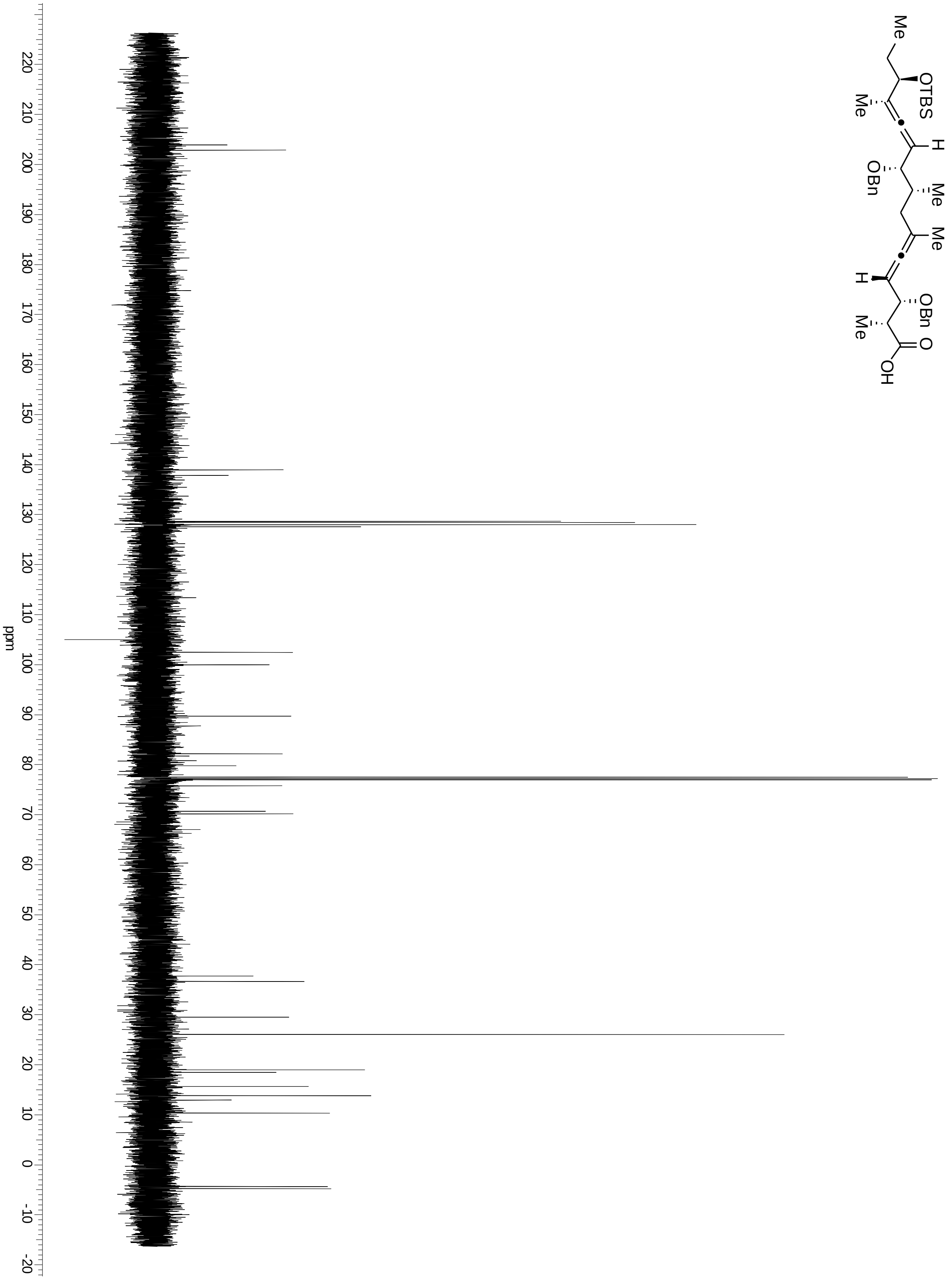
2.00
2.02
2.14
0.89
0.97
1.16
1.23
3.39
2.71
4.82
11.44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O
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OH
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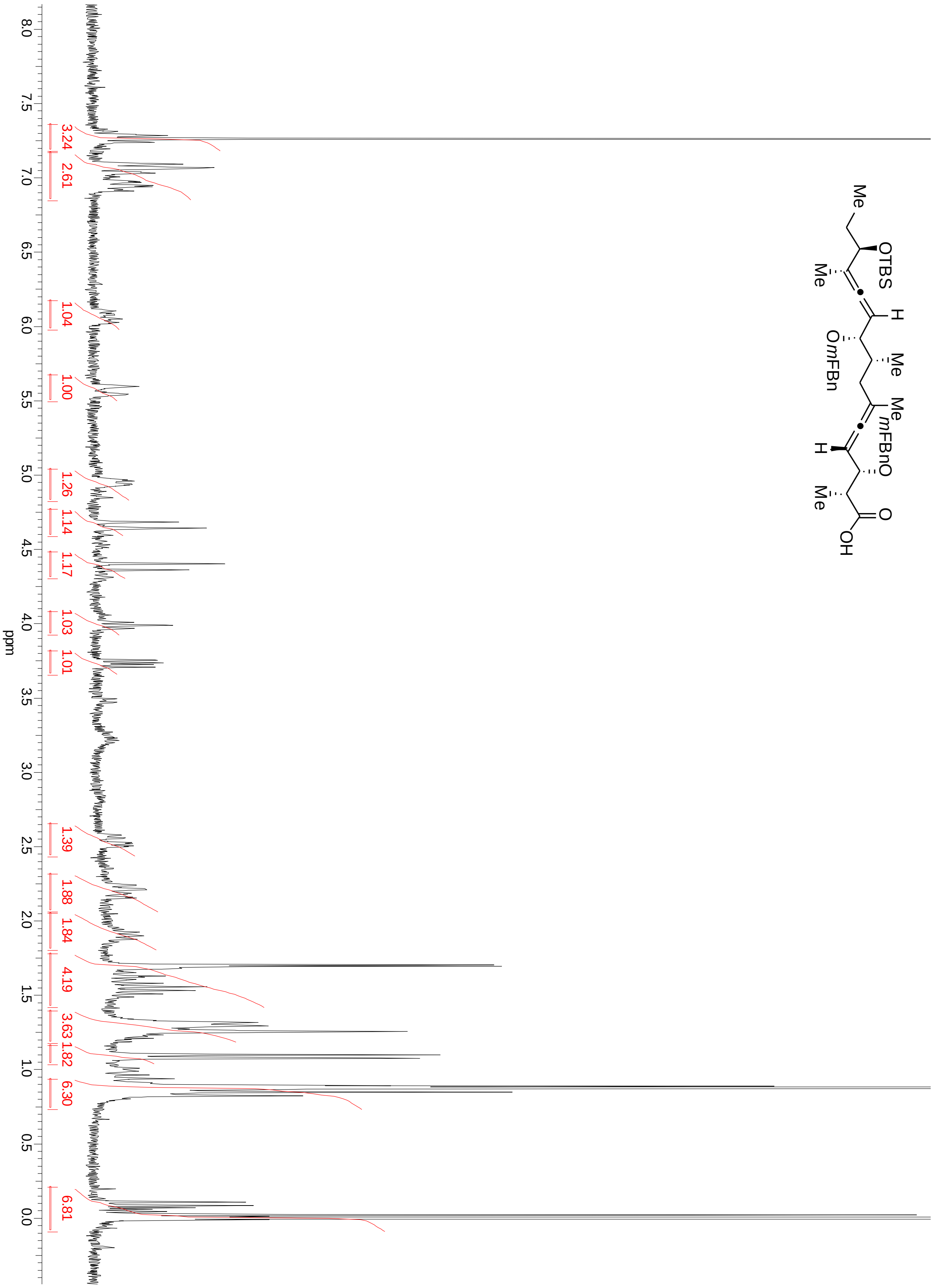
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7.0
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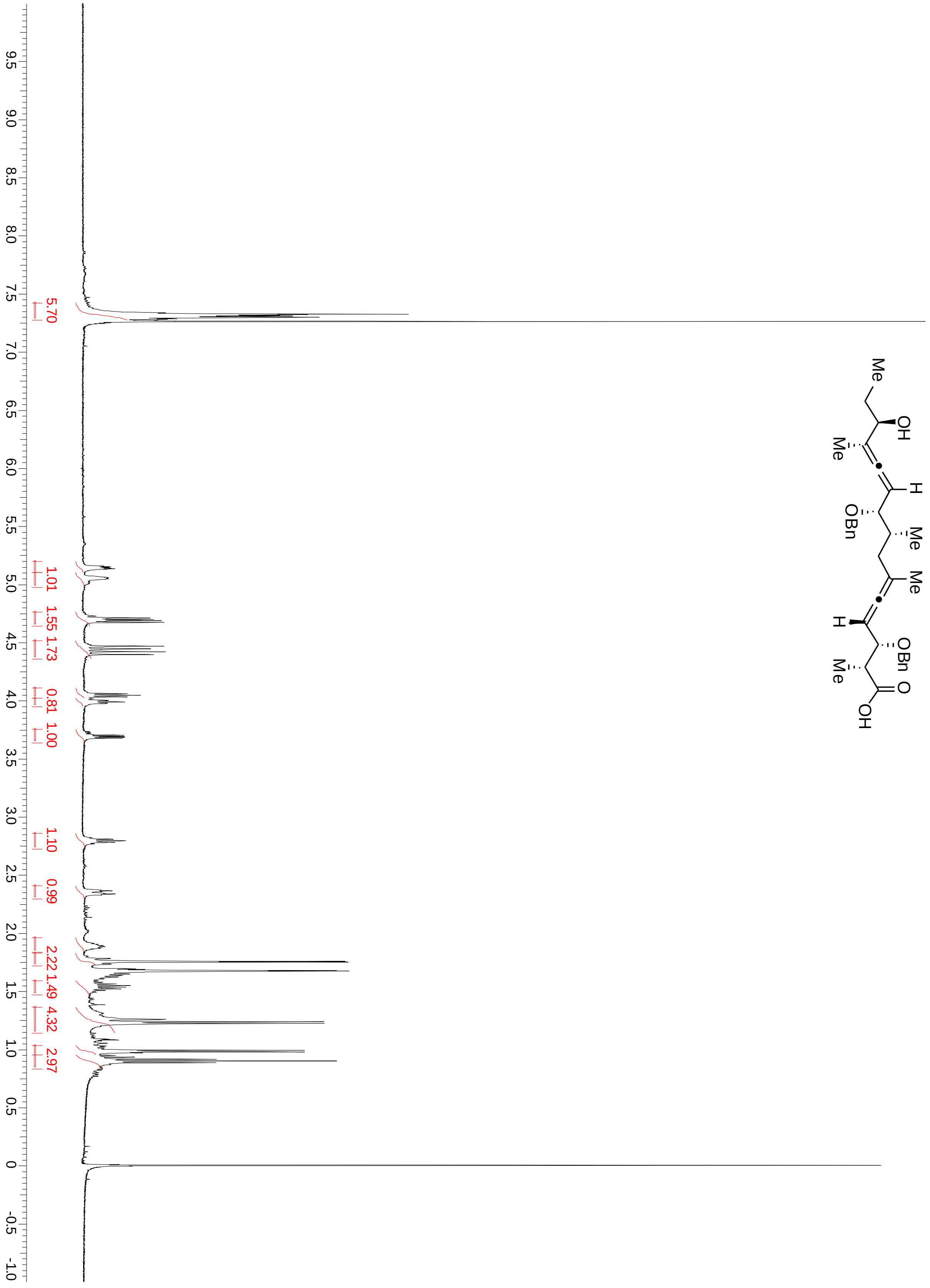
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
3.24
2.61
1.04
1.00
1.26
1.14
1.17
1.03
1.01
1.39
1.88
1.84
4.19
3.63
1.82
6.30
6.81
Me
OTBS
Me
H
O*m*FBn
Me
Me
*m*FBnO
H
Me
O
OH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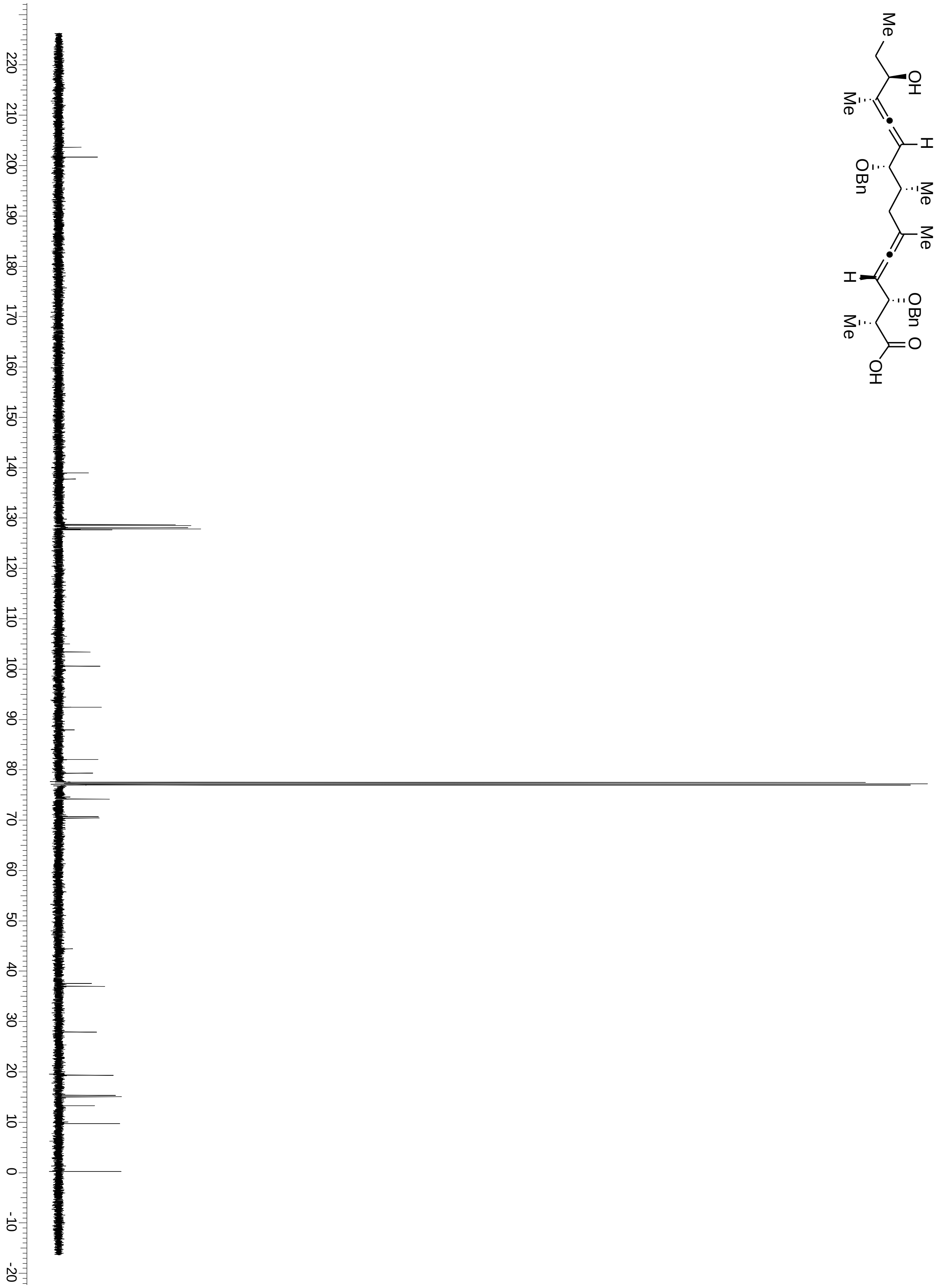
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
ppm
5.70
1.01
1.55
1.73
0.81
1.00
1.10
0.99
2.22
1.49
4.32
2.97
Me
OH
Me
H
OBn
Me
Me
H
OBn
O
Me
OH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OH
Me
H
OBn
Me
Me
H
OBn
O
Me
OH
220
210
200
190
180
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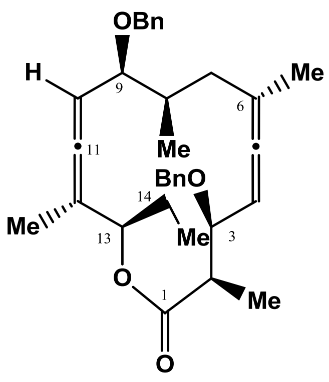
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm



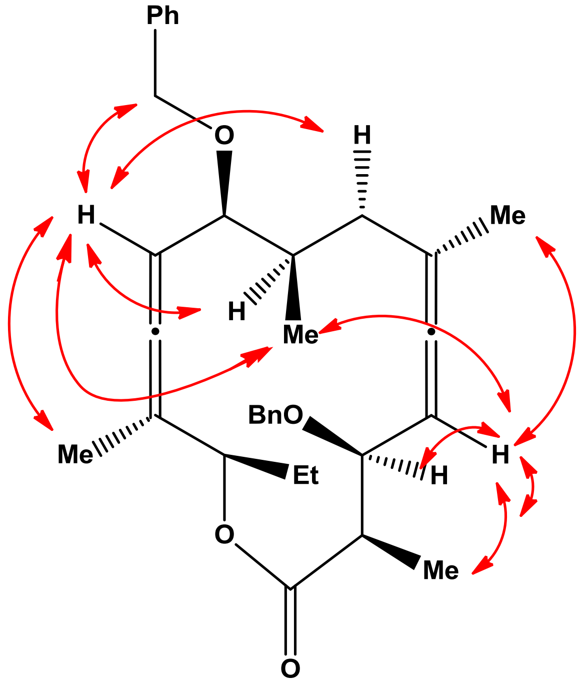
1H NMR chemical shifts (δ/ppm) with coupling constant (J/Hz)

2.78 (dq, $J_{H2,\ H3}$ = 8.4Hz, $J_{H2,\ 2\text{-}CH3}$ = 7.1Hz, **H2)**
1.26 (d, $J_{2\text{-}CH3,\ H2}$ = 7.1Hz, **2-CH$_3$)**
3.95 (dd, $J_{H3,\ H2}$ = 8.4Hz, $J_{H3,\ H4}$ = 4.0Hz, **H3)**
5.33 (m, $J_{H4,\ H3}$ = 4.0Hz, $J_{H4,6\text{-}CH3}$=2.9Hz,
$J_{H4,H7\alpha}$=2.7Hz, $J_{H4,\ H7\beta}$ = 3.4Hz, **H4)**
1.70 (d, **6-CH$_3$)**
1.61 (m, $J_{H7\beta,\ H8}$ = 5.4Hz, $J_{H7\beta,\ H7\alpha}$ = 15.4Hz,
$J_{H4,\ H7\beta}$ = 3.4Hz, **H7β)**
2.18 (m, $J_{H7\alpha,\ H8}$ = 5.7Hz, $J_{H7\alpha,\ H7\beta}$ = 15.4Hz,
$J_{H4,H7\alpha}$=2.7Hz, **H7α)**
1.96 (m, $J_{H7\alpha,\ H8}$ = 5.7Hz, $J_{H7\beta,\ H8}$ = 5.4Hz,
$J_{H8,\ 8\text{-}CH3}$ = 6.7Hz, $J_{H8,\ H9}$ = 7.2Hz, **H8)**
1.05 (d, $J_{8\text{-}CH3,\ H8}$ = 6.7Hz, **8-CH$_3$)**
3.74 (dd, $J_{H9,\ H8}$ = 7.2Hz, $J_{H9,\ H10}$= 7.8Hz, **H9)**
5.13 (m, $J_{H9,H10}$= 7.8Hz, $J_{H10,12\text{-}CH3}$ = 2.9Hz,
$J_{H10\text{-}H13}$=1.1Hz, **H10)**
1.71 (d, $J_{H10,12\text{-}CH3}$ = 2.9Hz, **12-CH$_3$)**
5.26 (dt, $J_{H10\text{-}H13}$=1.1Hz, $J_{H13,\ H14}$ = 6.8Hz, **H13)**
1.69, 1.67 (m, **14-CH$_2$)**
0.90 (t, $J_{14\text{-}CH3,\ H14}$ = 7.5Hz, **14-CH$_3$)**
4.64 (d, J=11.7Hz, **3-Bn-CH$_2$a)**
4.48 (d, J=11.7Hz, **3-Bn-CH$_2$b)**
4.54 (d, J=11.9Hz, **9-Bn-CH$_2$a)**
4.34 (d, J=11.9Hz, **9-Bn-CH$_2$b)**
7.22-7.35 (two phenyl)

13C NMR chemical shift (δ/ppm)

173.8 ----- C1
44.5 ----- C2
77.2 ----- C3
90.5 ----- C4
201.2 ----- C5
102.4 ----- C6
37.7 ----- C7
35.9 ----- C8
81.6 ----- C9
91.7 ----- C10
203.4 ----- C11
99.0 ----- C12
75.5 ----- C13
24.9 ----- C14
15.2 ----- 2-CH$_3$
20.2 ----- 6-CH$_3$
17.6 ----- 8-CH$_3$
14.0 ----- 12-CH$_3$
9.6 ----- 14-CH$_3$

NOESY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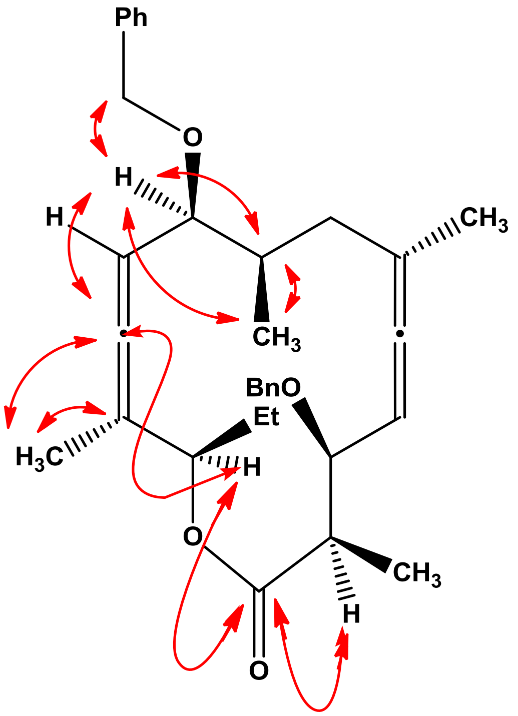
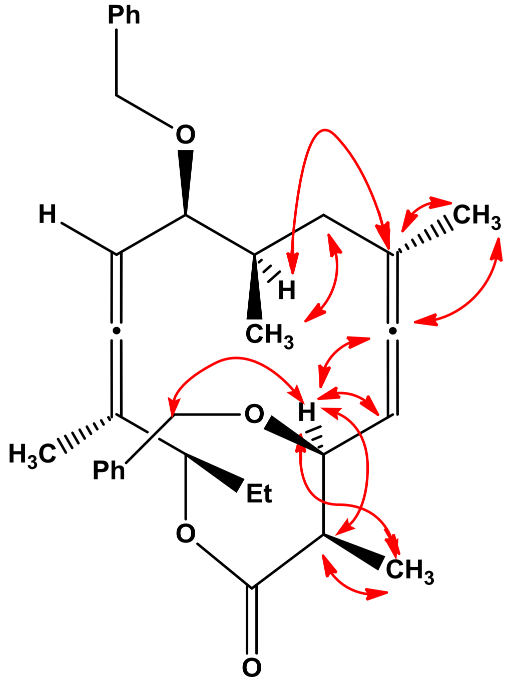
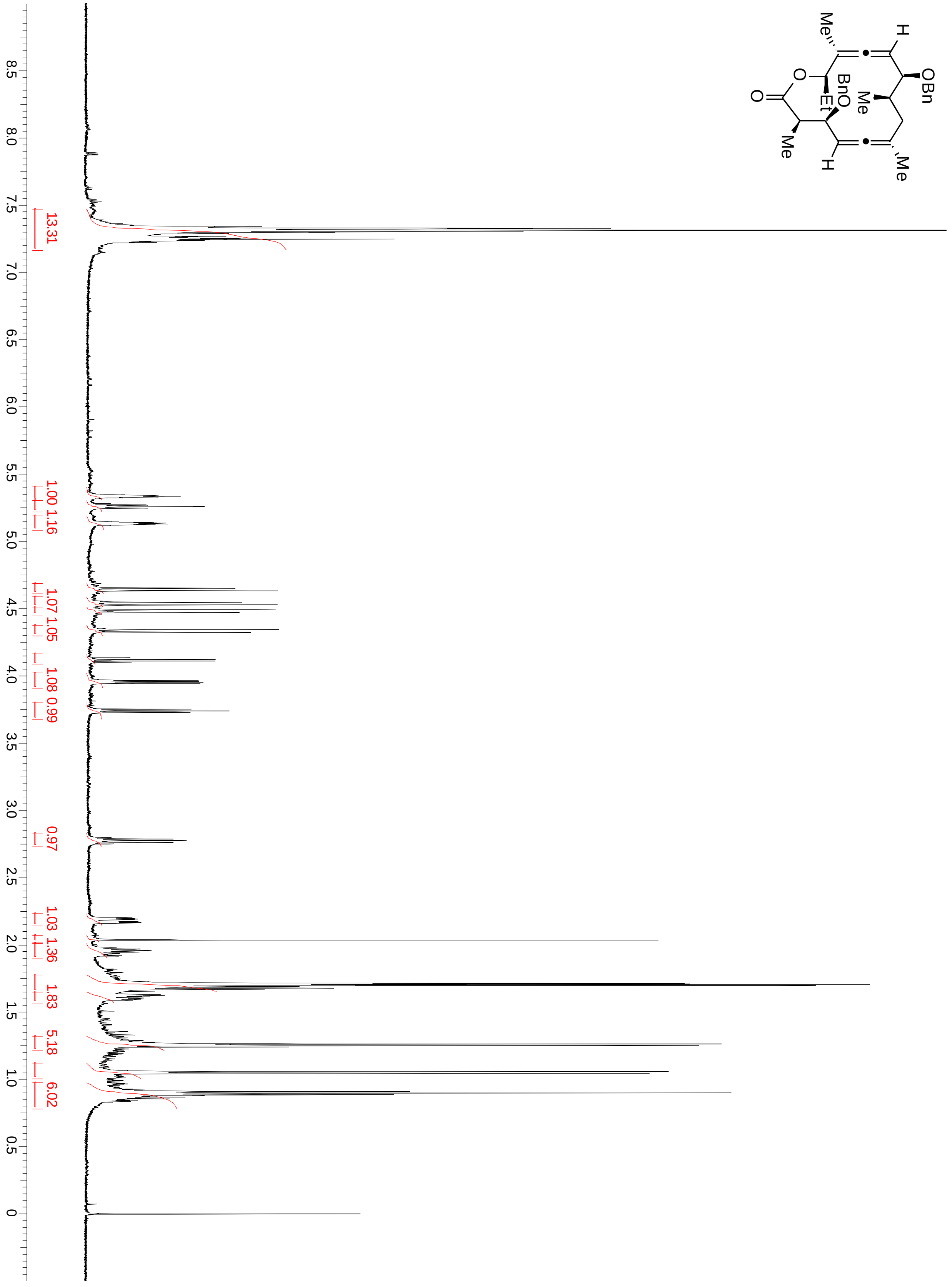
O
H
H
Me
H
Me
BnO
Et
H
H
Me
O
Me
O

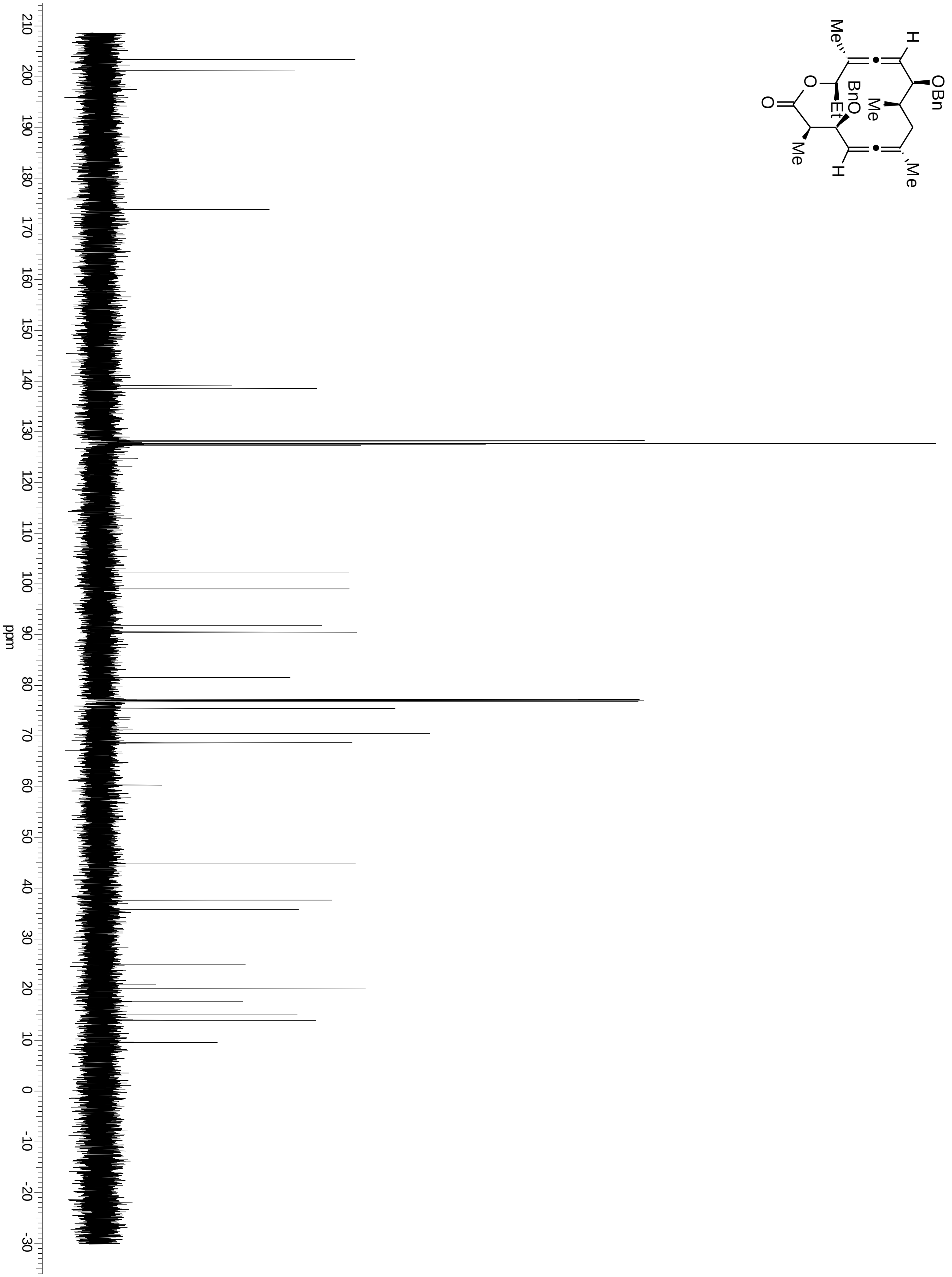
HMBC

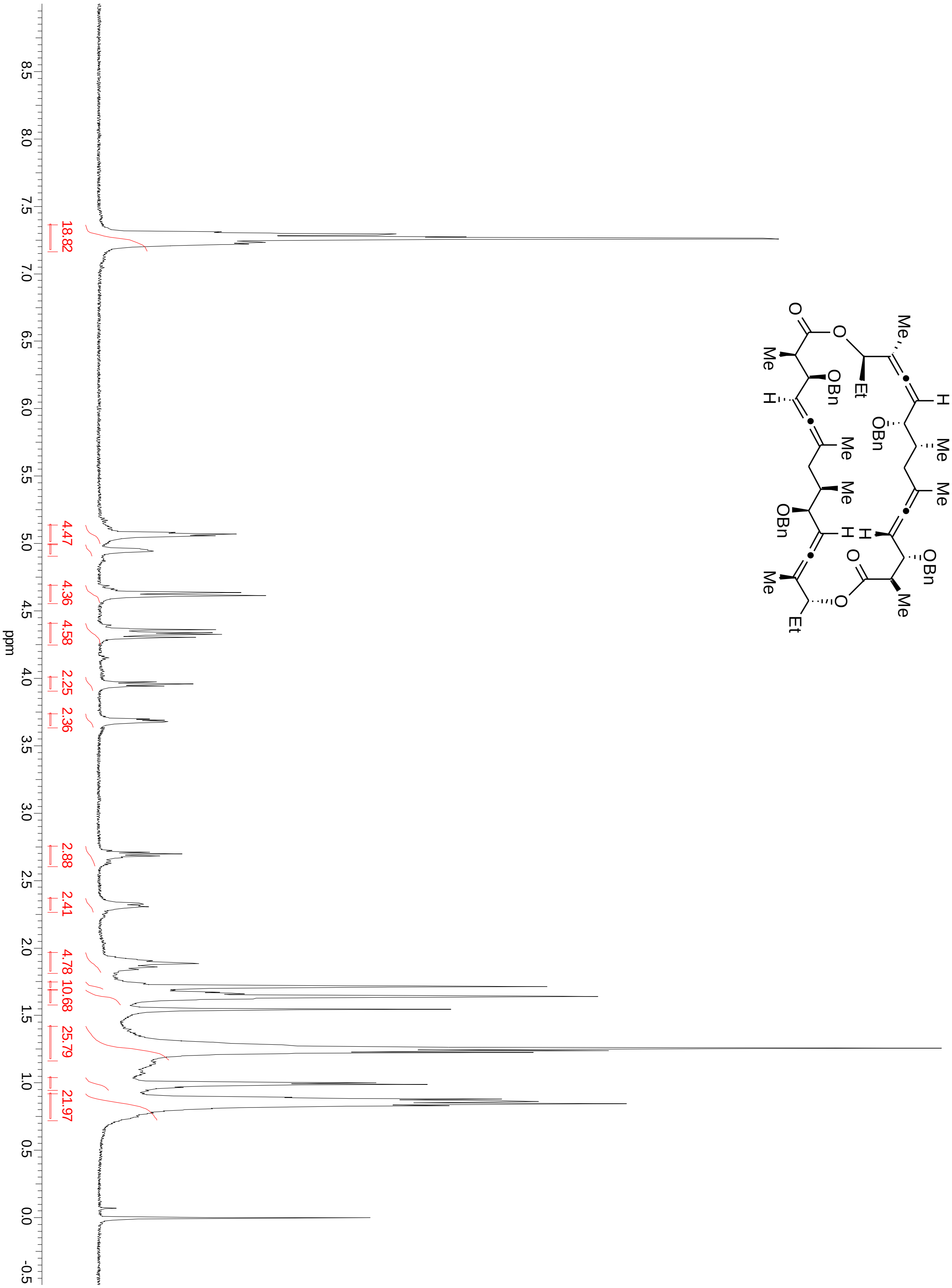

Ph
O
H
H
CH3
CH3
BnO
Et
H3C
H
O
CH3
H
O

HMBC


Ph
O
H
CH3
H
CH3
O
H
H3C
Ph
Et
O
CH3
O


H
OBn
Me
Me''
BnO
O
Et
O
Me
H
Me
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ppm
13.31
1.00
1.16
1.07
1.05
1.08
0.99
0.97
1.03
1.36
1.83
5.18
6.02


H
OBn
Me
BnO
Et
O
O
Me
H
Me
Me
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
ppm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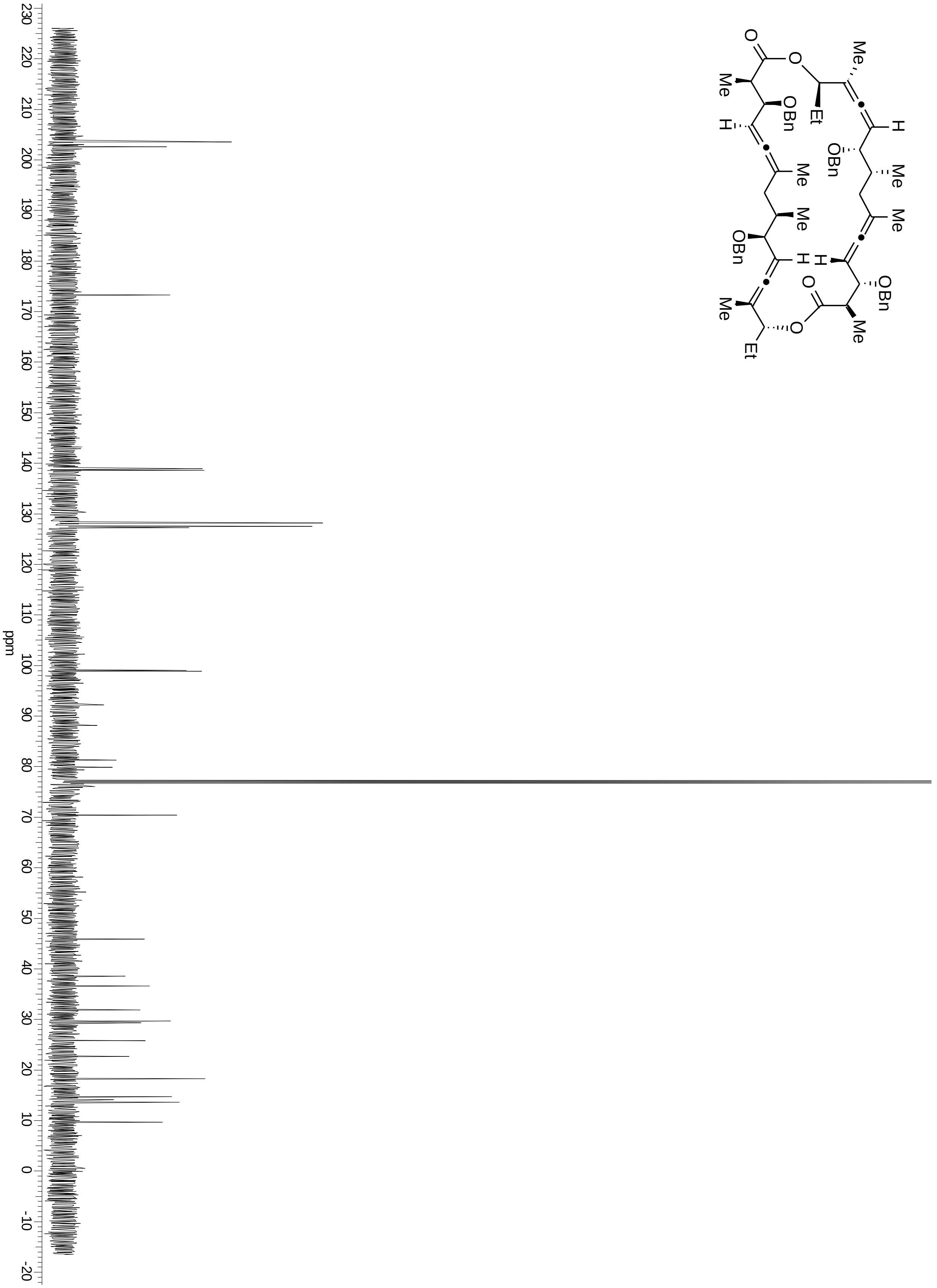
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm
18.82
4.47
4.36
4.58
2.25
2.36
2.88
2.41
4.78
10.68
25.79
21.97
Me
OBn
H
Et
O
Me
OBn
Me
Me
H
OBn
O
Me
Et
O
Me
OBn
H
Me
Me
H
OBn
O
Me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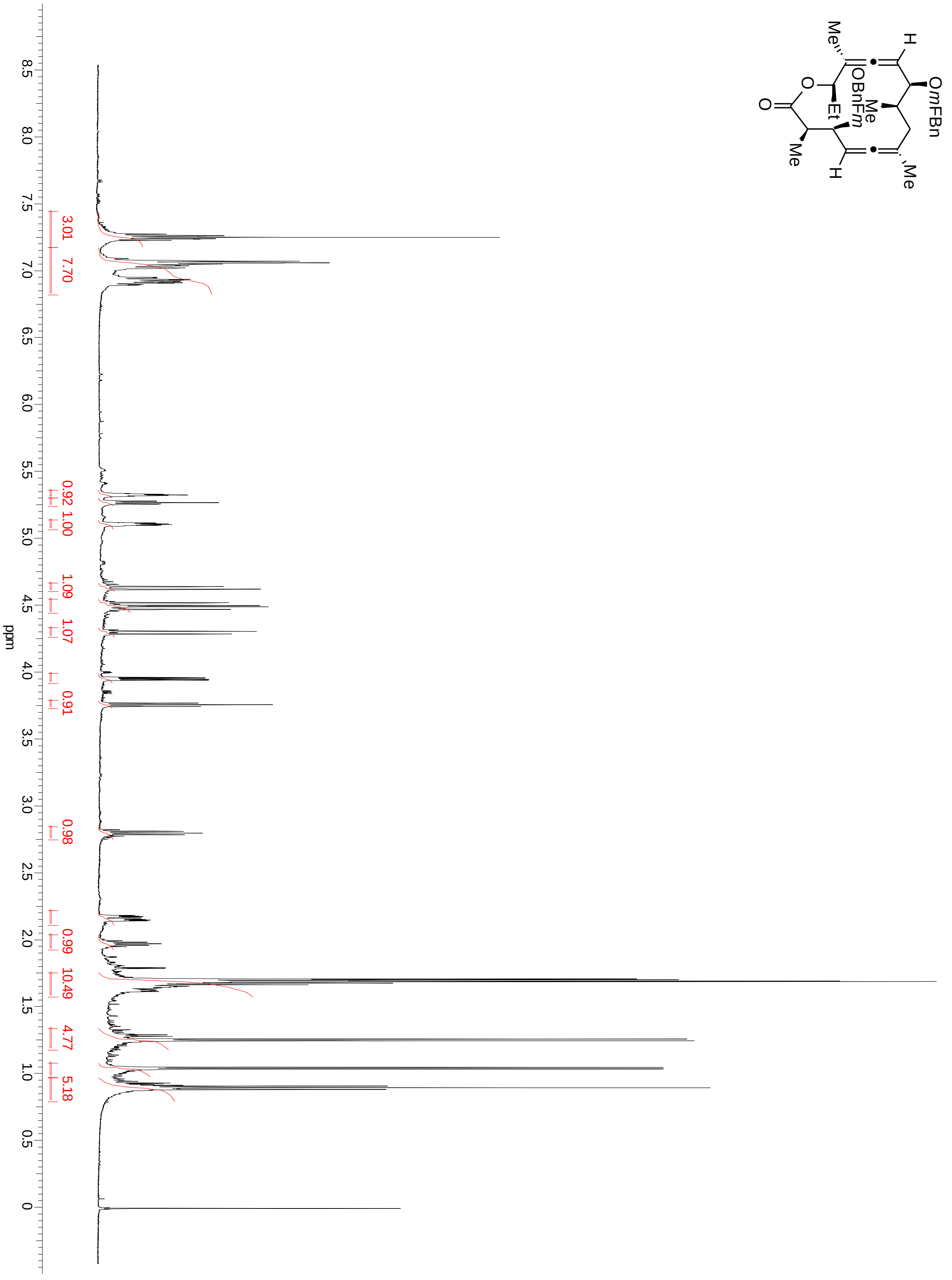
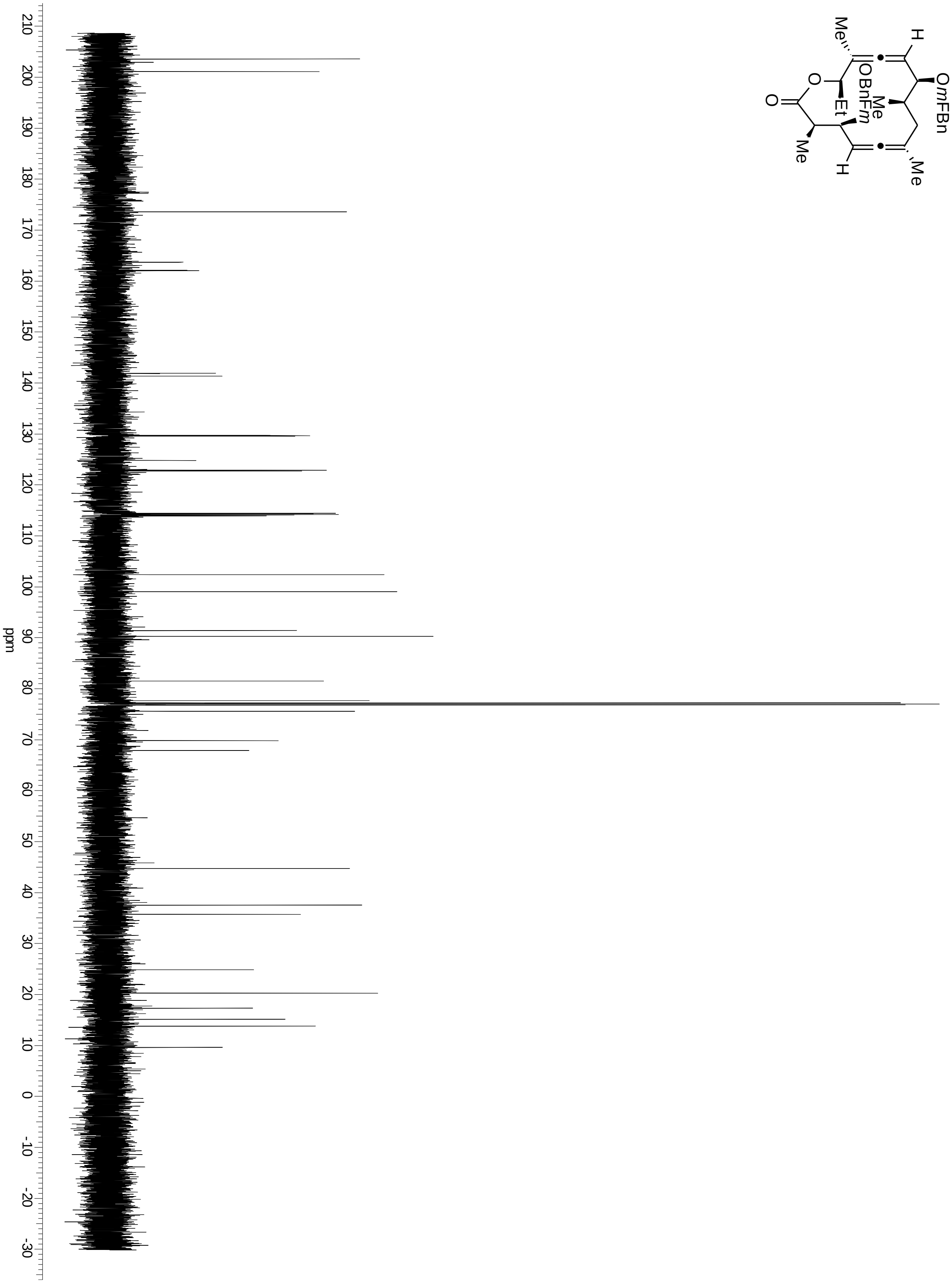
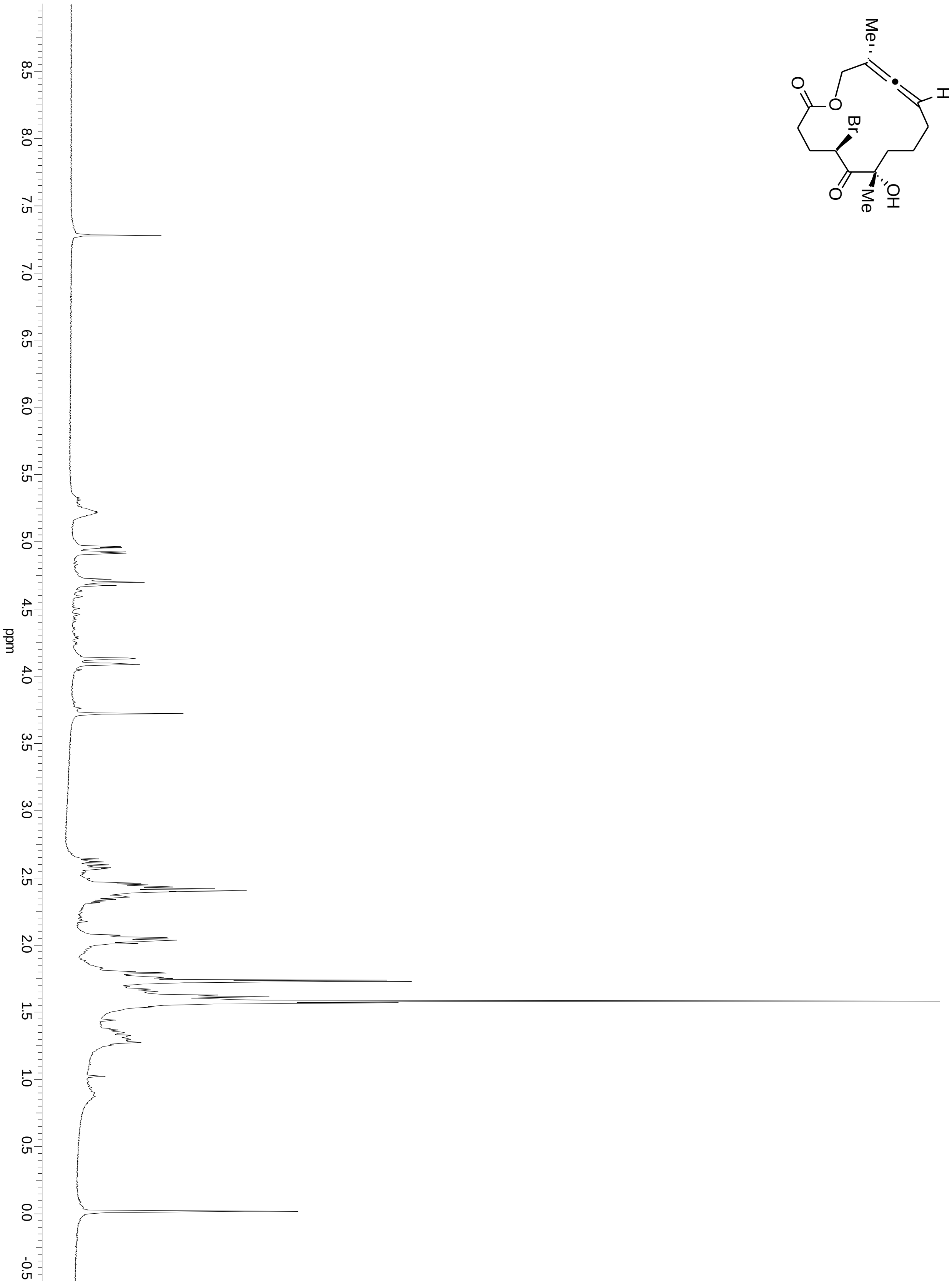
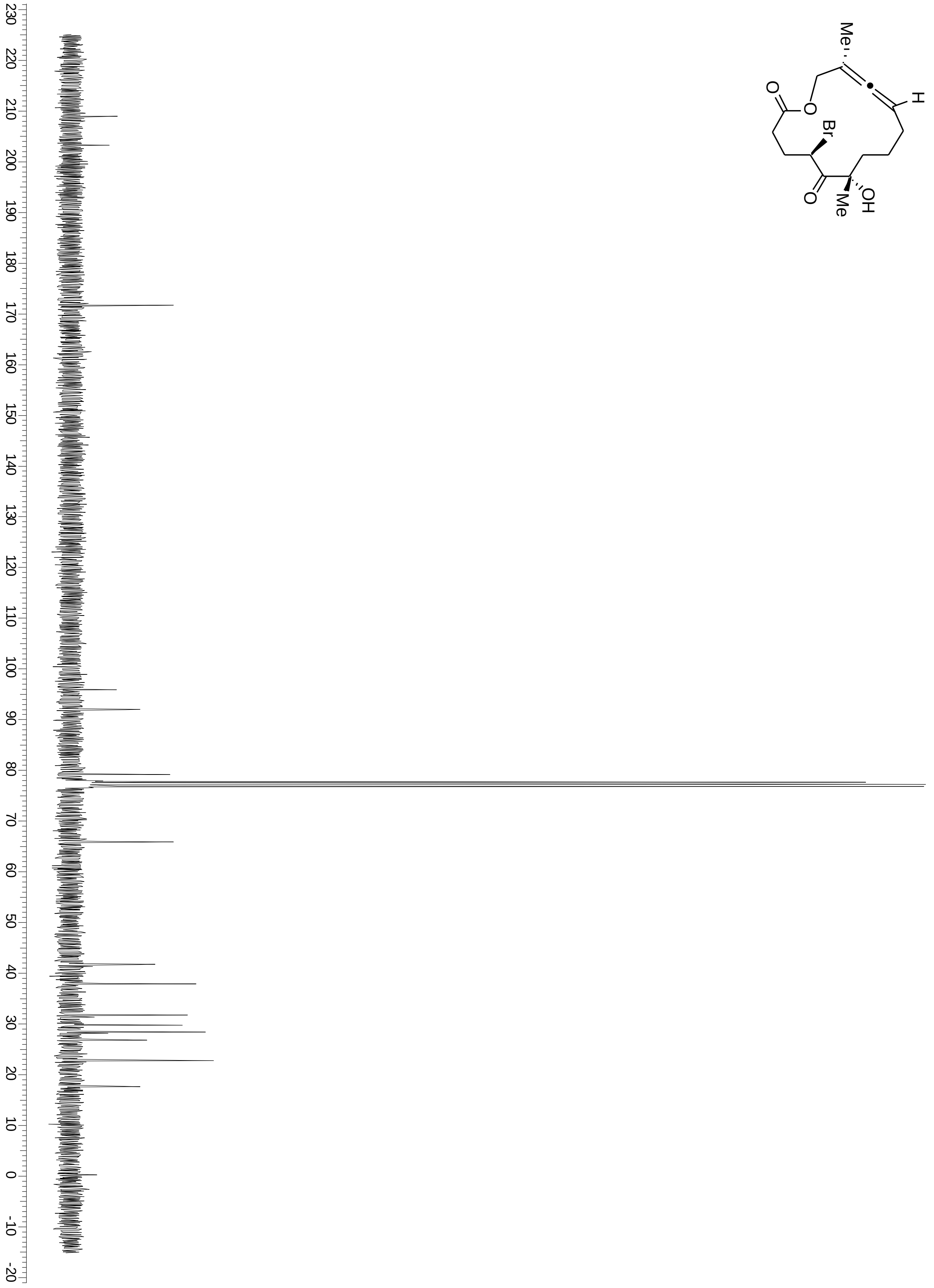
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm
O
O
Me
OBn
Et
H
Me
OBn
Et


H
OmFBn
Me
OBnFm
Et
Me
H
Me
O
O
Me
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ppm
3.01
7.70
0.92
1.00
1.09
1.07
0.91
0.98
0.99
10.49
4.77
5.18

H
OmFBn
Me
OBnFm
Me
Et
O
O
Me
H
Me
Me


210
200
190
180
170
160
150
140
130
120
110
100
90
ppm
80
70
60
50
40
30
20
10
0
-10
-20
-30


Me
H
O
O
Br
OH
Me
O
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm


Me
H
O
O
Br
O
OH
Me
ppm
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20

X-ray structure of 4.4

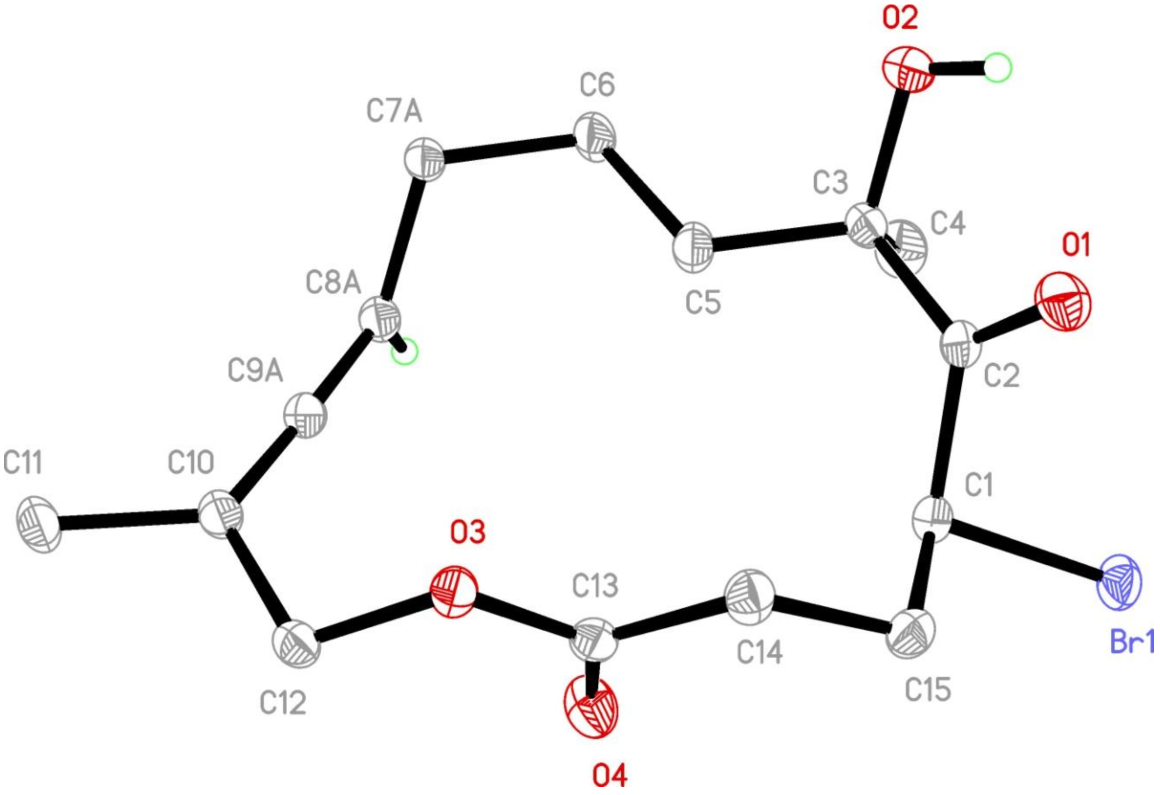

O2
C6
C7A
C3
C4
O1
C8A
C5
C2
C9A
C11
C10
C1
O3
C13
C14
Br1
C12
C15
O4

Table 1. Crystal data and structure refinement for brlact.

Identification code	brlact	
Empirical formula	$C_{15}H_{21}BrO_4$	
Formula weight	345.23	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 8.3053(4) Å	α= 107.384(1)°.
	b = 9.5418(5) Å	β= 100.426(1)°.
	c = 10.3999(5) Å	γ = 98.038(1)°.
Volume	756.85(7) Å^3	
Z	2	
Density (calculated)	1.515 Mg/m^3	
Absorption coefficient	2.726 mm^{-1}	
F(000)	356	
Crystal size	0.18 x 0.12 x 0.07 mm^3	

Theta range for data collection	2.11 to 30.55°.
Index ranges	-11<=h<=11, -13<=k<=13, -14<=l<=14
Reflections collected	9028
Independent reflections	4557 [R(int) = 0.0119]
Completeness to theta = 30.55°	98.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.746 and 0.646
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4557 / 125 / 212
Goodness-of-fit on F^2	1.001
Final R indices [I>2sigma(I)]	R1 = 0.0246, wR2 = 0.0612
R indices (all data)	R1 = 0.0264, wR2 = 0.0621
Largest diff. peak and hole	0.612 and -0.236 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3)
for brlact. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	3264(1)	2412(1)	122(1)	21(1)
O(1)	1630(1)	-303(1)	1048(1)	22(1)
O(2)	-501(1)	516(1)	2581(1)	26(1)
O(3)	6668(1)	1709(1)	5678(1)	16(1)
O(4)	6845(1)	3468(1)	4632(1)	24(1)
C(1)	3667(2)	1987(1)	1875(1)	16(1)
C(2)	2034(2)	984(1)	1828(1)	16(1)
C(3)	937(2)	1632(2)	2793(1)	18(1)
C(4)	404(2)	2989(2)	2475(2)	25(1)
C(5)	1941(2)	2013(2)	4290(1)	20(1)
C(6)	977(2)	2614(2)	5385(1)	22(1)
C(7A)	2032(2)	2893(2)	6848(1)	17(1)
C(8A)	3664(2)	3980(2)	7151(1)	17(1)
C(9A)	5142(2)	3708(2)	7538(1)	17(1)

C(7B)	2299(18)	3904(17)	6797(17)	11(2)
C(8B)	3221(16)	2817(16)	7232(17)	13(2)
C(9B)	4857(17)	3010(20)	7556(18)	13(2)
C(10)	6606(2)	3433(1)	7972(1)	17(1)
C(11)	7441(2)	3855(2)	9485(1)	21(1)
C(12)	7643(2)	2752(1)	6993(1)	17(1)
C(13)	6425(2)	2186(1)	4572(1)	16(1)
C(14)	5633(2)	856(1)	3282(1)	18(1)
C(15)	5162(2)	1228(2)	1952(1)	20(1)

Table 3. Bond lengths [Å] and angles [°] for brlact.

Br(1)-C(1)	1.9646(12)	C(7A)-C(8A)	1.5072(18)
O(1)-C(2)	1.2121(15)	C(7A)-H(7A)	0.9700
O(2)-C(3)	1.4210(15)	C(7A)-H(7B)	0.9700
O(2)-H(2)	0.8200	C(8A)-C(9A)	1.3069(18)
O(3)-C(13)	1.3514(15)	C(8A)-H(8A)	0.9300
O(3)-C(12)	1.4414(15)	C(9A)-C(10)	1.3090(17)
O(4)-C(13)	1.2037(16)	C(7B)-C(8B)	1.498(9)
C(1)-C(15)	1.5238(17)	C(7B)-H(7B1)	0.9700
C(1)-C(2)	1.5311(17)	C(7B)-H(7B2)	0.9700
C(1)-H(1)	0.9800	C(8B)-C(9B)	1.312(9)
C(2)-C(3)	1.5284(17)	C(8B)-H(8B)	0.9300
C(3)-C(4)	1.5309(19)	C(9B)-C(10)	1.402(14)
C(3)-C(5)	1.5356(17)	C(10)-C(11)	1.5058(17)
C(4)-H(4A)	0.9600	C(10)-C(12)	1.5089(17)
C(4)-H(4B)	0.9600	C(11)-H(11A)	0.9600
C(4)-H(4C)	0.9600	C(11)-H(11B)	0.9600
C(5)-C(6)	1.5282(18)	C(11)-H(11C)	0.9600
C(5)-H(5A)	0.9700	C(12)-H(12A)	0.9700
C(5)-H(5B)	0.9700	C(12)-H(12B)	0.9700
C(6)-C(7A)	1.5375(18)	C(13)-C(14)	1.5069(18)
C(6)-C(7B)	1.684(16)	C(14)-C(15)	1.5261(18)
C(6)-H(6A)	0.9700	C(14)-H(14A)	0.9700
C(6)-H(6B)	0.9700	C(14)-H(14B)	0.9700

C(15)-H(15A)	0.9700	C(15)-H(15B)	0.9700
C(3)-O(2)-H(2)	109.5	C(7B)-C(6)-H(6A)	135.4
C(13)-O(3)-C(12)	118.64(10)	C(5)-C(6)-H(6B)	109.5
C(15)-C(1)-C(2)	113.30(10)	C(7A)-C(6)-H(6B)	109.5
C(15)-C(1)-Br(1)	108.49(8)	C(7B)-C(6)-H(6B)	77.7
C(2)-C(1)-Br(1)	105.02(8)	H(6A)-C(6)-H(6B)	108.1
C(15)-C(1)-H(1)	110.0	C(8A)-C(7A)-C(6)	111.40(11)
C(2)-C(1)-H(1)	110.0	C(8A)-C(7A)-H(7A)	109.3
Br(1)-C(1)-H(1)	110.0	C(6)-C(7A)-H(7A)	109.3
O(1)-C(2)-C(3)	120.88(11)	C(8A)-C(7A)-H(7B)	109.3
O(1)-C(2)-C(1)	120.43(11)	C(6)-C(7A)-H(7B)	109.3
C(3)-C(2)-C(1)	118.69(10)	H(7A)-C(7A)-H(7B)	108.0
O(2)-C(3)-C(2)	109.26(10)	C(9A)-C(8A)-C(7A)	124.78(13)
O(2)-C(3)-C(4)	109.77(11)	C(9A)-C(8A)-H(8A)	117.6
C(2)-C(3)-C(4)	109.37(11)	C(7A)-C(8A)-H(8A)	117.6
O(2)-C(3)-C(5)	107.37(11)	C(8A)-C(9A)-C(10)	177.73(14)
C(2)-C(3)-C(5)	107.62(10)	C(8B)-C(7B)-C(6)	95.7(10)
C(4)-C(3)-C(5)	113.36(11)	C(8B)-C(7B)-H(7B1)	112.6
C(3)-C(4)-H(4A)	109.5	C(6)-C(7B)-H(7B1)	112.6
C(3)-C(4)-H(4B)	109.5	C(8B)-C(7B)-H(7B2)	112.6
H(4A)-C(4)-H(4B)	109.5	C(6)-C(7B)-H(7B2)	112.6
C(3)-C(4)-H(4C)	109.5	H(7B1)-C(7B)-H(7B2)	110.1
H(4A)-C(4)-H(4C)	109.5	C(9B)-C(8B)-C(7B)	124.0(13)
H(4B)-C(4)-H(4C)	109.5	C(9B)-C(8B)-H(8B)	118.0
C(6)-C(5)-C(3)	113.94(10)	C(7B)-C(8B)-H(8B)	118.0
C(6)-C(5)-H(5A)	108.8	C(8B)-C(9B)-C(10)	172.0(18)
C(3)-C(5)-H(5A)	108.8	C(9A)-C(10)-C(9B)	28.9(8)
C(6)-C(5)-H(5B)	108.8	C(9A)-C(10)-C(11)	122.87(12)
C(3)-C(5)-H(5B)	108.8	C(9B)-C(10)-C(11)	118.9(7)
H(5A)-C(5)-H(5B)	107.7	C(9A)-C(10)-C(12)	122.57(11)
C(5)-C(6)-C(7A)	110.75(10)	C(9B)-C(10)-C(12)	119.4(7)
C(5)-C(6)-C(7B)	109.6(5)	C(11)-C(10)-C(12)	114.46(10)
C(7A)-C(6)-C(7B)	35.0(5)	C(10)-C(11)-H(11A)	109.5
C(5)-C(6)-H(6A)	109.5	C(10)-C(11)-H(11B)	109.5
C(7A)-C(6)-H(6A)	109.5	H(11A)-C(11)-H(11B)	109.5

C(10)-C(11)-H(11C)	109.5	C(13)-C(14)-C(15)	115.32(11)
H(11A)-C(11)-H(11C)	109.5	C(13)-C(14)-H(14A)	108.4
H(11B)-C(11)-H(11C)	109.5	C(15)-C(14)-H(14A)	108.4
O(3)-C(12)-C(10)	113.95(10)	C(13)-C(14)-H(14B)	108.4
O(3)-C(12)-H(12A)	108.8	C(15)-C(14)-H(14B)	108.4
C(10)-C(12)-H(12A)	108.8	H(14A)-C(14)-H(14B)	107.5
O(3)-C(12)-H(12B)	108.8	C(1)-C(15)-C(14)	113.99(10)
C(10)-C(12)-H(12B)	108.8	C(1)-C(15)-H(15A)	108.8
H(12A)-C(12)-H(12B)	107.7	C(14)-C(15)-H(15A)	108.8
O(4)-C(13)-O(3)	124.17(12)	C(1)-C(15)-H(15B)	108.8
O(4)-C(13)-C(14)	126.73(12)	C(14)-C(15)-H(15B)	108.8
O(3)-C(13)-C(14)	109.04(10)	H(15A)-C(15)-H(15B)	107.6

Table 4. Anisotropic displacement parameters (Å^{2}x 10^3) for brlact. The anisotropic displacement factor exponent takes the form: $-2\pi^2$[h^2 a*^{2}U^{11} + ... + 2 h k a* b* U^{12}]

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	26(1)	22(1)	15(1)	8(1)	2(1)	0(1)
O(1)	24(1)	17(1)	22(1)	2(1)	5(1)	1(1)
O(2)	19(1)	33(1)	20(1)	2(1)	6(1)	-5(1)
O(3)	17(1)	17(1)	14(1)	5(1)	4(1)	3(1)
O(4)	26(1)	20(1)	23(1)	10(1)	0(1)	2(1)
C(1)	17(1)	18(1)	12(1)	4(1)	3(1)	2(1)
C(2)	16(1)	18(1)	12(1)	5(1)	2(1)	3(1)
C(3)	13(1)	22(1)	14(1)	3(1)	2(1)	2(1)
C(4)	23(1)	29(1)	24(1)	8(1)	4(1)	12(1)
C(5)	15(1)	30(1)	13(1)	4(1)	3(1)	7(1)
C(6)	13(1)	35(1)	14(1)	4(1)	3(1)	7(1)
C(7A)	12(1)	25(1)	14(1)	5(1)	3(1)	4(1)
C(8A)	15(1)	20(1)	14(1)	4(1)	3(1)	4(1)
C(9A)	16(1)	18(1)	13(1)	3(1)	3(1)	2(1)

C(7B)	11(2)	12(2)	11(2)	4(1)	3(1)	2(1)
C(8B)	13(2)	13(2)	12(2)	4(1)	3(1)	3(1)
C(9B)	13(2)	14(2)	13(2)	4(1)	3(1)	3(1)
C(10)	15(1)	20(1)	14(1)	4(1)	2(1)	4(1)
C(11)	18(1)	27(1)	16(1)	5(1)	0(1)	6(1)
C(12)	13(1)	20(1)	16(1)	5(1)	2(1)	4(1)
C(13)	13(1)	21(1)	16(1)	7(1)	4(1)	6(1)
C(14)	18(1)	20(1)	17(1)	4(1)	4(1)	7(1)
C(15)	18(1)	26(1)	16(1)	6(1)	6(1)	6(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10 ^{3}) for brlact.

	x	y	z	U(eq)
H(2)	-649	-130	1821	40
H(1)	3899	2923	2660	20
H(4A)	-375	3336	3014	37
H(4B)	-117	2704	1507	37
H(4C)	1371	3778	2707	37
H(5A)	2284	1117	4401	24
H(5B)	2945	2757	4450	24
H(6A)	-53	1896	5214	26
H(6B)	687	3545	5321	26
H(7A)	1405	3290	7526	21
H(7B)	2256	1948	6929	21
H(8A)	3617	4913	7055	20
H(7B1)	3014	4643	6568	13
H(7B2)	1725	4401	7480	13
H(8B)	2595	1952	7275	15
H(11A)	7647	2970	9690	32
H(11B)	6726	4314	10037	32

H(11C)	8483	4549	9692	32
H(12A)	8343	3553	6829	20
H(12B)	8376	2236	7435	20
H(14A)	4632	329	3427	22
H(14B)	6402	177	3156	22
H(15A)	6119	1881	1874	24
H(15B)	4912	307	1167	24

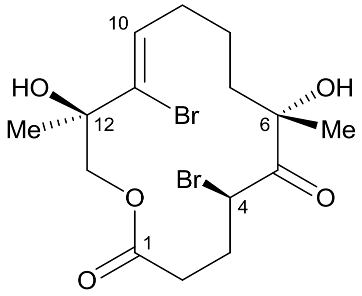
Table 6. Torsion angles [°] for brlact.

C(15)-C(1)-C(2)-O(1)	-46.99(15)
Br(1)-C(1)-C(2)-O(1)	71.25(13)
C(15)-C(1)-C(2)-C(3)	132.43(11)
Br(1)-C(1)-C(2)-C(3)	-109.33(10)
O(1)-C(2)-C(3)-O(2)	-0.42(16)
C(1)-C(2)-C(3)-O(2)	-179.84(10)
O(1)-C(2)-C(3)-C(4)	-120.59(13)
C(1)-C(2)-C(3)-C(4)	59.99(14)
O(1)-C(2)-C(3)-C(5)	115.86(13)
C(1)-C(2)-C(3)-C(5)	-63.56(14)
O(2)-C(3)-C(5)-C(6)	-59.93(15)
C(2)-C(3)-C(5)-C(6)	-177.44(11)
C(4)-C(3)-C(5)-C(6)	61.48(15)
C(3)-C(5)-C(6)-C(7A)	177.15(12)
C(3)-C(5)-C(6)-C(7B)	-145.5(5)
C(5)-C(6)-C(7A)-C(8A)	57.99(15)
C(7B)-C(6)-C(7A)-C(8A)	-36.9(9)
C(6)-C(7A)-C(8A)-C(9A)	-124.53(14)
C(7A)-C(8A)-C(9A)-C(10)	-65(4)
C(5)-C(6)-C(7B)-C(8B)	-69.2(10)
C(7A)-C(6)-C(7B)-C(8B)	29.2(6)
C(6)-C(7B)-C(8B)-C(9B)	131.0(17)
C(7B)-C(8B)-C(9B)-C(10)	32(13)
C(8A)-C(9A)-C(10)-C(9B)	63(4)
C(8A)-C(9A)-C(10)-C(11)	-28(4)
C(8A)-C(9A)-C(10)-C(12)	156(4)
C(8B)-C(9B)-C(10)-C(9A)	-28(11)
C(8B)-C(9B)-C(10)-C(11)	78(12)
C(8B)-C(9B)-C(10)-C(12)	-133(12)
C(13)-O(3)-C(12)-C(10)	100.16(12)
C(9A)-C(10)-C(12)-O(3)	-32.96(18)
C(9B)-C(10)-C(12)-O(3)	0.6(10)
C(11)-C(10)-C(12)-O(3)	150.63(11)
C(12)-O(3)-C(13)-O(4)	-6.90(17)
C(12)-O(3)-C(13)-C(14)	170.61(9)
O(4)-C(13)-C(14)-C(15)	-6.82(18)
O(3)-C(13)-C(14)-C(15)	175.75(10)
C(2)-C(1)-C(15)-C(14)	-64.76(14)
Br(1)-C(1)-C(15)-C(14)	179.04(9)
C(13)-C(14)-C(15)-C(1)	-71.60(14)

Table 7. Hydrogen bonds for brlact [Å and °].

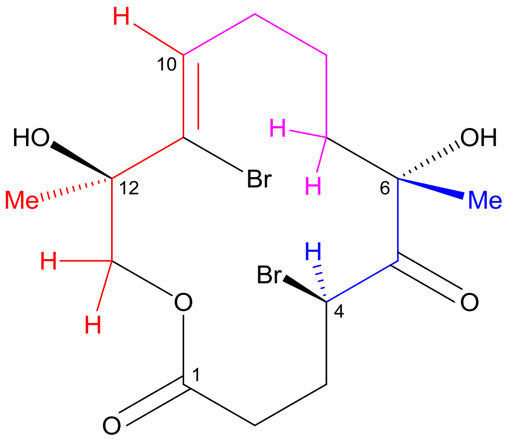
D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2)...O(1)	0.82	2.19	2.6364(14)	113.9
O(2)-H(2)...Br(1)#1	0.82	2.83	3.4865(10)	138.6

Symmetry transformations used to generate equivalent atoms: #1 -x,-y,-z



1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)	13C NMR chemical shift (δ/ppm)
2.70 (dddd, $^2J = 17.6$Hz, $^3J_{H2,\ H3} = 9.5$Hz, 2.5Hz, **H2**)	169.9 ----- C1
2.41 (dddd, $^2J = 17.6$Hz, $^3J_{H2,\ H3} = 9.5$Hz, 2.5Hz, **H2**)	31.3 ----- C2
2.35 (m, **H3**)	28.7 ----- C3
2.17 - 2.12 (m, **H3**)	42.1 ----- C4
4.64 (dd, $J_{H4,\ H3} = 9.1$Hz, $J_{H4,\ H3} = 5.7$Hz, **H4**)	208.1 ----- C5
1.57 (s, **6-CH$_3$**)	78.9 ----- C6
3.70 (s, **6-OH**)	36.6 ----- C7
1.70 - 1.60 (m, **H7β**, **H7α**)	22.0 ----- C8
1.93 - 1.85 (m, **H8**)	30.1 ----- C9
0.98 - 0.90 (m, **H8**)	132.1 ----- C10
2.37 (**H9**)	131.7 ----- C11
2.31 (**H9**)	64.5 ----- C12
6.04 (dd, J = 9.0, 6.6Hz, **H10**)	69.7 ----- C13
2.03 (s, **12-CH$_3$**)	26.9 ----- 6-CH$_3$
5.05 (d, $^2J = 13.3$ Hz, **H13**)	28.6 ----- 12-CH$_3$
4.18 (d, $^2J = 13.3$ Hz, **H13**)	

1d TOCSY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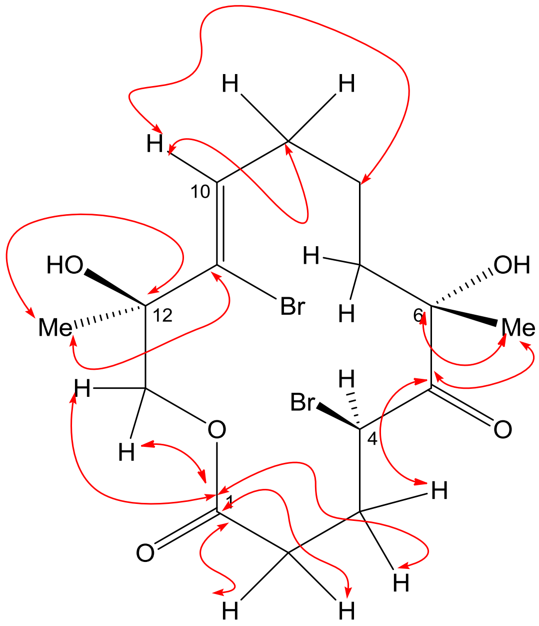
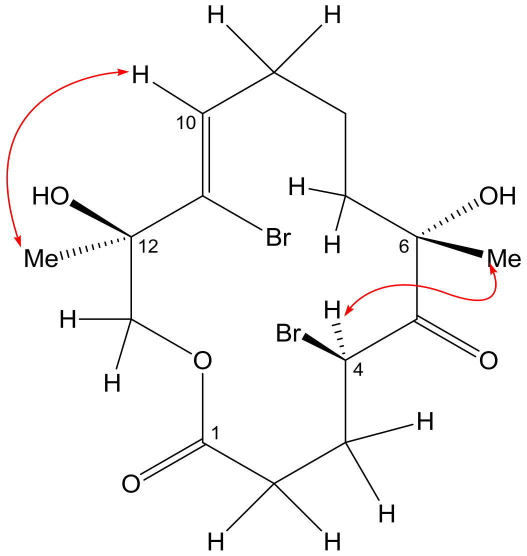
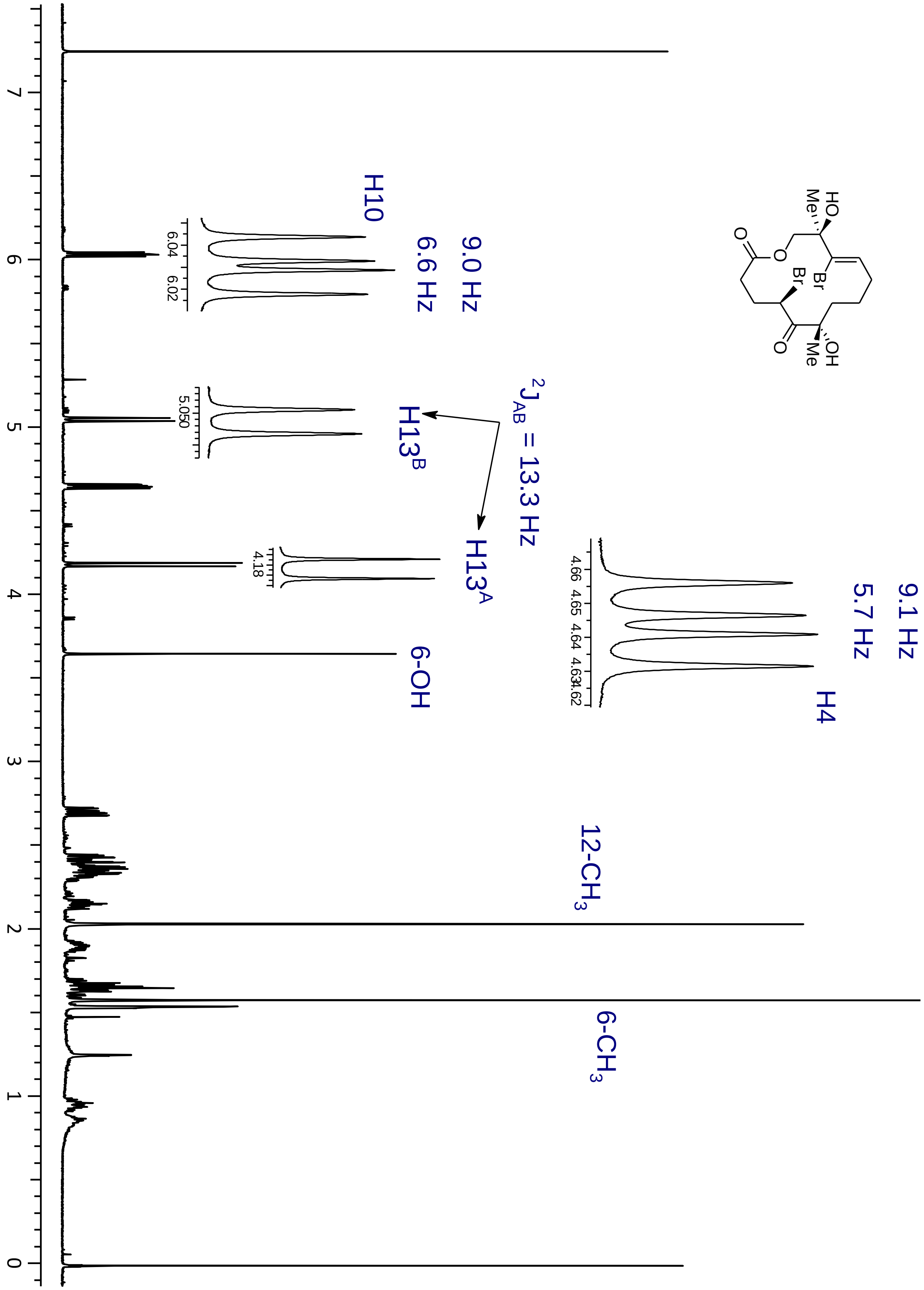
12
10
Br
1
4
6
OH
Me
O
O
O
Br

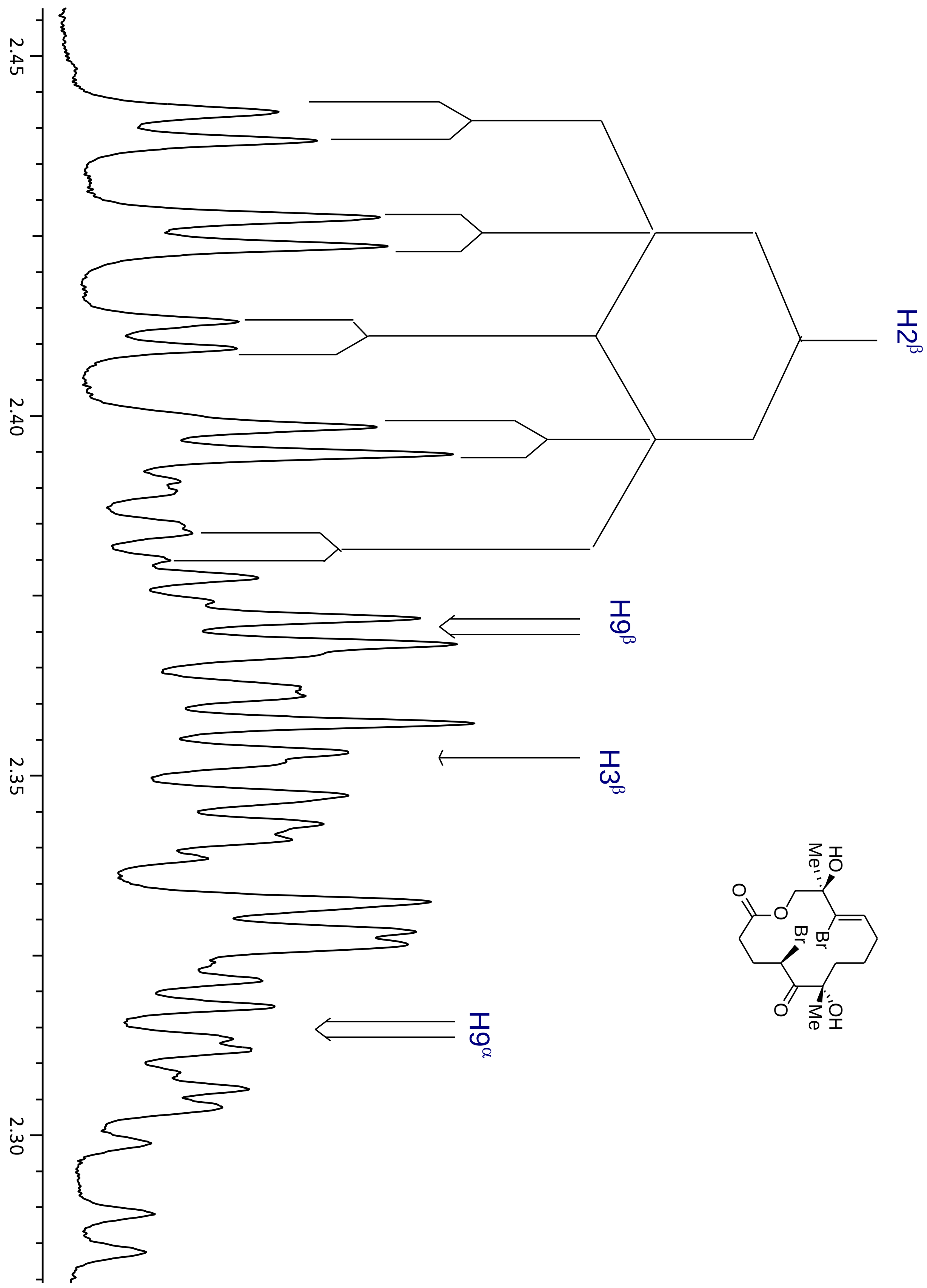
HMBC

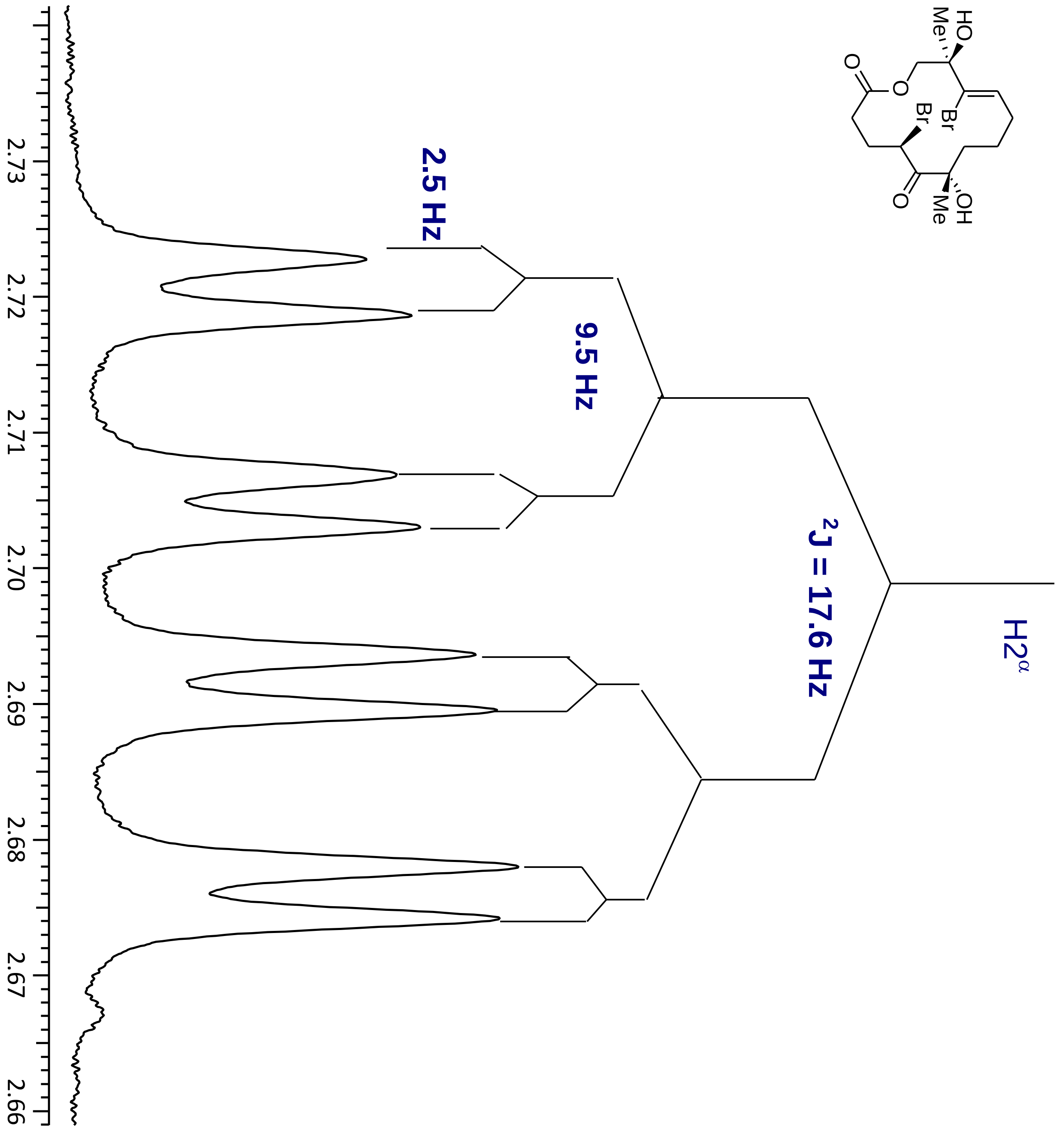

HO
Me
12
10
Br
1
4
6
OH
Me
O
O
O
Br

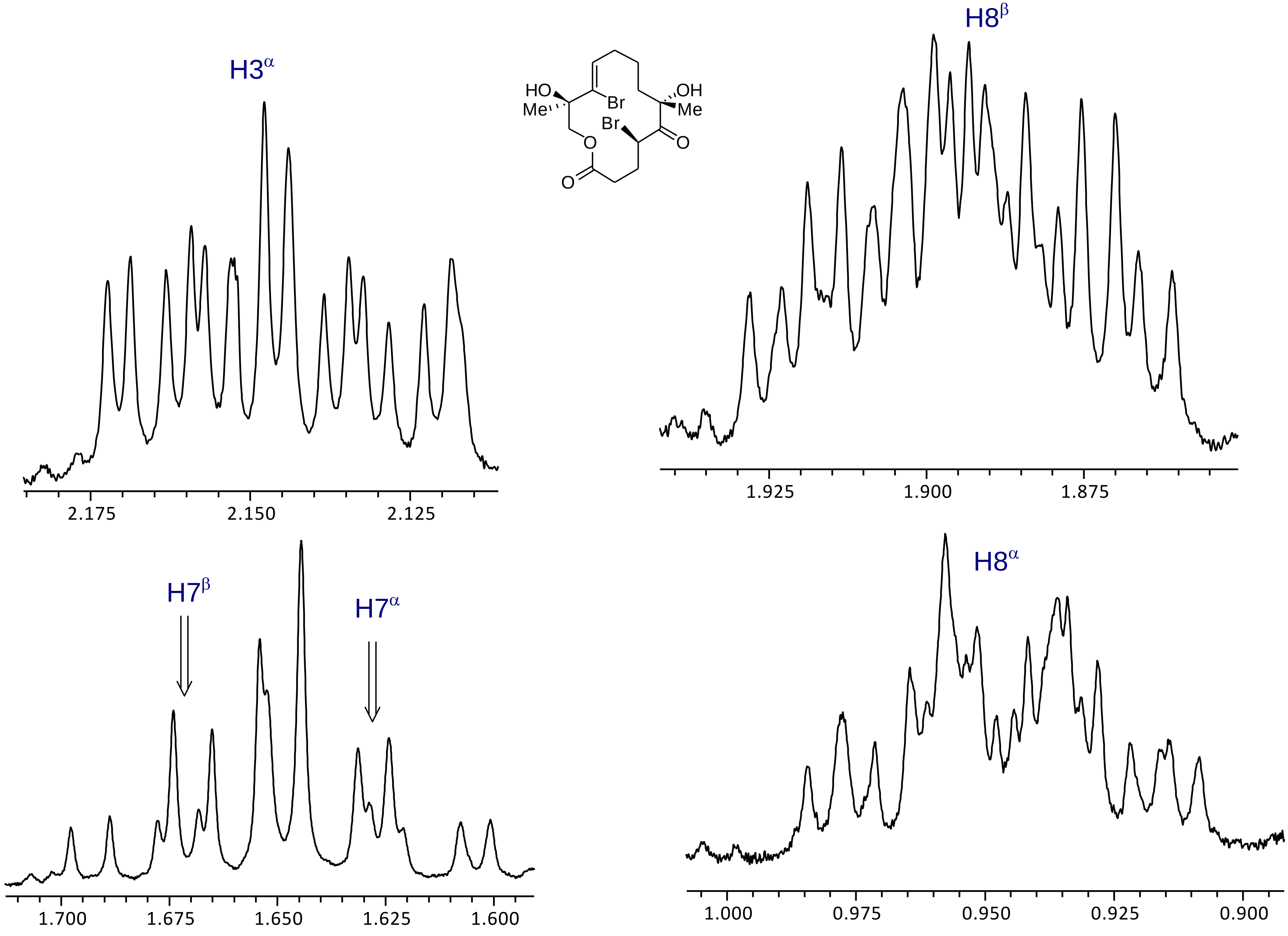
NOESY


HO
Me
12
10
Br
1
4
6
OH
Me
O
O
O
Br


HO
Me
O
O
Br
Br
O
OH
Me
H10
9.0 Hz
6.6 Hz
6.04
6.02
$^2J_{AB}$ = 13.3 Hz
H13^B
5.050
H13^A
4.18
9.1 Hz
5.7 Hz
H4
4.66
4.65
4.64
4.63
4.62
6-OH
12-CH$_3$
6-CH$_3$
7
6
5
4
3
2
1
0


2.45
2.40
2.35
2.30
H2β
H9β
H3β
H9α
HO
Me
Br
Br
O
O
O
OH
Me


2.5 Hz
9.5 Hz
²J = 17.6 Hz
H2α
2.73
2.72
2.71
2.70
2.69
2.68
2.67
2.66
HO
Me
Br
Br
O
O
O
OH
Me


H3$^{\alpha}$
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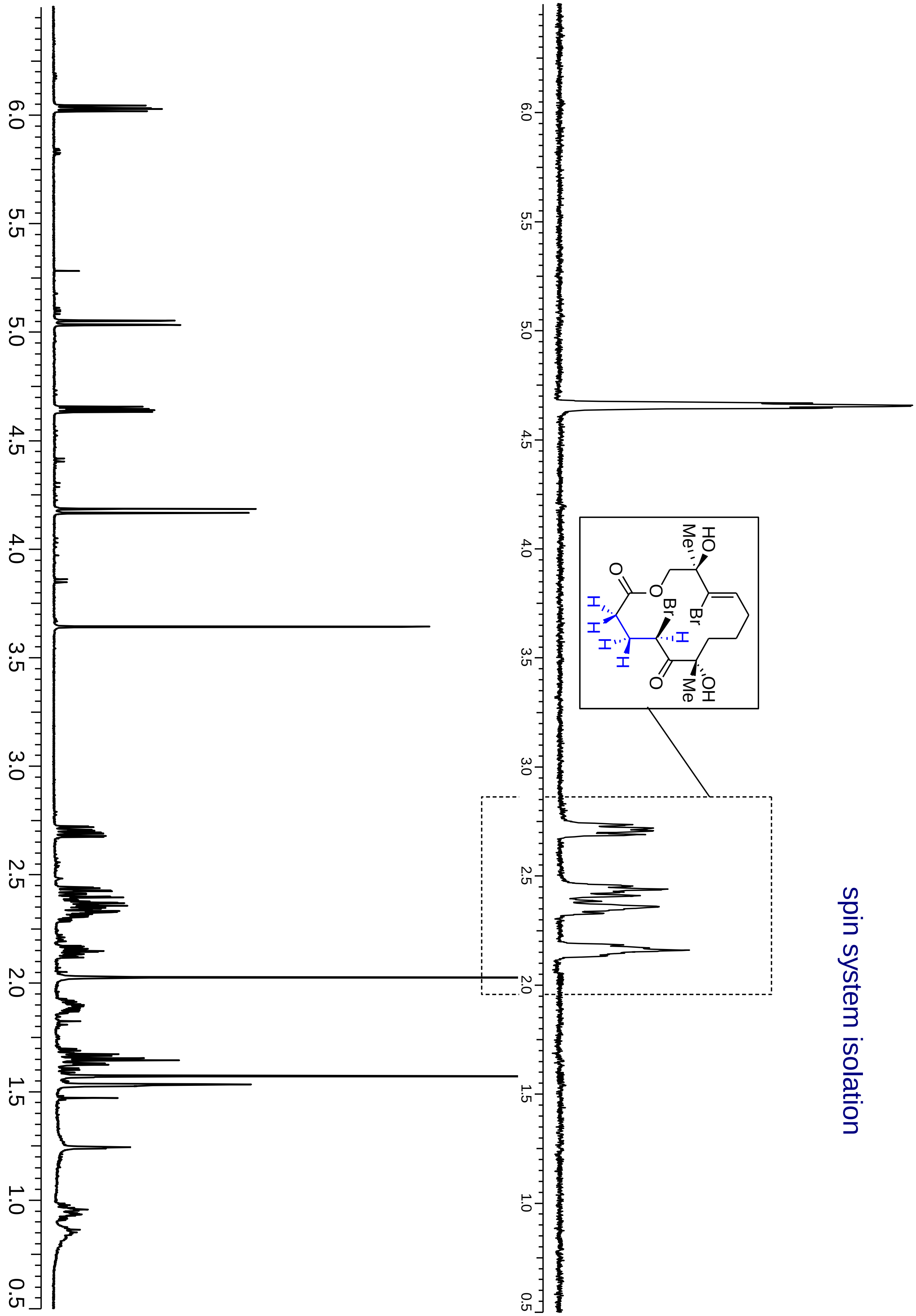
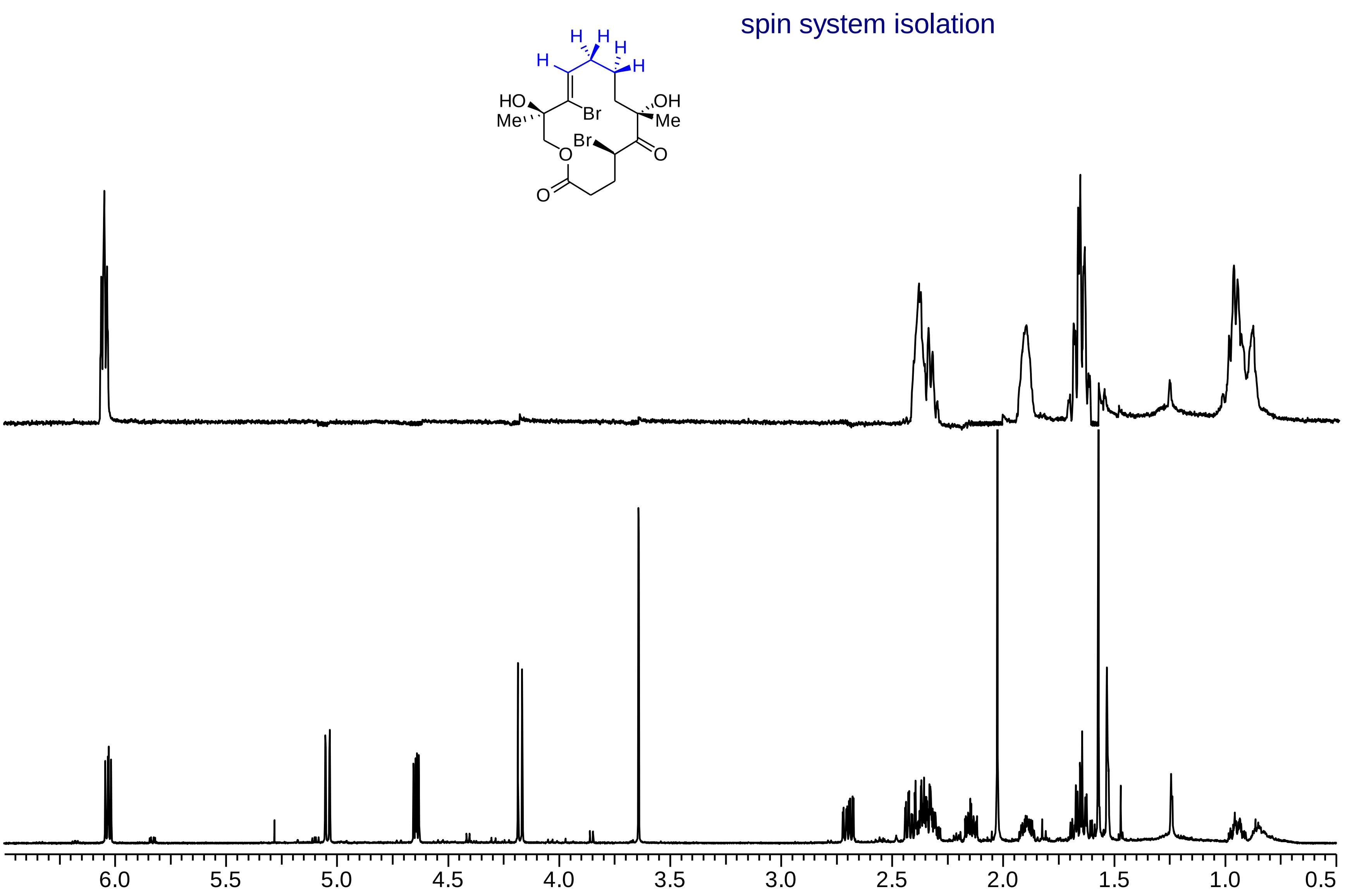
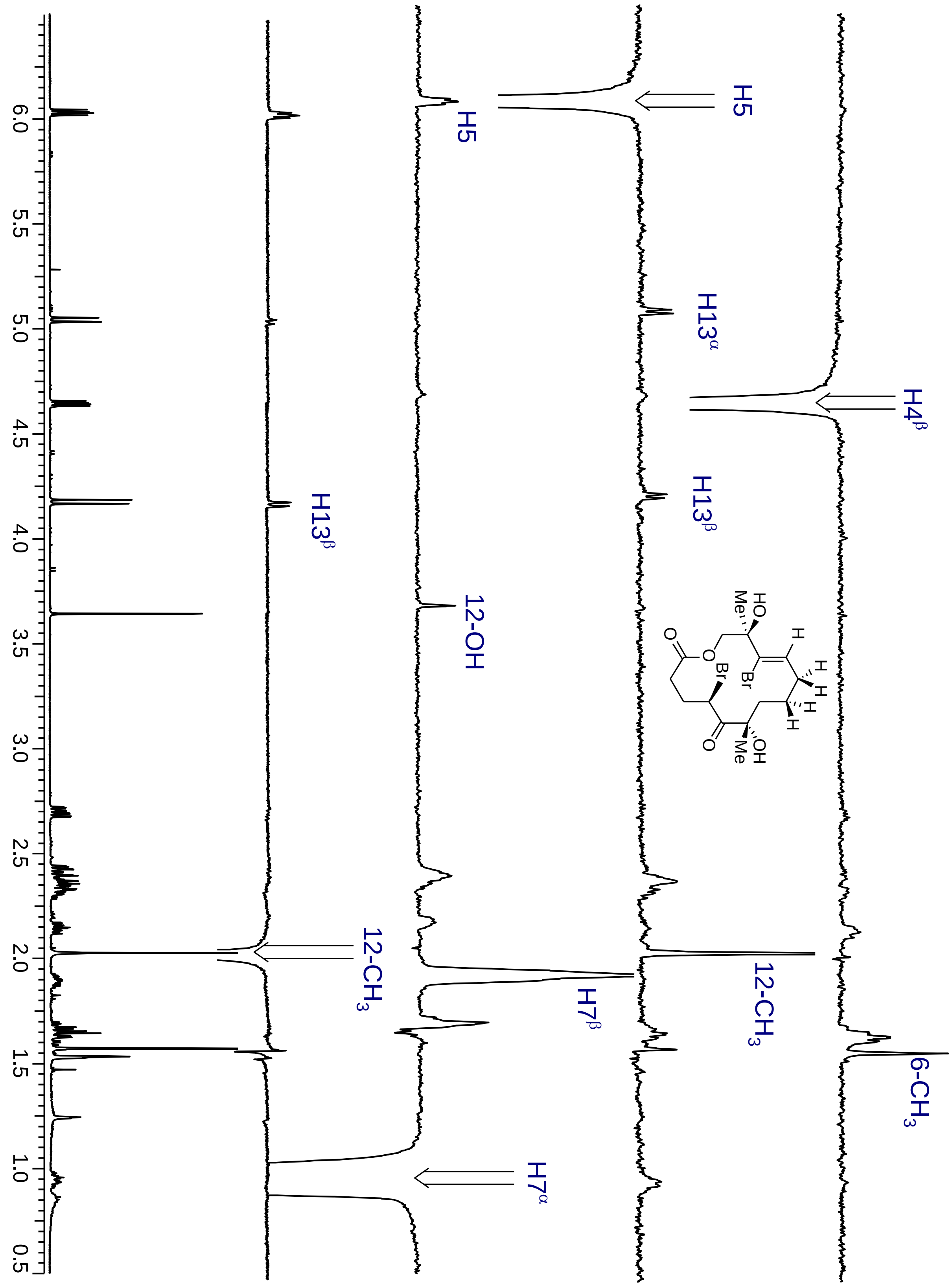
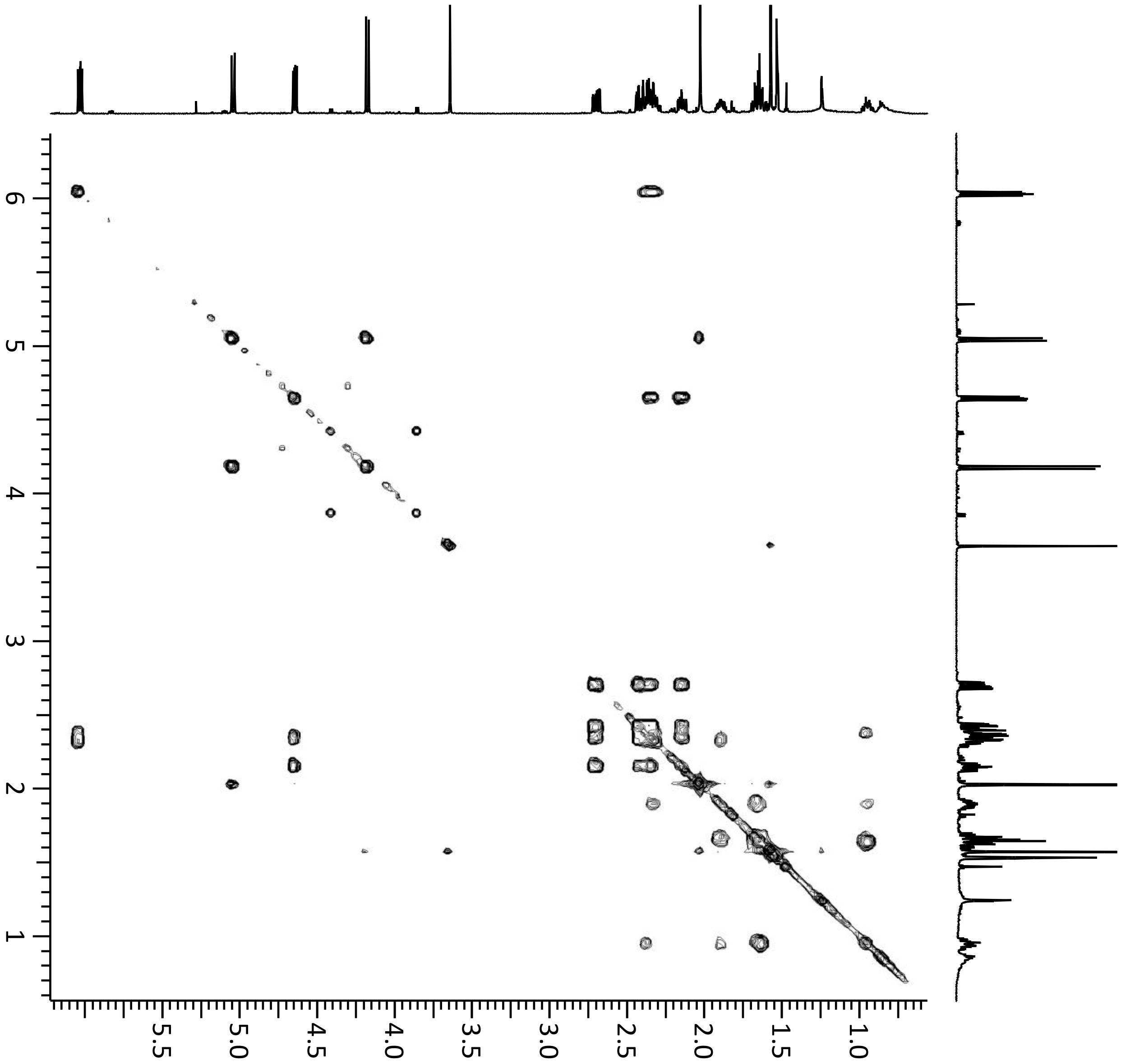
2.150
2.125
HO
Me
Br
Br
OH
Me
O
O
O
H8$^{\beta}$
1.925
1.900
1.875
H7$^{\beta}$
H7$^{\alpha}$
1.700
1.675
1.650
1.625
1.600
H8$^{\alpha}$
1.000
0.975
0.950
0.925
0.900


spin system isolation
HO
Me
Br
O
OH
Me
H
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

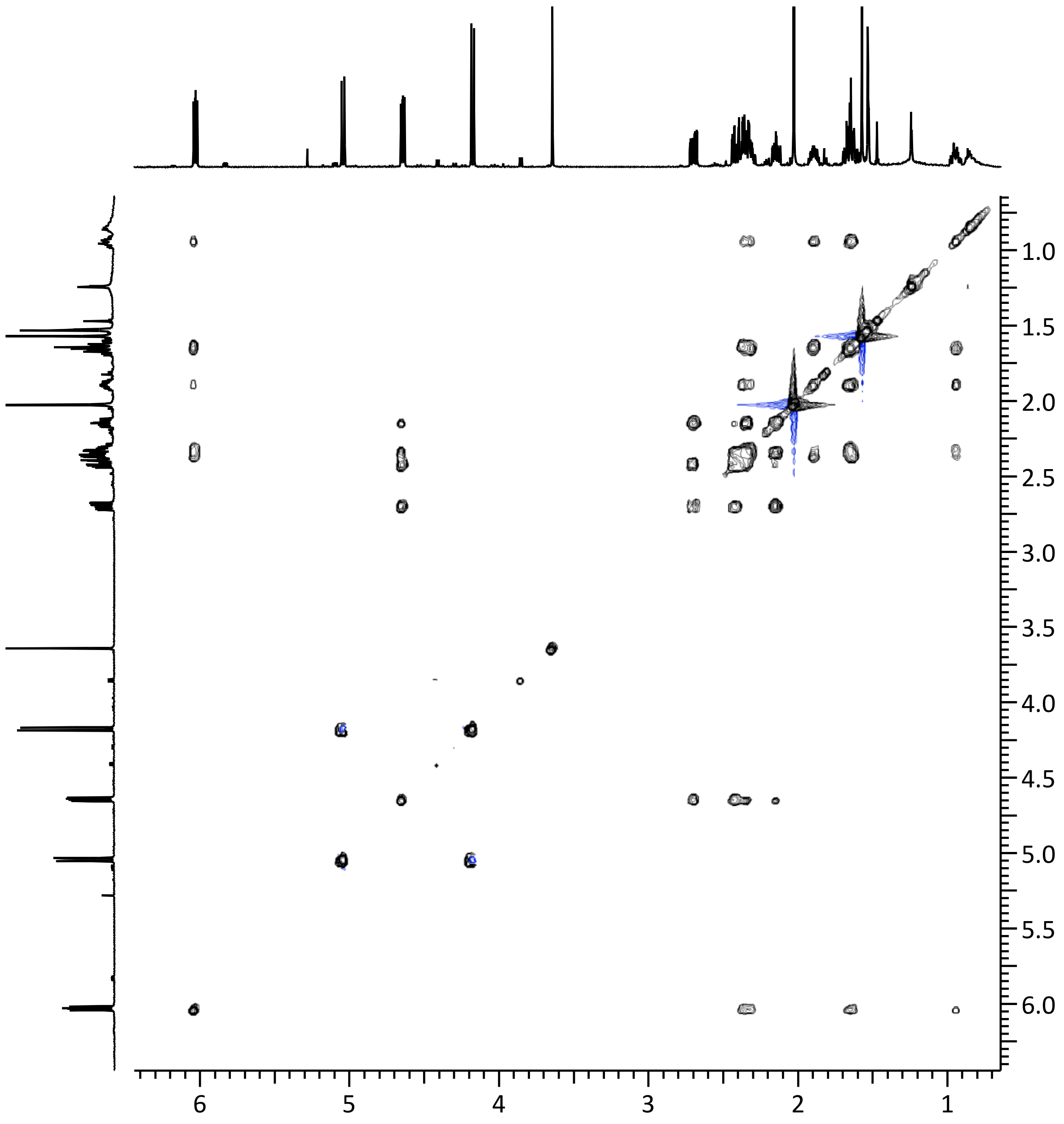

spin system isolation
H
H
H
H
H
HO
Me
Br
OH
Me
Br
O
O
O
O
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


H5
H13$^{\alpha}$
H4$^{\beta}$
H13$^{\beta}$
6-CH$_3$
12-CH$_3$
H5
12-OH
H7$^{\beta}$
H7$^{\alpha}$
H13$^{\beta}$
12-CH$_3$
HO
Me
H
H
H
H
H
Br
Br
O
O
O
OH
Me
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

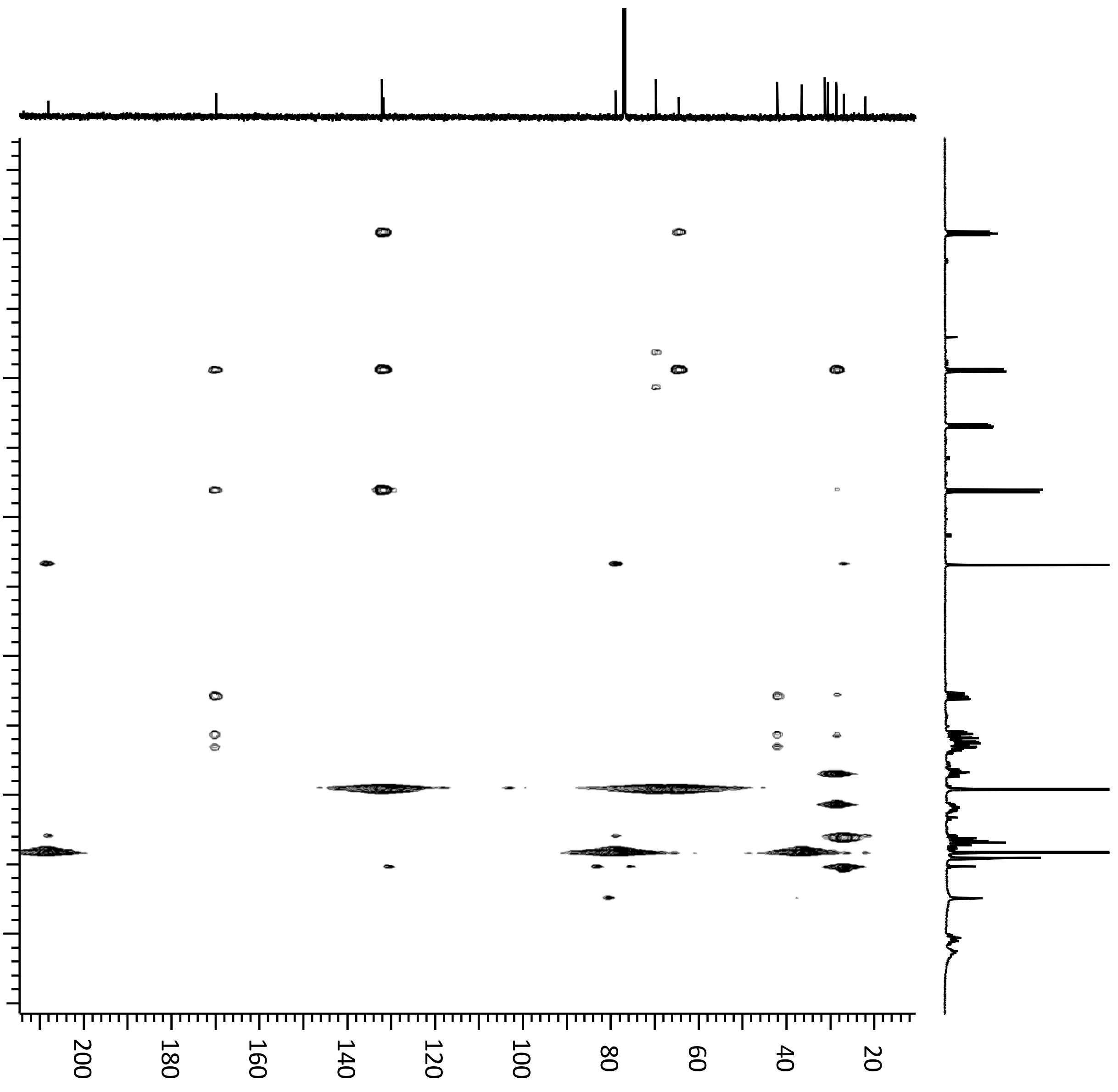
gCOSY

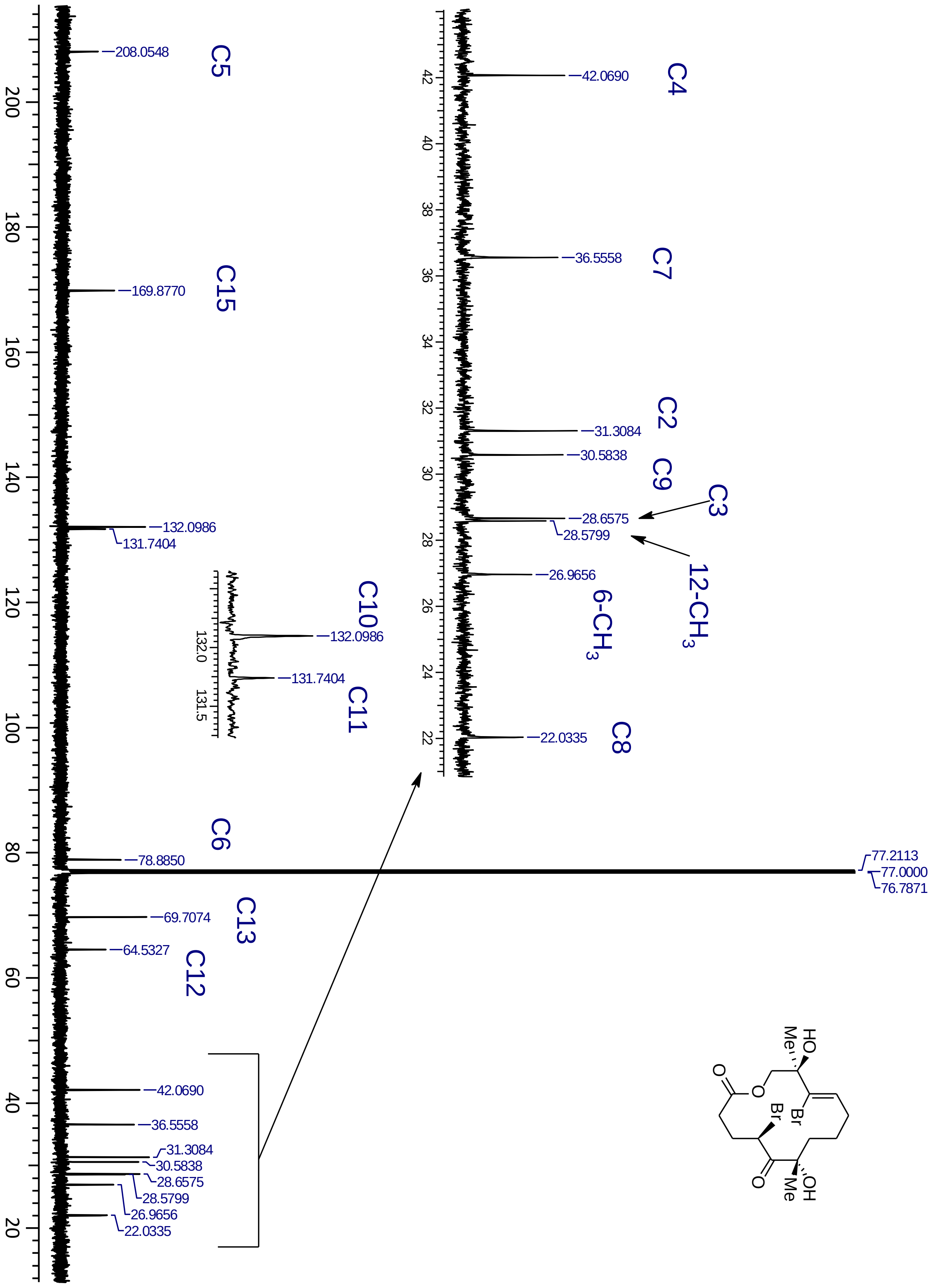


NOESY



HMBC

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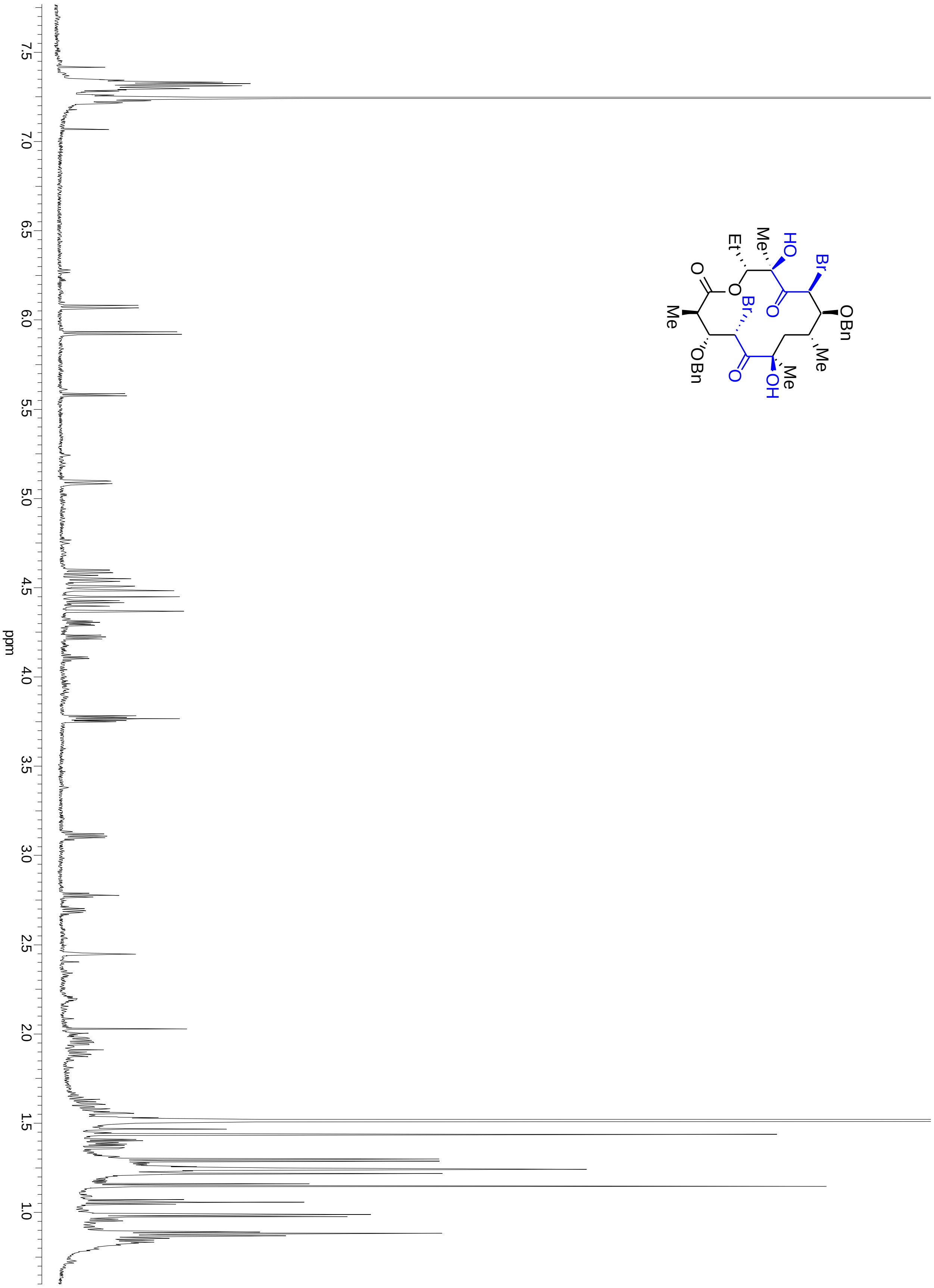
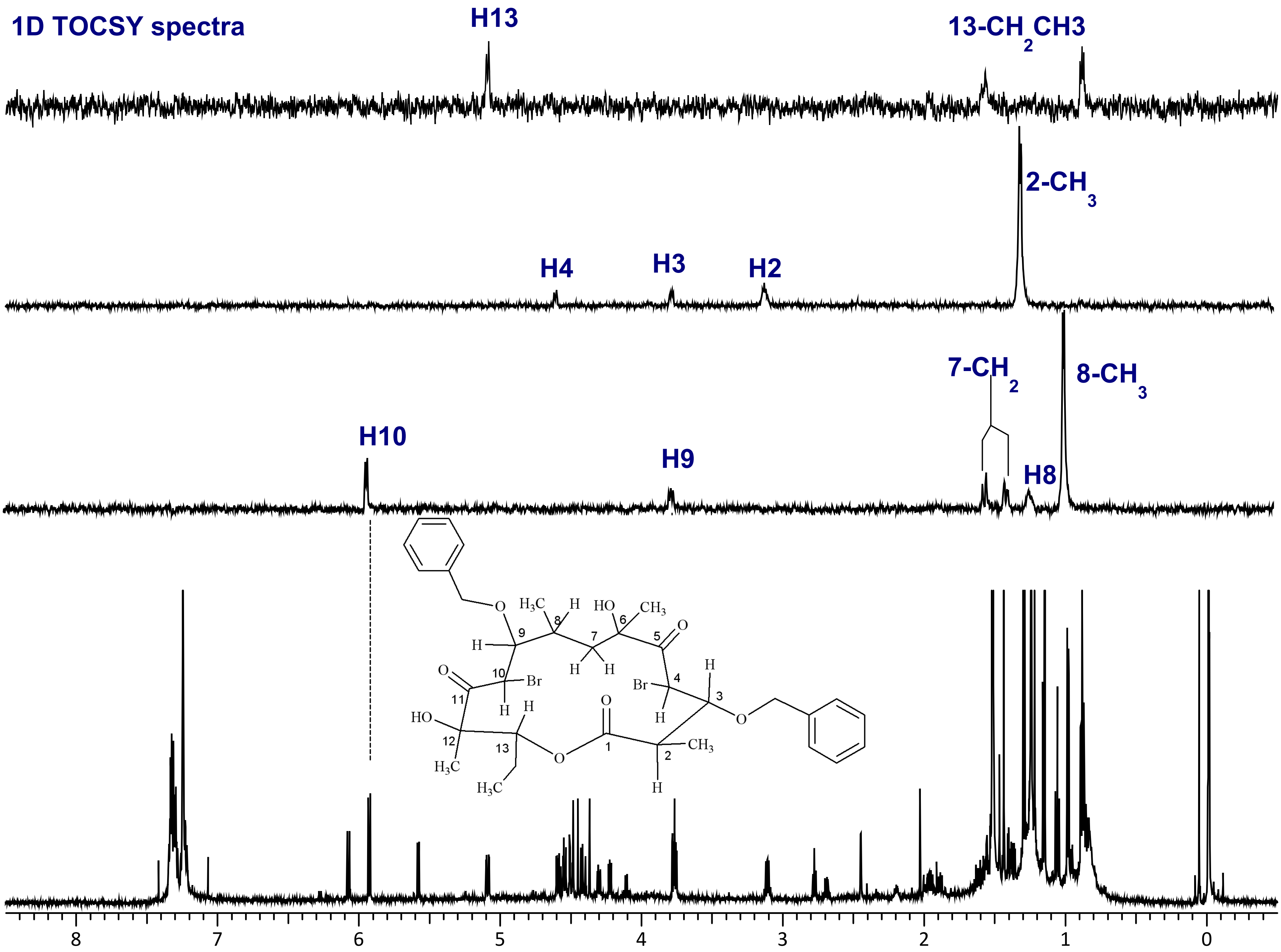
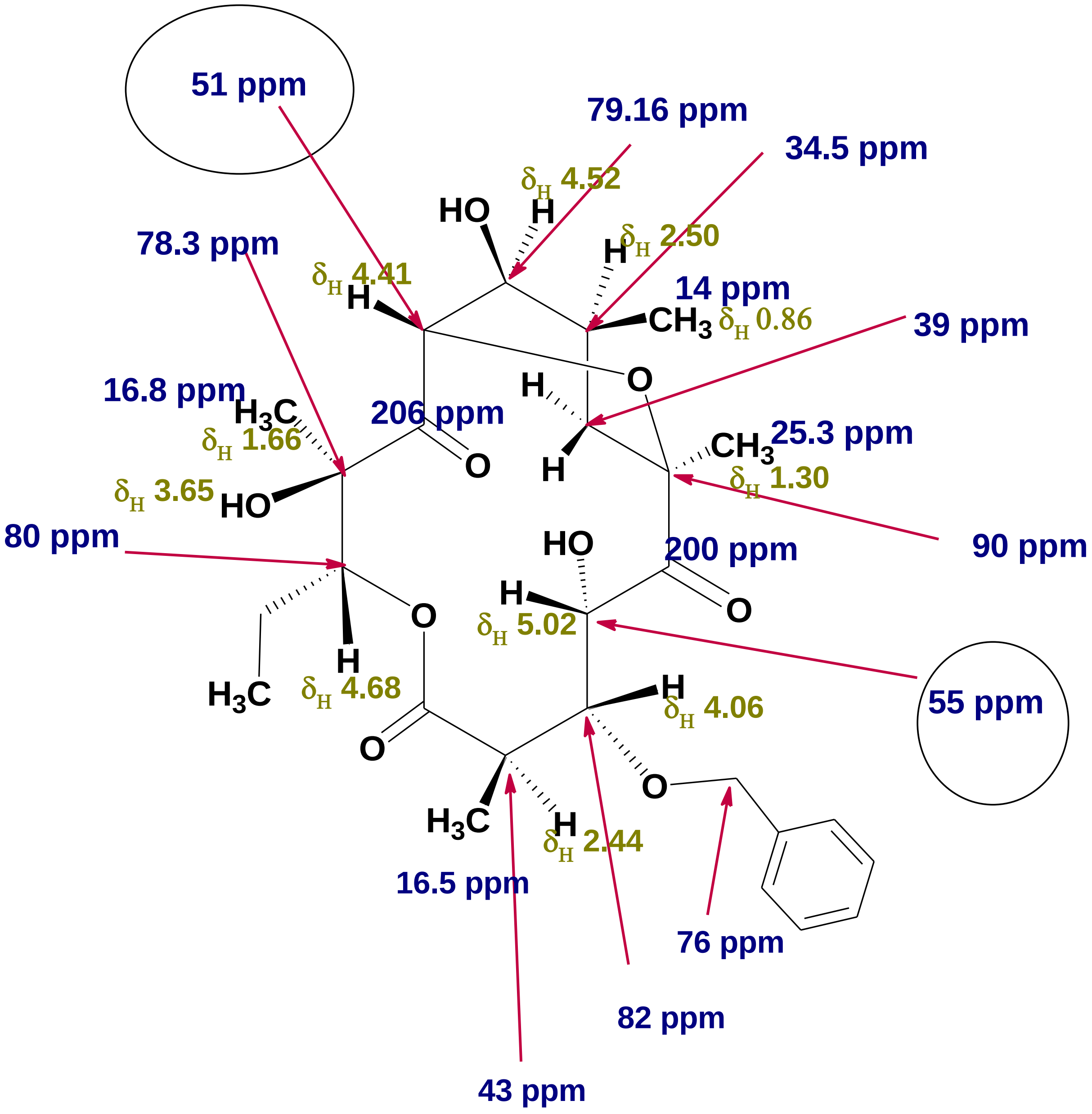
C5
169.8770
C15
132.0986
131.7404
C10
132.0986
131.7404
C11
132.0
131.5
78.8850
C6
77.2113
77.0000
76.7871
69.7074
C13
64.5327
C12
42.0690
36.5558
31.3084
30.5838
28.6575
28.5799
26.9656
22.0335
200
180
160
140
120
100
80
60
40
20
42.0690
C4
36.5558
C7
31.3084
C2
30.5838
C9
28.6575
C3
28.5799
12-CH3
26.9656
6-CH3
22.0335
C8
42
40
38
36
34
32
30
28
26
24
22
HO
Me
O
O
Br
Br
O
OH
Me



7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
OBn
Me
Br
HO
Me
Et
O
O
O
Br
Me
OBn
O
OH
Me
O


1D TOCSY spectra
H13
13-CH2CH3
2-CH3
H4
H3
H2
7-CH2
8-CH3
H10
H9
H8
8
7
6
5
4
3
2
1
0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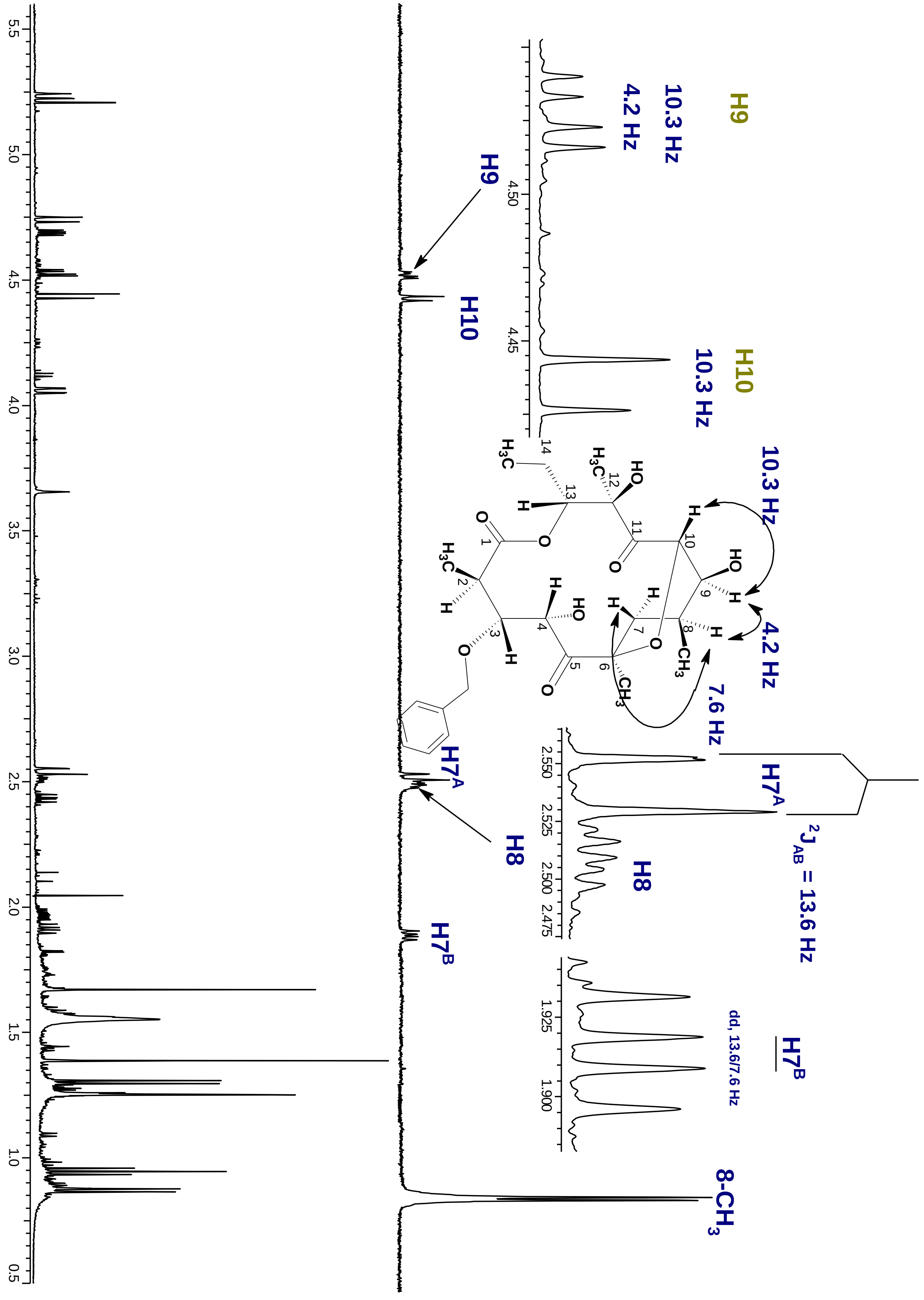
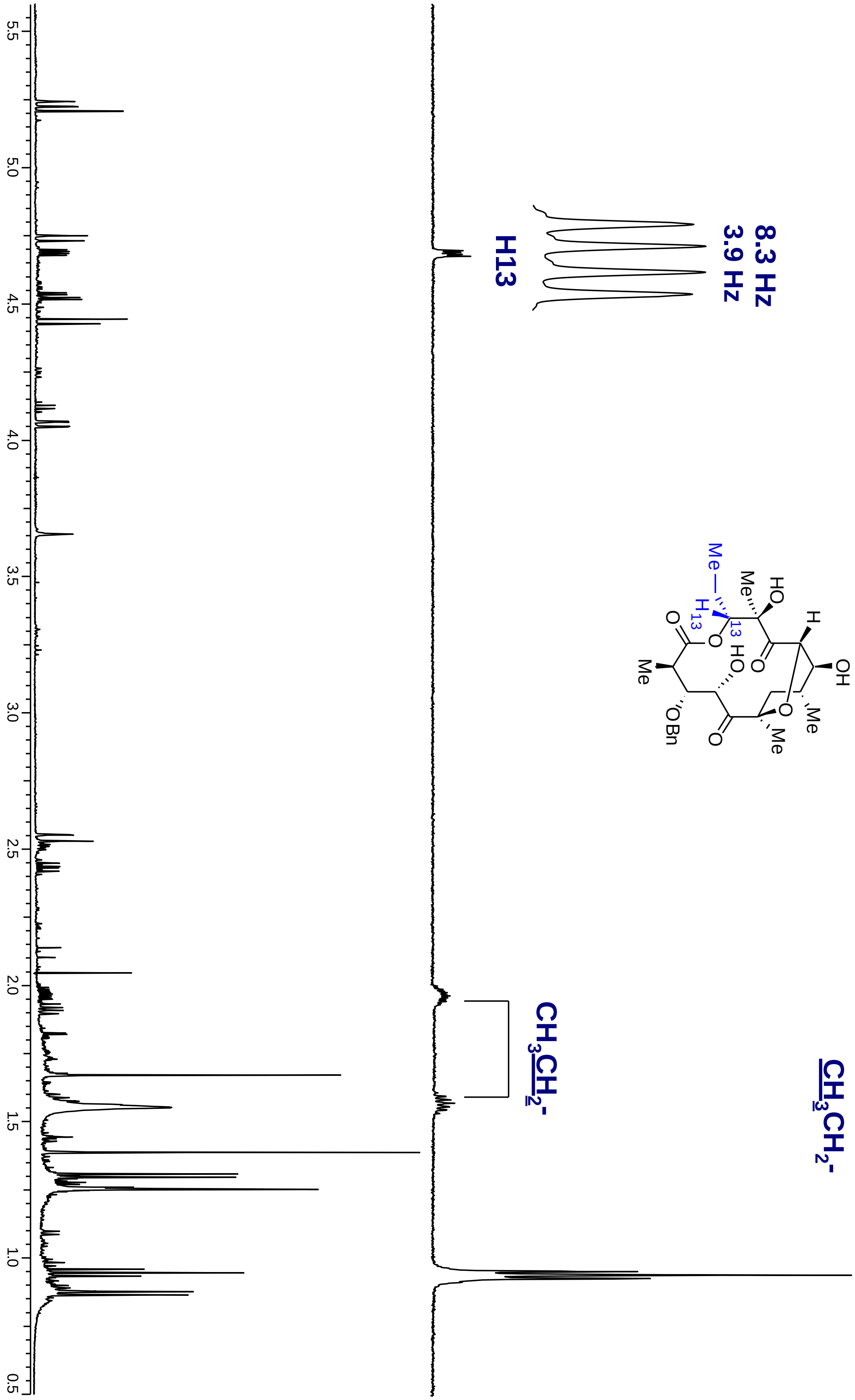
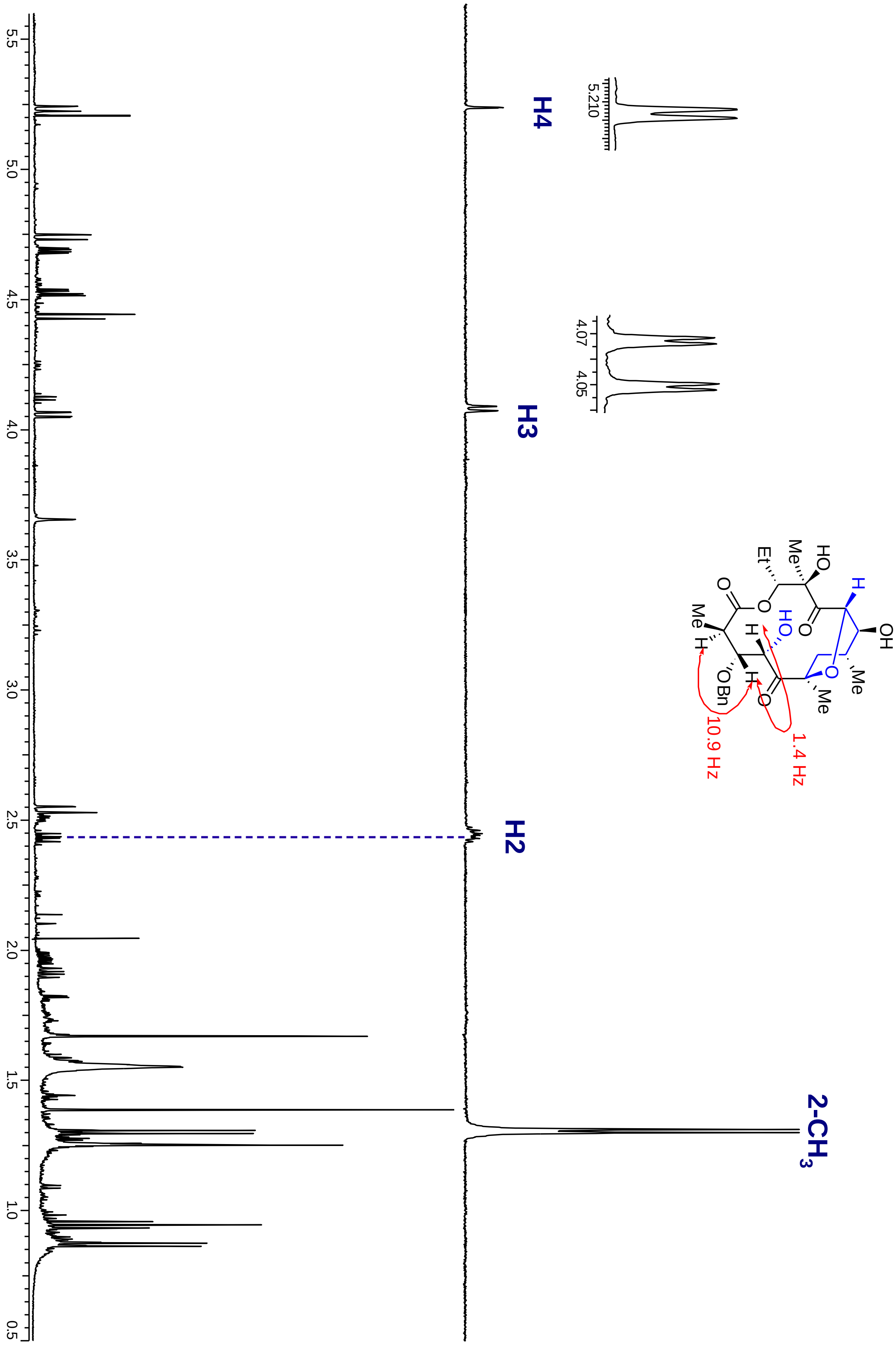
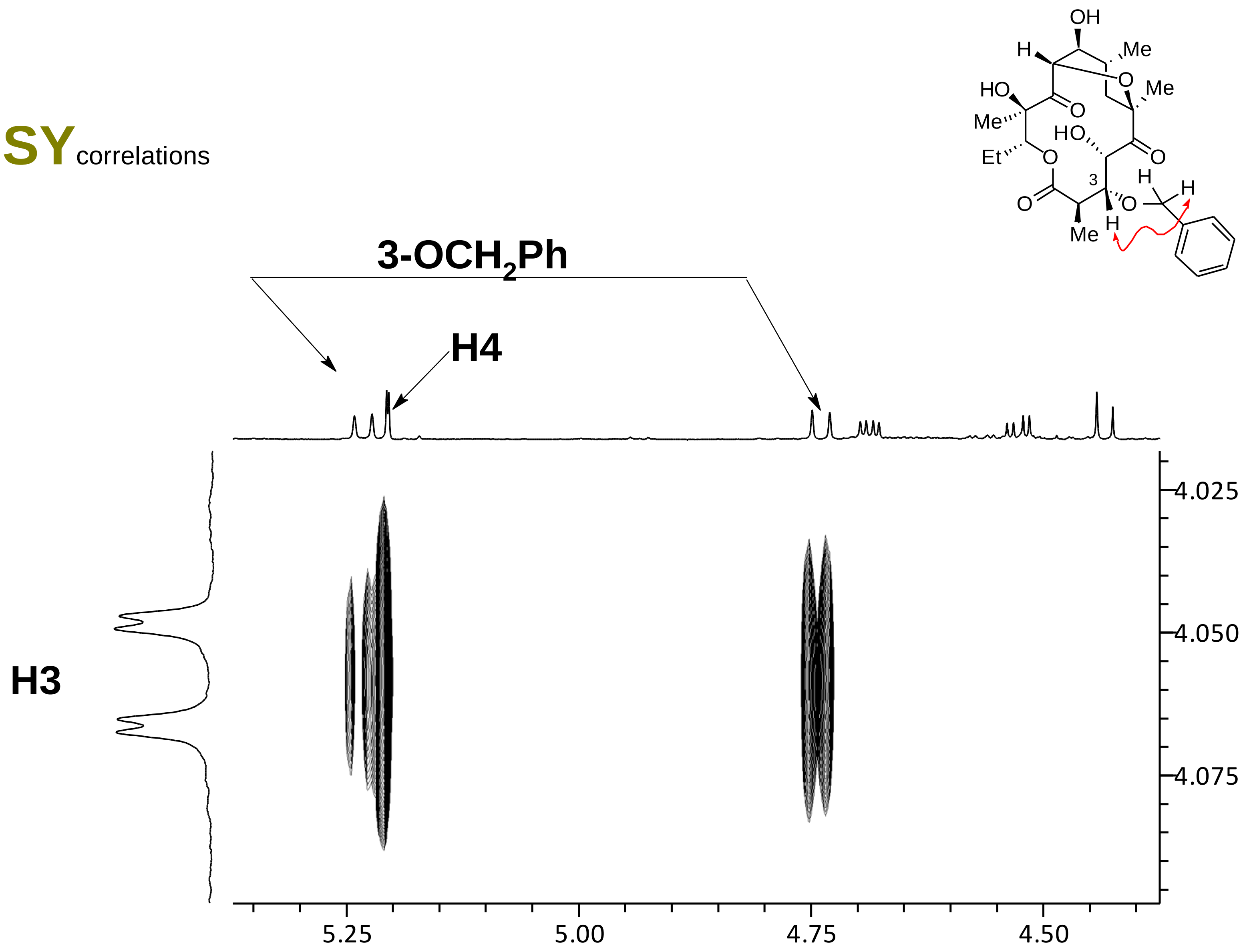
79.16 ppm
34.5 ppm
δH 4.52
HO
H
δH 2.50
H
78.3 ppm
δH 4.41
H
14 ppm
CH3 δH 0.86
39 ppm
16.8 ppm
H3C
δH 1.66
206 ppm
O
H
O
H
25.3 ppm
CH3
δH 1.30
δH 3.65
HO
80 ppm
HO
200 ppm
90 ppm
O
H
δH 5.02
O
H
H3C
δH 4.68
H
δH 4.06
55 ppm
O
O
H3C
H
δH 2.44
16.5 ppm
76 ppm
82 ppm
43 ppm

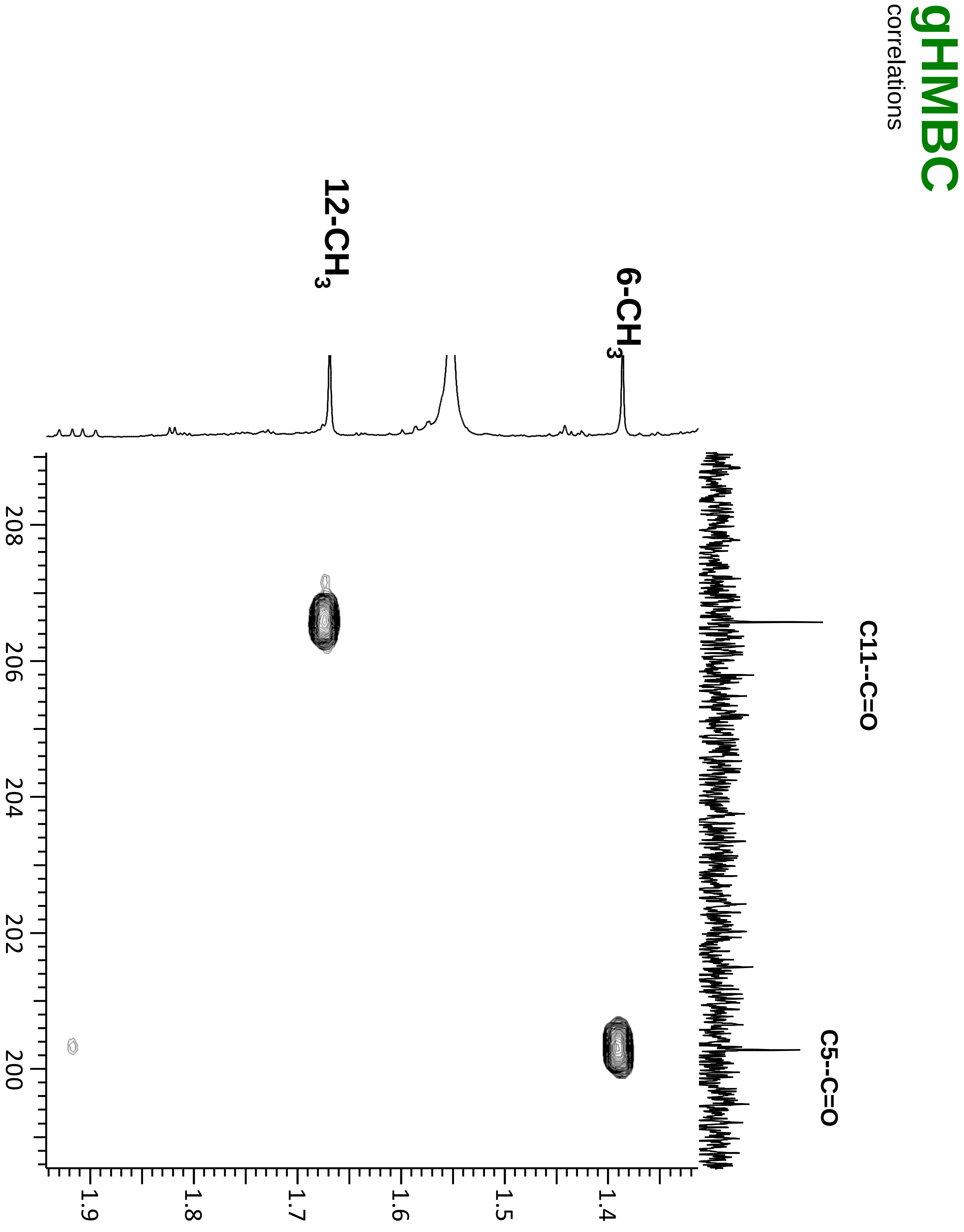

H9
10.3 Hz
4.2 Hz
4.50
4.45
H10
10.3 Hz
10.3 Hz
4.2 Hz
7.6 Hz
H7^A
$^2J_{AB}$ = 13.6 Hz
H8
2.550
2.525
2.500
2.475
H7^B
dd, 13.6/7.6 Hz
1.925
1.900
8-CH_3
H9
H10
H7^A
H8
H7^B
HO
H_3C
H_3C
H_3C
CH_3
CH_3
O
H
1
2
3
4
5
6
7
8
9
10
11
12
13
14
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


8.3 Hz
3.9 Hz
H13
CH3CH2-
CH3CH2-
HO
Me
Me
H13
13
O
O
HO
H
OH
Me
O
Me
O
Me
OBn
O
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

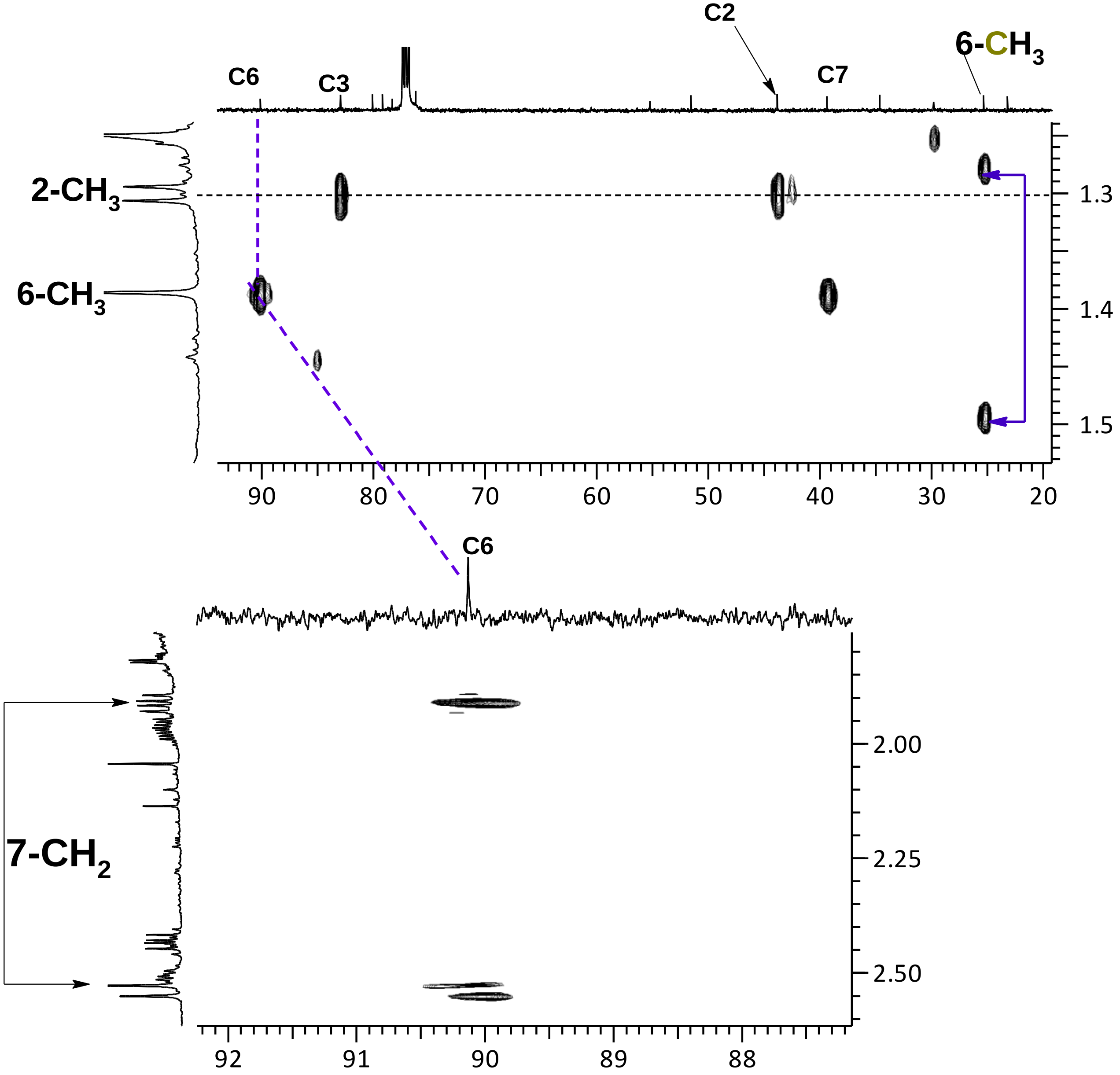

H4
5.210
H3
4.07
4.05
H2
2-CH$_3$
1.4 Hz
10.9 Hz
HO
Me
Et
O
OH
Me
Me
OBn
H
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

NOESY correlations

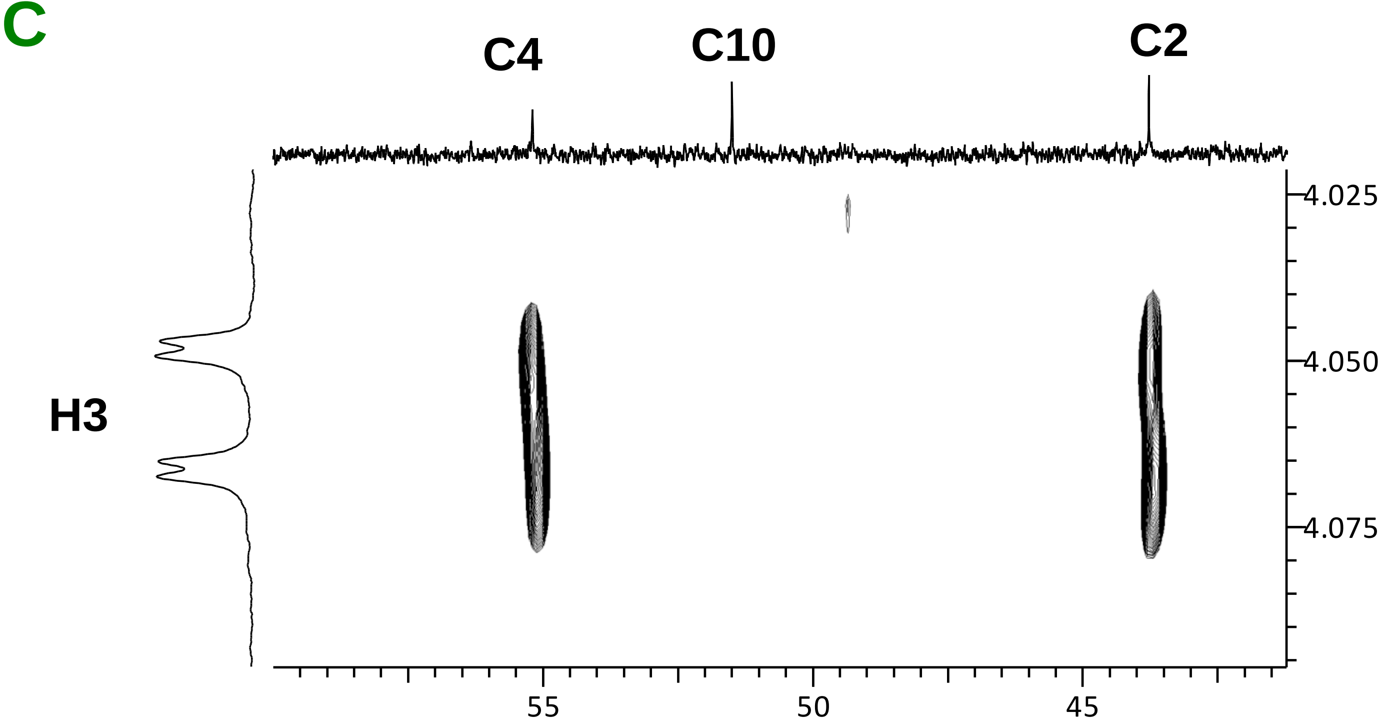



gHMBC
correlations
12-CH$_3$
6-CH$_3$
C11--C=O
C5--C=O
208
206
204
202
200
1.9
1.8
1.7
1.6
1.5
1.4

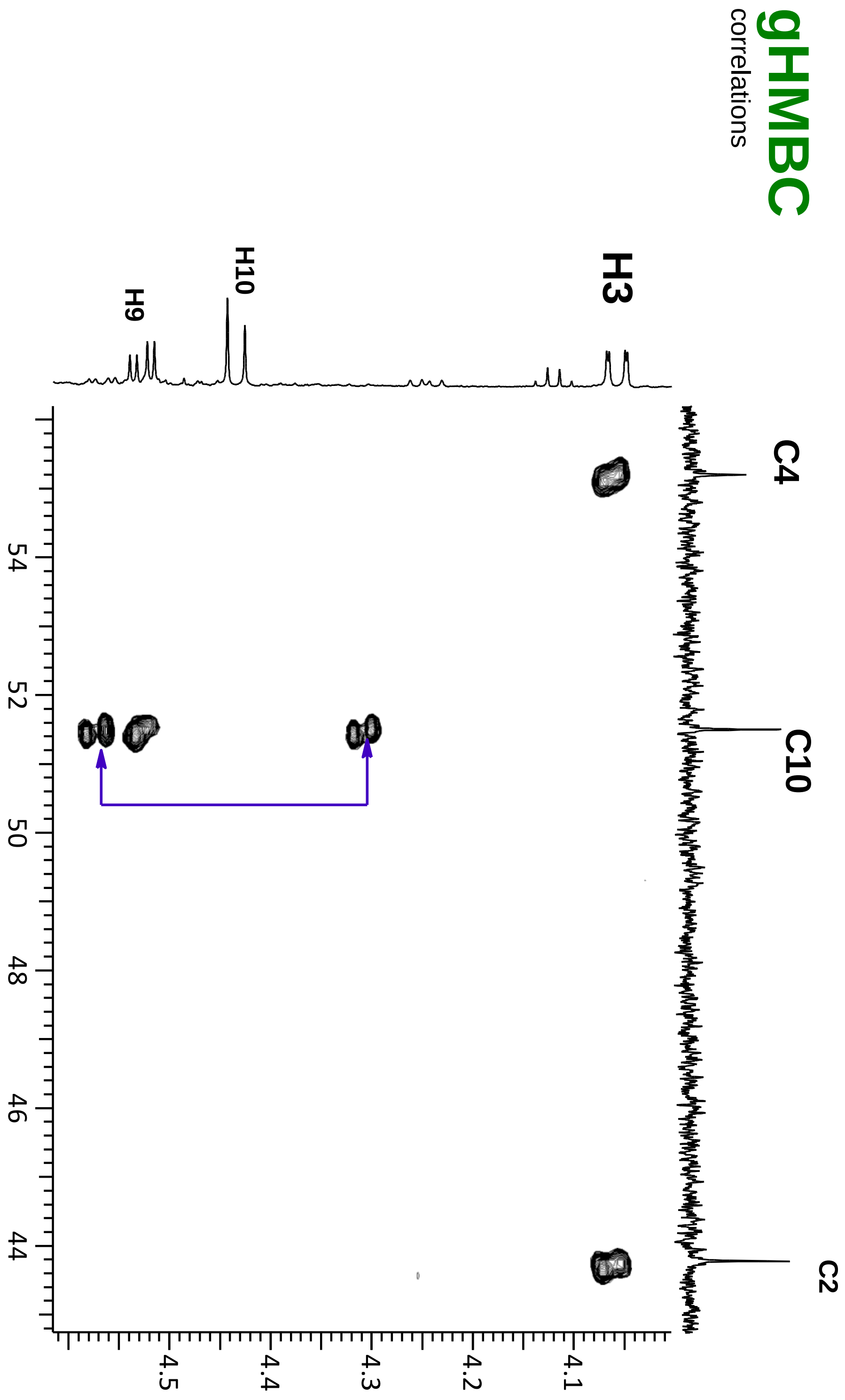
gHMBC
correlations

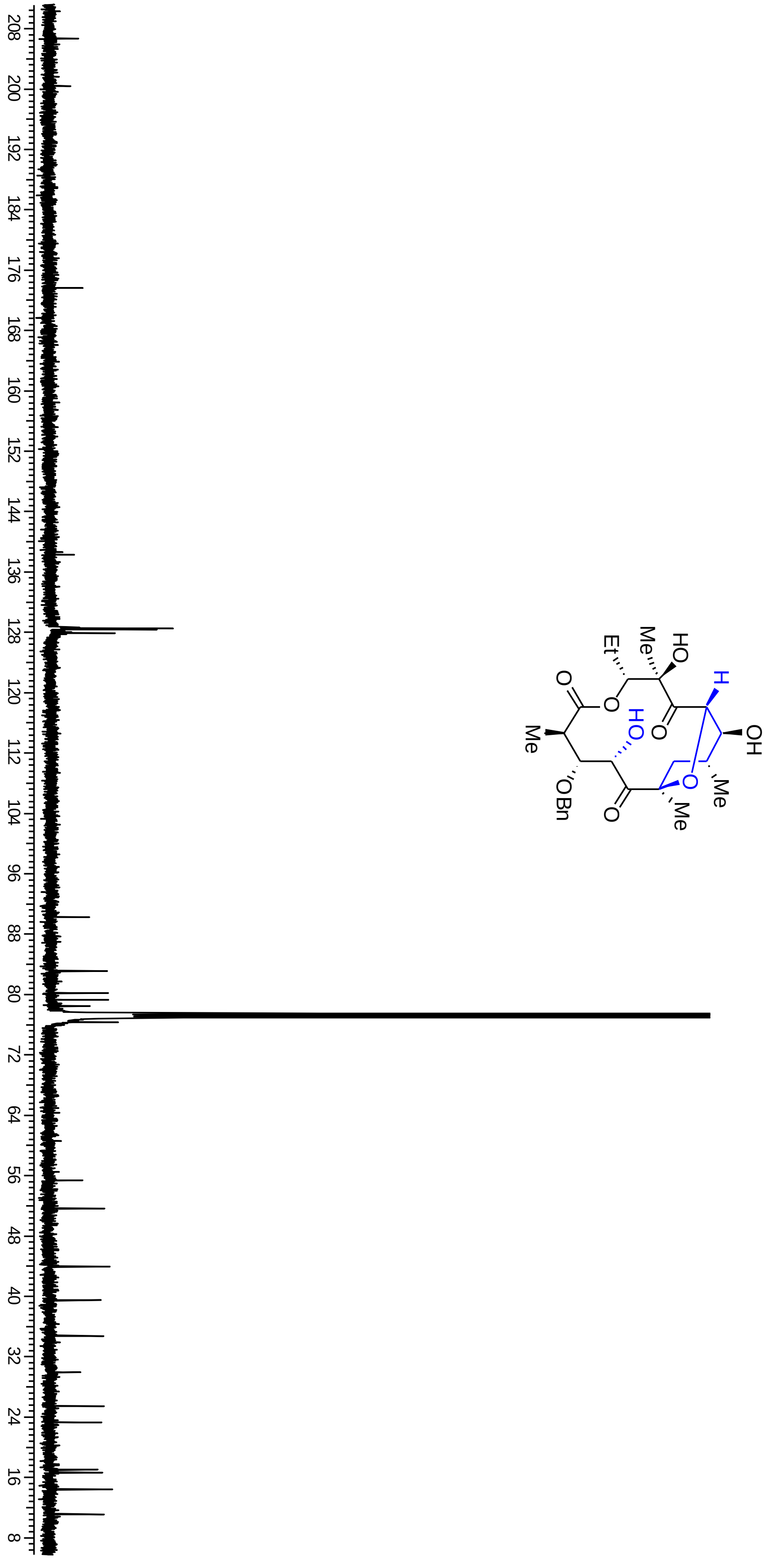


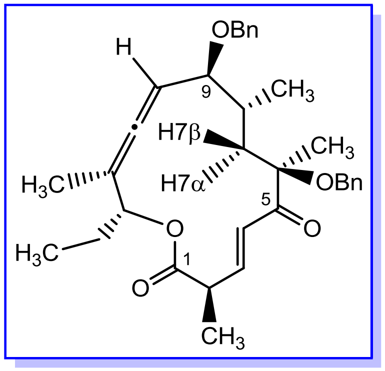
gHMBC
correlations



there is no more long range correlation to 55pm! only one between H3 and 55ppm)


gHMBC
correlations
H3
H10
H9
C4
C10
C2
54
52
50
48
46
44
4.5
4.4
4.3
4.2
4.1


208
200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
HO
Me
Et
O
O
O
HO
H
OH
Me
O
Me
O
OBn
Me



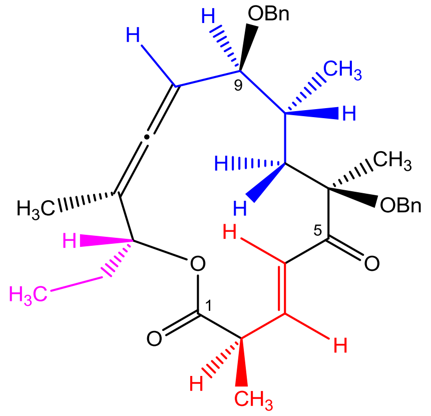
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

3.30 (ddd, $J_{H2,\ H3}$ = 8.2Hz, $J_{H2,\ 2\text{-}CH3}$ = 6.8Hz, $J_{H2,\ H4}$ = 1.1Hz, **H2**)
1.32 (d, $J_{2\text{-}CH3,\ H2}$ = 6.8Hz, **2-CH$_3$**)
6.73 (dd, $J_{H3,\ H4}$ = 15.7Hz, $J_{H3,\ H2}$ = 8.3Hz, **H3**)
7.05 (dd, $J_{H4,\ H3}$ = 15.7Hz, $J_{H4,\ H2}$ = 1.2Hz, **H4**)
1.44 (s, **6-CH$_3$**)
4.26 (d, J_{AB} = 11.5Hz, **6-OCH$_2$**)
4.56 (d, J_{AB} = 12.0Hz, **6-OCH$_2$**)
1.95 (dd, $J_{H7\alpha,\ H7\beta}$ = 14.2Hz, **H7β**)
1.48 (dd, $J_{H7\beta,\ H8}$ = 6.85 Hz, $J_{H7\beta,\ H7\alpha}$ = 14.4Hz, **H7α**)
1.41 (m, **H8**)
1.09 (d, $J_{8\text{-}CH3,\ H8}$ = 6.6Hz, **8-CH$_3$**)
3.24 (t, $J_{H9,\ H8}$ = 9.1Hz, **H9**)
4.24 (d, J_{AB} = 11.5Hz, **9-OCH$_2$**)
4.57 (d, J_{AB} = 11.0Hz, **9-OCH$_2$**)
4.62 (dq, J = 9.31Hz, **H10**)
1.82 (d, $J_{12\text{-}CH3,\ H10}$ = 2.9Hz, **12-CH$_3$**)
4.71 (ddd, $J_{H13,\ 14\text{-}CH2}$ = 6.6Hz, $J_{H13,\ H10}$ = 1.6Hz, **H13**)
1.68 - 1.78 (m, **14-CH$_2$**)
0.99 (t, $J_{14\text{-}CH3,\ 13\text{-}CH2}$ = 7.3Hz, **14-CH$_3$**)
7.48 - 7.17 (m, 8H, **Ph**)

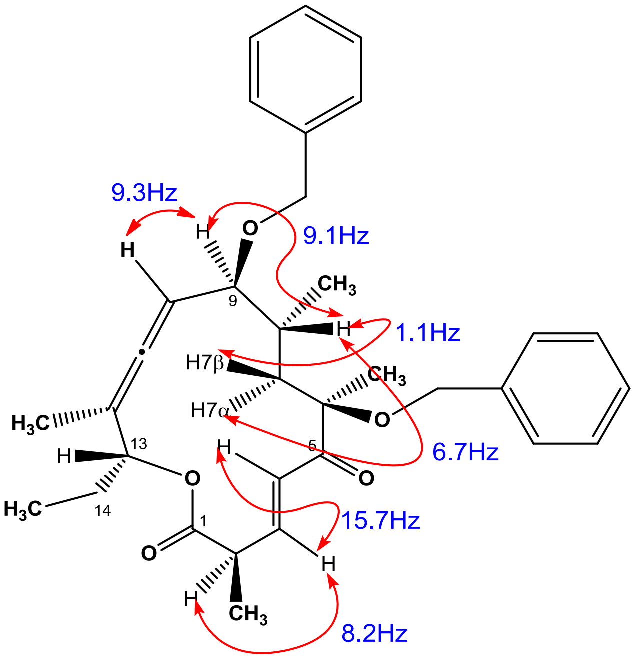
13C NMR chemical shift (δ/ppm)

171.9	-----	C1
42.3	-----	C2
145.3	-----	C3
127.8	-----	C4
202.1	-----	C5
84.9	-----	C6
41.4	-----	C7
33.5	-----	C8
83.0	-----	C9
93.9	-----	C10
202.6	-----	C11
101.1	-----	C12
75.8	-----	C13
26.4	-----	C14
14.6	-----	2-CH$_3$
17.4	-----	6-CH$_3$
19.9	-----	8-CH$_3$
18.0	-----	12-CH$_3$
9.7	-----	14-CH$_3$
66.3	-----	6-OCH$_3$
69.8	-----	9-OCH$_3$

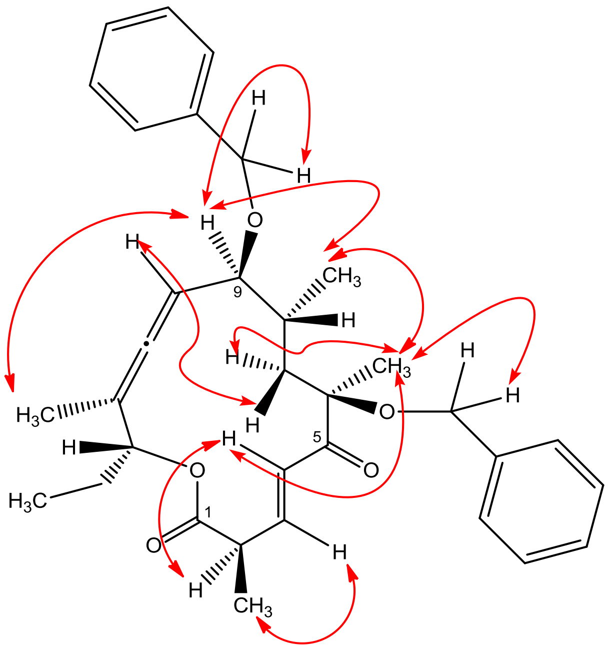
1d TOCSY


H
H
OBn
9
CH3
H
HIIII
H
CH3
OBn
5
O
H
H3C
H
H3C
O
1
O
H
CH3
H

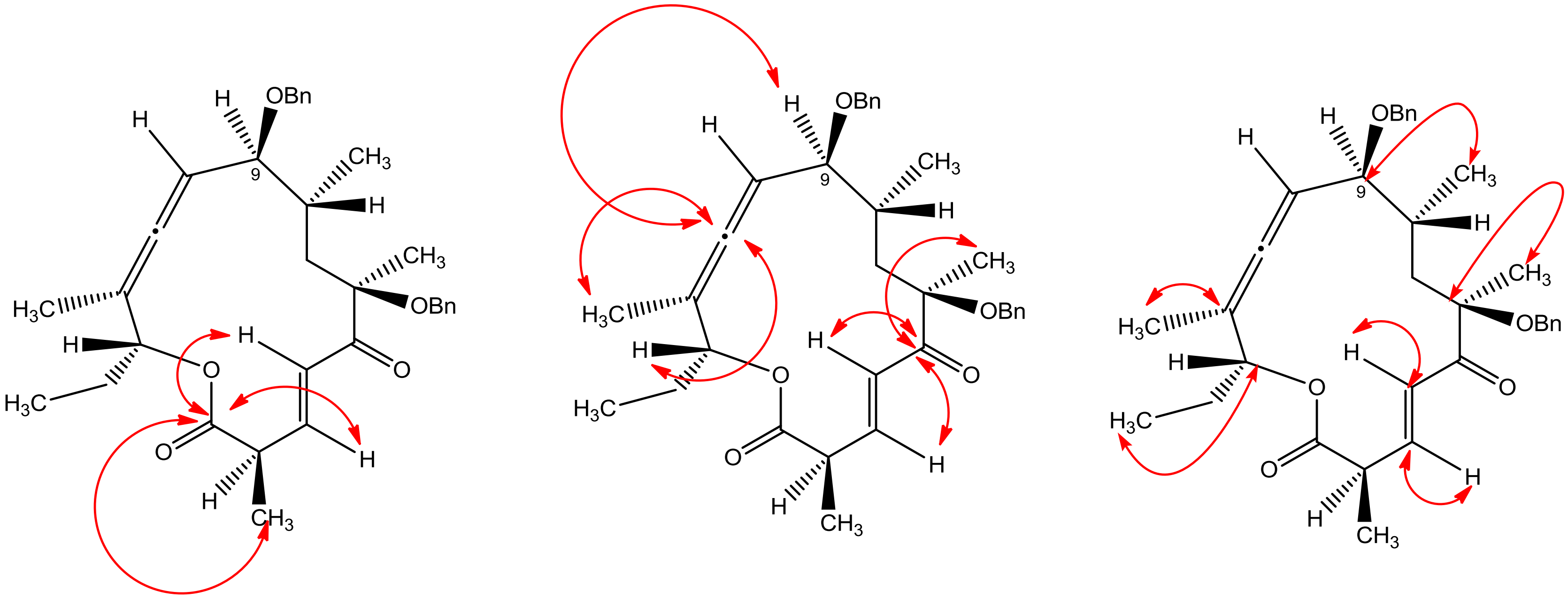
Coupling constant (J_{HH})

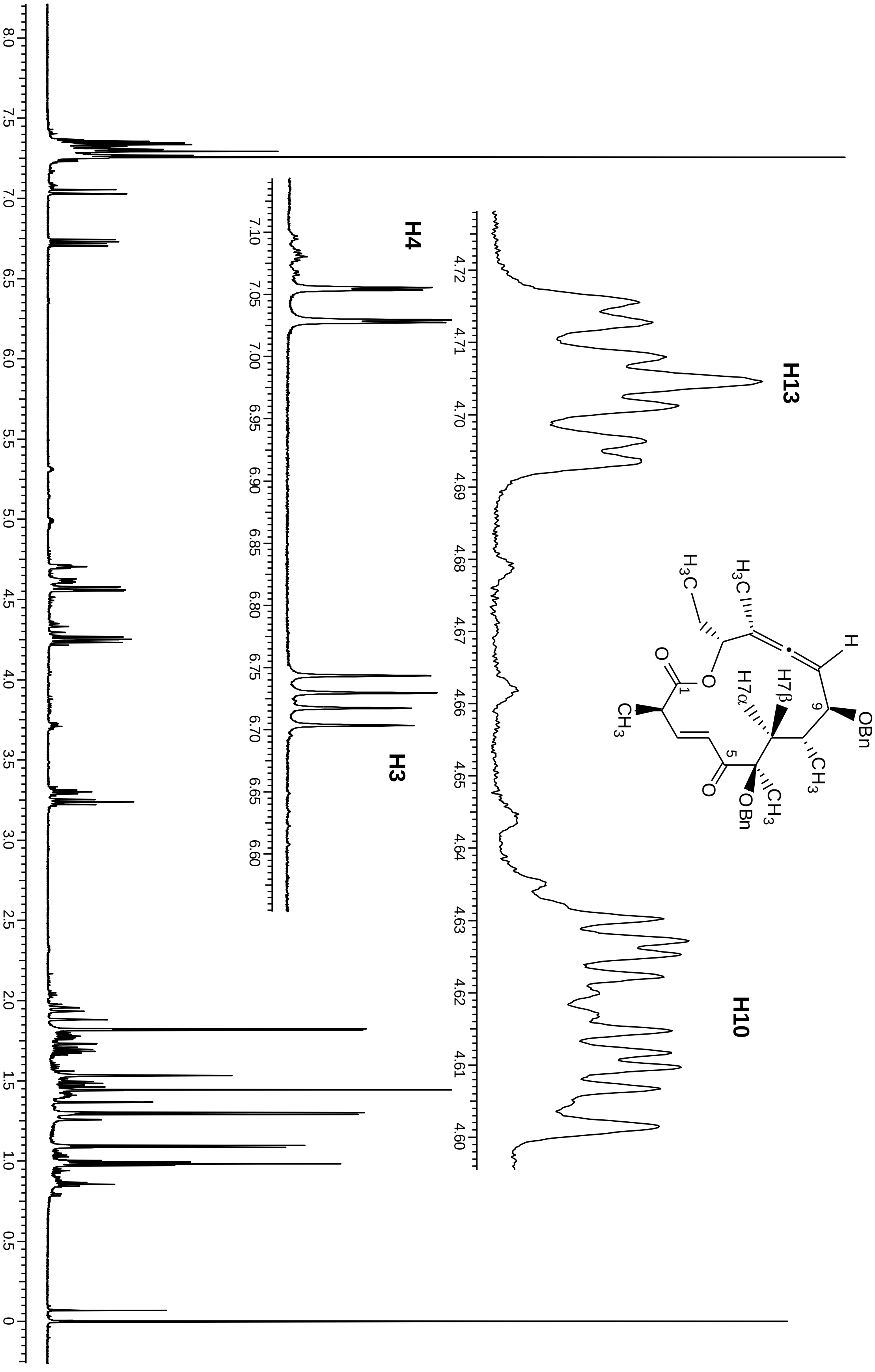

9.3Hz
H
H
O
9.1Hz
9
CH3
H
1.1Hz
H7β
H7α
CH3
O
6.7Hz
H3C
H
13
5
H
O
H3C
14
O
1
15.7Hz
O
H
H
CH3
8.2Hz

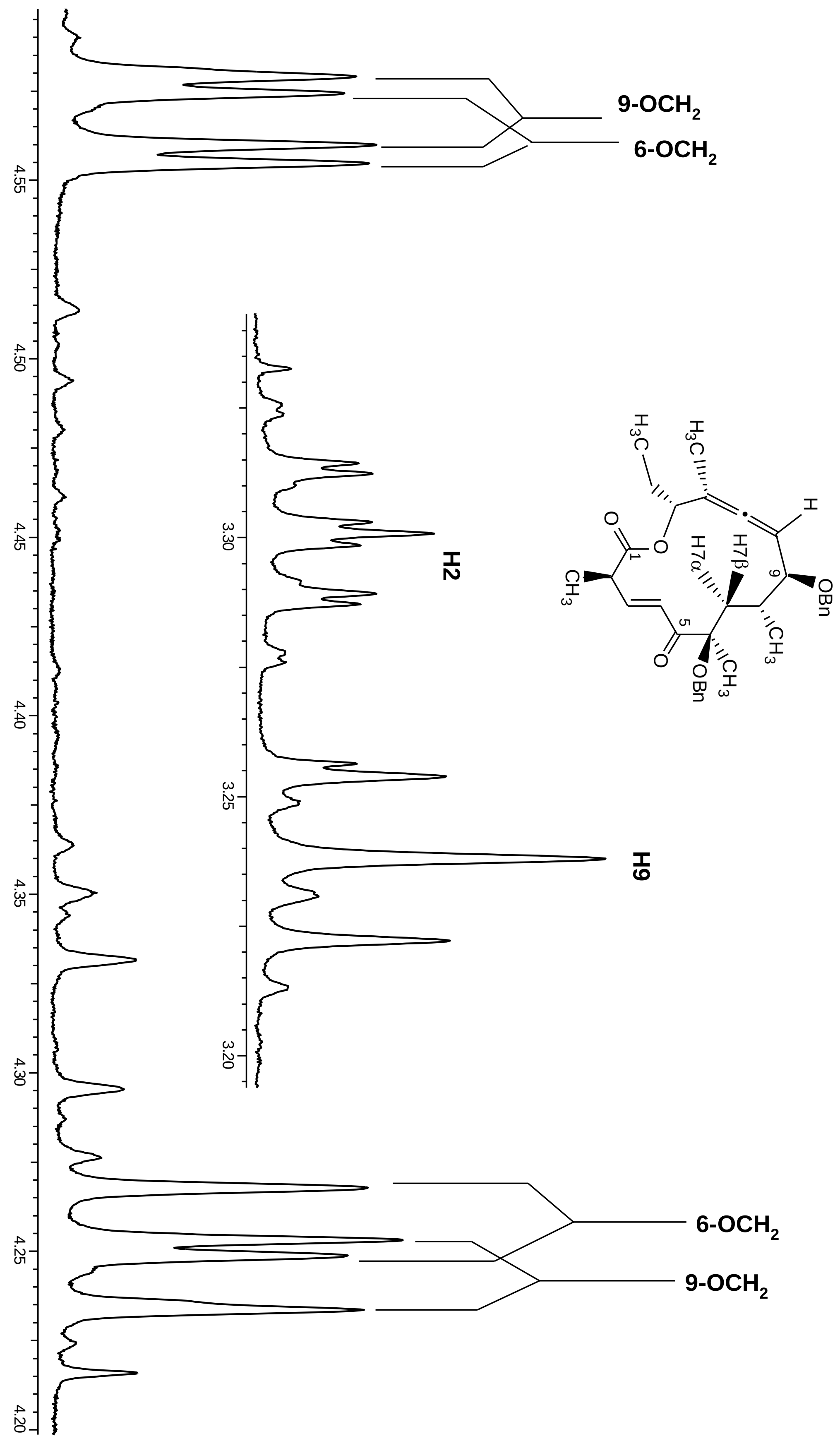
NOESY

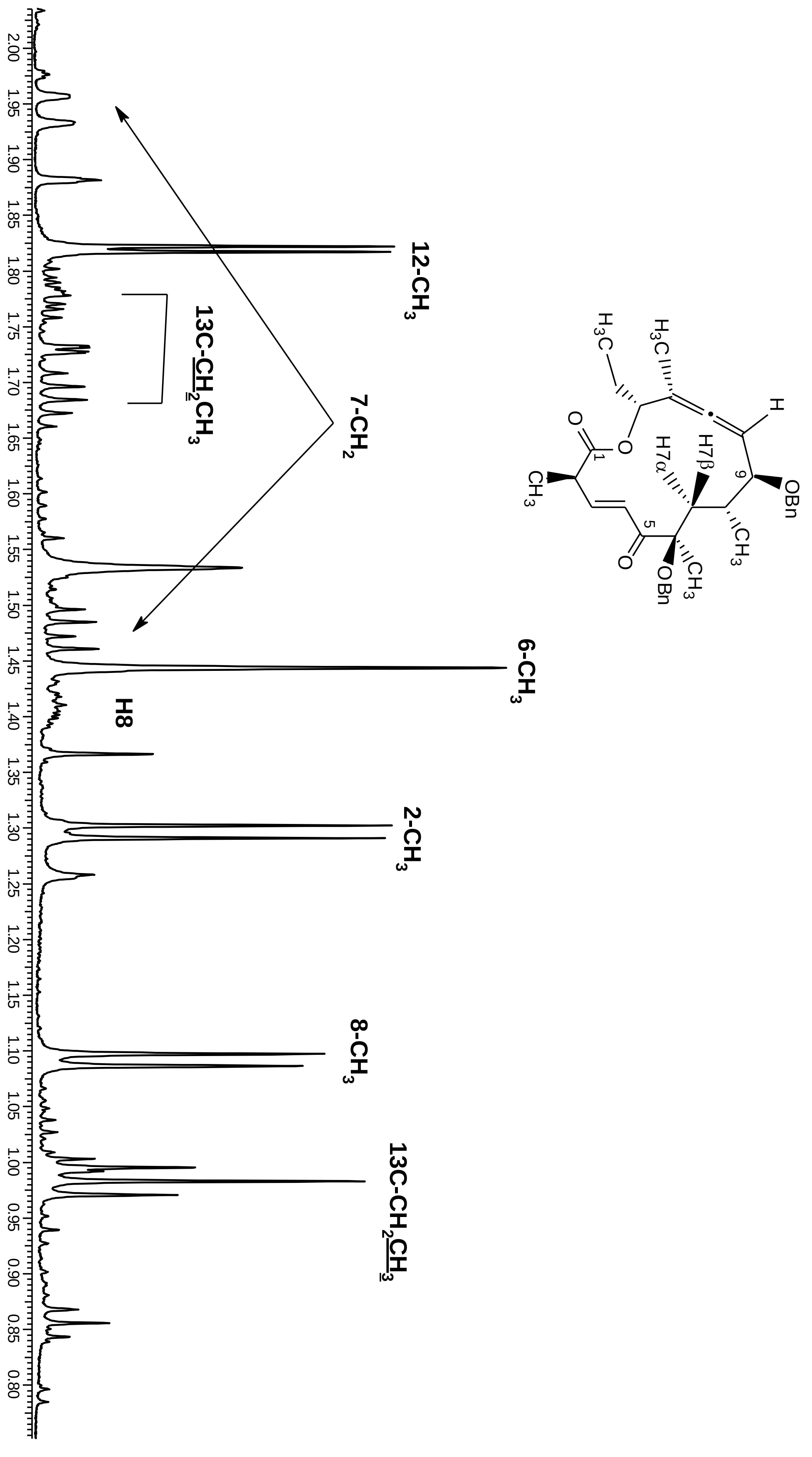

H
H
H
O
H
9
CH3
H
H
CH3
H
H
H3C
O
H
H
5
H3C
O
O
1
O
H
H
CH3

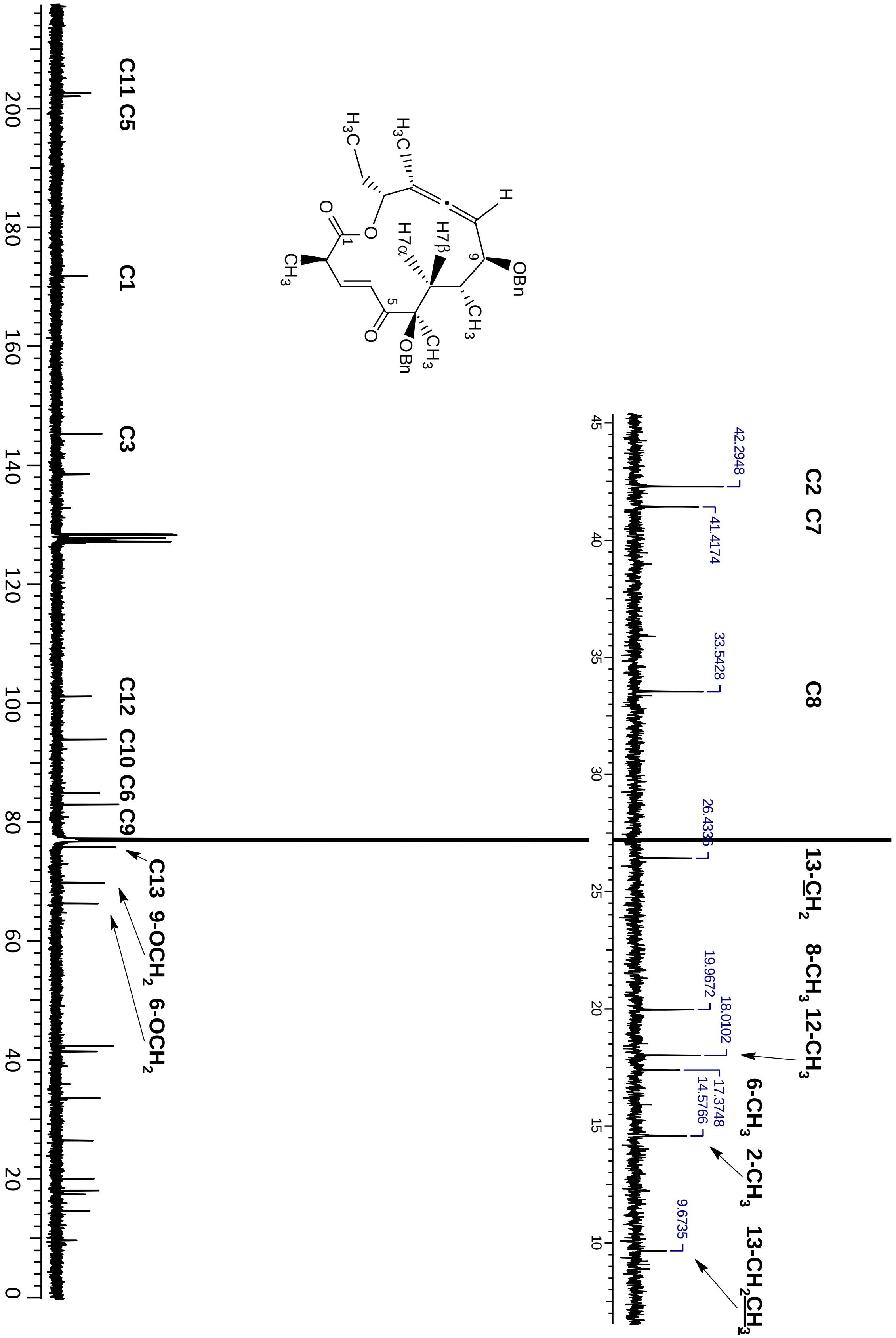
HMBC

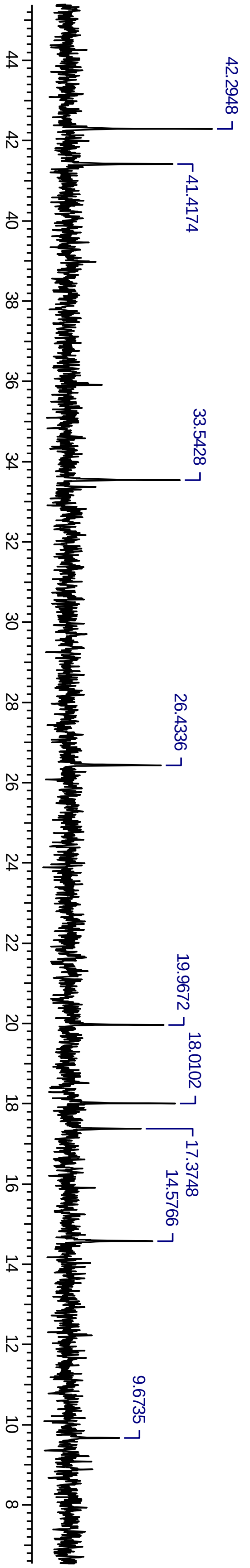

H
OBn
H
9
CH$_3$
H
CH$_3$
OBn
H$_3$C
H
O
H
O
H$_3$C
O
H
CH$_3$
H

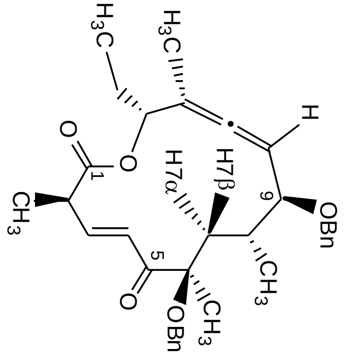

H13
H10
H4
H3
H
OBn
9
H7β
H7α
CH3
5
CH3
OBn
O
O
1
CH3
O
H3C
H3C
4.72
4.71
4.70
4.69
4.68
4.67
4.66
4.65
4.64
4.63
4.62
4.61
4.60
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
6.60
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

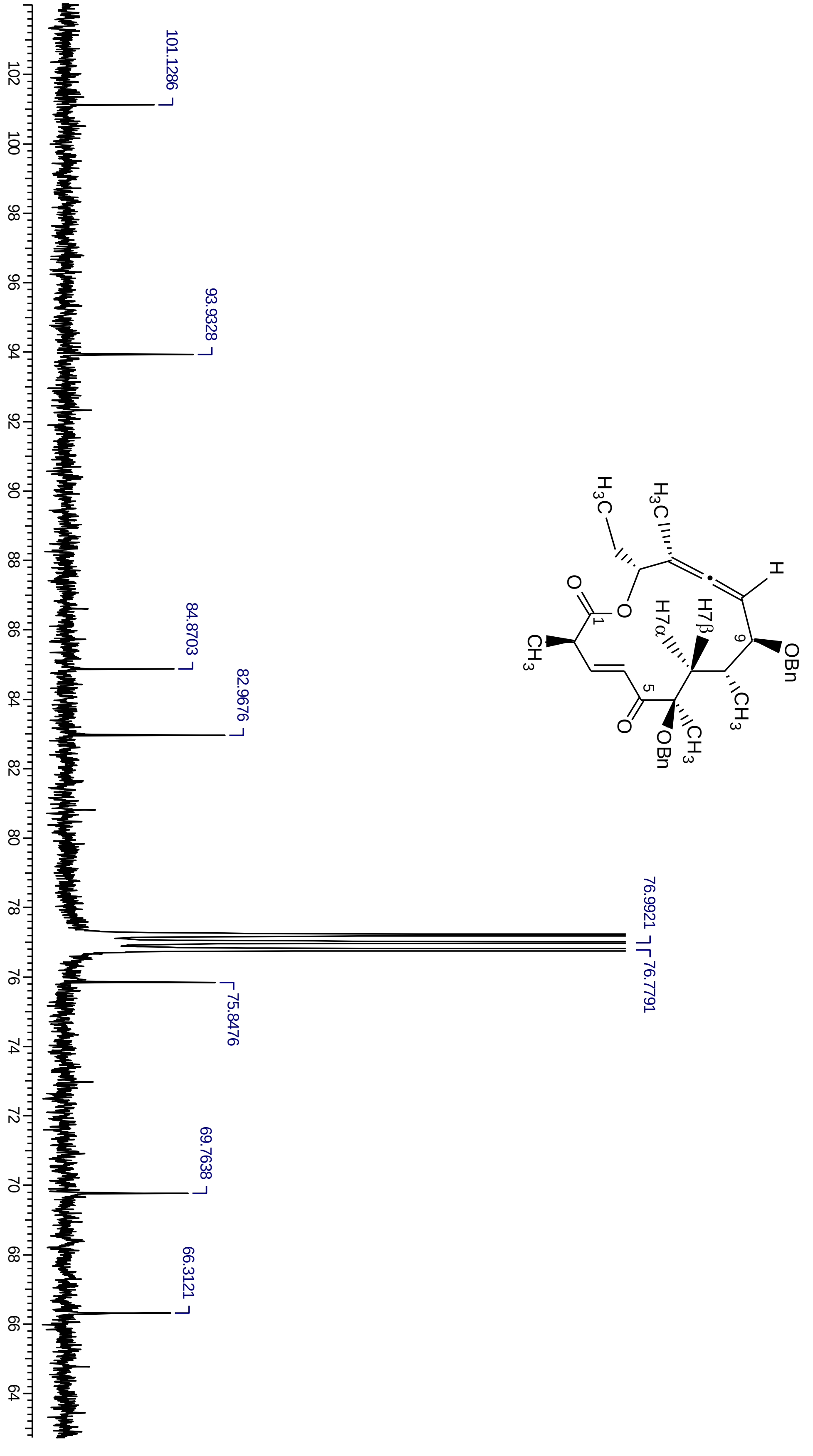

9-OCH$_2$
6-OCH$_2$
6-OCH$_2$
9-OCH$_2$
H2
H9
4.55
4.50
4.45
4.40
4.35
4.30
4.25
4.20
3.30
3.25
3.20
H$_3$C
H$_3$C
H
O
O
1
H7α
H7β
9
OBn
CH$_3$
5
CH$_3$
CH$_3$
O
OBn

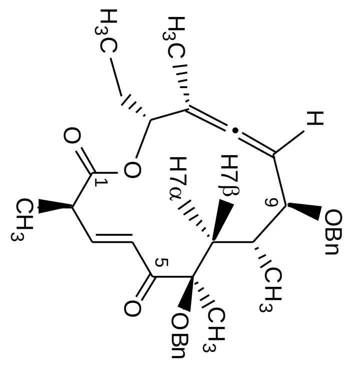

H
OBn
9
H7β
H7α
CH$_3$
5
O
OBn
CH$_3$
1
O
CH$_3$
O
H$_3$C
H$_3$C
12-CH$_3$
13C-CH$_2$CH$_3$
7-CH$_2$
6-CH$_3$
H8
2-CH$_3$
8-CH$_3$
13C-CH$_2$CH$_3$
2.00
1.95
1.90
1.85
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85
0.80

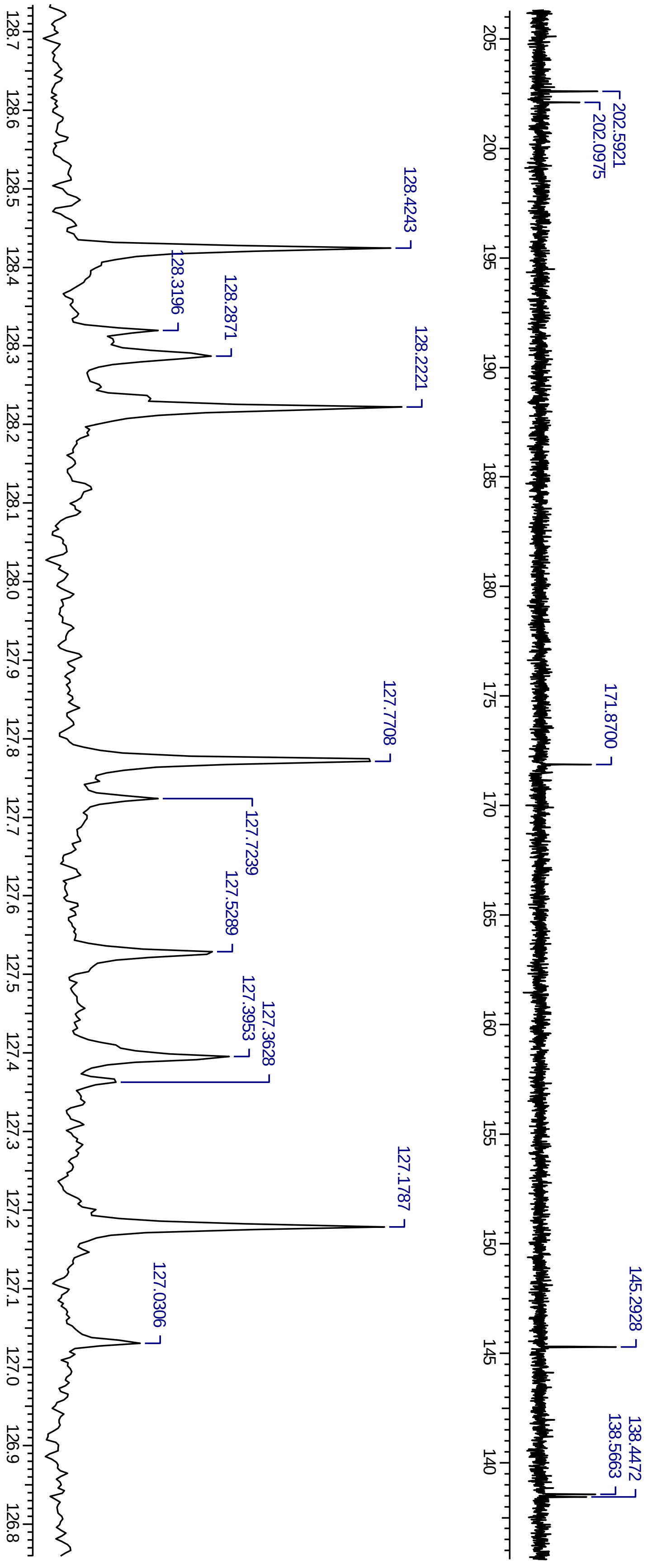

C11 C5
C1
C3
C12 C10 C6 C9
C13 9-OCH$_2$ 6-OCH$_2$
200
180
160
140
120
100
80
60
40
20
0
C2 C7
C8
13-CH$_2$ 8-CH$_3$ 12-CH$_3$
6-CH$_3$ 2-CH$_3$ 13-CH$_2$CH$_3$
42.2948
41.4174
33.5428
26.4336
19.9672
18.0102
17.3748
14.5766
9.6735
45
40
35
30
25
20
15
10
H$_3$C
H$_3$C
H
OBn
H7β
H7α
9
CH$_3$
O
O
1
CH$_3$
5
CH$_3$
O
OBn


42.2948
41.4174
33.5428
26.4336
19.9672
18.0102
17.3748
14.5766
9.6735
44
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8

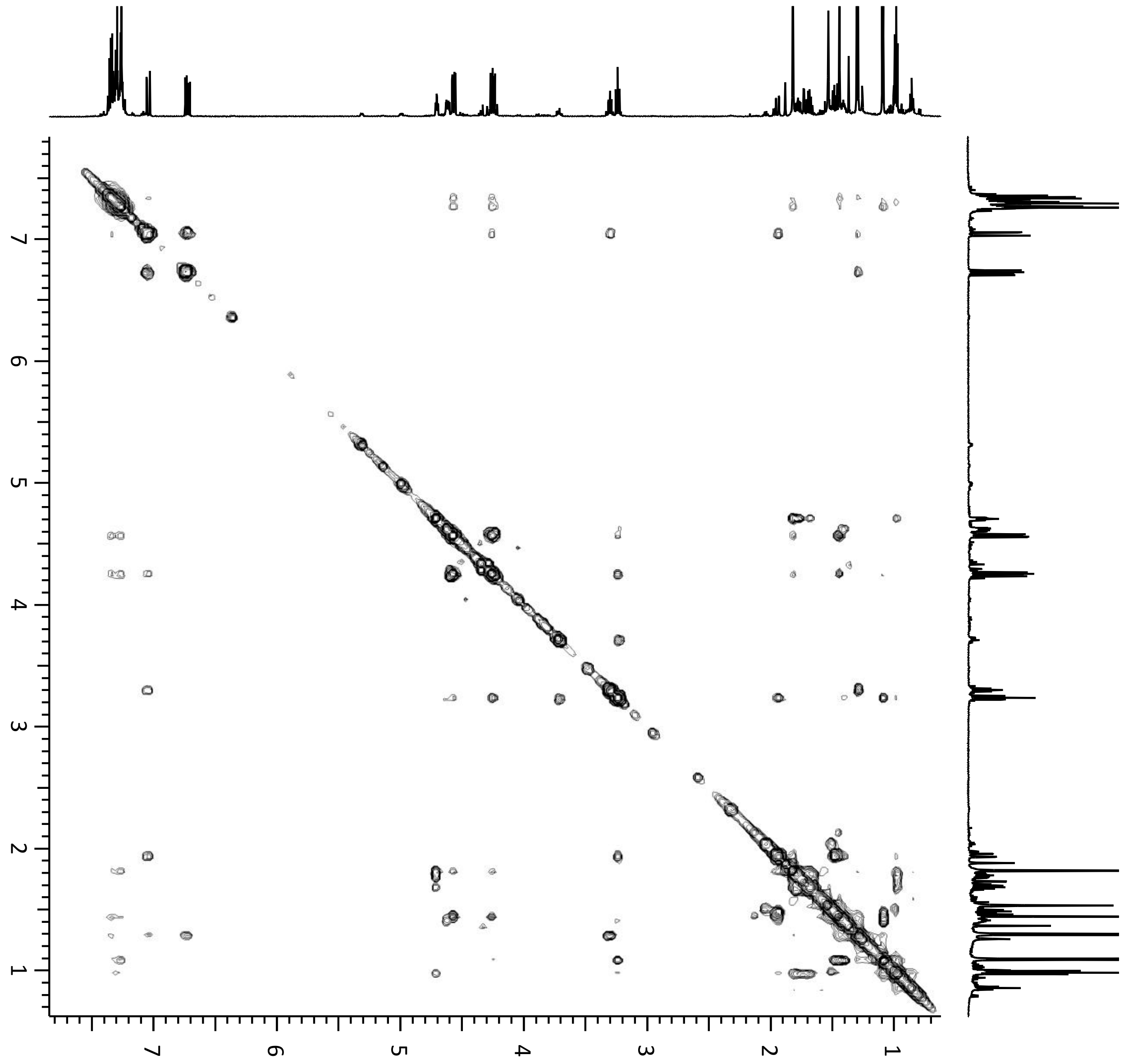

H$_3$C
H$_3$C
H
O
O
1
H7β
H7α
9
OBn
CH$_3$
5
O
OBn
CH$_3$
CH$_3$

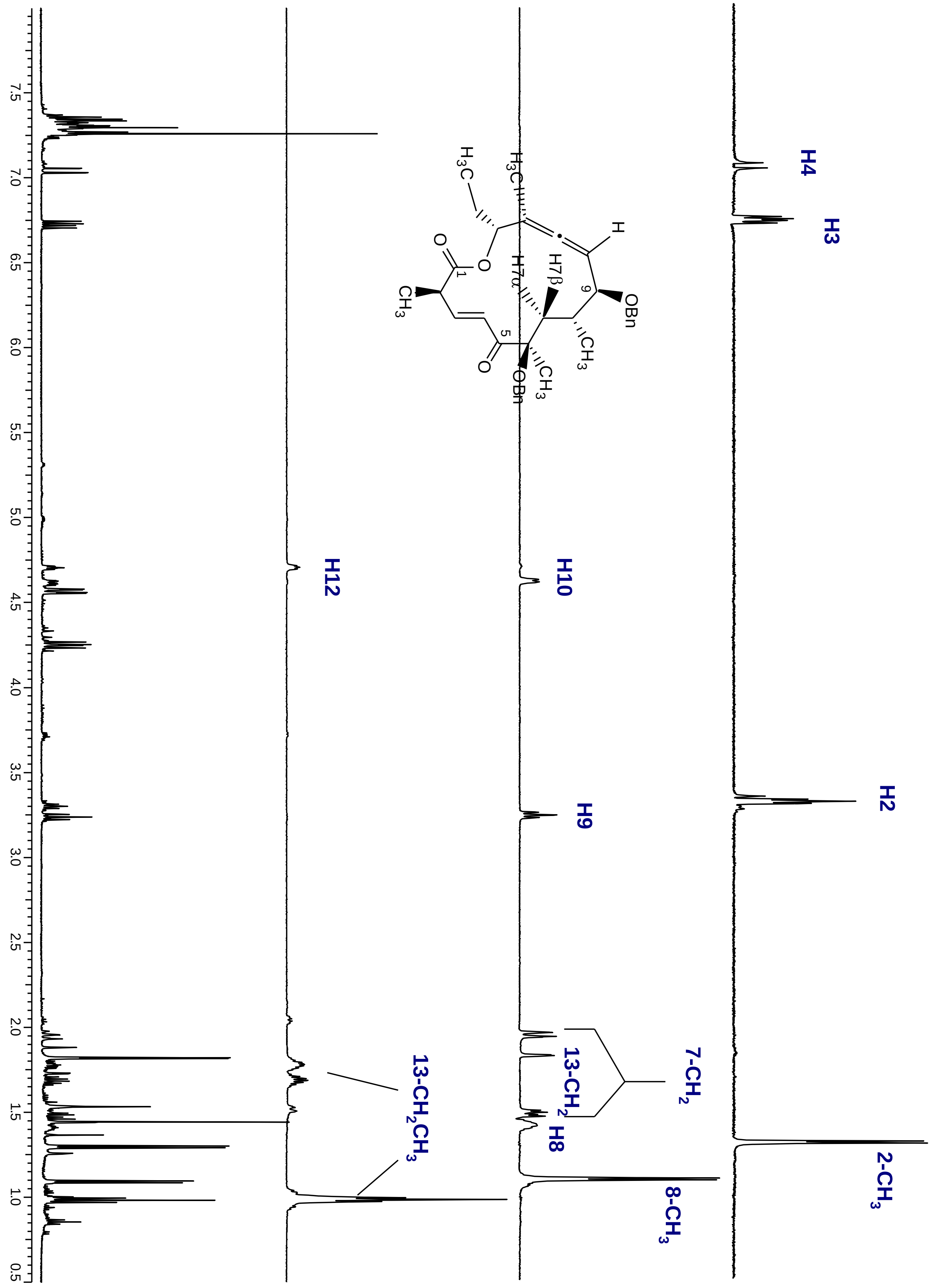

101.1286
93.9328
84.8703
82.9676
76.9921
76.7791
75.8476
69.7638
66.3121
102
100
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
66
64
H
OBn
9
H7β
H7α
CH3
CH3
OBn
5
O
1
O
O
CH3
H3C
H3C


H
OBn
9
CH_3
CH_3
OBn
5
O
H7β
H7α
H_3C
H_3C
O
1
O
CH_3

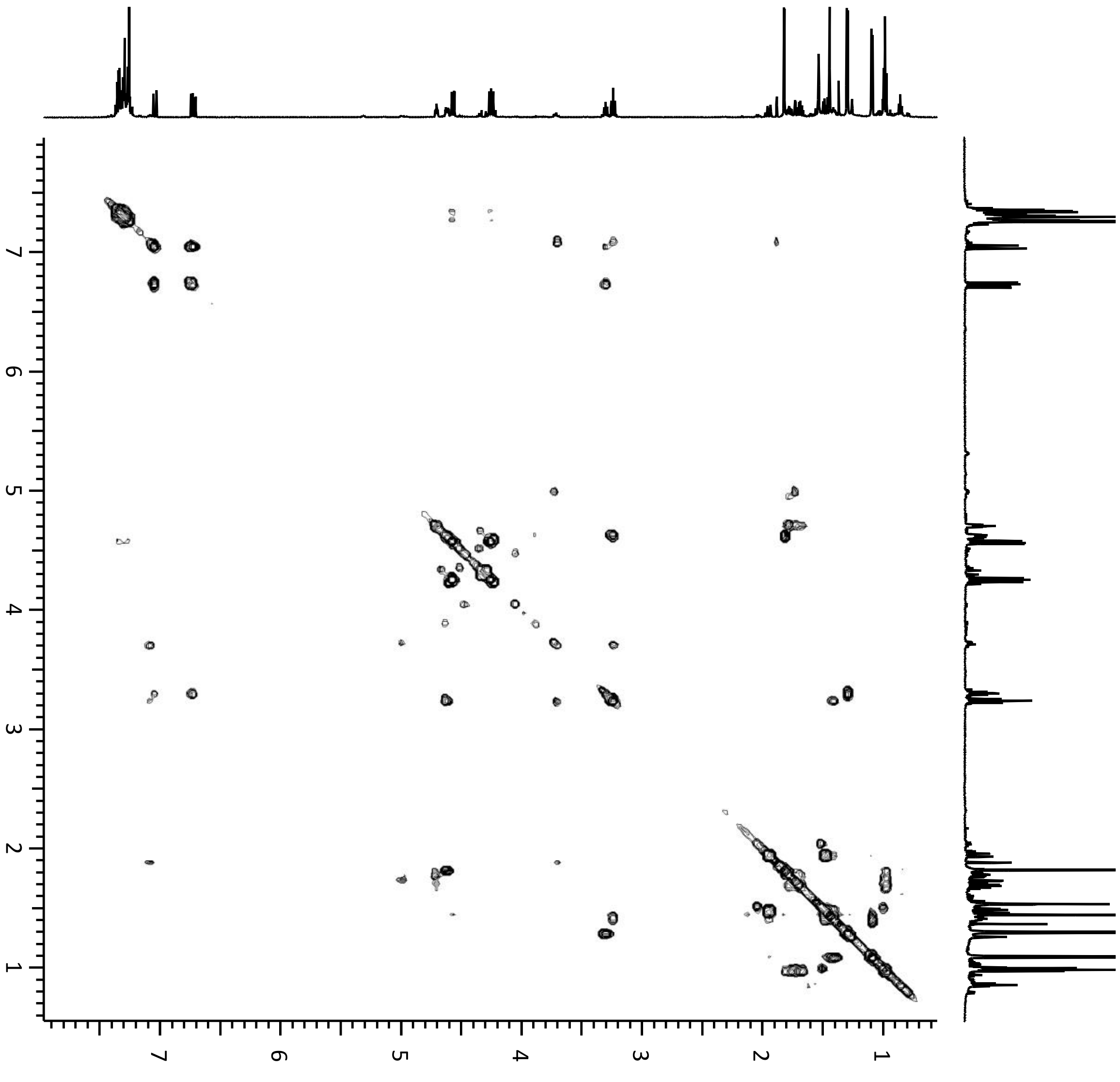

202.5921
202.0975
171.8700
145.2928
138.4472
138.5663
205
200
195
190
185
180
175
170
165
160
155
150
145
140
128.4243
128.3196
128.2871
128.2221
127.7708
127.7239
127.5289
127.3953
127.3628
127.1787
127.0306
128.7
128.6
128.5
128.4
128.3
128.2
128.1
128.0
127.9
127.8
127.7
127.6
127.5
127.4
127.3
127.2
127.1
127.0
126.9
126.8

NOESY



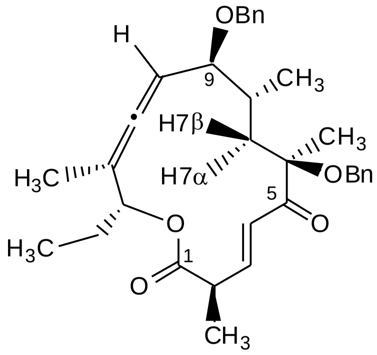

H4
H3
H2
2-CH_3
H12
H10
H9
7-CH_2
13-CH_2
H8
8-CH_3
13-CH_2CH_3
OBn
OBn
CH_3
CH_3
CH_3
H_3C
H_3C
H
H7β
H7α
O
O
O
1
5
9
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

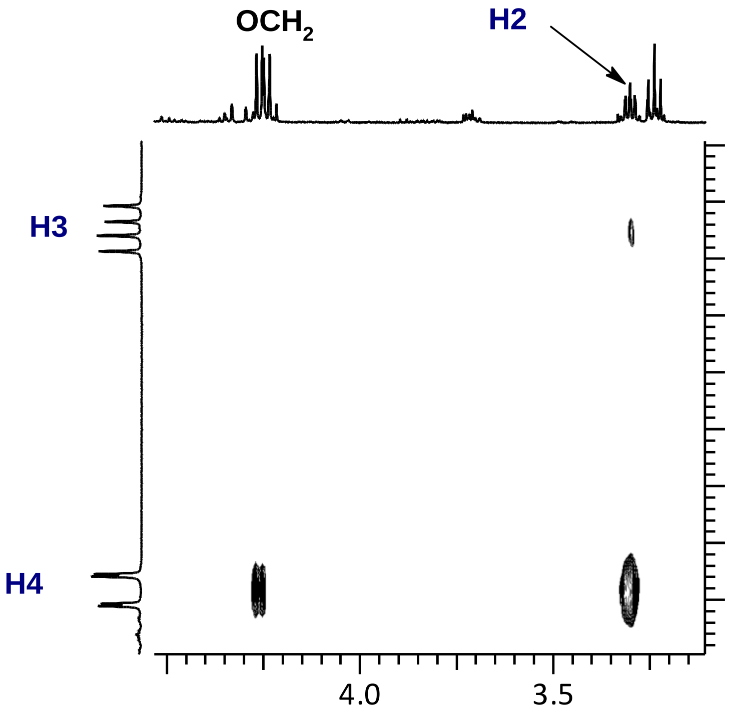
gCOSY

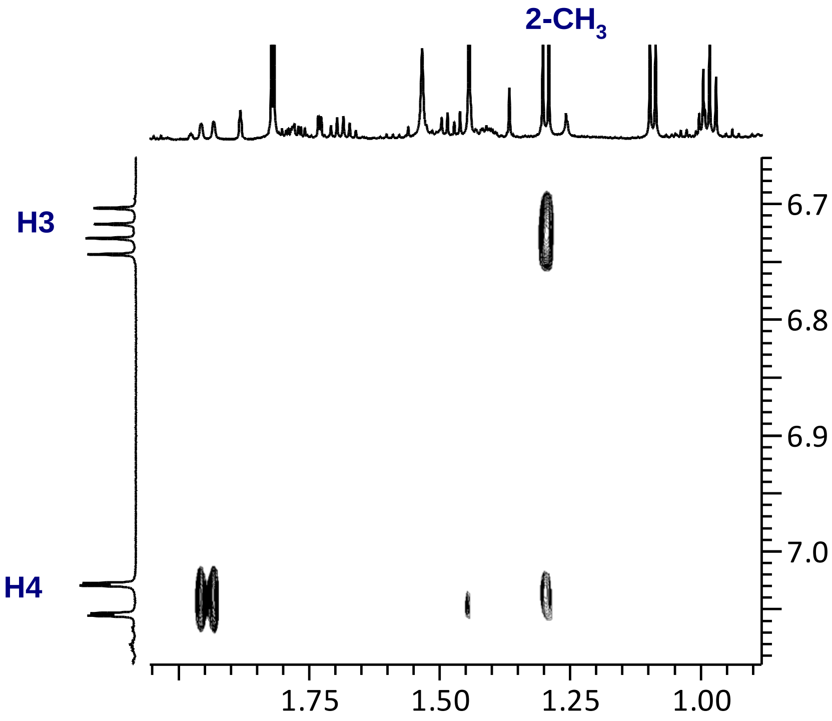


NOESY

expansions

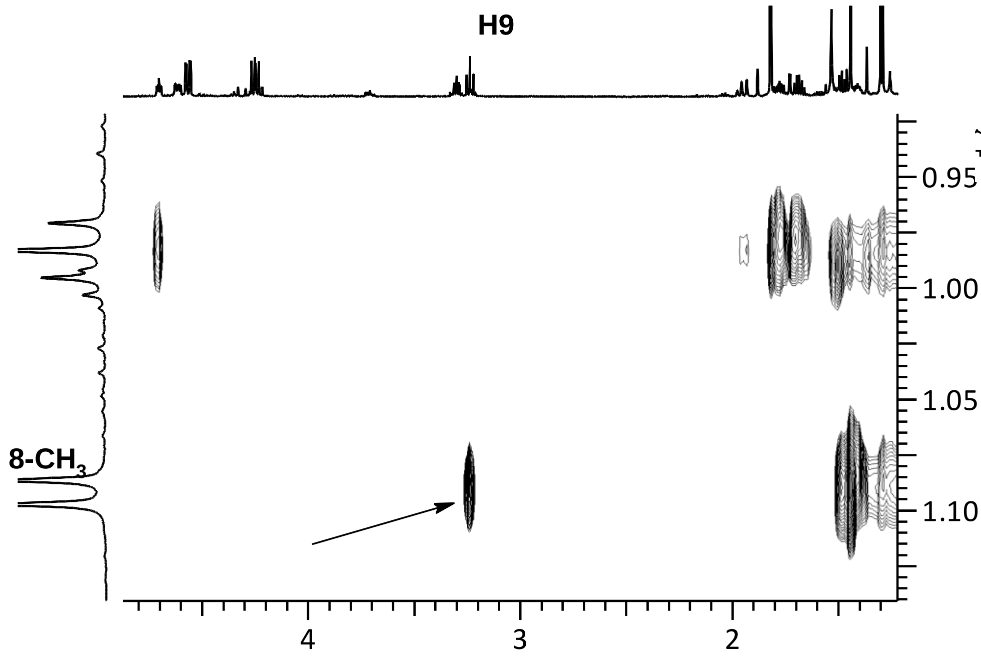

OBn
H
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
H3C
O
1
O
CH3

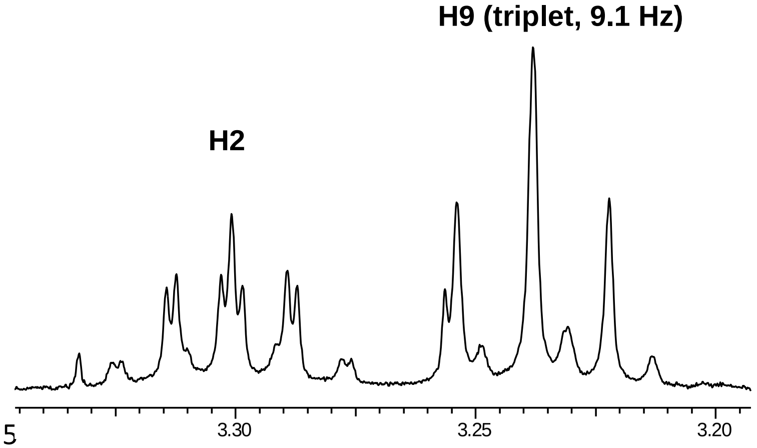


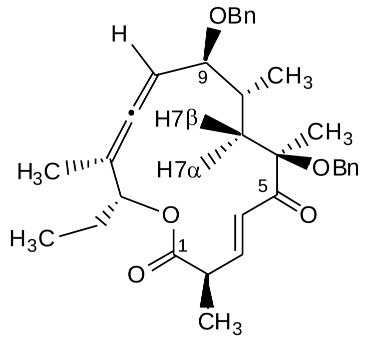


NOESY

expansion

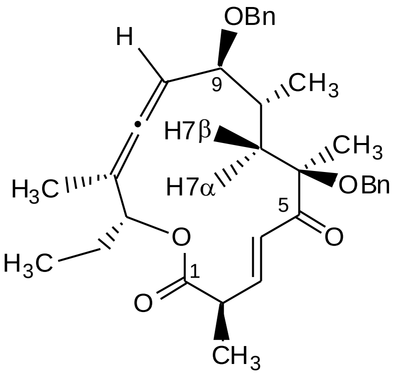


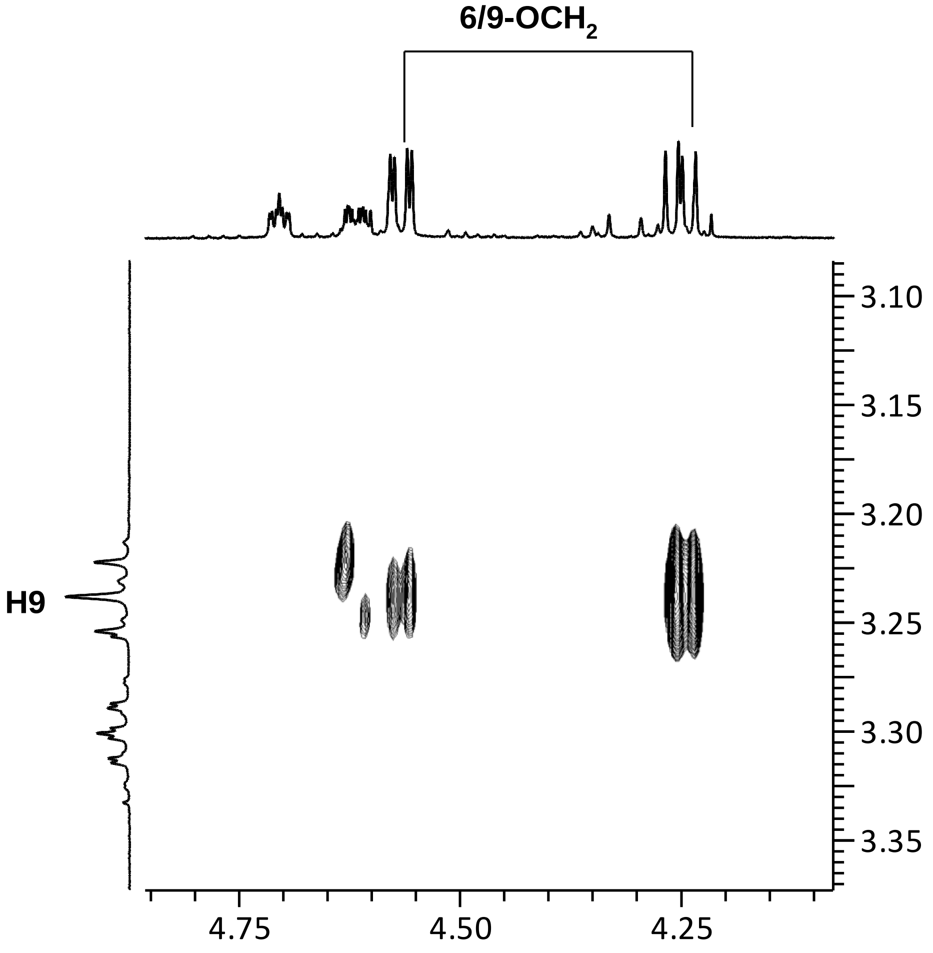



H
OBn
9
CH_3
H7β
CH_3
H7α
OBn
H_3C
5
O
O
H_3C
1
O
CH_3

NOESY

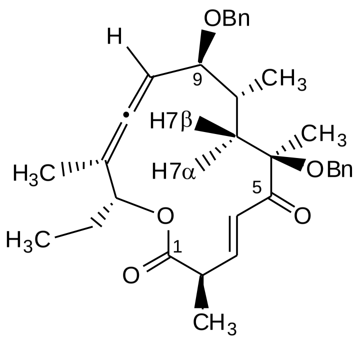
expansion

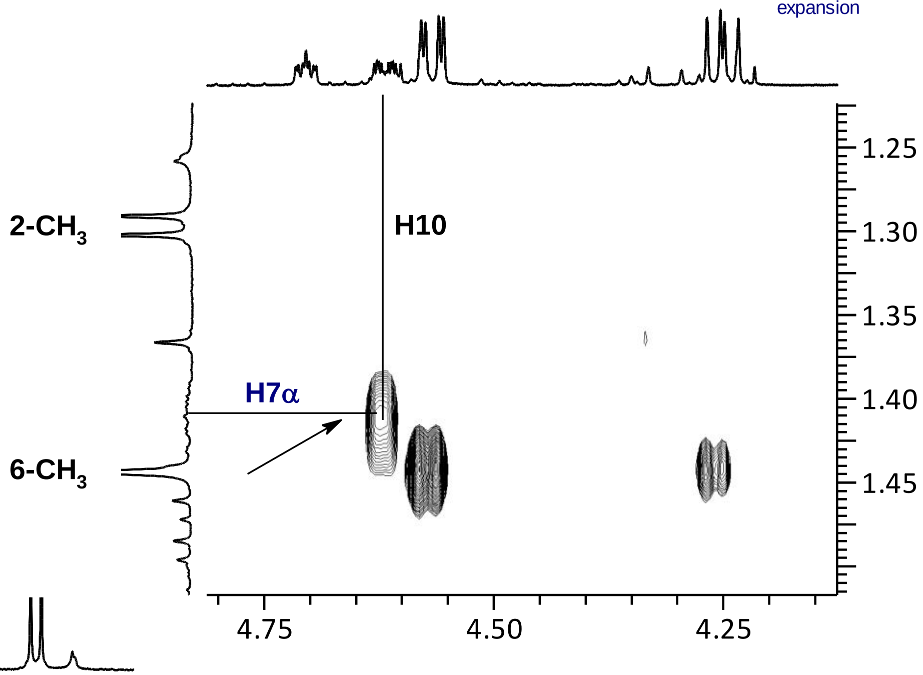

H
OBn
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
H3C
O
1
O
CH3

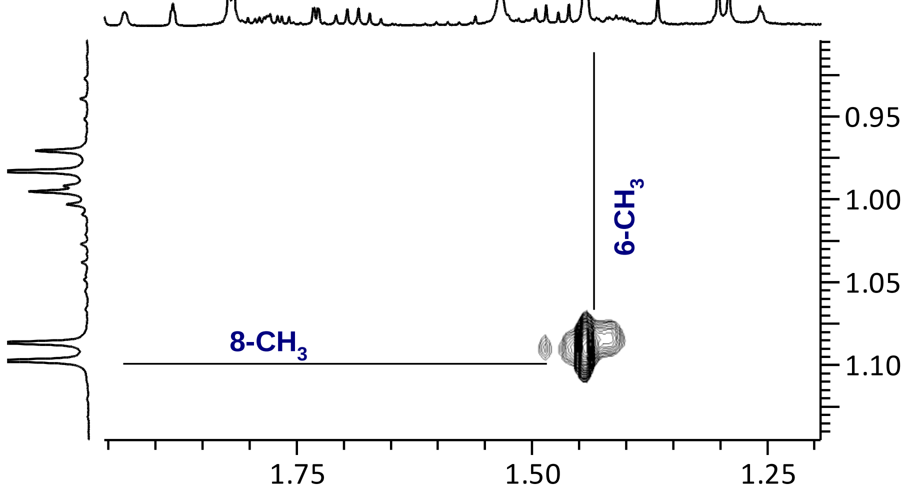


NOESY

expansion

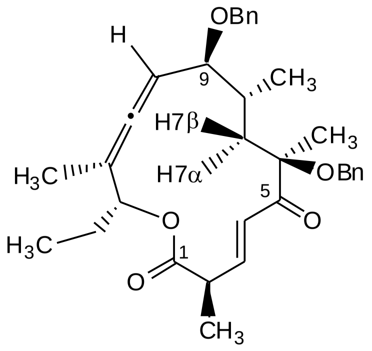

OBn
H
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
O
H3C
1
O
CH3



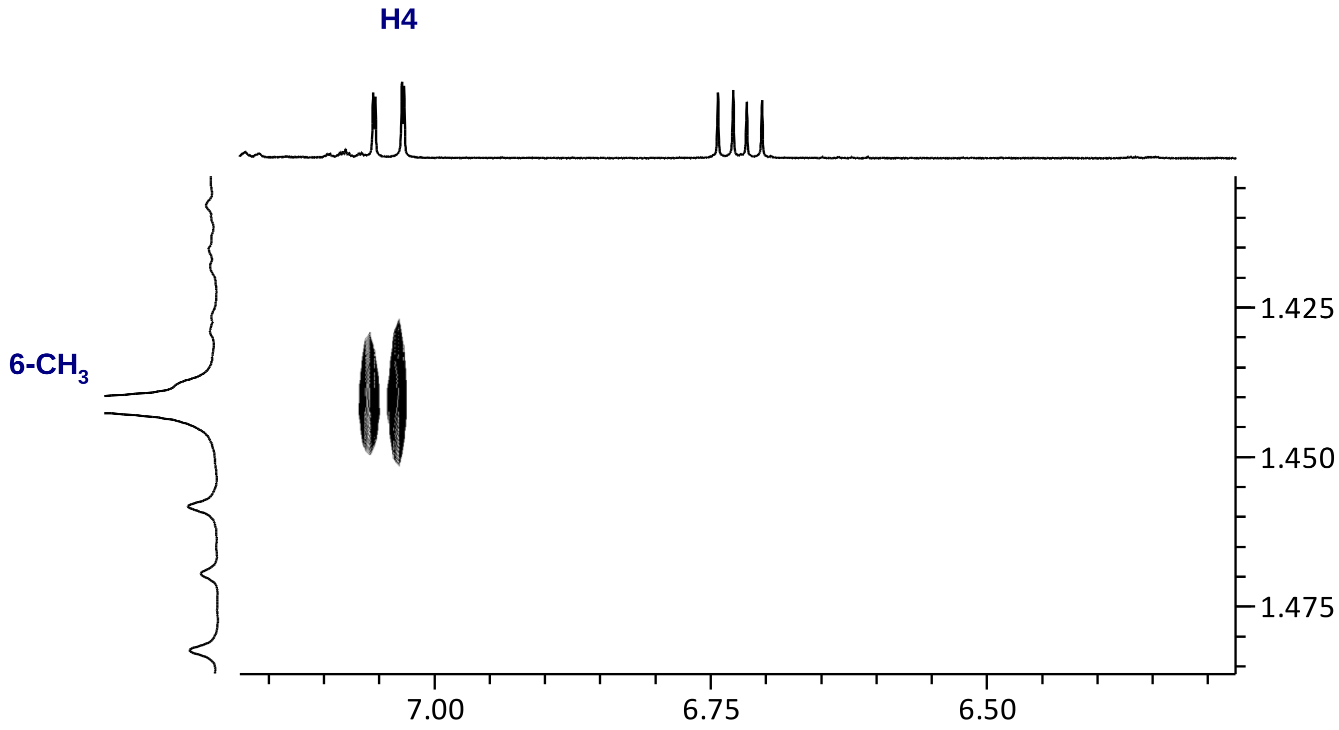


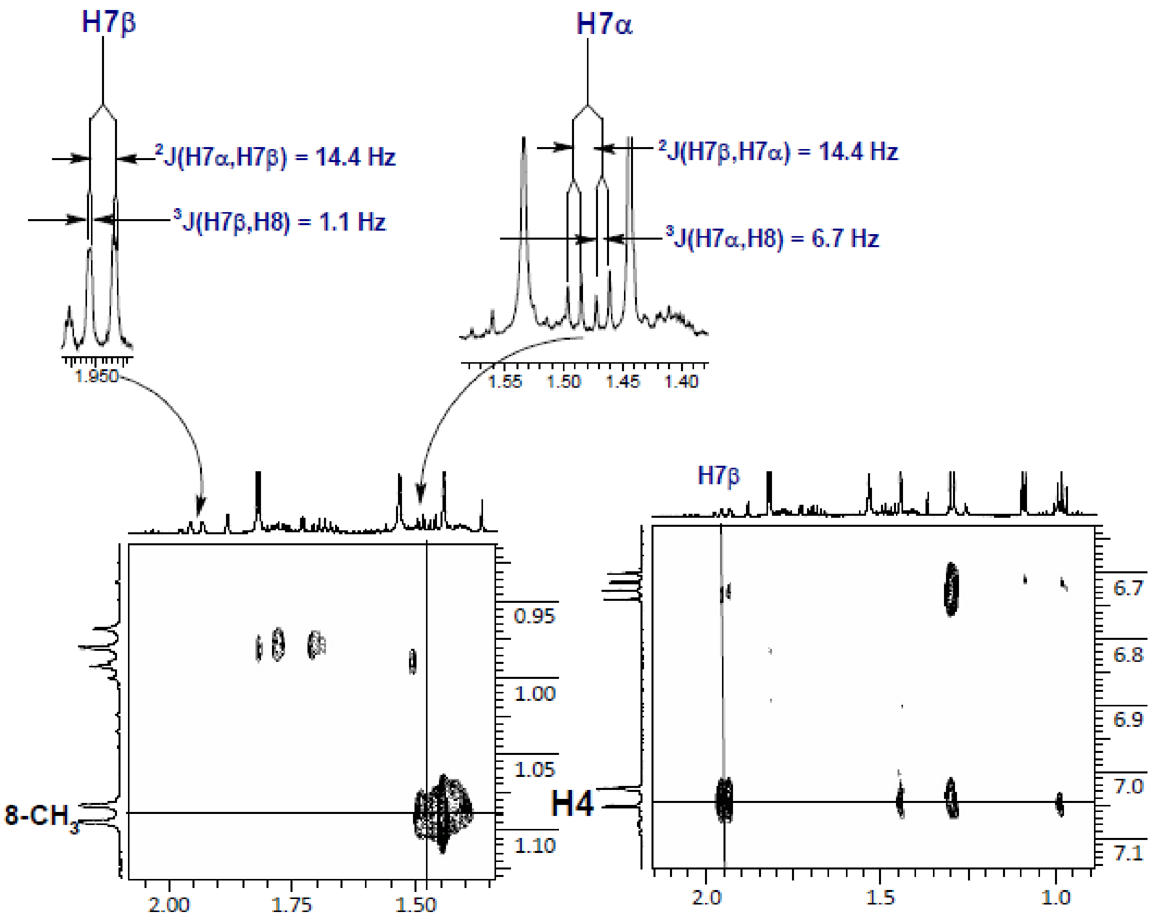
NOESY

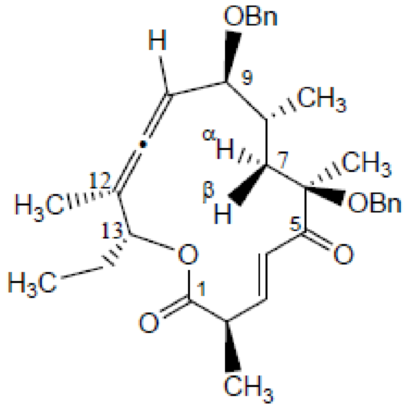
expansion

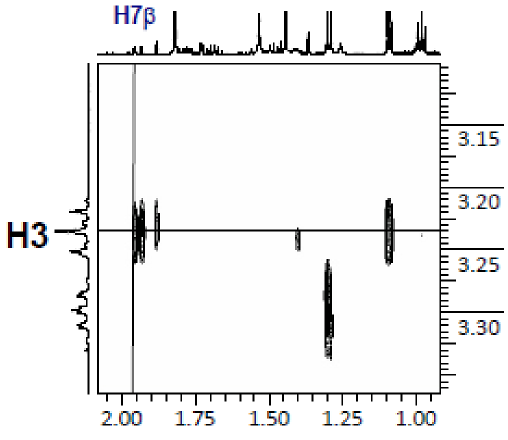


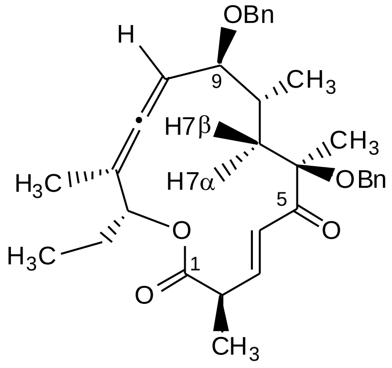
OBn
H
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
H3C
1
O
O
CH3

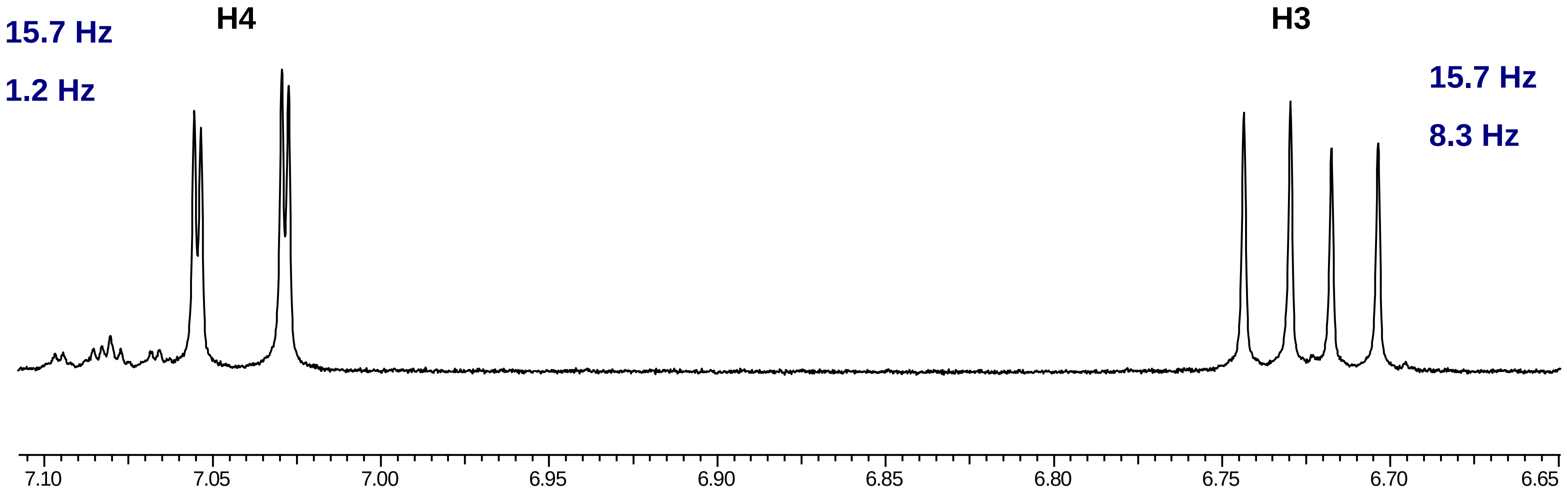



H7β
^2J(H7α,H7β) = 14.4 Hz
^3J(H7β,H8) = 1.1 Hz
1.950
H7α
^2J(H7β,H7α) = 14.4 Hz
^3J(H7α,H8) = 6.7 Hz
1.55
1.50
1.45
1.40
8-CH$_3$
0.95
1.00
1.05
1.10
2.00
1.75
1.50
H7β
H4
6.7
6.8
6.9
7.0
7.1
2.0
1.5
1.0


OBn
H
9
CH$_3$
α
H
7
CH$_3$
OBn
β
H
12
H$_3$C
13
5
O
O
1
H$_3$C
O
CH$_3$

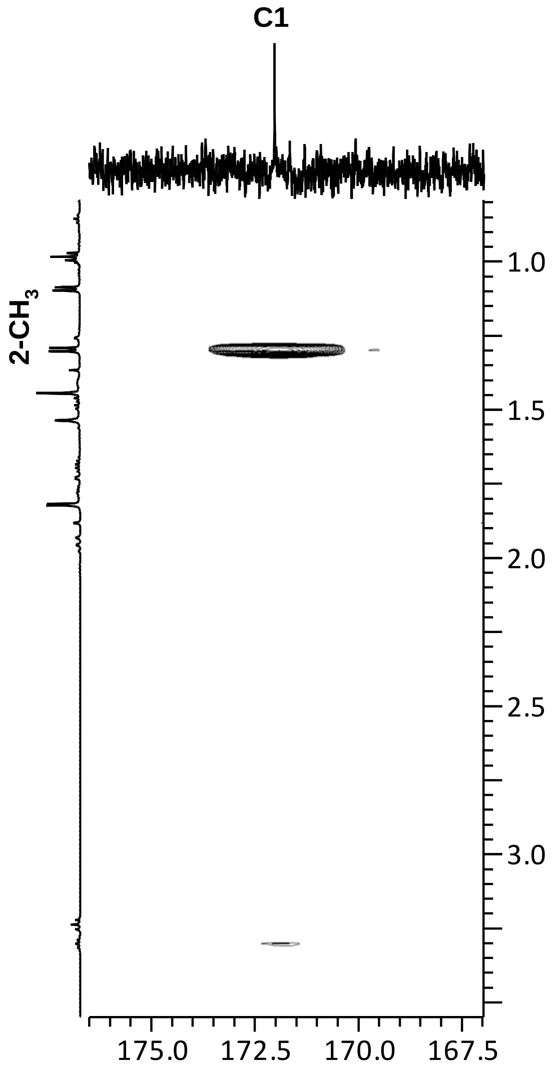

H7β
H3
3.15
3.20
3.25
3.30
2.00
1.75
1.50
1.25
1.00

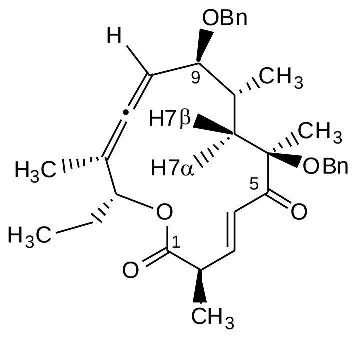

H
OBn
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
H3C
O
1
O
CH3

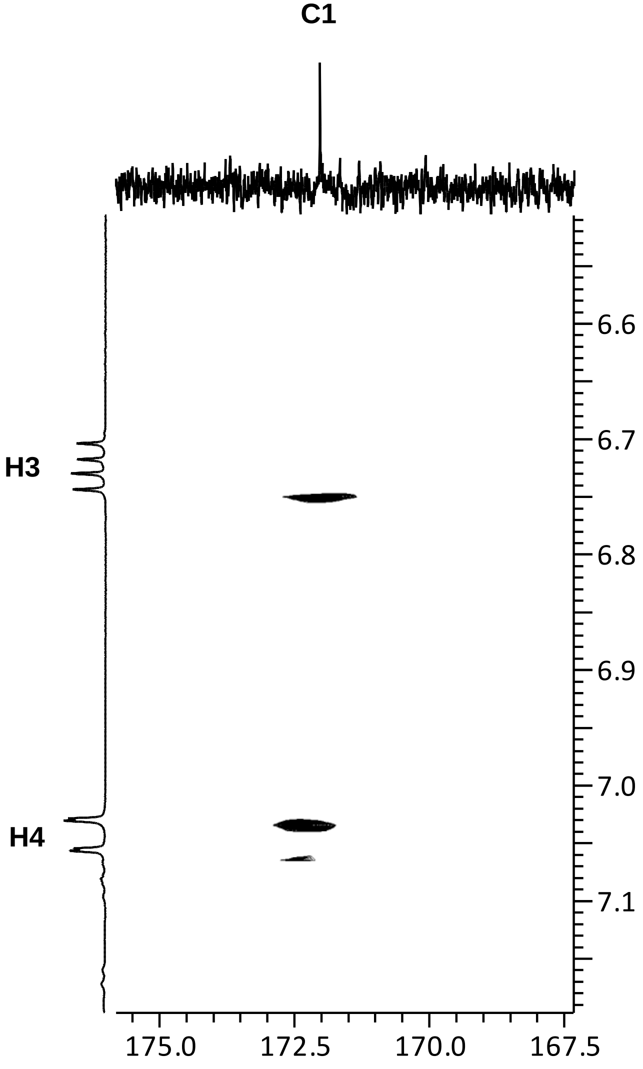

15.7 Hz
1.2 Hz
H4
H3
15.7 Hz
8.3 Hz
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65

gHMBC

correlations

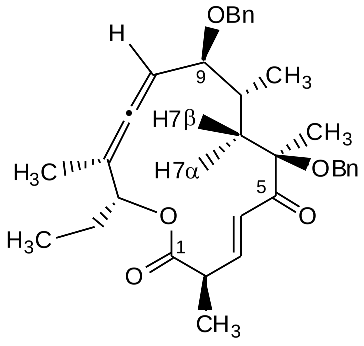


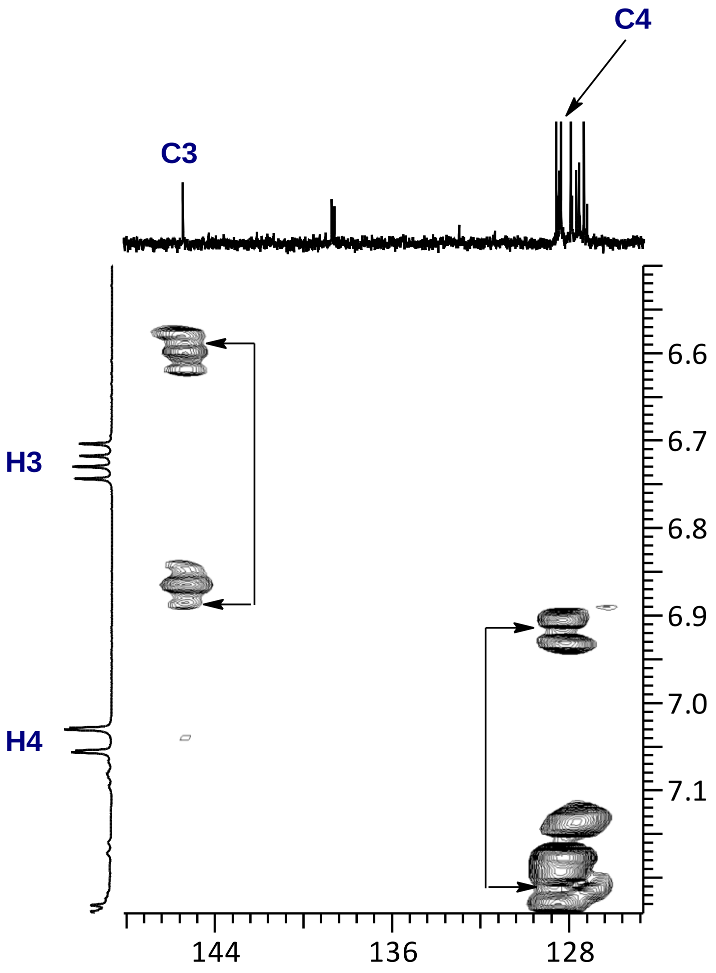

OBn
H
9
CH3
H7β
CH3
H7α
OBn
H3C
5
O
H3C
O
1
O
CH3



gHMBC

expansion

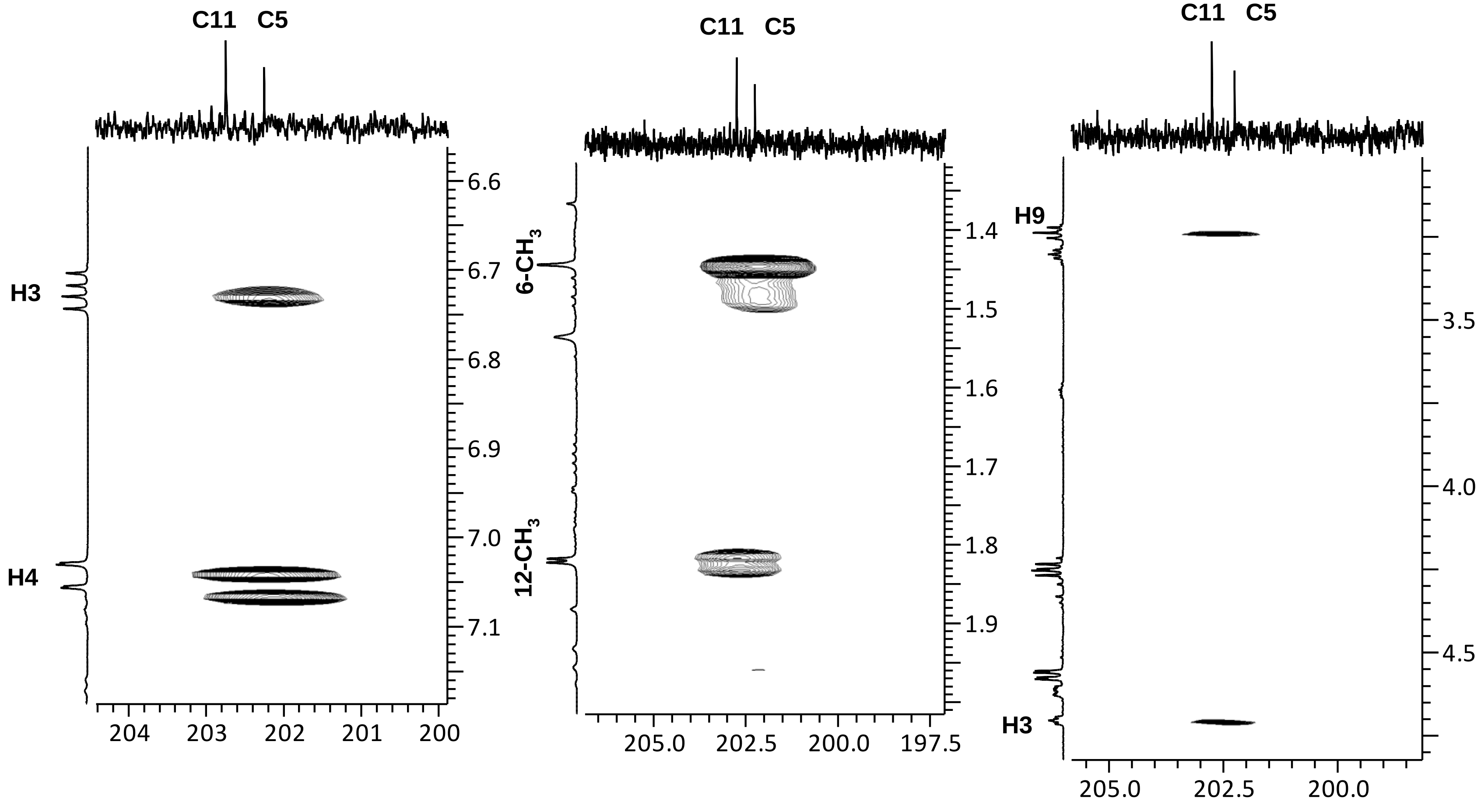

H
OBn
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
O
H3C
1
O
CH3

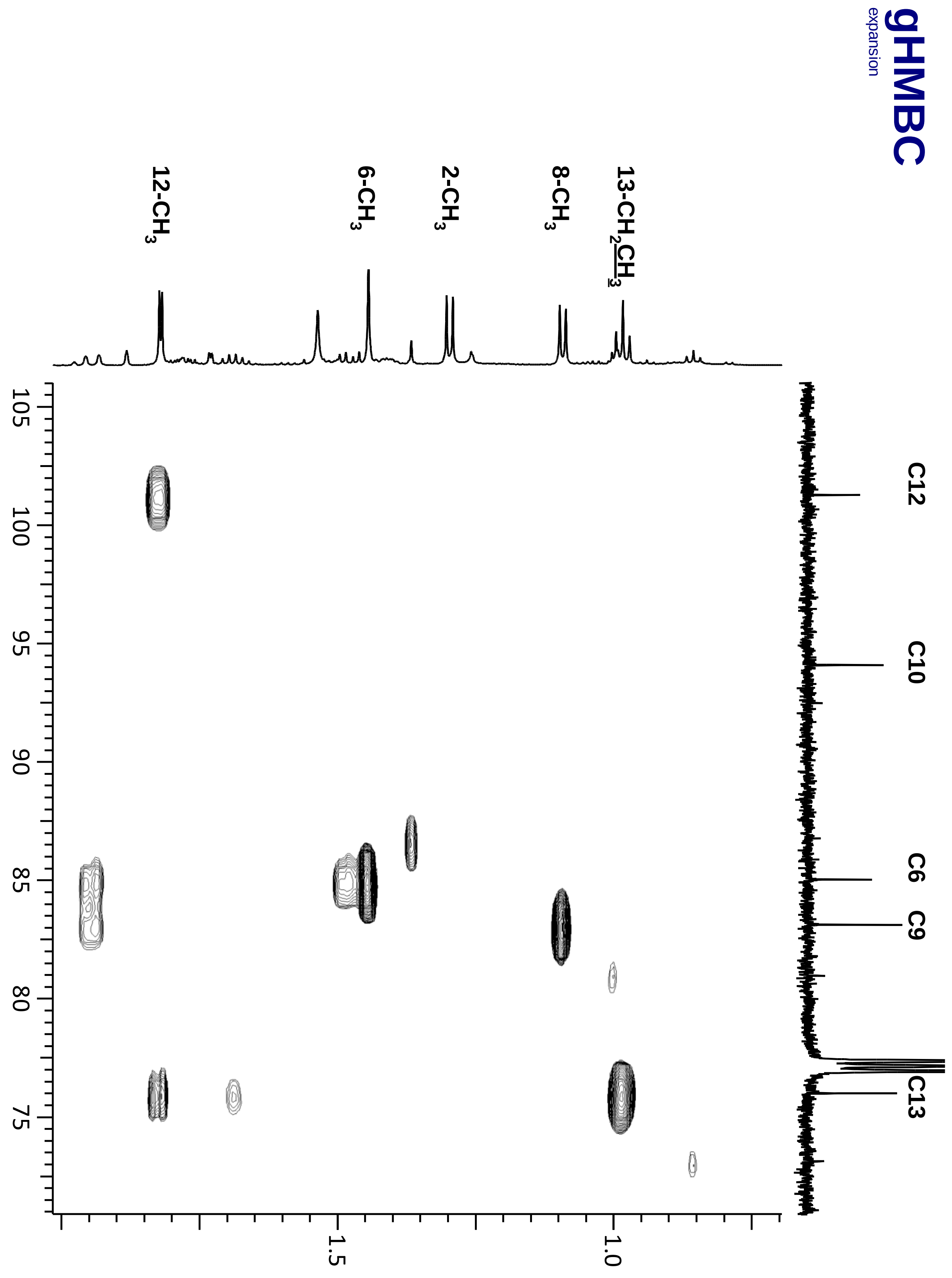


gHMBC

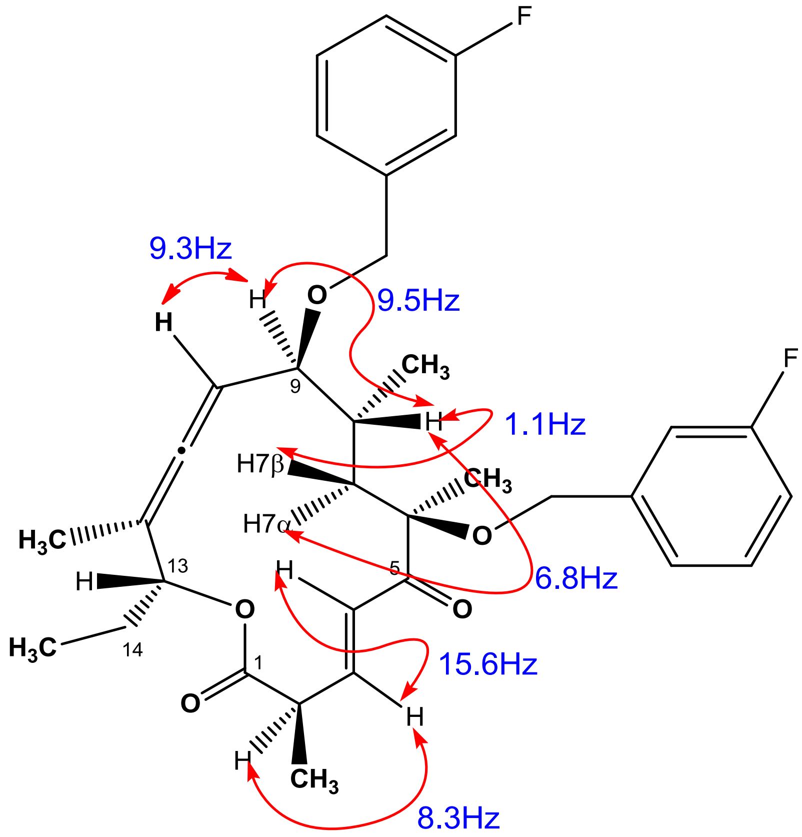
expansions

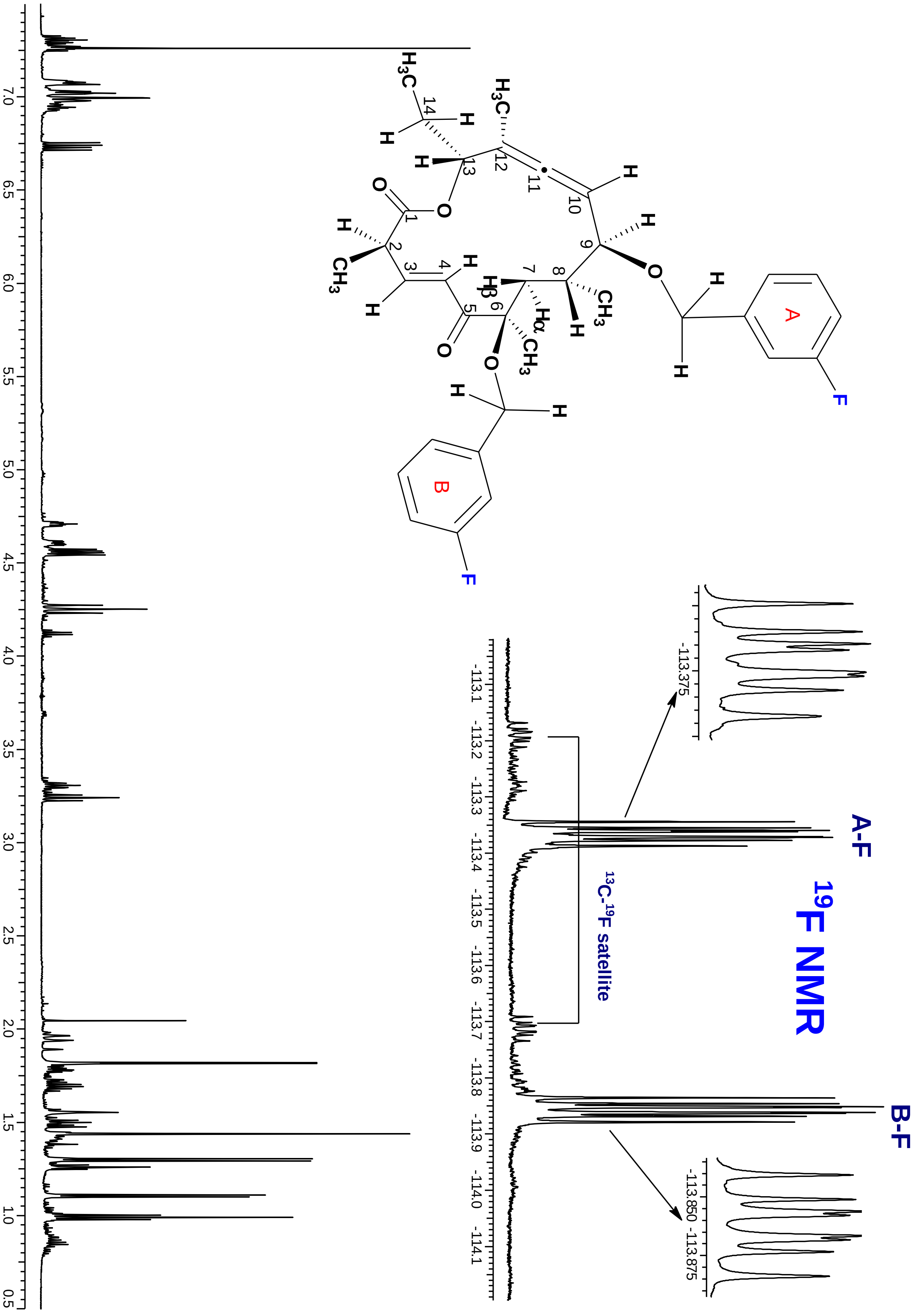
Assigments of CO groups



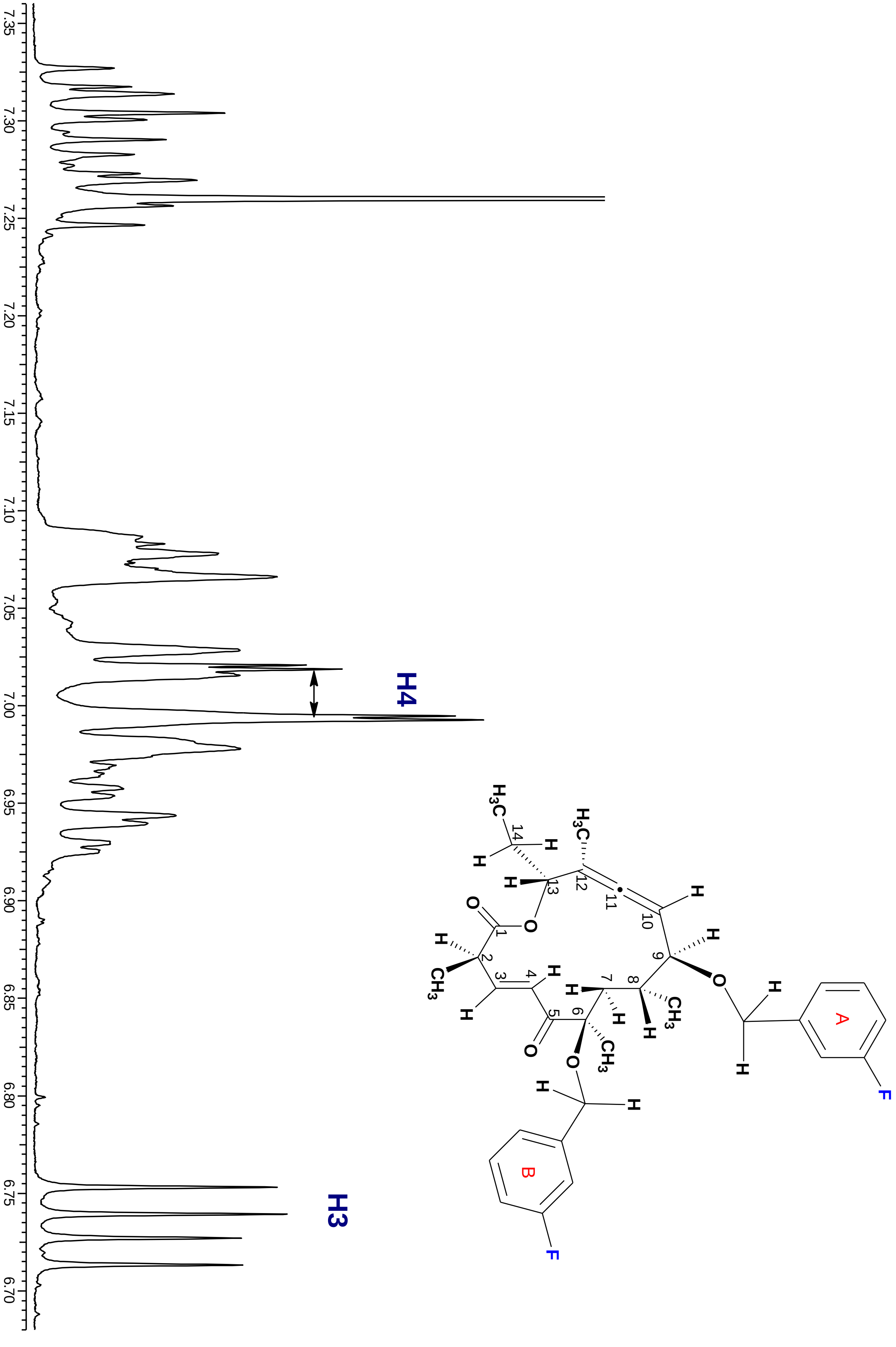

gHMBC
expansion
13-CH$_2$CH$_3$
8-CH$_3$
2-CH$_3$
6-CH$_3$
12-CH$_3$
C12
C10
C6
C9
C13
105
100
95
90
85
80
75
1.5
1.0

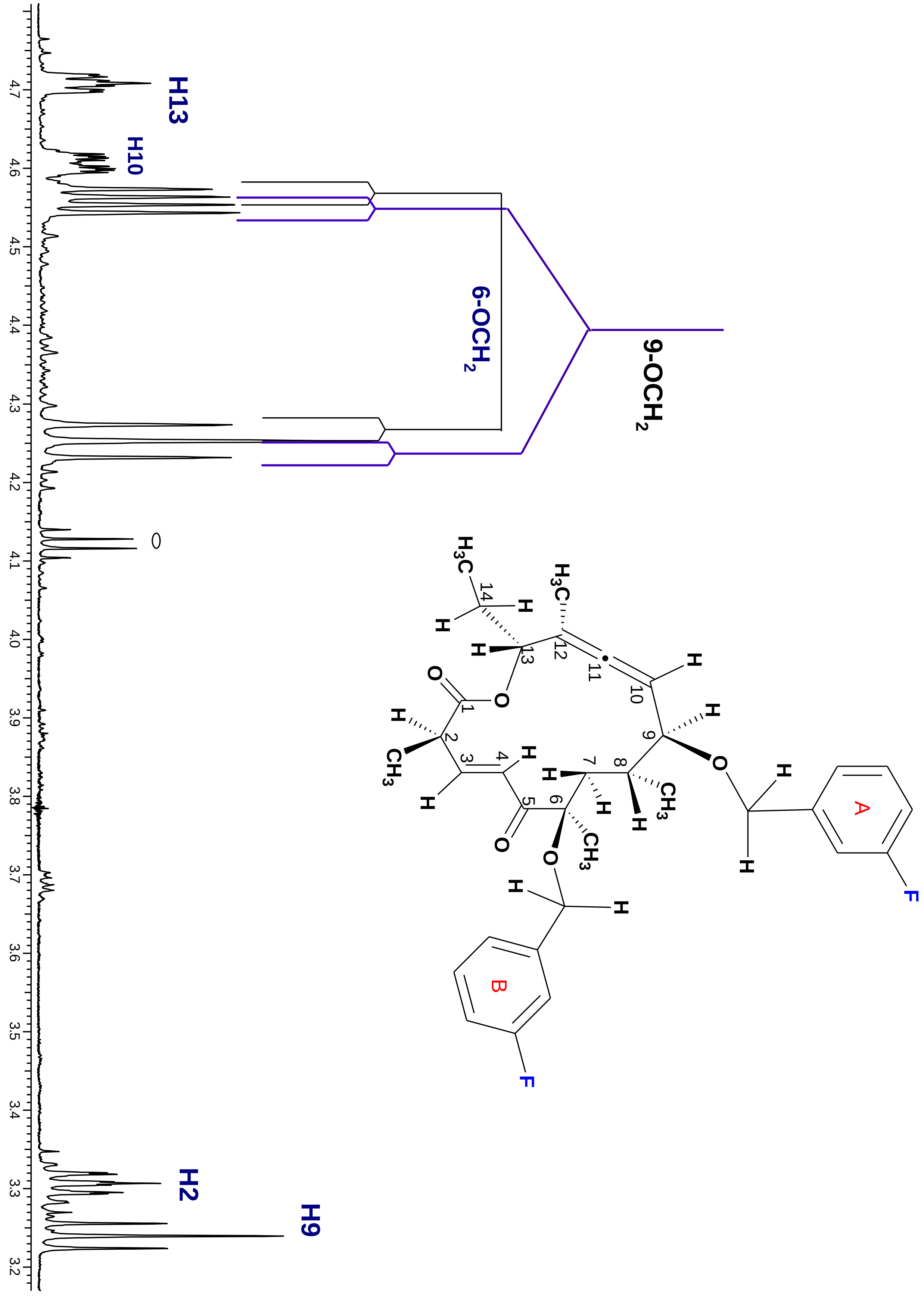
Coupling constant (J_{HH})

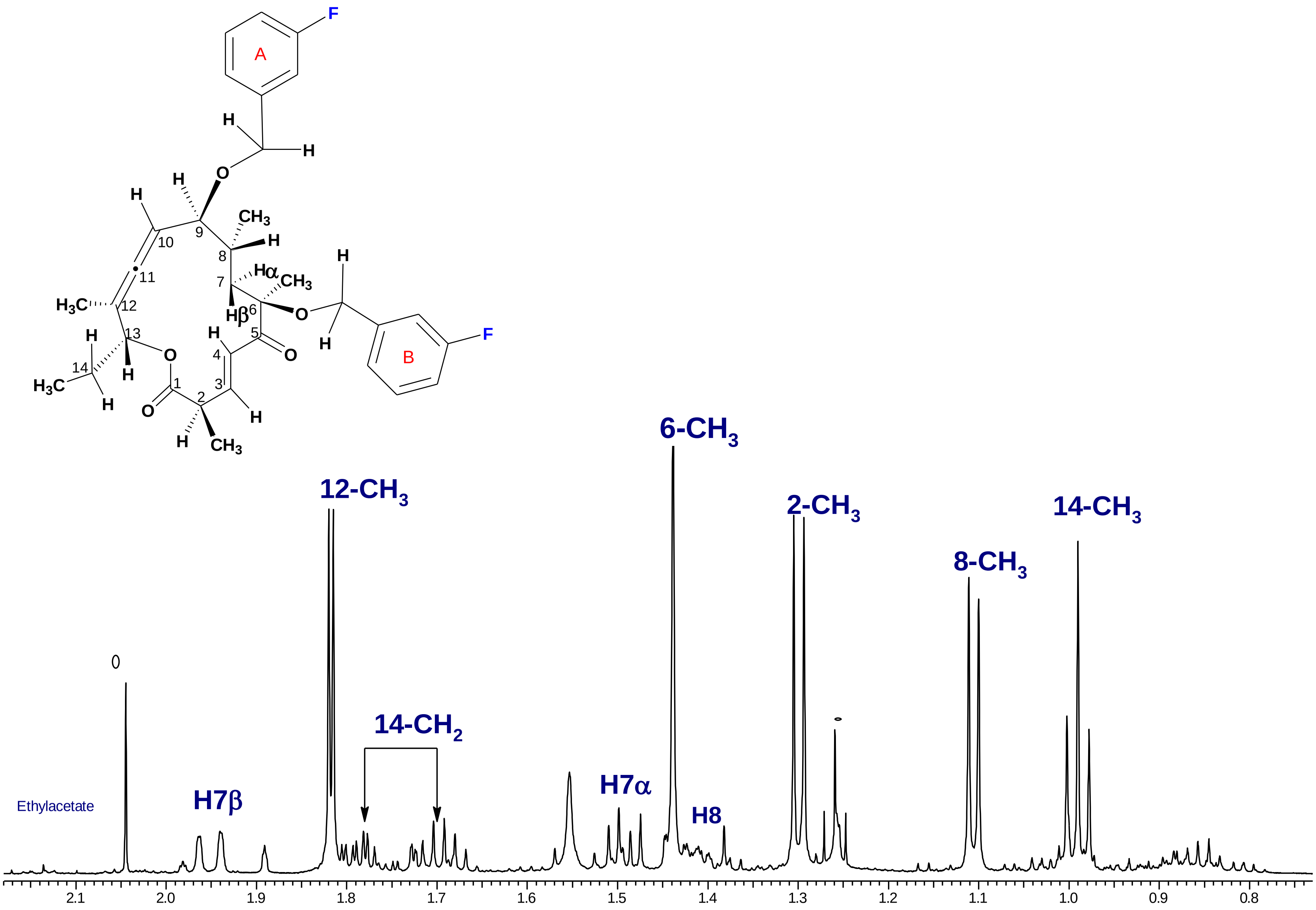

F
9.3Hz
9.5Hz
H
H
O
9
CH3
H
1.1Hz
F
H7β
CH3
H3C
H7α
O
13
5
6.8Hz
H
H
O
H3C
14
1
15.6Hz
O
H
H
CH3
8.3Hz



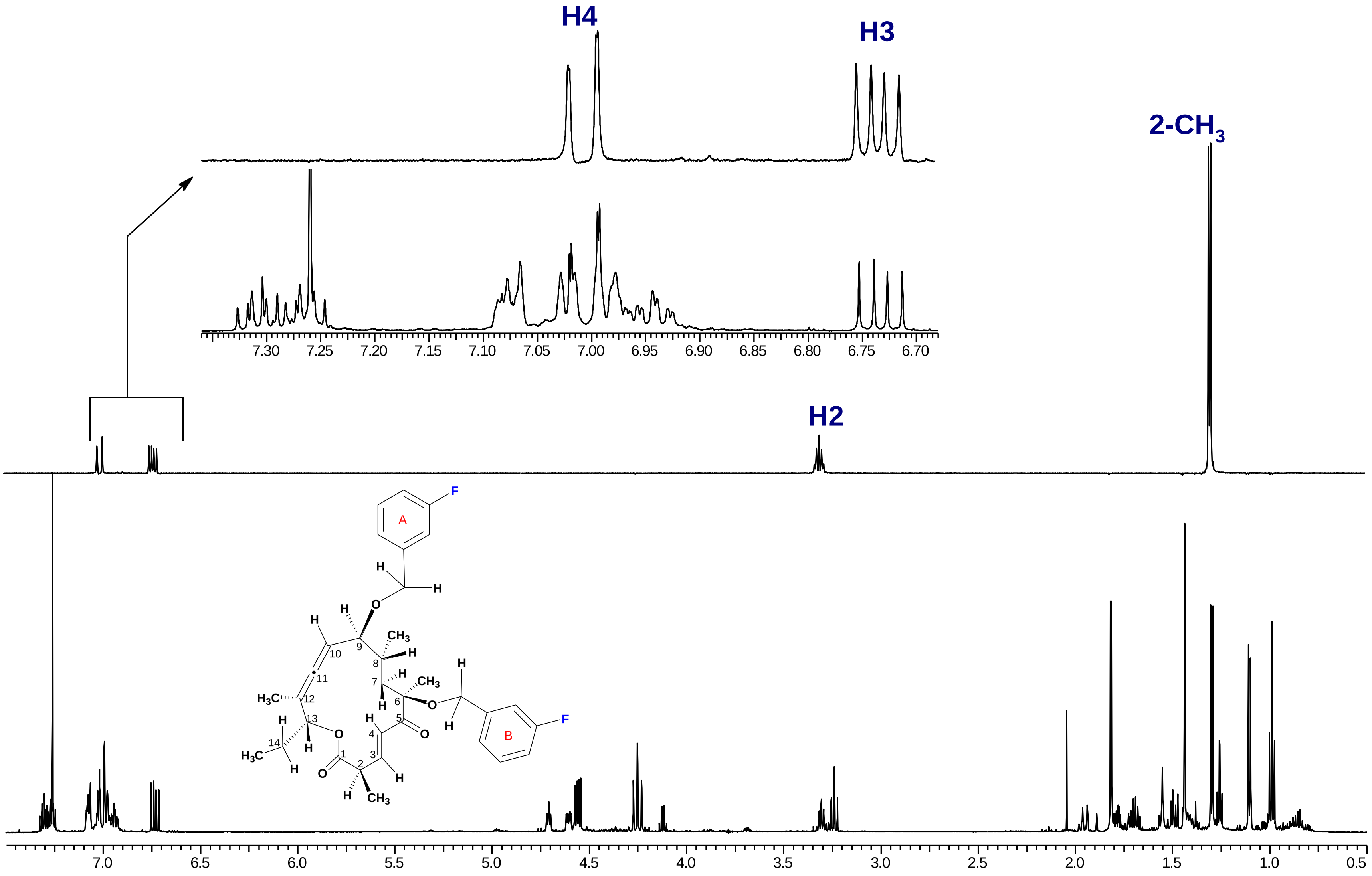
¹⁹F NMR
A-F
B-F
¹³C-¹⁹F satellite
-113.375
-113.850 -113.875
-113.1 -113.2 -113.3 -113.4 -113.5 -113.6 -113.7 -113.8 -113.9 -114.0 -114.1
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
A
B
F
F
H$_3$C
CH$_3$
O
H
Hα
Hβ
1 2 3 4 5 6 7 8 9 10 11 12 13 14

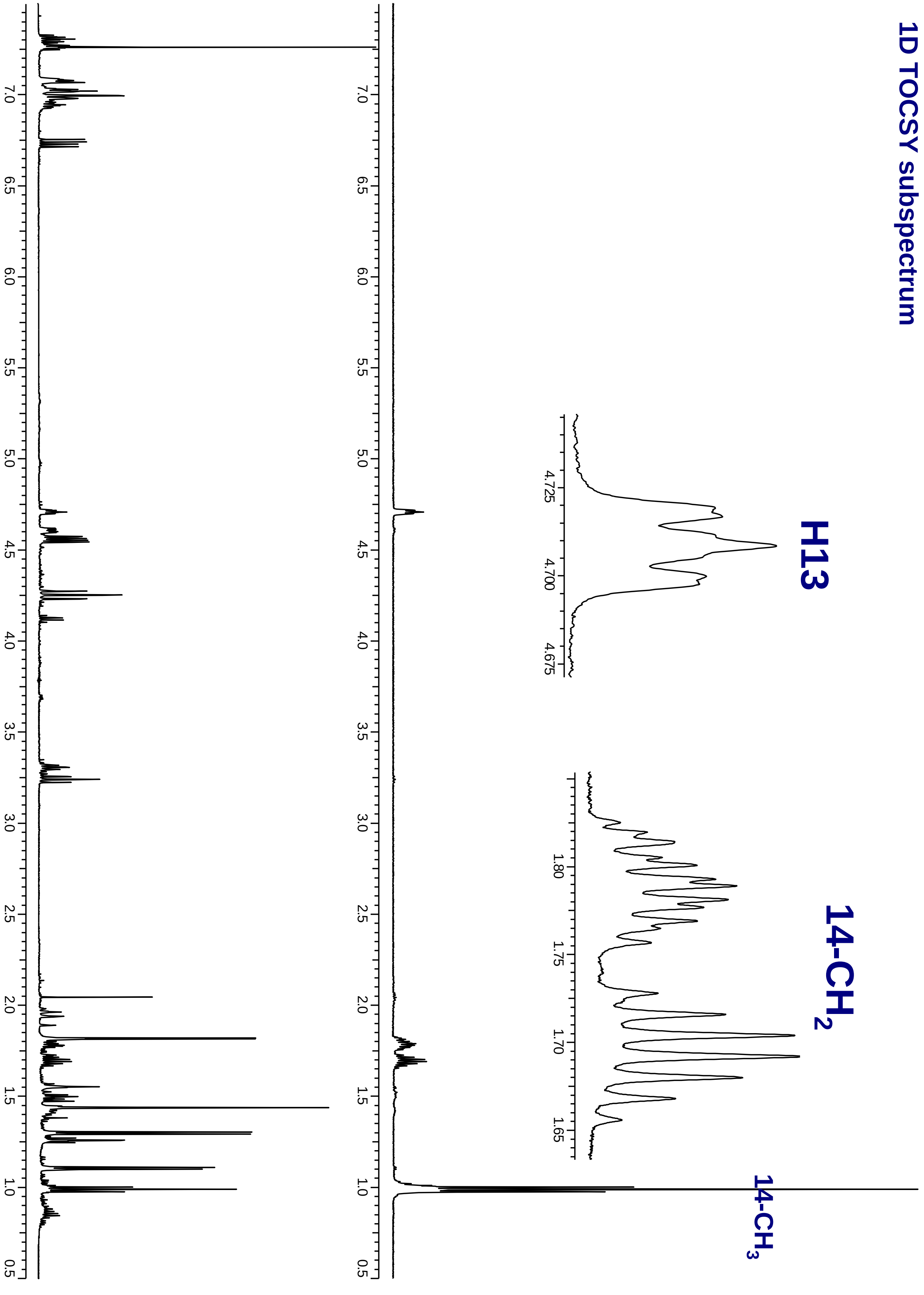

7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
H4
H3
A
B
F
F
1
2
3
4
5
6
7
8
9
10
11
12
13
14
O
CH$_3$
H$_3$C


H13
H10
6-OCH$_2$
9-OCH$_2$
0
H2
H9
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
A
B
F
F
O
O
O
O
H_3C
H_3C
CH_3
CH_3
CH_3
H
1
2
3
4
5
6
7
8
9
10
11
12
13
14

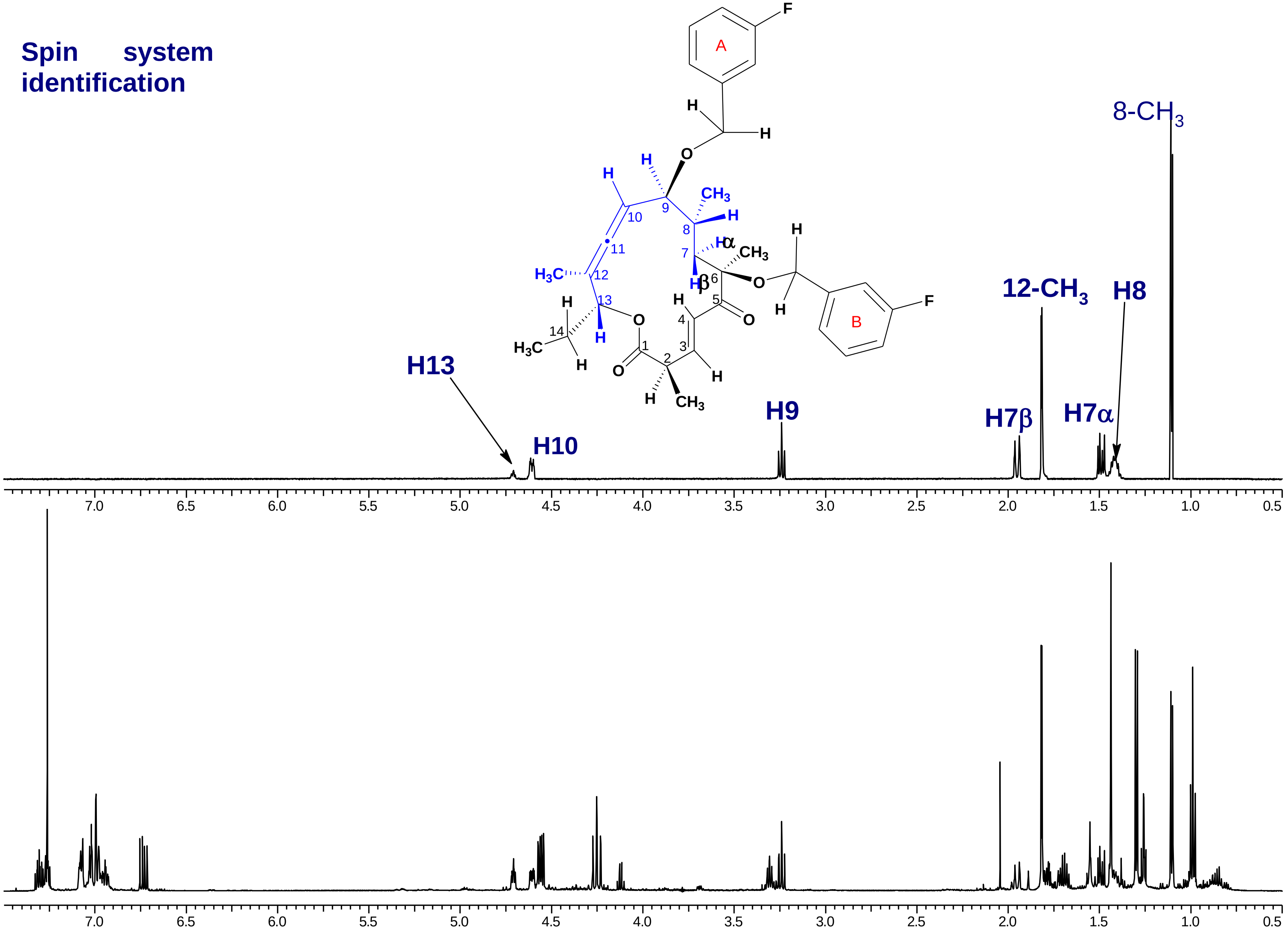

A
F
H
H
O
H
H
CH$_3$
H
10
9
8
11
7
Hα
CH$_3$
H
H$_3$C
12
Hβ
6
O
H
13
H
5
B
F
14
4
O
H$_3$C
H
1
3
O
H
2
O
H
H
CH$_3$
6-CH$_3$
12-CH$_3$
2-CH$_3$
14-CH$_3$
8-CH$_3$
0
14-CH$_2$
H7α
H7β
H8
Ethylacetate
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8

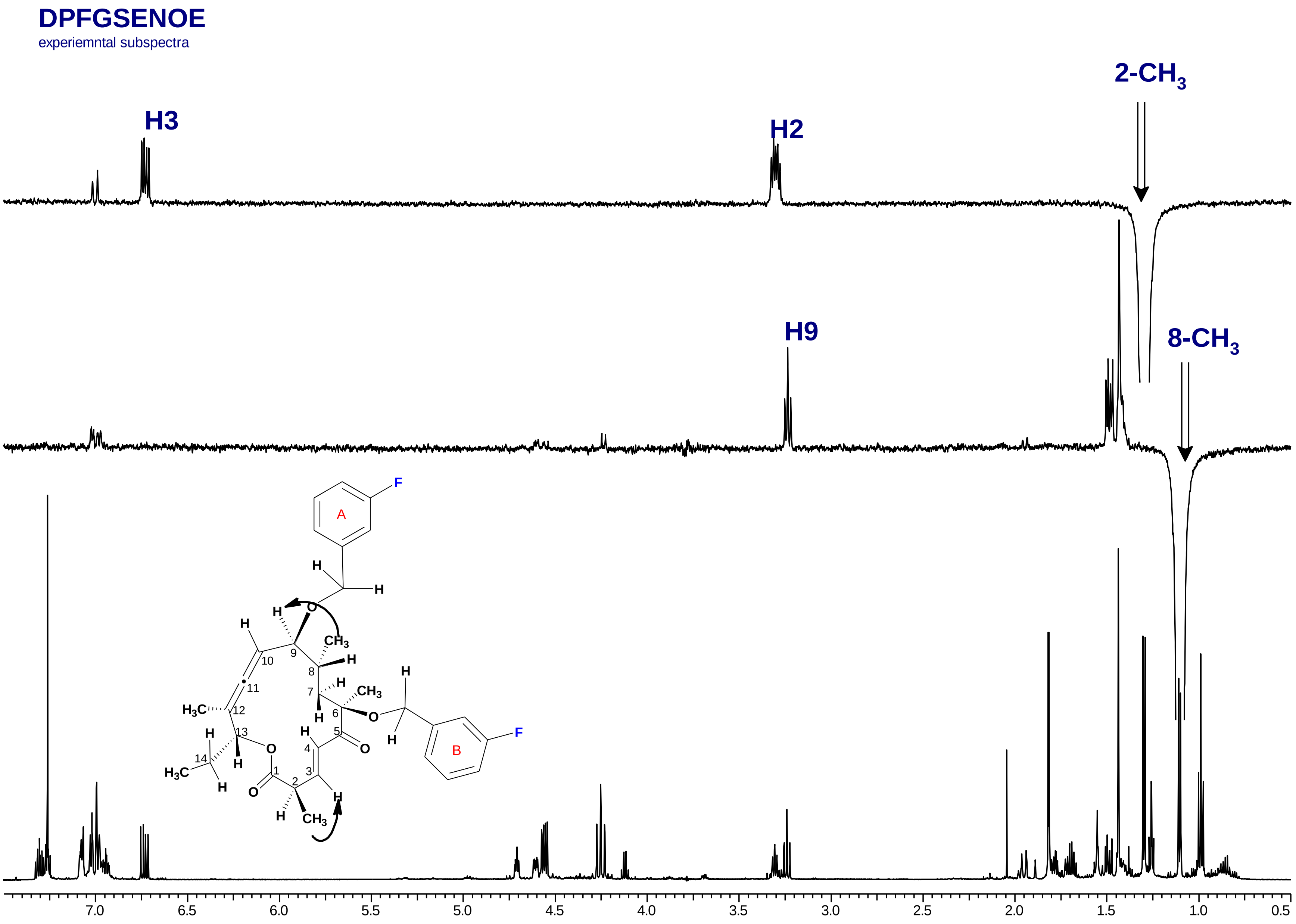
1D TOCSY subspectrum



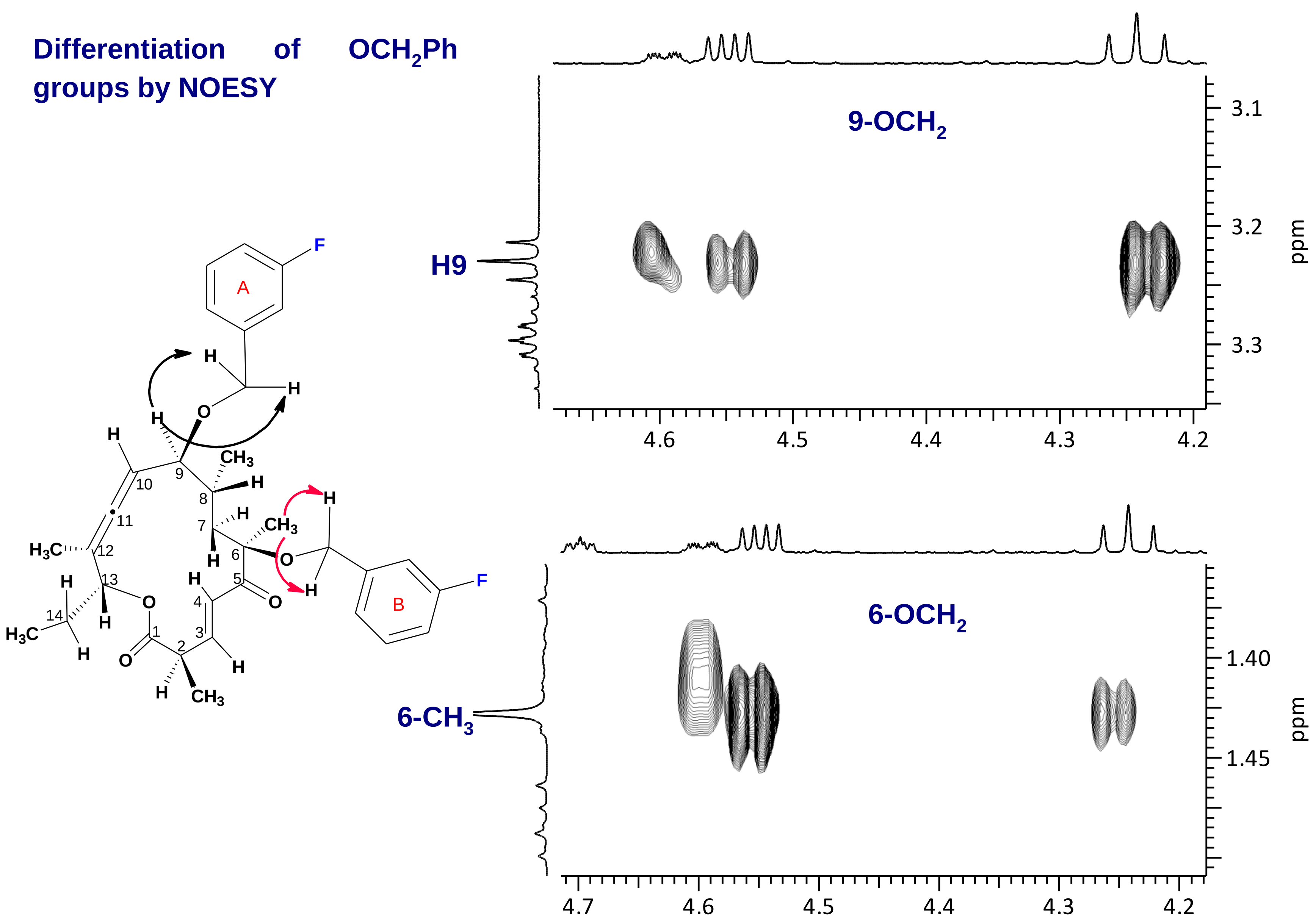

1D TOCSY subspectrum
H13
4.725
4.700
4.675
14-CH$_2$
1.80
1.75
1.70
1.65
14-CH$_3$
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

Spin system identification

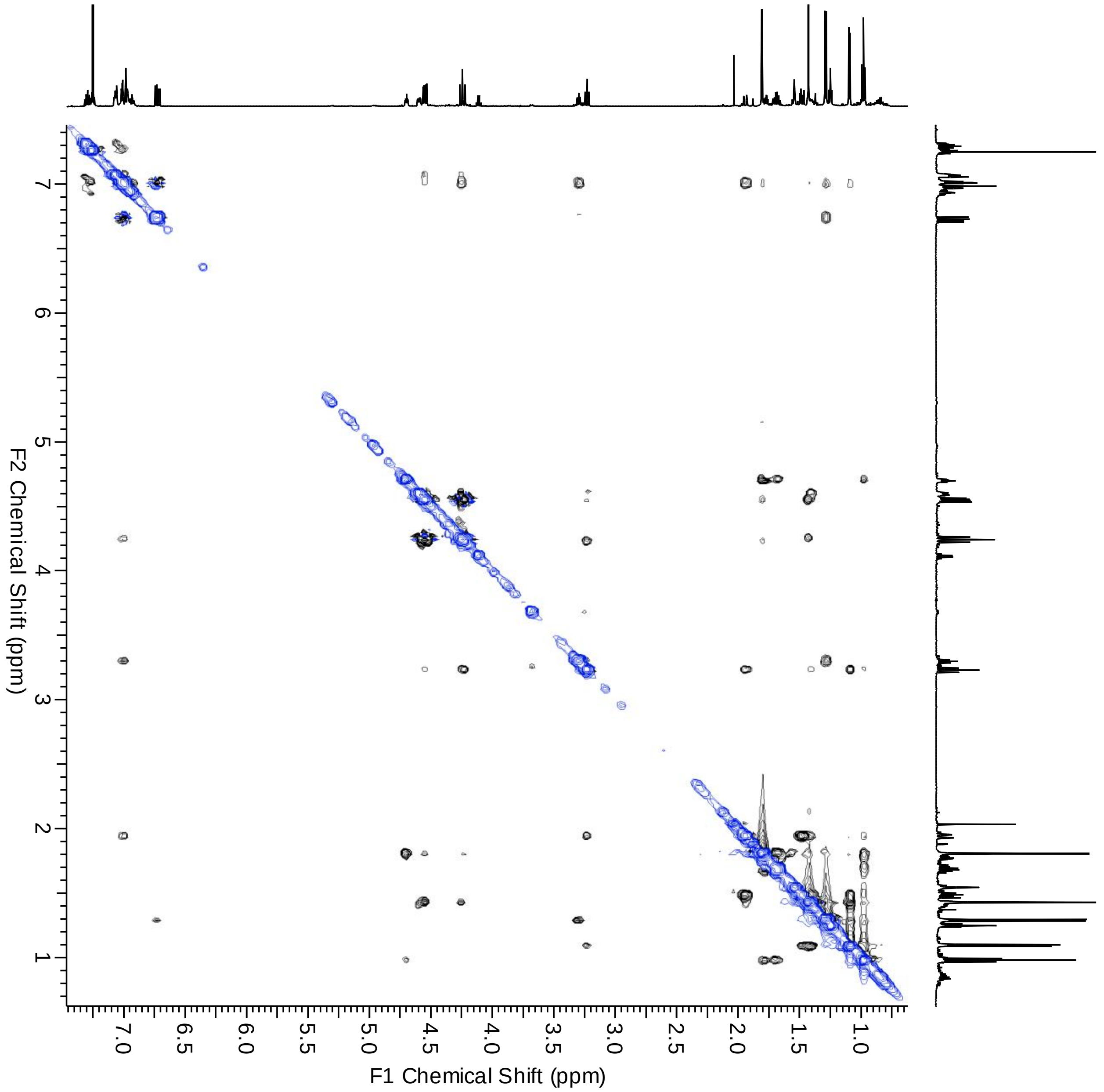



DPFGSENOE
experiemntal subspectra
H3
H2
2-CH3
H9
8-CH3
A
B
F
F
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

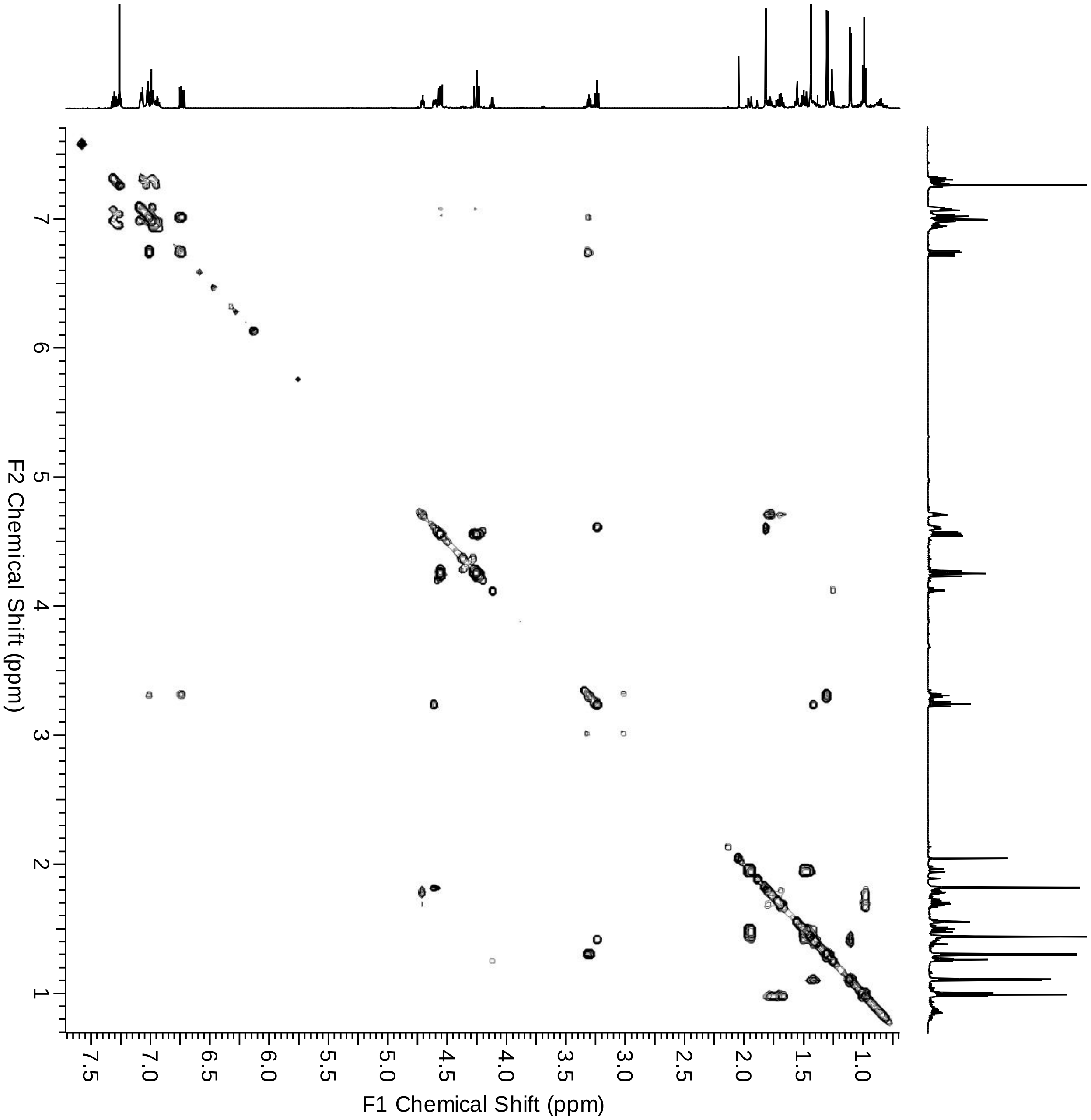
Differentiation of OCH_2Ph groups by NOESY



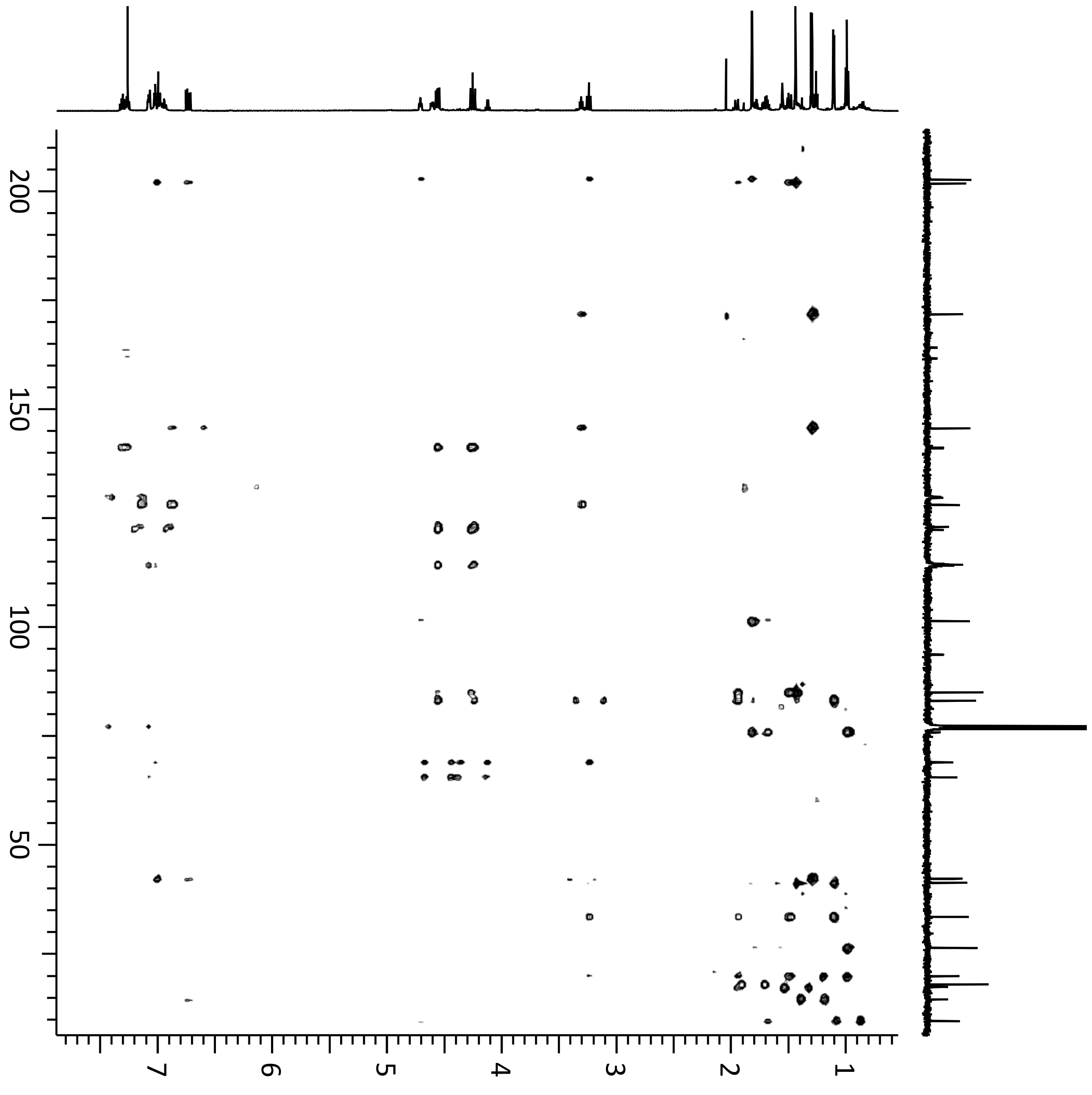
NOESY


F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
7
6
5
4
3
2
1

gCOSY

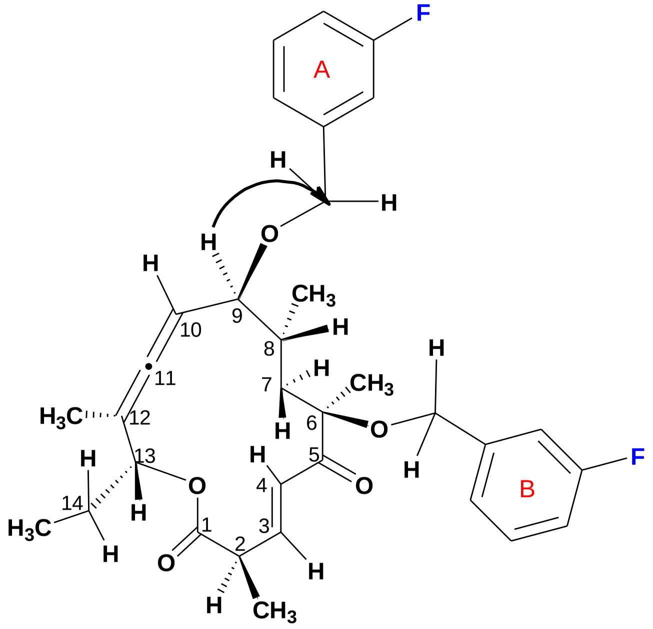

7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F1 Chemical Shift (ppm)
7
6
5
4
3
2
1
F2 Chemical Shift (ppm)

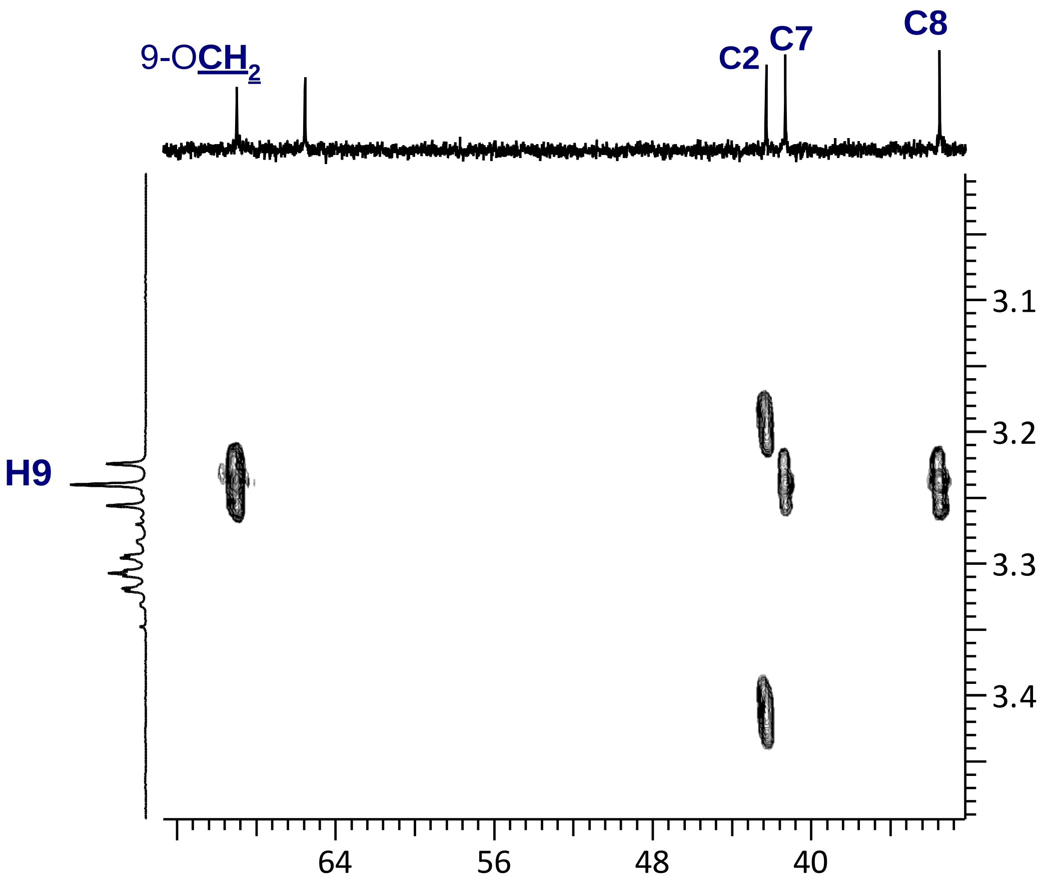
gHMBC



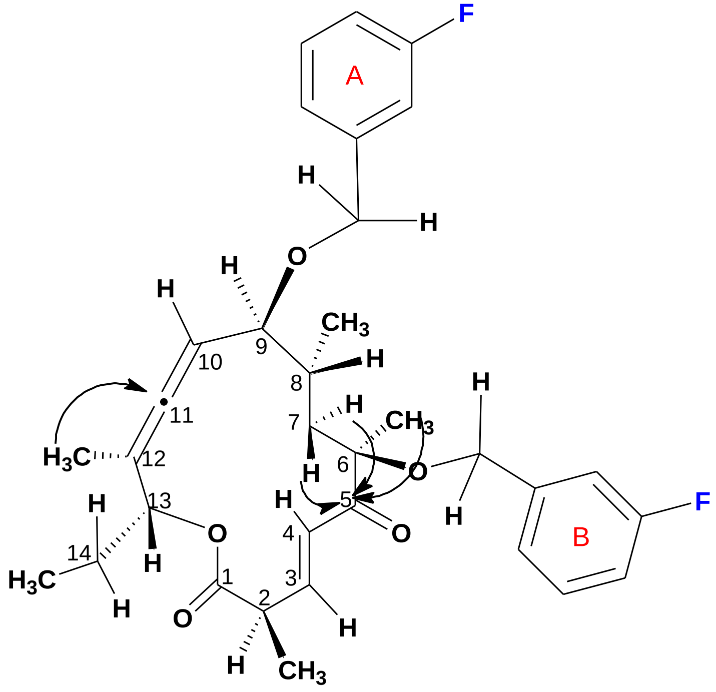
gHMBC

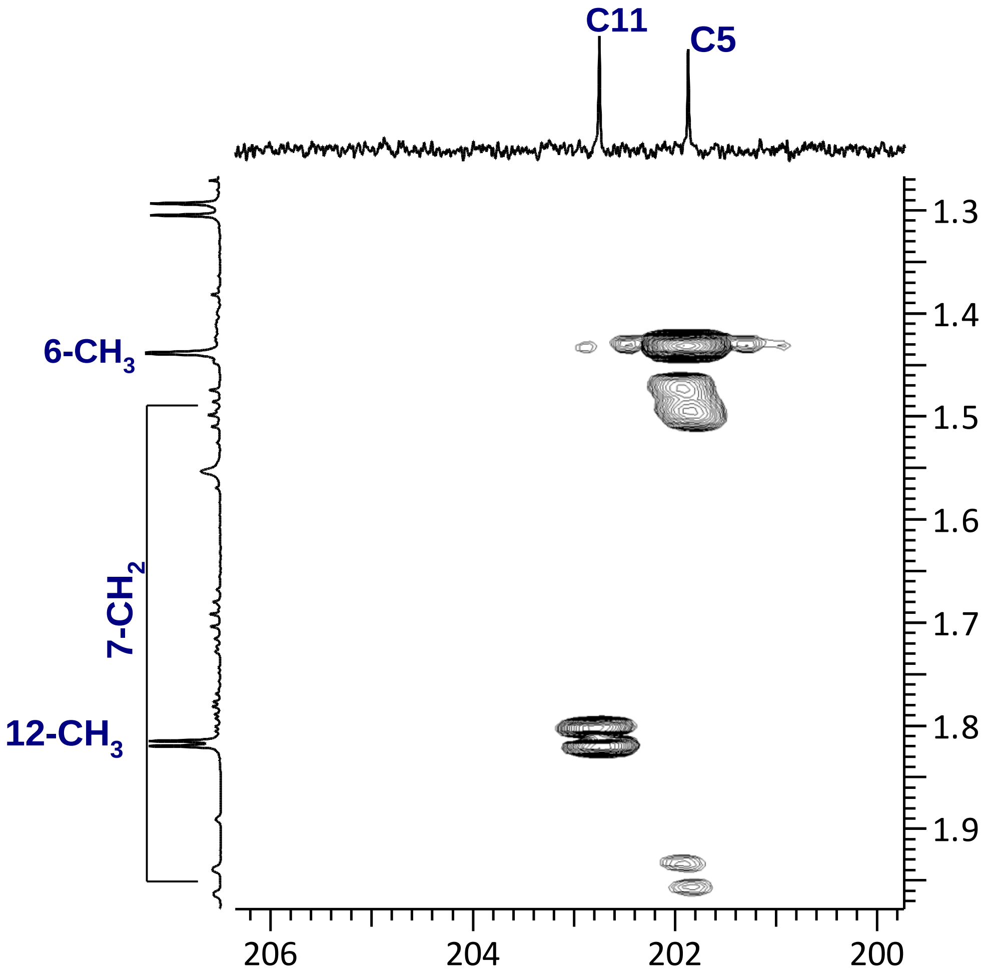
correlations

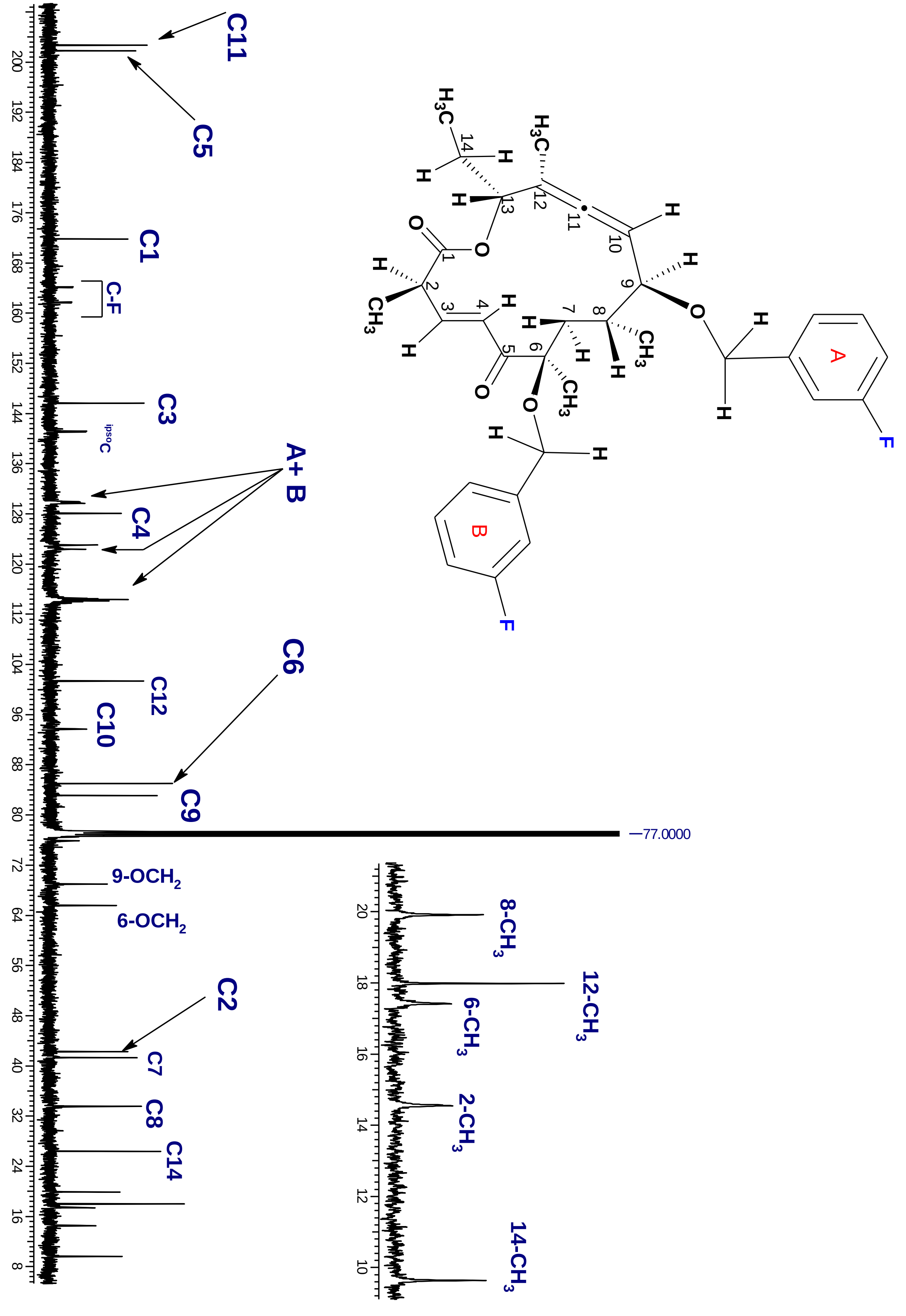

A
F
B
F
H
H
H
O
H
H
CH3
H
1
2
3
4
5
6
7
8
9
10
11
12
13
14
H3C
H3C
O
O
O
O
CH3
CH3
H
H
H
H
H
H
H
H
H

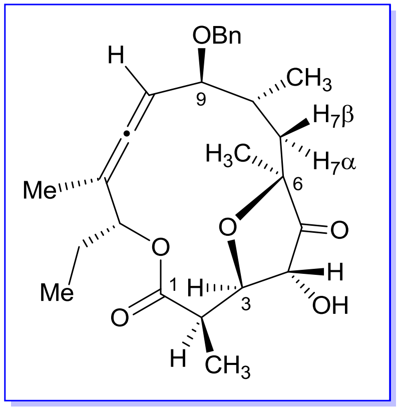


gHMBC
correlations


A
B
F
F
1
2
3
4
5
6
7
8
9
10
11
12
13
14
H
O
CH3
H3C




C11
C5
C1
C-F
C3
ipsoC
A+ B
C4
C12
C10
C6
C9
—77.0000
9-OCH$_2$
6-OCH$_2$
C2
C7
C8
C14
200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
8-CH$_3$
12-CH$_3$
6-CH$_3$
2-CH$_3$
14-CH$_3$
20
18
16
14
12
10
H$_3$C
14
H
13
12
11
10
9
8
7
6
5
4
3
2
1
O
CH$_3$
A
B
F



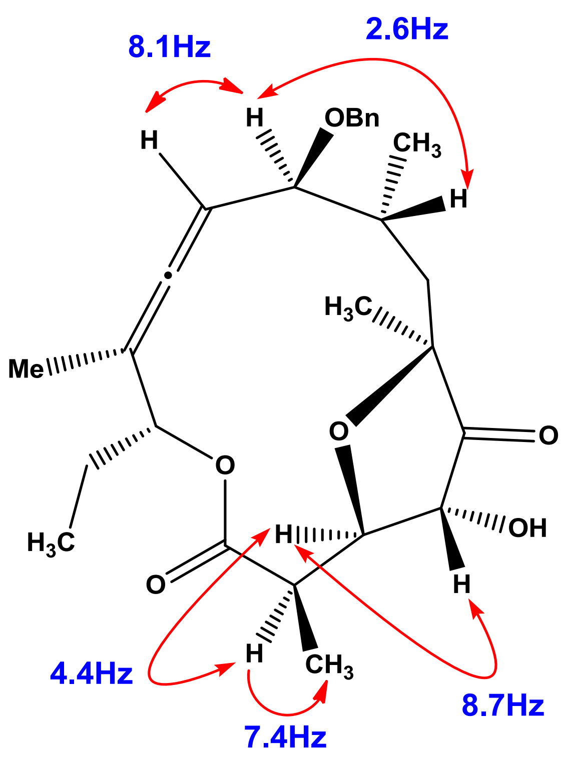
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

3.06 dq, $J_{H2,\ H3}$ = 4.4Hz, $J_{H2,\ 2\text{-}CH3}$ = 7.4Hz, **H2**)
1.35 (d, $J_{2\text{-}CH3,\ H2}$ = 7.4Hz, **2-CH_3**)
3.90 (dd, $J_{H3,\ H2}$ = 4.4Hz, $J_{H3,\ H4}$ = 8.7Hz, **H3**)
5.07 (d, $J_{H4,\ H3}$ = 8.7Hz, **H4**)
1.14 (s, **6-CH_3**)
1.65 (dd, $J_{H7\beta,\ H8}$ = 3.9 Hz, $J_{H7\beta,\ H7\alpha}$ = 15.0Hz, **H7β**)
1.87 (dd, $J_{H7\alpha,\ H8}$ = 5.8 Hz, $J_{H7\alpha,\ H7\beta}$ = 15.0Hz, **H7α**)
1.70 (m, **H8**)
0.88 (d, $J_{8\text{-}CH3,\ H8}$ = 7.0Hz, **8-CH_3**)
3.83 (dd, $J_{H9,\ H8}$ = 2.6Hz, $J_{H9,\ H10}$ = 8.1Hz, **H9**)
4.62, 4.36 (d, J_{AB} = 12.1Hz, **9-OCH_2**)
5.07 (qd, **H10**)
1.80 (d, **12-CH_3**)
5.60 (dd, $J_{H13,\ H14}$ = 6.3, 8.1Hz, **H13**)
1.65, 1.70 (m, **14-CH_2**)
0.93 (t, $J_{14\text{-}CH3,\ H14}$ = 7.6Hz, **14-CH_3**)

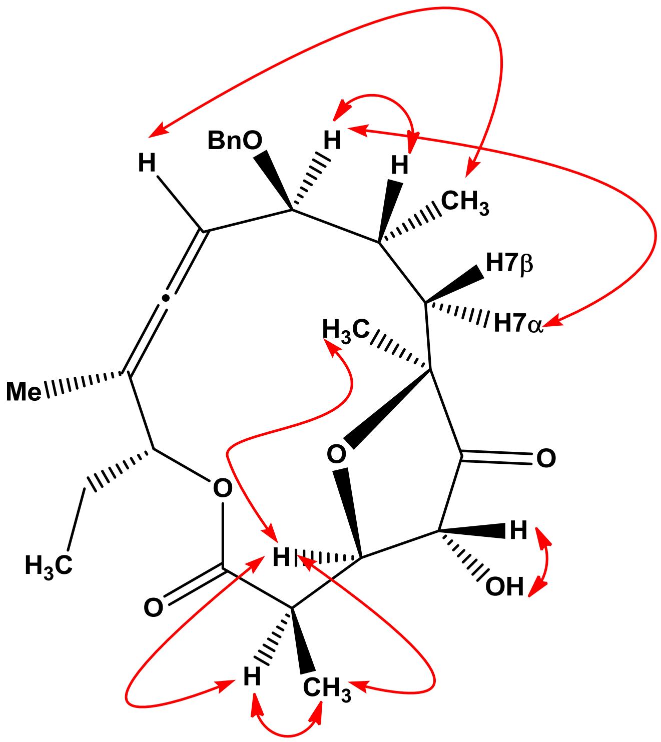
13C NMR chemical shift (δ/ppm)

171.0 ----- C1
40.2 ----- C2
79.7 ----- C3
72.5 ----- C4
218.9 ----- C5
83.4 ----- C6
42.9 ----- C7
34.0 ----- C8
82.3 ----- C9
90.7 ----- C10
206.6 ----- C11
99.0 ----- C12
76.6 ----- C13
24.3 ----- C14
13.8 ----- 2-CH_3
22.8 ----- 6-CH_3
14.5 ----- 8-CH_3
13.6 ----- 12-CH_3
10.0 ----- 14-CH_3
70.2 ----- 9-OCH_3

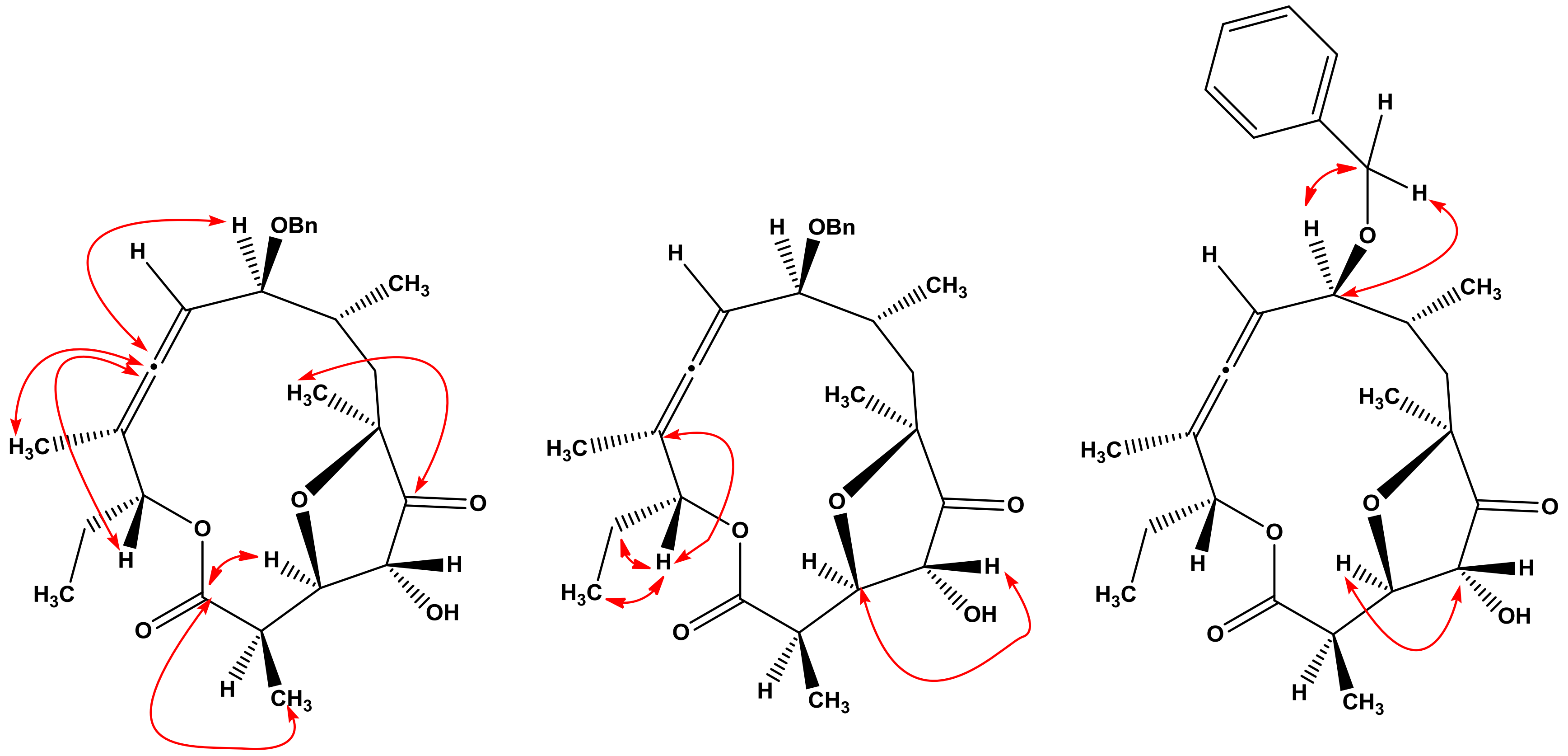
Coupling constant (J_{HH})


8.1Hz
2.6Hz
H
H
OBn
CH3
H
H3C
Me
O
O
O
H3C
H
OH
H
4.4Hz
H
CH3
7.4Hz
8.7Hz

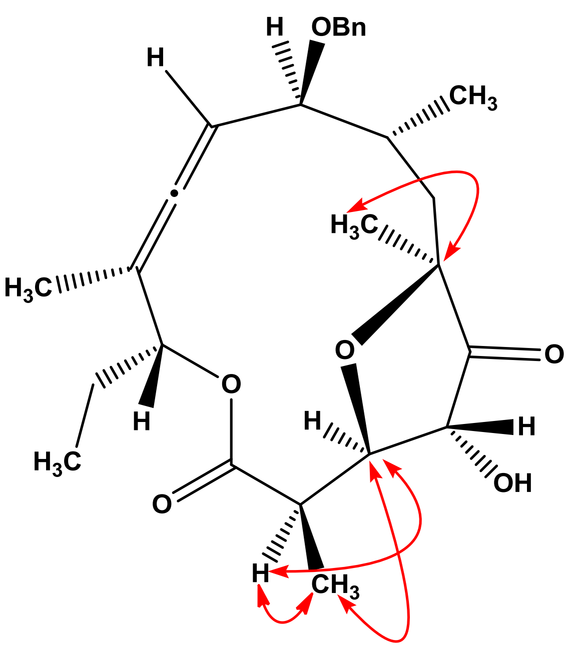
NOESY

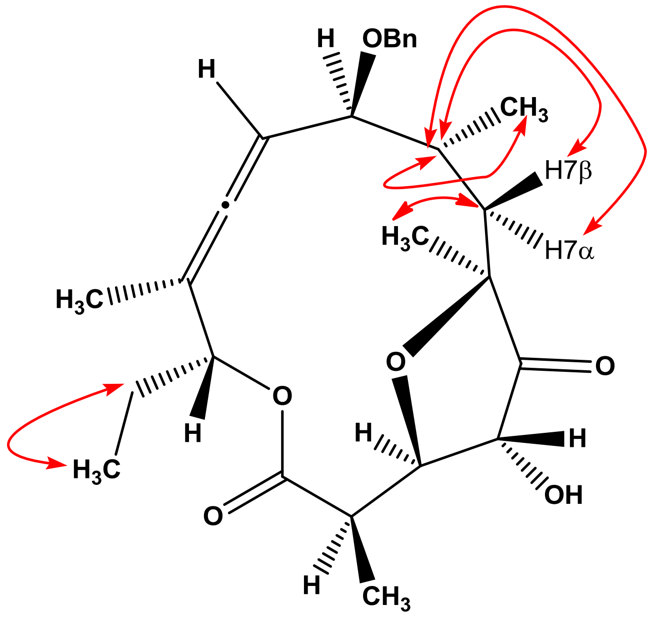

H
BnO
H
H
CH3
H7β
H7α
H3C
Me
O
O
O
H3C
O
H
H
OH
H
CH3

HMBC

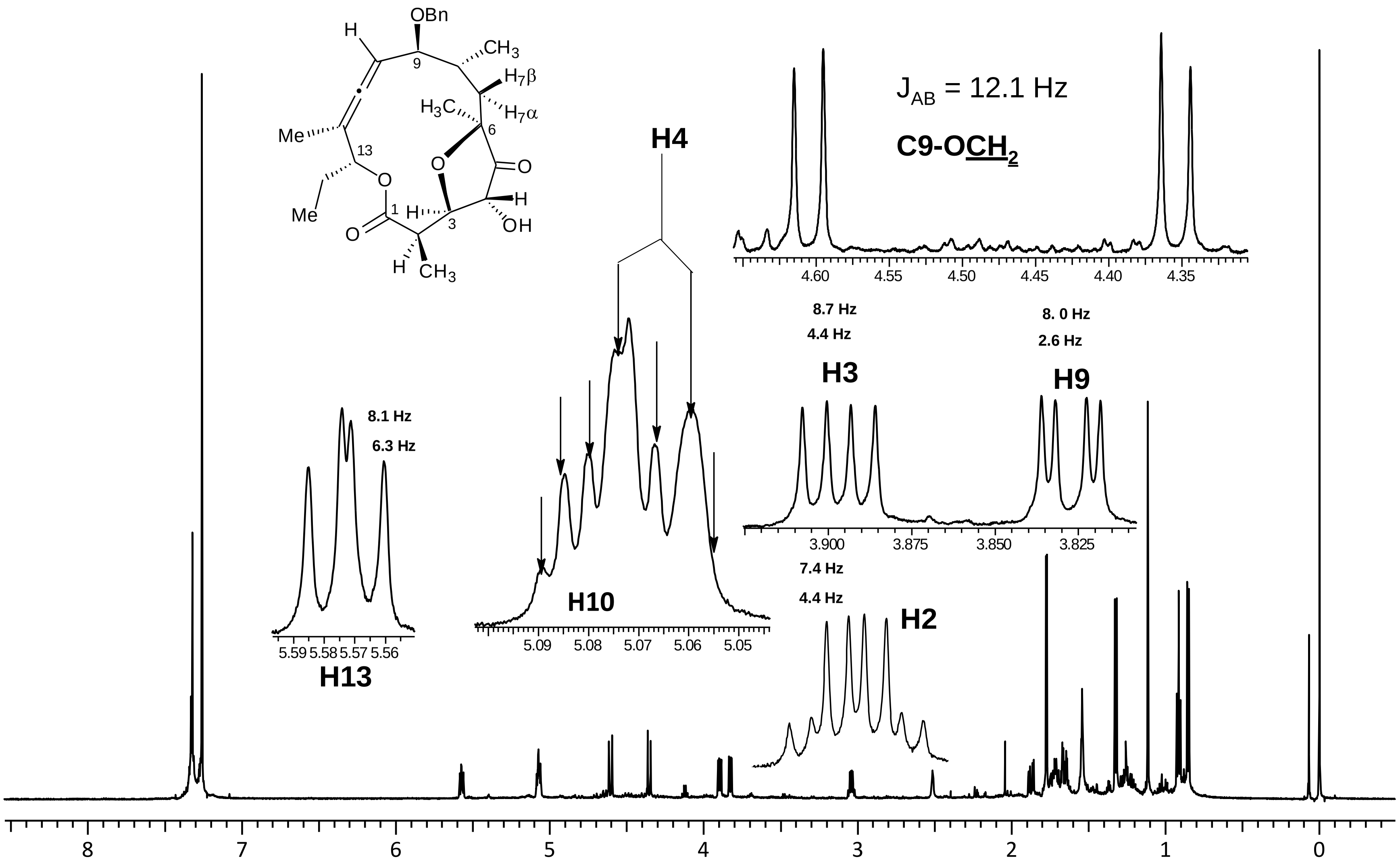

H
OBn
H
CH3
H3C
H3C
O
O
H
H
OH
H3C
O
H
CH3
H
H
OBn
H
CH3
H3C
H3C
O
O
H
H
OH
H3C
O
H
CH3
H
H
H
H
OBn
CH3
H3C
H3C
O
O
H
H
OH
H3C
O
H
CH3
H

HMBC

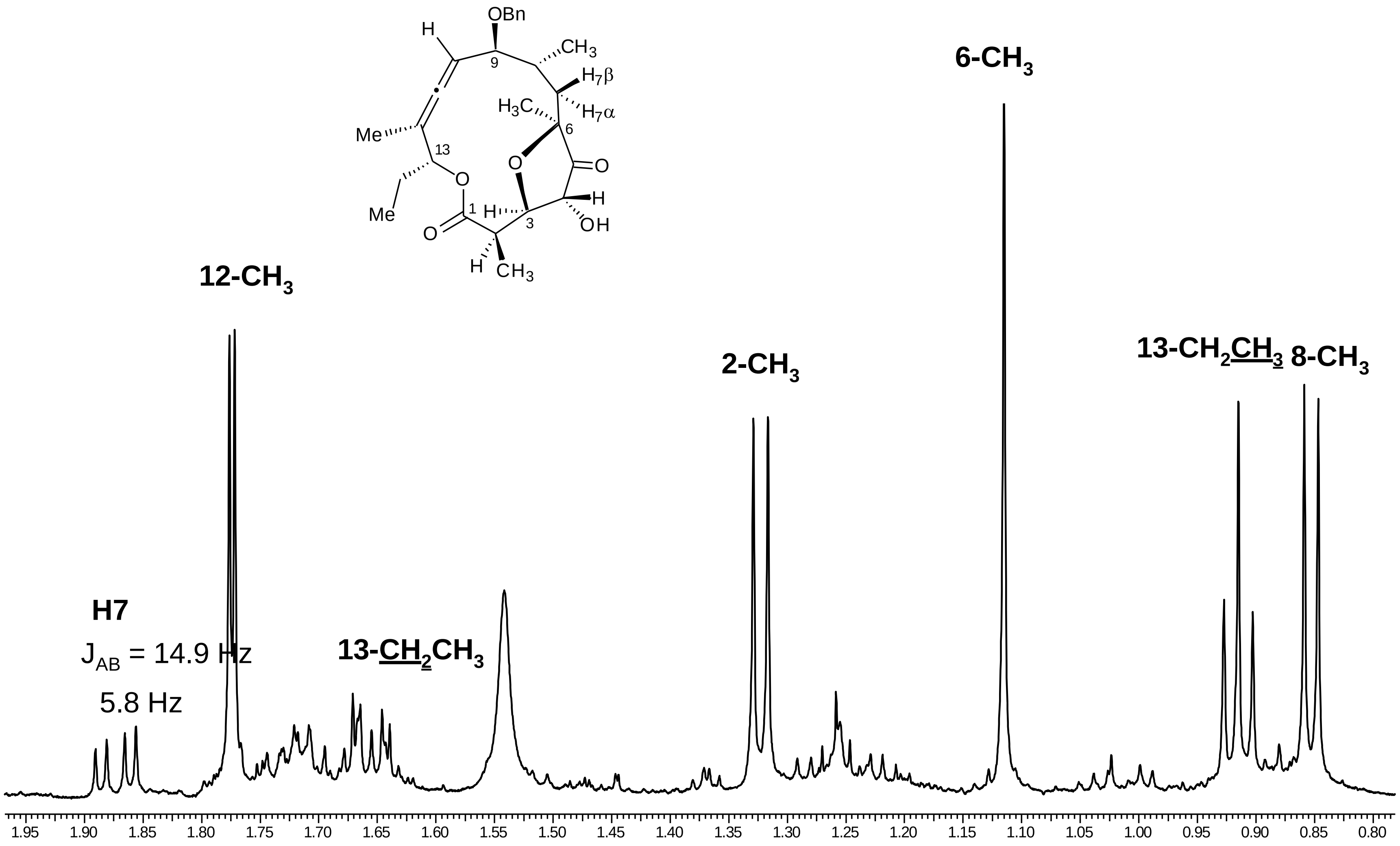

H
OBn
H
CH3
H3C
H3C
O
O
O
H
H3C
O
H
H
OH
H
CH3


H
OBn
H
CH3
H7β
H3C
H7α
H3C
O
O
O
H
H3C
O
H
H
OH
H
CH3

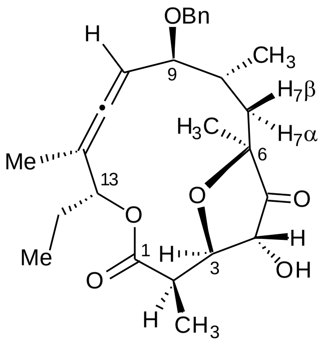
^{1}H NMR spectrum in $CDCl_3$

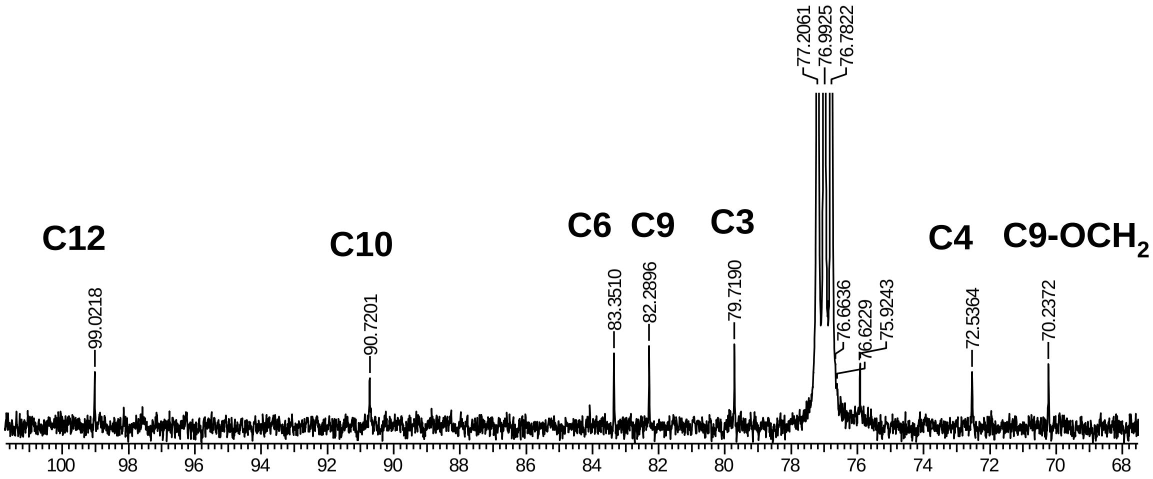


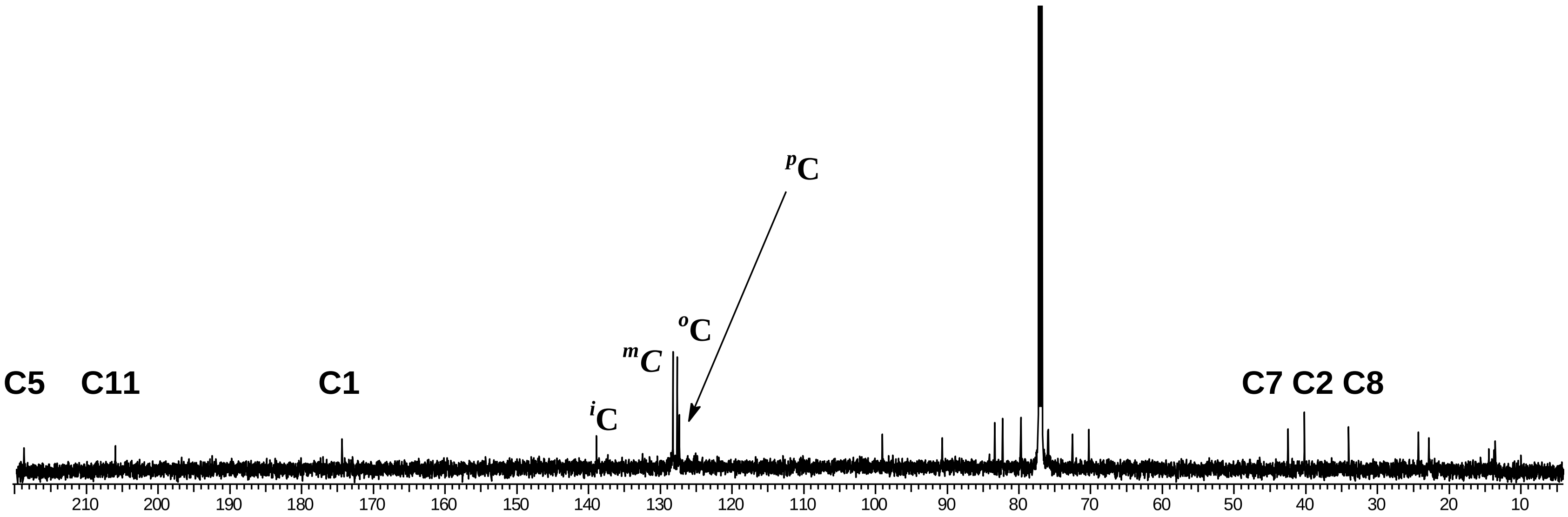
^{1}H NMR spectrum in CDCl$_3$



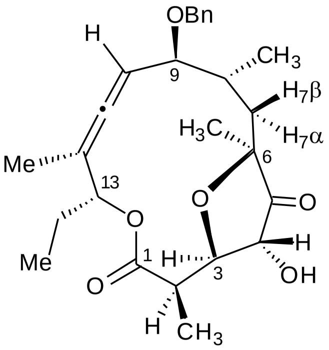
^{13}C NMR spectrum in CDCl$_3$


H
OBn
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3





^{13}C NMR spectrum in CDCl$_3$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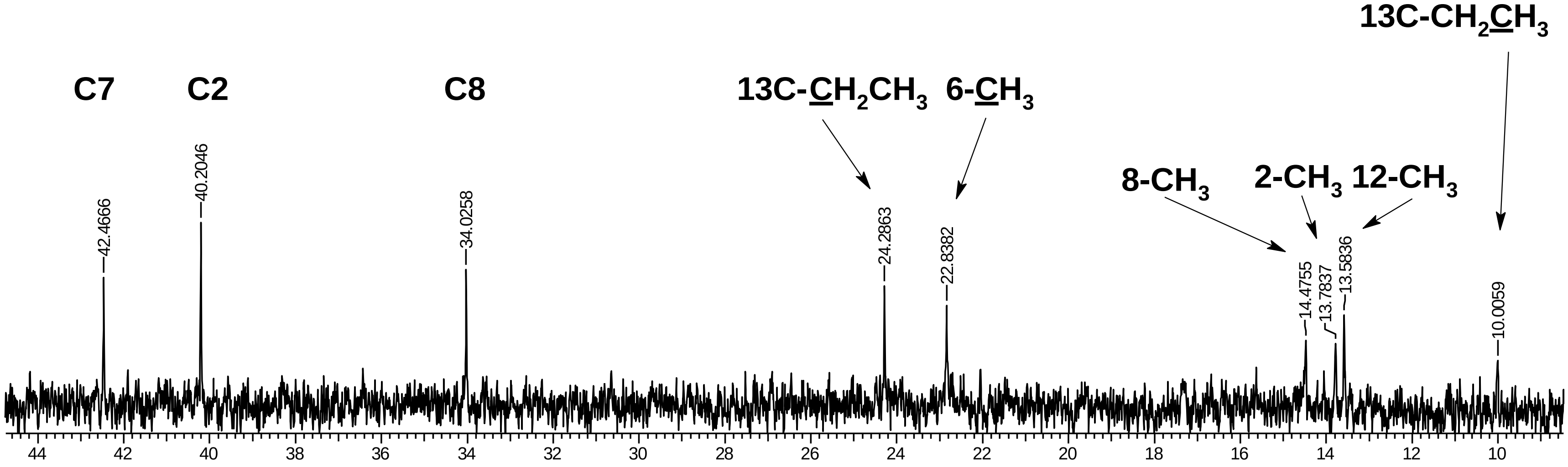
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
Me
1
H
3
H
OH
O
H
CH3



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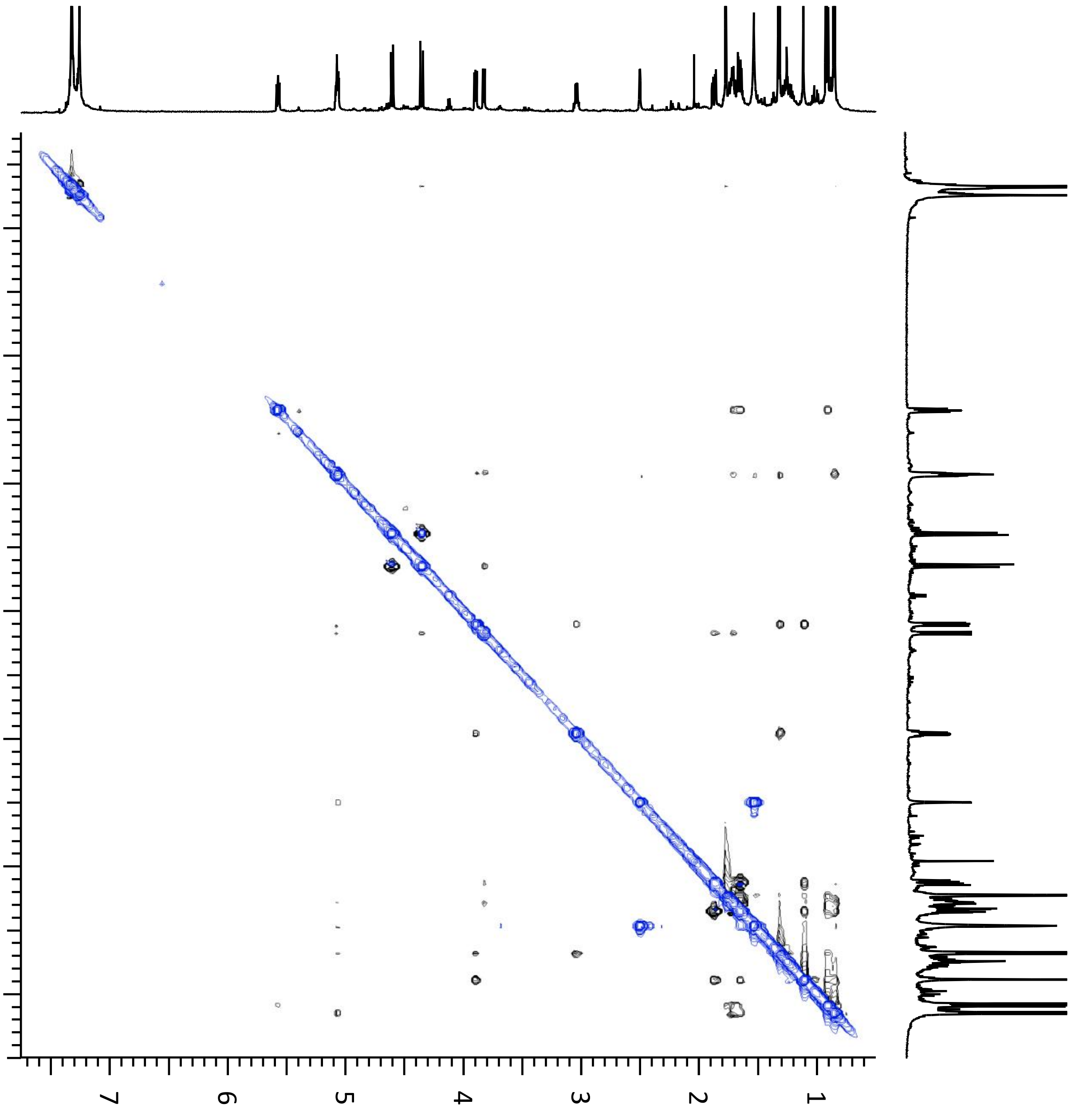
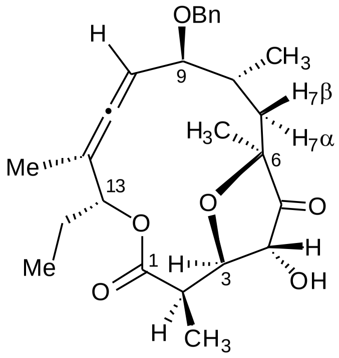
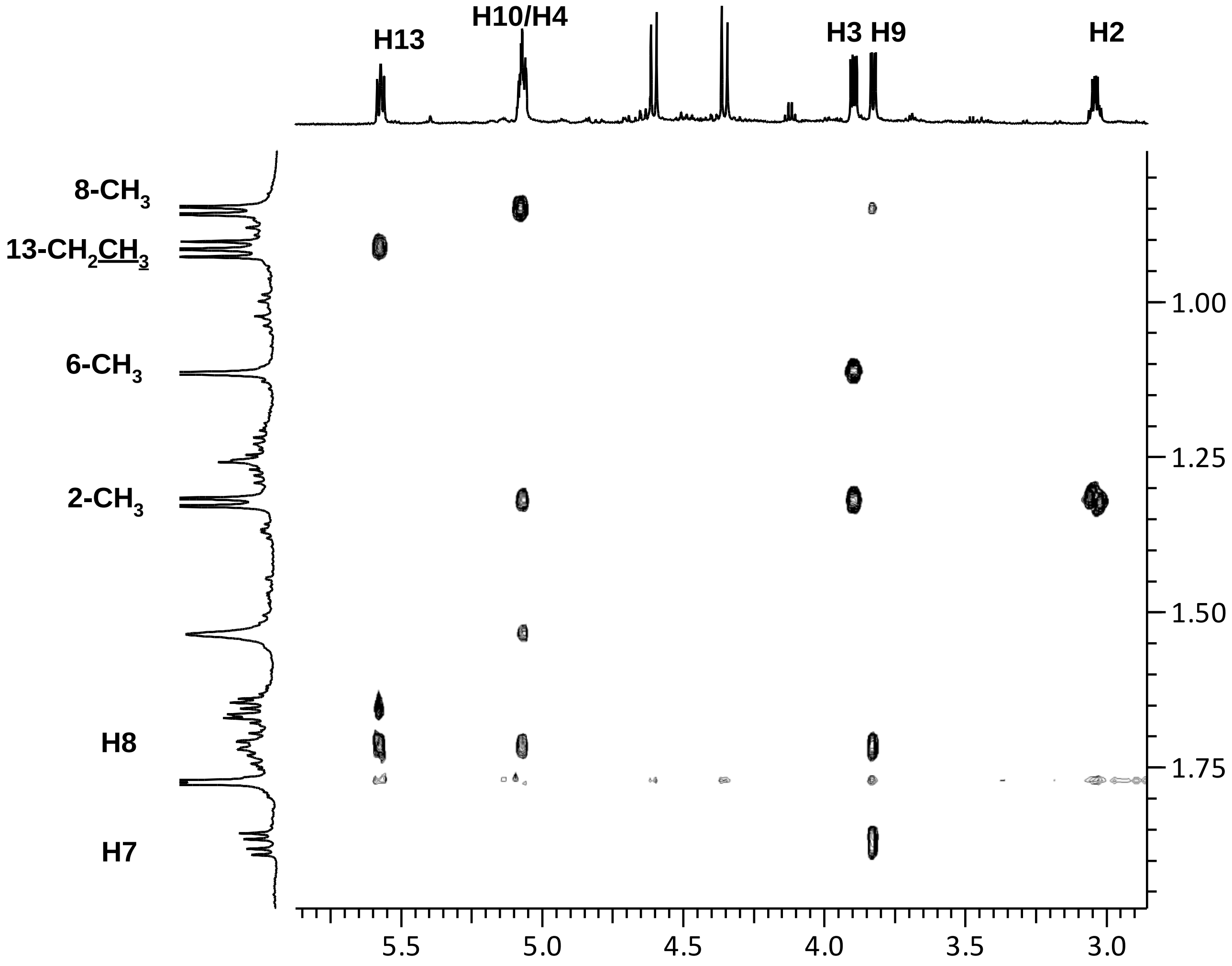


NOESY

expansion


OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3



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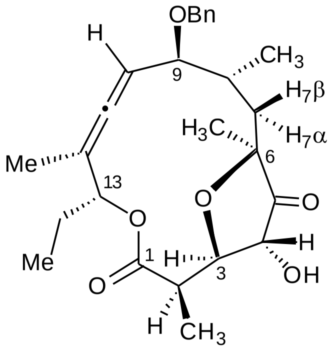
expansion


OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
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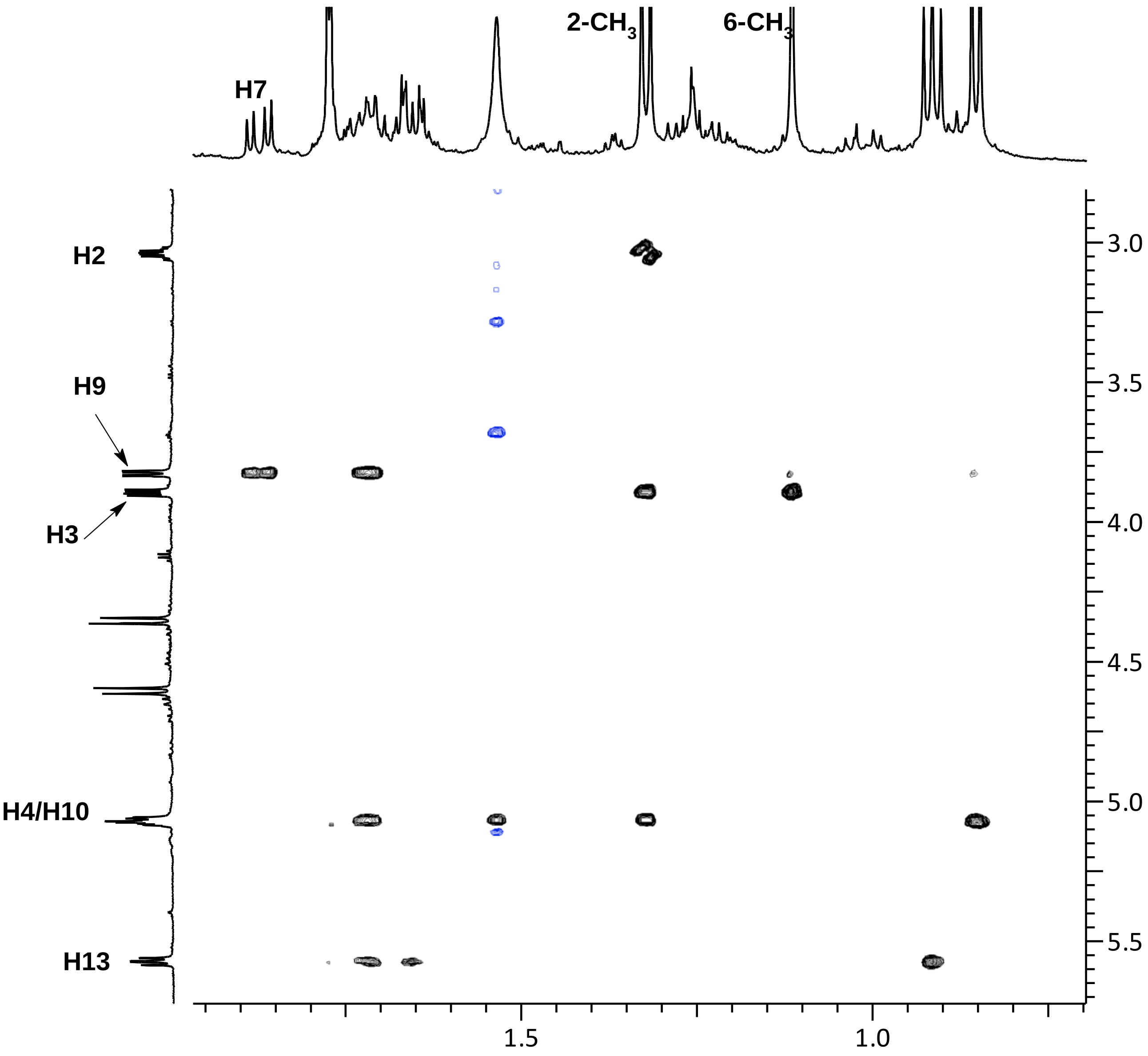
O
H
1
3
H
OH
Me
O
H
CH3



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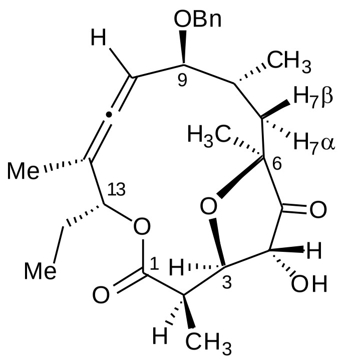
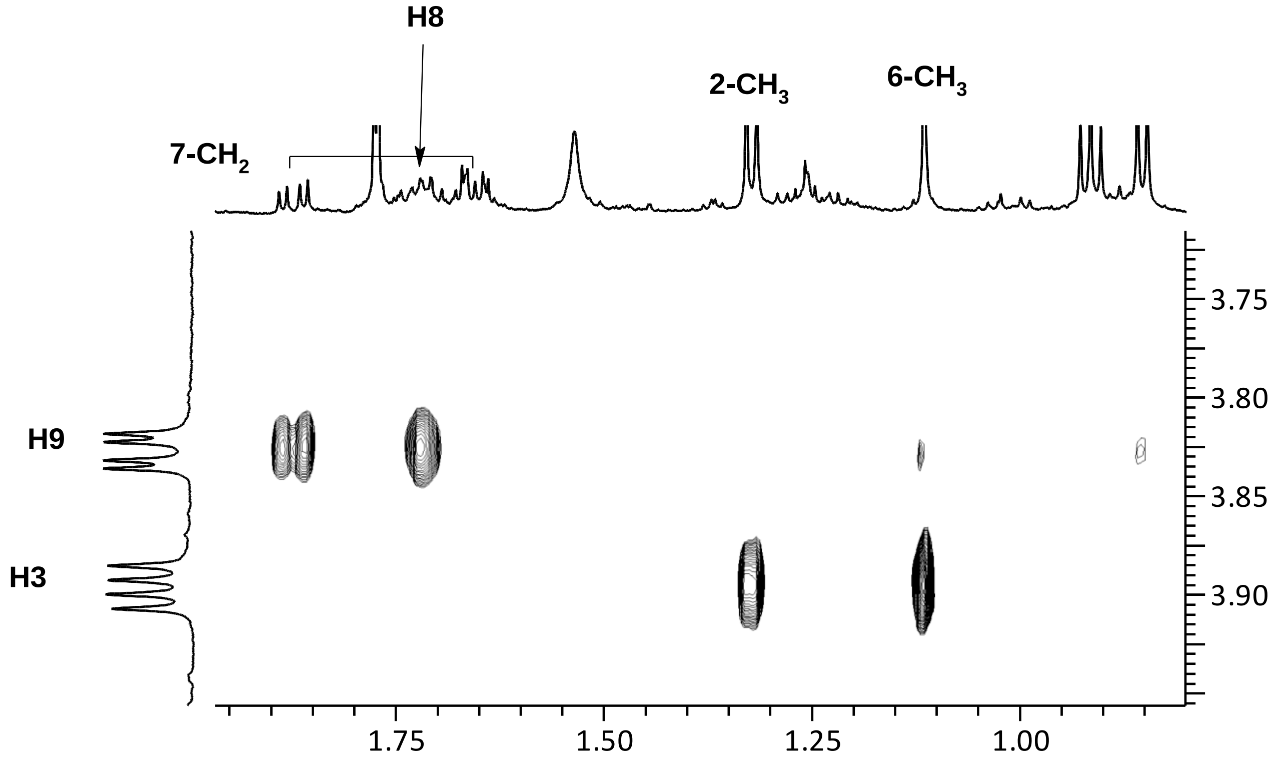
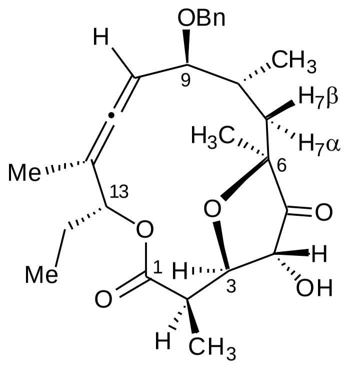
expansion

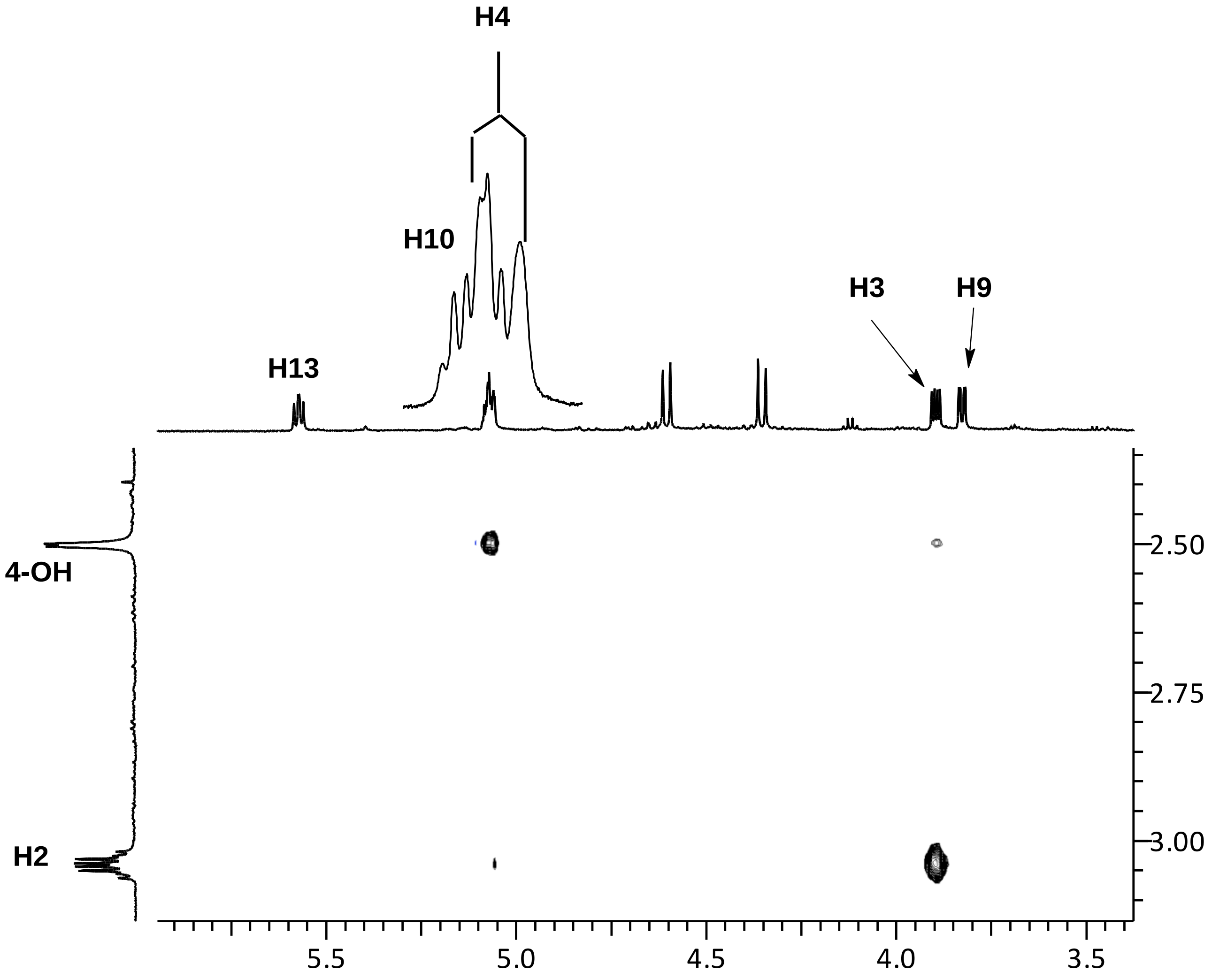

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
1
H
3
O
H
OH
Me
O
H
CH3



NOESY

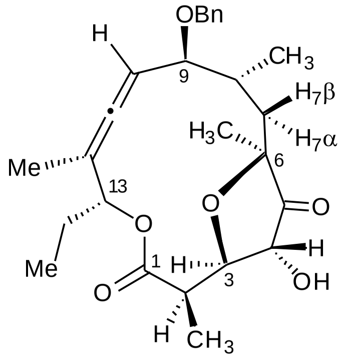
expansion

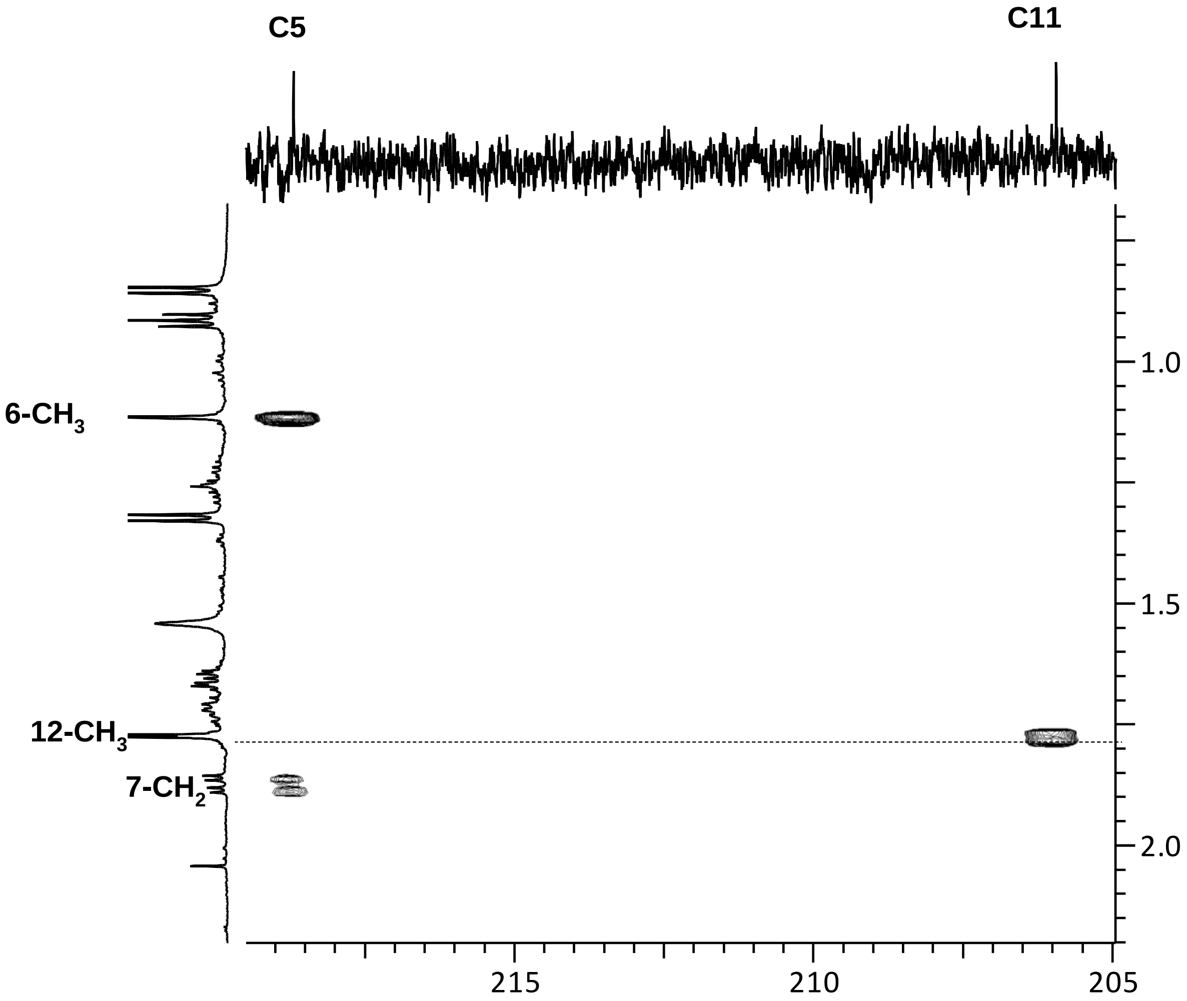

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3



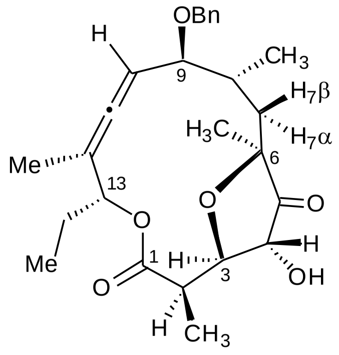
gHMBC

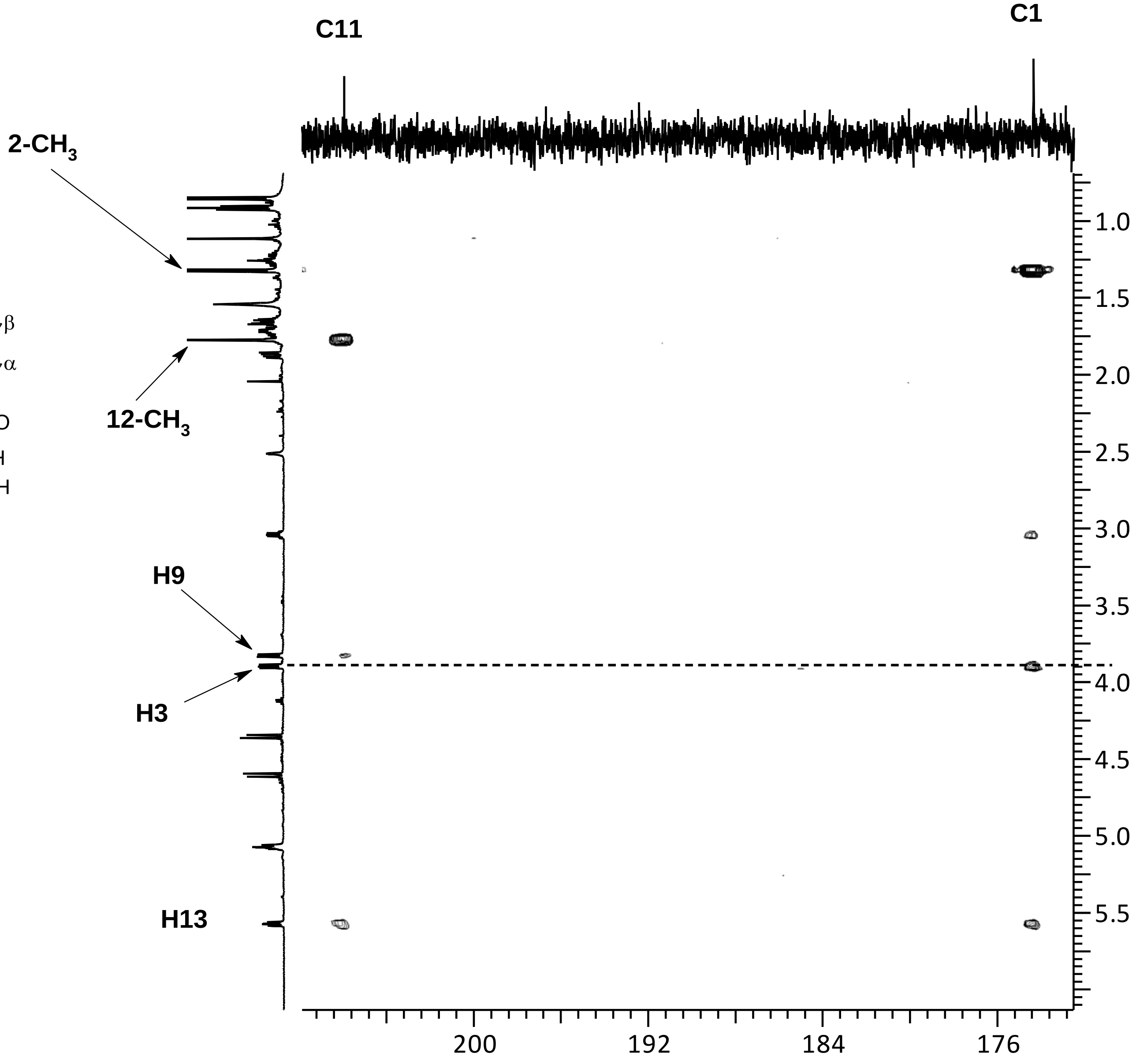
correlations


H
OBn
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
O
H
CH3
Me



gHMBC
correlations


H
OBn
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3



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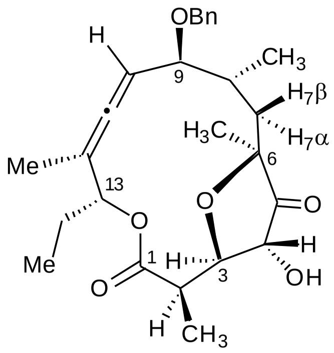
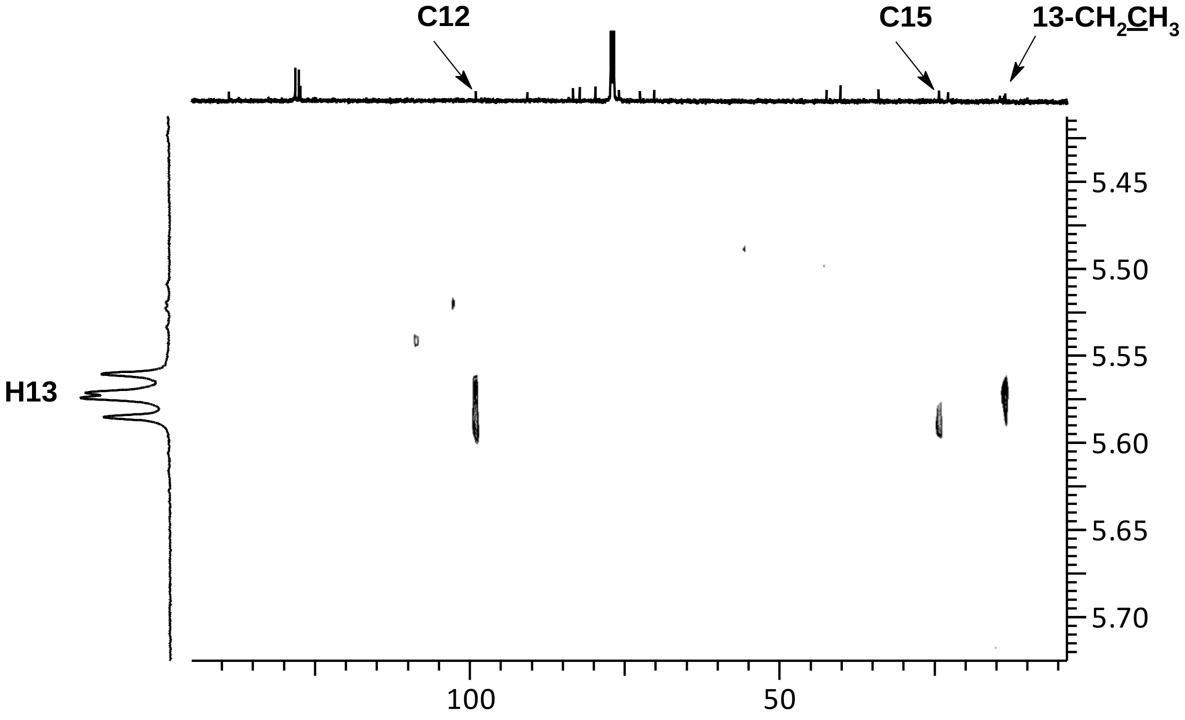
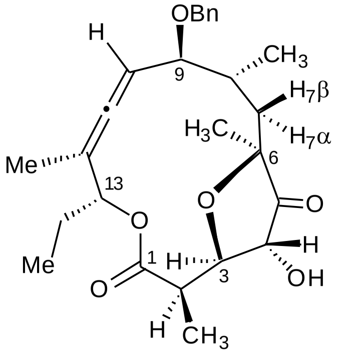
correlations

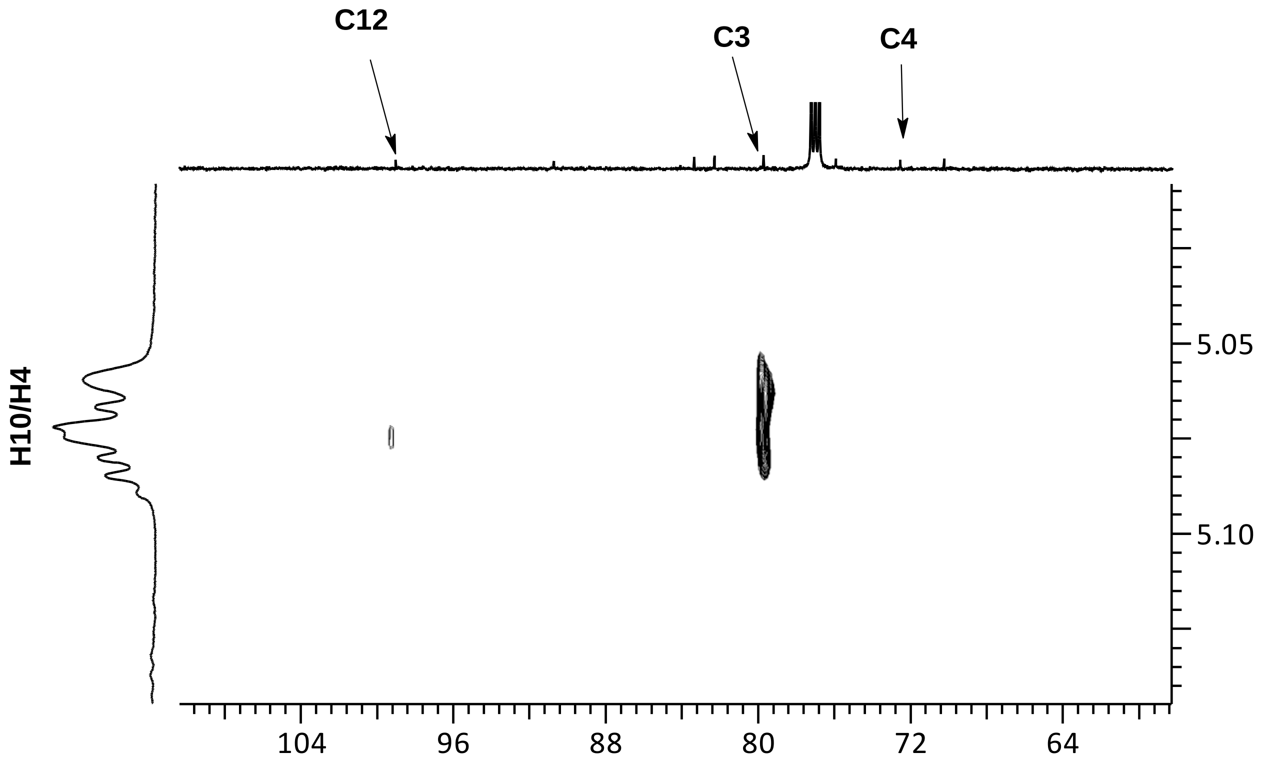

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
H
OH
1
H
3
Me
O
H
CH3



gHMBC

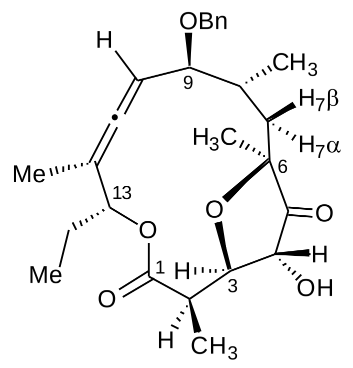
correlations

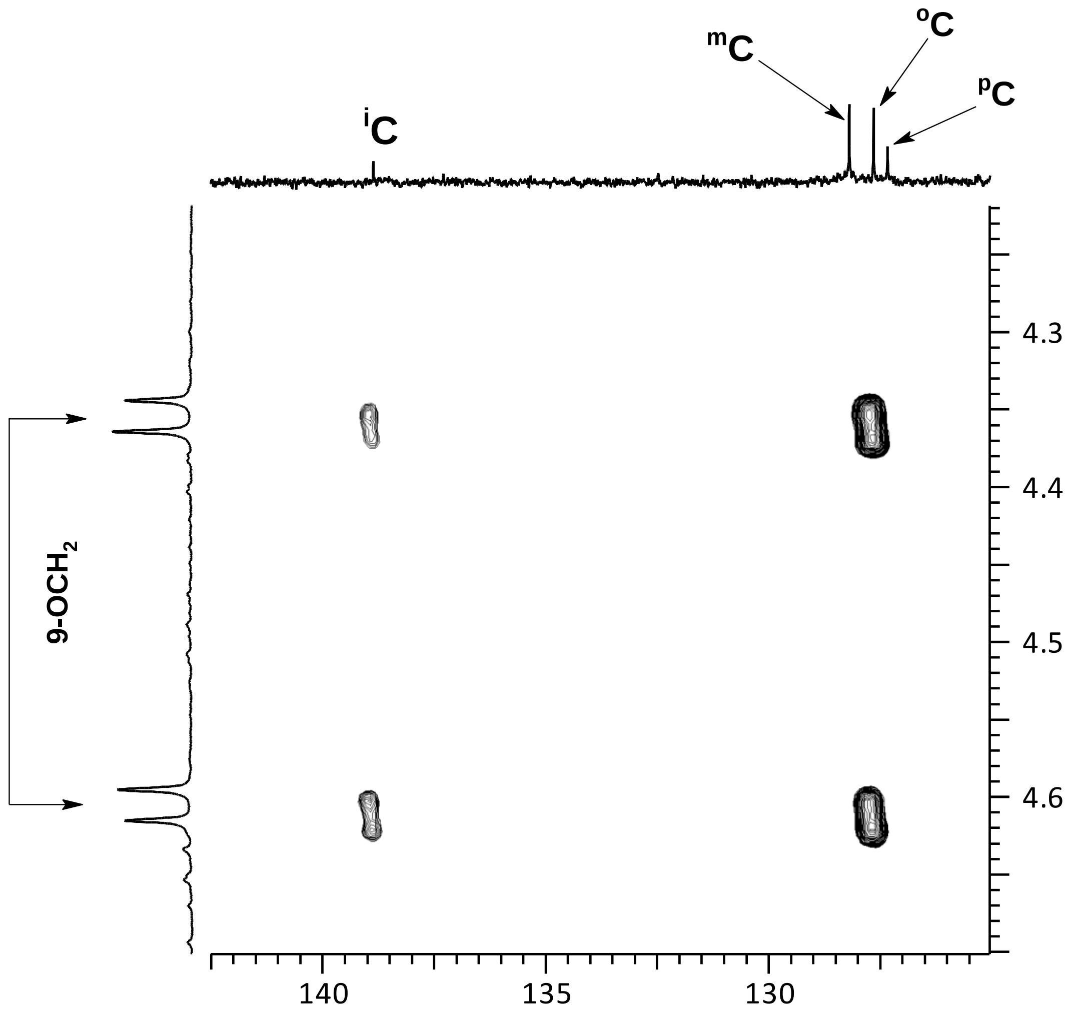

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3



gHMBC

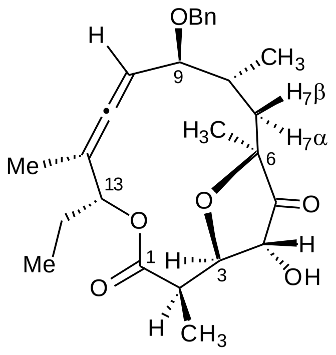
correlations

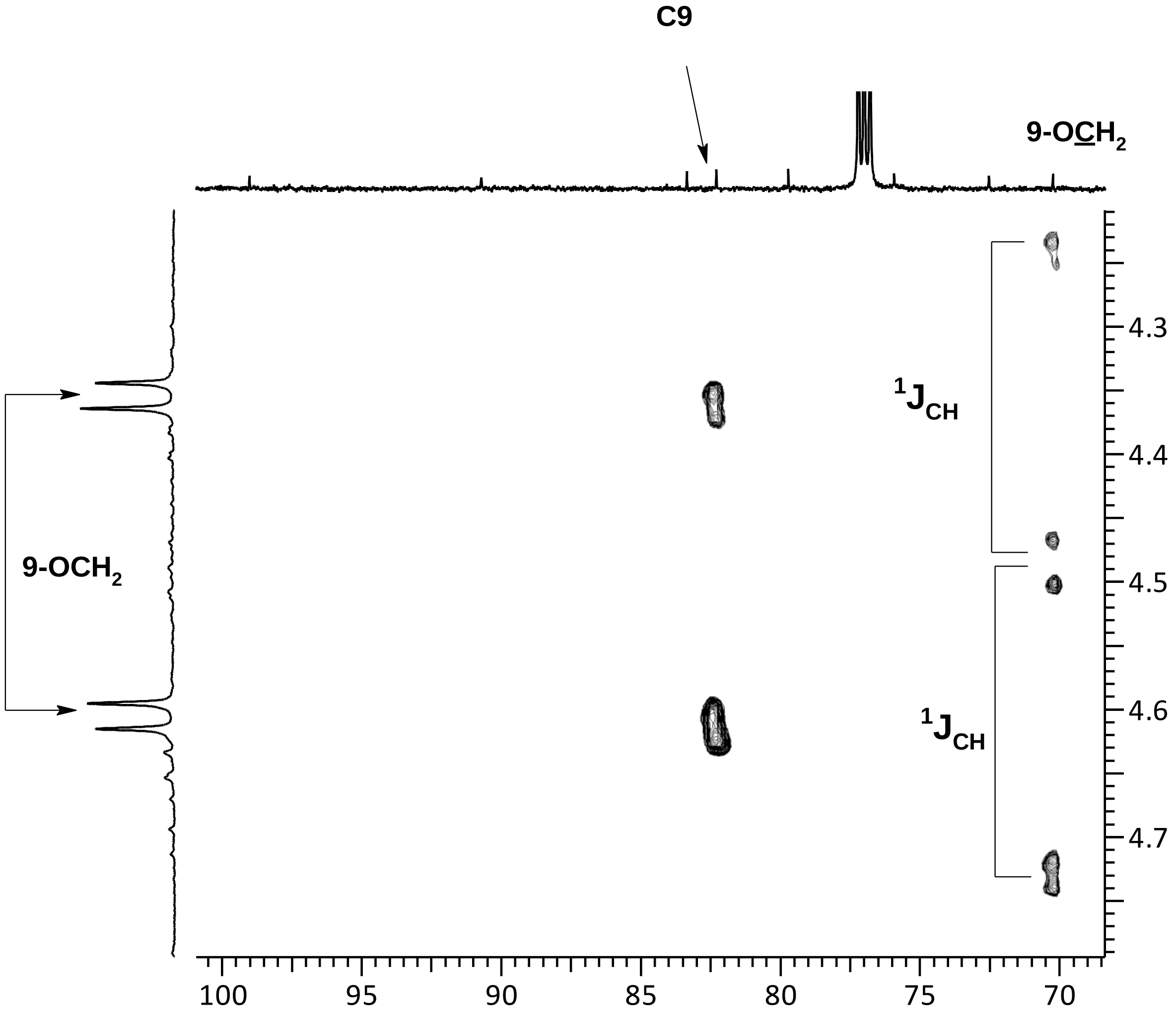

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
1
H
3
H
OH
Me
O
H
CH3



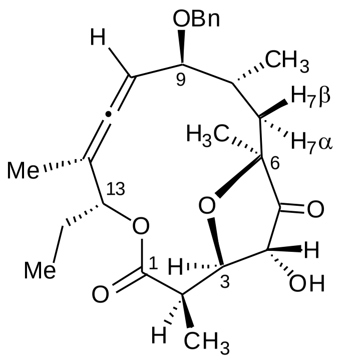
gHMBC

correlations

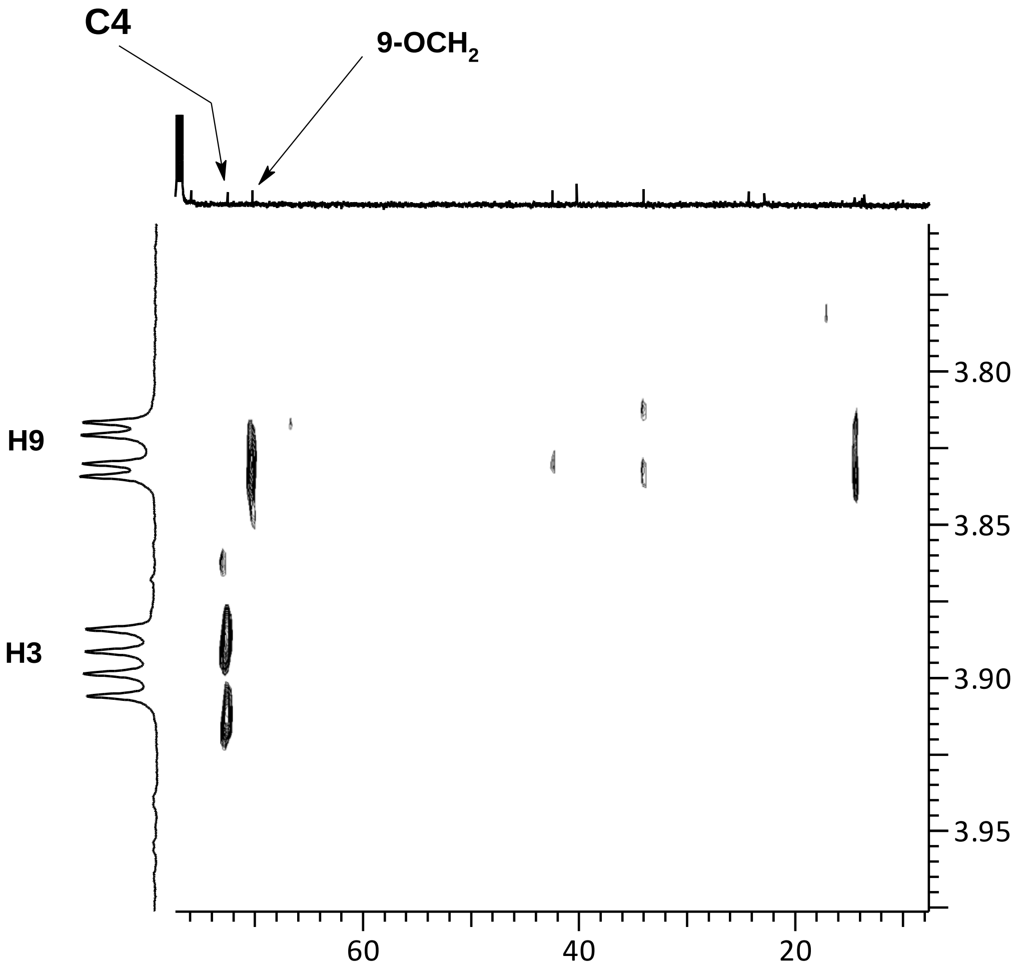

OBn
H
9
CH3
H7β
H7α
H3C
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3



gHMBC
correlations

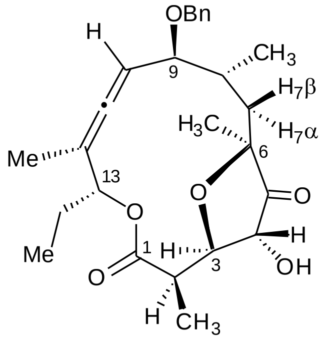


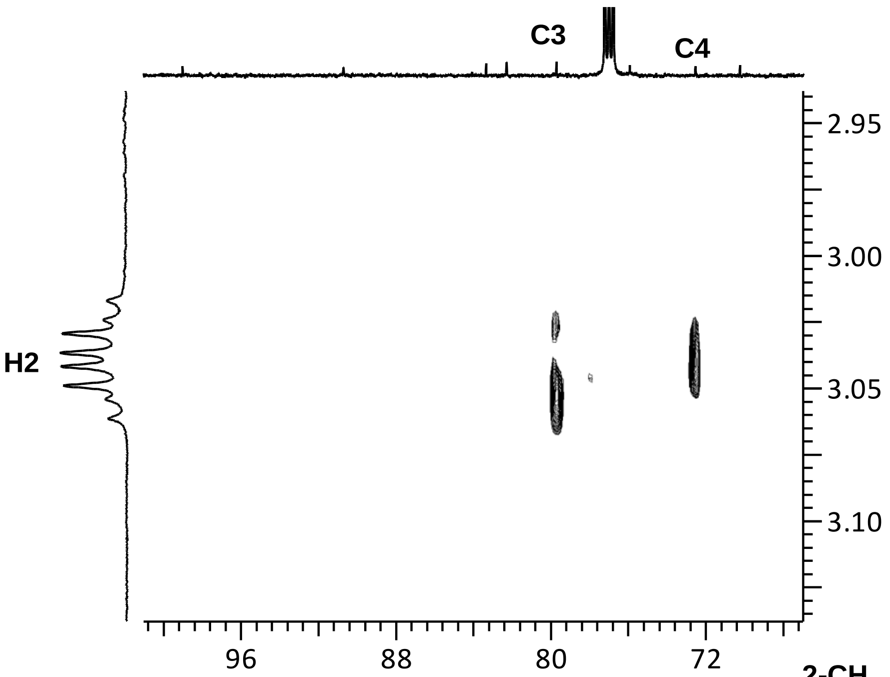
OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
1
H
3
O
H
OH
Me
O
H
CH3

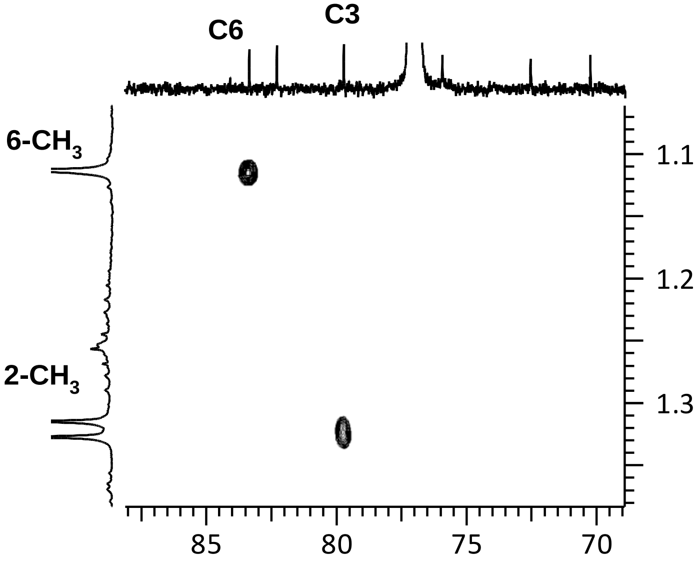


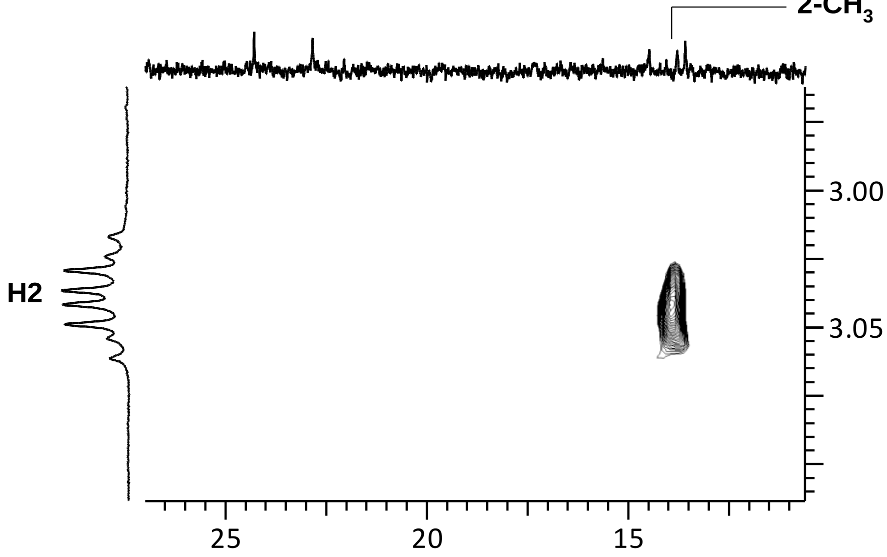
gHMBC

correlations


OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3

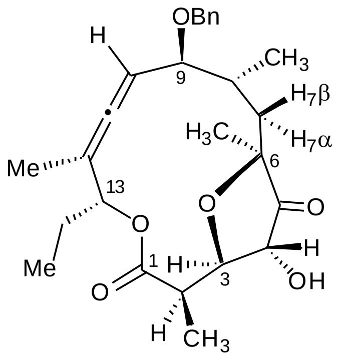


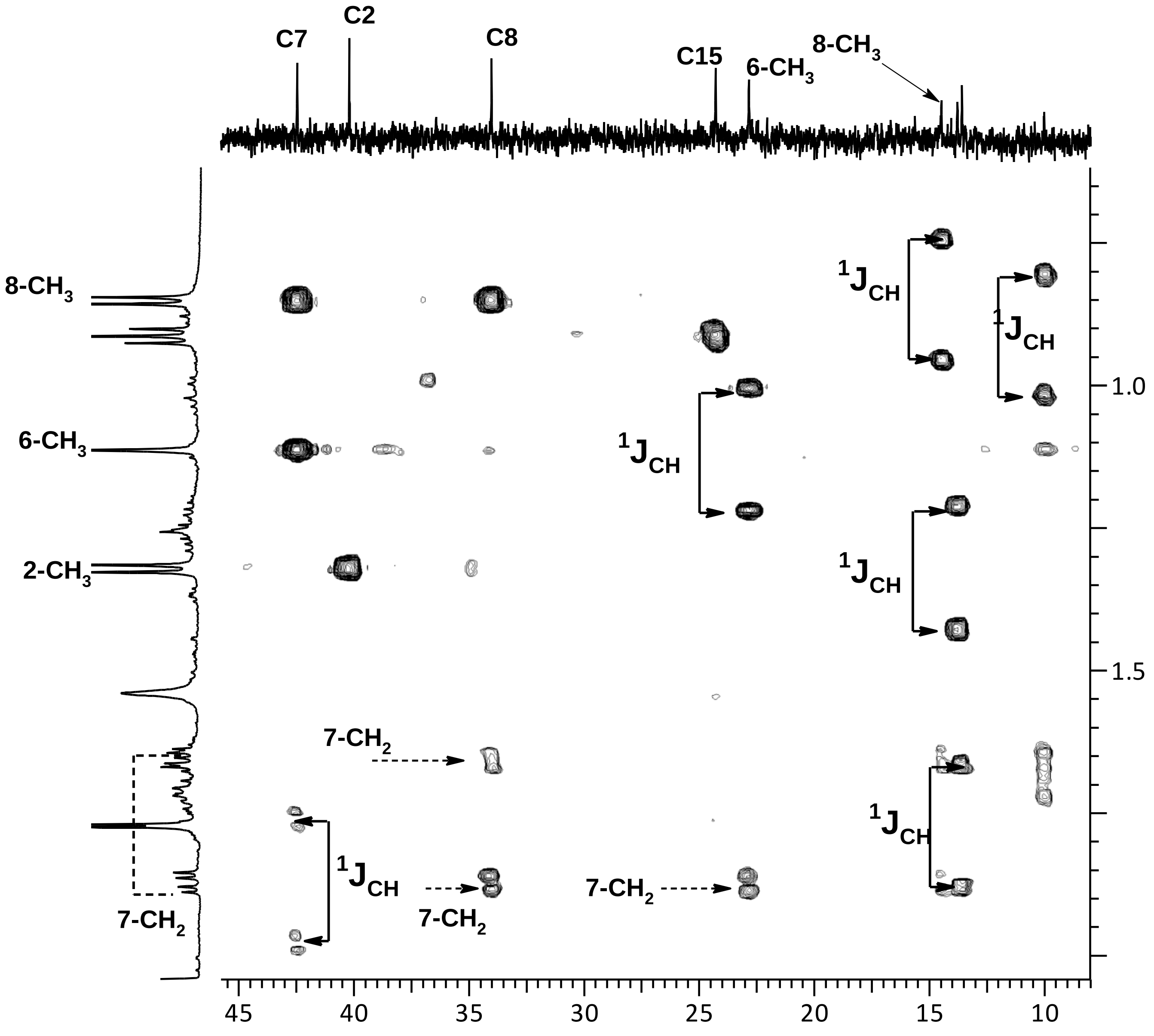




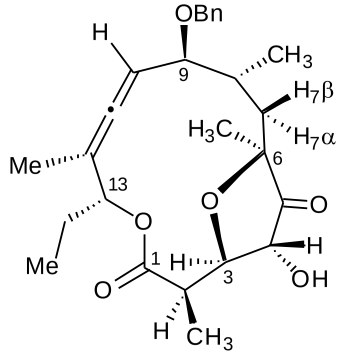
gHMBC

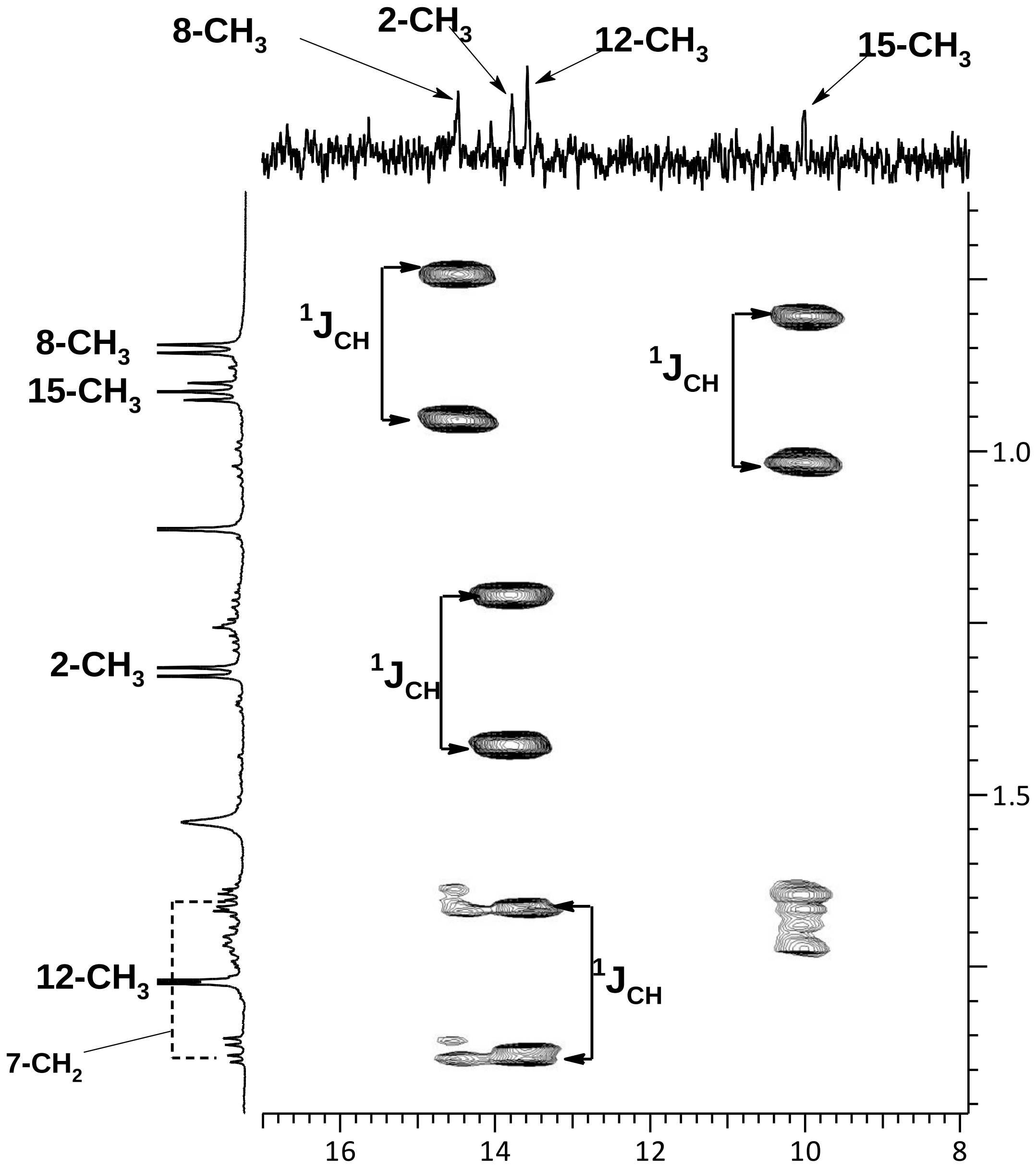
correlations


OBn
H
CH3
9
H7β
H3C
H7α
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH3



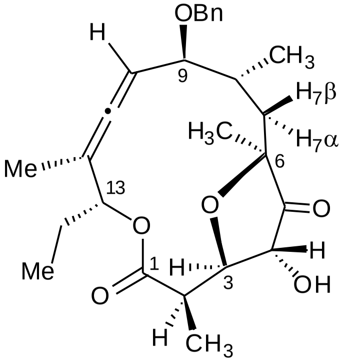
gHMBC
correlations

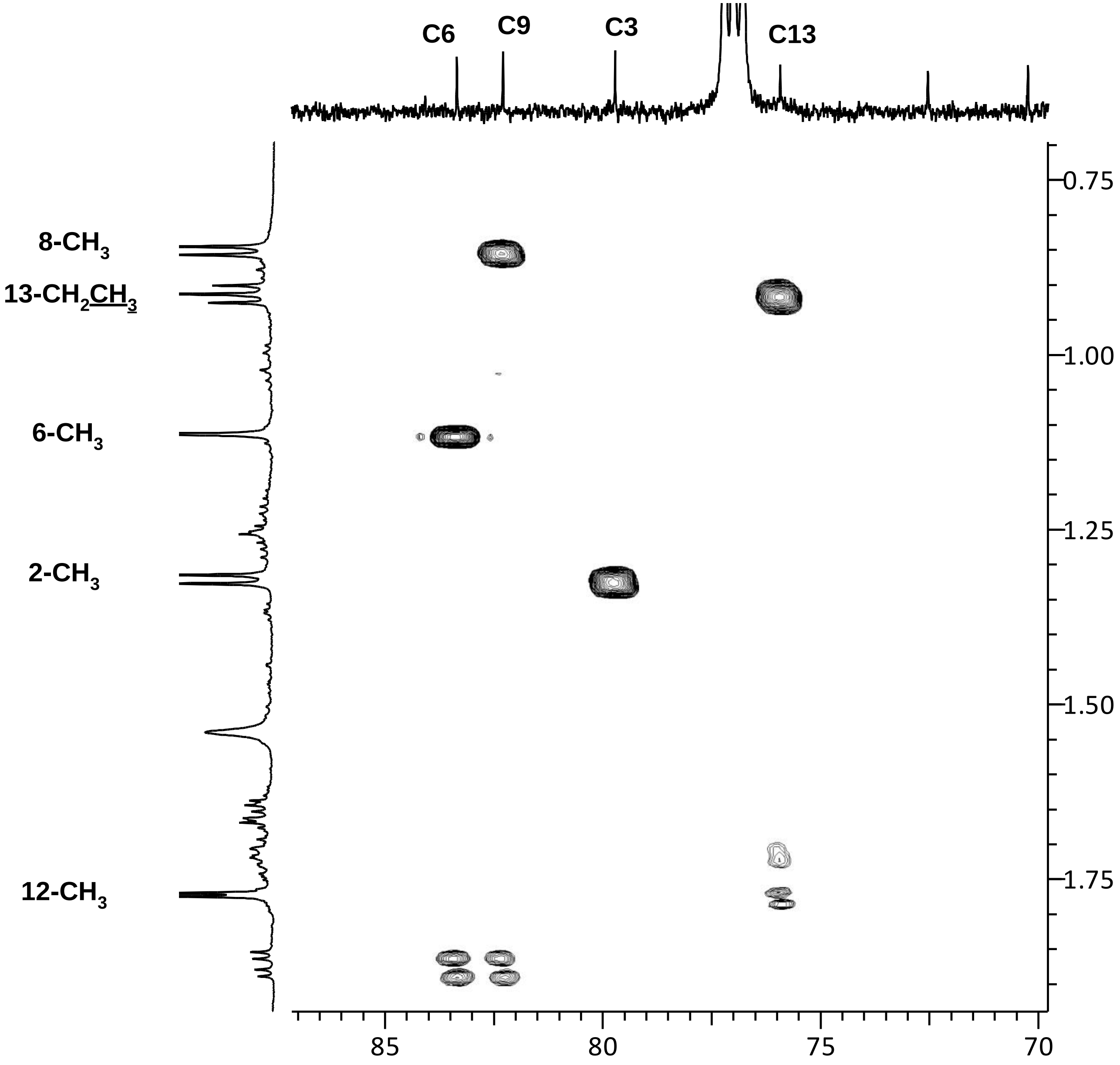

OBn
H
CH_3
$H_7\beta$
H_3C
$H_7\alpha$
9
6
Me
13
O
O
O
1
H
3
H
OH
Me
O
H
CH_3

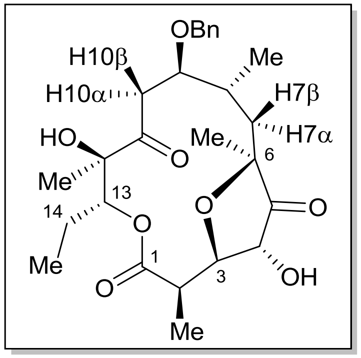


gHMBC

correlations

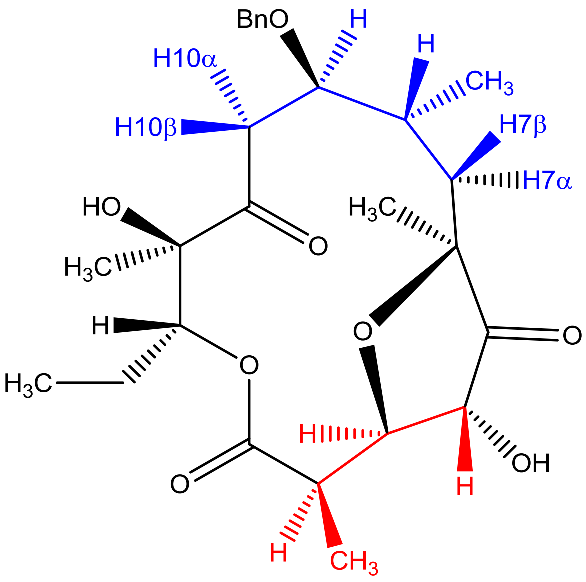

OBn
H
9
CH3
H7β
H7α
H3C
6
Me
13
O
O
O
H
1
3
OH
O
H
CH3



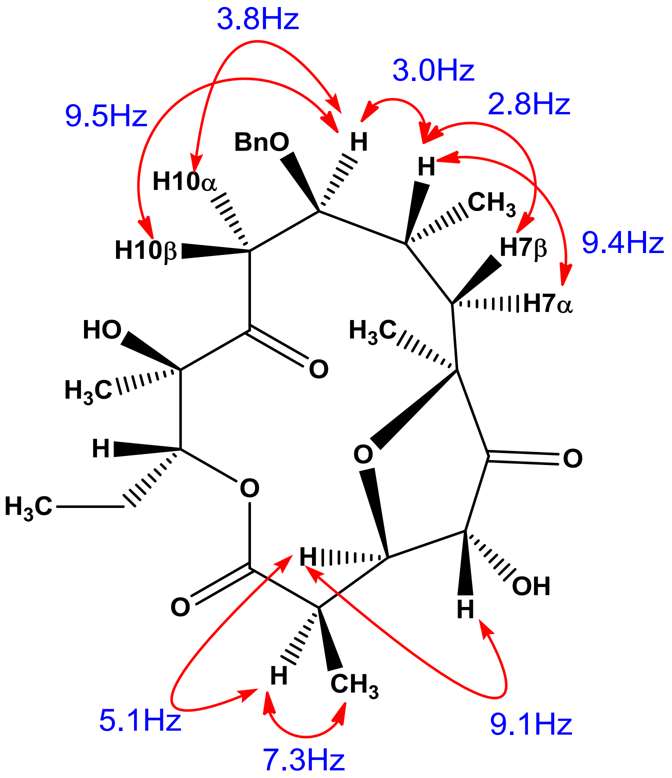


1H NMR chemical shifts (δ/ppm) w/ coupling constant (J/Hz)	13C NMR chemical shift (δ/ppm)
3.29 (dq, $J_{H2,\ H3}$ = 5.5Hz, $J_{H2,\ 2\text{-}CH3}$ = 7.4Hz, **H2**)	174.5 ----- C1
1.30 (d, $J_{2\text{-}CH3,\ H2}$ = 7.3Hz, **2-CH_3**)	42.0 ----- C2
3.96 (dd, $J_{H3,\ H2}$ = 5.1Hz, $J_{H3,\ H4}$ = 9.1Hz, **H3**)	73.1 ----- C3
4.58 (d, $J_{H4,\ H3}$ = 9.1Hz, **H4**)	79.8 ----- C4
1.20 (s, **6-CH_3**)	217.1 ----- C5
1.63 (dd, $J_{H7\beta,\ H8}$ = 2.8Hz, $J_{H7\beta,\ H7\alpha}$ = 15.0Hz, **H7**β)	83.9 ----- C6
1.75 (dd, $J_{H7\alpha,\ H8}$ = 9.4Hz, $J_{H7\alpha,\ H7\beta}$ = 15.0Hz, **H7**α)	41.2 ----- C7
1.65 (m, $J_{H8,\ 8\text{-}CH3}$ = 6.9Hz, **H8**)	35.9 ----- C8
0.86 (d, $J_{8\text{-}CH3,\ H8}$ = 6.9Hz, **8-CH_3**)	78.1 ----- C9
4.14 (dt, $J_{H9,\ H8}$ = 3.0Hz, $J_{H9,\ H10\alpha}$ = 3.8Hz, $J_{H9,\ H10\beta}$ = 9.5Hz, **H9**)	42.6 ----- C10
4.32, 4.49 (d, J_{AB} = 10.9Hz, **9-OCH_2**)	208.1 ----- C11
2.51 (d, $J_{H10\alpha,\ H9}$ = 3.8Hz, **H10**α)	93.2 ----- C12
3.41 (d, $J_{H10\beta,\ H9}$ = 9.5Hz, **H10**β)	76.2 ----- C13
1.47 (s, **12-CH_3**)	24.9 ----- C14
4.04 (dd, $J_{H13,\ H14}$ = 2.1, 10.4Hz, **H13**)	13.1 ----- 2-CH_3
1.26, 1.64 (m, **14-CH_2**)	23.5 ----- 6-CH_3
1.05 (t, $J_{14\text{-}CH3,\ H14}$ = 7.4Hz, **14-CH_3**)	17.6 ----- 8-CH_3
	21.0 ----- 12-CH_3
	11.3 ----- 14-CH_3
	73.2 ----- 9-OCH_3
	137.9 ----- ^{ipso}C
	129.0 ----- $^{ortho}C$
	128.3 ----- ^{meta}C
	128.0 ----- ^{para}C

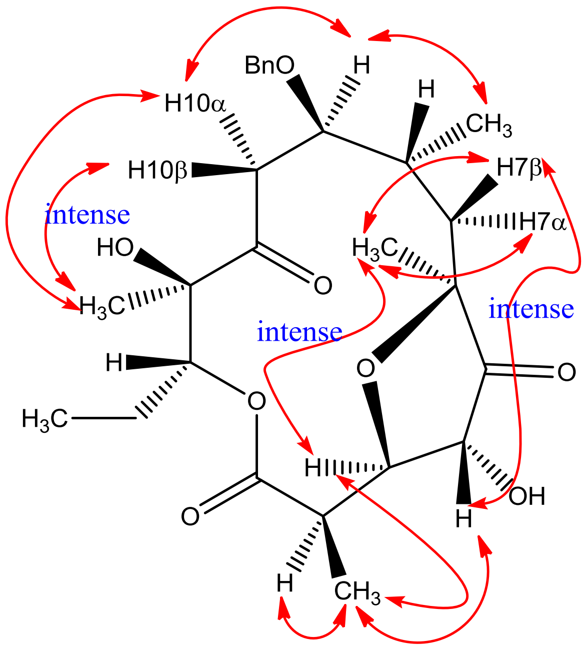
1d TOCSY

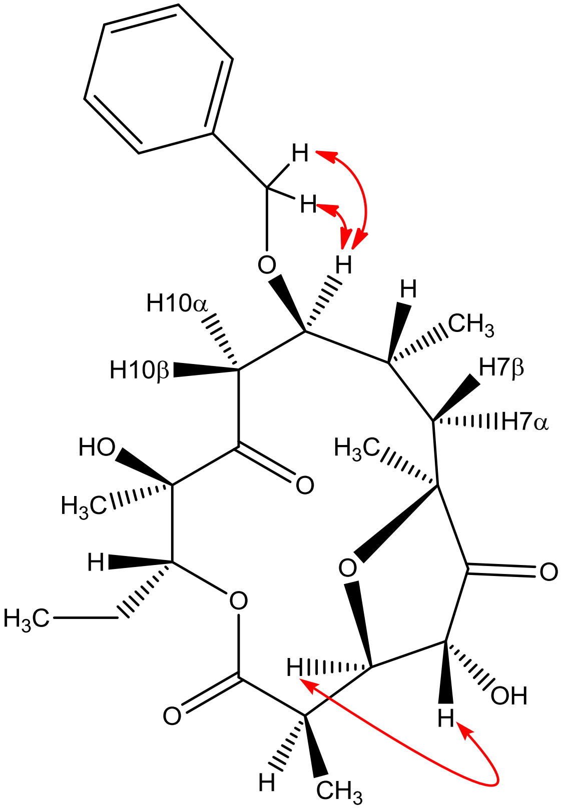

BnO
H
H10α
H
CH3
H10β
H7β
H7α
HO
H3C
O
H3C
H
O
O
H3C
O
HIIII
OH
O
H
H
CH3

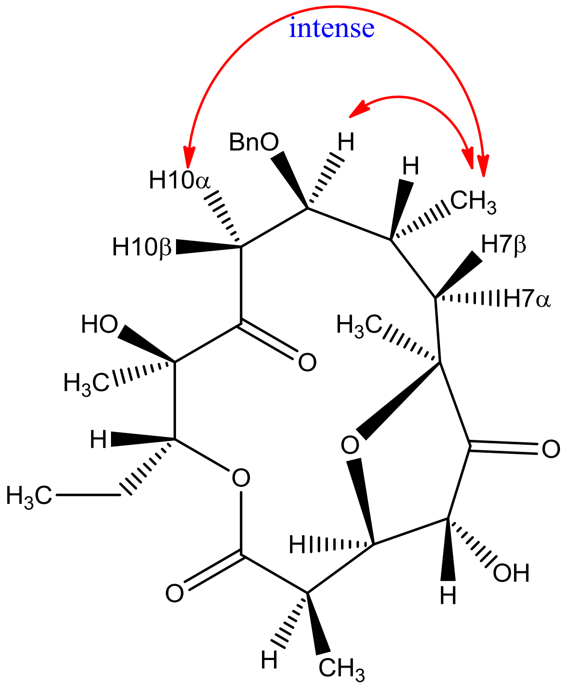
Coupling constant (J_{HH})


3.8Hz
3.0Hz
2.8Hz
9.5Hz
BnO
H
H10α
H
CH3
H10β
H7β
9.4Hz
H7α
HO
H3C
O
H3C
H
O
O
H3C
O
HIIII
OH
O
H
H
CH3
5.1Hz
7.3Hz
9.1Hz

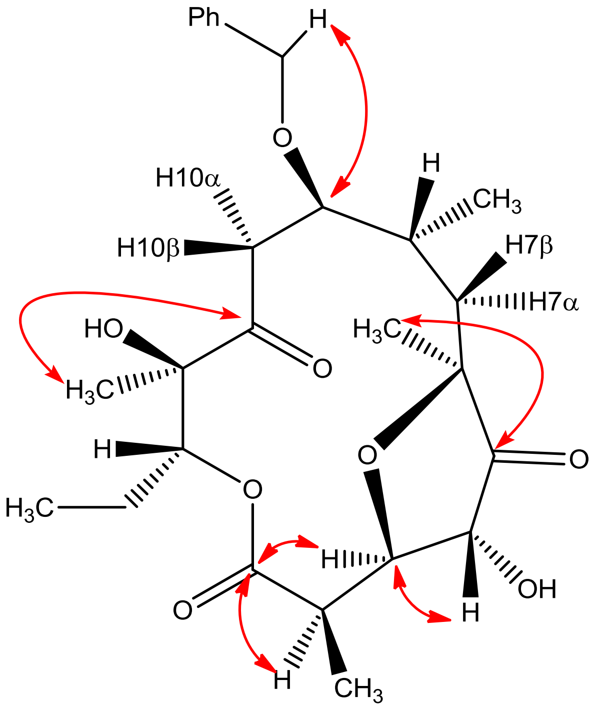
NOESY


H10α
BnO
H
H
CH$_3$
H10β
H7β
intense
HO
H$_3$C
H7α
H$_3$C
O
intense
intense
H
O
O
H$_3$C
O
H
OH
O
H
H
CH$_3$


H
H
O
H
H
H10α
CH$_3$
H10β
H7β
HO
H$_3$C
H7α
H$_3$C
O
H
O
O
H$_3$C
O
H
OH
O
H
H
CH$_3$


intense
BnO
H
H10α
H
CH$_3$
H10β
H7β
HO
H$_3$C
H7α
H$_3$C
O
H
O
O
H$_3$C
O
H
OH
O
H
H
CH$_3$

HMBC


Ph
H
O
H10α
H10β
H
CH$_3$
H7β
H7α
HO
H$_3$C
O
H$_3$C
H
O
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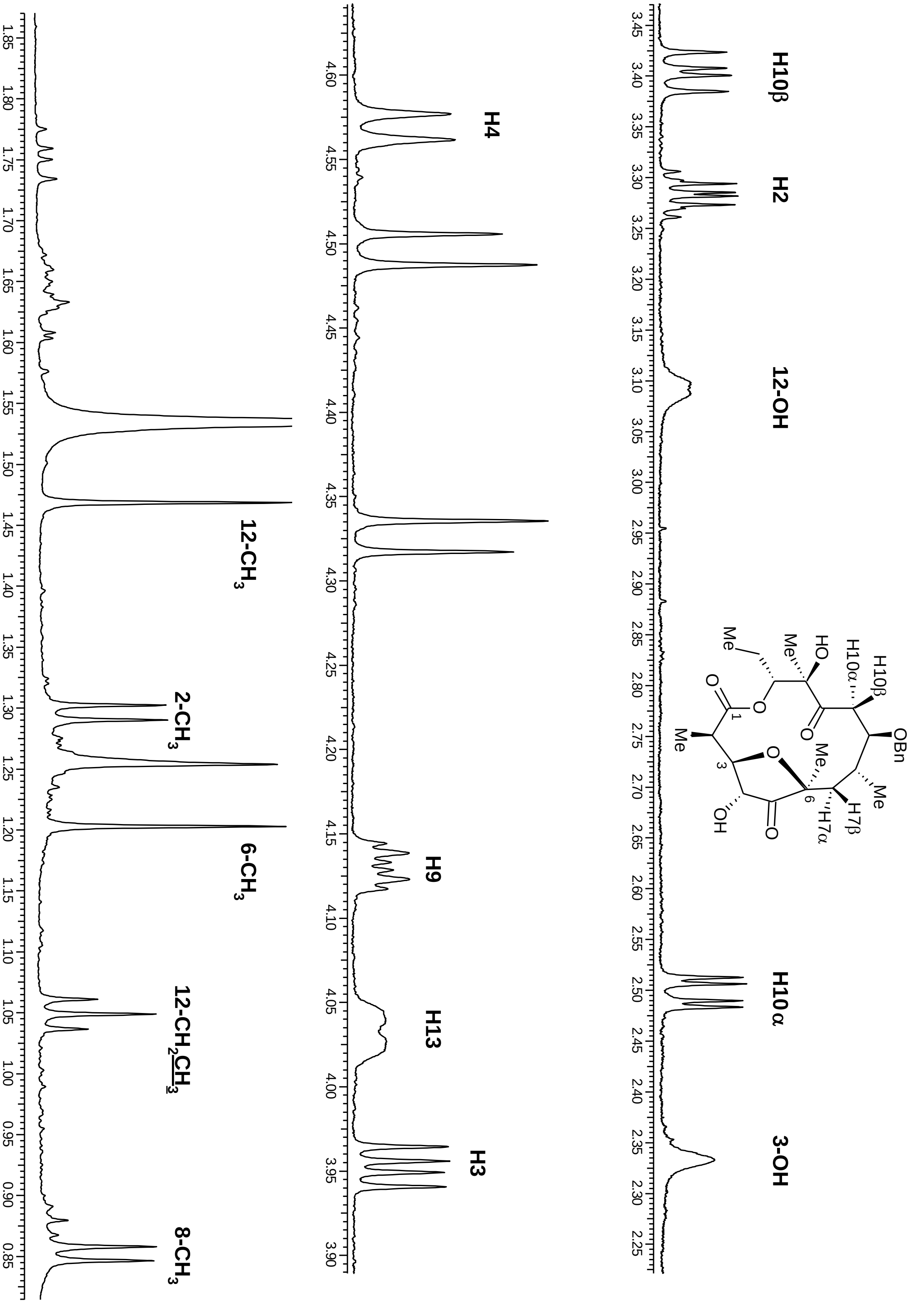
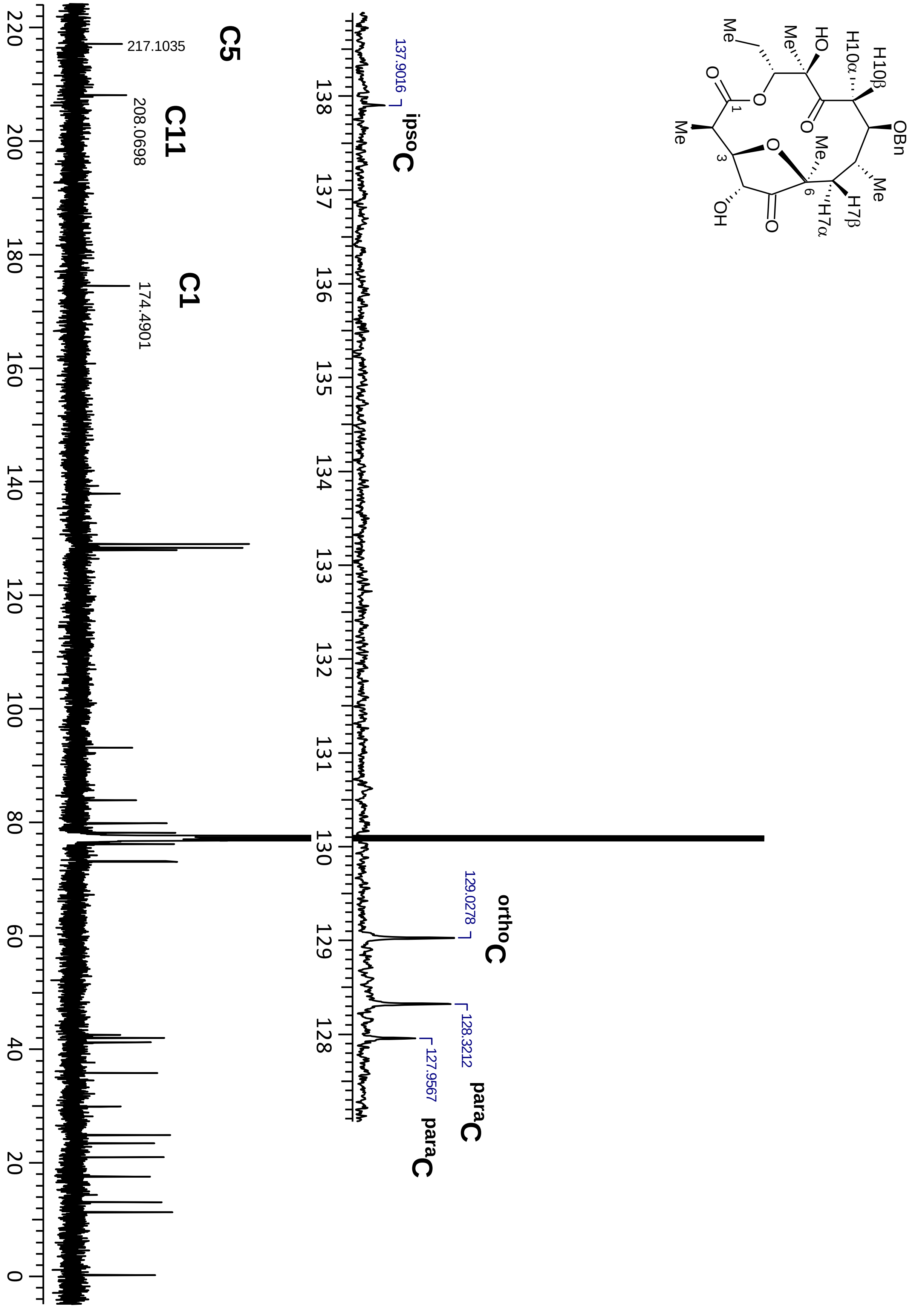
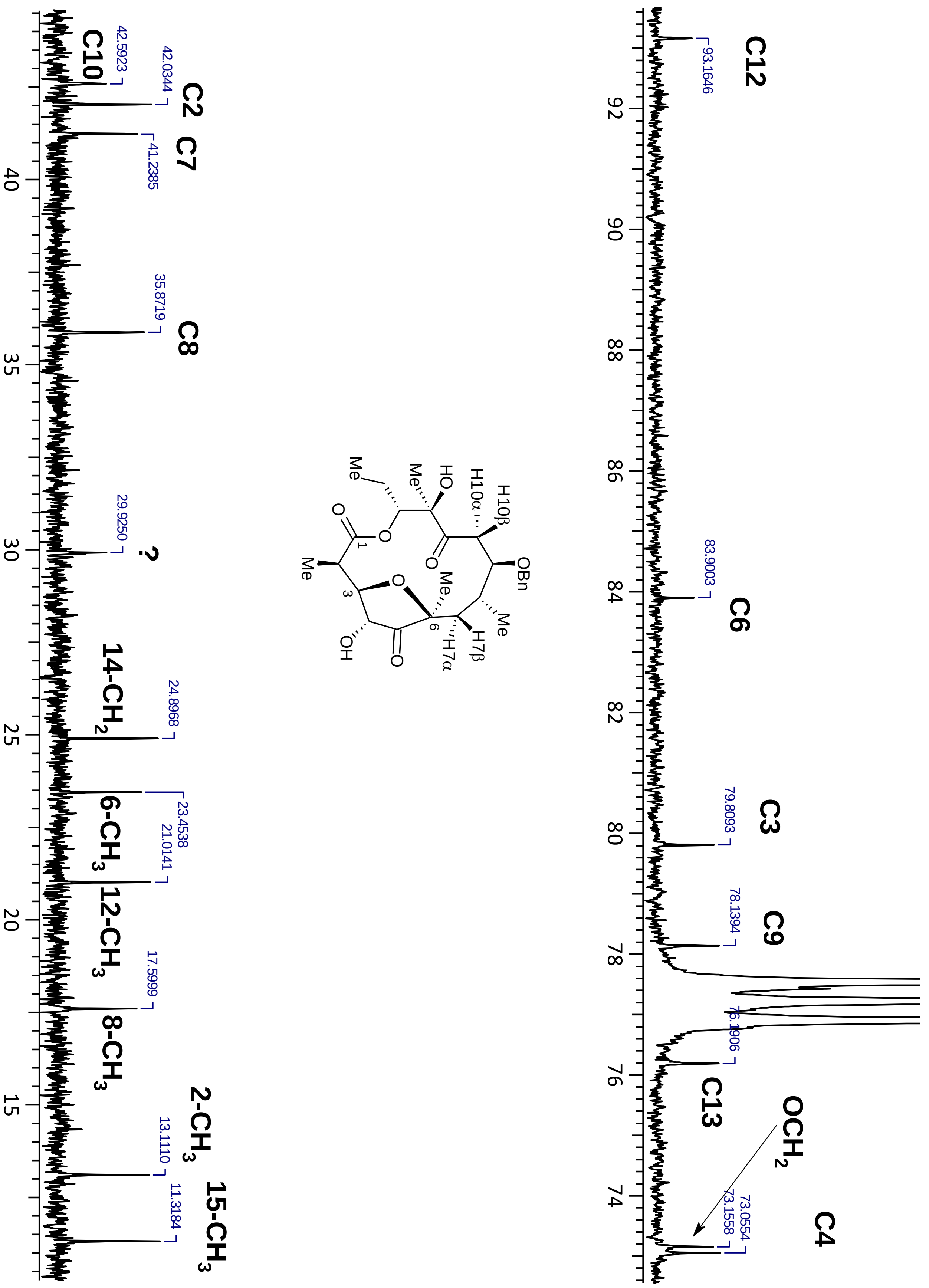
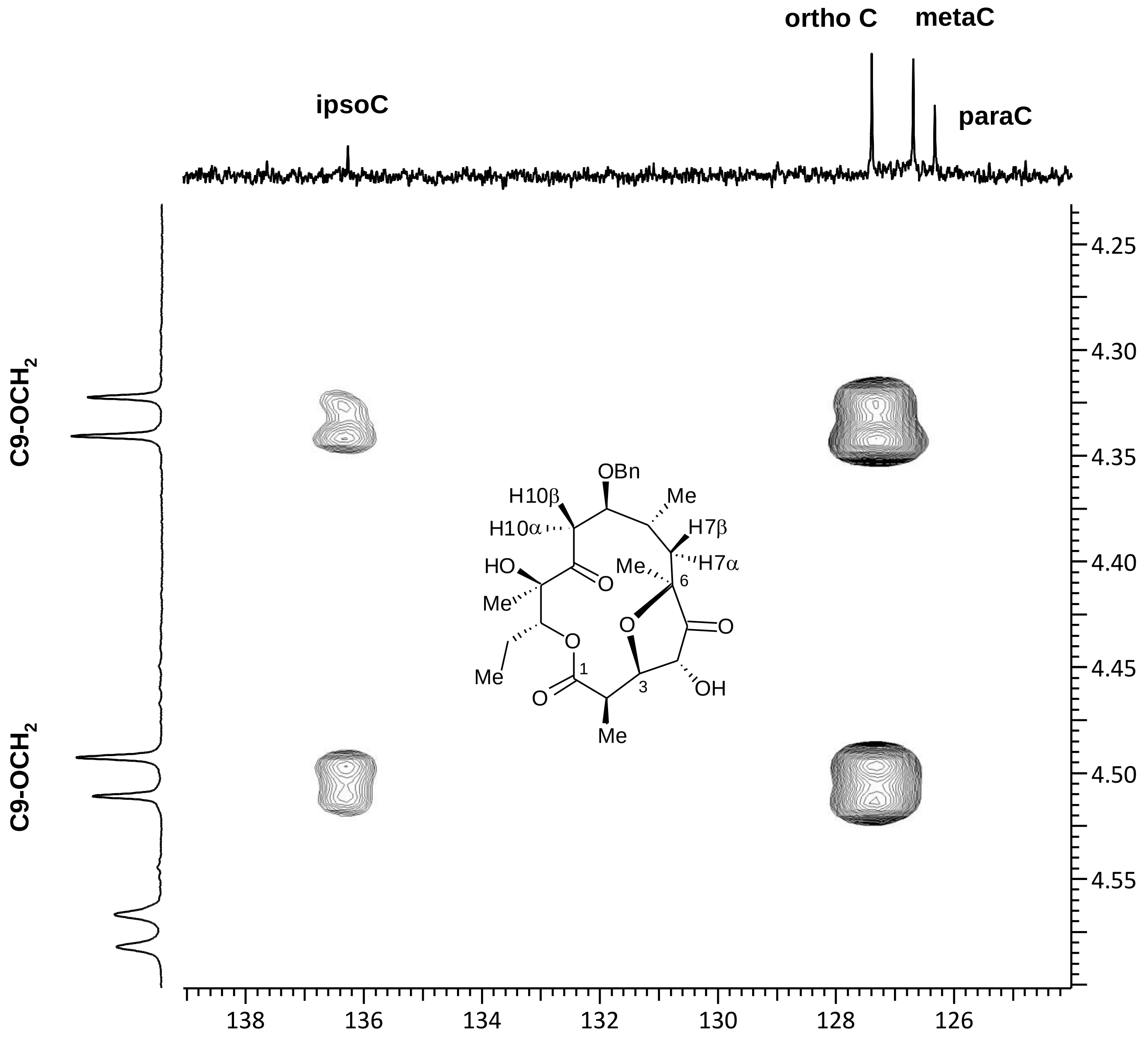
H$_3$C
O
HIII
OH
O
H
H
CH$_3$


H10β
H2
12-OH
H10α
3-OH
H4
H9
H13
H3
12-CH$_3$
2-CH$_3$
6-CH$_3$
12-CH$_2$CH$_3$
8-CH$_3$
H10β
H10α
HO
Me
Me
OBn
Me
H7β
H7α
Me
6
3
1
O
OH
Me
Me
3.45 3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 3.00 2.95 2.90 2.85 2.80 2.75 2.70 2.65 2.60 2.55 2.50 2.45 2.40 2.35 2.30 2.25
4.60 4.55 4.50 4.45 4.40 4.35 4.30 4.25 4.20 4.15 4.10 4.05 4.00 3.95 3.90
1.85 1.80 1.75 1.70 1.65 1.60 1.55 1.50 1.45 1.40 1.35 1.30 1.25 1.20 1.15 1.10 1.05 1.00 0.95 0.90 0.85

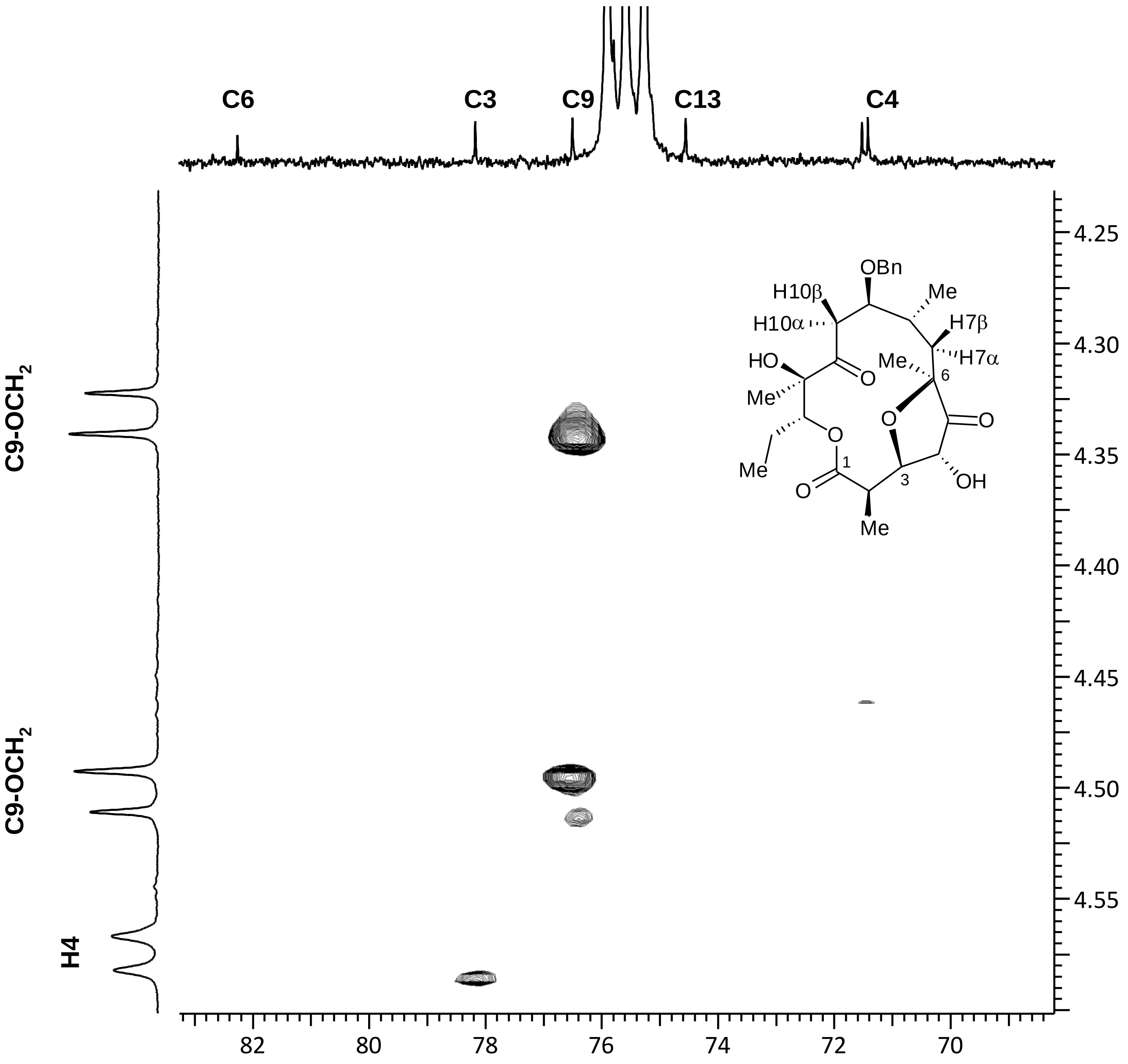

H10β
OBn
Me
H7β
H7α
H10α
HO
Me
O
O
Me
O
O
1
3
6
OH
Me
Me
217.1035
C5
208.0698
C11
174.4901
C1
137.9016
ipsoC
129.0278
orthoC
128.3212
paraC
127.9567
paraC
138
137
136
135
134
133
132
131
130
129
128
220
200
180
160
140
120
100
80
60
40
20
0


C12
93.1646
C6
83.9003
C3
79.8093
C9
78.1394
76.1906
C13
OCH2
73.1558
73.0554
C4
92
90
88
86
84
82
80
78
76
74
C10
42.5923
C2
42.0344
C7
41.2385
C8
35.8719
?
29.9250
14-CH2
24.8968
6-CH3
23.4538
12-CH3
21.0141
8-CH3
17.5999
2-CH3
13.1110
15-CH3
11.3184
40
35
30
25
20
15
H10β
H10α
OBn
HO
Me
Me
Me
Me
Me
H7β
H7α
OH
O
1
3
6

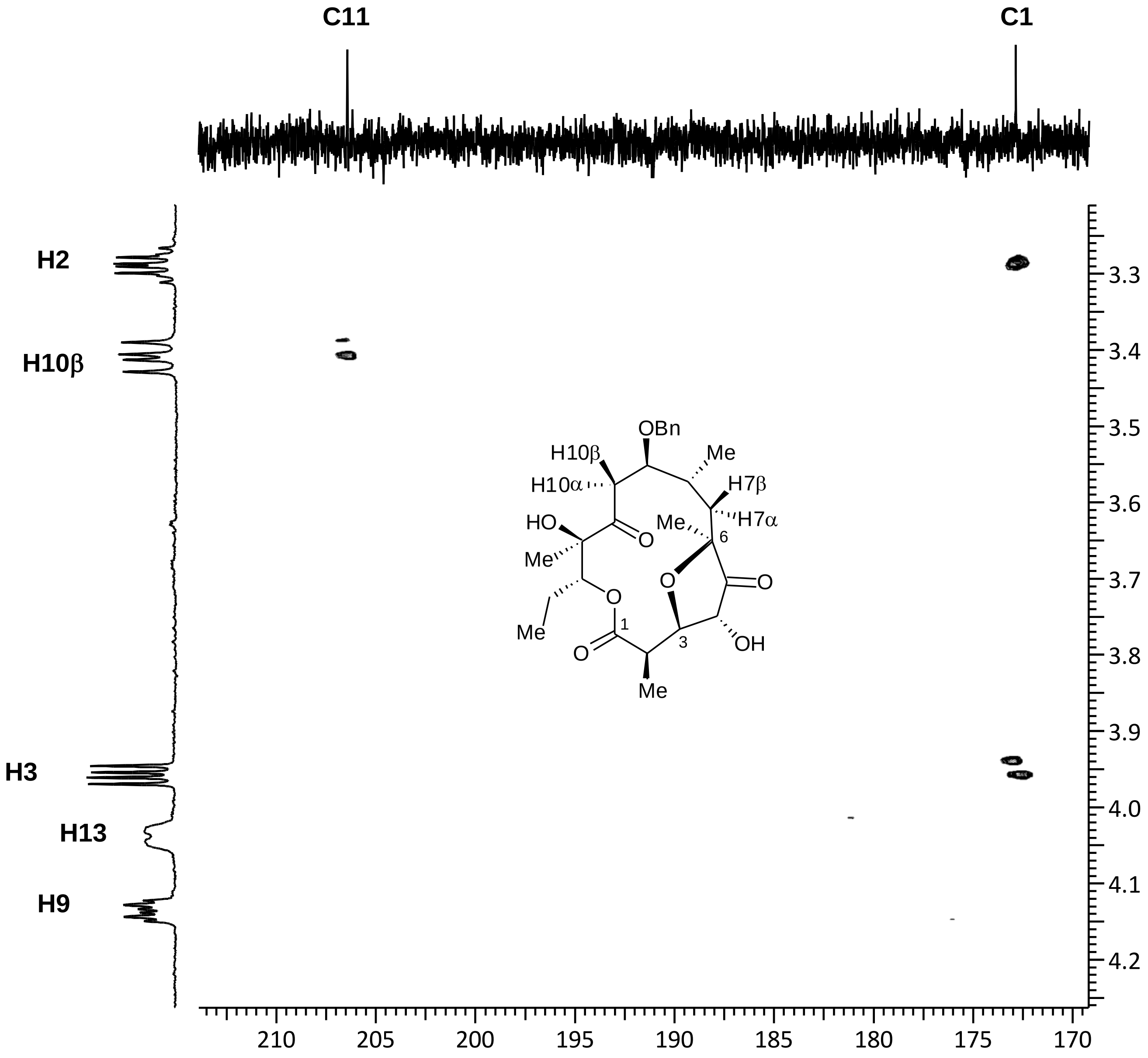
gHMBC

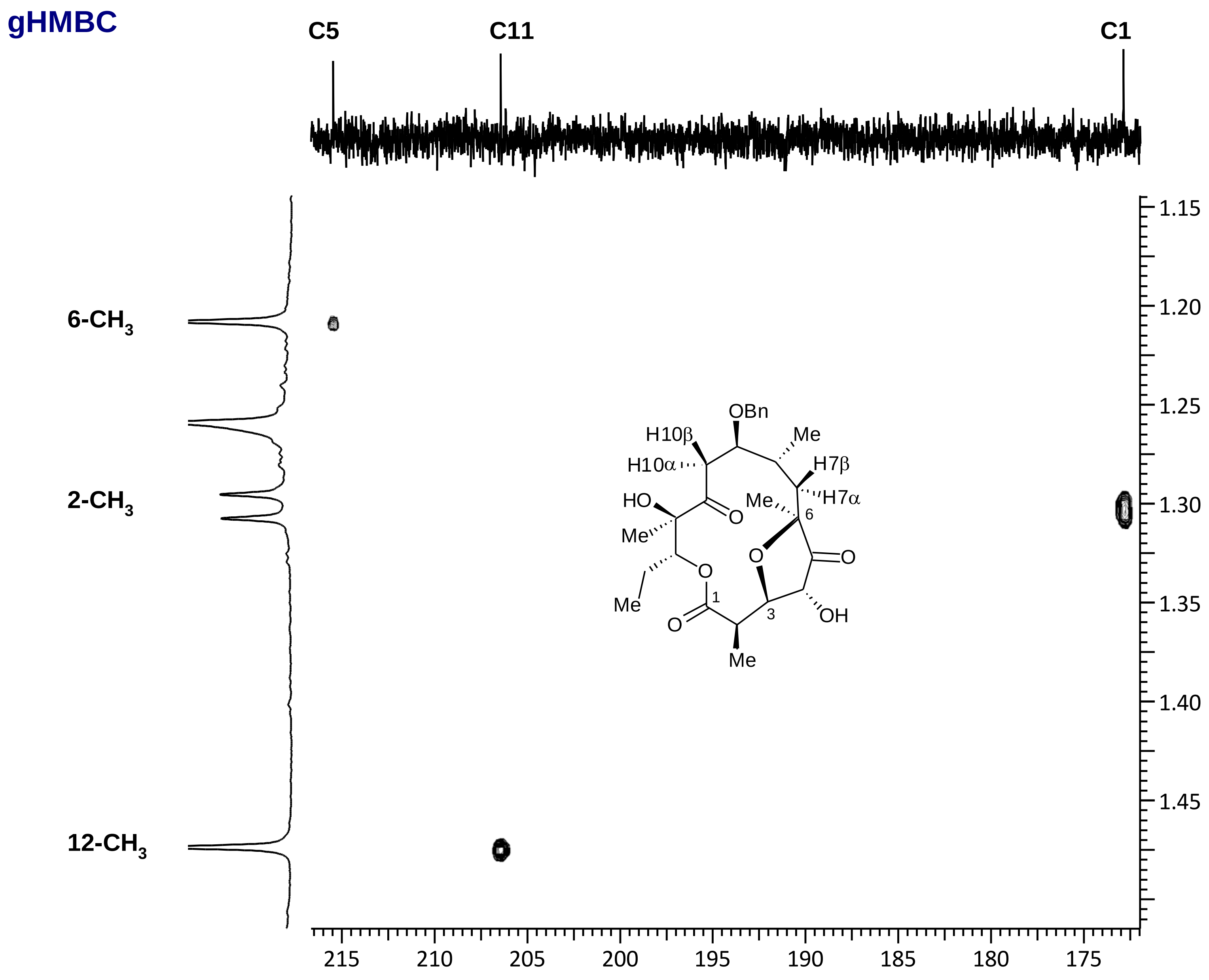

ipsoC
ortho C
metaC
paraC
C9-OCH$_2$
C9-OCH$_2$
OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
O
Me
O
O
1
3
OH
Me
O
Me
4.25
4.30
4.35
4.40
4.45
4.50
4.55
138
136
134
132
130
128
126

gHMBC

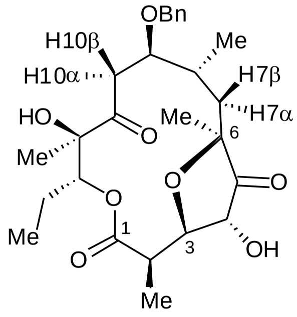

C6
C3
C9
C13
C4
C9-OCH$_2$
C9-OCH$_2$
H4
OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
O
Me
O
O
O
1
Me
3
OH
O
Me
4.25
4.30
4.35
4.40
4.45
4.50
4.55
82
80
78
76
74
72
70

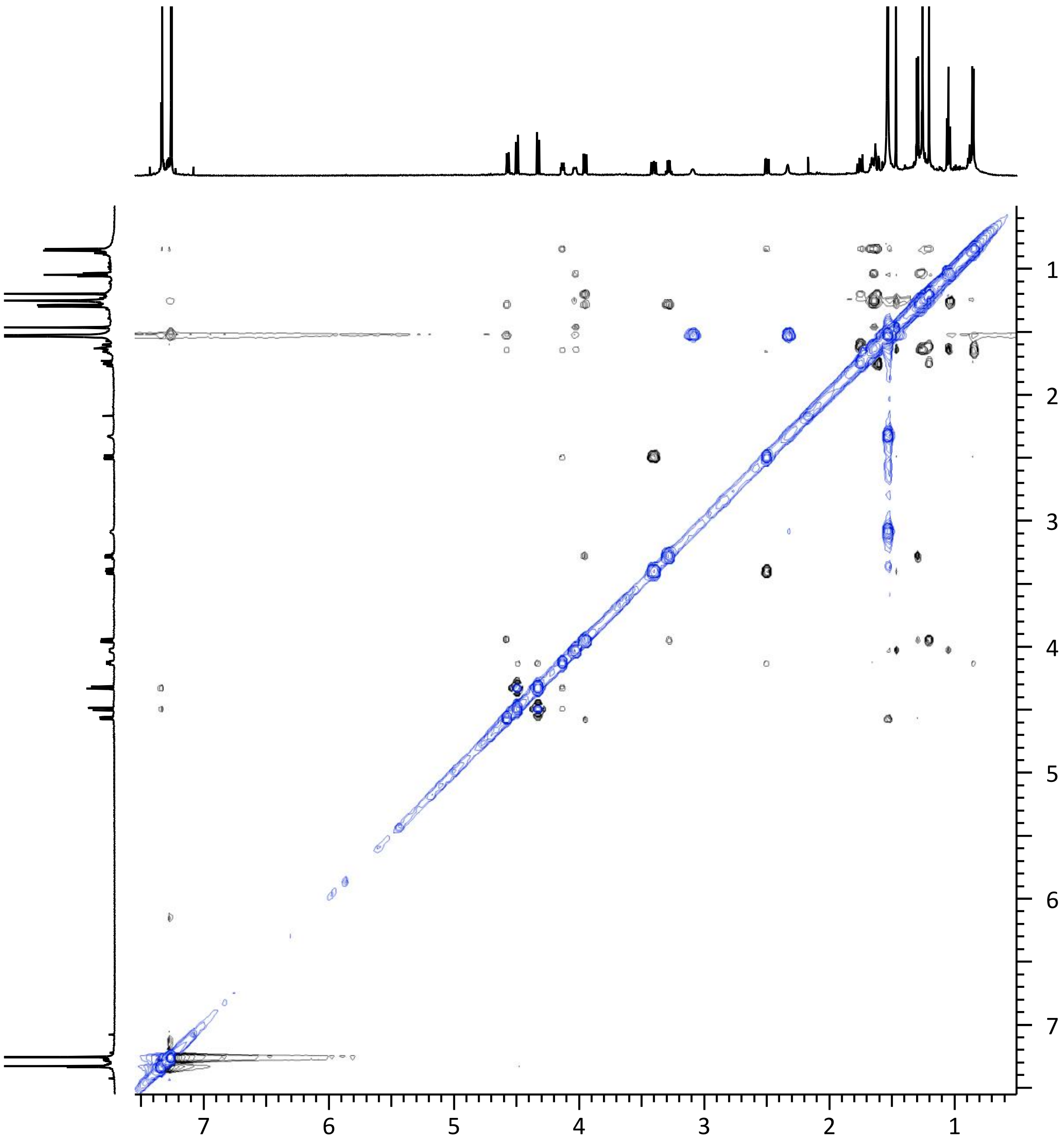
gHMBC


C11
C1
H2
H10β
H3
H13
H9
OBn
H10β
Me
H7β
H10α
H7α
HO
Me
6
Me
O
O
O
Me
O
1
3
OH
O
Me
Me
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
210
205
200
195
190
185
180
175
170

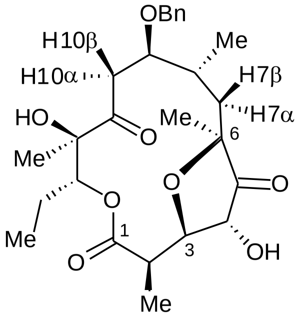

gHMBC
C5
C11
C1
6-CH$_3$
2-CH$_3$
12-CH$_3$
1.15
1.20
1.25
1.30
1.35
1.40
1.45
215
210
205
200
195
190
185
180
175
OBn
H10β
Me
H7β
H10α
H7α
HO
Me
O
6
Me
O
O
O
Me
1
3
OH
O
Me

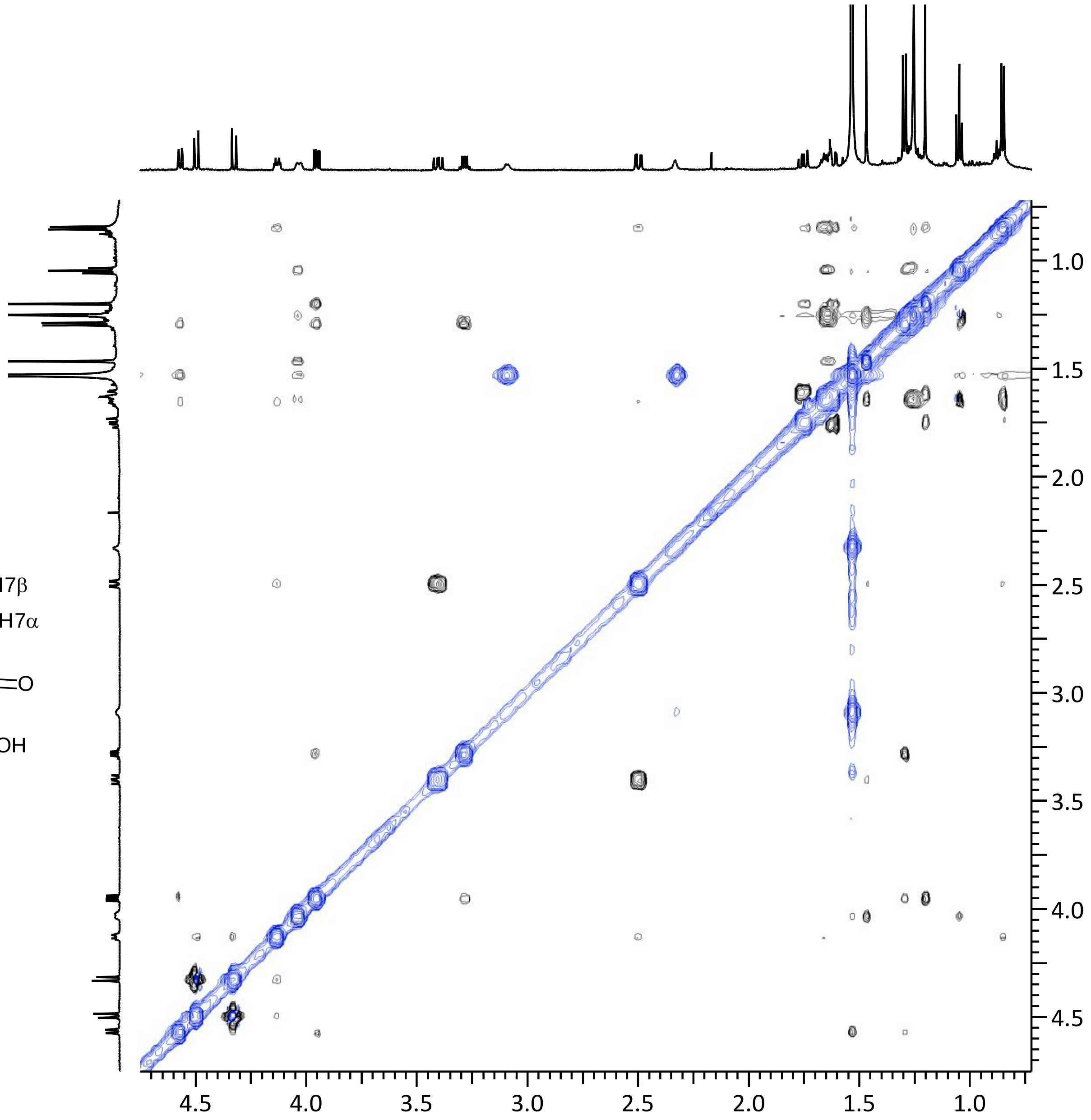
NOESY

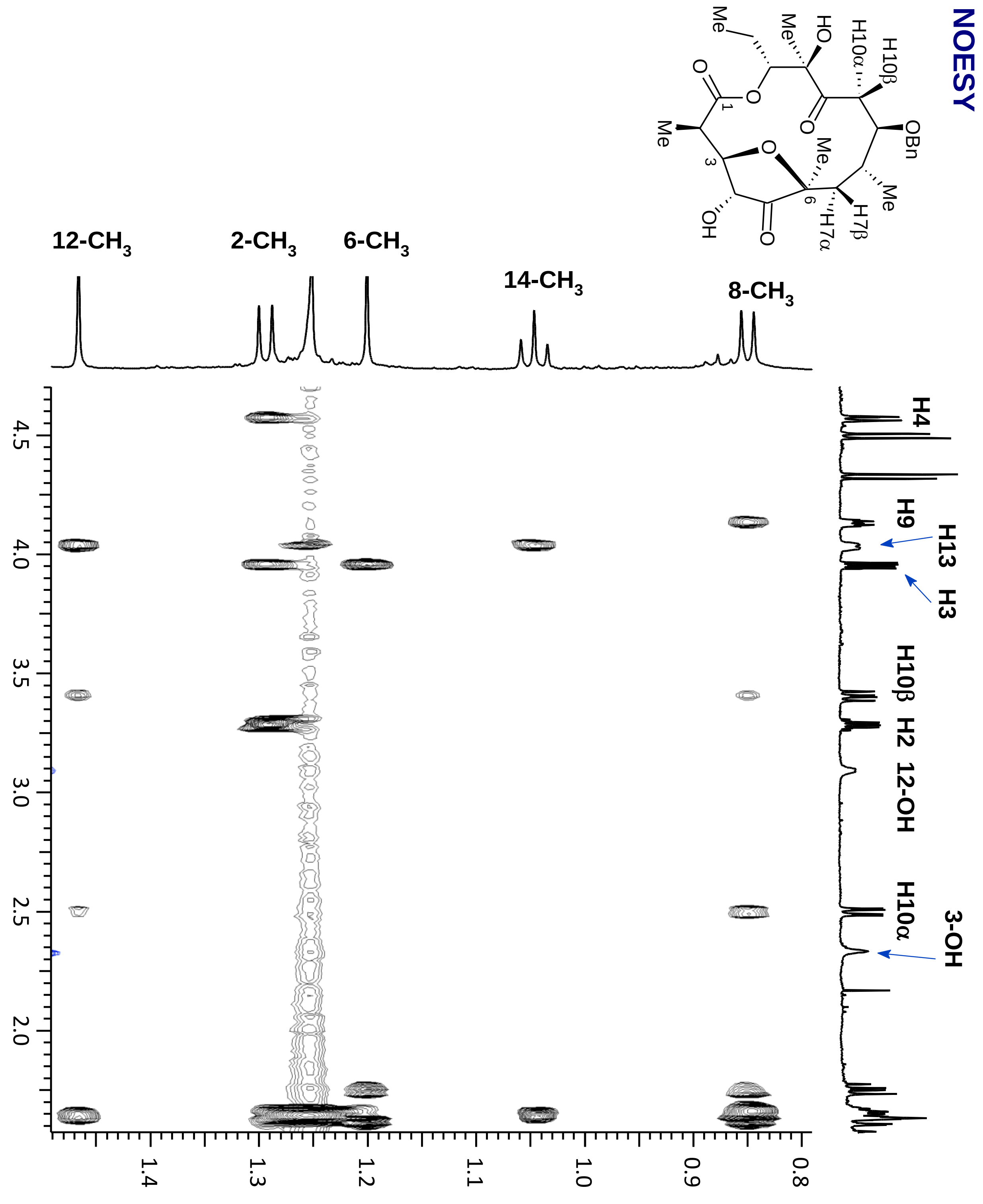

OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
O
O
Me
O
O
Me
1
3
OH
O
Me

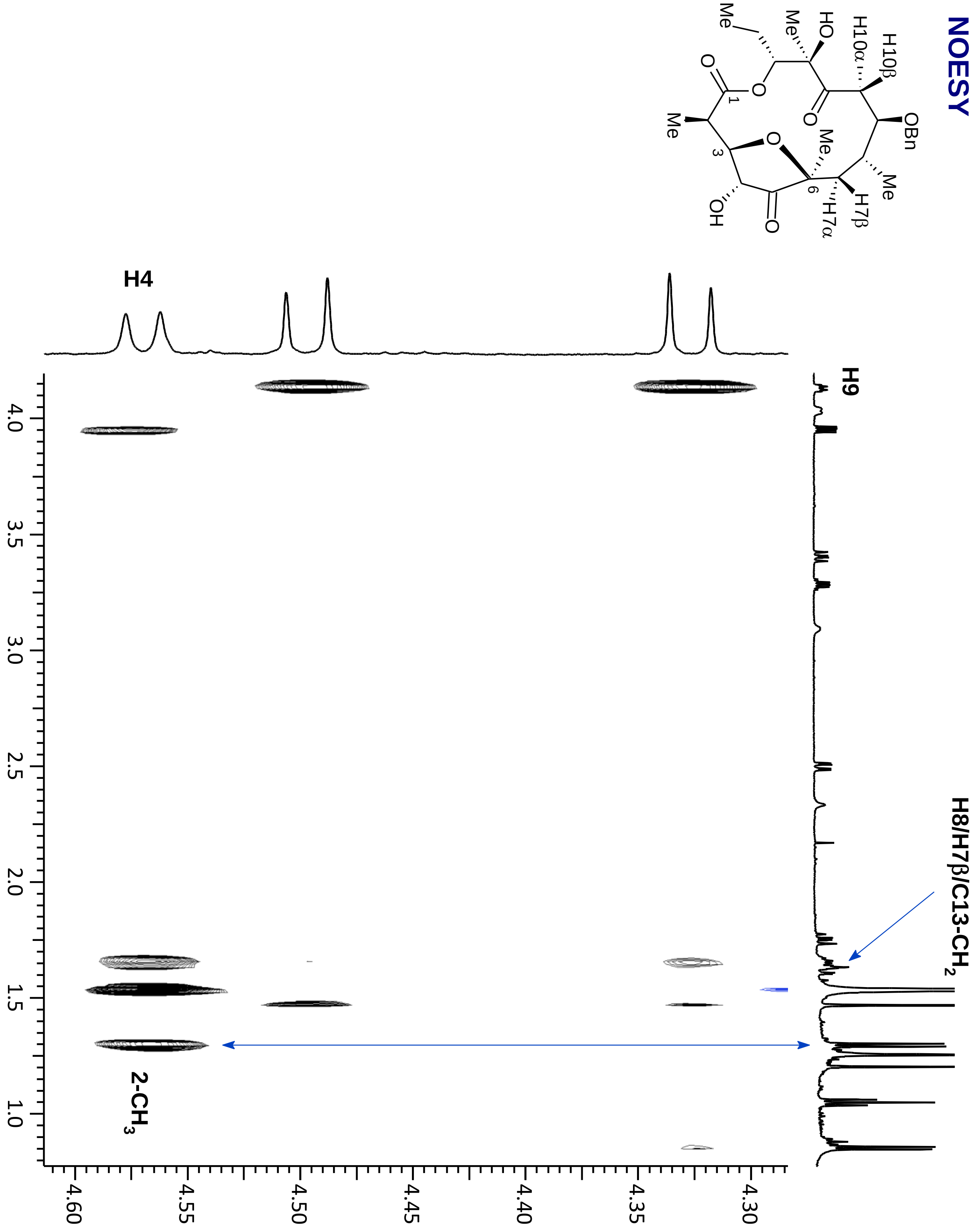

1
2
3
4
5
6
7
7
6
5
4
3
2
1

NOESY

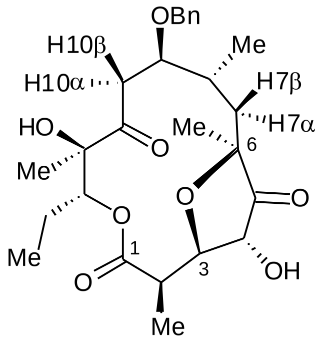

OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
O
Me
O
O
O
1
Me
3
OH
O
Me

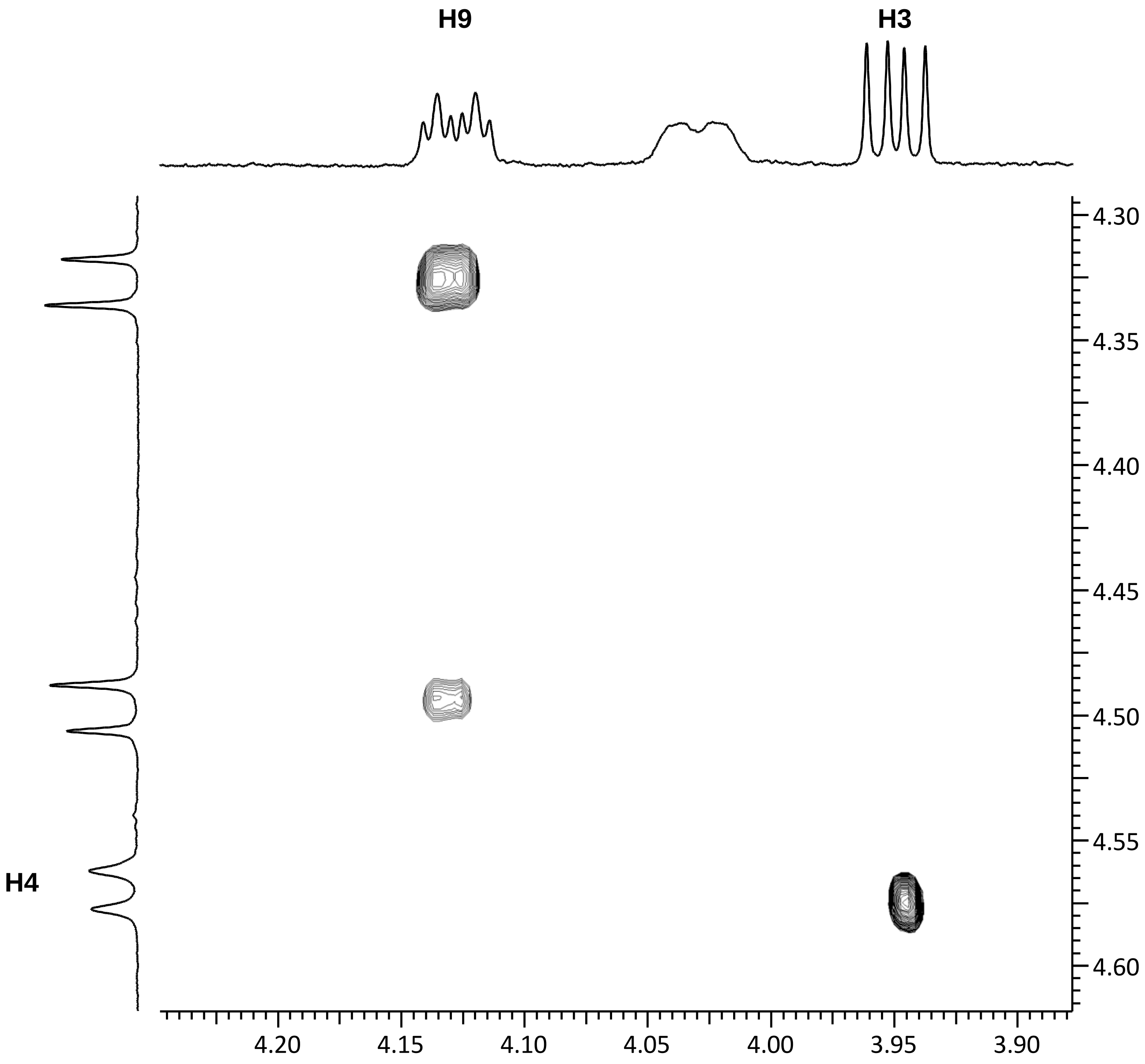

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


NOESY
H10β
H10α
HO
Me
Me
O
O
1
OBn
Me
O
Me
3
O
Me
6
H7β
H7α
O
OH
12-CH$_3$
2-CH$_3$
6-CH$_3$
14-CH$_3$
8-CH$_3$
H4
H9
H13
H3
H10β
H2
12-OH
H10α
3-OH
4.5
4.0
3.5
3.0
2.5
2.0
1.4
1.3
1.2
1.1
1.0
0.9
0.8

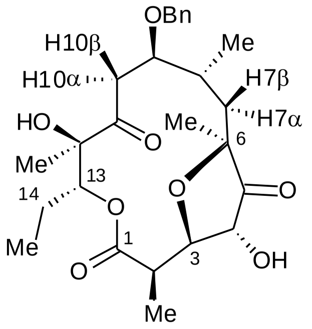

NOESY
H10β
H10α
HO
Me
Me
O
O
1
Me
O
3
OBn
Me
Me
6
H7β
H7α
O
OH
H4
H9
H8/H7β/C13-CH$_2$
2-CH$_3$
4.0
3.5
3.0
2.5
2.0
1.5
1.0
4.60
4.55
4.50
4.45
4.40
4.35
4.30

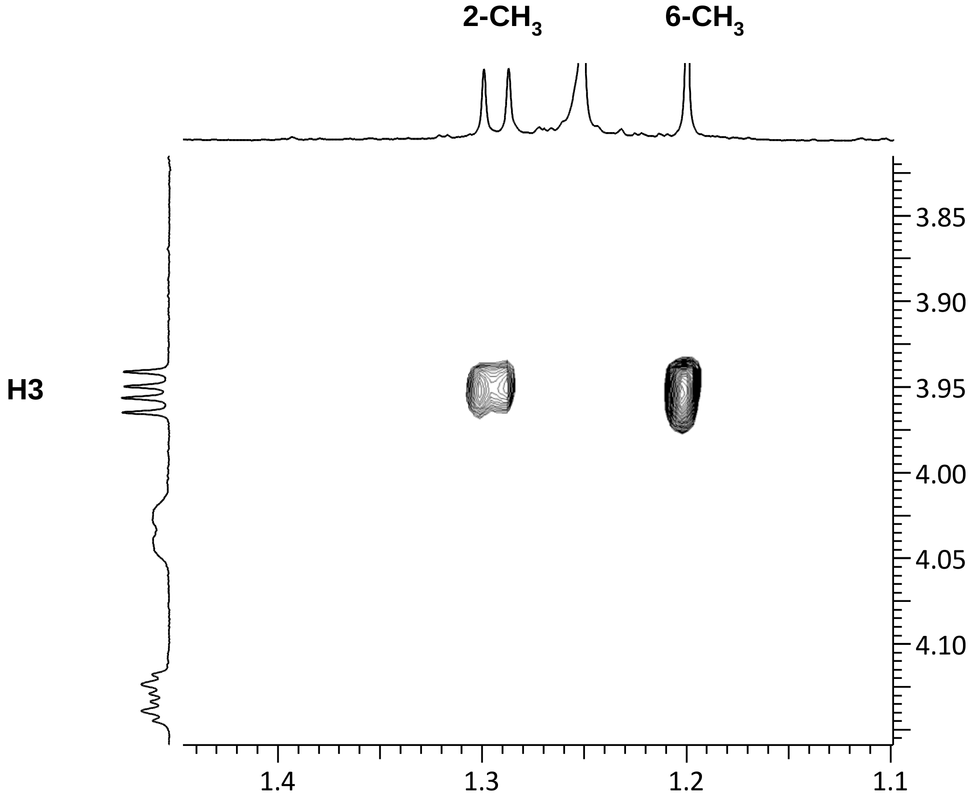
NOESY


OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
O
Me
O
O
Me
O
1
3
OH
O
Me

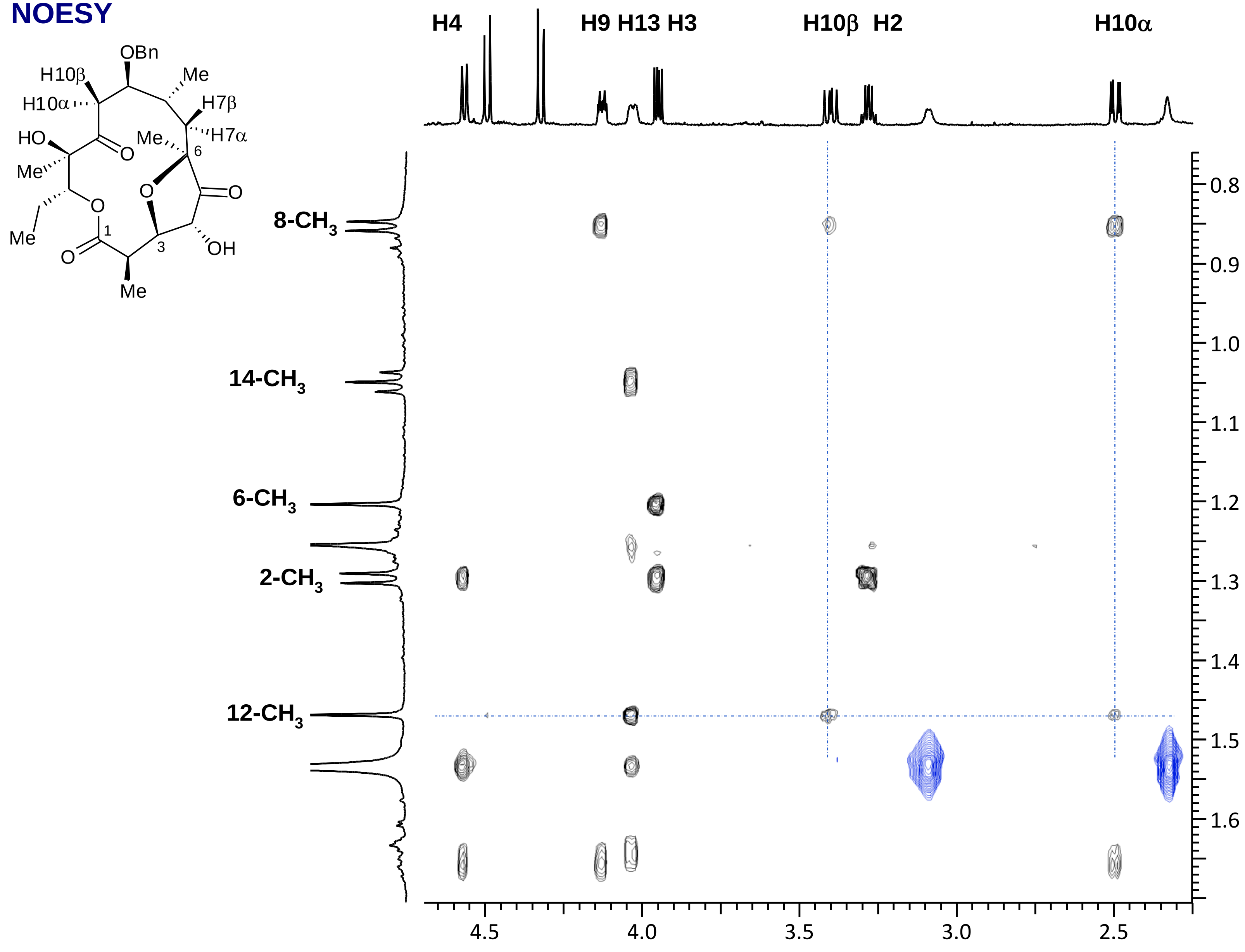

H9
H3
H4
4.30
4.35
4.40
4.45
4.50
4.55
4.60
4.20
4.15
4.10
4.05
4.00
3.95
3.90

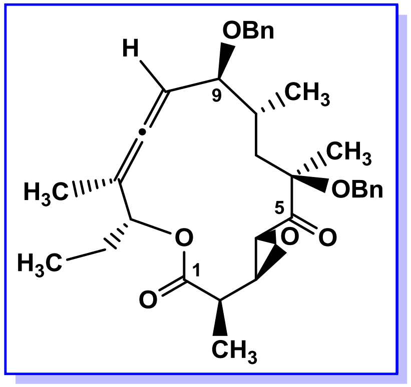
Observed NOESY cross peaks between H3 and 2-CH$_3$/6-CH$_3$


OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
Me
13
14
O
O
O
O
Me
1
3
OH
O
Me



NOESY


OBn
H10β
Me
H10α
H7β
HO
Me
H7α
6
O
Me
O
O
O
1
Me
3
OH
O
Me
H4
H9 H13 H3
H10β H2
H10α
8-CH$_3$
14-CH$_3$
6-CH$_3$
2-CH$_3$
12-CH$_3$
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
4.5
4.0
3.5
3.0
2.5



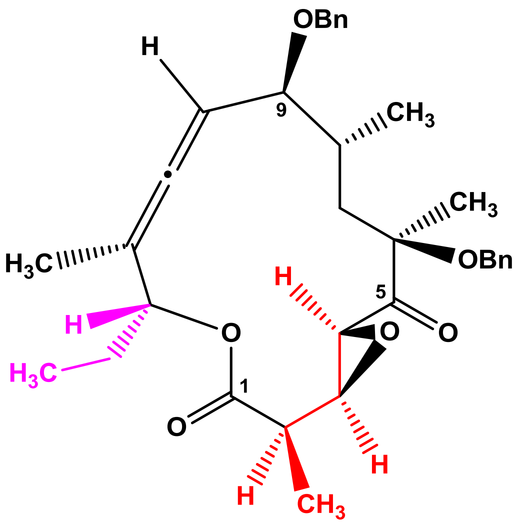
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

3.03 (qd, $J_{H2,\ H3}$ = 2.1Hz, $J_{H2,\ 2\text{-}CH3}$ = 6.7Hz, **H2**)
1.10 (d, $J_{2\text{-}CH3,\ H2}$ = 7.1Hz, **2-CH_3**)
3.37 (t, $J_{H3,\ H2}$ = 2.1Hz, **H3**)
3.97 (d, $J_{H4,\ H3}$ = 1.8Hz, **H4**)
1.62 (s, **6-CH_3**)
4.47 (s, **6-OCH_2**)
1.83 (dd, $J_{H7\beta,\ H8}$ = 10.2 Hz, $J_{H7\beta,\ H7\alpha}$ = 14.9Hz, **H7β**)
2.25 (dq, $J_{H7\alpha,\ H8}$ = 2.7Hz, $J_{H7\alpha,\ H7\beta}$ = 14.6Hz, **H7α**)
1.85 (m, **H8**)
1.14 (d, $J_{8\text{-}CH3,\ H8}$ = 6.5Hz, **8-CH_3**)
3.44 (t, $J_{H9,\ H8}$ = 8.8Hz, **H9**)
4.37 (d, J_{AB} = 11.7Hz, **9-OCH_2**)
4.64 (d, J_{AB} = 11.7Hz, **9-OCH_2**)
5.02 (dt, $J_{10,\ 12\text{-}CH3}$ = 2.6, $J_{H10,\ H9}$ = 9.1Hz, **H10**)
1.81 (d, $J_{12\text{-}CH3,\ H10}$ = 2.9Hz, **12-CH_3**)
4.94 (ddd, $J_{H13,\ H}$ = ? Hz, $J_{H13,\ 12\text{-}CH3}$ = ? Hz, **H13**)
1.80 - 1.77, 1.65 - 1.58 (m, **14-CH_2**)
0.91 (t, $J_{14\text{-}CH3,\ 13\text{-}CH2}$ = 7.3Hz, **14-CH_3**)
7.48 - 7.17 (m, 10H, **Ph**)

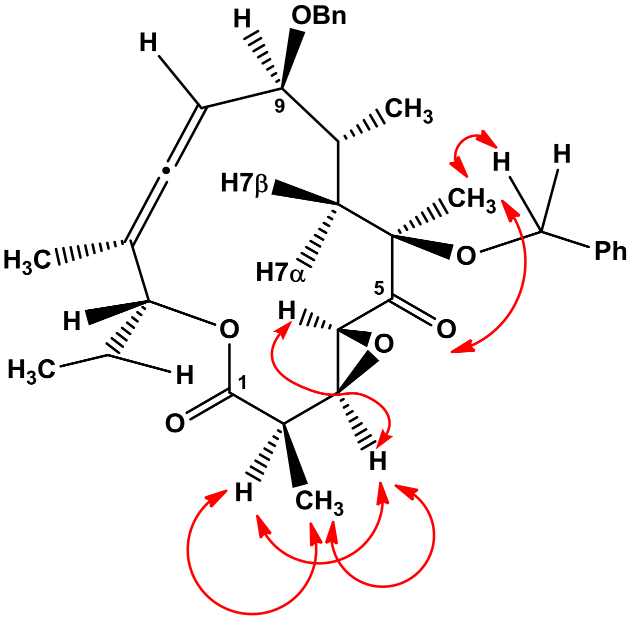
13C NMR chemical shift (δ/ppm)

170.4 ----- C1
39.5 ----- C2
59.4 ----- C3
52.4 ----- C4
206.2 ----- C5
83.8 ----- C6
34.7 ----- C7
40.1 ----- C8
83.8 ----- C9
94.3 ----- C10
202.1 ----- C11
100.8 ----- C12
76.8 ----- C13
26.3 ----- C14
11.4 ----- 2-CH_3
19.6 ----- 6-CH_3
17.9 ----- 8-CH_3
17.1 ----- 12-CH_3
9.8 ----- 14-CH_3
66.6 ----- 6-OCH_3
70.8 ----- 9-OCH_3

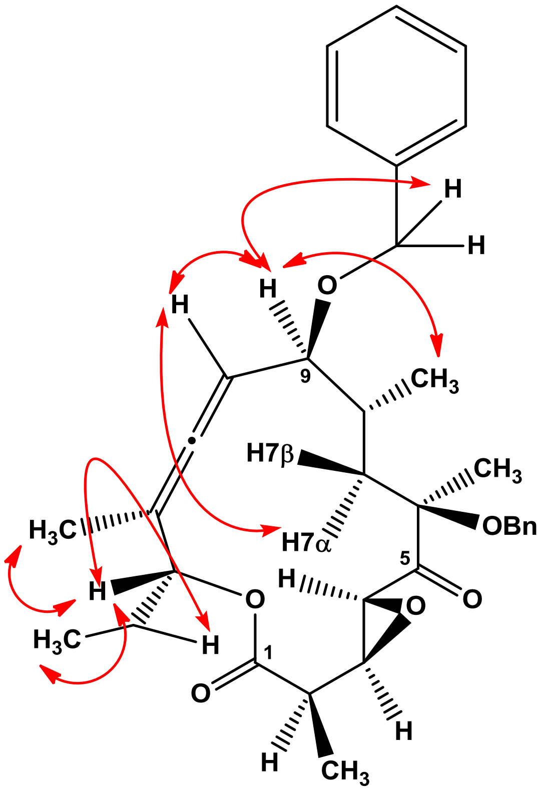
1d TOCSY


H
OBn
9
CH3
CH3
OBn
H3C
5
H
O
O
H
O
1
H3C
O
H
H
CH3

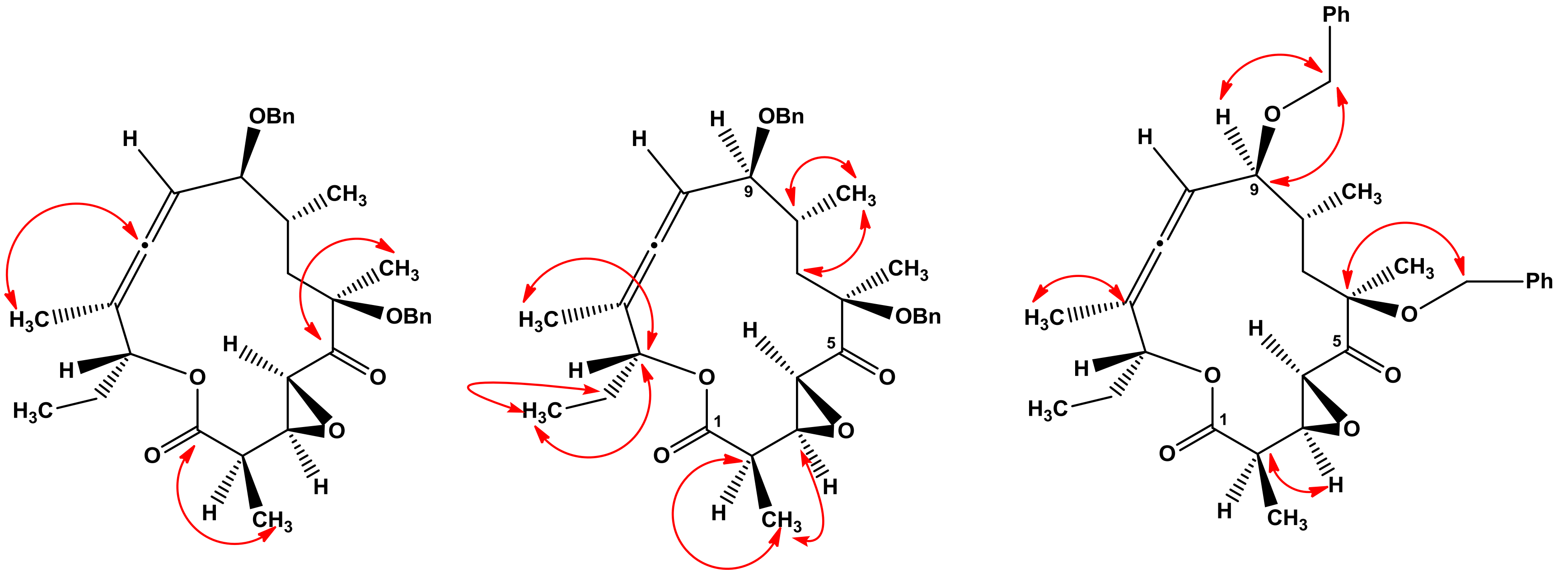
NOESY

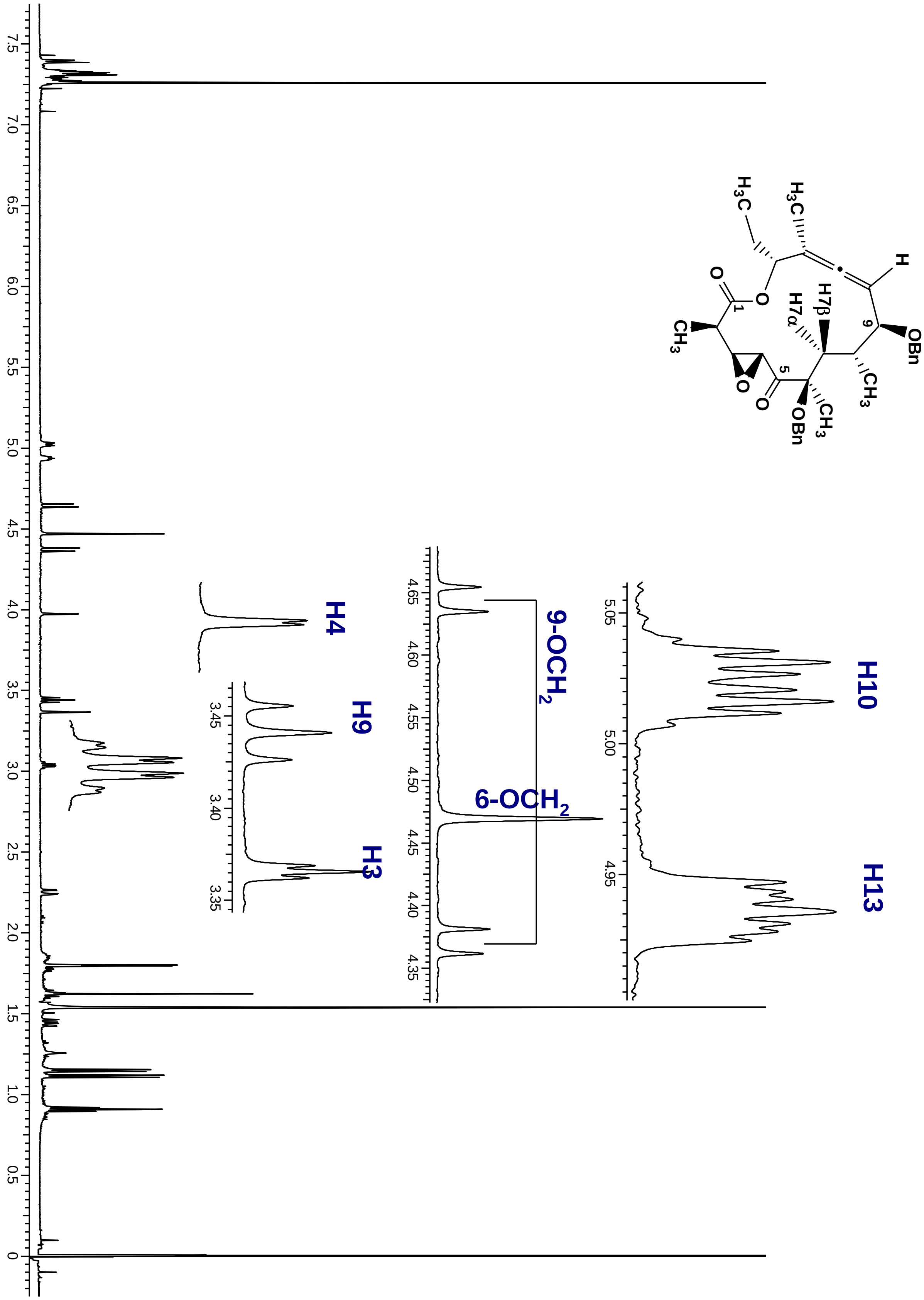

H
H
OBn
9
CH3
H7β
H
H
CH3
Ph
H3C
H7α
O
5
H
O
H
O
O
H3C
H
1
O
H
H
CH3

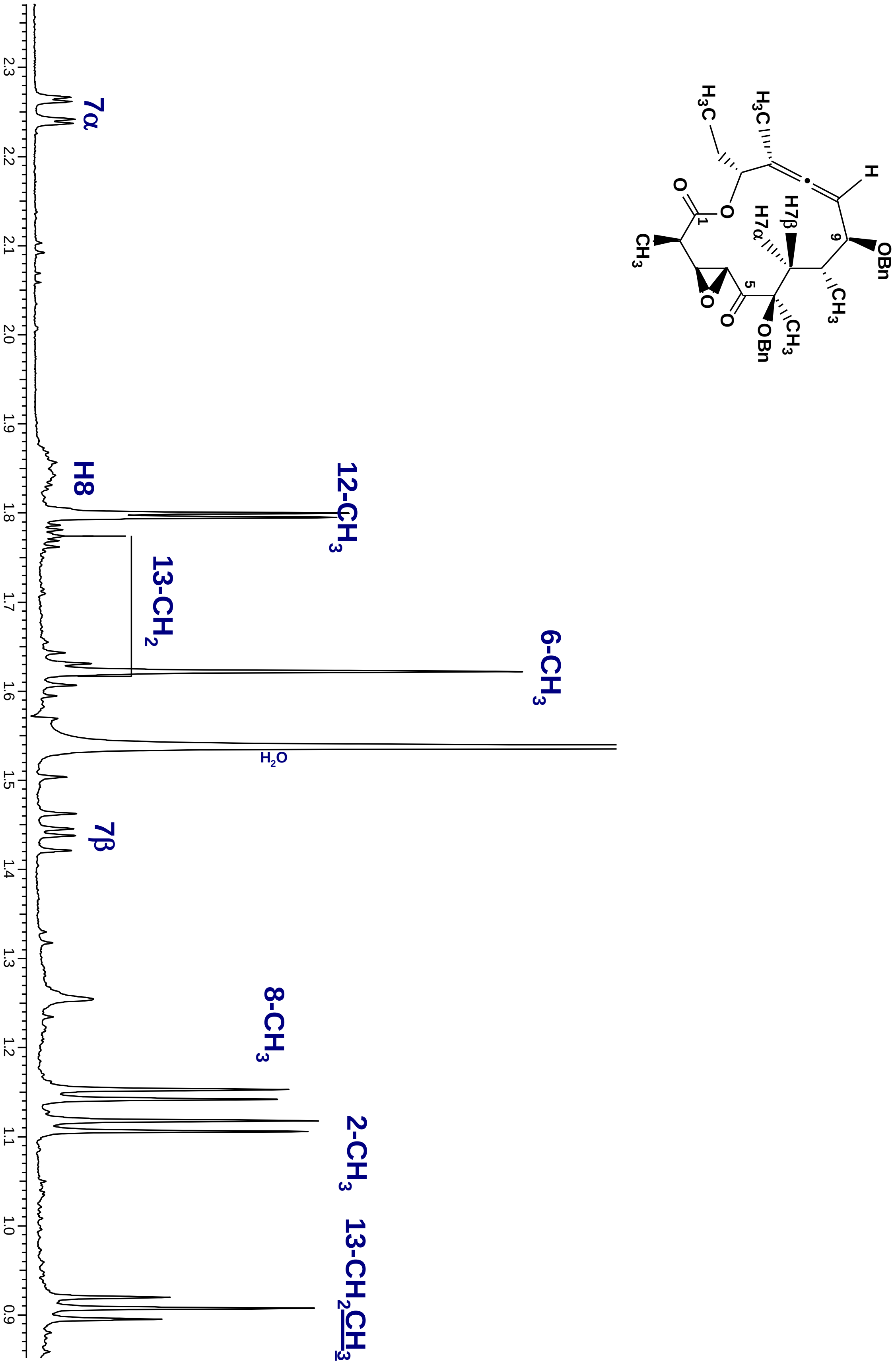
NOESY


H
H
H
H
O
9
CH3
H7β
CH3
H3C
H7α
OBn
5
H
H
O
O
H3C
H
1
O
O
H
H
CH3

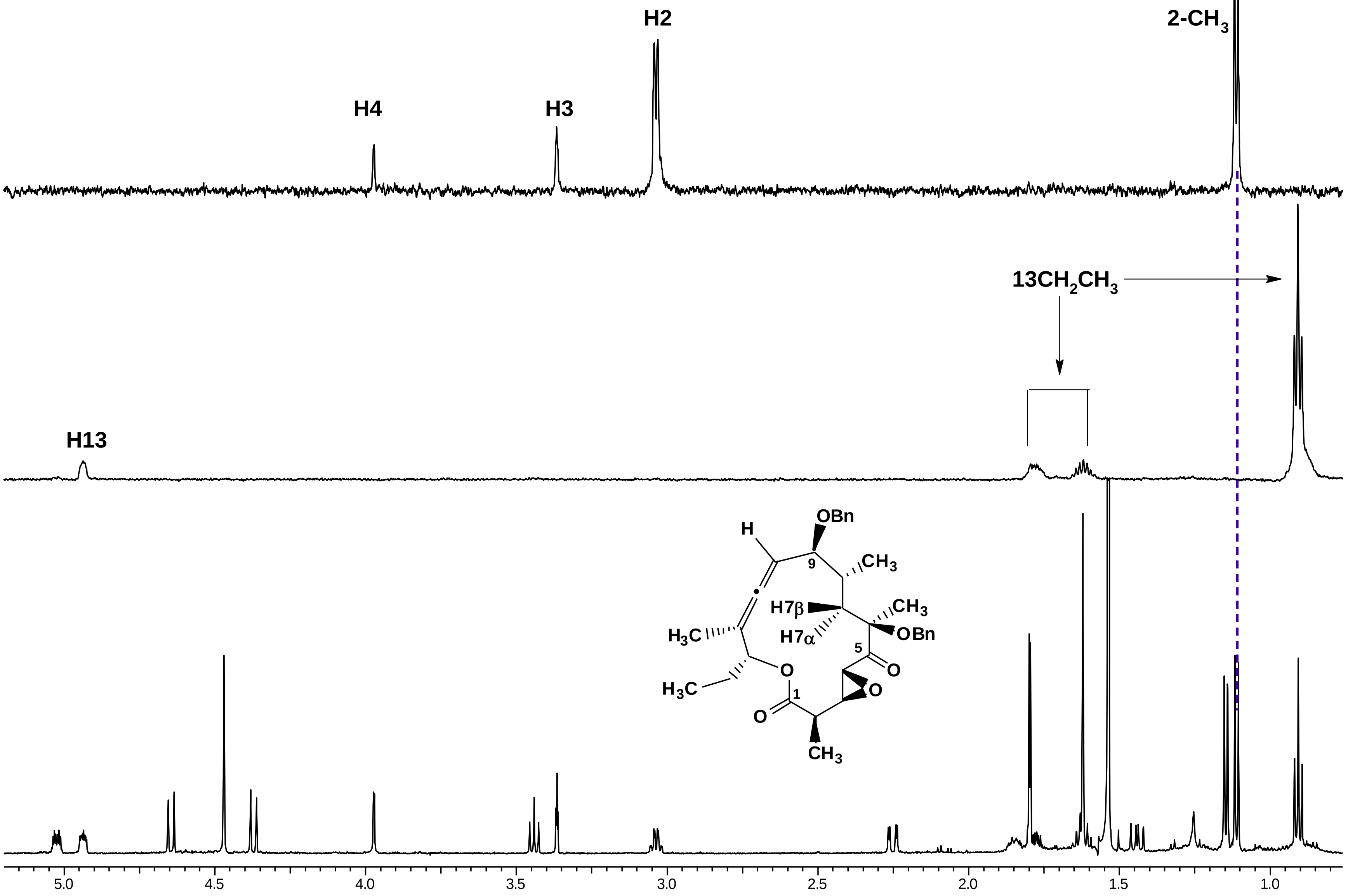
HMBC


H
OBn
CH3
CH3
OBn
H3C
H
O
H
O
H3C
O
H
CH3
H
H
H
OBn
9
CH3
CH3
OBn
H3C
H
O
H
5
O
H3C
1
O
O
H
CH3
H
Ph
H
O
H
9
CH3
CH3
Ph
O
H3C
H
O
H
5
O
H3C
1
O
O
H
H
CH3

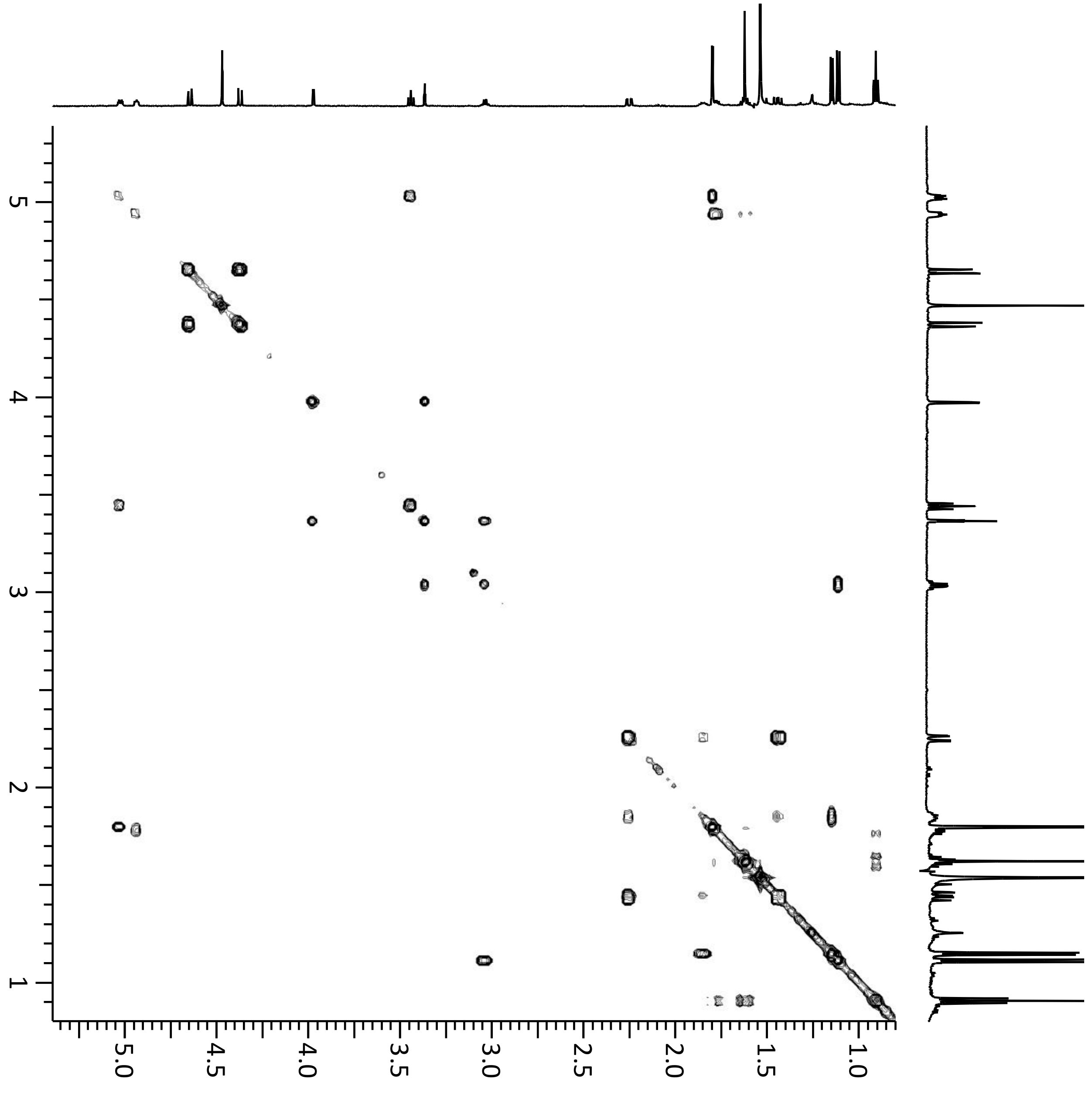

H10
H13
9-OCH$_2$
6-OCH$_2$
H4
H9
H3
H7β
H7α
OBn
OBn
CH$_3$
CH$_3$
CH$_3$
H$_3$C
H$_3$C
H
O
O
O
O
1
5
9
5.05
5.00
4.95
4.65
4.60
4.55
4.50
4.45
4.40
4.35
3.45
3.40
3.35
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0


H
OBn
9
CH3
H7β
H7α
5
CH3
OBn
O
H3C
H3C
O
1
O
CH3
O
7α
H8
12-CH3
13-CH2
6-CH3
H2O
7β
8-CH3
2-CH3
13-CH2CH3
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9

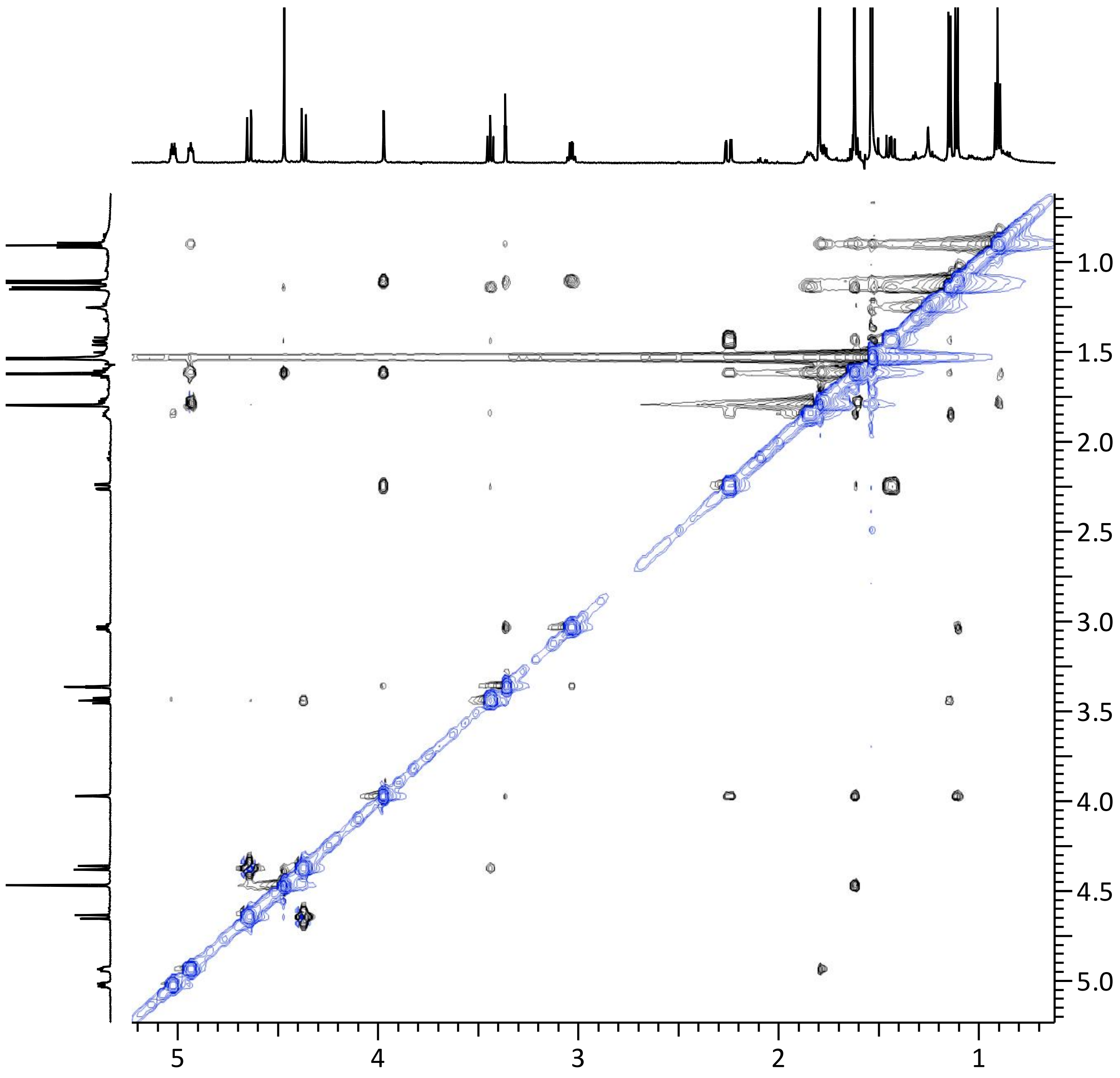
Spin system identification



gCOSY

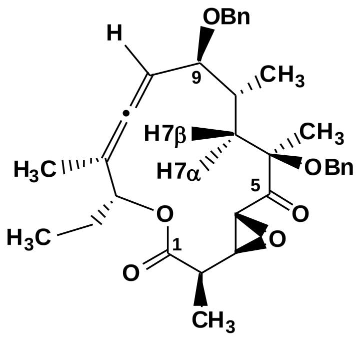


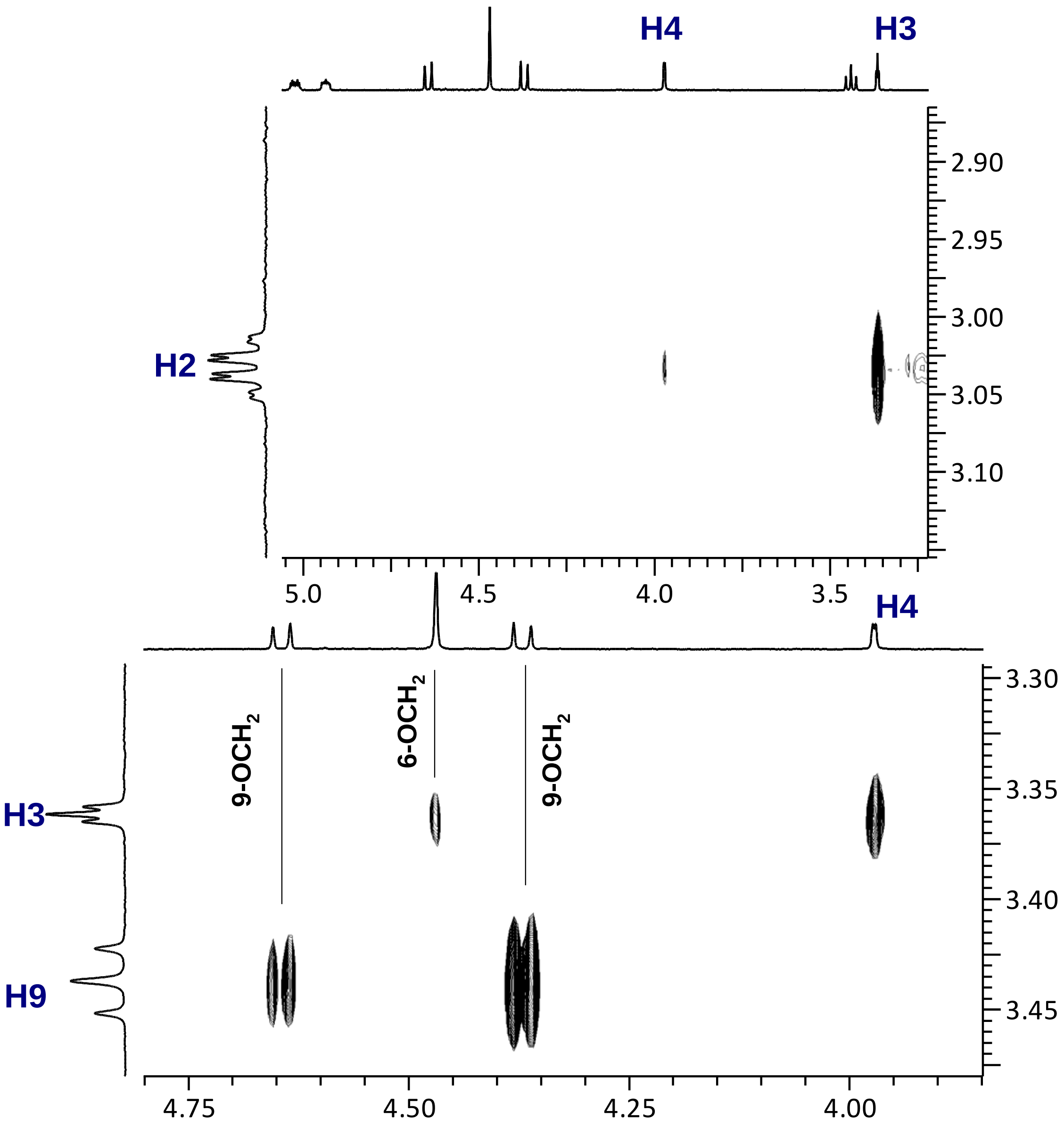
NOESY



NOESY

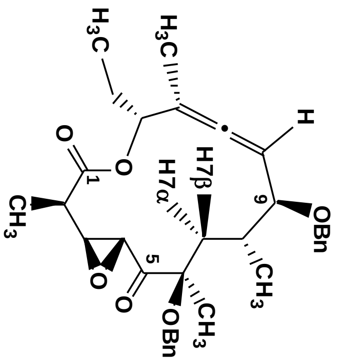
correlations

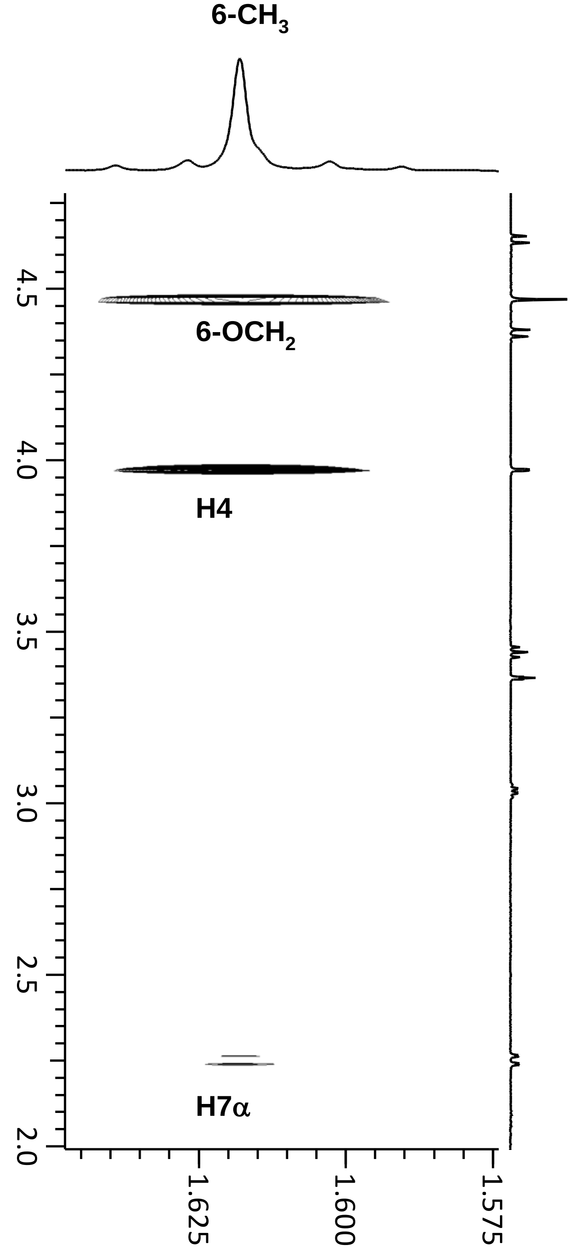

H
OBn
9
CH3
H7β
CH3
H7α
OBn
H3C
5
O
O
O
1
H3C
O
CH3

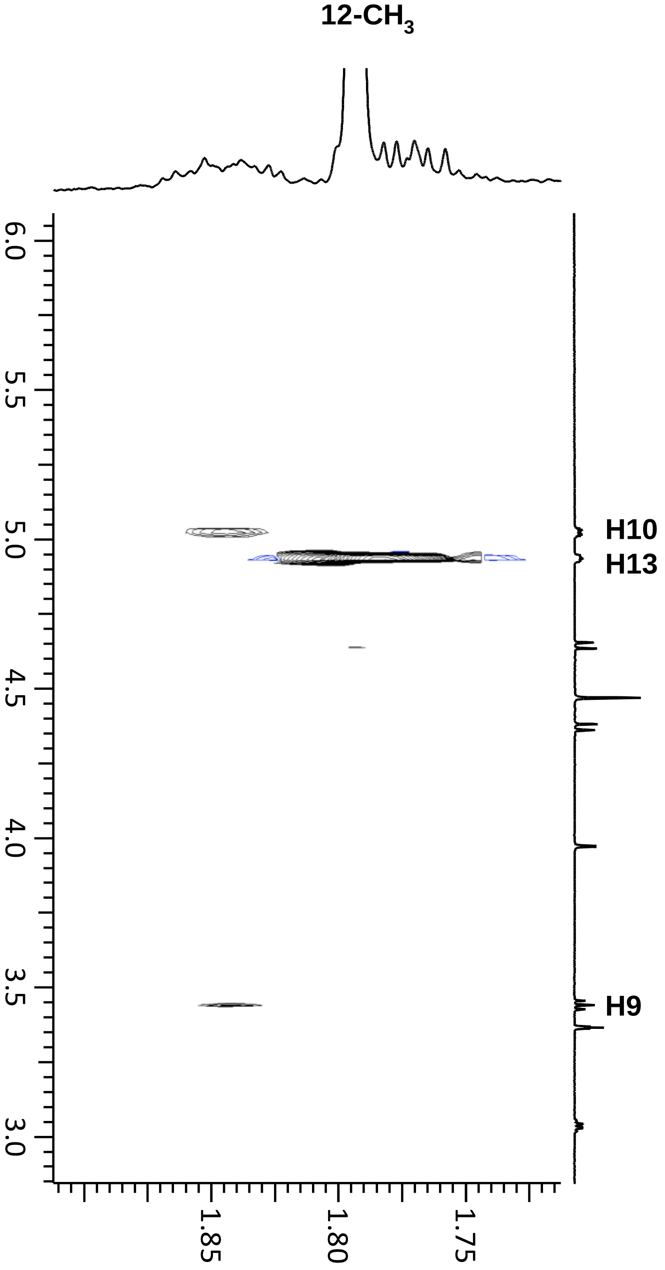


NOESY

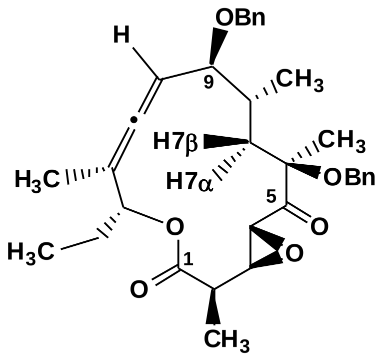
correlations

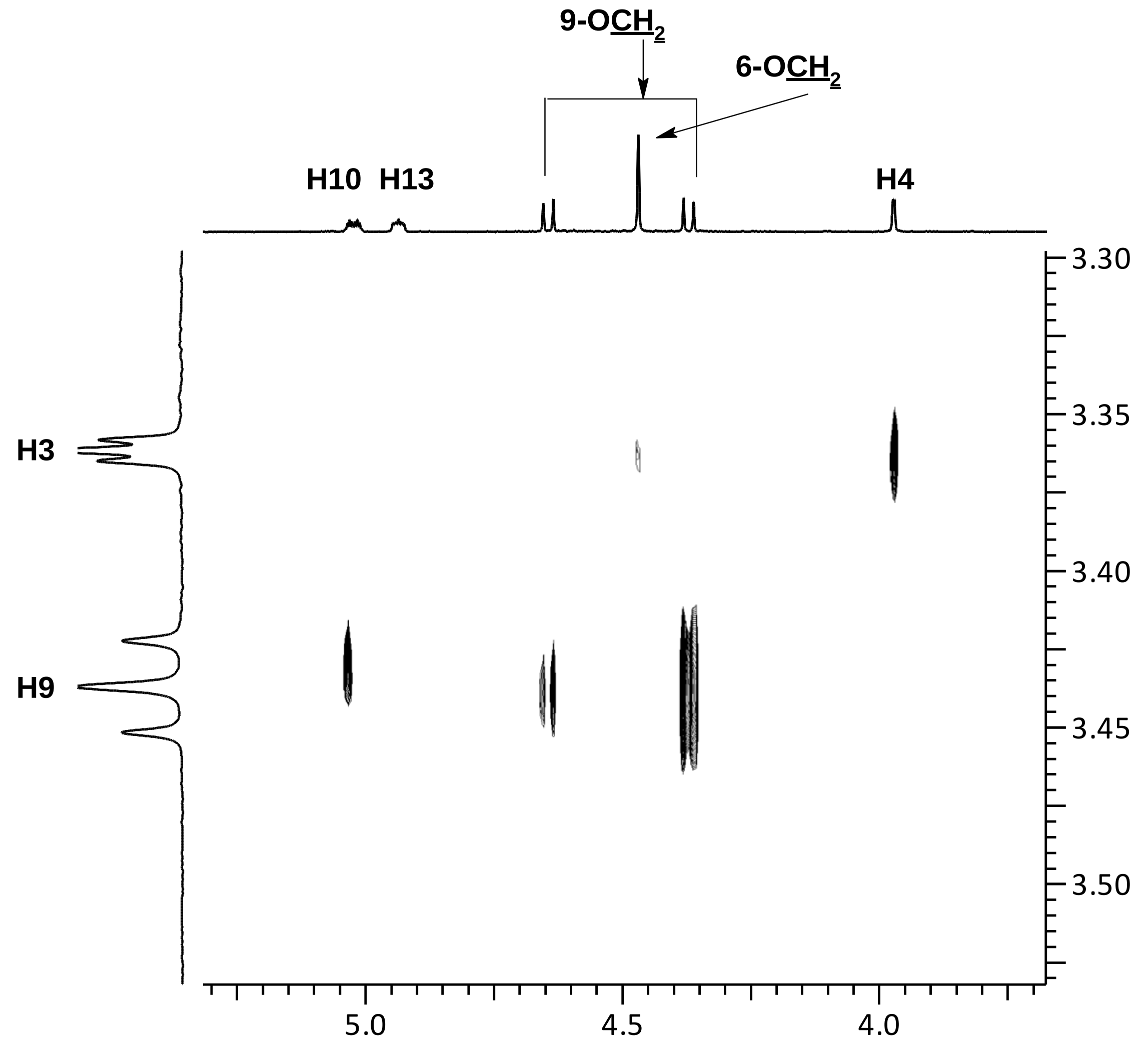

H
OBn
9
H7β
H7α
H₃C
H₃C
O
1
O
CH₃
O
5
O
CH₃
CH₃
OBn





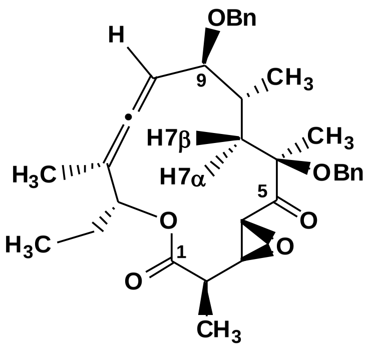
NOESY
correlations

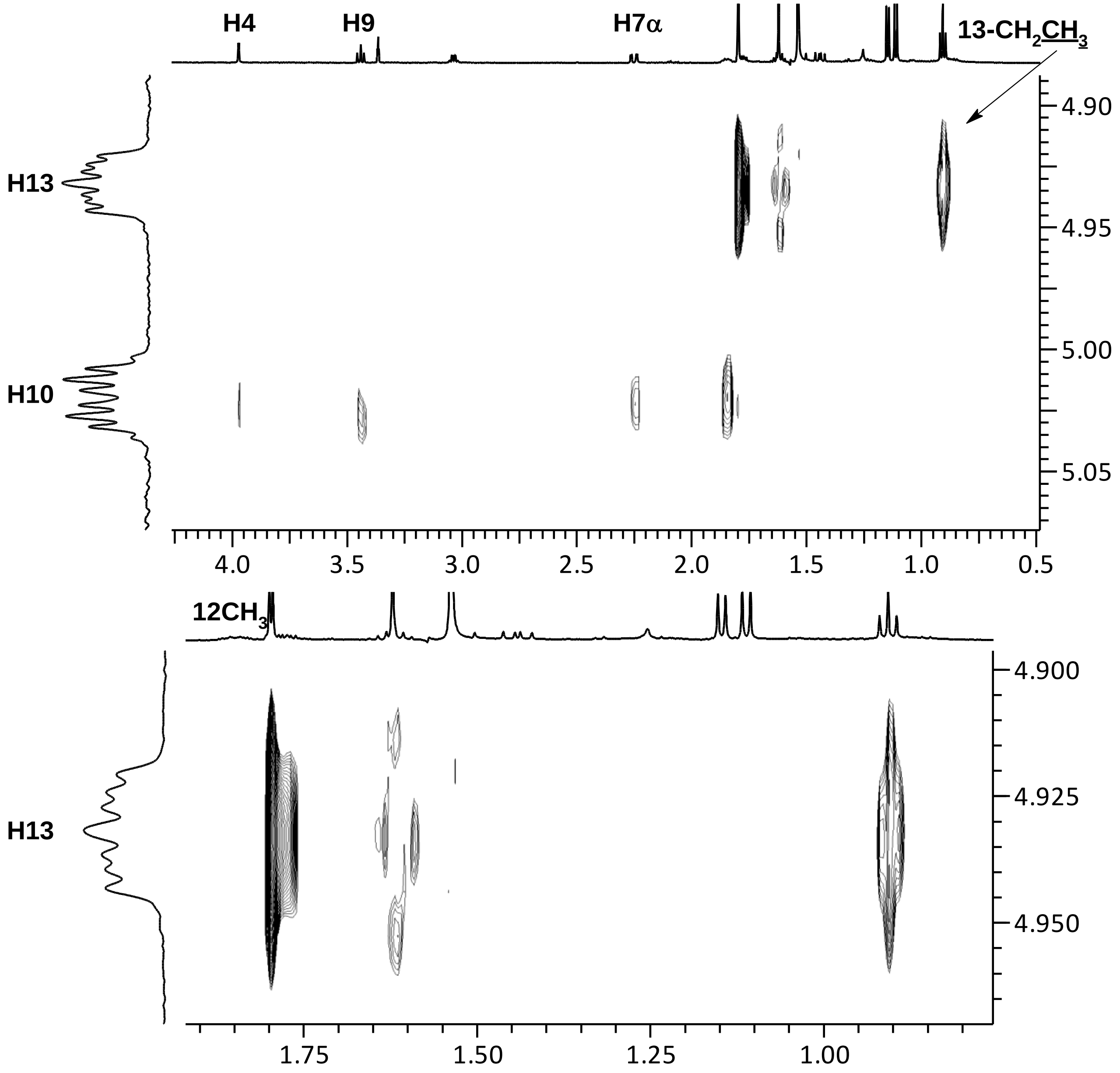

H
OBn
9
CH3
H7β
CH3
H7α
OBn
H3C
5
O
H3C
O
O
1
O
CH3



NOESY

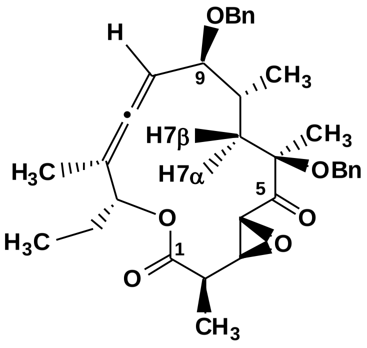
correlations

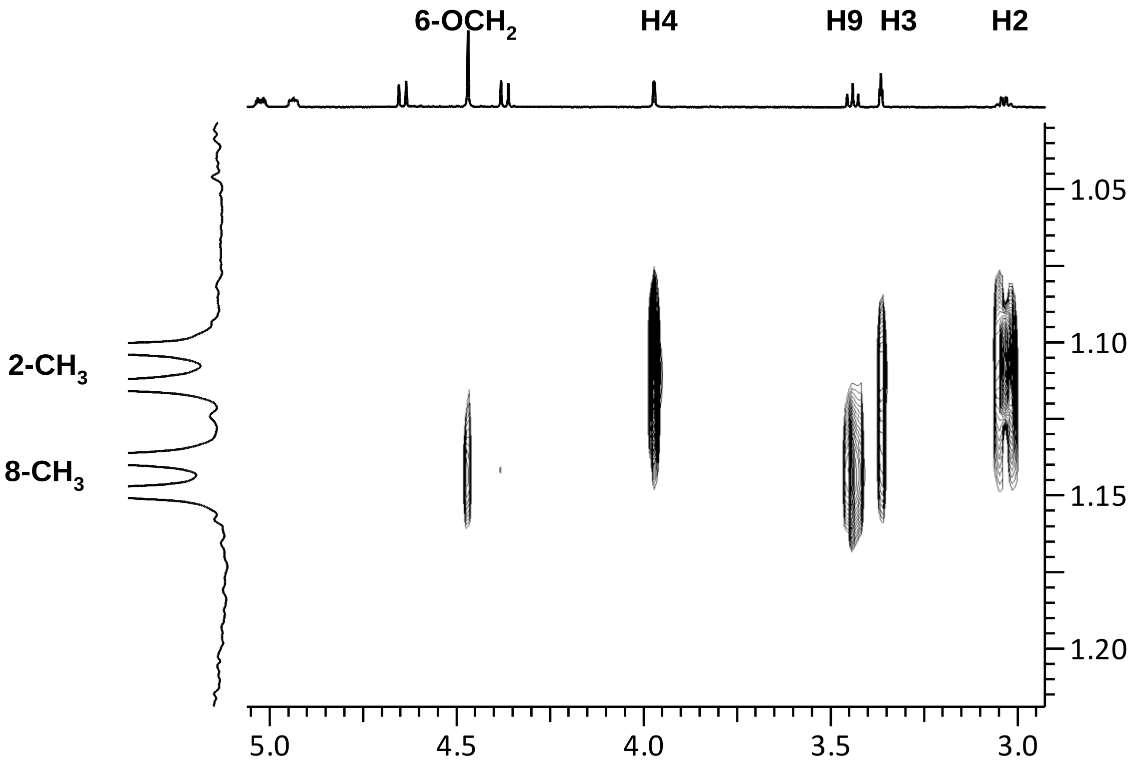

OBn
H
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
H3C
O
1
O
O
CH3

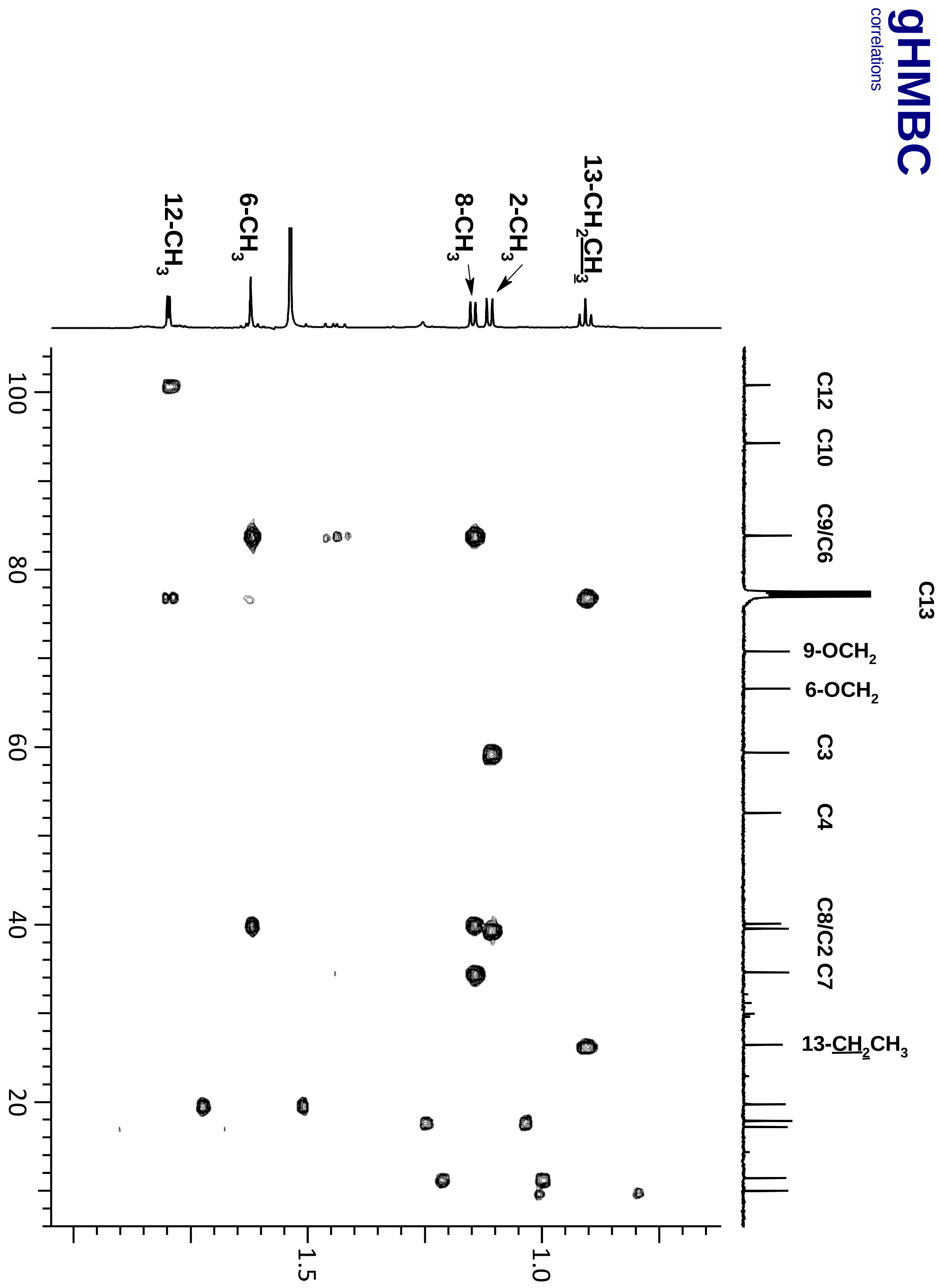


NOESY

correlations

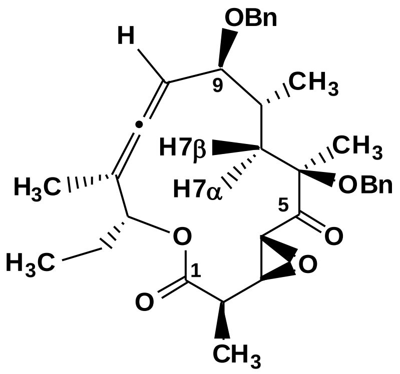

H
OBn
9
CH3
H7β
CH3
H3C
H7α
OBn
5
H3C
O
O
1
O
O
CH3



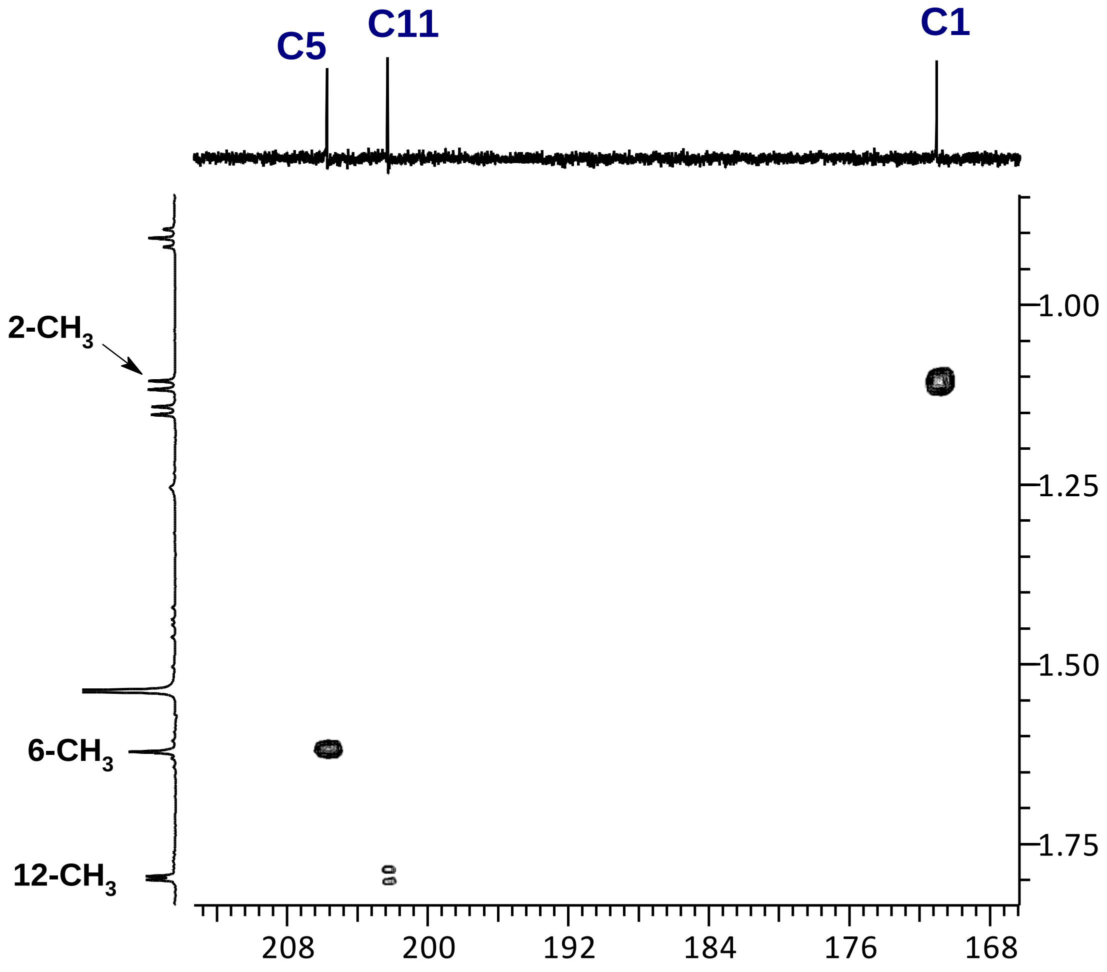

gHMBC
correlations
12-CH$_3$
6-CH$_3$
8-CH$_3$
2-CH$_3$
13-CH$_2$\underline{CH$_3$}
C12
C10
C9/C6
C13
9-OCH$_2$
6-OCH$_2$
C3
C4
C8/C2
C7
13-\underline{CH$_2$}CH$_3$
100
80
60
40
20
1.5
1.0

gHMBC

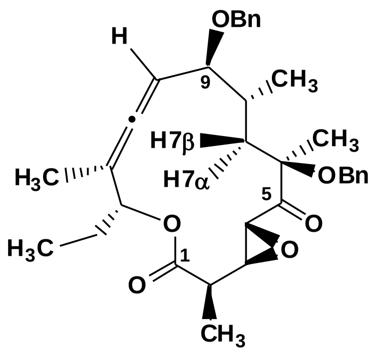
correlations

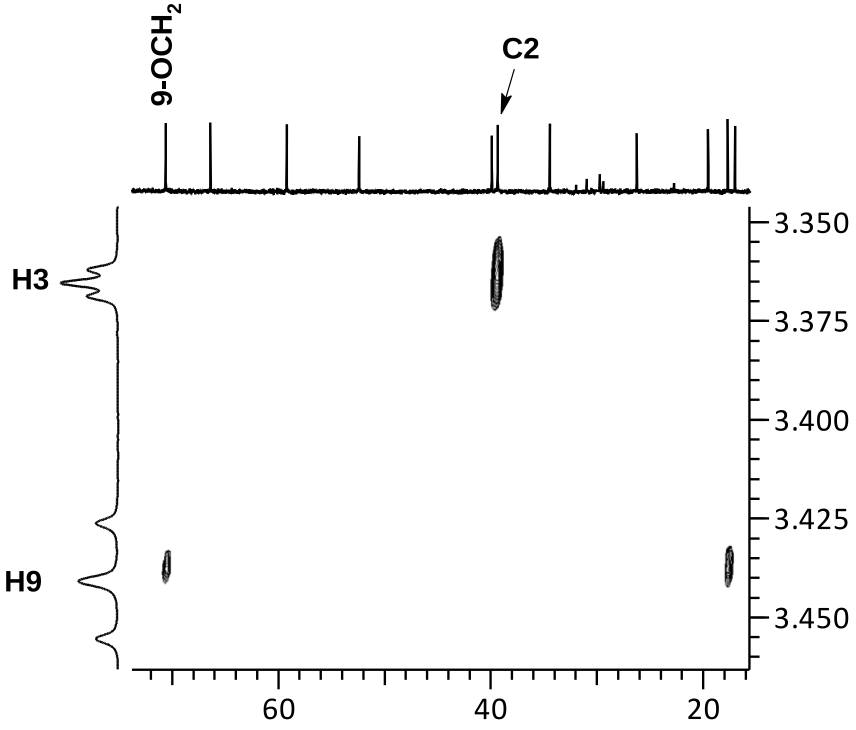


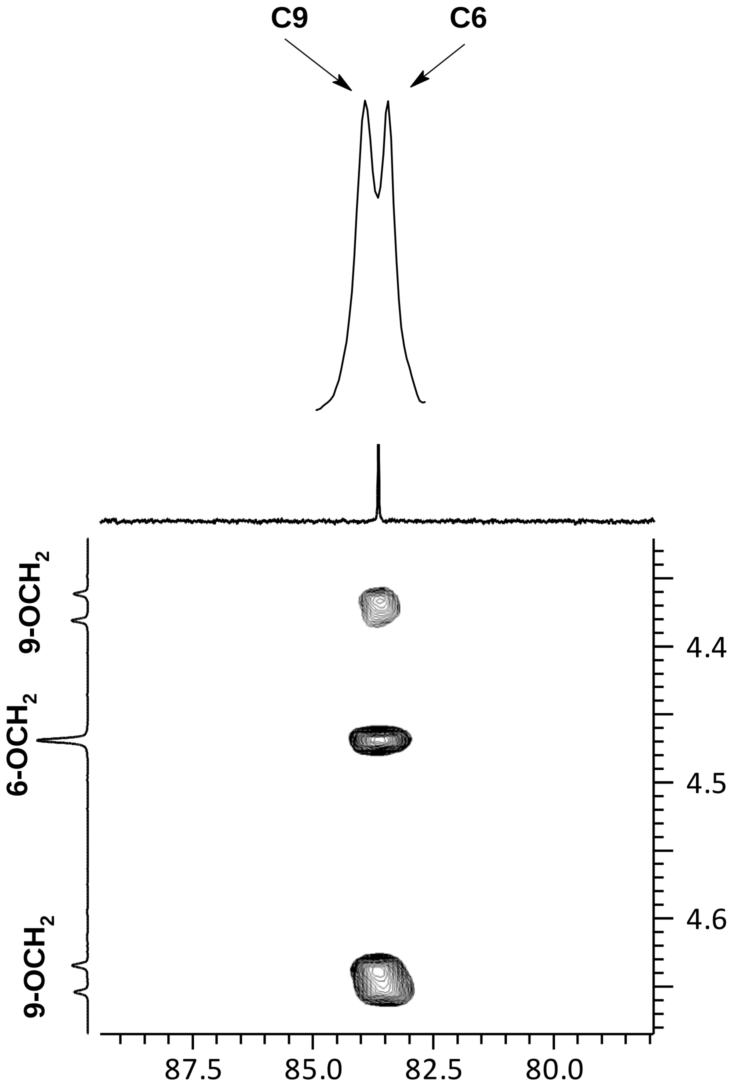
OBn
H
9
CH3
H7β
CH3
H7α
OBn
H3C
5
O
O
H3C
O
1
O
CH3

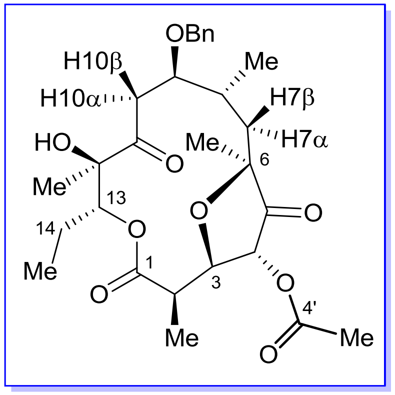


gHMBC
correlations


H
OBn
9
CH3
H7β
CH3
H3C
H7α
OBn
5
O
O
H3C
1
O
O
CH3

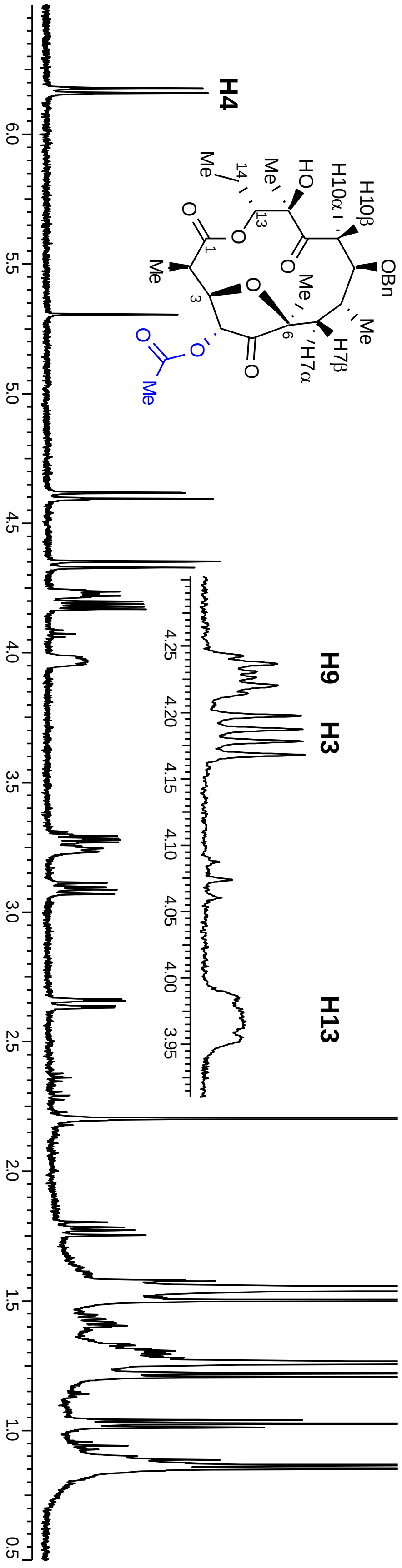


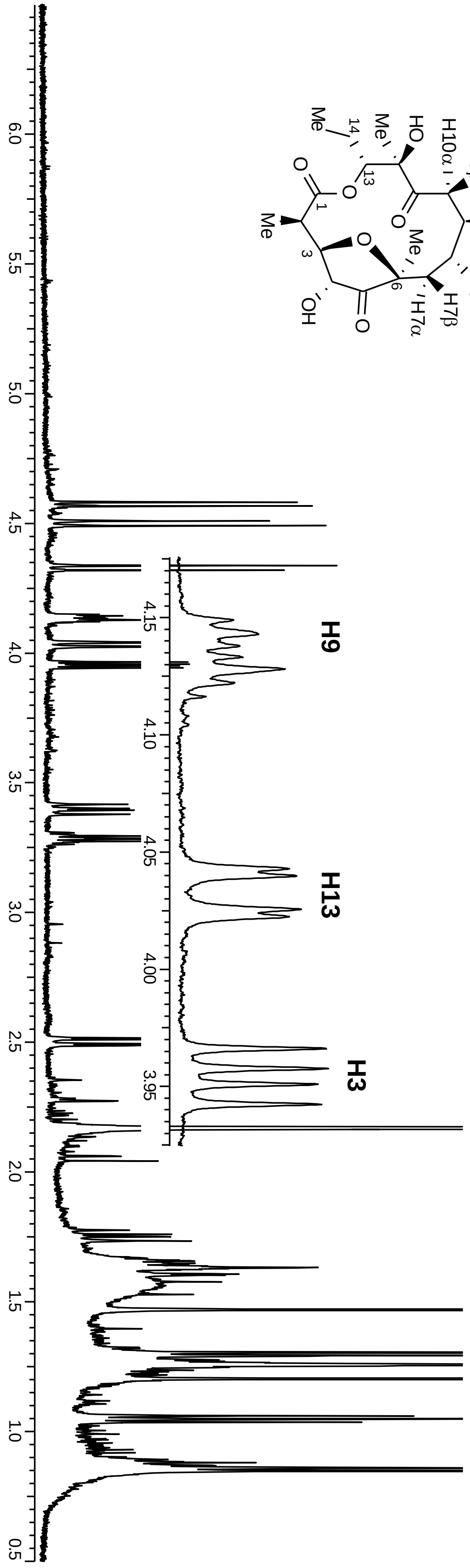


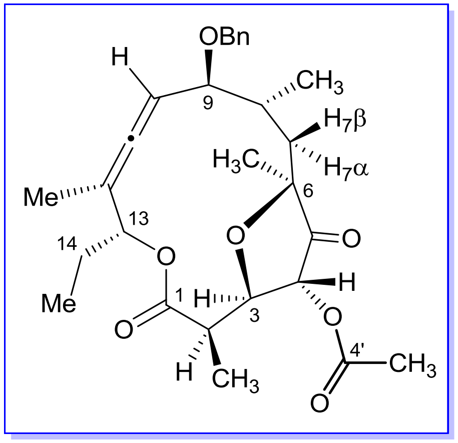


1H NMR chemical shifts (δ/ppm)
w/ coupling constant (J/Hz)

3.29 (dd, $J_{H2, H3}$ = 5.1Hz, $J_{H2, 2\text{-}CH3}$ = 7.3Hz, **H2**)
1.20 (d, $J_{2\text{-}CH3, H2}$ = 7.3Hz, **2-CH_3**)
4.18 (dd, $J_{H3, H2}$ = 5.1Hz, $J_{H3, H4}$ = 9.6Hz, **H3**)
6.18 (d, $J_{H4, H3}$ = 9.5Hz, **H4**)
2.20 (s, **4'-CH_3**)
1.26 (s, **6-CH_3**)
1.54 (dd, $J_{H7\beta, H8}$ = 2.2Hz, $J_{H7\beta, H7\alpha}$ = 15.2Hz, **H7**β)
1.75 (dd, $J_{H7\alpha, H8}$ = 9.9Hz, $J_{H7\alpha, H7\beta}$ = 15.2Hz, **H7**α)
1.52 (m, **H8**)
0.85 (d, $J_{8\text{-}CH3, H8}$ = 6.8Hz, **8-CH_3**)
4.23 (dt, $J_{H9, H10\alpha}$ = 3.2Hz, $J_{H9, H10\beta}$ = 8.3Hz, **H9**)
4.33, 4.59 (d, J_{AB} = 11.5Hz, **9-OCH_2**)
2.63 (dd, $J_{H10\alpha, H9}$ = 3.2Hz, $J_{H10\alpha, H10\beta}$ = 13.5Hz, **H10**α)
3.07 (dd, $J_{H10\beta, H9}$ = 8.3Hz, $J_{H10\beta, H10\alpha}$ = 13.5Hz, **H10**β)
1.60 (s, **12-CH_3**)
3.96 (dd, $J_{H13, H14}$ = 2.1, 10.0Hz, **H13**)
1.45 - 1.29 (m, **14-CH_2**)
1.02 (t, $J_{14\text{-}CH3, H14}$ = 7.1Hz, **14-CH_3**)

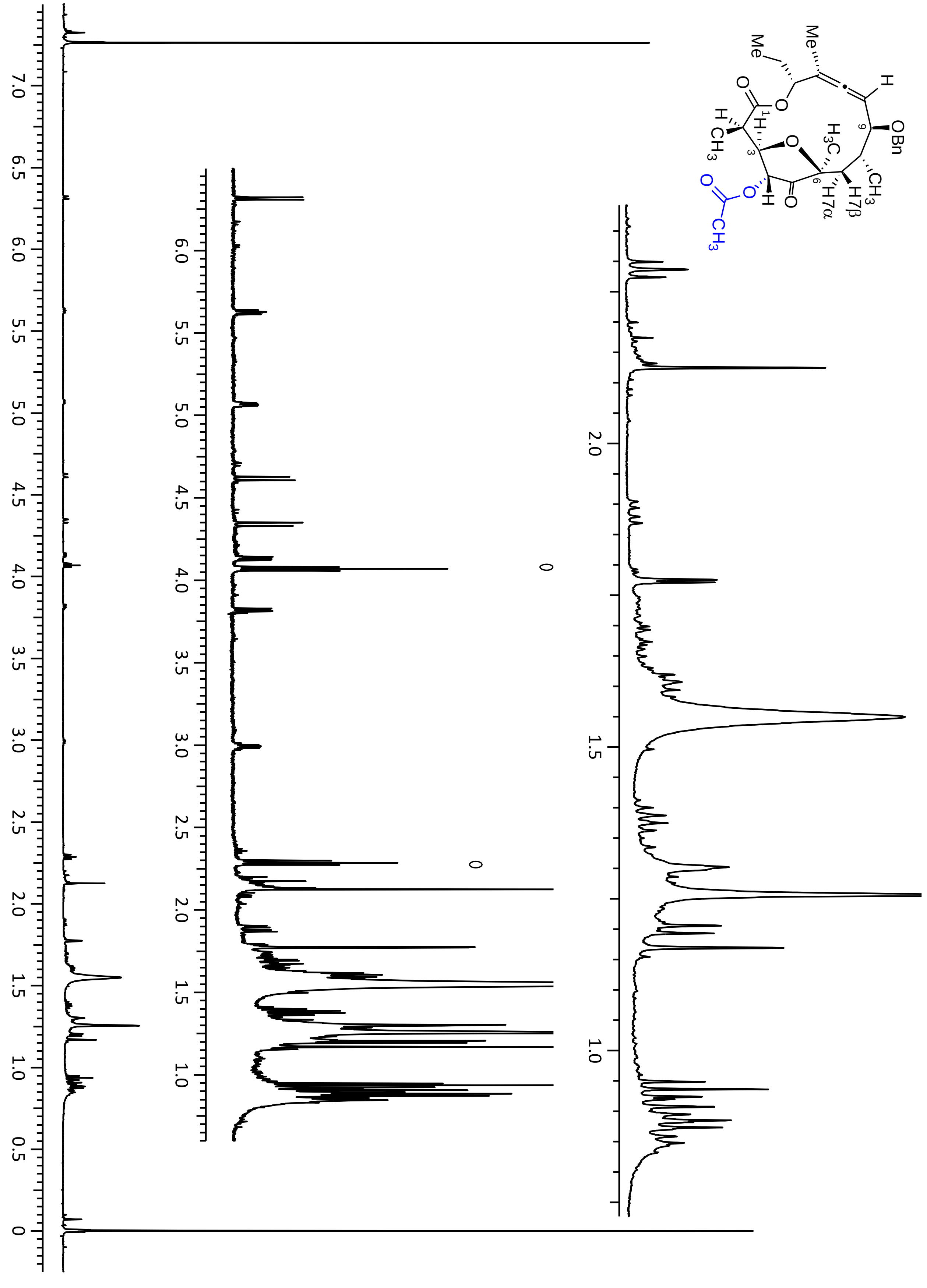

H4
H9
H3
H13
OBn
Me
H10β
H10α
HO
Me
14
13
O
1
Me
3
6
H7β
H7α
O
Me
4.25
4.20
4.15
4.10
4.05
4.00
3.95
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

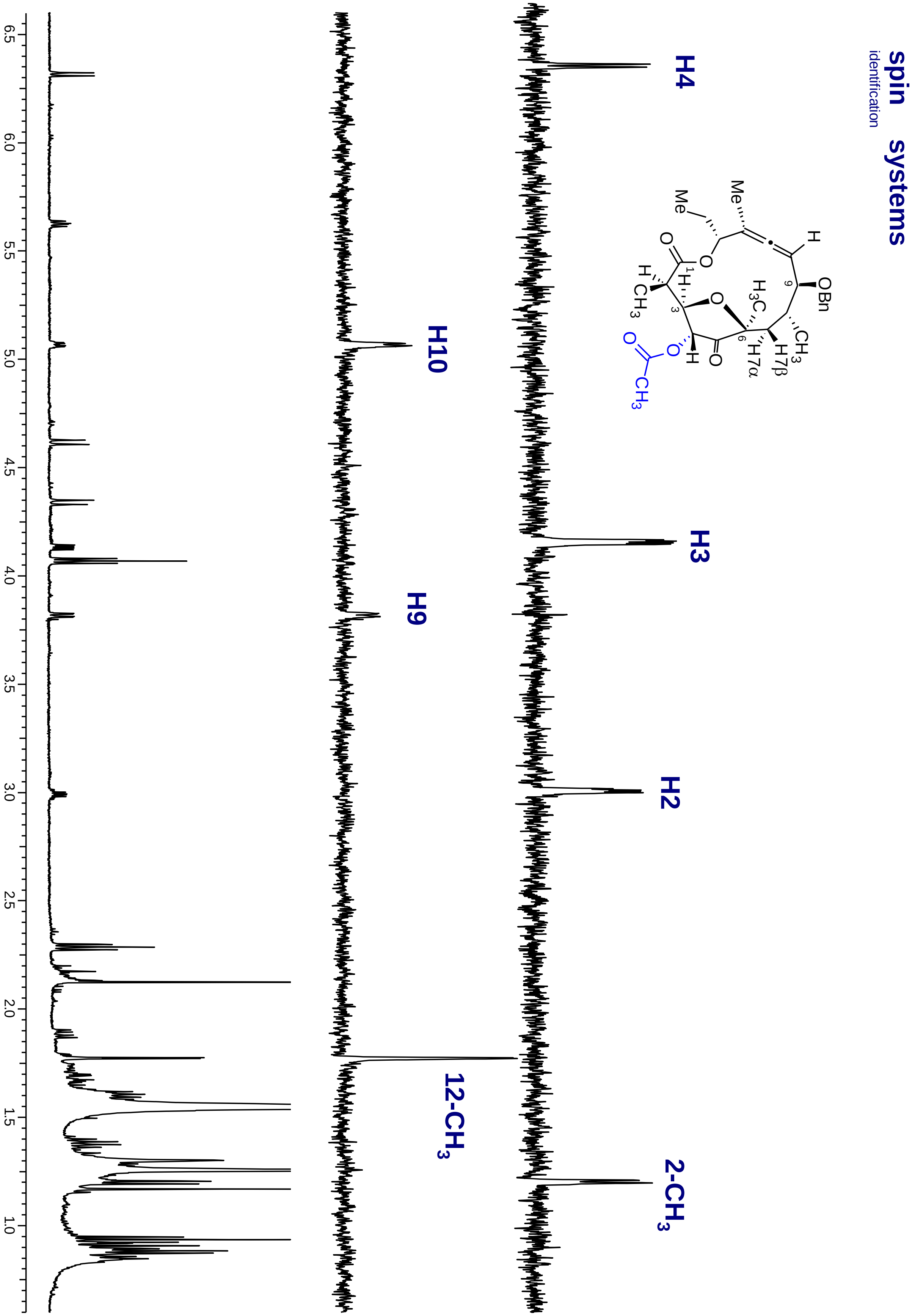

H9
H13
H3
H10β
H10α
HO
Me
14
13
OBn
Me
O
1
3
6
H7β
H7α
OH
O
4.15
4.10
4.05
4.00
3.95
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
14
O
O
O
H
1
H
3
Me
O
H
CH3
O
4'
CH3
O

1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

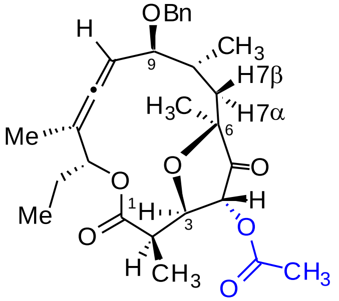
3.00 (dd, $J_{H2, H3}$ = 2.2Hz, $J_{H2, 2\text{-}CH3}$ = 8.3Hz, **H2**)
1.21 (d, $J_{2\text{-}CH3, H2}$ = 7.4Hz, **2-CH_3**)
4.13 (dd, $J_{H3, H2}$ = 4.2Hz, $J_{H3, H4}$ = 8.6Hz, **H3**)
6.31 (d, $J_{H4, H3}$ = 8.6Hz, **H4**)
2.13 (s, **4'-CH_3**)
1.17 (s, **6-CH_3**)
1.65 (m, **H7β**)
1.85 (m, **H7α**)
1.70 (m, **H8**)
0.88 (d, $J_{8\text{-}CH3, H8}$ = 7.1Hz, **8-CH_3**)
4.07 (dd, $J_{H9, H8}$ = 6.6Hz, $J_{H9, H10}$ = 2.0Hz, **H9**)
4.61, 4.33 (d, J_{AB} = 11.7Hz, **9-OCH_2**)
5.08 - 5.05 (m, **H10**)
1.77 (d, J = 2.7Hz,**12-CH_3**)
5.63 (t, $J_{H13, H14}$ = 9.0Hz, **H13**)
1.70 - 1.60 (m, **14-CH_2**)
0.93 (t, $J_{14\text{-}CH3, H14}$ = 7.3Hz, **14-CH_3**)

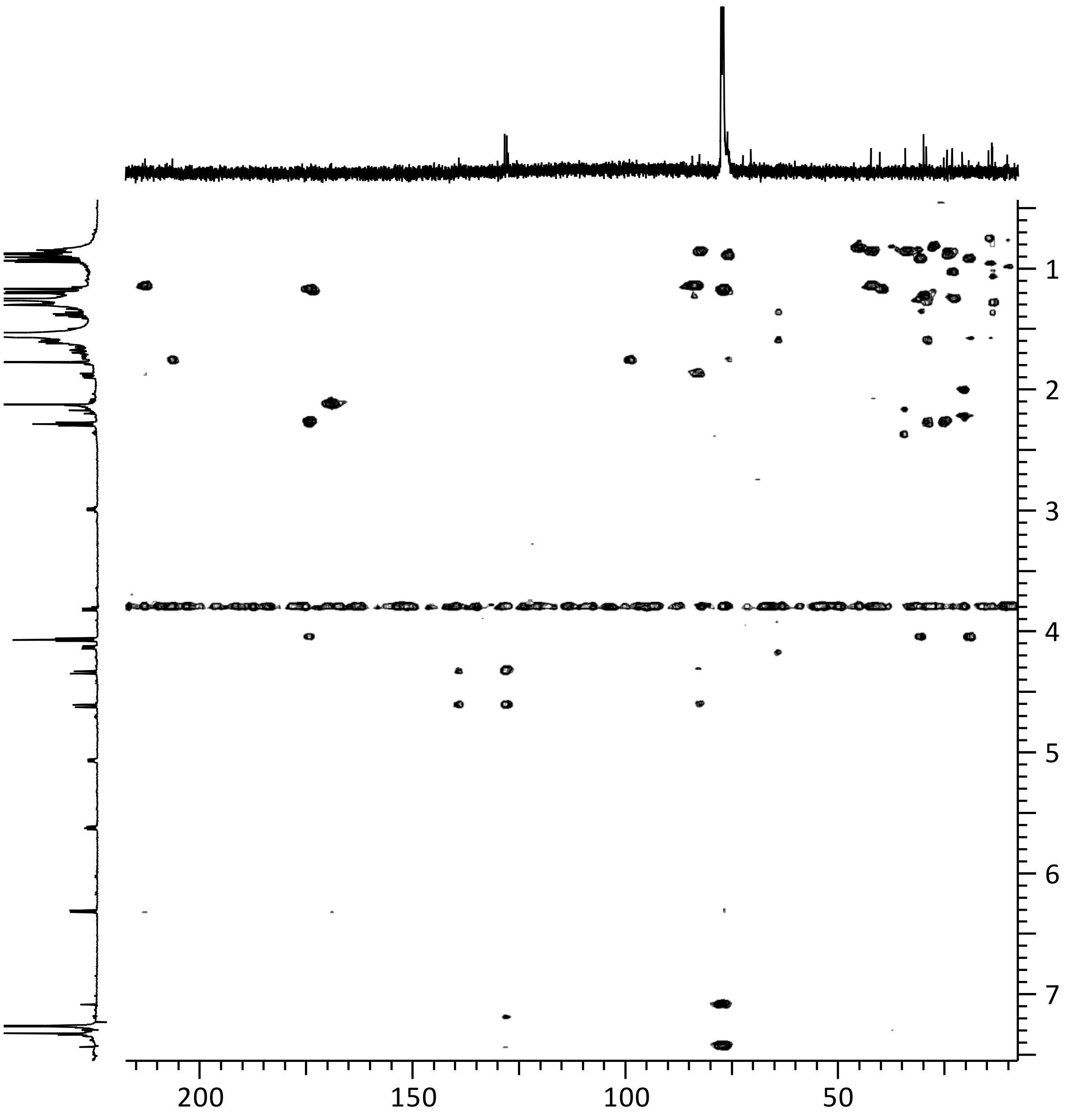

Me
Me
H
OBn
9
CH$_3$
H7β
H7α
6
O
H$_3$C
O
3
1
H
O
O
H
CH$_3$
O
O
CH$_3$
H
2.0
1.5
1.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0


spin systems
identification
H4
H3
H2
H10
H9
12-CH$_3$
2-CH$_3$
OBn
H7β
H7α
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

HMBC

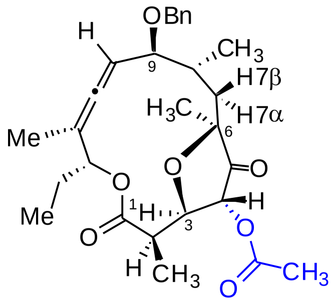
correlations

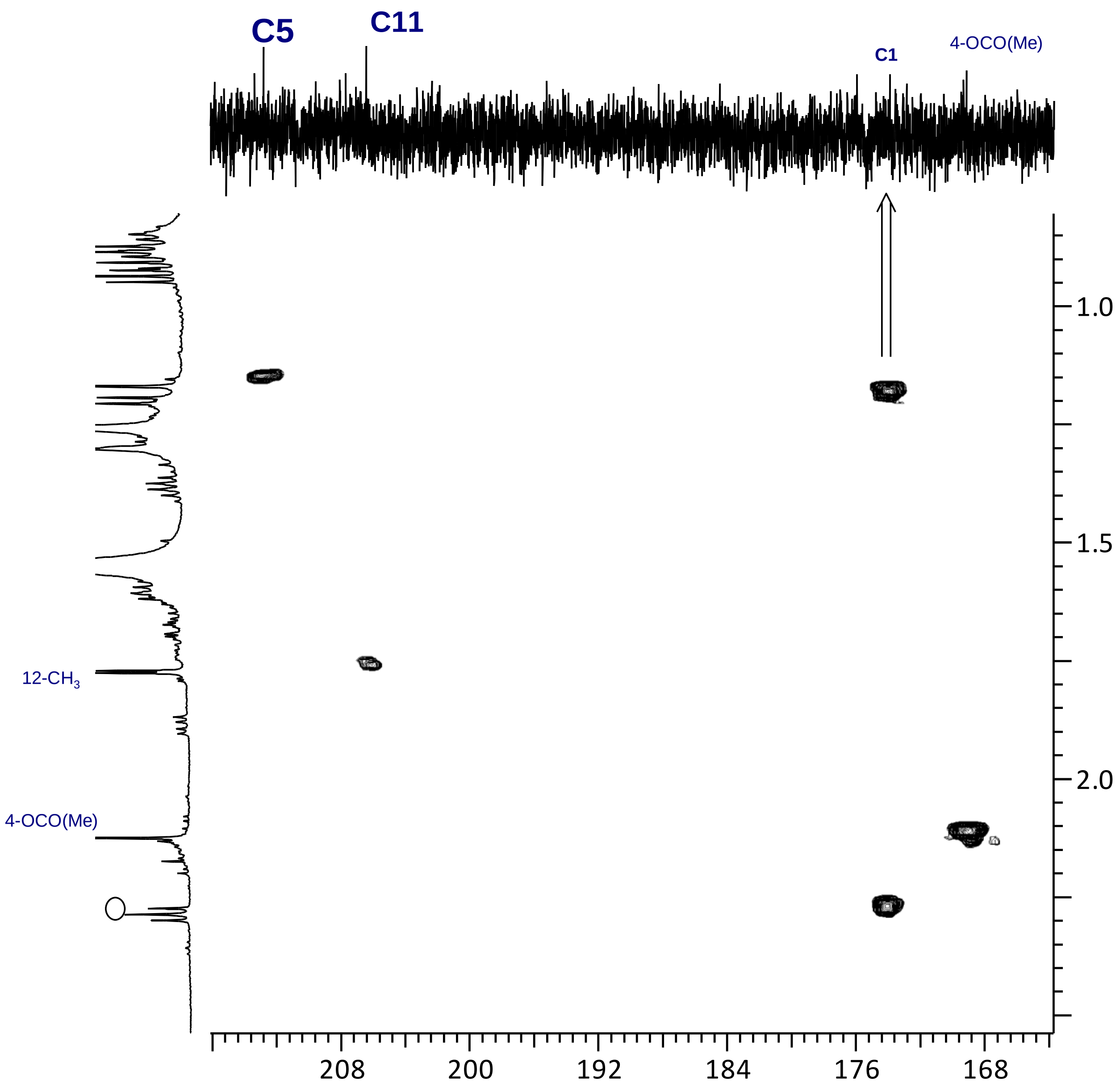

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
O
O
Me
1
H
3
O
H
O
O
CH3
H
CH3
O



HMBC

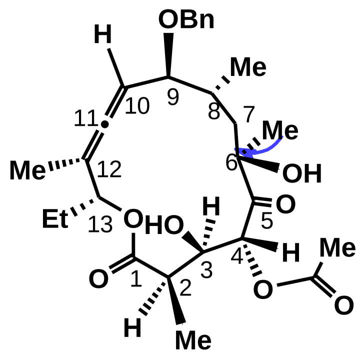
correlations

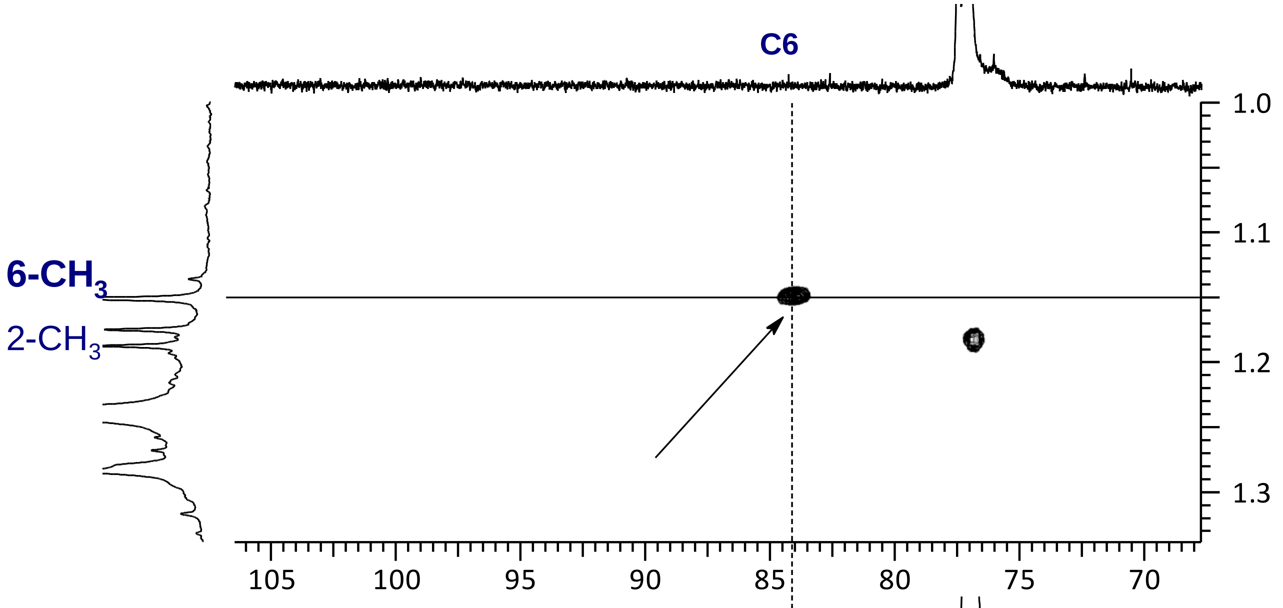

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
O
O
O
1
H
3
H
Me
O
H
CH3
O
CH3
O

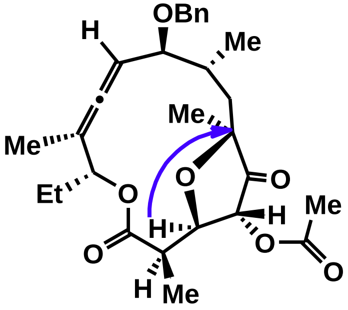


HMBC

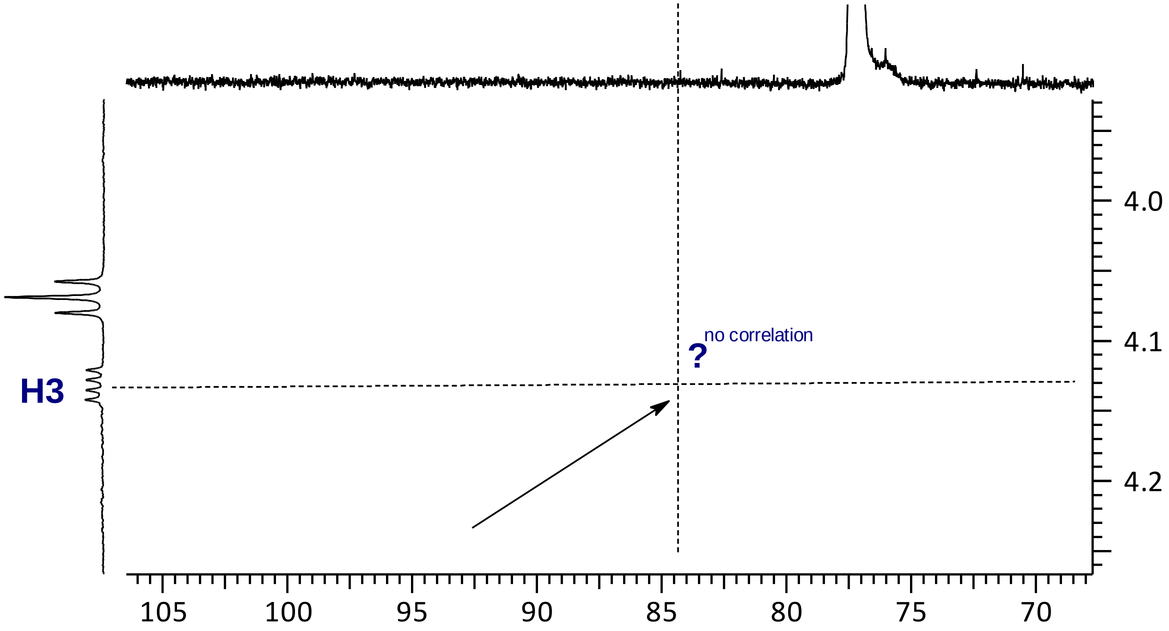
correlations

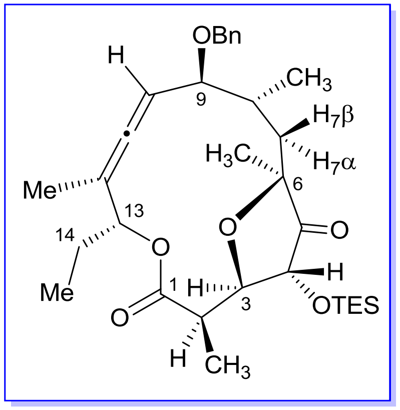

OBn
H
Me
Me
OH
Me
Et
HO
H
O
H
Me
O
O
H
Me
O
1
2
3
4
5
6
7
8
9
10
11
12
13




OBn
H
Me
Me
Me
O
O
Et
O
H
H
Me
O
H
Me
O
O

Expected correlation



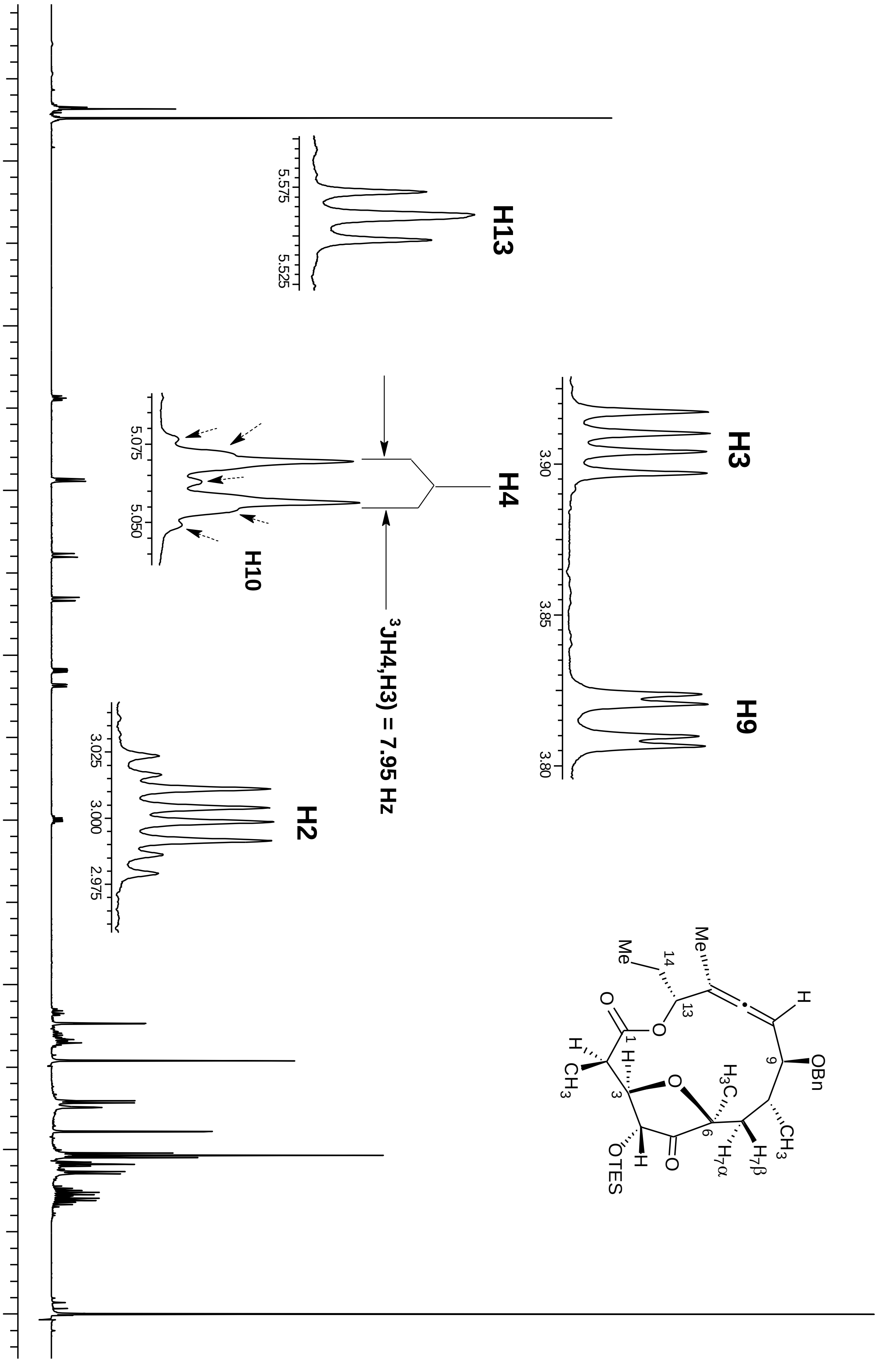


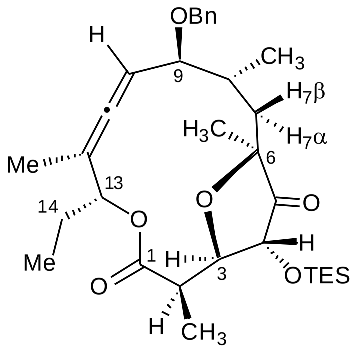
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

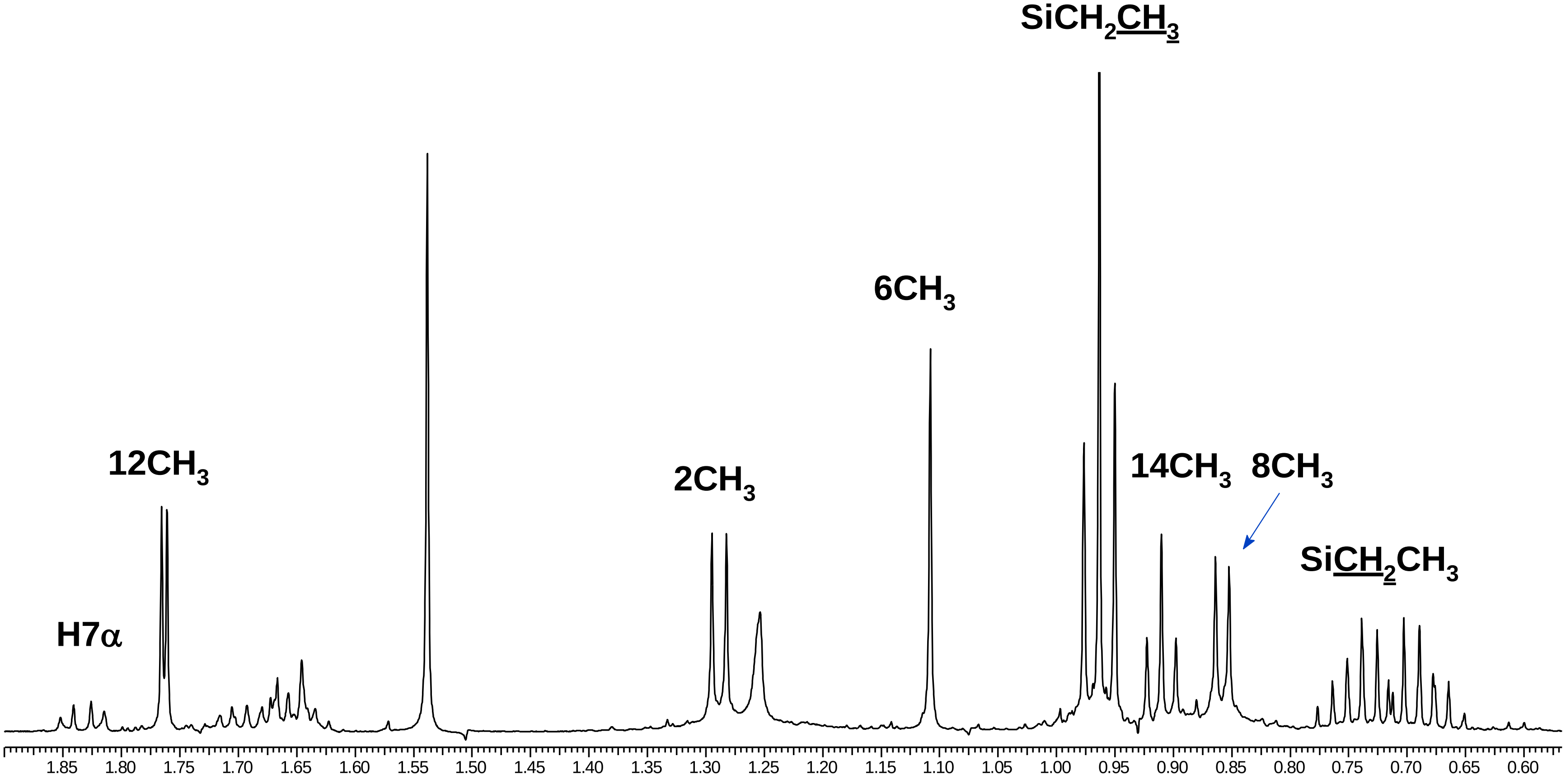
3.00 (dd, $J_{H2,\,H3}$ = 4.2Hz, $J_{H2,\,2\text{-}CH3}$ = 7.3Hz, **H2**)
1.30 (d, $J_{2\text{-}CH3,\,H2}$ = 7.3Hz, **2-CH$_3$**)
3.90 (dd, $J_{H3,\,H2}$ = 4.2Hz, $J_{H3,\,H4}$ = 8.0Hz, **H3**)
5.07 (d, $J_{H4,\,H3}$ = 8.0Hz, **H4**)
1.10 (s, **6-CH$_3$**)
1.65 (m, **H7β**)
1.85 (m, **H7α**)
1.70 (m, **H8**)
0.85 (d, $J_{8\text{-}CH3,\,H8}$ = 7.0Hz, **8-CH$_3$**)
3.83 (dd, $J_{H9,\,H8}$ = 8.3Hz, $J_{H9,\,H10}$ = 2.0Hz, **H9**)
4.60, 4.35 (d, J_{AB} = 12.0Hz, **9-OCH$_2$**)
5.07 - 5.05 (m, **H10**)
1.75 (d, J = 2.9Hz,**12-CH$_3$**)
5.55 (t, $J_{H13,\,H14}$ = 8.1Hz, **H13**)
1.70 - 1.60 (m, **14-CH$_2$**)
0.90 (t, $J_{14\text{-}CH3,\,H14}$ = 7.6Hz, **14-CH$_3$**)

13C NMR chemical shift (δ/ppm)

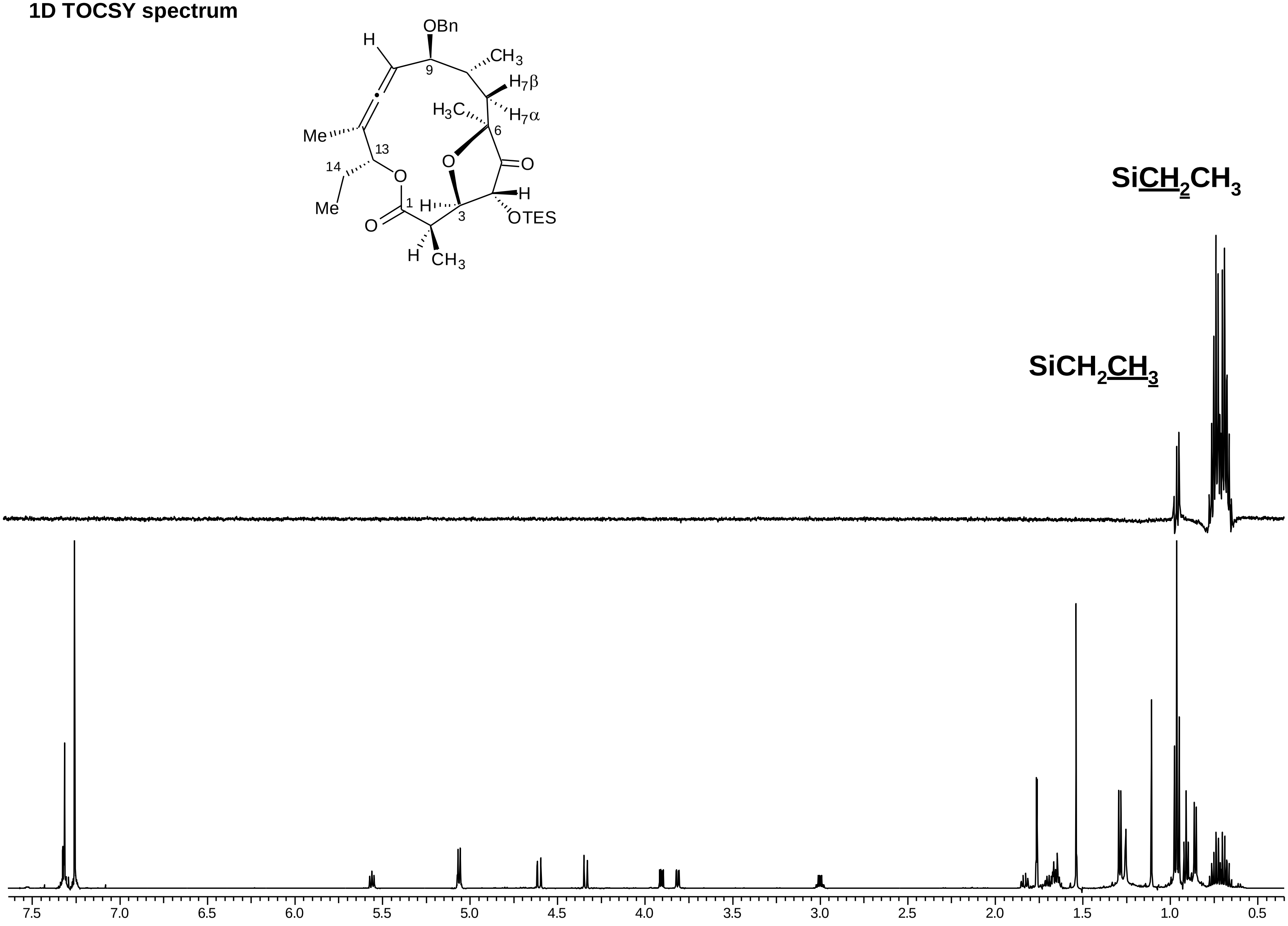
174.5 ----- C1
40.5 ----- C2
80.6 ----- C3
72.7 ----- C4
217.1 ----- C5
83.3 ----- C6
42.1 ----- C7
34.3 ----- C8
82.7 ----- C9
90.8 ----- C10
206.0 ----- C11
98.9 ----- C12
75.7 ----- C13
24.2 ----- C14
14.1 ----- 2-CH_3
22.5 ----- 6-CH_3
14.4 ----- 8-CH_3
13.6 ----- 12-CH_3
10.0 ----- 14-CH_3
70.4 ----- 9-OCH_3
6.8 ----- $SiCH_2\underline{CH_3}$
5.2 ----- $Si\underline{CH_2}CH_3$

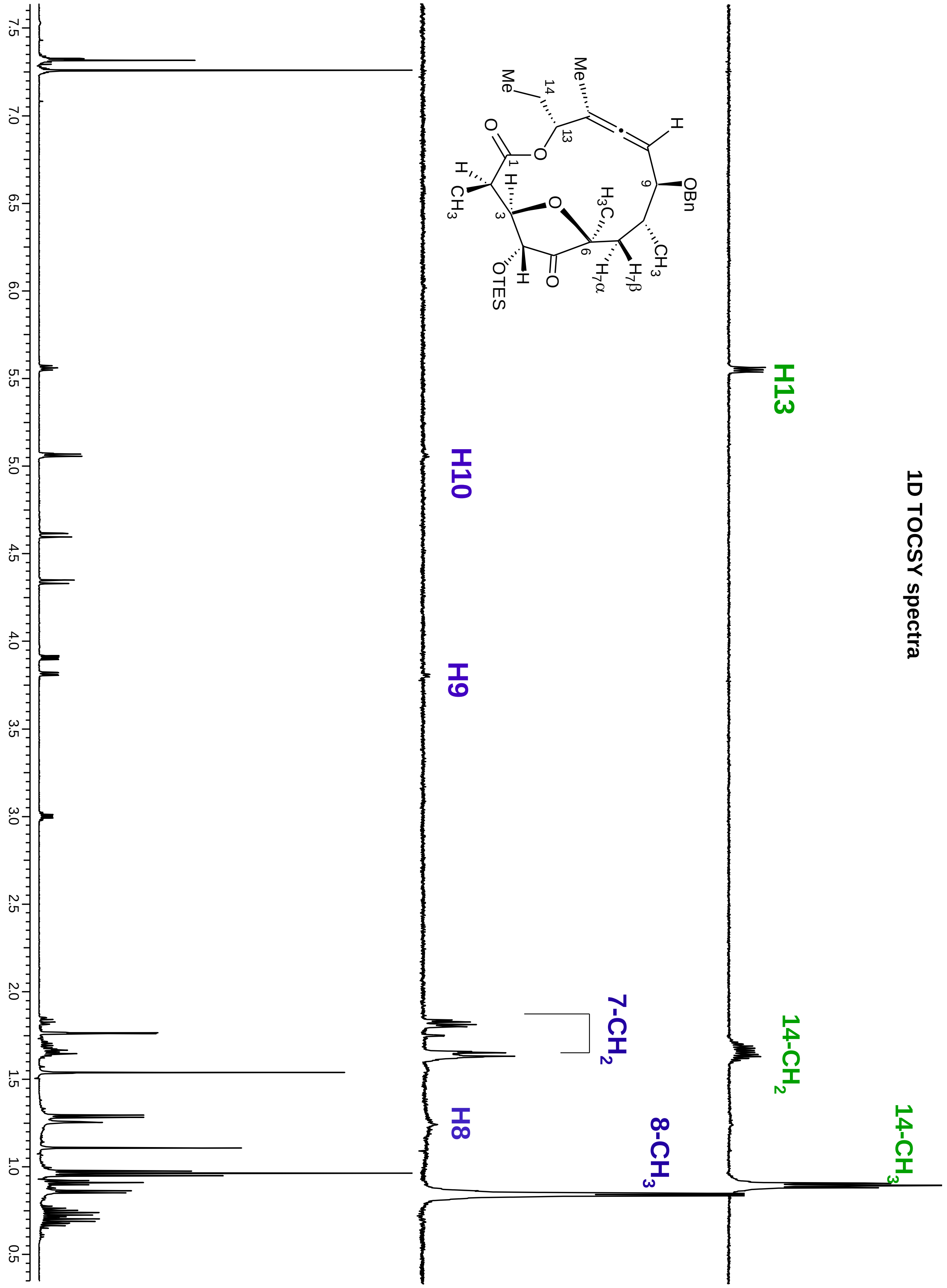

H13
5.575
5.525
H3
3.90
3.85
3.80
H9
H4
5.075
5.050
H10
³JH4,H3) = 7.95 Hz
H2
3.025
3.000
2.975
Me
14
Me
H
13
O
O
1
H
9
OBn
H3C
3
O
6
CH3
H7β
H7α
OTES
H
O
H
CH3
7
6
5
4
3
2
1
0


OBn
H
9
CH3
H7β
H7α
H3C
6
Me
13
14
O
O
O
1
H
3
H
OTES
Me
O
H
CH3


SiCH2CH3
6CH3
12CH3
2CH3
14CH3
8CH3
SiCH2CH3
H7α
1.85
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60

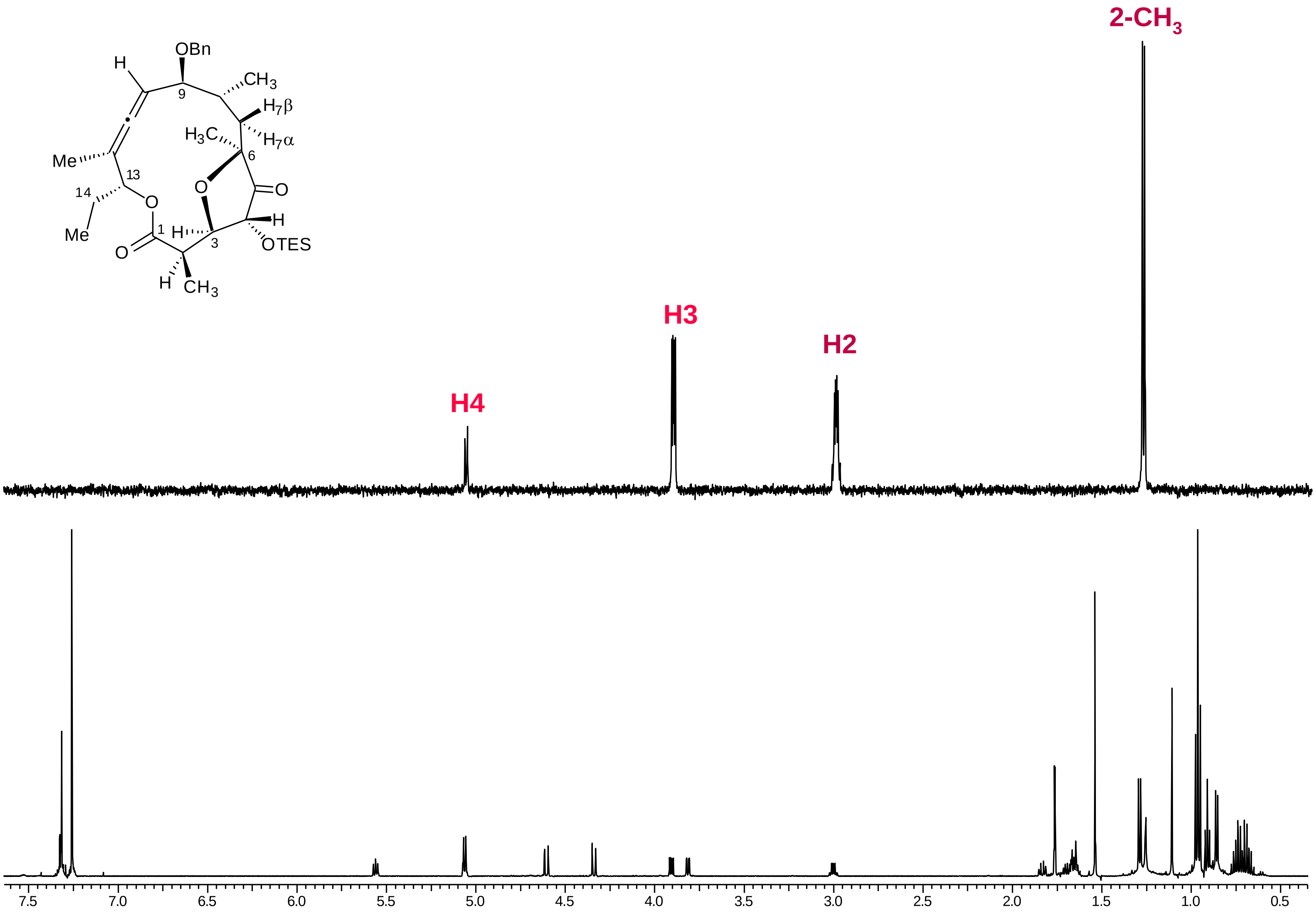
1D TOCSY spectrum





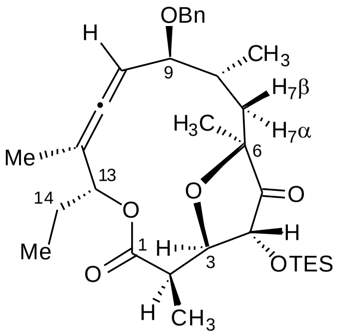
1D TOCSY spectra

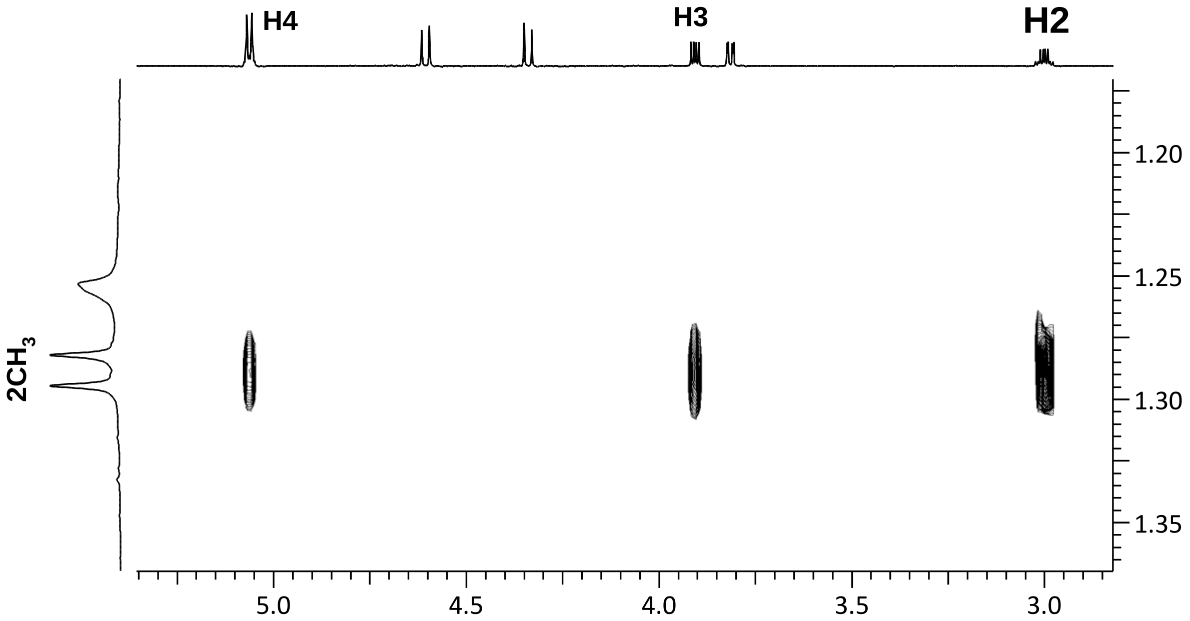
1D TOCSY spectra

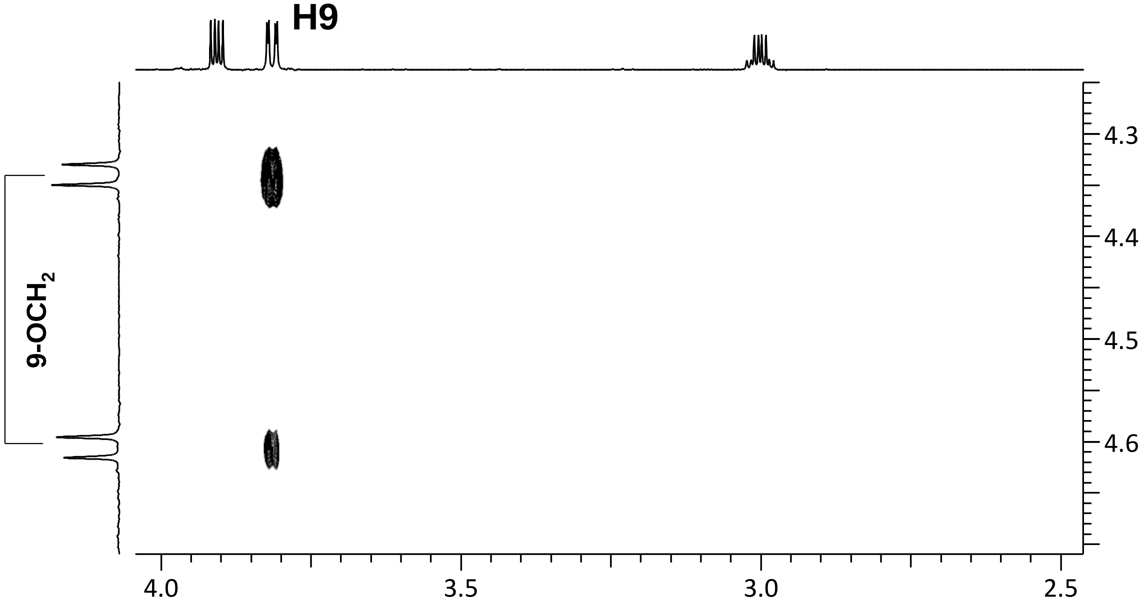


NOESY

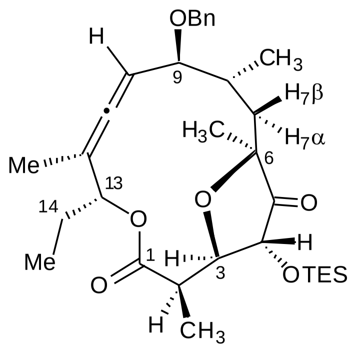
expansions

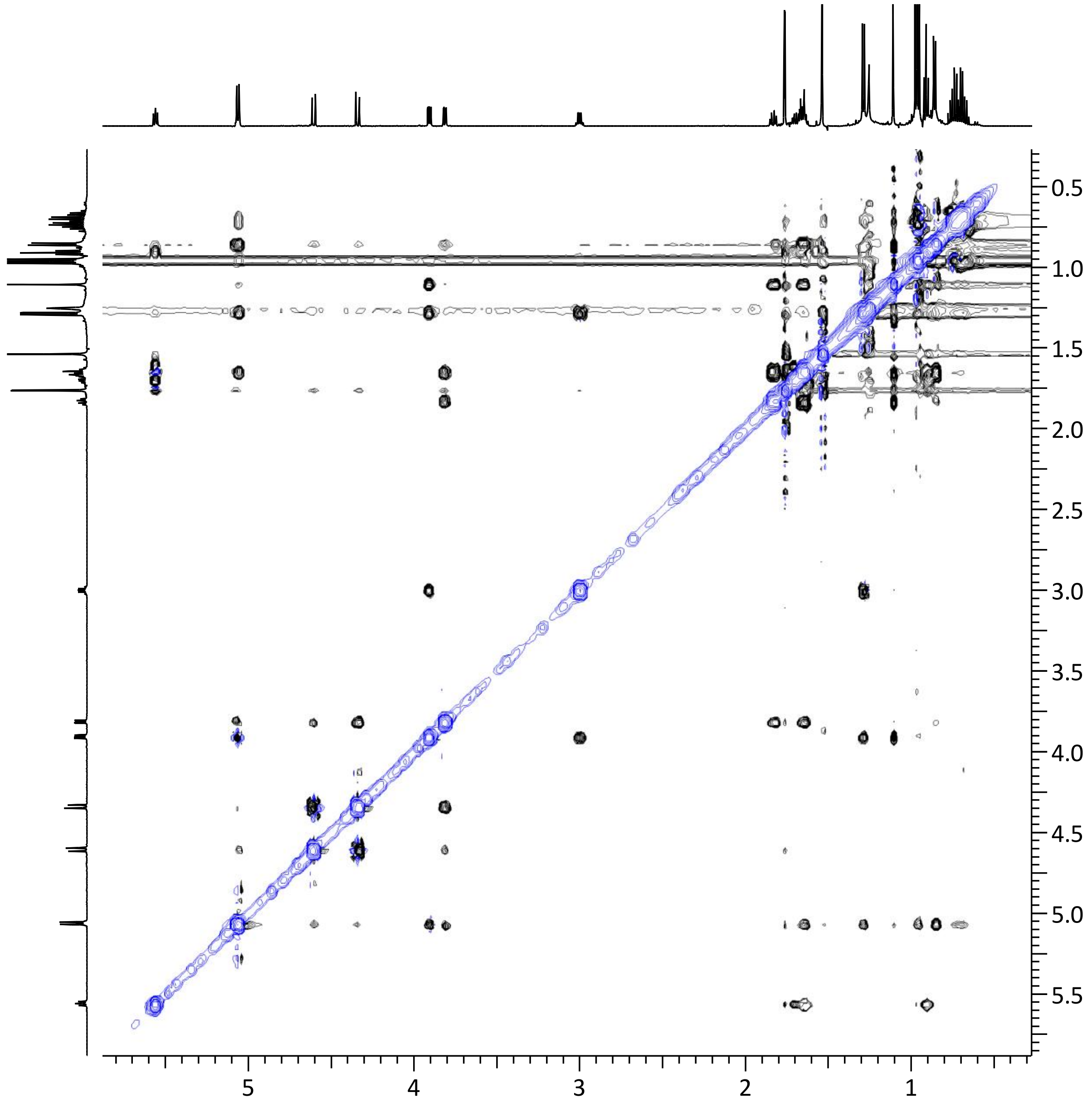

OBn
H
9
CH3
H7β
H7α
H3C
6
Me
13
14
O
O
O
Me
1
H
3
H
OTES
O
H
CH3



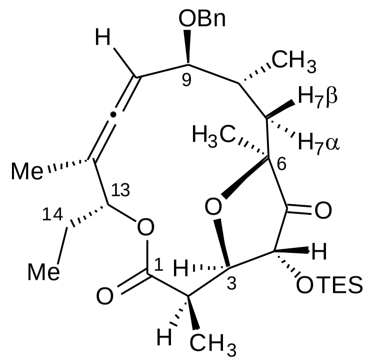


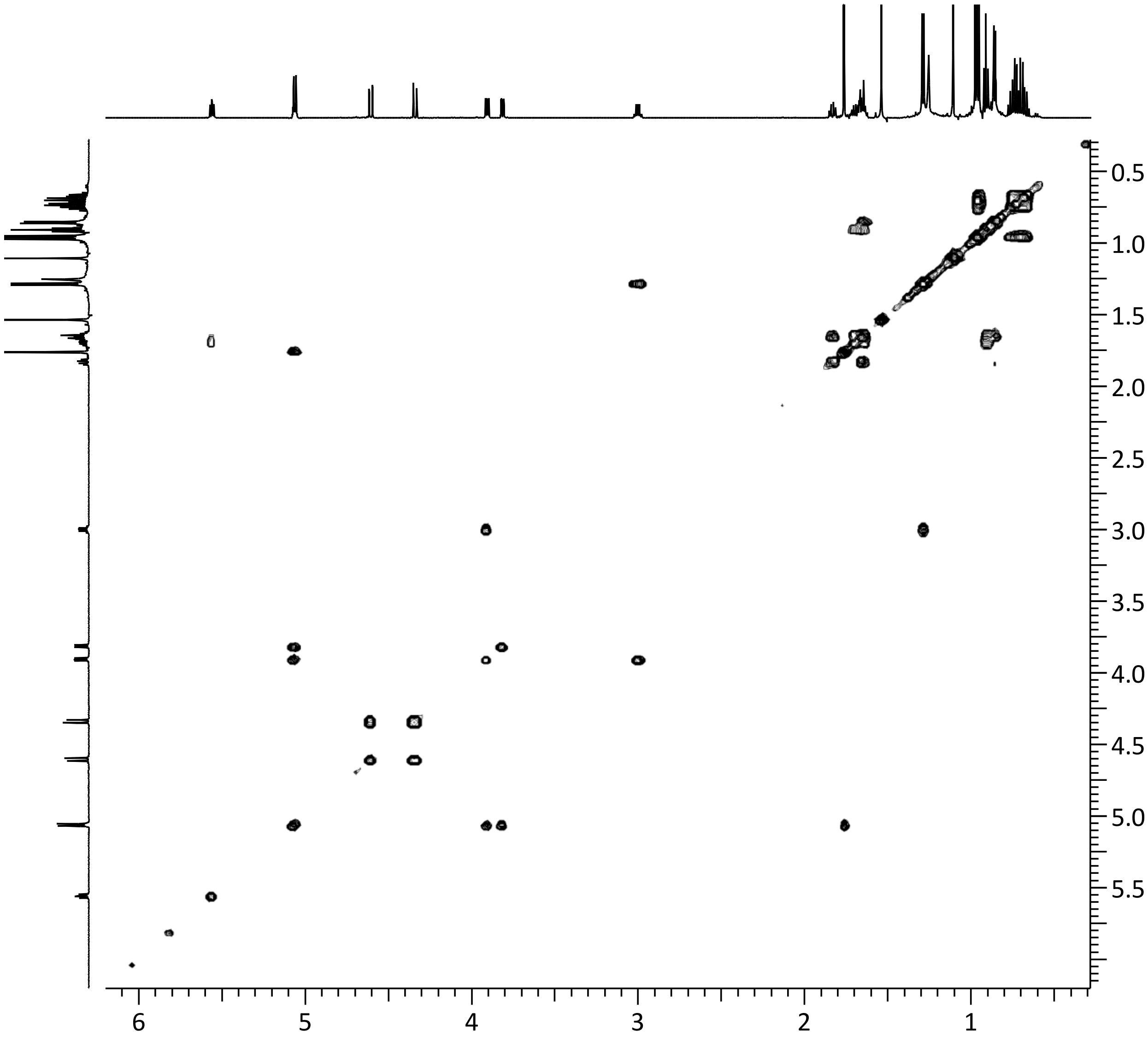
NOESY


OBn
H
9
CH3
H7β
H3C
H7α
6
O
Me
13
14
O
O
H
OTES
Me
1
H
3
O
H
CH3

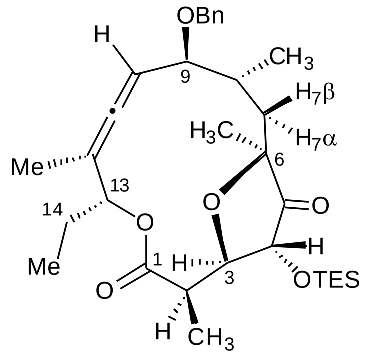

5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5

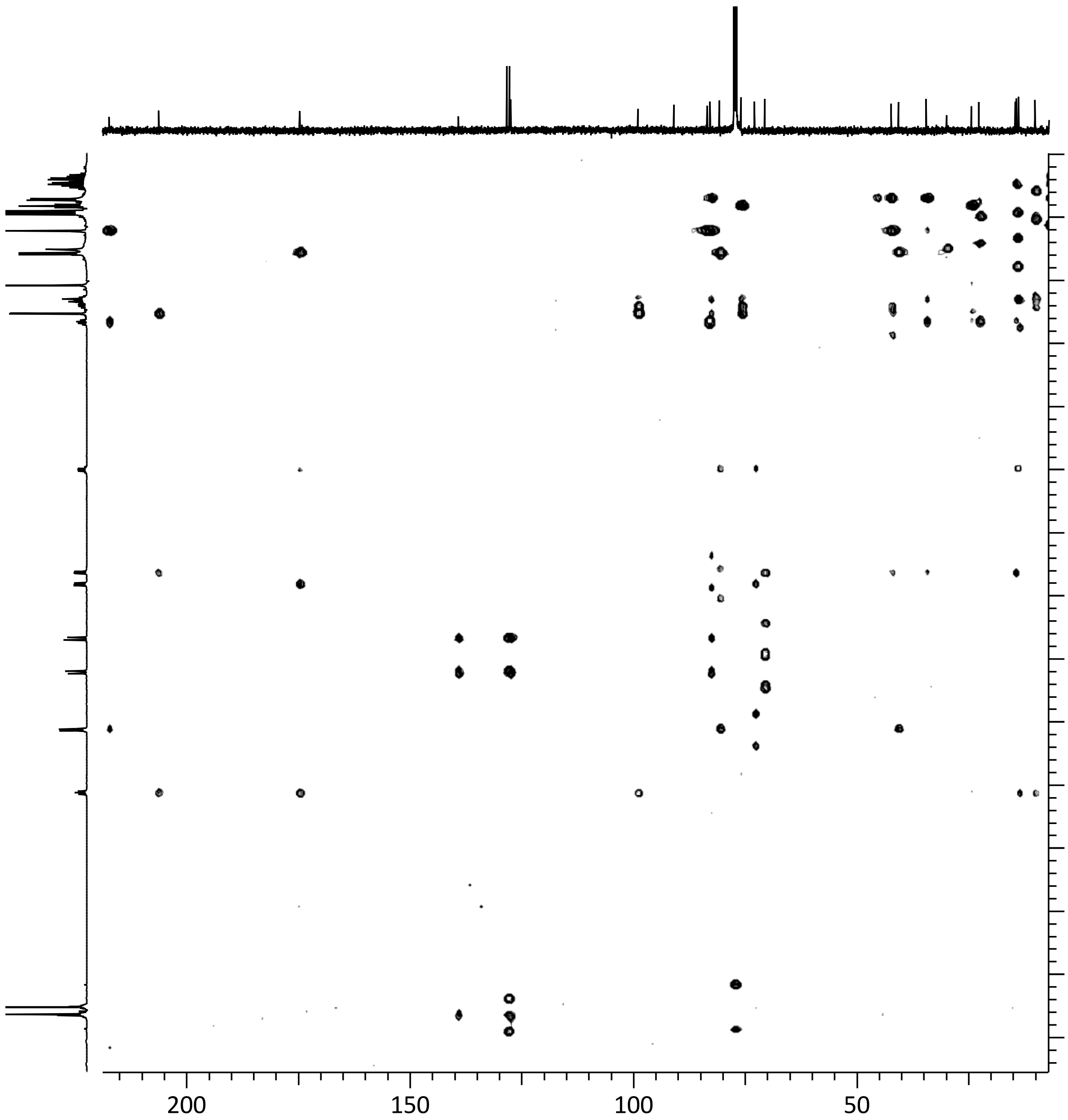
gCOSY


OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
14
O
O
O
1
H
3
H
OTES
Me
O
H
CH3

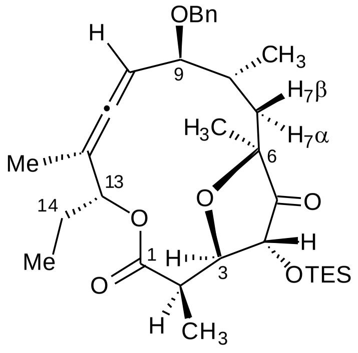

6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5

gHMBC

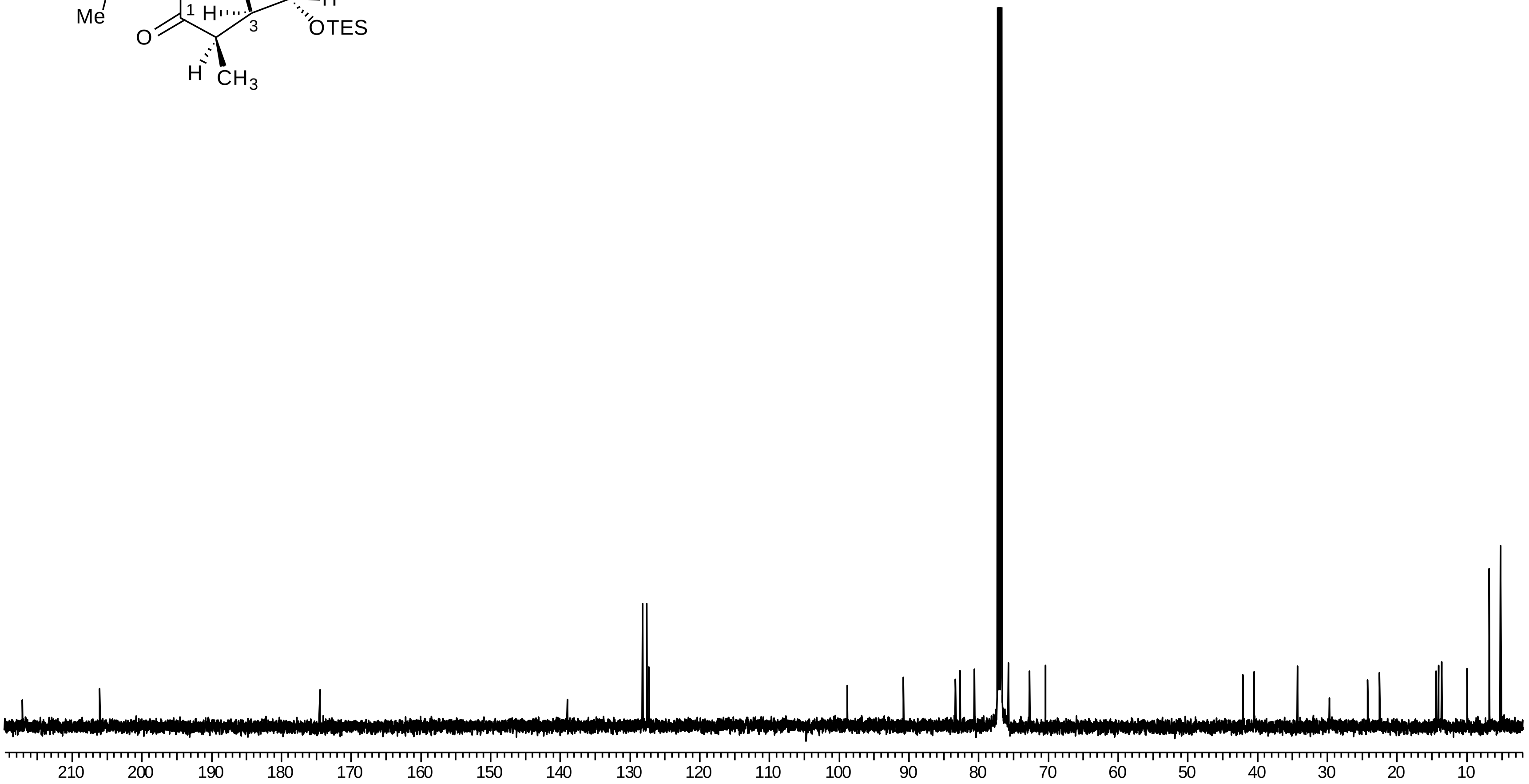

OBn
H
CH3
9
H7β
H3C
H7α
Me
6
13
14
O
O
O
Me
1
H
3
H
OTES
O
H
CH3


200
150
100
50
1
2
3
4
5
6
7

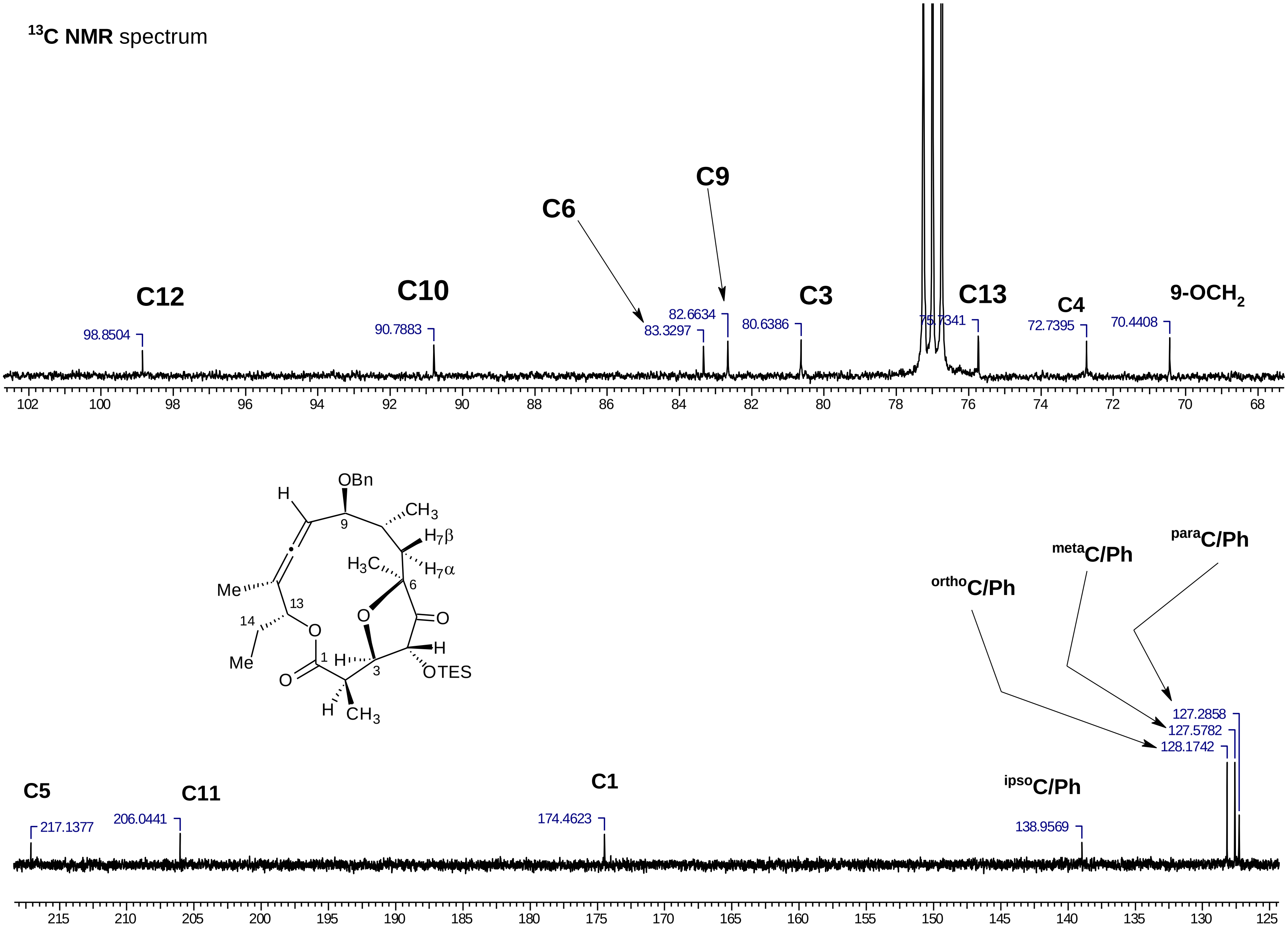
^{13}**C NMR** spectrum



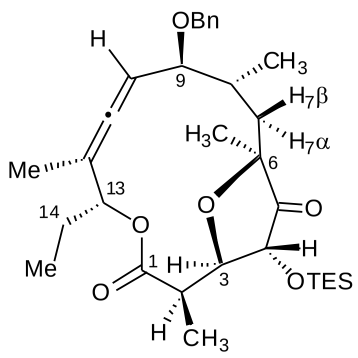
OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
14
O
O
O
Me
1
H
3
H
OTES
O
H
CH3

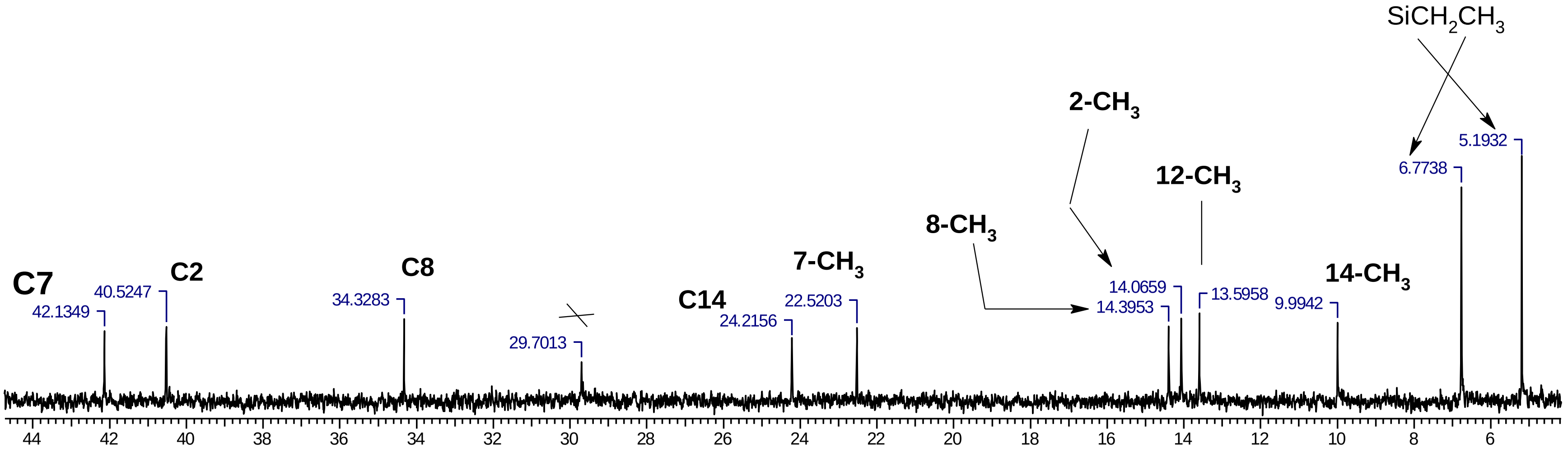


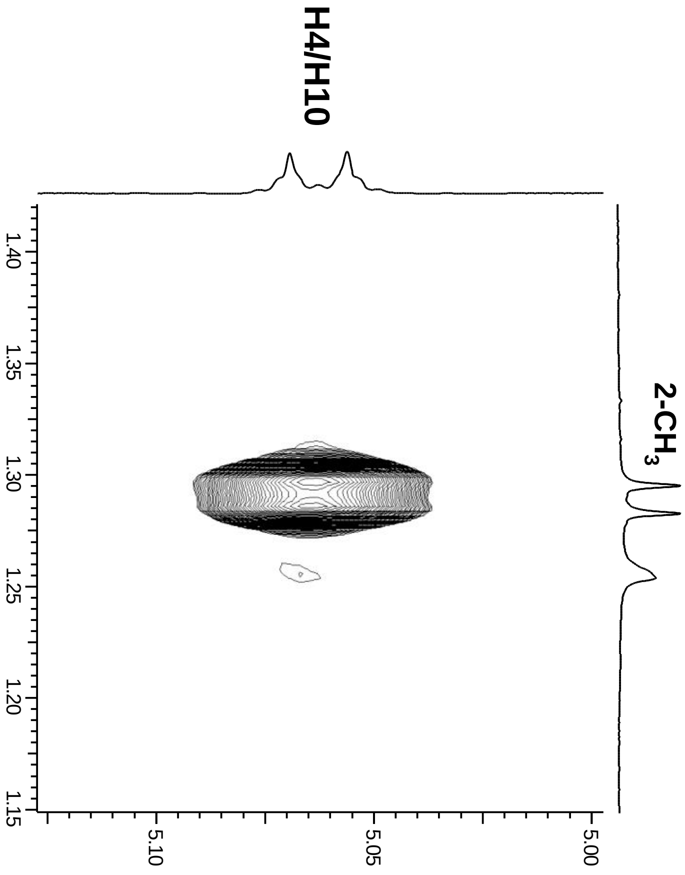
^{13}**C NMR** spectrum

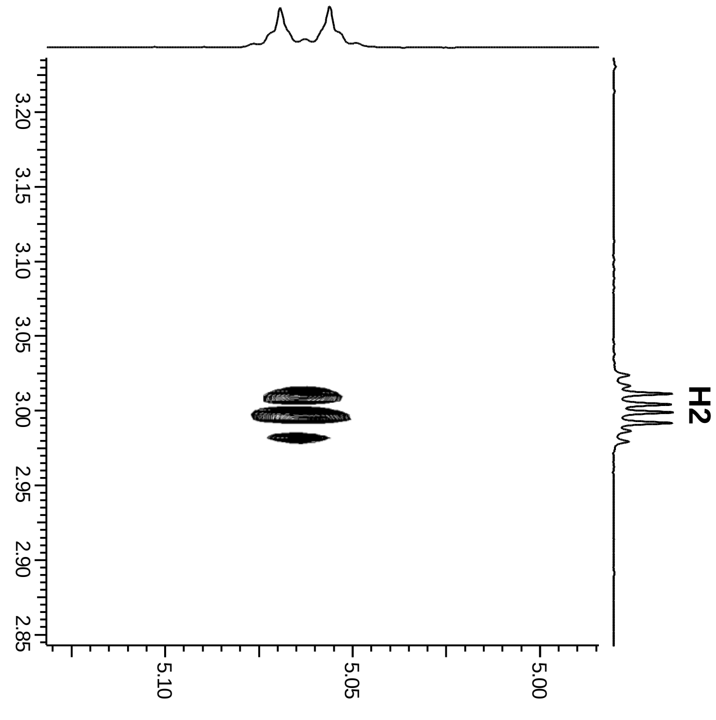


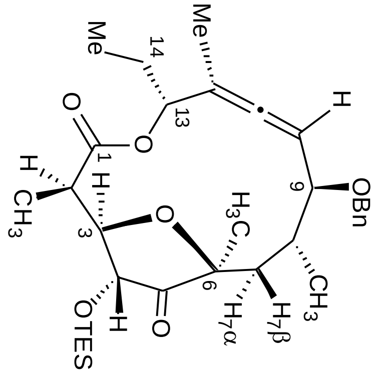
^{13}C NMR spectrum

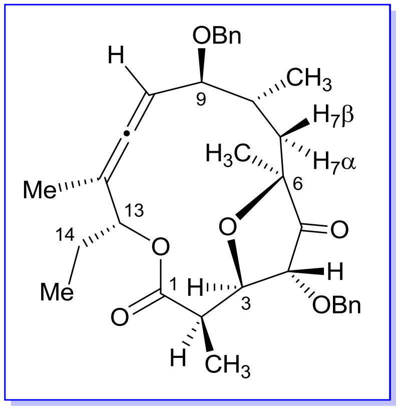

OBn
H
9
CH3
H7β
H3C
H7α
6
Me
13
14
O
O
O
1
H
3
H
OTES
Me
O
H
CH3




H4/H10
2-CH$_3$
1.40
1.35
1.30
1.25
1.20
1.15
5.10
5.05
5.00


H2
3.20
3.15
3.10
3.05
3.00
2.95
2.90
2.85
5.10
5.05
5.00


Me
14
Me
13
O
H
9
OBn
O
1
H
H$_3$C
CH$_3$
H
CH$_3$
3
O
6
H$_7$α
H$_7$β
O
H
OTES

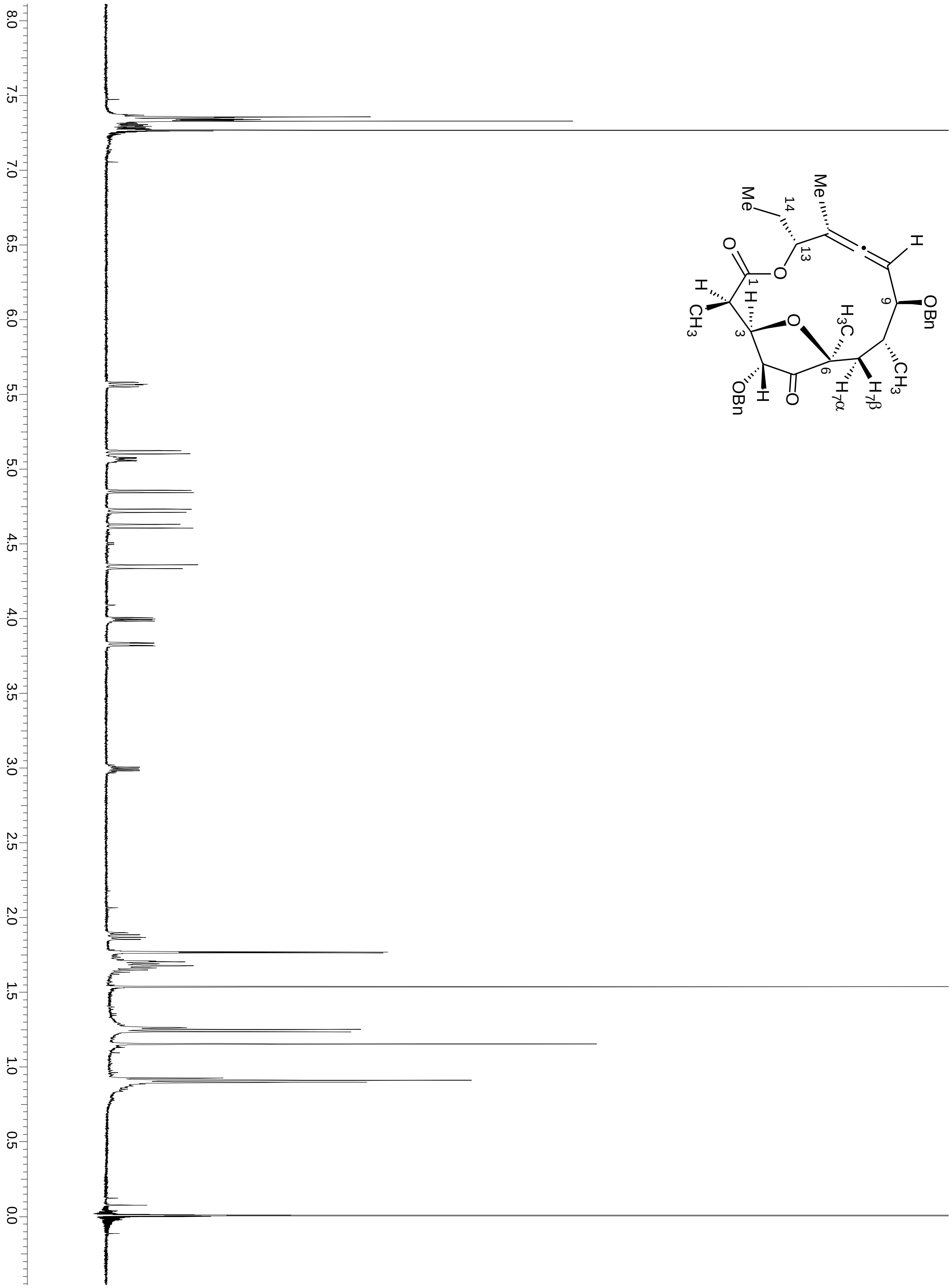


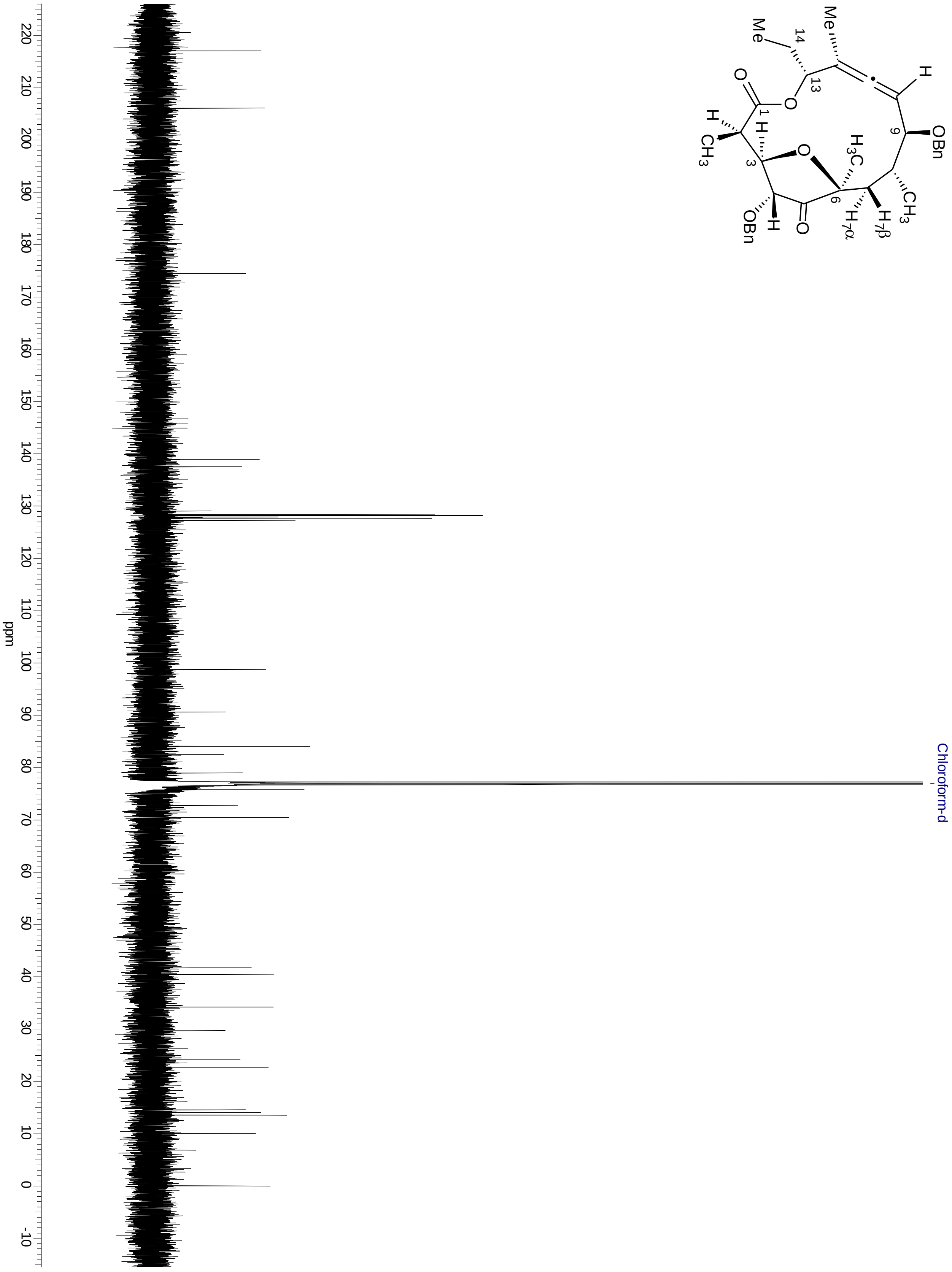
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

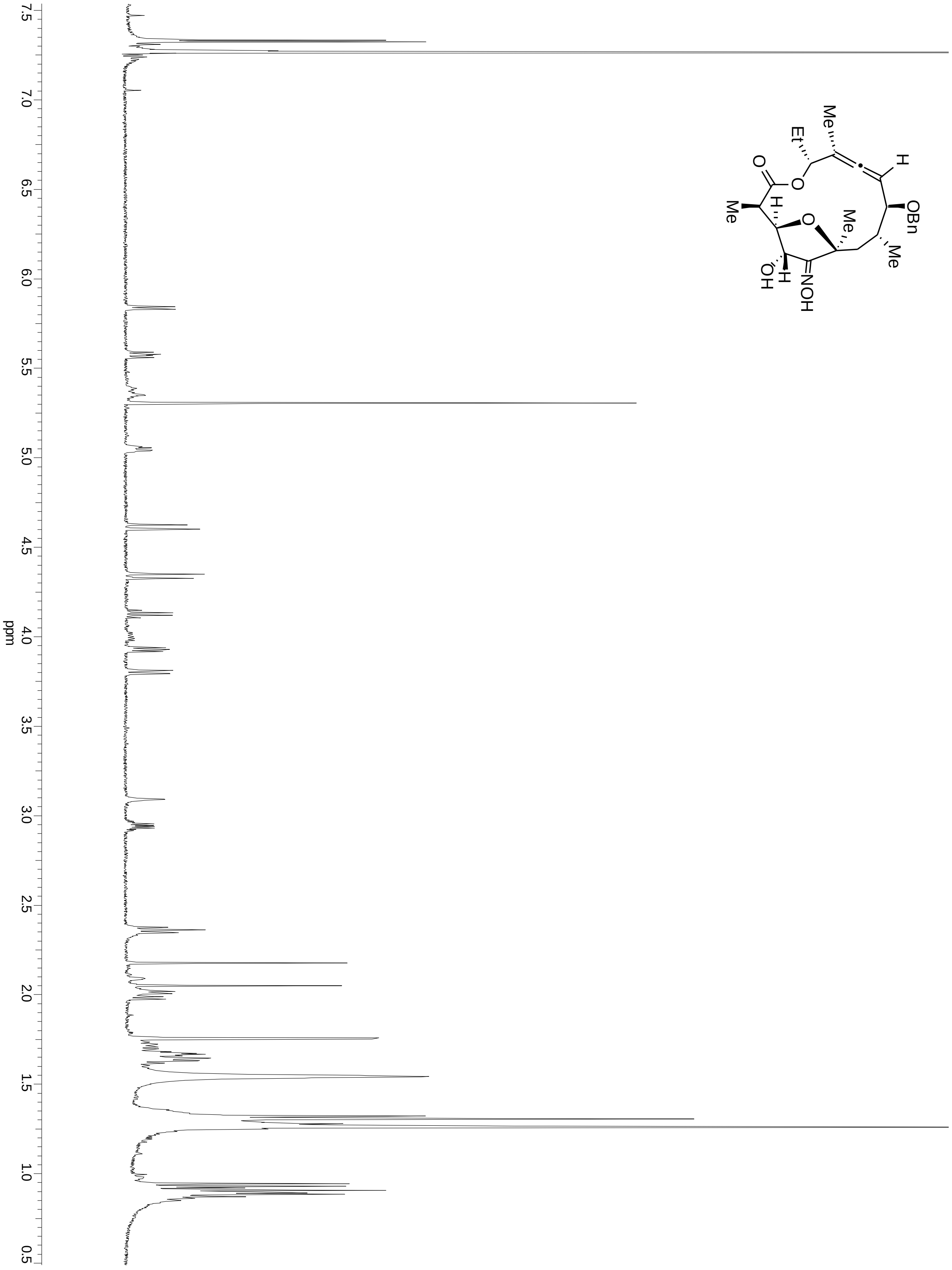
2.99 (dd, $J_{H2,\ H3}$ = 4.4Hz, $J_{H2,\ 2\text{-}CH3}$ = 7.6Hz, **H2**)
0.91 (d, $J_{2\text{-}CH3,\ H2}$ = 7.6Hz, **2-CH$_3$**)
4.00 (dd, $J_{H3,\ H2}$ = 4.4Hz, $J_{H3,\ H4}$ = 7.6Hz, **H3**)
4.86 (d, $J_{H4,\ H3}$ = 7.6Hz, **H4**)
1.15 (s, **6-CH$_3$**)
1.65 (m, **H7β**)
1.89 (dd, $J_{H7\alpha,\ H8}$ = 6.8, $J_{H7\alpha,\ H7\beta}$ = 15.7Hz, **H7α**)
1.72 - 1.62 (m, **H8**)
1.25 (d, $J_{8\text{-}CH3,\ H8}$ = 7.6Hz, **8-CH$_3$**)
3.83 (dd, $J_{H9,\ H8}$ = 8.6Hz, $J_{H9,\ H10}$ = 2.0Hz, **H9**)
5.12, 4.73, 4.63, 4.36 (d, J_{AB} = 11.0, 12.0 Hz, **4-OCH$_2$,9-OCH$_2$**)
5.07 (m, **H10**)
1.77 (d, J = 2.7Hz,**12-CH$_3$**)
5.56 (dd, $J_{H13,\ H14}$ = 6.4, 9.0Hz, **H13**)
1.72 - 1.62 (m, **14-CH$_2$**)
0.91 (t, $J_{14\text{-}CH3,\ H14}$ = 7.1Hz, **14-CH$_3$**)

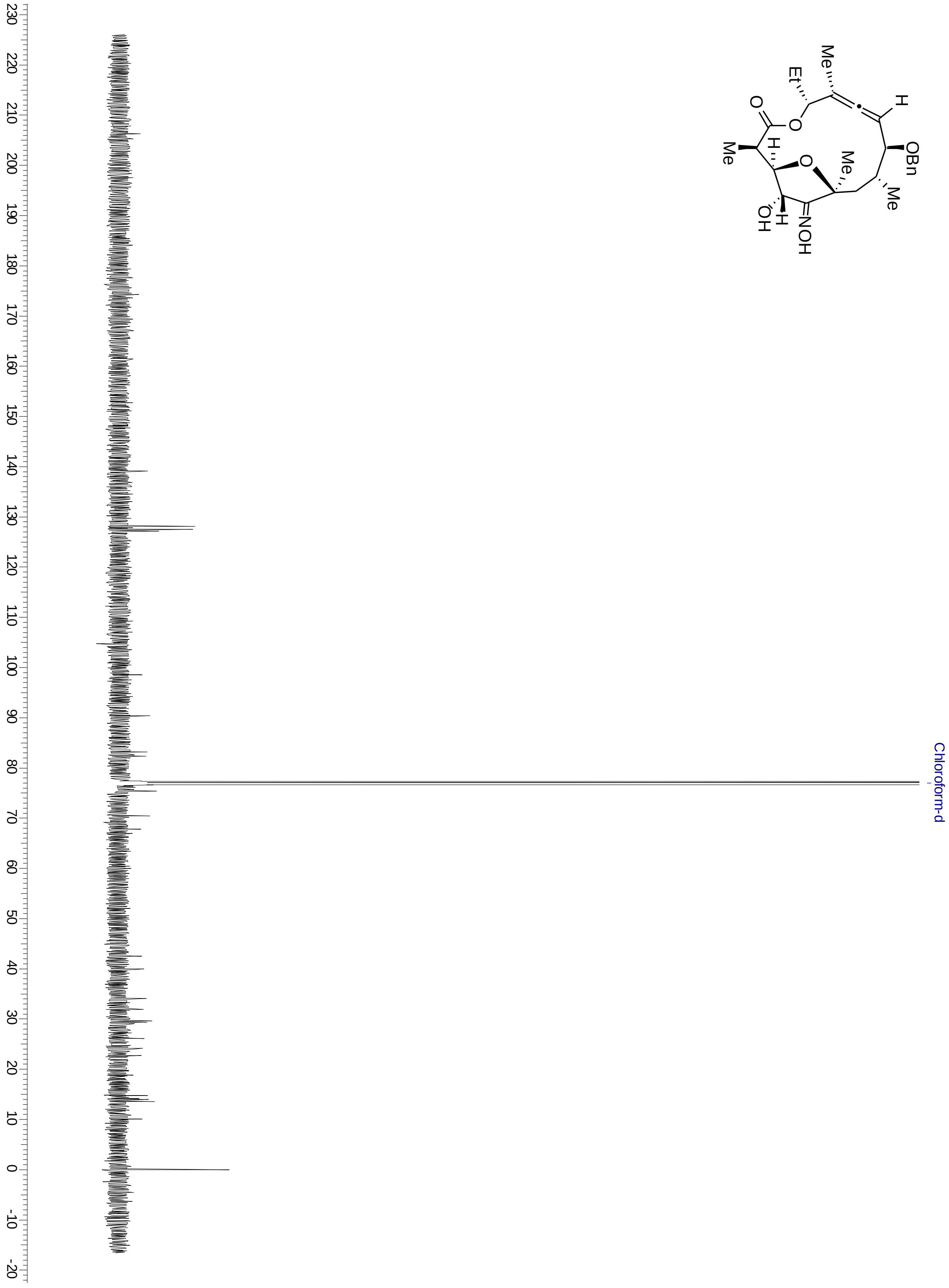
13C NMR chemical shift (δ/ppm)

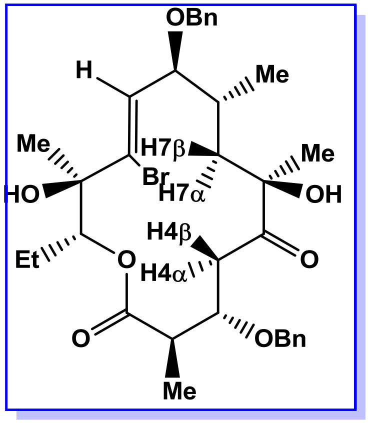
174.4 ----- C1
40.5 ----- C2
79.0 ----- C3
72.9 ----- C4
217.1 ----- C5
84.1 ----- C6
41.7 ----- C7
34.2 ----- C8
82.5 ----- C9
90.6 ----- C10
206.1 ----- C11
98.8 ----- C12
75.9 ----- C13
24.2 ----- C14
14.0 ----- 2-CH$_3$
22.6 ----- 6-CH$_3$
14.5 ----- 8-CH$_3$
13.6 ----- 12-CH$_3$
10.0 ----- 14-CH$_3$
70.4 ----- 9-OCH$_3$


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
Me
14
Me
13
O
O
1
H
H
CH3
3
O
OBn
H
O
6
H3C
H7α
H7β
CH3
9
OBn
H


Me
14
Me
13
O
O
1
H
CH3
H
3
O
OBn
H
O
6
H3C
H7α
H7β
CH3
9
OBn
H
Chloroform-d
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
ppm


7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Me
Et
O
O
H
Me
H
O
Me
OBn
Me
H
OH
NOH


Me
Et
O
H
OBn
Me
O
Me
O
H
Me
H
OH
NOH
Chloroform-d
ppm
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20



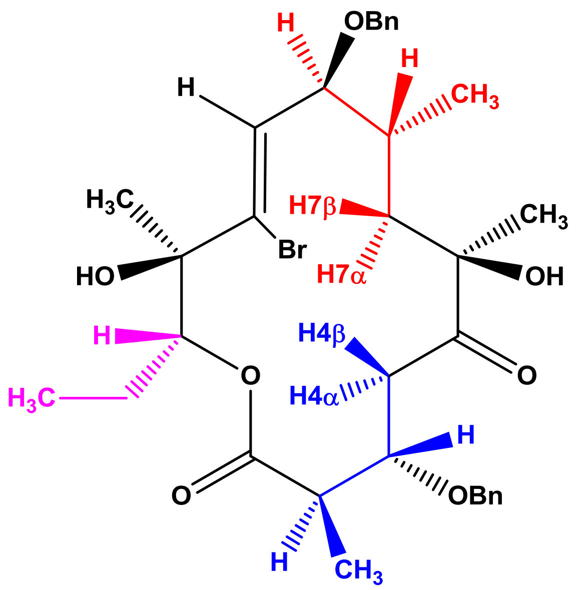
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

2.56 (quintet, $J_{H2,\ 2\text{-}CH3}$ = 6.9Hz, **H2**)
1.20 (d, $J_{2\text{-}CH3,\ H2}$ = 6.9Hz, **2-CH$_3$**)
4.34 (m, **H3**)
4.64, 4.52 (d, J_{AB} = 11.1Hz, **C3-OCH$_2$**)
3.11 (dd, $J_{H4\alpha,\ H4\beta}$ = 15.6Hz, $J_{H4\alpha,\ H3}$ = 6.4Hz, **H4α**)
2.47 (dd, $J_{H4\beta,\ H4\alpha}$ = 15.6Hz, $J_{H4\beta,\ H3}$ = 5.7Hz, **H4β**)
1.23 (s, **6-CH$_3$**)
1.79 (dd, $J_{H7\beta,\ H7\alpha}$ = 14.4Hz, **H7β**)
1.45 ($J_{H7\alpha,\ H8}$ = 5.90 Hz, $J_{H7\alpha,\ H7\beta}$ = 14.4Hz, **H7α**)
1.70-1.85 (m, **H8**)
1.05 (d, $J_{8\text{-}CH3,\ H8}$ = 6.6Hz, **8-CH$_3$**)
4.00 (dd, $J_{H9,\ H10}$ = 8.3Hz, $J_{H9,\ H8}$ = 6.1Hz, **H9**)
4.52, 4.40 (d, J_{AB} = 12.3Hz, **9-OCH$_2$**)
6.14 (d, J = 8.4Hz, **H10**)
1.44 (s, **12-CH$_3$**)
4.89 (dd, $J_{H13,\ H14}$ = 2.4, 10.9Hz, **H13**)
1.75-1.82, 1.47-1.55 (m, **14-CH$_2$**)
0.80 (t, $J_{14\text{-}CH3,\ H14}$ = 7.4Hz, **14-CH$_3$**)

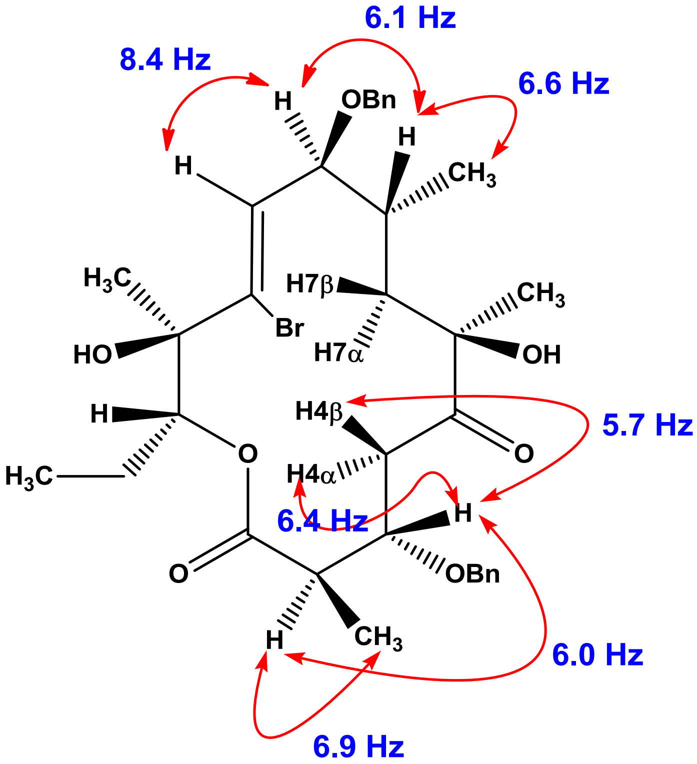
13C NMR chemical shift (δ/ppm)

174.6	-----	C1
42.5	-----	C2
76.1	-----	C3
44.8	-----	C4
212.7	-----	C5
79.4	-----	C6
41.3	-----	C7
35.0	-----	C8
84.0	-----	C9
131.2	-----	C10
134.2	-----	C11
77.1	-----	C12
79.3	-----	C13
24.4	-----	C14
12.6	-----	2-CH$_3$
26.8	-----	6-CH$_3$
19.6	-----	8-CH$_3$
25.1	-----	12-CH$_3$
11.1	-----	14-CH$_3$
73.3	-----	6-OCH$_3$
72.2	-----	9-OCH$_3$

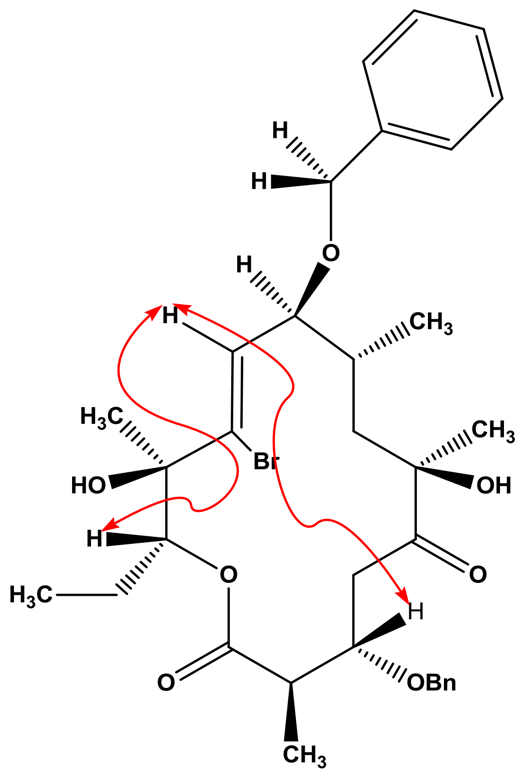
1d TOCSY


H
OBn
H
CH_3
H
H_3C
$H7\beta$
CH_3
Br
HO
$H7\alpha$
OH
H
$H4\beta$
H_3C
O
O
$H4\alpha$
H
O
OBn
H
CH_3

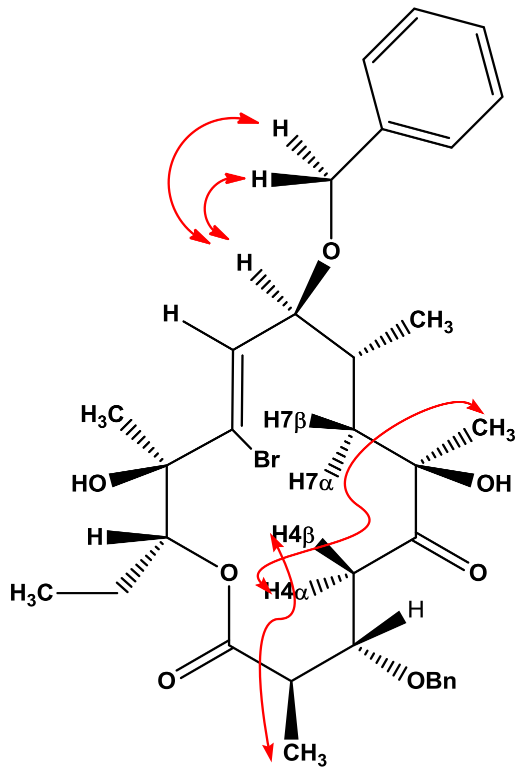
Coupling constant (J_{HH})


6.1 Hz
8.4 Hz
6.6 Hz
H
OBn
H
H
CH_3
H_3C
$H7\beta$
CH_3
Br
HO
$H7\alpha$
OH
H
$H4\beta$
5.7 Hz
H_3C
O
O
$H4\alpha$
H
6.4 Hz
O
OBn
H
CH_3
6.0 Hz
6.9 Hz

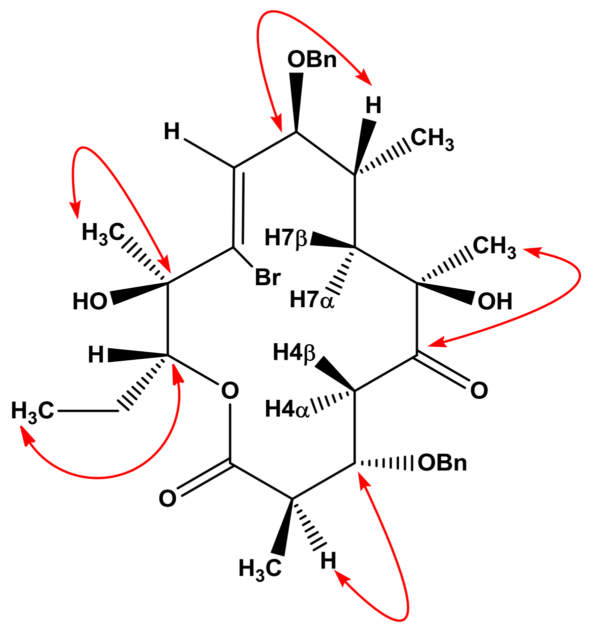
NOE

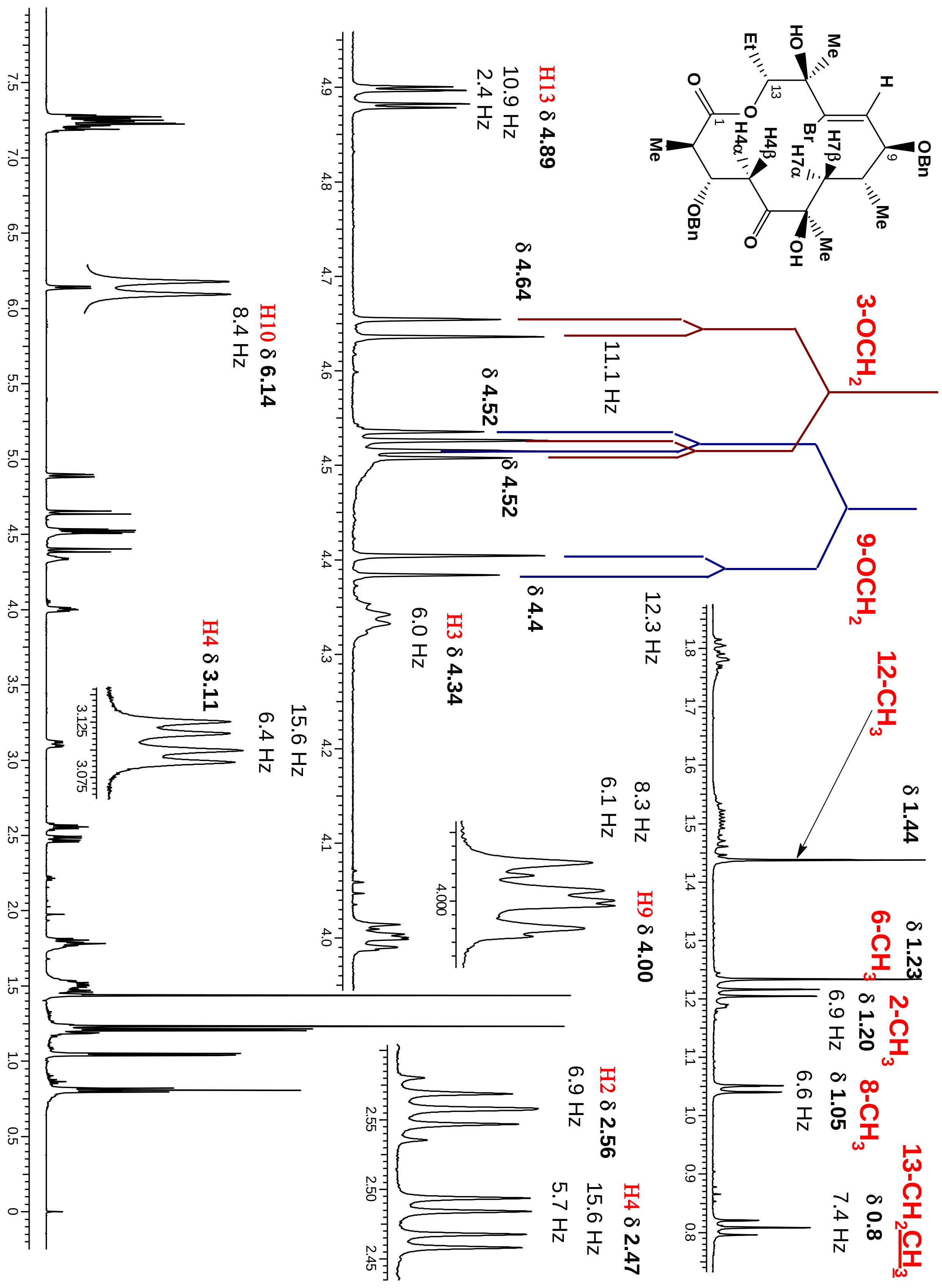


NOE

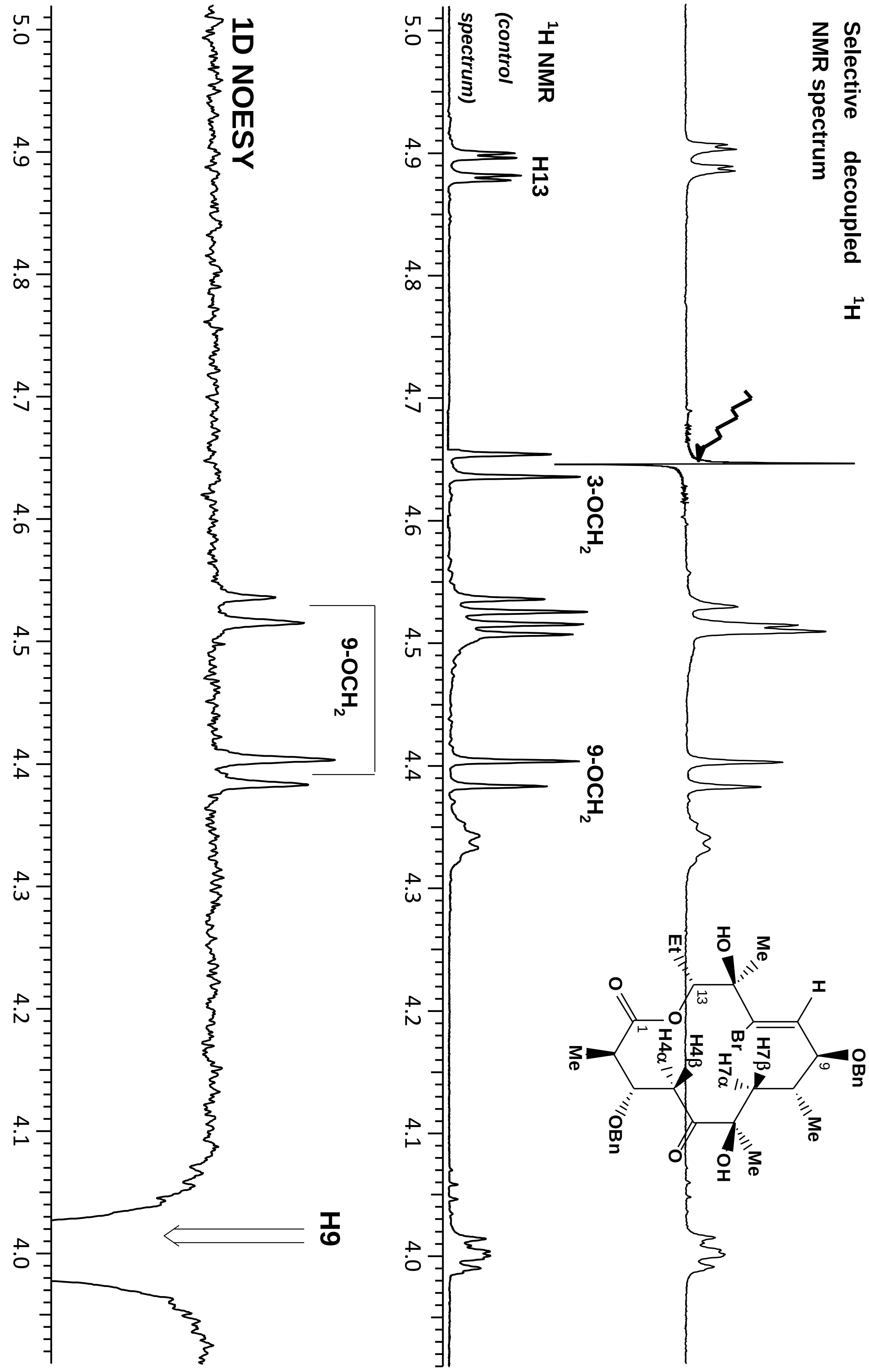

H7β
H7α
H4β
H4α

HMBC

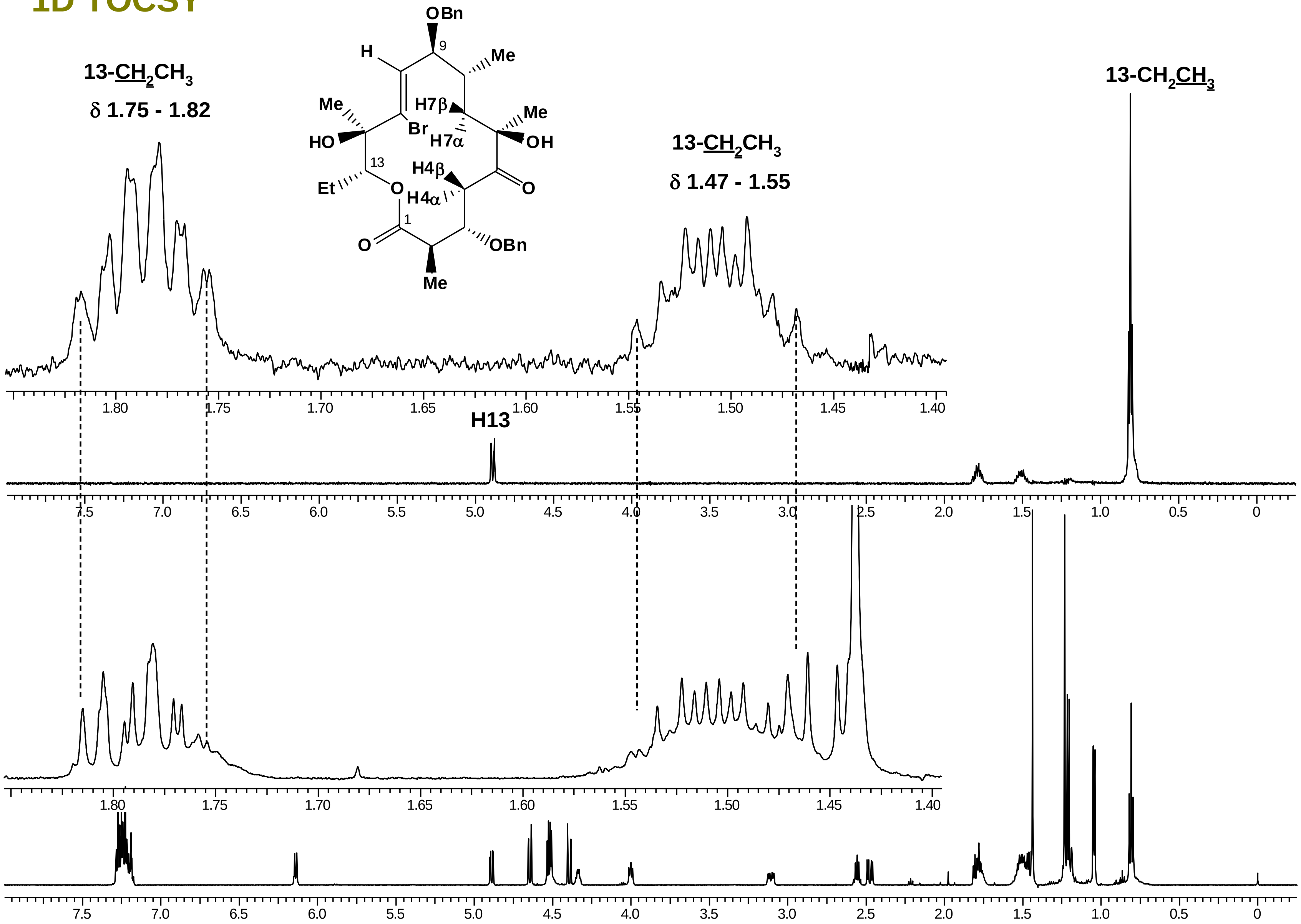

H7β
H7α
H4β
H4α

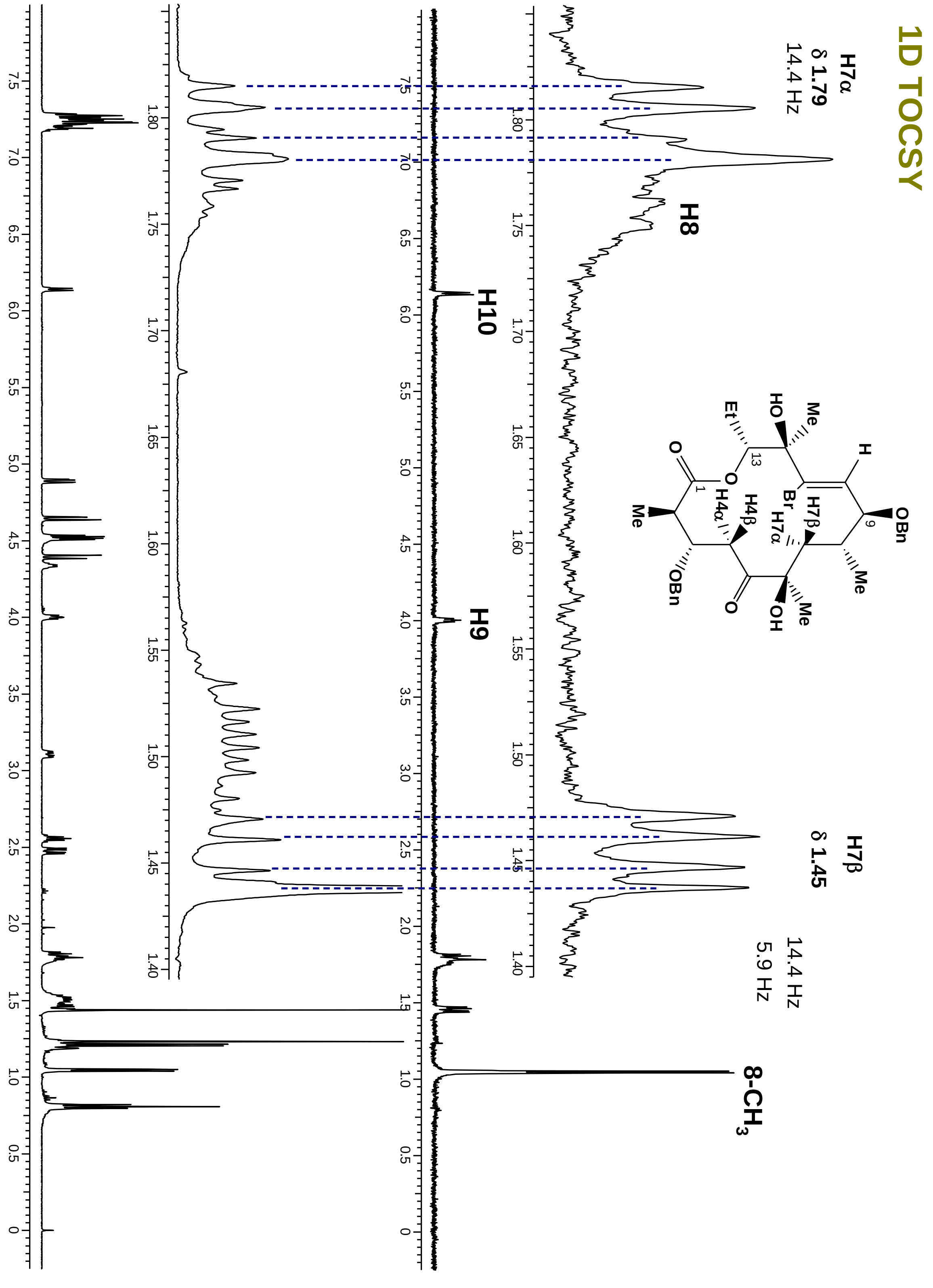


H
OBn
9
Me
Me
HO
Me
Et
13
O
O
1
Br
H7β
H7α
H4β
H4α
Me
OBn
O
OH
Me
3-OCH$_2$
9-OCH$_2$
12-CH$_3$
δ 1.44
δ 1.23
6-CH$_3$
2-CH$_3$
δ 1.20
6.9 Hz
8-CH$_3$
δ 1.05
6.6 Hz
13-CH$_2$CH$_3$
δ 0.8
7.4 Hz
H13 δ 4.89
10.9 Hz
2.4 Hz
δ 4.64
11.1 Hz
δ 4.52
δ 4.52
δ 4.4
12.3 Hz
H3 δ 4.34
6.0 Hz
H9 δ 4.00
8.3 Hz
6.1 Hz
4.000
H2 δ 2.56
6.9 Hz
H4 δ 2.47
15.6 Hz
5.7 Hz
H10 δ 6.14
8.4 Hz
H4 δ 3.11
15.6 Hz
6.4 Hz
3.125
3.075
2.55
2.50
2.45
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

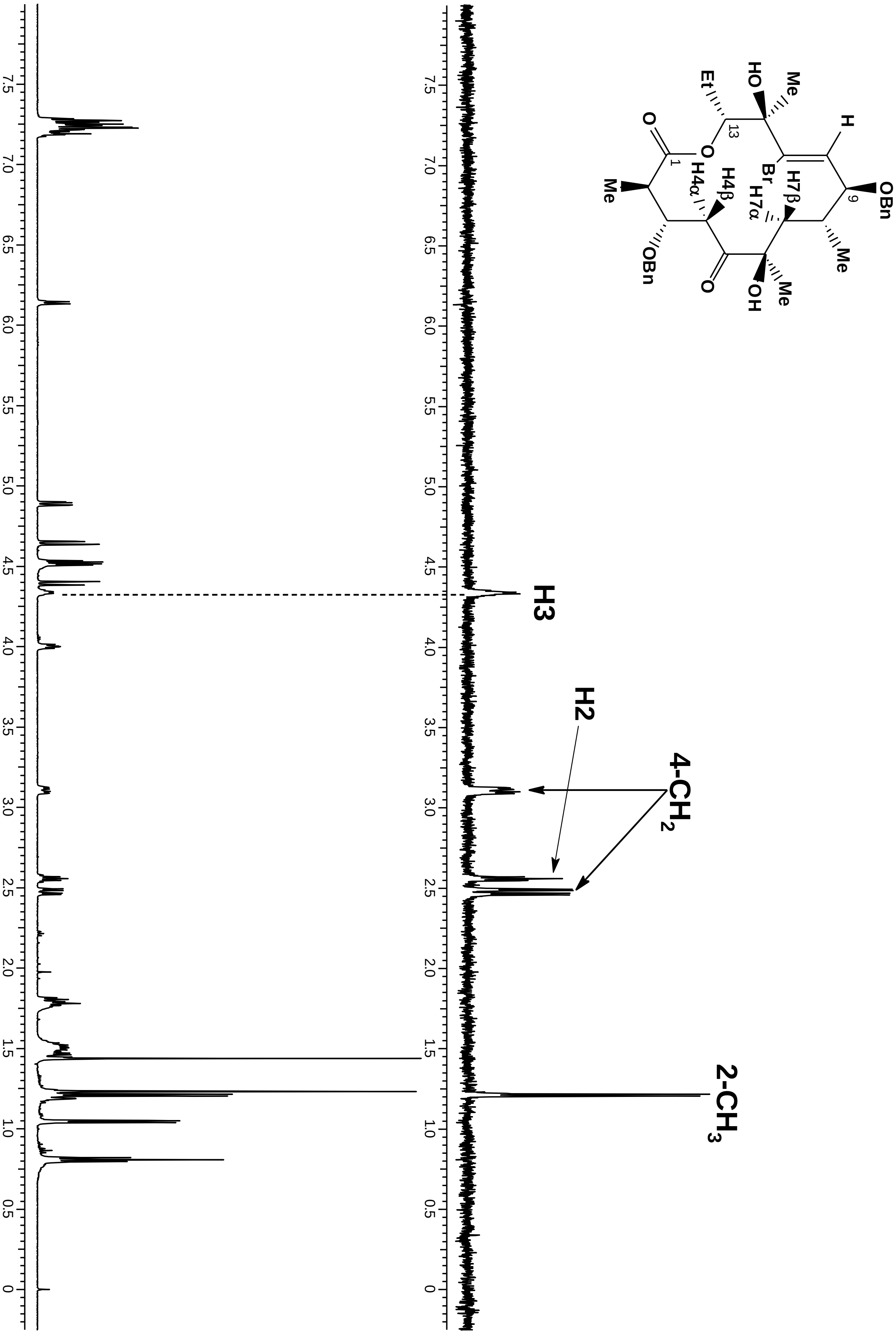

Selective decoupled ¹H NMR spectrum
¹H NMR
(control spectrum)
H13
3-OCH₂
9-OCH₂
1D NOESY
9-OCH₂
H9
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
Me
HO
Et
O
13
H
H7β
Br
H7α
OBn
9
Me
OH
Me
O
H4β
H4α
1
O
Me
OBn

1D TOCSY

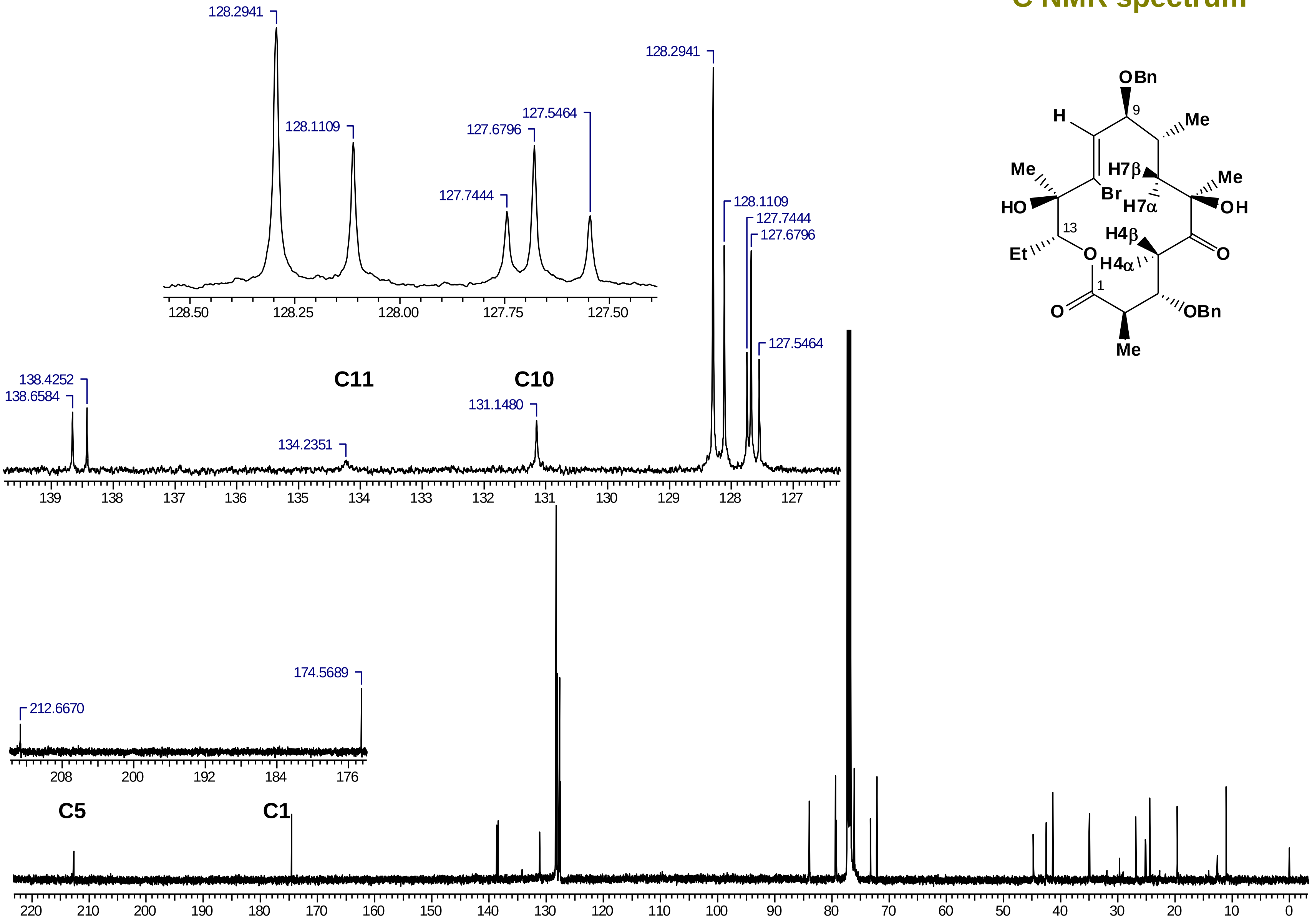

13-CH₂CH₃
δ 1.75 - 1.82
13-CH₂CH₃
δ 1.47 - 1.55
13-CH₂CH₃
H13
OBn
9
H
Me
Me
H7β
Me
HO
Br
H7α
OH
13
H4β
Et
O
H4α
O
1
OBn
O
Me
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0


1D TOCSY
H7α
δ 1.79
14.4 Hz
H8
H10
H9
H7β
δ 1.45
14.4 Hz
5.9 Hz
8-CH$_3$
H
OBn
9
Me
Me
OH
O
OBn
Me
O
1
O
13
Et
HO
Me
Br
H7β
H7α
H4β
H4α
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

1D TOCSY

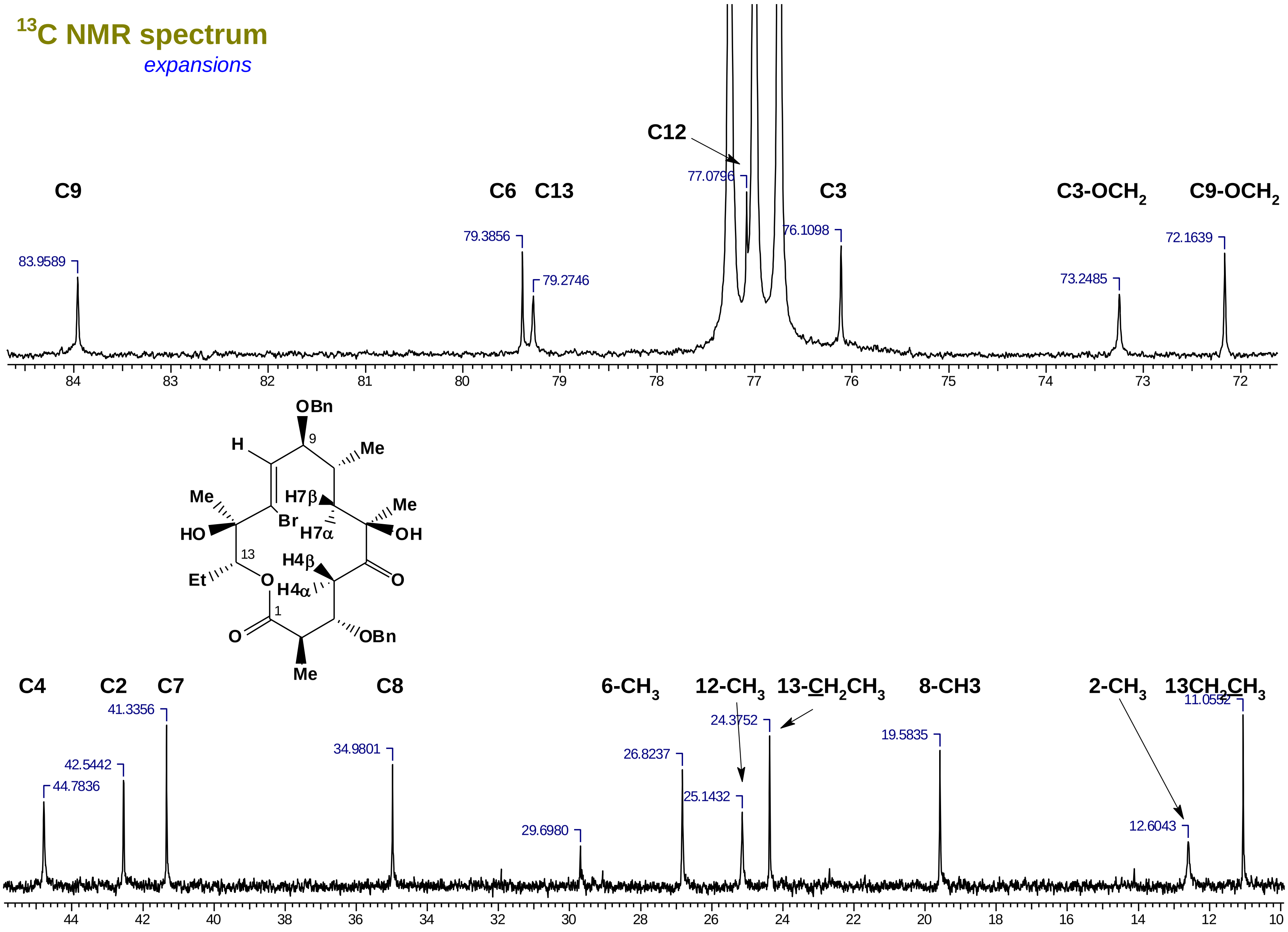

H3
H2
4-CH$_2$
2-CH$_3$
OBn
9
H
Me
Me
OH
HO
Me
Et
13
O
1
O
Me
OBn
O
Br
H7β
H7α
H4β
H4α
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

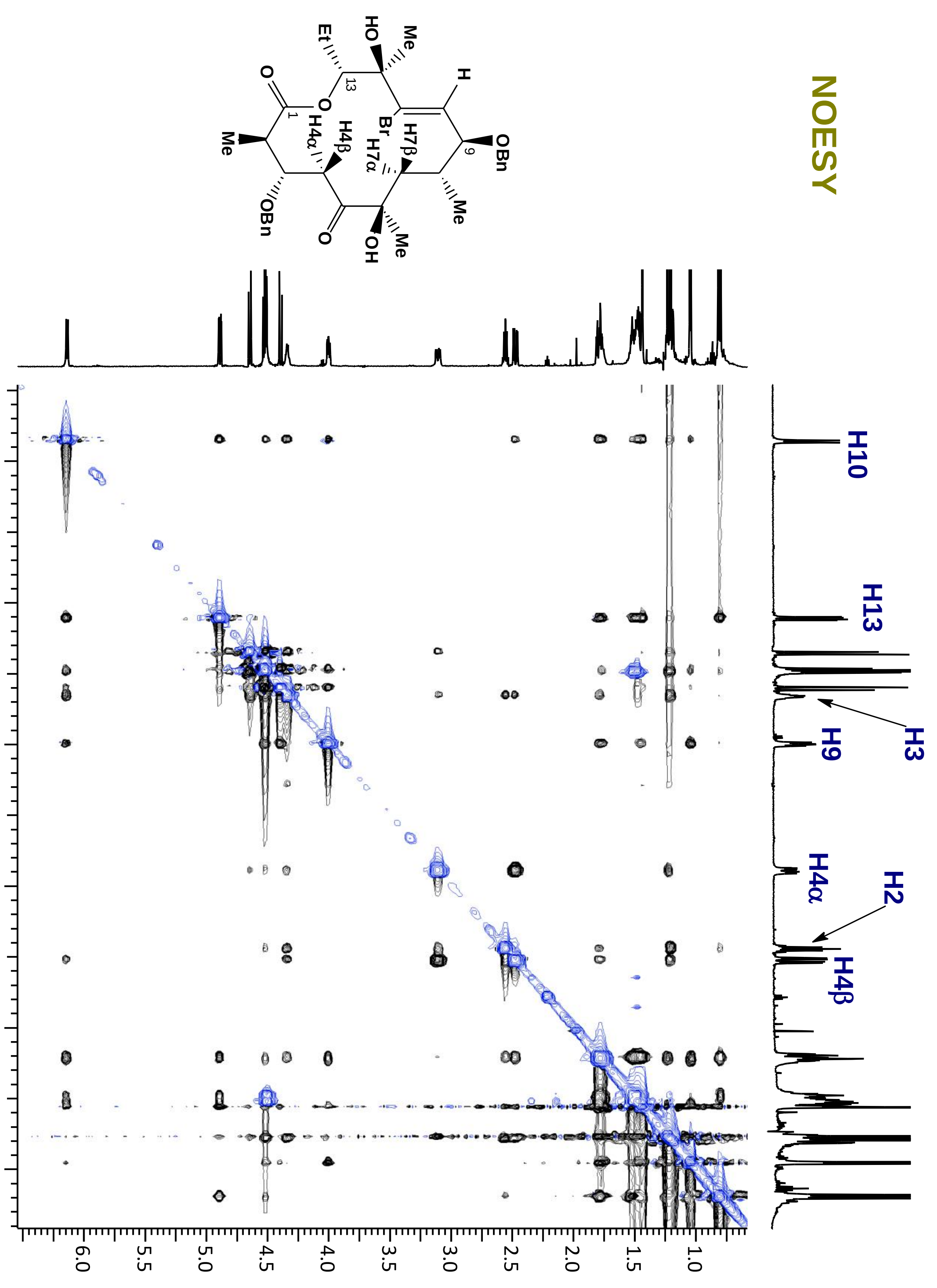
^{13}C NMR spectrum

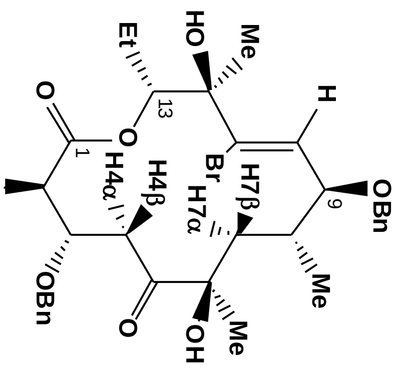


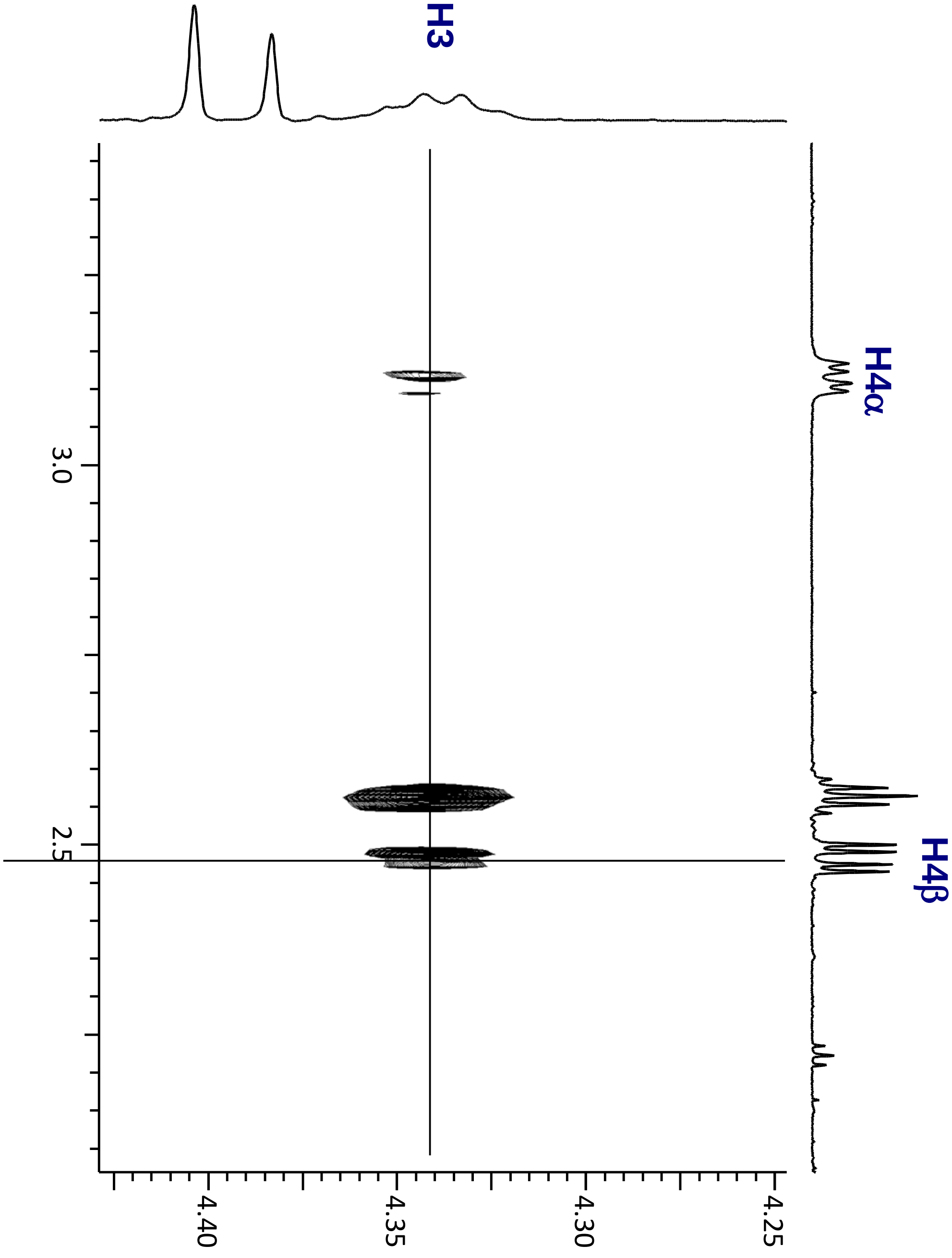
^{13}C NMR spectrum

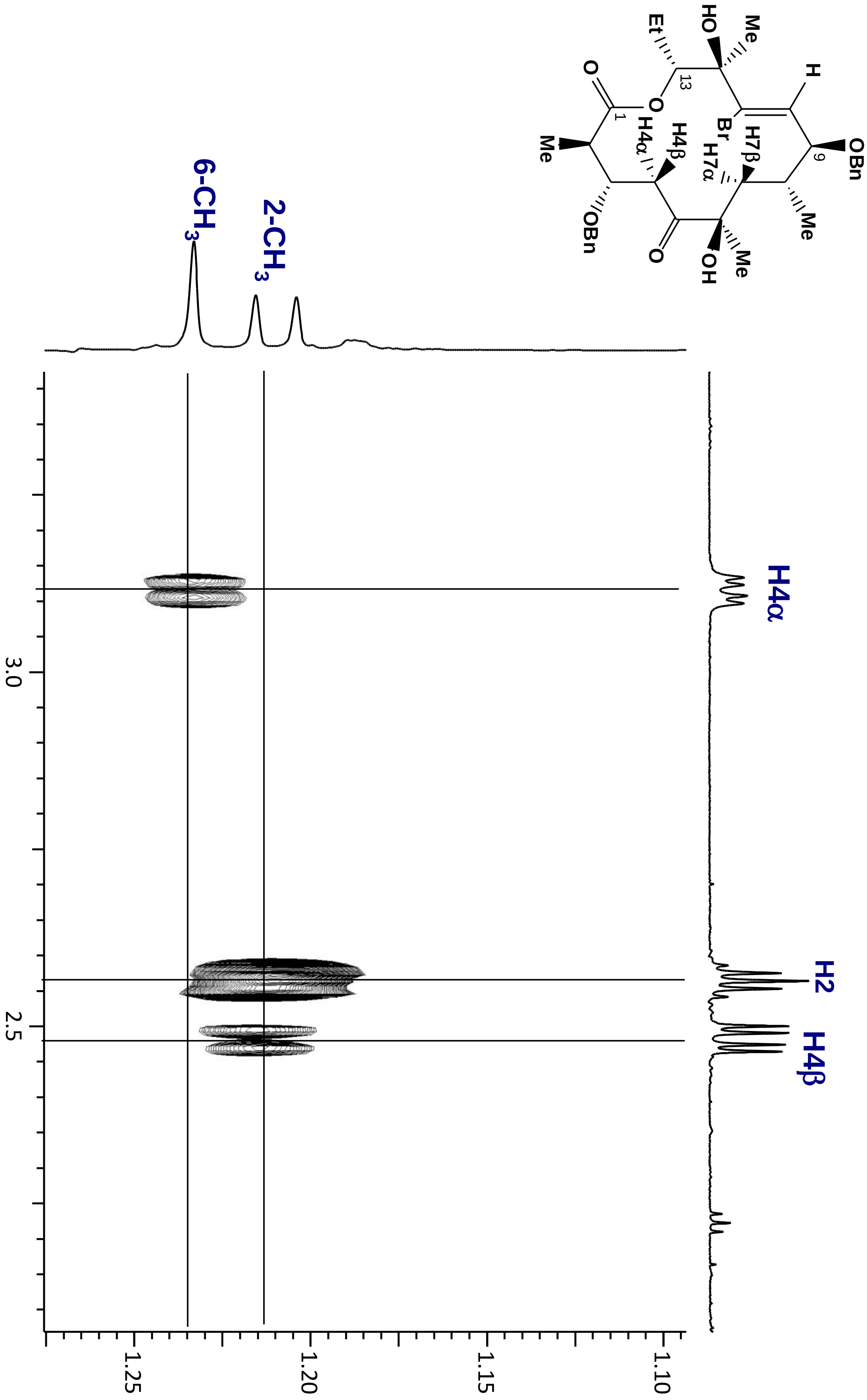
expansions

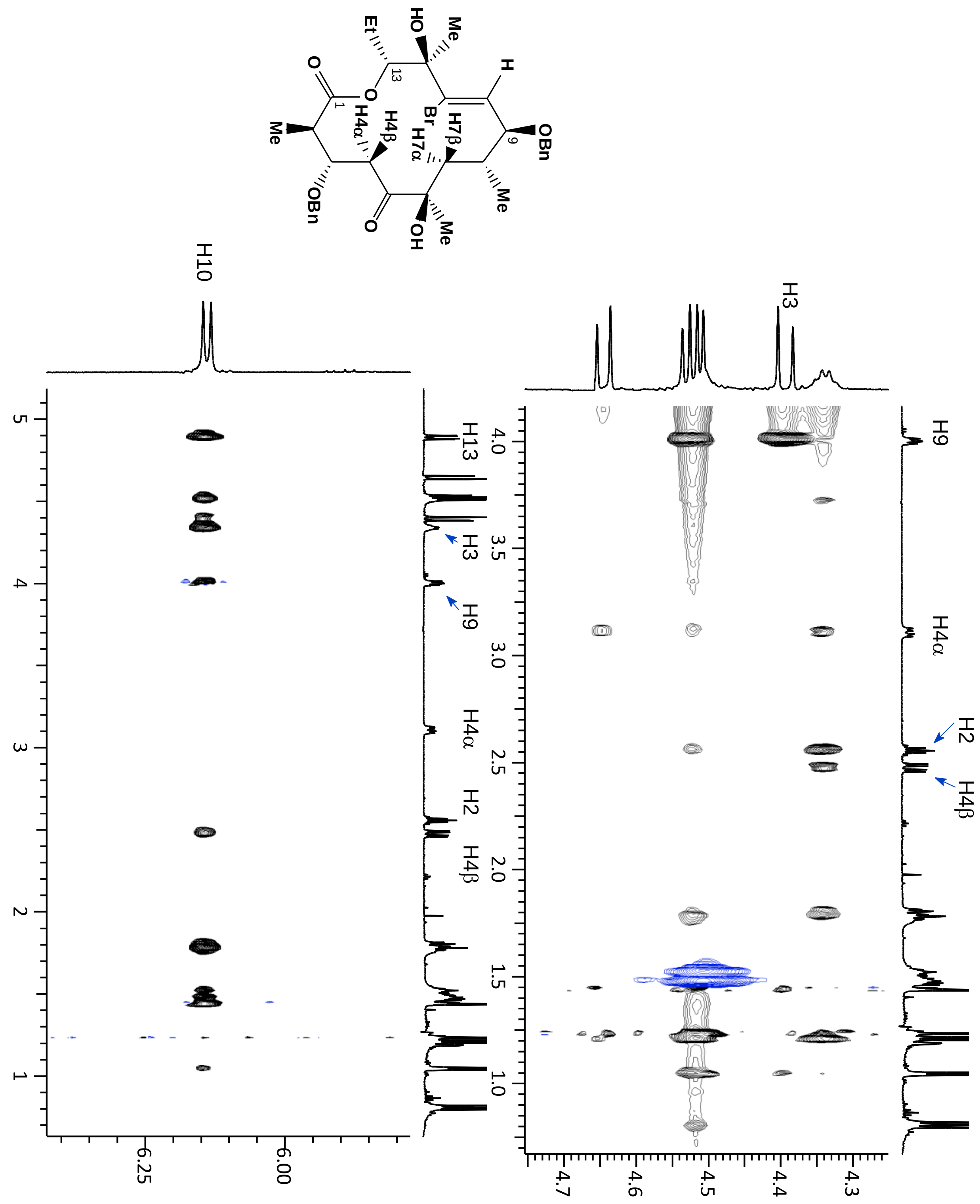


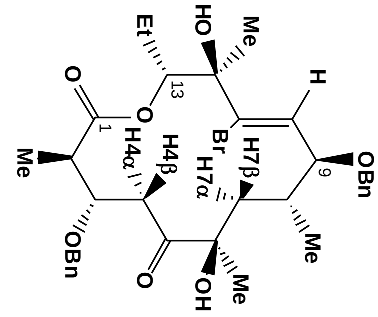

NOESY
Me
HO
Et
O
13
O
1
Me
H
Br
H7β
H7α
H4β
H4α
9
OBn
Me
OBn
O
OH
Me
H10
H13
H3
H9
H4α
H2
H4β
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
6
5
4
3
2
1


OBn
9
H
Me
HO
Et
13
O
O
1
Me
Br
H7β
H7α
H4β
H4α
Me
OH
O
OBn

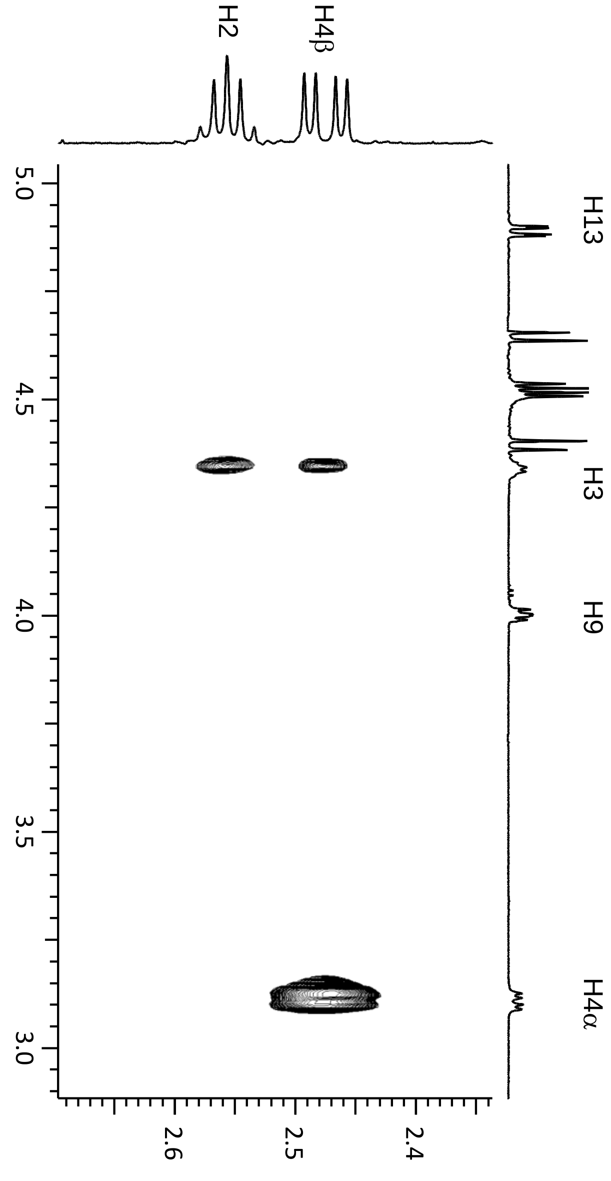

H3
H4α
H4β
3.0
2.5
4.40
4.35
4.30
4.25


Me
HO
Et
13
O
O
1
Me
H
Br
H7β
H7α
H4β
H4α
9
OBn
Me
Me
OH
O
OBn
6-CH$_3$
2-CH$_3$
H4α
H2
H4β
3.0
2.5
1.25
1.20
1.15
1.10

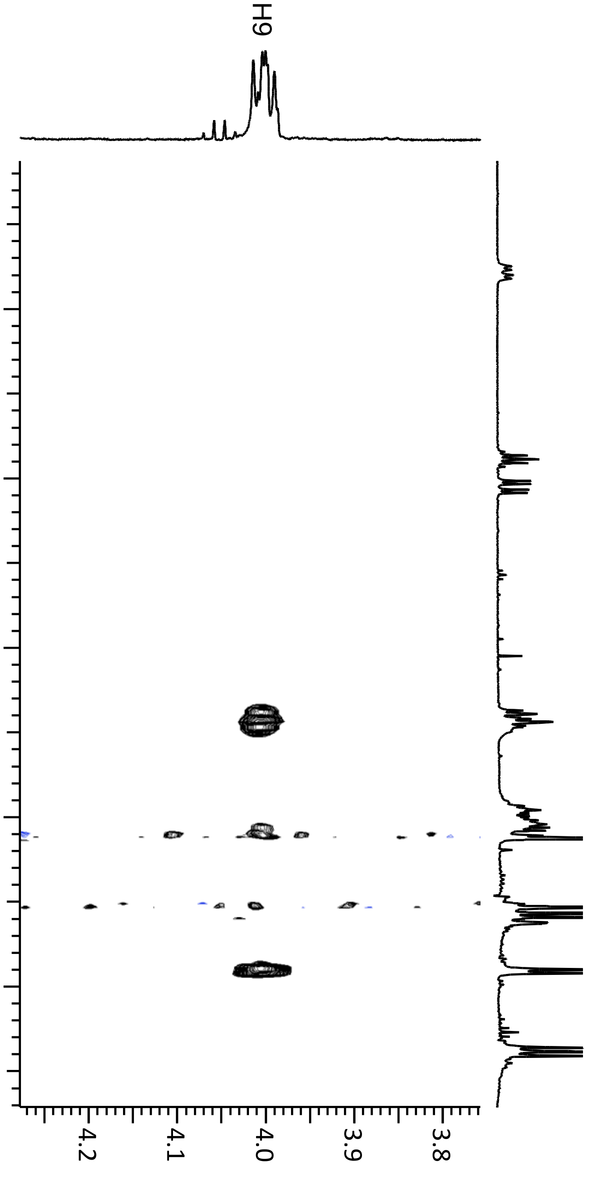

HO
Me
Et
13
O
O
1
H
Br
H7β
H7α
H4β
H4α
9
OBn
Me
Me
OBn
O
OH
Me
H10
H3
H9
H4α
H2
H4β
H13
H3
H9
H4α
H2
H4β
4.0
3.5
3.0
2.5
2.0
1.5
1.0
4.7
4.6
4.5
4.4
4.3
5
4
3
2
1
6.25
6.00



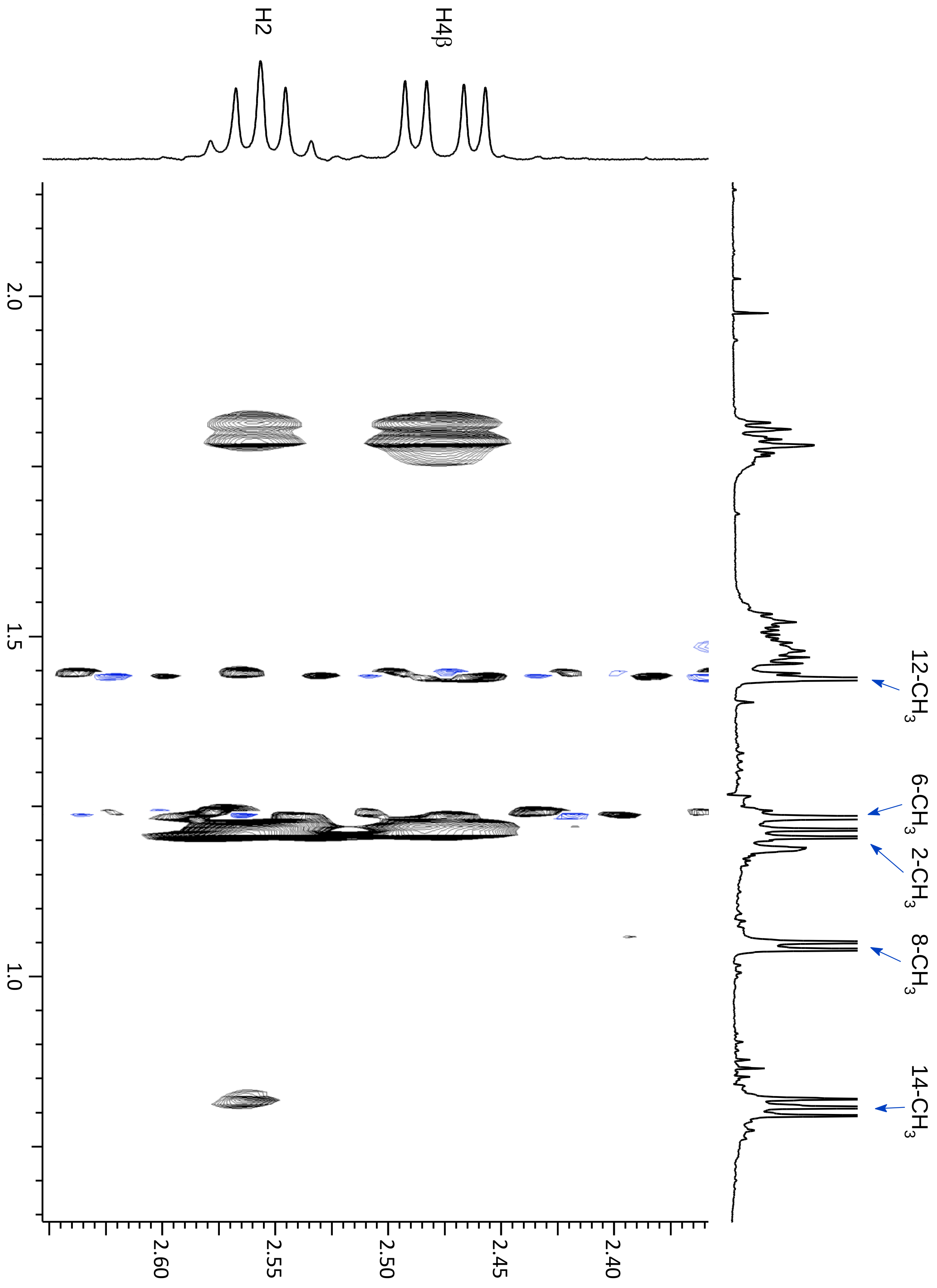
Me
HO
Et
H
O
13
O
1
Br
H7β
H7α
H4β
H4α
9
OBn
Me
Me
OBn
Me
OH
O

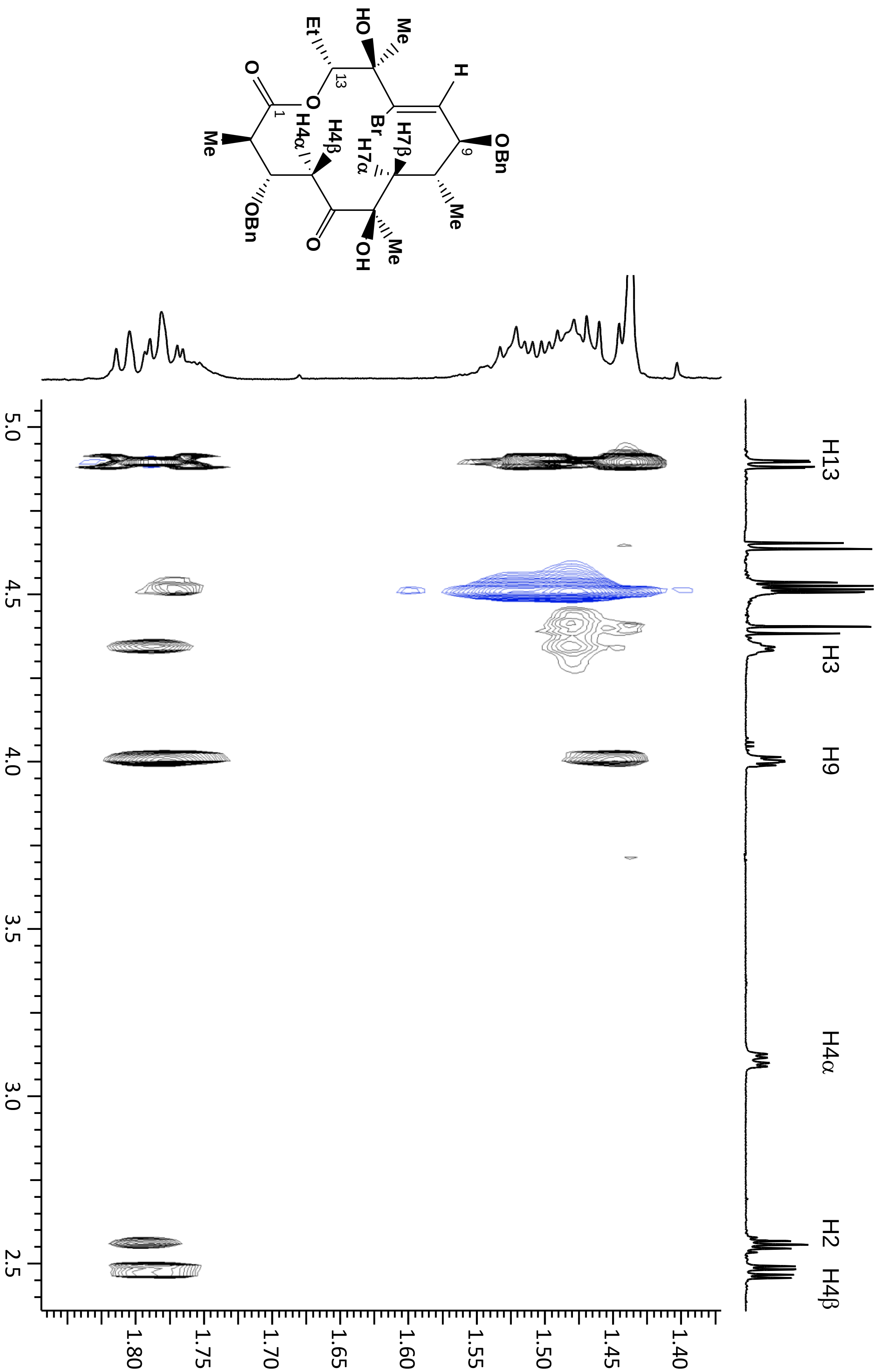


H2
H4β
H13
H3
H9
H4α
5.0
4.5
4.0
3.5
3.0
2.6
2.5
2.4

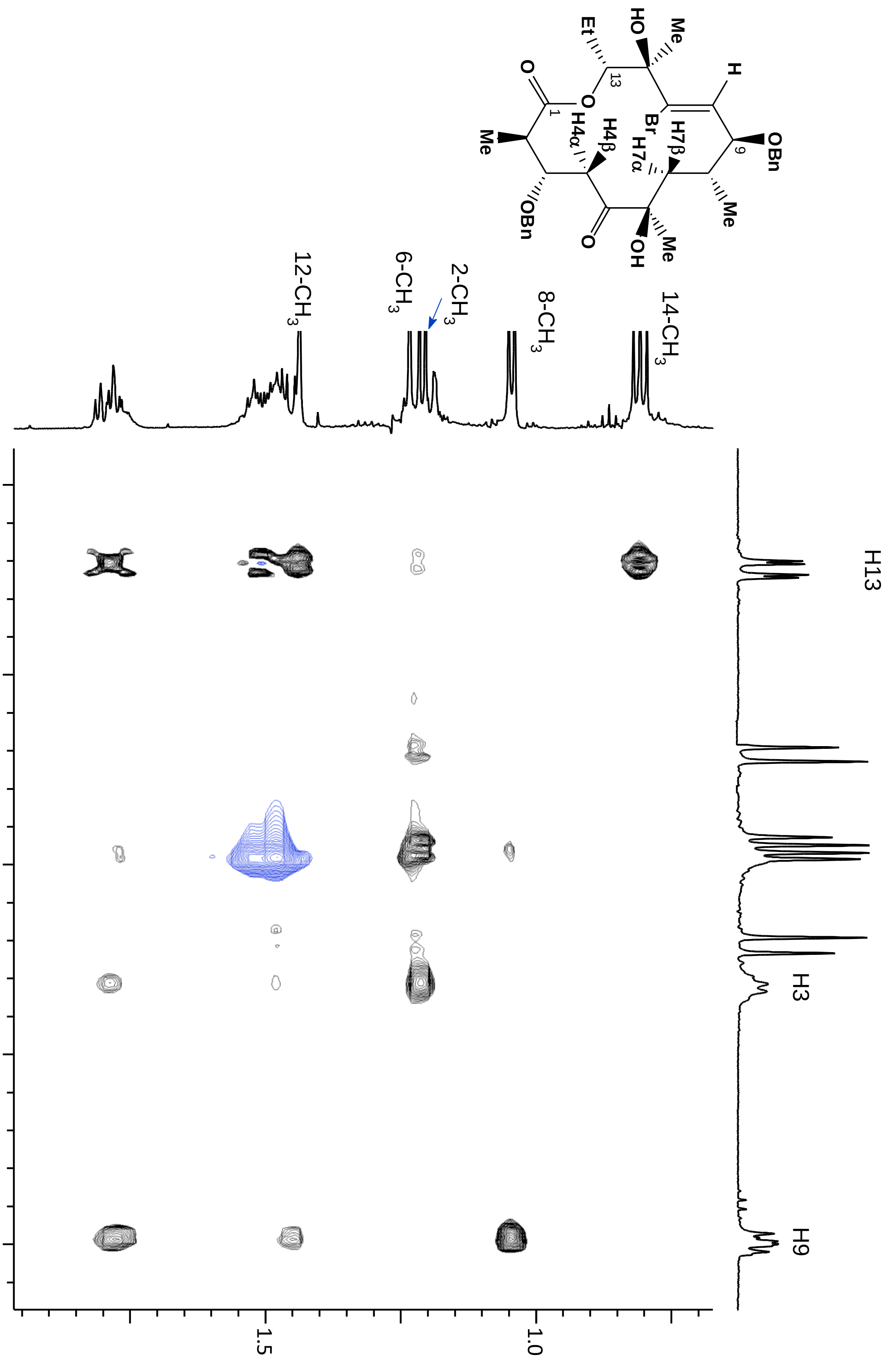


H9
3.0
2.5
2.0
1.5
1.0
4.2
4.1
4.0
3.9
3.8

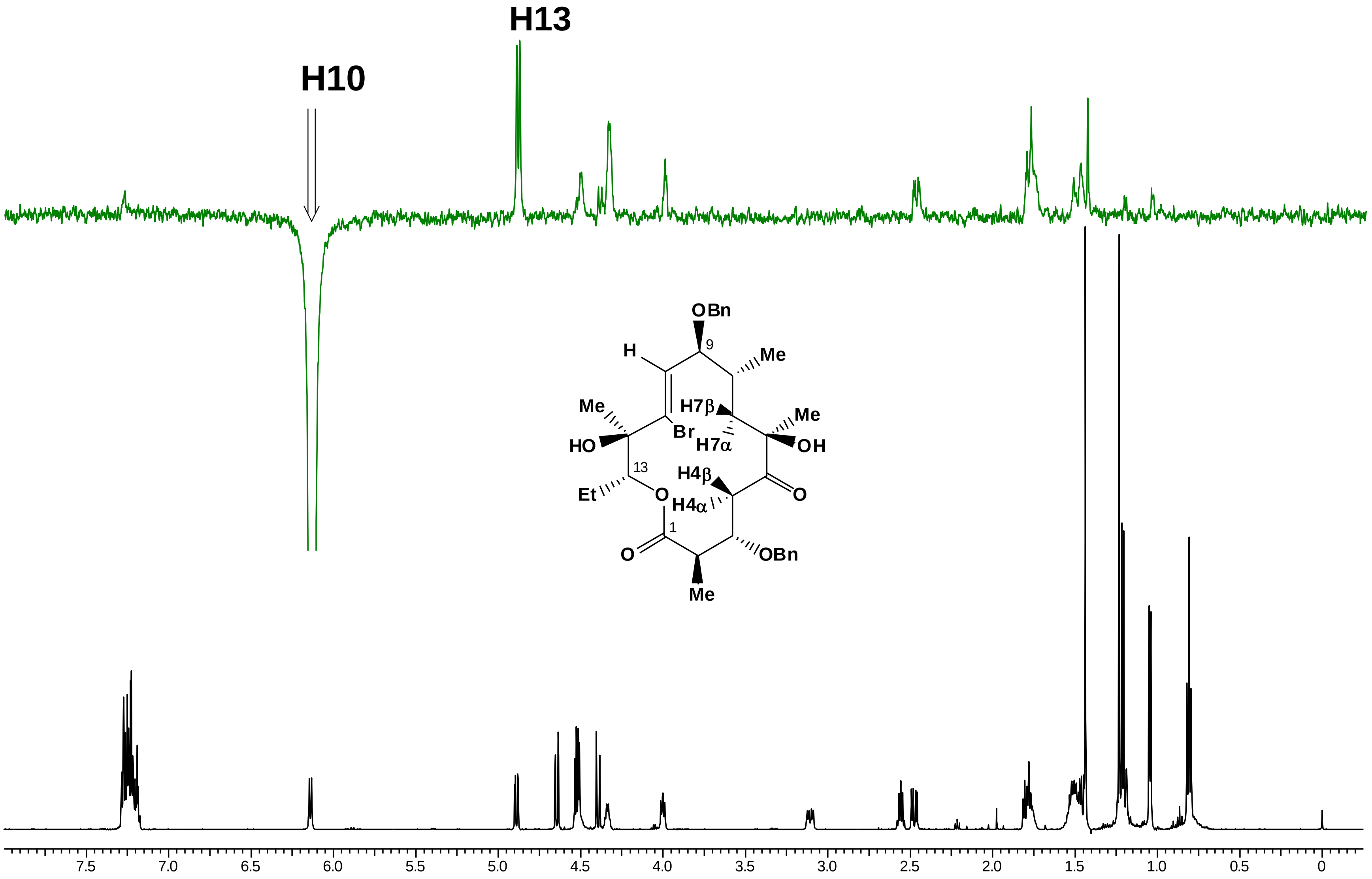

H2
H4β
12-CH_3
6-CH_3
2-CH_3
8-CH_3
14-CH_3
2.0
1.5
1.0
2.60
2.55
2.50
2.45
2.40

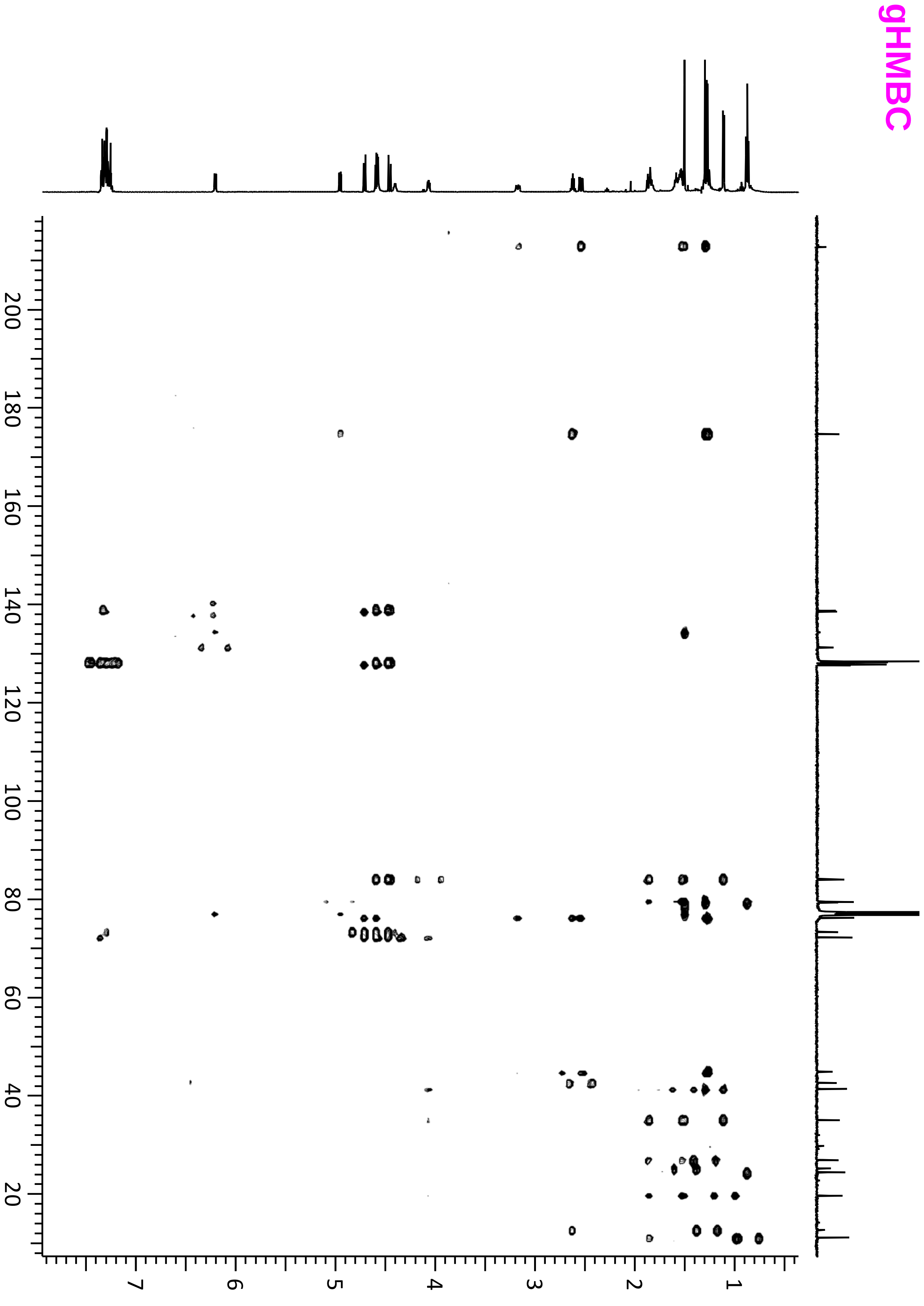


H
OBn
9
Me
Me
OH
O
HO
Me
Et
13
O
1
O
Me
OBn
H7β
H7α
Br
H4β
H4α
H13
H3
H9
H4α
H2
H4β
5.0
4.5
4.0
3.5
3.0
2.5
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40

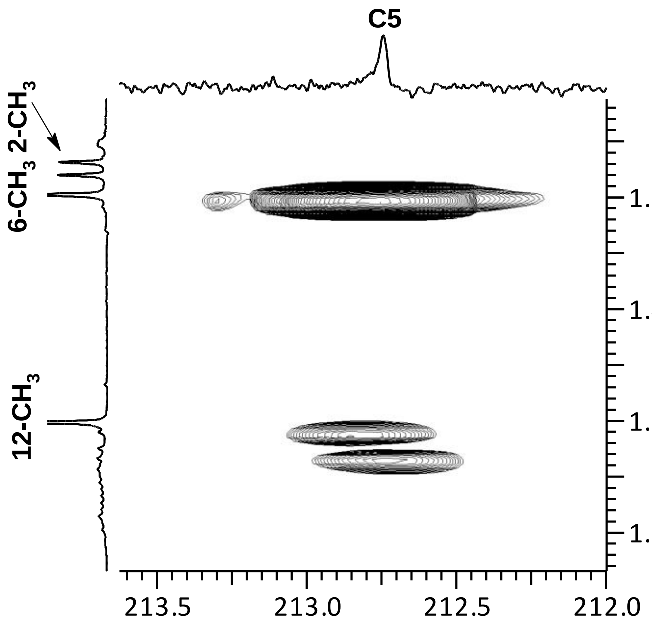

Et
HO
Me
H
OBn
9
13
O
1
Br
H7β
H7α
H4β
H4α
O
Me
OBn
O
OH
Me
Me
12-CH3
6-CH3
2-CH3
8-CH3
14-CH3
H13
H3
H9
5.00
4.75
4.50
4.25
4.00
1.5
1.0

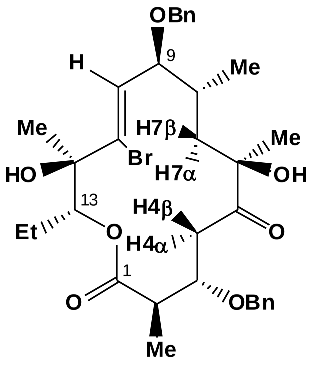
DPFGSENOE
subspectrum

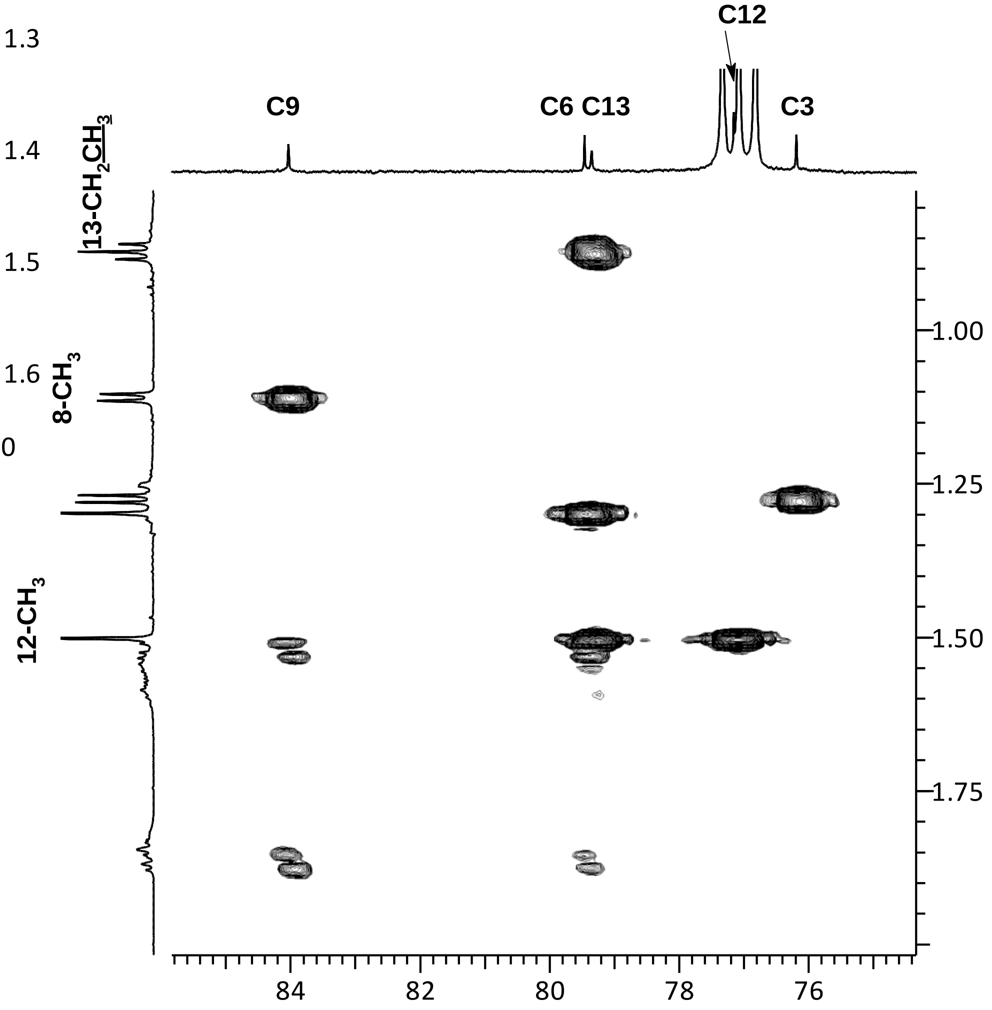


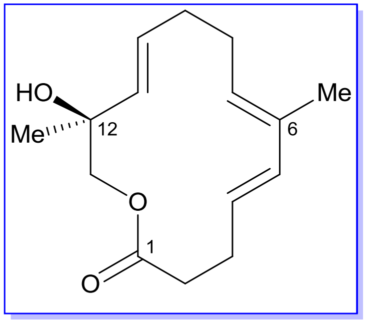

gHMBC

gHMBC expansions



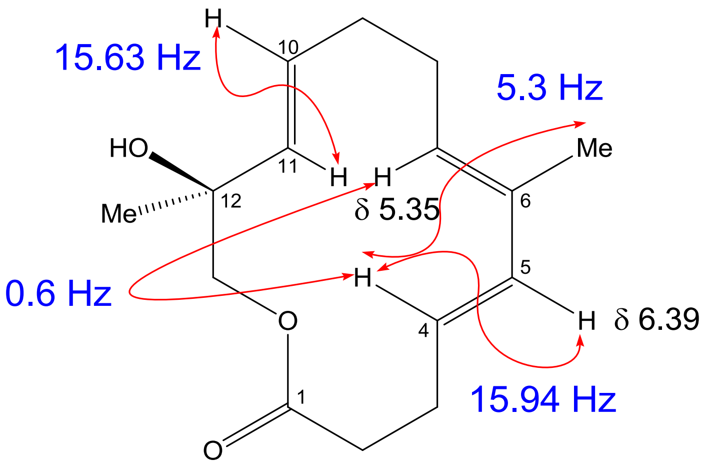

OBn
9
H
Me
H7β
Me
Me
HO
Br
H7α
OH
13
H4β
Et
O
H4α
O
1
O
OBn
Me



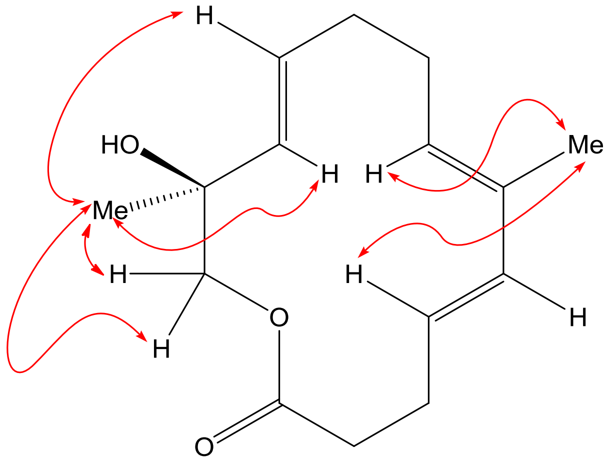


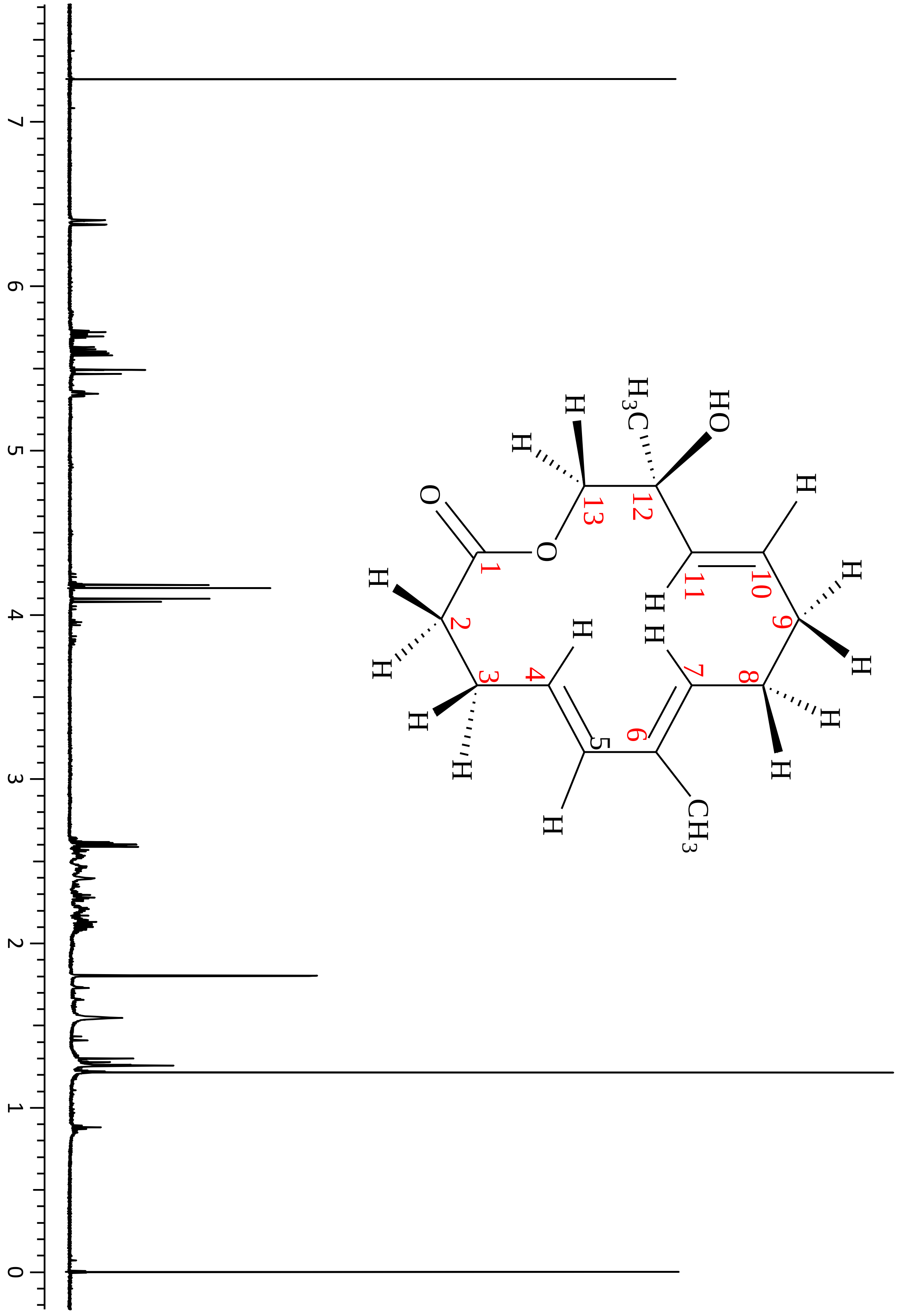
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)	**13C NMR chemical shift (δ/ppm)**
2.64 - 2.55 (m, **H2**)	173.45 ----- C1
2.55 - 2.41 (m, **H3**)	32.33 ----- C2
5.69 (dtd, $J_{H4,\ H5}$ = 15.94Hz, $J_{H4,\ 3\text{-}CH2}$ = 5.3Hz, $J_{H4,\ H7}$ = 0.6Hz, **H4**)	26.96 ----- C3
	126.70 ----- C4
6.39 (d= $J_{H5,\ H4}$ = 15.94Hz, H5)	127.62 ----- C5
1.80 (s, **6-CH$_3$**)	133.28 ----- C6
5.35 (t, $J_{H7,H8}$ = 8.64Hz, **H7**)	127.40 ----- C7
2.33 - 2.17 (m, **H8**)	27.62 ----- C8
2.17 - 2.04 (m, **H9**)	31.77 ----- C9
5.59 (ddd, $J_{H10,\ H11}$ = 15.63Hz, $J_{H10,\ H9}$ = 7.03Hz, **H10**)	129.37 ----- C10
1.21 (s, **12-CH$_3$**)	134.02 ----- C11
4.16 (d, ^2J = 11.3 Hz, **H13**)	25.59 ----- C12
4.08 (d, ^2J = 11.3 Hz, **H13**)	70.76 ----- C13
	20.34 ----- 6-CH$_3$
	25.59 ----- 12-CH$_3$

Coupling constant


15.63 Hz
H
10
HO
11
H
Me
12
H
δ 5.35
5.3 Hz
Me
6
0.6 Hz
H
5
O
4
H
δ 6.39
1
15.94 Hz
O

NOESY


H
HO
H
H
Me
Me
H
H
H
O
H
H
O


7
6
5
4
3
2
1
0
O
O
HO
H_3C
CH_3
H
1
2
3
4
5
6
7
8
9
10
11
12
13

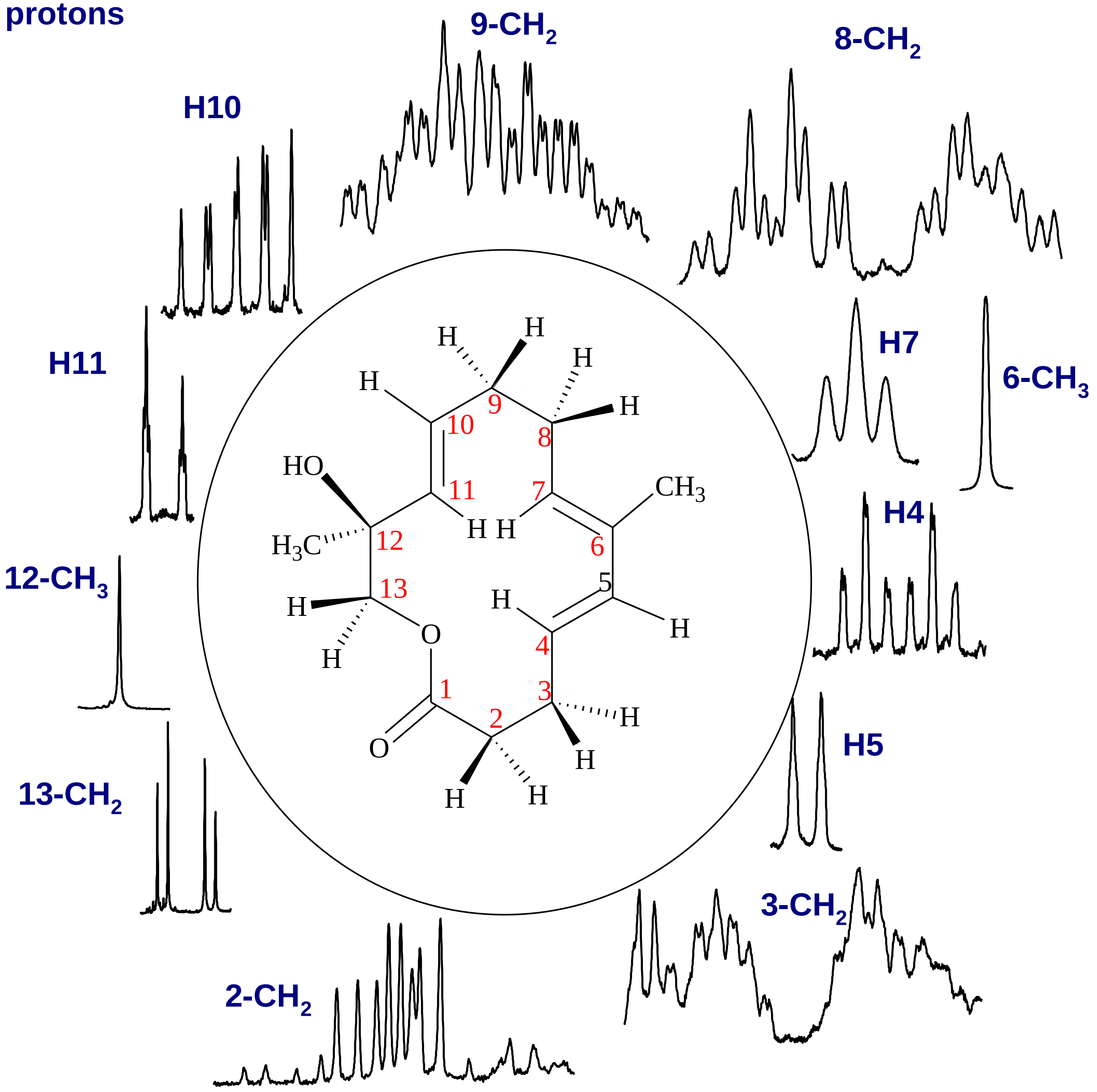
Multiplicity patterns of protons

$^3J(H4,H5) = 15.94$ Hz

$^3J(H4,3\text{-}CH_2) = 5.3$ Hz

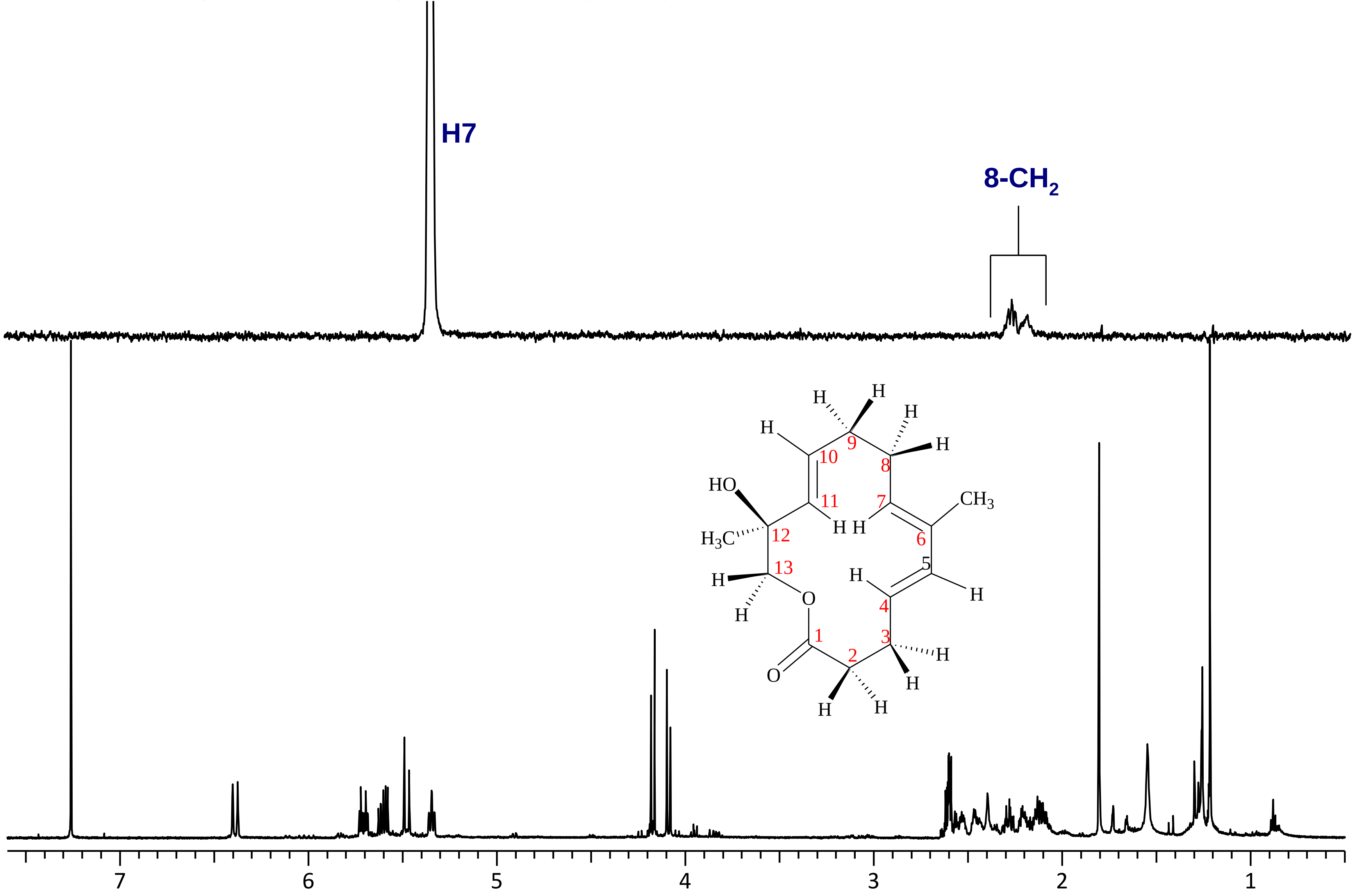
$^5J(H4,H7) = 0.6$ Hz

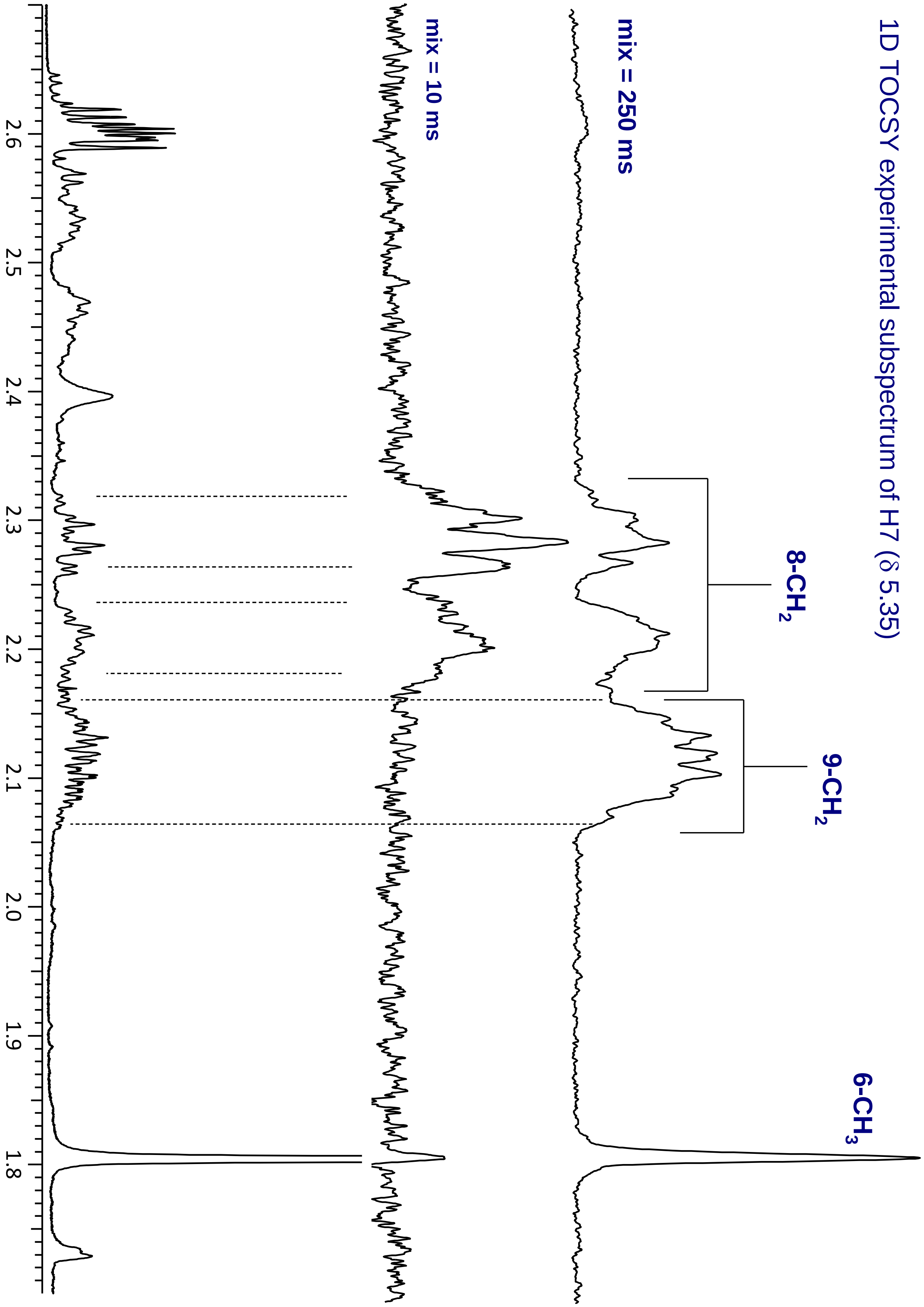
$^3J(H10,H11) = 15.63$ Hz

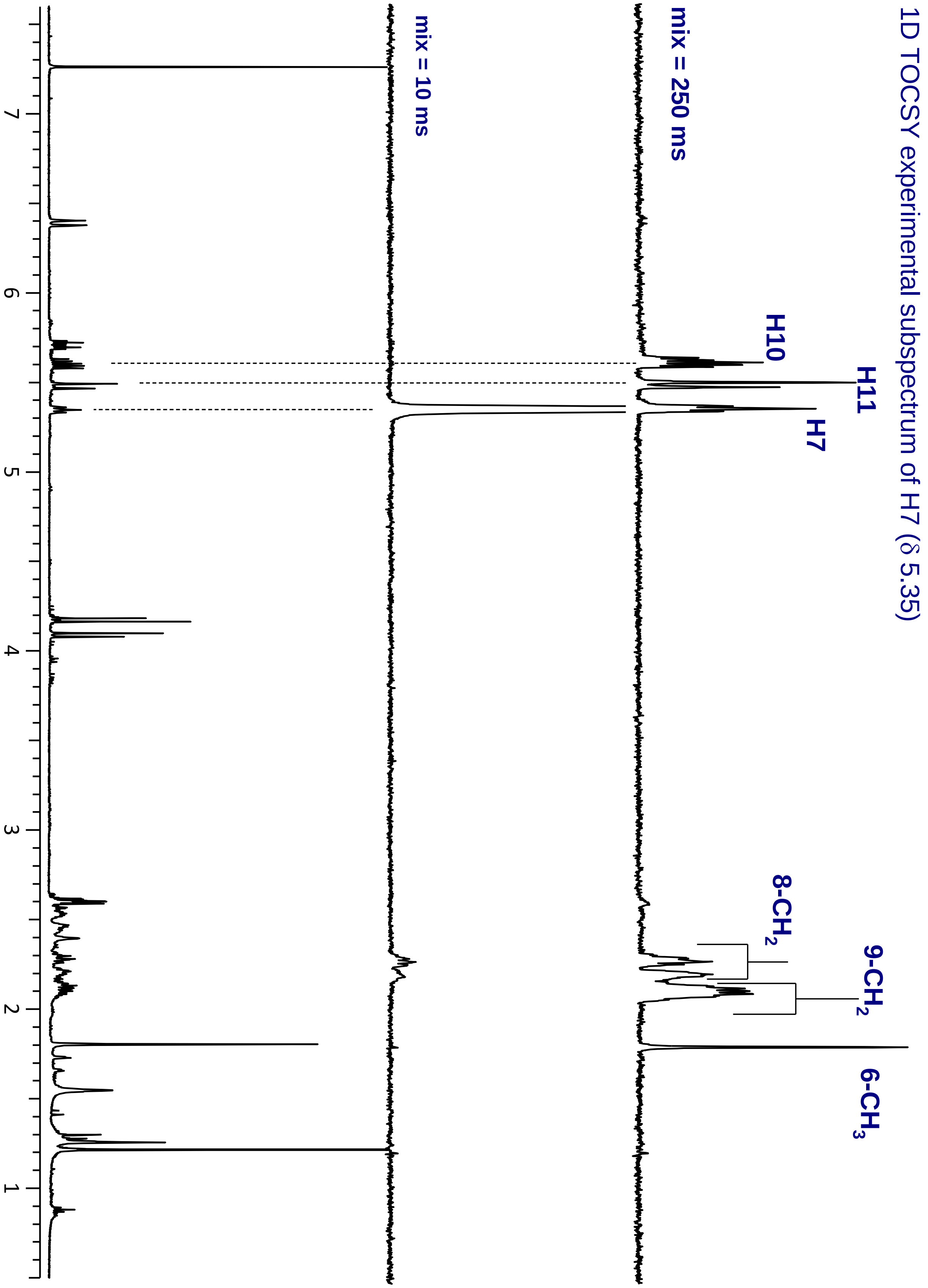


H10
9-CH$_2$
8-CH$_2$
H11
H7
6-CH$_3$
12-CH$_3$
H4
13-CH$_2$
H5
3-CH$_2$
2-CH$_2$
HO
H$_3$C
CH$_3$
O
1
2
3
4
5
6
7
8
9
10
11
12
13
H

1D TOCSY experimental subspectrum of H7 (δ 5.35)

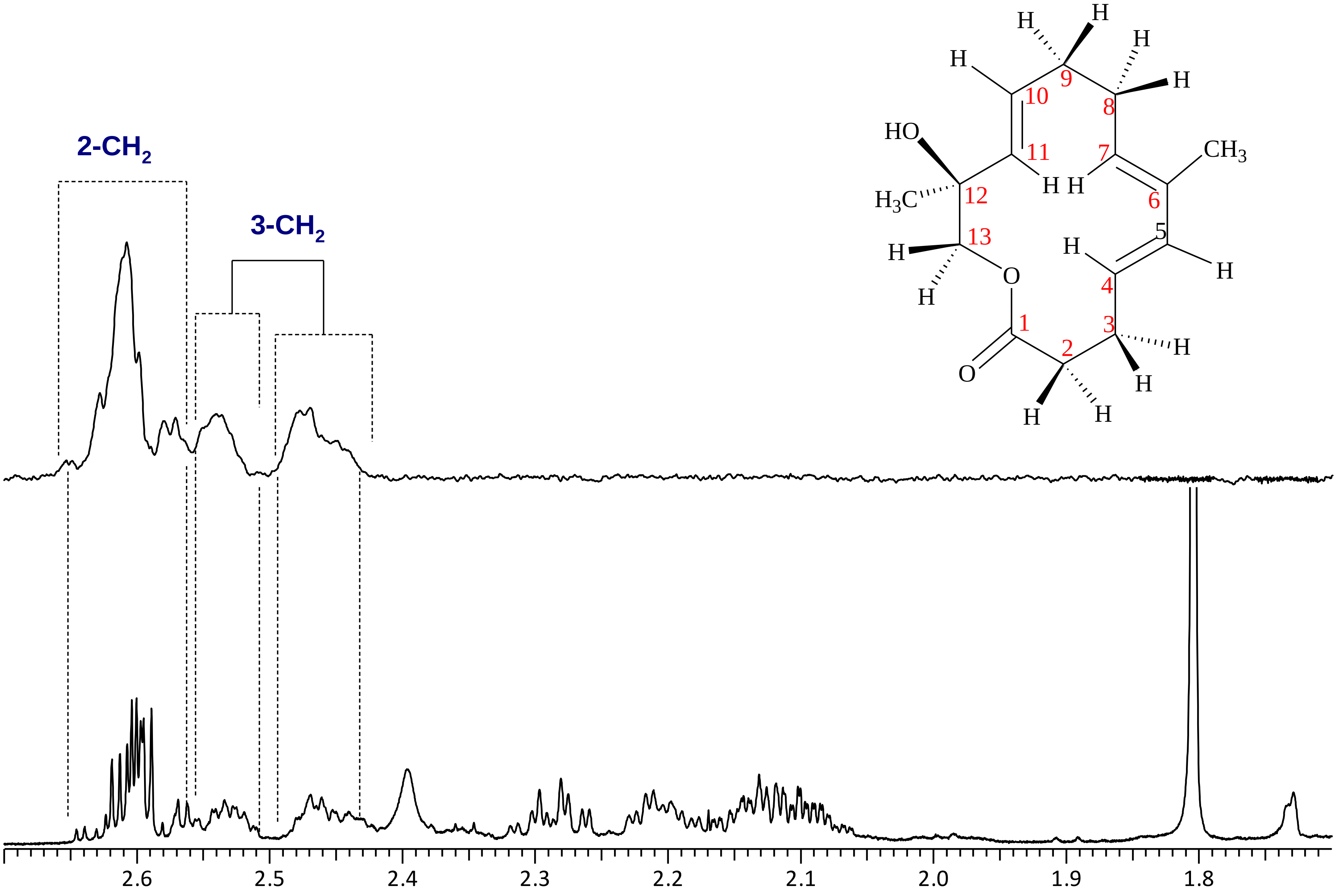



1D TOCSY experimental subspectrum of H7 (δ 5.35)
mix = 250 ms
mix = 10 ms
8-CH$_2$
9-CH$_2$
6-CH$_3$
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8

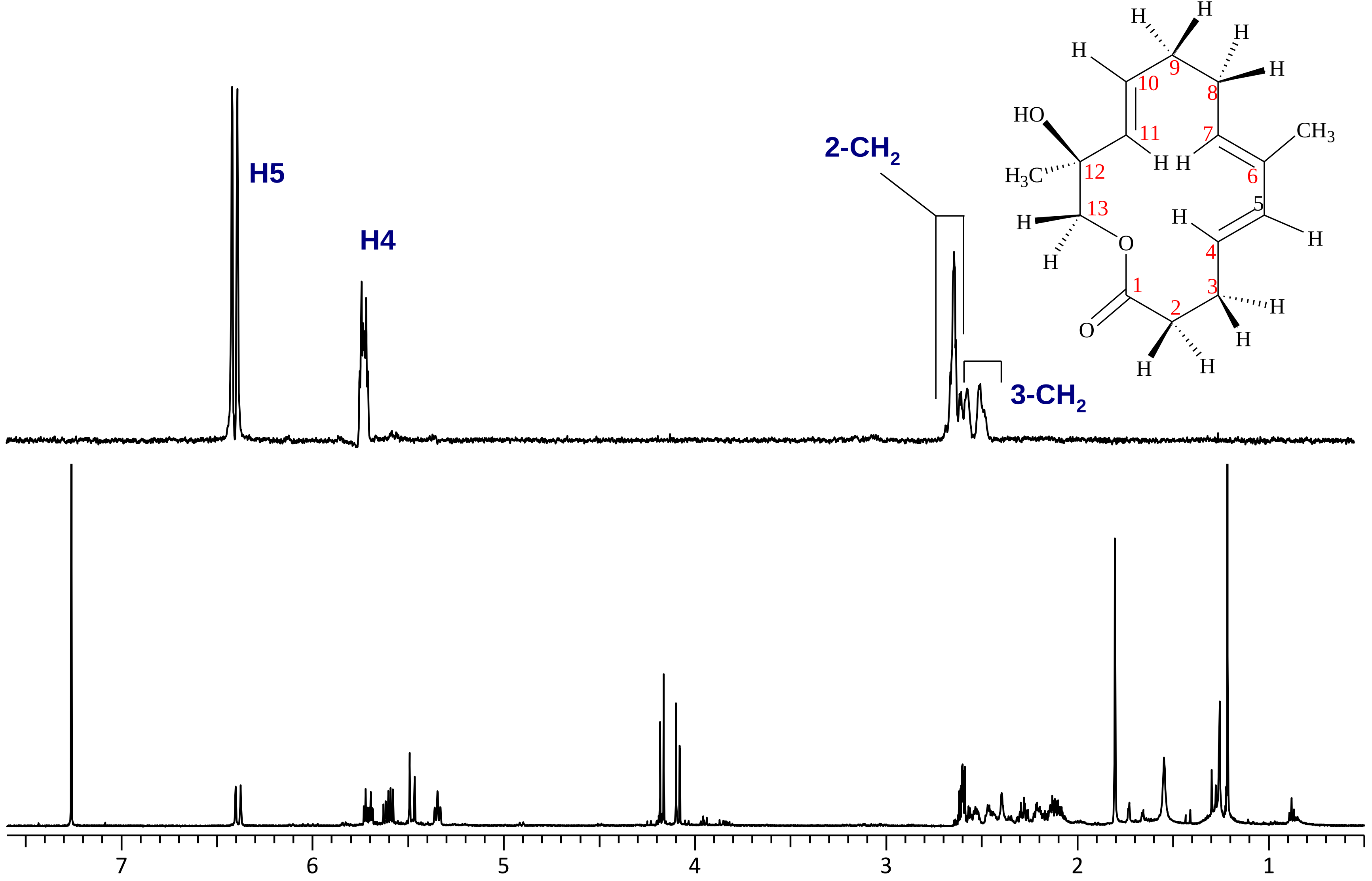


1D TOCSY experimental subspectrum of H7 (δ 5.35)

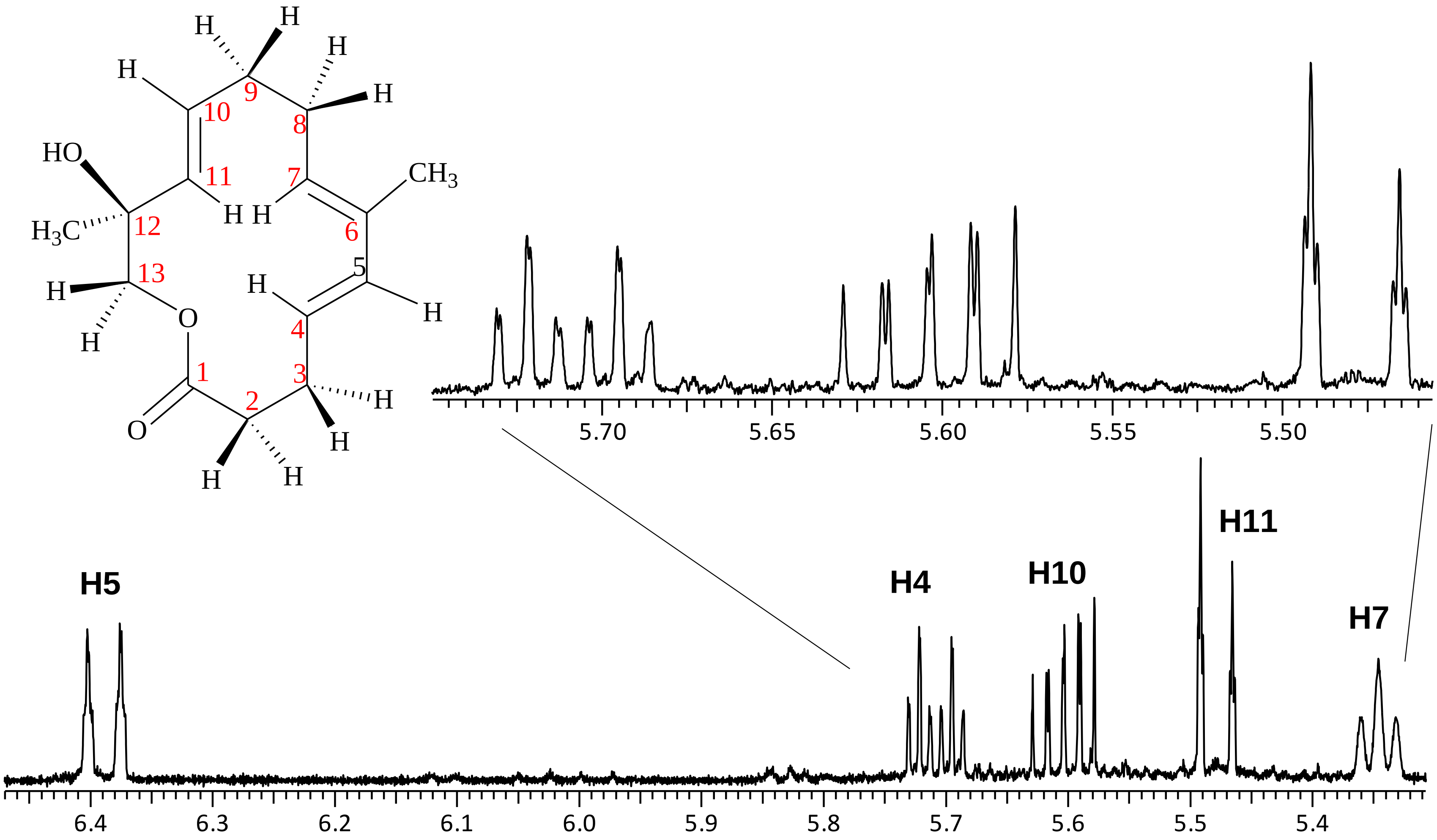
1D TOCSY experimental subspectrum of H5 (δ 6.39)

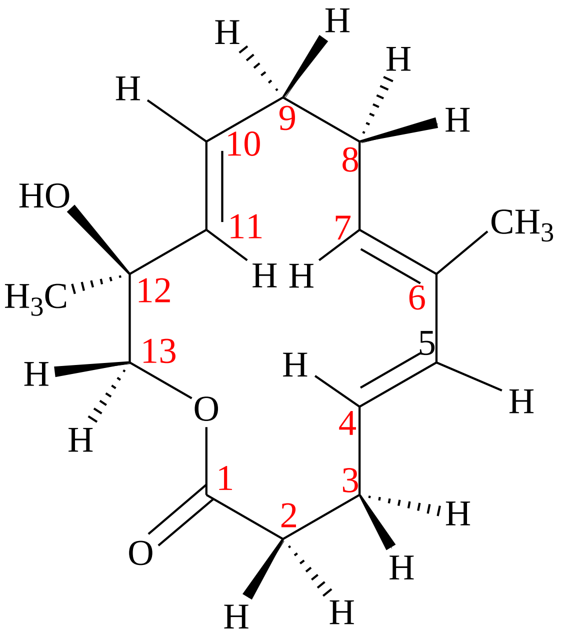


1D TOCSY experimental subspectrum of H5 (δ 6.39)

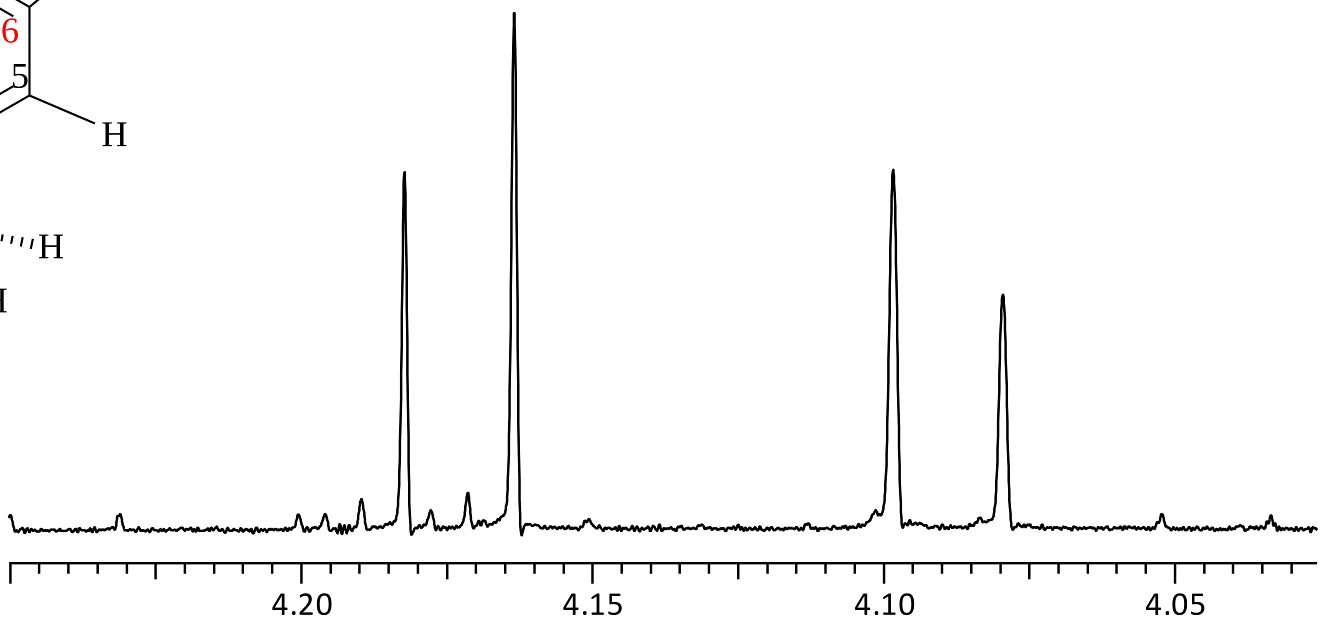


Resolution enhanced ^{1}H NMR spectrum

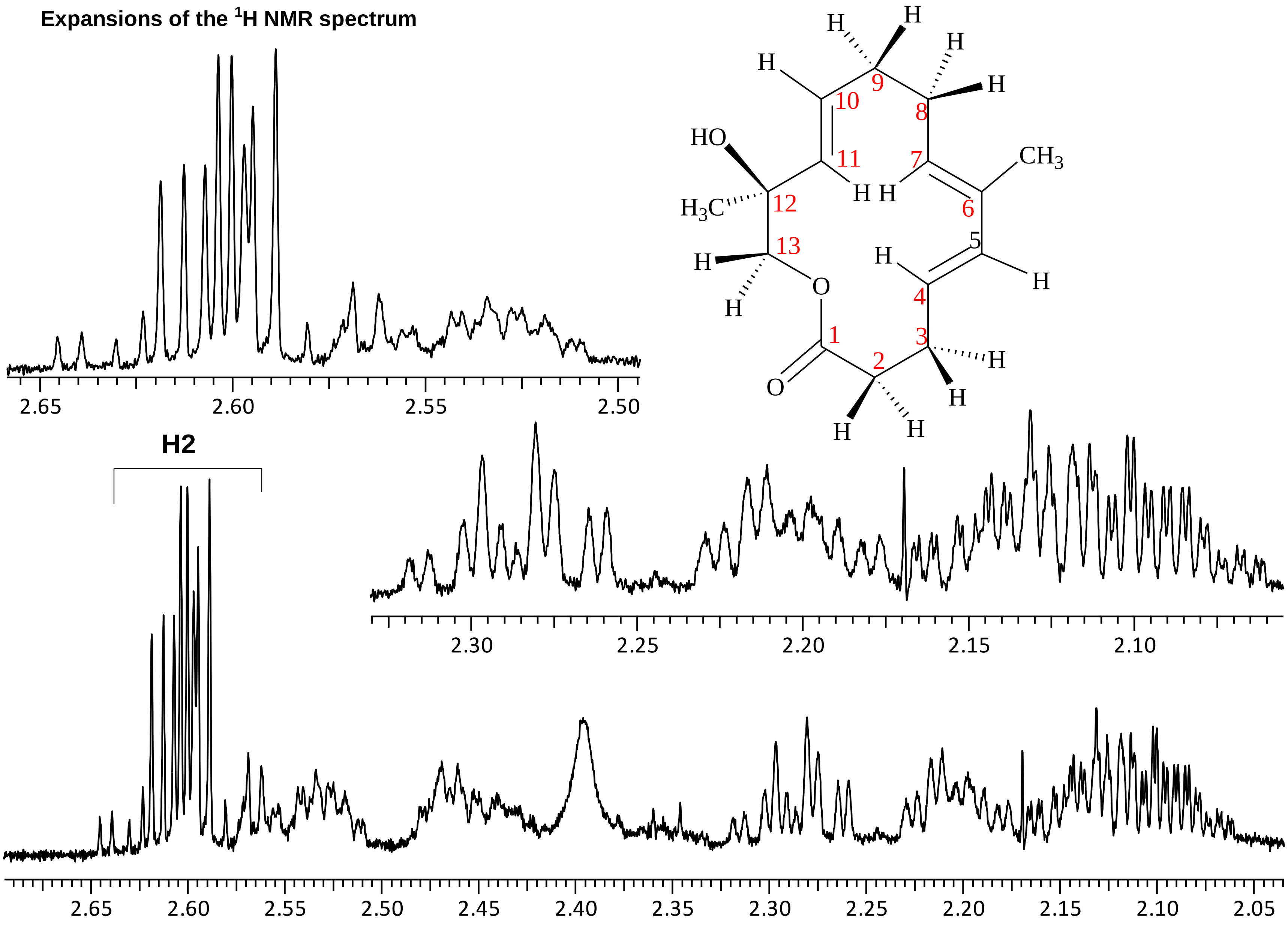


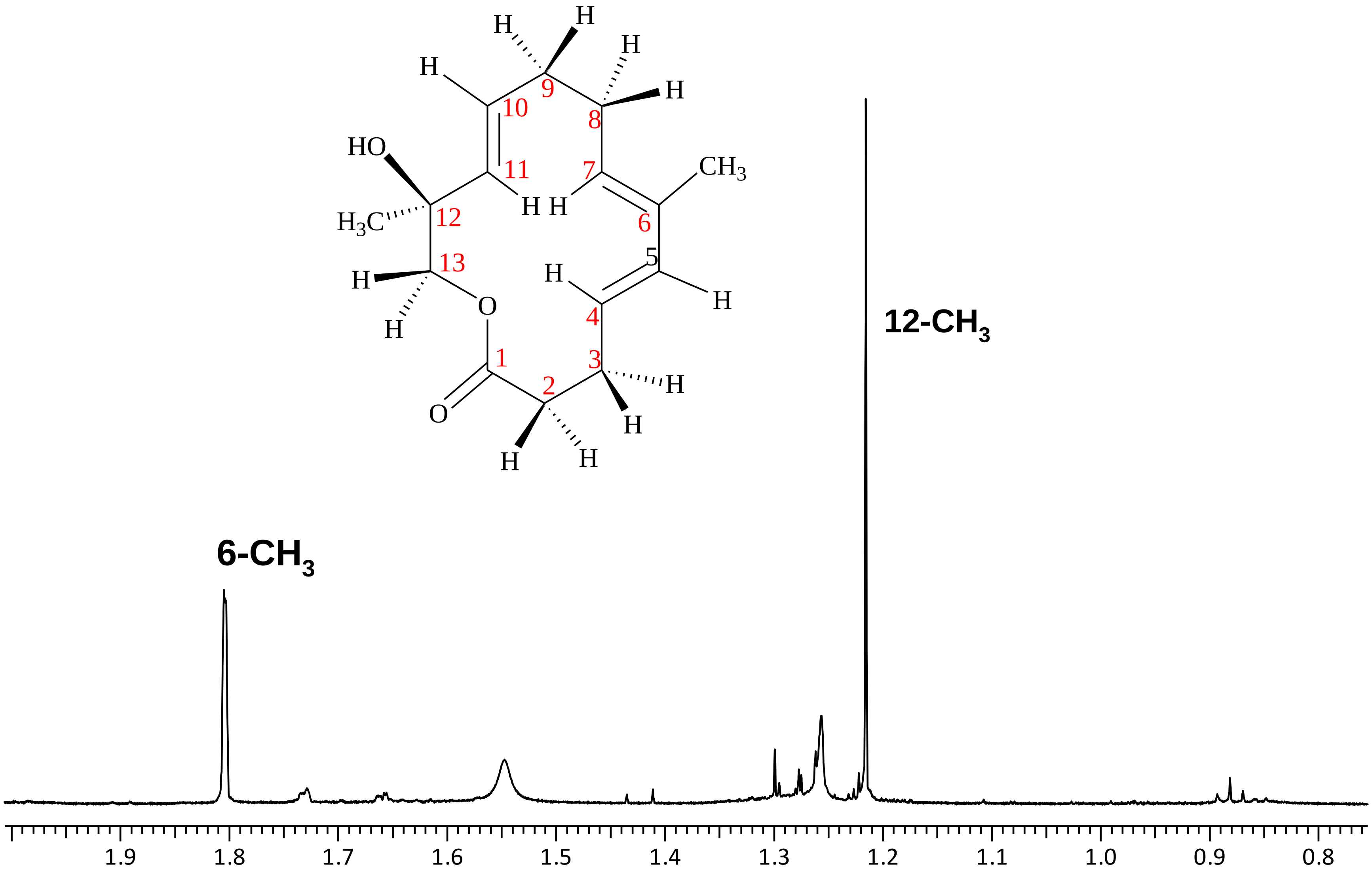

HO
H3C
1
2
3
4
5
6
7
8
9
10
11
12
13
O
CH3

$13\text{-}CH_2$



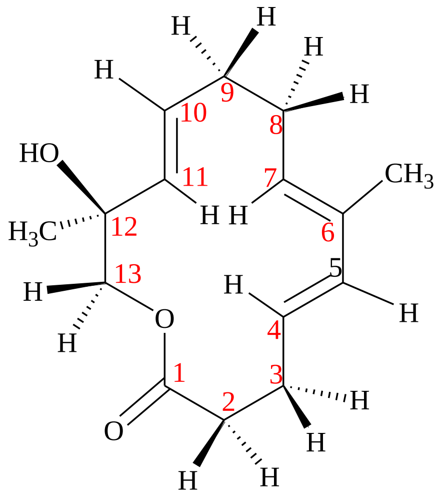
Expansions of the ^{1}H NMR spectrum



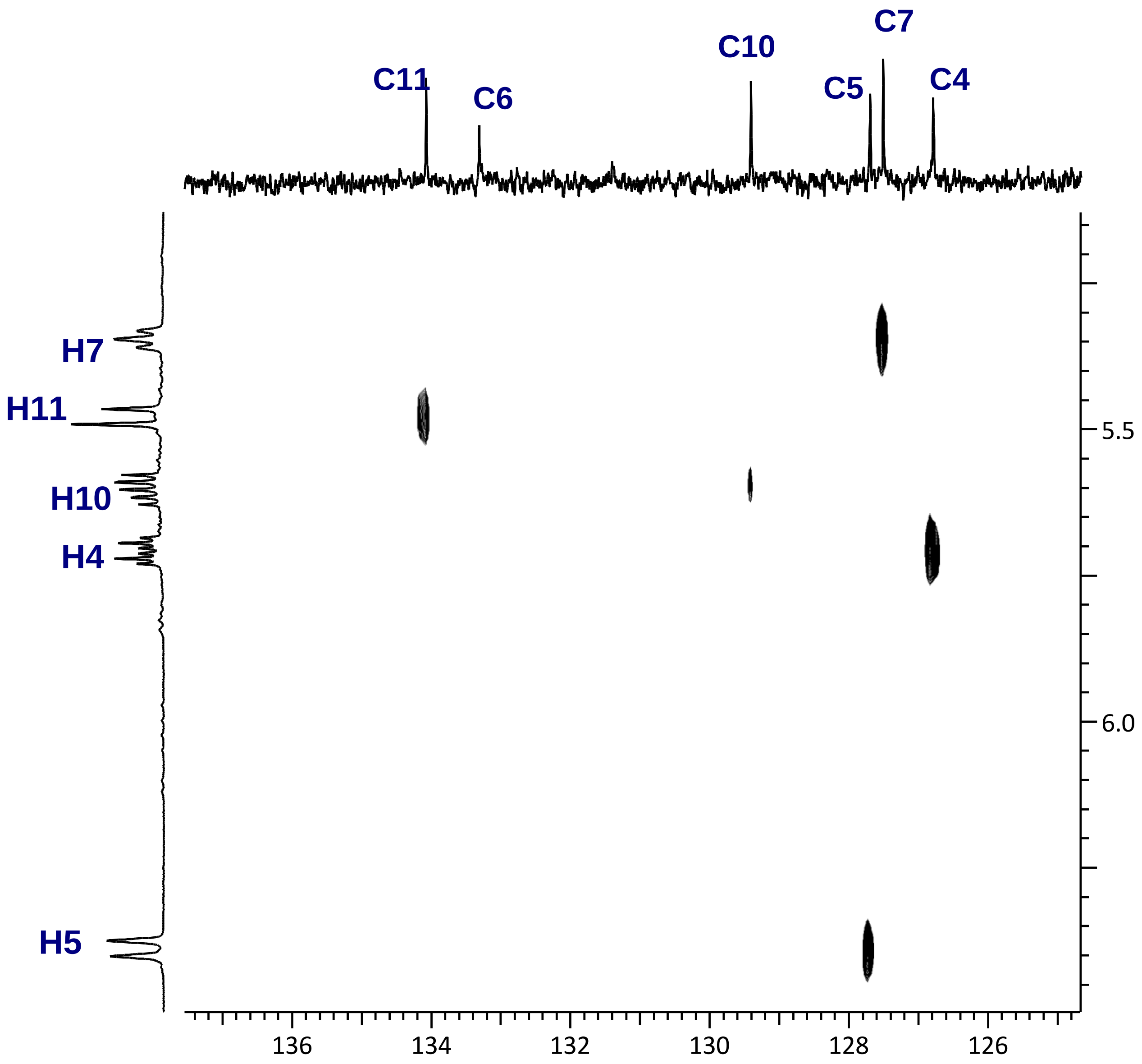

6-CH3
12-CH3
HO
H3C
O
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8

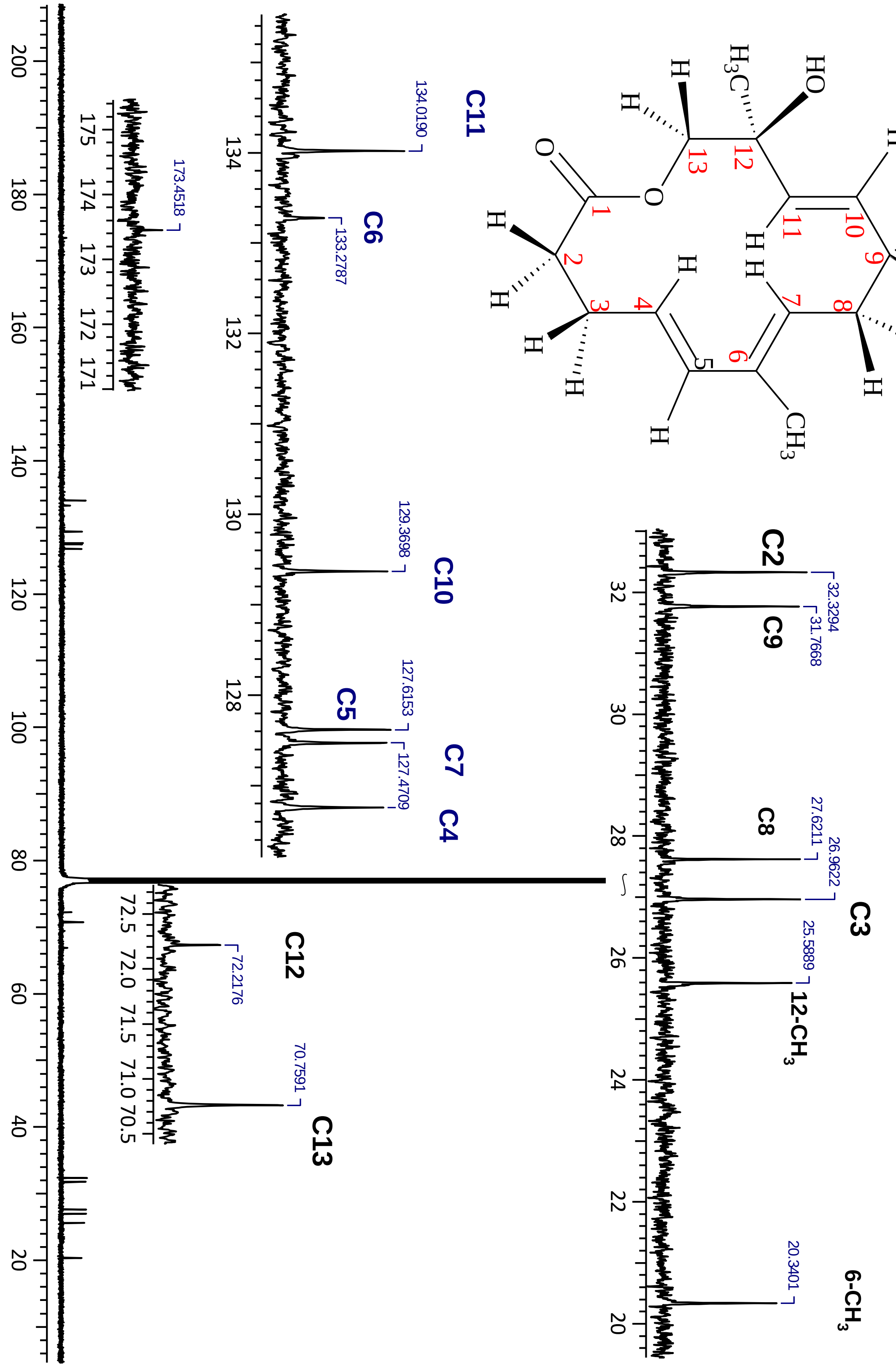
HETCOR

(aromatic region)

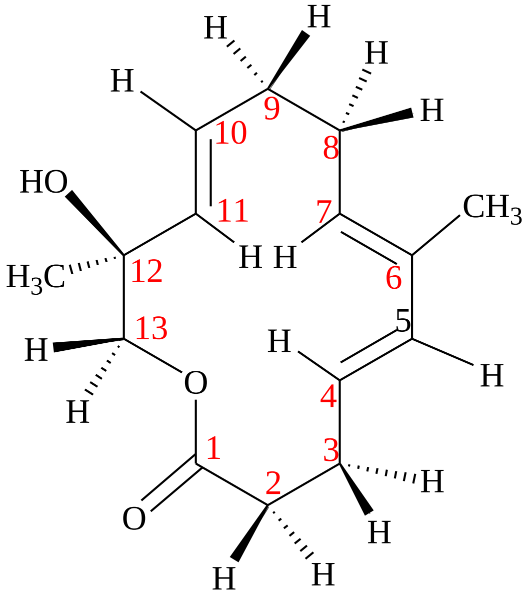


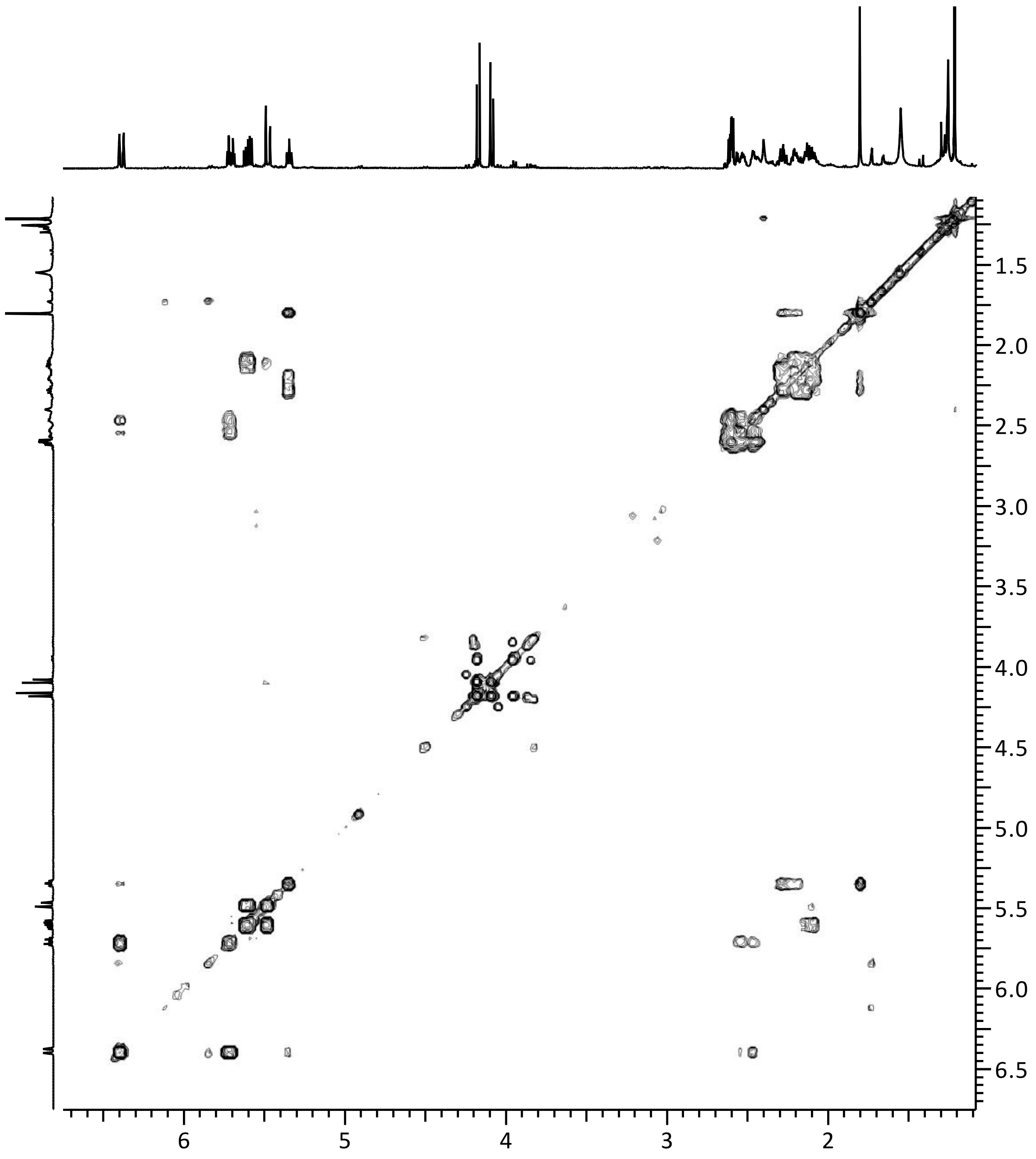
HO
H3C
CH3
O
1
2
3
4
5
6
7
8
9
10
11
12
13



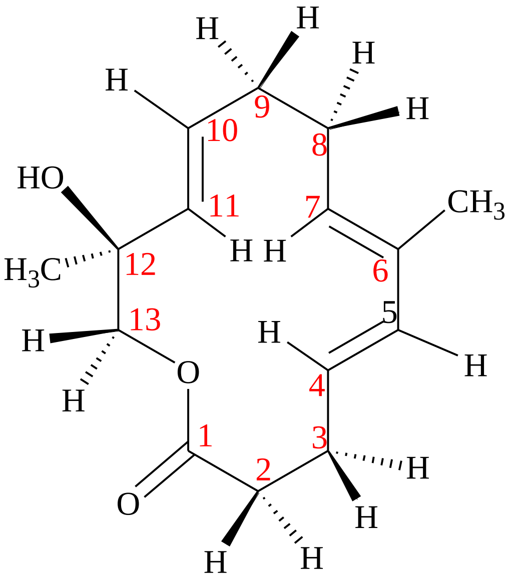

C2
32.3294
C9
31.7668
C8
27.6211
26.9622
C3
25.5889
12-CH$_3$
20.3401
6-CH$_3$
32
30
28
26
24
22
20
C11
134.0190
C6
133.2787
129.3698
C10
127.6153
C5
127.4709
C7
C4
134
132
130
128
173.4518
175
174
173
172
171
C12
72.2176
C13
70.7591
72.5
72.0
71.5
71.0
70.5
200
180
160
140
120
100
80
60
40
20
HO
H$_3$C
CH$_3$
O
O
1
2
3
4
5
6
7
8
9
10
11
12
13
H

gCOSY

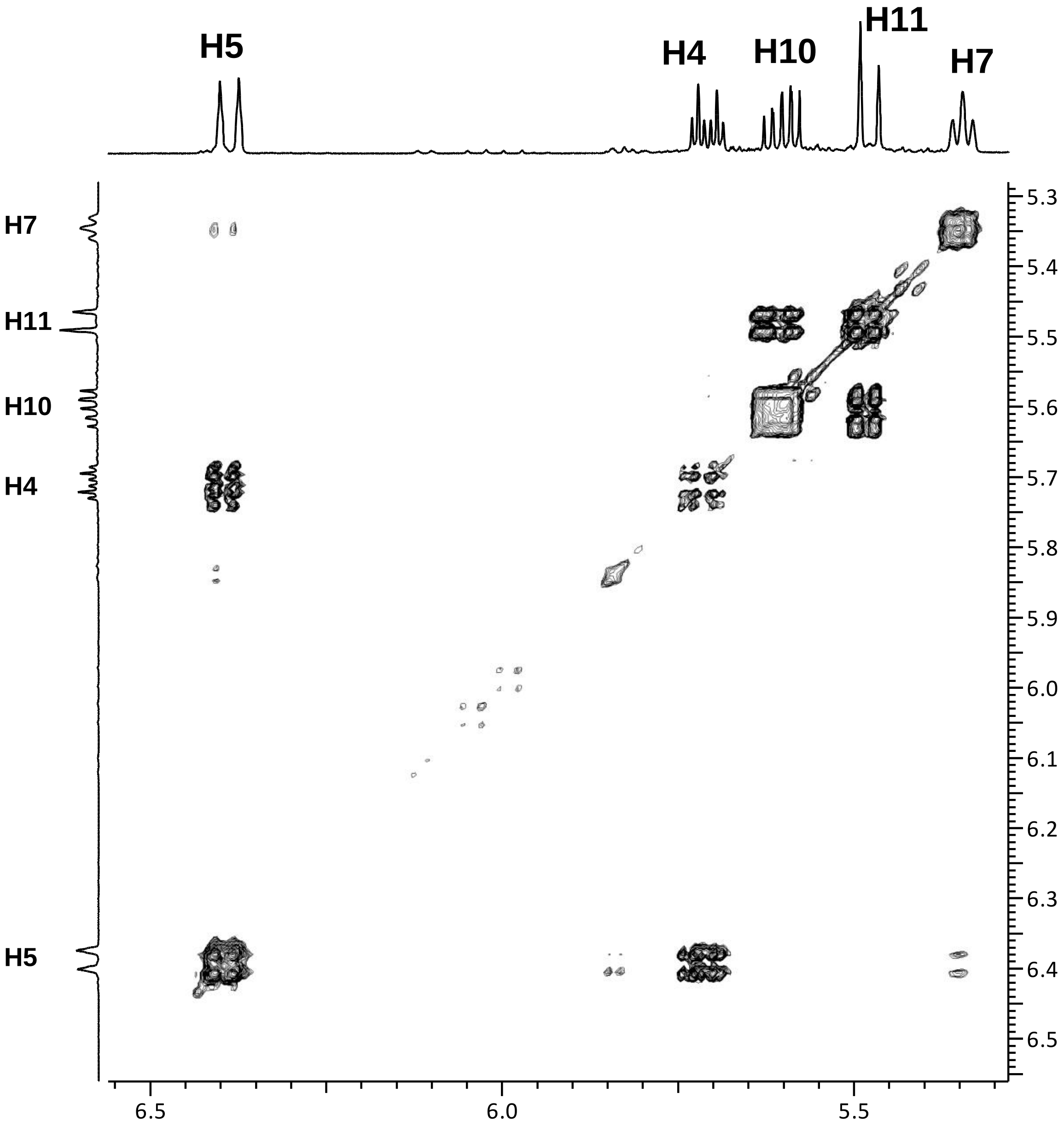

HO
H3C
H
H
H
H
H
H
H
H
H
H
H
H
H
H
O
O
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13


1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
6
5
4
3
2

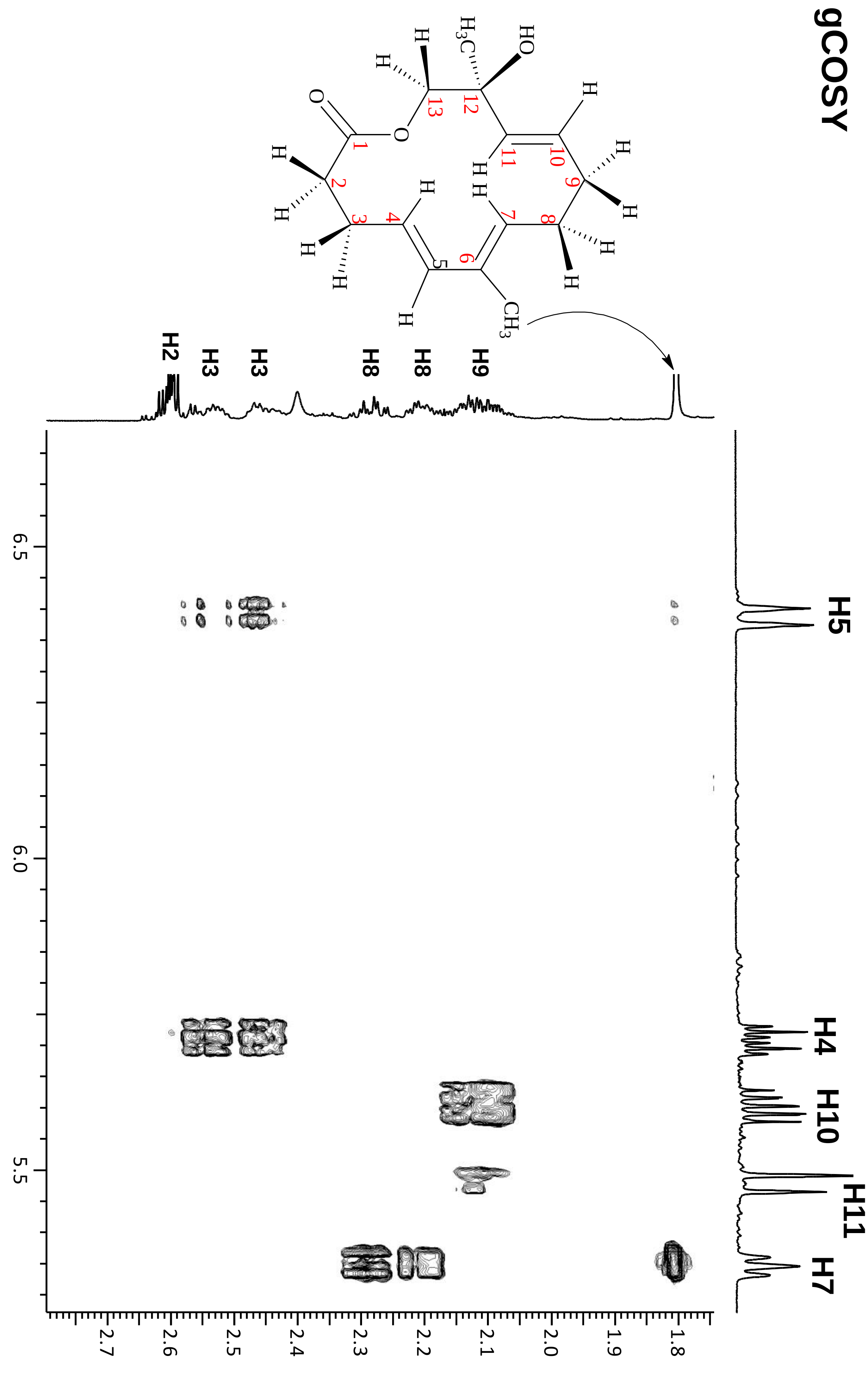
gCOSY



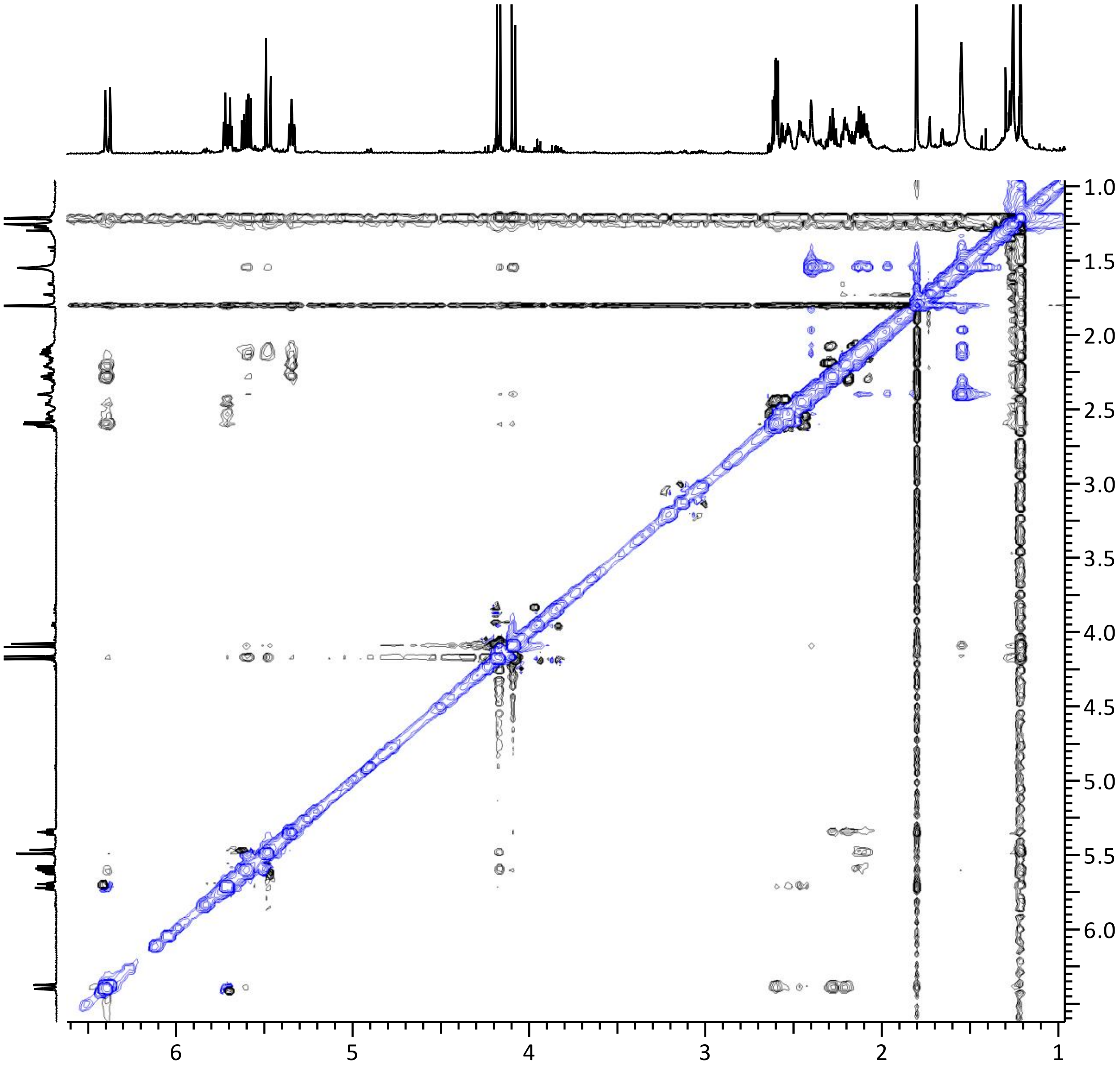
HO
H3C
H
H
H
H
H
H
H
H
H
H
H
H
H
CH3
O
O
1
2
3
4
5
6
7
8
9
10
11
12
13



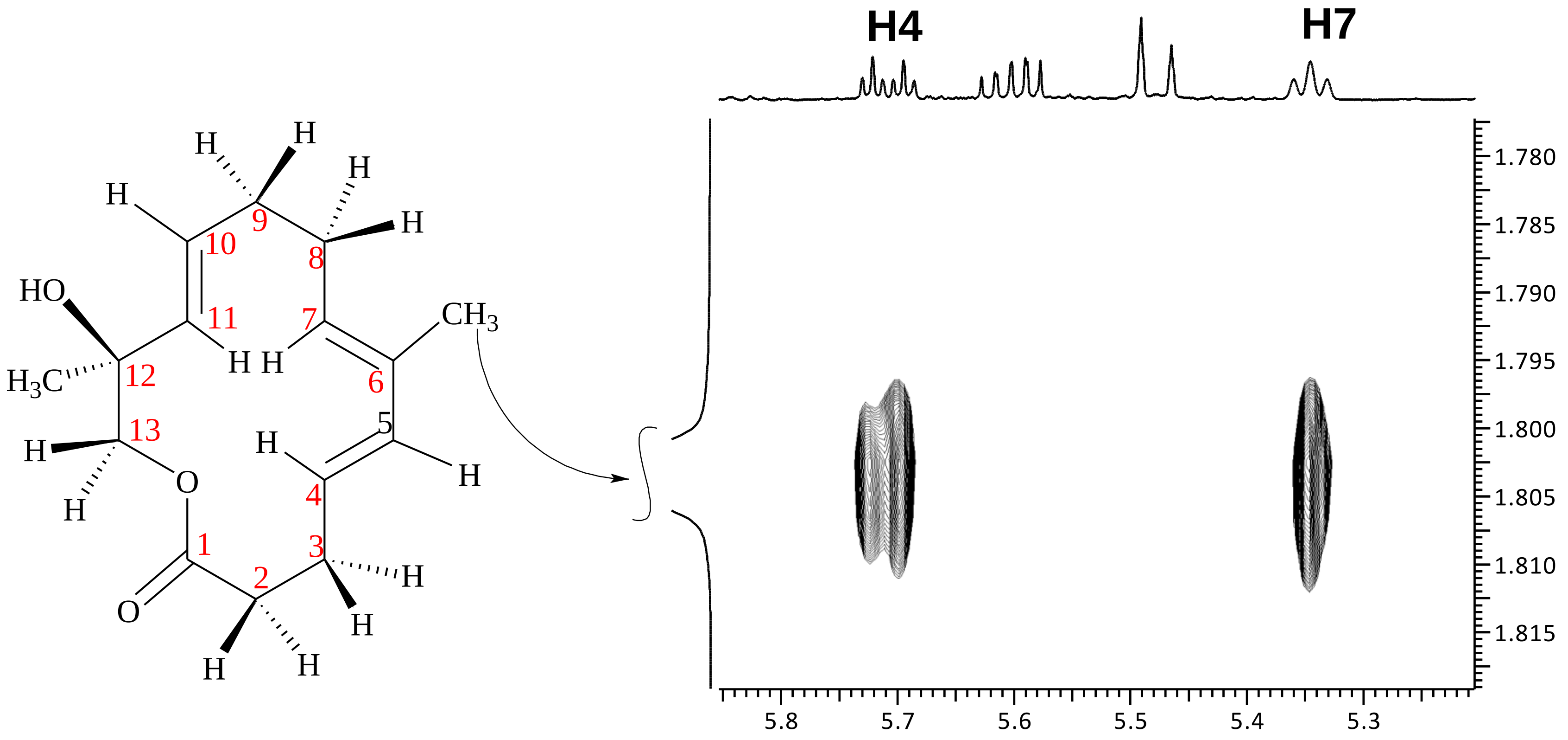
H5
H4
H10
H11
H7
H7
H11
H10
H4
H5
6.5
6.0
5.5
5.3
5.4
5.5
5.6
5.7
5.8
5.9
6.0
6.1
6.2
6.3
6.4
6.5


gCOSY
H2
H3
H3
H8
H8
H9
H5
H4
H10
H11
H7
6.5
6.0
5.5
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1
2
3
4
5
6
7
8
9
10
11
12
13
O
HO
H_3C
CH_3
H

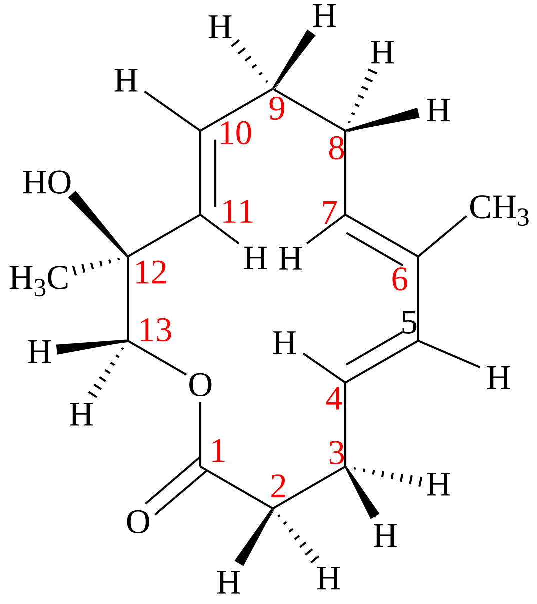
NOESY

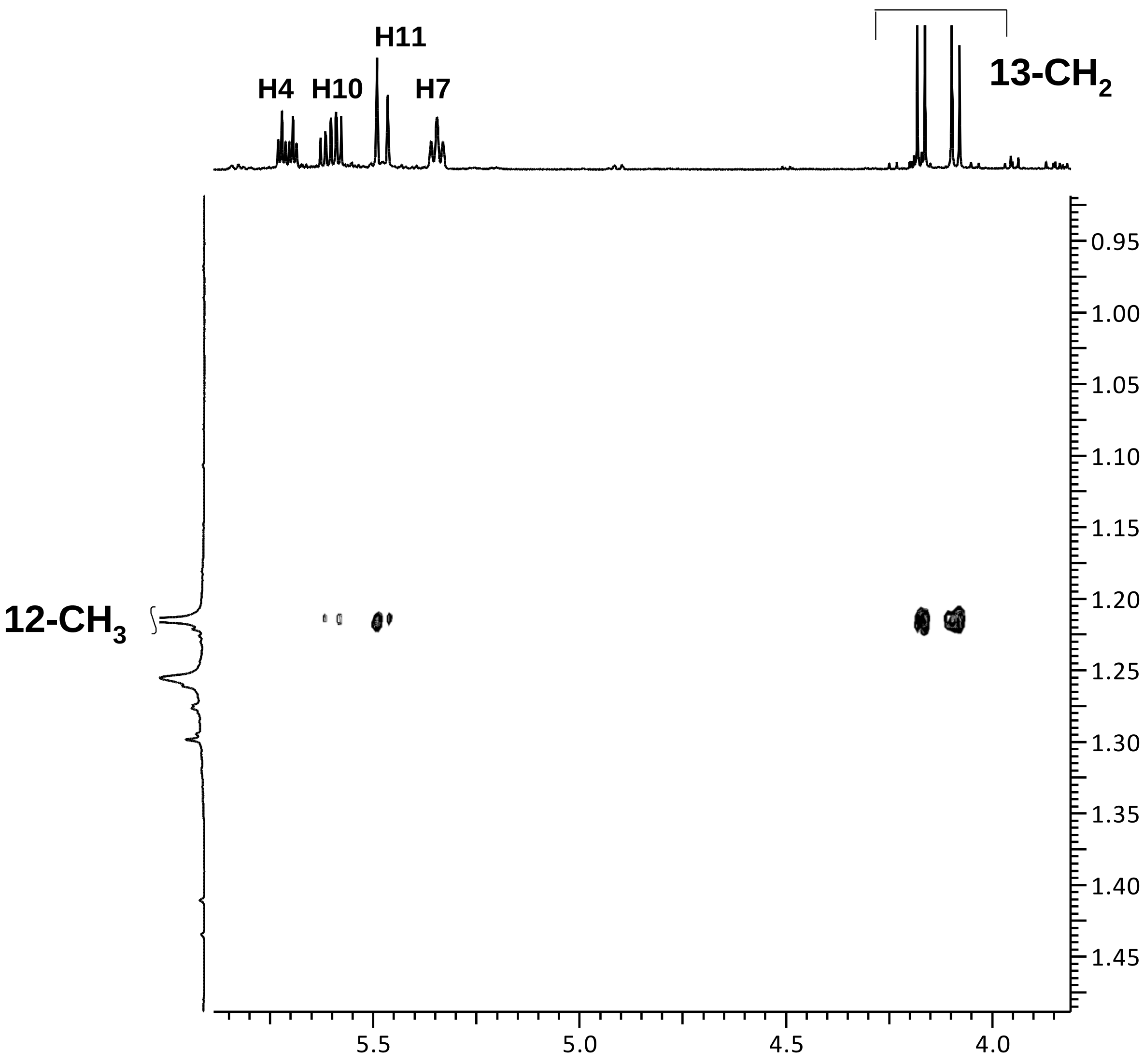


NOESY

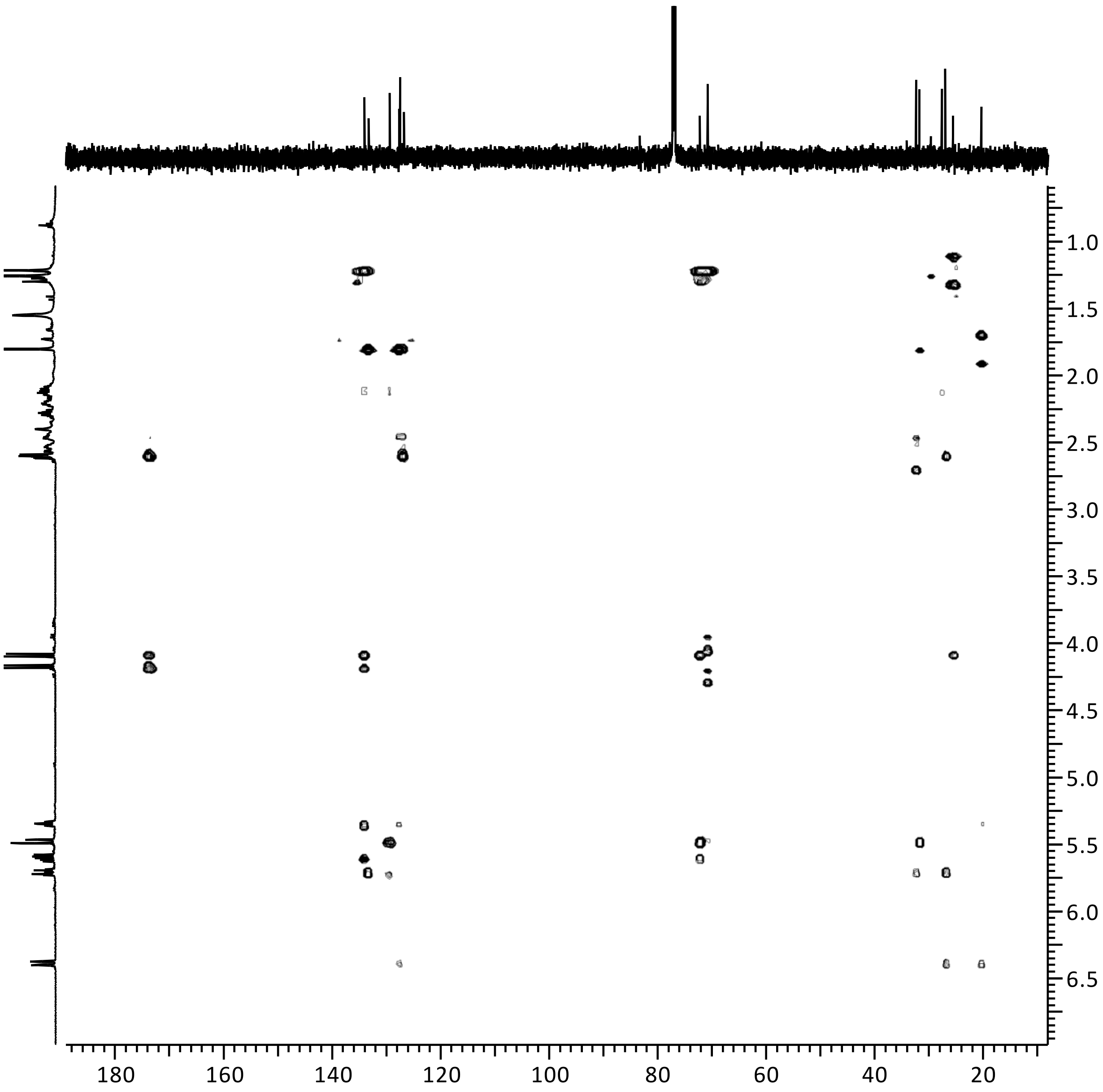

HO
H_3C
CH_3
O
1
2
3
4
5
6
7
8
9
10
11
12
13
H4
H7
5.8
5.7
5.6
5.5
5.4
5.3
1.780
1.785
1.790
1.795
1.800
1.805
1.810
1.815

NOESY

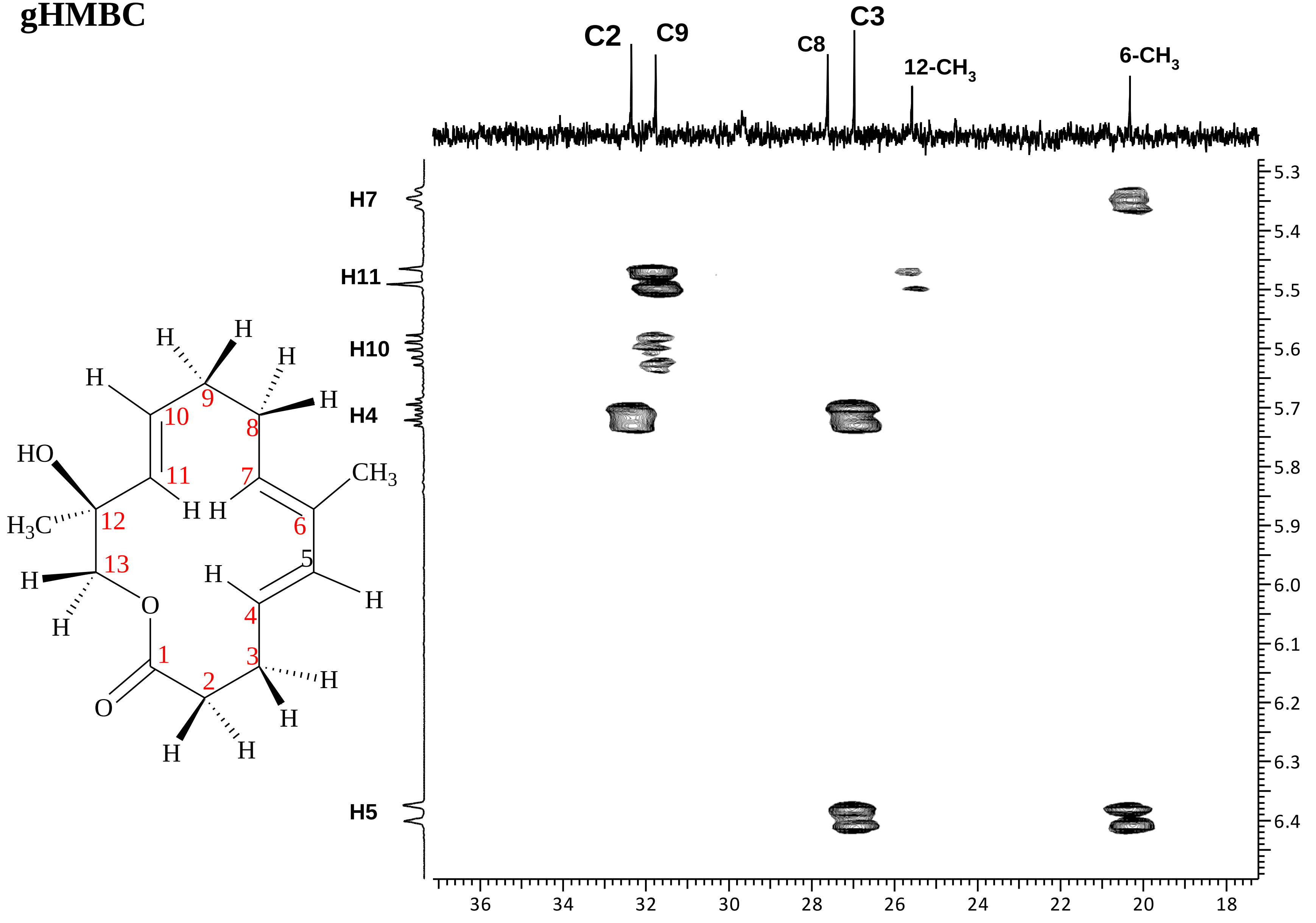

H
H
H
H
H
9
10
8
HO
11
7
CH3
H H
H3C
12
6
13
5
H
H
O
H
4
H
3
1
H
2
O
H
H
H


H11
H4
H10
H7
13-CH2
12-CH3
0.95
1.00
1.05
1.10
1.15
1.20
1.25
1.30
1.35
1.40
1.45
5.5
5.0
4.5
4.0

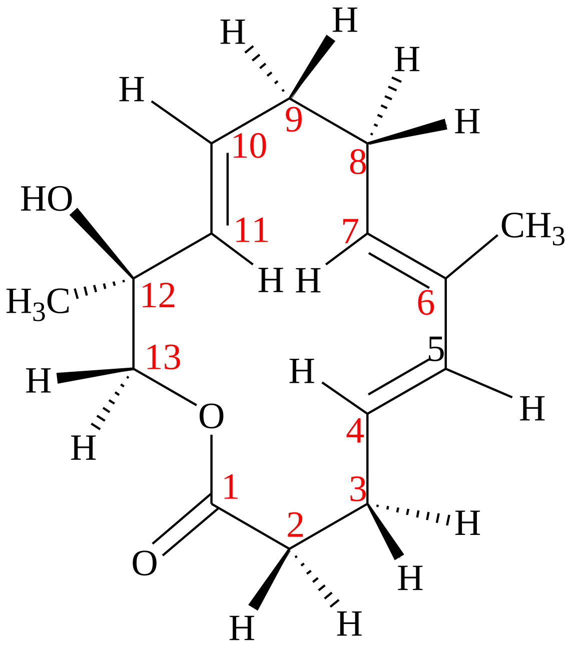
gHMBC

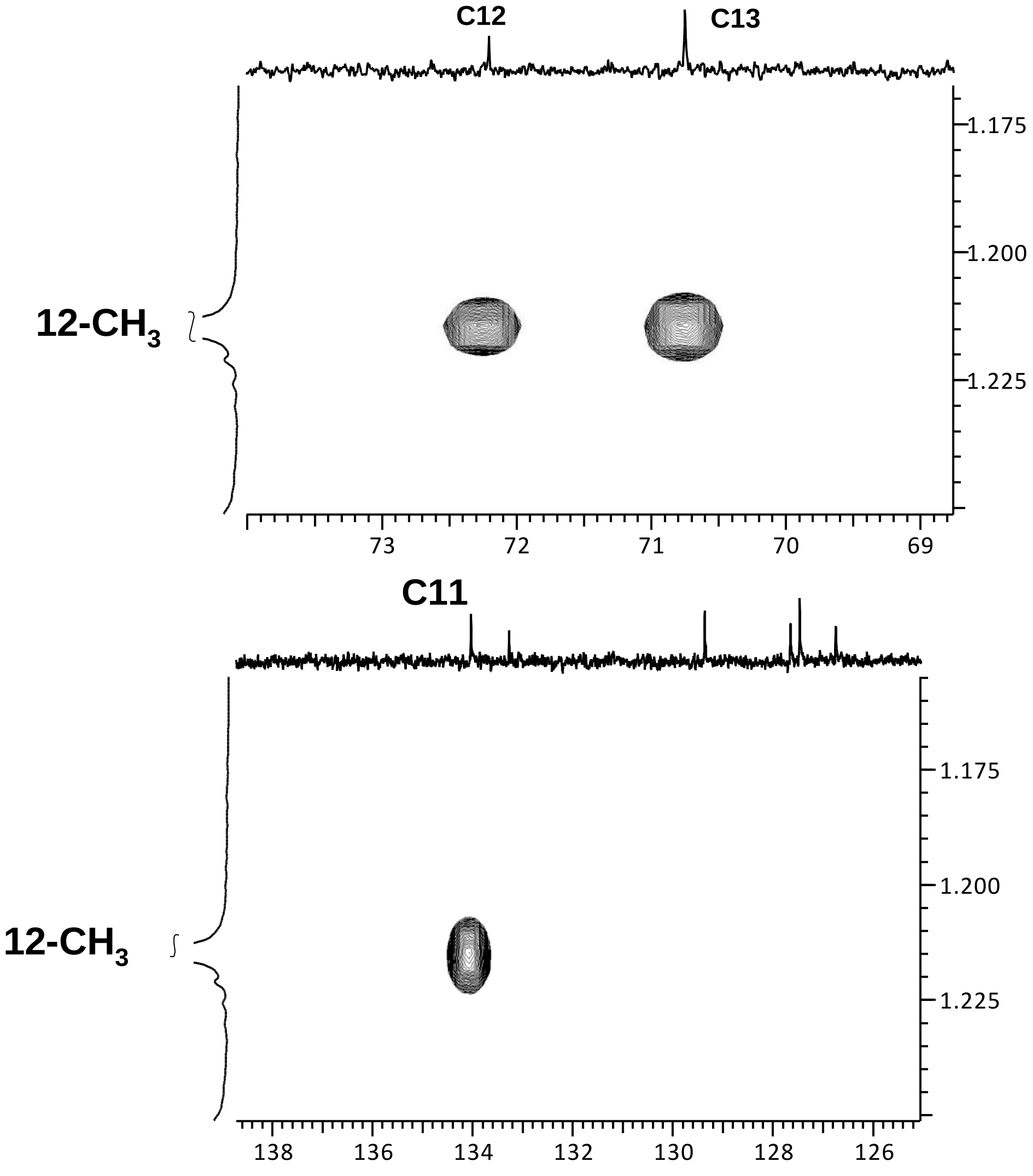


gHMBC

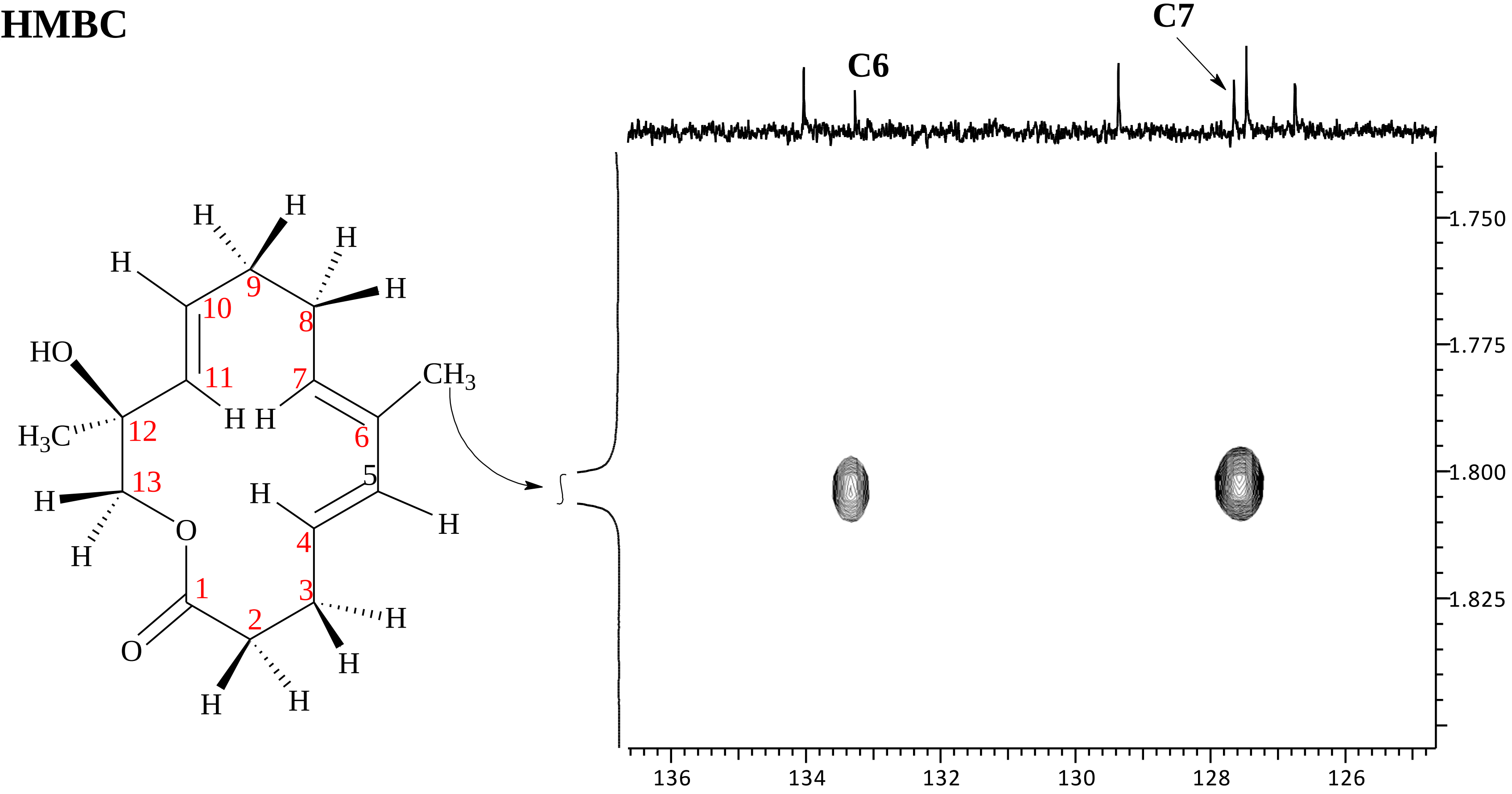

C2
C9
C8
C3
12-CH$_3$
6-CH$_3$
H7
H11
H10
H4
H5
5.3
5.4
5.5
5.6
5.7
5.8
5.9
6.0
6.1
6.2
6.3
6.4
36
34
32
30
28
26
24
22
20
18
HO
H$_3$C
CH$_3$
O
1
2
3
4
5
6
7
8
9
10
11
12
13

gHMBC

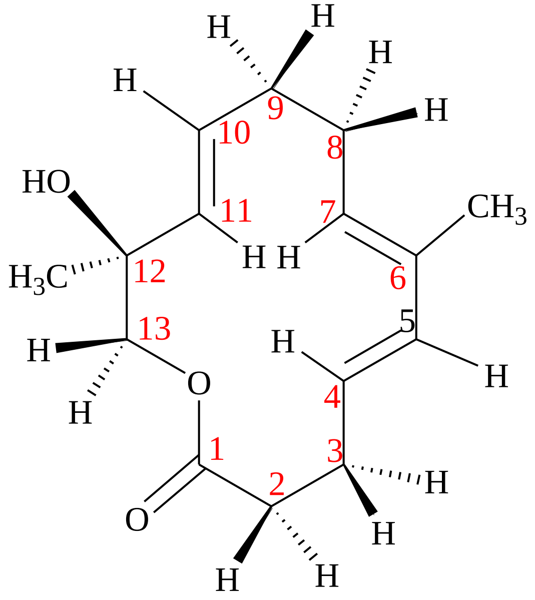

HO
H₃C
H
1
2
3
4
5
6
7
8
9
10
11
12
13
O
CH₃

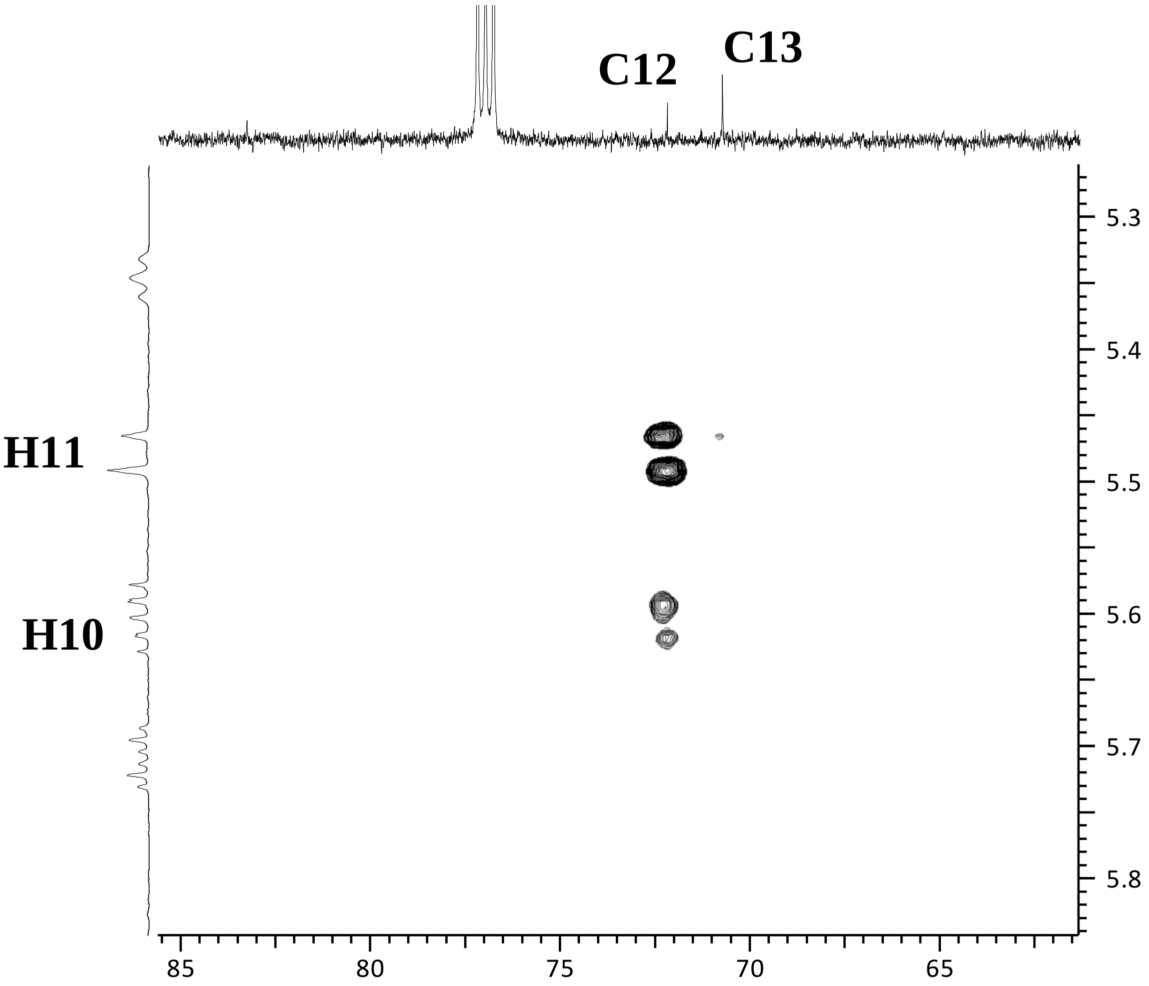

C12
C13
12-CH₃
1.175
1.200
1.225
73
72
71
70
69
C11
12-CH₃
1.175
1.200
1.225
138
136
134
132
130
128
126

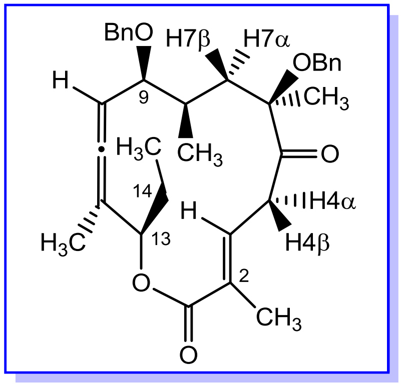
gHMBC


HO
H3C
O
O
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13
C6
C7
136
134
132
130
128
126
1.750
1.775
1.800
1.825

gHMBC

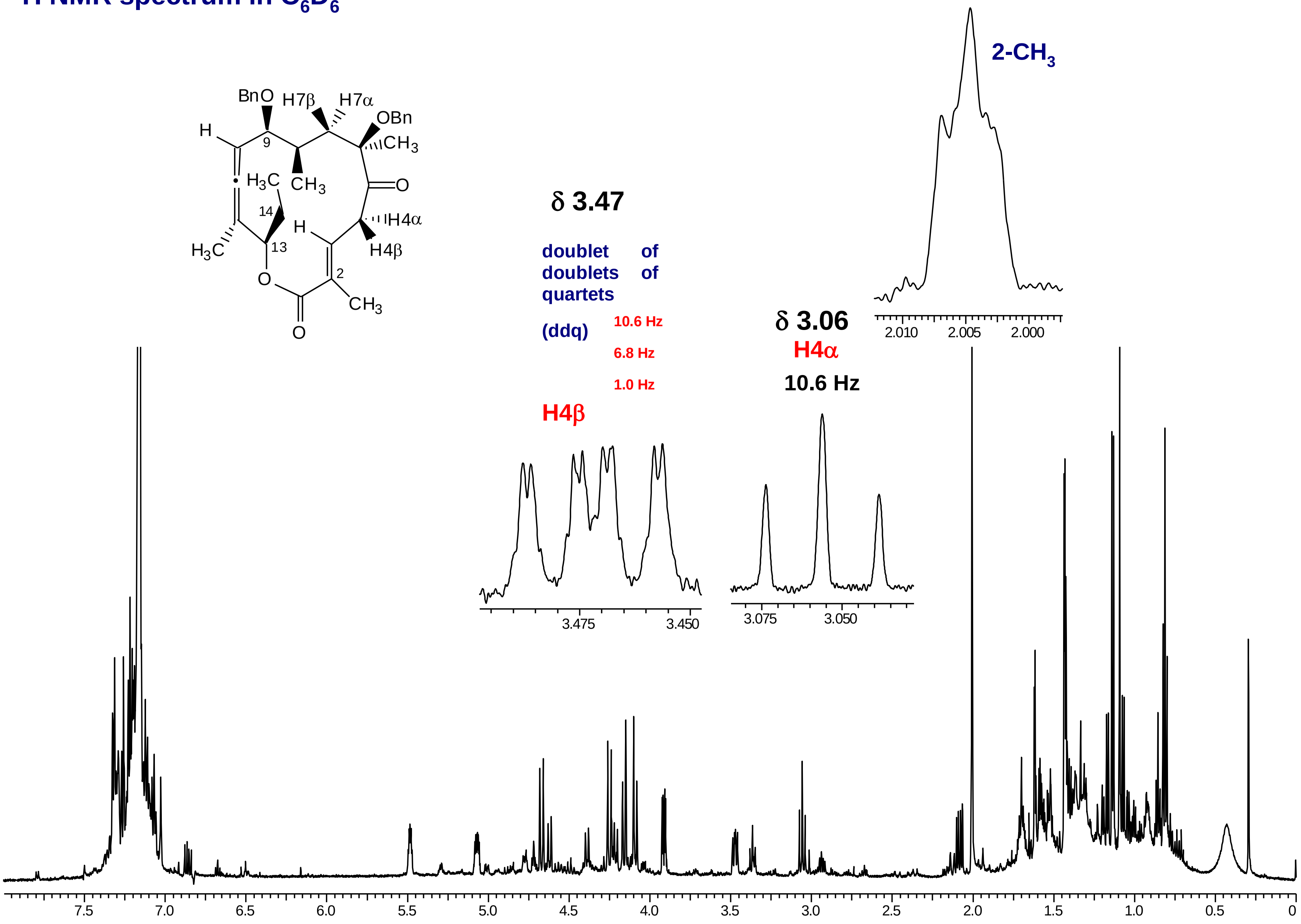

HO
H3C
H
H
O
O
H
H
H
H
H
H
H
H
H
H
H
H
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13

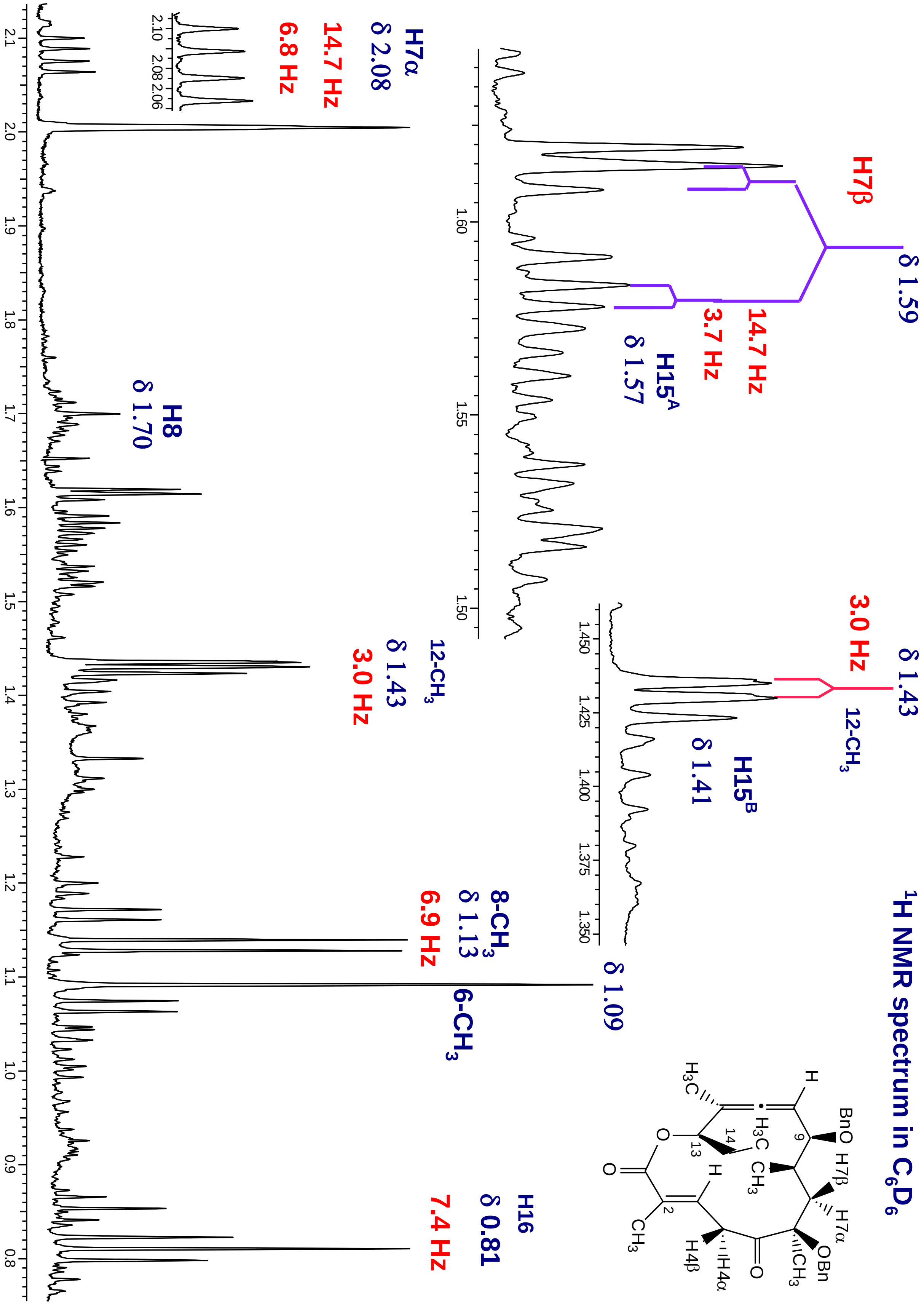

C12
C13
H11
H10
5.3
5.4
5.5
5.6
5.7
5.8
85
80
75
70
65

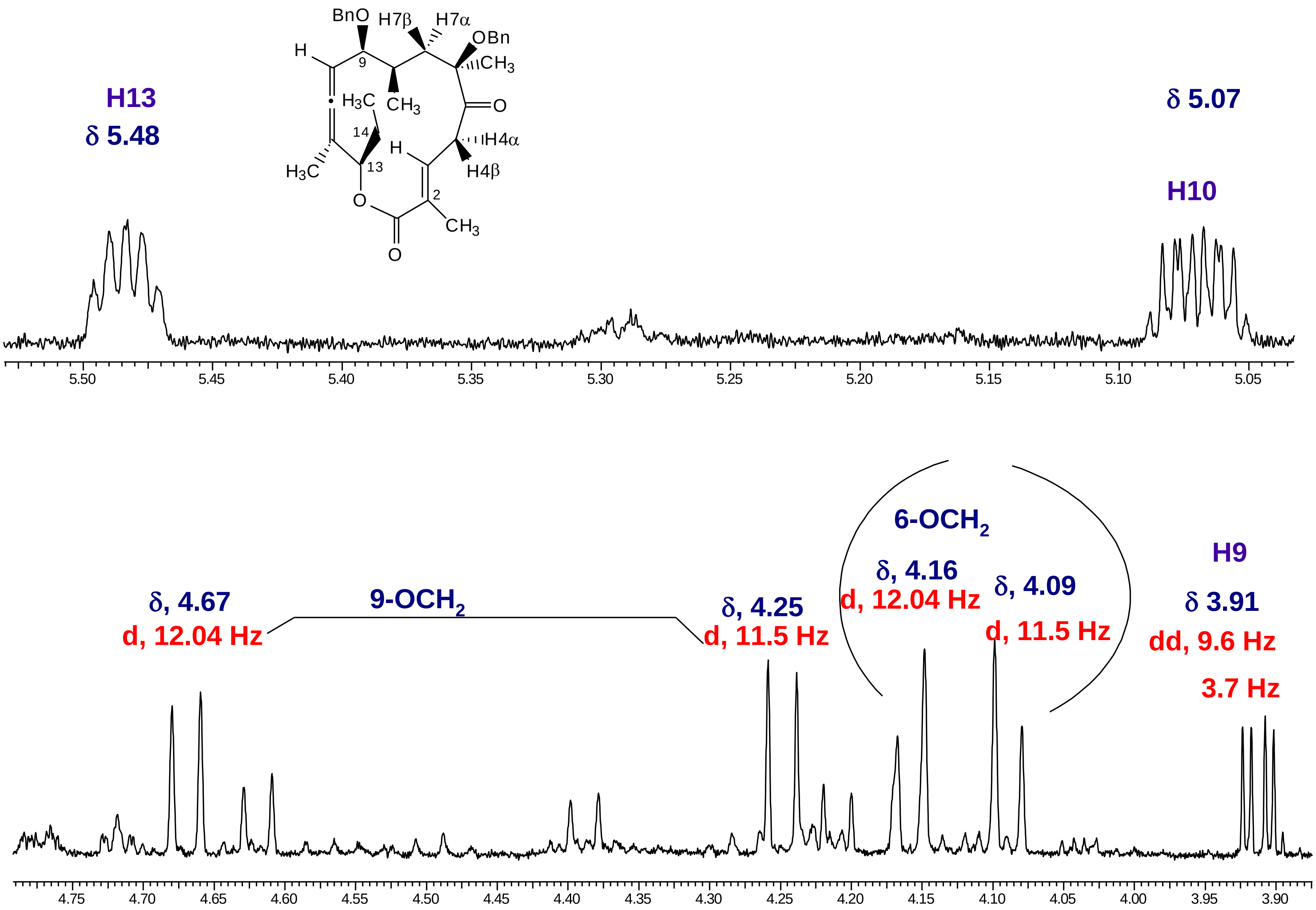


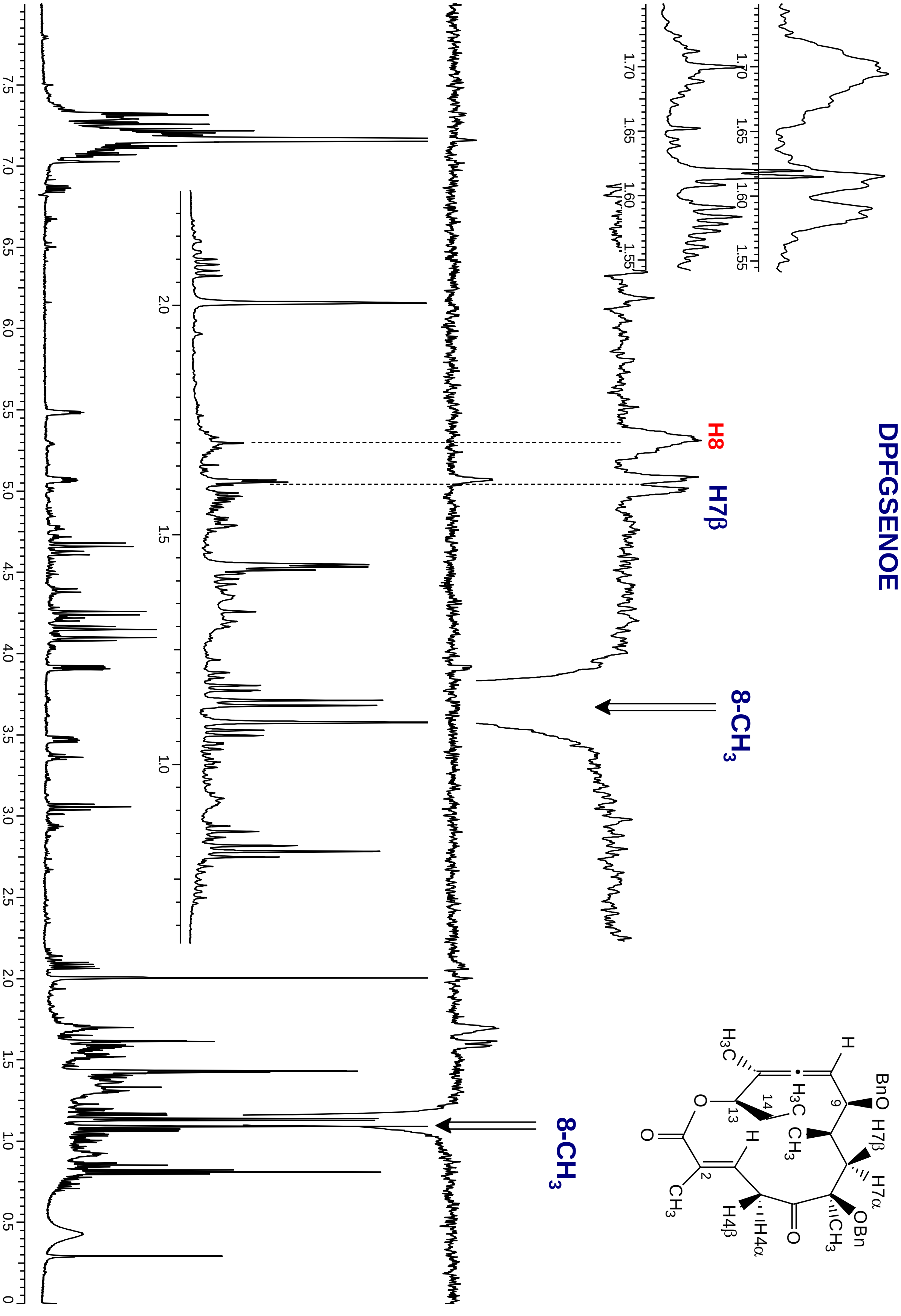
^{1}H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz), C_6D_6	^{13}C NMR chemical shift (δ/ppm), $CDCl_3$
2.00 (dt, J(2-CH$_3$,H3 = 1 Hz, J(2-CH$_3$-4-CH$_2$ = ~0.7 Hz, **2-CH$_3$**)	171.36 ----- C1
7.22 (ddq, **H3**)	165.89 ----- C2
3.06 (t, $J_{H4\alpha,\ H4\beta}$ = 10.6 Hz, **H4α**)	127.28 ----- C3
3.47 (ddq, $J_{H4\beta,\ H4\alpha}$ = 10.6 Hz, $J_{H4\beta,\ H3}$ = 6.8 Hz	41.98 ----- C4
$J_{H4\beta,\ 2\text{-}CH3}$ = 1.0 Hz, **H4β**)	208.77 ----- C5
1.09 (s, **6-CH$_3$**)	86.88 ----- C6
4.09 (d, J_{AB} = 11.6 Hz, **6-OCH$_2$**)	39.01 ----- C7
4.16 (d, J_{AB} = 11.6 Hz, **6-OCH$_2$**)	36.50 ----- C8
1.59 (dd, $J_{H7\alpha,\ H8}$ = 3.7 Hz, $J_{H7\alpha,\ H7\beta}$ = 14.7 Hz, **H7α**)	80.83 ----- C9
2.08 (dd, $J_{H7\beta,\ H8}$ = 6.8 Hz, $J_{H7\alpha,\ H7\beta}$ = 14.7 Hz, **H7β**)	99.84 ----- C10
1.70 (dddq, **H8**)	203.36 ----- C11
1.13 (d, $J_{8\text{-}CH3,\ H8}$ = 6.9 Hz, **8-CH$_3$**)	92.62 ----- C12
3.91 (dd, $J_{H9,\ H8}$ = 9.6 Hz, $J_{H9,\ H10}$ = 3.7 Hz, **H9**)	72.78 ----- C13
4.25 (d, J_{AB} = 12.2 Hz, **9-OCH$_2$**)	24.86 ----- C14
4.67 (d, J_{AB} = 12.2 Hz, **9-OCH$_2$**)	12.44 ----- 2-CH$_3$
5.05 - 5.07 (m, **H10**)	21.94 ----- 6-CH$_3$
1.43 (d, $J_{12\text{-}CH3,\ H10}$ = 3.0 Hz, **12-CH$_3$**)	16.35 ----- 8-CH$_3$
5.47 - 5.50 (m, **H13**)	15.67 ----- 12-CH$_3$
1.55 - 1.60, 1.37 - 1.42 (m, **14-CH$_2$**)	9.14 ----- 14-CH$_3$
0.81 (t, $J_{14\text{-}CH3,\ 13\text{-}CH2}$ = 7.4 Hz, **14-CH$_3$**)	64.76 ----- 6-OCH$_2$
7.48 - 7.10 (m, 10H, **Ph**)	69.88 ----- 9-OCH$_2$

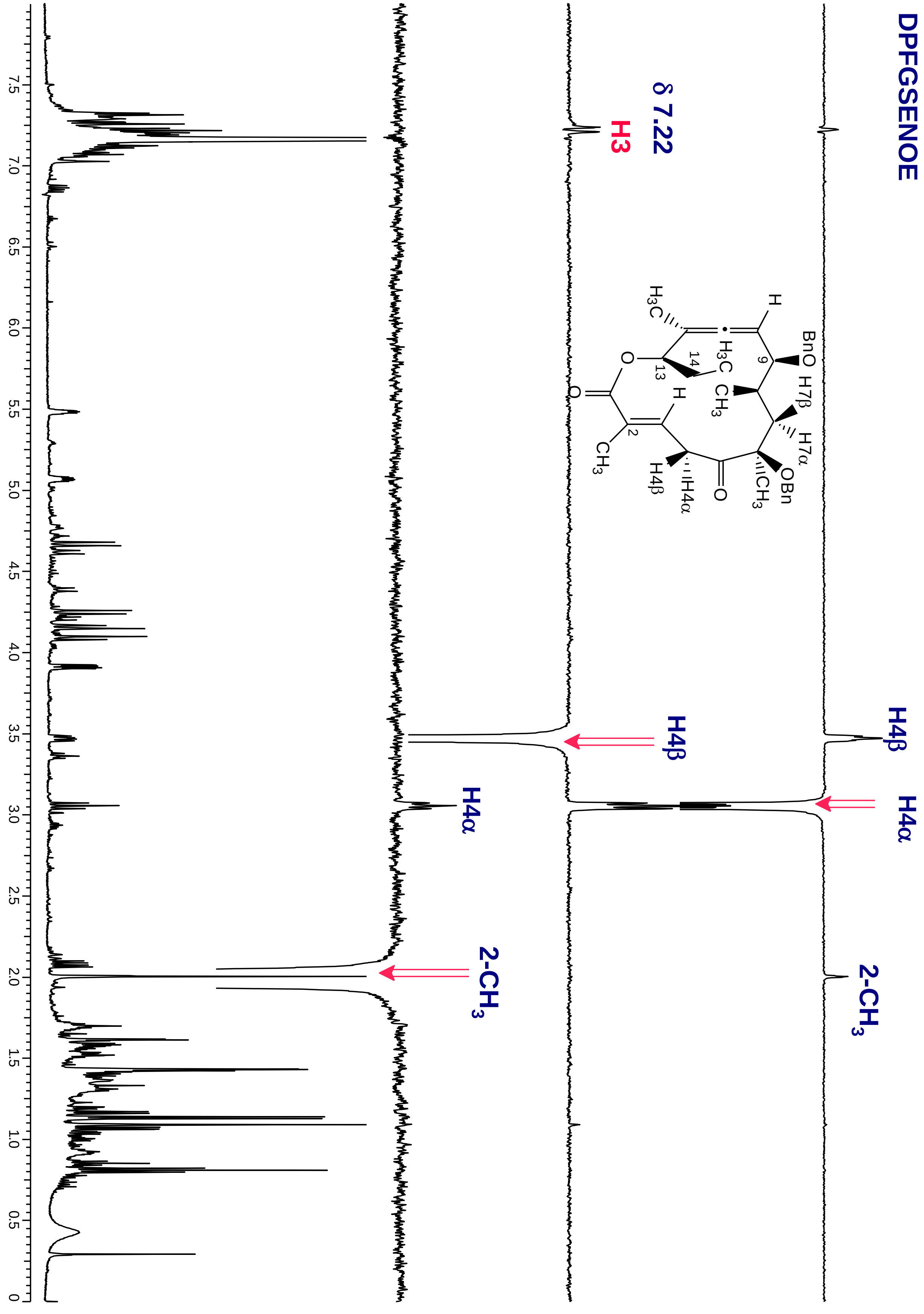
^1H NMR spectrum in C_6D_6



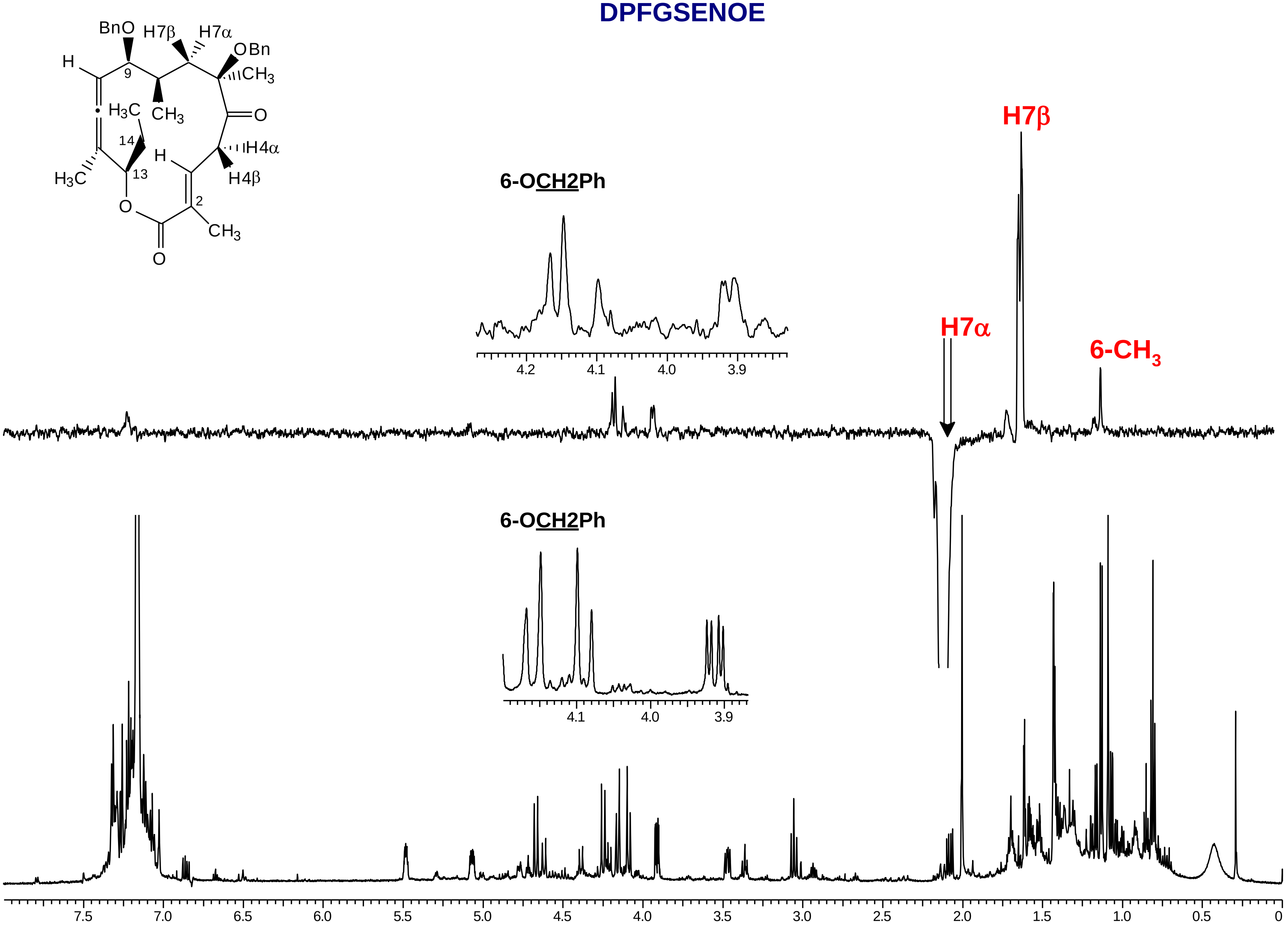

¹H NMR spectrum in C6D6
H
BnO
9
H7β
H7α
OBn
CH3
H3C
CH3
14
13
H
H3C
O
O
2
CH3
H4β
H4α
O
H7β
δ 1.59
14.7 Hz
3.7 Hz
H15A
δ 1.57
δ 1.43
3.0 Hz
12-CH3
H15B
δ 1.41
δ 1.09
H7α
δ 2.08
14.7 Hz
6.8 Hz
H8
δ 1.70
12-CH3
δ 1.43
3.0 Hz
8-CH3
δ 1.13
6.9 Hz
6-CH3
H16
δ 0.81
7.4 Hz
2.10
2.08
2.06
1.60
1.55
1.50
1.450
1.425
1.400
1.375
1.350
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8

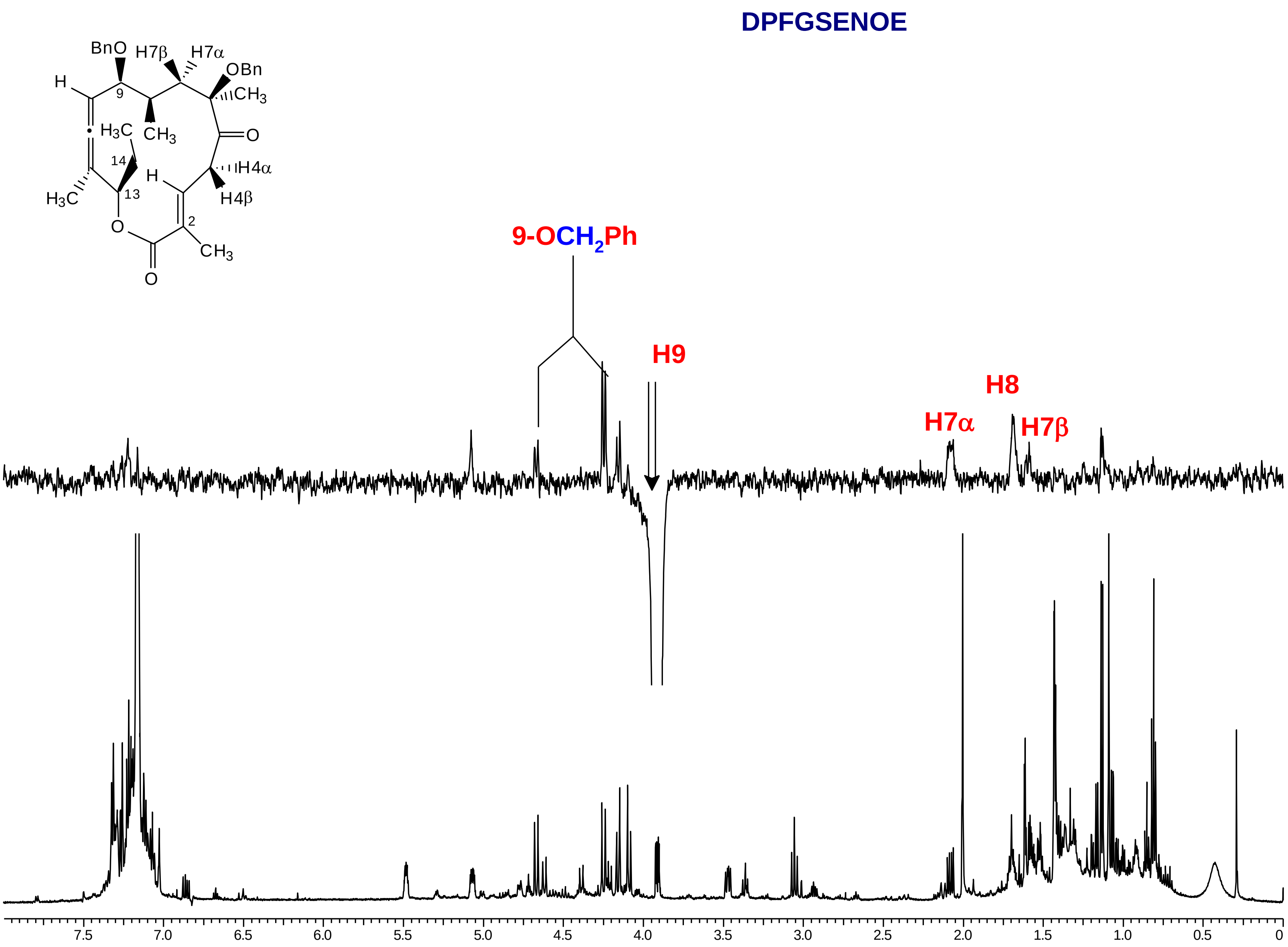

H13
δ 5.48
BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
H4α
H3C
13
H4β
2
O
CH3
O
δ 5.07
H10
5.50
5.45
5.40
5.35
5.30
5.25
5.20
5.15
5.10
5.05
6-OCH2
H9
δ, 4.16
d, 12.04 Hz
δ, 4.09
d, 11.5 Hz
δ 3.91
dd, 9.6 Hz
3.7 Hz
δ, 4.67
d, 12.04 Hz
9-OCH2
δ, 4.25
d, 11.5 Hz
4.75
4.70
4.65
4.60
4.55
4.50
4.45
4.40
4.35
4.30
4.25
4.20
4.15
4.10
4.05
4.00
3.95
3.90

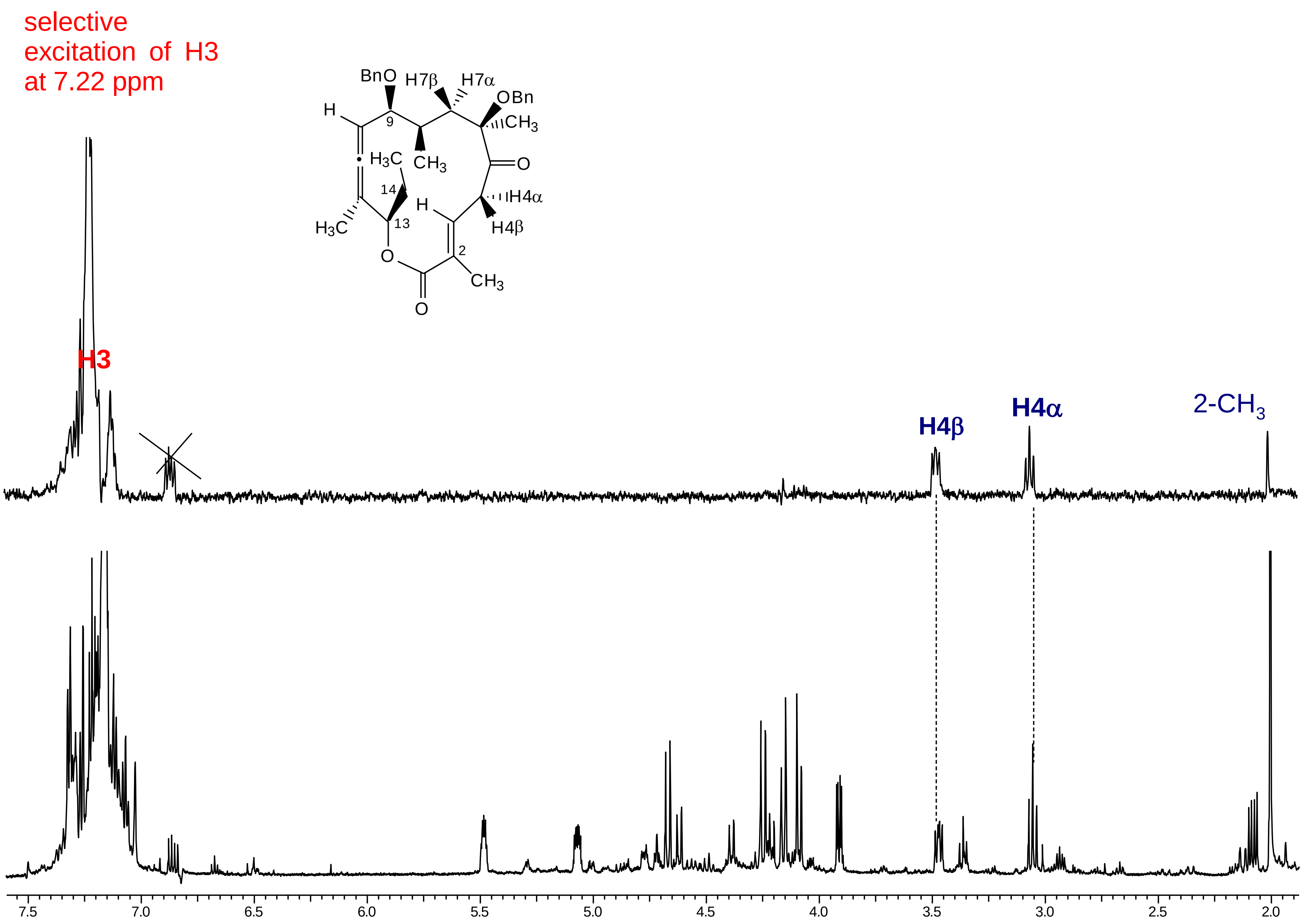

DPFGSENOE
H8
H7β
8-CH$_3$
8-CH$_3$
BnO
H7β
H7α
OBn
CH$_3$
H4α
H4β
H
H
H$_3$C
CH$_3$
H$_3$C
CH$_3$
O
O
O
9
14
13
2
1.70
1.65
1.60
1.55
2.0
1.5
1.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0


DPFGSENOE
δ 7.22
H3
H4β
H4α
2-CH_3
H4β
H4α
2-CH_3
BnO
H7β
H7α
OBn
CH_3
O
H
9
H_3C
CH_3
14
13
H_3C
H
O
O
2
CH_3
H4β
H4α
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

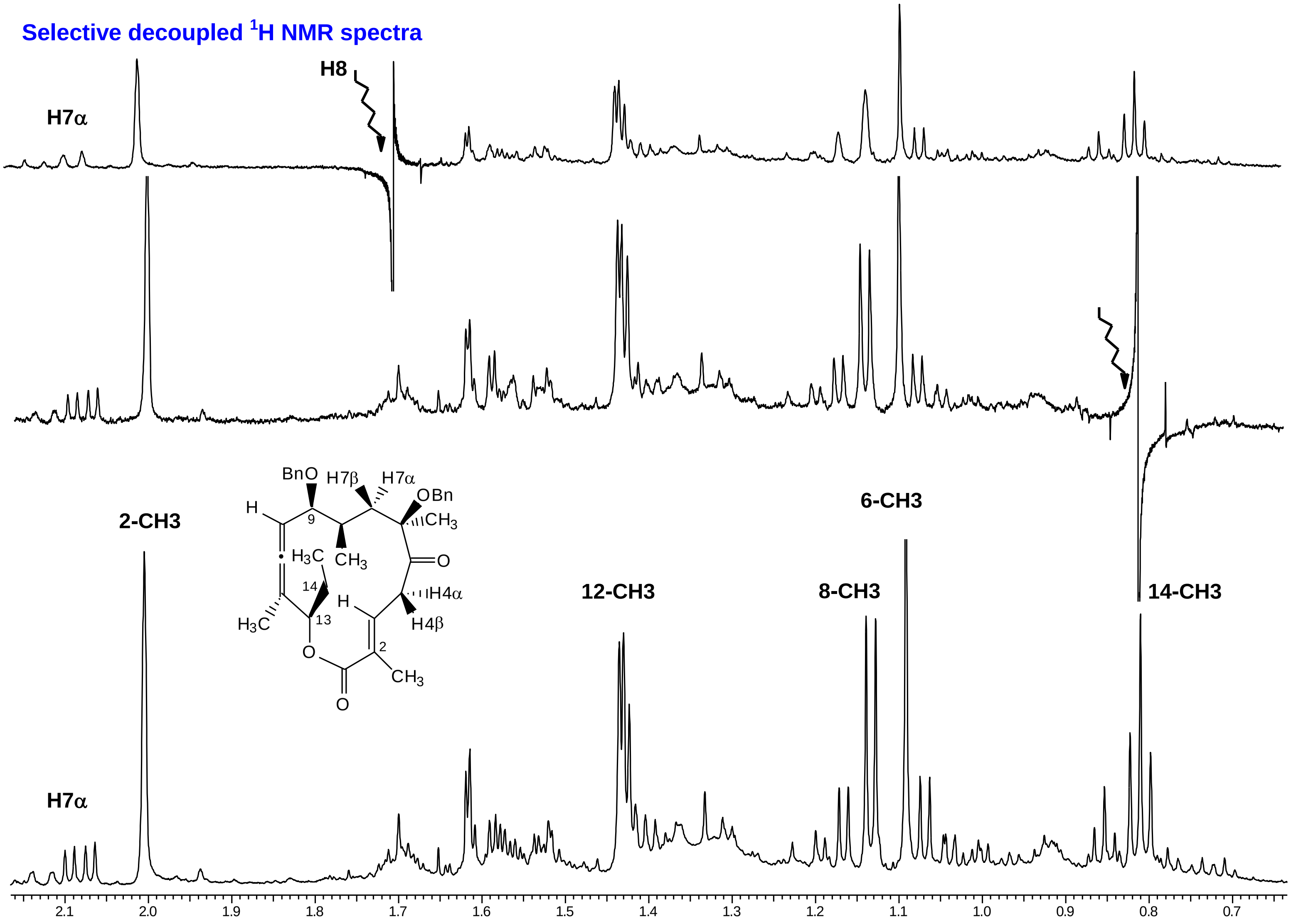
DPFGSENOE


BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
14
H
O
H4α
H4β
H3C
13
2
O
CH3
O
6-OCH2Ph
4.2
4.1
4.0
3.9
H7α
H7β
6-CH3
6-OCH2Ph
4.1
4.0
3.9
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

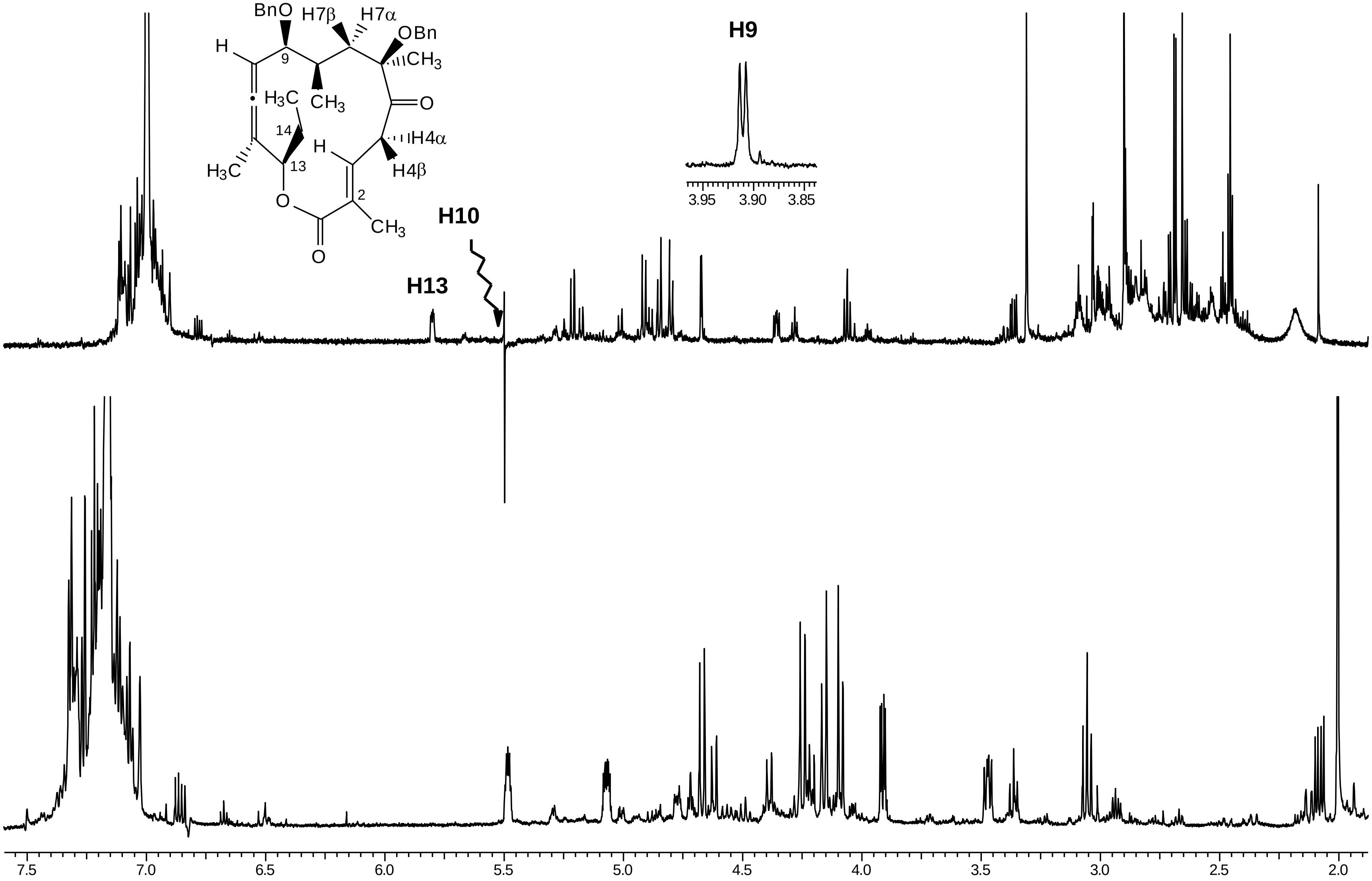

DPFGSENOE
BnO
H7β
H7α
OBn
H
9
CH3
H3C
CH3
O
14
H
IH4α
H4β
H3C
13
2
O
CH3
O
9-OCH2Ph
H9
H8
H7α
H7β
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

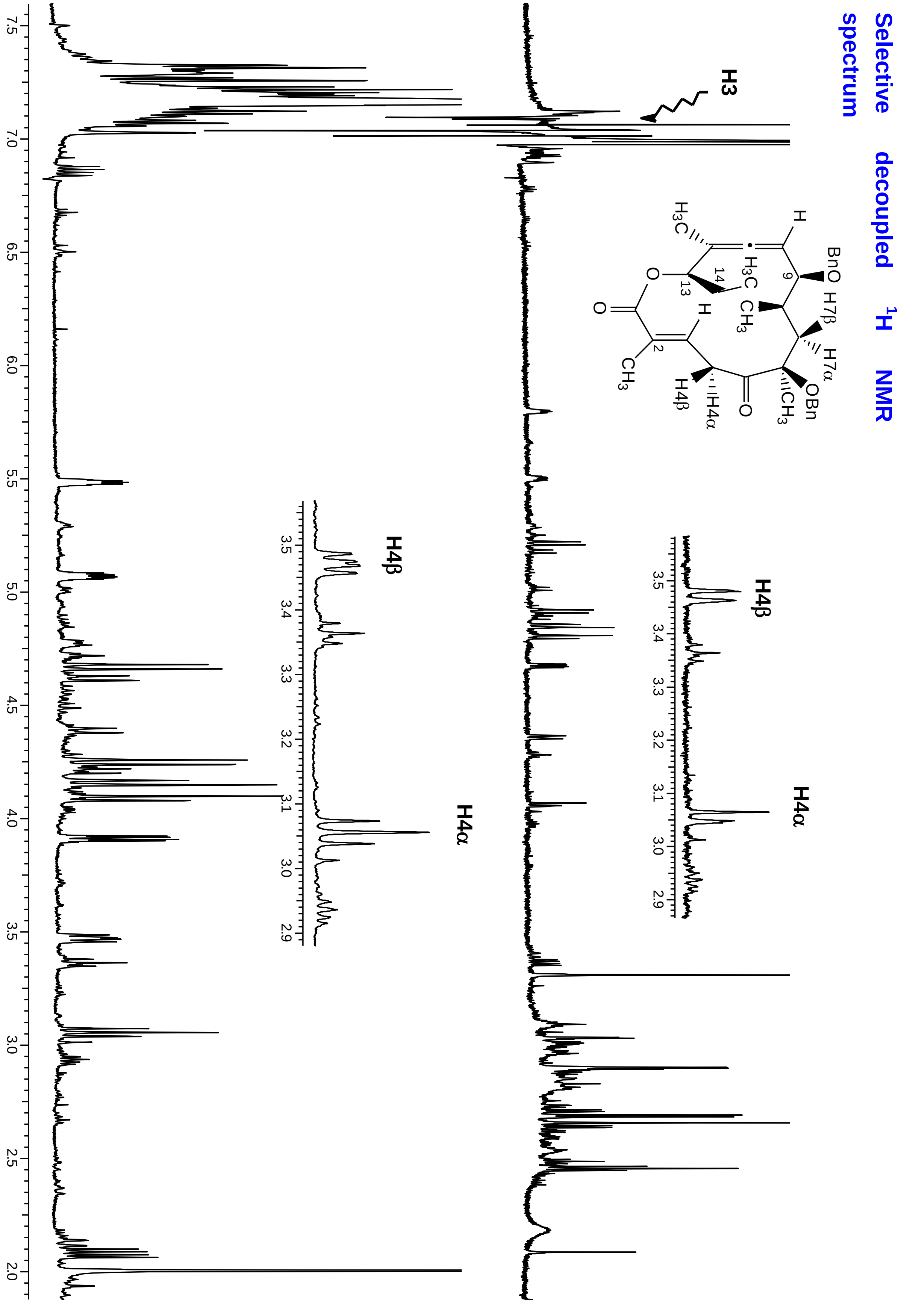

selective
excitation of H3
at 7.22 ppm
BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
IH4α
H4β
H3C
13
2
O
CH3
O
H3
H4β
H4α
2-CH3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0

Selective decoupled ^{1}H NMR spectra



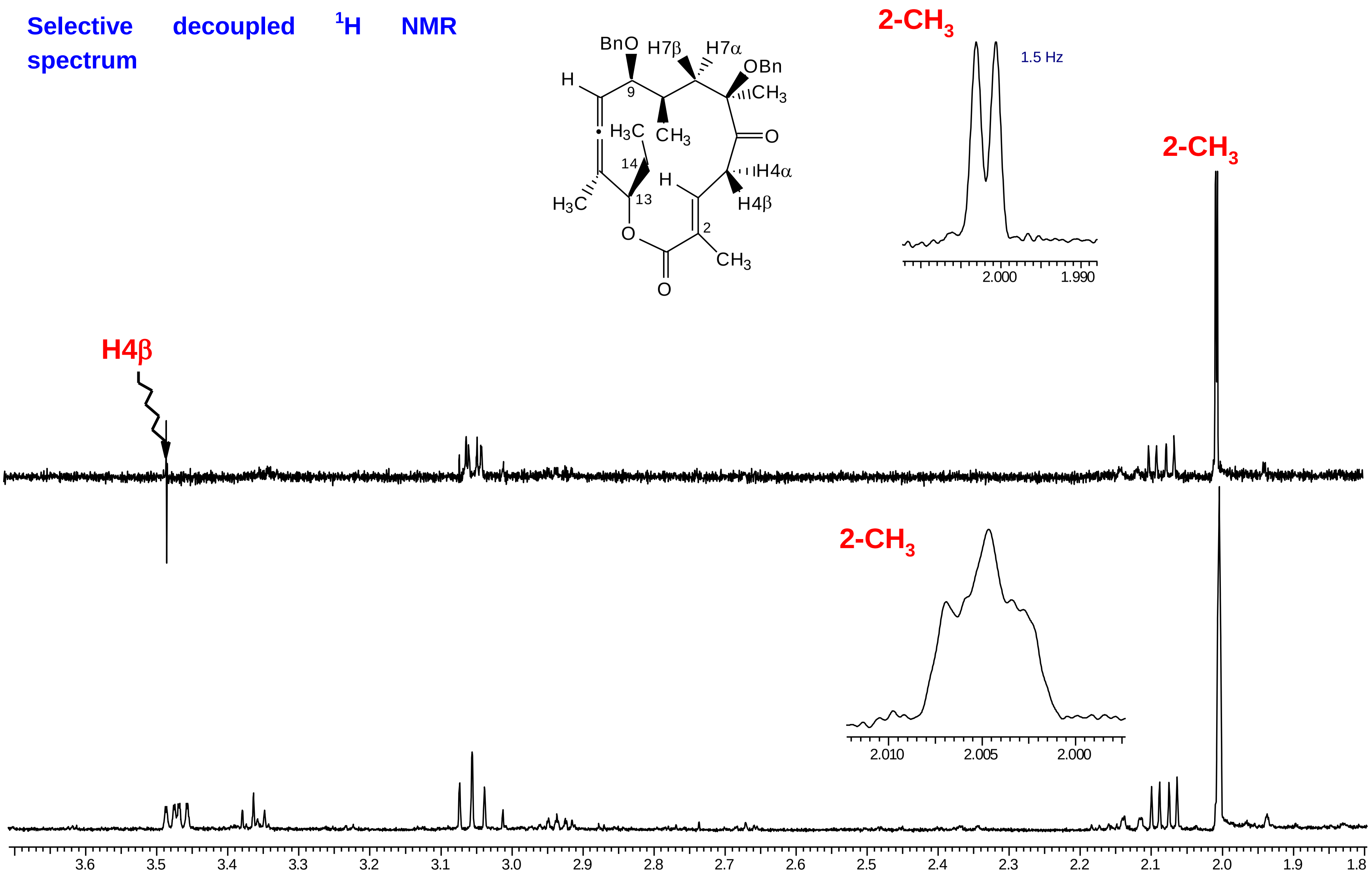
Selective decoupled ^{1}H NMR spectrum



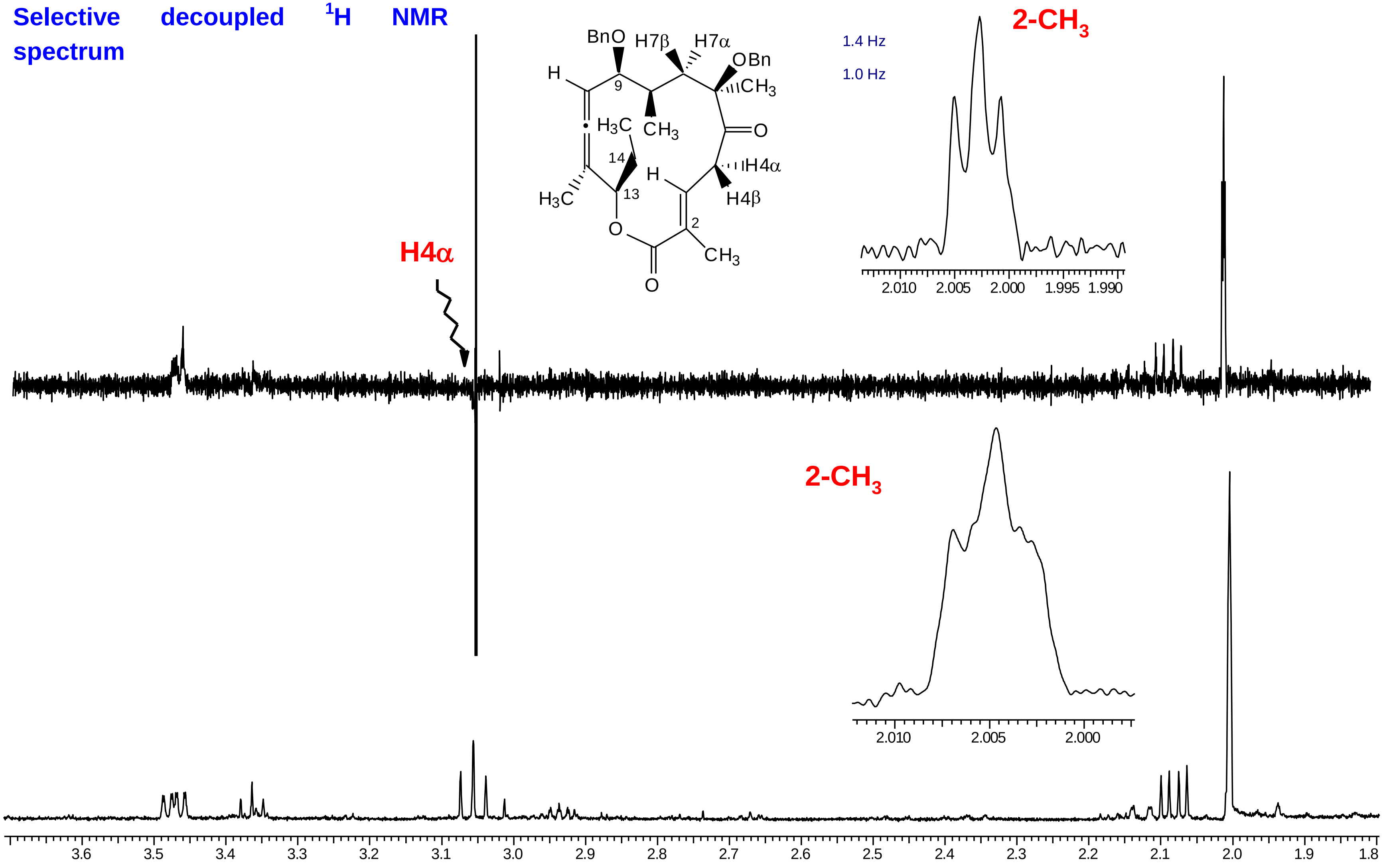


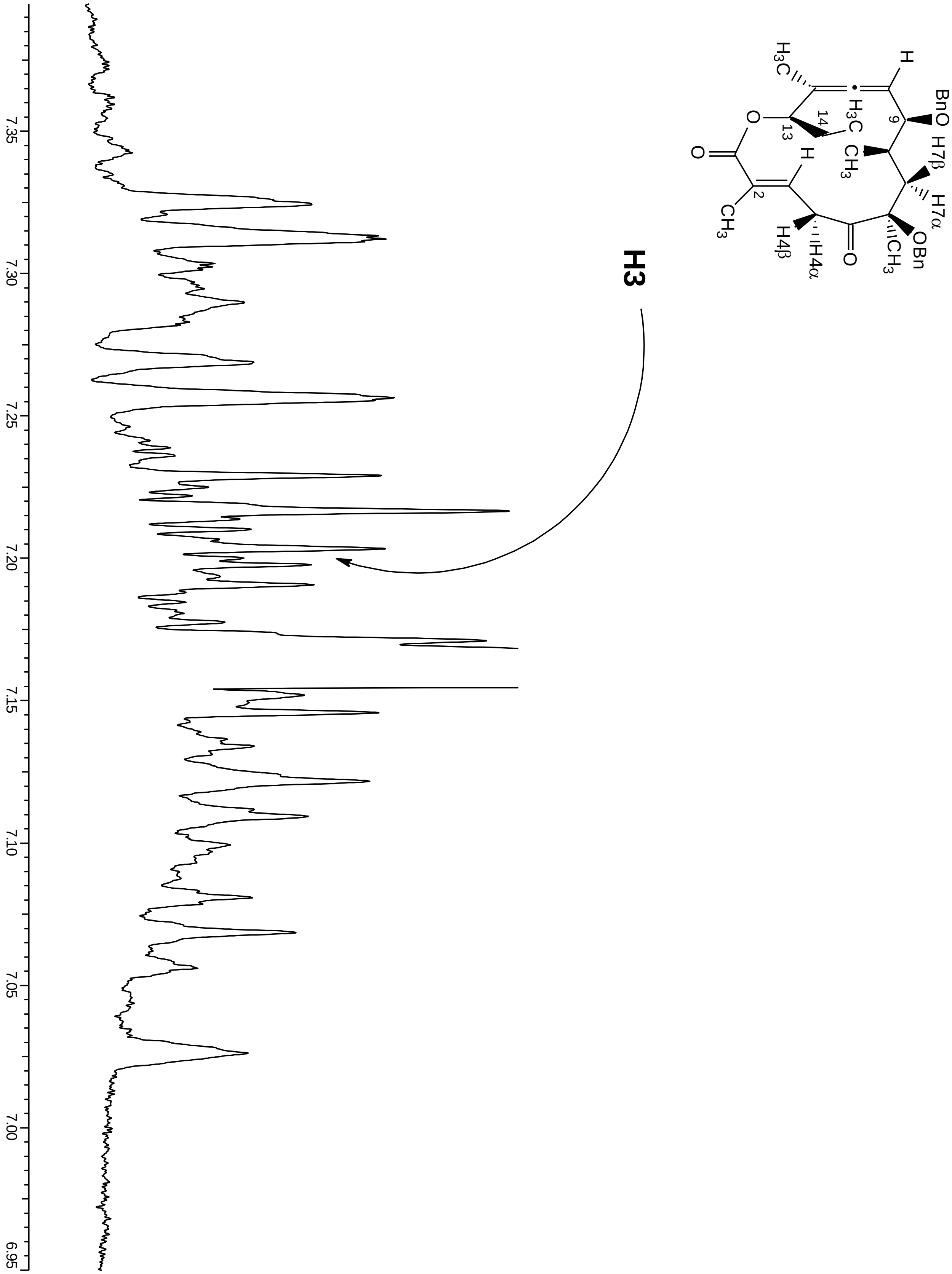
Selective decoupled ^1H NMR spectrum

Selective decoupled ^1H NMR spectrum

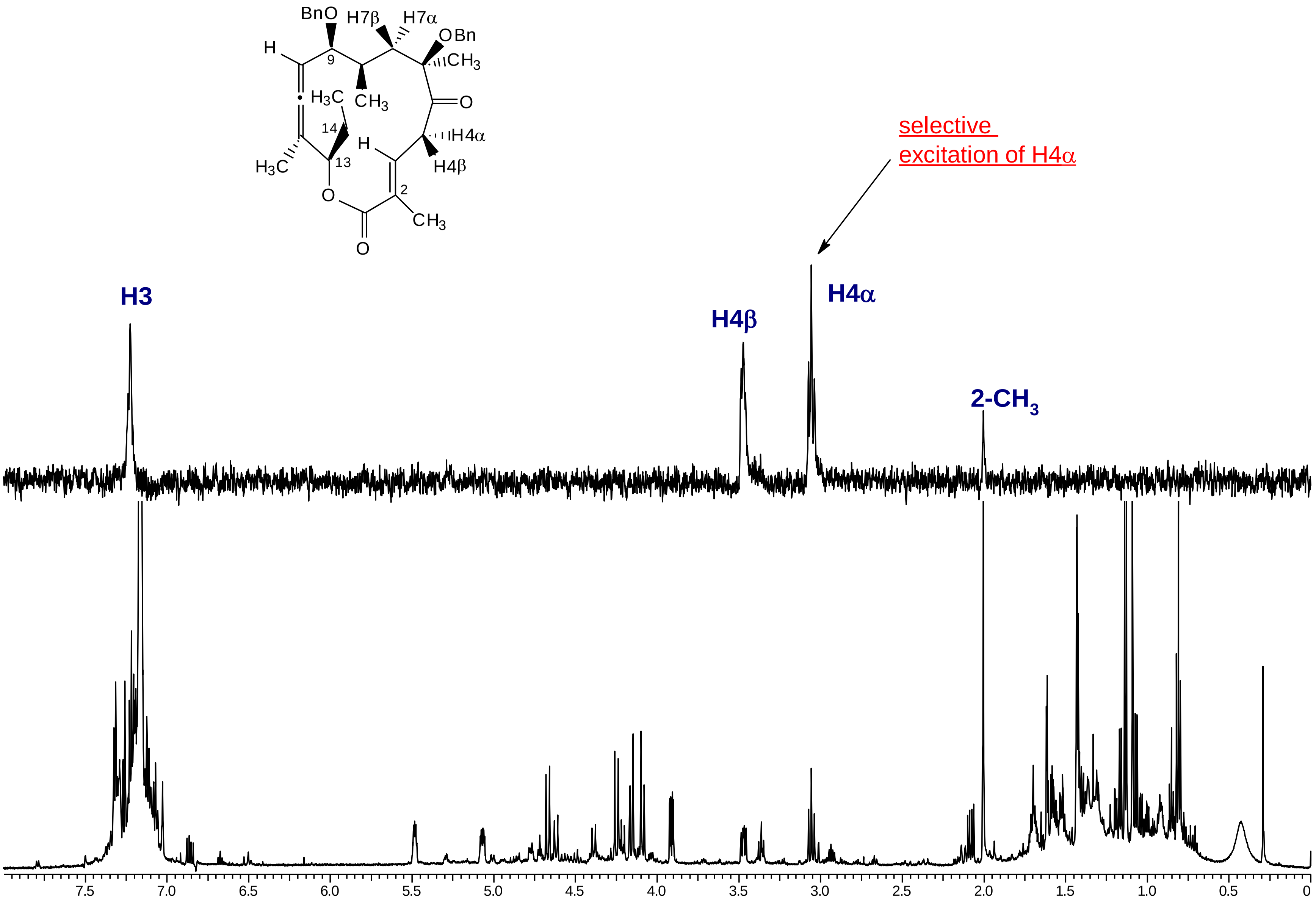


Selective decoupled ^{1}H NMR spectrum

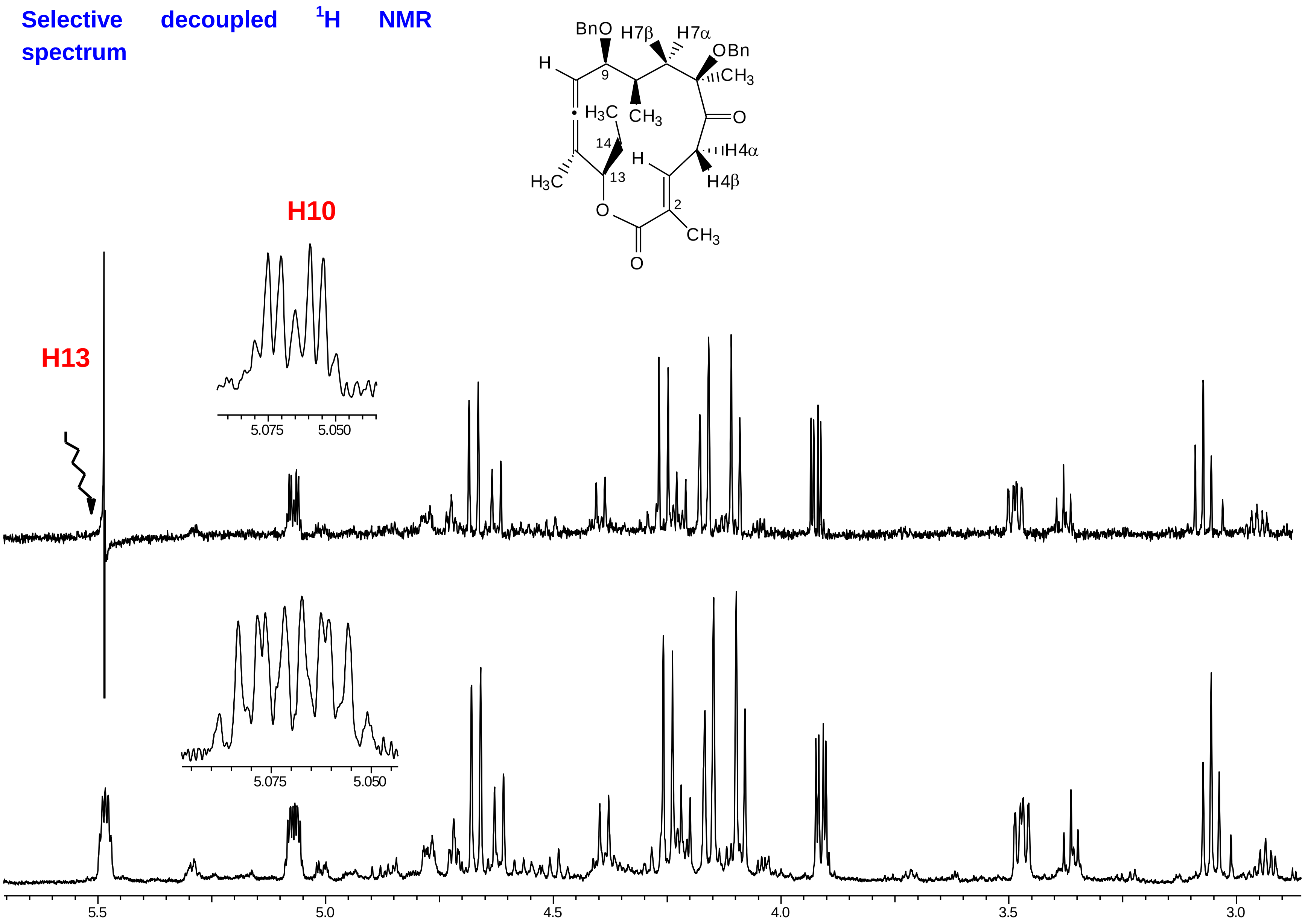



BnO
H7β
H7α
OBn
CH3
O
H
9
H3C
CH3
14
13
H3C
O
H
2
CH3
H4α
H4β
O
H3
7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
6.95

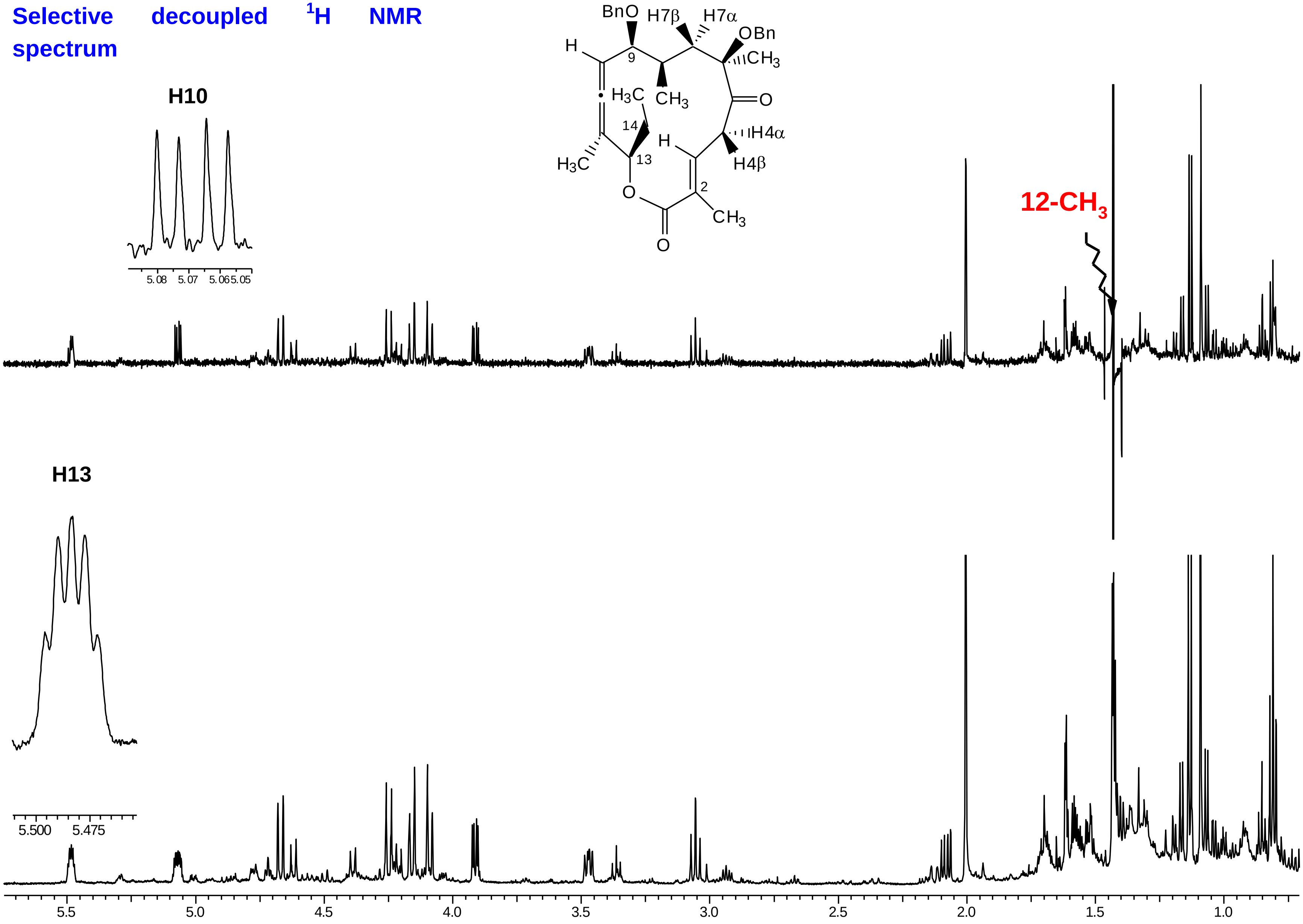
1D TOCSY


BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
H4α
H4β
H3C
13
2
O
CH3
O
selective excitation of H4α
H3
H4β
H4α
2-CH3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

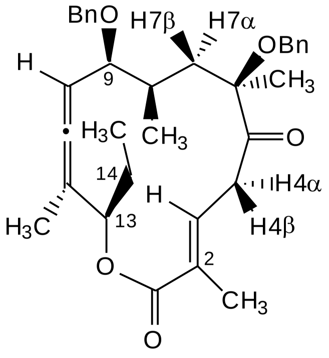
Selective decoupled ^{1}H NMR spectrum

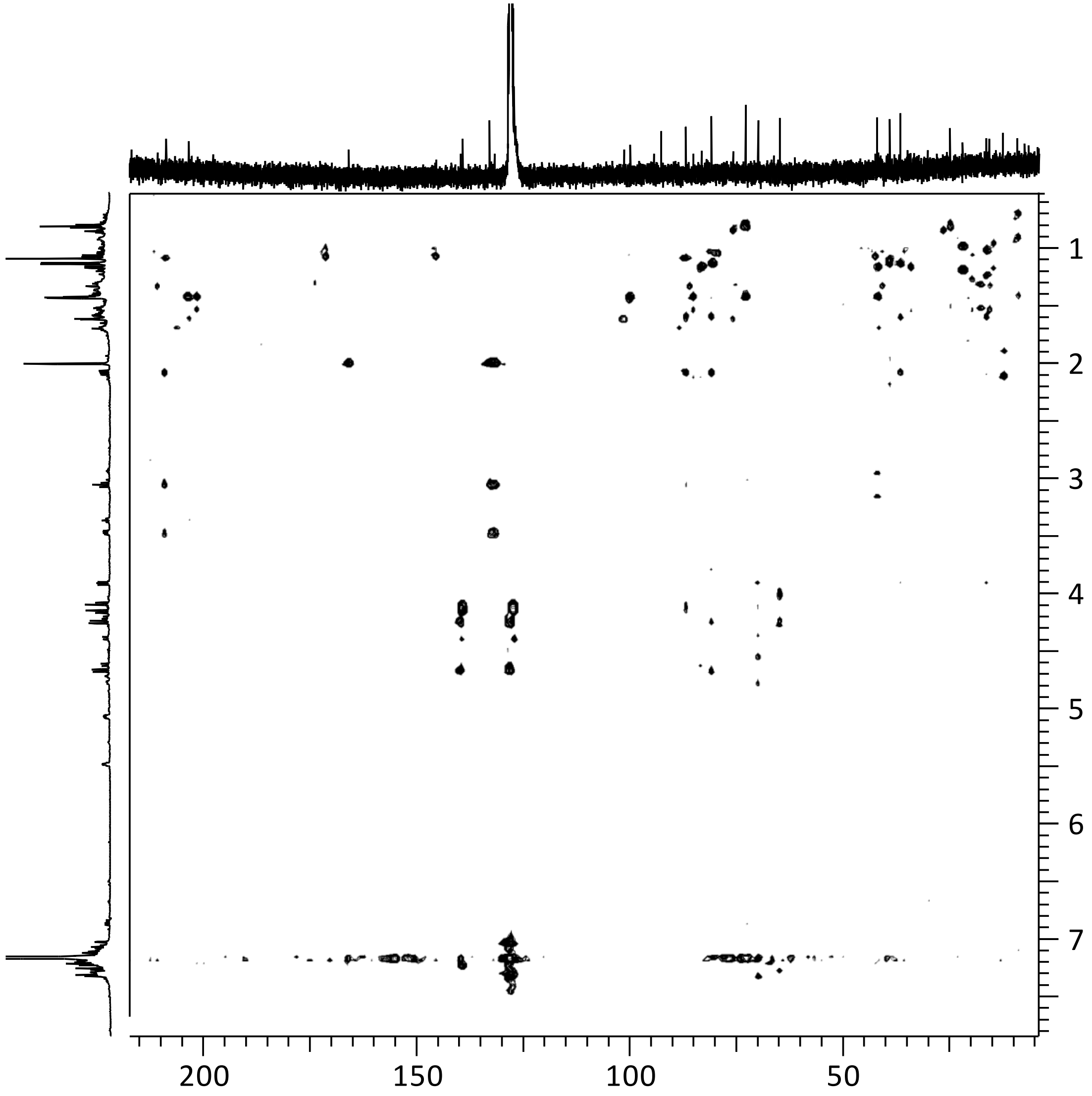


Selective decoupled ^{1}H NMR spectrum

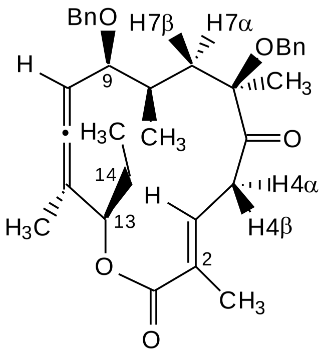


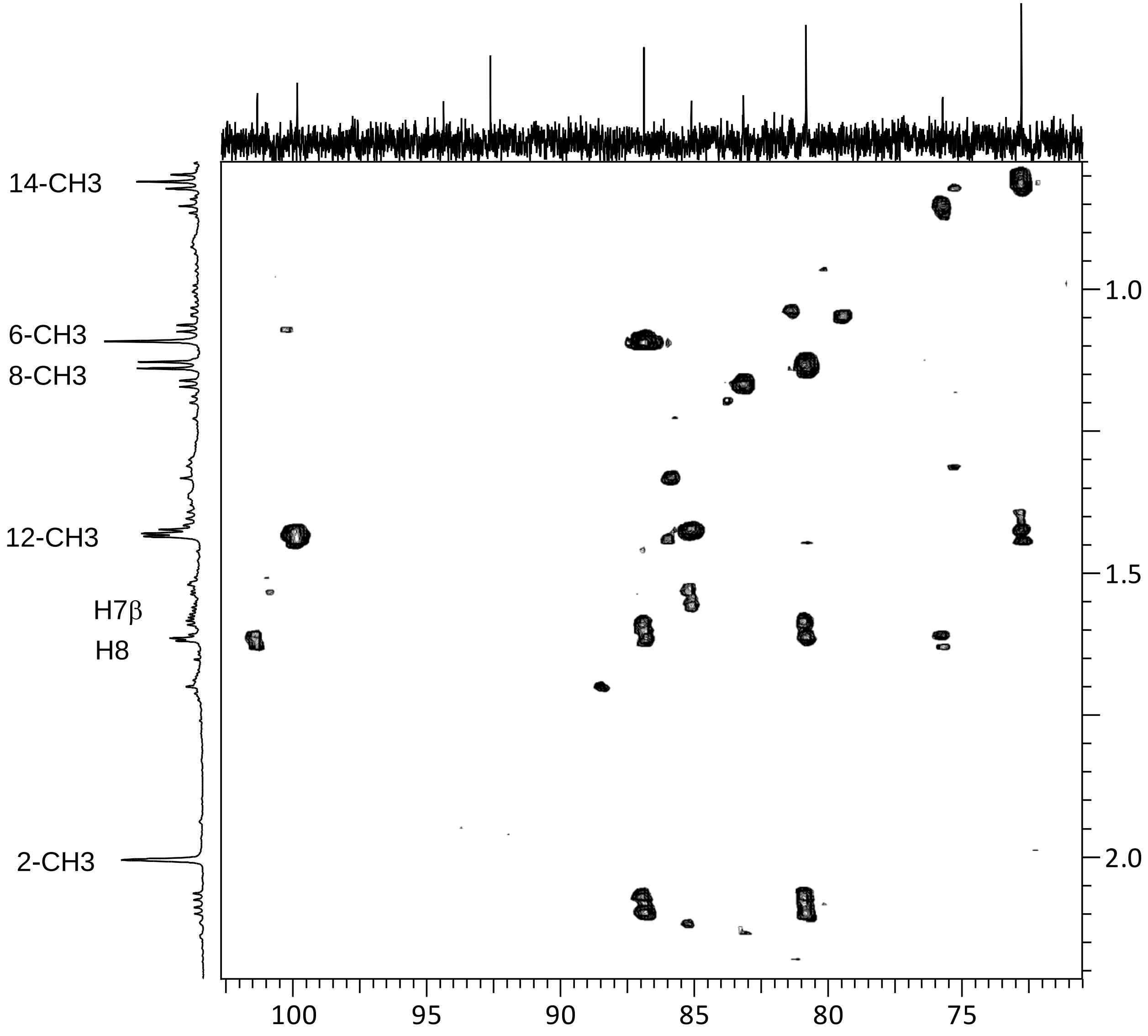
GHMBC


BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
H4α
H4β
H3C
13
O
2
CH3
O

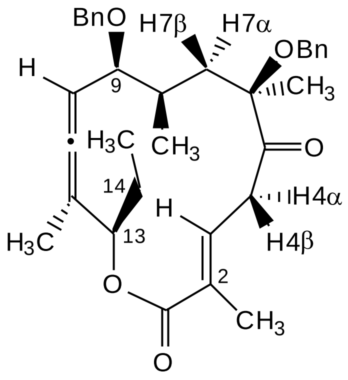

1
2
3
4
5
6
7
200
150
100
50

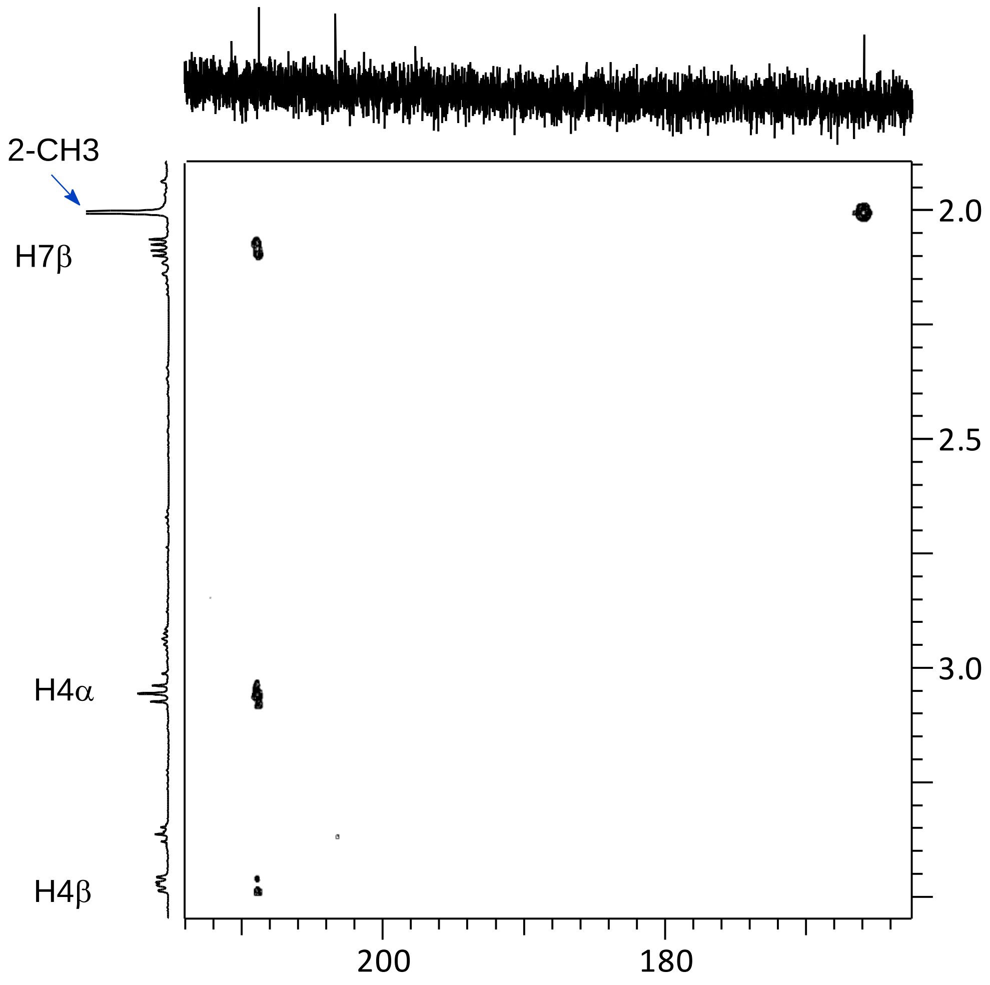
GHMBC


BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
H4α
H3C
13
H4β
2
O
CH3
O

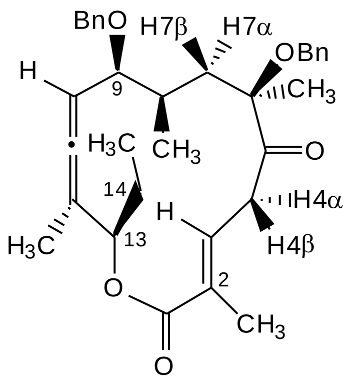

14-CH3
6-CH3
8-CH3
12-CH3
H7β
H8
2-CH3
100
95
90
85
80
75
1.0
1.5
2.0

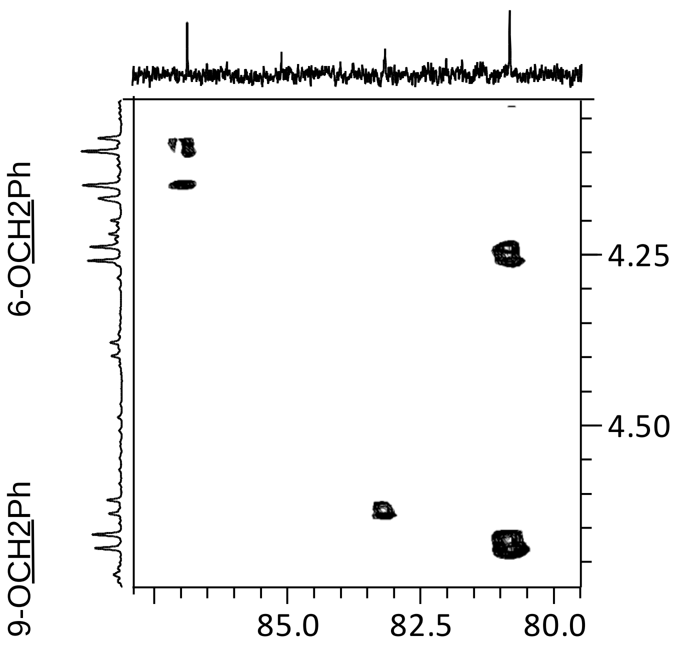
GHMBC

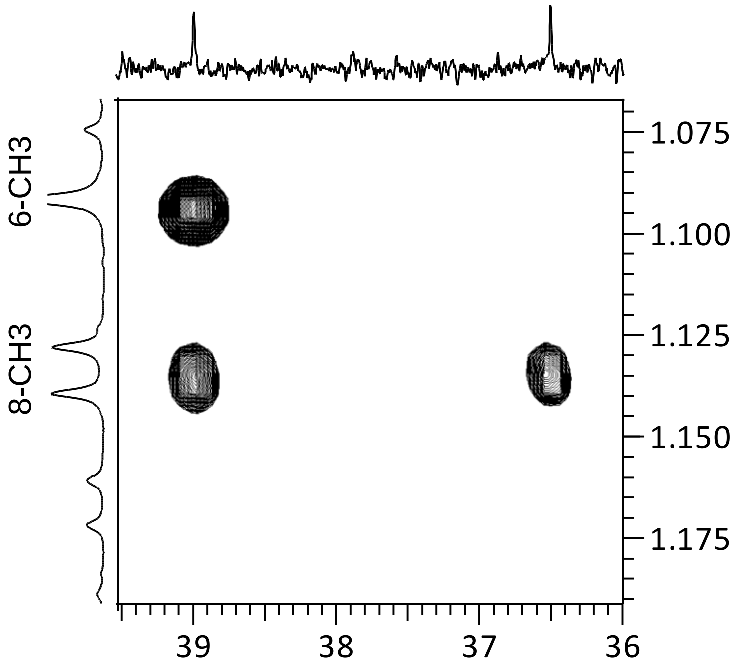

BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
H4α
H4β
H3C
13
2
O
CH3
O

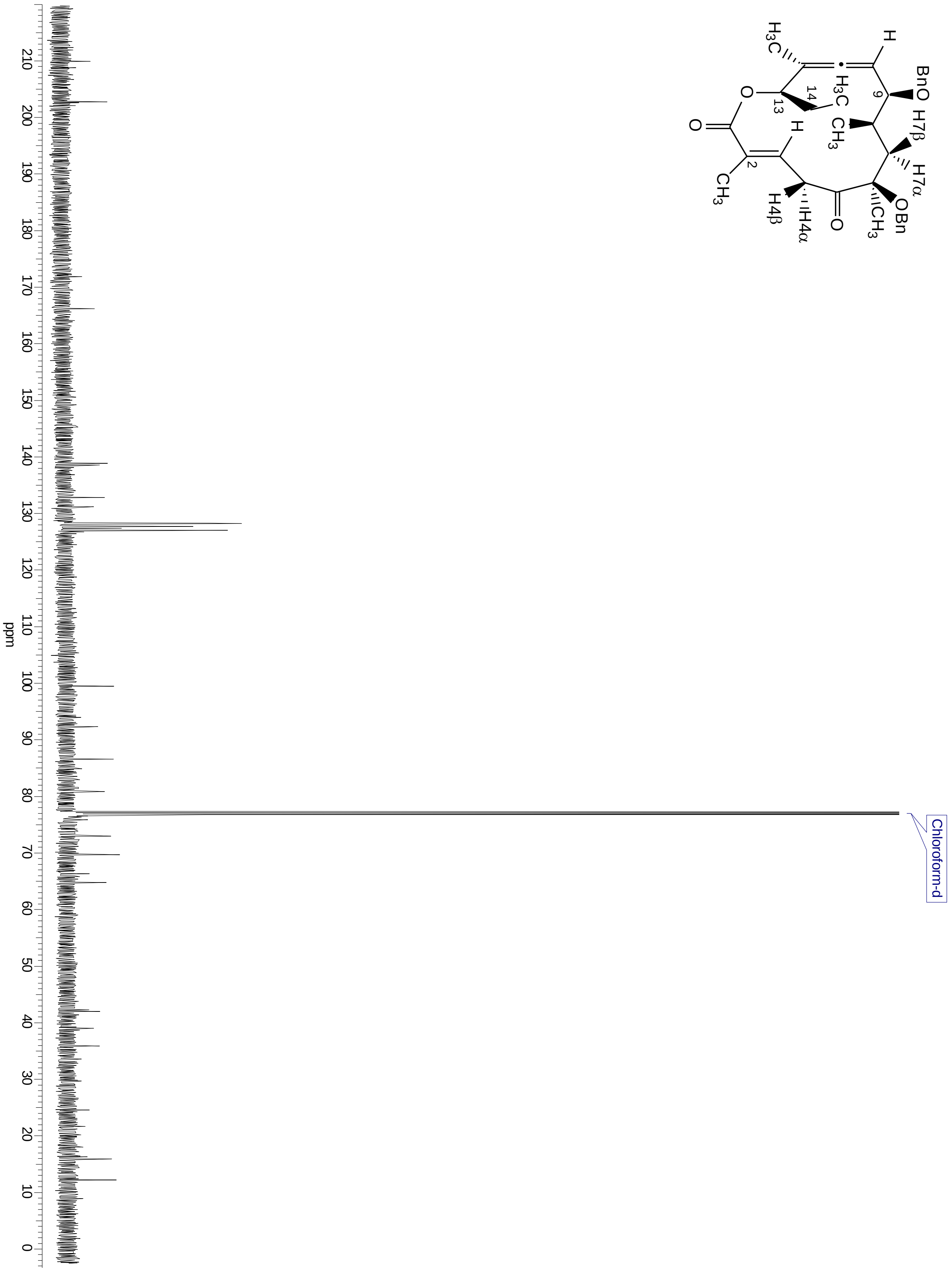

2-CH3
H7β
H4α
H4β
2.0
2.5
3.0
200
180

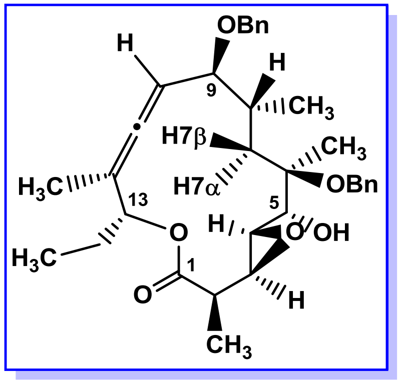
GHMBC


BnO
H7β
H7α
OBn
CH3
H
9
H3C
CH3
O
14
H
H4α
H4β
H3C
13
2
O
CH3
O


6-OCH2Ph
9-OCH2Ph
85.0
82.5
80.0
4.25
4.50


6-CH3
8-CH3
1.075
1.100
1.125
1.150
1.175
39
38
37
36


H
BnO
9
H7β
H7α
OBn
CH3
H3C
CH3
14
13
O
H
O
2
CH3
H4β
H4α
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
Chloroform-d



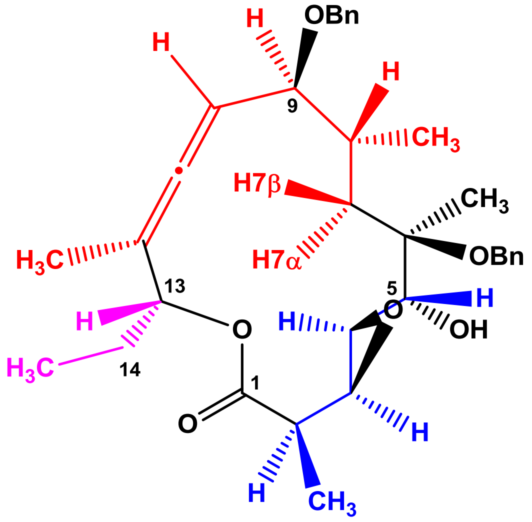
1H NMR chemical shifts (δ/ppm) coupling constant (J/Hz)

3.09 (dd, $J_{H2,\ H3}$ = 2.0Hz, $J_{H2,\ 2\text{-}CH3}$ = 7.3Hz, **H2**)
1.05 (d, $J_{2\text{-}CH3,\ H2}$ = 7.3Hz, **2-CH_3**)
3.86 (dd, $J_{H3,\ H2}$ = 2.0Hz, **H3**)
3.05 (dd, $J_{H4,\ H3}$ = 1.9Hz, **H4**)
4.26 (s, **H5**)
4.52 (d, J_{AB} = 11.0Hz, **6-OCH_2**)
4.45 (d, J_{AB} = 11.0Hz, **6-OCH_2**)
1.41 (s, **6-CH_3**)
1.45 (dd, $J_{H7\beta,\ H8}$ = 1.9 Hz, $J_{H7\beta,\ H7\alpha}$ = 14.8Hz, **H7β**)
1.68 (dd, $J_{H7\alpha,\ H8}$ = 11.2Hz, $J_{H7\alpha,\ H7\beta}$ = 14.8Hz, **H7α**)
1.87 - 1.78 (m, **H8**)
1.18 (d, $J_{8\text{-}CH3,\ H8}$ = 6.4Hz, **8-CH_3**)
3.29 (t, $J_{H9,\ H10}$ = 9.5Hz, **H9**)
4.68 (d, J_{AB} = 11.7Hz, **9-OCH_2**)
4.38 (d, J_{AB} = 11.7Hz, **9-OCH_2**)
4.92 (td, $J_{10,\ 12\text{-}CH3}$ = 2.9, $J_{H10,\ H9}$ = 9.5Hz, **H10**)
1.80 (d, $J_{12\text{-}CH3,\ H10}$ = 2.7Hz, **12-CH_3**)
4.96 (td, $J_{H13,\ H10}$ = 2.7 Hz, **H13**)
1.71 - 1.6 (m, **14-CH_2**)
0.92 (t, $J_{14\text{-}CH3,\ 14\text{-}CH2}$ = 7.3Hz, **14-CH_3**)
7.40 - 7.28 (m, 10H, **Ph**)

13C NMR chemical shift (δ/ppm)

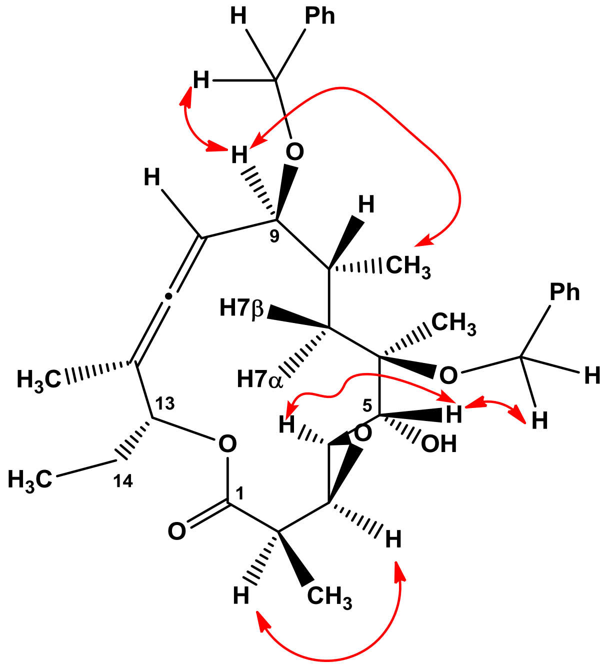
171.9 ----- C1
34.9 ----- C2
55.3 ----- C3
53.7 ----- C4
71.5 ----- C5
79.3 ----- C6
39.2 ----- C7
18.3 ----- C8
85.1 ----- C9
95.5 ----- C10
202.5 ----- C11
101.3 ----- C12
77.4 ----- C13
25.6 ----- C14
12.0 ----- 2-CH_3
19.7 ----- 6-CH_3
18.3 ----- 8-CH_3
17.0 ----- 12-CH_3
9.1 ----- 14-CH_3
64.0 ----- 6-OCH_2
70.6 ----- 9-OCH_2
136.7 ----- ^{ipso}C
128.7 ----- $^{ortho}C$
128.3 ----- ^{meta}C
127.8 ----- ^{para}C

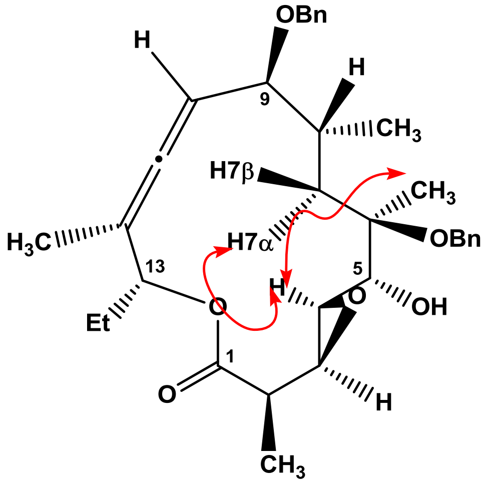
1d TOCSY

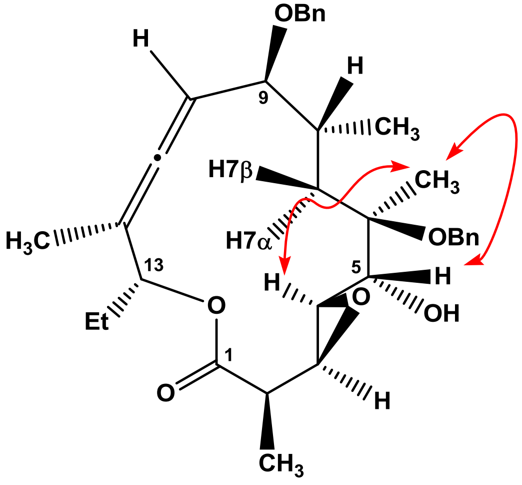


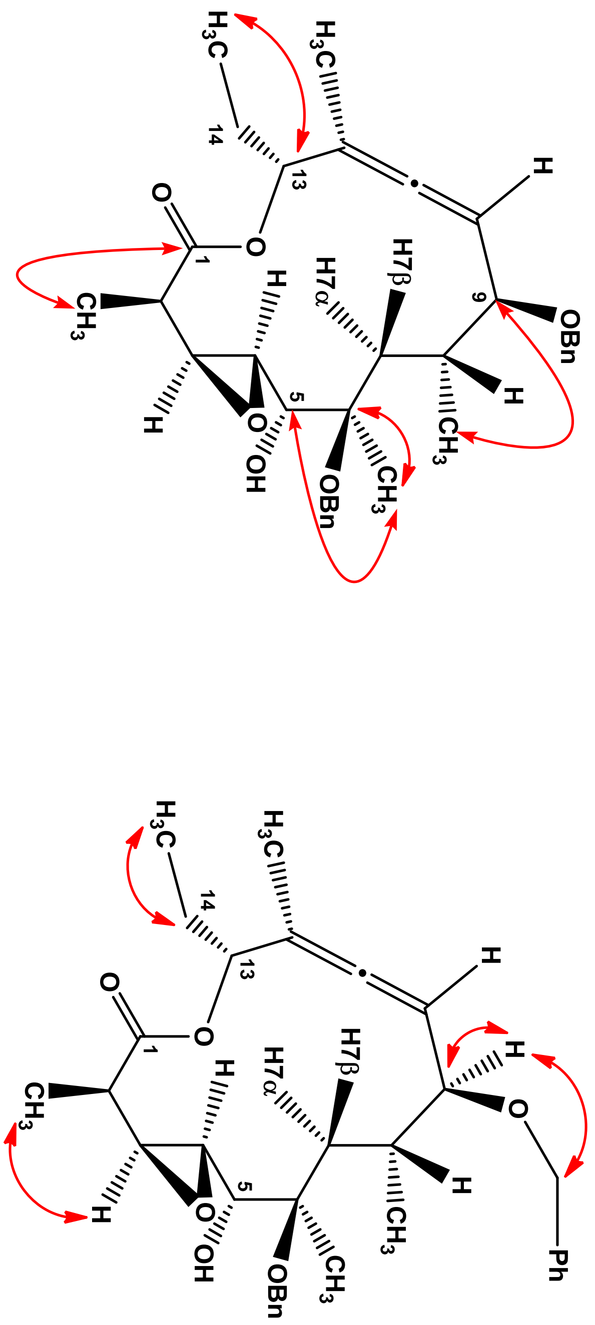
H
H
OBn
H
9
CH3
H7β
CH3
H3C
H7α
OBn
13
5
H
H
O
H
OH
H3C
14
O
1
O
H
H
CH3

NOESY

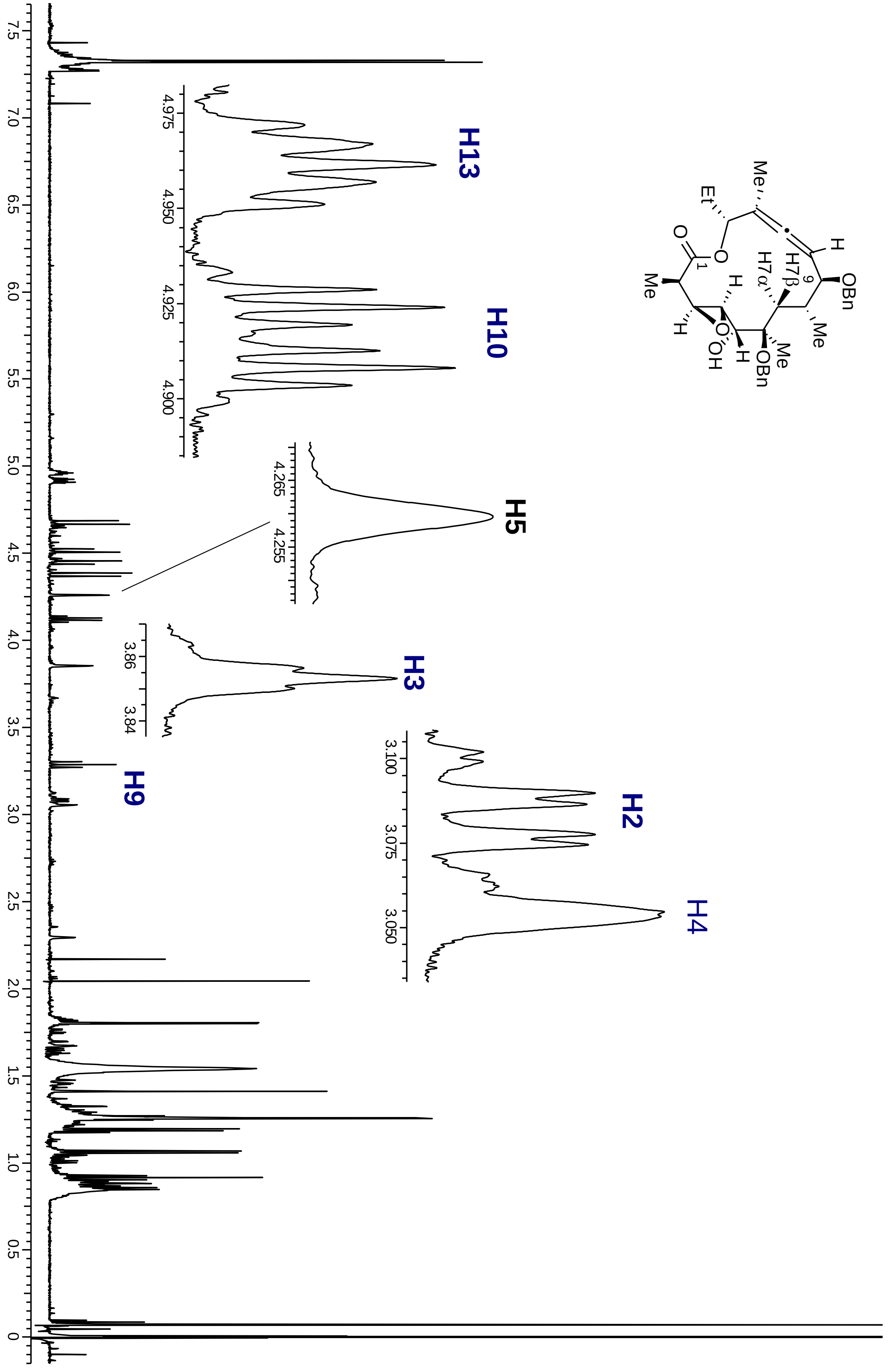

H
Ph
H
H
O
H
H
9
CH3
H7β
CH3
Ph
O
H
H3C
13
H7α
5
H
H
H
O
OH
H3C
14
O
1
O
H
H
CH3


OBn
H
9
H
CH3
H7β
CH3
H3C
H7α
OBn
13
5
H
O
OH
Et
O
1
O
H
CH3

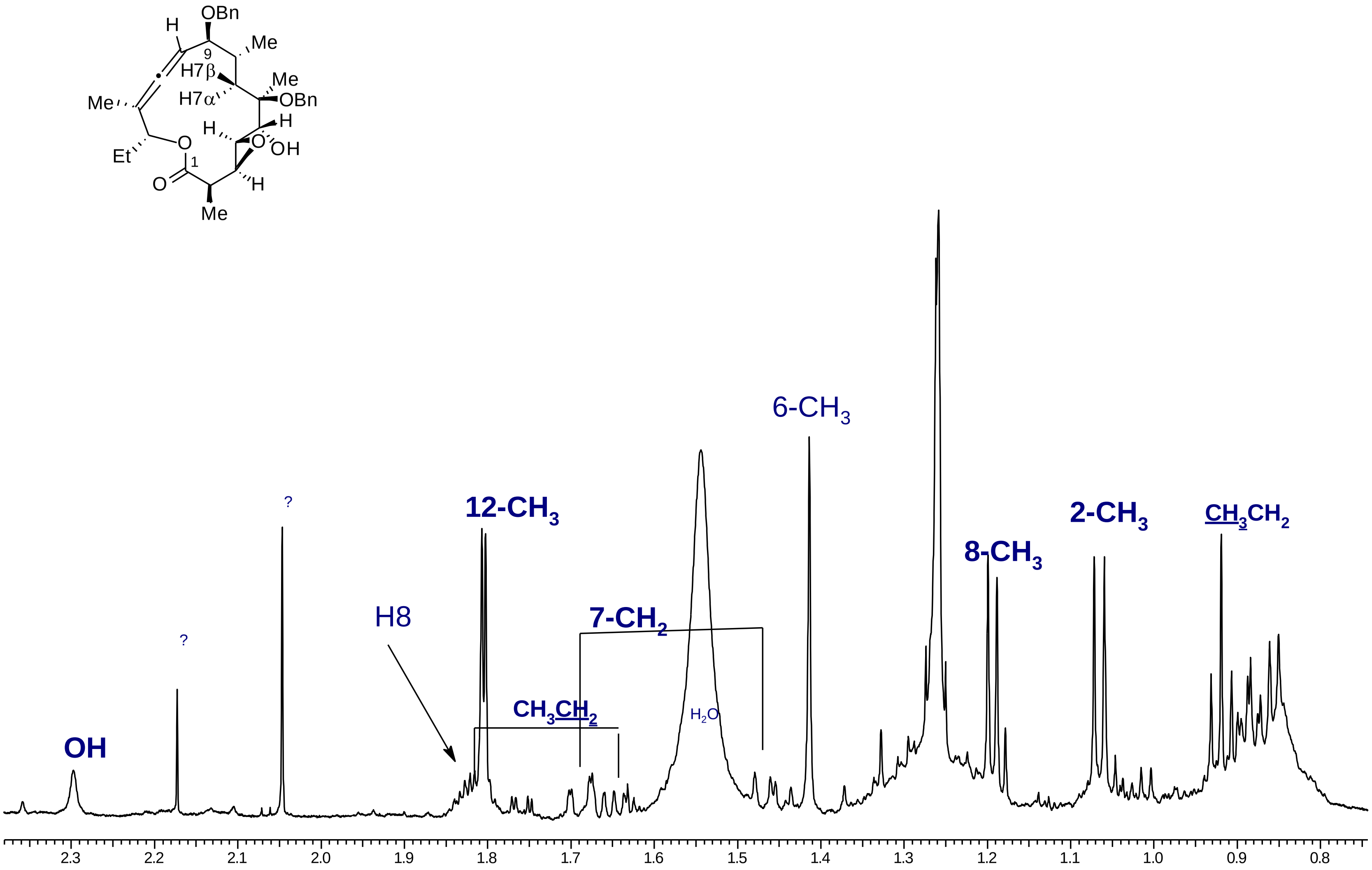

OBn
H
9
H
CH3
H7β
CH3
H3C
H7α
OBn
13
5
H
H
O
OH
Et
O
1
O
H
CH3


H3C
14
13
H3C
H
O
1
O
CH3
H
H7α
H7β
9
OBn
5
H
O
HO
OBn
CH3
CH3
H
Ph

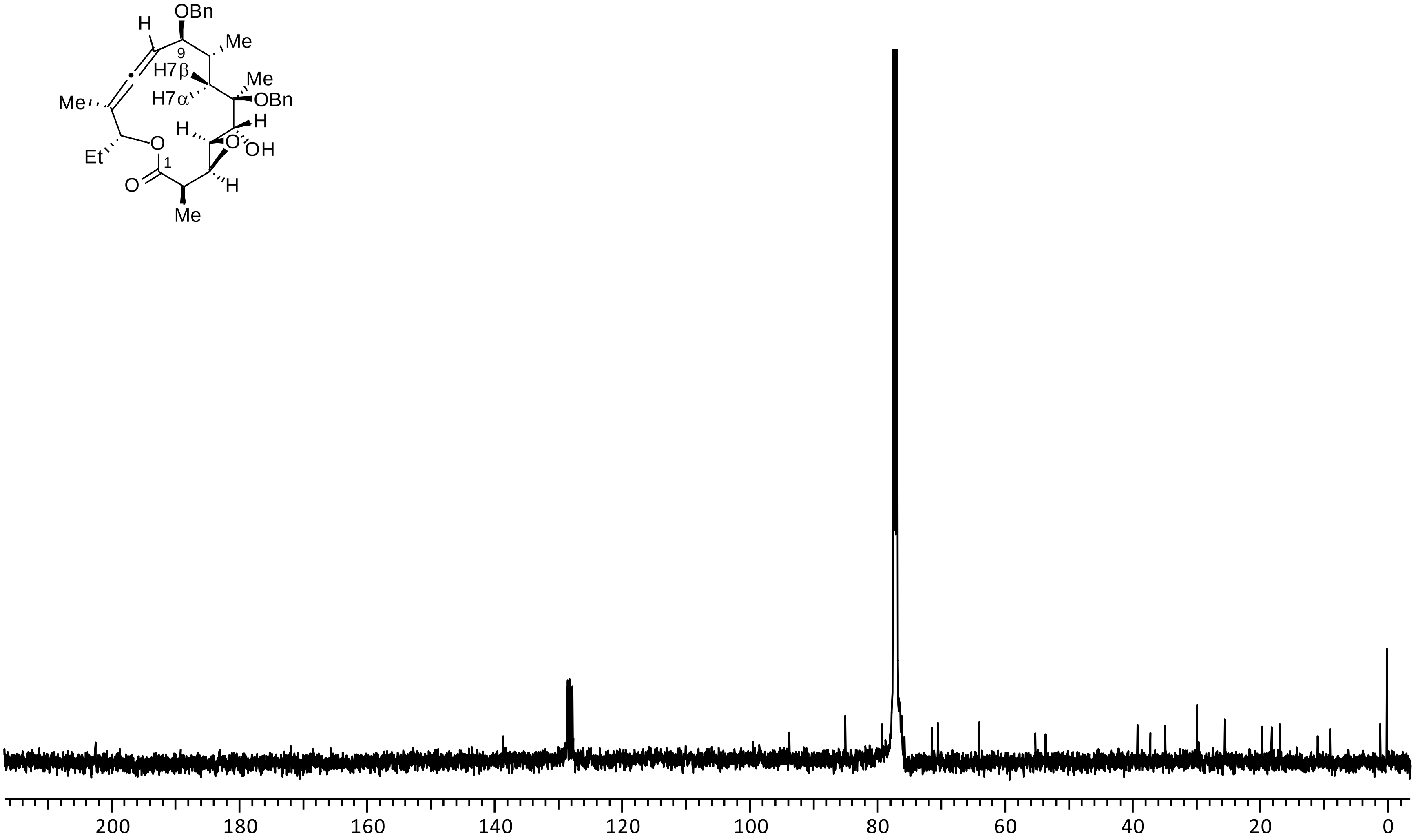
HMBC


Me
Et
O
1
H7β
9
H
OBn
Me
H7α
H
Me
OBn
OH
H
O
H
Me
H13
H10
H5
H3
H9
H2
H4
4.975
4.950
4.925
4.900
4.265
4.255
3.86
3.84
3.100
3.075
3.050
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

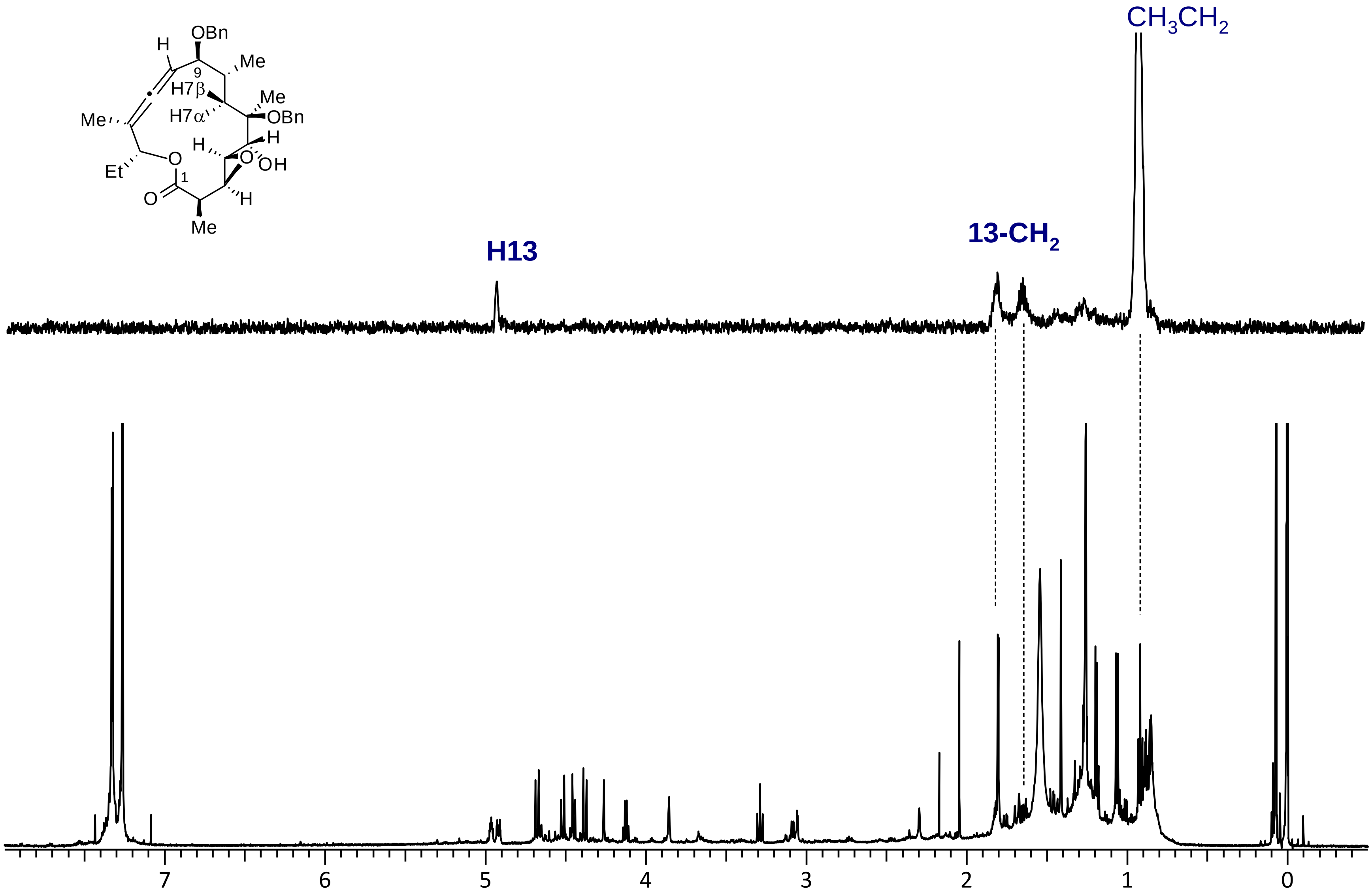
^1H NMR spectrum



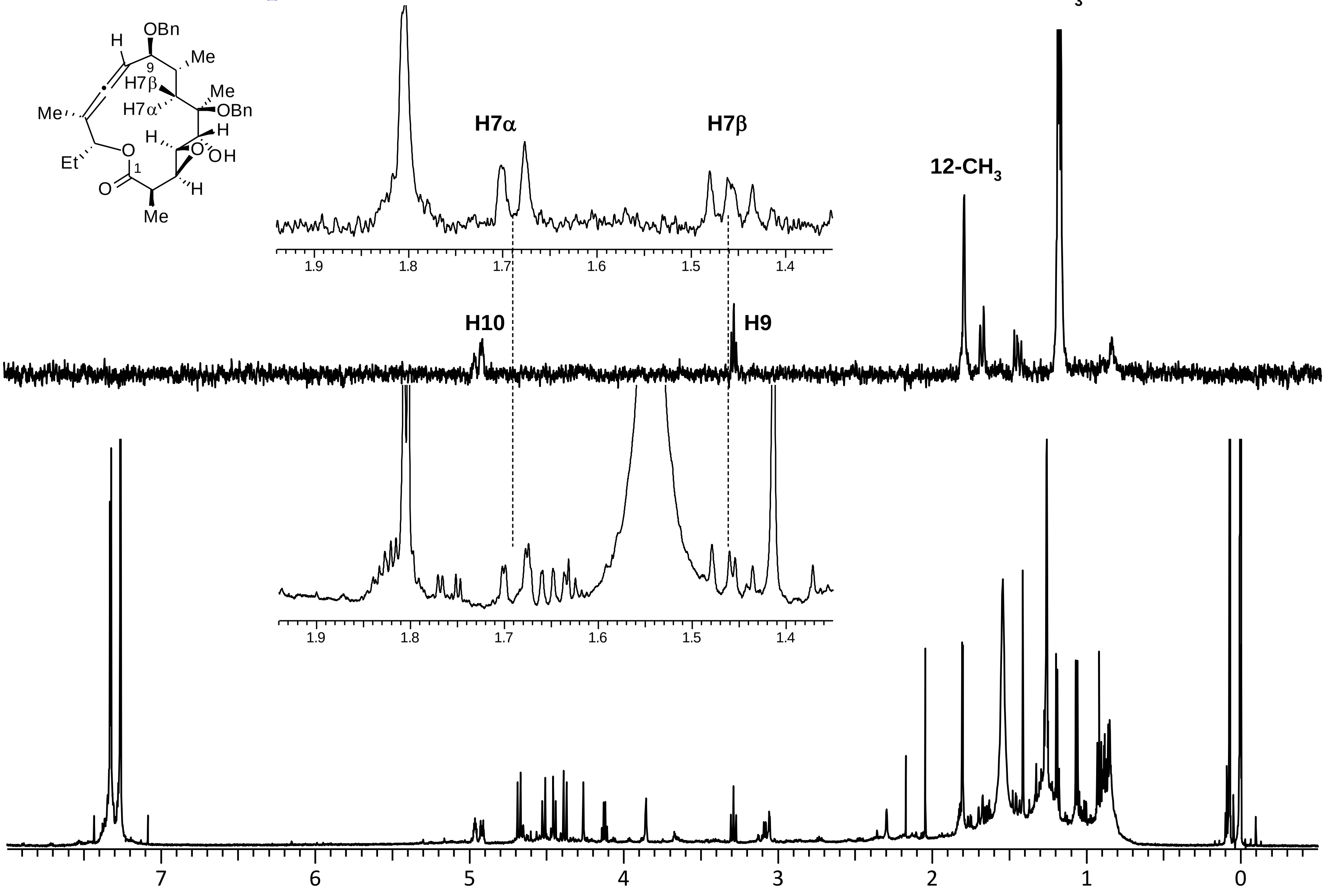
^{13}C NMR spectrum

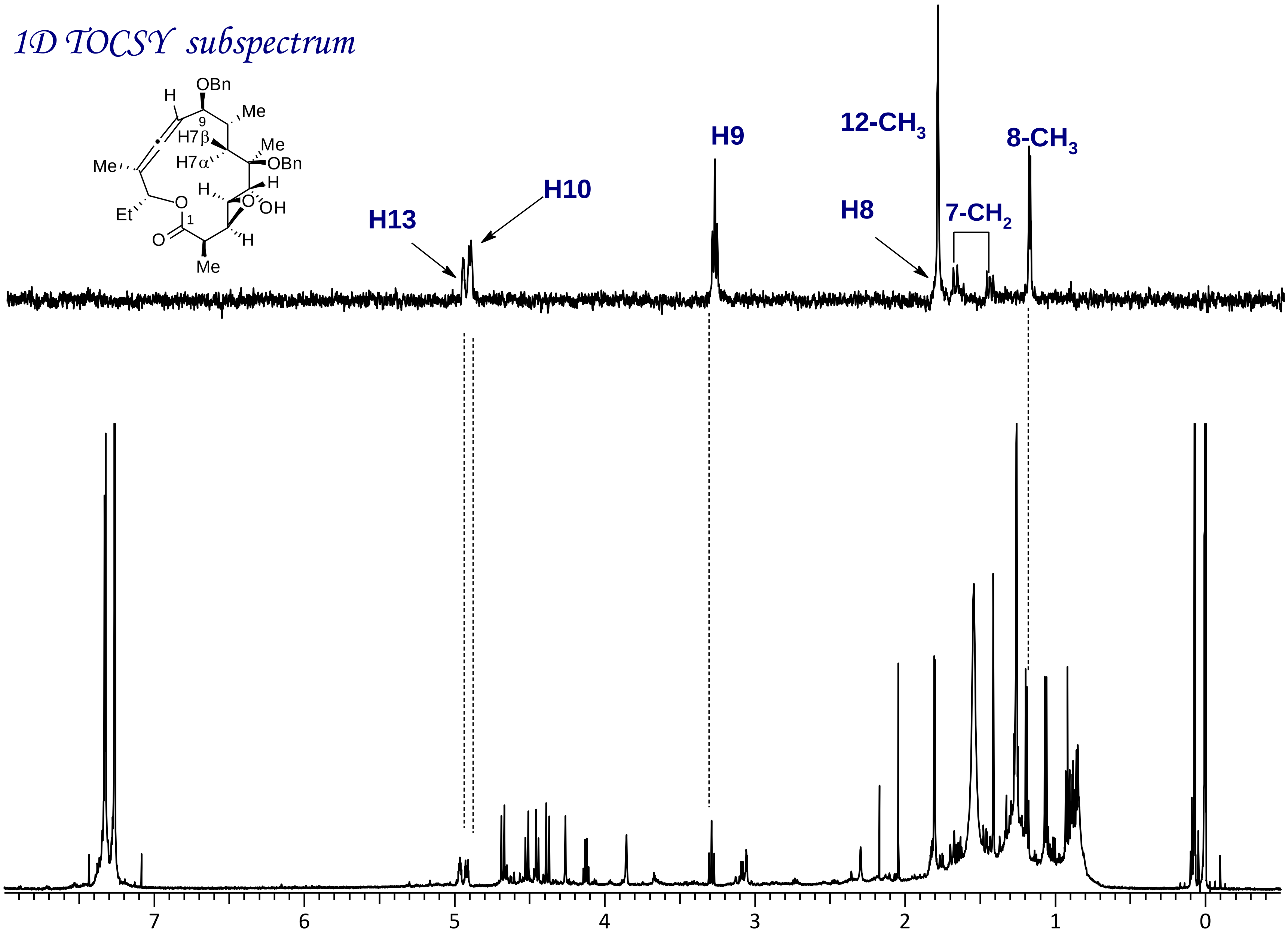


1D TOCSY subspectrum

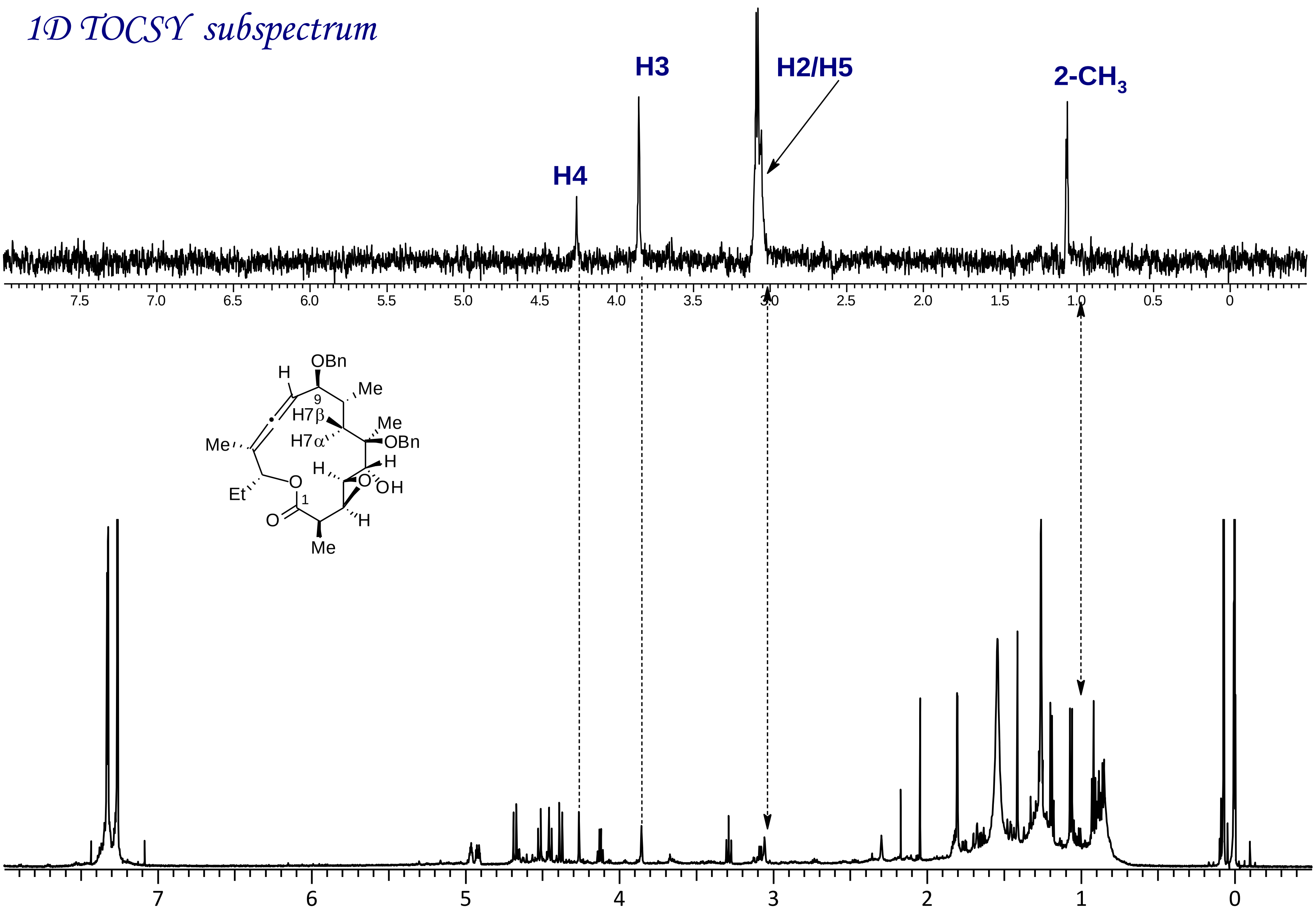


1D TOCSY subspectrum



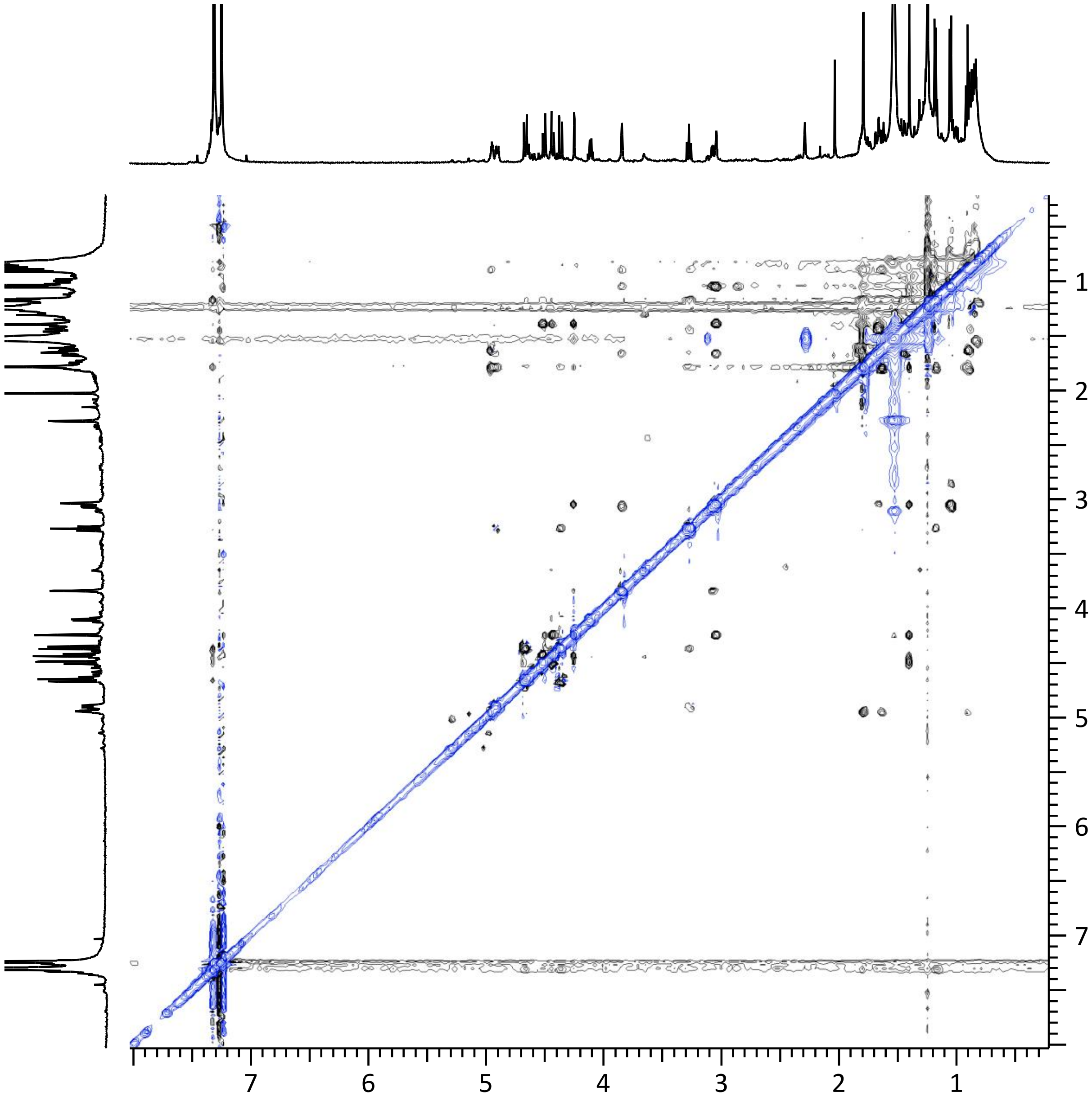


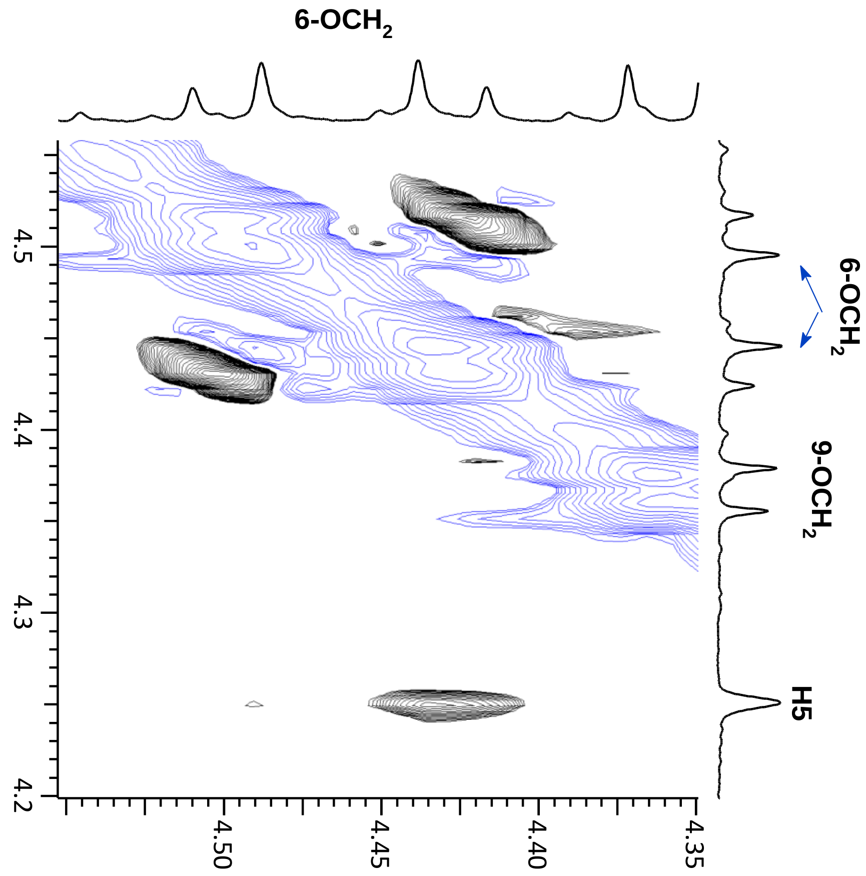
1D TOCSY subspectrum

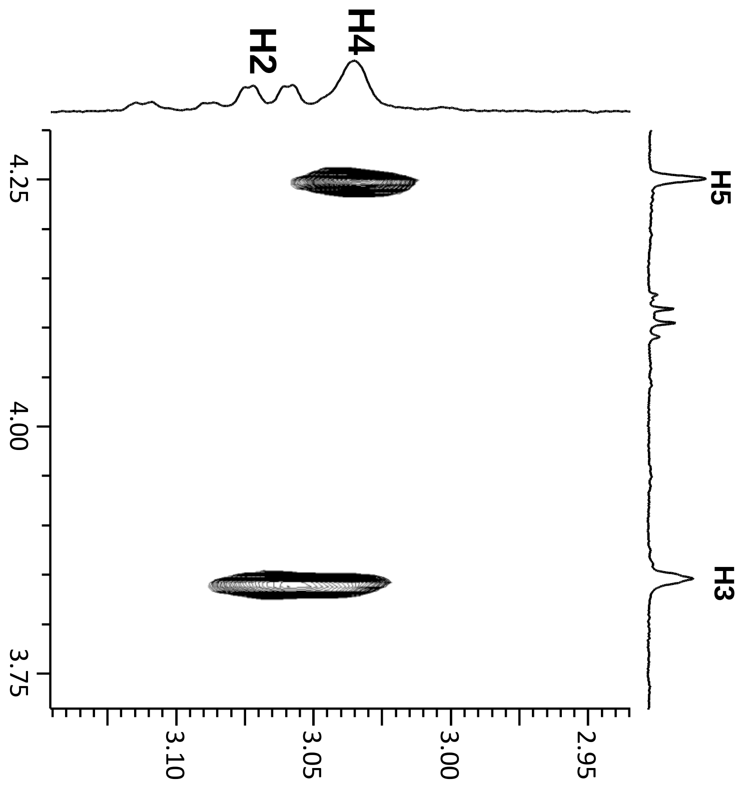


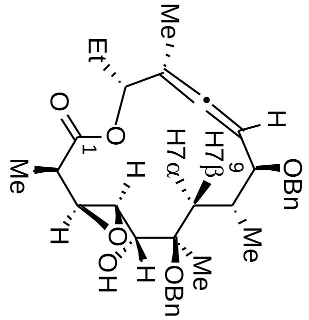
1D TOCSY subspectrum

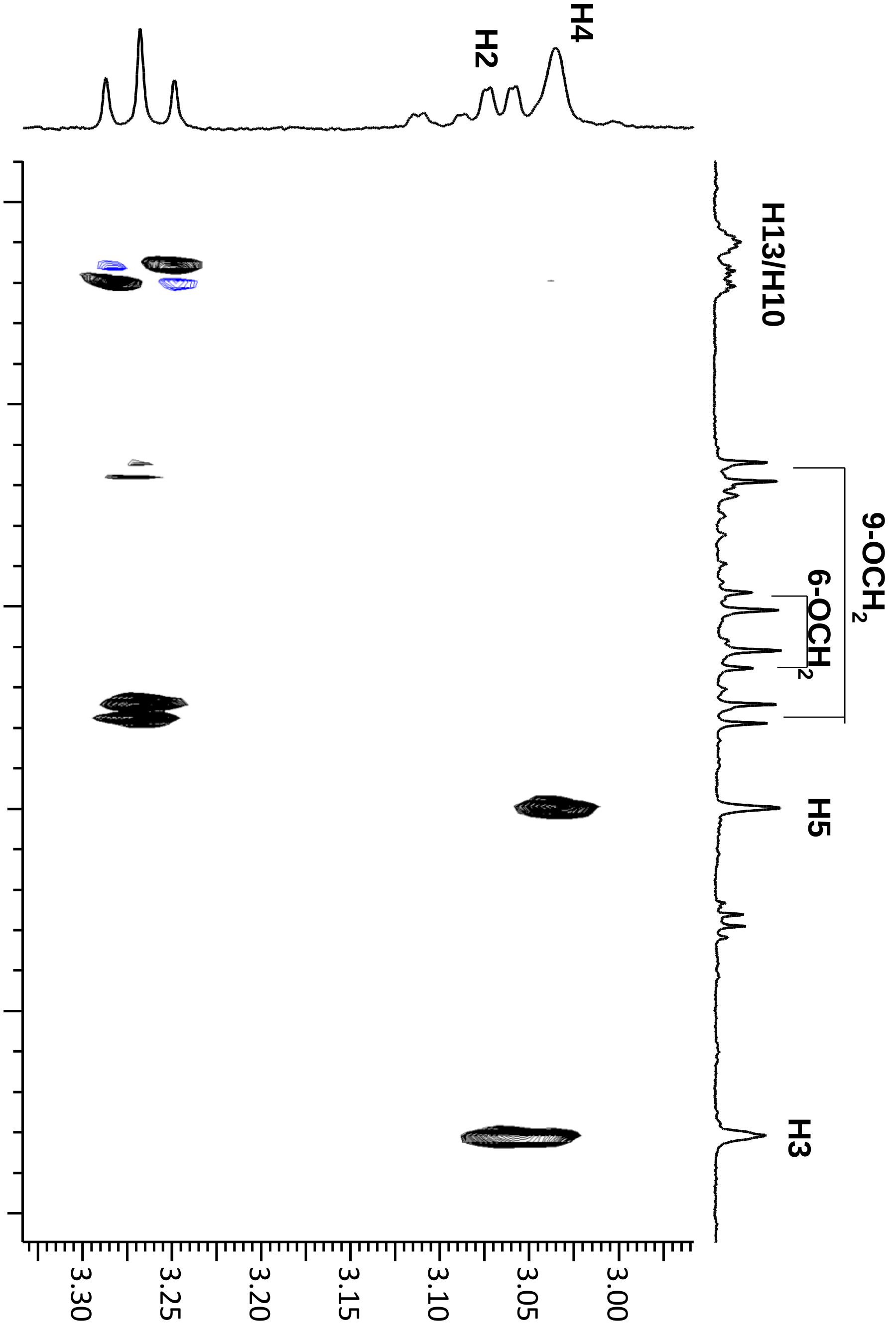
NOESY

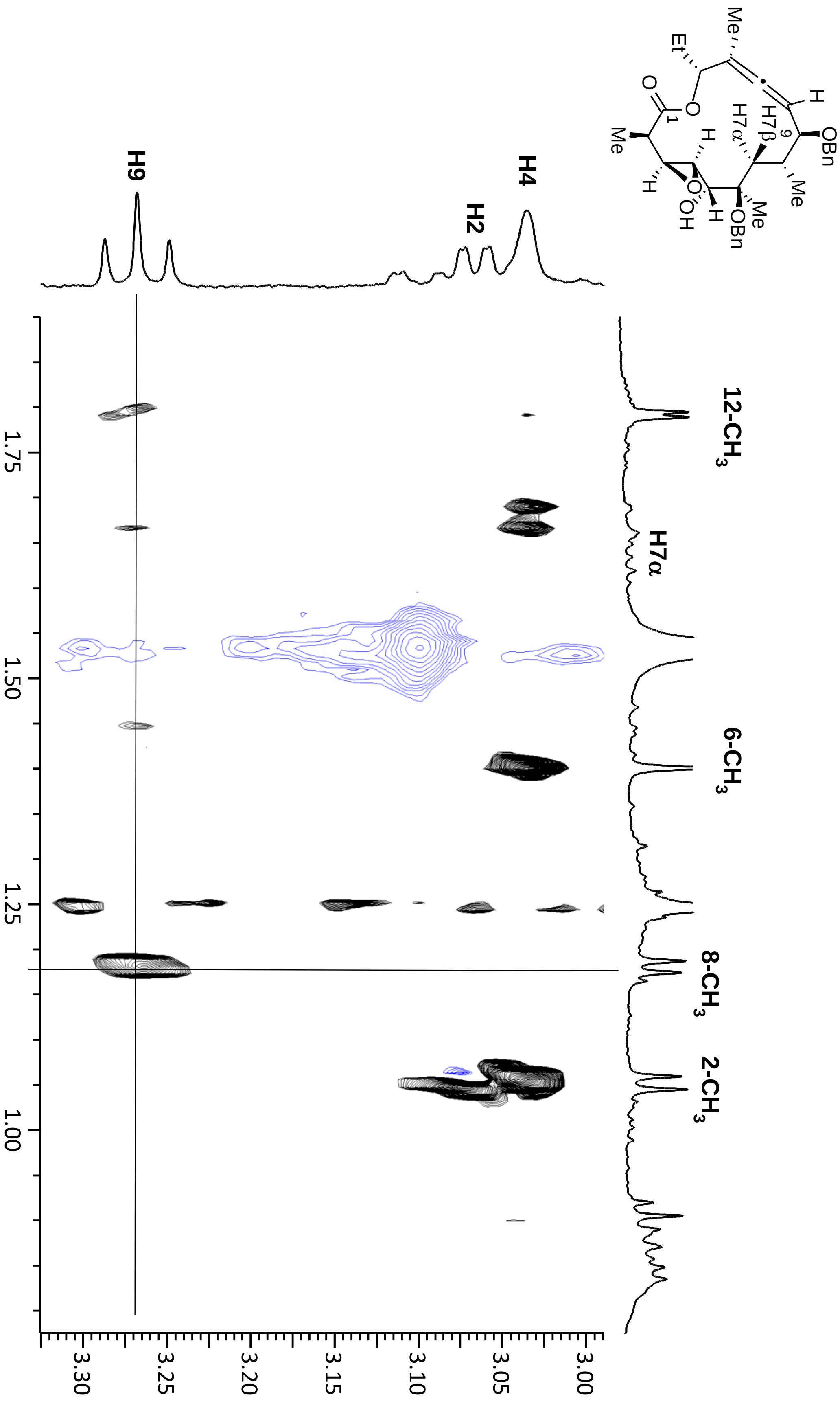


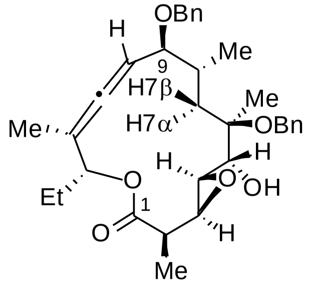

6-OCH$_2$
6-OCH$_2$
9-OCH$_2$
H5
4.5
4.4
4.3
4.2
4.50
4.45
4.40
4.35

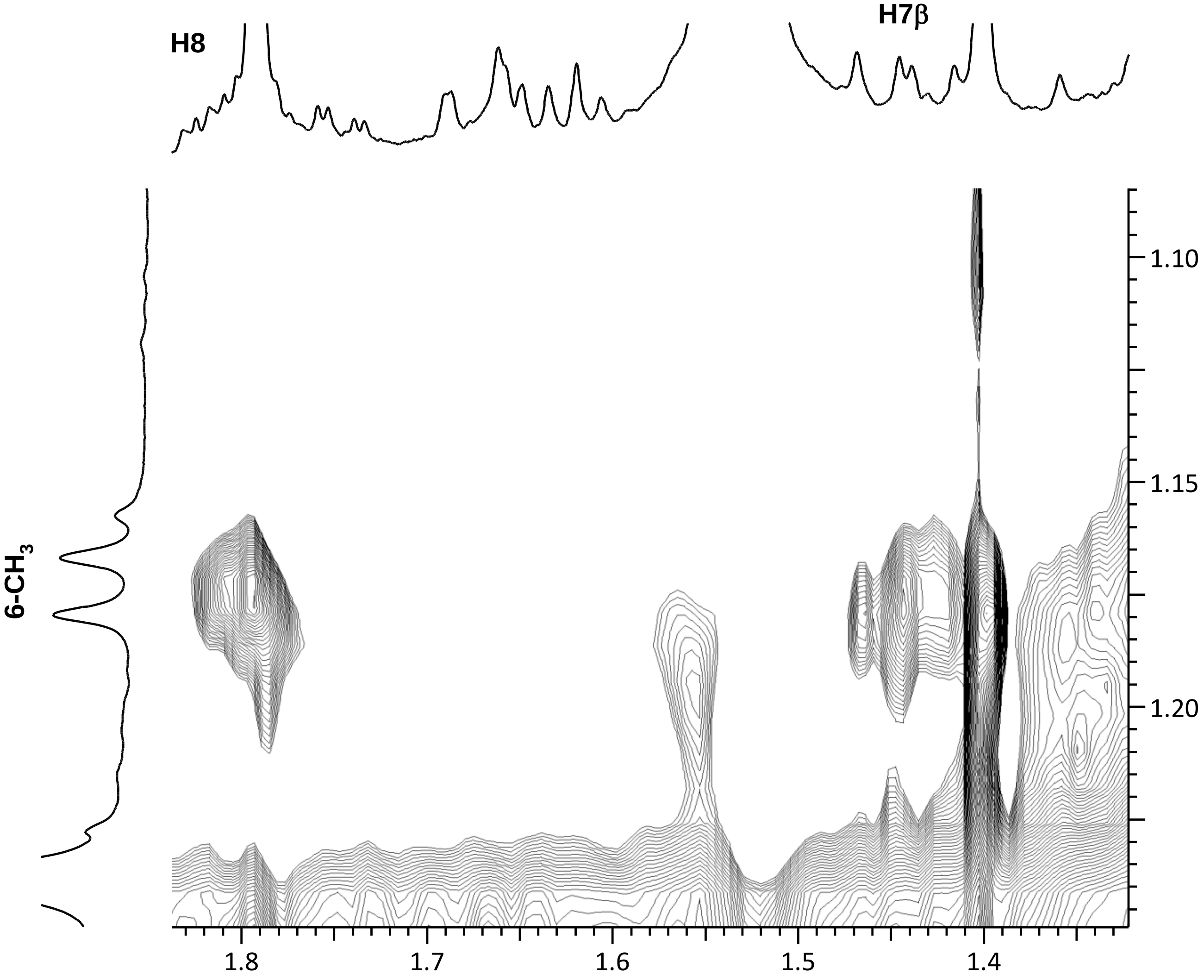

H2
H4
H5
H3
4.25
4.00
3.75
3.10
3.05
3.00
2.95

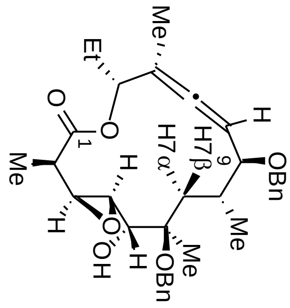

Me
Et
O
O
1
H7β
H7α
9
H
OBn
Me
Me
OBn
H
H
O
OH
H
Me

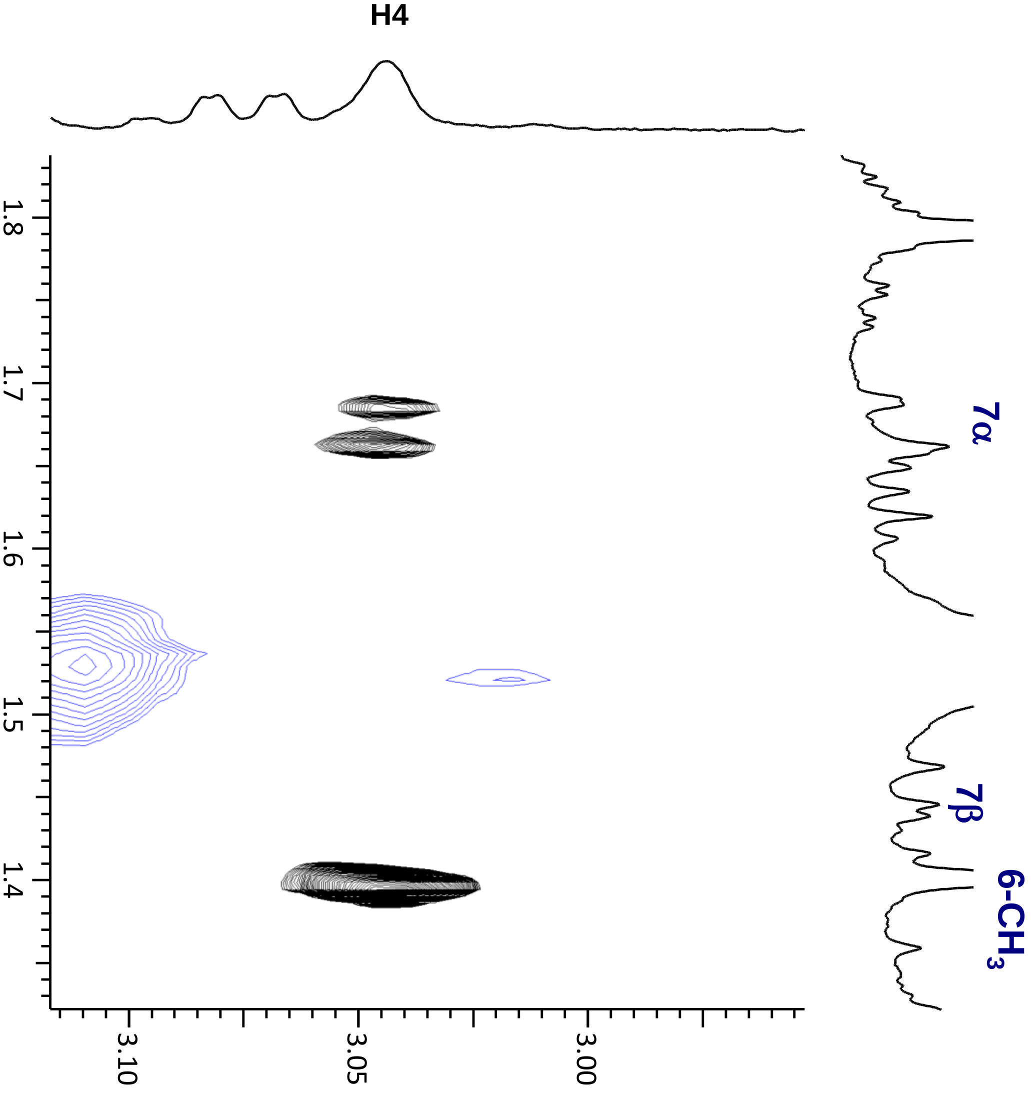

H9
H4
H2
H13/H10
9-OCH2
6-OCH2
H5
H3
5.0
4.5
4.0
3.30
3.25
3.20
3.15
3.10
3.05
3.00

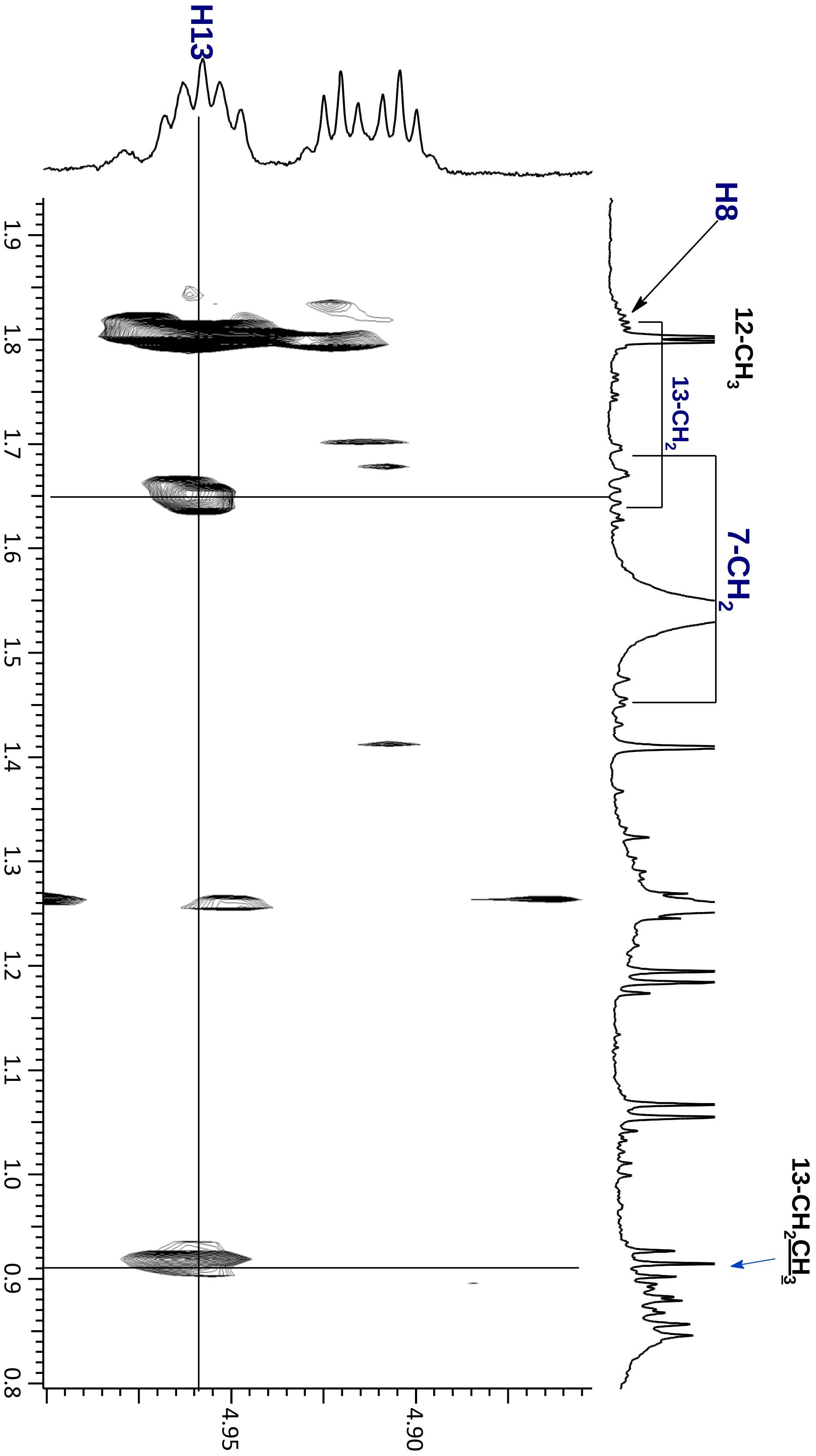

Me
Et
O
O
1
H
OBn
9
H7β
H7α
Me
H
Me
OBn
H
H
O
OH
Me
H9
H4
H2
12-CH$_3$
H7α
6-CH$_3$
8-CH$_3$
2-CH$_3$
1.75
1.50
1.25
1.00
3.30
3.25
3.20
3.15
3.10
3.05
3.00

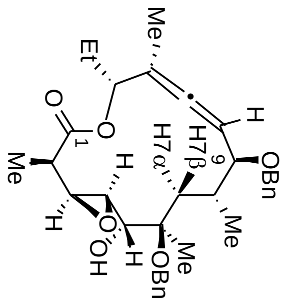

OBn
H
9
Me
H7β
Me
H7α
OBn
Me
H
H
O
OH
Et
O
1
O
H
Me

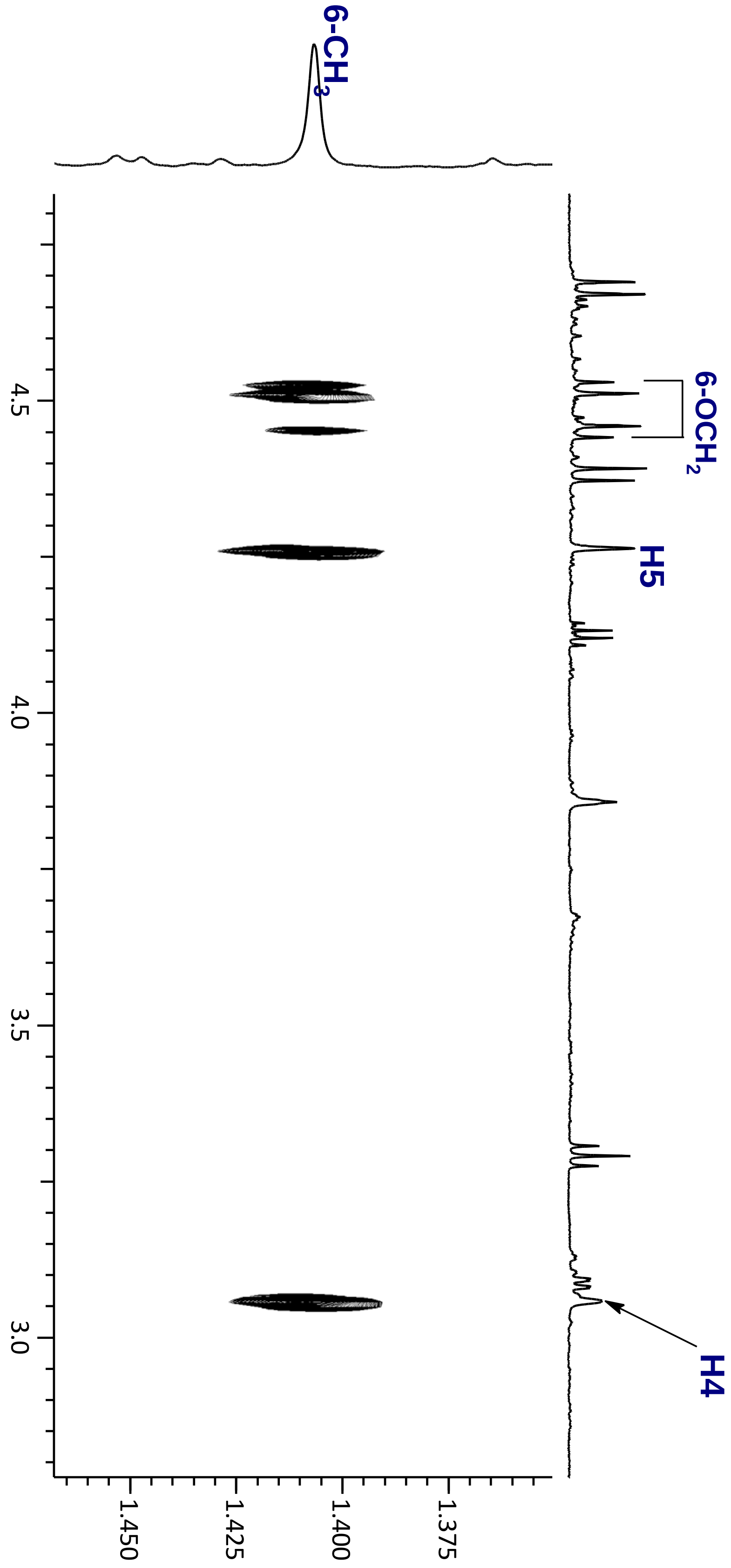

H8
H7β
6-CH3
1.10
1.15
1.20
1.8
1.7
1.6
1.5
1.4

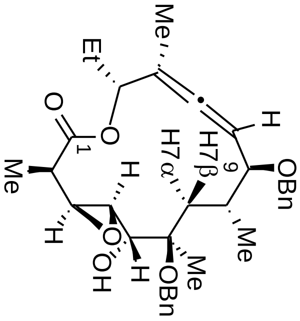

Me
Et
O
O
1
Me
H
H
O
OH
H
OBn
Me
Me
OBn
H
9
H7β
H7α

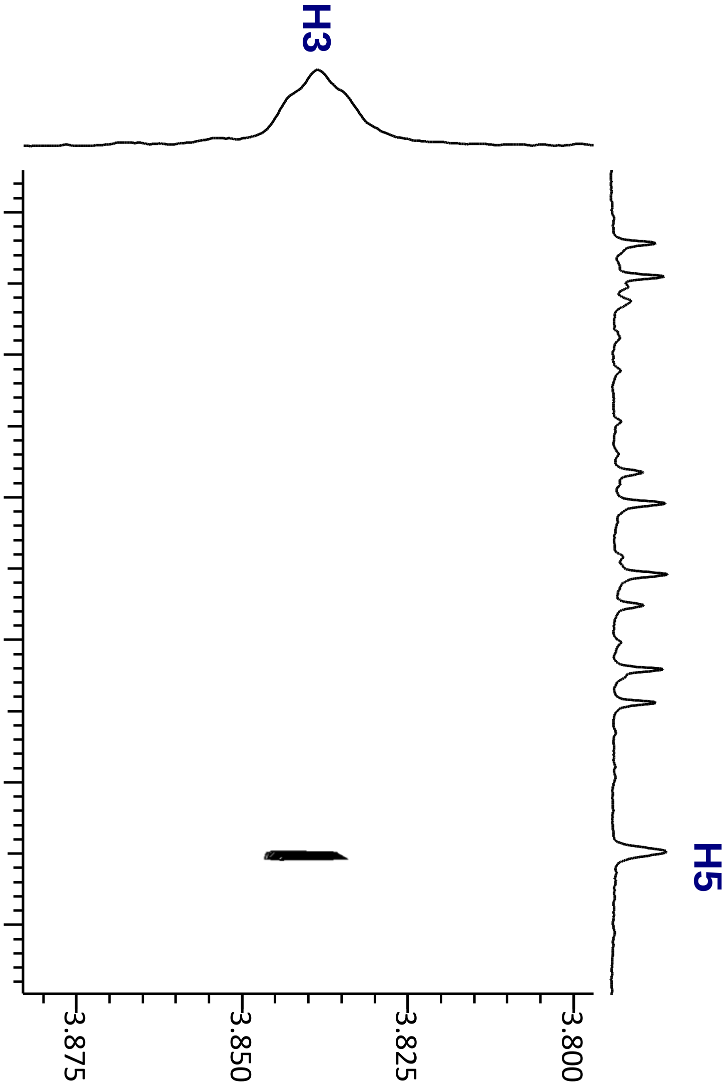

H4
7α
7β
6-CH$_3$
1.8
1.7
1.6
1.5
1.4
3.10
3.05
3.00

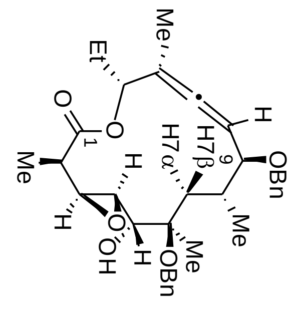

H13
H8
12-CH$_3$
13-CH$_2$
7-CH$_2$
13-CH$_2$CH$_3$
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
4.95
4.90

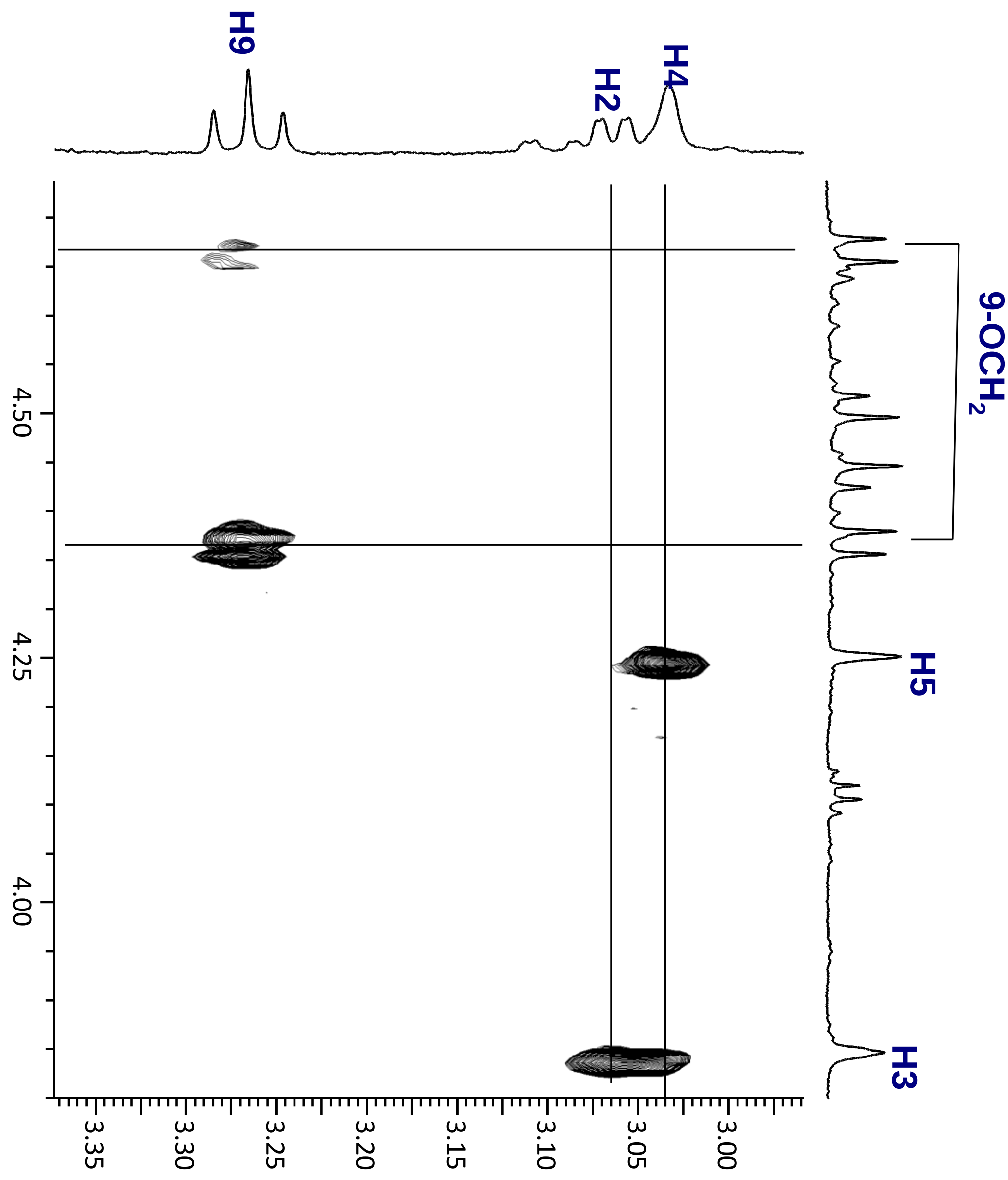

Me
Et
O
O
1
H
9
H7β
H7α
OBn
Me
Me
OBn
H
H
O
OH
Me
H

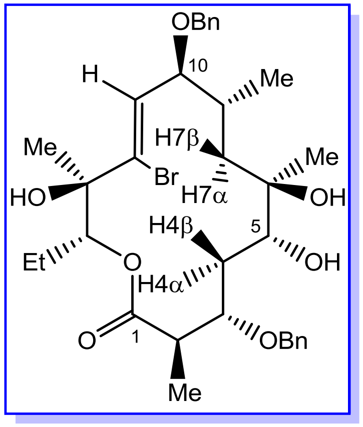

6-CH3
6-OCH2
H5
H4
4.5
4.0
3.5
3.0
1.450
1.425
1.400
1.375


Me
Et
O
O
1
Me
H
H
O
OH
H
OBn
Me
H7α
H7β
9
H
OBn
Me


H3
H5
4.7
4.6
4.5
4.4
4.3
4.2
3.875
3.850
3.825
3.800


Me
Et
O
O
1
Me
H
H7β
H7α
9
H
OBn
Me
Me
OBn
H
OH
O
H


H9
H2
H4
9-OCH$_2$
H5
H3
4.50
4.25
4.00
3.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00



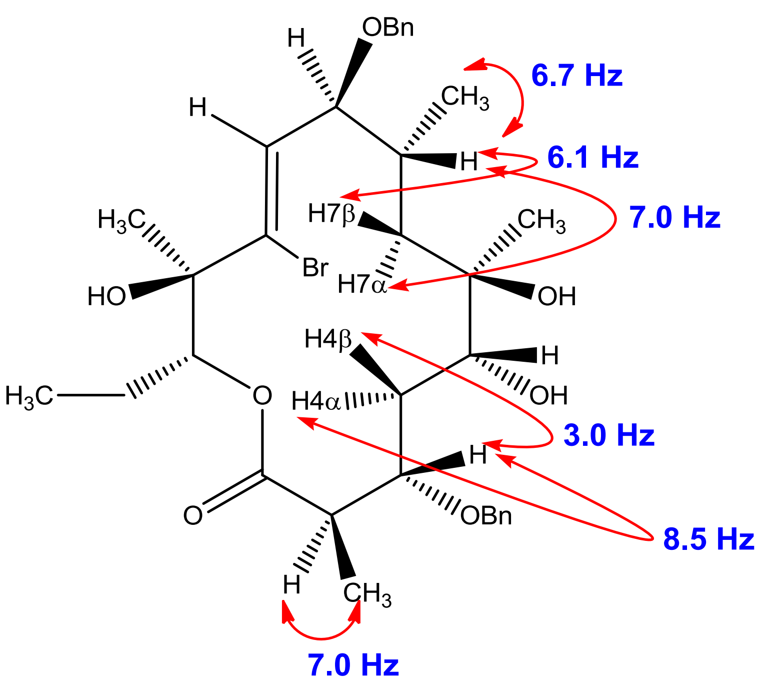
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

2.65 (qd, $J_{H2,\ 2\text{-}CH3}$ = 6.9Hz, $J_{H2,\ H3}$ = 13.4Hz, **H2**)
1.27 (d, $J_{2\text{-}CH3,\ H2}$ = 7.0Hz, **2-CH$_3$**)
4.36 (ddd. $J_{H3,\ H2}$ = 8.6Hz, $J_{H3,\ H4\alpha}$ = 6.2Hz, $J_{H3,\ H4\beta}$ = 2.9Hz, **H3**)
4.58, 4.51 (d, J_{AB} = 10.7Hz, **C3-OCH$_2$**)
1.83 (dd, $J_{H4\alpha,\ H4\beta}$ = 15.0Hz, $J_{H4\alpha,\ H3}$ = 8.5 Hz, **H4α**)
1.77 (dd, $J_{H4\beta,\ H4\alpha}$ = 15.0Hz, $J_{H4\beta,\ H3}$ = 3.0 Hz, **H4β**)
3.51 (dd, $J_{H5,\ H4\alpha}$ = 2.4 Hz, $J_{H5,\ H4\beta}$ = 7.0 Hz, **H5**)
1.28 (s, **6-CH$_3$**)
1.61 (dd, $J_{H7\beta,\ H8}$ = 6.1 Hz, $J_{H7\beta,\ H7\alpha}$ = 14.7Hz, **H7β**)
1.17 (dd, $J_{H7\alpha,\ H8}$ = 7.0 Hz, $J_{H7\alpha,\ H7\beta}$ = 14.9Hz, **H7α**)
2.31 (dt, $J_{H8,8\text{-}CH3}$ = 6.4Hz, **H8**)
1.12 (d, $J_{8\text{-}CH3,\ H8}$ = 6.7Hz, **8-CH$_3$**)
4.11 (dd, $J_{H9,\ H10}$ = 8.6Hz, $J_{H9,\ H8}$ = 6.6Hz, **H9**)
4.58, 4.47 (d, J_{AB} = 12.4Hz, **9-OCH$_2$**)
6.38 (d, $J_{H10,\ H9}$ = 8.6Hz, **H10**)
1.50 (s, **12-CH$_3$**)
4.88 (dd, $J_{H13,\ H14}$ = 2.3, 11.0Hz, **H13**)
1.73-1.85 (m, **14-CH$_2$**)
0.89 (t, $J_{14\text{-}CH3,\ H14}$ = 7.4Hz, **14-CH$_3$**)

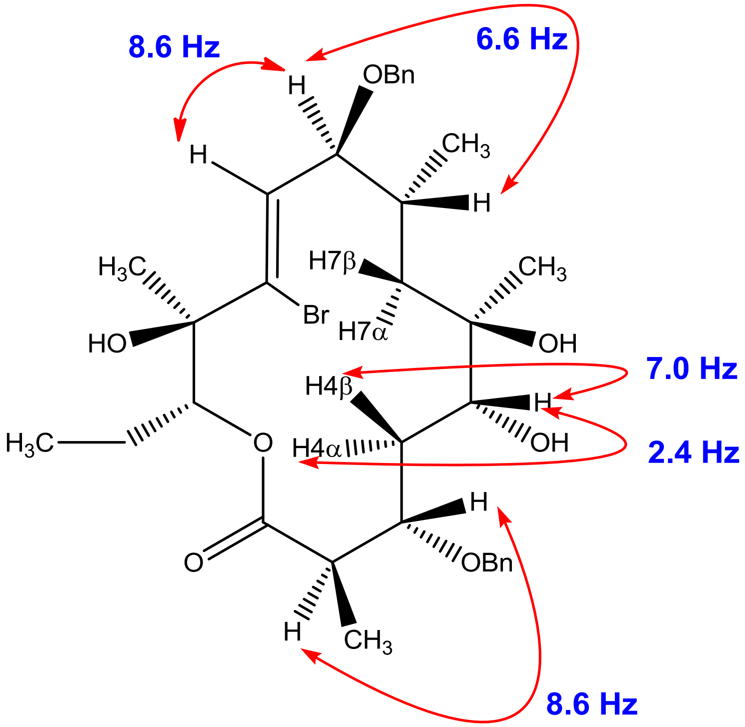
13C NMR chemical shift (δ/ppm)

176.8 ----- C1
44.8 ----- C2
78.2 ----- C3
35.9 ----- C4
75.2 ----- C5
73.7 ----- C6
40.8 ----- C7
34.6 ----- C8
84.7 ----- C9
131.4 ----- C10
133.0 ----- C11
77.6 ----- C12
81.3 ----- C13
24.7 ----- C14
12.4 ----- 2-CH$_3$
27.7 ----- 6-CH$_3$
20.0 ----- 8-CH$_3$
26.0 ----- 12-CH$_3$
11.4 ----- 14-CH$_3$
73.1 ----- 6-OCH$_3$
71.9 ----- 9-OCH$_3$
137.9, 139.0 ----- ^{ipso}C (C$_3$, C$_9$)
128.1, 128.0 ----- ^{ortho}C (C$_3$, C$_9$)
126.5, 128.3 ----- ^{meta}C (C$_3$, C$_9$)
127.9, 127.6 ----- ^{para}C (C$_3$, C$_9$)

Coupling constant (J_{HH})

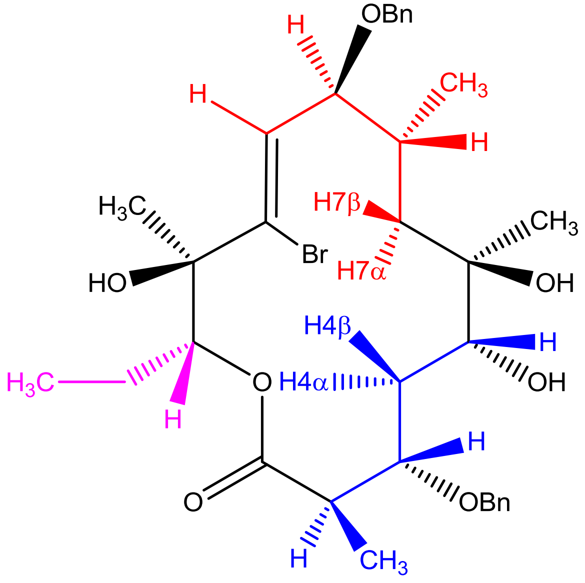


H
OBn
H
CH3
6.7 Hz
H
6.1 Hz
H3C
H7β
CH3
7.0 Hz
HO
Br
H7α
OH
H4β
H
H3C
O
H4α
OH
3.0 Hz
H
O
OBn
8.5 Hz
H
CH3
7.0 Hz

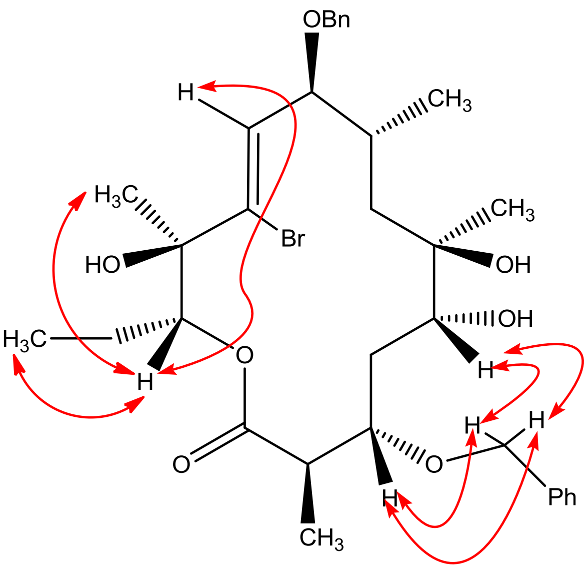


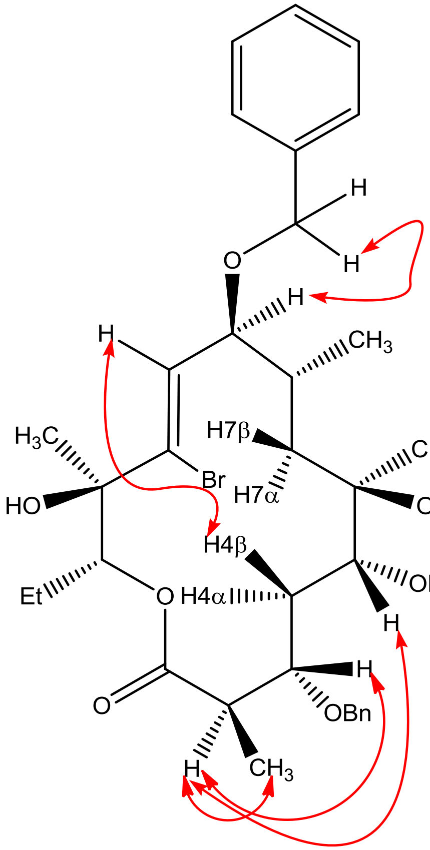
8.6 Hz
6.6 Hz
H
OBn
H
CH3
H
H3C
H7β
CH3
HO
Br
H7α
OH
7.0 Hz
H4β
H
H3C
O
H4α
OH
2.4 Hz
H
O
OBn
H
CH3
8.6 Hz

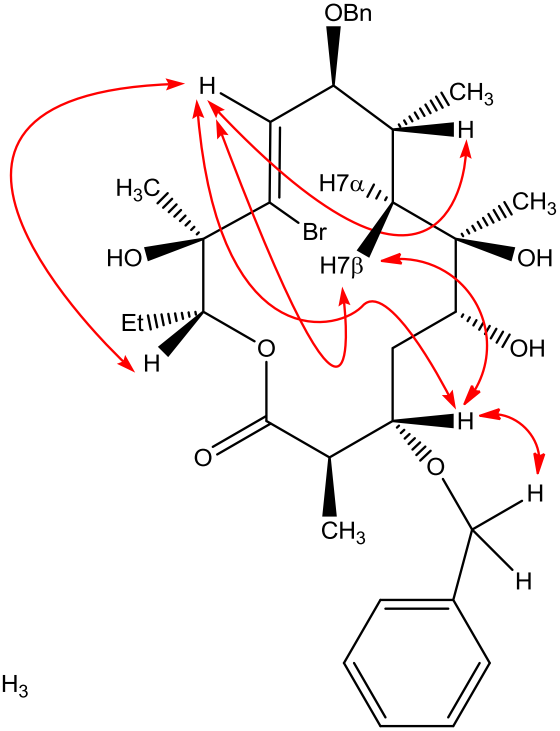
1d TOCSY


OBn
H
CH3
H
H
H7β
H3C
CH3
Br
H7α
HO
OH
H4β
H
H3C
O
H4α
OH
H
H
O
OBn
H
CH3

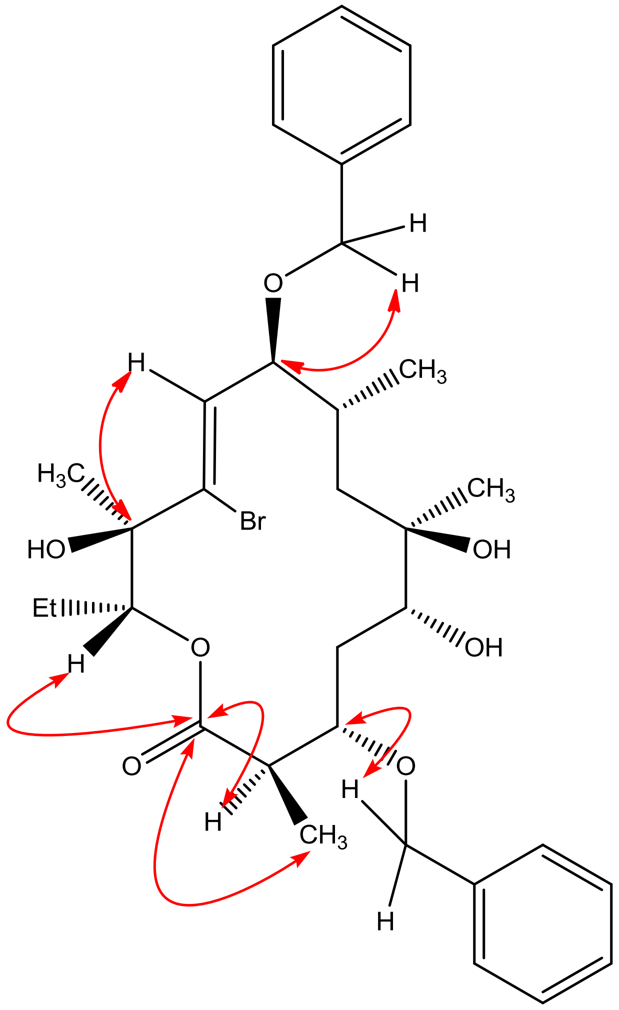
NOE

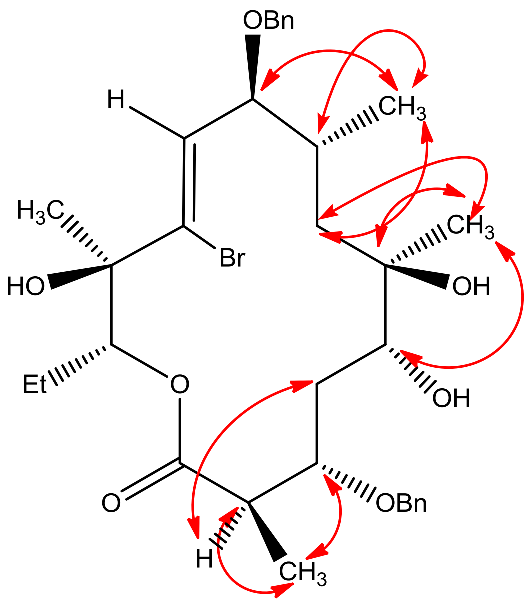

OBn
H
CH3
H3C
Br
CH3
HO
OH
H3C
OH
O
H
H
H
H
O
O
Ph
H
CH3

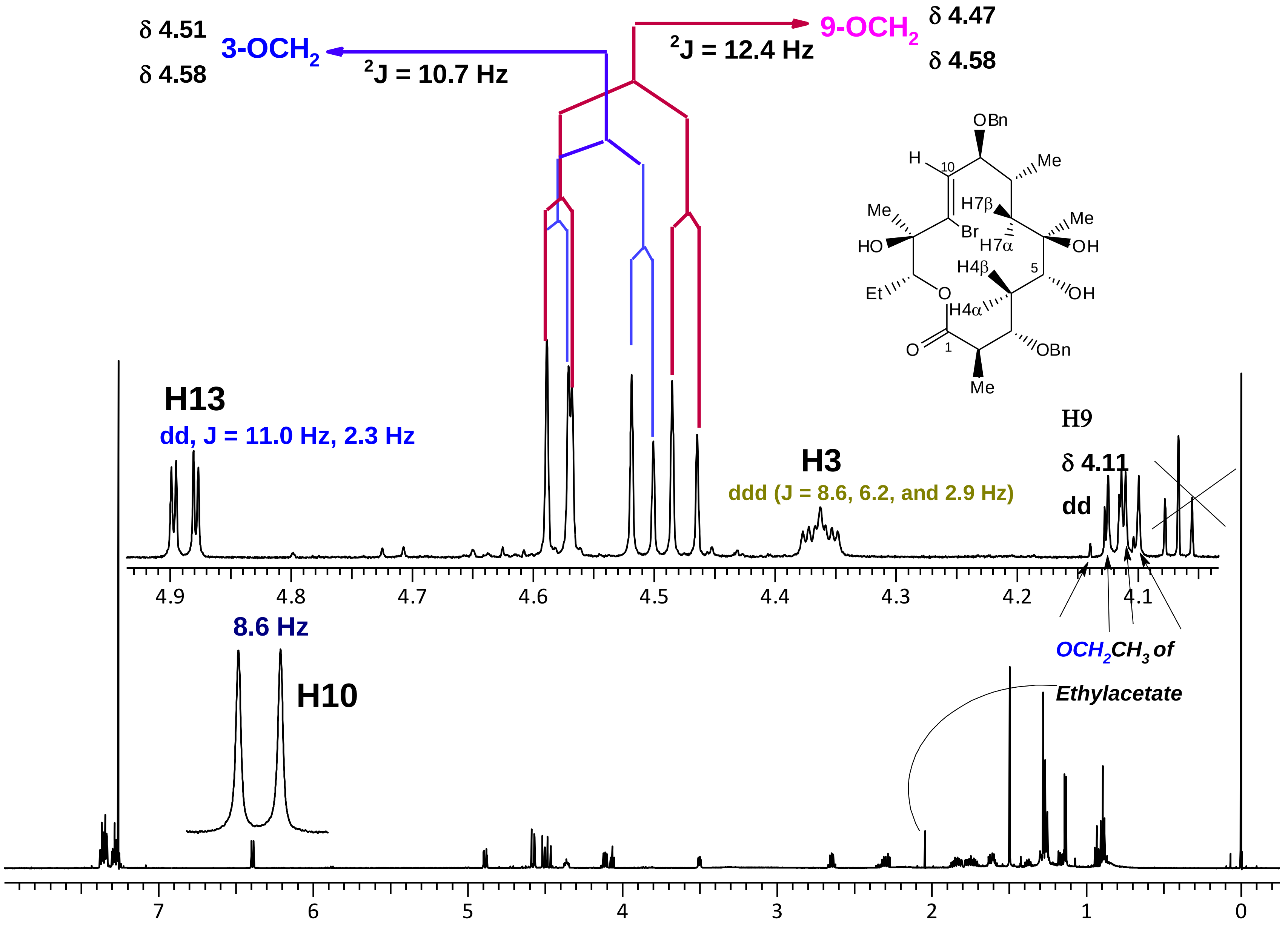

H
O
H
H
H
CH3
H7β
H3C
Br
CH3
H7α
HO
OH
H4β
OH
Et
O
H4α
H
H
O
OBn
H
CH3

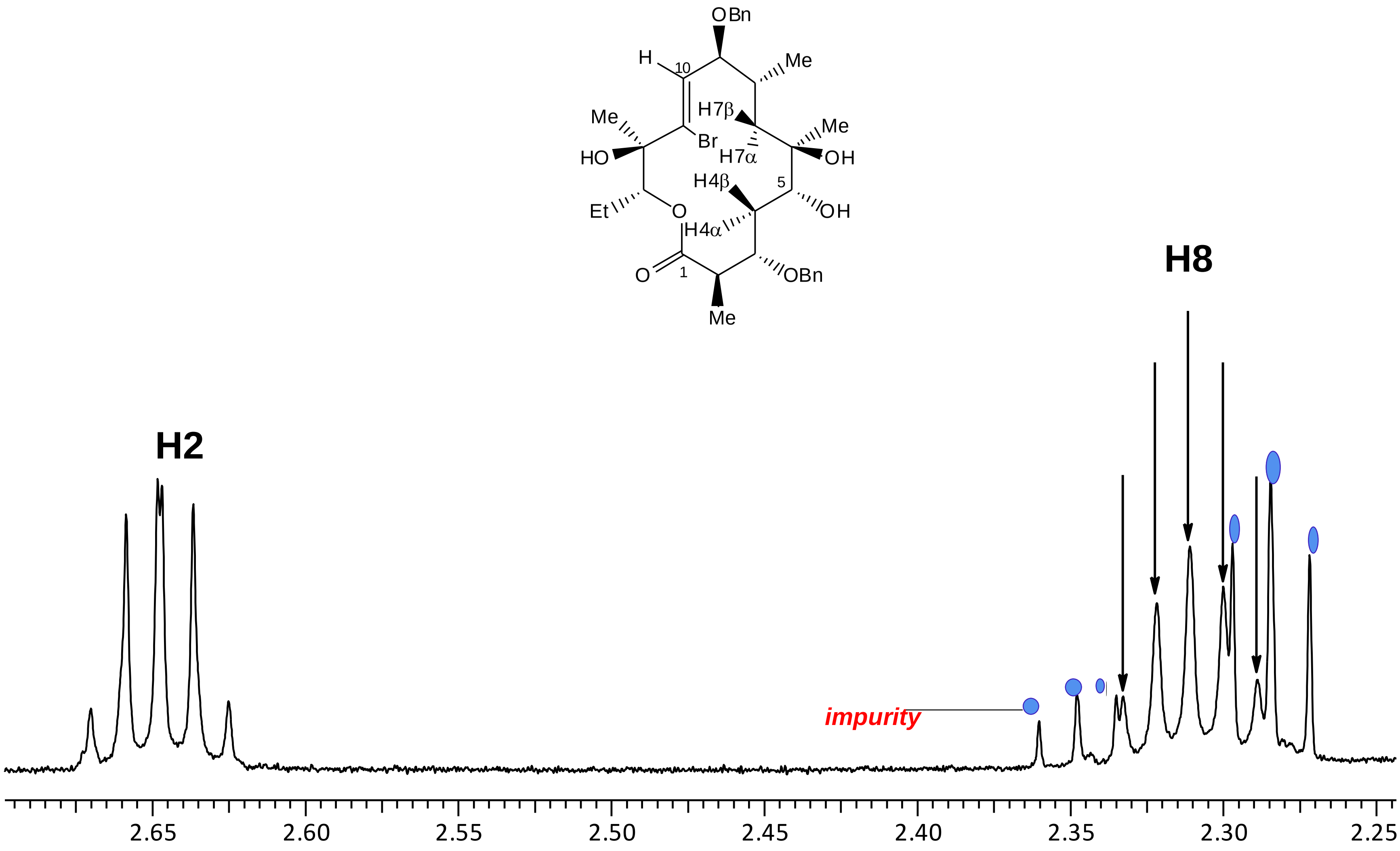

OBn
H
CH3
H
H7α
H3C
CH3
Br
HO
H7β
OH
Et
OH
O
H
H
O
O
H
CH3
H

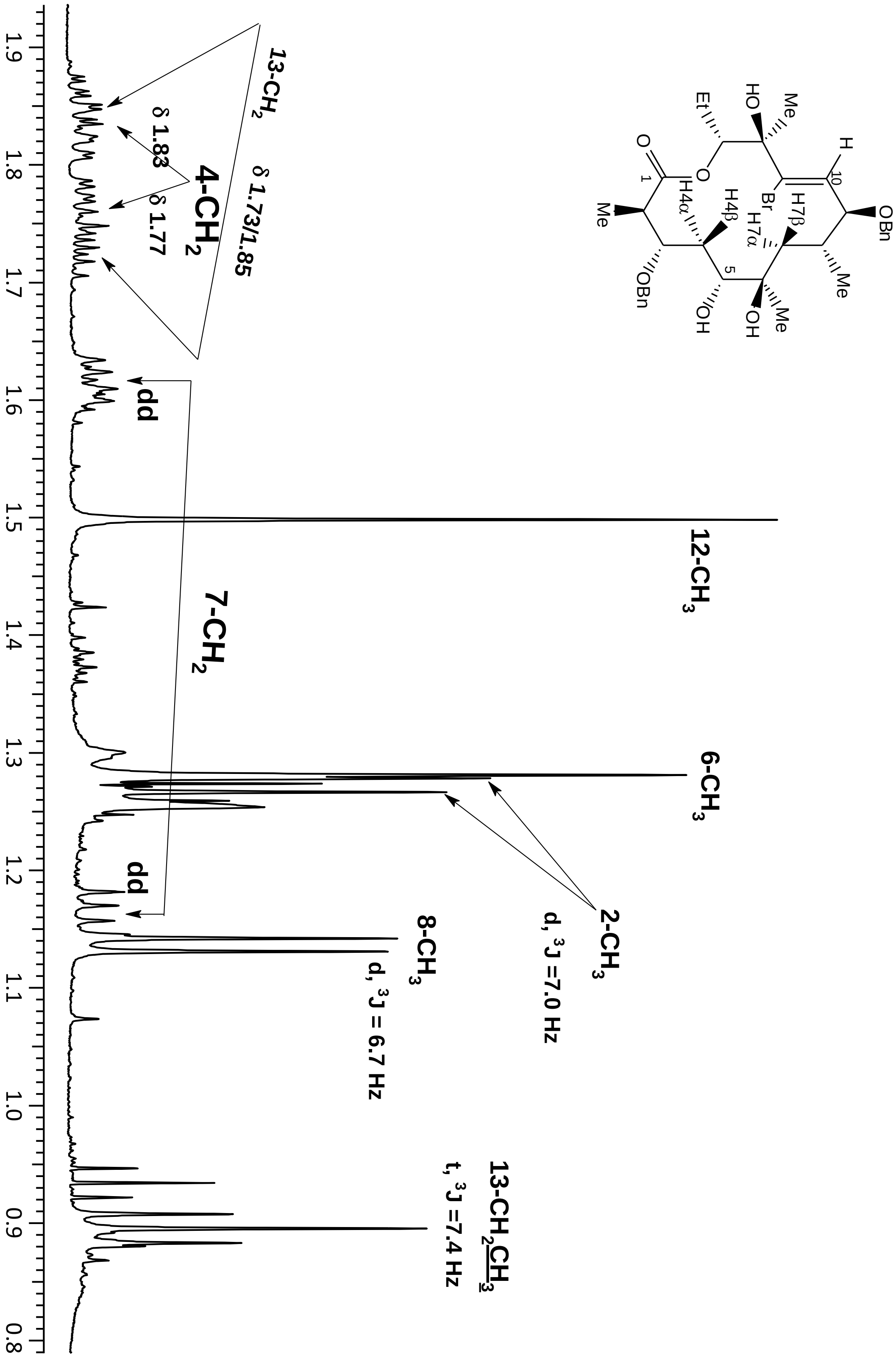
HMBC

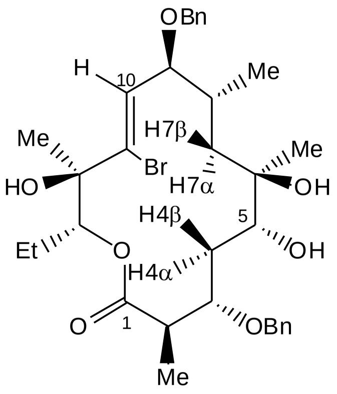

H
H
O
H
CH3
H3C
Br
CH3
HO
OH
Et
O
OH
H
O
O
H
H
CH3
H

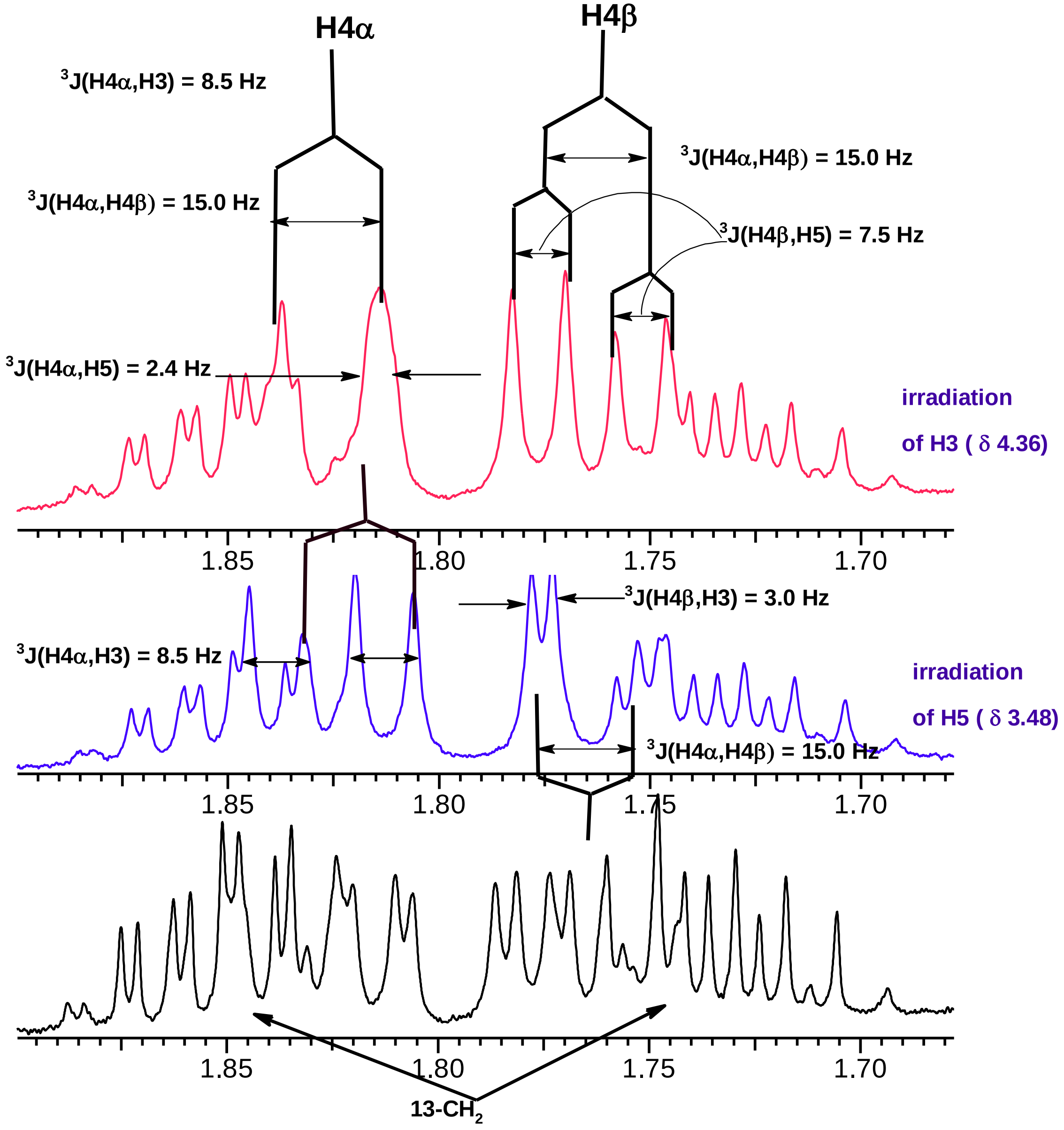

OBn
H
CH3
H3C
Br
CH3
HO
OH
Et
O
OH
O
OBn
H
CH3

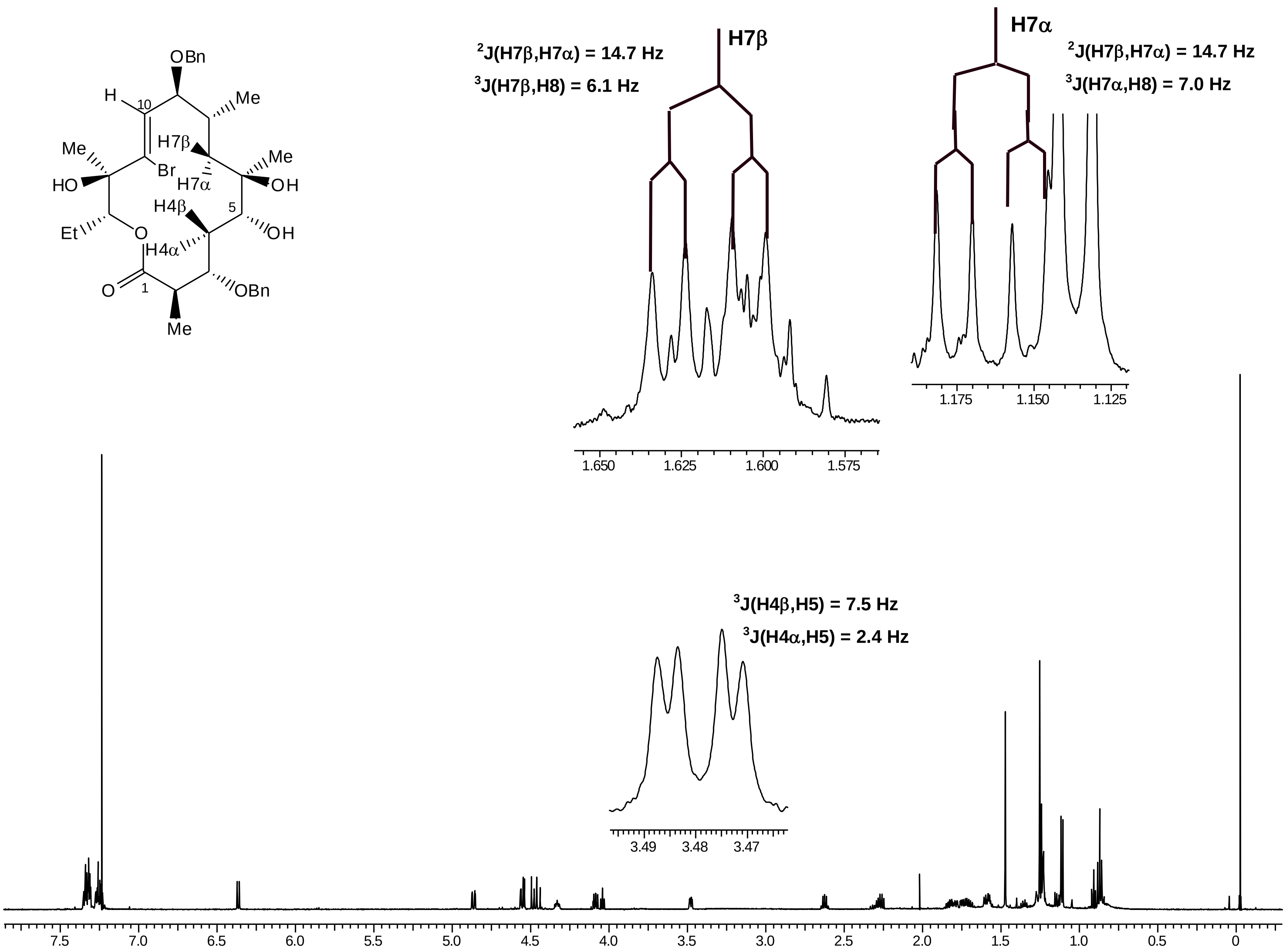

δ 4.51
δ 4.58
3-OCH$_2$
^{2}J = 10.7 Hz
^{2}J = 12.4 Hz
9-OCH$_2$
δ 4.47
δ 4.58
OBn
H
10
Me
H7β
Me
Me
Br
HO
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me
H13
dd, J = 11.0 Hz, 2.3 Hz
H3
ddd (J = 8.6, 6.2, and 2.9 Hz)
H9
δ 4.11
dd
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
8.6 Hz
H10
OCH$_2$CH$_3$ of
Ethylacetate
7
6
5
4
3
2
1
0


OBn
H
10
Me
H7β
Me
Br
Me
HO
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me
H8
H2
impurity
2.65
2.60
2.55
2.50
2.45
2.40
2.35
2.30
2.25

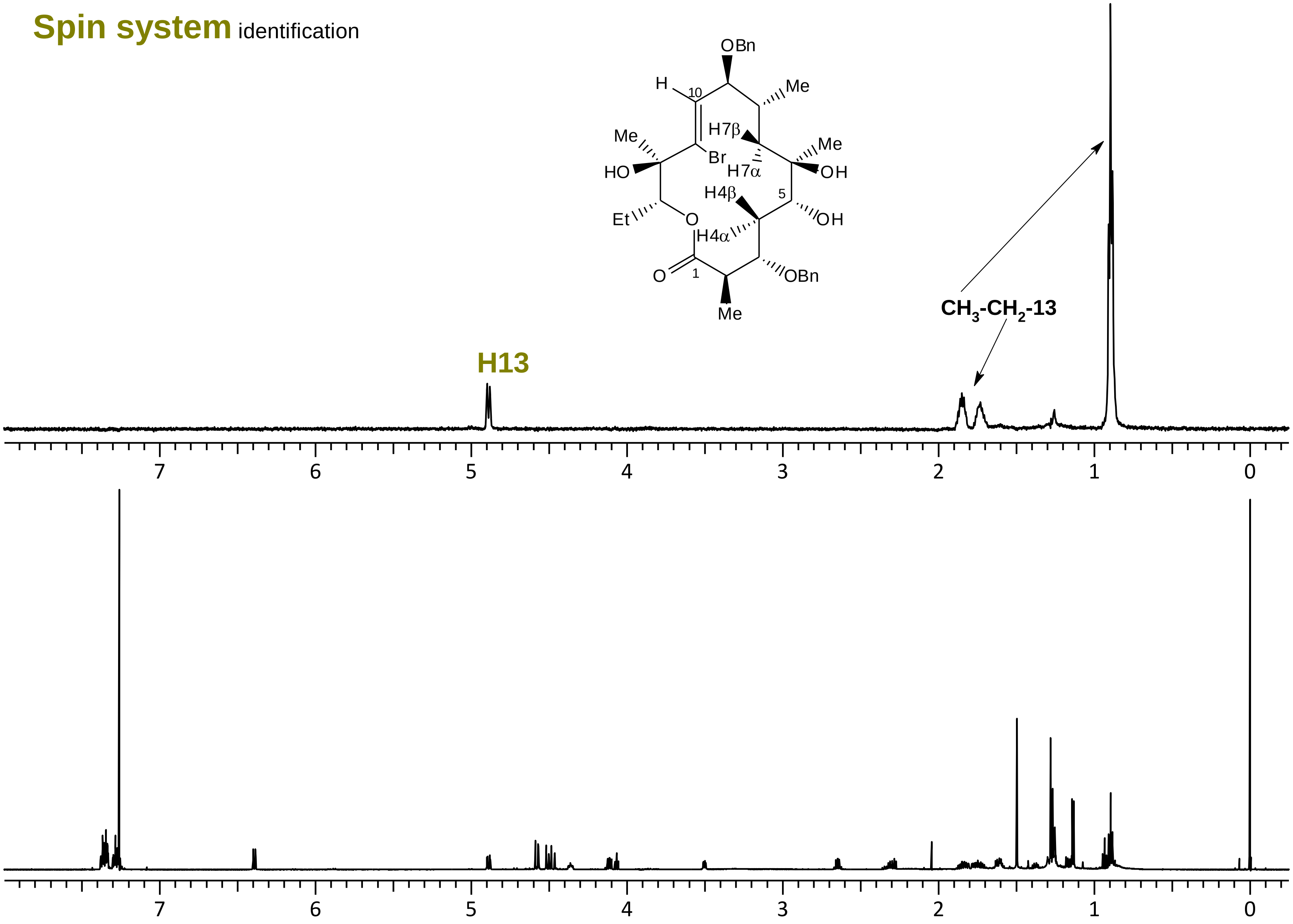

H
OBn
10
Me
HO
Me
Et
O
Br
H7β
H7α
H4β
H4α
Me
O
1
Me
5
OH
OH
OBn
13-CH$_2$
δ 1.73/1.85
4-CH$_2$
δ 1.83
δ 1.77
dd
12-CH$_3$
7-CH$_2$
6-CH$_3$
2-CH$_3$
d, ^{3}J =7.0 Hz
dd
8-CH$_3$
d, ^{3}J = 6.7 Hz
13-CH$_2$CH$_3$
t, ^{3}J =7.4 Hz
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8


OBn
H
10
Me
H7β
Me
Me
Br
HO
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me

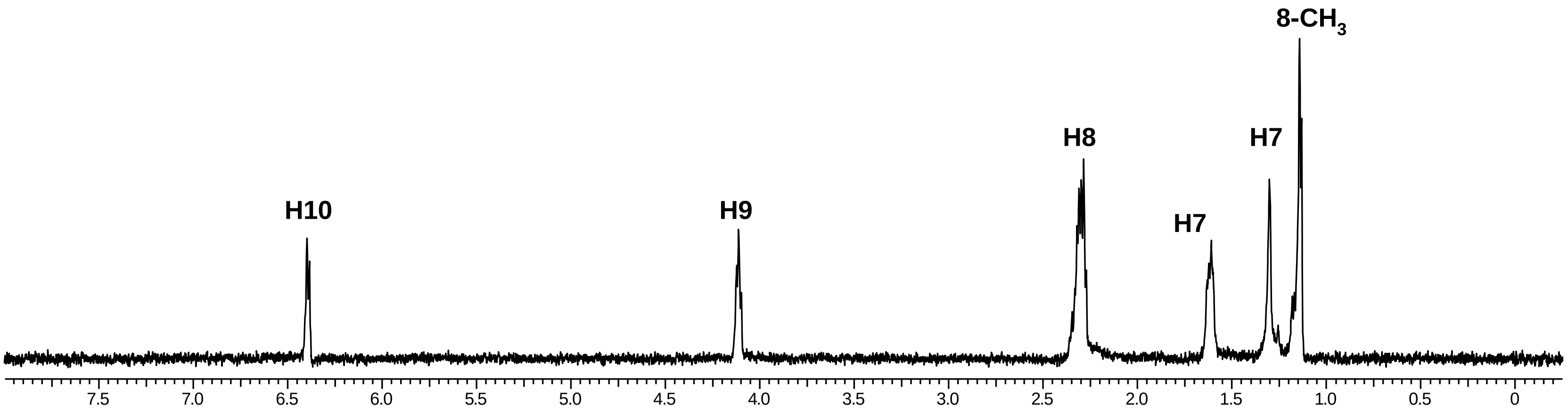

H4α
H4β
^3J(H4α,H3) = 8.5 Hz
^3J(H4α,H4β) = 15.0 Hz
^3J(H4α,H4β) = 15.0 Hz
^3J(H4β,H5) = 7.5 Hz
^3J(H4α,H5) = 2.4 Hz
irradiation
of H3 (δ 4.36)
1.85
1.80
1.75
1.70
^3J(H4β,H3) = 3.0 Hz
^3J(H4α,H3) = 8.5 Hz
irradiation
of H5 (δ 3.48)
^3J(H4α,H4β) = 15.0 Hz
1.85
1.80
1.75
1.70
1.85
1.80
1.75
1.70
13-CH$_2$

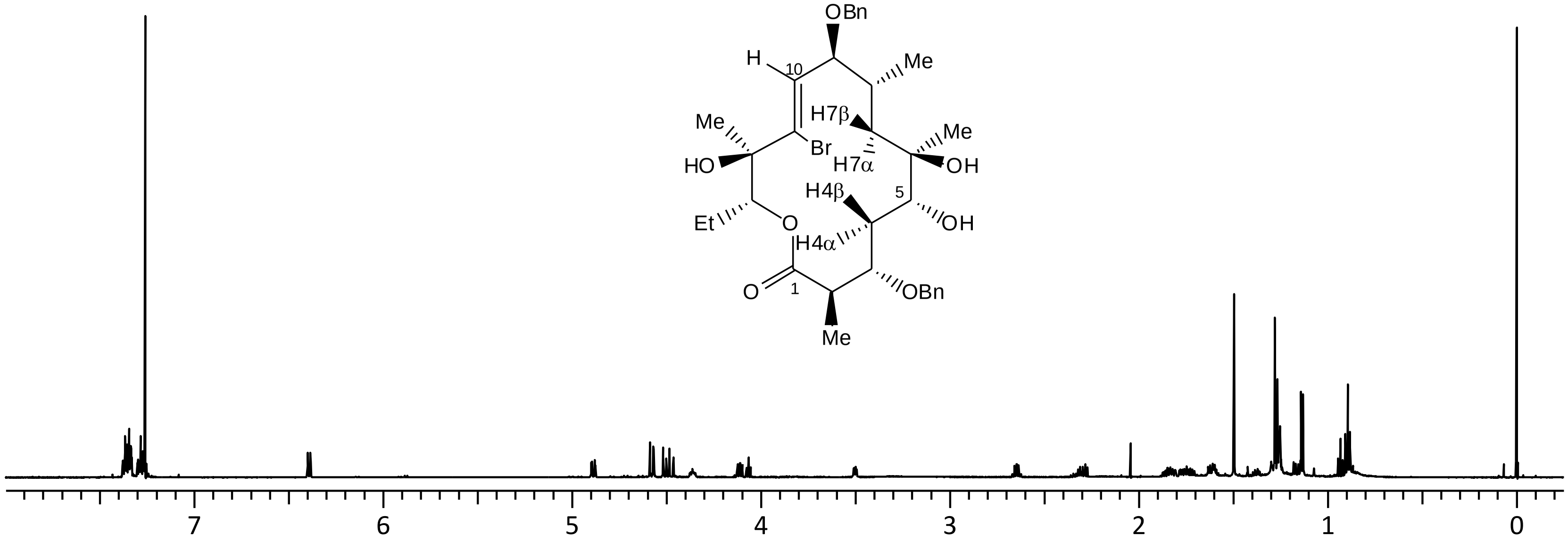

H7β
²J(H7β,H7α) = 14.7 Hz
³J(H7β,H8) = 6.1 Hz
H7α
²J(H7β,H7α) = 14.7 Hz
³J(H7α,H8) = 7.0 Hz
³J(H4β,H5) = 7.5 Hz
³J(H4α,H5) = 2.4 Hz
OBn
H
10
Me
H7β
Br
H7α
HO
OH
Et
O
H4β
5
H4α
OH
O
1
OBn
Me
1.650
1.625
1.600
1.575
1.175
1.150
1.125
3.49
3.48
3.47
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

Spin system identification

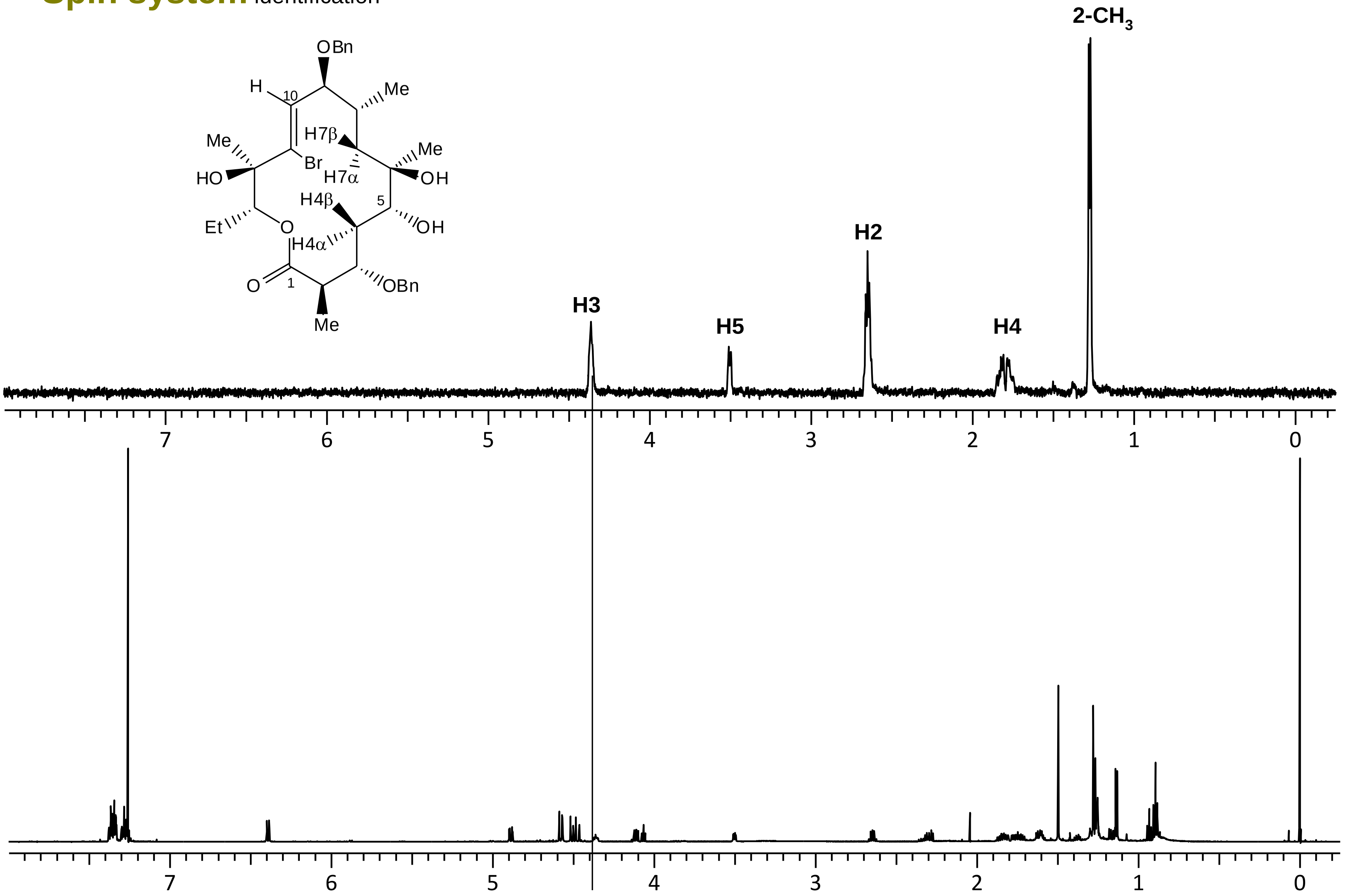


Spin system identification



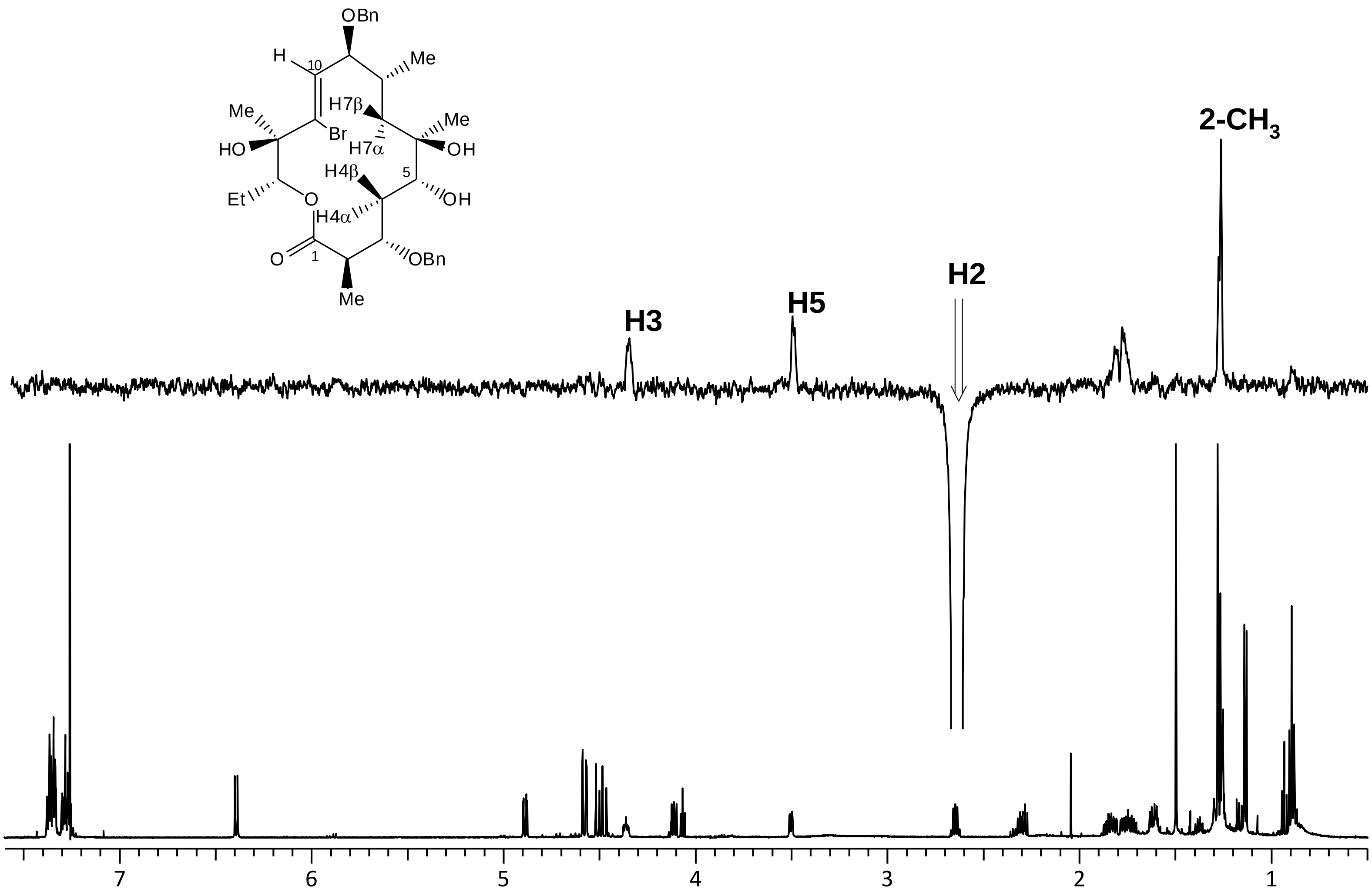


Spin system identification

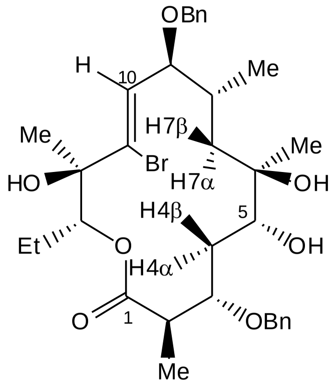


DPFGSENOE

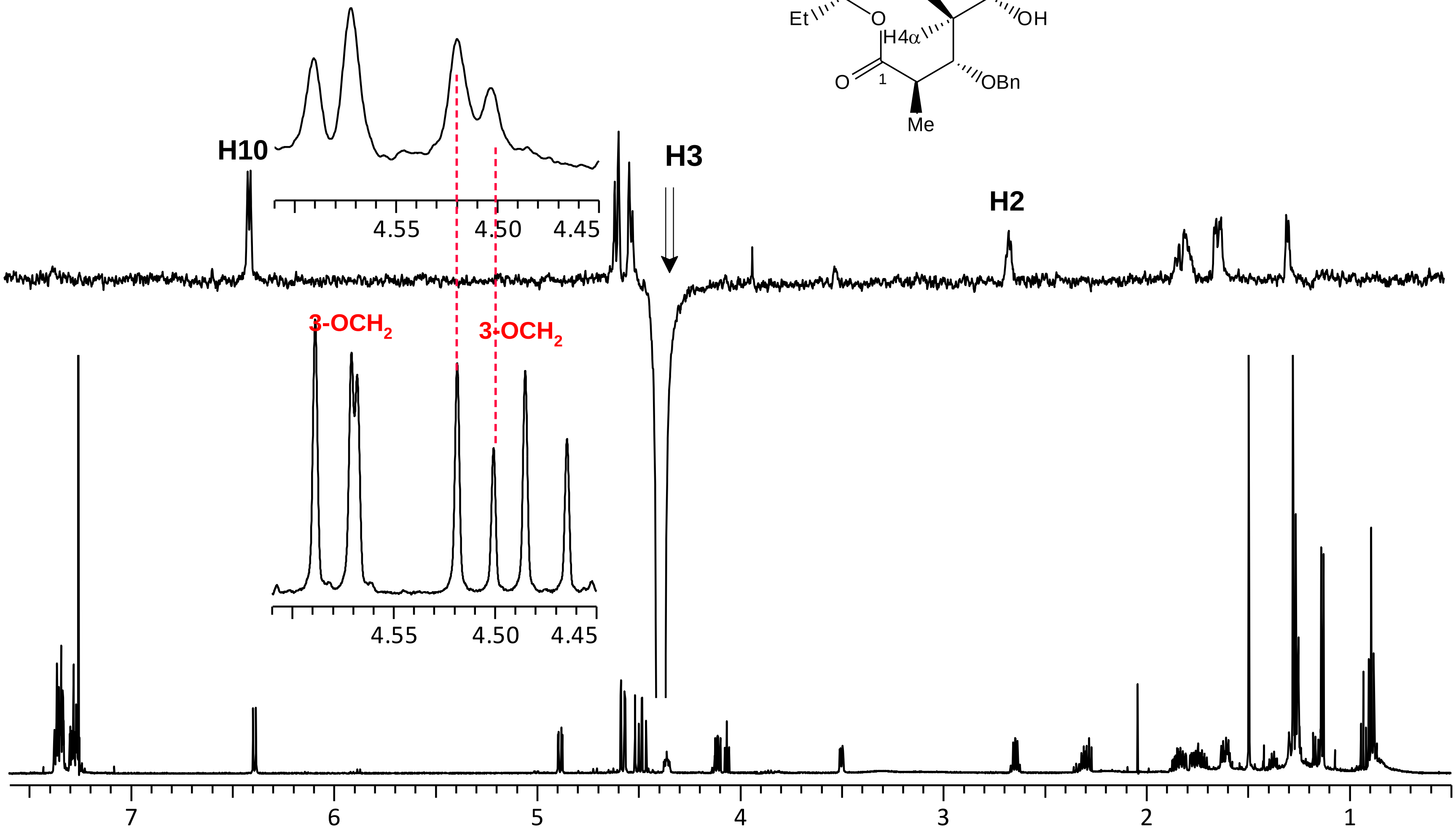
spectrum



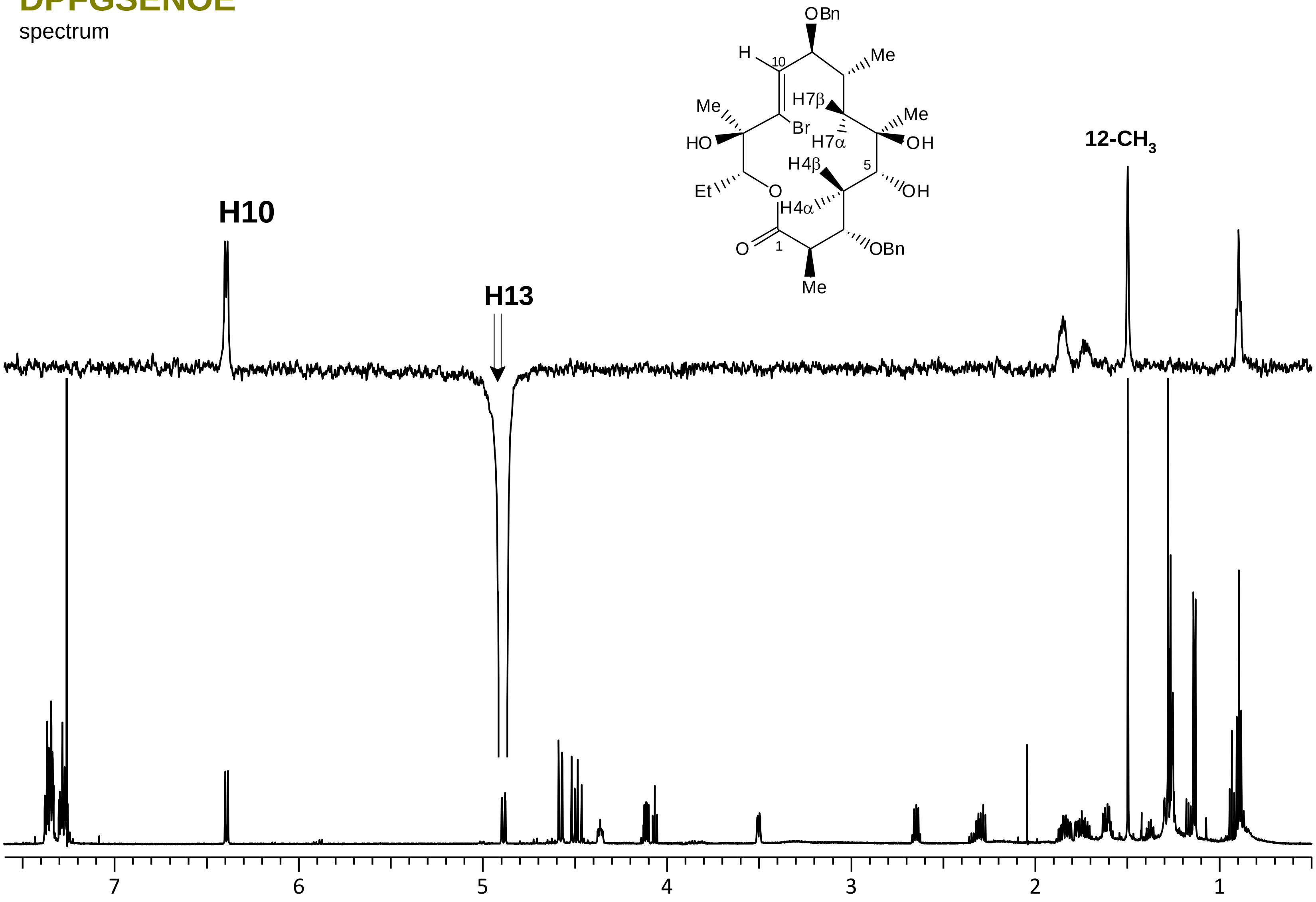
DPFGSENOE
spectrum



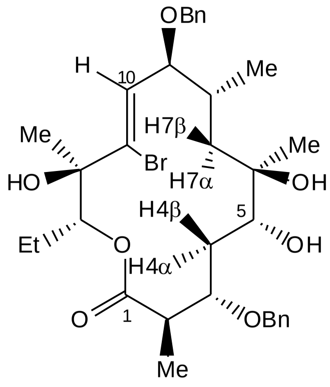
OBn
H
10
Me
H7β
Me
Me
HO
Br
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me

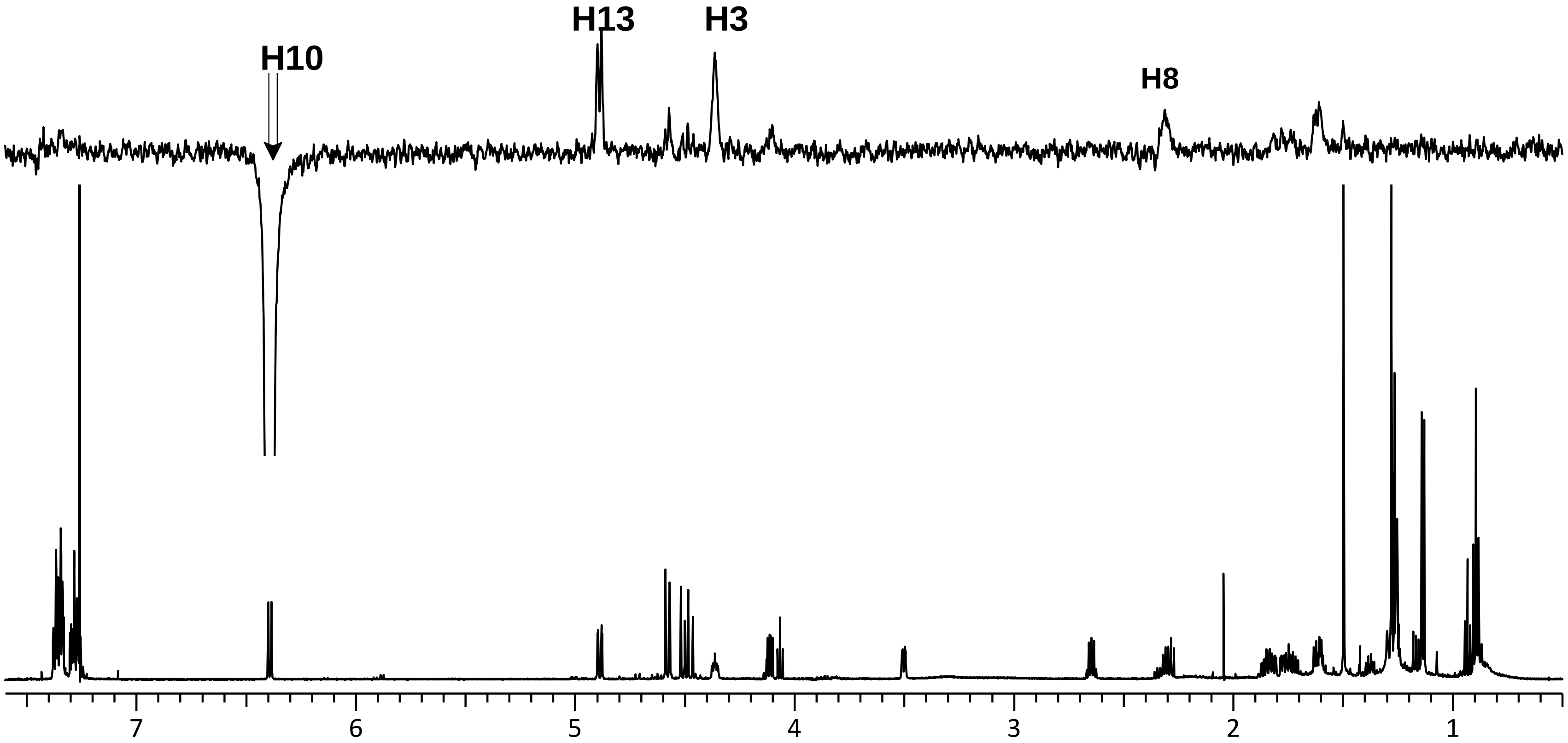


DPFGSENOE
spectrum



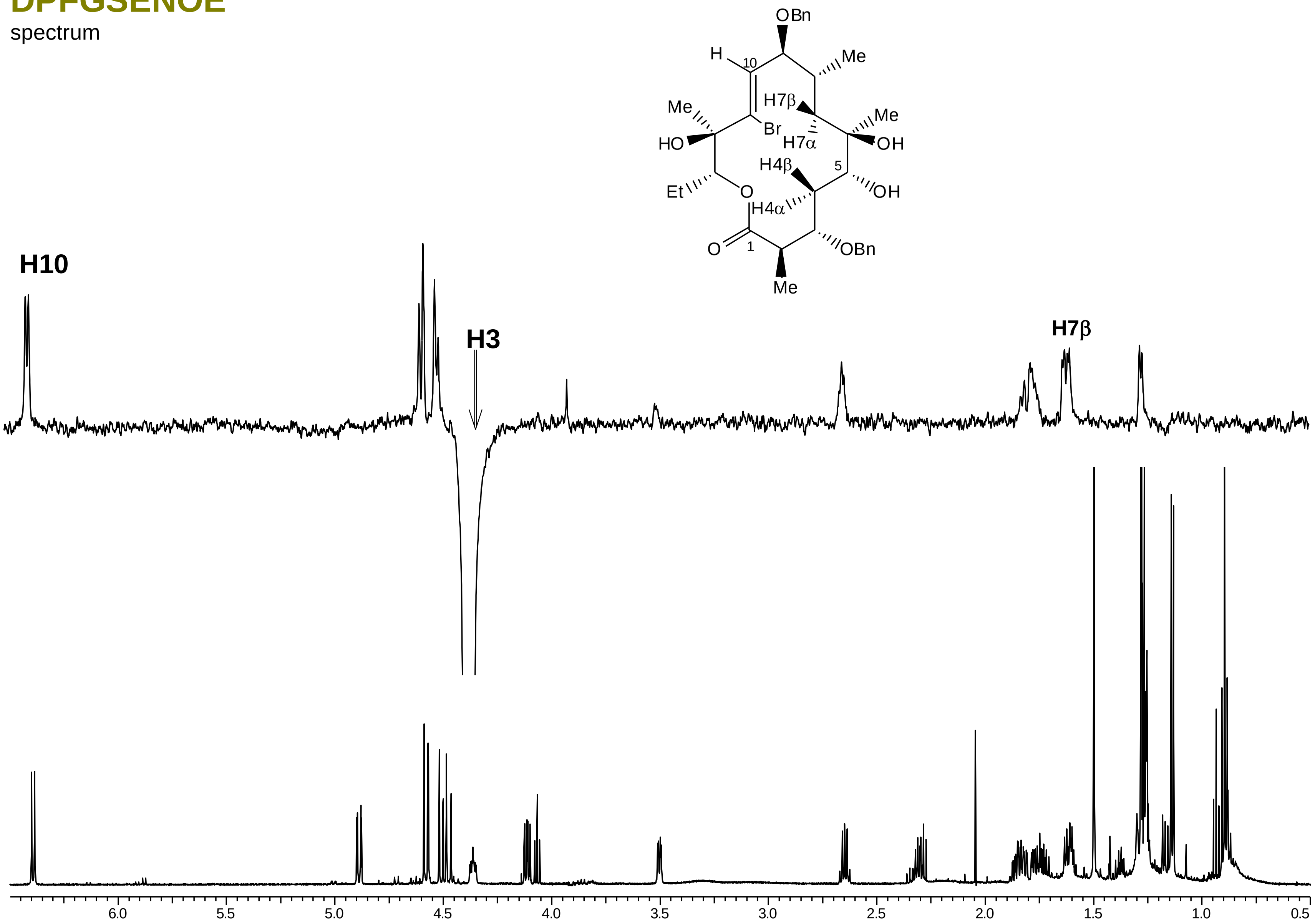
DPFGSENOE
spectrum

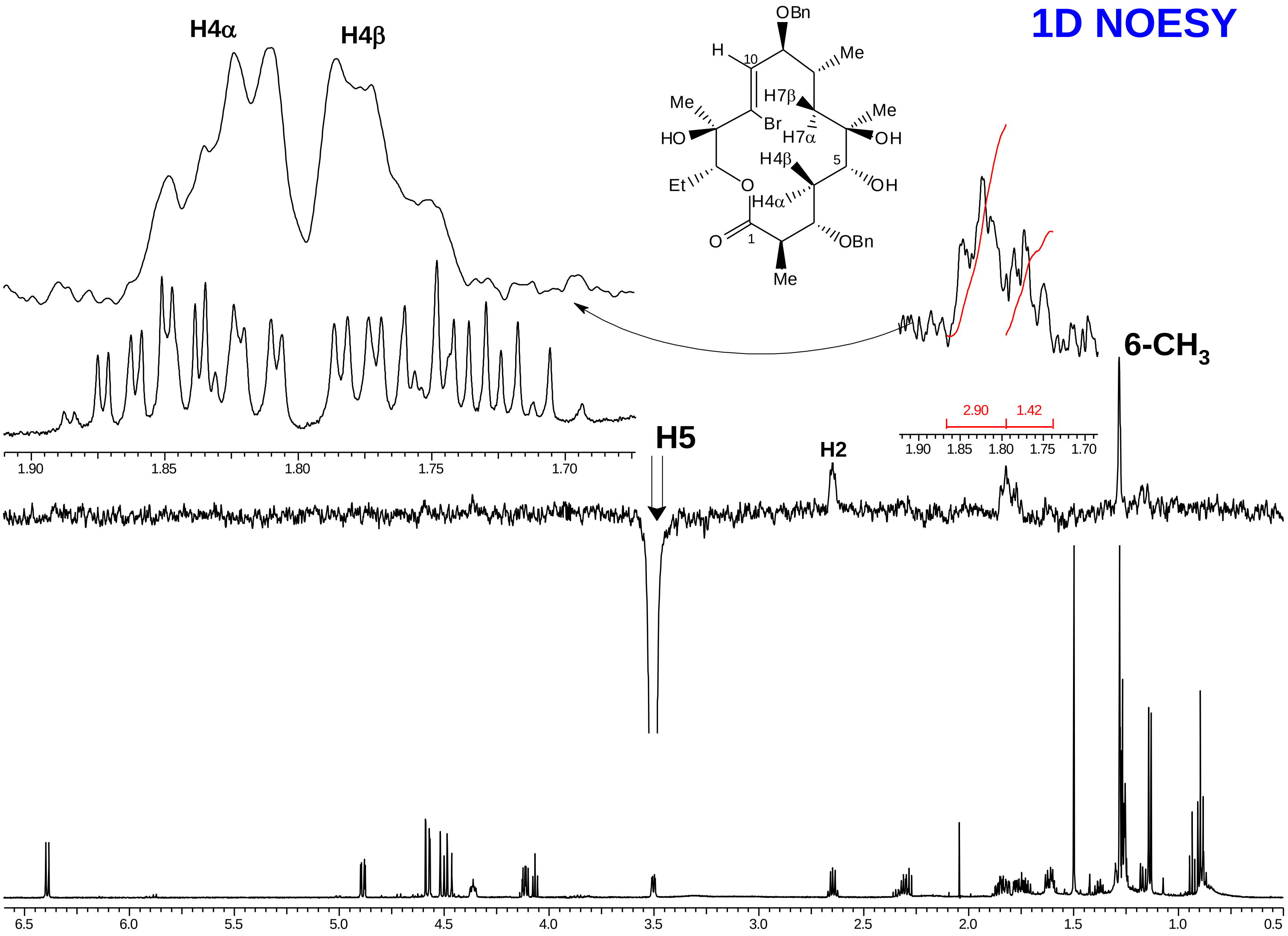

OBn
H
10
Me
H7β
Me
Me
Br
HO
H7α
OH
H4β
5
Et
O
OH
H4α
OBn
O
1
Me



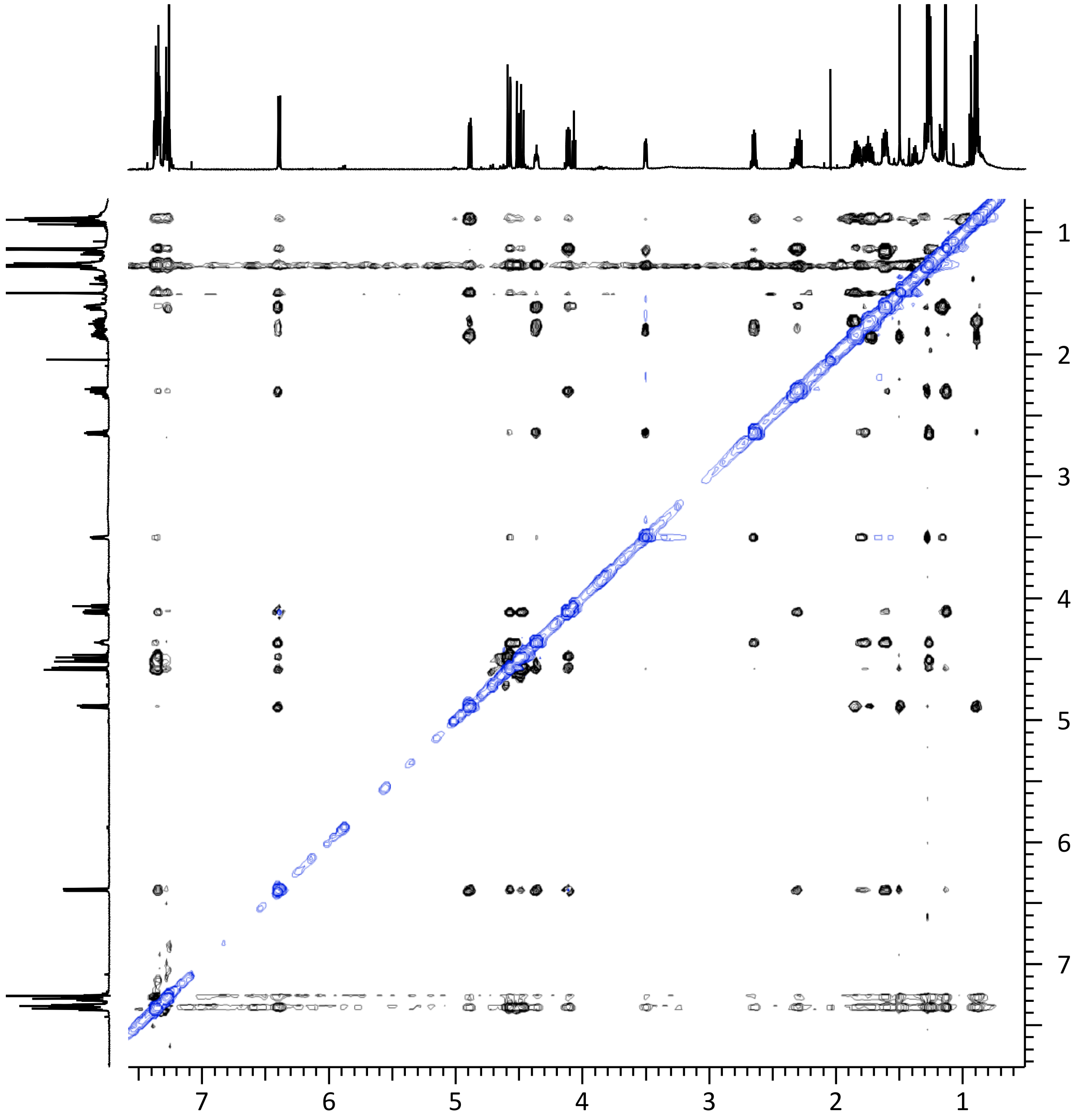
DPFGSENOE

spectrum

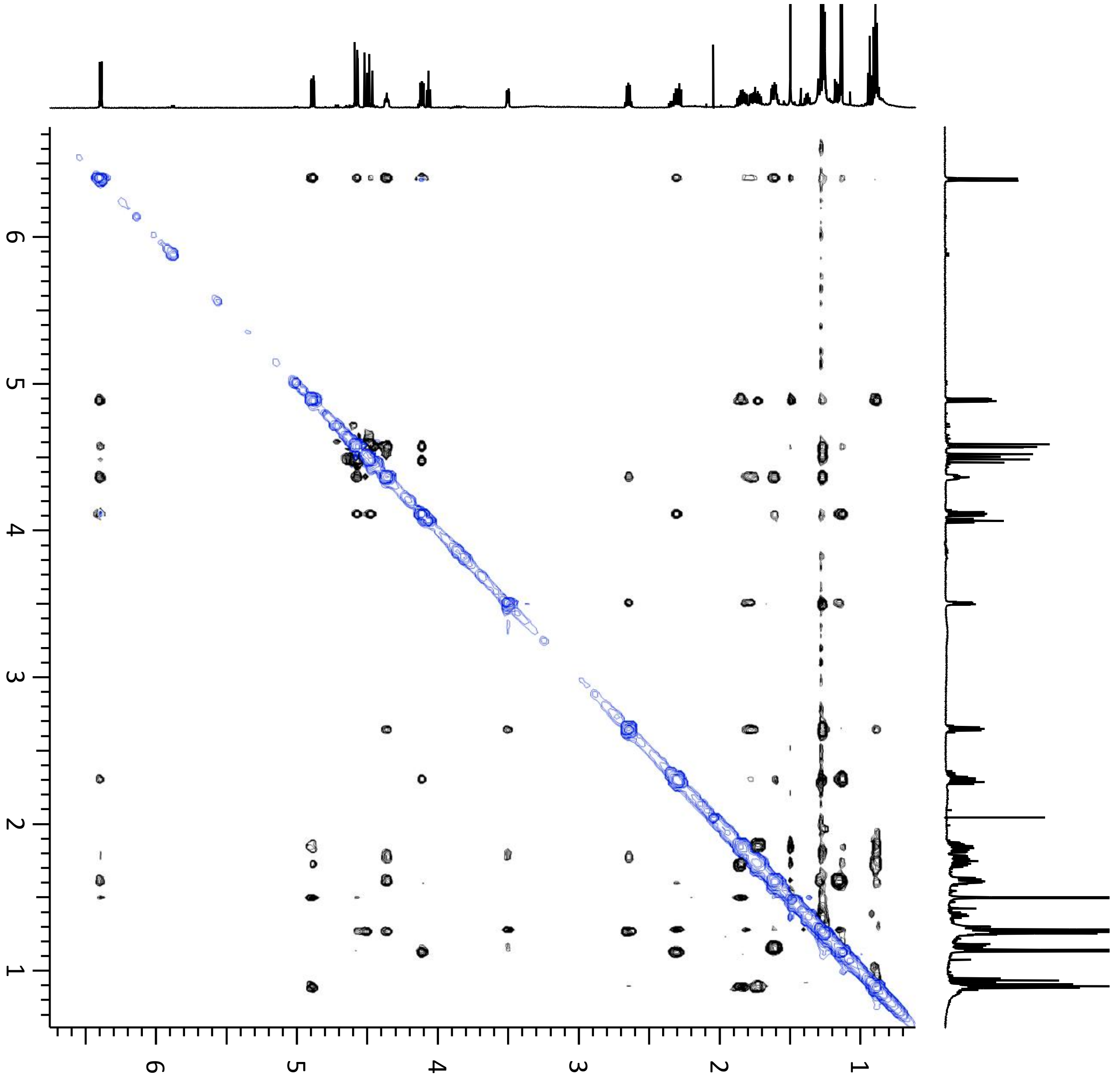



1D NOESY
H4α
H4β
OBn
H
10
Me
Me
H7β
Me
HO
Br
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me
6-CH$_3$
2.90
1.42
1.90
1.85
1.80
1.75
1.70
H5
H2
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

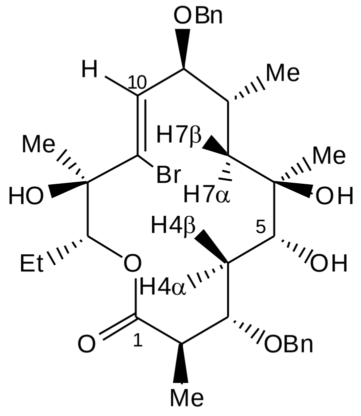
NOESY

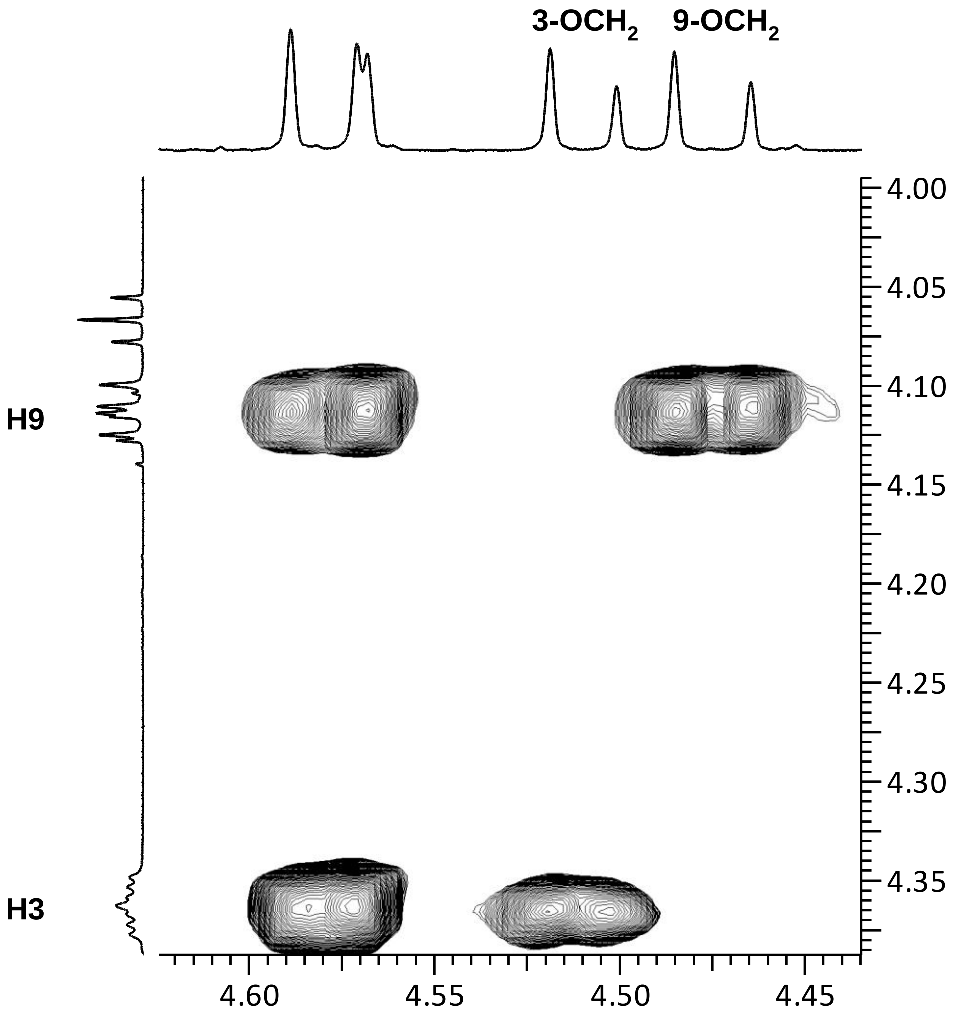


NOESY expansion

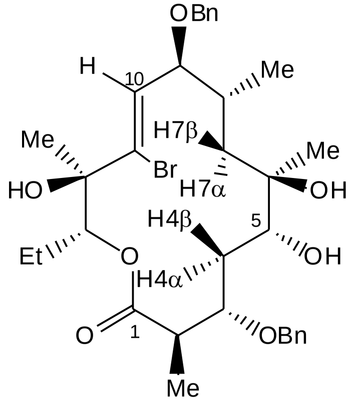


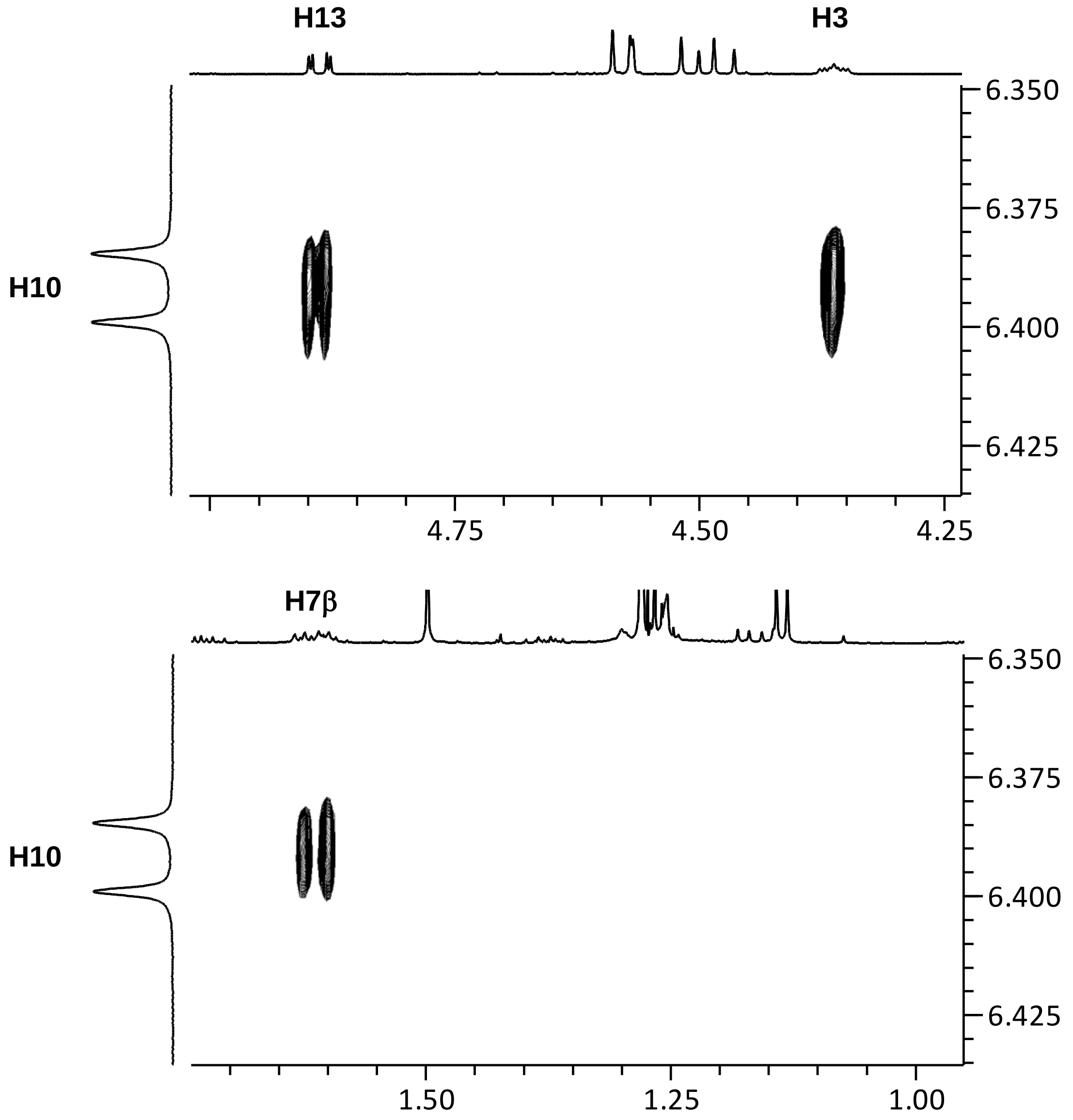
NOESY expansion


OBn
H
10
Me
Me
H7β
Me
HO
Br
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me

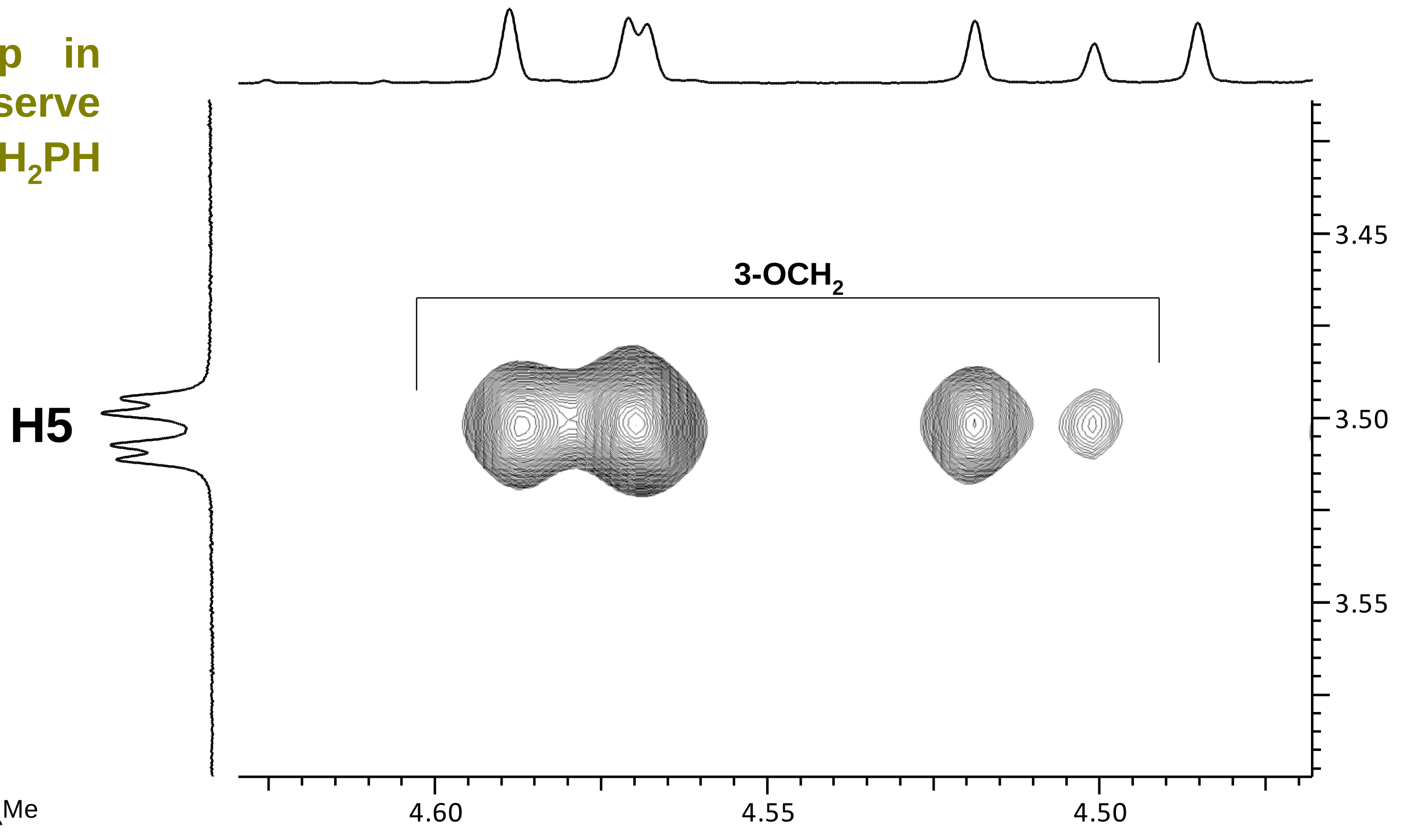


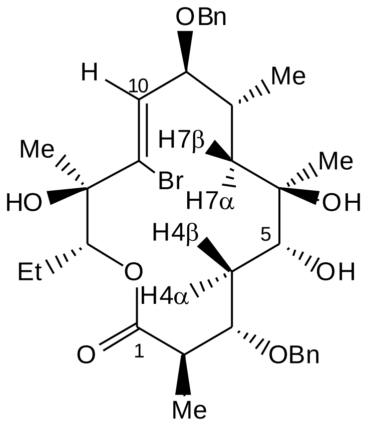
NOESY expansion


OBn
H
10
Me
Me
H7β
Me
Br
HO
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me

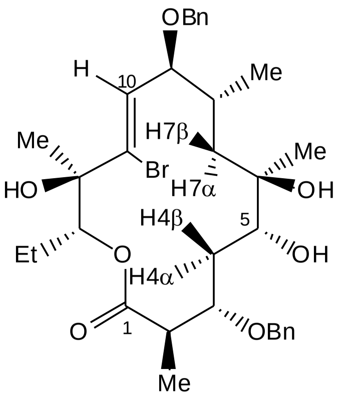


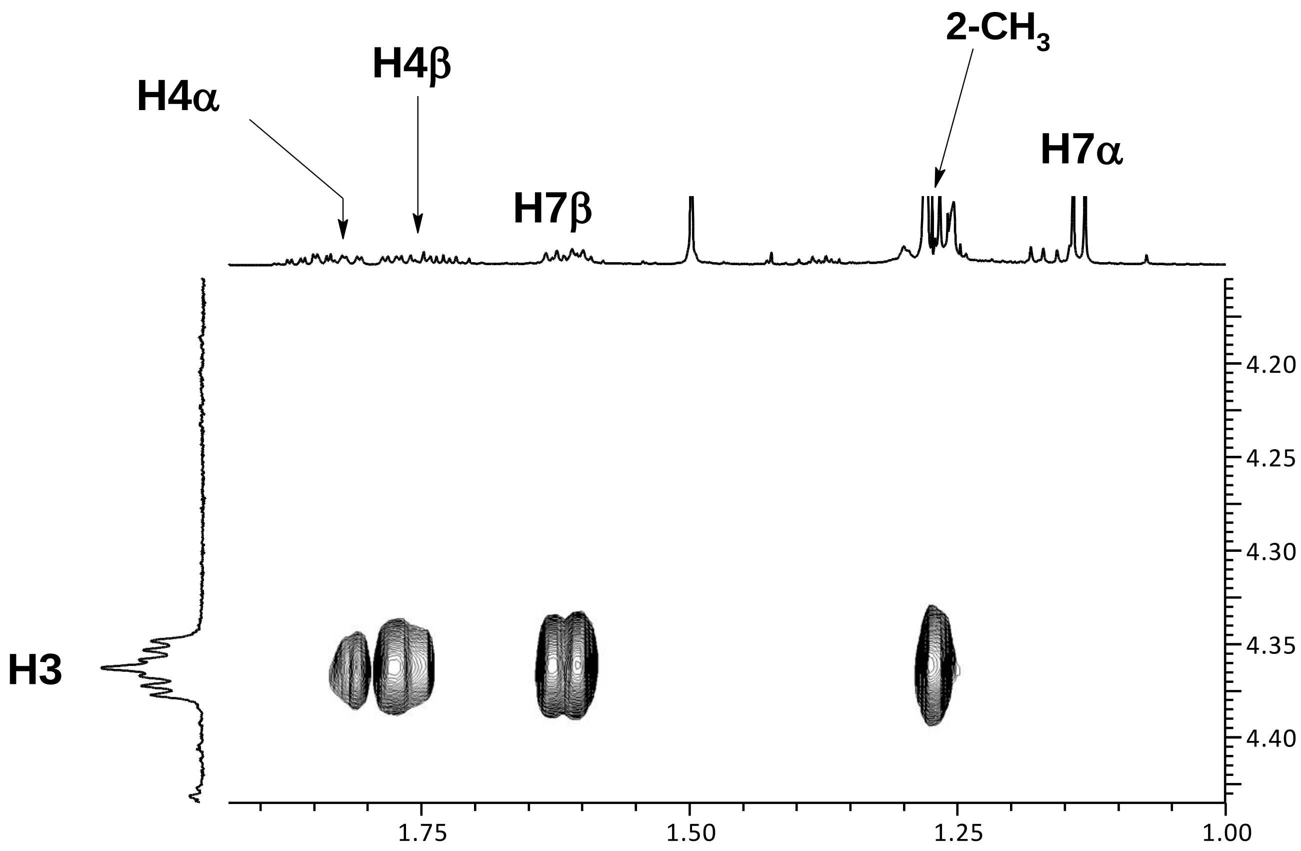
H5 has to up in order to observe nOe with 3-OCH$_2$PH (methylene protons);



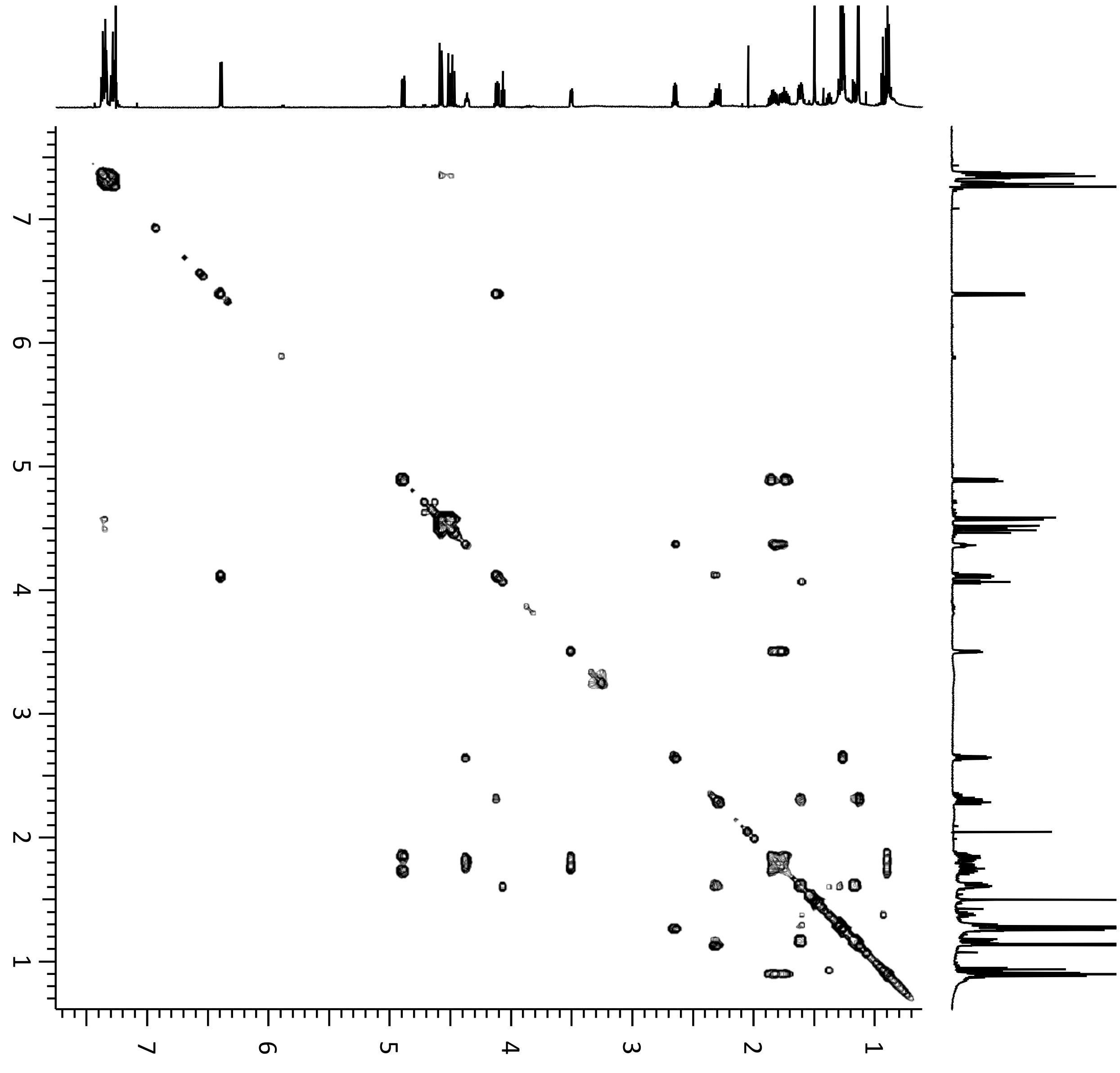

OBn
H
10
Me
Me
H7β
Me
HO
Br
H7α
OH
H4β
5
Et
O
H4α
OH
O
1
OBn
Me

NOESY expansion

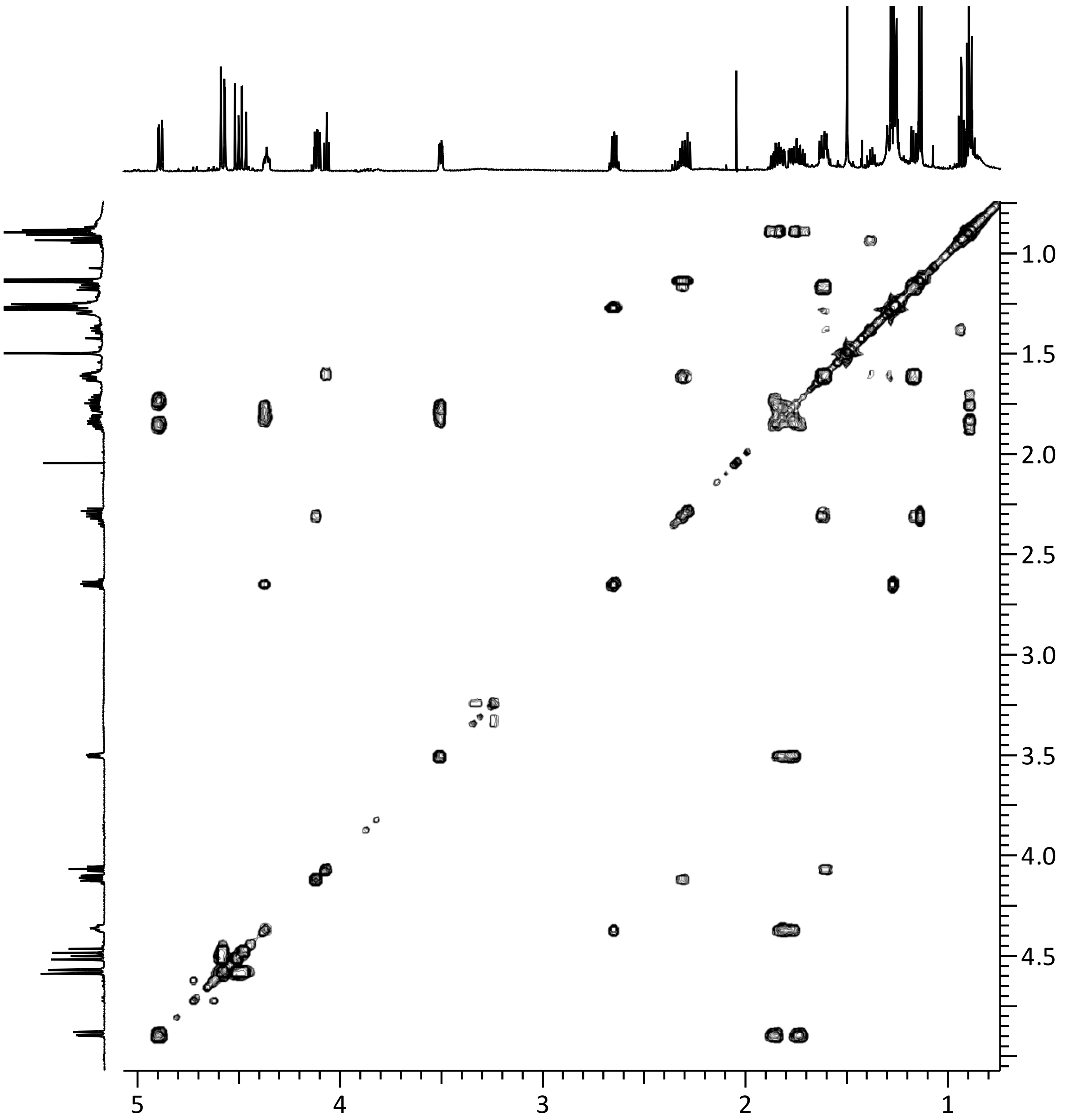

OBn
H
10
Me
Me
H7β
HO
Br
H7α
Me
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me



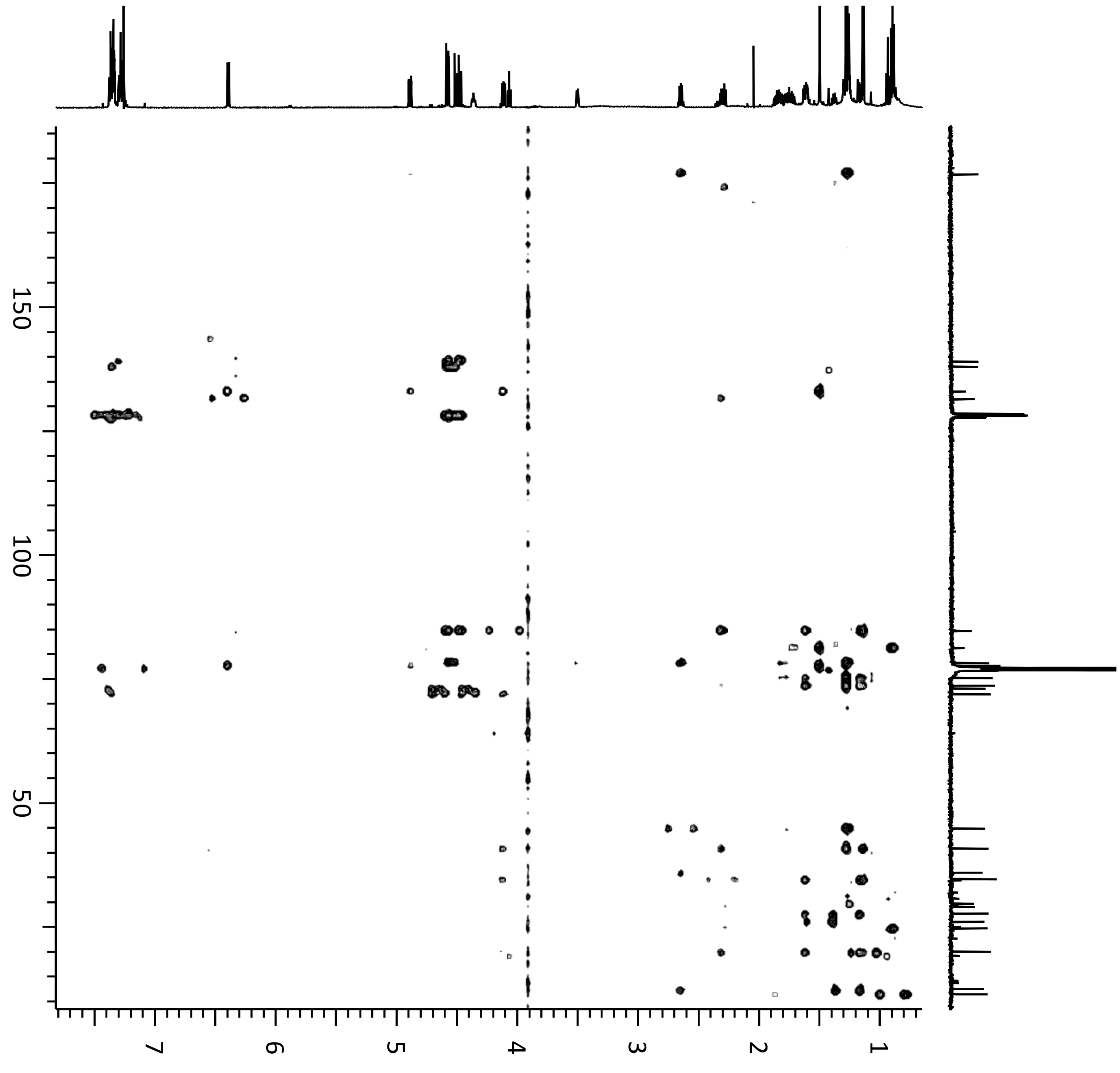
gCOSY



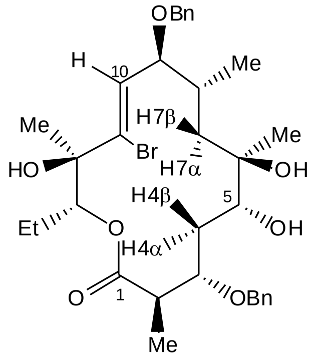
gCOSY **expansion**

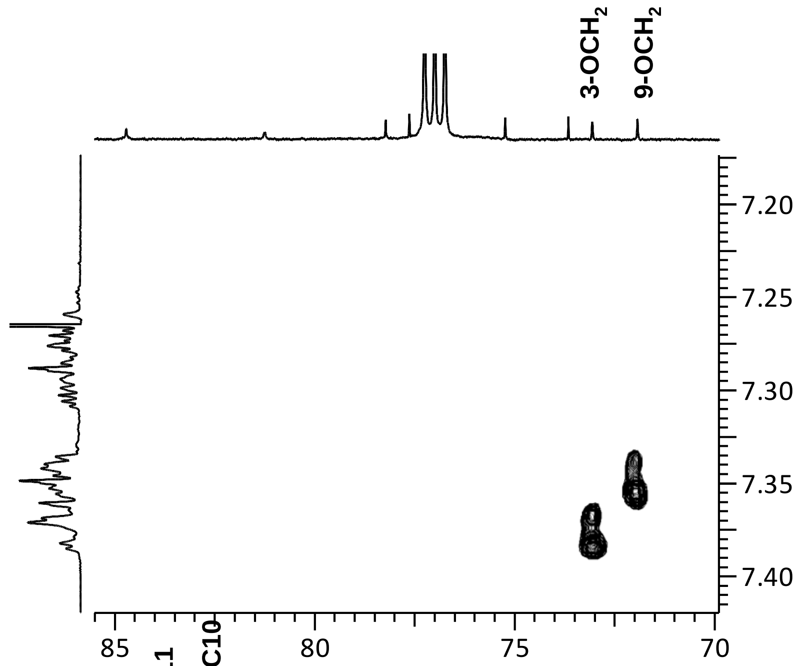


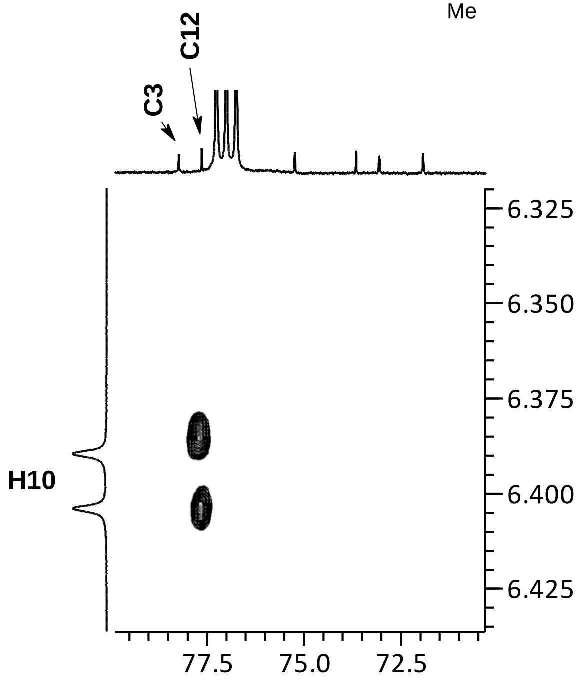
gHMBC

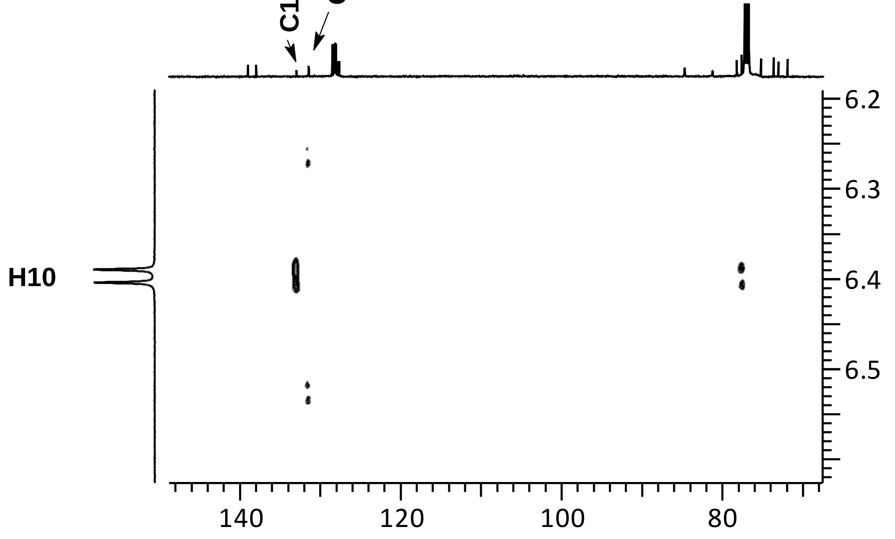


gHMBC expansions

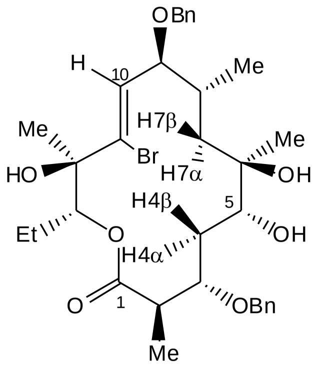

OBn
H
10
Me
Me
H7β
HO
Br
H7α
Me
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me

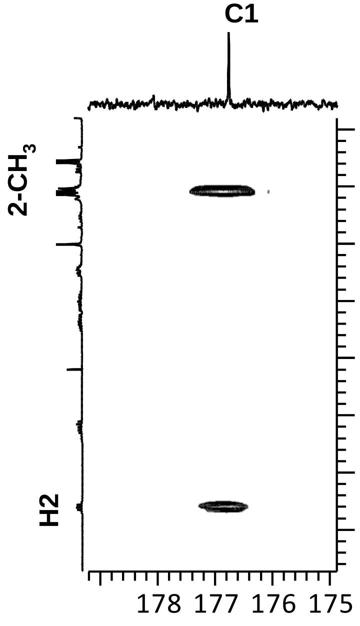


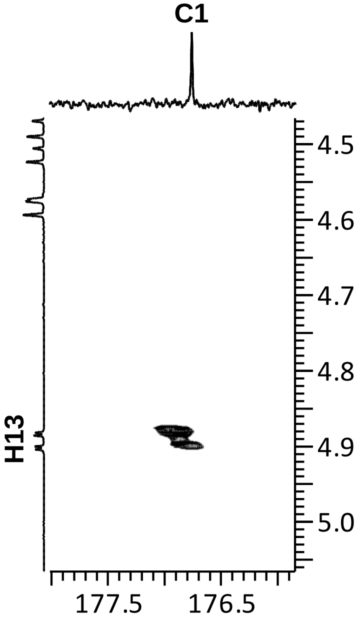


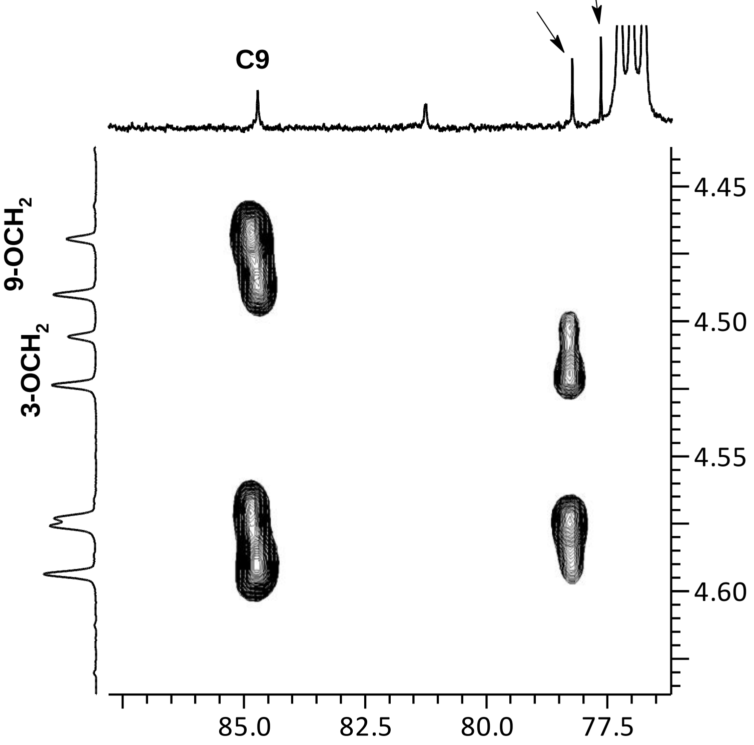


gHMBC expansions

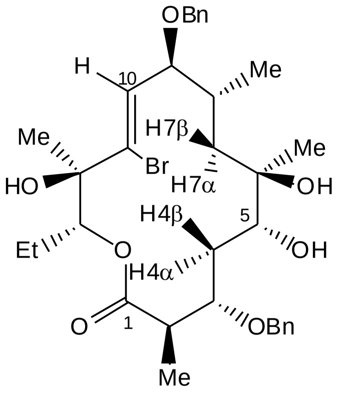

OBn
H
10
Me
H7β
Me
Me
Br
HO
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me

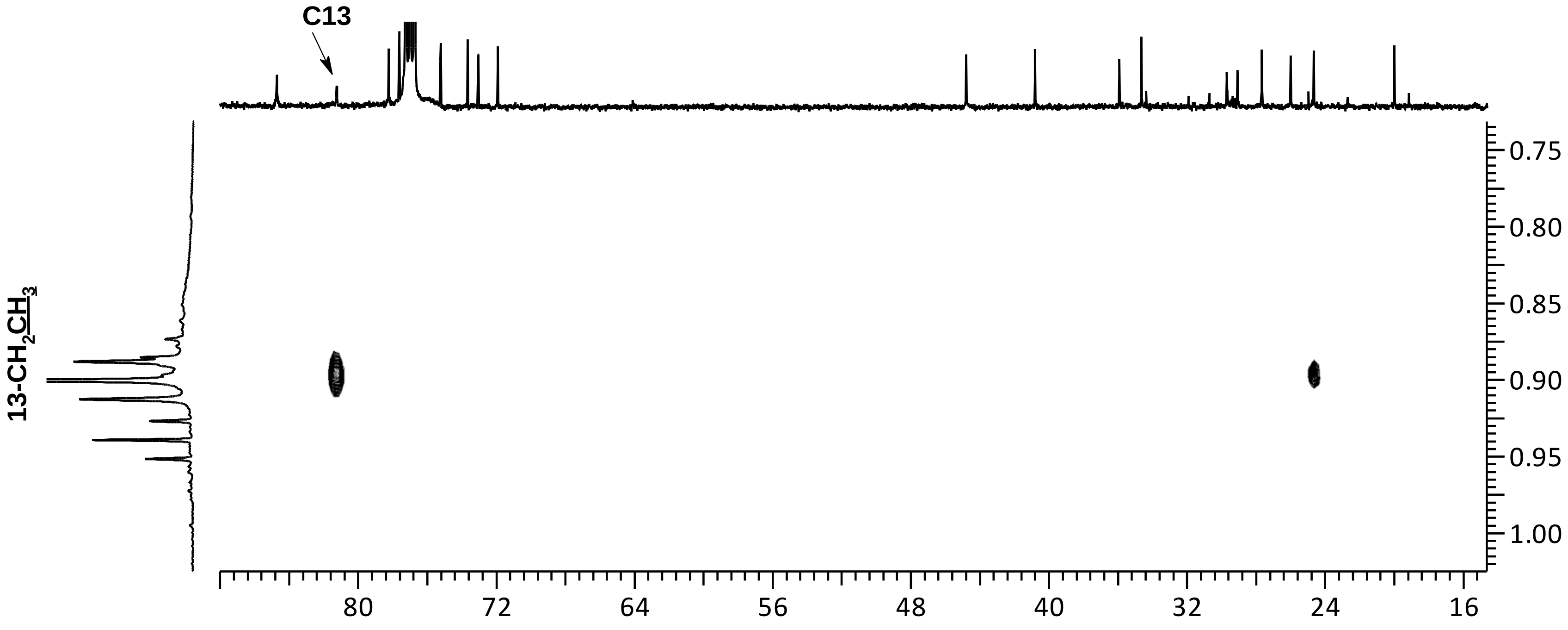




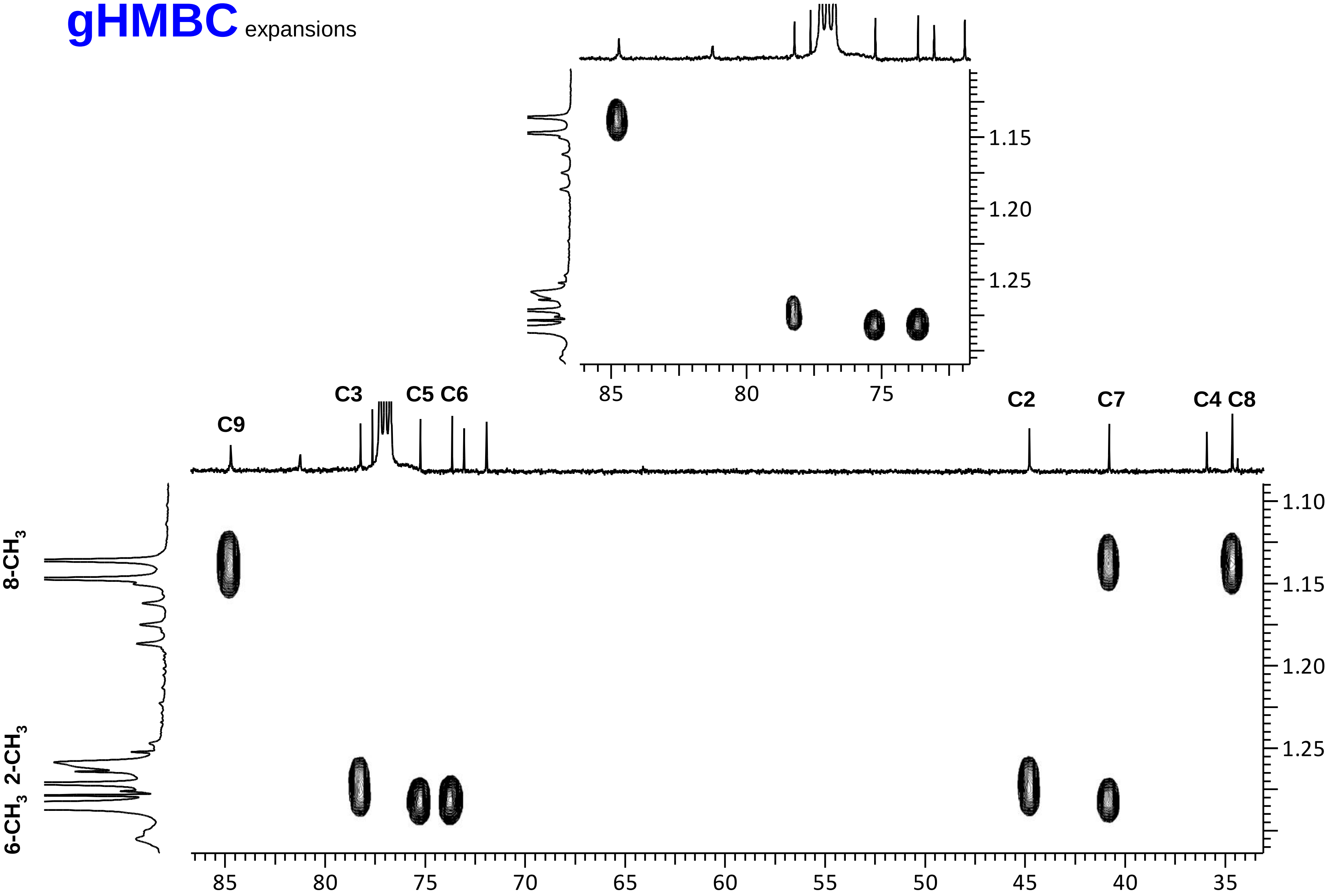


gHMBC expansion

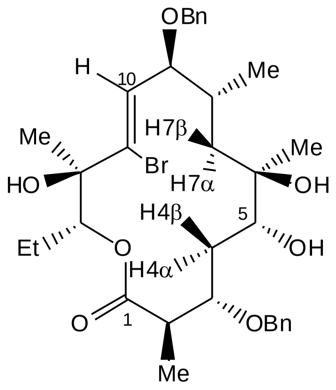

OBn
H
10
Me
H7β
Me
Me
Br
HO
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me



gHMBC expansions



gHMBC expansion


OBn
H
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Me
H7β
Me
Br
HO
H7α
OH
H4β
5
Et
O
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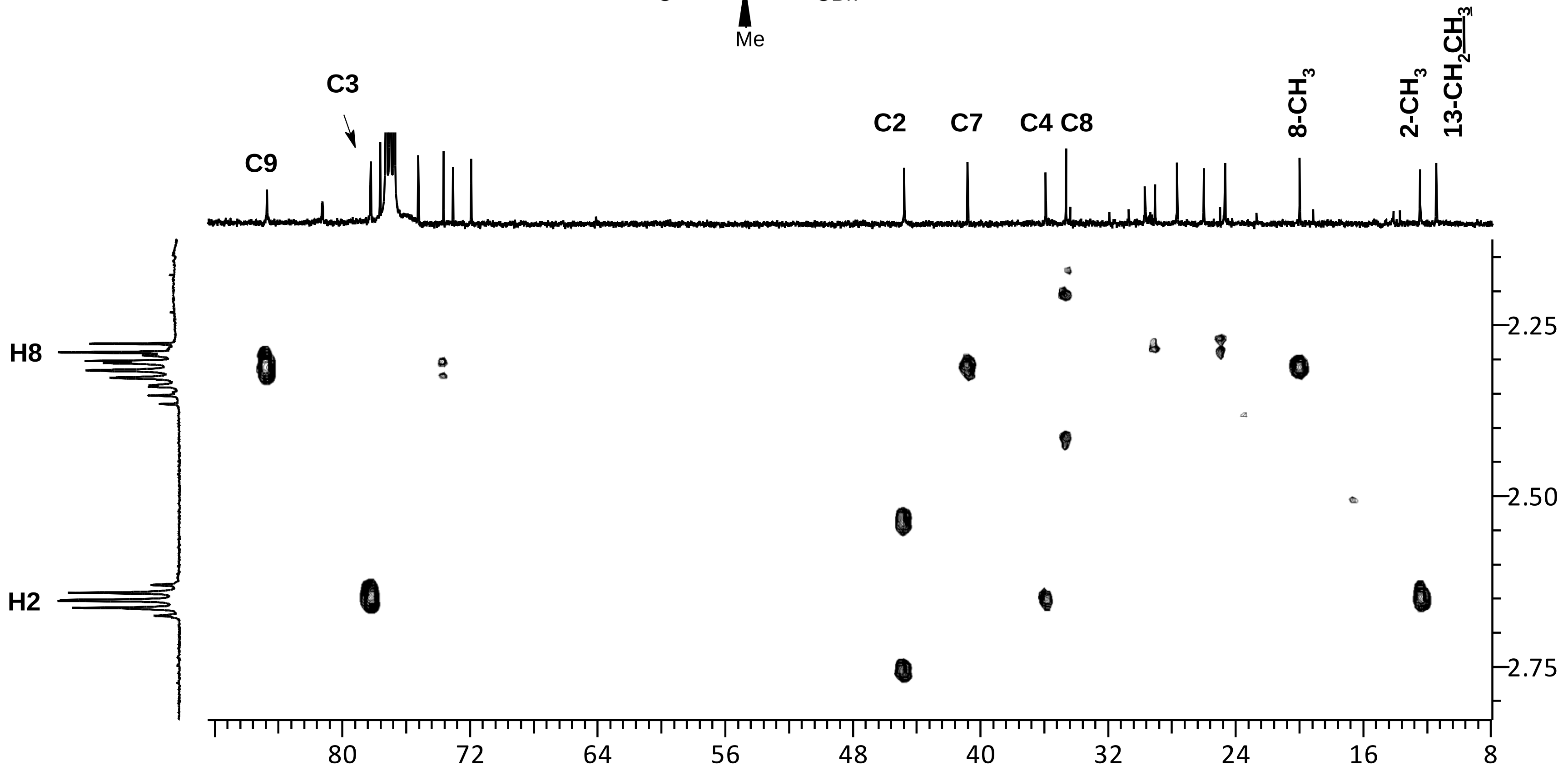
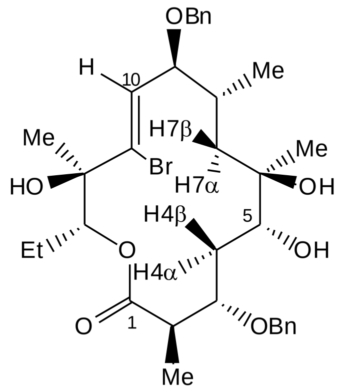
H4α
O
1
OBn
Me



gHMBC expansion


OBn
H
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H7β
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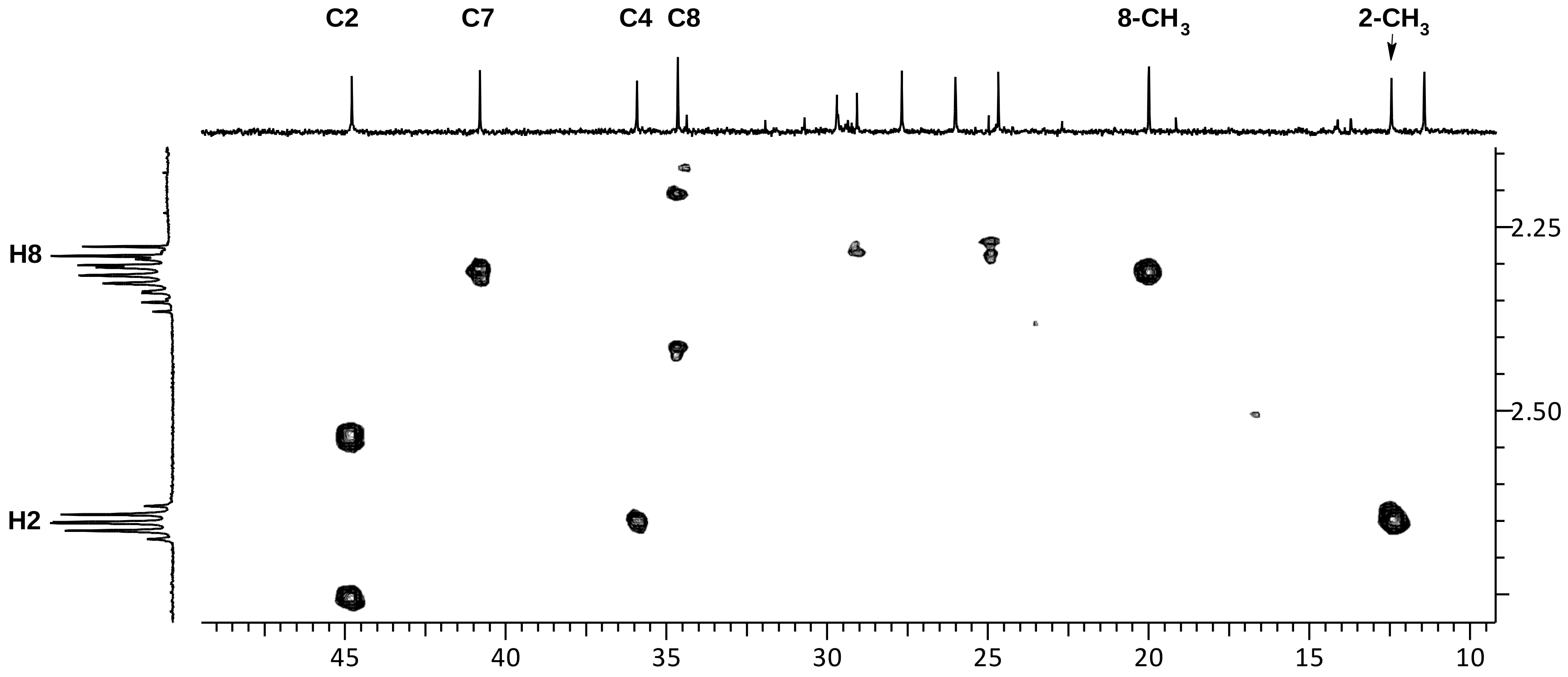
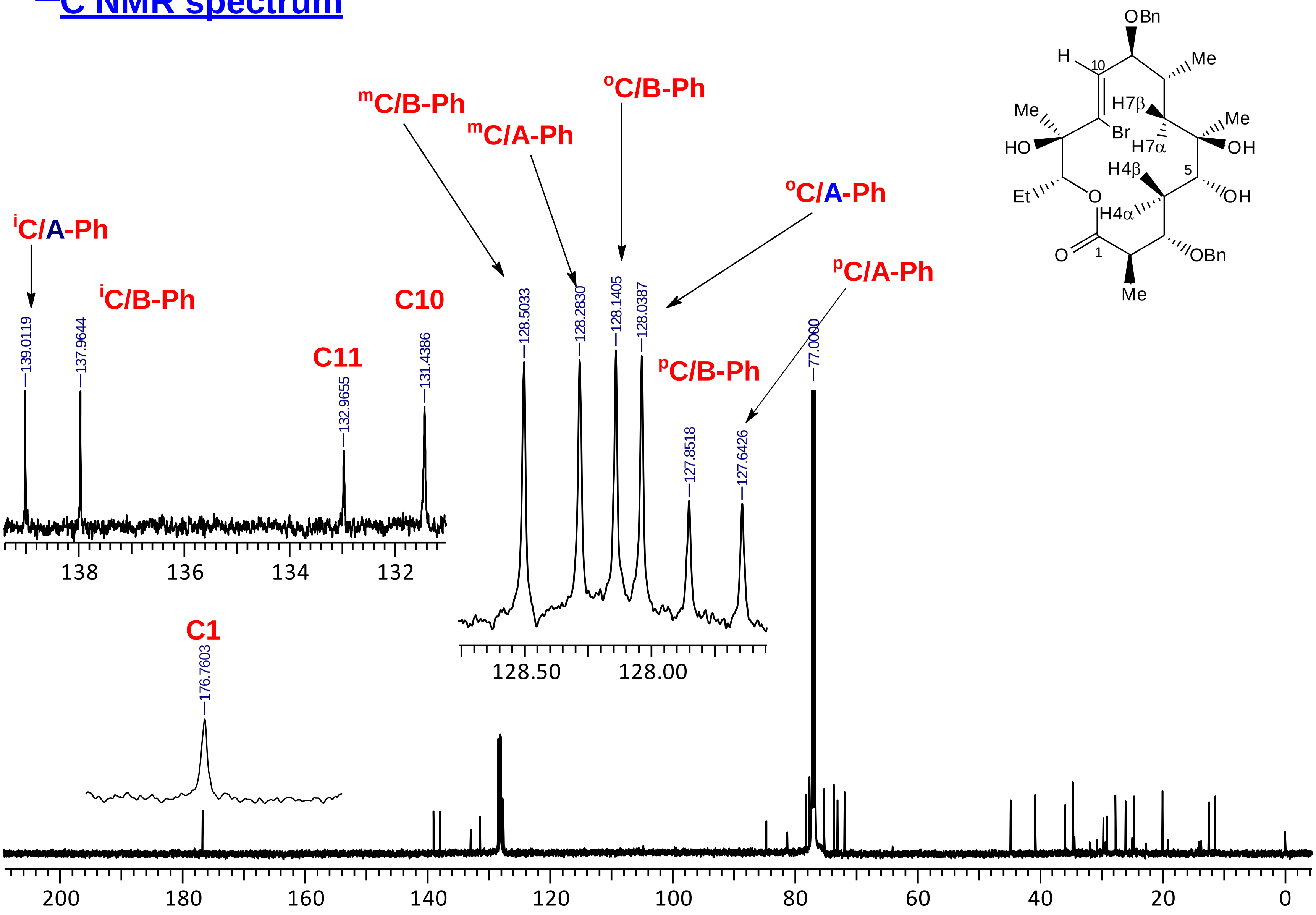
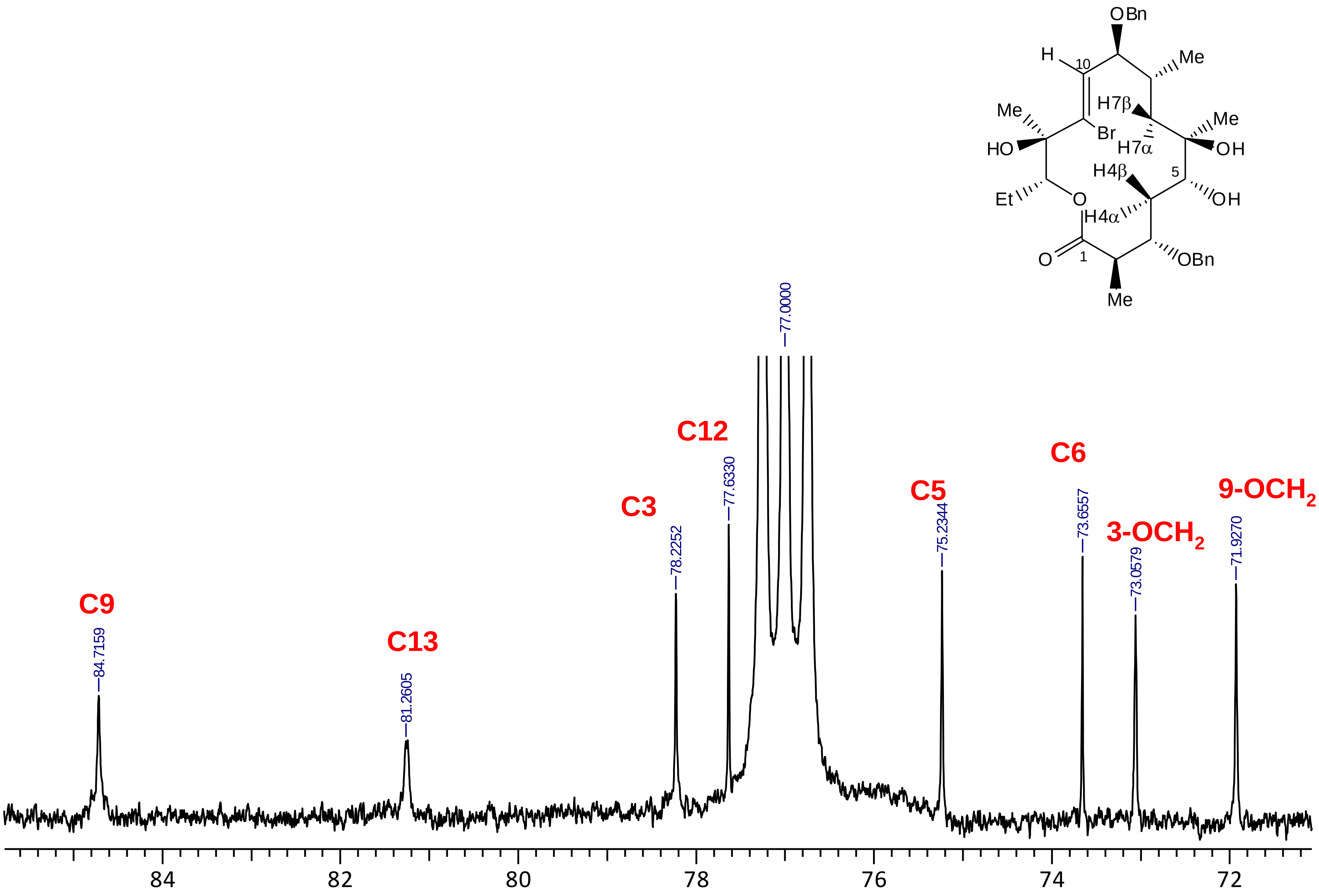
HO
Br
H7α
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me



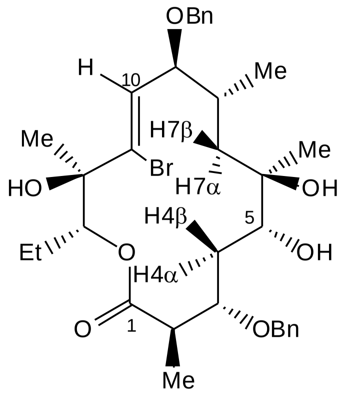
^{13}C NMR spectrum



^{13}C NMR spectrum



^{13}C NMR spectrum


OBn
H
10
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H7β
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HO
Br
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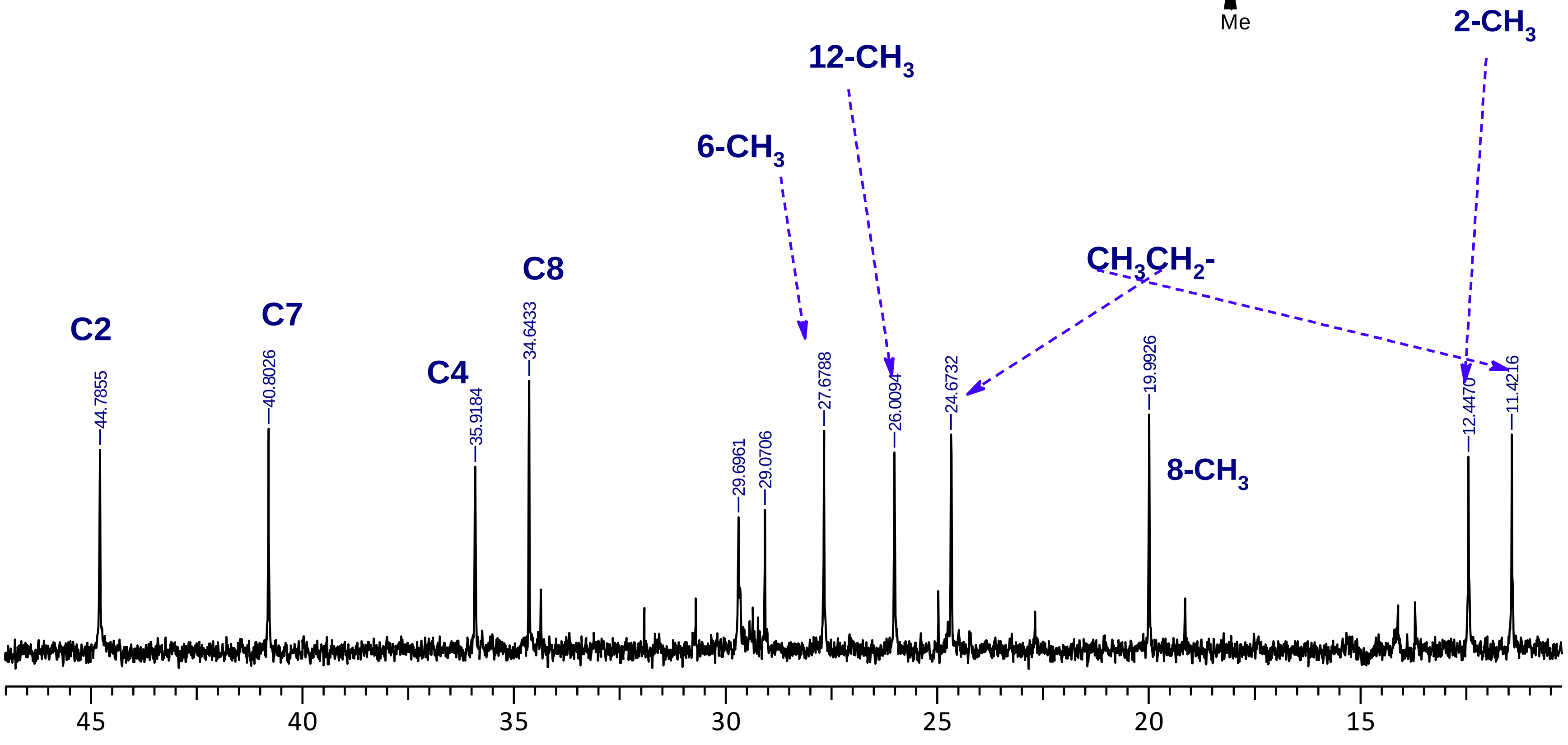
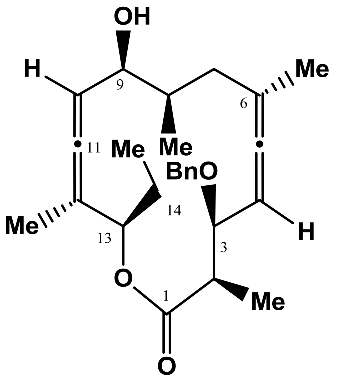
OH
H4β
5
Et
O
OH
H4α
O
1
OBn
Me





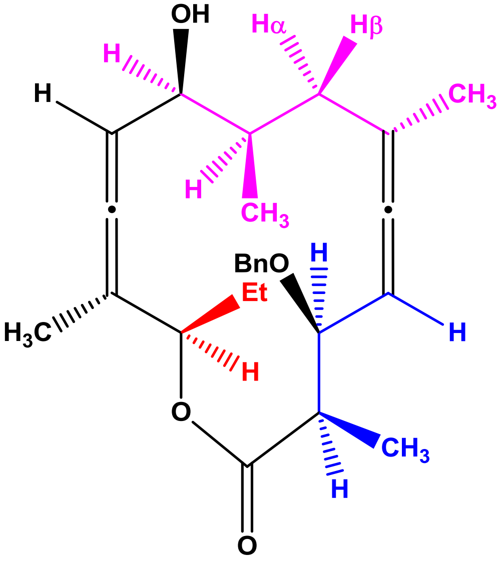
1H NMR chemical shifts (δ/ppm)
coupling constant (J/Hz)

2.77 (dq, $J_{H2, H3}$ = 8.7Hz, $J_{H2, 2\text{-}CH3}$ = 7.1Hz, **H2**)
1.27 (d, $J_{2\text{-}CH3, H2}$ = 7.3Hz, **2-CH_3**)
4.07 (dd, $J_{H3, H2}$ = 8.7Hz, $J_{H3, H4}$ = 3.4Hz, **H3**)
5.42 (m, $J_{H4, H3}$ = 3.4Hz, **H4**)
1.72 (d, $J_{6\text{-}CH3, H4}$ = 2.9Hz, **6-CH_3**)
1.45 (m, $J_{H7\beta, H7\alpha}$ = 16.1Hz, **H7β**)
2.17 (dt, $J_{H7\alpha, H8}$ = 6.4Hz, $J_{H7\alpha, H7\beta}$ = 16.1Hz, **H7α**)
1.80 - 1.85 (**m, H8**)
1.02 (d, $J_{8\text{-}CH3, H8}$ = 6.6Hz, **8-CH_3**)
3.80 (dd, $J_{H9, H8}$ = 5.1Hz, $J_{H9, H10}$ = 8.3Hz, **H9**)
5.27 (quartet, $J_{H10,H9}$ = 8.3Hz, $J_{H10,12\text{-}CH3}$ = 2.7Hz, **H10**)
1.62 (d, $J_{12\text{-}CH3, H10}$ = 2.7Hz, **12-CH_3**)
5.30 (t, J = 7.3Hz, **H13**)
1.60 - 1.70 (**m, 14-CH_2**)
0.88 (t, $J_{14\text{-}CH3, H14}$ = 7.6Hz, **14-CH_3**)
4.55 (d, J=11.5Hz, **3-OCH2Ph**)
4.62 (d, J=11.5Hz, **3-OCH2Ph**)

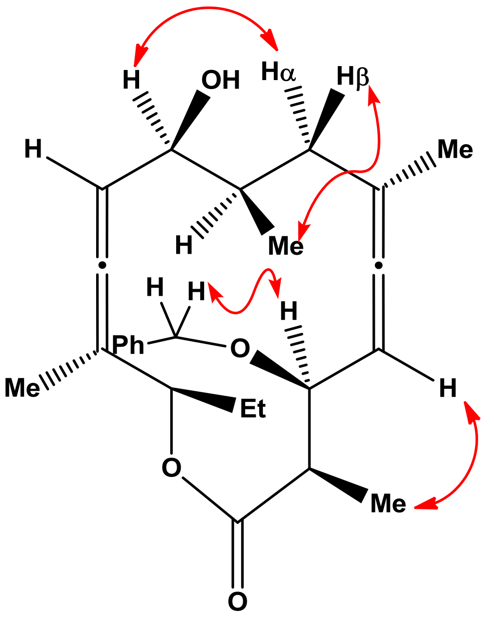
13C NMR chemical shift (δ/ppm)

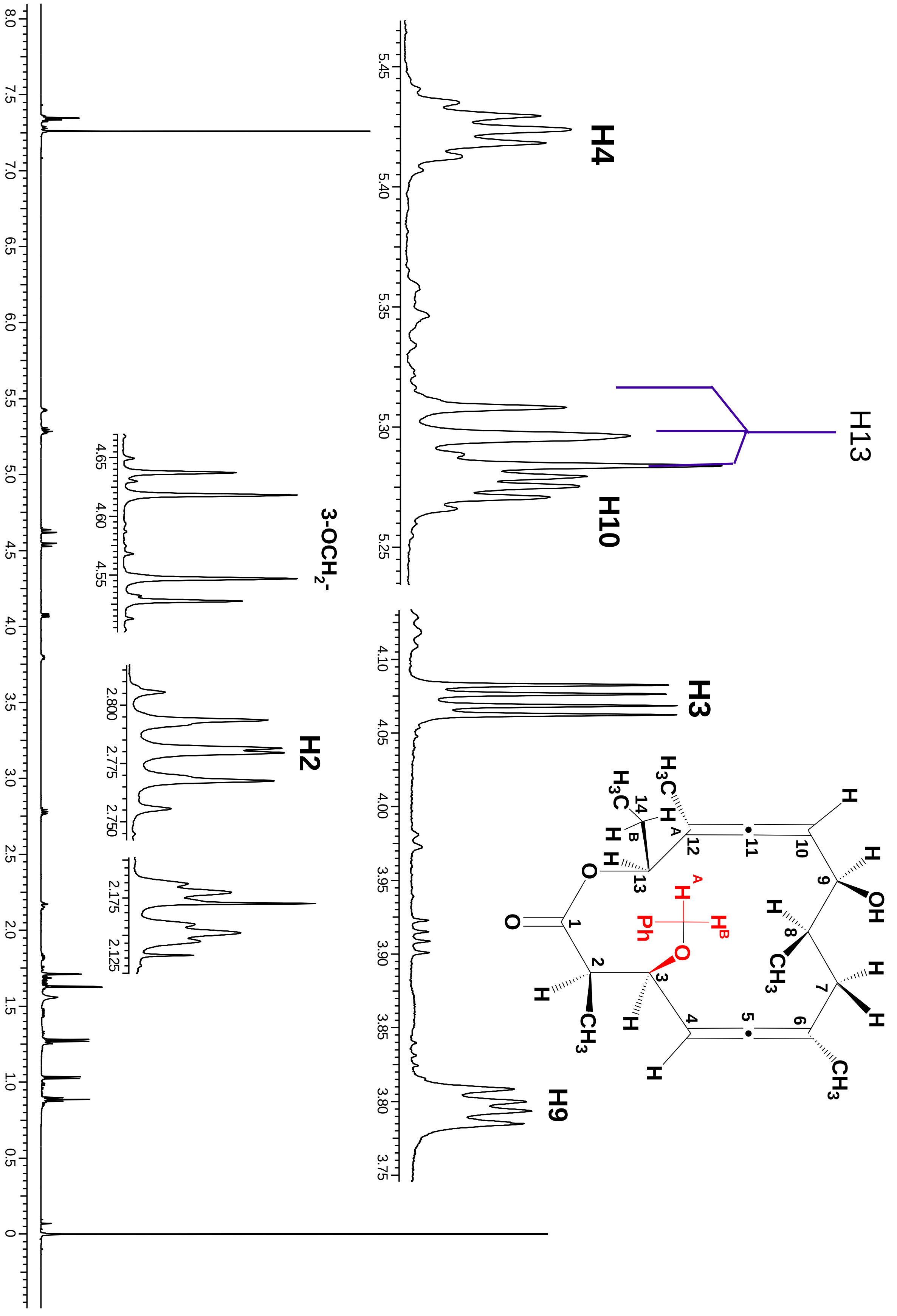
173.8 ----- C1
43.6 ----- C2
77.1 ----- C3
91.4 ----- C4
200.0 ----- C5
102.7 ----- C6
37.9 ----- C7
36.4 ----- C8
74.2 ----- C9
93.7 ----- C10
202.6 ----- C11
99.9 ----- C12
75.9 ----- C13
24.7 ----- C14
15.2 ----- 2-CH_3
20.6 ----- 6-CH_3
16.9 ----- 8-CH_3
12.5 ----- 12-CH_3
9.8 ----- 14-CH_3
138.5 ----- ^{ipso}C
128.3 ----- $^{ortho}C$
127.8 ----- ^{meta}C
127.5 ----- ^{para}C

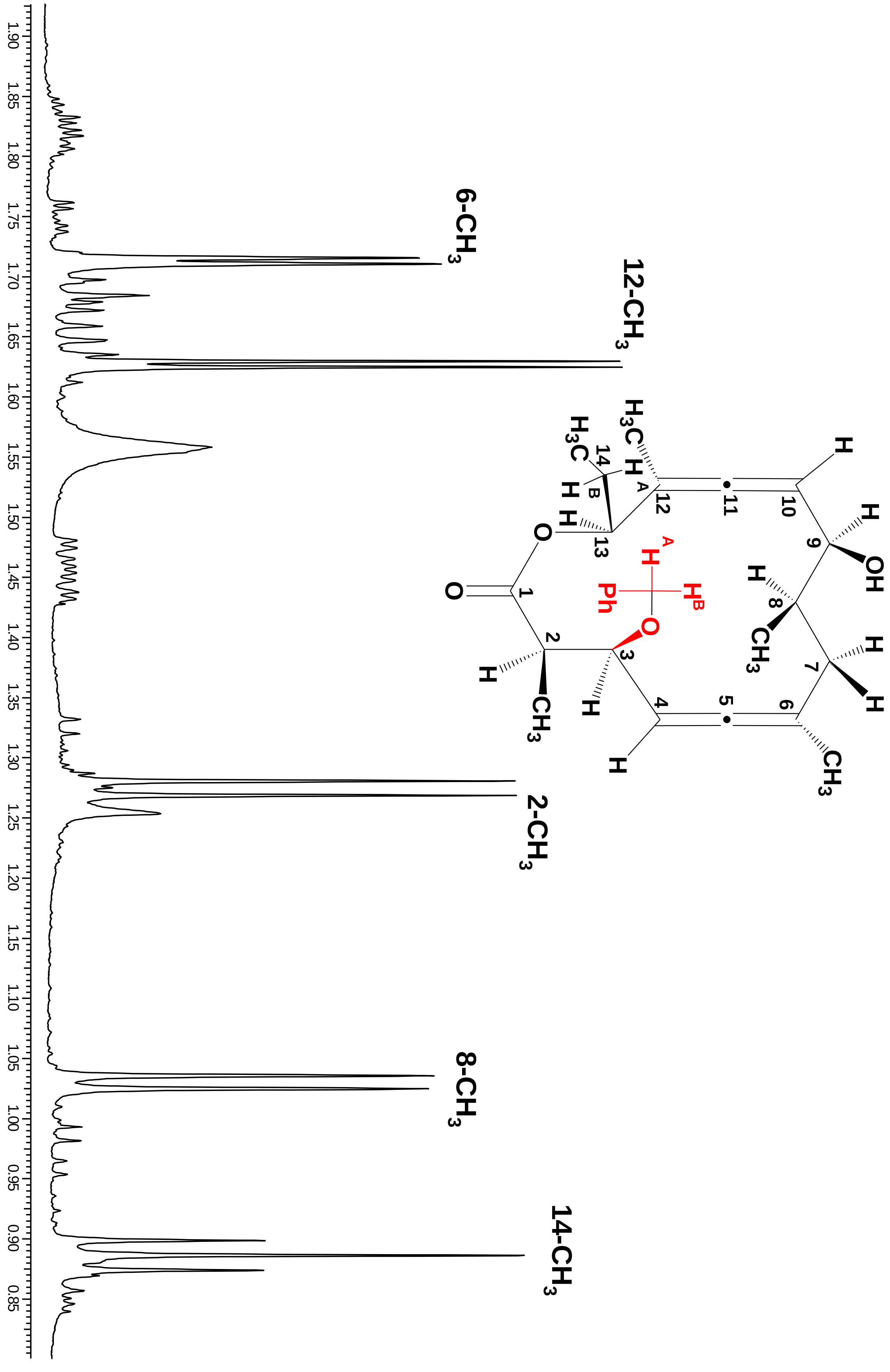
1D TOCSY

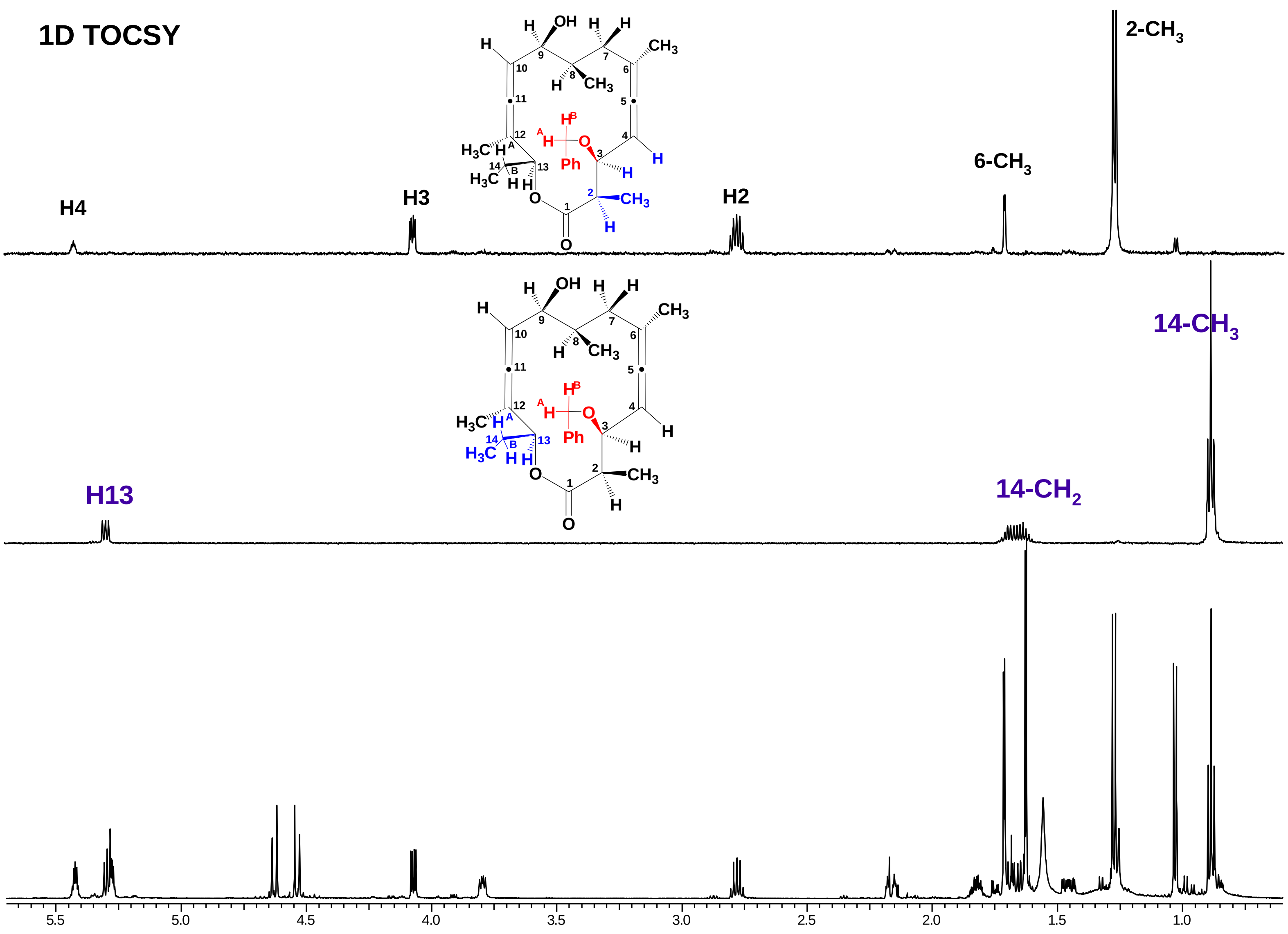

OH
H
Hα
Hβ
H
CH3
H
CH3
BnO
H
Et
H3C
H
H
O
CH3
H
O

NOESY

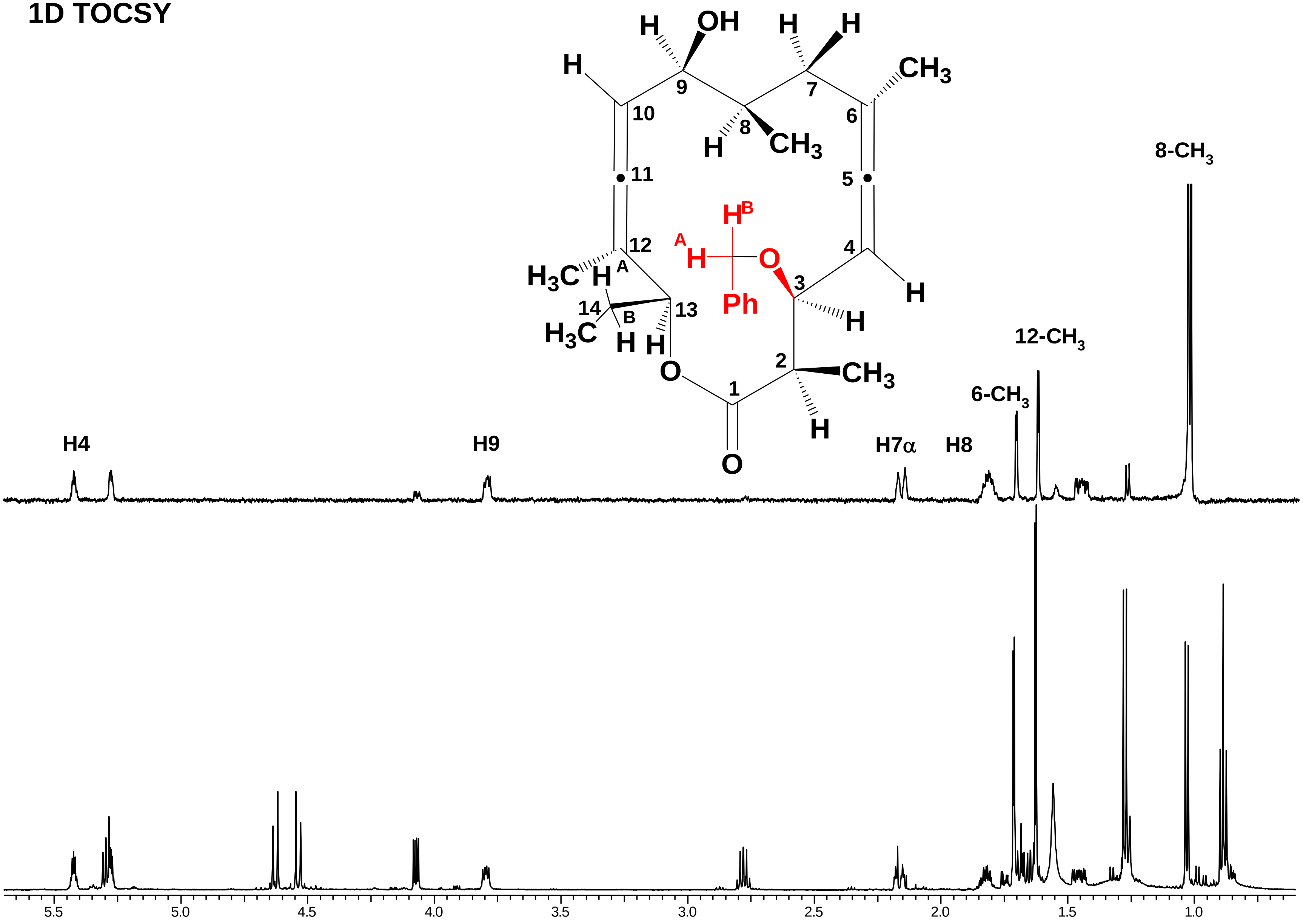

H
OH
Hα
Hβ
H
Me
H
Me
H
H
H
Ph
O
Me
H
Et
O
Me
O


H4
H13
H10
H3
H9
H2
3-OCH$_2$-
5.45
5.40
5.35
5.30
5.25
4.10
4.05
4.00
3.95
3.90
3.85
3.80
3.75
4.65
4.60
4.55
2.800
2.775
2.750
2.175
2.125
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
H
OH
CH$_3$
H$_3$C
H^A
H^B
Ph
O
1
2
3
4
5
6
7
8
9
10
11
12
13
14
A
B

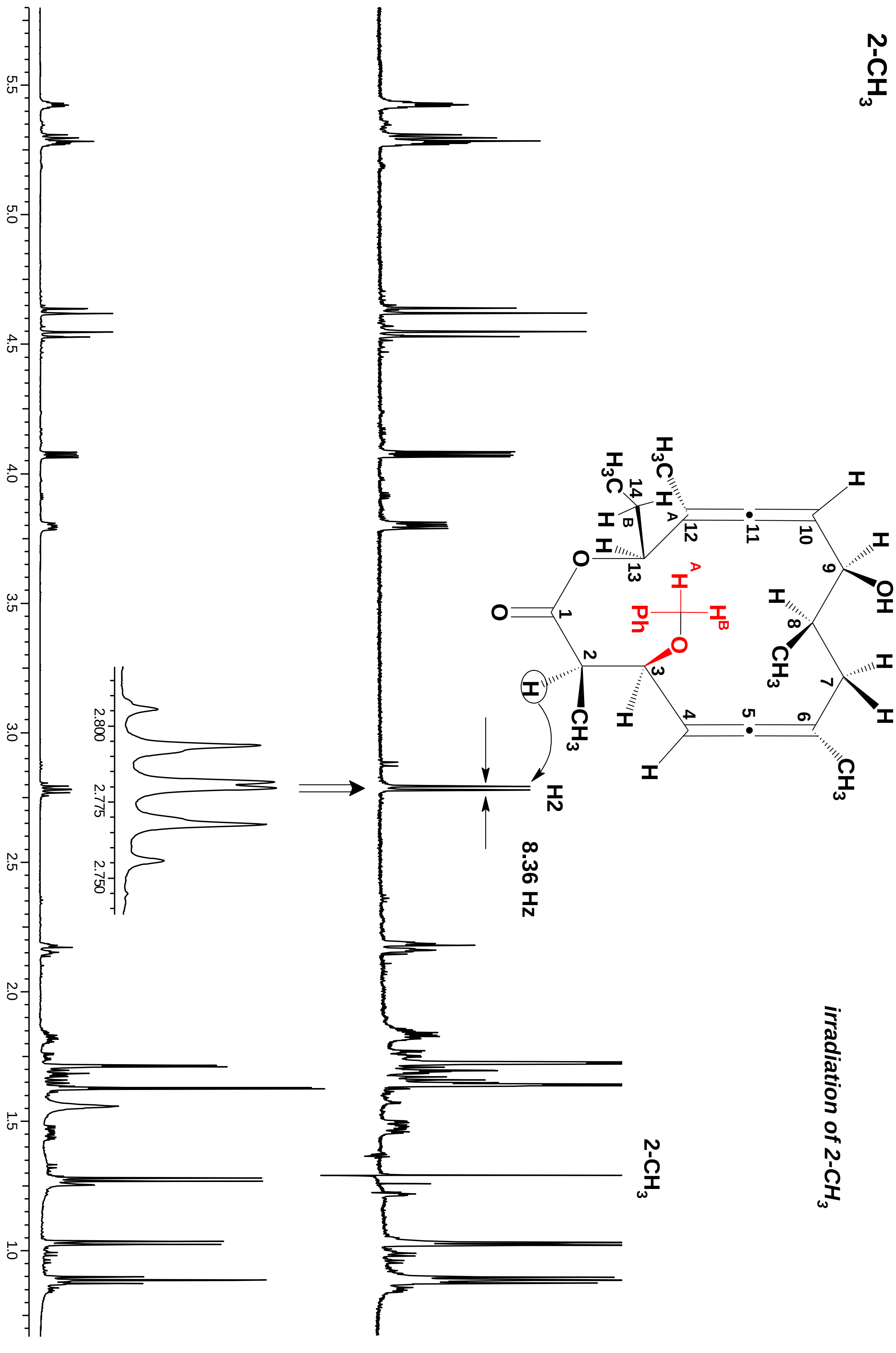

6-CH$_3$
12-CH$_3$
2-CH$_3$
8-CH$_3$
14-CH$_3$
1.90
1.85
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85
H$_3$C
H^A
H^B
Ph
OH
CH$_3$
O

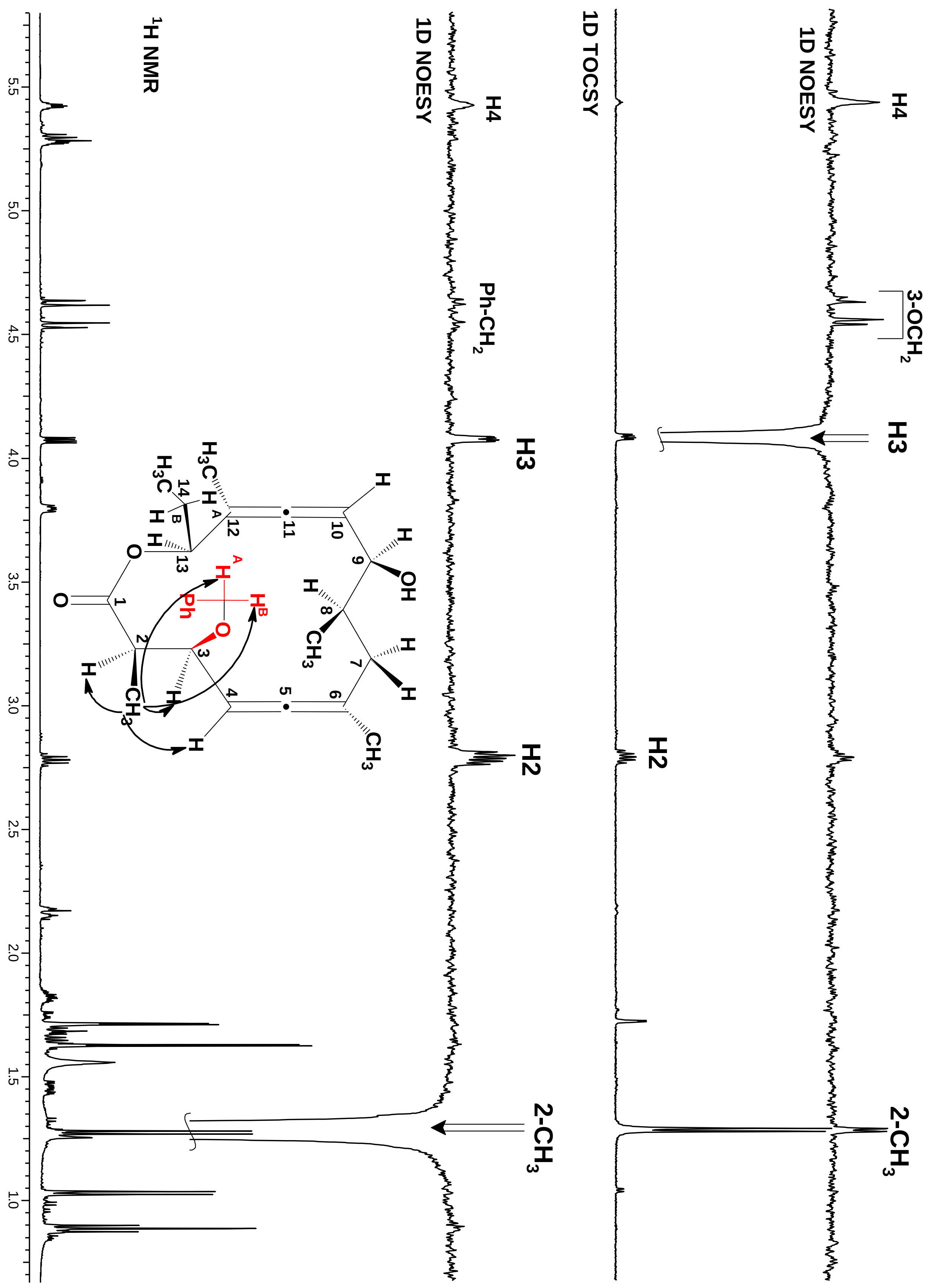

1D TOCSY
H4
H3
H2
6-CH$_3$
2-CH$_3$
H13
14-CH$_2$
14-CH$_3$
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

1D TOCSY

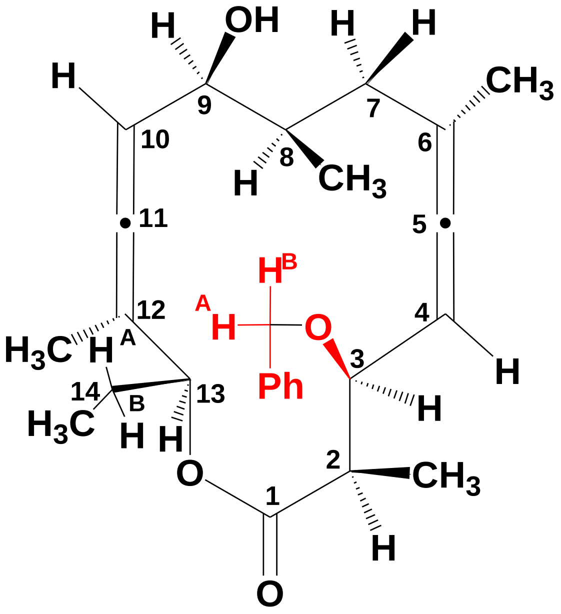


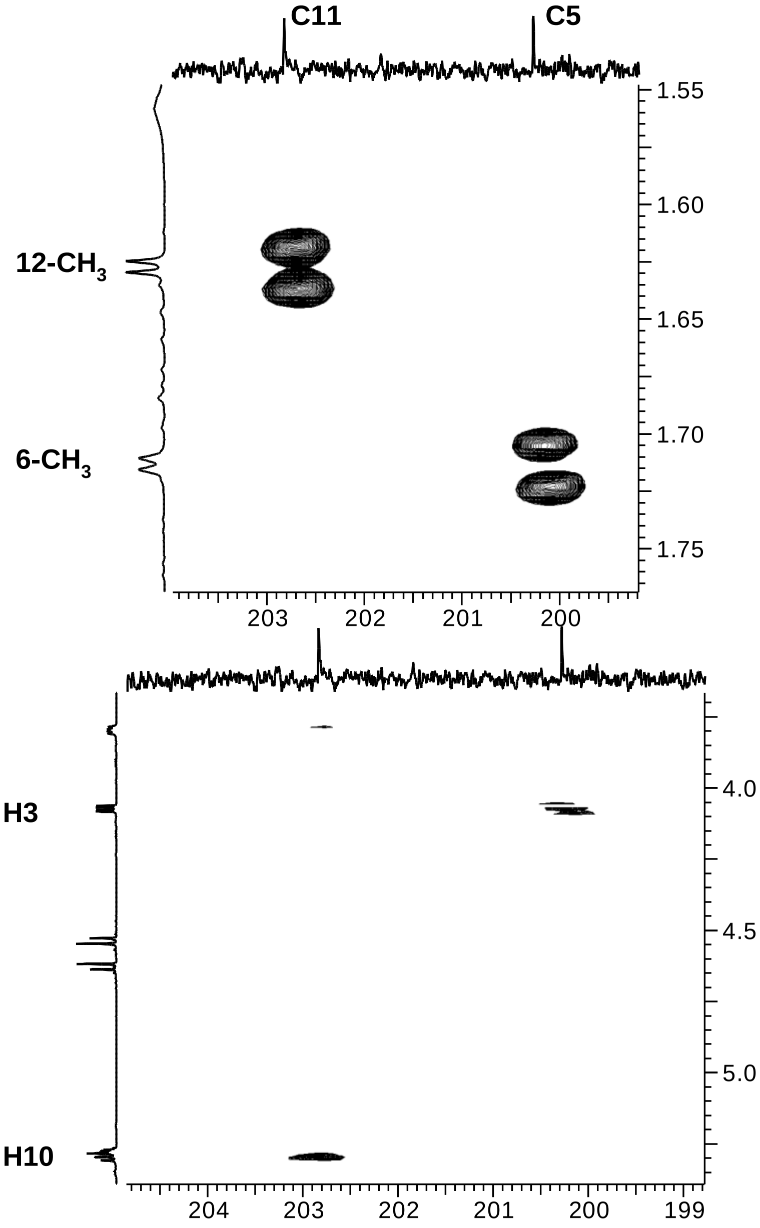
Selective decoupling of 2-CH$_3$




1D NOESY
H4
3-OCH$_2$
H3
H2
2-CH$_3$
1D TOCSY
H3
H2
2-CH$_3$
1D NOESY
H4
Ph-CH$_2$
H3
H2
2-CH$_3$
^{1}H NMR
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
H
OH
H
CH$_3$
H
H
CH$_3$
H$_3$C
H$_3$C
H
H
H
H
O
O
Ph
O
H^A
H^B
H
H
CH$_3$
H
1
2
3
4
5
6
7
8
9
10
11
12
13
14
A
B

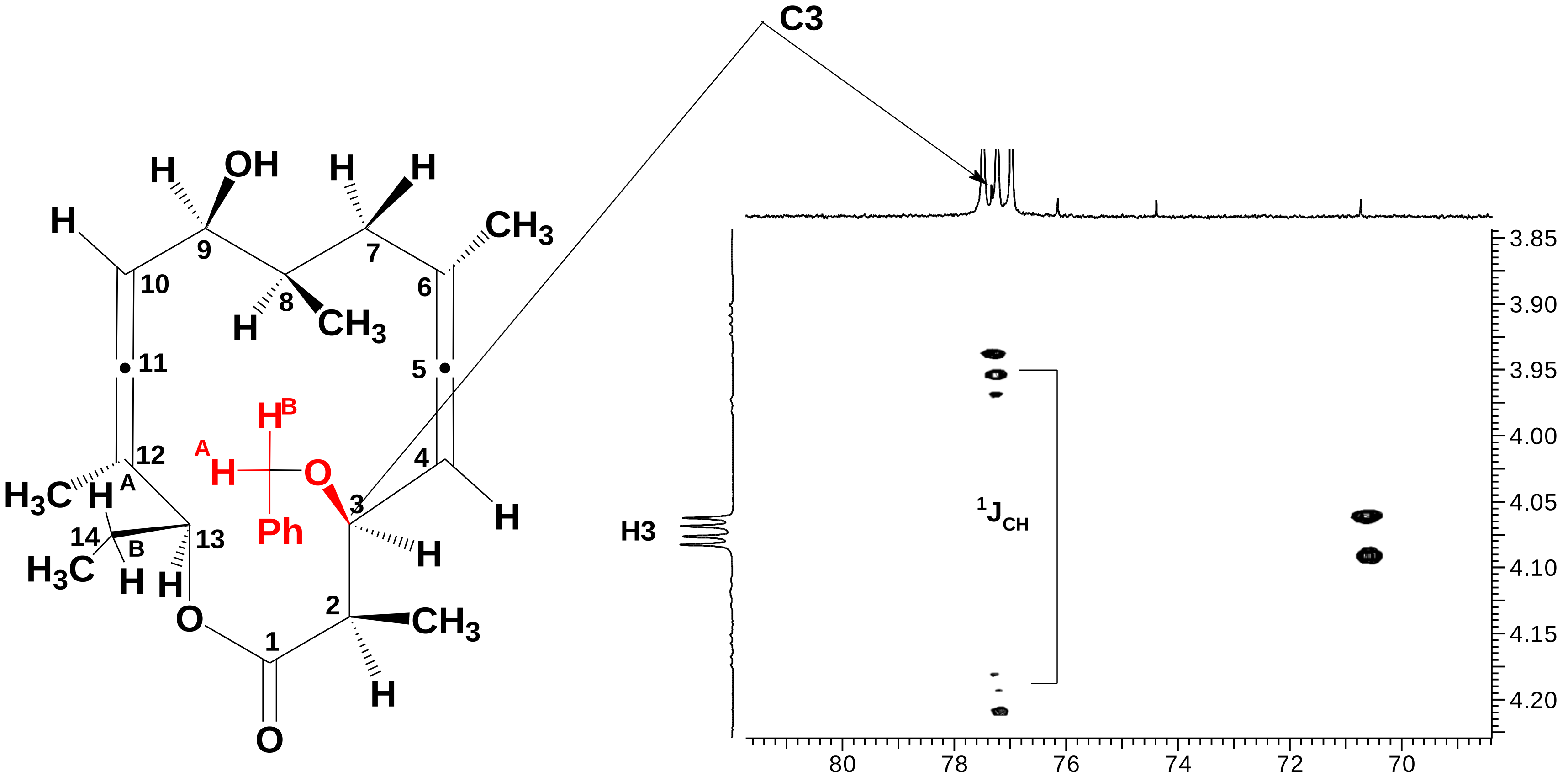
Long-range **gHMBC** correlations


H
OH
H
H
H
CH$_3$
9
7
6
10
8
H
CH$_3$
11
5
H^B
12
^{A}H
O
4
H$_3$C
H
A
3
H
Ph
14
B
13
H$_3$C
H
H
2
CH$_3$
O
1
H
O

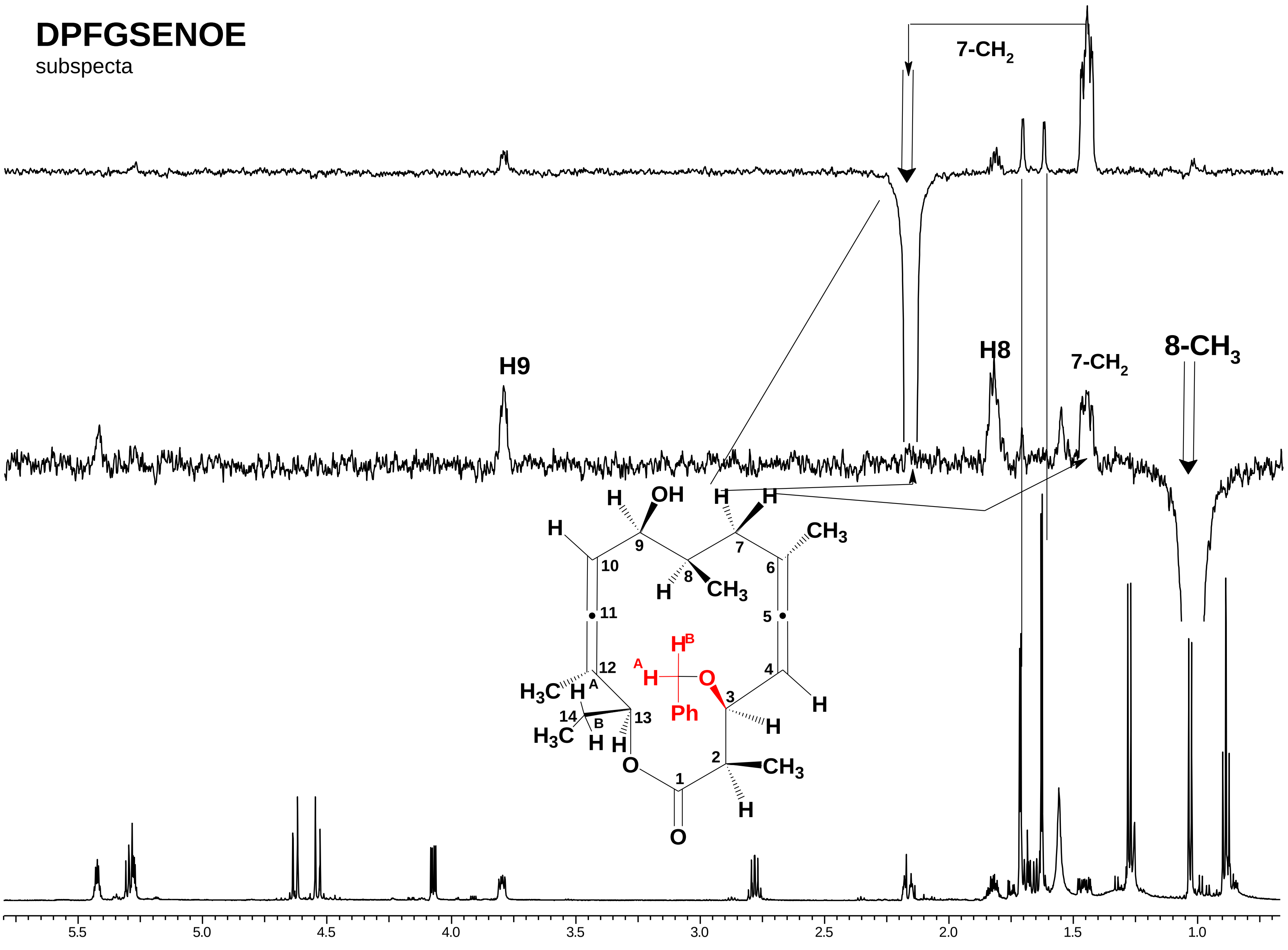


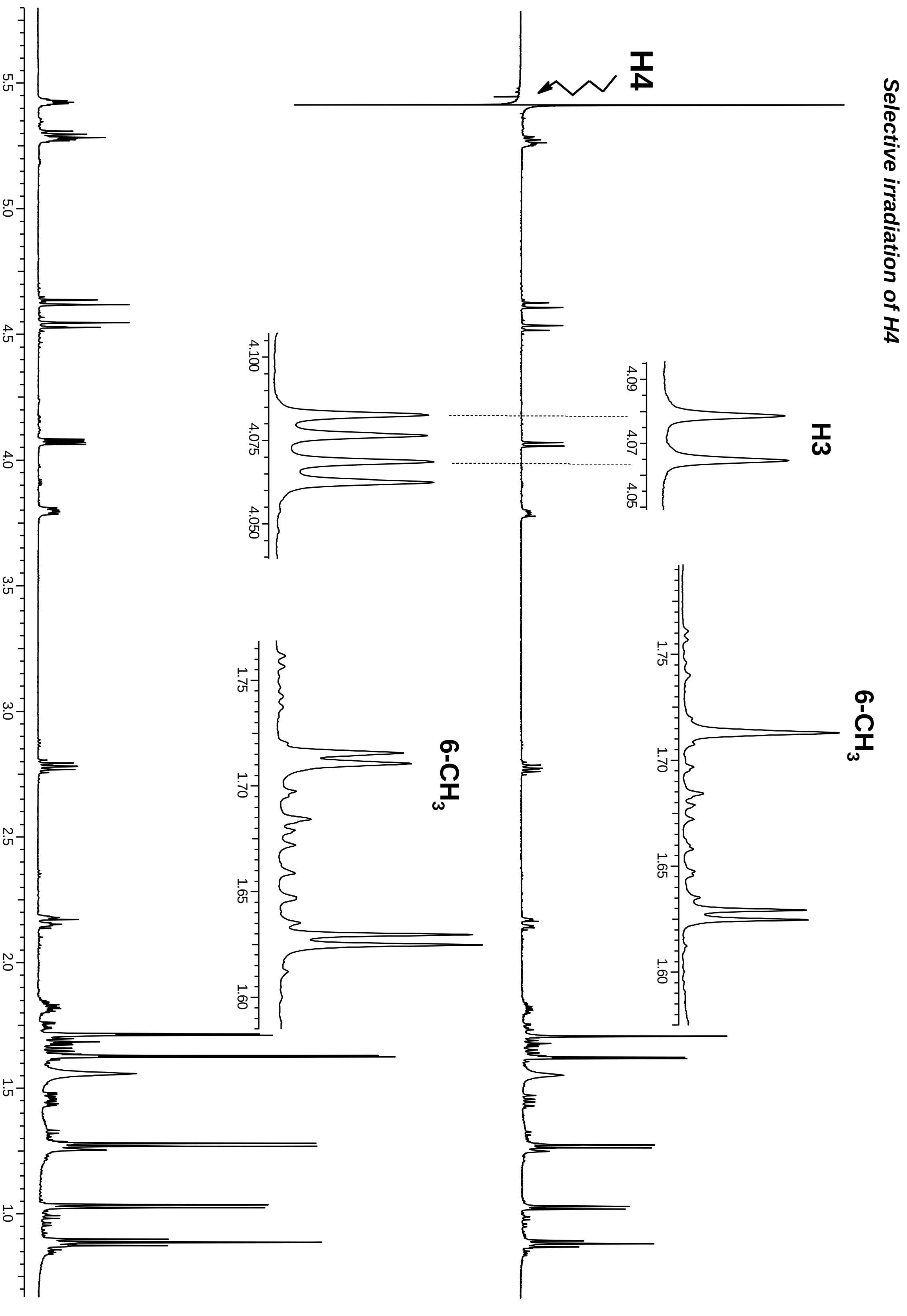
gHMBC

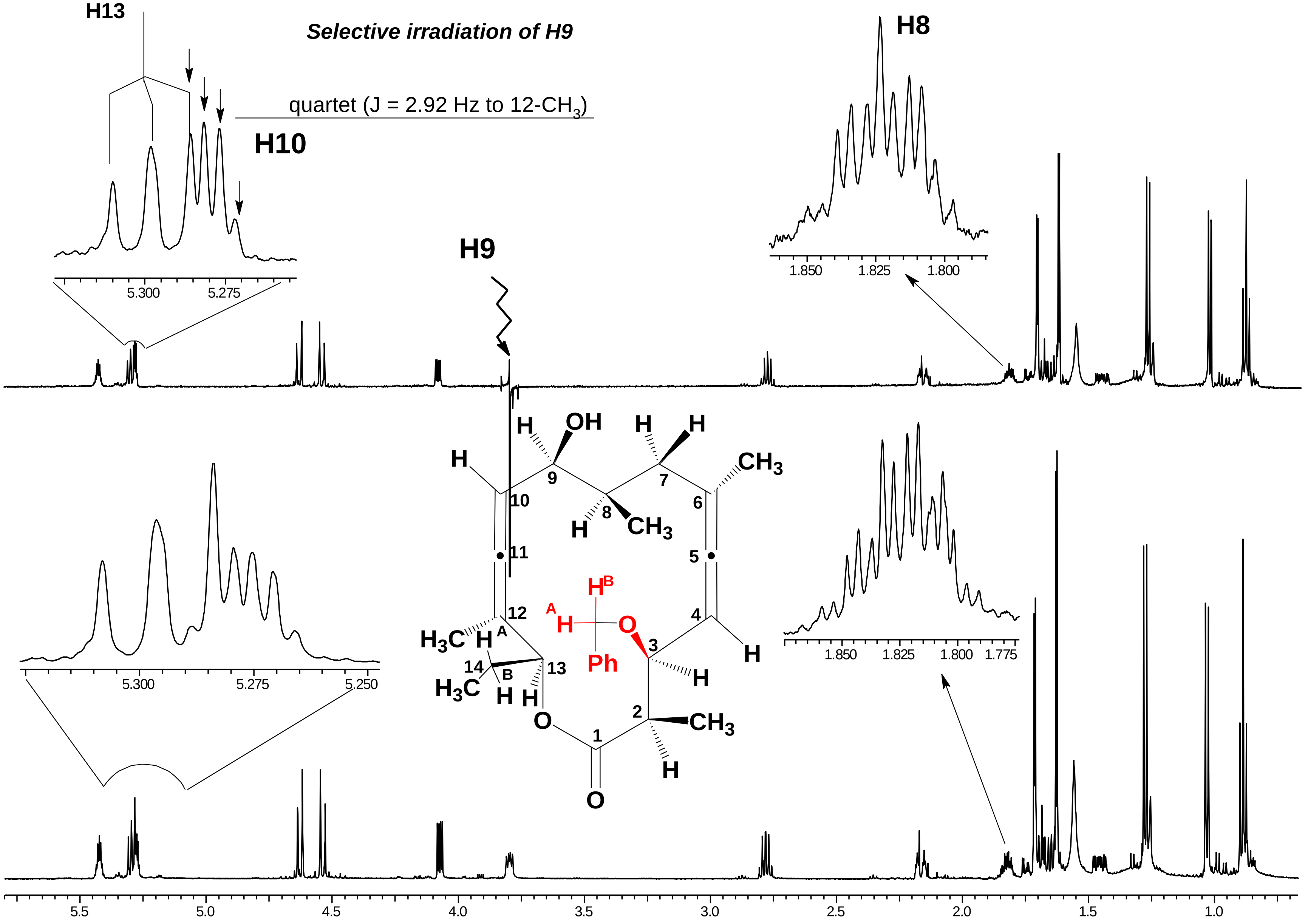
confirming of ***C3*** *assigment*



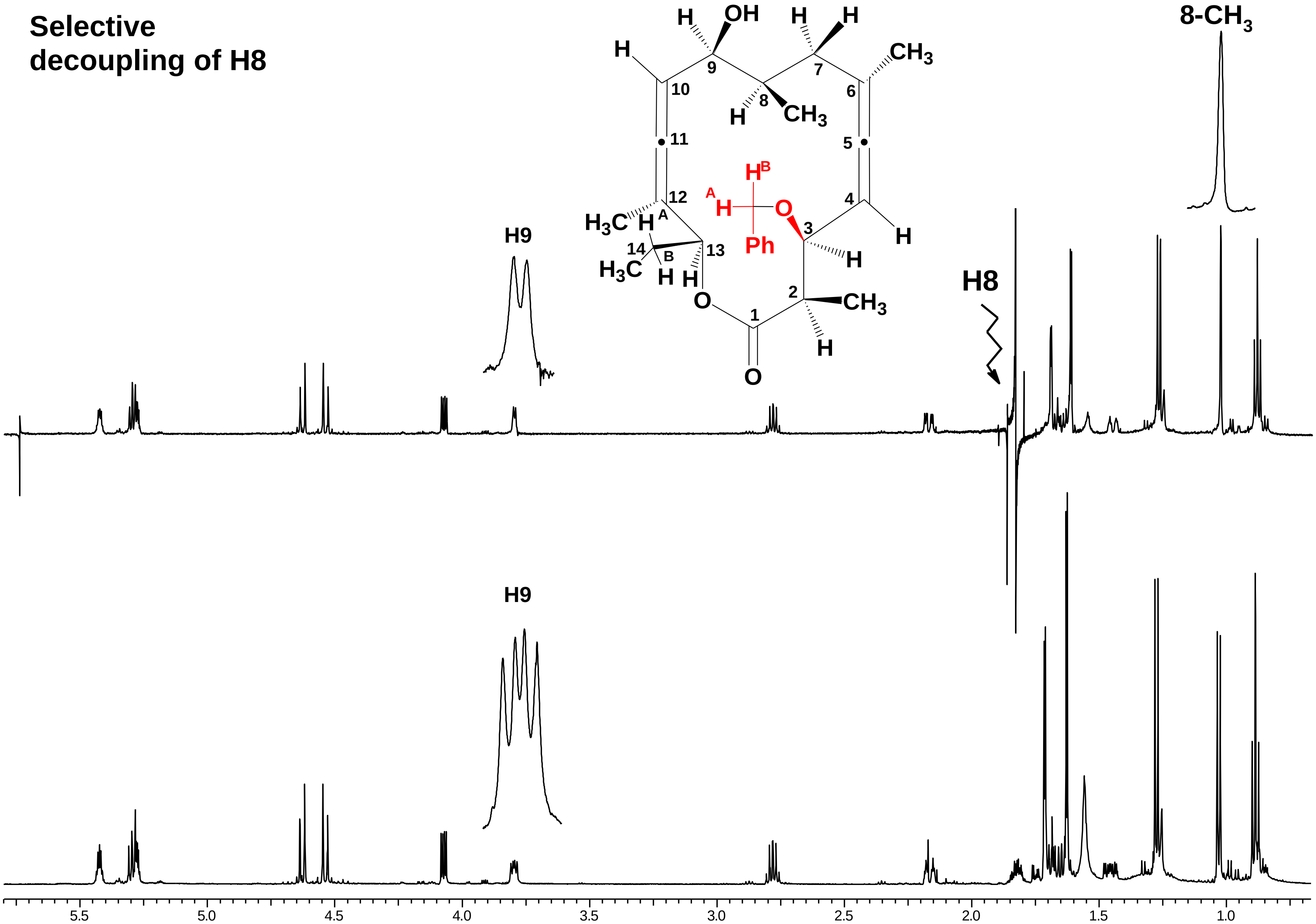
DPFGSENOE
subspecta

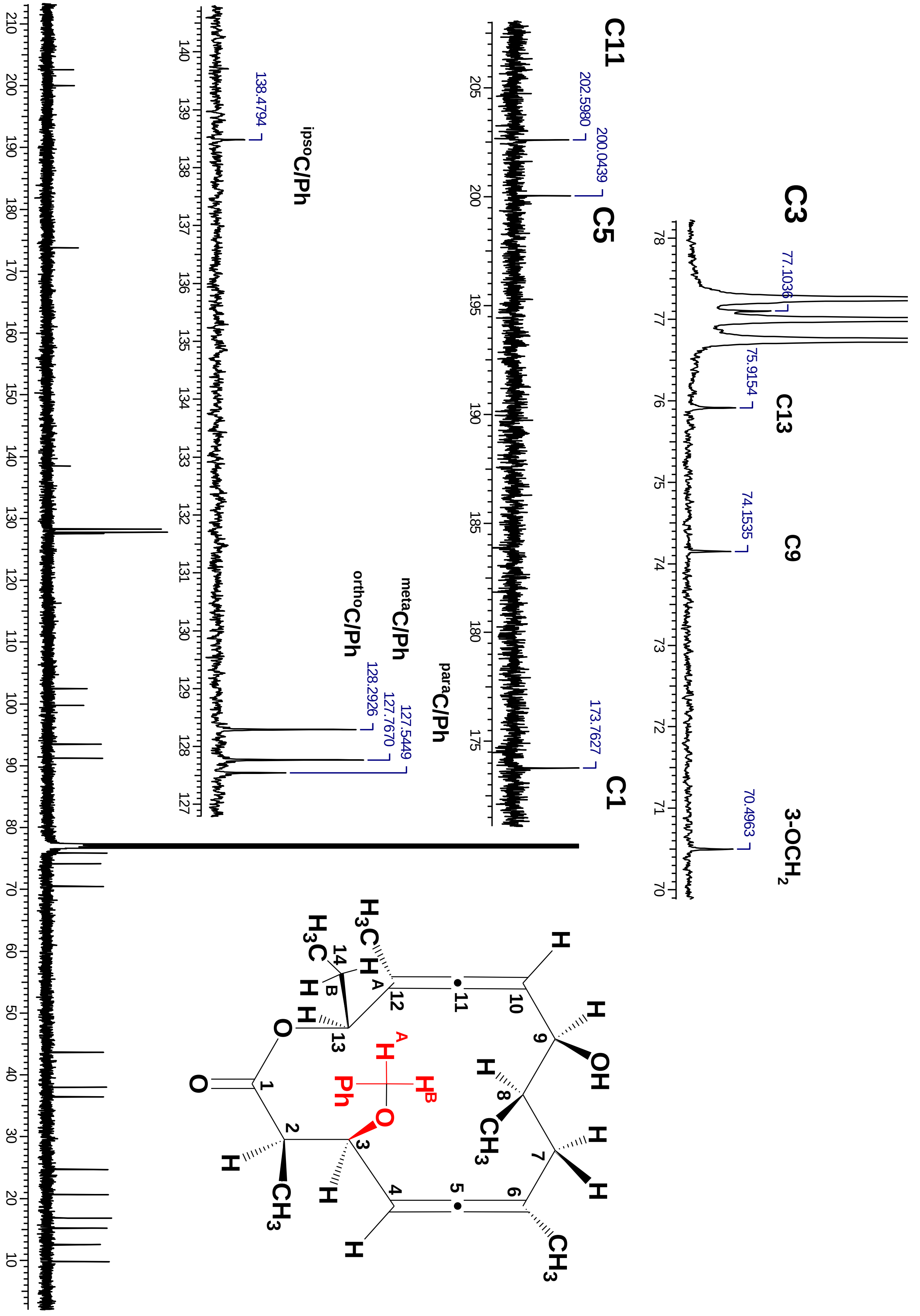



Selective irradiation of H4
H4
H3
6-CH$_3$
6-CH$_3$
4.09
4.07
4.05
4.100
4.075
4.050
1.75
1.70
1.65
1.60
1.75
1.70
1.65
1.60
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

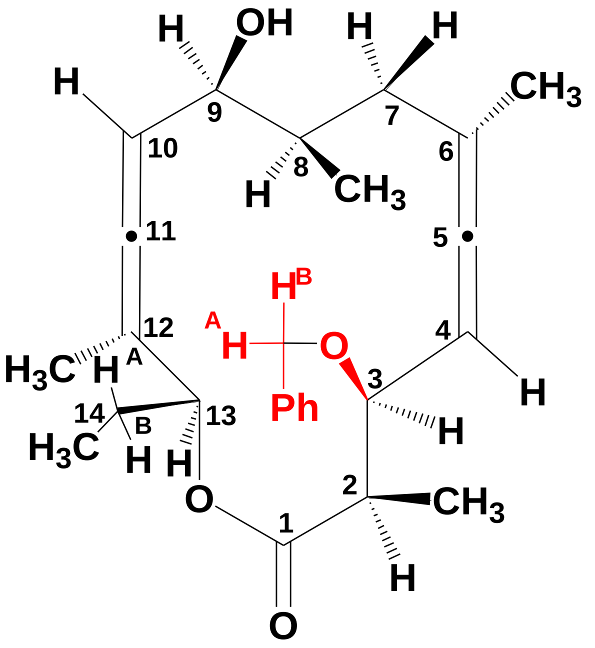

Selective irradiation of H9
H13
H10
quartet (J = 2.92 Hz to 12-CH3)
5.300
5.275
H9
H8
1.850
1.825
1.800
OH
H
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
A
B
H3C
O
Ph
5.250
1.775
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Selective decoupling of H8

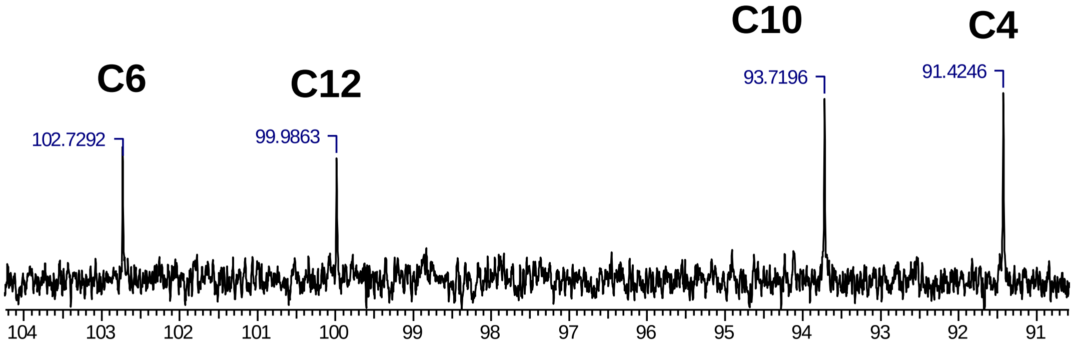


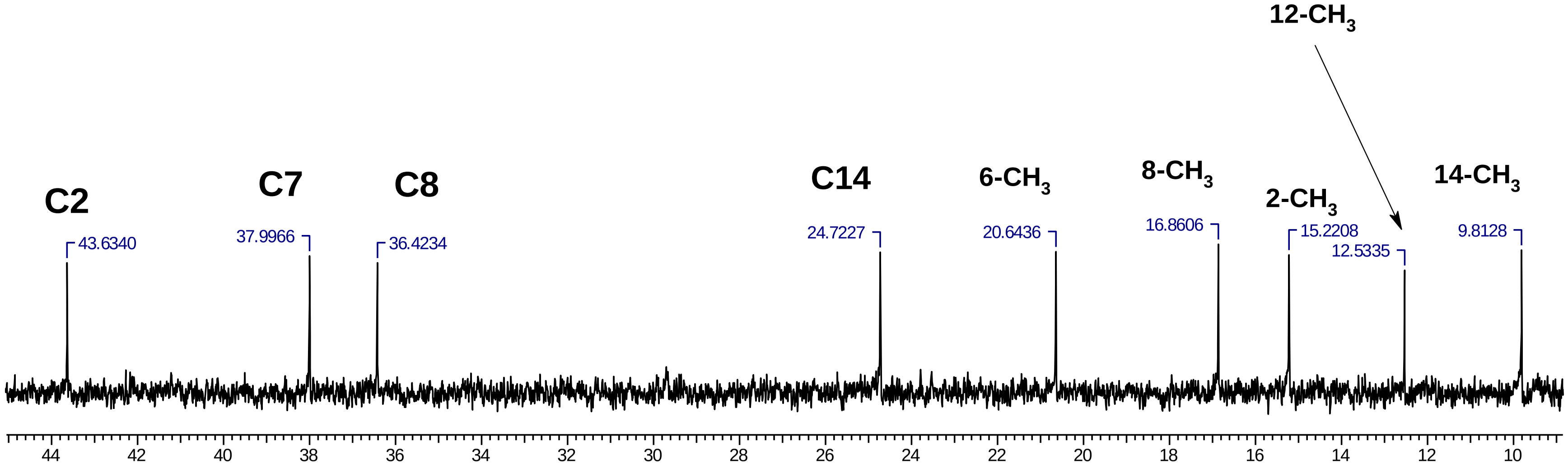

C3
77.1036
75.9154
C13
74.1535
C9
70.4963
3-OCH_2
C11
202.5980
200.0439
C5
173.7627
C1
138.4794
^{ipso}C/Ph
^{meta}C/Ph
^{ortho}C/Ph
^{para}C/Ph
128.2926
127.7670
127.5449
78
77
76
75
74
73
72
71
70
205
200
195
190
185
180
175
140
139
138
137
136
135
134
133
132
131
130
129
128
127
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
O
OH
CH_3
H_3C
H^A
H^B
Ph

^{13}C NMR spectrum in $CDCl_3$

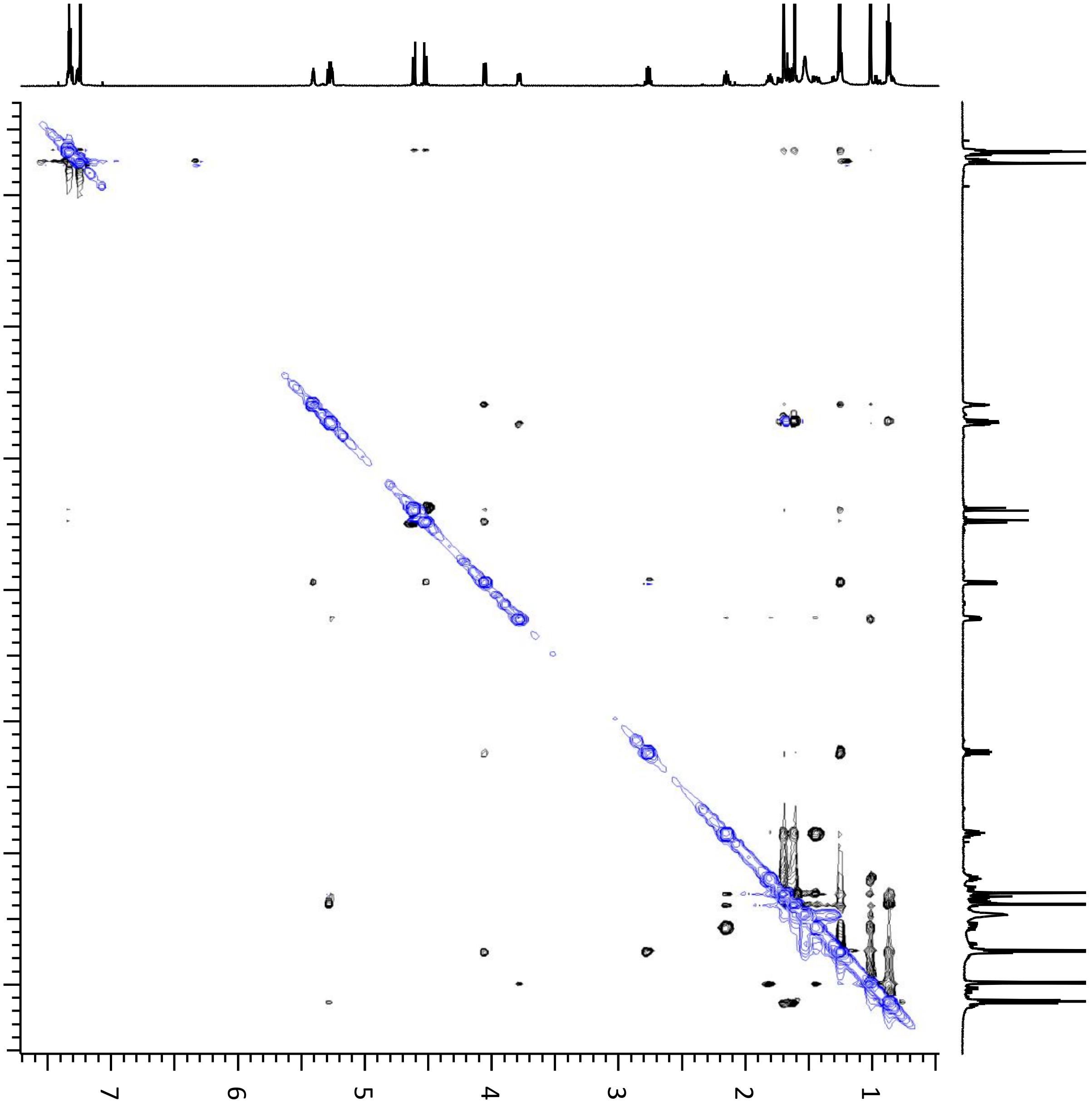


H
OH
H
H
CH3
H
9
7
10
6
8
H
CH3
11
5
HB
AH
O
12
4
H3C
H A
3
H
14
B
13
Ph
H
H3C
H
H
2
CH3
O
1
H
O



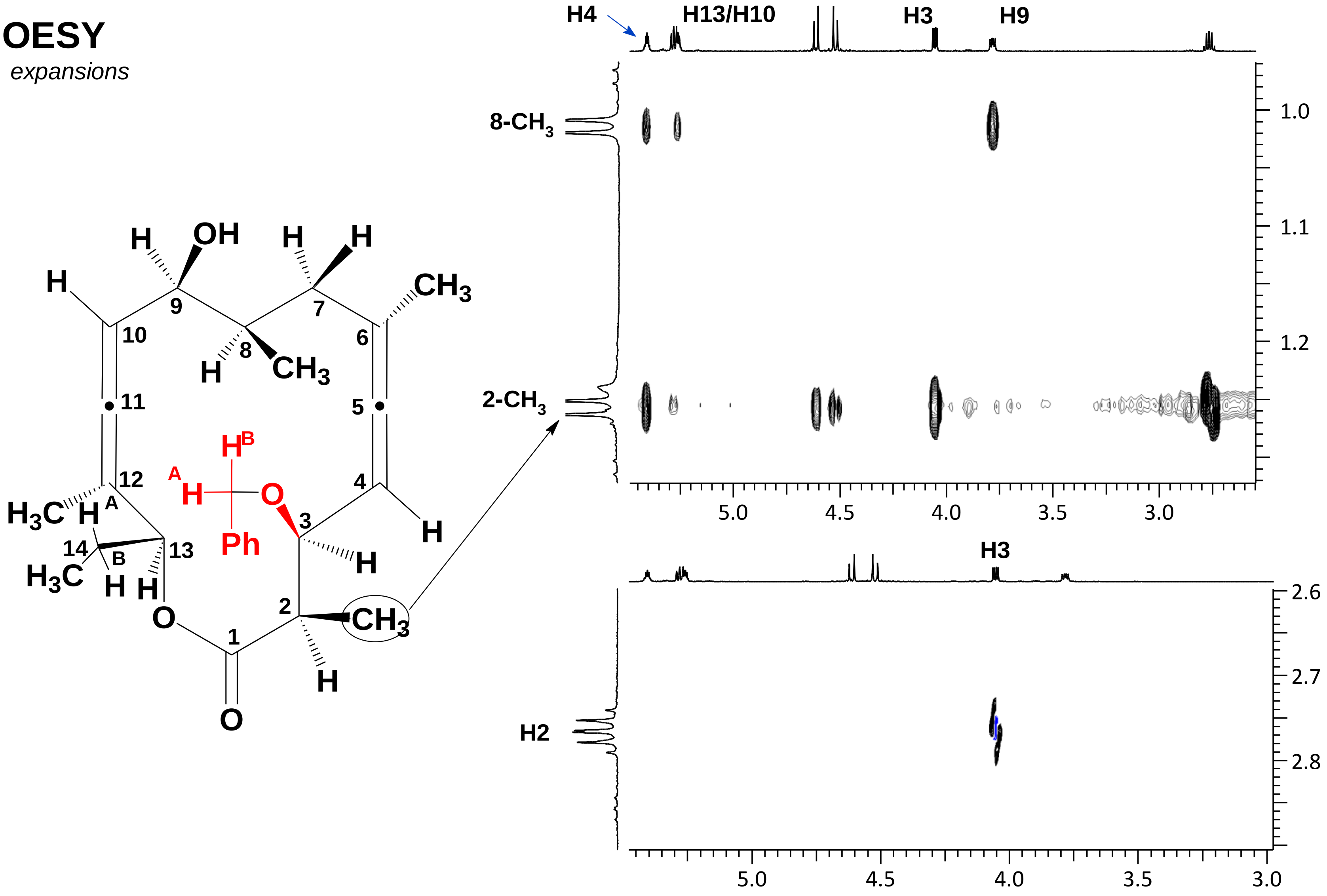


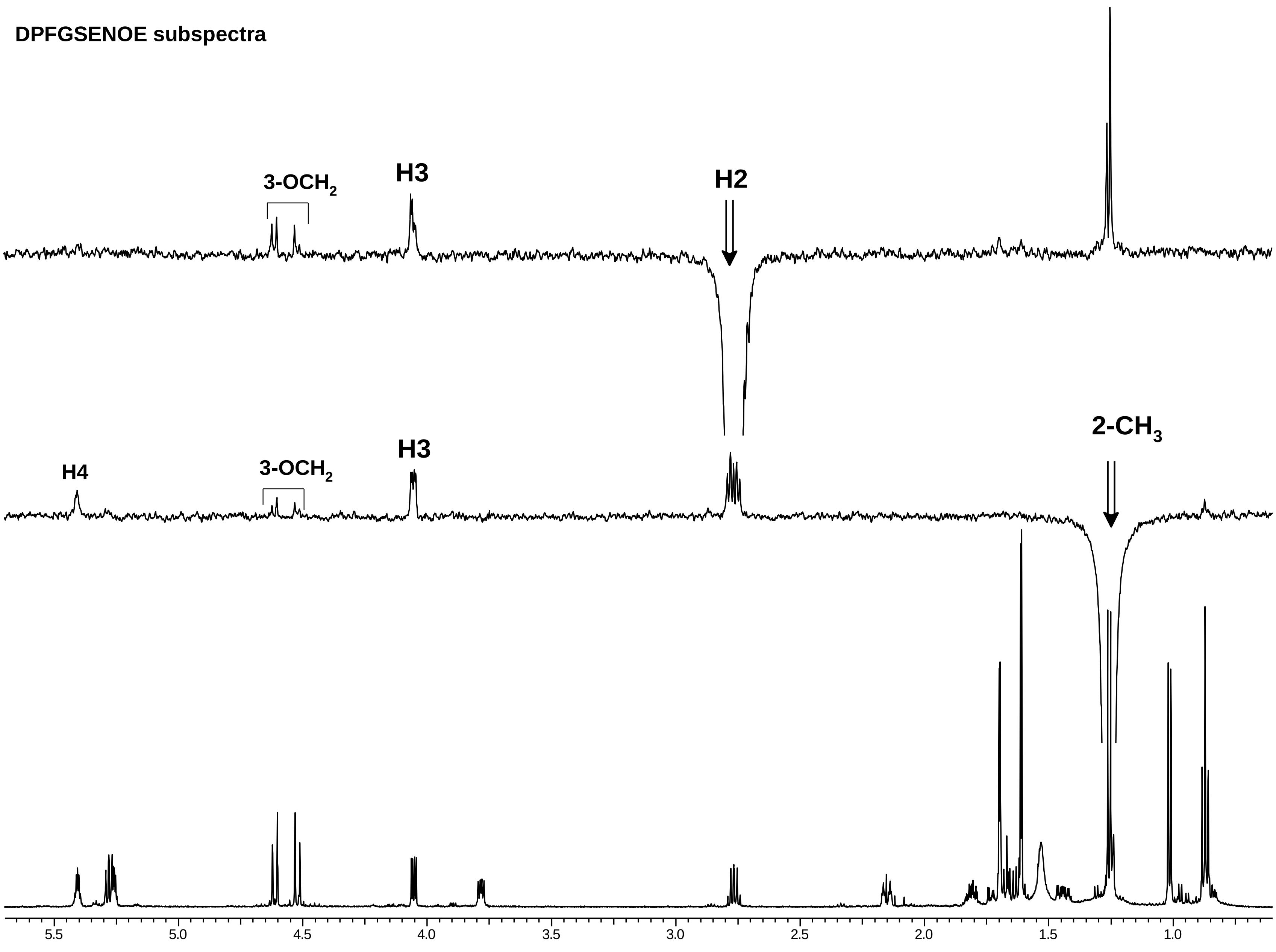
NOESY



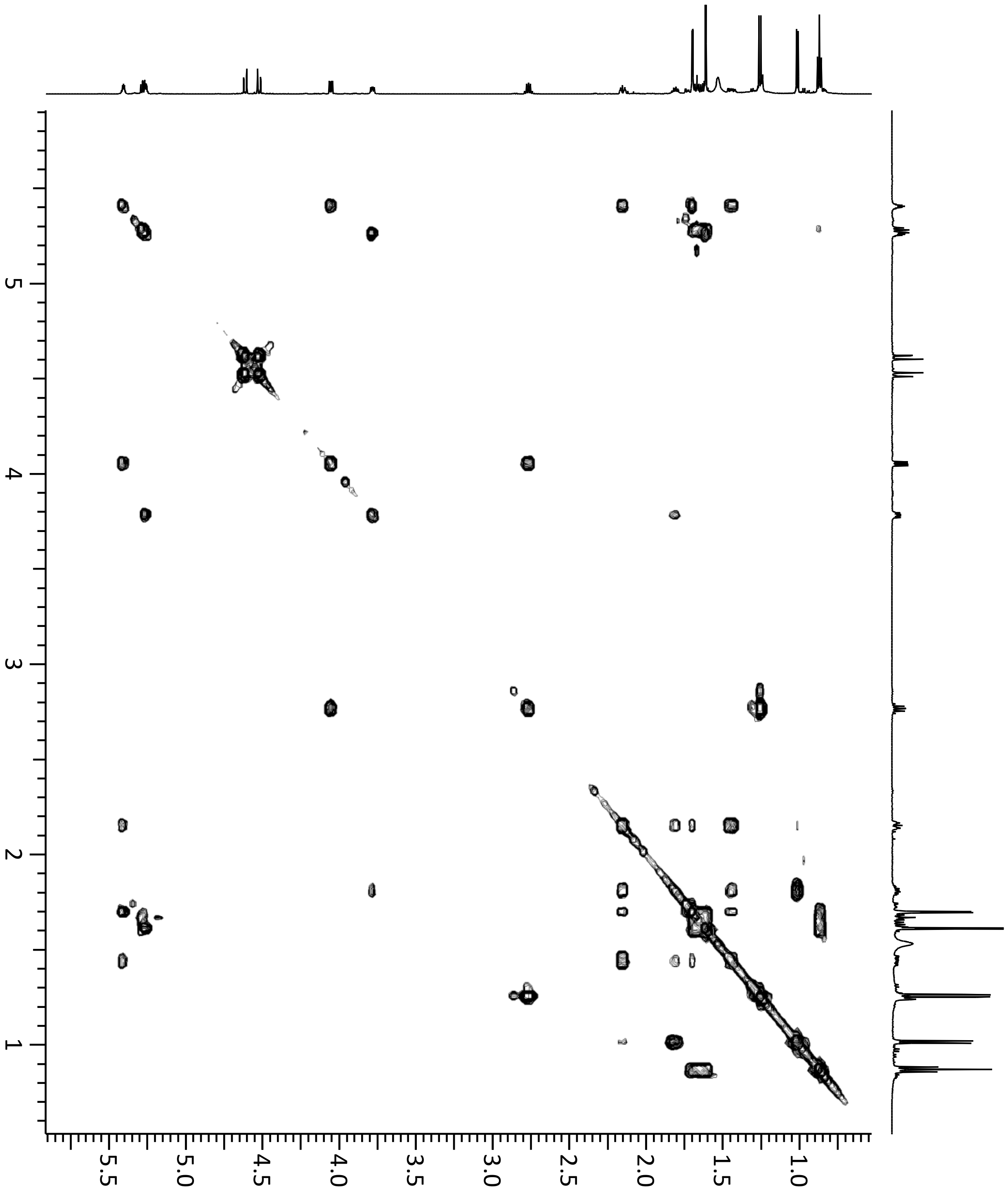
NOESY

expansions



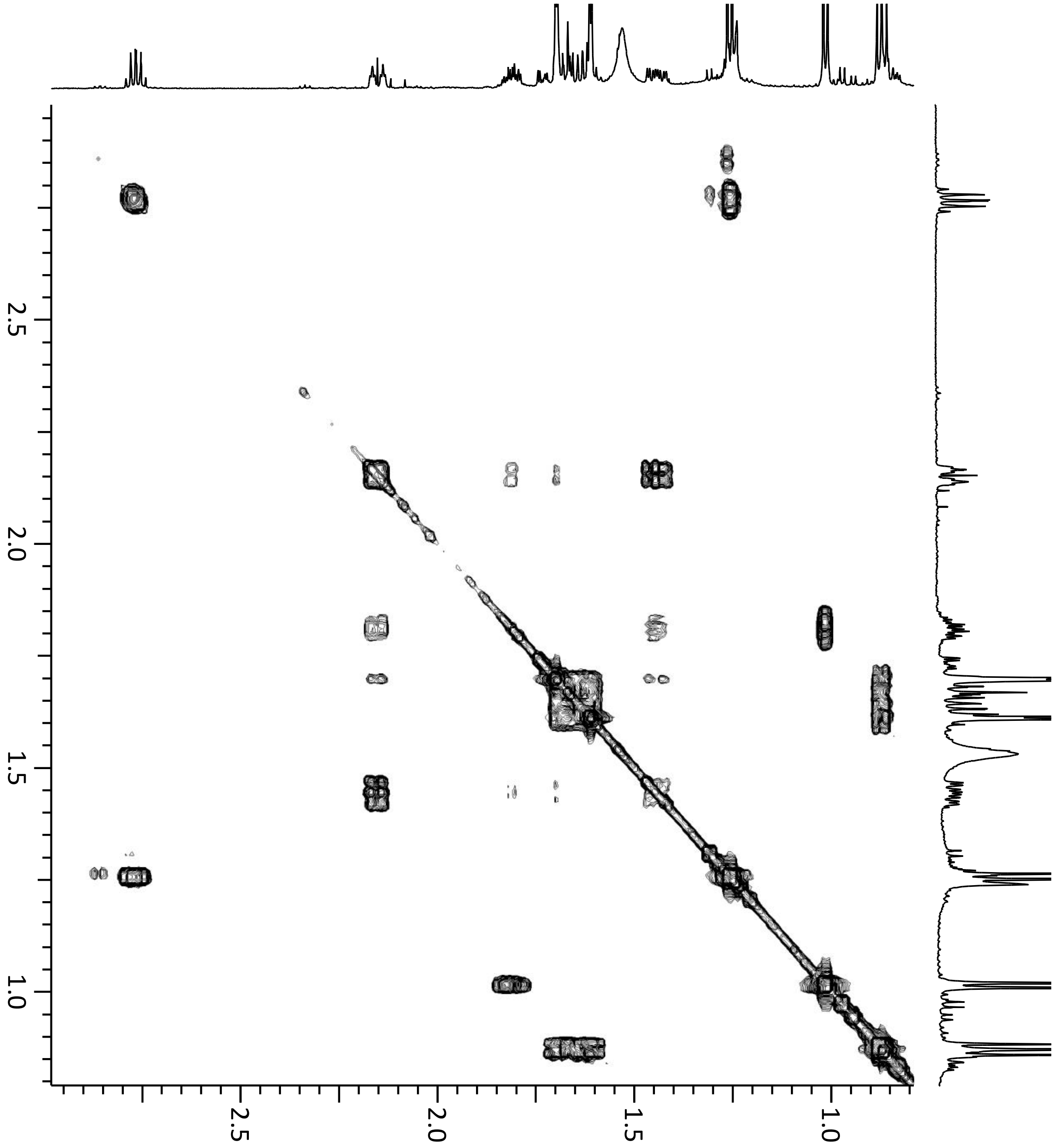

DPFGSENOE subspectra
3-OCH$_2$
H3
H2
H4
3-OCH$_2$
H3
2-CH$_3$
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

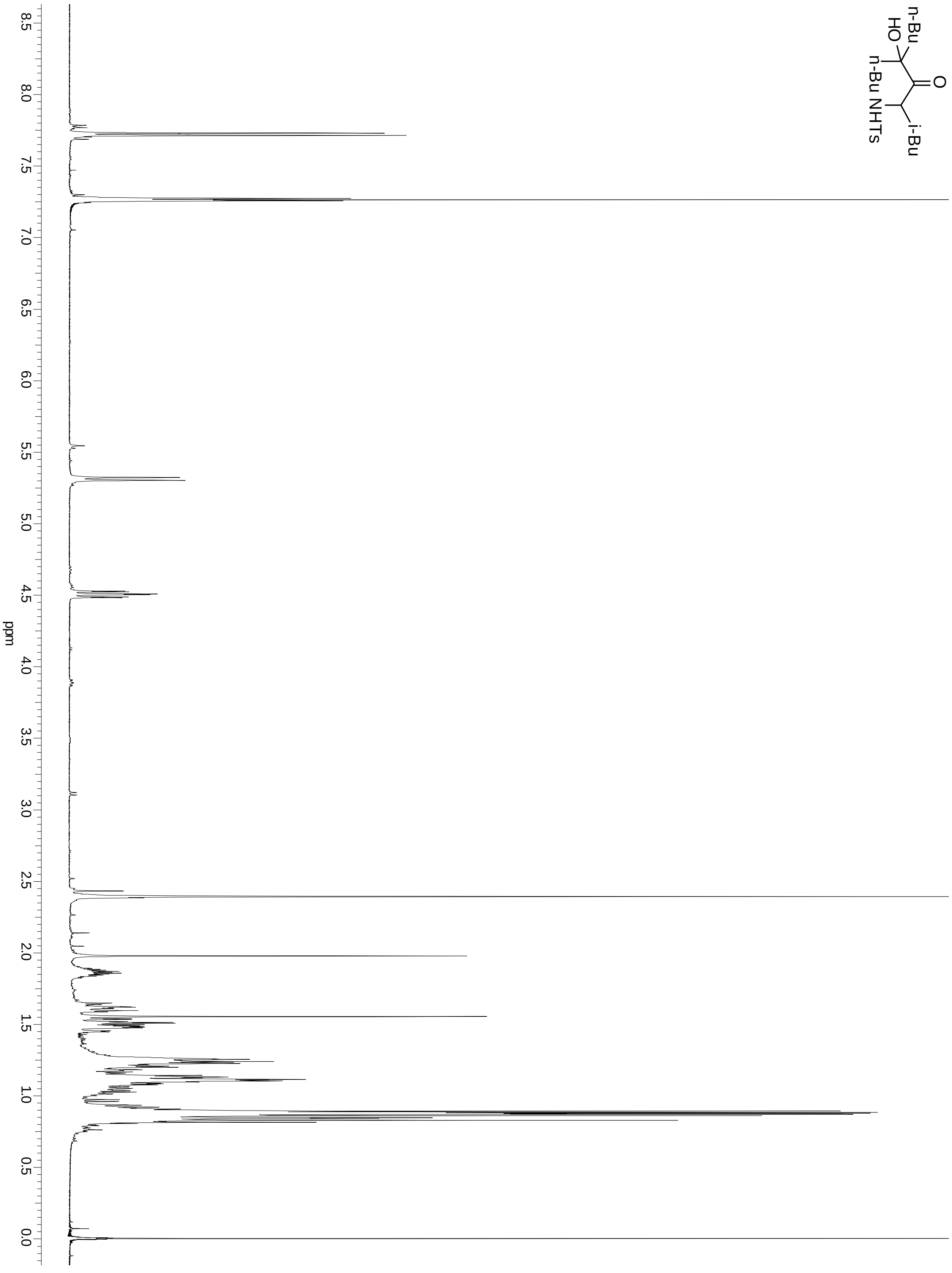
gCOSY

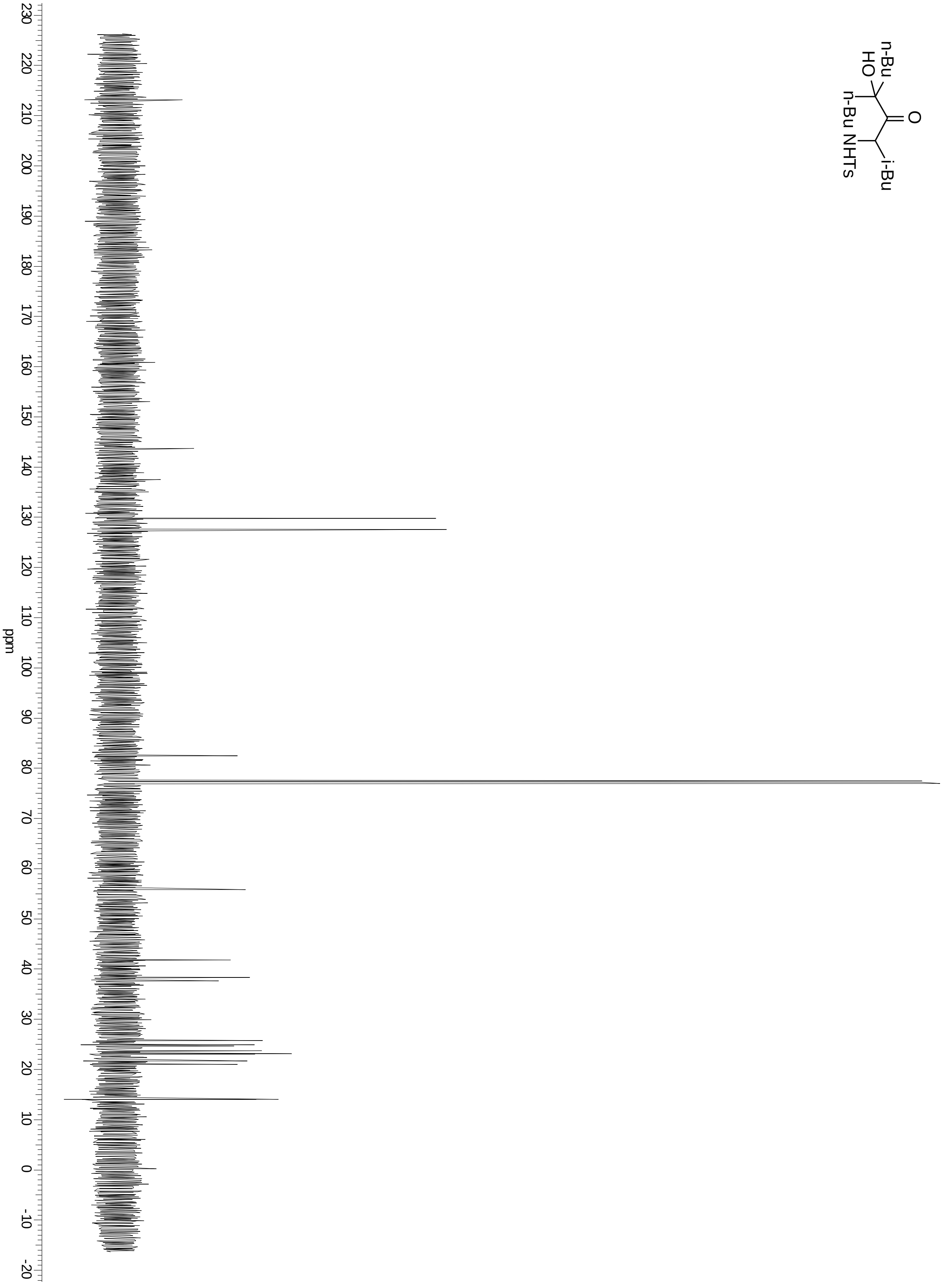


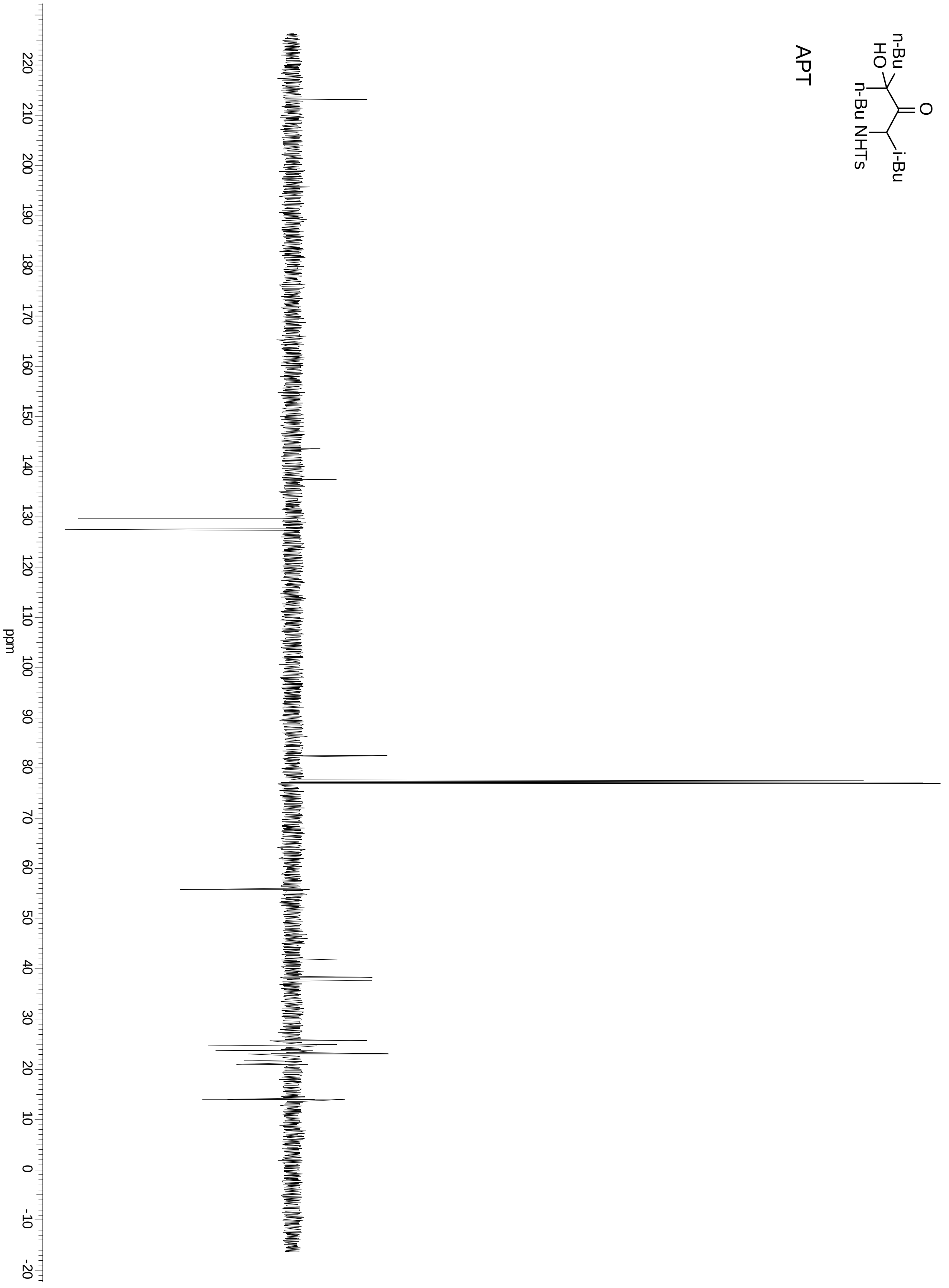
gCOSY

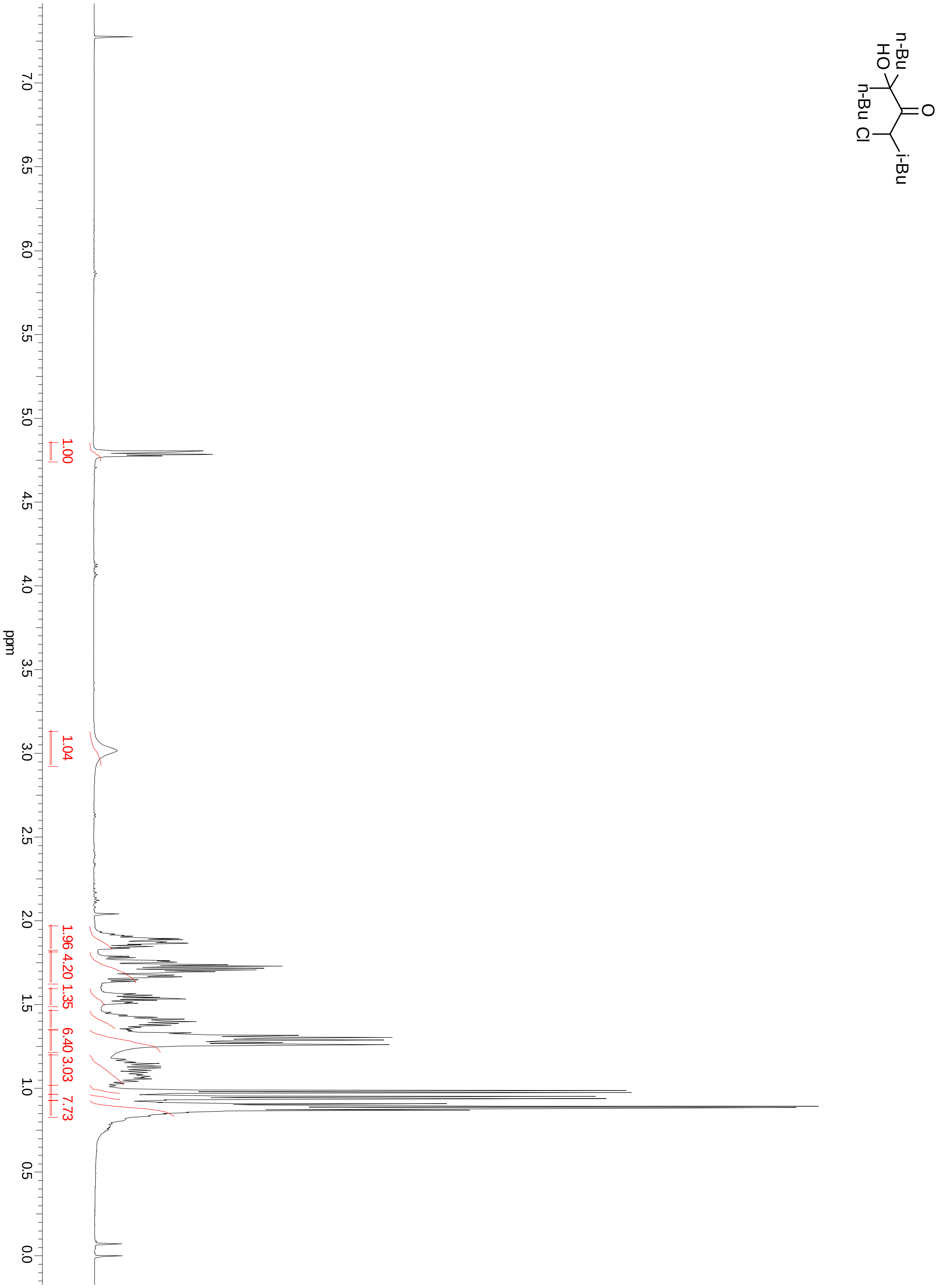
expansion

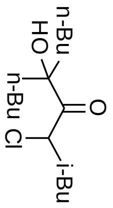


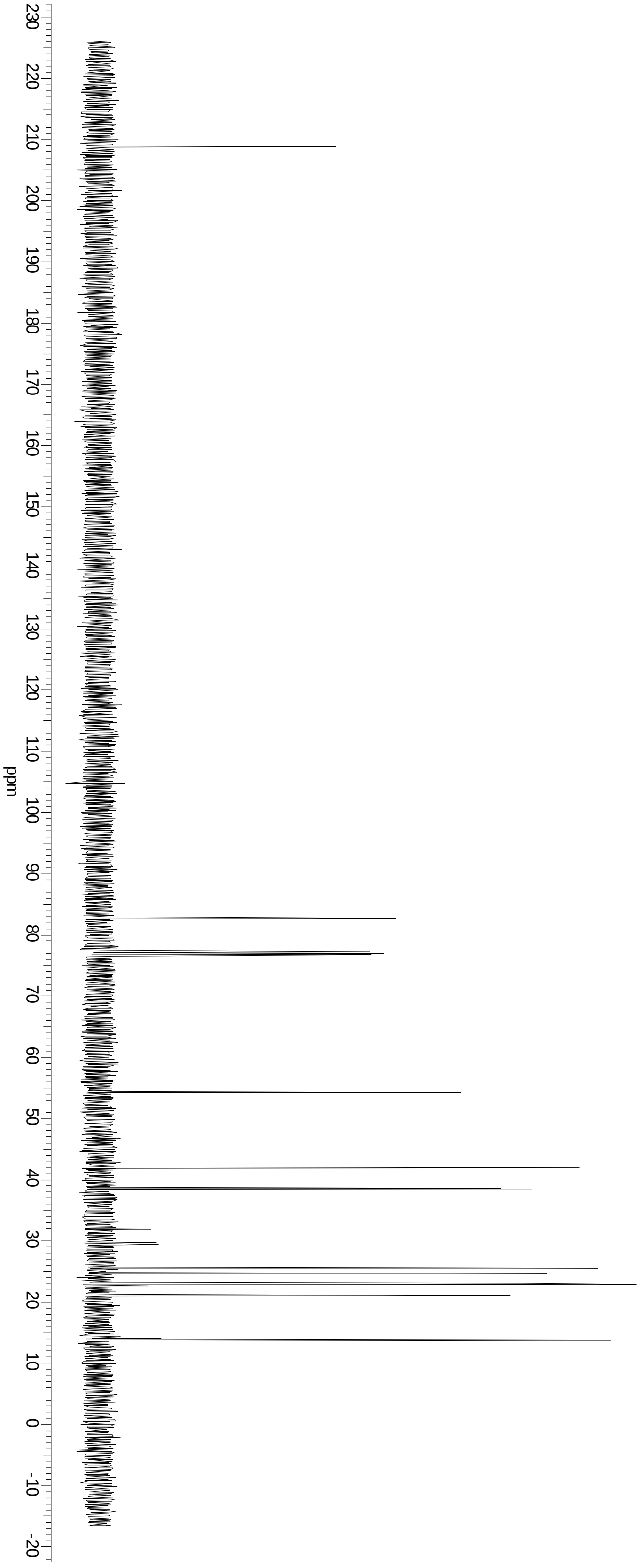

n-Bu
HO
O
n-Bu NHTs
i-Bu
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

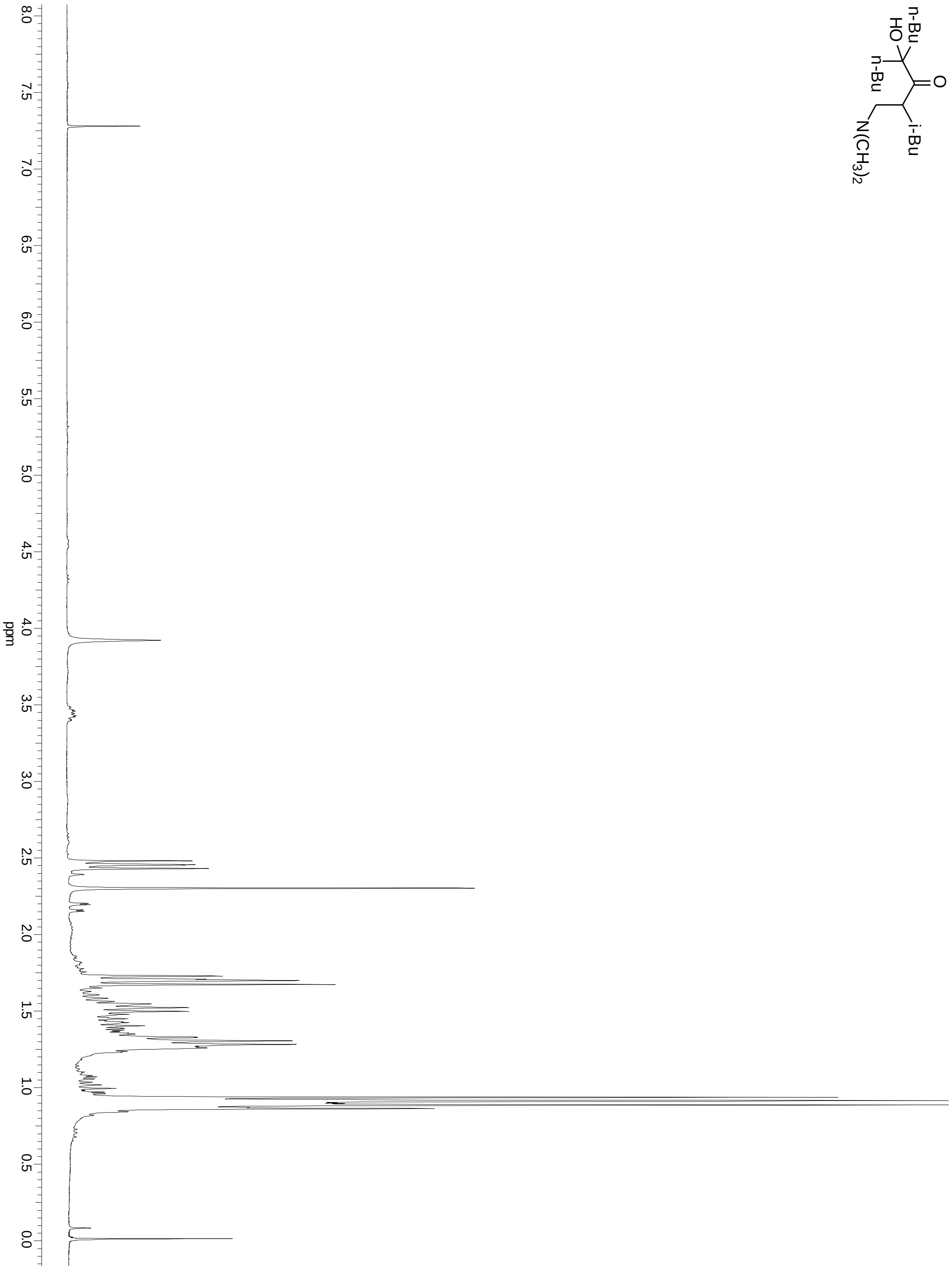

O
n-Bu
HO
i-Bu
n-Bu NHTs
ppm
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20

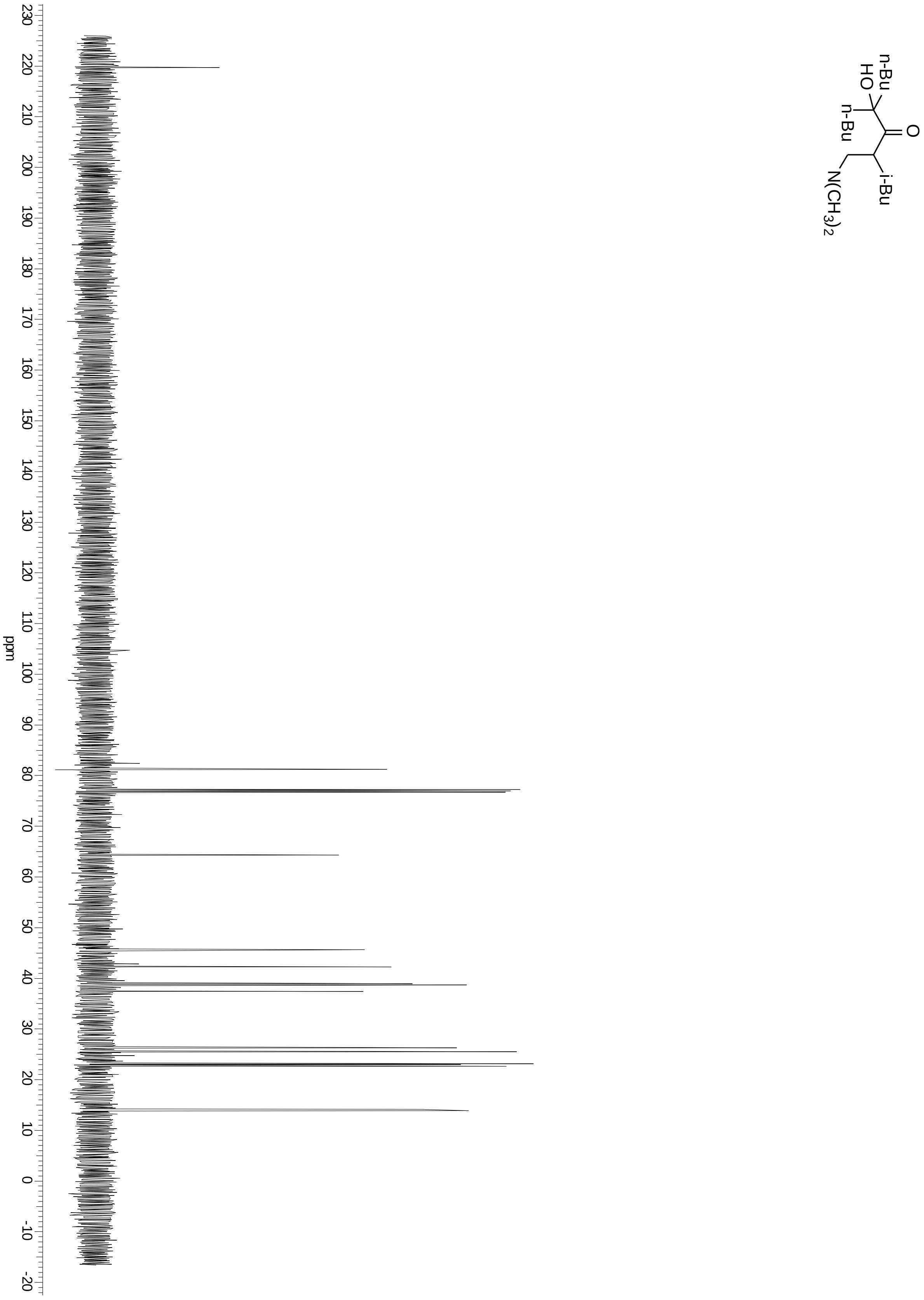

O
n-Bu
HO
i-Bu
n-Bu NHTs
APT
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm

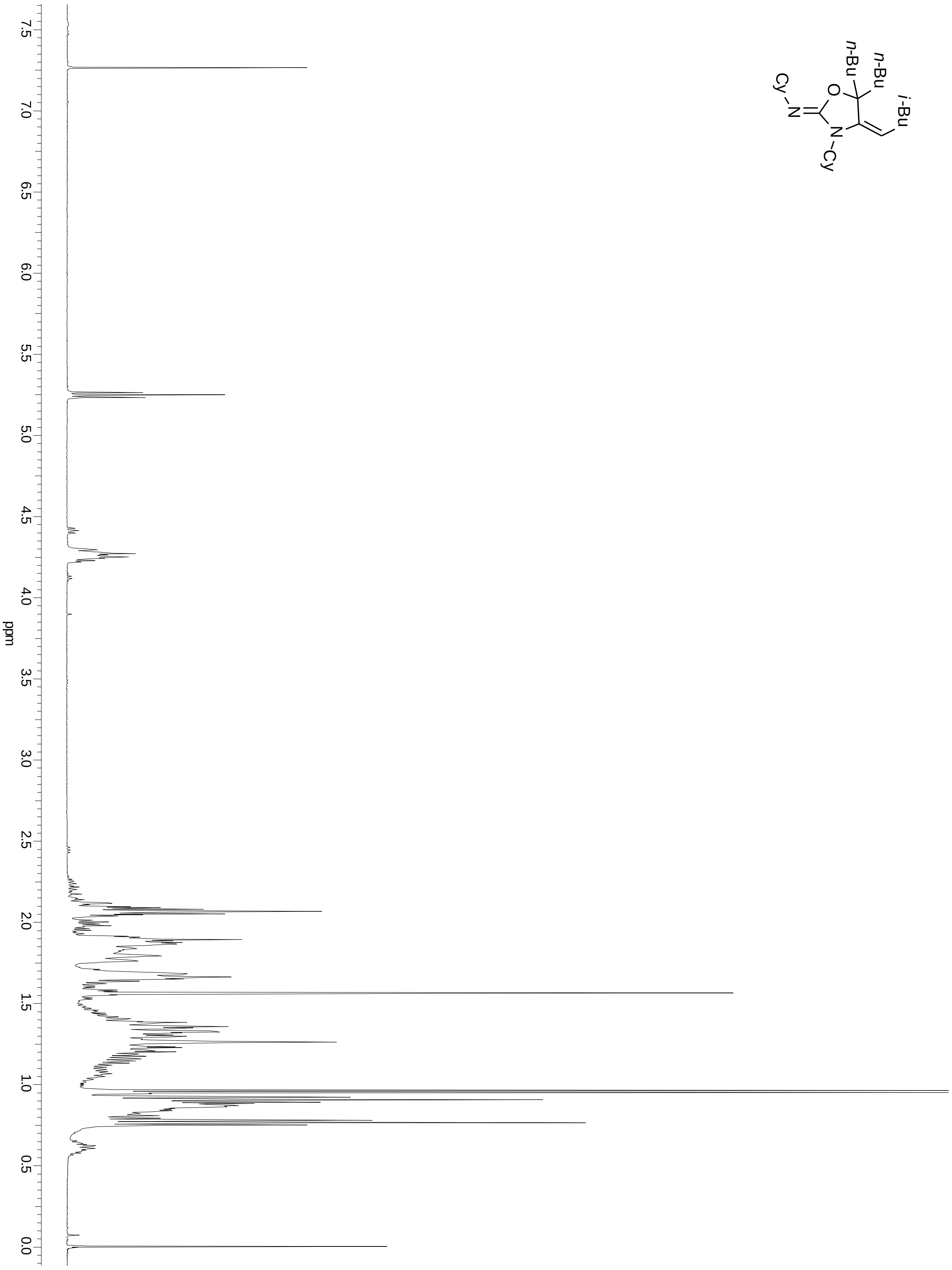

O
i-Bu
n-Bu
HO
n-Bu
Cl
1.00
1.04
1.96
4.20
1.35
6.40
3.03
7.73
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

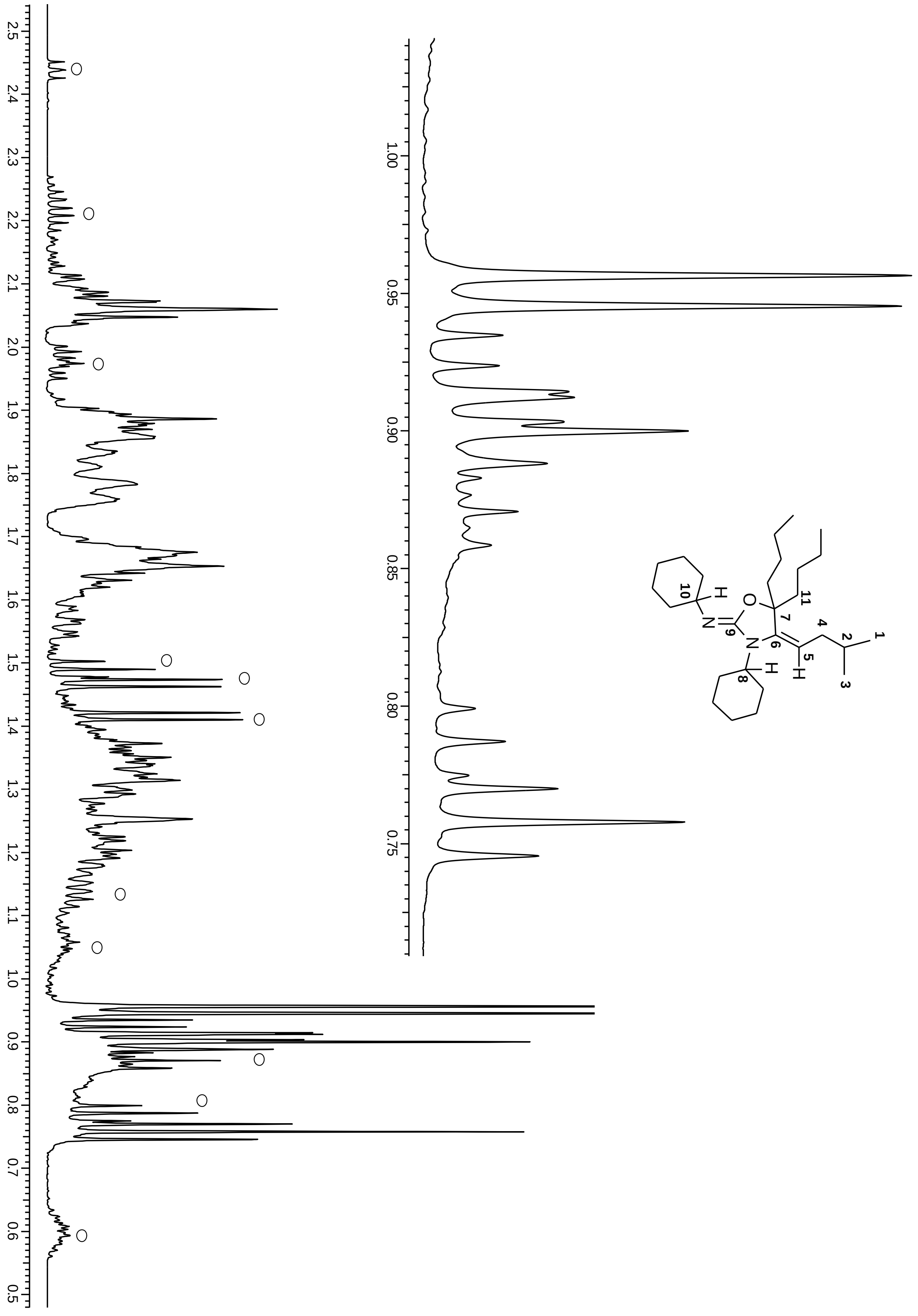

n-Bu
HO
O
n-Bu
Cl
i-Bu


230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm


O
n-Bu
HO
n-Bu
i-Bu
N(CH3)2
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

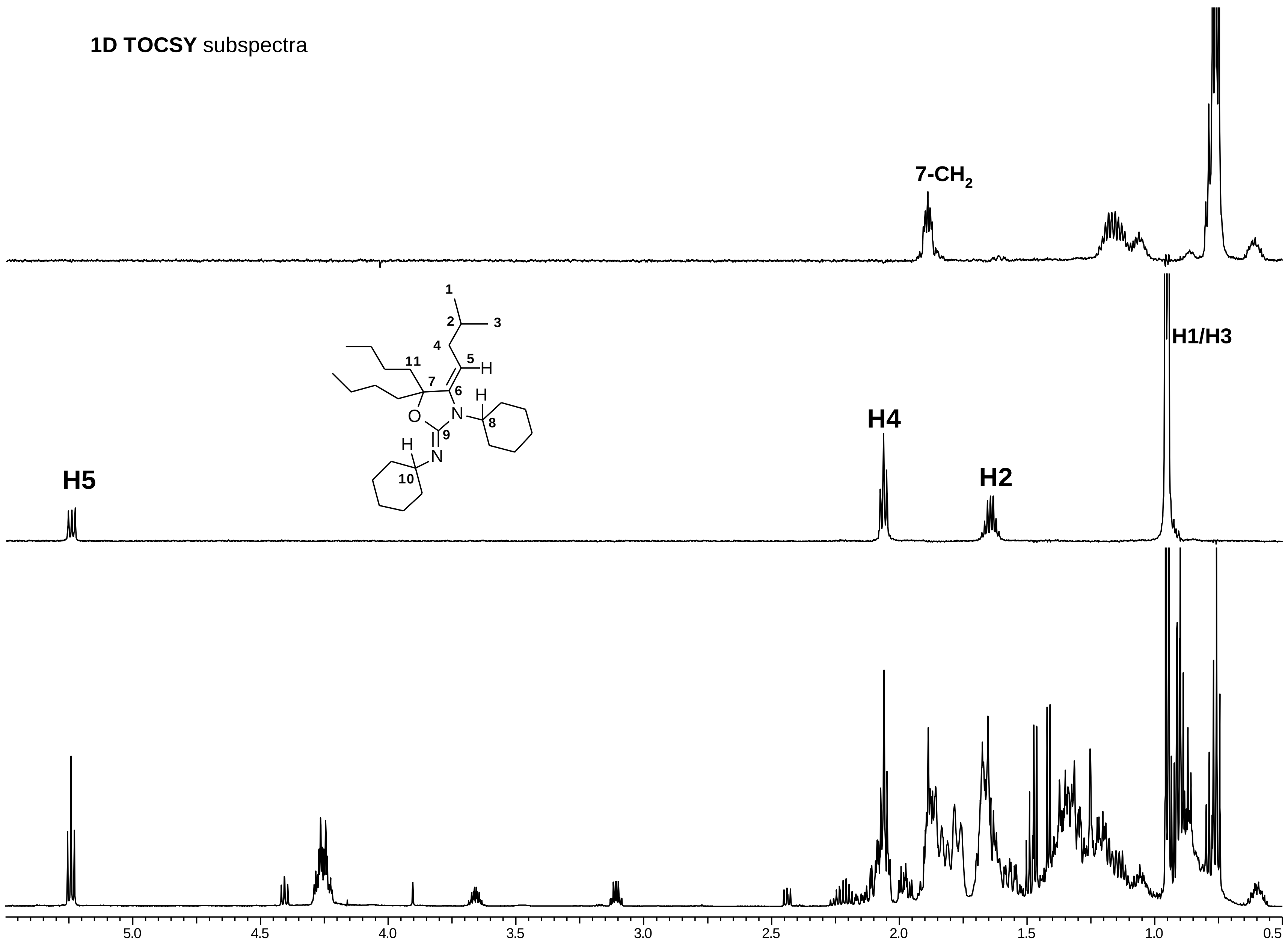

O
n-Bu
HO
n-Bu
i-Bu
N(CH3)2
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm


n-Bu
n-Bu
O
i-Bu
Cy
N
N
Cy
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

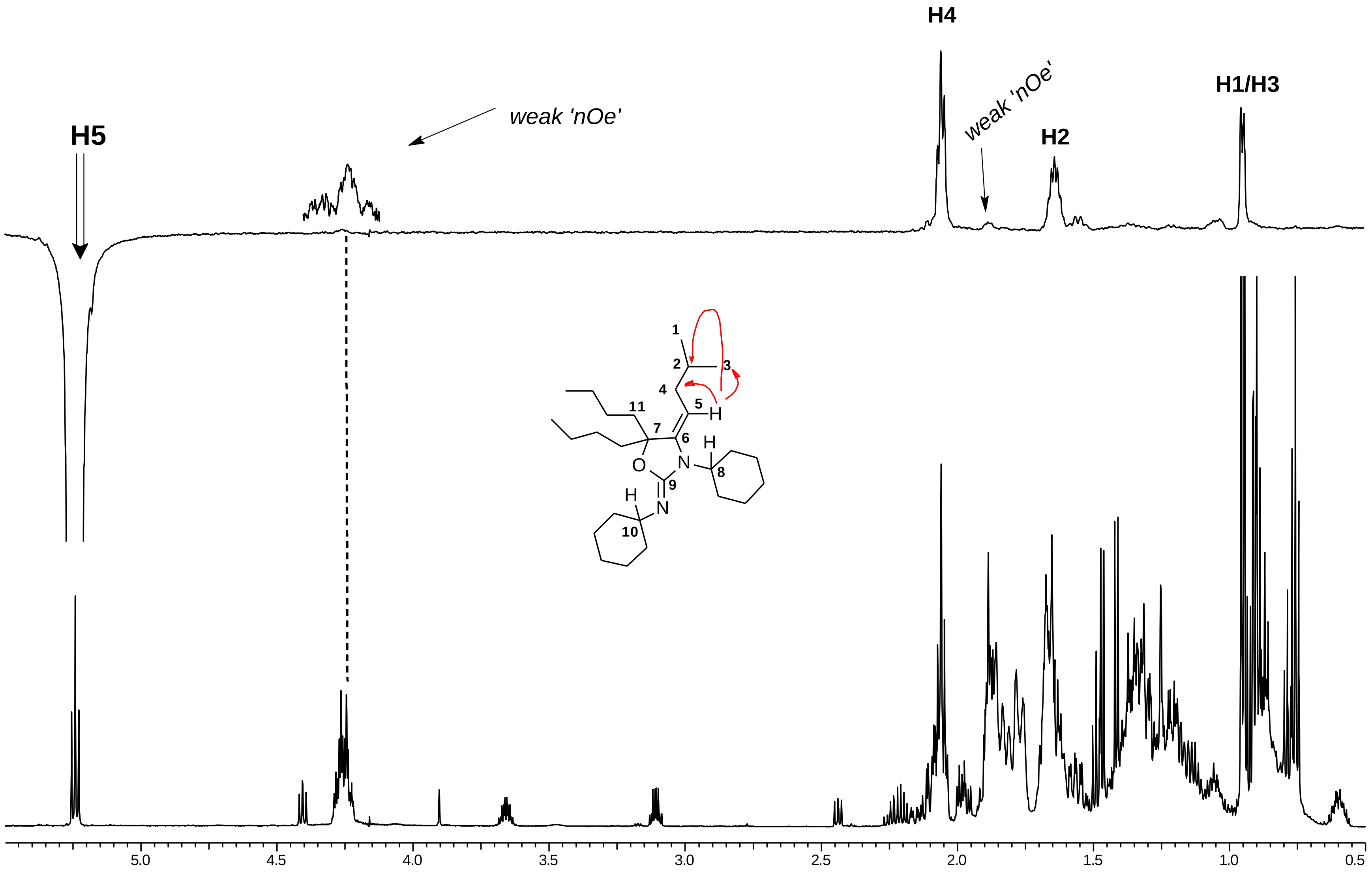


1.00
0.95
0.90
0.85
0.80
0.75
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
1
2
3
4
5
6
7
8
9
10
11
O
N
N
H
H
H

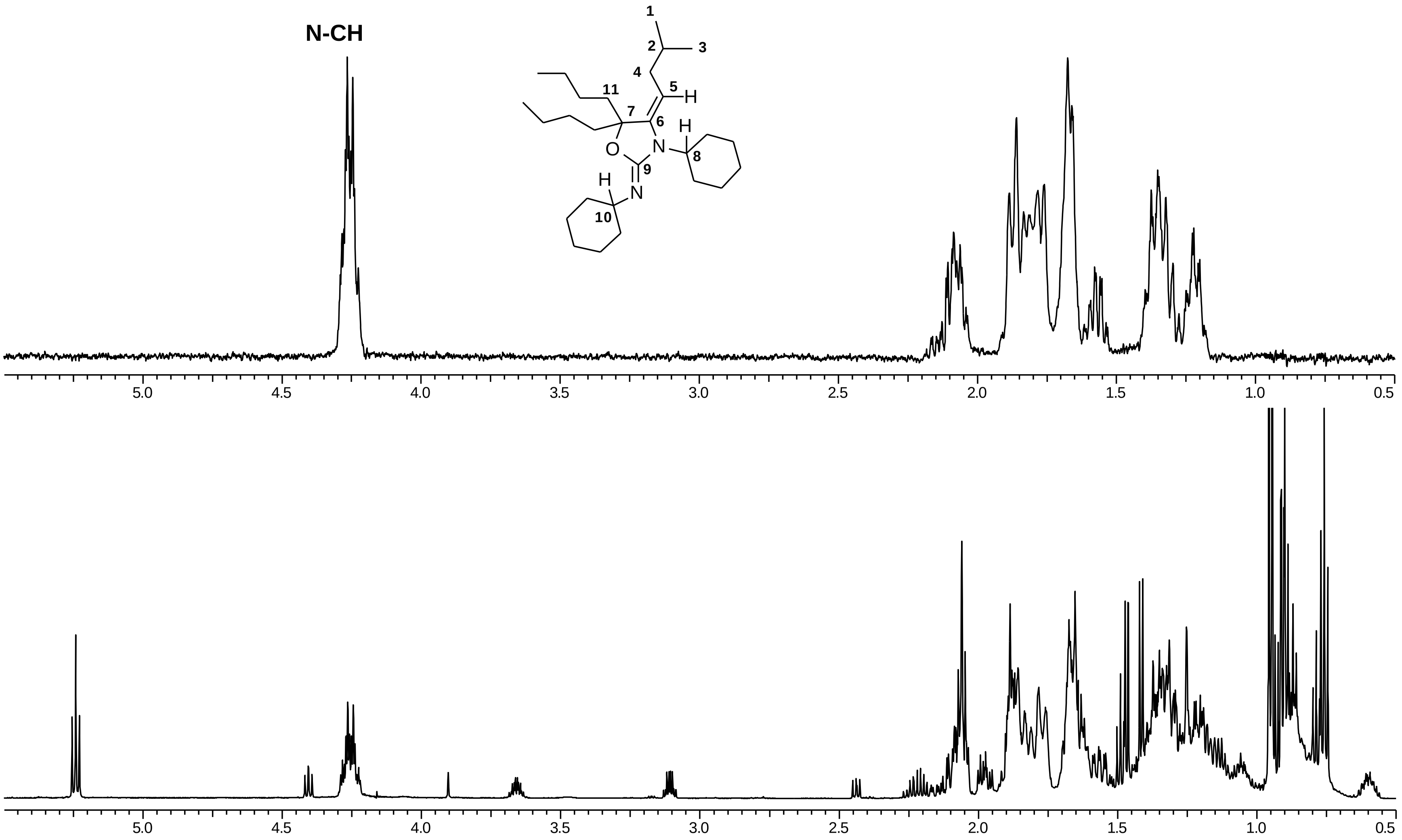
1D TOCSY subspectra

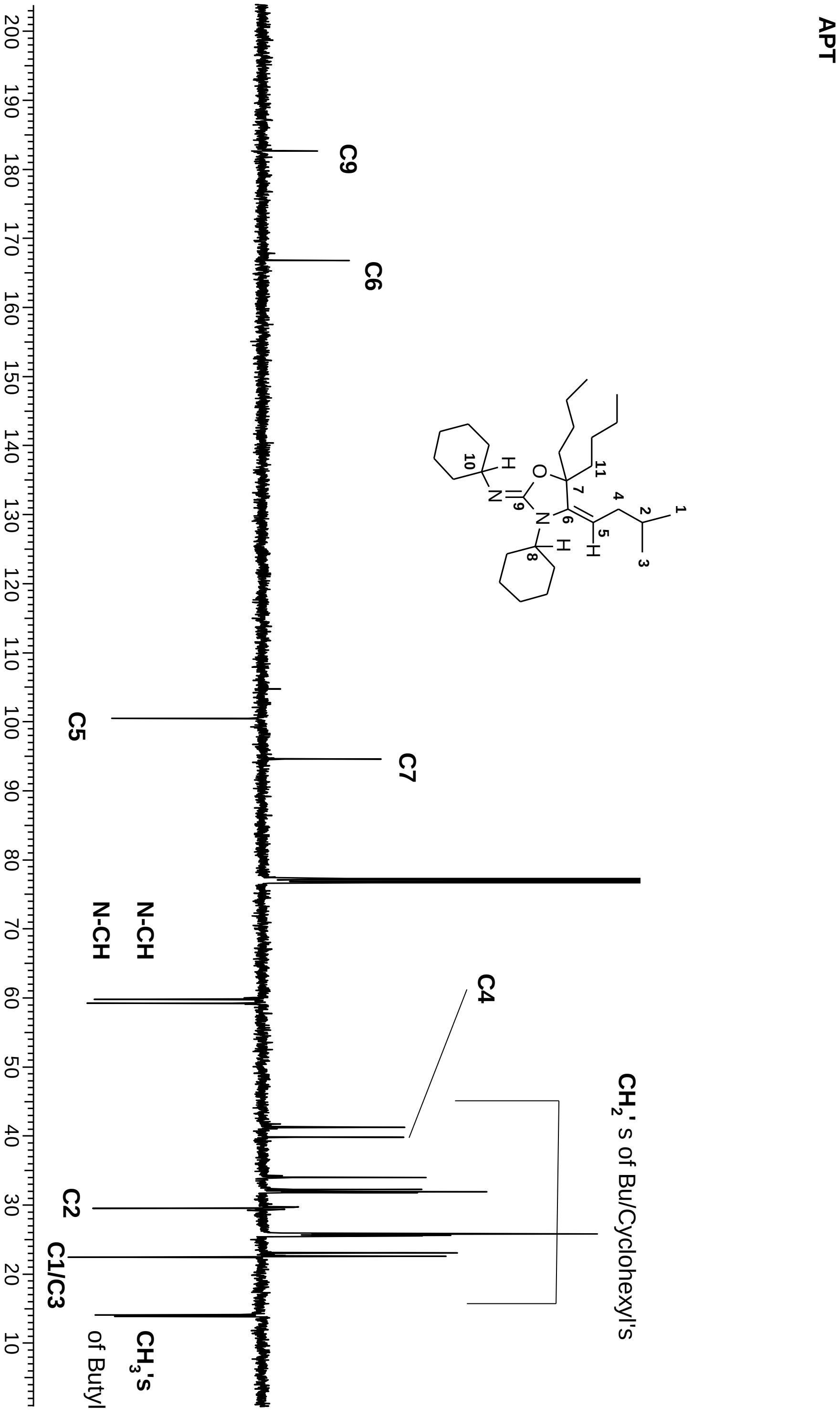


1D NOESY subspectrum

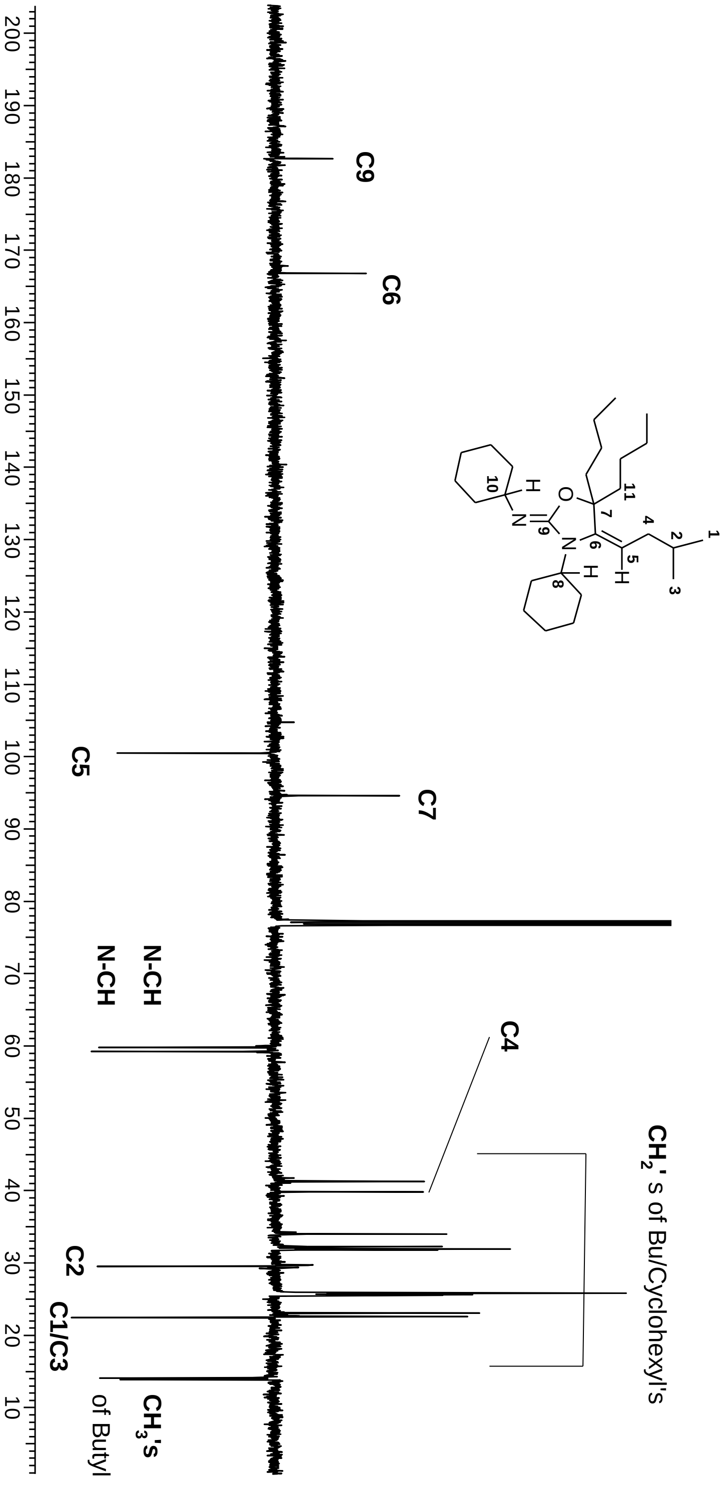


Cyclohexyl protons identification

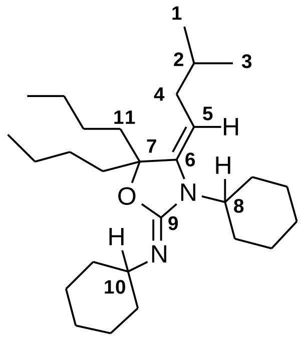


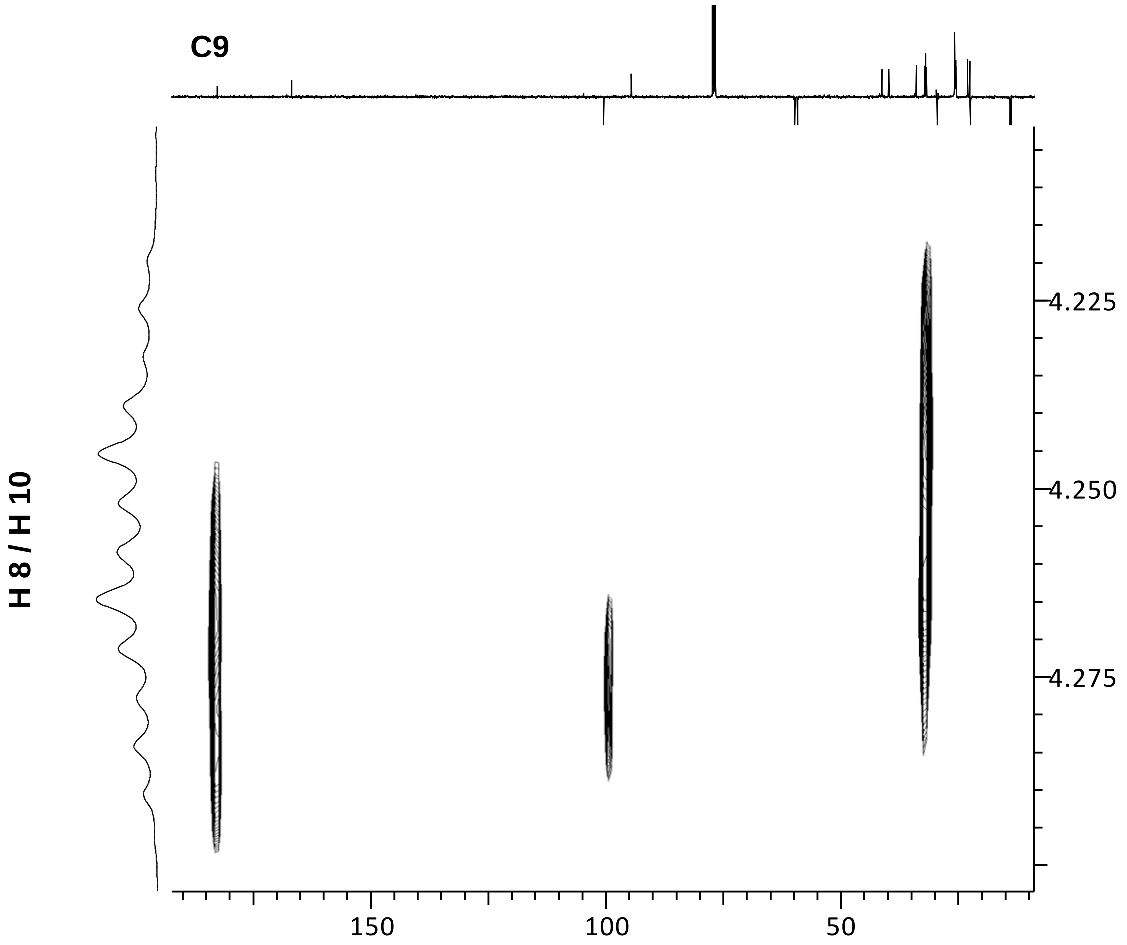

APT
C9
C6
C5
C7
N-CH
N-CH
C4
CH2's of Bu/Cyclohexyl's
C2
C1/C3
CH3's
of Butyl
1
2
3
4
5
6
7
8
9
10
11
O
N
N
H
H
H
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

APT

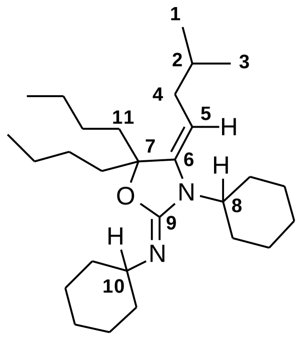

C9
C6
C5
C7
N-CH
N-CH
C4
CH$_2$' s of Bu/Cyclohexyl's
C2
C1/C3
CH$_3$'s
of Butyl
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
H
H
H
O
N
N

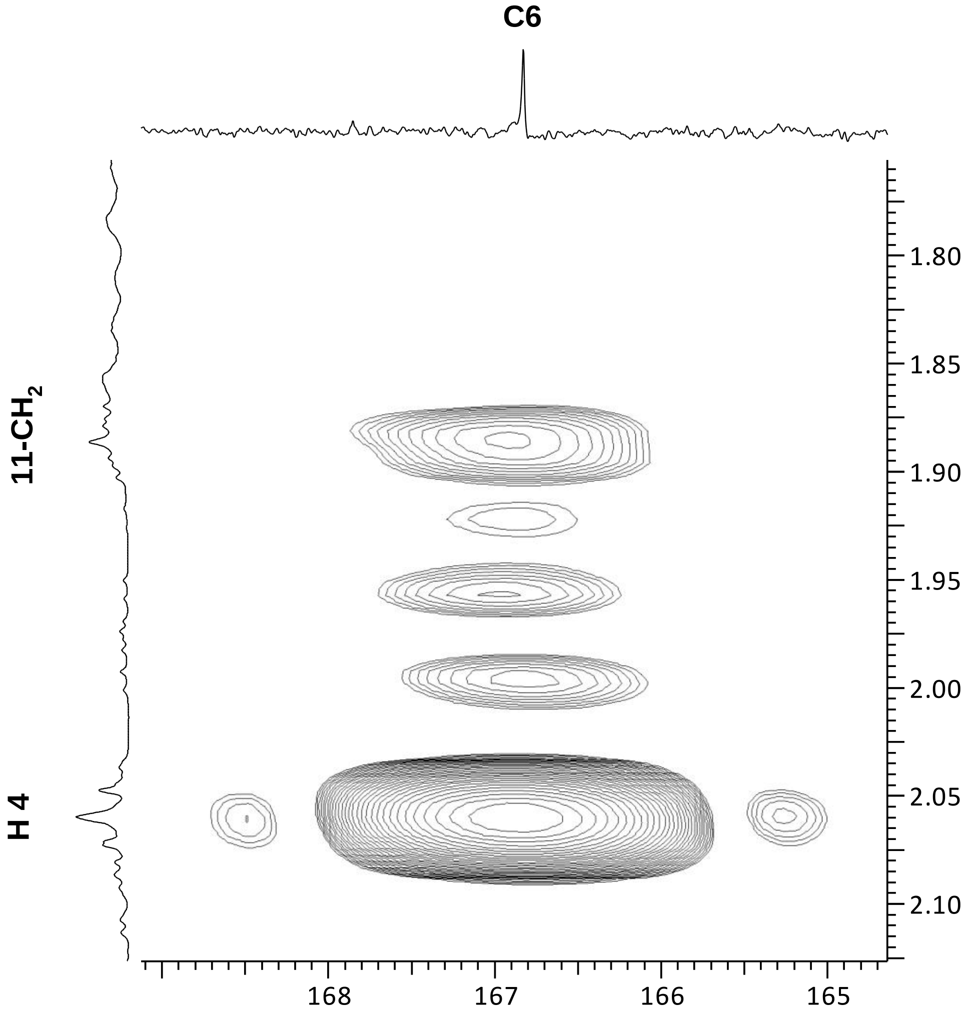
gHMBC

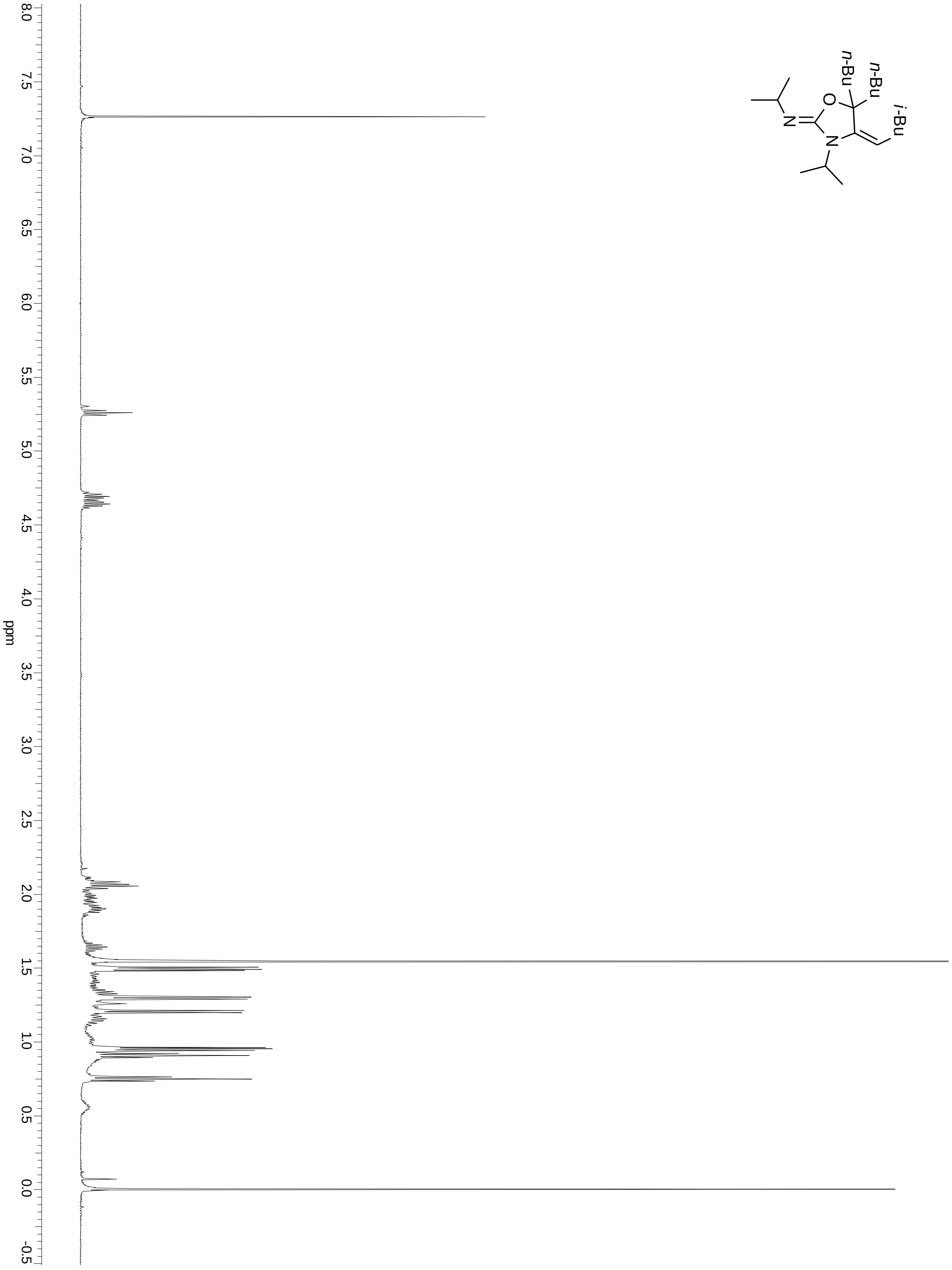

1
2
3
4
5
H
11
7
6
H
O
N
8
9
H
N
10

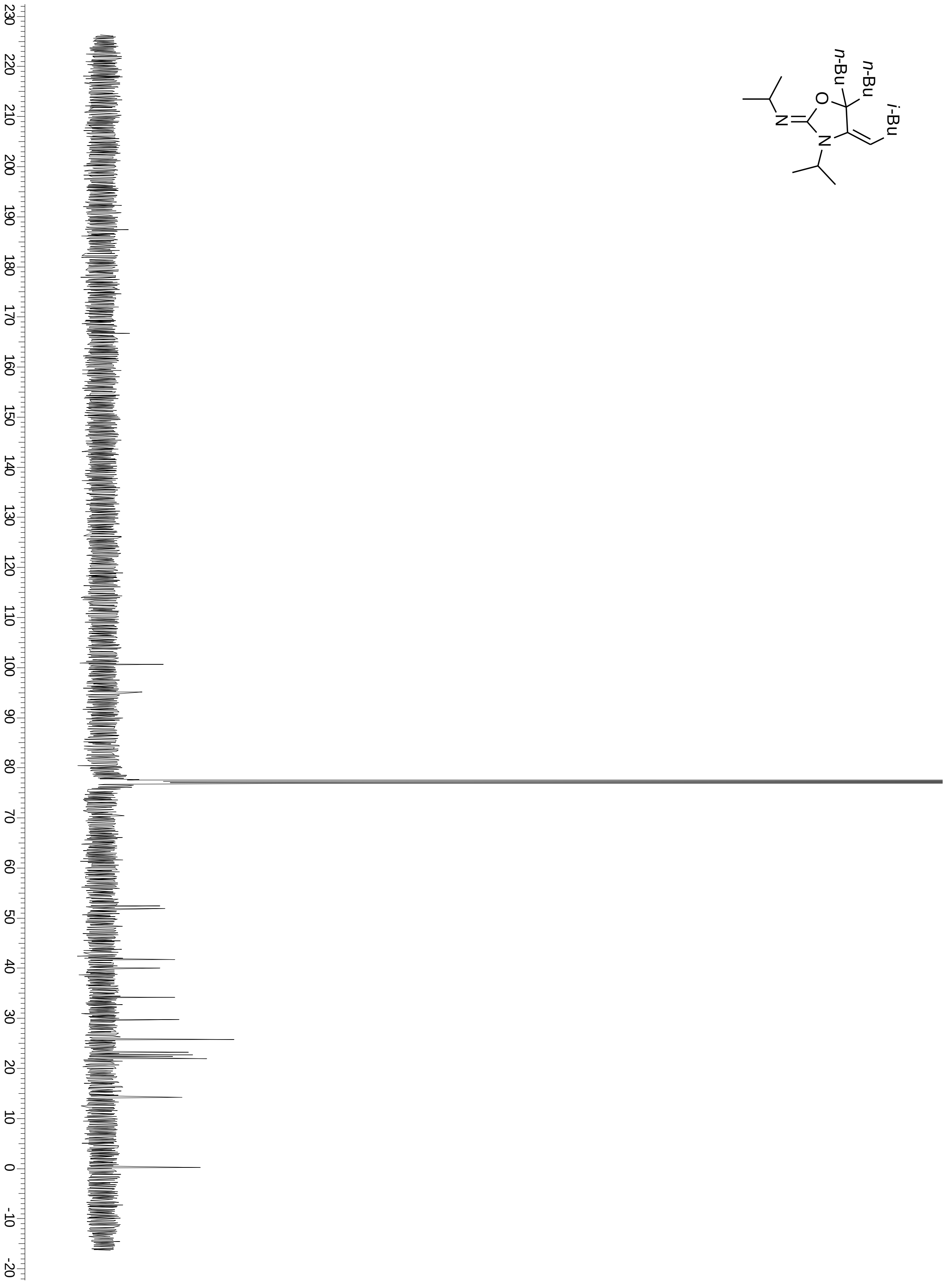


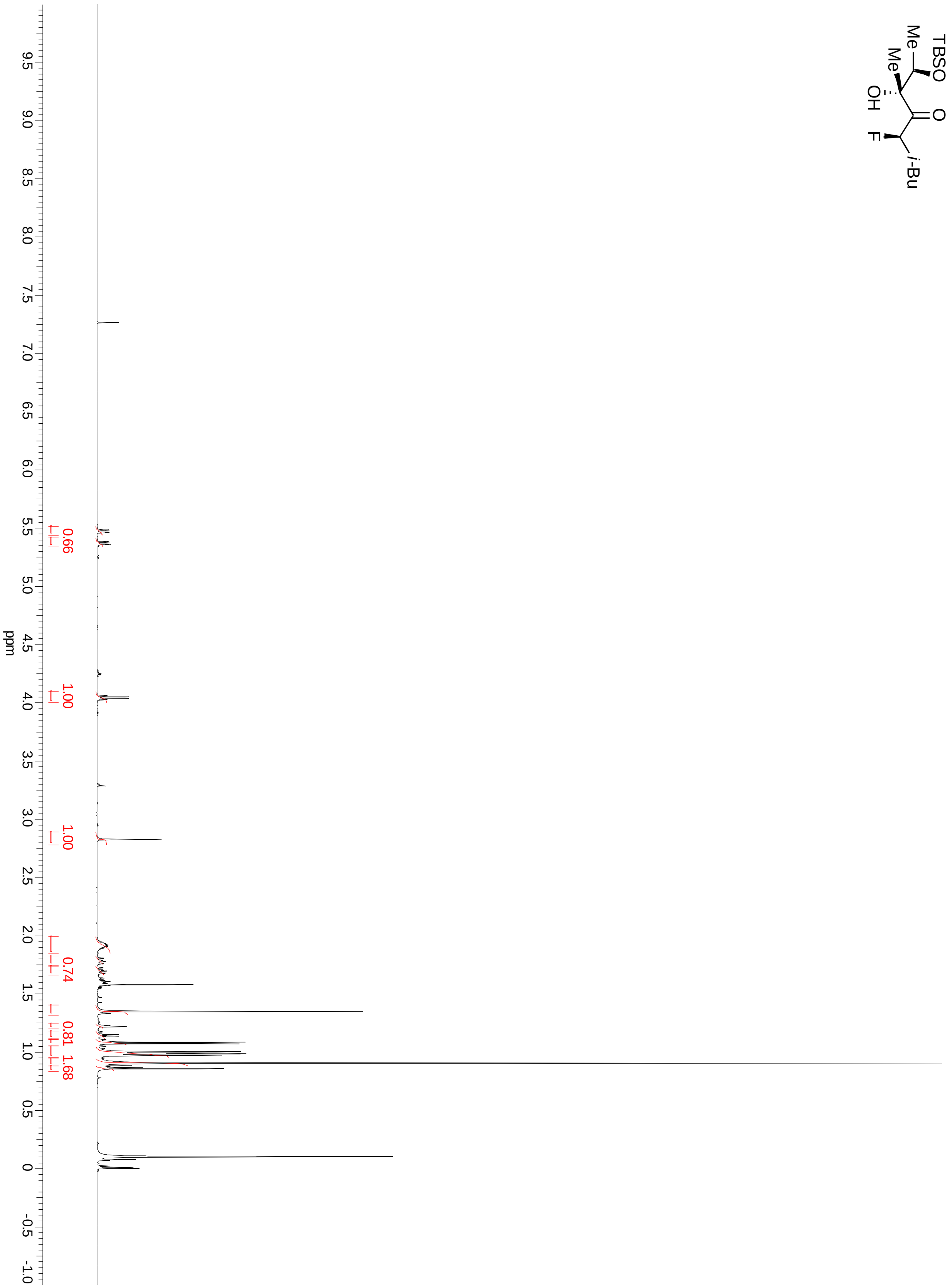
gHMBC

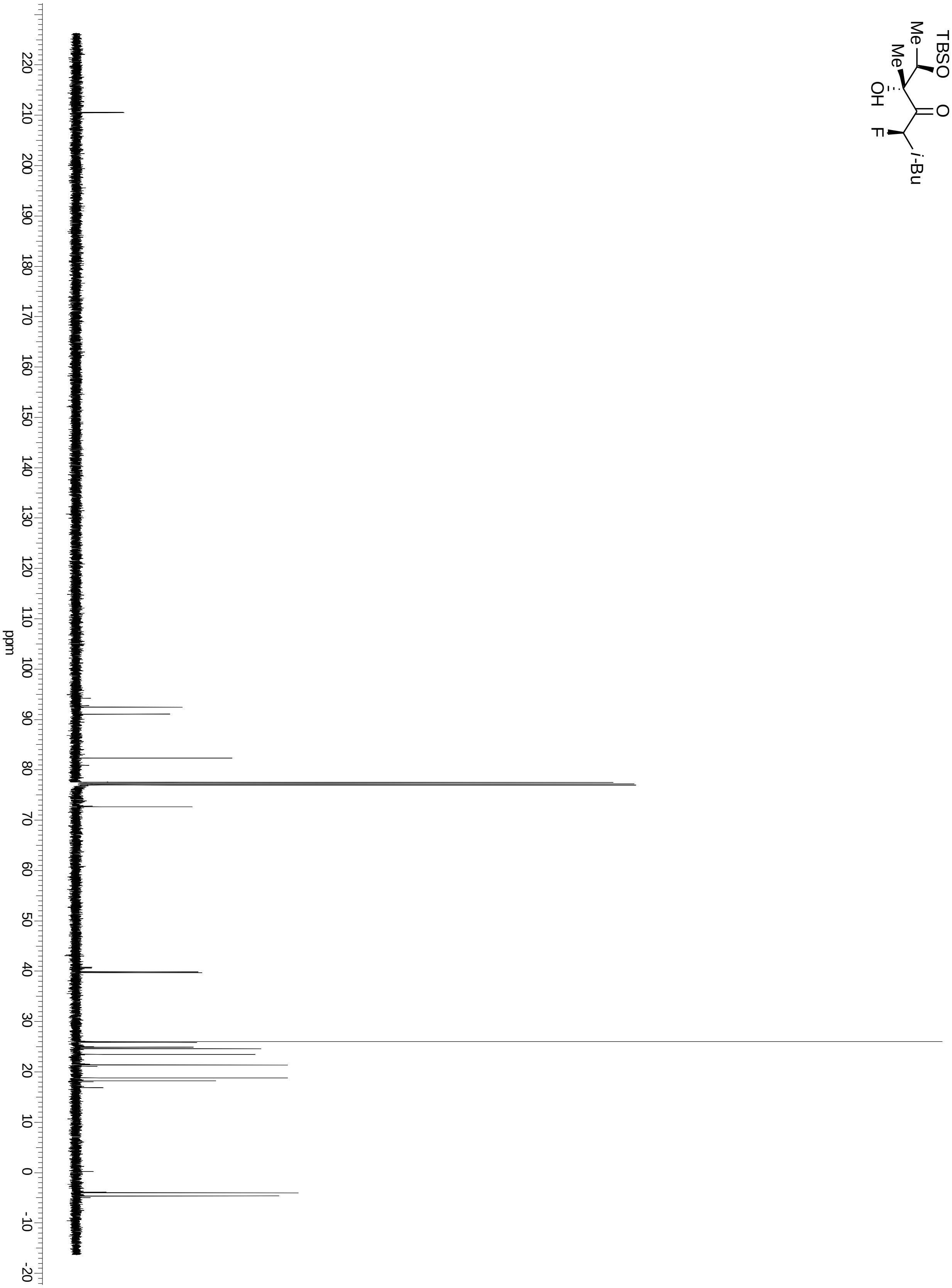

1
2
3
4
5
H
11
7
6
H
O
N
8
9
H
N
10

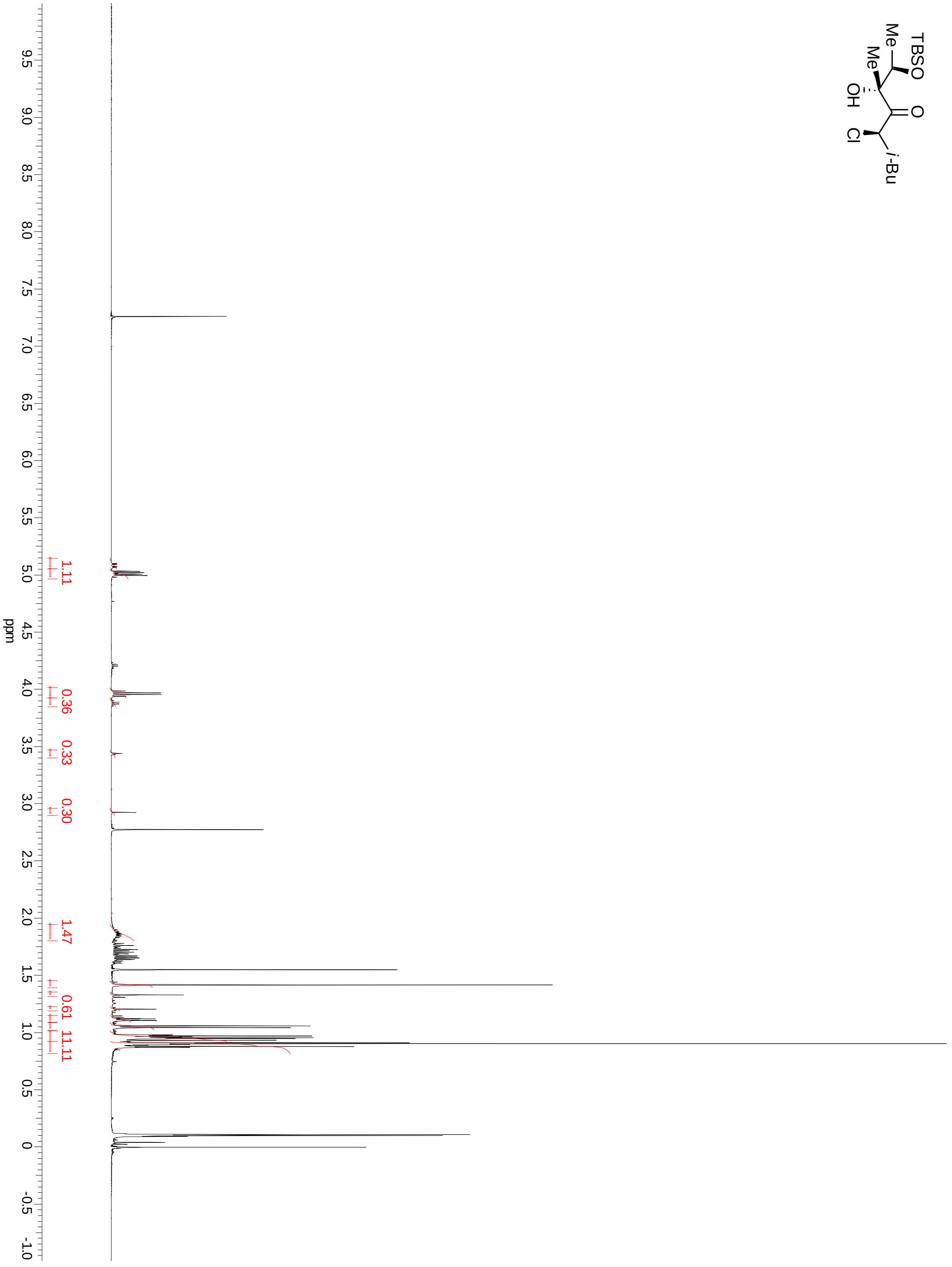

C6
11-CH$_2$
H 4
1.80
1.85
1.90
1.95
2.00
2.05
2.10
168
167
166
165

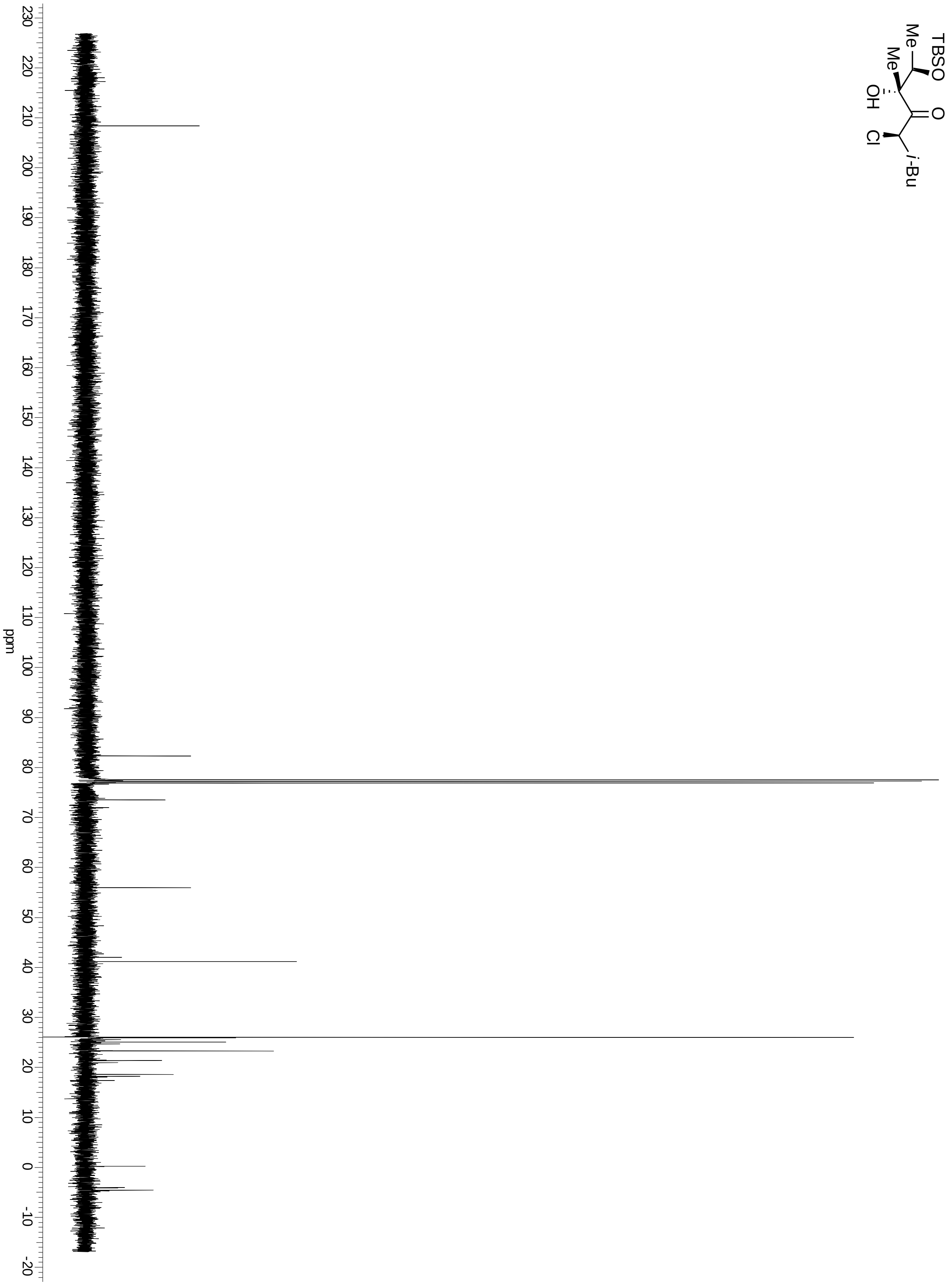

n-Bu
n-Bu
i-Bu
O
N
N
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm

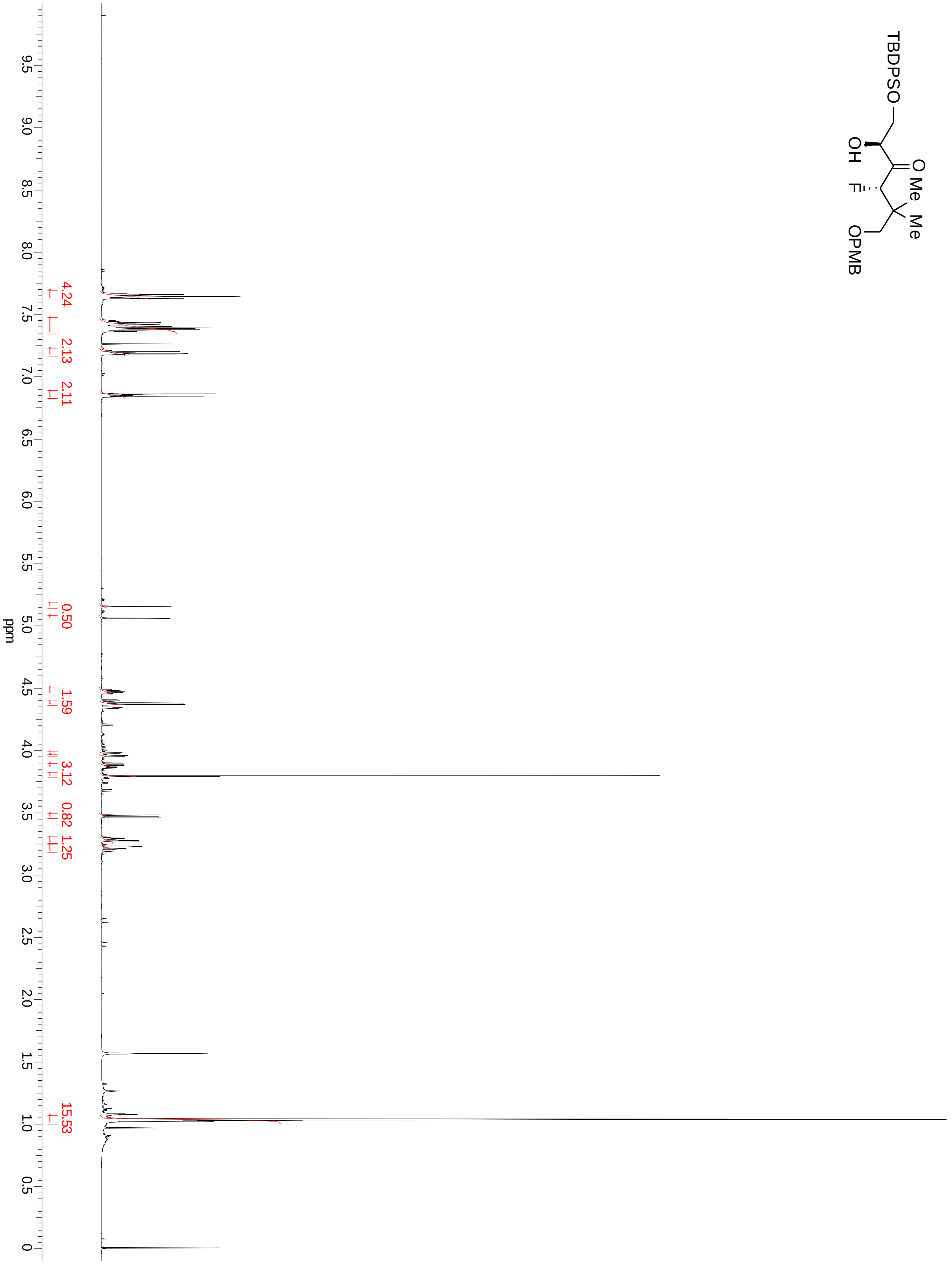

n-Bu
n-Bu
i-Bu
O
N
N
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm

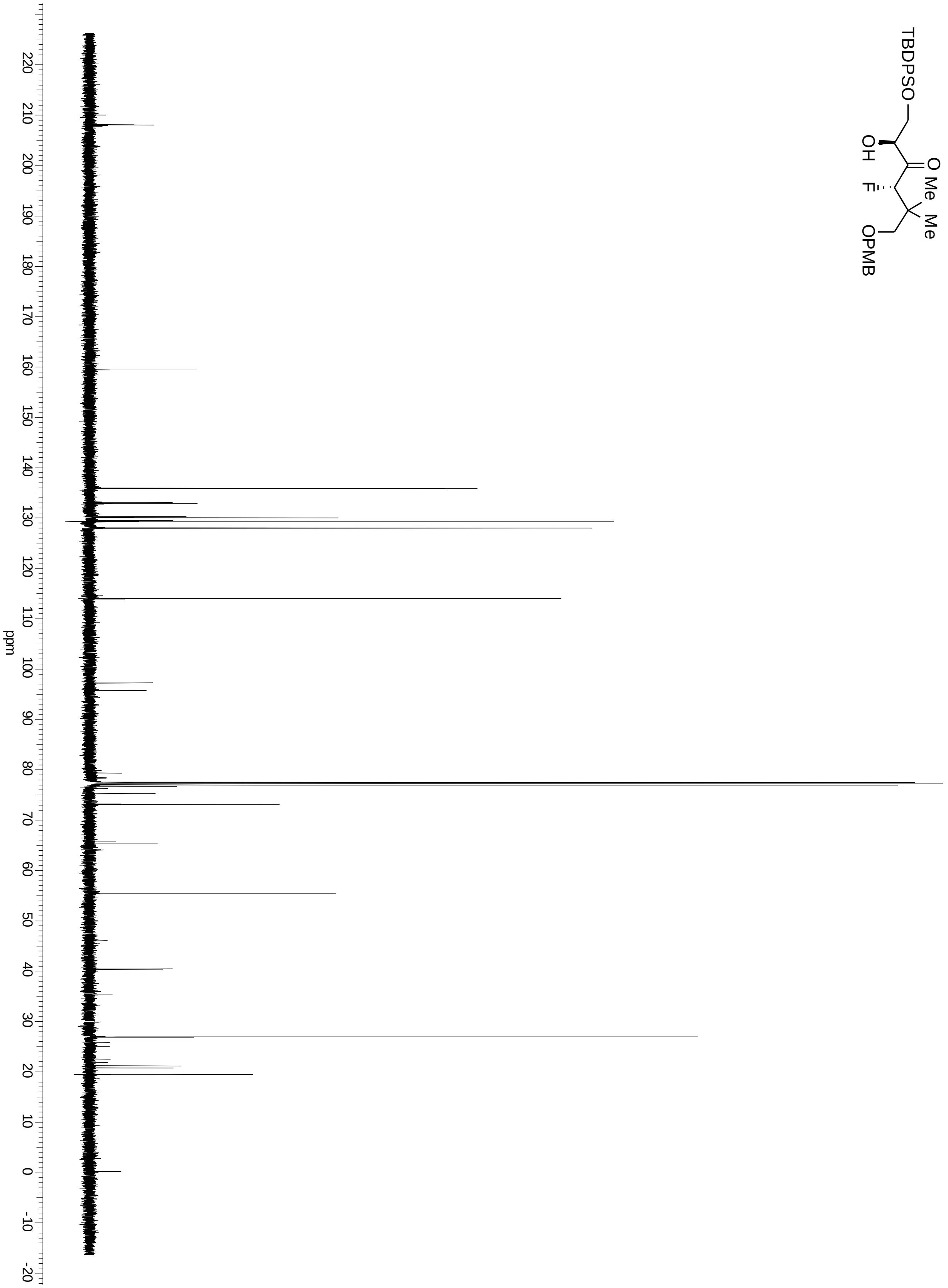

TBSO
O
Me
Me
OH
F
i-Bu
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
ppm
0.66
1.00
1.00
0.74
0.81
1.68

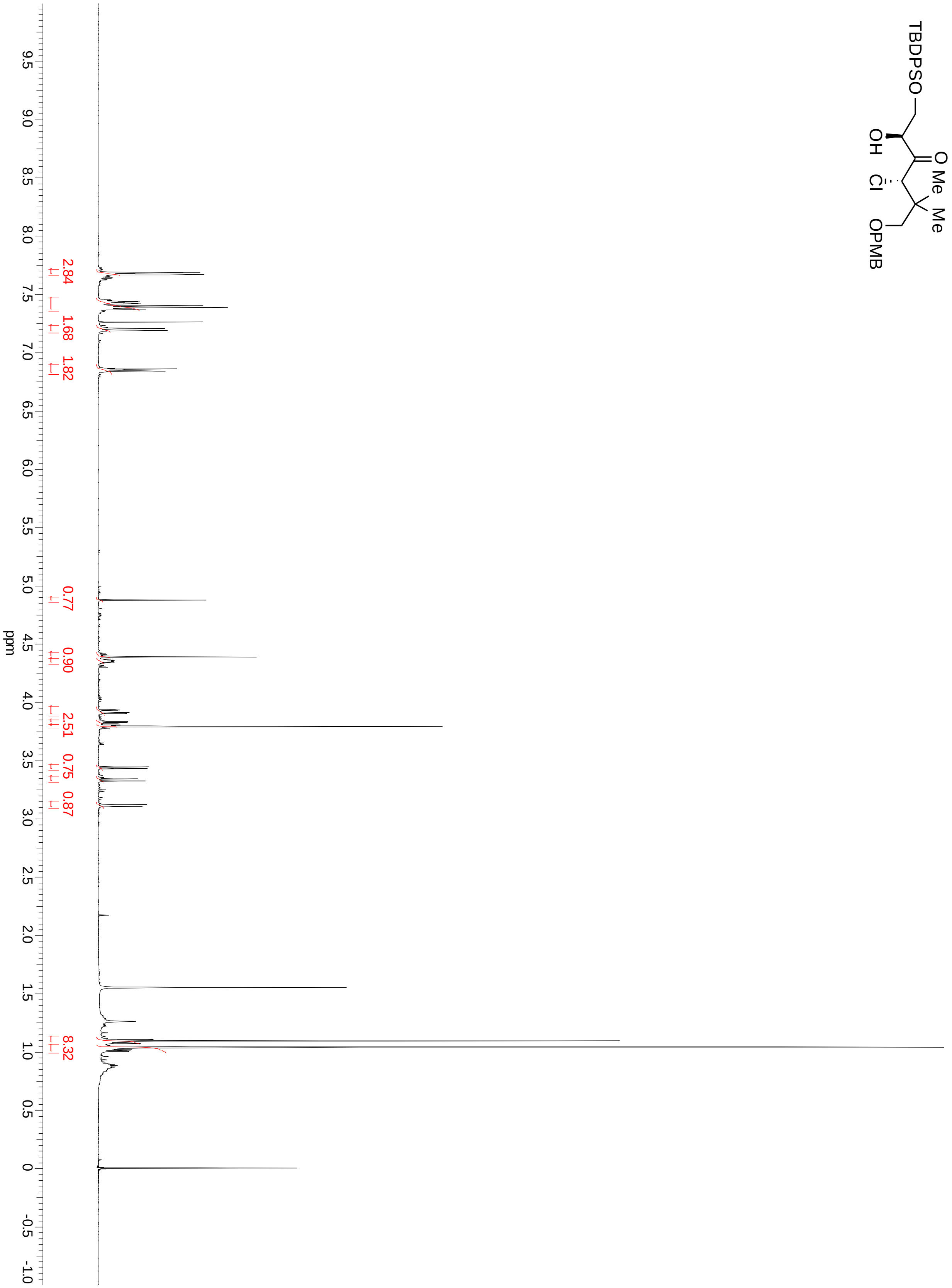

TBSO
Me
Me
O
OH
F
i-Bu
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm

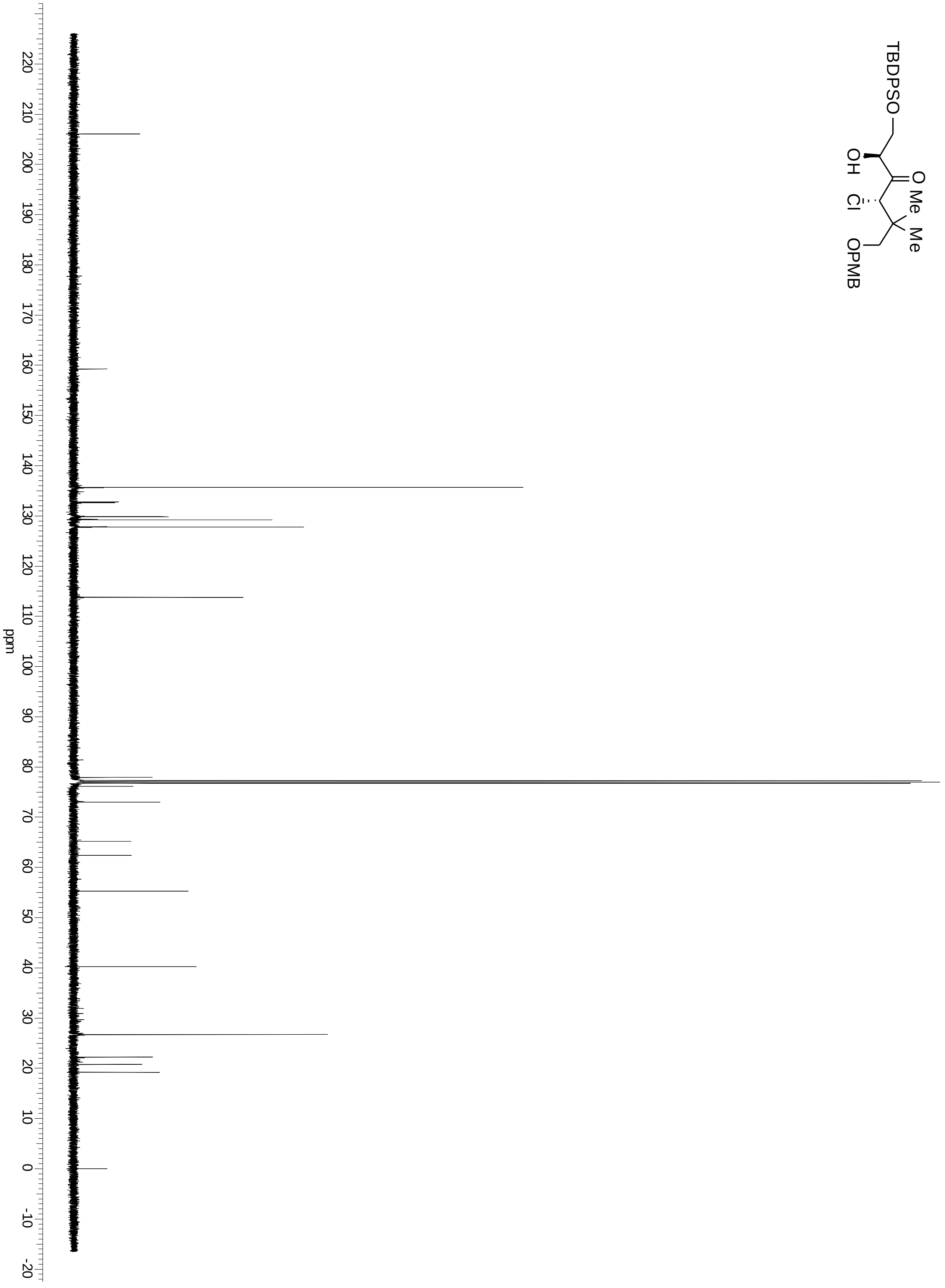

TBSO
Me
Me
OH
O
Cl
i-Bu
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
ppm
1.11
0.36
0.33
0.30
1.47
0.61
11.11

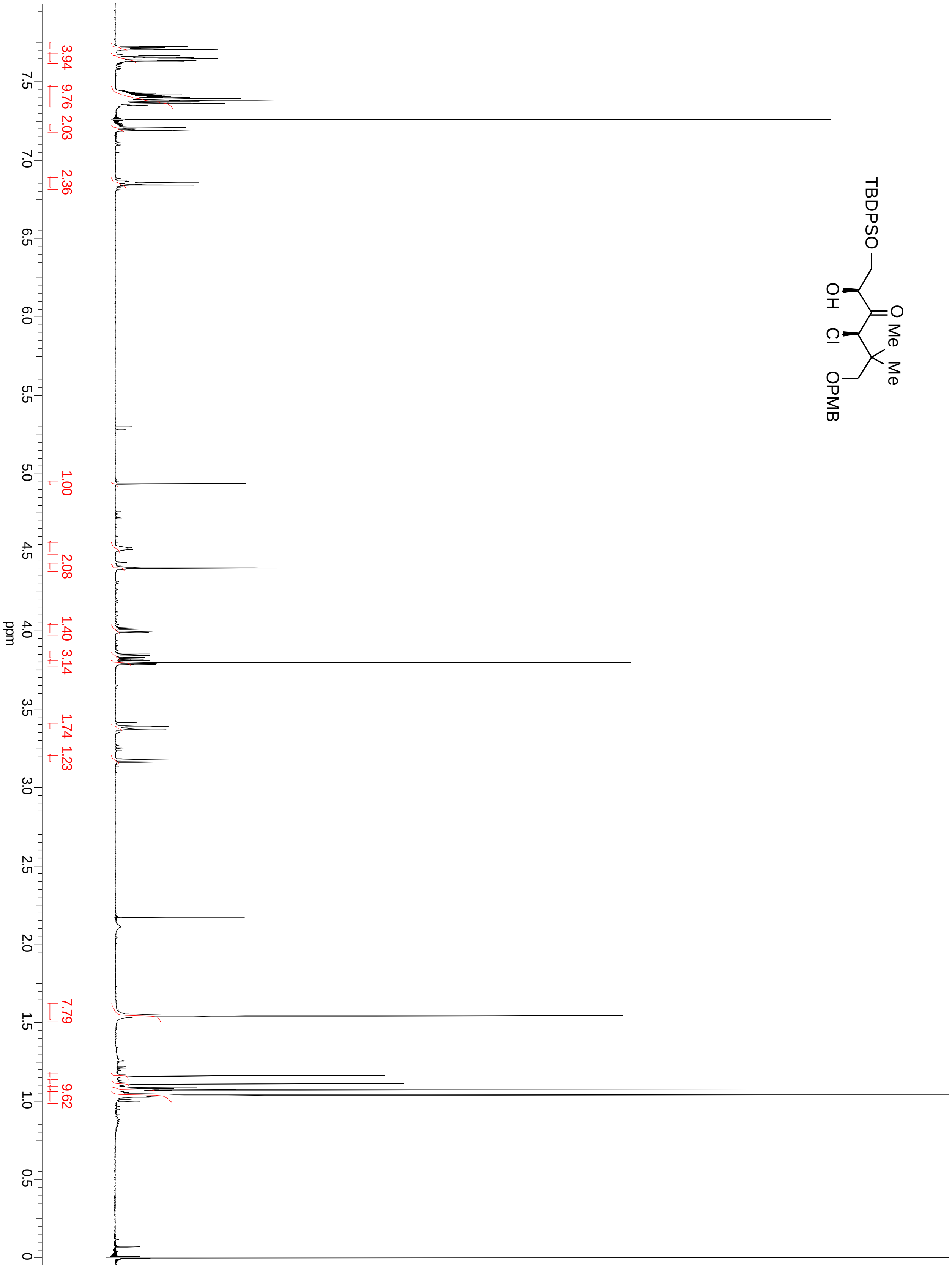

TBSO
O
Me
Me
OH
Cl
i-Bu
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm

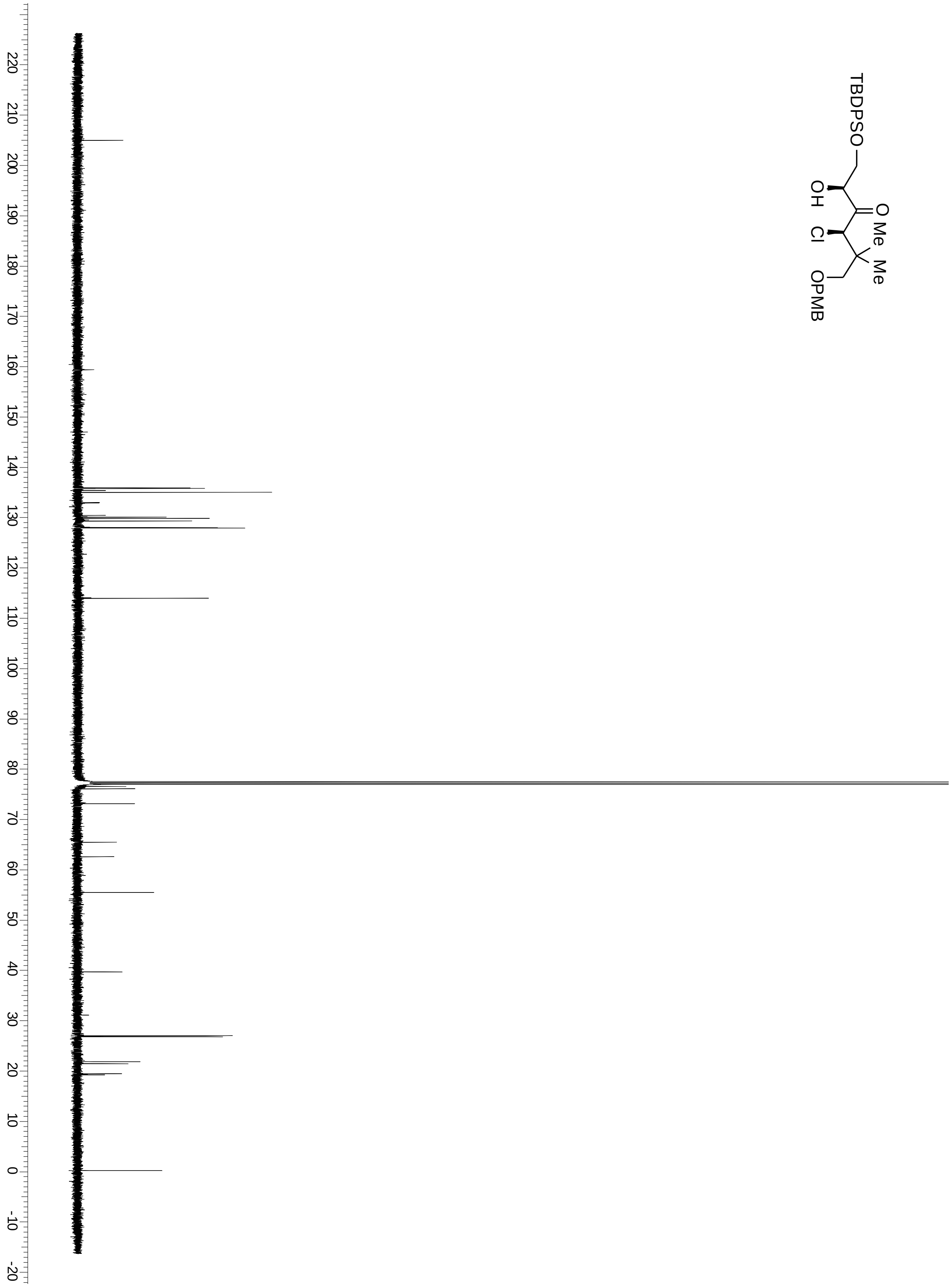

TBDPSO
O
Me
Me
OH
F
OPMB
4.24
2.13
2.11
0.50
1.59
3.12
0.82
1.25
15.53
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ppm

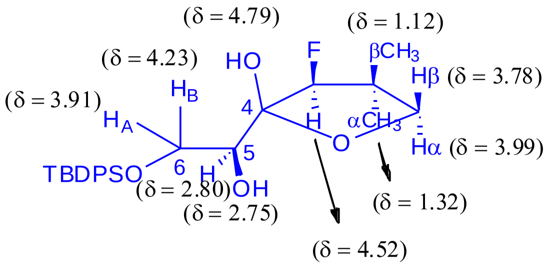

TBDPSO
O
Me
Me
OH
F
OPMB
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm


TBDPSO
O
Me
Me
OH
Cl
OPMB
2.84
1.68
1.82
0.77
0.90
2.51
0.75
0.87
8.32
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
ppm

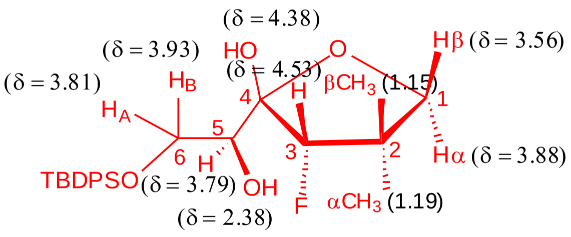

TBDPSO
O
Me
Me
OH
Cl
OPMB
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm


TBDPSO
OH
O
Cl
Me
Me
OPMB
3.94
9.76
2.03
2.36
1.00
2.08
1.40
3.14
1.74
1.23
7.79
9.62
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ppm

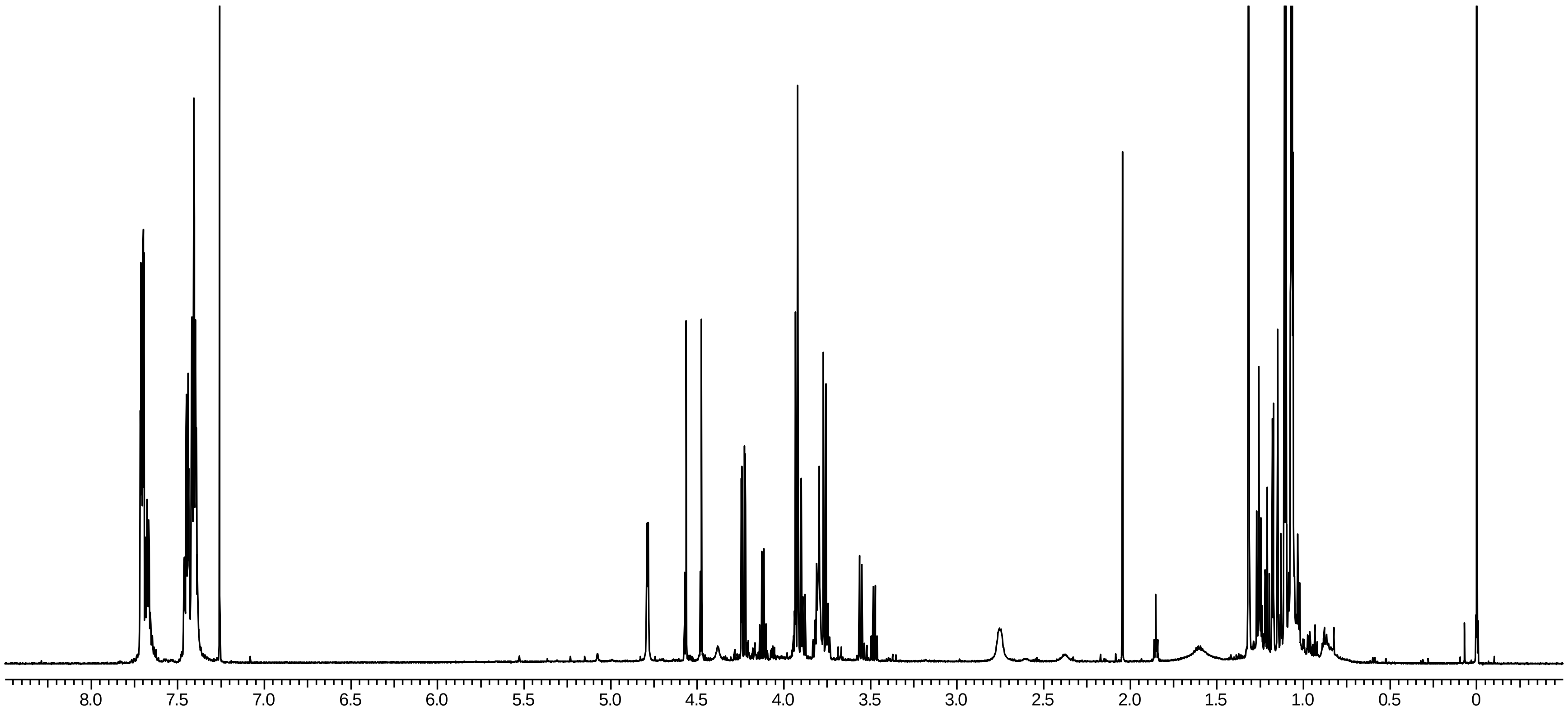

TBDPSO
O
Me
Me
OH
Cl
OPMB
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
ppm

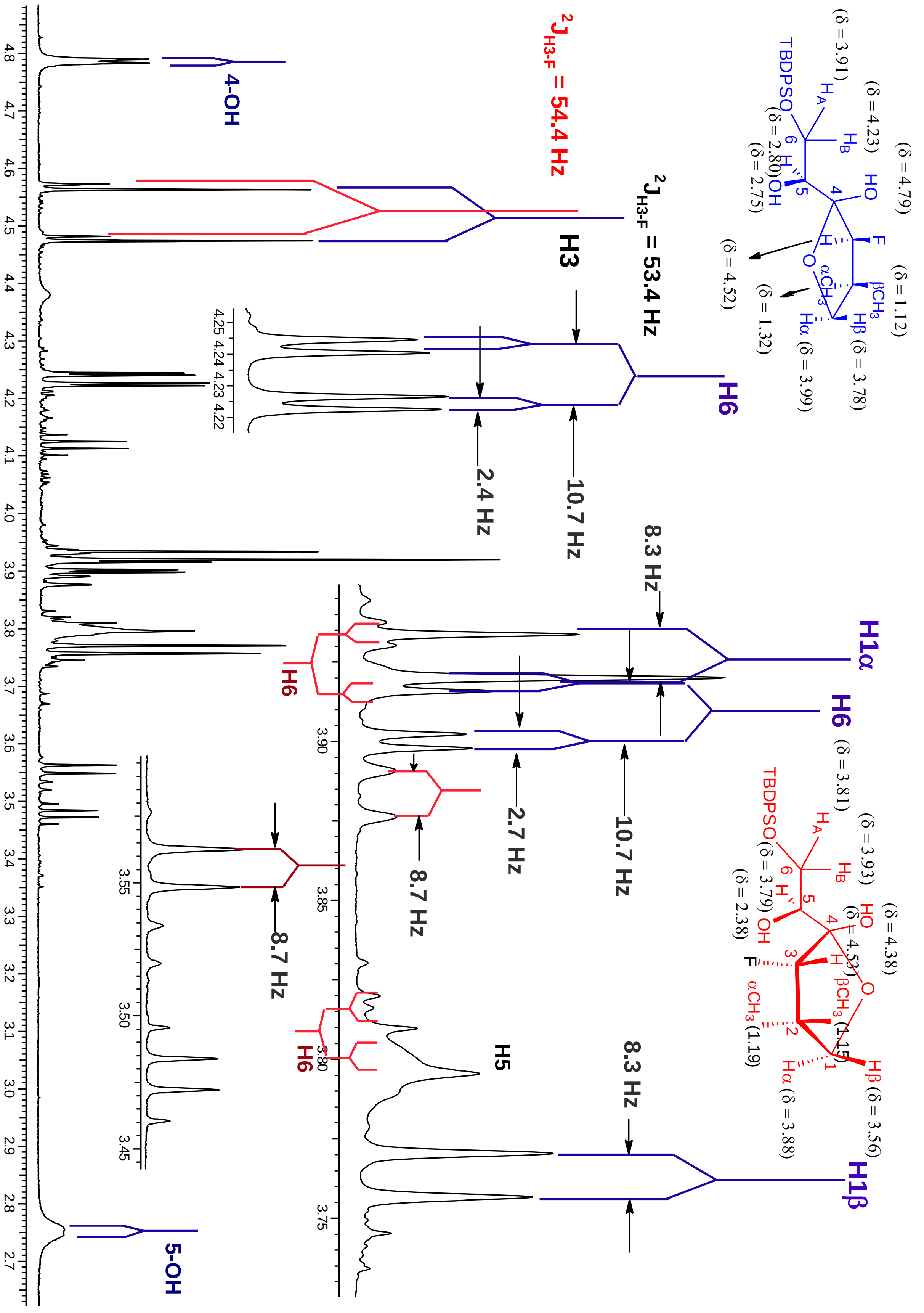

(δ = 4.79)
(δ = 1.12)
(δ = 4.23)
HO
F
βCH3
Hβ (δ = 3.78)
(δ = 3.91)
HB
HA
4
H
αCH3
O
Hα (δ = 3.99)
TBDPSO
6
H
5
(δ = 2.80)
OH
(δ = 2.75)
(δ = 1.32)
(δ = 4.52)

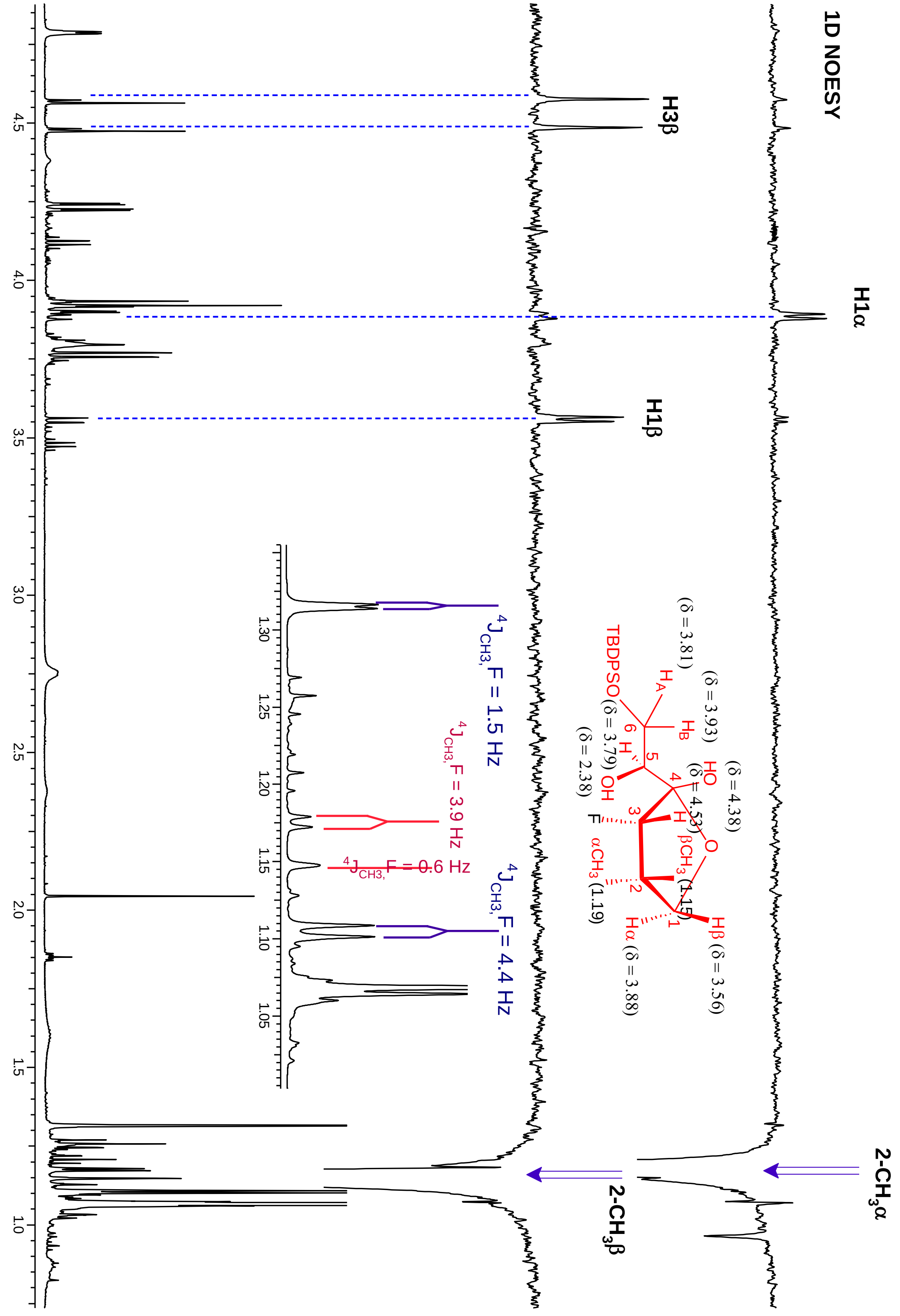
5.46

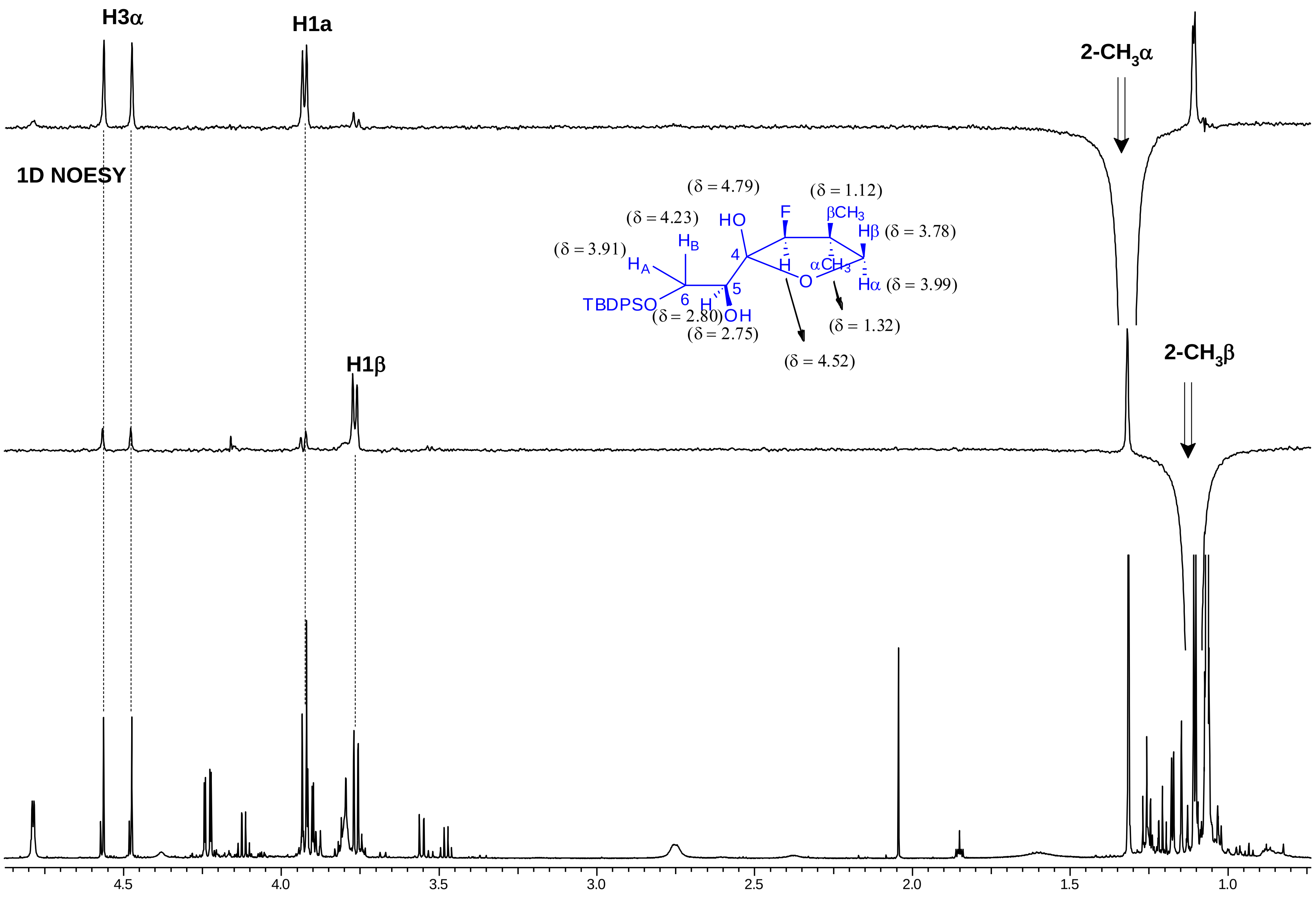

(δ = 4.38)
(δ = 3.93)
HO
O
Hβ (δ = 3.56)
(δ = 3.81)
HB
(δ = 4.53)
H
βCH3 (1.15)
1
HA
4
5
3
2
Hα (δ = 3.88)
6
H
TBDPSO
(δ = 3.79)
OH
F
αCH3 (1.19)
(δ = 2.38)

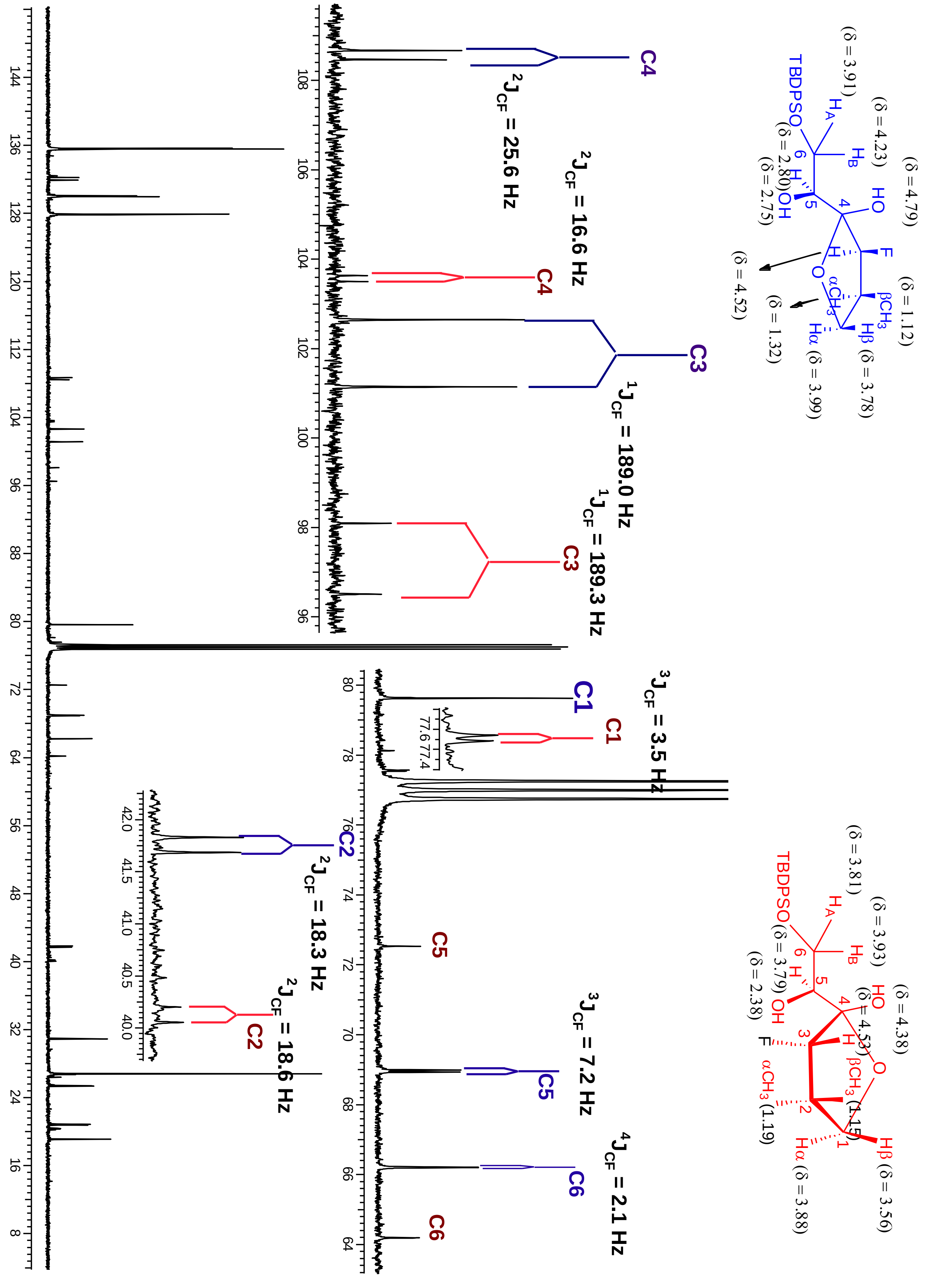
5.45

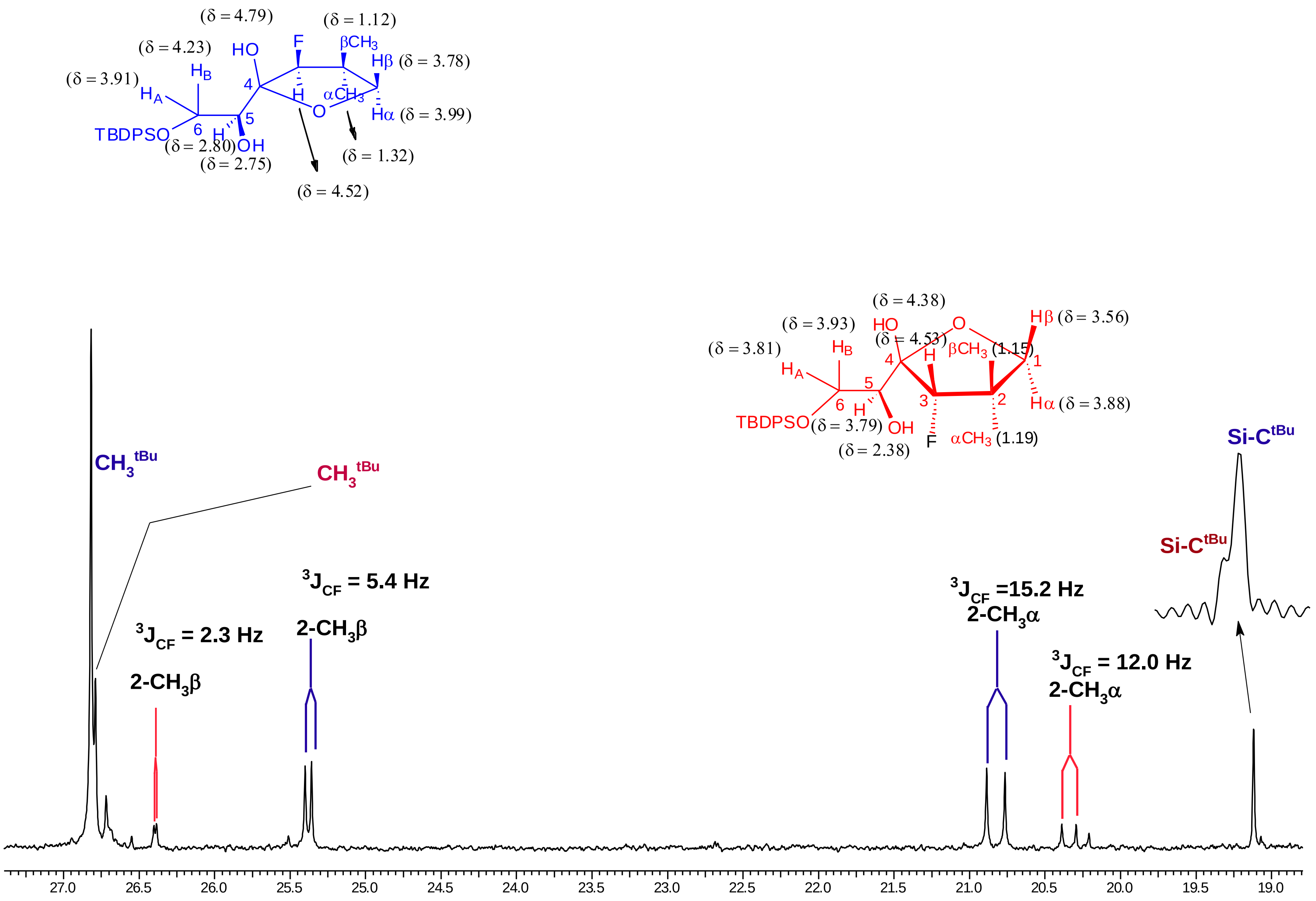

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

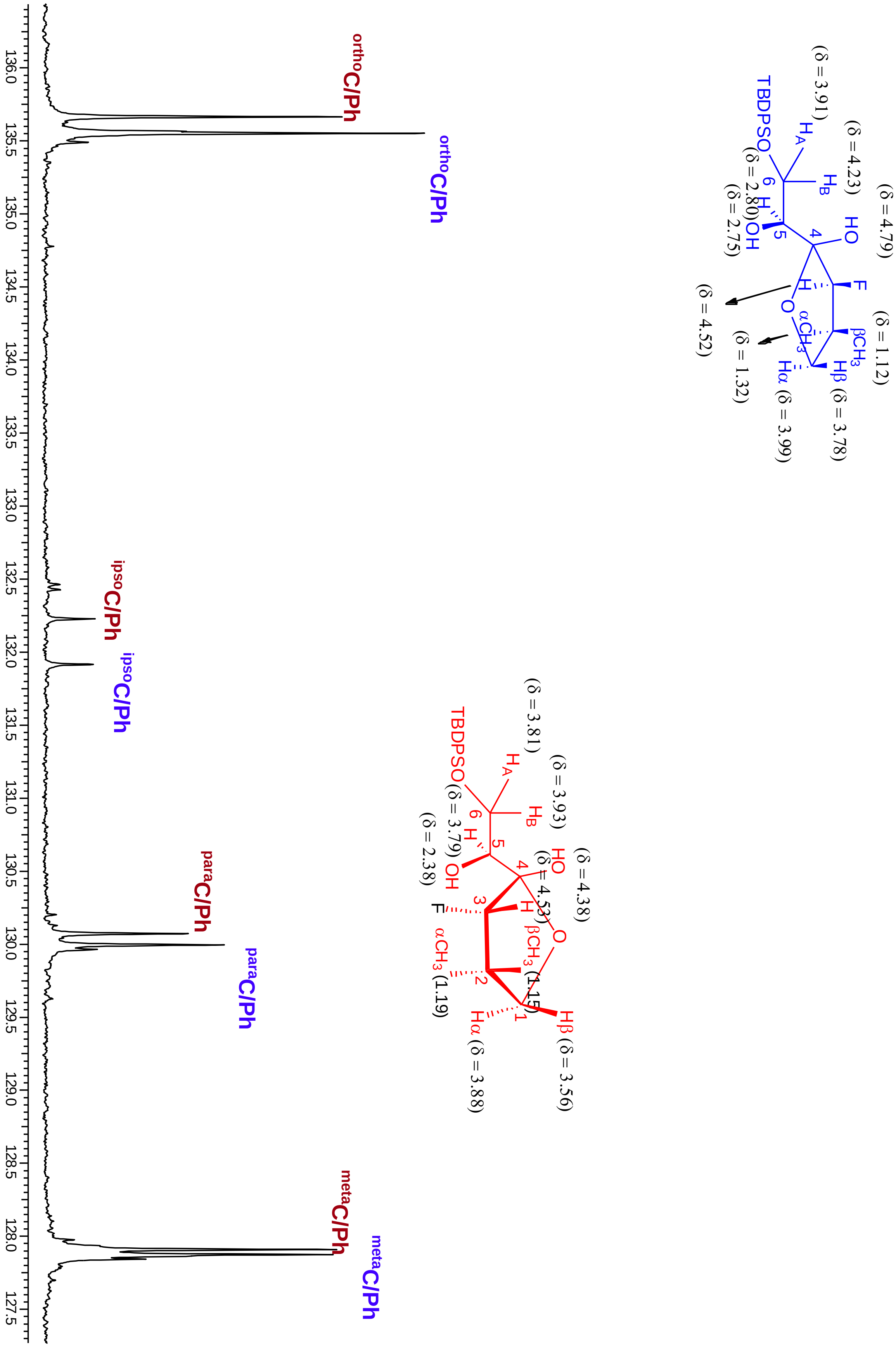

4-OH
$^{2}J_{H3-F}$ = 54.4 Hz
$^{2}J_{H3-F}$ = 53.4 Hz
H3
H6
10.7 Hz
2.4 Hz
4.25 4.24 4.23 4.22
8.3 Hz
H1α
H6
H6 (δ = 3.81)
10.7 Hz
2.7 Hz
8.7 Hz
8.7 Hz
3.90
3.85
3.80
3.75
3.55
3.50
3.45
H6
H6
H5
8.3 Hz
H1β
5-OH
TBDPSO
HA
HB
(δ = 3.91)
(δ = 4.23)
(δ = 4.79)
HO
F
βCH3
αCH3
Hβ (δ = 3.78)
Hα (δ = 3.99)
(δ = 1.12)
(δ = 1.32)
(δ = 4.52)
(δ = 2.80) OH
(δ = 2.75)
TBDPSO (δ = 3.79) OH
(δ = 3.93)
(δ = 4.38)
(δ = 4.53)
(δ = 2.38)
βCH3 (1.15)
αCH3 (1.19)
Hβ (δ = 3.56)
Hα (δ = 3.88)
4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7

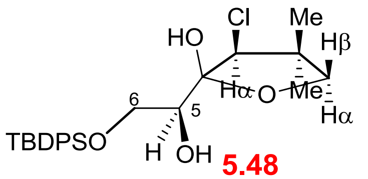

1D NOESY
H3β
H1α
H1β
2-CH$_3$α
2-CH$_3$β
(δ = 4.38)
(δ = 3.93)
(δ = 3.81)
H$_A$
H$_B$
HO
(δ = 4.53)
O
Hβ (δ = 3.56)
TBDPSO
6
5
4
3
2
1
H
H'
(δ = 3.79) OH
(δ = 2.38)
F
βCH$_3$ (1.15)
αCH$_3$ (1.19)
Hα (δ = 3.88)
$^4J_{CH3,}F$ = 1.5 Hz
$^4J_{CH3,}F$ = 3.9 Hz
$^4J_{CH3,}F$ = 0.6 Hz
$^4J_{CH3,}F$ = 4.4 Hz
1.30
1.25
1.20
1.15
1.10
1.05
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


H3α
H1a
2-CH3α
1D NOESY
H1β
2-CH3β
(δ = 4.79)
(δ = 1.12)
(δ = 4.23)
HO
F
βCH3
Hβ (δ = 3.78)
(δ = 3.91)
HB
4
HA
H
αCH3
O
Hα (δ = 3.99)
5
TBDPSO
6
H
(δ = 2.80) OH
(δ = 2.75)
(δ = 1.32)
(δ = 4.52)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

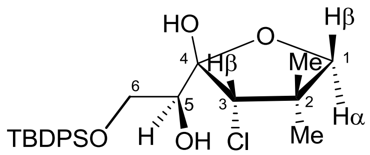

C4
2J_CF = 25.6 Hz
2J_CF = 16.6 Hz
C4
C3
1J_CF = 189.0 Hz
1J_CF = 189.3 Hz
C3
C1
C1
3J_CF = 3.5 Hz
77.6 77.4
C2
2J_CF = 18.3 Hz
2J_CF = 18.6 Hz
C2
42.0 41.5 41.0 40.5 40.0
C5
3J_CF = 7.2 Hz
C5
C6
4J_CF = 2.1 Hz
C6
TBDPSO
HA
HB
HO
F
βCH3
Hβ
Hα
αCH3
OH
(δ = 4.79)
(δ = 4.23)
(δ = 3.91)
(δ = 2.80)
(δ = 2.75)
(δ = 4.52)
(δ = 1.32)
(δ = 1.12)
(δ = 3.78)
(δ = 3.99)
(δ = 4.38)
(δ = 3.93)
(δ = 3.81)
(δ = 4.53)
(δ = 3.79)
(δ = 2.38)
(1.15)
(1.19)
(δ = 3.56)
(δ = 3.88)
144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8
108 106 104 102 100 98 96
80 78 76 74 72 70 68 66 64


(δ = 4.79)
(δ = 1.12)
(δ = 4.23)
HO
F
βCH3
Hβ (δ = 3.78)
(δ = 3.91)
HB
HA
4
H
αCH3
Hα (δ = 3.99)
5
O
TBDPSO
6
H
OH
(δ = 2.80)
(δ = 2.75)
(δ = 1.32)
(δ = 4.52)
(δ = 4.38)
(δ = 3.93)
HO
O
Hβ (δ = 3.56)
(δ = 4.53)
(δ = 3.81)
HB
H
βCH3 (1.15)
1
HA
4
5
3
2
Hα (δ = 3.88)
6
H
TBDPSO (δ = 3.79)
OH
F
αCH3 (1.19)
(δ = 2.38)
CH3 tBu
CH3 tBu
3JCF = 5.4 Hz
3JCF = 2.3 Hz
2-CH3β
2-CH3β
3JCF =15.2 Hz
2-CH3α
3JCF = 12.0 Hz
2-CH3α
Si-C tBu
Si-C tBu
27.0
26.5
26.0
25.5
25.0
24.5
24.0
23.5
23.0
22.5
22.0
21.5
21.0
20.5
20.0
19.5
19.0


(δ = 4.79)
(δ = 4.23)
(δ = 3.91)
HA
HB
HO
TBDPSO
6
5
4
(δ = 2.80)OH
(δ = 2.75)
F
H
O
αCH3
βCH3
(δ = 1.12)
Hβ (δ = 3.78)
Hα (δ = 3.99)
(δ = 1.32)
(δ = 4.52)
(δ = 4.38)
(δ = 3.93)
(δ = 3.81)
(δ = 4.53)
HO
HA
HB
TBDPSO(δ = 3.79) OH
(δ = 2.38)
F
αCH3 (1.19)
βCH3 (1.15)
Hβ (δ = 3.56)
Hα (δ = 3.88)
1
2
3
4
5
6
O
H
orthoC/Ph
orthoC/Ph
ipsoC/Ph
ipsoC/Ph
paraC/Ph
paraC/Ph
metaC/Ph
metaC/Ph
136.0
135.5
135.0
134.5
134.0
133.5
133.0
132.5
132.0
131.5
131.0
130.5
130.0
129.5
129.0
128.5
128.0
127.5



5.48

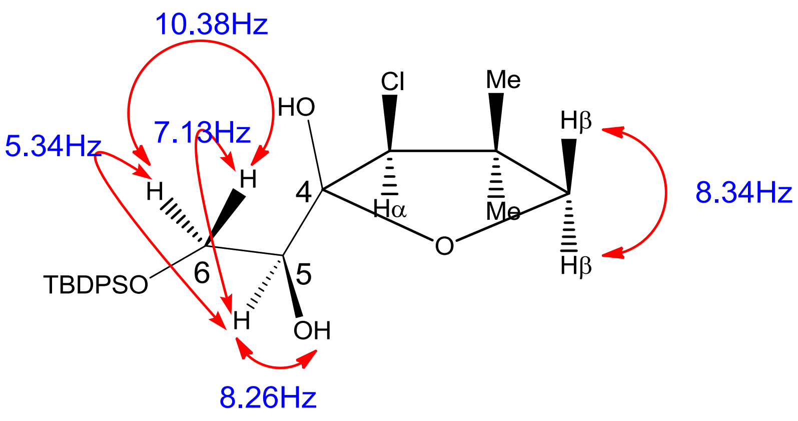


5.47

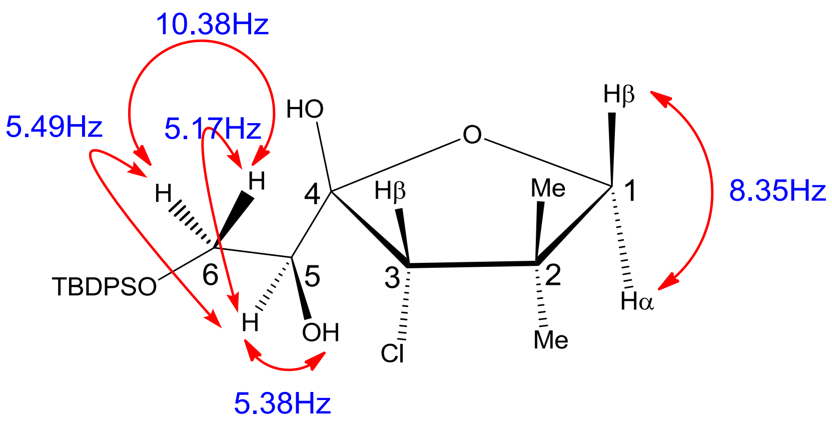
	1H NMR chemical shifts (δ/ppm) w/ coupling constant (J/Hz)
5.48	3.62 (d, JH1α, H1β = 8.34 Hz, H1α)
	3.98 (d, H1β)
	1.13 (s, 2-CH3α)
	1.29 (s, 2-CH3β)
	4.01 (d, H3α)
	4.82 (s, 4-OH)
	3.72 (ddd, JH5, OH = 8.26 Hz, H5)
	2.19 (d, 5-OH)
	3.83 (dd, H6)
	3.95 (dd, H6)
5.47	3.94 (d, JH1α, H1β = 8.35 Hz, H1α)
	3.59 (d, H1β)
	1.24 (s, 2-CH3α)
	1.11 (s, 2-CH3β)
	4.03 (d, H3β)
	4.56 (s, 4-OH)
	? (ddd, JH5, OH = 5.38 Hz, H5)
	2.56 (d, 5-OH)
	3.87 (dd, H6)
	3.93 (dd, H6)
	7.66 - 7.70 (m, orthoH/Ph)
	7.42 - 7.36 (m, metaH/Ph)
	7.43 - 7.47 (m, orthoH/Ph)

13C NMR chemical shift (δ/ppm)		**5.48**	**5.47**
C1	----	78.89	78.55
C2	----	41.82	41.81
C3	----	67.31	68.20
C4	----	105.17	104.56
C5	----	70.76	73.95
C6	----	64.31	64.45
$2CH_3^{\alpha}$	----	22.97	22.78
$2CH_3^{\beta}$	----	25.68	25.37
$CH_3^{t\text{-}Bu}$	----	19.10	19.11
$CH_3^{t\text{-}Bu}$	----	26.79	26.79
^{ipso}C	----	132.21	132.35
$^{ortho}C$	----	135.58	135.63
^{meta}C	----	127.87	127.67
^{para}C	----	130.02	130.03

Coupling constant (J_{HH})

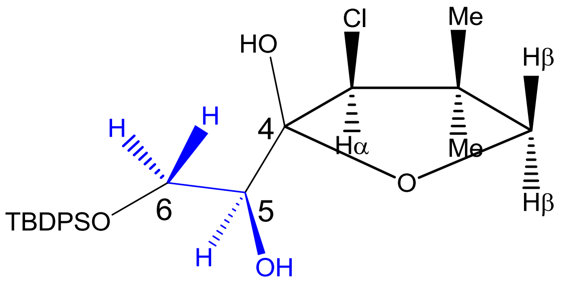

10.38Hz
5.34Hz
7.13Hz
HO
Cl
Me
Hβ
8.34Hz
H
H
4
Hα
Me
O
Hβ
TBDPSO
6
5
H
OH
8.26Hz

5.48

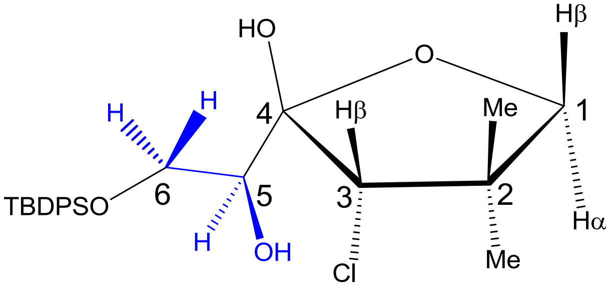

10.38Hz
5.49Hz
5.17Hz
HO
O
Hβ
8.35Hz
H
H
4
Hβ
Me
1
TBDPSO
6
5
3
2
Hα
H
OH
Cl
Me
5.38Hz

5.47

1d TOCSY

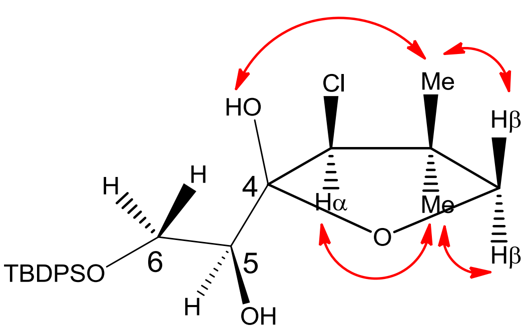

HO
Cl
Me
Hβ
H
H
4
Hα
Me
O
Hβ
TBDPSO
6
5
H
OH

5.48

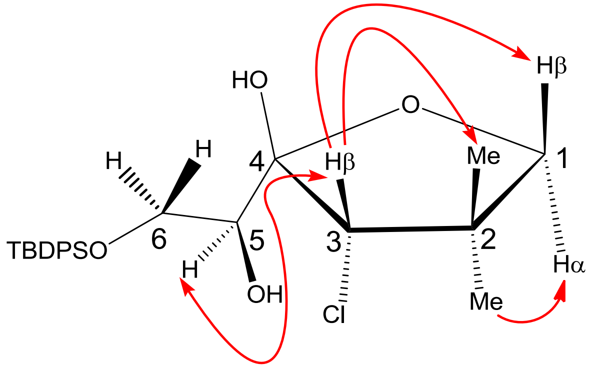

HO
O
Hβ
H
H
4
Hβ
Me
1
TBDPSO
6
5
3
2
Hα
H
OH
Cl
Me

5.47

NOESY

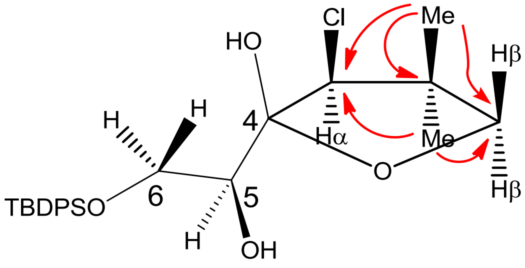

HO
Cl
Me
Hβ
H
H
4
Hα
Me
O
Hβ
TBDPSO
6
5
H
OH

5.48

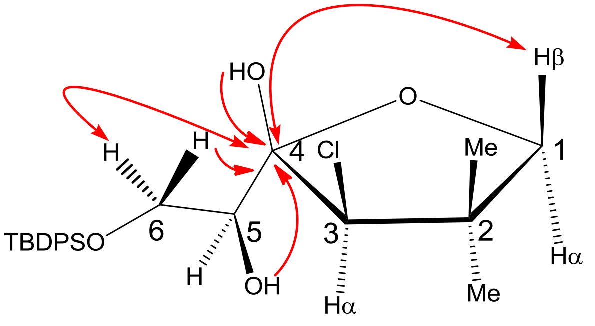

Hβ
HO
O
H
H
4
Hβ
Me
1
TBDPSO
6
5
3
2
H
OH
Hα
Cl
Me

5.47

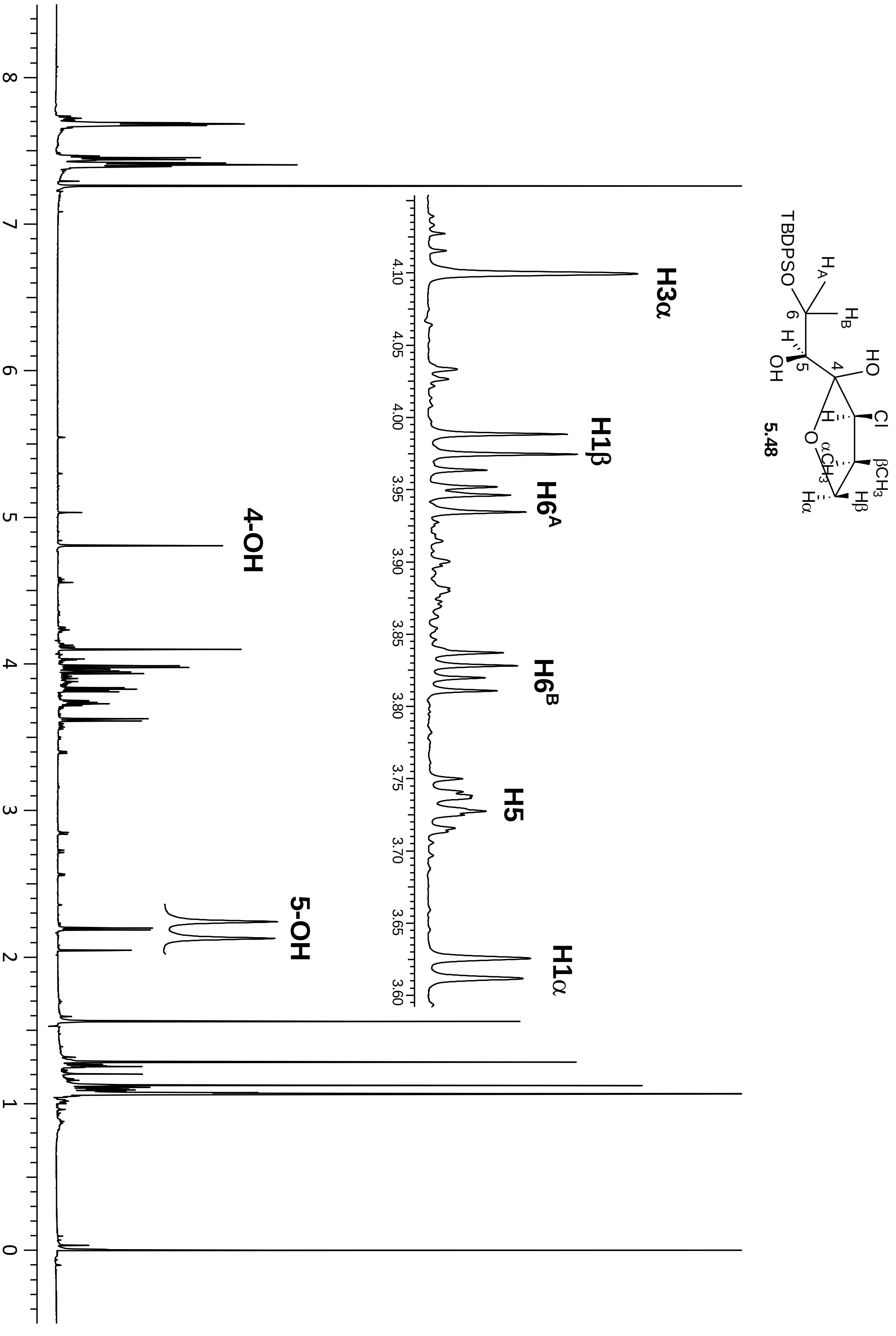
HMBC


Cl
Me
HO
Hβ
H
H
4
Hα
Me
O
Hβ
TBDPSO
6
5
H
OH

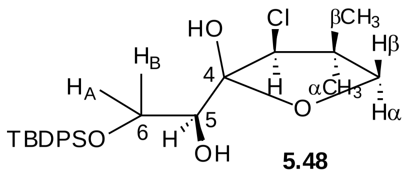
5.48


Hβ
HO
O
H
H
4
Cl
Me
1
TBDPSO
6
5
3
2
Hα
H
OH
Hα
Me

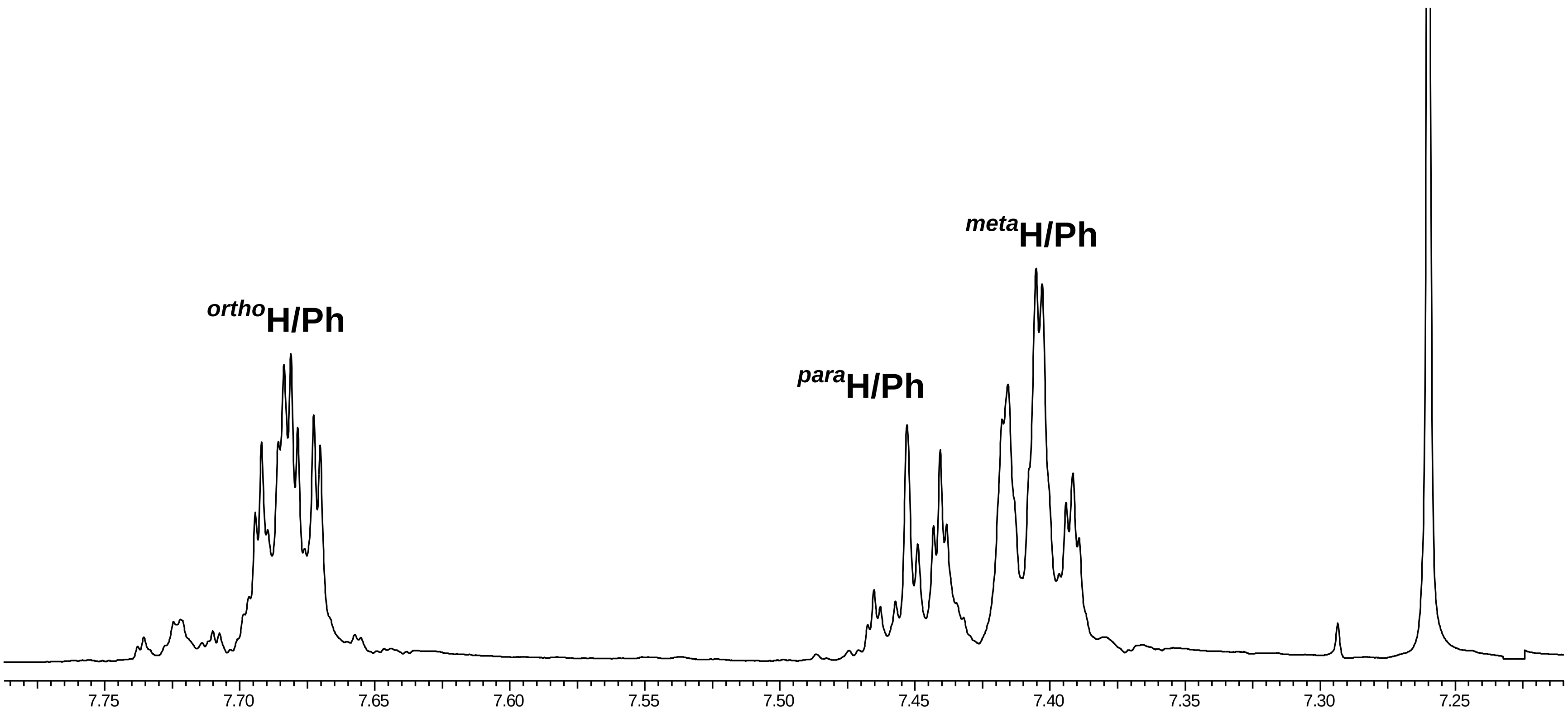
5.47

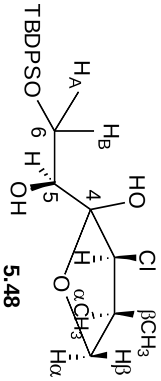

TBDPSO
H_A
H_B
6
H
5
OH
4
HO
H
O
Cl
βCH3
αCH3
Hβ
Hα
H3α
H1β
H6^A
H6^B
H5
H1α
4-OH
5-OH
4.10
4.05
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
8
7
6
5
4
3
2
1
0

5.48

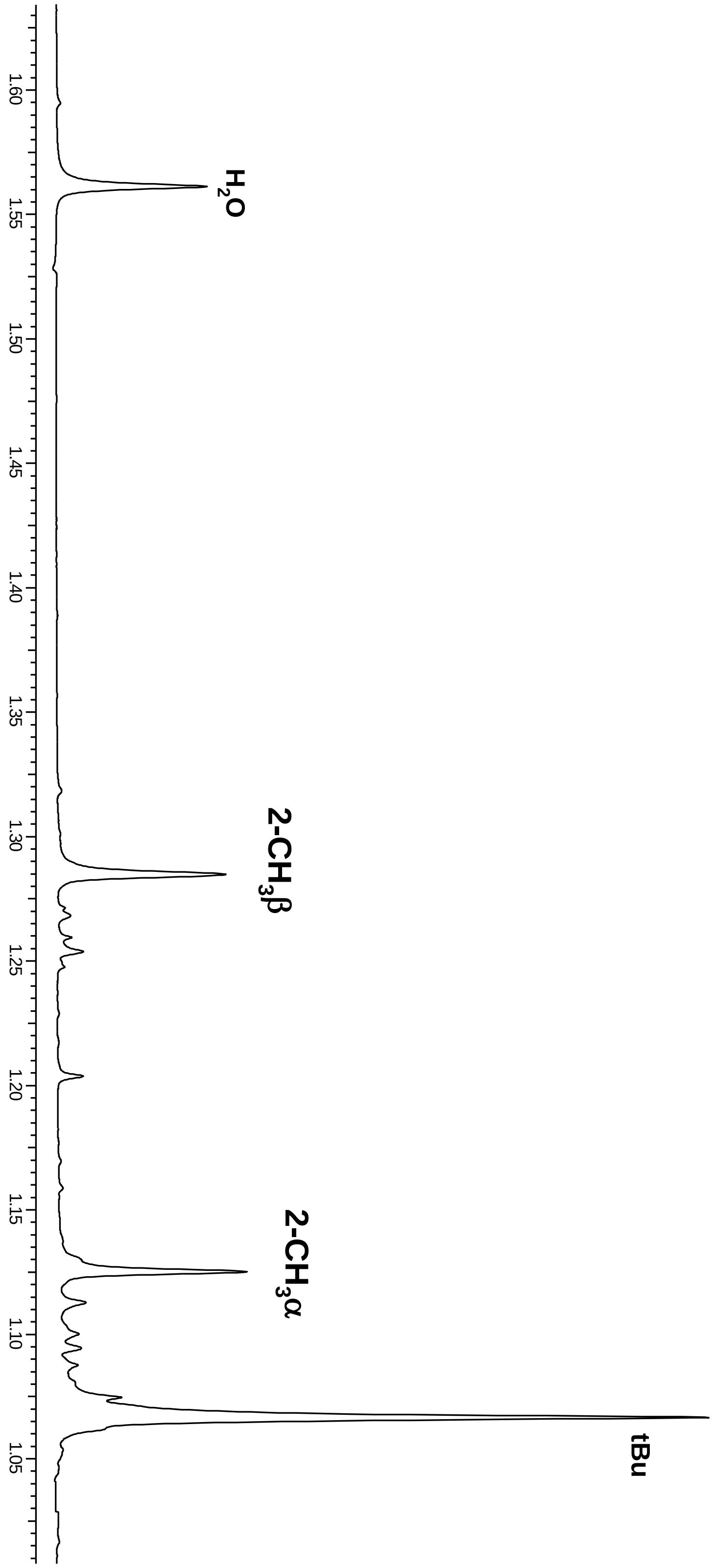

TBDPSO
H_A
H_B
6
H
5
OH
4
HO
Cl
H
O
βCH3
αCH3
Hβ
Hα

5.48

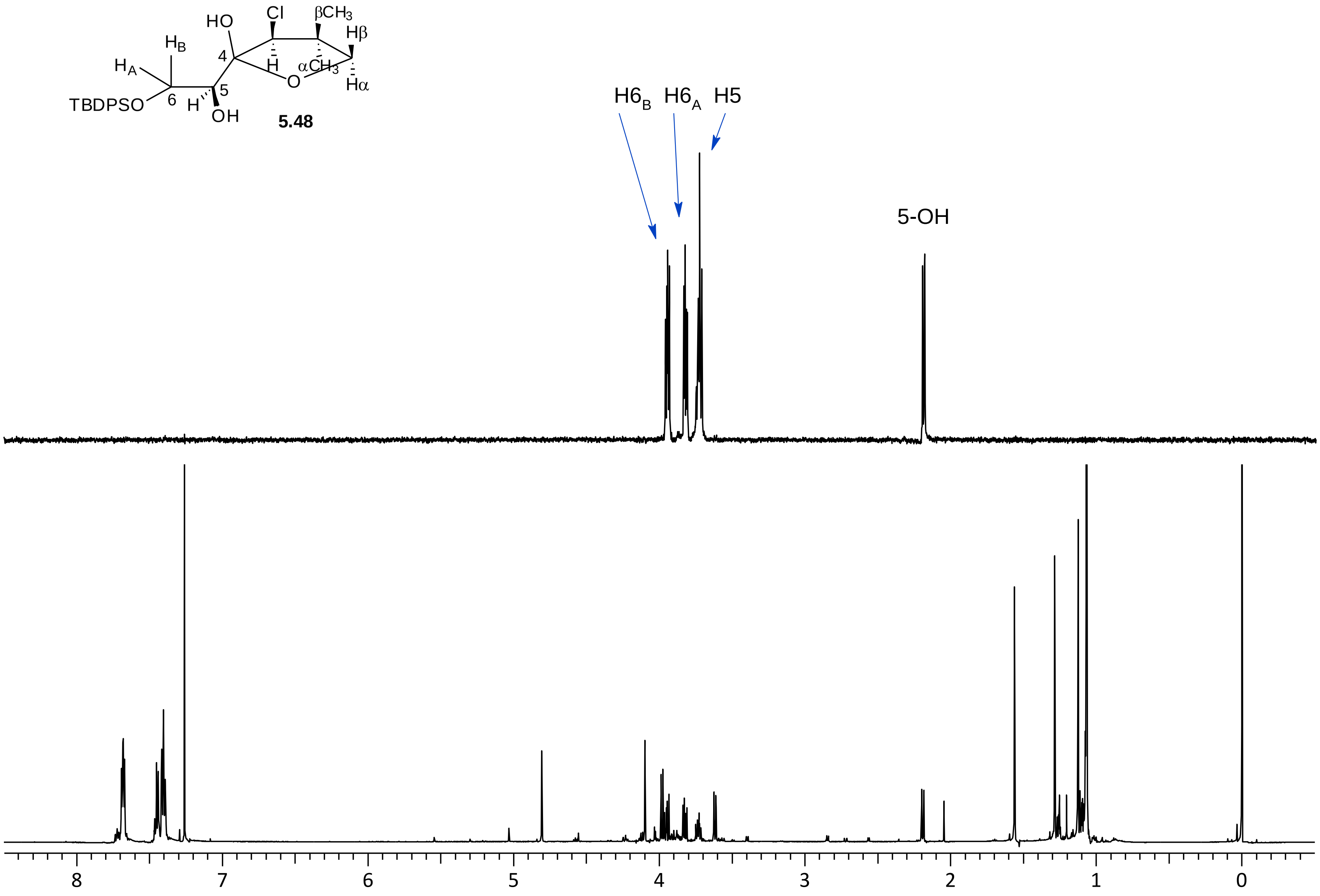

orthoH/Ph
paraH/Ph
metaH/Ph
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25


TBDPSO
H_A
H_B
6
H
5
OH
HO
4
H
Cl
O
αCH3
βCH3
Hβ
Hα

5.48

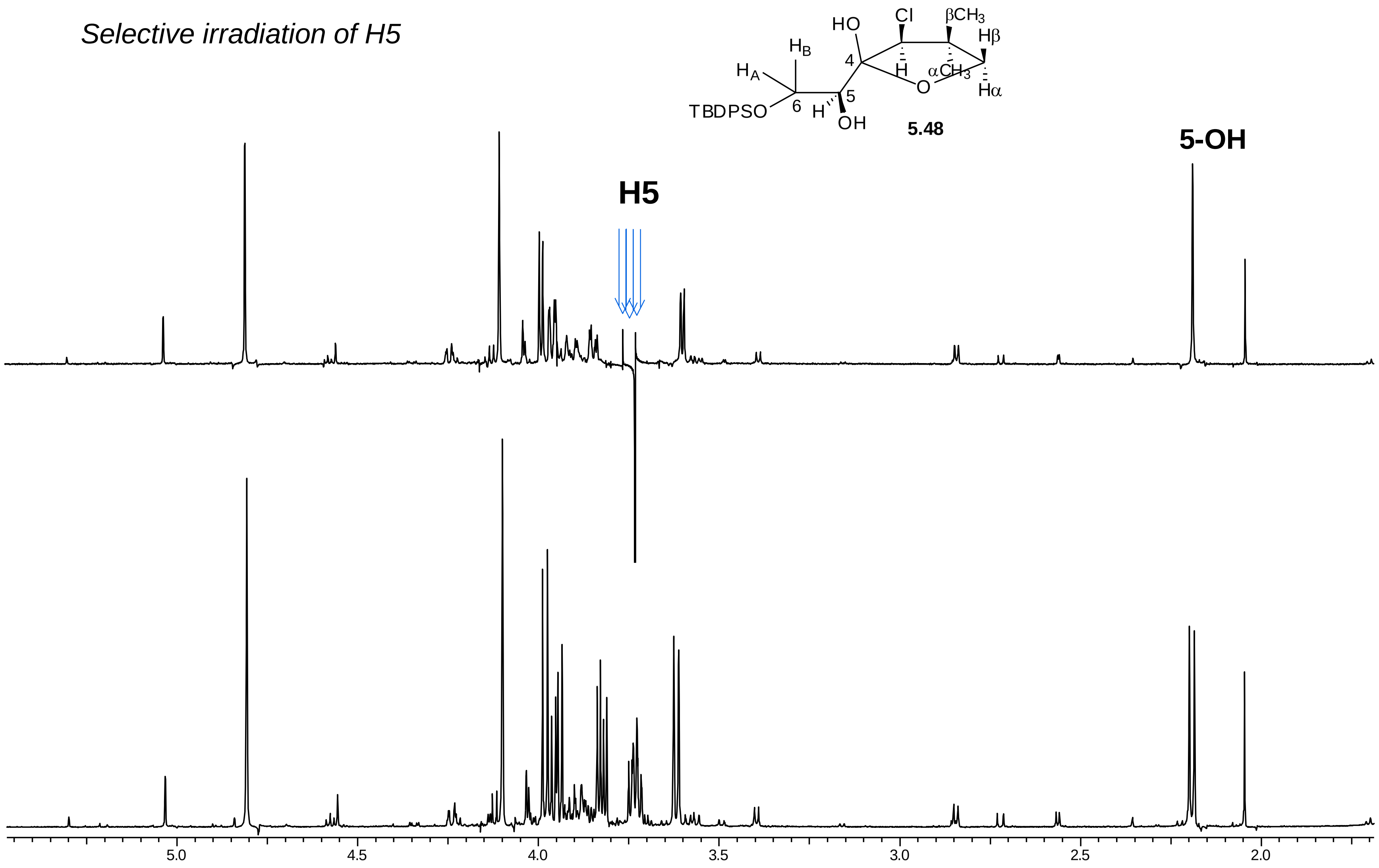

H2O
2-CH3β
2-CH3α
tBu
1.60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05

Magnetization transfer pathway (spin system identification)

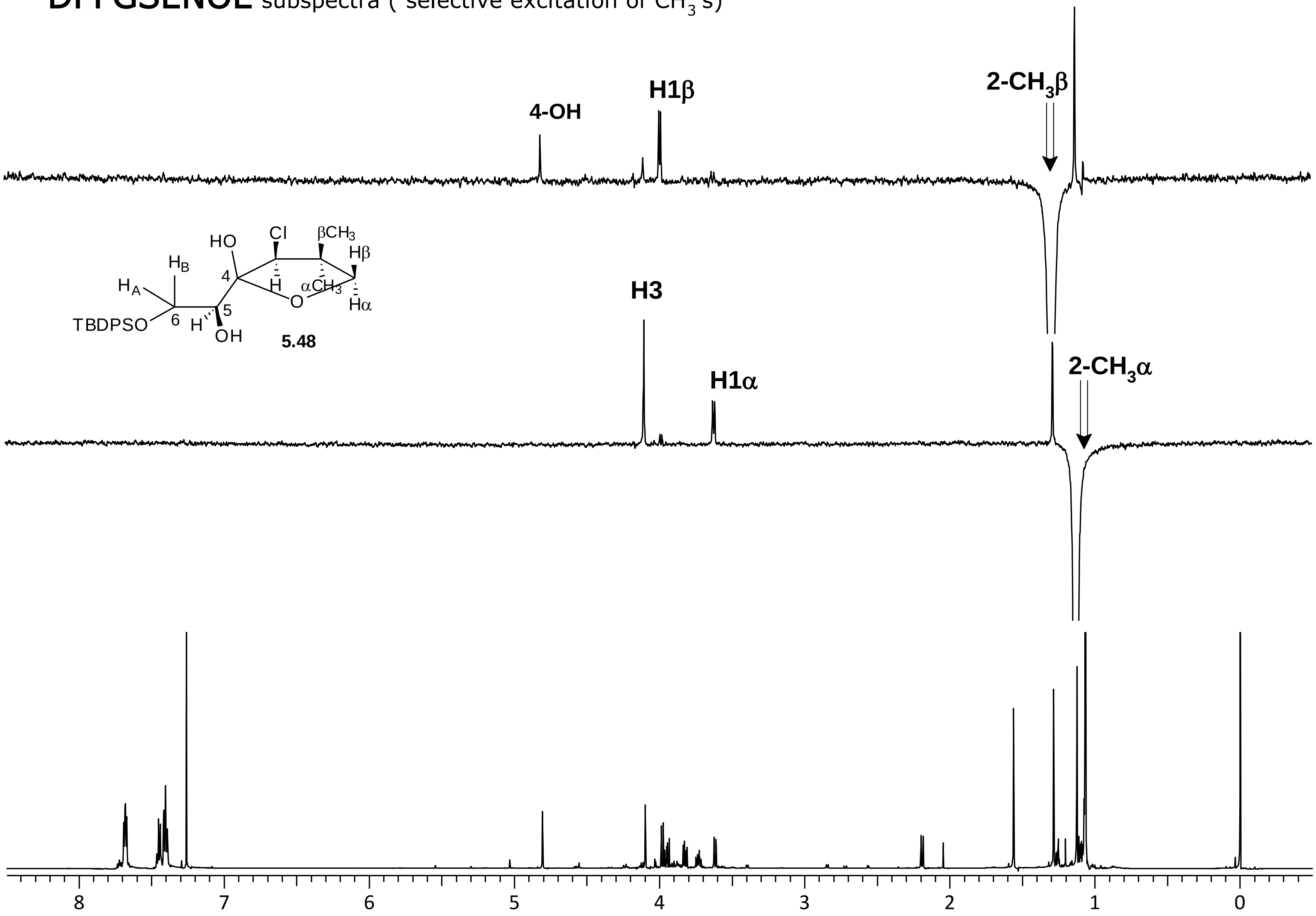


5.48

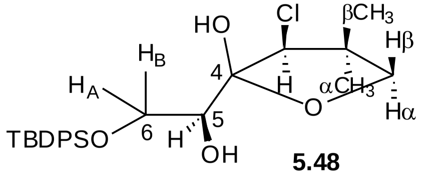
Selective irradiation of H5



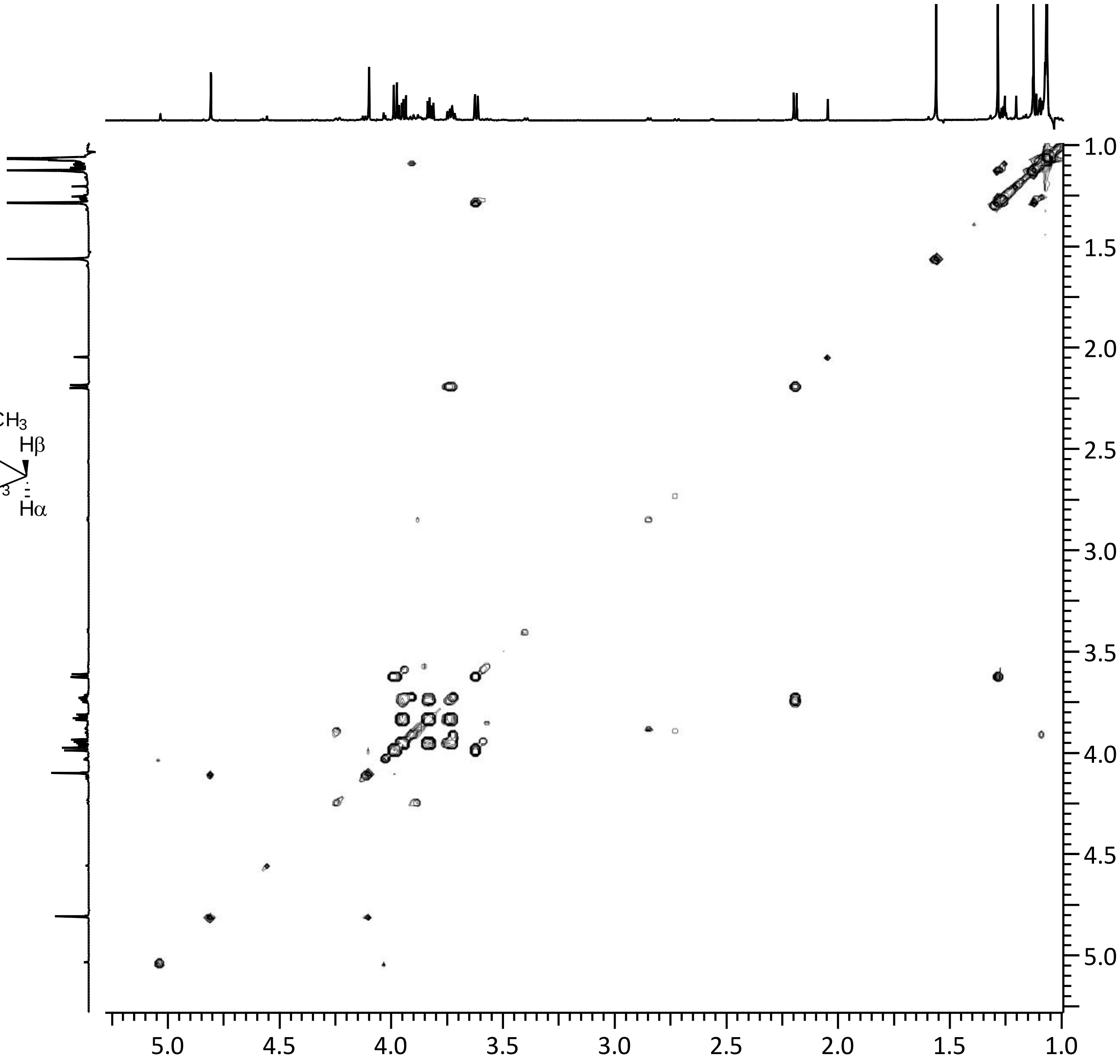
DPFGSENOE subspectra (selective excitation of CH_3's)



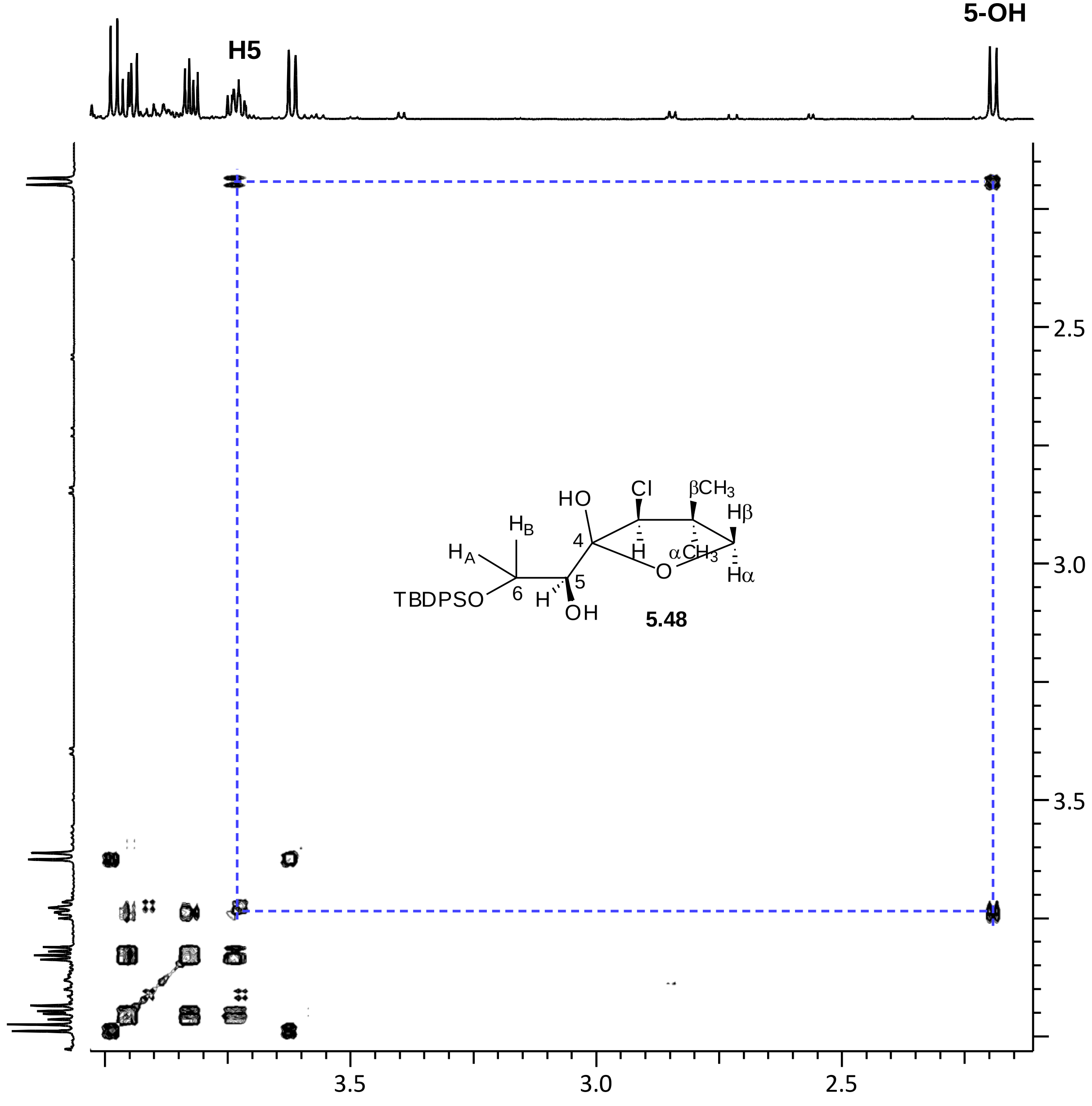
gCOSY


HO
Cl
βCH3
Hβ
HB
HA
4
H
αCH3
O
Hα
TBDPSO
6
H
5
OH

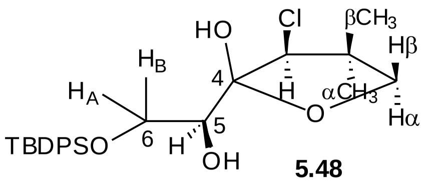
5.48


1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

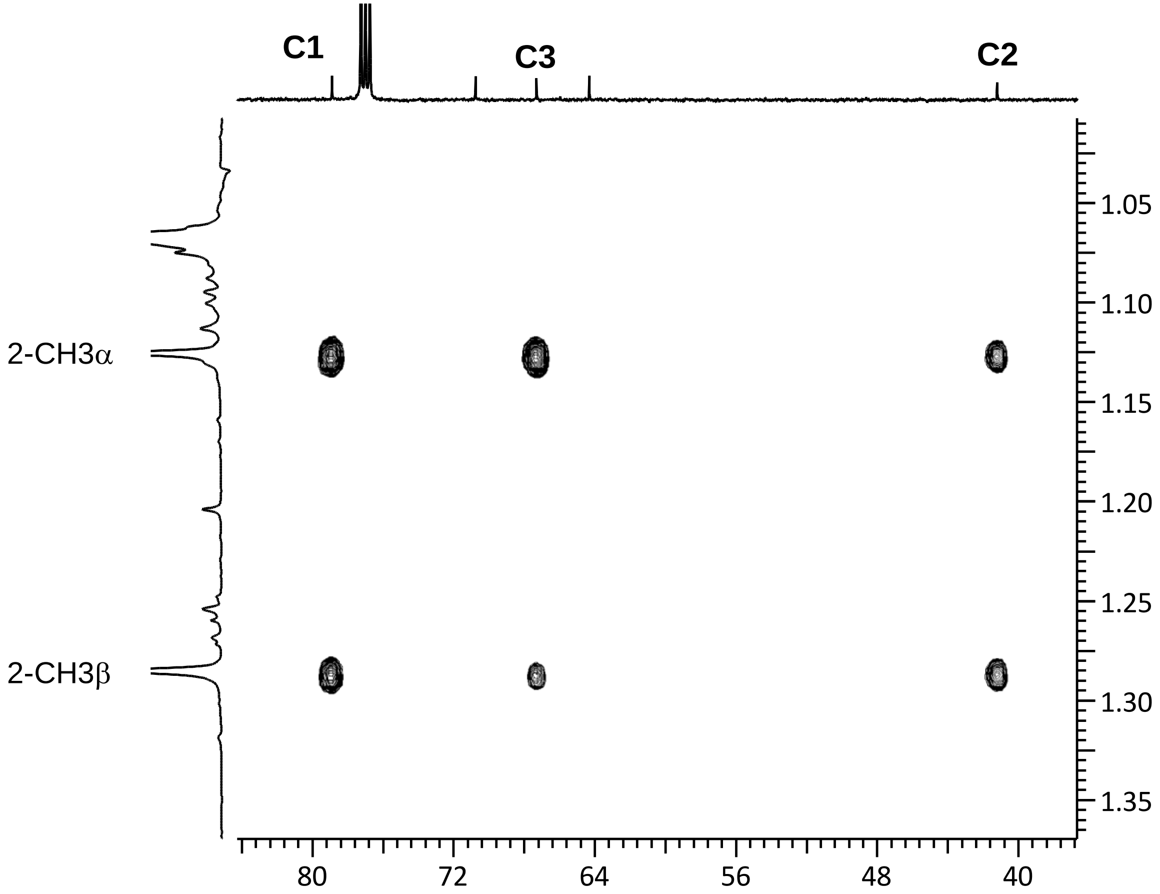
gCOSY


H5
5-OH
HO
Cl
βCH3
Hβ
HB
HA
4
H
αCH3
O
Hα
TBDPSO
6
H
5
OH
5.48
2.5
3.0
3.5
3.5
3.0
2.5

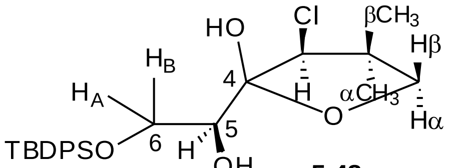
gHMBC


HO
Cl
βCH3
Hβ
HB
HA
4
H
αCH3
O
Hα
TBDPSO
6
H
5
OH

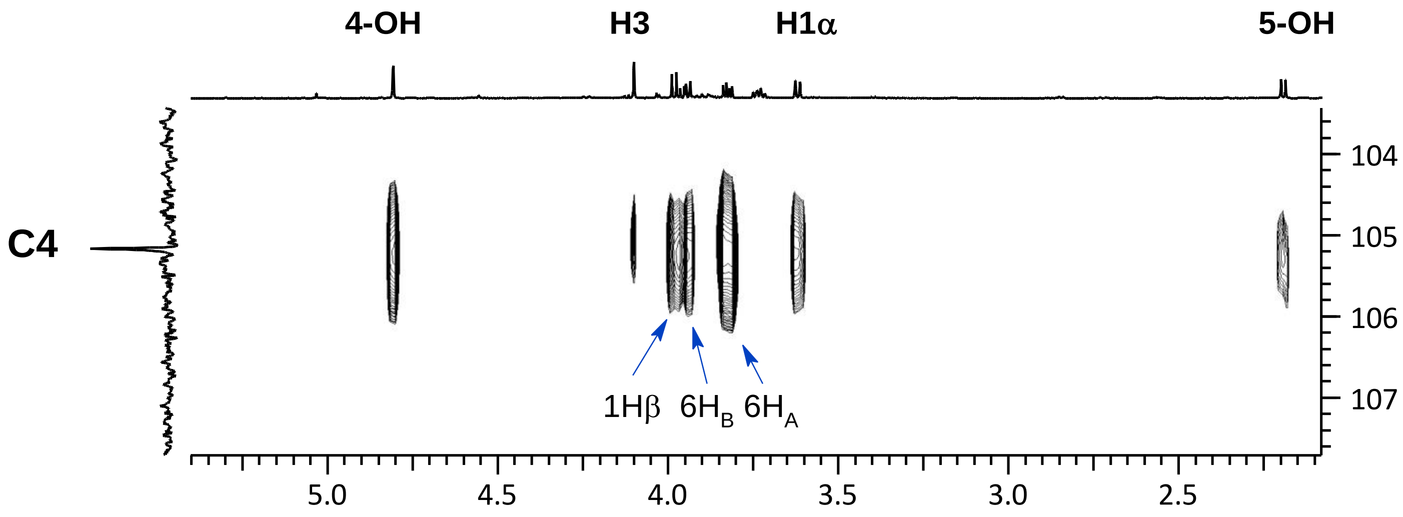
5.48


C1
C3
C2
2-CH3α
2-CH3β
1.05
1.10
1.15
1.20
1.25
1.30
1.35
80
72
64
56
48
40

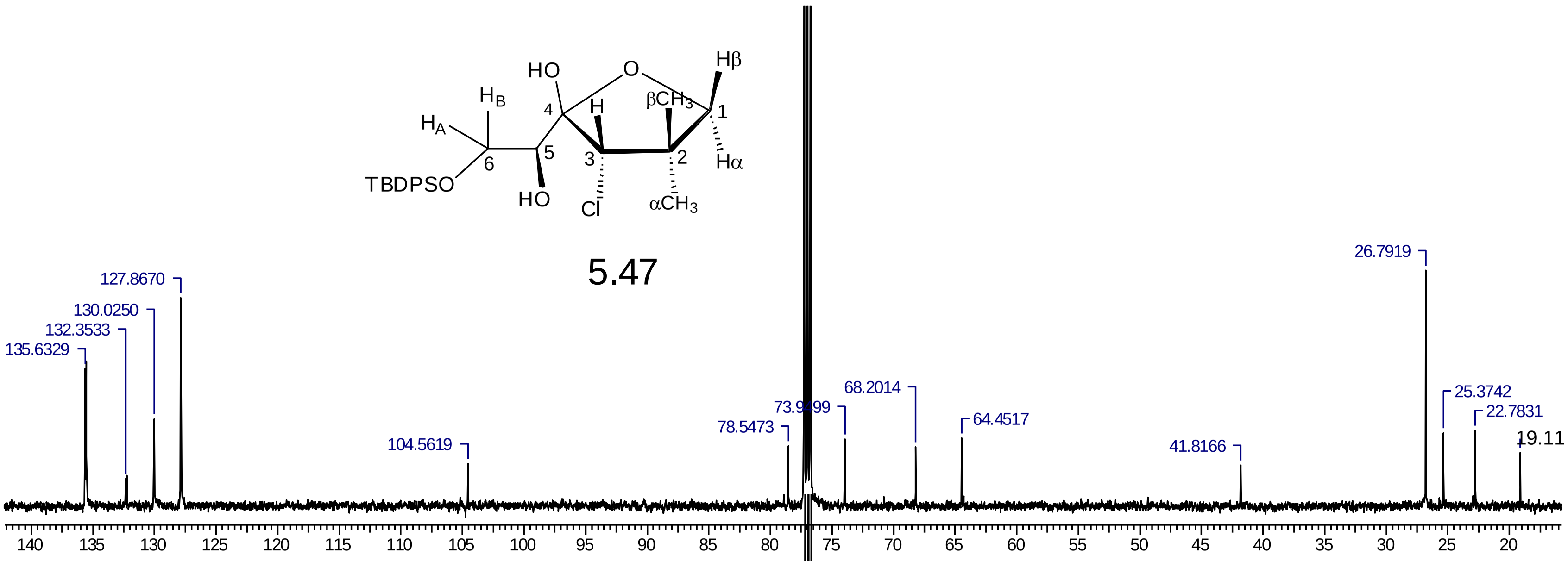
gHMBC


HO
Cl
βCH3
Hβ
HB
HA
4
H
αCH3
O
Hα
TBDPSO
6
H
5
OH

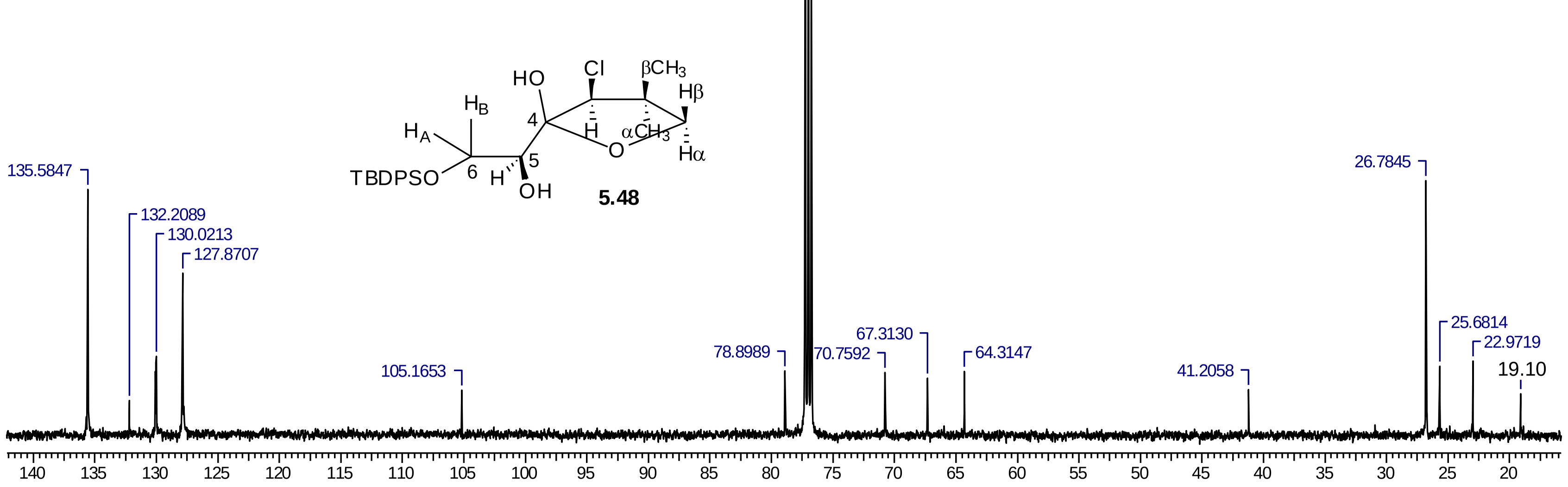
5.48


4-OH
H3
H1α
5-OH
C4
1Hβ
6HB
6HA
104
105
106
107
5.0
4.5
4.0
3.5
3.0
2.5

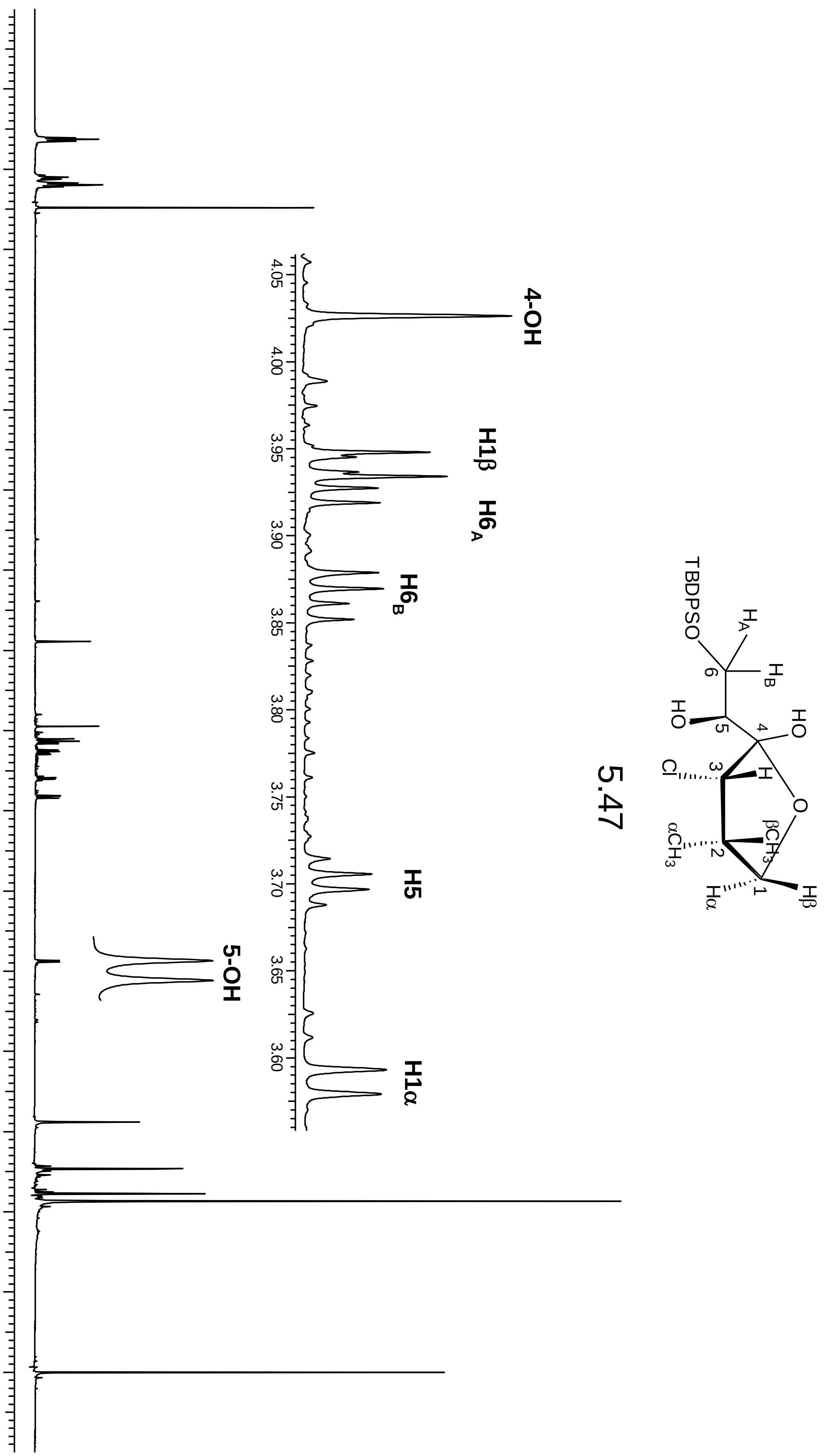
^{13}C NMR spectra



5.47

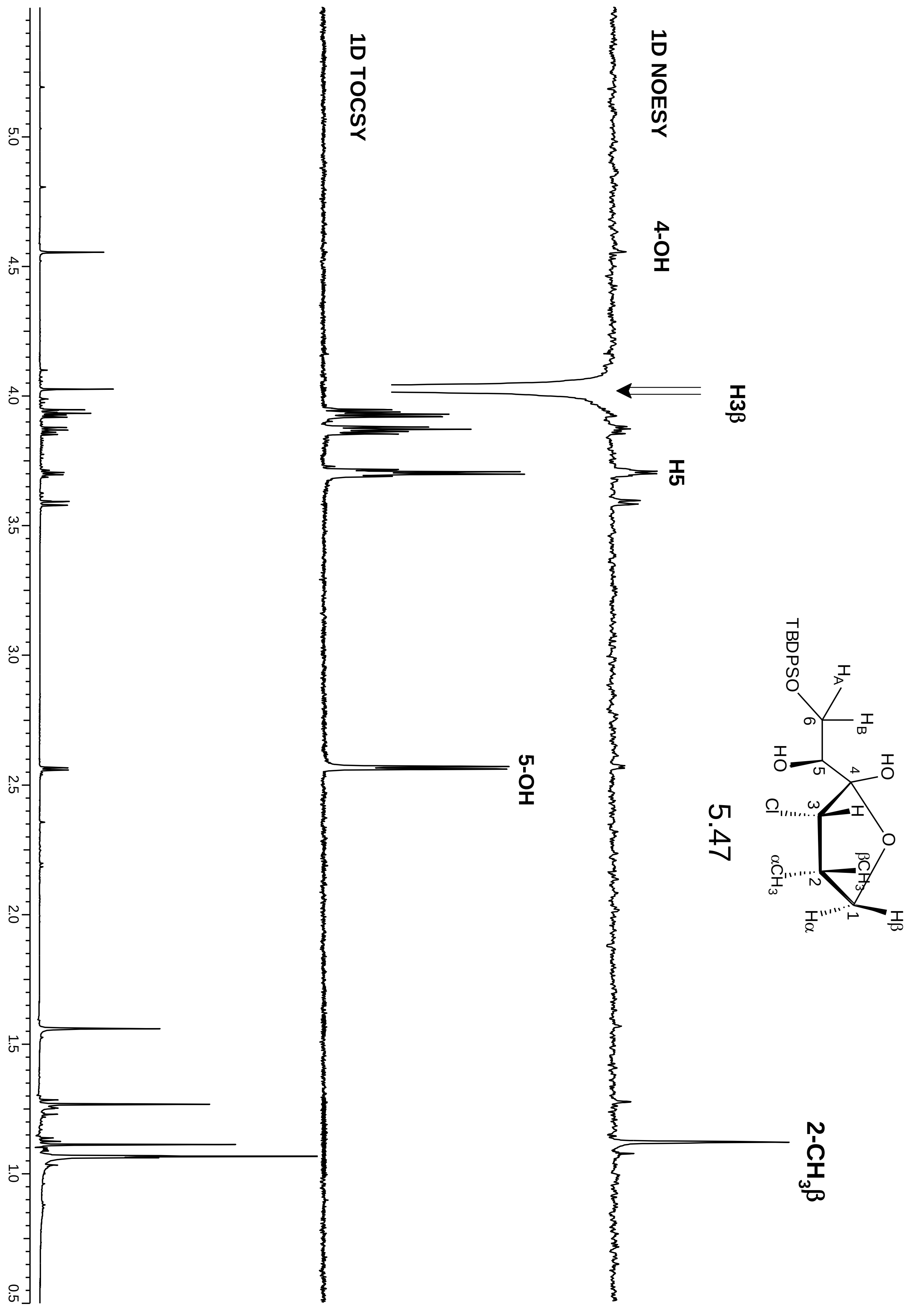


5.48

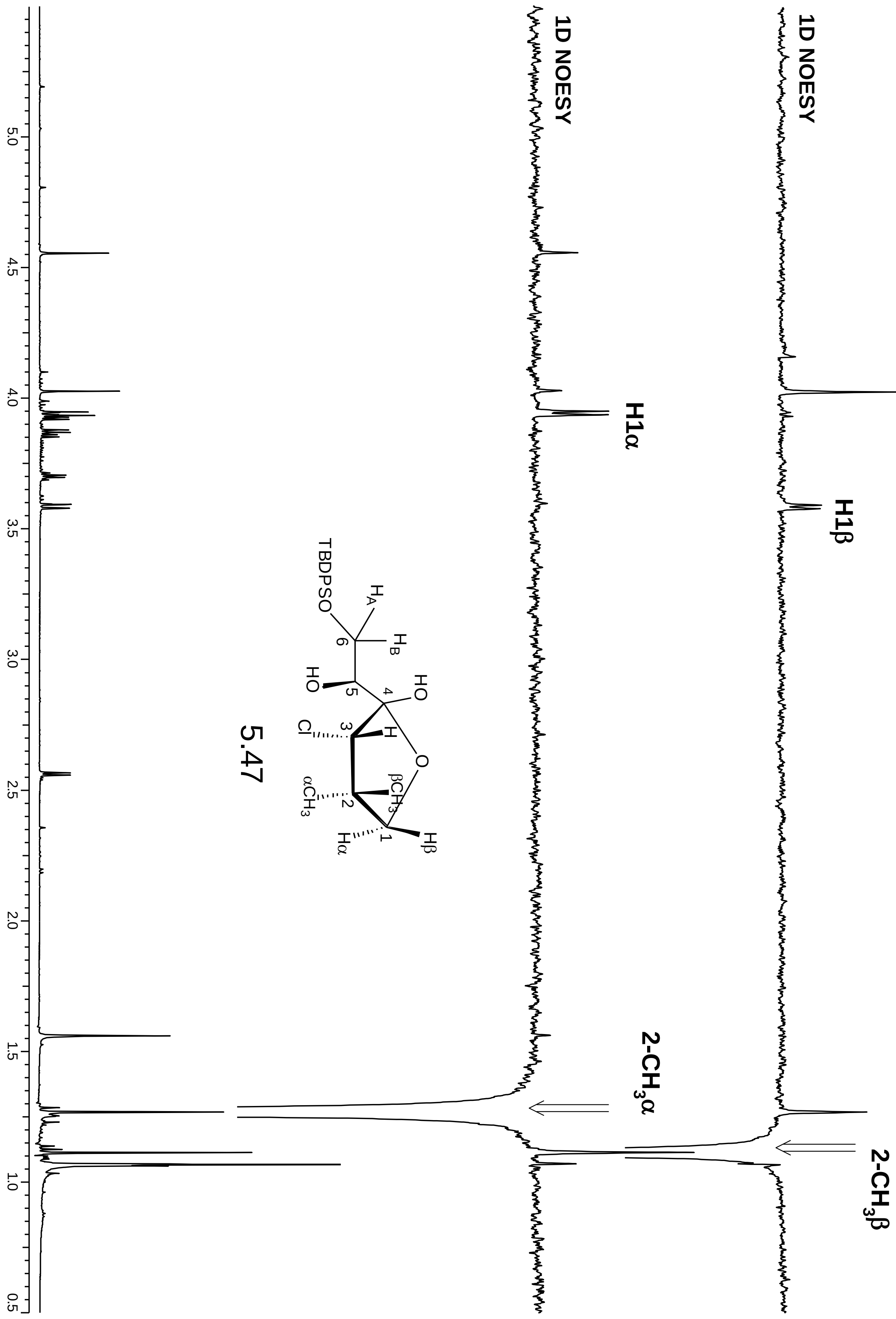


5.47

TBDPSO
H_A
H_B
6
HO
5
4
HO
O
3
H
Cl
2
βCH3
αCH3
1
Hβ
Hα
4-OH
H1β
H6A
H6B
H5
5-OH
H1α
4.05
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

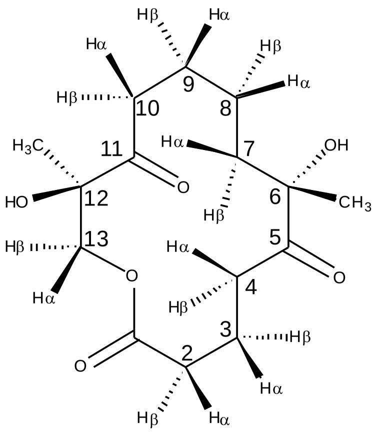

1D NOESY
4-OH
H3β
H5
2-CH$_3$β
1D TOCSY
5-OH
TBDPSO
H$_A$
H$_B$
6
5
4
3
2
1
HO
HO
H
O
Cl
βCH$_3$
αCH$_3$
Hα
Hβ
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

5.47


1D NOESY
H3β
H1β
2-CH$_3$β
1D NOESY
H1α
2-CH$_3$α
TBDPSO
H$_A$
H$_B$
6
HO
5
HO
4
O
3
H
Cl
2
βCH$_3$
αCH$_3$
1
Hβ
Hα
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

5.47

^{1}H and ^{13}C NMR Chemical shifts (δ/ppm) and Coupling constants (J/Hz)



Hβ
Hα
Hα
Hβ
9
Hβ
Hα
10
8
H$_3$C
11
Hα
7
OH
HO
12
O
6
CH$_3$
Hβ
Hβ
13
5
Hα
O
4
O
Hα
Hβ
3
Hβ
2
O
Hα
Hβ
Hα

H3α/H3β——1.91–1.95

H2α/H2βH/4β—2.31–2.40

H4α————2.62

6–CH$_3$———1.35

6–OH———3.94

H7α———1.74

H7β———1.52

H8α———1.62

H8β———0.54

H9α———1.25

H9β———2.19

H10α——2.56

H10β———2.50

12–CH$_3$——1.36

12–OH——4.18

H13α———4.43

H13β———4.16

^3J(H4α–H3α) = 6.22

^3J(H4α–H3β) = 9.41

^2J(H4α–H4β) = 18.40

^3J(H3α–H2α) = 5.45

^2J(H7α–H7β) = 13.91

^2J(H8α–H8β) = 14.02

^3J(H8α–H7α) = 3.27

^3J(H8α–H7β) = 12.65

^3J(H8β–H7α) = 13.12

^3J(H8β–H7β) = 3.67

^3J(H9α–H8α) = 3.63

^3J(H9α–H8β) = 9.78

^3J(H9β–H8α) = 6.64

^3J(H9β–H8β) = 9.78

^2J(H13α–H13β) = 11.71

^3J(H10α–H9α) = 2.27

^3J(H10α–H9β) = 12.30

^2J(H10α–H10β) = 18.65

C1------172.39

C2------32.33

C3------18.16

C4-----33.50

C5-----212.98

C6-----80.53

6-CH$_3$----25.88

C7--------39.72

C8--------21.83

C9--------21.61

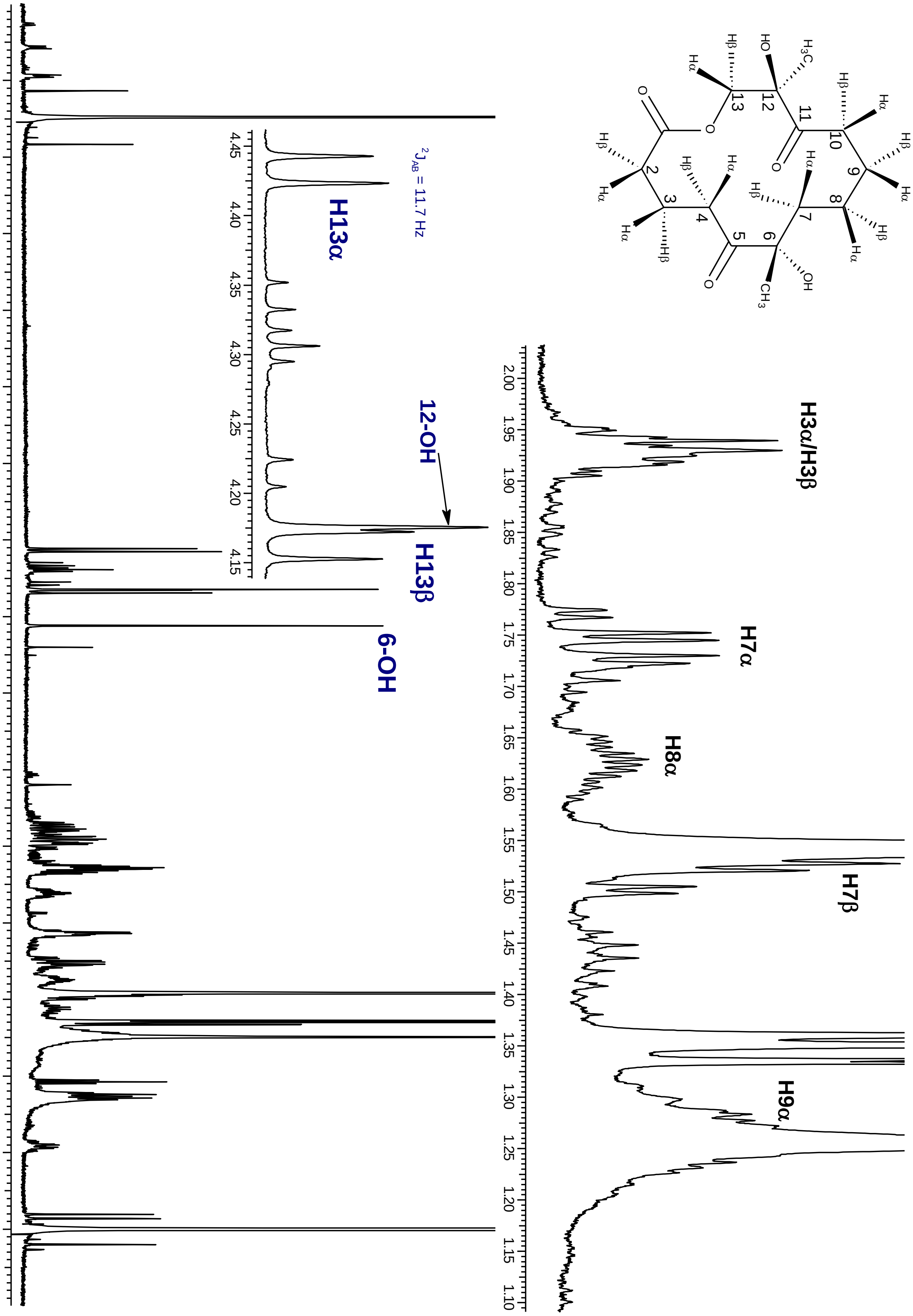
C10------33.59

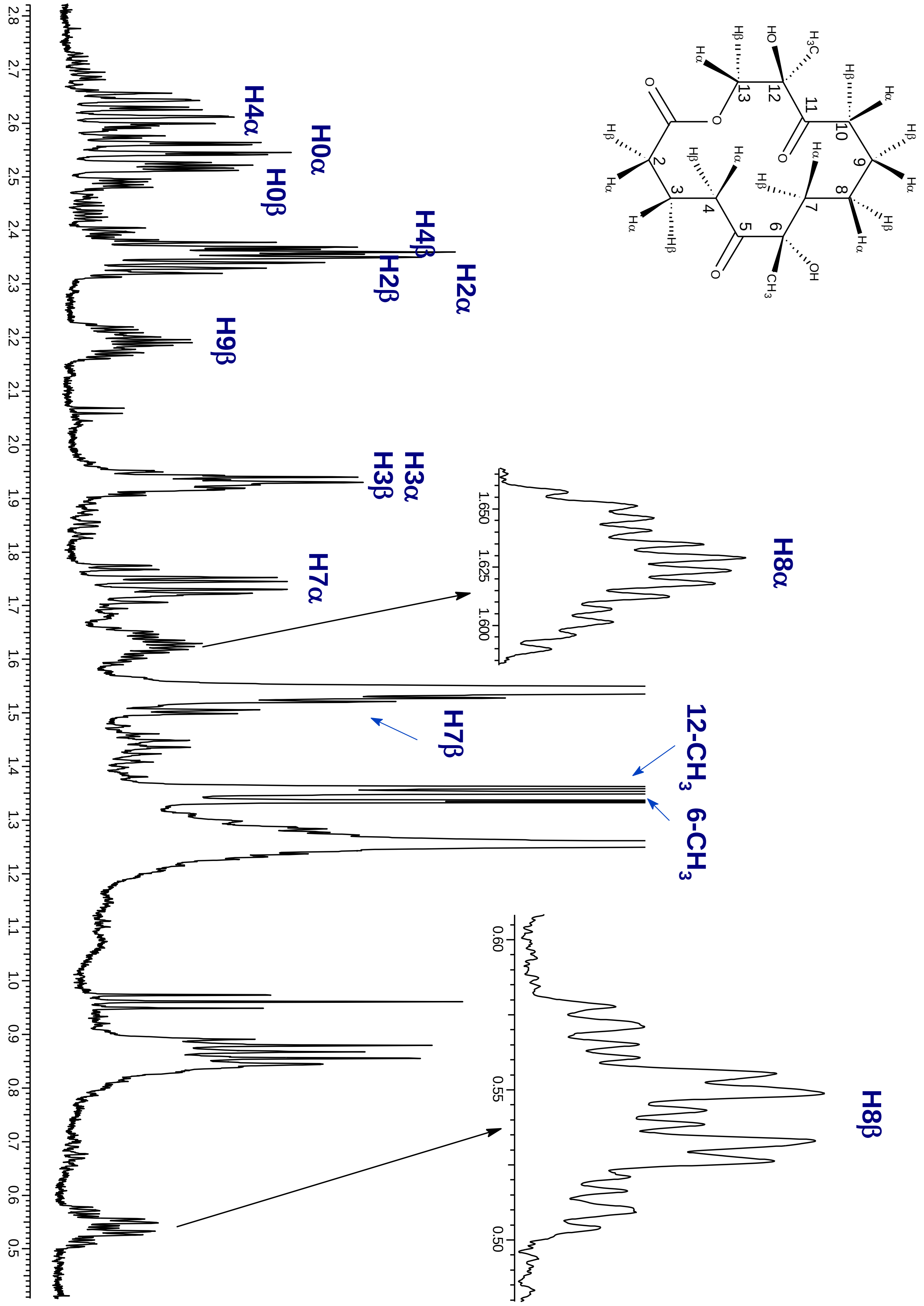
C11------210.01

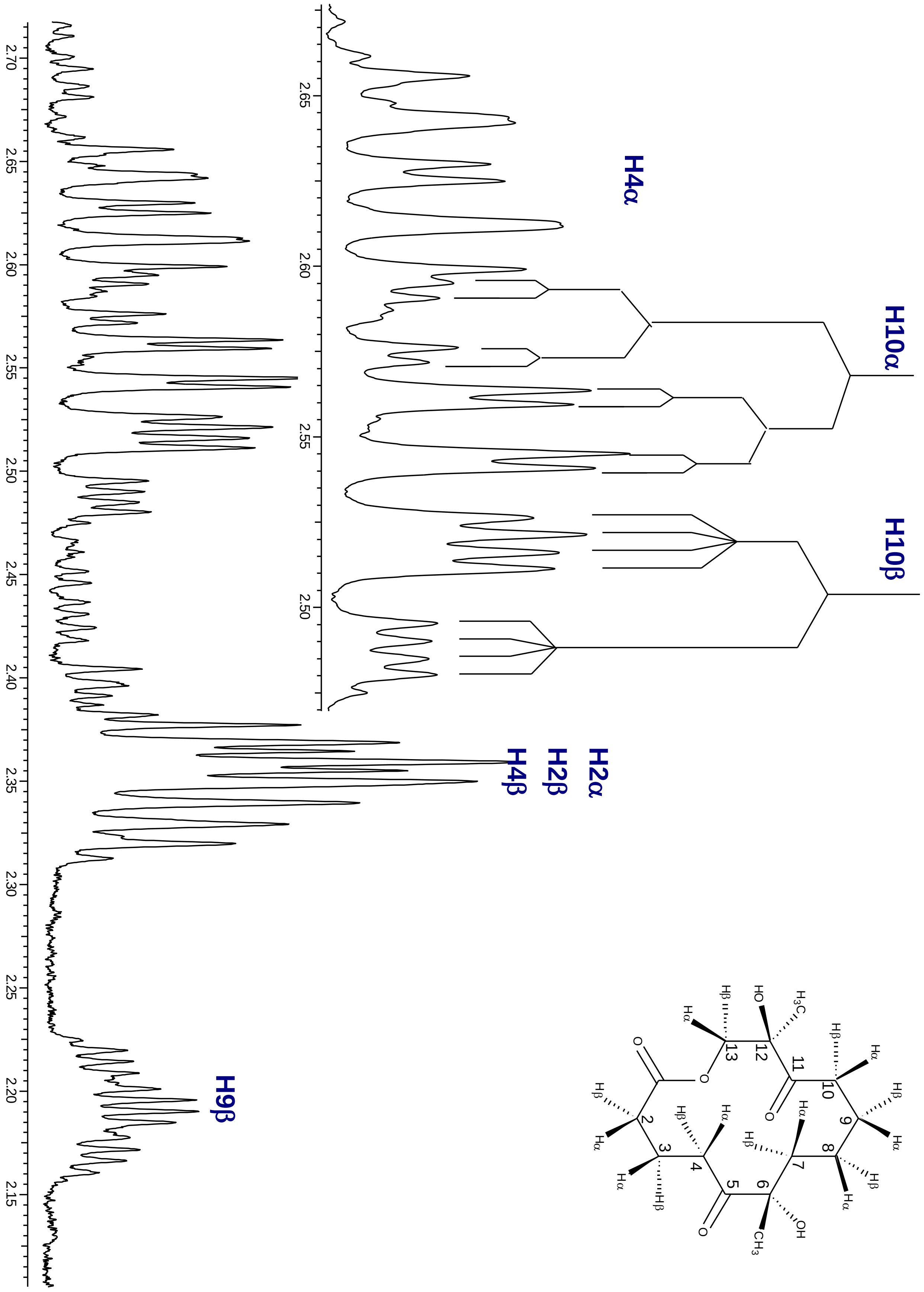
C12------78.44

12-CH$_3$----21.50

C13--------69.66

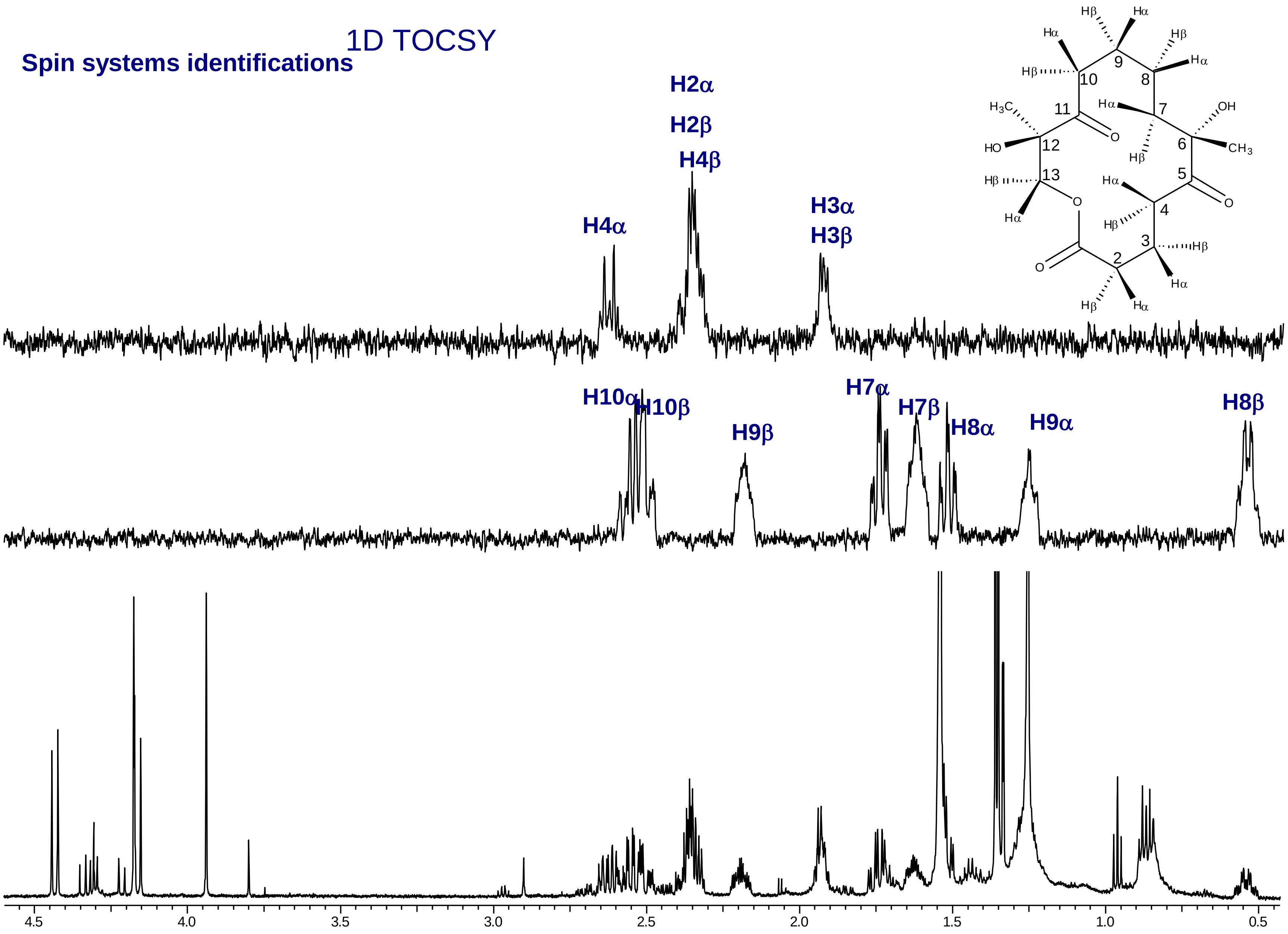

H3α/H3β
H7α
H8α
H7β
H9α
12-OH
H13α
H13β
6-OH
²J_AB = 11.7 Hz
2.00 1.95 1.90 1.85 1.80 1.75 1.70 1.65 1.60 1.55 1.50 1.45 1.40 1.35 1.30 1.25 1.20 1.15 1.10
4.45 4.40 4.35 4.30 4.25 4.20 4.15
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
H3C
HO
OH
CH3
O
Hα
Hβ
1 2 3 4 5 6 7 8 9 10 11 12 13


H4α
H0α
H0β
H4β
H2β
H2α
H9β
H3α
H3β
H7α
H8α
H7β
12-CH3
6-CH3
H8β
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
1.650
1.625
1.600
0.60
0.55
0.50
HO
OH
H3C
CH3
O
Hα
Hβ
2
3
4
5
6
7
8
9
10
11
12
13

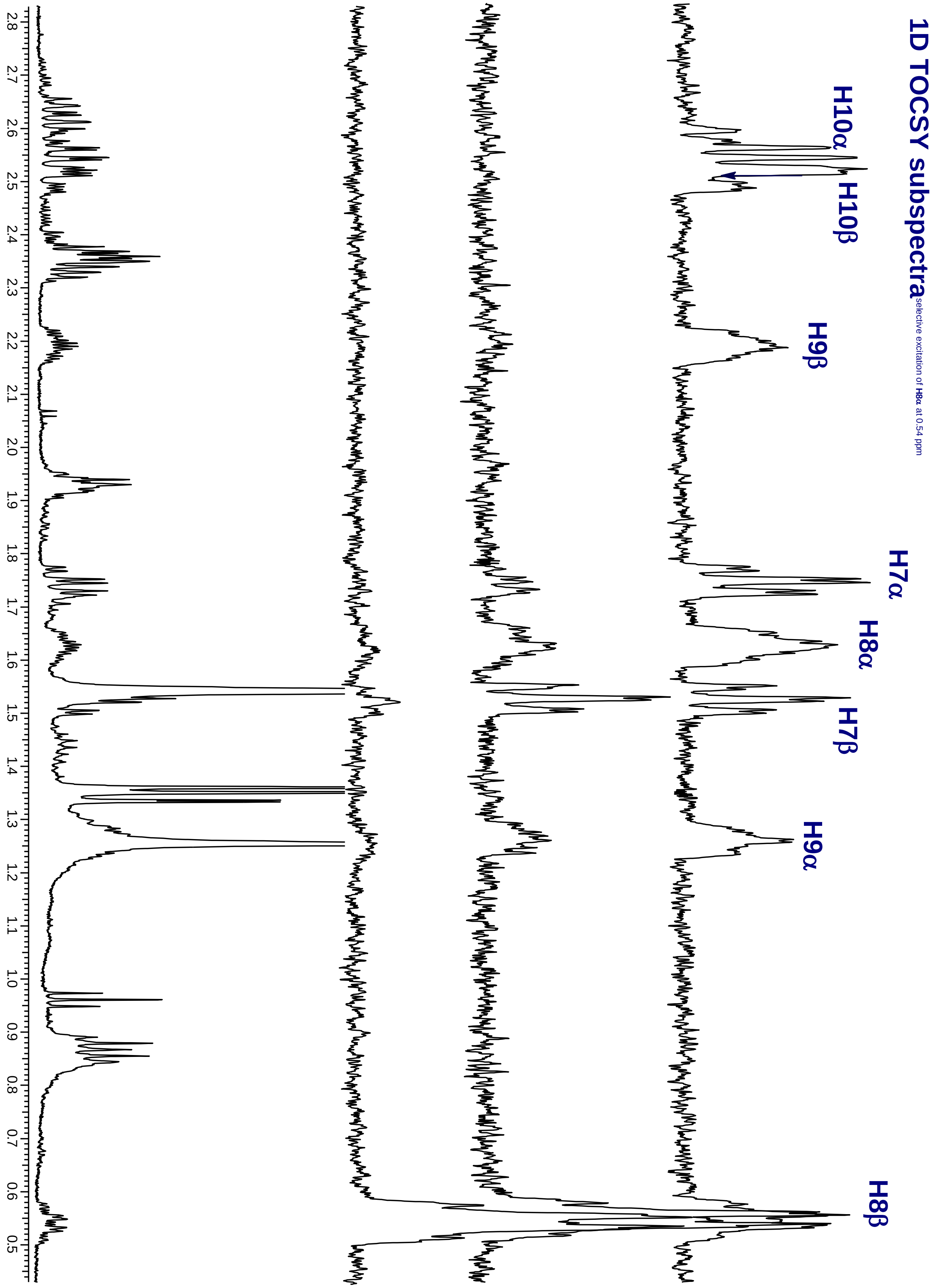

H4α
H10α
H10β
H2α
H2β
H4β
H9β
2.65
2.60
2.55
2.50
2.70
2.65
2.60
2.55
2.50
2.45
2.40
2.35
2.30
2.25
2.20
2.15
1
2
3
4
5
6
7
8
9
10
11
12
13
Hα
Hβ
OH
HO
CH$_3$
H$_3$C
O

1D TOCSY

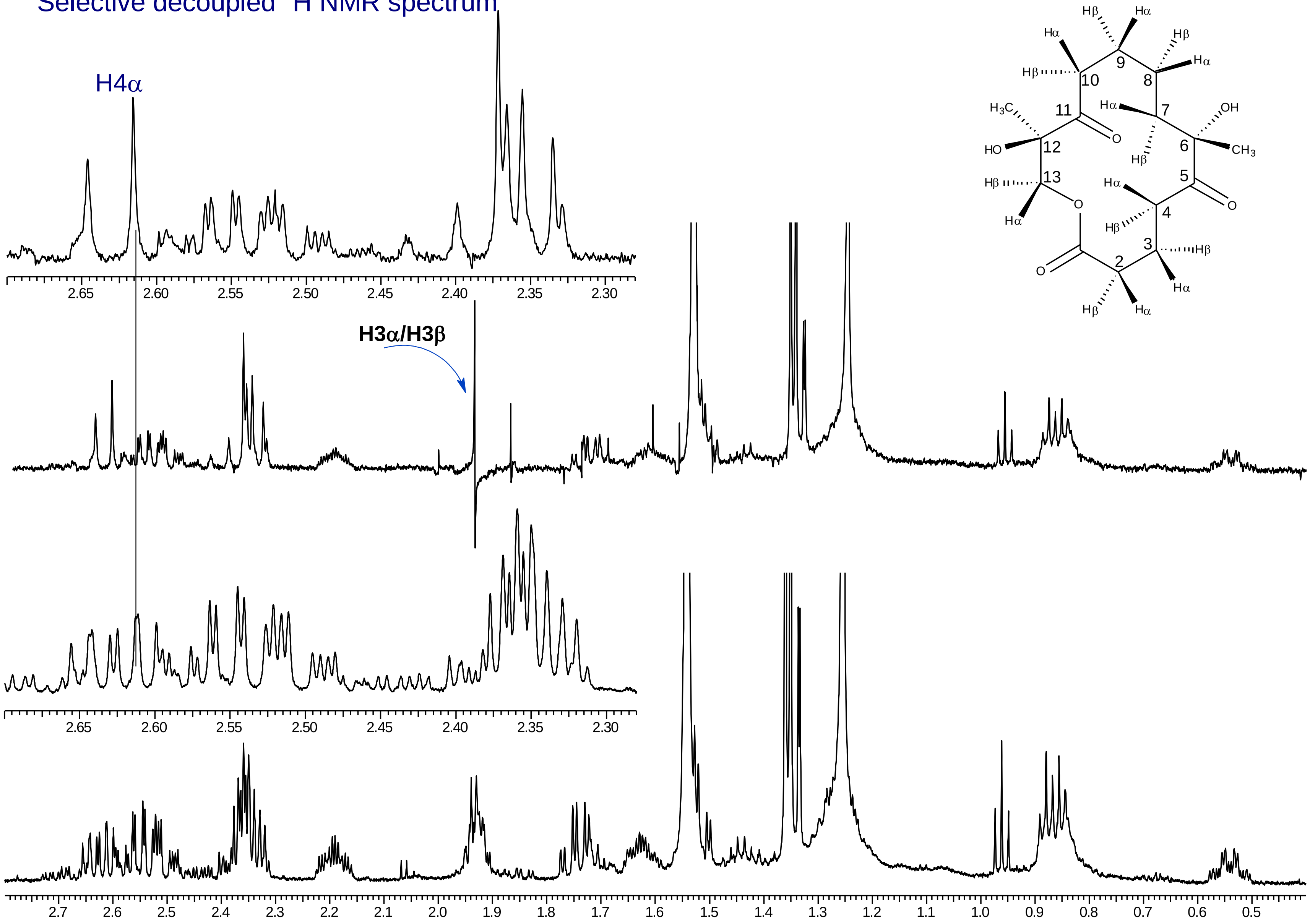
Spin systems identifications

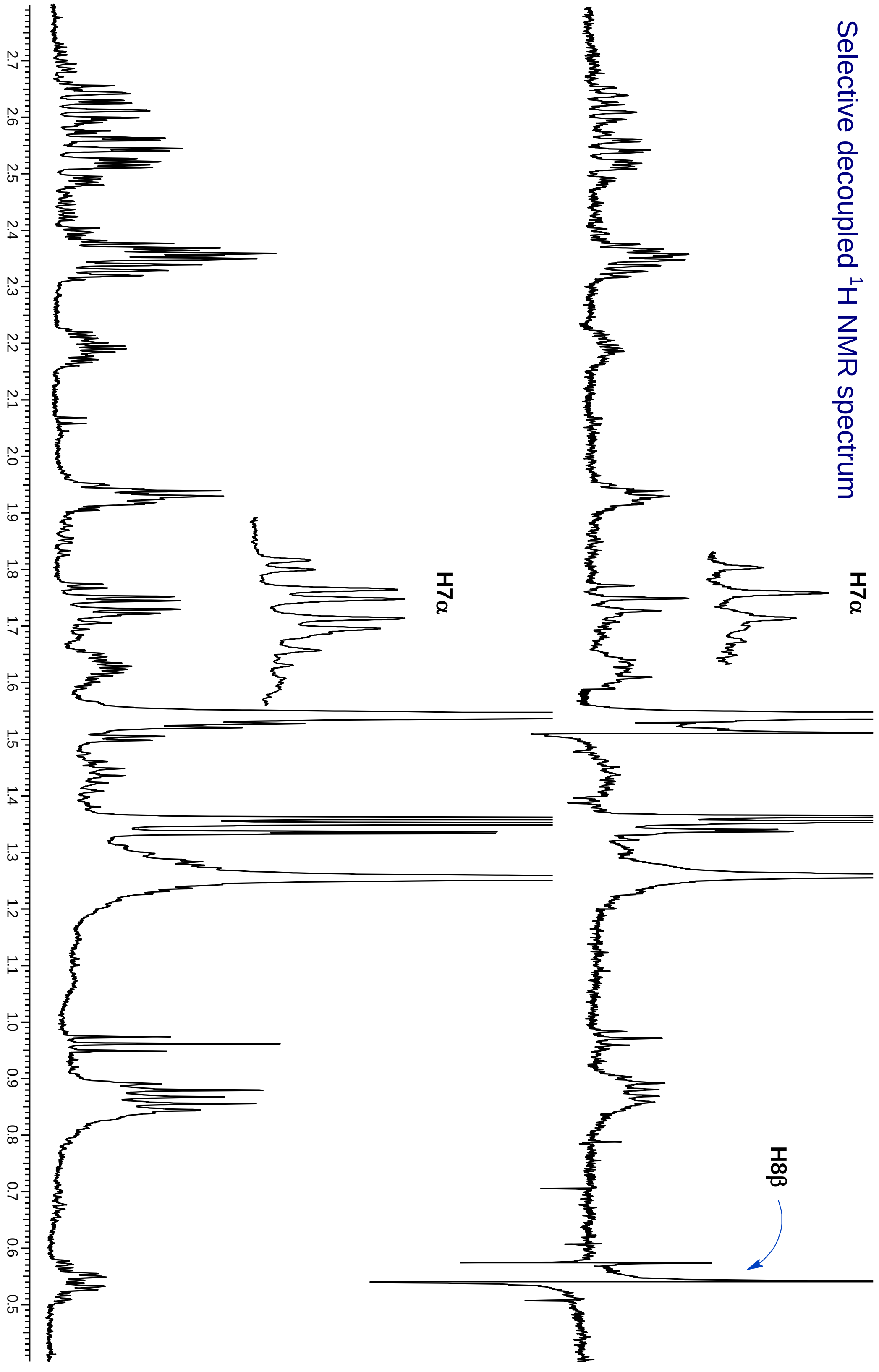


1D TOCSY subspectra selective excitation of **H8α** at 0.54 ppm

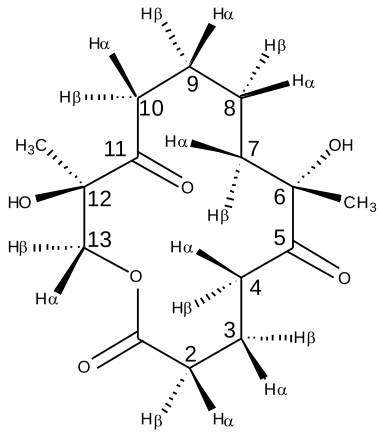


Selective decoupled ^{1}H NMR spectrum





Selective decoupled ^{1}H NMR spectrum



1
2
3
4
5
6
7
8
9
10
11
12
13
O
OH
HO
CH_3
H_3C
Hα
Hβ

C1------172.39

C2------32.33

C3------18.16

C4-----33.50

C5-----212.98

C6-----80.53

6-CH_3----25.88

C7--------39.72

C8--------21.83

C9--------21.61

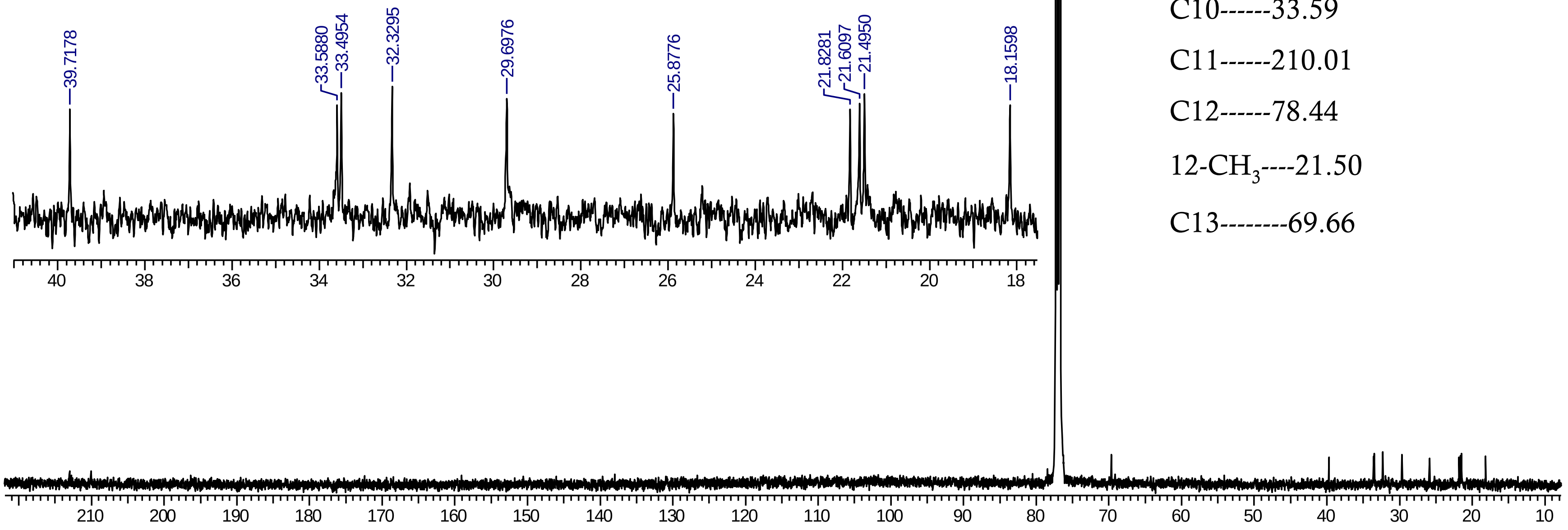
C10------33.59

C11------210.01

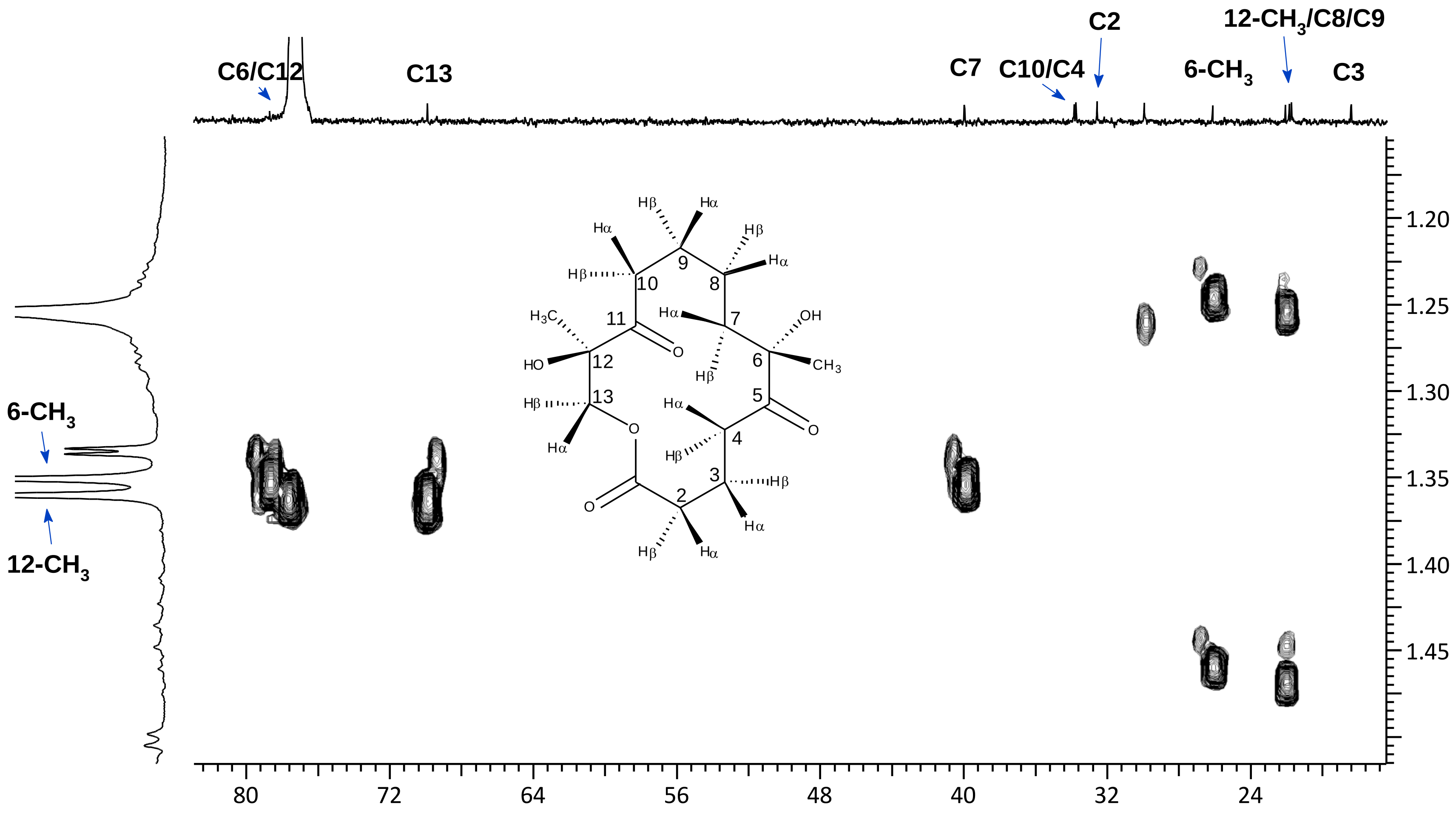
C12------78.44

12-CH_3----21.50

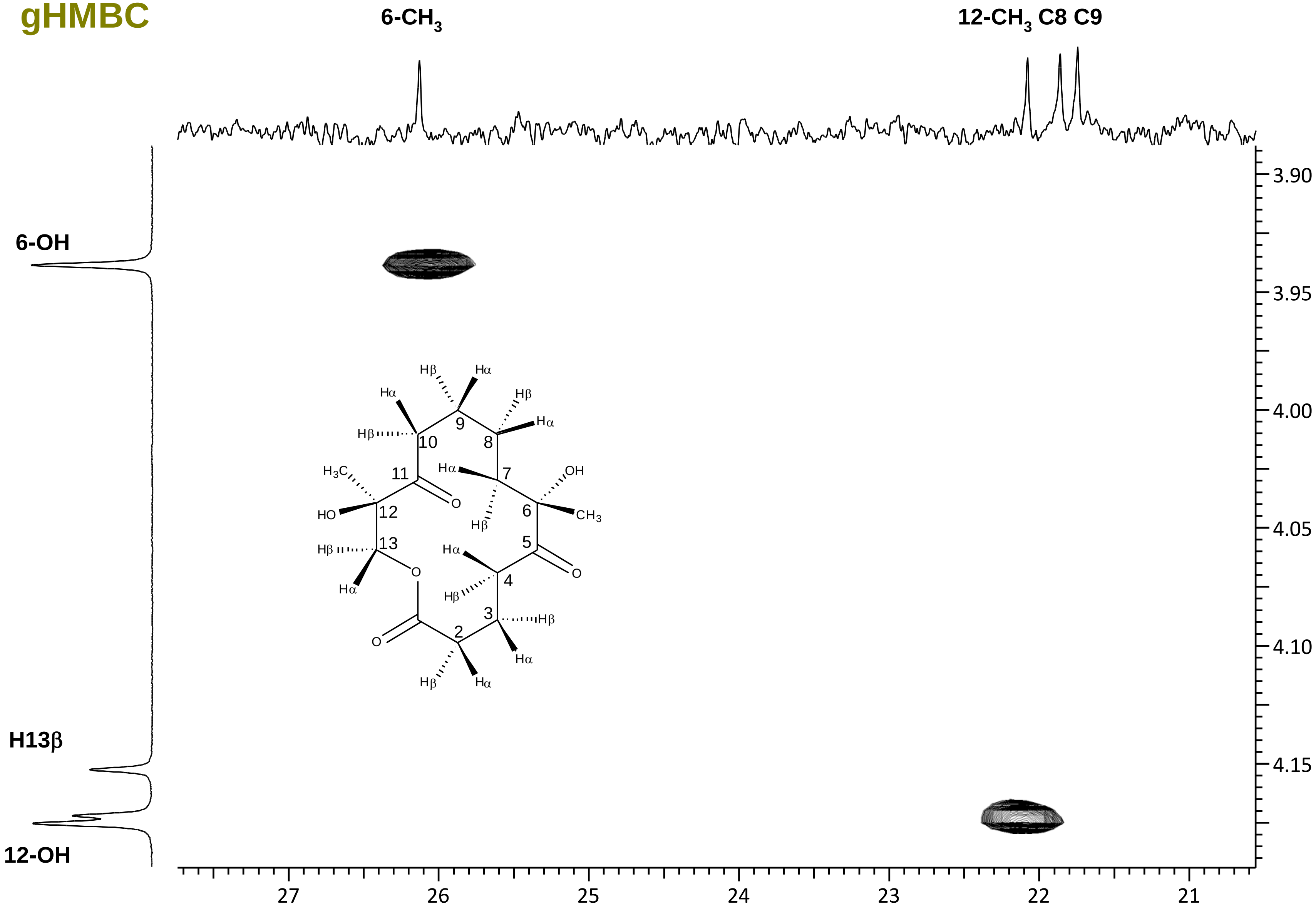
C13--------69.66



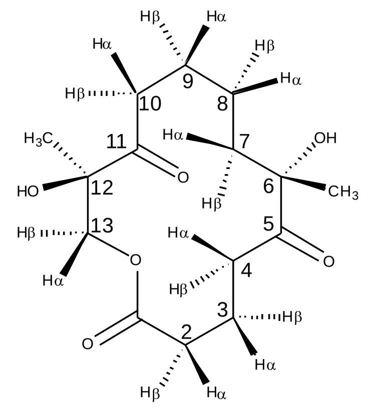
gHMBC

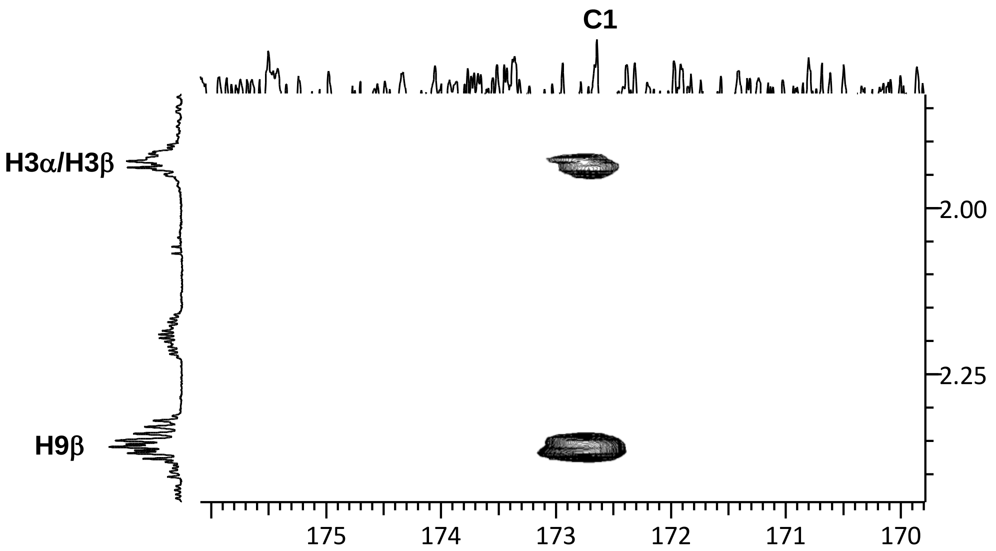

C6/C12
C13
C7
C10/C4
C2
6-CH$_3$
12-CH$_3$/C8/C9
C3
6-CH$_3$
12-CH$_3$
80
72
64
56
48
40
32
24
1.20
1.25
1.30
1.35
1.40
1.45
H3C
HO
OH
CH3
Hα
Hβ
O
2
3
4
5
6
7
8
9
10
11
12
13

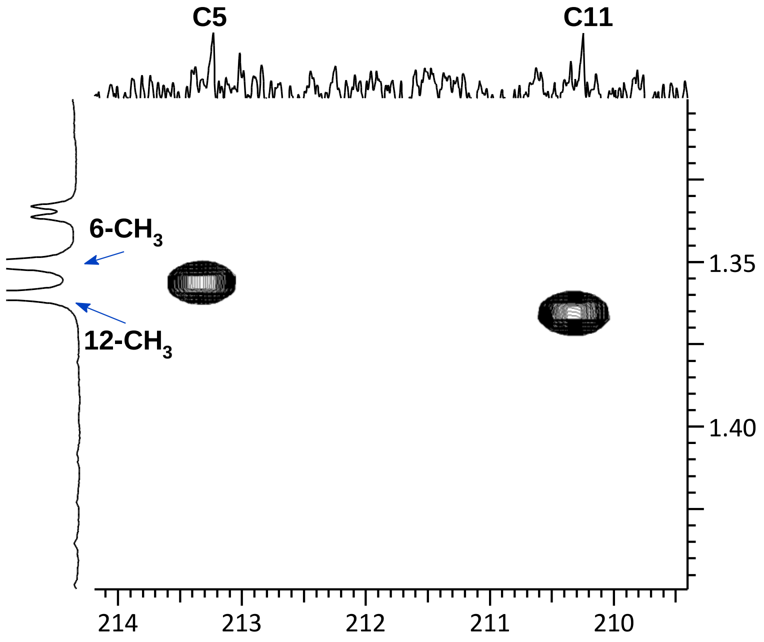
gHMBC

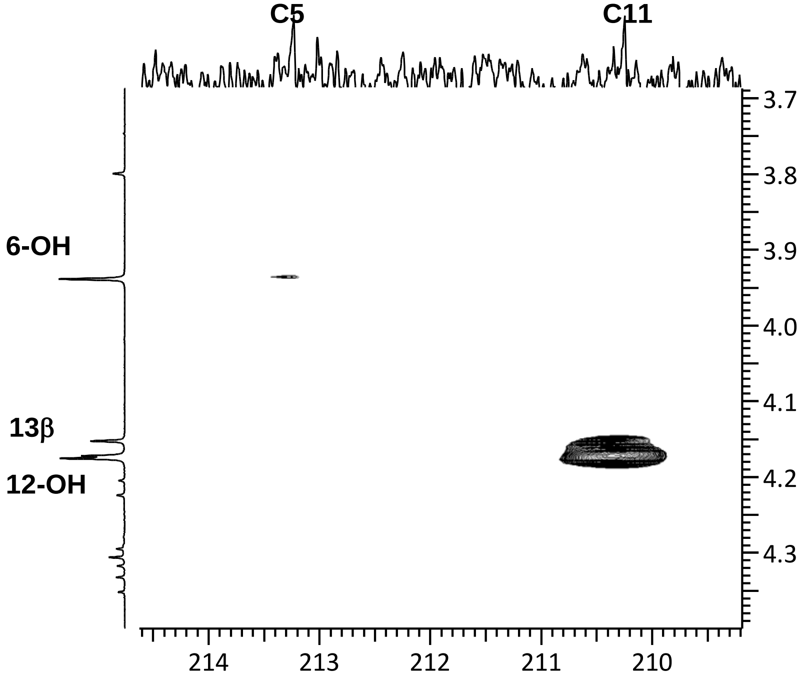

6-CH$_3$
12-CH$_3$ C8 C9
6-OH
H13β
12-OH
3.90
3.95
4.00
4.05
4.10
4.15
27
26
25
24
23
22
21
1
2
3
4
5
6
7
8
9
10
11
12
13
O
OH
CH$_3$
HO
H$_3$C
Hα
Hβ

gHMBC

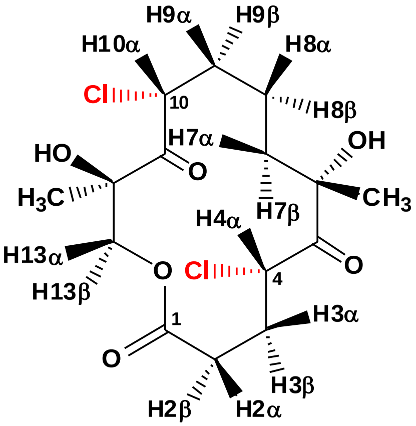

Hβ
Hα
Hα
Hβ
Hβ
Hα
10
9
8
H3C
11
Hα
7
OH
HO
12
O
Hβ
6
CH3
13
5
Hβ
Hα
O
O
4
Hα
Hβ
3
Hβ
O
2
Hα
Hβ
Hα


C1
H3α/H3β
H9β
2.00
2.25
175
174
173
172
171
170


C5
C11
6-CH3
12-CH3
1.35
1.40
214
213
212
211
210


C5
C11
6-OH
13β
12-OH
3.7
3.8
3.9
4.0
4.1
4.2
4.3
214
213
212
211
210

^{1}H and ^{13}C NMR Chemical shifts (δ/ppm) and Coupling constants (J/Hz)


H10α
H9α
H9β
H8α
Cl
10
H8β
H7α
OH
HO
O
H$_3$C
CH$_3$
H4α
H7β
H13α
O
Cl
4
O
H13β
H3α
1
O
H3β
H2β
H2α

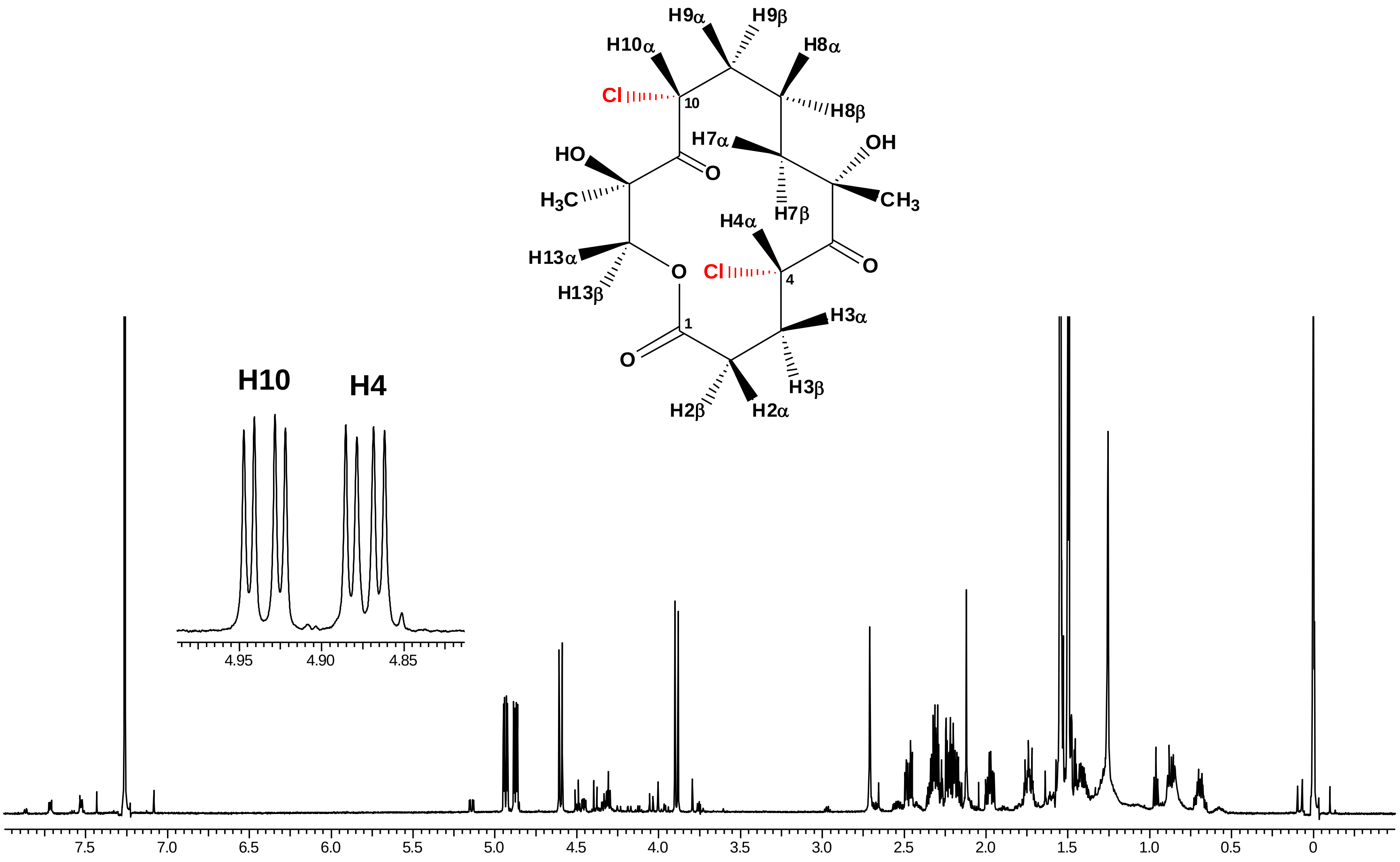
H2α————2.21
H2β————2.47
H3α————2.32
H3β————2.29
H4—————4.87
6–CH$_3$———1.49
6–OH———2.11
H7α———1.47
H7β———1.97
H8α———0.69
H8β———1.41
H9α———1.73
H9β———2.18
H10———4.93
12–CH$_3$——1.49
12–OH——2.71
H13α———3.88
H13β———4.59

C1------171.40
C2------30.84
C3------27.63
C4-----53.96
C5-----209.80
C6-----80.48
6-CH$_3$----29.85
C7--------41.62
C8--------21.67
C9--------33.20
C10------53.41
C11------207.54
C12------79.10
12-CH$_3$----24.32
C13--------71.43

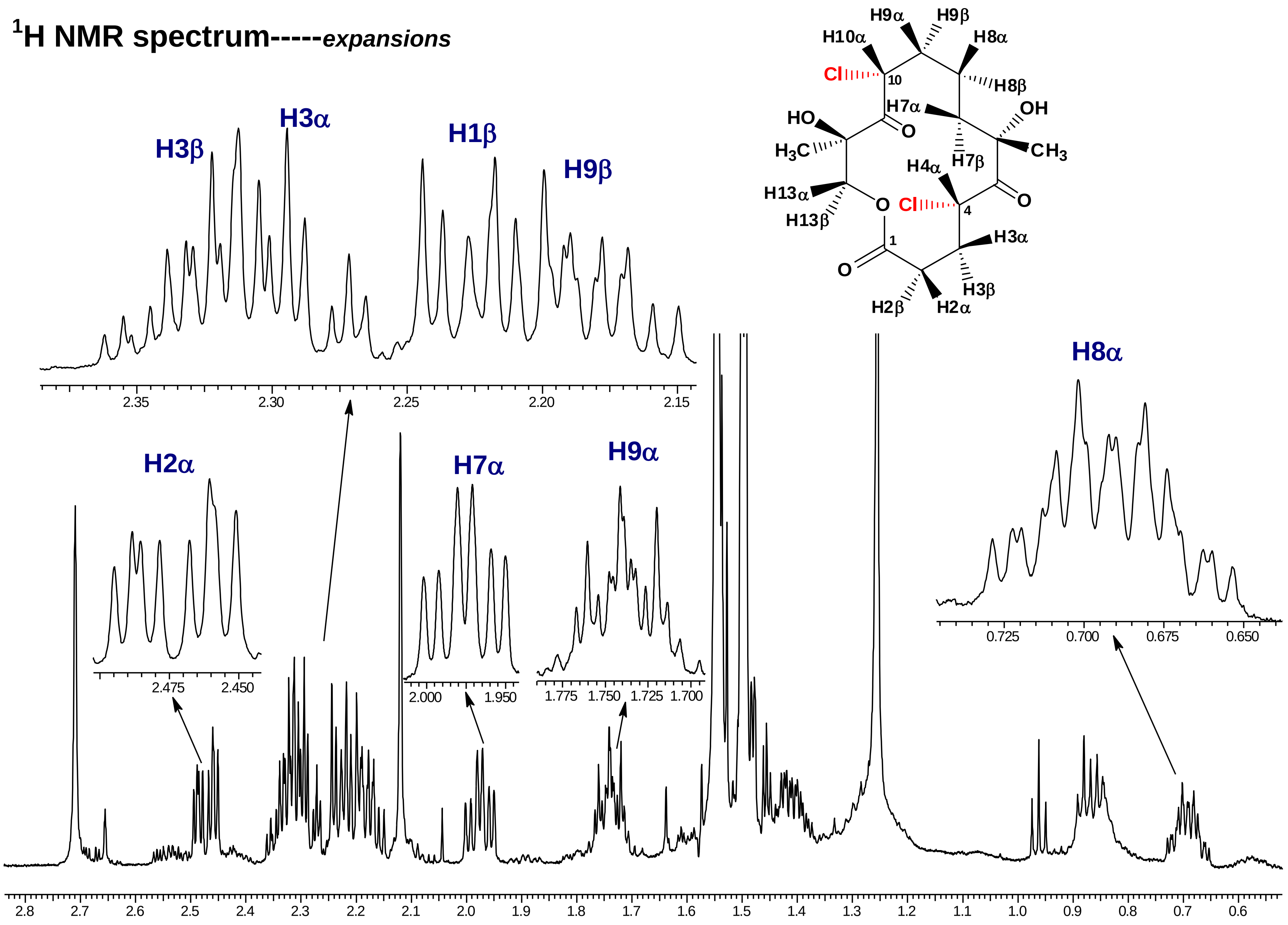
^3J(H4α–H3α) = 3.85
^3J(H4α–H3β) = 10.54
^2J(H3α–H3β) = 14.11
^3J(H3α–H2α) = 5.45
^3J(H3α–H2β) = 10.92
^2J(H2α–H2β) = 16.53
^3J(H2α–H3β) = 4.07
^3J(H2β–H3β) = 3.63
^3J(H10α–H9α) = 3.76
^3J(H10α–H9β) = 11.37
^2J(H9α–H9β) = 12.11

^3J(H9α–H8α) = 3.79
^3J(H9α–H8β) = 3.76
^3J(H9β–H8α) = 11.98
^3J(H9β–H8β) = 5.95
^2J(H8α–H8β) = 11.87
^3J(H8α–H7α) = 5.80
^3J(H8α–H7β) = 12.04
^3J(H8β–H7α) = 12.43
^3J(H8β–H7β) = 3.51
^2J(H7α–H7β) = 12.58
^2J(H13α–H13β) = 11.13

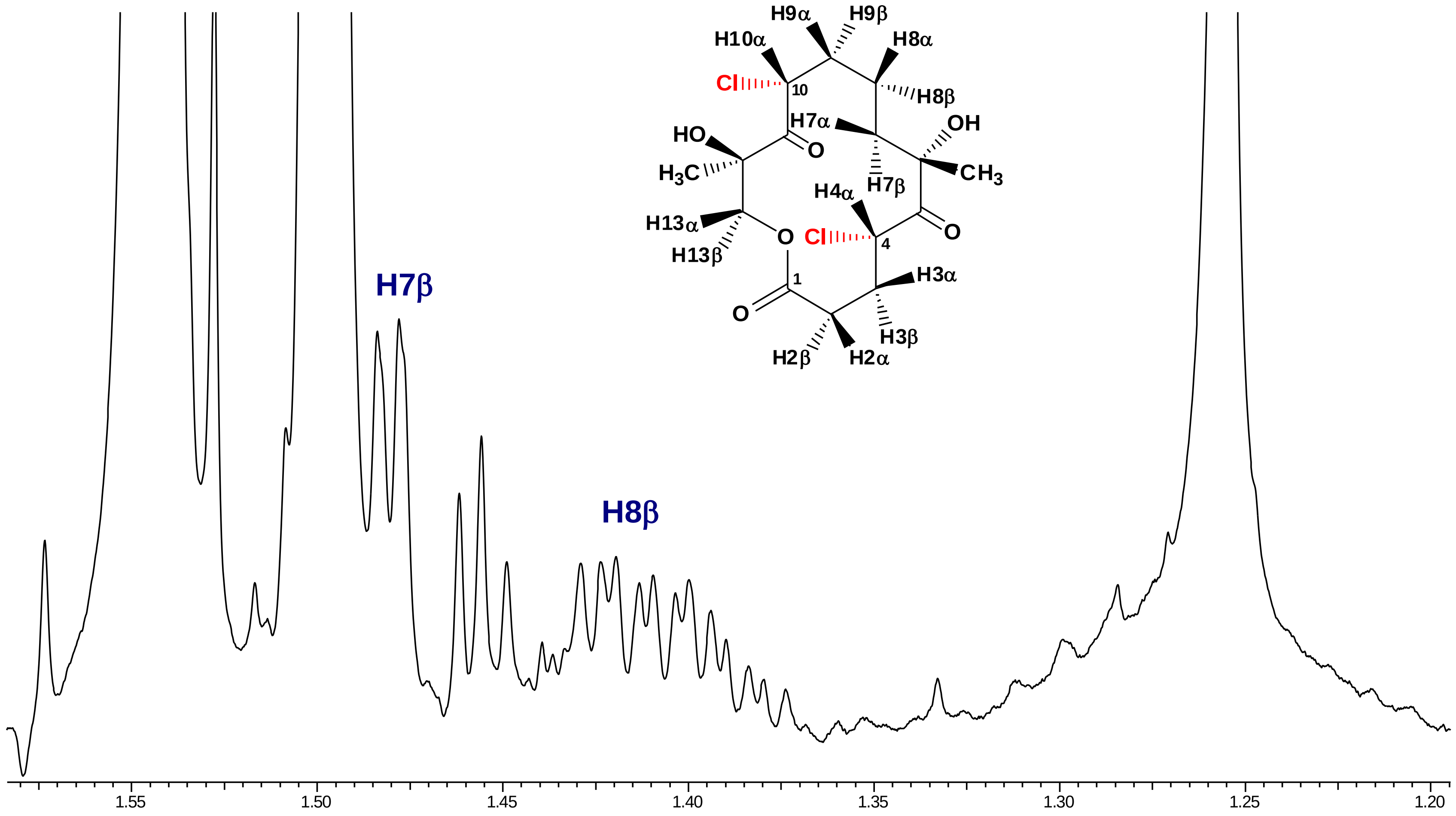
^1H NMR spectrum in $CDCl_3$



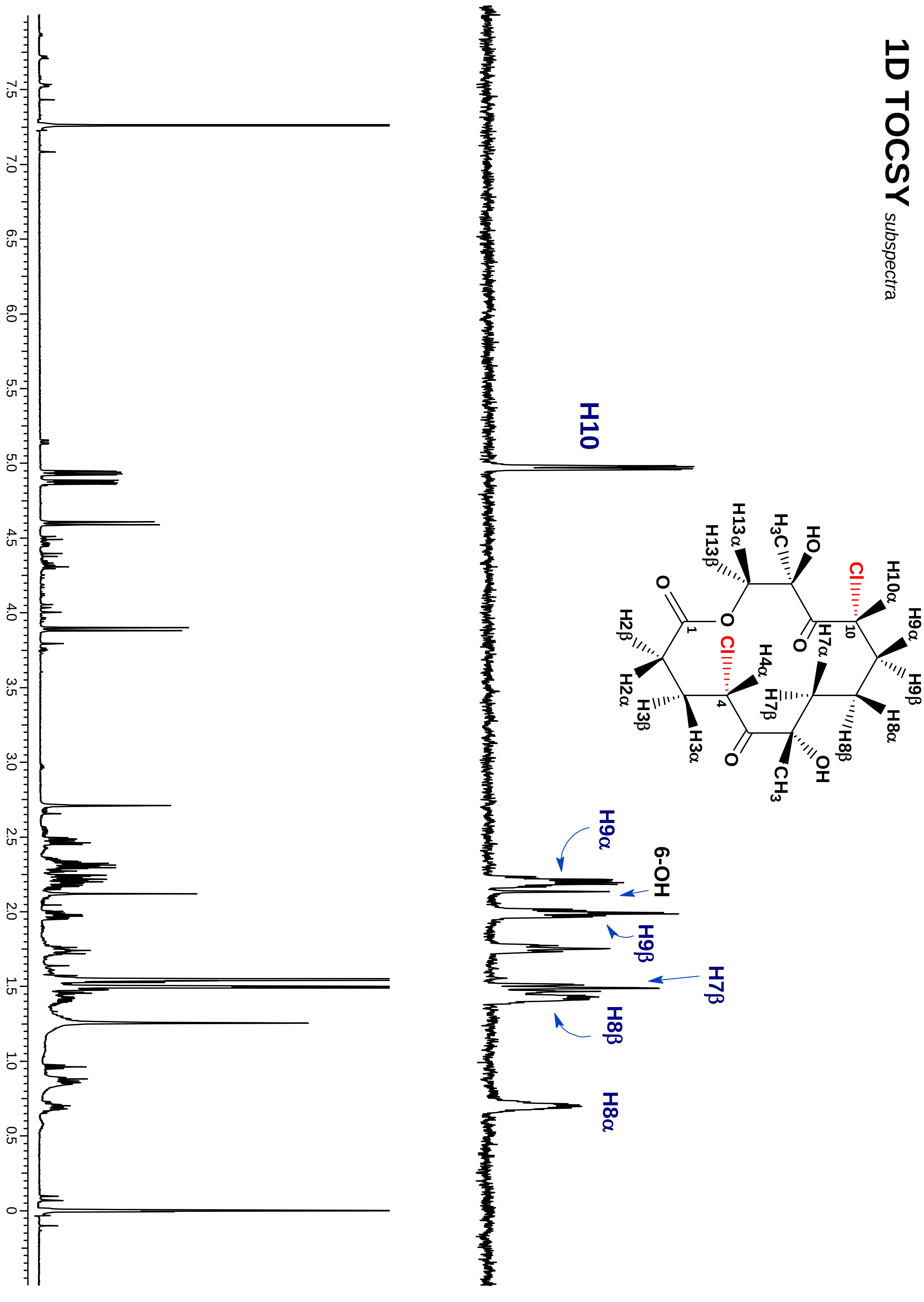
^{1}H NMR spectrum-----*expansions*



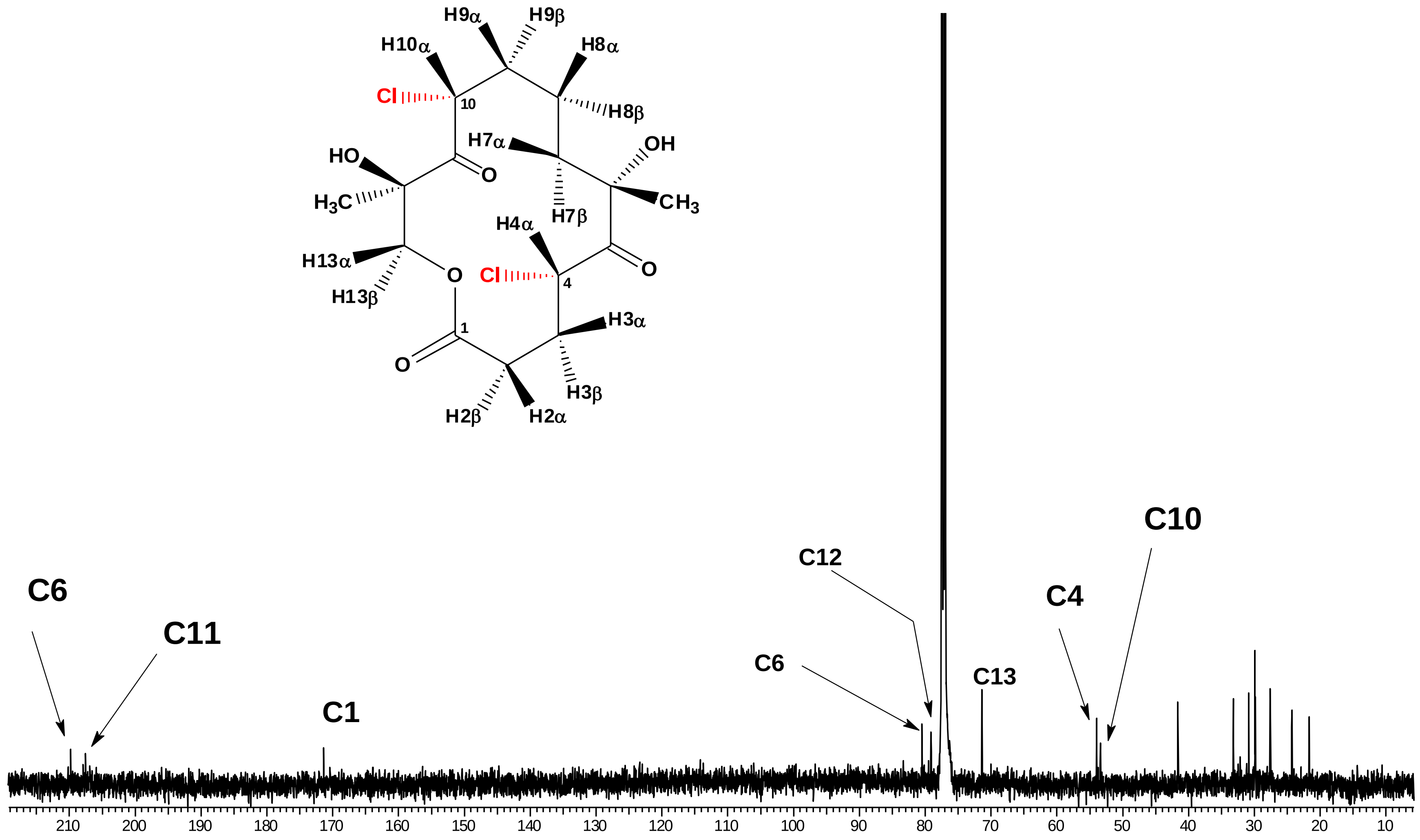
^{1}H NMR spectrum-----*expansion*



1D TOCSY *subspectra*

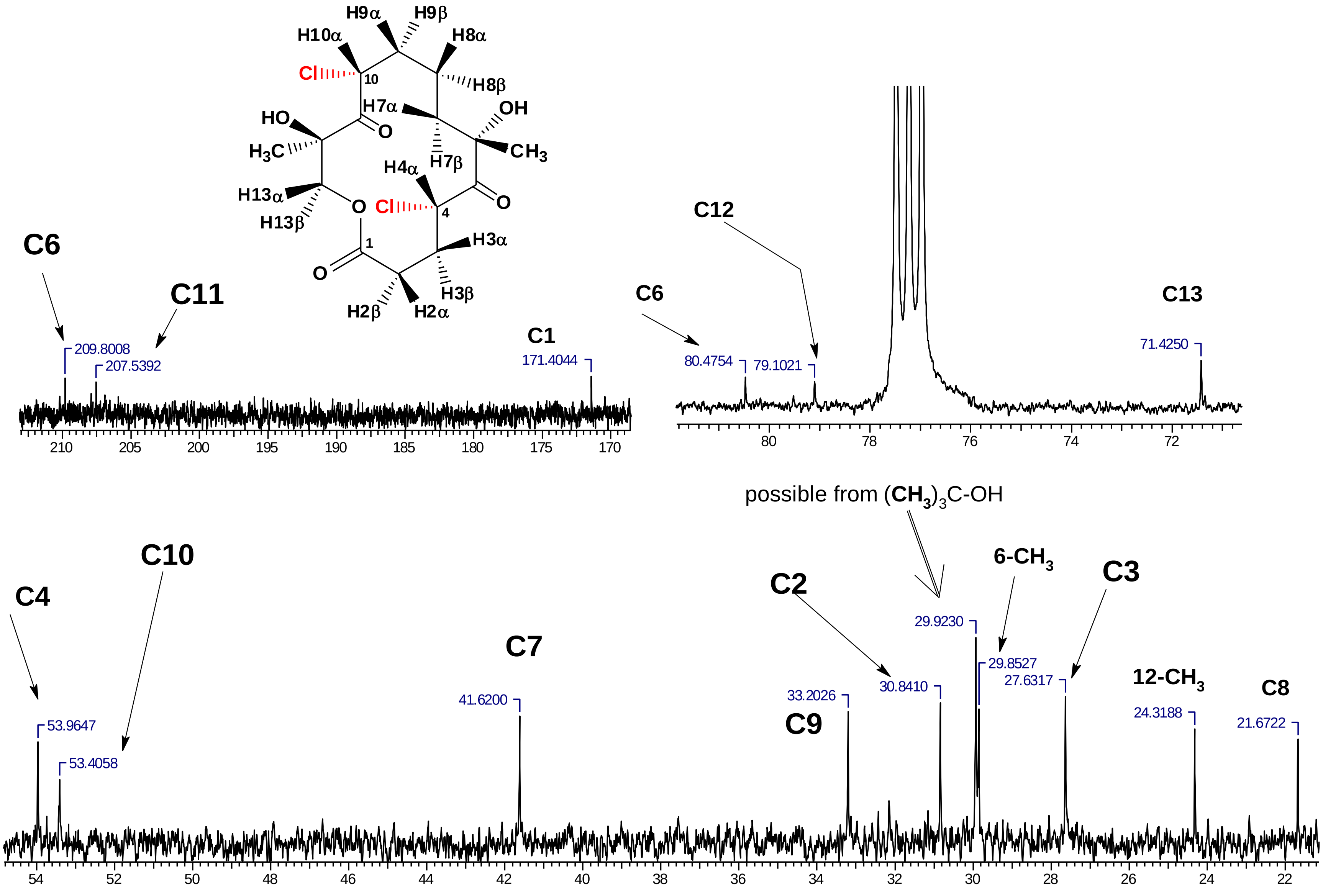


^{13}C NMR spectrum in $CDCl_3$

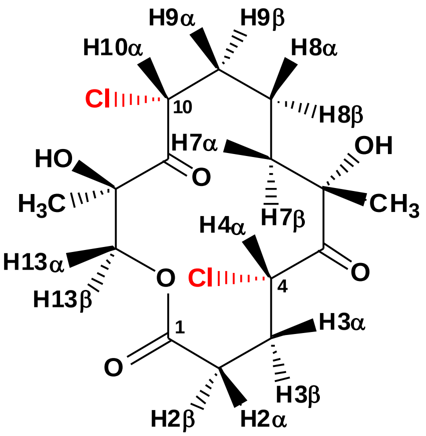


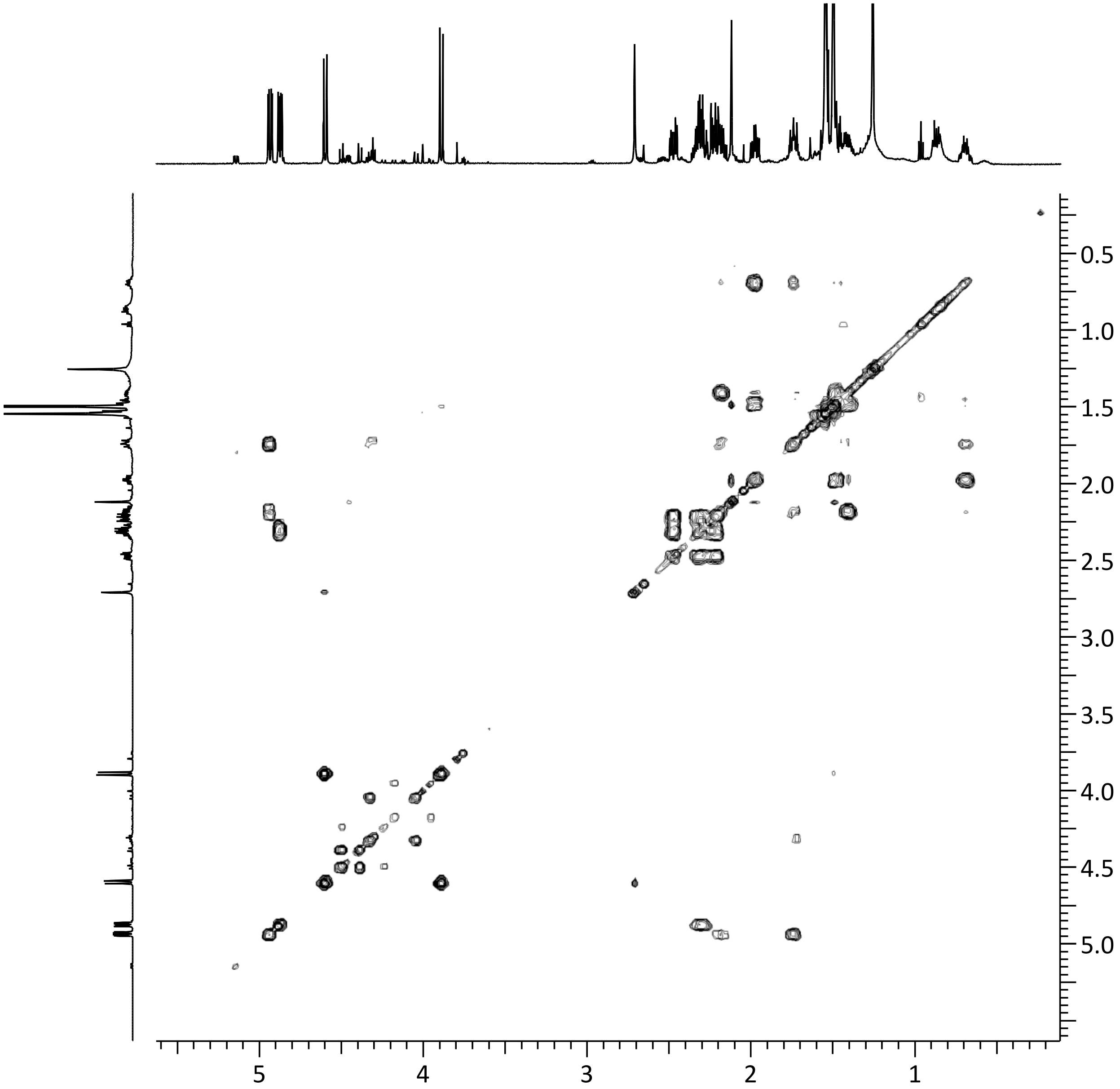
^{13}C NMR spectrum in $CDCl_3$

Expansions

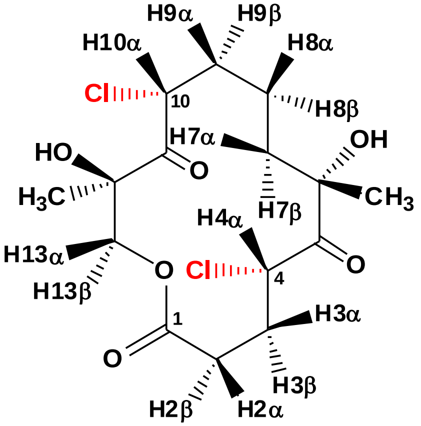


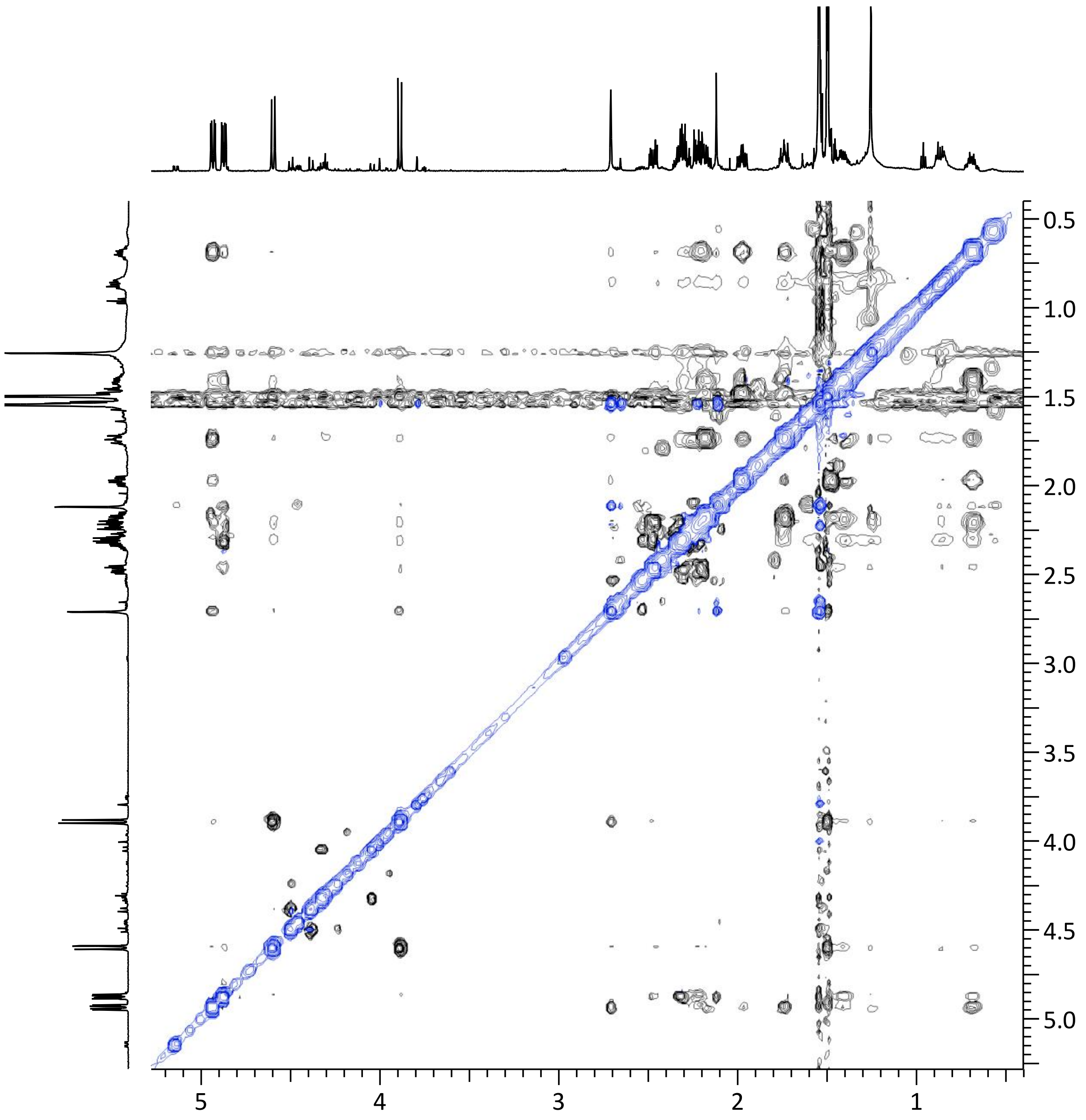
gCOSY


H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
H7β
CH3
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α


0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5
4
3
2
1

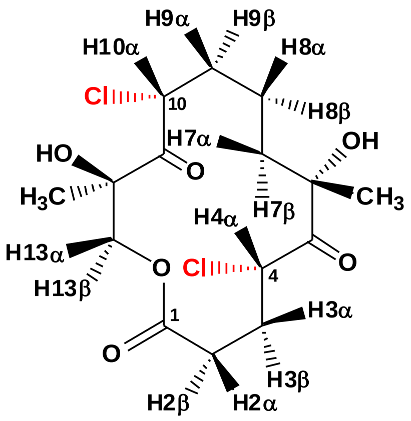
NOESY

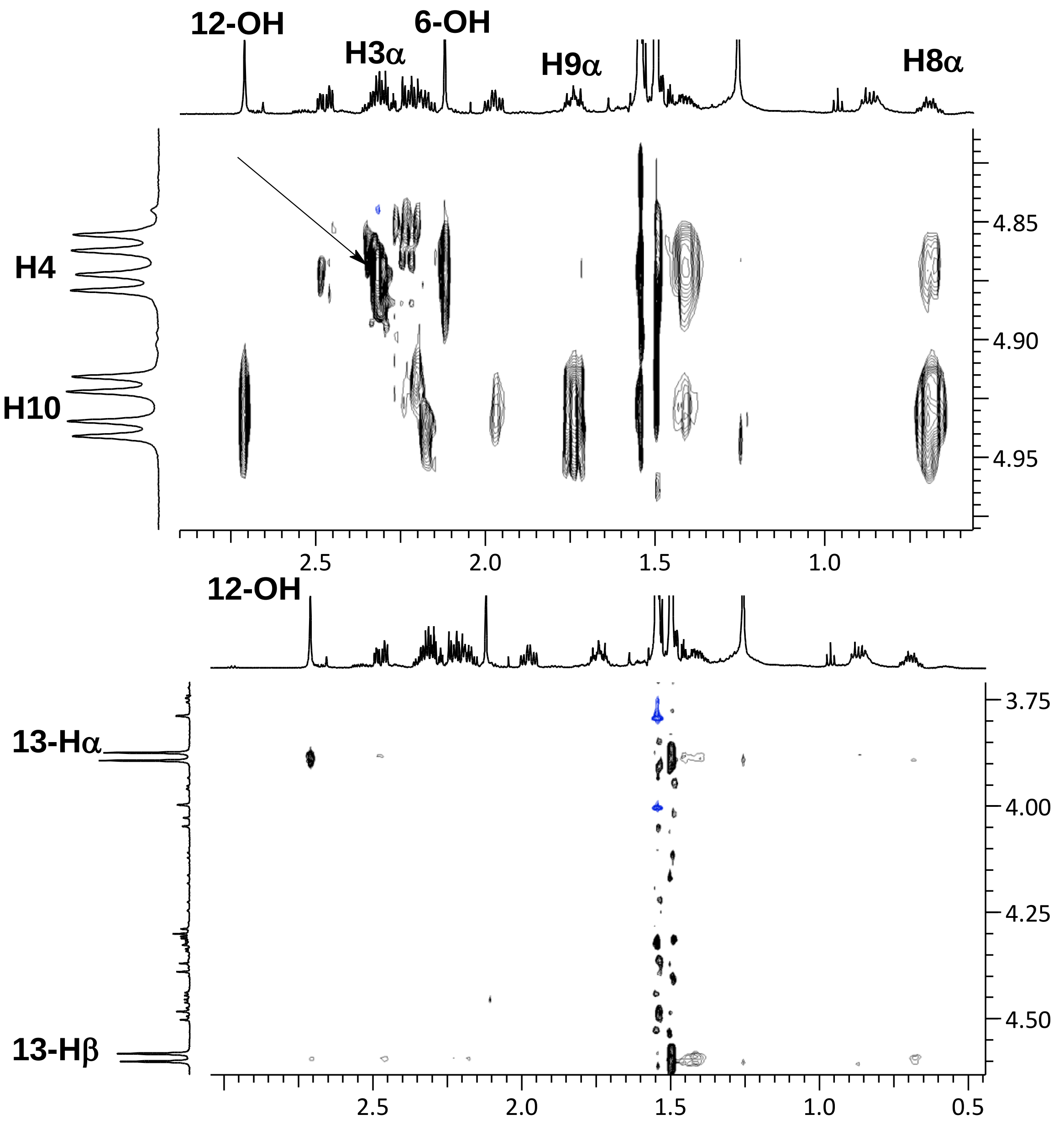

H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
H7β
CH3
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α


0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5
4
3
2
1

NOESY

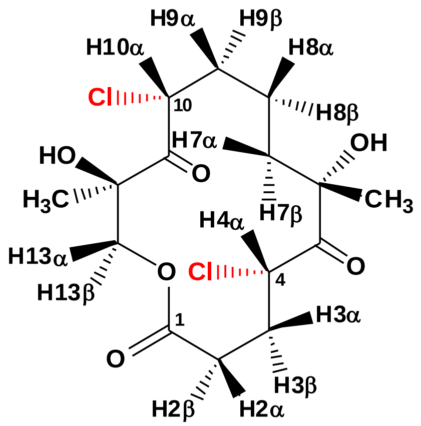
expansions

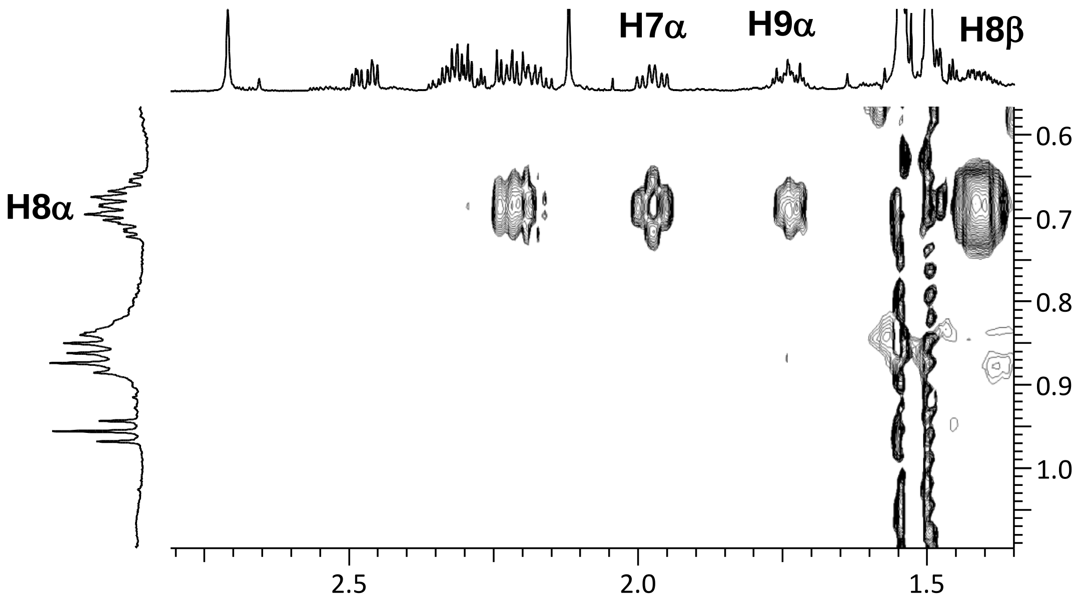

H10α
H9α
H9β
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
CH3
H7β
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α

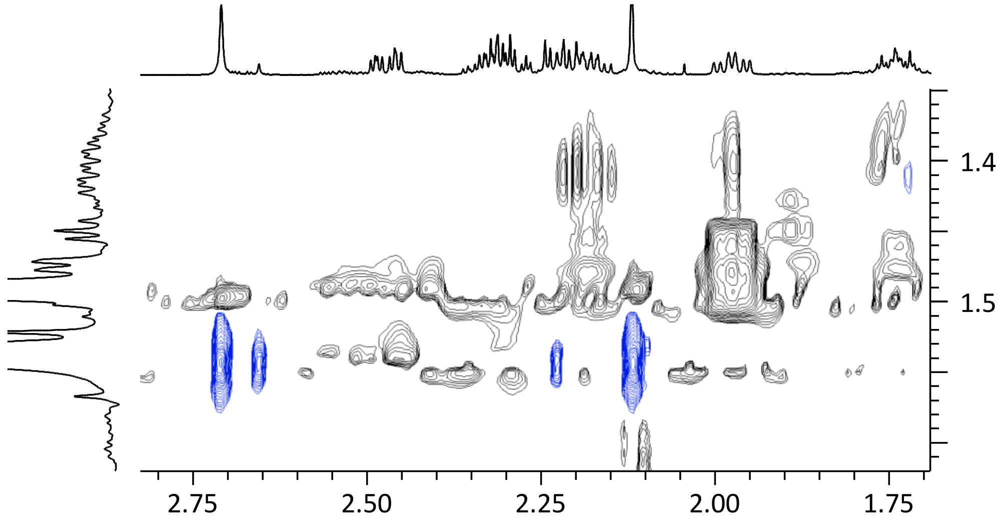


NOESY

expansions

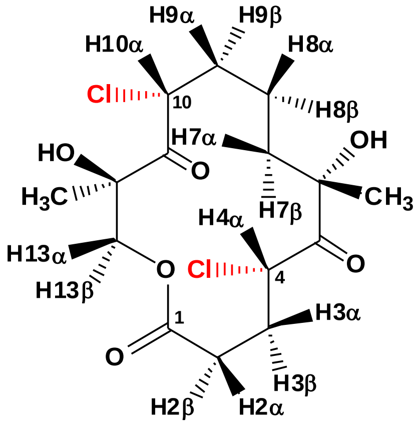

H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
CH3
H7β
H4α
H13α
O
Cl
4
O
H13β
H3α
1
O
H3β
H2β
H2α

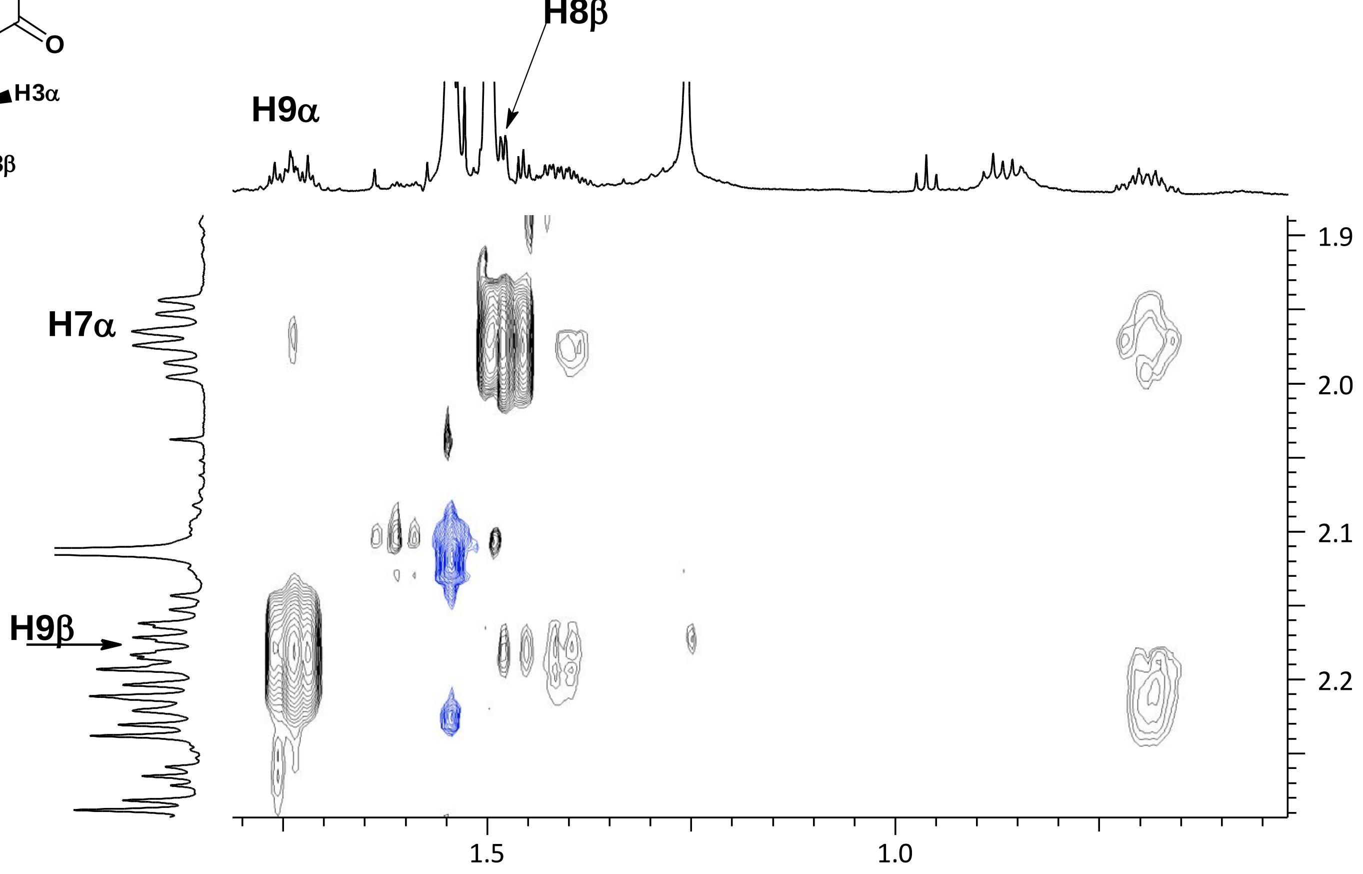




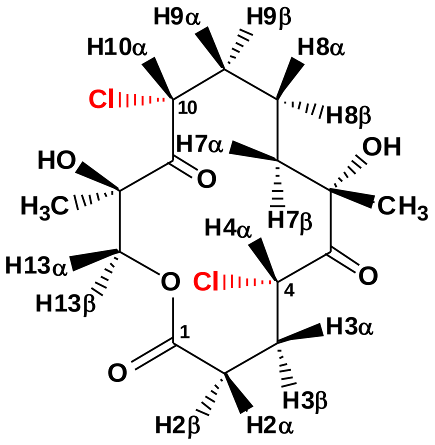
NOESY

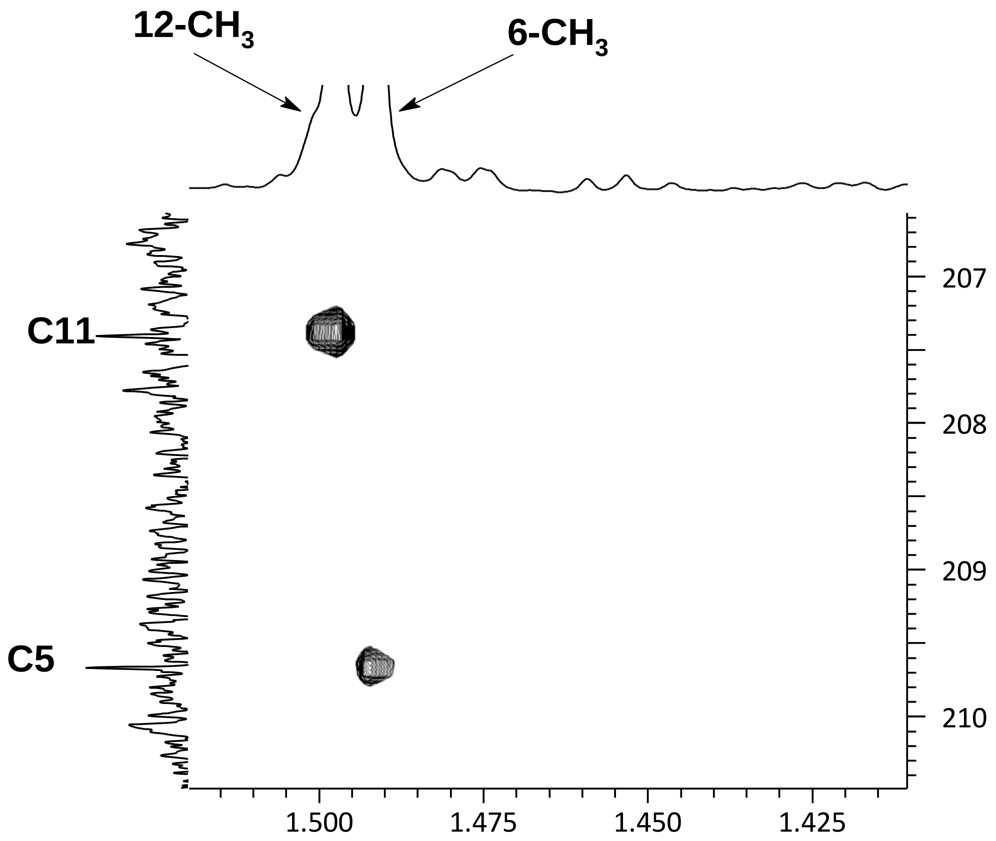
expansion


H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
H7β
CH3
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α

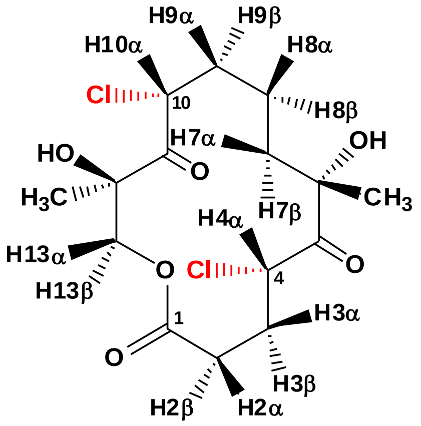


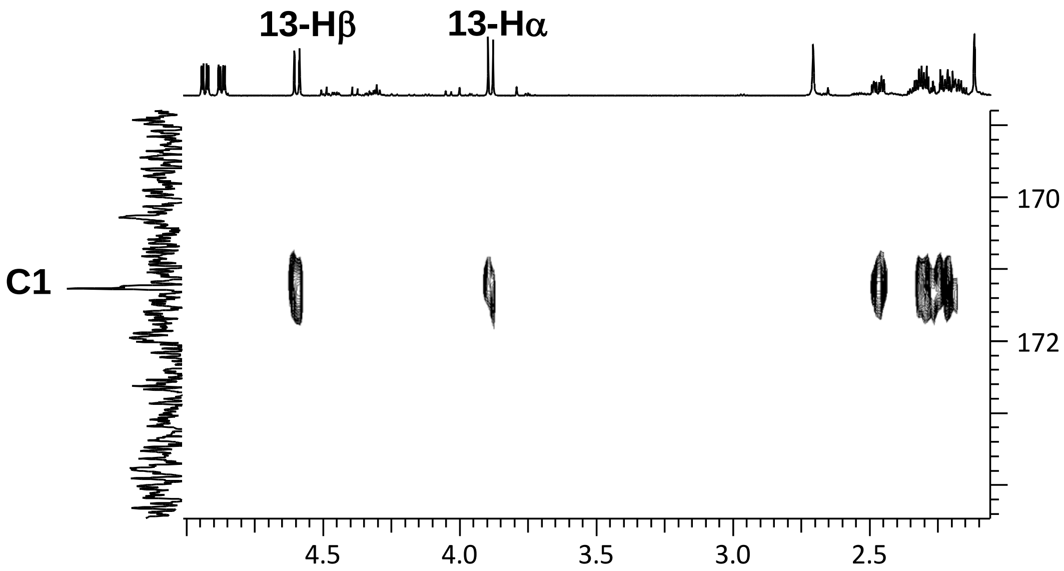
gHMBC *long-range correlations*

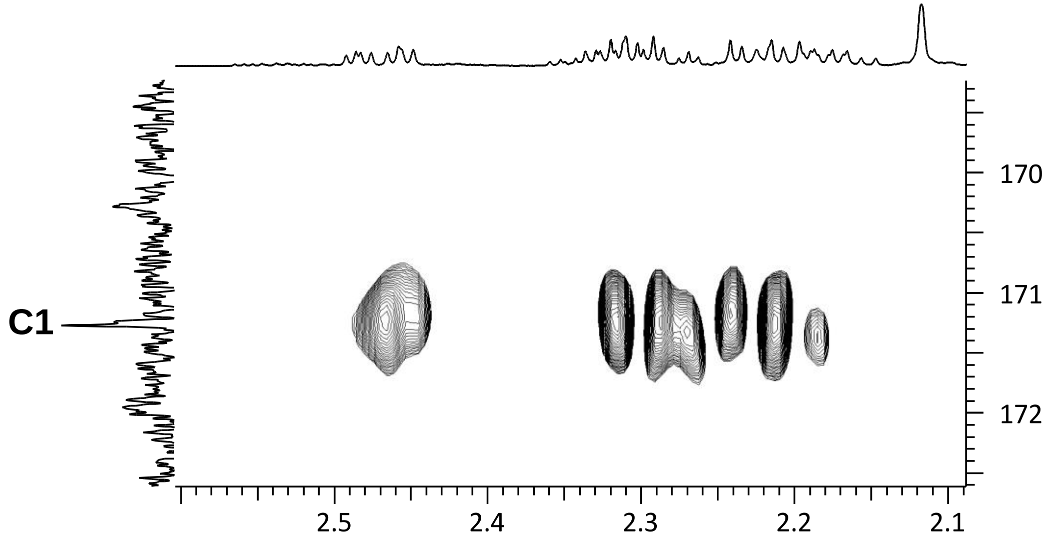

H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
H7β
CH3
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α



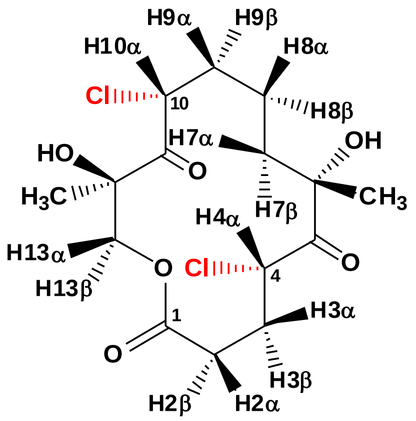
gHMBC *long-range correlations*

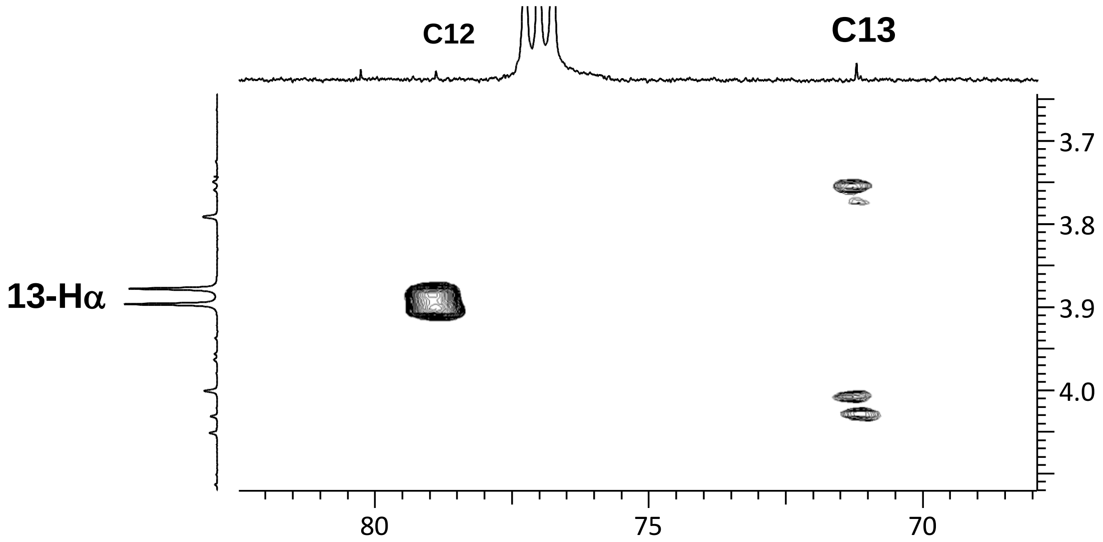

H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
CH3
H7β
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α

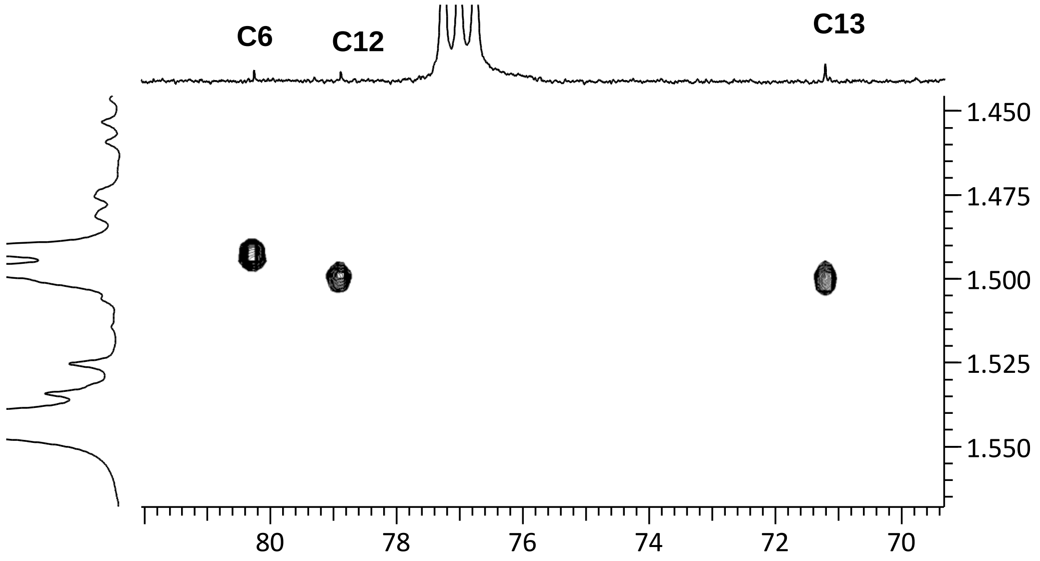


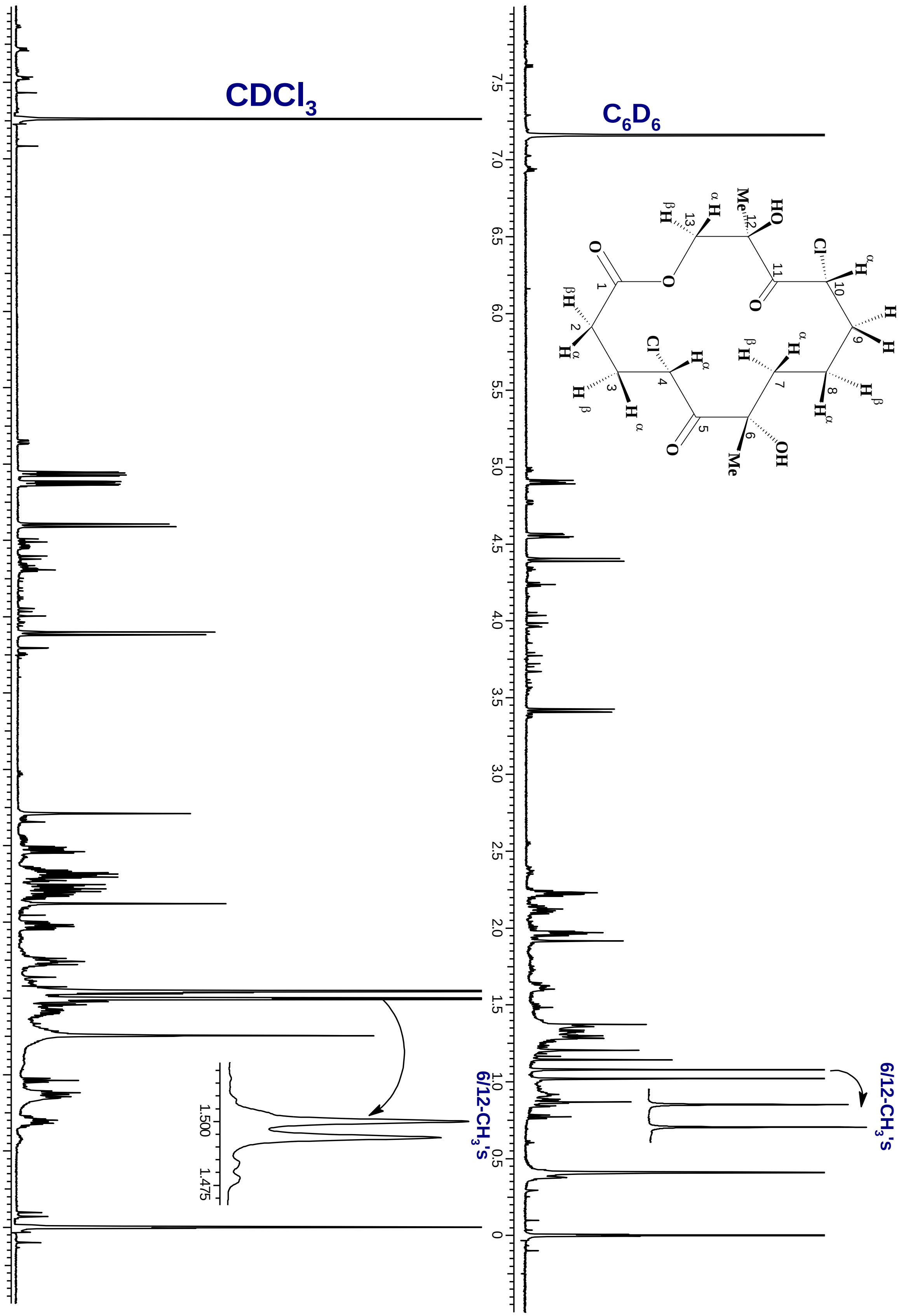


gHMBC *long-range correlations*

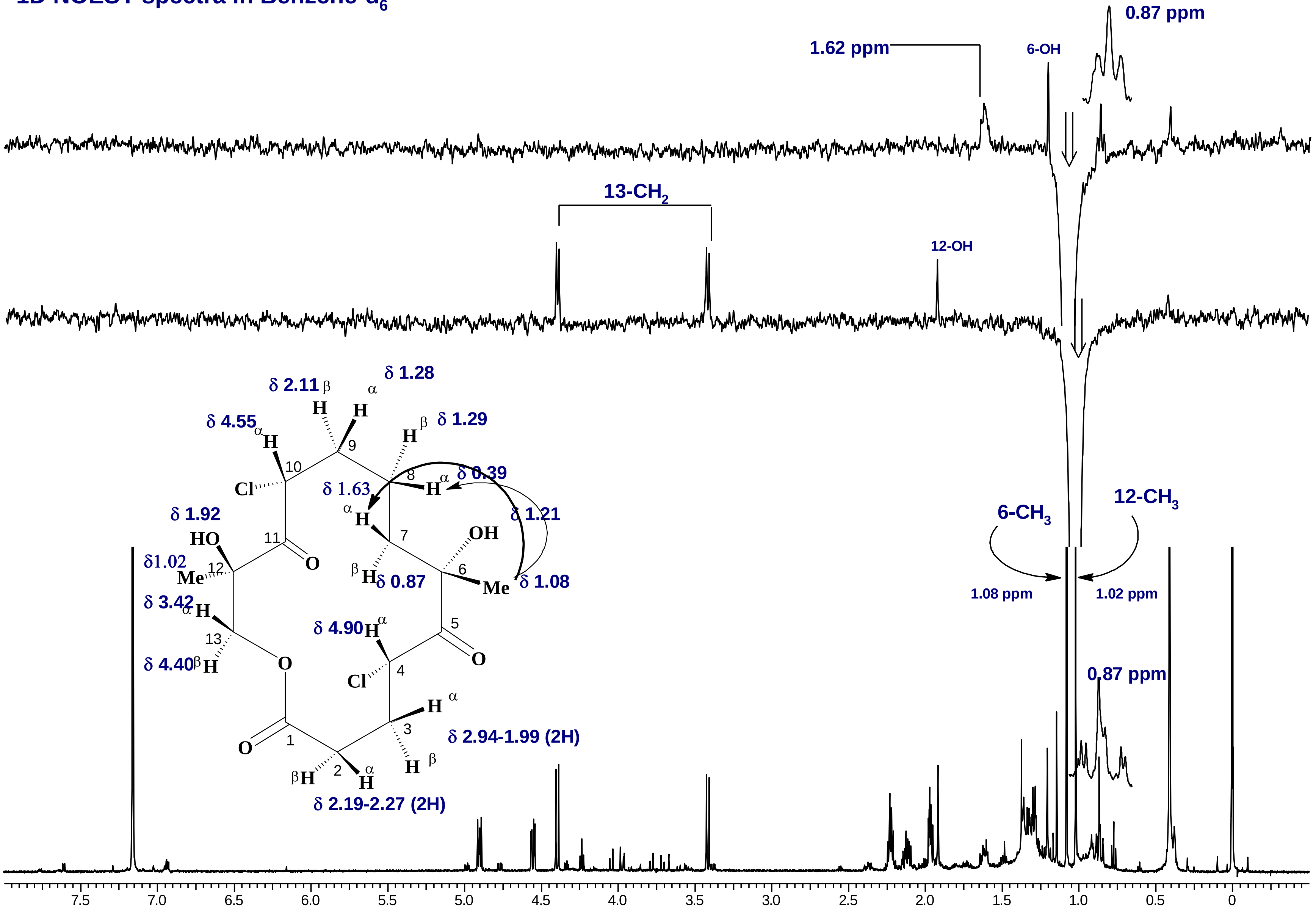

H9α
H9β
H10α
H8α
Cl
10
H8β
H7α
OH
HO
O
H3C
H7β
CH3
H4α
H13α
O
Cl
4
O
H13β
1
H3α
O
H3β
H2β
H2α



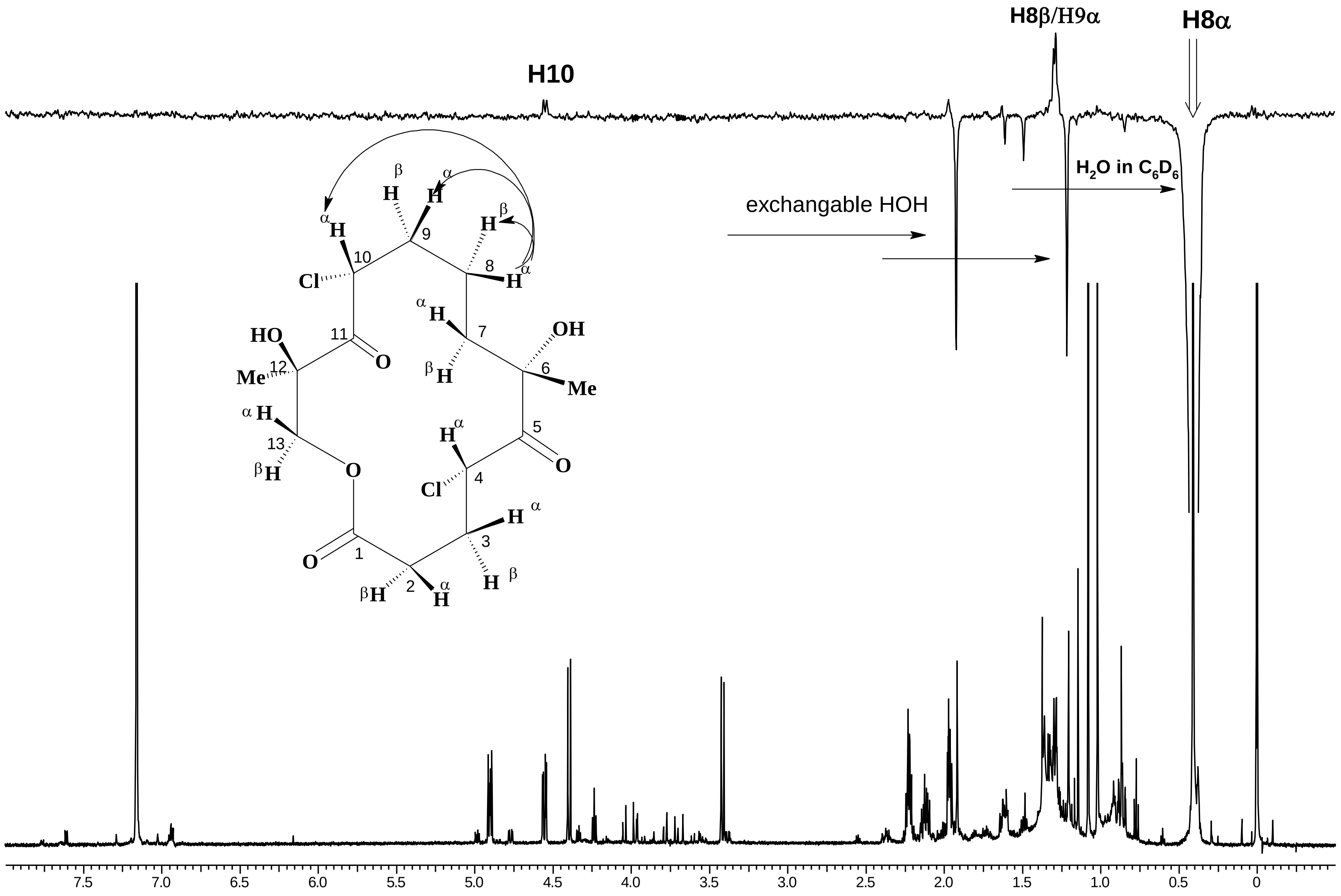



CDCl$_3$
C_6D_6
6/12-CH$_3$'s
6/12-CH$_3$'s
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1.500
1.475
HO
Me
OH
Me
Cl
Cl
O
O
O
O
1
2
3
4
5
6
7
8
9
10
11
12
13
H α
H β

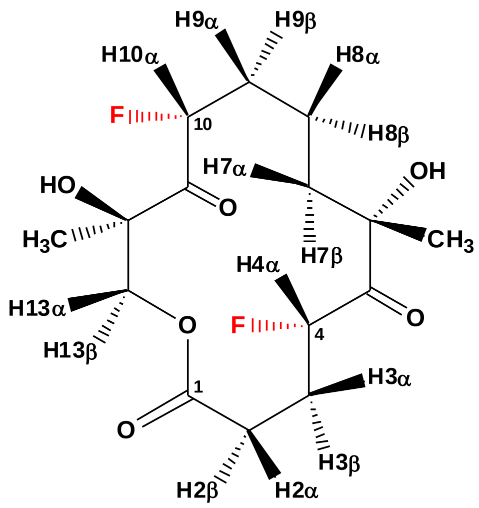
1D NOESY spectra in Benzene-d_6



1D NOESY spectrum in Benzene-d_6



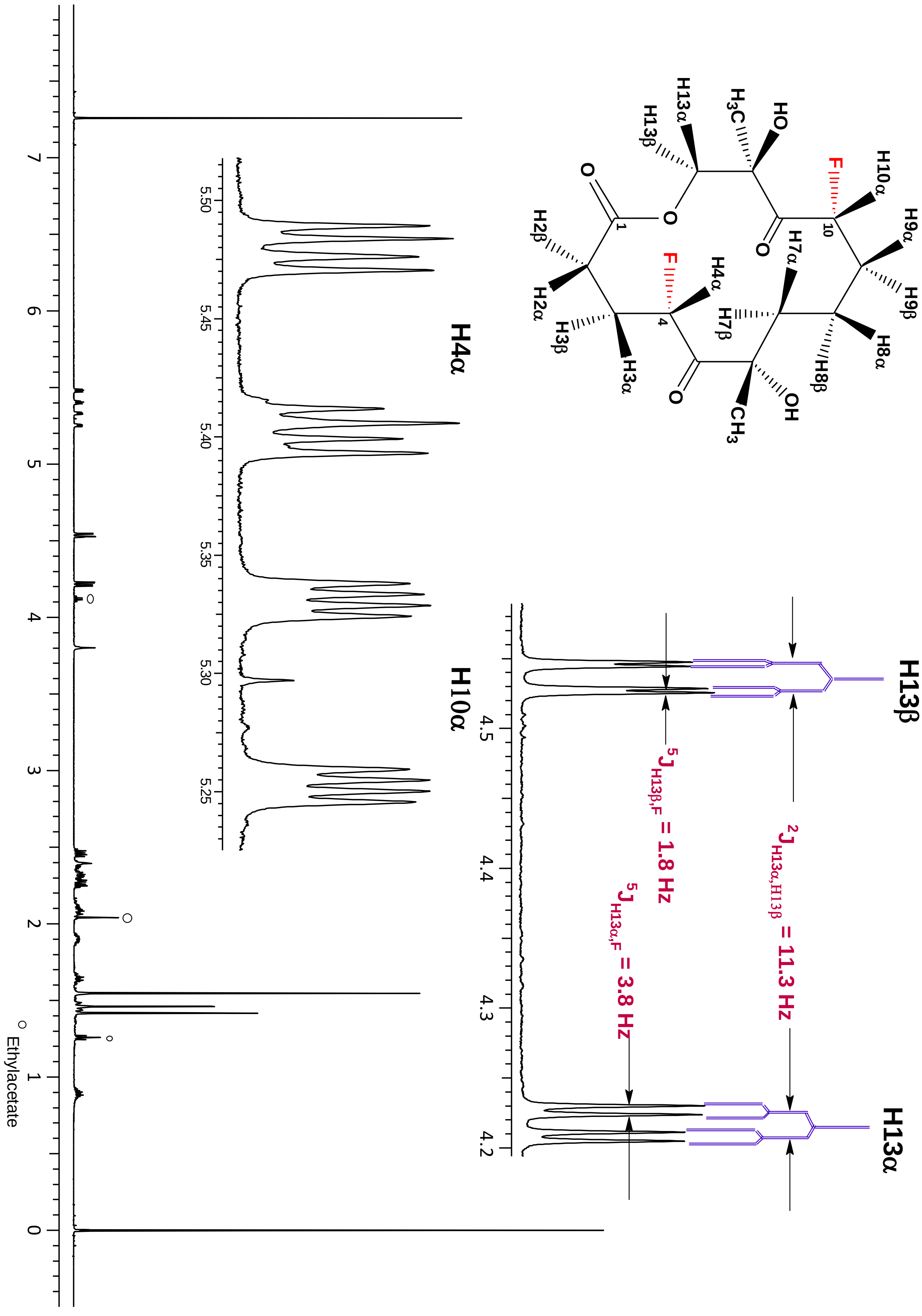
^{1}H and ^{13}C NMR Chemical shifts (δ/ppm) and Coupling constants (J/Hz)


H9α
H9β
H10α
H8α
F
10
H8β
H7α
OH
HO
O
H_3C
H7β
CH_3
H4α
H13α
O
F
4
O
H13β
1
H3α
O
H3β
H2β
H2α

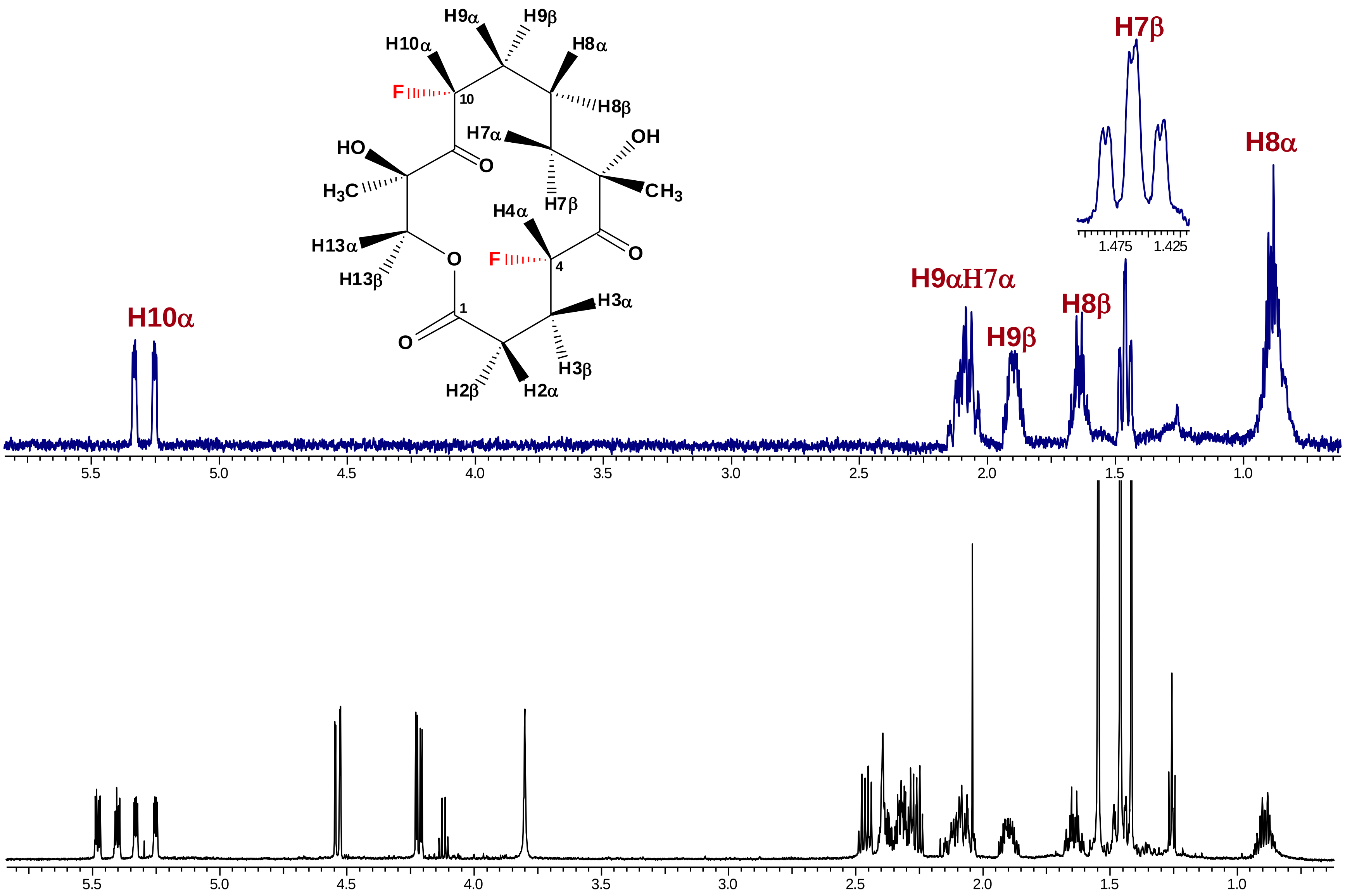
H2α———2.26
H2β———2.46
H3α———2.29
H3β———2.35
H4α———5.43
6–CH_3———1.46
6–OH———3.80
H7α———2.08
H7β———1.46
H8α———0.89
H8β———1.64
H9α———2.08
H9β———1.89
H10α——5.28
12–CH_3——1.46
12–OH——3.80
H13α———4.22
H13β———4.54

^{2}J(H2α–H2β) = 15.52
^{3}J(H2α–H3α) = 5.42
^{3}J(H2α–H3β) = 7.55
^{3}J(H2β–H3α) = 7.19
^{3}J(H2β–H3β) = 6.84
^{2}J(H3α–H3β) = 14.16
^{3}J(H3α–H4β) = 8.14
^{3}J(H3α–F) = 8.83
^{3}J(H3β–H4α) = 3.25
^{3}J(H3β–F) = 37.82
^{2}J(H4α–F) = 46.39
^{2}J(H7α–H7β) = 13.27
^{3}J(H7α–H8α) = 4.79
^{3}J(H7α–H8β) = 12.41
^{3}J(H7β–H8α) = 12.67
^{3}J(H7β–H8β) =3.74
^{2}J(H8α–H8β) = 12.23
^{3}J(H8α–H9α) = 4.54
^{3}J(H8α–H9β) = 12.12
^{3}J(H8β–H9α) = 12.60
^{3}J(H8β–H9β) = 4.44
^{2}J(H9α–H9β) = 14.49
^{3}J(H9α–F) = 39.50
^{3}J(H9α–H10α) = 2.69
^{3}J(H9β–F) = 10.13
^{3}J(H9β–H10α) = 5.73
^{2}J(H10α–F) = 47.05

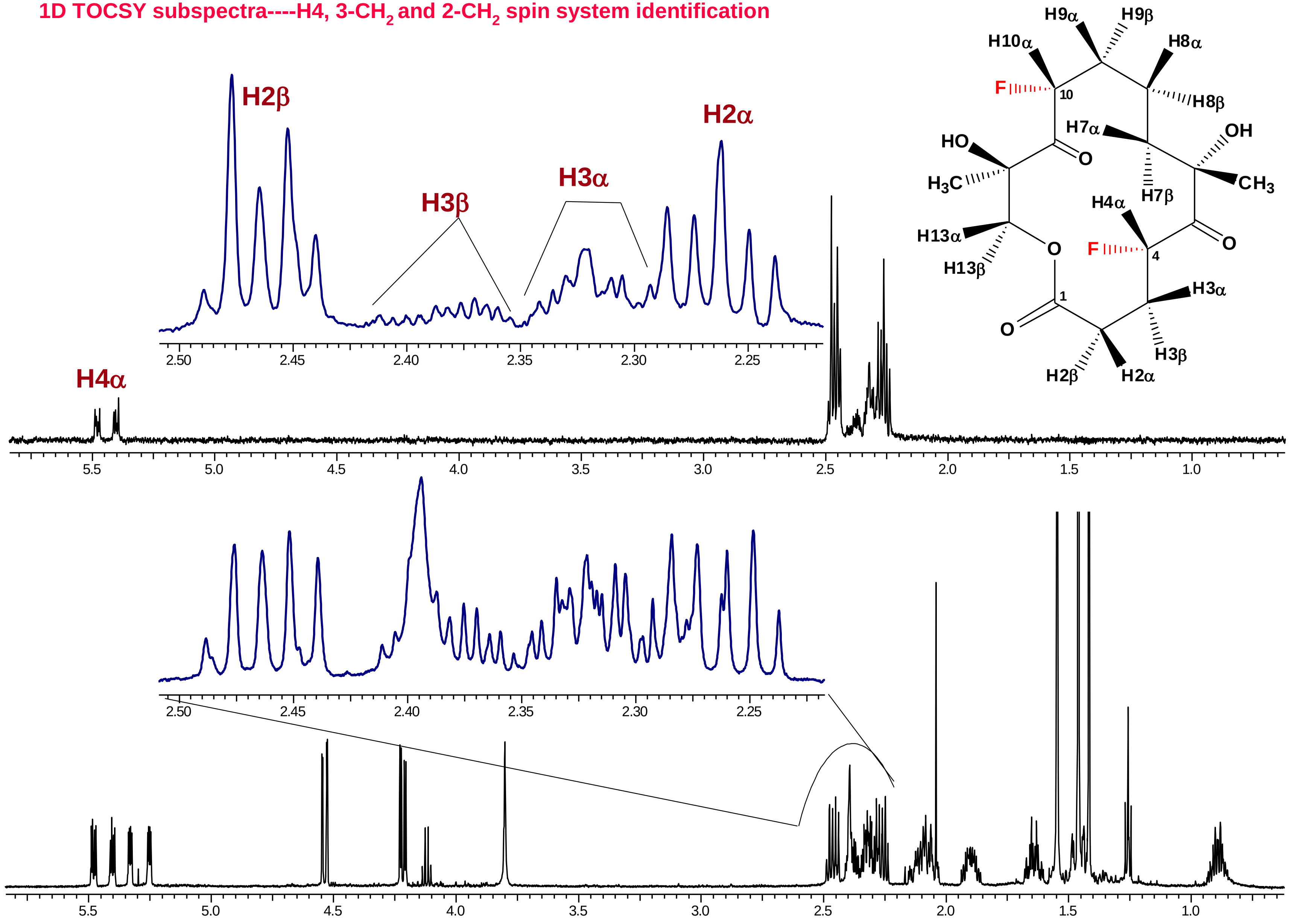
C1------171.74
C2------28.16
C3------24.99
C4-----90.91
C5-----209.92
C6-----80.11
6-CH_3----27.95
C7--------40.43
C8--------17.38
C9--------31.71
C10------94.93
C11------209.36
C12------78.49
12-CH_3----21.08
C13--------69.15


H13β
H13α
$^2J_{H13\alpha,H13\beta}$ = 11.3 Hz
$^5J_{H13\beta,F}$ = 1.8 Hz
$^5J_{H13\alpha,F}$ = 3.8 Hz
H4α
H10α
F
F
HO
OH
H_3C
CH_3
O
1
4
10
H2α
H2β
H3α
H3β
H4α
H7α
H7β
H8α
H8β
H9α
H9β
H10α
H13α
H13β
4.5
4.4
4.3
4.2
5.50
5.45
5.40
5.35
5.30
5.25
7
6
5
4
3
2
1
0
○ Ethylacetate

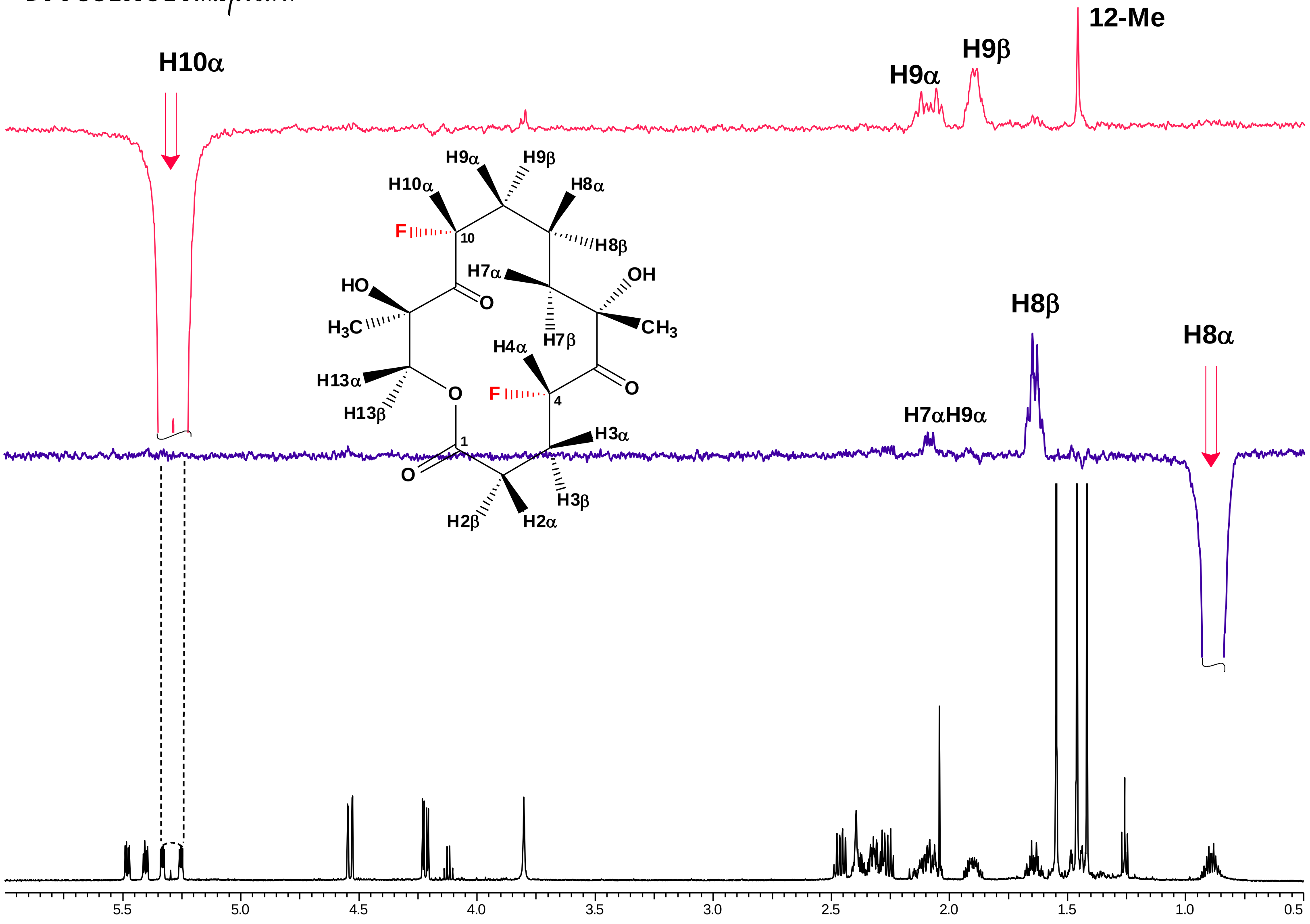
1D TOCSY subspectra----H10, 9-CH$_2$, 8-CH$_2$, and 7-CH$_2$ spin system identification



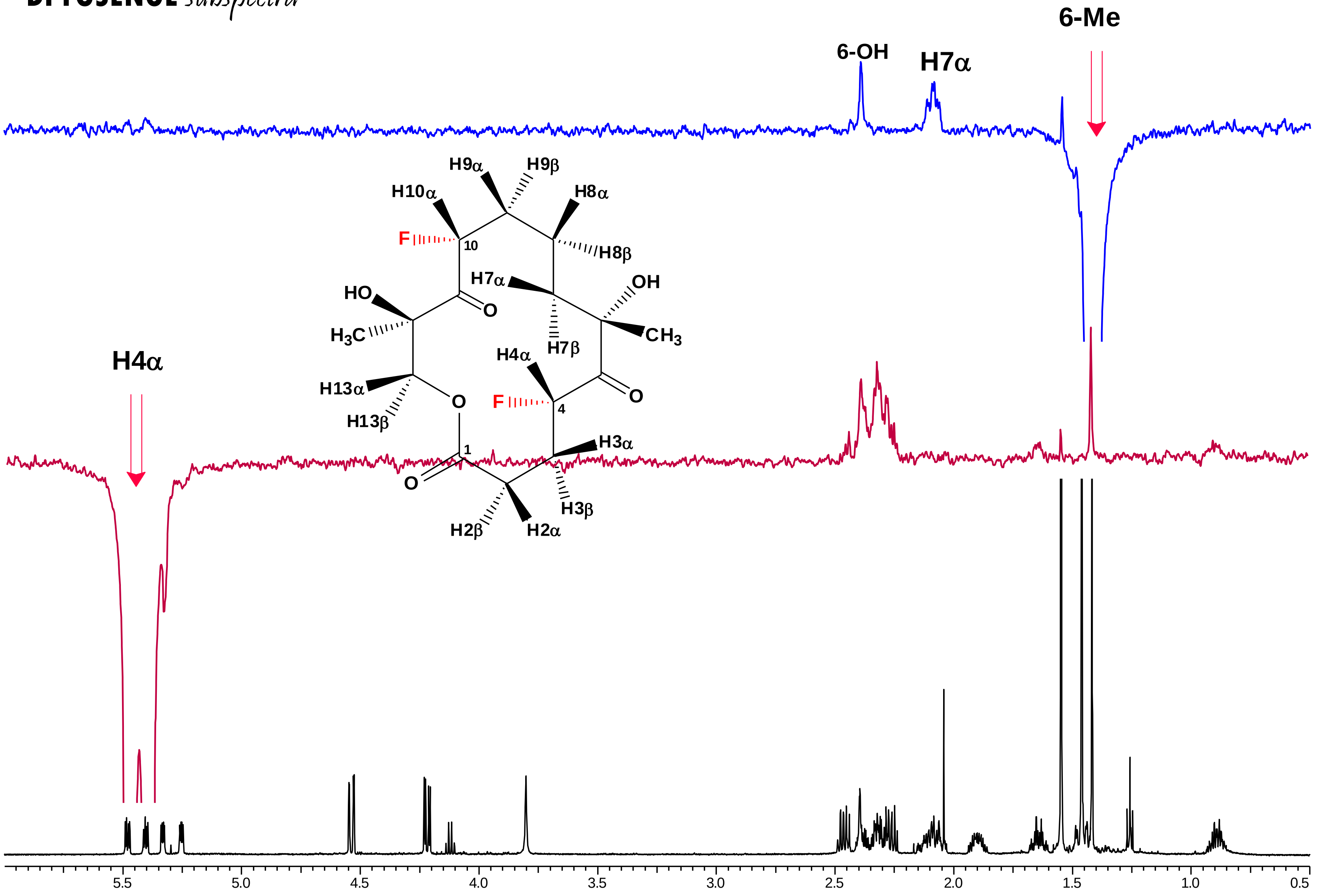
1D TOCSY subspectra----H4, 3-CH_2 and 2-CH_2 spin system identification



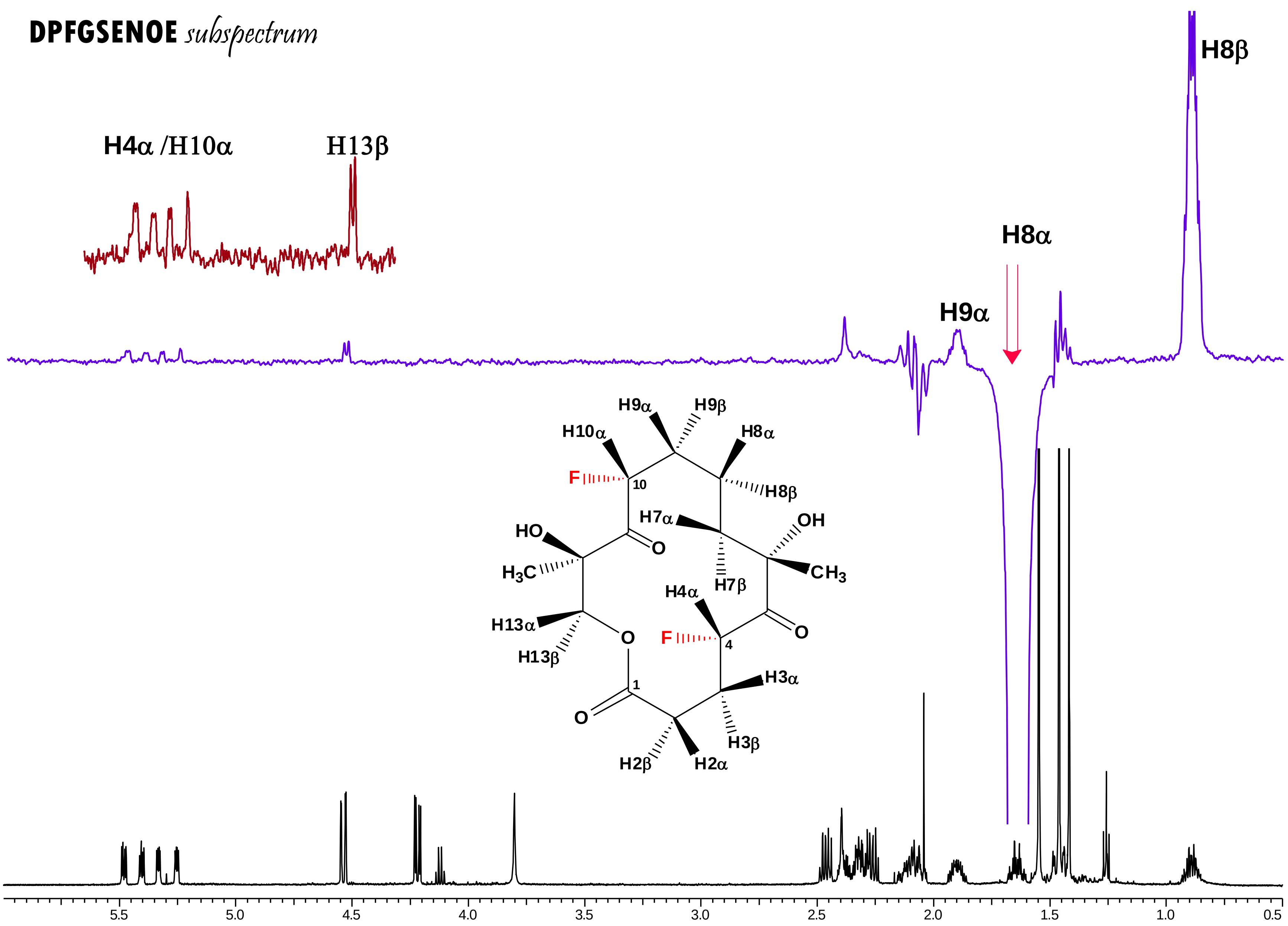
DPFGSENOE *subspectra*

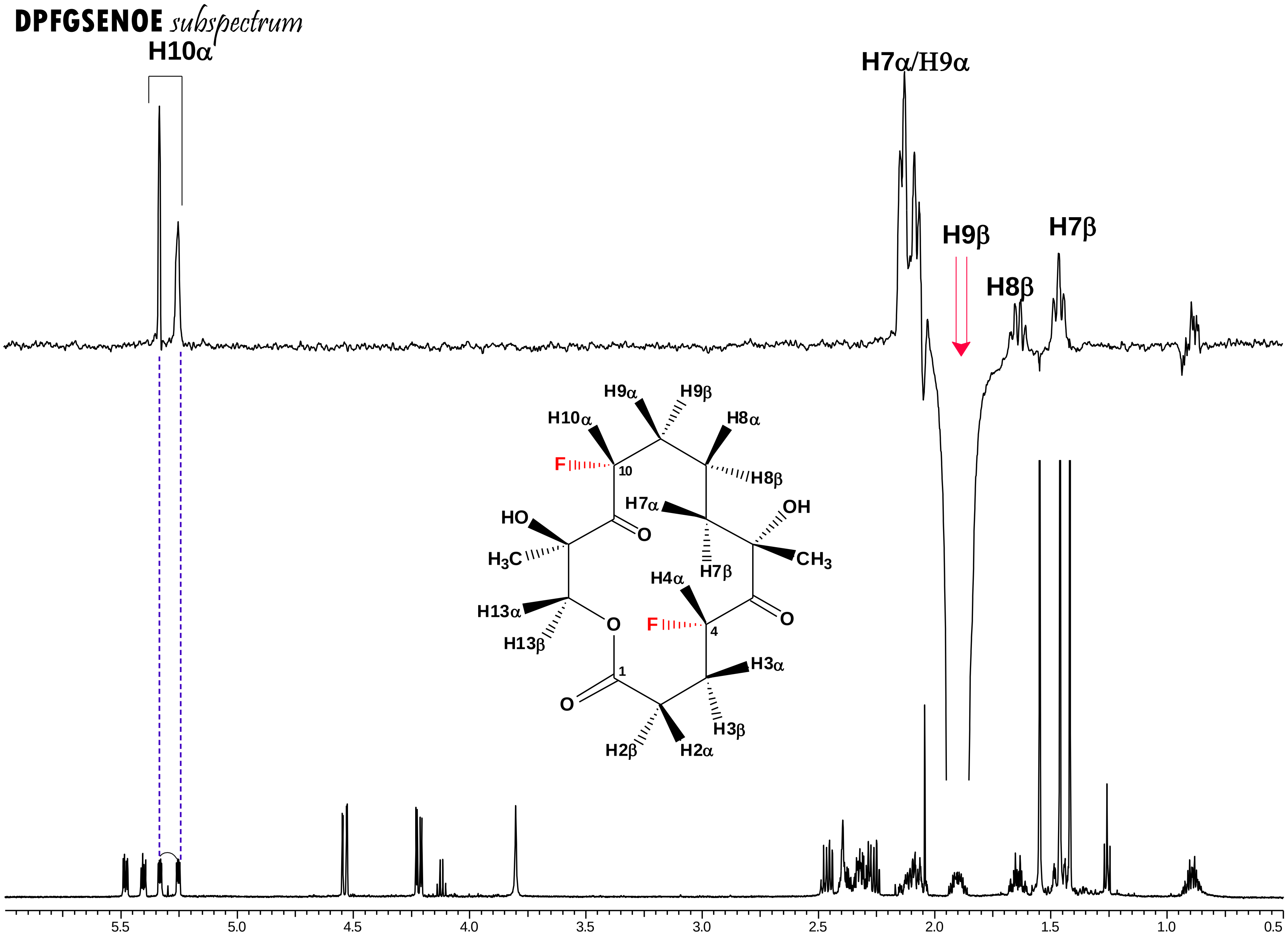


DPFGSENOE *subspectra*

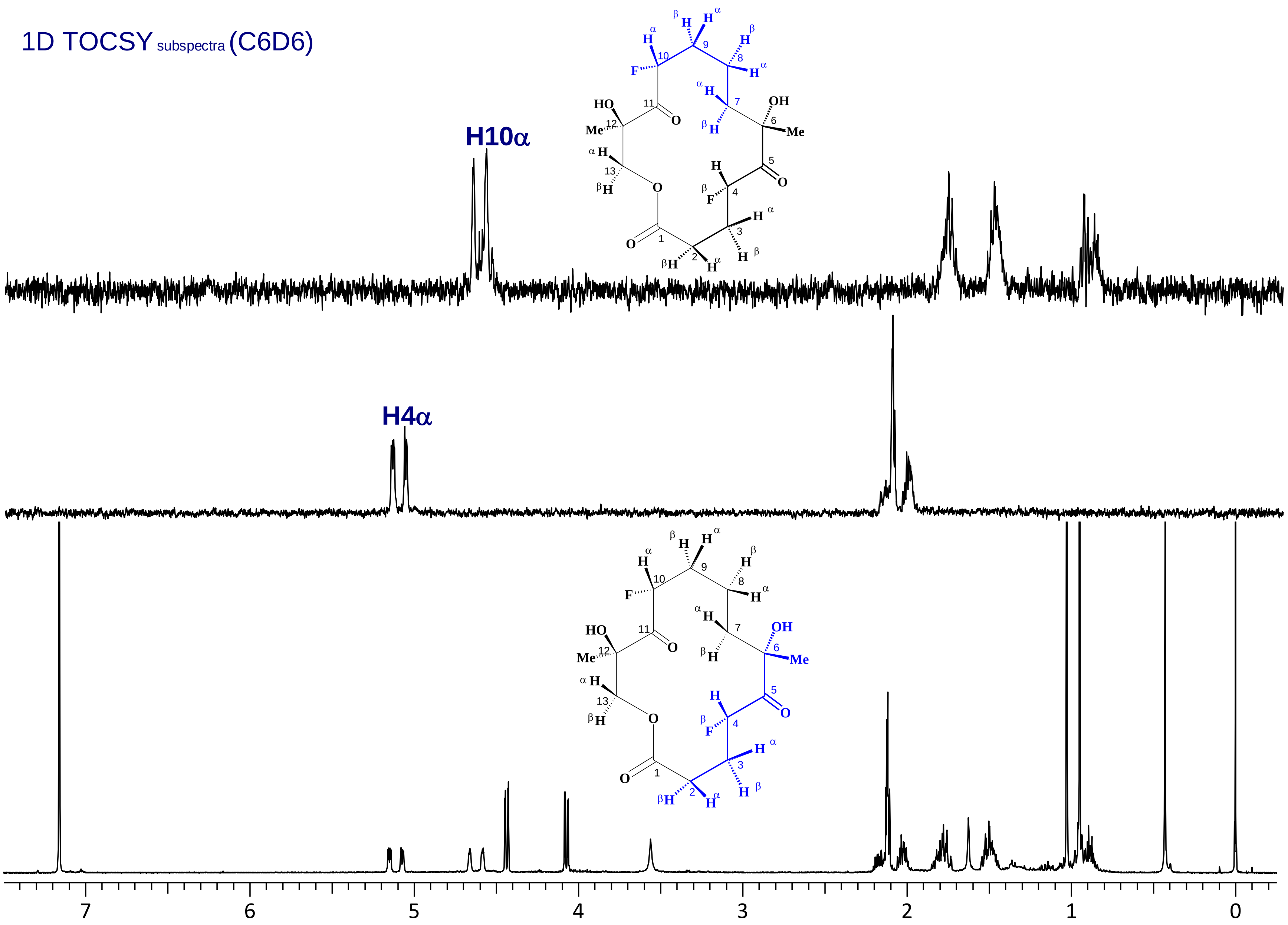


DPFGSENOE *subspectrum*

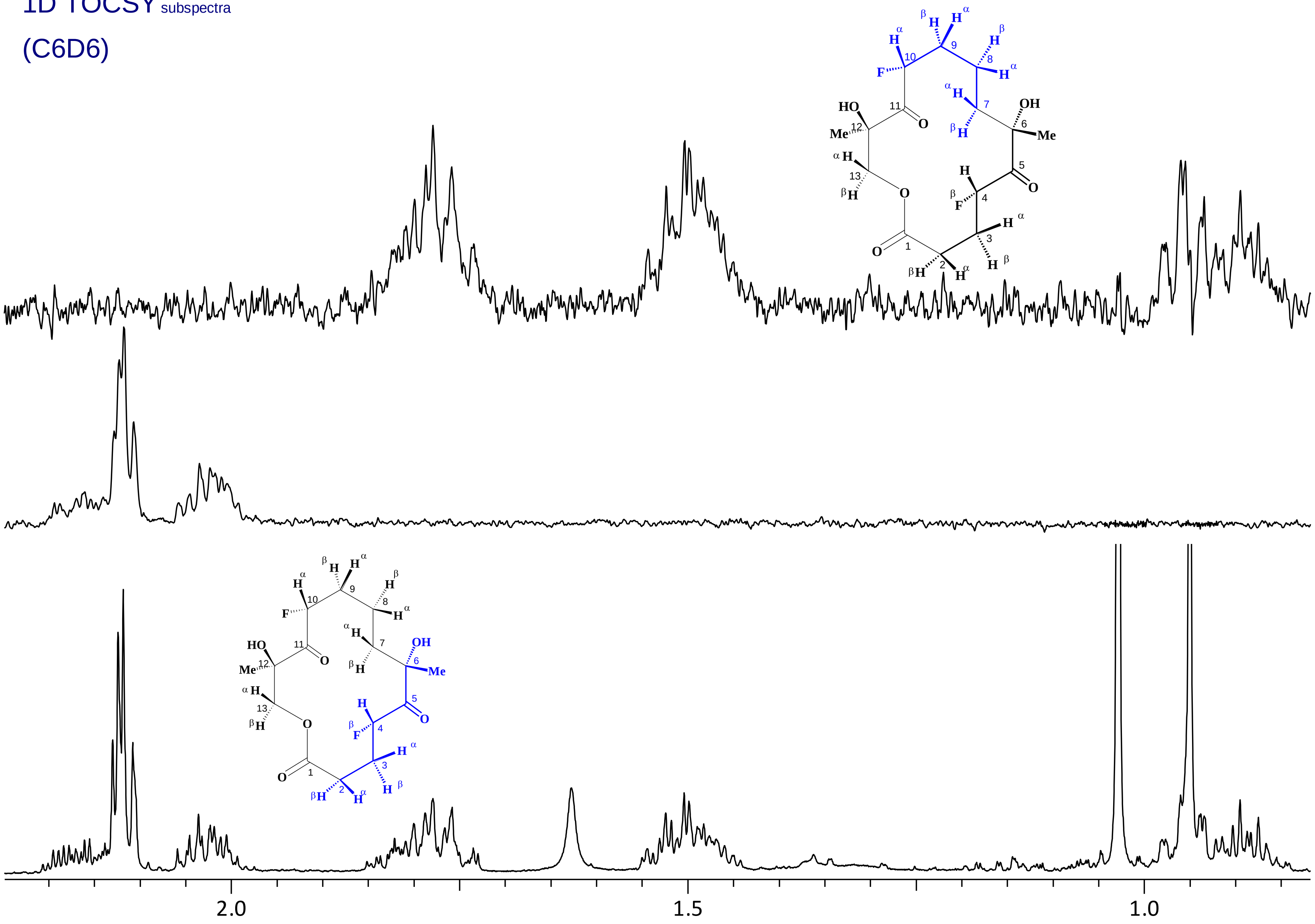


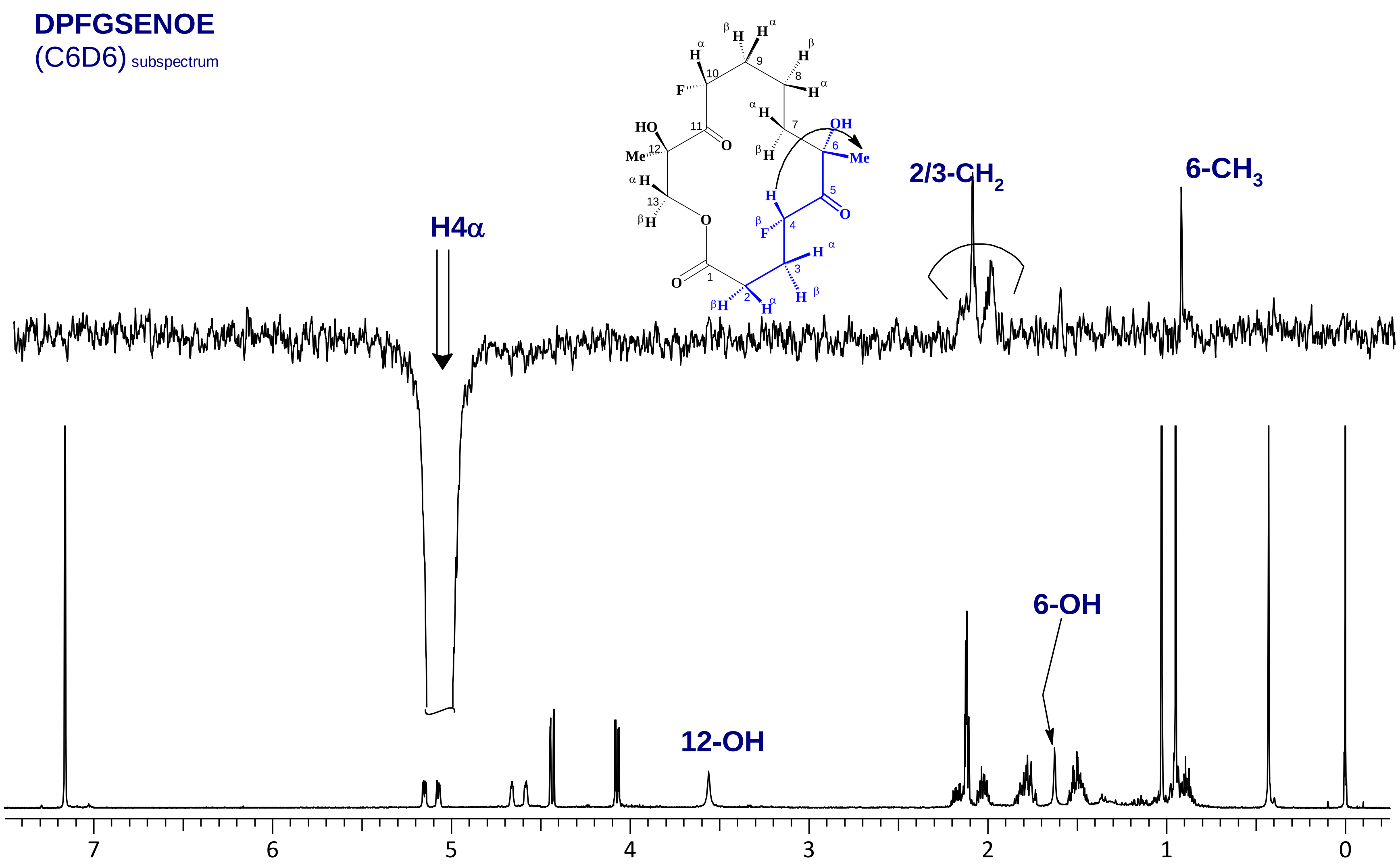

DPFGSENOE subspectrum
H10α
H7α/H9α
H9β
H8β
H7β
H9α
H9β
H10α
H8α
F
10
H8β
H7α
OH
HO
O
H3C
CH3
H7β
H4α
H13α
O
O
F
4
H13β
H3α
1
O
H3β
H2β
H2α
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

1D TOCSY subspectra (C6D6)

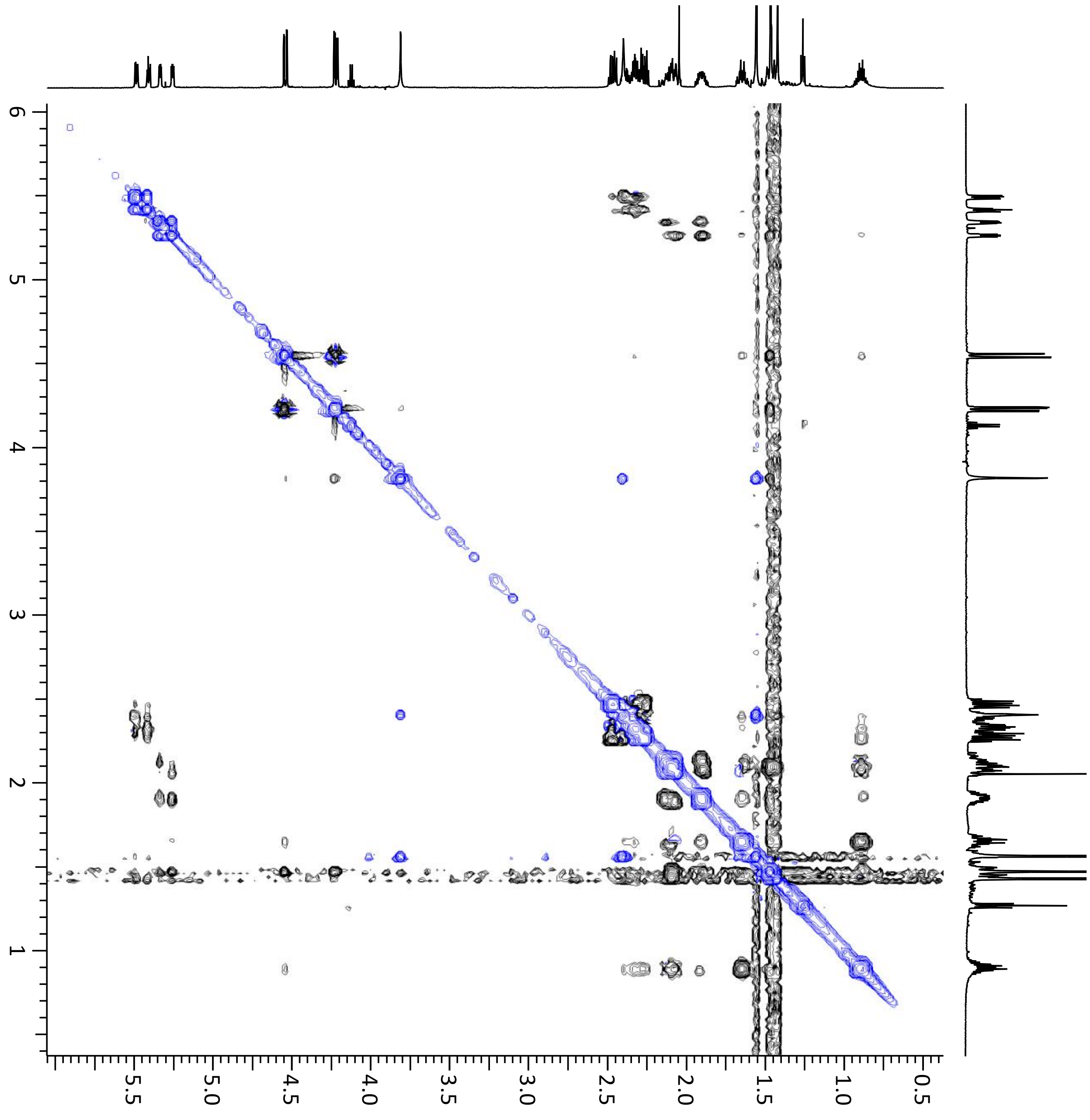


1D TOCSY $_{\text{subspectra}}^{\textit{expansions}}$
(C6D6)

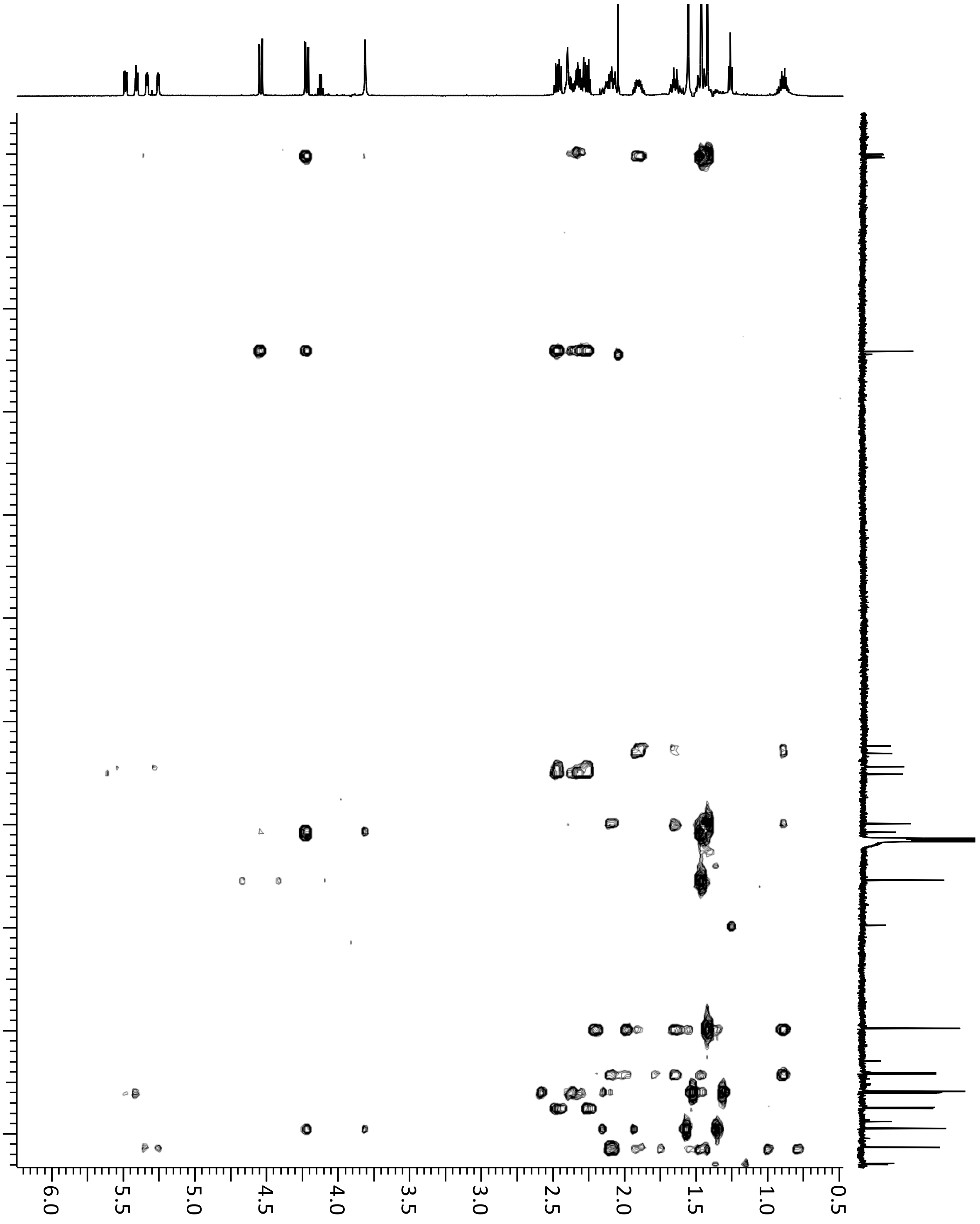



DPFGSENOE
(C6D6) subspectrum
H4α
2/3-CH$_2$
6-CH$_3$
12-OH
6-OH
HO
Me
F
O
OH
Me
F
H
1
2
3
4
5
6
7
8
9
10
11
12
13
α
β
7
6
5
4
3
2
1
0

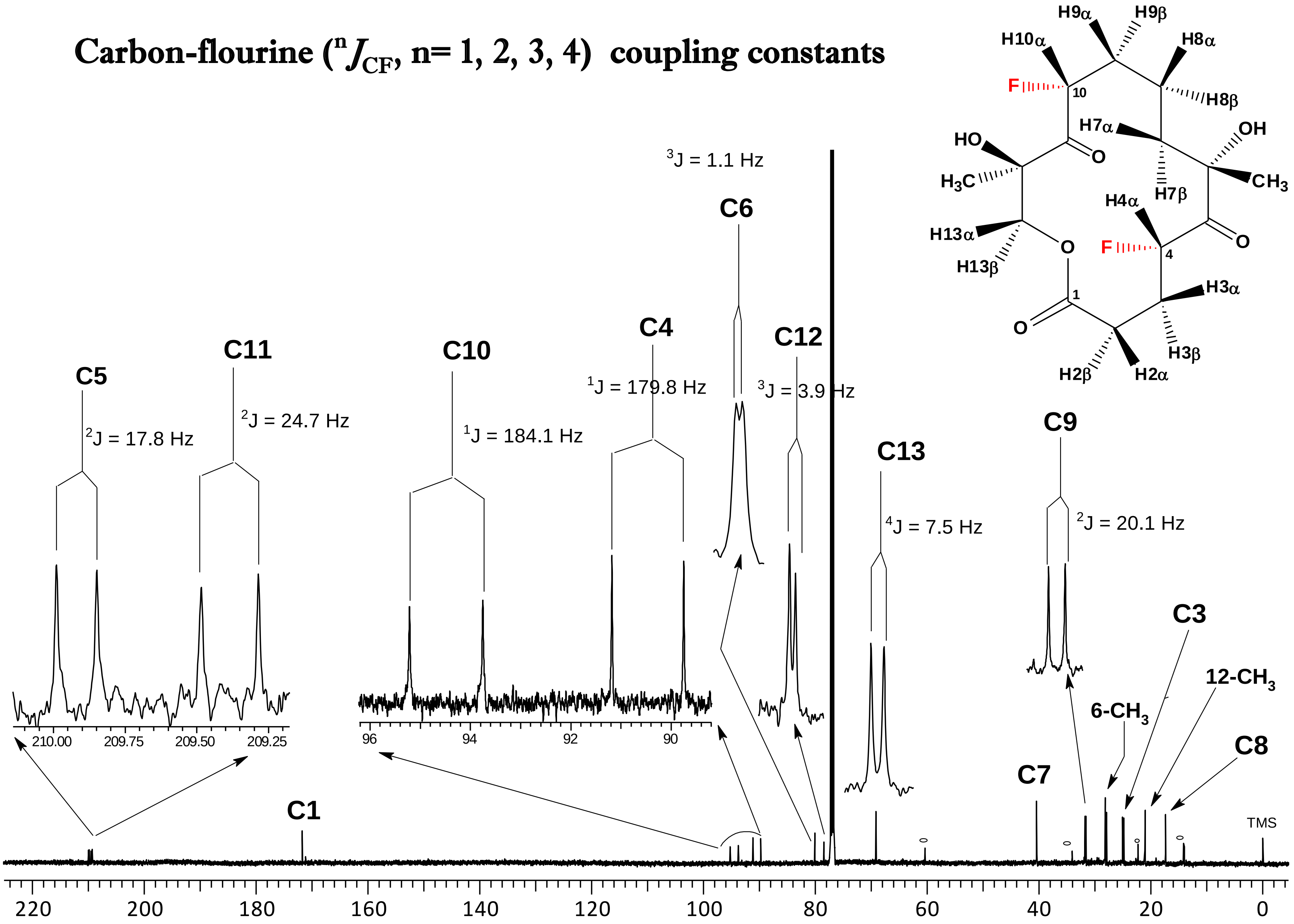
NOESY



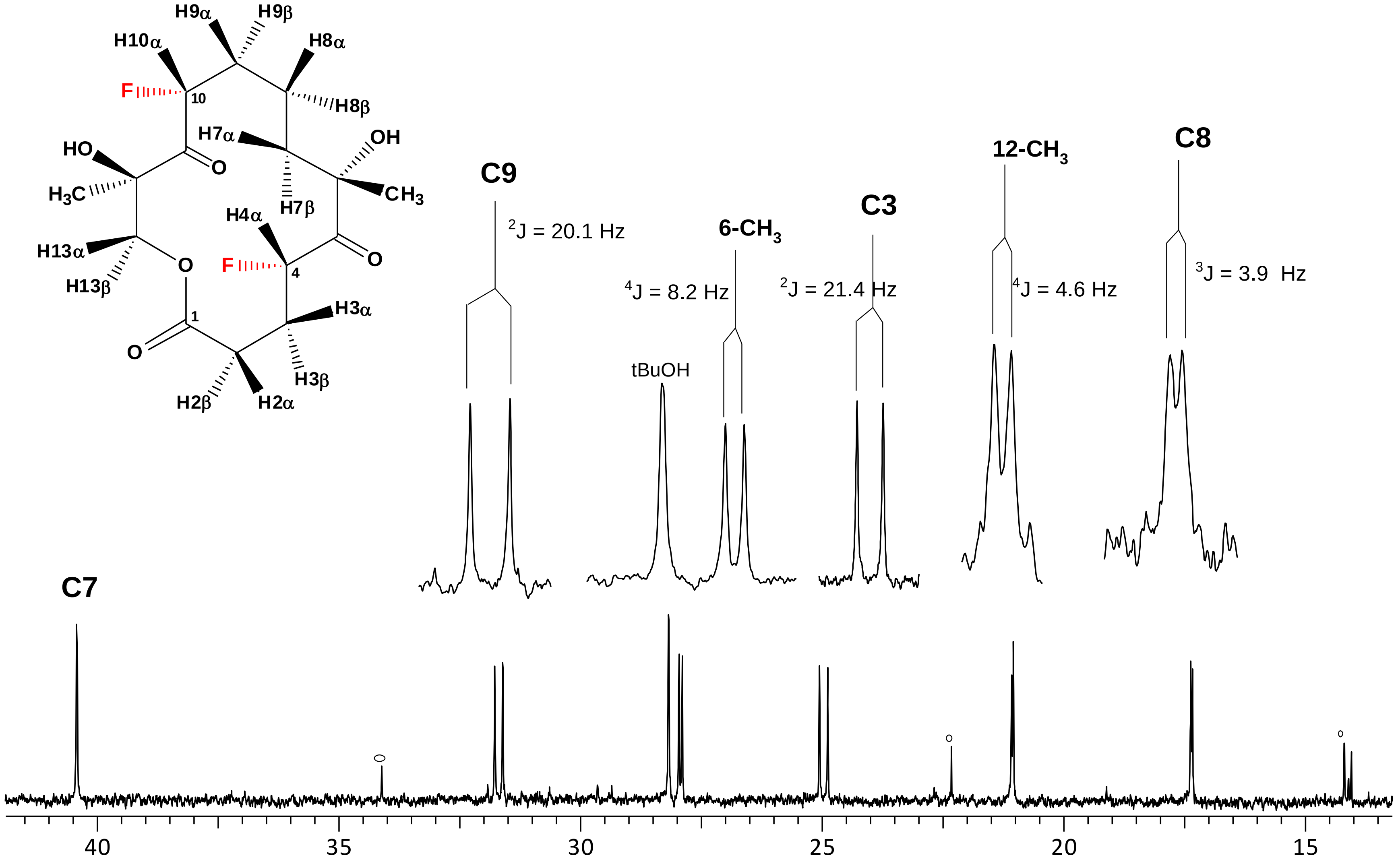
gHMBC

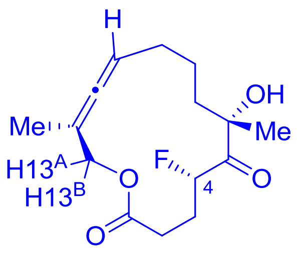

200
180
160
140
120
100
80
60
40
20
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

Carbon-flourine ($^{n}J_{CF}$, n= 1, 2, 3, 4) coupling constants

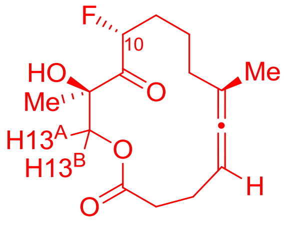


Carbon-flourine ($^nJ_{CF}$, n= 1, 2, 3, 4) coupling constants





5.52



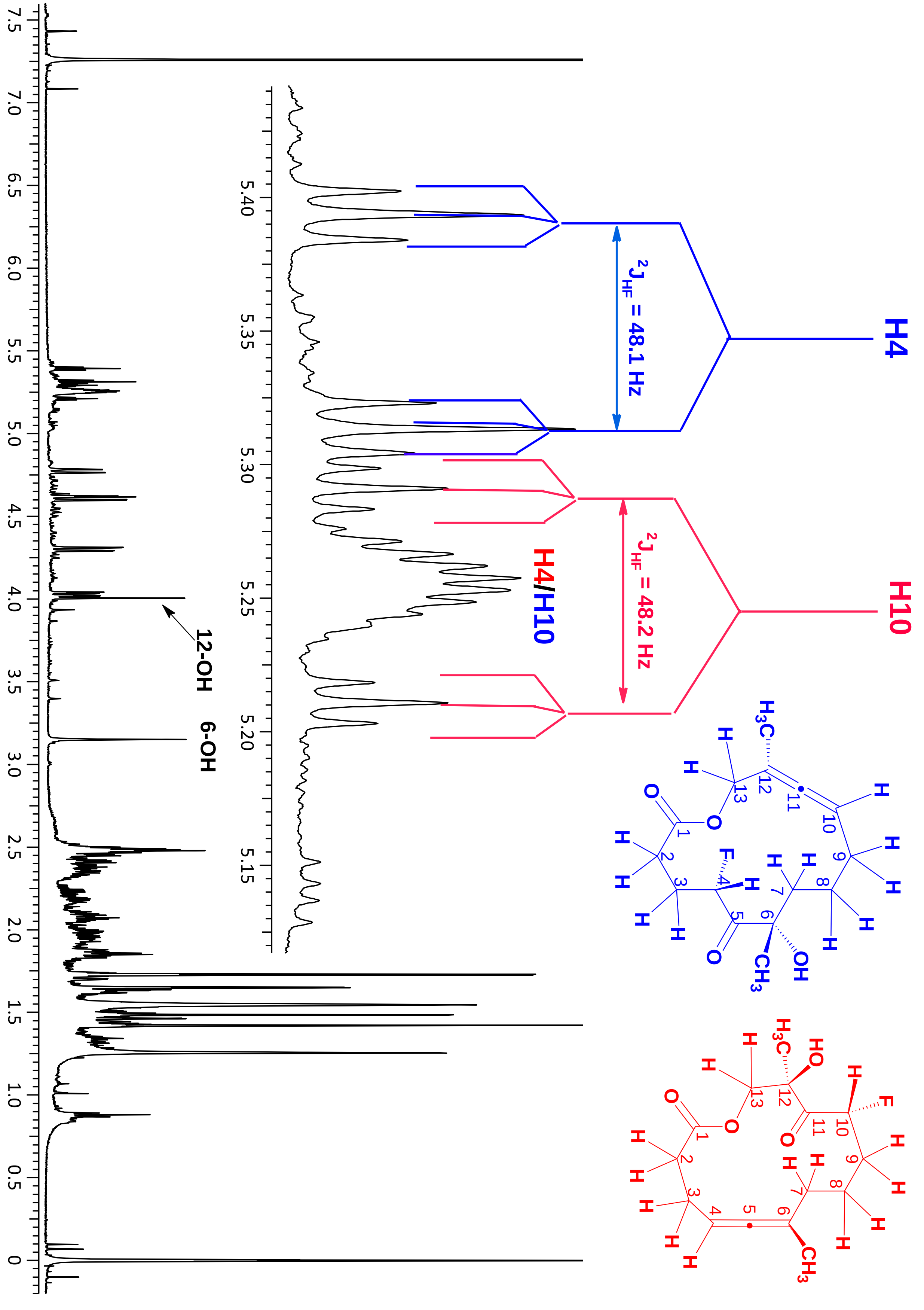
5.53

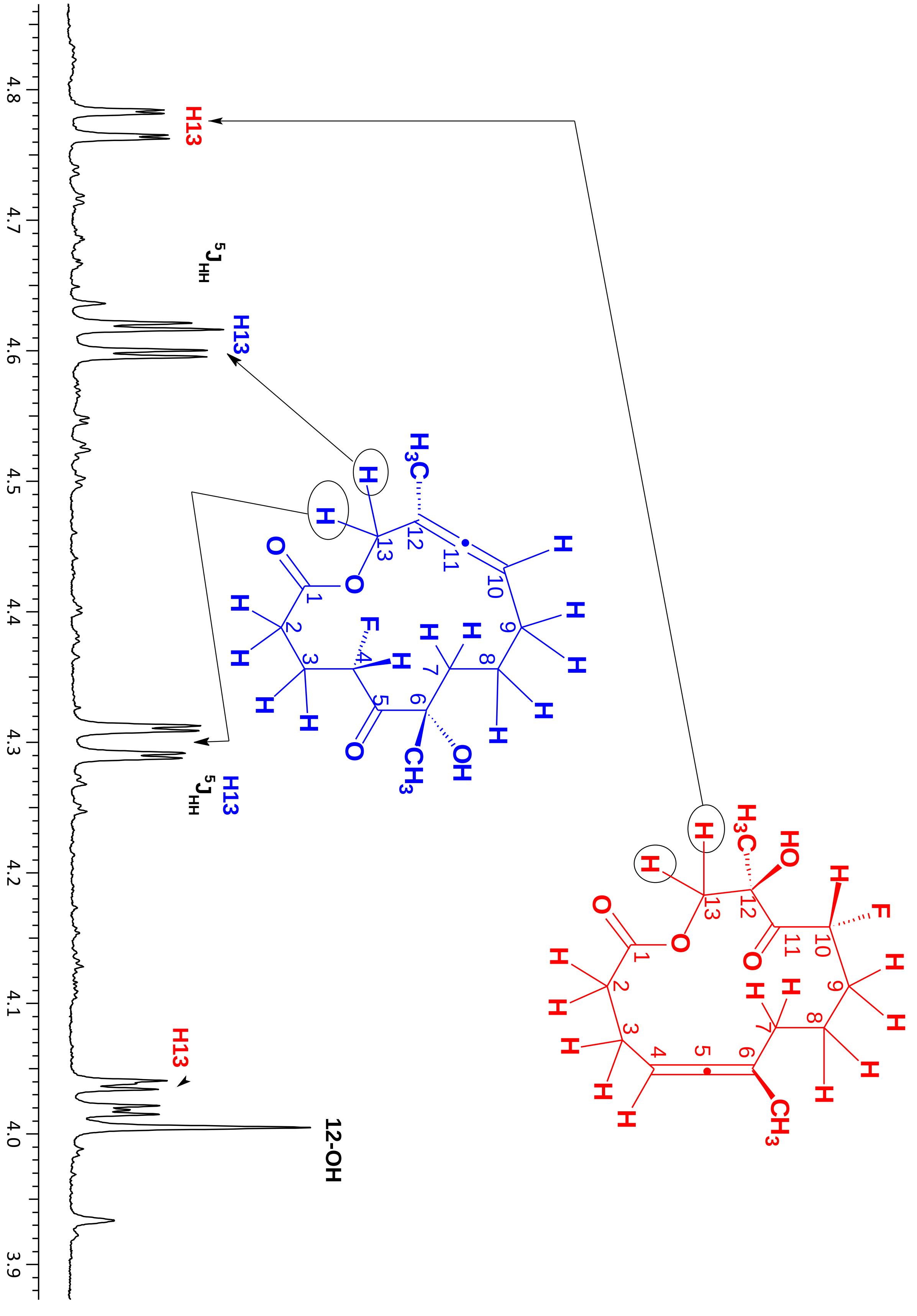
Observed chemical shifts (δ/ppm, $CDCl_3$) and coupling constants (Hz)

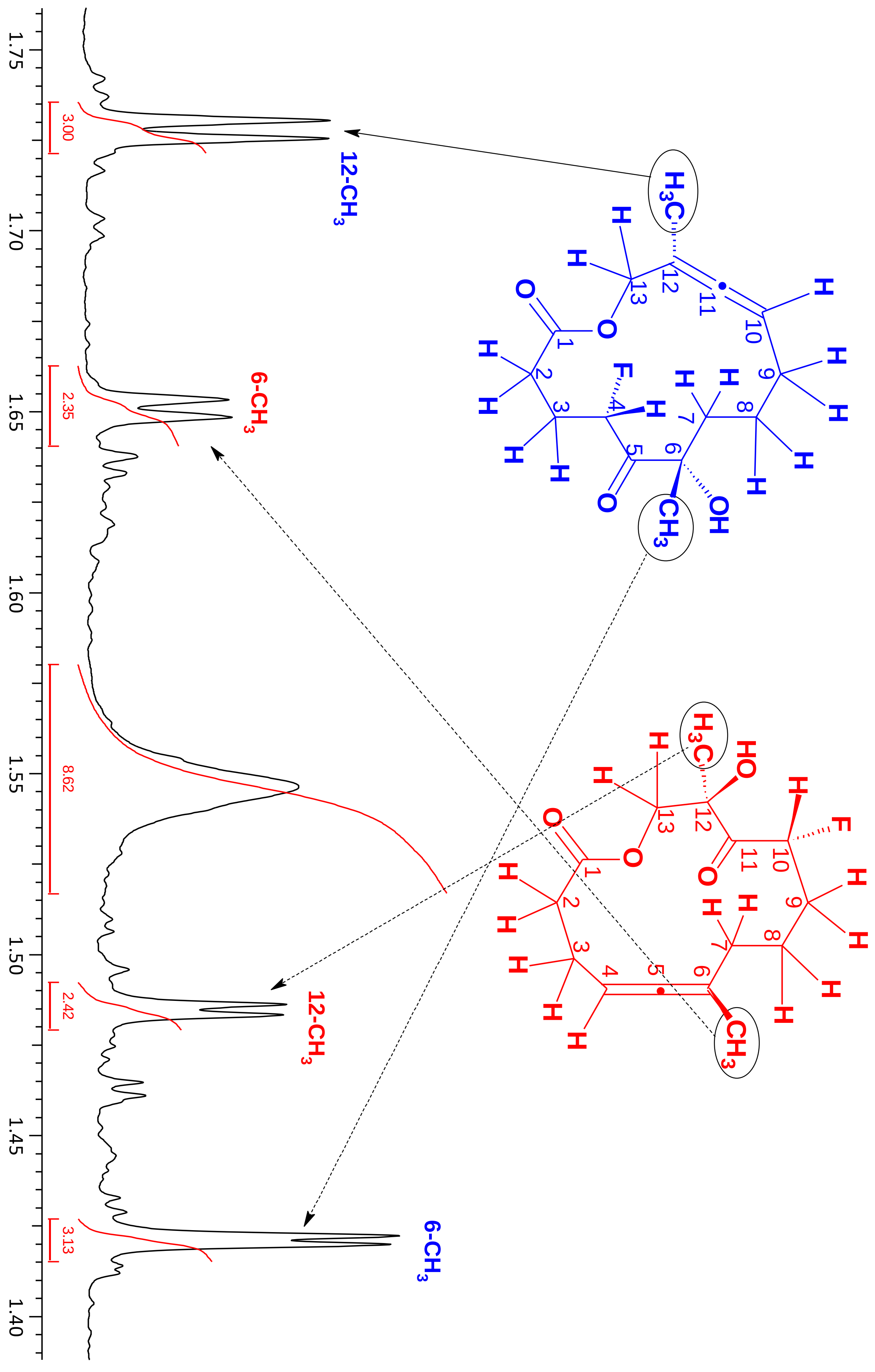
12-CH_3	1.73 (d, $J_{12\text{-}CH3,H10}$ = 2.97 Hz, 3H)	12-CH_3	1.48 (d, $J_{12\text{-}CH3,12\text{-}OH}$ = 1.8 Hz, 3H)
		12-OH	4.01 (br, 1H)
H4	5.35 (dt, $J_{H4,F}$ = 48.1 Hz, $J_{H4,\ 3\text{-}CH2}$ = 5.7 Hz, 1H)	6-CH_3	1.65 (d, $J_{6\text{-}CH3,\ H4}$ = 2.89 Hz, 3H)
6-CH_3	1.42 (d, J = 1.4 Hz, 3H)	H10	5.35(dt, JH10,F = 48.2 Hz, H10, 9-CH2 = 4.6 Hz, 1H)
6-OH	3.15 (br, s, 1H)		
$H13^A$	4.61 (dd, $J_{H13A,H13B}$ = 12.5 Hz, $J_{H13A,H10}$ = 2.97 Hz, 1H)	$H13^A$	4.77 (dd, $J_{H13A,H13B}$ = 11.5 Hz, $J_{H13A,?}$ = 3.9 Hz, 1H)
$H13^B$	4.30 (dd, $J_{H13B,H10}$ = 2.2 Hz, 1H)	$H13^B$	4.03 (dd, $J_{H13B,?}$ = 1.6 Hz, 1H)

7-CH_2 + 8-CH_2 + 9-CH_2 (from 1D TOCSY spectrum,
selective excitation of 12-CH3 at 1.73 ppm, mix = 360 ms)
1.30 - 1.37 (1H)
1.47 - 1.55 (1H)
1.60 - 1.67 (1H)
1.82 - 1.88 (1H)
1.95 - 2.02 (1H)
2.05 - 2.11 (1H)

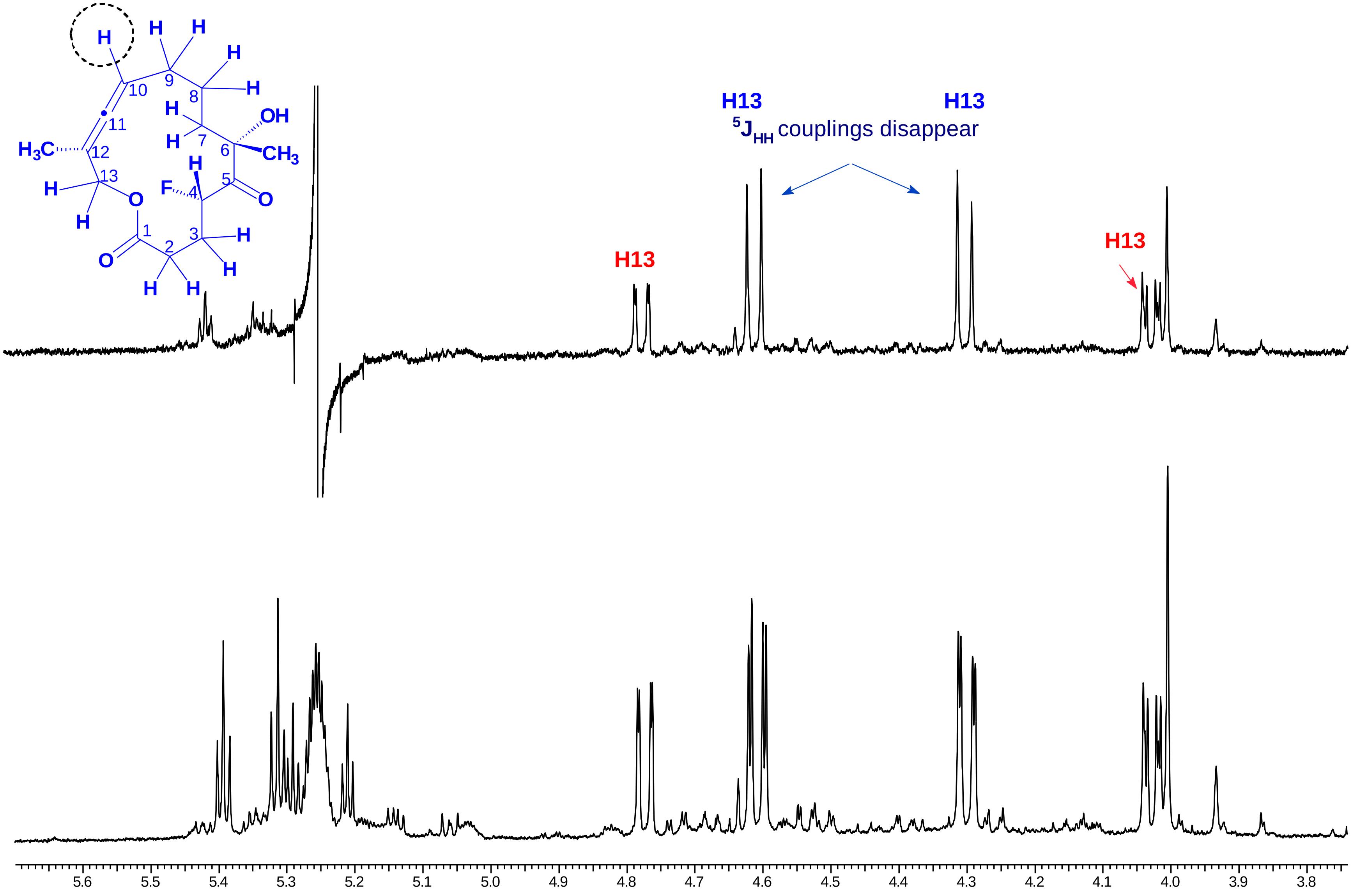
2.30 - 2.48 (2-CH_2 + 3-CH_2, 4H)


H4
H10
$^2J_{HF}$ = 48.1 Hz
$^2J_{HF}$ = 48.2 Hz
H4/H10
12-OH
6-OH
5.40
5.35
5.30
5.25
5.20
5.15
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0


H13
$^5J_{HH}$
H13
H13
$^5J_{HH}$
H13
12-OH
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
H_3C
F
OH
CH_3
O
HO
CH_3
1
2
3
4
5
6
7
8
9
10
11
12
13

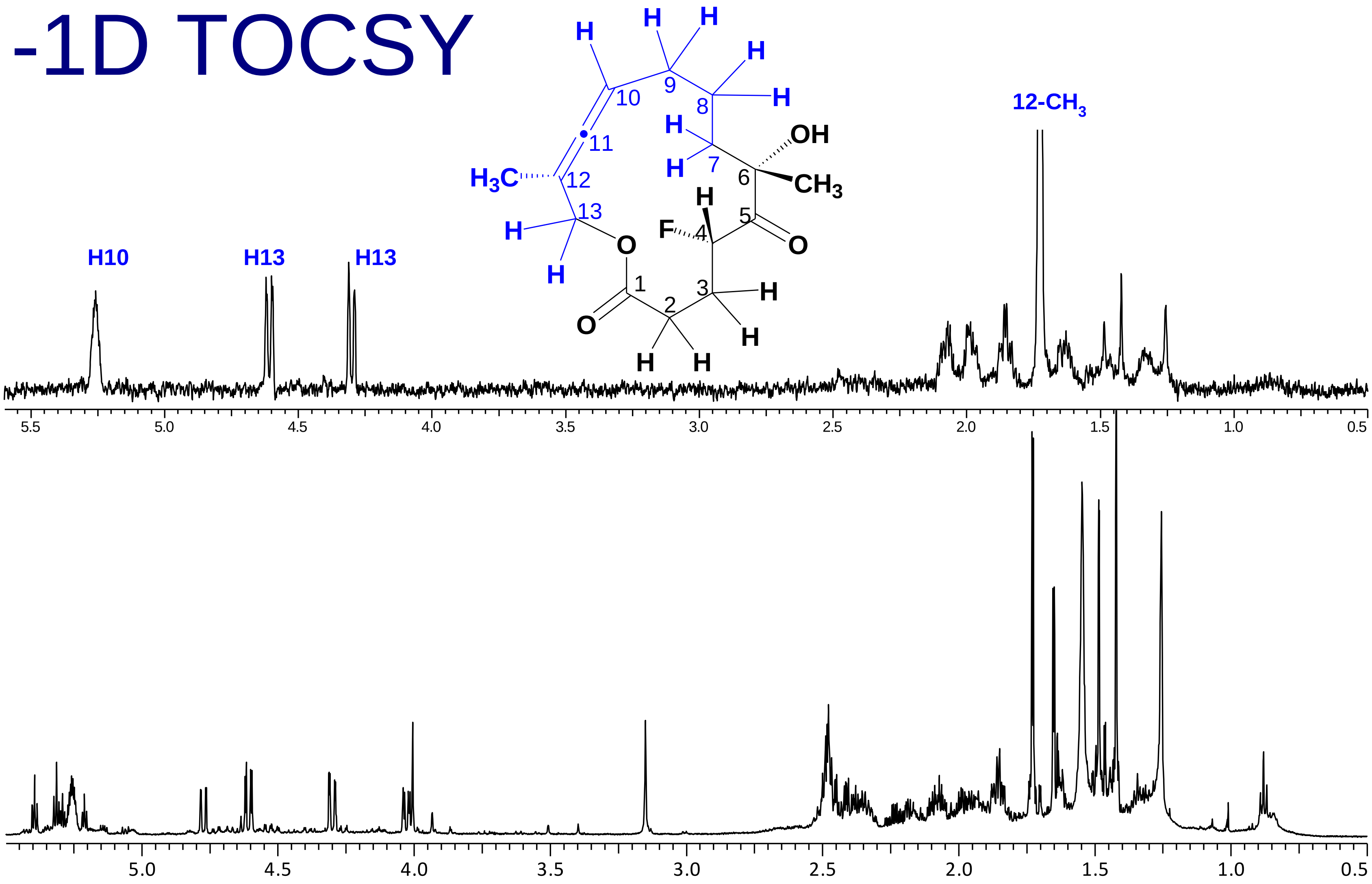

12-CH_3
6-CH_3
12-CH_3
6-CH_3
3.00
2.35
8.62
2.42
3.13
1.75
1.70
1.65
1.60
1.55
1.50
1.45
1.40
H_3C
CH_3
OH
HO
F
O
1
2
3
4
5
6
7
8
9
10
11
12
13

Selective decoupling of H10

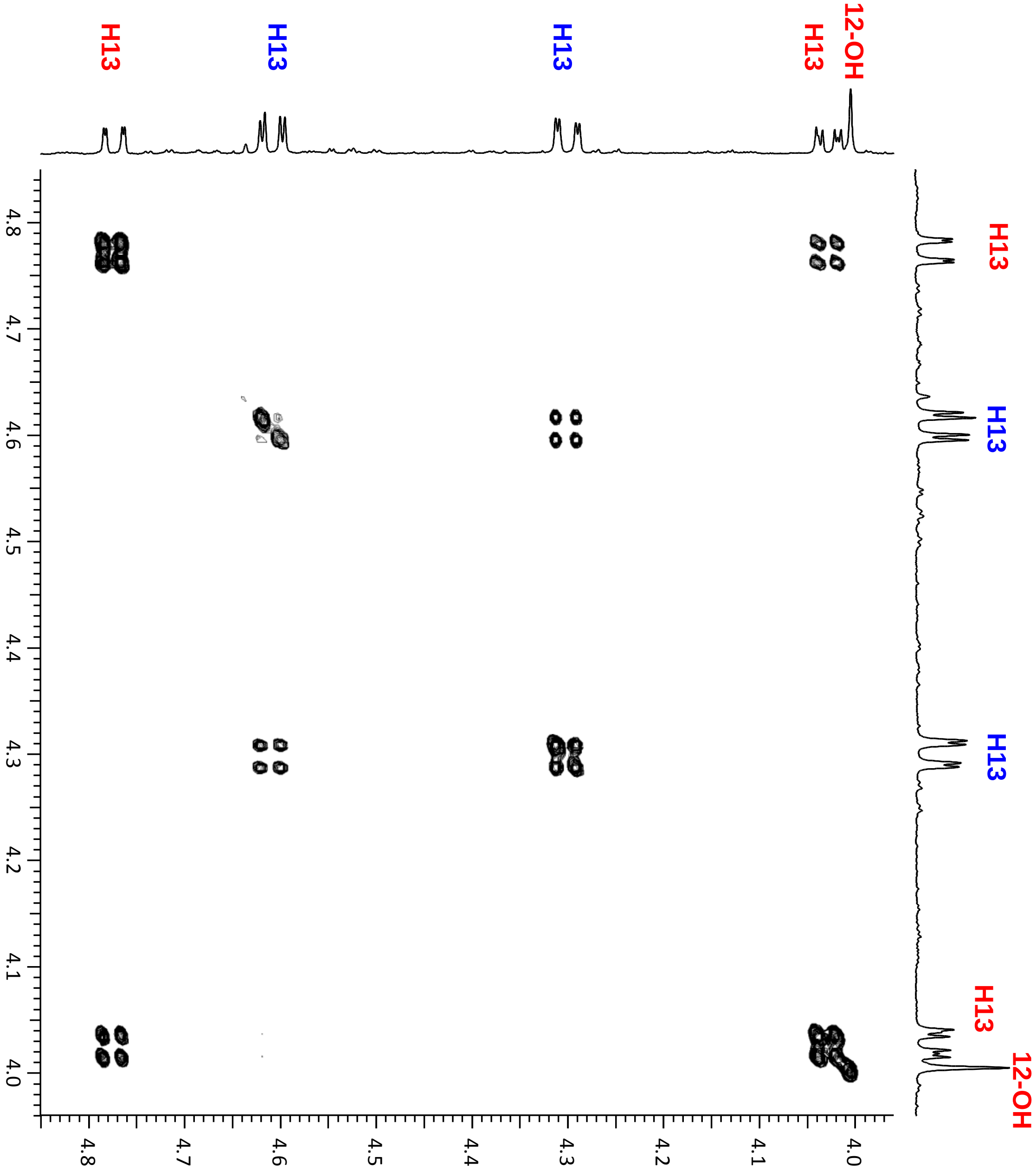


Spin system identification

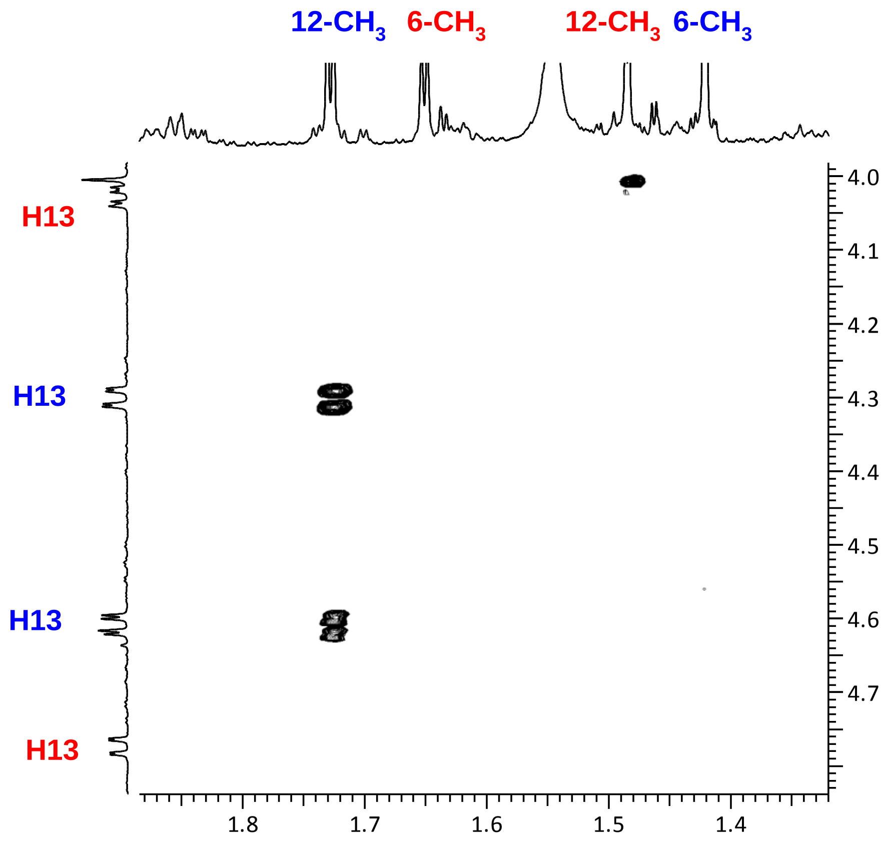
-1D TOCSY

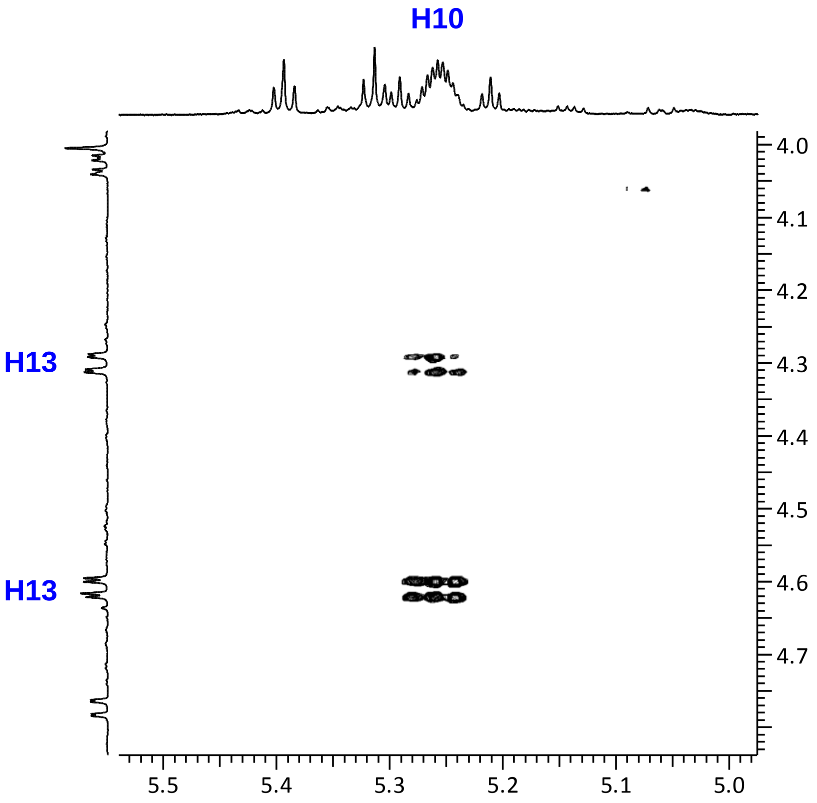


gCOSY


12-OH
H13
H13
H13
H13
H13
H13
H13
H13
12-OH
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0

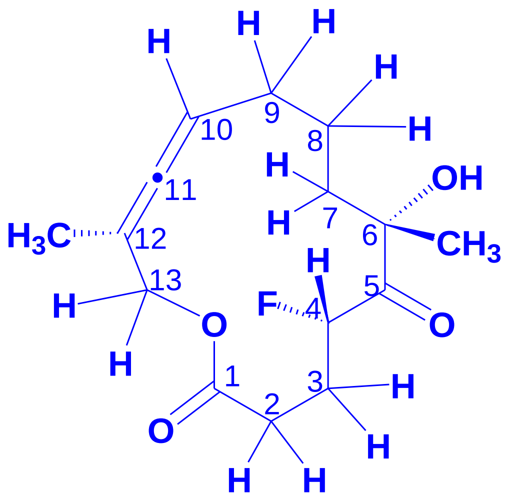
gCOSY

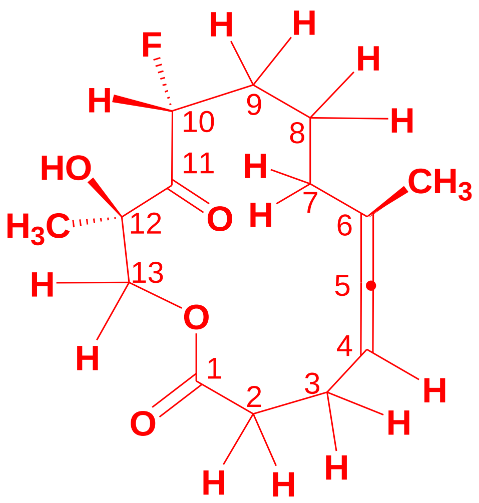

12-CH_3
6-CH_3
12-CH_3
6-CH_3
H13
H13
H13
H13
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
1.8
1.7
1.6
1.5
1.4

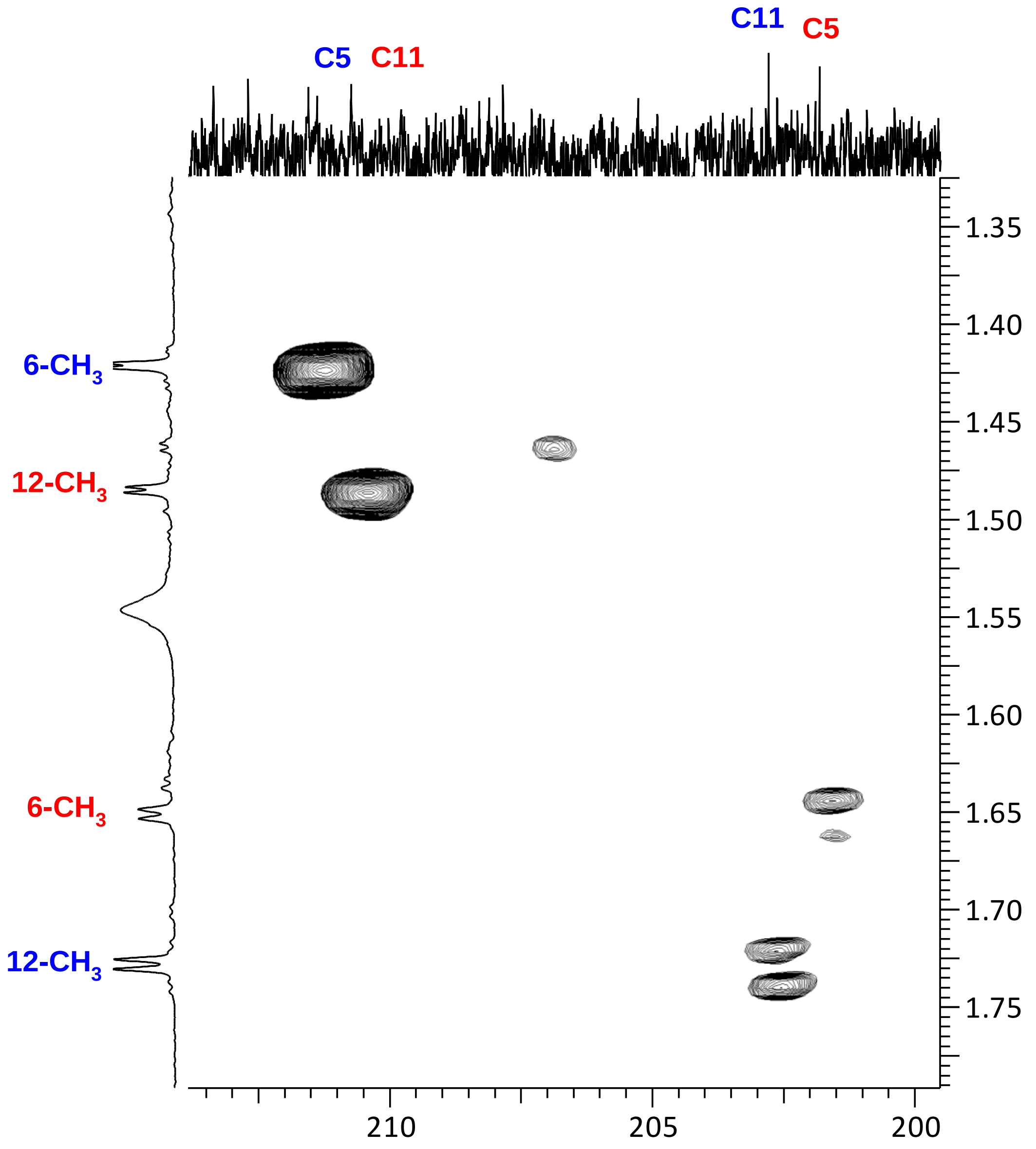

H10
H13
H13
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
5.5
5.4
5.3
5.2
5.1
5.0

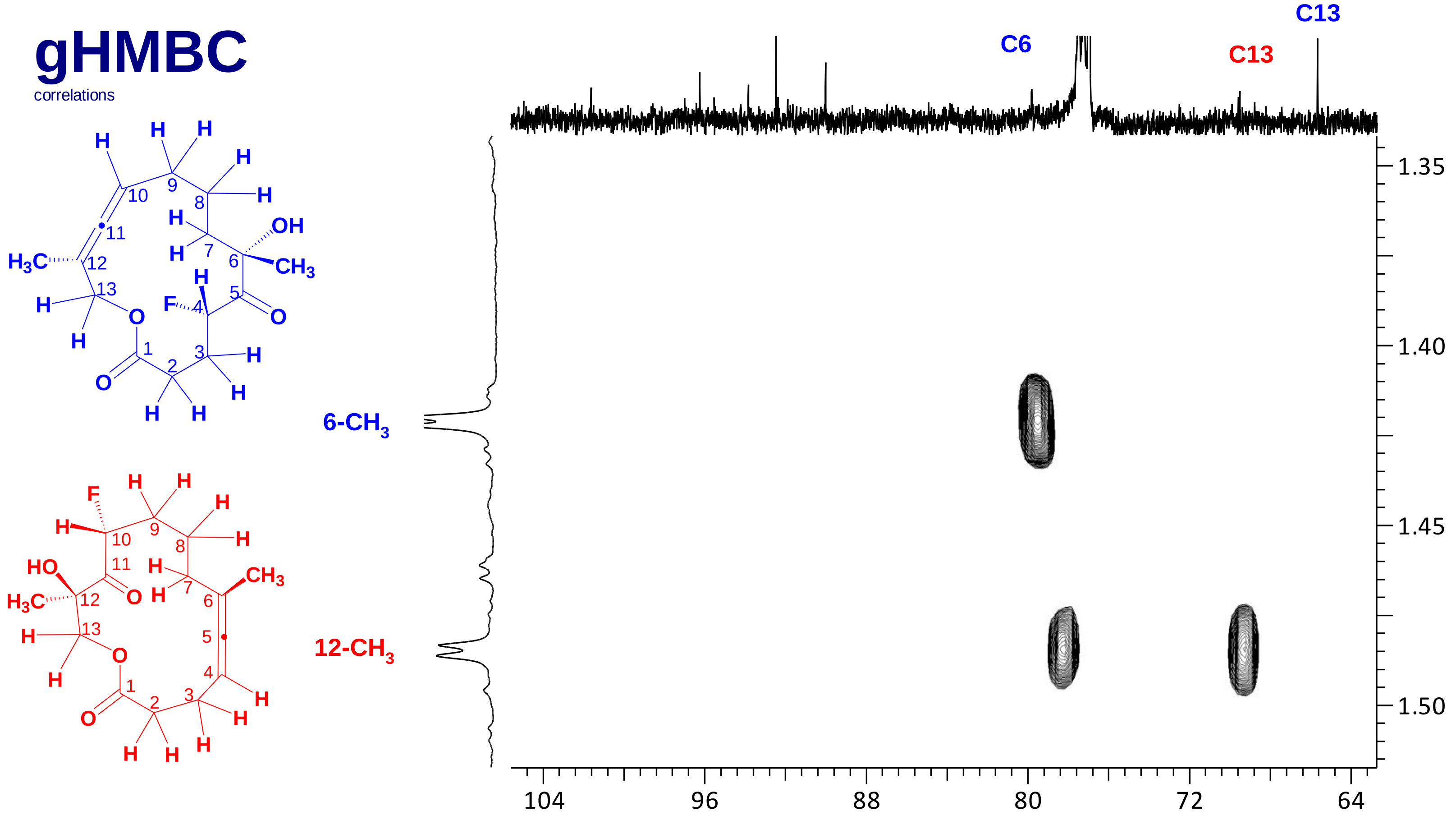
gHMBC

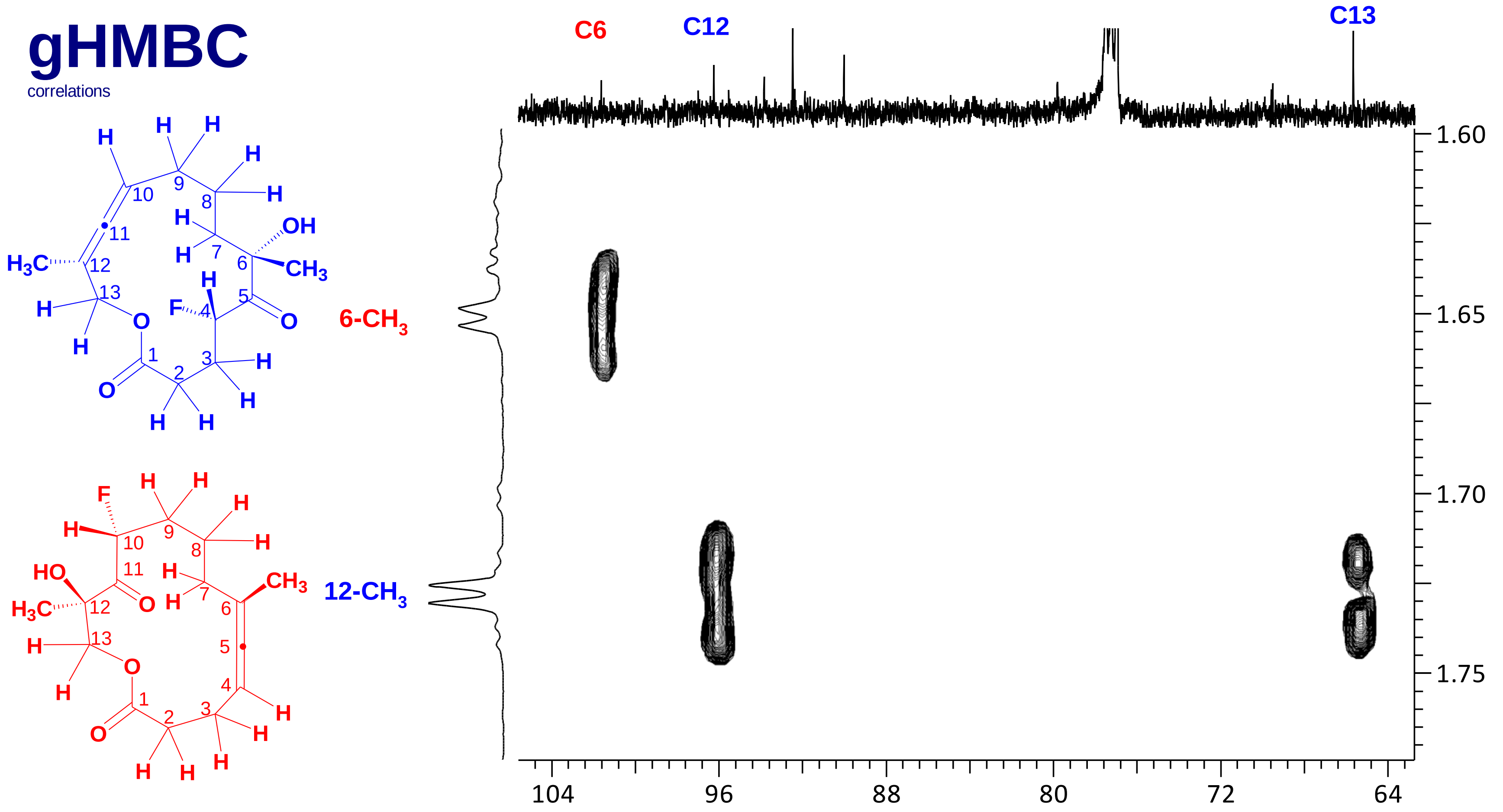
correlations


H
H
H
H
10
9
8
H
11
H
OH
H3C
12
H
7
6
CH3
H
13
H
F
4
5
O
O
H
1
3
H
2
O
H
H
H


F
H
H
H
H
10
9
8
H
11
HO
H
H3C
12
O
H
7
6
CH3
H
13
5
O
H
4
1
3
H
2
H
O
H
H
H

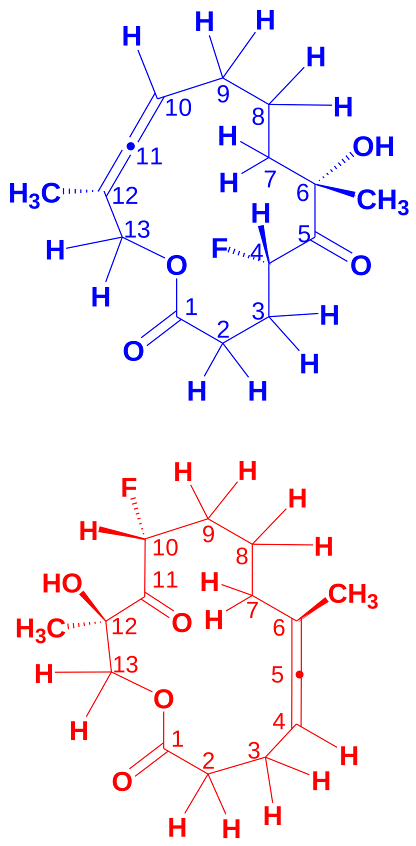



gHMBC
correlations
6-CH3
12-CH3
C6
C13
C13
104
96
88
80
72
64
1.35
1.40
1.45
1.50

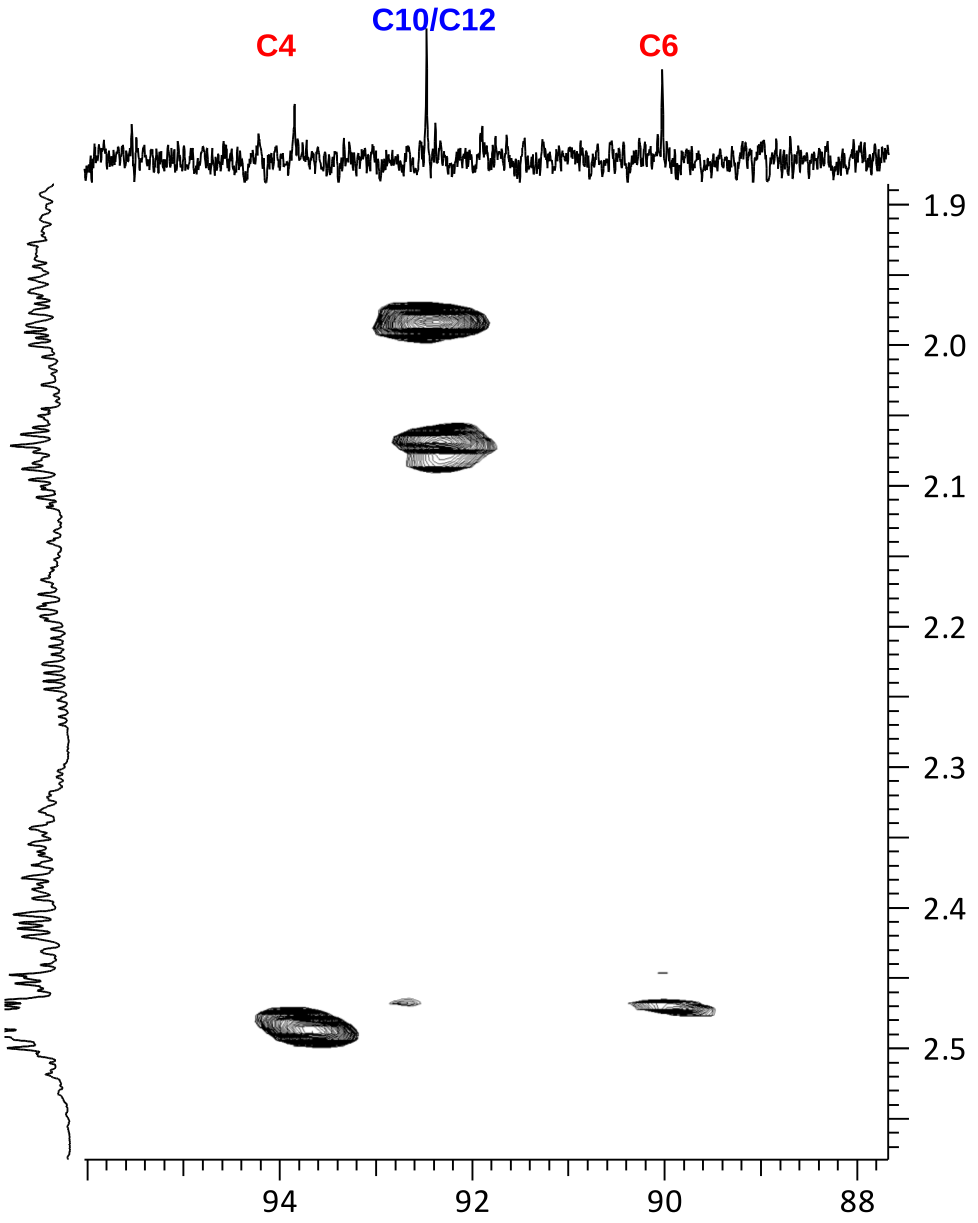

gHMBC
correlations
6-CH3
12-CH3
C6
C12
C13
104
96
88
80
72
64
1.60
1.65
1.70
1.75

gHMBC

correlations

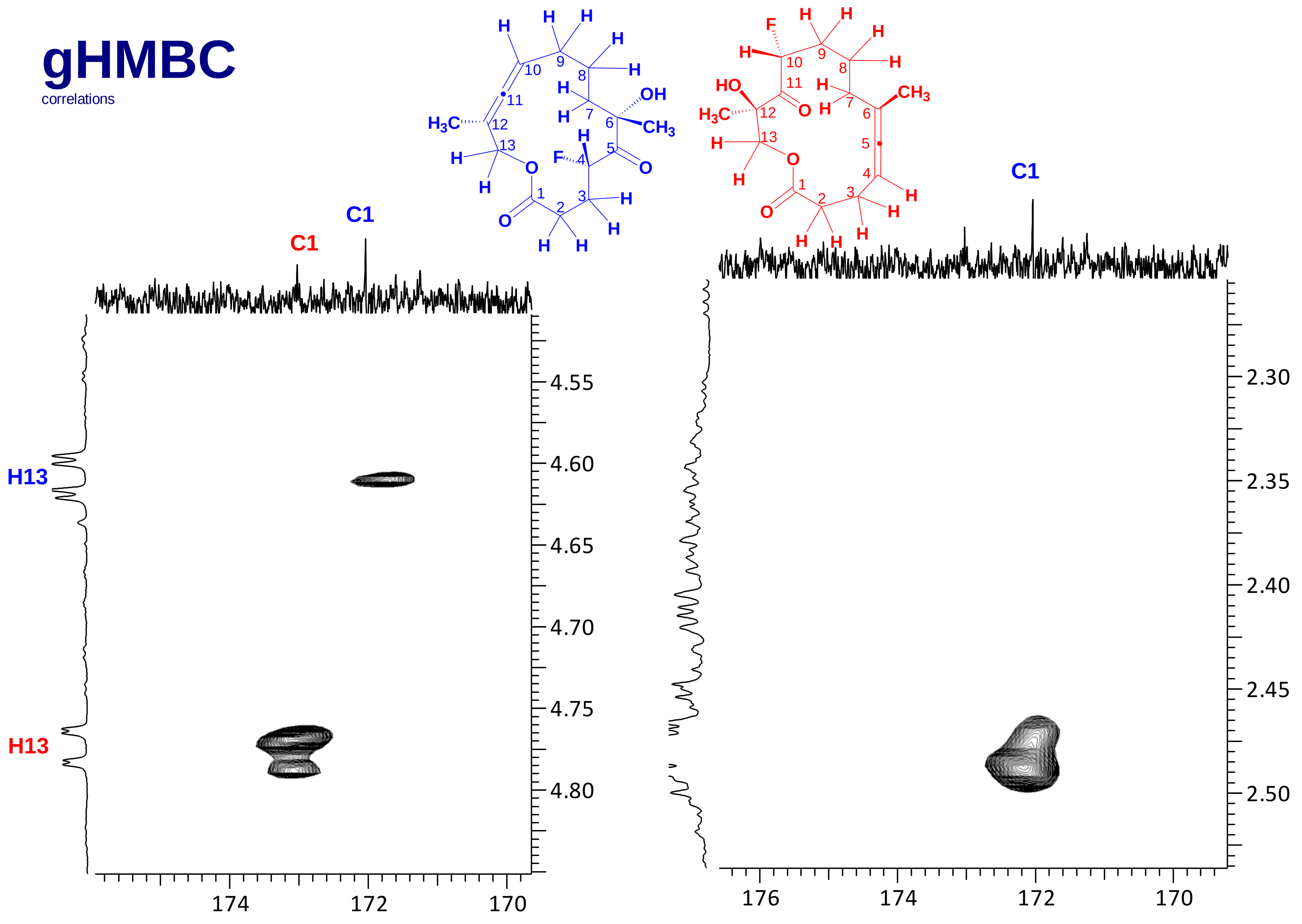


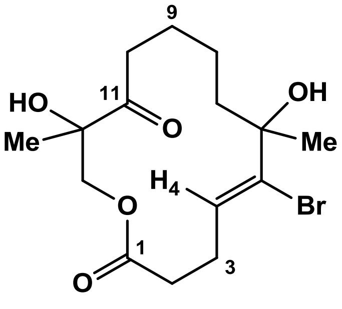
H
H
H
10
9
H
8
H
11
H
OH
H₃C
12
H
7
6
CH₃
13
H
5
H
F
4
O
O
H
1
3
H
O
2
H
H
H
F
H
H
H
9
10
8
H
HO
11
H
7
CH₃
H₃C
12
O
H
6
13
5
H
O
4
H
1
3
H
O
2
H
H
H



gHMBC

correlations

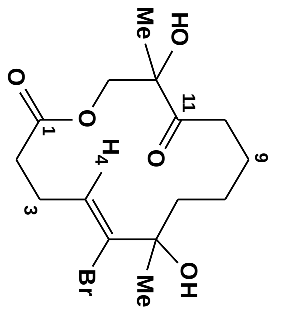




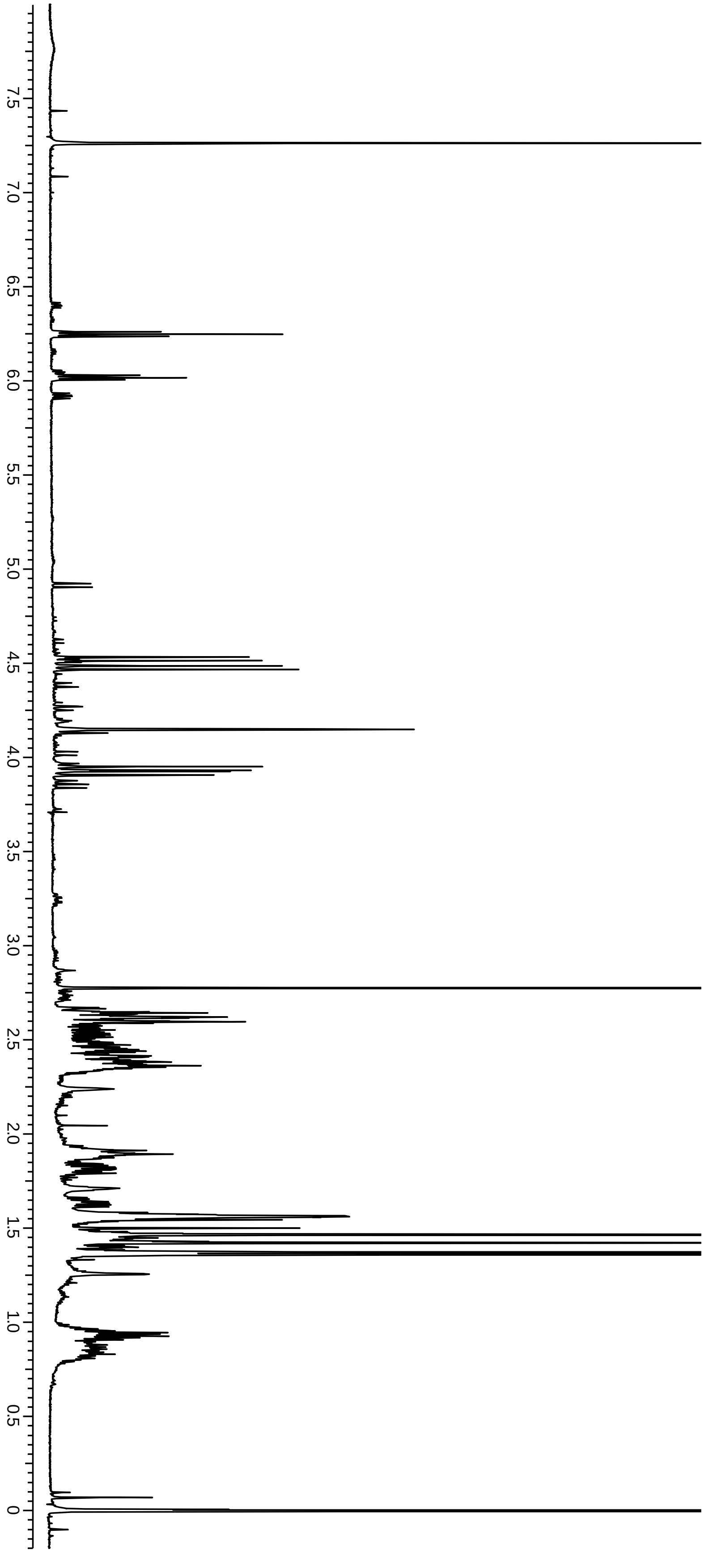
mixture of 2 isomers

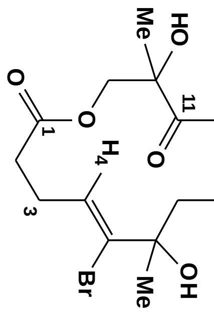
1H NMR chemical shifts (δ/ppm) with coupling constant (J/Hz)		
H2a	2.33 - 2.38	2.45 - 2.48
H2b	2.49 - 2.55	2.60 - 2.65
H3a	2.41 - 2.45	2.32 - 2.36
H3b	2.59 - 2.64	2.52 - 2.58
H4	6.25 (t, $J_{H4,\ 3\text{-}CH2}$ = 7.5 Hz)	6.02 (dd, $J_{H4,\ H3a}$ = 6.8 Hz) $J_{H4,\ H3b}$ = 7.6 Hz
6-CH$_3$	1.47 (s)	1.42 (s)
7-CH$_2$ + 8-CH$_2$ + 9-CH$_2$ + 10-CH$_2$		
	0.88 - 1.00	0.78 - 0.86
	1.38 - 1.43	0.89 - 0.97
	1.42 - 1.48	1.43 - 1.50
	1.78 - 1.84 (2H)	1.54 - 1.58
	1.87 - 1.93	1.60 - 1.66
	2.35 - 2.40	1.86 - 1.94
	2.62 - 2.67	2.37 - 2.42
		2.59 - 2.65
12-CH$_3$	1.38	1.36
12-OH	4.14	
H13^A	3.94 ($^2J_{AB}$ = 11.7 Hz)	3.92 ($^2J_{AB}$ = 11.6 Hz)
H13^B	4.48	4.52

13C NMR chemical shift (δ/ppm)	
C1	172.71 / 172.69
C2	36.33 / 36.22
C3	25.96 / 25.63
C4	126.47 / 125.68
C5	137.15 / 135.14
C6	76.82 / 76.45
6-CH$_3$	26.18 / 29.15
C7	40.83 / 39.70
C8	23.77 / 23.45
C9	25.49 / 24.31
C10	32.52 / 32.40
C11	211.66 / 211.58
C12	77.87
12-CH$_3$	20.78 / 20.74
C13	68.93

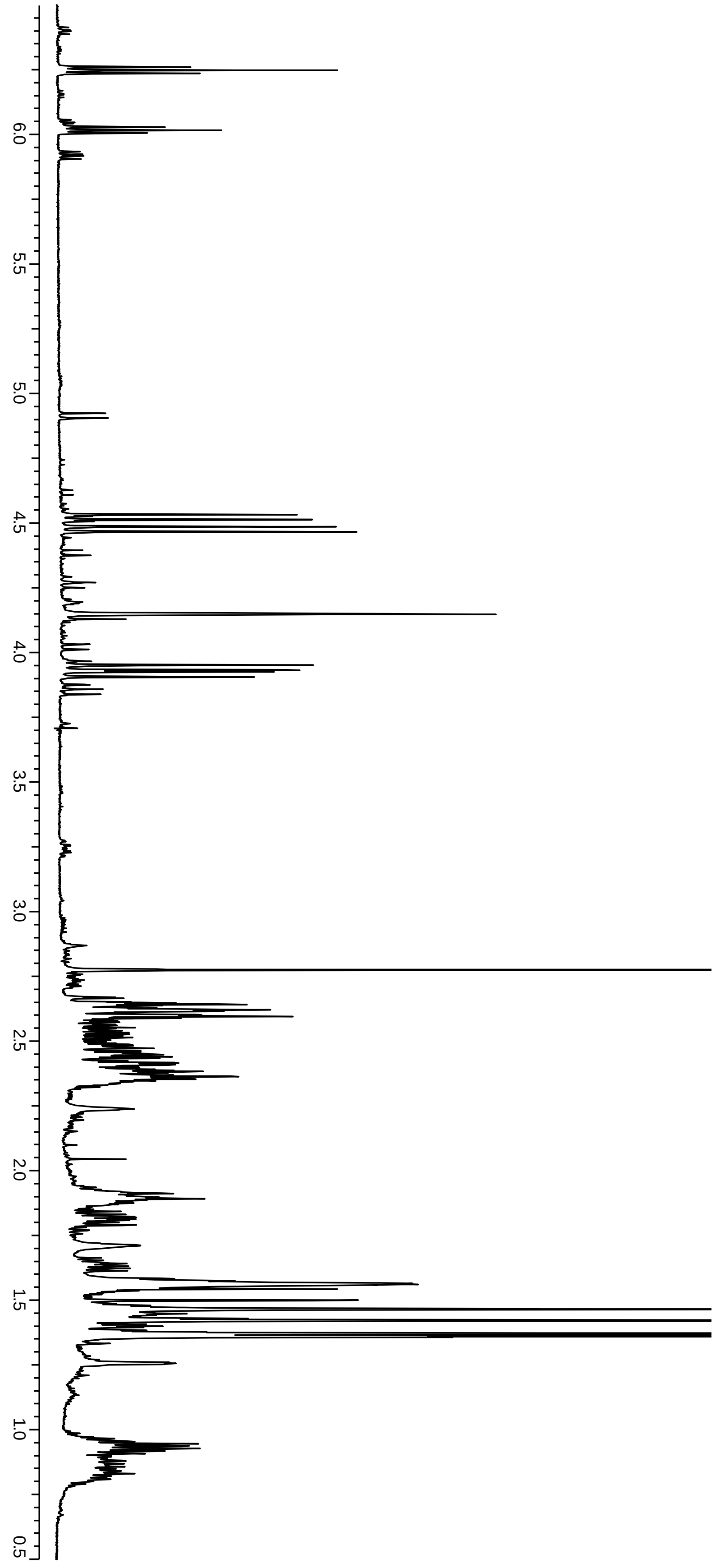

HO
Me
11
9
O
O
1
H4
O
3
OH
Me
Br

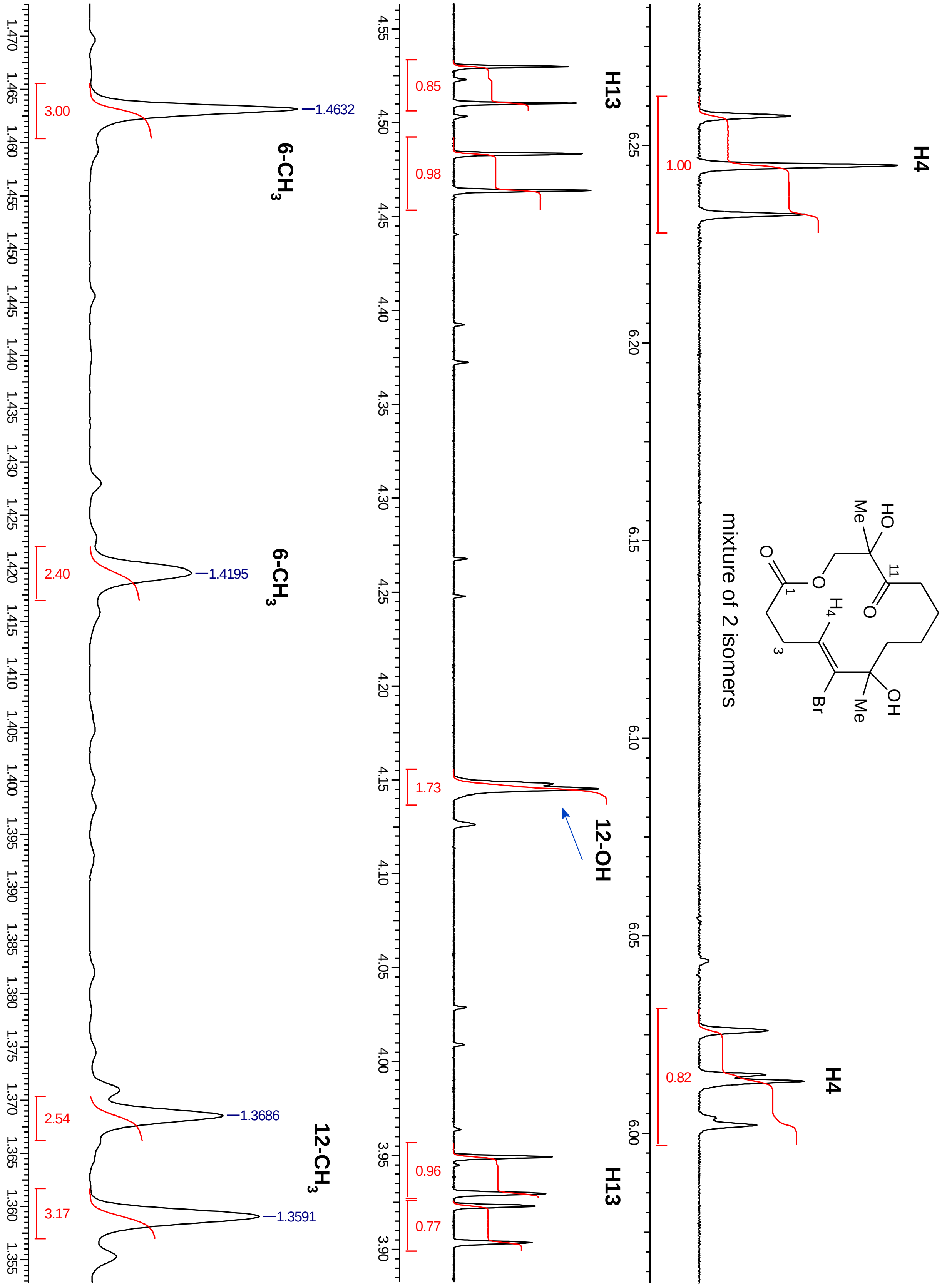
mixture of 2 isomers



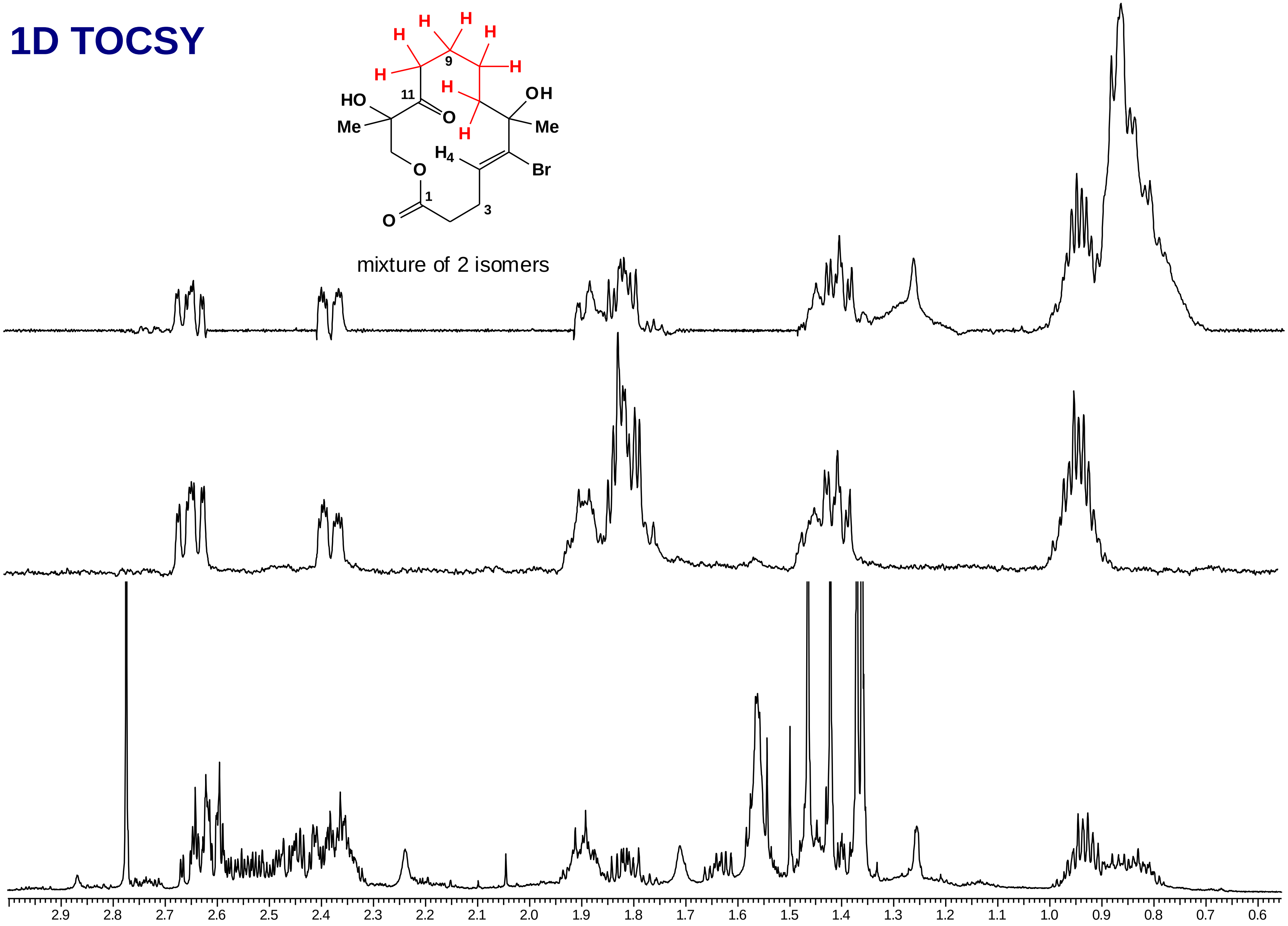

HO
Me
11
O
9
H4
O
1
3
OH
Me
Br

mixture of 2 isomers

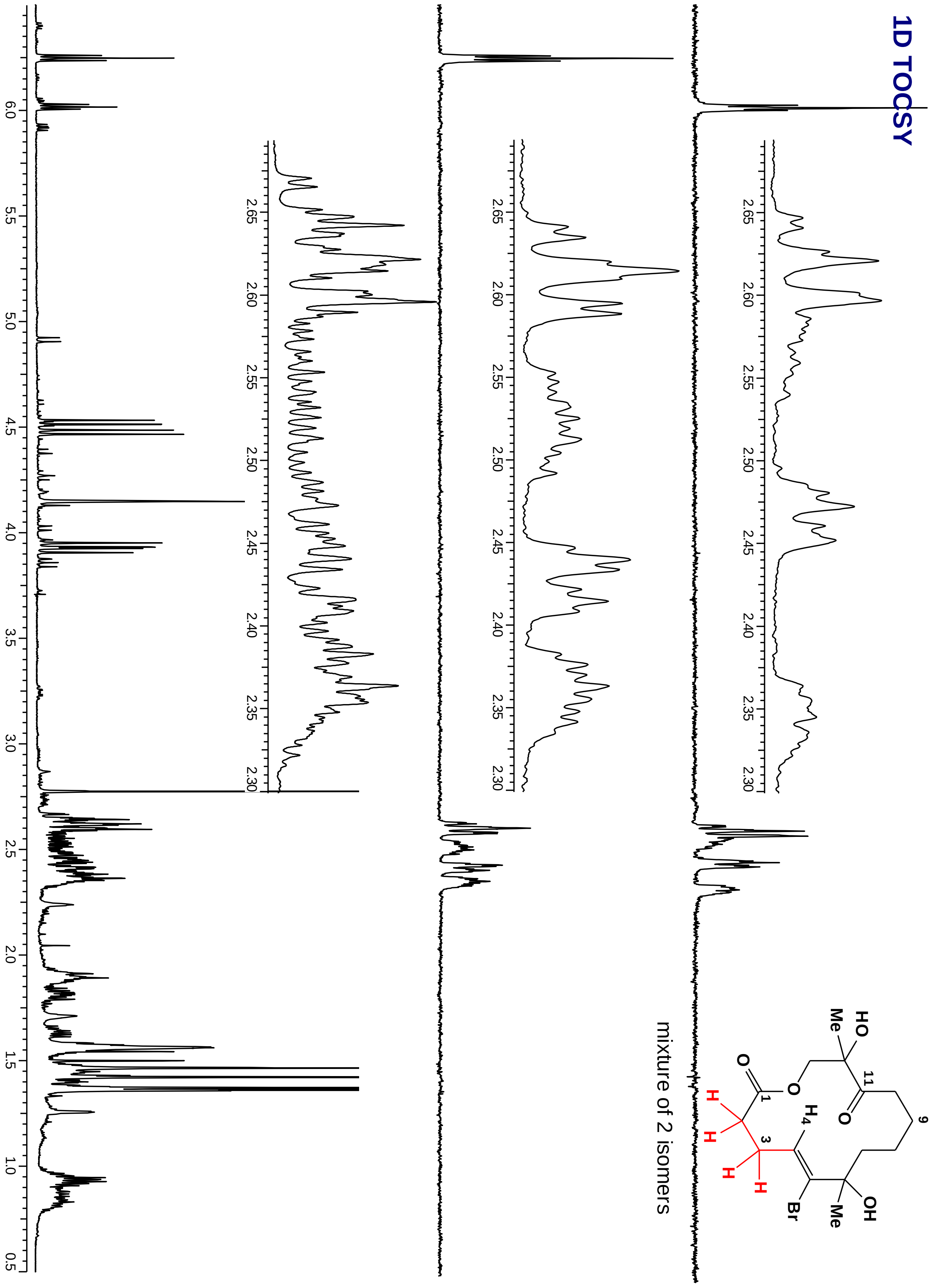


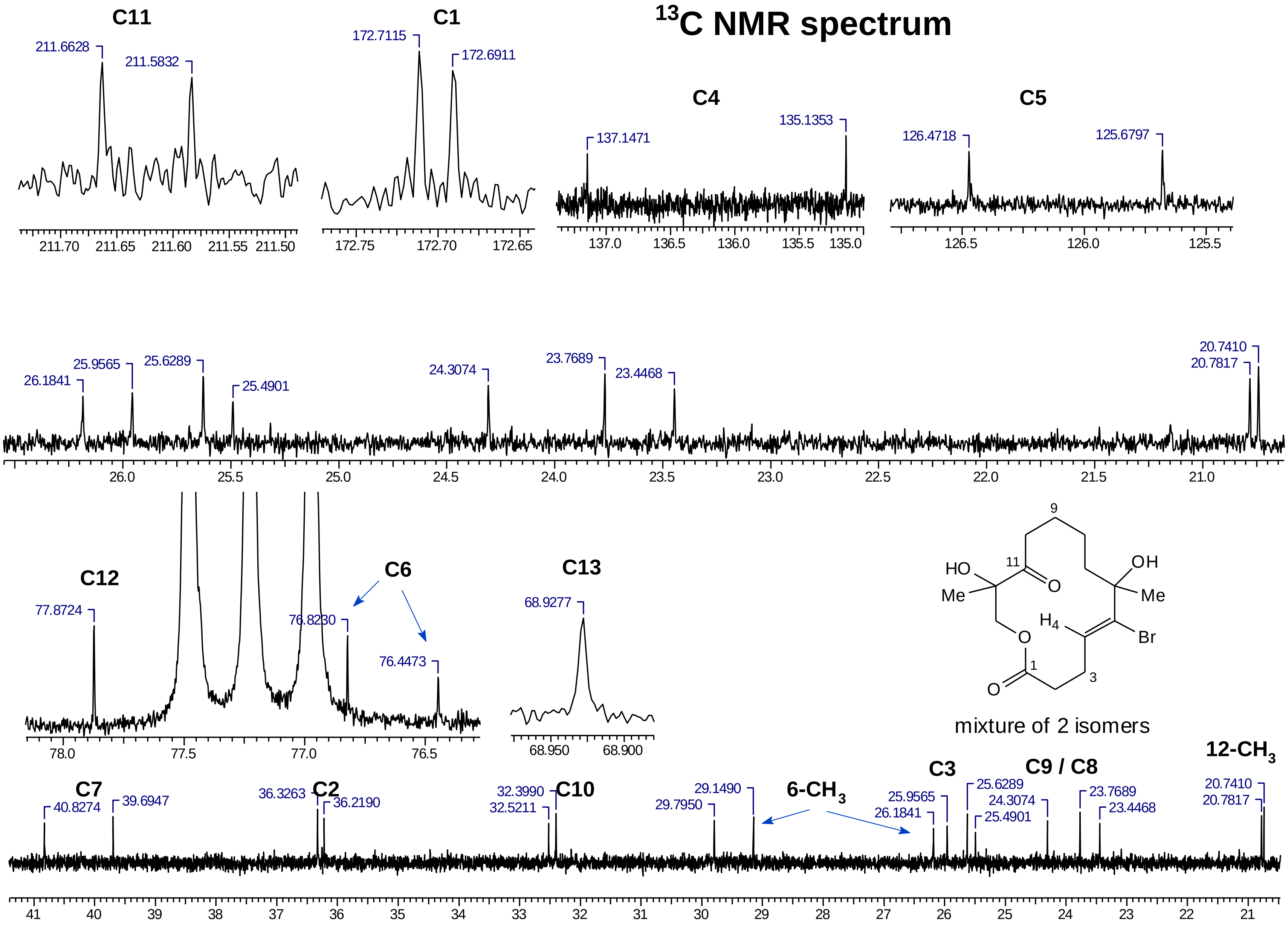

H4
1.00
H4
0.82
mixture of 2 isomers
HO
Me
O
O
11
9
O
H4
1
3
OH
Me
Br
H13
0.85
0.98
12-OH
1.73
H13
0.96
0.77
6-CH3
3.00
1.4632
6-CH3
2.40
1.4195
12-CH3
2.54
1.3686
3.17
1.3591
6.25
6.20
6.15
6.10
6.05
6.00
4.55
4.50
4.45
4.40
4.35
4.30
4.25
4.20
4.15
4.10
4.05
4.00
3.95
3.90
1.470
1.465
1.460
1.455
1.450
1.445
1.440
1.435
1.430
1.425
1.420
1.415
1.410
1.405
1.400
1.395
1.390
1.385
1.380
1.375
1.370
1.365
1.360
1.355

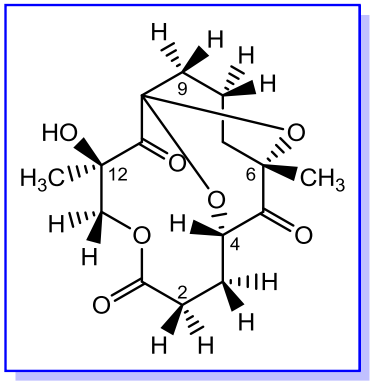
1D TOCSY



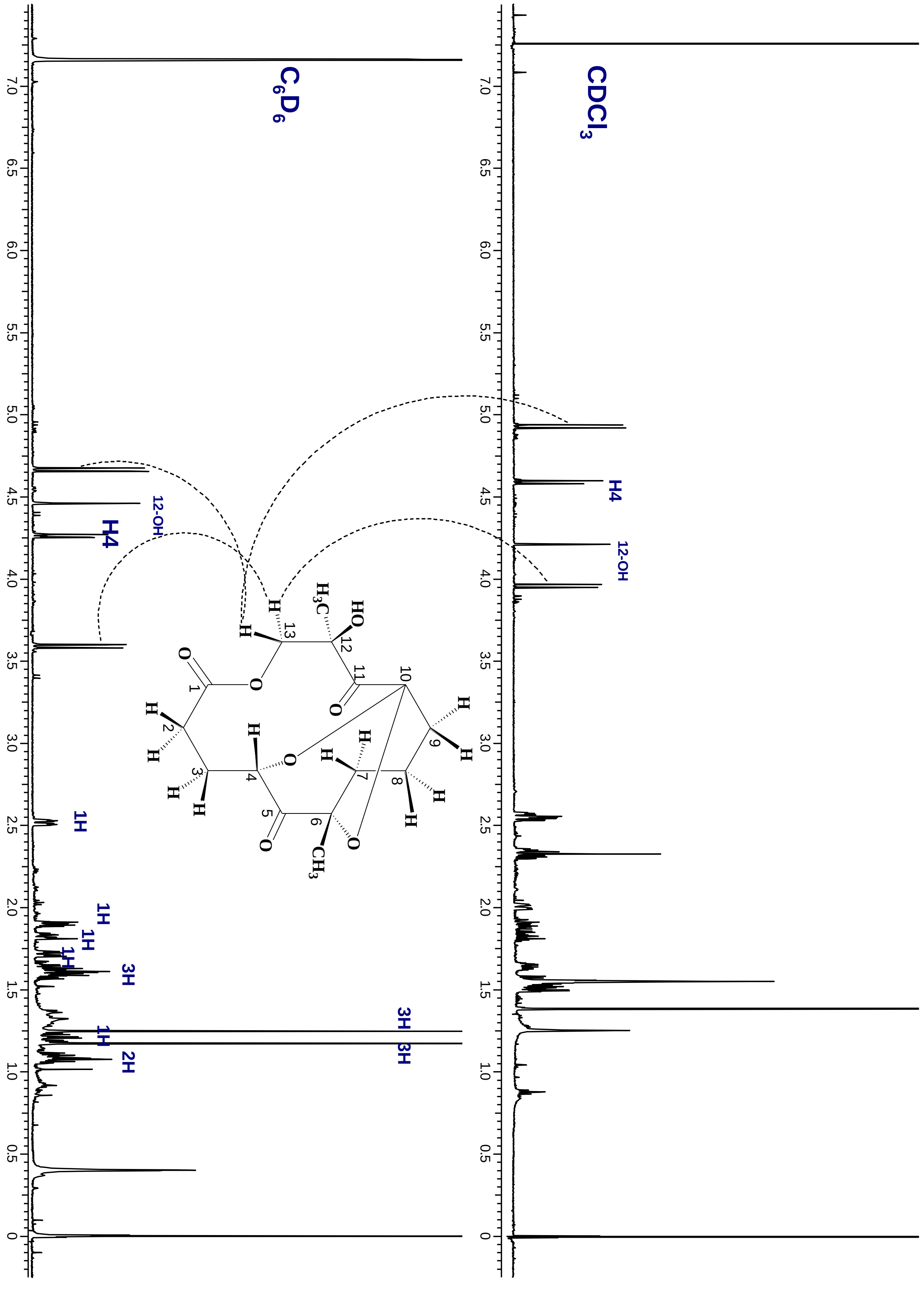
mixture of 2 isomers

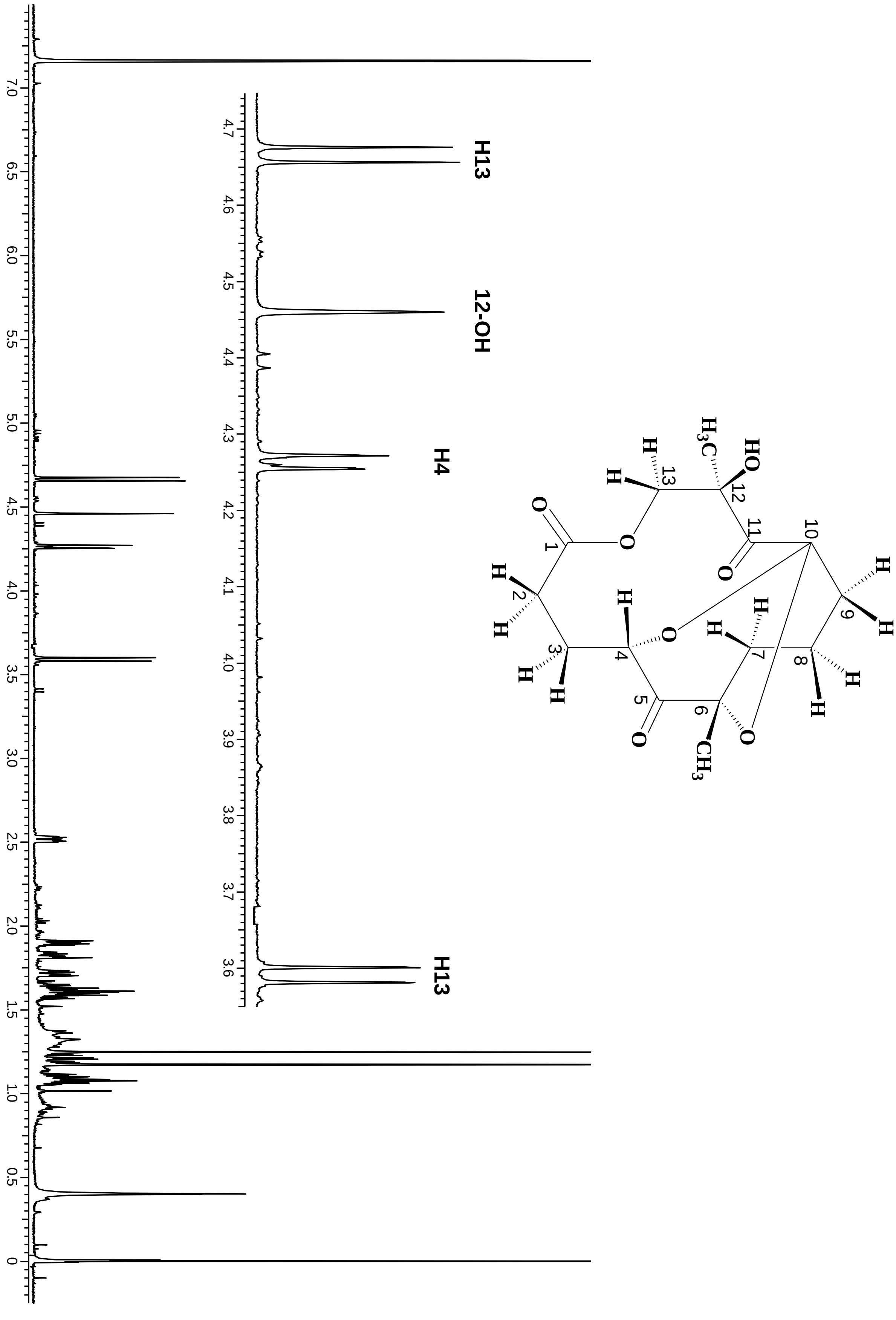

1D TOCSY
mixture of 2 isomers
HO
Me
O
O
11
O
9
1
H4
3
H
H
H
H
OH
Me
Br
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.65
2.60
2.55
2.50
2.45
2.40
2.35
2.30

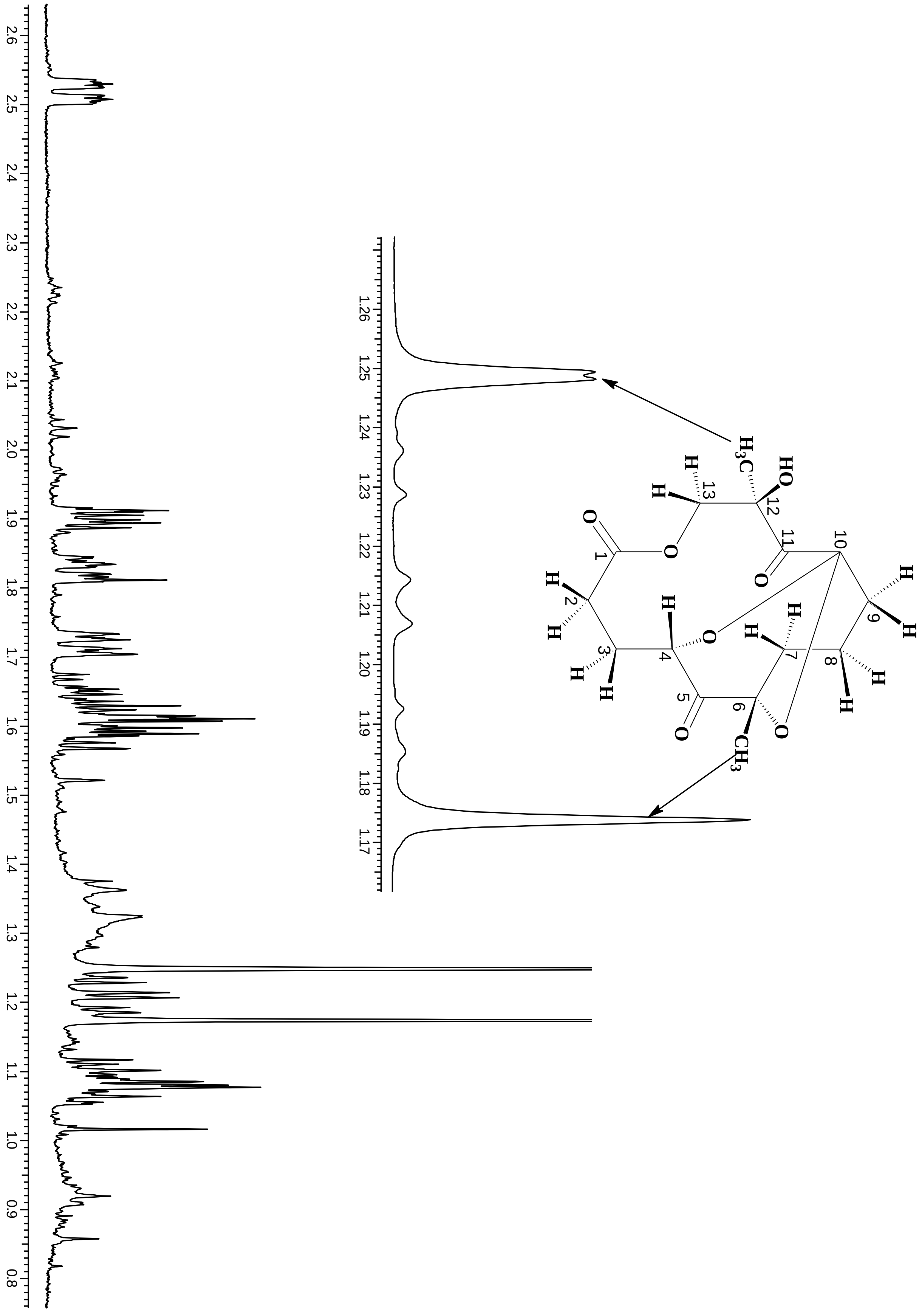

^{13}C NMR spectrum
C11
211.6628
211.5832
211.70 211.65 211.60 211.55 211.50
C1
172.7115
172.6911
172.75 172.70 172.65
C4
137.1471
135.1353
137.0 136.5 136.0 135.5 135.0
C5
126.4718
125.6797
126.5 126.0 125.5
26.1841
25.9565
25.6289
25.4901
24.3074
23.7689
23.4468
20.7410
20.7817
26.0 25.5 25.0 24.5 24.0 23.5 23.0 22.5 22.0 21.5 21.0
C12
77.8724
C6
76.8230
76.4473
78.0 77.5 77.0 76.5
C13
68.9277
68.950 68.900
HO
Me
11
O
9
OH
Me
H4
Br
O
1
O
3
mixture of 2 isomers
C7
40.8274
39.6947
36.3263
C2
36.2190
32.3990
32.5211
C10
29.1490
29.7950
6-CH3
25.9565
26.1841
C3
25.6289
24.3074
25.4901
C9 / C8
23.7689
23.4468
12-CH3
20.7410
20.7817
41 40 39 38 37 36 35 34 33 32 31 30 29 28 27 26 25 24 23 22 21



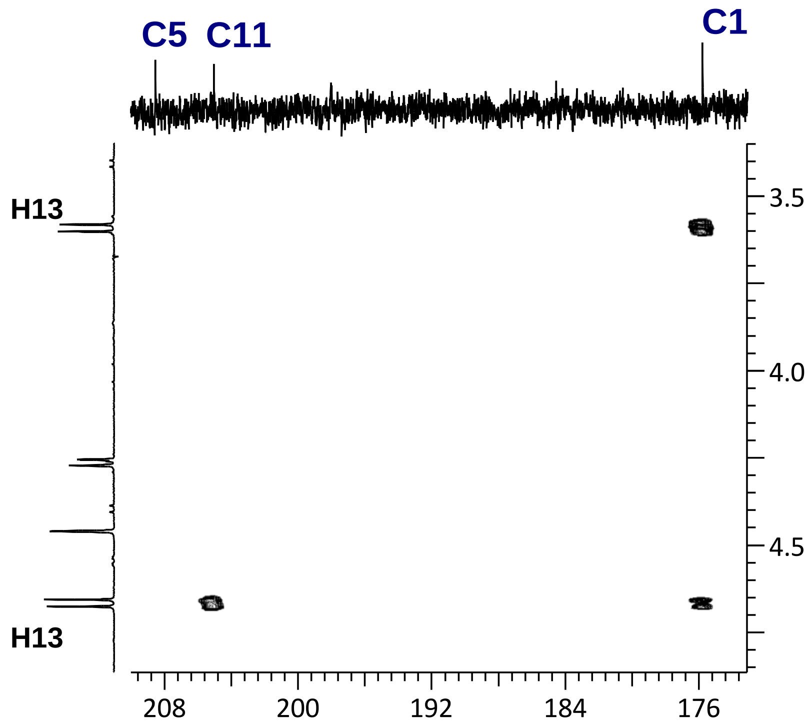
1H NMR chemical shifts (δ/ppm) with coupling constant (*J*/Hz), C6D6	**13C NMR chemical shift (δ/ppm)**
4.25 (d, $J_{H4,\ H3}$ = 9.5Hz, **H4)**	175.8 ----- C1
4.46 (s, **12-OH)**	32.2 ----- C2
4.66, 3.60 (2H, d, J = 11.8Hz, **H13)**	23.3 ----- C3
2.51 (1H, m, **H9**)	73.1 ----- C4
1.92 - 1.88 (1H, m, **H2**)	208.6 ----- C5
1.85 - 1.81 (1H, m, **H2**)	82.3 ----- C6
1.73 - 1.70 (1H, m, **H9**)	32.0 ----- C7
1.66 - 1.57 (3H, m, **H7**, **H3**)	16.3 ----- C8
1.25 (3H,s, **12-CH_3**)	29.9 ----- C9
1.17 (3H,s, **6-CH_3**)	100.0 ----- C10
1.24 - 1.19 (1H, m, **H7**)	205.0 ----- C11
1.12 - 1.05 (2H, m, **H8**)	83.0 ----- C12
	72.8 ----- C13
	24.1 ----- 6-CH_3
	23.6 ----- 12-CH_3

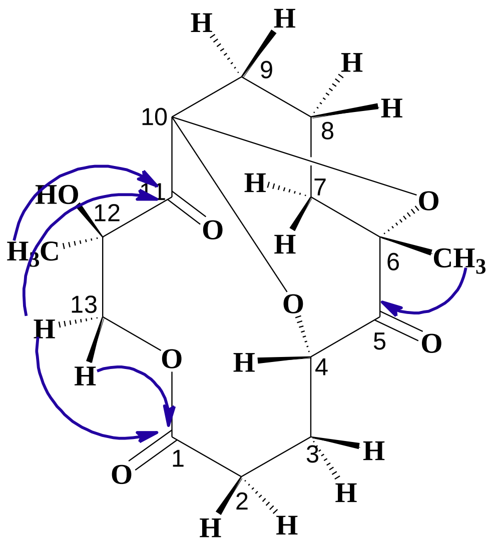

CDCl3
C6D6
H4
12-OH
H4
12-OH
1H
1H
1H
1H
3H
1H
2H
3H
3H
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
HO
H3C
H
H
O
O
O
O
O
O
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13

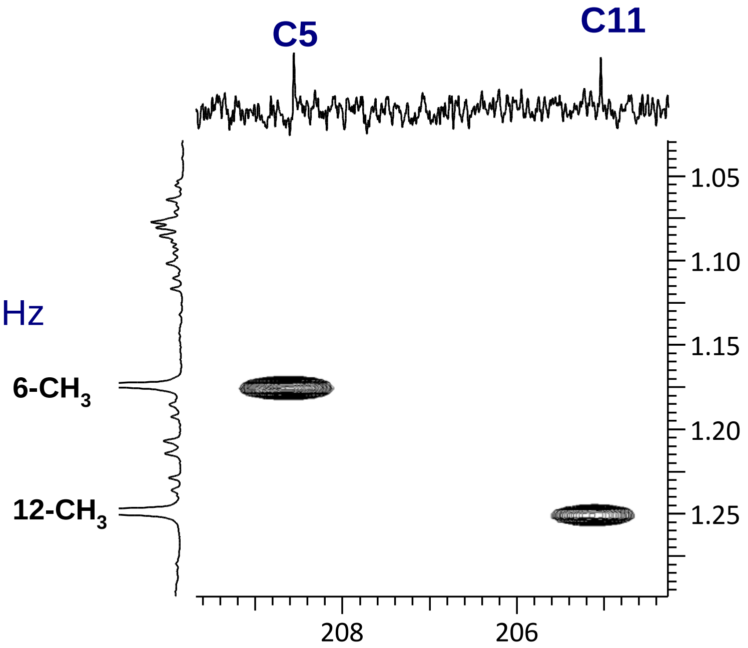

H13
12-OH
H4
H13
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
HO
H_3C
CH_3
O
H


2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
1.26
1.25
1.24
1.23
1.22
1.21
1.20
1.19
1.18
1.17
H3C
HO
CH3
O
H
1
2
3
4
5
6
7
8
9
10
11
12
13

GHMBC

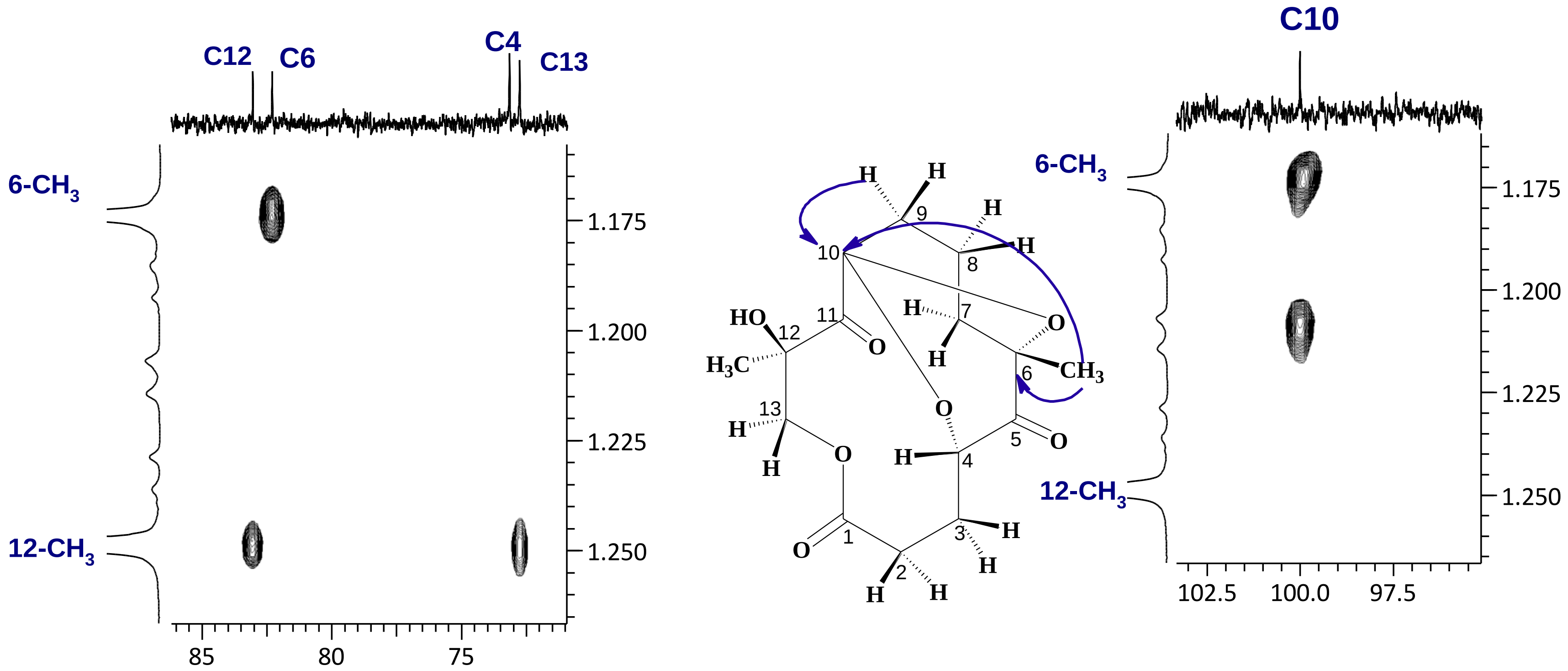



H
H
9
H
H
8
10
HO
11
12
O
H
7
O
H
H₃C
6
CH₃
13
O
H
O
5
O
H
4
H
O
1
3
H
H
2
H
H

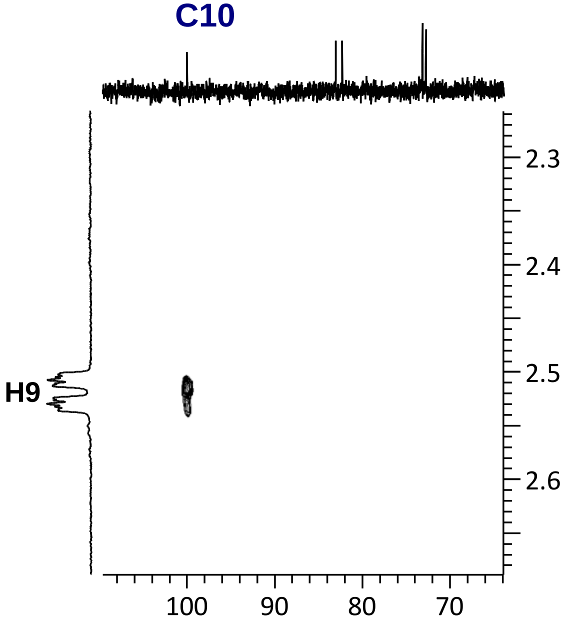


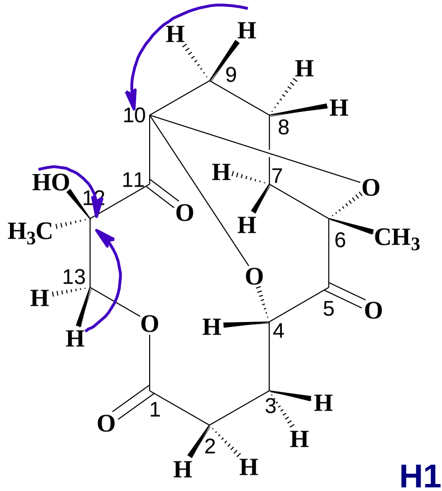
Long-range correlations in C_6D_6; $^1J_{CH}$ was set to 132 Hz

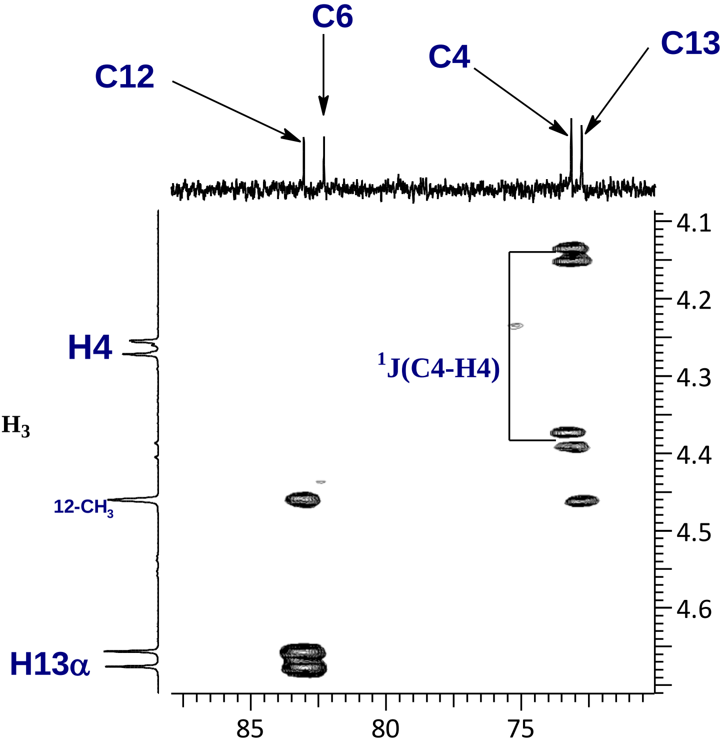
Long-range correlations in C_6D_6; $^1J_{CH}$ was set to 132 Hz



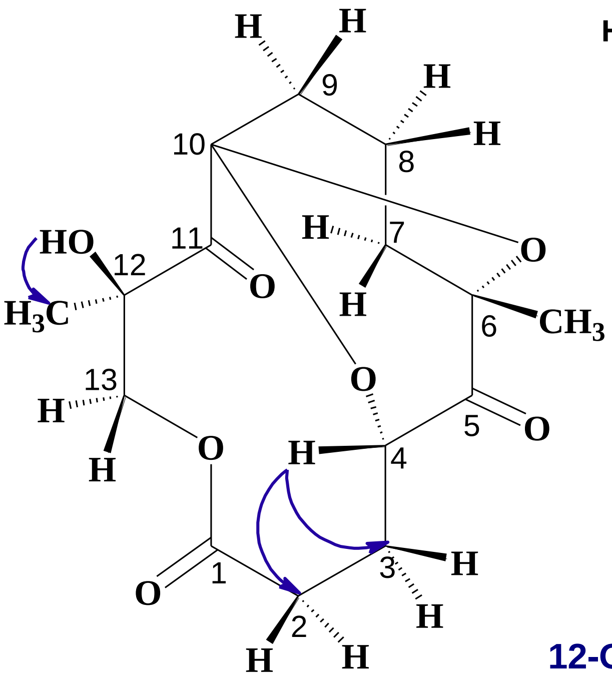
Long-range correlations in C_6D_6; $^1J_{CH}$ was set to 132 Hz

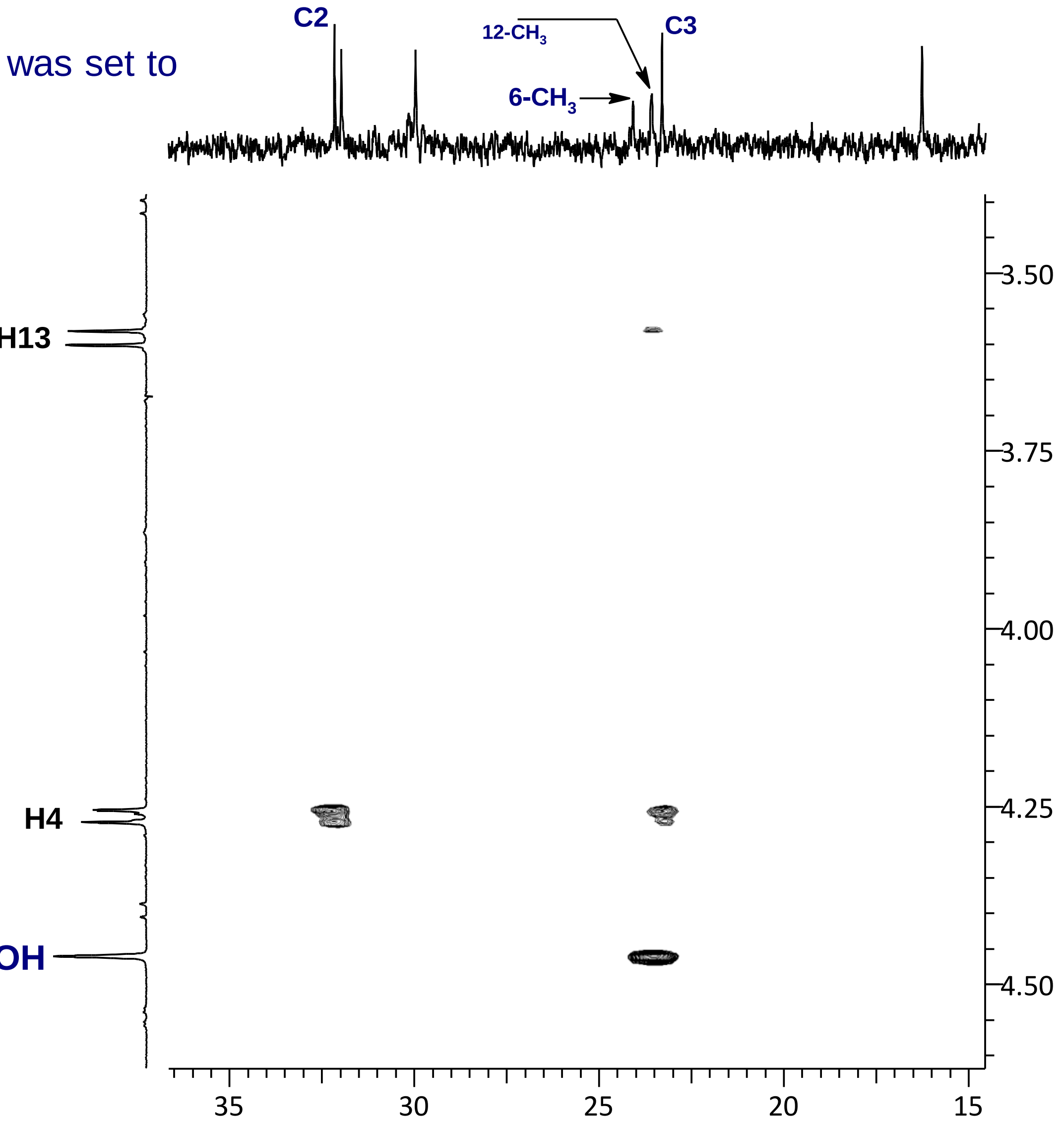



H
H
9
H
H
8
10
HO
11
12
H3C
O
H
7
O
H
6
CH3
13
O
H
5
O
H
O
H
4
3
H
O
1
H
2
H
H

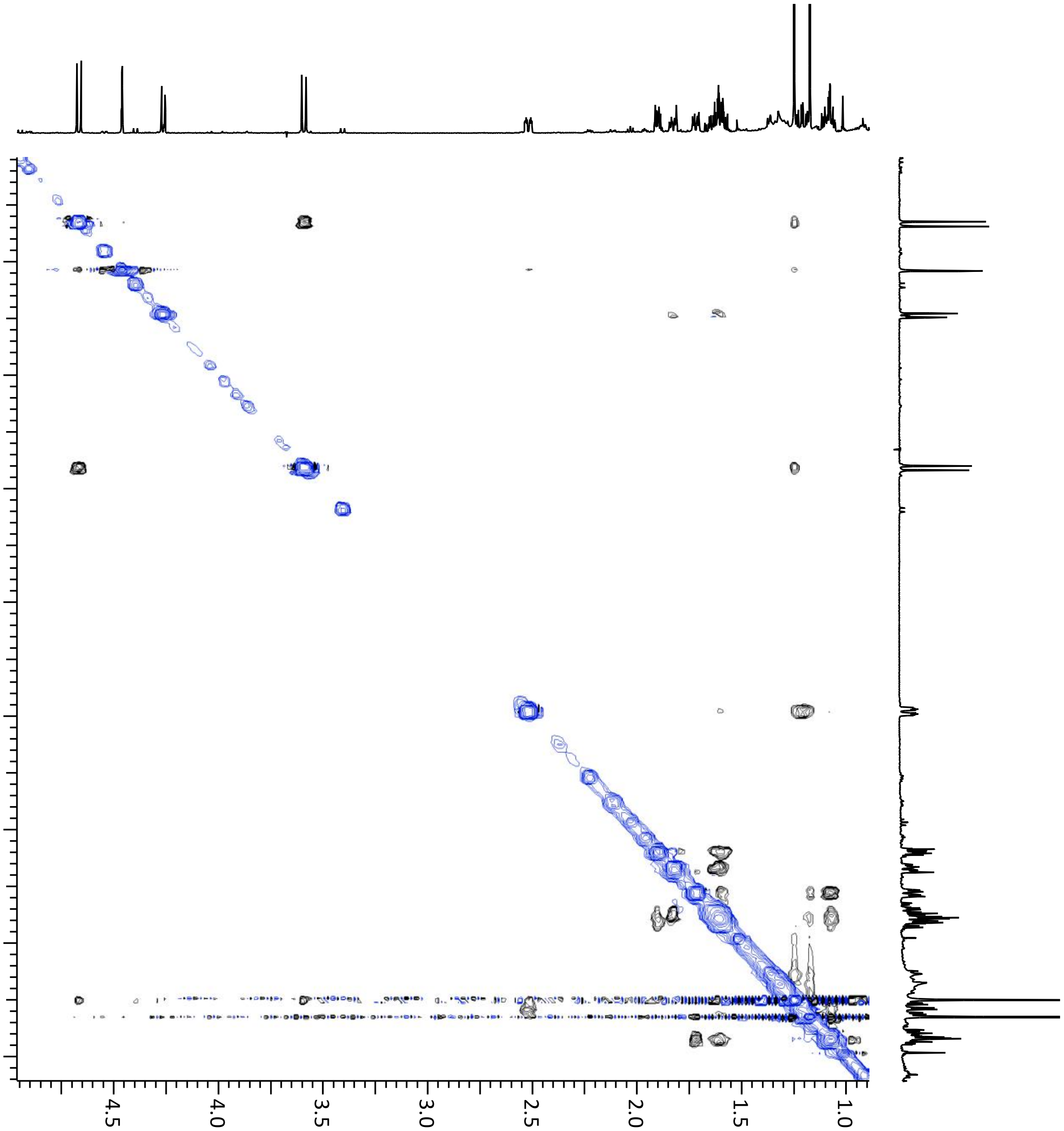


Long-range correlations in C_6D_6; $^1J_{CH}$ was set to 132 Hz


HO
12
11
10
9
8
7
6
5
4
3
2
1
13
O
H_3C
CH_3
H

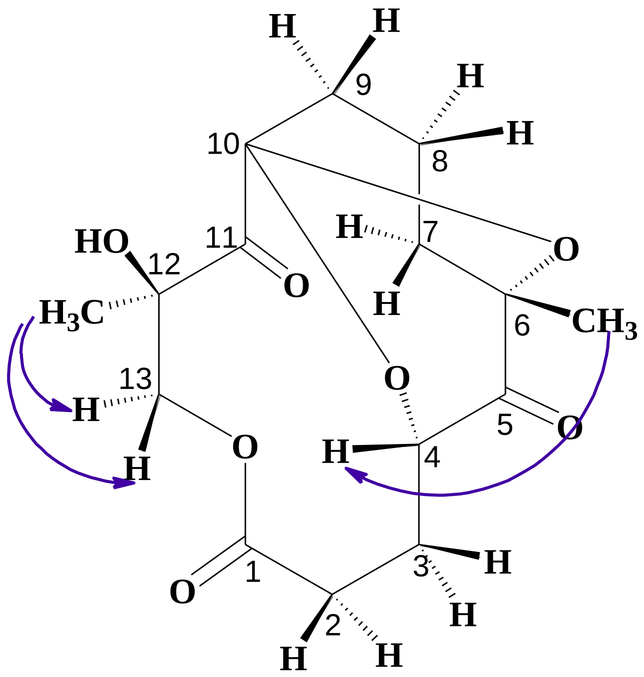


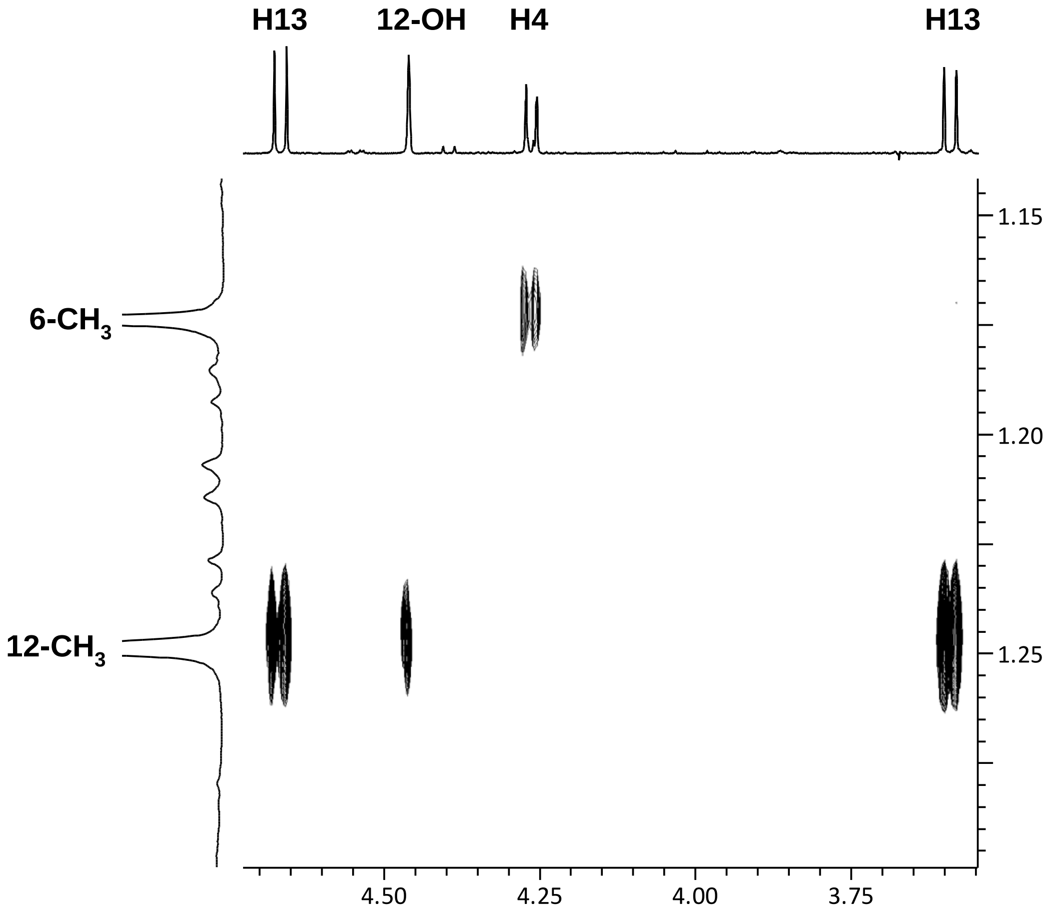
NOESY



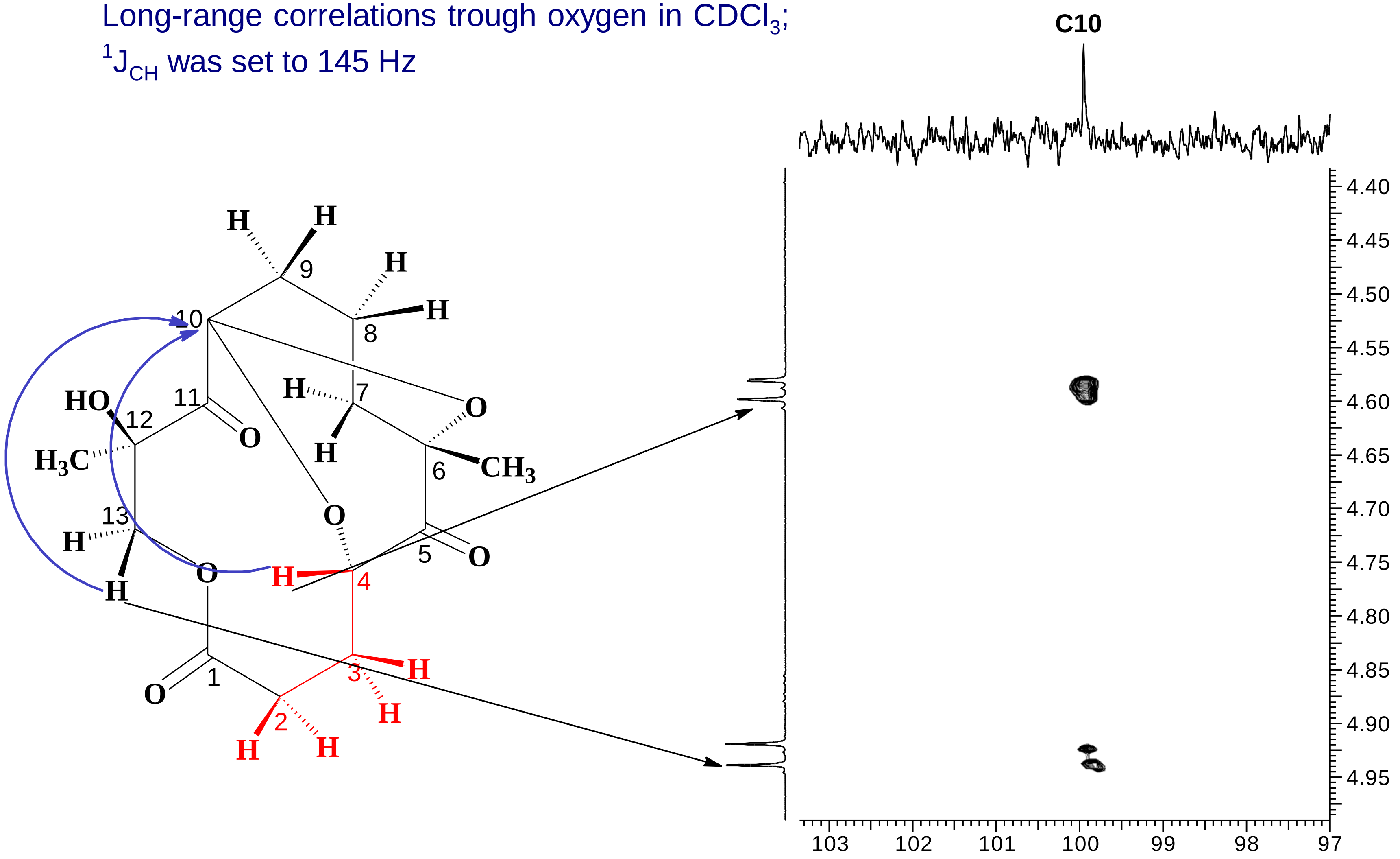
NOESY-----

expansion

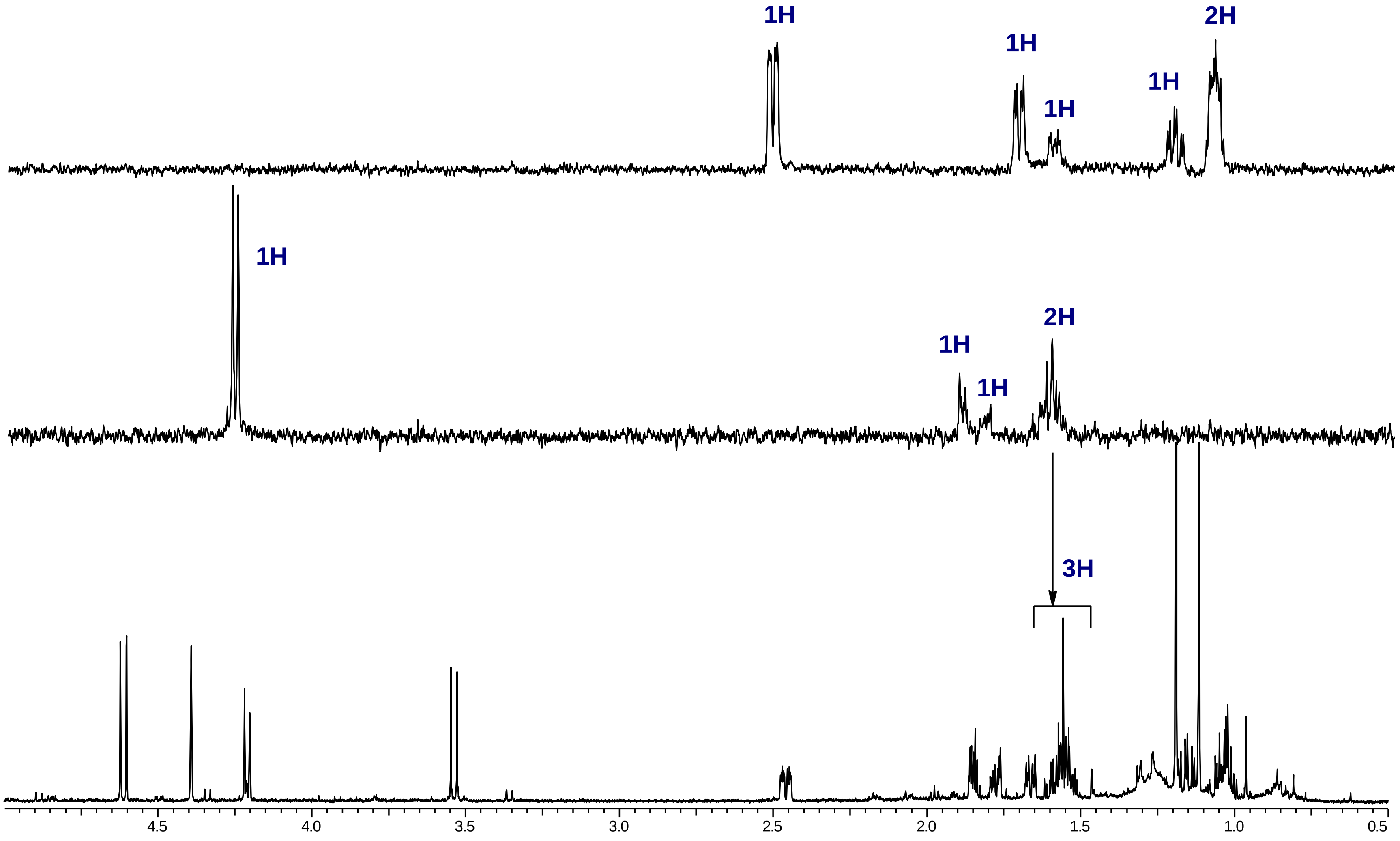

H
H
9
H
H
10
8
H
7
HO
11
12
O
O
H₃C
H
6
CH₃
13
O
H
O
5
O
H
4
H
H
3
O
1
H
2
H
H



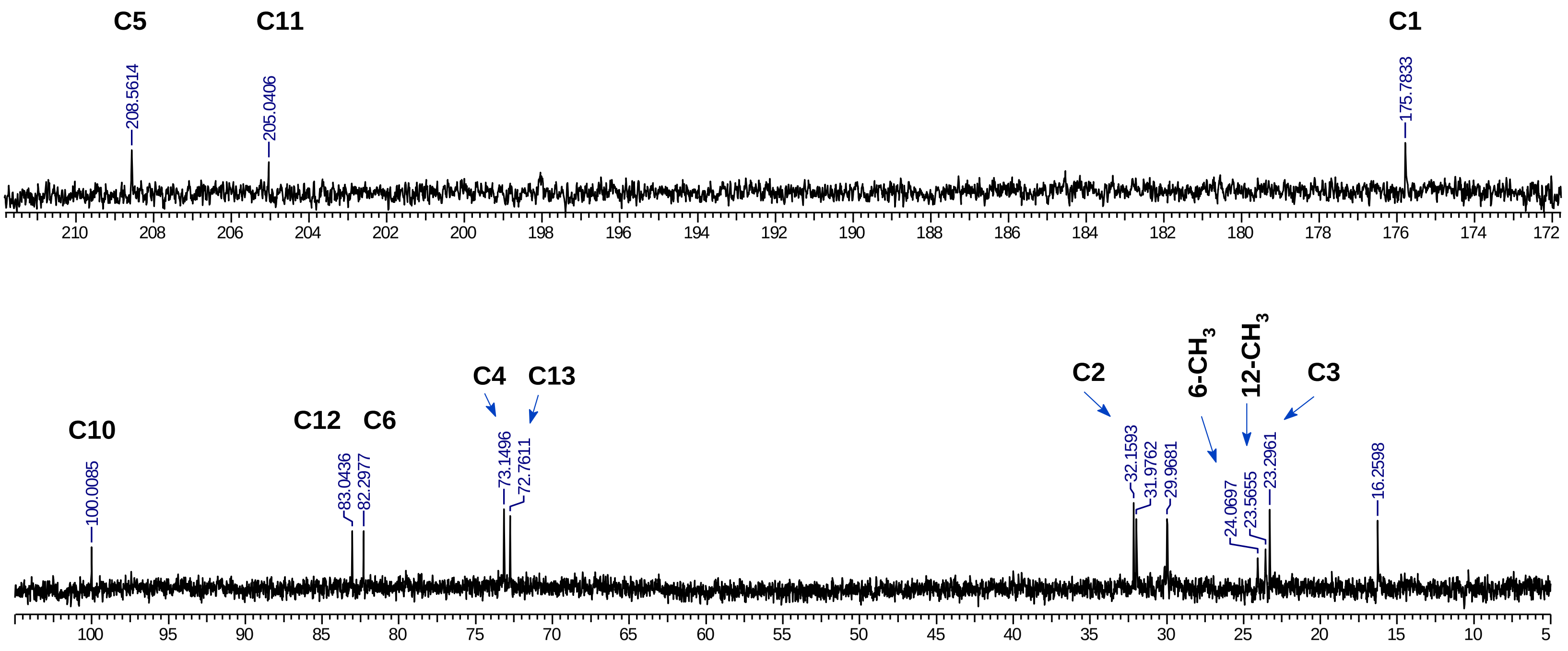
Long-range correlations trough oxygen in $CDCl_3$; $^1J_{CH}$ was set to 145 Hz

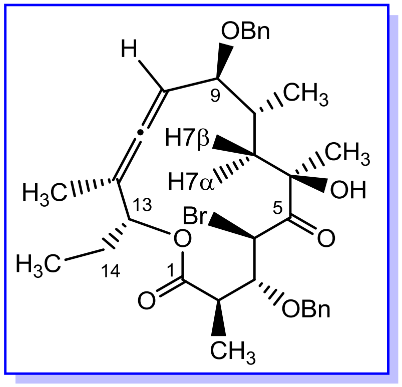


Spin system isolations



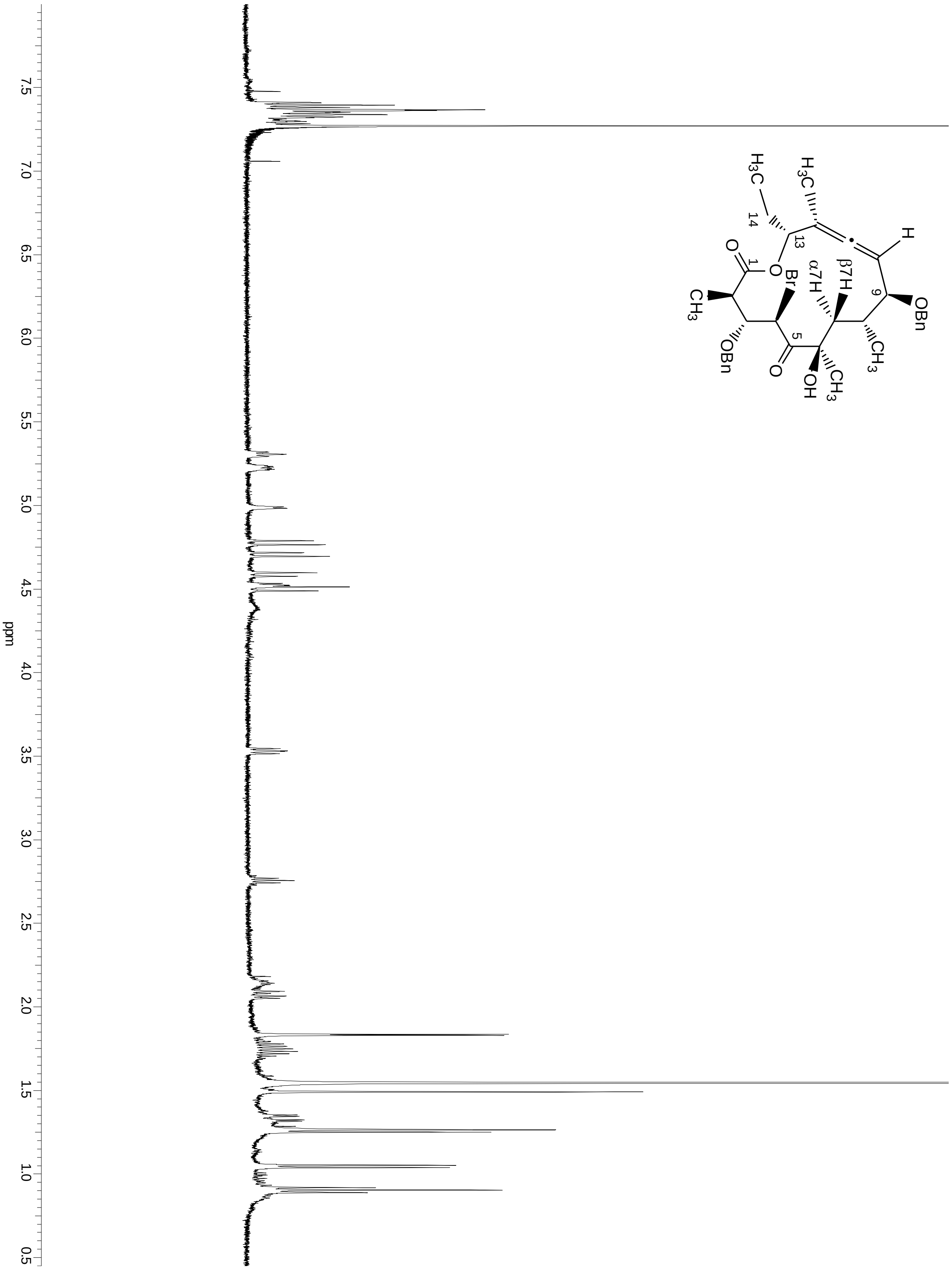
^{13}C NMR spectrum in C_6D_6

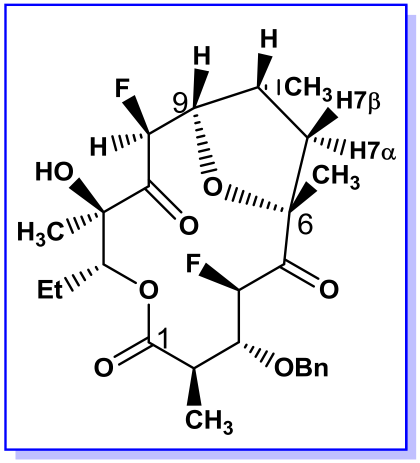




1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)

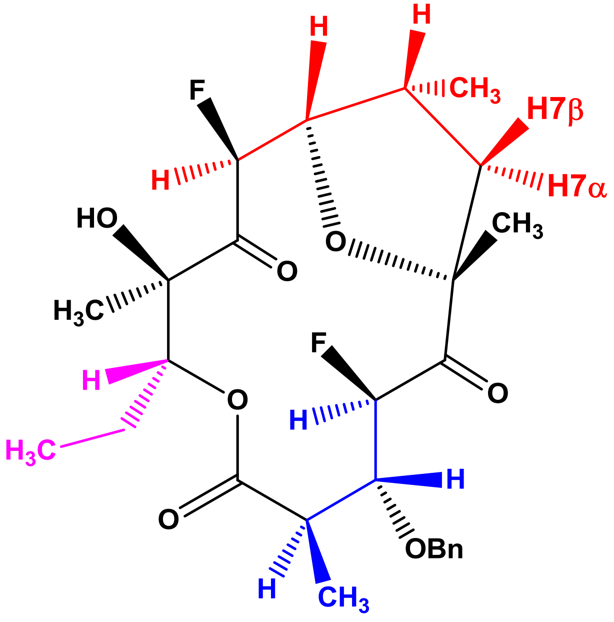
2.76 (quintet, $J_{H2,\ H3}$ = 6.6Hz, **H2**)
1.25 (d, $J_{2\text{-}CH3,\ H2}$ = 7.3Hz, **2-CH$_3$**)
3.53 (dd, $J_{H3,\ H4}$ = 8.6Hz, $J_{H3,\ H2}$ = 6.6Hz, **H3**)
4.98 (d, $J_{H4,\ H3}$ = 4.2Hz, **H4**)
1.49 (s, **6-CH$_3$**)
1.32 (dd, $J_{H7\beta,\ H7\alpha}$ = 14.2Hz, **H7β**)
2.08 (dd, $J_{H7\alpha,\ H8}$ = 6.1 Hz, $J_{H7\alpha,\ H7\beta}$ = 14.4Hz, **H7α**)
1.81 - 1.68 (m, **H8**)
1.05 (d, $J_{8\text{-}CH3,\ H8}$ = 6.9Hz, **8-CH$_3$**)
4.38 (m, **H9**)
4.70, 4.58 (d, J_{AB} = 10.5Hz, **9-OCH$_2$**)
4.76, 4.51 (d, J_{AB} = 12.0Hz, **9-OCH$_2$**)
5.25 - 5.21 (m, **H10**)
1.83 (d, $J_{12\text{-}CH3,\ H10}$ = 2.9Hz, **12-CH$_3$**)
5.31 (T, $J_{H13,\ 14\text{-}CH2}$ = 7.3Hz, **H13**)
2.18 - 2.11 (m, **14-CH$_2$**)
0.90 (t, $J_{14\text{-}CH3,\ 14\text{-}CH2}$ = 7.3Hz, **14-CH$_3$**)
7.42 - 7.29 (m, 8H, **Ph**)


ppm
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
H
OBn
9
CH_3
β7H
α7H
CH_3
OH
5
O
Br
OBn
CH_3
O
1
O
13
14
H_3C
H_3C

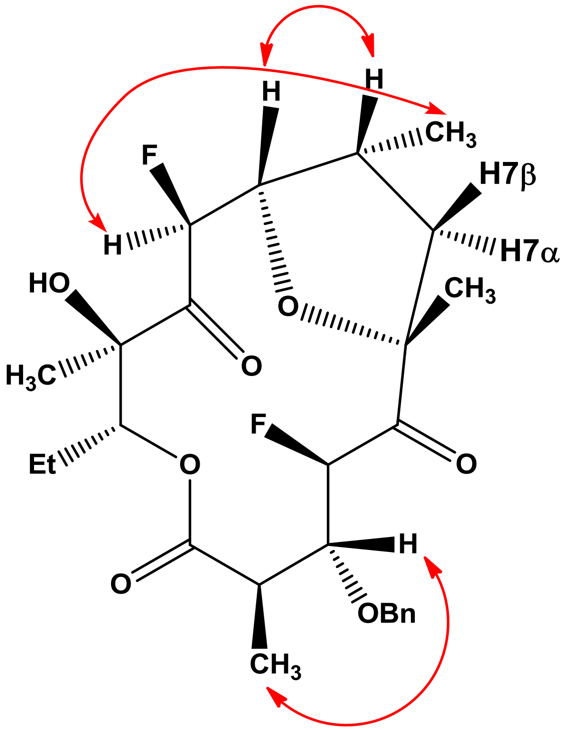


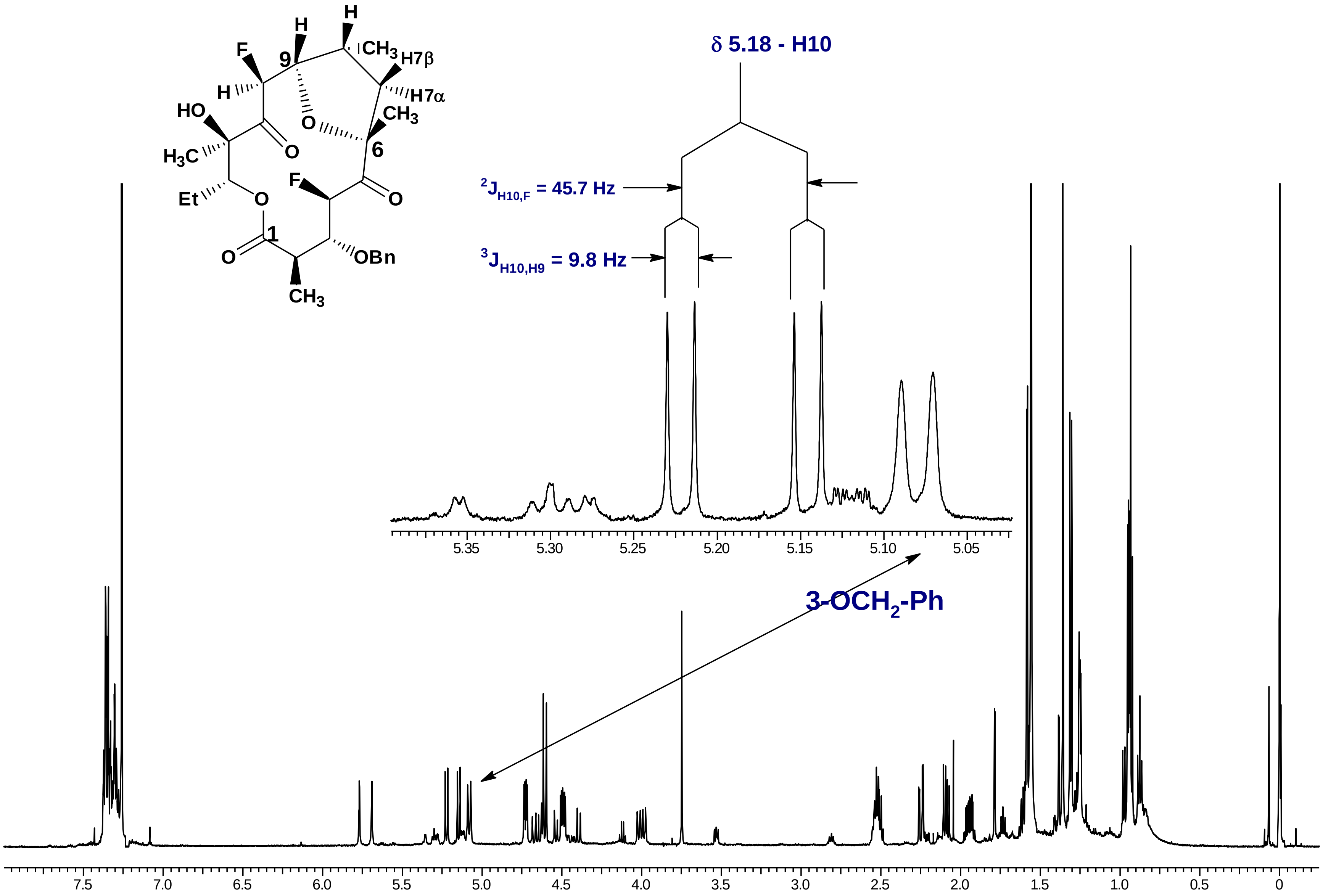
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)	13C NMR chemical shift (δ/ppm)
2.51 (dq, $J_{H2, H3}$ = 10.6Hz, **H2**)	172.9 ----- C1
1.31 (d, $J_{2\text{-}CH3, H2}$ = 7.3Hz, **2-CH$_3$**)	41.0 -- C2, J_{CF}=10.2Hz
4.01 (dd, $J_{H3, F}$ = 20.9Hz, $J_{H3, H2}$ = 11.2Hz, **H3**)	79.1 ----- C3
5.75 (dd, $J_{H4, F}$ = 46.9Hz, $J_{H4, H3}$ = 1.0Hz, **H4**)	91.4 -- C4, J_{CF}=178.6Hz
1.35 (s, **6-CH$_3$**)	202.7 -- C5, J_{CF}=14.9Hz
2.25 (dd, $J_{H7\alpha, H8}$ = 2.5 Hz, $J_{H7\alpha, H7\beta}$ = 13.6Hz, **H7α**)	89.2 ----- C6
2.09 (dd, $J_{H7\beta, H8}$ = 8.1Hz, $J_{H7\beta, H7\alpha}$ = 13.6Hz, **H7β**)	40.8 ----- C7
2.53 (dddq, **H8**)	33.6 ----- C8
0.95 (d, $J_{8\text{-}CH3, H8}$ = 7.1Hz, **8-CH$_3$**)	75.5 -- C9, J_{CF}=31.6Hz
4.49 (ddd, $J_{H9, H8}$ = 5.3Hz, $J_{H9, H10}$ = 9.8Hz, $J_{H9, F}$ = 2.6Hz, **H9**)	85.1 -- C10,J_{CF}=170.3Hz
4.61 (d, J_{AB} = 11.5Hz, **3-OCH$_2$**)	205.7 -- C11,J_{CF}=13.5Hz
5.08 (d, J_{AB} = 11.5Hz, **3-OCH$_2$**)	78.1 ----- C12
5.18 (dd, $J_{10, F}$ = 45.7, $J_{H10, H9}$ = 9.8Hz, **H10**)	79.5 ----- C13
1.58 (d, $J_{12CH3, 12\text{-}OH}$ = 2.5Hz, **12-CH$_3$**)	22.9 ----- C14
3.74 (s, **12-OH**)	16.4 ----- 2-CH$_3$
4.73 (ddd, $J_{H13, HA}$ = 3.6 Hz, $J_{H13, HB}$ = 9.1 Hz, **H13**)	24.8 ----- 6-CH$_3$
1.91 - 1.98, 1.56 - 1.63 (m, **14-CH$_2$**)	15.0 ----- 8-CH$_3$
0.93 (t, $J_{14\text{-}CH3, 13\text{-}CH2}$ = 7.6Hz, **14-CH$_3$**)	16.2 ----- 12-CH$_3$
7.36 (^{ortho}H / **Ph**)	10.6 ----- 14-CH$_3$
7.34 (^{meta}H / **Ph**)	74.5 ----- 3-O$\underline{C}$H$_2$PH
7.30 (^{para}H / **Ph**)	137.8 ----- ^{ipso}C
	128.6 ----- ^{ortho}C
	128.3 ----- ^{meta}C
	127.8 ----- ^{para}C

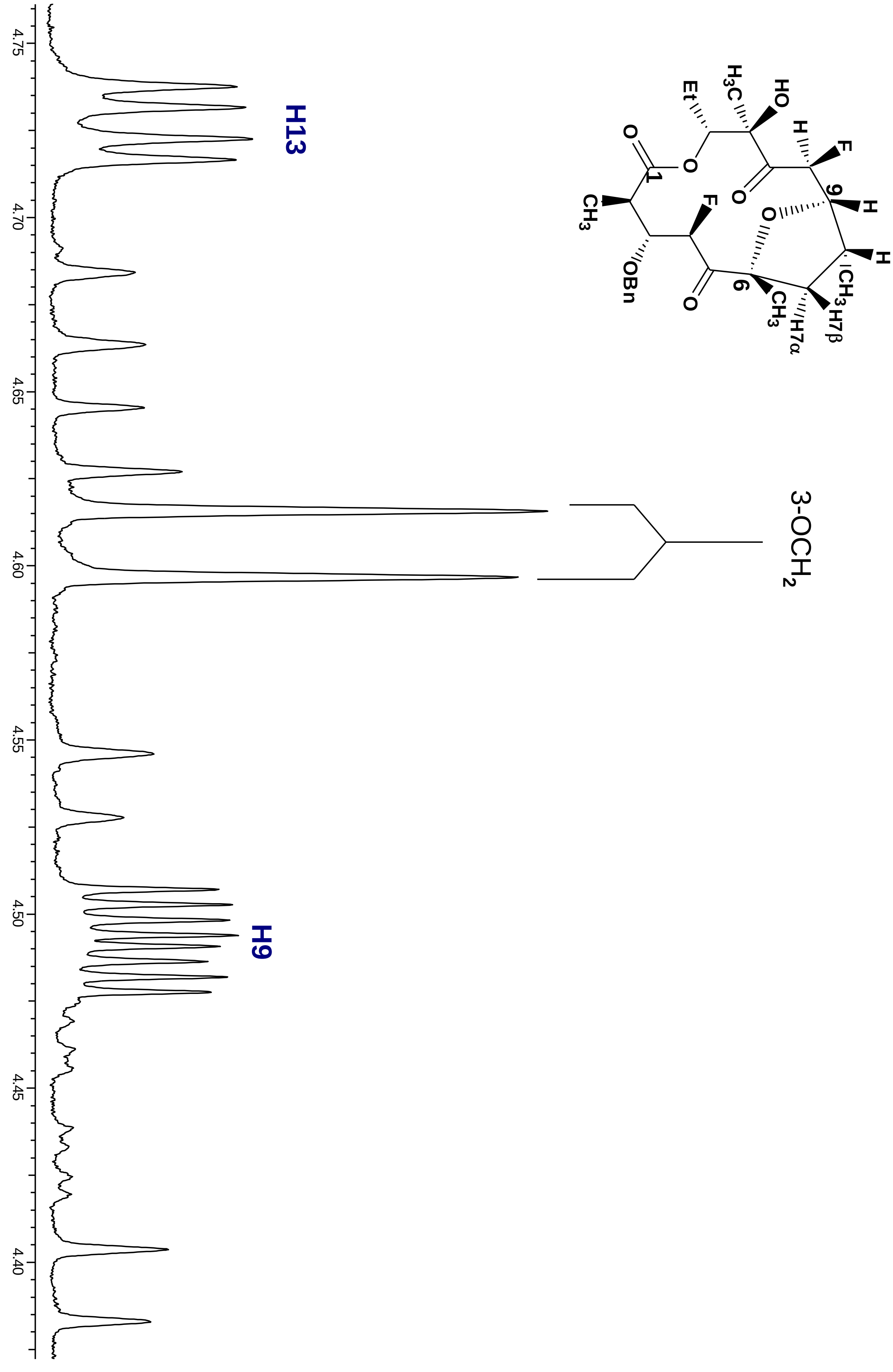
1d TOCSY

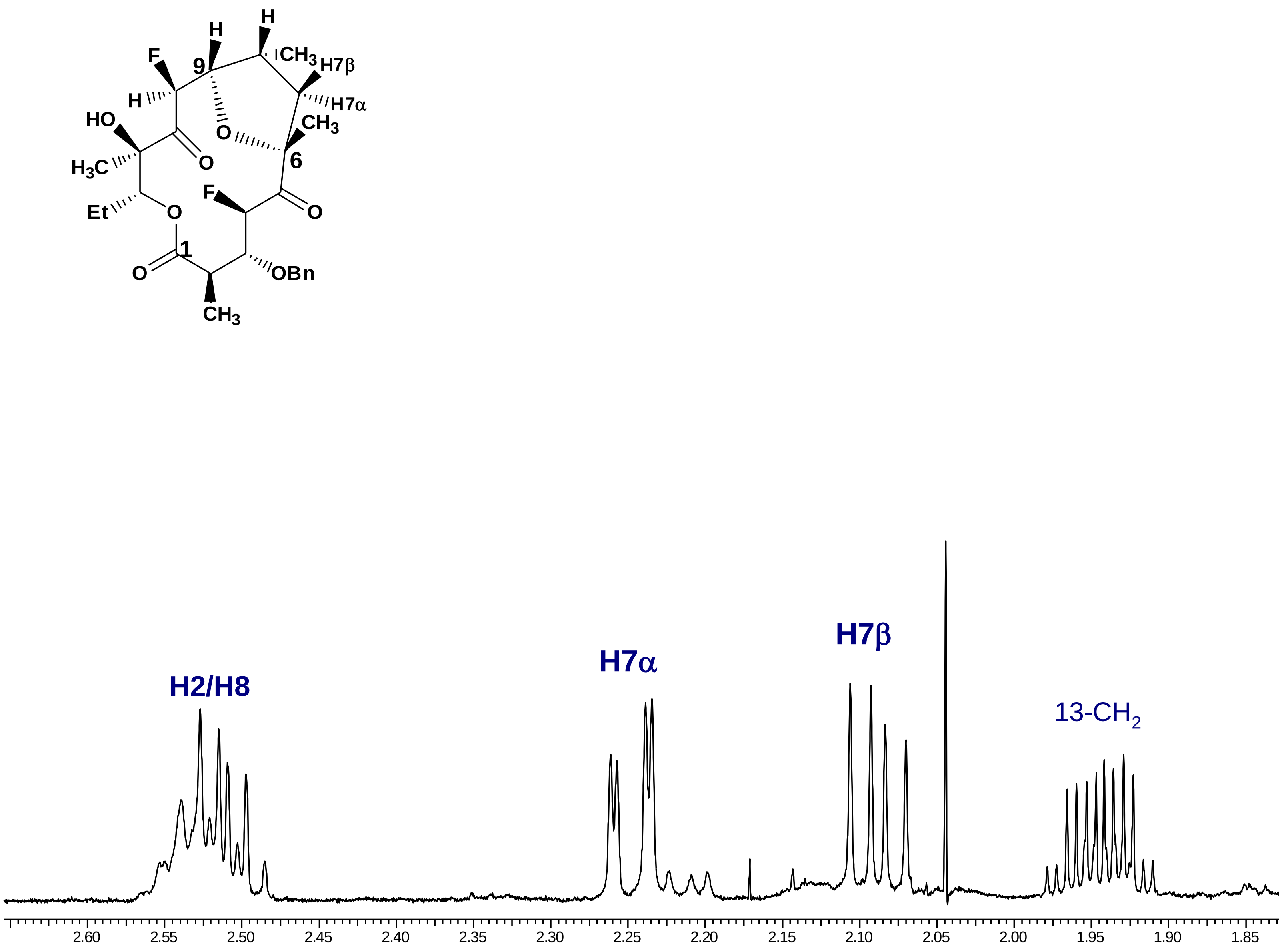

H
H
F
CH$_3$
H7β
H
H7α
HO
O
O
CH$_3$
H$_3$C
F
H
O
O
H
H$_3$C
H
O
OBn
H
CH$_3$

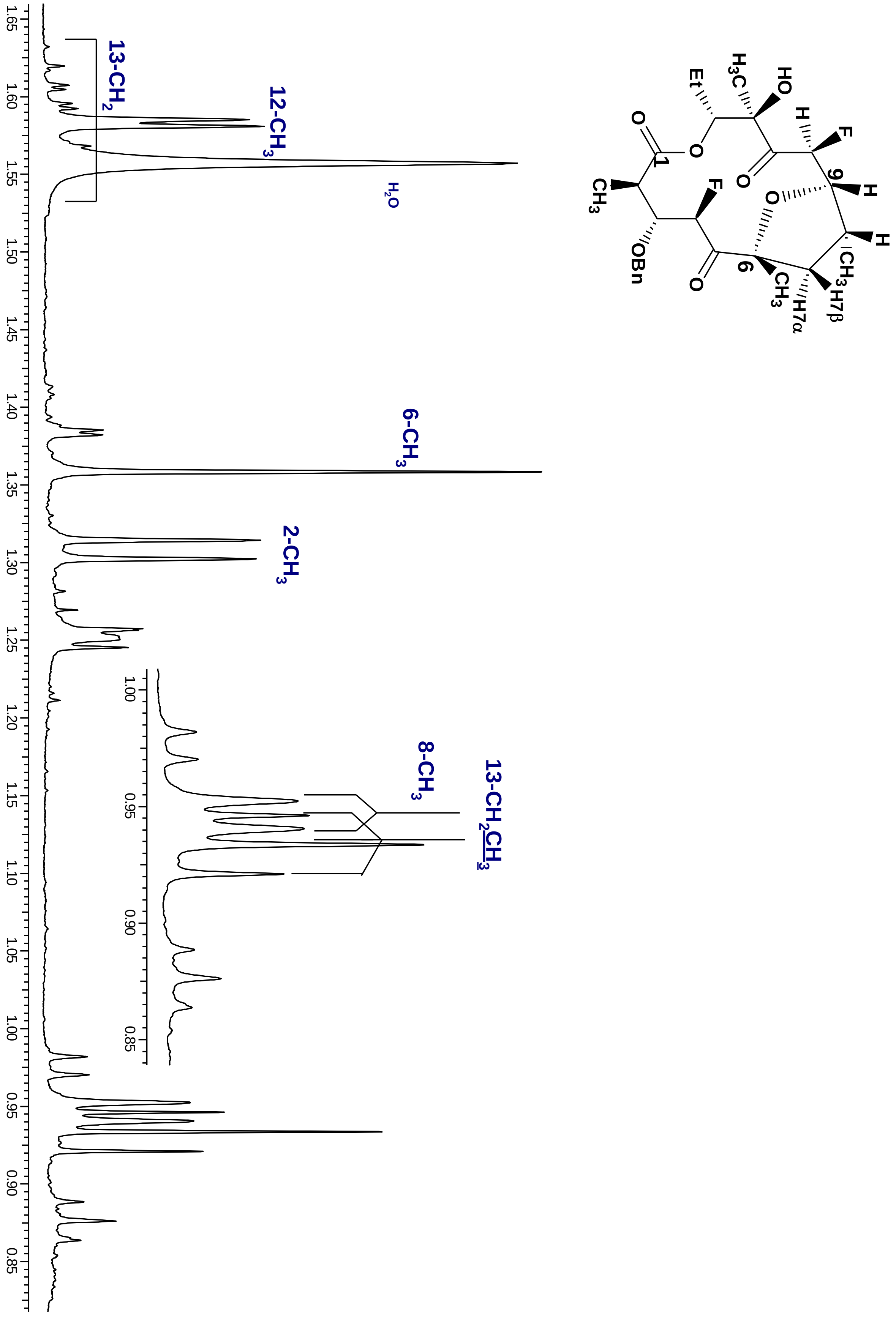
NOESY


H
H
F
CH$_3$
H7β
H
H7α
HO
O
CH$_3$
H$_3$C
O
F
Et
O
O
H
O
OBn
CH$_3$

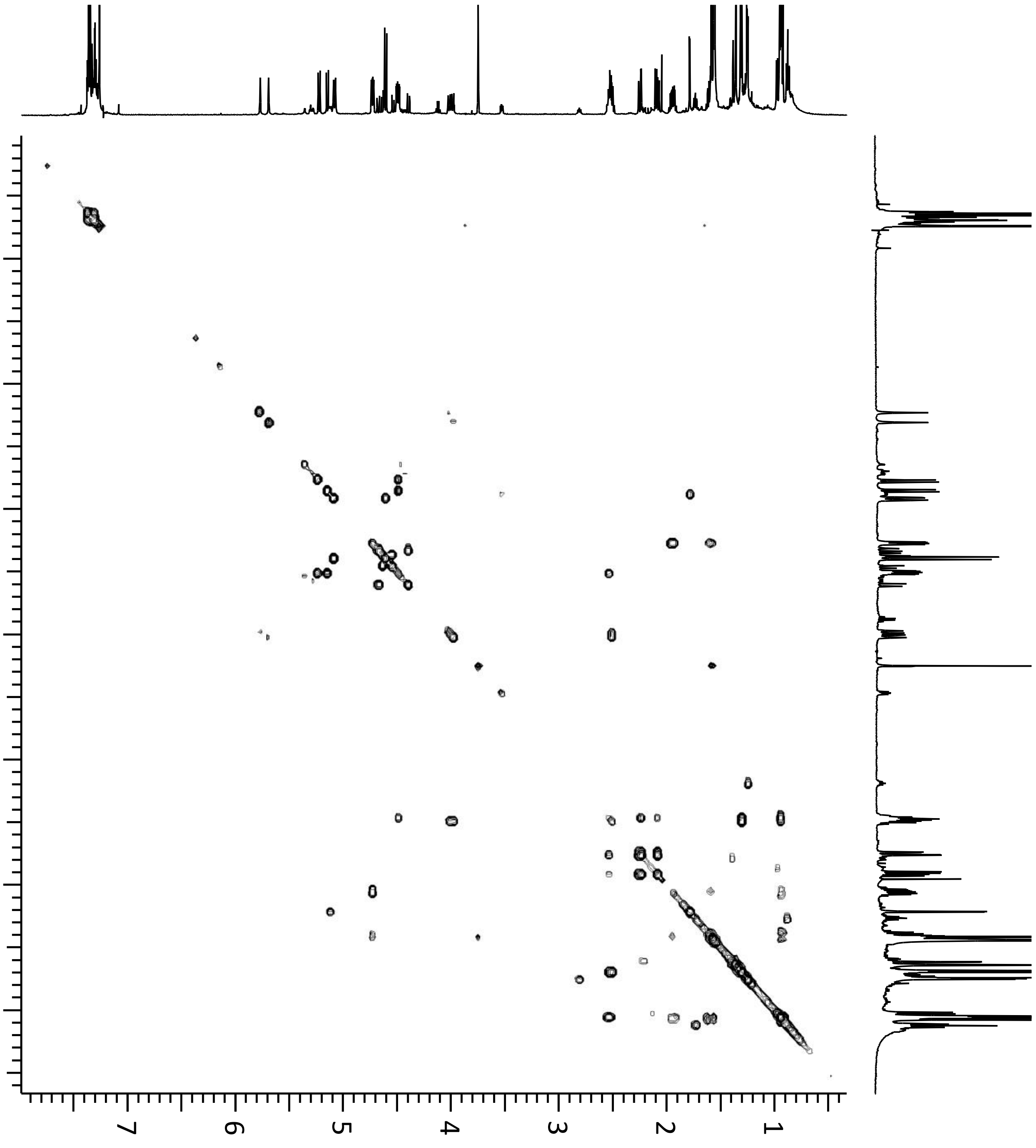

HO
H
F
9
H
H
CH3
H7β
H7α
CH3
O
H3C
O
6
Et
O
F
O
1
O
OBn
CH3
δ 5.18 - H10
²J_H10,F = 45.7 Hz
³J_H10,H9 = 9.8 Hz
5.35
5.30
5.25
5.20
5.15
5.10
5.05
3-OCH2-Ph
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0


HO
H3C
Et
H
F
O
1
9
H
O
F
H
CH3
CH3
H7β
H7α
6
CH3
OBn
O
O
O
3-OCH2
H13
H9
4.75
4.70
4.65
4.60
4.55
4.50
4.45
4.40


F
H
9
H
CH3
H7β
H7α
HO
H
O
CH3
6
H3C
O
F
O
Et
O
1
O
OBn
CH3
H2/H8
H7α
H7β
13-CH2
2.60
2.55
2.50
2.45
2.40
2.35
2.30
2.25
2.20
2.15
2.10
2.05
2.00
1.95
1.90
1.85

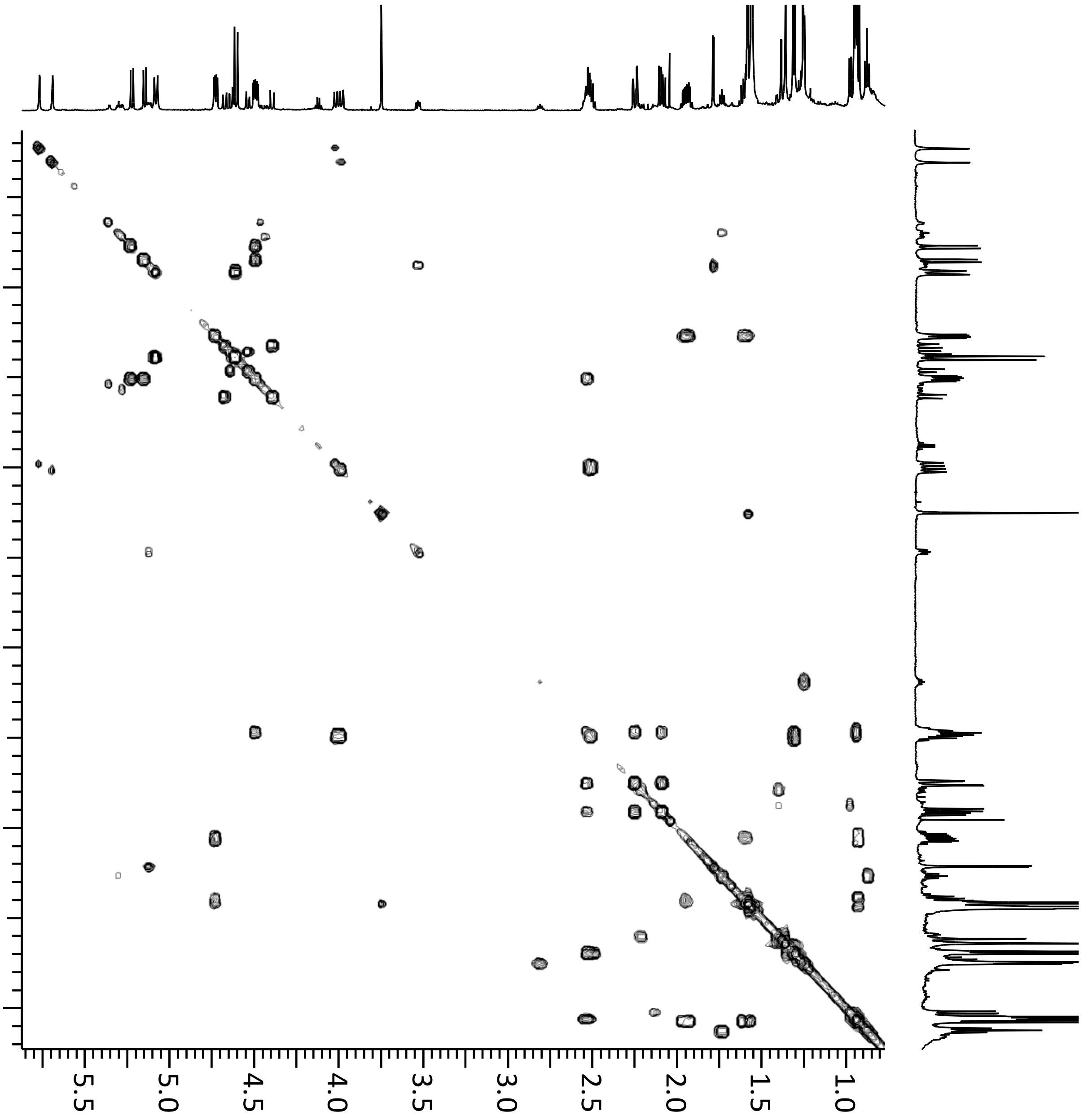

HO
H_3C
Et
H
F
9
H
H
O
O
O
1
CH_3
F
OBn
O
6
CH_3
CH_3
H7β
H7α
13-CH_2
12-CH_3
H_2O
6-CH_3
2-CH_3
8-CH_3
13-$CH_2\underline{CH_3}$
1.65
1.60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85

gCOSY

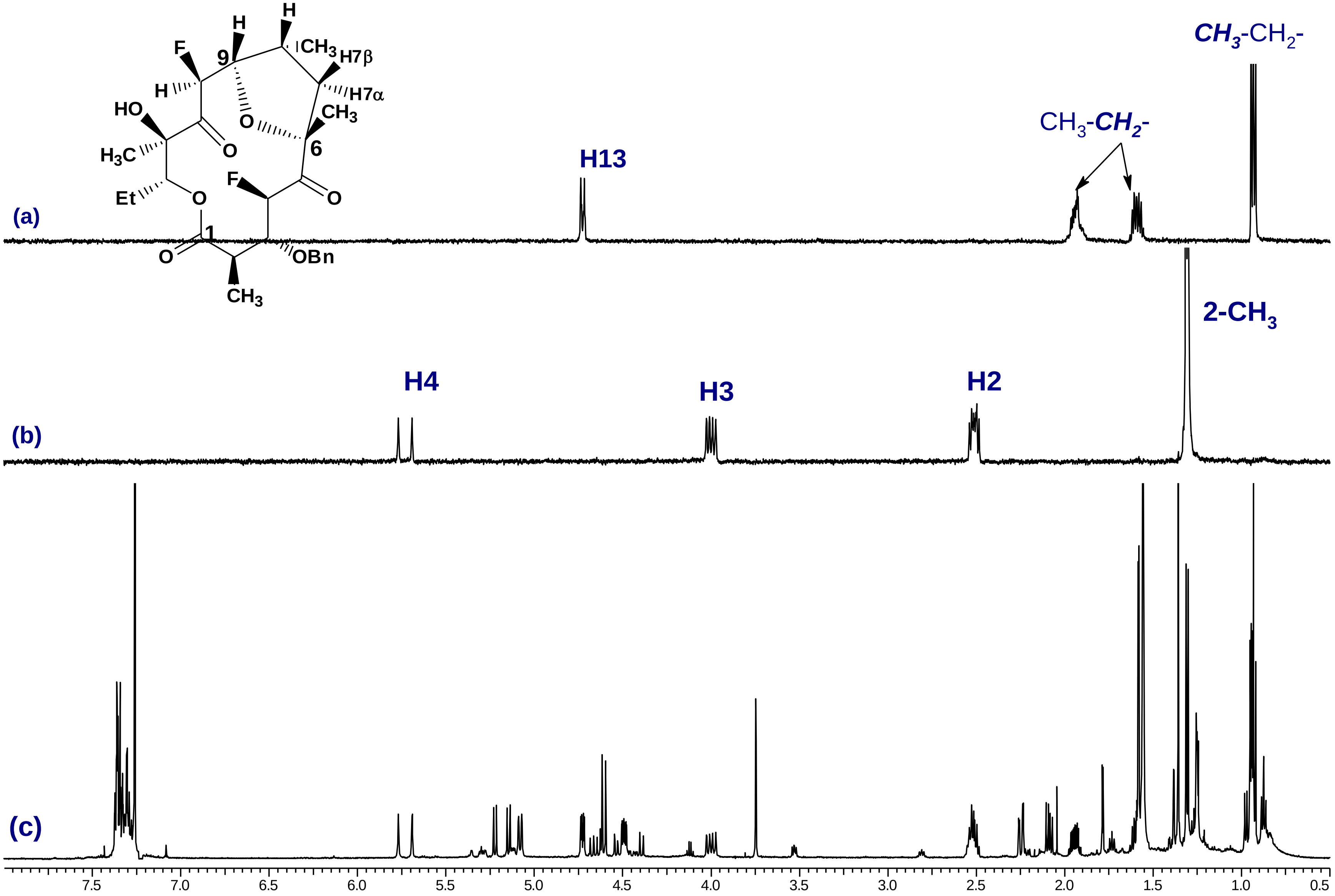


gCOSY

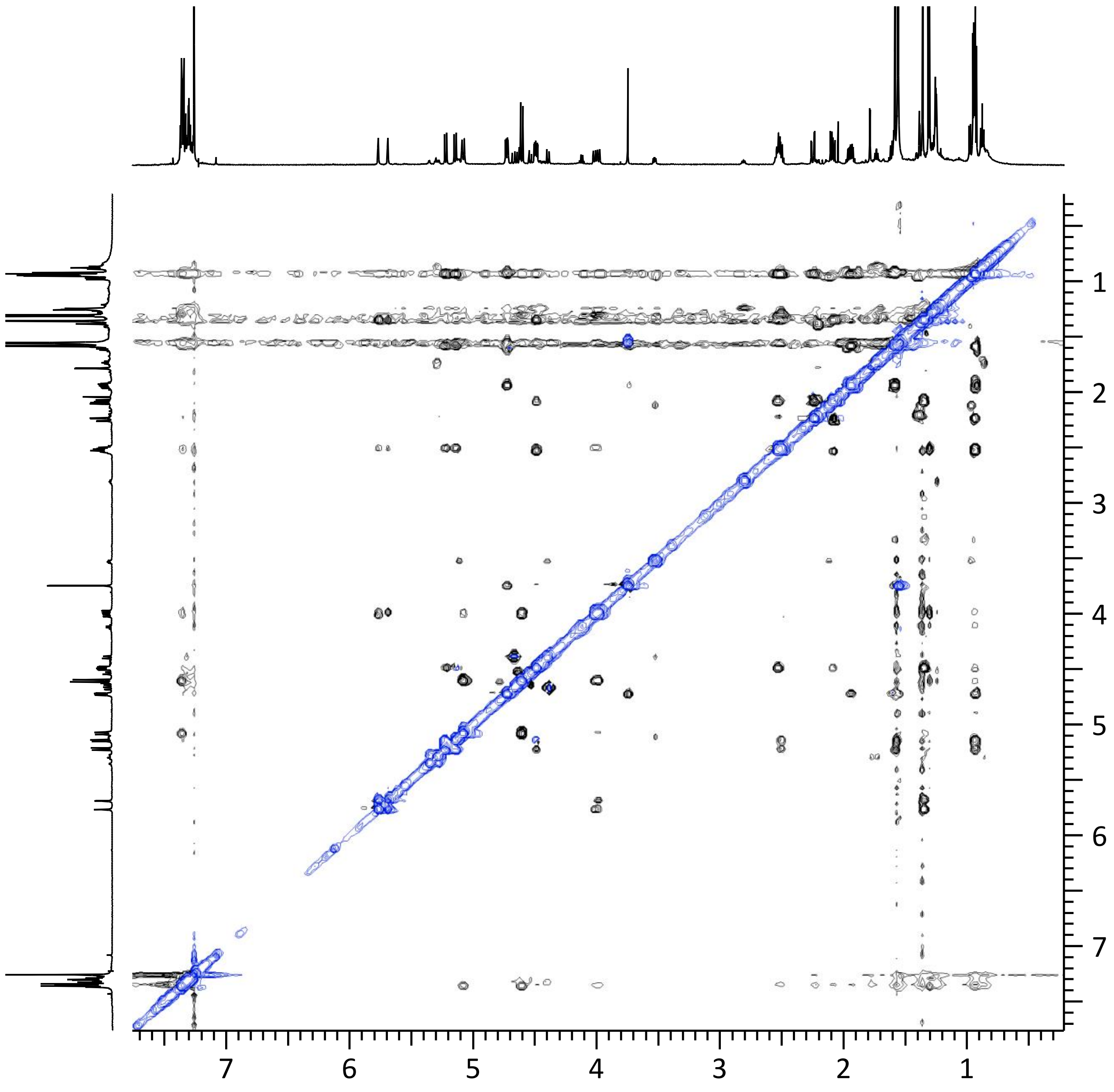
aliphatic region



1D TOCSY experimental subspectra (a-b); c- control ^{1}H NMR spectrum

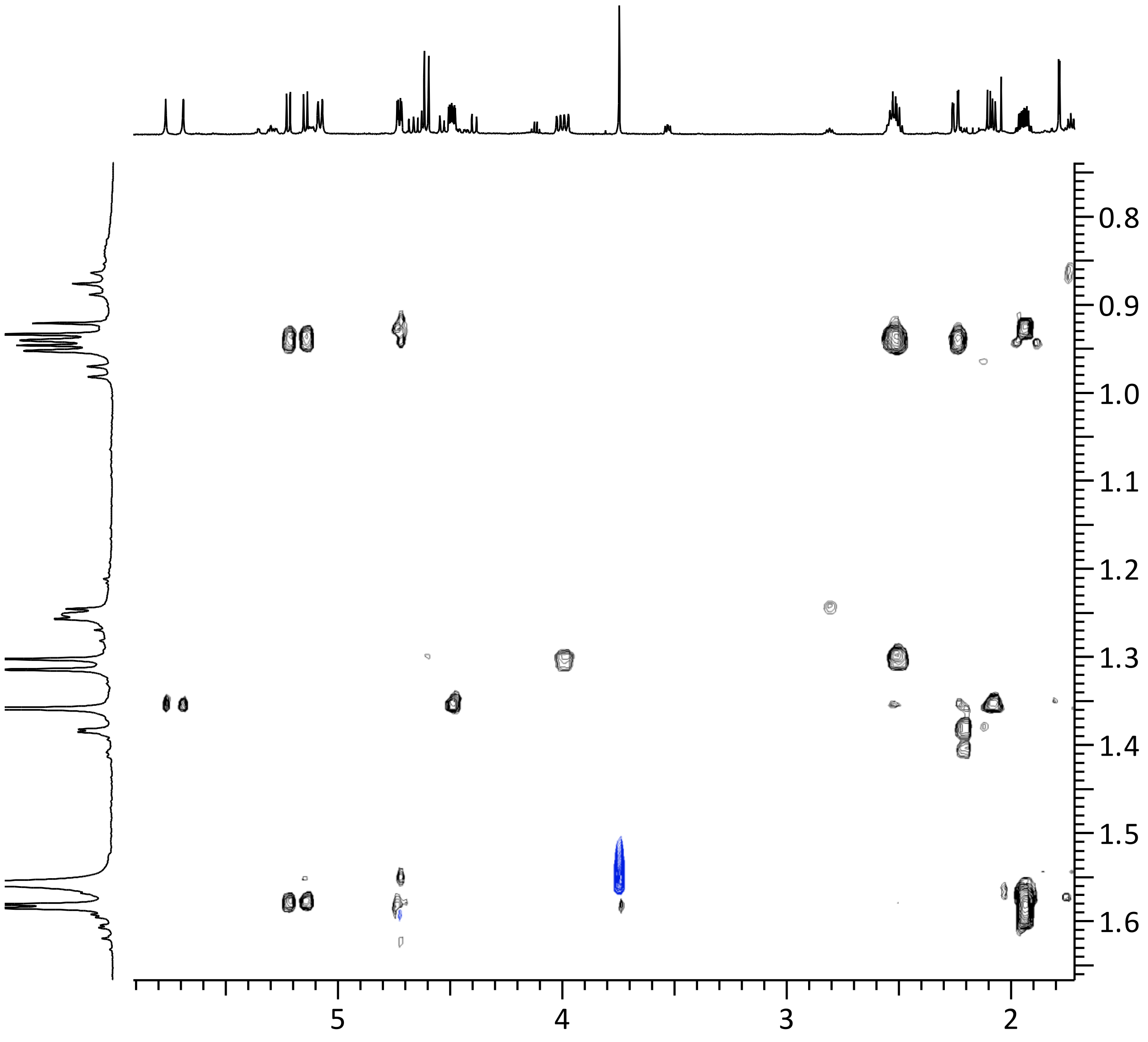


NOESY

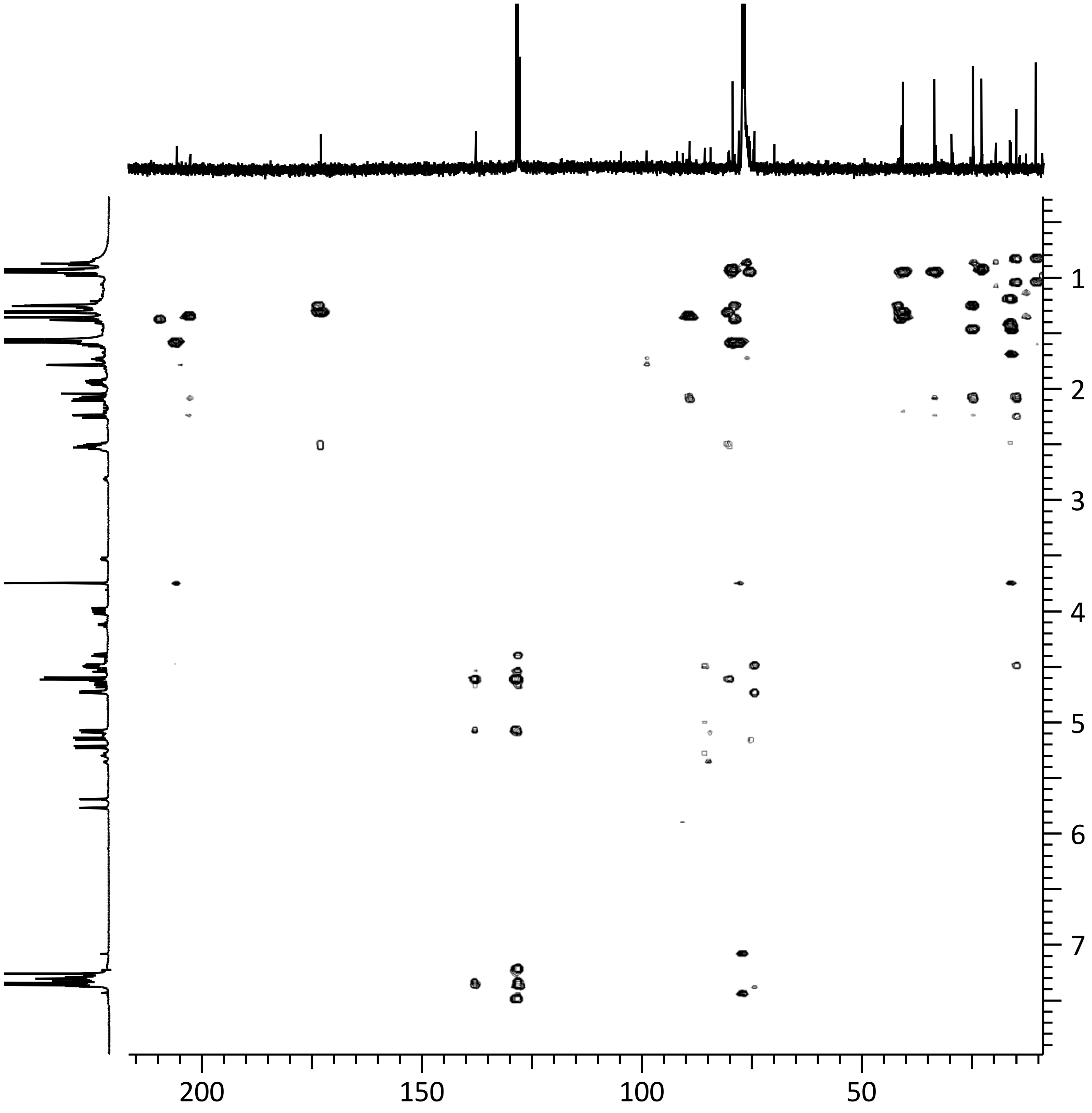


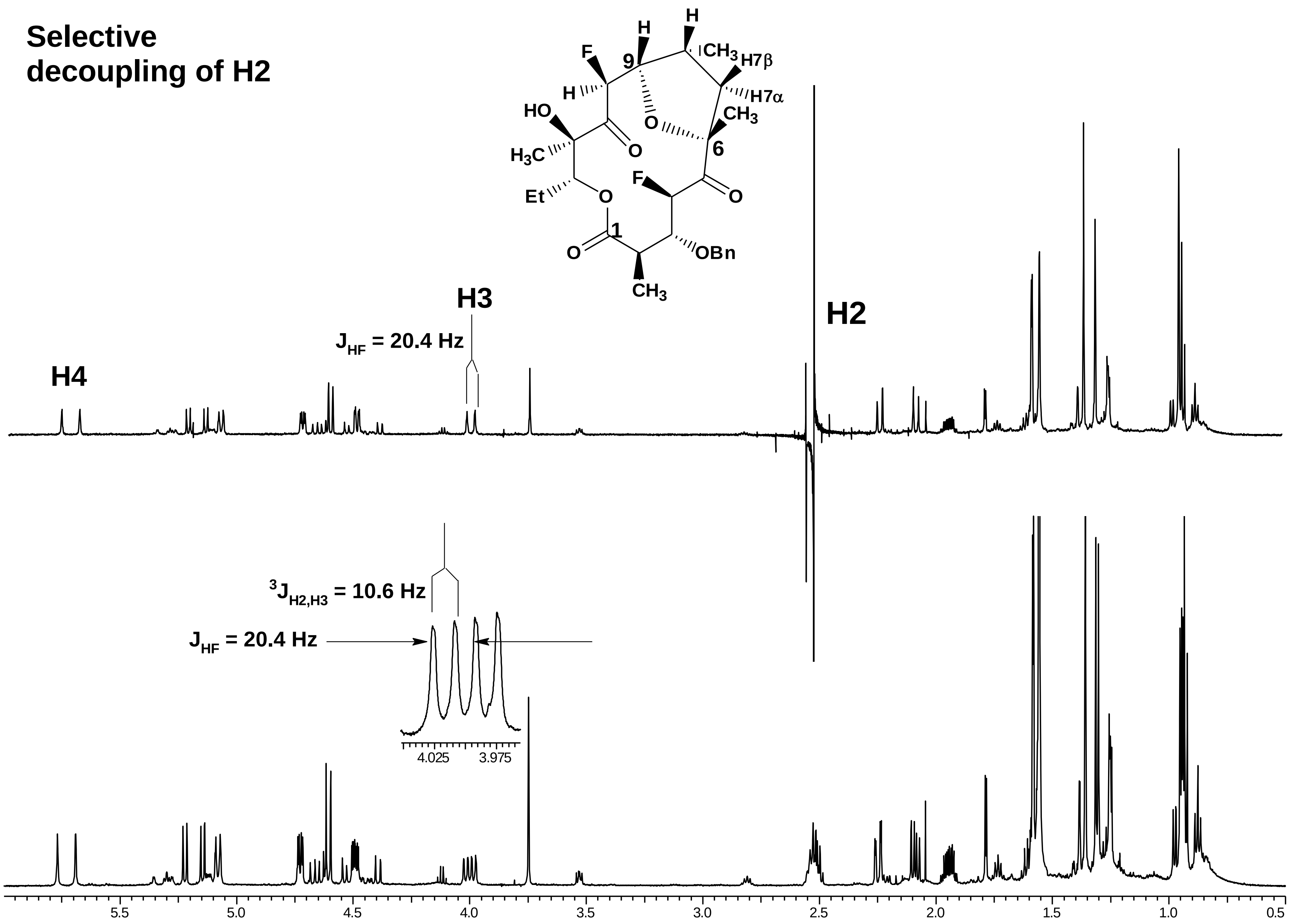
NOESY

expansion

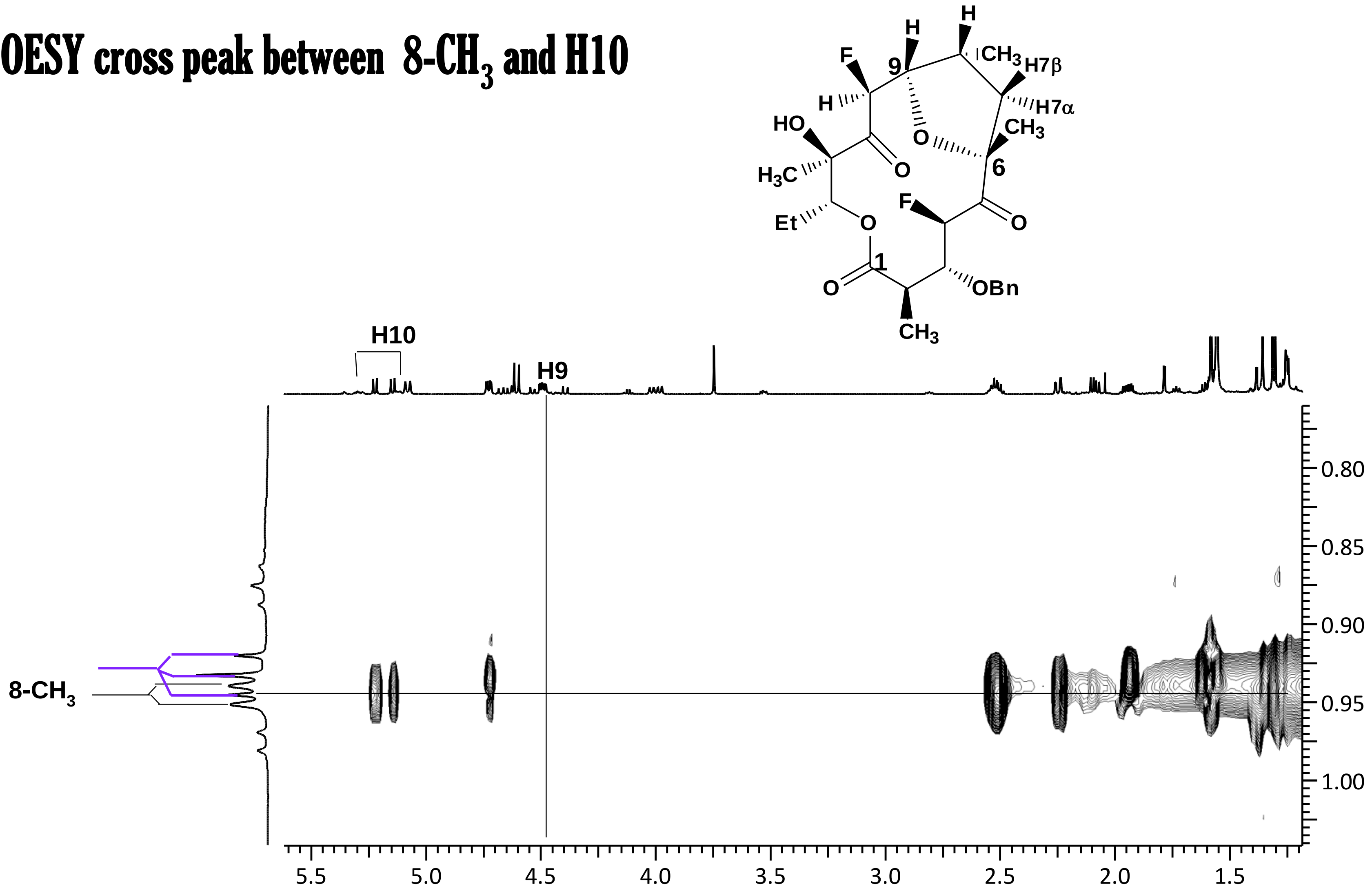


gHMBC

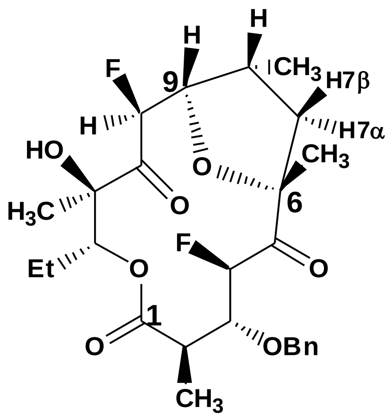


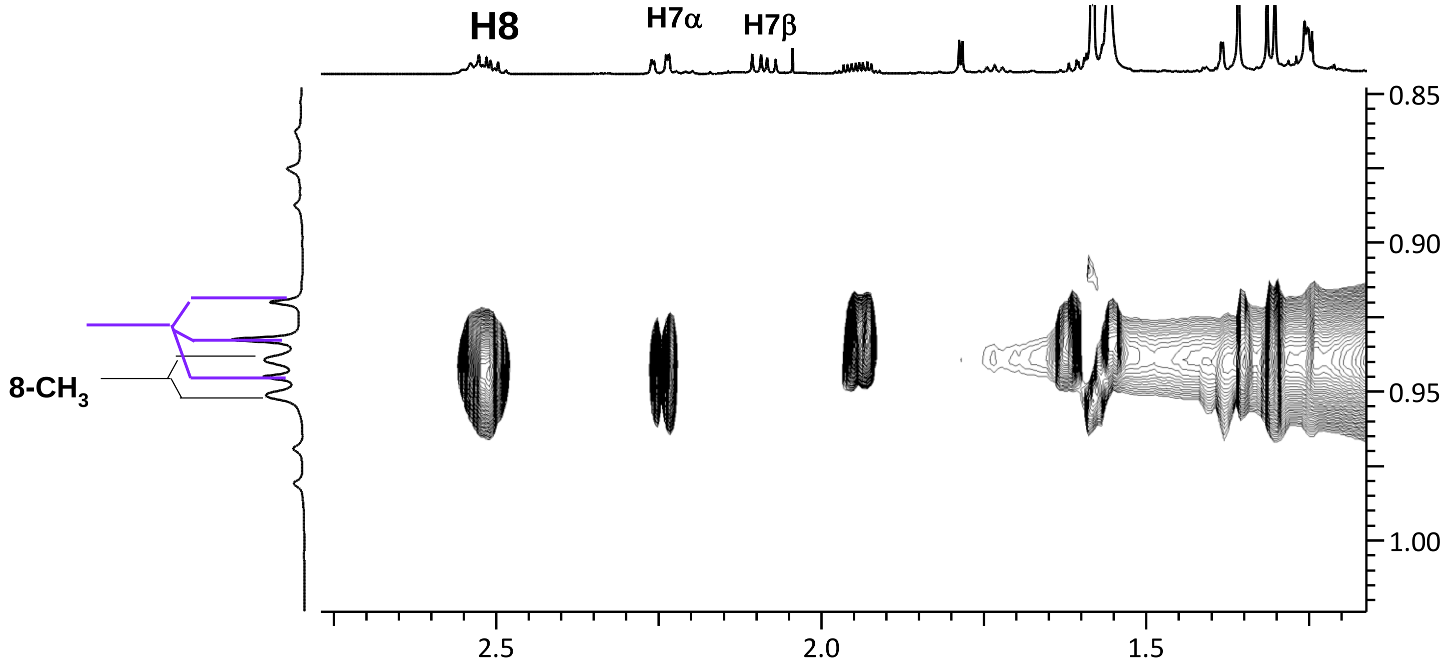

Selective decoupling of H2
H4
H3
J_{HF} = 20.4 Hz
H2
$^3J_{H2,H3}$ = 10.6 Hz
J_{HF} = 20.4 Hz
4.025
3.975
F
H
H
CH3
H7β
H7α
CH3
H
HO
9
O
O
6
H3C
F
O
Et
O
1
O
OBn
CH3
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

NOESY cross peak between 8-CH_3 and H10

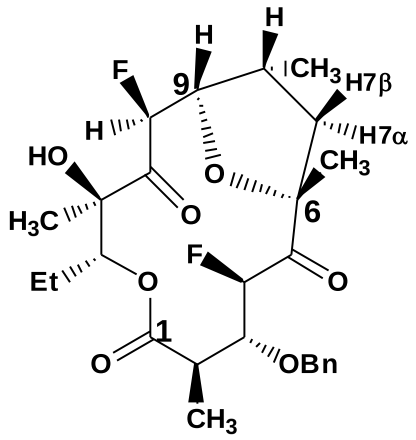


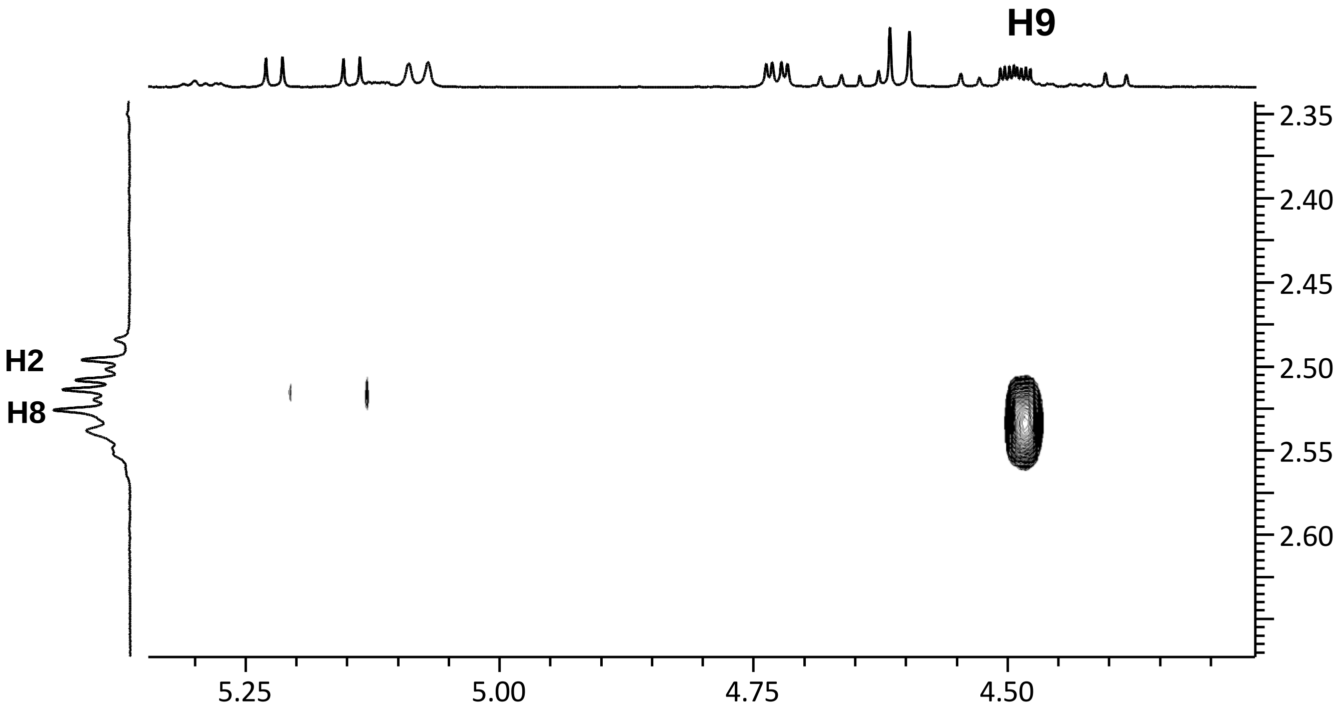
NOESY cross peaks between 8-CH$_3$ and others


H
H
F
9
CH3
H7β
H
H7α
HO
O
CH3
H3C
O
6
F
Et
O
O
1
O
OBn
CH3

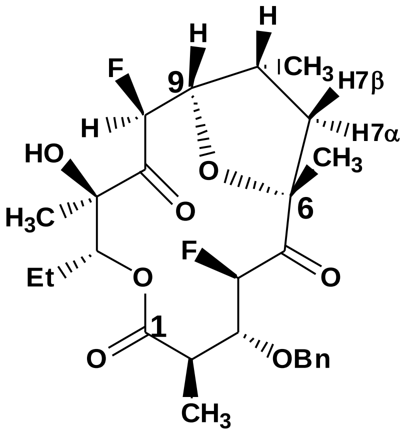


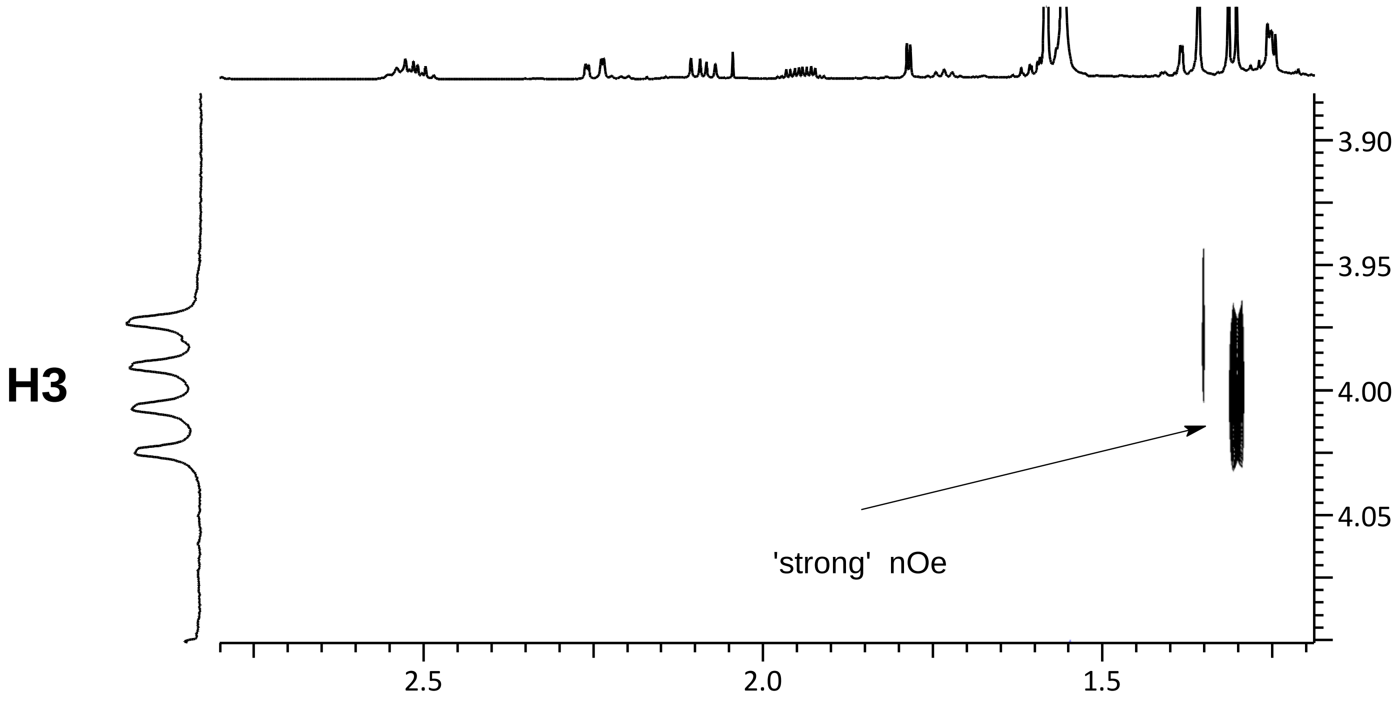
NOESY cross peak between H8 and H9

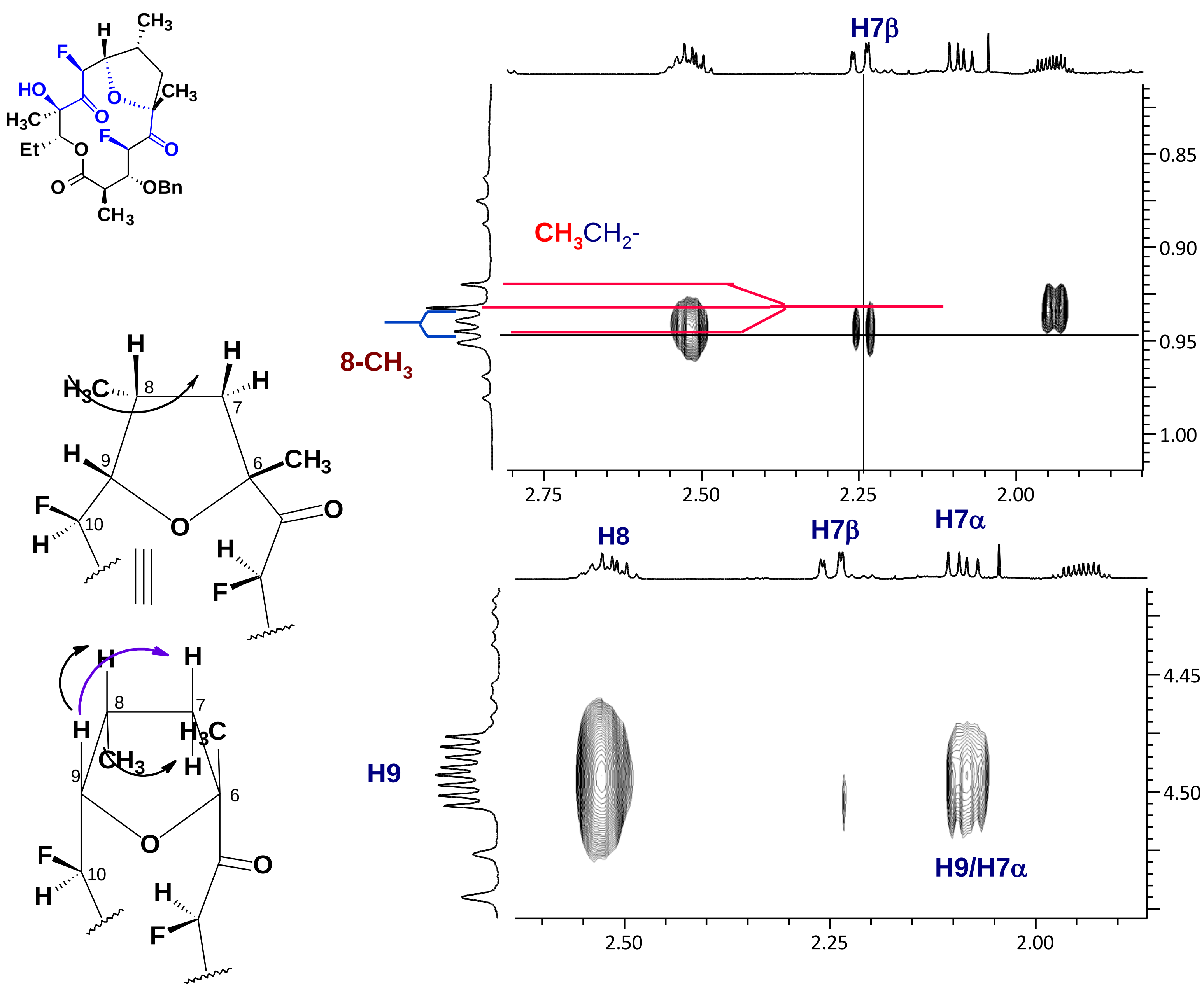

F
9
H
H
CH3
H7β
H7α
H
HO
O
CH3
6
H3C
O
F
Et
O
O
1
O
OBn
CH3

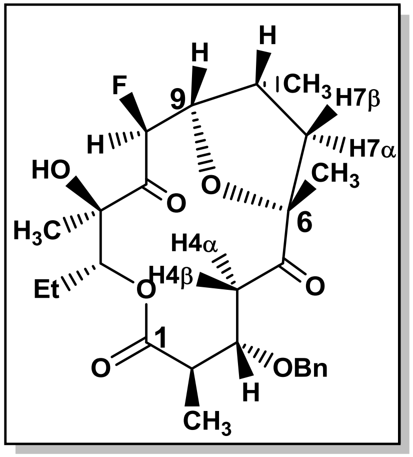


NOESY cross peak between H3 and 2-CH$_3$


F
9
H
H
CH3
H7β
H7α
CH3
6
O
H
HO
O
H3C
O
F
Et
O
O
1
O
OBn
CH3

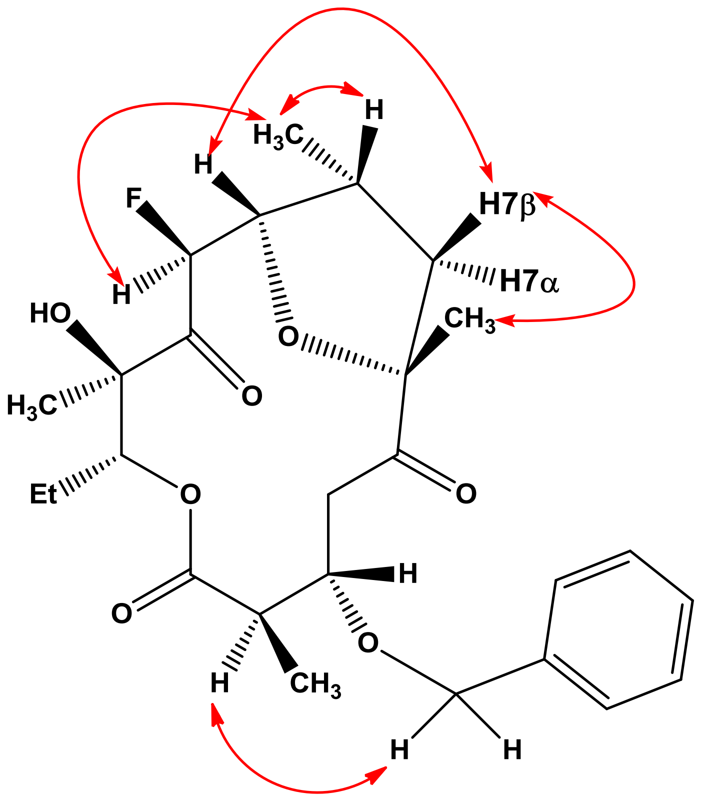



H
CH3
F
HO
O
CH3
H3C
O
F
O
Et
O
O
OBn
CH3
H
H
H3C
8
H
7
H
9
6
CH3
F
10
O
O
H
H
F
H
H
8
7
H
H3C
CH3
H
9
6
O
F
10
O
H
H
F
H7β
CH3CH2-
8-CH3
0.85
0.90
0.95
1.00
2.75
2.50
2.25
2.00
H8
H7β
H7α
H9
4.45
4.50
H9/H7α
2.50
2.25
2.00

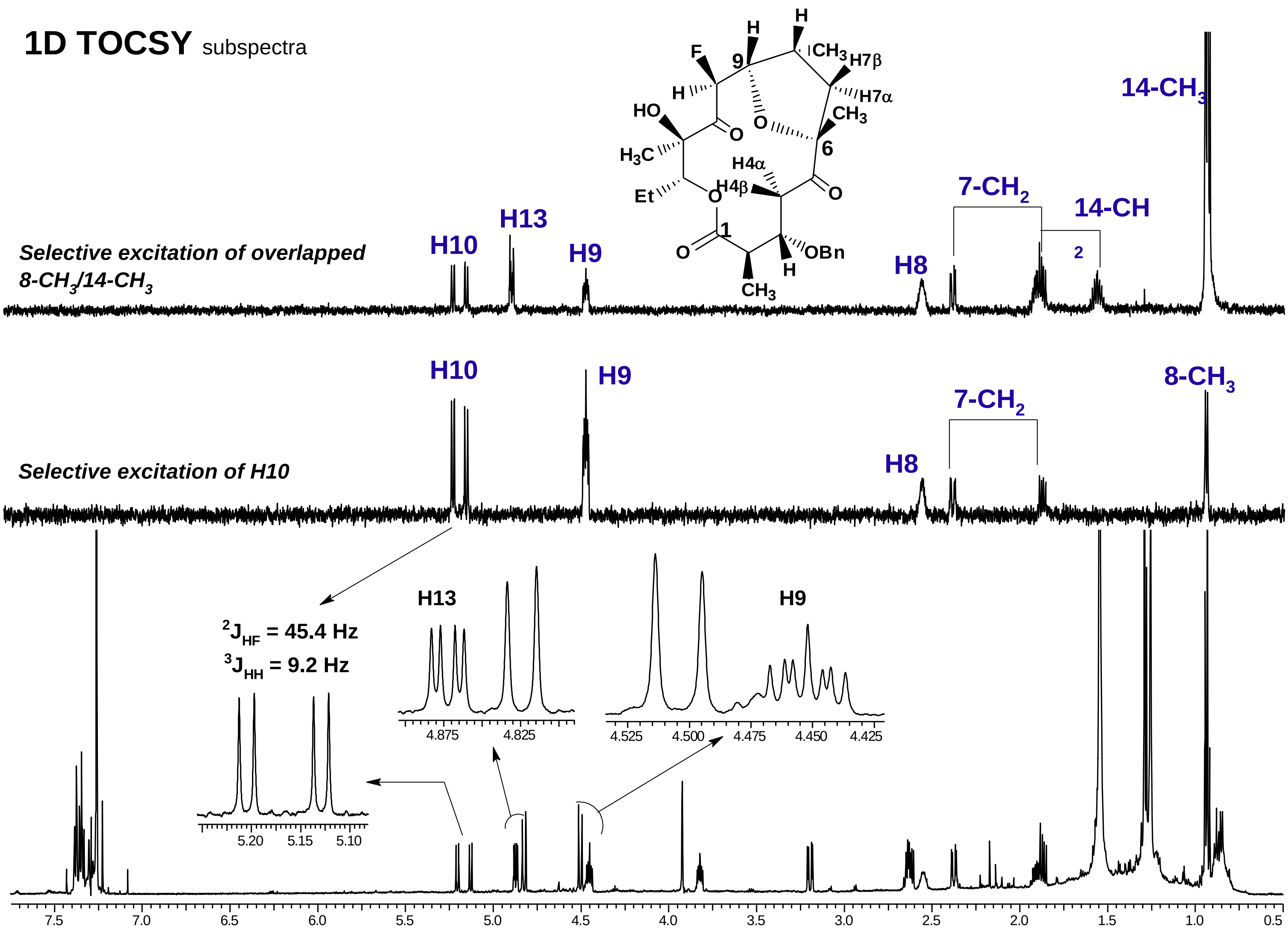


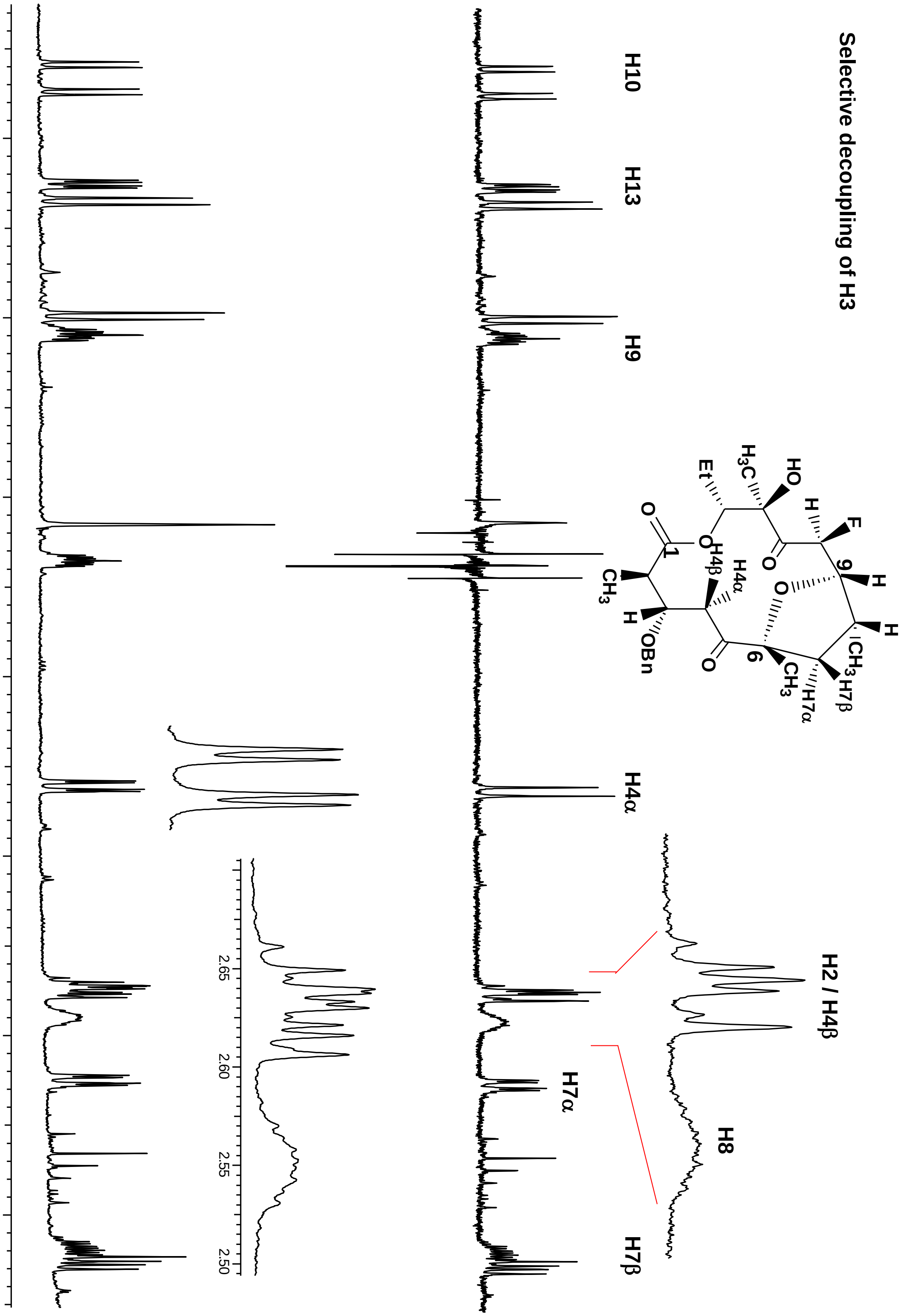
1H NMR chemical shifts (δ/ppm) & coupling constant (J/Hz)	13C NMR chemical shift (δ/ppm)
2.61 (q, $J_{H2, H3}$ = 9.4Hz, **H2**)	173.9 ----- C1
1.29 (d, $J_{2\text{-}CH3, H2}$ = 7.1Hz, **2-CH_3**)	44.2 ----- C2
3.82 (ddd, $J_{H3, H2}$ = 9.4Hz, $J_{H3, H4\beta}$= 3.3Hz, $J_{H3, H4\alpha}$= 5.9Hz **H3**)	76.9 ----- C3
4.50 (d, J_{AB} = 11.4Hz, **3-OCH_2**)	35.7 ----- C4
4.82 (d, J_{AB} = 11.4Hz, **3-OCH_2**)	207.2 ----- C5
3.19 (dd, $J_{H4\alpha,H4\beta}$= 14.2Hz, $J_{H4\alpha,H3}$= 5.9Hz, **H4α**)	90.1 ----- C6
2.62 (dd, $J_{H4\alpha,H3}$ = 3.3Hz, **H4β**)	39.9 ----- C7
1.29 (s, **6-CH_3**)	34.9 ----- C8
1.87 (dd, $J_{H7\beta, H8}$ = 7.5 Hz, $J_{H7\beta, H7\alpha}$ = 13.3Hz, **H7β**)	77.3 -- C9
2.38 (dd, $J_{H7\alpha, H8}$ = 3.6Hz, $J_{H7\alpha, H7\beta}$ = 13.3Hz, **H7α**)	87.6 -- C10,J_{CF}=173.1Hz
2.51 (m, **H8**)	205.9 -- C11,J_{CF}=13.6Hz
0.94 (d, $J_{8\text{-}CH3, H8}$ = 7.4Hz, **8-CH_3**)	78.8 ----- C12
4.45 (ddd, $J_{H9, H8}$ = 5.6Hz, $J_{H9, H10}$ = 9.2Hz, $J_{H9, F}$ = 3.6Hz, **H9**)	79.4 ----- C13
5.15 (dd, $J_{10, F}$ = 45.7, $J_{H10, H9}$ = 9.8Hz, **H10**)	23.4 ----- C14
1.54 (s, **12-CH_3**)	24.8 ----- 2-CH_3
3.92 (s, **12-OH**)	16.1 ----- 6-CH_3
4.87 (dd, $J_{H13, HA}$ = 2.6 Hz, $J_{H13, HB}$ = 11.2 Hz, **H13**)	14.7 ----- 8-CH_3
1.91 - 1.98, 1.56 - 1.63 (m, **14-CH_2**)	17.1 ----- 12-CH_3
0.94 (t, $J_{14\text{-}CH3, 13\text{-}CH2}$ = 7.4Hz, **14-CH_3**)	11.0 ----- 14-CH_3
7.39 - 7.28 (m, 5H, **Ph**)	71.6 ----- 3-O$\underline{C}H_2$PH
	137.8 ----- ^{ipso}C
	128.4 ----- $^{ortho}C$
	128.3 ----- ^{meta}C
	127.8 ----- ^{para}C

NOESY

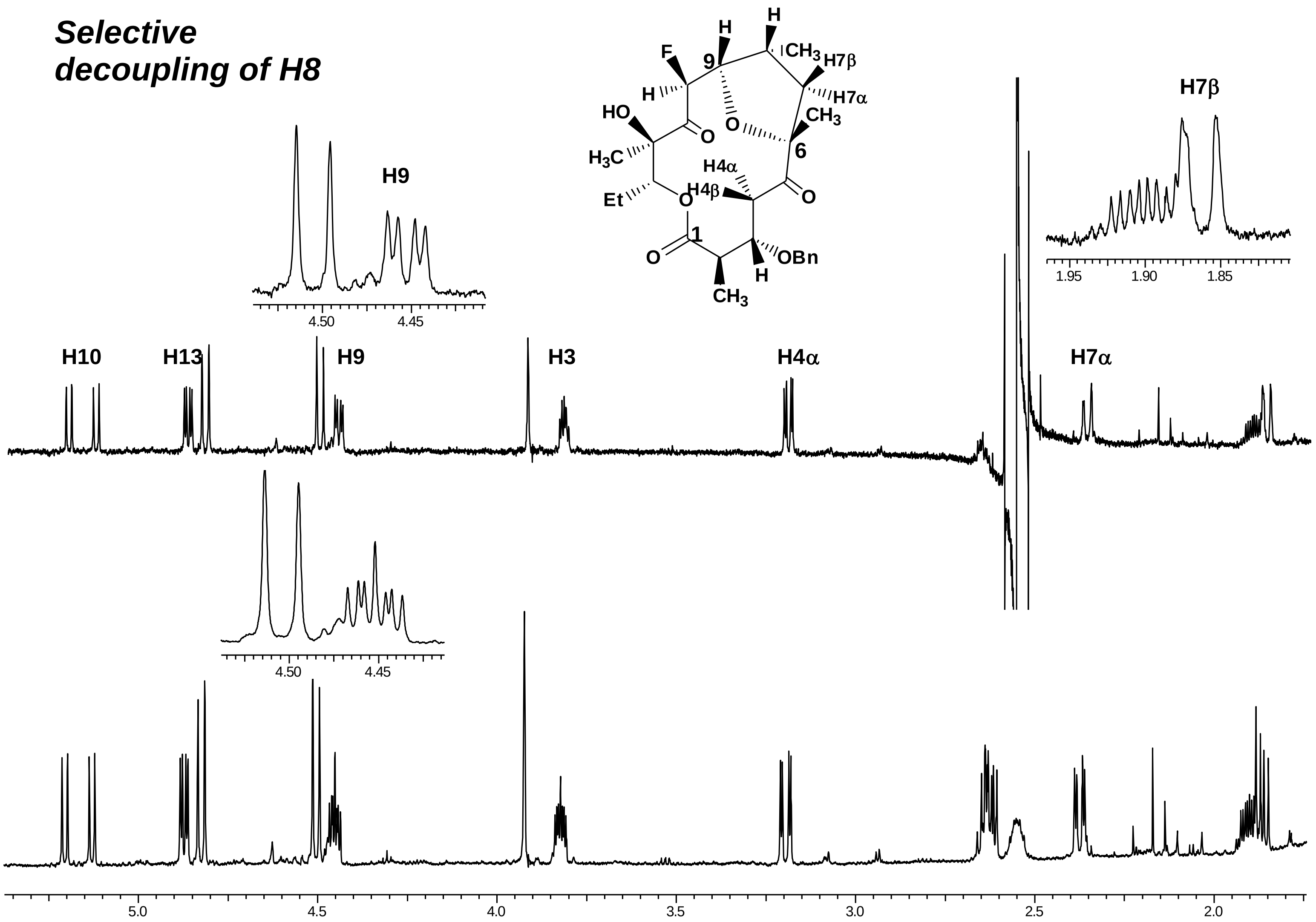

H
H₃C
H
H
F
H7β
H
H7α
HO
CH₃
O
O
H₃C
Et
O
O
O
H
O
O
H
CH₃
H
H

1D TOCSY subspectra



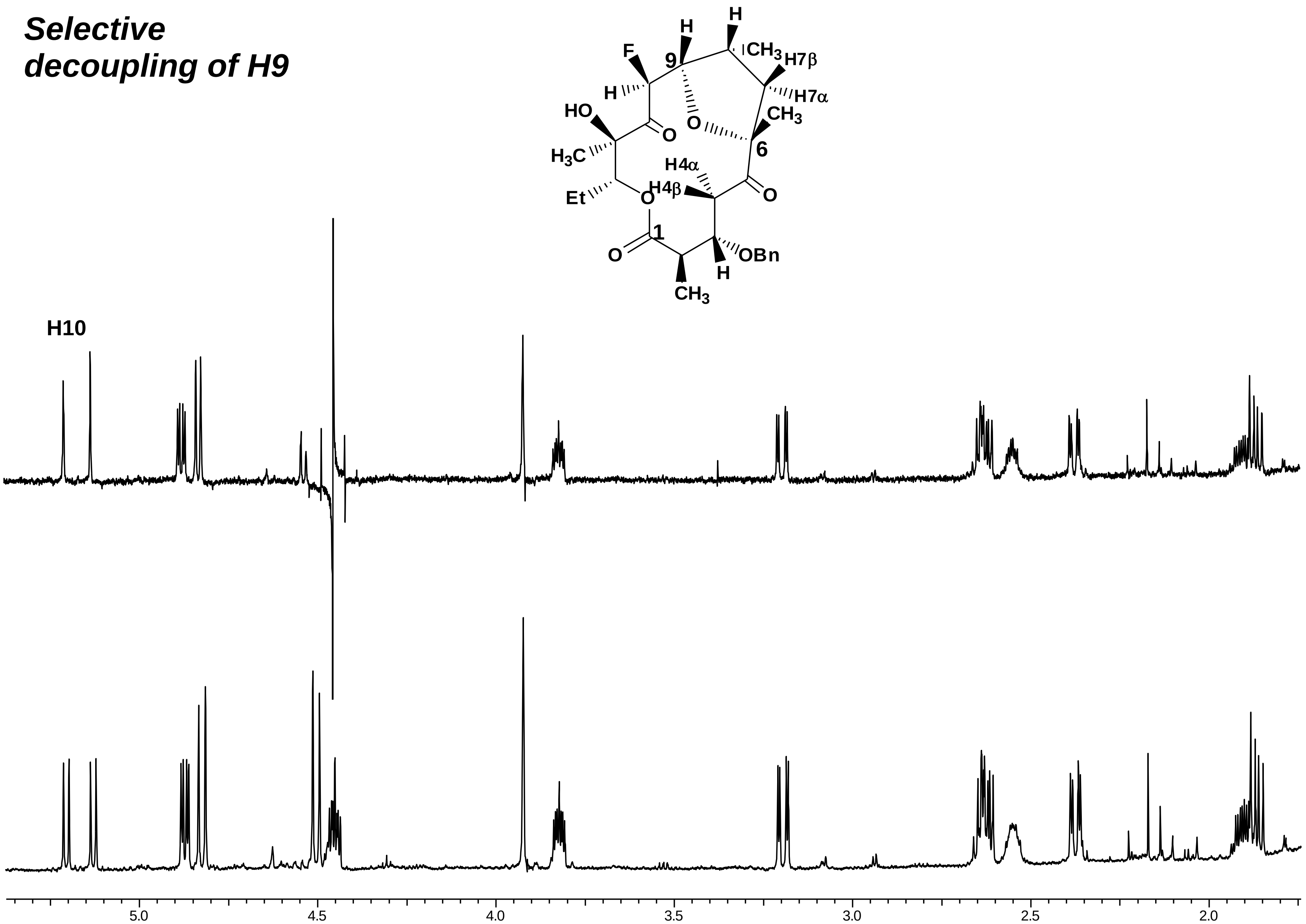


Selective decoupling of H3

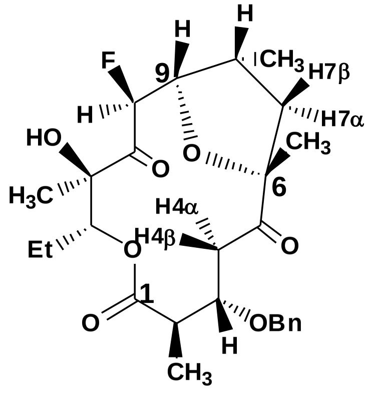


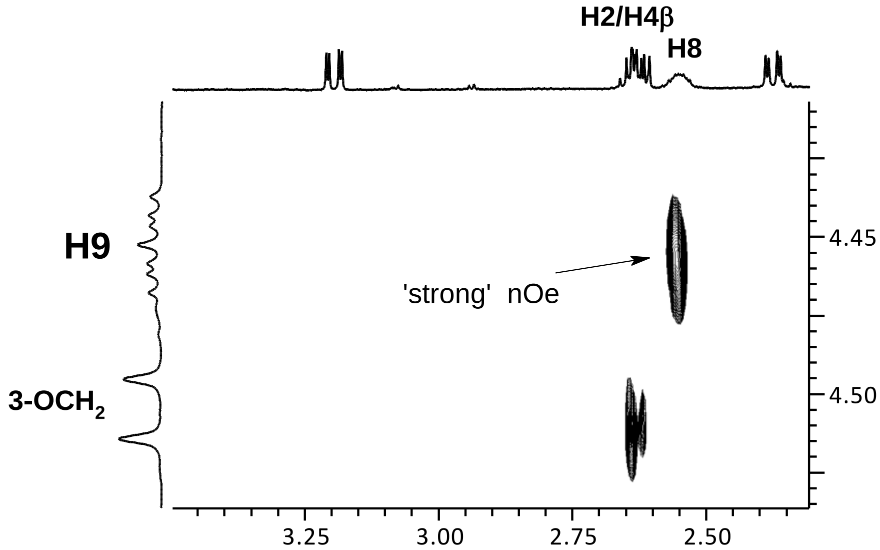
Selective decoupling of H8

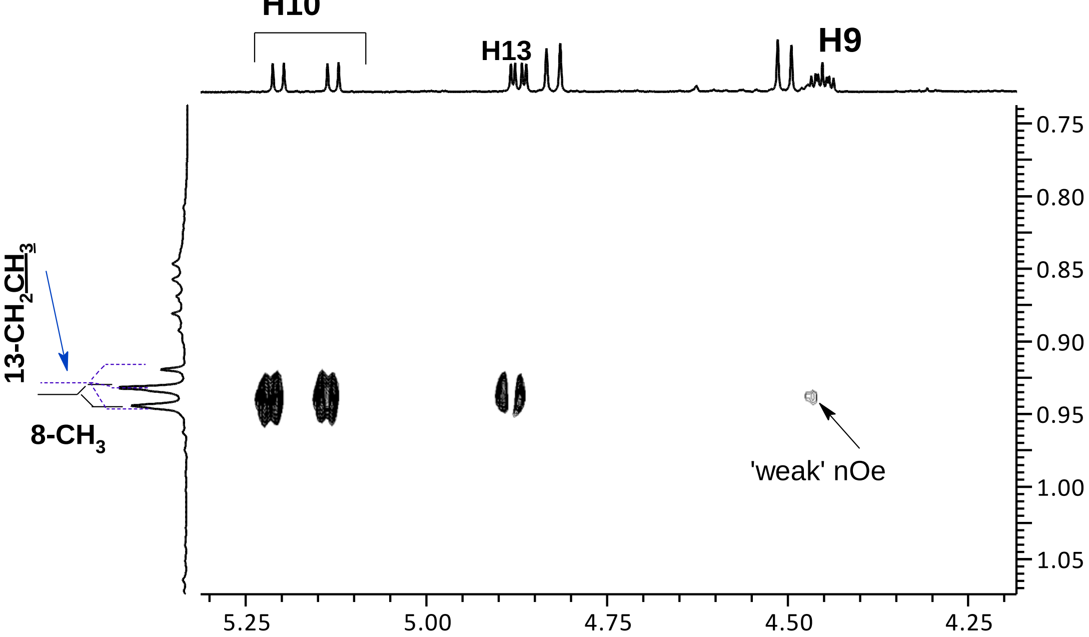
Selective decoupling of H9



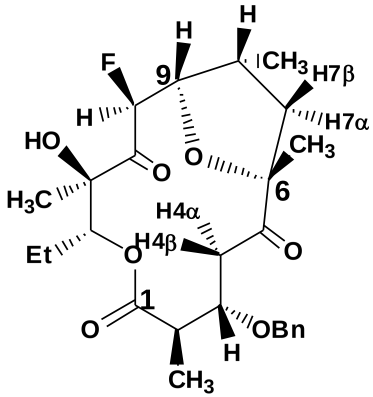
NOESY

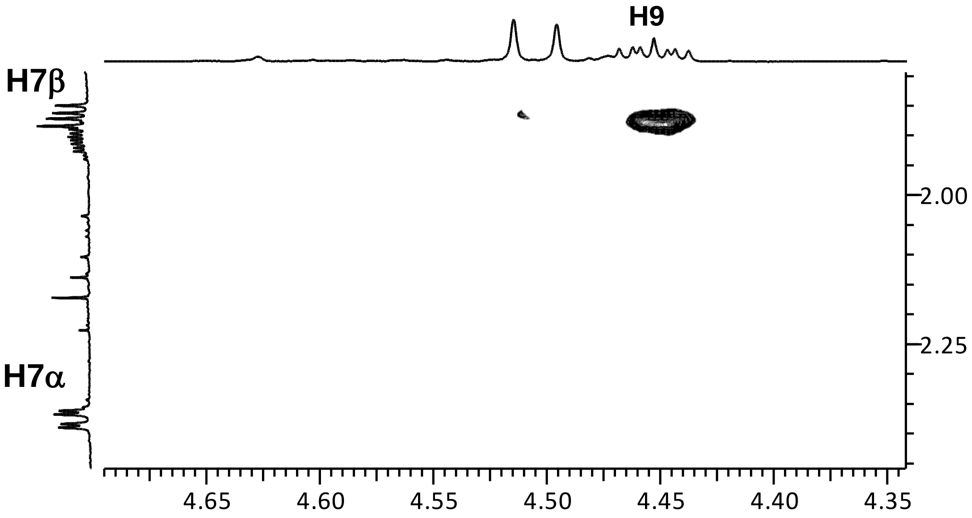

F
H
9
H
CH3
H7β
H7α
CH3
6
O
HO
H3C
H
O
H4α
H4β
Et
O
1
O
OBn
H
CH3
O

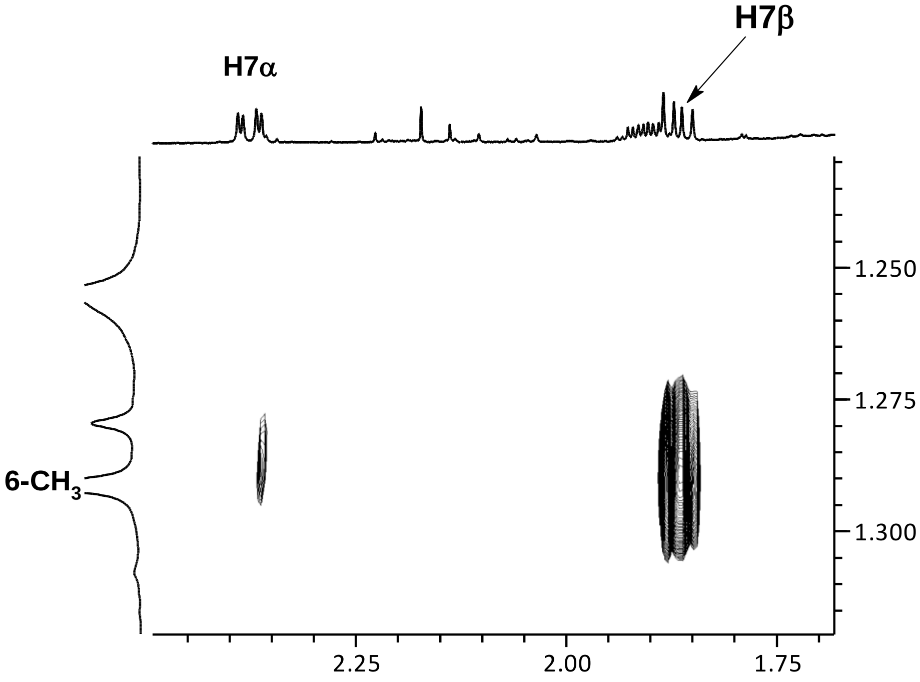

H2/H4β
H8
H9
3-OCH2
'strong' nOe
3.25
3.00
2.75
2.50
4.45
4.50

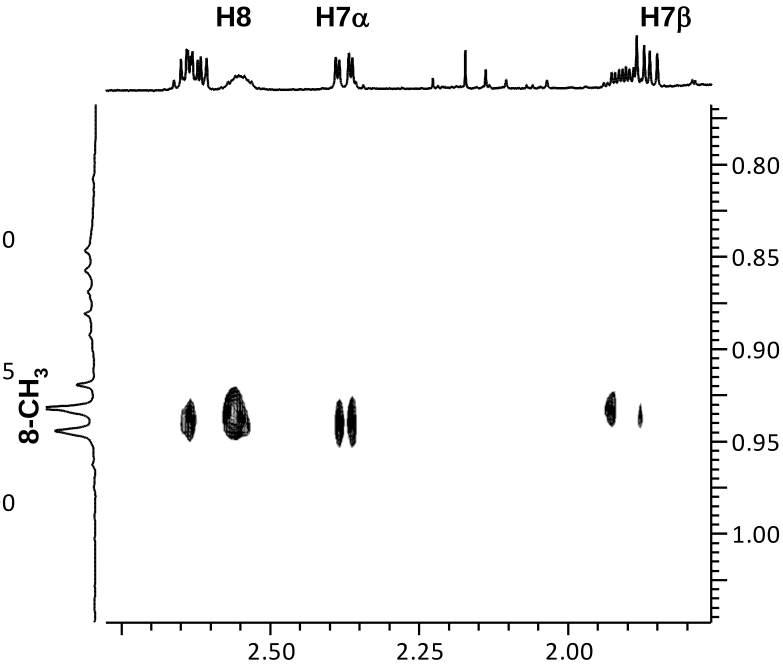

H10
H13
H9
13-CH2CH3
8-CH3
'weak' nOe
5.25
5.00
4.75
4.50
4.25
0.75
0.80
0.85
0.90
0.95
1.00
1.05

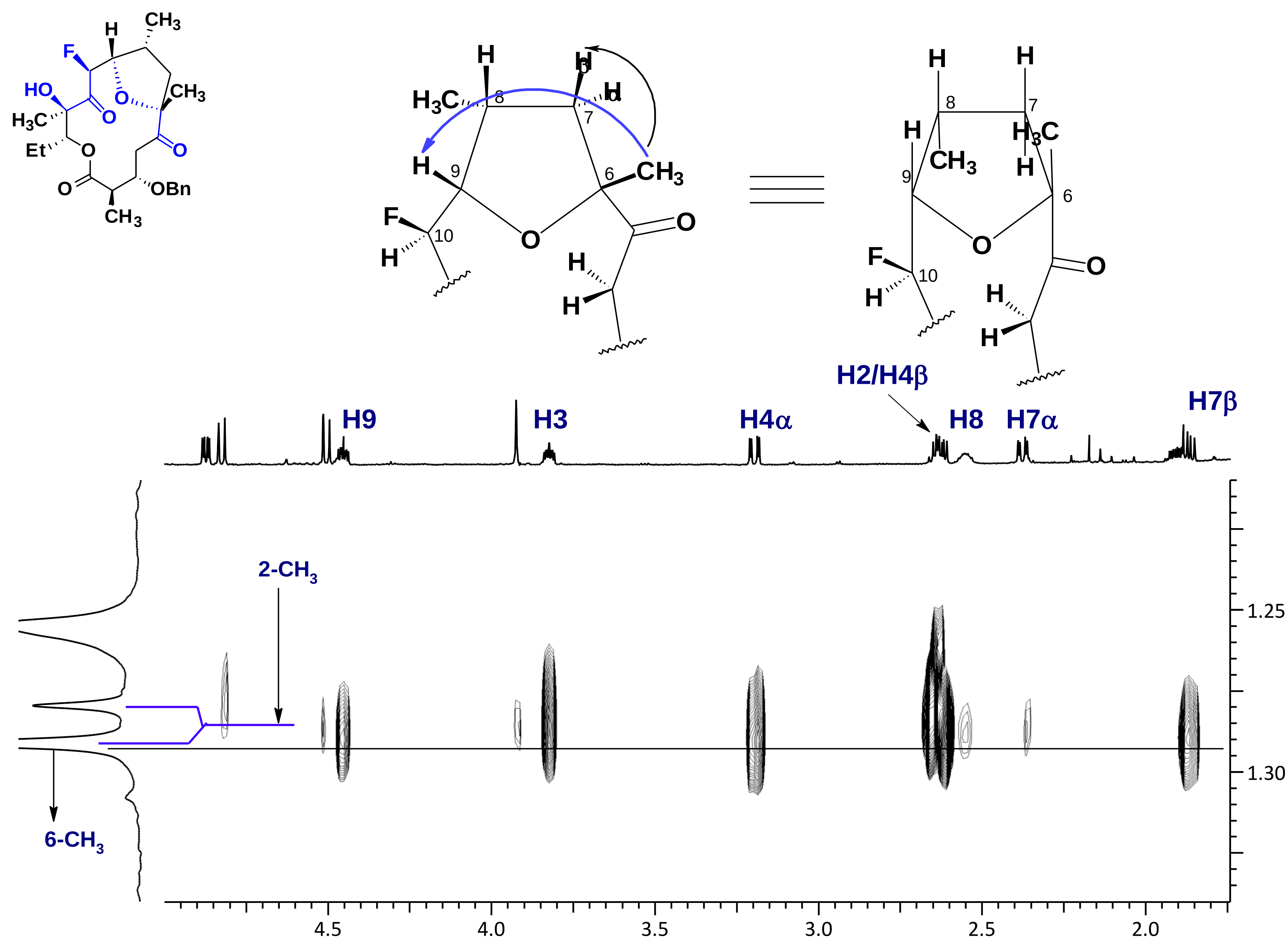
NOESY

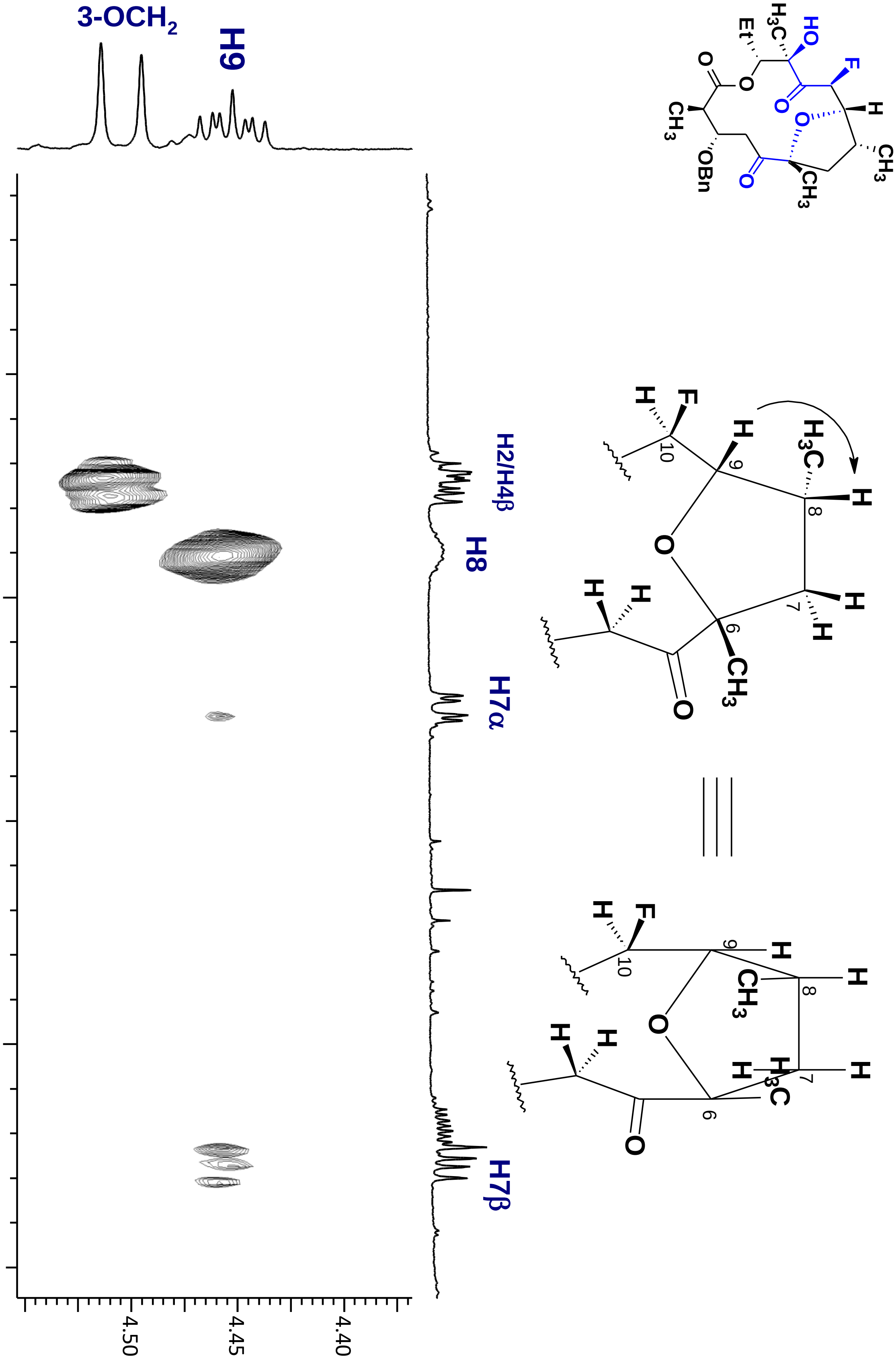

F
H
9
H
CH3
H7β
H7α
H
HO
O
O
CH3
6
H3C
H4α
H4β
Et
O
O
1
O
OBn
H
CH3

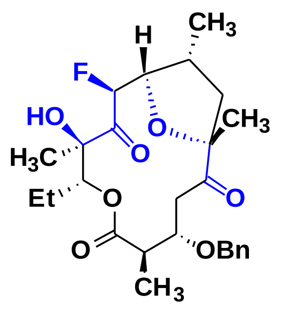

H9
H7β
H7α
2.00
2.25
4.65
4.60
4.55
4.50
4.45
4.40
4.35

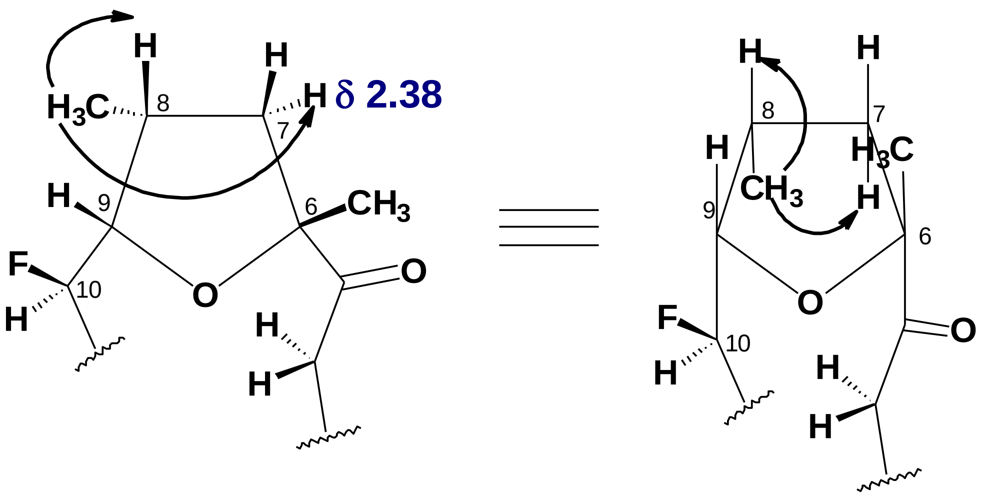

H7α
H7β
6-CH3
1.250
1.275
1.300
2.25
2.00
1.75

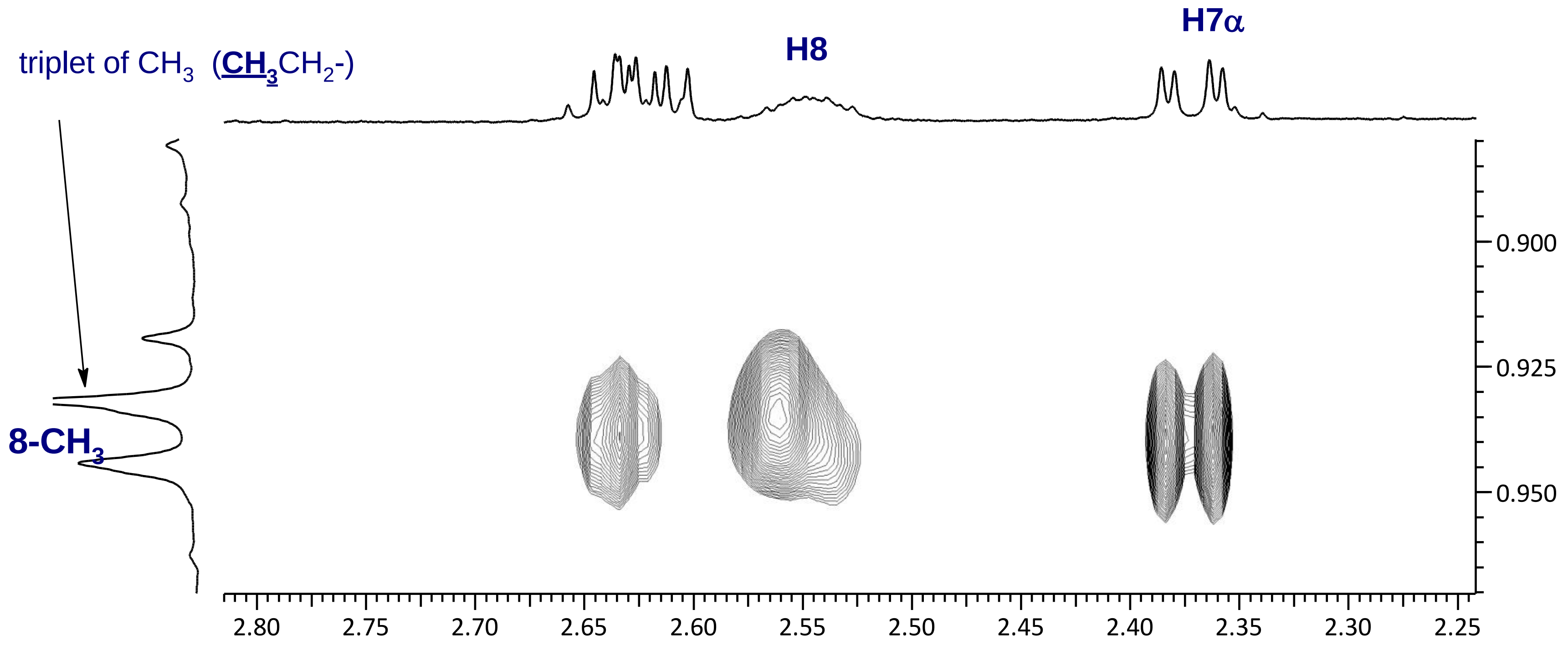

H8
H7α
H7β
8-CH3
0.80
0.85
0.90
0.95
1.00
2.50
2.25
2.00

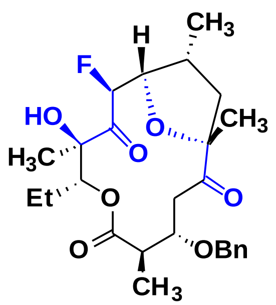

F
H
CH3
HO
O
CH3
H3C
O
Et
O
O
O
OBn
CH3
H
H3C
8
H
H
7
H
9
6
CH3
F
10
O
O
H
H
H
≡
H
H
8
7
H
H3C
CH3
9
H
6
O
F
10
O
H
H
H
H2/H4β
H9
H3
H4α
H8
H7α
H7β
2-CH3
6-CH3
4.5
4.0
3.5
3.0
2.5
2.0
1.25
1.30

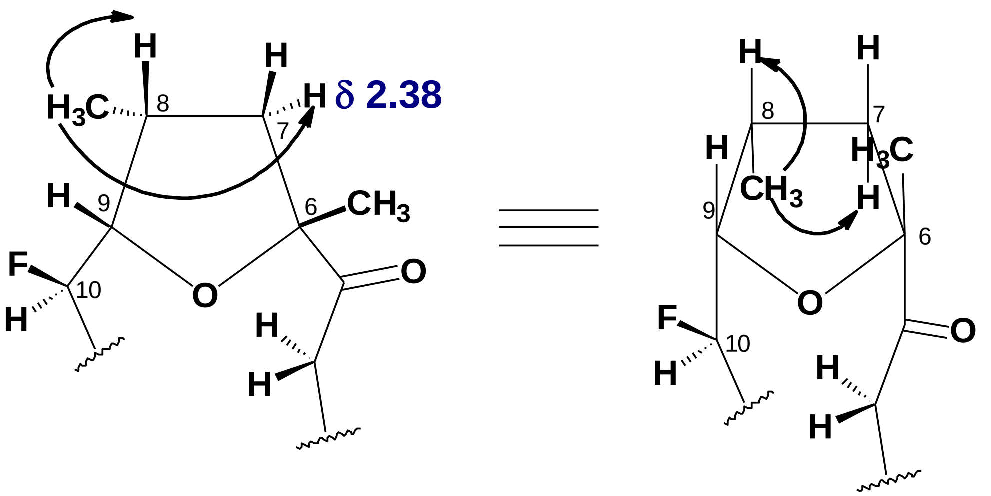

3-OCH$_2$
H9
H2/H4β
H8
H7α
H7β
2.5
2.0
4.50
4.45
4.40
HO
F
H
CH$_3$
H$_3$C
Et
O
OBn
CH$_3$
6
7
8
9
10

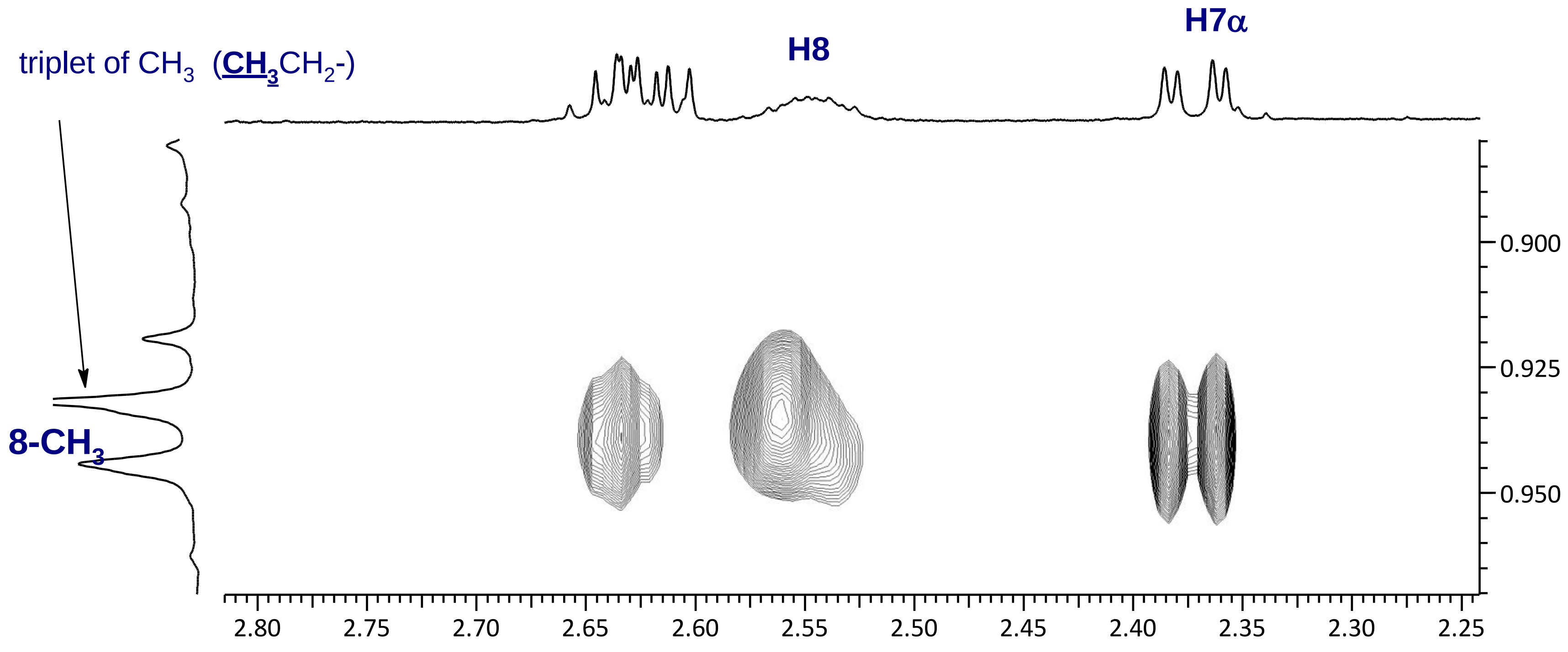

CH3
H
F
HO
H3C
Et
O
O
O
CH3
O
OBn
O
CH3

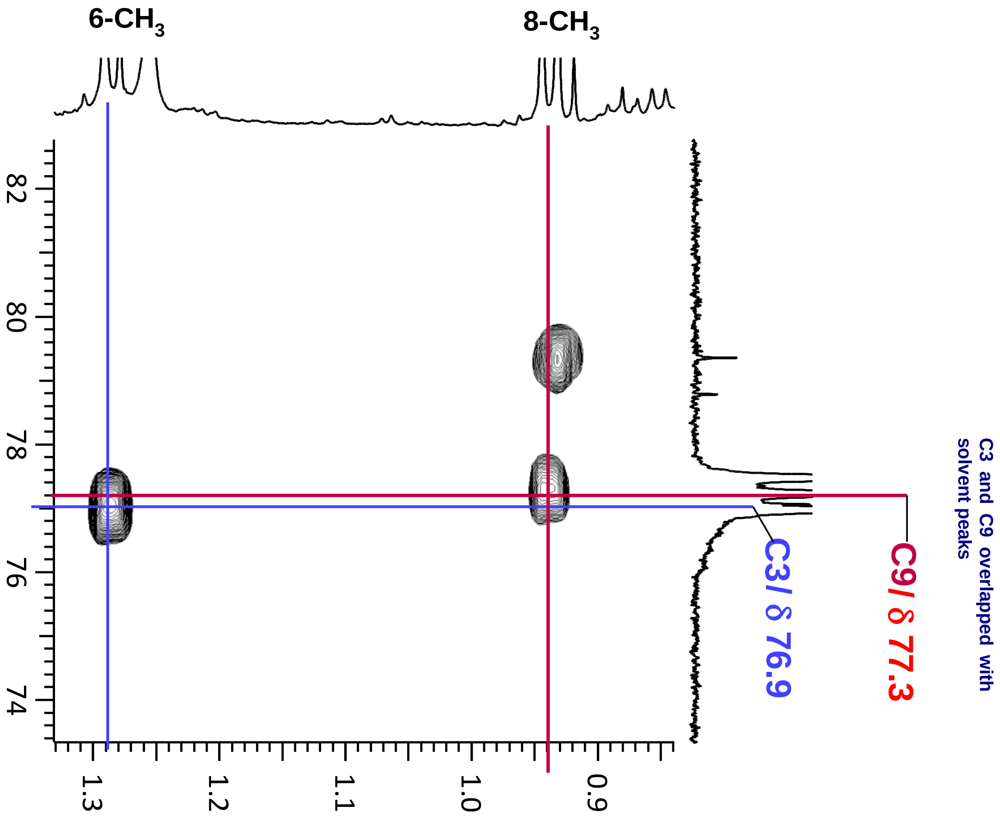

H3C
H
8
H
7
H δ 2.38
H
9
6
CH3
F
10
H
O
O
H
H
H
H
8
7
H3C
H
9
CH3
H
6
F
10
H
O
O
H
H

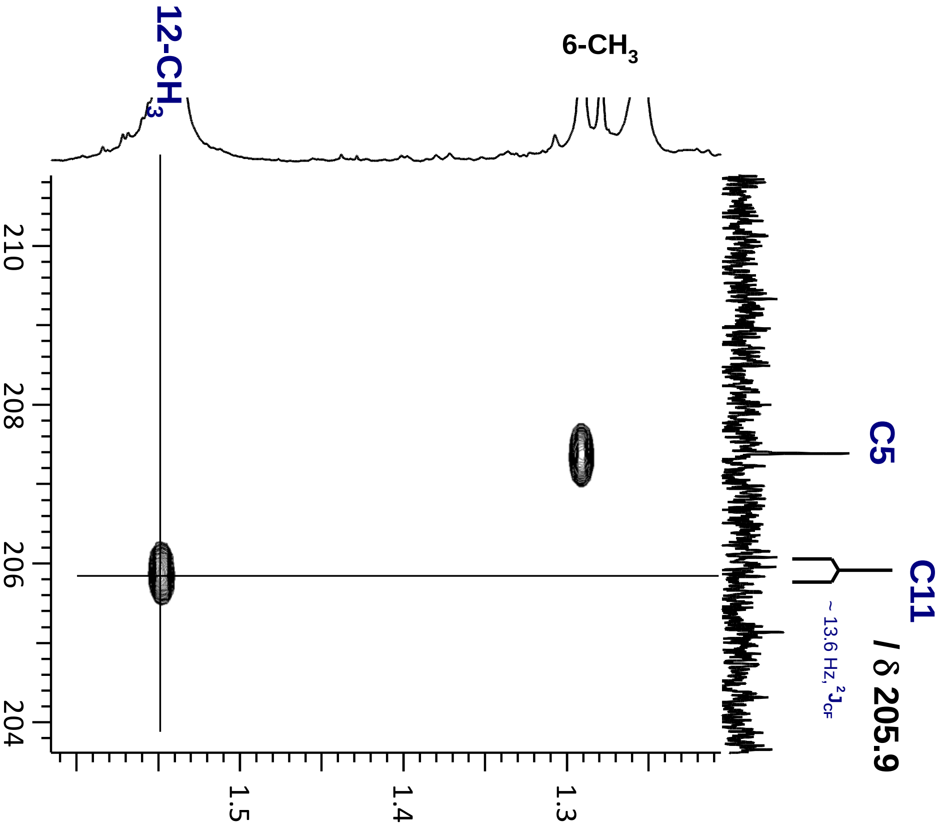

triplet of CH3 (CH3CH2-)
H8
H7α
8-CH3
0.900
0.925
0.950
2.80
2.75
2.70
2.65
2.60
2.55
2.50
2.45
2.40
2.35
2.30
2.25

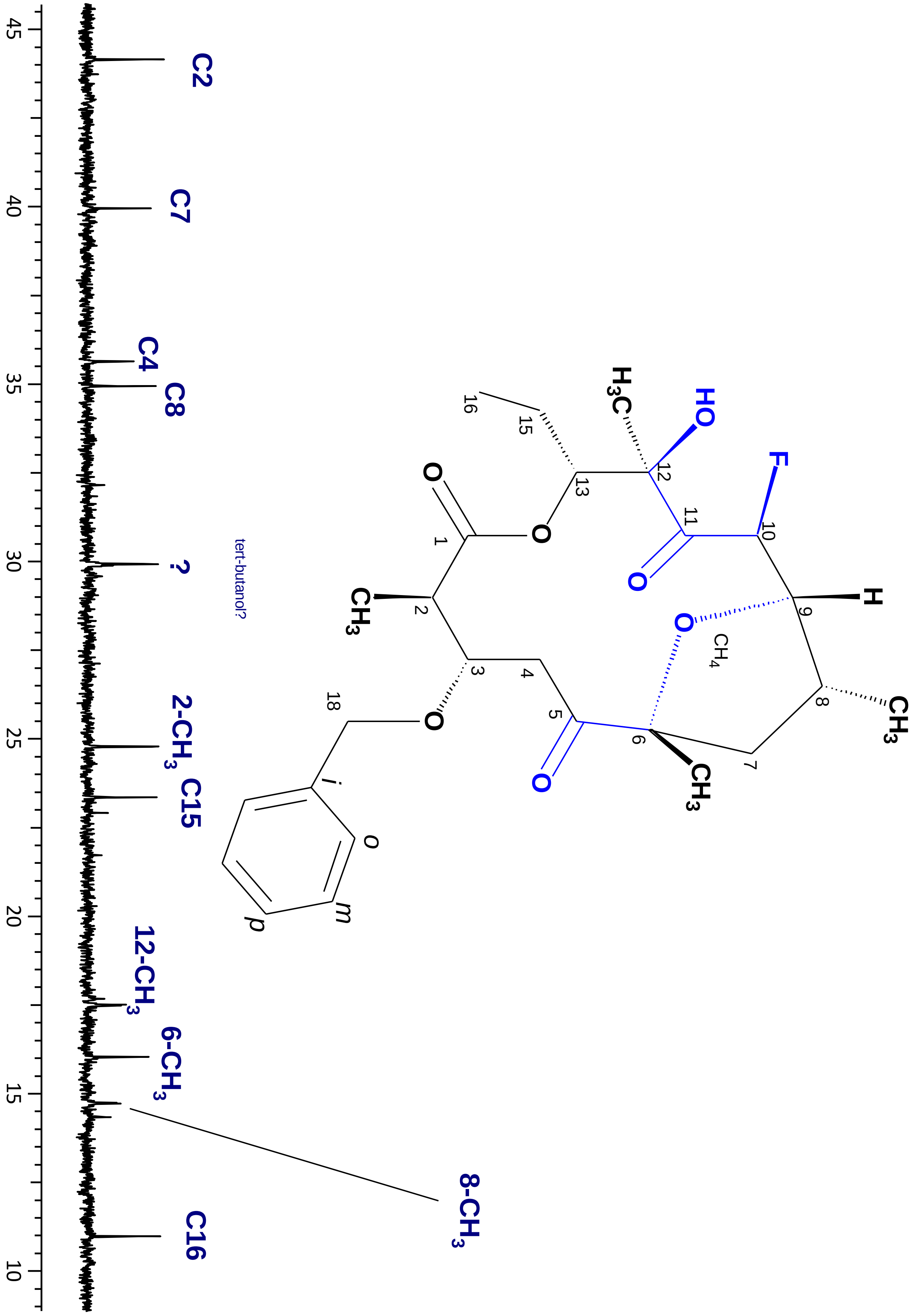

H
CH3
F
HO
O
CH3
H3C
O
Et
O
O
OBn
CH3

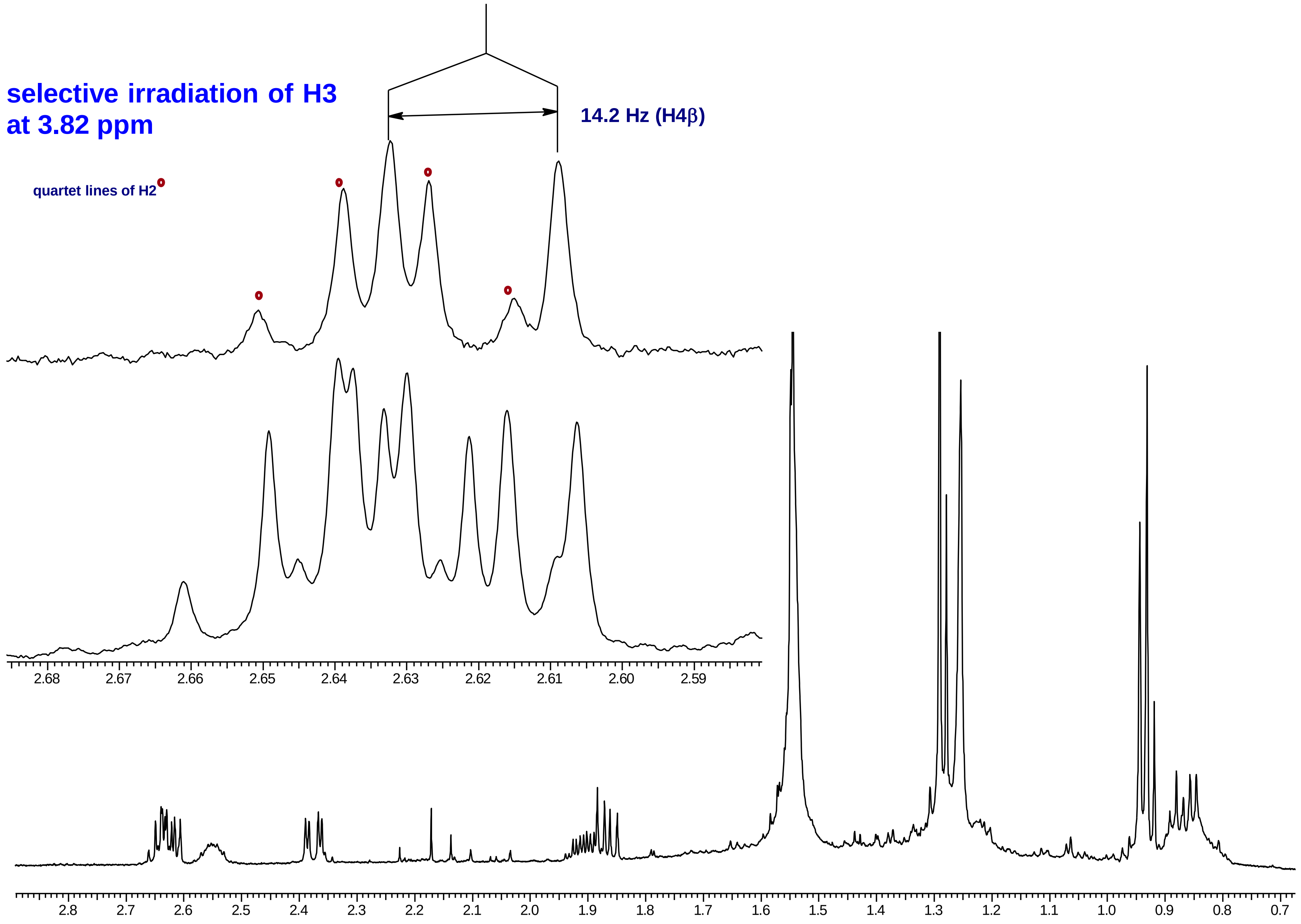

H
H
H3C
8
7
H δ 2.38
H
9
6
CH3
F
10
O
O
H
H
H
H
≡
H
H
8
7
H
H3C
9
CH3
H
6
F
10
O
O
H
H
H


triplet of CH3 (CH3CH2-)
H8
H7α
8-CH3
0.900
0.925
0.950
2.80
2.75
2.70
2.65
2.60
2.55
2.50
2.45
2.40
2.35
2.30
2.25


6-CH$_3$
8-CH$_3$
C3 and C9 overlapped with solvent peaks
C9/ δ 77.3
C3/ δ 76.9
82
80
78
76
74
1.3
1.2
1.1
1.0
0.9


12-CH$_3$
6-CH$_3$
C5
C11 / δ 205.9
~ 13.6 Hz, $^2J_{CF}$
210
208
206
204
1.5
1.4
1.3


C2
C7
C4
C8
?
tert-butanol?
2-CH$_3$
C15
12-CH$_3$
6-CH$_3$
8-CH$_3$
C16
45
40
35
30
25
20
15
10
F
HO
H$_3$C
O
O
O
O
O
O
CH$_3$
CH$_3$
CH$_3$
CH$_3$
H
CH$_4$
1
2
3
4
5
6
7
8
9
10
11
12
13
15
16
18
i
o
m
p


selective irradiation of H3
at 3.82 ppm
quartet lines of H2
14.2 Hz (H4β)
2.68
2.67
2.66
2.65
2.64
2.63
2.62
2.61
2.60
2.59
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7

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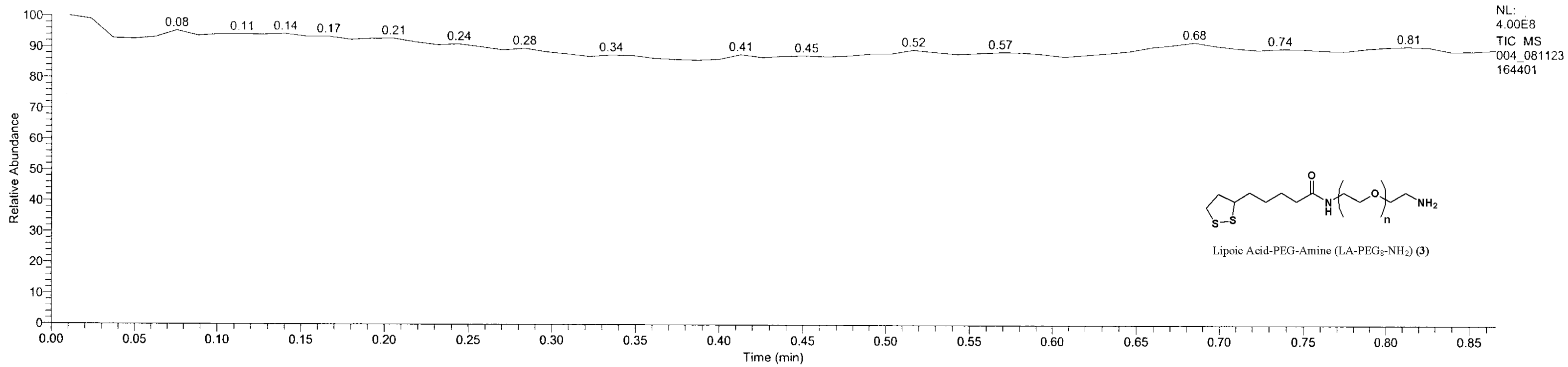
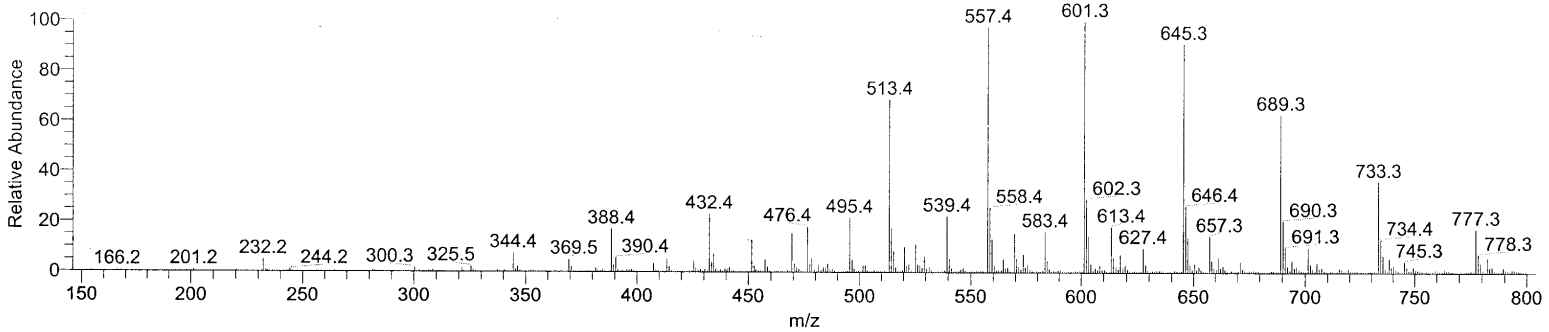
RT: 0.00 - 0.87



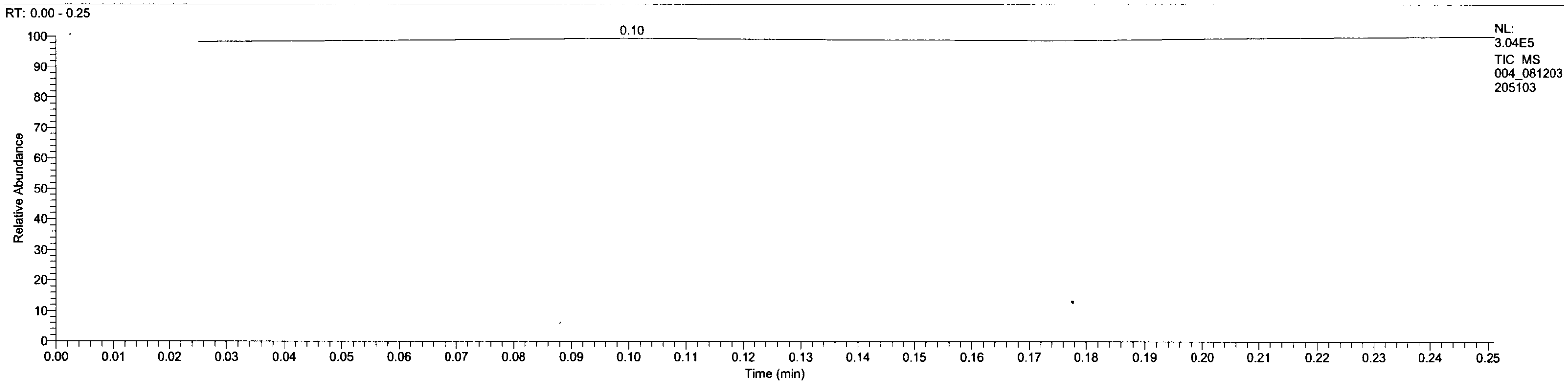
Lipoic Acid-PEG-Amine (LA-PEG$_8$-NH$_2$) **(3)**

004_081123164401#1-40 RT: 0.01-0.52 AV: 40 NL: 2.64E7
T: + c ms [150.00-800.00]

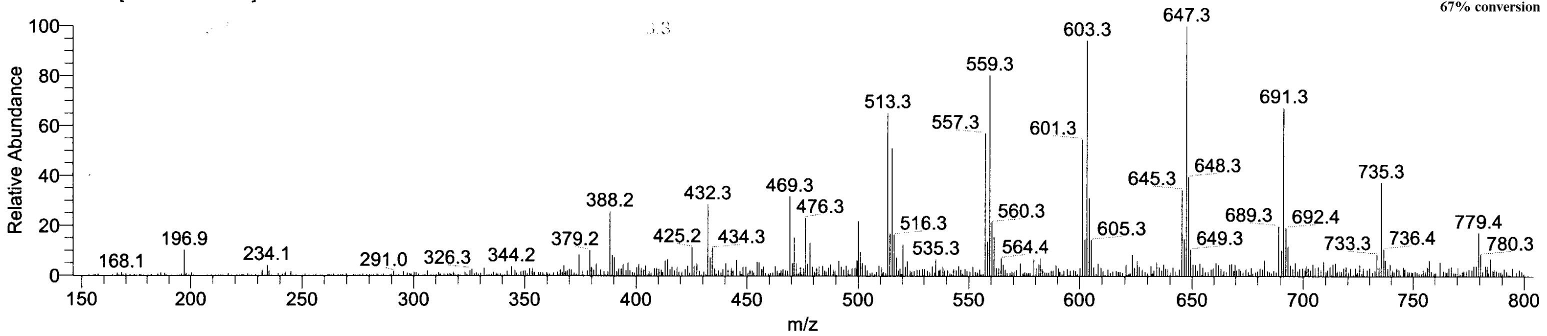


```
004_081123164401#1-40 RT: 0.01-0.52 AV: 40
T: + c ms [ 150.00-800.00]
m/z= 150.00-500.00
m/z           Intensity    Relative
  432.43        6057648.5 100.00
  495.44        5682525.7  93.81
  476.43        4760604.8  78.59
  388.44        4453938.2  73.53
  469.41        4070761.0  67.20
  451.47        3351374.5  55.32
  344.40        1930992.0  31.88
  434.42        1909039.1  31.51
  478.43        1478813.8  24.41
  390.43        1463203.4  24.15
```

D:\Michael\004_081203205103 12/03/2008 08:51:03 PM



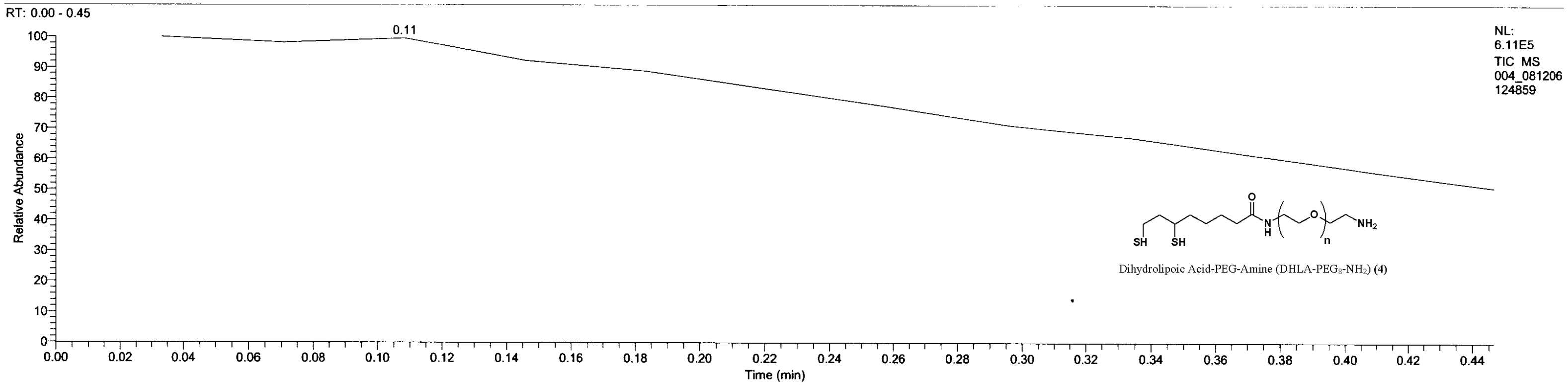
004_081203205103#1-7 RT: 0.03-0.25 AV: 7 NL: 1.31E4
T: + c Full ms [150.00-800.00]



Time = 4 h
67% conversion

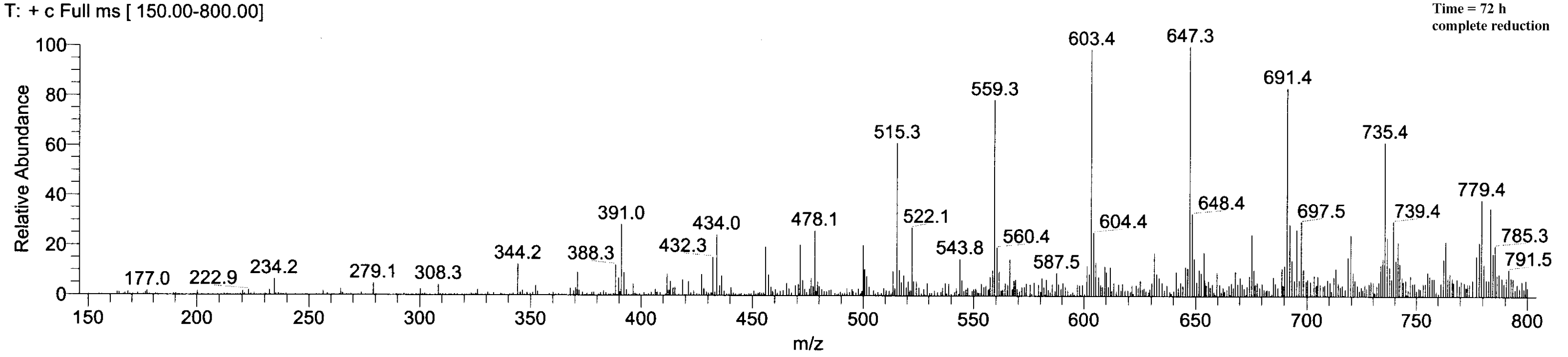
```
004_081203205103#1-7 RT: 0.03-0.25 AV: 7
T: + c Full ms [ 150.00-800.00]
m/z= 150.00-500.00
m/z          Intensity    Relative
  469.31          4144.3 100.00
  432.31          3739.0  90.22
  388.20          3356.3  80.99
  476.30          3023.9  72.96
  499.97          2856.3  68.92
  471.21          1985.9  47.92
  478.36          1720.6  41.52
  425.19          1485.1  35.84
  434.31          1437.1  34.68
  379.20          1314.6  31.72
```

D:\Michael\004_081206124859 12/06/2008 12:48:59 PM

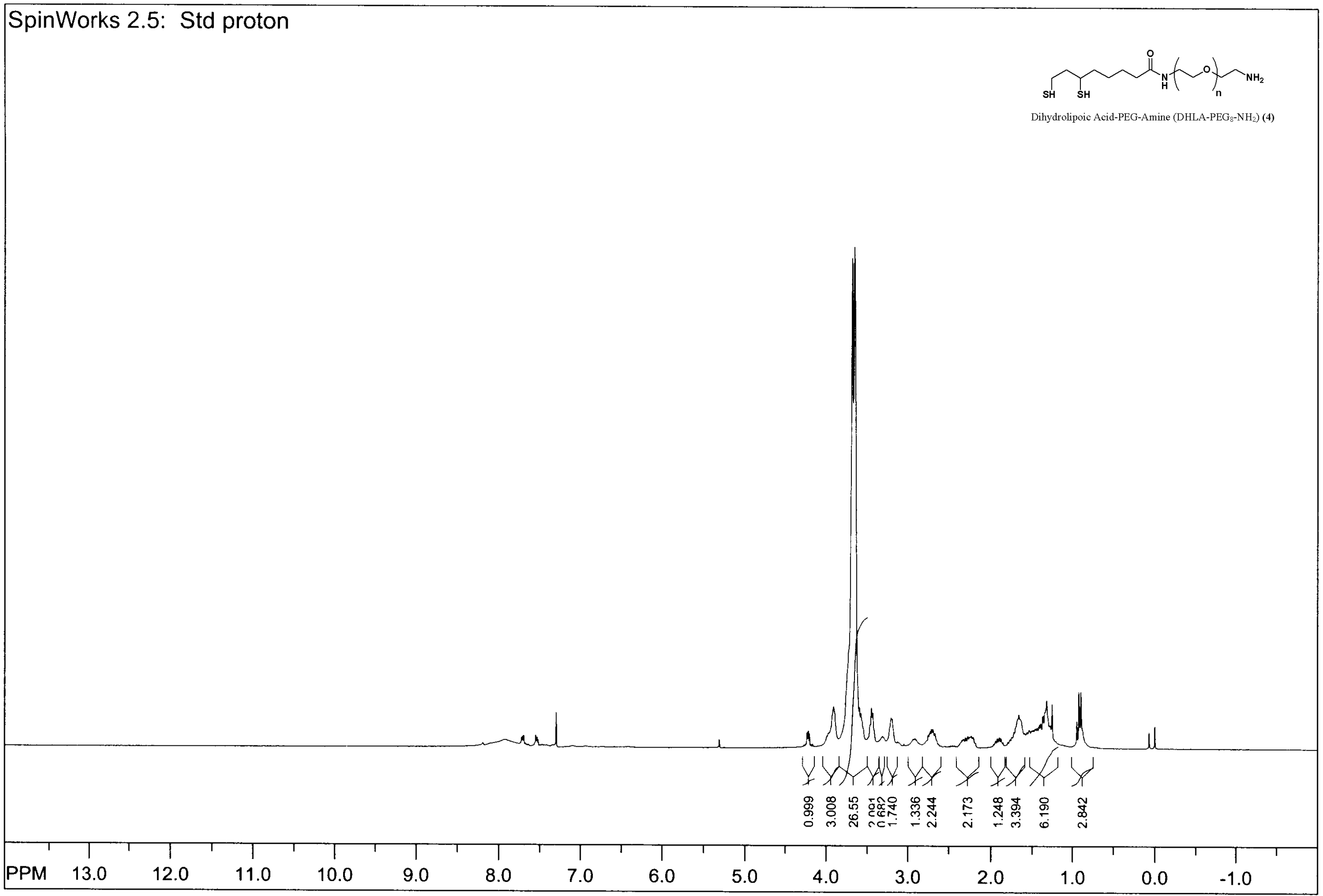


Dihydrolipoic Acid-PEG-Amine (DHLA-PEG$_8$-NH$_2$) (4)

004_081206124859#1-12 RT: 0.03-0.45 AV: 12 NL: 1.67E4
T: + c Full ms [150.00-800.00]



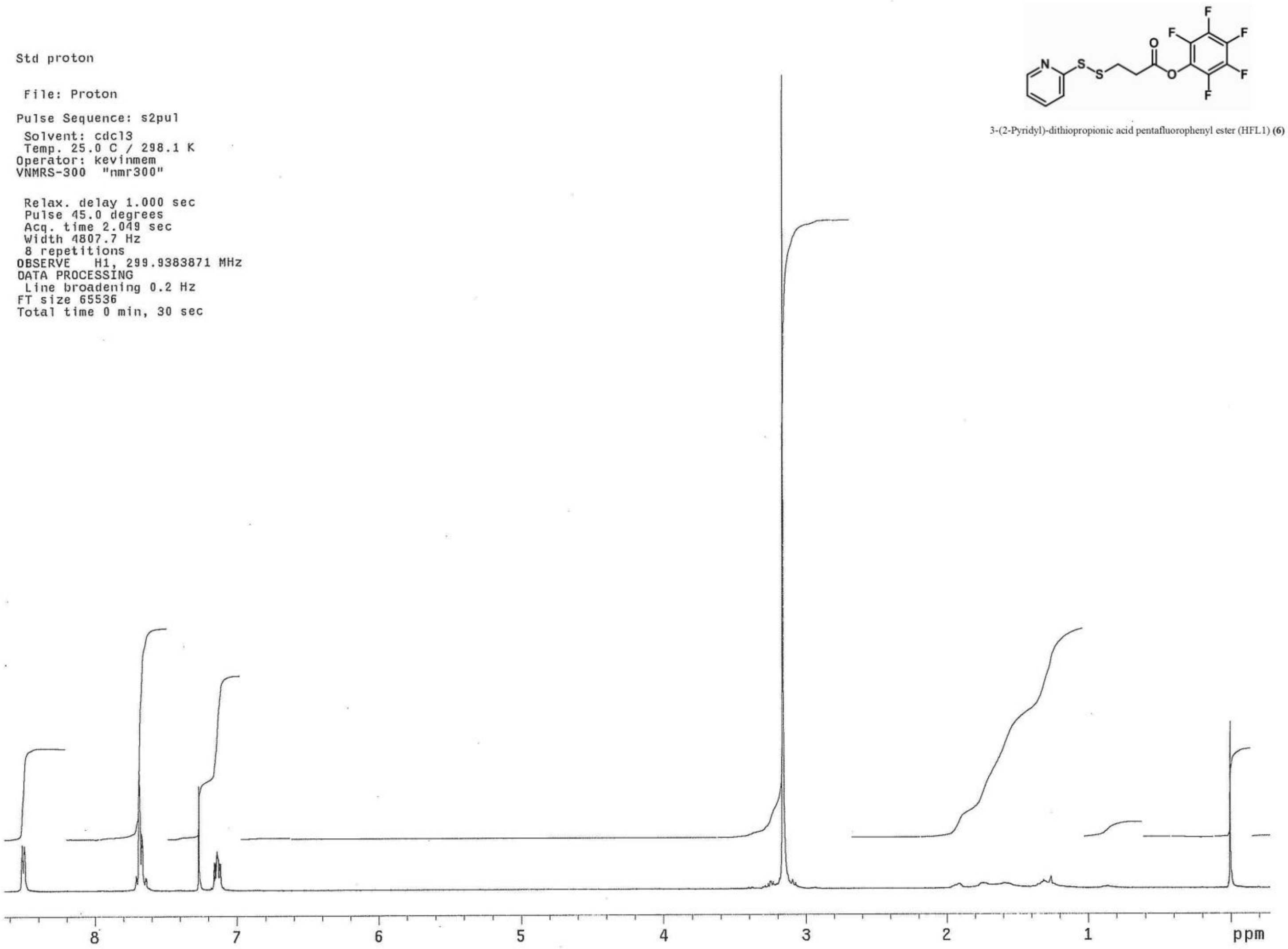
```
004_081206124859#1-12 RT: 0.03-0.45 AV: 12
T: + c Full ms [ 150.00-800.00]
m/z=  150.00-500.00
m/z          Intensity    Relative
  391.02          4719.2 100.00
  478.10          4321.8  91.58
  434.04          4056.1  85.95
  471.38          3384.5  71.72
  499.89          3342.2  70.82
  455.98          3235.1  68.55
  432.30          2548.7  54.01
  344.23          2056.0  43.57
  388.34          2013.2  42.66
  371.15          1521.3  32.24
```



SpinWorks 2.5: Std proton

Dihydrolipoic Acid-PEG-Amine (DHLA-PEG$_8$-NH$_2$) **(4)**

```
file: C:\Documents and Settings\Michael Drahl\My Documents\Rutgers - New Brunswick\2008 - 2009\Resefreq. of 0 ppm: 299.938377 MHz300\011-5.fid\fid  block# 1 expt: "s2pul"
transmitter freq.: 299.940183 MHz                                    processed size: 65536 complex points
time domain size: 19698 points                                       LB:   0.200   GB: 0.0000
width:  4807.69 Hz = 16.028837 ppm = 0.244070 Hz/pt                  Hz/cm:  192.307    ppm/cm:   0.64115
number of scans: 8
```



```
Std proton

 File: Proton

Pulse Sequence: s2pul
 Solvent: cdcl3
 Temp. 25.0 C / 298.1 K
Operator: kevinmem
VNMRS-300  "nmr300"

 Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 2.049 sec
 Width 4807.7 Hz
 8 repetitions
OBSERVE   H1, 299.9383871 MHz
DATA PROCESSING
 Line broadening 0.2 Hz
FT size 65536
Total time 0 min, 30 sec
```

3-(2-Pyridyl)-dithiopropionic acid pentafluorophenyl ester (HFL1) **(6)**

CURRICULUM VITAE

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September 1996 – December 2000	Grand Canyon University Phoenix, AZ Subject: Chemistry Degree Earned: B.S.
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