SYNTHESIS OF STRUCTURALLY SIMPLIFIED BRYOSTATIN ANALOGUES: PROBING THE UNDERLYING BIOLOGY AND PROGRESSING TOWARDS MORE 'DRUG LIKE' COMPOUNDS

by

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STATEMENT OF DISSERTATION APPROVAL

The following faculty members served as the supervisory committee chair and members for the dissertation of **Mark Edmund Petersen**. Dates at right indicate the members' approval of the dissertation.

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ABSTRACT

Bryostatin 1 is a highly complex marine natural product originally isolated by Pettit in the 1960s. Since its structural elucidation in 1982 bryostatin 1 has attracted considerable attention for the treatment of several human diseases such as cancer, HIV, and Alzheimer's. Bryostatin 1 exerts it effect by binding to and activating Protein Kinase C (PKC) isozymes with nanomolar affinity. Bryostatin 1 is unique among the many known activators in that it is nontumor promoting. Contrastingly, the Phorbal ester PMA, which shares the same binding pocket, is one of the most potent tumor promoters known.

Despite intense medical interest, the development of bryostatin 1 as a therapeutic has been impeded by its extremely low natural abundance. To address this problem numerous groups have developed elegant syntheses of the natural bryostatins. Another and perhaps more attractive solution however is the synthesis of simplified bryostatin analogues. Towards this end the Keck group synthesized the analogue Merle 23, which in cell assays demonstrated either a PMA like response or a bryostatin like response depending on the cell line. This paradoxical behavior illustrates the complexity of PKC activation as therapeutic strategy, and Merle 23 provides a valuable tool for probing the subtle differences between tumor promoting and nontumor promoting PKC ligands. Described within is the scaled synthesis of Merle 23 and it use for further probing the biological consequence of PKC activation at the transcriptional level. Merle 23 as well

as two less lipophilic analogues Merle 35 and Merle 37 are also shown to be potent activators of latent HIV reservoirs.

Central to the Keck group's analogue work is identifying strategies by which the synthetic burden can be reduced. In order to simplify the synthesis of new analogues the use of simple aromatic building blocks as surrogates for the A and B ring pyrans was explored. Using phenyl rings to replace the pyrans resulted in an analogue that failed to maintain high affinity binding in spite of it still containing all of the elements previously believed to be responsible for binding.

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STANDARD LIST OF ABBREVIATIONS

Δv	geometrical mean of the distances between the two outer and the two inner signals in an AB spin system (in NMR)
$[\alpha]_D^{20} =$	specific rotation [expressed without units; units, deg mL/(g dm), are understood]
Å	Ångström
Ac	acetyl
AcOH	Acetic acid
BBr_3	boron tribromide
BF_3OEt_2	boron trifluoride etherate
9-BBN	9-borabicyclo(3.3.1)nonane
BINOL	(1,1'-binapthalene)-2,2'-diol
BOM	benzyloxymethyl
Bn	benzyl
Bu	butyl
t-Bu	*tert*-butyl
n-Bu	butyl
n-BuLi	*n*-butyl lithium
°C	degrees Celsius
CAA	catalytic asymmetric allylation

calcd	calculated
$CDCl_3$	deuterated chloroform
CDI	carbonyldiimidizole
$CHCl_3$	chloroform
CH_2Cl_2	dichloromethane
COSY	correlation spectroscopy
d	day(s); doublet (spectral)
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarization transfer
DIBAL-H	diisobutylaluminun hydride
DIPEA	diisopropylethylamine
DIPA	diisopropyamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EtOAc	ethyl acetate
Et_2O	diethylether
Et_3N	triethylamine

er	enantiomeric ratio
equiv	evuivelent(s)
g	gram(s)
h	hour(s)
HRMS	high-resolution mass spectrum
Hz	hertz
IC_{50}	50% inhibitory concentration
iPr_2NH	diisopropylamine
IR	infrared
J	coupling constant (in NMR)
KH	potassium hydride
K_i	binding affinity
LDA	Lithium diisopropyl amide
LIDBB	Lithium ditertbutylbiphenyl
M	moles per liter
*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MeCN	acetonitrile
MeOH	methanol
$MgSO_4$	magnesium sulfate
MHz	megahertz

m	minute(s)
MMPP	magnesium monoperoxyphthalate hexahydrate
MNBA	2-methyl-6-nitrobenzoic anhydride
mol	mole(s)
mp	melting point
M/Z	mass to charge ration (mass spec)
$NaBH_4$	sodium borohydride
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
Na_2SO_4	sodium sulfate
NH_4Cl	ammonium chloride
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
Ph	phenyl
PKC	protein kinase C
PMB	*p*-methoxybenzyl
ppm	parts per million (in NMR)
PPTs	pyridinium *p*-toluenesulfonate
i-Pr	isopropyl
Py	pyridine

prenyl	3-methyl-2-buten-1-yl
q	quartet (spectral)
R_f	retention factor (in chromatography)
rt	room temperature
s	singlet (NMR); second(s)
SO_3•Pyr	sulfur trioxide pyridine complex
t	triplet (spectra)
TBAF	tetrabutylammonium fluoride
t-Bu	tertiary butyl
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TES	triethylsilyl
Tf	trifluoromethanesulfonoyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N*,*N*,*N*,*N*-tetramethyl-1,2-ethylenediamine
TMS	trimethylsilyl, tetramethylsilane
TNFα	tumor necrosis factor alpha
TPAP	tetrapropylammonium perruthenate
TsOH	*p*-toluenesulfonic acid

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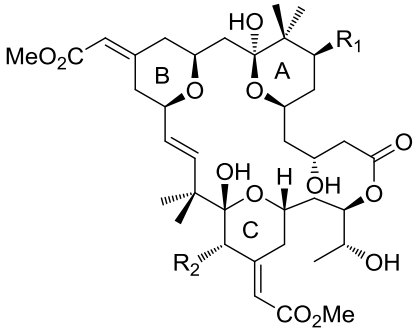
CHAPTER 1

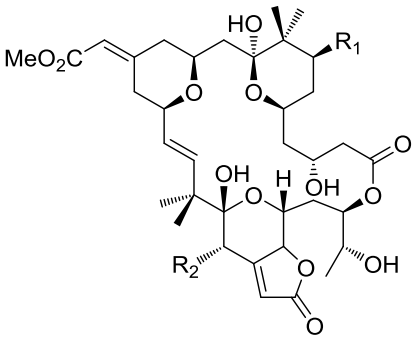
SCALED SYNTHESIS OF THE BRYOSTATIN ANALOGUE MERLE 23, AND IDENTIFYING A STRATEGY FOR SOLUBILIZING ANALOGUES

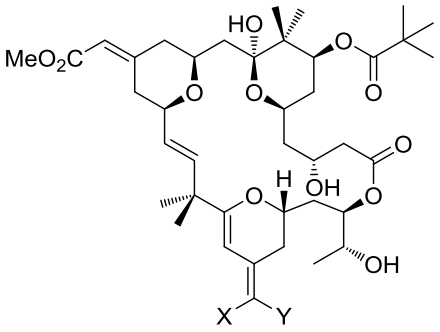
Introduction to Bryostatins

Bryostatins are a family of 20 marine macrolides isolated in 1970 from the bryozoan *Bugula neritina*, collected from the Gulf of Mexico.[1] Crude extracts from *Bugula neritina*, a moss like animal, which grows worldwide and is known to attach to the hull of ships, first attracted attention when they were shown to have considerable cytotoxicity against the murine p388 lymphocytic leukemia cell line, as well as moderate effects against other NCI 60 cancer cell lines. The active component of these extracts, bryostatin 1 (Bryo 1), was purified and characterized in 1982.[2] Since the initial collection and isolation, 19 other bryostatins,[3] as well as another closely related natural product,[4] have been discovered (Figure 1.1). All bryostatins contain three pyran rings A, B, and C embedded within a 20-membered ring, and most differ only in their C7 (R_1) and C20 (R_2) ester substituents. Bryostatins 3, 19, and 20 contain an additional ring that results from the fusion of the C21 exocyclic enoate to the C-ring. Bryostatins 16 and 17 lack both the C20 ester substituent and the C19 hemi-ketal, while bryostatin 18 is missing only the C20



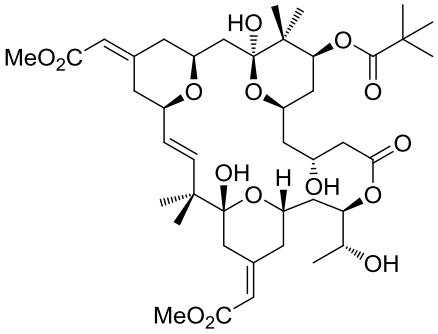
bryostatin 1	R_1 = OAc	R_2 = OCO(CH)$_4$Pr
bryostatin 2	R_1 = OH	R_2 = OCO(CH)$_4$Pr
bryostatin 4	R_1 = OCO*t*-Bu	R_2 = OCO*n*-Pr
bryostatin 5	R_1 = OCO*t*-Bu	R_2 = OAc
bryostatin 6	R_1 = OCO*n*-Pr	R_2 = OAc
bryostatin 7	R_1 = OAc	R_2 = OAc
bryostatin 8	R_1 = OCO*n*-Pr	R_2 = OCO*n*-Pr
bryostatin 9	R_1 = OAc	R_2 = OCO*n*-Pr
bryostatin 10	R_1 = OCO*t*-Bu	R_2 = H
bryostatin 11	R_1 = OAc	R_2 = H
bryostatin 12	R_1 = OCO(CH)$_4$Pr	R_2 = OCO*n*-Pr
bryostatin 13	R_1 = OCO*n*-Pr	R_2 = H
bryostatin 14	R_1 = OCO*t*-Bu	R_2 = OH
bryostatin 15	R_1 = OAc	R_2 = OCO(CH)$_4$ CH(OH)Et



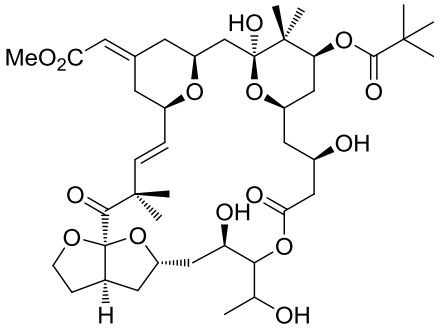


bryostatin 3	R_1 = OAc	R_2 = OCO(CH)$_4$Pr
bryostatin 19	R_1 = OCO*t*-Bu	R_2 = OCO*n*-Pr
bryostatin 20	R_1 = OCO*t*-Bu	R_2 = H

bryostatin 16	Y = CO_2Me	X = H
bryostatin 17	Y = H	X = CO_2Me



bryostatin 18



neristatin

Figure 1.1. The bryostatin family

ester. Neristatin is another member of the bryostatin family; however, it contains a rearranged C- ring.

Bryostatin and cancer

As a result of bryostatin 1's potent antineoplastic properties against the murine p388 lymphocytic leukemia cell line, it has been the focus of numerous in-vitro and in-vivo studies. In 2002 Pettit et al. published a comprehensive review of these studies.[5] In spite of potent activity in cell assays and mouse models, bryo 1 has shown limited activity in clinical trials as a single agent. To date only one patient, a 41-year-old woman who had stage 4 follicular small-cell-cleaved non-Hodgkin's lymphoma, has been cured by bryo 1.[6] More promising has been the use of bryostatin in combination with other anticancer treatments such as paclitaxel,[7] vincristine,[8] interleukin II,[9] fludarabine,[10] and dolastatin 10,[11] among others. In the approximately 80 completed or ongoing clinical trials, bryo 1 has been so potent that only 1 mg has been required for an entire 6-week cycle, and the only negative side effect observed was mild to severe myalgia.[12] To some degree the observed synergism with other oncotic agents can be attributed to bryo 1's ability to activate T lymphocytes, platelets and neutrophils,[13] thus enhancing the body's inherent ability to fight cancer and diminish the often severe side effects of other chemotherapeutics.

Bryostatin and HIV

Concomitant with bryostatin 1 potentiating the immune system is its ability to activate latent HIV reservoirs. HIV/AIDS is a global pandemic that through highly active

antiretroviral therapy (HAART) has become a manageable yet chronic disease.[14] There exist two significant drawbacks to HAART therapies. First is the expense and logistics of distributing a lifetime supply of the drugs to the areas of world that are most in need, and second is that if HAART is discontinued the disease will return. The HIV virus establishes residency in $CD4^+$ memory T cells, which contain an integrated yet transcriptionally silent infection allowing it to evade treatment by HAART therapy.[15] Bryostatin 1 has not only been shown to induce transcription of viral DNA, but also to downregulate the expression of the HIV-1 co-receptors CD4 and CXCR4, preventing de-novo infection.[15] Thus, bryo 1 is an attractive agent for achieving a total cure when combined with a HAART regiment in a strategy referred to as "Shock and Kill."[16] The Wender group has recently demonstrated in addition to bryo 1 simplified analogues are also capable of activating latent HIV reservoirs in vitro.[17]

Bryostatin and neuroregeneration/ neurodegenerative disorders

Another emerging area of bryostatin research is in the field of neuroregeneration and the treatment of neurodegenerative diseases. Alkon and co-workers have demonstrated that treatment with bryostatin 1 stimulates synaptogenesis, presynaptic ultrastructural specialization, and protein synthesis leading to enhanced spatial learning and memory in rats.[18] Bryo 1 is also capable of preventing neuronal loss by maintaining and restoring synapses when administered within 24 h following induced cerebral ischemia/ hypoxia in rats.[19] Alzheimer's is a neurodegenerative disease that is characterized by memory loss. In the early stages of the disease, learning and storage of recent information are affected most, followed by the gradual decay of long-term memory. In 2004, bryostatin 1 was

shown to reverse an Alzheimer's phenotype in AD double-transgenic mouse model at nanomolar concentration,[20] and has since been advanced into phase II clinical trials.

Bryostatin and protein kinase C

All of the advantageous biological properties reported for the bryostatins are a direct result of their ability to bind to the protein kinase C (PKC) family of signaling enzymes with nanomolar affinity. The PKC family of kinases consists of 10 isozymes, which play a central role in signaling cascades that regulate many cellular functions such as mitogenesis, differentiation, cell proliferation, and apoptosis.[21] All PKCs possess a highly conserved C-terminal kinase domain and a pseudosubstrate which occupies the catalytic pocket when the enzyme is inactive (Figure 1.2).[22] Differences in the C1 and C2 regulatory domains divide the 10 isozymes into three subclasses based upon their requirement for second messengers. Conventional PKCs respond to both diacylglycerol (DG) and calcium, novel PKCs contain a mutated C2 domain that no longer requires calcium, and atypical PKCs lack a C2 domain along with a mutated C1 domain making them insensitive to both DG and calcium. The C1 and C2 regulatory domains have multiple functions. In addition to maintaining the enzyme in an autoinhibited conformation when not active, they mediate protein-protein interactions targeting the enzyme to specific cellular membranes. Binding of DG anchors the enzyme to a membrane and produces a conformational change removing the pseudosubstrate to give the catalytically competent enzyme. Due to their central role in cell signaling, disregulation of PKCs occurs in many human cancers as well as other diseases.

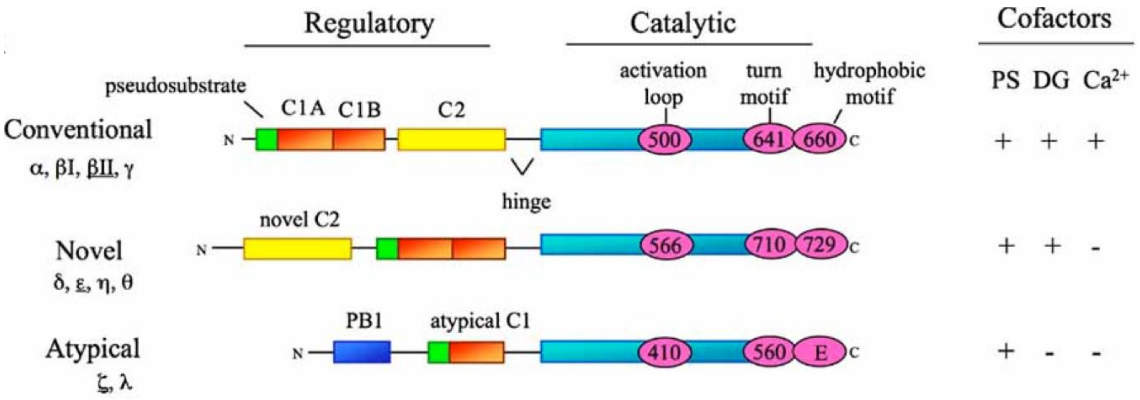


Figure 1.2. PKC isozymes

Tumor promoting vs. nontumor promoting PKC ligands

In addition to binding DG, the C1 domain is also the binding pocket for the tumor promoting natural product phorbol-12-myristate-13-acetate (PMA) (Figure 1.3). Bryostatin 1 also binds to the C1 domain inducing PKC activation; however in contrast to PMA, bryostatin 1 is antitumorigenic. Furthermore, bryo 1 will block any effects it itself does not induce in a dose dependent manner, therefore functioning to antagonize PMA when co-administered. Several other natural and synthetic compounds are known to bind the PKC C1 domain and display a range of tumor promoting ability (Figure 1.3).[23] Although the exact structural features that determine tumor-promoting ability are somewhat nebulous, a few trends exist. The most distinct trend is that tumor-promoting ligands often contain a long hydrocarbon chain whereas nontumor promoting ligands either lack that chain or have it replaced by a shorter more polar group, i.e., PMA vs. prostratin. It is also important to observe that high affinity binding to PKC does not correlate well with tumor promotion. The co-crystal structure of phorbol 13-acetate bound to the C1 domain indicates that binding of a ligand occurs in a hydrophilic cleft and completes a hydrophobic protein surface (Figure 1.4).[24] This protein surface then

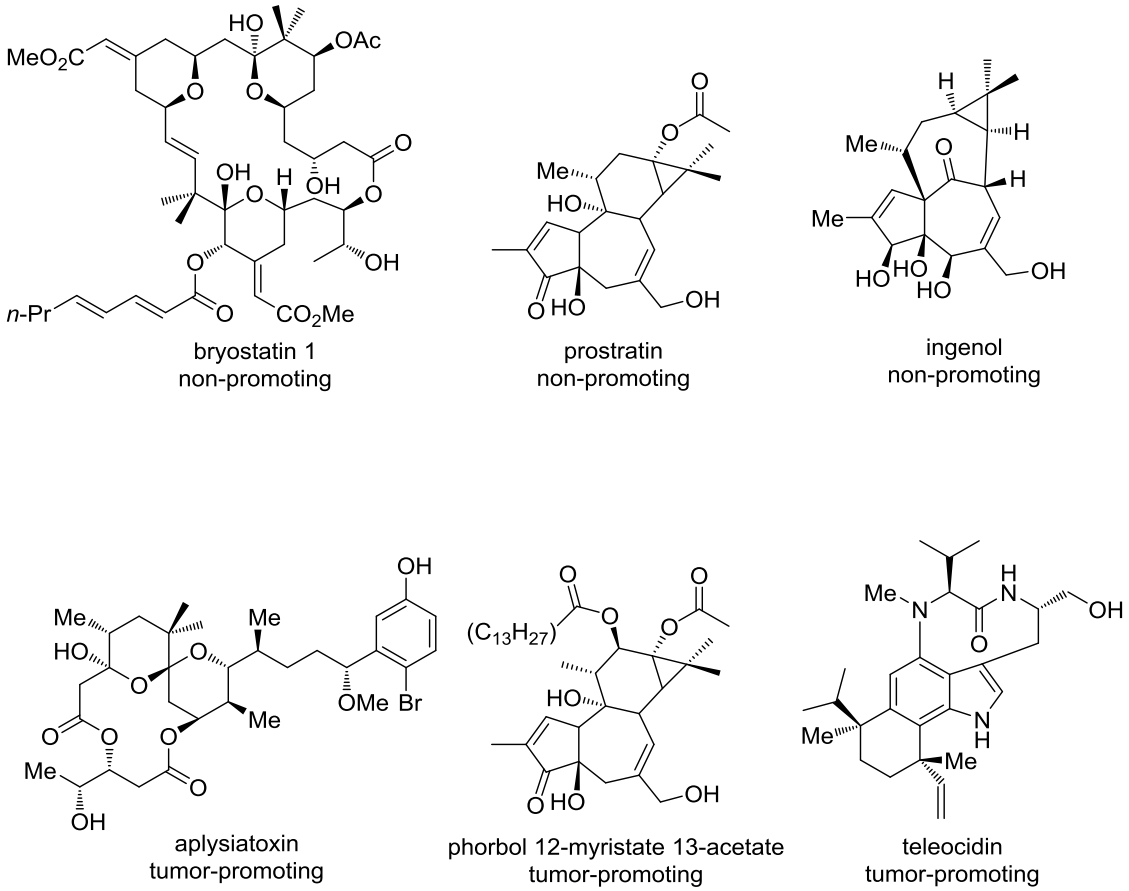


Figure 1.3. PKC C1 domain ligands

interacts with other proteins and cell membranes. Thus, a reasonable hypothesis is that tumor-promoting ability is not dictated by binding to PKC, but rather by the interactions of the newly formed surface with other cellular components. Understanding these interactions is of paramount importance for the successful development of PKC activators as pharmaceuticals. The bryostatins are extremely interesting in this regard as they appear to be naturally optimized for inducing the beneficial effects of PKC activation without being tumor promoting.

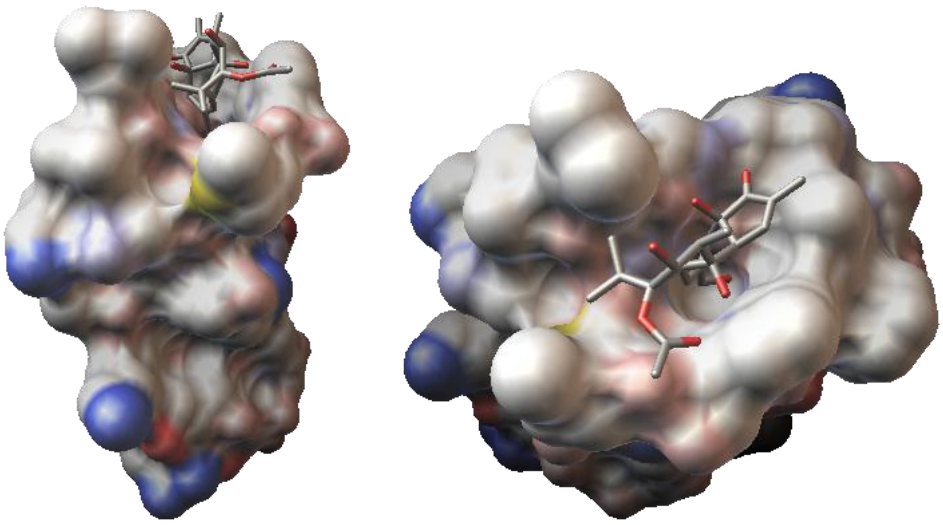


Figure 1.4. Phorbol 13-acetate bound to the C1 domain (PDB id: 1PTR)

The supply problem

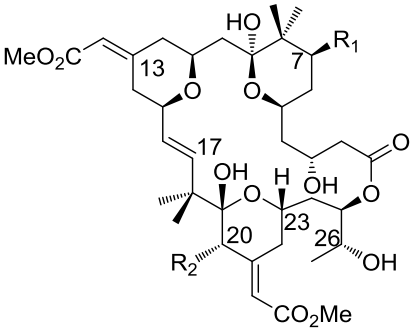
Despite intense interest in bryostatin 1 as a therapeutic for treatment of many serious human ailments, the issue of limited supply remains a significant obstacle. Following the initial screening of *Bugula neritina* a second much larger scale collection was undertaken. This harvest yielded 18 grams of bryostatin 1 from approximately 13,000 kilograms of organism. To date, this 18 grams had been sufficient to supply all clinical trials and research efforts; however, continuing to obtain bryo 1 from its natural source is both costly and environmentally unsustainable. Due to the intriguing biology and developing clinical relevance of bryo 1 many approaches have been pursued to solve the problem of limited supply. In conjunction with the Scripps Institution of Oceanography, CalBioMarine Technologies attempted to aquaculture *Bugula neritina* with high nutrient serums to increase the organism's natural production.[25] Although this attempt failed, it did help to identify the symbiotic bacterium *Candidatus* Endobugula sertula (*E. sertula*) as the true source of bryostatins.[26] Direct culture of *E. sertula* has also been unsuccessful,

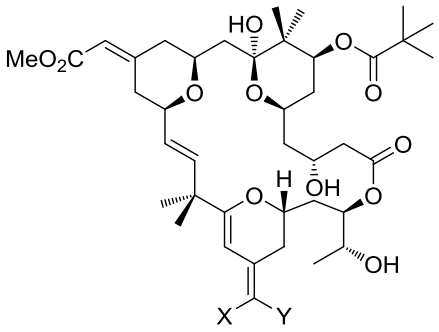
presumably because bryostatins are only produced as a chemical defense against predation. The final approach taken by Dr. Haygood's team at Scripps was the 2007 identification of the putative bryostatin polyketide synthase gene cluster in *E. sertula*;[27] but there have yet to be any subsequent reports concerning the expression of these genes in a suitable host. As an alternative approach, chemical synthesis has attracted attention from numerous groups and resulted in the syntheses of bryostatins 2[28], 3[29], 7[30], 9[31], and 16[32]. Bryostatin 1 finally succumbed to total synthesis by the Keck group in 2010.[33] Each of these syntheses represents a tremendous accomplishment in synthetic chemistry; however, all of them still fall short as a means of providing significant amounts of material for biological and clinical study. Currently, the most feasible solution to the supply problem may be the synthesis of structurally simplified analogues. This approach has the added benefit of potentially improving upon the biological profile and substrate specificity of compounds that were not naturally designed for medical use.

Initial Analogue Work

Early analogue work was pioneered by a collaboration between George Pettit, Peter Blumberg and Paul Wender who compared the structural features of natural and semi-synthetic bryostatins to identify the pharmacophoric elements (Figure 1.5).[34] First, by looking at the natural bryostatins it is clear that changing the ester substituents at C7 and C20 has little effect on PKC affinity; however, if the C20 ester and C19 ketal are missing the binding affinity drops by two orders of magnitude, i.e., bryostatins 16 and 17.

Comparison of the semisynthetic analogues (Figure 1.6) indicated that hydrogenation of bryostatin 2 at the C13-C30 olefin as well as on the C20 ester side chain had a minimal





bryostatin 16 Y = CO_2Me X = H K_i = 118 ± 2 nM
bryostatin 17 Y = H X = CO_2Me K_i = 188 ± 7 nM

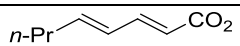
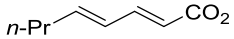
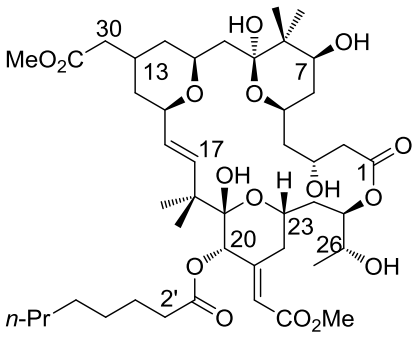
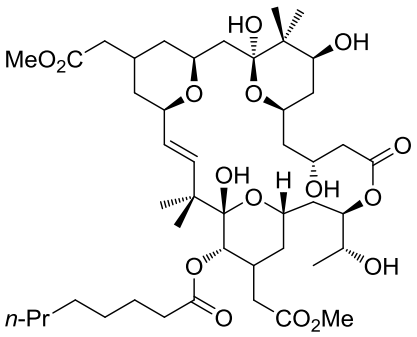
Bryostatin	R_1	R_2	K_i (nM)
1	O_2CCH_3		1.35 ± 0.17
2	OH		5.86 ± 1.13
4	$O_2CC(CH_3)_3$	$O_2CH_2CH_2CH_3$	1.30 ± 0.19
5	$O_2CC(CH_3)_3$	O_2CCH_3	1.04 ± 0.10
6	$O_2CH_2CH_2CH_3$	O_2CCH_3	1.18 ± 0.29
7	O_2CCH_3	O_2CCH_3	0.84 ± 0.07
8	$O_2CH_2CH_2CH_3$	$O_2CH_2CH_2CH_3$	1.72 ± 0.10
9	O_2CCH_3	$O_2CH_2CH_2CH_3$	1.31 ± 0.00
10	$O_2CC(CH_3)_3$	H	1.56 ± 0.16

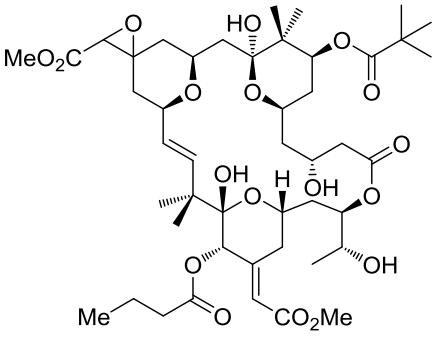
Figure 1.5. K_i values for natural bryostatins



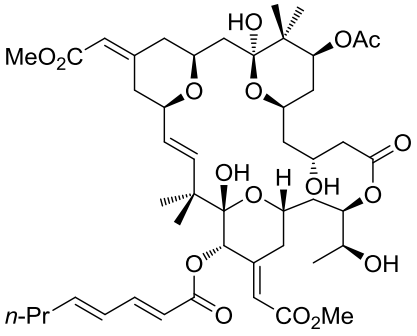
(13, 30, 2', 3', 4', 5'-Hexahydro)-bryostatin 2;
K_i = 9.61 ± 0.94 nM



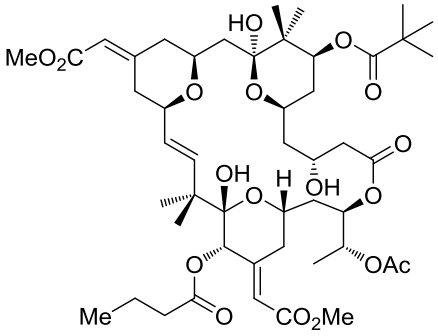
(13, 30, 21, 34, 2', 3', 4', 5'-Octahydro)-bryostatin 2;
K_i = 473 ± 96 nM



(13, 30-epoxy)-bryostatin 4;
K_i = 0.54 ± 0.07 nM



(26-epi)- bryostatin 1
K_i = 32.6 ± 0.60 nM



(26-Acetoxy)-bryostatin 4;
K_i >> 100 nM

Figure 1.6. Semisynthetic bryostatin analogues

effect on binding. However, further hydrogenation of the C21- C34 exocyclic enoate had a much more dramatic effect. The C13-C30 olefin was also manipulated through epoxidation, which showed minimal effects implying that substituents on the B- ring are unlikely to play any substantial role in determining PKC affinity. Inversion of the C26 stereocenter of bryostatin 1 or acetylating it on bryostatin 4 both had detrimental effects.

These comparisons indicate that the functionalities surrounding the C-ring are the major factors in binding to PKC, and of particular importance are the C26 oxygen, an olefin at C21- C34, and a C19 ketal in combination with an ester at C20. The identity of the C20 ester does however appear to be flexible, and is therefore an attractive position for synthetic diversification.

Utilizing computer modeling to compare the energy-minimized structure of bryostatin 1 to diacylglycerol (DAG), and phorbol-12-myristate-13-acetate (PMA), Wender and Co-workers were able to make spatial correlations between the C26 hydroxyl, C19 ketal and C1 carbonyl to that of an oxygen atom triad on the other ligands (Figure 1.7). The A-, and B-ring portion of the molecule was then hypothesized to function as a ‘spacer domain’ to hold the three pharmacophoric oxygen’s on the C-ring ‘binding domain’ in the correct position.

Wender’s Analogue Design

Wender’s original analogues called for significant changes to the A- and B-ring portion while leaving the C-ring ‘binding domain’ completely intact. In general, the retrosynthetic plan was to disconnect the novel B-ring through a trans-acetylation macrocyclization followed by an esterification to separate the two halves of the analogue

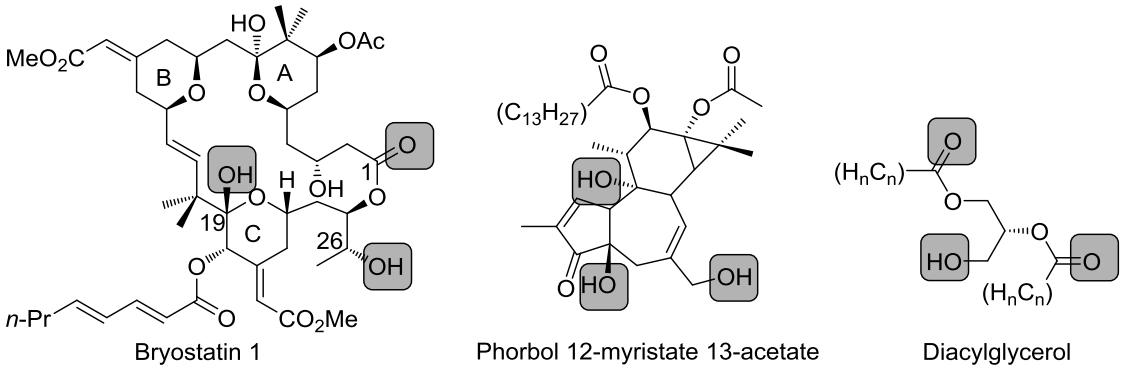


Figure 1.7. PKC ligand binding hypothesis

(Figure 1.8). This strategy was chosen to allow for the utilization of multiple spacer domains in a combinatorial approach.

Many of Wender's early analogues are represented in Figure 1.9, and a few additional ones will be discussed later. Through the synthesis of these analogues, several new structure-function relationships were probed. First was the role of the C3 hydroxyl group that when absent (**1.4**) or inverted (**1.6**) binding affinity suffered by ~100 fold.[34c, 35] This was hypothesized to be due to the loss of the internal hydrogen bonding network created by the C3 hydroxyl, C19 ketal, and the pyran oxygen of the B-ring. Similar to what was observed for (26-acetoxy)-bryostatin 4, when the C26 hydroxyl was acylated, binding was almost completely inhibited.[35a] Analogues **1.8-1.11** demonstrate that removal of the A- ring was well tolerated; however, better PKC affinity was achieved when the sterically bulky *t*-Butyl group was used at R_1 as opposed to a hydrogen.[34c, 36] Diversification at C20 yielded some interesting results. While the C20 was amenable to a variety of long hydrocarbon chains and a phenyl group, introducing an acetate ester had a detrimental effect on Wender's analogues. In contrast, this modification had no effect on the bryostatins 1 vs 7.[37] Finally, analogue **1.15** demonstrated that a macrocyclic structure was

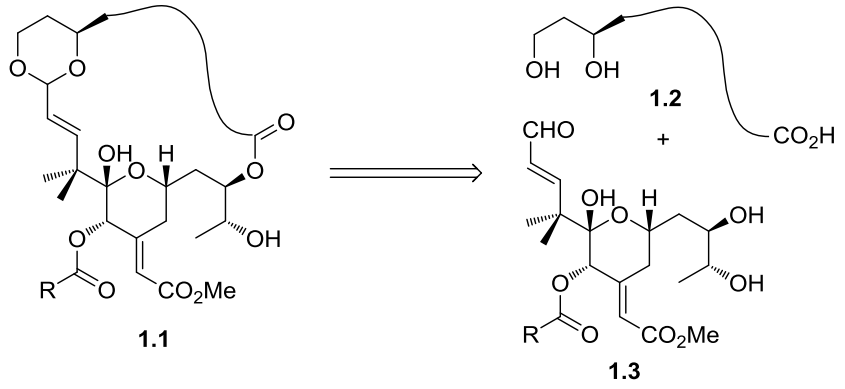
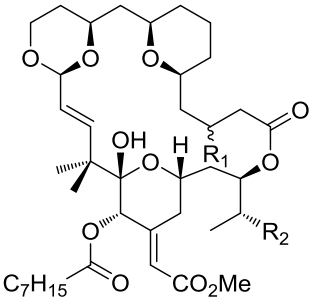


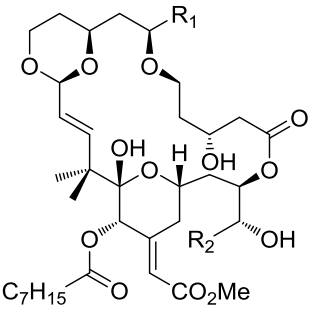
Figure 1.8. Wender's analogue strategy

necessary, presumably to help arrange the three pharmacophoric oxygens correctly.[34c] In addition to obtaining K_i values, analogues **1.5** and **1.11** were evaluated for activity against a number of human cancer cell lines (Figure 1.9).[35b] In general, analogue **1.5** yielded similar activity to that of bryostatin 1. Analogue **1.11** demonstrated reduced growthinhibition indicating that while the A-ring may not be responsible for PKC binding it still plays a role in biological potency.

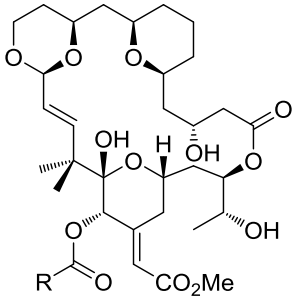
In subsequent publications, the Wender group revealed a first- and second- generation synthesis as well as biological evaluation against the NCI panel of 60 human cancer lines for their most potent analogue **1.16**, which has subsequently been named 'picolog' (Figure 1.10).[38] In general, picolog demonstrates similar growth inhibitory effects as bryostatin 1 and in some cell lines such as MOLT-4 human acute lymphoblastic leukemia and NCI-H460 human lung cancer it was orders of magnitude more potent. The only difference between picolog and other analogues is the lack of a C27 methyl group, and as the most potent and easily synthesized analogue, it has become Wender's most well-known and studied analogue.[39]



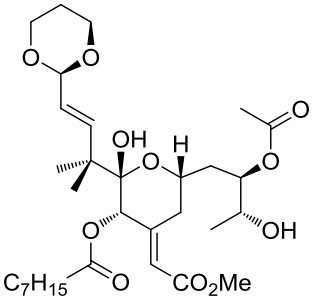
1.4: R_1 = H, R_2 = OH; K_i = 297 nM
1.5: R_1 = αOH, R_2 = OH; K_i = 3.4 nM
1.6: R_1 = βOH, R_2 = OH; K_i = 285 nM
1.7: R_1 = αOH, R_2 = OAc; K_i > 10000 nM



1.8: R_1 = H, R_2 = Me; K_i = 47 nM
1.9: R_1 = *t*-Bu, R_2 = Me; K_i = 8.3 nM
1.10: R_1 = H, R_2 = H; K_i = 8.0 nM
1.11: R_1 = *t*-Bu, R_2 = H; K_i = 6.5 nM



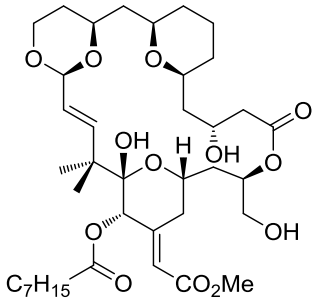
1.12: R = C_7H_{27}; K_i = 1.5 nM
1.13: R = CH_3; K_i = 232 nM
1.14: R = Ph; K_i = 7 nM



1.15: K_i > 10000 nM

		Analogue, GI_{50} in ng/mL	
Cell Type	Cell Line	**1.5**	**1.11**
Pancreas	BXPC-3	6	80
Lung	NCI-H460	120	3100
Pharynx	FADU	1.8	290
Prostate	DU-145	170	3000

Figure 1.9. Initial Wender analogues and growth inhibitory values



1.16

K_i = 0.25 nM

Figure 1.10. 'Picolog'

Keck's Analogue Design

The Keck group has had a long-standing program directed at the total synthesis of bryostatin 1. Towards this end, a novel reaction termed 'pyran-annulation' was developed to access the A- and B-ring pyrans. This methodology allows for the convergent union of two aldehydes across the four-carbon allyl stannane/silane **1.18** resulting in a stereo-defined 2,6- tetrahyropyran (Figure 1.11).[40] The first step in the process is the catalytic asymmetric allylation of the first aldehyde **1.17** with stannane **1.18**, which is available from commercial sources in three steps.[41] The resulting chiral β-hydroxyallylsilane **1.19** is then treated with the second aldehyde **1.20** under Lewis acidic conditions to give the 2,6-tetrahydropyran through a proposed six-member transition state. When this methodology is utilized in an iterative fashion bis-pyran **1.25**, which represents a simplified spacer domain, can be accessed rapidly.

Pyran annulation approach to analogues

The development of the pyran annulation allowed for the synthesis of the group's first analogue by Dr. Anh Truong.[42] The retrosynthetic plan was to use sequential annulation

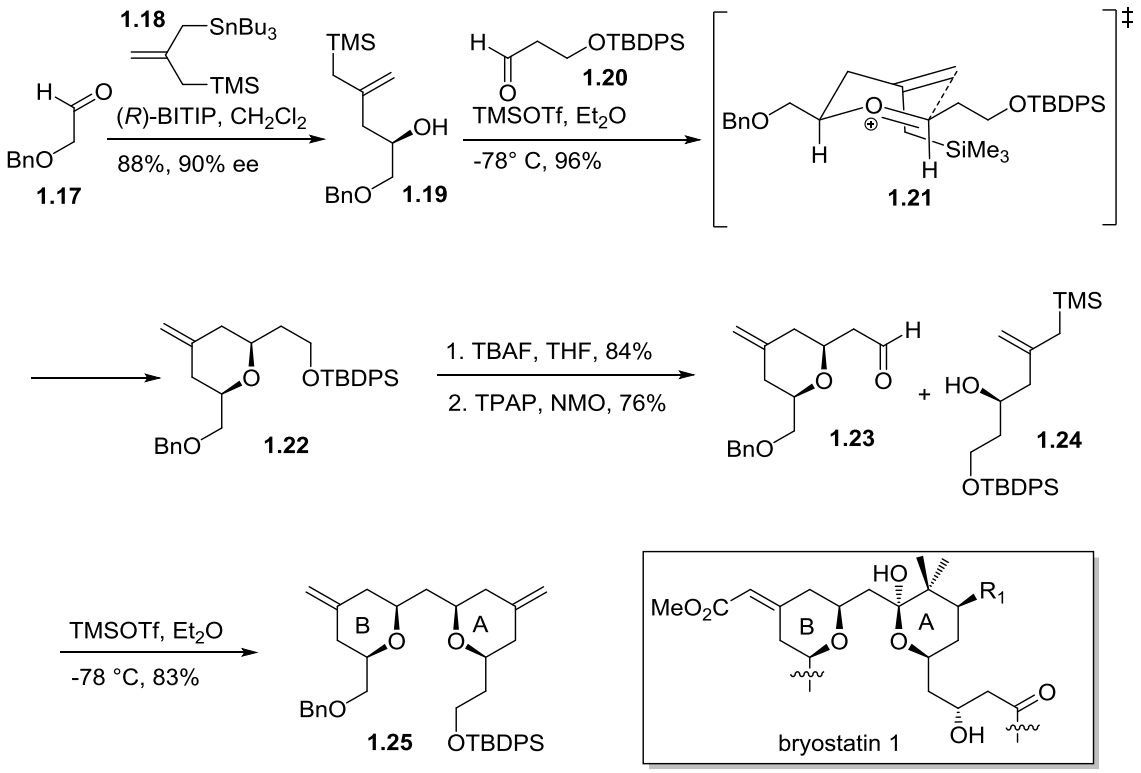


Figure 1.11. Pyran annulation strategy

reactions to construct the A- and B-rings. The C-ring would then come from linear precursor **1.30** which itself was elaborated from (R)-(+)-isobutyl lactate **1.31** through a series of chelation controlled allylation reactions (Figure 1.12). Analogue **1.26** contains all three oxygen hypothesized to dictate PKC binding, but was simplified at C20, and C21 of the C-ring as well as the replacement of the high oxygenated A- and B-rings with pyrans containing only an exocyclic methylene. This design was chosen for three reasons: first, to test the applicability of the pyran annulation to complex substrates; second, at that time, the role of the C20, and C21 substituents were still not well-defined; and finally to examine the use of simplified pyrans as surrogates for the bryostatin A- and B-rings.

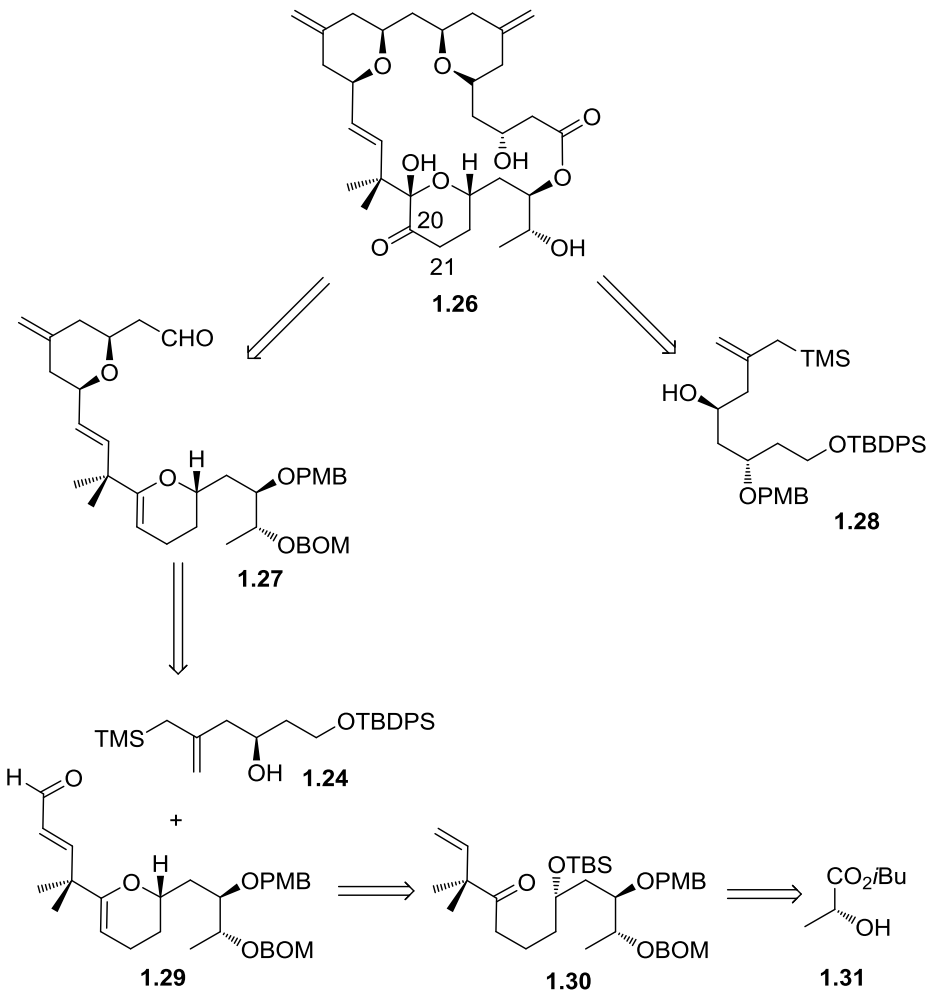


Figure 1.12. First analogue retrosynthesis

In the forward direction (Figure 1.13), commercially available (R)-(+)-isobutyl lactate provided the first stereocenter, which was protected as the BOM ether, followed by DIBAl-H reduction to the aldehyde. The first 1,2 chelation-controlled allylation proceeded in high yield to give homoallylic alcohol **1.33** as a single, NMR observable, diastereomer.[43] The free alcohol was protected as the PMB ether and the olefin was cleaved with ozone to provide aldehyde **1.34**. The C23 stereocenter was established through a 1,3 chelation-controlled allylation[44] which accessed the homoallylic alcohol as

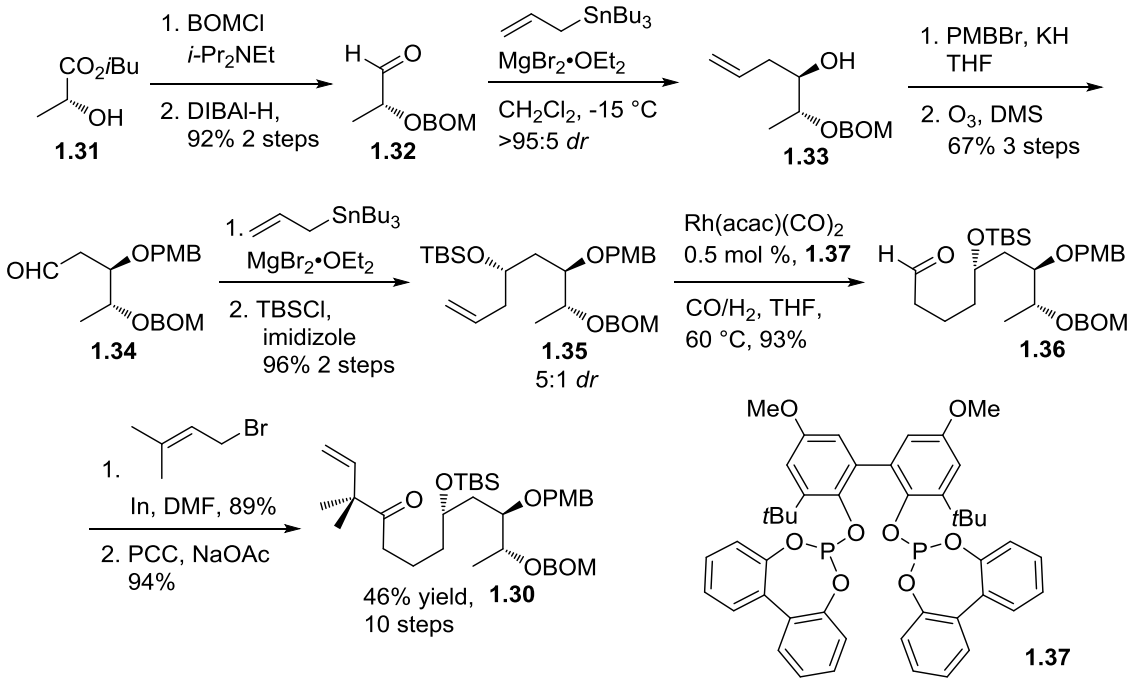


Figure 1.13. Synthesis of the C16-C27 segment

a 5:1 mixture of diastereomers, which were separable following protection of the alcohol with TBSCl. The terminal olefin was converted to the one carbon homologated aldehyde using Buchwald's hydroformylation conditions.[45] Prenyl indium addition[46] was used to install the gem-dimethyl and the resulting racemic alcohol was oxidized using PCC[47] to give **1.30** in 10 steps and ~46% yield.

With an efficient route to olefin **1.30** in hand, attention was turned to elaborating **1.30** to the desired C-ring aldehyde **1.41** for the first pyran annulation. The first generation plan (Figure 1.14) was to remove the TBS protecting group, close the C- ring, and then oxidize the resulting glycal olefin to give ketone **1.39**. Cleavage of the terminal olefin was accomplished with ozone. However, the C17 aldehyde was slow to react in subsequent transformations. Wender and Trost[32] have also reported similar observations for both the C17 aldehyde and C16-C17 terminal olefin.

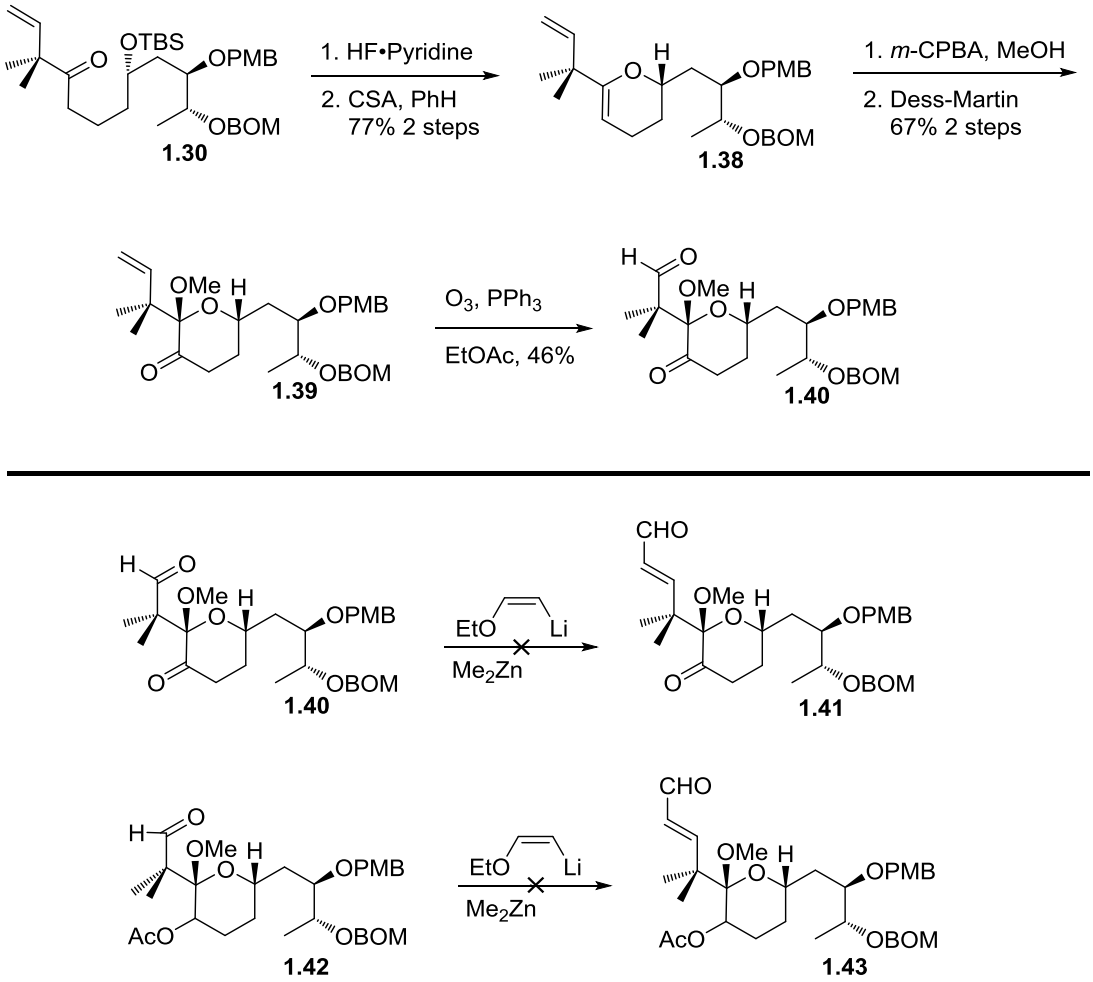


Figure 1.14. Attempted functionalization of the C17 aldehyde

In order to circumvent the reactivity issue, the C17 aldehyde was extended prior to cyclization (Figure 1.15). Cleavage of olefin **1.30** proceeded with a dramatically improved yield over the cyclized compound **1.39** and a Horner-Wadsworth-Emmons olefination with a thioester phosphonate provided the α,β-unsaturated thioester **1.44**.[48] Cleavage of the TBS protecting group followed by dehydrative cyclization accessed glycal **1.45**. Finally, the thioester was reduced to the aldehyde with DIBAl-H to give C-ring **1.46** in a remarkable 27% overall yield from (R)-(+)-isobutyl lactate. Unfortunately, this C-ring could not be oxidized further prior to pyran annulation due to the tendency of

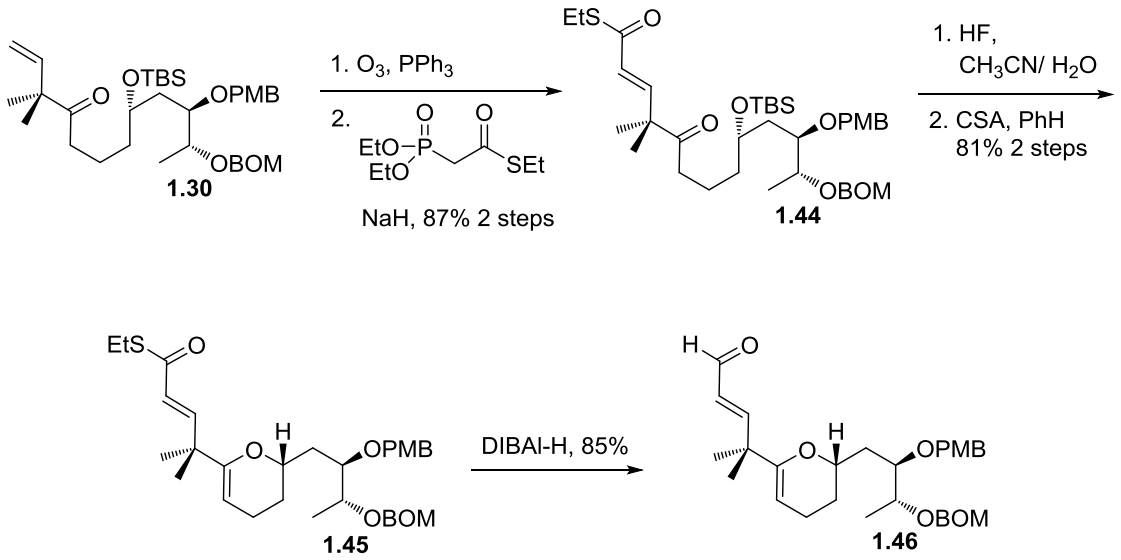


Figure 1.15. Completing the C-ring

the C20 alcohol to undergo Michael addition into the α,β-unsaturated thiolester, as well as poor selectivity for reducing the thiol ester in the presence of a C20 ketone. Attempts to use the C-ring as the β-hydroxyallyl silane halve were also meet with limited success due to the instability of the glycal to catalytic asymmetric allylation conditions.

Pyran annulation of the C-ring aldehyde **1.46** with β-hydroxyallyl silane **1.24** provided **1.47** (Figure 1.16), which after removal of the TBDPS group and oxidation to an aldehyde, under Ley conditions,[49] was ready for the second pyran annulation with β-hydroxyallyl silane **1.28**. The C-ring of coupled product **1.48** was oxidized with *m*-CPBA, the C1 TBDPS was removed and the free alcohols at C1 and C20 were both oxidized.[49] Further oxidation of the C1 aldehyde using Pinnick conditions,[50] and deprotection of the PMB ether gave seco-acid **1.50**, which was cyclized using Yamaguchi conditions.[51] Finally, simultaneous deprotection of the BOM ether and methyl ketal was achieved in one step with $LiBF_4$.[52] Analogue **1.26**, which contains the three, proposed,

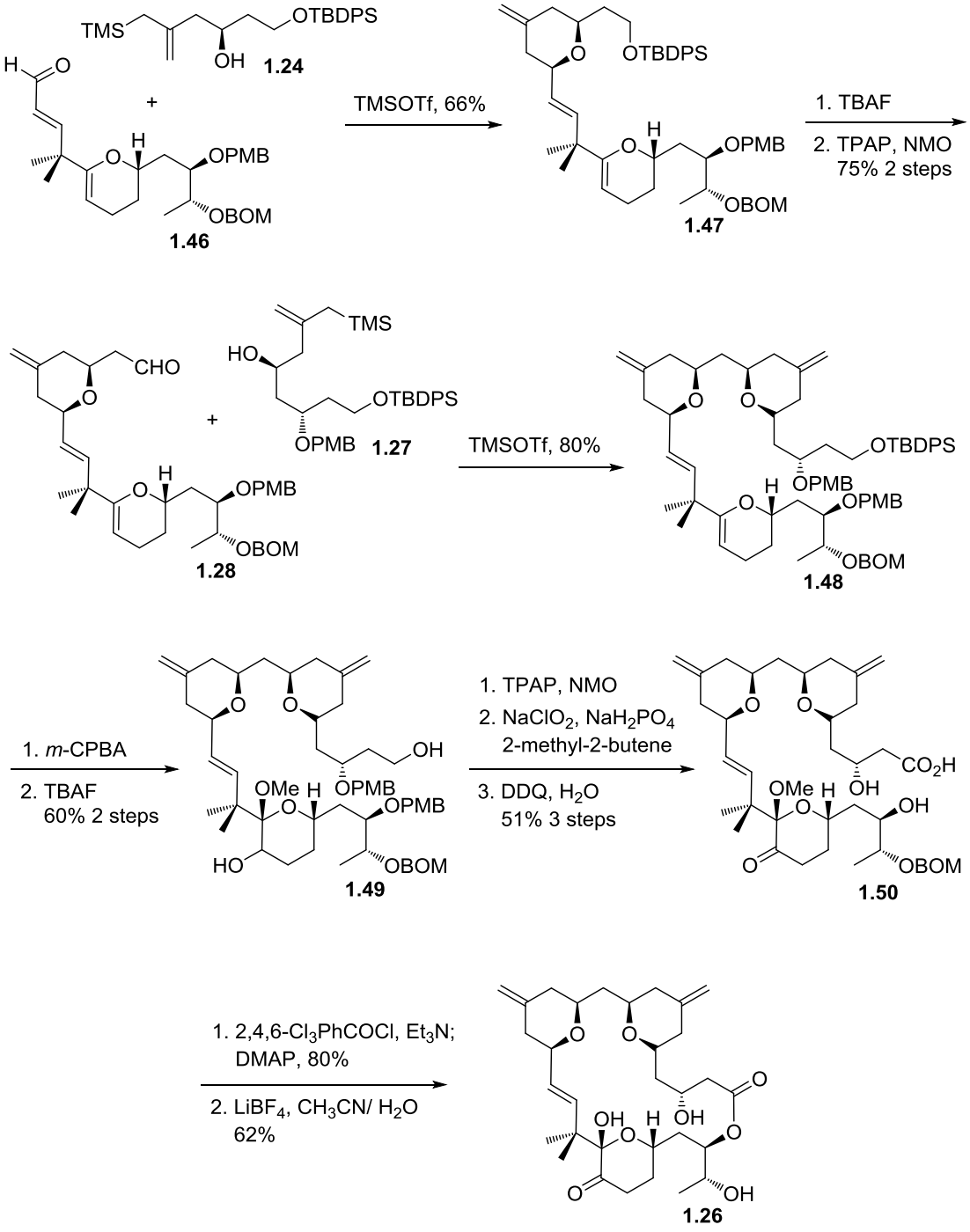


Figure 1.16. Sequential pyran annulation approach to analogue **1.26**

pharmacophoric oxygens, was sent to Dr. Peter Blumberg at the NIH for biological testing regarding binding affinity. Analogue **1.26** exhibited a K_i of 546 nM for purified PKC-α. Such a dramatically higher K_i compared to that of bryostatin 1 (K_i = 1.35 nM) or Wender's analogues indicates that the C20 ester and C21 enoate play a critical role in PKC binding.

Carina 1

At this stage the project was continued by Dr. Carina Sanchez who installed the missing C-ring functionalities. To accomplish this, Dr. Sanchez started with TBS protection of the C3 alcohol on intermediate **1.51**. The C21 enoate was added through an aldol reaction with methyl glyoxylate followed by elimination. The exocyclic enoate was obtained as a single isomer in which olefin geometry was dictated by developing 1,3-allylic strain from the vicinal ketone.[53] Luche reduction and esterification with benzoic anhydride provided a separable 4:1 mixture of diastereomers. Global deprotection completed the analogue 'Carina 1' (Figure 1.17), which exhibited a K_i = 0.70 nM confirming the necessity of the C21 enoate and a C20 ester in order to maintain single digit nanomolar affinity for PKC.

Second-generation route to pyran analogues

Building on the success of Carina 1, Dr. Matt Kraft and Dr. Wei Li designed a more convergent route in order to create a series of bryostatin analogues that would further probe the role of the C20 ester and C21 exocyclic enoate (Figure 1.18).[54] The two major improvements in this route are use of an oxidized C-ring, which avoided complications

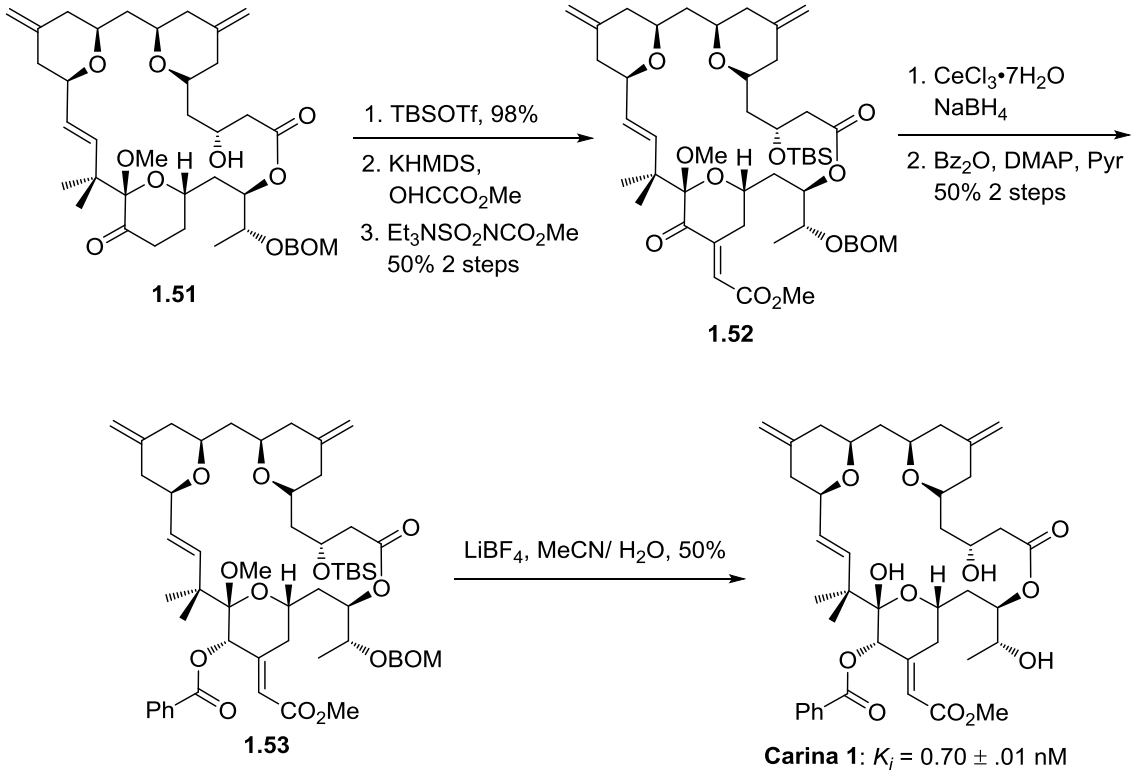


Figure 1.17. Synthesis of Carina 1

associated with the acid sensitive glycal, and the convergent union of two equally complex subunits. Both modifications allowed for greater throughput of material compared to the previous completely linear route.

Synthesis of the A- and C-ring fragments was divided between Dr. Kraft, C-ring, and Dr. Wei, A-ring. The C-ring, up to intermediate **1.45**, was made in a linear fashion as previously described. Glycal **1.45** was epoxidized with *m*-CPBA. *In-situ* opening of the epoxide through oxonium ion formation and trapping by MeOH addition to the axial position gave a C20 alcohol that was oxidized to ketone **1.58** using Ley conditions[49] (Figure 1.19). Low yields in these steps were attributed to the tendency of the intermediate C20 alcohol to undergo Michael addition into the proximal α,β-unsaturated

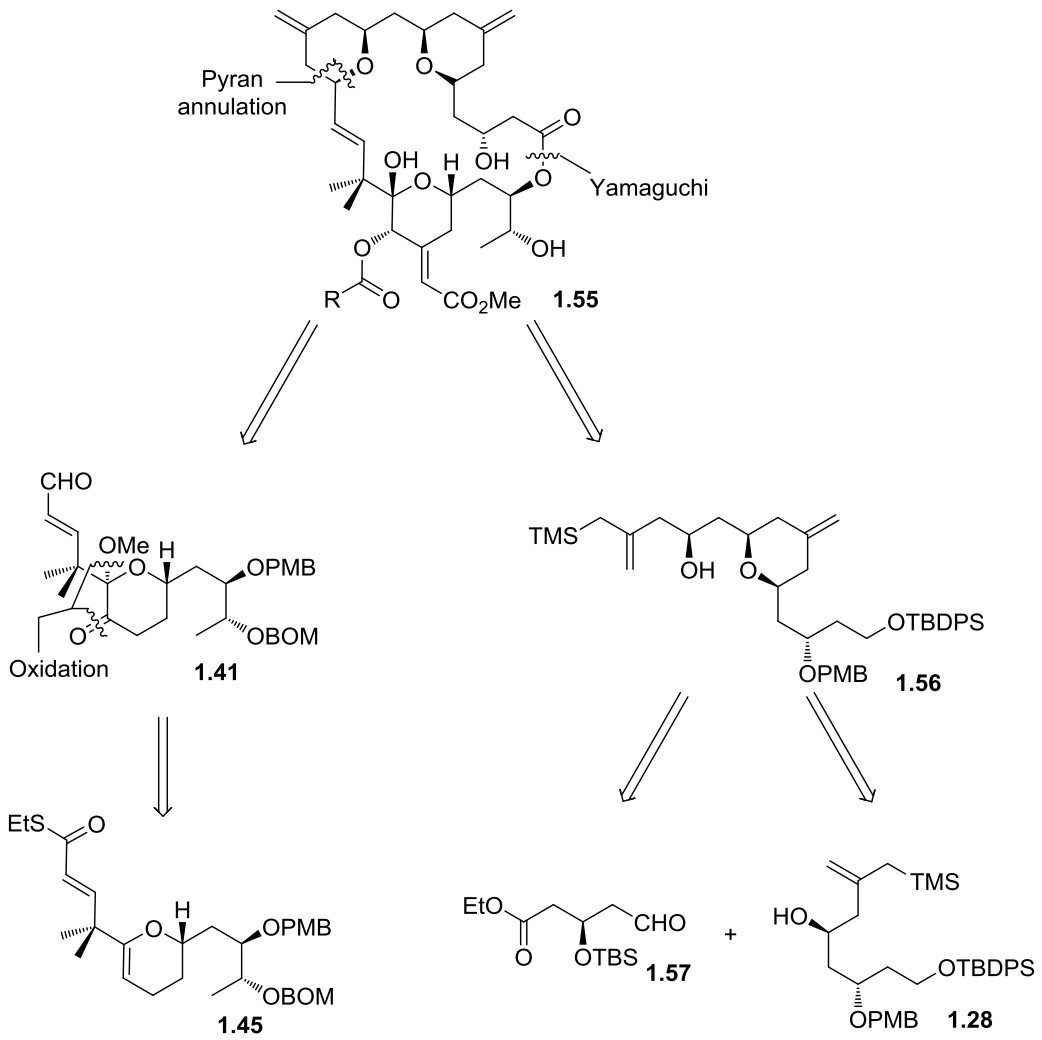


Figure 1.18. Second-generation retrosynthesis

thioester. The PMB protecting group was then exchanged for a TBS in order to avoid a difficult purification encountered in the previous route when both PMB ethers were removed prior to macrolactonization. The final step was to selectively reduce the thioester to an aldehyde in the presence of the C20 ketone. After extensive screening of conditions, Dr. Kraft discovered that conditions developed in the Evans group (Lindlar's catalyst, Et_3SiH, 1-hexene)[55] were able to accomplish the task in high yield.

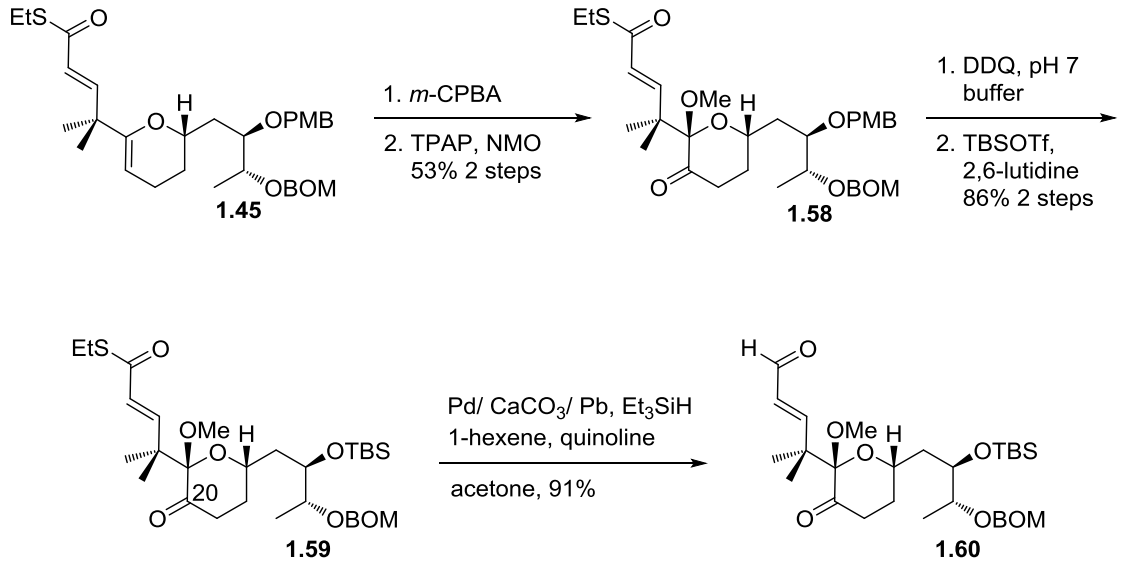


Figure 1.19. Synthesis of second-generation C-ring

Synthesis of the A-ring portion (Figure 1.20) commenced with Michael addition of benzyl alcohol into acrylonitrile, followed by a Reformatsky reaction with ethyl bromoacetate.[56] Keto-ester **1.62** was then subjected to a Noyori asymmetric hydrogenation to give chiral alcohol **1.63** in 95% ee.[57] The C11 free alcohol was protected as the TBS ether, the benzyl grouped cleaved, and the resulting C9 alcohol was oxidized to the aldehyde. Pyran annulation of aldehyde **1.57** with the known β-hydroxyallylsilane **1.28** provided the A-ring in excellent yield and as a single diastereomer. Removal of the C13 TBS protecting group resulted in a mixture of the mono alcohol **1.66** and the diol **1.65** from loss of the C1 TBDPS, which could be selectively replaced. The free C13 alcohol was then protected as a TMS ether and a Bunnelle reaction was used to access the desired β-hydroxyallylsilane **1.56**.[58]

Pyran annulation between C-ring aldehyde **1.60** and A-ring β-hydroxyallylsilane **1.56** formed the B-ring in excellent yield and was a stunning demonstration of how powerful

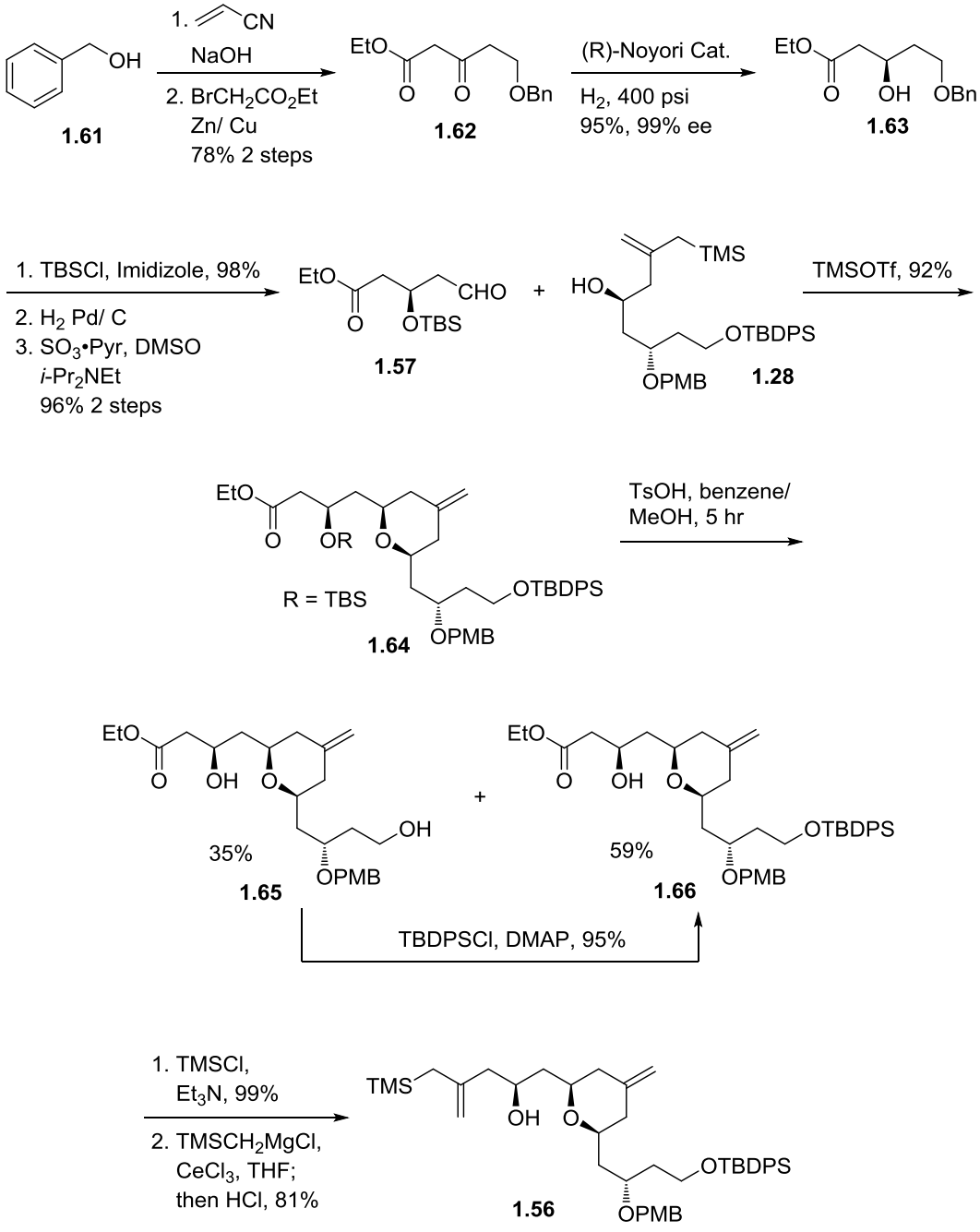


Figure 1.20. Synthesis of A-ring β-hydroxyallylsilane **1.56**

and general this reaction is (Figure 1.21). Selective removal of the primary TBDPS group at C1 in the presence of the C25 TBS was accomplished with TBAF in AcOH/DMF,[59] and the free alcohol was oxidized to the acid over two steps. Cleavage of the C25 TBS with HF•Pyr provided the seco-acid, which was carried directly into the Yamaguchi macrolatonization.[51]

Once again the C21 enoate was added through an aldol reaction with methyl glyoxylate followed by elimination (Figure 1.22). Luche reduction with a *dr* = 4:1, esterification with the appropriate anhydride, and global deprotection completed two new

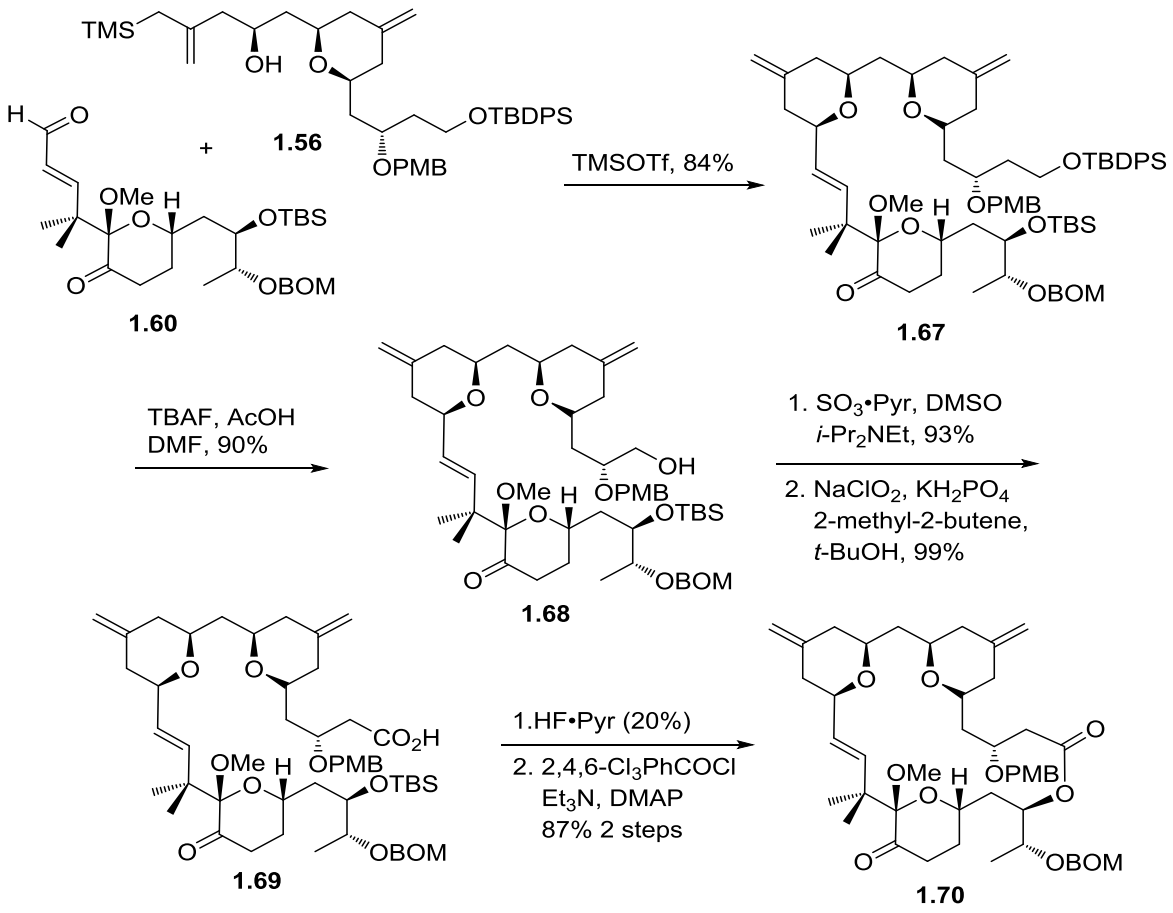


Figure 1.21. Synthesis of macrolactone **1.70**

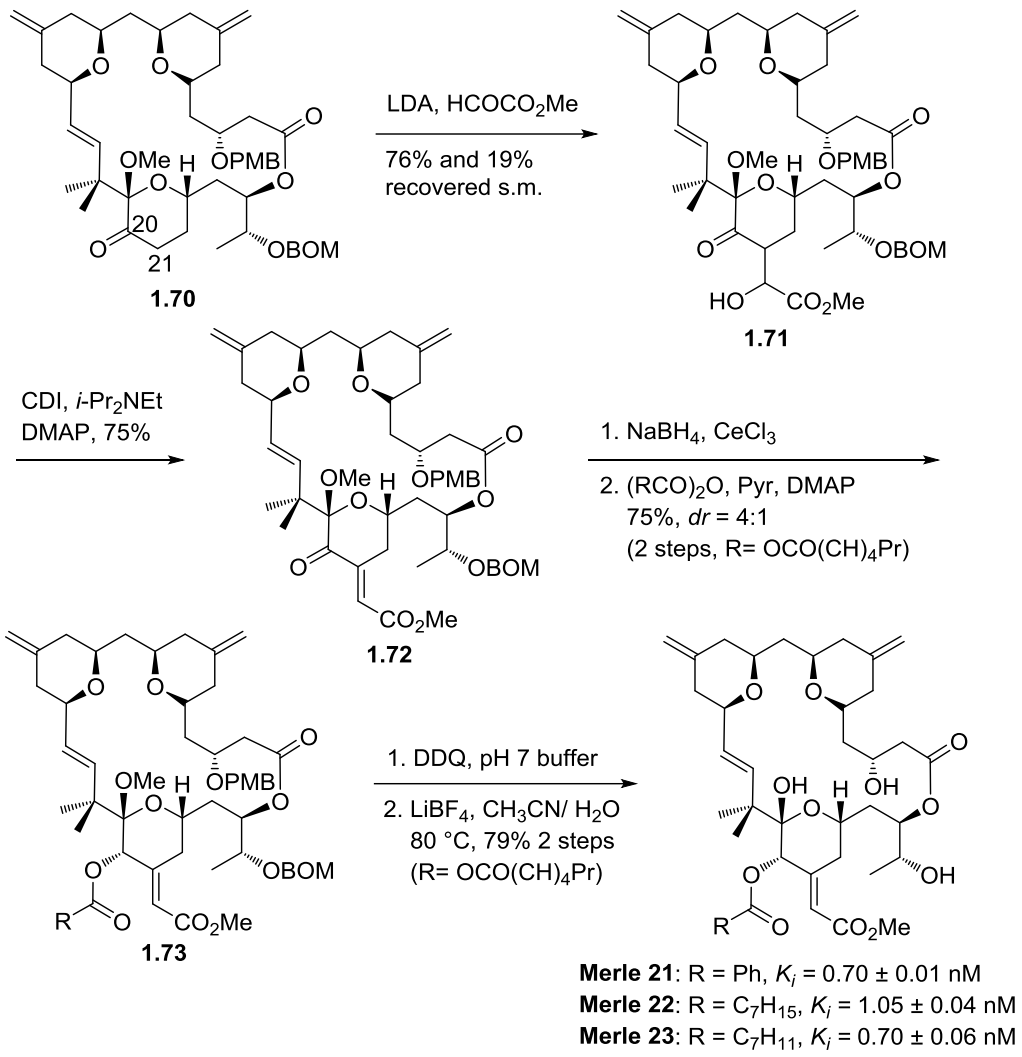


Figure 1.22. Completion of Merle 21, 22, and 23

analogues, Merle 22 and Merle 23, as well as Carina 1 (Merle 21). All three analogues exhibited K_i values comparable to that of bryostatin 1 (K_i = 1.35 ± 0.17 nM).

Waiting to install the C20 ester and C21 enoate until the end allowed for the individual assessment of each of these groups' contribution to binding affinity to be done without significant deviations to the synthetic route (Figure 1.23). The C20 ketone could be reduced to give either the axial or equatorial alcohol by using $NaBH_4$ or L-selectride. Esterification with the fully saturated version of the bryostatin 1 side chain, followed by

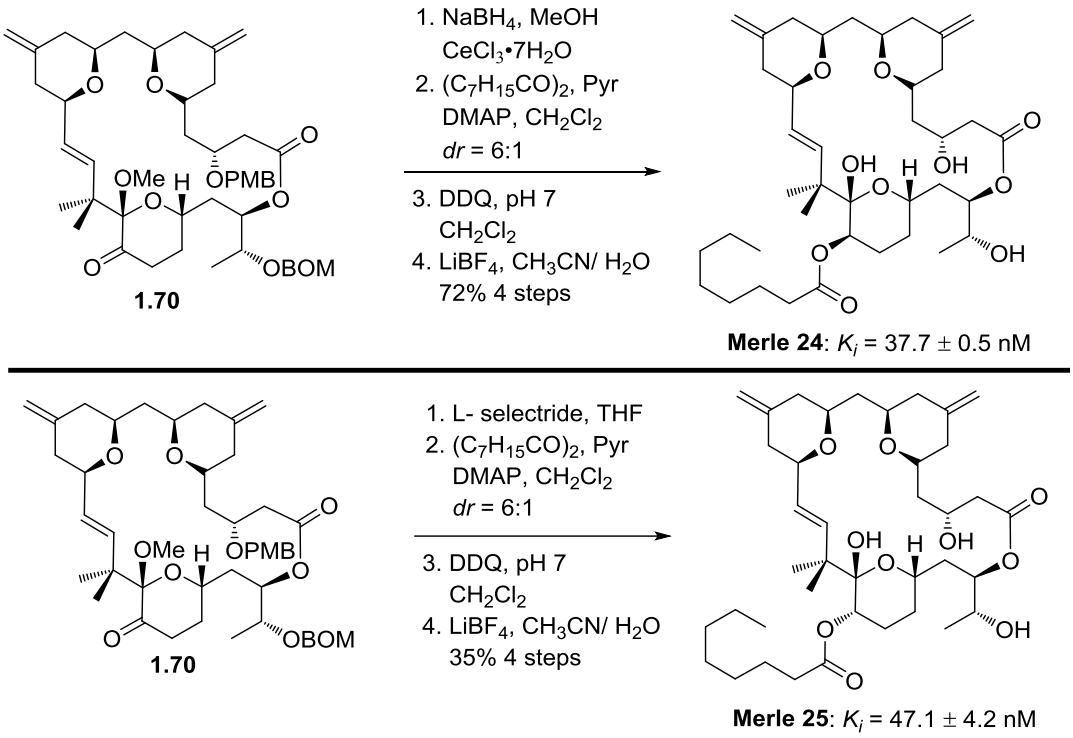


Figure 1.23. Synthesis Merle 24, and 25

global deprotection provided two new analogues, Merle 24 and Merle 25. Both of the new analogues had significantly improved binding as compared to analogue **1.26** but still fell well short of the high binding affinities observed for Merle 21-23 or the natural bryostatins. Additionally, by also comparing with Bryostatin 10 (Figure 1.5), which lacks a C20 ester but contains a C21 enoate, it appears that an analogue can tolerate removing one of the C-ring substituents but not both. Of the two substituents, the C21 enoate seems to have a greater effect.

Biology of Merle 23

Merle 23 differs from bryostatin 1 at four positions across the A- and B-rings (Figure 1.24). The C7 acetate and the C13 enoate are both replaced by exocyclic methylenes, and

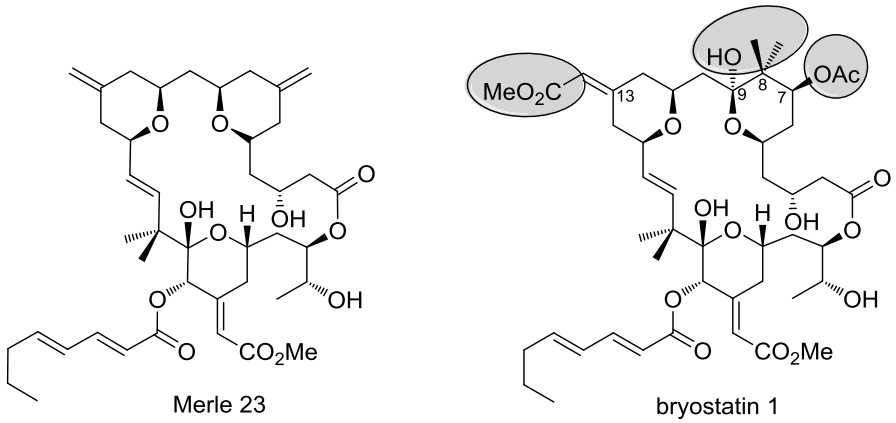


Figure 1.24. Structural differences

the C8 gem-dimethyl and C9 alcohol are omitted. If the A- and B-rings are to serve merely as a spacer domain holding the three-pharmacophoric oxygens in place then these changes should not affect bryostatins 1's highly attractive biology.

Merle 21-23 all represent potent PKC ligands with binding affinities equal to or greater than Bryostatin 1; however, since strong binding does not solely dictate tumor promoting vs. nontumor promoting behavior it was prudent to examine whether or not the Merle compounds were true bryostatin mimics biologically. To accomplish this goal, a more extensive collaboration was formed with Peter Blumberg's group at the NIH. The new analogues were tested in a number of human cell lines in which tumor promoting PMA and nontumorigenic bryostatin 1 have contrasting effects. The first test was the U937 leukemia cell attachment and proliferation assay. In U937 cells PMA inhibits proliferation and induces attachment, while bryostatin 1 has little effect on either. Furthermore, bryostatin 1 will antagonize the response induced by PMA in a dose dependent manner.[60] In this assay (Figure 1.25), Merle 23 (purple bars) displays PMA like behavior in that it both inhibits proliferation and induces attachment. These results

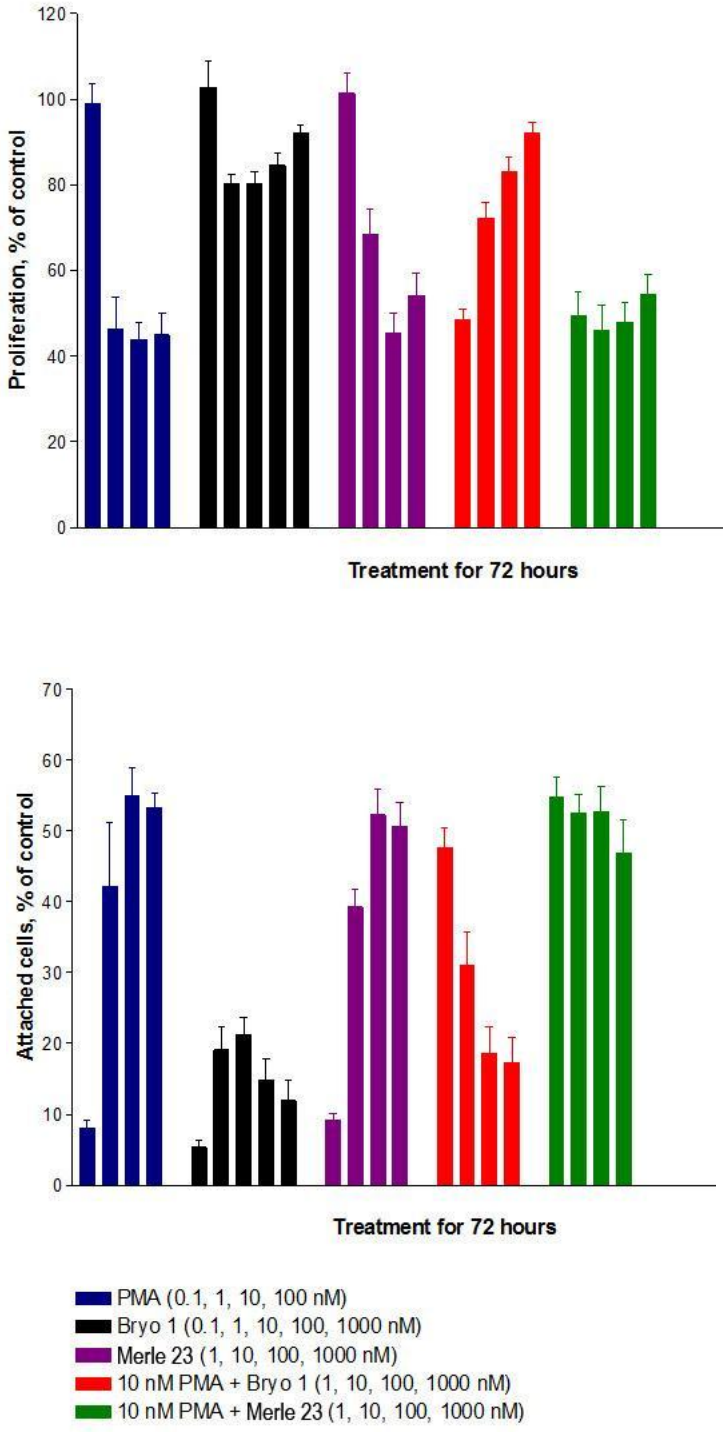
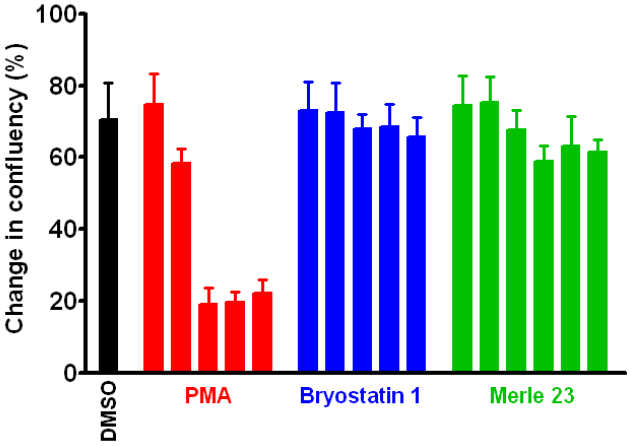


Figure 1.25. U937 proliferation (top) and attachment (bottom)

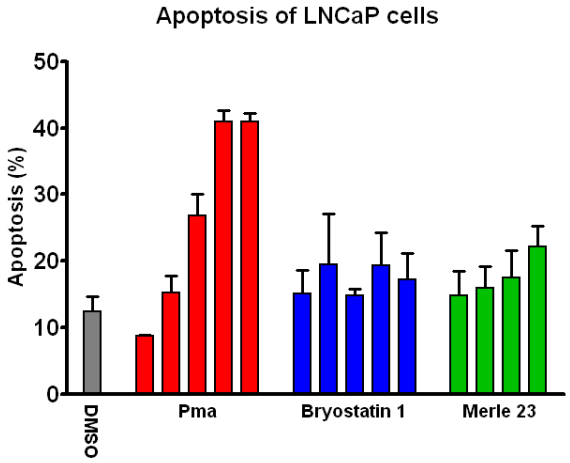
were mirrored when cell growth was measured in the K562 chronic myelogenous leukemia cell line indicating that the A- and B-rings do not simply act as a spacer domain, but instead play a critical role in the analogue's biological profile.

In the LNCaP human prostate cancer cell line, PMA inhibits proliferation and induces apoptosis while Merle 23, like Bryostatin 1, fails to induce either response (Figures 1.26, and 1.27).[61] Additional studies using the LNCaP cell line have demonstrated that the biological response of Merle 23 in LNCaP cells was more complex, showing either a bryo 1 like, PMA like, or a novel response depending on what biological end point was observed. When Merle 23 was co-administered with the proteasome inhibitor MG-132 it, like PMA, inhibited proliferation and induced apoptosis while bryostatin 1 was unaffected by the presence of MG-132. When phosphorylation of known PKC substrates MARCKS and PKD1 were observed, Merle 23 showed duration of response intermediate between that of bryostatin 1 and PMA, but closer to the long activation time seen for PMA. Activation, down regulation, and translocation of PKC isozymes, alpha and delta, was another observable endpoint in which all three compounds exhibit distinct responses. Merle 23 was the least potent compound for activation of PKC delta through phosphorylation at Ser299; however Merle 23 showed intermediate potency for phosphorylation at Tyr311. Merle 23 was the most efficient down-regulator of PKC delta, but the least efficient down-regulator of PKC alpha. Bryostatin 1 induced a biphasic down-regulation of PKC delta and was the most potent against PKC alpha, while PMA down-regulated both with equal efficiency. Translocation of PKC isoforms to either internal membranes or the plasma membrane dictates what substrates are available for



PMA: 0.1, 1, 10, 100, 1000 nM
Bryostatin 1: 0.1, 1, 10, 100, 1000 nM
Merle 23: 0.1, 1, 10, 100, 1000, 10000 nM

Figure 1.26. LNCaP proliferation assay



PMA: 0.1, 1, 10, 100, 1000 nM
Bryostatin 1: 0.1, 1, 10, 100, 1000 nM
Merle 23: 1, 10, 100, 1000 nM

Figure 1.27. LNCaP apoptosis assay

phosphorylation and therefore what signaling pathways are activated. Merle 23 demonstrated a PMA like response of translocating PKC alpha and epsilon to the plasma membrane, but showed bryo 1 like behavior in directing PKC delta and PKD1 primarily to internal membranes. Our current understanding of Merle 23 is that it is not simply bryostatin 1 or PMA like but that it is a distinct compound with a unique biological profile that provides an invaluable tool for understanding the role of PKC isozymes in cellular processes. Unfortunately, at this point all of the material previously synthesized had been consumed, and for continued testing of Merle 23 a larger scale synthesis was needed.

Results and Discussion

Scaled synthesis of Merle 23

In order to produce substantial quantities of Merle 23, a third-generation approach (Figure 1.28) was developed. For the third approach, we wanted to utilize a fully functionalized C-ring in the B-ring pyran annulation so that difficult manipulations on a complex substrate could be avoided. The new fully functionalized C- ring was envisioned to come from linear precursor **1.75** through an olefinic ester metathesis reaction developed by the Rainier group,[62] followed by a series of stereo- and regio-selective oxidations. Metathesis precursor **1.75** would then be tracked back to two nearly equal size fragments **1.76** and **1.77** that could be united through an EDCI mediated coupling. The A-ring β-hydroxyallylsilane **1.56** was produced on gram scale following the route previously described by Dr. Li (Figure 1.20) without any deviation and therefore will not be discussed further.

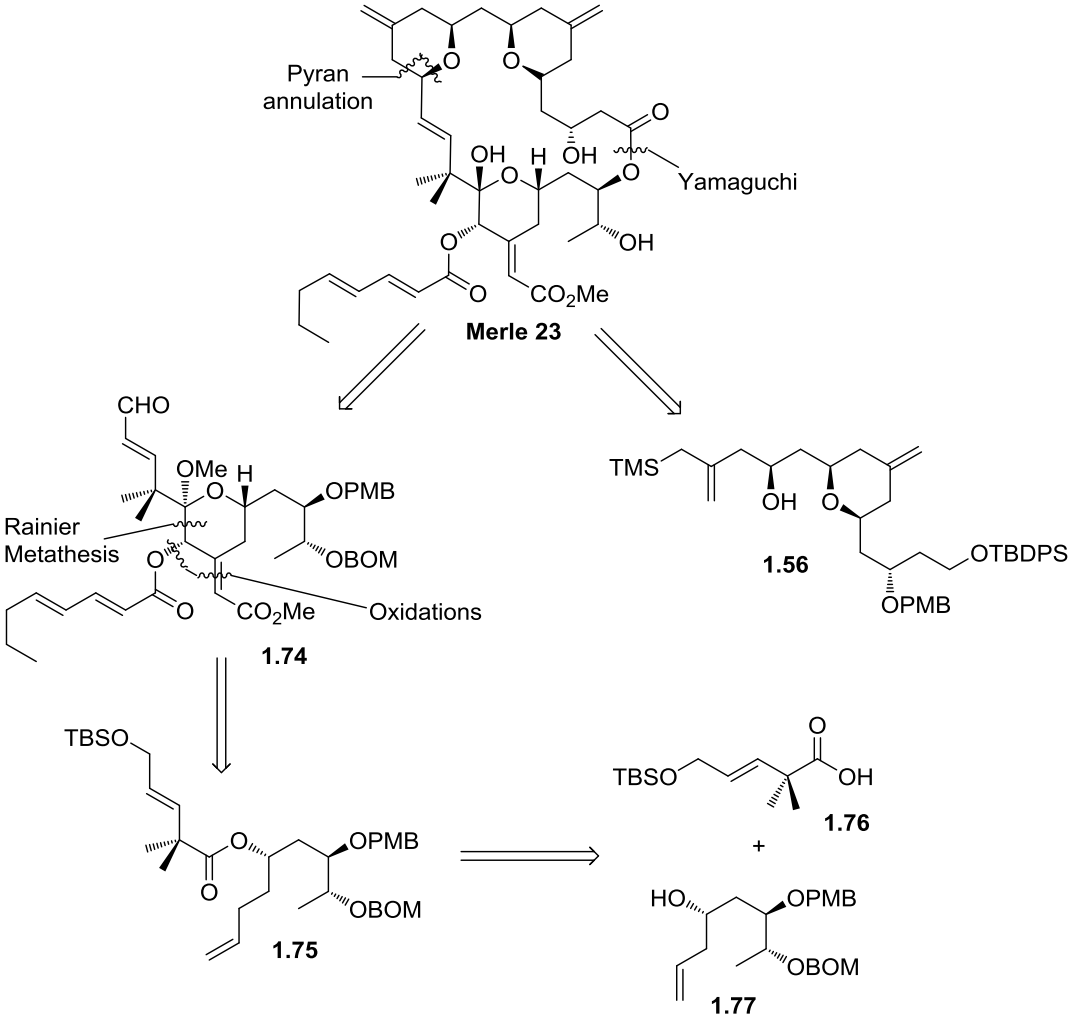


Figure 1.28. Third-generation retrosynthesis

The 3rd-generation C-ring also began with (R)-(+)-isobutyl lactate, which was elaborated to homoallyl alcohol **1.77** through the same series of chelation controlled allylations described previously (Figure 1.13). At this point, rather than expanding linearly, **1.77** was coupled to acid **1.76**, which was constructed in six steps from methyl isobutyrate (Figure 1.29). Deprotonation with LDA and alkylation with allyl bromide provided terminal olefin **1.79** in 70 % yield on a 1-mole scale. Wohl-Ziegler radical bromination afforded the primary allyl bromide **1.80**,[63] which was displaced with KOAc.

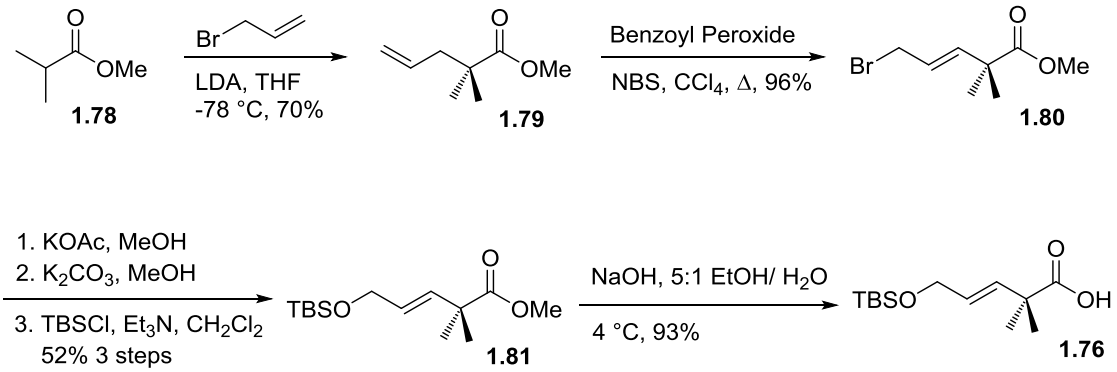


Figure 1.29. Synthesis of acid **1.76**

Removal of the acetate through transesterification with K_2CO_3/MeOH accessed the free alcohol, which was protected with TBSCl, and hydrolysis of the methyl ester under basic conditions provided acid fragment **1.76**. Having produced each C-ring fragment on large-scale, attention was focused on coupling and elaborating to glycal **1.85**.

Acid piece **1.76** was coupled to alcohol **1.77** with EDCI to provide ester **1.82** in 92 % yield (Figure 1.30). A three-step hydroboration,[64] oxidation,[65] and Wittig-olefination[66] sequence was employed to prepare the one carbon homologated olefin **1.75**. Use of the Rainer metathesis reaction[62] to form glycal **1.85** was facilitated by the presence of the C18 *gem*-dimethyl, which directs the titanium catalyst towards reacting with the olefin first, whereas reaction with the ester first leads to olefination without cyclization. The 3rd-generation route for the C-ring accessed gram quantities of glycal **1.85** in 11 linear steps, a 3-step improvement over the previous routes. Furthermore, having a TBS protected alcohol at C15, as opposed to the previously used thioester, allowed for functionalization of the glycal. The unsaturated thioester (Figure 1.19) was prone to cyclization though Michael addition of the C20 alcohol when the olefin was oxidized.

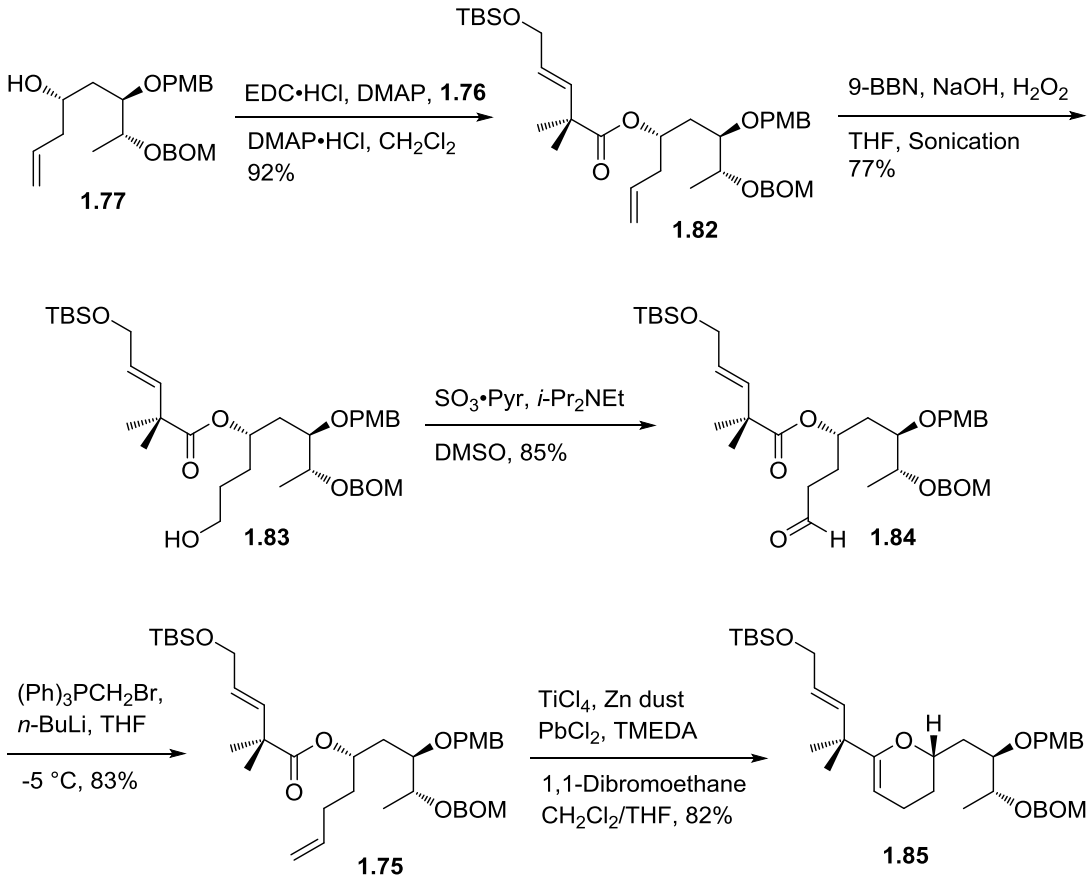


Figure 1.30. Synthesis of glycal **1.85**

Analogous to previous routes, glycal **1.85** was epoxidized with magnesium monoperoxy phthalate (MMPP) with *in-situ* opening of the epoxide, followed by oxidation of C20 to the ketone using Ley conditions[49] (Figure 1.31). Aldol addition of methyl glyoxylate at C21 with K_2CO_3 in MeOH allowed for *in-situ* elimination to provide exocyclic enoate **1.87** in one-step,[38b] and reduction using Luche conditions[67] gave the axial alcohol as a single diastereomer by ^{1}H NMR. The high level of selectivity during the reduction can be rationalized by considering the C20-C21 portion of the ring as highly planar, and approach of the hydride from the face opposite the bulky substituent at C19.[53] This level of selectivity is a good improvement over the 4:1 selectivity observed

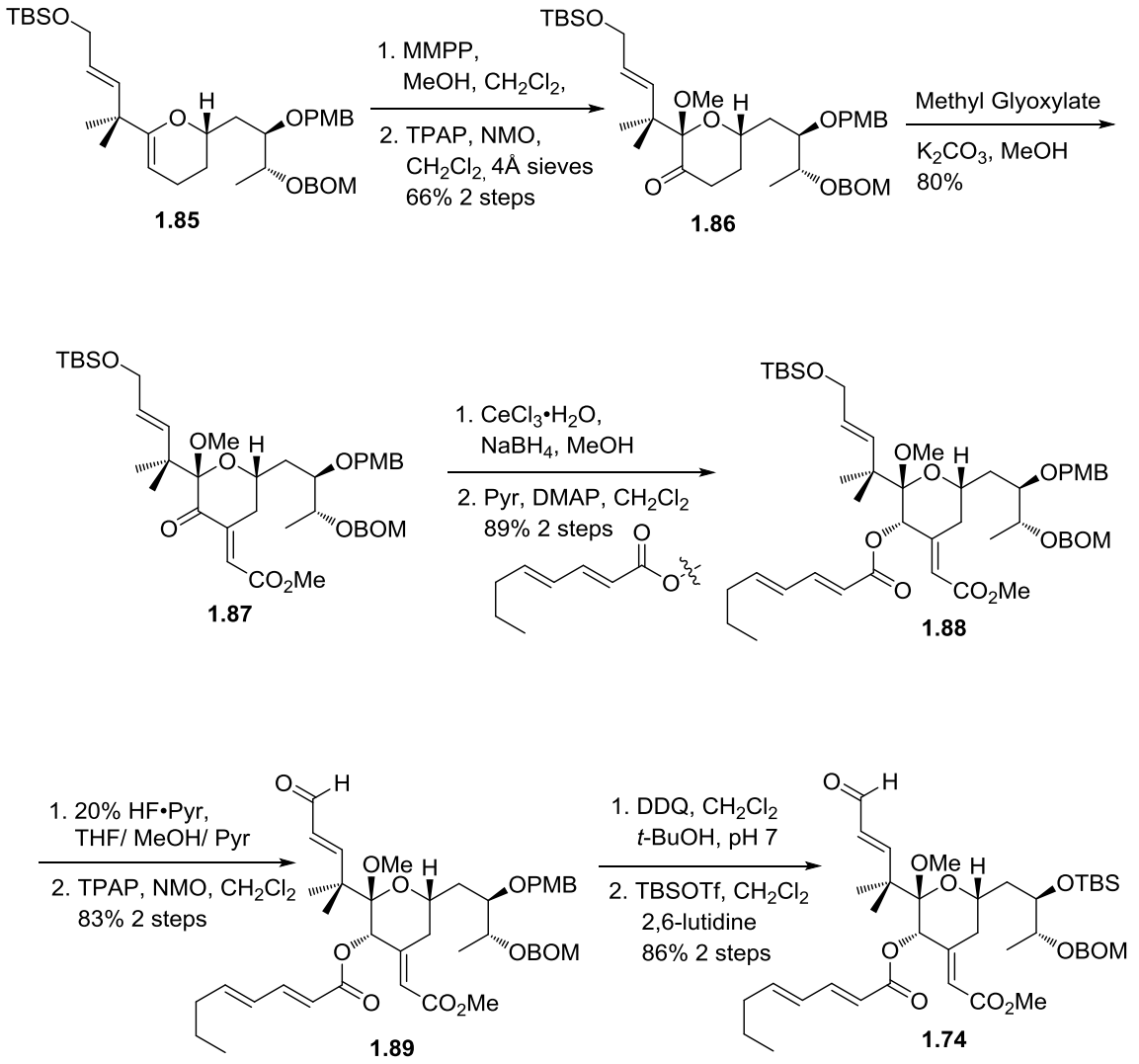


Figure 1.31. Functionalization of the C-ring

when the reduction is carried on the macrocylcic structure **1.72** (Figure 1.22). Subsequent esterification with (2*E*,4*E*)-octa-2,4-dienoic anhydride provided the fully functionalized C-ring **1.88**. Cleavage of the C15 TBS ether was accomplished with HF•Pyr, and the resulting alcohol was oxidized to an aldehyde. Finally, protecting group exchange of PMB for TBS was done to avoid later complications when the PMB containing A-ring was introduced.

Pyran annulation between the A- and C-ring fragments proceeded in 75 % yield to give the full carbon skeleton **1.91**, and at this point the synthesis intercepted known steps to finish the analogue (Figure 1.32). Selective removal of the C1 TBDPS,[59] double oxidation of the resulting alcohol to the C1 acid,[50, 65] and Yamaguchi macrolactonization[51] accessed macrolactone **1.73**. Deprotection over two steps to remove first the PMB then BOM ether and methyl ketal[52] provided Merle 23 in 28 linear steps, 50 total. The 3rd-generation route only represents a 3-step improvement over the previous route; however, the true advantage was the convergent route to the fully functionalized C-ring. The new C-ring route resolved issues associated with functionalizing the C-ring with the thioester present at C15 allowing for quantities in excess of 10 grams to be prepared. The other serious advantage is that after the pyran annulation to form the B-ring only 7 steps remained to finish the analog as opposed to 11. Through the 3rd-generation route, 15 mg of Merle 23 have been produced to date as well as significant amounts of materials that could readily be converted to Merle 23 or to other new analogues.

Transcriptional response of Merle 23

Building on the previous result where Merle 23 was seen to act like the phorbol ester PMA when tested for cell proliferation and attachment in U937 cells, but bryostatin 1 like when looking at cell growth and TNFα induced apoptosis in LNCaP cells, we sought to examine the effects of all three agents from a more comprehensive view.[68] Using qPCR, we examined the effects of the compounds as a function of dose and time on the levels of mRNA expression for 18 genes in both U937 and LNCaP cells. Each gene was selected

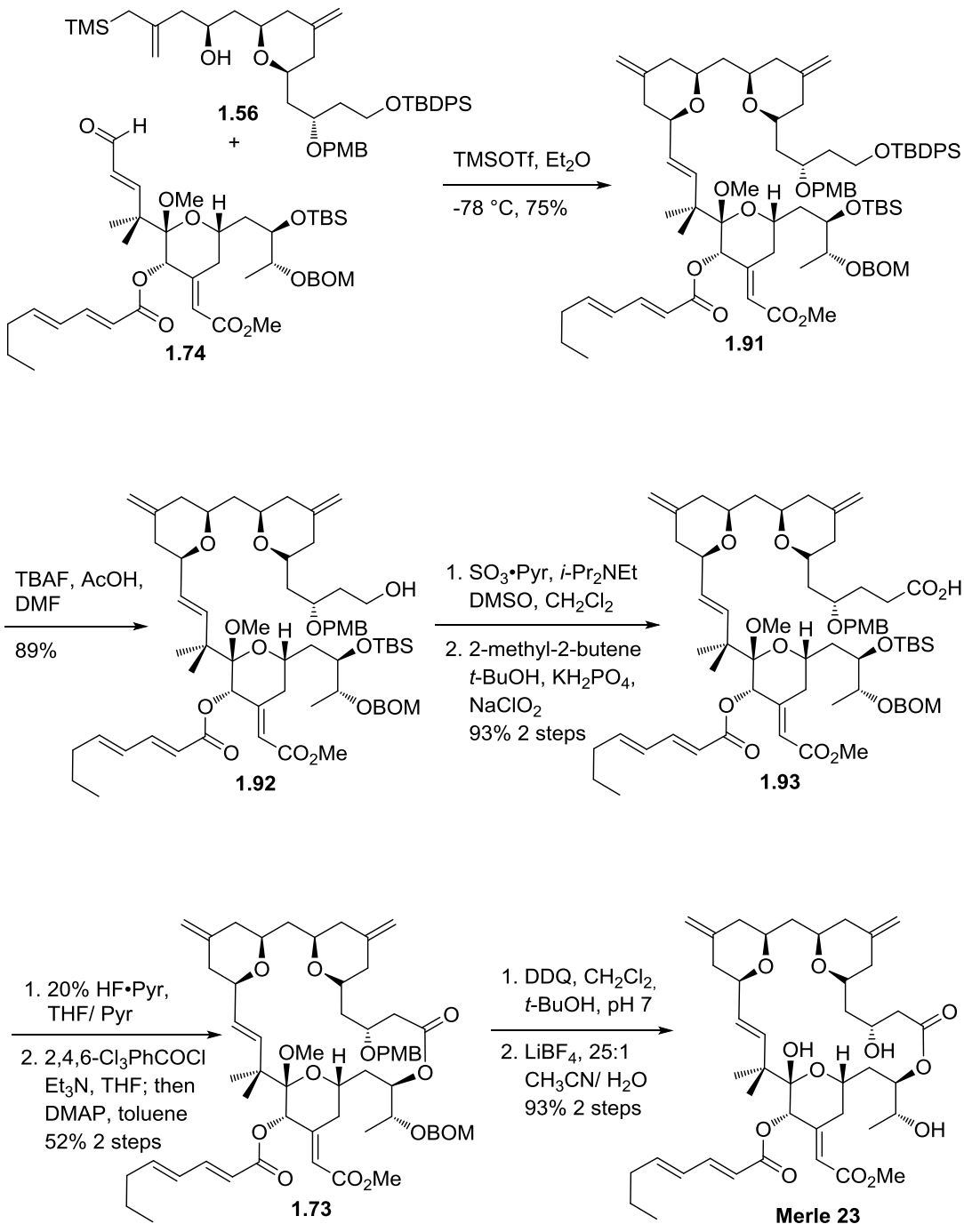


Figure 1.32. Completion of Merle 23

based on its marked regulation by PMA as described by Caino,[69] as well as their relevance to known growth regulating pathways. We sought to answer three questions: how do each of the three compounds compare in the activation of individual genes? Does each gene show a similar pattern of response to the compounds? Finally, how do the patterns of response compare and contrast for the two cell lines given that a different biological outcome is reached?

For the LNCaP cells the time course for modulation of all 18 genes at 2, 6, 12, and 24 h windows is illustrated in (Figure 1.33) at 100 nM PMA, a fully effective dose. The maximal response for most genes examined was observed at 6 h or later. For several genes (e.g., CXCL8, CCL2, TNFa, TRAF1, BIRC3), elevated levels were maintained past the 24 h time frame, while others (e.g., ALOXE3, ETS2, SMAD6) had largely returned to baseline. Next, the pattern of response to PMA was compared to that of bryostatin 1 and Merle 23 (Figure 1.34 and 1.35). At the 2 h time point, the average PMA response had risen to 26-fold over the control. Bryostatin 1 had induced only 52% of the PMA response while Merle 23 induced 73%. After 6 h the PMA response had climbed 87-fold over control. Bryostatin 1 now only exhibited 11% of this response and Merle 23 58%. At the 12 and 24 h time points PMA continued to display elevated mRNA levels of 71- and 83-fold over control. The bryostatin 1 response fell to 6.9% of PMA at 12 h then rebounded slightly up to 9.8% at 24 h. The Merle 23 response followed a similar pattern dropping to 23% before rebounding to 35% of the PMA response.

To answer the first question “how do each of the three compounds compare in the activation of individual genes?” We concluded that each compound induced an overall

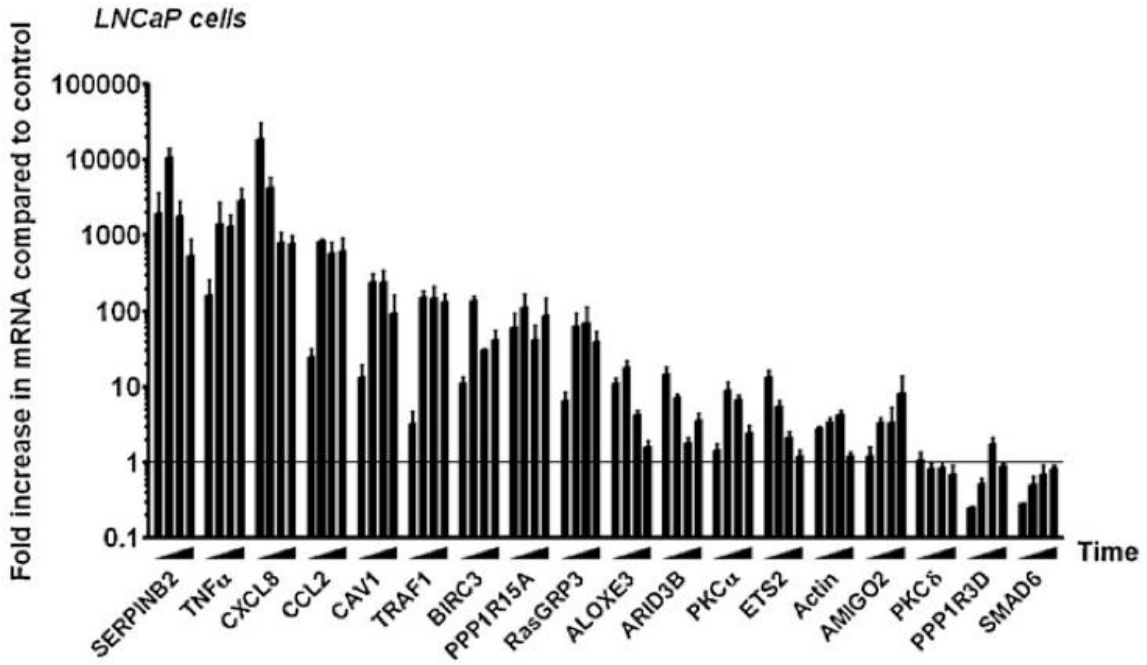


Figure 1.33. Activation of genes by PMA

similar pattern of response; however, the degree of transience was dramatically different. The second question can be answered by observing that the degree of transience was not conserved for the different genes. In particular, SERPINB2 showed dramatic induction by bryostatin 1 at 2 h, which was entirely lost by 24 h. Contrastingly, PPP1R15A experienced an approximate 10-fold increase that was maintained through the entire 24 h time course. It is also important to once again note that the transient response of bryostatin 1 was not due to instability as bryo 1 was capable of antagonizing the effects of PMA throughout the full 24 h when co-administered.

Merle 23 demonstrated contrasting biological outcomes in U937 cells where it resembled PMA in its ability to inhibit proliferation and induce attachment whereas in LNCaP cells it was bryostatin 1 like, not inhibiting proliferation nor inducing apoptosis. Therefore we compared the pattern of transcriptional response in these two cell lines to better understand the underlying reason. Of the 18 genes previously used, only the 8 that

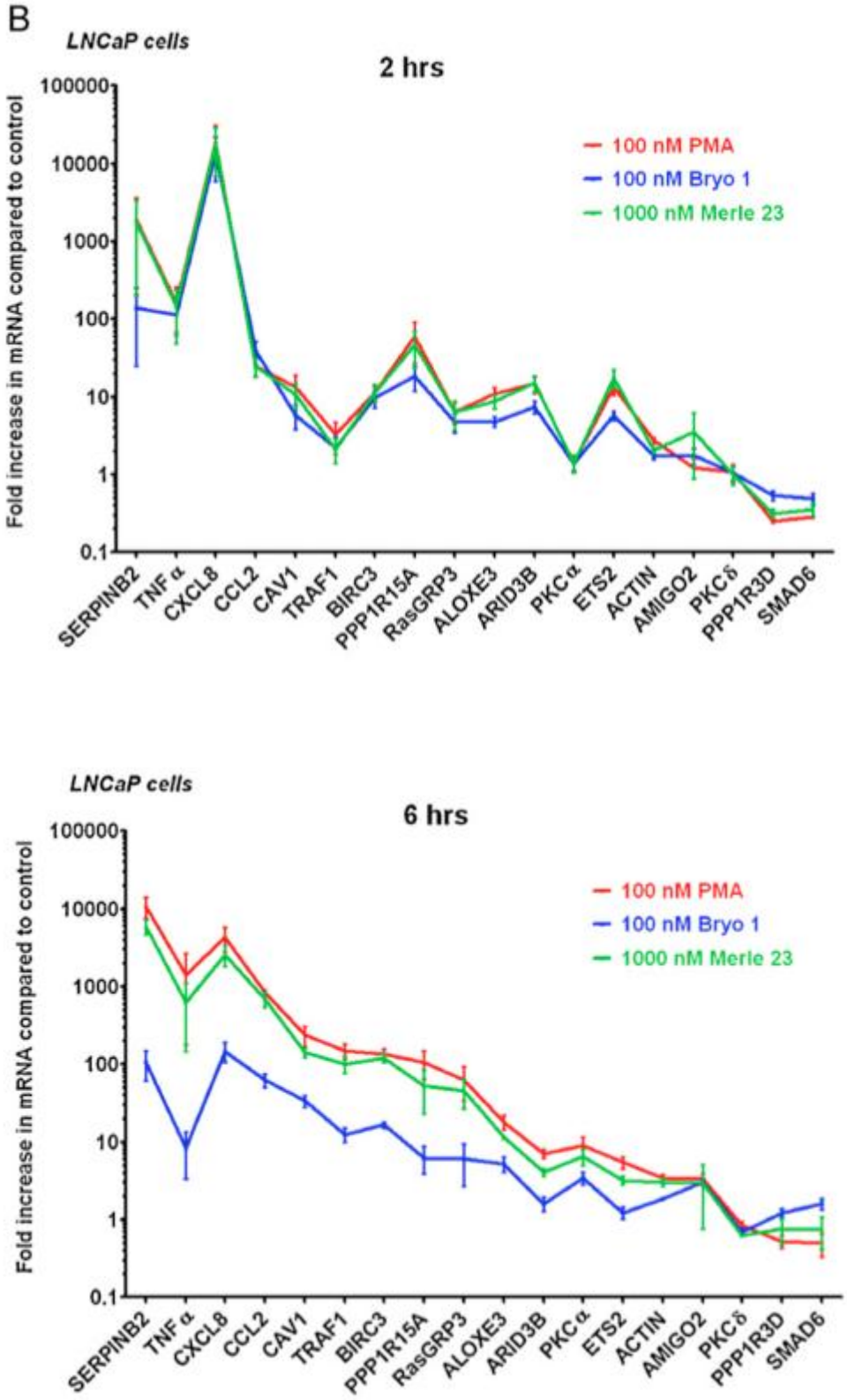


Figure 1.34. Transcriptional response of LNCaP Cells at 2, and 6 h

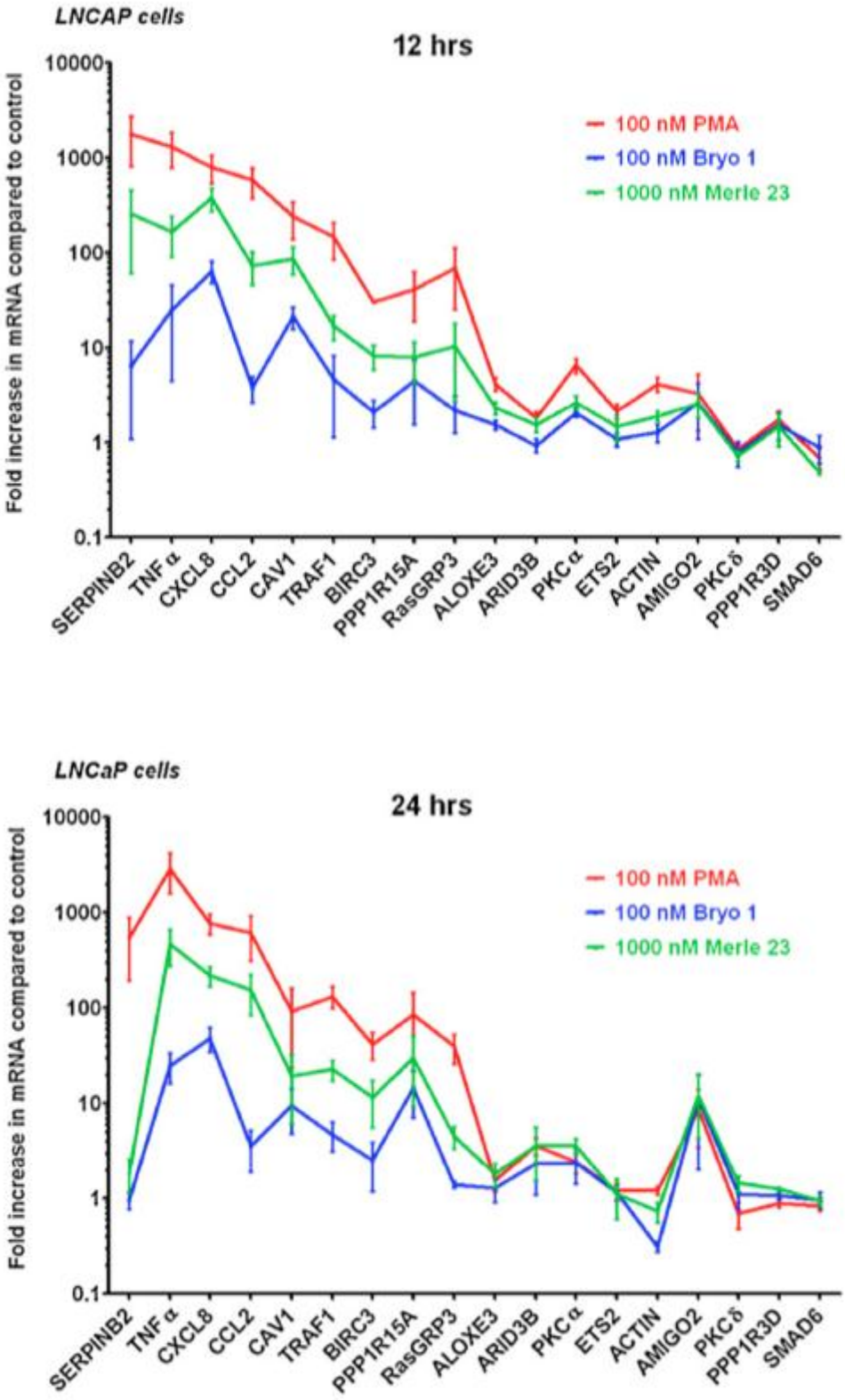


Figure 1.35. Transcriptional response in LNCaP cells at 12, and 24 h

showed the most significant response were used again (Figure 1.36, and 1.37). The first difference of note is that in U937 cells the maximum level of stimulation was not as high as for LNCaP cells. The average changes in gene response due to PMA were 4.8, 9.1, and 17-fold at 2, 8, and 24 h compared to average increases of 26- and 83-fold at 2 and 24 h in LNCaP cells. Likewise, the response to bryo 1 and Merle 23 were considerably reduced. The bryostatin 1 responses were 75.4%, 43.4% and 11.5% of the PMA response, and the corresponding values for Merle 23 were 45.4%, 60.9% and 48.2%, respectively.

To adress the third question, the two cell lines showed dramatically different levels of activation in response to the three compounds, suggesting that the different biological outcome may be a result of the cells sensitivity to PKC activation. U937 cells having a smaller range seem to be more susceptible to modest changes in degree and duration of mRNA activation.

The major difficulty in understanding the rationale for a bryostatin like response compared to PMA are the multiple mechanistic differences such as isozyme translocalization, modification, and down regulation. These studies sought to look at the net effect of these factors on what genes are activated and for how long. For both cell lines, the dominant difference in PMA vs. bryostatin like behavior was the degree of transiency following the initial activation. Merle 23 gave a transiency level intermediate between that of PMA and bryostatin for both cell lines. We now believe that tumor-promoting behavior is likely a result of two factors: first, the duration of gene activation induced by the ligand and second, the specific cell's ability to tolerate the increase.

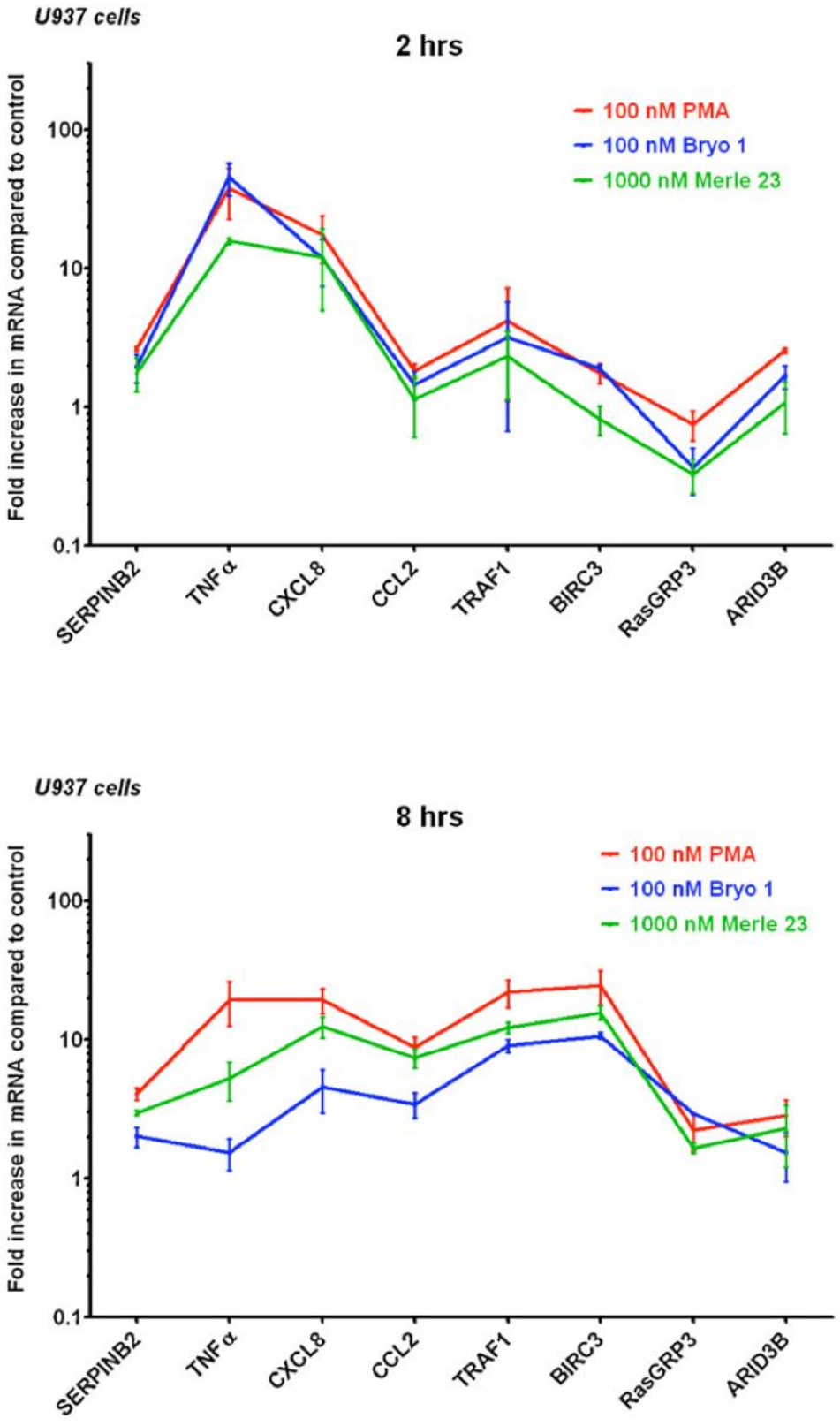


Figure 1.36. Transcriptional response in U937 cells at 2, and 8 h

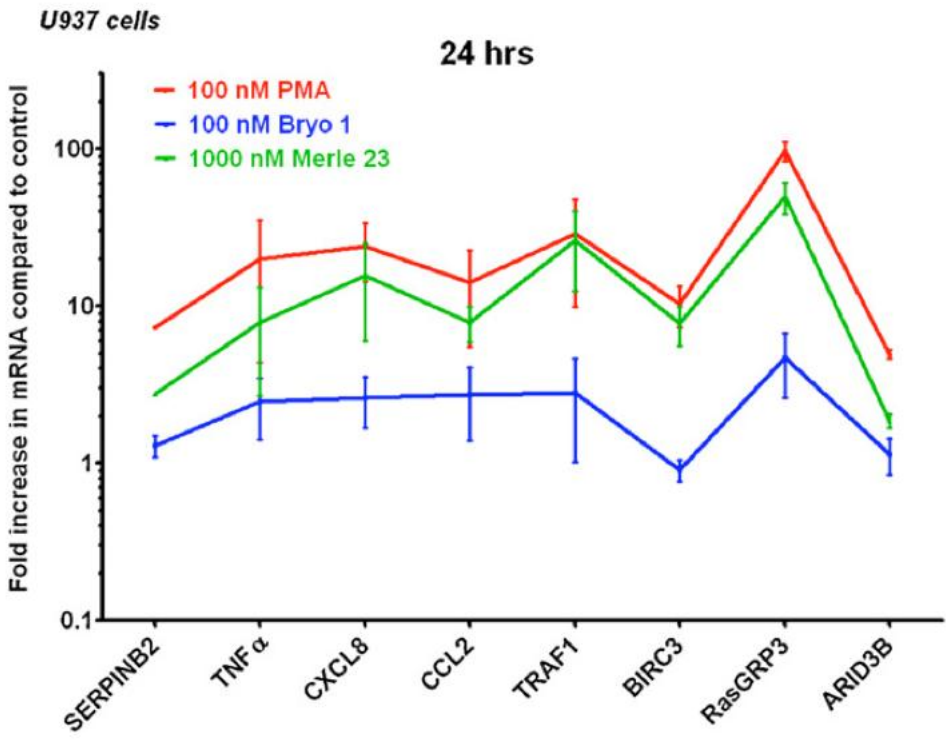


Figure 1.37. Transcriptional response in U937 cells at 24 h

Merle 23 and HIV

Having a sufficient supply of Merle 23, we looked to explore new areas in which bryostatin 1 has previously demonstrated beneficial effects. To evaluate Merle 23's ability to activate latent HIV, we submitted both Merle 23 and bryostatin 7, synthesized by Dr. Poudel, to Dr.Kazmierski's HIV Medicinal Chemistry group at GlaxoSmithKline. Both compounds were exposed to a Human Osteosarcoma (HOS) long terminal repeat (LTR) stimulation assay. The concept of the assay is that HOS cells are transfected with a luciferase reporter under the control of the HIV LTR promoter. Therefore, compounds that stimulate the HIV LTR promoter will produce the luciferase enzyme in a dose dependent manner. Both Merle 23 and bryostatin 7 display potent EC50's with Merle 23 being slightly more potent but having a lower maximal response (Table 1.1). Both

Table 1.1. HIV activation data

Compound	EC50 (uM)	Max signal (% of control)	Conc (uM) At max	CCEC50
Merle 23	0.002	166	0.07	39.57
Bryostatin 7	0.006	250	0.07	6.24
Bryostatin 1	0.002	187	2.51	>5
Prostratin	1.08	274	27.78	>50

compounds have minimal cytotoxicity with CCEC50's in the low micro molar range, which is very similar to bryostatin 1. Prostratin, which is also in development as an activator, is three orders of magnitude less potent. Based on these initial findings GSK has expressed interest in conducting further studies aimed at determining pharmacokinetic properties.

Identifying a Strategy for Solubilizing Analogues

Owing to an inability to detect low levels of bryostatin 1 in patients there exist very little pharmacokinetic data in humans. Mouse studies conducted with a rapid i.v. injection of [C26-^{3}H] labeled bryo 1 revealed a two-compartment model of plasma disappearance with half-lives of 1.05 and 22.97 h. Bryo 1 was widely distributed with particularly high levels in the lungs, liver, gastrointestinal tract, and fatty tissue. Radioactivity associated with the intact compound was observed for up to 24 h and it was eliminated primarily through urinary secretion.[70] In the clinic, the low aqueous solubility of bryo 1 necessitated infusion initially in 60% ethanol, which caused venous sclerosis. A second-generation formulation of polyethylene glycol, ethanol, and tween (PET

60/30/10 v/v) also caused significant injection site issues that were not believed to be associated with bryostatin itself.[71] Rather than dealing with problems related to formulating an i.v. injection a couple of groups have explored making structural modifications to bryo 1 in order to increase the aqueous solubility.

The first attempt at modifying bryo 1 structurally to improve aqueous solubility was the synthesis of 26-succinylbryostatin 1 by Kraft (Figure 1.38).[71] Addition of the carboxylate increased solubility by 100-fold. Unfortunately this compound was no longer a good ligand for PKC because the requisite C26 oxygen was now blocked. Additionally, the compound showed very little biological activity, and what was observed was most likely the result of the succinate side chain undergoing hydrolysis and reverting back to bryostatin 1.

In the course of the Wender group's analogue work they recognized that the C20 position might be a more attractive position to attach a solubilizing group. To test the feasibility of using this position for tuning pharmacokinetic properties, they developed a strategy based on a late stage diversification of a C20 aniline with a variety of different

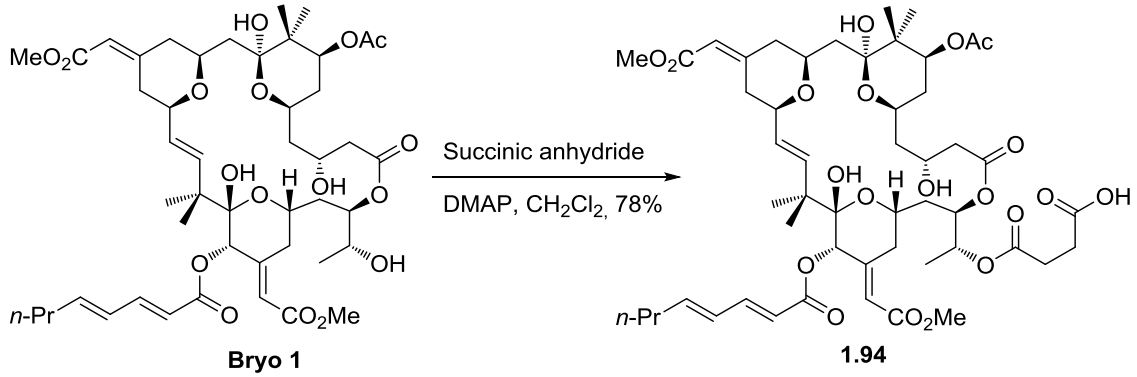


Figure 1.38. 26-succinylbryostatin 1

anhydrides (Figure 1.39).[72] The aniline linkage was chosen due to difficulties encountered when directly esterifying the C20 alcohol with more complex anhydrides. All analogues synthesized demonstrated double-digit nanomolar PKC affinity; however, none were tested for biological activity.

Synthesis and biology of Merle 35 and Merle 36

Merle 23 has demonstrated interesting biology and considerable promise as an activator of latent HIV. Unfortunately, Merle 23 is even more lipophilic than bryostatin 1, and if it were to ever progress in its development, a strategy by which aqueous solubility could be enhanced would need to be developed. Based on knowledge of structure function relationships as well as Wender's success using the C20 position, we also chose to use C20 for attaching solubilizing groups. In contrast to previous work, we felt that attaching directly to the C20 alcohol, rather than through an aniline appendage, would minimize negative effects on both binding and the biological activity of the analogs.

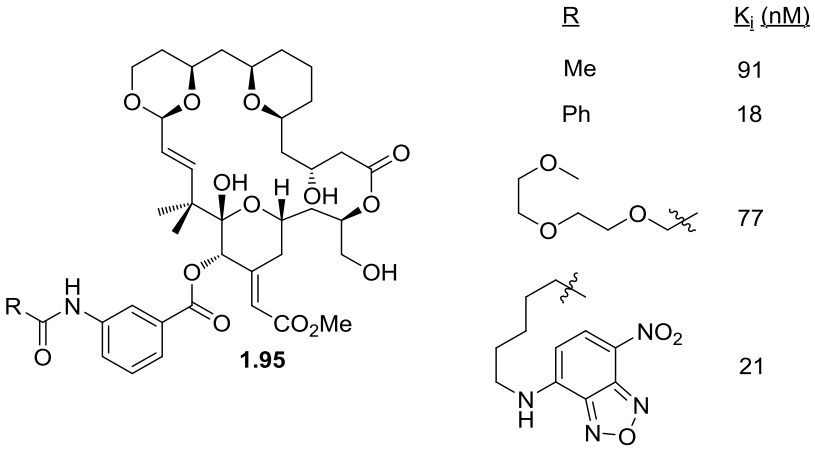


Figure 1.39. Wender's C20 analogues

The first analogue we targeted was Merle 35 where the (2*E*,4*E*)-octa-2,4-dienoic ester was to be replaced by an acetate. This simple modification is analogous to the difference between bryostatin 1 and bryostatin 7, which is less lipophilic and which we have already demonstrated to have comparable binding and biology.[73] The synthesis began with Luche reduction of previously used ketone **1.87**, followed by esterification with acetic anhydride. Removal of the C15 TBS protecting group, oxidation to the aldehyde, and exchange of the PMB protecting group for a TBS provided the acetate containing C-ring (Figure 1.40).

Pyran annulation with the A-ring gave the full carbon skeleton, and the TBDPS group was removed. Oxidation of the free alcohol to the acid, as previously described, was followed by TBS removal and macrolactonization. The PMB, BOM and methyl ketal groups were removed in two steps to furnish Merle 35 (Figure 1.41).

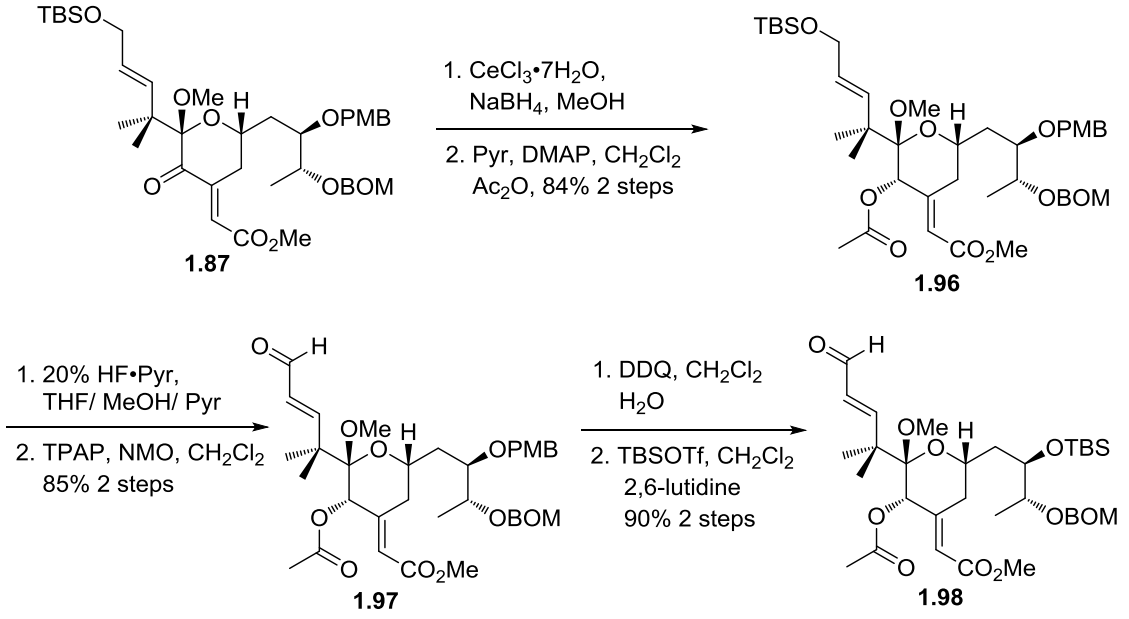


Figure 1.40. Synthesis of acetate containing C-ring

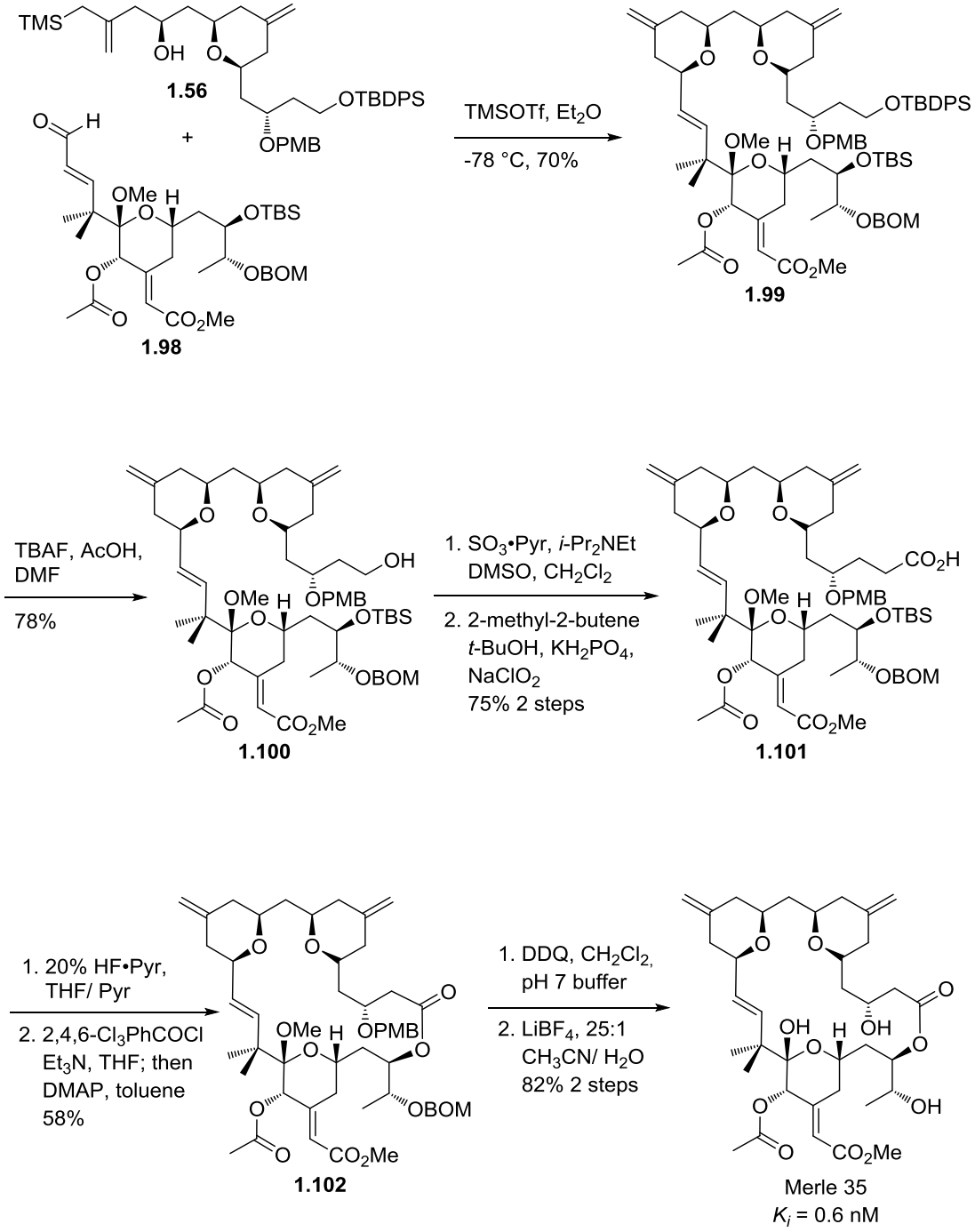


Figure 1.41. Synthesis of Merle 35

Merle 35 was not only designed as a less lipophilic analogue but was also envisioned as a late stage intermediate for more dramatic changes. The first solubilizing group we wanted to install was a carboxylic acid. To accomplish this the C20 acetate of macrocycle **1.102** was removed with K_2CO_3/MeOH and the free alcohol was esterified with succinic anhydride.[74] The free carboxyl was protected with a BOM group to help facilitate efficient purification of the C3 alcohol after PMB removal with DDQ. The final deprotection with $LiBF_4$ afforded Merle 36, which was converted to the morpholine salt (Figure 1.42).

Both new analogues were submitted to the Blumberg group to test if these changes would affect their biology. Merle 35 was even more potent than Merle 23 with a K_i = 0.6

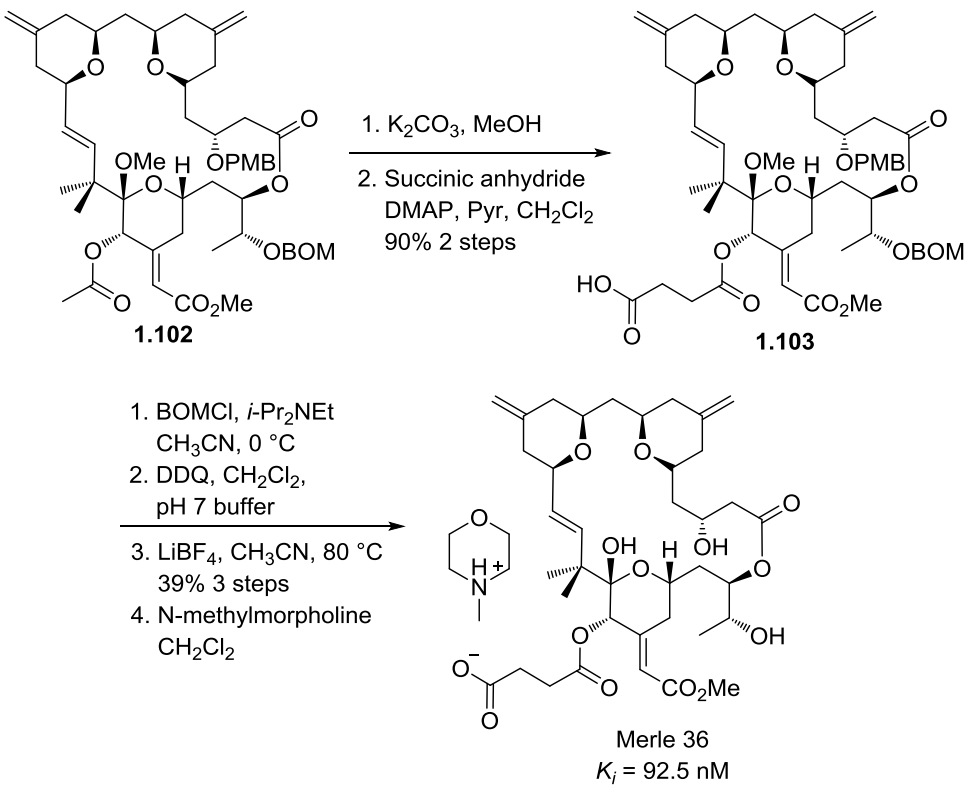


Figure 1.42. Synthesis of Merle 36

nM, and Merle 36 was nearly 100 times less potent with a K_i = 92.5 nM. In the U937 proliferation assay Merle 35 behaved like Merle 23 in that it inhibited proliferation and was unable to antagonize the action of PMA (Figure 1.43). In contrast Merle 36 failed to induce a biological response until the highest compound concentration (5 μM) was reached. A possible explanation for this is that the negatively charged carboxylate was preventing the compound from traversing the cell membrane efficiently.

Synthesis and biology of Merle 37

To avoid having a negative charge directly on the analogue, we decided instead to utilize a quaternary ammonium salt. Once again the acetate ester of macrocycle **1.102**

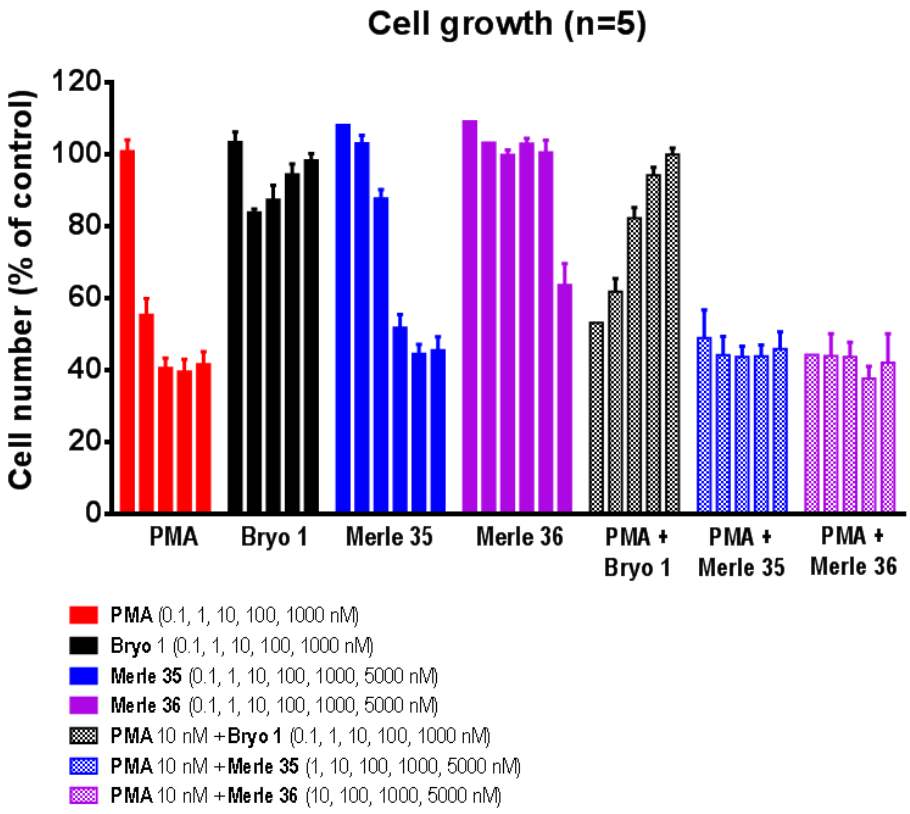


Figure 1.43. U937 cell assay with Merle 35 and 36

was cleaved; however, esterification with the previously used anhydride/DMAP procedure failed to effectively couple 6-(dimethylamino)hexanoic acid to the C20 alcohol. This problem was fixed by using a Shiina esterification,[75] and the analogue could be deprotected using our standard two-step protocol (Figure 1.44).

Merle 37 (M37), submitted as the TFA salt, has a K_i = 5.08 nM and was biologically active demonstrating Merle 23 like behavior albeit two orders of magnitude less potent (Figure 1.45). Lower potency has also been observed for bryostatin 7 compared to bryostatin 1 and may be a due to reduced membrane association and increased cytosolic concentration as implicated by translocation studies performed with bryostatin 7[73]. Merle 37 also resembled PMA in its ability to induce TNFα secretion (Figure 1.46).

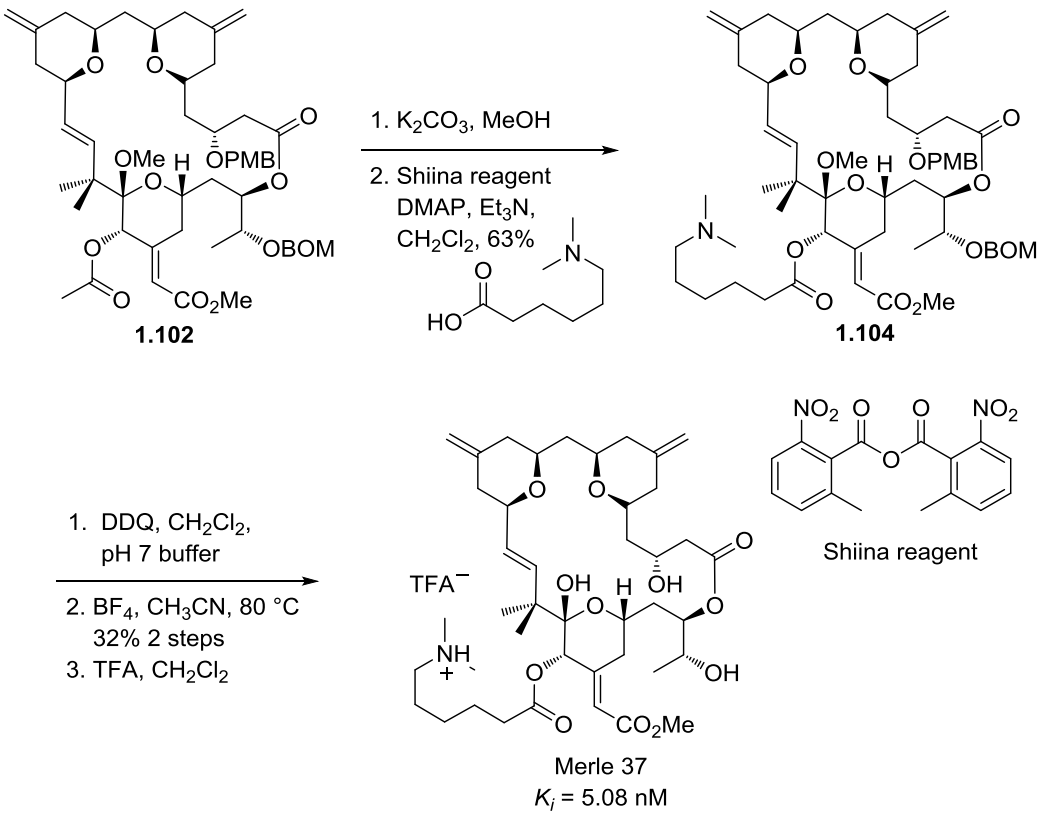


Figure 1.44. Synthesis of Merle 37

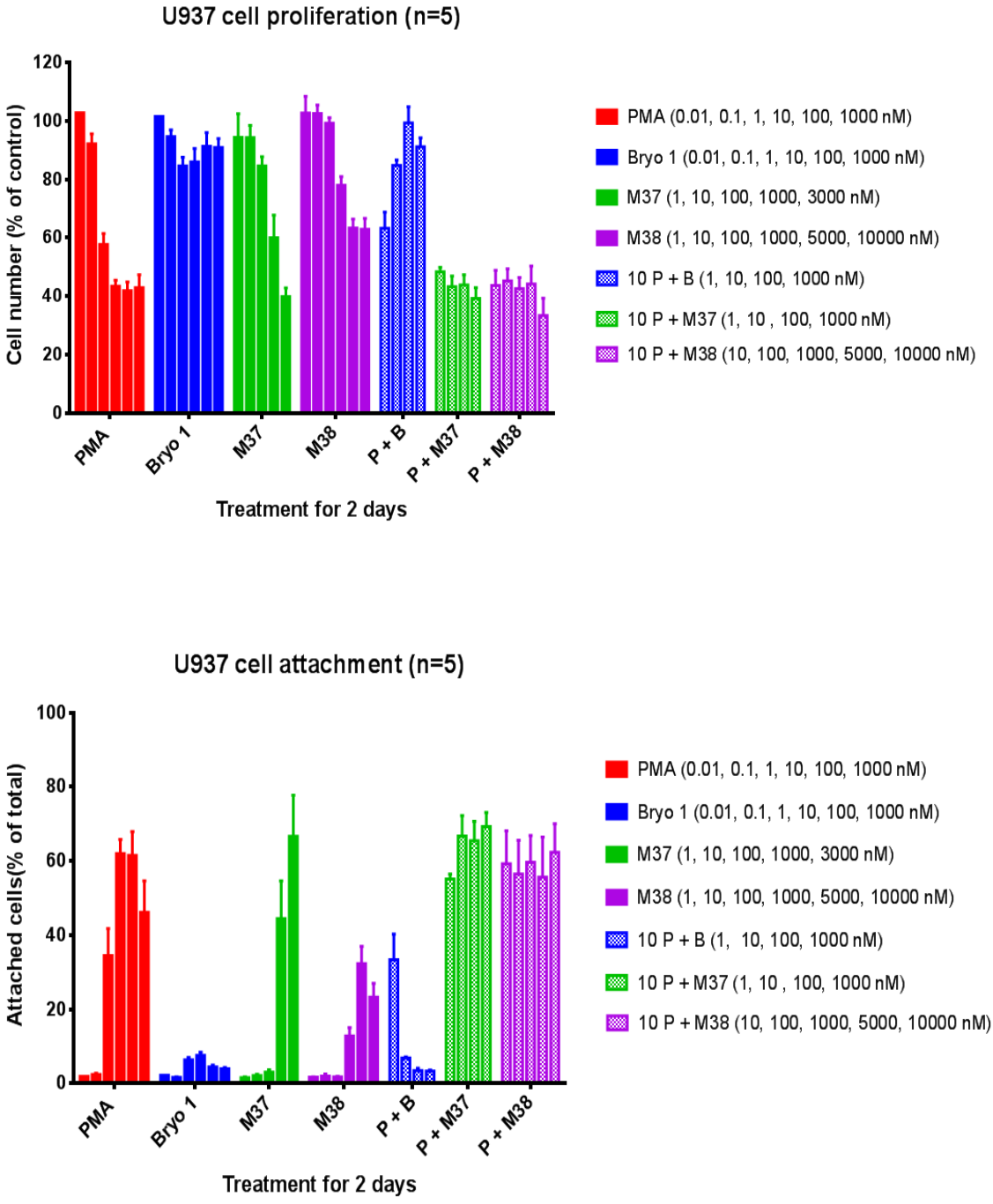


Figure 1.45. U937 proliferation and attachment assay

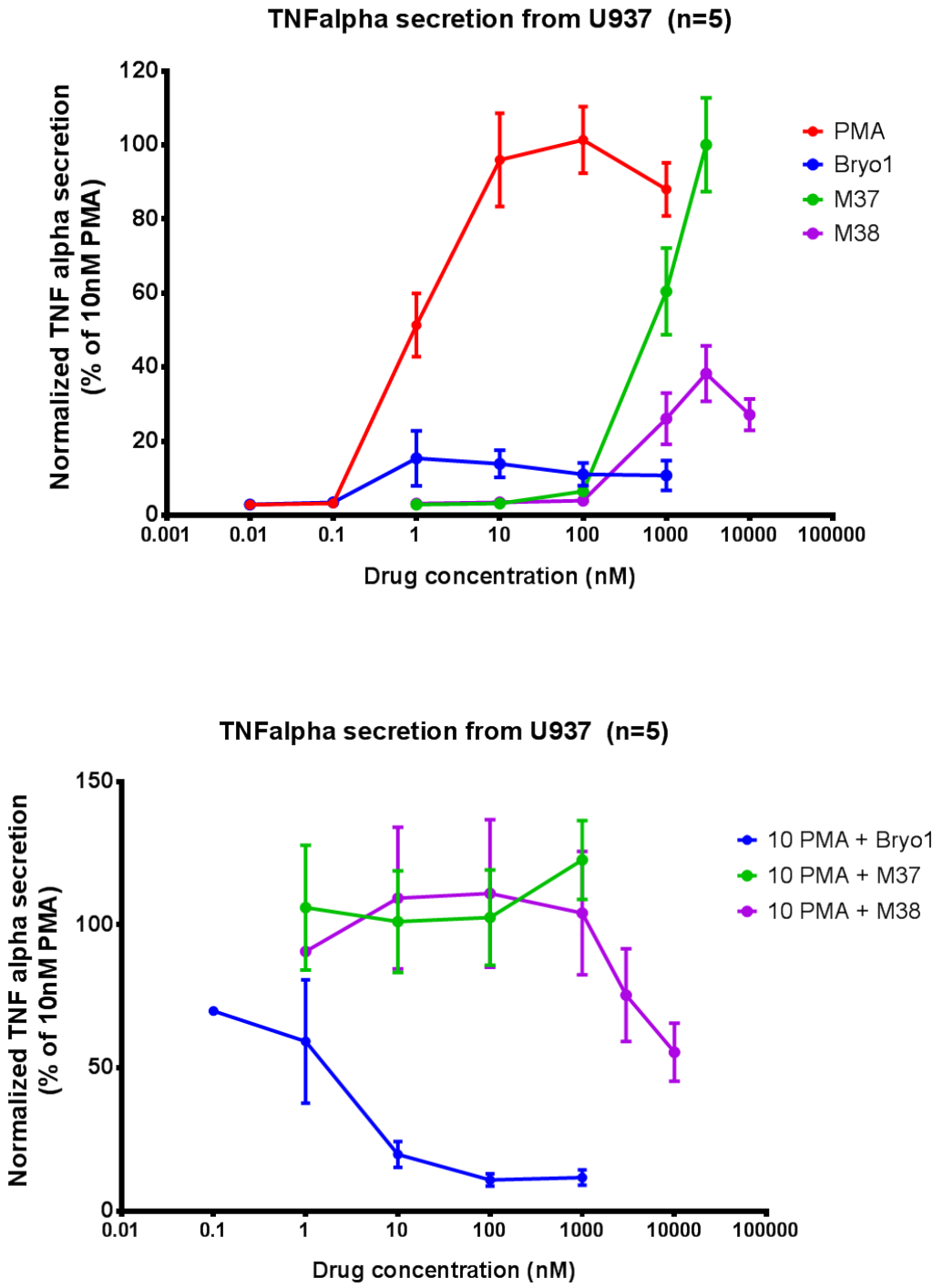


Figure 1.46. TNF secretion from U937 cells

Comparison of relative lipophilicity

After having successfully developed a strategy for attaching solubilizing groups to our bis-pyran scaffold that maintained binding and similar biology we wanted to evaluate how dramatic a change in lipophilicity the acetate and amine produced. To achieve this goal we chose to use the reverse phase HPLC method[76] relating retention time to the relative lipophilicity (Figure 1.47). This method is not a highly accurate way of calculating Log P values; however, it was ideal for us because it only requires ~ 0.01 mg of compound and provides us an excellent way to compare our analogues to each other. As expected Merle 23 was more lipophilic than bryo 1, and Merle 37 was by far the lest lipophilic compound. Interestingly, simply exchanging the eight carbon 20 side chain for an a acetate had a very dramatic effect for both the Merle and bryostatin series indicating that this modification alone may be sufficient to improve pharmacokinetic properties.

Activation of latent HIV by Merle 35 and 37

After having demonstrated that modification of the C20 ester endowed Merles 35 and Merle 37 with dramatically improved aqueous solubility while maintaining single digit K_i values for PKC, and without changing the biological response in U937 cells, the two new analogues were submitted to GSK for HIV activation analysis. Merle 35 activated HIV in Jurkat cells with an EC50 = 10 nM and cell cytotoxicity 50 (CC50) = 50 nM. Merle 37 had an EC50 = 80 nM and a CC50 = 550 nM. Both compounds compare very favorably to prostratin which as an EC50 = 130 nM and CC50 = 370 nM. Merle 23 however is still the best compound in the Jurkat cells with an EC50 = 0.2 nM and a CC50 = 5.0 nM.

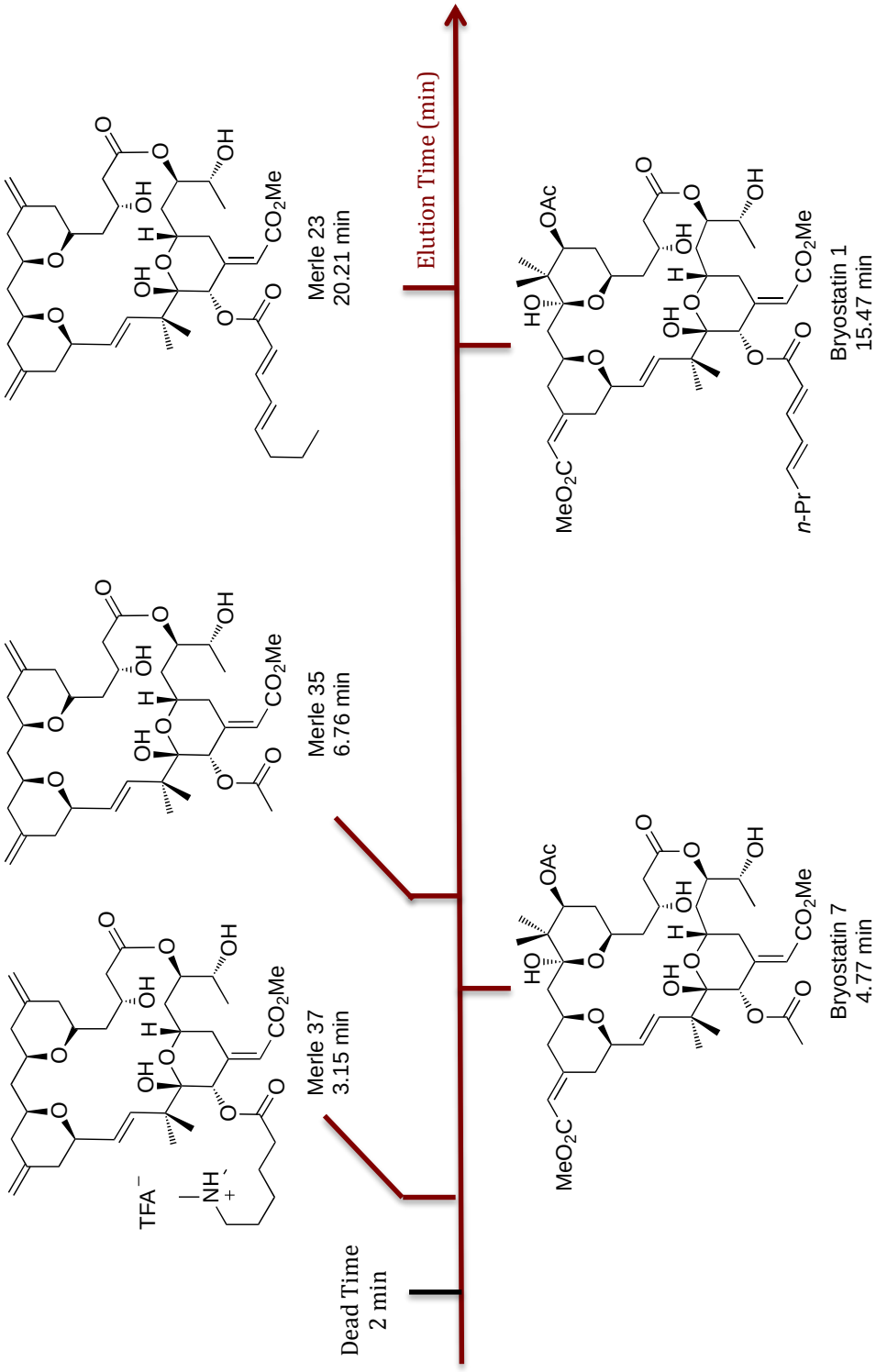


Figure 1.47. Relative lipophilicity of analogues

Conclusions

Merle 23 is a valuable tool for understanding the fundamental difference between tumor promoting and nontumor promoting PKC ligands. The scaled synthesis utilized a more convergent strategy that allowed for approximately 15 mg of Merle 23 to be synthesized to date as well as significant quantities of intermediate compounds that can be readily advanced to Merle 23 or other analogues. The most significant of the improvements was the use of a fully functionalized C-ring that circumvented the need for challenging manipulations on complex and delicate advanced intermediates. Use of a Rainier metathesis reaction facilitated a multigram synthesis of the C-ring in a highly efficient and convergent manner. The Merle 23 produced through this route has already helped to expand our understanding of bryostatin related biology through transcriptional response studies, and is sufficient in quantity to conduct the first direct tumor promotion studies in mice. Additionally, we have demonstrated Merle 23's potent activity in the HOS-LTR HIV activation cell assay.

The large-scale C-ring synthesis also enabled us to amend solubilizing groups to the Merle bis-pyran scaffold through the C20 ester. Merle 35 and 37 both demonstrate reduced lipophilicity while maintaining single digit nanomolar affinity for PKC and Merle 23 like biology in the U937 proliferation and attachment assay.

Experimental Section

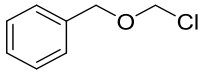
General experimental procedures

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals* (Perrin, Armarego, and Perrin, Pergamon: Oxford, 1996).[77]

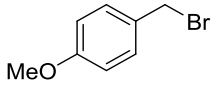
Diisopropylamine, diisopropylethylamine, pyridine, triethylamine, EtOAc, and CH_2Cl_2, were distilled from CaH_2. Reagent grade DMF, DMSO and acetone were purchased, stored over 4Å molecular sieves and used without further purification. Et_2O, THF, and toluene were distilled from Na under an atmosphere of N_2. MeOH was distilled from dry Mg turnings. The titer of *n*-BuLi was determined by the method of Eastham and Watson.[78] $TiCl_4$ was distilled prior to use. Zn was activated with aqueous HCl solution prior to use. All other reagents were used without further purification. Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR). Thin layer chromatography was performed on Merck Kieselgel 60 Å F_{254} plates or Silicycle 60Å F_{254} eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid, or an aqueous potassium permanganate solution. Flash column chromatography was performed with Silicycle Flash Silica Gel 40 – 63 μm or Silicycle Flash Silica Gel 60 – 200 μm, slurry packed with hexanes in glass columns. Glassware for reactions was oven dried at 125 °C and cooled under a dry atmosphere prior to use. Liquid reagents and solvents were introduced by oven-dried syringes through septum-sealed flasks under a nitrogen atmosphere. Nuclear magnetic resonance spectra were acquired at 300, 500 MHz for ^1H and 75, 125 MHz for ^{13}C. Chemical shifts for proton nuclear magnetic resonance (^1H NMR) spectra are reported in parts per million relative to the signal of residual $CDCl_3$ at 7.27 ppm. Chemicals shifts for carbon nuclear magnetic resonance (^{13}C NMR and DEPT) spectra are reported in parts per million relative to the centerline of the $CDCl_3$ triplet at 77.23 ppm. Chemical shifts of the unprotonated carbons (‘C’) for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The

abbreviations s, d, dd, ddd, dddd, dq, t, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, triplet and multiplet respectively. Optical rotations (Na D line) were obtained using a microcell with 1 dm path length. Specific rotations ($[\alpha]_D^{20}$, Unit: °cm^2/g) are based on the equation $\alpha = (100 \cdot \alpha)/(l \cdot c)$ and are reported as unit-less numbers where the concentration c is in g/l00 mL and the path length l is in decimeters. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry at The University of Utah on a double focusing high-resolution mass spectrometer. Compounds were named using ChemDraw 12.0.0.

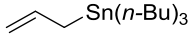
Synthesis of reagents



Preparation of ((chloromethoxy)methyl)benzene: To a stirring solution of paraformaldehyde (9.6 g, 319 mmol, 1 equiv) and benzyl alcohol (33.0 mL, 319 mmol, 1 equiv) in a 250 mL three-neck rb flask equipped with a dropping funnel, at 0 ºC, was added thionyl chloride (23.2 mL, 319 mmol, 1 equiv) dropwise over a period of 30 min. The reaction was warmed to rt, stirred for an additional 1 h, then diluted with pentane (200 mL). The solution of ((chloromethoxy)methyl)benzene was washed with brine (2 × 50 mL), dried over $MgSO_4$, and concentrated. The crude ((chloromethoxy)methyl)benzene (50 g, 320 mmol, 100%) was obtained as an off white liquid which was used without purification. 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.40 (s, 5H), 5.57 (s, 2H), 4.78 (s, 2H).



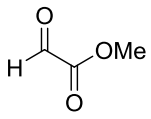
Preparation of 1-(bromomethyl)-4-methoxybenzene: To a stirring solution of phosphorus tribromide (7.50 mL, 80.0 mmol, 0.5 equiv) in Et_2O (150 mL) in a 500 mL three-neck rb flask equipped with a dropping funnel, at 0 ºC, was added 4-methoxybenzyl alcohol dropwise over a period of 45 min. The reaction was warmed to rt, stirred for an additional 1 h, then quenched by pouring over a stirring solution of saturated aqueous sodium bicarbonate and ice. The aqueous phase was extracted with Et_2O (3 × 30 mL), the combined organic phases were dried over $MgSO_4$, and concentrated. The crude 1-(bromomethyl)-4-methoxybenzene (32.9 g, 164 mmol, 100%) was obtained as a white liquid, solid at -20 ºC, which was used without purification. 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.34 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 4.52 (s, 2H), 3.82 (s, 2H).



Preparation of allyltributylstannane: To a stirring suspension of magnesium turnings (14.8 g, 609 mmol, 1.3 equiv) and iodine (160 mg) in THF (450 mL) at reflux in a 2 L three-neck rb flask equipped with a dropping funnel and an efficient reflux condenser was added a premixed solution of allyl bromide (50.1 mL, 579 mmol, 1.2 equiv) and tributyltin chloride (129 mL, 475 mmol, 1 equiv) in THF (75 mL) dropwise over a period of 2 h. The reaction mixture was maintained at reflux for an additional 16 h, then cooled to rt, filtered through a coarse glass frit and partitioned between 10% EtOAc/hexanes (1 L) and saturated aqueous $NaHCO_3$ solution (200 mL). The phases were separated and the organic layer was washed with brine (2 × 200 mL), dried over Na_2SO_4, and concentrated. The crude allytributylstannane was purified by

distillation using a 1-piece distillation apparatus with a 3.0 × 14.0 cm vigruex column. Pure allytributylstannane (151.2 g, 455 mmol, 95%) as a clear liquid was stored in two 100 mL Aldrich sure-seal bottles: bp 90 ºC at 0.2 mm Hg, 300 MHz ^{1}H NMR ($CDCl_3$) δ 6.03- 5.86 (m, 1H), 4.79 (d, J = 16.9 Hz, 1H), 4.65 (d, J = 9.5 Hz, 1H), 1.79 (d, J= 8.8 Hz, 2H), 1.57- 1.43 (m, 6H), 1.39- 1.24 (m, 6H), 0.91 (t, J = 7.2 Hz, 9 H), 0.90 (q, J = 7.2 Hz, 6H).

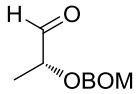
Preparation of magnesium bromide diethyletherate: To a stirring suspension of magnesium turnings (15.5 g, 638 mmol, 1.1 equiv) in Et_2O (600 mL) at reflux in a 1 L three-neck rb flask equipped with a dropping funnel and an efficient reflux condenser was added 1,2-dibromoethane (50.0 mL, 580 mmol, 1 equiv) dropwise over a period of 3 h. The reaction was heated at reflux for an additional 2 h, cooled to rt, and filtered through a coarse glass frit under N_2. The filtrate was sealed with a septum and placed in a -20 ºC freezer overnight. The Et_2O was removed via cannula, the grey crystals were triturated with Et_2O (4 × 100 mL), and the now off-white crystals were kept under vacuum (0.2 mm Hg) overnight. Magnesium bromide diethyletherate (127 g, 493 mmol, 85%) was stored in amber bottles under N_2 and in a desiccator.



Preparation of methyl glyoxylate: To a stirring solution of dimethyl maleate (5.00 mL, 39.9 mmol, 1 equiv) in CH_2Cl_2 (50 mL) in a 250 mL rb flask, at -78 ºC, was passed O_3 until a blue color developed. Excess O_3 was purged with O_2, dimethyl sulfide (3.20 mL, 43.3 mmol, 1.1 equiv) was added and the solution was allowed to warm

to rt overnight. The solution was concentrated, and the crude methyl glyoxylate was distilled under aspirator pressure using a 1-piece distillation apparatus with a 5 cm column. Pure methyl glyoxylate was weighed, then immediately diluted with THF to make a cloudy 3M solution, which was used directly and not stored.

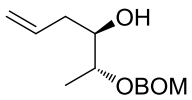
Experimental procedures for Merle 23



Preparation of (*R*)-2-((benzyloxy)methoxy)propanal (1.32):[79] To a stirring solution of (*R*)-(+)- isobutyl lactate (14.6 mL, 85.6 mmol, 1 equiv) and DIEA (40.0 mL, 229.6 mmol, 2.7 equiv) in a 500 mL rb flask, at 0 ºC, was added ((chloromethoxy)methyl)benzene (27.0 mL, 194.8 mmol, 2.3 equiv). The reaction mixture was allowed to warm to rt as it stirred overnight, then quenched with an aqueous 1.0 M HCl solution (15 mL), and partitioned between Et_2O (1000 mL) and water (100 mL). The phases were separated and the aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 7.5 × 22.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 125 mL fractions. The product containing fractions (12-25) were concentrated to give protected (*R*)-(+)-isobutyl lactate as an impure yellow oil that was taken on without further purification.

The aforementioned lactate ester in a 1 L rb flask was taken up in CH_2Cl_2 (500 mL), and cooled to -78 °C. A solution of DIBAL 1.0 M in hexanes (103 mL, 103 mmol, 1.2 equiv) was added down the side of the flask using a syringe pump (20 mL/h). The reaction mixture was stirred at -78 °C for an additional 1 h, then quenched with MeOH

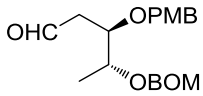
(50 mL) added by a syringe pump (25 mL/hr). The cloudy solution was warmed to rt, poured into a stirring aqueous sodium potassium tartrate solution (500 mL), and kept at rt overnight. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 10.0 × 18.0 cm silica gel column, eluting with 8% EtOAc/hexanes, collecting 125 mL fractions. The product containing fractions (24-65) were concentrated to give pure (*R*)-2-((benzyloxy)methoxy)propanal (**1.32**) (15.07 g, 90 s%, 2 steps) as clear oil. R_f = 0.50 (30% EtOAc/hexanes); 300 MHz ^{1}H NMR ($CDCl_3$) δ 9.66 (s, 1H), 7.44–7.25 (m, 5H), 4.88 (s, 2H), 4.69 (ABq, *J* = 11.8, Δν = 14.5 Hz, 2H), 4.13 (dq, *J* = 6.7, 1.2 Hz, 1H), 1.34 (d, *J* = 7.0 Hz, 3H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 202.5, 137.4, 128.6, 127.9, 127.8, 94.2, 78.2, 70.0, 15.3.



Preparation of (2*R*,3*R*)-2-((benzyloxy)methoxy)hex-5-en-3-ol (1.33):[79]

To a stirring solution of aldehyde **1.32** (7.28 g, 38.3 mmol, 1 equiv) in CH_2Cl_2 (300 mL) in a 500 mL rb flask, at rt, was added $MgBr_2 \bullet Et_2O$ (19.3 g, 74.8 mmol, 2 equiv). The cloudy solution was stirred for 5 min, then cooled to -15 °C. After 15 min at -15 °C a solution of allyl tributyltin (19.5 g, 58.7 mmol, 1.5 equiv) in CH_2Cl_2 (10 mL) was added slowly via cannula over 30 min. The transfer was made complete with two CH_2Cl_2 (3 mL) washes, and the flask allowed to warm slowly to rt as it stirred overnight. The reaction was quenched by carefully pouring into a stirring three-part mixture of CH_2Cl_2 (200 mL), a saturated aqueous $NaHCO_3$ solution (200 mL), and a saturated aqueous KF solution (200 mL). The biphasic mixture was stirred vigorously for 2 h, the phases were

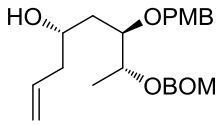
separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, and concentrated to a viscous oil. The oil was taken up in Et_2O (200 mL) and stirred with an insoluble solid mixture of KF/Celite (1:1, 15 g) for 2 h. KF/Celite was removed by filtration, Et_2O was removed by rotavap, and the resulting crude product was purified by flash column chromatography using a 7.5 × 20.0 cm silica gel column, eluting with 5-10% EtOAc/Hexanes, collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (19-38) were concentrated to provide pure homoallyl alcohol **1.33** (8.07 g, 89%) as a colorless oil. R_f = 0.40 (30% EtOAc/Hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.42-7.27 (m, 5H), 5.92 (dddd, *J* = 17.1, 10.2, 7.0, 6.4 Hz, 1H), 5.18-5.10 (m, 2H), 4.86 (ABq, *J* = 7.0, Δν = 20.9 Hz, 2H), 4.66 (ABq, *J* = 11.84, Δν = 10.0 Hz, 2H), 3.68 (dq, *J* = 6.2, 6.2 Hz, 1H), 3.56 (ddd, *J* = 7.7, 5.7, 4.1, 1H), 2.66 (s, 1H), 2.43-2.34 (m, 1H), 2.31-2.17 (m, 1H), 1.24 (d, *J* = 6.7, 3H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 137.8, 134.9, 128.7, 128.1, 128.0, 117.7, 94.1, 77.3, 74.4, 70.0, 37.9, 16.9.



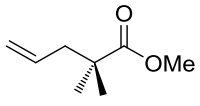
Preparation of (3*R*,4*R*)-4-((benzyloxy)methoxy)-3-((4-methoxybenzyl)oxy)pentanal (1.34):[79] To a 1 L rb flask was added 35% by wt. KH in mineral oil (3.93 g, 98.3 mmol, 1.5 equiv). The majority of the excess mineral oil was removed by pipet, and the KH was triturated with hexanes (3 × 100 mL) under a strict N_2 atmosphere. THF (400 mL) was added, and the flask was cooled to 0 °C. Homoallylic alcohol **1.33** (15.5 g, 65.5 mmol, 1 equiv) was added via cannula, and the solution was warmed to rt, and allowed to stir for 20 min prior to the addition of PMBBr (14.1 mL,

98.3 mmol, 1.5 equiv) dropwise via cannula. The reaction mixture was stirred at rt for 1 h, quenched with NH_4OH (100 mL), and stirred overnight. The solution was transferred to a separatory funnel and extracted with Et_2O (3 × 200 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 10.0 × 15.0 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 125 mL fractions. The product containing fractions (9-20) were concentrated to give the crude PMB protected alcohol as a yellow oil. R_f = 0.51 (30% EtOAc/hexanes).

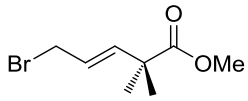
The yellow oil was taken up in a 4:1 solution of CH_2Cl_2/MeOH (400 mL), $NaHCO_3$ (31.45 g, 374.4 mmol, 6.7 equiv.) was added, and the resulting solution was cooled to -78 °C. While stirring, O_3 was bubbled through the solution until a faint blue color was observed. Excess O_3 was purged by bubbling O_2 through the solution for 20 min, DMS (45 mL, 608 mmol, 11 equiv) was added, and this solution was allowed to stir overnight at rt. The solution was filtered through a medium glass frit, concentrated, and purified by flash column chromatography using a 7.5 × 30.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (19-34) were concentrated to give pure aldehyde **1.34** (19.43 g, 83%, 2 steps) as a yellow oil. R_f = 0.38 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.75 (t, *J* = 2.0 Hz, 1H), 7.41-7.29 (m, 5H), 7.23 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 4.79 (ABq, *J* = 7.7, Δν = 21.6 Hz, 2H), 4.59 (s, 2H), 4.53 (ABq, *J* = 11.2 Hz, Δν = 11.2 Hz, 2H), 4.05-3.96 (m, 2H), 3.79 (s, 3H), 2.70-2.58 (m, 2H), 1.20 (d, J = 6.6, 3H); ^{13}C NMR ($CDCl_3$) δ 201.6, 159.6, 138.0, 130.3, 129.8, 128.7, 128.1, 128.0, 114.1, 93.8, 75.7, 73.2, 72.4, 70.0, 55.5, 45.5, 15.2.



Preparation of (4*S*,6*R*,7*R*)-7-((benzyloxy)methoxy)-6-((4-methoxybenzyl)oxy)oct-1-en-4-ol (1.73):[79] To a stirring solution of aldehyde **1.34** (3.56 g, 9.93 mmol, 1 equiv) in CH_2Cl_2 (80 mL), at rt, was added $MgBr{\bullet}Et_2O$ (5.13 g, 19.9 mmol, 2 equiv). The cloudy solution was stirred at rt for 5 min, then cooled to -15 °C. After 25 min a solution of allyltributyltin (4.98 g, 14.9 mmol, 1.5 equiv) in THF (10 mL) was added over 15 min via cannula, and this solution was maintained at -15 °C for 8 h. The completed reaction mixture was poured into stirring saturated aqueous $NaHCO_3$ solution (50 mL) and saturated aqueous KF solution(50 mL). This solution was stirred for 3 h, diluted with CH_2Cl_2 (100 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 5.0 × 15.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 18 x 150 mm test tube fractions. The product containing fractions (15-54) were concentrated to give pure homoallyl alcohol **1.73** (3.44 g, 87%) as an inseparable 7:1 mixture of diastereomers, and as a clear oil. R_f = 0.25 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.41-7.28 (m, 5H), 7.26 (d, *J* = 9.3 Hz, 2H), 6.86 (d, *J* = 9.3 Hz, 2H), 5.87-5.76 (m, 1H), 5.11 (d, *J* = 13.6 Hz, 2H), 4.84 (q, *J* = 14.0, 6.8 Hz, 2H), 4.64-4.60 (m, 2H), 4.61 (d, *J* = 11.0 Hz, 1H) 4.52 (d, *J* = 11.0 Hz, 1H), 4.00 (quintet, *J* = 5.9, 1H), 3.87-3.81 (m, 1H), 3.79 (s, 3H), 3.77-3.70 (m, 1H) 2.34 (s, 1H), 2.27-2.17 (m, 2H), 1.68-1.62 (m, 2H), 1.20 (d, *J* = 5.9 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.5, 138.1, 135.2, 130.8, 129.9, 128.7, 128.1, 127.9, 118.0, 114.1, 94.0, 78.8, 78.5, 74.1, 69.8, 68.0, 55.5, 42.7, 36.6, 15.7.

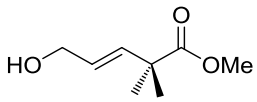


Preparation of Methyl 2,2-dimethylpent-4-enoate (1.79):[33] To a stirring solution of diisopropylamine (169 mL, 1.25 mol, 1.1 equiv) in THF (800 mL) in a 2 L rb flask, at -78 °C, was added a 2.5 M solution of *n*-BuLi (500 mL, 1.250 mol, 1.1 equiv.) in hexanes dropwise by cannula. The reaction was allowed to warm to rt and stir for 30 min before being returned to -78 °C. Methyl isobutyrate (130 mL, 1.13 mol, 1 equiv) in THF (100 mL) was added slowly via cannula, and the mixture was stirred for 1.5 h at -78 °C. The solution was brought to 0 °C, stirred for 30 min, and a solution of allylbromide (113 ml, 1.31 mol, 1.2 equiv) in THF (100 mL) was added via cannula. This solution was allowed to reach rt as it stirred overnight. The crude reaction mixture was filtered through a medium glass frit, concentrated, and distilled under atmospheric pressure to give pure olefin **1.79** (119 g, 70%) as clear oil. BP = 133 °C, R_f = 0.75 (10 % EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.75-5.66 (m, 1H), 5.04 (dd, *J* = 1.3,1.3 Hz, 1H), 5.03-5.00 (m, 1H), 3.64 (s, 3H), 2.26 (dt, *J* = 7.4, 1.1 Hz, 2H), 1.16 (s, 6H).



Preparation of (*E*)-methyl 5-bromo-2,2-dimethylpent-3-enoate (1.80):[33] To a stirring solution of olefin **1.79** (119.4 g, 839.6 mmol, 1 equiv), and N-bromosuccinimide (190 g, 1.07 mol, 1.3 equiv) in CCl_4 (700 mL) in a 1 L rb flask equipped with a reflux condenser was added benzoyl peroxide (5.0 g, 21 mmol, 0.25 equiv). The flask was submerged in a preheated 105 °C oil bath, and heated at reflux for 2 h. Another portion of benzoyl peroxide (1 g) was added, and the reaction mixture was heated at reflux for an additional 1 h. The yellow solution was cooled to rt, filtered

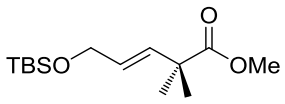
through a coarse glass frit, concentrated, and purified by flash column chromatography using a 9.5 × 17.0 cm silica gel column, eluting with 5% EtOAc/hexanes, and collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (7-30) were concentrated to give a crude allyl bromide **1.80** (177.3 g, 96%) as yellow oil. R_f = 0.48 (5% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.97 (d, *J* = 15.1 Hz, 1H), 5.74 (dt, *J* = 15.1 Hz, 1H), 3.95 (dd, *J* = 7.4, 1.1 Hz, 2H), 3.68 (s, 3H), 1.31 (s, 6H).



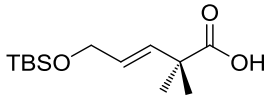
Preparation of (*E*)-methyl 5-hydroxy-2,2-dimethylpent-3-enoate (1.105): To a stirring solution of allyl bromide **1.80** (177.3 g, 801.8 mmol, 1 equiv) in MeOH (1.0 L) in a 2 L rb flask, at rt, was added KOAc (400.0 g, 4.075 mol, 5 equiv). The flask was equipped with a reflux condenser and heated at 50 °C for 2 days. The reaction was concentrated to approximately 100 mL, filtered through a medium glass frit washing with EtOAc (500 mL). The filtrate was transferred to a separatory funnel, washed with brine (3 × 200 mL), dried over Na_2SO_4, and concentrated to give the crude allylacetate as a brown oil, R_f = 0.22 (25 % EtOAc/hexanes).

This brown oil was taken up in MeOH (1.0 L), K_2CO_3 (115.0 g, 0.832 mol, 1 equiv) was added, and the white solution was stirred for 20 h at rt. The mixture was concentrated to approximately 100 mL, and partitioned between EtOAc (500 mL) and water (500 mL). The organic layer was washed with water (2 × 200 mL), dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 9.5 × 18.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (11-35) were concentrated to give pure

allyl alcohol **1.105** (66.04 g, 52 %) as a clear oil. Rf = 0.10 (25 % EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.89 (dt, J = 15.7, 1.3 Hz, 1H), 5.69 (dt, J = 15.7, 5.5 Hz, 1H), 4.15 (dd, J = 5.7, 1.3 Hz, 2H), 3.67 (s, 3H), 1.74 (s, 1H), 1.31 (s, 6H).

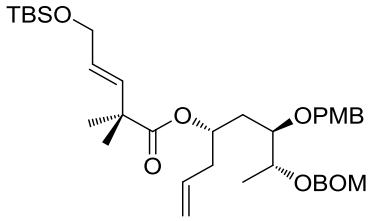


Preparation of (*E*)-methyl 5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoate (1.81):[33] To a stirring solution of allyl alcohol **1.105** (29.37 g, 185.6 mmol, 1 equiv) in CH_2Cl_2 (500 mL) in a 2 L rb flask, at rt, was added triethylamine (50.0 mL, 360 mmol, 2 equiv), followed by TBSCl (50.80 g, 337.0 mmol, 1.9 equiv). After 20 h at rt the mixture was diluted with Et_2O (1 L), and quenched with saturated aqueous NH_4Cl solution (250 mL). The phases were separated, and the organic layer was washed with brine (2 × 200 mL), dried over Na_2SO_4, and concentrated. Purification was accomplished by flash column chromatography using a 9.5 × 18.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (3-22) were concentrated to give pure TBS ether **1.81** (56.65 g, 99%) as a yellow oil. R_f = 0.80 (25% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.85 (dt, *J* = 15.6, 1.6 Hz, 1H), 5.60 (dt, *J* = 15.8, 5.4 Hz, 1H) 4.19 (dd, *J* = 5.4, 1.9, 2H), 3.67 (s, 3H), 1.31 (s, 6H), 0.91 (s, 9H), 0.07 (s, 6H).



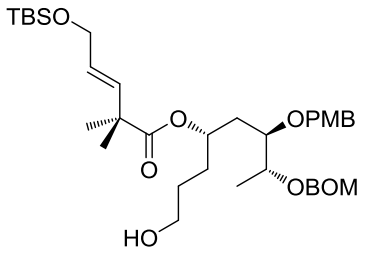
Preparation of (*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoic acid (1.76):[33] To a stirring solution of methyl ester **1.81** (35.14 g, 132.7 mmol, 1 equiv) in a 5:1 mixture of EtOH/H_2O (300 mL) in 1 L rb flask, at 0 °C,

was added NaOH (21.0 g, 525 mmol, 4 equiv) in a single portion. This solution was stirred at 0 °C for 16 h, quenched at 0° C with glacial acetic acid (20 mL), and partitioned between EtOAc (500 mL) and brine (500 mL). The organic layer was washed with brine (2 × 200 mL), dried over Na_2SO_4, and concentrated. Purification was accomplished by flash column chromatography using a 9.5 × 6.0 cm silica gel column, eluting with 5-20% EtOAc/hexanes, collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (8-22) were concentrated to give acid **1.76** (31.89 g, 93% yield) as a yellow oil. R_f = 0.20 (25% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.87 (dt, *J* = 15.6, 1.8 Hz, 1H), 5.65 (dt, *J* = 15.6, 5.0 Hz, 1H), 4.20 (dd, *J* = 5.0, 1.8 Hz, 2H) 1.33 (s, 6H), 0.91 (s, 9H), 0.07 (s, 6H).



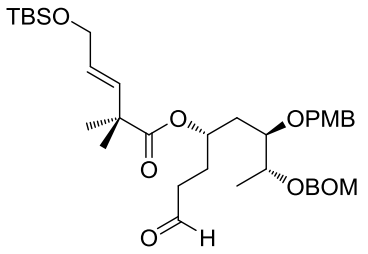
Preparation of (*E*)-(4*R*,6*R*,7*R*)-7-((benzyloxy)methoxy)-6-((4-methoxybenzyl)oxy)oct-1-en-4-yl 5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoate (1.82):[33] To a stirring solution of acid **1.76** (8.10 g, 31.3 mmol, 2 equiv) in CH_2Cl_2 (20 mL) in a 100 mL rb flask, at rt, was added DMAP (3.23 g, 26.5 mmol, 1.5 equiv), DMAP•HCl (2.80 g, 17.6 mmol, 1 equiv), and EDC•HCl (6.76 g, 35.3 mmol, 2 equiv). After 5 min, a solution of alcohol **1.77** (7.06 g, 17.6 mmol, 1 equiv) in CH_2Cl_2 (3 mL) was added via cannula. CH_2Cl_2 (2 mL) was used to rinse the alcohol flask and was added to the reaction mixture, which was allowed to stir overnight at rt. The reaction mixture was partitioned between 50% EtOAc/hexanes (50 mL) and brine (50 mL). The organic layer was washed with brine (2 × 25 mL), dried over Na_2SO_4, and

concentrated. Purification was accomplished by flash column chromatography using a 5.0 × 10.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 18 × 150 mm test tube fractions. The product containing fractions (7-27) were concentrated to give ester **1.82** (10.34 g, 92%) as a yellow oil. R_f = 0.58 (35% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.29 (m, 5H), 7.27 (d, *J* = 8.9 Hz, 2H), 6.86 (d, *J* = 8.9 Hz, 2H), 5.86 (dt, *J* = 15.6, 1.9 Hz, 1H), 5.79-5.69 (m, 1H), 5.60 (dt, *J* = 15.6, 5.4 Hz, 1H), 5.24-5.16 (m, 1H), 5.08-5.02 (m, 2H), 4.80 (Aβq, *J* = 7.2 Hz, Δν = 17.8 Hz, 2H), 4.62 (Aβq, *J* = 11.9 Hz, Δν = 20.1 Hz, 2H), 4.52 (d, *J* = 10.7 Hz, 1H), 4.35 (d, *J* = 10.7 Hz, 1H), 4.16 (dd, *J* = 5.0, 1.6 Hz, 2H), 3.95 (dq, *J* = 6.2, 6.2 Hz, 1H), 3.79 (s, 3H), 3.42 (ddd, *J* = 10.3, 5.0, 2.1 Hz, 1H), 2.41-2.26 (m, 2H), 1.84-1.77 (m, 1H), 1.70-1.63 (m, 1H) 1.29 (d, *J* =2.7 Hz, 6H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 11.7 Hz, 9H), 0.09 (d, *J* = 24.4, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 176.0, 159.6, 138.2, 134.9, 133.8, 130.8, 130.1, 128.8, 128.2, 128.1, 128.0, 118.3, 114.2, 93.6, 73.5, 73.3, 70.6, 69.7, 64.1, 55.6, 44.3, 39.8, 34.7, 26.3, 26.0, 25.5, 25.2, 18.7, 18.3, 15.5, -3.3.



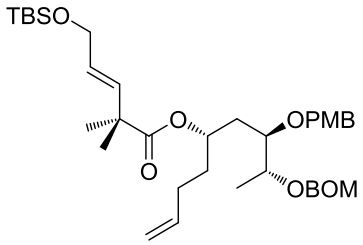
Preparation of (*E*)-(4*R*,6*R*,7*R*)-7-((benzyloxy)methoxy)-1-hydroxy-6-((4-methoxybenzyl)oxy)octan-4-yl 5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoate (1.83):[33] To a stirring solution of olefin **1.82** (10.34g, 16.13 mmol, 1 equiv) in THF (80 mL), in a 250 mL rb flask, at rt, was added a 0.5 M solution of 9-Borabicyclo[3.3.1]nonane (96.8 mL, 48.4 mmol, 3.0 equiv) in THF. The reaction

mixture was stirred at rt for 10 min, sonicated in a water bath at 60 Hz for 45 min, then cooled to 0 °C. A 2 M aqueous solution of NaOH (40 mL) was added slowly followed by the careful addition of a 30% aqueous solution of H_2O_2 (20 mL). After 1 h the mixture was diluted with EtOAc (100 mL) and water (100 mL), the phases were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 5.0 × 10.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 18 × 150 mm test tube fractions. The product containing fractions (12-35) were concentrated to give alcohol **1.83** (8.18 g, 77% yield) as a clear oil. R_f = 0.21 (30% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.29 (m, 5H), 7.27 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 8.0 Hz, 2H), 5.86 (dt, *J* = 15.8, 2.0 Hz, 1H), 5.61 (dt, *J* = 15.6, 5.0 Hz, 1H), 5.21-5.14 (m, 1H), 4.80 (Aβq, *J* = 7.4 Hz, Δν = 18.1 Hz, 2H), 4.62 (Aβq, *J* = 11.9 Hz, Δν = 19.8 Hz, 2H), 4.53 (d, *J* = 10.4 Hz, 1H), 4.36 (d, *J* = 10.4 Hz, 1H), 4.17 (dd, *J* = 5.0, 1.6 Hz, 2H), 3.96 (dq, *J* = 6.1, 6.1 Hz, 1H), 3.79 (s, 3H), 3.61 (t, *J* = 6.5 Hz, 2H), 3.42 (ddd, *J* = 10.4, 4.9, 2.1 Hz, 1H), 1.81 (ddd, *J* = 14.5, 9.8, 2.4, 1H), 1.68-1.50 (m, 6H), 1.30 (d, *J* = 2.2, 6H), 1.18 (d, *J* = 7.2 Hz, 3H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 176.4, 159.7, 138.2, 135.0, 130.8, 130.2, 128.8, 128.3, 128.1, 128.0, 114.2, 93.7, 77.8, 73.5, 73.3, 71.4, 69.8, 64.1, 62.8, 55.6, 44.4, 35.1, 31.5, 28.5, 26.3, 25.5, 25.2, 18.8, 15.5, -4.8.



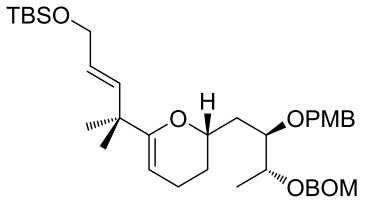
Preparation of (*E*)-(4*R*,6*R*,7*R*)-7-((benzyloxy)methoxy)-6-((4-methoxybenzyl)oxy)-1-oxooctan-4-yl 5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoate (1.84):[33] To a stirring solution of alcohol **1.83** (8.18 g, 12.4 mmol, 1 equiv) in CH_2Cl_2 (124 mL, 0.1 M) in 250 mL rb flask, at 0 °C, was added *i*-Pr_2EtN (15.2 mL, 86.9 mmol, 7 equiv) and DMSO (8.80 mL, 124 mmol, 10 equiv). The reaction mixture was stirred for 10 min, then SO_3•Pyr complex (7.90 g, 49.6 mmol, 4 equiv) was added in three equal aliquots 5 min apart. The cloudy solution was stirred at 0 °C for 1 h, then quenched by the addition of saturated aqueous $NaHCO_3$ solution (50 mL). The biphasic solution was stirred for 10 min, partitioned between water (100 mL) and CH_2Cl_2 (100 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 5.0 × 12.5 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 18 × 150 mm test tube fractions. The product containing fractions (16-45) were concentrated to give aldehyde **1.84** (6.91 g, 85%) as a clear oil. R_f = 0.40 (30% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1H), 7.39-7.33 (m, 5H), 7.27 (d, *J* = 9.1 Hz, 2H), 6.86 (d, *J* = 9.1 Hz, 2H), 5.86 (dt, *J* = 15.9, 1.5 Hz, 1H), 5.62 (dt, *J* = 15.6, 4.9 Hz, 1H), 5.20-5.14 (m, 1H), 4.80 (Aβq, *J* = 7.1 Hz, Δν = 19.7 Hz, 2H), 4.62 (Aβq, *J* = 11.9 Hz, Δν = 19.8 Hz, 2H), 4.52 (d, *J* = 10.6 Hz, 1H), 4.34 (d, 10.6 Hz, 1H), 4.17 (dd, *J* = 4.9, 1.7 Hz, 2H), 3.97 (dq, *J* = 4.9, 6.3 Hz, 1H), 3.80 (s, 3H), 3.42 (ddd, *J* = 10.4, 4.7, 2.1 Hz, 1H), 2.44 (t, *J* = 7.7, 2H), 2.00-1.92 (m, 1H), 1.89-1.79 (m,

2H), 1.66-1.50 (m, 1H), 1.31 (s, *J* = 2.9 Hz, 6H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.6, 176.3, 159.6, 138.2, 134.6, 130.7, 130.2, 128.8, 128.6, 128.1, 128.0, 114.2, 93.7, 77.6, 73.2, 73.1, 70.9, 69.8, 64.0, 55.6, 44.3, 40.1, 35.1, 27.6, 26.3, 25.5, 25.3, 18.7, 15.4, -4.8.



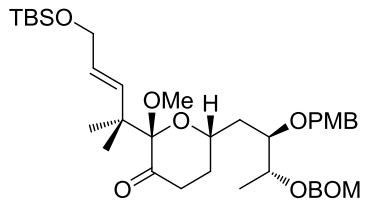
Preparation of (*E*)-(5*R*,7*R*,8*R*)-8-((benzyloxy)methoxy)-7-((4-methoxybenzyl)oxy)non-1-en-5-yl 5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoate (1.75):[33] To a stirring solution of methyltriphenylphosphonium bromide (7.52 g, 21.0 mmol, 2 equiv) in THF (105 mL) in a 250 mL rb flask, at -5 °C, was added *n*-BuLi (5.47 mL, 13.7 mmol, 1.3 equiv). The yellow solution was warmed to rt and stirred for 30 min, then returned to -5 °C, and aldehyde **1.84** (6.91 g, 10.5 mmol, 1 equiv) was added slowly via cannula along with a THF rinse (5 mL). After 5 min the reaction mixture was quenched by the addition of an aqueous pH 7 buffer (100 mL), and the aqueous layer was extracted with 25% EtOAc/hexanes. The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 5.0 × 10.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 18 × 150 mm test tube fractions. The product containing fractions (9-30) were concentrated to give olefin **1.75** (5.72 g, 83%) as a clear oil. R_f = 0.70 (35% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.40-7.30 (m, 5H), 7.28 (d, *J* = 9.0 Hz, 2H), 6.68 (d, *J* = 9.0 Hz, 2H), 5.88 (dt, *J* = 15.7, 1.5 Hz, 1H), 5.83-5.73 (m, 1H), 5.62 (dt, *J* = 15.7, 5.2 Hz, 1H), 5.22-5.15 (m, 1H), 5.03-4.93 (m, 2H), 4.80 (Aβq, *J* = 7.1 Hz, Δν = 17.1 Hz, 2H), 4.62 (Aβq, *J*

= 11.8 Hz, Δν = 21.4 Hz, 2H), 4.52 (d, *J* = 10.5 Hz, 1H), 4.38 (d, *J* = 10.5 Hz, 1H), 4.17 (dd, *J* = 5.0, 1.5 Hz, 2H), 3.95 (dq, *J* = 4.8, 6.4 Hz, 1H), 3.80 (s, 3H), 3.42 (ddd, *J* = 10.3, 4.9, 2.2 Hz, 1H), 2.05 (q, *J* = 8.2 Hz, 2H), 1.84-1.77 (m, 1H), 1.73-1.59 (m ,3H), 1.31 (d, *J* = 2.8 Hz, 6H), 1.18 (d, *J* = 6.3 Hz, 3H), 0.91 (s, 9H), 0.064 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 176.2, 159.6, 138.2, 138.2, 134.8, 130.8, 130.2, 128.8, 128.3, 128.1, 128.0, 115.2, 114.1, 93.6, 77.8, 73.5, 73.3, 71.3, 69.7, 64.1, 55.6, 44.3, 35.2, 34.6, 29.7, 26.3, 25.5, 25.3, 18.7, 15.5, -4.8.



Preparation of (((*E*)-4-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-4-methylpent-2-en-1-yl)oxy)(*tert*-butyl)dimethylsilane (1.85):[33] To a stirring solution of $TiCl_4$ (13.6 mL, 124 mmol, 15 equiv) in CH_2Cl_2 (500 mL) in a 2 L three neck rb flask equipped with a reflux condenser and a 250 mL dropping funnel, at 0 °C, was added THF (70 mL). The solution turned yellow and was stirred for 10 min prior to the addition of TMEDA (119.0 mL, 798.7 mmol, 96 equiv) dropwise via cannula, which turned the solution brown. This mixture was warmed to rt, stirred for 30 min, and then $PbCl_2$ (4.63 g, 16.7 mmol, 2 equiv) and activated zinc dust (19.58 g, 299.4 mmol, 36 equiv) were added in a single portion causing the reaction mixture to transition to a deep blue color. A premixed solution of olefin **1.75** (5.45 g, 8.32 mmol, 1 equiv) and 1,1-dibromoethane (12.1 mL, 133 mmol, 16 equiv) in CH_2Cl_2 (100 mL) was added dropwise using the 250 mL addition funnel. This suspension was heated at reflux for 2 h, then cooled to 0 °C,

and quenched by the slow addition of saturated aqueous K_2CO_3 solution (100 mL). The resulting black mud was filtered through a 3 cm pad of alumina, washing with CH_2Cl_2 (500 mL). The filtrate was concentrated to a yellow solid, which was suspended in 50% EtOAc/hexanes (250 mL) and filtered through a coarse glass frit. The filtrate was concentrated to a yellow oil and purified by flash column chromatography using a 9.5 × 12.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 125 mL Erlenmeyer flask fractions. The product containing fractions (4-18) were concentrated to give glycal **1.85** (4.26 g, 82%) as a clear oil. R_f = 0.39 (5% EtOAc/Toluene). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 5H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.75 (dt, *J* = 15.6, 1.5 Hz, 1H), 5.52 (dt, *J* = 15.8, 5.5 Hz, 1H), 4.83 (Aβq, *J* = 7.0 Hz, Δν = 6.3 Hz, 2H), 4.64 (Aβq, *J* = 11.7 Hz, Δν = 11.5 Hz, 2H), 4.62 (d, *J* = 10.9 Hz, 1H), 4.55 (dd, *J* = 4.3, 2.6 Hz, 1H), 4.52 (d, *J* = 10.9, 1H), 4.14 (dd, *J* = 5.6, 1.6 Hz, 2H), 4.04-3.08 (m, 1H), 3.95 (dq, *J* = 5.3, 6.2 Hz, 1H), 3.83 (ddd, *J* = 10.4, 4.9, 2.0 Hz, 1H), 3.80 (s, 3H), 2.12-2.03 (m, 1H), 2.01-1.94 (m, 1H), 1.83-1.71 (m, 2H), 1.65-1.57 (m, 1H), 1.35-1.2 (m, 1H), 1.20 (d, *J* = 6.4 Hz, 3H), 1.17 (d, *J* = 4.3, 6H), 0.90 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.6, 159.4, 139.0, 138.4, 131.3, 129.8, 128.8, 128.2, 128.0, 126.1, 114.2, 93.8, 93.4, 78.2, 74.3, 73.8, 71.9, 69.8, 64.7, 55.6, 40.7, 36.6, 28.5, 26.4, 26.3, 26.1, 20.7, 18.8, 16.0, -4.3.

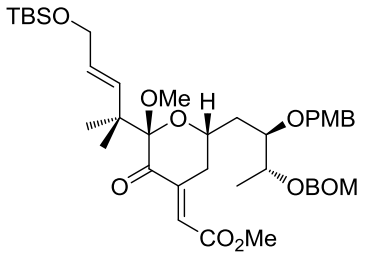


Preparation of (2*S*,6*S*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((*tert*-

butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxydihydro-2*H*-pyran-3(4*H*)-one (1.86):[33] To a stirring solution of glycal **1.85** (1.11 g, 1.78 mmol, 1 equiv) in CH_2Cl_2 (20 mL) in a 100 mL rb flask, at 0 °C, was added MeOH (8 mL) and $NaHCO_3$ (0.370 g, 4.40 mmol, 2.5 equiv). After 10 min, MMPP (2.11 g, 3.42 mmol, 1.9 equiv) was added and stirring continued for an additional 1 h at 0 °C. The reaction mixture was quenched by the addition of a saturated aqueous $NaHCO_3$ solution (10 mL), partitioned between EtOAc (20 mL) and water (20 mL), and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated to give the crude alcohol as a clear oil which was taken on without further purification. R_f = 0.21 (20% EtOAc/ hexanes)

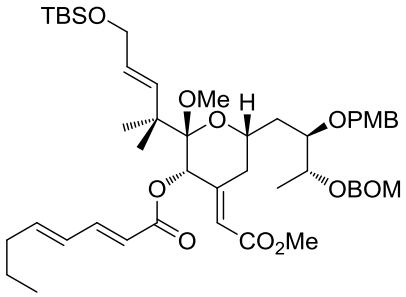
The crude alcohol was taken up in CH_2Cl_2 (30 mL), and powdered 4 Å molecular sieves (600 mg) were added. TPAP (0.062 g, 0.177 mmol, 0.1 equiv) and NMO (0.624 g, 5.32 mmol, 3 equiv) were added in a single portion, and the black suspension was stirred at rt for 1 h. The reaction mixture was diluted with EtOAc (20 mL) and filtered through a 3 cm plug of florisil washing with copious amounts of EtOAc. The filtrate was concentrated and purified by flash column chromatography using a 2 × 8.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (7-25) were concentrated to give ketone **1.86** (0.789 g, 66%) as a clear oil. R_f = 0.30 (20% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.34 (m, 5H), 7.21 (d, *J* = 9.2 Hz, 2H), 6.84 (d, *J* = 9.2 Hz, 2H), 5.97 (dt, *J* = 16.1, 1.7, 1H), 5.50 (dt, *J* = 15.8, 5.1 Hz, 1H), 4.85 (Aβq, *J* = 6.8 Hz, Δν = 7.7 Hz, 2H), 4.66 (s, 2H), 4.62 (d, *J* = 10.9 Hz, 1H), 4.44 (d, *J* = 10.9 Hz, 1H), 4.16-4.07 (m, 2H), 4.13 (dd, *J* = 14.2, 7.3 Hz, 2H), 3.88 (ddd, *J* = 10.2, 4.8, 1.9 Hz, 1H), 3.79 (s, 3H), 3.23 (s, 3H), 2.43

(dd, *J* = 8.6, 5.7 Hz, 2H), 1.98-1.85 (m, 3H), 1.69-1.59 (m, 1H), 1.41 (s, 3H) 1.21 (d, *J* = 6.7 Hz, 3H), 1.13 (s, 3H) 1.09 (s, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.8, 159.6, 138.2, 136.5, 130.9, 129.6, 128.8, 128.2, 128.1, 128.0, 114.2, 104.4, 93.7, 77.3, 72.8, 72.4, 70.3, 69.8, 64.4, 55.6, 52.5, 44.3, 37.9, 36.6, 30.4, 26.3, 23.1, 18.7, 15.1, -4.8.



Preparation of (*E*)-methyl 2-((2*S*,6*S*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxy-3-oxodihydro-2*H*-pyran-4(3*H*)-ylidene)acetate (1.87):[33] To a stirring solution of ketone **1.86** (514 mg, 0.766 mmol, 1 equiv) in MeOH (11.0 mL) in a 25 mL rb flask, at rt, was added K_2CO_3 (529 mg, 3.83 mmol, 5 equiv) and a 3 M solution of freshly distilled methyl glyoxylate (1.3 mL, 380 mmol, 5 equiv) in THF. After 1 h, during which time the solution developed a vibrant yellow color, it was quenched by the addition of saturated aqueous NH_4Cl solution (10 mL), and the aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 2.5 × 13.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 12 × 75 mm test tube fractions. The product containing fractions (9-37) were concentrated to give ester **1.87** (431 g, 76%) as a yellow oil. R_f = 0.30 (20% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.35 (m, 5H), 7.18 (d, *J*

= 8.4 Hz, 2H), 6.82 (d, *J* = 84 Hz, 2H), 6.53 (dd, *J* = 3.2, 1.7 Hz, 1H), 5.81 (dt, *J* = 16.1, 14 Hz, 1H), 5.40 (dt, *J* = 15.8, 5.0 Hz, 1H), 4.85 (Aβq, *J* = 7.3 Hz, Δν = 9.7 Hz, 2H), 4.66 (s, 2H), 4.61 (d, *J* = 10.9 Hz, 1H), 4.41 (d, *J* = 10.9 Hz, 1H), 4.15-4.10 (m, 2H), 4.07-4.04 (m, 2H), 3.91-3.86 (m, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.31 (dt, *J* = 18.3, 1.8 Hz, 1H), 3.21 (s, 3H), 2.85 (ddd, *J* = 18.7, 12.6, 3.3 Hz, 1H), 1.96 (ddd, *J* = 14.8, 9.2, 2.4 Hz, 1H), 1.78-1.72 (m, 1H), 1.21 (d, *J* = 6.7 Hz, 3H), 1.10 (s, 3H), 1.04 (s, 3H), 0.90 (s, 9H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.8, 166.2, 159.4, 148.2, 138.1, 134.8, 130.6, 129.3, 128.8, 128.7, 128.0, 127.9, 122.7, 114.0, 104.8, 93.6, 76.9, 72.4, 71.8, 69.7, 69.5, 64.1, 55.4, 52.2, 52.0, 44.7, 36.2, 36.1, 26.1, 22.5, 22.0, 18.5, 14.7, -5.0

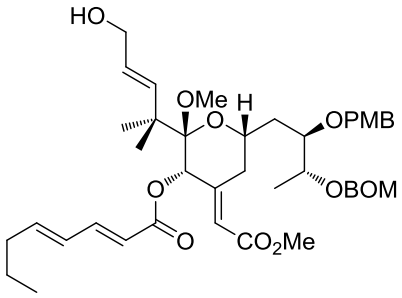


Preparation of (2*E*,4*E*)-(2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octa-2,4-dienoate (1.88):[80] To a stirring solution of ketone **1.87** (740 mg, 0.999 mmol, 1 equiv) in MeOH (100 mL) in a 250 mL rb flask, at rt, was added $CeCl_3 \bullet 7H_2O$ (7.45 g, 20.0 mmol, 20 equiv), and the suspension was stirred until the $CeCl_3 \bullet 7H_2O$ was completely solvated before being cooled to -42 °C. $NaBH_4$ (378 mg, 10.0 mmol, 10 equiv) was added in a single portion. The reaction mixture was stirred at -42 °C for 1 h, then diluted with 40% EtOAc/hexanes (50 mL), and

quenched by the addition of saturated aqueous NH_4Cl solution (30 mL). The aqueous layer was extracted with 40% EtOAc/hexanes (3 × 50 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over Na_2SO_4, and concentrated to give the intermediate alcohol as a clear oil. R_f = 0.10 (20% EtOAc/hexanes)

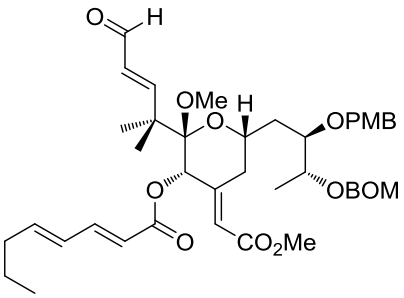
The crude alcohol was taken up in CH_2Cl_2 (25 mL), and DMAP (611 mg, 5.00 mmol, 5 equiv), pyridine (2.42 mL, 30.0 mmol, 30 equiv) and (2*E*,4*E*)-octa-2,4-dienoic anhydride (5.25 g, 20.0 mmol, 20 equiv) were added. This solution was stirred at rt for 20 h before first quenching with MeOH (5 mL), stirring for 30 min, then quenching with saturated aqueous $NaHCO_3$ solution (20 mL). The phases were separated, and the aqueous layer was extracted with 40% EtOAc/hexanes (3 × 25). The combined organic layers were washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated. Purification was accomplished by flash column chromatography using a 2.0 × 7.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (5-22) were concentrated to give ester **1.88** (654 mg, 74%) as a clear oil. R_f = 0.38 (20% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.33 (m, 5 H), 7.21 (d, *J* = 9.0 Hz, 2H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.18-6.10 (m, 2H), 5.96 (d, *J* = 15.8 Hz, 1H), 5.91 (s, 1H), 5.75 (d, *J* = 15.8 Hz, 1H), 5.48 (s, 1H), 5.37 (dt, *J* = 15.9, 5.7 Hz, 1H), 4.86 (Aβq, *J* = 6.6 Hz, Δν = 6.5 Hz, 2H), 4.68 (Aβq, *J* = 11.8 Hz, Δν = 9.1 Hz, 2H), 4.62 (d, *J* = 11.1 Hz, 1H), 4.44 (d, *J* = 11.1 Hz, 1H), 4.15-4.01 (m, 4H), 3.90 (ddd, *J* = 10.1, 4.4, 2.0 Hz, 1H), 3.79 (s, 3H), 3.68 (s, 3H), 3.51 (dd, *J* = 15.4, 2.5 Hz, 1H), 3.26 (s, 3H), 2.36-2.24 (m, 1H), 2.19-2.12 (m, 2H) 1.92 (ddd, *J* = 14.2, 9.6, 2.1 Hz, 1H), 1.74 (ddd, *J* = 14.2, 9.6, 2.1 Hz, 1H), 1.59 (s, 1H), 1.47 (q, *J* = 7.2 Hz, 2H), 1.23 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 2.5 Hz, 6H), 0.91 (t, *J* = 7.2 Hz, 3H), 0.90

(s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 166.9, 165.8, 159.5, 152.9, 146.6, 145.8, 138.3, 138.2, 130.9, 129.6, 128.8, 128.7, 128.1, 128.0, 125.2, 118.9, 117.7, 114.1, 103.0, 93.7, 77.2, 72.8, 72.3, 72.1, 69.8, 68.6, 64.9, 55.6, 51.9, 51.4, 46.1, 36.7, 35.4, 32.9, 26.4, 24.6, 23.9, 22.2, 18.8, 15.2, 14.0, -4.7.



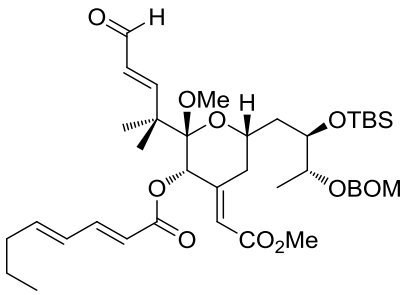
Preparation of (2*E*,4*E*)-(2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-hydroxy-2-methylpent-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octa-2,4-dienoate (1.106):[80] To a stirring solution of TBS ether **1.88** (114 mg, 0.132 mmol, 1 equiv) in a 5:4:1 solution of THF/MeOH/pyridine (2.6 mL) in a plastic bottle, at 0 °C, was added HF•Pyr (20%, 1 mL). The reaction mixture was stirred at 0 °C for 10 min, then at rt for 2 h, then quenched by pipetting it into a stirring solution of 50% EtOAc/hexanes (10 mL) and saturated aqueous $NaHCO_3$ solution (10 mL). The phases were separated and the aqueous layer was extracted with 50% EtOAc/hexanes (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 1.5 × 8.5 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (10-38) were concentrated to give pure alcohol **1.106** (92.0 mg, 93%) as a yellow oil. R_f = 0.50 (50% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.35 (m, 5H), 7.21 (d, *J* = 8.6 Hz, 2H) 6.84 (d, *J* = 8.6 Hz, 2H), 6.20-6.16 (m, 2H),

5.99 (d, *J* = 15.9 Hz, 1H), 5.91 (s, 1H), 5.75 (d, *J* = 15.6 Hz, 1H), 5.51 (s, 1H), 5.46 (dt, *J* = 15.8, 6.1 Hz, 1H), 4.87 (Aβq, *J* = 6.6 Hz, Δν = 5.9 Hz, 2H), 4.68 (s, 2H), 4.62 (d, *J* = 11.0 Hz, 1H), 4.43 (d, *J* = 11.0 Hz, 1H), 4.15 (dq, *J* = 6.4, 4.6 Hz, 1H), 4.10-4.03 (m, 1H), 4.02-3.99 (m, 2H), 3.92 (ddd, *J* = 10.2, 4.6, 2.2 Hz, 1H), 3.79 (s, 3H), 3.68 (s, 3H), 3.50 (dd, *J* = 15.6, 2.3 Hz, 1H), 3.25 (s, 3H), 2.35 (ddd, *J* = 13.4, 11.8, 1.8 Hz, 1H), 2.19-2.14 (m, 2H), 1.94 (ddd, *J* = 14.5, 9.8, 2.0 Hz, 1H), 1.75 (ddd, *J* = 14.0, 10.2, 2.5 Hz, 1H), 1.62 (s,1H), 1.47 (q, *J* = 7.5 Hz, 3H), 1.23 (d, *J* = 6.0 Hz, 3H), 1.12 (d, *J* = 15.6 Hz, 6H), 0.93 (t, *J* = 7.5 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 167.0, 165.9, 159.6, 152.8, 147.1, 146.4, 140.1, 138.2, 130.8, 129.6, 128.8, 128.6, 128.2, 128.1, 125.2, 118.7, 117.7, 114.2, 103.0, 93.7, 77.0, 72.6, 72.1, 72.1, 69.8, 68.7, 64.6, 55.6, 51.8, 51.6, 46.4, 36.6, 35.5, 33.0, 24.4, 24.2, 22.2, 15.0, 14.1.



Preparation of (2*E*,4*E*)-(2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)tetrahydro-2*H*-pyran-3-yl octa-2,4-dienoate (1.88):[80] To a stirring solution of alcohol **1.106** (177 mg, 0.24 mmol, 1 equiv) in CH_2Cl_2 (8 mL) in 25 mL rb flask, at rt, was added powdered 4 Å molecular sieves (300 mg), followed by TPAP (11.0 mg, 0.02 mmol, 0.1 equiv) and NMO (83.0 mg, 0.705 mmol, 3 equiv) in a single portion. After 1 h the reaction mixture was diluted with EtOAc (10 mL) and filtered through a 3 cm pad of florisil washing with copious

amounts of EtOAc. The filtrate was concentrated to a dark oil, and purified by flash column chromatography using a 1.5 × 9.5 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (10-40) were concentrated to give aldehyde **1.88** (157.0 mg, 89%) as a clear oil. R_f = 0.66 (50% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.44 (d, *J* = 7.8 Hz, 1H), 7.37-7.34 (m, 4H), 7.33-7.29 (m, 1H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.16-6.11 (m, 2H), 5.90 (dd, *J* = 16.5, 7.4 Hz, 1H), 5.89 (s, 1H), 5.60 (s, 1H), 5.57 (d, *J* = 15.2 Hz, 2H), 4.86 (Aβq, *J* = 7.0 Hz, Δν = 10.9 Hz, 2H), 4.67 (s, 2H), 4.63 (d, *J* = 10.9 Hz, 1H), 4.41 (d, *J* = 10.9 Hz, 1H), 4.17 (dd, *J* = 6.2, 4.5 Hz, 1H), 4.16-4.12 (m, 1H), 3.90 (ddd, *J* = 10.0, 4.4, 1.9 Hz, 1H), 3.79 (s, 3H), 3.81-3.78 (m, 1H), 3.69 (s, 3H), 3.50 (dd, *J* = 16.4, 2.0 Hz, 1H), 3.28 (s, 3H), 2.44 (dd, *J* = 14.9, 11.3 Hz, 1H), 2.16 (q, *J* = 7.0 Hz, 2H), 1.97 (ddd, *J* = 14.4, 9.7, 1.9 Hz, 1H), 1.78 (ddd, *J* = 14.2, 10.2, 2.6 Hz, 1H), 1.47 (q, *J* = 7.7 Hz, 2H), 1.23 (d, *J* = 6.5 Hz, 3H), 1.16 (s, 3H), 1.13 (s, 3H), 0.93 (t, *J* = 7.4, 3H); 125 MHz ^{13}C NMR (CDCl3); 194.7, 166.8, 166.4, 165.2, 159.4, 152.1, 147.4, 146.6, 137.9, 130.5, 129.4, 128.6, 128.3, 127.9, 127.9, 127.2, 117.8, 117.4, 114.0, 102.8, 93.6, 76.7, 72.2, 71.8, 70.7, 69.7, 69.0, 55.5, 51.4, 47.6, 46.4, 36.3, 35.3, 33.4, 23.7, 22.3, 22.0, 14.7, 13.9

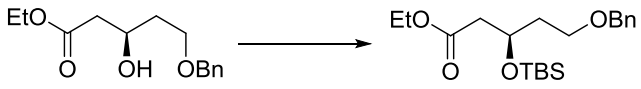


Preparation of (2*E*,4*E*)-(2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-

methoxy-2-oxoethylidene)-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)tetrahydro-2*H*-pyran-3-yl octa-2,4-dienoate (1.74):[80] To a stirring solution of PMB ether **1.88** (177 mg, 0.236 mmol, 1 equiv) in CH_2Cl_2 (3.4 mL) and *t*-BuOH (2.4 mL) in a 25 mL rb flask, at rt, was added a 1 M aqueous pH 7 buffer (2.4 mL). The mixture was cooled to 0 °C and DDQ (134 mg, 0.590 mmol, 2.5 equiv) was added in a single portion. This reddish mixture was stirred for 1 h at 0 °C, then another portion of DDQ (134 mg, 0.590 mmol, 2.5 equiv) was added, and stirring continued for an additional 1 h. The reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, and concentrated to give the crude alcohol as a red oil which was taken on to the next step without further purification.

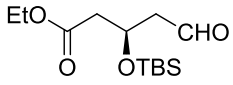
The crude alcohol was taken up in CH_2Cl_2 (4.7 mL), cooled to 0 °C, and 2,6-lutidine (165 μL, 1.42 mmol, 6 equiv) was added followed by TBSOTf (136 μL, 0.590 mmol, 2.5 equiv). The reaction mixture was stirred for 30 min at 0 °C, then quenched first with MeOH (200 μL) and then with a saturated aqueous $NaHCO_3$ solution (1 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 10.5 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (5-45) were concentrated to give TBS ether **1.74** (150.2 mg, 86%) as a yellow oil. R_f = 0.58 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.43 (d, *J* = 7.9 Hz, 1H), 7.34-7.30 (m, 5H), 7.29-7.26 (m, 1H), 6.18-6.06 (m, 2H), 5.93-5.86 (m, 2H), 5.66 (s, 1H), 5.56 (d, *J* = 15.1 Hz, 1H), 4.79 (Aβq, *J* = 7.1 Hz, Δν = 6.3 Hz, 2H), 4.62 (s, 2H),

4.17-4.07 (m, 2H), 3.89-3.76 (m, 1H), 3.67 (s, 3H), 3.50 (dd, *J* = 16.6, 2.7 Hz, 1H), 3.39 (s, 3H), 2.42 (dd, *J* = 16.1, 11.9 Hz, 1H), 2.14 (q, *J* = 7.4 Hz, 2H), 2.04 (ddd, *J* = 14.4, 8.8, 2.7, 1H), 1.64 (ddd, *J* = 14.1, 8.6, 3.0 Hz, 1H), 1.45 (sextet, *J* = 7.4 Hz, 2H), 1.19 (s, 3H), 1.15 (s, 3H), 1.13 (s, 3H), 0.91 (t, *J* = 7.4 Hz, 3H), 0.87 (s, 9H), 0.06 (d, *J* = 10.5 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 195.0, 167.1, 166.6, 165.5, 152.4, 147.7, 146.8, 138.1, 128.8, 128.5, 128.1, 128.1, 127.5, 117.9, 117.6, 103.1, 93.5, 75.3, 70.5, 70.5, 69.7, 69.2, 51.9, 51.5, 47.7, 38.9, 35.5, 33.7, 26.2, 23.7, 22.5, 22.2, 18.4, 14.0, 14.0, 1.4, -3.6, -4.3.



Preparation of (*R*)-ethyl 5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)pentanoate (1.107):[80] To a stirring solution of alcohol **1.63**, provided by Dr. Li (213 mg, 0.844 mmol, 1 equiv), in DMF (1 mL) in a 10 mL rb flask, at rt, was added imidazole (69 mg, 1.0 mmol, 1.2 equiv) and TBSCl (140 mg, 0.928 mmol, 1.1 equiv). After 3 h the solution was diluted with 40% EtOAc/hexanes (5 mL), and quenched with water (5 mL). The phases were separated and the organic layer was washed with water (3 × 3 mL), dried over Na_2SO_4, and concentrated. Purification was accomplished by flash column chromatography using a 1.5 × 12.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (9-22) were concentrated to give pure silyl ether **1.107** (254 mg, 85%) as a clear oil. R_f = 0.60 (20% EtOAc/hexanes); (500 MHz ^{1}H NMR ($CDCl_3$) δ 7.29-7.26 (m, 5H), 4.43 (Aβq, *J* = 12.0 Hz, Δν = 13.4 Hz, 2H), 4.26 (quintet, *J* = 6.1 Hz, 1H), 4.11-4.00 (m, 2H), 3.49 (t, *J* = 6.5 Hz, 2H), 2.42 (d, *J* = 6.6 Hz, 2H), 1.79 (q, *J* = 6.7 Hz, 2H), 1.19 (t, *J* = 6.7 Hz, 3H), 0.80 (s, 9H), 0.00 (d, *J* =

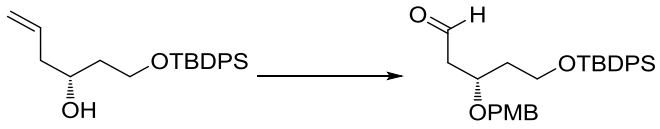
6.7 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.9, 138.8, 128.6, 127.9, 127.8, 73.2, 67.3, 66.8, 60.6, 43.3, 37.7, 26.1, 18.3, 14.5, -4.4, -4.5.



Preparation of (*R*)-ethyl 3-((*tert*-butyldimethylsilyl)oxy)-5-oxopentanoate (1.57):[54] To a stirring solution of benzyl ether **1.107** (1.37 g, 3.74 mmol, 1 equiv) in EtOAc (20 mL) in a 100 mL rb flask, at 0 °C, was added palladium on carbon (50 mg, 10% wt.). The flask was equipped with a hydrogen balloon and allowed to stir at rt for 4 days. The black solution was filtered through a plug of celite washing with copious amounts of EtOAc, and concentrated to give a crude alcohol R_f = 0.10 (20% EtOAc/hexanes) as a dark oil that was used without purification.

The crude alcohol was taken up in CH_2Cl_2 (40 mL), cooled to 0 °C, and *i*-Pr_2EtN (4.6 mL, 26 mmol, 7 equiv) and DMSO (2.65 mL, 37.4 mmol, 10 equiv) were added. The mixture was stirred for 10 min, then SO_3•Pyr complex (2.38 g, 14.9 mmol, 4 equiv) was added in three equal aliquots 5 min apart. The cloudy solution was stirred at 0 °C for 1.5 h, then quenched by the addition of saturated aqueous $NaHCO_3$ solution (20 mL). The biphasic solution was stirred for 10 min, partitioned between water (50 mL) and CH_2Cl_2 (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 2.0 × 9.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 13 × 150 mm test tube fractions. The product containing fractions (14-30) were concentrated to give aldehyde **1.57** (839 mg, 82%) as a clear oil. R_f = 0.35 (20% EtOAc/hexanes). 500 MHz ^1H NMR ($CDCl_3$) δ 9.81 (Aβq, *J* = 1.5 Hz, Δν = 2.0 Hz, 1H),

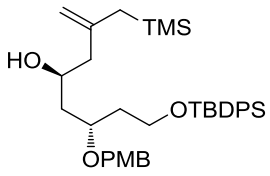
4.64 (dq, *J* = 5.6, 6.4 Hz, 1H), 4.14-4.09 (m, 2H), 2.67 (ddd, *J* = 16.8, 5.7, 2.2 Hz, 1H), 2.62 (ddd, *J* = 16.8, 5.7, 2.2 Hz, 1H), 2.56 (dd, *J* = 15.1, 6.3 Hz, 1H), 2.53 (dd, *J* = 15.1, 6.3 Hz, 1H), 1.27 (t, *J* = 7.7 Hz, 3H), 0.86 (s, 9H), 0.09 (d, *J* = 1.5 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.5, 171.2, 65.4, 61.0, 51.3, 43.0, 26.0, 18.2, 14.5, -4.5.



Preparation of (*S*)-5-((*tert*-butyldiphenylsilyl)oxy)-3-((4-methoxybenzyl)oxy)pentanal (1.108):[80] To a stirring solution of homoallyl alcohol, provided by Dr. Li (1.99 g, 5.62 mmol, 1 equiv), in THF (50 mL) in a 250 mL rb flask, at rt, was added PMBBr (4.0 mL, 27 mmol, 5 equiv) and Et_3N (11.9 mL, 85.3 mmol, 15 equiv). The mixture was cooled to -78 °C, KHMDS (0.5 M, 33.4 mL, 16.7 mmol, 3 equiv) was added dropwise by syringe, and this solution was stirred for 1 h at -78 °C then for 1.5 h at -15 °C. The reaction mixture was then quenched by the addition of saturated aqueous NH_4OH solution (20 mL) and allowed to stir overnight at rt. The phases were separated and the aqueous layer was extracted with Et_2O (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, concentrated and filtered through a 3 cm plug of silica to give the crude PMB ether (2.09 g) as a yellow oil.

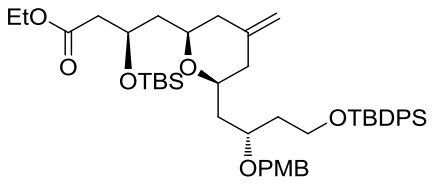
The crude oil was taken up in a 4:1 solution of CH_2Cl_2/MeOH (75 mL), $NaHCO_3$ (1.85 g, 22.0 mmol, 5 equiv) was added, and the mixture was cooled to -78 °C. A stream of O_3 was bubbled through the solution until a light blue color developed (~3 min.). Excess O_3 was purged by bubbling O_2 through the solution for 15 min. PPh_3 (2.3 g, 8.8 mmol, 2 equiv) was added and this mixture was stirred at rt for 1 h. The cloudy solution was filtered through a medium glass frit, concentrated, and purified by flash column

chromatography using a 4.0 × 9.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 13 × 150 mm test tube fractions. The product containing fractions (12-49) were concentrated to give aldehyde **1.108** (2.02 g, 75%, 2 steps) as a clear oil. R_f = 0.25 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.76 (t, *J* = 2.2 Hz, 1H), 7.71-7.65 (m, 4H), 7.48-7.38 (m, 6H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 4.47 (s, 2H), 4.20 (quintet, *J* = 6.0 Hz, 1H), 3.88-3.81 (m, 1H), 3.81 (s, 3H), 3.79-3.73 (m, 1H), 2.69 (ddd, *J* = 16.3, 7.1, 2.8 Hz, 1H), 2.63 (ddd, *J* = 16.3, 7.1, 2.8 Hz, 1H), 1.99-1.75 (m, 2H), 1.08 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 102.0, 159.6, 135.9, 133.9, 133.9, 130.1, 129.9, 129.8, 129.7, 128.1, 114.2, 71.6, 71.4, 60.4, 55.6, 48.9, 37.5, 27.2, 19.5.



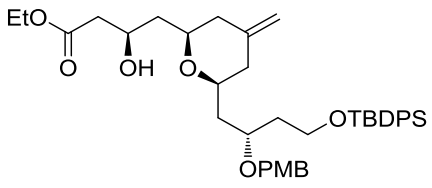
Preparation of (4*S*,6*S*)-8-((*tert*-butyldiphenylsilyl)oxy)-6-((4-methoxybenzyl)oxy)-2-((trimethylsilyl)methyl)oct-1-en-4-ol (1.28):[54] To a stirring solution of aldehyde **1.108** (1.92 g, 4.04 mmol, 1 equiv) in CH_2Cl_2 (32 mL) in a 100 mL rb flask, at rt, was added $MgBr•Et_2O$ (2.08 g, 8.06 mmol, 2 equiv) in a single portion. The cloudy mixture was cooled to -78 °C and stirred for 30 min. Trimethyl(2-((tributylstannyl)methyl)allyl)silane (2.87 g, 6.88 mmol, 1.7 equiv) was added in CH_2Cl_2 (5 mL) along with a CH_2Cl_2 (2 mL) rinse. This solution was maintained at -78 °C for 5 h, then quenched by the addition of saturated aqueous $NaHCO_3$ solution (15 mL) and brine (20 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4,

concentrated, and purified by flash column chromatography using a 4.0 × 11.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 13 × 150 mm test tube fractions. The product containing fractions (21-54) were concentrated to give β-hydroxyallylsilane **1.28** (1.60 g, 65%) as a clear oil. R_f = 0.51 (25% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.65 (m, 4H), 7.46-7.36 (m, 6H), 7.20 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.65 (d, *J* = 8.6 Hz, 2H), 4.47 (s, 2H), 4.03-3.94 (m, 2H), 3.80 (s, 3H), 3.84-3.71 (m, 2H), 2.65 (d, *J* = 2.5 Hz, 1H), 2.12 (dd, *J* = 13.7, 8.1 Hz, 1H), 2.06 (dd, *J* = 13.7, 81 Hz, 1H), 1.92 (dq, *J* = 12.8, 6.1 Hz, 1H), 1.78 (dq, *J* = 13.8, 6.1 Hz, 1H), 1.71-1.58 (m, 2H), 1.53 (Aβq, *J* = 13.4 Hz, Δν = 14.7 Hz, 2H), 1.06 (s, 9H), 0.03 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.5, 144.9, 136.0, 134.2, 134.1, 131.0, 130.0, 129.9, 128.0, 128.0, 114.2, 110.3, 74.2, 71.7, 66.4, 60.9, 55.6, 47.1, 41.0, 37.5, 27.2, 27.1, 19.5, -1.0.



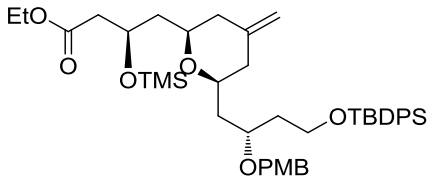
Preparation of (3*R*)-ethyl 3-((*tert*-butyldimethylsilyl)oxy)-4-(6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)butanoate (1.64):[80] To a stirring solution of β-hydroxyallylsilane **1.28** (2.48 g, 4.10 mmol, 1.2 equiv) and aldehyde **1.57** (839 mg, 3.06 mmol, 1 equiv) in Et_2O (30 mL) in a 100 mL rb flask, at -78 °C, was added a 1.0 M TMSOTf solution (3.7 mL, 3.7 mmol, 1.2 equiv) in THF dropwise by syringe. After1 h at -78 °C the reaction mixture was quenched first by *i*-Pr_2EtN (1 mL) then with saturated aqueous $NaHCO_3$ solution (10 mL). The mixture was warmed to rt, the phases were separated, and the aqueous layer was extracted with

Et_2O (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 2.0 × 10.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 12 × 75 mm test tube fractions. The product containing fractions (15-52) were concentrated to give pyran **1.64** (2.31 g, 95%) as a clear oil. R_f = 0.62 (20% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.67 (m, 4H), 7.46-7.36 (m, 6H), 7.17 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 4.70 (d, *J* = 10.8 Hz, 2H), 4.41 (Aβq, *J* = 10.8 Hz, Δν = 25.4 Hz, 2H), 4.38-4.33 (m, 1H), 4.12-3.99 (m, 2H), 3.91-3.85 (m, 1H), 3.82-3.75 (m, 2H), 3.79 (s, 3H), 3.54-3.47 (m, 1H), 3.46-3.40 (m, 1H), 2.54-2.45 (m, 2H), 2.23 (d, *J* = 13.1 Hz, 1H), 2.16 (d, *J* = 13.1 Hz, 1H), 1.99-1.89 (m, 2H), 1.89-1.77 (m, 3H), 1.69-1.61 (m, 3H), 1.18 (t, *J* = 13.1 Hz, 3H), 1.06 (s, 9H), 0.86 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.9, 159.3, 144.9, 135.9, 134.2, 134.1, 131.1, 129.8, 129.6, 127.9, 127.9, 114.0, 108.8, 75.2, 75.1, 73.1, 71.9, 67.0, 60.8, 60.5, 55.6, 44.1, 42.9, 42.6, 41.4, 41.3, 37.9, 27.2, 26.1, 19.5, 18.4, 14.4, -4.1, -4.5.



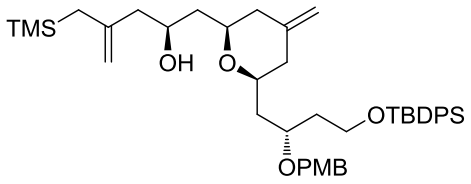
Preparation of (3*R*)-ethyl 4-(6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-3-hydroxybutanoate (1.66):[80] To a stirring solution of TBS ether **1.64** (685 mg, 0.867 mmol, 1 equiv.) in benzene (28 mL) and MeOH (12 mL) in a 100 mL rb flask, at rt, was added *p*-toluenesulfonic acid (331 mg, 1.73 mmol, 2 equiv). After stirring for 3 h at rt the reaction mixture was quenched by the addition of Et_3N (2 mL), and

concentrated. Purification was accomplished by flash column chromatography using a 2.0 × 13.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 12 × 75 mm test tube fractions. Fractions (5-18) were concentrated to give starting material **1.64** (308 mg, 45%) as a clear oil, and product containing fractions (20-35) were concentrated to give alcohol **1.66** (319 mg, 54%) as a clear oil that was carried on without complete characterization. R_f = 0.30 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.69-7.65 (m, 4H), 7.45-7.36 (m, 6H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 4.71 (d, *J* = 11.0 Hz, 2H), 4.39 (Aβq, *J* = 11.2 Hz, Δν = 41.2 Hz, 2H), 4.28-4.21 (m, 1H), 4.14 (ddd, *J* = 14.5, 7.2, 2.4 Hz, 2H), 3.80 (s, 3H), 3.79-3.72 (m, 4H), 3.56-3.48 (m, 2H), 2.52 (dd, *J* = 15.7, 7.5 Hz, 1H), 2.44 (dd, *J* = 15.7, 7.5 Hz, 1H), 2.22 (d, *J* = 13.4 Hz, 1H), 2.16 (d, *J* = 13.4 Hz, 1H), 1.99 (t, *J* = 12.4 Hz, 1H), 1.92 (t, *J* = 12.4 Hz, 1H), 1.85-1.62 (m, 6H), 1.24 (t, *J* = 6.9 Hz, 3H), 1.06 (s, 9H).



Preparation of (3*R*)-ethyl 4-(6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-3-((trimethylsilyl)oxy)butanoate (1.109):[80] To a stirring solution of alcohol **1.66** (391 mg, 0.579 mmol, 1 equiv) in CH_2Cl_2 (10 mL) in a 50 mL rb flask, at rt, was added TMSCl (0.11 mL, 0.87 mmol, 1.5 equiv) and Et_3N (0.24 mL, 1.7 mmol, 3 equiv). After 3.5 h the reaction mixture was quenched with water (50 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash

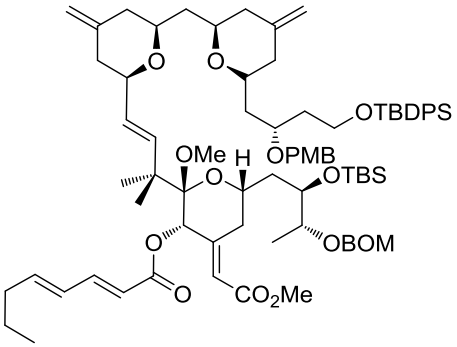
column chromatography using a 1.5 × 8.5 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (6-26) were concentrated to give pure silyl ether **1.109** (415 mg, 97%) as a yellow oil. R_f = 0.60 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.68 (m, 4H), 7.47-7.37 (m, 6H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 4.72 (dd, *J* = 8.2, 1.6 Hz, 2H), 4.42 (Aβq, *J* = 10.8 Hz, Δν = 28.2 Hz, 2H), 4.37 (dt, *J* = 6.5, 65 Hz, 1H), 4.15-4.02 (m, 2H), 3.90 (dt, *J* = 5.9, 6.0 Hz, 1H), 3.82-3.76 (m, 2H), 3.80 (s, 3H), 3.56-3.48 (m, 1H), 3.44-3.37 (m, 1H), 2.50 (d, *J* =1.3 Hz, 1H), 2.49 (s, 1H), 2.27 (d, *J* = 13.1 Hz, 1H), 2.18 (d, *J* = 13.1 Hz, 1H), 1.94 (q, *J* = 12.8 Hz, 2H), 1.89-1.79 (m, 3H), 1.68-1.61 (m, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), 1.08 (s, 9 H), 0.13 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 177.8, 159.3, 144.9, 135.9, 134.2, 131.4, 129.9, 129.6, 128.0, 114.1, 108.9, 75.2, 75.2, 72.9, 71.9, 66.9, 60.9, 60.6, 55.6, 44.4, 43.1, 42.6, 41.4, 41.2, 38.0, 27.2, 19.5, 14.5, 0.6.



Preparation of (*S*)-1-((2*R*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-4-((trimethylsilyl)methyl)pent-4-en-2-ol (1.56):[80] Powdered $CeCl_3$•$7H_2O$ (674 mg, 1.81 mmol, 10 equiv) in a 15 mL rb flask was dried at 170 °C under a vacuum of 0.3 mm of Hg for 16 h. The dry $CeCl_3$ has a slight grey appearance. The flask was cooled to rt, flushed with N_2, THF (1.5 mL) was added, and the thick suspension was stirred for 2 h during which time a tan color developed. While the $CeCl_3$ and THF stirred

a 1 M solution of the $TMSCH_2MgCl$ was prepared: Mg turnings (125 mg) along with a single crystal of I_2 were heated using a heat gun in a flame dried 25 mL 2 neck rb flask equipped with a reflux condenser. After purple vapors filled the flask THF (4.6 mL) was added in single portion, and was brought to reflux using the heat gun. $TMSCH_2Cl$ (0.4 mL) was added dropwise to the reddish brown THF solution along with continuous heating. The THF solution first turned clear and then developed a mild metallic silver color at which point heating was discontinued. The self-maintained reaction solution was stirred for 1.5 h, during which time a deep grey color developed and most of the Mg was consumed. The flask containing the $CeCl_3$ was cooled to -78 °C and the 1 M $TMSCH_2MgCl$ (1.81 mL, 1.81 mmol, 10 equiv) solution was added in a single portion. This solution was stirred at -78 °C for 1 h, a brown color developed, then ethyl ester **1.109** (135 mg, 0.181 mmol, 1 equiv) was added along with a THF (0.5 mL) rinse. This solution was stirred at -78 °C for 2 h then allowed to reach rt as it stirred overnight. The mixture was transferred to 25 mL rb flask, diluted with THF (10 mL), and cooled to -78 °C. To this vigorously stirred solution was added a 1 N aqueous HCl solution 5 drops at a time until only a single spot, blue in PMA, was observed by TLC. The acidic solution was immediately quenched with a saturated aqueous $NaHCO_3$ solution (5 mL) and allowed to warm to rt. This solution was partitioned between CH_2Cl_2 (10 mL) and water (5 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 1.5 × 11.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (8-27) were concentrated to give β-hydroxyallylsilane **1.56** (98 mg, 76%) as a

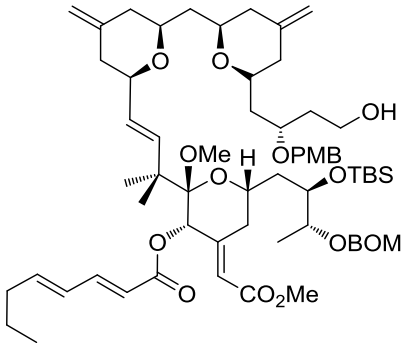
clear oil. R_f = 0.55 (30% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.69-7.65 (m, 4H), 7.45-7.36 (m, 6H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.70 (dd, *J* = 10.7, 1.6 Hz, 2H), 4.64 (d, *J* = 14.3 Hz, 2H), 4.39 (Aβq, *J* = 11.1 Hz, Δν = 30.8 Hz, 2H), 3.99-3.93 (m, 1H), 3.79 (s, 3H), 3.79-3.74 (m, 3H), 3.52-3.51 (m, 3H), 2.25-2.14 (m, 3H), 2.08-1.90 (m, 3H), 1.79 (q, *J* = 6.5 Hz, 2H), 1.69-1.57 (m, 4H), 1.55 (s, 2H), 1.05 (s, 9H), 0.02 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.4, 144.8, 144.3, 135.9, 134.2, 134.2, 131.3, 129.9, 129.8, 129.7, 128.0 114.1, 110.2, 109.2, 79.5, 75.7, 73.0, 71.7, 69.8, 60.7, 55.6, 46.8, 42.8, 42.1, 41.4, 41.3, 37.7, 27.3, 27.1, 19.5, -1.0.



Preparation of (2*E*,4*E*)-(2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octa-2,4-dienoate (1.91): To a stirring solution of aldehyde **1.74** (24.1 mg, 0.032 mmol, 1 equiv) and silane **1.56** (33.5 mg, 0.047 mmol, 1.5 equiv) in Et_2O (1.0 mL) in a 5 mL rb flask, at -78 °C, was added a 1.0 M TMSOTf solution (1.0 M, 47 μL, 0.047 mmol, 1.5 equiv) in THF dropwise by syringe. After 9 h the reaction mixture was quenched first with *i*-Pr_2NEt (0.2 mL), stirred for 10 min, then quenched with saturated

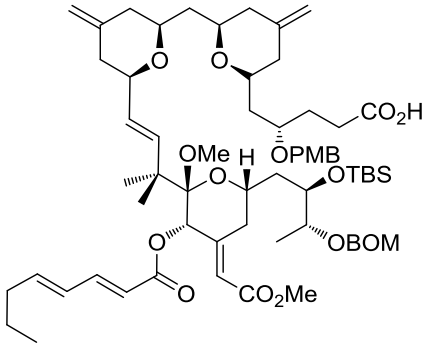
aqueous $NaHCO_3$ solution (1 mL). The mixture was warmed to rt, the phases were separated, and the aqueous layer was extracted with Et_2O (3 × 1 mL). The combined organic layers were concentrated, dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 8.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (11-23) were concentrated to give pyran **1.91** (33.2 mg, 75%) as a clear oil. R_f = 0.44 (20% EtOAc/hexanes); $[\alpha]_D^{20}$ = +7.3 (c =1.28, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.65 (m, 4H), 7.45 (m, 10H), 7.30 (m, 1H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.18 (s, 1H), 6.17-6.12 (m, 1H), 5.96 (d, *J* = 15.2 Hz, 1H), 5.94-5.89 (m, 1H), 5.79 (d, *J* = 15.2 Hz, 1H), 5.61 (s, 1H), 5.40 (dd, *J* = 15.9, 5.8 Hz, 1H), 4.81 (s, 2H), 4.71 (d, *J* = 10.8 Hz, 2H), 4.64 (s, 2H), 4.62 (s, 1H), 4.54 (s, 1H), 4.39 (Aβq, *J* = 10.4 Hz, Δν = 31.2 Hz, 2H), 4.13-4.04 (m, 2H), 3.93-3.78 (m, 3H), 3.79 (s, 3H), 3.67 (s, 3H), 3.58-3.41 (m, 5H), 3.31 (s, 3H), 2.40-2.31 (m, 1H), 2.24 (t, *J* = 11.7 Hz, 2H), 2.19-2.10 (m, 3H), 2.03-1.85 (m, 6H), 1.82-1.73 (m, 3H), 1.67-1.53 (m, 6H), 1.49-1.42 (m, 2H), 1.17 (d, *J* = 6.7 Hz, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.06 (s, 9H), 0.92 (t, *J* = 6.9 Hz, 3H), 0.88 (s, 9H), 0.08 (d, *J* = 7.8, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 166.8, 165.9, 159.4, 153.2, 146.9, 146.9, 145.6, 145.2, 138.5, 138.3, 136.0, 135.9, 134.2, 134.2, 131.3, 129.9, 129.8, 129.7, 128.7, 128.2, 128.0, 127.4, 118.8, 117.4, 114.1, 109.1, 108.8, 102.9, 93.4, 79.3, 75.2, 75.2, 75.1, 75.0, 72.9, 72.2, 71.8, 70.4, 69.6, 68.5, 60.7, 55.6, 51.9, 51.4, 46.2, 43.1, 42.7, 41.6, 41.3, 41.0, 40.6, 38.9, 38.1, 35.4, 33.4, 27.3, 27.2, 26.2, 24.3, 22.2, 19.5, 18.4, 14.2, 14.0, -3.7, -4.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 51.8, 51.3, 27.2, 26.1, 24.2, 14.0, 13.9, -3.8, -4.4; CH_2 δ 109.1, 108.8, 93.4, 72.2, 69.6, 60.7, 43.1, 42.6, 41.4, 41.2, 41.0, 40.6, 38.9, 35.4, 33.4, 22.2; CH δ 146.9, 145.9, 138.4, 135.9, 129.9, 129.7,

128.7, 128.1, 128.0, 127.4, 118.8, 117.4, 114.1, 79.3, 75.2, 75.1, 75.1, 75.0, 72.9, 71.7, 70.4, 68.5; C_0 δ 166.8, 165.9, 159.4, 153.2, 146.9, 145.6, 145.2, 138.3, 134.2, 134.2, 131.3, 129.8, 128.7, 102.9, 51.9, 46.2, 27.2, 19.5, 18.4, 14.2; IR (neat) 2933, 2857, 1720, 1643, 1613, 1514, 1407, 1428, 1382, 1360, 1248, 1108, 1042 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{81}H_{114}O_{14}NaSi_2$ (M+Na) 1389.7661, found 1389.7661.



Preparation of (2*E*,4*E*)-(2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octa-2,4-dienoate (1.92): To a stirring solution of TBDPS ether **1.91** (50.8 mg, 0.037 mmol, 1 equiv) in DMF (742 μL) in a 4 mL reaction vial, at rt, was added a premixed solution of 1 M TBAF in THF (37 μL, 0.037 mmol, 1 equiv) and 1 M AcOH in DMF (37 μL, 0.037 mmol, 1 equiv). The transfer was made complete by washing with DMF (2 × 50 μL), and the solution was stirred for 20 h, then diluted with 40% EtOAc/hexanes (1 mL), and quenched with water (1 mL). The aqueous layer was extracted with 40% EtOAc/hexanes (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 8.0 cm silica gel column, eluting

with 10% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (11-23) were concentrated to give alcohol **1.92** (38 mg, 89%) as a clear oil. R_f = 0.20 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +4.0 (c =1.00, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.34 (m, 4H,) 7.31-7.48 (m, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.24-6.15 (m, 2H), 5.98 (d, *J* = 16.2 Hz, 1H), 5.93 (s, 1H), 5.80 (d, *J* = 14.7 Hz, 1H), 5.61 (s, 1H), 5.40 (dd, *J* = 15.5, 5.9 Hz, 1H), 4.82 (s, 2H), 4.75-4.71 (m, 2H), 4.67-4.65 (m, 2H), 4.65-4.56 (m, 2H), 4.48 (Aβq, *J* = 10.7 Hz, Δν = 20.1 Hz, 2H), 4.15-4.05 (m, 2H), 3.92-3.84 (m, 2H), 3.80 (s, 3H), 3.76-3.67 (m, 3H), 3.68 (s, 3H), 3.57-3.41 (m, 5H), 3.32 (s, 3H), 2.46-2.32 (m, 1H), 2.29-2.13 (m, 5H), 2.04-1.86 (m, 6H), 1.83-1.68 (m, 2H), 1.68-1.56 (m, 6H), 1.47 (q, *J* = 7.5 Hz, 2H), 1.18 (d, *J* = 7.4 Hz, 3H), 1.12 (d, *J* = 2.9 Hz, 6H), 0.93 (t, *J* = 7.1 Hz, 3H), 0.88 (s, 9H), 0.08 (d, *J* = 7.0 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 166.8, 165.9, 159.7, 153.2, 146.9, 146.1, 144.8, 144.6, 138.9, 138.2, 130.7, 129.8, 128.7, 128.2, 128.0, 127.3, 118.8, 117.4, 114.3, 109.0, 102.8, 93.4, 79.6, 77.6, 77.4, 77.1, 75.6, 75.2, 75.2, 72.2, 71.7, 70.3, 69.7, 68.5, 60.6, 55.6, 52.0, 51.4, 46.2, 43.0, 41.9, 41.6, 41.1, 41.0, 40.6, 38.8, 37.0, 35.4, 33.4, 26.2, 24.4, 24.2, 22.2, 18.4, 14.1, -3.7, -4.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.6, 51.9, 51.3, 26.1, 24.3, 24.2, 14.1, 14.0, -3.7, -4.3; CH_2 δ 109.0, 93.4, 72.1, 69.6, 60.5, 43.0, 41.9, 41.6, 41.1, 41.0, 40.5, 38.8, 36.9, 35.4, 33.4, 22.2; CH δ 146.9, 146.1, 138.9, 129.8, 128.7, 128.1, 128.0, 127.3, 118.7, 117.4, 114.3, 79.6, 77.6, 75.6, 75.1, 71.7, 70.3, 68.5; C_0 δ 166.8, 165.9, 159.7, 153.2, 144.8, 144.6, 138.2, 130.7, 102.8, 77.1, 77.2, 46.2, 18.4; IR (neat) 3493, 3070, 2936, 1719, 1642, 1514, 1463, 1435, 1381, 1359, 1302, 1249, 1108, 1043 1042 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{65}H_{96}O_{14}NaSi$ (M+Na) 1151.6487, found 1151.6487.

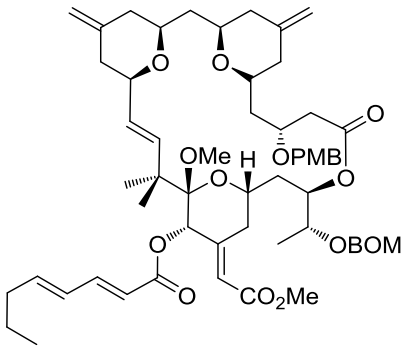


Preparation of (*R*)-4-((2*S*,6*R*)-6-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-3-((2*E*,4*E*)-octa-2,4-dienoyloxy)tetrahydro-2*H*-pyran-2-yl)-3-methylbut-1-en-1-yl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-3-((4-methoxybenzyl)oxy)butanoic acid (1.93): To a stirring of alcohol **1.92** (33.4 mg, 0.029 mmol, 1 equiv) in CH_2Cl_2 (300 μL) in a 4 mL reaction vial, at 0 °C, was added *i*-Pr_2NEt (36 μL, 0.210 mmol, 7 equiv), DMSO (20 μL, 0.290 mmol, 10 equiv), and SO_3•Pyr (18 mg, 0.12 mmol, 4 equiv) in a single portion. This solution was stirred at 0 °C for 75 min, then quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated, and aqueous layer was extracted with 40% EtOAc/hexanes (3 × 3 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting clear oil with run through a 1.5 × 2 cm plug of silica with EtOAc, and concentrated to give the crude aldehyde (33.1 mg), which was carried on without characterization.

To a stirring solution of the aforementioned aldehyde in 2-methyl-2-butene (400 μL) and *t*-BuOH (400 μL) in a 5 mL rb flask, at rt, was added a 1.25 M aqueous KH_2PO_4 solution (140 μL). This solution was cooled to -10 °C in an ethylene glycol/ CO_2 bath, and $NaClO_2$ (16 mg, 0.14 mmol, 5 equiv) was added in a single portion. The reaction

mixture was stirred vigorously for 1.5 h then quenched with a 0.05 M aqueous pH 4 buffer solution (1 mL). The phases were separated, and aqueous layer with extracted with Et_2O (3 x 3 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 1.5 × 12.0 cm silica gel column, eluting with 3% MeOH/CH_2Cl_2, collecting 10 × 75 mm test tube fractions. The product containing fractions (4-13) were concentrated to give acid **1.93** (30.7 mg, 93 %, 2 steps) as a clear oil. R_f = 0.32 (10% MeOH/ 40% EtOAc/ 50% hexanes); $[\alpha]_D^{20}$ = +12.9 (c =1.14, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.34 (m, 4H), 7.32-7.27 (m, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.21-6.16 (m, 2H), 6.00 (d, *J* = 15.9 Hz, 1H), 5.93 (s, 1H), 5.80 (d, *J* = 15.5 Hz, 1H), 5.62 (s, 1H), 5.41 (dd, *J* = 16.3, 6.4 Hz, 1H), 4.85-4.80 (m, 2H), 4.75-4.70 (m, 2H), 4.67 (d, *J* = 3.8 Hz, 2H), 4.65-4.58 (m, 2H), 4.57 (d, *J* = 10.5 Hz, 1H), 4.45 (d, *J* = 10.5 Hz, 1H), 4.16-4.04 (m, 3H), 3.87 (dt, *J* = 10.9, 6.3 Hz, 1H), 3.80 (s, 3H), 3.81-3.78 (m, 1H), 3.68 (s, 3H), 3.58-3.39 (m, 5H), 3.32 (s, 3H), 2.62 (t, *J* = 5.2 Hz, 2H), 2.29-2.20 (m, 2H), 2.19-2.12 (m, 2H), 2.05-1.87 (m, 4H), 1.83-1.66 (m, 4H), 1.64-1.55 (m, 2H), 1.50-1.42 (m, 2H), 1.28-1.25 (m, 3H), 1.18 (d, *J* = 6.5 Hz, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 0.93 (t, *J* = 71. Hz, 3H), 0.88 (s, 9H), 0.09-0.07 (m, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 166.9, 165.9, 159.7, 153.3, 147.0, 146.1, 144.7, 144.5, 139.2, 138.1, 130.4, 129.9, 129.8, 128.8, 128.8, 128.7, 128.2, 128.0, 127.2, 118.7, 117.4, 114.3, 114.2, 109.1, 102.8, 93.3, 79.7, 77.6, 77.4, 77.1, 75.2, 75.2, 75.1, 73.2, 72.5, 71.8, 70.3, 69.6, 68.5, 55.6, 52.0, 51.5, 46.2, 43.0, 42.0, 41.4, 41.1, 41.0, 40.6, 40.3, 38.8, 35.4, 33.4, 30.0, 26.3, 26.2, 24.3, 24.3, 22.2, 18.4, 14.1, 14.0, 1.4, -3.7, -4.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.6, 52.0, 51.5, 26.2, 24.3, 24.3, 14.1, 14.0, -3.9, -4.4; CH_2 δ 109.1, 93.2, 72.5, 69.6, 43.0, 42.2, 41.4, 41.1, 41.0, 40.6, 40.3, 38.8, 35.4, 33.5,

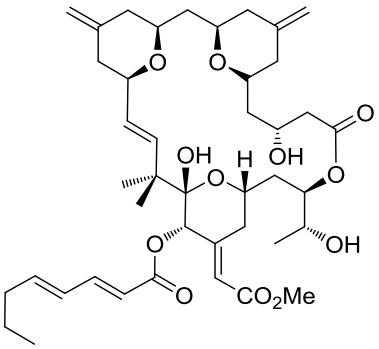
30.0, 22.2; CH δ 147.0, 146.1, 139.2, 129.8, 128.7, 128.2, 128.0, 127.2, 118.7, 117.4, 114.2, 79.7, 75.2, 75.1, 73.1, 71.7, 70.2, 68.5; CH_0 δ 166.9, 165.9, 159.7, 153.4 144.7, 144.5, 138.1, 130.4, 129.8, 128.8, 128.7, 128.7, 114.2, 102.8, 77.6, 77.4, 77.1, 46.2, 26.2, 18.4, 1.4; IR (neat) 2933, 2856, 1716, 1643, 1614, 1514, 1463, 1435, 1381, 1360, 1303, 1249, 1173, 1107, 1082, 1041, 836 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{65}H_{94}O_{15}NaSi$ (M+Na) 1165.6518, found 1165.6520.



Preparation of (2*E*,4*E*)-(1*R*,3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*S*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-5,25-dimethylene-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1$^{3,7.111,15}$]nonacos-8-en-12-yl octa-2,4-dienoate (1.73):[54] To a stirring solution of TBS ether **1.93** (11.6 mg, 0.0101 mmol, 1 equiv) in a solution of 9:1 THF/Pyr in a 2 mL plastic vial, at rt, was added HF•Pyr (20%, 253 μL, 25 mL/mmol of silyl ether) using a needleless plastic syringe. This solution was stirred for 2 days then quenched by pipetting into a stirring mixture of EtOAc (20 mL) and brine (20 mL). The phases were separated, the organic layer was washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated to give the seco-acid as a yellow oil that was used without further purification.

To a stirring solution of the aforementioned seco-acid in THF (373 μL) in a 2 mL vial, at 0 °C, was added Et_3N (8.5 μL, 0.061 mmol, 6 equiv) and a 1 M solution of trichlorobenzyl chloride (30 μL, 0.030 mmol, 3 equiv) in THF. After 5 min, the reaction mixture was warmed to rt and stirring was continued for an additional 3 h. The reaction mixture was diluted with a solution of 3:1 toluene/THF (6 mL) and taken up into a 10 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (25 mg, 0.20 mmol, 20.0 equiv) in toluene (6.7 mL) in 50 mL rb flask, at 40 °C, over a period of 12 h. The residual contents of the syringe were rinsed into the flask with toluene (0.5 mL) and stirring was continued for an additional 2 h. The reaction mixture was cooled to rt, diluted with 30% EtOAc/hexanes (10 mL) and washed with water (3 × 10 mL) and with brine (10 mL). The organic phase was dried over Na_2SO_4, concentrated, and purified by flash column chromatography with a 0.5 × 6.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 6.0 × 50 mm test tube fractions. The product containing fractions (11-26) were combined and concentrated under reduced pressure to provide macrolactone **1.73** as a white foam (5.4 mg, 52 % over 2 steps): R_f = 0.44 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.41-7.33 (m, 4H), 7.31-7.24 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.24 (d, *J* = 15.8 Hz, 1H), 6.18-6.14 (m, 2H), 5.98 (d, *J* = 1.7 Hz, 1H), 5.77 (d, *J* = 15.6 Hz, 1H), 5.59 (ddd, *J* = 11.6 4.3, 2.5 Hz, 1H), 5.33 (dd, *J* = 15.8, 8.6 Hz, 1H), 5.25 (s, 1H), 4.83 (Aβq, *J* = 7.3 Hz, Δν = 12.0 Hz, 2H), 4.76-4.73 (m, 2H), 4.71 (s, 2H), 4.65 (Aβq, *J* = 12.0 Hz, Δν = 15.7 Hz, 2H), 4.49 (s, 2H), 4.22-4.15 (m, 1H), 3.99-3.92 (m, 2H), 3.75 (s, 3H), 3.73-3.70 (m, 2H), 3.68 (s, 3H), 3.35-3.47 (m, 1H), 3.40-3.33 (m, 2H), 3.11 (s, 3H), 3.11-3.06 (m, 1H), 2.59 (dd, *J* = 15.6, 2.4 Hz, 1H), 2.48 (dd, *J* = 15.5, 10.1 Hz, 1H), 2.30 (d, *J* = 14.0 Hz, 1H),

2.22-1.82 (m, 12H), 1.79-1.72 (m, 1H), 1.56 (dd, *J* = 13.8, 6.9 Hz, 1H), 1.50-1.41 (m, 3H), 1.09 (s, 3H), 1.08 (s, 3H), 1.07 (d, J = 6.9 Hz, 3H), 0.92 (t, J = 6.9, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.2, 167.0, 165.6, 159.3, 151.7, 146.7, 145.9, 144.6, 144.5, 142.0, 138.1, 131.0 , 129.6, 128.6, 128.6, 128.1, 127.8, 125.5, 119.4, 118.7, 113.9, 109.1, 109.0, 103.5, 93.7, 81.5, 76.5, 76.4, 76.3, 75.3, 75.3, 73.2, 72.1, 70.7, 69.8, 67.3, 55.4, 52.8, 51.3, 45.3, 44.2, 43.1, 42.0, 41.5, 41.1, 41.0, 35.3, 34.7, 31.0, 29.9, 26.3, 22.0, 20.3, 15.2, 13.9.

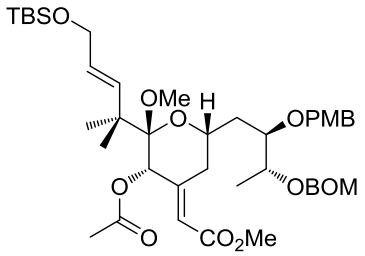


Preparation of Merle 23:[54] To a stirring solution of protected Merle 23 (2.0 mg, 0.0020 mmol, 1 equiv) in CH_2Cl_2 (440 µl) in a 4 mL reaction vial, at 0 °C, was added 1 M aqueous pH 7 buffer (300 µL), and DDQ (4.0 mg, 0.020 mmol, 10 equiv). After 2 h the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 3mL). The combined organic layers were washed with brine (2 × 1 mL), dried over Na_2SO_4, concentrated, and used without further purification.

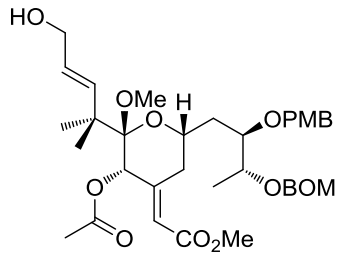
To the aforementioned analog in a 4 mL reaction vial was added a 0.25 M solution of $LiBF_4$ (270 µL, 0.0900 mmol, 45.0 equiv) in 25:1 CH_3CN/H_2O. The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 12 h. After cooling to rt

the reaction mixture was poured into a stirring solution of EtOAc (5 mL), and quenched with saturated aqueous $NaHCO_3$ solution (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, concentrated, and purified by flash column chromatography with a 0.5 × 6 cm silica gel column, eluting with 35% EtOAc/hexanes, collecting 6 × 50 mm test tube fractions. The product containing fractions (9-18) were combined and concentrated under reduced pressure to provide Merle 23 (1.4 mg, 93%, 2 steps) as a white foam: R_f = 0.13 (30% EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 6.18-6.15 (m, 2H), 6.02 (d, *J* = 1.7 Hz, 1H), 5.80 (dd, *J* = 15.2, 6.4 Hz, 2H), 5.33 (dd, *J* = 15.8, 8.6 Hz, 1H), 5.27 (s, 1H), 5.26-5.22 (m, 1H), 5.21 (s, 1H), 4.75-4.69 (m, 4H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.26-4.18 (m, 1H), 4.11-3.99 (m, 2H), 3.86-3.80 (m, 1H), 3.76-3.65 (m, 1H), 3.68 (s, 3H), 3.56 (ddd, *J* = 11.1, 7.5, 2.3 Hz, 1H), 3.53-3.46 (m, 1H), 3.41 (dd, *J* = 11.1, 11.1 Hz, 1H), 2.53-2.41 (m, 3H), 2.19-1.80 (m, 12H), 1.65-1.42 (m, 8H), 1.24 (d, *J* = 6.7 Hz, 3H), 1.14 (s, 3H), 1.01 (s, 3H), 0.93 (t, *J* = 7.4, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.4, 167.3, 165.8, 152.3, 146.5, 145.6, 144.0, 143.5, 138.9, 129.9, 128.6, 119.8, 118.9, 109.3, 108.7, 99.2, 80.2, 79.7, 77.8, 76.5, 74.2, 73.9, 70.5, 68.8, 64.7, 51.3, 45.1, 43.3, 42.8, 42.4, 41.6, 41.0, 40.9, 40.3, 36.1, 35.3, 31.6, 25.0, 22.1, 20.0, 20.0, 13.9.

Experimental procedures for Merle 35,36, and 37



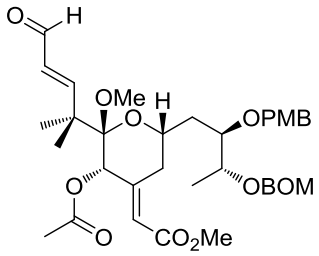
methyl(*E*)-2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxytetrahydro-4*H*-pyran-4-ylidene)acetate (1.96):[33] This compound was prepared from ketone **1.87** in same manner as **1.88** using acetic anhydride at 0 °C for 3h. (84% yield, 2 steps, as a clear oil). R_f = 0.46 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.34 (m, 4H), 7.33-7.29 (m, 1H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.84 (d, *J* = 8.3 Hz, 2H), 6.00 (d, *J* = 15.9 Hz, 1H), 5.89 (s, 1H), 5.46-5.32 (m, 2H), 4.86 (ABq, *J* = 7.1 Hz, Δν = 4.1 Hz, 2H), 4.71-4.65 (m, 2H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.42 (d, *J* = 10.8 Hz, 1H), 4.15-4.09 (m, 3H), 4.08-4.00 (m, 1H), 3.90 (ddd, *J* = 10.2, 4.6, 2.0 Hz, 1H), 3.79 (s, 3H), 3.69 (s, 3H), 3.52 (dd, *J* = 15.4, 2.7 Hz, 1H), 3.24 (s, 3H), 2.30 (ddd, *J* = 14.1, 11.7, 1.6 Hz, 1H), 2.03 (s, 3H), 1.92 (ddd, *J* = 14.3, 9.8, 1.8 Hz, 1H), 1.73 (ddd, *J* = 13.3, 10.3, 2.6 Hz, 1H), 1.23 (d, *J* = 6.4, 3H), 1.11 (s, 6H), 0.90 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.5, 166.7, 159.4, 152.5, 138.4, 138.1, 130.8, 129.5, 129.5, 129.4, 128.7, 128.2, 128.0, 127.9, 124.6, 117.7, 114.1, 114.0, 102.9, 93.6, 77.0, 72.7, 72.3, 72.2, 69.7, 68.6, 64.7, 55.5, 55.5, 51.8, 51.8, 51.4, 46.0, 36.6, 32.7, 26.3, 26.3, 24.9, 23.5, 21.6, 21.6, 15.1, 15.0, -4.8, -4.8.



methyl (*E*)-2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-hydroxy-2-methylpent-3-en-2-yl)-2-methoxytetrahydro-4*H*-pyran-4-ylidene)acetate (1.110):[33]

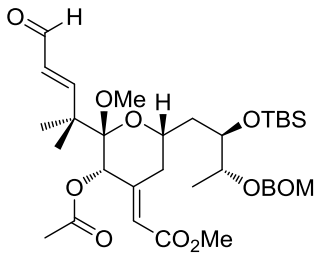
To a stirring solution of TBS-ether **1.96** (110 mg, 0.140 mmol, 1 equiv) in a 5:4:1 solution of THF/ MeOH/ pyridine in a plastic bottle, at 0 °C, was added HF•Pyr (20%, 1.1 mL). The mixture was stirred at 0 °C for 10 min, then at rt for 2 h, and then quenched by pipetting it into a stirring mixture of 50% EtOAc/hexanes (10 mL) and saturated aqueous $NaHCO_3$ solution (10 mL). The phases were separated and the aqueous layer was extracted with 50% EtOAc/hexanes (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 5.0 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (6-27) were combined and concentrated under reduced pressure to provide pure alcohol **1.110** (85 mg, 91% yield) as a clear oil: R_f = 0.29 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.34 (m, 4H), 7.34-7.29 (m, 1H), 7.21 (d, *J* = 8.9 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 5.99 (d, *J* = 16.0 Hz, 1H), 5.88 (s, 1H), 5.50 (dt, *J* = 15.6, 5.8 Hz, 1H), 5.43 (s, 1H), 4.86 (ABq, *J* = 96. Hz, Δν = 7.0 Hz, 2H), 4.68 (s, 2H), 4.62 (d, *J* = 10.9 Hz, 1H), 4.42 (d, *J* = 10.9 Hz, 1H), 4.14 (dt, *J* = 12.7, 6.4 Hz, 1H), 4.09-4.02 (m, 3H), 3.90 (ddd, *J* = 9.9, 4.3, 1.7 Hz, 1H), 3.79 (s, 3H), 3.70 (s, 3H), 3.47 (dd, *J* = 15.7, 2.4 Hz, 1H), 3.23 (s, 3H), 2.34 (ddd, *J* = 14.3, 12.0, 1.6 Hz, 1H), 2.05 (s, 3H), 1.93 (ddd, *J* = 14.3, 10.0, 1.7 Hz 1H), 1.73 (ddd, *J*

= 13.0, 10.0 2.1 Hz, 1H), 1.53 (s, 1H), 1.22 (d, *J* = 6.4 Hz, 3H), 1.13 (s, 3H), 1.10 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.7, 166.8, 159.4, 159.4, 152.5, 139.8, 138.1, 138.1, 130.7, 129.5, 129.5, 128.7, 128.0, 128.0, 125.1, 117.5, 114.1, 114.0, 102.7, 93.6, 76.9, 72.5, 72.3, 72.0, 69.7, 68.6, 64.4, 55.5, 51.6, 51.5, 46.3, 36.4, 33.0, 24.3, 24.2, 21.6, 14.9.



(*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)dihydro-2*H*-pyran-4(3*H*)-ylidene)acetate (1.97):[33] To a stirring solution of alcohol **1.110** (85 mg, 0.127 mmol, 1 equiv) in CH_2Cl_2 (4.2 mL), at rt, was added powdered 4 Å molecular sieves (110 mg), followed by TPAP (4.00 mg, 0.013 mmol, 0.1 equiv) and NMO (45.0 mg, 0.381 mmol, 3 equiv) in a single portion. After 1 h the reaction mixture was diluted with EtOAc (10 mL), and filtered through a 3 cm pad of florisil washing with copious amounts of EtOAc. The filtrate was concentrated to a dark oil, and purified by flash column chromatography using a 1.5 × 3.0 cm silica gel column, eluting with 30% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (2-8) were combined and concentrated under reduced pressure to provide **1.97** (79 mg, 93% yield) as a clear oil: R_f = 0.54 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.52 (d, *J* = 7.9 Hz, 1H), 7.38-7.34 (m, 4H), 7.33-7.28 (m, 2H), 7.21 (d, *J* = 9.7 Hz, 2H), 6.84 (d, *J* = 9.1 Hz, 2H), 5.94 (dd, *J* = 16.0, 8.0 Hz, 1H), 5.89 (s, 1H), 5.42 (s, 1H), 4.86 (Aβq, *J* = 13.9 Hz, Δν = 11.9 Hz, 2H),

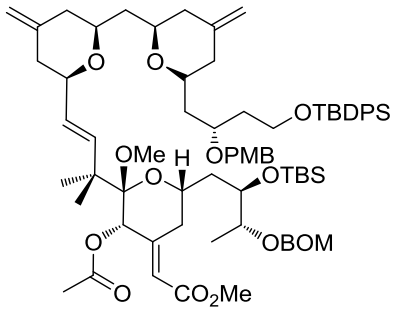
4.67 (s, 2H), 4.63 (d, *J* = 11.0 Hz, 1H), 4.40 (d, *J* = 11.0 Hz, 1H), 4.17 (dt, *J* = 10.8, 6.1 Hz, 1H), 4.14-4.06 (m, 1H), 3.89 (ddd, *J* = 10.1, 4.7, 2.0 Hz, 1H), 3.79 (s, 3H), 3.70 (s, 3H), 3.54 (dd, *J* = 16.6, 3.1 Hz, 1H), 3.26 (s, 3H), 2.37 (ddd, *J* = 14.2, 12.0, 1.8 Hz, 1H), 1.98 (ddd, *J* = 14.4, 9.7, 2.0 Hz, 1H), 1.91 (s, 3H), 1.78 (ddd, *J* = 14.1, 10.3, 2.8 Hz, 1H), 1.24 (d, *J* = 6.6 Hz, 3H), 1.16 (s, 3H), 1.14 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.8, 169.0, 167.1, 166.4, 159.5, 151.4, 138.0, 130.5, 129.5, 128.7, 128.0, 127.9, 127.0, 118.1, 114.2, 114.1, 102.6, 93.6, 77.6, 77.3, 77.0, 76.7, 72.3, 71.8, 71.5, 69.8, 69.2, 55.5, 51.6, 51.5, 47.6, 36.6, 32.9, 24.1, 21.9, 21.5, 14.7.



(*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)dihydro-2*H*-pyran-4(3*H*)-ylidene)acetate (1.98): To a stirring solution of PMB ether **1.97** (79 mg, 0.118 mmol, 1 equiv) in CH_2Cl_2 (3.0 mL) and water (30 μL) in a 10 mL rb flask, at rt, was added DDQ (40.0 mg, 0.177 mmol, 1.5 equiv) in a single portion. The reaction solution was stirred at rt for 30 min, then pippeted directly onto a 1.5 × 10.0 silica gel column eluting with 25% EtOAc/hexanes, and collecting 10 × 74 mm test tube fractions. The product containing fractions (19-45) were combined and concentrated under reduced pressure to provide the crude alcohol (61 mg, 94% yield) as a yellow oil, which was used immediately in the next step.

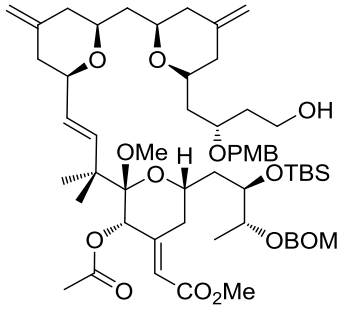
The crude alcohol was taken up in CH_2Cl_2 (4.0 mL), cooled to 0 °C, and 2,6-lutidine

(82.0 μL, 0.708 mmol, 6 equiv) was added followed by TBSOTf (68.0 μL, 0.295 mmol, 2.5 equiv). After 30 min the reaction mixture was quenched first with MeOH (200 μL), and then with a saturated aqueous $NaHCO_3$ solution (1 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 7.5 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (4-11) were combined and concentrated under reduced pressure to provide **1.98** (70.5 mg, 96% yield or 90% yield over 2 steps) as a clear oil: R_f = 0.60 (40% EtOAc/hexanes); $[\alpha]_D^{20}$ = +2.0 (c =2.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.50 (d, *J* = 7.6 Hz, 1H), 7.33-7.30 (m, 4H), 7.28-7.24 (m, 2H), 5.92 (dd, *J* = 15.8, 7.6 Hz, 1H), 5.89 (s, 1H), 5.48 (s, 1H), 4.78 (Aβq, *J* = 6.9 Hz, Δν = 7.0 Hz, 2H), 4.61 (s, 2H), 4.12-4.06 (m, 2H), 3.85 (dt, *J* = 10.7, 6.3 Hz, 2H), 3.67 (s, 3H), 3.53 (ddd, *J* = 16.0, 2.8, 0.8 Hz, 1H), 3.38 (s, 3H), 2.35 (ddd, *J* = 15.6, 11.6, 1.8 Hz, 1H), 2.03 (ddd, *J* = 14.2, 8.7, 2.5 Hz, 1H), 1.91 (s, 3H), 1.16 (d, *J* = 6.4 Hz, 3H), 1.15 (s, 3H), 1,13 (s, 3H), 0.86 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.8, 169.1, 167.1, 166.5, 151.6, 138.0, 128.7, 128.7, 128.1, 128., 127.3, 118.2, 102.6, 93.4, 75.3, 71.2, 70.5, 69.6, 69.3, 52.1, 51.5, 47.6, 38.8, 33.1, 26.1, 26.0, 23.9, 22.0, 21.5, 18.4, 13.9, -2.7, -3.7, -4.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 52.1, 51.5, 26.1, 26.0, 23.9, 22.0, 21.5, 13.9, -2.7, -3.7, -4.4; CH_2 δ 93.4, 69.6, 38.8, 33.1; CH δ 194.8, 167.1, 128.7, 128.0, 127.3, 118.2, 75.3, 71.2, 70.5, 69.3; C δ 169.1, 166.5, 151.6, 138.0, 102.6, 47.6, 18.4; IR (neat) 2953, 2933, 2890, 2858, 2721, 1751, 1721, 1689, 1465, 1436 cm^{-1}; HRMS (ESI/ APCI) calcd for $C35H54O_{10}NaSi$ (M+Na) 685.3486, found 685.3395.



(*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxydihydro-2*H*-pyran-4(3*H*)-ylidene)acetate (1.99): To a stirring solution of aldehyde **1.98** (49.0 mg, 0.074 mmol, 1 equiv) and silane **1.56** (57.0 mg, 0.080 mmol, 1.1 equiv) in E_2O (1.85 mL) in a 10 mL rb flask, at -78 °C, was added a 1.0 M TMSOTf solution (96 μL, 0.096mmol, 1.3 equiv) in THF dropwise by syringe. After 4 h the reaction mixture was quenched first with *i*-Pr_2NEt (0.2 mL), stirred for 10 min, and then quenched with a saturated aqueous $NaHCO_3$ solution (1 mL). The mixture was warmed to rt, the phases were separated, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 9.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (6-19) were concentrated to give bis-pyran **1.99** (67.0 mg, 70%) as a clear oil. R_f = 0.59 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +5.0 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.66 (m, 4H), 7.47-7.33 (m, 10H), 7.32-7.28 (m, 1H), 7.19 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 2H), 5.97 (d, *J* = 16.1 Hz, 2H), 5.91 (s, 1H), 5.56 (s, 1H), 5.42 (dd, *J* = 16.1, 5.9 Hz, 1H), 4.81 (s, 1H), 4.72 (d, *J* = 10.6 Hz, 2H), 4.65

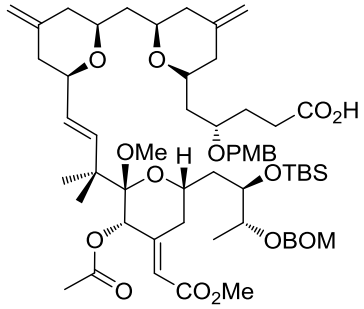
(s, 2H), 4.64 (s, 1H), 4.56 (s, 1H), 4.42 (Aβq, J = 10.8 Hz, Δν = 8.2 Hz, 2H), 4.14-4.05 (m, 2H), 3.96-3.90 (m, 1H), 3.89-3.68 (m, 5H), 3.80 (s, 3H), 3.69 (s, 3H), 3.61-3.43 (m, 4H), 3.32 (s, 3H), 2.55-2.34 (m, 1H), 2.33-2.13 (m, 4H), 2.08 (s, 3H), 2.06-1.87 (m, 6H), 1.85-1.74 (m, 2H), 1.72-1.56 (m, 3H), 1.18 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 3.7 Hz, 6H), 1.07 (s, 9H), 0.89 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.5, 166.7, 159.3, 153.0, 145.0, 144.4, 138.3, 138.1, 135.9, 134.1, 134.1, 131.2, 129.8, 129.6, 128.6, 128.0, 127.9, 127.0, 117.1, 114.0, 109.1, 108.7, 102.7, 93.3, 79.1, 77.6, 77.3, 77.0, 75.1, 75.1, 74.9, 72.9, 72.2, 71.7, 70.3, 69.6, 68.5, 60.6, 55.5, 51.7, 51.4, 46.2, 43.1, 42.6, 41.5, 41.2, 41.0, 40.5, 38.8, 38.0, 33.6, 27.2, 26.2, 24.3, 24.0, 21.5, 20.9, 19.4, 18.4, 14.1, -3.8, -4.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 51.7, 51.4, 27.2, 26.2, 24.3, 24.0, 20.9, 14.0, -3.8, -4.4, CH_2 δ 109.1, 108.7, 93.3, 72.2, 69.6, 60.6, 43.1, 42.6, 41.5, 41.2, 41.0, 40.5, 38.8, 38.0, 33.6; CH δ 138.3, 138.1, 135.9, 129.8, 129.6, 128.6, 128.0, 127.9, 127.0, 117.0, 114.0, 79.1, 75.1, 75.0, 74.9, 72.9, 71.7, 70.3, 68.5; C δ 169.5, 166.7, 159.3, 153.0, 145.0, 144.4, 138.1, 134.1, 134.1, 131.2, 102.7, 77.6, 77.3, 77.0, 46.2, 21.5, 19.4, 18.4; IR (neat) 2937, 2890, 2858, 1748, 1721, 1653, 1513, 1465; cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{75}H_{106}O_{14}NaSi_2$ (M+Na) 1309.7019, found 1309.7013.



(*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-

2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxydihydro-2*H*-pyran-4(3*H*)-ylidene)acetate (1.100): To a stirring solution of TBDPS ether **1.99** (44.0 mg, 0.034 mmol, 1 equiv) in DMF (683 μL) in a 4 mL reaction vial, at rt, was added a premixed solution of 1 M TBAF in THF (34 μL, 0.034 mmol, 1 equiv) and 1 M AcOH in DMF (34 μL, 0.034 mmol, 1 equiv). The transfer was made complete by washing with DMF (2 × 50 μL), and the reaction mixture was stirred for 20 h before being diluted with 40% EtOAc/hexanes (1 mL), and quenched with water (1 mL). The aqueous layer was extracted with 40% EtOAc/hexanes (3 x 3 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 6.0 cm silica gel column, eluting with 25% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (4-9) were concentrated to give alcohol **1.100** (28 mg, 78%) as a clear oil. R_f = 0.27 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +9.0 (c =1.00, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.32 (m, 4H), 7.32-7.28 (m, 1H), 7.26 (d, *J* = 9.4 Hz, 2H), 6.88 (d, *J* = 9.4 Hz, 2H), 5.98 (d, *J* = 16.0 Hz, 1H), 5.90 (s, 1H), 5.55 (s, 1H), 5.41 (dd, *J* = 16.0, 5.8 Hz, 1H), 4.80 (s, 2H), 4.73 (s, 2H), 4.66 (s, 1H), 4.64 (s, 2H), 4.59 (s, 1H), 4.48 (Aβq, *J* = 10.9 Hz, Δν = 16.5 Hz, 2H), 4.13-4.04 (m, 2H), 3.93-3.82 (m, 2H), 3.80 (s, 3H), 3.76-3.69 (m, 2H), 3.69 (s, 3H), 3.56-3.40 (m, 4H), 3.32 (s, 3H), 2.37 (ddd, *J* = 15.8, 11.7, 1.5 Hz, 1H), 2.28-2.15 (m, 4H), 2.08 (s, 3H), 2.06-1.87 (m, 8H), 1.82-1.55 (m, 6H), 1.17 (d, *J* = 6.8 Hz, 3H), 1.10 (d, *J* = 5.1 Hz, 6H), 0.08 (s, 9H), 0.84 (s, 3H), 0.07 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.6, 166.7, 159.6, 153.0, 144.7, 144.5, 138.6, 138.2, 130.7, 129.8, 128.7, 128.1, 127.9, 127.0, 117.1, 114.2, 109.1, 109.0, 102.7, 93.3, 79.4, 75.5, 75.5, 75.2, 75.1, 75.0, 72.1, 71.8, 70.4, 69.6, 68.6, 60.5, 55.6, 51.7, 51.4, 46.2, 43.0, 41.9, 41.5, 41.1,

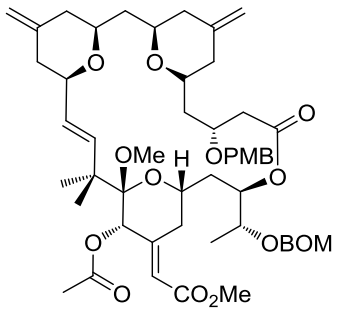
41.0, 40.6, 38.8, 37.0, 33.6, 26.2, 24.3, 24.0, 21.6, 18.4, 14.0, -3.8, -4.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.6, 51.7, 51.4, 26.2, 24.3, 24.0, 21.6, 14.0, -3.8, -4.4; CH_2 δ 109.1, 109.0, 93.3, 72.1, 69.6, 60.5, 43.0, 41.9, 41.5, 41.1, 41.0, 40.6, 38.8, 37.0, 33.6; CH δ 138.6, 129.8, 128.7, 128.1, 127.9, 127.0, 117.1, 114.2, 79.4, 75.5, 75.5, 75.2, 75.1, 75.0, 71.8, 70.4, 68.8; C δ 169.6, 166.7, 159.6, 153.0, 144.7, 144.5, 138.2, 130.7, 102.7, 46.2, 18.4; IR (neat) 3482, 3070, 2938, 2858, 1748, 1721, 1654, 1613, 1514, 1463 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{59}H_{88}O_{14}NaSi$ (M+Na) 1071.5841, found 1071.5835.



(*R*)-4-((2*S*,6*R*)-6-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,3*S*,6*S*,*E*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-2-yl)-3-methylbut-1-en-1-yl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-3-((4-methoxybenzyl)oxy)butanoic acid (1.101): To a stirring of alcohol **1.100** (32.4 mg, 0.031 mmol, 1 equiv) in CH_2Cl_2 (300 μL) in a 1 mL reaction vial, at 0 °C, was added *i*-Pr_2NEt (38 μL, 0.21 mmol, 7 equiv), DMSO (22 μL, 0.31 mmol, 10 equiv), and SO_3•Pyr (20 mg, 0.12 mmol, 4 equiv) in a single portion. After 75 min the mixture was quenched with saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated, and the aqueous layer was extracted with 40% EtOAc/hexanes (3 × 3 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced

pressure. The resulting clear oil was run through a 1.5 × 4.5 cm plug of silica eluting with 20% EtOAc/hexanes, and concentrated to give the crude aldehyde (24.5 mg), which was carried on immediately without characterization.

To a stirring solution of the aforementioned aldehyde in 2-methyl-2-butene (400 μL) and *t*-BuOH (400 μL) in a 5 mL rb flask, at rt, was added a 1.25 M aqueous KH_2PO_4 solution (140 μL). This solution was cooled to -10 °C in an ethylene glycol/ CO_2 bath, and $NaClO_2$ (15 mg, 0.14 mmol, 5 equiv) was added in a single portion. The reaction mixture was stirred vigorously for 1.5 h then quenched with a 0.05 M aqueous pH 4 buffer solution (1 mL). The phases were separated, and aqueous layer was extracted with Et_2O (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 1.5 × 10.0 cm silica gel column, eluting with 3% MeOH/CH_2Cl_2, collecting 10 × 75 mm test tube fractions. The product containing fractions (5-11) were concentrated to give acid **1.101** (31.0 mg, 75%, 2 steps) as a clear oil. R_f = 0.48 (10% MeOH/ 40% EtOAc/ 50% hexanes). Decomposition of this acid could be observed within a few hours so it was used immediately in the next reaction without complete characterization.

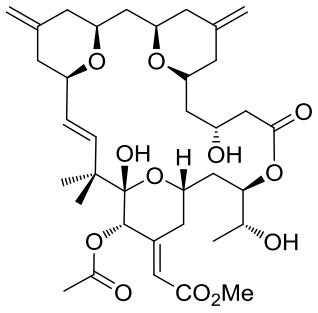


(*E*)-methyl 2-((1*R*,3*S*,7*R*,11*S*,12*S*,15*S*,17*R*,21*R*,23*S*,*E*)-12-acetoxy-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-21-((4-

methoxybenzyl)oxy)-10,10-dimethyl-5,25-dimethylene-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-13-ylidene)acetate (1.102): To a stirring solution of TBS ether **1.101** (31.0 mg, 0.029 mmol, 1 equiv) in a solution of 9:1 THF/Pyr in a 2 mL plastic vial, at rt, was added 20% HF•Pyr (729 μL, 25 mL/mmol of silyl ether) using a needleless plastic syringe. This solution was stirred for 2 days then quenched by pipetting into a stirring solution of EtOAc (20 mL) and brine (20 mL). The phases were separated and organic layer was washed with a brine (2 × 10 mL), dried over Na_2SO_4, and concentrated to give the seco-acid as a yellow oil that was used without further purification.

To a stirring solution of the aforementioned seco-acid in THF (1.0 μL) in 2 mL vial, at 0 °C, was added Et_3N (24 μL, 0.174 mmol, 6 equiv) and a 1 M solution of trichlorobenzoyl chloride (87 μL, 0.030 mmol, 3 equiv) in THF. After 5 min, the solution was warmed to rt and stirring was continued for an additional 3 h. The reaction mixture was diluted with toluene (9 mL) and taken up into a 10 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (71 mg, 0.58 mmol, 20.0 equiv) in toluene (19 mL) in a 100 mL rb flask, at 40 °C, over a 12 h period. The residual contents of the syringe were rinsed into the flask with toluene (1 mL) and stirring was continued for an additional 2 h. The reaction mixture was cooled to rt, diluted with 30% EtOAc/hexanes (50 mL) and washed with water (3 × 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography using a 1.5 × 5.5 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing fractions (26-37) were combined and concentrated under reduced pressure to

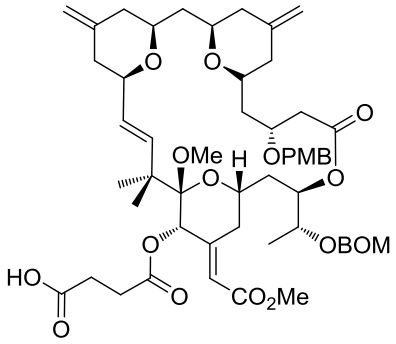
provide macrolactone **1.102** as a clear oil (15.6 mg, 58% over 2 steps): R_f = 0.40 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +32 (c = 0.2, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.40-7.32 (m, 5H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.82 (d, *J* = 8.3 Hz, 2H), 6.23 (d, *J* = 16.3 Hz, 1H), 5.95 (s, 1H), 5.60-5.54 (m, 1H), 5.34 (dd, *J* = 15.6, 8.5 Hz, 1H), 5.16 (s, 1H), 4.82 (Aβq, *J* = 7.7 Hz, Δν = 10.8 Hz, 2H), 4.76 (s, 2H), 4.71 (s, 2H), 4.64 (Aβq, *J* = 11.8 Hz, Δν = 17.0 Hz, 2H), 4.48 (s, 2H), 4.22-4.14 (m, 1H), 4.00-3.91 (m, 2H), 3.75 (s, 3H), 3.70 (s, 3H), 3.73-3.65 (m, 2H), 3.54-3.47 (m, 2H), 3.40-3.32 (m, 2H), 3.09 (s, 3H), 2.58 (dd, *J* = 15.0, 1.8 Hz, 1H), 2.47 (dd, *J* = 15.4, 9.8 Hz, 2H), 2.31 (d, *J* = 13.2 Hz, 1H), 2.22-1.82 (m, 11H), 2.06 (s, 3H), 1.78-1.71 (m, 2H), 1.09 (d, *J* = 5.1 Hz, 6H), 1.06 (d, *J* = 6.2 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.3, 169.4, 167.0, 159.4, 155.4, 144.6, 141.9, 138.2, 131.0, 129.7, 128.7, 127.1, 127.9, 125.8, 119.6, 114.0, 109.2, 109.1, 103.4, 93.8, 81.6, 76.6, 76.5, 76.4, 75.4, 74.0, 73.2, 72.2, 70.8, 69.9, 67.4, 55.5, 53.7, 52.9, 51.5, 45.3, 44.3, 43.2, 42.0, 41.6, 41.2, 41.1, 34.8, 31.1, 26.4, 21.8, 20.3, 15.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 52.9, 51.5, 26.4, 21.8, 20.3, 15.3; CH_2 δ 109.2, 109.1, 93.8, 72.2, 69.9, 44.3, 43.2, 42.0, 41.6, 41.2, 41.1, 34.8, 31.1; CH δ 141.9, 129.7, 128.7, 128.1, 127.9, 125.8, 119.6, 114.0, 81.6, 76.6, 76.5, 76.4, 75.4, 74.0, 73.2, 70.8, 67.4; C δ 172.3, 169.4, 167.0, 159.4, 155.4, 144.6, 138.2, 131.0, 103.4, 53.7, 45.3; IR (neat) 3070, 3027, 1722, 1652, 1514, 1435, 1370, 1300, 1234, 1156, 1089, 1042 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{53}H_{70}O_{14}Na$ (M+Na) 953.4663, found 953.4670.



Preparation of Merle 35: To a stirring solution of protected Merle 35 (1.5 mg, 0.0017 mmol, 1 equiv) in CH_2Cl_2 (340 µL) in a 2 mL vial, at 0 °C, was added 1 M aqueous pH 7 buffer solution (190 µL), and DDQ (4.0 mg, 0.020 mmol, 10 equiv). After 2 h the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL). The organic layers were combined, washed with brine (2 × 1 mL), dried over Na_2SO_4, concentrated, and used without further purification.

To the aforementioned analogue in a 4 mL reaction vial was added a 0.25 M solution of $LiBF_4$ (306 µL, 0.077 mmol, 45.0 equiv) in 25:1 CH_3CN/H_2O. The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 10 h. After cooling to rt the reaction mixture was poured into a stirring solution of EtOAc (5 mL), and quenched with saturated aqueous $NaHCO_3$ solution (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 0.5 × 6 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 6 × 50 mm test tube fractions. The product containing fractions (14-19) were combined and concentrated under reduced pressure to provide Merle 35 (1.0 mg, 82%, 2 steps) as white foam: R_f = 0.33 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = -7.0 (c =0.13, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.99 (d, *J* = 1.7 Hz, 1H), 5.79 (d, *J* = 15.9 Hz, 1H), 5.33 (dd, *J* = 15.9, 8.3 Hz, 1H), 5.26

(s, 1H), 5.22 (ddd, *J* = 12.1, 5.7, 3.0, 1H), 5.14 (s, 1H), 4.78-4.69 (m, 4H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.22 (ddd, *J* = 13.5, 11.4, 2.6 Hz, 1H), 4.10-3.99 (m, 2H), 3.82 (ddd, *J* = 12.5, 12.5, 5.2 Hz, 1H), 3.71 (d, *J* = 1.9 Hz, 1H), 3.69 (s, 3H), 3.56 (ddd, *J* = 11.1, 7.5, 2.1 Hz, 1H), 3.53-3.46 (m, 1H), 3.44-3.37 (m, 1H), 2.52-2.41 (m, 2H), 2.19-1.80 (m, 8H), 1.68-1.40 (m, 8H), 1.27 (s, 3H), 1.24 (d, *J* = 6.4 Hz, 3H), 1.15 (s, 3H), 1.02 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 175.5, 169.6, 167.3, 152.1, 144.1, 143.6, 138.9, 130.2, 120.0, 109.3, 108.8, 99.2, 80.2, 79.8, 77.9, 76.6, 74.7, 74.0, 70.6, 68.9, 64.8, 51.3, 45.1, 43.5, 42.9, 42.5, 41.7, 41.1, 41.0, 40.4, 36.2, 31.6, 25.1, 21.8, 20.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.3, 25.1, 21.8, 20.1; CH_2 δ 109.3, 108.8, 43.5, 42.9, 42.5, 41.7, 41.1, 41.0, 40.4, 36.2, 31.6; CH δ 138.9, 130.2, 120.0, 80.2, 79.8, 77.9, 76.6, 74.7, 74.0, 70.6, 68.9, 64.8; C δ 172.5, 169.6, 167.3, 152.1, 144.1, 143.6, 99.2; HRMS (ESI/ APCI) calcd 699.3356 for $C_{36}H_{52}O_{12}Na$ (M+Na), found 699.3365.

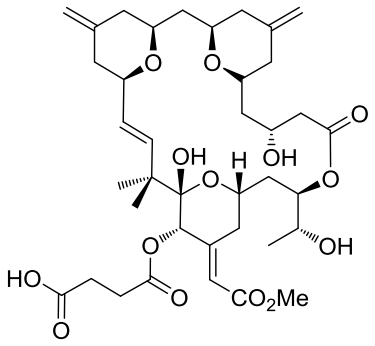


Preparation of 4-(((1*R*,3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-5,25-dimethylene-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl)oxy)-4-oxobutanoic acid (1.103): To a stirring solution of macrolactone **1.102** (4.2 mg, 0.0045 mmol, 1 equiv) in

MeOH (0.9 mL) in a 4 mL vial, at rt, was added K_2CO_3 (6 mg, 0.045 mmol, 10 equiv). The reaction mixture was stirred at rt for 45 min, and then quenched by pipetting into a mixture of CH_2Cl_2 (5 mL) and saturated aqueous NH_4Cl solution (5 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were combined, washed with brine (2 × 3 mL), dried over Na_2SO_4, and concentrated. The crude alcohol was used without further purification.

To a stirring solution of the aforementioned alcohol in a 1 mL conical vial, at rt, was added CH_2Cl_2 (45 μL), followed by succinic anhydride (5 mg, 0.045 mmol, 10 equiv) and DMAP (7 mg, 0.54 mmol, 12 equiv). The reaction mixture was stirred at rt for 20 h, and then quenched by pipetting into a mixture of EtOAc (5 mL) and a 0.5 M aqueous pH 4 acetate buffer solution (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 0.5 × 6 cm silica gel column, eluting with 3% MeOH/CH_2Cl_2, collecting 6 × 50 mm test tube fractions. The product containing fractions (4-5) were combined and concentrated under reduced pressure to provide acid **1.103** (4.0 mg, 90%, 2 steps) as a clear oil: R_f = 0.53 (2:1 toluene/dioxane and 3% AcOH); $[\alpha]_D^{20}$ = +3.2 (c =0.2, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.41-7.32 (m, 4H), 7.32-7.26 (m, 1H), 7.20 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.25 (d, *J* = 15.9 Hz, 1H), 5.93 (d, *J* = 2.0 Hz, 1H), 5.58 (ddd, *J* = 12.2, 4.6, 2.6 Hz, 1H), 5.35 (dd, *J* = 16.1, 9.2 Hz, 1H), 5.18 (s, 1H), 4.82 (Aβq, *J* = 13.6 Hz, Δν = 12.0 Hz, 2H), 4.78-4.74 (m, 2H), 4.71 (s, 2H), 4.65 (Aβq, *J* = 14.3 Hz, Δν = 21.3 Hz, 2H), 4.47 (s, 2H), 4.21-4.15 (m, 2H), 4.00-3.93 (m, 2H), 3.75 (s, 3H), 3.73-7.67 (m, 2H), 3.69 (s, 3H), 3.54-3.48 (m, 2H), 3.40-3.33 (m, 1H), 3.09 (s, 3H), 2.76-2.55 (m, 6H), 2.51-2.43

(m, 1H), 2.38-2.29 (m, 1H), 2.23-1.83 (m, 9H), 1.79-1.70 (m, 1H), 1.67-1.60 (m 1H), 1.56 (ddd, *J* = 14.4, 7.7, 1.3 Hz, 1H), 15.0-1.40 (m, 1H), 1.10 (s, 3H), 1.09 (s, 3H), 1.06 (d, *J* = 6.6 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.3, 170.7, 167.0, 159.4, 151.2, 144.6, 144.6, 141.9, 138.2, 131.1, 129.7, 128.7, 128.2, 127.9, 125.8, 119.7, 114.0, 109.2, 109.1, 103.4, 93.8, 81.6, 76.6, 76.5, 76.5, 75.5, 74.5, 73.3, 72.2, 70.8, 69.9, 67.4, 55.5, 52.9, 51.4, 45.3, 44.3, 43.2, 42.0, 41.6, 41.2, 41.2, 41.1, 34.7, 31.0, 30.0, 29.7, 26.5, 23.0, 20.4, 15.2, 14.4, 1.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 52.9, 51.4, 26.5, 15.2, 1.3; CH_2 δ 109.2, 109.1, 93.8, 72.2, 69.9, 44.3, 43.2, 42.0, 41.6, 41.2, 41.2, 41.1, 34.7, 31.0, 31.0, 29.7; CH δ 141.9, 129.7, 128.7, 128.2, 127.9, 125.8, 119.7, 114.0, 81.6, 77.5, 76.6, 76.5, 75.5, 74.5, 73.3, 70.8, 67.4; C δ 172.3, 170.7, 167.0, 159.4, 151.2, 144.6, 144.6, 138.2, 131.1, 23.0, 20.4, 14.4; IR (neat) 2934, 1726, 1608, 1382, 1247, 1152, 1041 cm^{-1}; HRMS (ESI/ APCI) calcd 1011.4717 for $C_{55}H_{72}O_{16}Na$ (M+Na), found 1011.4731.



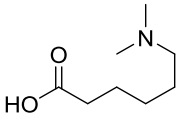
Preparation of Merle 36: To a stirring solution of carboxylate **1.103** (3.5 mg, 0.0035 mmol, 1 equiv) in CH_3CN (350 μL) in a 1 ml vial, at 0 ºC, was added a 1 M solution of *i*-Pr_2NEt (7.1 μL, 0.0071 mmol, 2 equiv) in CH_3CN, and a 1 M solution of BOMCl (5 μL, 0.005 mmol, 1.3 equiv) in CH_3CN. After 30 min the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5

mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The crude product was taken on without purification.

To a stirring solution of the aforementioned product in CH_2Cl_2 (500 μL) in a 4 mL reaction vial, at 0 ºC, was added 1 M aqueous pH 7 buffer solution (278 μl), and DDQ (6.0 mg, 0.026 mmol, 10 equiv). After 2 h the reaction was quenched with a saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were combined, washed with brine (2 × 1 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was used without further purification.

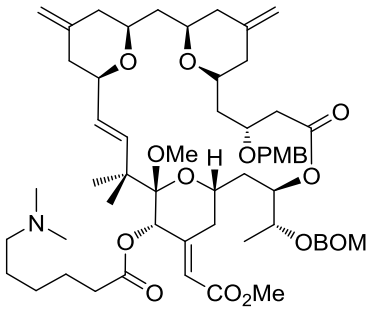
To the aforementioned protected analog in a 4 mL reaction vial was added a 0.25 M solution of $LiBF_4$ (450 μL, 0.113 mmol, 45.0 equiv) in 25:1 CH_3CN/H_2O. The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 12 h. After cooling to rt the reaction mixture was poured into a stirring solution of EtOAc (5 mL), and quenched with saturated aqueous $NaHCO_3$ solution (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Purification was accomplished using a 10 × 20 cm preparatory chromatography plate eluting with 5% EtOAc/hexanes. The dominant band was cut out, stirred with EtOAc for 20 min, filtered, and concentrated under reduced pressure to provide Merle 36 (1.0 mg, 39%, 3 steps) as a clear oil: R_f = 0.25 (10% MeOH/ 40% EtOAc/ 50% hexanes); $[\alpha]_D^{20}$ = -15.0 (c = 0.07, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.98 (d, *J* = 1.9 Hz, 1H), 5.79 (d, *J* = 15.9 Hz, 1H), 5.33 (dd, *J* = 15.9, 8. Hz, 1H), 5.29 (s, 1H), 5.22 (ddd, *J* = 12.1, 5.7, 2.9 Hz, 1H), 5.16 (s, 1H), 4.78-4.69 (m. 4H), 4.48 (d, *J* = 12.1 Hz, 1H), 4.21 (ddd, *J* = 11.9, 11.9, 2.8 Hz 1H), 4.08-3.99

(m, 2H), 3.82 (t, *J* = 6.5 Hz, 1H), 3.73-3.67 (m, 1H), 3.69 (s, 3H), 3.56 (ddd, *J* = 10.5, 7.3, 2.2 Hz, 1H), 3.53-3.46 (m, 1H), 3.44-3.37 (m, 1H), 2.80-2.57 (m, 4H), 2.53-2.40 (m, 2H), 2.19-1.79 (m, 14H), 1.67-1.58 (m, 2H), 1.52 (d, *J* = 14.9 Hz, 1H), 1.26 (s, 3H), 1.24 (d, *J* = 6.2 Hz, 3H), 1.16 (s, 3H); 125MHz ^{13}C NMR ($CDCl_3$) δ 174.7, 172.5, 170.9, 167.3, 151.8, 144.1, 143.6, 138.9, 130.1, 120.2, 109.4, 108.8, 99.2, 80.2, 79.8, 77.9, 76.6, 75.1, 74.0, 70.6, 69.0, 64.8, 51.4, 45.1, 43.4, 42.9 42.5, 41.6, 41.1, 41.0, 40.4, 36.1, 31.5, 30.0, 28.5, 25.0, 20.1, 14.4, 1.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.4, 25.0, 20.1, 1.3; CH_2 δ 109.4, 108.8, 43.4, 42.9, 42.5, 41.6, 41.1, 41.0, 40.4, 36.1, 31.5, 30.0, 28.5; CH δ 138.9, 130.1, 120.2, 80.2, 79.8, 77.9, 76.6, 75.1, 74.0, 70.6, 69.0, 64.8; C δ 174.7, 172.5, 170.9, 167.3, 151.8, 144.1, 143.6, 99.6, 45.1, 14.4; IR (neat) 3254, 2923, 1735, 1718, 1231 cm^{-1}; HRMS (ESI/ APCI) calcd 757.3411 for $C_{38}H_{54}O_{14}Na$ (M+Na), found 757.3420.



Preparation of 6-(dimethylamino)hexanoic acid: To a stirring solution of 6-aminohexanoic acid (1.49 g, 11.4 mmol, 1 equiv) in a 37% aqueous formaldehyde solution (2.10 mL, 28.4 mmol, 2.5 equiv) in a 25 mL 2 neck rb flask equipped with a reflux condenser, at rt, was added 90% aqueous formic acid (2.30 mL, 56.8 mmol, 5 equiv). The reaction mixture was heated at reflux for 16 h, cooled to rt, transferred to a 100 mL rb flask, and diluted with water (5 mL). This aqueous solution was shell frozen then lyophilized to give a thick orange sludge that was taken up in acetone (10 mL), acidified with 1 M HCl (2 mL), and left in a -20 ºC freezer overnight. The precipitate was collected by vacuum filtration to give pure 6-(dimethylamino)hexanoic acid as the

hydrochloride salt (1.61 g, 8.27 mmol, 73%) and as an off white solid: Mp = 104 ºC (108 ºC lit); 500 MHz ^{1}H NMR (D_2O) δ 3.12 (dd, *J* = 8.6, 8.4 Hz, 2H), 2.86 (s, 6H), 2.36 (t, *J* = 7.6 Hz, 2H), 1.73 (ddt, *J* = 15.3, 9.7, 5.8 Hz, 2H), 1.63 (quintet, *J* = 7.3 Hz, 2H), 1.38 (d, *J* = 7.3 Hz, 2H); 125MHz ^{13}C NMR (D_2O) δ 57.8, 42.7, 34.3, 25.2, 24.1, 23.8.

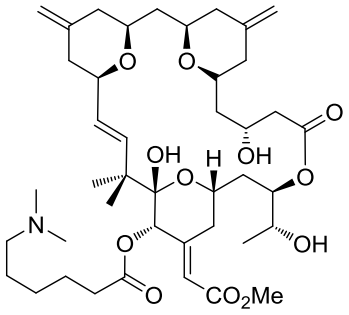


Preparation of (1*R*,3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-5,25-dimethylene-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl 6-(dimethylamino)hexanoate (1.104): To a stirring solution of macrolactone **1.102** (8.0 mg, 0.0079 mmol, 1 equiv) in MeOH (1.58 mL) in a 4 mL vial, at rt, was added K_2CO_3 (11 mg, 0.079 mmol, 10 equiv). After 45 min the reaction mixture was quenched by pipetting into a mixture of CH_2Cl_2 (10 mL) and saturated aqueous NH_4Cl solution (10 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated. The crude alcohol was used without further purification.

The aforementioned alcohol was transferred to a 0.5 mL conical vial and dried under vacuum. Meanwhile, to a stirring solution of 6-(dimethylamino)hexanoic acid (3.1 mg, 0.016 mmol, 2 equiv) in CH_2Cl_2 (158 µL) in a 4 ml vial, at rt, was added 2-methyl-6-

nitrobenzoic anhydride (6.00 mg, 0.017 mmol, 2.2 equiv), and DMAP (0.20 mg, 0.0016 mmol, 0.2 equiv). This solution was stirred for 30 min at rt then added to the conical vial containing the alcohol washing with an additional 50 µL of CH_2Cl_2. After 4 h, the reaction mixture was quenched by pipetting into a mixture of EtOAc (5 mL) and a 0.5 M aqueous pH 10 carbonate buffer solution (5 mL). The phases were separated, and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 0.5 × 6 cm silica gel column, eluting with 5% MeOH/CH_2Cl_2, collecting 6 × 50 mm test tube fractions. The product containing fractions (4-16) were combined and concentrated under reduced pressure to provide amine **1.104** (5.2 mg, 63%, 2 steps) as clear oil: R_f = 0.22 (10% MeOH/$CHCl_3$); $[\alpha]_D^{20}$ = +29.5 (c =0.5, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.41-7.32 (m, 4H), 7.31-7.28 (m, 1H), 7.21 (d, *J* = 9.3 Hz, 2H), 6.82 (d, *J* = 9.3 Hz, 2H), 6.22 (d, *J* = 15.5 Hz, 1H), 5.95 (s, 1H), 5.57 (ddd, *J* = 12.1, 4.7, 2.9 Hz, 1H), 5.34 (dd, *J* = 15.8, 8.4 Hz, 1H), 5.17 (s, 1H), 4.82 (Aβq, *J* = 13.1 Hz, Δν = 11.0 Hz, 2H), 4.77-4.74 (m, 2H), 4.71 (s, 2H), 4.64 (Aβq, *J* = 20.1 Hz, Δν = 16.3 Hz, 2H), 4.48 (s, 2H), 4.21-4.14 (m, 1H), 3.99-3.92 (m, 2H), 3.81-3.65 (m, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 3.53-3.47 (m, 1H), 3.36 (dddd, *J* = 10.8, 10.8, 2.8, 2.0 Hz, 1H), 3.09 (s, 3H), 2.57 (dd, *J* = 15.8, 2.6 Hz, 1H), 2.47 (dd, *J* = 15.8, 9.9 Hz, 1H), 2.34-2.27 (m, 2H), 2.22 (s, 6H), 2.21-2.05 (m, 4H), 2.01-1.91 (m, 3H), 1.91-1.82 (m, 3H), 1.78-1.71 (m, 1H), 1.68-1.59 (m, 3H), 1.56 (dd, *J* = 13.5, 7.0 Hz, 1H), 1.52-1.41 (m, 3H), 1.36-1.28 (m, 3H), 1.26 (s, 2H), 1.09 (s, 3H), 1.07 (s, 3H), 1.06 (d, *J* = 7.0 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.3, 172.1, 167.1, 159.4, 151.5, 144.6, 144.6, 141.9, 138.2, 131.1, 129.7, 128.7, 128.2, 127.9, 125.8, 119.6, 114.0, 109.2, 109.1, 103.4, 93.8, 81.6, 76.6, 76.5, 76.4, 75.4, 73.9, 73.3, 72.2, 70.8, 69.9,

67.3, 59.8, 55.5, 52.9, 51.4, 45.7, 45.3, 44.3, 43.1, 42.0, 41.6, 41.2, 41.2, 41.0, 34.8, 31.1, 30.0, 27.5, 27.2, 26.5, 24.8, 20.3, 15.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 52.9, 51.4, 45.7, 26.5, 20.3, 15.3; CH_2 δ 109.2, 109.1, 93.8, 72.2, 69.9, 59.8, 44.3, 43.1, 42.0, 41.6, 41.2, 41.2, 41.0, 34.8, 31.1, 30.0, 27.5, 27.2, 24.8; CH δ 414.9, 129.7, 128.7, 128.2, 127.9, 125.8, 119.6, 114.0, 81.6, 76.6, 76.5, 76.4, 75.4, 73.9, 73.3, 70.8, 67.3; C δ 172.3, 172.1, 167.1, 159.4, 151.5, 144.6, 144.6, 138.2, 131.1, 103.4, 45.3; IR (neat) 2938, 1734, 1653, 1514, 1456 cm^{-1}; HRMS (ESI/ APCI) calcd 1030.5886 for $C_{59}H_{84}NO_{14}Na$ (M+Na), found 1030.5902.



Preparation of Merle 37: To a stirring solution of protected Merle 37 (5.0 mg, 0.005 mmol, 1 equiv) in CH_2Cl_2 (980 µl) in a 2 mL reaction vial, at 0 °C, was water (10 µl), and DDQ (5.6 mg, 0.024 mmol, 5 equiv). After 2 h the reaction mixture was quenched with a saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, washed with brine (2 × 5 mL), dried over Na_2SO_4, and concentrated. The crude product was used without further purification.

To the aforementioned analog in a 4 mL reaction vial was added a 0.25 M solution of $LiBF_4$ (882 µL, 0.220 mmol, 45.0 equiv) in 25:1 CH_3CN/H_2O. The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 10 h. After cooling to rt the reaction mixture was pipetted directly on to a 0.5 × 6 cm silica gel column, eluting with

10% MeOH/$CHCl_3$. Further purification was accomplished using a 10 × 20 cm preparatory chromatography plate eluting with 20% MeOH/$CHCl_3$. The dominant band was cut out, stirred with 20% MeOH/$CHCl_3$ for 20 min, filtered, and concentrated under reduced pressure to provide Merle 37 (1.2 mg, 32%, 2 steps) as a white foam: R_f = 0.21 (20% MeOH/$CHCl_3$); $[\alpha]_D^{20} = +17.0$ (c = 0.10, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.98 (s, 1H), 5.78 (d, *J* = 16.2 Hz, 2H), 5.33 (d, *J* = 16.2 Hz, 2H), 5.27 (s, 1H), 5.22 (ddd, *J* = 11.9, 5.4, 2.9 Hz, 1H), 5.14 (s, 1H), 4.75-4.69 (m, 4H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.25-4.17 (m, 1H), 4.08-3.99 (m, 2H), 3.82 (dd, *J* = 6.3, 5.7 Hz, 1H), 3.72-3.67 (m, 1H), 3.69 (s, 3H), 3.59-3.52 (m, 1H), 3.52-3.46 (m, 1H), 3.44-.337 (m, 1H), 3.01 (d, *J* = 7.6 Hz, 2H), 2.53-2.36 (m, 2H), 2.41 (s, 6H), 2.36-2.23 (m, 2H), 2.19-1.91 (m, 8H), 1.91-1.80 (m, 2H), 1.71-1.56 (m, 4H), 1.44 (d, *J* = 14.3 Hz, 1H), 1.41-1.33 (m, 4H), 1.26 (s, 3H), 1.24 (d, *J* = 6.5 Hz, 3H), 1.14 (s, 3H), 1.01 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.4, 172.0, 167.3, 152.1, 144.1, 143.6, 138.9, 130.2, 120.0, 109.4, 108.8, 99.2, 80.2, 79.8, 77.9, 76.6, 74.7, 73.9, 70.4, 69.0, 64.8, 51.4, 46.1, 45.1, 43.5, 42.9, 42.5, 41.6, 41.1, 41.1, 40.4, 36.1, 34.6, 31.7, 30.0, 26.7, 25.2, 24.5, 20.1, 20.0; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.4, 25.2, 20.1, 20.0; CH_2 δ 109.4, 108.8, 46.1, 43.5, 42.9, 42.5, 41.6, 41.1, 41.1, 40.4, 36.1, 34.6, 31.7, 30.0, 26.7, 24.5; CH δ 138.9, 130.2, 120.0, 80.2, 79.8, 77.9, 76.6, 74.7, 73.9, 70.4, 69.0, 64.8; C δ 172.4, 172.0, 167.3, 152.1, 144.1, 143.6, 99.2, 45.1; IR (neat) 3455, 3326, 2975, 2939, 2858, 1735, 1653, 1465 cm^{-1}; HRMS (ESI/ APCI) calcd 776.4580 for $C_{42}H_{65}NO_{12}Na$ (M+Na), found 776.4593.

HPLC Conditions: HPLC was conducted using Rainnin Dynamax model SD-200 solvent pumps, a Waters symmetry C18 150 × 4.6 μm column, and a Rainnin model RI-1

refractive index detector. 10 μL aliquots of a 1-mg/mL stock sample solution in acetonitrile were injected, eluting with thoroughly degassed 80% acetonitrile/water at a flow rate of 1 mL/min and a column pressure of 1740 psi.

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CHAPTER 2

DEVELOPMENT OF AN AROMATIC A-, B-RING SYSTEM

Evaluation A-, B-ring Structure Function Relationships

To date, the combination of the Keck and Wender groups have synthesized over 100 analogues. The Wender group has focused primarily on determining structural features that contribute to high affinity binding, while the Keck group has chosen to explore the structural features that endow bryostatin 1 with its unique biological profile.

Wender's initial work identified a heteroatom triad on the C-ring that was required for high affinity binding. The A- and B-rings were then hypothesized to function merely as a "spacer domain" that held those atoms in the correct spatial orientation. The synthesis of Merle 23 by the Keck group and subsequent biological evaluation by the Blumberg group has demonstrated that the A- and B-rings are not merely a "spacer domain' but that in fact they endow bryostatin 1 with its unique biology. Merle 23 differs from bryo 1 in that the C7 acetate, C8 gem dimethyl, C9 alcohol and C13 exocyclic enoate have all been replaced by exocyclic olefins at C7 and C13 (Figure 2.1). These changes resulted in Merle 23 displaying a biological profile distinct from that of bryo 1 and the phorbal ester PMA in U937 and LNCaP cell assays among others.

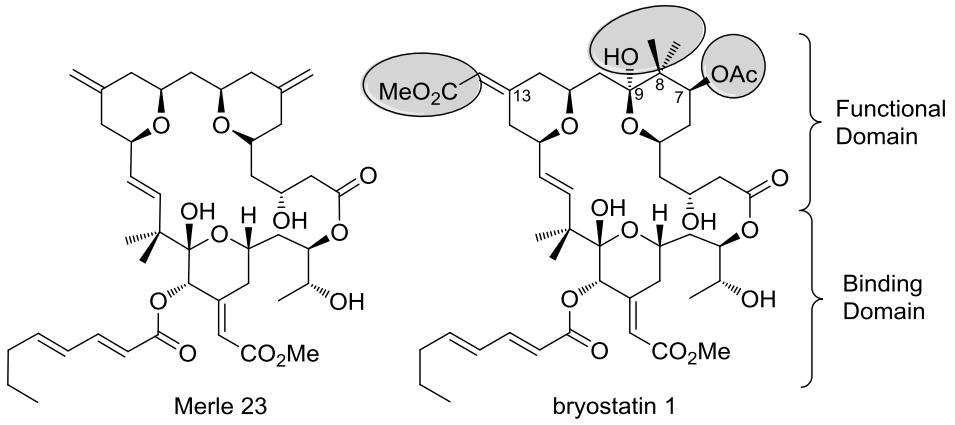


Figure 2.1. Structural comparison of Merle 23 and Bryo 1

In order to better understand the role of the A-, B-ring functionality in determining a bryostatin like response, the Keck group undertook the task of examining each of the four positions by systematically reinstalling each of the four missing functional groups on Merle 23 or by deleting them from bryo 1. These efforts resulted in the synthesis of seven new analogues that possess some combination of the natural bryostatin functionality (Figure 2.2). All analogues were initially tested in the U937 proliferation and attachment assay, in which Merle 23 was PMA like, for either bryo 1 like or PMA like behavior. Adding the C7 acetate, Merle 27[1], to the Merle 23 bis-pyran ring system failed to induce a switch to bryo 1 like behavior, but the combination of the C7 acetate with the C13 enoate, Merle 33[2], did. Merle 28[3], which lacks only the B-ring enoate while maintaining the entire A-ring, behaved very similar to Bryo 1; however, if the B -ring is completely omitted (Merle 29u)[4] the compound reverts to PMA like behavior. Furthermore, replacing the B-ring with a more flexible ester linkage resulted in an analogue that would spontaneously undergo ring expansion to make Merle 29d. Merle 29 u and d were obtained in an approximate 1:7 ratio that was separable by preparatory plate

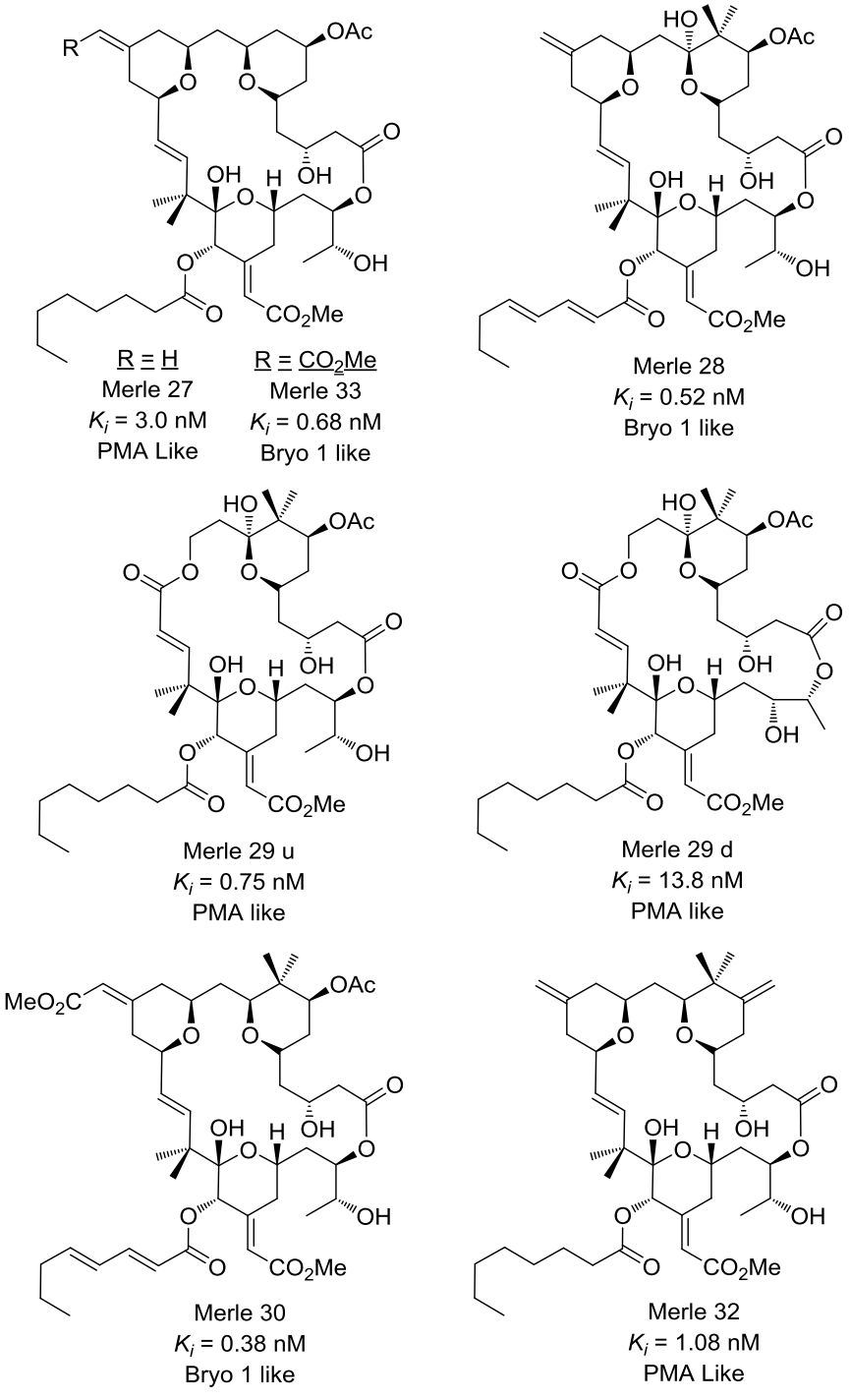


Figure 2.2. A-, B- ring analogues

chromatography, but during cell assays, conversion of Merle 29u to Merle 29d was observed in the form of decreasing biological potency. Merle 30[5], which is missing the C9 ketal exhibited bryo 1 like activity, and Merle 32[6], with only the gem-dimethyl added back, was PMA like. The synthesis of this set of analogues revealed that the structural requirements for bryo like behavior are more complex than just a single functional group being responsible. However, there does exist a clear trend in that analogues containing two or more polar moieties tend to be bryo 1 like provided that both A- and B-rings rings are present.

The polarity hypothesis

Our current understanding is that bryostatins and analogues bind to PKC mainly though interactions with the C-ring while the A- and B-rings form a hydrophobic surface on the protein. The entire assemble then undergoes translocation during which time interactions of the hydrophobic surface with membranes, other PKC domains, and other proteins are critical for determining the biological response. By systematically removing functional groups from bryostatin or adding them to Merle 23, the Keck group was able to show that, while no single group dictated biological outcome in U937 cells, there was a dependence on the overall polarity of the A-, B-ring region as determined by ClogP calculated using Chem Draw 12 (Figure 2.3).

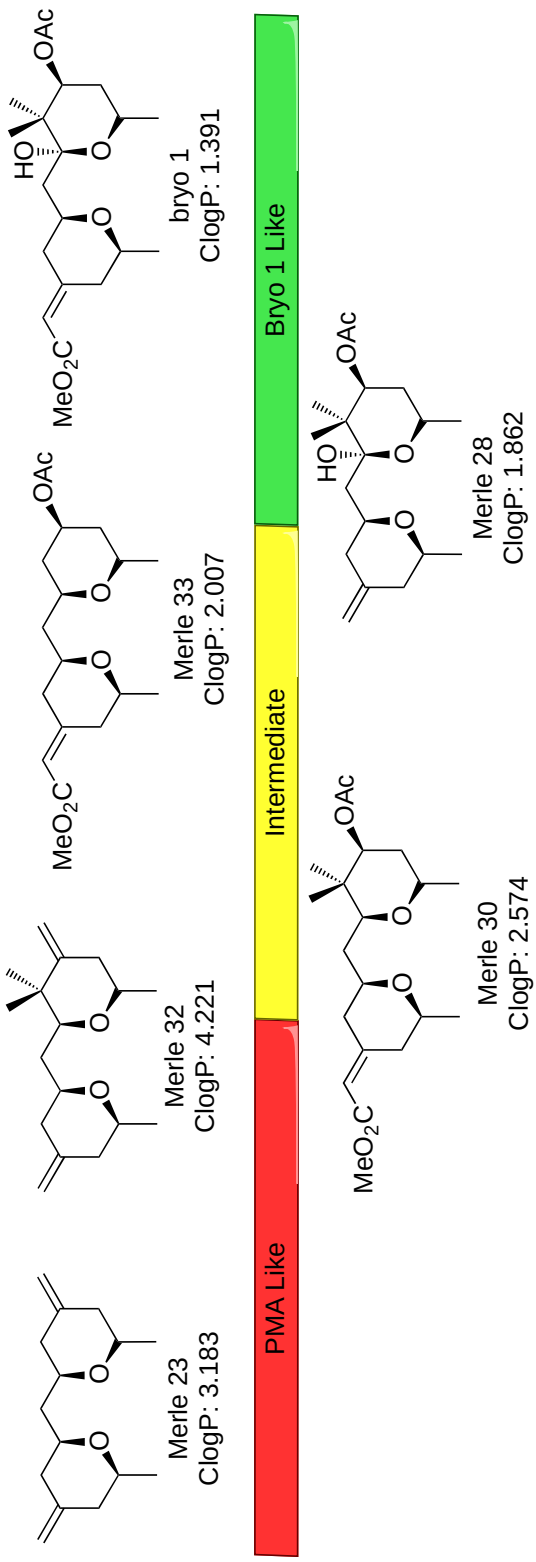


Figure 2.3. Polarity hypothesis

Transcriptional Response of A-, B-ring Analogues

To better understand the subtle biological differences between the set of A- and B-ring analogues, they were examined with regard to their transcriptional response in both U937 and LNCaP cells.[7] Figure 2.4 represents the mean increase in gene induction for SERPINB2, TNFα, CXCL8, CCL2, TRAF1, and BIRC3 at 8 h following exposure to 1000 nM of the indicated compounds in both cell lines. In spite of the fact that all analogues are bryo 1 like in the LNCaP proliferation assay, they show a range of activity from PMA like to almost bryo 1 like in the transcription assay. This trend correlates well

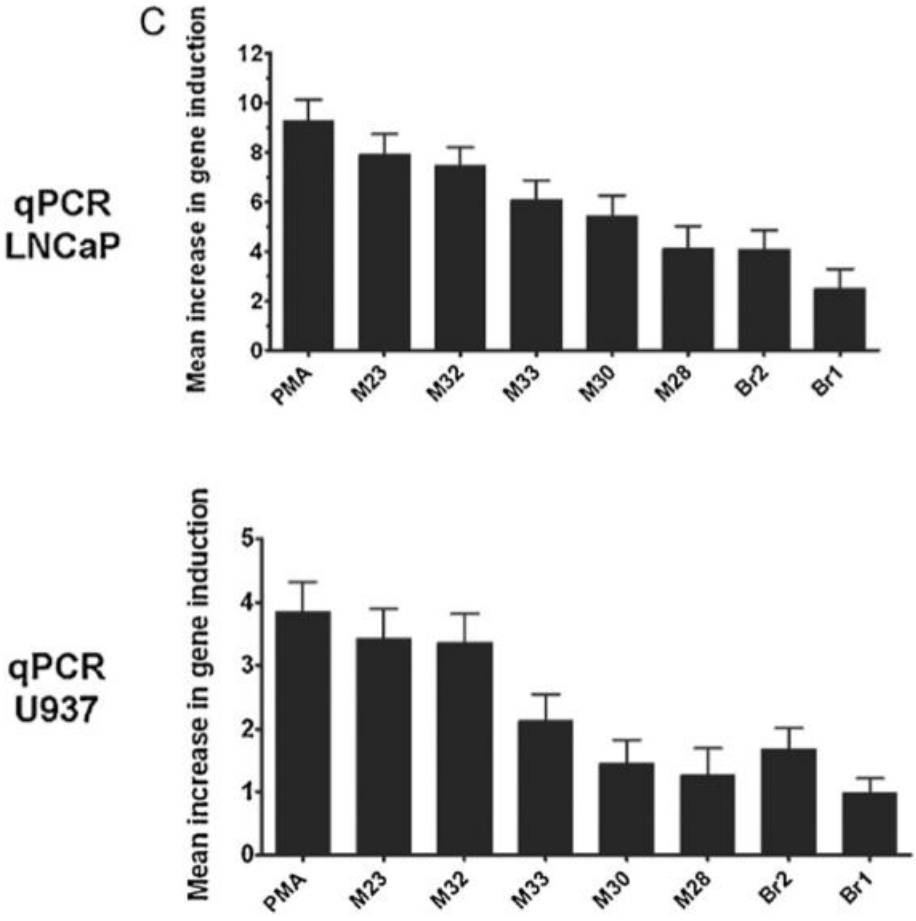


Figure 2.4. Transcriptional response of analogues

with the analogues ClogP value giving some validation to the polarity hypothesis. Additionally, just as was observed in Chapter 1, the LNCaP cells have a much larger scope of activation presumably making them less sensitive to PKC activation and explaining why all analogues show bryo 1 like proliferation. Contrastingly, the U397 cells appear to have a distinct break at about a two-fold increase in activation that correlates well with which analogues are PMA like in the proliferation and attachment assay (Figure 2.5).

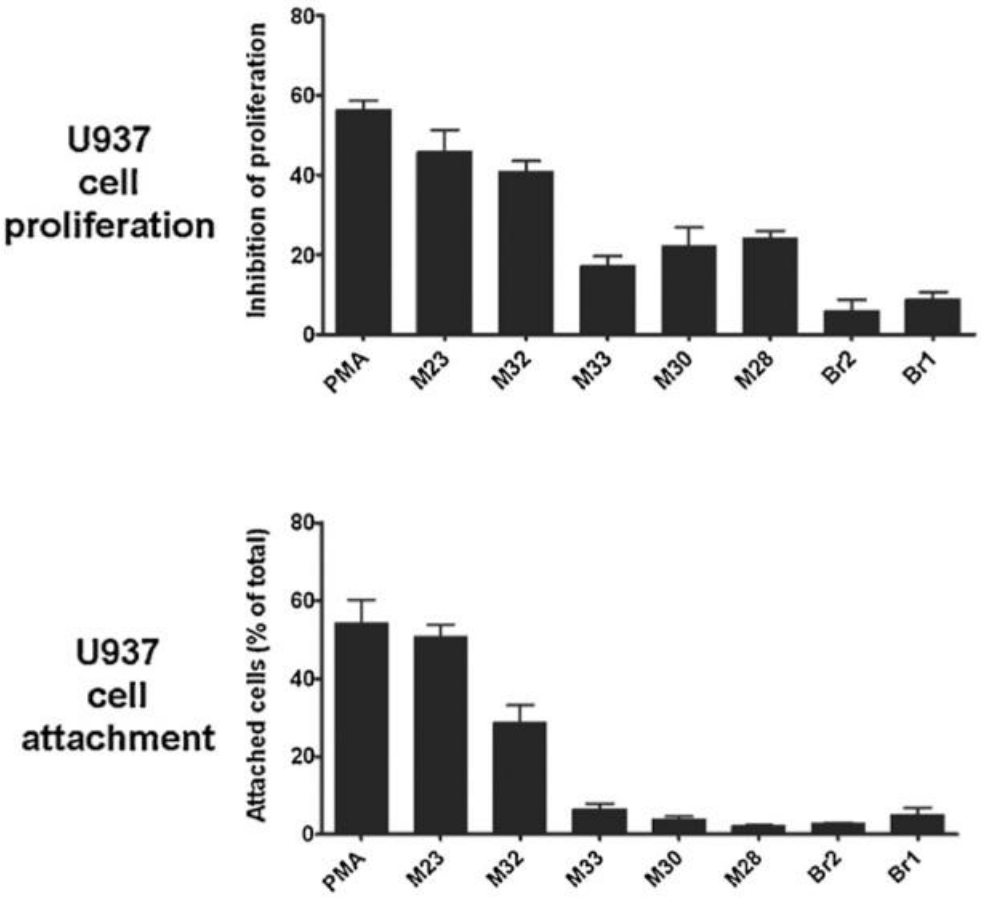


Figure 2.5. U937 proliferation and attachment

The analogues were also tested for their ability to induce TNFα secretion in both LNCaP and U937 cells (Figure 2.6). TNFα secretion is of particular interest as it has been identified as an important contributor to PMA induced cell growth inhibition in LNCaP cells.[8] All analogues induce dramatically less TNFα secretion than PMA at concentrations of 1, 10, 100, and 1000 nM, which is consistent with their bryo 1 like behavior with regard to growth inhibition. Mirroring the qPCR results, the U937 cells show a difference between the PMA like analogues Merle 23 and 32 and the bryo 1 like analogues Merle 28, 30, and 33.

The qPCR transcriptional studies and TNFα secretion analysis support the idea that modulating the polarity across the A- and B-rings will endow analogues with a variety of biological responses that span the range from PMA to bryo 1. The new goal thus became to develop a method by which polarity could be adjusted in a more simplistic way than altering all four of the groups examined earlier.

Design and Synthesis of Merle's 34 and 38

The initial concept for a more adaptable A-, B-ring 'functional domain' was based on attaching esters of varying carbon chain length at the C7 and C13 positions to control lipophilicity. This approach had the added advantage of being directly amenable to the group's well-established pyran annulation chemistry. The first analogue chosen to test the feasibility of this strategy was Merle 34, which has a calculated partition coefficient (ClogP) equal to 1.36 resulting from a C7 acetate and C13 propionate. Merle 38 was also targeted because it would come from an intermediate in the Merle 34 synthesis, and would also be a more polar top half than bryo 1 (Figure 2.7).

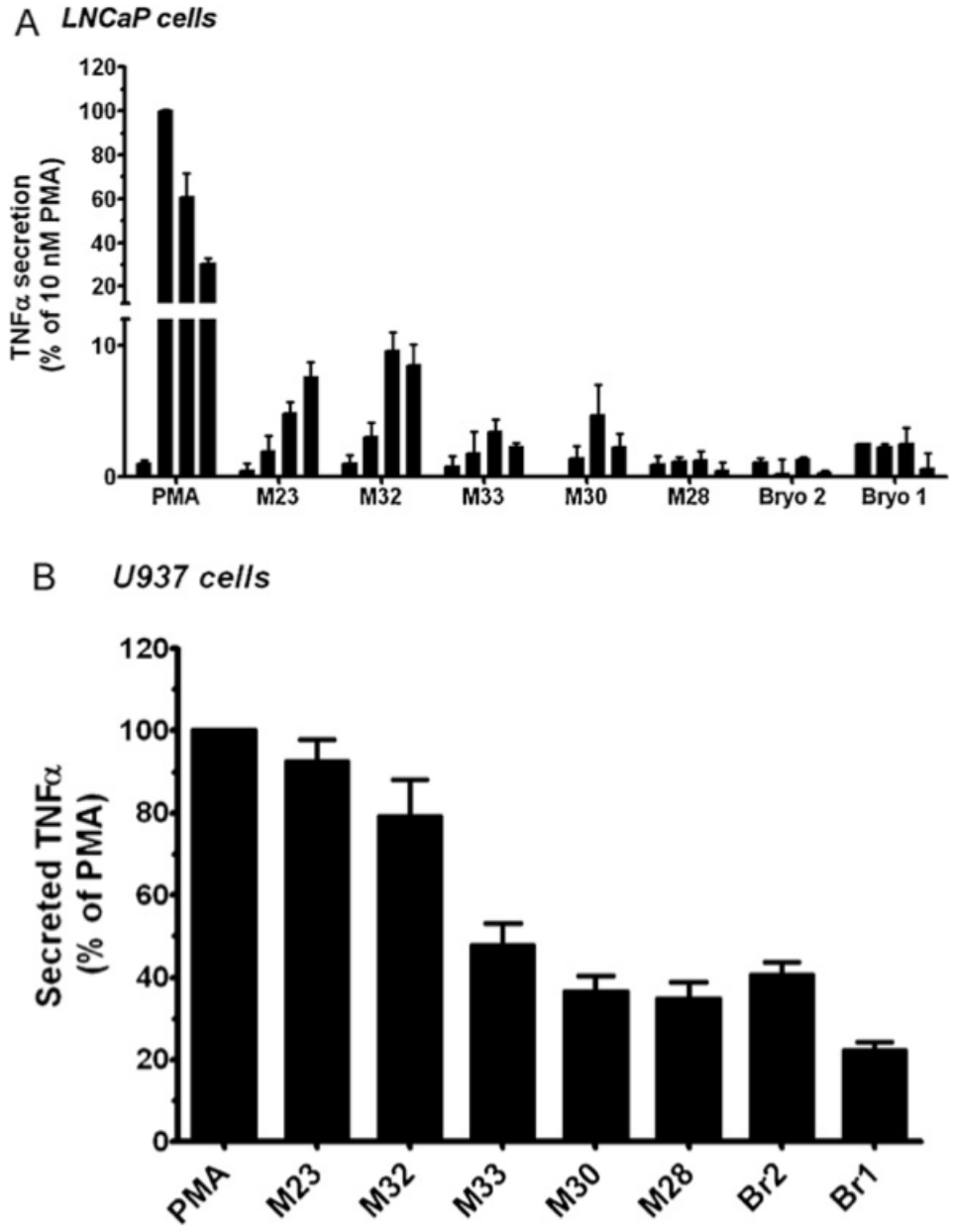


Figure 2.6. TNFα secretion by analogues.

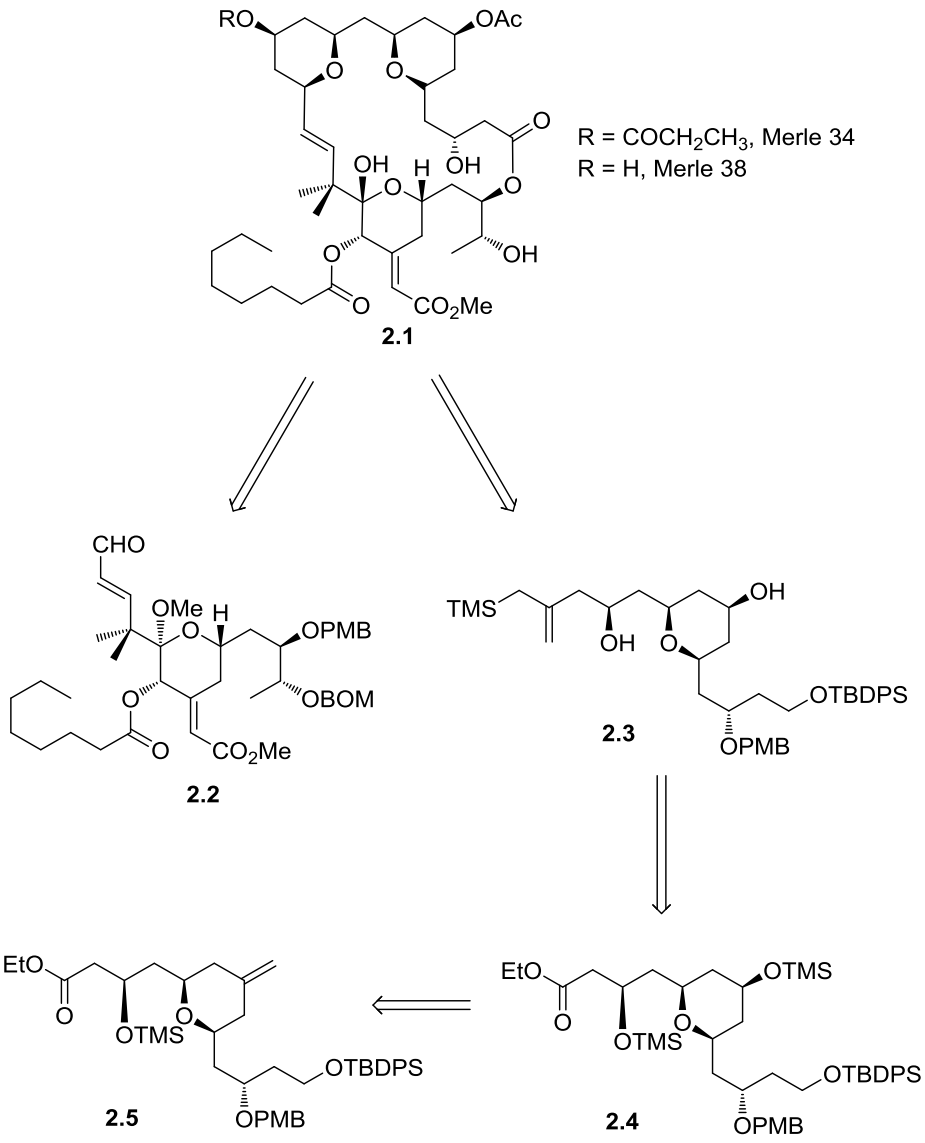


Figure 2.7. Retrosynthesis of Merle 34 and 38

Dr. Rudra designed and executed the synthesis of these two analogues. From a retrosynthetic perspective Merle 34 and 38 were envisioned to come from the convergent union of A-ring β-hydroxyallyl silane **2.3** and fully functionalized C-ring **2.2** described in Chapter 1 (Figure 2.7). The A-ring subunit would then come from bis-TMS protected **2.4**, which is derived from the previously used intermediate **2.5**.

Starting from intermediate **2.5**, available in 18 total steps following the route originally developed by Dr. Li (Chapter 1, Figure **1.56**), Dr. Rudra first cleaved the C7 olefin with ozone and reduced the resulting ketone to an alcohol with $NaBH_4$ (Figure 2.8). Alcohol **2.6** was obtained as a single diastereomer resulting from axial attack of the hydride. Protection with TMSCl was followed by a Bunnelle reaction[9] accessed β-hydroxyallyl silane **2.3**.

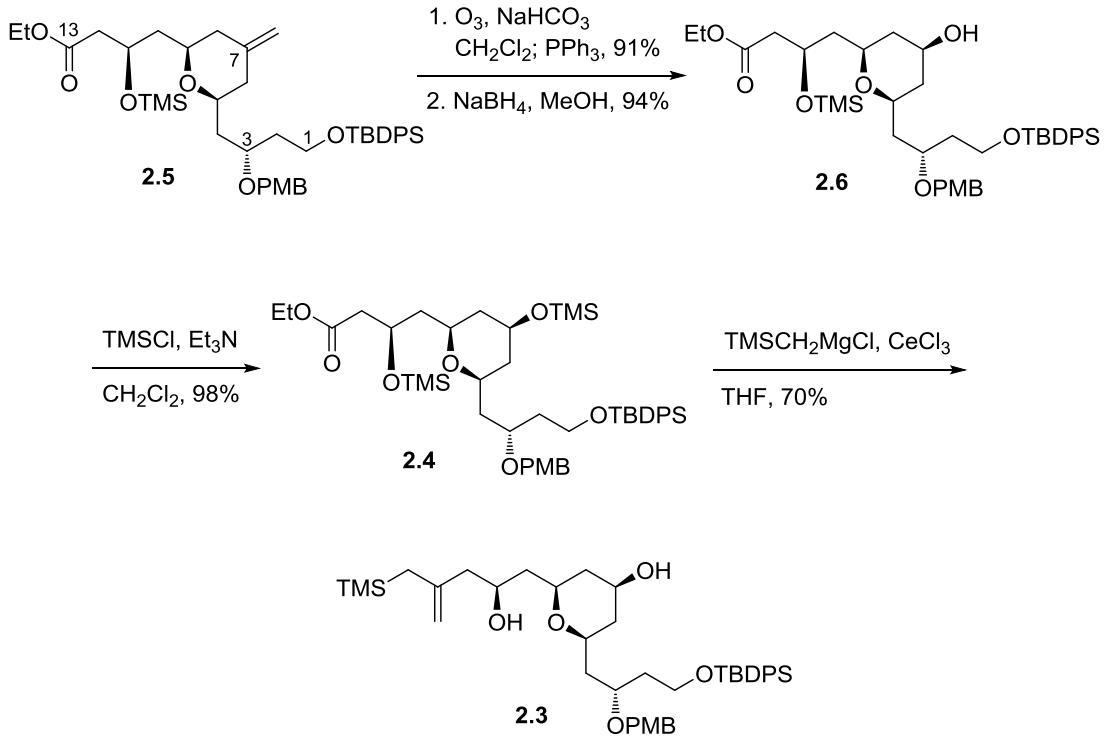


Figure 2.8. A-ring synthesis

The A- and C-ring portions were joined using a pyran annulation reaction forming the B-ring and providing the full carbon skeleton (Figure 2.9). The C1 TBDPS group was removed and the compound advanced to macrolactone **2.8** as previously described for other analogues. At this point, the B-ring olefin could be cleaved by the slow addition of O_3 in a solution of CH_2Cl_2 and the ketone was reduced with $NaBH_4$ to the equatorial alcohol in the 7:1 mixture of diastereomers. Merle 34 was accessed by esterification of the C13 alcohol with propionic anhydride followed by global deprotection, while Merle 38 resulted from the global deprotection of intermediate **2.9** (Figure 2.10).

Merle 34 and 38 have binding affinities of 16.2 and 13.2 nM respectively, which implies that C13 substituents with an (*S*) configuration have a negative effect on binding.

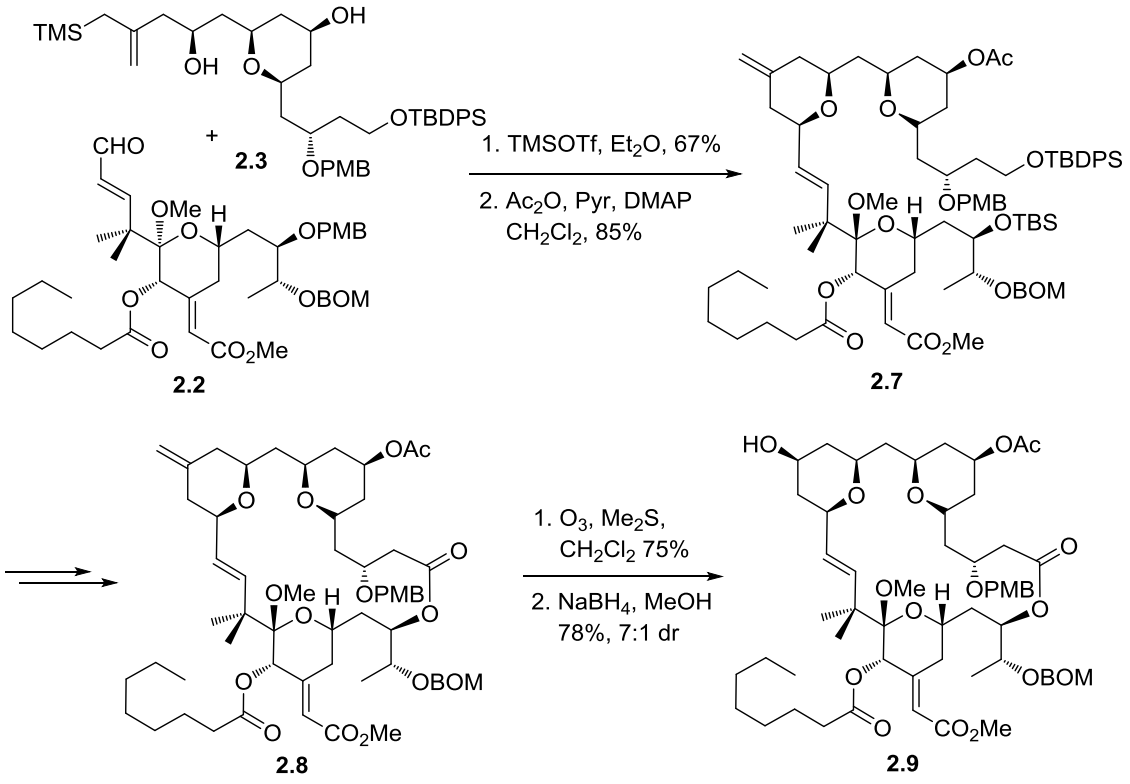


Figure 2.9. Coupling and elaboration to the macrolactone

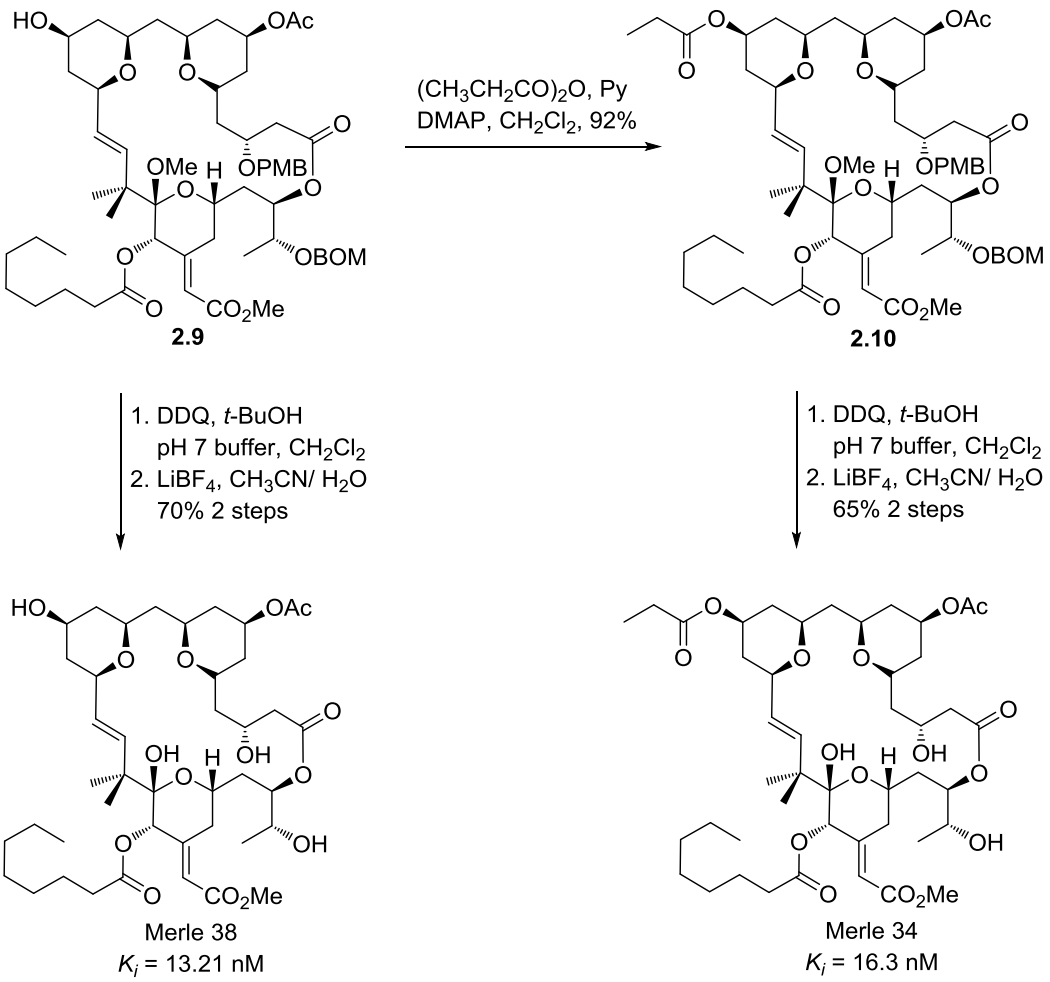


Figure 2.10. Completion of Merle 34 and 38

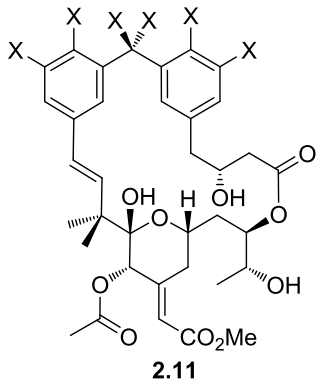
Contrastingly, loss of binding is not observed in a series of dioxirane B-ring analogues made by the Wender group with various substituents in the (*S*) configuration.[10] When tested in the U937 assay, both analogues were intermediate between PMA and bryo 1, and both were approximately 100-fold less potent than bryo 1 and other analogues. Merle 34 was remarkably similar to Merle 33, the C7 acetate and C13 enoate analogue, except for being less potent. These results suggest that having an sp3 center at C13 is disadvantageous for binding and biological potency; however, the opposite C13 diastereomer was not tested.

Results and Discussion

Design of aromatic A- and B-ring analogues

Based on our modest success with using esters and alcohols at C7 and C13 we were encourage to continue trying to modulate polarity on the A- and B-rings, and thereby, control the resulting biology. A significant shortcoming of previous approaches was that they required many steps, making each analogue a significant challenge. We therefore sought to discover a new strategy that would facilitate the rapid assembly of highly diverse A- and B-rings from simple building blocks in a convergent or 'Lego' like fashion.

Switching from pyran rings to aromatic rings was an attractive way to simplify the synthesis of A-, B-ring analogues (Figure 2.11). Aromatic rings would have several advantages. First, they do not contain any stereocenters, which will greatly simplify their synthesis. Second, there are numerous diverse aromatic building blocks commercially available that would require minimal functional group manipulations. Finally, they can be easily diversified further through cross-coupling methodologies should any of the 'X' labeled position be an aryl triflate or halide.



2.11

Figure 2.11. Hypothetical aromatic analogues

Synthesis of Merle 40

To test the ability of aromatic rings to act as surrogates for the natural pyran rings we set out to synthesize the simplest and most unadorned A-, B-ring system (Figure 2.12). The new analogue, Merle 40, was believed to be obtainable by splitting the molecule in half using a Heck reaction between B-ring bromide **2.14** and C-ring olefin **2.12**, and using a Yamaguchi esterification between a C26 alcohol and a C1 acid. This strategy was intended to be flexible allowing the Heck and esterification reactions to be done in either order. The sole top-half stereocenter at C3 would come from a catalytic asymmetric allylation (CAA) and the A-, and B-rings would be joined using a Suzuki cross coupling reaction. The fully functionalized C-ring was to come from known glycal **2.13**[11] through a series of regio- and stereo-specific oxidations. At the outset, we were aware that due to the low reactivity of the C16-C17 olefin, particularly in cross metathesis reactions,[12] that this was a rather aggressive and likely challenging approach; however, since the majority of the chemistry leading to olefin **2.12** was already developed it was worth pursuing.

Synthesis of the top half commenced by coupling B-ring benzyl bromide **2.15** with the Molander salt derived from 3-formylbenzeneboronic acid (Figure 2.13).[13] Aldehyde **2.17** was homo-elongated using a Wittig reaction with triphenyl-(methoxymethyl)-phosphonium chloride followed by acidic work-up of the resulting enol-ether to give to homobenzyl aldehyde.[14] Aldehyde **2.18** was highly unstable necessitating its immediate use in the subsequent CAA reaction, which accessed alcohol **2.19** in modest yields but excellent enantioselectivity. The BOM group was chosen so that only one global deprotection step would be needed at the end of the synthesis. The terminal olefin of

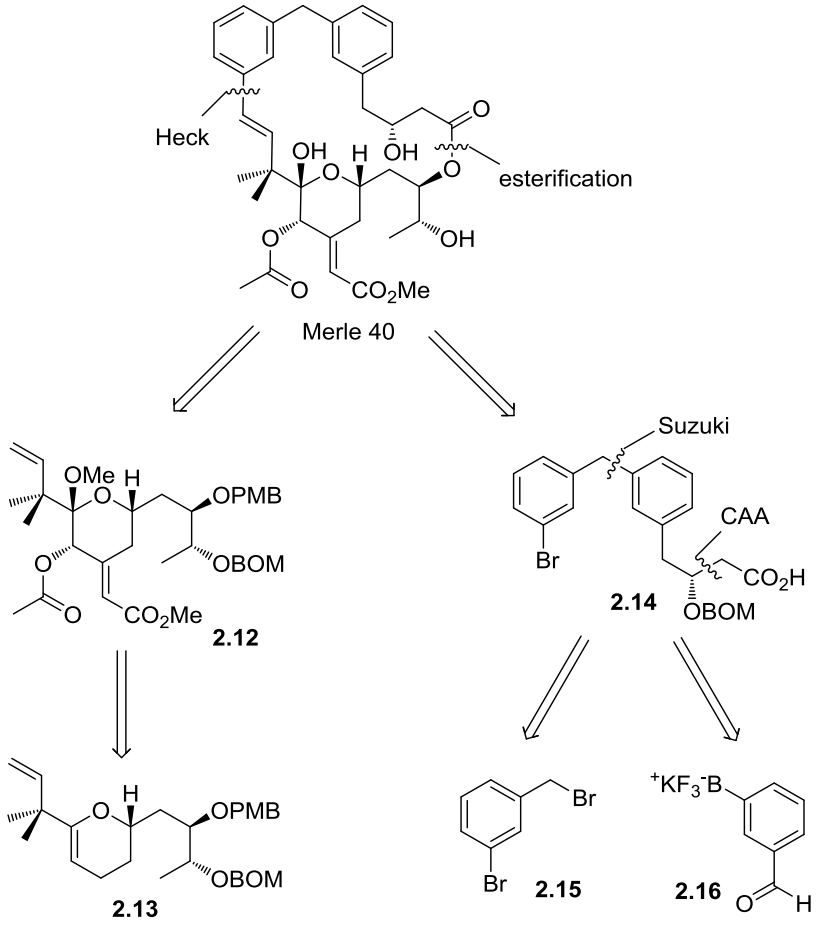


Figure 2.12. First generation retrosynthesis

2.19 was cleaved with ozone and oxidized to the acid[15] completing the synthesis of the desired A-, B-ring system in merely 7 total steps compared to the 19 required for the simplest of the bis-pyran analogues.

The route to the C-ring, originally developed up through ketone **2.27** by Dr. Troung,[11] started with chiral alcohol **2.21** that had been synthesized in excess of 70 grams in chapter 1 (Figure 2.14). TBS protection of the free alcohol was followed by hydroformylation using Buchwald's ligand **2.26**.[16] Prenyl indium addition[17] installed the gem-dimethyl moiety and the resulting alcohol was oxidized to the ketone.[18]

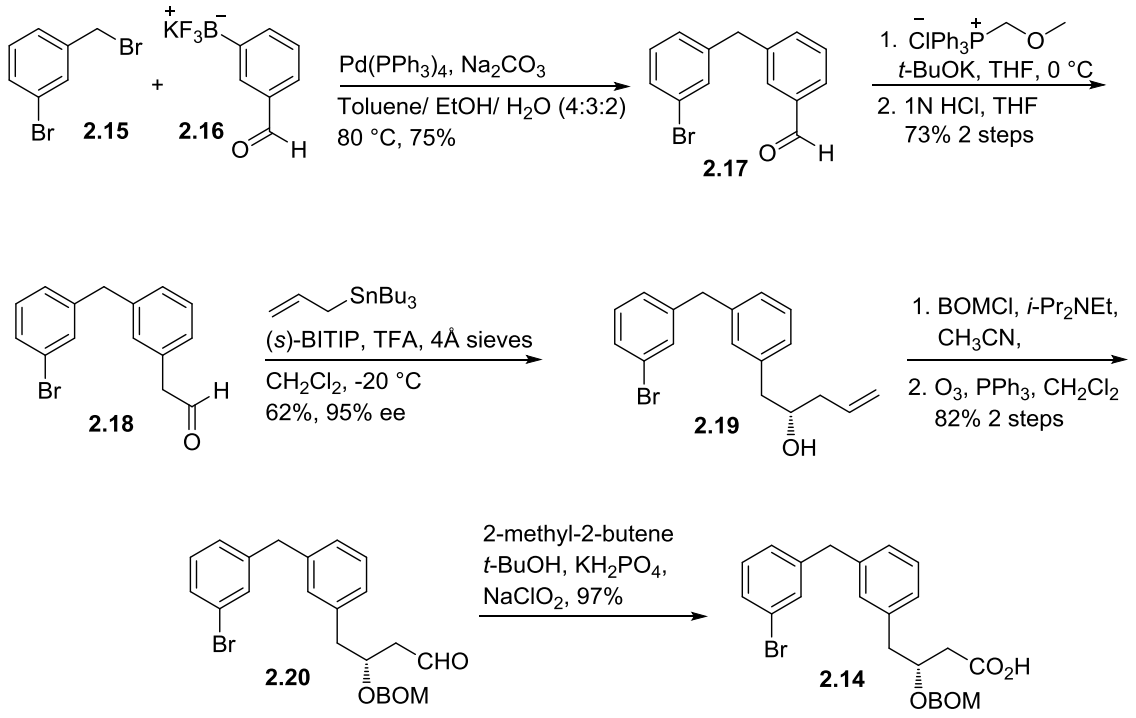


Figure 2.13. Synthesis of aromatic A-, B-rings

Removal of the C23 TBS ether and cyclizing under dehydrating conditions accessed glycal **2.13**.

Glycal **2.13** was first oxidized with MMPP in MeOH so that the epoxide was opened *in-situ* and trapped exclusively as the anomeric methyl ketal (Figure 2.15). The free alcohol at C20 was immediately oxidized to the ketone using Dess-Martin conditions.[19] The exocyclic enoate was installed through an aldol addition and *in-situ* elimination resulting in a single olefin isomer that was dictated by developing A-1,3 strain with the vicinal ketone.[20] The final two steps were the reduction of the C20 ketone and esterification of the free alcohol with acetic anhydride. The acetate ester was chosen because it had already been demonstrated that the acetate can be removed late stage and replaced with other esters.[21]

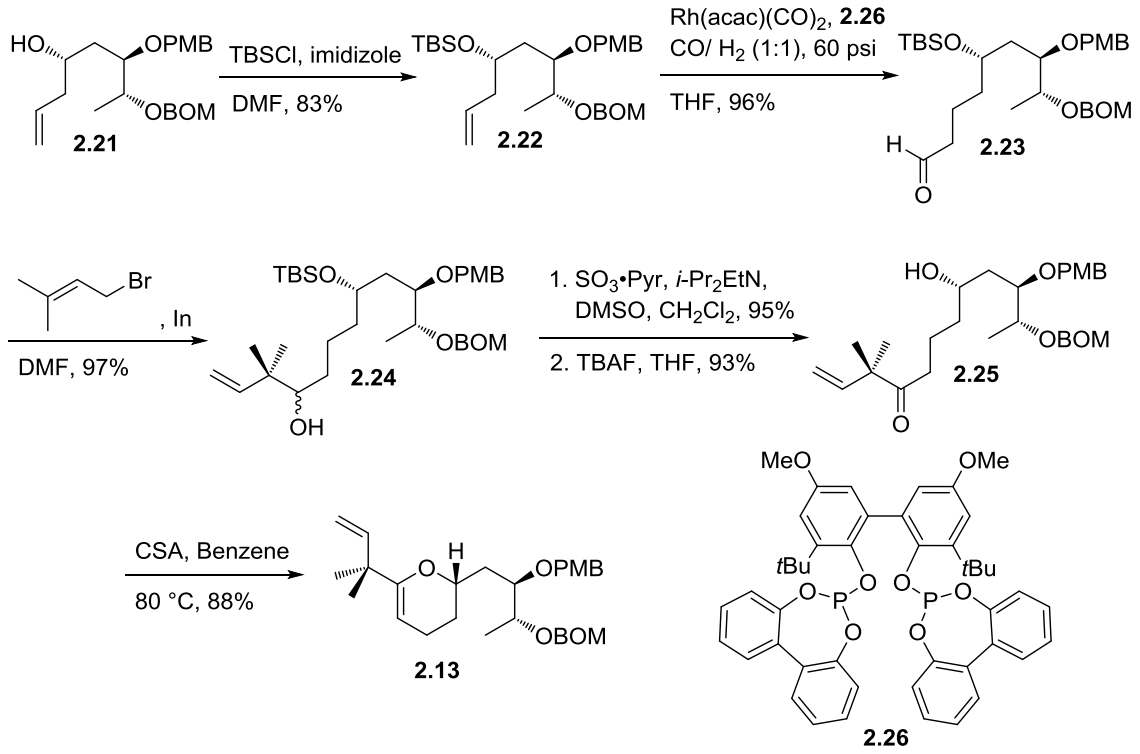


Figure 2.14. Synthesis of glycal **2.13**

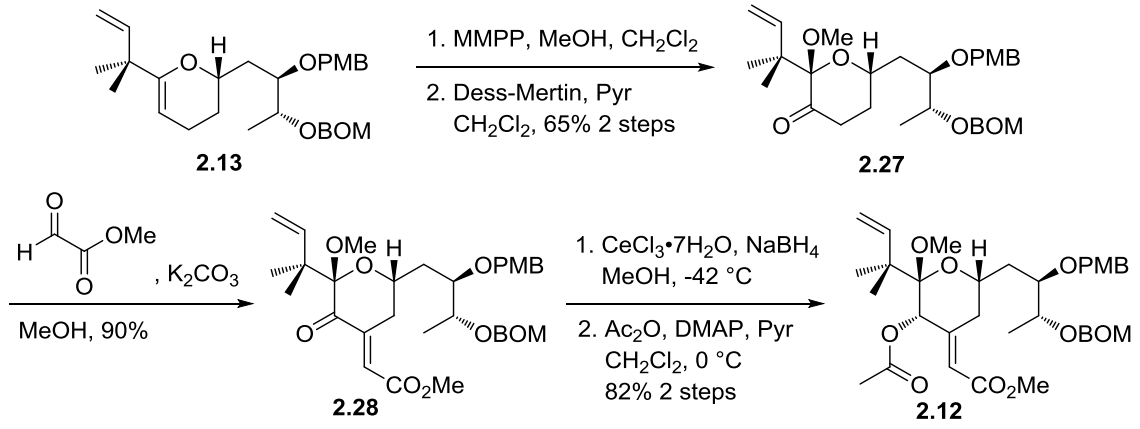


Figure 2.15. Completion of the fully functionalized C-ring

Having successfully synthesized both fragments, focus was directed towards coupling them. Although this route was designed to be flexible allowing the Heck and esterification reactions to be conducted in either order, we suspected that the Heck would work better if performed first. Rather than screening catalyst conditions on the valuable fully functionalized C-ring, bromobenzene and intermediate **2.27** were used as a model reaction (Figure 2.16). We were pleased to discover that the first set of conditions attempted were very effective at carrying out this reaction with good yield and the product was obtained exclusively as the E-isomer.[22]

Unfortunately, this result did not translate to the desired substrate for which multiple problems were observed (Figure 2.17). The first issue discovered was that the fully functionalized C-ring was slightly unstable to prolonged exposure to strong bases at elevated temperature leading to internalization of the exocyclic enoate **2.31**. Similar sensitivities to basic conditions have also been reported by Wender[10] and Evans.[20] The Heck reaction also failed to proceed when simplified C-ring **2.27** was used. Recovery of the de-brominated B-ring indicates that oxidative addition is occurring but the reaction is stalling out during the migratory insertion step. This failure was rationalized as being due to the increased sterics, compared to bromobenzene, as well as the C1 acid

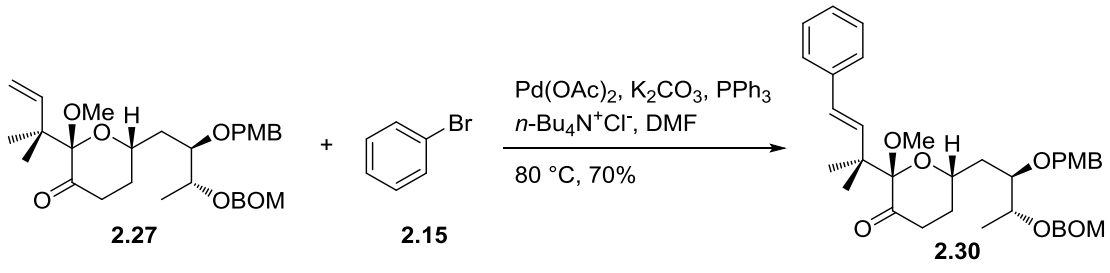


Figure 2.16. Heck model reaction

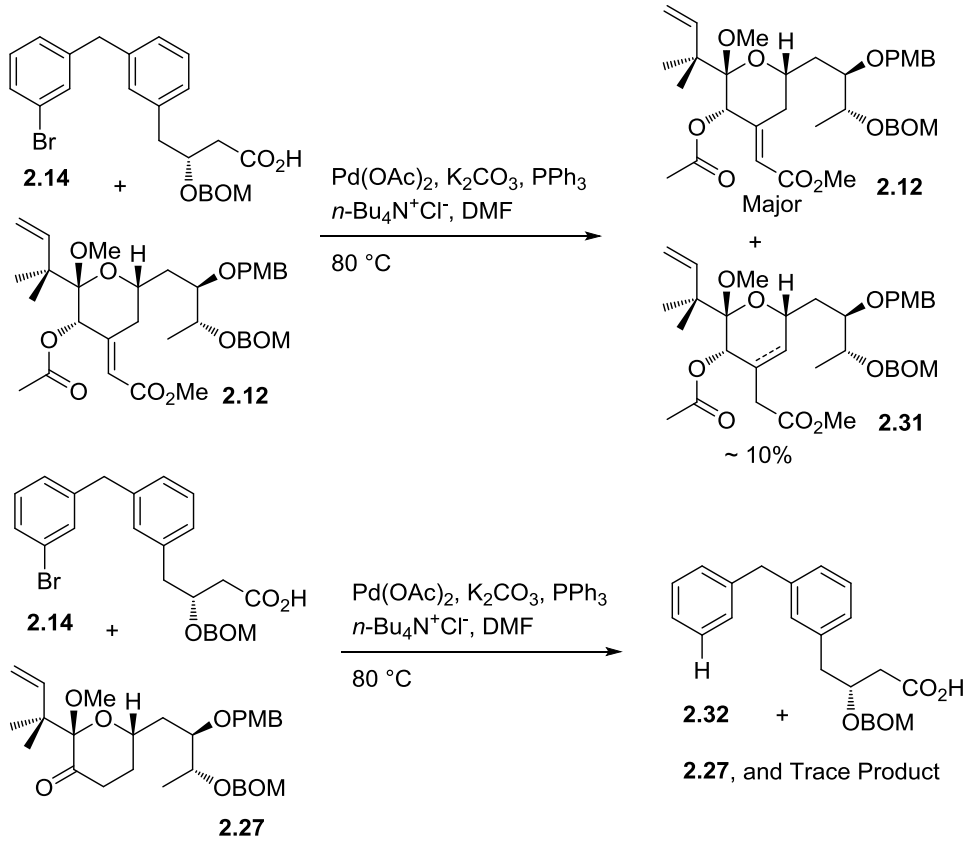
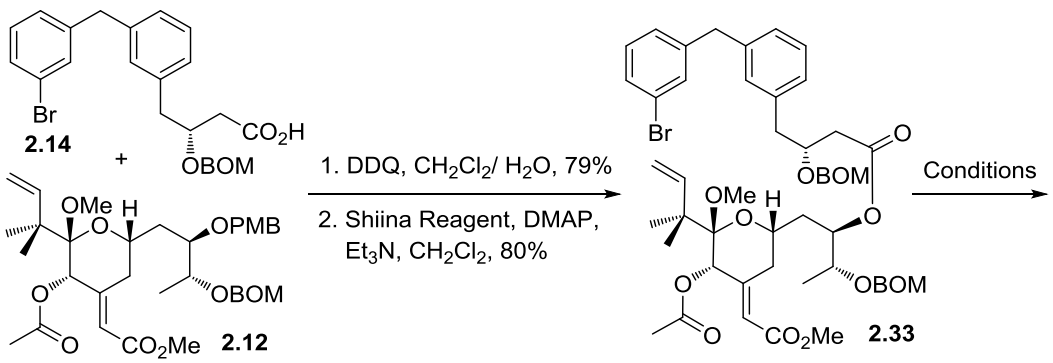


Figure 2.17. Heck reaction with Jeffery conditions

possibly acting as a chelating ligand effectively blocking a necessary coordination site on palladium.

At this point, performing the esterification first followed by an intramolecular Heck was explored. Removal of the PMB protecting group followed by coupling using Shiina's reagent[23] afforded **2.33** in excellent yield (Figure 2.18). The Heck reaction under Jeffery conditions again only produced the de-brominated product along with significant C-ring isomerization/decomposition. Exchanging K_2CO_3 for less basic $KHCO_3$ or KOAc failed to remedy stability issues, and oxidative addition failed with amine bases. Replacing the solvent with toluene, acetonitrile, or THF also resulted in



Conditions	Result
Jeffery w/ K_2CO_3, KOAc, $KHCO_3$	Debromination/ C-ring isomerization
Jeffery w/ amine basses	No oxidative addition
$Pd(P(t\text{-}Bu)_3)_2$, Et_3N, DMF	No oxidative addition
$Pd(OAc)_2$, SPHOS, Et_3N	Debromination
$Pd(P(t\text{-}Bu)_3)_2$, $Pd_2(dba)_3$, Cy_2NMe, Toluene, rt	Trace product/ debrominated

Figure 2.18. Attempted intramolecular Heck reaction

failure to undergo oxidative addition. By now it had become apparent that the triphenyl phosphine ligands were not electron donating enough to accomplish this reaction under mildly basic conditions. Using $Pd(P(t\text{-}Bu)_3)_2$ with Et_3N in DMF gave no reaction. Using the Buchwald ligand SPHOS[24] and Et_3N the catalyst underwent oxidative addition to give the de-brominated product, with minimal decomposition or isomerization. Unfortunately, changing the base, temperature, and solvent failed to facilitate the insertion step with this catalyst. In 2001, the Fu group reported the use of $Pd/P(t\text{-}Bu)_3$ in a 1:1 ratio, and Cy_2NMe as a versatile catalyst for the Heck reaction of aryl bromides with sterically encumbered olefins at rt.[25] Under these conditions some

product formation was finally observed albeit only in trace amounts. Heating, long reaction times, and high catalyst loadings did not provide any improvement. A possible explanation for observing minimal product formation but complete de-bromination is that migratory insertion is taking place but that the compound cannot adapt a conformation that would allow for β-hydride elimination and therefore undergoes β-aryl elimination instead.

An attractive alternative to the Heck reaction would be use either a Stille or Suzuki cross coupling reaction. Both reaction types would extend the reaction one carbon further away from the C18 gem-dimethyl alleviating the need to have a bulky Pd species at C17. However, since both the Stille and Suzuki reactions would require additional functional group manipulations to access a vinyl stannane or borane the Heck was given one final consideration. Based on previous results, the intermolecular reaction was going to be necessary as well as not having C1 at the acid oxidation state. Additionally, the C3 BOM group was replaced with a TES group because it would still be labile to the $LiBF_4$ deprotection conditions, but could also be removed selectively should the need arise. Following protection of the C3 alcohol with TESCl the terminal olefin **2.34** was cleaved with O_3, and reduced to the primary alcohol (Figure 2.19). Both the Jeffery and Fu conditions were attempted on this substrate. The Jeffery conditions gave no product but the Fu conditions gave the product in yields consistently in the 70% range along with recovery of unreacted C-ring. Increasing reaction times or using up to a three-fold excess of the bromide failed to drive the reaction any further. Considering the complexity of these substrates and the known inert nature of the C16-C17 olefin this reaction is remarkable efficient, and being able to conduct the reaction at

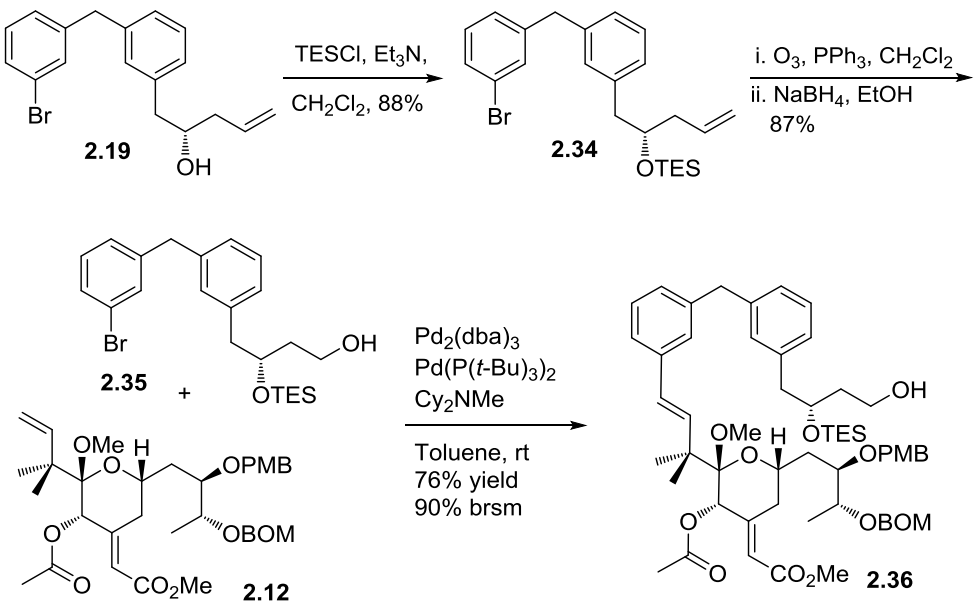


Figure 2.19. Successful Heck reaction

rt in only 16 h avoided all of the C-ring stability issues previous observed using harsher conditions.

Completion of the analog from this stage only required five steps (Figure 2.20). Oxidation of alcohol **2.36** to the acid over 2 steps provided carboxylate **2.37**. Removal of the PMB ether with DDQ gave an intermediate seco-acid, which required extensive purification prior to macrolactonization due to decomposition if any DDQ side products were present when forming the mixed anhydride.[26] Macrolatonization proceeded in good yield to provide analogue precursor **2.38**. Unfortunately, exposure of **2.38** to our standard $LiBF_4$ at 80 °C deprotection conditions completely destroyed the compound yielding no usable material. This is not the first analogue to be unstable under these conditions. Dr. Kraft and Dr. Chavez both encountered similar issues while trying to remove the BOM group from Merle 29 and 31 (Figure 2.21), respectively. Dr. Kraft was able to solve this problem by first removing the BOM by transfer hydrogenation

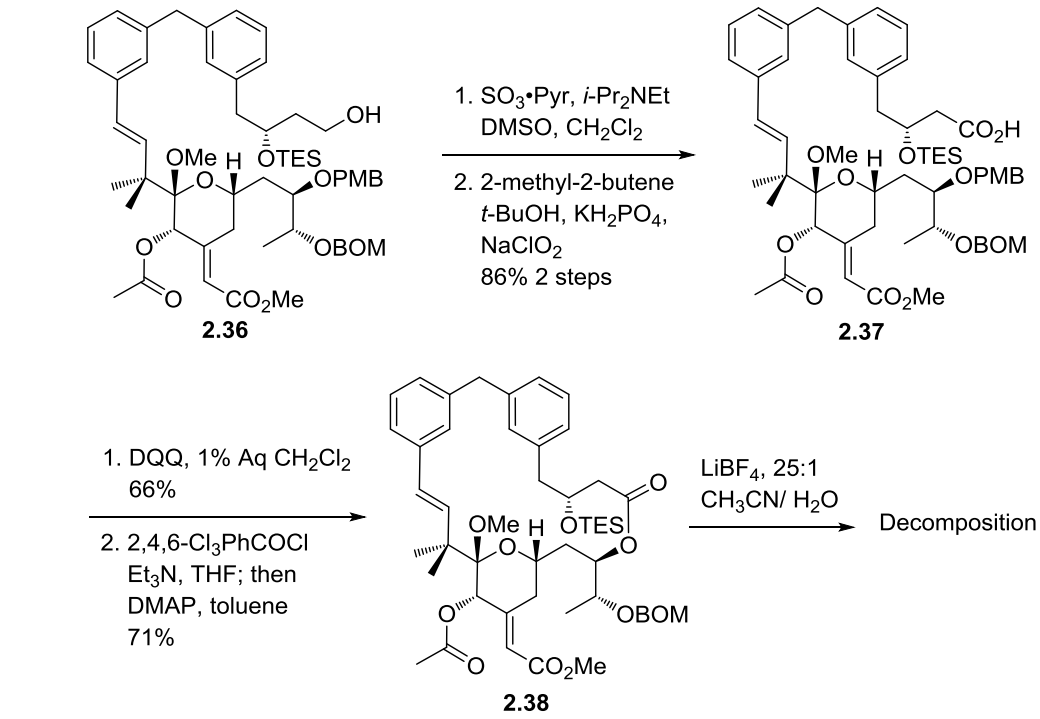


Figure 2.20. Attempted completion of Merle 40

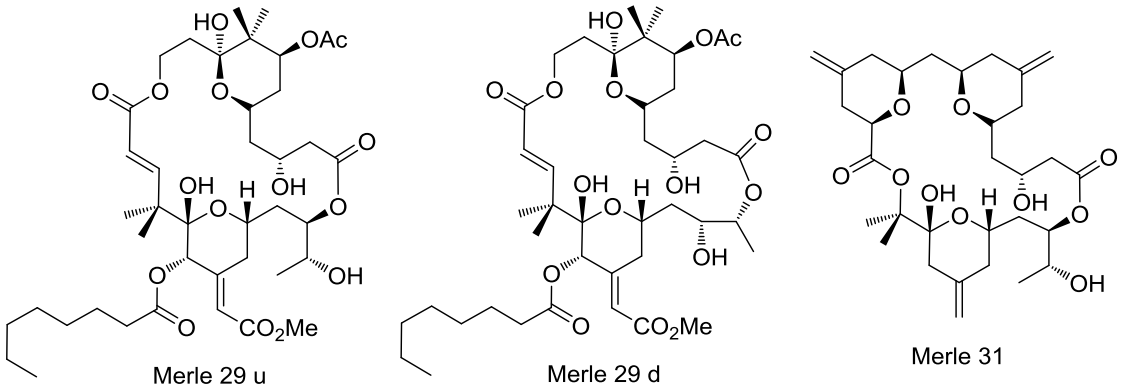


Figure 2.21. Other analogues unstable to $LiBF_4$

followed by cleavage of a C3 BPS with HF•Pyr and the C19 methyl ketal with $LiBF_4$ at 50 °C.[4] Conducting the reactions in this order resulted in the major product being the ring expanded compound Merle 29d. However, doing the hydrogenation last decomposed the analogue. Dr. Chavez was able to deprotect the BOM group with BF_3•OEt_2/DMS in dichloromethane after first cleaving a C3 PMB with DDQ and hydrolyzing the methyl ketal with $LiBF_4$ at 50 °C.

Transfer hydrogenation[27] of the new bis-aromatic analog resulted in reduction of the C16-C17 olefin along with considerable decomposition (Figure 2.22). Removal of the TES and methyl ketal was accomplished efficiently with aqueous HF in acetonitrile; however, exposure to the previously used BF_3•OEt_2 conditions provided only the eliminated product **2.41**. Bromocatechol borane at -78 °C[28] was also tried to no avail.

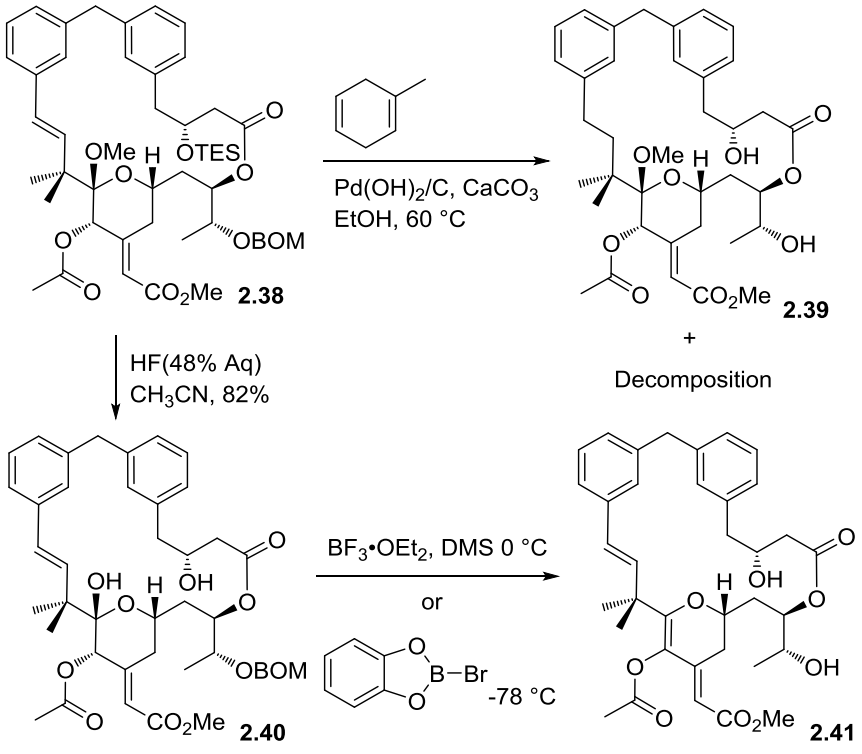


Figure 2.22. Alternative deprotections

Considering that the C21 exocyclic enoate would not be stable to dissolving metal conditions and that screening for a specific ‘goldilocks’ Lewis acid or hydrogenation catalyst would be time consuming and not likely to yield a successful result, attention was focused on removing the BOM at an earlier stage.

The major challenge associated with earlier removal of the BOM was selectivity in the presence of the PMB and methyl ketal groups. This immediately ruled out using Lewis acidic or hydrogenation conditions. The only example of selectivity favoring BOM cleavage over PMB was with the use of Lithium 4,4’-ditertbutylbiphenyl (LiDBB) by Roush and co-workers;[29] however, for our purposes this reaction would need to be done prior to installment of C21 enoate. To accommodate all these constraints glycal **2.13** was chosen as the best place to attempt BOM removal (Figure 2.23). The dissolving metal reaction proceeded in modest yield along with recovered starting material and a product lacking both the BOM and PMB. Nevertheless, the free alcohol could be protected as the TBS ether **2.42** and advanced.

Strangely, the epoxidation/ methanolysis/ oxidation sequence provided the methyl ketal as a 2.5:1 mixture of diastereomers rather than the near exclusive axial methyl ketal that was observed with a BOM group at C26. Presumable, the axial methyl ketal is the thermodynamic product. Therefore, the observed mixture likely resulted from kinetic control. Inserting a thermodynamic isomerization step with monochloroacetic acid rectified the problem giving **2.43** as the sole product without loss of yield after Dess-Martin oxidation.[20] Installation of the C21 enoate and C20 acetate proceeded smoothly to give the C26 TBS C-ring **2.44**. Heck coupling of the new C-ring did not progress as far but gave the same yield as before based on recovered C-ring.

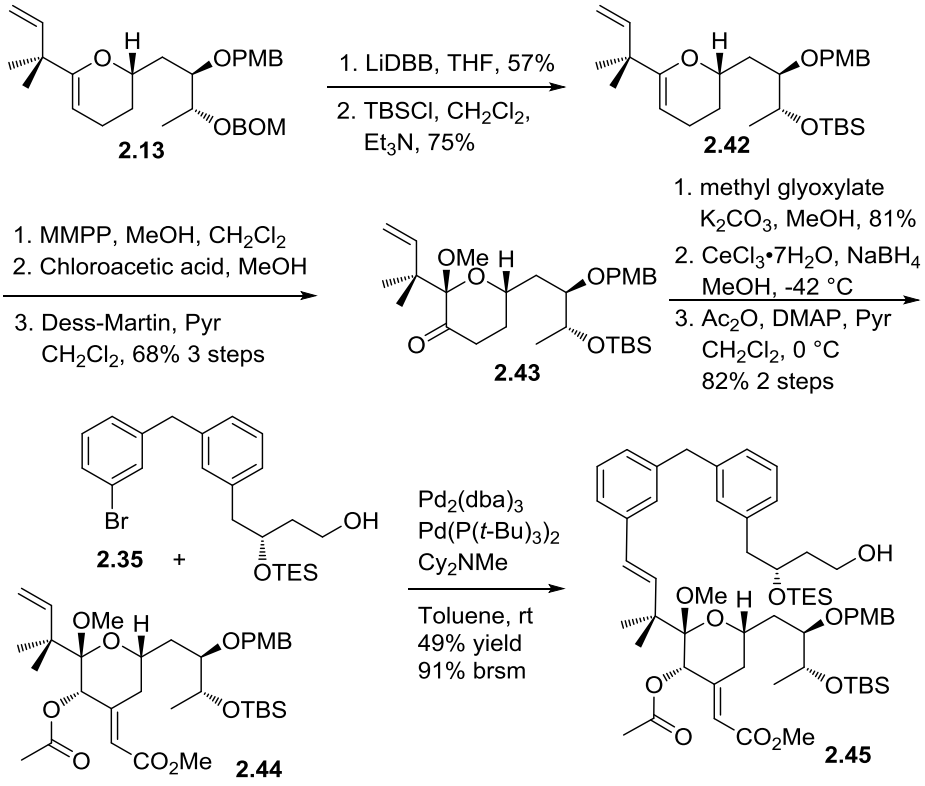


Figure 2.23. Replacing BOM with TBS

Alcohol **2.45** was oxidized to the acid, the PMB group removed, and the compound was cyclized using Yamaguchi conditions[26] as described previously (Figure 2.24). Aqueous HF had previously been used to successfully remove the TES and methyl ketal. However, trying to remove all three protecting groups under these conditions gave a mixture of three products in approximately equal proportion. The eliminated product **2.41** could be separated by column chromatography, and the ring expanded compound **2.47** could be separated by successive elution on a TLC plate with 30% acetone/benzene. Unfortunately, the desired analogue was not stable to successive elution on a preparatory plate resulting in decomposition and reintroduction of the eliminated compound.

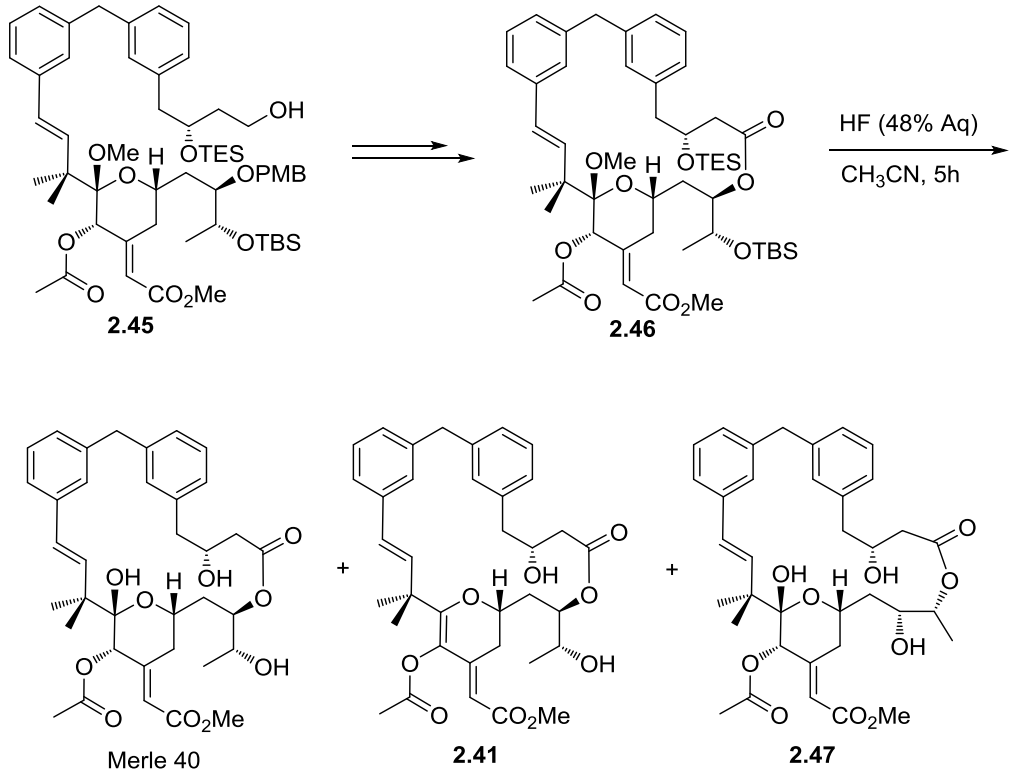


Figure 2.24. Attempted deprotection

To avoid the ring expanded compound, deprotection of the TES and methyl ketal first followed by cleavage of the TBS under buffered HF•Pyr conditions was attempted (Figure 2.25). Complete removal of the ketal required 2 h at which point the reaction was quenched, and this fortuitously yielded only two products. Along with the TBS protected compound **2.48**, Merle 40 was obtained contaminated with only trace amounts of **2.47**. Deprotection of **2.48** with the buffered conditions required >24 h and resulted in a 1:1 mixture of Merle 40 and ring expanded **2.47**. However, having obtained sufficient quantities of Merle 40 in the first reaction it was sent to the Blumberg group for evaluation.

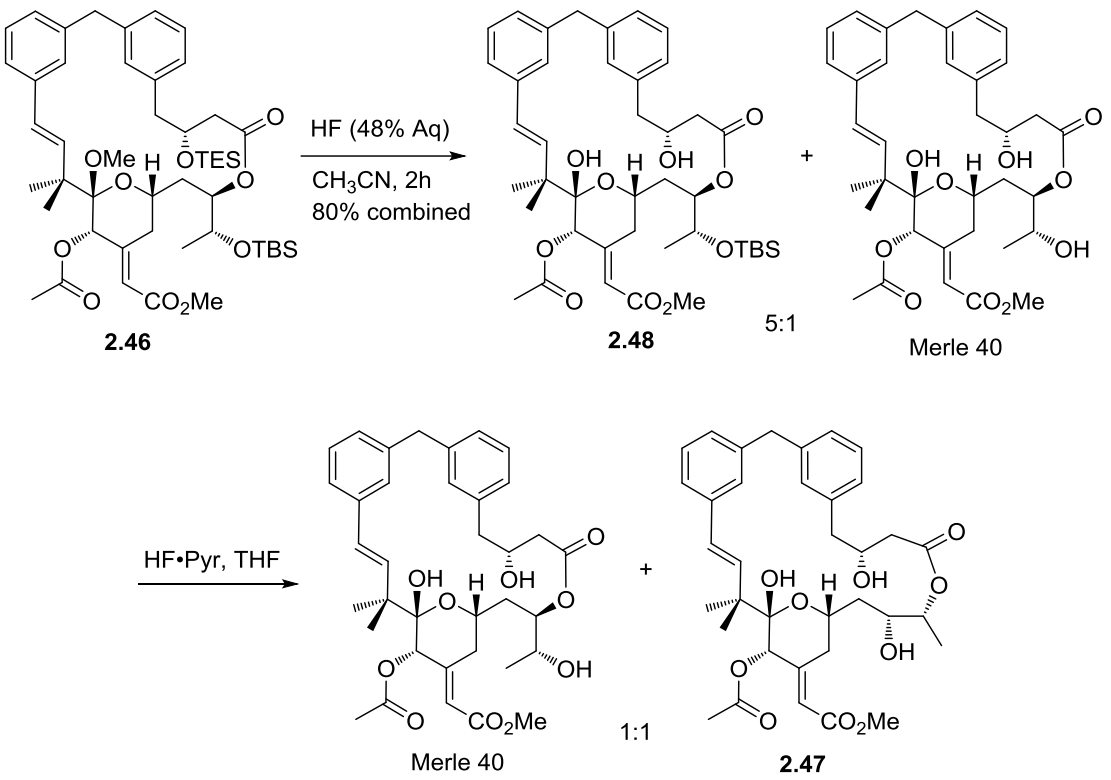


Figure 2.25. Successful deprotection of Merle 40

Biological evaluation of Merle 40

Surprisingly, Merle 40 displayed a K_i = to 971 nM. Such a result was entirely unexpected considering that the entire C-ring 'binding domain' was intact, and that even if the analogue was undergoing ring expansion during the assay that should only result in a minor loss in binding similar to what was seen for Merle 29u and d. Merle 40 also displayed a dramatic loss in biological potency in U937 cells, where no response was observed until 20 μM was applied (Figure 2.26). The lack of potency is represented even more dramatically in Toledo cells, which experience growth inhibition in response to both PMA and bryo 1 (Figure 2.27). In Toledo cells, Merle 40 is four orders of magnitude less potent than either PMA or bryostatin 1.

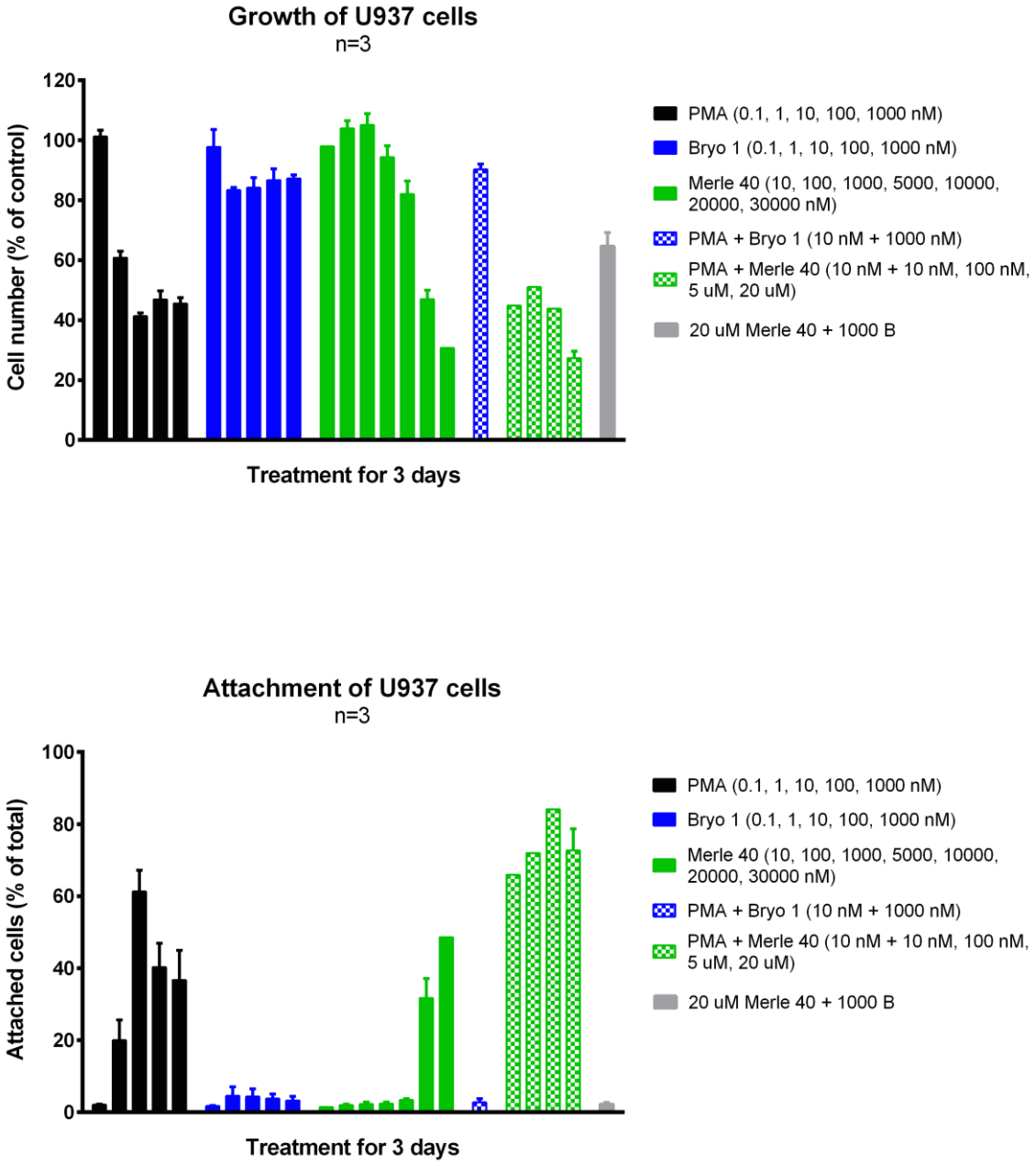


Figure 2.26. U937 proliferation and attachment assay

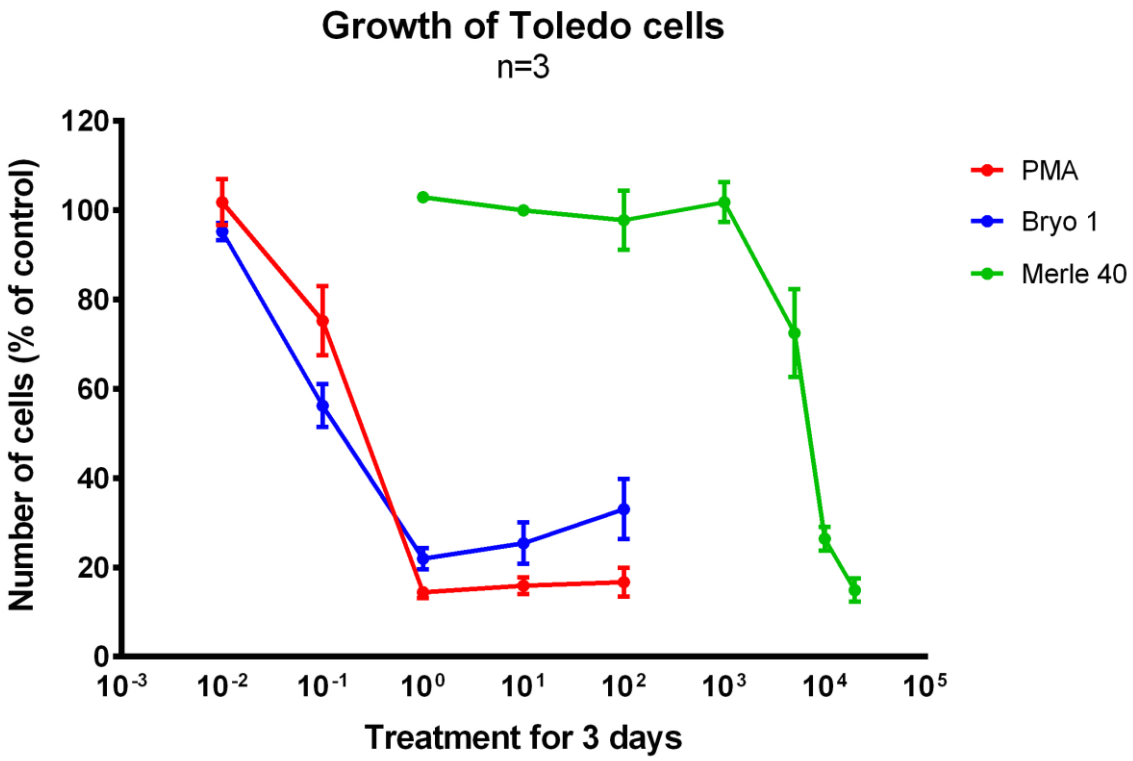


Figure 2.27. Growth inhibition of Toledo cells.

Despite Merle 40's lack of potency and therefore limited potential as a medicinal compound it does provide the opportunity to learn more about the subtleties of bryo 1's structure function relationships. The two most distinct differences are the lack of the internal hydrogen-bonding network, present in bryo 1 and other analogues, and the aromatic rings being flat and more rigid than pyrans. Simple plastic models indicated that Merle 40 was at least capable of adapting a near identical conformation as seen in the bryo 1 crystal structure without considerable strain. Bryostatins and analogues are known to have highly rigid structures due to a strong internal hydrogen-bonding network (Figure 2.28).[30] The loss of this network may allow for greater flexibility and for a different, nonbinding, conformation to dominate Merle 40's solution structure.

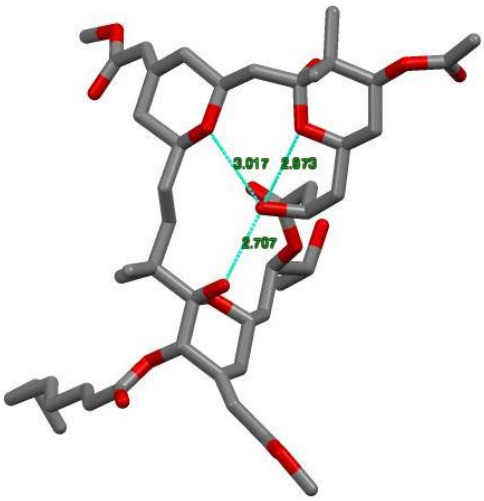


Figure 2.28. Bryostatin 1 crystal structure

Support for hydrogen bonding being required to maintain a proper binding conformation can be found by comparison with other known analogues (Figure 2.29). The binding affinity of Wender's analogue **2.49** drops from 3.4 nM to 297 nM when the C3 hydroxyl is removed in compound **2.50**. This is the same order of magnitude in binding loss that was observed with Merle 40, and was hypothesized, using computer modeling, to be a direct result of the internal hydrogen-bonding network not holding the C-ring in the proper conformation.[31] Similarly bryostatin 16, which lacks the C19 hemiketal, also losses binding affinity by two orders of magnitude. The H-bonding network may also be very important for preorganizing the ligand thus minimizing the entropy of binding, and without this preorganization entropy becomes a significant factor. To further explore the conformational changes in Merle 40 that resulted in such a dramatic change in binding we are currently pursuing both crystallizing the analogue as well as determining the NMR solution structure.

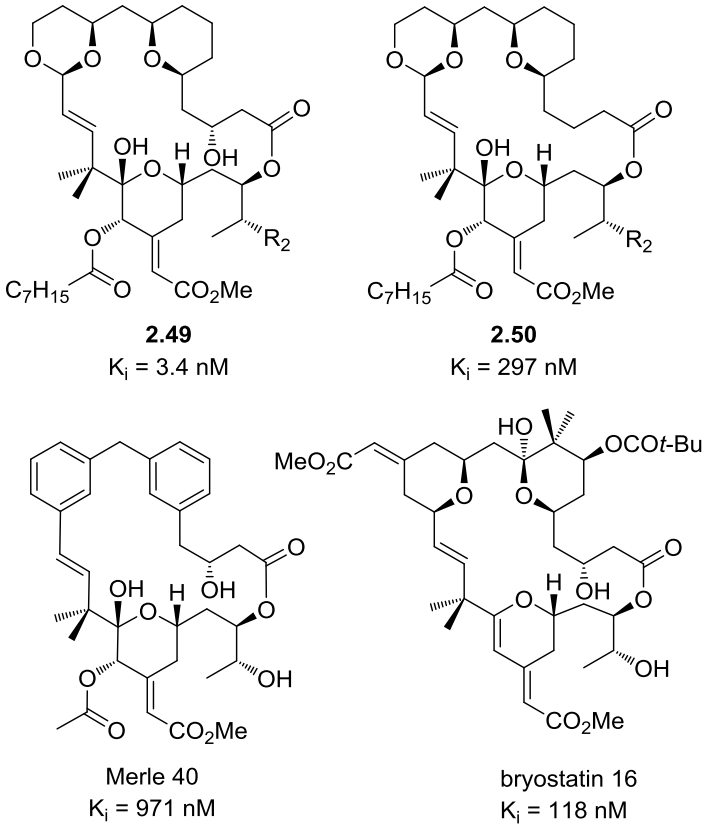


Figure 2.29. K_i of analogues lacking the internal H-bonding network

Future direction

In addition to evaluating the conformational differences between Merle 40 and high affinity PKC ligands, two new analogues have been proposed to further probe the role of the internal hydrogen-bonding network (Figure 2.30). Analogue **2.51** will utilize a pyran A-ring so that the role of the B-ring can be more directly examined. Based on our experience with Merle 29u and d, this analogue is expected to still suffer from a propensity to ring expand but may restore high affinity binding. If binding is in fact restored then the second analogue **2.52**, which contains a pyridine B-ring, could be used to directly explore the role of the hydrogen-bonding network with regard to compound stability. Even if the B-ring analogue **2.51** fails to correct the binding affinity issue, the

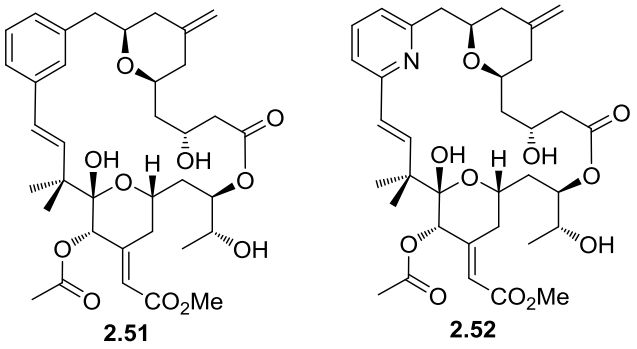


Figure 2.30. Proposed analogues

pyridine analogue **2.52** may still succeed at solving both the binding and ring expansion/ C19 elimination problems. Should **2.52** not resolve these issues then it is unlikely that anything other than a pyran type heterocycle could be used as the B-ring; however, if the pyridine is an acceptable modification then the project could move forward by replacing both the A- and B-rings with substituted pyridines.

Conclusion

In order to develop a simplified platform upon which the polarity hypothesis could be rigorously tested a new analogue, Merle 40 was synthesized. The C-ring portion was accessed in only 19 steps, and the novel A-, B-ring system was constructed in a mere 7 steps. The two fragments were combined through a Heck insertion, which is the only example of the C16-C17 olefin, of a fully functionalized C-ring, being utilized with good yield. Unfortunately Merle 40 is not a stable compound and is not a good ligand for PKC with a K_i = 971 nM. In spite of its shortcomings Merle 40 does however provide a great opportunity to learn more about the structural features that make bryostatin 1 such an amazing natural product. Additionally, insights gained from the

structural analysis will likely be highly valuable for the future design of simplified analogues. Based on this work as well as the work of former Keck group members and other groups our understanding of bryostatin structure function relationships has been advanced considerably (Figure 2.31).

Experimental Section

General experimental procedures

Diisopropylamine, diisopropylethylamine, pyridine, triethylamine, EtOAc, and CH_2Cl_2 were distilled from CaH_2. Reagent grade DMF, DMSO and acetone were purchased, stored over 4Å molecular sieves and used without further purification. Et_2O, THF, and toluene were distilled from Na under an atmosphere of N_2. MeOH was distilled from dry Mg turnings. The titer of *n*-BuLi was determined by the method of Eastham and Watson.[2] $TiCl_4$ was distilled prior to use. Zn was activated with aqueous HCl solution prior to use. All other reagents were used without further purification.

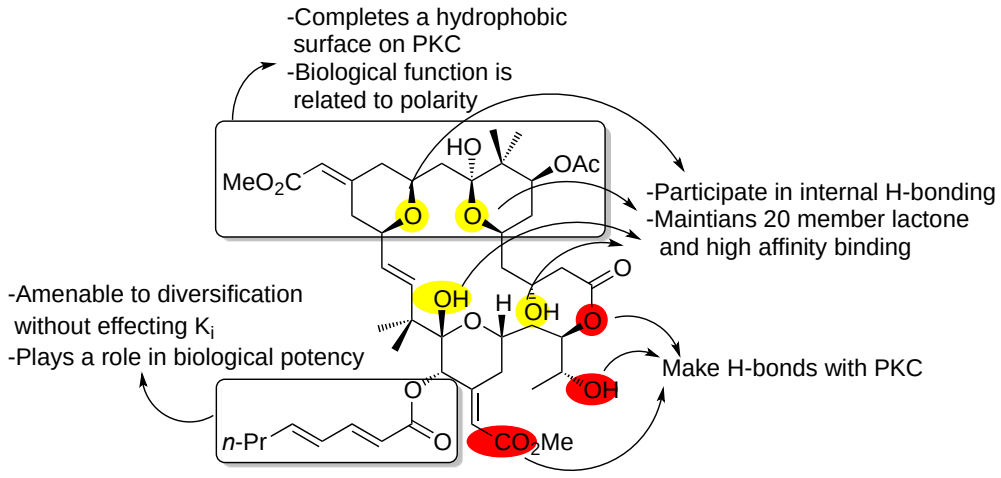
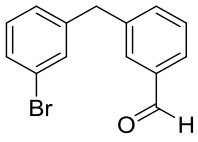


Figure 2.31. Bryostatin 1 structure function relationships

Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR). Thin layer chromatography was performed on Merck Kieselgel 60 Å F_{254} plates or Silicycle 60Å F_{254} eluting with the solventindicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid, or an aqueous potassium permanganate solution. Flash column chromatography was performed with Silicycle Flash Silica Gel 40 – 63 µm or Silicycle Flash Silica Gel 60 – 200 µm, slurry packed with hexanes in glass columns. Glassware for reactions was oven dried at 125 °C and cooled under a dry atmosphere prior to use. Liquid reagents and solvents were introduced by oven-dried syringes through septum-sealed flasks under a nitrogen atmosphere. Nuclear magnetic resonance spectra were acquired at 300, 500 MHz for ^{1}H and 75, 125 MHz for ^{13}C. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal of residual $CDCl_3$ at 7.27 ppm. Chemicals shifts for carbon nuclear magnetic resonance (^{13}C NMR and DEPT) spectra are reported in parts per million relative to the centerline of the $CDCl_3$ triplet at 77.23 ppm. Chemical shifts of the unprotonated carbons ('C') for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The abbreviations s, d, dd, ddd, dddd, dq, t, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, triplet and multiplet, respectively. Optical rotations (Na D line) were obtained using a microcell with 1 dm path length. Specific rotations ($[\alpha]_D^{20}$, Unit: $°cm^2/g$) are based on the equation $\alpha = (100 \cdot \alpha)/(l \cdot c)$ and are reported as unit-less numbers where the concentration c is in g/l00 mL and the path length l is in decimeters. Mass spectrometry was performed at the mass spectrometry facility of the Department

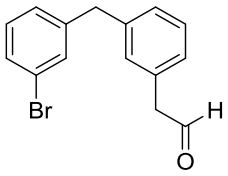
of Chemistry at The University of Utah on a double focusing high-resolution mass spectrometer. Compounds were named using ChemDraw 12.0.0.

Experimental procedures for Merle 40



Preparation of 3-(3-bromobenzyl)benzaldehyde 2.17: To a stirring biphasic solution of 1-bromo-3-(bromomethyl)benzene (1.15 g, 4.59 mmol, 1 equiv) and potassium tetrafluoroborate salt **2.16** (0.975 g, 4.59 mmol, 1 equiv) in a 4:3:2 mixture of toluene/EtOH/H_2O (46 mL) in a 100 mL rb flask equipped with a reflux condenser, at rt, was added Na_2CO_3 (0.937 g, 9.18 mmol, 2 equiv) and $Pd(PPh_3)$ (0.132 g, 0.115 mmol, 0.025 equiv). The reaction mixture was stirred at reflux (80 °C oil bath) for 2 h, cooled to rt, and then partitioned between EtOAc (50 mL) and water (50 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 3.5 × 8.0 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (22-49) were combined and concentrated under reduced pressure to provide **2.17** (0.955 g, 75% yield) as a clear oil: R_f = 0.42 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 10.0 (s, 1H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.71 (s, 1H), 7.51-7.43 (m, 2H), 7.39-7.33 (m, 2H), 7.18 (t, *J* = 7.2 Hz, 1H), 7.13 (d, *J* = 7.2 Hz, 1H), 4.03 (s, 2H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 192.5, 142.7, 141.6, 137.0, 135.3, 132.1, 130.5, 130.0, 129.9, 129.6, 128.5, 127.8, 123.0, 41.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ none; CH_2 δ 41.4; CH δ 192.5, 135.3, 132.1, 130.5, 130.0, 129.9, 129.6, 128.5, 127.8; C δ 142.7, 141.6, 137.0,

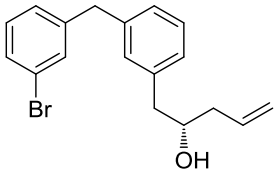
123.0; IR (neat) 3050, 3026, 2911, 2900, 2780, 1725, 1590, 1560, 1478 cm^{-1}; HRMS (ESI/ APCI) calcd 296.9891 and 298.9871 for $C_{14}H_{11}BrONa$ found 296.9892 and 298.9875.



Preparation of 2-(3-(3-bromobenzyl)phenyl)acetaldehyde 2.18: To a stirring solution of potassium *tert*-butoxide (283 mg, 2.52 mmol, 2 equiv) in THF (25 mL) in a 50 mL rb flask, at 0 °C, was added (methoxymethyl) triphenylphosphonium chloride (775 mg, 2.52 mmol, 2 equiv). The mixture immediately turned deep red and was stirred for 2 h. To this solution, at 0 °C, was added aldehyde **2.17** (347 mg, 1.26 mmol, 1 equiv) dropwise by syringe, and stirring was continued for 30 min. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution (15 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, and concentrated to provide the crude enol ether as 1.4:1 mixture of E/Z isomers, which was used without purification.

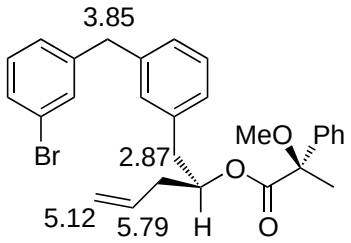
To a stirring solution of the aforementioned enol ether in THF (25 mL) in a 50 mL rb flask, at rt, was added a 1 M aqueous HCl solution. The homogenous solution was stirred for 24 h at rt, then quenched with saturated aqueous $NaHCO_3$ solution (15 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 2.0 × 7.0 cm silica gel column, eluting with 9% EtOAc/hexanes, collecting 10 × 75 mm test tube fractions. The product containing

fractions (9-25) were combined and concentrated under reduced pressure to provide **2.18** (266 mg, 73% yield) as a clear oil: R_f = 0.39 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.75 (s, 1H), 7.43-7.29 (m, 3H), 7.22-7.07 (m, 4H), 7.05 (s, 1H), 3.96 (s, 2H), 3.67 (s, 2H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 199.6, 143.3, 141.3, 132.5, 132.2, 130.4, 130.3, 129.6, 129.5, 128.3, 127.9, 127.8, 122.9, 50.7, 41.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ none; CH_2 δ 50.7, 41.6; CH δ 199.6, 132.2, 130.4, 130.3, 129.6, 129.5, 128.3, 127.9, 127.8, 122.9; C δ 143.3, 141.3, 132.5, 122.9; ; IR (neat) 3056, 3026, 2909, 2824, 2725, 1946, 1723, 1591, 1567, 1475, 1426, 1311, 1183, 1071, 777, 704 cm^{-1}; HRMS (ESI/ APCI) calcd 311.0047 and 313.0027 for $C_{15}H_{13}BrONa$ found 311.0047 and 313.0032.

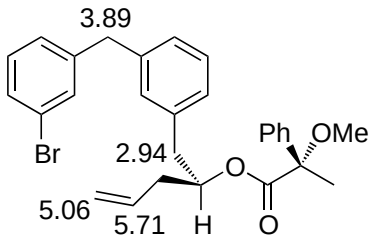


Preparation of (*S*)-1-(3-(3-bromobenzyl)phenyl)pent-4-en-2-ol 2.19: To a stirring solution of (*S*)-Binol (106 mg, 0.369 mmol, 0.4 equiv) and powdered 4 Å molecular sieves (369 mg) in CH_2Cl_2 (3.69 mL) in a 25 ml 2-neck rb flask, at rt, was added a 1.0 M solution of Ti(*i*-OPr)$_4$ (171 μL, 0.2 equiv) in CH_2Cl_2 and a freshly prepared 0.1 M solution of TFA (28 μL, 1.3 equiv) in CH_2Cl_2. This suspension was heated at 40 °C for 1 h, during which time a deep red color developed. After cooling to rt. aldehyde **2.18** (266 mg, 0.922 mmol, 1 equiv) in CH_2Cl_2 (500 μL) was added via cannula. An additional aliquot of CH_2Cl_2 (200 μL) was used to complete the transfer, and the mixture was cooled to -78 °C. After 30 min, allyl tributylstannane (373 μL, 1.20 mmol, 1.3 equiv) was added dropwise, and stirring was continued for 15

min at -78 °C. Stirring was discontinued, the reflux condenser was replaced with a septa, and the flask was stored in a -20 °C. After 3 days the reaction mixture was quenched, at -20 °C by the addition of saturated aqueous $NaHCO_3$ solution (0.5 mL) then filtered through a 3 cm pad of celite® into a 50 mL Erlenmeyer flask. Saturated aqueous $NaHCO_3$ solution (10 mL) was added along with additional CH_2Cl_2 (10 mL), and the biphasic solution was stirred for 1 h. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine (2 × 5 mL), dried over Na_2SO_4, concentrated to a red oil, and purified by flash column chromatography using a 2.0 × 10.0 cm silica gel column, eluting with 8% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (21-38) were combined and concentrated under reduced pressure to provide **2.19** (189 mg, 62% yield) as a yellow oil: R_f = 0.25 (20% EtOAc/hexanes); $[\alpha]_D^{20}$ = +15.0 (c =1.0, $CHCl_3$) @ 95% ee; 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.35 (m, 2H), 7.31-7.28 (m, 1H), 7.21-7.17 (m, 1H), 7.17-7.11 (m, 2H), 7.10-7.06 (m, 2H), 5.90 (dddd, *J* = 13.1, 11.2, 7.4, 7.1 Hz, 1H), 5.21-5.16 (m, 2H), 3.97 (s, 2H), 3.91 (ddd, *J* = 15.1, 7.5, 4.6 Hz, 1H), 2.83 (dd, *J* = 13.7, 4.9 Hz, 1H), 2.73 (dd, *J* = 13.7, 8.1 Hz, 1H), 2.40-2.34 (m, 1H), 2.29-2.22 (m, 1H), 1.73 (s, OH); 125 MHz ^{13}C NMR ($CDCl_3$) δ 143.7, 140.7, 139.0, 134.9, 132.2, 130.3, 130.3, 129.5, 129.1, 127.8, 127.7, 127.4, 122.8, 118.5, 71.9, 43.5, 41.7, 41.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ none; CH_2 δ 118.5, 43.5, 41.7, 41.5; CH δ 134.9, 132.2, 130.3, 130.3, 129.5, 129.1, 127.8, 127.7, 127.4, 71.9; C δ 143.7, 140.7, 139.0, 122.8; IR (neat) 3402, 3058, 3016, 2918, 1640, 1606, 1592, 1567, 1486 cm^{-1}; HRMS (ESI/ APCI) calcd 353.0517 and 355.0497 for $C_{18}H_{19}BrONa$ found 353.0520 and 355.0502.

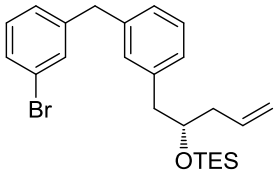


(*R*)-MTPA ester



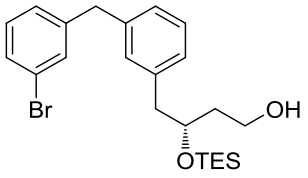
(*S*)-MTPA ester

Mosher ester analysis[32] confirms (*S*) orientation of the C3 alcohol



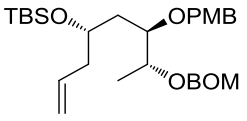
Preparation of (*S*)-((1-(3-(3-bromobenzyl)phenyl)pent-4-en-2-yl)oxy)triethylsilane 2.34: To a stirring solution of alcohol **2.19** (222 mg, 0.670 mmol, 1 equiv) in CH_2Cl_2 (6.7 mL) in a 25 mL rb flask, at rt, was added Et_3N (280 μL, 2.01 mmol, 3 equiv) followed by TESCl (169 μL, 1.01 mmol, 1.5 equiv). After 7 h the reaction mixture was quenched with water (10 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash column chromatography using a 2.0 × 8.0 cm silica gel column, eluting with 10% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (6-16) were combined and concentrated under reduced pressure to provide **2.34** (285 mg, 95% yield) as a clear oil: R_f = 0.71 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +8.9 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36-7.32 (m, 2H), 7.21 (t, *J* = 8.2 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.14-7.11 (m, 1H), 7.07-6.99 (m, 3H), 5.87 (dddd, *J* = 17.3, 10.3, 7.4, 7.4 Hz, 1H), 5.08-5.01 (m, 2H), 3.92 (s, 2H), 3.89 (ddd, *J* = 12.9, 7.0, 5.5 Hz, 1H), 2.75 (dd, *J* = 13.9, 5.3 Hz,

1H), 2.65 (dd, *J* = 13.9, 5.3 Hz, 1H), 2.28-2.17 (m, 2H), 0.87 (t, *J* = 7.9 Hz, 9H), 0.50-0.40 (m, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 143.8, 140.1, 139.9, 135.2, 132.1, 130.6, 103.1, 129.4, 128.6, 128.0, 127.8, 126.9, 122.7, 117.4, 73.5, 43.7, 42.0, 41.7, 7.1, 5. 0; 125 MHz DEPT ($CDCl_3$) CH_3 δ 7.1; CH_2 δ 117.4, 43.7, 42.0, 41.7, 5.0; CH δ 135.2, 132.1, 130.6, 130.1, 129.4, 128.6, 128.0, 127.8, 126.9, 73.5; C δ 143.8, 140.1, 139.9, 122.7; IR (neat) 2953, 2911, 2875, 1640, 1594, 1568, 1475, 1424 cm^{-1}; HRMS (ESI/ APCI) calcd 467.1382 and 469.1361 for $C_{24}H_{33}BrOSiNa$ found 467.1389 and 469.1367.



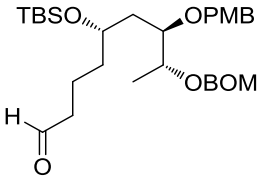
Preparation of (*R*)-4-(3-(3-bromobenzyl)phenyl)-3-((triethylsilyl)oxy)butan-1-ol 2.35: Into a stirring solution of olefin **2.34** (155 mg, 0.348 mmol, 1 equiv) in CH_2Cl_2 (6.9 mL) in a 50 mL rb flask, at - 78 °C, was bubbled a steady stream of O_3 until a faint blue color developed, approximately 3 min. Flushing with O_2 for 15 min purged excess ozone, then PPh_3 (183 mg, 0.696 mmol, 2 equic) was added, and the reaction mixture was allowed to warm to rt as it stirred for 3 h. This solution of crude aldehyde was cooled to 0 °C, and ethanol (3.5 mL) was added followed by $NaBH_4$ (6.6 mg, 0.17 mmol, 0.5 equiv). After 1 h the mixture was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 2.0 × 7.0 cm silica gel column, eluting with 10% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing

fractions (10-29) were combined and concentrated under reduced pressure to provide **2.35** (137 mg, 88% yield) as a clear oil: R_f = 0.42 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -2.0 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36-7.32 (m, 2H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.16 (t, *J* = 6.9 Hz, 1H), 7.13-7.10 (m, 2H), 7.05-7.01 (m, 2H), 6.98 (s, 1H), 4.13 (ddd, *J* = 12.9, 6.3 Hz, 1H), 3.93 (s, 2H), 3.86 (ddd, *J* = 12.3, 8.2, 4.3 Hz, 1H), 3.75-3.69 (m, 1H), 2.87 (dd, *J* = 13.3, 6.0 Hz, 1H), 2.75 (dd, *J* = 13.1, 7.1 Hz, 1H), 2.44 (s, 1H), 1.81-1.74 (m, 1H), 1.65-1.58 (m, 1H), 0.93 (t, *J* = 8.2 Hz, 9H), 0.55 (ddd, *J* = 15.0, 7.4, 4.1 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 143.6, 140.4, 139.2, 132.1, 130.3, 130.1, 129.4, 128.8, 127.8, 127.1, 122.8, 73.4, 60.5, 43.9, 41.7, 37.9, 7.0, 5.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 7.1; CH_2 δ 60.5, 43.9, 41.7, 37.9, 5.0; CH δ 132.1, 130.3, 130.1, 129.4, 128.8, 127.8, 127.8, 127.1, 73.4; C δ 143.6, 140.4, 139.2, 122.8; IR (neat) 3386, 2951, 2910, 2875, 1593, 1568, 1474 cm^{-1}; HRMS (ESI/ APCI) calcd 471.1331 and 473.1310 for $C_{23}H_{33}BrO_2SiNa$ found 471.1339 and 473.1323.



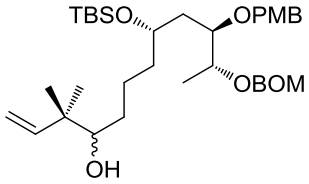
Preparation of (5*R*,6*R*,8*S*)-8-allyl-6-((4-methoxybenzyl)oxy)-5,10,10,11,11-pentamethyl-1-phenyl-2,4,9-trioxa-10-siladodecane 2.22:[11] To a stirring solution of alcohol (3.71 g, 9.26 mmol, 1 equiv), and imidazole (2.52 g, 37.1 mmol, 4 equiv), in DMF (46 mL), in a 100 mL rb flask, at rt, was added TBSCl (2.79 g, 18.5 mmol, 2 equiv). This solution was stirred for 20 h, quenched with water (20 mL), and then diluted with 30% EtOAc/hexanes (50 mL). The phases were separated and the aqueous layer was extracted with 30% EtOAc/hexanes (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, concentrated and the 7:1 diastereomers were

separated by flash column chromatography using a 5.0 × 19.0 cm silica gel column, eluting with 3% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (83-290) were combined and concentrated under reduced pressure to provide **2.22** (3.95 g, 83% yield) as a pure diastereomer and as a clear oil: R_f = 0.27 (5% EtOAc/ 35% toluene/ 60% hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.34 (m, 4H), 7.32-7.28 (m, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.83 (dddd, *J* = 13.4, 13.4, 6.8, 7.2 Hz, 1H), 5.04 (dd, *J* = 13.7, 2.0 Hz, 2H), 4.81 (Aβq, *J* = 7.3 Hz, Δν = 9.6 Hz, 2H), 4.63 (Aβq, *J* = 11.8 Hz, Δν = 18.7 Hz, 2H), 4.59 (d, *J* = 10.9 Hz, 1H), 4.47 (d, *J* = 10.9 Hz, 1H), 4.05-3.93 (m, 2H), 3.80 (s, 3H), 3.66 (ddd, *J* = 8.4, 4.5, 3.0 Hz, 1H), 2.31-2.25 (m, 2H), 1.69-1.58 (m, 2H), 1.18 (d, *J* = 6.7 Hz, 3H), 0.90 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.4, 138.3, 135.0, 131.3, 129.4, 128.7, 128.1, 127.9, 117.3, 114.1, 93.5, 78.2, 73.2, 72.2, 69.6, 69.4, 55.6, 43.2, 37.6, 26.2, 18.4, 15.4, -3.5, -4.1.



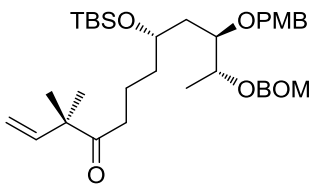
Preparation of (5*S*,7*R*,8*R*)-8-((benzyloxy)methoxy)-5-((*tert*-butyldimethylsilyl)oxy)-7-((4-methoxybenzyl)oxy)nonanal 2.23:[11] To a stirring solution of olefin **2.22** (3.01 g, 5.85 mmol, 1 equiv) in THF (11.7 mL) in a high pressure Parr reaction vessel, at rt and open to air, was added phosphite ligand **2.26** (92 mg, 0.12 mmol, 0.02 equiv), and $Rh(acac)(CO)_2$ (8.0 mg, 0.03 mmol, 0.005 equiv). The pressure gage was secured and the reaction vessel was purged with N_2 three times then with a 1:1 gaseous mixture of CO_2/H_2 three times. The vessel was then pressurized

to 60 psi with CO_2/H_2, and heated at 60 °C for 20 h. Pressure was carefully released, and then the solution was transferred to a 100 mL rb flask rinsing with copious amounts of EtOAc, and then concentrated. Purification was accomplished by flash column chromatography using a 5.0 × 14.0 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (89-190) were combined and concentrated under reduced pressure to provide **2.23** (3.03 g, 95% yield) as a clear oil: R_f = 0.30 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.74 (s, 1H), 7.38-7.34 (m, 4H), 7.33-7.29 (m, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.81 (Aβq, *J* = 7.4 Hz, Δν = 12.0 Hz, 2H), 4.64 (Aβq, *J* = 12.0 Hz, Δν = 18.9 Hz, 2H), 4.60 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 10.8 Hz, 1H), 4.03 (ddd, *J* = 12.8, 6.4, 4.8 Hz, 1H), 3.95-3.88 (m, 1H), 3.80 (s, 3H), 3.64 (ddd, *J* = 9.3, 4.7, 2.5 Hz, 1H), 2.40 (ddd, *J* = 8.9, 7.3, 1.6 Hz, 2H), 1.75-1.46 (m, 6H), 1.19 (d, *J* = 6.3 Hz, 3H), 0.90 (s, 9H), 0.60 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 202.8, 159.4, 138.2, 131.2, 129.4, 128.7, 128.1, 128.0, 114.1, 93.5, 78.3, 73.0, 72.2, 69.6, 69.6, 55.6, 44.3, 37.8, 37.4, 26.2, 18.4, 17.5, 15.2, -3.6, -4.1.

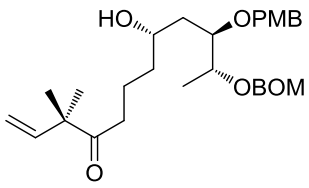


Preparation of (8*S*,10*R*,11*R*)-11-((benzyloxy)methoxy)-8-((*tert*-butyldimethylsilyl)oxy)-10-((4-methoxybenzyl)oxy)-3,3-dimethyldodec-1-en-4-ol 2.24:[11] To a stirring solution of aldehyde **2.23** (3.03 g, 5.56 mmol, 1 equiv) and indium powder (1.92 g, 16.7 mmol, 3 equiv) in DMF (28 mL) in a 100 ml rb flask, at rt, was added prenyl bromide (1.92 mL, 16.7 mmol, 3 equiv). After 1 h the reaction

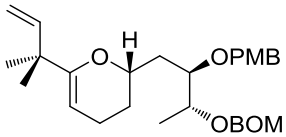
mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL). The phases were separated and the aqueous layer was extracted with a 30% EtOAc/hexanes solution (2 × 10 mL). The aqueous layer was acidified with a 1 M aqueous HCl solution (5 mL) and extracted once more. The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 5.0 × 10.0 cm silica gel column, eluting with 10-20% EtOAc/hexanes, collecting 18 × 150 mm test tube fractions. The product containing fractions (8-45) were combined and concentrated under reduced pressure to provide **2.24** (3.32 g, 97% yield) as a clear oil: R_f = 0.54 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.34 (m, 4H),7.32-7.28 (m, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 5.81 (dd, *J* = 16.9, 10.5 Hz, 1H), 5.09 (d, J = 10.5 Hz, 1H), 5.05 (d, *J* = 17.8 Hz, 1H), 4.81 (Aβq, *J* = 6.9 Hz, Δν = 10.4 Hz, 2H), 4.63 (Aβq, *J* = 11.4 Hz, Δν = 19.6 Hz, 2H), 4.59 (d, *J* = 11.0 Hz, 1H), 4.47 (d, *J* = 11.0 Hz, 1H), 4.05-3.98 (m, 1H), 3.95-3.87 (m, 1H), 3.80 (s, 3H), 3.65 (ddd, *J* = 11.5, 4.0, 2.8 Hz, 1H), 3.23 (dd, *J* = 9.9, 6.4 Hz, 1H), 1.71-1.42 (m, 8H), 1.38-1.26 (m, 1H), 1.18 (d, *J* = 6.2 Hz, 3H), 1.00 (s, 6H), 0.89 (s, 9H), 0.06 (dd, *J* = 5.5, 2.6 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.4, 145.7, 138.3, 131.3, 129.4, 128.7, 128.1, 127.9, 114.0, 113.5, 93.5, 78.5, 78.5, 78.4, 78.4, 73.3, 72.2, 70.1, 69.9, 69.6, 55.6, 41.9, 38.6, 38.6, 37.7, 32.0, 31.9, 26.3, 23.4, 22.6, 22.5, 22.3, 22.3, 18.4, 15.5, -3.4, -4.1.



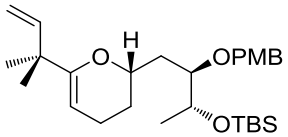
Preparation of (8*S*,10*R*,11*R*)-11-((benzyloxy)methoxy)-8-((*tert*-butyldimethylsilyl)oxy)-10-((4-methoxybenzyl)oxy)-3,3-dimethyldodec-1-en-4-one 2.53:[11] To a stirring solution of alcohol **2.24** (3.32 g, 5.40 mmol, 1 equiv) in CH_2Cl_2 (54 mL) in a 100 mL rb flask, at 0 °C, was added *i*-Pr_2NEt (6.60 mL, 37.8 mmol, 7 equiv), DMSO (3.85 mL, 54.0 mmol, 10 equiv), and SO_3•Pyr (3.44 g, 21.6 mmol, 4 equiv) in a single portion. The reaction mixture was stirred at 0 °C for 1 h, then quenched with saturated aqueous $NaHCO_3$ solution (30 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, concentrated and the purified by flash column chromatography using a 5.0 × 12.0 cm silica gel column, eluting with 10% EtOAc/hexanes, and collecting 18 × 150 mm test tube fractions. The product containing fractions (11-43) were combined and concentrated under reduced pressure to provide **2.53** (2.84 g, 89% yield) as a faint yellow oil: R_f = 0.48 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.33 (m, 4H), 7.32-7.28 (m, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.90 (dd, *J* = 17.3, 11.4 Hz, 1H), 5.13 (dd, *J* = 13.8, 3.0 Hz, 2H), 4.81 (Aβq, *J* = 7.4 Hz, Δν = 10.3 Hz, 2H), 4.63 (Aβq, *J* = 11.8 Hz, Δν = 21.5 Hz, 2H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.01 (ddd, *J* = 12.3, 5.8, 4.8 Hz, 1H), 3.92-3.86 (m, 1H), 3.80 (s, 3H), 3.64 (ddd, *J* = 9.1, 4.6, 2.4 Hz, 1H), 2.42 (ddd, *J* = 7.0, 7.0, 3.7 Hz, 2H), 1.68 (ddd, *J* = 14.0, 8.5, 2.6 Hz, 1H), 1.61-1.52 (m, 3H), 1.47-1.40 (m, 2H), 1.21 (s, 6H), 1.18 (d, *J* = 6.6 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 213.1, 159.4, 142.9, 138.3, 131.3, 129.8, 128.7, 128.1, 127.9, 114.5, 114.0, 93.5, 78.3, 73.2, 72.2, 69.8, 69.6, 55.6, 51.0, 38.0, 37.8, 37.5, 26.3, 23.8, 19.6, 18.4, 15.4, -3.5, -4.1.



Preparation of (8*S*,10*R*,11*R*)-11-((benzyloxy)methoxy)-8-hydroxy-10-((4-methoxybenzyl)oxy)-3,3-dimethyldodec-1-en-4-one 2.25:[11] To a stirring solution of silyl ether **2.53** (488 mg, 0.796 mmol, 1 equiv) in THF (3.98 mL) in a 25 mL rb flask, at rt, was added a 1.0 M solution of TBAF (3.19 mL, 3.19 mmol, 4 equiv) in THF. The reaction was stirred for 20 h, and then another aliquot of TBAF (1 mL) was added. After an additional 6 h the reaction was quenched with saturated aqueous NH_4Cl solution (10 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 2.5 × 9.0 cm silica gel column, eluting with 30% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (21-61) were combined and concentrated under reduced pressure to provide **2.25** (368 mg, 93% yield) as a clear oil: R_f = 0.16 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.33 (m, 4H), 7.32-7.28 (m, 1H), 7.25 (d, *J* = 9.1 Hz, 2H), 6.86 (d, *J* = 9.1 Hz, 2H), 5.91 (dd, *J* = 17.5, 10.4 Hz, 1H), 5.14 (ddd, 14.7, 3.8, 0.9 Hz, 2H), 4.84 (Aβq, *J* = 13.6 Hz, Δν = 11.3 Hz, 2H), 4.63 (s, 2H), 4.61 (d, *J* = 11.1 Hz, 1H), 4.50 (d, *J* = 11.1 Hz, 1H), 4.00 (ddd, *J* = 6.3, 6.3, 5.4 Hz, 1H), 3.80 (s, 3H), 3.75-3.68 (m, 2H), 2.53-2.41 (m, 2H), 1.65-1.59 (m, 4H), 1.41-1.34 (m, 2H), 1.22 (s, 6H), 1.19 (d, *J* = 6.5 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 213.5, 159.6, 142.8, 138.1, 130.7, 130.0, 129.9, 128.7, 128.1, 128.0, 114.5, 114.1, 94.0, 78.5, 74.1, 72.7, 69.8, 68.4, 55.5, 51.0, 37.6, 37.4, 37.0, 23.8, 20.1, 15.8.



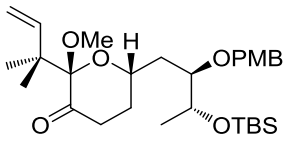
Preparation of (*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-6-(2-methylbut-3-en-2-yl)-3,4-dihydro-2*H*-pyran 2.13:[11] To a stirring solution of hydroxyketone **2.25** (192 mg, 0.385 mmol, 1 equiv) in benzene (7.7 mL) in a 25 mL rb flask, at rt, was added 4 Å molecular sieves (5) and CSA (6.0 mg, 0.02 mmol, 0.05 equiv). The flask was fitted with an efficient reflux condenser then heated in a 85 °C oil bath. After 3 h the solution was cooled to rt, quenched with pyridine (200 μL), concentrated, and purified by flash column chromatography using a 2.0 × 8.5 cm silica gel column, eluting with 8% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (11-30) were combined and concentrated under reduced pressure to provide **2.13** (163 mg, 88% yield) as a clear oil: R_f = 0.60 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.34 (m, 4H), 7.33-7.29 (m, 1H), 7.26 (d, *J* = 8.9 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 5.94 (dd, *J* = 18.5, 11.0 Hz, 1H), 5.02 (dd, *J* = 17.6, 1.5 Hz, 1H), 4.94 (dd, *J* = 10.7, 1.5 Hz, 1H), 4.84 (Aβq, *J* = 6.7 Hz, Δν = 5.6 Hz, 2H), 4.65 (Aβq, *J* = 11.8 Hz, Δν = 7.2 Hz, 2H), 4.62 (d, *J* = 10.9, 1H), 4.58 (dd, *J* = 4.6, 2.8 Hz, 1H), 4.53 (d, *J* = 10.9 Hz, 1H), 4.05-3.94 (m, 2H), 3.86 (ddd, *J* = 10.7, 5.1, 2.3 Hz, 1H), 3.80 (s, 3H), 2.14-2.05 (m, 1H), 2.03-1.91 (m, 1H), 1.90-1.73 (m, 2H), 1.61 (ddd, *J* = 14.5, 10.6, 2.5 Hz, 1H), 1.56-1.46 (m, 1H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.18 (s, 3H), 1.17 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.5, 159.1, 146.5, 138.2, 131.2, 130.0, 129.8, 128.7, 128.1, 127.9, 114.1, 114.0, 111.1, 93.6, 93.4, 77.8, 73.9, 73.6, 71.7, 69.6, 55.6, 41.6, 36.3, 28.4, 25.8, 25.6, 20.6, 15.7.



Preparation of *tert*-butyl(((2*R*,3*R*)-3-((4-methoxybenzyl)oxy)-4-((*S*)-6-(2-methylbut-3-en-2-yl)-3,4-dihydro-2*H*-pyran-2-yl)butan-2-yl)oxy)dimethylsilane 2.42: To a stagnant solution of 4,4-ditertbutylbiphenyl (1.0 g, 3.75 mmol) in THF (7.5 mL) in a 25 mL rb flask under an Ar atmosphere, at 0 °C, was added freshly cut Li wire, washed with hexanes then Et_2O (~60 mg). This solution was maintained at 0 °C in an ultrasonication bath for 2 h resulting in a deep blue/green solution. The 0.5 M LiDBB solution was then added dropwise to a stirring solution of BOM ether **2.13** (224 mg, 0.466 mmol, 1 equiv) in THF (4.6 mL) in a 25 mL rb flask, at -78 °C, until a deep blue color persisted. After 1 h the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl solution (5 mL) then warmed to rt. The phases were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 2.0 × 12.0 cm silica gel column, eluting first with 100 mL of 5% EtOAc/hexanes to wash off 4,4-ditertbutylbiphenyl, then with 20% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (58-85) were combined and concentrated under reduced pressure to provide the pure alcohol (95 mg, 57% yield) as a clear oil: R_f = 0.33 (30% EtOAc/hexanes)

To a stirring solution of the aforementioned alcohol in CH_2Cl_2 (2.64 mL) in a 25 mL rb flask, at rt, was added TBSCl (80.0 mg, 0.528 mmol, 2 equiv), and imidazole (72.0 mg, 1.06 mmol, 4 equiv). This solution was stirred for 20 h, and then quenched with water (5 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4,

concentrated, and purified by flash column chromatography using a 1.5 × 10.0 cm silica gel column, eluting with 5% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (3-11) were combined and concentrated under reduced pressure to provide **2.42** (94 mg, 75% yield) as a clear oil: R_f = 0.66 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +29.4 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.27 (d, *J* = 8.9 Hz, 2H), 6.89 (d, *J* = 8.9 Hz, 2H), 5.97 (dd, *J* = 17.6, 10.7 Hz, 1H), 5.04 (dd, *J* = 17.5, 1.3 Hz, 1H), 4.95 (dd, *J* = 10.8, 1.5 Hz, 1H), 4.59 (d, *J* = 10.5 Hz, 1H), 4.58 (s, 3H), 4.51 (d, *J* = 10.5 Hz, 1H), 4.01-3.95 (m, 2H), 3.81 (s, 3H), 3.75 (ddd, *J* = 11.1, 4.8, 1.8 Hz, 1H), 2.10 (dddd, *J* = 6.7, 9.9, 9.9, 2.5 Hz, 1H), 2.03-1.94 (m, 1H), 1.85 (ddd, *J* = 14.5, 10.7, 1.9 Hz, 1H), 1.78-1.71 (m, 1H), 1.51 (ddd, *J* = 14.0, 11.1, 2.9 Hz, 2H), 1.20 (s, 3H), 1.18 (s, 3H), 1.12 (d, *J* = 6.3 Hz, 3H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.4, 159.3, 146.6, 131.5, 129.5, 114.0, 110.9, 93.1, 79.1, 73.1, 71.8, 68.4, 55.5, 41.6, 35.0, 28.6, 26.1, 25.8, 25.4, 20.9, 18.3, 17.7, -4.5, -4.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 26.1, 25.8, 25.4, 17.7, -4.5, -4.6; CH_2 δ110.9, 73.1, 35.0, 28.6, 20.9; CH δ 146.6, 129.5, 114.0, 93.1, 79.1, 71.8, 68.4; C δ 159.4, 159.3, 131.5, 41.6, 18.3; IR (neat) 2956, 2929, 2856, 1663, 1613, 1587, 1515, 1463 cm^{-1}; HRMS (ESI/ APCI) calcd 497.3036 for $C_{28}H_{46}O_4SiNa$ found 497.3059.



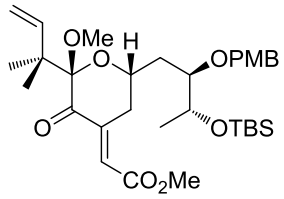
Preparation of (2*S*,6*S*)-6-((2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-2-(2-methylbut-3-en-2-yl)dihydro-2*H*-pyran-3(4*H*)-one 2.43: To a stirring solution of glycal **2.42** (94.0 mg, 0.198 mmol, 1 equiv) in CH_2Cl_2 (2.8 mL) in a 25 mL rb flask, at 0

°C, was added MeOH (1.0 mL), $NaHCO_3$ (33.0 mg, 0.392 mmol, 2 equiv), and MMPP (196 mg, 0.392 mmol, 2 equiv). After 1 h the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (5 mL), warmed to rt, and the phases were separated. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were thoroughly dried over $MgSO_4$, and concentrated to give the crude alcohol as a clear oil, which was used without purification.

To a stirring solution of the aforementioned crude alcohol in MeOH (20 mL) in a 50 mL rb flask, at 0 °C, was added monochloroacetic acid (19.0 mg, 0.198 mmol, 1 equiv). This solution was stirred for 45 min, and then quenched with saturated aqueous $NaHCO_3$ solution (20 mL). The phases were separated and the aqueous layer was extracted with 50% EtOAc/hexanes (3 × 20 mL). The combined organic layers were thoroughly dried over $MgSO_4$, and concentrated to give a crude alcohol as a clear oil, which was used without purification.

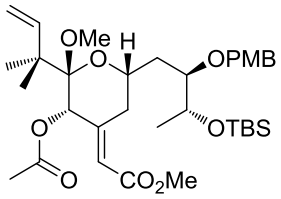
To a stirring solution of Dess-Martin periodinane (168 mg, 0.396 mmol, 2 equiv) in CH_2Cl_2 (1.2 mL) in a 25 rb flask, at 0 °C, was added pyridine (160 μL, 1.98 mmol, 10 equiv). The aforementioned alcohol was added as a solution in CH_2Cl_2 (0.5 mL) via cannula. An additional 0.5 mL aliquot of CH_2Cl_2 was used to complete the transfer. The solution was warmed to rt, stirred for 4 h, and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 1.5 × 10.0 cm silica gel column, eluting with 5% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (16-41) were

combined and concentrated under reduced pressure to provide **2.43** (69 mg, 68% yield over 3 steps) as a clear oil: R_f = 0.60 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +21.1 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.23 (d, *J* = 8.9 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.21 (dd, *J* = 17.7, 10.8 Hz, 1H), 5.02 (dd, *J* = 9.3, 1.4 Hz, 1H), 4.99 (dd, *J* = 2.6, 1.4 Hz, 1H), 4.61 (d, *J* = 10.8 Hz, 1H), 4.44 (d, *J* = 10.8 Hz, 1H), 4.19-4.09 (m, 2H), 3.80 (s, 3H), 3.77 (ddd, *J* = 10.9, 4.6, 2.1 Hz, 1H), 3.28 (s, 3H), 2.54-2.40 (m, 2H), 1.97-1.90 (m, 3H), 1.57 (ddd, *J* = 14.2, 11.0, 3.2 Hz, 1H), 1.16 (s, 3H), 1.12 (d, *J* = 5.9 Hz, 3H), 1.11 (s, 3H), 0.91 (s, 9H), 0.09 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.5, 159.4, 144.5, 131.0, 129.4, 114.1, 112.6, 104.0, 78.9, 72.0, 69.4, 67.1, 55.5, 52.2, 45.1, 37.7, 35.1, 30.5, 26.0, 22.5, 22.3, 18.3, 17.1, -4.5, -4.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 52.2, 26.0, 22.5, 22.3, 17.1, -4.5, -4.6; CH_2 δ 112.6, 72.0, 37.7, 35.1, 30.5; CH δ 144.5, 129.4, 114.1, 78.9, 69.4, 67.1; C δ 207.5, 159.4, 131.0, 104.0, 45.1, 18.3; IR (neat) 2954, 2890, 1725, 1612, 1514, 1463 cm^{-1}; HRMS (ESI/ APCI) calcd 543.3118 for $C_{29}H_{48}O_6SiNa$ found 543.3125.



Preparation of (*E*)-methyl 2-((2*S*,6*S*)-6-((2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-2-(2-methylbut-3-en-2-yl)-3-oxodihydro-2*H*-pyran-4(3*H*)-ylidene)acetate 2.54: To a stirring solution of ketone **2.43** (69 mg, 0.133 mmol, 1 equiv) in MeOH (1.33 mL) in a 15 mL rb flask, at rt, was added K_2CO_3 (92 mg, 0.663 mmol, 5 equiv) followed by a 3.0 M solution of freshly distilled methyl glyoxylate in THF (221 μL, 0.663 mmol, 5

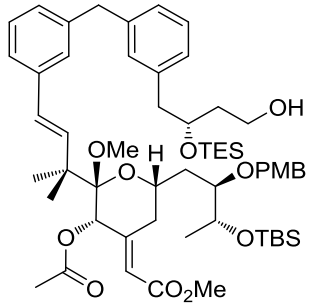
equiv). The solution rapidly developed a vibrant yellow color, and after 1 h it was quenched with saturated aqueous NH_4Cl solution (5 mL). The phases were separated and the aqueous layer was extracted with Et_2O (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 1.5 × 12.0 cm silica gel column, eluting with 12% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (4-13) were combined and concentrated under reduced pressure to provide **2.54** (64 mg, 81% yield) as an intense yellow oil: R_f = 0.59 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -75.3 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.20 (d, *J* = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.58 (s, 1H), 6.05 (dd, *J* = 17.8, 11.2 Hz, 1H), 4.97 (dd, *J* = 5.3, 1.1 Hz, 1H), 4.95 (dd, *J* = 12.0, 1.4 Hz, 1H), 4.60 (d, *J* = 11.3 Hz, 1H), 4.42 (d, *J* = 11.3 Hz, 1H), 4.21-4.11 (m, 2H), 3.79 (s, 3H), 3.80-3.75 (m, 1H), 3.76 (s, 3H), 3.31 (ddd, 18.8, 1.9, 1.9 Hz, 1H), 3.27 (s, 3H), 2.88 (ddd, *J* = 18.8, 12.7, 3.4 Hz, 1H), 2.01 (ddd, *J* = 14.5, 9.4, 1.9 Hz, 1H), 1.68 (ddd, *J* = 13.8, 10.3, 2.9 Hz, 1H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.10 (s, 3H), 1.05 (s, 3H), 0.91 (s, 9H), 0.09 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.7, 166.3, 159.4, 148.4, 143.4, 130.9, 129.2, 123.1, 114.1, 113.4, 104.7, 78.6, 71.6, 69.1, 67.1, 55.5, 52.3, 52.0, 45.8, 36.3, 35.0, 26.0, 22.3, 21.9, 18.2, 17.0, -4.4, -4.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 52.3, 52.0, 26.0, 22.3, 17.1, -4.4, -4.6; CH_2 δ 113.4, 71.6, 36.3, 35.0; CH δ143.4, 129.2, 123.1, 114.1, 78.6, 69.1, 67.1; C δ 197.7, 166.3, 159.4, 148.4, 130.9, 104.7, 45.8, 18.2; IR (neat) 2955, 2931, 2858, 1725, 1706, 1613, 1514, 1463 cm^{-1}; HRMS (ESI/ APCI) calcd 613.3173 for $C_{32}H_{50}O_8SiNa$ found 613.3184



Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-2-(2-methylbut-3-en-2-yl)dihydro-2*H*-pyran-4(3*H*)-ylidene)acetate 2.44: To a stirring solution of ketone **2.54** (32.0 mg, 0.054 mmol, 1 equiv) in MeOH (3.6 mL) in a 15 mL rb flask, at rt, was added $CeCl_3 \bullet 7H_2O$ (404 mg, 1.08 mmol, 20 equiv). This suspension was stirred until complete solvation of the cerium, then cooled to -42 °C, and $NaBH_4$ (20 mg, 0.54 mmol, 10 equiv) was added in a single portion. After 1 h the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl solution (5 mL), then warmed to rt. The phases were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, and concentrated to give the crude alcohol as a clear oil.

To a stirring solution of the aforementioned crude alcohol in CH_2Cl_2 (1.8 mL) in a 10 mL rb flask, at 0 °C, was added pyridine (65 μL, 0.81 mmol, 15 equiv), DMAP (6.7 mg, 0.05 mmol, 1 equiv), and acetic anhydride (26.0 μL, 0.270 mmol, 5 equiv). After 3 h the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL), and warmed to rt. The phases were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 1.5 × 11.0 cm silica gel column, eluting with 8% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (10-40) were combined and concentrated under reduced pressure to provide **2.44** (28 mg, 82% yield)

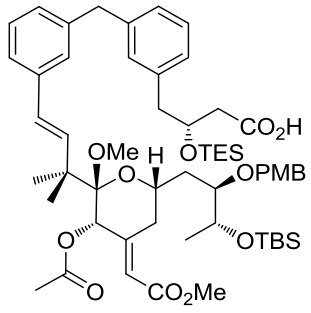
as a clear oil: R_f = 0.58 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -8.8 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.21 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.25 (dd, *J* = 17.6, 11.1 Hz, 1H), 5.89 (s, 1H), 5.36 (s, 1H), 4.86 (d, *J* = 17.6 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.60 (d, *J* = 11.1 Hz, 1H), 4.41 (d, *J* = 11.1 Hz, 1H), 4.13 (dq, *J* = 4.5, 6.2 Hz, 1H), 4.01 (dddd, *J* = 13.8, 13.8, 2.8, 2.8 Hz, 1H), 3.80 (s, 3H), 3.78 (dd, *J* = 4.4, 2.5 Hz, 1H), 3.69 (s, 3H), 3.51 (dd, *J* = 15.5, 2.2 Hz, 1H), 3.27 (s, 3H), 2.30 (ddd, *J* = 14.4, 11.9, 1.5 Hz, 1H), 2.00 (s, 3H), 1.97 (dd, *J* = 13.7, 9.7 Hz, 1H), 1.65 (ddd, *J* = 14.0, 11.2, 2.5 Hz, 1H), 1.14 (d, *J* = 6.0 Hz, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 0.93 (s, 9H), 0.10 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.4, 166.7, 159.4, 152.6, 146.5, 131.0, 129.3, 117.8, 114.0, 108.7, 102.9, 78.8, 72.4, 71.9, 68.6, 67.3, 55.5, 51.7, 51.3, 46.8, 35.4, 32.6, 26.1, 24.5, 22.7, 21.6, 18.3, 17.2, -4.4, -4.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 51.7, 51.3, 26.1, 24.5, 22.7, 17.2, -4.4, -4.6; CH_2 δ 108.7, 71.9, 35.4, 32.6; CH δ 146.5, 129.3, 117.8, 114.0, 78.8, 72.4, 68.6, 67.3; C δ 169.4, 166.7, 159.4, 152.6, 131.0, 102.9, 46.8, 18.3; IR (neat) 2954, 2856, 1748, 1721, 1667, 1613, 1514, 1464 cm^{-1}; HRMS (ESI/ APCI) calcd 657.3435 for $C_{34}H_{54}O_9SiNa$ found 657.3434.



Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-4-(3-(3-((*R*)-4-hydroxy-2-((triethylsilyl)oxy)butyl)benzyl)phenyl)-2-methylbut-3-en-2-

yl)-2-methoxydihydro-2*H*-pyran-4(3*H*)-ylidene)acetate 2.45: A 4 mL reaction vial was charged with a stir bar, olefin **2.44** (40.0 mg, 0.063 mmol, 1 equiv), bromide **2.35** (34.0 mg, 0.076 mmol, 1.2 equiv), $Pd_2(dba)_3$ (1.40 mg, 0.0016 mmol, 0.025 equiv), and $Pd(P(t\text{-}Bu)_3)_2$ (1.60 mg, 0.0032 mmol, 0.05 equiv). The vial was fitted with septum cap, evacuated, and refilled with N_2. Toluene (500 μL) was added followed by Cy_2NMe (16.0 μL, 0.076 mmol, 1.2 equiv), and the solution was degassed by three consecutive freeze/vacuum (10 min) then thaw cycles. After the final thaw the N_2 line was removed, and the vial cap was wrapped with parafilm. Within 30 min the solution transitioned from a deep purple color to very dark green indicating that it was progressing. After 20 h, at rt, the almost black solution was pipeted directly on to a 1.5 × 11.0 cm silica gel column, eluting first with 10% EtOAc/hexanes (100 mL), then with 15% EtOAc/hexanes (100 mL), and finally with 20% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The recovered C-ring containing fractions (14-25) were combined and concentrated under reduced pressure to provide olefin **2.44** (17 mg, 42% yield). The product containing fractions (40-72) were combined and concentrated under reduced pressure to provide **2.45** (31 mg, 49% yield) as a clear oil: R_f = 0.31 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -6.9 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.25-7.16 (m, 5H), 7.05-6.96 (m, 5H), 6.84 (d, *J* = 9.1 Hz, 2H), 6.58 (d, *J* = 16.9 Hz, 1H), 6.17 (d, *J* = 16.9 Hz, 1H), 5.85 (s, 1H), 5.48 (s, 1H), 4.59 (d, *J* = 11.3 Hz, 1H), 4.38 (d, *J* = 11.3 Hz, 1H), 4.20-4.00 (m, 3H), 3.93 (s, 2H), 3.89-3.79 (m, 1H), 3.79 (s, 3h), 3.74-3.67 (m, 2H), 3.64 (s, 3H), 3.46 (d, *J* = 15.8 Hz, 1H), 3.27 (s, 3H), 2.86 (dd, *J* = 13.1, 6.1 Hz, 1H), 2.73 (dd, *J* = 13.1, 7.8 Hz, 1H), 2.44 (s, 1H), 2.36 (dd, *J* = 13.8, 13.8 Hz, 1H), 2.00 (dd, *J* = 14.3, 10.3 Hz, 1H), 1.82 (s, 3H), 1.80-1.72 (m, 1H), 1.66 (ddd, *J* = 14.0, 10.6,

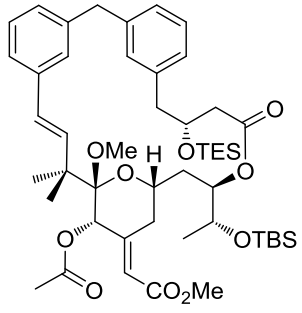
2.3 Hz, 1H), 1.63-1.55 (m, 1H), 1.22 (s, 3H), 1.20 (s, 3H), 1.15 (d, *J* = 6.4 Hz, 3H), 0.98-0.87 (m, 18H), 0.60-0.50 (m, 6H), 0.11 (d, *J* = 3.5 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.7, 166.7, 159.4, 152.8, 141.4, 141.4, 138.9, 138.5, 138.3, 130.9, 130.3, 129.3, 128.8, 128.6, 127.6, 127.5, 127.5, 127.4, 127.1, 127.1, 123.5, 114.0, 102.9, 78.8, 73.5, 71.9, 71.9, 68.5, 67.2, 60.5, 55.5, 51.4, 51.3, 46.5, 44.0, 42.0, 37.8, 35.3, 33.2, 26.1, 26.0, 24.5, 23.2, 21.4, 18.3, 17.2, 7.1, 5.1, -4.4, -4.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 51.4, 51.3, 26.1, 26.0, 24.5, 23.2, 21.4, 17.2, 7.1, -4.4, -4.5; CH_2 δ 71.9, 60.5, 44.0, 42.0, 37.8, 35.3, 33.2, 5.1; CH δ 138.5, 130.3, 129.3, 128.8, 128.6, 127.6, 127.5, 127.5, 127.4, 127.1, 127.1, 123.5, 114.0, 78.8, 73.5, 71.9, 68.5, 67.2; C δ 169.7, 166.7, 159.4, 152.8, 141.4, 141.4, 138.9, 138.3, 130.9, 102.9, 46.5, 18.3; IR (neat) 3476, 2955, 1746, 1721, 1601, 1514, 1462 cm^{-1}; HRMS (ESI/ APCI) calcd 1025.5606 for $C_{57}H_{86}O_{11}Si_2Na$ found 1025.5616.



Preparation of (*R*)-4-(3-(3-((*E*)-3-((2*S*,3*S*,6*S*,*E*)-3-acetoxy-6-((2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-2-yl)-3-methylbut-1-en-1-yl)benzyl)phenyl)-3-((triethylsilyl)oxy)butanoic acid 2.55: To a stirring of alcohol **2.45** (12.0 mg, 0.012 mmol, 1 equiv) in CH_2Cl_2 (120 μL) in a 2 mL reaction vial, at 0 °C, was added *i*-Pr_2NEt (15.0 μL, 0.084 mmol, 7 equiv), DMSO (8.0 μL,

0.120 mmol, 10 equiv), and SO_3•Pyr (8.0 mg, 0.048 mmol, 4 equiv) in a single portion. This solution was stirred at 0 °C for 45 min, and then quenched with a saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated, and aqueous layer was extracted with 40% EtOAc/hexanes (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. The resulting clear oil was run through a 1.5 × 4.5 cm plug of silica with 20% EtOAc/hexanes, and concentrated to give the crude aldehyde (11.0 mg).

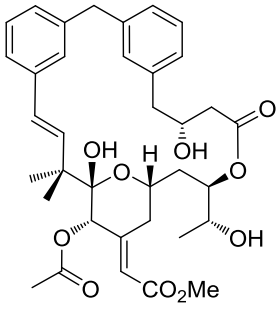
To a stirring solution of the aforementioned aldehyde in 2-methyl-2-butene (171 μL) and *t*-BuOH (171 μL) in a 5 mL rb flask, at rt, was added a 1.25 M aqueous solution of KH_2PO_4 (60.0 μL). This solution was cooled to -10 °C in an ethelene glycal/CO_2 bath, and $NaClO_2$ (80% by wt., 5.0 mg, 0.06 mmol, 5 equiv) was added in a single portion. The reaction was stirred vigorously for 1.5 h, and then quenched with a 0.05 M aqueous pH 4 buffer solution (1 mL). The phases were separated, and aqueous layer was extracted with Et_2O (3 × 3 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated. Purification was accomplished by flash column chromatography using a 1.5 × 10.0 cm silica gel column, eluting with 30% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. The product containing fractions (2-8) were concentrated to give acid **2.55** (10.0 mg, 82%, 2 steps) as a clear oil that was used without characterization. R_f = 0.67 (10% MeOH/ 40% EtOAc/ 50% hexanes).



Preparation of Protected Merle 40 2.46: To a stirring solution of PMB ether **2.55** (10.0 mg, 0.0098 mmol, 1 equiv) in CH_2Cl_2 (250 μL) and water (2.5 μL) in a 5 mL rb flask, at rt, was added DDQ (3.3 mg, 0.015 mmol, 1.5 equiv). After 30 min the reaction was pipeted directly onto a 1.5 × 8.0 cm silica gel column, eluting with 25% EtOAc/hexanes, and collecting 10 × 75 mm test tube fractions. Product containing fractions (8-18) were concentrated to give seco-acid (7.0 mg, 80%) as a clear oil. R_f = 0.61 (10% MeOH/ 40% EtOAc/ 50% hexanes).

To stirring a solution of the aforementioned seco-acid in THF (260 μL) in 2 mL vial, at 0 °C, was added Et_3N (6.5 μL, 0.047 mmol, 6 equiv) and a 1.0 M solution of trichlorobenzoyl chloride (23 μL, 0.023 mmol, 3 equiv) in THF. After 5 min, the mixture was warmed to rt and stirring was continued for an additional 3 h. The reaction mixture was diluted with toluene (2.6 mL) and taken up into a 5 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (19.0 mg, 0.156 mmol, 20.0 equiv) in toluene (5.2 mL) at 40 °C over 12 h. The residual contents of the syringe were rinsed into the flask with toluene (1 mL) and stirring was continued for an additional 2 h. The reaction mixture was cooled to rt, diluted with 30% EtOAc/hexanes (25 mL) and washed with water (3 × 10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography using a 0.5 × 5.5 cm

silica gel column, eluting with 5% EtOAc/hexanes, and collecting 6 × 50 mm test tube fraction. The product containing fractions (4-13) were combined and concentrated under reduced pressure to provide macrolactone **2.46** as a clear oil (4.0 mg, 58% over 2 steps) along with a compound resulting from elimination of the C19 methyl ketal (1.0 mg, 14% yield). Data for protected Merle 40 **2.46**: R_f = 0.69 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = + 5.0 (c =1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.24-7.12 (m, 5H), 7.06-7.02 (m, 2H), 7.00 (d, *J* = 7.1 Hz, 1H), 6.24 (s, 2H), 5.90 (s, 1H), 5.32 (s, 1H), 5.05 (d, *J* = 9.3 Hz, 1H), 4.26 (ddd, *J* = 12.0, 6.5, 6.5 Hz, 1H), 4.00-3.95 (m, 1H), 3.93 (q, *J* = 14.0 Hz, 2H), 3.76-3.68 (m, 1H), 3.60 (s, 3H), 3.50 (d, *J* = 15.1 Hz, 1H), 2.95 (s, 3H), 2.82 (dd, *J* = 13.9, 4.8 Hz, 1H), 2.68 (dd, *J* = 13.9, 7.1 Hz, 1H), 2.24-2.16 (m, 2H), 2.10-2.02 (m, 2H), 2.07 (s, 3H), 1.79 (ddd, *J* = 14.5, 10.1, 4.6 Hz, 1H), 1.25 (s, 3H), 1.11 (s, 3H), 1.08 (d, *J* = 6.3 Hz, 3H), 0.93 (t, *J* = 8.2 Hz, 9H), 0.93 (s, 9H), .057 (q, *J* = 8.2 Hz, 6H), 0.15 (s, 3H), 0.11 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.7, 169.4, 166.7, 152.0, 141.9, 141.9, 141.7, 138.5, 138.0, 137.5, 130.7, 128.6, 128.3, 127.9, 127.8, 127.0, 126.9, 126.0, 125.3, 103.3, 72.2, 70.1, 68.8, 67.7, 51.5, 51.3, 45.9, 43.5, 42.2, 41.4, 34.1, 31.8, 26.0, 24.3, 23.1, 21.5, 18.3, 17.8, 7.1, 5.0, -4.5, -4.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.5, 51.3, 26.0, 23.1, 21.5, 17.8, 7.1, -4.5, -4.6; CH_2 δ 43.5, 42.2, 41.4, 34.1, 5.1; CH δ 137.5, 130.7, 128.6, 128.3, 127.9, 127.8, 127.0, 126.9, 126.0, 125.3, 72.2, 70.1, 68.8, 67.7; C δ 170.7, 169.4, 166.7, 152.0, 141.9, 141.7, 138.5, 138.0, 103.3, 45.9, 18.3; IR (neat) 2954, 2884, 1743, 1719, 1661, 1602, 1472 cm^{-1}; HRMS (ESI/ APCI) calcd 901.4718 for $C_{49}H_{74}O_{10}Si_2Na$ found 901.4732.



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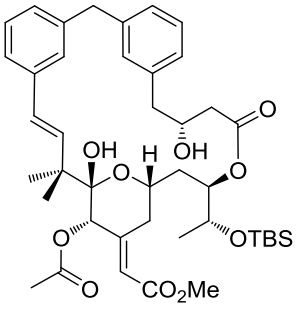

Preparation of Merle 40 and 2.48:

To a stirring solution of analog precursor **2.46** (4.2 mg, 0.0048 mmol, 1 equiv) in CH_3CN (432 μL) and water (48 μL) in a 2 mL plastic vial, at rt, was added a 48% aqueous HF solution (19 μL) using a plastic ependorf pipet. After 2 h the reaction mixture was quenched by the addition of a 1:1 mixture of EtOAc and a saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using a 0.5 × 5.0 cm silica gel column, eluting with 25% EtOAc/hexanes collecting 6 × 50 mm test tube fractions. The C26 TBS protected Merle 40 containing fractions (14-20) were combined and concentrated to give **2.48** (2.4 mg, 67% yield) as a clear oil, and then the eluent was changed to 65% EtOAc/hexanes. The product containing fractions (32-40) were combined and concentrated under reduced pressure to provide Merle 40 (0.5 mg, 15% yield) as a clear oil.

Data for **2.48**. R_f = 0.72 (10% MeOH/ 40% EtOAc/ 50% hexanes); $[\alpha]_D^{20}$ = + 4.8 (c = 0.24, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.27-7.23 (m, 2H), 7.20-7.11 (m, 3H), 7.10-7.06 (m, 1H), 6.99-6.92 (m, 2H), 6.42 (d, *J* = 17.1 Hz, 1H), 6.37 (d, *J* = 17.1 Hz, 1H), 5.99 (s, 1H), 5,31 (s, 1H), 5.14 (s, 1H), 5.08 (dd, *J* = 10.8, 4.4 Hz, 1H), 4.24-4.16 (m, 1H), 3.95 (Aβq, *J* = 15.2 Hz, Δν = 12.5 Hz, 2H), 3.83-3.75 (m, 1H), 3.68 (s, 3H),

3.65-3.57 (m, 2H), 2.93 (dd, *J* = 14.2, 5.7 Hz, 1H), 2.82 (d, *J* = 8.7 Hz, 1H), 2.69 (dd, *J* = 13.5, 3.4 Hz, 1H), 2.36 (dd, *J* = 16.2, 7.9 Hz, 1H), 2.23 (dd, *J* = 15.2, 3.8 Hz, 1H), 2.09 (s, 3H), 1.91 (dd, *J* = 13.3, 13.3 Hz, 1H), 1.28 (s, 3H), 1.15 (s, 3H), 1.14 (d, *J* = 6.3 Hz, 3H), 0.94 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.3, 169.5, 166.9, 151.7, 142.1, 141.9, 137.5, 136.6, 135.3, 131.2, 130.7, 128.8, 128.8, 128.4, 127.6, 127.5, 127.0, 125.1, 119.9, 98.7, 74.5, 71.8, 68.2, 67.7, 65.7, 51.4, 45.9, 43.1, 42.2, 39.2, 34.0, 31.4, 26.0, 24.6, 21.6, 20.7, 18.3, 17.9, -4.4, -4.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.4, 26.0, 24.6, 21.6, 20.7, 17.9, -4.4, -4.5; CH_2 δ 43.1, 42.2, 39.2, 34.0, 31.4; CH δ 135.3, 131.2, 130.7, 128.8, 128.8, 128.4, 127.6, 127.6, 127.5, 127.0, 125.1, 119.9, 74.5, 71.8, 68.2, 67.7, 65.8; C δ 171.3, 169.5, 166.9, 151.7, 142.1, 141.9, 137.5, 136.6, 98.7, 45.9, 18.3; IR (neat) 3492, 2930, 2857, 1722, 1660, 1462 cm^{-1}; HRMS (ESI/ APCI) calcd 773.3697 for $C_{42}H_{58}O_{10}SiNa$ found 773.6704.

Data for Merle 40. R_f = 0.40 (10% MeOH/ 40% EtOAc/ 50% hexanes); $[\alpha]_D^{20}$ = -11.0 (c = 0.5, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.22-7.02 (m, 7H), 6.96 (d, *J* = 7.1 Hz, 1H), 6.42 (d, *J* = 16.3 Hz, 1H), 6.39 (d, *J* = 16.3 Hz, 1H), 6.00 (s, 1H), 5.16 (s, 1H), 5.12 (s, 1H) 5.08 (ddd, *J* = 8.7, 6.5, 1.9 Hz, 1H), 4.19-4.11 (m, 1H), 3.98-3.93 (m, 1H), 3.93 (s, 2H), 3.91-3.81 (m, 1H), 3.69 (s, 3H), 3.66-3.61 (m, 1H), 2.88(d, *J* = 5.9 Hz, 2H), 2.79 (s, 2H), 2.38 (dd, *J* = 15.8, 6.5 Hz, 1H), 2.27 (dd, *J* = 15.4, 5.0 Hz, 1H), 2.09 (s, 3H), 1.93 (ddd, *J* = 14.5, 11.7, 2.2 Hz, 1H), 1.85-1.78 (m, 1H), 1.72 (d, *J* = 5.5 HZ, 1H), 1.30 (s, 3H), 1.26 (s, 3H), 1.24 (d, *J* = 6.4 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.1, 169.5, 166.9, 151.0, 142.4, 142.3, 137.5, 137.0, 135.5, 130.6, 129.0, 128.9, 128.7, 127.4, 127.3, 126.9, 124.9, 120.1, 98.7, 74.9, 74.3, 69.4, 69.1, 65.9, 51.4, 45.8,

42.6, 42.3, 39.2, 36.6, 31.3, 24.7, 21.7, 20.8, 20.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.4, 24.7, 21.7, 20.8, 20.1; CH_2 δ 42.6, 42.3, 39.2, 36.6, 31.3; CH δ 135.5, 130.6, 129.0, 128.9, 128.7, 127.4, 127.3, 126.9, 124.9, 120.1, 74.9, 74.3, 69.4, 69.1, 65.9; C δ 172.1, 169.5, 166.9, 151.0, 142.4, 142.3, 137.5, 137.0, 98.7, 45.8; IR (neat) 3470, 3016, 2960, 2888, 2840, 1746, 1730, 1600, 1510 cm^{-1}; HRMS (ESI/ APCI) calcd 659.2832 for $C_{36}H_{44}O_{10}Na$ found 659.2841.

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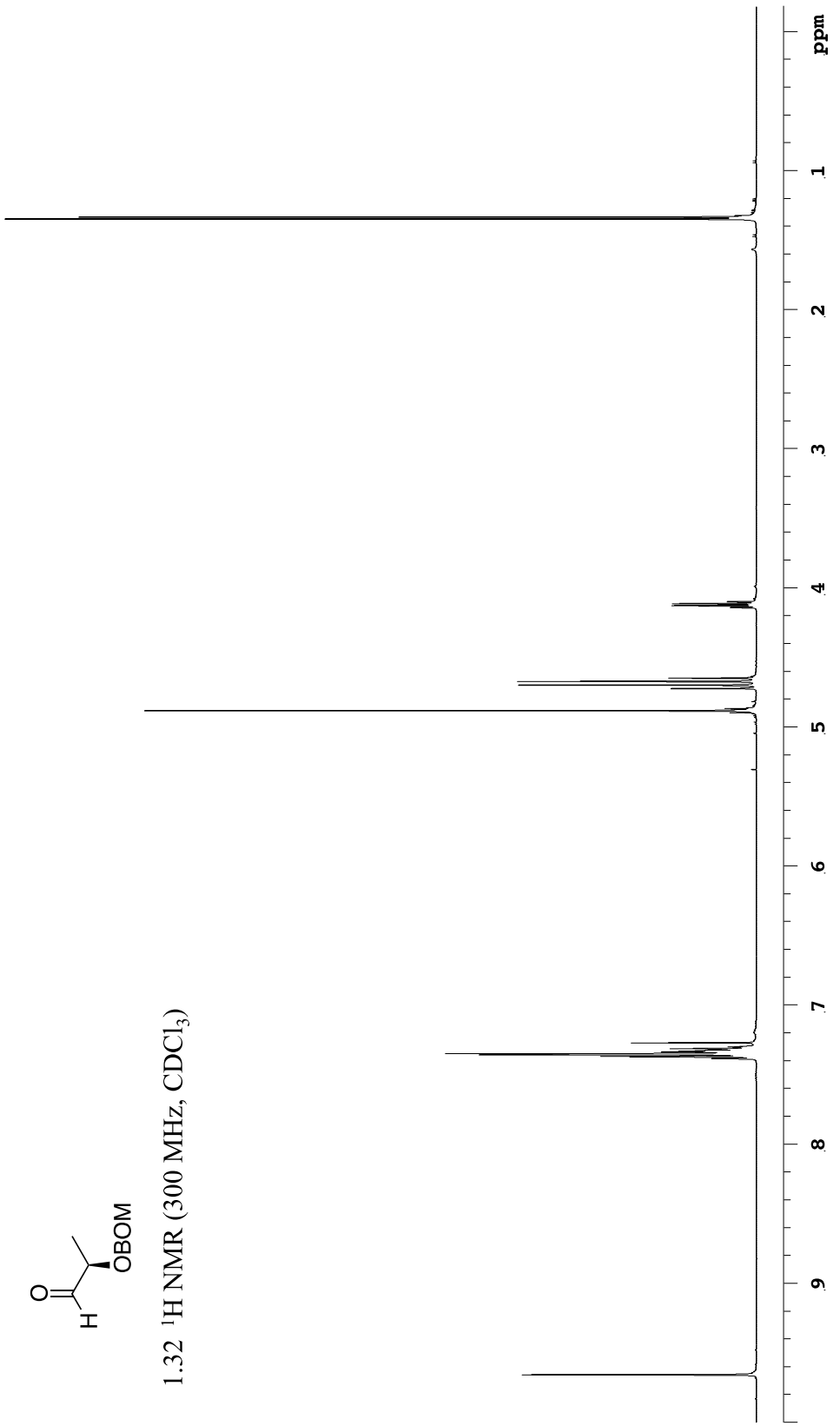
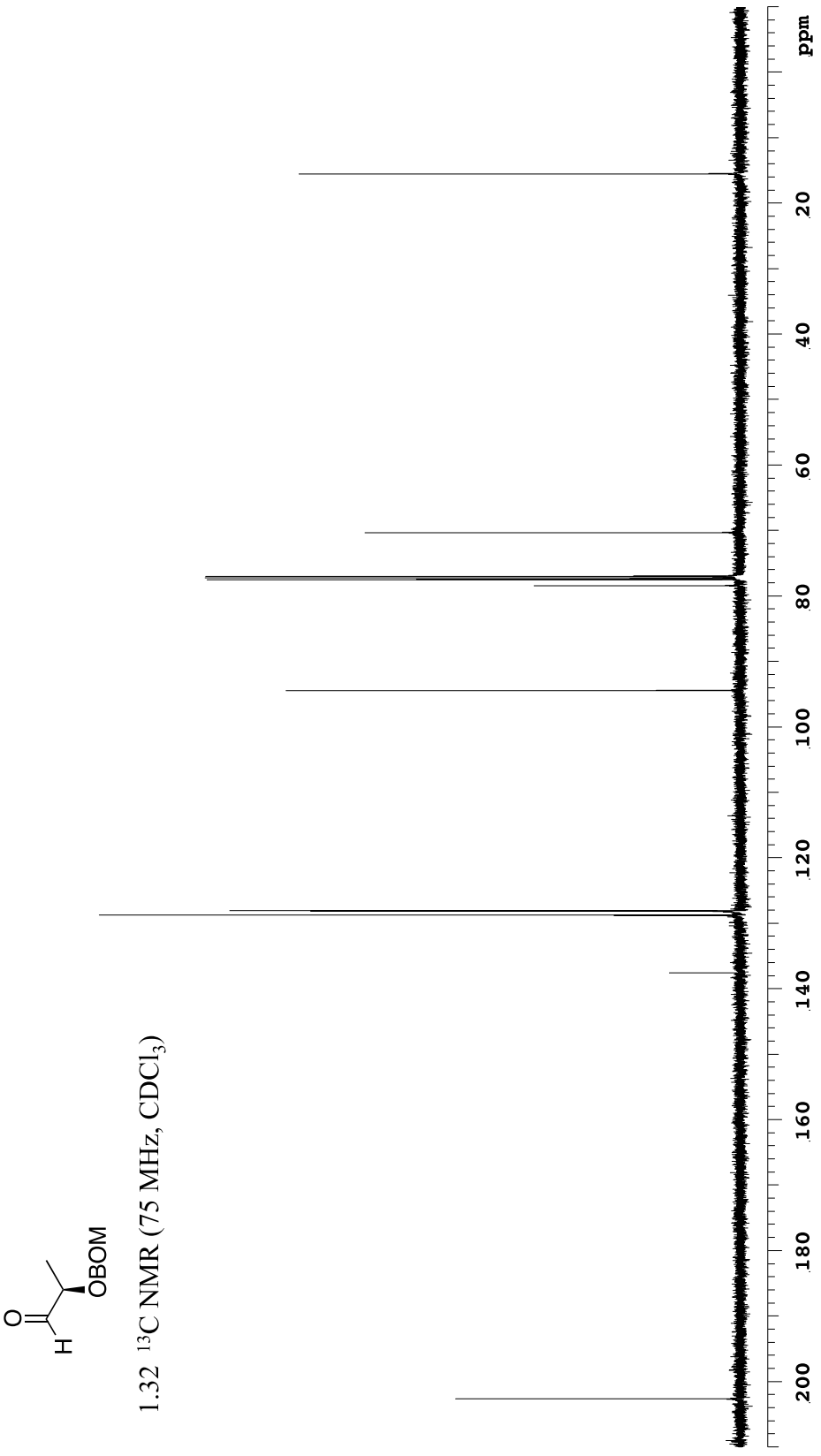
APPENDIX A

^{1}H, ^{13}C, AND DEPT SPECTRA FOR CHAPTER 1



1.32 ^{1}H NMR (300 MHz, $CDCl_3$)



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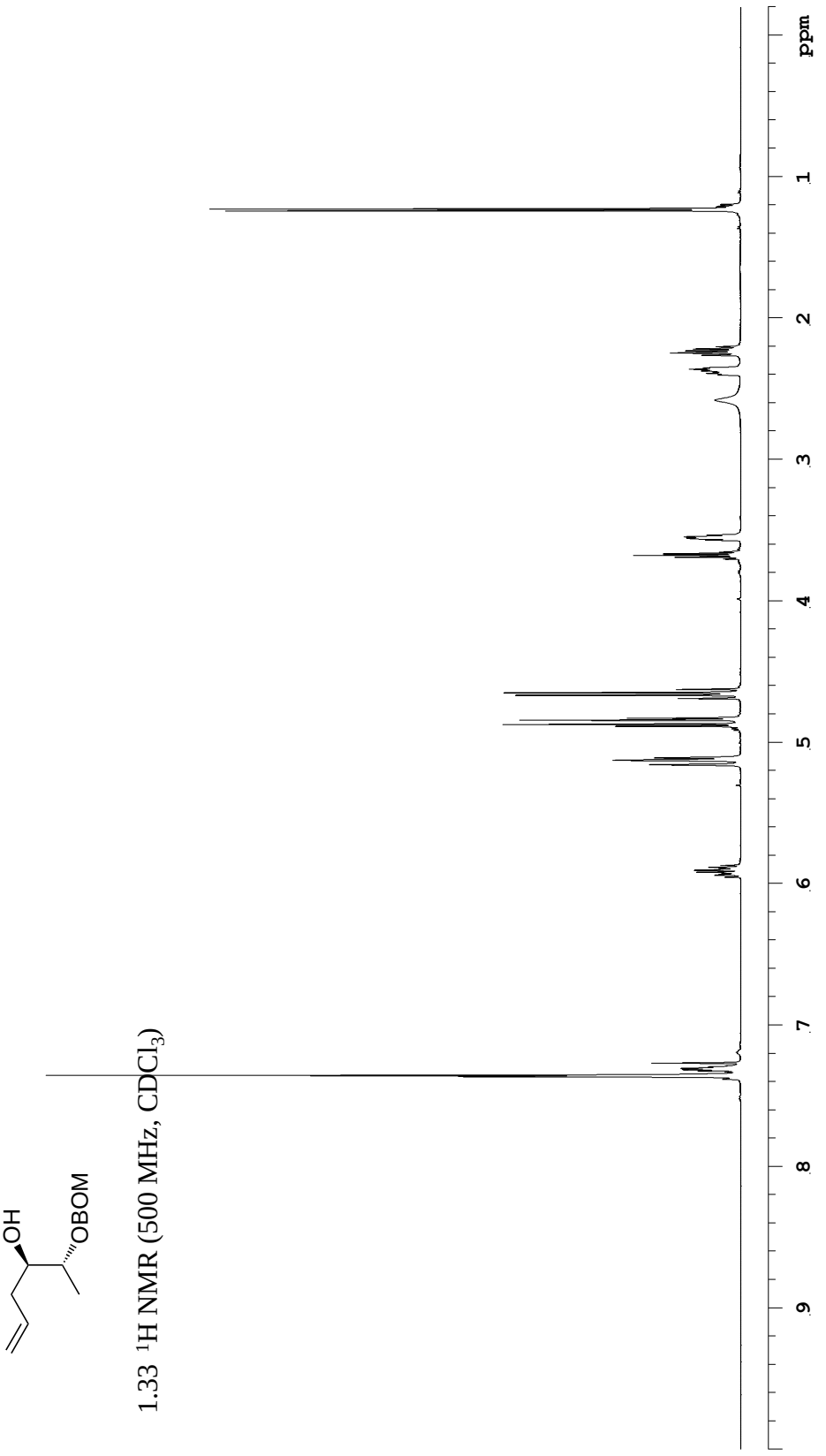

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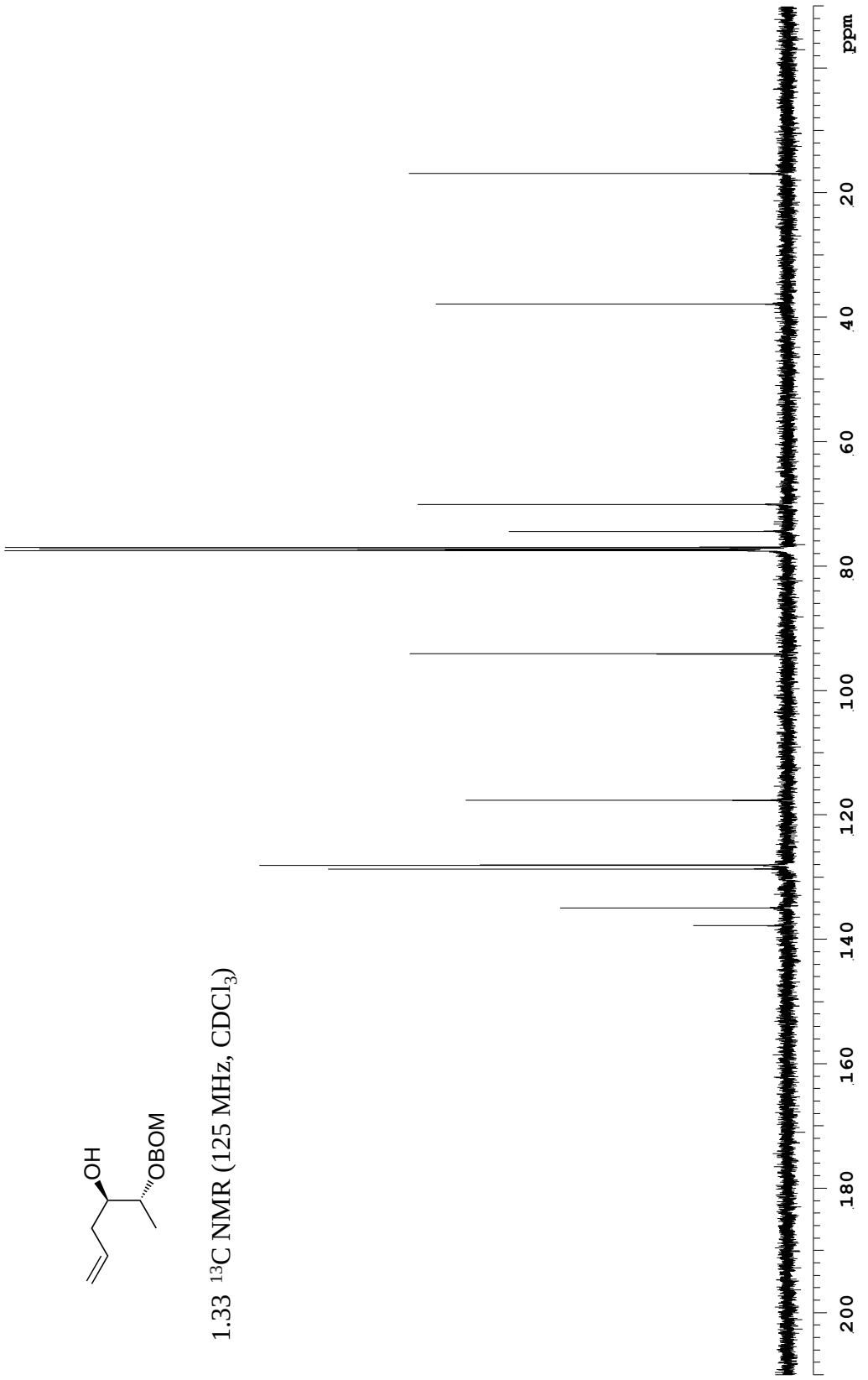

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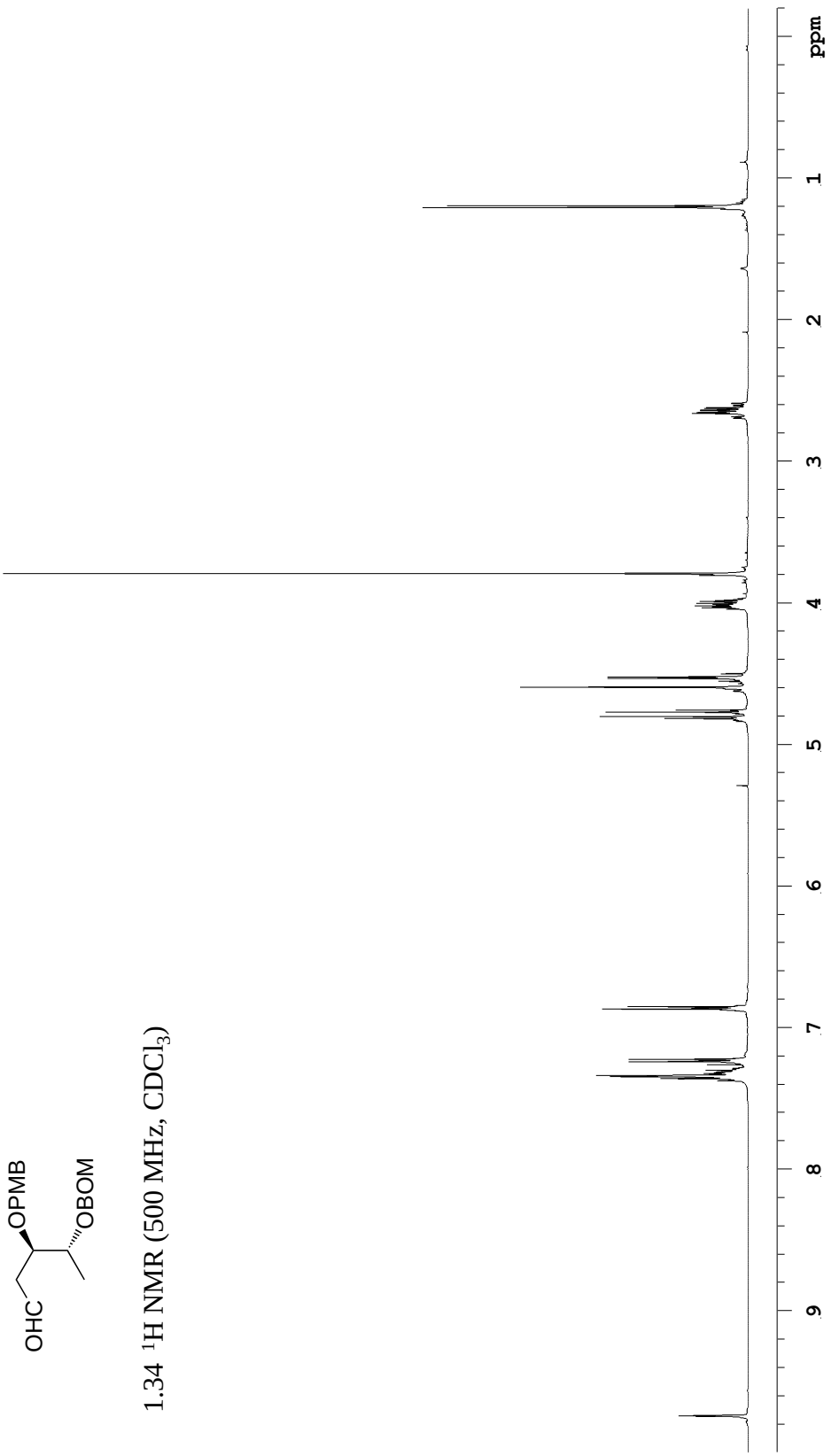

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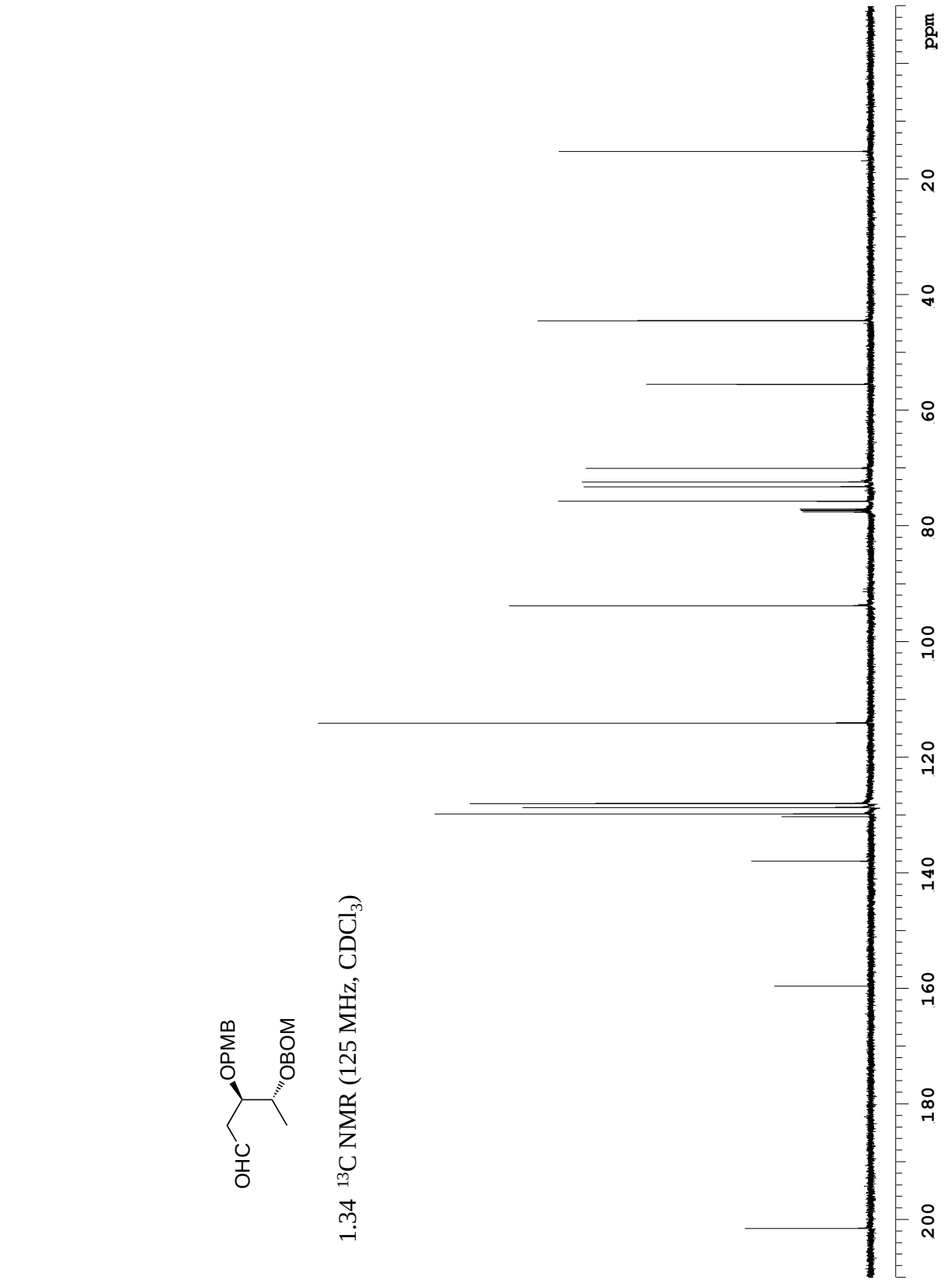

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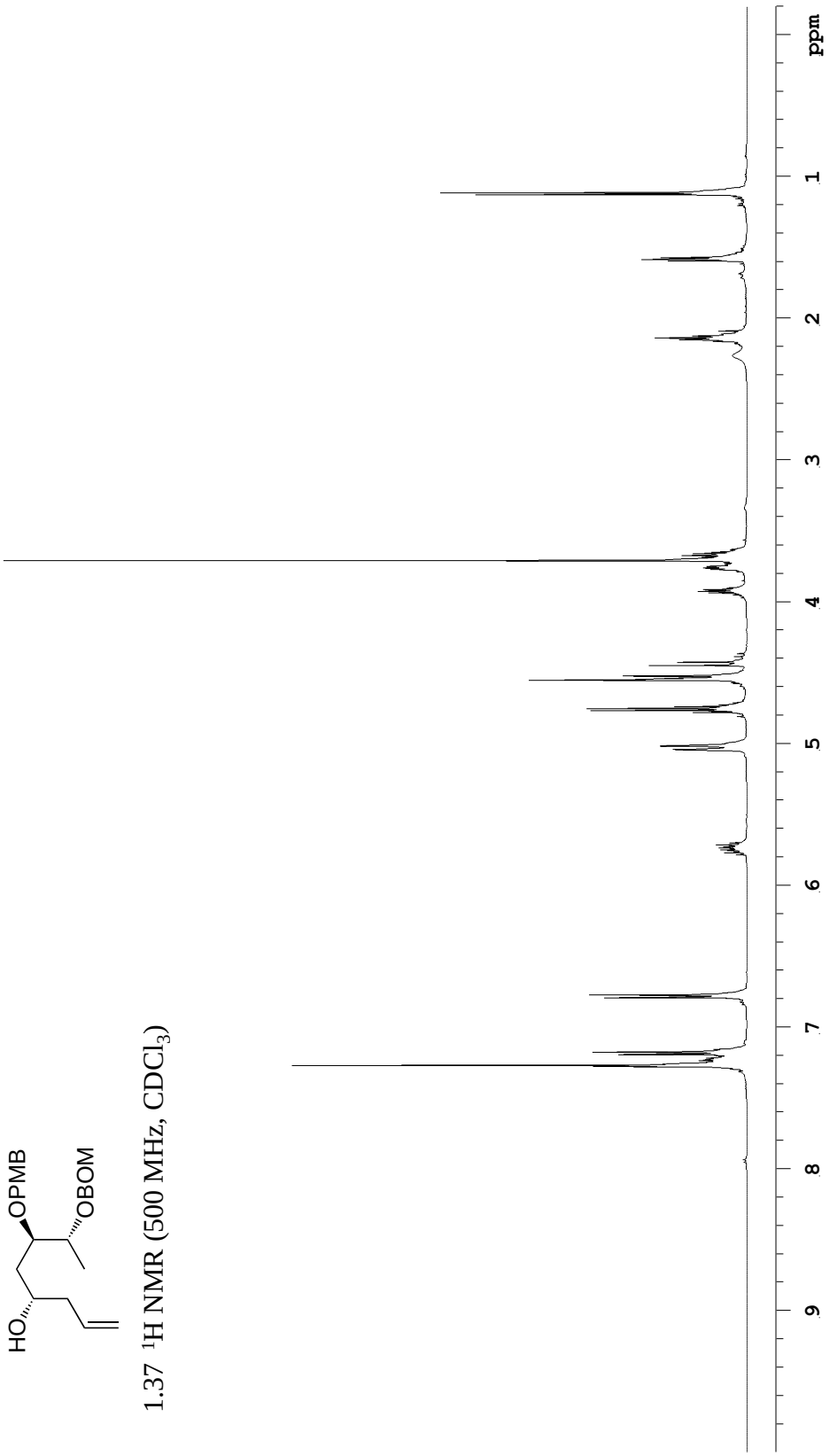

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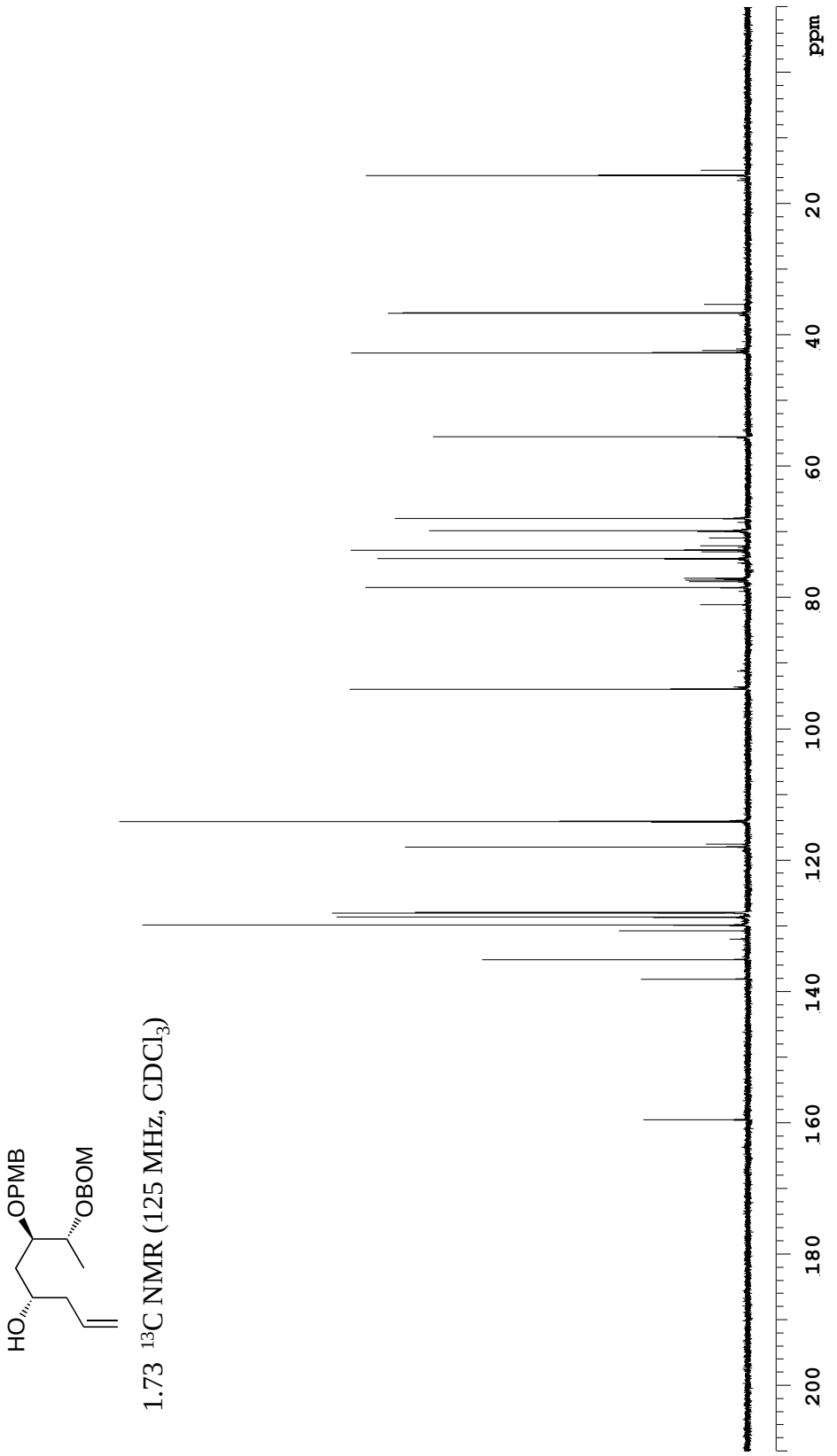

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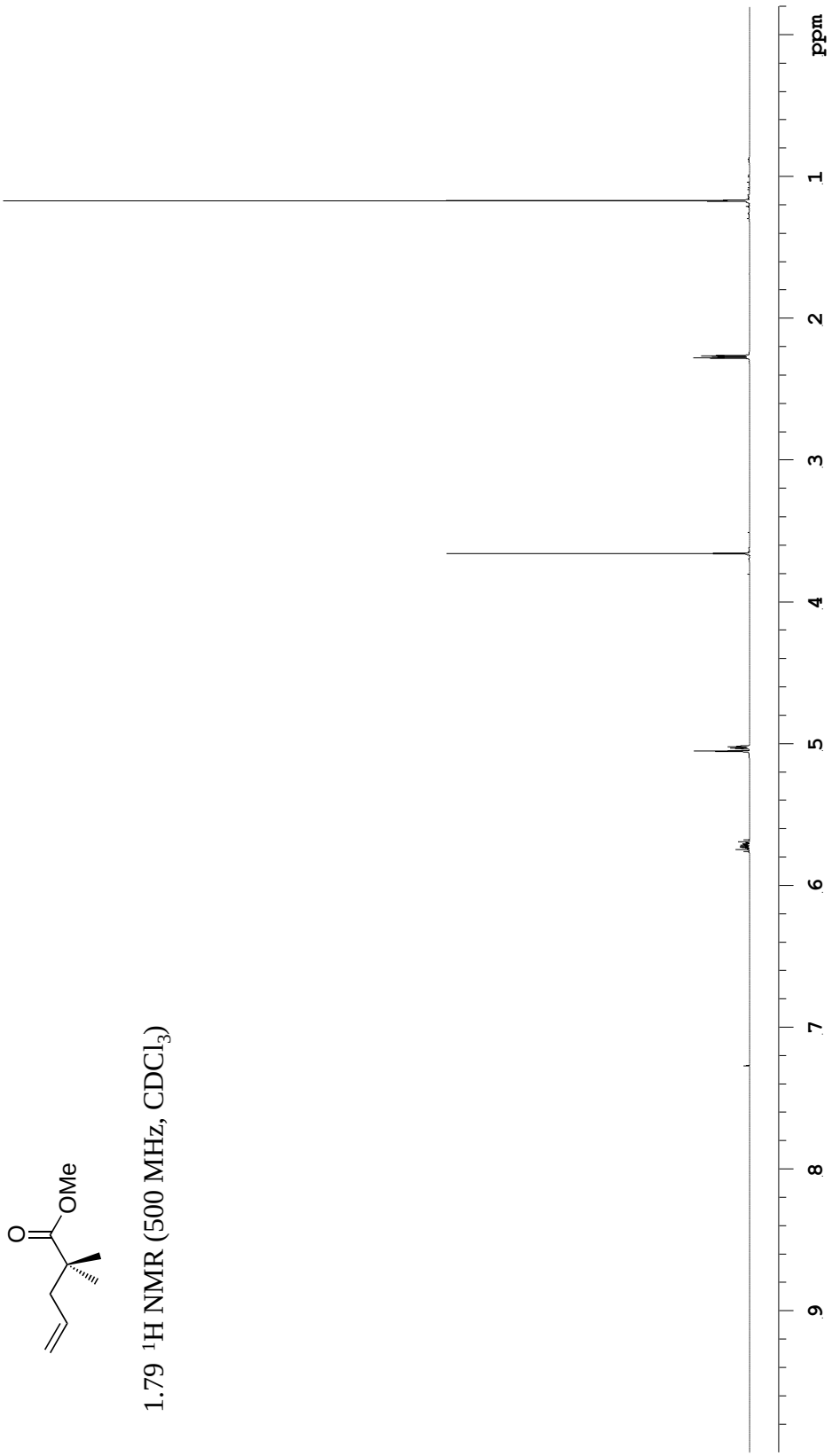

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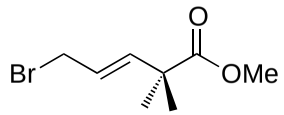

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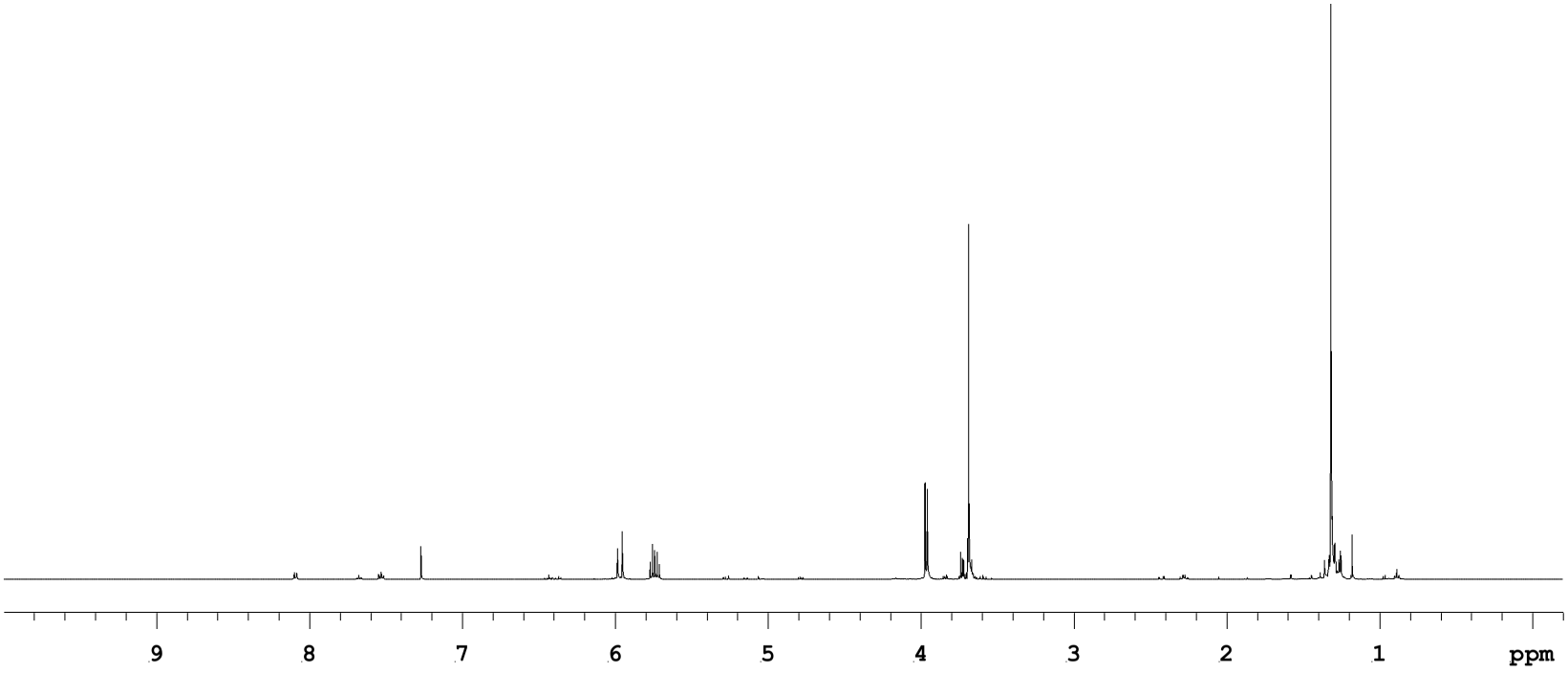

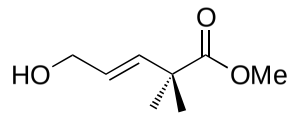

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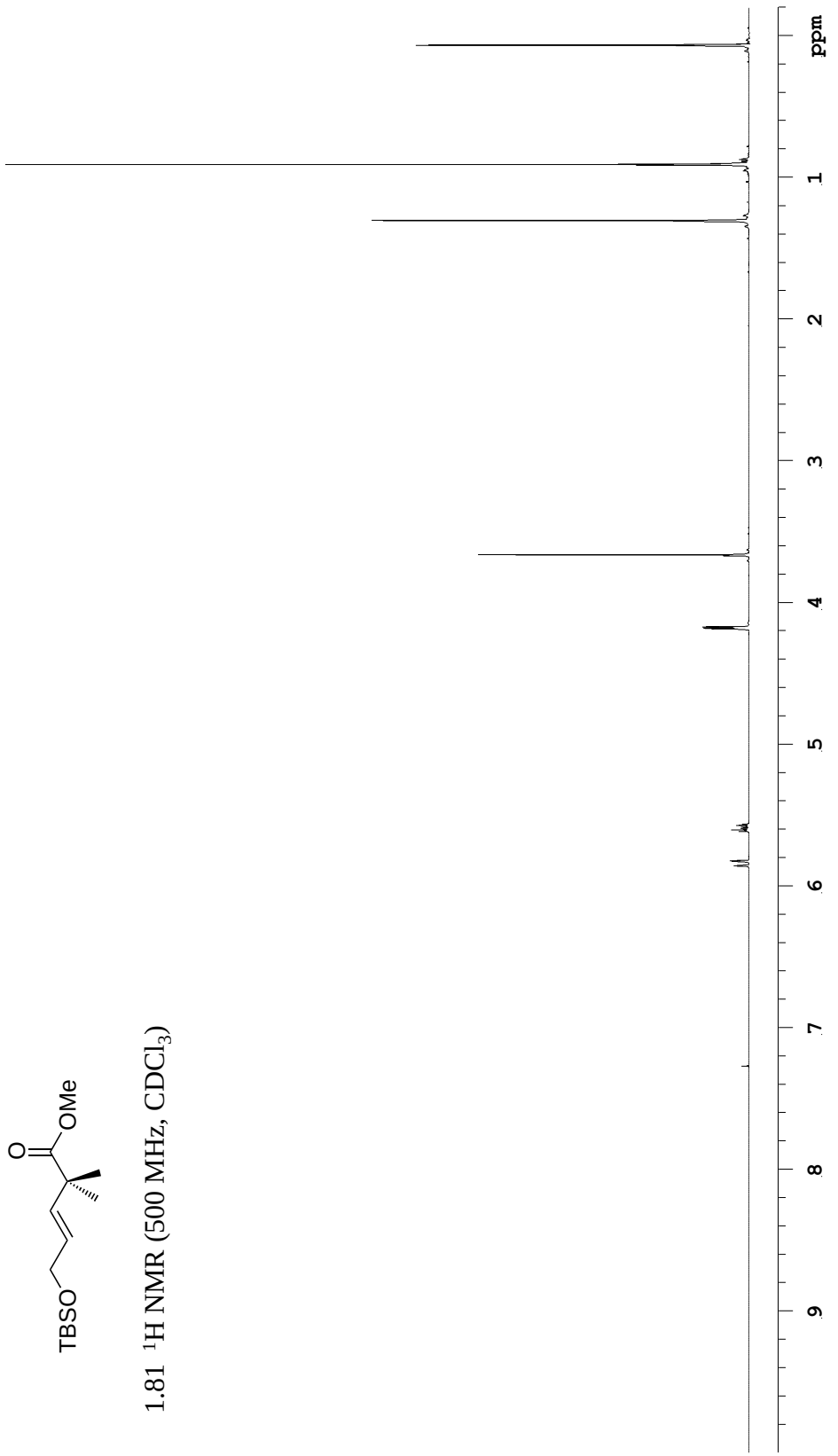

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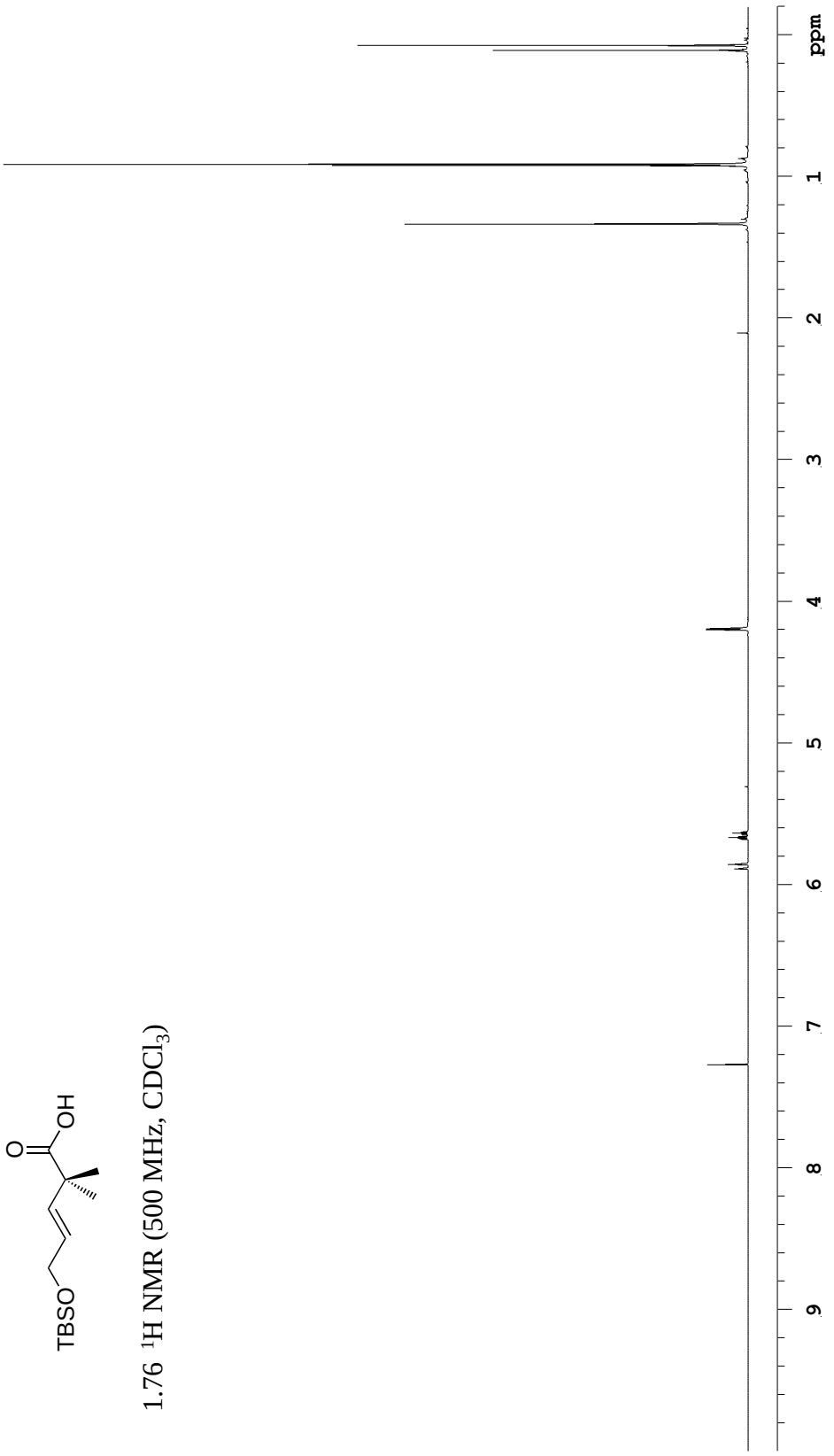

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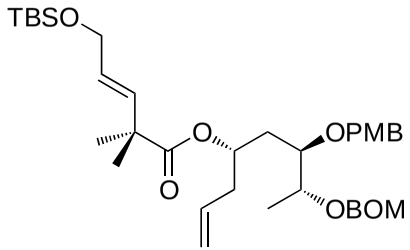

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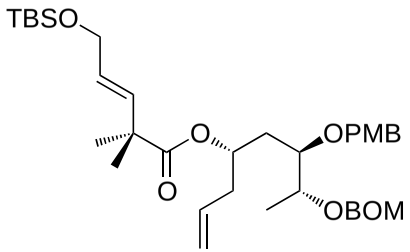

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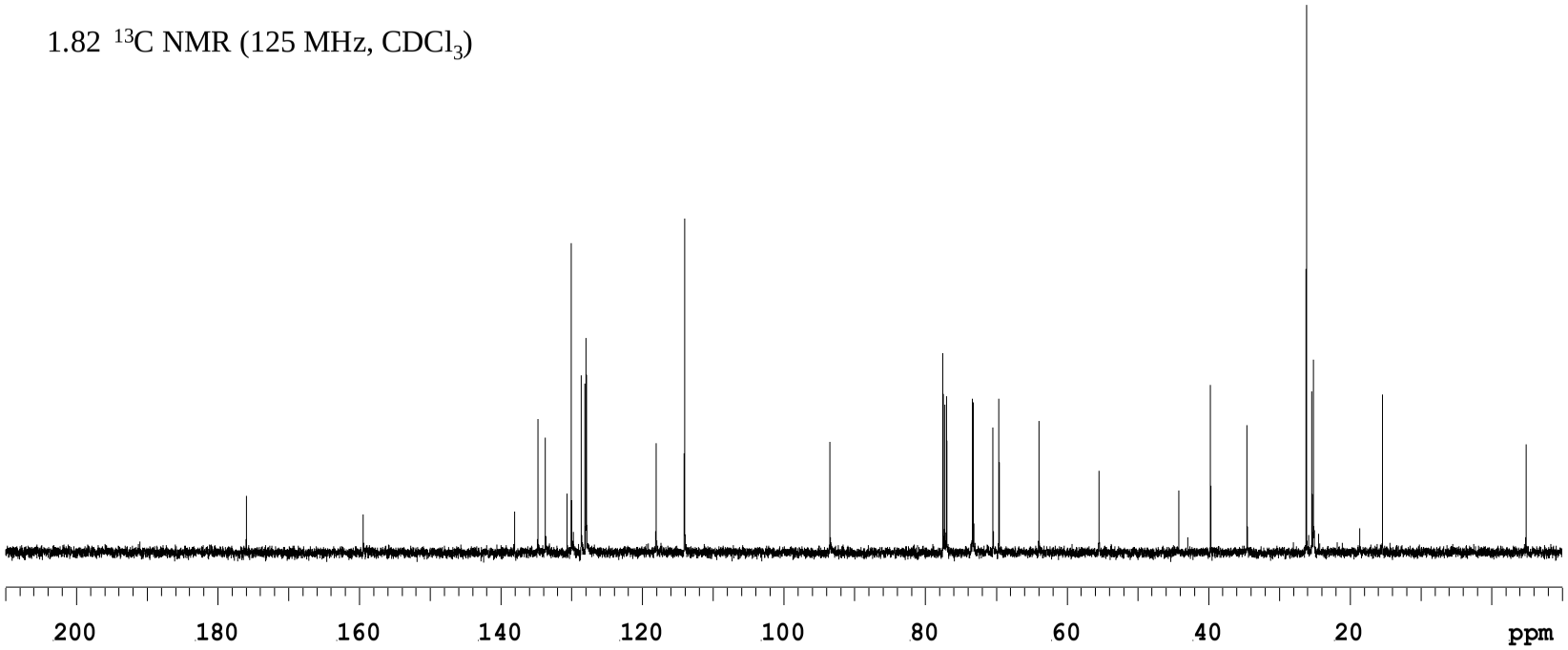

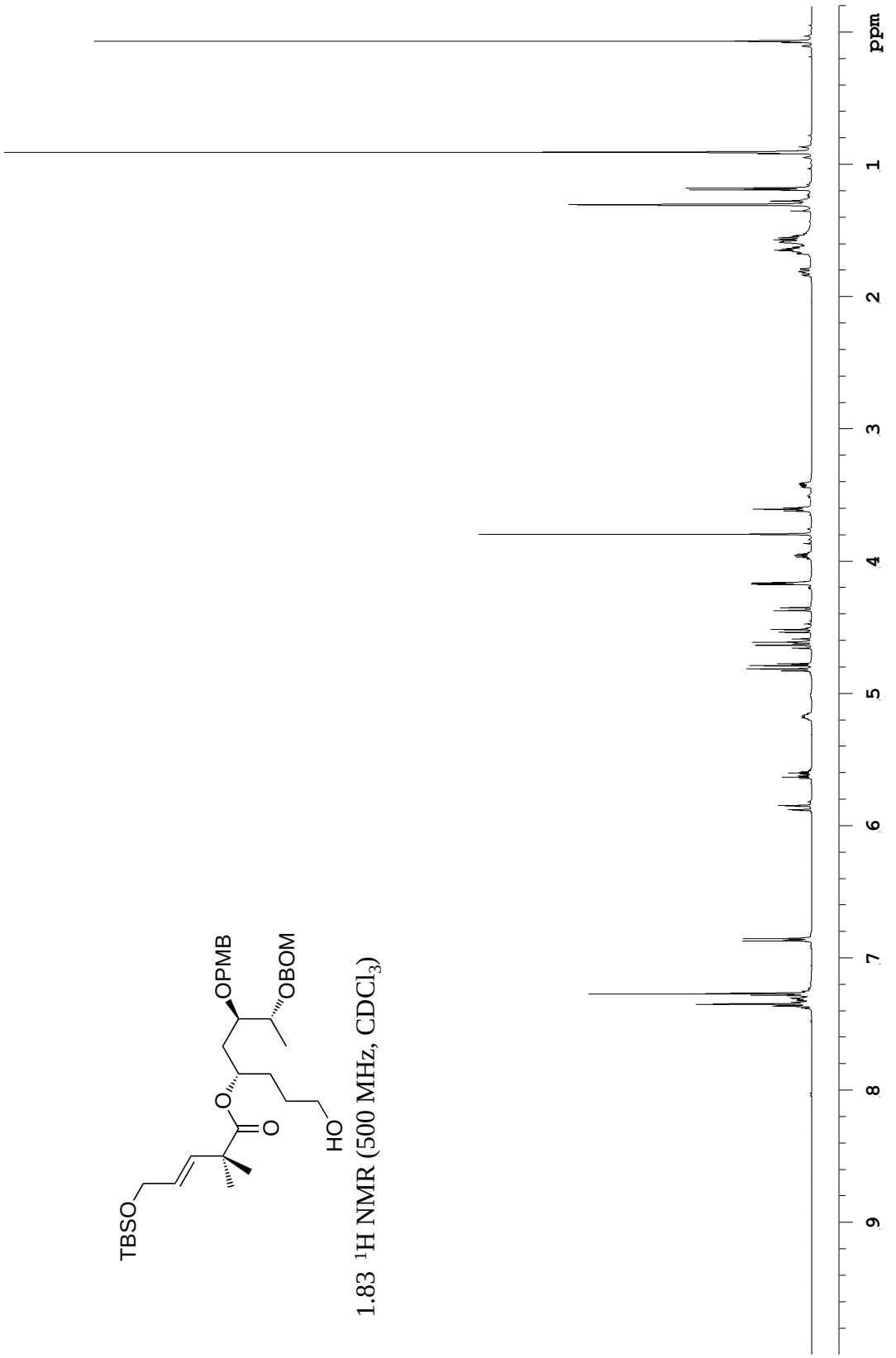

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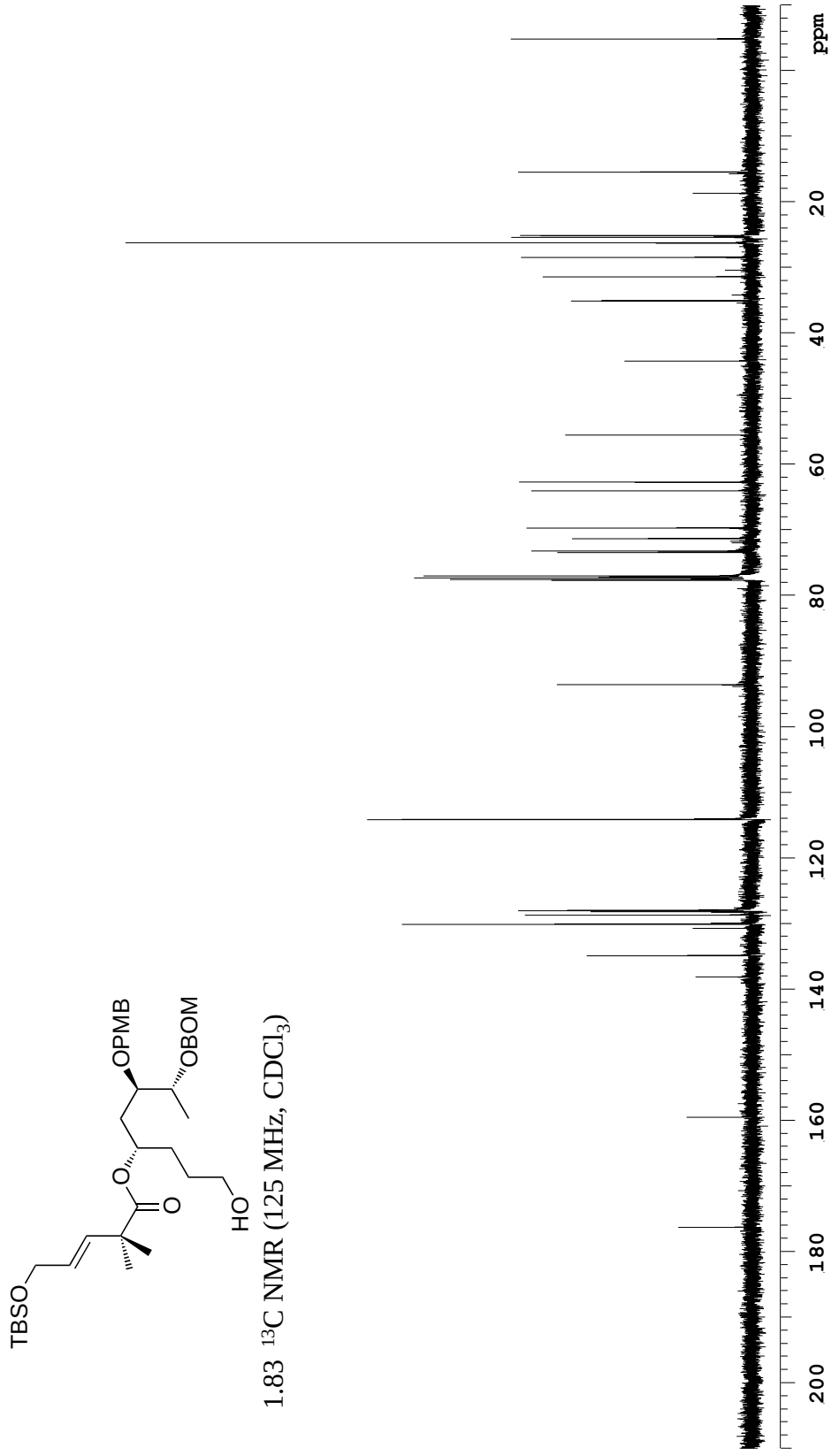
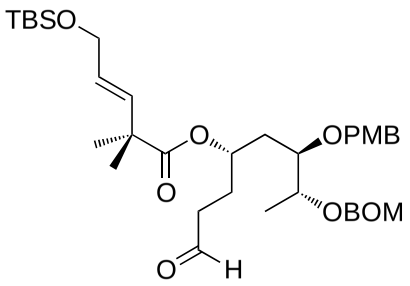
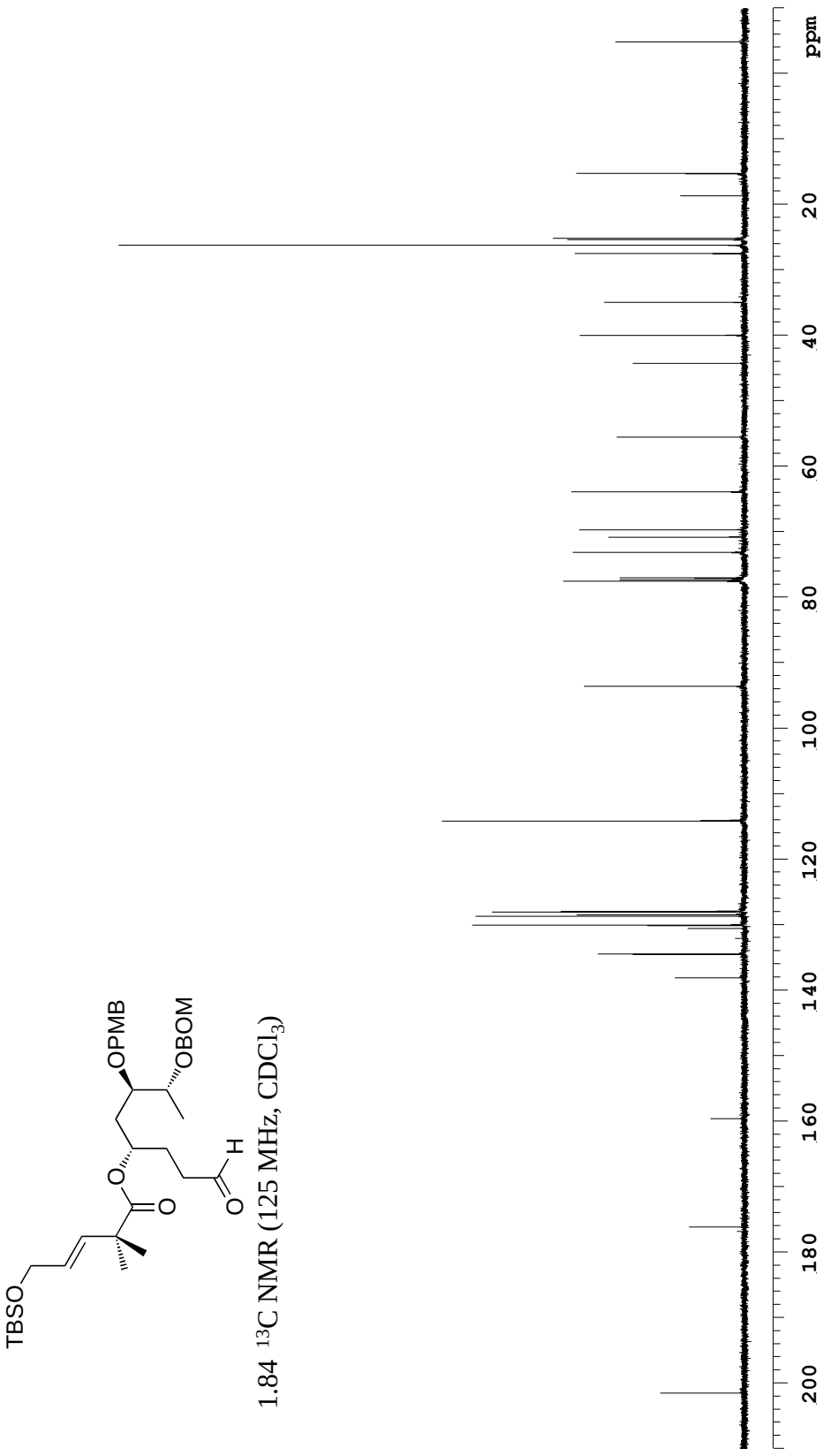


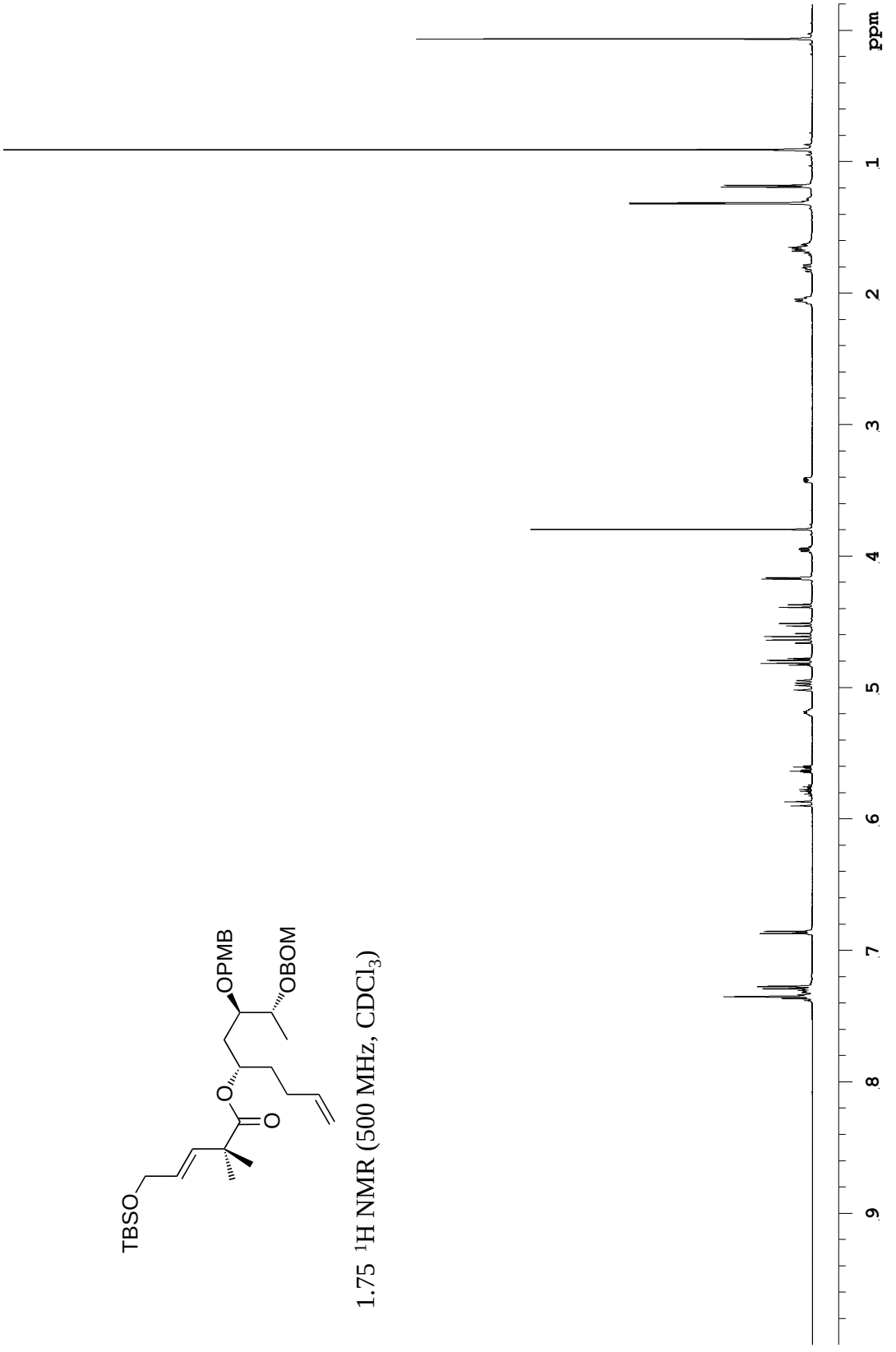
1.83 ^{13}C NMR (125 MHz, $CDCl_3$)



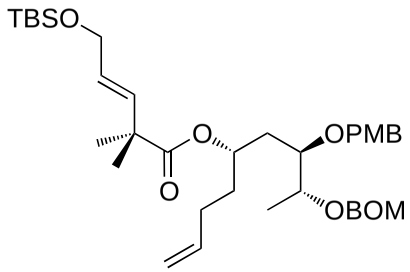
1.84 ^{1}H NMR (500 MHz, $CDCl_3$)



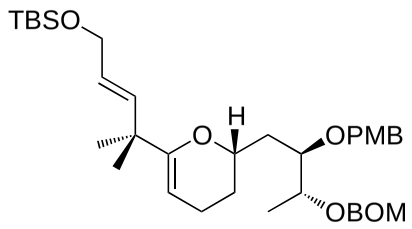
1.84 ^{13}C NMR (125 MHz, $CDCl_3$)



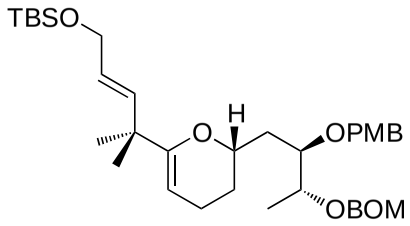
1.75 ^{1}H NMR (500 MHz, $CDCl_3$)



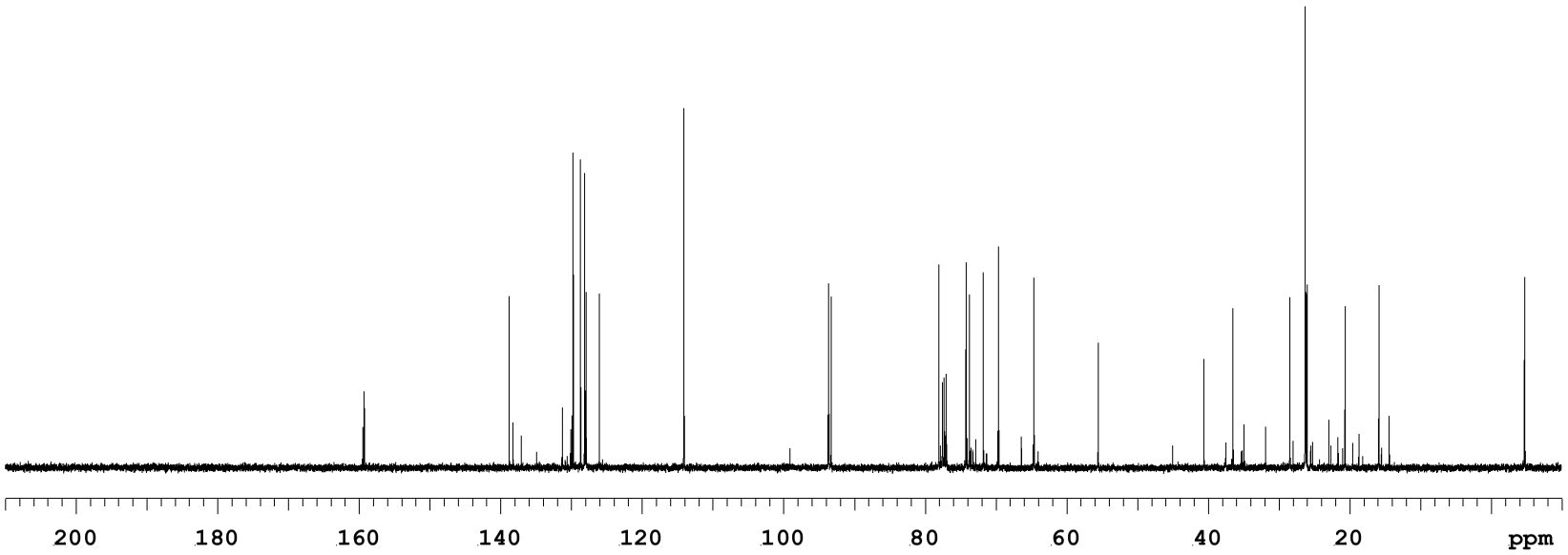
1.75 ^{13}C NMR (125 MHz, $CDCl_3$)

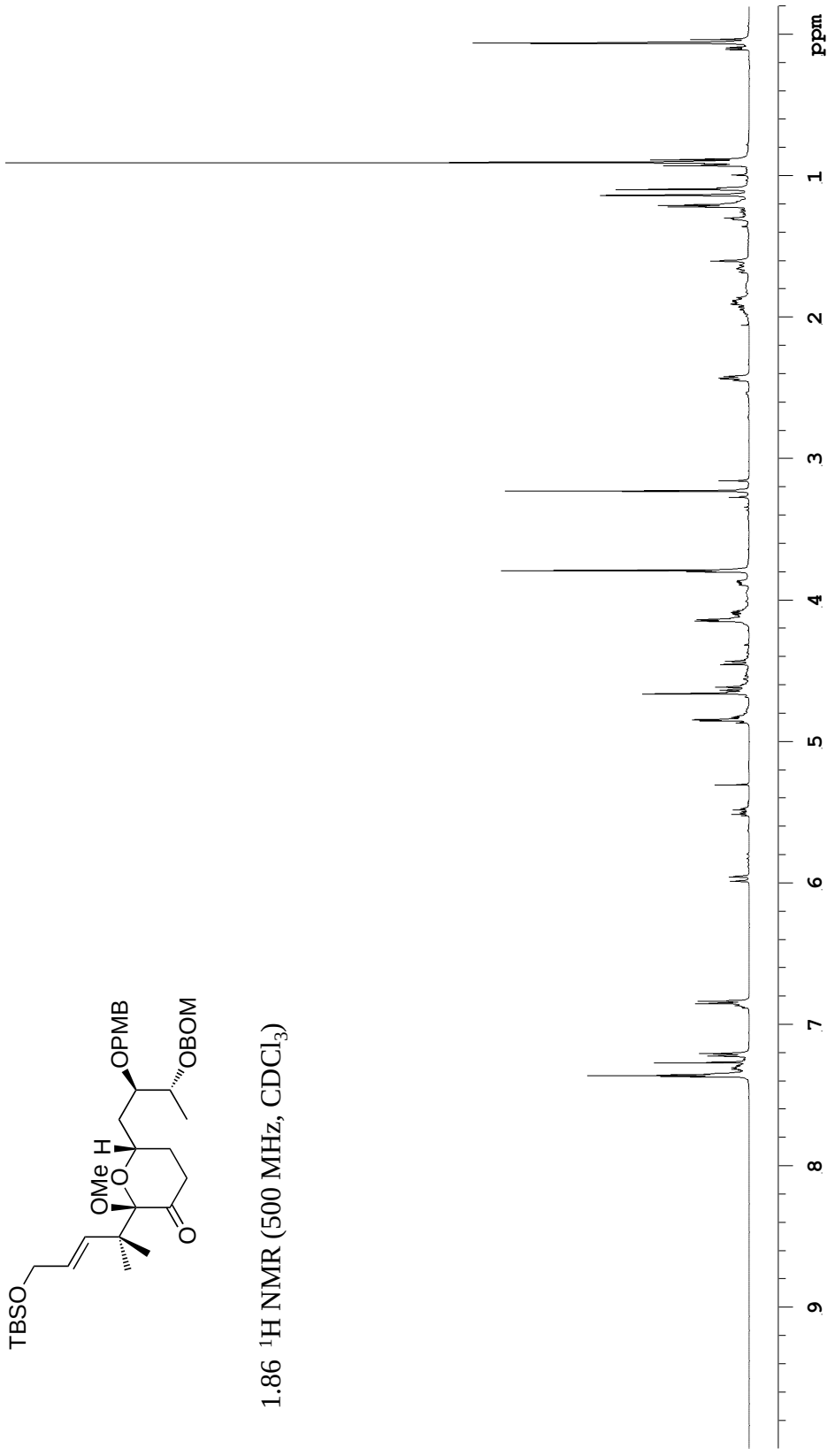


1.85 ^{1}H NMR (500 MHz, $CDCl_3$)

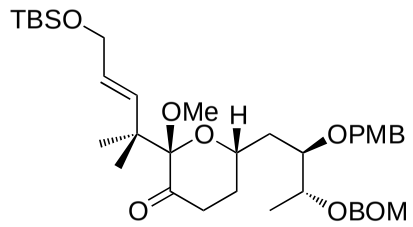


1.85 ^{13}C NMR (125 MHz, $CDCl_3$)

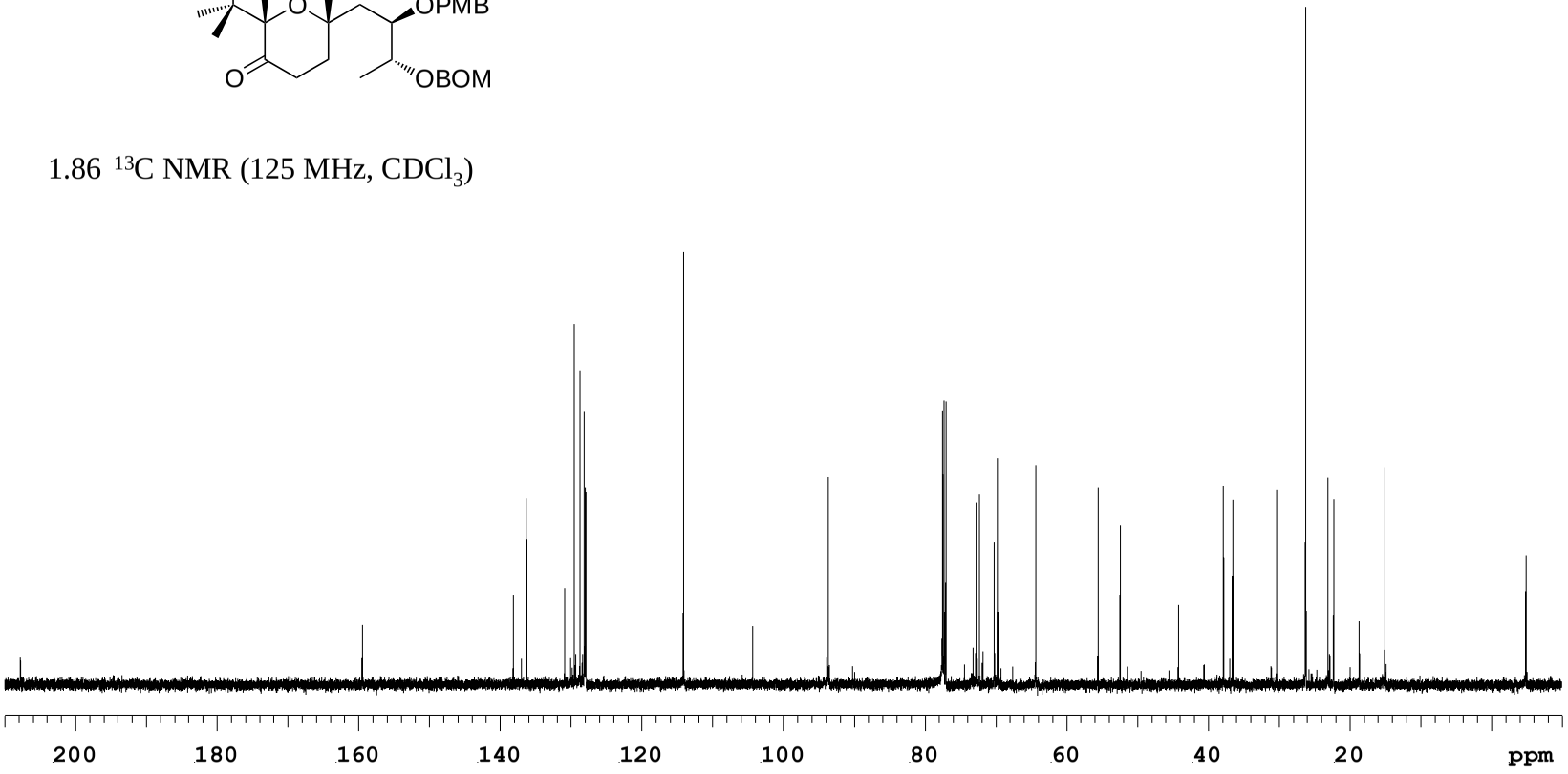


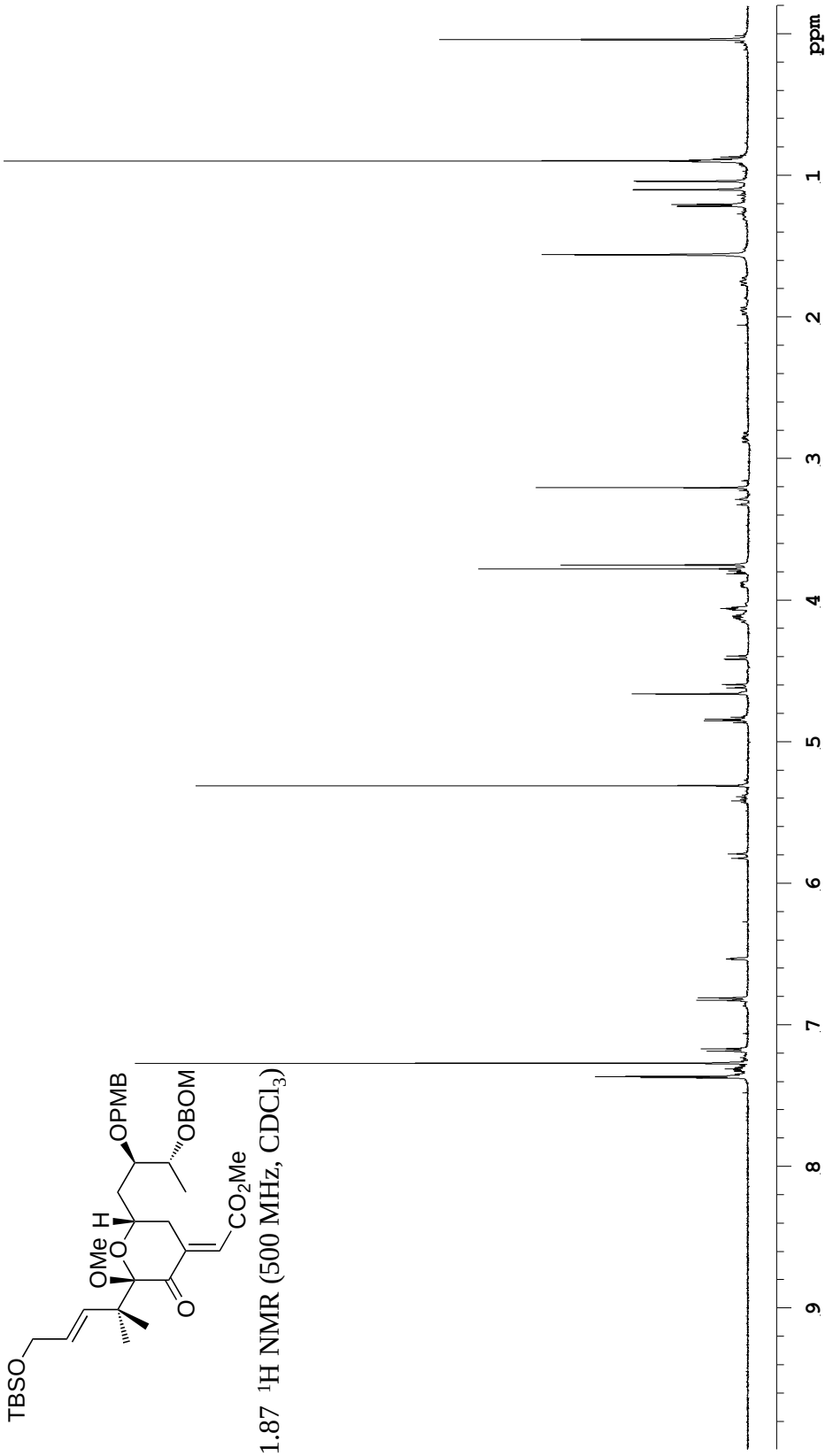


1.86 ^{1}H NMR (500 MHz, $CDCl_3$)

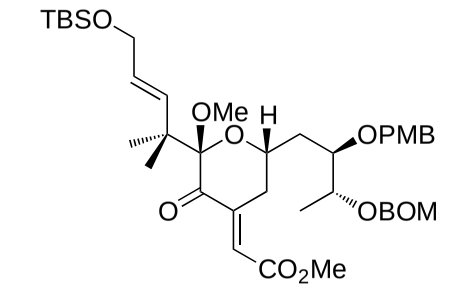


1.86 ^{13}C NMR (125 MHz, $CDCl_3$)

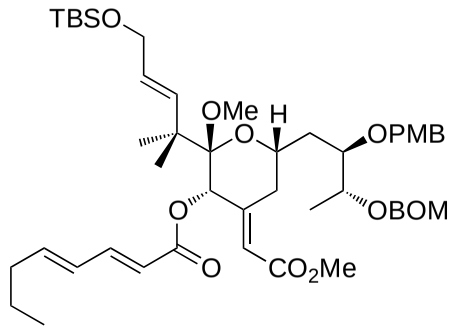




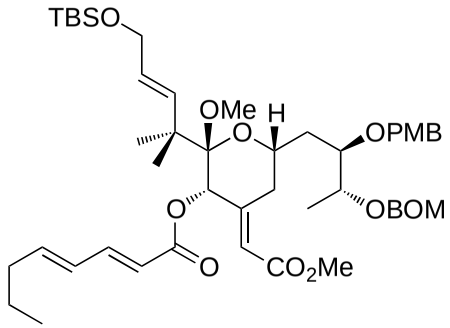
1.87 ^{1}H NMR (500 MHz, $CDCl_3$)



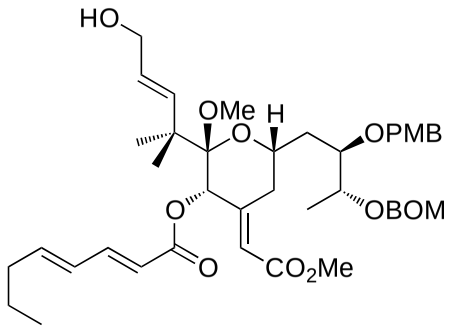
1.87 ^{13}C NMR (125 MHz, $CDCl_3$)



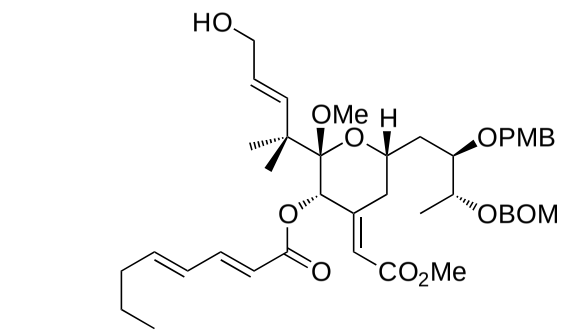
1.88 ^{1}H NMR (500 MHz, $CDCl_3$)



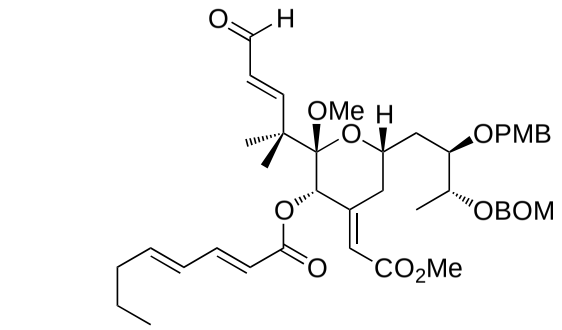
1.88 ^{13}C NMR (125 MHz, $CDCl_3$)



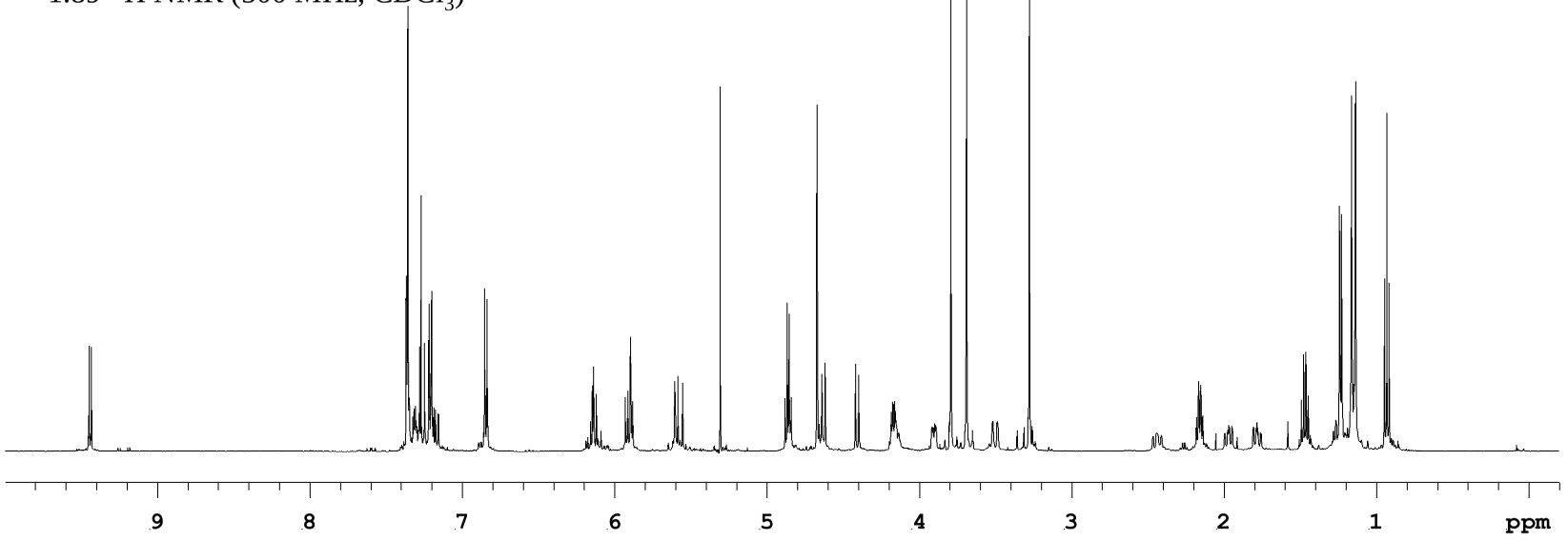
1.106 ^{1}H NMR (500 MHz, $CDCl_3$)

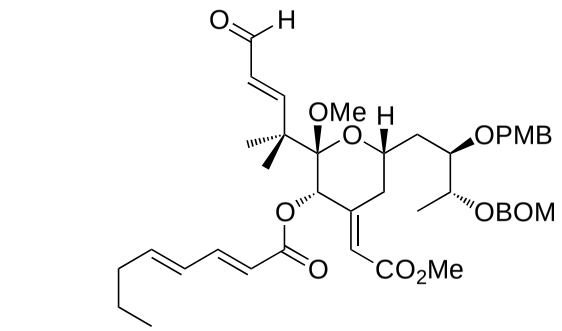


1.106 ^{13}C NMR (125 MHz, $CDCl_3$)

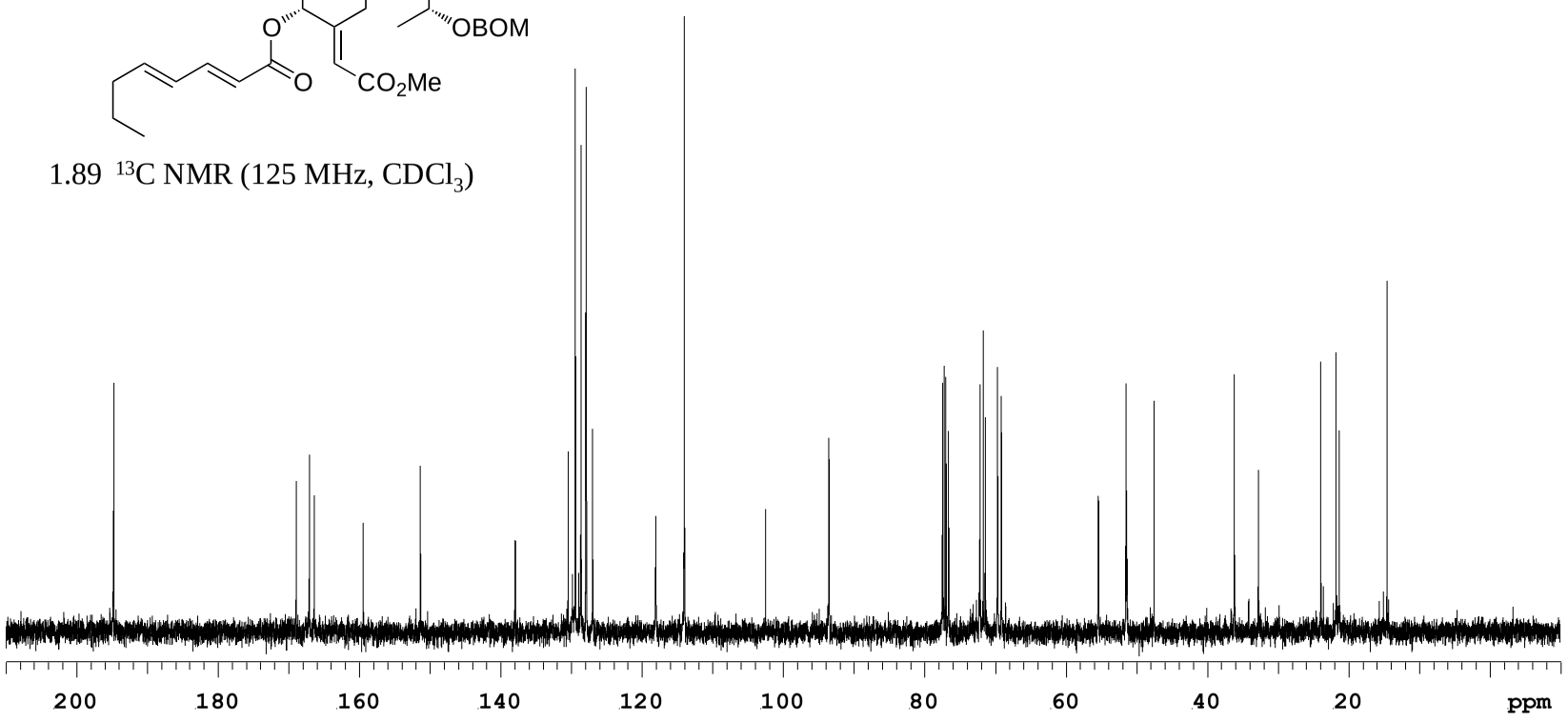


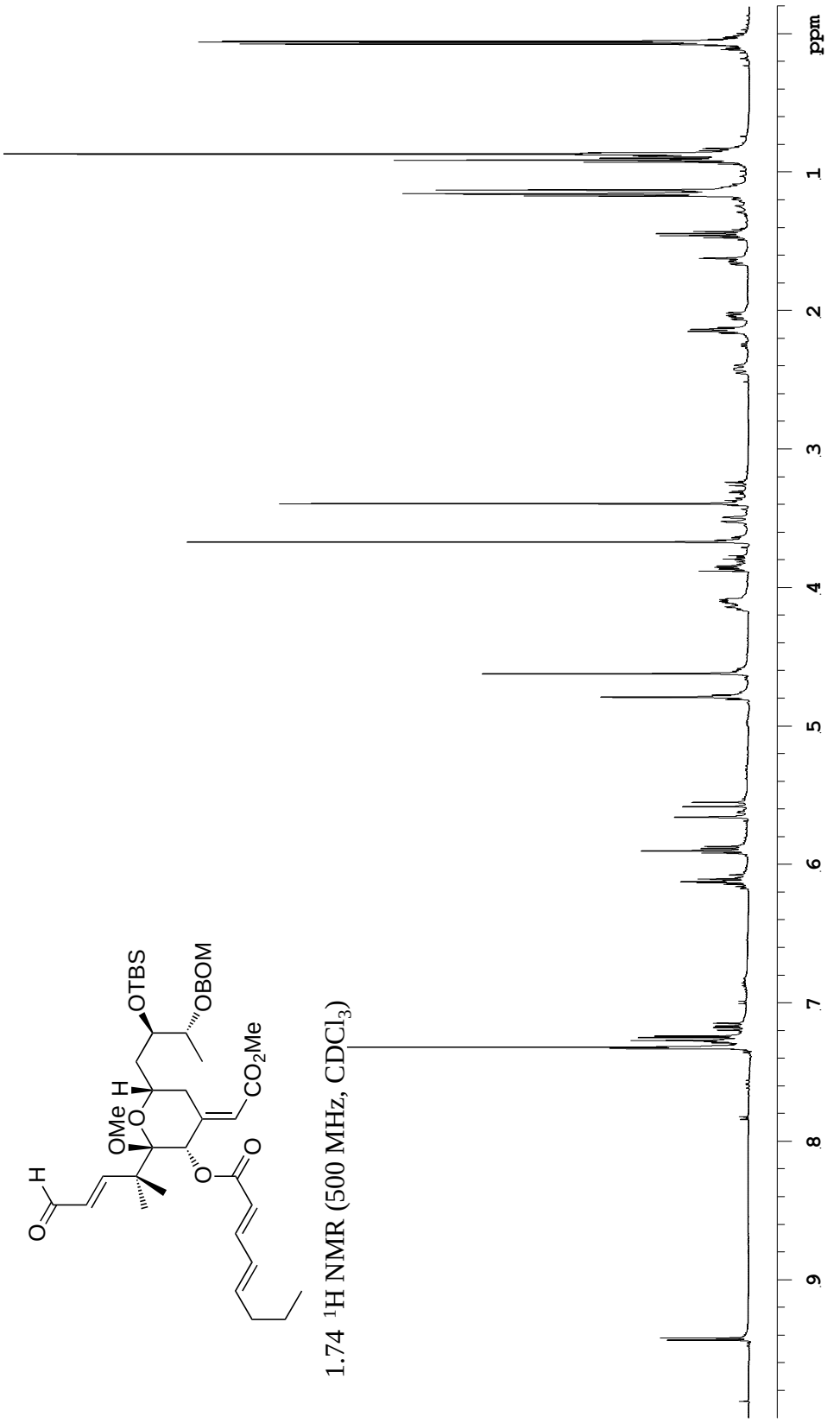
1.89 ^{1}H NMR (500 MHz, $CDCl_3$)



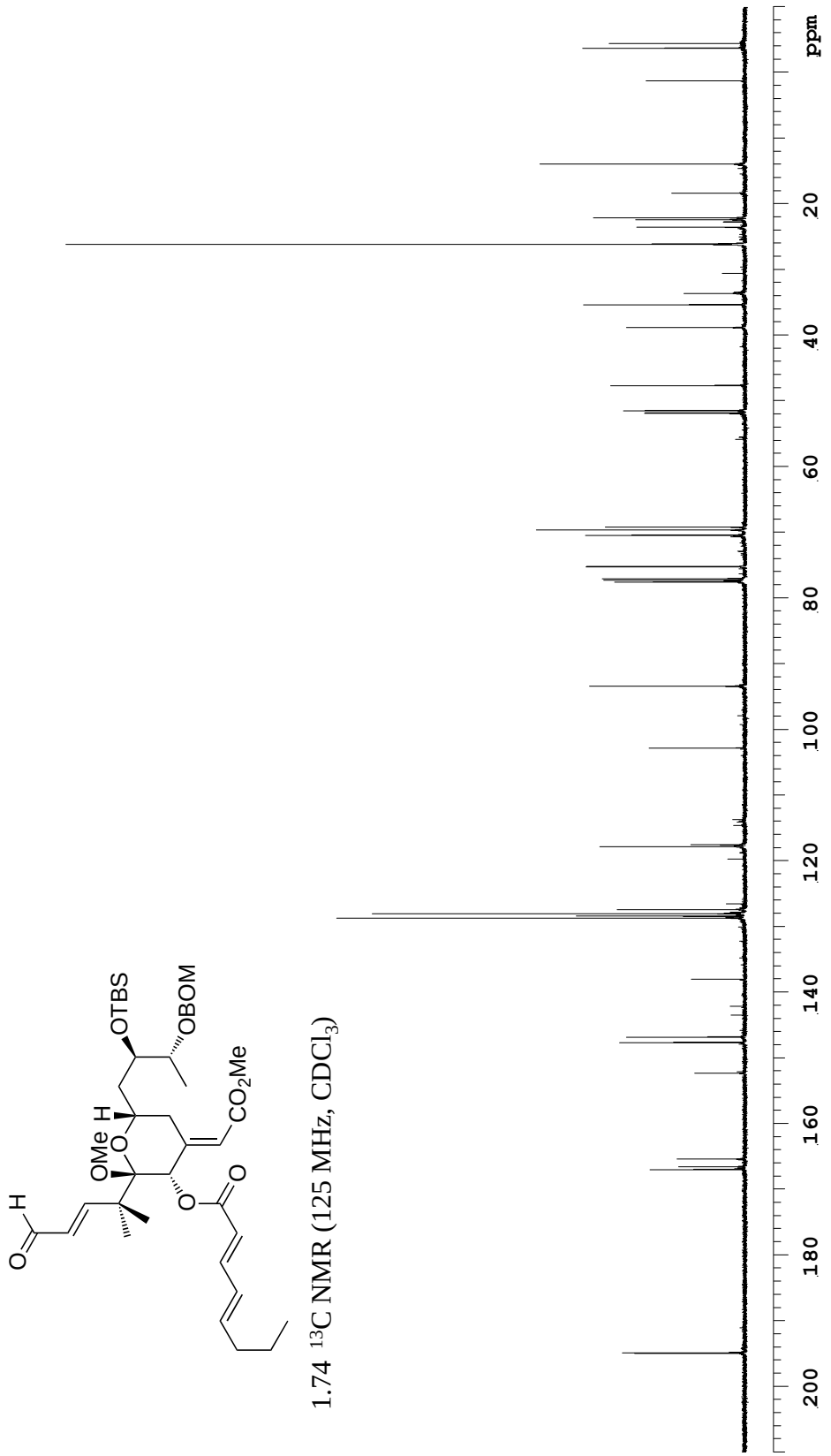


1.89 ^{13}C NMR (125 MHz, $CDCl_3$)

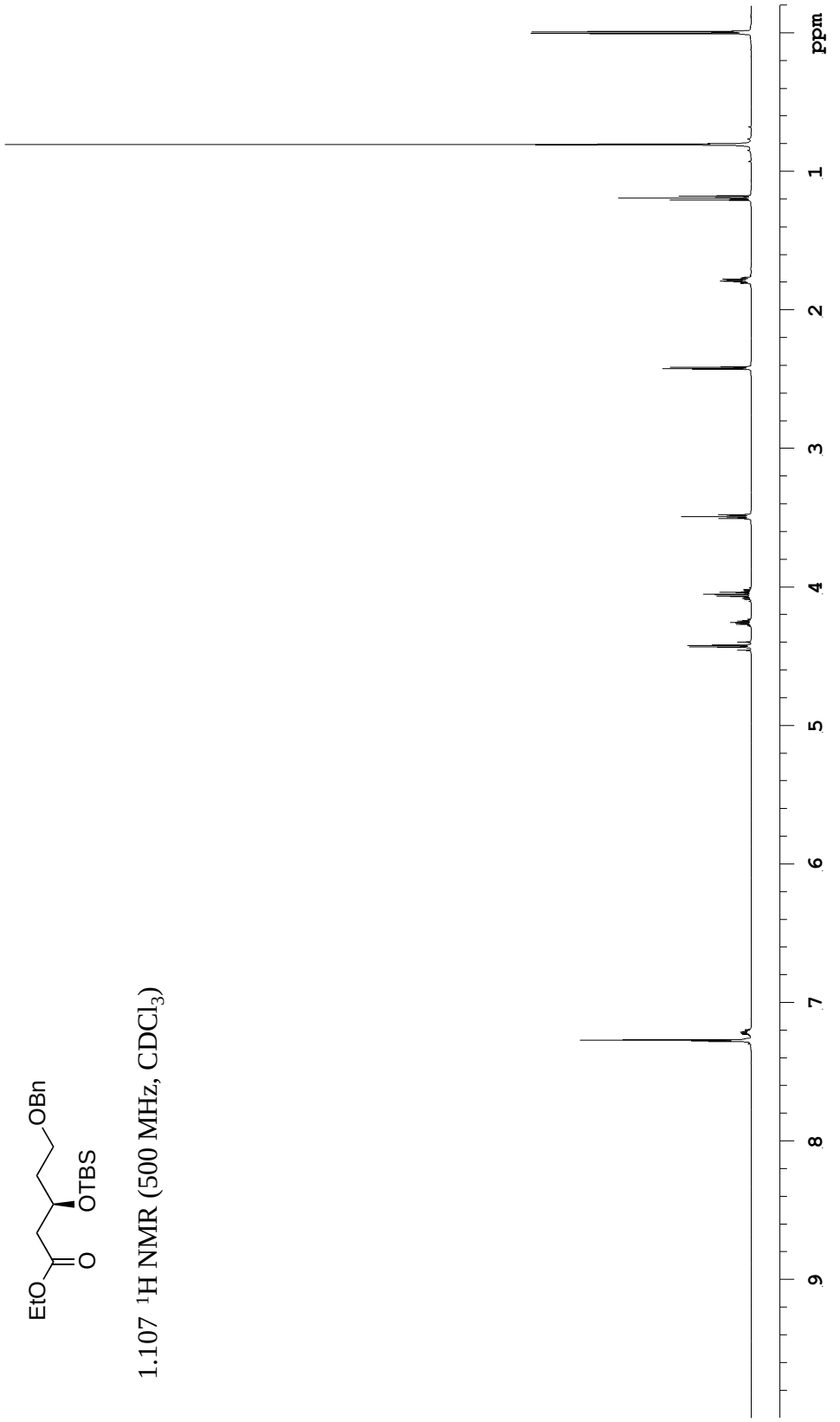




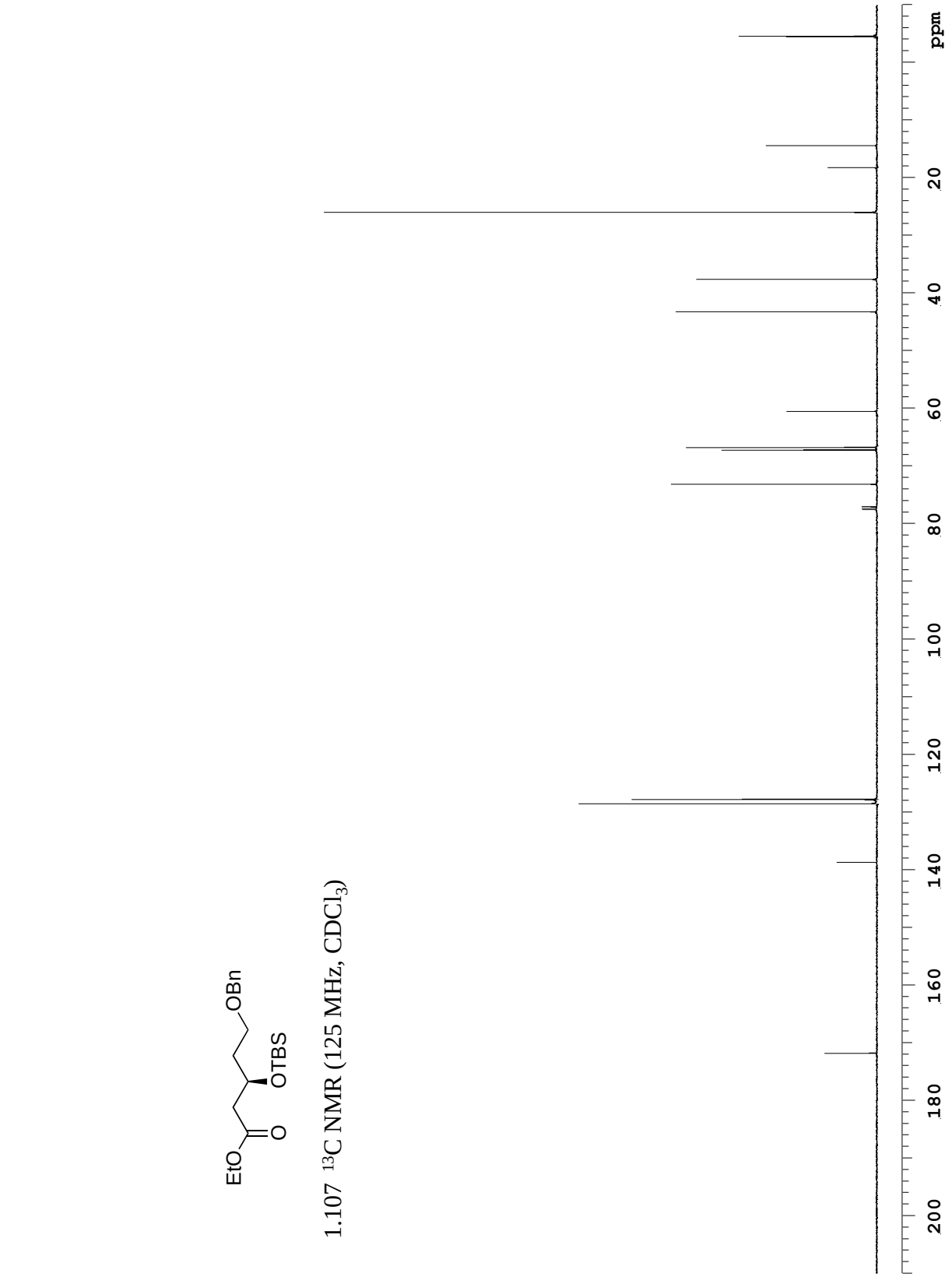
1.74 ^{1}H NMR (500 MHz, $CDCl_3$)



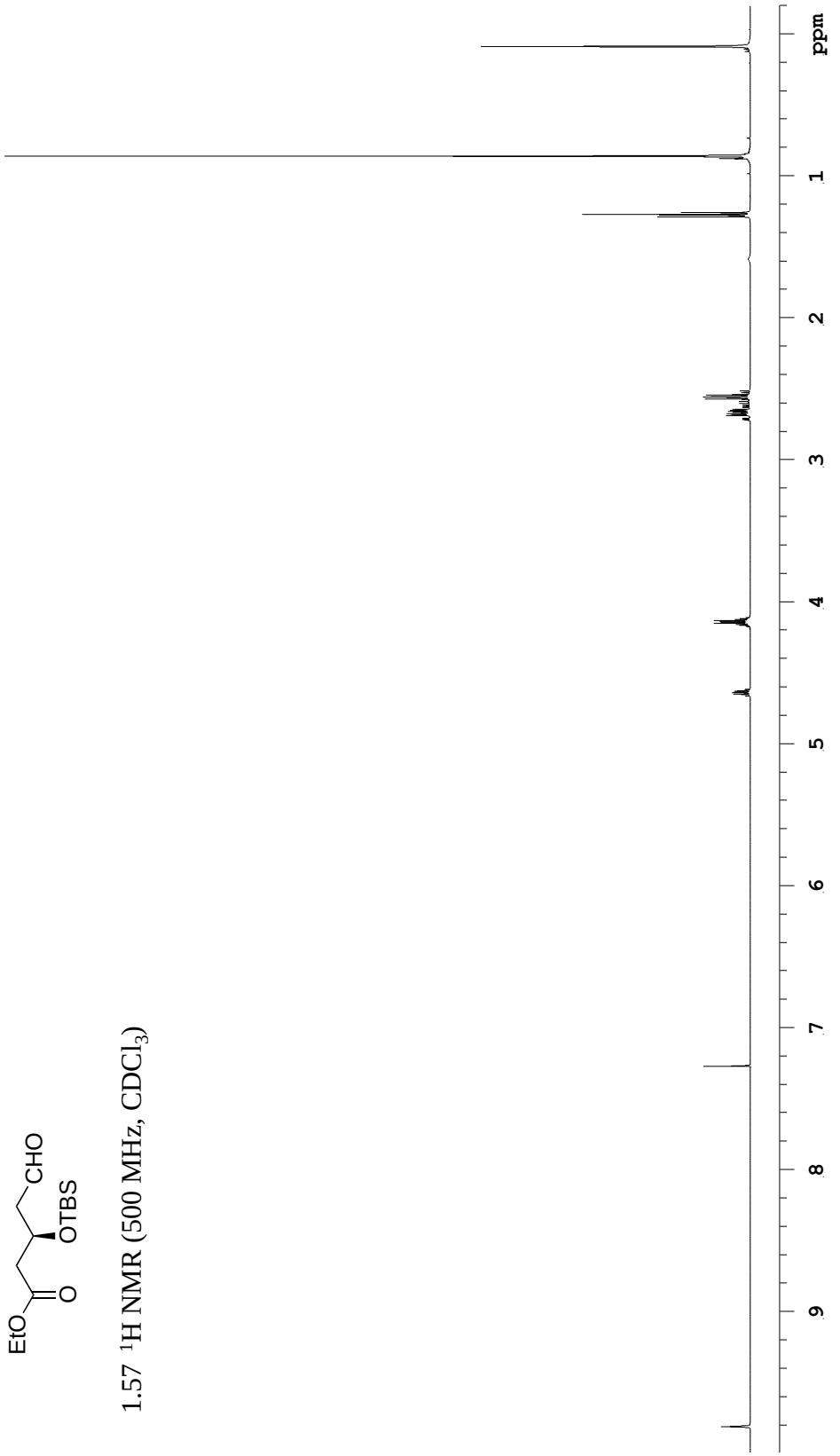
1.74 ^{13}C NMR (125 MHz, $CDCl_3$)



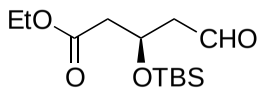
1.107 ^{1}H NMR (500 MHz, $CDCl_3$)



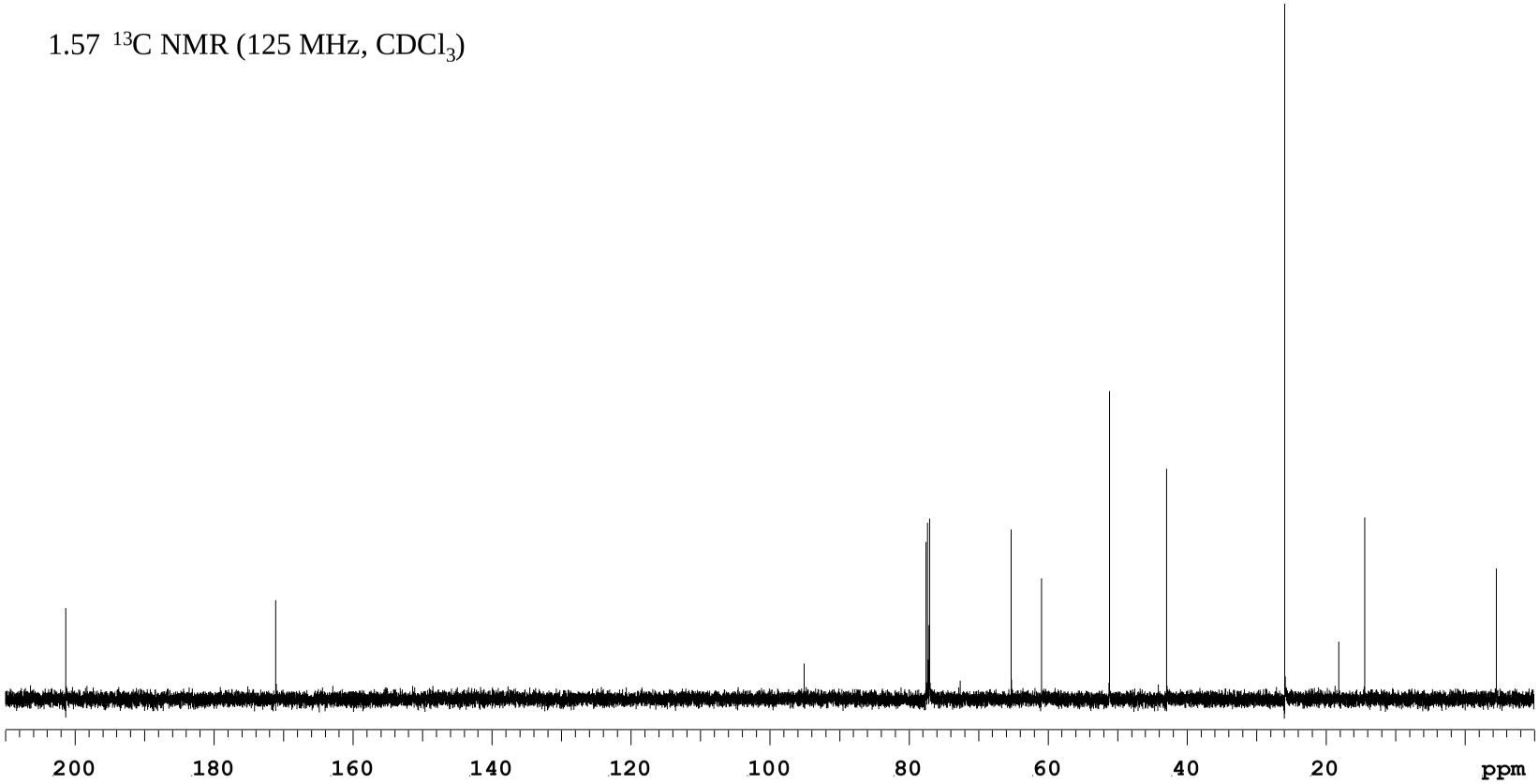
1.107 ^{13}C NMR (125 MHz, $CDCl_3$)

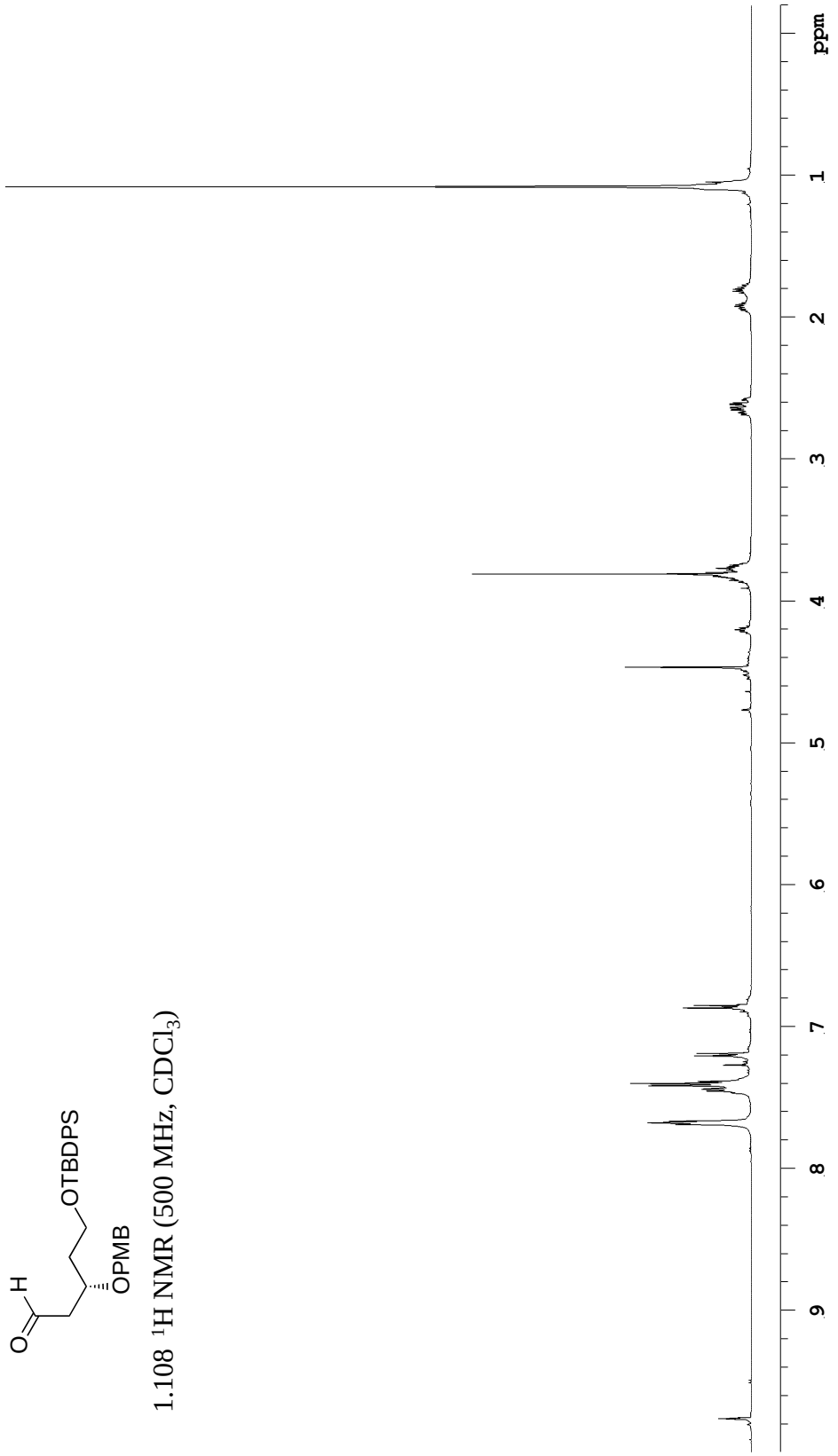


1.57 ^{1}H NMR (500 MHz, $CDCl_3$)

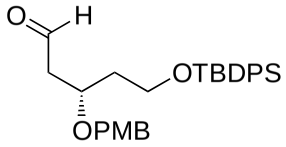


1.57 ^{13}C NMR (125 MHz, $CDCl_3$)

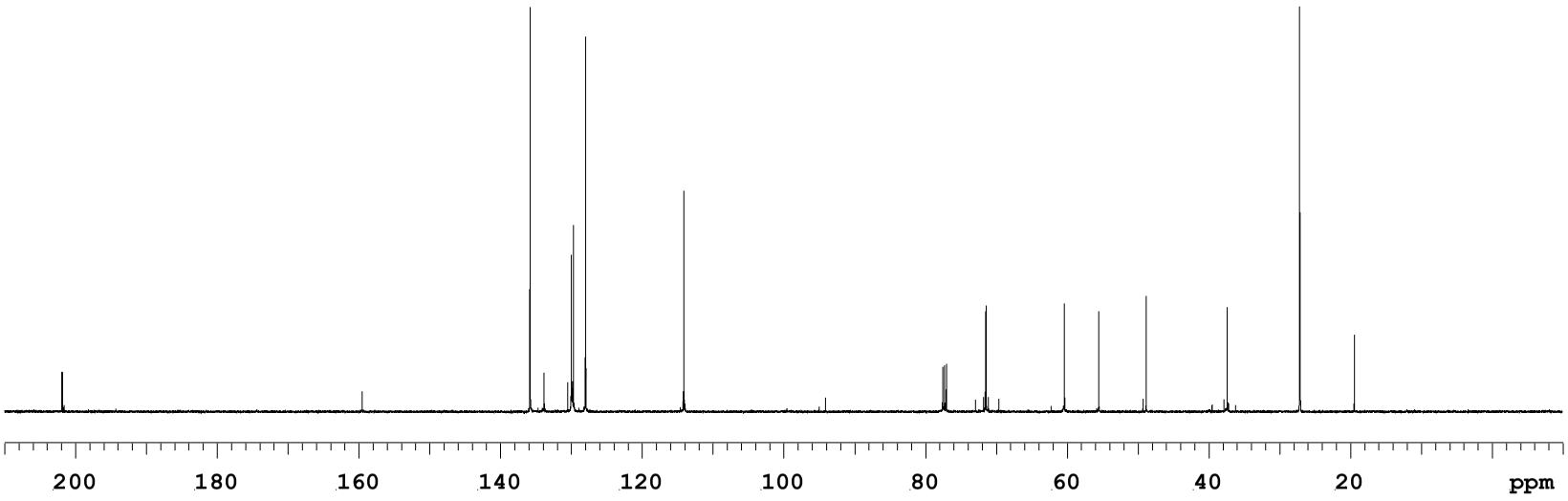


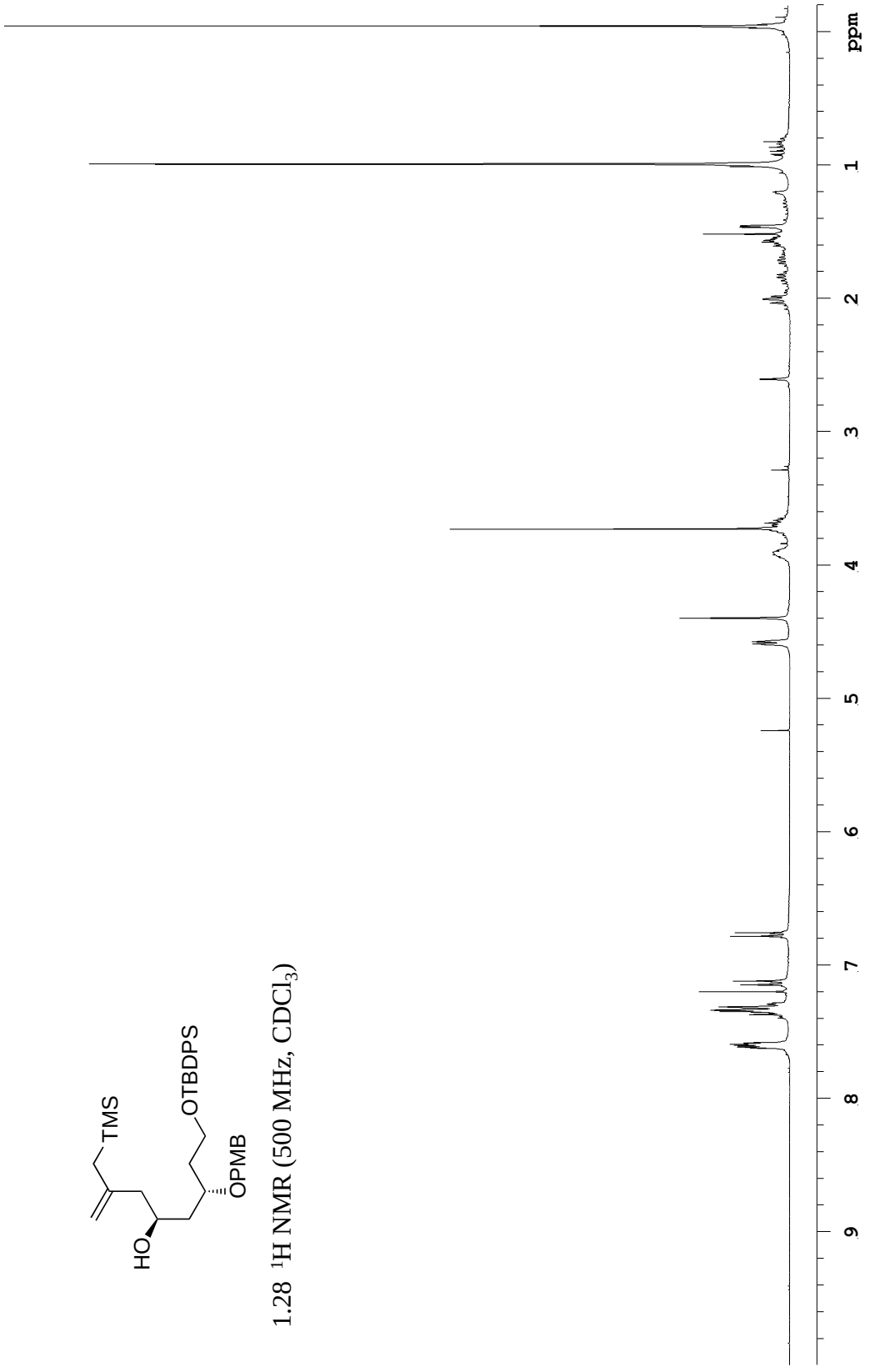


1.108 ^{1}H NMR (500 MHz, $CDCl_3$)

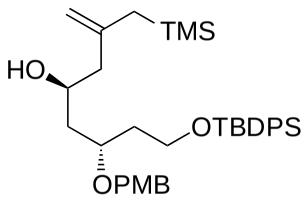


1.108 ^{13}C NMR (125 MHz, $CDCl_3$)

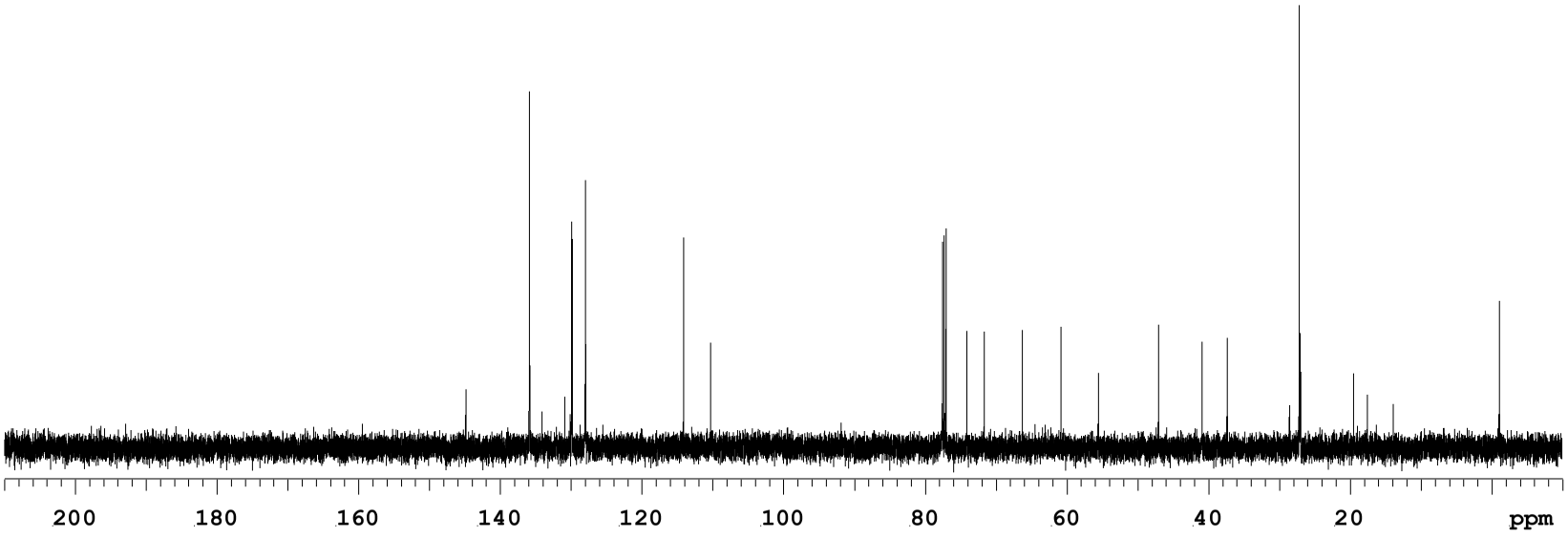


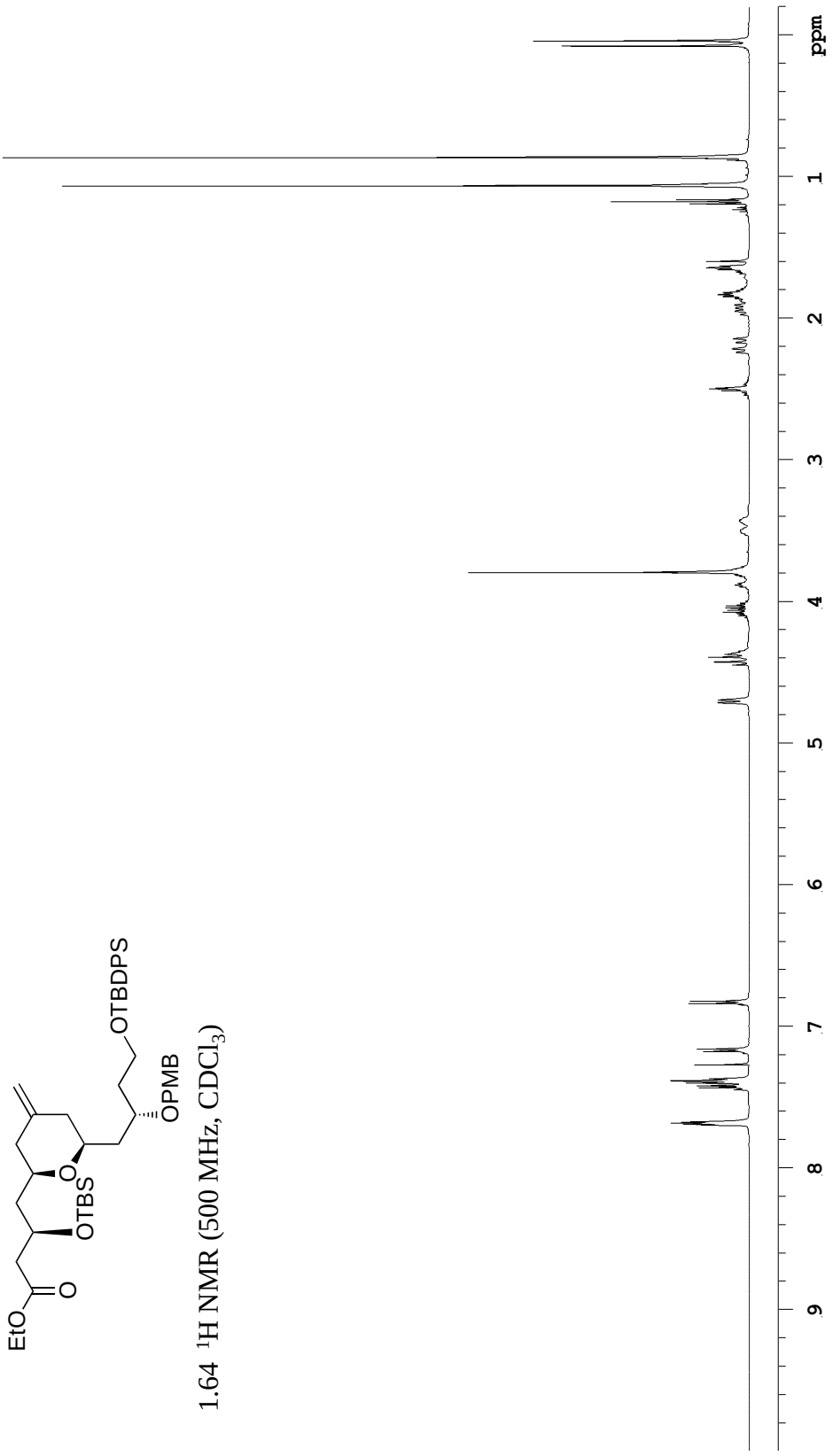


1.28 ^{1}H NMR (500 MHz, $CDCl_3$)

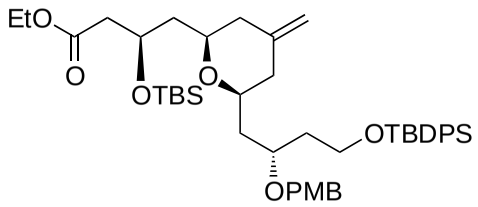


1.28 ^{13}C NMR (125 MHz, $CDCl_3$)

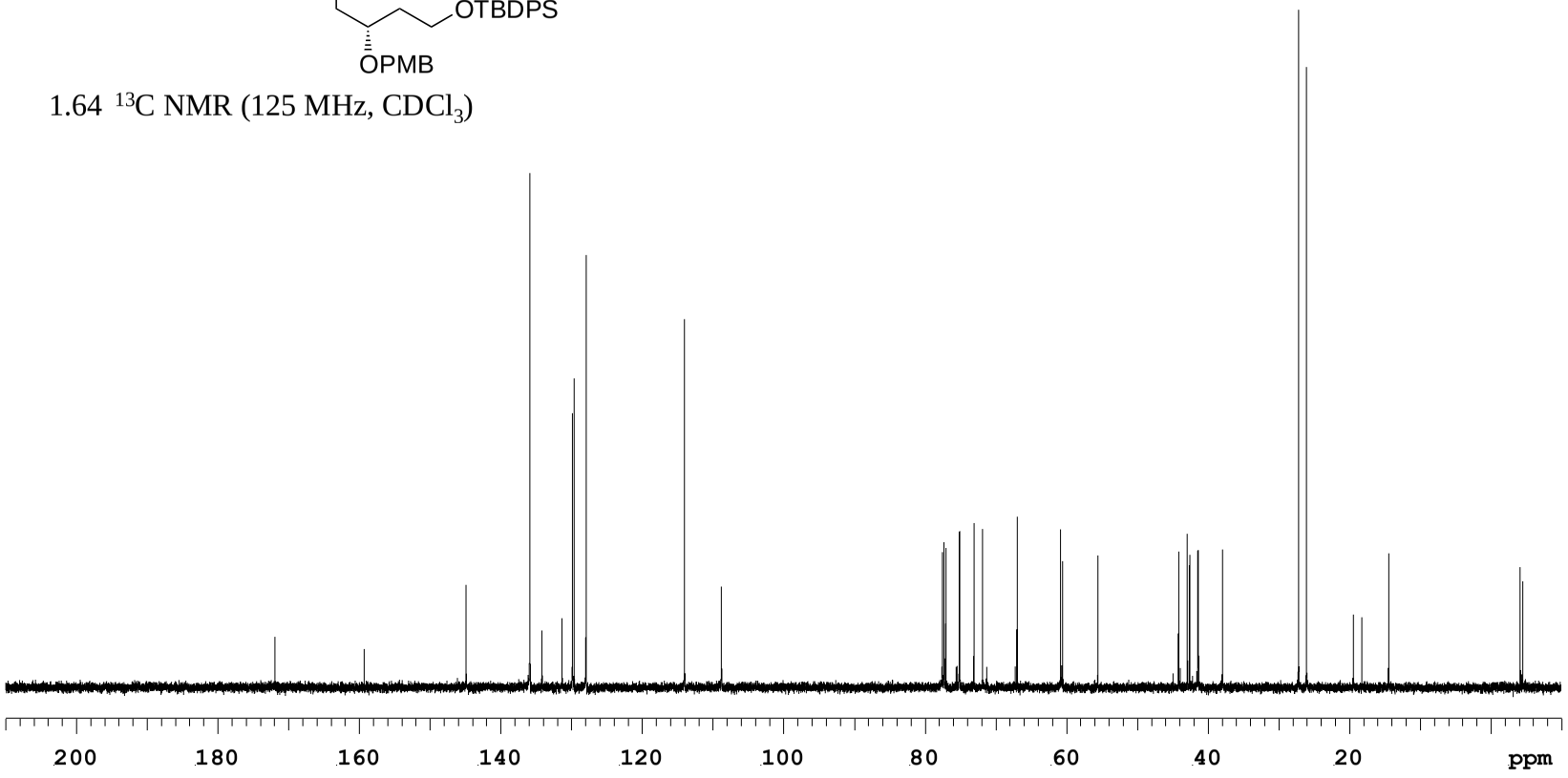


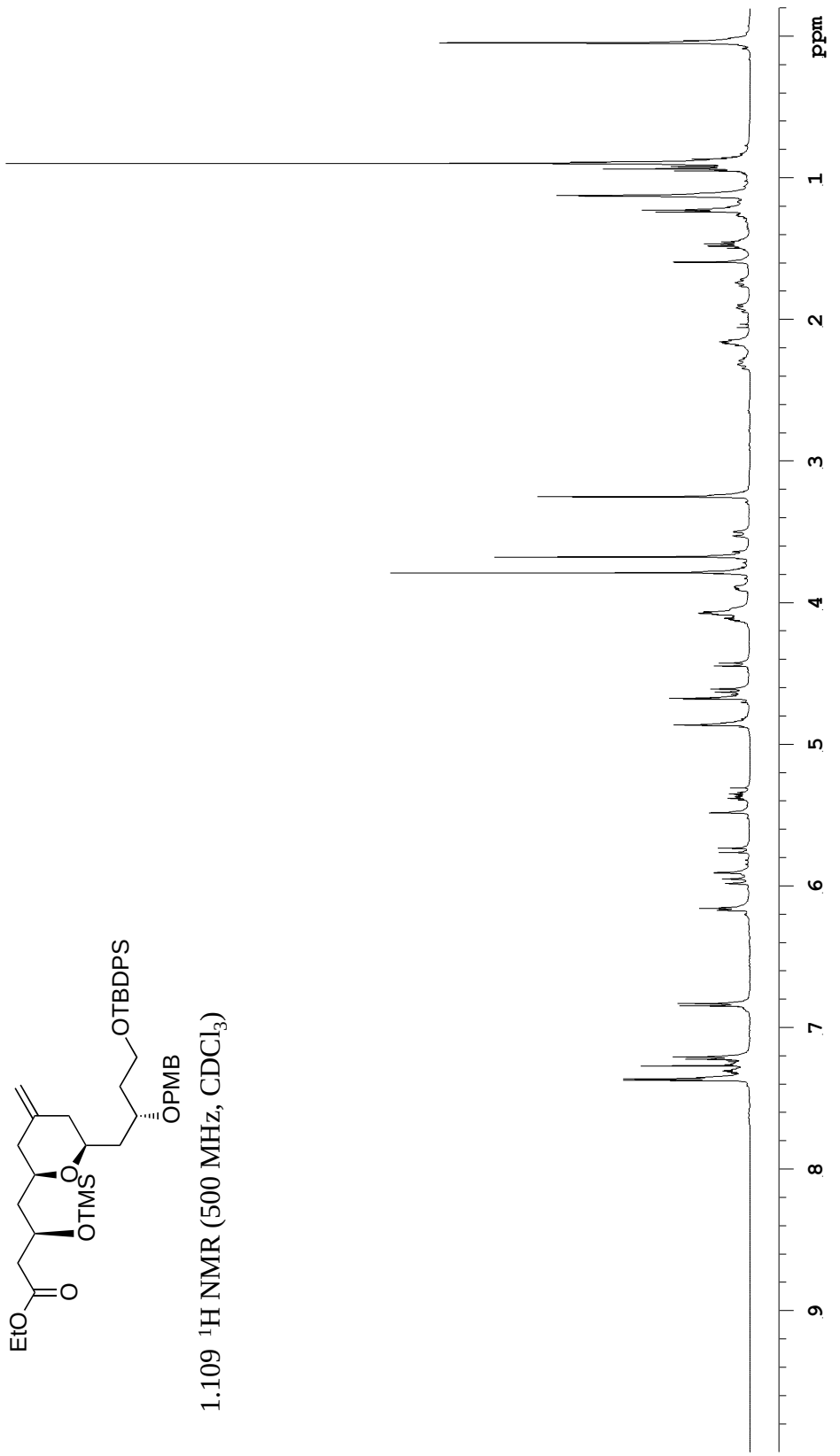


1.64 ^{1}H NMR (500 MHz, $CDCl_3$)

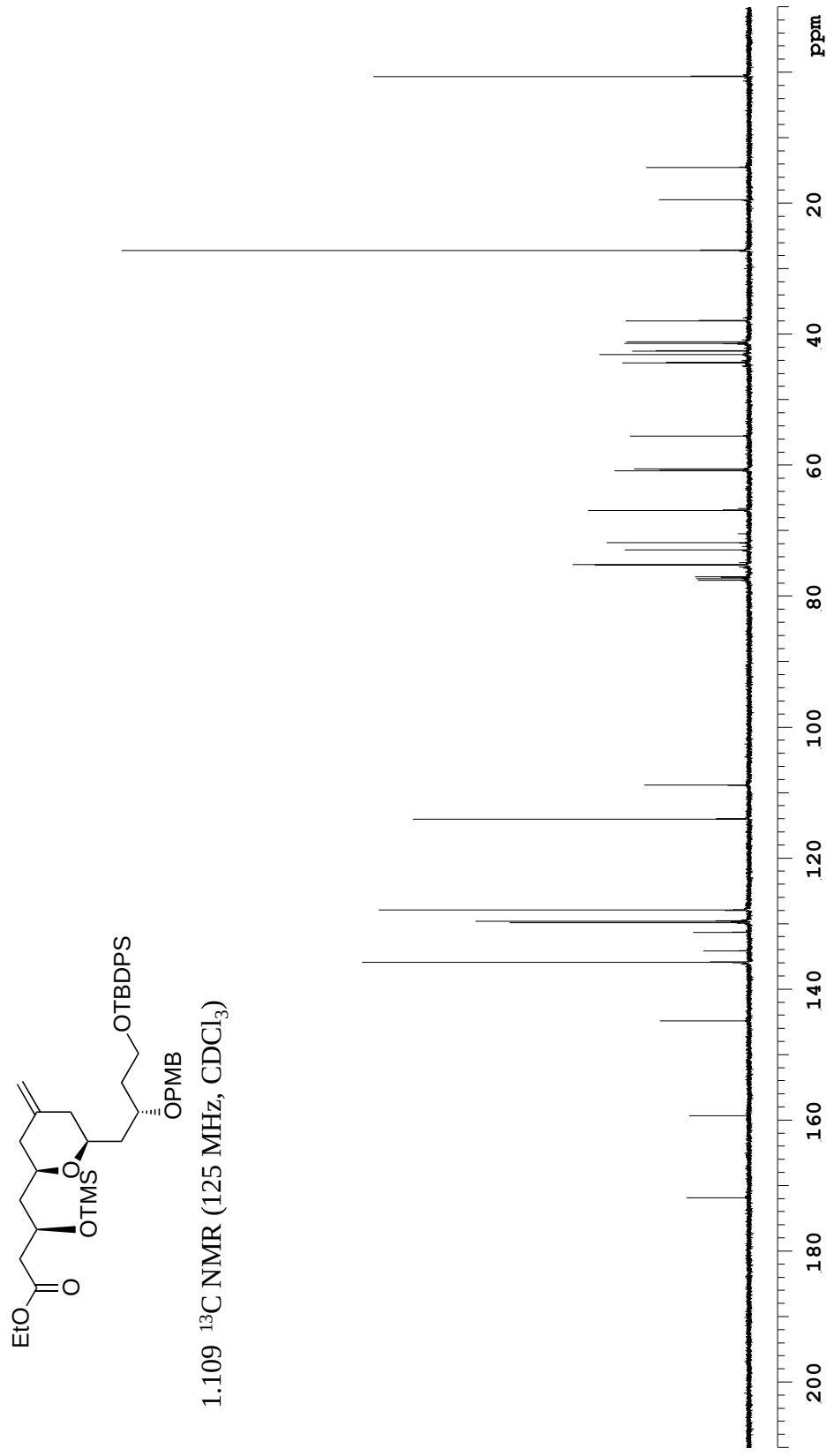


1.64 ^{13}C NMR (125 MHz, $CDCl_3$)

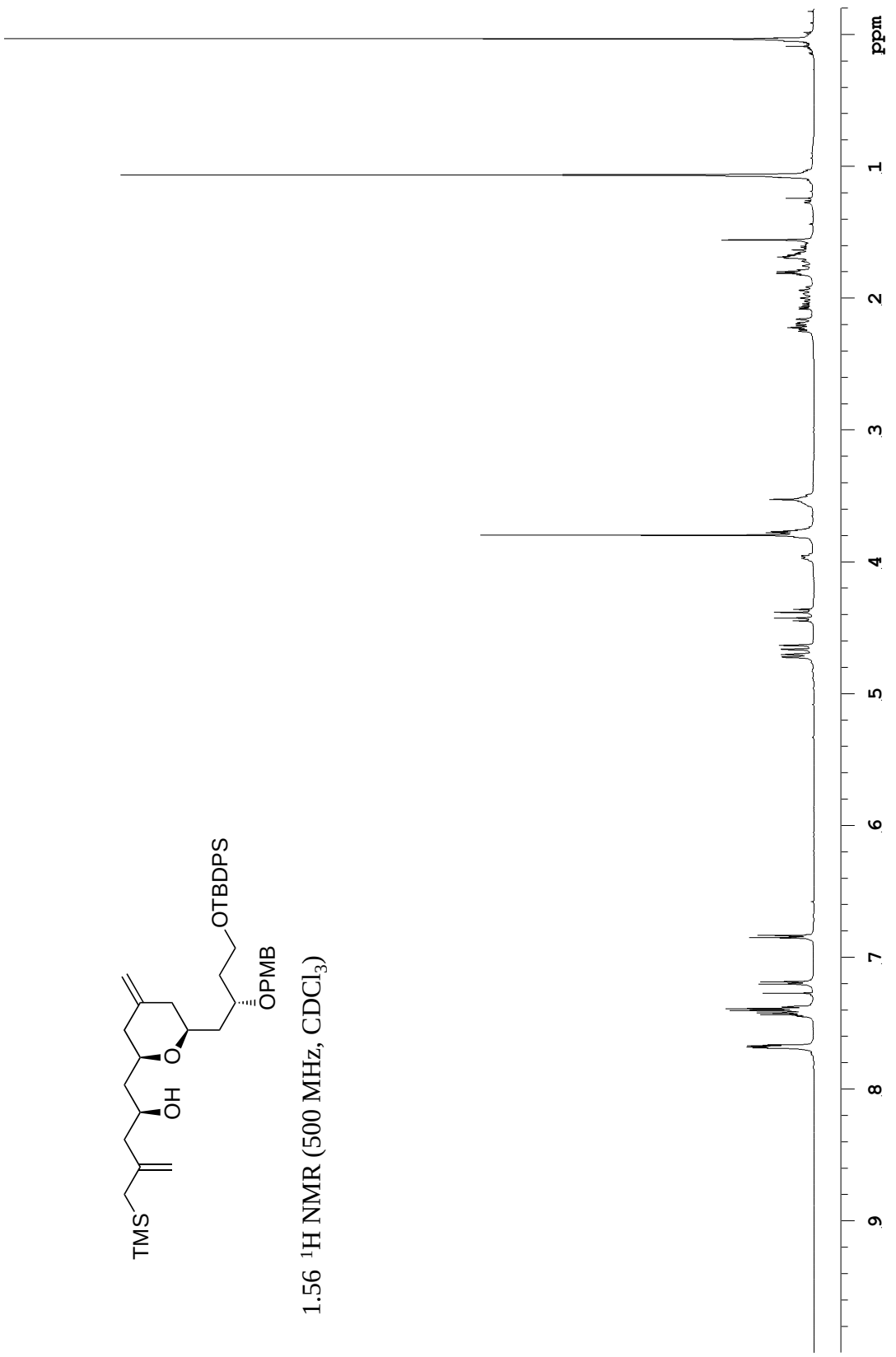




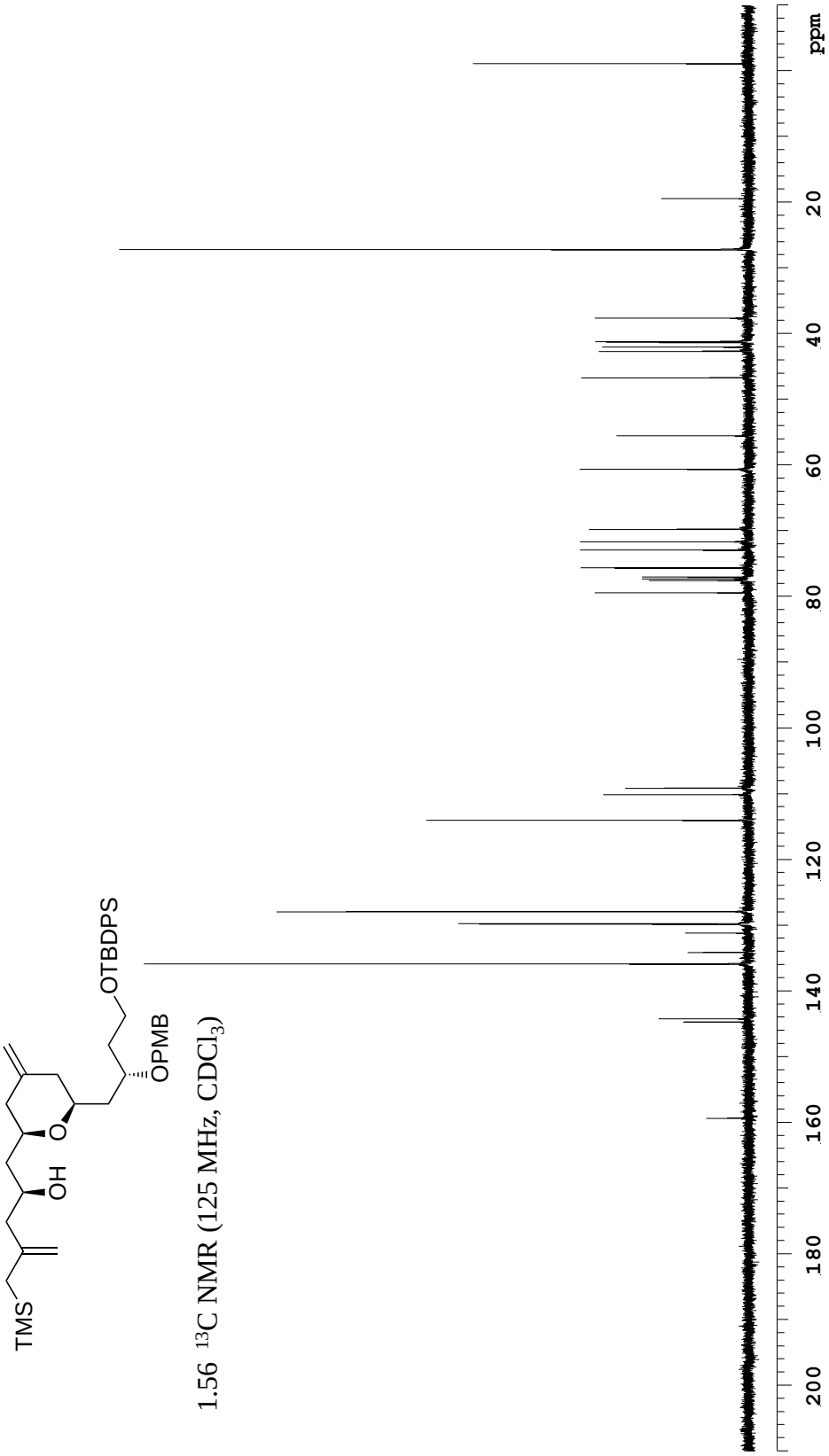
1.109 ^{1}H NMR (500 MHz, $CDCl_3$)



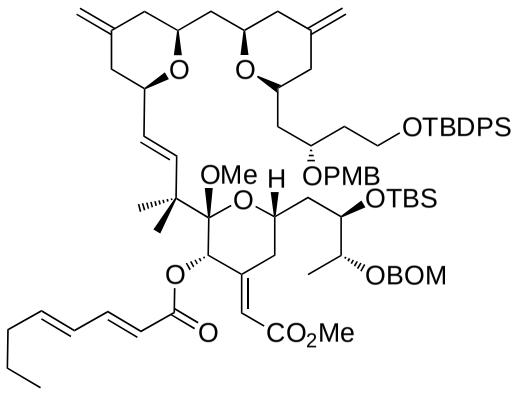
1.109 ^{13}C NMR (125 MHz, $CDCl_3$)



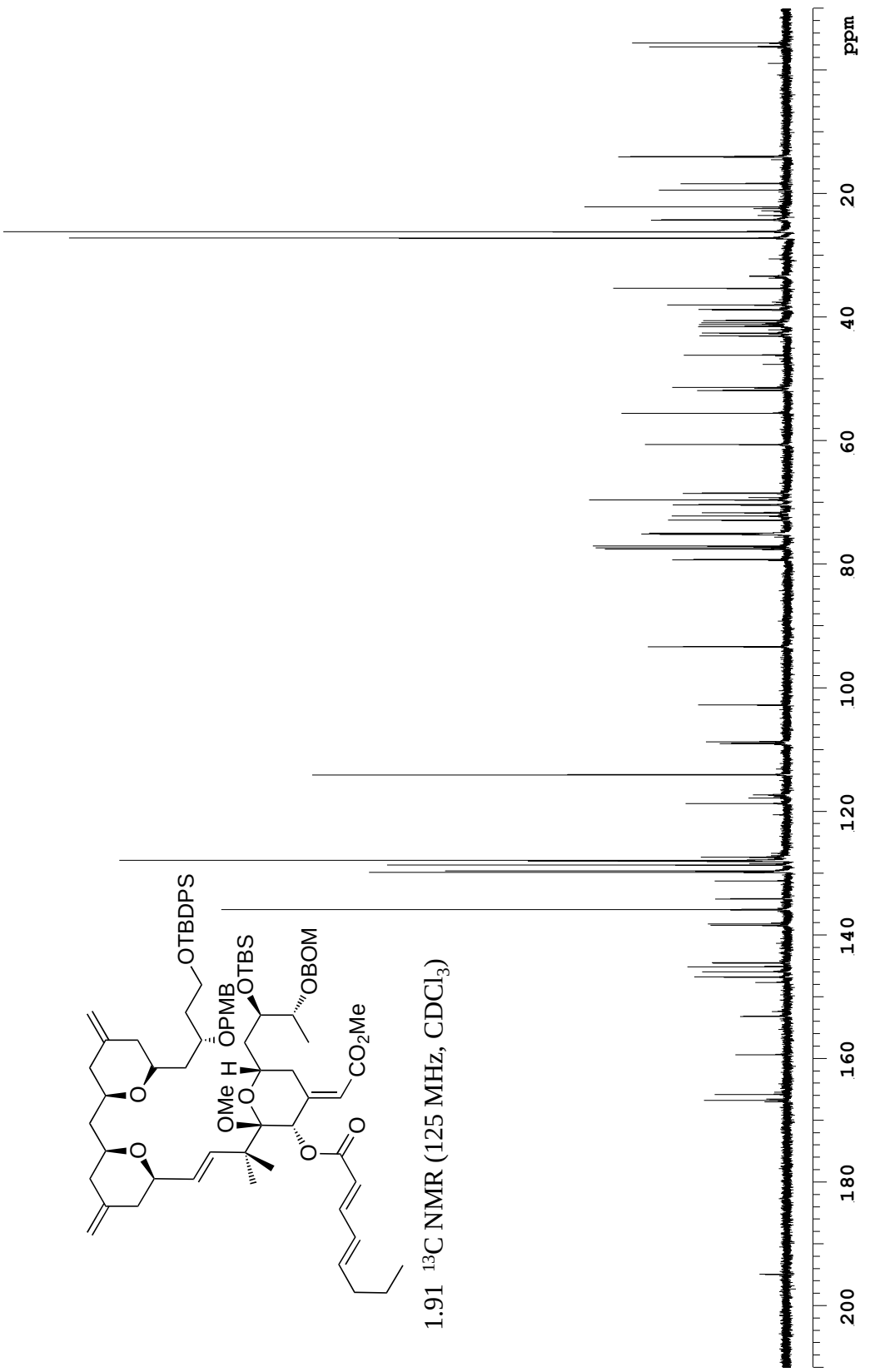
1.56 ^{1}H NMR (500 MHz, $CDCl_3$)



1.56 ^{13}C NMR (125 MHz, $CDCl_3$)

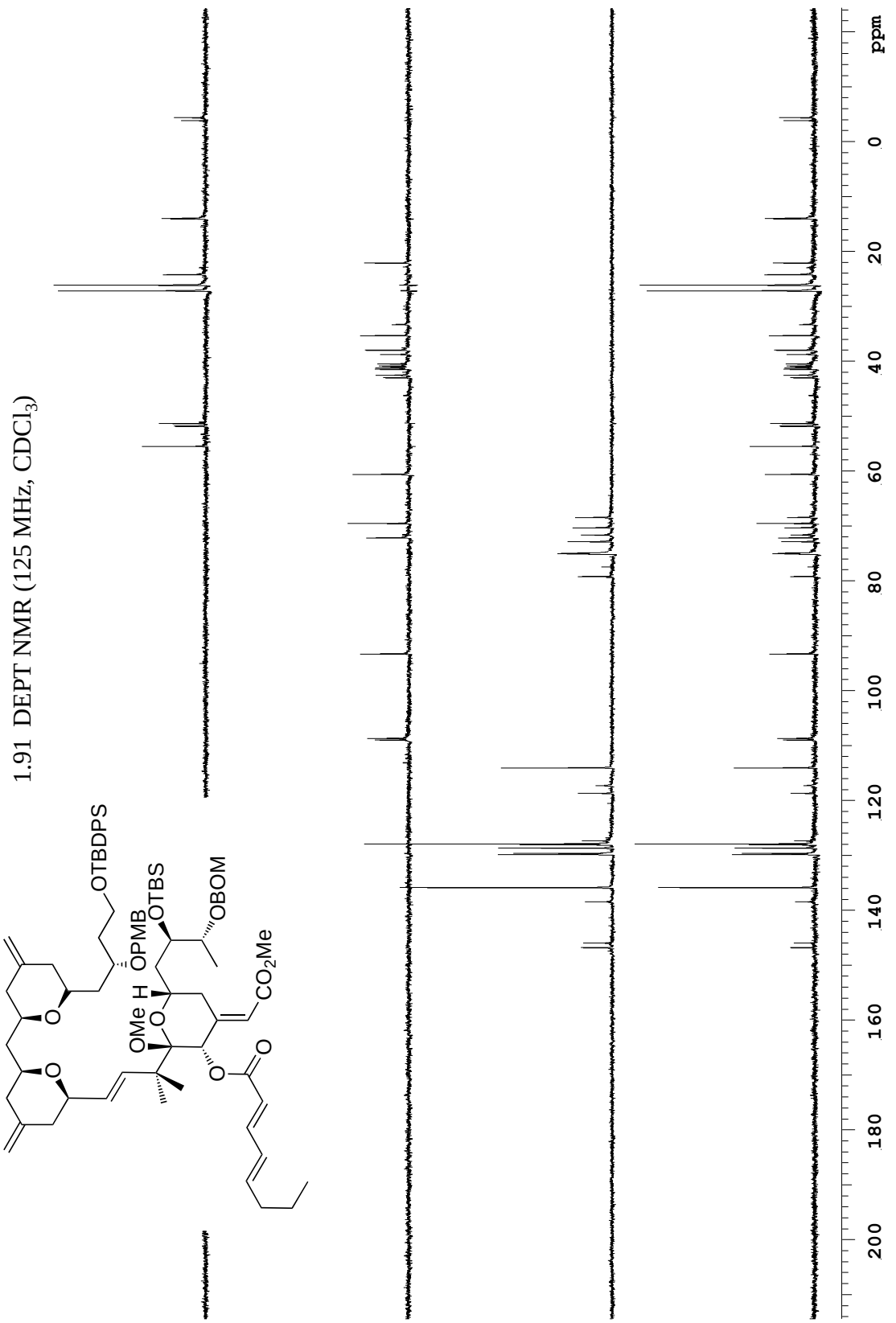


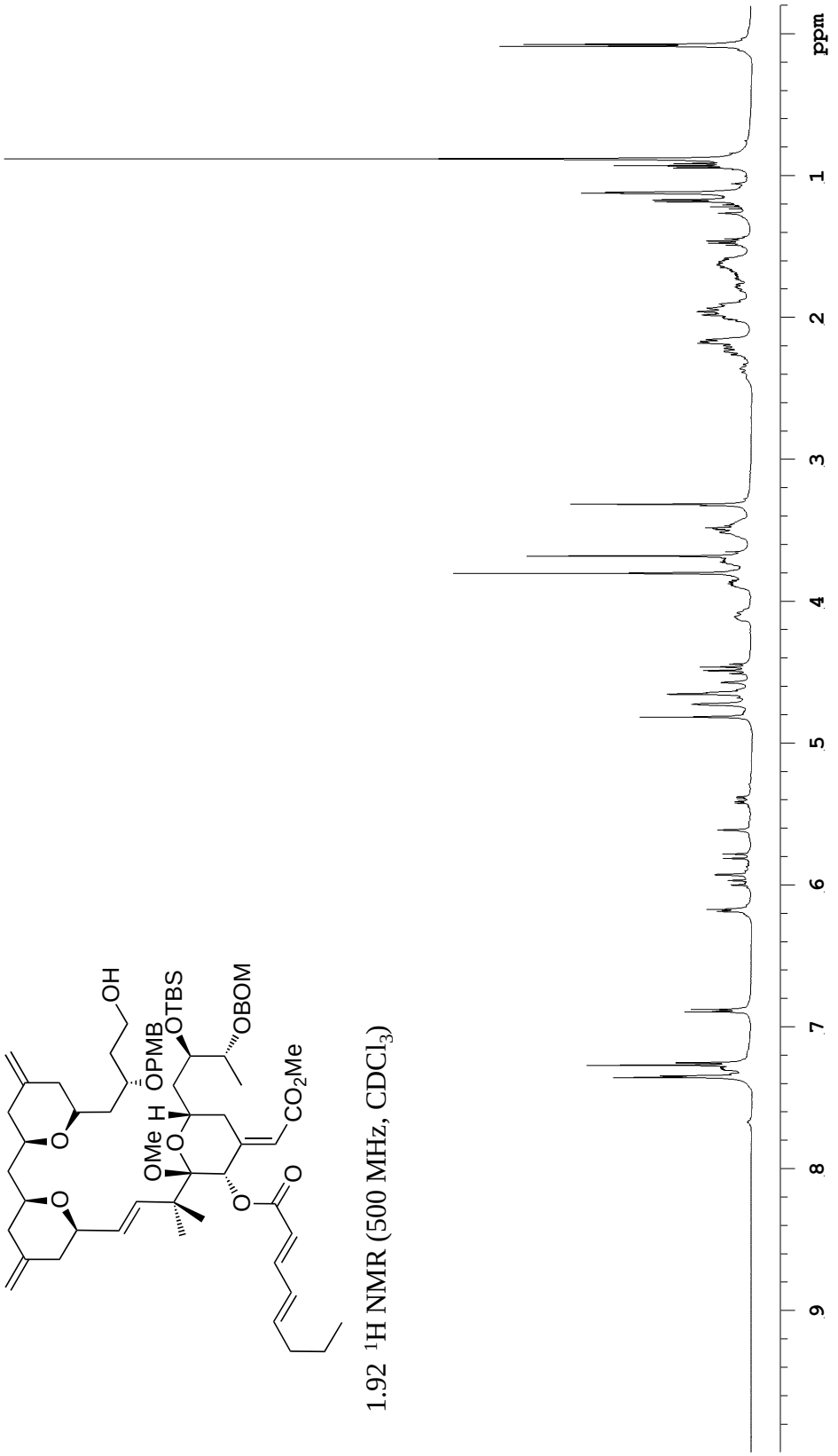
1.91 ^{1}H NMR (500 MHz, $CDCl_3$)



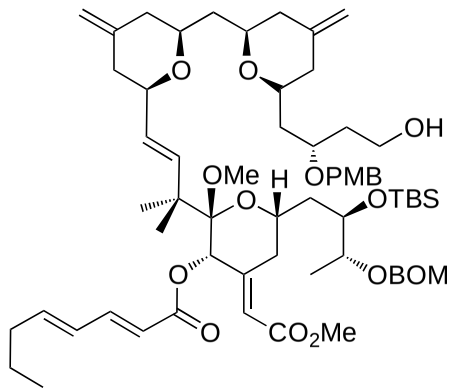
1.91 ^{13}C NMR (125 MHz, $CDCl_3$)

1.91 DEPT NMR (125 MHz, $CDCl_3$)

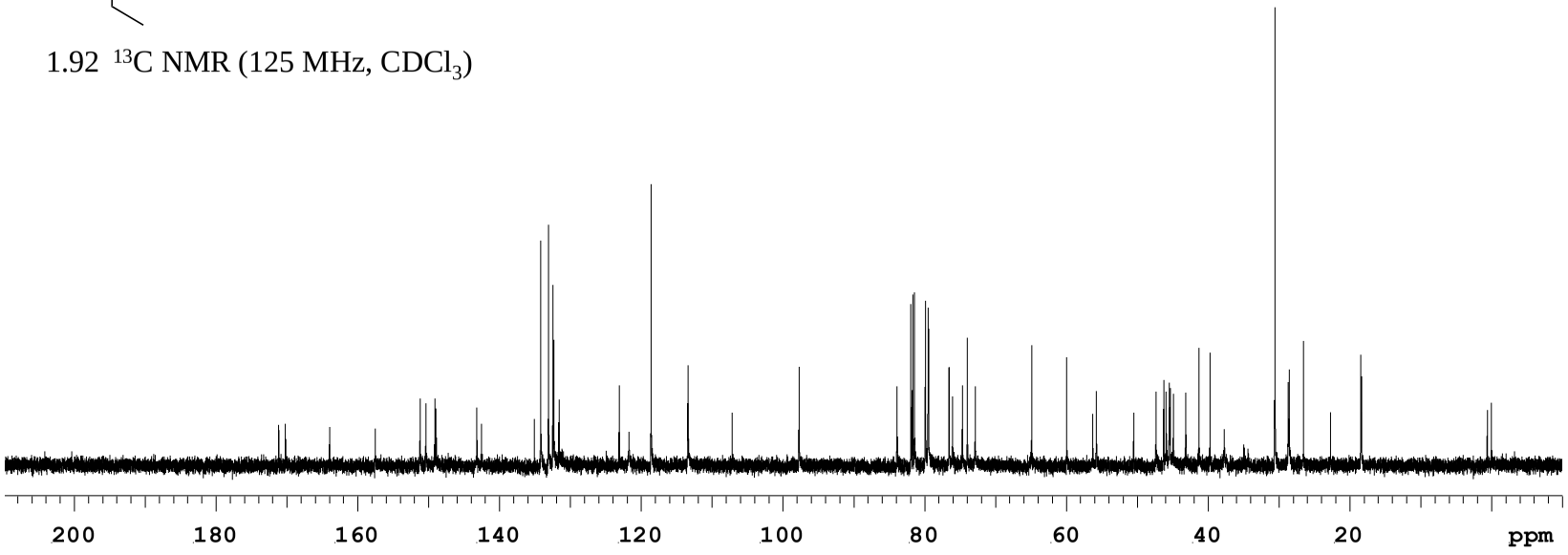


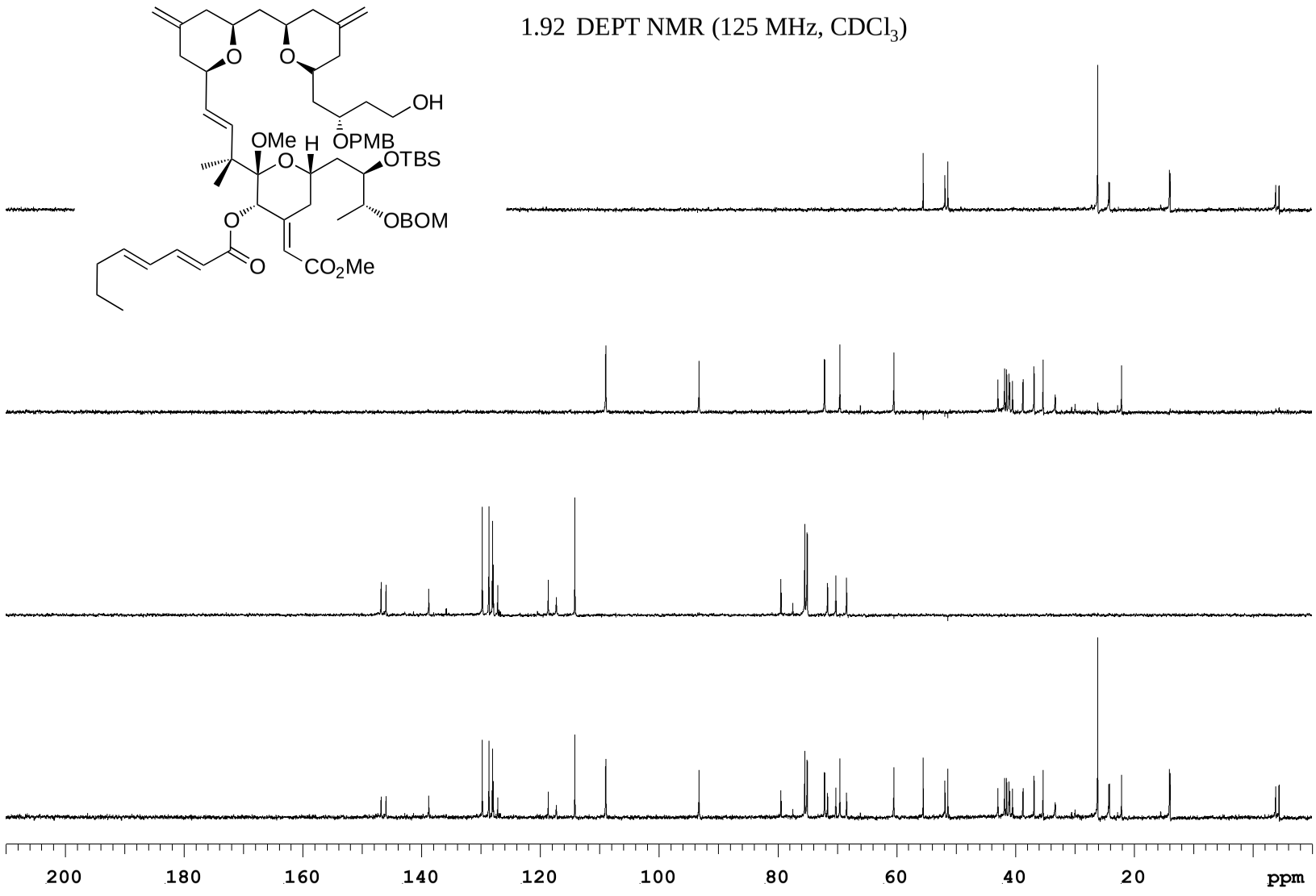


1.92 ^{1}H NMR (500 MHz, $CDCl_3$)

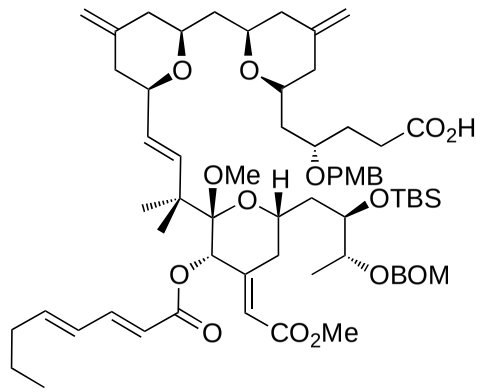


1.92 ^{13}C NMR (125 MHz, $CDCl_3$)

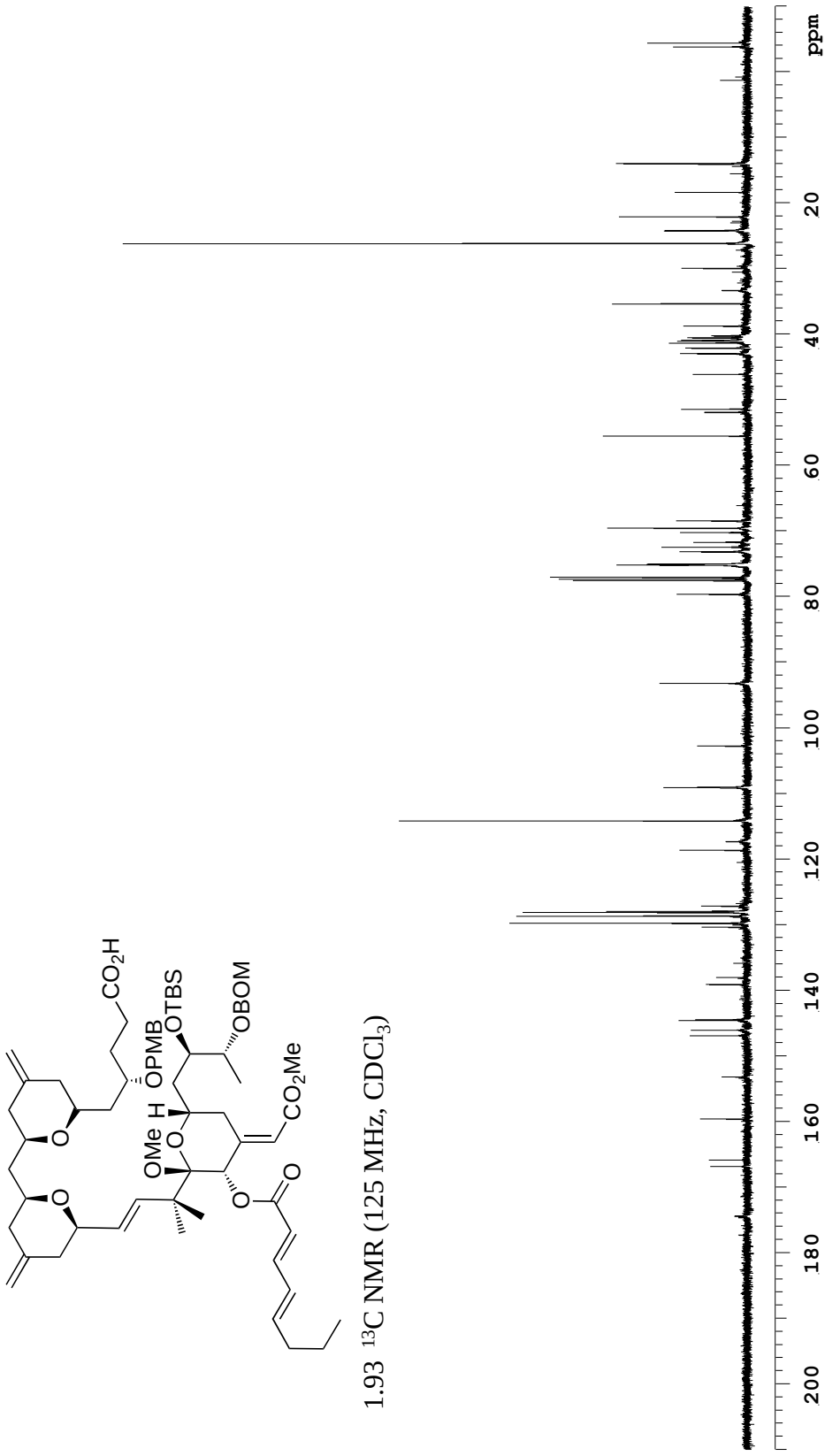




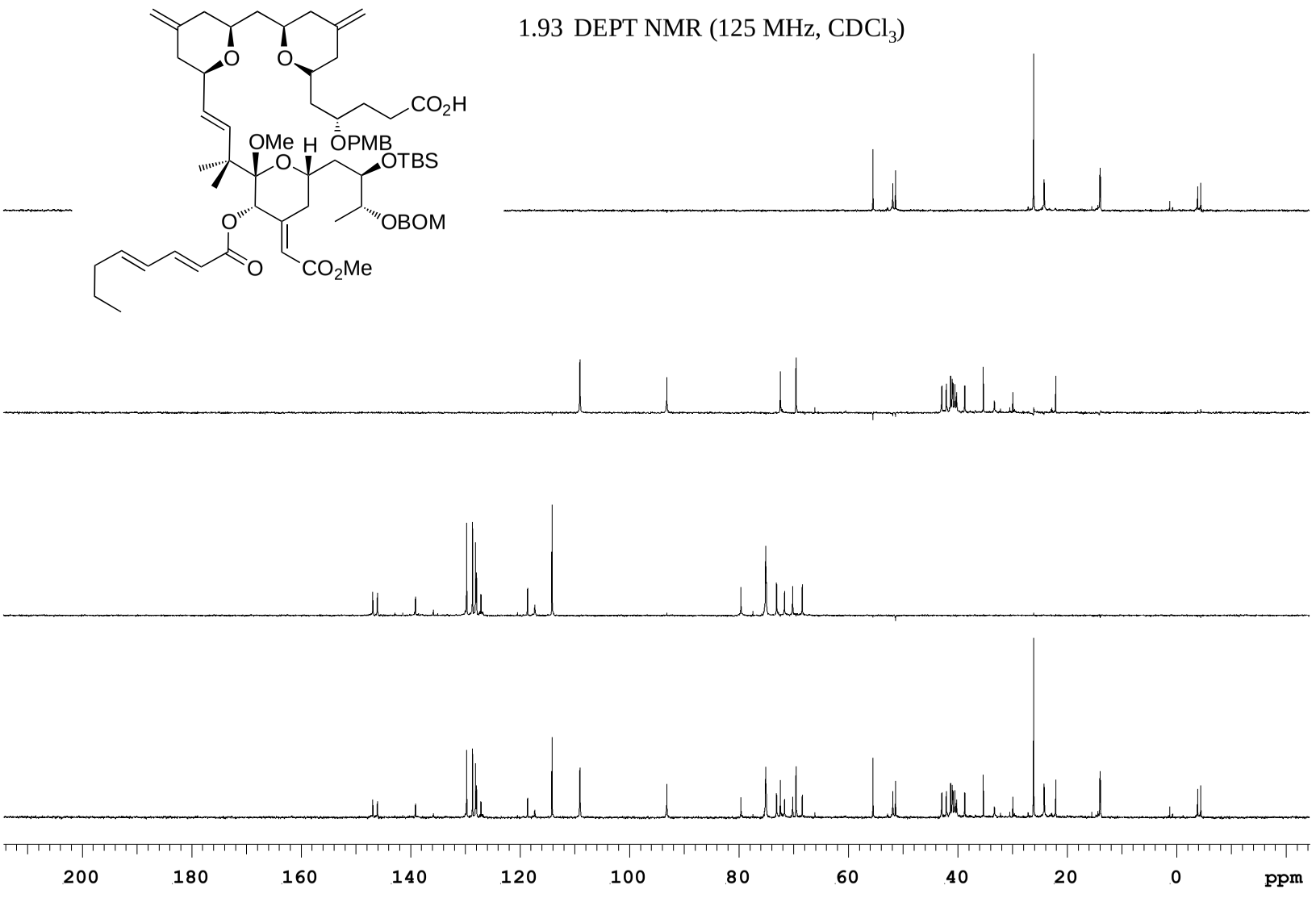
1.92 DEPT NMR (125 MHz, $CDCl_3$)



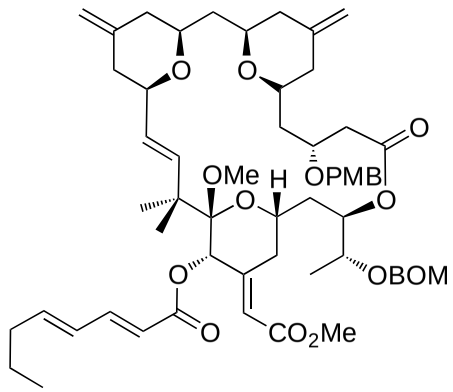
1.93 ^{1}H NMR (500 MHz, $CDCl_3$)



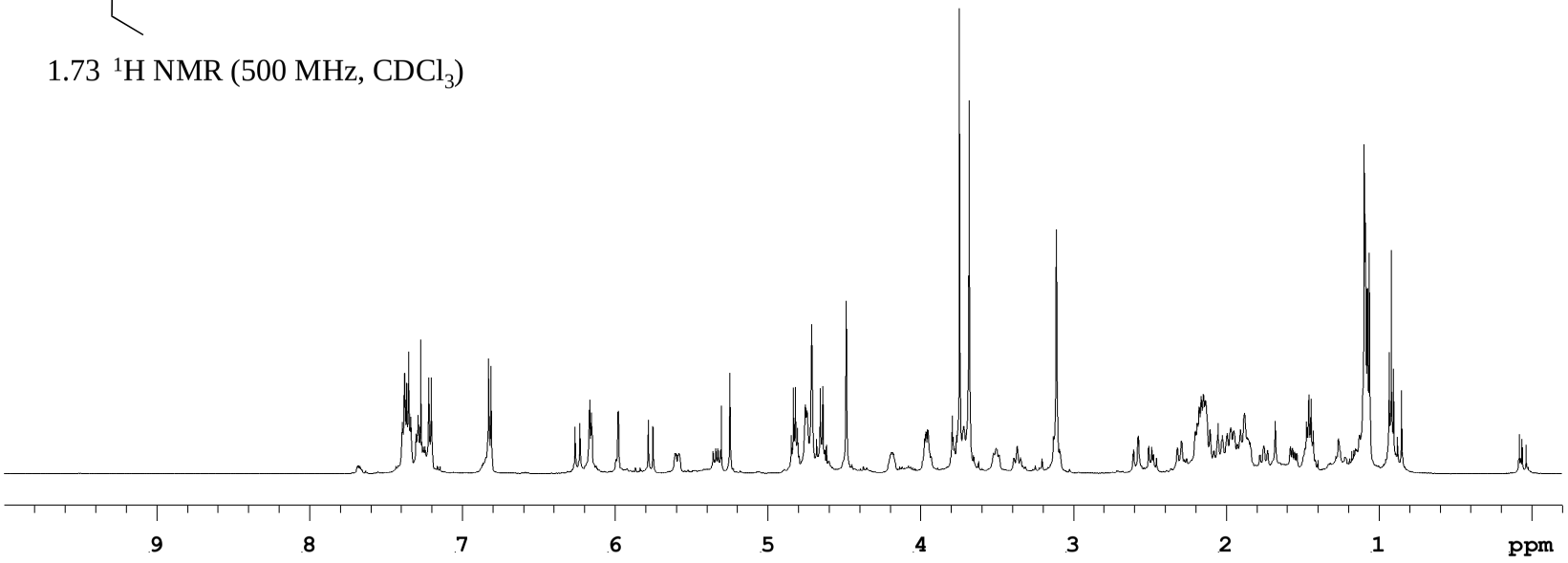
1.93 ^{13}C NMR (125 MHz, $CDCl_3$)

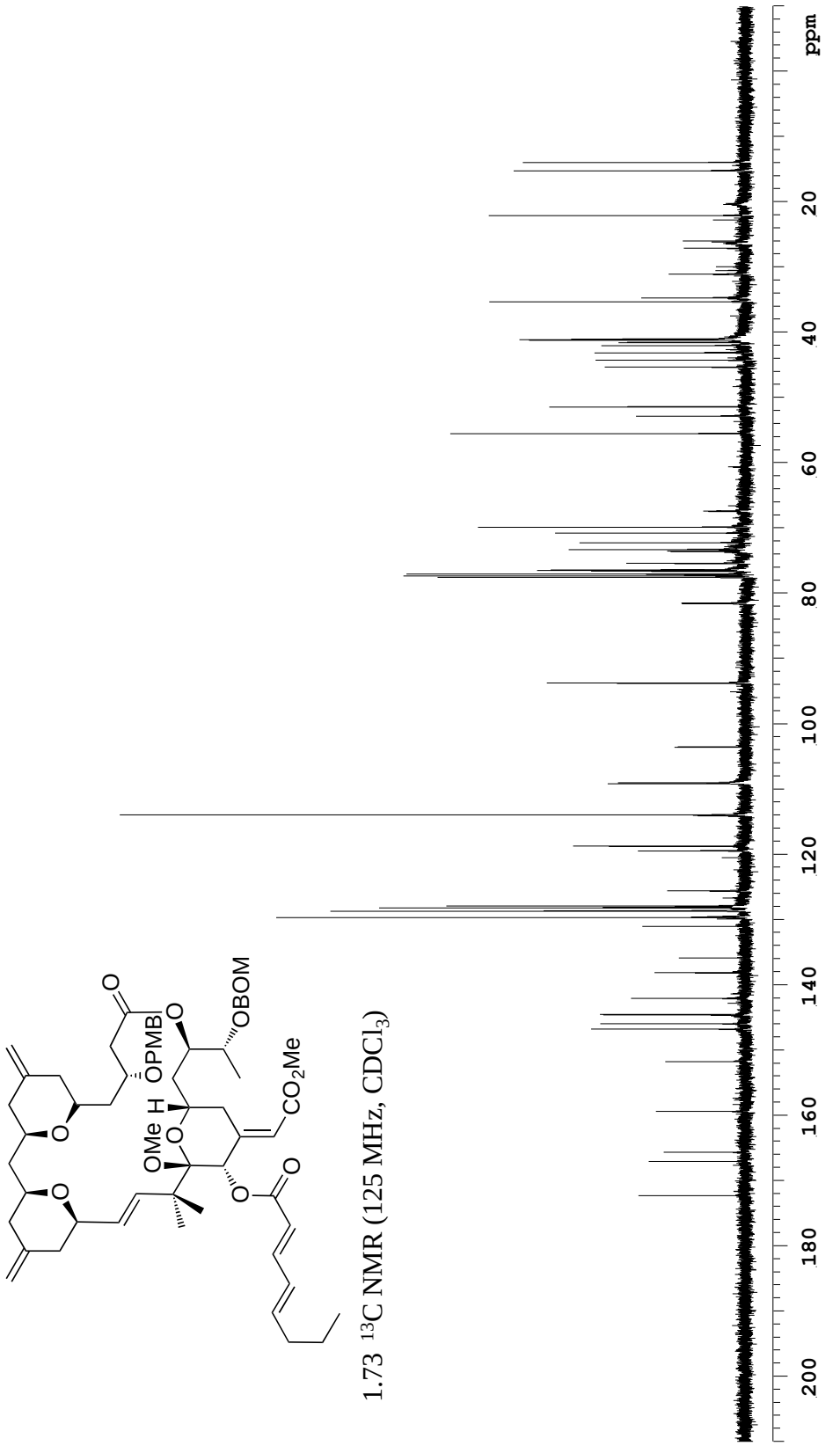


1.93 DEPT NMR (125 MHz, $CDCl_3$)

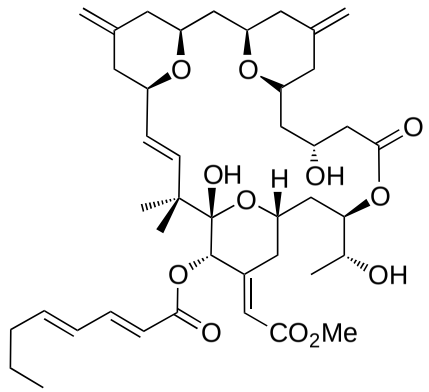


1.73 ^{1}H NMR (500 MHz, $CDCl_3$)

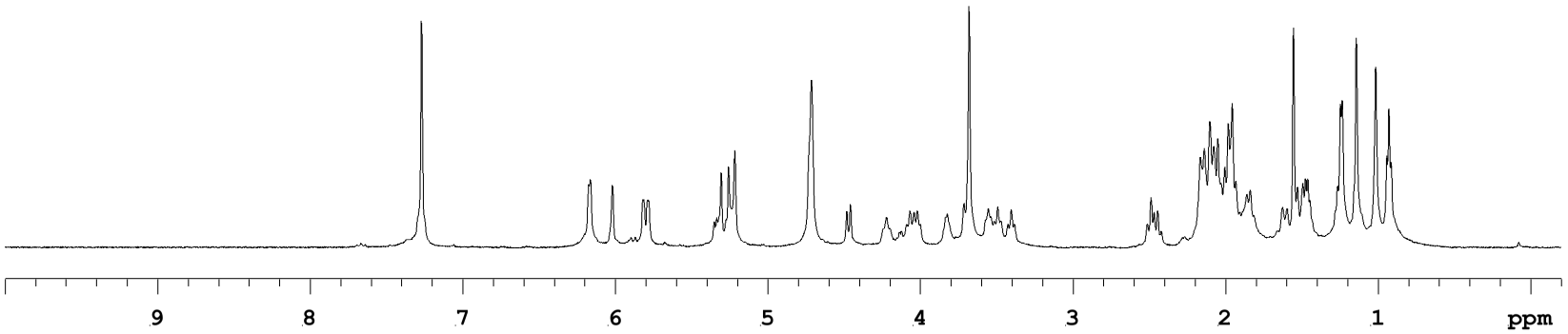


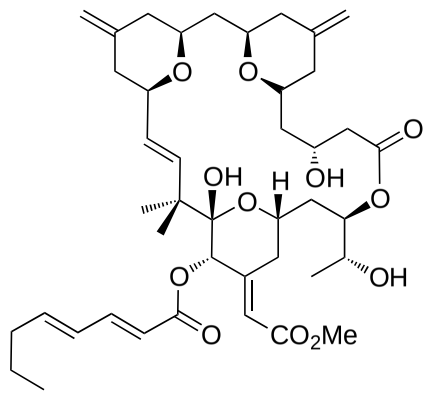


1.73 ^{13}C NMR (125 MHz, $CDCl_3$)

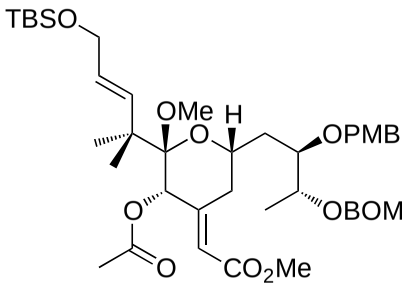


Merle 23 ^{1}H NMR (500 MHz, $CDCl_3$)

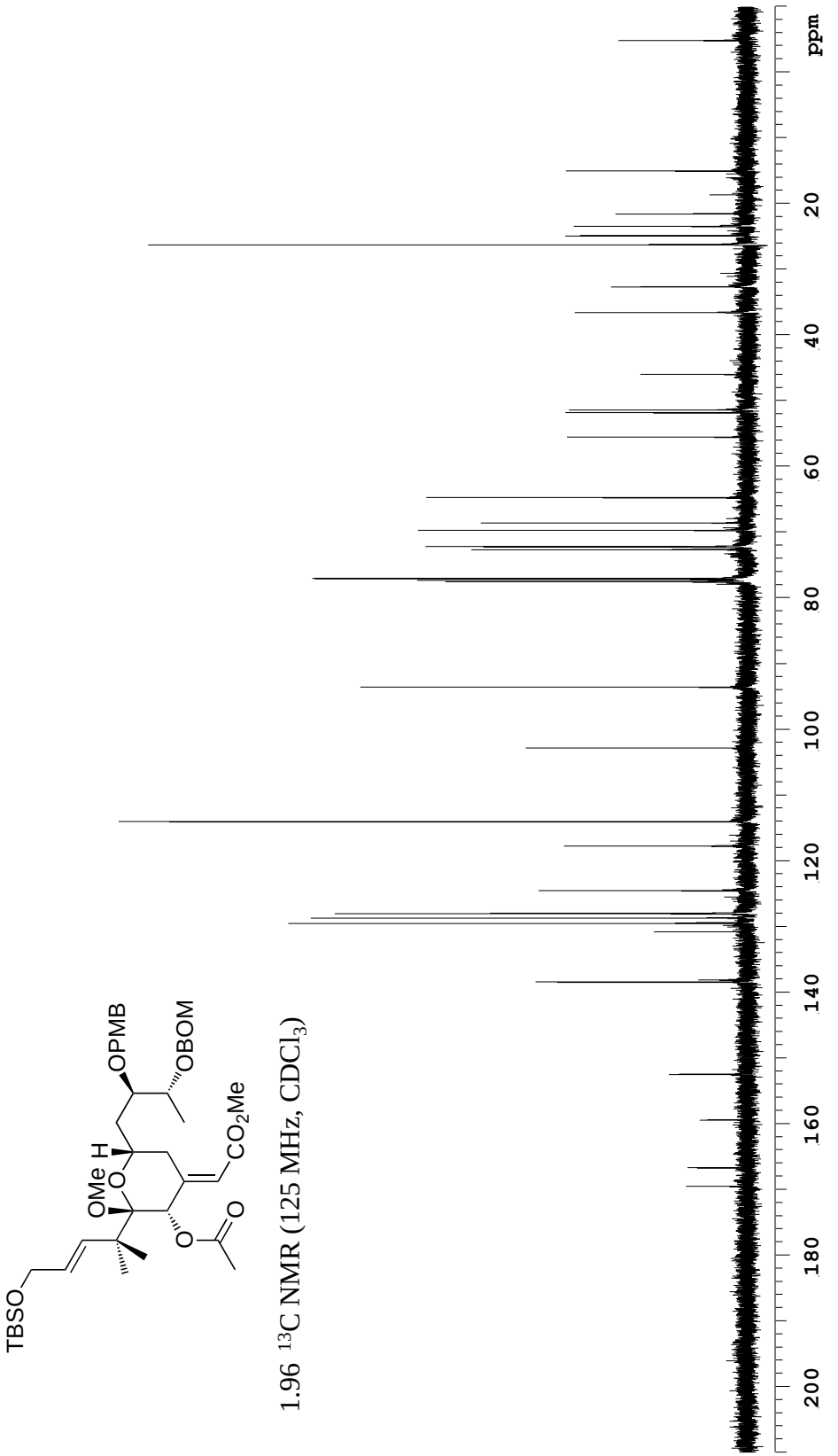




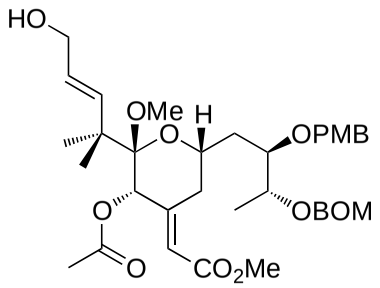
Merle 23 ^{13}C NMR (125 MHz, $CDCl_3$)



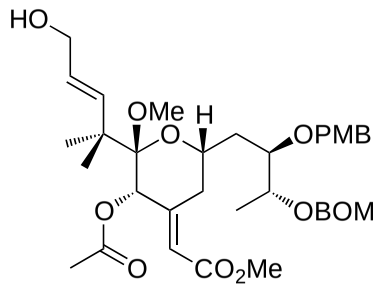
1.96 ^{1}H NMR (500 MHz, $CDCl_3$)



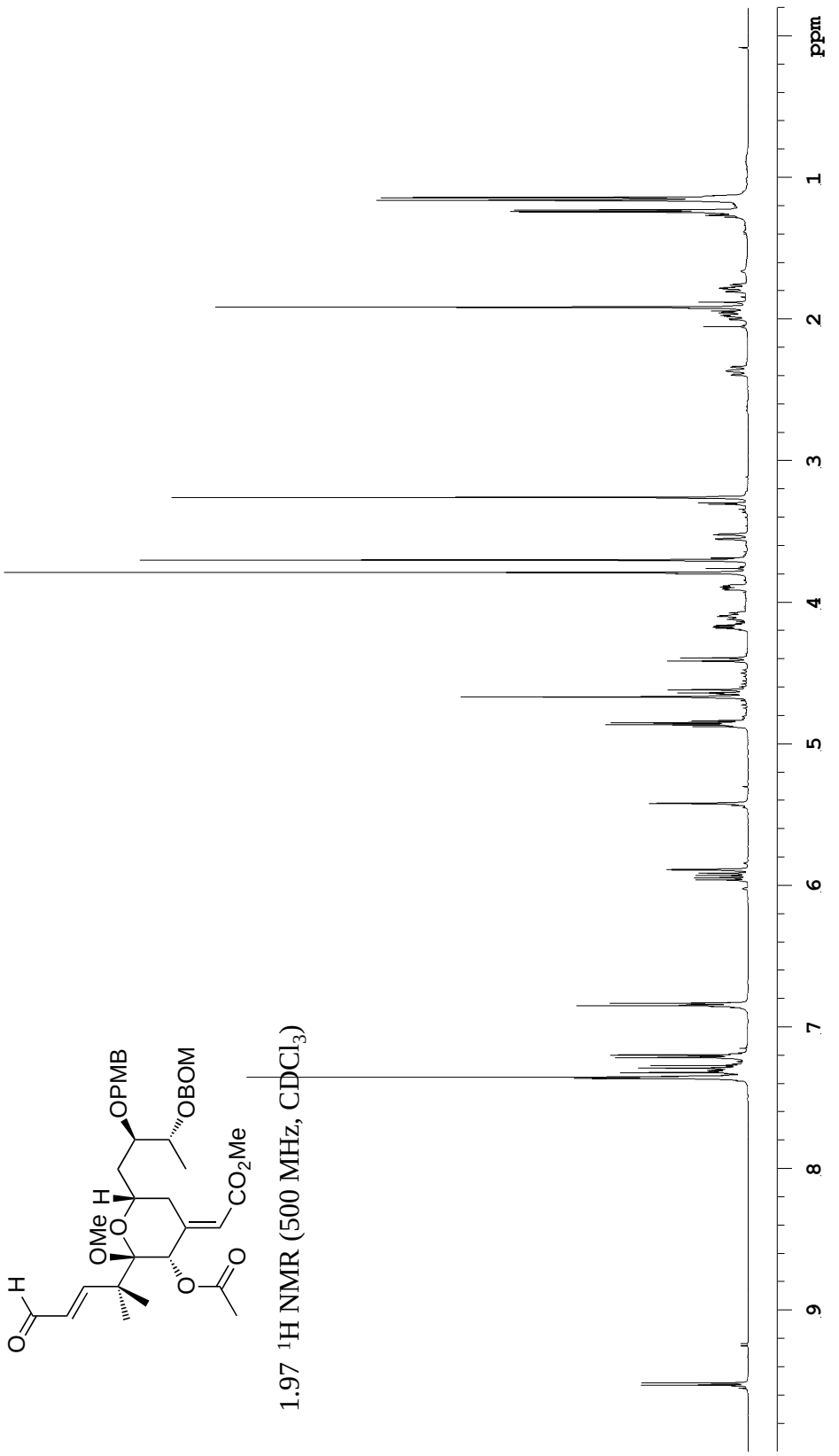
1.96 ^{13}C NMR (125 MHz, $CDCl_3$)



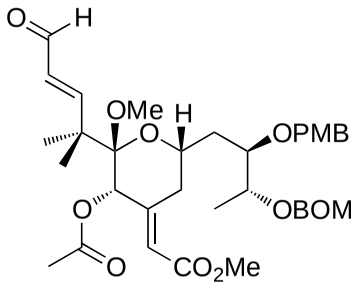
1.110 ^{1}H NMR (500 MHz, $CDCl_3$)



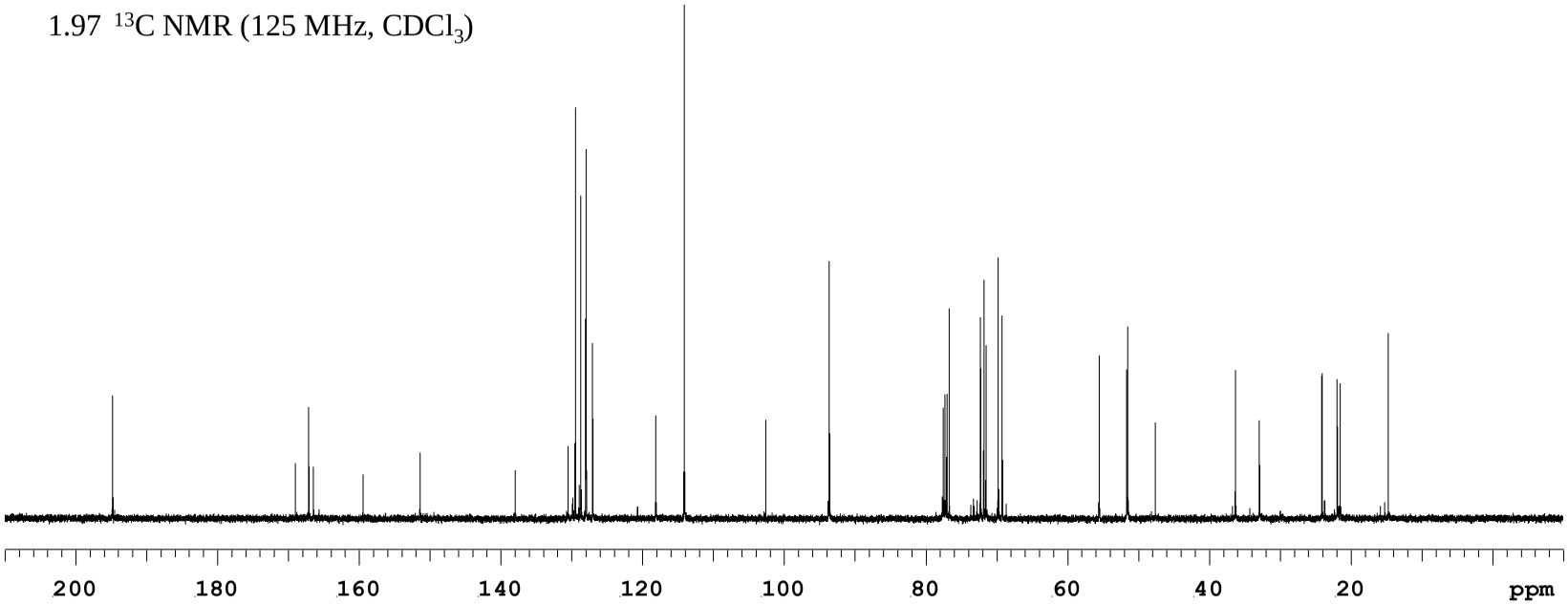
1.110 ^{13}C NMR (125 MHz, $CDCl_3$)

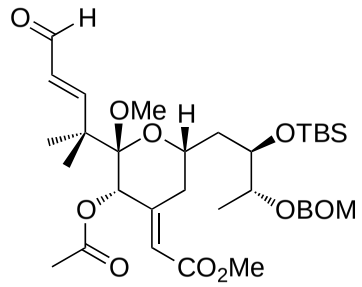


1.97 ^{1}H NMR (500 MHz, $CDCl_3$)

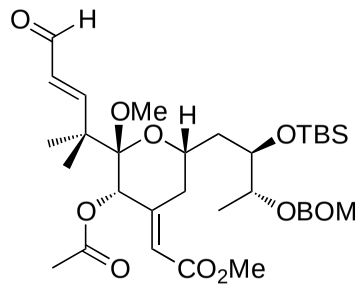


1.97 ^{13}C NMR (125 MHz, $CDCl_3$)



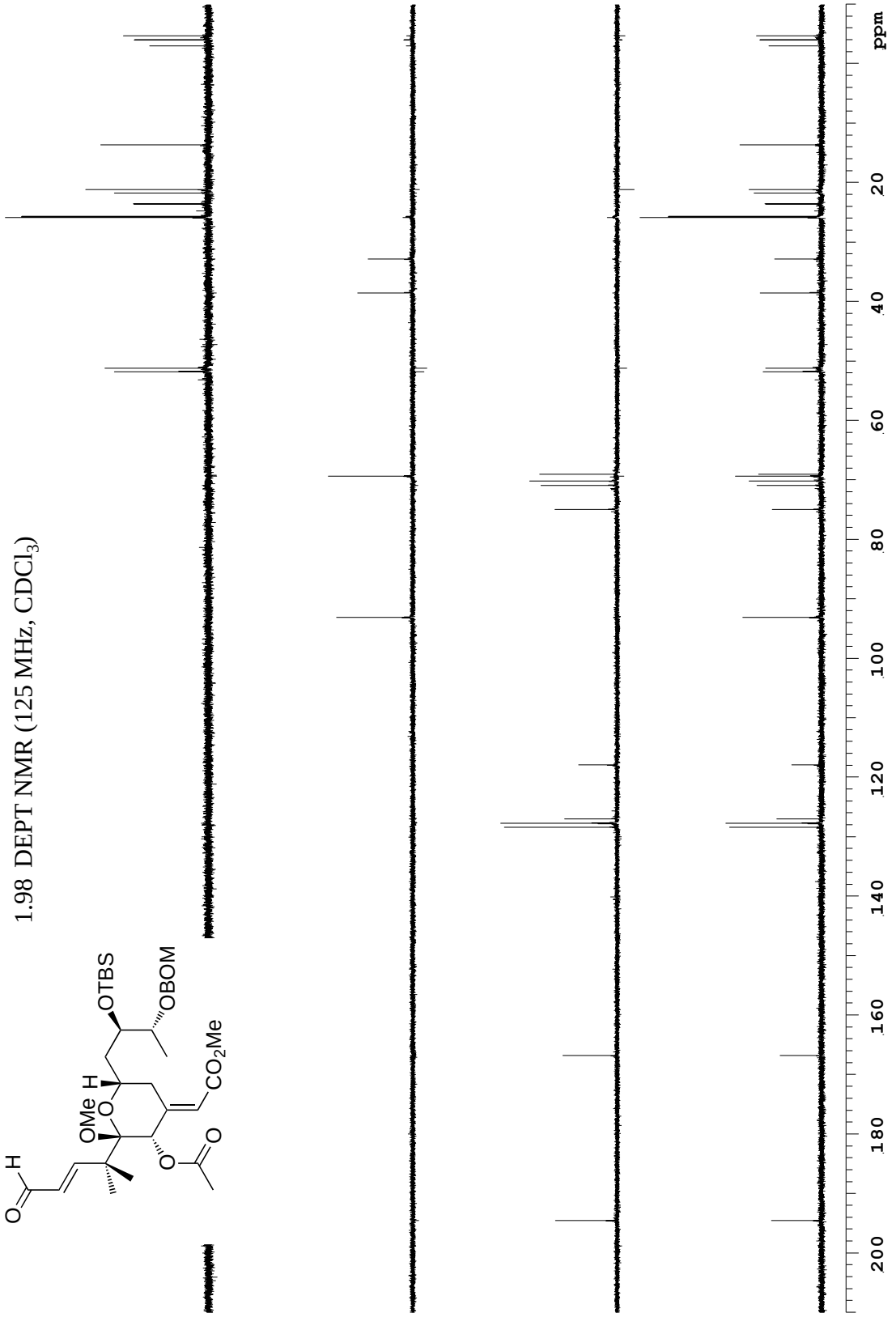


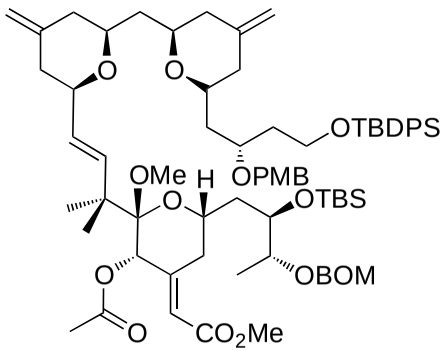
1.98 ^{1}H NMR (500 MHz, $CDCl_3$)



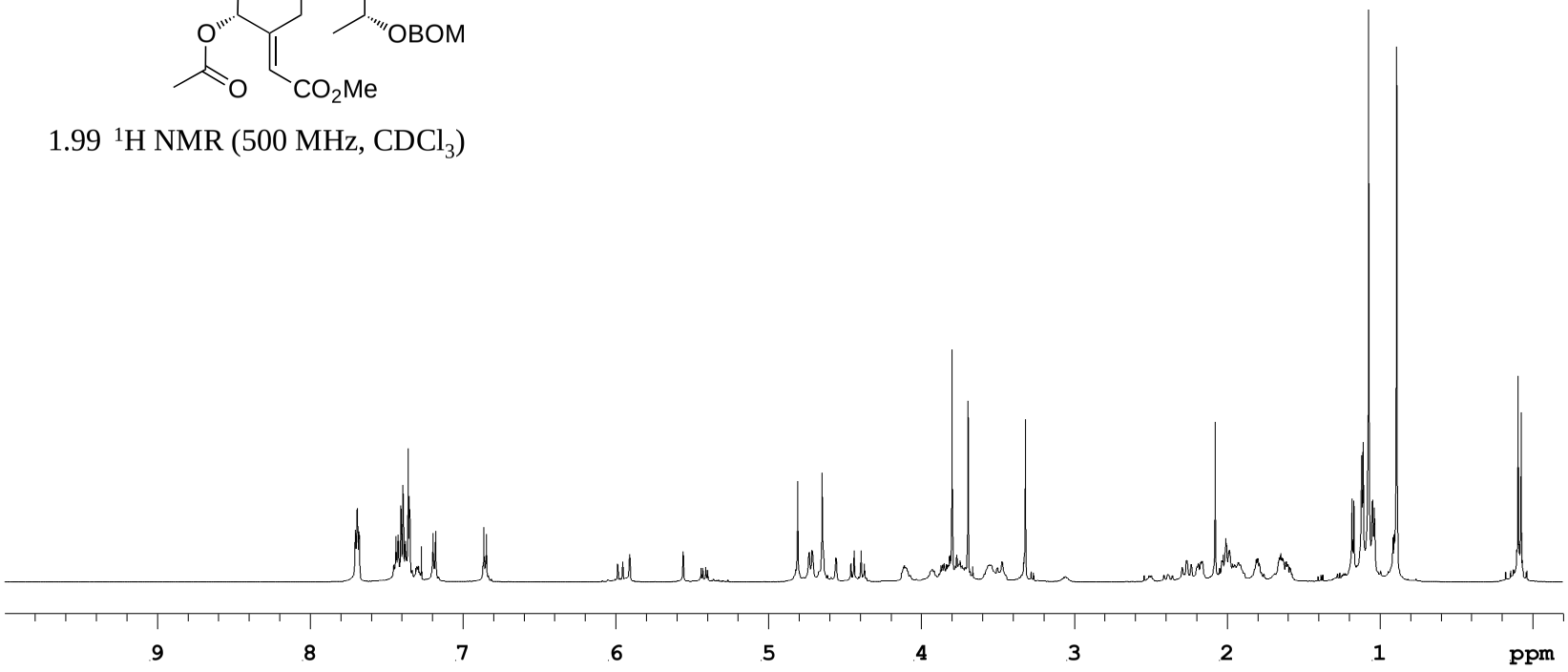
1.98 ^{13}C NMR (125 MHz, $CDCl_3$)

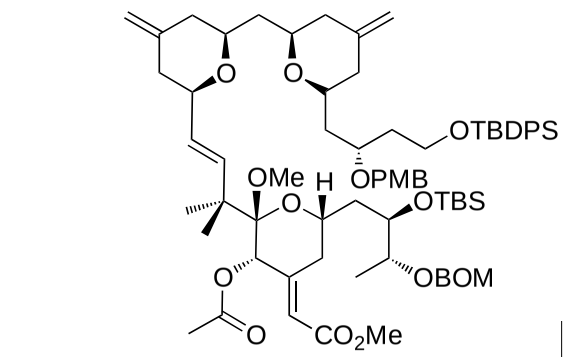
1.98 DEPT NMR (125 MHz, $CDCl_3$)





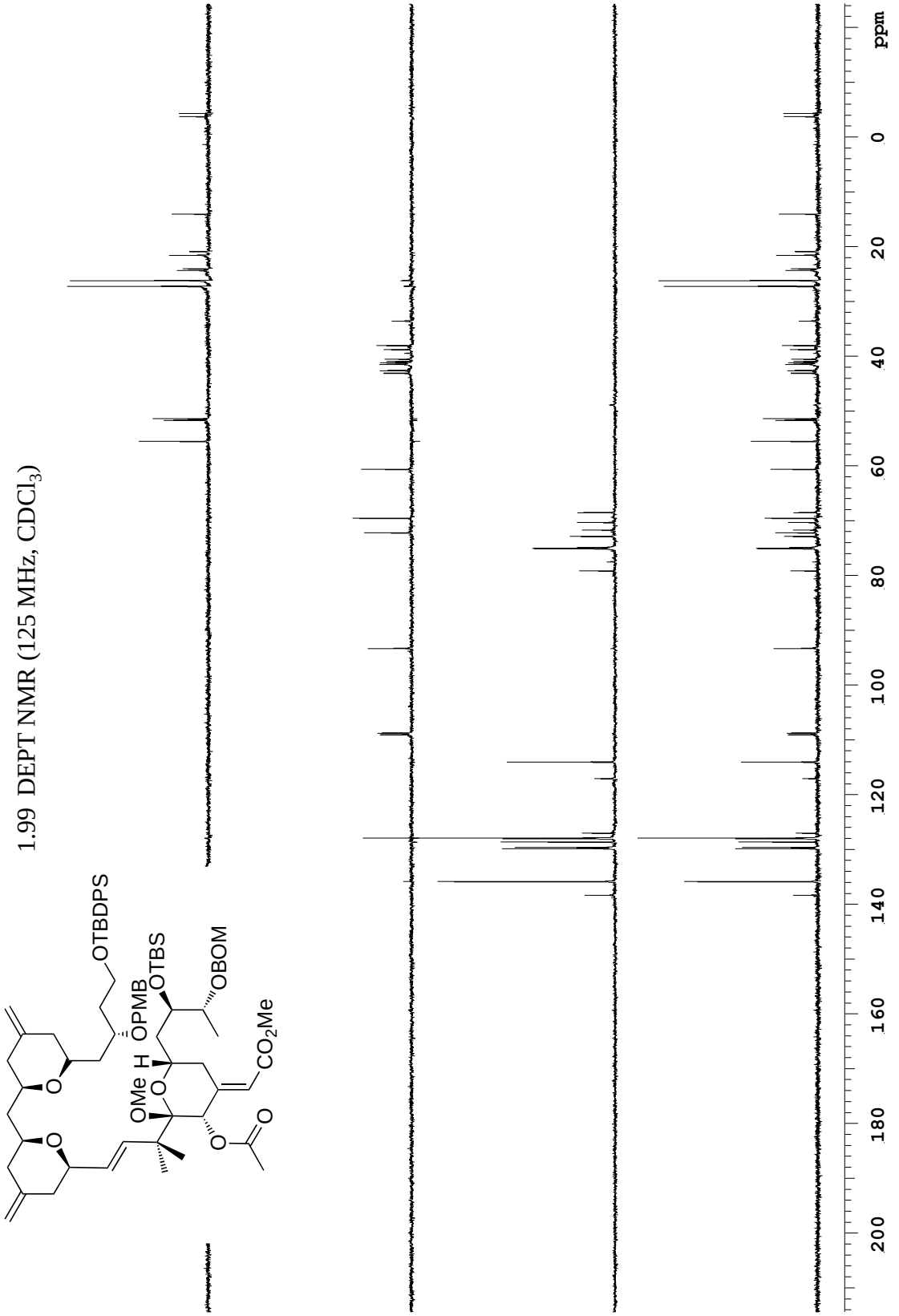
1.99 ^{1}H NMR (500 MHz, $CDCl_3$)

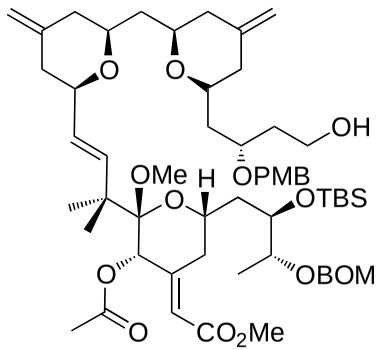




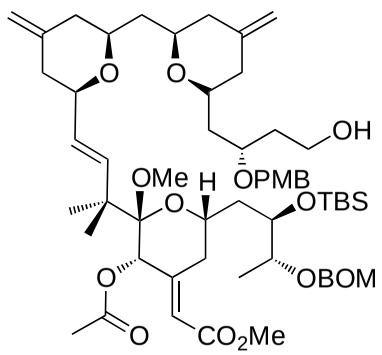
1.99 ^{13}C NMR (125 MHz, $CDCl_3$)

1.99 DEPT NMR (125 MHz, $CDCl_3$)

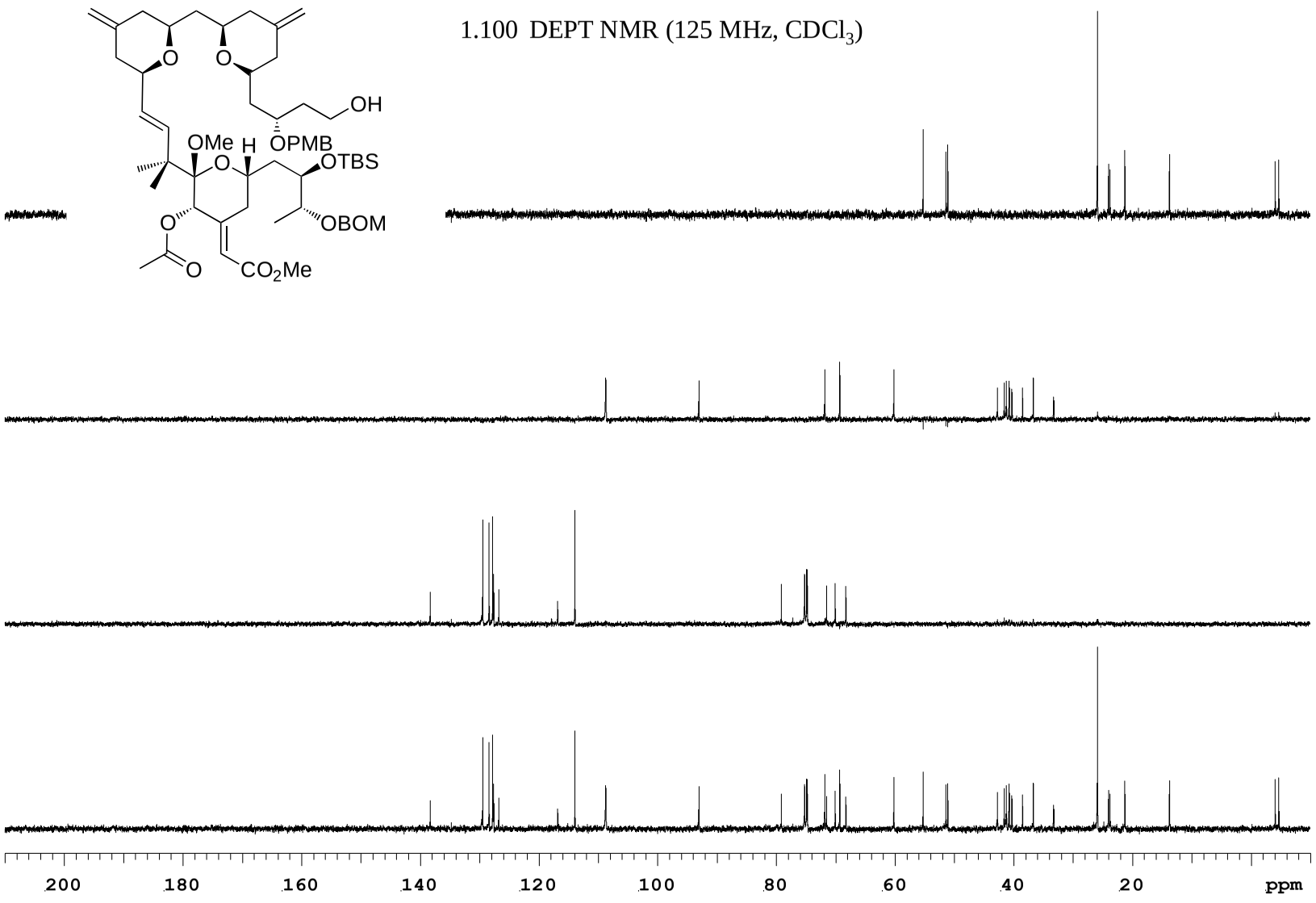




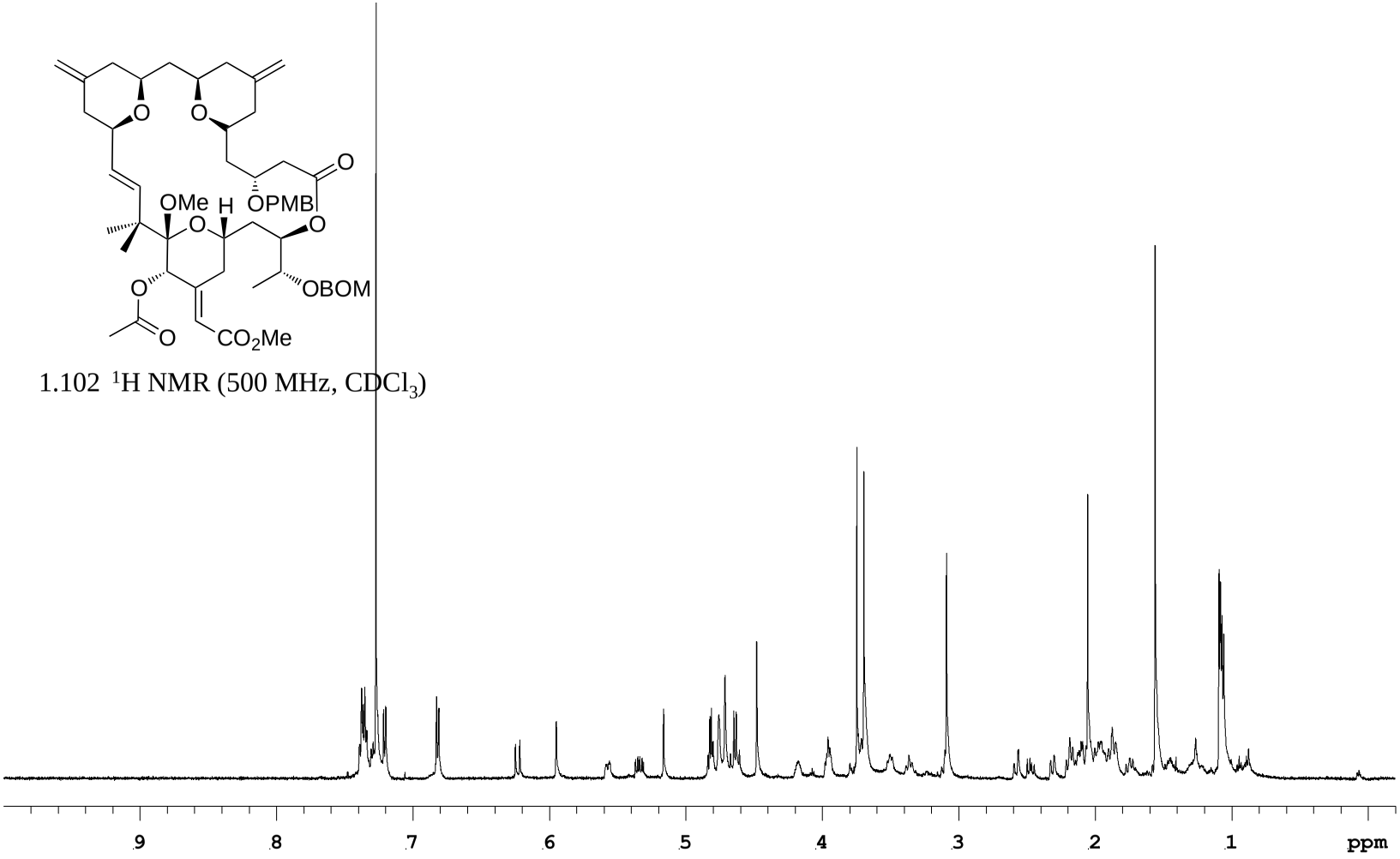
1.100 ^{1}H NMR (500 MHz, $CDCl_3$)



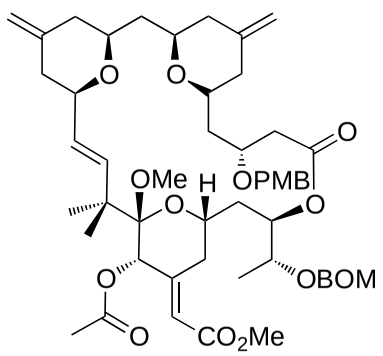
1.100 ^{13}C NMR (125 MHz, $CDCl_3$)



1.100 DEPT NMR (125 MHz, $CDCl_3$)

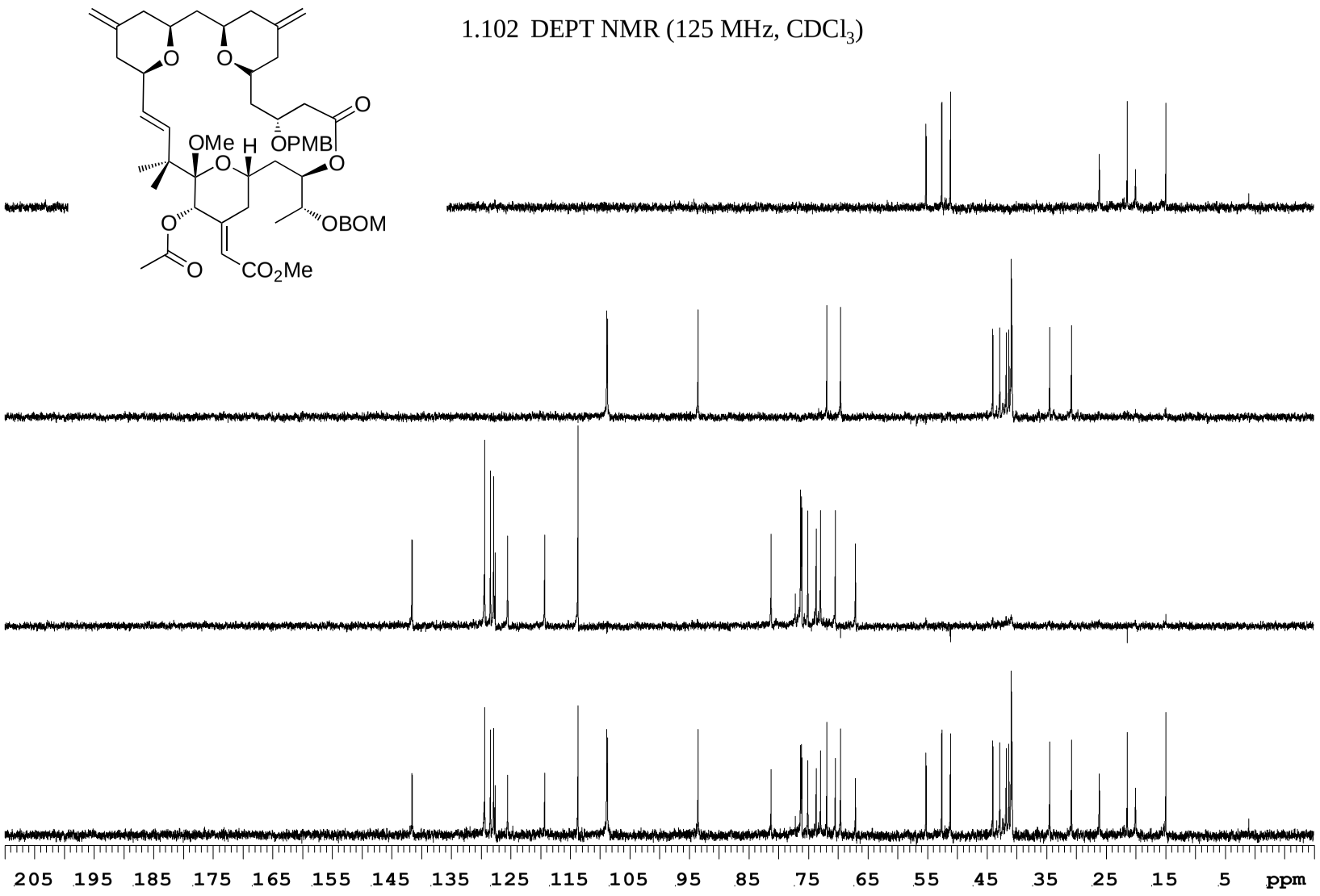


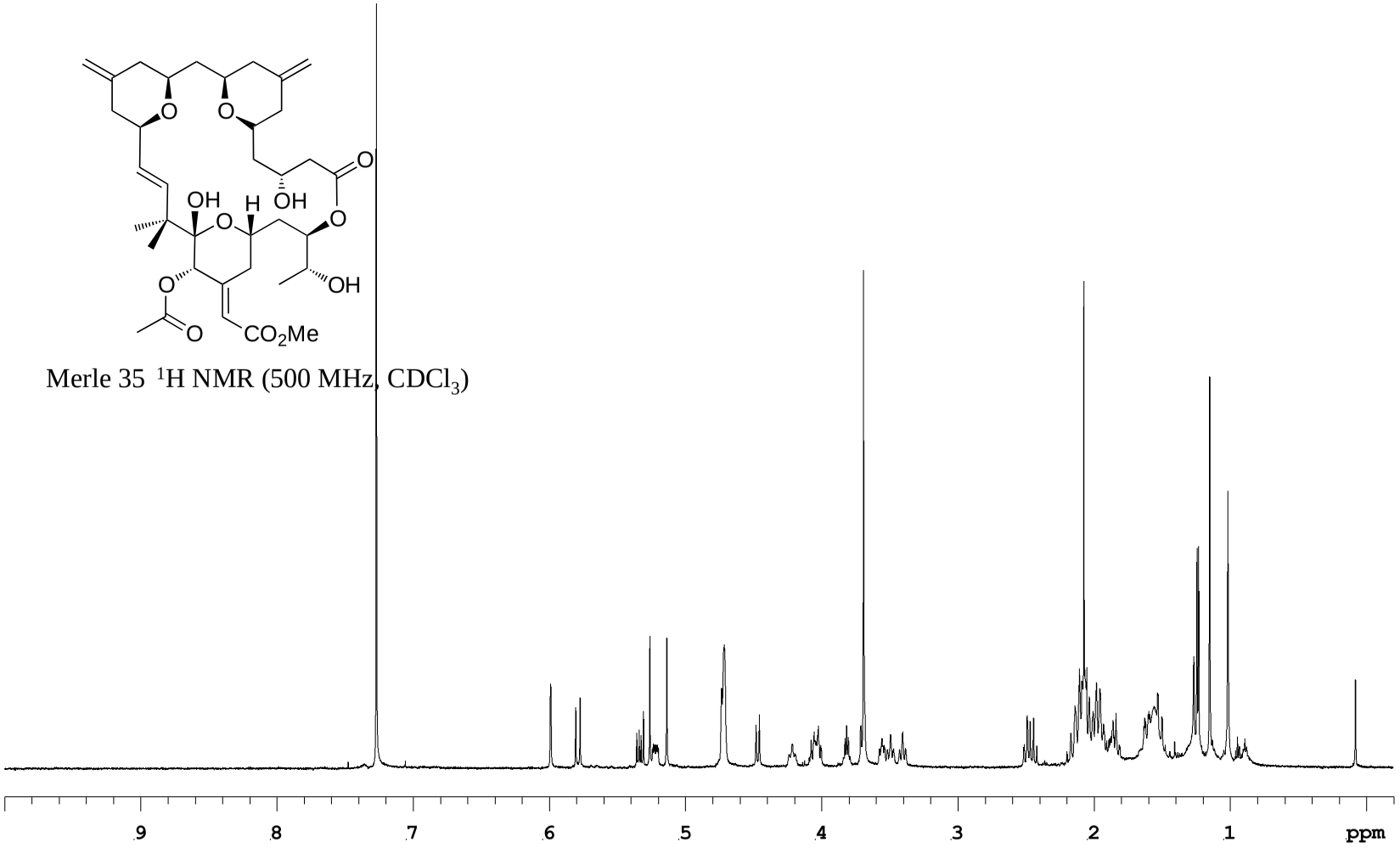
1.102 ^{1}H NMR (500 MHz, $CDCl_3$)



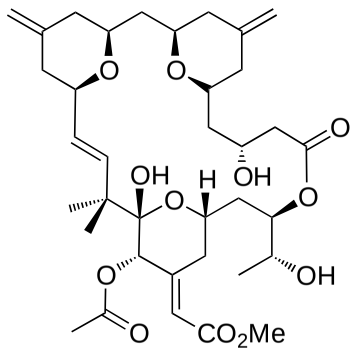
1.102 ^{13}C NMR (125 MHz, $CDCl_3$)

1.102 DEPT NMR (125 MHz, $CDCl_3$)

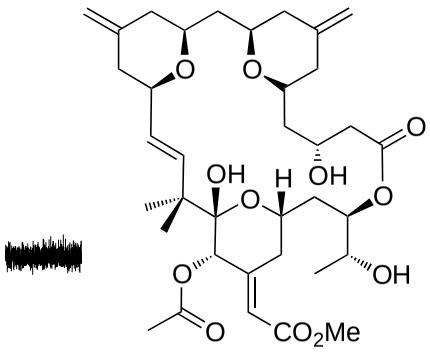




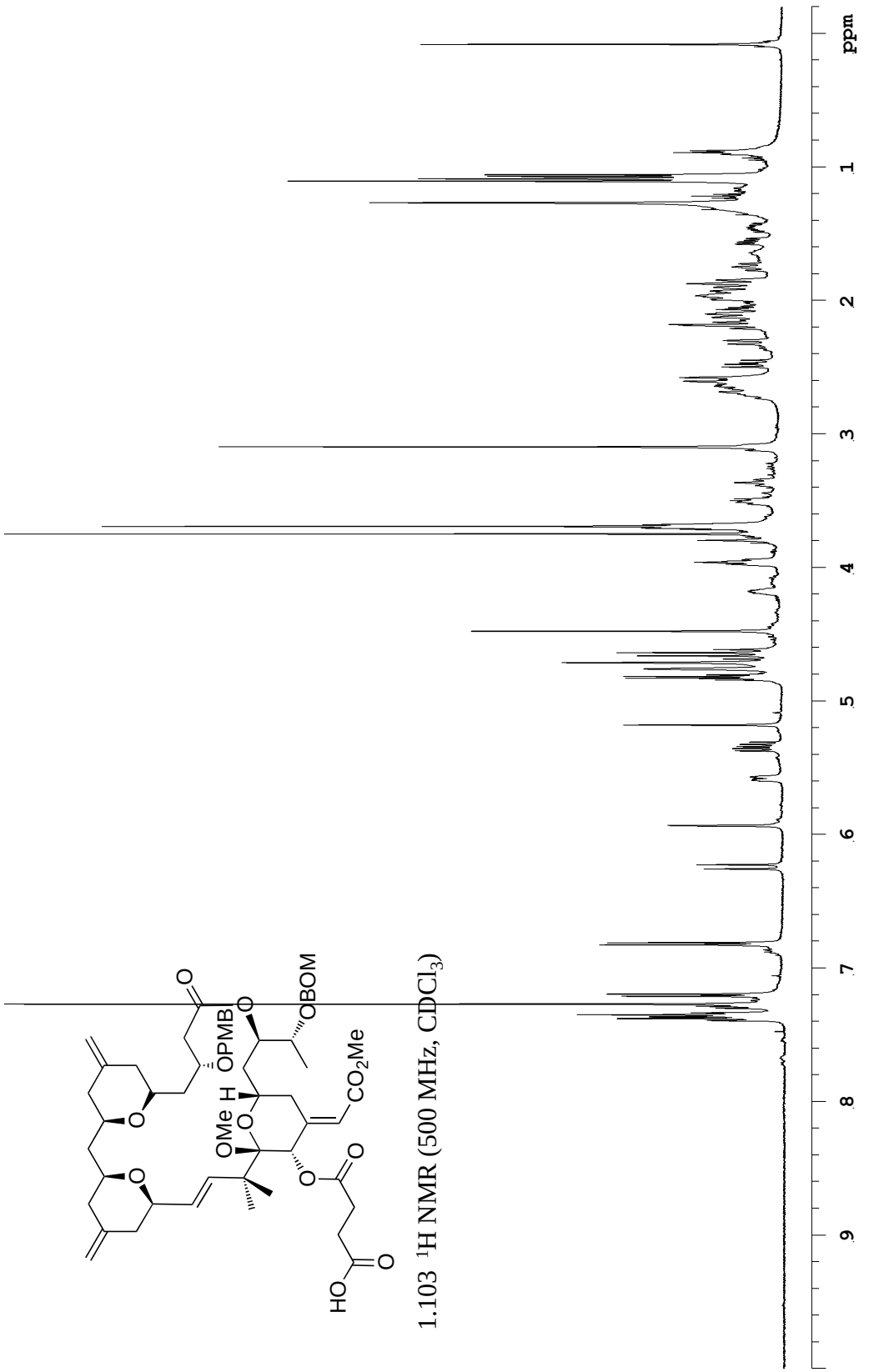
Merle 35 ^{1}H NMR (500 MHz, $CDCl_3$)



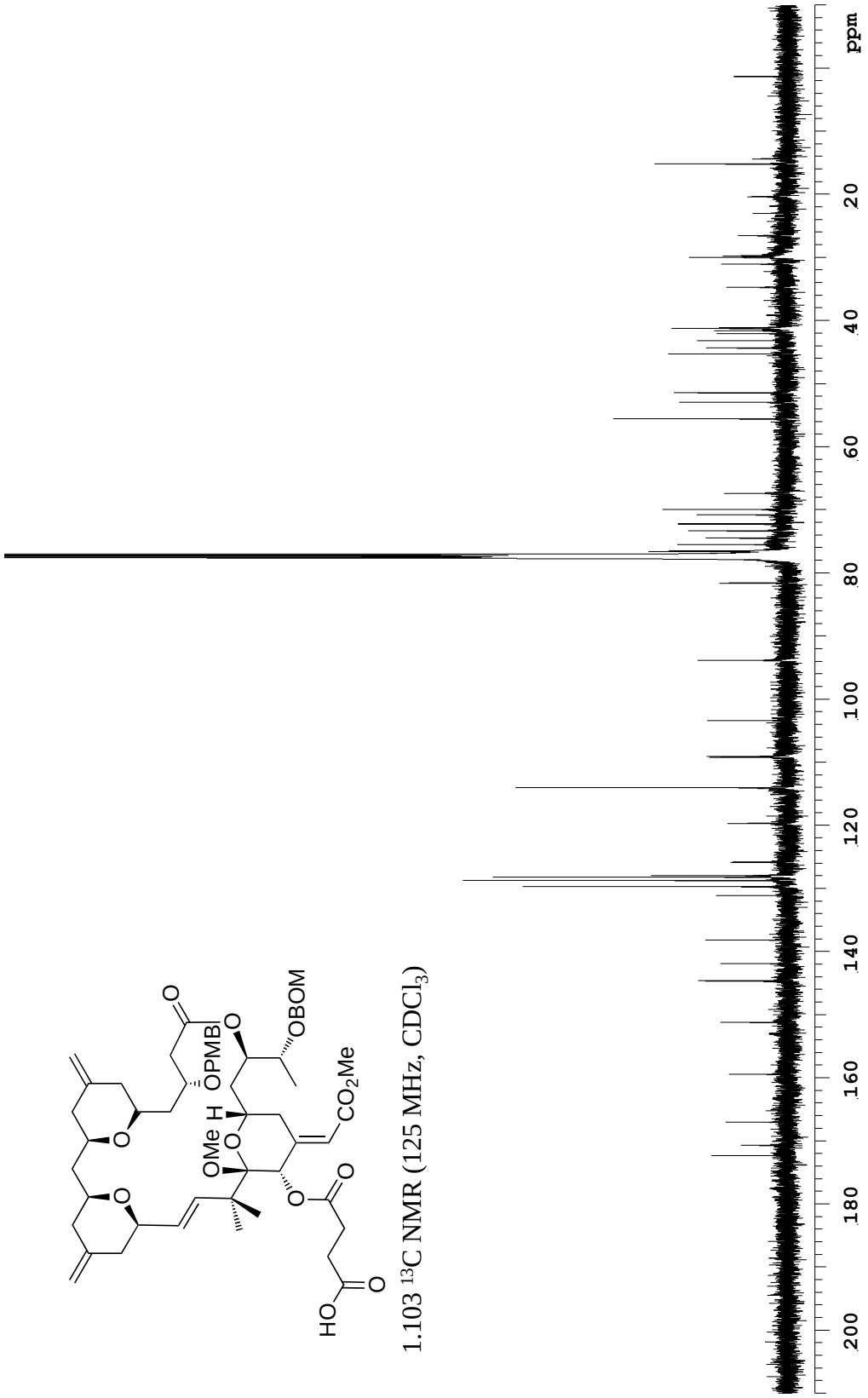
Merle 35 ^{13}C NMR (125 MHz, $CDCl_3$)



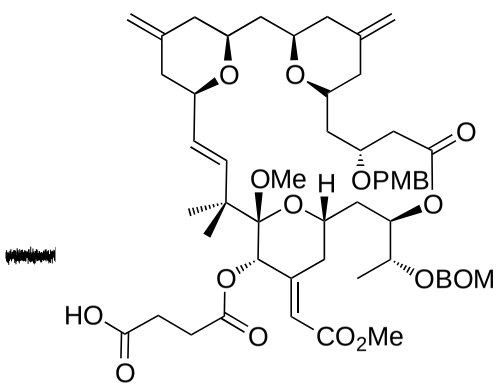
Merle 35 DEPT NMR (125 MHz, $CDCl_3$)



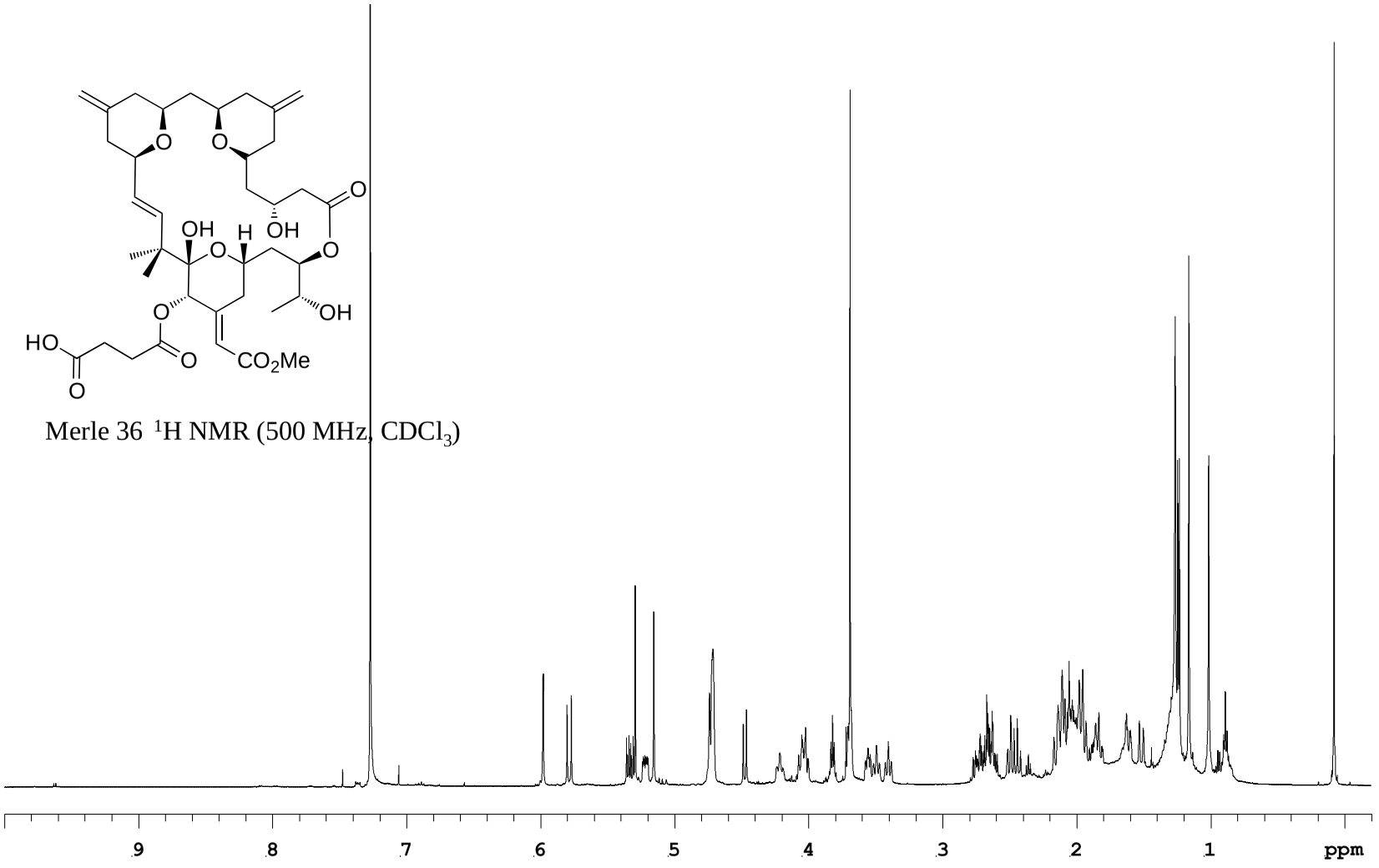
1.103 ^{1}H NMR (500 MHz, $CDCl_3$)



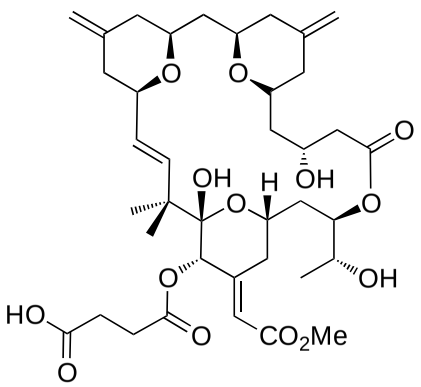
1.103 ^{13}C NMR (125 MHz, $CDCl_3$)



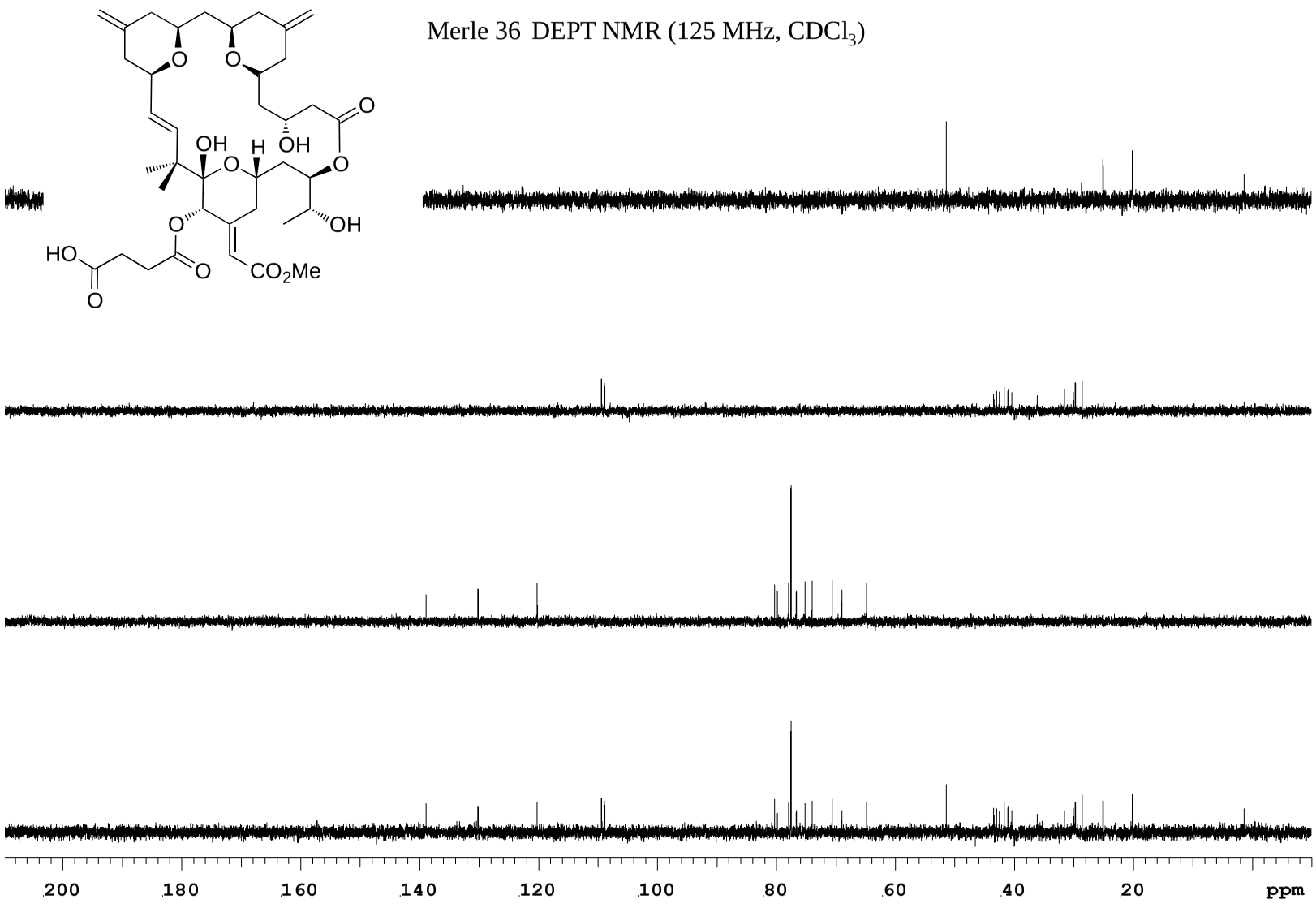
1.103 DEPT NMR (125 MHz, $CDCl_3$)

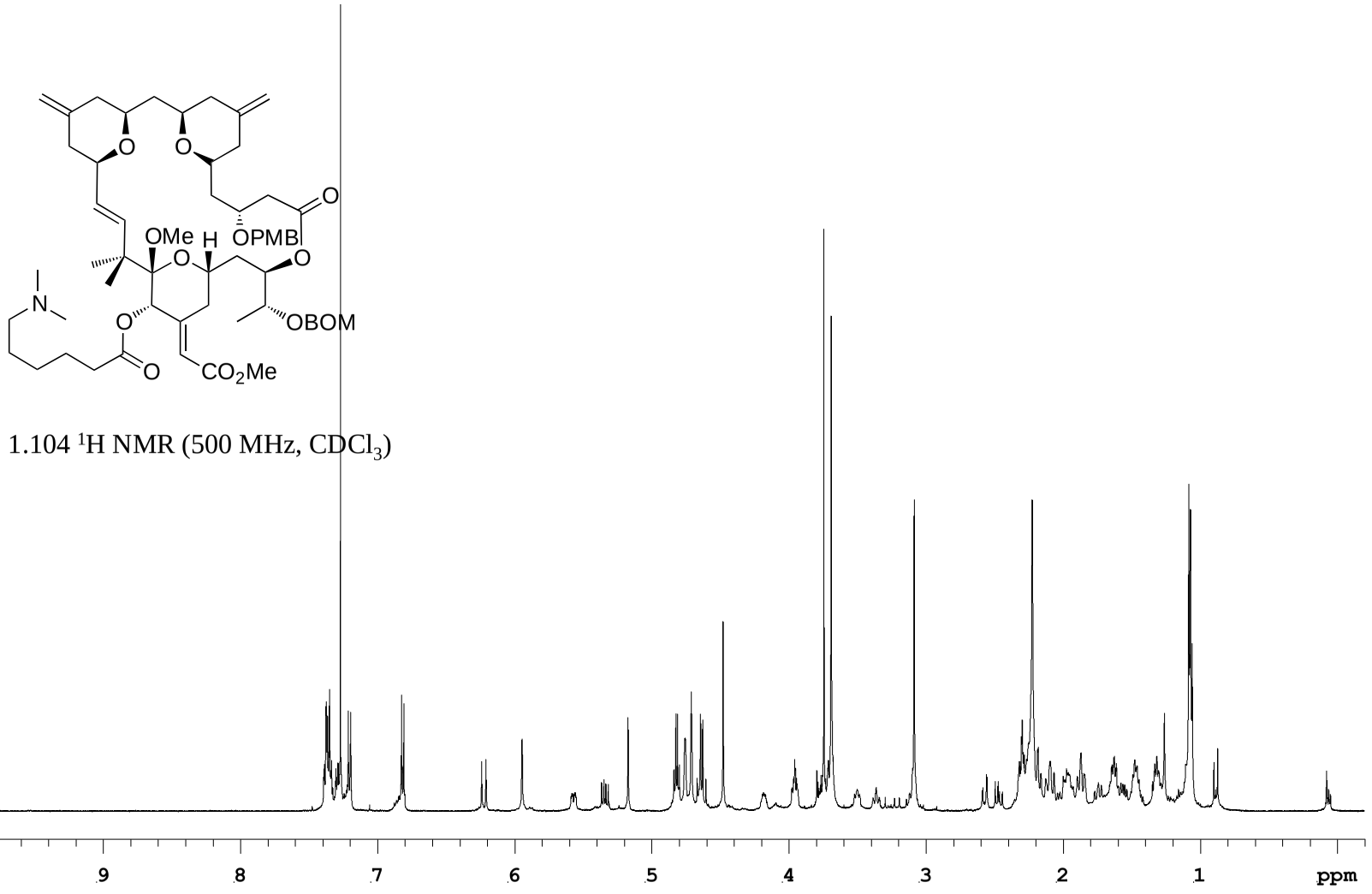


Merle 36 ^{1}H NMR (500 MHz, $CDCl_3$)

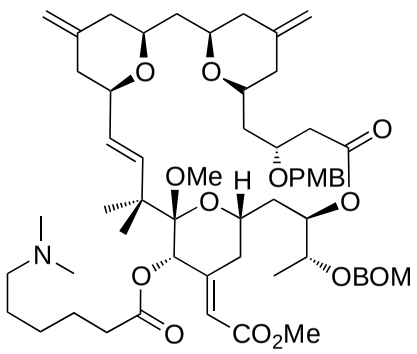


Merle 36 ^{13}C NMR (125 MHz, $CDCl_3$)

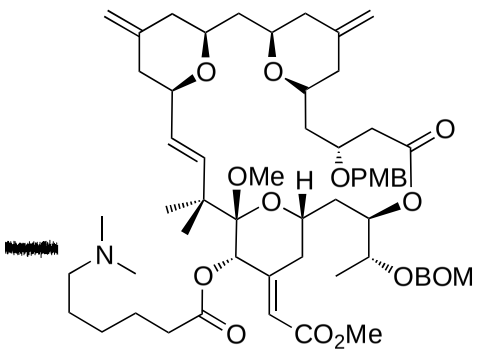

Merle 36 DEPT NMR (125 MHz, CDCl3)
OH
H
OH
O
O
O
O
O
O
OH
HO
O
O
CO2Me
200
180
160
140
120
100
80
60
40
20
ppm



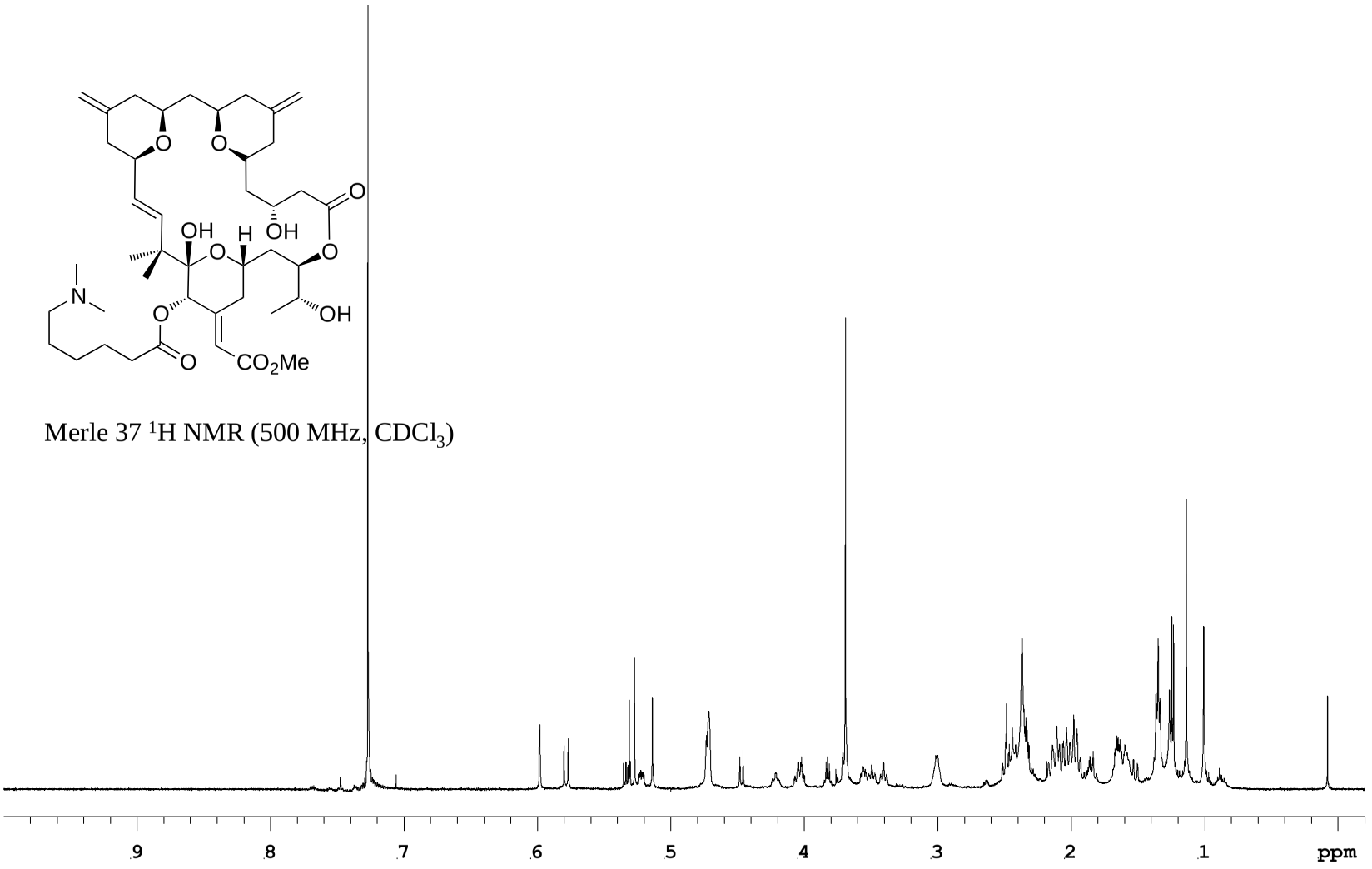
1.104 ^{1}H NMR (500 MHz, $CDCl_3$)



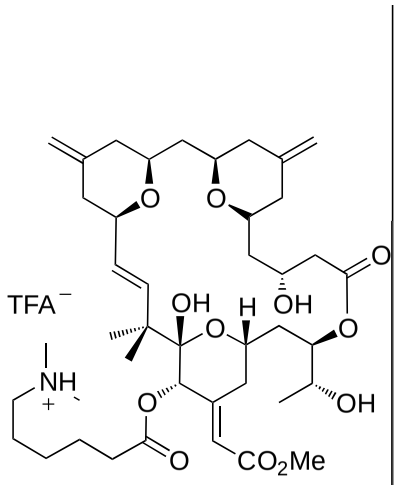
1.104 ^{13}C NMR (125 MHz, $CDCl_3$)



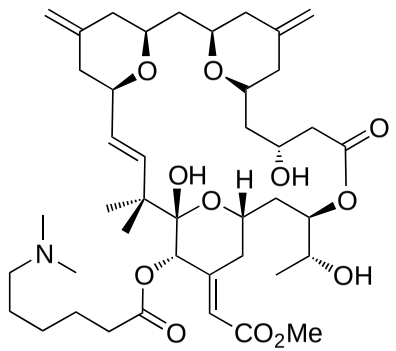
1.104 DEPT NMR (125 MHz, $CDCl_3$)



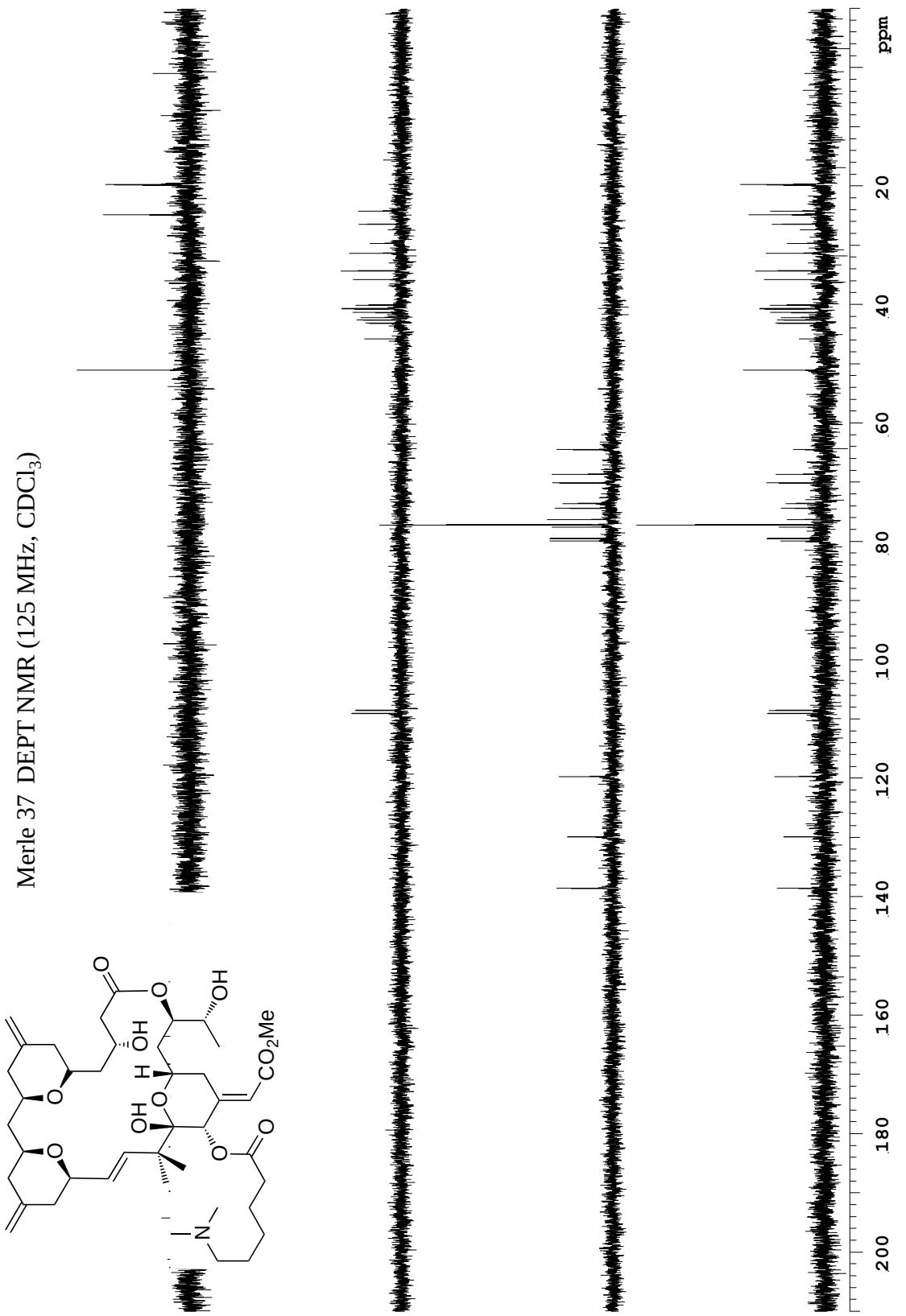
Merle 37 ^{1}H NMR (500 MHz, $CDCl_3$)



Merle 37 ^{1}H NMR (500 MHz, $CDCl_3$)

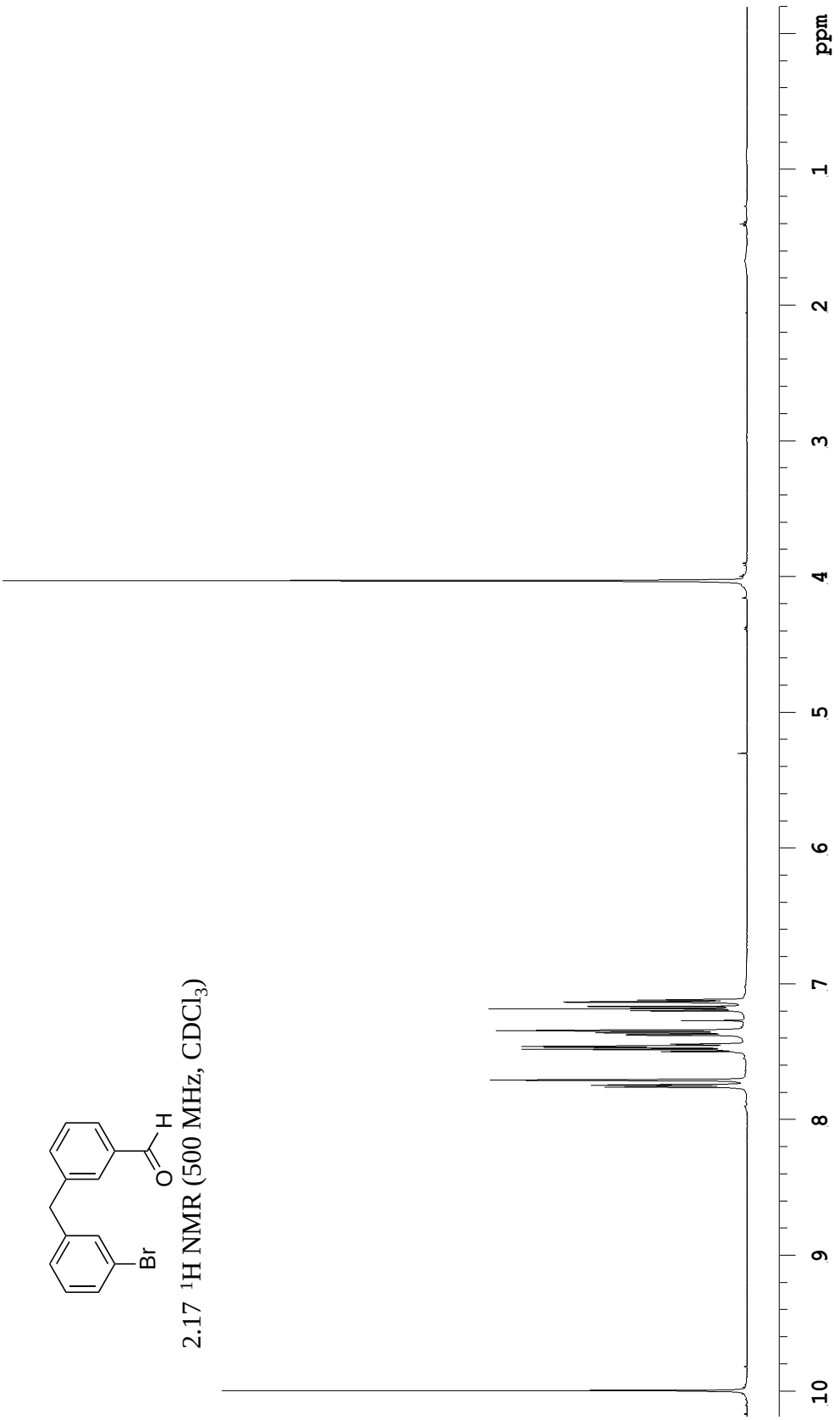


Merle 37 ^{13}C NMR (125 MHz, $CDCl_3$)

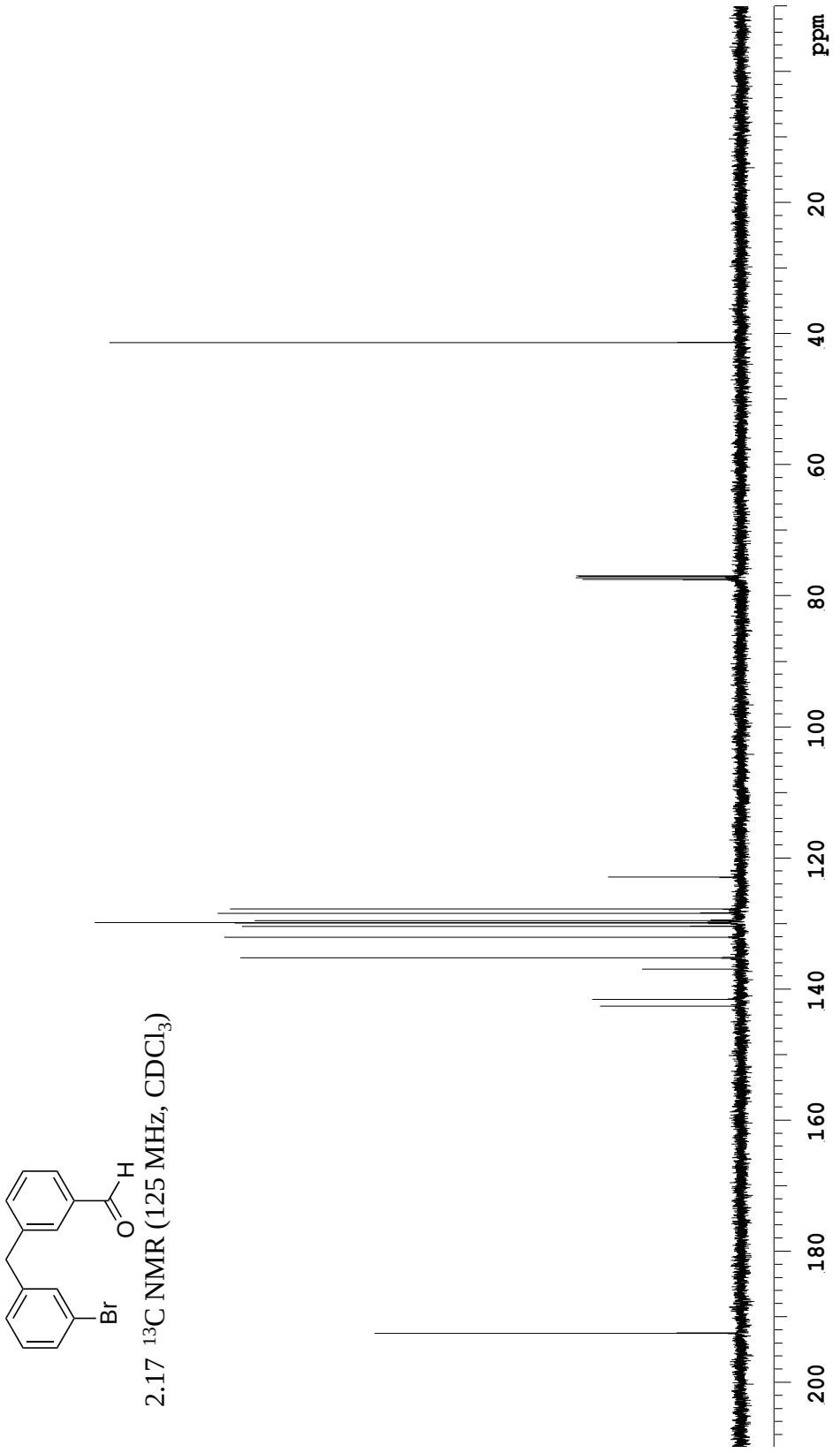

Merle 37 DEPT NMR (125 MHz, $CDCl_3$)
ppm
20
40
60
80
100
120
140
160
180
200
CO_2Me
OH
OH
H
OH
O
N

APPENDIX B

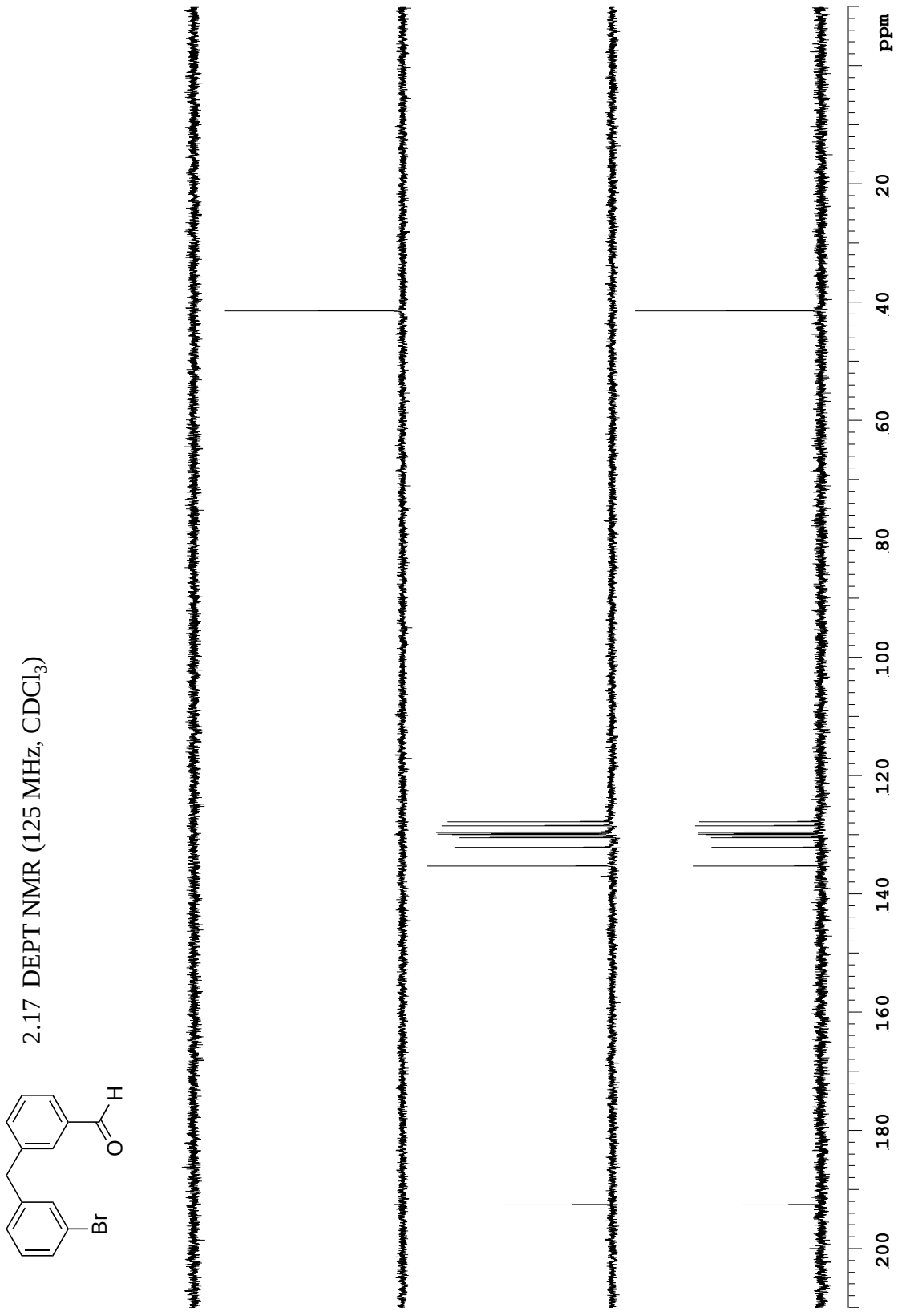
^{1}H, ^{13}C, AND DEPT SPECTRA FOR CHAPTER 2



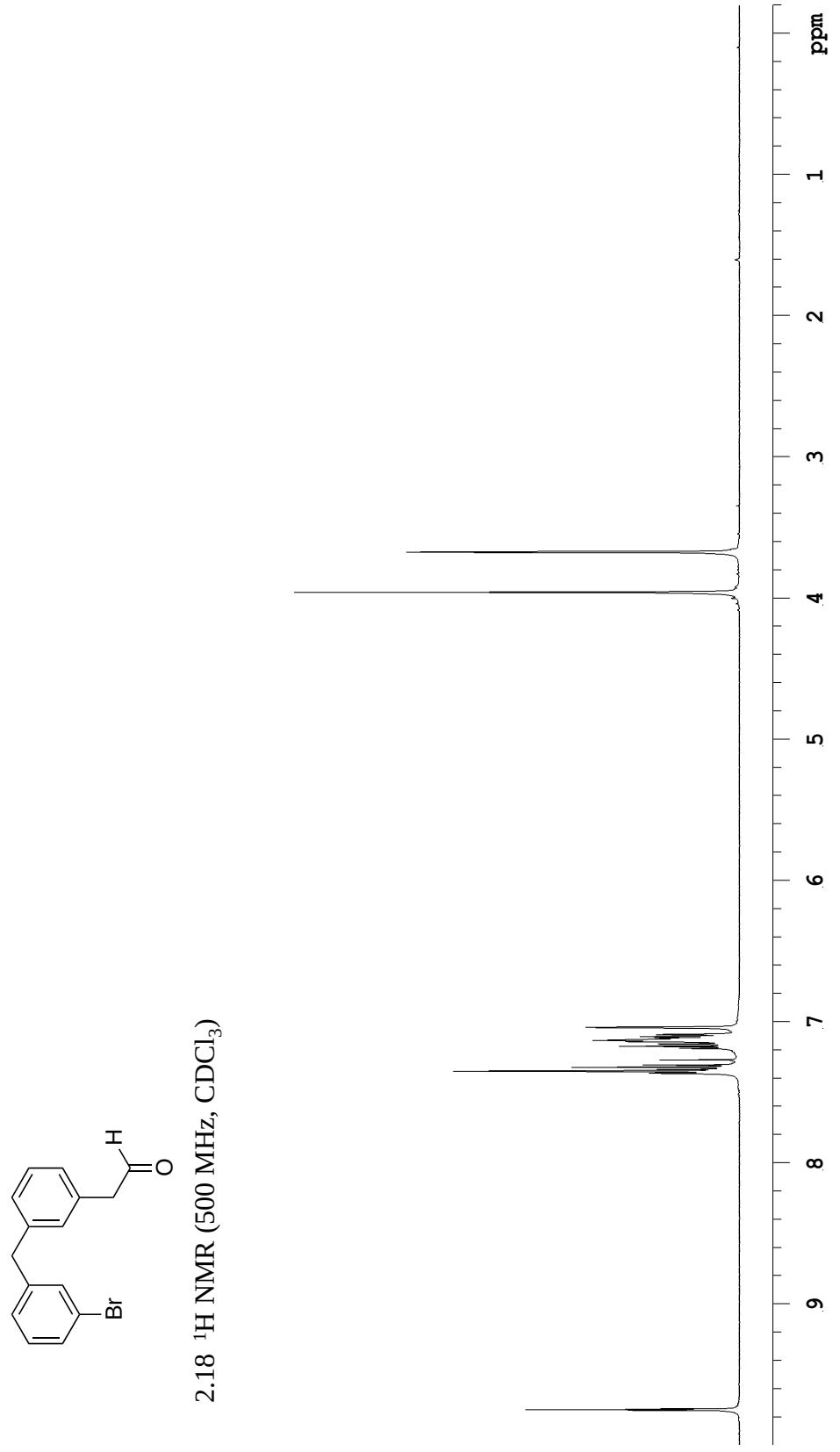
2.17 ^{1}H NMR (500 MHz, $CDCl_3$)



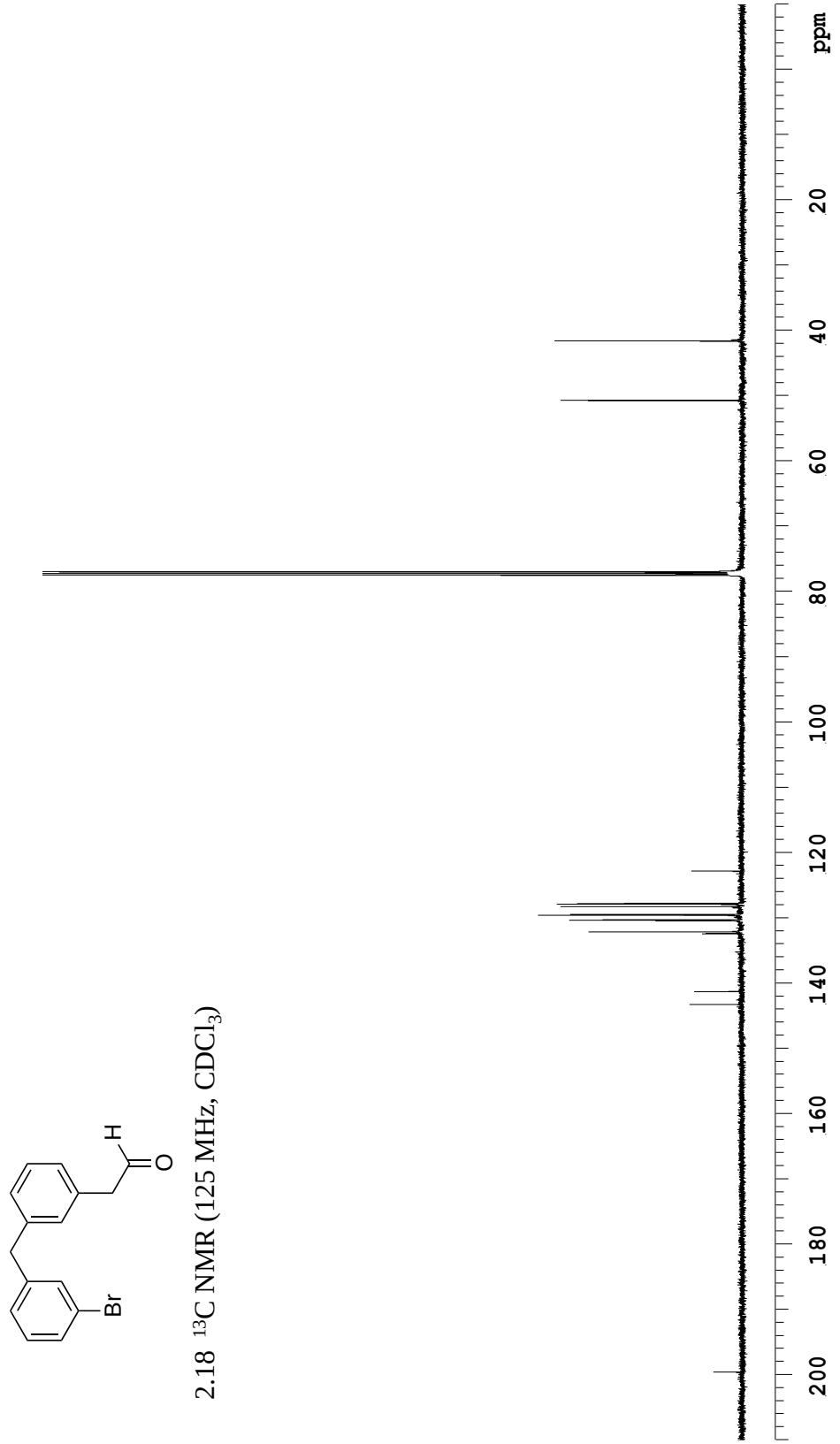
2.17 ^{13}C NMR (125 MHz, $CDCl_3$)



2.17 DEPT NMR (125 MHz, $CDCl_3$)

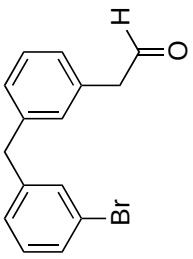


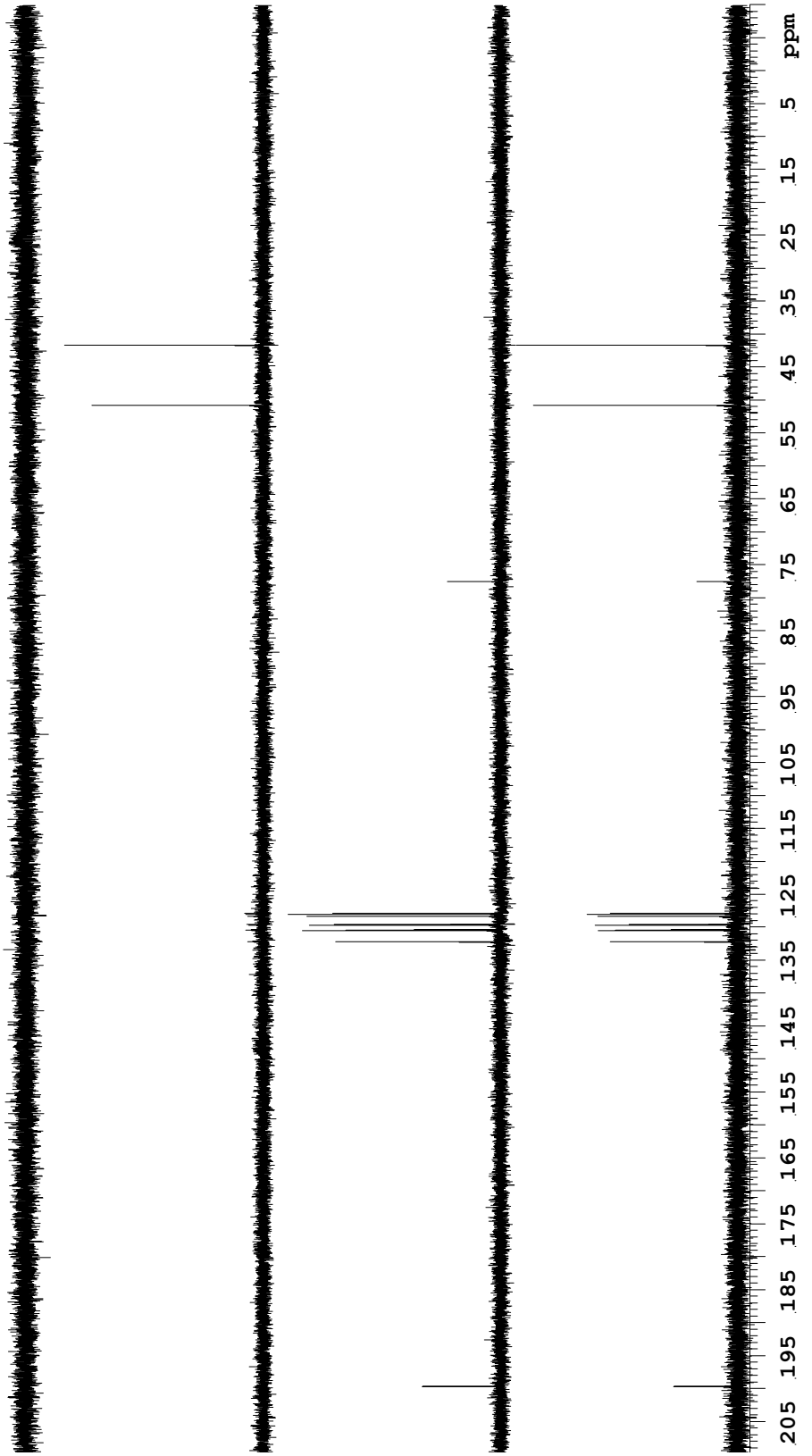
2.18 ^{1}H NMR (500 MHz, $CDCl_3$)

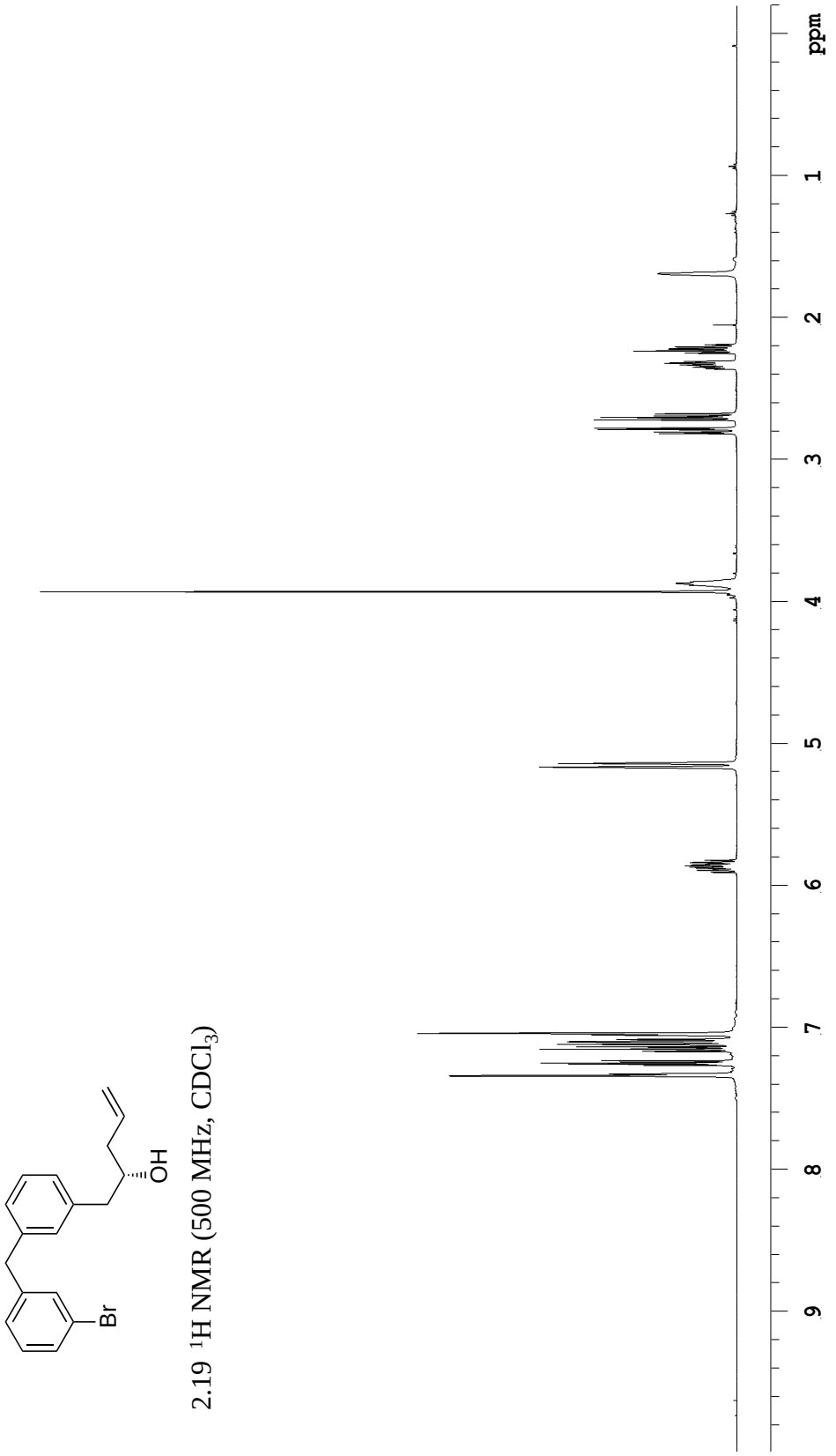


2.18 ^{13}C NMR (125 MHz, $CDCl_3$)

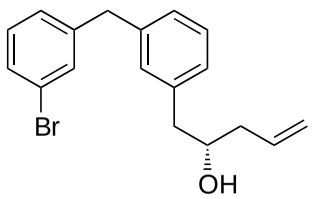
2.18 DEPT NMR (125 MHz, $CDCl_3$)



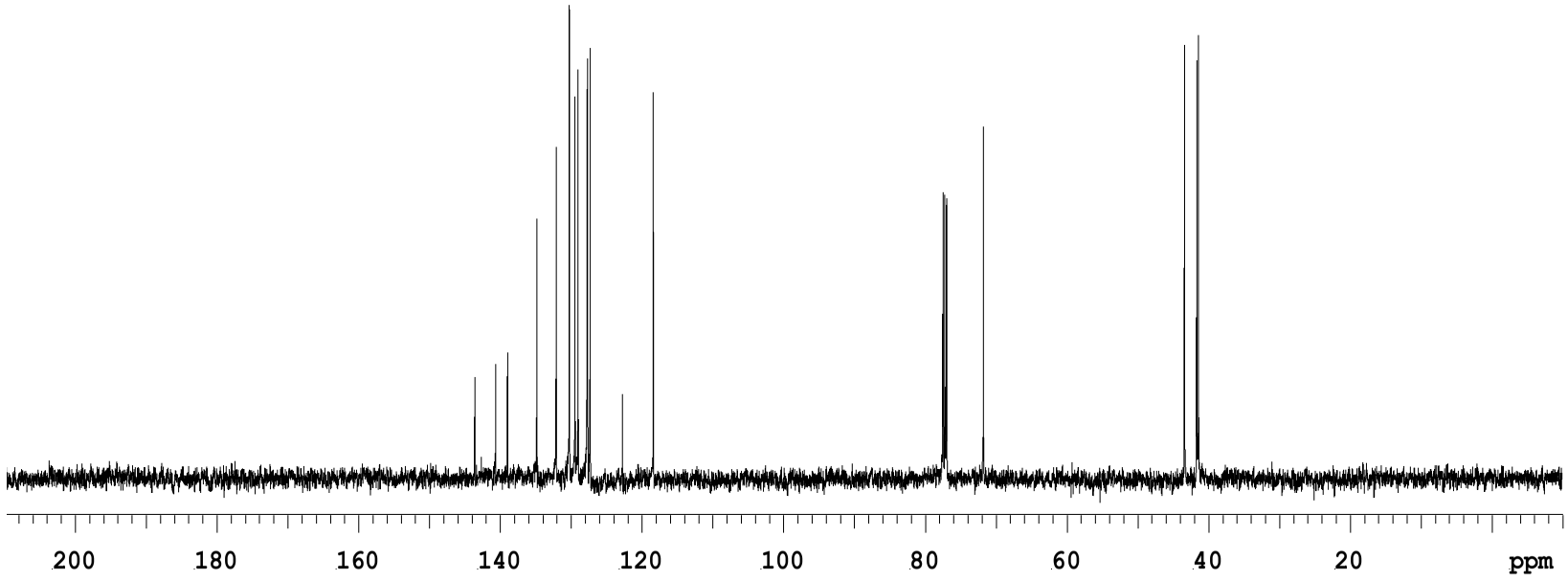




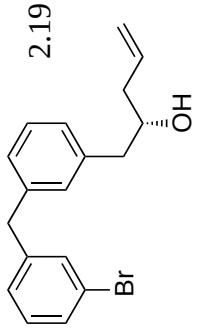
2.19 ^{1}H NMR (500 MHz, $CDCl_3$)

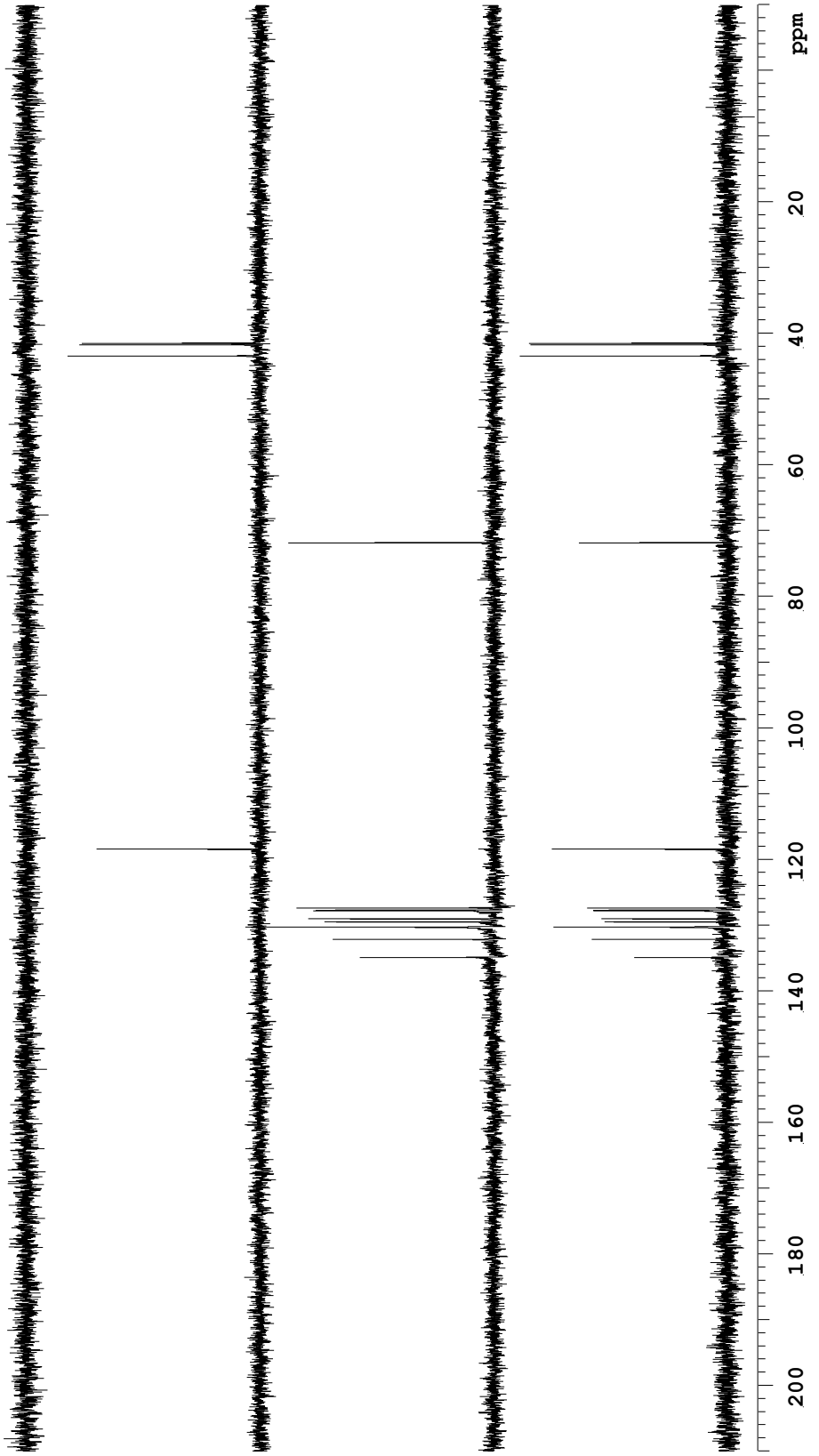


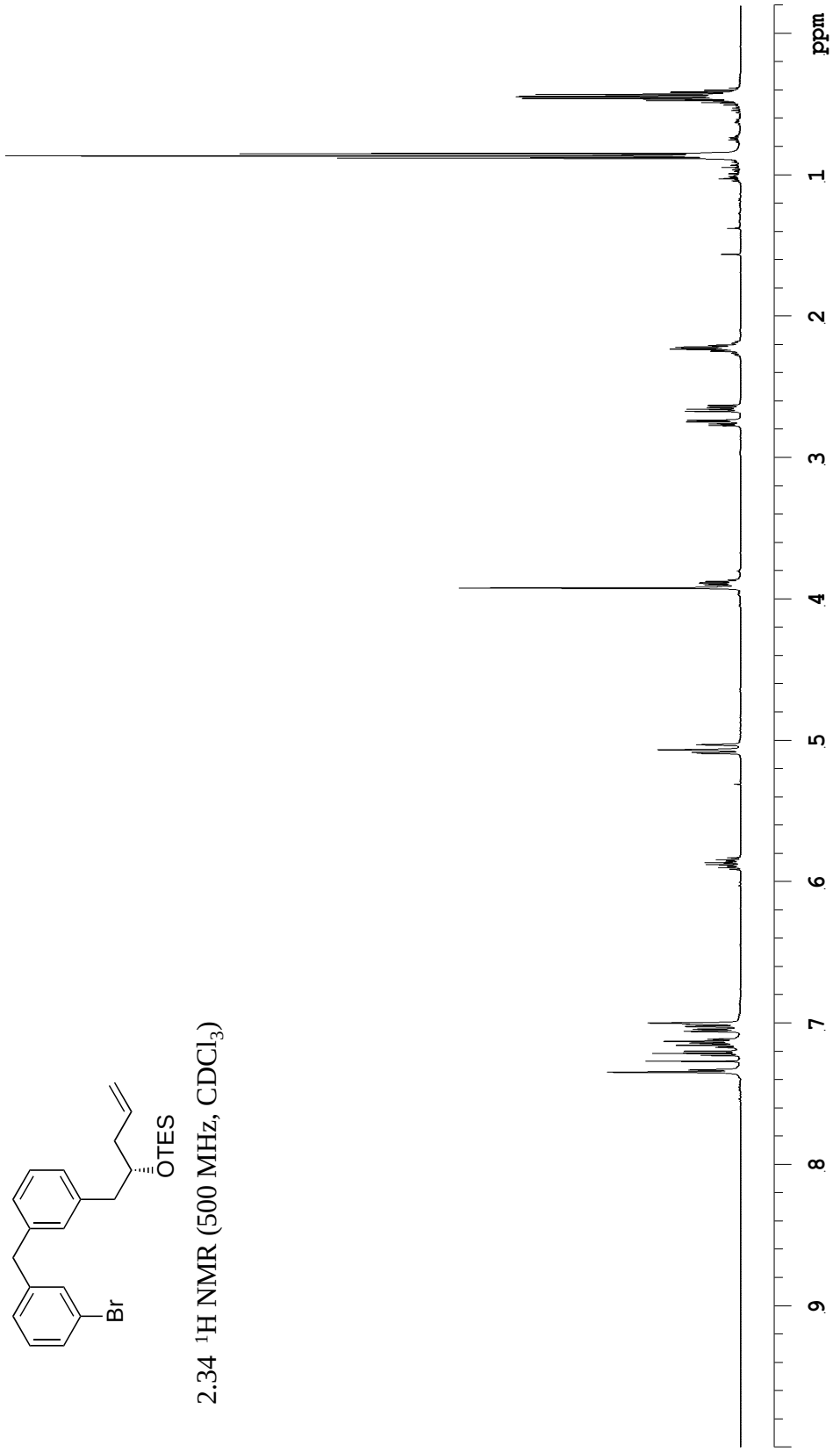
2.19 ^{13}C NMR (125 MHz, $CDCl_3$)



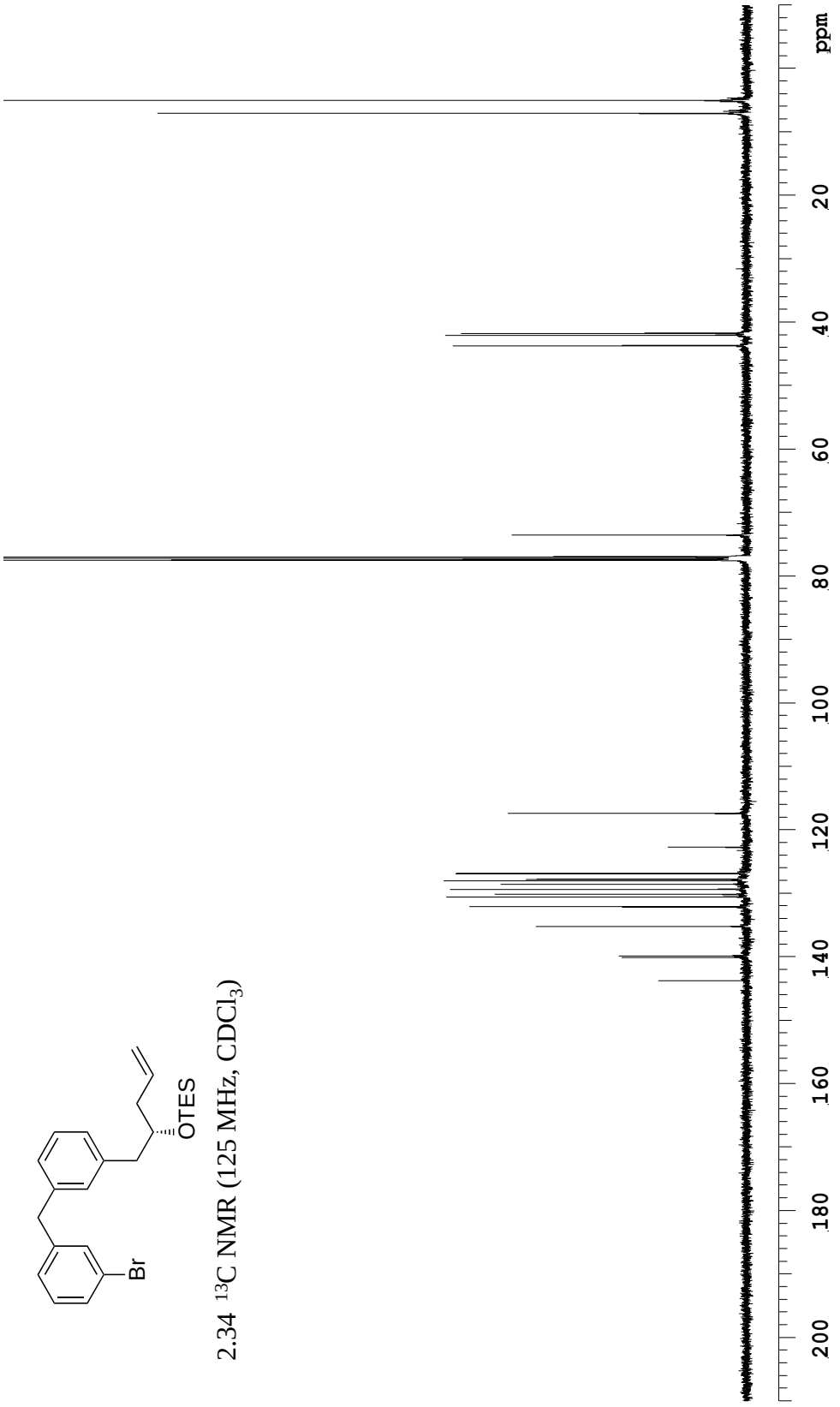
2.19 DEPT NMR (125 MHz, $CDCl_3$)





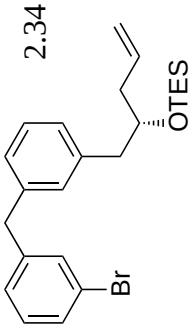


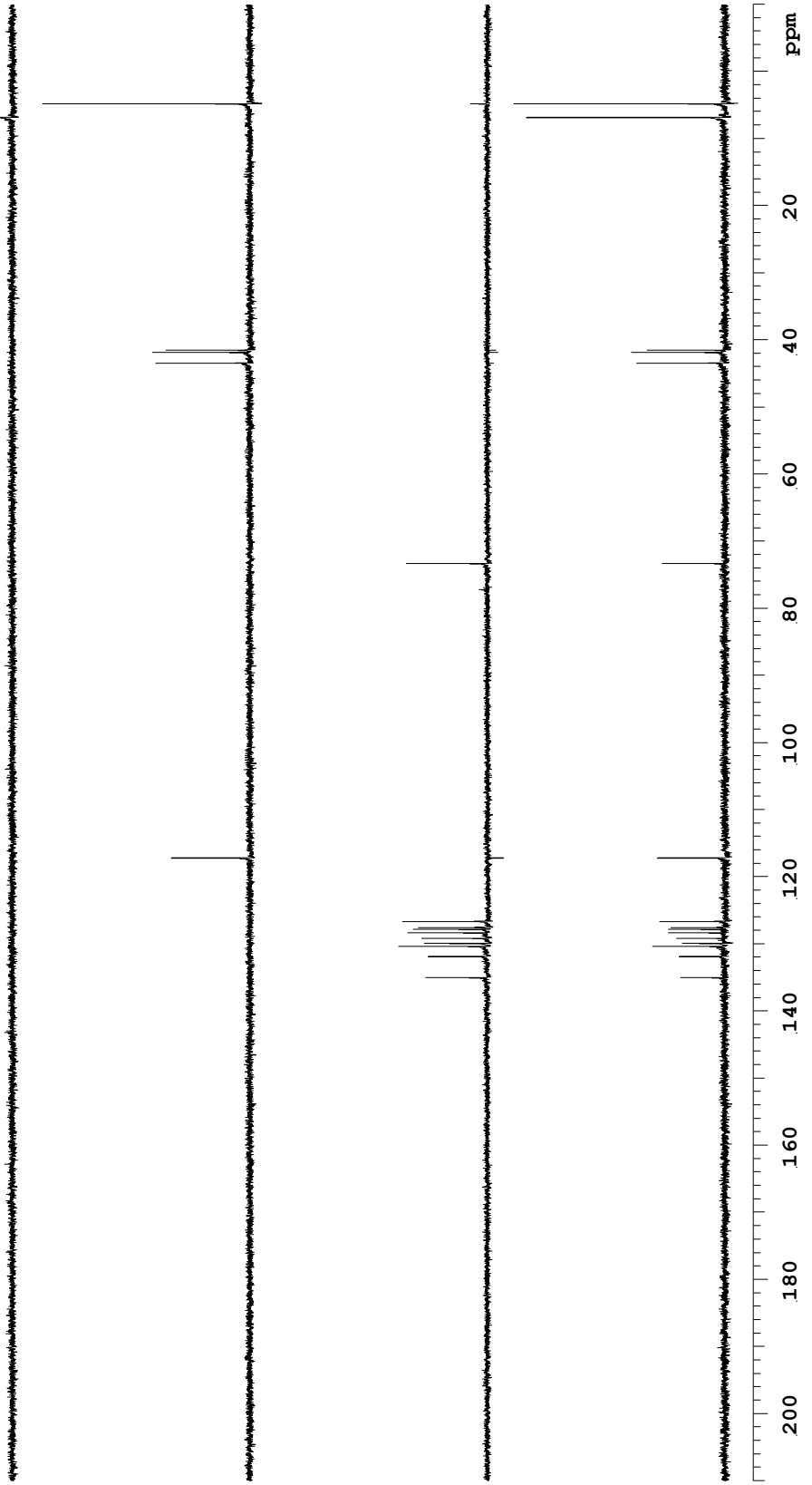
2.34 ^{1}H NMR (500 MHz, $CDCl_3$)

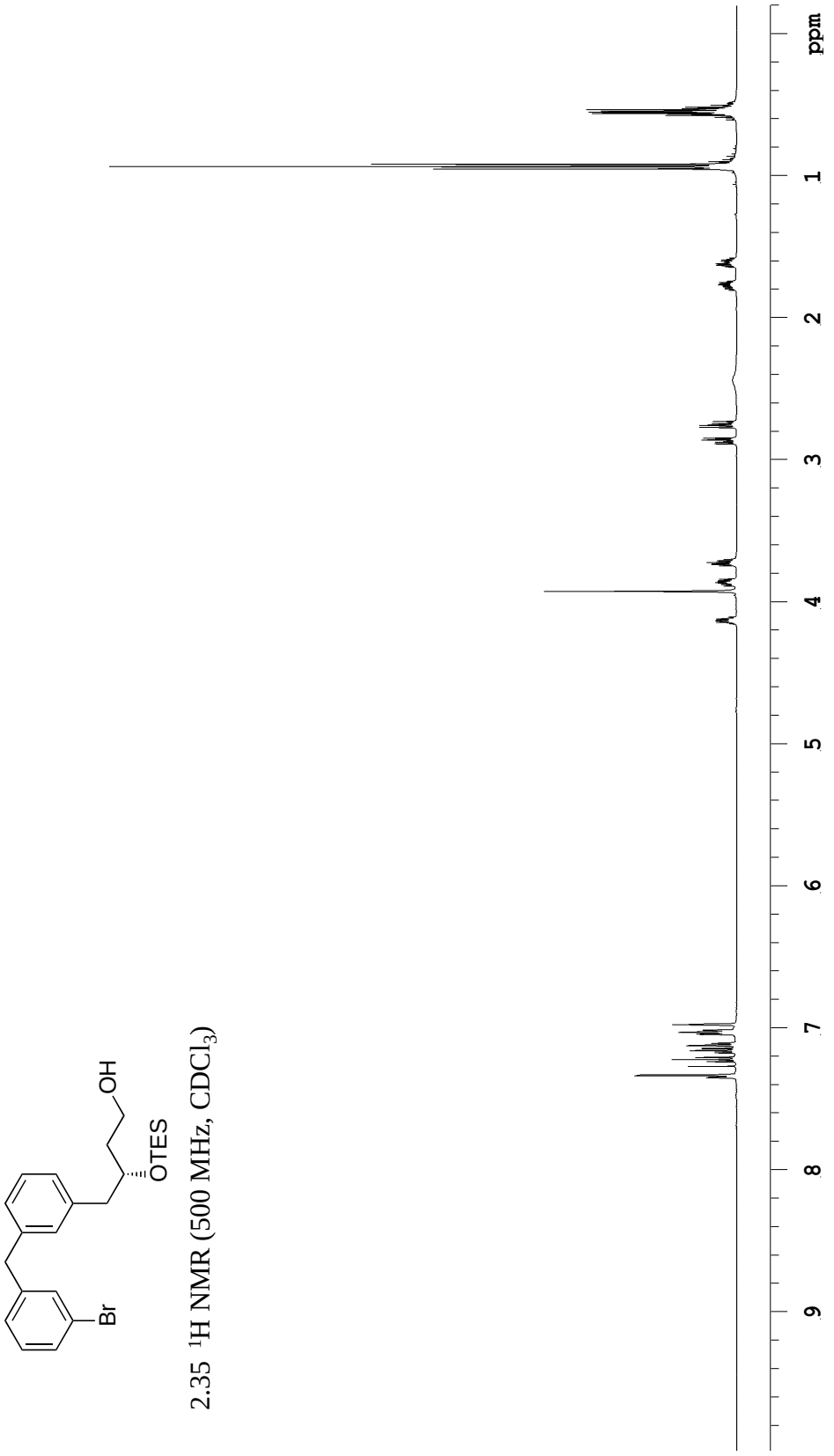


2.34 ^{13}C NMR (125 MHz, $CDCl_3$)

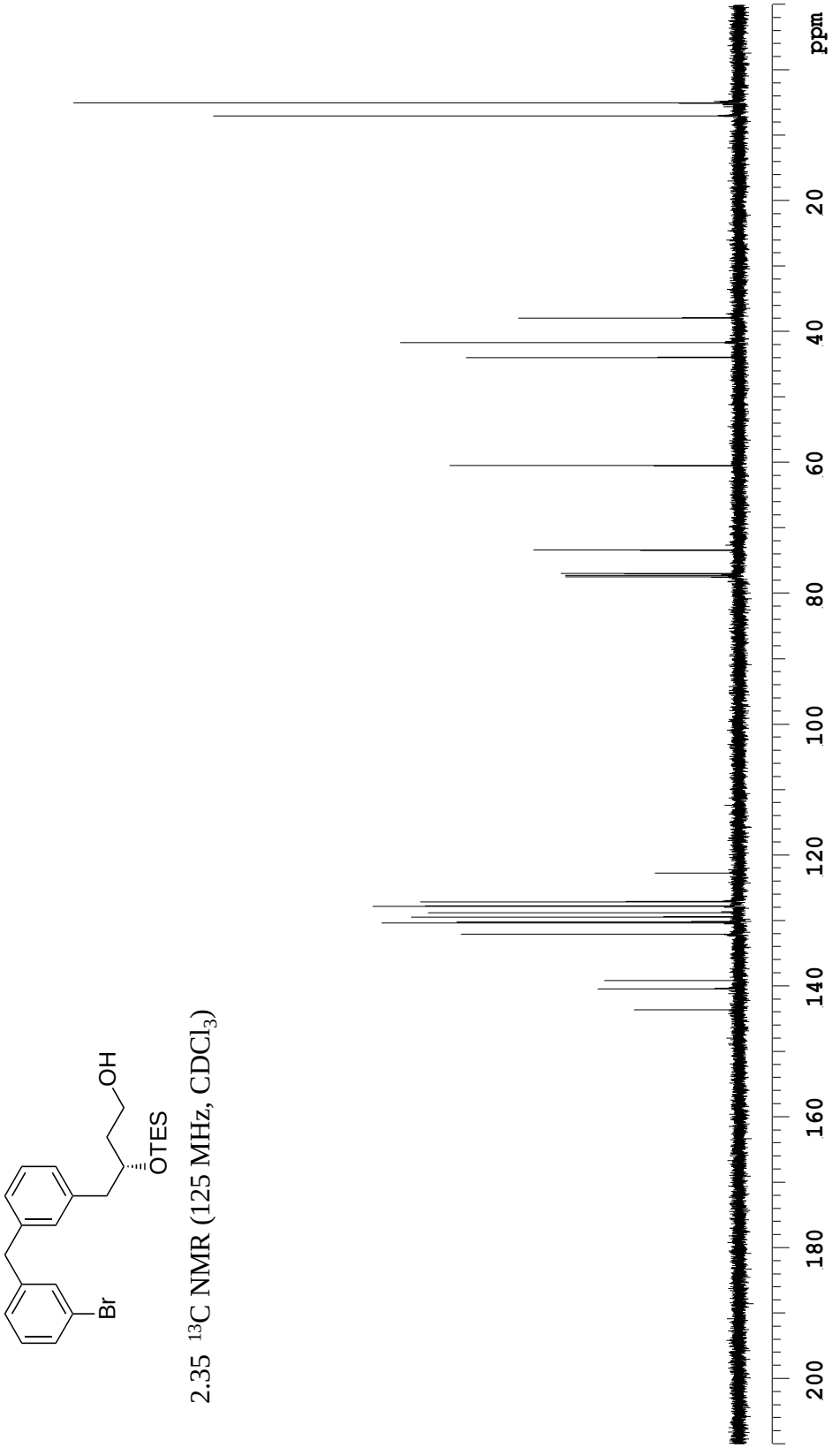
2.34 DEPT NMR (125 MHz, $CDCl_3$)



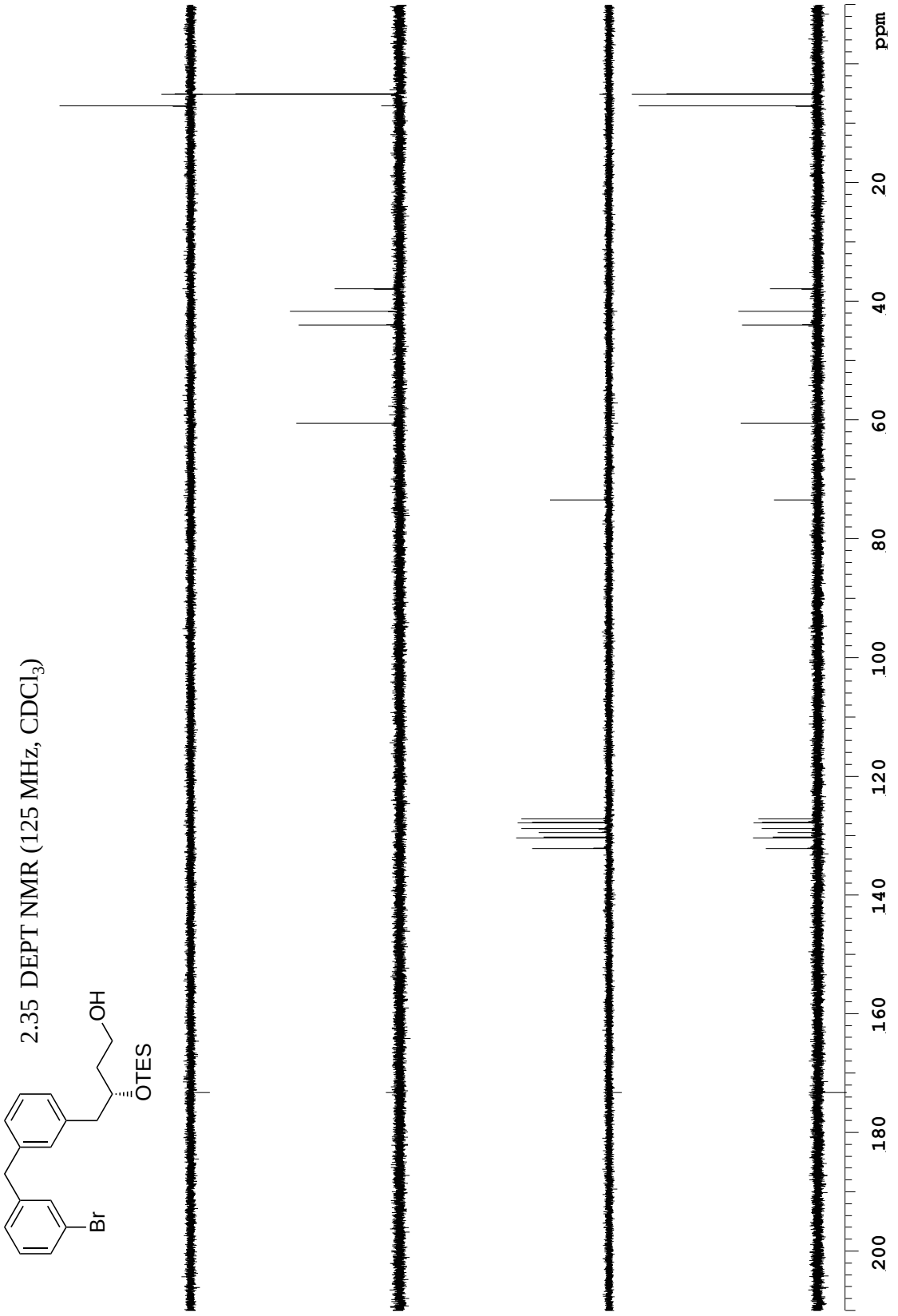


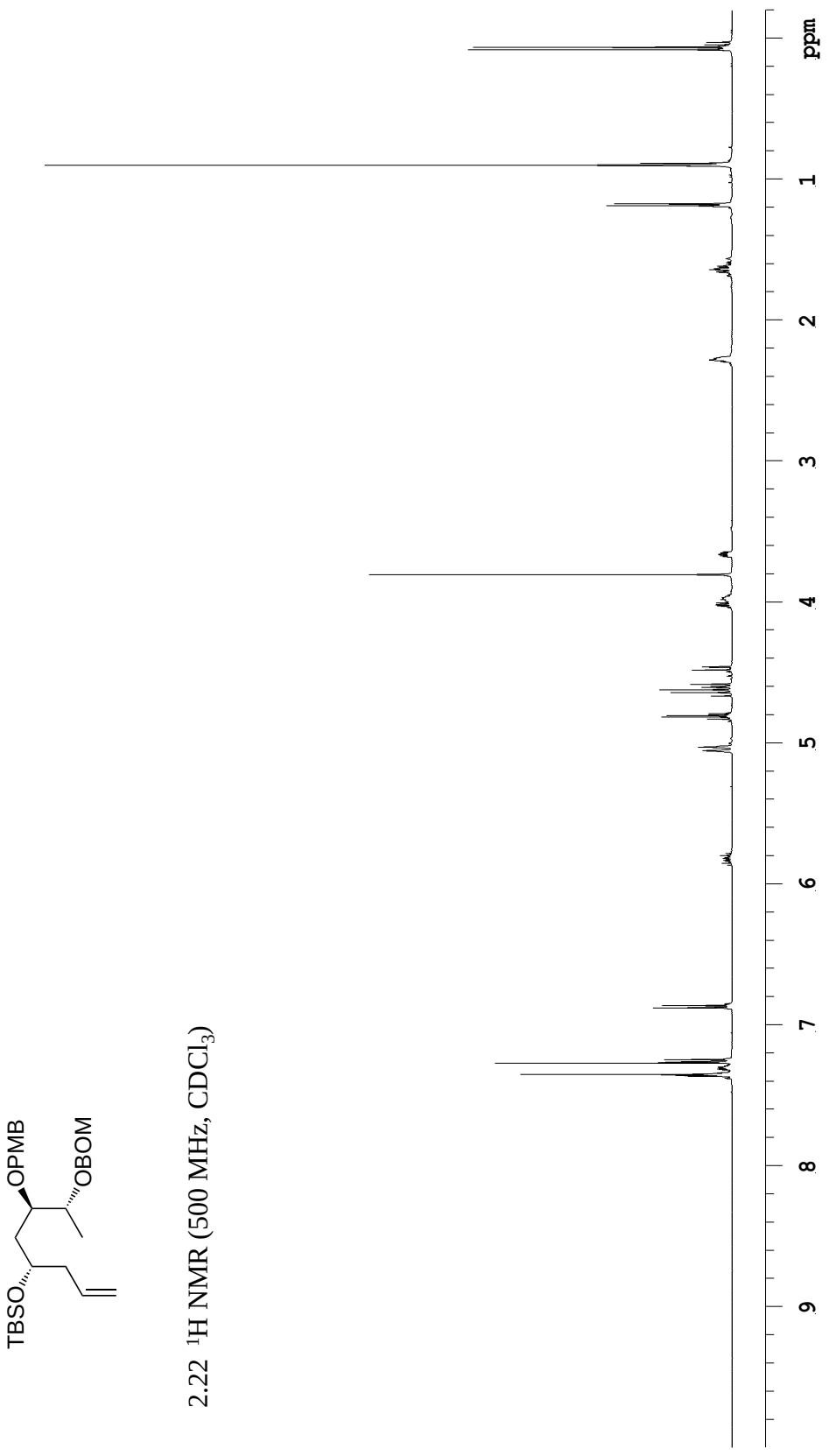


2.35 ^{1}H NMR (500 MHz, $CDCl_3$)

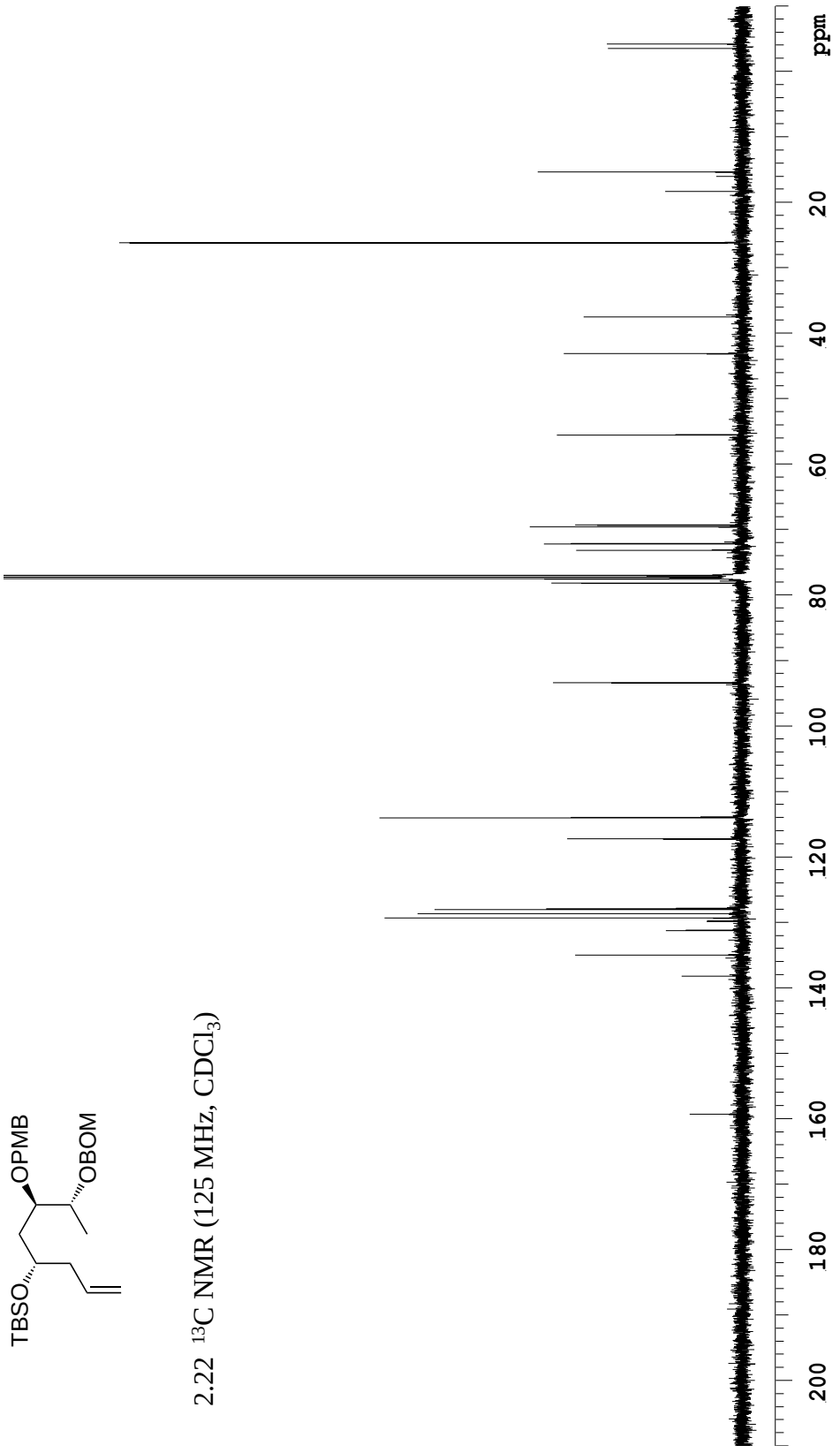


2.35 ^{13}C NMR (125 MHz, $CDCl_3$)

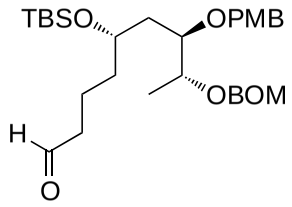

2.35 DEPT NMR (125 MHz, $CDCl_3$)
OH
OTES
Br
200
180
160
140
120
100
80
60
40
20
ppm



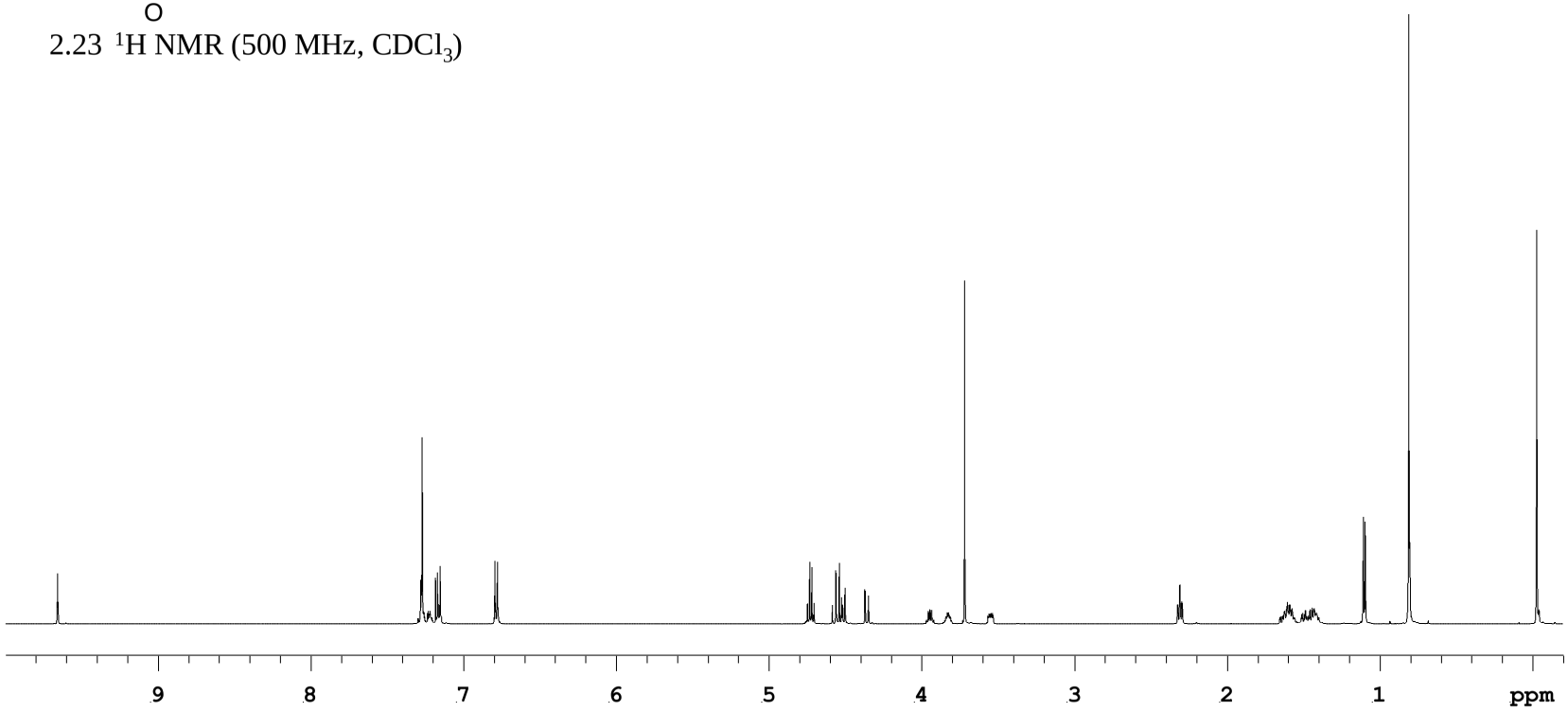
2.22 ^{1}H NMR (500 MHz, $CDCl_3$)

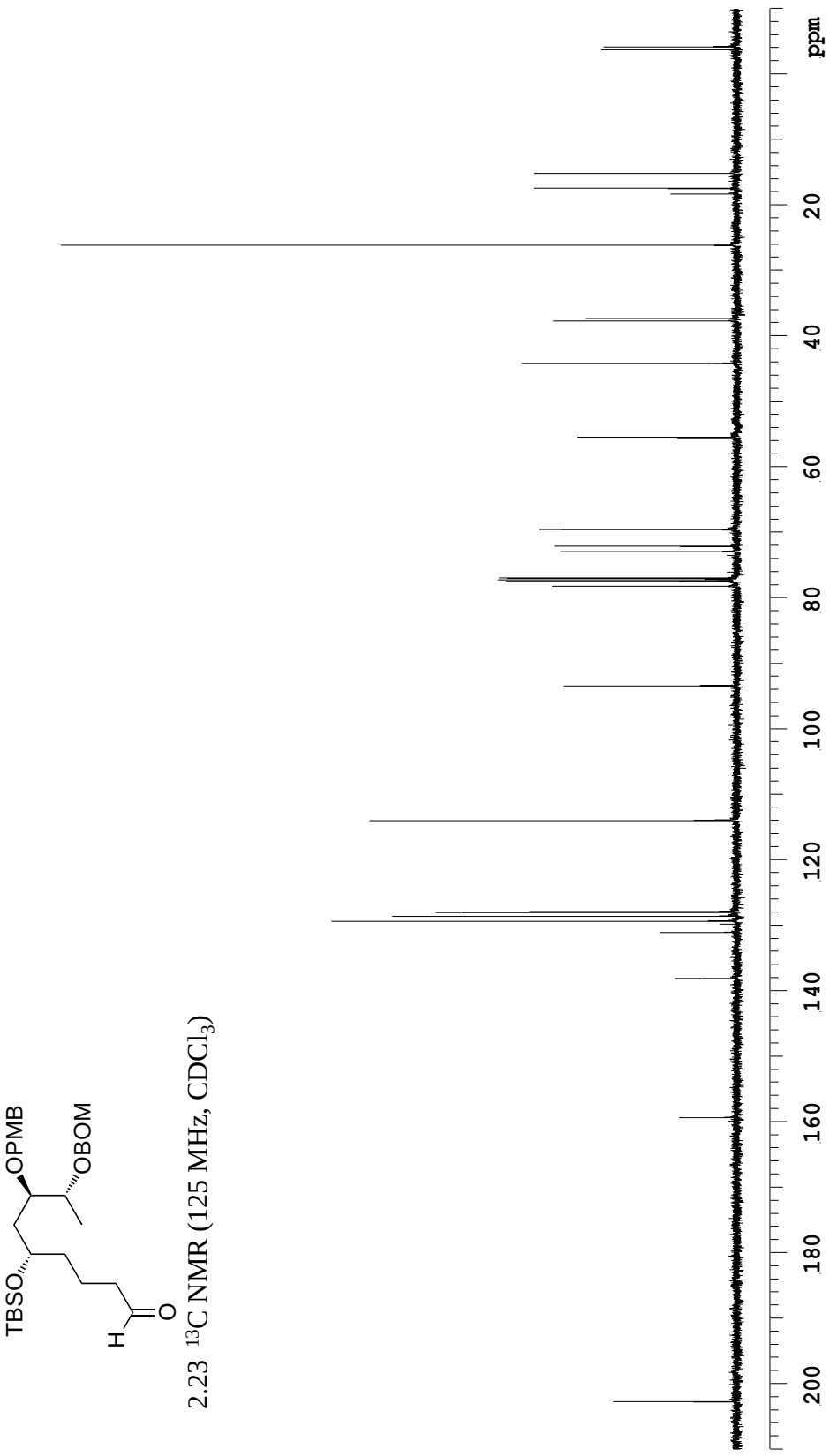


2.22 ^{13}C NMR (125 MHz, $CDCl_3$)

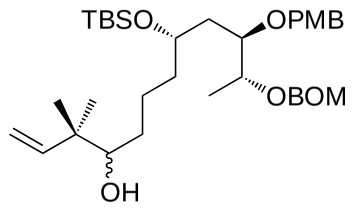


2.23 ^{1}H NMR (500 MHz, $CDCl_3$)

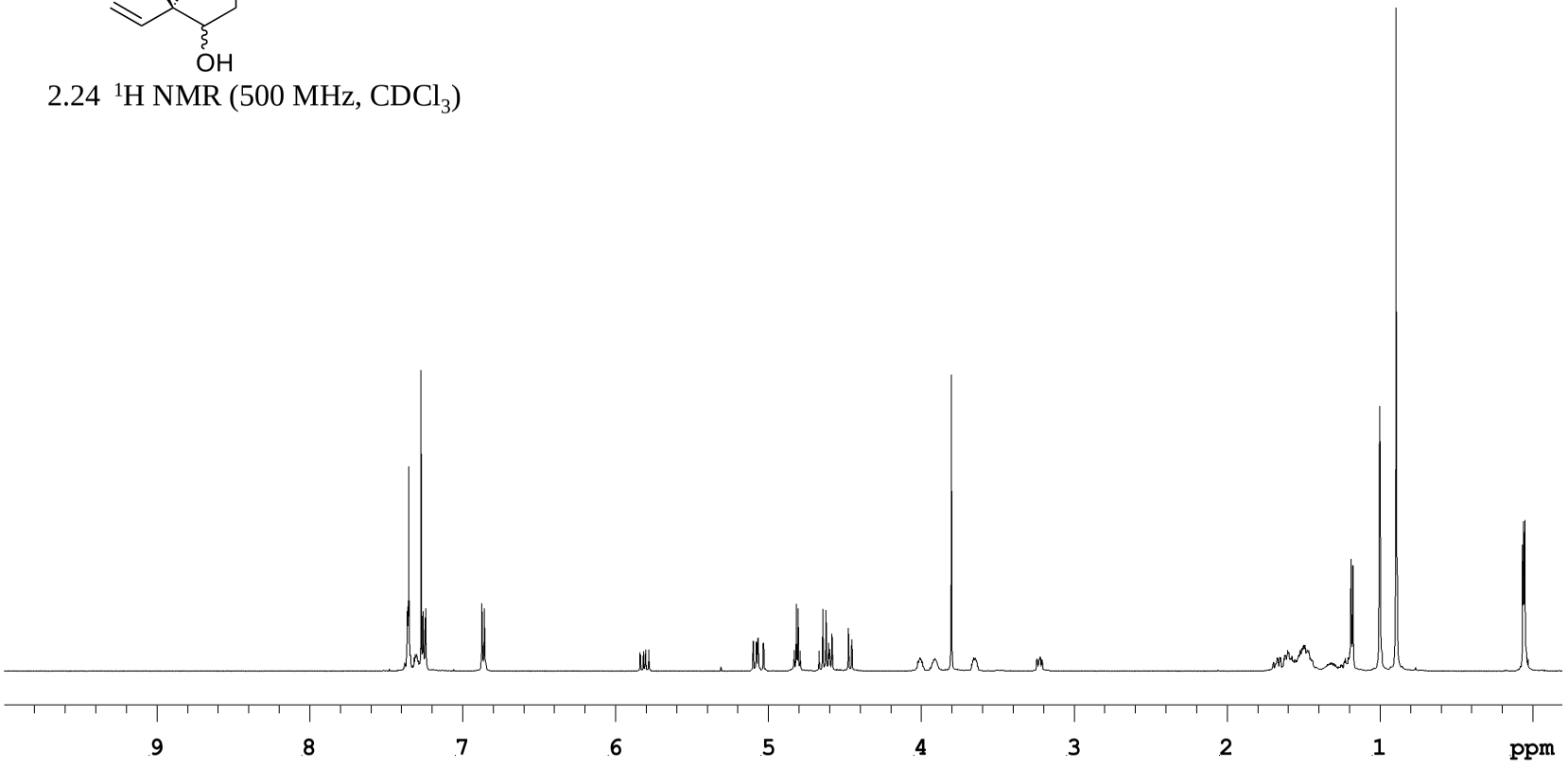


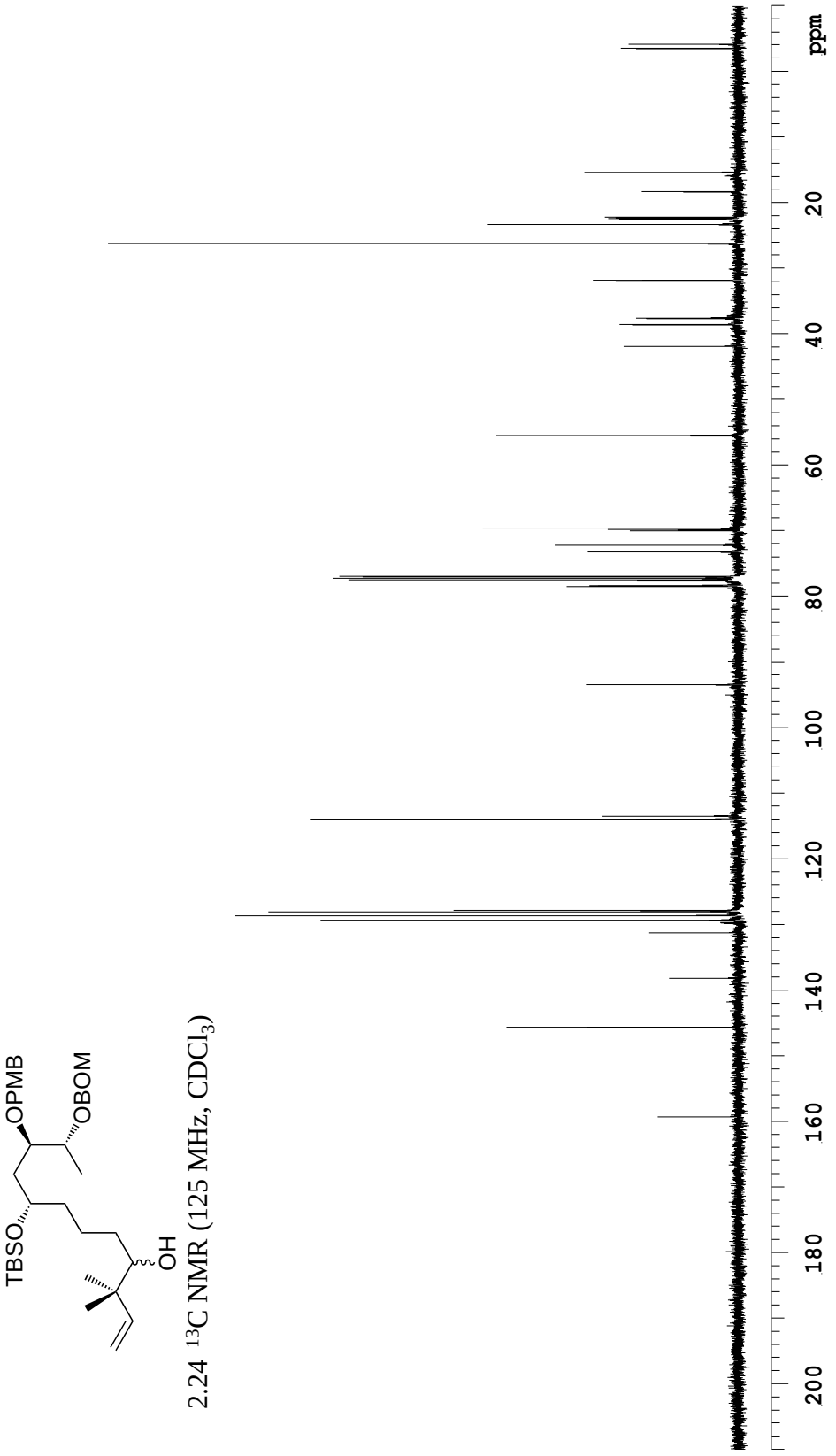


2.23 ^{13}C NMR (125 MHz, $CDCl_3$)

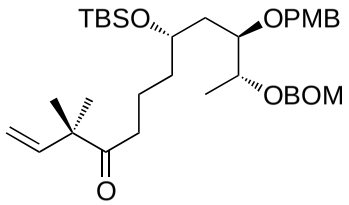


2.24 ^{1}H NMR (500 MHz, $CDCl_3$)

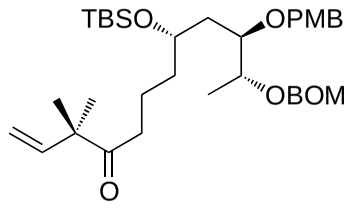




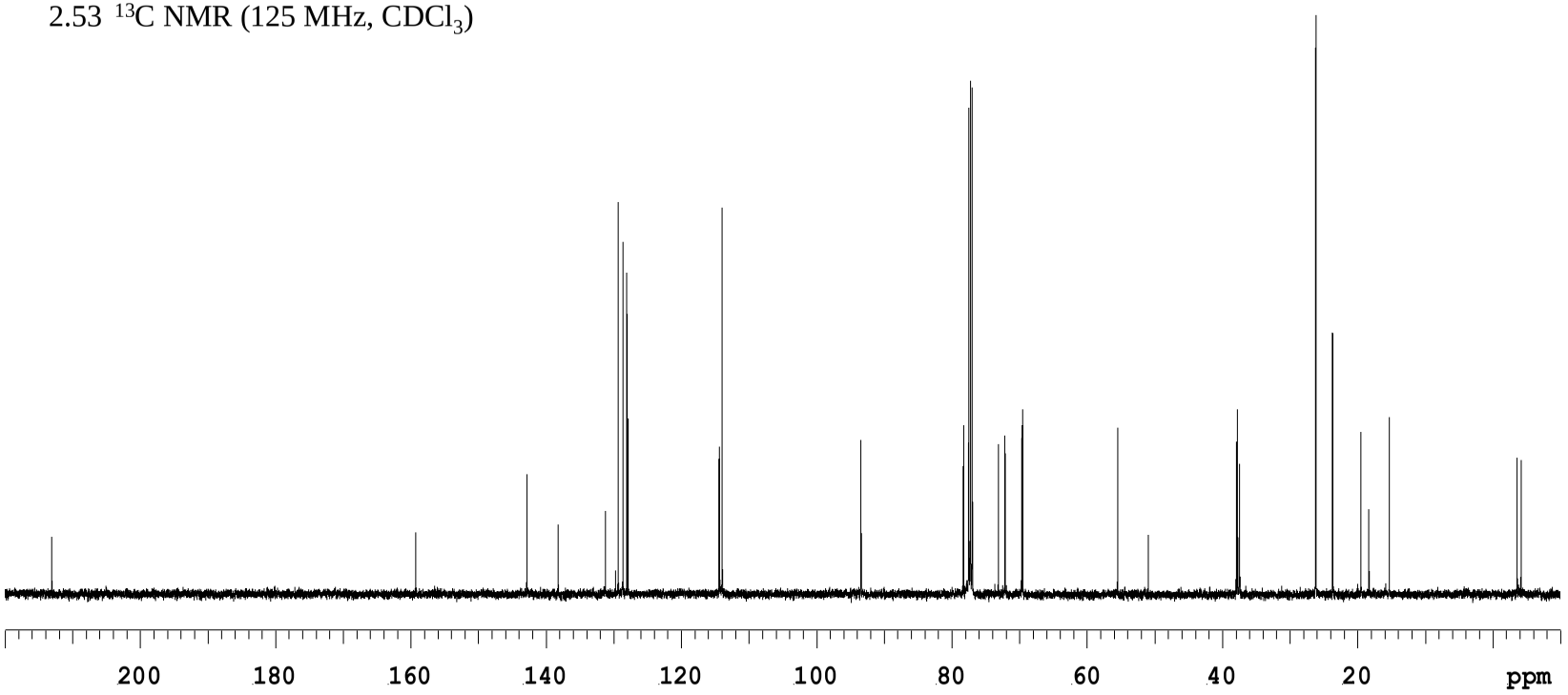
2.24 ^{13}C NMR (125 MHz, $CDCl_3$)

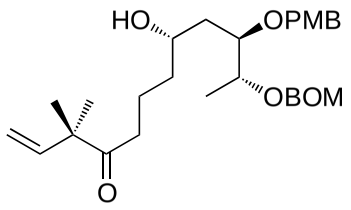


2.53 ^{1}H NMR (500 MHz, $CDCl_3$)

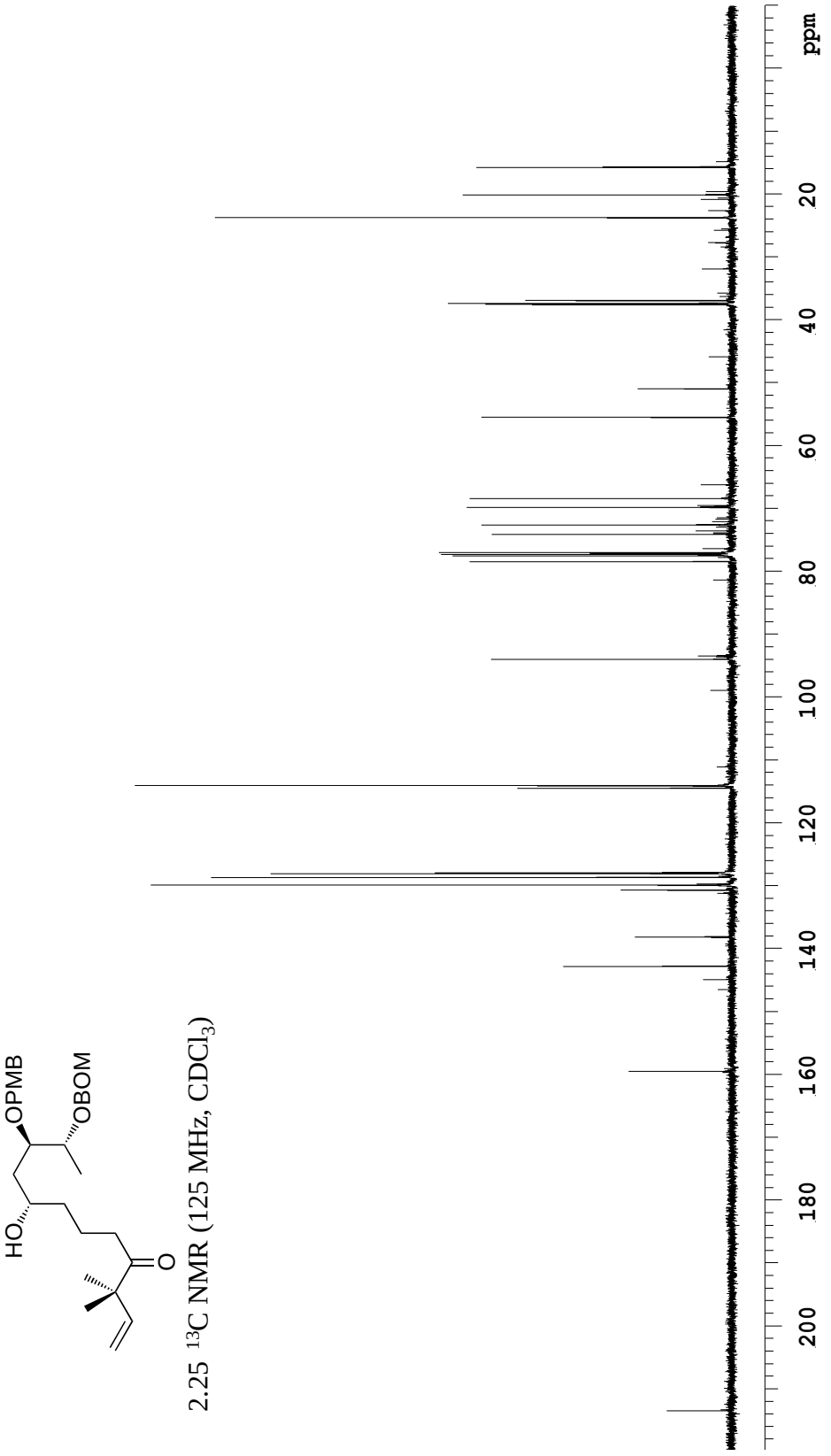


2.53 ^{13}C NMR (125 MHz, $CDCl_3$)

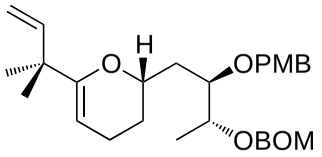




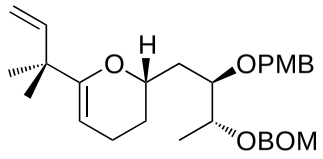
2.25 ^{1}H NMR (500 MHz, $CDCl_3$)



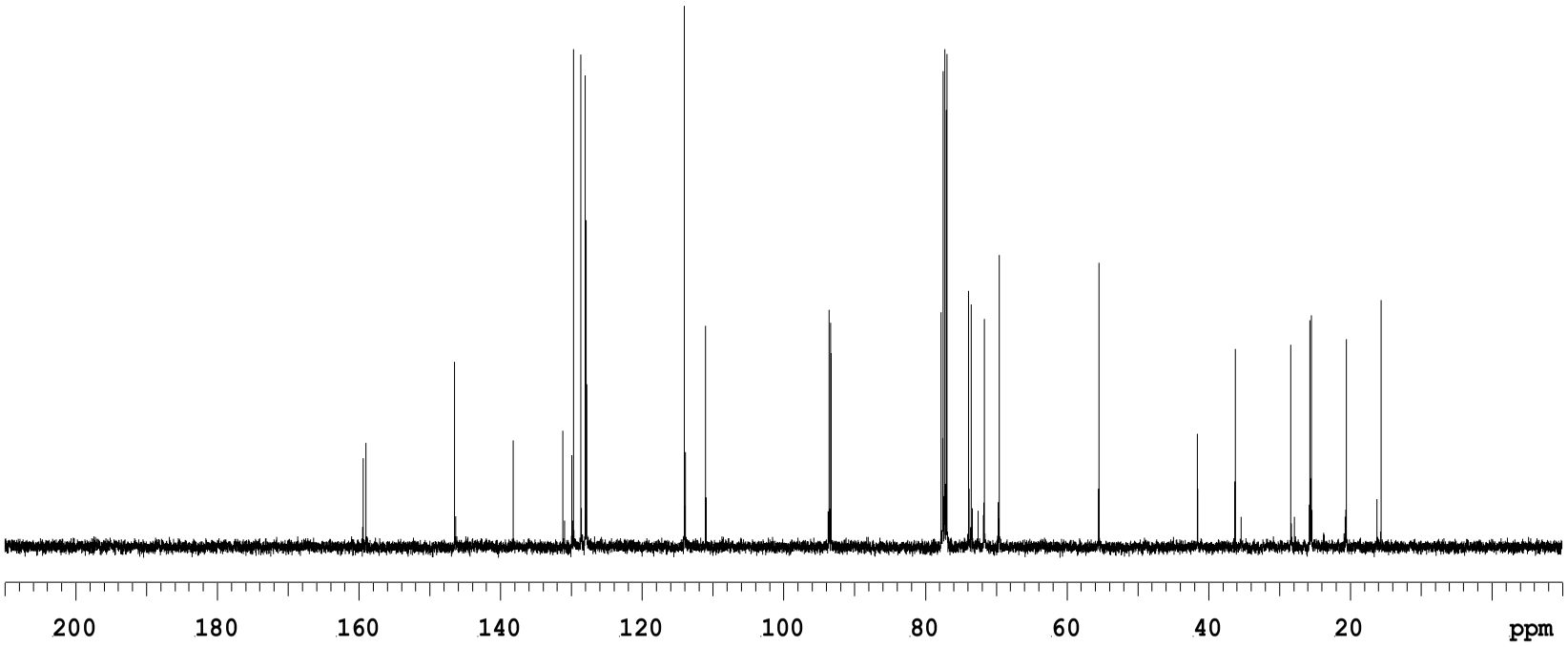
2.25 ^{13}C NMR (125 MHz, $CDCl_3$)

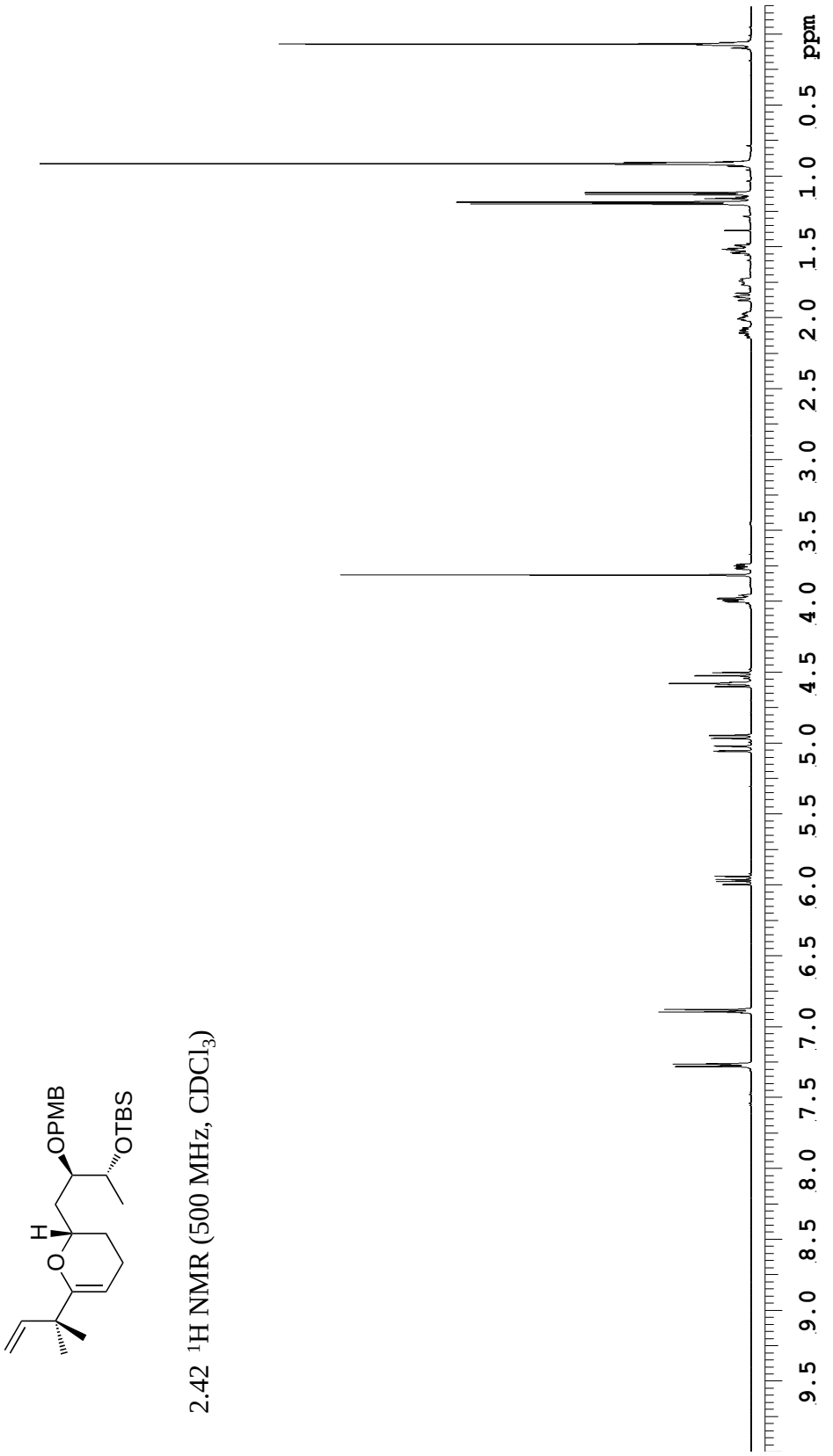


2.13 ^{1}H NMR (500 MHz, $CDCl_3$)

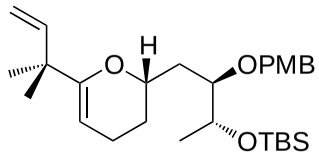


2.13 ^{13}C NMR (125 MHz, $CDCl_3$)

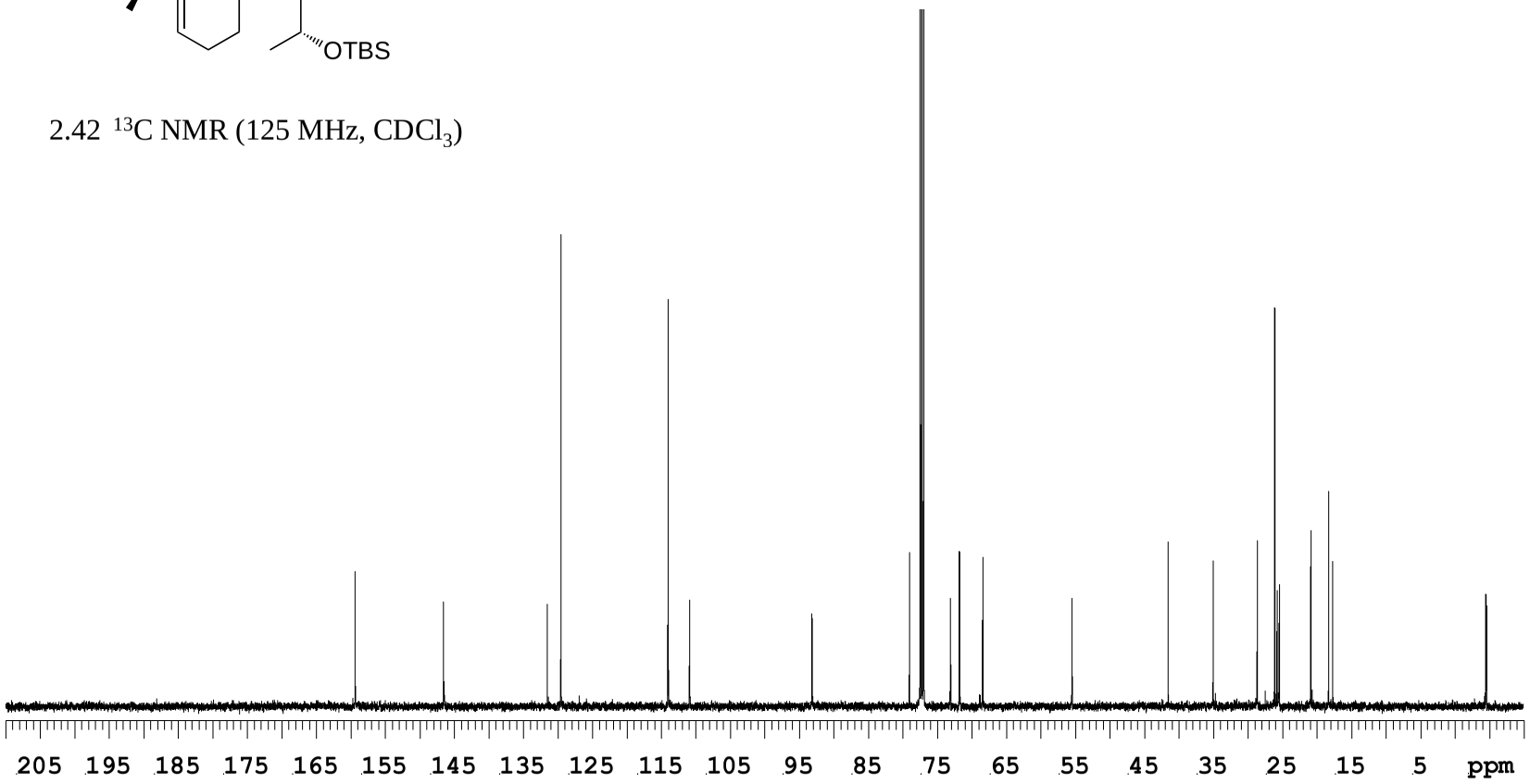


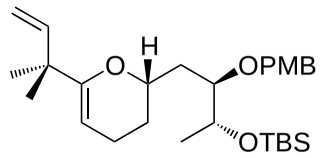


2.42 ^{1}H NMR (500 MHz, $CDCl_3$)

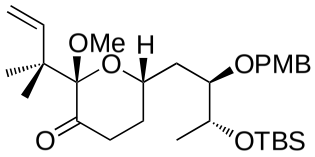


2.42 ^{13}C NMR (125 MHz, $CDCl_3$)

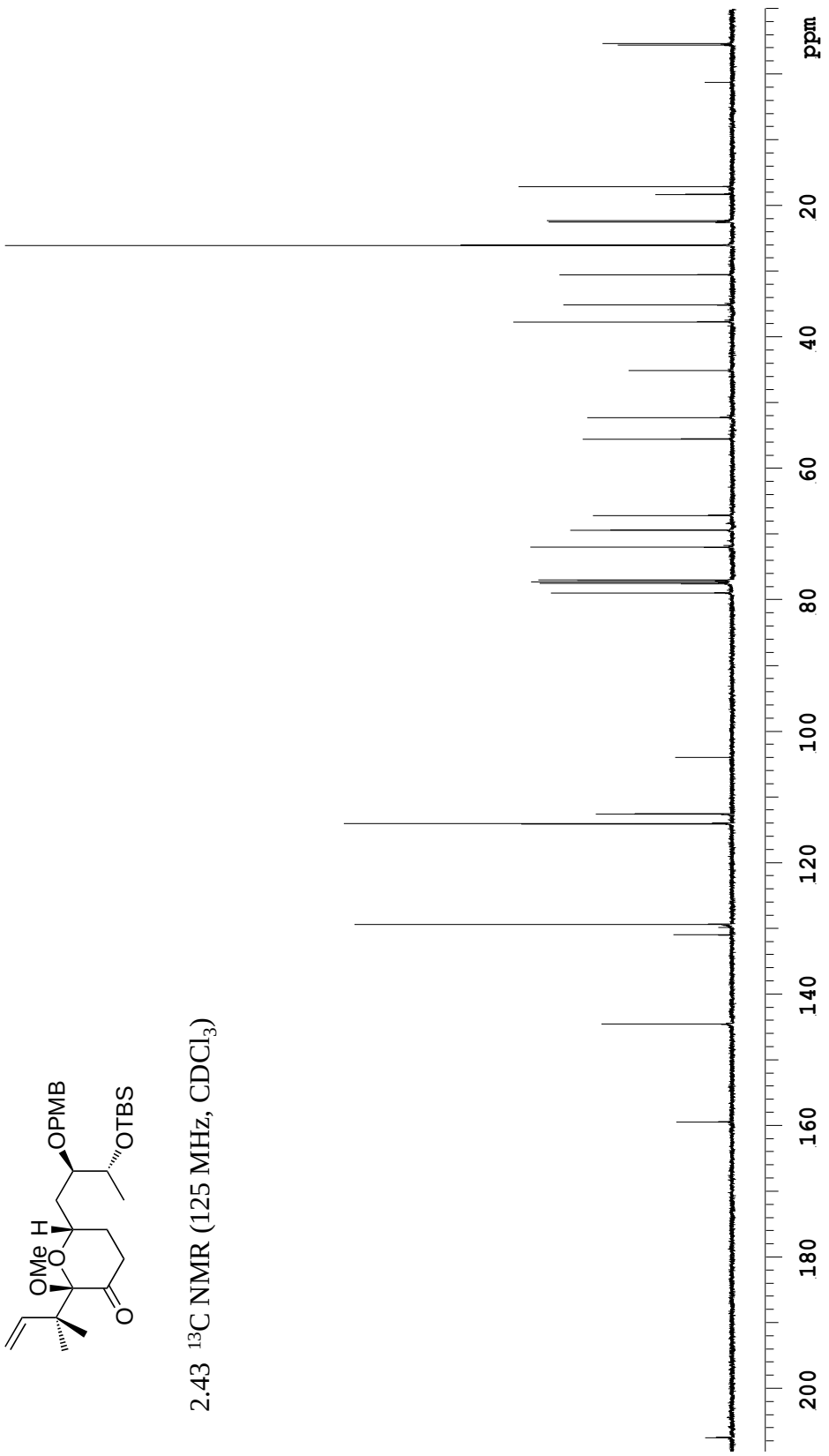




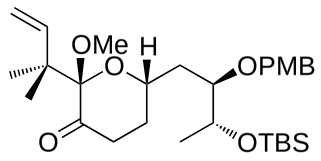
2.42 DEPT NMR (125 MHz, $CDCl_3$)



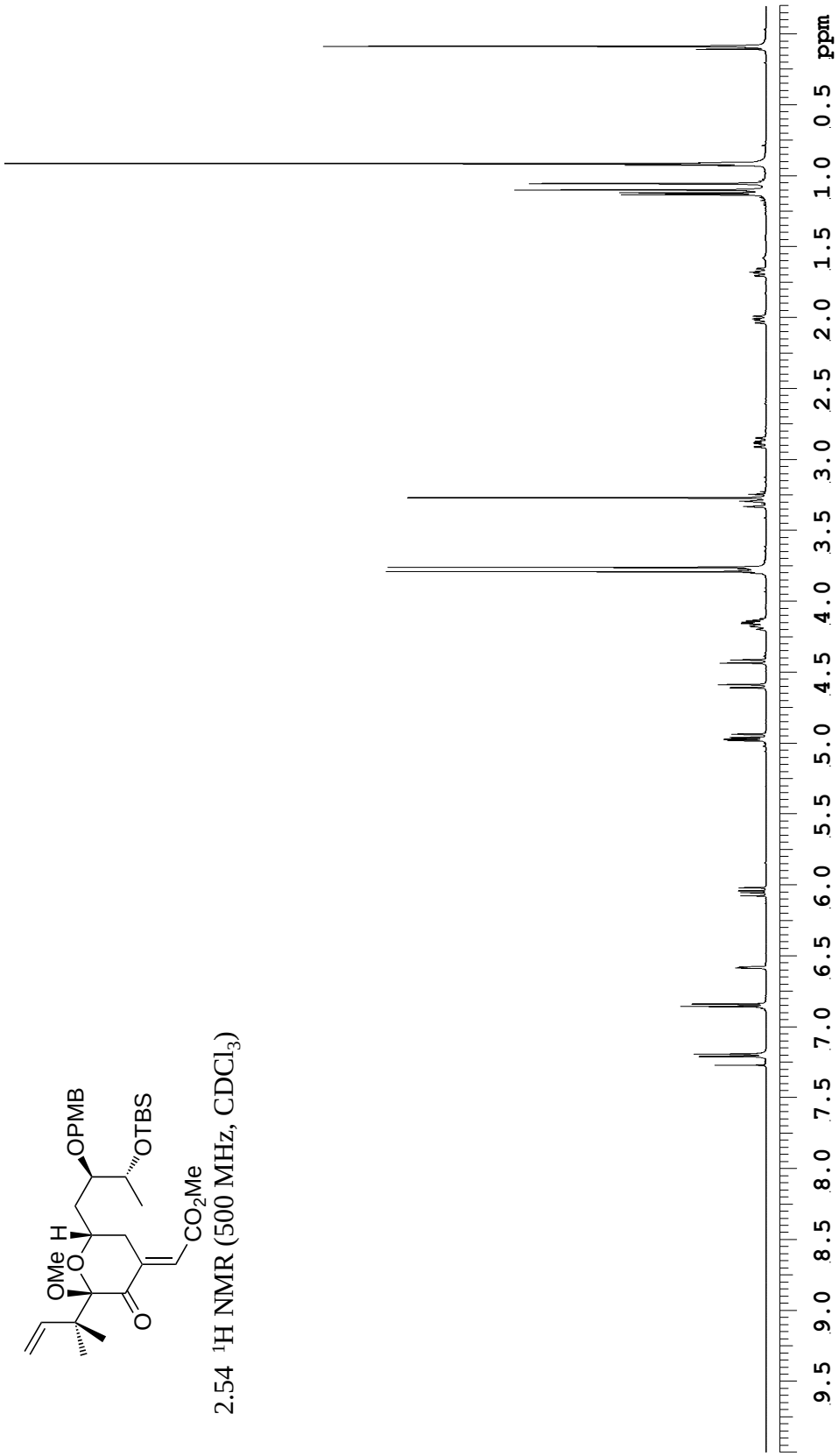
2.43 ^{1}H NMR (500 MHz, $CDCl_3$)



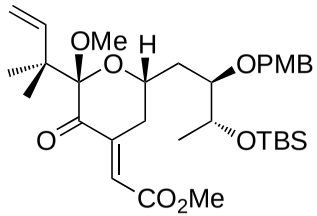
2.43 ^{13}C NMR (125 MHz, $CDCl_3$)



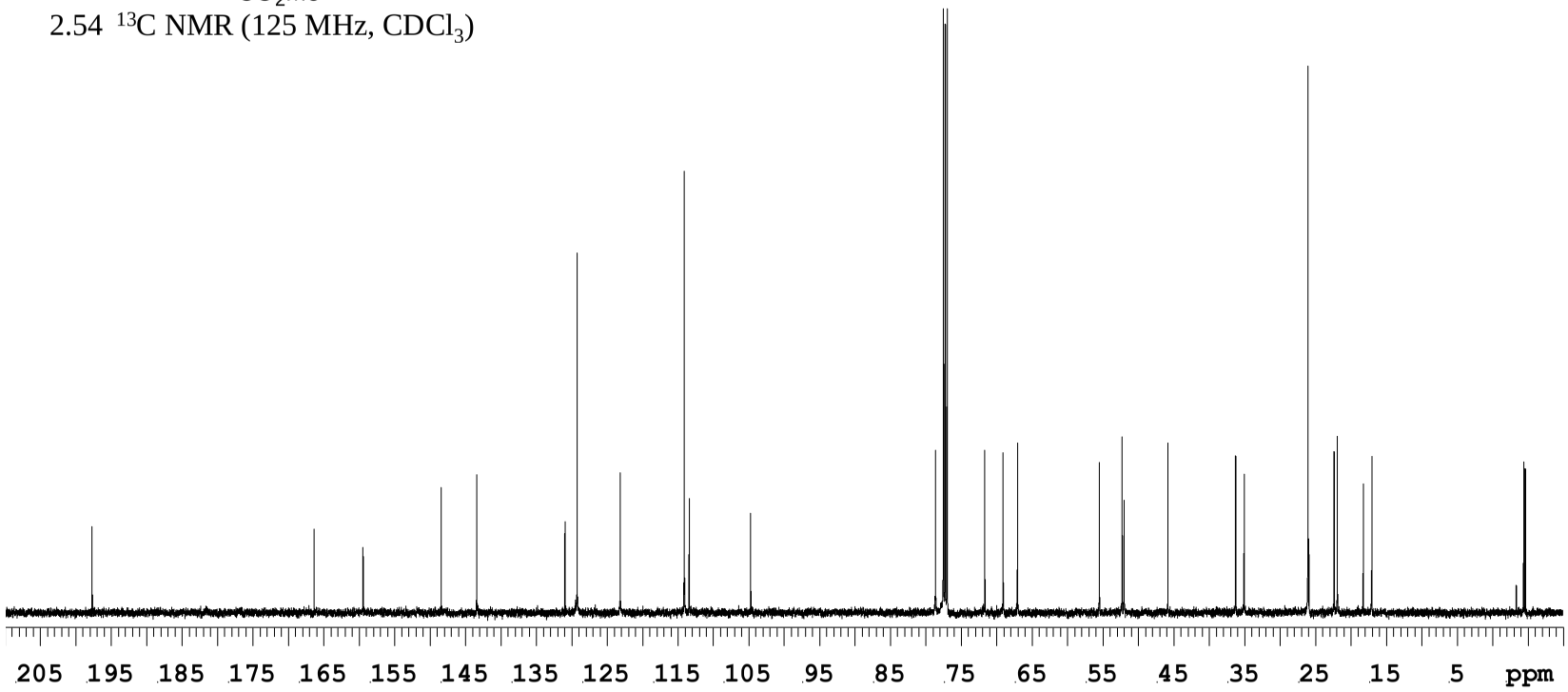
2.43 DEPT NMR (125 MHz, $CDCl_3$)

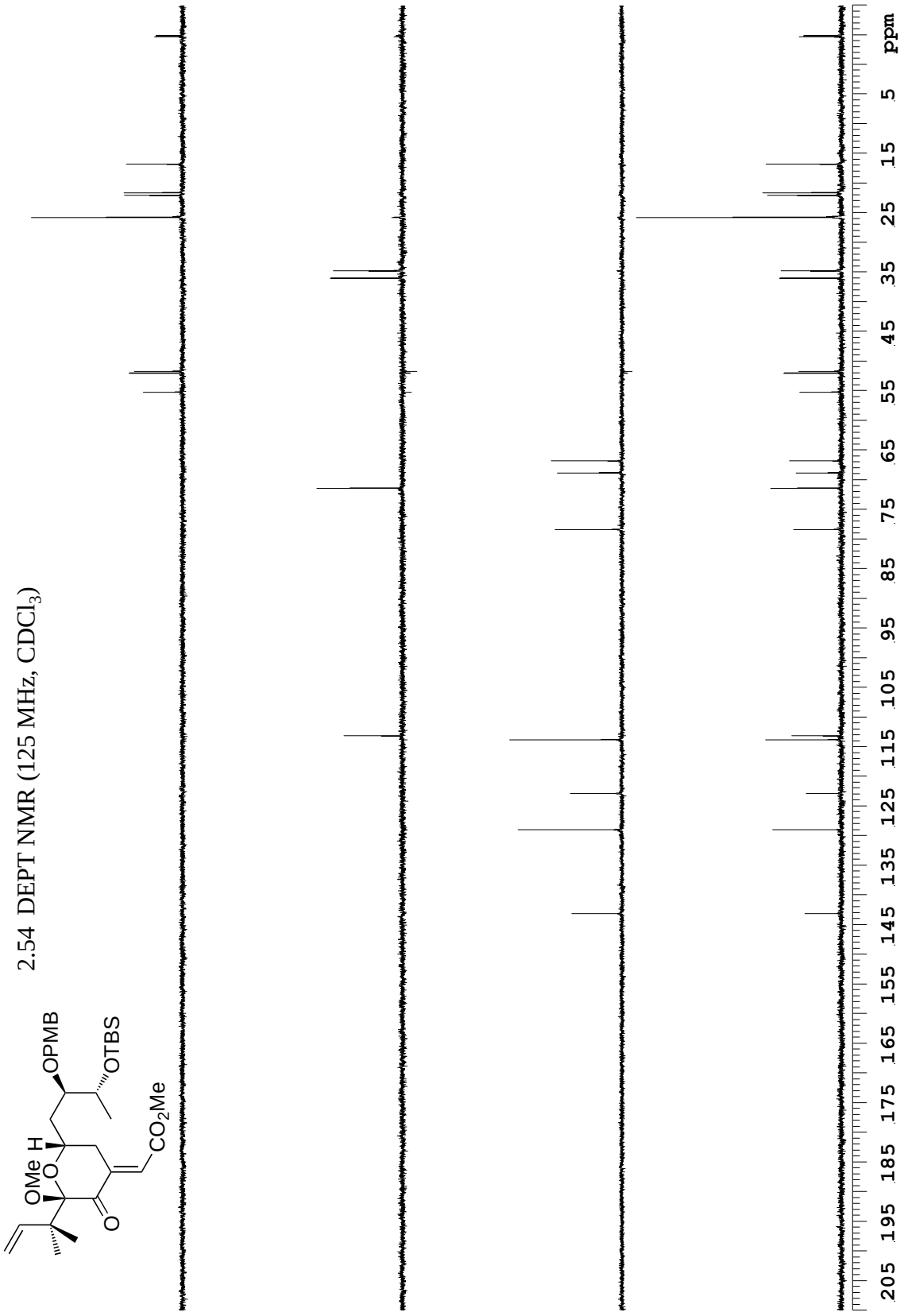


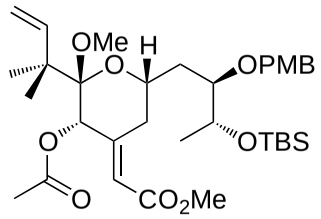
2.54 ^{1}H NMR (500 MHz, $CDCl_3$)



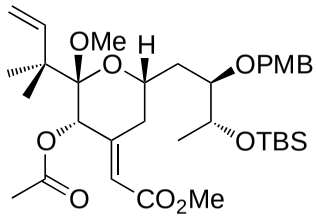
2.54 ^{13}C NMR (125 MHz, $CDCl_3$)



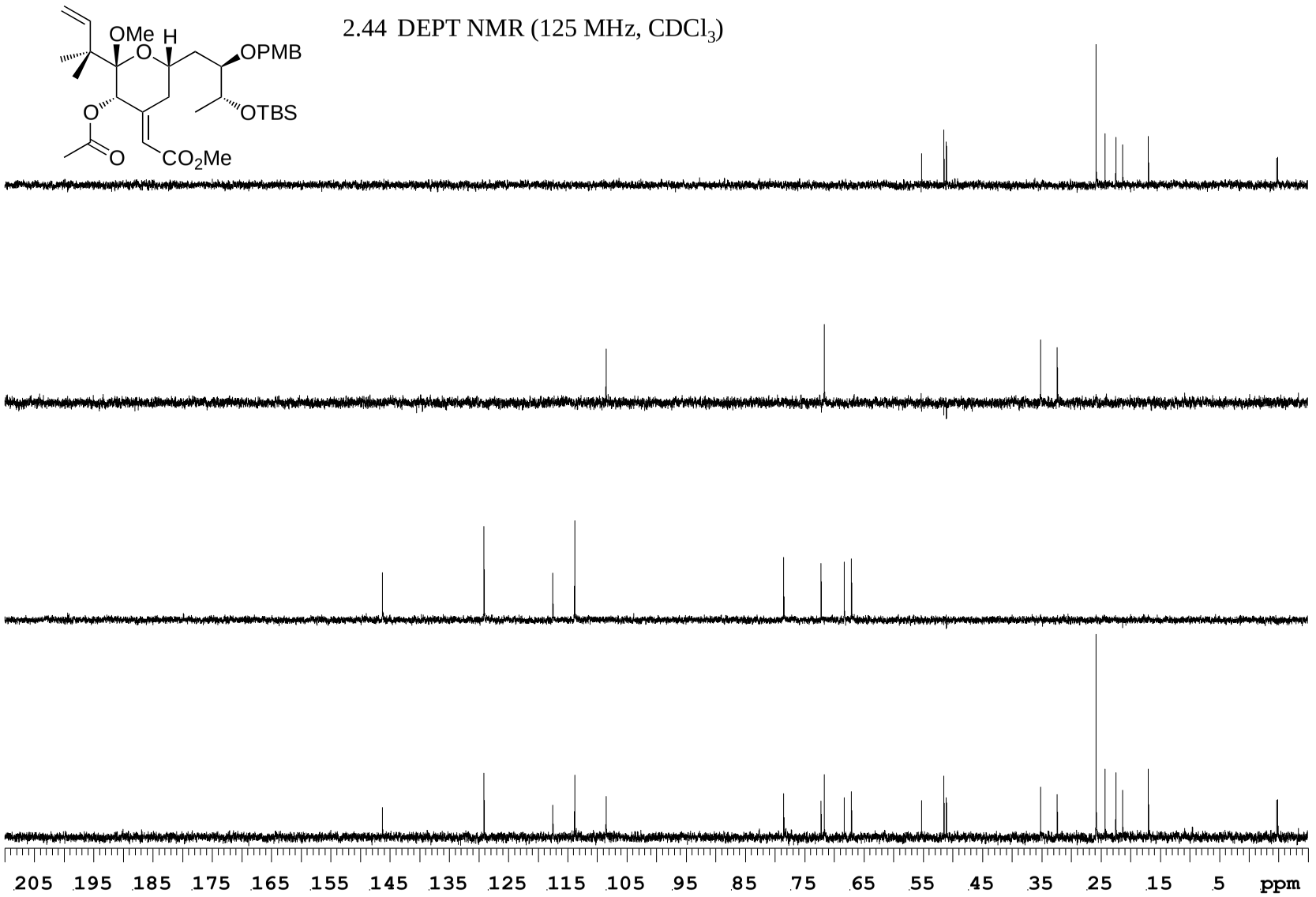

2.54 DEPT NMR (125 MHz, CDCl3)
OMe H
O
OPMB
OTBS
CO2Me
O
205 195 185 175 165 155 145 135 125 115 105 95 85 75 65 55 45 35 25 15 5
ppm



2.44 ^{1}H NMR (500 MHz, $CDCl_3$)

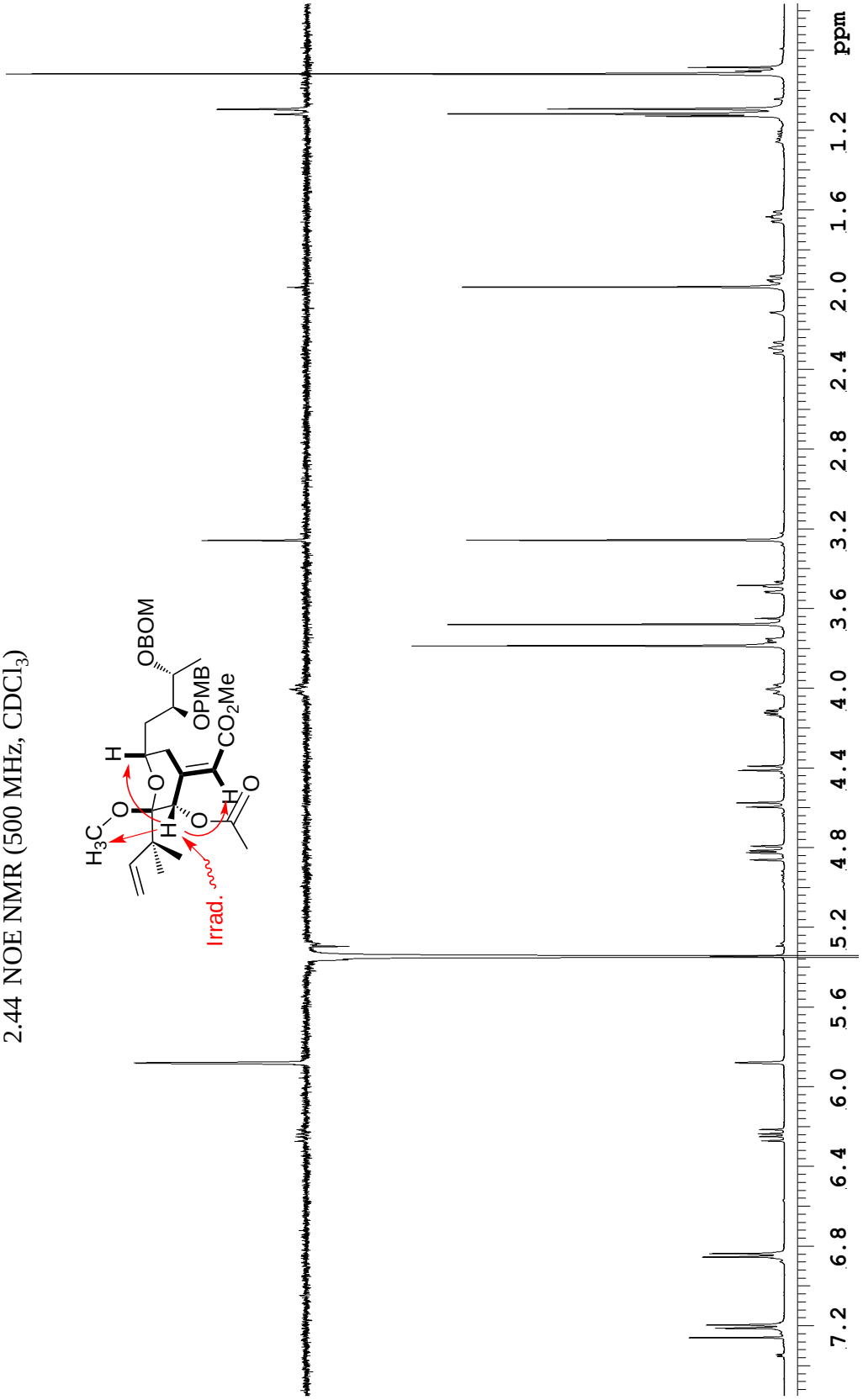


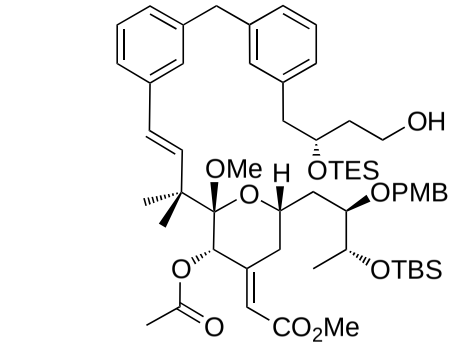
2.44 ^{13}C NMR (125 MHz, $CDCl_3$)



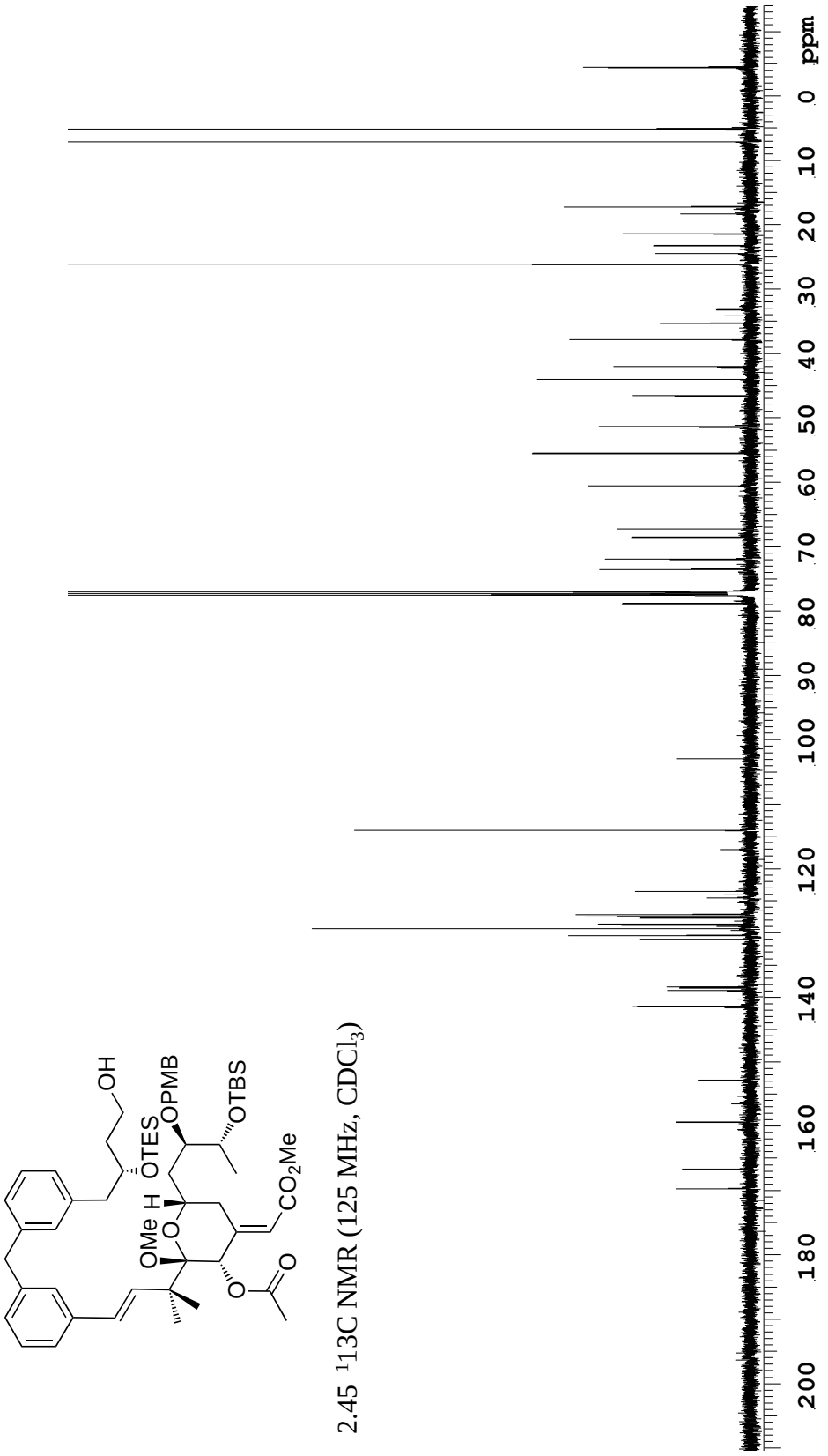
2.44 DEPT NMR (125 MHz, $CDCl_3$)

2.44 NOE NMR (500 MHz, $CDCl_3$)



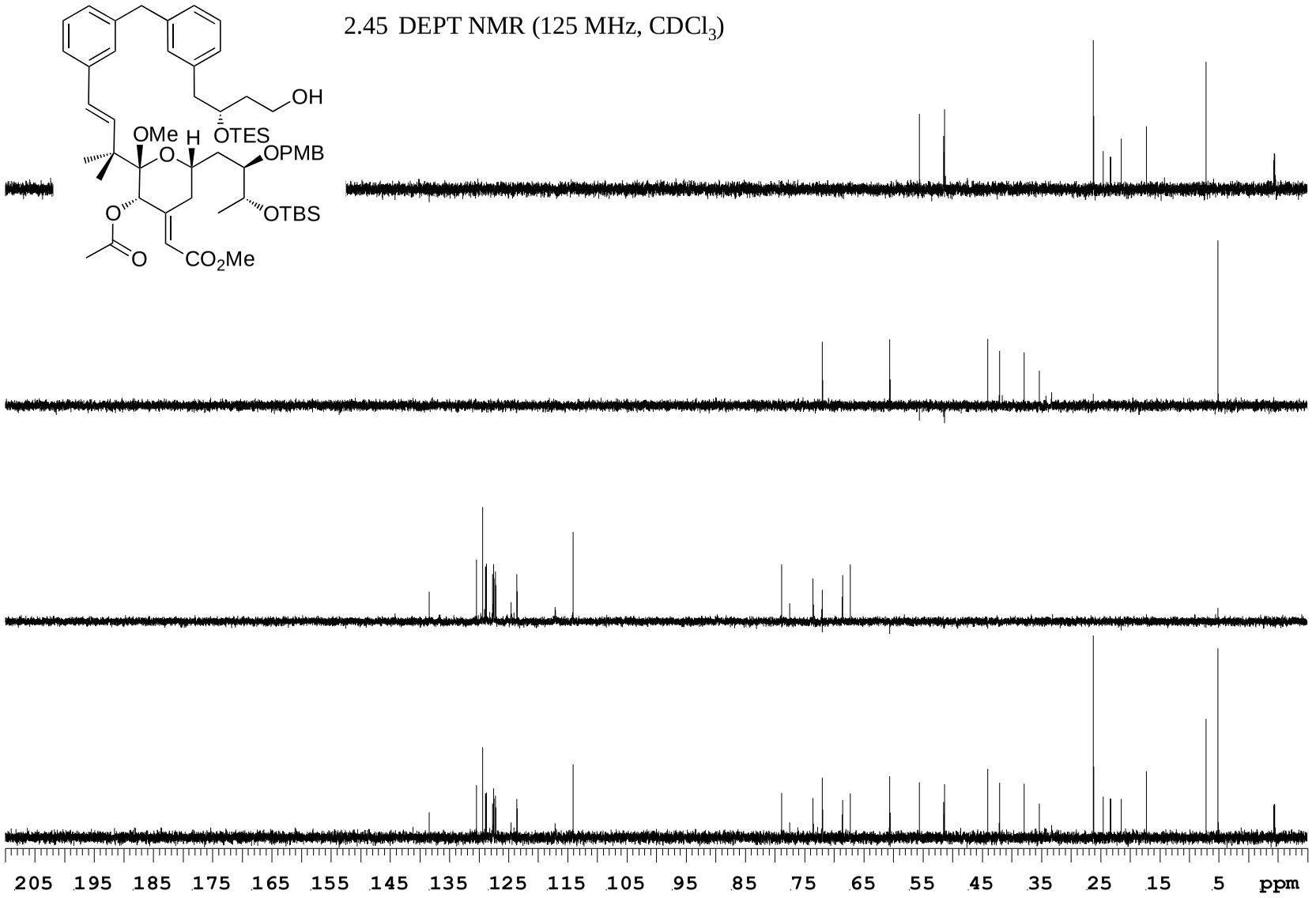


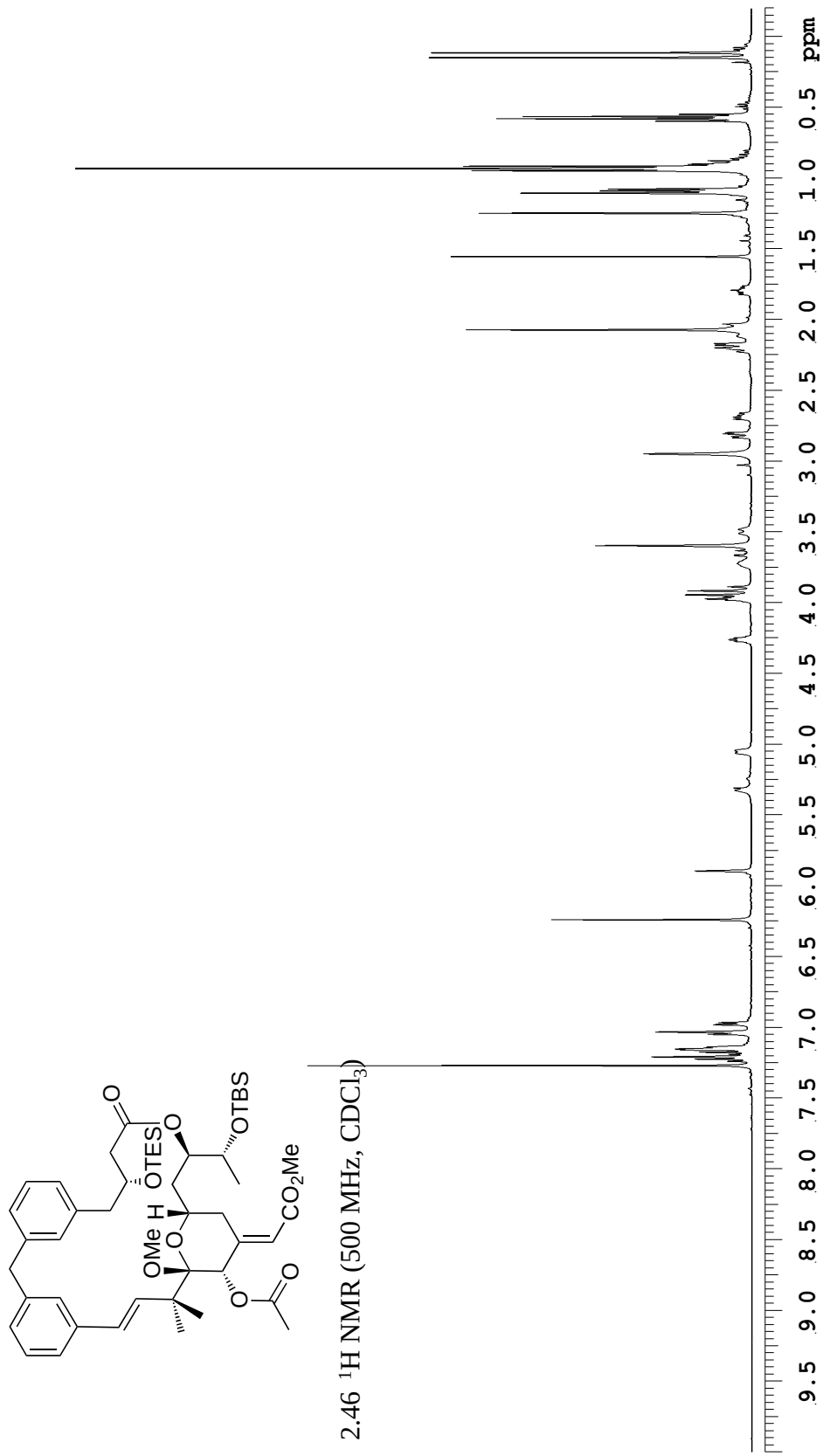
2.45 ^{1}H NMR (500 MHz, $CDCl_3$)



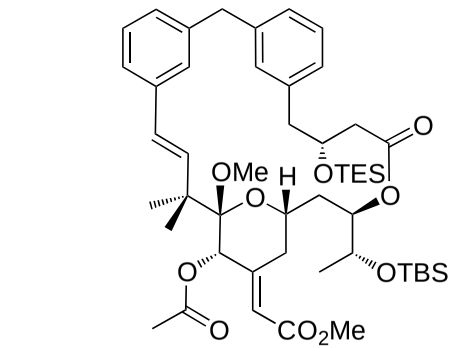
2.45 ^{1}13C NMR (125 MHz, $CDCl_3$)

2.45 DEPT NMR (125 MHz, $CDCl_3$)

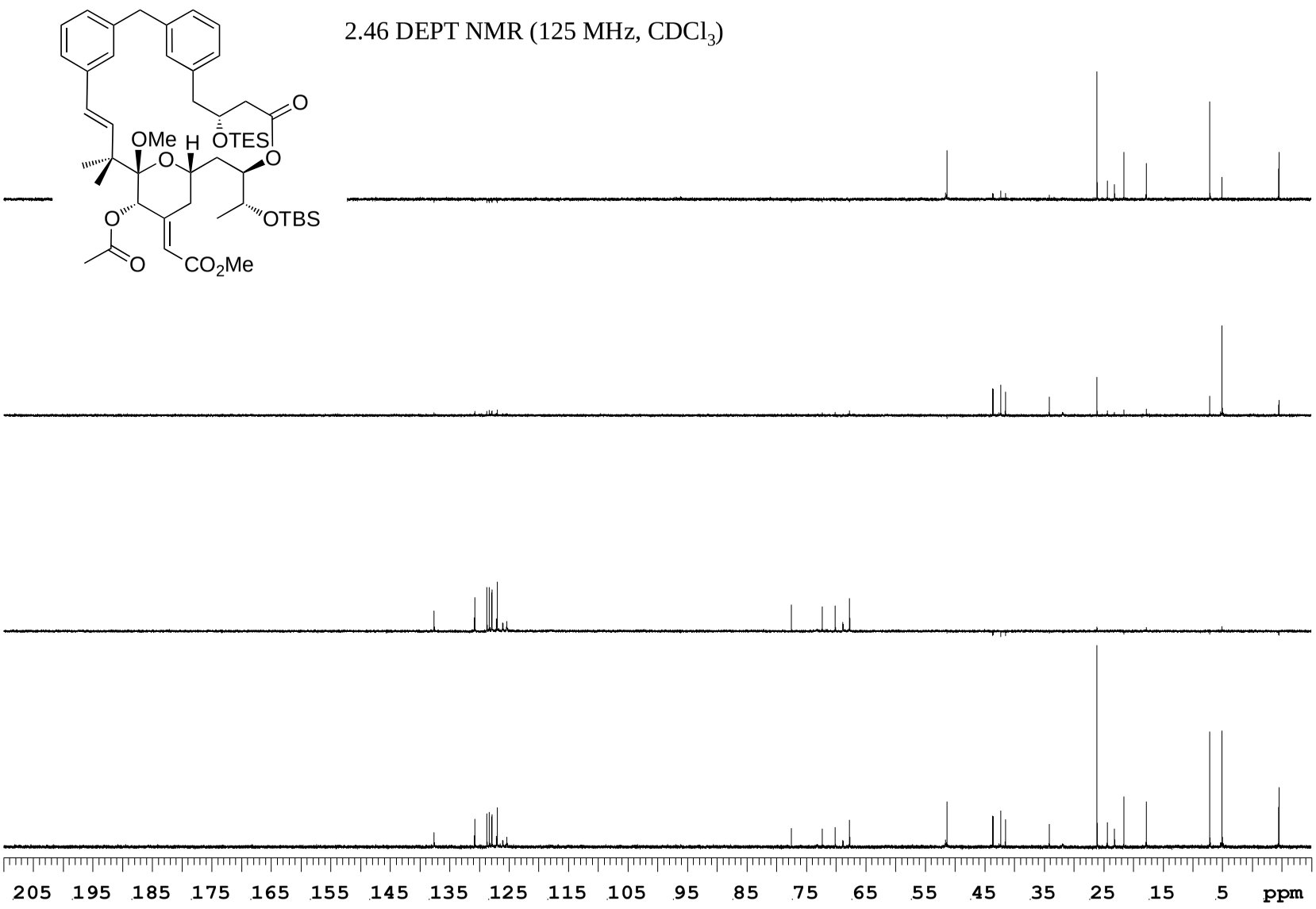




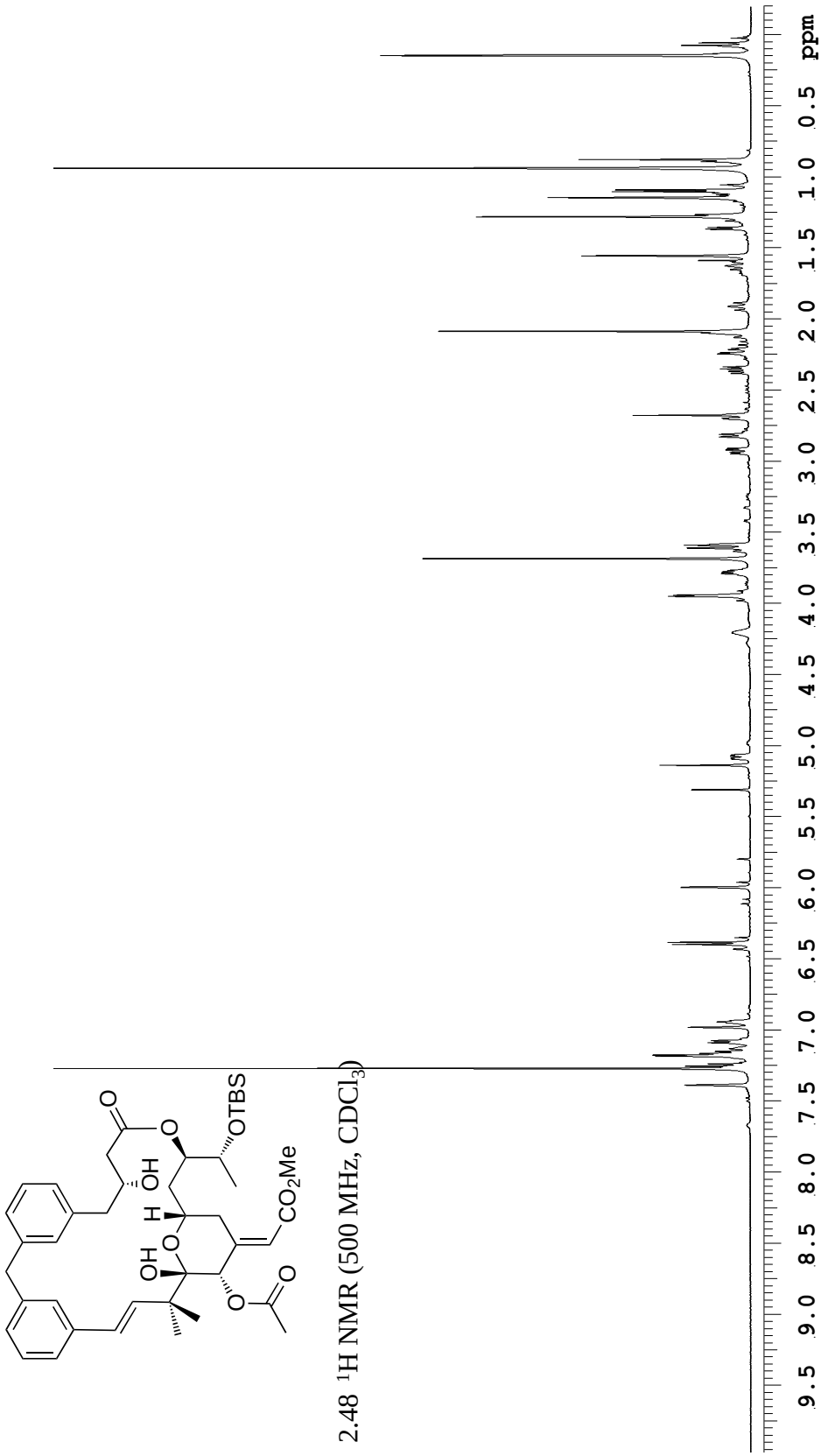
2.46 ^{1}H NMR (500 MHz, $CDCl_3$)



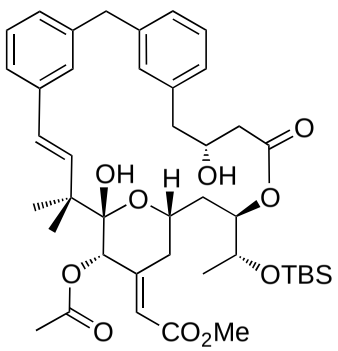
2.46 ^{1}13C NMR (125 MHz, $CDCl_3$)



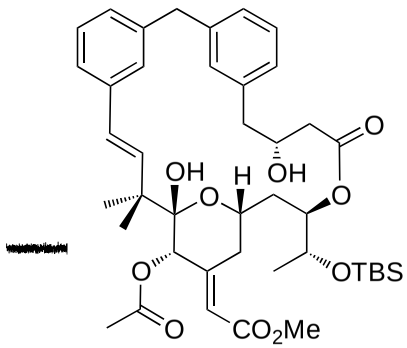
2.46 DEPT NMR (125 MHz, $CDCl_3$)



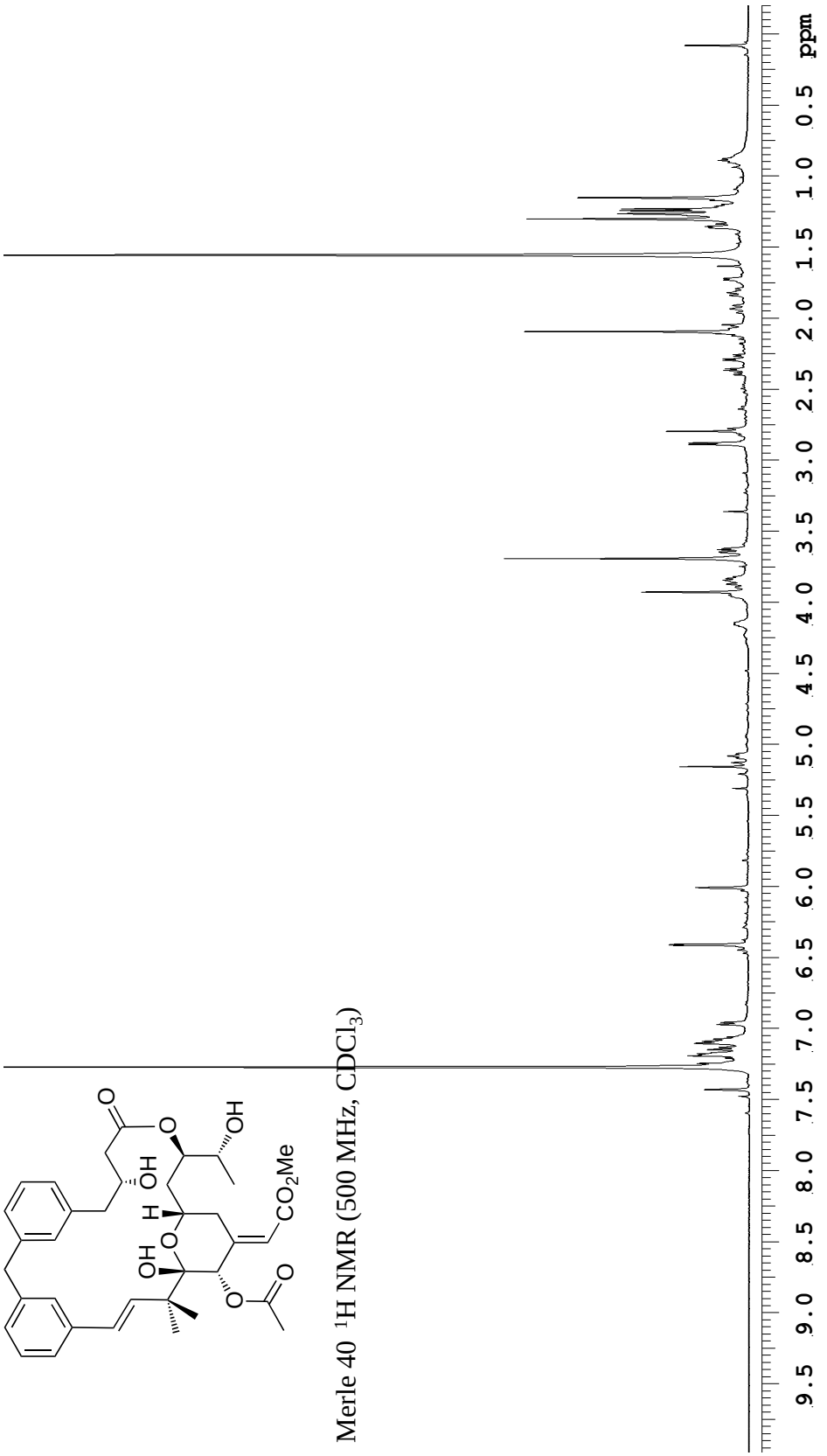
2.48 ^{1}H NMR (500 MHz, $CDCl_3$)



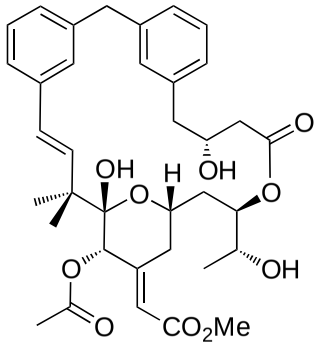
2.48 ^{1}13C NMR (125 MHz, $CDCl_3$)



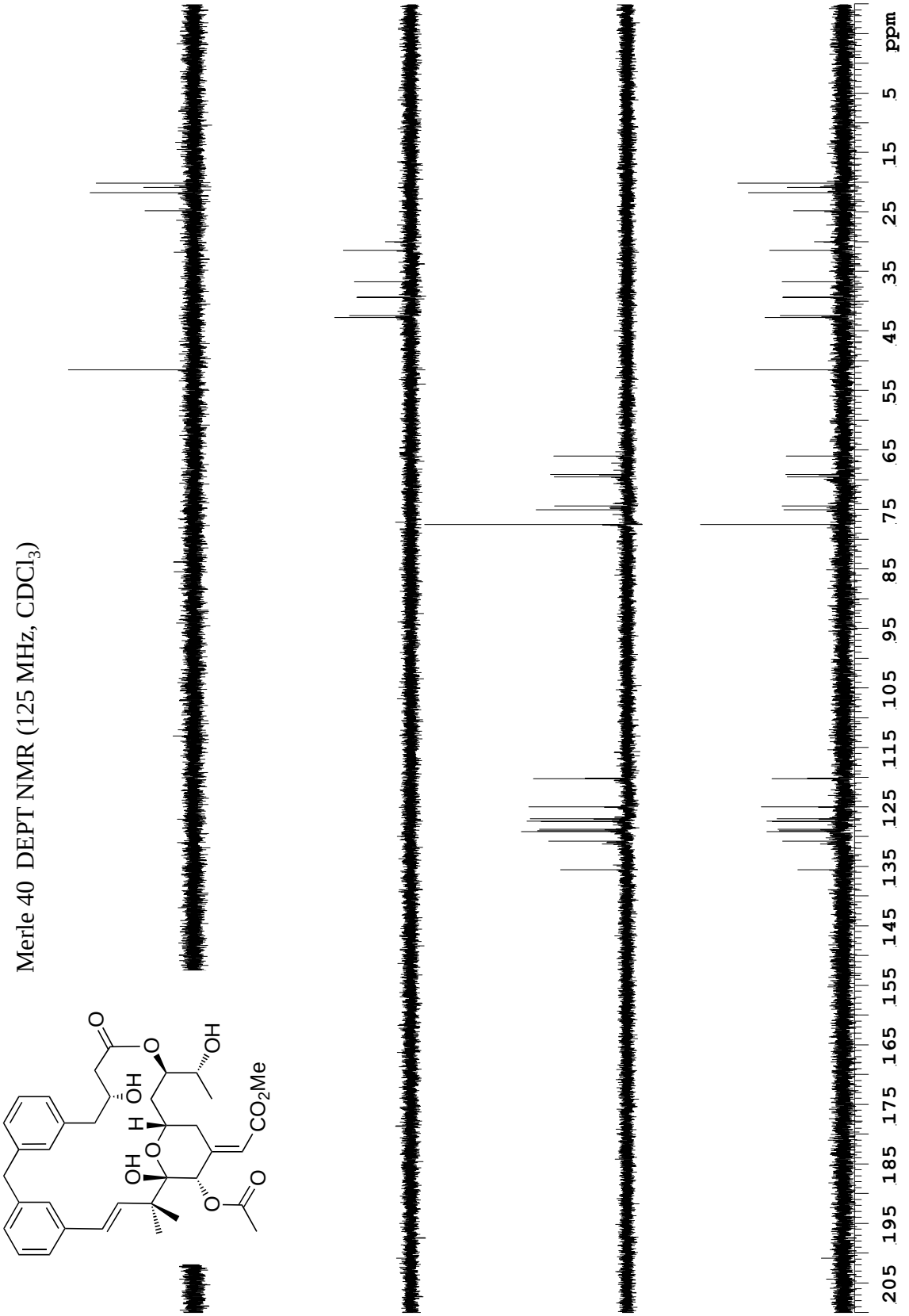
2.48 DEPT NMR (125 MHz, $CDCl_3$)



Merle 40 ^{1}H NMR (500 MHz, $CDCl_3$)



Merle 40 ^{1}13C NMR (125 MHz, $CDCl_3$)


Merle 40 DEPT NMR (125 MHz, $CDCl_3$)
ppm
205 195 185 175 165 155 145 135 125 115 105 95 85 75 65 55 45 35 25 15 5
OH
H
OH
O
O
O
O
O
CO_2Me