SYNTHESIS OF DIACYL GLYCEROL LACTONE-BASED BRYOSTATIN ANALOGS: PROGRESS TOWARDS A SIMPLIFIED BINDING DOMAIN THAT RETAINS THE BIOLOGICAL PROPERTIES OF THE NATURAL PRODUCT

by

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STATEMENT OF DISSERTATION APPROVAL

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ABSTRACT

Bryostatin 1 is a large macrocyclic polyketide natural product that was isolated from the marine bryozoan *Bugula neritina*. Bryostatin was found to have many interesting biological properties stemming from its extremely high affinity for protein kinase C (PKC) isozymes. Bryostatin 1 has attracted increased attention due to its anticancer and immune system stimulating properties. In recent years, bryostatin 1 has been found to have properties that may lead to effective treatments for Alzheimer's disease and HIV. Several high affinity PKC ligands are potent tumor promoters; however, bryostatin 1 does not display any of these properties and even antagonizes the effects of phorbol esters, which are potent tumor promoters.

Due to the continued interest in bryostatin 1, attention has been focused on the synthesis of simplified analogs in order to study structure-function relationships. Most of this work has focused on the synthesis of analogs with simplified AB top-halfs, while very little attention has been applied towards the synthesis of analogs with simplified C-ring binding domains. Extensive work has been conducted on the synthesis of diacylglycerol (DAG) lactone analogs, which are simple high affinity PKC ligands. The focus of this work involves the substitution of a DAG lactone for the C-ring in a bryostatin analog, resulting in the synthesis of 3 bryostatin analogs. Biological evaluation indicates that significant binding affinity in the first analog was lost as compared to the natural product even though all structural elements were present that are thought to be required for good affinity.

Molecular modeling studies indicate that the planer conformation of the top-half of the natural product was lost in these new analogs, resulting in a conformation unfavorable for effective binding. The binding domain orientation of the analog was reversed, resulting in a slight increase in affinity, but at the cost of lower stability under biological conditions, due to the diester linkage used to assemble the first two analogs. Building upon these results, a third-generation analog with increased lipophilicity and lacking the diester linker was synthesized. The third-generation analog had a much higher affinity for PKC as well as an improved biological profile as compared to the first 2 analogs.

TABLE OF CONTENTS

STANDARD LIST OF ABBREVIATIONS

$\Delta\nu$	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
$BF_3{\bullet}OEt_2$	boron trifluoride etherate
9-BBN	9-borabicyclo(3.3.1)nonane
BINOL	(1,1'-binapthalene)-2,2'-diol
BITIP	BINOL titanium tetraisopropoxide
BOM	benzyloxymethyl
Bn	benzyl
Bu	butyl
t-Bu	*tert*-butyl
n-Bu	butyl
n-BuLi	*n*-butyl lithium
Bz	Benzoyl

°C	degrees Celsius
CAA	catalytic asymmetric allylation
calcd	calculated
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
CH_2Cl_2	dichloromethane
COSY	correlation spectroscopy
d	day(s); doublet (spectral)
DAG	diacylglycerol
DBU	1,8-Diazabicycloundec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarization transfer
DET	diethyl tartrate
DIBALH	diisobutylaluminun hydride
DIPEA	diisopropylethylamine
DIPA	diisopropylamine
DMAP	4-dimethylaminopyridine
DMB	dimethoxybenzyl
DMF	N, N-dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
dt	doublet of triplets (spectral)

EDA	ethylenediamine
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethylether
Et_3N	triethylamine
er	enantiomeric ratio
equiv	equivalent(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
KHMDS	potassium hexamethyldisilazide
K_i	binding affinity
LDA	lithium diisopropyl amide
M	moles per liter
*m*CPBA	*m*-chloroperoxybenzoic acid

Me	methyl
MeCN	acetonitrile
MeOH	methanol
$MgSO_4$	magnesium sulfate
MHz	megahertz
m	minute(s)
mL	milliliter
MMPP	magnesium monoperoxyphthalate hexahydrate
mol	mole(s)
mp	melting point
MsCl	methanesulfonyl chloride
m/z	mass to charge ratio (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
q	quartet (spectral)
quint	quintet (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	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
THP	tetrahydropyranyl

TLC	thin layer chromatography
TMEDA	*N,N,N,N*-tetramethyl-1,2-ethylenediamine
TMS	trimethylsilyl, tetramethylsilane
TNFα	tumor necrosis factor alpha
TPAP	tetrapropylammonium perruthenate
Tr	trityl
TsOH	*p*-toluenesulfonic acid

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CHAPTER 1

SYNTHESIS OF A VERSATILE DIACYLGLYCEROL LACTONE MOTIF AMENABLE TO THE SYNTHESIS OF SIMPLIFIED BRYOSTATIN ANALOGS

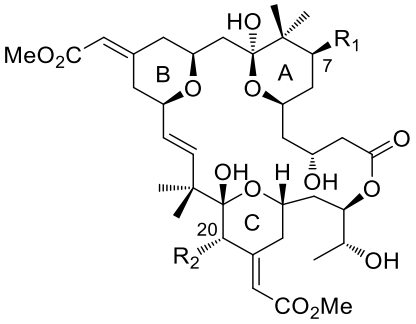
Summary

Bryostatin 1 is a macrocyclic marine natural product isolated from the marine bryozoan *Bugula* neritina in the search for antineoplastic natural products. Since its isolation, Bryostatin 1 has been in over 80 clinical trials for the treatment of various cancers. More recently, bryostatin has shown promise in the treatment of HIV-AIDS, stroke, and Alzheimer's disease. The impressive biological properties of bryostatin 1 stem from its nanomolar affinity for protein kinase C (PKC). Unlike many PKC activators, bryostatin 1 is not a tumor promoter and even reverses the effects of certain tumor promoters. In the search for potent bryostatin analogs, many modifications of the natural product have been made, but few have focused on the simplification of the binding domain. Diacylglycerols (DAGs) are the endogenous ligand for PKC. Building upon the structure of DAGs, Marquez and Coworkers produced a series of high affinity DAG lactones. The results section of this chapter describes the synthesis of a DAG lactone binding domain amenable to the synthesis of bryostatin analogs bearing this structural motif.

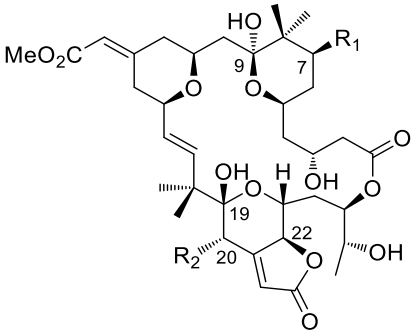
The Bryostatins and Related Natural Products

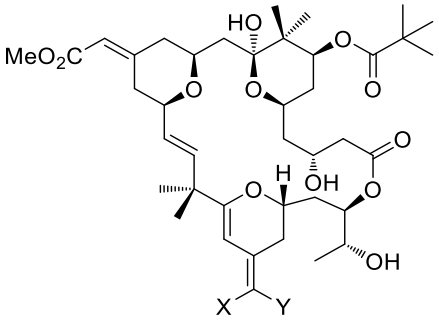
The bryostatins are a series of 20 macrolactone natural products that were first discovered in 1970 by Pettit and coworkers while screening marine organisms for antineoplastic compounds.[1] The bryostatins were isolated from the marine bryozoan *Bugula neritina*, a sessile marine animal, which commonly attaches itself to the hulls of ships and docks.[2] Further studies of extracts from this organism indicated considerable activity against murine p388 lymphocytic leukemia as well as activity against 60 other NCI cancer cells.[3] The active component of these extracts, bryostatin 1, was isolated and fully characterized in 1982.[2] The bryostatin family of natural products is characterized by the presence of a 20-membered macrolactone with 3 pyran rings, which are situated within it (Figure **1.1**). The 3 pyran rings are named A, B, and C; the natural products differ at C7 (R1) and C20 (R2) with various esters connected to these points.[4] Three members of the bryostatin family (3, 19, and 20) have an additional oxygenation at C22, which is esterified with the C21 enoate to form a fused butyrolactone with the C-ring.[4] The C20 ester substituent and C19 hemi-ketal are both missing in bryostatins 16 and l7 while bryostatin 18 only lacks the C20 ester.[4] Neristatin is a bryostatin type of natural product with a highly modified C-ring consisting of a [3.3.2] fused bicyclofuranone scaffold.[5] The complexity and the wide range of biological effects of these natural products have produced much interest in the isolation of the natural product and the preparation of simplified analogs that retain the biological activity of the natural product.

Bugula neritina was initially thought to produce bryostatin. However, later work determined that the endosymbiotic bacterium *Candidus endobugula sertula* secretes this product.[6] Bryostatin is thought to discourage predation of the larvae of the host organism



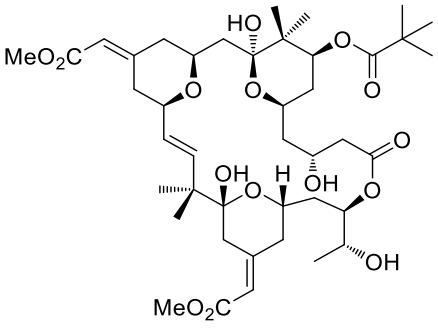
bryostatin 1	R_1 = OAc	R_2 = OCO$(CH)_4$Pr
bryostatin 2	R_1 = OH	R_2 = OCO$(CH)_4$Pr
bryostatin 4	R_1 = OCO*t*-Bu	R_2 = OCO*n*-Pr
bryostatin 5	R_1 = OCO*t*-Bu	R_2 = OAc
bryostatin 6	R_1 = OCO*n*-Pr	R_2 = OAc
bryostatin 7	R_1 = OAc	R_2 = OAc
bryostatin 8	R_1 = OCO*n*-Pr	R_2 = OCO*n*-Pr
bryostatin 9	R_1 = 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



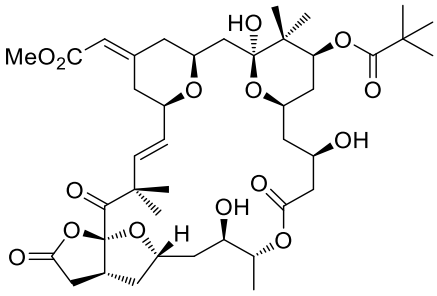


bryostatin 3	R_1 = OAc	R_2 = OCO$(CH)_4$Pr
bryostatin 19	R_1 = OCOt-Bu	R_2 = OCO*n*-Pr
bryostatin 20	R_1 = OCOt-Bu	R_2 =H

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



bryostatin 18



neristatin 1

Figure 1.1. Bryostatin Natural Products

B. neritina.[7] The bryostatin content of *B. neritina* was found to be extremely low, with isolation yields typically around 10^{-6} percent.[3] Isolation of relevant quantities of bryostatin is rendered difficult due to the miniscule amount that is found in the organism. Extraction of 10000 gallons of wet *B. neritina* yielded 18 g of bryostatin 1 after extensive purification[8]; this relatively small amount of bryostatin has enabled over 80 clinical trials of bryostatin against various cancers as a single agent and in combination with other chemotherapeutic agents[3] due to its extremely high affinity for PKC.

However, continued isolation of bryostatin from wild *B. neritina* is not feasible for continued studies. In order to overcome these supply issues, studies of aquaculture of *B. neritina* were initiated by the now defunct CalBioMarine Technologies[9]; these studies appeared to be successful, but were never commercially used. Another interesting result is the identification of the bryA gene cluster, which is believed to be responsible for the biosynthesis of the bryostatins.[10] Further work in this area has been hampered by the large size of the gene cluster and uncertainties in the regulation of gene expression making expression and biosynthesis in a suitable host organism difficult. Even if successful, however, this biosynthetic approach would produce "bryostatin 0", a bryostatin with considerably less oxidation than the flagship compound bryostatin 1.[10]

Bryostatin 1 and Its Anticancer Properties

Bryostatin was initially found to have a high activity against murine p388 lymphocytic leukemia cells; this activity has resulted in many *in vitro* and *in vivo* studies as summarized by Pettit.[3] Bryostatin 1 was found to double the life expectancy of mice with p388 leukemia, and caused remission in mice with B16 melanoma and M5 ovary

carcinoma.[11] As a single agent, bryostatin has not proven to be an effective drug in clinical trials, in contrast to its potent effects in cell culture and murine cancer models. On the other hand, studies using bryostatin in combination with other chemotherapeutic drugs have shown promise. Studies conducted with vincristine,[12] paclitaxel,[13] cytarabine,[14] dolastatin,[15] and others are among the over 80 clinical trials to date.[3] Bryostatin is an extremely potent drug; only 1 mg is needed for 6 weeks of treatment.[12] During these studies, bryostatin was found to be relatively nontoxic; the only dose-limiting side effect attributed to bryostatin itself was severe myalgia (muscle pain).[16] Injection site reactions were also a considerable problem, but this was a result of the ethanol formulations required to solubilize it, and not bryostatin itself.[17] Contributing to bryostatin's appeal as a chemotherapeutic is that it has demonstrated immunostimulatory properties such as promoting the production of proinflammatory cytokines.[18] Bryostatin was also shown to promote the growth of normal bone marrow progenitor cells,[19] and the activation of neutrophils, platelets,[20] and T-lymphocytes.[21] These properties are a stark contrast to most chemotherapeutic agents, which are generally highly toxic, and indicate that bryostatin may be a useful drug when used in combination with other therapeutics to support natural immune response during chemotherapy.

Bryostatin 1 and Neurodegenerative Conditions

Bryostatin has been shown to have impressive effects upon several neurological conditions. Dr. Daniel Alkon of the Banchette Rockefeller Institute of Neuroscience has conducted many studies of PKC activators, most especially bryostatin 1 on murine models of stroke[22] and Alzheimer's disease.[23] Bryostatin treatment improves the

cognitive ability of rats.[24] Bryostatin treatment of aged ischemic rats promotes synaptogenesis, neurogenesis, reduces apoptosis, and improves cognitive abilities of the treated animals.[25] Bryostatin treatment is thought to cause activation of protein kinase C (PKC), its subsequent downregulation, and resultant de-novo synthesis of PKC, along with attenuation of the inflammatory response; this may account for the results observed in rats.[26]

Alzheimer's Disease (AD) is thought to be the result of the accumulation of amyloid plaques in brain tissue with resulting neuronal death.[27] These plaques result from an abnormal degradation pathway of amyloid precursor protein (APP) to form the toxic amyloid β.[28] Protein kinase signaling is believed to be crucial for the normal degradation of APP into sAPP-α.[29] In a transgenic murine model of AD, bryostatin and other PKC activators are known to increase the production of α-secretase, which is responsible for the production of sAPP-α from APP.[30] Bryostatin has also demonstrated the ability to reduce the formation of amyloid plaques in a mouse model of AD.[30] Based on these preclinical results, the FDA approved a phase II clinical trial in 2009 to evaluate the effectiveness of bryostatin in treating AD. The results of this work have not been published to date. Recently, treatment of a patient with severe early onset AD with bryostatin resulted in improvement of speech, limb spasticity, attentional focus, and swallowing.[31]

Bryostatin 1 and HIV Activation

Current treatments for HIV-AIDs reduce the viral load to undetectable levels and allow for the management of the disease.[32] Drugs used in highly active antiretroviral

therapy (HAART) have many long-term side effects and compliance with such a treatment regime can be problematic.[32] HAART kills the active virus, but dormant viral DNA remains incorporated in CD4+ T-cells, resulting in a latent viral reservoir that results in the return of the disease if therapy is discontinued.[33] Discovery of drugs that activate the dormant virus, thus allowing current treatment options to clear the patient of the disease, are a current area of HIV research.[33] Bryostatin treatment of cultured latent infected lymphocytic cells resulted in viral activation via PKC, MAPK, and NF-κB pathways.[34] The HIV receptors CD4 and CXCR4 are downregulated by bryostatin treatment, preventing infection of new cells.[34] Recently, Wender[35] has reported the synthesis of structurally simplified bryostatin analogs that are potent activators of HIV in cell culture. Bryostatin 1 could be effectively used with current HAART therapy in a strategy known as "shock and kill" to effectively cure HIV infection.[32]

Bryostatin 1 and PKC Activation

The multitude of biological effects of bryostatin 1 stem from its nanomolar binding affinity towards the cell signaling enzyme protein kinase C (PKC). Dysfunction of PKC is implicated in many disease processes such as cancer,[36] and neurological conditions.[37] PKC enzymes, when suitably activated, serve to phosphorylate the amino acid residues serine and threonine in other proteins. PKCs are involved in cellular signaling cascades, differentiation, apoptosis, and proliferation.[38]

The PKC family of kinases consists of at least 10 different isozymes that are divided into three classes based upon regulatory domain structure and the requirement for a cofactor.[39] The three classes consist of classical (cPKC), novel (nPKC), and atypical

(aPKC). These isozymes are present in differing amounts depending on cellular or tissue types.[40] The phospholipid, phosphotidyl-L-serine is required by all PKCs.[41] Classical PKCs (α, β_I, β_{II}, γ) also require endogenous 1,2-diacylglycerol (DAG) and calcium. Novel PKCs (δ, ε, η, θ) are only DAG dependent, while atypical PKCs (ξ, τ) require neither.

The PKCs are enzymes that are composed of a regulatory domain and a catalytic domain (Figure **1.2**). The C-terminal catalytic domain is highly conserved amongst isozymes,[42] while the N-terminal (regulatory) domain is highly variable dependent on isozyme type.[39] A pseudo-substrate is bound to the catalytic site when the enzyme is in its resting state; this motif is present at the N-terminus[43]. The cysteine-rich regulartory domains termed C1 and C2 are involved with ligand binding and interaction with cellular membranes.[44] The C1 domain of PKC's binds to secondary messenger ligands such as diacylglycerols or phobol esters. The C1 domain can be expressed as a single domain or a pair of domains usually labled C1a and C1b, depending on the isozyme. The C1 domain consists of approximately 50 amino acids and has 4 cysteine residues which coordinate 2 zinc cations[45]. The C1 domains of aPKC's do not bind phorbol esters due to structural modifications. Classical PKCs and novel PKCs also have a C2 domain which is thought to be involved in binding calcium and anionic phospholipids. The C3 domain is involved in binding ATP, while the C4 domain is the catalytic site of the enzyme. The V domains are variable sequences depending on isozyme type.

Diacylglycerols or DAGs are endogenous intercellular messengers that are produced upstream by hydrolysis of phosphatidylinositol 2,5-bisphosphate by phosphor-lipase C, which is in turn activated by signaling from G-protein-coupled receptors.[46] In

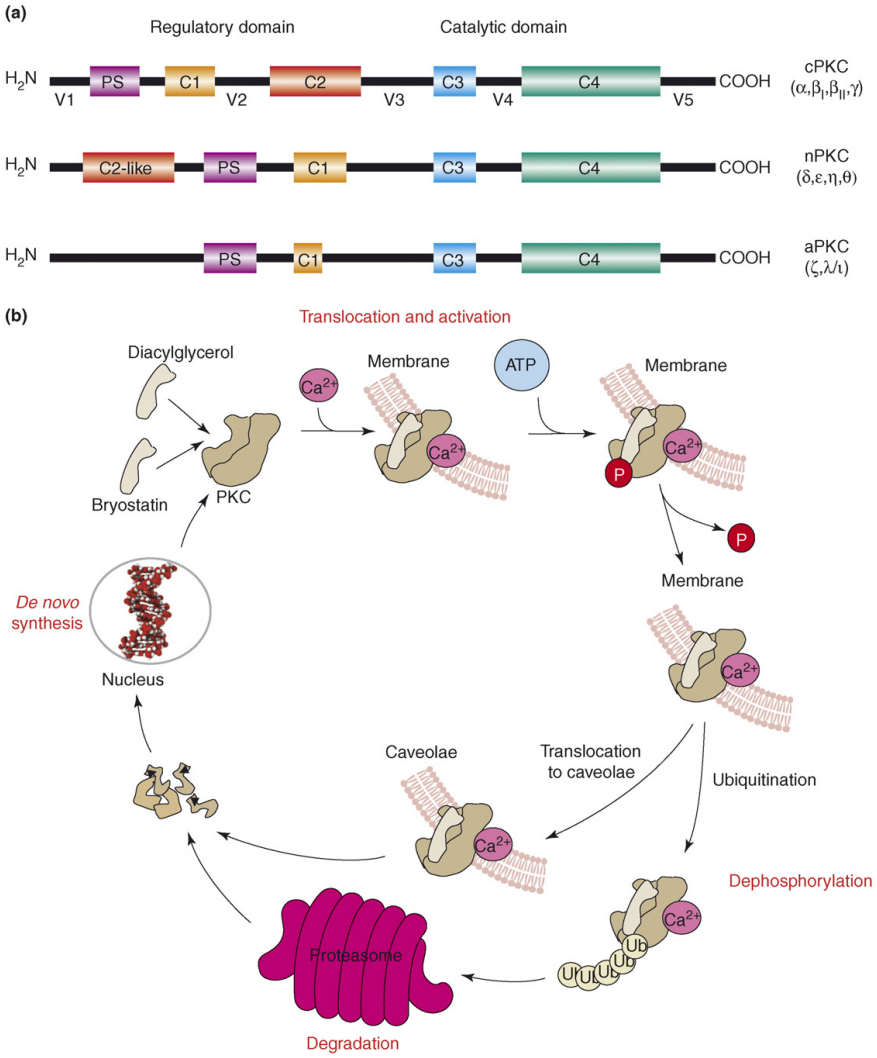


Figure 1.2 Protein Kinase C Structure and Activation
(used with permission from ref 28a).

the DAG-dependent cPKCs, and nPKCs, DAG binds to the C1b domain, resulting in a conformational change, thereby removing the pseudo substrate from the active site. The activated PKC translocates from the cytosol to cellular membranes. ATP and calcium bind, and the holoenzyme phosphorylates its specific protein substrates, continuing the signaling cascade. The activated enzyme is then subject to downregulation by several different pathways, including ubiquitinylation and caveolae-dependent degradation.[47]

PKC Binding Hypotheses

Initial studies into the mode of ligand binding to the C1 domain of PKC were undertaken by the Wender group. Through computer modeling, Wender reasoned that the C1 ester, C19 ketal, and C26 alcohol were involved in binding to the C1 domain of PKC (Figure **1.3**).[48] A crystal structure of the C1 domain of PKC δ bound to phorbol 12-myristrate 13-acetate (PMA) demonstrated that threonine 242, leucine 251, and glycine 253 are involved in binding to the PKC ligand.[49] Building upon these results, Keck embarked in another modeling study based upon experimental results gleaned from previous analog work and docking of the ligand to the C1b domain (Figure **1.4**).[50] This study showed that the C21 enoate and not the C19 ketal is involved in binding.

The C19 ketal was found to be involved in an intramolecular hydrogen bonding network between the C3 alcohol and A and B ring pyran oxygens. The network holds the pharmacophore elements of bryostatin 1 in proper position for binding to the C1 domain of PKC. The C1 ester is bound to glutamine 257, the C21 enoate carbonyl is bound to glycine 253, while the C26 alcohol is bound to leucine 251 and threonine 242. The C9 ketal was found to bind to methionine 239 and assist in anchoring the ligand to the

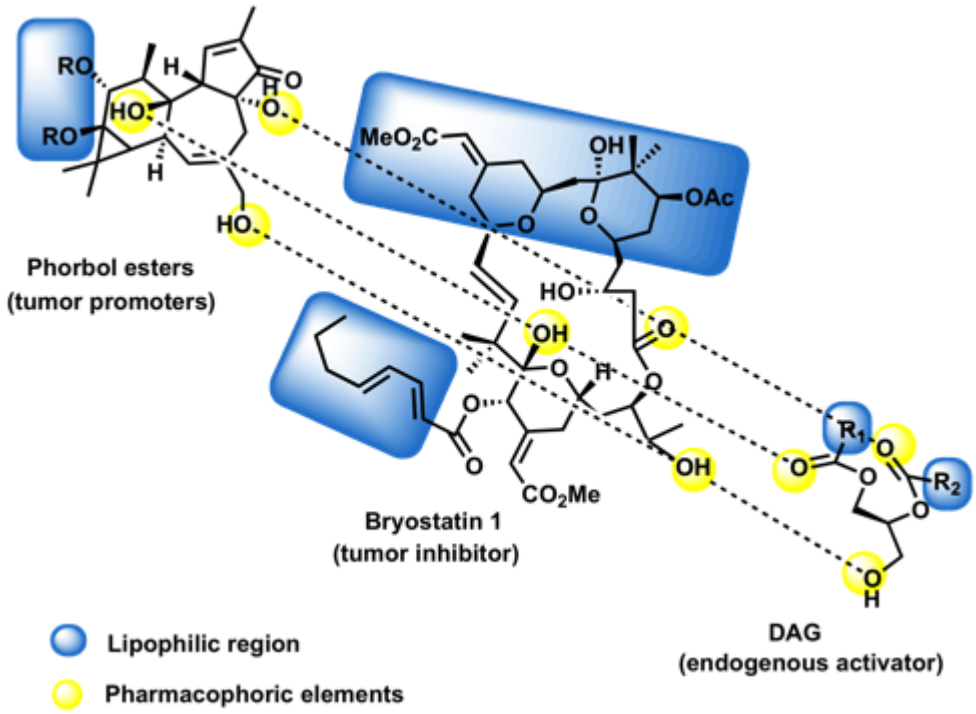


Figure 1.3 Wender's Binding Hypothesis (Adapted from ref 60b).

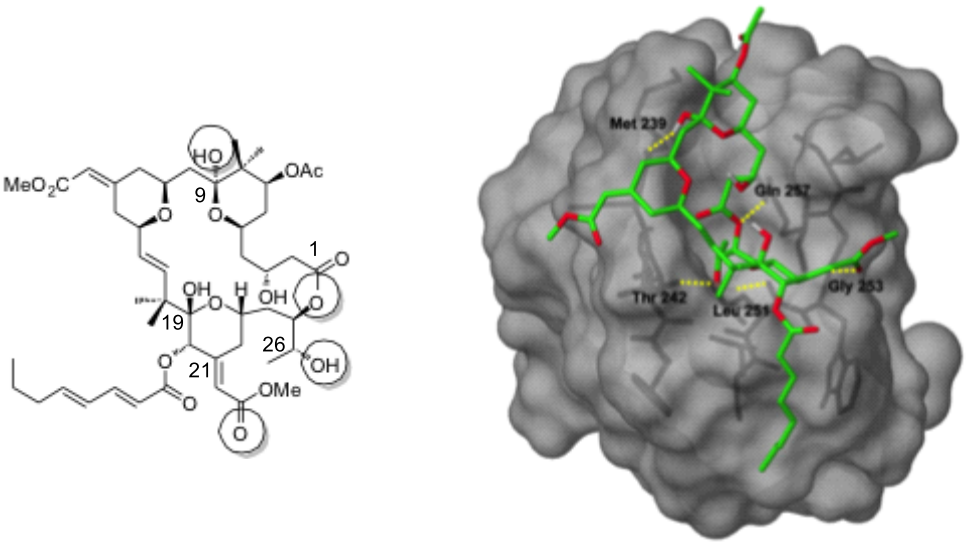


Figure 1.4 Keck's Binding Hypothesis (used with permission from ref 50).

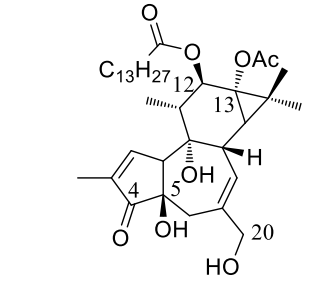
enzyme.

Tumor Promoting and Nontumor Promoting PKC Ligands

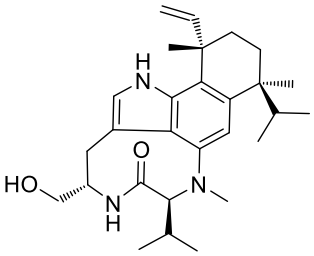
The resin obtained from the shrub *Croton tiglium* has long been recognized as a potent irritant and poison.[51] Early studies into the composition of this substance yielded a complex mixture of esters of the terpene phorbol. Animal studies of mixtures of these esters determined that these compounds sensitized mice towards carcinogens applied to the skin. The most studied member of the phorbol ester family is phorbol-12-myristate-13-acetate (PMA) (Figure **1.5**). PMA is a very potent activator of PKC with nanomolar (K_i = 0.55 nM) binding affinity to the C1 domain of PKC.[52]

Several other natural products that are tumor-promoting PKC activators have been isolated from diverse sources (Figure **1.5**). Teleocidin was isolated from *Streptomyces mediocidicus*.[53] Aplysitoxin, isolated from the sea hare *Stylocheilus longicauda*, is a spiroketal natural product that also binds PKC (3.0 nM for PKCδ) and is highly tumor promoting.[54] Aplysiatoxin is a tumor promoter with a very high affinity for PKCδ (3.0 nM).

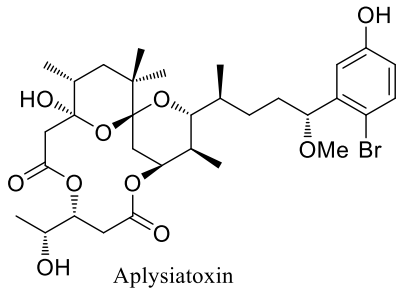
Several compounds with high affinity for PKC isozymes, but which lack the tumor promoting properties of the phorbol esters and related natural products, are in clinical trials (Figure **1.6**). The phorbol type terpene 12-deoxyphorbol 13-acetate (prostratin) was isolated from the mamala tree, *Homalanthus nutans*.[55] The bark of the tree was used by Samoan natives to treat hepatitis, and further studies of this compound indicated that it has a high affinity for PKC (12.5 nM). Prostratin has been experimentally demonstrated to activate latent HIV[56]. Prostratin was determined to have the same



Phorbol-12-myristate-13-acetate (PMA)

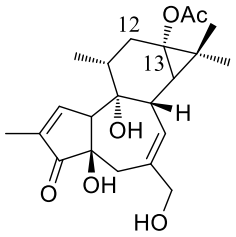


Teleocidin

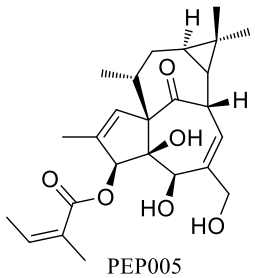


Aplysiatoxin

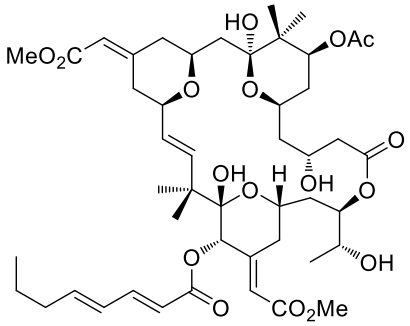
Figure 1.5 Tumor Promoters



Prostratin



PEP005



Bryostatin 1

Figure 1.6 Nontumor Promoting PKC Ligands

tricyclic structure as PMA, but lacks functionality at C12, and is nontumor promoting.

The natural product PEP005, isolated from the petty spurge, *Euphorbia peplus*, is a member of the ingenol family of terpenes, which are structurally related to the phorbol esters.[57] PEP005 is a very potent PKC ligand with sub nanomolar affinity for PKC. PEP005 is not a tumor promoter and was approved for the treatment of actinic keratosis by the FDA. Bryostatin 1, the most studied member of the bryostatin family, is a potent PKC ligand and is nontumor promoting. Bryostatin when given in the presence of PMA antagonizes and reverses the effects of PMA in a dose-dependent manner.[58]

The difference between tumor promoting and nonpromoting ligands is believed to be dependent upon differences in polarity of side chains attached to the top section of the PKC ligand. Ligands with nonpolar, long side chains, like those attached to PMA, tend to be tumor promoting. Ligands with no side chain or very short side chains tend to be nontumor promotors like prostratin. Interestingly, the binding constant to PKC has little correlation to tumor promotion properties. Bryostatin, a nontumor promoter, has a single digit nanomolar affinity for PKC, while PMA, a high affinity ligand, is a tumor promoter.

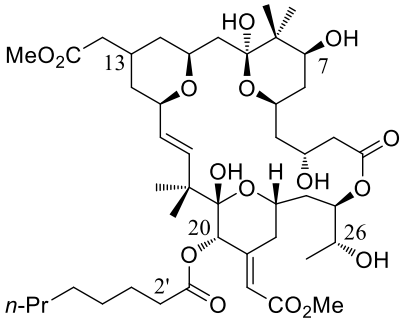
The crystal structure of PMA bound to PKC[49] indicates that the most polar portion of the ligand binds to the C1 domain. The rest of the ligand rests on top of the C1 domain, forming a hydrophobic surface on the enzyme. This lipophilic ligand/ enzyme complex then interacts with internal cellular membranes. These interactions are thought to be the reason for the differing effects of PKC ligands.

Early Structural Modifications of Bryostatin

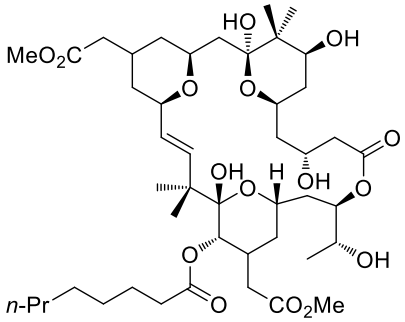
Early studies on the structure-activity relationships of the bryostatins focused upon making derivatives of the natural product to determine which structural elements are important for PKC binding. Upon comparing the natural bryostatins, it was noticed that the various ester groups at C7 and C20 had minor effects on binding. The lack of the C19 acetal and C20 ester in bryostatins 16 and 17 resulted in a large decrease in affinity. In a collaboration between Blumberg, Pettit, and Wender, bryostatin 2, which lacks the C7 ester, was successively hydrogenated to determine if any of the unsaturations were important for binding (Figure **1.7**).[59]

Hydrogenation of the C20 side chain as well as the C13 enoate slightly decreased the affinity for PKC. However, further hydrogenation reduced the C21 enoate, and the resulting compound had a binding affinity for PKC 3 orders of magnitude lower than that of the natural product. Inversion or acylation of the C26 alcohol produced analogs with a low binding affinity. Finally, epoxidation of the C13 enoate on bryostatin 4 did not significantly alter binding affinity.

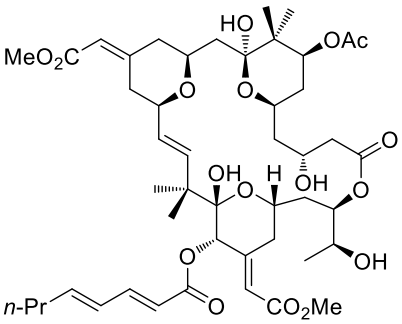
From these studies, it was determined that alteration of the A- and B-ring functionality of bryostatin had minimal effects on binding. On the other hand, changes of hydrogen bonding groups on the C-ring were very detrimental to binding. The only variability on the C-ring that did not significantly affect the binding was the nature of the C20 ester. A computer modeling study was conducted by Wender and coworkers, which showed homology between several PKC ligands; this work demonstrated that 1,2-diacylglycerols, PMA, and bryostatin all have hydrogen bonding domains which interact with the binding site on the C1 domain of PKC. All three ligands contain three



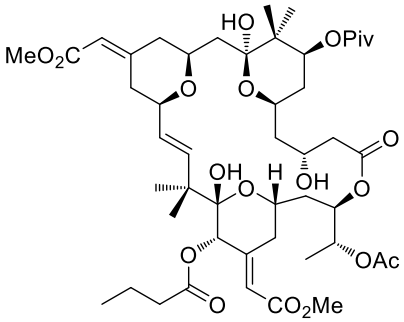
13, 30, 2', 3', 4', 5'-Hexahydrobryostatin 2
K_i = 9.61 nM



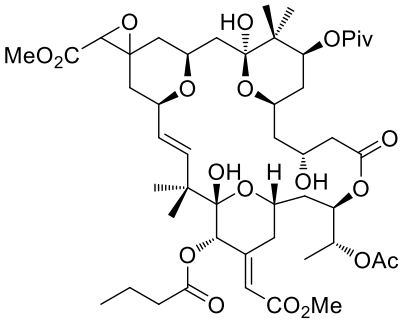
13, 30, 21, 34, 2', 3', 4', 5'-Octahydrobryostatin 2
K_i = 473 nM



26-epi-Bryostatin 1
K_i = 32.6 nM



26-Acetoxybryostatin 4
K_i > 100 nM



13, 30-Epoxybryostatin 4
K_i = 0.54 nM

Figure 1.7 Semi-synthetic Bryostatin Analogs

oxygenated functionalities, which were proposed to interact with the C1 domain (Figure **1.8**).[48] The upper portions of these molecules were hypothesized to serve as spacer domains that interact with cellular membranes and influence the biological activities of these ligands and position the C-ring pharmacophores into position for binding.

Wender's Analog Work

Paul Wender hypothesized that the C-ring of bryostatin, which he termed the "recognition domain", is the key portion of the molecule in relation to its binding affinity for PKC.[49, 60] The top portion of the molecule served as a spacer domain to position the pertinent hydrogen bonding pharmacophores in the C-ring into position to bind with PKC.Wender incorporated this idea into his synthesis of highly potent bryostatin analogs, with a fully functionalized C-ring and simplified A- and B-rings (Figure **1.9**).[60] The synthesis of these provided much information on structure-activity relationships of the bryostatins.

Analog **1.1** retained much of the affinity for PKC, but had a drastically simplified AB top-half for ease of synthesis. Elimination of the C3 alcohol in **1.2** resulted in the decrease of binding affinity by several orders of magnitude. Elimination of the A- ring in **1.3** and attachment of a *tert*-butyl group at C9 resulted in a compound that still retained high affinity for PKC. Complete elimination of the macrocyclic lactone in **1.4** resulted in almost complete loss of activity, demonstrating that the macrolactone is a requirement for PKC affinity.Analog **1.5**, lacks the C27 methyl group and retains high binding affinity. This analog, **1.5**, was called "picolog" due to its subnanomolar affinity for PKC[61]; upon reevaluation, this compound was later found to have a K_i of 3.1 nM.[62] Picolog was

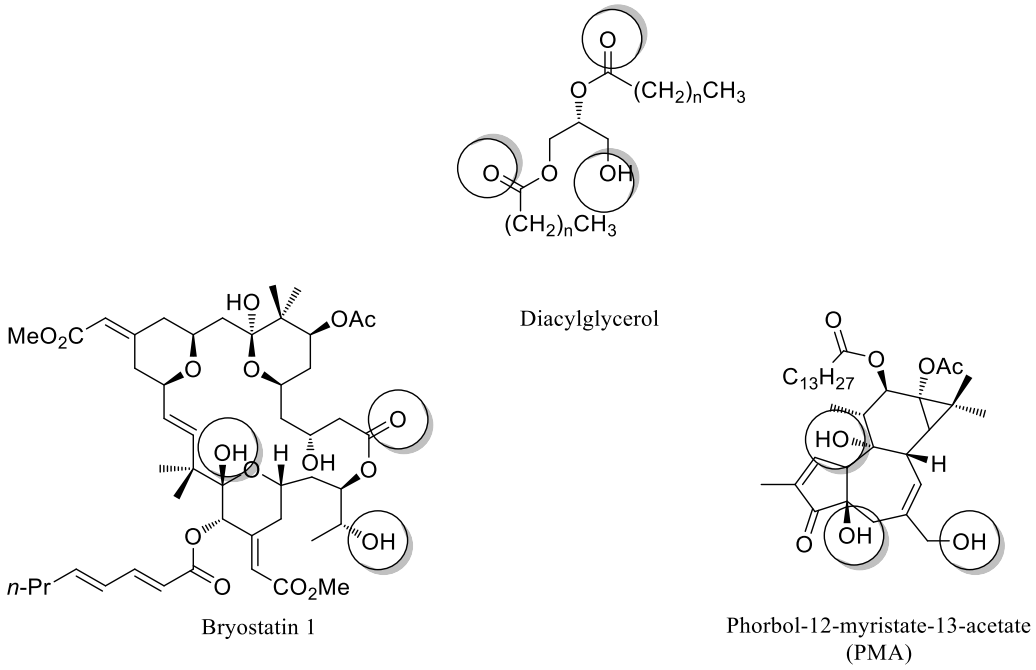


Figure 1.8 PKC Ligand Pharmacophores

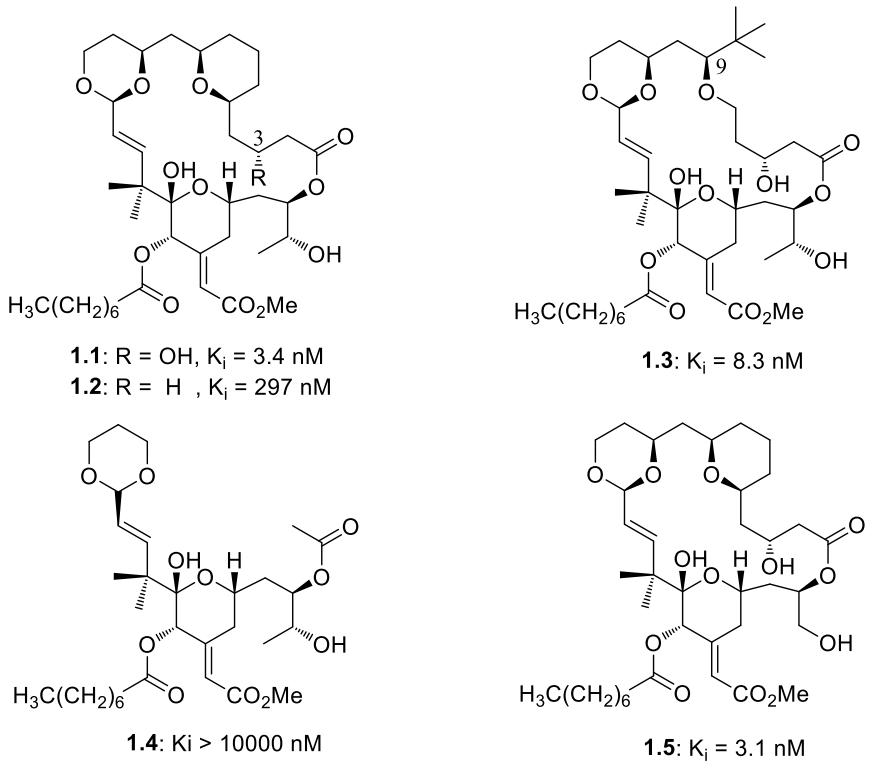


Figure 1.9 Wender's Bryostatin Analogs

evaluated in the NCI 60 tumor cell panel and was generally found to be as potent as bryostatin 1. This indicates that removal of the C26 methyl group has little effect on binding.

Keck's Analog Work

The key reaction in the Keck lab bryostatin program is the pyran annulation, which was developed by Dr. Covel for the synthesis of 2,6-disubstituted tetrahydropyrans in the context of complex polyketide natural product synthesis.[63] The substrate for this reaction can be prepared in a single step by catalytic asymmetric allylation of an aldehyde **1.6** with a bifunctional stannane **1.7** to produce a β-hydroxyallylsilane **1.8**[64] (Figure **1.10**). Pyran annulation of **1.8** with a second aldehyde **1.9** is carried out under Lewis acidic conditions (TMSOTf at −78 ^{o}C) to yield a tetrahydropyran **1.10** as a single diastereomer. The pyran annulation is a powerful method for the rapid synthesis of bryostatin like compounds. For example, removal of TBDPS group of **1.10** and subsequent oxidation of the primary alcohol provides an aldehyde **1.11**, which is suitable for a second pyran annulation with another β-hydroxyallylsilane **1.12**. Using this method, a simplified bryostatin spacer domain **1.13** was prepared in only 8 steps from commercially available compounds.

Early Keck Group Analogs

The Keck group has directed much effort towards the synthesis of bryostatin 1 and simplified analogs. The development of the pyran annulation enabled the disconnection of bryostatin-like compounds into simplified fragments. The Keck group's

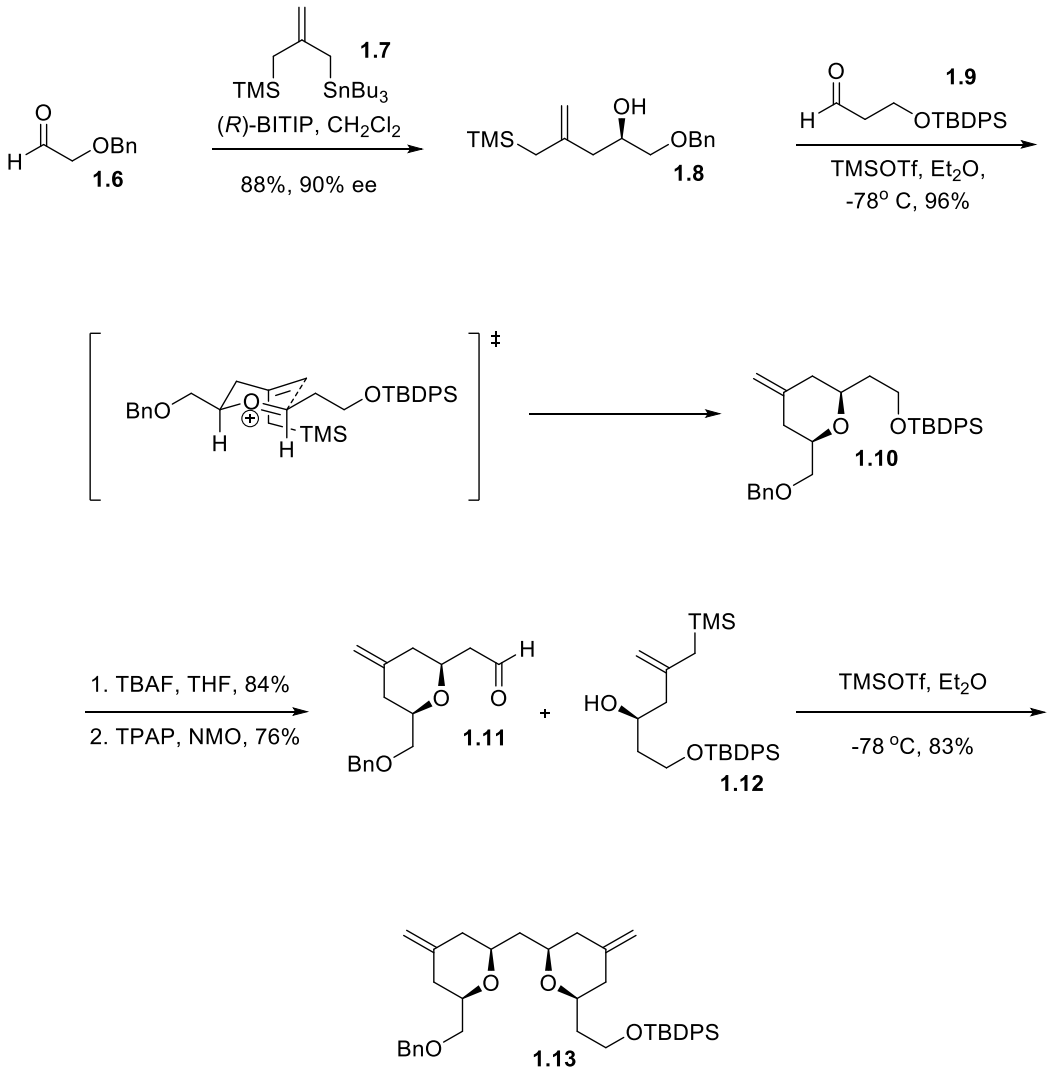


Figure 1.10 The Pyran Annulation

first bryostatin analog was synthesized by Dr. Ahn Truong.[65]At that time, the function of the C20 ester and enoate remained unknown, so the C21 enoate was omitted and C20 was left at the ketone oxidation state. This analog was designed so that further functionalization would allow for the installation of all elements of the natural bryostatinC-ring at a late stage. The retrosynthesis of this analog is shown in Figure **1.11**.

The synthesis began with the BOM protection of commercially available (R)-(+)-isobutyl lactate **1.20**, followed by partial reduction to aldehyde **1.21** (Figure **1.12**). 1, 2-Chelation controlled allylation of aldehyde **1.21** provided alcohol **1.22** as a single diastereomer via ^{1}HNMR.[66] PMB ether formation followed by ozonolysis provided aldehyde **1.23**. 1,3-Chelation controlled allylation[67] and subsequent protection of the newly formed alcohol as the TBS ether provided olefin **1.24** as a 5:1 mixture of diastereomers. Hydroformylation of **1.24** using Buchwald's conditions[68] provided the homoelongated aldehyde **1.25** in high yield. Prenyl indium addition[69] followed by oxidation with PCC[70] gave ketone **1.19** in an overall yield of 46% over 3 steps.

The completion of the C-ring fragment (Figure **1.13**) was achieved by ozonolysis of **1.19** and olefination to form thiol ester **1.28**.[71] TBS deprotection and dehydration of the resulting hemiketal yielded glycal **1.29**. Selective reduction yielded aldehyde **1.17**. Pyran annulation with β-hydroxyallylsilane **1.18**, afforded pyran **1.31**. TBDPS deprotection and oxidation under Ley conditions[72] afforded aldehyde **1.16** (Figure **1.14**). Pyran annulation with β-hydroxyallylsilane **1.15** yielded tricycle **1.32** bearing all of the carbons of the target compound.

Functionalization of the C-ring was accomplished first by epoxidation and *in-situ* opening with methanol, followed by Ley[72] and Pinnick[73] oxidations to the carboxylic

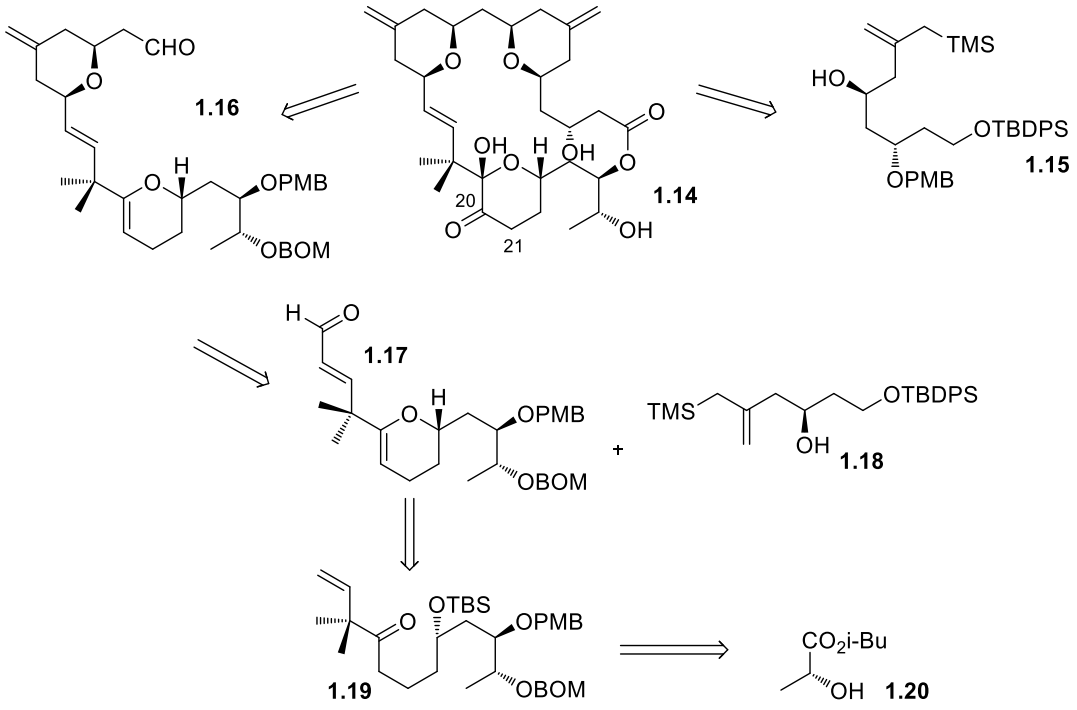


Figure 1.11 Retrosynthesis of Ahn's Analog

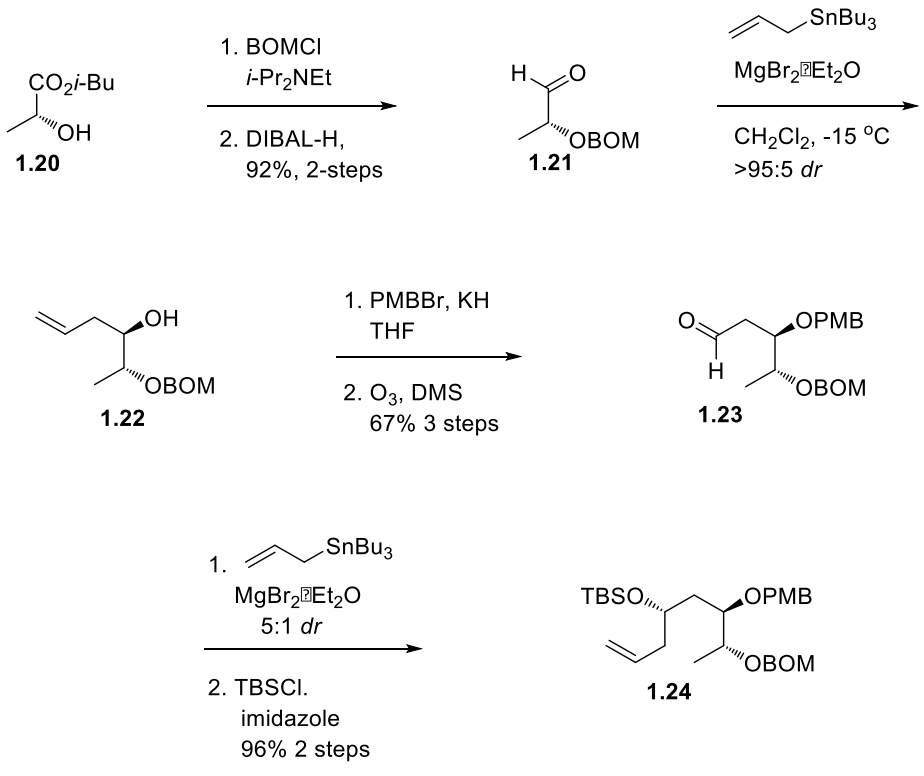


Figure 1.12 Synthesis of the C-ring Fragment **1.24**

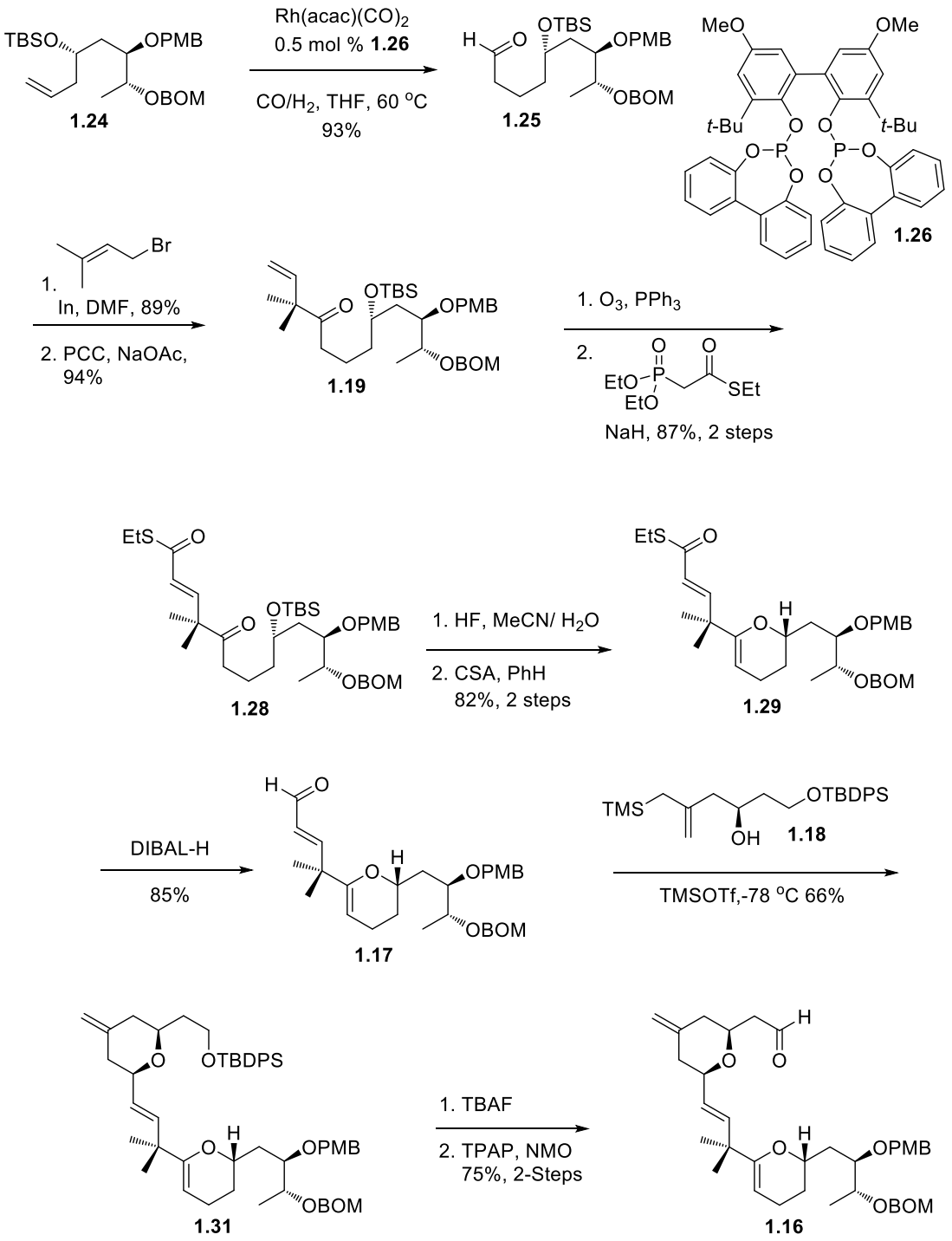


Figure 1.13 Elaboration of Anh's Analog

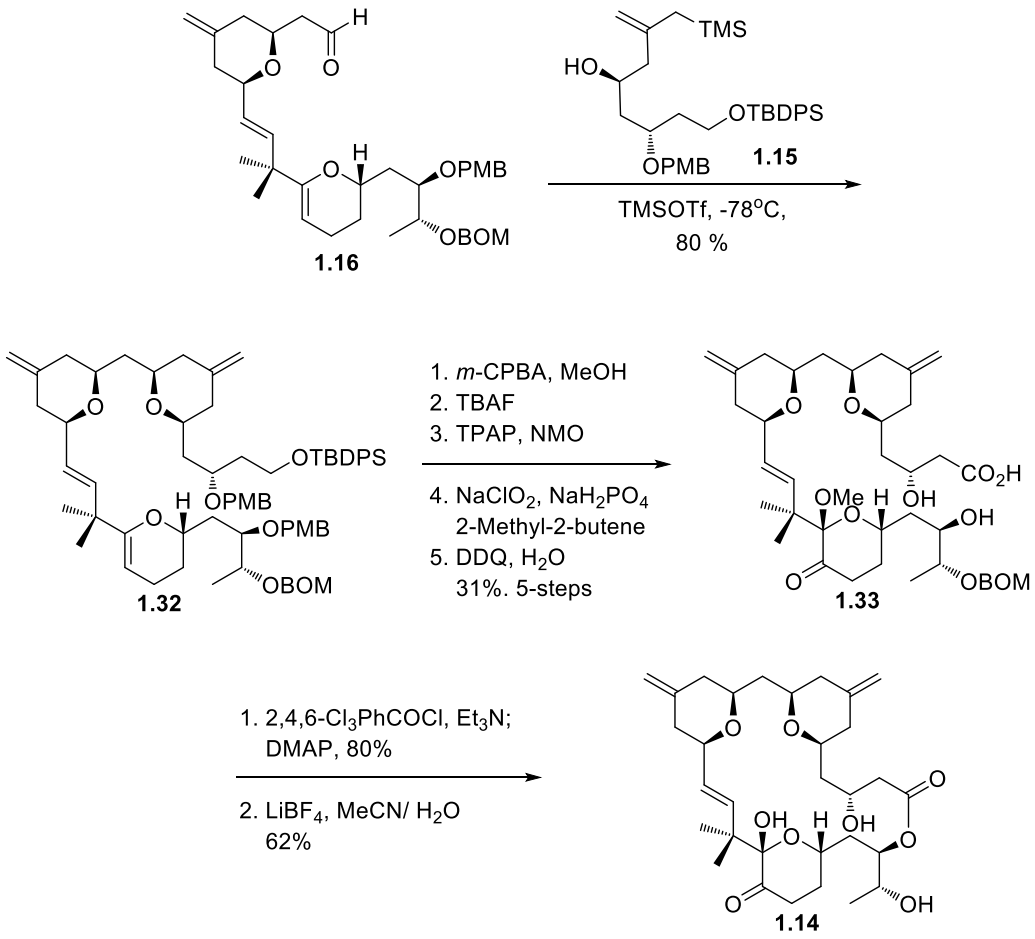


Figure 1.14 Completion of Ahn's Analog

acid, and finally PMB deprotection produced *seco*-acid **1.33** in 31% yield over 5 steps. Completion of the analog **1.14** was achieved via Yamaguchi macrolactonization[74] and subsequent BOM and methyl ketal deprotection using Lipshutz's conditions.[75] Even though the analog contains the 3 crucial pharmacophores as determined by Wender, the analog demonstrated low binding affinity for PKC α, 546 nM. This compound demonstrates the requirement of the C20 ester and C21 enoate for high binding affinity.

Dr. Carina Sanchez finished the functionalization of Ahn's analog via attachment of the C21 enoate and C20 ester (Figure **1.15**). Protection of C3 of **1.34** as a TBS ether followed by an aldol reaction with methyl glyoxylate and subsequent dehydration with Burgess reagent[76] produced enoate **1.35**. Luche reduction[77] of the C20 ketone followed by acylation gave C20 ester **1.36**. Deprotection of the C26 BOM ether and C20 ketal was once again accomplished with Lipshutz's conditions ($LiBF_4$, MeCN/ H_2O)[75] yielding analog **1.37**. Analog **1.37** has a high affinity for PKC α, 0.7 nM, indicating that the C20 ester and C21 enoate are required for high affinity.

Merle 23, a Highly Potent PKC Activator

Graduate students Matt Kraft and Wei Li developed a more convergent route for the assembly of bryostatin analogs, resulting in the synthesis of Merles 21, 22, 23, which only differed with respect to the C20 ester. All showed similar binding affinities for PKCα which were essentially identical to that of bryostatin 1. Merle 23, which bears the natural C-ring of bryostatin 1, was found to be the most active of this series with a K_i of 0.7 nM. Further improvements to the route were made by Tom Cummins and Mark Petersen, which allowed for the synthesis of biologically significant quantities of the

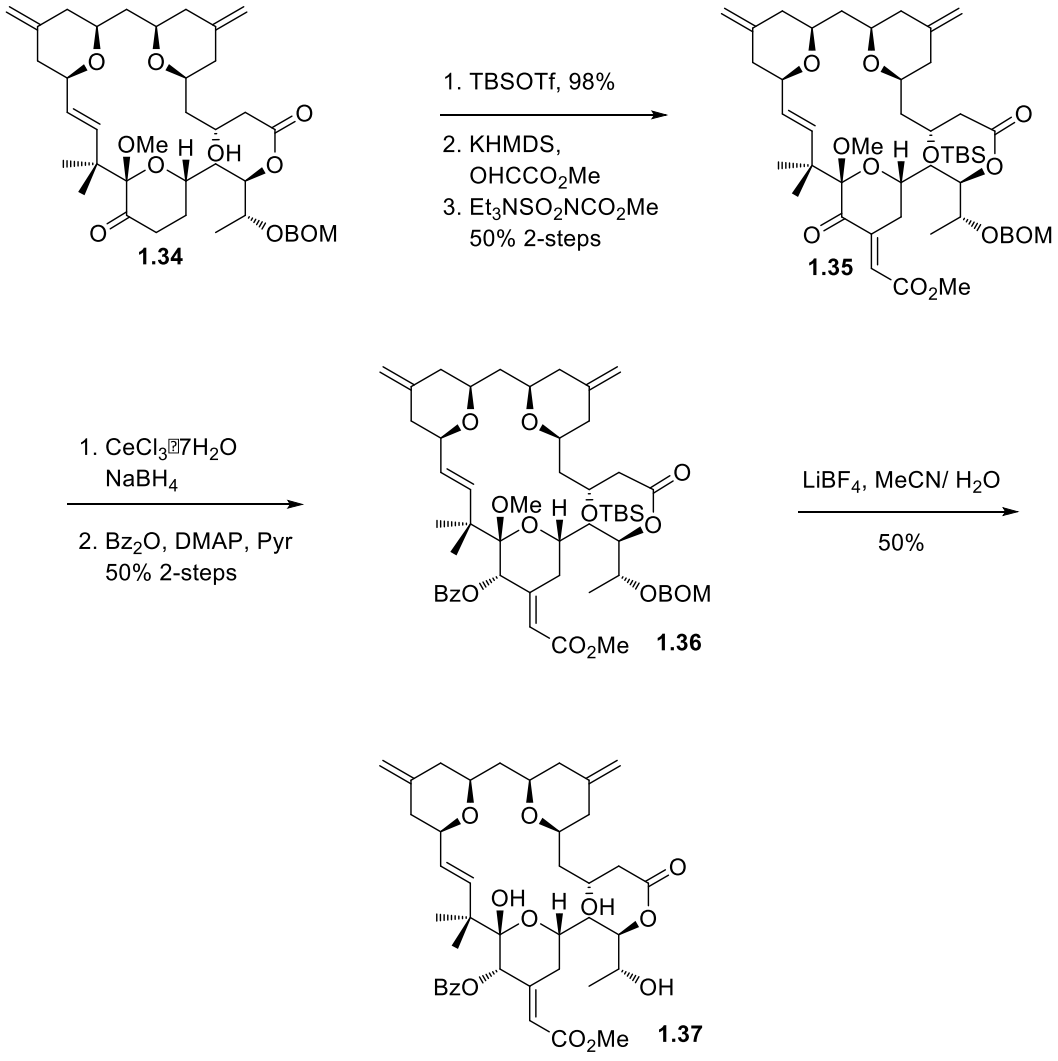


Figure 1.15 Synthesis of Carina's Analog **1.37**

compound as well as many other analogs. The final route is considerably more convergent and efficient; the retrosynthesis is shown in Figure **1.16**.

Synthesis of the fully functionalized C-ring fragment began with the alkylation of the enolate of methyl isobutyrate **1.44** with allyl bromide, followed by Wohl-Zeigler bromination (Figure **1.17**).[78] Displacement of the bromide of **1.46** with potassium acetate followed by transesterification and protection of the free alcohol with a TBS ether gave ester **1.47**. Hydrolysis of **1.47** afforded carboxylic acid **1.40**. Even though this route was low yielding, large quantities of carboxylic acid **1.40** could be produced inexpensively. Alcohol **1.41** was produced via the same allylation procedure as described in Figure **1.12**. Union of the two pieces was accomplished via Keck-Boden modified Steglich esterification conditions[79] to produce ester **1.48**. Homoelongation of **1.48** began with hydroboration/ oxidation[80] to produce alcohol **1.49**. Parikh-Doering oxidation[81] followed by Wittig olefination[82] afforded olefin **1.39** in good overall yield. The Rainier metathesis reaction[83] was then used to cyclize olefin **1.39** to glycal **1.50**, allowing for the production of gram quantities of this intermediate.

Functionalization of glycal **1.50** proceeded via epoxidation with magnesium monoperoxy phthalate, followed by *in-situ* epoxide opening with methanol (Figure **1.18**). Oxidation of the intermediate alcohol via Ley conditions[72] gave C20 ketone **1.51**. Aldol reaction of **1.51** with methyl glyoxylate under mild conditions produced enoate **1.52** with good selectivity.[76] Luche reduction[77] of enoate **1.52** proceeded with high selectivity due to the bulky substituent at C19. Esterification of the intermediate alcohol gave ketal **1.53**. Deprotection of the C15 TBS followed by subsequent oxidation[72] provided aldehyde **1.54**. Protective group exchange of the C25 PMB for TBS was accomplished in high

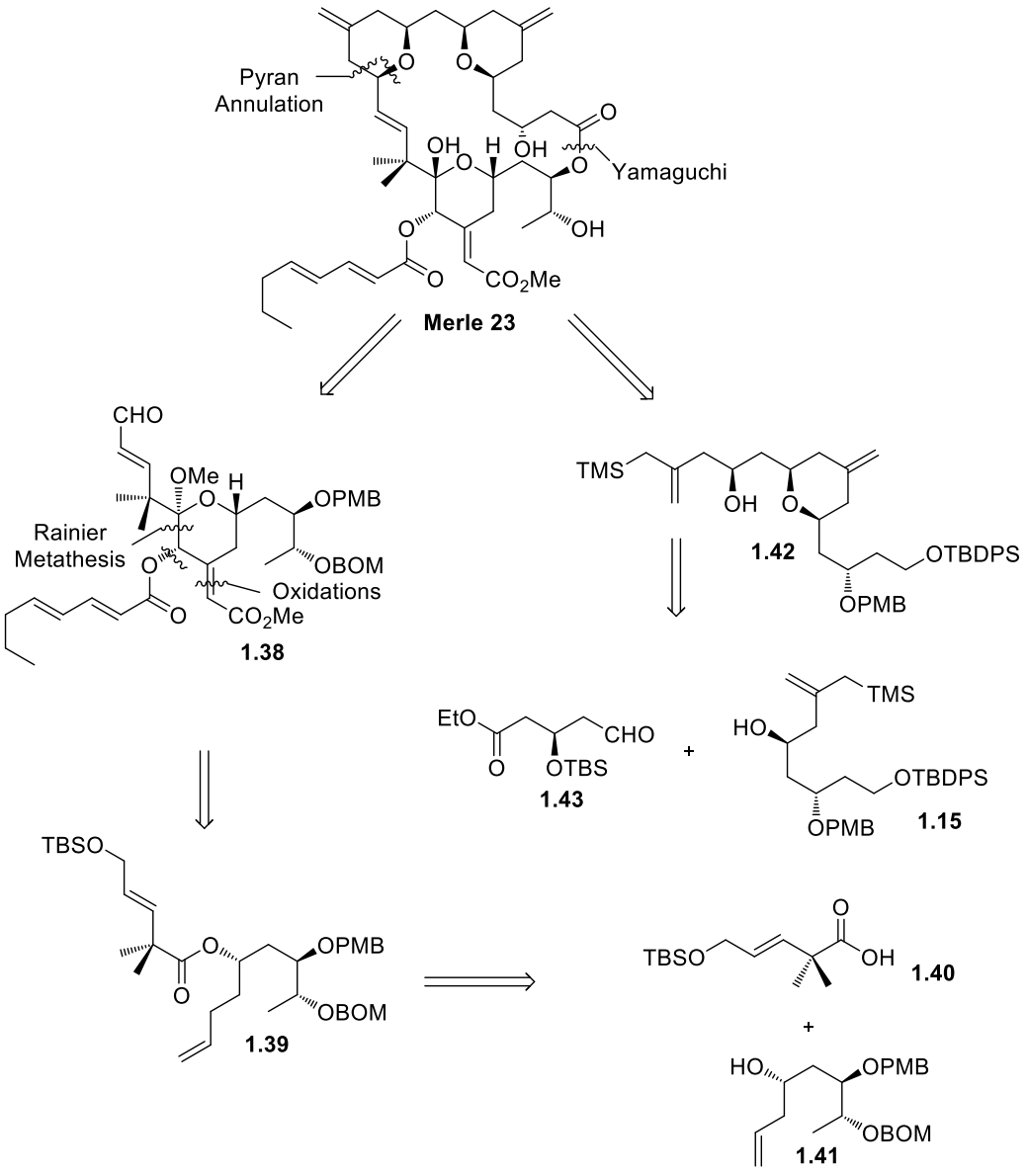


Figure 1.16 Retrosynthesis of Merle 23

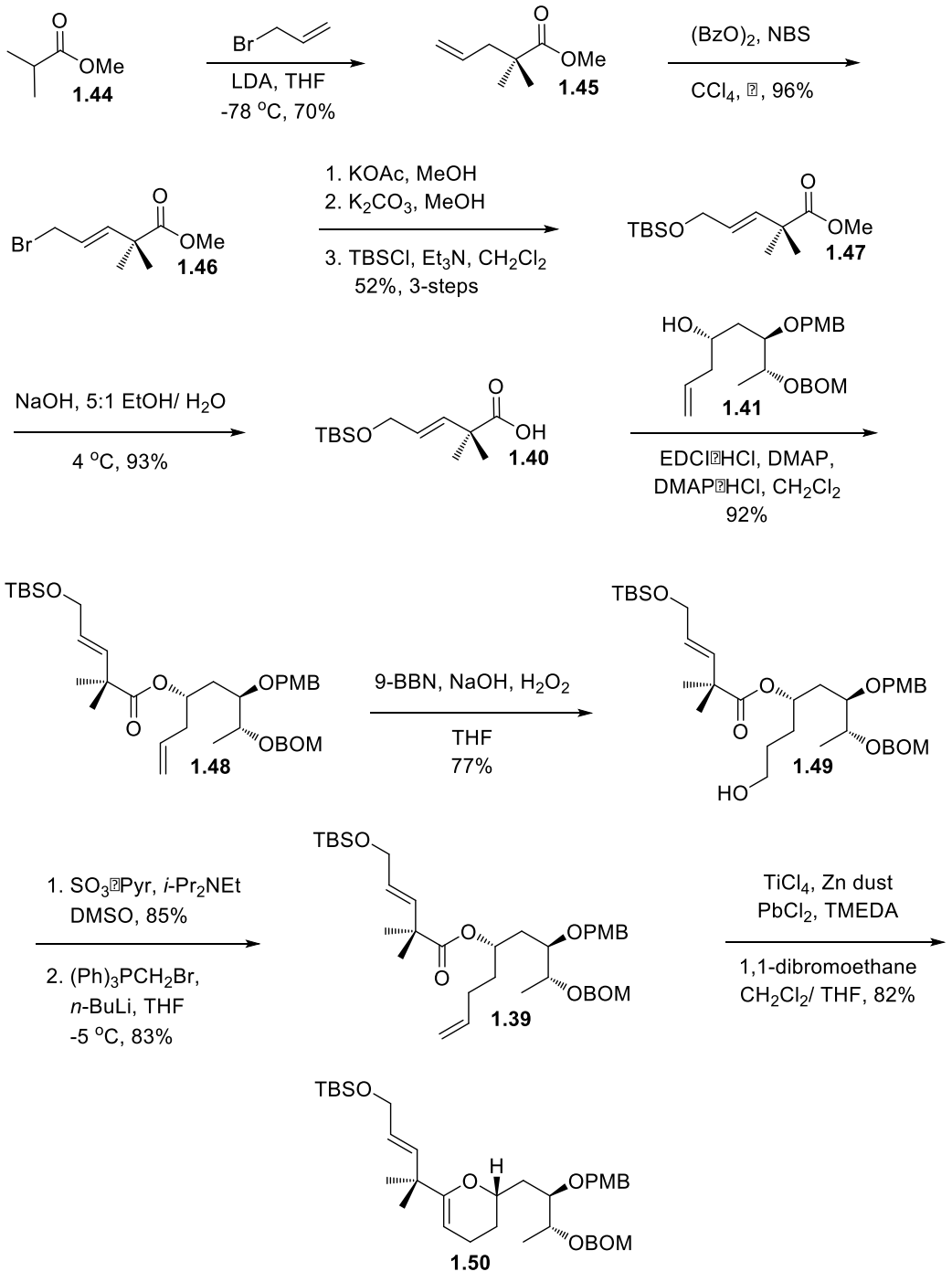


Figure 1.17 Synthesis of Glycal **1.50**

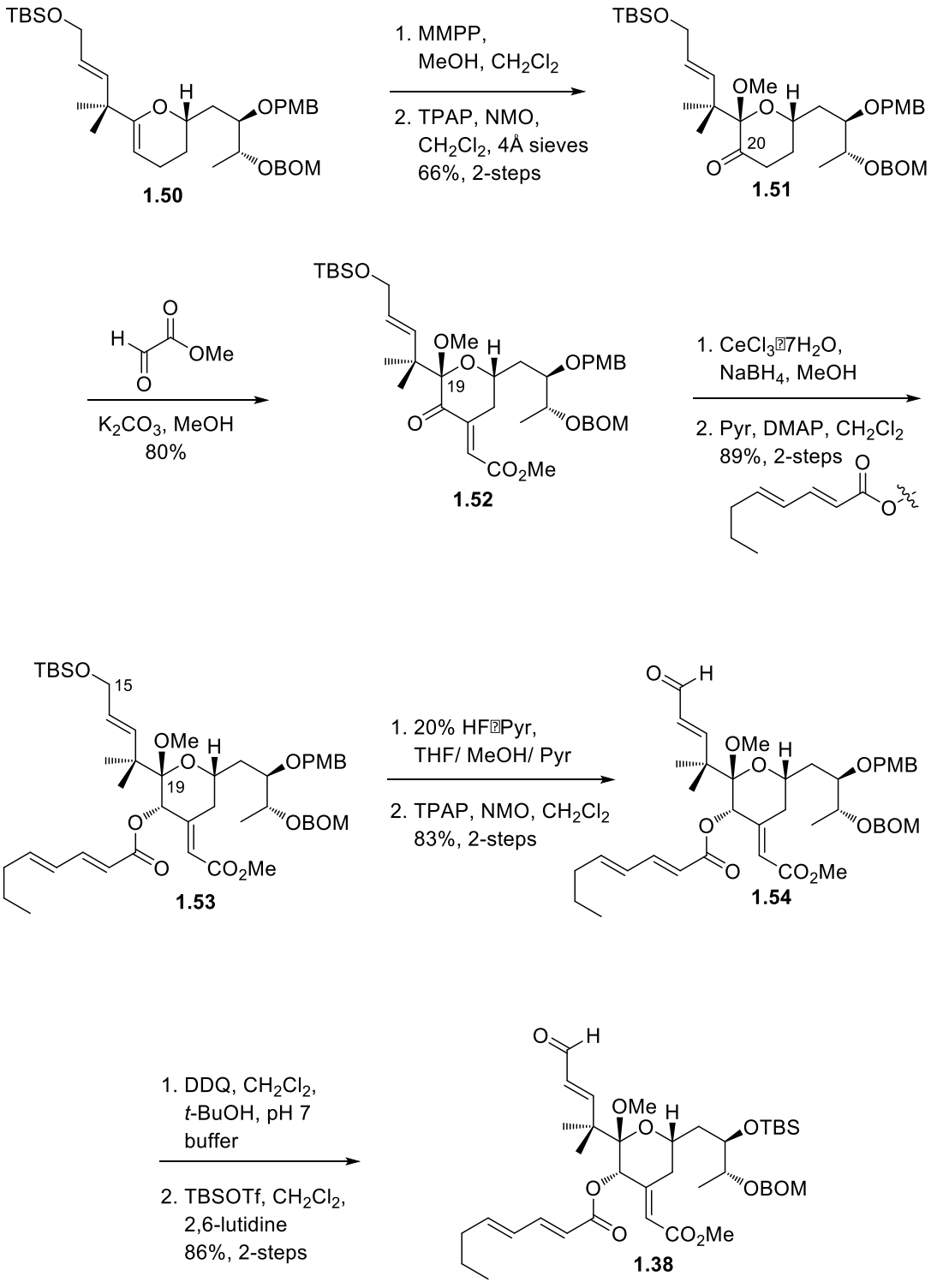


Figure 1.18 Completion of Fully Functionalized C-ring **1.38**

yield to give the fully functionalized C-ring fragment **1.38**.

The synthesis of A-ring fragment **1.42** began with mono protection of 1,3-propanediol as the TBDPS ether, followed by oxidation of the free alcohol to the aldehyde (Figure **1.19**). Catalytic asymmetric allylation of aldehyde **1.9** with allylstannane using (*R*)-BITIP catalyst afforded homoallylic alcohol **1.56** in excellent yield and a 97% ee.[64]

Protection of the free hydroxyl group of alcohol **1.56** as the PMB ether, followed by ozonolysis, produced aldehyde **1.57** in moderate yield. 1,3-chelation controlled allylation of aldehyde **1.57** with stannane **1.7** gave β-hydroxyallylsilane **1.15** as a single diastereomer in moderate yield.[84] Synthesis of the functionalized aldehyde for the pyran annulation began with the Michael addition of benzyl alcohol into acrylonitrile **1.58** (Figure **1.20**). Blaise reaction[85] of the resulting nitrile with ethyl bromoacetate gave keto-ester **1.59**. Noyori hydrogenation[86] produced the β-hydroxy ester **1.60** in excellent yield with a 99% ee. TBS protection of the free alcohol, removal of the benzyl ether via hydrogenolysis, and Parikh-Doering[81] oxidation produced aldehyde **1.43**.

Finally, pyran annulation of aldehyde **1.43** with β-hydroxyallylsilane 1.**15** gave pyran **1.62** in good yield and as a single diastereomer.[87] Removal of the C11 TBS group in the presence of the C1 TBDPS group of **1.62** was not selective enough for producing large quantities of advanced intermediates. However, deprotection of both silyl groups followed by selective re-protection of C1 provided access to secondary alcohol **1.63** (Figure **1.21**). Protection of the free alcohol in **1.64** as the TMS ether, followed by a Bunnelle reaction,[88] provided β-hydroxyallylsilane **1.42** in good overall yield. Union of the two fragments was achieved by pyran annulation, producing the tricyclic compound

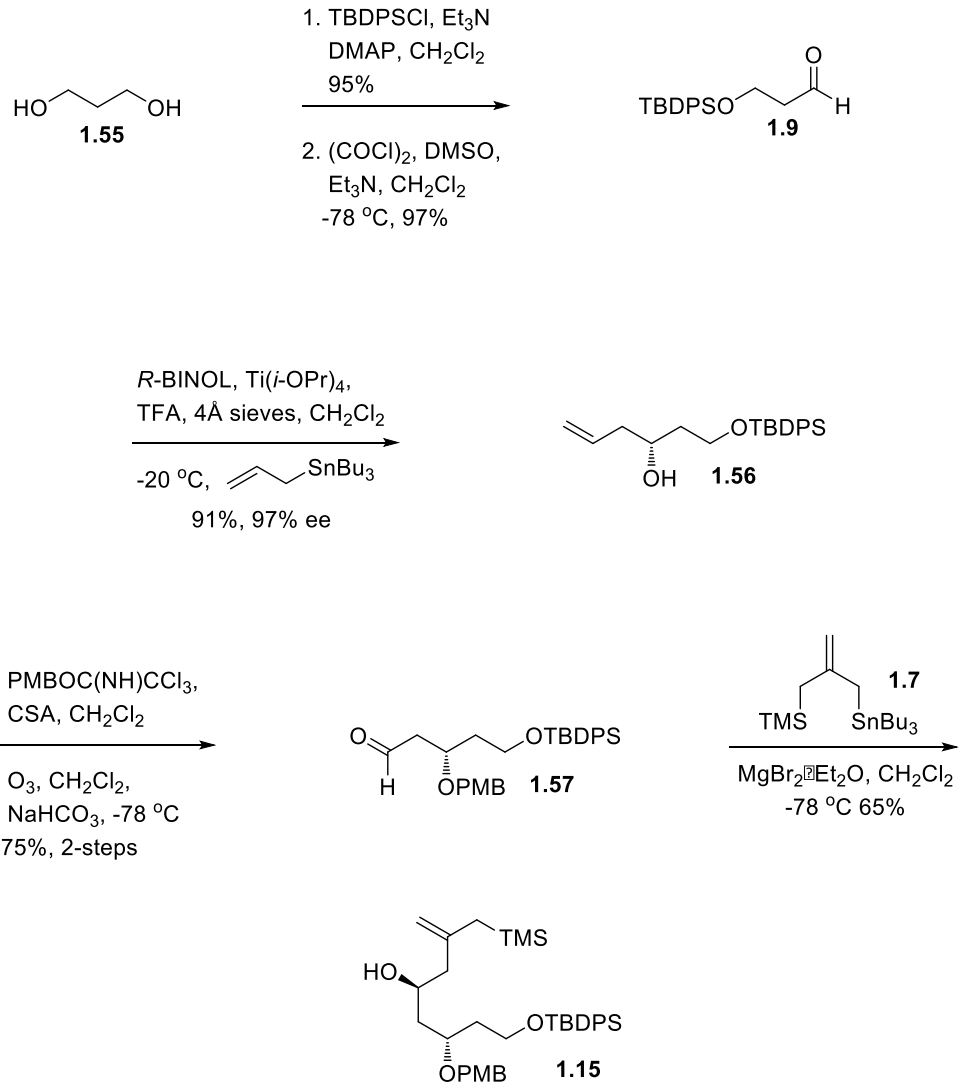


Figure 1.19 Synthesis of A-ring β-hydroxyallylsilane

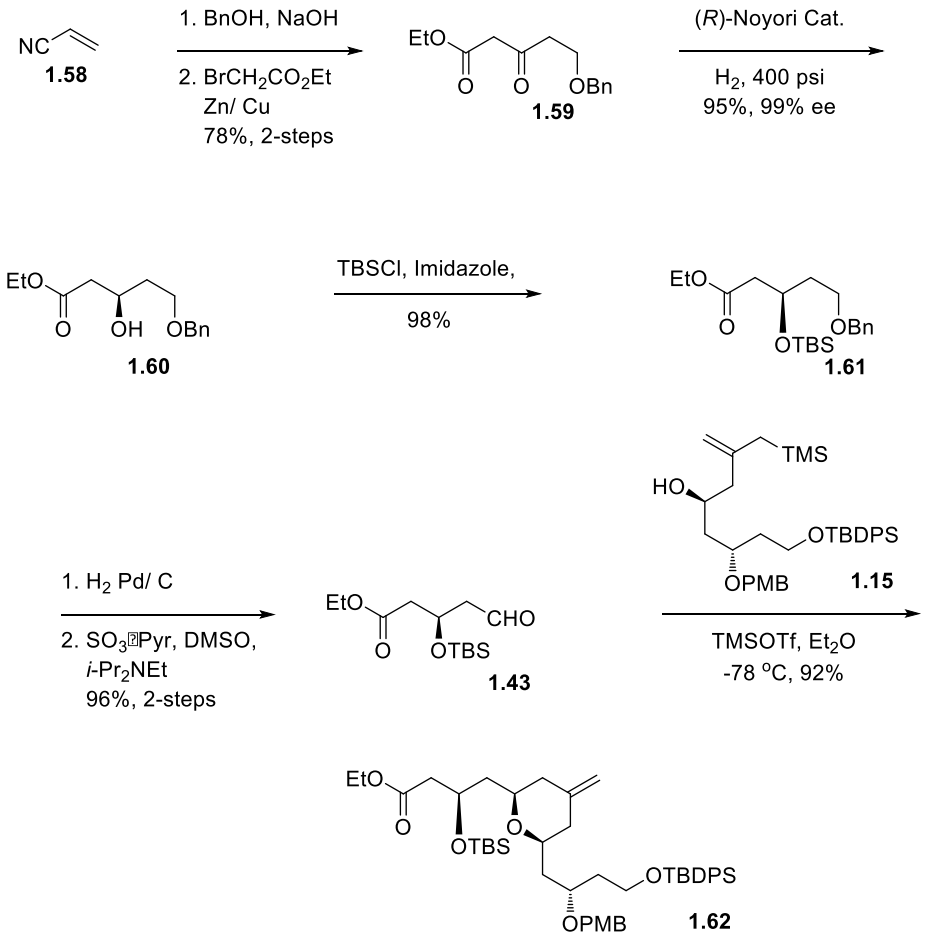


Figure 1.20 Completion of A-ring Pyran **1.62**

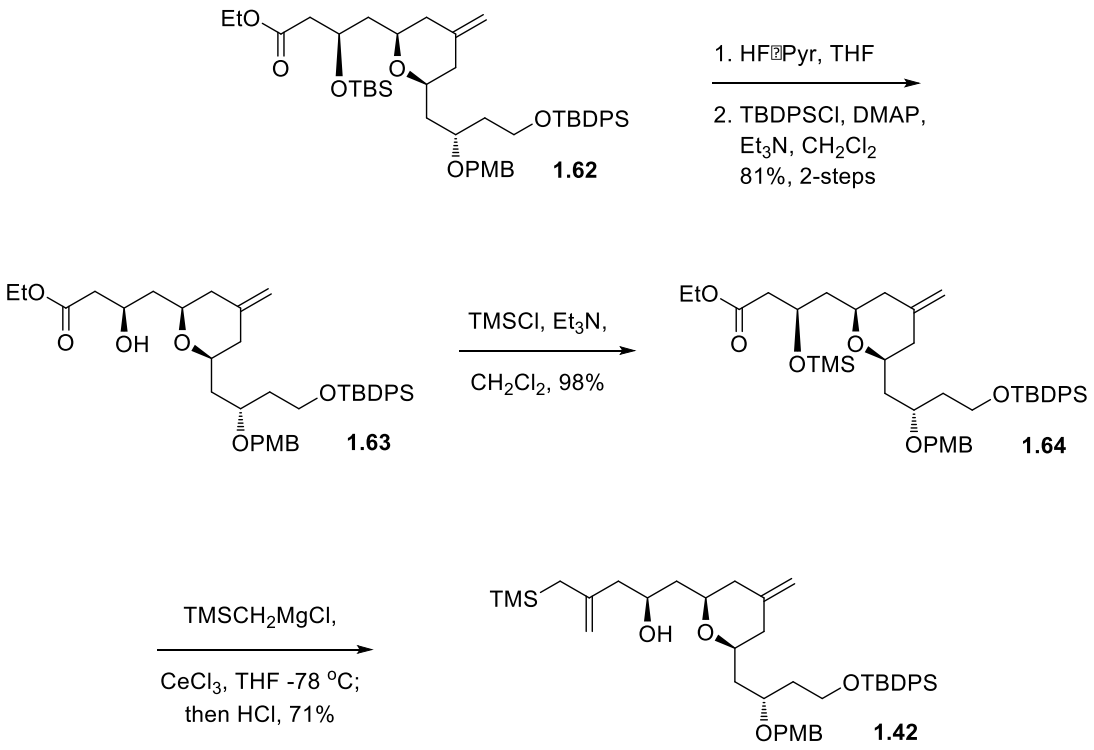


Figure 1.21 Completion of β-hydroxyallylsilane **1.42**

1.65 in good yield and as a single diastereomer (Figure **1.22**).[87]

Functionalization of C1 was achieved by selective deprotection of the C1 TBDPS in the presence of the C25 secondary TBS with AcOH/ TBAF, affording alcohol **1.66** (Figure **1.23**).[89] Parikh-Doering[81] and Pinnick[73] oxidations produced the C1 carboxylic acid **1.67**. Deprotection of the C25 TBS group was accomplished with HF•pyridine and Yamaguchi macrolactonization[74] was used to cyclize the *seco*-acid to give macrolactone **1.68** in moderate yield. Deprotection of the C3 PMB group was accomplished with buffered DDQ and both the C19 methyl ketal and C26 BOM groups were cleanly removed with $LiBF_4$ in MeCN/ H_2O[75] to give Merle 23.

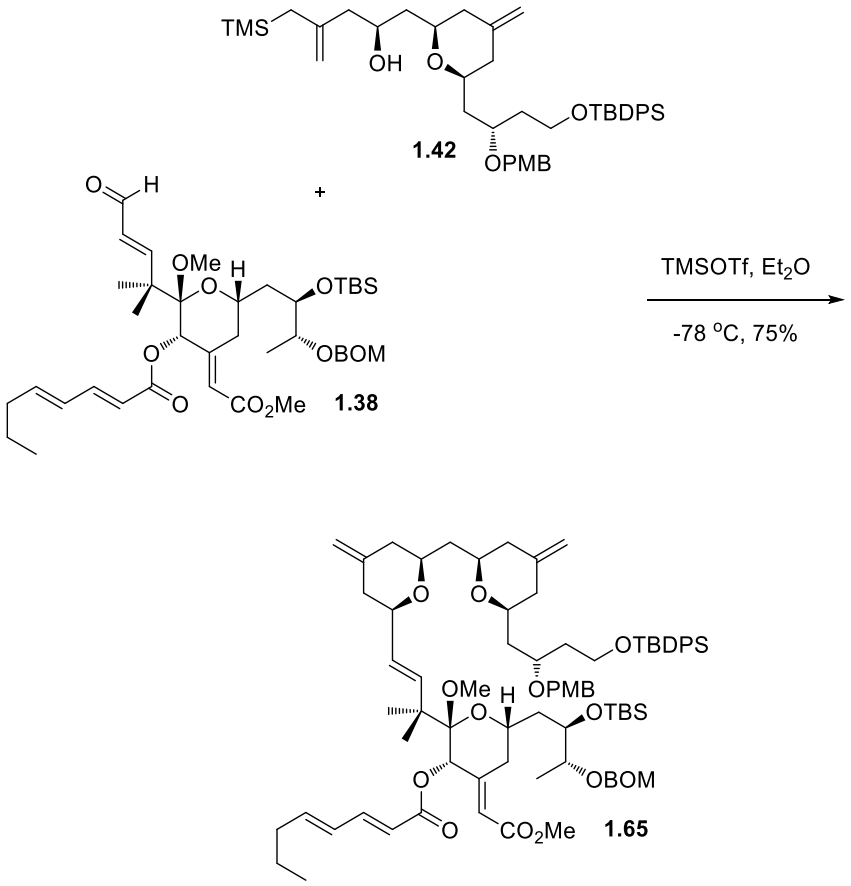


Figure 1.22 Pyran Annulation

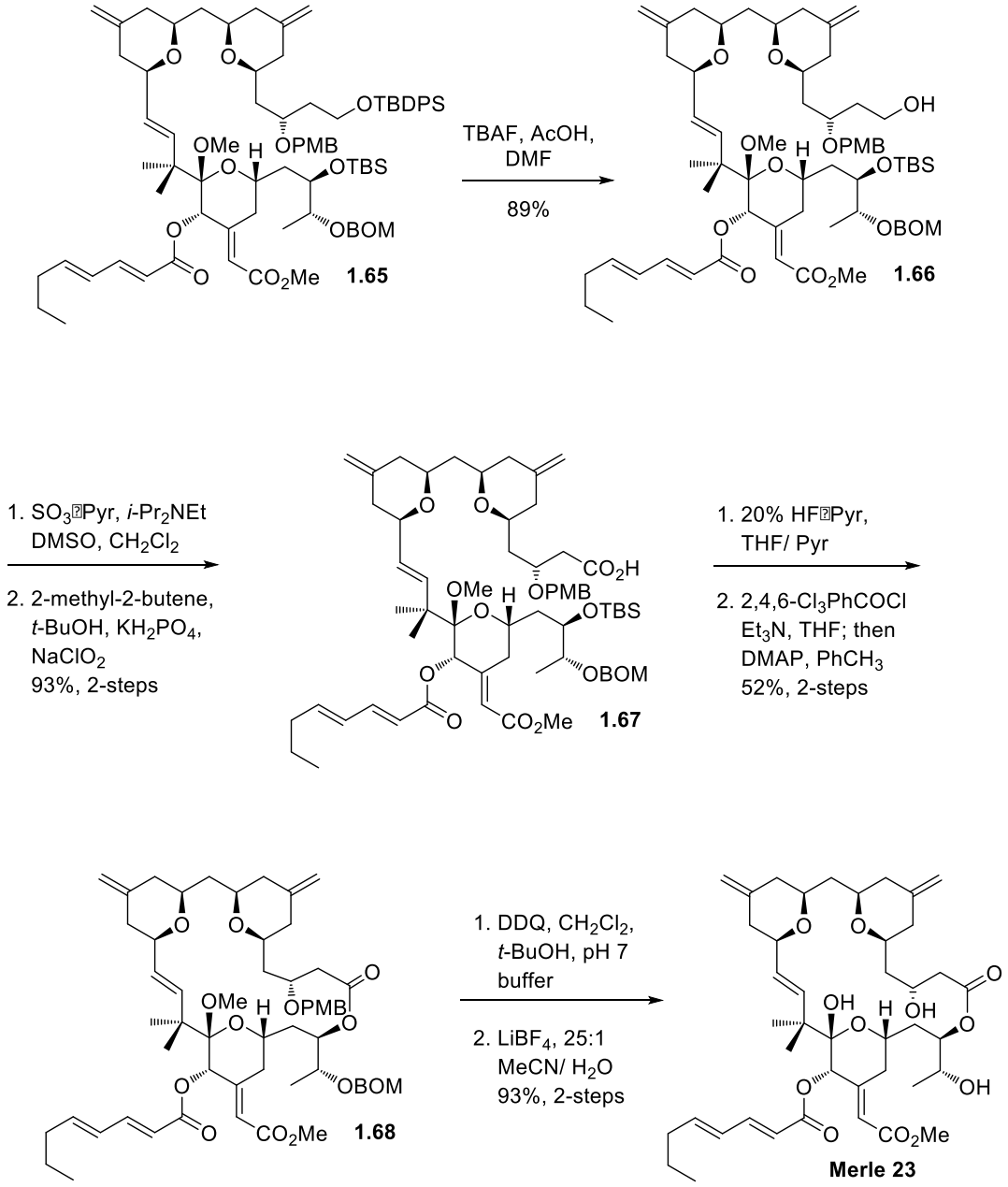
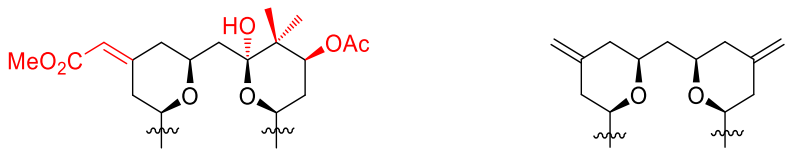


Figure 1.23 Completion of Merle 23

Biology of Merle 23

An assumption was made that Merle 23 and similar compounds would exhibit similar biological activity to that of bryostatin 1 since all C-ring pharmacophores are held into the same position in Merle 23 by the spacer domain as in bryostatin 1. In Merle 23, the C8 *gem*-dimethyl and C9 ketal are deleted, and the C7 acetate and C13 enoate are replaced by methylene groups (Figure **1.24**). Up until this point, biological evaluation of bryostatin analogs had solely focused on PKC binding affinity and activity in cancer cell lines. Could deletion of substituents on the spacer domain dramatically change a PKC ligands biological activity?

Both Merle 23 and bryostatin 1 are high affinity ligands for PKC, K_i = 0.70 nM,[90] and K_i = 1.35 nM, respectively; however, binding affinity for PKC does not correlate with tumor promoting ability. Bryostatin 1 does not cause tumor promotion and counteracts the effects of PMA, a potent tumor promoter. Would simplified bryostatin analogs act like bryostatin or would they simply act like PMA and cause tumor promotion? A vital collaboration with Dr. Peter Blumberg at the NIH was formed with the Keck group to investigate this biological question and facilitate the rational design of future analogs.



Bryostatin 1 Merle 23

Figure 1.24 Bryostatin 1 and Merle 23 Top Halves.

The response of U937 leukemia cells towards PKC ligands has been used to differentiate between tumor promoting and nonpromoting ligands (Figure **1.25**).[91] Phorbol esters such as PMA induce attachment of U937 cells and inhibit proliferation. Bryostatin 1 has a much less dramatic effect. Bryostatin 1 also antagonizes and reverses the effects of PMA in a dose-dependent manner. Merle 23 was shown to exhibit identical effects as those of PMA in the proliferation and attachment assays.[90] Results in K562 leukemia cells are similar to those seen in the U937 cells.

Results with Merle 23 in the LNCaP human prostate cancer cell line were different from those of PMA.[92] PMA induces apoptosis, TNF α secretion, and inhibits proliferation, while bryostatin 1 and Merle 23 failed to illicit these responses (Figure **1.26**). Upon further examination, the effects of Merle 23 in LNCaP cells were found to be very complex depending on the PKC isozyme and experimental conditions (Figure **1.26**). It was found that PMA causes translocation of PKC δ to the cell membrane, but Merle 23 and bryostatin 1 tended to cause translocation to internal membranes. In the presence of the protease inhibitor MG-132, Merle 23 displays PMA-like behavior in LNCaP cells, whereas bryostatin 1 behavior is unaffected. Differential effects were also seen with respect to activation, translocation, and downregulation of specific PKC isozymes. From these studies, it is apparent that Merle 23 is a distinct compound from either bryostatin 1 or PMA in its biological effects in LNCAP cells.

The Polarity Hypothesis

After the biological evaluation of Merle 23, the Keck group turned its attention towards examining the effect of substitution in the A-B ring region of bryostatin 1 in an

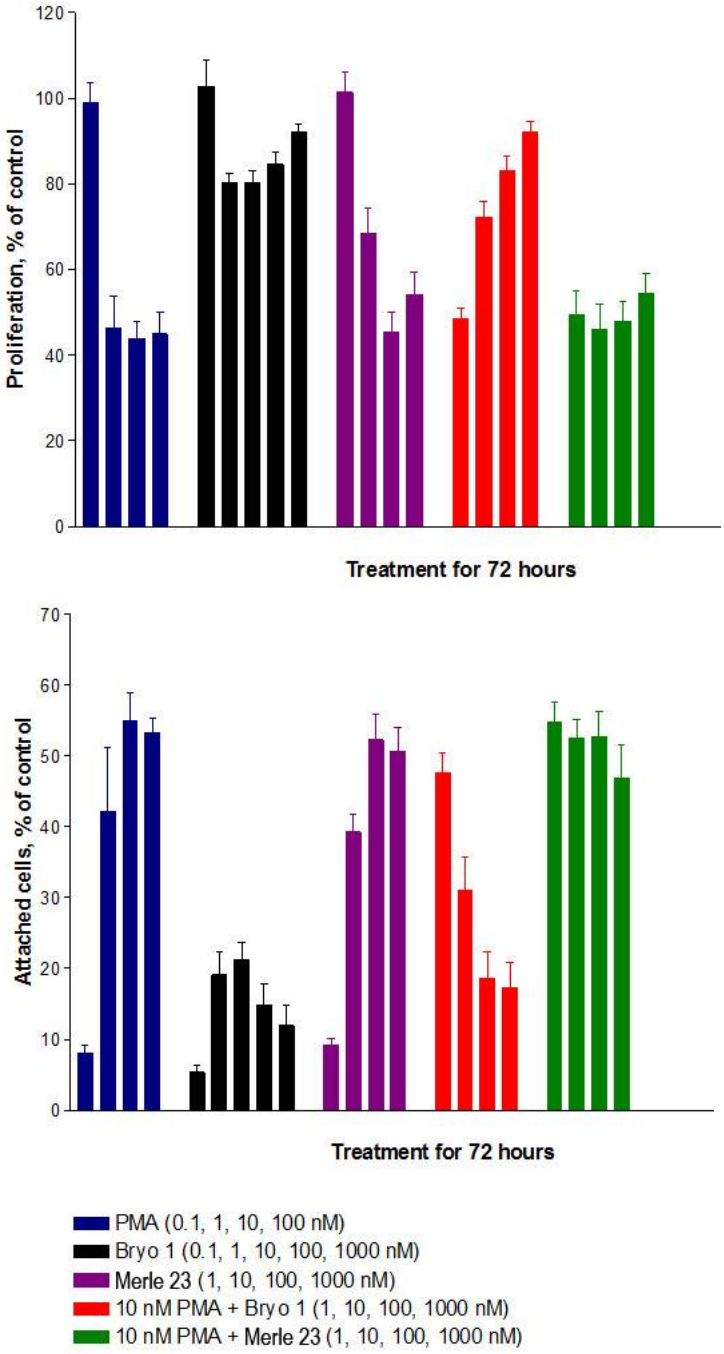
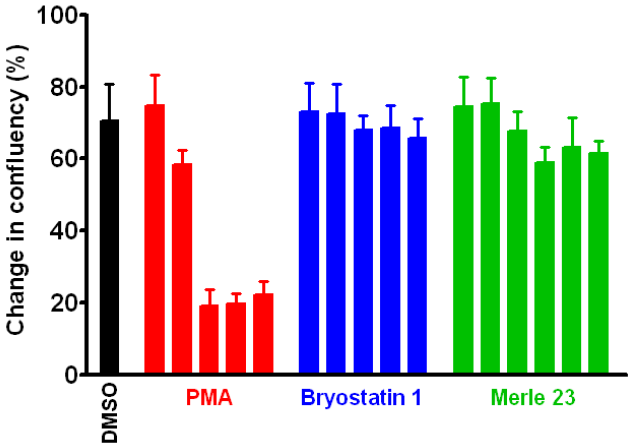
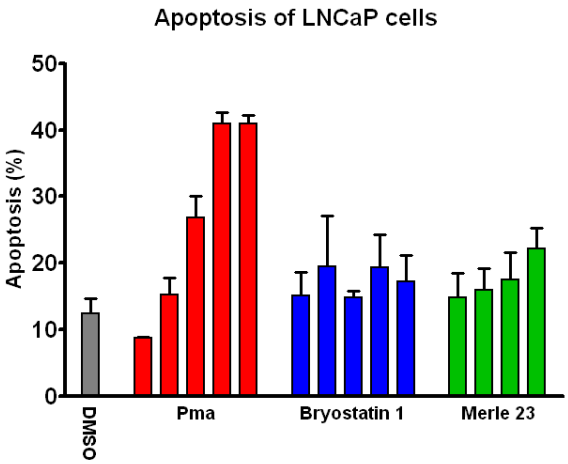


Figure 1.25 Effects of PKC Ligands in U937 Cells



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

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

LNCaP Apoptosis Assay

Figure 1.26 Effects of PKC Ligands in LNCaP Prostate Cancer Cells

attempt to define what characteristics influence if a PKC ligand behaves like bryostatin or PMA. A series of analogs were synthesized wherein the A- and B-ring substituents were systematically removed from bryostatin 1 or introduced in Merle 23, and the response in U937 leukemia cells measured (Figure **1.27**).[93] From this study, it was determined that no single substituent acted as a 'switch' that would determine if an analog would behave like PMA or Bryostatin. However, it was observed that analogs that bore two or more polar groups on the top-half tend to behave more "bryo-like" in assays, but the more lipophilic analogs tend to act more PMA-like. From these studies, it is clear that the top-half of bryostatin does not merely serve as a "spacer domain", but has dramatic influences upon the biology of these compounds. The C-ring of bryostatin is thought to interact with the binding domain of PKC, while the top-half is thought to produce a hydrophobic surface on top of the C1 domain. The ligand-enzyme complex then translocates to cellular membranes. Interaction of the hydrophobic surface of the PKC-ligand complex with these membranes is thought to dictate biological activity. The C-ring motif in bryostatin 1 is very complex, requiring 20 linear steps to synthesize it. Substitution of a suitable surrogate for the C-ring that is less complicated to synthesize would be highly desirable for the synthesis of new PKC activators.

Diacylglycerols and Marquez Lactones

1,2-Diacyl glycerols (DAG) are the endogeneous ligands for PKC signaling. Binding of extracellular ligands to G-coupled protein receptors initiates signaling cascades that result in phospholipase C (PLC) hydrolyzing membrane bound phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate

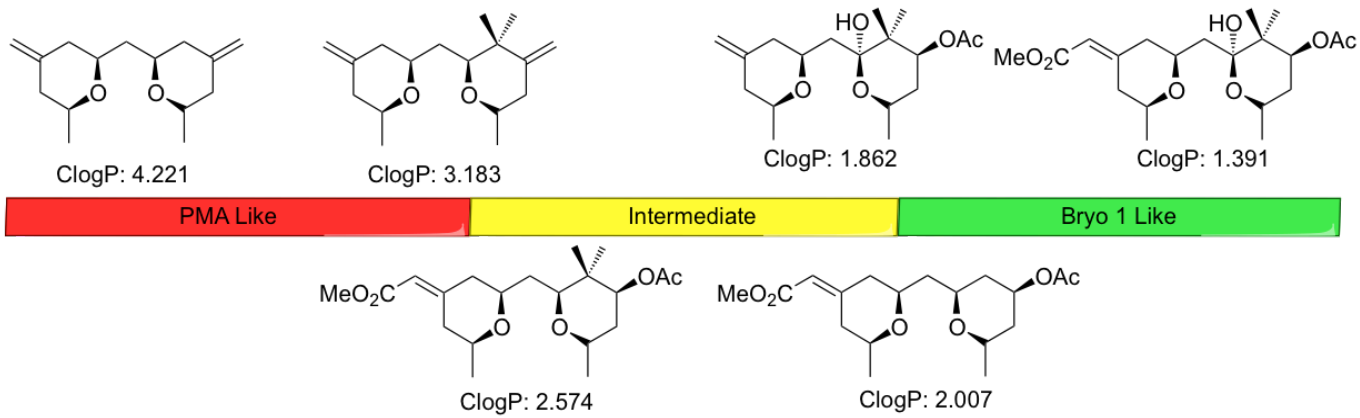
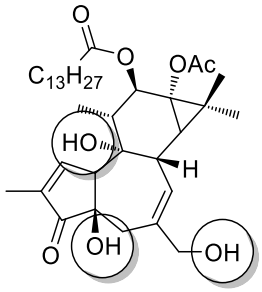


Figure 1.27 Polarity Hypothesis

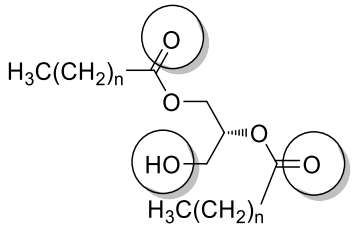
(IP_3). IP_3 causes the release of Ca^{+2}, then DAG, in combination with Ca^{+2}, activates PKC, which then phosphorylates threonine or serine residues of target proteins.

In order to study structure-activity relationships, Victor Marquez and coworkers at the NCI chose to study DAG analogs since synthesis of large numbers of these compounds was much more feasible than modifying potent PKC ligands such as bryostatins or phorbol esters. Early models[60] of PKC-ligand interactions identified a three point pharmacophore domain on the ligand that was responsible for binding (Figure **1.28**). Despite the similarities of the pharmacophores, PMA is 1000 times more potent than DAG.

In order to increase the binding affinity of DAGs, Marquez reasoned that constraining the DAG pharmacophore into a 5-membered ring would reduce the entropic penalty for ligand binding.[94] In order to accomplish this, an extra carbon was added to the DAG scaffold. The preferred configuration of natural DAGs is (*S*); the (*R*) enantiomer is basically inactive (Figure **1.29**). Cyclization of (*S*) DAG results in the orientation of the lactone carbonyl, which is crucial to binding, changing to an inactive configuration. Cyclization of (*R*) DAG results in a lactone with the carbonyl in the proper orientation for



Phorbol-12-myristate-13-acetate (PMA)



Diacylglycerol

Figure 1.28 Three Point Pharmacophores

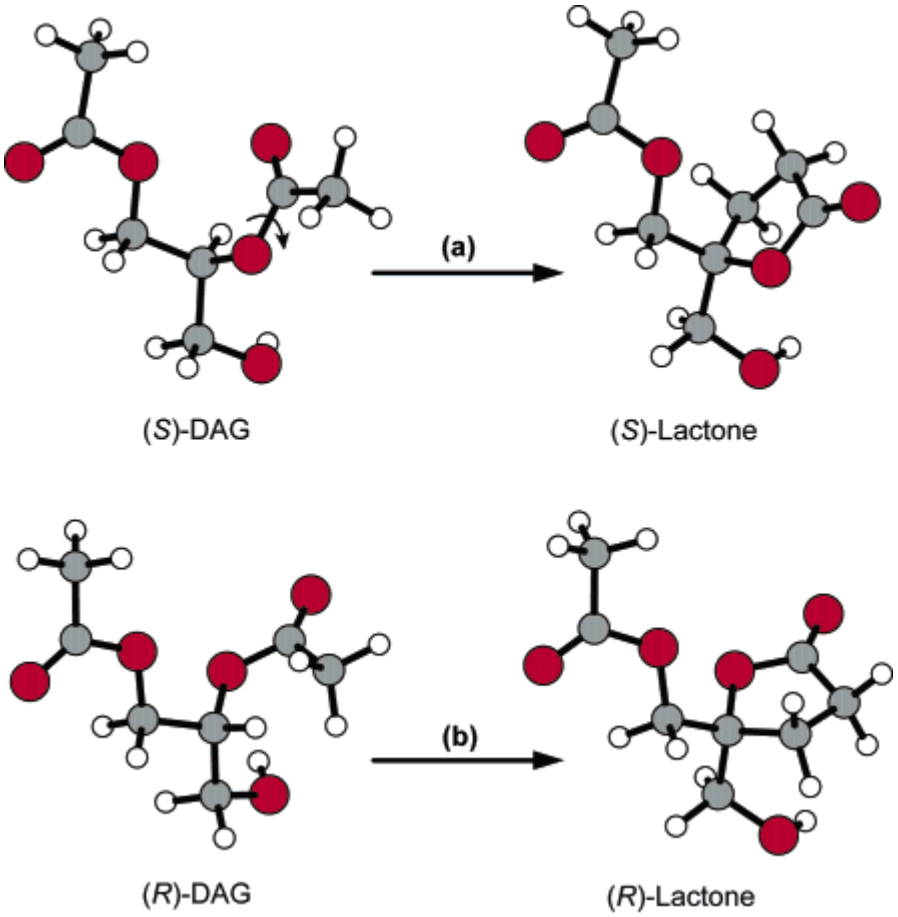
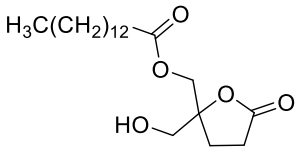
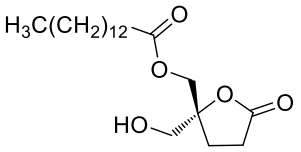


Figure 1.29 DAG Lactone Configuration Rational (used with permission from ref 89a). Evaluation of simple DAG lactones revealed that they are fairly potent PKC

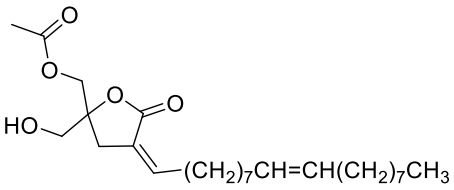
effective binding with PKC. Evaluation of simple DAG lactones revealed that they are fairly potent PKC ligands.[95] It was observed that the pure (*R*)-enantiomers were twice as potent as the racemic compounds; the racemate was used for expediency of synthesis in many cases (Figure **1.30**).[95] Addition of an enoate side chain to the α-postion of the lactone increased the binding affinity by an order of magnitude.[95] Modification of the enoate side chain from a linear to a branched structure, along with the exchange of the



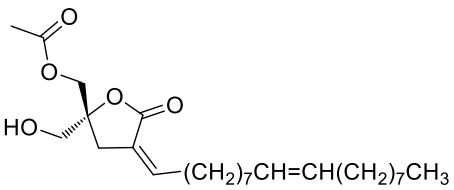
K_i = 138 +/- 24 nM



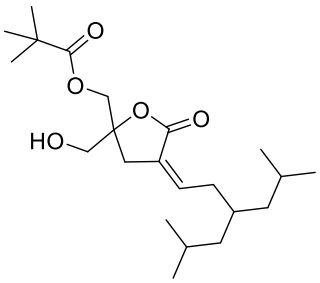
K_i = 96 +/- 3.1 nM



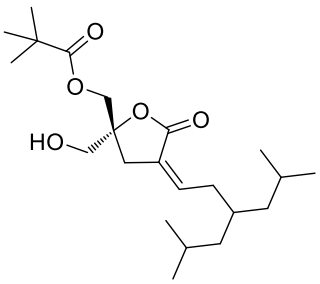
K_i = 24 +/- 2.9 nM



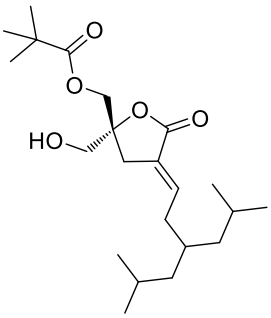
K_i = 12 +/- 0.4 nM



K_i = 2.9 +/- 0.4 nM



K_i = 1.5 +/- 0.2 nM



K_i = 1.5 +/- 0.2 nM

Figure 1.30 Acyclic DAG Lactones

acetate for a pivalate group at the esterified hydroxymethyl group, increased binding by another order of magnitude.[96] It was also found that the *Z*-enoate was more potent than the *E*-enoate by a factor of 2.

A short asymmetric synthesis of the most potent DAG analog was developed by Marquez and Coworkers[97]; this route is shown in Figure **1.31**. Mono protection of commercially available diol **1.69** gave alcohol **1.70** in moderate yield. Sharpless asymmetric epoxidation of allylic alcohol **1.71** gave the desired epoxide in high yield with an ee of 96%. Protection of the free alcohol as a benzyl ether followed by addition of lithium acetylide and Lindlar reduction gave allylic alcohol **1.73** in high overall yield. Hydroboration, and oxidation of the intermediate organoborane with PCC, gave lactone **1.74** in moderate yield.

Aldol reaction of **1.74** with 5-methyl-3-(2-methylpropyl)hexanal, followed by mesylation-elimination gave a nearly 50:50 mixture of *E*/*Z* isomers. Removal of the trityl group, acylation with pivaloyl chloride, and removal of the benzyl group gave DAG lactone **1.76**. Biological evaluation of DAG lactones indicates that they have potent anticancer properties against leukemia and colon cancer cell lines.[98] DAG lactone 1.76 induces apoptosis in LNCaP cells, but is selective towards PKCα, unlike PMA, which activates both PKCα and PKCδ.[99] 1.76 also selectively translocates PKCδ to nuclear membranes, unlike PMA, while PKCα is exclusively translocated to the plasma membrane. Lactone **1.76** has also demonstrated considerable activity towards the K562 leukemia and colo205 colon cancer cell lines.[100] These studies potentially indicate that DAG lactones are not mimics of PMA *in-vitro*. Synthesis of hybrid DAG lactones bearing a simplified bryostatin top-half scaffold would allow for further structure-

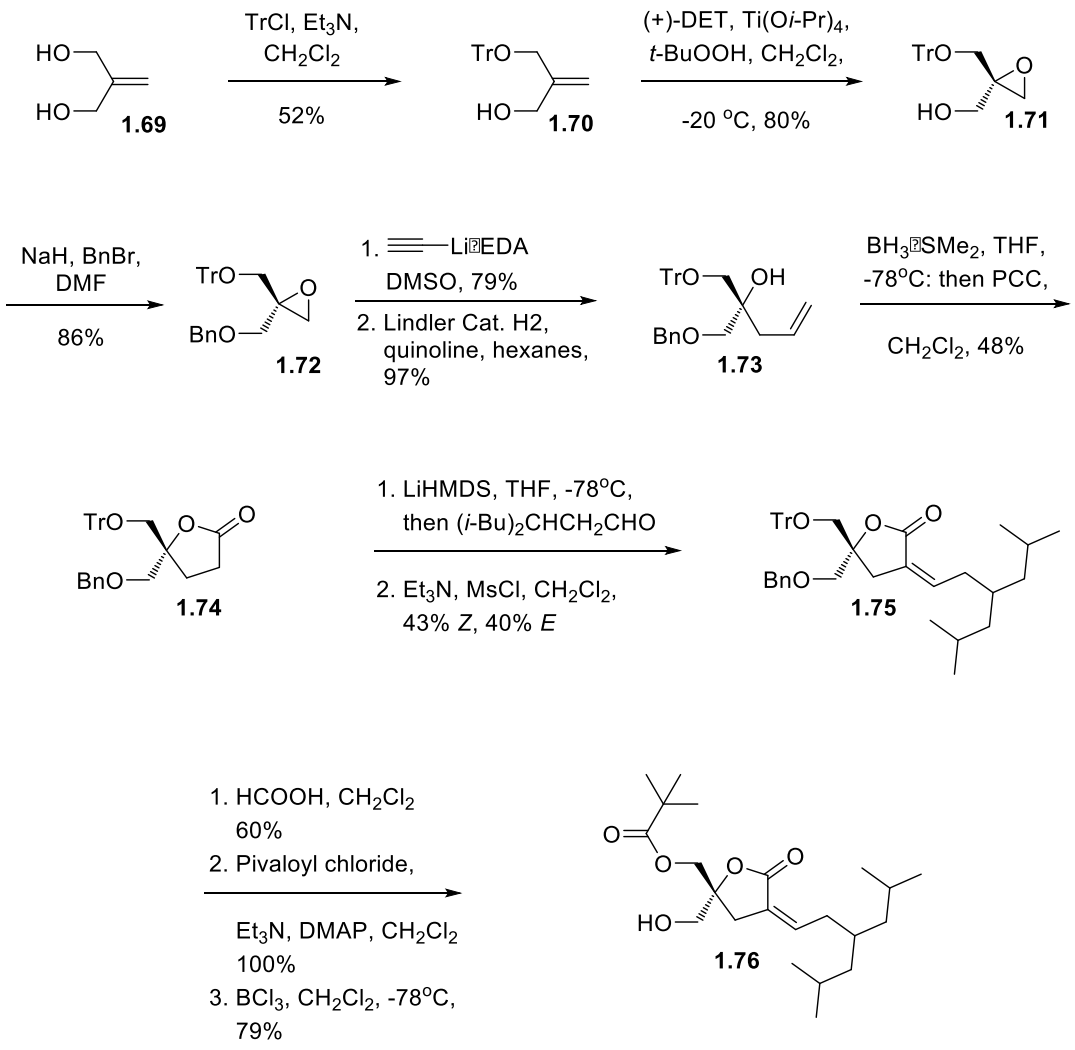


Figure 1.31 Optimized Marquez DAG Lactone Synthesis

functionality relationships to be determined.

Results and Discussion

Synthesis of a Diacylglycerol Lactone C-ring

In the search for simplified bryostatin analogs that retain the high binding affinity of the natural compound, it was decided to substitute the C-ring binding motif of the simplified analog Merle 23 for a scaffold that retained the high binding affinity of the original compound. The diacylglycerol lactones, extensively studied by Marquez and coworkers, were chosen to be the C-ring surrogate due to their high affinity for PKC and simplicity of construction. The retrosynthesis for the first- generation analog is shown in Figure **1.32**. The synthesis of the C-ring domain was based upon modifications of the optimized Marquez route[97] (Figure **1.31**) to allow for late stage alteration and functionalization.

Commencement of the synthesis of the functionalized C-ring surrogate began with a tandem aldol reaction-Horner-Wadsworth Emmons olefination of diethyl phosphonoacetate to yield acrylate **1.84** (Figure **1.33**).[101] Since acrylate **1.84** is readily polymerizable, it was immediately protected as the trityl ether, yielding protected acrylate ester **1.82** in 97% yield on a 35g scale.[102] Reduction of ester **1.82** to the corresponding alcohol **1.81** was conducted with DiBAL-H. Sharpless epoxidation[103] with L-(+)-diethyl tartrate and $Ti(i\text{-}OPr)_4$ produced epoxy alcohol **1.85** with an ee of 90% on a gram scale. Protection of the free hydroxy group as the benzyloxy methyl (BOM) ether yielded epoxide **1.80**. Nucleophilic ring opening of the epoxide using a lithium acetylide ethylenediamine complex provided propargylic alcohol **1.86** in 88% yield. Lindlar

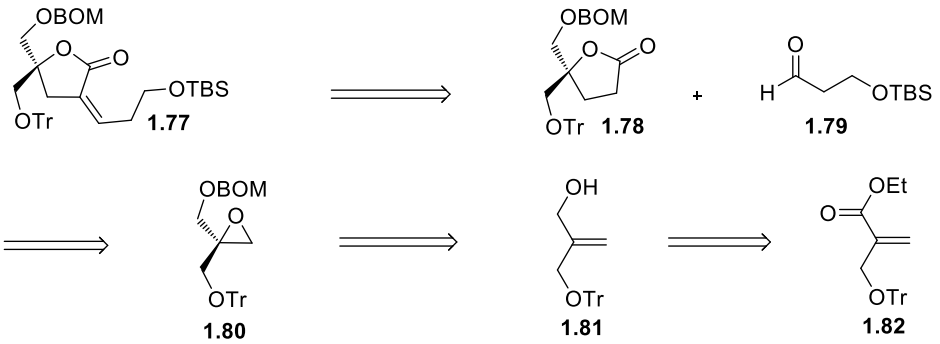


Figure 1.32 C-Ring Retrosynthesis

hydrogenation[104] of propargylic alcohol afforded allylic alcohol **1.87**.

Hydroboration-oxidation of the allylic alcohol produced diol **1.88**. Diol **1.88** was then oxidized to the corresponding hydroxy acid via sequential Parikh-Doering and Pinnick oxidations. Cyclization of hydroxy acid was achieved by formation of the mixed anhydride with benzoyl chloride, followed by spontaneous cyclization to produce lactone **1.89**.[105]

This route was successful; however, we realized that lactone **1.89** could be produced by a much shorter route commencing with the nucleophilic ring opening of protected epoxide **1.80** with the enolate of diethyl malonate, followed by spontaneous ring closure to produce diester **1.90 (**Figure **1.34)**.[106] Diester **1.90** can be hydrolyzed to the corresponding β-keto acid which was decarboxylated at 80 ^{o}C in toluene in the presence of quinoline.[107] This route shortens the synthesis of lactone **1.89** by 3 steps and provides lactone in 85% yield overall from epoxide **1.80** The functionalization of the alpha position of lactone **1.89** proved difficult to accomplish in a selective manner.[97] Enolization of lactone **1.89** with LDA at −78 ^{o}C followed by the aldol reaction with bifunctional aldehyde **1.79** produced a mixture of aldol product diastereomers, which

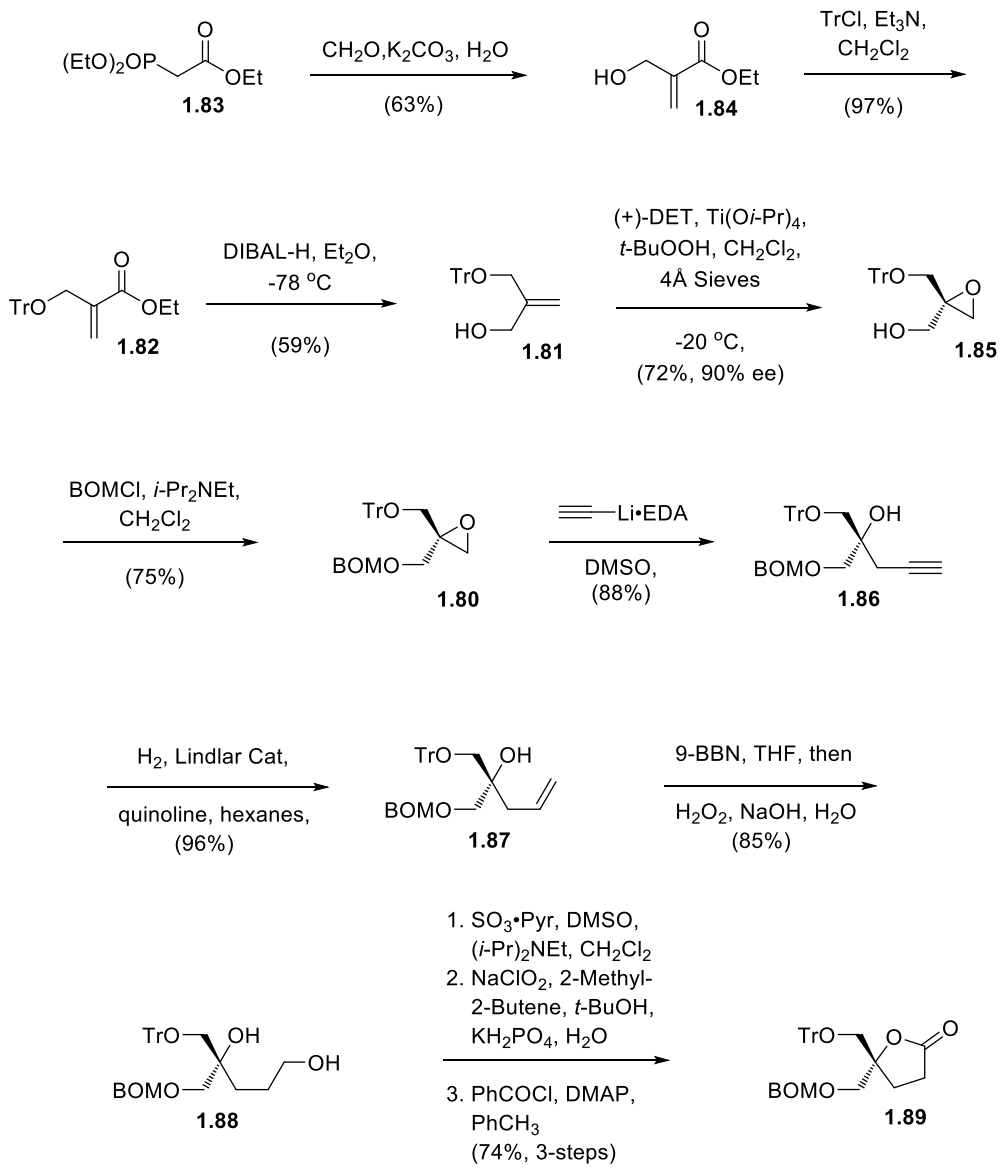


Figure 1.33 First-Generation C-ring Lactone Synthesis

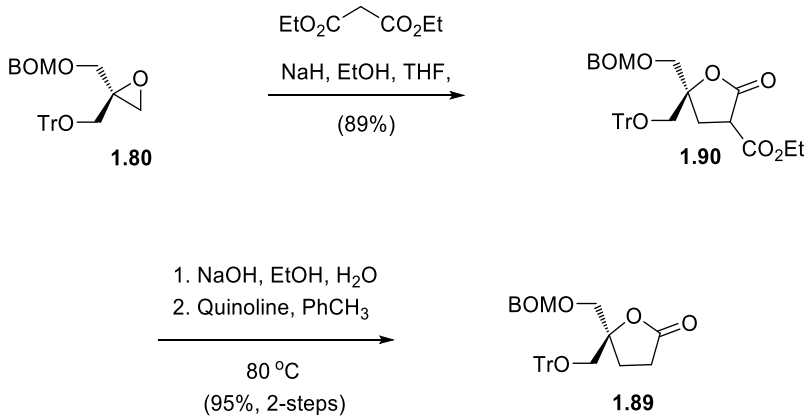


Figure 1.34 Second-Generation C-ring Lactone Synthesis

were not further characterized (Figure **1.35**). One-pot mesylation yield and elimination with DBU yielded a 1.2:1 mixture of *Z*/ *E* enoate products in 55%, separable viacolumn chromatography.

In order to improve selectivity for the desired Z-enoate, another approach for olefination was evaluated (Figure **1.36**). Lactone **1.89** was deprotonated with LDA at −78 ^{o}C to form the enolate and then reacted with diethyl chlorophosphate to form the enol phosphate which was exposed to LDA to cause rearrangement to the C- phosphonate **1.91**.[108] This phosphonate was not readily isolated as a pure compound and was therefore used without characterization.

To further advance phosphonate **1.91**, we sought to use a *Z*-selective Horner-Wadsworth-Emmons type olefination for which examples have been reported in the literature. The results of a screen of conditions for selective olefination are reported in Figure **1.36**. Deprotonation of phosphonate **1.91** with LDA at −78 ^{o}C in the presence of 18-crown-6 and subsequent addition of aldehyde **1.9** lead to a 1:2 *Z*/ *E* mixture of

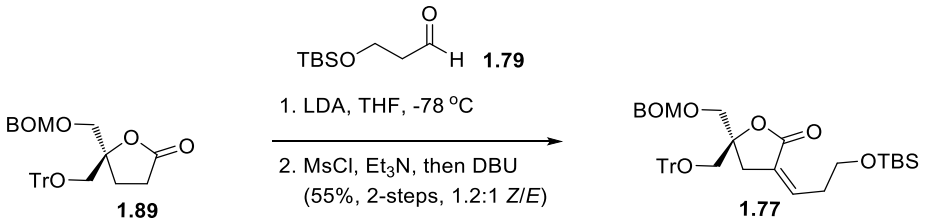
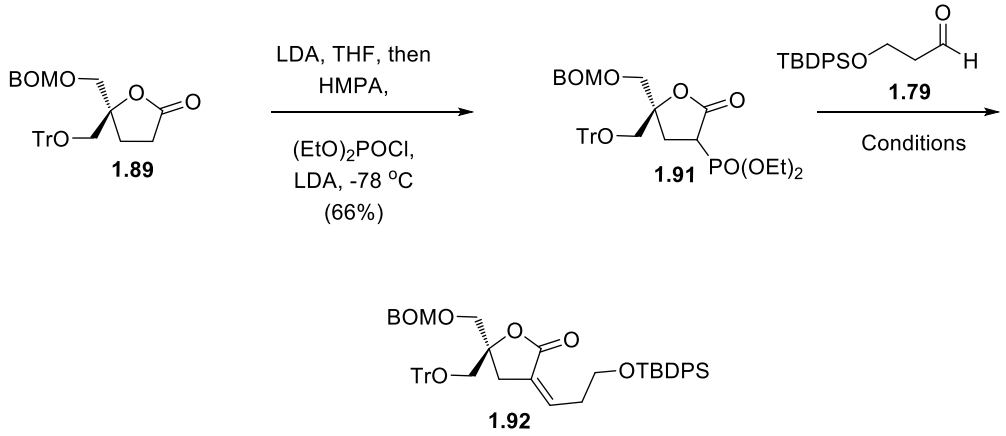


Figure 1.35 Synthesis of Enoate **1.77**



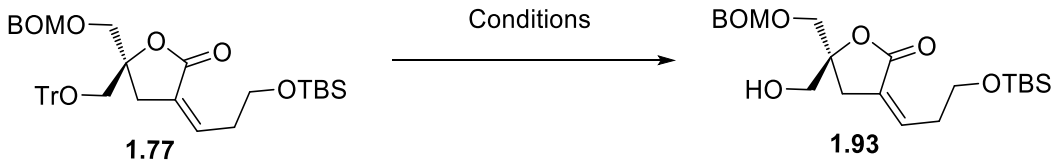
Conditions	Result
LDA, 18-crown-6, THF, −78 °C	1:2 *Z*/ *E*
KHMDS, 18-crown-6, THF, −78 °C	Decomposition
LiCl, DBU, MeCN, r.t.	1:3 *Z*/ *E*
K_2CO_3, 18-crown-6, THF	10:1 *Z*/ *E* 40% yield, 31% elimination

Figure 1.36 Olefination Experiments

enoates.[109] A *Z*- selective olefination using KHMDS and 18-crown-6 was evaluated in this context.[110] These conditions lead to the decomposition of the starting phosphonate **1.91**.

Roush conditions[111] using LiCl, DBU in MeCN, lead to a 1:3 *Z*/ *E* mixture of enoates. The best conditions that we identified were K_2CO_3, and 18-crown-6 in THF,[112] which produced a 10:1 mixture of *Z*/ *E* products. The desired *Z*-enoate was obtained in 40% yield with a 31% yield of the C3 TBS eliminated product. However, these conditions were not found to be acceptable to produce the desired *Z*-enoate product on a preparative scale. The only *Z*-selective conditions that were found caused much elimination of the terminal OTBS group of the olefinated product. The previously mentioned aldol/ dehydration sequence (Figure **1.35**) was found to be more scalable and therefore was used to produce gram quantities of the desired enoate **1.77** after chromatographic separation.

In order to couple the lactone fragment with the AB-ring system of Merle 23, the trityl group of enoate **1.77** needed to be selectively removed in the presence of a primary BOM ether and a primary homoallylic TBS ether. The results of the deprotection campaign are shown in Figure **1.37**. Formic acid in ether[113] resulted in TBS deprotection, but not the desired trityl deprotection. Use of Me_2AlCl in CH_2Cl_2[114] resulted in cleavage of both the trityl and BOM groups. Reaction with $BF_3 \bullet Et_2O$ in CH_2Cl_2[115] resulted in cleavage of both the trityl and TBS groups. Exposure to triethylsilane and TMSOTf in CH_2Cl_2[116] afforded the desired trityl deprotected compound **1.93**, but in low yield. It was found that a 50:50 mixture of TFA/ TFAA in CH_2Cl_2[117] removed the trityl group at 0 ^oC without removing the sensitive homoallylic TBS or the BOM group.



Conditions	Results
HCOOH, Et_2O, rt	TBS deprotection
Me_2AlCl, CH_2Cl_2, rt	Trityl and BOM deprotection
BF_3•Et2O, CH_2Cl_2, rt	TBS and trityl deprotection
Et_3SiH, TMSOTf, CH_2Cl_2, rt	Trityl deprotection, 49% yield
TFA/TFAA, CH_2Cl_2, 0 ^oC	Trityl deprotection, 81% yield

Figure 1.37 Trityl Deprotection

Conclusions

The scale up synthesis of the A-ring hydroxyallylsilane **1.42**, based upon improvements of the previous route developed for the synthesis of Merle 23 allowed for the synthesis of many bryostatin analogs. Several improvements were made to the Marquez route for the synthesis of DAG lactones, including starting from low cost precursor, and an efficient malonic ester addition-cyclization route to form the lactone skeleton from a chiral epoxide. Attempts were made at selective *Z*-olefination of the lactone; however, these were unsuccessful due to facile elimination of the γ-alkoxy group of the enoate after formation. The efficient synthesis of the DAG lactone core was used to provide material for the synthesis of the 3-bryostatin analogs described in Chapter 2.

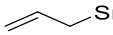
Experimental Section

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 Baclawski and Kofron.[118] Ti(*i*-OPr)$_4$ was distilled 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 60Å F254 plates or Silicycle 60Å F254 eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid, a solution of ninhydrin in 1-butanol, a solution of p-anisaldehyde in ethanol acidified with sulfuric acid, an aqueous potassium permanganate solution, or a solution of ceric ammonium molybdate, acidified with sulfuric acid. 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. Enantiomeric excess (ee) were determined using a Rainin Dynamax HPLC with a Knauer variable wavelength detector set at 254 nm, using a Chiracel OD-H column. Nuclear magnetic resonance spectra were acquired at 300, 500 MHz for 1H and 75, 125 MHz for 13C. 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 or $(CH_3)_4Si$ at 0.00 ppm. Chemicals shifts for carbon nuclear magnetic resonance (13C 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 13C NMR spectrum. The abbreviations s, d, dd, ddd, dddd, ddddd dt, quint, t, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, doublet of doublet of doublet of doublet of doublets, doublet of triplets, quintet, triplet, and multiplet, respectively. Optical rotations (Na D line) were obtained using a microcell with a 1 dm path length. Specific rotations ([α], Unit: °cm2/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 time of flight (TOF) high-resolution mass spectrometer. Compounds were named using ChemBioDraw 14.0.0.

Synthesis of Reagents

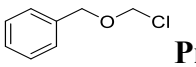


Preparation of allyltributylstannane: To a 2000 mL 3-neck round bottomed flask was added magnesium turnings (19.3 g, 792 mmol, 1.25 equiv); a magnetic stir bar, reflux condenser, and addition funnel were then attached. The system was put under vacuum and flame dried. Nitrogen was admitted after cooling to rt. THF (500 mL) was added to the flask followed by several I_2 crystals. The mixture was stirred

and heated to reflux. A solution of allyl bromide (66 mL, 760 mmol, 1.2 equiv) and chlorotributyltin (172 mL, 634 mmol, 1.0 equiv) in THF (100 mL) was added dropwise over 1.5 h to the stirred mixture. The solution turned to a cloudy gray color and most of the magnesium had dissolved after 2 h. The mixture was then heated at reflux for 20 h. After cooling, the mixture was filtered and the filter cake was washed with 9:1 hexanes/ EtOAc (400 mL). The combined organic phase was then washed with saturated aqueous $NaHCO_3$ solution (200 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was distilled under vacuum, collecting between 105 ^oC – 120 ^oC at 0.35 mmHg. The resulting allyltributylstannane was transferred to 2 Aldrich Sure-Seal bottles for storage (205g, 98%). 300 MHz ^{1}HNMR ($CDCl_3$) 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), 1.49–1.24 (m, 6H), 0.91 (t, J = 7.2 Hz, 9H), 0.90 (q, J =7.2 Hz, 6H).

Bu_3SnH **Preparation of tributyltin hydride:** A 2000 mL 3-neck flask was fitted with a reflux condenser, addition funnel, and stir bar. The flask was flame dried under vacuum and cooled to rt. $LiAlH_4$ (28.0 g, 737 mmol, 2.0 equiv) was added under a stream of N_2. Et_2O (1000 mL) was then added and the mixture was heated to reflux. Chlorotributyltin (100 mL, 367 mmol, 1.0 equiv) was added dropwise through the addition funnel to the mixture over 30 min. The mixture was heated at reflux for 1.5 h and then cooled in an ice bath. Crystals of Na_2SO_4•$10H_2O$ were added slowly until the exothermic reaction had subsided; a substantial excess of the salt was then added. The mixture was filtered through a 6 × 3 cm bed of Celite™ and the Celite™ was washed with Et_2O (100 mL). The solvent was evaporated and the crude product was distilled

under reduced pressure, collecting the fraction boiling between 70 °C – 90 °C at 1.0 mm Hg. The tributyltin hydride was stored in an Aldrich Sure-Seal bottle (101 g, 94%). 300 MHz ^{1}HNMR ($CDCl_3$) δ 5.30 (s, 1H), 1.70–1.60 (m, 6H), 1.43–1.30 (m, 12H), 0.93 (t, *J* = 12.5 Hz, 9H).

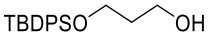


Preparation of benzyloxymethyl chloride (BOMCl): A dry 250 mL flask was fitted with a stir bar and addition funnel. Paraformaldehyde (8.26 g, 275 mmol, 1.0 equiv) and benzyl alcohol (28.5 mL, 275 mmol, 1.0 equiv) were added to the flask. After cooling to 0 °C, $SOCl_2$ (20 mL, 275 mmol, 1.0 equiv) was added through the addition funnel dropwise over 25 min to the stirred mixture. The mixture was then warmed to rt and stirred for 1 h. The reaction mixture was quenched with pentane (200 mL) and extracted with brine (2 × 100 mL). After drying over $MgSO_4$, the solution was filtered and concentrated to yield the product as a colorless oil (40.2 g, 94%). 300 MHz ^{1}HNMR ($CDCl_3$) δ 7.40 (s, 5H), 5.57 (s, 2H), 4.78 (s, 2H).

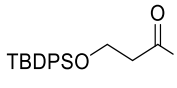
Preparation of magnesium bromide diethyletherate: A modified 1000 mL 3-neck flask bearing a fritted filter fused to one of the necks was fitted with a reflux condenser and addition funnel. The filter had a ground glass joint and a vacuum adaptor, which was sealed off respectively with a 1000 mL flask and rubber septum. The apparatus was flame dried under vacuum after adding magnesium (15.5 g, 638 mmol, 1.1 equiv). After cooling to rt, Et_2O (600 mL) was added and the mixture was heated to reflux. To the stirred mixture was added 1,2-dibromoethane (50 mL, 580 mmol, 1.0 equiv) dropwise over 3 h. The mixture was refluxed for 2 h and filtered through the side arm under positive pressure while still hot. The solution was flushed with N_2, capped, and allowed to crystallize in a –20 °C freezer overnight. The mother liquor was removed by

cannulation and the resulting crystals washed with Et_2O (4 × 100 mL). The product was then dried under high vacuum overnight, yielding a gray colored solid (114 g, 76%).

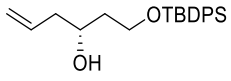
Experimental Procedures for A-Ring β-Hydroxyallylsilane **1.64**



Preparation of 3-((*tert*-butyldiphenylsilyl)oxy)propan-1-ol (1.94): A 2000 mL round bottomed flask was fitted with a magnetic stir bar and a septum. CH_2Cl_2 (1350 mL) was added to the flask, followed by *tert*-butyldiphenylsilyl chloride (60 mL, 230 mmol, 1.0 equiv). 1,3-propanediol **12** (82 mL, 1130 mmol, 4.9 equiv), triethylamine (63 mL, 450 mmol, 2.0 equiv), and DMAP (1.39 g, 11 mmol, 0.05 equiv) were then subsequently added. The solution was stirred at rt for 3 days, then quenched with water (500 mL). The layers were separated and the organic layer was washed with water (2 × 250 mL). The solution was then washed once again with brine (100 mL) and dried with Na_2SO_4. The solution was filtered through a fritted funnel and concentrated under reduced pressure to yield an oil that crystallized upon cooling in a −20 ^oC freezer overnight. The product was purified via flash chromatography using a 12 × 30 cm silica gel column eluting with 10 % EtOAc/hexanes, collecting 125 mL fractions. Product containing fractions (6−36) were combined and concentrated, producing a colorless oil (73.6 g, 96 %) that crystallized upon cooling. R_f = 0.32 (25 % EtOAc/ hexanes); mp: 41 ^oC – 43 ^oC; 300 MHz ^{1}HNMR ($CDCl_3$) δ 7.71−7.68 (m, 4H), 7.46−7.38 (m, 6H), 3.89−3.81 (m, 4H), 2.39 (t, J = 5.4 Hz, 1H), 1.82 (quint, J = 5.7 Hz, 2H) 1.07 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 135.8, 133.4, 130.0, 128.0, 63.6, 62.3, 34.4, 27.0, 19.3.



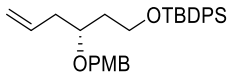
Preparation of 3-((*tert*-butyldiphenylsilyl)oxy)propanal (1.9): To a 2000 mL round bottomed flask equipped with a stir bar was added CH_2Cl_2 (960 mL) and oxalyl chloride (13.1 mL, 149 mmol, 1.6 equiv). The mixture was chilled to −78 ^oC, and a solution of dimethyl sulfoxide (20 mL, 280 mmol, 3.0 equiv) in CH_2Cl_2 (30 mL) was added dropwise via cannula to the stirring solution. The solution was stirred for 1.5 h at −78 ^oC and then alcohol **1.94** (30.3 g, 96.4 mmol, 1.0 equiv) in CH_2Cl_2 (150 mL) was added via cannula; CH_2Cl_2 (10 mL) was used to complete the transfer. After 1 h, triethylamine (67 mL, 480 mmol, 5.0 equiv) was added. After stirring for an additional h, aqueous pH 7.0 phosphate buffer (0.1 M, 197 mL) was then added and the mixture was allowed to warm to rt. The mixture was washed with water (2 × 300 mL) and with brine (500 mL). The solution was dried over Na_2SO_4, filtered, and concentrated to give a yellow oil. The product was purified by column chromatography using a 12 × 30 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 125 mL fractions. Product containing fractions 12−22 were concentrated, yielding a nearly colorless oil (25.5 g, 82 %) that crystallized upon cooling. R_f = 0.76 (25 % EtOAc/hexanes.); mp 46 ^oC – 49 ^oC; 300 MHz ^{1}HNMR ($CDCl_3$) δ 9.83 (t, J = 2.2 Hz, 1H), 7.69−7.66 (m, 4H), 7.46−7.38 (m, 6H), 4.04 (t, J = 6.0 Hz, 2H), 2.62 (dt, J = 6.0, 2.4 Hz, 2H), 1.05 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 202.3, 135.8, 133.5, 130.1, 128.0, 58.5, 46.6, 27.0, 19.4.



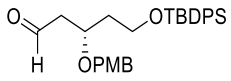
Preparation of (*R*)-1-((*tert*-butyldiphenylsilyl)oxy)hex-5-en-3-ol (1.56): A 1000 mL 3-neck flask was fitted with a magnetic stir bar, reflux condenser, and rubber septa to close the other two necks. The flask was flame dried under vacuum and cooled under N_2. Powdered 4Å molecular sieves (53.0 g), (*R*)-BINOL (6.50 g, 22.8

mmol, 0.40 equiv), and CH_2Cl_2 (230 mL) were added to the flask under a stream of N_2. Ti(*i*-OPr)$_4$ (1.0 M in CH_2Cl_2, 11.4 mL, 11.4 mmol, 0.20 equiv) was added followed by TFA (1.0 M in CH_2Cl_2, 0.24 mL, 0.24 mmol, 0.004 equiv), resulting in a dark red color. The mixture was heated at reflux for 1 h, before a solution of aldehyde **1.9** (17.8 g, 57.0 mmol, 1.0 equiv) in CH_2Cl_2 (25 mL) was then added via cannula; CH_2Cl_2 (5.0 mL) was used to complete the transfer. After stirring at rt for 0.5 h, the mixture was cooled to −78 ^oC and allyltributyltin (23 mL, 74.3 mmol, 1.3 equiv) was added via syringe pump over 0.5 h. After stirring for 10 min at −78 ^oC, the mixture was transferred to a −20 ^oC freezer. After 1 week, the mixture was removed from the freezer and immediately poured into a 0 ^oC solution of saturated aqueous $NaHCO_3$ (230 mL). After 1 h of stirring, the mixture was filtered through a 9 × 2 cm bed of Celite™ and washed with CH_2Cl_2 (3 × 100 mL). The organic phase was separated from the aqueous phase and the aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated, leaving a thick-red colored oil. The product was purified via column chromatography using a 12 × 30 cm column of silica gel, eluting with 5 % EtOAc/hexanes, collecting 100 mL fractions. Fractions 25−58 where concentrated, yielding the homoallylic alcohol (18.7 g, 93 %) as a light yellow oil. R_f = 0.48 (25 % EtOAc/hexanes); 300 MHz ^1H NMR ($CDCl_3$) δ 7.70−7.61 (m, 4H), 7.46−7.39 (m, 6H), 5.86 (dddd, *J* = 14.4, 10.5, 7.2, 7.2 Hz, 1H), 5.15−5.09 (m, 2H), 4.03−3.94 (m, 1H), 3.92−3.80 (m, 2H), 3.26 (d, *J* = 2.5 Hz, 1H), 2.32−2.25 (m, 2H), 1.76−1.69 (m, 2H), 1.06 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 135.8, 135.7, 135.2, 130.0, 128.0, 117.6, 71.1, 63.5, 42.2, 38.0, 27.0, 19.2. The enantiomeric excess was determined using a 4.6 × 150 mm Chiralcel OD-H column (1.0 % *i*-PrOH/Hexanes; 0.40 mL/min); t_r (major) = 9.71

min, t_r (minor) = 11.87 min; 95% ee.



Preparation of (*R*)-*tert*-butyl((3-((4-methoxybenzyl)oxy)hex-5-en-1-yl)oxy)diphenylsilane (1.95): A 250 mL single neck flask was fitted with a septum and a magnetic stir bar. Toluene (100 mL), followed by homoallylic alcohol **1.56** (3.5 g, 9.7 mmol, 1.0 equiv) were added to the flask. Scandium (III) triflate (48.4 mg, 0.1 mmol, 0.01 equiv) was added, and a solution of 4-methoxybenzyltrichloroacetimidate (5.50 g, 19.6 mmol, 2.0 equiv) in toluene (10 mL) was transferred to the stirring solution of homoallylic alcohol **1.56** at 0 ^{o}C. The ice bath was removed and the mixture was stirred for an additional 6 h at rt. The solvent was removed under reduced pressure, and the semi-solid residue was triturated with 9:1 hexanes/ether (200 mL), and then filtered through Celite™. The Celite™ was then washed with of 9:1 hexanes/ether (5 × 10 mL) and then the solvent was removed under reduced pressure. The product was purified on a 5 × 20 cm silica gel column, eluting with 5 % EtOAc/hexanes, collecting 30 mL fractions. Product containing fractions 10–31 were concentrated, leaving the product (4.2 g, 90 %) as a yellow oil. R_f = 0.63 (25 % EtOAc/hexanes). 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.73–7.67 (m, 4H), 7.47–7.37 (m, 6H), 7.24 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 5.88 (dddd, *J* = 14.1, 10.2, 6.9, 6.9 Hz, 1H), 5.16–5.07 (m, 2H), 4.48 (ABq, *J* = 11.1 Hz, Δν = 33.8 Hz, 1H), 3.89–3.84 (m, 1H), 3.82 (s, 3H), 3.81–3.72 (m, 2H), 2.36 (t, *J* = 6.6 Hz, 2H), 1.81 (q, *J* = 6.6 Hz, 2H), 1.1 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 135.8, 135.1, 134.1, 131.1, 129.9, 129.5, 127.8, 117.2, 113.9, 75.2, 71.0, 60.7, 55.4, 37.2, 27.1, 19.4.

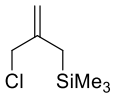


Preparation of (*S*)-5-((*tert*-butyldiphenylsilyl)oxy)-3-((4-methoxybenzyl)oxy)pentanal (1.57): A solution of PMB ether **1.95** (10.1 g, 21.3 mmol,

1.0 equiv) in 80:20 CH_2Cl_2/ MeOH (300 mL) was prepared in a 500 mL single neck flask. A stir bar followed by sodium bicarbonate (10.1 g, 90.2 mmol, 4.2 equiv) were added to the flask containing the PMB ether. The solution was chilled to −78 ^oC and ozone was passed into the solution until the solution turned to a permanent slate gray color. Excess ozone was then flushed with oxygen for 5 min, triphenylphosphine (10.6 g, 40.4 mmol, 1.9 equiv) was added, and the solution was allowed to stir at rt overnight. The solution was filtered through a coarse fritted funnel, and concentrated to a thick oil. The product was purified via flash chromatography on a 5 × 30 cm silica gel column, eluting with 5 % EtOAc/ hexanes, switching to 10 % EtOAc/hexanes once the product began to elute, collecting 30 mL fractions. Product containing fractions 65−112 were concentrated, yielding the product (6.1 g, 60 %) as a yellowish oil. R_f = 0.38 (25 % EtOAc/hexanes). 300 MHz ^1H NMR ($CDCl_3$) δ 9.76 (t, *J* = 2.1 Hz, 1H), 7.70−7.65 (m, 4H) 7.49−7.36 (m, 6H), 7.20 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 4.47 (s, 2H), 4.20 (quint, *J* = 6.6 Hz, 2H), 3.90−3.82 (m, 1H), 3.81 (s, 3H), 3.79−3.73 (m, 1H), 2.66 (ddd, *J* = 16.2, 6.9, 2.4 Hz, 1H), 2.58 (ddd, *J* = 16.5, 5.1, 2.1 Hz, 1H), 2.00−1.87 (m, 1H), 1.86−1.73 (m, 1H), 1.1 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 201.8, 159.4, 135.8, 133.8, 130.4, 129.9, 129.6, 127.9, 114.0, 71.4, 71.3, 60.3, 55.5, 48.8, 37.3, 27.1, 19.4.

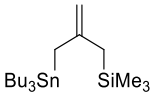
Preparation of trichloro(2-(chloromethyl)allyl)silane (1.96):[119] A 1000 mL 3-neck flask was fitted with a magnetic stir bar, pressure equalizing addition funnel, reflux condenser, and a septum was fitted in the remaining neck. The flask was dried under vacuum with a flame, and N_2 was admitted after cooling to rt. Copper (I) chloride (0.158 g, 1.6 mmol, 0.01 equiv) was added followed by Et_2O (300 mL) to the

flask. Triethylamine (28.0 mL, 201 mmol, 1.26 equiv) was added, turning the solution to a yellow-green color. A solution of 3-chloro-2-(chloromethyl)propene (20.0 g, 160 mmol, 1.0 equiv), and trichlorosilane (20.0 mL, 198 mmol, 1.24 equiv) in Et_2O (50 mL) was prepared in the addition funnel. This solution was then added over a period of 4 h to the stirred reaction mixture in the flask; a white colored suspension soon formed. After stirring overnight, the solution was filtered through a fritted funnel into a dry flask and the filter cake was washed with Et_2O (50 mL). The solvent was carefully removed on a rotary evaporator using a cold water bath. The product was filtered and distilled under water aspirator pressure, collecting the fraction boiling between 70 ^oC – 90 ^oC as a colorless fuming liquid (28.8 g, 81 %). 300 MHz ^1H NMR ($CDCl_3$) δ 5.35 (s, 1H), 5.16 (s, 1H), 4.14 (d, J = 1.2 Hz, 2H), 2.55 (d, J = 0.9 Hz, 2H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 136.4, 119.0, 49.0, 29.8.



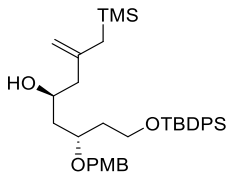
Preparation of (2-(chloromethyl)allyl)trimethylsilane (1.97): A 1000 mL single neck flask was fitted with a magnetic stir bar and a septum. Et_2O (500 mL) was added and the flask was flushed with N_2. Trichloro(2-(chloromethyl)allyl)silane **1.96** (35.0 g, 156 mmol, 1.0 equiv) was transferred via cannula to the flask. After chilling to −78 ^oC, a solution of MeMgBr (3.0 M, 185 mL, 549 mmol, 3.5 equiv) in ether was slowly added via syringe. After 1 h at −78 ^oC, the solution was warmed to rt, and stirred overnight; during this time, the solution became cloudy and subsequently cleared and white crystals formed. The solution was then slowly poured into a solution of saturated aqueous NH_4Cl (500 mL), which was previously chilled in an ice bath. The upper organic phase was then washed with brine (250 mL). After drying over $MgSO_4$ and filtering, the

solution was carefully concentrated with a rotary evaporator, using a cold water bath to minimize the loss of the volatile product. The product was distilled under vacuum, collecting the fraction boiling between 20 °C – 23 °C at 1.3 mm Hg. A colorless oil was obtained (15.8 g, 62 %). 300 MHz ^{1}H NMR ($CDCl_3$) δ 5.01 (d, *J* = 1.5 Hz, 1H), 4.76 (d, *J* = 1.2 Hz, 1H), 3.98 (d, *J* = 1.2 Hz, 2H), 1.70 (d, *J* = 0.9 Hz, 2H), 0.05 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 143.2, 112.5, 50.1, 23.8, −1.3.



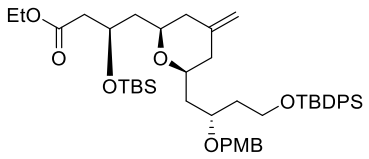
Preparation of trimethyl(2-((tributylstannyl)methyl)allyl)silane (1.7): A 50 mL single necked flask was fitted with a stir bar and rubber septum. A solution of LDA was prepared by first adding THF (7.0 mL), and diisopropylamine (1.0 mL 7.1 mmol, 1.2 equiv) to the flask, chilling to 0 °C, and finally adding a solution of n-BuLi (2.5 M, 2.65 mL, 6.6 mmol, 1.1 equiv) dropwise with stirring. After stirring for 10 min, Bu_3SnH (1.62 mL, 6.1 mmol, 1.0 equiv) was added, turning the solution to a milky yellow color. At this point, the Teflon stir bar turned to a black color. The solution was chilled to −78 °C after stirring at rt for 30 min, and 2-(chloromethyl)allyl)trimethylsilane **1.97** (1.00 g, 6.20 mmol, 1.02 equiv) was added via cannula, followed by THF (2.0 mL) to rinse the cannula. The reaction mixture was stirred overnight after warming to rt. The solution was poured into ice water (25 mL), extracted with Et_2O (3 × 25 mL), washed with brine (25 mL), and dried over $MgSO_4$. After filtering and concentrating, the product was distilled using a Kugelrohr apparatus at 1.5 mmHg. The fraction boiling between 125 – 140 °C oven temperature was collected as a light yellow oil (2.26 g, 87 %). 300 MHz ^{1}H NMR ($CDCl_3$) δ 4.24–4.36 (m, 1H), 4.26–4.19 (m, 1H), 1.74 (s, 2H), 1.57–1.44 (m, 7H), 1.44 (s, 2H), 1.32 (sextet, *J* = 7.5 Hz, 9H), 0.91 (t, *J* = 7.5 Hz, 16H), 0.06 (s, 9H); 75

MHz ^{13}C NMR ($CDCl_3$) δ 147.7, 103.1, 29.4, 27.6, 21.9, 14.0, 9.7, 9.0, −1.1.



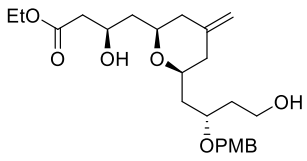
Preparation of (4*S*,6*S*)-8-((tert-butyldiphenylsilyl)oxy)-6-((4-methoxybenzyl)oxy)-2-((trimethylsilyl)methyl)oct-1-en-4-ol (1.15): A solution of aldehyde **1.57** (9.50 g, 19.3 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (200 mL) in a 500 mL flask under N_2. After cooling to −78 ^oC, $MgBr_2 \bullet Et_2O$ (9.96 g, 38.6 mmol, 2.0 equiv) was added in one portion. The resulting yellow solution was stirred for 15 min at −78 ^oC. Freshly distilled stannane **1.7** (9.66 g, 23.2 mmol, 1.2 equiv) was added dropwise via syringe to the reaction mixture, and the reaction was complete by TLC after 40 min of stirring. The reaction mixture was first quenched with a solution of saturated aqueous $NaHCO_3$ (83 mL) and then water (412 mL) was added. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (200 mL) and dried over $MgSO_4$. After filtration, the solution was concentrated to obtain a clear oil that was purified via column chromatography on a 6 × 30 cm silica gel column eluting with 5 % EtOAc/hexanes. Three columns were required to remove all tin compounds and impurities with very similar R_f values to obtain the product as a clear oil (7.9 g, 66 %). R_f = 0.43 (25 % EtOAc/hexanes). 300 MHz ^1H NMR ($CDCl_3$) δ 7.71−7.66 (m, 4H), 7.49−7.39 (m, 6H), 7.22 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.67 (dd, *J* = 6.9, 2.1 Hz, 2H), 4.49 (s, 2H), 4.05−3.95 (m, 2H), 3.85−3.71 (m, 1H), 3.81 (s, 3H), 2.69 (d, *J* = 2.1 Hz, 1H), 2.18−2.02 (m, 2H), 2.01−1.87 (m, 1H), 1.80 (quint, *J* = 6.3 Hz, 2H), 1.66 (ddd, *J* = 8.4, 8.4, 3.9 Hz, 2H), 1.55 (d, *J* = 2.4 Hz, 2H), 1.29 (d, *J* = 3.0 Hz, 2H), 1.08 (s, 9H), 0.04 (s, 9H); 75

MHz ^{13}C NMR ($CDCl_3$) δ 159.4, 144.8, 135.8, 134.0, 130.8, 129.8, 129.8, 127.9, 114.0, 110.2, 74.0. 71.6, 66.2, 60.7, 55.5, 46.9, 40.8, 37.2, 27.1, 27.0, 19.4, −1.2.



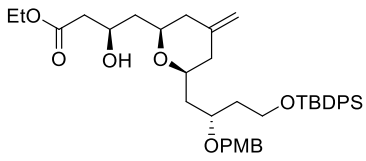
Preparation of (3*R*)-ethyl 3-((*tert*-butyldimethylsilyl)oxy)-4-((2*R*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)butanoate (1.62): A solution of hydroxyallylsilane **1.15** (5.40 g, 8.9 mmol, 1.0 equiv) and aldehyde **1.61** provided by Kevin McGowan (2.70 g, 9.85 mmol, 1.1 equiv) was prepared in Et_2O (90 mL) in a 250 mL flask equipped with a stir bar and septum. After cooling to −78 ^{o}C, a solution of TMSOTf in Et_2O (1.04 M, 10.3 mL, 10.7 mmol, 1.2 equiv) was added dropwise. The reaction was complete after 25 min based on TLC; *i*-Pr_2NEt (10 mL) was added to quench the reaction mixture at −78 ^{o}C. After 5 min, the flask was transferred to a 0 ^{o}C bath and a solution of saturated aqueous $NaHCO_3$ (50 mL) was added. After 20 min, the organic phase was separated, and the aqueous phase was extracted with Et_2O (3 × 50 mL). After drying over $MgSO_4$, and filtering, the solution was concentrated. The product was purified via flash chromatography using a 5 × 20 cm column of silica gel, eluting with 5 % EtOAc/hexanes, collecting 30 mL fractions. Fractions 17−32 contained pure product, and fractions 33−47 had to be re-purified via column chromatography. A light yellow oil (6.25 g, 89 %) was obtained upon concentration of the pure fractions. R_f = 0.63 (25 % EtOAc/hexanes). 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.72−7.66 (m, 4H), 7.48−7.35 (m, 6H), 7.18 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 4.71 (d, *J* = 4.8 Hz, 2H), 4.42 (ABq, *J* = 10.8 Hz, Δν = 15.7 Hz, 2H), 4.38−4.33 (m, 1H), 4.14−3.97 (m, 2H), 3.92−

3.83 (m, 1H), 3.82–3.77 (m, 2H), 3.79 (s, 3H), 3.58–3.38 (m, 2H), 2.52 (d, *J* = 3.3 Hz, 1H), 2.50 (d, *J* = 1.2 Hz, 1H), 2.24 (d, *J* = 12.6 Hz, 1H), 2.16 (d, *J* = 12.9 Hz, 1H), 1.93 (q, *J* = 12.9 Hz, 2H), 1.89–1.77 (m, 3H), 1.70–1.61 (m, 3H), 1.18 (t, *J* = 7.2 Hz, 3H), 1.07 (s, 9H), 0.87 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 171.9, 159.2, 144.8, 135.8, 134.1, 134.0, 131.2, 129.8, 129.6, 129.7, 113.9, 108.7, 75.1, 75.0, 73.0, 71.8, 66.9, 60.7, 60.5, 55.5, 44.0, 42.8, 42.5, 41.3, 41.3, 37.8, 27.1, 26.0, 19.4, 18.2, 14.3, –4.2, –4.3.



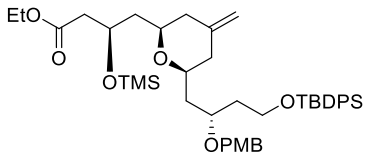
Preparation of ethyl (*R*)-3-hydroxy-4-((2*R*,6*S*)-6-((*S*)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)butanoate (1.97): To a 50 mL polyethylene vial was added **1.62** (914 mg, 1.16 mmol, 1.0 equiv), followed by 9:1 THF/ pyridine (11.6 mL 0.1 M). To the stirring solution, 20% HF•Pyr (29 mL, 25 mL/ mmole) was added. After stirring for 6 h, the reaction mixture was quenched by pipetting the solution into a stirring solution of saturated aqueous $NaHCO_3$ (300 mL). After the effervesence subsided, the solution was extracted with EtOAc (3 × 50 mL), washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 3 × 15 cm silica gel column, eluting with a gradient of 25 – 100% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 40–76 were concentrated to yield the product (448 mg, 89%) as a clear oil. R_f = 0.14 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = +38.2° (c = 1.70, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.28 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 4.73 (s, 2H), 4.49 (ABq, *J* = 11.5 Hz, Δν = 43.0 Hz, 2H), 4.27–4.20 (m, 1H), 4.15 (q, *J* = 6.5 Hz, 2H), 3.80 (s, 3H), 3.80–3.74 (m,

2H), 3.71–3.66 (m, 2H), 3.54–3.46 (m, 2H), 2.52 (dd, *J* = 15.5, 7.5 Hz, 1H), 2.44 (dd, *J* = 15.5, 5.5 Hz, 1H), 2.23 (d, *J* = 13 Hz, 1H), 2.19 (d, *J* = 13 Hz, 1H), 1.97 (q, *J* = 11.5 Hz, 2H), 1.90–1.83 (m, 1H), 1.78–1.65 (m, 5H), 1.26 (t, *J* = 6.5 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.0, 159.4, 143.6, 130.5, 129.8, 114.0, 109.3, 78.2, 75.6, 74.1, 71.5, 67.6, 60.6, 59.7, 55.3, 42.4, 42.0, 41.8, 41.4, 41.0, 40.9, 36.6, 14.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.3, 14.3; CH_2 δ 109.2, 71.5, 60.6, 59.7, 42.4, 41.8, 41.4, 41.0, 40.9, 36.6; CH δ 129.8, 114.0, 78.2, 75.6, 74.1, 67.6; C δ 172.0, 159.4, 143.6, 130.5; IR (neat) 3448, 3073, 2939, 1733, 1653, 1613, 1586, 1514, 1465, 1421, 1372, 1328, 1302, 1248, 1176, 1086, 1035, 892, 822 cm^{-1}; HRMS (ESI) calcd 459.2359 for (M+Na), found 459.2367.



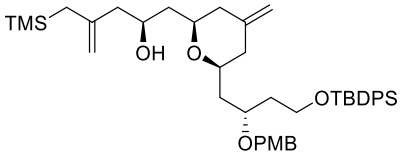
Preparation of (*R*)-ethyl 4-((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-3-hydroxybutanoate (1.63): To a stirring solution of diol **1.97** (441 mg, 1.01 mmol, 1.0 equiv) and DMAP (43 mg, 0.35 mmol, 0.35 equiv) in CH_2Cl_2 (10.1 mL, 0.1 M) in a 25 mL flask under N_2 was added *i*-Pr_2NEt (350 μL, 2.0 mmol, 2.0 equiv). TBDPSCl (260 μL, 1.0 mmol, 1.0 equiv) was then added dropwise. After stirring overnight, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with CH_2Cl_2 (3 × 25 mL), washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified on a 3 × 15 cm silica gel column, eluting with a gradient of 5 – 25% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 6–82 were combined and concentrated to yield the product (597 mg, 88%) as a clear oil. R_f = 0.30 (25 % EtOAc/hexanes). 500

MHz ^{1}H NMR ($CDCl_3$) δ 7.73–7.64 (m, 4H), 7.47–7.35 (m, 6H), 7.22 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 7.5 Hz, 2H), 4.74 (d, *J* = 9.5 Hz, 2H), 4.42 (ABq, *J* = 11.5 Hz, Δν = 40.2 Hz, 2H), 4.31–4.22 (m, 1H), 4.16 (ddd, *J* = 7.5, 7.5, 2.5 Hz, 2H), 3.81 (s, 3H), 3.84–3.75 (m, 4H), 3.59–3.49 (m, 2H), 2.55 (dd, *J* = 15.5, 7.5 Hz, 1H), 2.47 (dd, *J* = 15.5, 5.5 Hz, 1H), 2.25 (d, *J* = 12.5 Hz, 1H), 2.19 (d, *J* = 13 Hz, 1H), 2.01 (t, *J* = 12 Hz, 1H), 1.95 (t, *J* = 12 Hz, 1H), 1.88–1.62 (m, 6H), 1.26 (t, *J* = 7.5 Hz, 3H), 1.09 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.1, 159.2, 143.9, 135.8, 134.0, 131.0, 129.8, 129.7, 127.8, 113.9, 109.2, 78.3, 75.4, 72.6, 71.5, 67.8, 60.6, 60.5, 55.4, 42.5, 42.1, 41.9, 37.4, 27.0, 19.3, 14.3.



Preparation of (*R*)-ethyl 4-((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-3-((trimethylsilyl)oxy)butanoate (1.64): A solution of ethyl ester **1.63** (274 mg, 0.42 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (7.0 mL) in a 100 mL flask. Et_3N (180 μL, 1.3 mmol, 3.0 equiv) and TMSCl (80 μL, 0.64 mmol, 1.5 equiv) were then added. After stirring for 3 h, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was then extracted with CH_2Cl_2 (3 × 10mL). The combined organic layers were washed with brine (10 mL), the organic phase was dried over $MgSO_4$, filtered, and concentrated. The resulting oil was purified on a 3 × 10 cm silica gel column with 10 % EtOAc/hexanes, collecting 5 mL fractions. Fractions 4–12 were combined and concentrated to yield the product (317 mg, 96 %) as a colorless oil. R_f = 0.53 (25 % EtOAc/ hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ

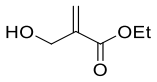
7.73–7.65 (m, 4H), 7.48–7.36 (m, 6H), 7.17 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.71 (d, *J* = 11.0 Hz, 2H), 4.41 (ABq, *J* = 10.5 Hz, Δν = 27.7 Hz, 2H), 4.39-4.33 (m, 1H), 4.16–4.02 (m, 2H), 3.91–3.85 (m, 1H), 3.84–3.75 (m, 2H), 3.80 (s, 3H), 3.55-3.47 (m, 1H), 3.43–3.36 (m, 1H), 2.50 (d, *J* = 7.0 Hz, 1H), 2.48 (s, 1H), 2.26 (d, *J* = 13.5 Hz, 1H), 2.17 (d, *J* = 13 Hz, 1H), 1.93 (q, *J* = 13 Hz, 1H), 1.88–1.79 (m, 3H), 1.69–1.61 (m, 3H), 1.20 (t, *J* = 7.5 Hz, 3H), 1.06 (s, 9H), 0.11 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.7, 159.2, 144.7, 135.8, 134.1, 131.2, 129.7, 129.5, 127.8, 113.9, 108.7, 75.1, 72.8, 71.7, 66.8, 60.7, 60.4, 55.4, 44.2, 43.0, 42.4, 41.3, 41.1, 37.8, 27.1, 19.3, 14.4, 0.50.



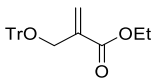
Preparation of (*S*)-1-((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)-4-((trimethylsilyl)methyl)pent-4-en-2-ol (1.42): A 10 mL flask was equipped with a magnetic stir bar, and $CeCl_3 \bullet 7H_2O$ (674 mg, 1.81 mmol, 10.0 equiv) was added. The flask was heated with stirring at 170 ^{o}C at 0.05 mm Hg for 16 h. The resulting anhydrous $CeCl_3$ was cooled under vacuum, and subsequently stirred with THF (1.5 mL) for 2 h. Meanwhile, a 25 mL 2-neck flask was connected to a reflux condenser and the condenser was connected to a vacuum adaptor. A stir bar and magnesium turnings (125 mg, 5.14 mmol, 28.4 equiv) were added and the second neck was closed with a rubber septum. The flask was flame dried under vacuum; after cooling and admitting N_2, an iodine crystal was added and the flask was heated until purple vapors appeared. THF (4.6 mL) was added and the mixture was heated to reflux. $TMSCH_2Cl$, (400 μL, 2.87 mmol, 15.9 equiv) was added dropwise. The solution soon became colorless and then darker

after refluxing for 1.5 h, indicating Grignard reagent formation. The concentration of the solution was estimated to be 1.8 M based on the amount of $TMSCH_2Cl$ used. The slurry of $CeCl_3$ was chilled to −78 °C and the Grignard reagent (1.80 mL, 1.8 mmol, 10 equiv) was added dropwise to the $CeCl_3$ slurry. After stirring for 1 h at −78 °C, TMS ether **1.64** (135 mg, 0.18 mmol, 1.0 equiv) in THF (0.5 mL) was added. The solution was allowed to warm up to rt after stirring for 2 h at −78 °C. After stirring overnight, the solution was diluted with THF (10 mL), chilled to −78 °C, and aqueous 1.0 M HCl was added five drops at a time until the spot with R_f = 0.63 via TLC was the predominant product. The mixture was then quenched by the addition of saturated aqueous Rochelle salt solution (10 mL), and stirred for 1 h. The resulting solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was washed with brine (10 mL) and dried over $MgSO_4$. After filtration and concentration, the crude product was purified on a 3 × 10 cm silica gel column, eluting with 5 % EtOAc/hexanes, collecting 5 mL fractions. Fractions 9–38 contained the product (62 mg, 48 %). R_f = 0.63 (30 % EtOAc/hexanes). 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.74–7.69 (m, 4H), 7.48–7.39 (m, 6H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 4.74 (d, *J* = 9.0 Hz, 2H), 4.69 (d, *J* = 16.5 Hz, 2H), 4.45 (ABq, *J* = 11 Hz, Δν = 30.1 Hz, 2H), 4.04–3.98 (m, 1H), 3.81 (s, 3H), 3.85–3.77 (m, 3H), 3.62–3.52 (m, 3H), 2.26 (d, *J* = 13.5 Hz, 1H), 2.25 (d, *J* = 13.5 Hz, 1H), 2.20 (d, *J* = 13 Hz, 1H), 2.10 (dd, *J* = 14, 6.0 Hz, 1H) 2.05–1.94 (m, 2H), 1.84 (q, *J* = 6.5 Hz, 2H), 1.77–1.64 (m, 4H), 1.60 (s, 2H), 1.10 (s, 9H), 0.07 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.2, 144.6, 144.1, 135.8, 134.0, 134.0, 131.1, 129.7, 129.6, 127.8, 113.9, 110.0, 109.0, 79.3, 75.5, 72.8, 71.5, 69.6, 60.5, 55.4, 46.6, 42.6, 41.9, 41.2, 41.1, 37.5, 27.1, 19.3, −1.2.

Synthetic Procedures for Protected DAG Lactone **1.77**

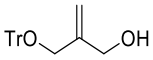


Preparation of Ethyl 2-(hydroxymethyl)acrylate (1.84): A 3-neck 250 mL flask was equipped with a large magnetic stir bar and a reflux condenser. Water (40 mL), paraformaldehyde (17.2 g, 573 mmol, 4.2 equiv), and aqueous H_3PO_4 (1.0 M, 1.53 mL, 1.53 mmol, 0.01 equiv) were added. The mixture was heated at reflux for 2.5 h at which time most of the solids had dissolved. After cooling to rt, triethyl phosphonoacetate **17** (27.0 mL, 136 mmol, 1.0 equiv) was added. The solution was stirred for 5 min, before a solution of K_2CO_3 (20.7 g, 150 mmol, 1.1 equiv) in water (22 mL) was added dropwise, keeping the temperature between 35 ^oC and 40 ^oC by immersing the flask in a tepid water bath. After an additional 5 min of stirring, the mixture was placed in a 0 ^oC bath and then diluted with Et_2O (40 mL) and brine (40 mL). The biphasic solution was separated, and the aqueous phase was extracted with Et_2O (3 × 40 mL). The combined organic phases were washed with brine (2 × 50 mL) and dried over $MgSO_4$. After filtering and concentrating, the resulting liquid was distilled at 0.6 mmHg, collecting the fraction boiling between 49 ^oC – 88 ^oC as a clear liquid, (13.2 g, 74%). The product was immediately used in the next step. 300 MHz ^1H NMR ($CDCl_3$) δ 6.17 (q, J = 1.2 Hz, 1H), 5.77 (q, J = 1.2 Hz, 1H), 4.22 (d, J = 6.0 Hz, 2H), 4.14 (q, J = 7.2 Hz, 2H), 3.36 (t, J = 6.3 Hz, 1H), 1.22 (t, J = 7.2 Hz, 3H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 166.3, 139.7, 125.1, 61.8, 60.8, 14.1.



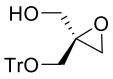
Preparation of Ethyl 2-((trityloxy)methyl)acrylate (1.82): Acrylate ester **1.84**, (11.4 g, 88 mmol, 1.0 equiv), CH_2Cl_2 (137 mL, 0.64 M), and pyridine (8.80 mL, 114 mmol, 1.3 equiv) were combined in a 500 mL flask fitted with a

stir bar and rubber septum under N_2. After cooling to 0 ^oC, trityl chloride (26.6 g, 95 mmol, 1.1 equiv) was added. The flask was removed from the ice bath and stirred overnight at rt. After quenching by the addition of saturated aqueous NH_4Cl solution (150 mL), the mixture was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic layers were washed with brine (100 mL). The organic phase was then dried over $MgSO_4$, filtered, and concentrated to yield a yellow crystalline solid, which was recrystallized from a minimum amount of boiling EtOH to yield the product (31.2 g, 97 %) as an off-white colored crystalline material. R_f = 0.59 (50 % EtOAc/hexanes); mp: 94 – 96 ^oC; 300 MHz ^1H NMR ($CDCl_3$) δ 7.48–7.42 (m, 6H), 7.34–7.20 (m, 9H), 6.35 (d, *J* = 1.8 Hz, 1H), 6.21 (d, *J* = 1.8 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 2H), 1.24 (t, *J* = 7.2 Hz, 3H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 166.0, 144.1, 138.1, 128.8, 128.1, 127.3, 124.6, 87.3, 62.4, 60.8, 14.3.



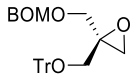
Preparation of 2-((trityloxy)methyl)prop-2-en-1-ol (1.81): A solution of acrylate ester **1.82** (9.0 g, 24 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (100 mL) in a 250 mL flask and chilled to −78 ^oC under an atmosphere of N_2. DIBAL-H (1.0 M, 73 mL, 73 mmol, 3.0 equiv) was added via syringe. After stirring at −78 ^oC for 1 h, the mixture was quenched by the slow addition of MeOH (1.0 mL), followed by pouring into a saturated aqueous Rochelle's salt solution (100 mL) in a 1000 mL Erlenmeyer flask. The solution was filtered through a 6 × 3 cm Celite™ after 3 h of stirring. The mixture was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified on a 5 × 10 cm silica gel column, eluting with 10 % EtOAc/hexanes, collecting 30 mL fractions. After concentrating fractions 28– 56, a white crystalline solid was obtained (6.8

g, 85 %). R_f = 0.31 (25 % EtOAc/hexanes); mp: 78 – 80 °C; 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.47–7.42 (m, 6H), 7.34–7.20 (m, 9H), 5.26 (d, *J* = 0.9 Hz, 1H), 5.17 (d, *J* = 1.2 Hz, 1H), 4.13 (d, *J* = 6.3 Hz, 2H), 3.70 (s, 2H), 1.75 (t, *J* = 6.3 Hz, 1H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 145.9, 144.1, 128.8, 128.1, 127.3, 112.2, 87.3, 65.6, 65.0.



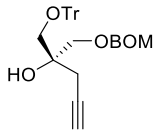
Preparation of (*S*)-(2-((trityloxy)methyl)oxiran-2-yl)methanol (1.85): A 50 mL flask was equipped with a stir bar and 4Å molecular sieves (7.2 g). The flask was chilled to −20 °C after adding CH_2Cl_2 (12 mL). L-(+)-diethyl tartarte (1.0 M in CH_2Cl_2, 1.74 mL, 1.74 mmol, 0.12 equiv), and Ti(*i*-OPr)$_4$ (1.0 M in CH_2Cl_2 , 1.45 mL, 1.45 mmol, 0.10 equiv) were added via syringe. After stirring for 10 min, *t*-BuOOH in decane (5.5 M, 5.3 mL, 29 mmol, 2.0 equiv) was added. After stirring for 0.5 h, a solution of allylic alcohol **1.81** (4.80 g, 14.5 mmol, 1.0 equiv) in CH_2Cl_2 (12 mL) was added. The mixture was stirred overnight in a −20 °C freezer after which time no more conversion to product was noted via TLC. The mixture was warmed to 0 °C, H_2O (4.0 mL) was added, and the mixture was stirred for 1 h. Brine (2.0 mL) and aqueous NaOH solution (50 %, 1.0 mL) were added and the mixture was stirred for an additional h. The mixture was diluted with $CHCl_3$ (25 mL) and H_2O (25 mL) and filtered through a 6 × 4 cm bed of Celite™. The organic phase was separated and the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL). The combined organic phases were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified on a 3 × 15 cm silica gel column, eluting with 10 % EtOAc/hexanes, collecting 30 mL fractions. Fractions 30–64 were combined and concentrated to yield a white crystalline solid (3.6 g, 72 %). R_f = 0.45 (50 % EtOAc/hexanes); mp: 120–122 °C; EtOAc/hexanes); $[\alpha]_D^{20}$ = −16

(c = 1.3, $CHCl_3$); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.43 (d, *J* = 8.1 Hz, 6H), 7.32–7.16 (m, 9H), 3.79 (ABq, *J* = 12.3 Hz, Δν = 52 Hz, 2H), 3.27 (t, *J* = 12.3 Hz, 2H), 2.84 (d, *J* = 4.8 Hz, 1H), 2.68 (d, *J* = 4.5 Hz, 1H), 2.23 (s, 1H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 143.7, 128.7, 128.0, 127.3, 86.9, 64.5, 62.1, 59.2, 48.9. The enantiomeric excess was determined using a 4.6 × 150 mm Chiralcel OD-H column (5.0 % *i*-PrOH/ hexanes; 0.50 mL/min); t_r (major) = 13.5 min, t_r (minor) = 18.6 min; 90 % ee.



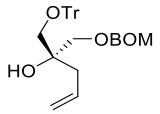
Preparation of (*S*)-2-(((benzyloxy)methoxy)methyl)-2-((trityloxy)methyl)oxirane (1.80): A solution of epoxy-alcohol **1.85** (2.01 g, 5.80 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (58 mL, 0.1 M) in a 100 mL flask equipped with a magnetic stir bar. *i*-Pr_2NEt (2.5 mL, 14.5 mmol, 2.5 equiv) was added, followed by BOM-Cl (1.80 mL, 13.3 mmol, 2.3 equiv) to the stirring solution. After stirring overnight, the mixture was quenched with saturated aqueous $NaHCO_3$ solution (60 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were washed with brine (60 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified on a 5 × 20 cm silica gel column using 10 % EtOAc/hexanes, collecting 30 mL fractions. Fractions 18–32 were combined and concentrated, yielding an oil that crystallized upon chilling overnight in a −20 °C freezer (2.0 g, 75 %). R_f = 0.47 (25 % EtOAc/hexanes); $[\alpha]_D^{20}$ = −8.04 ° (c = 3.65, $CHCl_3$); mp: 75 – 86 °C; 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.47–7.41 (m, 6H), 7.36–7.19 (m, 14H), 4.71 (s, 2H), 4.51 (s, 2H), 3.83 (ABq, *J* = 11.4 Hz, Δν = 9.75 Hz, 2H), 3.29 (q, *J* = 10.5 Hz, 2H), 2.78 (dd, *J* = 15, 4.8 Hz, 2H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 143.9, 137.8, 128.9, 128.6, 128.2, 128.1, 127.9, 127.3, 94.9, 86.9, 69.6, 68.0, 64.1, 58.1, 49.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 94.9, 69.6, 68.0, 64.1, 49.4;

CH δ 128.9, 128.6, 128.2, 128.1, 127.9, 127.3; C δ 143.9, 137.8, 86.9, 58.1; IR (neat) 3060, 3031, 2932, 2880, 1597, 1491, 1449, 1213, 1166, 1104, 1073, 1048, 900, 747, 703, 631 cm^{-1}; HRMS (ESI) calcd 489.2042 for $C_{31}H_{30}O_4Na$ (M+Na), found 489.2047.

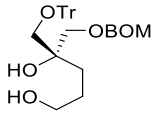


Preparation of (*S*)-1-((benzyloxy)methoxy)-2-((trityloxy)methyl)pent-4-yn-2-ol (1.86): A solution of epoxide **1.80** (2.7 g, 5.8 mmol, 1.0 equiv) was prepared in DMSO (14 mL) in a 50 mL flask under N_2. Lithium acetylide ethylenediamine complex (1.34 g, 14.5 mmol, 2.5 equiv) was weighed out in a dry box and transferred to the stirring solution of the epoxide. After 2 h, the solution was cooled in an ice bath and slowly quenched by the addition of saturated aqueous NH_4Cl solution (25 mL). The solution was then extracted with CH_2Cl_2 (3 × 25 mL). The organic phase was then dried over $MgSO_4$, filtered, and concentrated. The product was dissolved in the minimum amount of $CHCl_3$, and purified on a 5 × 20 cm silica gel column, eluting with 10 % EtOAc/hexanes, collecting 30 mL fractions. Fractions 10–38 were concentrated, yielding the product (2.5 g, 88 %) as a colorless oil. R_f = 0.26, (25 % EtOAc/ hexane); $[\alpha]_D^{20}$ = –5.55 ^{o} (c = 12.2, $CHCl_3$); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.47–7.41 (m, 6H), 7.34–7.20 (m, 14H), 4.72 (s, 2H), 4.53 (s, 2H), 3.73 (ABq, *J* = 10.2 Hz, Δν = 8.8 Hz, 2H), 3.24 (s, 2H), 2.74 (s, 1H), 2.60 (d, *J* = 2.4 Hz, 2H), 1.95 (t, *J* = 2.4 Hz, 1H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 143.8, 137.7, 128.9, 128.6, 128.1, 128.0, 128.0, 127.3, 95.3, 86.9, 80.0, 73.4, 71.3, 71.3, 69.7, 65.4, 25.7; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 95.4, 71.4, 69.8, 65.5, 25.8; CH δ 129.0, 128.7, 128.2, 128.1, 128.0, 127.4; C δ 143.8, 137.7, 86.9, 80.0, 73.4, 71.3; IR (neat) 3556, 3454, 3295, 3060, 3031, 2936, 2881, 2119, 1597, 1492,

1449, 1047, 991, 903, 767, 746, 702, 633 cm^{-1}; HRMS (ESI) calcd 515.2198 for $C_{33}H_{32}O_4Na$ (M+Na), found 515.2202.

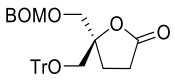


Preparation of (*S*)-1-((benzyloxy)methoxy)-2-((trityloxy)methyl)pent-4-en-2-ol (1.87): A solution of homopropargylic alcohol **1.86** (1.99 g, 4.0 mmol, 1.0 equiv) was prepared in 50% EtOAc/hexanes (35 mL) in a 100 mL 3-neck flask. The center neck of the flask was fitted with a valve connected to a balloon filled with H_2. The other 2 necks were closed with rubber septa. Lindlar's catalyst containing 5 % Pd (0.90 g) and quinoline (0.84 mL, 7.3 mmol, 1.8 equiv) were then added and the flask was flushed with H_2. The reaction was complete by TLC after 2 h of stirring. The mixture was filtered through a 3 × 2 cm bed of Celite™ and concentrated. The crude product was purified on a 3 × 20 cm silica gel column, eluting with 10 % EtOAc/hexanes, collecting 10 mL fractions. Fractions 12–30 were concentrated, yielding the product (1.91 g, 96 %) as a colorless oil. R_f = 0.33 (25 % EtOAc/hexanes); $[\alpha]_D^{20}$ = –2.5° (c = 0.96, $CHCl_3$); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.46–7.41 (m, 6H), 7.36–7.19 (m, 14H), 5.72 (dddd, *J* = 14.7, 9.9, 7.2, 7.2 Hz, 1H), 5.08–5.02 (m, 1H), 5.02–4.95 (m, 1H), 4.72 (s, 2H), 4.53 (s, 2H), 3.65 (ABq, *J* = 9.9 Hz, Δν = 33.7 Hz, 2H), 3.12 (s, 2H), 2.58 (s, 1H), 2.38 (d, *J* = 7.2 Hz, 2H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 143.9, 137.7, 133.2, 128.9, 128.7, 128.1, 128.0, 127.3, 118.7, 95.4, 86.8, 73.6, 72.2, 69.7, 65.7, 39.6; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 118.7, 95.4, 72.2. 69.7, 65.7, 39.6; CH δ 133.2, 128.9, 128.7, 128.1, 128.0, 127.3; C δ 143.9, 137.7, 86.8, 73.6; IR (neat) 3563, 3456, 3062, 3030, 2935, 2880, 1641, 1597, 1492, 1449, 1046, 701 cm^{-1}; HRMS (ESI) calcd 517.2355 for $C_{33}H_{34}O_4Na$ (M+Na), found 517.2350.



Preparation of (*S*)-5-((benzyloxy)methoxy)-4-((trityloxy)methyl)pentane-1,4-diol (1.88): A solution of homoallylic alcohol **1.87** (1.91 g, 3.86 mmol, 1.0 equiv) was prepared in THF (19 mL) in a 100 mL flask under N_2 with stirring. A solution of 9-BBN (0.5 M, 19.0 mL, 11.6 mmol, 3.0 equiv) was added and the mixture was stirred for 15 min. The mixture was then placed in an ultrasonic bath for 1 h, after which time no further conversion to product was noticeable via TLC. The mixture was chilled in an ice bath and aqueous NaOH (2.0 M, 10 mL) was carefully added. Aqueous H_2O_2 (30%, 5.0 mL) was then carefully added in small portions to prevent loss of the reaction mixture due to the exothermic nature of the oxidation of the organoborane. After stirring for 1 h at rt, the mixture was extracted with EtOAc (3 × 30 mL) and then washed with brine (50 mL). The organic phase was then dried over $MgSO_4$, filtered, and concentrated. The product was then purified on a 5 × 20 cm silica gel column, eluting with a 10–35 % gradient of EtOAc/ hexanes, collecting 10 mL fractions. Fractions 96–120 were combined and concentrated to yield the product (1.61 g, 81 %) as a viscous oil. R_f = 0.28 (25 % EtOAc/hexanes); $[\alpha]_D^{20}$ = –2.2° (c = 2.1, $CHCl_3$); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.44–7.41 (m, 6H), 7.32–7.21 (m, 14H), 4.72 (s, 2H), 4.53 (s, 2H) 3.69 (ABq, *J* = 9.9 Hz, Δν = 46.5 Hz, 2H), 3.69 (t, *J* = 6.0 Hz, 2H), 3.14 (dd, *J* = 12.9, 8.5 Hz, 2H), 2.96 (s, 1H), 2.19 (s, 1H), 1.70–1.61 (m, 2H), 1.53–1.45 (m, 2H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 143.9, 137.7, 128.9, 128.7, 128.1, 128.0, 128.0, 127.3, 95.5, 86.8, 73.6, 72.4, 69.8, 65.4, 63.4, 31.5, 26.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 95.5, 72.4, 69.8, 65.4, 63.4, 31.5, 26.2; CH δ 129.0, 128.7, 128.1, 128.1, 128.0, 127.3; C δ 143.9, 137.7, 86.8, 73.6; IR (neat) 3387, 3086, 3060, 2934, 2878, 1597, 1491, 1449, 1049, 746, 701 cm^{-1};

HRMS (ESI) calcd 535.2498 for $C_{33}H_{36}O_5Na$ (M+Na), found 535.2450.

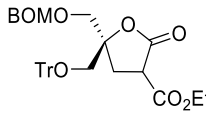


Preparation of (*S*)-5-(((benzyloxy)methoxy)methyl)-5-((trityloxy)methyl)dihydrofuran-2(3*H*)-one (1.89): A solution of diol **1.88** (1.32 g 2.58 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (26 mL) in a 100 mL flask fitted with a magnetic stir bar and septum under N_2. After chilling to 0 ^oC, *i*-Pr_2NEt (3.20 mL, 18.1 mmol, 7.0 equiv), and DMSO (1.80 mL, 25.8 mmol, 10.0 equiv) were added to the stirred solution. $SO_3 \bullet$ Pyr (1.65 g, 10.3 mmol, 4.0 equiv) was added in 4 portions over 20 min. After 1 h, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (50 mL), and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated to yield a yellow colored oil that was used in the next step without purification.

A solution of the crude aldehyde from the previous step was prepared in *t*-BuOH (36 mL) in a 250 mL flask, 2-methyl-2-butene (36 mL) was added, along with an aqueous solution of KH_2PO_4 (1.25 M, 12.4 mL). After cooling to −10 ^oC, $NaClO_2$, (80 %, 1.45 g, 16.1 mmol, 5.0 equiv) was added in 4 portions over 20 min. The reaction mixture was then allowed to warm to rt overnight. The reaction mixture was then extracted with Et_2O (3 × 25 mL), and the combined organic phases were washed with brine (50 mL). The organic phase was then dried over $MgSO_4$, filtered, and then concentrated to yield the crude carboxylic acid as a viscous yellow oil. The product was then purified on a 2.5 × 15 cm silica gel column, eluting with 3% MeOH/ CH_2Cl_2, collecting 10 mL fractions. Fractions 22−48 contained a mixture of the carboxylic acid and the desired lactone and were concentrated to yield an oil which was used in the subsequent step without

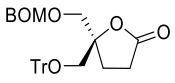
characterization (1.08g, 80 %, 2-steps).

The crude product from the previous step (0.20 g, 0.38 mmol, 1.0 equiv) was dissolved in toluene (58 mL, 0.006 M) in a 100 mL flask. DMAP (0.27 g, 2.2 mmol, 6.1 equiv) was added, followed by the slow addition of benzoyl chloride (60 μL, 0.5 mmol, 1.3 equiv). The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ solution (10 mL) after stirring overnight. After separating the organic phase, the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (10 mL) and dried over $MgSO_4$. The mixture was filtered and the solvent removed. The product was purified on a 2.5 × 20 cm silica gel column, eluting with a gradient from 10 – 75 % EtOAc/hexanes, collecting 5 mL fractions. Fractions 28–38 were concentrated to yield the product (0.15 g, 79 %) as a viscous oil. R_f = 0.65 (50 % EtOAc/ hexanes); $[\alpha]_D^{20}$ = + 6.6 ^{o} (c = 4.6, $CHCl_3$); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.46–7.39 (m, 6H), 7.36–7.20 (m, 14H), 4.72 (s, 2H), 4.53 (s, 2H), 3.69 (ABq, J = 10.5 Hz, Δν = 37.4 Hz, 2H), 3.24 (ABq, J = 9.9 Hz, Δν = 38.3 Hz, 2H), 2.62 (t, J = 8.7 Hz, 2H), 2.15–1.94 (m, 2H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 177.1, 143.3, 137.5, 128.6, 128.4, 128.0, 127.9, 127.8, 127.2, 94.7, 87.0, 86.4, 70.5, 69.6, 66.2, 29.1, 26.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 94.7, 70.5, 69.6, 66.2, 29.1, 26.5; CH δ 128.6, 128.4, 128.0, 127.9, 127.8, 127.2; C δ 177.1, 143.3, 137.5, 87.0, 86.4; IR (neat) 3060, 3031, 2938, 2879, 1779, 1597, 1492, 1450, 1414, 1381, 1048, 747, 704, 634 cm^{-1}; HRMS (ESI) calcd 531.2147 for $C_{33}H_{32}O_5Na$ (M+Na), found 531.2148.



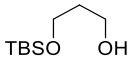
Preparation of Ethyl (5*S*)-5-(((benzyloxy)methoxy)methyl)-2-

oxo-5-((trityloxy)methyl)tetrahydrofuran-3-carboxylate (1.90): To a 500 mL flask containing 60% NaH (6.84 g, 171 mmol, 9.0 equiv), and a stir bar under N_2 was slowly added EtOH (190 mL, 0.1 M) at 0 ^oC. After 20 min, the ice bath was removed and diethyl malonate (29 mL, 190 mmol, 10.0 equiv) was added. After stirring for 30 min at rt, a solution of epoxide **1.80** (8.90 g, 19.0 mmol, 1.0 equiv) in THF (30 mL) was added via cannula, THF (20 mL) was used to complete the transfer. After stirring for 8 days at rt, the reaction was quenched with saturated aqueous NH_4Cl solution (250 mL), and diluted with H_2O (200 mL). The solution was then extracted with 50% EtOAc/ hexanes (3 × 100 mL), washed with brine (2 × 100 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was then purified on a 5 × 20 cm silica gel column, eluting with 15 % EtOAc/ hexanes, collecting 30 mL fractions. Fractions 18–82 contained the product as well as diethyl malonate. The product was heated under high vacuum to remove the remaing diethyl malonate, yielding the product as a mixture of two diastereomers (9.87 g, 89%). Used without characterization.



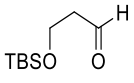
Preparation of (*S*)-5-(((benzyloxy)methoxy)methyl)-5-((trityloxy)methyl)dihydrofuran-2(3*H*)-one (1.89): A solution of diester **1.90** (3.0 g, 5.2 mmol, 1.0 equiv) was prepared in EtOH (52 mL, 0.1 M) in a 100 mL flask. To the stirred solution, aqueous NaOH (1.0 M, 12.9 mL, 12.9 mmol, 2.5 equiv) was added. After stirring for 24 h, the solution was concentrated and then quenched with a solution of AcOH (2.0 mL) in brine (50 mL). The solution was then extracted with EtOAc (3 × 25 mL), washed with brine (50 mL), dried over Na_2SO_4, filtered, and then concentrated and evaporated with toluene to remove any remaining AcOH. The crude carboxylic acid was used as is (2.82 g, 99%)

To a stirring solution of the previously produced carboxylic acid (2.82g, 5.1 mmol, 1.0 equiv) in toluene (102 mL, 0.05 M) in a 250 mL flask was added quinoline (730 μL, 6.1 mmol, 1.2 equiv). A reflux condenser was attached and the mixture was flushed with N_2 and heated at 80 ^oC overnight. After cooling to rt, the solution was washed with aqueous HCl (1.0 M, 2 × 50 mL), saturated aqueous $NaHCO_3$ solution (100 mL), brine (100 mL), and dried over Na_2SO_4. After filtration and concentration, the product was purified on a 3 × 15 cm silica gel column, eluting with 25% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 8–24 were combined and concentrated to yield the product (2.33 g, 90%) as a clear oil spectroscopically identical as material produced in the preceeding manner. R_f = 0.65 (50 % EtOAc/ hexanes); $[\alpha]_D^{20}$ = +6.6 ^{o} (c = 4.6, $CHCl_3$); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.46–7.39 (m, 5H), 7.36–7.20 (m, 15H), 4.72 (s, 2H), 4.53 (s, 2H), 3.69 (ABq, J = 10.5 Hz, Δν = 37.4 Hz, 2H), 3.24 (ABq, J = 9.9 Hz, Δν = 38.3 Hz, 2H), 2.62 (t, J = 8.7 Hz, 2H), 2.15–1.94 (m, 2H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 177.1, 143.3, 137.5, 128.6, 128.4, 128.0, 127.9, 127.8, 127.2, 94.7, 87.0, 86.4, 70.5, 69.6, 66.2, 29.1, 26.5.

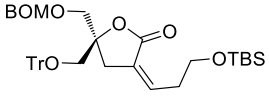


Preparation of 3-((tert-butyldimethylsilyl)oxy)propan-1-ol (1.97): A solution of 1,3-propanediol (18 mL, 250 mmol, 5.0 equiv) and TBSCl (7.54 g, 50 mmol, 1.0 equiv) in CH_2Cl_2 (294 mL, 0.17 M) was prepared in a 500 mL flask under N_2 to which was subsequently added DMAP (305 mg, 2.5 mmol, 0.05 equiv), along with Et_3N (14.0 mL, 100 mmol, 2.0 equiv). The reaction mixture was stirred overnight at rt and quenched with a solution of saturated aqueous $NaHCO_3$ (100mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL), washed with brine (100mL), dried over $MgSO_4$,

filtered, and concentrated. The product was purified on a 5 × 20 cm silica gel column, eluting with 10 % EtOAc/hexanes, collecting 30 mL fractions. Fractions 12–46 were concentrated to give the product (8.4 g, 88%) as a colorless oil. R_f = 0.53 (50% EtOAc/ hexanes). 300 MHz ^{1}H NMR ($CDCl_3$) δ 3.86–3.74 (m, 4H), 2.78–2.68 (m, 1H), 1.77 (quint, *J* = 5.4 Hz, 2H), 0.89 (s, 9H), 0.08 (s, 6H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 63.1, 62.6, 34.3, 26.1, 18.4, –5.3.



Preparation of 3-((tert-butyldimethylsilyl)oxy)propanal (1.79): To a stirring solution of alcohol **1.97** (4.24 g, 22.3 mmol, 1.0 equiv) in CH_2Cl_2 (223 mL, 0.1 M) contained in a 500 mL flask under N_2 at 0 °C was added *i*-Pr_2NEt (27 mL, 156 mmol, 7.0 equiv), followed by DMSO (15.8 mL, 223 mmol, 10.0 equiv) After stirring for 10 min, SO_3 • Pyr (14.2 g, 89 mmol, 4.0 equiv) was added in 4 equal portions 5 min apart over 20 min. After stirring for 1 h at 0 °C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (150 mL). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phase was washed with brine (250 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 4 × 10 cm silica gel column, eluting with 10 % EtOAc/ hexanes, collecting 30 mL fractions. Fractions 6–10 were concentrated to yield the product (3.7 g, 89 %) as a light yellow oil. R_f = 0.59 (25% EtOAc/hexanes). 300 MHz ^{1}H NMR ($CDCl_3$) δ 9.80 (t, *J* = 2.1 Hz, 1H), 3.99 (t, *J* = 6.0 Hz, 2H), 2.60 (dt, *J* = 6.3, 2.1 Hz, 2H), 0.88 (s, 9H), 0.07 (s, 6H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 202.3, 57.6, 46.8, 26.0, 18.4, –5.3.

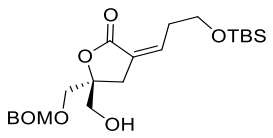


Preparation of (*S*,*Z*)-5-(((benzyloxy)methoxy)methyl)-3-

(3-((tert-butyldimethylsilyl)oxy)propylidene)-5-((trityloxy)methyl)dihydrofuran-2(3H)-one (1.77): A 1.0 M solution of LDA in THF was prepared by adding *i*-Pr_2NH, (0.77 mL, 5.5 mmol, 1.1 equiv) to a 5.0 mL volumetric flask under N_2.The solution was chilled to −78 ^oC, and n-BuLi (2.5 M, 2.0 mL, 5.0 mmol, 1.0 equiv) was then added dropwise. After 5 min, the mixture was transferred to a 0 ^oC bath and THF added to make 5 mL of solution. THF (2.0 mL) was added to a 15 mL flask under N_2, which was chilled to −78 ^oC, and the previously prepared solution of LDA (1.0 M, 0.52 mL, 0.52 mmol, 1.1 equiv) was added. A solution of lactone **1.89** (238 mg, 0.47 mmol, 1.0 equiv) in THF (1.0 mL) was then added via cannula to the LDA containing solution; the transfer was completed with THF (1.7 mL). After stirring for 45 min at −78 ^oC, a solution of aldehyde **1.79** (133 mg, 0.71 mmol, 1.5 equiv) in THF (1.0 mL) was added. THF (1.0 mL) was used to complete the transfer of the aldehyde containing solution. The reaction mixture was stirred for 2 h, allowing the −78 ^oC bath to expire. The reaction mixture was then quenched with saturated aqueous NH_4Cl solution (25 mL), extracted with EtOAc (3 × 15 mL), and washed with brine (50 mL). After drying over Na_2SO_4, the solution was filtered and concentrated. The product was purified on a 2 × 10 cm silica gel column, eluting with 25% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 6−12 contained the product, which was used as is without further characterization.

The aldol product (326 mg) was taken up in CH_2Cl_2 (4.7 mL, 0.1 M) contained in a 15 mL flask under N_2. The stirring solution was cooled to 0 ^oC. Et_3N (200 μL, 1.40 mmol, 3.0 equiv) was added to the solution, followed by MsCl (68 μL, 0.75 mmol, 1.6 equiv) After stirring for 10 min, DBU (210 μL, 1.40 mmol, 3.0 equiv) was added dropwise. After 15 min, all starting material was consumed via TLC; the reaction mixture

was then quenched with a solution of saturated aqueous $NaHCO_3$ (50 mL). After extraction with CH_2Cl_2 (3 × 15 mL), the organic phase was washed with brine (50 mL) and dried over Na_2SO_4. The solution was filtered and concentrated, and the resulting oil was purified on a 2 × 15 cm silica gel column, eluting with 10% EtOAc/ hexanes, collecting 5.0 mL fractions. Fractions 12–22 contained the desired Z-isomer (150 mg, 47%) R_f = 0.64 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = –1.73 ^{o} (c = 1.62, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) 7.48–7.46 (m, 4H), 7.36–7.32 (m, 14H), 7.20–7.16 (m, 4H), 6.31 (dddd, *J* = 9.0, 4.0, 1.5, 1.5 Hz, 1H), 4.70 (s, 2H), 4.53 (s, 2H) 3.75–3.67 (m, 4H), 3.25 (ABq, *J* = 10 Hz, Δν = 61.2 Hz, 2H), 3.06–3.01 (m, 1H), 2.97–2.93 (m, 1H), 2.81 (d, *J* = 16.5 Hz, 1H), 2.72 (d, *J* = 18.5 Hz, 1H), 0.91 (s, 9H), 0.08 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.2, 143.6, 140.4, 137.7, 128.8, 128.8, 128.6, 128.1, 127.9, 127.3, 126.3, 94.9, 87.1, 83.0, 70.2, 69.7, 65.9, 62.3, 34.1, 31.4, 26.1, 18.6, –5.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 26.1, –5.1; CH_2 δ 94.9, 70.2, 69.7, 65.9, 62.3, 34.1, 31.4; CH δ 140.4, 128.8, 128.6, 128.1, 127.9, 127.3, 126.3 C δ 169.2, 143.6, 137.7, 128.8, 87.1, 83.0, 18.6; IR (neat) 3058, 2953, 2928, 2857, 1758, 1491, 1449, 1363, 1256, 1201, 1178, 1095, 1047, 992, 941, 836, 777, 746, 700, 668, 632 cm^{-1}; HRMS (ESI) calcd 701.3280 for $C_{42}H_{50}O_6SiNa$ (M+Na), found 701.3284.



Preparation of (*S*,*Z*)-5-(((benzyloxy)methoxy)methyl)-3-(3-((tert-butyldimethylsilyl)oxy)propylidene)-5-(hydroxymethyl)dihydrofuran-2(3H)-one (1.93): To a stirring solution of **1.77** (80 mg, 0.12 mmol, 1.0 equiv) in CH_2Cl_2 (2.4 mL, 0.05 M) at 0 ^{o}C was added a TFA/TFAA solution in CH_2Cl_2 (0.5 M, 720 μL,

0.36 mmol, 3.0 equiv) The reaction mixture turned bright yellow and was stirred at 0 ^{o}C for 45 min. The reaction mixture was quenched with Et_3N (0.34 mL, 2.4 mmol, 20 equiv) After 5 min, the mixture was evaporated with methanol (3 × 25 mL), followed by toluene (10 mL) The product was purified on a 2 × 13 cm silica gel column, eluting with 25% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 6–24 were concentrated to yield the product (45.5 mg, 69%) as a colorless oil. R_f = 0.14 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = –8.1 ^{o} (c = 0.61, MeOH); 500 MHz ^{1}H NMR ($CDCl_3$) 7.37–7.27 (m, 5H), 6.35 (dddd, *J* = 9.5, 4.5, 2.0, 2.0 Hz, 1H), 4.77 (s, 2H), 4.60 (s, 2H), 3.75–3.64 (m, 6H), 2.93 (dd, *J* = 13.5, 6.0 Hz, 2H), 2.83 (dd, *J* = 16.5, 2.5 Hz, 1H), 2.78 (dd, *J* = 14.5, 2.0 Hz, 1H), 2.25 (s, 1 H), 0.90 (s, 9H), 0.06 (s, 6H). δ 125 MHz ^{13}C NMR ($CDCl_3$) δ; 169.2, 141.8, 137.7, 128.7, 128.1, 128.0, 125.7, 95.2, 83.5, 70.0, 69.7, 65.4, 62.3, 33.2, 31.4, 26.1, 18.5, 5.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 26.1, –5.1; CH_2 δ 95.2, 70.0, 69.7, 65.4, 62.3, 33.2, 31.4; CH δ 141.8, 128.7, 128.1, 128.0; C δ 169.2, 137.7, 125.7, 83.5, 18.5; IR (neat) 3443, 3033, 2954, 2930, 2885, 2858, 1755, 1672, 1575, 1498, 1472, 1463, 1381, 1364, 1257, 1200, 1177, 1101, 1046, 939, 837, 778 cm^{-1}; HRMS (ESI) calcd 459.2179 for $C_{23}H_{36}O_6SiNa$ (M+Na), found 459.2181.

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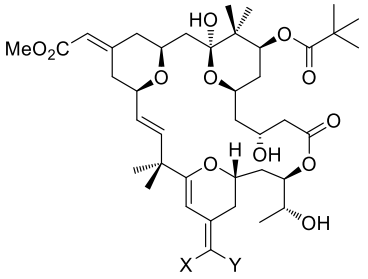
CHAPTER 2

SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-DAG LACTONE C-RING BRYOSTATIN ANALOGS

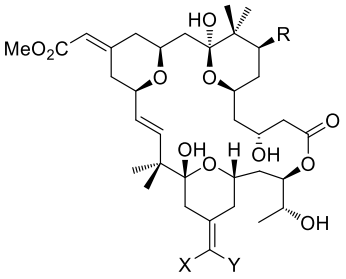
Introduction to C-Ring Analogs

Several natural products in the bryostatin family consist of C-ring varients. Several natural C20 esters analogs exist; these variations have little effect on the binding potency. Bryostatins 16, 17 are missing the C19 ketal and C20 ester functionality which have been replaced by an olefin (Figure **2.1**).[1] The binding affinity of these compounds is reduced by 3 orders of magnitude. Bryostatin 16 has the same *E* geometry of the C21 enoate as bryostatin 1 and has a binding affinity of 118 nM. The enoate of bryostatin 17 has a *Z* geometry, resulting in an approximately 2 fold loss of affinity.

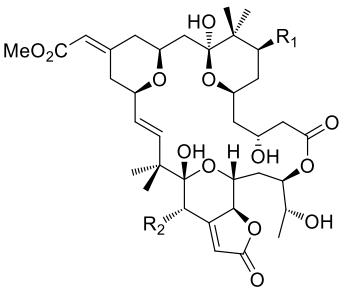
Bryostatins 10, 13, and 18 are all C20 deoxygenated; relatively little binding affinity is lost in bryostatin 10.[2] Bryostatin 18 has a Z C21 enoate, resulting in a minor loss of binding affinity.[1] Bryostatins 3, 19, 20 contain a C22 oxygenated C-ring, where the enoate has cyclized onto the new C22 alcohol.[3] The binding constant for bryostatin 3 is known, K_i = 2.8 nM, indicating very little loss of affinity in this series. Neristatin, a related natural product to bryostatin, contains a highly modified C-ring and still retains a high affinity for PKC, K_i = 21.2 nM.[4] Neristatin has been shown to be bryostatin 1-like in



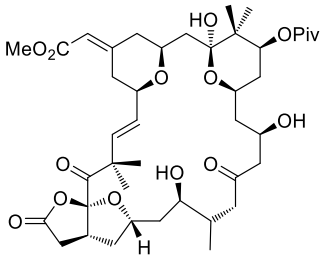
Bryostatin 16 Y = CO_2Me X = H
K_i = 118
Bryostatin 17 Y = CO_2Me X = H
K_i = 188



Bryostatin 10 R = OCO*t*-Bu X = H Y = CO_2Me
K_i = 1.6 nM
Bryostatin 13 R = OCO*n*-Pr X = H Y = CO_2Me
K_i = ?
Bryostatin 18 R = OPiv X = CO_2Me Y = H
K_i = 4.8 nM



Bryostatin 3 R_1 = OAc R_2 = $OCO(CH)_4Pr$
K_i = 2.8 nM
Bryostatin 19 R_1 = OCO*t*-Bu R_2 = OCO*n*-Pr
K_i = ?
Bryostatin 20 R_1 = OCO*t*-Bu R_2 = H
K_i = ?

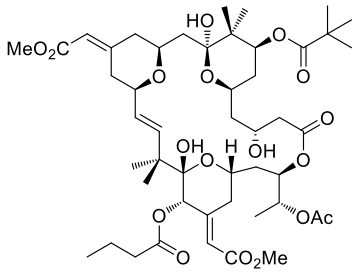


Neristatin
K_i = 21.2 nM

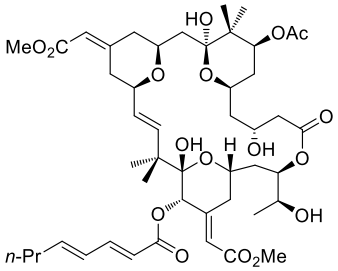
Figure 2.1 Natural Bryostatin C-Ring Analogs

U937 leukemia cells; this indicates that molecules with bryostatin like top-halfs probably will retain bryostatin-like properties as long as the lower half has a high affinity for PKC.

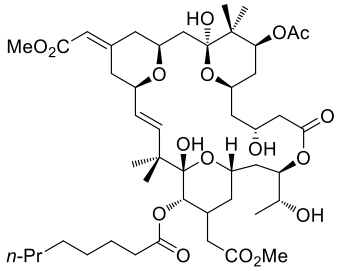
In comparison to analogs of the AB-ring system of bryostatin, relatively few synthetic C-ring analogs of the natural product have been produced to date (Figure **2.2**). The collaboration between Pettit and Blumberg, mentioned previously (Figure **1.5**), resulted in several C-ring modifications.[5] Inversion of the C26 alcohol resulted in the loss of affinity by 2 orders of magnitude, while esterification resulted in a loss of affinity greater then 3 orders of magnitude. Hydrogenation of the C22 enoate resulted in dramatic



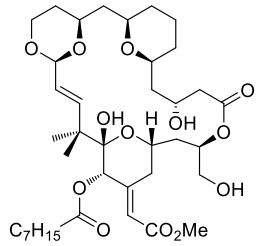
(26-Acetoxy)-bryostatin 4
K_i > 100 nM



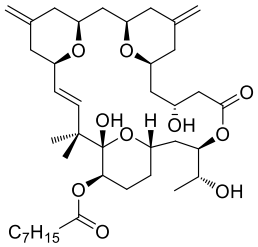
(26-epi)-bryostatin 1
K_i = 32.6 +/- 0.6 nM



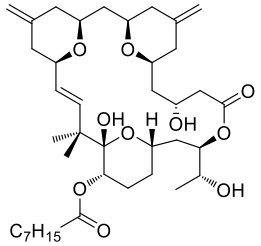
(13 ,30 ,21, 34, 2', 3', 4', 5'-Octahydro)-
bryostatin 2
K_i = 473 +/- 96 nM



Picolog
K_i = 3.1 nM



Merle 24
K_i = 37.7 +/- 0.5 nM



Merle 25
K_i = 47.1 +/- 4.2 nM

Figure 2.2 Synthetic C-Ring Analogs

loss of affinity. Wender's most studied analog, "Picolog" is missing the C27 methyl group and still retains high affinity.[6] Keck's Merle 24 and 25 are both missing the C22 enoate, resulting in a loss of binding affinity by 2 orders of magnitude.[7] Inversion of the C20 ester in Merle 25 results in some loss of affinity versus Merle 24. The synthesis of potent bryostatins remains difficult due to the length of the C-ring synthesis. Very little work to date has been conducted with respect to replacing the C-ring of bryostatin analogs with a suitable surrogate that retains the same binding affinity of the natural product.

Design of Merle's 39, and 47, 48

In the search for simplified bryostatin analogs, the Keck group turned its attention towards the synthesis of simplified C-ring domains that retain the high binding affinity of bryostatin 1. The Marquez diacylglycerol lactones have high binding affinity and are relatively simple to construct compared to the C-ring of the natural product.[8] Interchanging the C-ring of bryostatin for a Marquez type lactone could possibly yield a simplified analog retaining the affinity of the natural product. For simplicity of synthesis, the Merle 23 type top-half was chosen, since the biology of this top-half is well explored and would make comparison of the biological results straightforward.[9] A Marquez lactone scaffold with high binding affinity was chosen to serve as the C-ring.[10] The only remaining questions were how to attach the lactone piece to the top-half and the chirality of gamma carbon of the lactone.

DAG lactones are thought to bind in two different orientations termed sn-*1* and sn-*2* modes (Figure **2.3**).[11] Simulation of binding of DAG lactones by the Marquez group

using Autodock 2.4, using the crystal structure of PMA bound to the C1b domain of PKC δ, determined that two different binding modes are possible. The binding modes differ with respect to which carbonyl is bound to PKC. The sn-*1* mode is depicted on the left and the sn-*2* mode is depicted on the right, respectively. The sn-*1* mode is thought to be the primary mode of binding for DAGs, while the sn-*2* mode is more energetically favorable for DAG lactones. In this binding model, only one carbonyl is involved in an interaction with the protein. Synthesis of DAG analogs missing the side chain ester result in compounds with a binding affinity of a 100 fold less then the parent compound; this indicates that both carbonyls are important in binding.[12]

A series of DAG lactones were synthesized as the racemates that incorporated the ester side chain and the enoate into a macrocyclic structure.[13] These compounds retained much of the binding affinity of the open chain compounds (Figure **2.4**). These compounds would have been more potent if they were constructed from the (*R*) lactone. The 22-membered macrolactone was found to be the most potent of these compounds. Larger and smaller lactone sizes resulted in the loss of binding affinity. Contraction seemed to result in the sharpest decrease in affinity, but expansion seen to decrease the affinity only slightly.

The results of these studies indicate that the two possible attachment modes in the diester analog could lead to active analogs (Figure **2.5**). The proper chirality of the lactone in the analog with respect to PKC binding was unknown. It was assumed that the optimum chirality determined by Marquez in his studies on DAG lactones would be the optimum chirality needed for good binding affinity in these bryostatin/DAG hybrid analogs. Marquez determined that the binding affinity of the (*R*)-lactone was 2-fold better

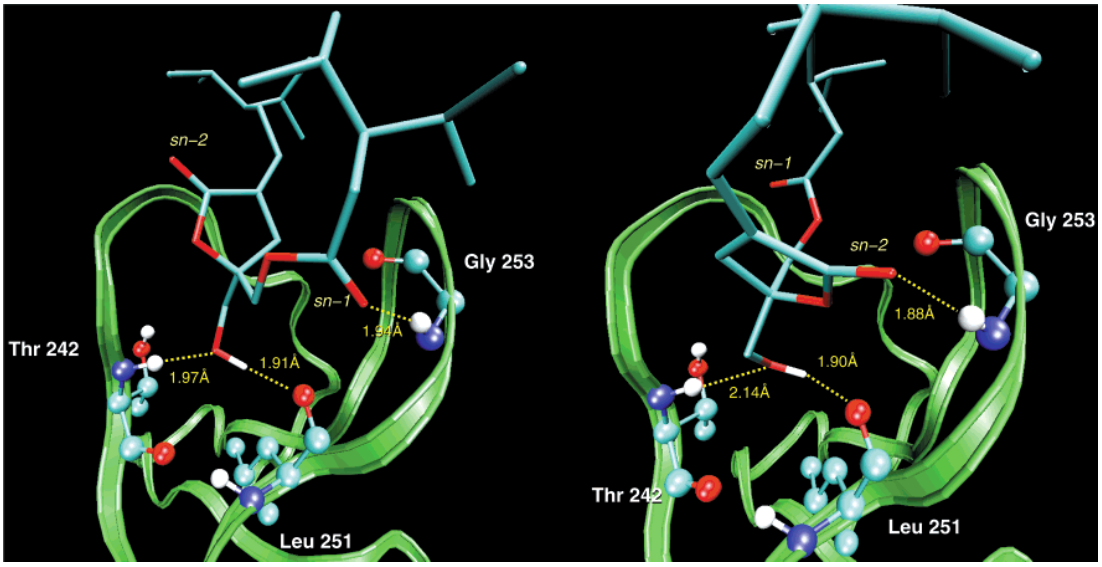


Figure 2.3 DAG Lactone Binding Modes (used with permission from 11).

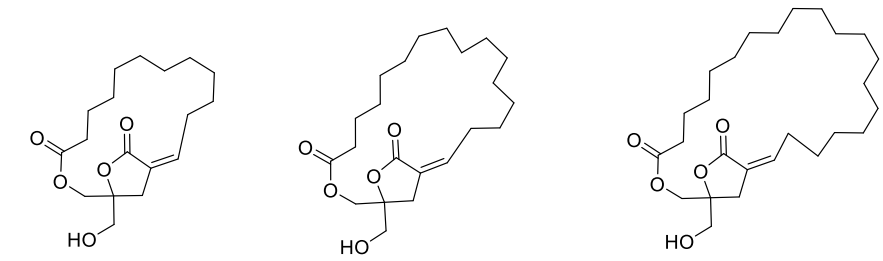


Figure 2.4 Macrocyclic DAG Lactones

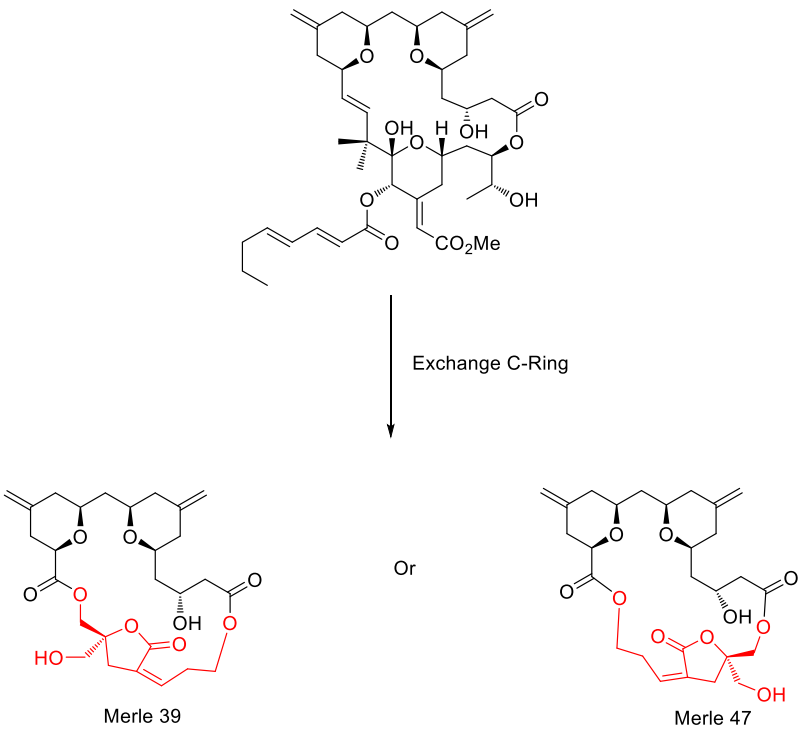


Figure 2.5 C-Ring Orientations

then that of the racemate (Figure **2.6**).[10]

Results and Discussion

Synthesis of Merle 39

The first analog based upon the Marquez lactone C-Ring motif was Merle 39. Merle 39 was based upon a modular C1/ C16 diester top-half to allow for alteration of orientation if binding affinity of the first compound was unsatisfactory. The retrosynthesis of Merle 39 is shown in Figure **2.7**. The protecting group scheme was chosen to allow for selective removal of protecting groups at the proper stage. A Yamaguchi macrolactonization was proposed to form the macrolactone, while a Steglich

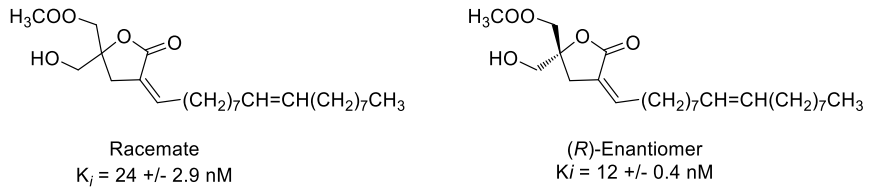


Figure 2.6 Comparison of Racemic and Enantiopure DAG Lactones

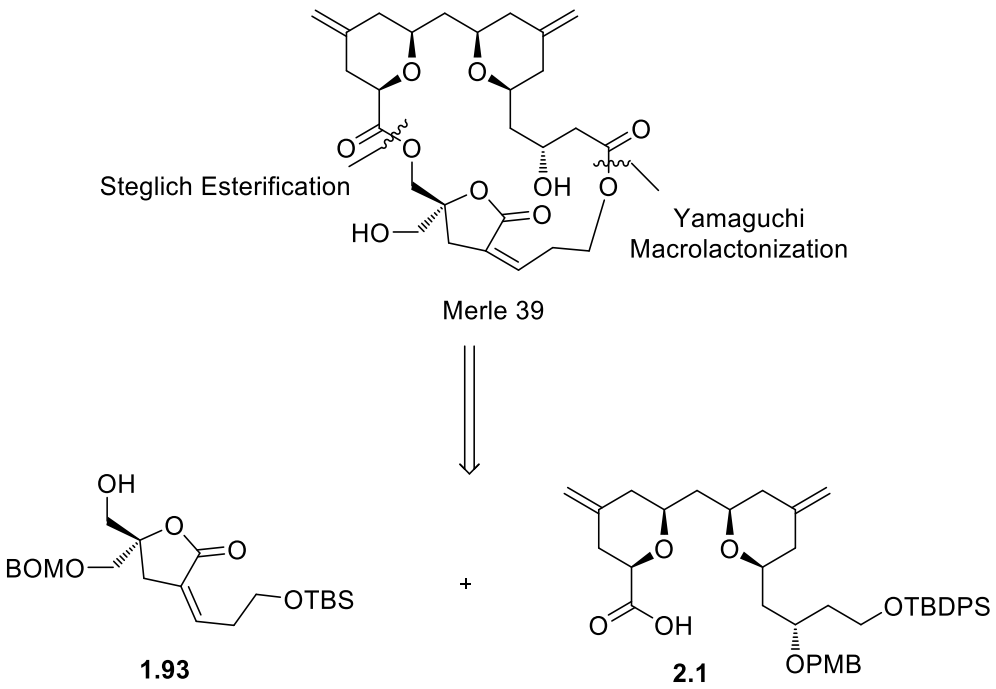


Figure 2.7 Retrosynthesis of Merle 39

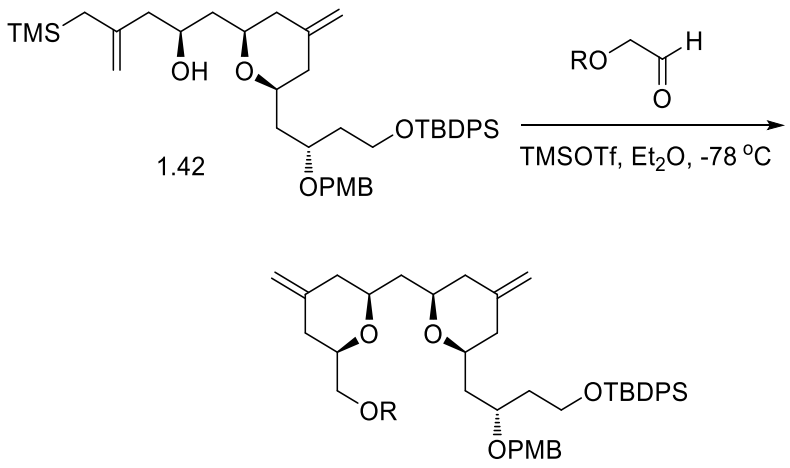
esterification was envisioned to be used to couple the AB bis-pyran acid **2.1** and C-ring alcohol **1.93**.

Scaled A-ring Synthesis and Elaboration

In order to produce material for the synthesis of new bryostatin analogs, a program was undertaken to produce multigram quantities of hydroxyallylsilane **1.15**. The route used is the same as in Figure **1.17** and therefore is not repeated here.[14] In total, approximately 60 grams of the required hydroxyallylslilane were produced. This hydroxylallylsilane was coupled with aldehyde **1.43**, generously provided by Kevin McGowan to yield pyran **1.62** (Figure **1.18**). Elaboration to the B-ring precursor hydroxyallylsilane **1.42** was the same as in Figure **1.19** and will not be described here.

Elaboration of hydroxyallylsilane **1.42** was envisioned to proceed via pyran annulation with a protected glycolaldehyde[15] derivative (Figure **2.8**). A screen of protected glycolaldehydes determined that the TBS protected compound **2.2** gave a high yield of pyran product **2.3**, which was spectroscopically identical to that produced by Dr. Sherry Chavez, by a different route.[16] NOESY experiments determined that the new stereocenter was of the desired configuration. Elaboration to carboxylic acid **2.1** was accomplished by selective TBS deprotection,[17] Parikh-Doering[18] and Pinnick oxidations[19] provided the desired carboxylic acid in high yield (Figure **2.9**). Coupling of AB bis pyran carboxylic acid **2.1** with C-ring alcohol **1.93** was accomplished with Keck Boden-modified Steglich esterification conditions.[20] This esterification produced the tricyclic ester **2.4** in good yield, allowing the synthesis of Merle 39 to progress.

Typically, the C1 TBDPS group in Keck group analogs is deprotected in the



R	Yield (%)
PMB	43%
3,4-DMB	53%
Bz	Decomposition
BOM	46%
THP	44%
TBS	93%

Figure 2.8 B-Ring Pyran Annulation

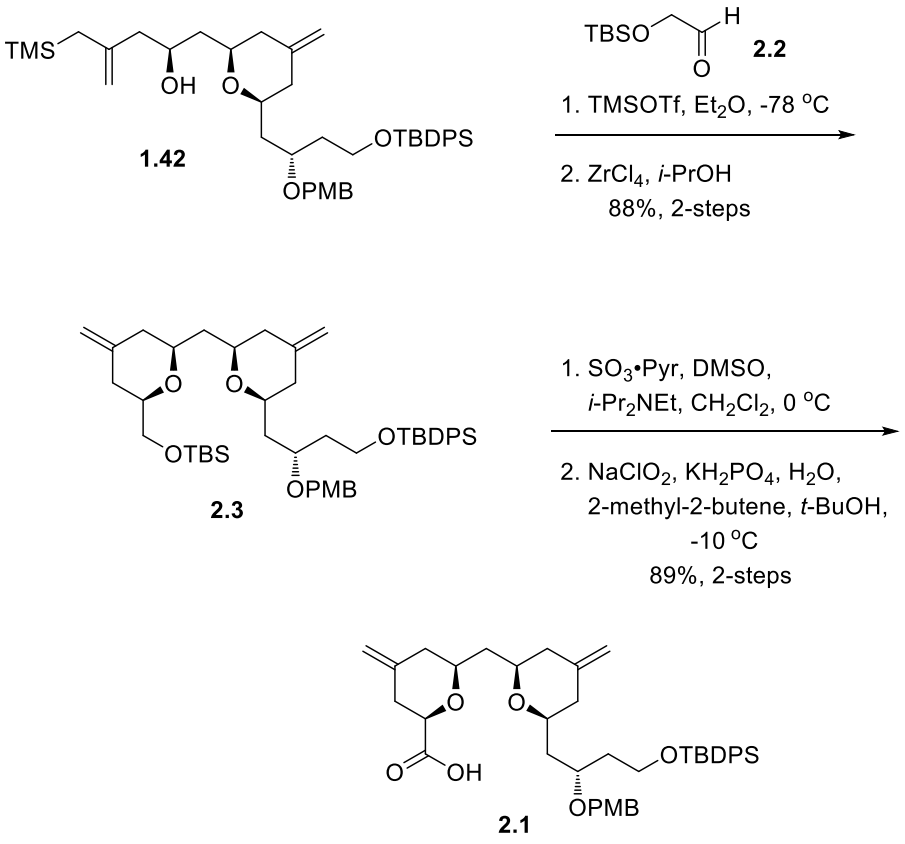


Figure 2.9 Elaboration of AB-Top-Half

presence of the C26 secondary TBS group using a mixture of TBAF, AcOH in DMF.[21] Deprotection of the C1 TBDPS group in the presence of the C26 primary TBS proved difficult and was not amendable for scale up due to poor selectivity (Figure **2.10**).

Due to its reactivity, it was decided to deprotect the C26 TBS group and reprotect it as an ethoxyethyl ether (EE) which is orthogonal to conditions that are used for TBDPS deprotection. Deprotection of the C26 TBDPS group on **2.4** was accomplished by the conditions developed by Corey[22] using 3:1:1 AcOH, H_2O, THF at 45 ºC. This yielded the C26 alcohol in 74% yield on a 100 mg scale. Protection of the C26 alcohol was accomplished with ethyl vinyl ether in CH_2Cl_2 with PPTS.[23] The C1 TBDPS was then removed under the standard TBAF, AcOH, DMF conditions in good yield affording alcohol **2.7**.[21]

The C1 hydroxyl group was functionalized by consecutive Parikh-Doering[18] and Pinnick[19] oxidations to provide the C1 carboxylic acid **2.8** in an 82% yield over 2-steps (Figure **2.11**). Deprotection of the C26 ethoxy ethyl group was accomplished by stirring with MeOH and catalytic PPTS, giving the C26 alcohol in 51% yield.[24] Cyclization of the resulting *seco*-acid was accomplished by Yamaguchi conditions,[25] producing the macrolactone **2.9** in 57% yield. Deprotection of the C3 PMB and C26 BOM ether of **2.9** was initially accomplished with bromocatechol borane in CH_2Cl_2 at −78 ºC in the presence of K_2CO_3 (Figure **2.12**).[26] Separation of Merle 39 from reaction byproducts via column chromatography or preparative TLC proved to be exceedingly difficult. It was later found that a 2-step sequence where the PMB group was removed with DDQ[27] followed by BOM deprotection with $LiBF_4$ in MeCN/ H_2O at 80 ºC[28] provided a product that was readily purifiable in 73% yield over two steps.

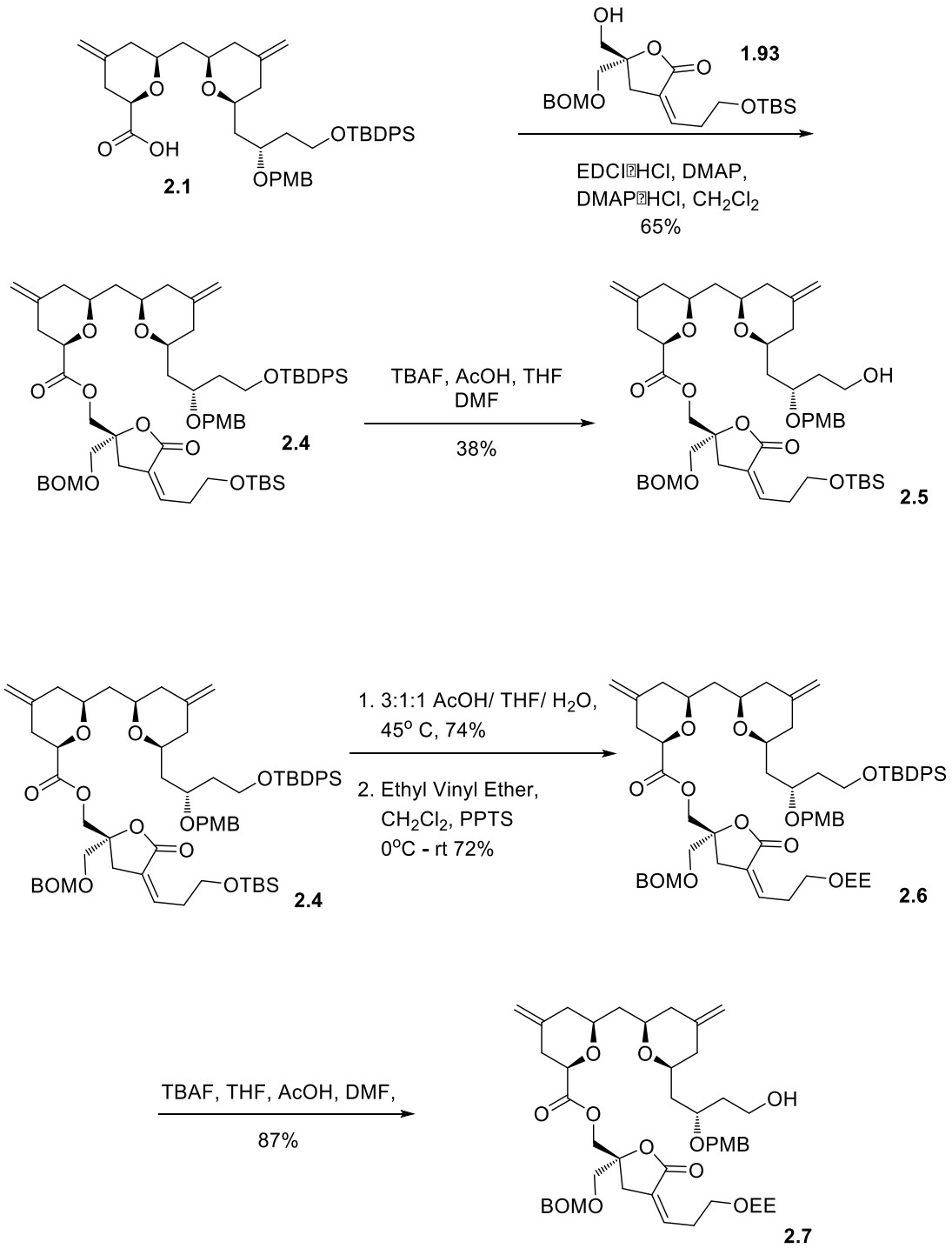


Figure 2.10 Fragment Coupling and C1 Deprotection

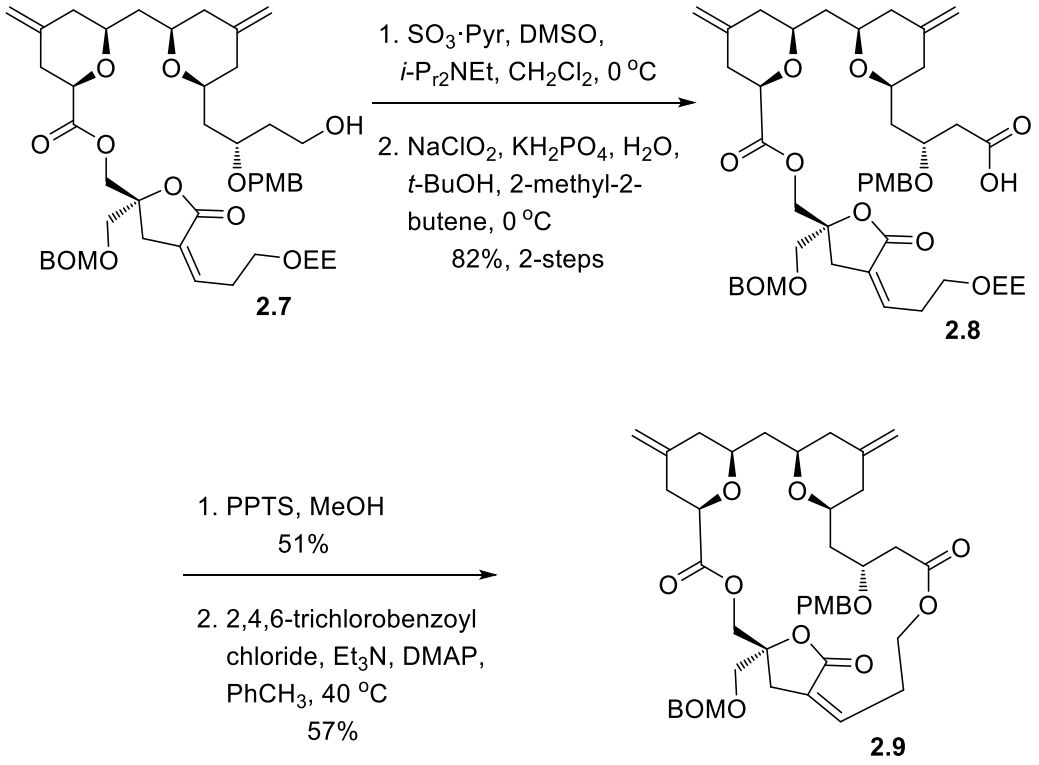


Figure 2.11 Functionalization of C1 and Macrolactonization

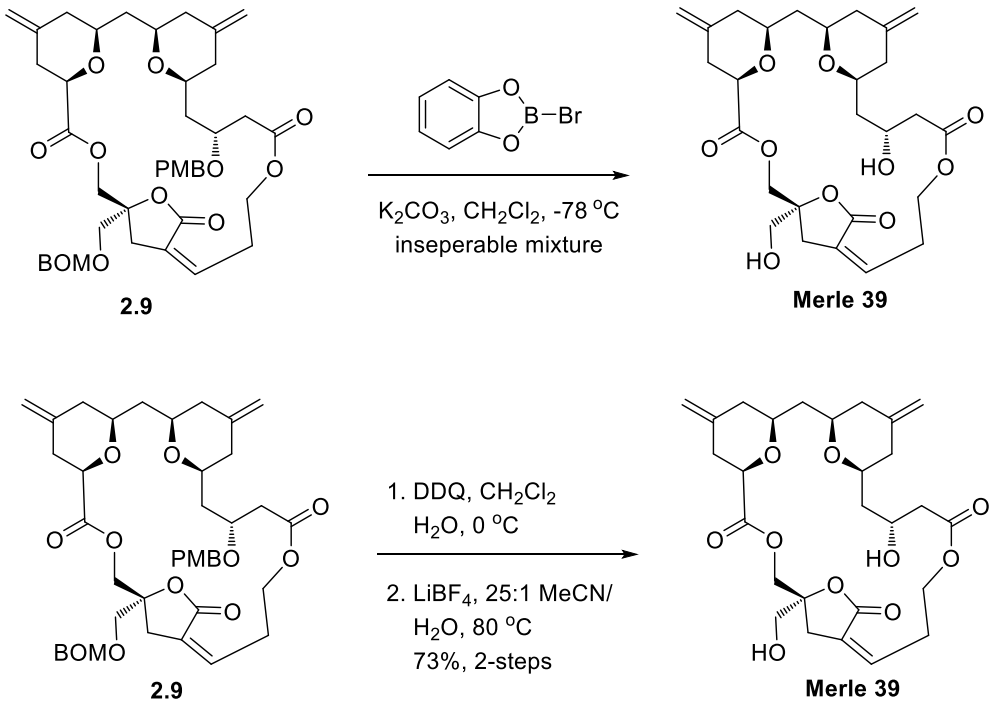


Figure 2.12 Completion of Merle 39

Biological Evaluation of Merle 39

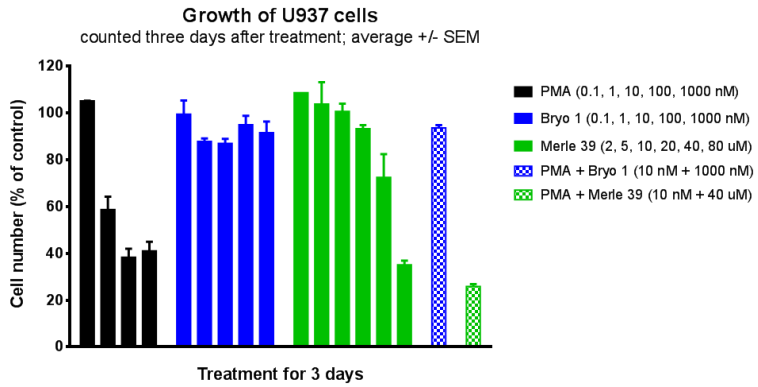
Merle 39 was determined to have a binding constant of 6985 nM for PKC α, indicating a dramatic loss of affinity. Merle 39 is over 5 orders of magnitude less potent than the model compound Merle 23. Merle 39 exhibits a dramatic loss of potency in U937 cells (Figure **2.13**).

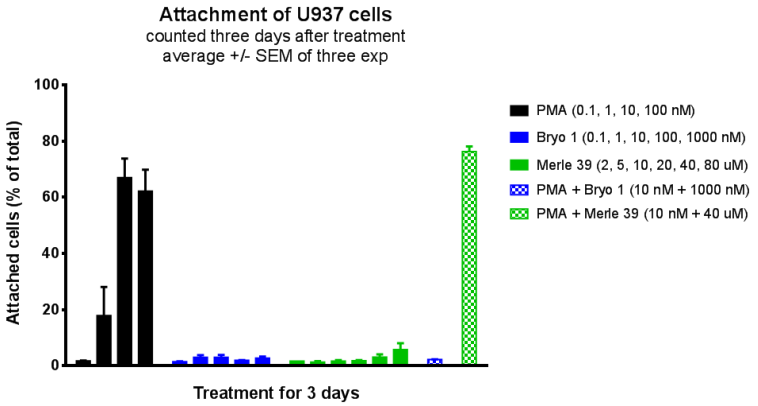
In the attachment assay, it appears PMA like and inhibits growth; the EC_{50} is 34.5 μM. Merle 39 at very high doses weakly promotes attachment and TNF α secretion in U937 cells, indicating PMA-like behavior. These results could be due to instability of the compound under biological conditions due to the diester linkage. Merle 39 is only weakly active with respect to the inhibition of Toledo leukemia cells as compared to the potent inhibitors PMA and bryostatin, with an EC50 value of 9.35 μM (Figure **2.14**).

From the biological results, Merle 39 is dramatically less potent then Merle 23, but displays a similar biological activity profile at high concentrations. Due to the diester linkage, Merle 39 is considerably more polar then Merle 23. The CLog P was calculated using ChemBioDraw version 14.0.0.118 and found to be −1.48, whereas Merle 23 has a CLog P of 3.06. From this information, Merle 39 appears to be unoptimized with respect to lipohilicity and possibly the orientation of the C-ring.

Synthesis of Merle 47

Due to Merle 39's low binding affinity to PKC, it was decided to test the hypothesis that the orientation of the DAG lactone C-ring in Merle 39 was not oriented in the correct position to bind to the C1 domain of PKC with high affinity. To test this hypothesis, it was decided to reverse the manner in which the ester groups are attached to





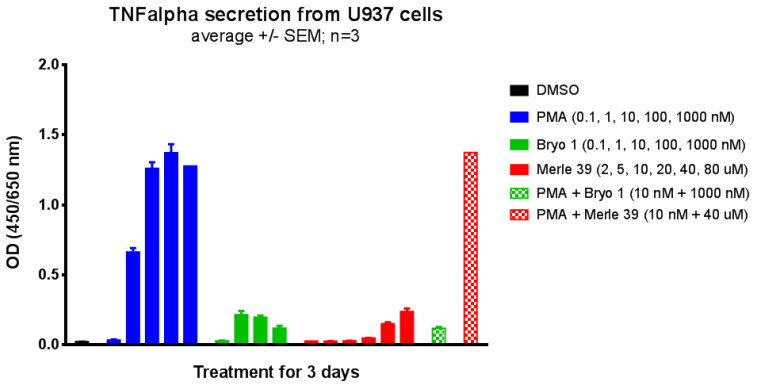


Figure 2.13 Biological Evaluation of Merle 39 in U937 Cells

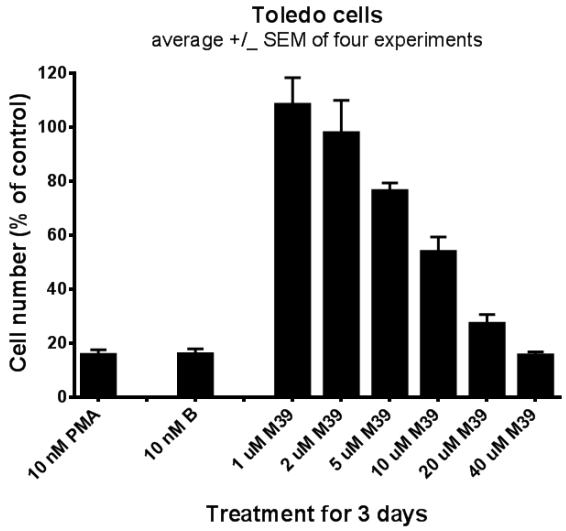


Figure 2.14 Activity of Merle 39 in Toledo Cells

the AB-top-half of the analog. Reversal of the C-ring attachment points would change orientation of the top-half with respect to the C-ring, but not the relative stereochemistry. The resultant changes are shown in Figure **2.15**. The effects of this alteration on the biological properties of the analog were unknown.

The synthesis of Merle 47 commenced with TBS deprotection of lactone **1.77**, giving alcohol **2.10** in high yield (Figure **2.16**).[29] Esterification of alcohol **2.10** with AB-ring carboxylic acid **2.1** proved to be difficult (Figure **2.17**). Standard Keck-Boden-modified Steglich conditions[20] provided the tricyclic ester **2.11** in low overall yields. The low yields were attributed to a facile elimination of the resulting ester under basic conditions. Modified Yamaguchi esterification[30] resulted in somewhat better yields and allowed the synthesis to progress. The C1 TBDPS group of **2.11** was deprotected with HF•pyridine to give the primary alcohol **2.12** in high yield. Sequential Parikh-Doering[18]

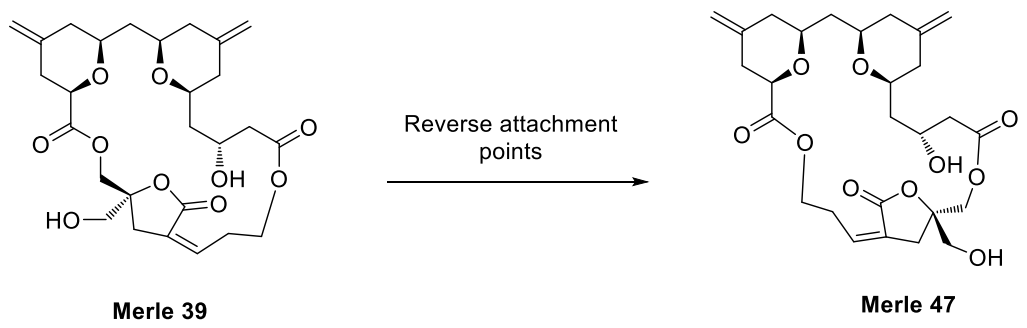


Figure 2.15 Design of Merle 47

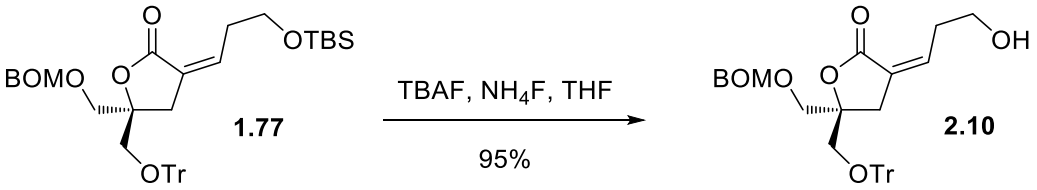


Figure 2.16 TBS Deprotection

and Pinnick[19] oxidations produced C1 carboxylic acid **2.13** in excellent yield over two steps. Deprotection of the C26 trityl ether with formic acid in ether[31] provided the *seco*-acid which was used without characterization (Figure **2.18**). Yamaguchi macrolactonization[25] gave the desired macrolactone **2.14** in moderate yield over two steps. Deprotection of the C3 PMB ether with DDQ[27] and deprotection of the BOM group with $LIBF_4$ in MeCN/ H_2O at 80 ^oC[28] gave Merle 47 in moderate yield over two steps.

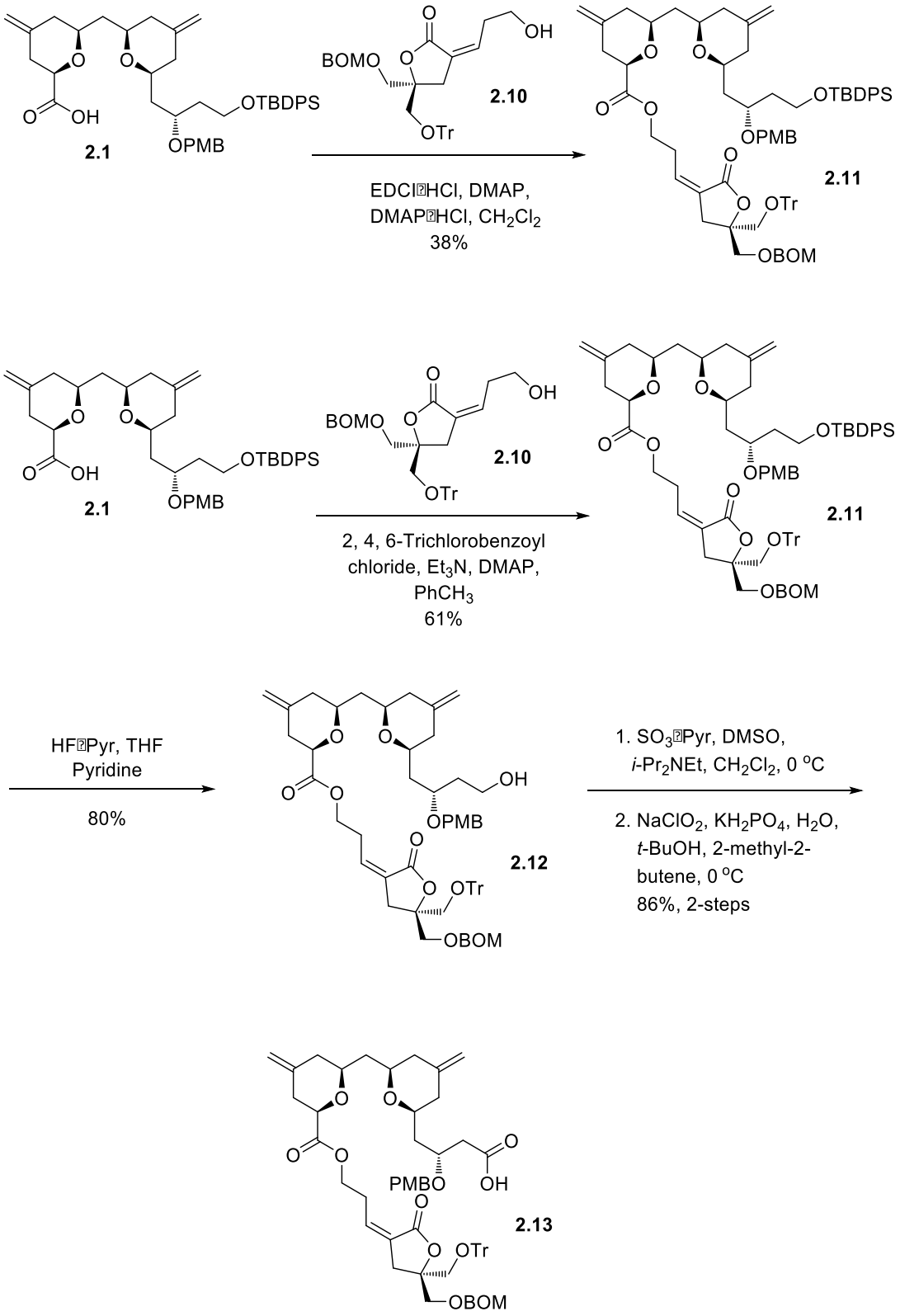


Figure 2.17 Esterification Optimization

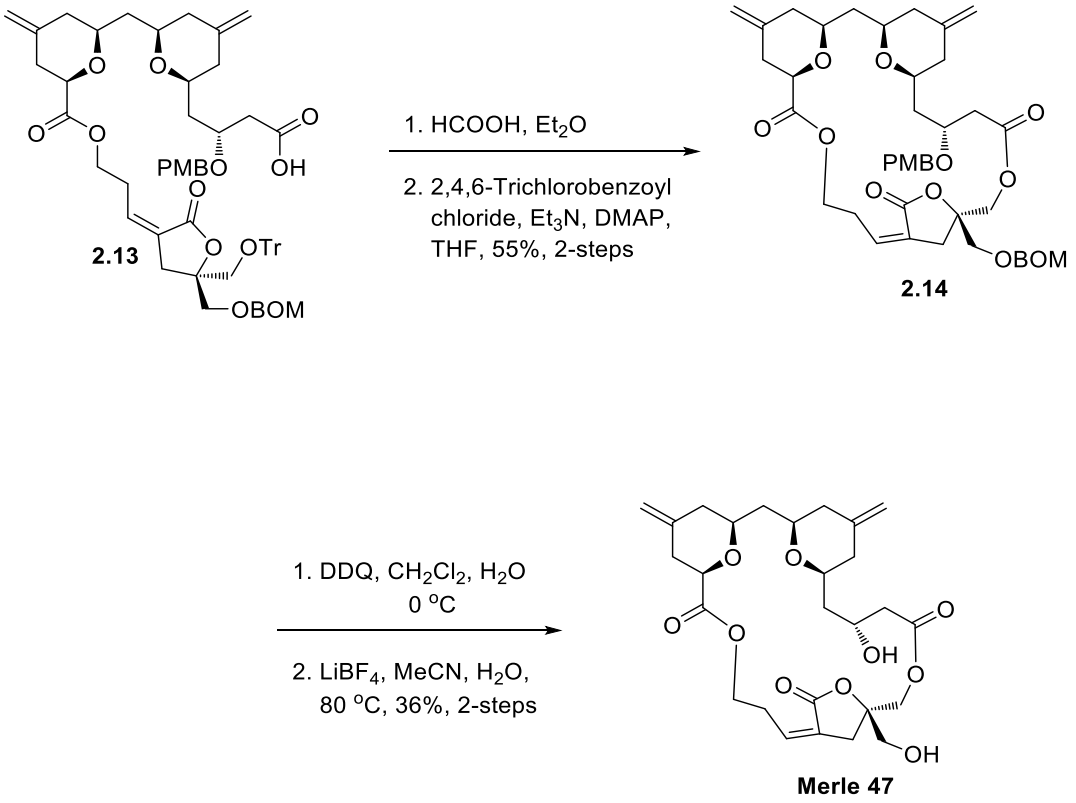


Figure 2.18 Completion of Merle 47

Biological Evaluation of Merle 47

Merle 47 was evaluated for PKC binding affinity and found to have a K_i of 4934 nM. This is a 400 nM improvement over Merle 39. This indicates that the orientation of the DAG lactone in Merle 47 is possibly more favorable compared to Merle 39. The overall orientation of the top-half of Merle 47 has changed with respect to Merle 39, while retaining the original chirality at the gamma carbon of the butyrolactone. Biological evaluation of Merle 47 was made difficult due to the inherent instability of this compound due to the facile elimination of the C16 ester due to its connection to the C-ring enoate. The compound was less potent in all cell culture assays then Merle 39; the compound demonstrated ambiguous results in cell cultures at concentrations that were

evaluated.

The overall polarity of Merles 39 and 47 was substantially increased due to the ester linkage on the left-hand side of these molecules as compared to Merle 23. The increased polarity of these analogs likely decreased the biological activity of these compounds with respect to Merle 23. Reintroduction of the olefin linkage and *gem*-dimethyl group present in Merle 23 would decrease the polarity and likely improve the biological profile of the resulting analog.

Design and Synthesis of Merle 48

Building upon the results of Merle 39, and 47, a third generation DAG lactone analog was designed. Much of the loss of binding affinity in Merle's 39 and 47 was attributed to the low lipophilicity of these compounds. The bryostatin family of natural products contains a *gem*-dimethyl group at C18 and *trans*-olefin centered between C16-C17. Merle 23, a potent bryostatin analog with a simplified top-half, also bears these functionalities. Reintroduction of these functionalities into a DAG lactone analog would increase the lipophilicity and thus improve the binding affinity. The rational for the design of Merle 48 is shown in Figure **2.19**. Merle 48 is based upon Merle 47 which is more potent then our initial analog Merle 39. The enoate side chain of Merle 47 would be amendable to reintroduction of these functionalities. The enoate side chain was designed to retain the 22-membered macrolactone of the previous two analogs. Elimination of the C16 ester in Merle 48 would also circumvent the stability problems seen during the biological evaluation of Merle 47.

Retrosynthesis of Merle 48 consisted of disconnection of the C1 ester and B-ring

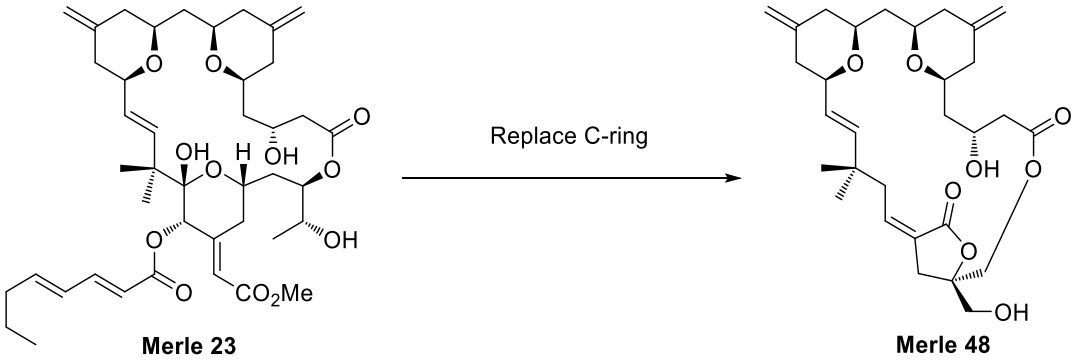


Figure 2.19 Merle 48 Design Rational

pyran to give known β-hydroxyallyl silane **1.42** and aldehyde **2.15** (Figure **2.20**) This disconnection allows for the efficient construction of the B-ring of the analog via pyran annulation. Pyran annulations of fully functionalized C-ring aldehydes and β-hydroxy-allylsilane **1.42** are well known and would be expected to provide the desired product in high yield.[32]

Disconnection of the enoate side chain on **2.15** would give lactone **2.17**, which would be easily accessible from known lactone **1.89**. Aldehyde **2.16** could be produced from known ester **1.47**. Ester **1.47** was previously used in the synthesis of bryostatin analogs bearing the natural C-ring.[33] Commencement of the synthesis of Merle 48 began with an aldol reaction of the lithium enolate of methyl isobutryate with known aldehyde **1.79** to give aldol product **2.18** (Figure **2.21**). Mesylation and elimination of **2.18** using conditions developed by Kevin McGowan provided ester **1.47**. Reduction of ester **1.47** with $LiAlH_4$ and subsequent Parikh-Doering oxidation[18] gave aldehyde **2.19**.

Wittig olefination[34] of aldehyde **2.19** with (methoxymethyl)triphenyl phosphonium chloride in THF with KO*t*-Bu gave enol ether **2.20** in high yield. Attempted hydrolysis of enol ether **2.21** with HCl, H_2O, THF or PPTS, MeOH, H_2O gave

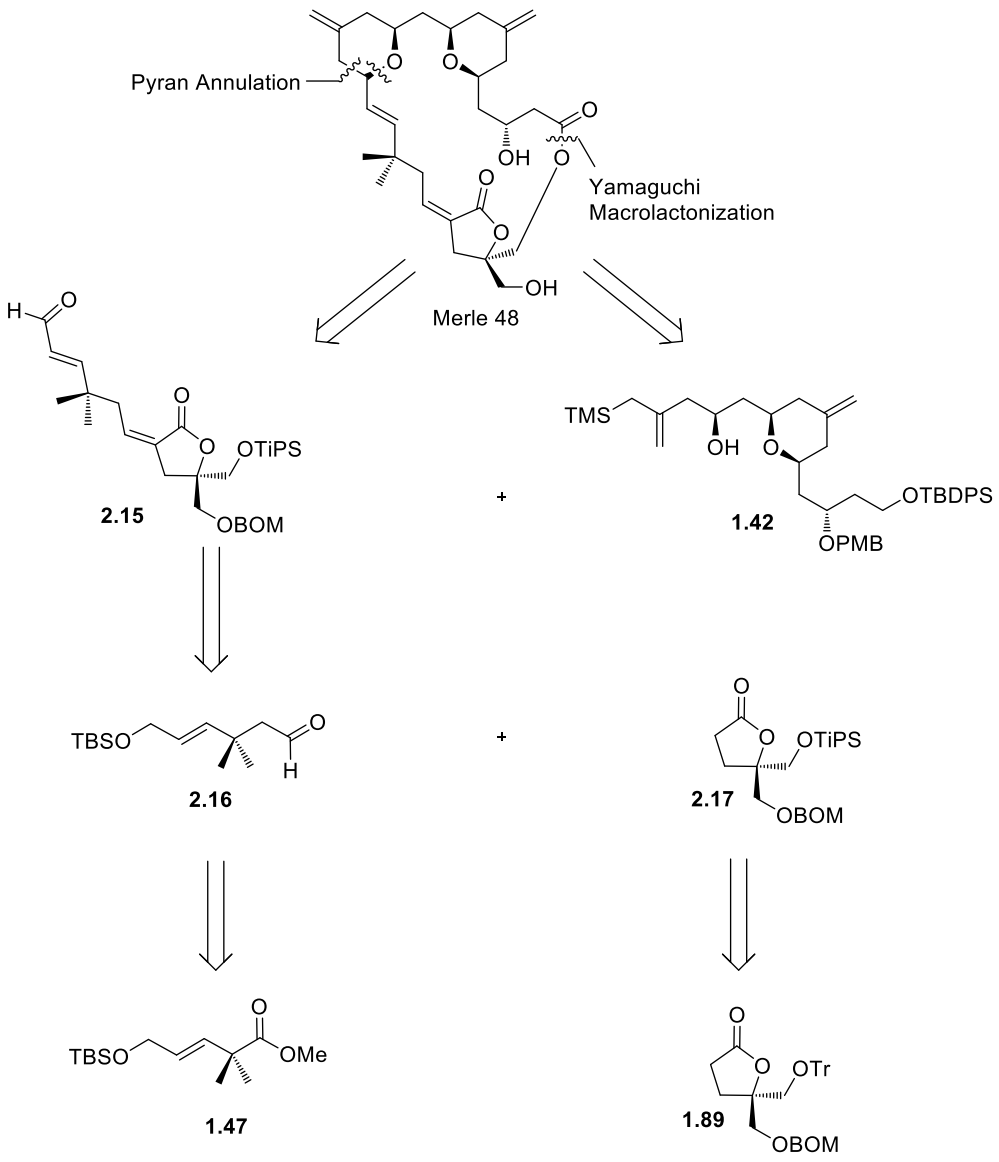


Figure 2.20 Retrosynthesis of Merle 48

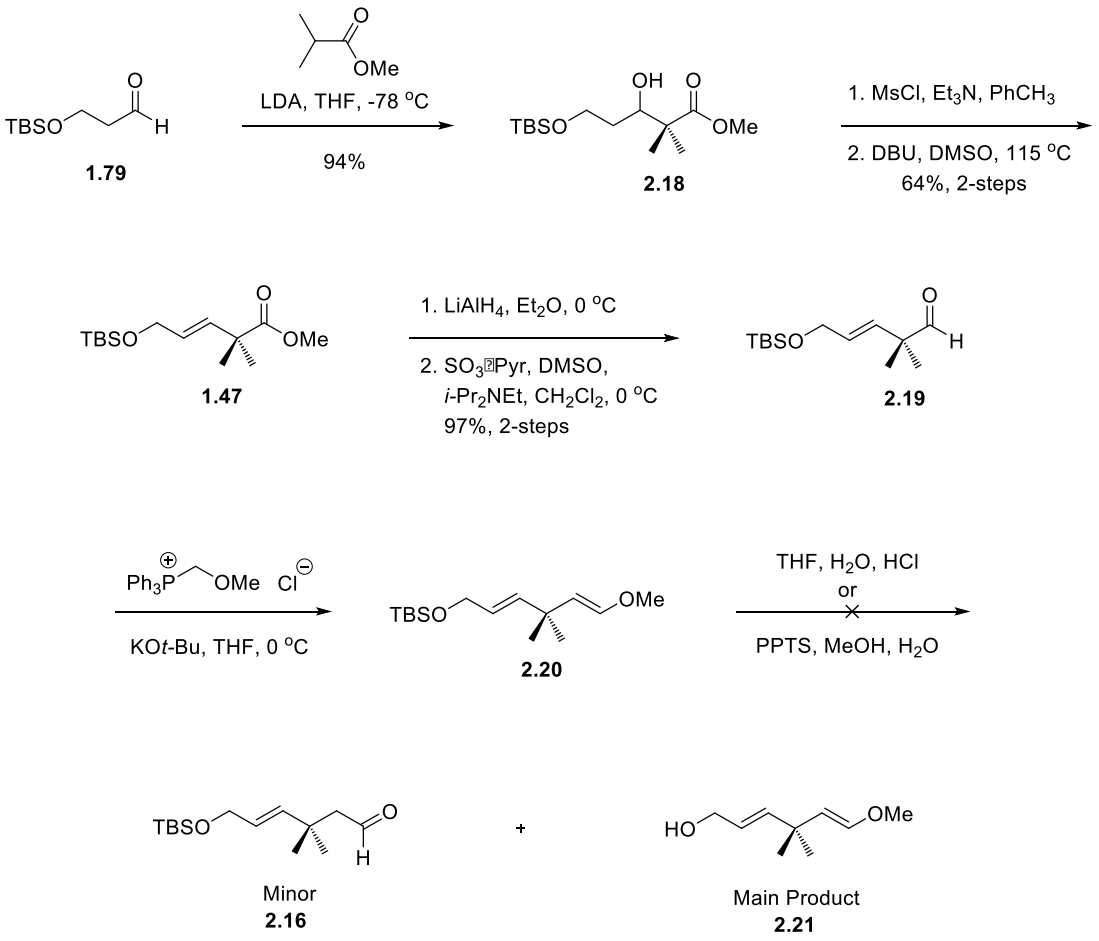


Figure 2.21 Attempted Wittig Olefination

mainly the deprotected alcohol **2.21**, not the desired aldehyde **2.16**. In order to circumvent these selectivity issues, aldehyde **2.19** was olefinated with methyl triphenylphosphonium bromide in THF with KO*t*-Bu to give olefin **2.22** in high yield (Figure **2.22**).[35]

Hydroboration/ oxidation of olefin **2.22** with 9-BBN gave alcohol **2.23** in good yield.[36] Oxidation of this alcohol under Parikh-Doering conditions[18] gave aldehyde **2.16**. Synthesis of lactone **2.17** commenced with deprotection of the trityl group of **1.89** with TFA/ TFAA to give alcohol **2.14** in high yield.[37] Alcohol **2.14** was protected as the TIPS ether with TIPSOTf[38] to give lactone **2.17** in high yield.

Deprotonation of lactone **2.17** with LDA, aldol condensation with aldehyde **2.16**, and finally dehydration gave enoate **2.24** as a 1:1.2 mixture of *Z* and *E* enoates in good yield. Improvement in selectivity was needed in order to produce material for the synthesis to progress. Reaction of the enolate of lactone **2.17** with diethyl chlorophosphate, followed by base-induced rearrangement of the intermediate enol phosphate, gave the corresponding phosphonate which was used without characterization (Figure **2.23**).[39]

Treatment of the intermediate phosphonate with KHMDS, 18-crown-6 at −78 ^{o}C, followed by addition of aldehyde **2.16** and rapid warming to rt lead to enoate **2.24** as a 5:1 Z/ E mixture of enoates in moderate yield.[40] Deprotection of the TBS group was accomplished with 3:1:1 AcOH/ THF/ H_2O at 45 ^{o}C, providing alcohol **2.25** in good yield.[22] Parikh-Doering oxidation[18] of alcohol **2.25** gave aldehyde **2.15** in moderate yield.

Coupling of aldehyde **2.15** and β-hydroxyallylsilane **1.42** was accomplished via pyran annulation,[32] providing tricycle **2.26** in good yield as a single diastereomer via

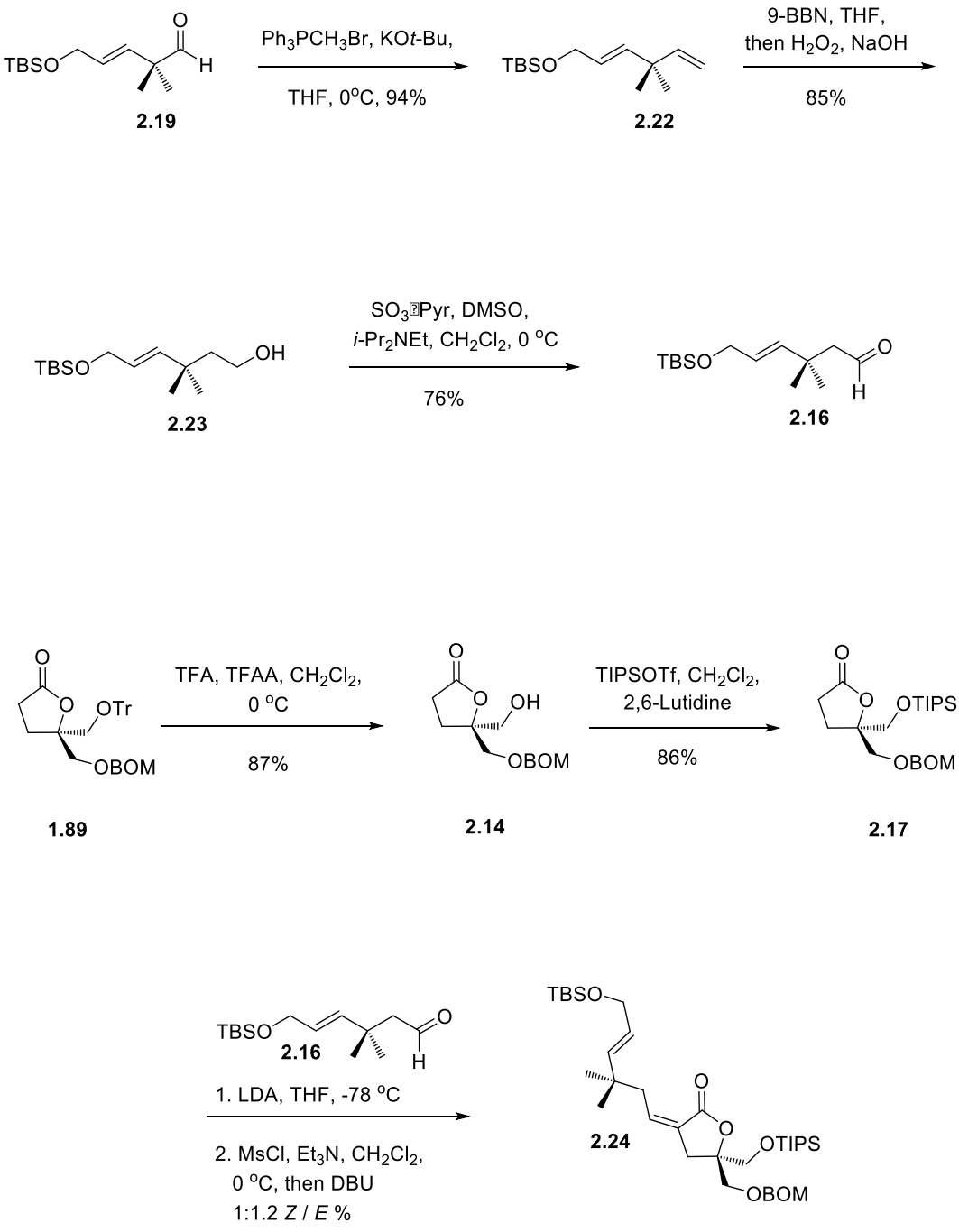


Figure 2.22 Initial Olefination

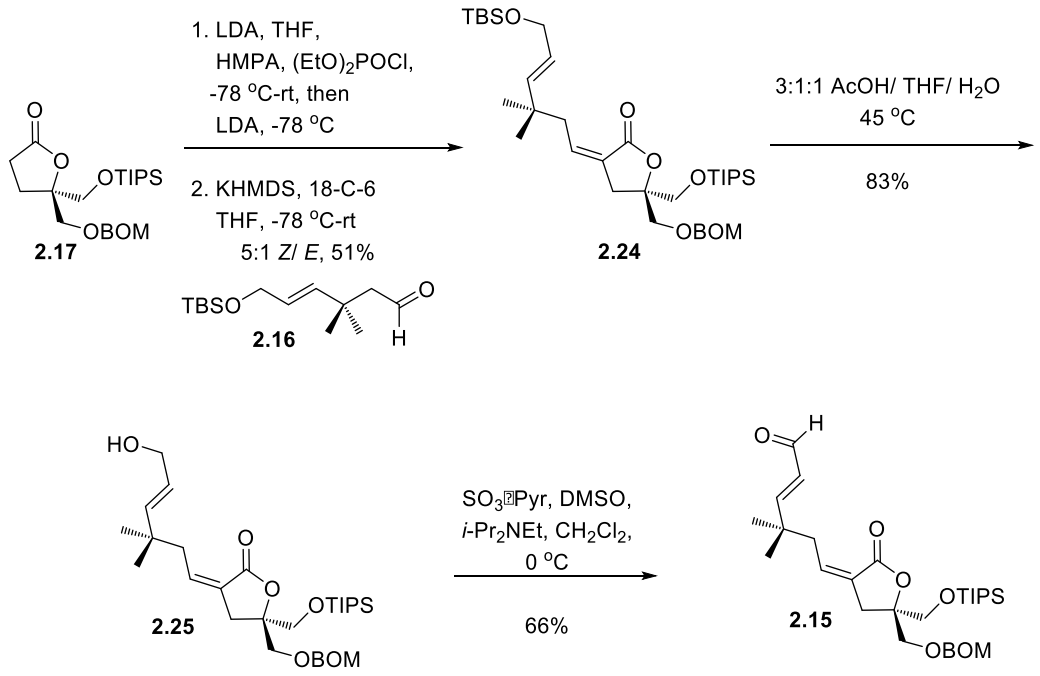


Figure 2.23 Completion of C-ring Aldehyde **2.16**

1HNMR (Figure **2.24**). Attempted deprotection of the C1 TBDPS of **2.26** with TBAF, AcOH, DMF led to the removal of the C26 TIPS group.[21]

Deprotection of the C1 TBDPS was accomplished with HF•Pyridine at 0 °C, providing C1 alcohol **2.27** in moderate yield. Parikh-Doering[18] and subsequent Pinnick[19] oxidation gave the C1 carboxylic acid **2.28** in acceptable yield over 2-steps. Removal of the C26 TIPS group was accomplished by stirring the carboxylic acid in HF•pyridine at rt for 2 days. The crude *seco* acid was cyclized by Yamaguchi macrolactonization,[25] yielding macrolactone **2.29** in moderate yield over 2-steps. Completion of Merle 48 was accomplished by deprotection of the C3 PMB with DDQ[27] at 0 °C, followed by

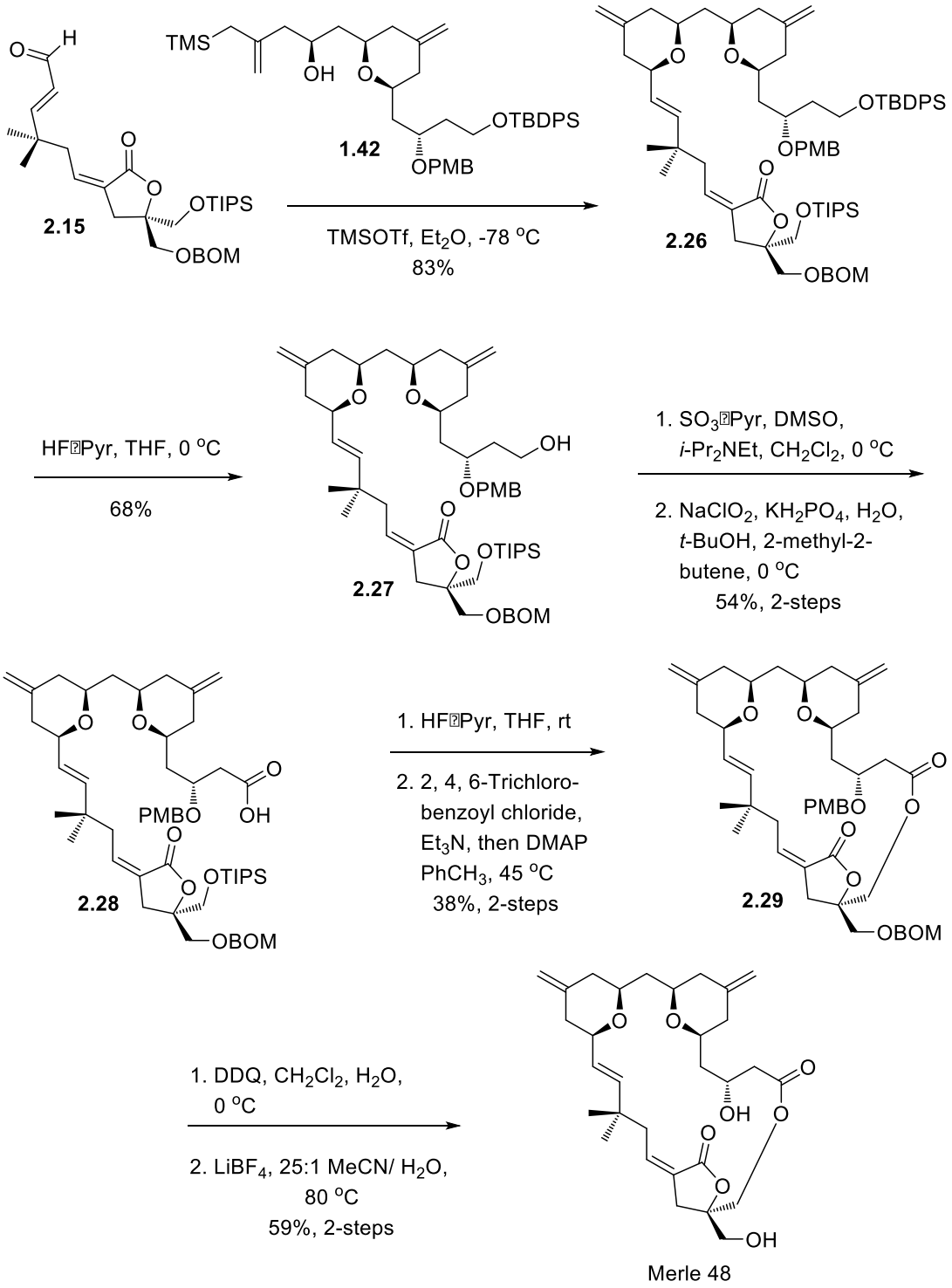


Figure 2.24 Completion of Merle 48

deprotection of the C26 BOM acetal with $LiBF_4$ in MeCN/ H_2O at 80 ^oC.[28] Merle 48 was obtained in moderate yield over 2-steps.

Biological Evaluation of Merle 48

Merle 48 was found to have a binding constant of 363 nM towards PKCα. This is a 13-fold increase from that of Merle 47. The dramatic increase in affinity can be attributed to the increased lipophilicity imparted by the deletion of the C16 ester, along with the reinstallation of the C16-C17 olefin, as well as the C18 *gem*-dimethyl group. In U937 cells, Merle 48 is Merle 23-like, it inhibits proliferation and induces attachment, but is considerably less potent then PMA (Figure **2.25**). Bryostatin was found to reverse these effects of Merle 48. Merle 48 was found to induce the secretion of TNF α at high concentrations of the ligand (Figure **2.26**).

In the Toledo cell system, Merle 48 was found to inhibit growth similarly to PMA and bryostatin 1, but at much higher concentrations (Figure **2.27**). Merle 48 was 2 orders of magnitude more potent then Merle 47 in this assay. The results from these assays indicate that Merle 48 is much more stable under biological conditions. These results are promising, due to the dramatic increase in binding relative to Merles 39 and 47.

Molecular Modeling Studies

Molecular modeling studies of Merles 39, 47, and 48 were conducted by Dr. Megan Peach of the NIH. A conformational search of the three analogs was conducted using by the program MacroModel.[41] These modeling studies indicate that the hydrogen bonding network present between the C3 and C19 hydroxyls of bryostatin and previous

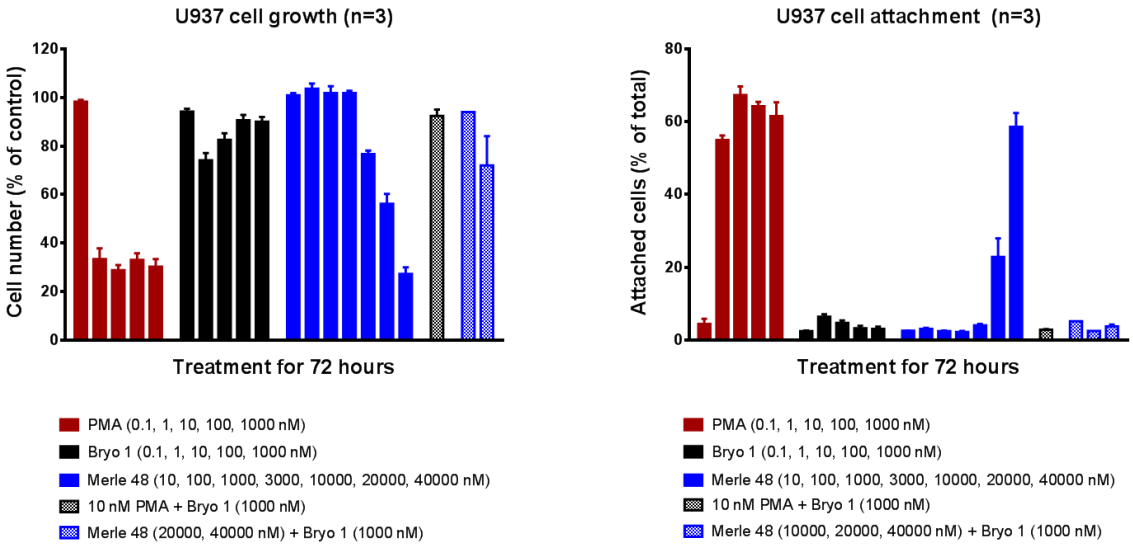


Figure 2.25 Biological Evaluation of Merle 48 in U937 Cells

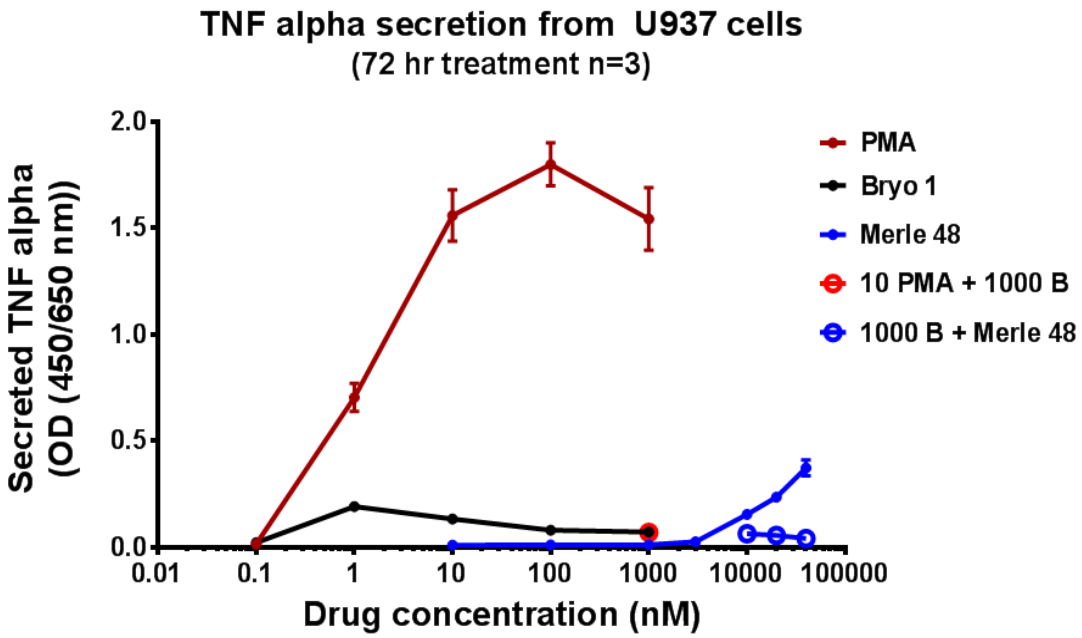


Figure 2.26 TNF Secretion in U937 Cells

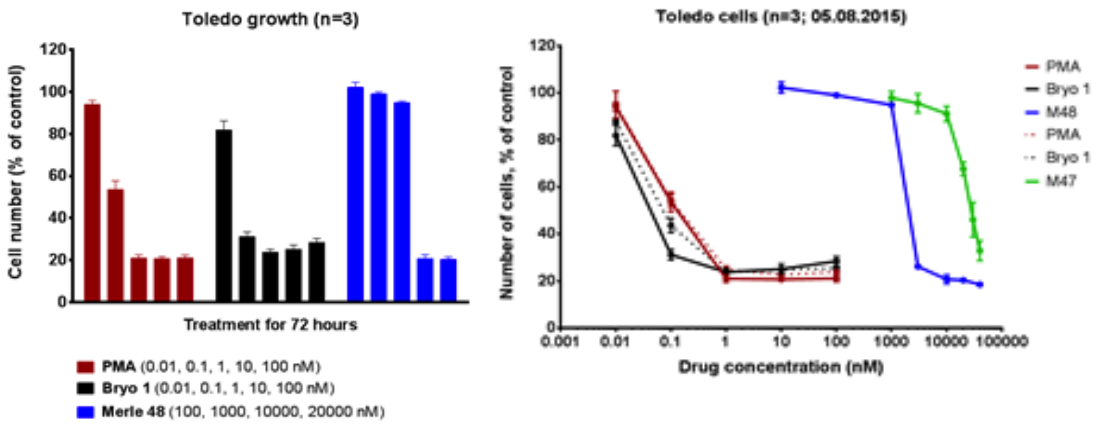


Figure 2.27 Biological Results in Toledo Cells

analogs was lost upon substitution of the natural C-ring for the DAG lactone. This results in the nearly perpendicular conformation of the A and B rings as opposed to the coplanar conformation seen in the natural product (Figure **2.28**). This study suggests that membrane orientation and penetration of these compounds may be much different then bryostatin 1. Bryostatins and previous analogs have highly rigid structures due to this intramolecular hydrogen bonding network.

Docking studies with the C1b domain of PKC were conducted with the program GOLD, version 5.2.2 to model the interaction between the ligand and protein.[42] These studies indicate that only Merle 39 has a reasonable binding mode with the C1b domain of PKC (Figure **2.29**). In Merle 39, the DAG lactone binds to threonine 242, leucine 251, and glycine 253 in a sn-*2* fashion. Modeling studies of Merles 47 and 48 indicate that a reasonable mode of binding does not exist due to the reversed orientation of the top-half in relation to the C-ring. The starting point in these docking studies is the crystal structure of PMA bound to the C1b domain of PKC. PMA has a much different structure then DAG lactones; these differences could result in the discrepancy between

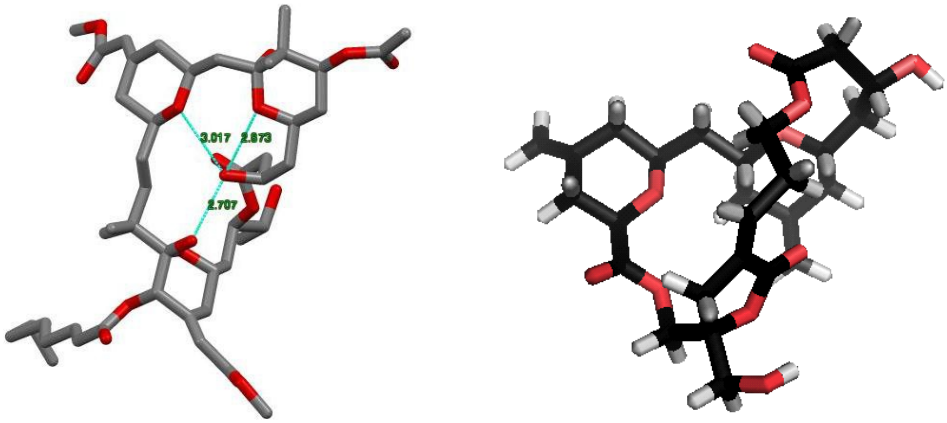


Figure 2.28 Crystal Structure of Bryo 1 and Energy Minimized Structure of Merle 39

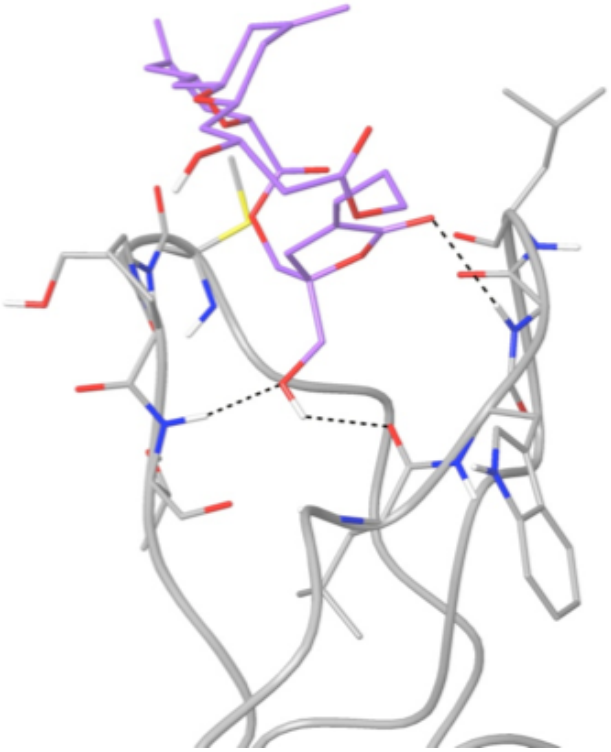
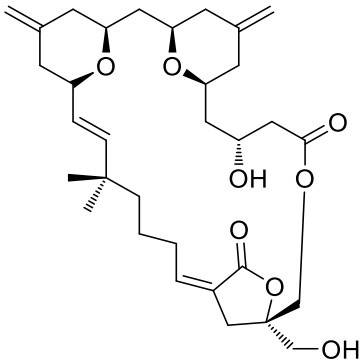


Figure 2.29 Interaction of Merle 39 with the C1 Domain of PKCδ

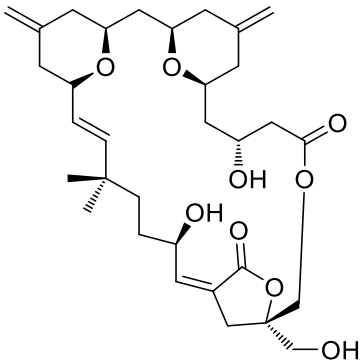
the modeling study and the actual results observed for Merle 48. No crystal structures of a DAG lactone bound to the C1 domain have been reported to date.

Future Direction

Modeling studies on Merles 39, 47, 48 indicated that the intramolecular hydrogen bonding network present in bryostatin 1 and Merle 23 to have been disrupted. The coplanarity of the AB ring system was also shown to be significantly altered by the introduction of the DAG lactone. Expansion of this ring system by two carbons should result in the restoration of the co-planarity of the AB rings and restoration of the hydrogen bonding network in analog **2.30** (Figure **2.30**). Marquez has reported a small loss in affinity for 26 membered macrocyclic DAG lactones as opposed to the 22 membered systems.[13] The conformational preferences of this system should negate any detrimental effects of the ring expansion. Further adjustment of conformational preference could be achieved by reintroduction of the internal hydrogen bonding network in analog **2.31**.



Analog **2.30**



Analog **2.31**

Figure 2.30 Proposed Analogs

Conclusion

In order to synthesize bryostatin analogs with a simplified C-ring scaffold, new analogs Merle 39, 47, 48 were synthesized. A DAG lactone core was inserted in the place of the natural C-ring. Merle 39 was found to be a weak PKC ligand, and biologically mimicked Merle 23 in several assays. Merle 47, which was a reversed version of Merle 39, was found to have a slightly higher binding affinity. Merle 47 was unstable biologically, making biological evaluation difficult. Both Merles 39 and 47 are very polar compounds compared to past high affinity analogs. Merle 48 was the result of an attempt to increase the lipophilicity of DAG lactone analogs. A large increase in binding was observed in this compound, likely a result of its increased lipophilicity. Molecular modeling studies indicated that the DAG scaffold significantly changes the conformation of the AB-top-half due to the loss of the internal hydrogen bonding network; this could account for the relatively poor binding affinity of these compounds.

Experimental Section

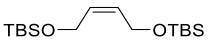
General Experimental Procedures

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 Baclawski and Kofron.[114] Ti(*i*-OPr)$_4$ was distilled prior to use. All other reagents were used without further purification. Yields were calculated for material judged homogenous by thin layer

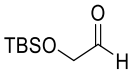
chromatography and nuclear magnetic resonance (NMR). Thin layer chromatography was performed on Merck Kieselgel 60Å F254 plates or Silicycle 60Å F254 eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid, a solution of ninhydrin in 1-butanol, a solution of p-anisaldehyde in ethanol acidified with sulfuric acid, an aqueous potassium permanganate solution, or a solution of ceric ammonium molybdate, acidified with sulfuric acid. 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. Enantiomeric excess (ee) were determined using a Rainin Dynamax HPLC with a Knauer variable wavelength detector set at 254 nm, using a Chiracel OD-H column. Nuclear magnetic resonance spectra were acquired at 300, 500 MHz for 1H and 75, 125 MHz for 13C. 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 or $(CH_3)_4Si$ at 0.00 ppm. Chemicals shifts for carbon nuclear magnetic resonance (13C 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 13C NMR spectrum. The abbreviations s, d, dd, ddd, dddd, ddddd dt, quint, t, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, doublet of doublet of doublet of doublet of doublets, doublet of triplets, quintet, triplet,

and multiplet, respectively. Optical rotations (Na D line) were obtained using a microcell with a 1 dm path length. Specific rotations ([α], Unit: °cm2/g) are based on the equation α = (100·α)/(*l*·*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 time of flight (TOF) high-resolution mass spectrometer. Compounds were named using ChemBioDraw 14.0.0.

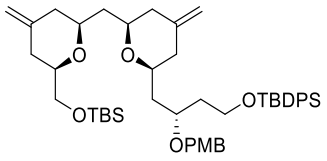
Experimental Procedures for Merle 39



Preparation of (*Z*)-2,2,3,3,10,10,11,11-octamethyl-4,9-dioxa-3,10-disiladodec-6-ene (2.32): A 500 mL flask was equipped with a stir bar and imidazole (9.3 g, 137 mmol, 4.0 equiv) and DMAP (1.64 g, 13.4 mmol, 0.4 equiv) were then added. After flushing with N_2, CH_2Cl_2 (150 mL) was added, followed by *cis*-1,4-but-2-enediol (3.0 g, 34 mmol, 1.0 equiv). After cooling to 0 ^oC, a solution of TBSCl (12.3 g, 81.6 mmol, 2.4 equiv) in CH_2Cl_2 (135 mL) was added via cannula to the stirring solution. The reaction mixture was stirred overnight at rt, and then quenched with water (300 mL), extracted with CH_2Cl_2 (3 × 30 mL), and washed with brine (200 mL). After drying over $MgSO_4$, the solution was filtered and concentrated. The crude product was then purified via column chromatography on a 5 × 25 cm silica gel column, eluting with 2% EtOAc/hexanes, collecting 30 mL fractions. Fractions 6–24 were combined and concentrated to yield the product (10.2 g, 94%). R_f = 0.76 (25% EtOAc/hexanes). 300 MHz ^1H NMR ($CDCl_3$) δ 5.57 (t, *J* = 3.3 Hz, 2H), 4.24 (dd, *J* = 3.3, 0.9 Hz, 4H), 0.91 (s, 18H), 0.07 (s, 12H): 75 MHz ^{13}C NMR ($CDCl_3$) δ 130.4, 59.8, 26.1, 18.5, −5.0.

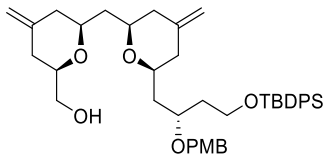


Preparation of 2-((tert-butyldimethylsilyl)oxy)acetaldehyde (2.2): To a 100 mL flask with a stir bar was added **2.32** (2.95 g, 9.3 mmol, 1.0 equiv) and CH_2Cl_2 (50 mL, 0.2 M). After cooling to −78 °C, O_3 was passed into the solution until a persistent blue color appeared in the solution and the solution was flushed with O_2, and this process was repeated twice. After flushing out the remaining O_3, PPh_3 (2.93 g, 11.2 mmol, 1.2 equiv) was added. The dry ice bath was allowed to expire overnight. The solution was then concentrated and the crude product purified on a 5 × 10 cm silica gel column. The product was eluted with 5% EtOAc/hexanes, collecting 30 mL fractions. Fractions 2−16 contained the desired product. The product was then distilled using a Kughelrohr apparatus, using house vacuum, 40 mmHg, bath temperature 125 °C, yielding the product aldehyde (1.95 g, 61%) as a colorless oil. R_f = 0.53 (25% EtOAc/hexanes). 300 MHz ^{1}H NMR ($CDCl_3$) δ 9.71 (d, *J* = 0.9 Hz, 1H), 4.22 (t, *J* = 0.6 Hz, 2H), 0.93 (s, 9H), 0.11 (s, 6H): 75 MHz ^{13}C NMR ($CDCl_3$) δ 202.6, 69.8, 26.0, 18.6, −5.2.



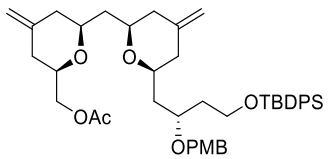
Preparation of *tert*-butyl((*S*)-4-((2*S*,6*R*)-6-(((2*S*,6*R*)-6-(((tert-butyldimethylsilyl)oxy)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3-((4-methoxybenzyl) oxy)butoxy)diphenylsilane (2.3): To a 25 mL flask fitted with a stir bar was added silane **1.42** (262 mg, 0.367 mmol, 1.0 equiv) and aldehyde **2.2** (319 mg, 1.83 mmol, 5.0 equiv). The flask was flushed with N_2 and Et_2O (10.5 mL, 0.035 M) was added. After cooling to −78 °C, a solution of TMSOTf in Et_2O (610 μL of 0.9 M, 0.55 mmol, 1.5 equiv) was added dropwise to the stirring solution. After 3 h, the reaction

mixture was quenched by dropwise addition of *i*-Pr_2NEt (1.0 mL). After 5 min, saturated aqueous $NaHCO_3$ solution (5.0 mL) was added and the mixture was stirred until it had reached rt. The mixture was extracted with EtOAc (3 × 10 mL), the organic phase was washed with brine (25 mL), and dried over Na_2SO_4. The solution was filtered and concentrated. The crude product was purified on a 3 × 10 cm silica gel column, eluting with 4 % EtOAc/hexanes, collecting 10 mL fractions. Fractions 4–14 were concentrated to yield the product (272 mg, 93%) which was spectroscopically identical to that reported by Chavez. R_f = 0.44 (10% EtOAc /hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71–7.68 (m, 4H), 7.46–7.38 (m, 6H), 7.20 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 8.0 Hz, 2H), 4.73 (dd, *J* = 8.0, 1.5 Hz, 2H), 4.69 (d, *J* = 1.5 Hz, 1H), 4.60 (d, *J* = 1.5 Hz, 1H), 4.43 (ABq, *J* = 11 Hz, Δν = 30.4 Hz, 2H), 3.98–3.93 (m, 1H), 3.85–3.80 (m, 1H), 3.81 (s, 3H), 3.78–3.75 (m, 1H), 3.71 (dd, *J* = 10, 5.0 Hz, 1H), 3.59–3.48 (m, 4H), 3.40–3.36 (m, 1H), 2.28 (d, *J* = 13 Hz, 3H), 2.18 (d, *J* = 13.5 Hz, 1H), 2.03–1.90 (m, 5H), 1.86–1.73 (m, 2H), 1.70–1.58 (m, 3H), 1.08 (s, 9H), 0.91 (s, 9H), 0.07 (d, *J* = 2.0 Hz, 6H): 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.3, 145.0, 144.4, 135.8, 134.1, 131.2, 129.8, 129.6, 127.9, 114.0, 109.0, 108.7, 79.0, 75.1, 75.0, 74.9, 72.7, 72.2, 66.8, 60.6, 55.5, 43.0, 42.6, 41.5, 41.0, 40.9, 38.1, 37.7, 27.1, 26.2, 19.4, 18.6, −5.0. HRMS (ESI) calcd 821.4609 for $C_{48}H_{70}O_6NaSi_2$ (M+Na); found 821.4614.



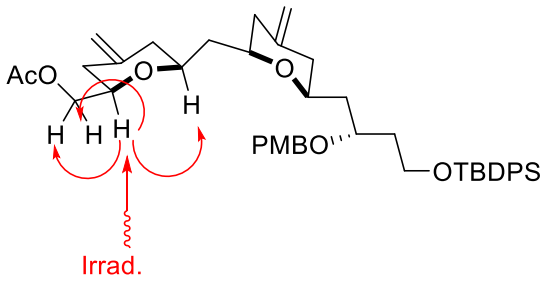
Preparation of ((2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)methanol (2.33): To a

stirring solution of **2.3** (220 mg, 0.275 mmole, 1.0 equiv) in *i*-PrOH (6.9 mL 0.04 M) contained in a 15 mL flask under N_2 was added $ZrCl_4$ (32 mg, 0.14 mmol, 0.5 equiv). The reaction mixture was stirred at rt for 60 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (25 mL) and extracted with EtOAc (3 × 25 mL), then washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 3 × 10 cm silica gel column, eluting with a gradient of 10 – 30% EtOAc/hexanes, collecting 10 mL fractions. Fractions 16–28 were concentrated to yield the product alcohol (132 mg, 70%) as a colorless oil. R_f = 0.58 (25% EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70–7.76 (m, 4H), 7.45–7.37 (m, 6H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 4.73 (dd, *J* = 11, 2.0 Hz, 2H), 4.69 (s, 1H), 4.47 (s, 1H), 4.42 (ABq, *J* = 10.5 Hz, Δν = 41.7 Hz, 2H), 3.96–3.82 (m, 1H), 3.84–3.80 (m, 1H), 3.81 (s, 3H), 3.77–3.73 (m, 1H), 3.65–3.63 (m, 1H), 3.60–3.49 (m, 3H), 3.48–3.39 (m, 2H), 2.27 (t, *J* = 13 Hz, 2H), 2.17 (d, *J* = 13 Hz, 1H), 2.08 (q, *J* = 12 Hz, 3H), 2.02–1.90 (m, 4H), 1.86–1.73 (m, 2H), 1.69–1.57 (m, 4H), 1.07 (s, 9H): 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.3, 144.8, 143.7, 135.8, 134.1, 131.2, 129.8, 129.6, 127.9, 114.0, 109.5, 108.8, 78.7, 75.2, 75.0, 74.9, 72.6, 72.0, 66.2, 60.6, 55.5, 42.9, 42.5, 41.4, 41.1, 40.8, 37.9, 36.5, 27.1, 19.4, 14.1; HRMS (ESI) calcd 707.3744 for $C_{42}H_{56}O_6NaSi$ (M+Na), found 707.3755.

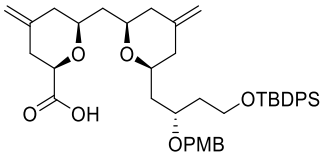


Preparation of ((2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl acetate (2.34): To

a stirring solution of alcohol **2.33** (36 mg, 0.053 mmol, 1.0 equiv) in pyridine (1.75 mL, 0.03 M) contained in a 5 mL flask under N_2 was added Ac_2O (150 μL, 1.6 mmol, 30 equiv). The reaction mixture was stirred overnight at rt and quenched with saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified on a 2 × 10 cm silica gel column, eluting with 15% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 6–12 were concentrated to yield the product (36 mg, 92%) as a colorless oil. R_f = 0.76 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = +3.2° (c = 0.76, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70–7.67 (m, 4H), 7.45–7.37 (m, 6H), 7.19 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 4.72 (d, *J* = 10 Hz, 2H), 4.69 (s, 1H), 4.59 (s, 1H). 4.42 (ABq, *J* = 10.5 Hz, Δν = 37.8 Hz, 2H), 4.11 (dd, *J* = 12, 6.5 Hz, 1H), 4.06 (dd, *J* = 12, 4.0 Hz, 1H), 3.94–3.92 (m, 1H), 3.83–3.80 (m, 1H), 3.80 (s, 3H), 3.77–3.74 (m, 1H), 3.57–3.50 (m, 3H), 3.47–3.46 (m, 1H), 2.27 (t, *J* = 15.5 Hz, 2H), 2.17 (d, *J* = 12.5 Hz, 2H), 2.06 (s, 3H), 2.02–1.91 (m, 6H), 1.84–1.74 (m, 3H), 1.67–1.58 (m, 3H), 1.06 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.2, 159.4, 145.0, 143.4, 135.9, 134.1, 131.2, 129.8, 129.6, 127.9, 114.0, 109.8, 108.7, 76.1, 75.3, 75.1, 74.9, 72.7, 72.2, 67.1, 60.6, 55.5, 42.8, 42.5, 41.4, 41.0, 40.4, 38.0, 37.0, 27.2, 21.1, 19.4. 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.2, 21.1; CH_2 δ 109.8, 108.7, 72.2, 67.1, 60.6, 42.8, 42.5, 41.4, 41.0, 40.4, 38.0, 37.0; CH δ 135.9, 129.8, 129.6, 127.9, 114.0, 76.1, 75.3, 75.1, 74.9, 72.7; C δ 171.2, 159.4, 145.0, 143.4, 134.1, 131.2, 19.4; IR (neat) 3071, 2939, 2858, 1740, 1684, 1653, 1558, 1515, 1472, 1428, 1362, 1248, 1111, 1040, 823, 703 cm^{-1}; HRMS (ESI) calcd 749.3850 for $C_{44}H_{58}O_7NaSi$ (M+Na), found 749.3856.



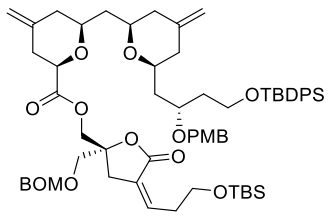
Key 1D nOe Correlations



Preparation of (2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylic acid (2.1): A solution of alcohol **2.33** (131 mg, 0.19 mmol, 1.0 equiv) was prepared in CH_2Cl_2 (1.9 mL, 0.1 M) in a 10 mL flask under N_2. After cooling to 0 ^oC, *i*-Pr_2NEt (230 μL, 1.33 mmol, 7.0 equiv) was added, followed by DMSO (140 μL, 1.9 mmol, 10 equiv). After 10 min, SO_3•Pyr (121 mg, 0.76 mmol, 4.0 equiv) was added in 4 equal portions over 20 min to the stirring solution. The reaction mixture was stirred for 1 h at 0 ^oC and then quenched with saturated aqueous $NaHCO_3$ solution (25 mL), extracted with EtOAc (3 × 10 mL), washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified through a 3 × 10 cm silica gel column, collecting 10 mL fractions. The product containing fractions were concentrated to yield the aldehyde (127 mg, 97%) which was used immediately for the subsequent oxidation step.

A solution of the previously prepared aldehyde (127 mg, 0.190 mmol, 1.0 equiv) in *t*-BuOH (2.65 mL) was prepared in a 10 mL flask, 2-methyl-2-butene (2.65 mL) was

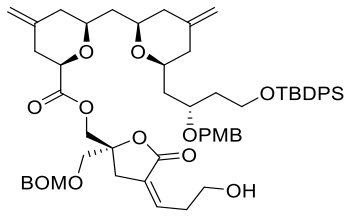
added, along with an aqueous solution of KH_2PO_4 (1.35 M. 930 μL, 6.0 equiv) to the stirring solution. The reaction mixture was cooled to −10 °C, powdered 80% $NaClO_2$ (106 mg, 5.0 equiv) was added. The reaction mixture was stirred overnight, allowing the cooling bath to expire. The mixture was then quenched with pH 4.0 acetate buffer (0.1 M, 15 mL). After extracting with EtOAc (3 × 10 mL), the organic phase was washed with brine (25 mL), dried over Na_2SO_4, filtered, and then concentrated. The product was purified on a 3 × 10 cm silica gel column, eluting with a 60:15:1 toluene/ EtOAc/ AcOH mixture, collecting 5 mL fractions. Fractions 6–16 were concentrated to yield the carboxylic acid product (119 mg, 92%) as a colorless oil. R_f = 0.21 (60:15:1 toluene /EtOAc/ AcOH); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71–7.68 (m, 4H), 7.46–7.38 (m, 6 H), 7.19 (dd, *J* = 8.5, 2.0 Hz, 2H), 6.86 (dd, *J* = 8.5, 2.0 Hz, 2H), 4.77 (d, *J* = 21 Hz, 2H), 4.74 (s, 1H), 4.66 (s, 1H), 4.38 (ABq, *J* = 10.5 Hz, Δν = 35.6 Hz, 2H), 3.94–3.88 (m, 2H), 3.85–3.79 (m, 1H), 3.81 (s, 3H), 3.78–3.73 (m, 1H), 3.70–3.65 (m, 1H), 3.56–3.52 (m, 1H), 3.46–3.41 (m, 1H), 2.62 (d, *J* = 13 Hz, 1H), 2.37–2.31 (m, 1H), 2.26–2.17 (m, 3H), 2.08–1.91 (m, 5H), 1.88–1.81 (m, 1H), 1.80–1.73 (m, 1H), 1.72–1.60 (m, 3H), 1.07 (s, 9H): 125 MHz ^{13}C NMR ($CDCl_3$) δ 173.2, 159.3, 144.4, 141.4, 135.8, 134.0, 131.1, 129.8, 129.6, 127.9, 114.0, 111.3, 109.1, 76.1, 76.1, 75.1, 74.6, 72.7, 71.9, 60.6, 55.5, 42.6, 42.4, 41.3, 41.1, 39.8, 37.8, 37.2, 27.1, 19.4; HRMS (ESI) calcd 721.3537 for $C_{42}H_{54}O_7NaSi$ (M+Na), found 721.3553.



Preparation of ((*R*, *Z*)-2-

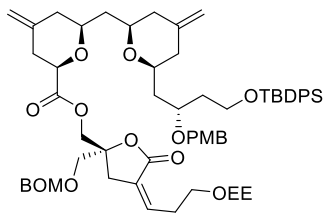
(((benzyloxy)methoxy)methyl)-4-(3-((tert-butyldimethylsilyl)oxy)propylidene)-5-oxotetrahydrofuran-2-yl)methyl (2R,6S)-6-(((2R,6S)-6-((S)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylate (2.4): A stirring solution of carboxylic acid **2.1** (91 mg, 0.13 mmol, 1.0 equiv) and alcohol **1.93** (68 mg, 0.16 mmol, 1.2 equiv) was prepared in CH_2Cl_2 (1.3 mL, 0.1 M) in a 5 mL flask under N_2. After cooling to 0 ^oC, EDCI•HCl (100 mg, 0.52 mmol, 4.0 equiv), DMAP (48 mg, 0.39 mmol, 3.0 equiv) and DMAP•HCl (41 mg, 0.26 mmol, 2.0 equiv) were then added. After warming to rt, the reaction mixture was stirred for 6 h. The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ solution (25 mL), extracted with CH_2Cl_2 (3 × 15 mL), washed with brine (25 mL) and dried over Na_2SO_4. After filtration and concentration, the product was purified on a 3 × 10 cm silica gel column, eluting with a gradient consisting of 100 mL 10% EtOAc/ hexanes, 300 mL 25% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 36–64 were concentrated to yield the product (135 mg, 93%) as a colorless oil. R_f = 0.44 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +7.5^o (c = 0.85, $PhCH_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70–7.68 (m, 5H), 7.45–7.33 (m, 10H), 7.18 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 6.36 (t, *J* = 7.5 Hz, 1H), 4.76 (s, 2H), 4.73 (d, *J* = 4.0 Hz, 2H), 4.71 (s, 1H), 4.62(s, 1H), 4.60 (s, 2H), 4.40 (ABq, *J* = 10.5 Hz, Δν = 42.7 Hz, 2H), 4.28 (ABq, *J* = 12 Hz, Δν = 64.9 Hz, 2H), 3.92 (dd, *J* = 12, 2.5 Hz, 2H), 3.83–3.79 (m, 1H), 3.80 (s, 3H), 3.77–3.71 (m, 4H), 3.67 (dd, *J* = 15, 10 Hz, 2H), 3.62–3.50 (m, 2H), 3.50–3.42 (m, 1H), 3.06–2.97 (m, 1H), 2.92–2.84 (m, 1H), 2.85 (d, *J* = 16.5 Hz, 1H), 2.75 (d, *J* = 16.5 Hz, 1H), 2.44 (d, *J* = 13.5 Hz, 1H), 2.30–2.23 (m, 3H), 2.18 (t, *J* = 12 Hz, 1H), 2.09–1.90 (m, 5H), 1.86–1.72 (m, 3H), 1.71–1.59 (m, 4H), 1.06 (s, 9H), 0.90

(s, 9H), 0.07 (s, 6H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.3, 168.5, 159.3, 144.8, 142.1, 142.0, 137.7, 135.8, 134.1, 134.0, 131.2, 129.8, 129.5, 128.7, 128.1, 127.9, 125.0, 114.0, 110.8, 108.8, 95.2, 81.2, 76.6, 75.6, 75.0, 74.6, 72.8, 72.0, 70.1, 69.6, 66.0, 62.2, 60.6, 55.5, 42.6, 42.5, 41.4, 41.1, 39.9, 37.9, 37.6, 33.8, 31.4, 27.2, 26.1, 19.4, 18.5, −5.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.3, 27.2, 26.1, −5.1; CH_2 δ 110.8, 108.8, 95.2, 72.0, 70.1, 69.6, 66.0, 62.2, 60.6, 42.6, 42.5, 41.4, 41.1, 39.9, 37.9, 37.6, 33.8, 31.4; CH δ 170.3, 142.1, 135.8, 129.8, 129.5, 128.7, 128.1, 127.9, 114.0, 76.6, 75.6, 75.0, 74.6, 72.8; C δ 168.5, 159.3, 144.8, 142.0, 137.7, 134.1, 134.0, 131.2, 125.0, 81.2, 19.4, 18.5; IR (neat) 2933, 2857, 1762, 1653, 1612, 1514, 1472, 1429, 1362, 1249, 1173, 1106, 1048, 893, 836, 777, 737, 702 cm^{-1}; HRMS (ESI) calcd for $C_{65}H_{88}O_{12}Si_2Na$ (M+Na), 1139.5712 found 1139.5618.



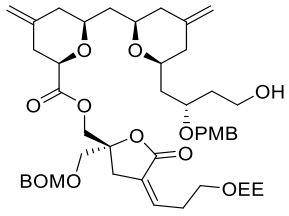
Preparation of ((*R*, *Z*)-2-(((benzyloxy)methoxy)methyl)-4-(3-hydroxypropylidene)-5-oxotetrahydrofuran-2-yl)methyl (2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylene tetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylate (2.5): A solution of **2.4** (175 mg, 0.157 mmol, 1.0 equiv) was prepared in 3:1:1 AcOH/ THF/ H_2O (15.7 mL, 0.01 M) in a 25 mL flask. The stirring solution was heated at 45 ^oC for 1.5 h, after which the reaction was complete. The reaction mixture was diluted with brine (100 mL), and extracted with EtOAc (3 × 25 mL). After drying over Na_2SO_4, and filtering, the solution was concentrated. The product was purified on a 2 × 15 cm silica

gel column, eluting with a gradient of 25–50% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 35–72 were concentrated to yield the product (117 mg, 74%) as a colorless oil. R_f = 0.28 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +9.7° (c = 0.57, CH_2Cl_2); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70–7.67 (m, 5H), 7.45–7.30 (m, 10H), 7.18 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 6.33 (dddd, *J* = 12, 7.5, 2.0, 2.0 Hz, 1H), 4.77 (s, 2H), 4.74–4.72 (m, 3H), 4.61 (s, 1H), 4.57 (s, 2H), 4.40 (ABq, *J* = 10.5 Hz, Δν = 45.6 Hz, 2H), 4.30 (ABq, *J* = 12 Hz, Δν = 42 Hz, 2H), 3.93 (dd, *J* = 11.5, 2.0 Hz, 1H), 3.94–3.88 (m, 1H), 3.84–3.80 (m, 1H), 3.80 (s, 3H), 3.77–3.72 (m, 3H), 3.68 (s, 2H), 3.61–3.56 (m, 1H), 3.56–3.50 (m, 1H), 3.49–3.43 (m, 1H), 2.96 (dd, *J* = 13.5, 6.5 Hz, 2H), 2.82 (ABq, *J* = 19 Hz, Δν = 33.9 Hz, 2H), 2.43 (d, *J* = 13.5 Hz, 1H), 2.31–2.23 (m, 3H), 2.17 (d, *J* = 13.5 Hz, 1H), 2.09–2.04 (m, 2H), 2.02–1.90 (m, 3H), 1.84–1.74 (m, 2H), 1.69–1.61 (m, 3H), 1.06 (s, 9H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.3, 169.0, 159.3, 144.7, 142.0, 140.9, 137.6, 135.8, 134.0, 131.1, 129.8, 129.5, 128.7, 128.1, 127.9, 127.9, 126.3, 114.0, 110.8, 108.9, 95.2, 81.6, 76.6, 75.6, 75.1, 74.6, 72.8, 72.1, 70.1, 69.6, 66.1, 61.9, 60.6, 55.5, 42.6, 42.4, 41.3, 41.1, 39.8, 37.9, 37.5, 33.9, 31.1, 27.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.1; CH_2 δ 110.8, 108.9, 95.2, 72.1, 70.1, 69.6, 66.1, 61.9, 60.6, 42.6, 42.4, 41.3, 39.8, 37.9, 37.5, 33.9, 31.1, 27.1; CH δ 140.8, 135.8, 129.8, 129.5, 128.7, 128.1, 127.9, 114.0, 76.6, 75.6, 75.1, 74.6, 72.8; C δ 170.3, 169.0, 159.3, 144.7, 142.0, 137.6, 134.0, 131.1, 126.3, 81.6, 70.1; IR (neat) 3496, 3070, 2939, 2888, 2857, 1760, 1671, 1654, 1612, 1514, 1472, 1428, 1363, 1248, 1173, 1111, 1048, 895, 823, 739, 703 cm^{-1}; HRMS (ESI) calcd 1025.4847 for $C_{59}H_{74}O_{12}NaSi$ (M+Na), found 1025.4835.



Preparation of ((2*R*,*Z*)-2-(((benzyloxy)methoxy)methyl)-4-(3-(1-ethoxyethoxy)propylidene)-5-oxotetrahydrofuran-2-yl)methyl (2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylate (2.6): A solution of alcohol **2.5** (38.2 mg, 0.038 mmol, 1.0 equiv) in a 4:1 mixture of CH_2Cl_2/ ethyl vinyl ether (3.80 mL, 0.01 M) was prepared in a 10 mL flask under N_2. After cooling to 0 ^oC, PPTS (1.0 mg, 0.004 mmol, 0.1 equiv) was added, and the mixture was then stirred at 0 ^oC for 1 h. The reaction mixture was warmed to rt, and stirred for 1 h. The reaction mixture was quenched with brine (10 mL), extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 1 × 10 cm silica gel column, eluting with a gradient of 100 mL 15% EtOAc, followed by 100 mL 25% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 18–30 where concentrated to yield the product (32 mg, 78%) as a colorless oil. R_f = 0.77 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = + 6.5^o (c = 1.6, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) 7.70–7.67 (m, 5H), 7.45–7.28 (m, 10H), 7.18 (d, *J* = 8.5 Hz, 2H) 6.86 (d, *J* = 8.5 Hz, 2H), 6.34 (t, *J* = 7.0 Hz, 1H), 4.76 (s, 2H), 4.75–4.68 (m, 4H), 4.61 (s, 1H), 4.59 (s, 2H), 4.40 (ABq, *J* =10.5 Hz, Δν = 43.8 Hz, 2H), 4.28 (ABq, *J* = 12.5 Hz, Δν = 64.5 Hz, 2H), 3.94–3.88 (m, 2H), 3.84–3.81 (m, 1H), 3.80 (s, 3H), 3.77–3.72 (m, 1H), 3.70–3.64 (m, 4H), 3.63–3.51 (m, 4H), 3.50–3.44 (m, 2H), 3.09–3.02 (m, 1H), 2.98–2.91 (m, 1H), 2.85 (d, *J* = 16.5 Hz,

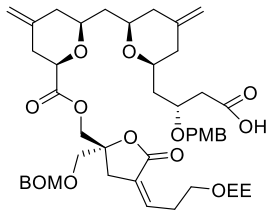
1H), 2.75 (d, *J* = 16.5 Hz, 1H), 2.43 (d, *J* = 13 Hz, 1H), 2.30–2.15 (m, 4H), 2.06 (ddd, *J* = 14, 8.5, 6.5 Hz, 1H), 2.02–1.94 (m, 2H), 1.91 (d, *J* = 12.5 Hz, 1H), 1.80 (ddddd, *J* = 13, 13, 13, 6.5, 6.5 Hz, 2H), 1.73–1.63 (m, 4H), 1.31 (d, *J* = 5.0 Hz, 3H), 1.21 (t, *J* = 6.5 Hz, 3H), 1.06 (s, 9H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.3, 168.5, 159.3, 144.8, 142.0, 141.7, 137.6, 135.8, 134.1, 131.1, 129.8, 129.5, 128.7, 128.1, 127.9, 127.9, 125.3, 114.0, 110.8, 108.8, 99.8, 95.2, 81.3, 76.6, 75.6, 75.0, 74.6, 72.8, 72.0, 70.1, 69.5, 66.0, 64.0, 61.3, 60.6, 55.5, 42.6, 42.5, 41.3, 41.0, 39.9, 37.9, 37.6, 33.7, 28.6, 27.2, 20.1, 19.4, 15.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.1, 20.1, 15.5; CH_2 δ 110.8, 108.9, 95.1, 72.1, 70.1, 69.5, 66.0, 64.0, 61.3, 60.6, 42.6, 42.5, 41.4, 41.0, 39.9, 37.9, 37.6, 33.7, 28.6; CH δ 141.7, 135.8, 129.8, 129.5, 128.7, 128.1, 127.9, 127.9, 114.0, 99.8, 76.6, 75.6, 75.0, 74.6, 72.8; C δ 170.3, 168.5, 159.3, 144.8, 142.0, 137.6, 134.1, 131.1, 125.3, 81.3, 19.4; IR (neat) 2939, 2887, 1761, 1656, 1612, 1513, 1428, 1378, 1248, 1172, 1110, 1049, 894, 823, 738, 703 cm^{-1}.



Preparation of ((2*R*,*Z*)-2-(((benzyloxy)methoxy)methyl)-4-(3-(1-ethoxyethoxy)propylidene)-5-oxotetrahydrofuran-2-yl)methyl (2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylate (2.7): A solution of **2.6** (90.3 mg, 0.084 mmol, 1.0 equiv) was prepared in DMF (1.70 mL, 0.05 M) in a 5 mL flask. A solution of AcOH in DMF (1.0 M, 84 μL, 1.0 equiv) and TBAF in THF (1.0 M, 84 μL, 1.0 equiv) were mixed together and added to the reaction mixture. The mixture was stirred at rt

overnight, and then quenched with a solution of saturated aqueous $NaHCO_3$ (5.0 mL) and diluted with brine (10 mL). After extraction with EtOAc (3 × 10 mL), the solution was dried over Na_2SO_4, filtered, and concentrated. The crude product was then purified on a 1 × 10 cm silica gel column, eluting with a gradient of 25 % –60 % EtOAc/ hexanes, collecting 5 mL fractions. Fractions 50–90 were concentrated to yield the product (70.1 mg, 100%) as a colorless oil. R_f = 0.15 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +9.5° (c = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) 7.36–7.29 (m, 5H), 7.26 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.34 (t, *J* = 7.0 Hz, 1H), 4.77 (s, 2H), 4.77 (s, 1H), 4.72 (s, 2H), 4.70 (q, *J* = 5.5 Hz, 1H), 4.67 (s, 1H), 4.59 (s, 2H), 4.47 (ABq, *J* = 10.5 Hz, Δν = 26.5 Hz, 2H), 4.28 (ABq, *J* = 11.5 Hz, Δν = 42.0 Hz, 2H), 3.95 (dd, *J* = 12.5, 2.5 Hz, 1H), 3.90–3.85 (m, 1H), 3.83–3.81 (m, 1H), 3.80 (s, 3H), 3.75–3.62 (m, 6H), 3.50–3.44 (m, 3H), 3.08–3.02 (m, 1H), 3.00–2.94 (m, 1H,), 2.89–2.71 (m, 2H), 2.45 (d, *J* = 13 Hz, 1H), 2.25 (q, *J* = 13 Hz, 2H), 2.18 (d, *J* = 13.5 Hz, 2H), 2.07–2.01 (m, 2H), 1.99–1.94 (m, 3H), 1.92–1.89 (m, 1H), 1.80–1.70 (m, 2H), 1.68–1.63 (m, 2H), 1.31 (d, *J* = 5.5 Hz, 3H), 1.21 (t, *J* = 6.5 Hz, 3H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.3, 168.5, 159.6, 144.4, 142.1, 141.7, 137.6, 130.6, 129.7, 128.7, 128.1, 125.3, 114.2, 110.8, 109.1, 99.9, 95.2, 81.3, 76.7, 75.8, 75.4, 75.3, 74.8, 72.0, 70.1, 69.5, 66.1, 64.0, 61.3, 60.4, 55.5, 42.5, 41.7, 41.4, 40.9, 39.9, 37.6, 36.8, 33.8, 31.2, 28.6, 20.1, 15.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 20.1, 15.5; CH_2 δ 110.8, 109.1, 95.2, 72.0, 70.1, 69.5, 66.1, 64.0, 61.3, 60.4, 42.5, 41.7, 41.4, 40.9, 39.9, 37.6, 36.8, 33.8, 31.2, 28.6; CH δ 141.7, 129.7, 128.7, 128.1, 114.2, 99.9, 76.7, 75.8, 75.4, 75.3, 74.8; C δ 170.3, 168.5, 159.6, 144.4, 142.1, 137.6, 130.6, 125.3, 81.3; IR (neat) 3478, 2940, 2886, 1760, 1653, 1612, 1514, 1441, 1378, 1249,

1173, 1108, 1048, 898, 822, 747, 700 cm^{-1}; HRMS (ESI) calcd 859.4245 for $C_{47}H_{64}O_{13}Na$ (M+Na), found 859.4240.

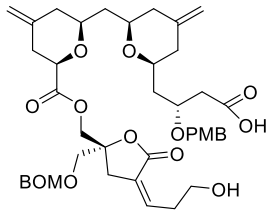


Preparation of (3*R*)-4-((2*S*,6*R*)-6-(((2*S*,6*R*)-6-((((2*R*,*Z*)-2-(((benzyloxy)methoxy)methyl)-4-(3-(1-ethoxyethoxy)propylidene)-5-oxotetra hydrofuran-2-yl)methoxy)carbonyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3-((4-methoxybenzyl)oxy) butanoic acid (2.8): To a stirring solution of **2.7** (17.1 mg, 0.02 mmol, 1.0 equiv) in CH_2Cl_2 (200 μL, 0.1 M) in a 5 mL via was added *i*-Pr_2NEt (20 μL, 0.13 mmol, 7.0 equiv), followed by DMSO (10 μL, 0.19 mmol, 10.0 equiv). The solution was chilled to 0 ^oC and SO_3•Pyr (12 mg, 0.08 mmol, 4.0 equiv) was added in one portion. After stirring for 1.5 h at 0 ^oC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (2.0 mL). After extraction with EtOAc (3 × 5 mL), the solution was dried over Na_2SO_4, filtered, and concentrated. The crude aldehyde was purified on a plug of silica gel in a Pasteur pipette, eluting with 25% EtOAc/ hexanes. The crude aldehyde was used as is without characterization.

The aldehyde (0.02 mmol, 1.0 equiv) was taken up in *t*-BuOH (270 μL) in a 5 mL vial. To a stirring solution of aldehyde, 2-methyl-2-butene (270 μL) was added, followed by an aqueous KH_2PO_4 solution (1.25 M, 90 μL, 0.11 mmol, 6.0 equiv). The rapidly stirred mixture was chilled to −10 ^oC in a MeOH/ ice bath. To the cold solution 80% $NaClO_2$ (11 mg, 0.1 mmol, 5.0 equiv) was added in one portion. After stirring for 1 h at −10 ^oC, the reaction was quenched with aqueous pH 4.0 acetate buffer (0.1 M, 5.0 mL).

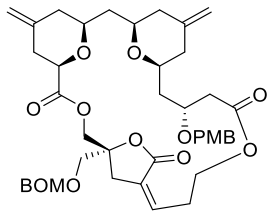
After extracting with EtOAc (4 × 5.0 mL), the solution was dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 0.5 × 5 cm Pasteur pipette silica gel column, eluting with 80/20/1 toluene/ EtOAc/ AcOH, collecting 1.0 mL fractions. Fractions 4–14 were concentrated to yield the product (14 mg, 82%) as a colorless oil. R_f = 0.37 (80/ 20/ 1 $PhCH_3$/ EtOAc/ AcOH); $[\alpha]_D^{20}$ = +14° (c = 0.45, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) 7.38–7.28 (m, 5H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.34 (ddd, *J* = 5.0, 5.0, 2.0 Hz, 1H), 4.77 (s, 2H), 4.74 (q, *J* = 5.0 Hz, 1H), 4.73–4.69 (m, 4H), 4.60 (s, 2H), 4.49 (ABq, *J* = 11 Hz, Δν = 60 Hz, 2H), 4.32 (dd, *J* = 13, 4.5 Hz, 1H), 4.28 (dd, *J* = 15, 3.5 Hz, 1H), 4.09–4.07 (m, 1H), 3.94 (dd, *J* = 11.5, 2.0 Hz, 1H), 3.81–3.80 (m, 1H), 3.80 (s, 3H), 3.72–3.62 (m, 3H), 3.58–3.54 (m, 1H), 3.52–3.46 (m, 2H), 3.43–3.37 (m, 1H), 3.10–2.92 (m, 2H), 2.81 (ABq, *J* = 16.5 Hz, Δν = 40.8 Hz, 2H), 2.63 (d, *J* = 5.0 Hz, 1H), 2.45 (d, *J* = 12.5 Hz, 1H), 2.27–2.19 (m, 3H), 2.15 (d, *J* = 12.5 Hz, 1H), 2.08–2.02 (m, 1H), 1.96 (q, *J* = 12 Hz, 3H), 1.79 (dd, *J* = 14, 8.0 Hz, 1H), 1.70 (ddd, *J* = 10, 5.0, 5.0 Hz, 1H), 1.65 (ddd, *J* = 11, 5.5, 5.0 Hz, 1H), 1.32 (t, *J* = 5.5 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 173.8, 170.8, 168.6, 159.5, 144.3, 141.8, 141.3, 137.6, 130.4, 129.6, 128.7, 128.1, 125.5, 114.1, 111.0, 109.1, 99.8, 95.2, 81.4, 76.6, 75.9, 75.1, 75.0, 73.3, 72.0, 70.1, 69.6, 66.5, 64.0, 61.3, 55.5, 42.7, 42.0, 41.2, 41.0, 40.1, 40.0, 37.5, 33.9, 28.5, 20.0, 15.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 20.0, 15.5; CH_2 δ 111.0, 109.1, 95.2, 72.0, 70.1, 69.6, 66.5, 64.0, 61.3, 42.7, 42.0, 41.2, 40.1, 40.0, 37.5, 33.9, 28.5; CH δ 141.3, 129.6, 128.7, 128.1, 114.1, 99.8, 76.6, 75.9, 75.1, 75.0, 73.3, 41.0; C δ 173.8, 170.8, 168.6, 159.5, 144.3, 141.8, 137.6, 130.4, 125.5, 81.4; IR (neat) 2938, 1761, 1684, 1653, 1514, 1379, 1249, 1173, 1108, 1047, 899, 823, 740, 699

cm^{-1}; HRMS (ESI) calcd 873.4037 for $C_{47}H_{62}O_{14}Na$ (M+Na), found 873.4047.



Preparation of (*R*)-4-((2*S*,6*R*)-6-(((2*S*,6*R*)-6-((((*R*,*Z*)-2-(((benzyloxy)methoxy)methyl)-4-(3-hydroxypropylidene)-5-oxotetrahydrofuran-2-yl)methoxy)carbonyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3-((4-methoxybenzyl)oxy)butanoic acid (2.35): To a stirring solution of carboxylic acid **2.8** (46 mg, 0.05 mmol, 1.0 equiv) in MeOH (5.4 mL, 0.01 M) in a 10 mL flask under N_2 was added PPTS (6.4 mg, 0.03 mmol, 0.47 equiv). After stirring overnight, the reaction mixture was diluted with brine (25 mL), and extracted with EtOAc (3 × 10 mL). After drying over Na_2SO_4, the solution was filtered and concentrated. The crude product was purified on a 1 × 10 cm silica gel column, eluting with 50 mL 50 % EtOAc/ hexanes and then 50/ 40/ 10 hexanes/ EtOAc/ MeOH, collecting 5 mL fractions. Fractions 12–42 were concentrated to yield the *seco*-acid (39.2 mg, 93%) as a colorless oil. R_f = 0.21 (50/ 40/ 10 Hexanes/ EtOAc/ MeOH); $[\alpha]_D^{20}$ = +12.1° (c = 0.860, MeOH); 500 MHz ^{1}H NMR ($CDCl_3$) 7.38–7.29 (m, 5H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.34 (t, *J* = 7.5 Hz, 1H), 4.78 (m, 3H), 4.75–4.72 (m, 2H), 4.68 (s, 1H), 4.61 (s, 2H), 4.49 (ABq, *J* = 10.5 Hz, Δν = 52.2 Hz, 2H), 4.32 (ABq, *J* = 12 Hz, Δν = 27.9 Hz, 2H), 4.11–4.08 (m, 1H), 3.95 (dd, *J* = 12, 2.5 Hz, 1H), 3.80 (s, 3H), 3.82–3.74 (m, 2H), 3.68 (dd, *J* = 11, 1.0 Hz, 2H), 3.58–3.52 (m, 1H), 3.47 (t, *J* = 10.5 Hz, 1H), 3.42–3.35 (m, 3H), 3.10 (dddd, *J* = 13.5, 7.0, 7.0, 7.0 Hz, 1H), 3.00–2.98 (m, 3H), 2.63 (d, *J* = 6.0 Hz, 2H), 2.45 (d, *J* = 13 Hz, 1H), 2.36 (s, 1H), 2.29–2.20

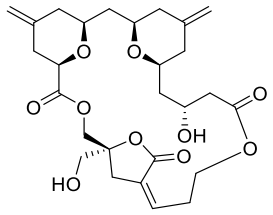
(m, 3H), 2.16 (d, *J* = 13 Hz, 1H), 2.08–2.02 (m, 1H), 1.97 (q, *J* = 10.5 Hz, 2H), 1.79 (dd, *J* = 13, 8.0 Hz, 1H), 1.72–1.62 (m, 2H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.7, 169.3, 159.5, 144.2, 141.9, 140.4, 137.6, 130.4, 129.6, 129.3, 128.7, 128.4, 128.1, 125.5, 114.1, 111.0, 109.2, 95.2, 81.8, 76.7, 75.9, 75.3, 74.9, 73.6, 72.1, 70.2, 69.8, 66.7, 61.9, 55.5, 42.8, 42.0, 41.2, 41.1, 40.3, 39.9, 37.6, 34.0, 30.9; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5; CH_2 δ 111.0, 109.2, 95.2, 72.1, 70.2, 69.8, 66.7, 61.9, 42.8, 42.0, 41.2, 41.1, 40.3, 39.9, 37.6, 34.0, 30.9; CH δ 140.4, 129.6, 128.7, 128.4, 128.1, 114.1, 76.7, 75.9, 75.3, 74.9, 73.6; C δ 170.7, 169.3, 159.5, 144.2, 141.9, 137.6, 130.4, 129.3, 125.5, 81.8; IR (neat) 3467, 3072, 3032, 2936, 2891, 1757, 1673, 1654, 1613, 1586, 1514, 1498, 1455, 1439, 1421, 1399, 1374, 1302, 1249, 1174, 1108, 1081, 1045, 898, 848, 822 cm^{-1}; HRMS (ESI) calcd 801.3462 for $C_{43}H_{54}O_{13}Na$ (M+Na), found .



Preparation of (1*R*,3*S*,7*R*,11*R*,21*R*,23*S*,*Z*)-11-(((benzyloxy)methoxy)methyl)-21-((4-methoxybenzyl)oxy)-5,25-dimethylene-9,12,18,27,28-pentaoxatetracyclo[21.3.1.13,7.111,14]nonacos-14-ene-8,13,19-trione (2.9): To a 100 mL flask under N_2 was added toluene (14.5 mL), followed by 2,4,6-trichlorobenzoyl chloride (230 μL of 0.1 M, 0.023 mmol, 1.0 equiv), Et_3N (460 μL of 0.1 M, 0.046 mmol, 2.0 equiv), and DMAP (23 μL of 0.1 M, 0.002 mmol, 0.1 equiv). All of these solutions were prepared in toluene. A solution of *seco*-acid **2.35** (17.8 mg, 0.02 mmol, 1.0 equiv) in toluene (14.5 mL) was added via syringe pump at 0.75 mL/ h to the stirring solution at 40 ^{o}C. The addition was complete after 20 h. The transfer was completed by washing the syringe with toluene (1.0 mL) and adding this to the reaction

mixture via syringe pump at 0.75 mL/ h. After stirring at 40 ^{o}C for 3 h, the reaction mixture was cooled to rt and quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The mixture was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 1 × 10 cm silica gel column, eluting with 25% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 17–37 were concentrated to the yield the product (9.3 mg, 54%) as a colorless oil. R_f = 0.56 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +27^{o} (c = 0.69, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) 7.39–7.31 (m, 5H), 7.22 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 6.28 (t, *J* = 7.0 Hz, 1H), 4.81 (s, 1H), 4.80 (s, 2H), 4.75 (s, 2H), 4.69 (s, 2H), 4.62 (s, 2H), 4.41 (s, 2H), 4.27 (ddd, *J* = 15, 8.0, 3.5 Hz, 1H), 4.16–4.09 (m, 3H), 3.81 (s, 3H), 3.74 (dd, *J* = 12, 2.5 Hz, 1H), 3.60 (t, *J* = 11 Hz, 1H), 3.43 (t, *J* = 11 Hz, 1H), 3.27 (t, *J* = 11 Hz, 1H), 3.18–3.10 (m, 1H), 2.83 (dd, *J* = 17, 2.5 Hz, 1H), 2.76 (dd, *J* = 15.5, 2.5 Hz, 1H), 2.72 (d, *J* = 17 Hz, 1H), 2.56 (dd, *J* = 15.5, 10.5 Hz, 1H), 2.46 (d, *J* = 13 Hz, 1H), 2.27 (t, *J* = 12.5 Hz, 1H), 2.20 (t, *J* = 11.5 Hz, 2H), 2.06–2.00 (m, 2H), 1.97–1.92 (m, 2H), 1.80 (m, 1H), 1.75–1.69 (m, 2H), 1.66–1.61 (m, 2H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.5, 170.0, 168.8, 159.5, 143.9, 141.6, 138.1, 137.6, 130.6, 129.5, 128.8, 128.2, 127.3, 114.1, 111.3, 109.5, 95.3, 82.3, 77.8, 77.5, 76.6, 75.9, 74.8, 74.4, 73.0, 70.3, 69.9, 67.8, 64.0, 55.5, 43.0, 42.2, 41.7, 41.3, 39.6, 37.6, 34.3, 27.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5; CH_2 δ 111.3, 109.5, 95.3, 73.0, 70.3, 69.9, 67.8, 64.0, 43.0, 42.2, 41.7, 41.3, 39.6, 37.6, 34.3, 27.2; CH δ 138.1, 129.5, 128.8, 128.2, 114.1, 77.8, 76.6, 75.9, 74.9, 74.4; C δ 171.5, 170.0, 168.8, 159.5, 143.9, 141.6, 137.6, 130.6, 127.3, 82.3, 77.5; IR (neat) 3073, 2929, 2856, 1819, 1760, 1739, 1692, 1674, 1613, 1581, 1550, 1514, 1454, 1440, 1405, 1369, 1326, 1301, 1249, 1207, 1178, 1083, 1044, 987, 899 cm^{-1};

HRMS (ESI) calcd 783.3356 for $C_{43}H_{52}O_{12}Na$ (M+Na), found 783.3356.

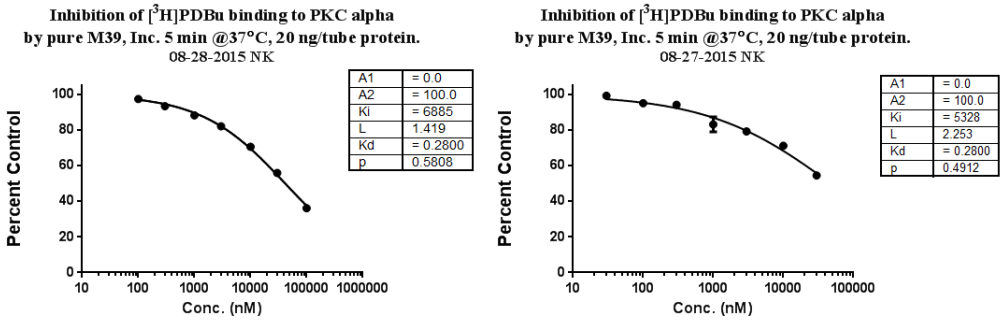


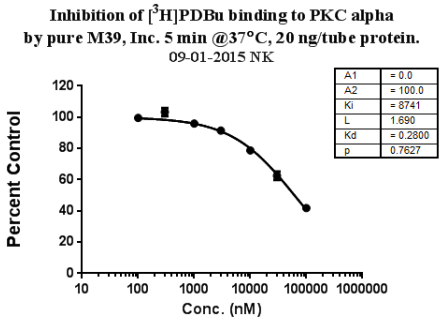
Preparation of Merle 39 (1*R*,3*S*,7*R*,11*R*,21*R*,23*S*,*Z*)-21-hydroxy-11-(hydroxymethyl)-5,25-dimethylene-9,12,18,27,28-pentaoxatetracyclo [21.3.1.13,7.111,14]nonacos-14-ene-8,13,19-trione: To a stirring solution of macrolactone **2.9** (6.9 mg, 0.01 mmol, 1.0 equiv) in CH_2Cl_2 (900 μL, 0.01 M) contained in a 5 mL vial at 0 ^oC was added H_2O (20 μL), followed by DDQ (21 mg, 0.1 mmol, 10.0 equiv). The reaction mixture was stirred at 0 ^oC for 2 h and then quenched with a solution of saturated aqueous $NaHCO_3$ (10 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was used without purification.

To the previously described crude alcohol contained in a 5 mL vial, a solution of $LiBF_4$ in 25:1 MeCN/ H_2O (0.25 M, 1.62 mL, 0.41 mmol, 45 equiv) was added. After flushing with N_2, the vial was capped and heated at 80 ^oC overnight with stirring. After cooling to rt, the solvent was evaporated and the reaction mixture was quenched with brine (10 mL), and extracted with EtOAc (3 × 10 mL). After drying over Na_2SO_4, the solution was filtered and concentrated. The product was purified on a 1 × 5 cm silica gel column, eluting with 60% EtOAc/ pentane, collecting 1 mL fractions. Fractions 11–56 were concentrated to yield the product (3.4 mg, 73% over 2-steps) as a colorless oil. R_f = 0.40 (50/ 40/ 10 hexanes/ EtOAc/ MeOH); $[\alpha]_D^{20} = +6.2^o$ (c = 0.065, $CHCl_3$); 500 MHz ^1H NMR ($CDCl_3$) 6.29 (t, *J* = 7.5 Hz, 1H), 4.83 (d, *J* = 18 Hz, 2H), 4.74 (s, 2H), 4.65 (d,

J = 12 Hz, 1H), 4.38 (ddd, *J* = 15, 8.0, 3.5 Hz, 1H), 4.20–4.17 (m, 1H), 4.14 (d, *J* = 12 Hz, 2H), 3.82 (dd, *J* = 12.5, 2.5 Hz, 1H), 3.79–3.76 (m, 1H), 3.70 (dd, *J* = 12, 6.5 Hz, 1H), 3.55–3.49 (m, 1H), 3.46–3.41 (m, 2H), 3.32–3.25 (m, 2H), 2.92 (dd, *J* = 16.5, 3.0 Hz, 1H), 2.85–2.79 (m, 1H), 2.71 (dd, *J* = 15.5, 1.5 Hz, 1H), 2.46 (d, *J* = 13 Hz, 1H), 2.41 (dd, *J* = 16, 10.5 Hz, 1H), 2.30 (d, *J* = 14.5 Hz, 1H), 2.24 (d, *J* = 12.5 Hz, 1H), 2.18 (d, *J* = 13.5 Hz, 2H), 2.05–1.92 (m, 5H), 1.79–1.66 (m, 4H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 173.6, 173.3, 170.2, 143.8, 141.8, 138.7, 127.7, 111.0, 109.5, 83.8, 76.8, 76.8, 75.2, 67.8, 67.2, 65.1, 64.2, 42.6, 42.4, 41.2, 41.2, 39.7, 37.6, 33.2, 29.9, 27.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 111.0, 109.5, 67.2, 65.1, 64.2, 42.6, 42.4, 41.2, 41.2, 39.7, 37.6, 33.2, 29.9, 27.4; CH δ 138.7, 76.8, 75.2, 67.8; C δ 173.6, 173.3, 170.2, 143.8, 141.8, 127.7, 83.8; IR (neat) 3488, 2924, 1755, 1652, 1408, 1368, 1327, 1262, 1179, 1104, 907, 733 cm^{-1}; HRMS (ESI) calcd 543.2206 for $C_{27}H_{36}O_{10}Na$ (M+Na), found 543.2216.

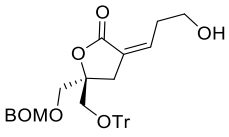
Binding Assay for Merle 39 Towards PKC α





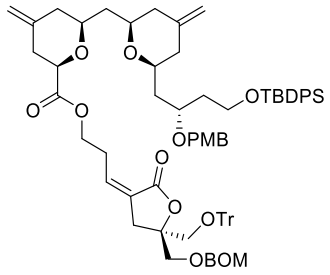
M39	date	Kd (nM)	Ki (nM)	p value	
	8/27/2015	0.28	5328	0.4912	
	8/28/2015	0.28	6885	0.5808	
	9/1/2015	0.28	8741	0.7627	
		Mean	6984.667	986.5077	SEM

Experimental Procedures for Merle 47



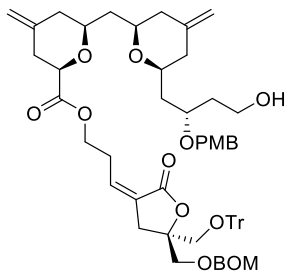
Preparation of (*S*,*Z*)-4-(((benzyloxy)methoxy)methyl)-2-(3-hydroxypropylidene)-4-((trityloxy)methyl)cyclopentan-1-one (2.10): To a solution of lactone **1.93** (150 mg, 0.22 mmol, 1.0 equiv) in THF (4.40 mL, 0.05 M) contained in a 15 mL polyethylene centrifuge tube was added NH_4F (41 mg, 1.1 mmol, 5.0 equiv). TBAF (1.0 M, 1.11 mL, 1.11 mmol, 5.0 equiv) was added. After stirring for 1.5 h, the reaction mixture was quenched with brine (20 mL) and extracted with EtOAc (3 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 2 × 10 cm silica gel column, eluting with 35% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 18–44 were concentrated to yield the product as a colorless oil (118 mg, 95%). R_f = 0.40 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +0.48° (c = 1.67, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.44–7.43 (m, 6H), 7.39–7.24 (m, 14H), 6.28 (dddd, *J* = 7.5, 4.5, 2.0, 2.0 Hz, 1H), 4.72 (s, 2H), 4.53 (s, 2H), 3.83–3.74 (m, 2H), 3.70 (q, *J* = 10 Hz, 2H), 3.34 (d, *J* = 9.5 Hz, 1H), 3.17 (d, *J* = 11.5 Hz, 1H), 3.10–3.04 (m, 1H), 3.00–2.94 (m, 1H), 2.83 (dd, *J* = 14, 2.0 Hz, 1H), 2.74 (dd, *J* = 16.5, 2.0 Hz, 1H), 1.78 (s, 1H). 125

MHz ^{13}C NMR ($CDCl_3$) δ 169.9, 143.6, 139.4, 137.7, 128.8, 128.6, 128.1, 128.1, 128.0, 127.7, 127.4, 95.0, 87.2, 83.5, 70.2, 69.8, 65.9, 62.1, 34.2, 31.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 95.0, 70.2, 69.8, 65.9, 62.1, 34.2, 31.2; CH δ 139.4, 128.8, 128.6, 128.1, 128.0, 127.4; C δ 169.9, 143.6, 137.7, 127.7, 87.2, 83.5; IR (neat) 3472, 3087, 2928, 2876, 1754, 1672, 1491, 1449, 1370, 1214, 1172, 1078, 1045, 992, 944, 900, 747, 700 cm^{-1}; HRMS (ESI) calcd 587.2410 for $C_{36}H_{36}O_6Na$ (M+Na), found 587.2420.



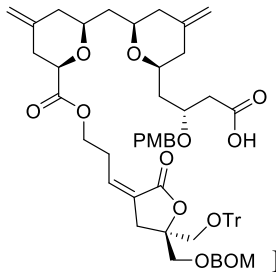
Preparation of (*Z*)-3-((*R*)-5-(((benzyloxy)methoxy)methyl)-2-oxo-5-((trityloxy)methyl)dihydrofuran-3(2H)-ylidene)propyl (2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-((tert-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylate (2.11): To a stirring solution of carboxylic acid **2.1** (117 mg, 0.167 mmol, 1.0 equiv) in $PhCH_3$ (1.67 mL, 0.1 M) in a 10 mL flask under N_2 was added Et_3N (93 μL, 0.67 mmol, 4.0 equiv). After stirring for 5 min, 2,4,6-trichlorobenzoyl chloride (40 μL, 0.23 mmol, 1.4 equiv) was added. The reaction mixture was stirred for 1 h. A solution of alcohol **2.10** (165 mg, 0.29 mmol, 1.8 equiv) and DMAP (36 mg, 0.29 mmol, 1.8 equiv) in $PhCH_3$ (500 μL) was added. $PhCH_3$ (2 × 250 μL) was used to complete the transfer. The reaction mixture was stirred for 1 h, and then quenched with saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with EtOAc (3 × 10 mL), washed with brine (15 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 2 × 15 cm silica gel column,

eluting with 15% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 14–32 were concentrated to yield the product as a colorless oil (127 mg, 61%). R_f = 0.48 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +2.48° (c = 1.62); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70–7.67 (m, 4H), 7.45–7.37 (m, 12H), 7.35–7.23 (m, 16H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 6.13 (t, *J* = 7.0 Hz, 1H), 4.73 (d, *J* = 4.0 Hz, 2H), 4.71 (s, 2H), 4.61 (s, 2H), 4.52 (s, 2H), 4.41 (ABq, *J* = 10.5 Hz, Δν = 41.2 Hz, 2H), 4.25 (ddddd, *J* = 13.5, 10.5, 10.5, 6.5, 6.5 Hz, 2H), 3.94–3.91 (m, 2H), 3.85–3.81 (m, 1H), 3.80 (s, 3H), 3.77–3.73 (m, 1H), 3.68 (ABq, *J* = 10.5 Hz, Δν = 22.9 Hz, 2H), 3.63–3.60 (m, 1H), 3.58–3.51 (m, 1H), 3.50–3.45 (m, 1H), 3.32 (d, *J* = 10 Hz, 1H), 3.17 (d, *J* = 9.5 Hz, 1H), 3.20–3.14 (m, 1H), 3.13–3.09 (m, 1H), 2.79 (d, *J* = 16.5 Hz, 1H), 2.71 (d, *J* = 16.5 Hz, 1H), 2.48 (d, *J* = 12.5 Hz, 1H), 2.31 (d, *J* = 13 Hz, 1H), 2.27–2.23 (m, 2H), 2.17 (d, *J* = 13.5 Hz, 1H), 2.10 (ddd, *J* = 14.0, 8.0, 5.5 Hz, 1H), 2.01 (t, *J* = 12 Hz, 2H), 1.93 (t, *J* = 12 Hz, 1H), 1.84–1.74 (m, 2H), 1.72–1.62 (m, 4H), 1.07 (s, 9H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.9, 169.1, 159.3, 144.8, 143.5, 142.3, 137.7, 137.4, 135.8, 134.0, 131.1, 129.8, 129.5, 128.8, 128.6, 128.1, 128.0, 127.9, 127.9, 127.4, 114.0, 110.6, 108.8, 95.0, 87.2, 83.4, 77.0, 75.7, 75.0, 74.6, 72.8, 72.1, 70.2, 69.8, 66.0, 63.9, 60.6, 55.5, 42.6, 42.5, 41.4, 41.1, 39.9, 38.0, 37.7, 34.0, 27.2, 27.2, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.2; CH_2 δ 110.6, 108.8, 95.0, 72.1, 70.2, 69.8, 66.0, 63.9, 60.6, 42.6, 42.5, 41.4, 41.1, 39.9, 38.0, 37.7, 34.0, 27.2; CH δ 137.4, 135.8, 129.8, 129.5, 128.8, 128.6, 128.1, 128.0, 127.9, 127.4, 114.0, 77.0, 75.7, 75.0, 74.6, 72.8; C δ 170.9, 169.1, 159.3, 144.8, 143.5, 142.3, 137.7, 134.0, 131.1, 127.9, 87.2, 83.4, 19.4; IR (neat) 2936, 1758, 1611, 1513, 1449, 1428, 1362, 1248, 1174, 1110, 1046, 898, 823, 745, 703, 632 cm^{-1}; HRMS (ESI) calcd 1267.5943 for $C_{78}H_{88}O_{12}NaSi$ (M+Na), found 1267.5942.



Preparation of (*Z*)-3-((*R*)-5-(((benzyloxy)methoxy)methyl)-2-oxo-5-((trityloxy)methyl)dihydrofuran-3(2H)-ylidene)propyl (2*R*,6*S*)-6-(((2*R*,6*S*)-6-((*S*)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-carboxylate (2.12): A solution of ester **2.11** (25 mg, 0.02 mmol, 1.0 equiv) was prepared in THF (200 μL, 0.1 M) in a 2 mL polyethylene vial. To the stirring solution, a 20% HF•pyridine solution in pyridine (500 μL) (25 mL/ mmol) was added. After stirring for 2.5 h, the reaction mixture was quenched with brine (10 mL) and saturated aqueous $NaHCO_3$ solution (5 mL). The mixture was extracted with EtOAc (4 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 1 × 10 cm silica gel column, eluting with 45% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 6–22 were concentrated to yield the product as a clear oil (16.1 mg, 80%). R_f = 0.37 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +2.1° (c = 0.59); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.42–7.40 (m, 6H), 7.35–7.23 (m, 16H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.14 (t, *J* = 7.0 Hz, 1H), 4.77–4.73 (m, 2H), 4.71 (s, 2H), 4.69 (d, *J* = 16 Hz, 2H), 4.52 (s, 2H), 4.47 (ABq, *J* = 10.5 Hz, Δν = 19.5 Hz, 2H), 4.30–4.22 (m, 2H), 3.95 (dd, *J* = 11.5, 2.5 Hz, 1H), 3.91–3.86 (m, 1H), 3.84–3.81 (m, 1H), 3.80 (s, 3H), 3.75–3.71 (m, 1H), 3.68 (q, *J* = 6.0 Hz, 2H), 3.59–3.55 (m, 1H), 3.51–3.44 (m, 2H), 3.32 (d, *J* = 10 Hz, 1H), 3.22–3.15 (m, 1H), 3.16 (d, *J* = 10 Hz, 1H), 3.13–3.08 (m, 1H), 2.80 (dd, *J* = 16.5, 2.0 Hz, 1H), 2.72

(d, J = 16 Hz, 1H), 2.51 (d, J = 13 Hz, 1H), 2.30–2.23 (m, 3H), 2.19 (d, J = 12.5 Hz, 1H), 2.10–1.90 (m, 5H), 1.81–1.64 (m, 5H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.9, 169.1, 159.5, 144.4, 143.6, 142.4, 137.7, 137.4, 130.6, 129.7, 128.8, 128.7, 128.7, 128.1, 128.0, 128.0, 127.4, 114.2, 110.6, 109.1, 95.0, 87.2, 83.4, 77.0, 75.8, 75.5, 75.4, 74.9, 72.0, 70.2, 69.8, 66.0, 63.9, 60.5, 55.5, 42.5, 41.7, 41.4, 41.0, 40.0, 37.7, 36.8, 34.1, 27.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5; CH_2 δ 110.6, 109.1, 95.0, 72.0, 70.2, 69.8, 66.0, 63.9, 60.5, 42.5, 41.7, 41.4, 41.0, 40.0, 37.7, 36.8, 34.1, 27.2; CH δ 137.4, 129.7, 128.8, 128.7, 128.1, 128.0, 127.4, 114.2, 77.0, 75.8, 75.5, 75.4, 74.9; C δ 170.9, 169.1, 159.5, 144.4, 143.6, 142.4, 137.7, 130.6, 128.7, 128.0, 87.2, 83.4; IR (neat) 3503, 2936, 2884, 1756, 1684, 1652, 1616, 1516, 1490, 1448, 1362, 1249, 1175, 1078, 1045, 898, 748, 704, 633 cm^{-1}; HRMS (ESI) calcd 1029.4765 for $C_{62}H_{70}O_{12}Na$ (M+Na), found 1029.4774.

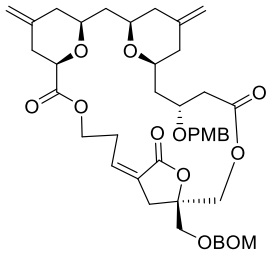


Preparation of (*R*)-4-((2*S*,6*R*)-6-(((2*S*,6*R*)-6-(((*Z*)-3-((*R*)-5-(((benzyloxy)methoxy)methyl)-2-oxo-5-((trityloxy)methyl)dihydrofuran-3(2H)-ylidene)propoxy)carbonyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3-((4-methoxybenzyl)oxy)butanoic acid (2.13): To a stirring solution of alcohol **2.12** (25 mg, 0.03 mmol, 1.0 equiv) in CH_2Cl_2 (250 μL, 0.1 M) contained in a 5 mL vial under N_2 was added *i*-Pr_2NEt (30 μL, 0.18 mmol, 7.0 equiv), followed by DMSO (20 μL, 0.3 mmol, 10 equiv). After cooling to 0 ^{o}C, SO_3•Pyr (16 mg, 0.10 mmol, 4.0 equiv) was added. The reaction mixture was stirred for 2 h at 0 ^{o}C and quenched with saturated aqueous $NaHCO_3$ solution (5 mL). After dilution with

brine (5 mL), the mixture was extracted with EtOAc (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. After evaporation with $PhCH_3$ (3 × 2 mL), the crude aldehyde was used without purification for the subsequent Pinnick oxidation.

A solution of the previously described aldehdye (0.025 mmol, 1.0 equiv) in *t*-BuOH (360 μL, 14.3 mL/ mmol) and 2-methyl-2-butene (360 μL, 14.3 mL/ mmol) was prepared in a 5 mL vial. After the addition of a solution of aqueous KH_2PO_4 (1.25 M, 120 μL, 0.15 mmol, 6.0 equiv), the reaction mixture was cooled to −10 °C in an ice/ MeOH bath. $NaClO_2$ 80% (14.1 mg, 0.13 mmol, 5.0 equiv) was added in 1 portion to the rapidly stirred mixture. The cooling bath was allowed to expire overnight. The reaction mixture was quenched with aqueous pH 4.0 acetate buffer (0.1 M, 5 mL) and brine (5 mL). After extraction with EtOAc (3 × 5 mL), the organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude carboxylic acid was purified on a 1 × 10 cm silica gel column, eluting with 80/20/1 $PhCH_3$/ EtOAc/ AcOH, collecting 5 mL fractions. Fractions 4–12 were concentrated and evaporated with $PhCH_3$ (3 × 5 mL) to remove residual AcOH, yielding the product as a colorless oil (22 mg, 86% over 2 steps). R_f = 0.35 (80/20/1 $PhCH_3$/ EtOAc/ AcOH); $[\alpha]_D^{20}$ = + 6.33° (c = 1.08); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.42–7.41 (m, 6H), 7.33–7.29 (m, 10H), 7.27–7.23 (m, 6H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.14 (t, *J* = 7.0 Hz, 1H), 4.78 (s, 1H), 4.74–4.69 (m, 5H), 4.55 (d, *J* = 11 Hz, 1H), 4.52 (s, 2H), 4.45 (d, *J* = 10.5 Hz, 1H), 4.30–4.23 (m, 2H), 4.10 (dddd, *J* = 11.5, 7.5, 5.5, 5.5 Hz, 1H), 3.98 (dd, *J* = 11.5, 2.5 Hz, 1H), 3.79 (s, 3H), 3.69 (q, *J* = 11 Hz, 2H), 3.54–3.42 (m, 3H), 3.32 (d, *J* = 10 Hz, 1H), 3.24–3.19 (m, 1H), 3.17 (d, *J* = 9.5 Hz, 1H), 3.11–3.06 (m, 1H), 2.80 (d, *J* = 16.5 Hz, 1H), 2.72 (d, *J* = 15.5 Hz, 1H), 2.69–2.61 (m, 2H), 2.51 (d, *J* = 8.0 Hz, 1H), 2.27–2.22 (m, 3H), 2.15 (q, *J* = 12.5 Hz, 1H), 2.08 (dd, *J* = 14.5, 7.0 Hz, 1H),

2.03 (t, *J* = 12.5 Hz, 1H), 1.96 (t, *J* = 12 Hz, 2H), 1.82 (ddd, *J* = 15, 8.5, 2.0 Hz, 1H), 1.73–1.62 (m, 3H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 174.5, 171.3, 169.1, 159.5, 144.3, 143.5, 142.3, 137.6, 137.3, 130.3, 129.7, 128.8, 128.7, 128.4, 128.1, 128.1, 128.0, 127.4, 114.1, 110.7, 109.1, 95.0, 87.2, 83.4, 76.8, 75.7, 75.1, 74.9, 73.2, 72.0, 70.2, 69.8, 65.9, 64.0, 55.5, 42.8, 41.9, 41.2, 41.0, 40.2, 39.9, 37.7, 34.1, 27.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5; CH_2 δ 110.7, 109.1, 95.0, 72.0, 70.2, 69.8, 65.9, 64.0, 42.8, 41.9, 41.2, 41.0, 40.2, 39.9, 37.7, 34.1, 27.2; CH δ 137.4, 129.7, 128.8, 128.7, 128.1, 128.0, 127.4, 114.1, 76.8, 75.7, 75.1, 74.9, 73.2 ;C δ 174.5, 171.3, 169.1, 159.5, 144.3, 143.5, 142.3, 137.6, 130.3, 128.4, 128.1, 87.2, 83.4; IR (neat) 3065, 2959, 2894, 1756, 1711, 1653, 1612, 1514, 1449, 1369, 1249, 1176, 1080, 1043, 900, 824, 748, 701 cm^{-1}; HRMS (ESI) calcd 1043.4558 for $C_{26}H_{68}O_{13}Na$ (M+Na), found 1043.4554.

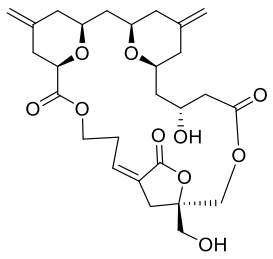


Preparation of (1*R*,3*S*,7*R*,16*R*,21*R*,23*S*,*Z*)-16-(((benzyloxy)methoxy)methyl)-21-((4-methoxybenzyl)oxy)-5,25-dimethylene-9,15,18,27,28-pentaoxatetracyclo[21.3.1.13,7.113,16]nonacos-12-ene-8,14,19-trione (2.14): To a 5 mL vial containing carboxylic acid **2.13** (22 mg, 0.02 mmol, 1.0 equiv) was added 50:50 Et_2O/ HCOOH (2.3 mL, 105 mL/ mmol). The vial was flushed with N_2 and the mixture stirred for 3 h at rt. The solvent was evaporated and the crude product was azeotroped with $PhCH_3$ (3 × 5 mL). The product was purified on a 1 × 10 cm silica gel column, eluting with 50/45/5 hexanes/ EtOAc/ MeOH, collecting 5 mL fractions. Fractions 4–22 were concentrated, yielding the product as a clear oil which was

used without further characterization.

The previously prepared *seco*-acid was dried overnight under high vacuum in a 5 mL vial. The *seco*-acid was taken up in THF (820 μL, 0.03 M) and Et_3N (20 μL, 0.13 mmol, 6.0 equiv) was added to the stirred solution under N_2. After cooling to 0 ^oC, a solution of 2,4,6-trichlorobenzoyl chloride in THF (0.1 M, 660 μL, 0.066 mmol, 3.0 equiv) was added dropwise. After stirring for 5 min at 0 ^oC, the mixture was then stirred at rt for 3 h. The resulting solution was taken up in $PhCH_3$ (14 mL) in a 25 mL gas-tight syringe. This solution was then added over 16 h via syringe pump at 0.875 mL/ h to a stirring solution of DMAP (54 mg, 0.44 mmol, 20 equiv) in $PhCH_3$ (14 mL) contained in a 50 mL flask under N_2 that was heated at 40 ^oC. This produced a final reactant concentration of 0.0008 M. After the addition was complete, $PhCH_3$ (1.0 mL) was taken up in the syringe and added over 1 h to complete the transfer. After stirring for 3 h at 40 ^oC, the reaction mixture was quenched with brine (50 mL), extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified on a 1 × 10 cm silica gel column, eluting with 50% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 2–7 were concentrated to yield the product as a colorless oil (9.2 mg, 55% over 2 steps). R_f =0.52 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +16.1^o (c = 0.460); 500 MHz ^1H NMR ($CDCl_3$) δ 7.35–7.28 (m, 5H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 6.17 (t, *J* = 8.0 Hz, 1H), 4.95 (d, *J* = 6.0 Hz, 1H), 4.78 (d, *J* = 8.5 Hz, 1H), 4.78 (s, 1H), 4.72–4.71 (m, 2H), 4.66 (d, *J* = 11.0 Hz, 1H), 4.64 (s, 1H), 4.58 (d, *J* = 16.5 Hz, 2H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 2H), 4.39 (d, *J* = 11 Hz, 1H), 4.19–4.14 (m, 1H), 4.01 (d, *J* = 11.5 Hz, 1H), 4.00–3.95 (m, 1H), 3.88 (ddd, *J* = 11.5, 11.5, 2.0 Hz, 2H), 3.81 (s, 3H), 3.67 (d, *J* = 10 Hz, 1H), 3.63–3.57 (m, 1H), 3.55–3.49 (m, 1H), 3.38–

3.34 (m, 2H), 2.89 (d, *J* = 15.5 Hz, 1H), 2.81 (dd, *J* = 15.5, 4.0 Hz, 1H), 2.59 (d, *J* = 13 Hz, 1H), 2.55 (d, *J* = 16 Hz, 1H), 2.45 (dd, *J* = 14.5, 9.5 Hz, 1H), 2.34 (d, *J* = 13.5 Hz, 1H), 2.27 (t, *J* =12 Hz, 1H), 2.21–2.18 (m, 1H), 2.15 (t, *J* = 12 Hz, 2H), 1.96 (t, *J* = 12.5 Hz, 2H), 1.88 (t, *J* = 12.5 Hz, 2H), 1.70 (dd, *J* = 8.0, 4.0 Hz, 2H), 1.63–1.60 (m, 1H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.4, 168.8, 159.5, 144.4, 142.4, 138.5, 137.8, 130.4, 129.4, 128.7, 128.1, 128.0, 128.0, 127.9, 114.2, 110.6, 109.1, 94.6, 81.5, 77.6, 76.2, 74.9, 74.7, 73.0, 72.4, 69.9, 66.6, 65.3, 62.6, 55.5, 43.1, 42.4, 41.4, 41.2, 40.8, 40.4, 37.0, 34.7, 26.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5; CH_2 δ 110.6, 109.1, 94.6, 72.4, 69.9, 66.6, 65.3, 62.3, 62.5, 43.1, 42.4, 41.4, 41.2, 40.8, 40.4, 37.0, 34.7, 26.5; CH δ 138.5, 129.4, 128.7, 128.0, 114.1, 77.6, 76.2, 74.9, 74.7, 73.0; C δ 170.4, 168.8, 159.5, 144.4, 142.4, 137.8, 130.4, 128.1, 128.0, 127.9, 81.5. IR (neat) 2940, 2886, 1764, 1742, 1612, 1514, 1380, 1249, 1176, 1120, 1047, 892, 821, 740 cm^{-1}: HRMS (ESI) calcd 783.3356 for $C_{43}H_{52}NaO_{15}$ (M+Na), found 783.3367.



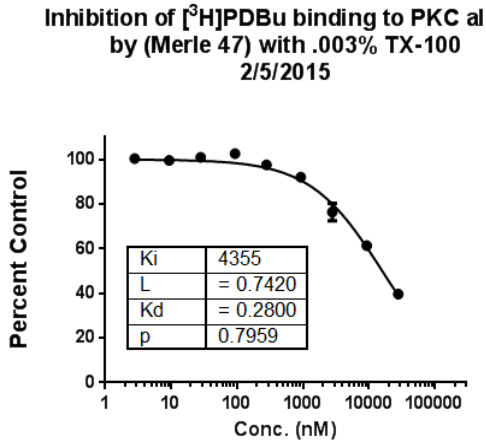
Preparation of Merle 47 (1*R*,3*S*,7*R*,16*S*,21*R*,23*S*,*Z*)-21-hydroxy-16-(hydroxymethyl)-5,25-dimethylene-9,15,18,27,28-pentaoxatetracyclo [21.3.1.13,7.113,16]nonacos-12-ene-8,14,19-trione: To a stirring solution of macrolactone **2.14** (5.3 mg, 0.007 mmol, 1.0 equiv) in CH_2Cl_2 (700 μL, 0.01 M) contained in a 5 mL vial was added H_2O (20 μL). After cooling to 0 ^oC, DDQ (16.1 mg, 0.07 mmol, 10 equiv) was added. After stirring at 0 ^oC for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (25 mL) and extracted with CH_2Cl_2

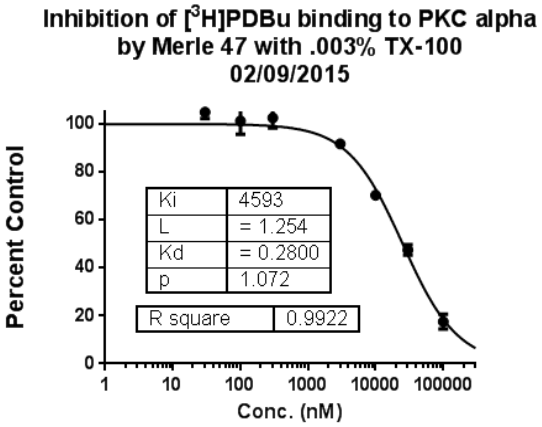
(3 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. After drying under high vacuum for 1 hour, the crude product was used as is for the subsequent deprotection step.

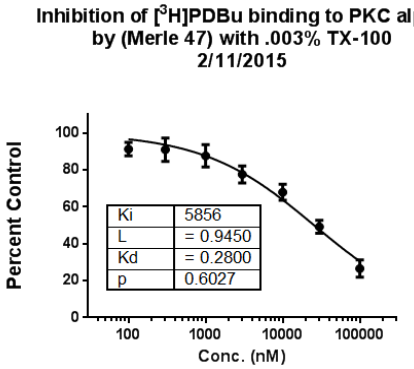
To the aforementioned product contained in a 5 mL vial, a solution of $LiBF_4$ in 25:1 MeCN/ H_2O (0.25 M, 1.26 mL, 45 equiv) was added. The vial was flushed with N_2, capped, and stirred at 80 ^oC overnight. After cooling to rt, the reaction mixture was quenched with brine (10 mL), and extracted with EtOAc (3 × 10 mL). After drying over Na_2SO_4, the solution was filtered and concentrated to dryness. The crude product was purified on a 1 × 5 cm silica gel column, eluting with 50% EtOAc/ hexanes, collecting 1 mL fractions. Fractions 9–50 contained the desired product. The product was purified a second time by the same procedure to yield the desired macrolactone as an oil (1.3 mg, 36% 2-steps). R_f = 0.08 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = –6.2^o (c = 0.065); 500 MHz ^1H NMR ($CDCl_3$) δ 6.28 (t, *J* = 7.0, Hz, 1H), 4.61 (dd, *J* = 10, 1.5 Hz, 2H), 4.57 (s, 2H), 4.50 (d, *J* = 12 Hz, 1H), 4.45–4.41 (m, 1H), 4.36–4.30 (m, 1H), 4.24 (ddd, *J* = 10.5, 10.5, 2.5 Hz, 1H), 4.07 (d, *J* = 12 Hz, 1H), 3.87 (dd, *J* = 11.5, 2.5 Hz, 1H), 3.72–3.70 (m, 2H), 3.63 (t, *J* = 10 Hz, 1H), 3.53–3.48 (m, 1H), 3.43 (t, *J* = 11.5 Hz, 2H), 2.92 (d, *J* = 16 Hz, 1H), 2.87 (t, *J* = 5.0 Hz, 1H), 2.77 (dd, *J* = 13.5, 4.5 Hz, 2H), 2.69 (d, *J* = 16 Hz, 1H), 2.53 (d, *J* = 13 Hz, 1H), 2.48 (dd, *J* = 14, 9.5 Hz, 1H), 2.42 (d, *J* = 6.0 Hz, 1H), 2.37–2.29 (m, 2H), 2.18 (t, *J* = 9.0 Hz, 2H), 2.07–1.97 (m, 3H), 1.78–1.73 (m, 1H), 1.70–1.62 (m, 3H). 125 MHz ^{13}C NMR ($CDCl_3$) δ; 170.4, 168.8, 143.6, 141.9, 139.2, 127.7, 111.0, 109.6, 83.0, 77.5, 77.0, 76.3, 76.1, 75.5, 65.8, 63.4, 63.2, 43.2, 43.0, 41.9, 41.3, 41.2, 40.6, 37.0, 34.0, 26.9; 125 MHz DEPT ($CDCl_3$) CH_3 δ; CH_2 δ 111.0, 109.6, 77.5, 75.4, 65.8, 63.4, 63.2, 43.2, 41.9, 41.4, 41.2, 40.6, 37.0, 26.9; CH δ 139.2, 77.0, 76.3, 76.1,

75.4, 65.8; C δ 170.4, 168.8, 143.6, 141.9, 127.7, 83.0. IR (neat) 3430, 2943, 1754, 1742, 1581, 1382, 1257, 1194, 1081, 1030, 896, 753 cm^{-1}; HRMS (ESI) calcd for $C_{27}H_{36}NaO_{10}$ (M+Na) 543.2206, found 543.2207.

Binding Assay for Merle 47 Towards PKC α

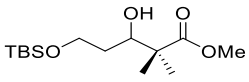




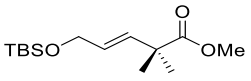


Compound	date	Kd (nM)	Ki (nM)	p	
M47	2/5/2015	.28 + .02	4355	0.8	
	2/9/2015	.28 + .02	4593	1.1	
	2/11/2015	.28 + .02	5856	0.6	
		Mean	4935	466	SEM

Experimental Procedures for Merle 48

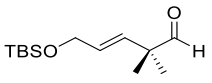


Preparation of methyl 5-((tert-butyldimethylsilyl)oxy)-3-hydroxy-2,2-dimethylpentanoate (2.18): To a solution of *i*-Pr_2NH (3.0 mL, 21.5 mmol, 1.1 equiv) in THF (15 mL) contained in a 100 mL flask under N_2 at −78 °C was added a solution of n-BuLi (2.5 M, 8.6 mL, 21.5 mmol, 1.1 equiv). The solution was stirred at rt for 30 min. After cooling the solution to −78 °C, a solution of methyl isobutyrate (2.2 mL, 19.5 mmol, 1.0 equiv) in THF (5 mL) was added via cannula. The solution was stirred for 1.5 h at −78 °C, after which a solution of aldehyde **1.79** (4.4 g, 23.4 mmol, 1.2 equiv) in THF (5 mL) was added via cannula. The reaction mixture was stirred at −78 °C for 2 h. The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The mixture was then extracted with 50% EtOAc/ hexanes (3 × 15 mL). The organic phase was washed with brine (2 × 25 mL) and dried over Na_2SO_4. After filtration, the solvent was evaporated. The crude product was purified on a 4 × 10 cm silica column, eluting with 10% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 22−58 were combined and concentrated to yield the product (5.9 g, 94%) as a colorless oil: R_f = 0.54 (25 % EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 3.94 (ddd, *J* = 8.0, 6.0, 3.0 Hz, 1H), 3.89 (dd, *J* = 10, 5.0 Hz, 1H), 3.85−3.81 (m, 1H), 3.70 (s, 3H), 3.43 (d, J = 3.0 Hz, 1H), 1.59 (t, *J* = 4.5 Hz, 2H), 1.20 (s, 3H), 1.17 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 178.0, 76.3, 62.5, 52.1, 47.3, 33.9, 26.1, 21.5, 20.8, 18.4, −5.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 52.1, 26.1, 21.5, 20.7, −5.3; CH_2 δ 62.9, 33.8; CH δ 76.3; C δ 178.0, 47.3, 18.4; IR (neat) 3508, 2954, 2857, 1732, 1471, 1388, 1256, 1090 cm^{-1}; HRMS (ESI) calcd 313.1811 for $C_{14}H_{30}O_4NaSi$ (M+Na), found 313.1809.



Preparation of methyl (*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enoate (1.47): To a stirring solution of ester **2.18** (5.9 g, 20 mmol, 1.0 equiv) in toluene (81 mL, 0.25 M) contained in a 250 mL flask under N_2 was added Et_3N (5.6 mL, 40 mmol, 2.0 equiv); MsCl (1.9 mL, 24 mmol, 1.2 equiv) was then slowly added. A white precipitate immediately formed; the reaction mixture was then stirred overnight at rt. The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ solution (150 mL). The mixture was then extracted with 50% EtOAc/ hexanes (3 × 25 mL). The organic phase was then washed with brine (100 mL) and dried over Na_2SO_4. After filtration, the solution was concentrated and the crude mesylate used as is.

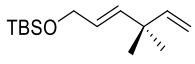
To a stirring solution of crude mesylate in DMSO (200 mL, 0.1 M) contained in a 500 mL flask fitted with a reflux condenser under N_2 was added DBU (7.5 mL, 50 mmol, 2.5 equiv). The reaction mixture was heated at 115 ^{o}C overnight. After cooling to rt, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (250 mL) and H_2O (250 mL). The mixture was extracted with 50% EtOAc/ hexanes (3 × 100 mL). The organic phase was washed with brine (200 mL) and dried over Na_2SO_4. After filtration and concentration, the product was purified on a 4 × 11 cm silica gel column, eluted with 5% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 28–44 were combined and concentrated to yield the product (3.5 g, 64%) as a colorless oil: R_f = 0.76 (25 % EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) 5.84 (dt, *J* = 15.5, 2.0 Hz, 1H), 5.59 (dt, *J* = 15.5, 5.0 Hz, 1H), 4.18 (dd, *J* = 5.0, 1.5 Hz, 2H), 3.67 (s, 3H), 1.31 (s, 6H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 177.1, 134.8, 128.0, 64.0, 52.2, 44.1, 26.2, 25.2, 18.6, –4.9.



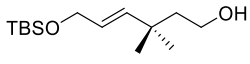
Preparation of (*E*)-5-((tert-butyldimethylsilyl)oxy)-2,2-dimethylpent-3-enal (2.19): To a stirring solution of ester **1.47** (3.24 g, 8.7 mmol, 1.0 equiv) in Et_2O (43 mL, 0.2 M) contained in a 100 mL flask under N_2 at 0 ^oC was added $LiAlH_4$ (362 mg, 9.6 mmol, 1.1 equiv). After stirring for 1 h, the reaction mixture was quenched by the slow addition of EtOAc (5 mL), followed by a saturated aqueous solution of Rochelle salts (50 mL). After stirring for 30 min, the mixture was extracted with 50% EtOAc/ hexanes (3 × 25 mL), dried over Na_2SO_4, filtered, and concentrated. The crude alcohol was used as is for the subsequent oxidation step.

To a stirring solution of the aforementioned alcohol (2.90 g, 8.7 mmol, 1.0 equiv) in CH_2Cl_2 (87 mL, 0.1 M) contained in a 250 mL flask under N_2 was added DMSO (6.2 mL, 87 mmol, 10.0 equiv) and *i*-Pr_2NEt (10.6 mL, 61 mmol, 7.0 equiv). After cooling to 0 ^oC, SO_3•Pyr (5.54 g, 35 mmol, 4.0 equiv) was added to the stirring solution. After stirring for 1 h at 0 ^oC, the reaction was quenched with saturated aqueous $NaHCO_3$ solution (100 mL). After stirring at rt for 20 min, the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic phase was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The crude aldehyde was purified on a 4 × 10 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 10 mL fractions. Product bearing fractions 16–36 were combined and concentrated to yield the aldehyde (2.78 g, 97%, over 2 steps) as a colorless oil: R_f = (25 % EtOAc/hexanes); 500 MHz ^1H NMR ($CDCl_3$) δ 9.38 (s, 1H), 5.64 (s, 2H), 4.20 (t, *J* = 1.0 Hz, 2H), 1.20 (s, 6H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 202.8, 131.4, 131.3, 63.8, 59.9, 48.4, 26.2, 21.6, 18.6, −5.0; 125 MHz DEPT ($CDCl_3$) CH_3 δ 26.2, 21.7, −4.9; CH_2 δ 63.9; CH δ 202.8,

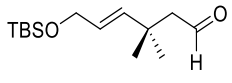
131.5, 131.3; C δ 48.6, 18.7; IR (neat) 2930, 2858, 1730, 1472, 1362, 1255, 1107, 837 cm^{-1}: HRMS (ESI) calcd 265.1600 for $C_{13}H_{26}O_2SiNa$ (M+Na), found 265.1608.



Preparation of (*E*)-tert-butyl((4,4-dimethylhexa-2,5-dien-1-yl)oxy)dimethylsilane (2.22): To a 250 mL flask was added KO*t*-Bu (1.72 g, 15.3 mmol, 1.8 equiv), followed by methyl triphenylphosphonium bromide (6.1 g, 17 mmol, 2.0 equiv). After flushing with N_2, the mixture was cooled to 0 ^oC and THF (75 mL) was added. The yellow mixture was stirred at 0 ^oC for 30 min. A solution of aldehyde **2.19** (2.06 g, 8.5 mmol, 1.0 equiv) in THF (5 mL) was then added via cannula, using THF (5.0 mL) to complete the transfer. After stirring at rt for 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl solution (100 mL). The mixture was extracted with 50% EtOAc/ hexanes (3 × 25 mL). The organic phase was washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The crude olefin was purified on a 2 × 10 cm silica gel column, eluting with 10 % EtOAc/ hexanes, collecting 5 mL fractions. Fractions 2–14 were combined and concentrated to yield the desired product (1.92 g, 94 %). R_f = 0.88 (10 % EtOAc/ hexanes); 500 MHz ^1H NMR ($CDCl_3$) δ 5.83 (dd, *J* = 17, 10 Hz, 1H), 5.64 (dt, *J* = 15.5, 1.5 Hz, 1H), 5.48 (dt, *J* = 10.5, 5.5 Hz, 1H), 4.94 (ddd, *J* = 17.5, 10.5, 1.0 Hz, 2H), 4.17 (dd, *J* = 5.5, 1.5 Hz, 2H), 1.12 (s, 6H), 0.92 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 147.3, 139.3, 126.1, 110.8, 64.4, 59.9, 27.1, 26.2, 18.7, −4.8; 125 MHz DEPT ($CDCl_3$) CH_3 δ 27.1, 26.2, −4.8; CH_2 δ 110.7, 64.4; CH δ 147.3, 139.3, 126.1; C δ 59.9, 18.7; IR (neat) 3084, 2958, 2929, 2858, 1637, 1472, 1386, 1361, 1255, 1106, 1068, 973, 914, 837, 775, 665 cm^{-1}; HRMS (ESI) calcd 263.1807 for $C_{14}H_{28}ONaSi$ (M+Na), found 263.1805.

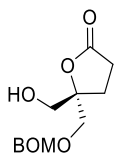


Preparation of (E)-6-((tert-butyldimethylsilyl)oxy)-3,3-dimethylhex-4-en-1-ol (2.23): To a stirring solution of olefin **2.22** (1.60 g, 6.65 mmol, 1.0 equiv) in THF (27 mL, 0.25 M) contained in a 100 mL flask was added a solution of 9-BBN in THF (0.5 M, 20 mL, 10.0 mmol, 1.5 equiv). The reaction mixture was then placed in an ultrasonic bath for 1 h. The reaction mixture was quenched with H_2O (50 mL), followed by an aqueous solution of NaOH (1 M, 50 mL) and chilled in an ice bath; 30% aqueous H_2O_2 (20 mL) was then added in small portions. The mixture was stirred for 1 h at 0 ^oC. The mixture was diluted with brine (50 mL), followed by extraction with 50% EtOAc/ hexanes (3 × 25 mL). The organic phase was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 4 × 15 cm silica gel column, eluting with 5% EtOAc/ hexanes until fraction 96 was reached; after this the product was eluted with 25% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 110−138 were combined and concentrated to yield the product (1.46 g, 85%) as a colorless oil: R_f = 0.10 (10 % EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.66 (dt, *J* = 16, 1.5 Hz, 1H), 5.48 (dt, *J* = 16, 5.5 Hz, 1H), 4.15 (dd, *J* = 5.0, 1.5 Hz, 2H), 3.65 (t, *J* = 7.0 Hz, 2H), 1.62 (t, *J* = 7.5 Hz, 2H), 1.33 (s, 1H), 1.04 (s, 6H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 140.6, 126.0, 64.3, 60.4, 45.7, 35.0, 27.7, 26.2, 18.6, −4.9; 125 MHz DEPT ($CDCl_3$) CH_3 δ 27.7, 26.2, −4.9; CH_2 δ 64.3, 60.4, 45.6; CH δ 140.6, 125.9; C δ 35.0, 18.6; IR (neat) 3356, 2956, 2857, 1472, 1387, 1362, 1255, 1107, 1060, 1024, 975, 836, 776 cm^{-1}; HRMS (ESI) calcd 281.1913 for $C_{14}H_{30}O_2NaSi$ (M+Na), found 281.1915.



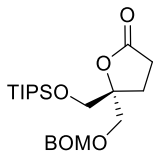
Preparation of (*E*)-6-((tert-butyldimethylsilyl)oxy)-3,3-

dimethylhex-4-enal (2.16): To a 50 mL flask, alcohol **2.23** (500 mg, 1.93 mmol, 1.0 equiv) was added. After flushing with N_2, CH_2Cl_2 (19 mL, 0.1 M) was added. The solution was chilled to 0 ^oC, and *i*-Pr_2NEt (2.4 mL, 14 mmol, 7.0 equiv) was added, followed by DMSO (1.4 mL, 19 mmol, 10.0 equiv). To the solution, SO_3•Pyr (1.23 g, 7.7 mmol, 4.0 equiv) was added. The reaction mixture was then stirred at 0 ^oC for 1 h. The reaction mixture was then quenched with a solution of saturated aqueous $NaHCO_3$ (50 mL). After stirring for 15 min, the mixture was extracted with CH_2Cl_2 (2 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified on a 2 × 15 cm silica gel column, eluting with 5% EtOAc/ hexanes, collecting 5 mL fractions. Product containing fractions 10−15 were combined and concentrated to yield the product (377 mg, 76%) as a colorless oil: R_f = 0.40 (10% EtOAc/ hexanes); 500 MHz ^1H NMR ($CDCl_3$) δ 9.73 (t, *J* = 1.8 Hz, 1H), 5.75 (dt, *J* = 15.5, 1.5 Hz, 1H), 5.53 (dt, *J* = 15.5, 4.5 Hz, 1H), 4.17 (dd, *J* = 5.0, 1.5 Hz, 2H), 2.34 (d, *J* = 3.0 Hz, 2H), 1.16 (s, 6H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 203.5, 138.4, 127.0, 64.0, 55.3, 35.3, 27.9, 26.2, 18.6, −4.9; 125 MHz DEPT ($CDCl_3$) CH_3 δ 27.9, 26.2, −4.9; CH_2 δ 64.0, 55.2; CH δ 203.5, 138.3, 127.0; C δ 35.3, 18.6; IR (neat) 2958, 2930, 2857, 2731, 1724, 1472, 1388, 1375, 1255, 1108, 1068, 976, 837, 776, 666 cm^{-1}; HRMS (ESI) calcd 279.1756 for $C_{14}H_{28}O_2NaSi$ (M+Na), found 279.1760.



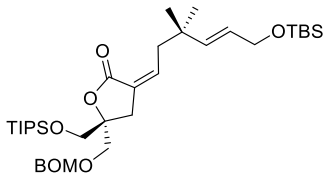
Preparation of (S)-5-(((benzyloxy)methoxy)methyl)-5-(hydroxymethyl)dihydrofuran-2(3H)-one (2.14): A stirring solution of lactone **1.89** (750 mg, 1.48 mmol, 1.0 equiv) in CH_2Cl_2 (30 mL, 0.05 M) contained in a 50 mL flask at

0 °C was prepared under N_2. To this solution, a 50:50 TFA/ TFAA solution in CH_2Cl_2 (1.0 M, 4.44 mL, 4.44 mmol, 3.0 equiv) was added dropwise, turning the solution bright yellow in color. After stirring for 30 min at 0 °C, Et_3N (4.1 mL, 30 mmol, 20 equiv) was added and the mixture was stirred for 5 min. The solution was then transferred to a 250 mL flask and evaporated with MeOH (3 × 75 mL). The resulting oil was dissolved in $PhCH_3$ (2.0 mL) and purified on a 3 × 15 cm silica gel column. The column was eluted with 25% EtOAc/ hexanes (300 mL), 50% EtOAc/ hexanes (200 mL), and 75% EtOAc/ hexanes (100mL), collecting 10 mL fractions. Fractions 46–64 were combined and concentrated to yield the product (342 mg, 87%) as a light yellow oil: R_f = 0.29 (50% EtOAc/ hexanes); $[\alpha]_D^{20} = +2.18^\circ$ (c = 6.0); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36–7.34 (m, 4H), 7.32–7.30 (m, 1H), 4.77 (dd, *J* = 9.5, 6.5 Hz, 1H), 4.60 (s, 2H), 3.77 (d, *J* = 12 Hz, 1H), 3.69 (ABq, *J* = 10.5 Hz, Δν = 36.3 Hz, 2H), 3.62 (d, *J* = 14.0 Hz, 1H), 2.71 (s, 1H), 2.64 (ddd, *J* = 14, 10, 8.0 Hz, 2H), 2.22–2.16 (m, 1H), 2.13–2.07 (m, 1H). 125 MHz ^{13}C NMR ($CDCl_3$) δ 177.6, 137.6, 128.7, 128.1, 128.0, 95.1, 87.6, 70.2, 70.0, 65.4, 29.4, 25.7; 125 MHz DEPT ($CDCl_3$) CH_3 δ ; CH_2 δ 95.1, 70.2, 70.0, 65.4, 29.4, 25.7; CH δ 128.7, 128.1, 128.0; C δ 177.6, 137.6, 87.6; IR (neat) 3454, 2940, 1765, 1605, 1461, 1414, 1382, 1208, 1170, 1110, 1043, 946, 736, 699 cm^{-1}; HRMS (ESI) calcd 289.1052 for $C_{14}H_{18}O_5Na$ (M+Na), found 289.1052.



Preparation of (R)-5-(((benzyloxy)methoxy)methyl)-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-2(3H)-one (2.17): To a stirring solution of alcohol **2.14** (933 mg, 3.5 mmol, 1.0 equiv) in CH_2Cl_2 (35 mL, 0.1 M) contained in a

100 mL flask under N_2 was added 2,6-lutidine (1.2 mL, 11 mmol, 3.0 equiv), followed by TIPSOTf (1.4 mL, 5.3 mmol, 1.5 equiv). The reaction mixture was stirred overnight at rt, quenched with saturated aqueous $NaHCO_3$ solution (50 mL), and extracted with CH_2Cl_2 (3 × 15 mL). The organic phase was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 3 × 10 cm silica gel column, eluting with 15% EtOAc/ hexanes, collecting 10 mL fractions. Fractions 14–30 were combined and concentrated to yield the product (1.28 g, 86%) as a colorless oil: R_f = 0.48 (25% EtOAc/ hexanes); $[\alpha]_D^{20} = -1.03^{\circ}$ (c = 1.95); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38–7.29 (m, 5H), 4.78 (dd, *J* = 10, 6.5 Hz, 2H), 4.61 (s, 2H), 3.81 (ABq, *J* = 10.5 Hz, Δv = 28 Hz, 2H), 3.71 (dd, *J* = 17, 10.5 Hz, 2H), 2.68–2.57 (m, 2H), 2.25 (ddd, *J* = 13.5, 10.5, 7.0 Hz, 1H), 2.14–2.05 (m, 1H), 1.16–1.09 (m, 3H), 1.07 (d, *J* = 7.0 Hz, 18H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 177.2, 137.8, 128.7, 128.1, 128.0, 95.2, 87.4, 70.6, 69.9, 66.7, 29.5, 26.1, 18.1, 12.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 18.1; CH_2 δ 95.2, 70.6, 69.9, 66.7, 29.5, 26.1; CH δ 128.7, 128.1, 128.0, 12.1; C δ 177.2, 137.8, 87.4; IR (neat) 2943, 2866, 1781, 1463, 1382, 1207, 1171, 1116, 1049, 949, 883, 811, 738, 696 cm^{-1}; HRMS (ESI) calcd 445.2386 for $C_{23}H_{38}O_5SiNa$ (M+Na), found 445.2394.

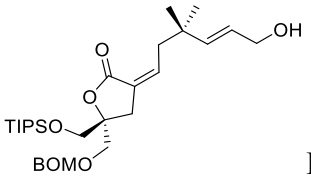


Preparation of (R,Z)-5-(((benzyloxy)methoxy)methyl)-3-((E)-6-((tert-butyldimethylsilyl)oxy)-3,3-dimethylhex-4-en-1-ylidene)-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-2(3H)-one (2.24): To a solution of lactone **2.17** (150 mg, 0.36 mmol, 1.0 equiv) in THF (3.6 mL, 0.1 M) in a 10 mL flask at −78 °C was added a solution of LDA (1.0 M, 400 μL, 0.4

mmol, 1.1 equiv). After stirring at −78 °C for 0.5 h, a solution 1M in HMPA and 1M in $(EtO)_2POCl$ in THF (470 μL, 0.47 mmol, 1.3 equiv) was added to the reaction mixture. After stirring for 5 min, the reaction mixture was then warmed to rt and stirred for 1 h. After cooling the reaction mixture to −78 °C, a solution of LDA (1.0 M, 790 μL, 0.79 mmol, 2.2 equiv) was added. The reaction mixture was stirred at −78 °C for 1.5 h. The reaction was then quenched at −78 °C with a solution of AcOH (1.0 mL) in Et_2O (5.0 mL). The mixture was diluted with Et_2O (50 mL) and filtered through a 4 × 1 cm pad of Celite™. The solution was then concentrated to yield the phosphonate as a mixture of diastereomers which were used without further characterization.

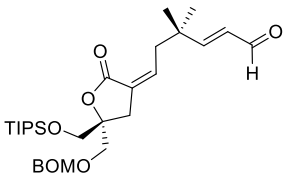
To the aforementioned phosphonate (65 mg, 0.12 mmol, 1.0 equiv) contained in a 15 mL flask, 18-crown-6 (159 mg, 0.60 mmol. 5.0 equiv) was added. The flask was flushed with N_2 and THF (3.0 mL, 0.04 M) was added. After cooling to −78 °C, a solution of KHMDS in toluene (0.5 M, 270 μL, 0.13 mmol, 1.1 equiv) was added dropwise to the stirring mixture. After stirring for 30 min at −78 °C, a solution of aldehyde **2.16** (37 mg, 0.15 mmol, 1.2 equiv) in THF (500 μL) was added dropwise; the transfer was completed with THF (500 μL). The reaction mixture was promptly placed in a 30 °C water bath and stirred overnight at rt. The reaction mixture was quenched with saturated aqueous NH_4Cl solution (10 mL), extracted with EtOAc (3 × 10 mL), washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated. 1H NMR analysis of the crude product indicated a 5:1 ratio of *Z* to *E* enoates by integration of the *B*-enoate proton peaks. The product was purified on a 2 × 10 cm silica gel column, eluting with 5% EtOAc/ hexanes, collecting 5 mL fractions. The product containing fractions where combined and concentrated to yield the product (79 mg, 51% over 2-steps) as a colorless

oil: R_f = 0.29 (10% EtOAc/ hexanes); $[\alpha]_D^{20}$ = −0.69° (c = 1.73, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37−7.29 (m, 5H), 6.15 (dddd, *J* = 9.5, 5.5, 2.0, 2.0 Hz, 1H), 5.63 (ddd, *J* = 15.5, 1.5, 1.5 Hz, 1H), 5.48 (ddd, *J* = 16, 5.5, 5.5 Hz, 1H), 4.78 (dd, *J* = 8.5, 6.5 Hz, 2H), 4.60 (s, 2H), 4.17 (dd, *J* = 5.5, 1.5 Hz, 2H), 3.77 (ABq, *J* = 10.5 Hz, Δv = 23.8 Hz, 2H), 3.69 (d, *J* = 1.5 Hz, 2H), 2.90 (dd, *J* = 17, 2.0 Hz, 1H), 2.81 (q, *J* = 7.5 Hz, 1H), 2.75−2.67 (m, 2H), 1.15−1.04 (m, 27 H), 0.92 (s, 9H), 0.08 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.3, 140.9, 139.8, 137.8, 128.6, 128.1, 128.0, 126.5, 126.4, 95.2, 83.5, 70.0, 69.8, 65.9, 64.4, 39.8, 36.5, 33.5, 27.3, 26.2, 18.6, 18.1, 12.1, −4.8; 125 MHz DEPT ($CDCl_3$) CH_3 δ 27.2, 26.2, 18.1, −4.8; CH_2 δ 95.2, 70.0, 69.8, 65.9, 64.4, 39.9, 33.5; CH δ 140.9, 139.8, 128.6, 128.1, 128.0, 126.5, 12.1; C δ 169.3, 137.8, 126.4, 83.5, 26.5, 18.6; IR (neat) 2957, 2866, 1761, 1671, 1463, 1367, 1254, 1117, 1049, 882, 837, 777, 684 cm^{-1}; HRMS (ESI) calcd 683.4139 for $C_{37}H_{64}O_6NaSi_2$ (M+Na), found 683.4139.



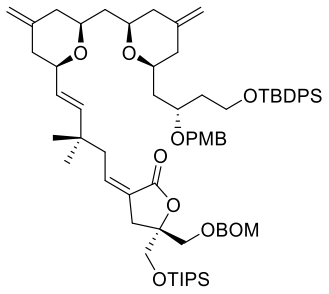
Preparation of (R,Z)-5-(((benzyloxy)methoxy)methyl)-3-((E)-6-hydroxy-3,3-dimethylhex-4-en-1-ylidene)-5-(((triisopropylsilyl)oxy)methyl) dihydrofuran-2(3H)-one (2.25): To a 25 mL flask containing enoate **2.24** (133 mg, 0.20 mmol, 1.0 equiv) was added 3:1:1 AcOH/ THF/ H_2O (10 mL). The flask was stoppered and stirred in an oil bath at 45 °C for 1 h. Upon cooling to rt, the reaction mixture was slowly added to a stirring saturated aqueous $NaHCO_3$ solution (100 mL). The mixture was extracted with EtOAc (3 × 15 mL), washed with saturated aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL). The organic phase was then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified on a 2 × 10 cm silica gel column, eluting

with 25% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 10 –25 were combined and concentrated to yield the product (91 mg, 83%) as a colorless oil: R_f = 0.15 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = –5.4° (c = 0.50, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38–7.29 (m, 5H), 6.14 (dddd, *J* = 10, 7.5, 5.0, 2.5 Hz, 1H), 5.69 (dd, *J* = 15.5, 1.5 Hz, 1H), 5.56 (dt, *J* = 16, 5.5 Hz, 1H), 4.78 (dd, *J* = 8.5, 6.0 Hz, 1H), 4.60 (s, 2H), 4.12 (dd, *J* = 6.0, 1.0 Hz, 2H), 3.77 (ABq, *J* = 10.5 Hz, Δv = 21 Hz, 2H), 3.69 (dd, *J* = 13, 11 Hz, 2H), 2.91 (dd, *J* = 16, 2.0 Hz, 1H), 2.85 (dddd, *J* = 15.5, 8.0, 3.5, 2.0 Hz, 1H), 2.74 (dd, *J* = 16, 2.0 Hz, 1H), 2.67 (dddd, *J* = 15, 9.5, 7.5, 2.0 Hz, 1H), 1.12–1.07 (m, 3H), 1.05 (s, 18 H), 1.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.4, 141.8, 140.5, 137.8, 128.7, 128.1, 128.0, 126.8, 126.1, 95.2, 83.6, 70.0, 69.8, 65.9, 64.1, 39.7, 36.7, 33.5, 27.3, 27.1, 18.1, 12.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 27.3, 27.1, 18.1; CH_2 δ 95.2, 70.0, 69.8, 65.9, 64.1, 39.7, 33.5; CH δ 141.8, 140.5, 128.7, 128.1, 128.0, 126.1, 12.1; C δ 169.4, 137.8, 126.1, 83.6, 36.7; IR (neat) 3464, 2943, 2866, 1757, 1684, 1653, 1464, 1383, 1368, 1210, 1170, 1117, 1046, 973, 882, 801, 734, 682 cm^{-1}; HRMS (ESI) calcd 569.3274 for $C_{31}H_{50}O_6NaSi$ (M+Na), found 569.3273.



Preparation of (2E,6Z)-6-((R)-5-(((benzyloxy)methoxy)methyl)-2-oxo-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-3(2H)-ylidene)-4,4-dimethylhex-2-enal (2.15): To a solution of alcohol **2.25** (91 mg, 0.17 mmol, 1.0 equiv) in CH_2Cl_2 (3.3 mL, 0.05 M) under N_2, contained in a 15 mL flask, was added DMSO (120 μL, 1.7 mmol, 10 equiv), followed by *i*-Pr_2NEt (210 μL, 1.2 mmol, 7.0 equiv). After cooling to 0 °C, SO_3•Pyr (108 mg, 0.68 mmol, 4.0 equiv) was

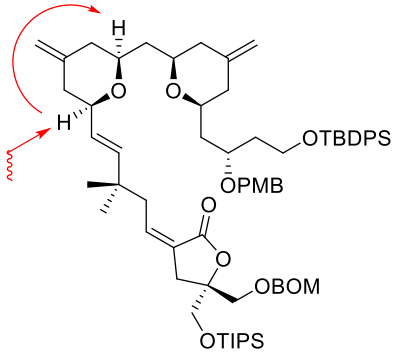
added. The reaction mixture was stirred at 0 °C for 1 h and then quenched with saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 2 × 10 cm silica column, eluting with 15% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 8–20 were combined and concentrated to yield the product (60 mg, 66%) as a colorless oil: R_f = 0.38 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = −59.4° (c = 1.75, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.53 (d, *J* = 8.0 Hz, 1H), 7.37−7.30 (m, 5H), 6.81 (d, *J* = 15.5 Hz, 1H), 6.08−6.03 (m, 2H), 4.77 (s, 2H), 4.59 (s, 2H), 3.77 (dd, *J* = 14, 10.5 Hz, 2H), 3.68 (dd, *J* = 12.5, 10.5 Hz, 2H), 2.98 (dd, *J* = 15, 8.0 Hz, 1H), 2.91 (d, *J* = 16.5 Hz, 1H), 2.81 (dd, *J* = 15, 7.5 Hz, 1H), 2.75 (d, *J* = 16 Hz, 1H), 1.16 (s, 6H), 1.12−1.04 (m, 21H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.5, 169.2, 166.7, 138.0, 137.8, 130.1, 128.7, 128.3, 128.1, 128.0, 95.2, 83.9, 70.0, 69.9, 66.0, 38.9, 38.5, 33.4, 26.4, 26.3, 18.1, 12.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 26.4, 26.3, 18.1; CH_2 δ 95.2, 70.0, 69.9, 66.0, 38.9, 33.4; CH δ 194.5, 166.7, 138.0, 130.1, 128.7, 128.3, 128.1, 12.1; C δ 169.2, 137.8, 128.0, 83.9, 38.2; IR (neat) 2944, 2867, 1757, 1693, 1464, 1369, 1117, 1047, 883, 803, 739 cm^{-1}; HRMS (ESI) calcd 567.3118 for $C_{31}H_{48}O_6NaSi$ (M+Na), found 567.3116.



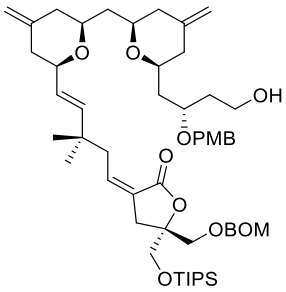
Preparation of (R,Z)-5-(((benzyloxy)methoxy)methyl)-3-((E)-5-((2R,6S)-6-(((2R,6S)-6-((S)-4-((tert-butyldiphenylsilyl)oxy)-2-((4methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-

pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3,3-dimethylpent-4-en-1-ylidene)-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-2(3H)-one (2.26): To a stirring solution of hydroxyallylsilane **1.42** (66 mg, 0.092 mmol, 1.0 equiv) and aldehyde **2.15** (60 mg, 0.11 mmol, 1.2 equiv) in Et_2O (2.3 mL, 0.04 M) contained in a 10 mL flask under N_2 at −78 °C was added a solution of TMSOTf in Et_2O (1.0 M, 120 μL, 0.12 mmol, 1.3 equiv). The reaction mixture was stirred at −78 °C for 3 h, and then quenched with *i*-Pr_2NEt (500 μL). After stirring for 5 min at −78 °C, the solution was added to saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 2 × 10 cm silica column, eluting with 10% EtOAc/ hexanes, collecting 5 mL fractions. Product containing fractions, 8–22 were combined and concentrated to yield the product (90 mg, 83%) as a colorless oil: R_f = 0.83 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = +1.56° (c = 3.4, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71–7.68 (m, 4H), 7.45–7.35 (m, 10H), 7.32–7.29 (m, 1H), 7.20 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.0 Hz, 2H), 6.12 (t, *J* = 7.5 Hz, 1H), 5.63 (d, *J* = 16 Hz, 1H), 5.45 (dd, *J* = 15.5, 5.5 Hz, 1H), 4.78 (s, 2H), 4.73 (d, *J* = 11.5 Hz, 2H), 4.66 (s, 1H), 4.61 (s, 2H), 4.55 (s, 1H), 4.43 (ABq, *J* = 10.5 Hz, Δv = 35.1 Hz, 2H), 3.98–3.93 (m, 1H), 3.85–3.83 (m, 1H), 3.80 (s, 3H), 3.78 (ABq, *J* = 10 Hz, Δv = 20.2 Hz, 2H), 3.76–3.73 (m, 1H), 3.69 (s, 2H), 3.64–3.61 (m, 1H), 3.59–3.55 (m, 1H), 3.51–3.45 (m, 1H), 2.90 (d, *J* = 16 Hz, 1H), 2.81–2.69 (m, 2H), 2.73 (d, *J* = 16.5 Hz, 1H), 2.30 (d, *J* = 23.5 Hz, 1H), 2.27 (d, *J* = 24 Hz, 1H), 2.18 (t, *J* = 15.5 Hz, 2H), 2.07–2.01 (m, 3H), 1.98–1.91 (m, 2H), 1.85–1.73 (m, 2H), 1.70–1.61 (m, 4H), 1.08–1.03 (m, 36H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.3, 159.3, 145.0, 144.3, 140.7, 140.6, 137.8, 135.8, 134.1, 134.0, 131.2, 129.8, 129.6, 128.6, 128.1, 128.0, 128.0, 127.9, 126.8, 114.0, 109.0, 108.7, 95.2,

83.5, 79.2, 75.1, 75.0, 74.8, 72.8, 72.3, 70.0, 69.8, 66.0, 60.6, 55.5, 43.1, 42.6, 41.5, 41.4, 41.2, 40.4, 39.8, 38.0, 36.6, 33.5, 27.2, 27.1, 27.0, 19.4, 18.1, 12.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.2, 18.1; CH_2 δ 109.1, 108.7, 95.2, 72.3, 70.0, 69.8, 66.0, 60.6, 43.1, 42.6, 41.5, 41.4, 41.3, 40.4, 39.8, 38.1, 33.5; CH δ 140.7, 140.6, 135.8, 129.8, 129.6, 128.7, 128.1, 128.0, 128.0, 127.9, 114.0, 79.2, 75.1, 75.0, 74.8, 72.8, 12.1; C δ 169.3, 159.3, 145.0, 144.3, 137.8, 134.1, 134.0, 131.2, 83.5, 36.6, 19.4; IR (neat) 3072, 2941, 1761, 1613, 1514, 1464, 1428, 1362, 1248, 1110, 846, 730, 703 cm^{-1}; HRMS (ESI) calcd 1191.6753 for $C_{71}H_{100}O_{10}NaSi_2$ (M+Na), found 1191.6760.



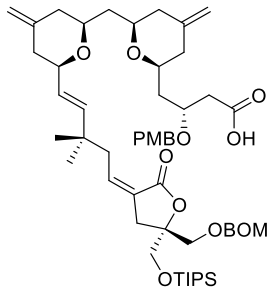
Key 1D nOe Correlations



Preparation of (R,Z)-5-(((benzyloxy)methoxy)methyl)-3-((E)-5-((2R,6S)-6-(((2R,6S)-6-((S)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3,3-dimethylpent-4-en-1-ylidene)-5-(((triisopropylsilyl)oxy)methyl) dihydrofuran-2(3H)-one (2.25): To a stirring solution of ester **2.26** (59 mg, 0.05 mmol,

1.0 equiv) in THF (1.0 mL, 0.05 M) contained in a 10 mL polyethylene vial at 0 °C was added a solution of 20% HF•Pyr in pyridine (1.25 mL, 25 mL/ mmol). The solution was stirred at 0 °C for 5 h, after which it was quenched by slowly pipetting it into stirring saturated aqueous $NaHCO_3$ solution (30 mL). The mixture was extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude alcohol was purified on a 2 × 10 cm silica gel column, eluting with 30% EtOAc/ hexanes, collecting 5 mL fractions. Concentration of fractions 10–36 yielded the product (32 mg, 68%) as a colorless oil: R_f = 0.48 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = –0.88° (c = 0.57, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37–7.28 (m, 5H), 7.26 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.12 (t, *J* = 8.0 Hz, 1H), 5.66 (d, *J* = 16.5 Hz, 1H), 5.45 (dd, *J* = 15.5, 5.5 Hz, 1H), 4.77 (s, 2H), 4.74 (m, 2H), 4.69 (s, 1H), 4.60 (s, 2H), 4.60 (s, 1H), 4.48 (q, *J* = 10 Hz, 2H), 4.02–3.97 (m, 1H), 3.85–3.80 (m, 1H), 3.80 (s, 3H), 3.76–3.73 (m, 1H), 3.77 (ABq, *J* = 10.5 Hz, Δv = 20.9 Hz, 2H), 3.68 (s, 2H), 3.58–3.53 (m, 1H), 3.52–3.43 (m, 2H), 2.90 (d, *J* = 17 Hz, 1H), 2.80 (dd, *J* = 16, 8.0 Hz, 1H), 2.73 (d, *J* = 19 Hz, 1H), 2.69 (dd, *J* = 15.5, 7.5 Hz, 1H), 2.36 (s, 1H), 2.29–2.18 (m, 4H), 2.06–1.92 (m, 6H), 1.80 (dddd, *J* = 14.5, 9.0, 8.5, 2.5 Hz, 1H), 1.75–1.62 (m, 4H), 1.10–1.03 (m, 27H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.3, 159.6, 144.7, 144.4, 140.7, 140.6, 137.9, 130.7, 129.7, 129.6, 128.7, 128.1, 128.0, 126.7, 114.2, 109.0, 108.9, 95.2, 83.6, 79.4, 77.4, 75.5, 75.3, 75.1, 72.1, 70.1, 69.8, 66.0, 60.5, 55.5, 43.0, 41.8, 41.5, 41.1, 40.5, 39.8, 36.9, 36.6, 33.6, 27.1, 27.1, 18.1, 12.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.1, 27.0, 18.1; CH_2 δ 109.0, 108.9, 95.2, 72.1, 70.0, 69.8, 66.0, 60.5, 43.0, 41.8, 41.5, 41.1, 40.5, 39.8, 36.8, 33.5; CH δ 140.7, 140.6, 129.7, 129.6, 128.6, 128.1, 114.1, 79.4, 77.4, 75.5, 75.2, 75.2, 12.1; C δ 169.3, 159.6, 144.7, 144.4, 137.9, 130.7, 126.7, 83.6, 36.6; IR (neat) 3488, 2942, 1758,

1652, 1612, 1514, 1464, 1366, 1248, 1048, 884, 804, 739, 684 cm^{-1}; HRMS (ESI) calcd 953.5575 for $C_{55}H_{82}O_{10}NaSi$ (M+Na), found 953.5592.

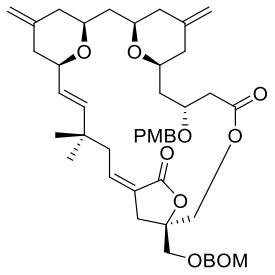


(R)-4-((2S,6R)-6-(((2S,6R)-6-((1E,5Z)-5-((R)-5-(((benzyloxy)methoxy)methyl)-2-oxo-5-(((triisopropylsilyl)oxy)methyl)dihydrofuran-3(2H)-ylidene)-3,3-dimethylpent-1-en-1-yl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3-((4-methoxybenzyl)oxy)butanoic acid (2.28): To a stirring solution of alcohol **2.27** (37 mg, 0.04 mmol, 1.0 equiv) in CH_2Cl_2 (800 μL, 0.05 M) contained in a 5 mL vial under N_2 was added *i*-Pr_2NEt (70 μL, 0.40 mmol, 10.0 equiv), followed by DMSO (20 μL, 0.28 mmol, 7.0 equiv). The mixture was cooled to 0 ^oC, and SO_3•Pyr (26 mg, 0.16 mmol, 4.0 equiv) was added. After stirring at 0 ^oC for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (25 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was then dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 1 × 10 cm silica gel column, eluting with 20% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 6–14 were concentrated to yield the intermediate aldehyde as a colorless oil which was used without further characterization (28 mg, 75%).

To a stirring solution of the aforementioned aldehyde (28 mg, 0.030 mmol, 1.0 equiv) in *t*-BuOH (430 μL, 14.3 mL/ mmol) and 2-methyl-2-butene (430 μL, 14.3 mL/ mmol) contained in a 5 mL vial was added an aqueous solution of KH_2PO_4 (1.25 M, 144 μL, 0.18 mmol, 6.0 equiv). The rapidly stirred reaction mixture was cooled to −5 ^oC, and

80% $NaClO_2$ (17 mg, 0.15 mmol, 5.0 equiv) was added. The reaction mixture was stirred at 0 ^oC for 1.5 h and then quenched with aqueous pH 4.0 acetate buffer (0.1 M, 25 mL). After extraction with EtOAc (3 × 10 mL), the solution was dried over Na_2SO_4, filtered, and concentrated. The resulting product was purified on a 2 × 15 cm silica gel column, eluting with 40% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 8–28 were combined and concentrated to yield the product as a colorless oil (15 mg, 54%). R_f = 0.48 (20/ 5/ 0.3 $PhCH_3$/ EtOAc/ AcOH); $[\alpha]_D^{20}$ = +9.4^o (c = 0.74, $CHCl_3$); 500 MHz ^{1}H NMR (C_6D_6) δ 7.29 (dd, *J* = 7.5, 3.0 Hz, 4H), 7.16 (t, *J* = 7.0 Hz, 2H), 7.07 (t, *J* = 10 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.00 (t, *J* = 8.0 Hz, 1H), 5.69 (d, *J* = 16 Hz, 1H), 5.55 (dd, *J* = 16.5, 6.0 Hz, 1H), 4.74 (s, 2H), 4.65 (d, *J* = 4.0 Hz, 2H), 4.55 (s, 2H), 4.51 (ABq, *J* = 11Hz, Δv = 54.2 Hz, 2H), 4.48 (s, 2H), 3.81 (dd, *J* = 10, 5.5 Hz, 1H), 3.69–3.62 (m, 2H), 3.67 (d, *J* = 3.0Hz, 2H), 3.54–3.47 (m, 2H), 3.51 (d, *J* = 5.5 Hz, 2H), 3.31 (s, 3H), 3.02 (dd, *J* = 14.5, 7.5 Hz, 1H), 2.86 (dd, *J* = 14.5, 7.0 Hz, 1H), 2.69 (dd, *J* = 16.5, 2.0 Hz, 1H), 2.50–2.42 (m, 3H), 2.18 (t, *J* = 14 Hz, 2H), 2.12–2.03 (m, 3H), 1.97–1.76 (m, 5H), 1.66–1.54 (m, 2H), 1.04–1.00 (m, 27H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 174.5, 169.3, 159.6, 144.5, 144.3, 140.8, 140.7, 137.8, 130.3, 129.7, 128.7, 128.1, 128.0, 128.0, 126.7, 114.2, 109.1, 109.0, 95.2, 83.6, 79.3, 75.3, 75.2, 75.0, 72.9, 72.4, 70.1, 69.8, 66.0, 55.5, 43.0, 42.0, 41.4, 41.3, 41.0, 40.6, 40.0, 39.8, 36.7, 33.6, 27.1, 27.1, 18.1, 12.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.1, 27.0, 18.1; CH_2 δ 109.1, 109.0, 95.2, 72.4, 70.0, 69.8, 66.0, 43.0, 41.9, 41.4, 41.3, 41.0, 40.6, 40.0, 39.8, 33.5; CH δ 140.7, 140.6, 129.7, 128.6, 128.1, 128.0, 128.0, 114.1, 79.3, 75.2, 75.2, 75.0, 72.9, 12.1; C δ 174.5, 169.3, 159.6, 144.5, 144.3, 137.8, 130.3, 126.7, 83.6, 36.7; IR (neat) 2943, 2867, 1758, 1712, 1653, 1613, 1514, 1464, 1367, 1249, 1172, 1112, 1046, 884, 805, 739 cm^{-1}; HRMS (ESI) calcd

967.5368 for $C_{55}H_{80}O_{11}NaSi$ (M+Na), found 967.5378.

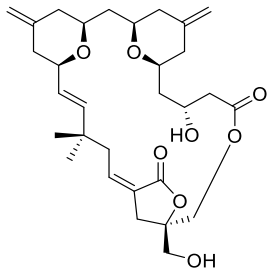


(1R,3S,7R,8E,12Z,16R,21R,23S)-16-(((benzyloxy)methoxy)methyl)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-5,25-dimethylene-15,18,27,28-tetraoxatetracyclo[21.3.1.13,7.113,16]nonacosa-8,12-diene-14,19-dione (2.29): To a stirring solution of acid **2.28** (0.037 mmol, 1.0 equiv) in THF (740 μL, 0.05 M) contained in a 10 mL polyethylene vial at rt was added a 20% solution of HF•Pyr in pyridine (925 μL, 25 mmol/ mL). The mixture was stirred at rt for 2 days, then quenched by pouring into aqueous pH 4.0 acetate buffer (0.1 M, 25 mL). The mixture was extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified on a 1 × 10 cm silica gel column, eluting with 3% MeOH/ CH_2Cl_2, collecting 5 mL fractions. Fractions 4–16 were concentrated to yield the product as a colorless oil which was used without characterization for the subsequent macrolactonization.

To a stirring solution of the aforementioned *seco*-acid in THF (1.2 mL, 0.03 M) contained in a 10 mL flask under N_2 was added a solution of Et_3N in THF (1.0 M, 220 μL, 0.22 mmol, 6.0 equiv). The reaction mixture was cooled to 0 ^oC, and a solution of 2,4,6-trichlorobenzoyl chloride in THF, (1.0 M, 110 μL, 0.11 mmol, 3.0 equiv) was added. The reaction mixture was stirred at 0 ^oC for 5 min and warmed to rt. The reaction mixture was then stirred at rt for 3 h. The resulting solution was taken up into $PhCH_3$ (10 mL) in a 25 mL gas-tight syringe and added over 16 h via syringe pump to a stirring

solution of DMAP (90 mg, 0.74 mmol, 20.0 equiv) in $PhCH_3$ (27 mL) contained in a 100 mL flask under N_2 at 40 °C, achieving a final concentration of 0.001 M in substrate. After 16 h, $PhCH_3$ (1.0 mL) was used to complete the transfer of the reactant. The reaction mixture was stirred for an additional h at 40 °C, then cooled to rt, quenched with brine (50 mL), and extracted with EtOAc (3 × 10 mL). After drying over Na_2SO_4, the solution was filtered and concentrated. The product was purified on a 1 × 12 cm silica gel column, eluting with 15% EtOAc/ hexanes, collecting 5 mL fractions. Fractions 12–24 were combined and concentrated to yield the product as a colorless oil (11 mg, 38%). R_f = 0.25 (25% EtOAc/ hexanes); $[\alpha]_D^{20}$ = + 22.4° (c = 0.42, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36–7.28 (m, 5H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.17 (t, *J* = 8.5 Hz, 1H), 5.59 (d, *J* = 17 Hz, 1H), 5.40 (dd, *J* = 16, 4.0 Hz, 1H), 4.90 (d, *J* = 6.5 Hz, 1H), 4.78 (d, *J* = 6.5 Hz, 1H), 4.72–4.70 (m, 3H), 4.65 (s, 1H), 4.62 (ABq, *J* = 12 Hz, Δv = 35.9 Hz, 2H), 4.46 (ABq, *J* = 11 Hz, Δv = 38.5 Hz, 2H), 4.09 (m, 1H), 4.01 (d, *J* = 11.5 Hz, 1H), 3.81 (s, 3H), 3.73–3.71 (m, 1H), 3.69 (q, *J* = 10.5 Hz, 2H), 3.50–3.41 (m, 2H), 3.36–3.32 (m, 1H), 2.81–2.74 (m, 3H), 2.62–2.54 (m, 3H), 2.26 (d, *J* = 13.5 Hz, 2H), 2.21 (d, *J* = 13.5 Hz, 1H), 2.15 (d, *J* = 13.5 Hz, 1H), 2.01–1.89 (m, 5H), 1.79–1.69 (m, 2H), 1.11 (s, 3H), 1.06 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.7, 168.6, 159.5, 144.6, 144.5, 141.4, 137.8, 137.8, 130.5, 129.5, 129.0, 128.7, 128.0, 128.0, 125.8, 114.1, 109.0, 108.9, 94.9, 81.4, 78.5, 77.4, 75.5, 75.4, 73.5, 71.8, 69.9, 65.1, 55.5, 43.7, 42.3, 41.3, 41.3, 41.2, 41.0, 40.3, 37.9, 34.8, 22.6, 14.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 14.3; CH_2 δ 109.0, 108.9, 94.9, 71.8, 69.9, 65.1, 43.6, 42.3, 41.3, 41.3, 41.2, 41.0, 34.8, 22.5; CH δ 141.4, 137.8, 129.5, 129.0, 128.7, 128.1, 128.0, 114.1, 78.5, 77.5, 75.5, 75.4, 73.5; C δ 170.7, 168.6, 159.5, 144.6, 144.5, 137.8, 130.5, 125.8, 81.4, 37.9; IR (neat) 3057, 2916,

1762, 1744, 1707, 1675, 1632, 1576, 1562, 1496, 1446, 1343, 1220, 1077, 1007, 946, 867, 809, 698 cm^{-1}; HRMS (ESI) calcd 793.3928 for $C_{46}H_{58}O_{10}Na$ (M+Na), found 793.3939.

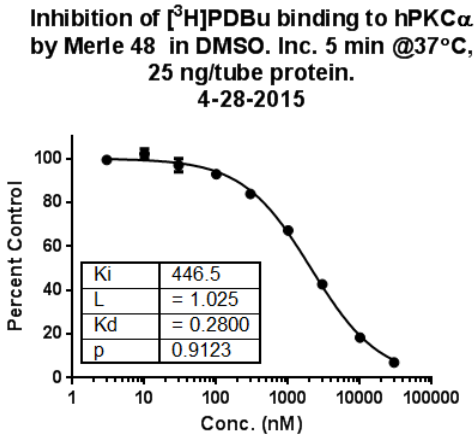


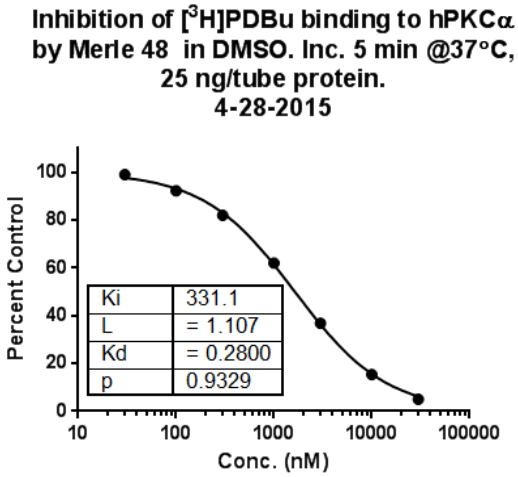
Preparation of Merle 48 (1R,3S,7R,8E,12Z,16R,21R,23S)-21-hydroxy-16-(hydroxymethyl)-10,10-dimethyl-5,25-dimethylene-15,18,27,28-tetraoxatetracyclo[21.3.1.13,7.113,16]nonacosa-8,12-diene-14,19-dione: To a stirring solution of macrolactone **2.29** (9.0 mg, 0.012 mmol, 1.0 equiv) in CH_2Cl_2 (1.2 mL, 0.01 M) contained in a 5 mL vial was added H_2O (30 μL). After cooling to 0 ^oC, DDQ (27 mg, 0.12 mmol, 10.0 equiv) was added and the reaction mixture was stirred at 0 ^oC for 1 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), then extracted with CH_2Cl_2 (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The crude alcohol was used without purification for the subsequent reaction.

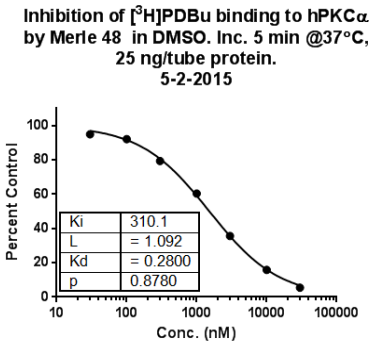
To the previously prepared alcohol contained in a 5 mL vial, a solution of $LiBF_4$ in 25:1 MeCN/ H_2O (0.25 M, 2.0 mL, 45.0 equiv) was added. The vial was flushed with N_2, capped, and heated at 80 ^oC overnight. After cooling to rt, the reaction mixture was concentrated to dryness and taken up in EtOAc (10 mL). The solution was partitioned with brine (25 mL) and subsequently extracted with EtOAc (2 × 10 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified on a 1 × 11 cm silica gel column, eluting with 35% EtOAc/ pentane,

collecting 5 mL fractions. Fractions 20–48 were combined and concentrated to yield the macrolide as a colorless oil (3.8 mg, 59% over 2 steps). R_f = 0.36 (50% EtOAc/ hexanes); $[\alpha]_D^{20}$ = –27° (c = 0.12, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 6.24 (t, *J* = 8.5 Hz, 1H), 5.58 (d, *J* = 16 Hz, 1H), 5.34 (dd, *J* = 16, 5.5 Hz, 1H), 4.78 (s, 2H), 4.75 (s, 2H), 4.56 (d, *J* = 11.5 Hz, 1H), 4.34 (m, 1H), 4.00 (d, *J* = 12 Hz, 1H), 3.70–3.63 (m ,4H), 3.44–3.36 (m, 3H), 3.32 (t, *J* = 12 Hz, 1H), 2.88 (d, *J* = 15 Hz, 1H), 2.82 (dd, *J* = 14, 4.5 Hz, 1H), 2.59 (d, *J* = 16 Hz, 1H), 2.45 (dd, *J* = 9.5, 4.0 Hz, 1H), 2.34 (d, *J* = 13 Hz, 1H), 2.20–2.14 (m, 4H), 2.03–1.95 (m, 3H), 1.90 (t, *J* = 11.5 Hz, 1H), 1.77 (t, *J* = 14.5 Hz, 1H), 1.64–1.59 (m, 2H), 1.12 (s, 3H), 1.10 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 170.1, 169.0, 143.8, 143.6, 141.7, 139.4, 128.3, 126.1, 109.6, 109.4, 82.7, 79.8, 76.1, 75.3, 75.1, 66.0, 65.7, 62.0, 44.6, 43.8, 42.7, 41.8, 41.6, 41.3, 41.3, 40.3, 38.2, 34.6, 30.3, 25.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 30.3, 25.1; CH_2 δ 109.6, 109.4, 66.0, 62.0, 44.6, 43.8, 42.7, 41.8, 41.6, 41.3, 41.3, 40.3, 34.6; CH δ 141.7, 139.4, 128.3, 79.8, 76.1, 75.3, 75.1, 65.7; C δ 170.1, 169.0, 143.8, 143.6, 126.1, 82.7, 38.2; IR (neat) 3444, 2940, 1743, 1671, 1653, 1559, 1541, 1437, 1366, 1327, 1281, 1246, 1219, 1161, 1123, 1098, 1072, 1042, 1000, 981, 936, 893, 792 cm^{-1} HRMS (ESI) calcd 553.2777 for $C_{30}H_{42}O_8Na$ (M+Na), found 553.2778.

Binding Assay for Merle 48 Towards PKC α







Compound	Date	Kd nM PKCα	Ki (nM)	p value	protein
Merle 48	4/28/2015	.28 ± .02	446.5	0.91	25 ng
	4/30/2015	.28 ± .02	331.1	0.93	25 ng
	5/2/2015	.28 ± .02	310.1	0.88	25 ng
		Mean	362.6	42.4	SEM

Molecular Modeling Methods

Molecular modeling studies were conducted by Dr. Megan Peach of the NIH. Her methods for these studies are summarized here.

Methods

Conformational searching. The initial structures for Merle 39, Merle 47 , and Merle 48 were built based on the crystal structure of bryostatin 1[43] from the Cambridge

Structural Database (reference code BOKKIV) to keep the structure of the A + B rings consistent. All searches were performed using mixed torsional/large-scale low-mode sampling in MacroModel[41] with the OPLS 2005 forcefield[44] in octanol implicit solvent. During the searches, torsions were varied for 10,000 steps with enhanced sampling, but the chiral centers and double bonds were restricted to their crystal conformations. Low mode displacements were between 3 and 18 Å. After each step, the resulting structure was energy minimized to a gradient convergence of 0.05. The minimized structure was then compared to previously stored structures and either kept as a unique conformer or rejected as a duplicate, using a 0.75 Å RMSD cutoff to the heavy atoms in the central macrolide ring structure. A set of low-energy conformers for each structure, with energies with 3 kcal/mol of the global minimum, were passed on to the docking program.

Docking. The crystal structure of the C1b domain of PKCδ[45] was prepared for docking by adding hydrogen atoms and deleting the phorbol-13-acetate ligand. This was saved to a separate file to be used as a template for the similarity constraint (see below). Docking was done using the program GOLD, version 5.2.2,[42] which uses a genetic algorithm to optimize the set of interactions between the ligand and the protein. Default settings were used for the genetic algorithm. The binding site was defined as a sphere with a 10.0 Å radius, centered on the Nε atom of residue Gln 257. For each conformer, 20 docking runs were performed, with no early termination, using the GoldScore scoring function with an internal ligand energy offset. Free corners of ligand rings were allowed to flip above or below the plane of their neighboring atoms during docking, and intramolecular hydrogen bonds in the ligand were allowed to form. Torsion angle distributions were from the CSD. A template similarity constraint was added to bias the

conformation of docked ligands toward solutions where the acceptor atoms in the ligand were close in space to the acceptor atoms in bound phorbol-13-O-acetate from the crystal structure.

Energy minimization. A subset of diverse poses with fitness score > 40.0 were further refined by energy minimization in MacroModel,[2] using the OPLS_2005 forcefield and octanol implicit solvent. All atoms in the C1 domain were held fixed, while ligand atoms were free to move, and the complex was minimized using the Polak-Ribiere conjugate gradient scheme to a gradient convergence of 0.05. Hydrogen bonds in the docked poses were preserved using distance constraints.

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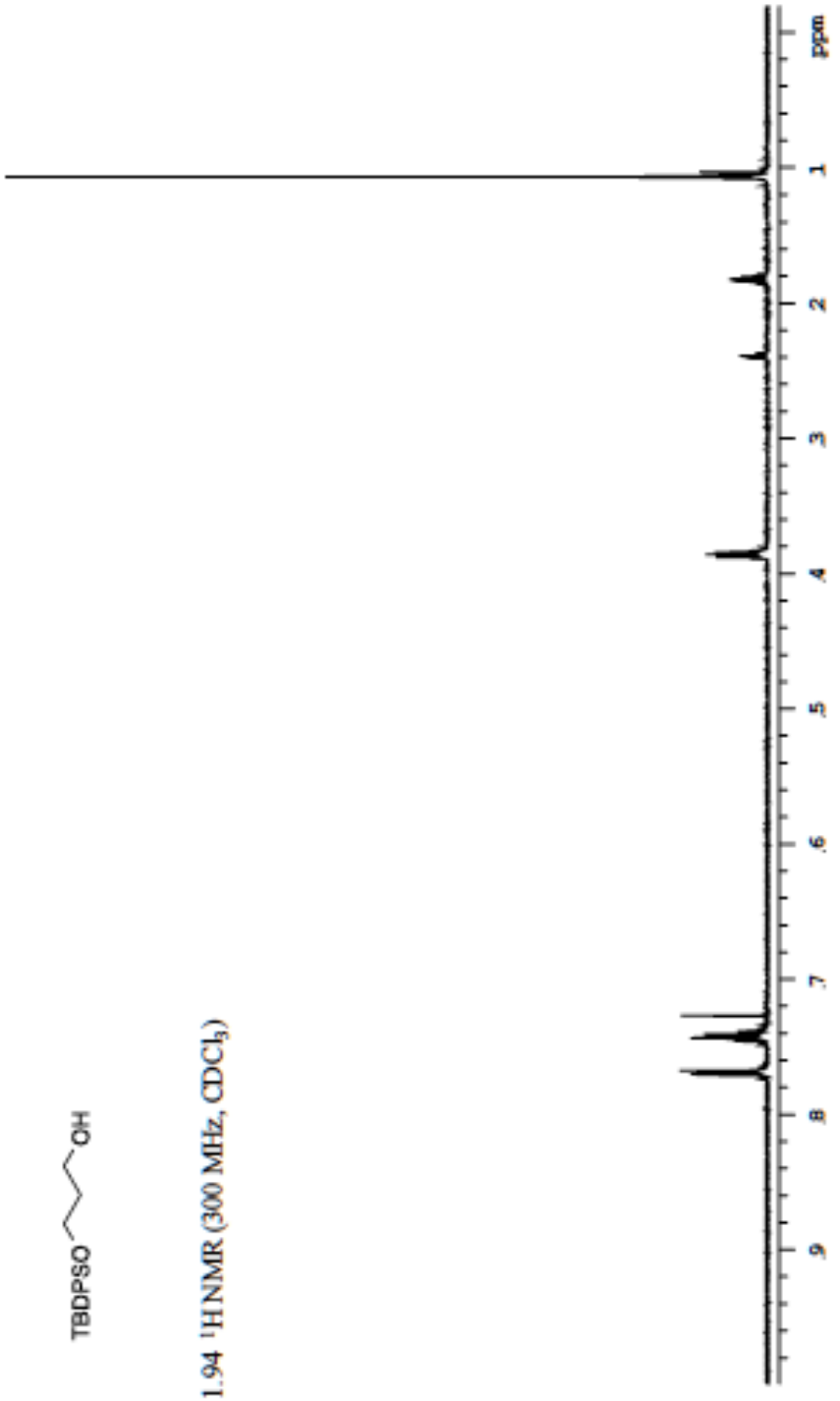
APPENDIX A

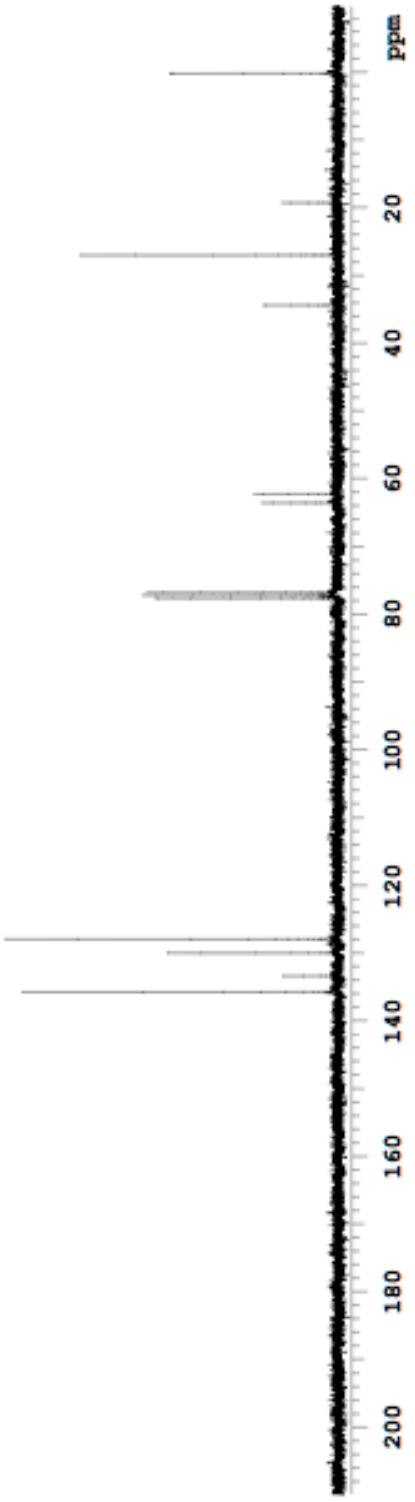
^{1}H AND ^{13}C SPECTRA FOR CHAPTER 1

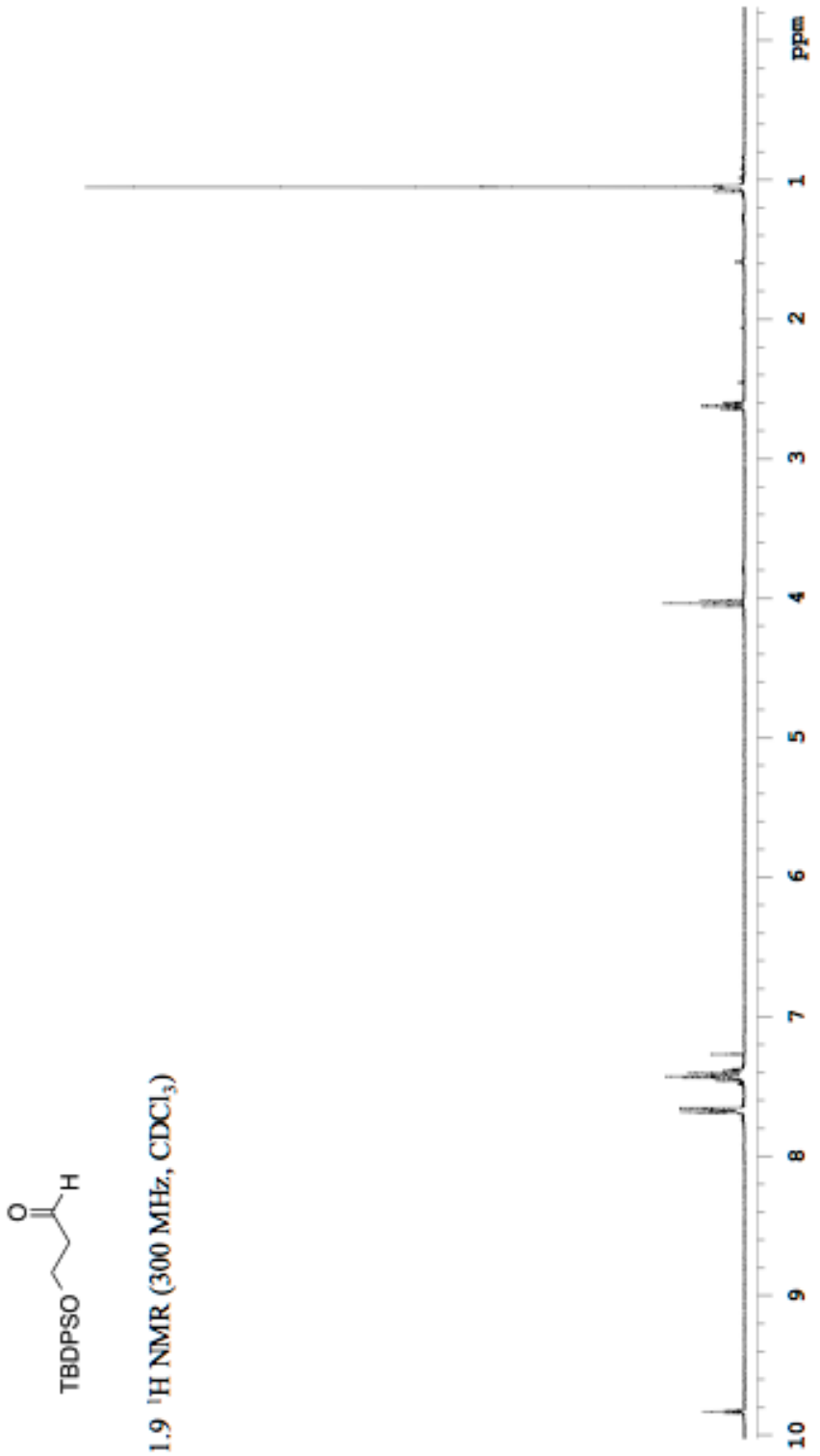


1.94 ^{1}H NMR (300 MHz, $CDCl_3$)

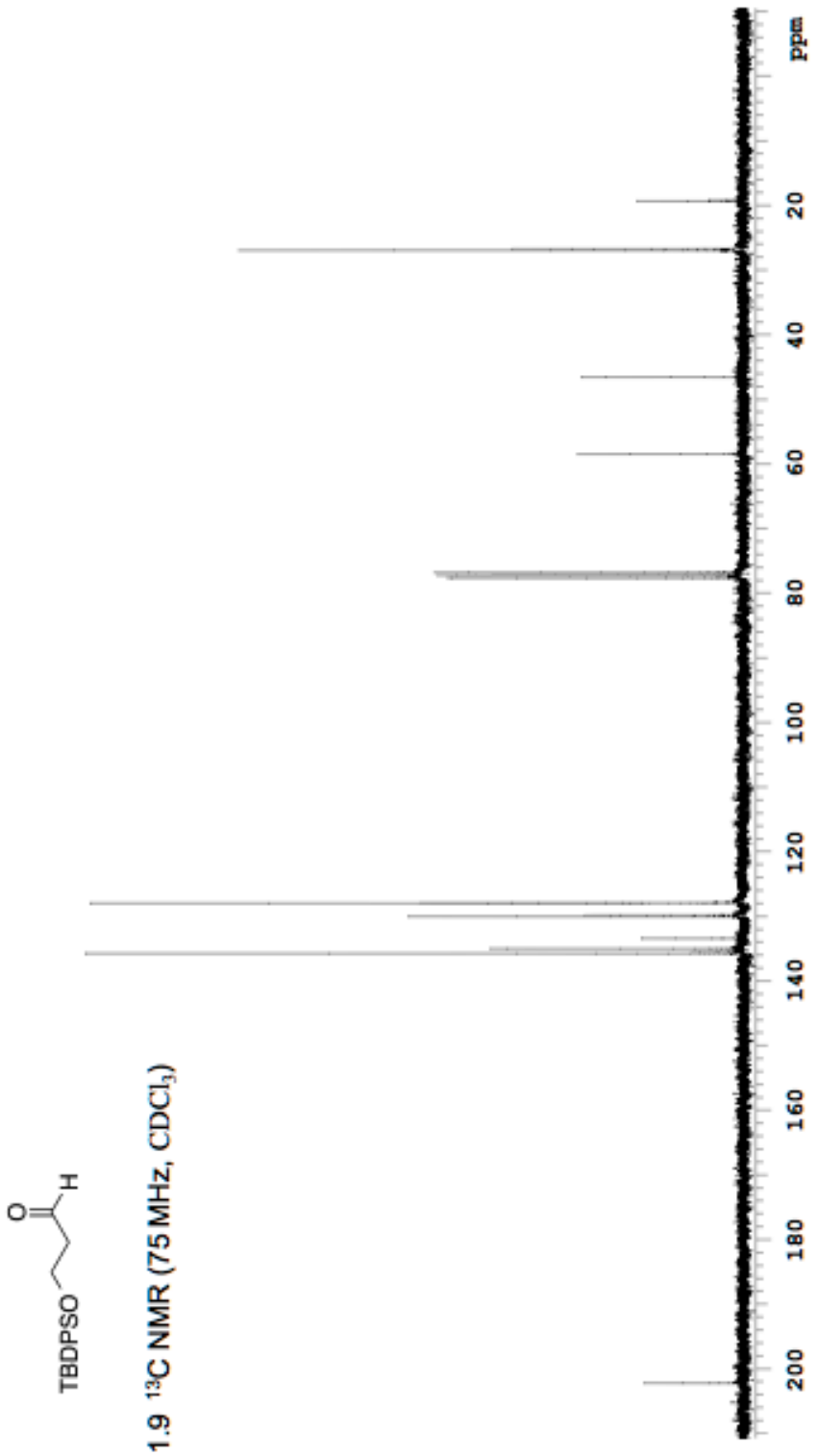
TBDPSO⟋⟍OH

1.94 ^{13}C NMR (75 MHz, $CDCl_3$)

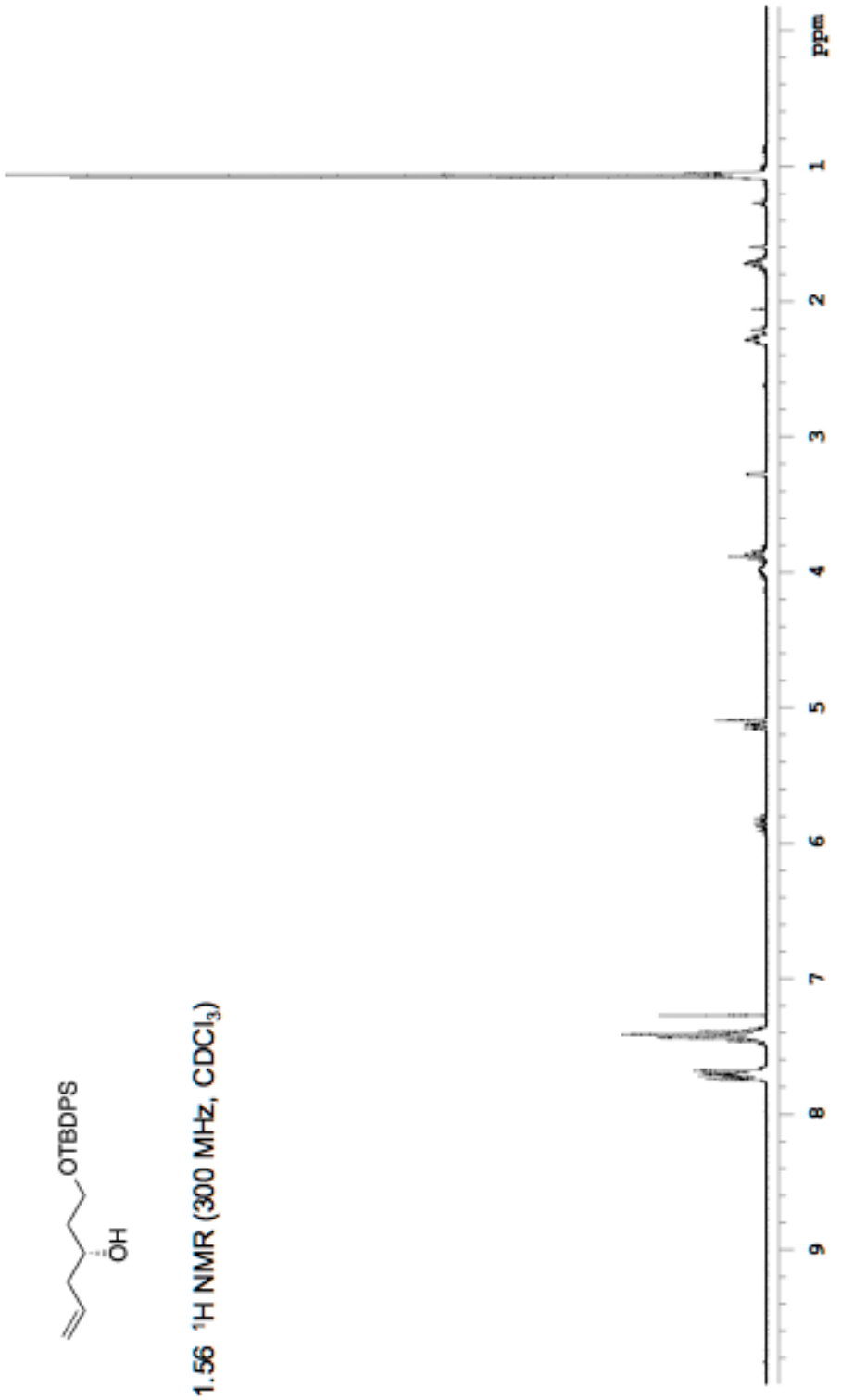




1.9 ^{1}H NMR (300 MHz, $CDCl_3$)



1.9 ^{13}C NMR (75 MHz, $CDCl_3$)



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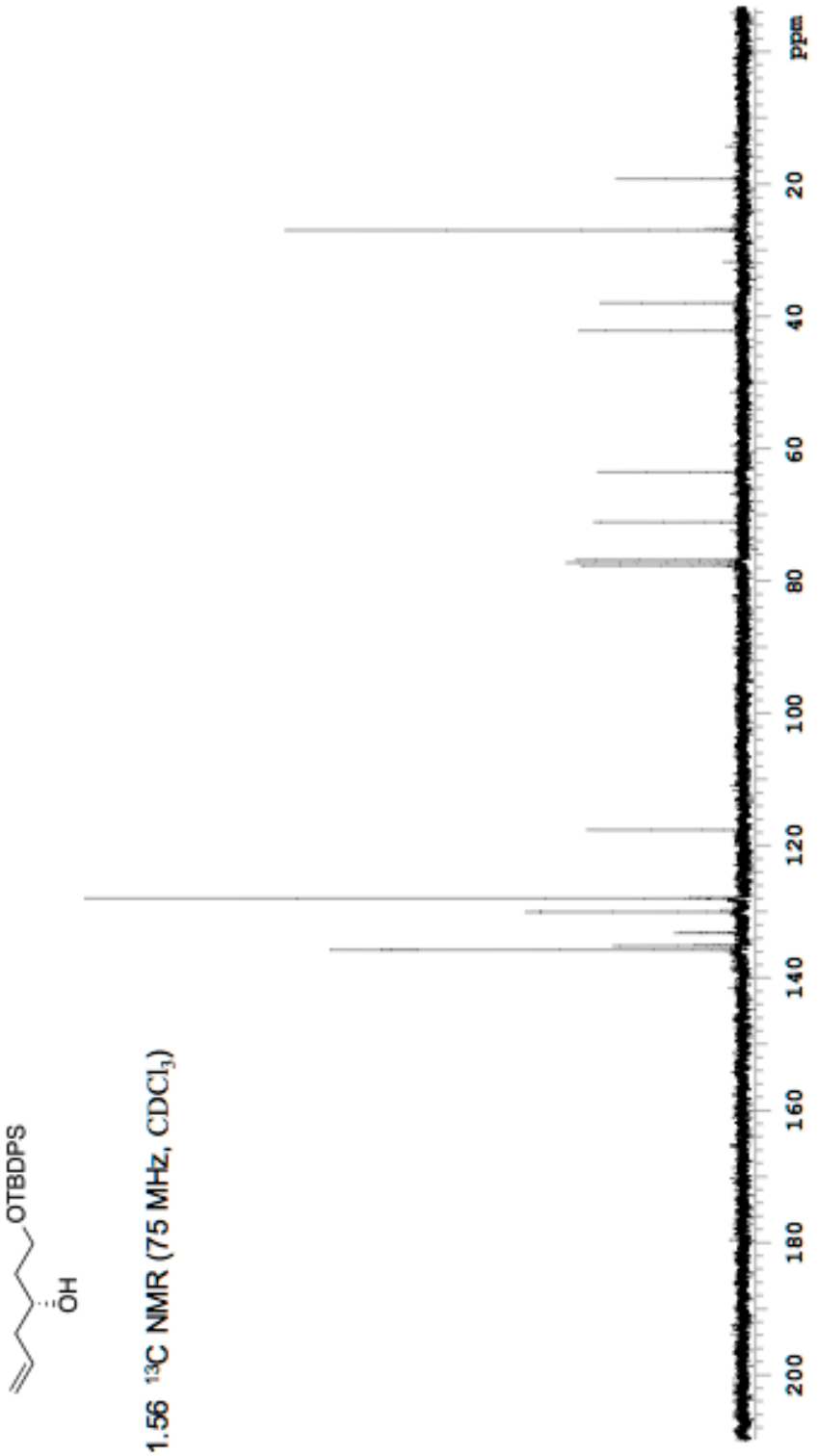
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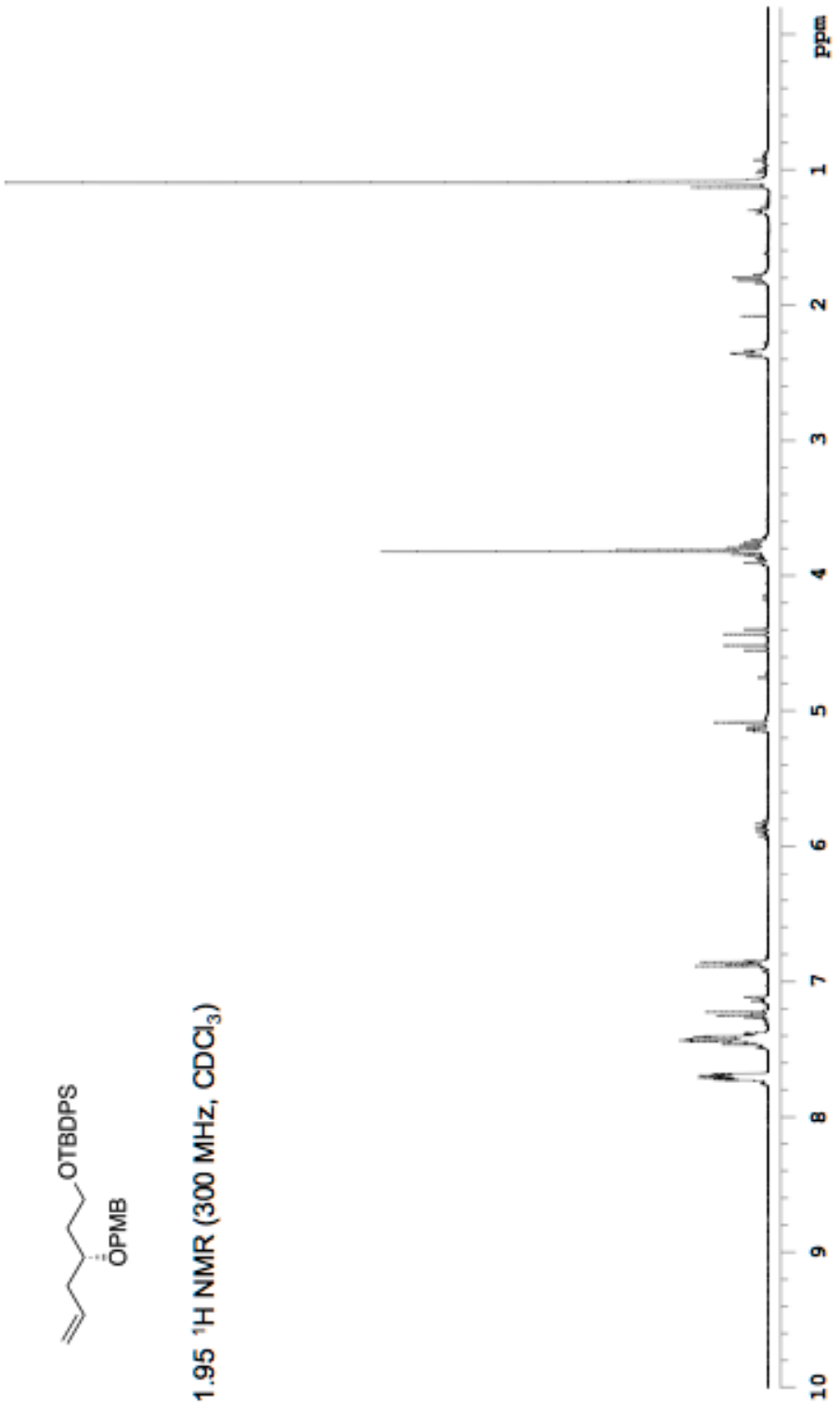
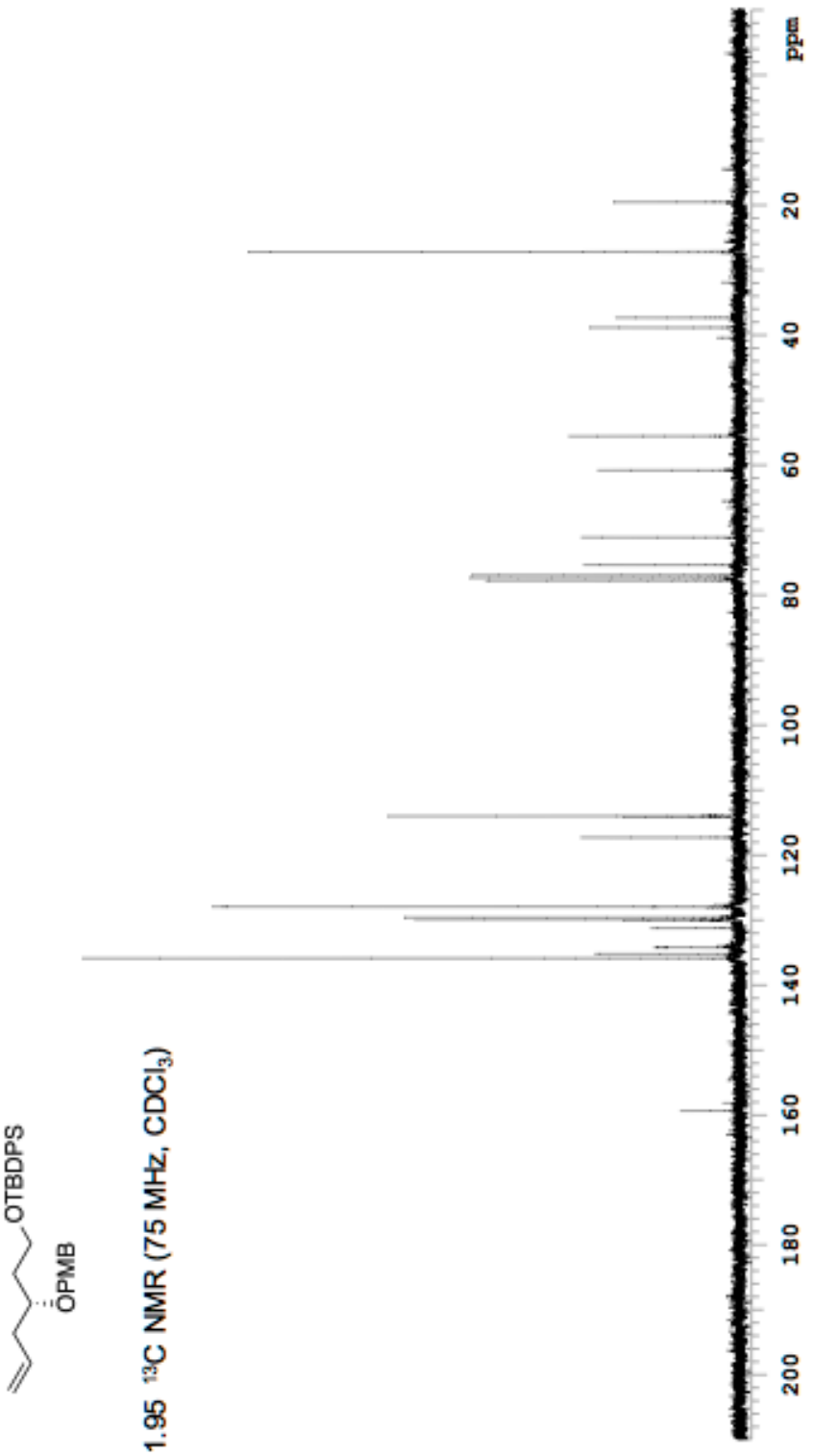
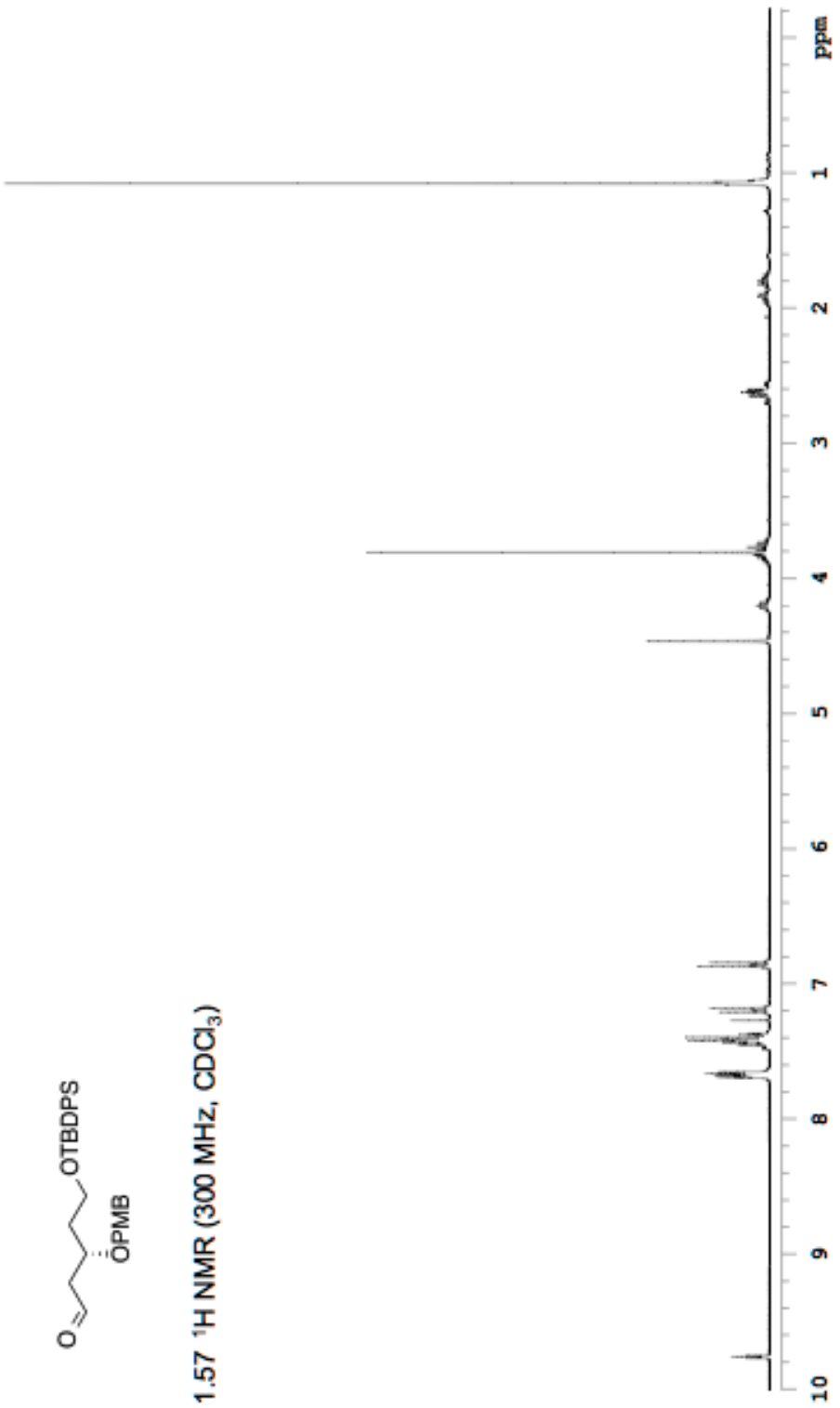
OH
1.56 ^{13}C NMR (75 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20


OTBDPS
OPMB
1.95 ¹H NMR (300 MHz, CDCl3)
ppm
10
9
8
7
6
5
4
3
2
1


OTBDPS
OPMB
1.95 ^{13}C NMR (75 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20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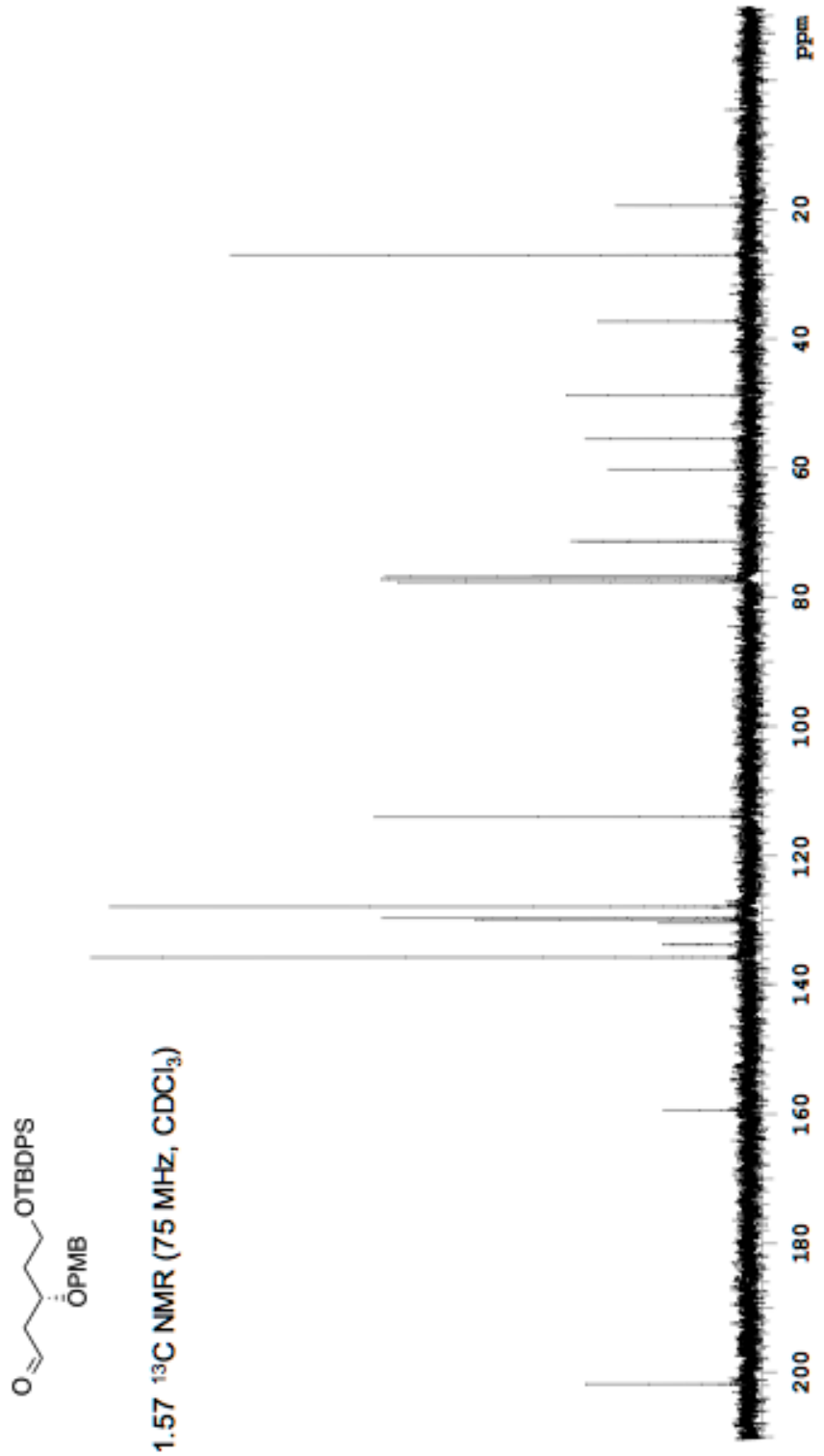
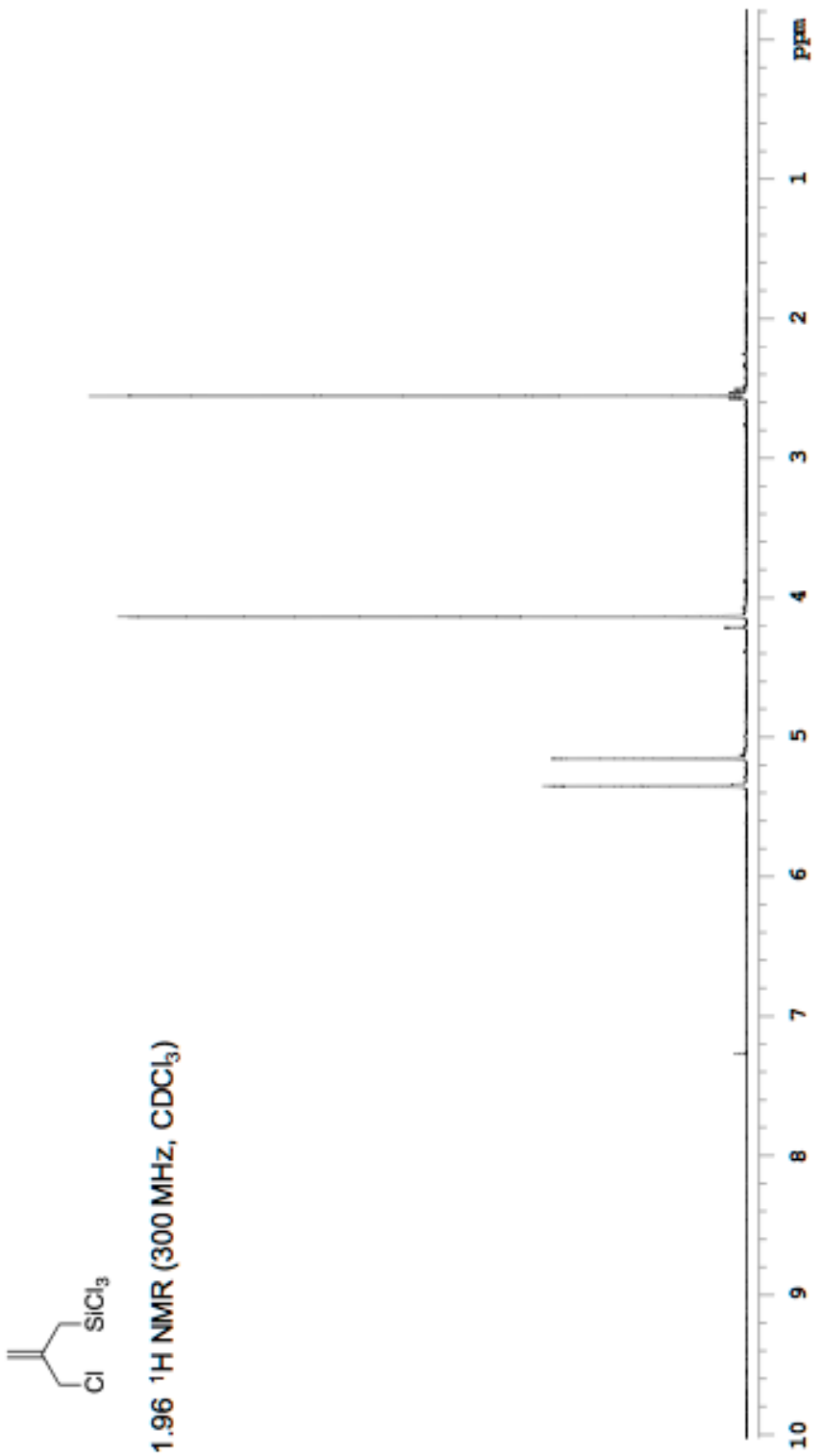
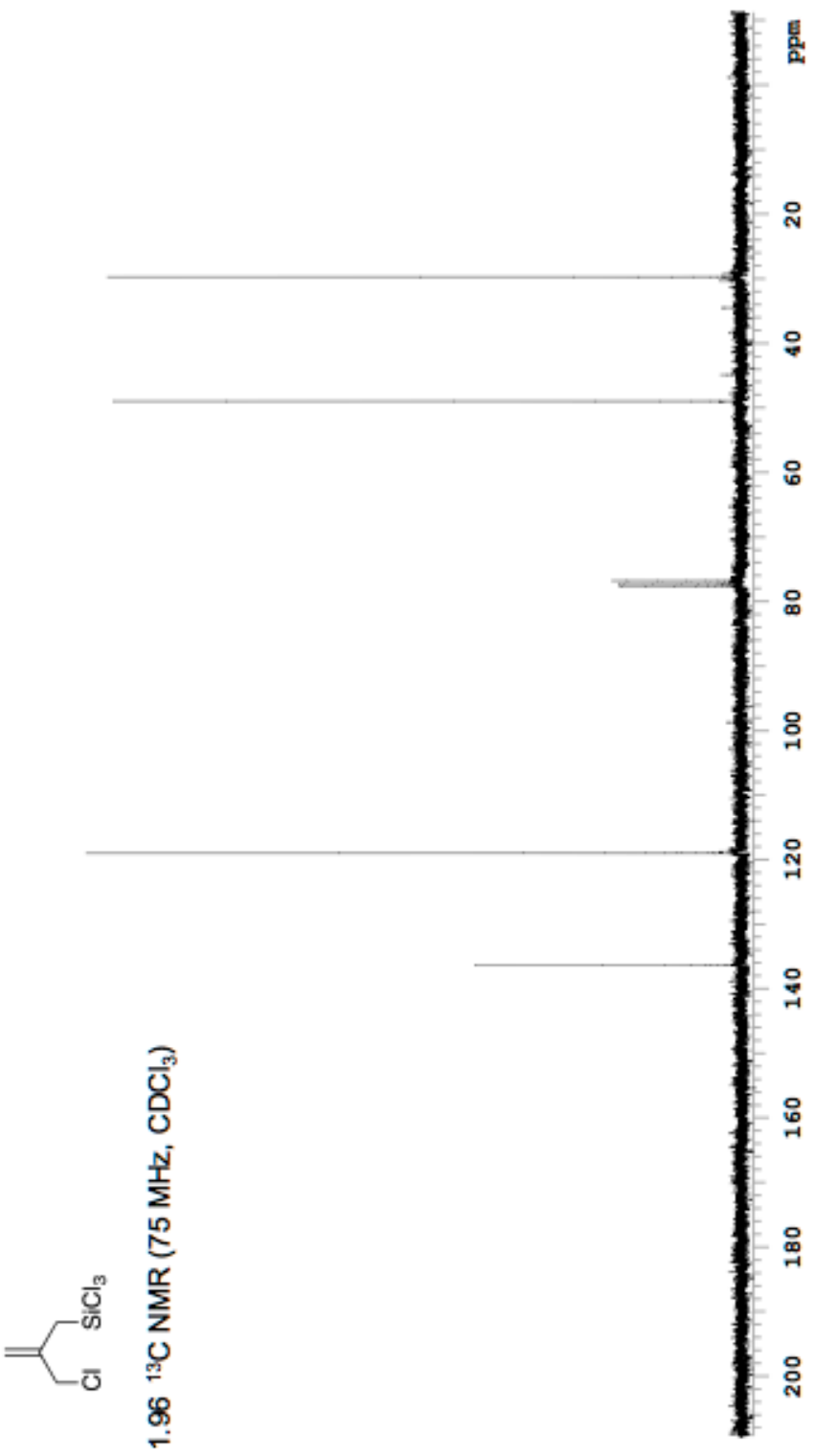
OPMB
O
1.57 ¹H NMR (300 MHz, CDCl3)
ppm
10
9
8
7
6
5
4
3
2
1


OTBDPS
OPMB
O
1.57 ^{13}C NMR (75 MHz, $CDCl_3$)
200
180
160
140
120
100
80
60
40
20
ppm


SiCl3
Cl
1.96 ¹H NMR (300 MHz, CDCl3)
ppm
10
9
8
7
6
5
4
3
2
1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SiCl3
1.96 13C NMR (75 MHz, CDCl3)
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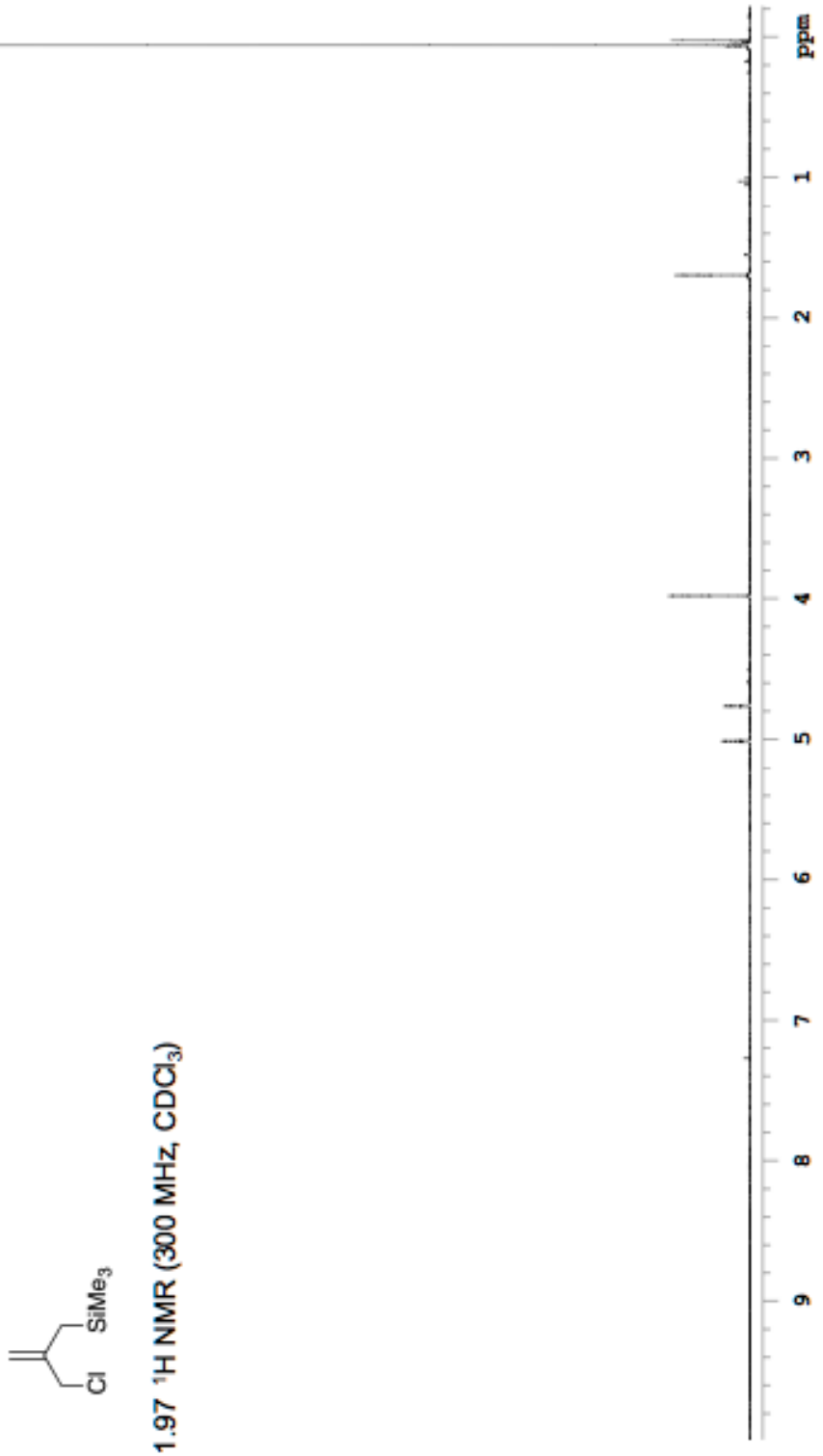
180
160
140
120
100
80
60
40
20
ppm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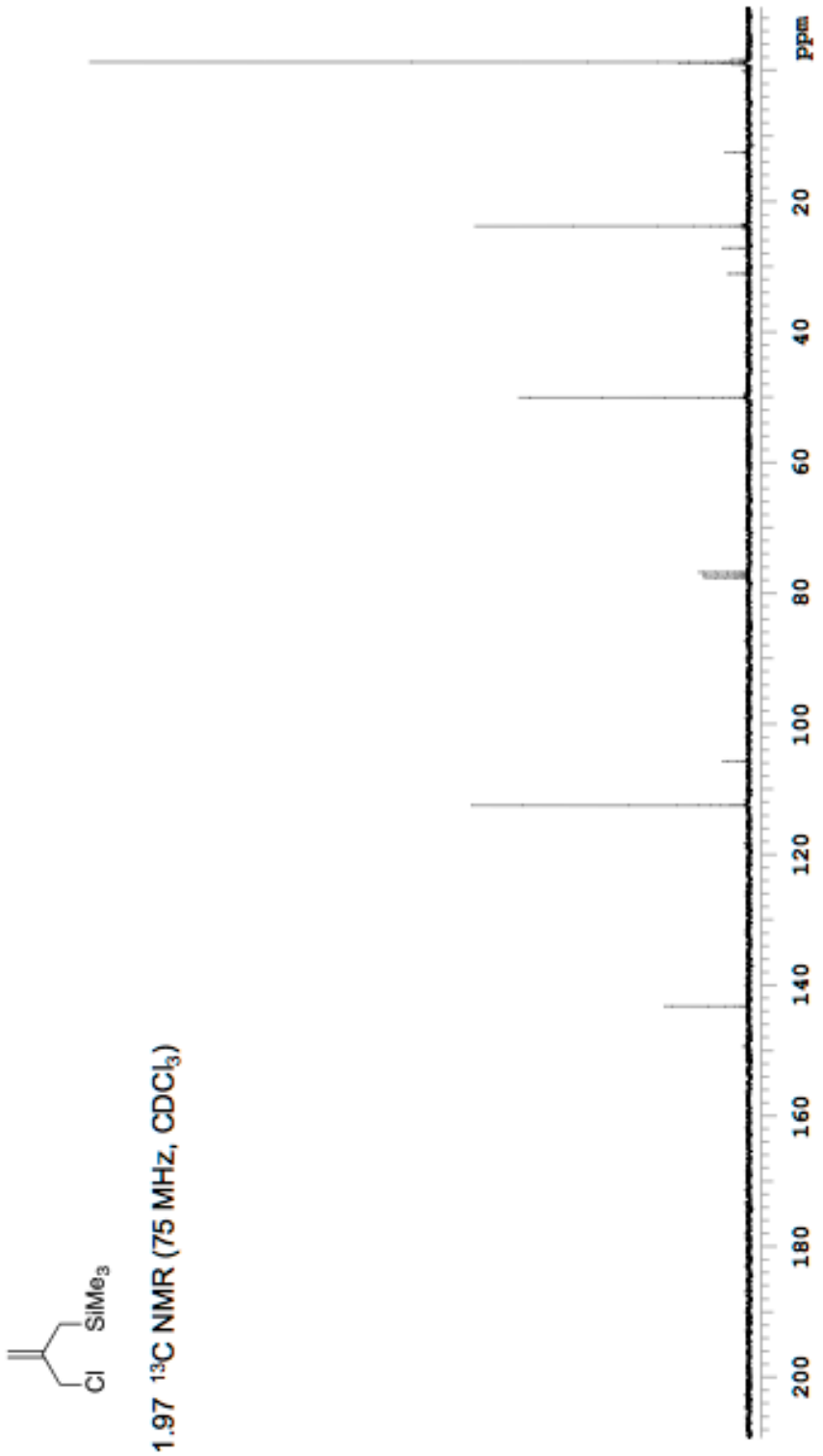
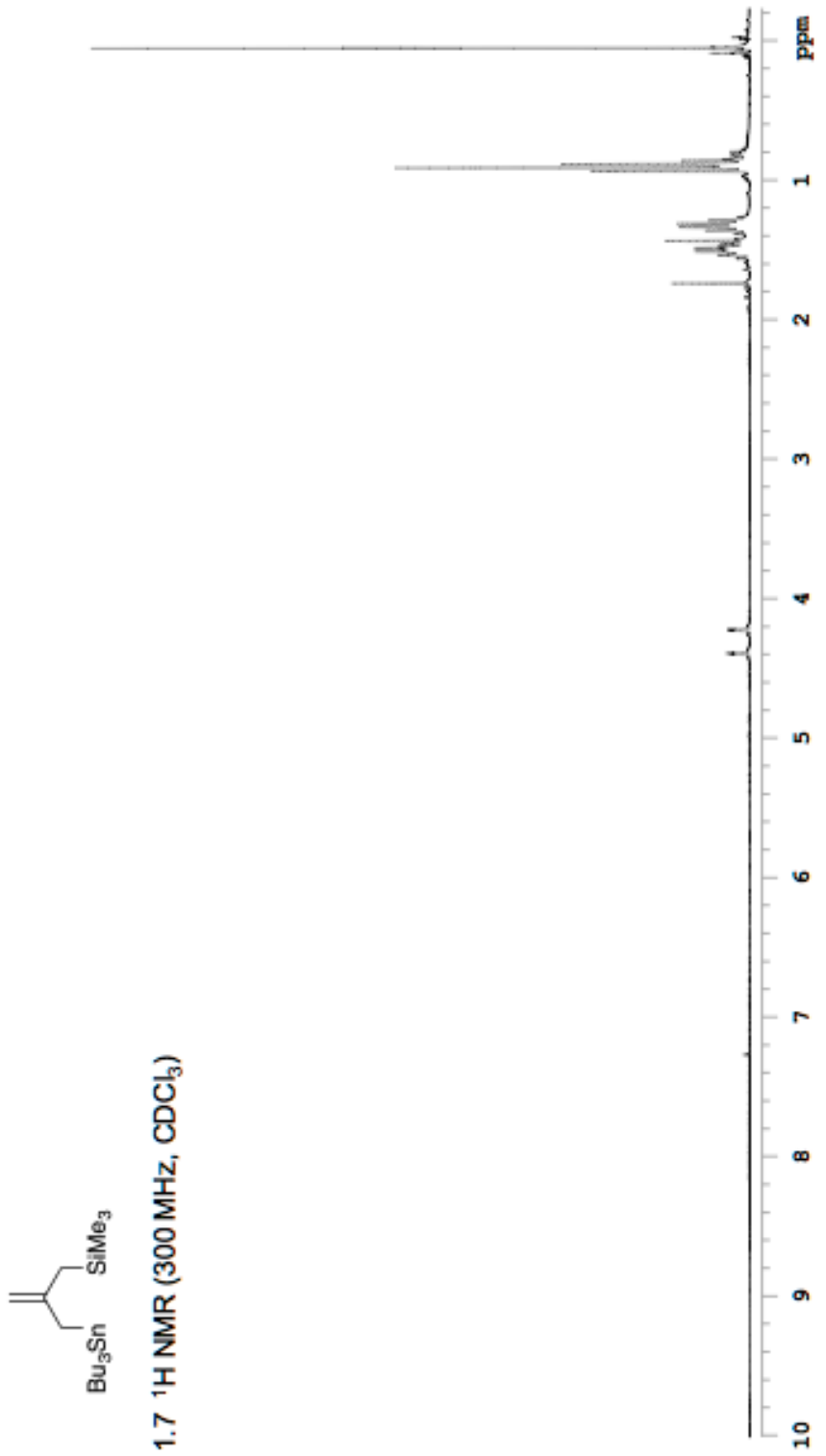
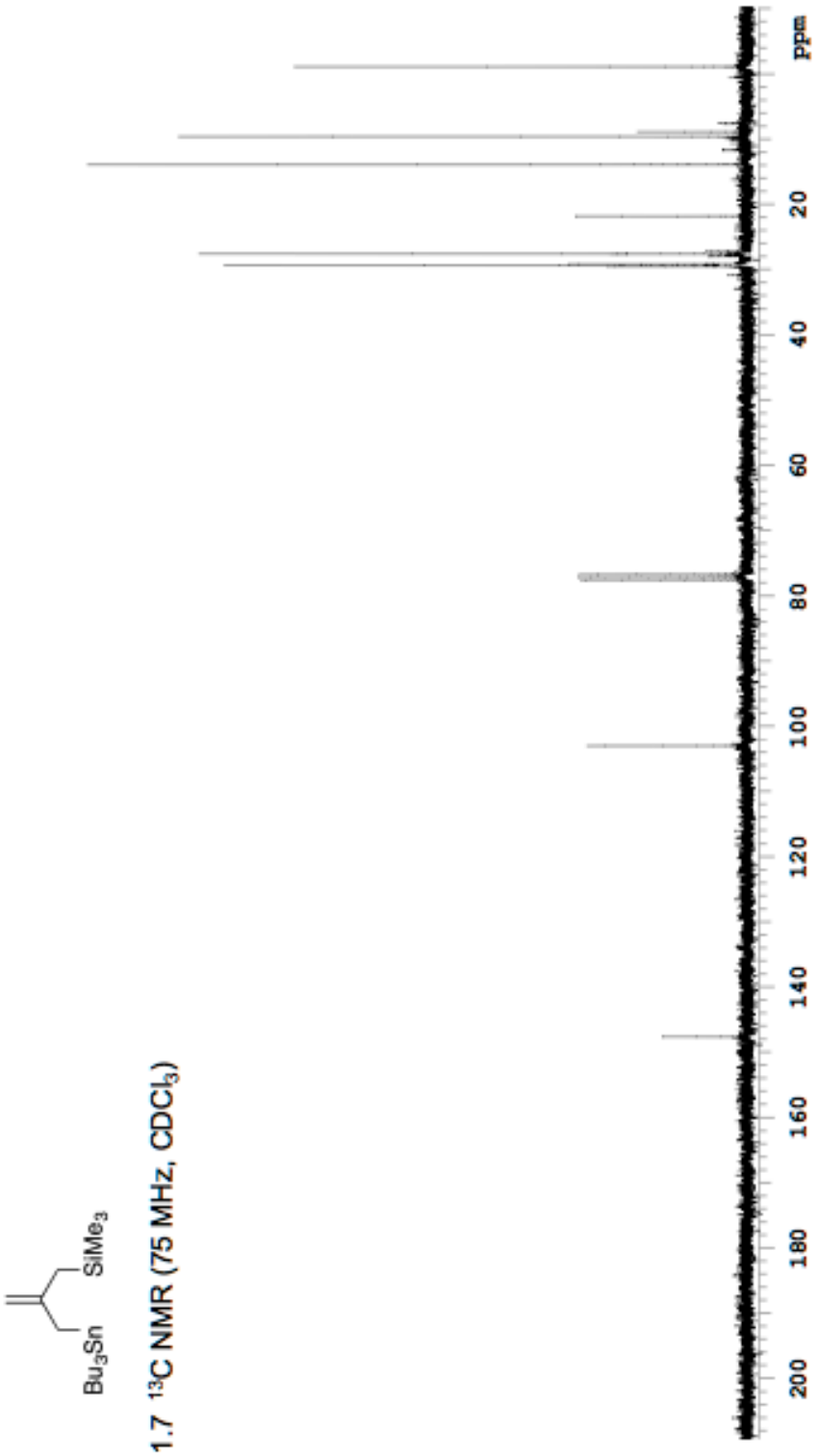
1.97 ¹H NMR (300 MHz, CDCl3)
ppm
1
2
3
4
5
6
7
8
9



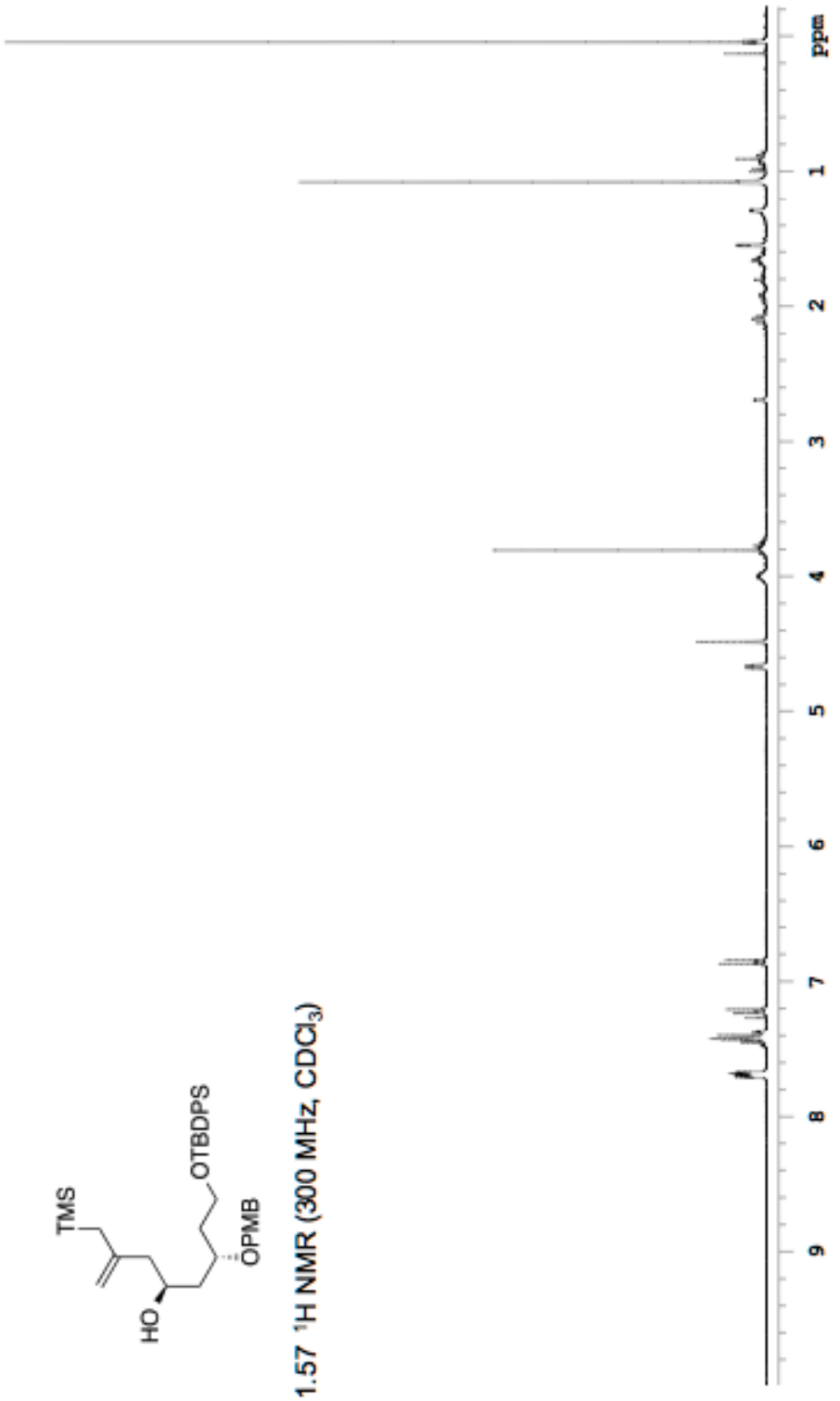
1.97 ^{13}C NMR (75 MHz, $CDCl_3$)



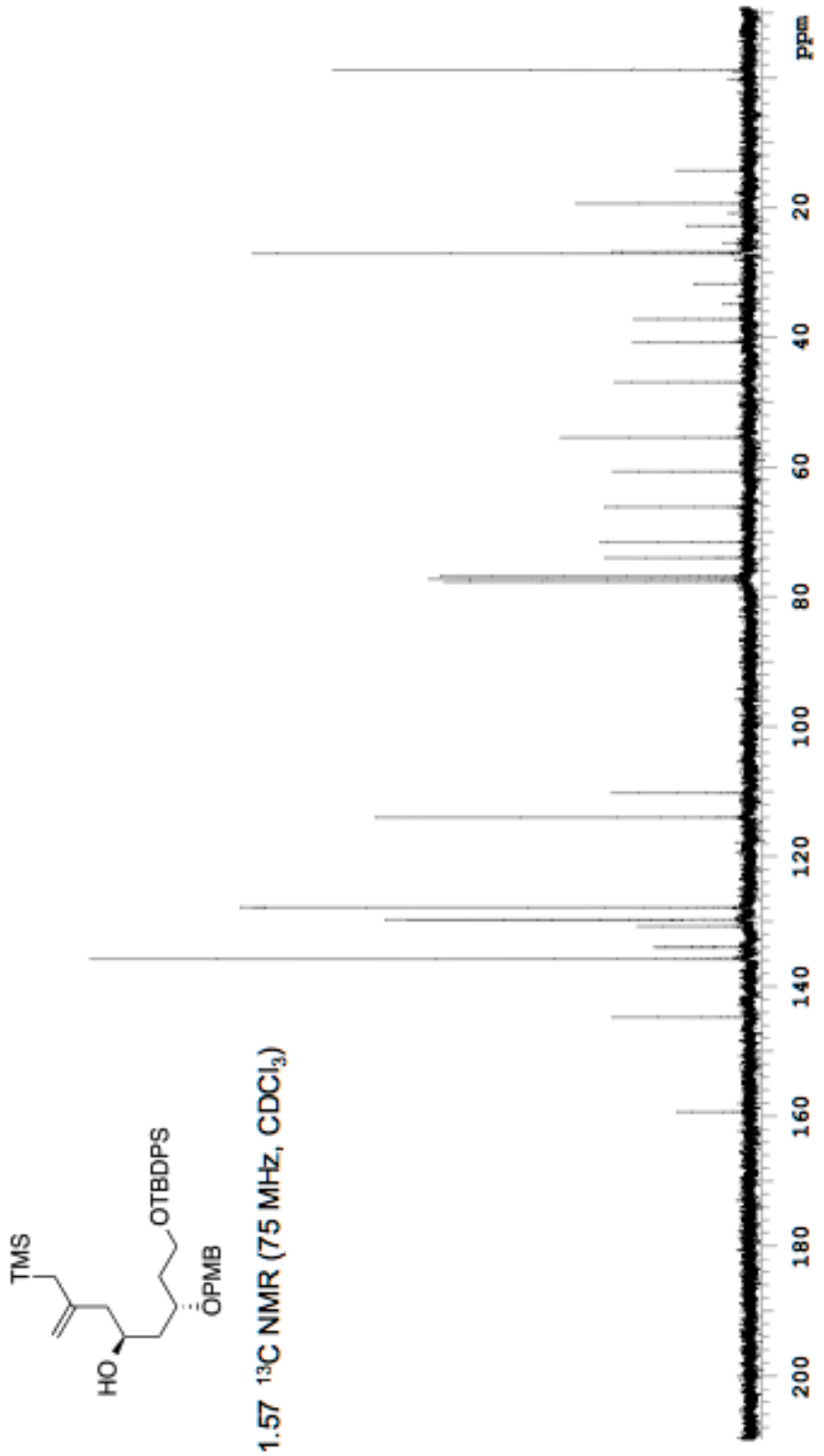
1.7 ^{1}H NMR (300 MHz, $CDCl_3$)



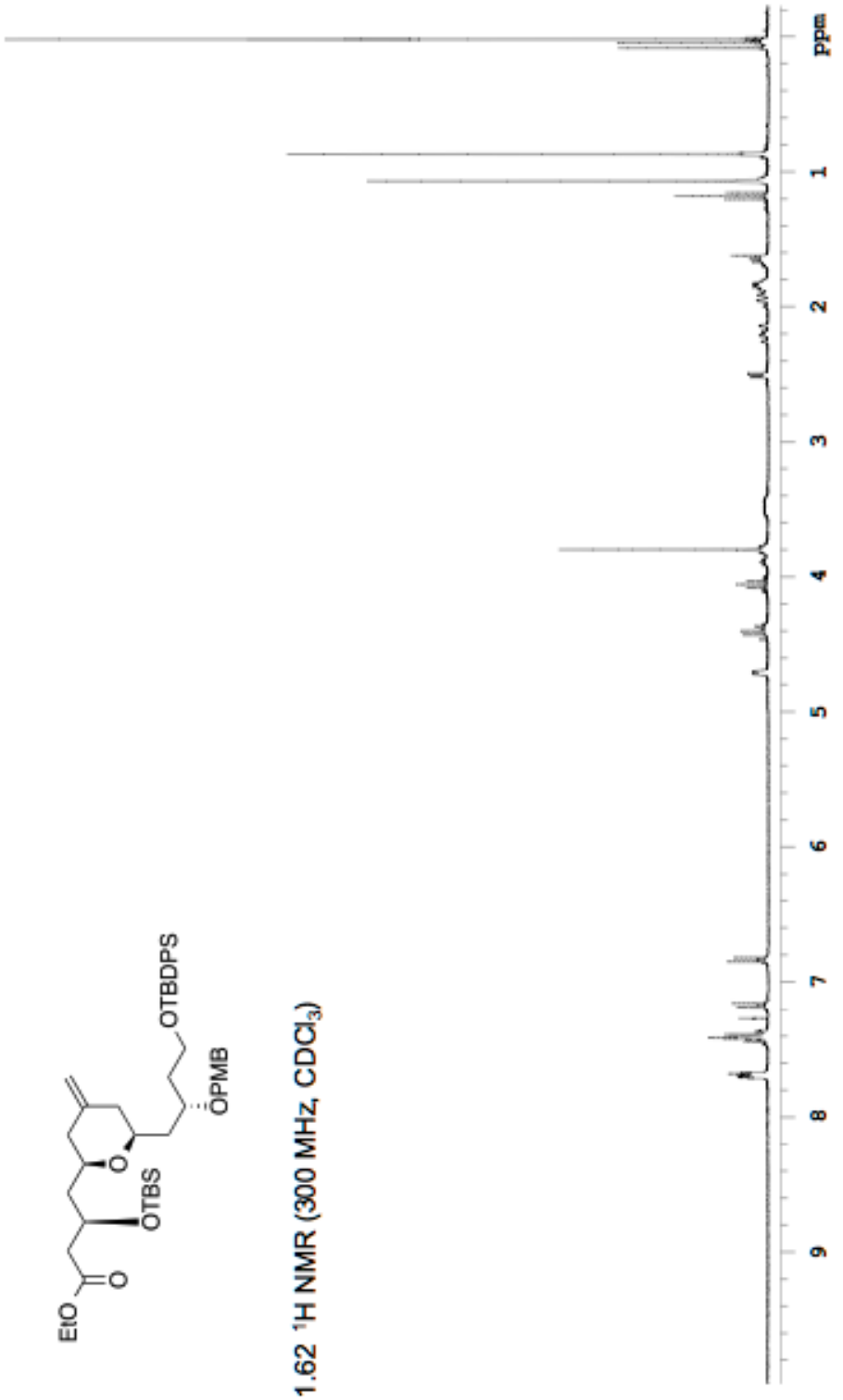
1.7 ^{13}C NMR (75 MHz, $CDCl_3$)



1.57 ^{1}H NMR (300 MHz, $CDCl_3$)



1.57 ^{13}C NMR (75 MHz, $CDCl_3$)



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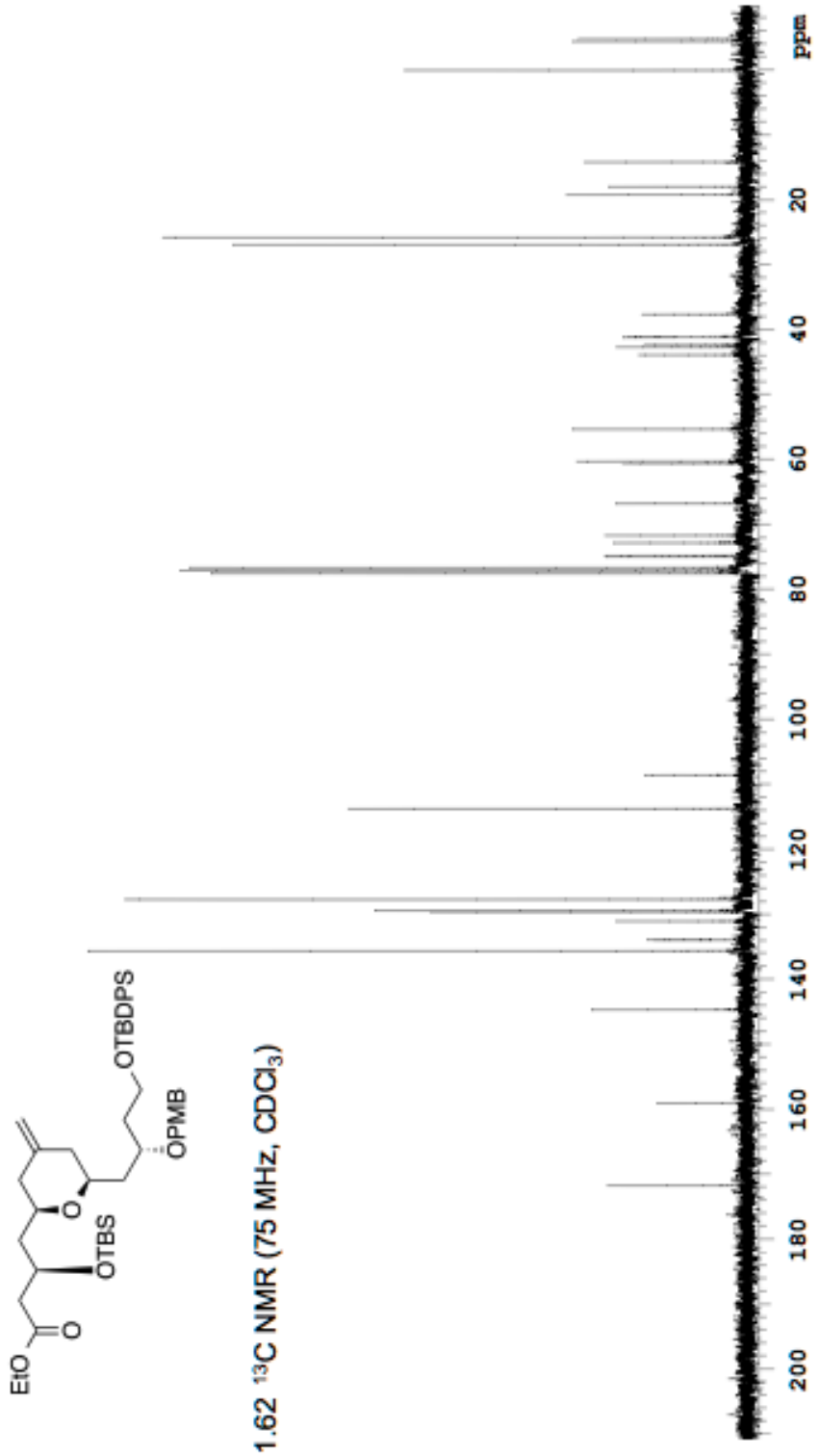
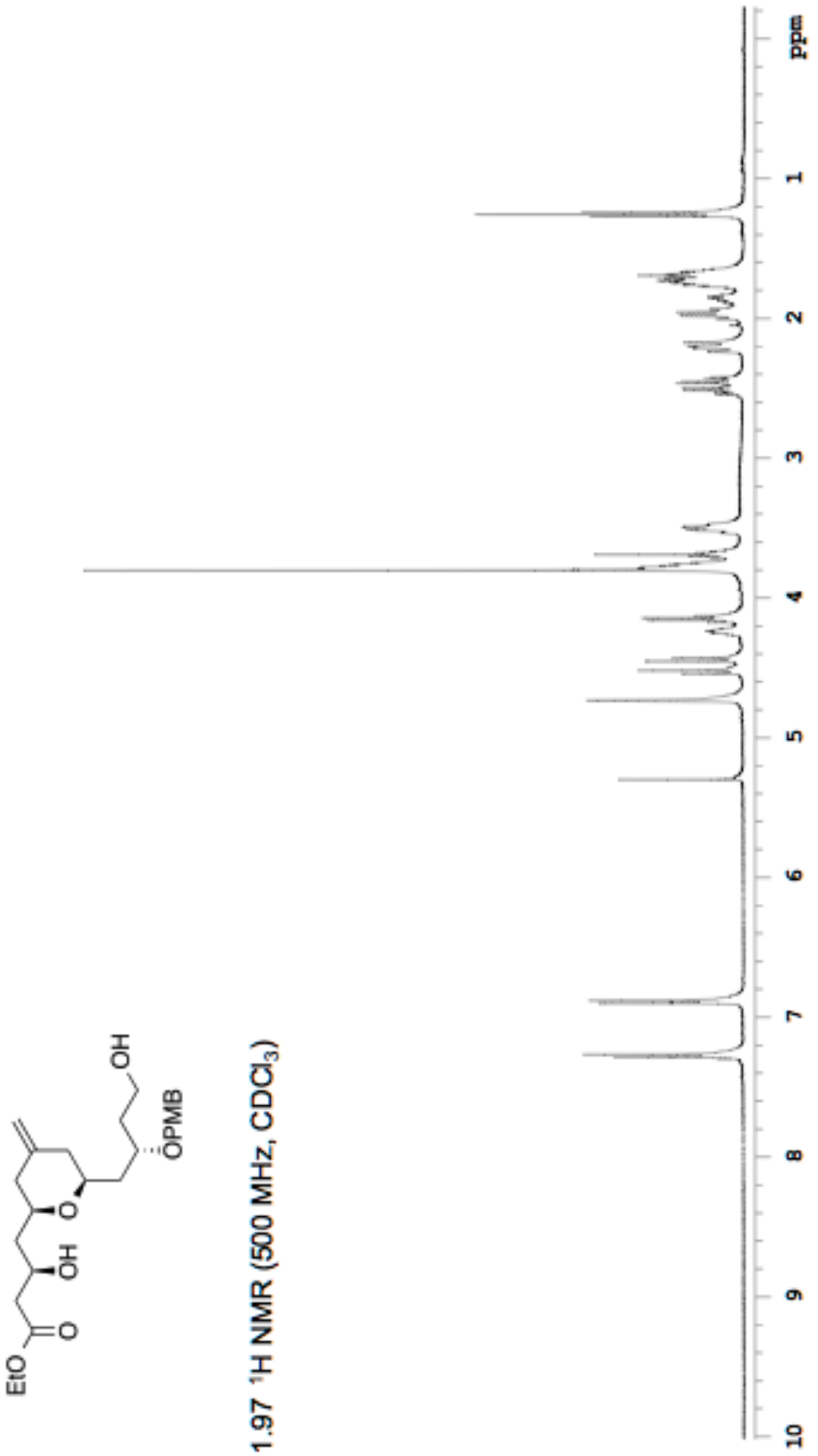


1.62 ^{13}C NMR (75 MHz, $CDCl_3$)



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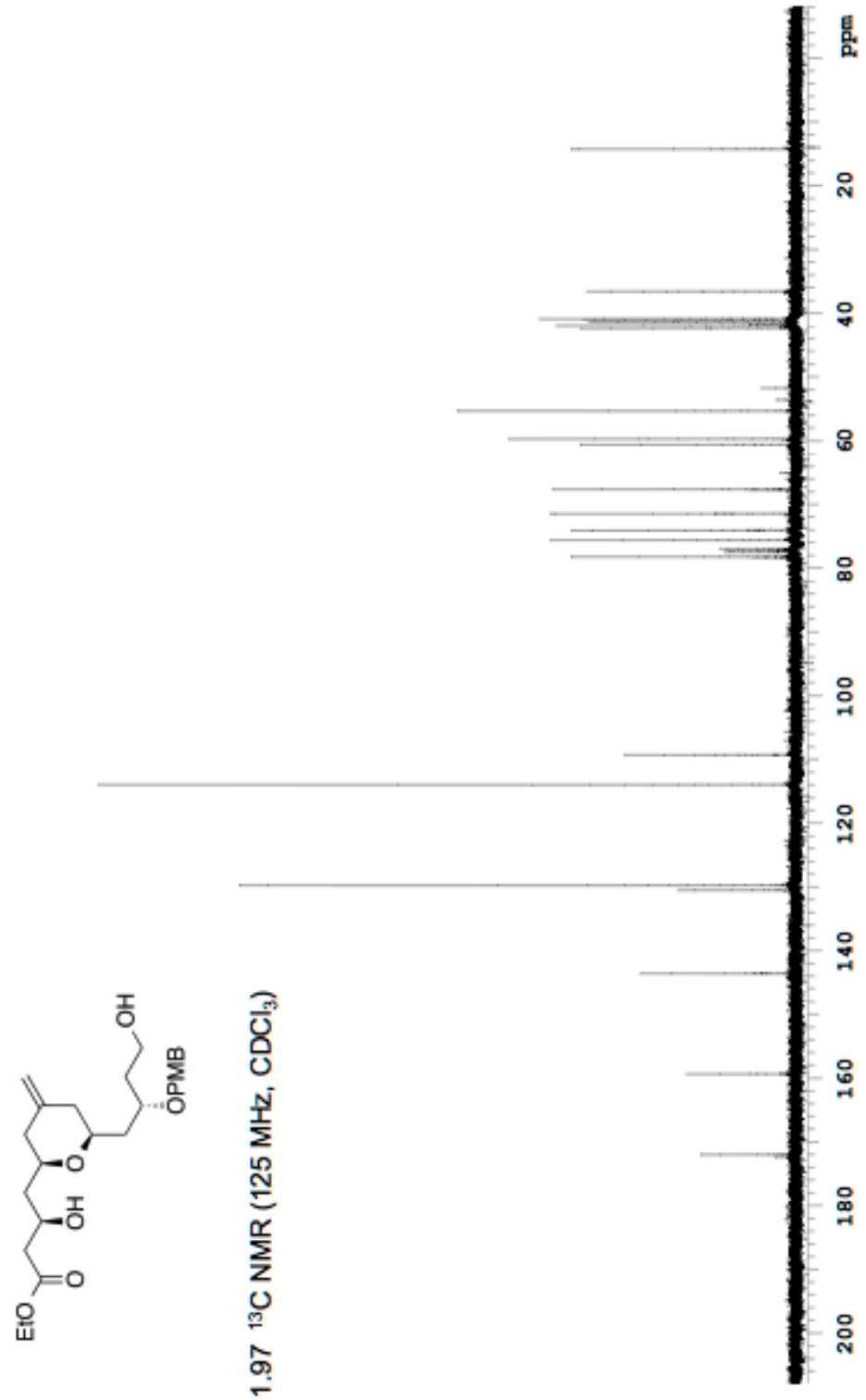
EtO
O
OH
O
OPMB
OH
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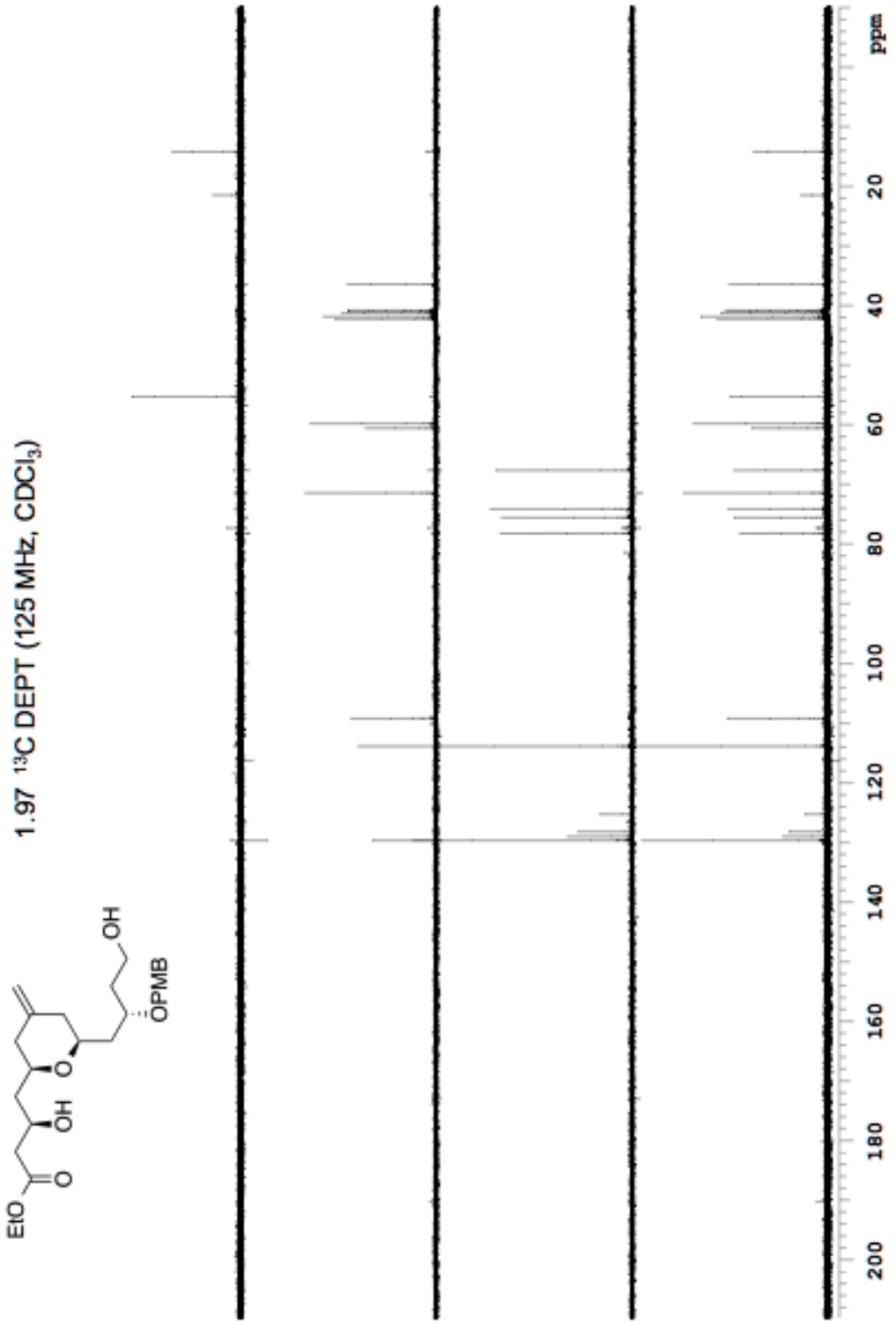
200
180
160
140
120
100
80
60
40
20
ppm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OH
O
OPMB
OH
ppm
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180
160
140
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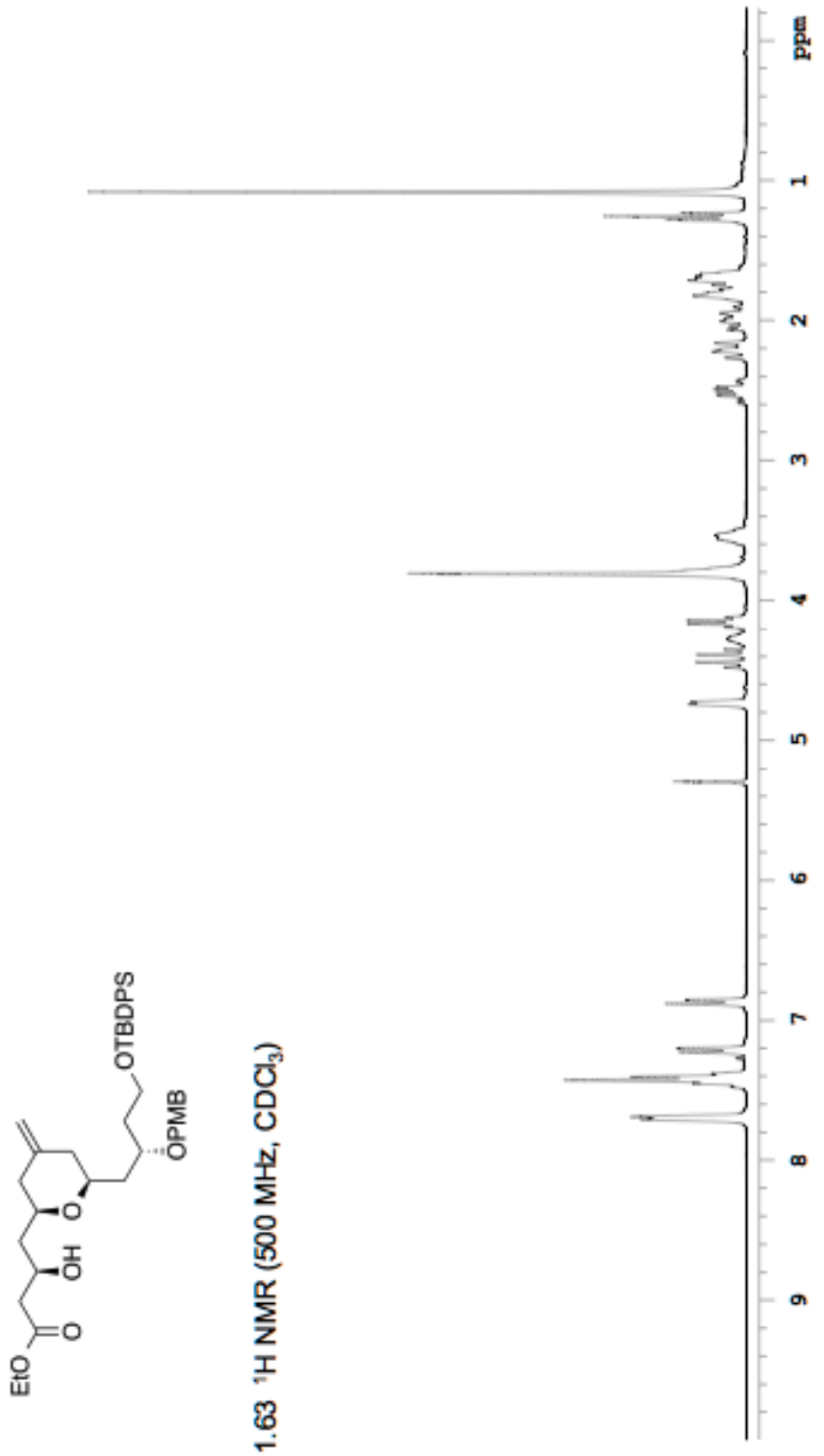
100
80
60
40
20



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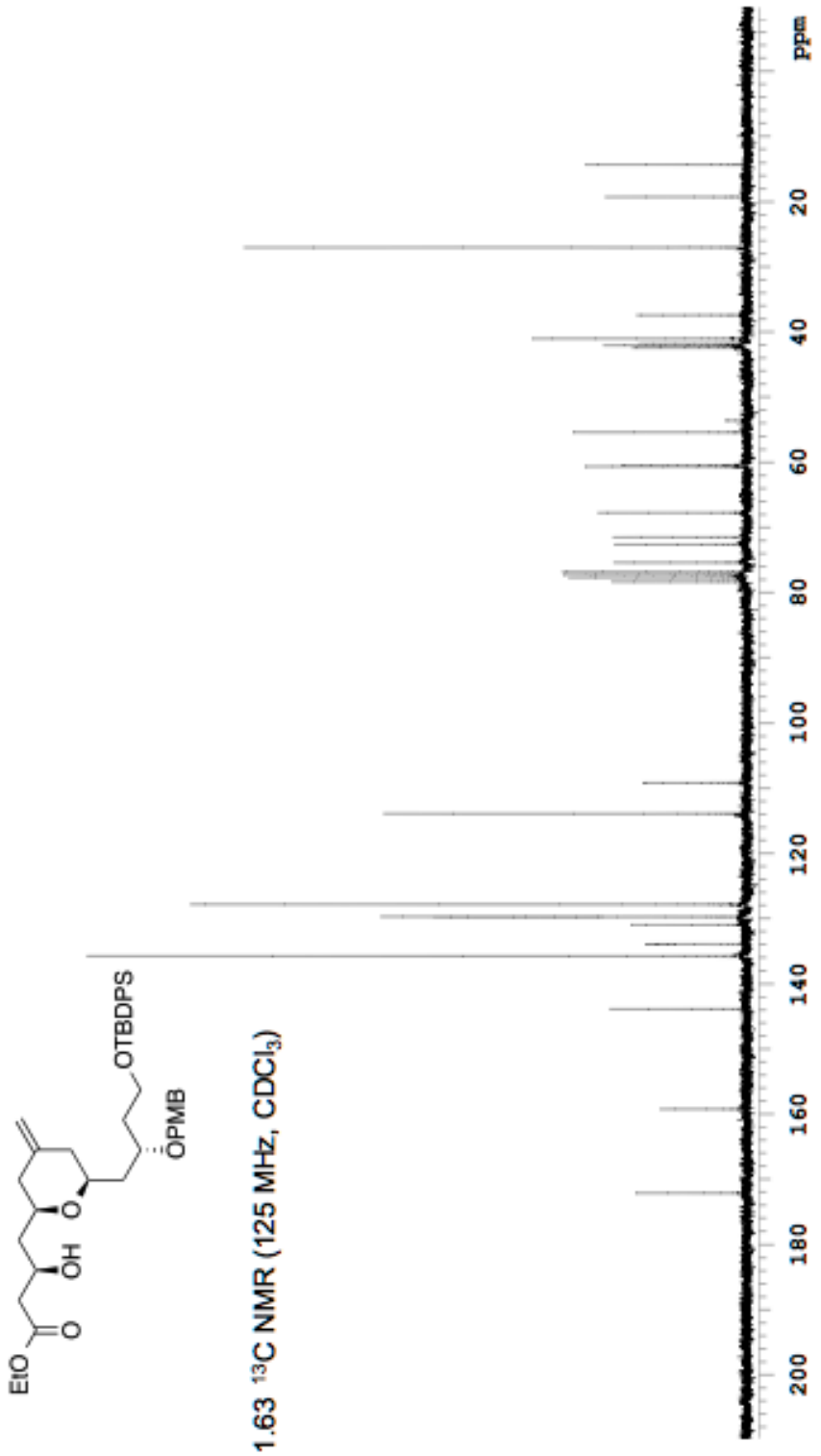

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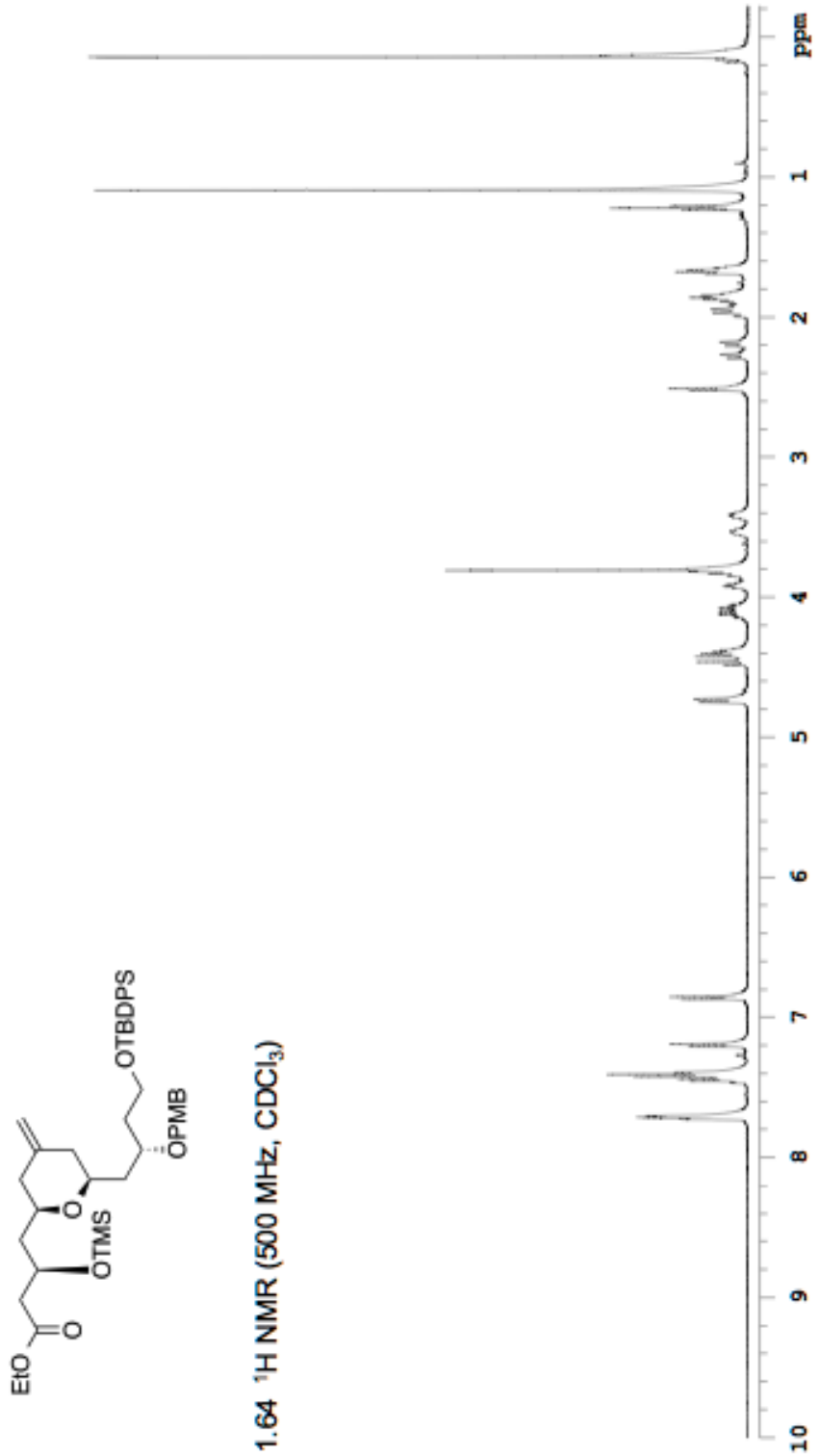

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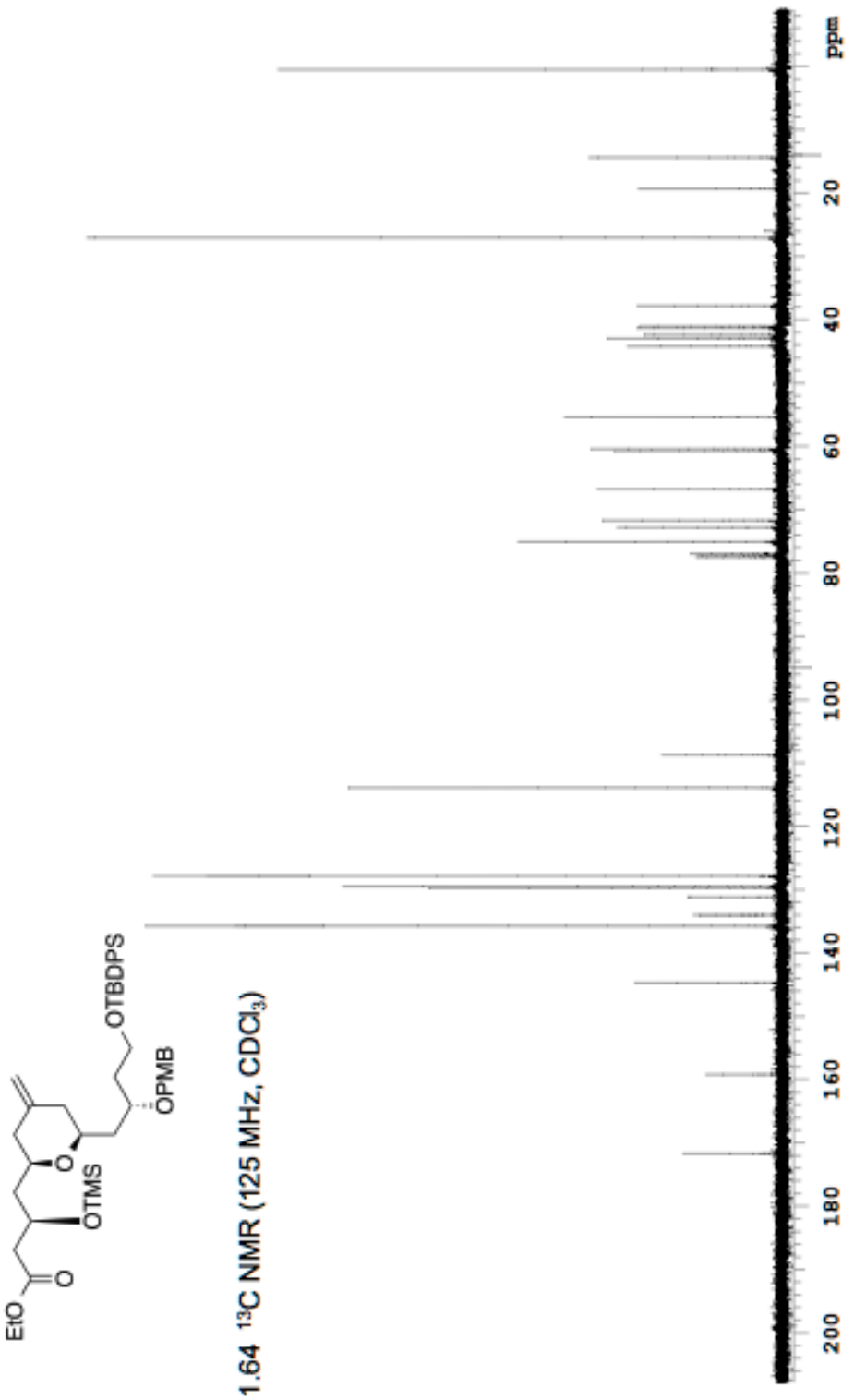
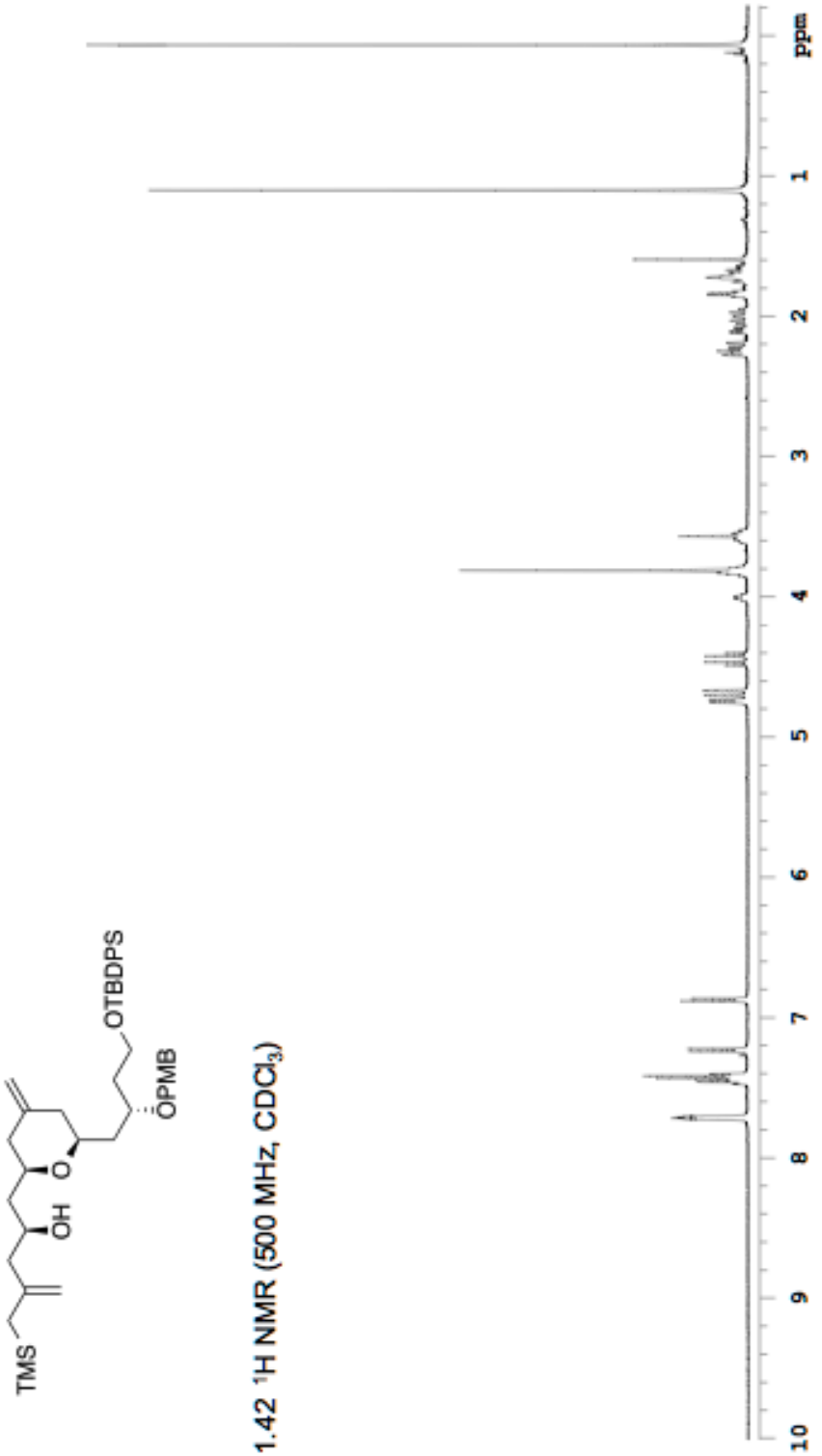


1.64 ^{13}C NMR (125 MHz, $CDCl_3$)



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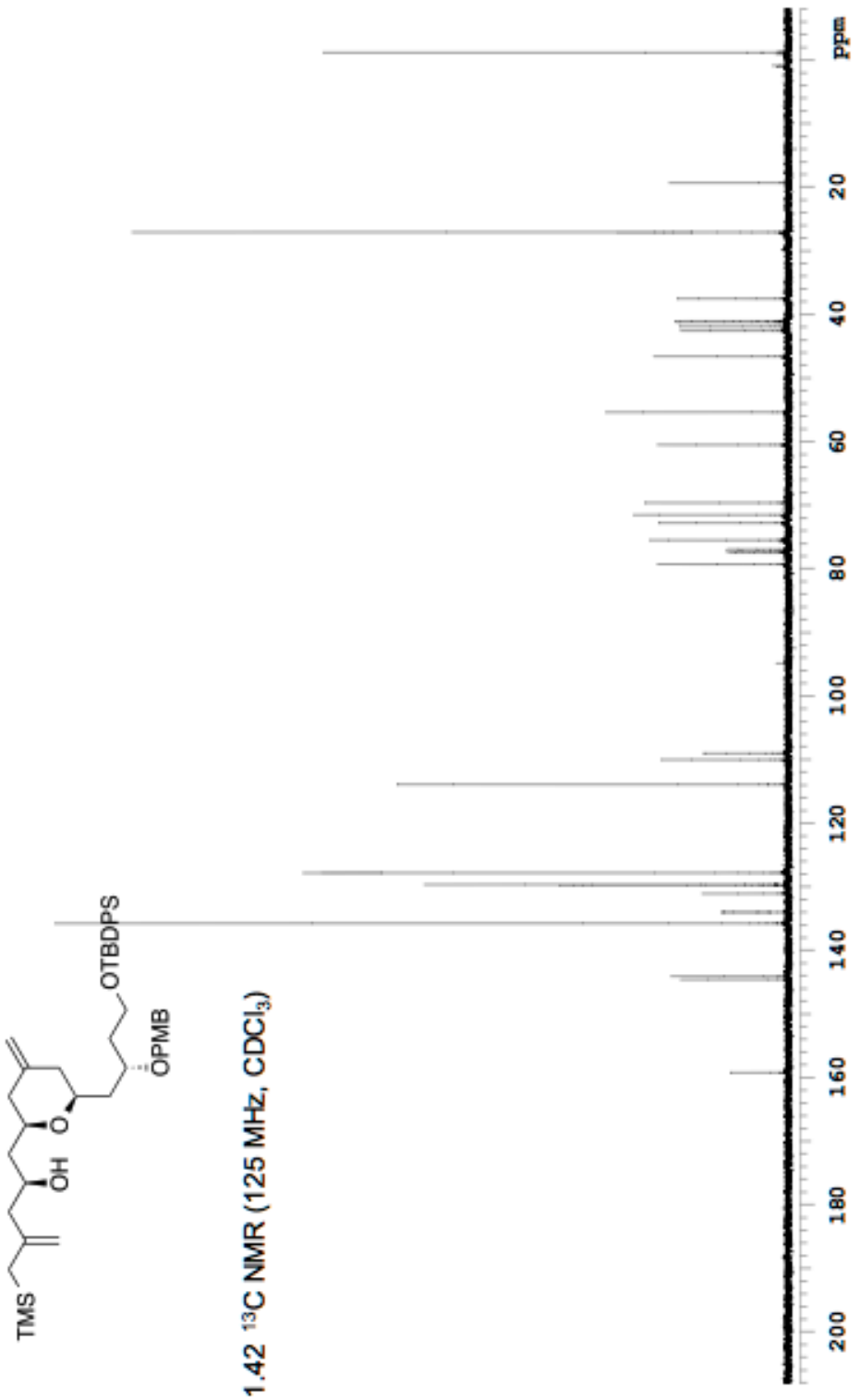
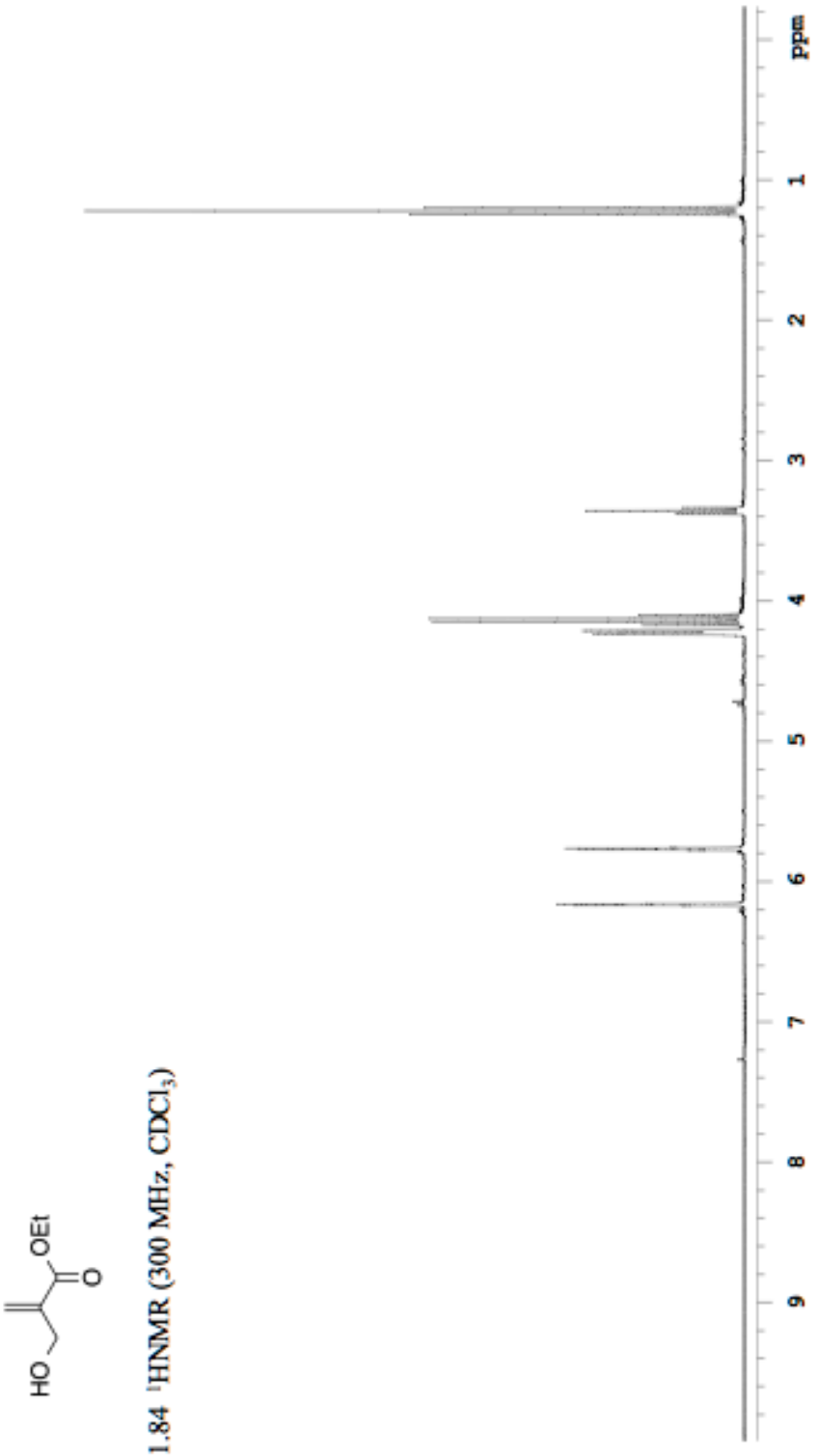

TMS
OH
O
OPMB
OTBDPS
1.42 ^{13}C NMR (125 MHz, $CDCl_3$)
200
180
160
140
120
100
80
60
40
20
ppm



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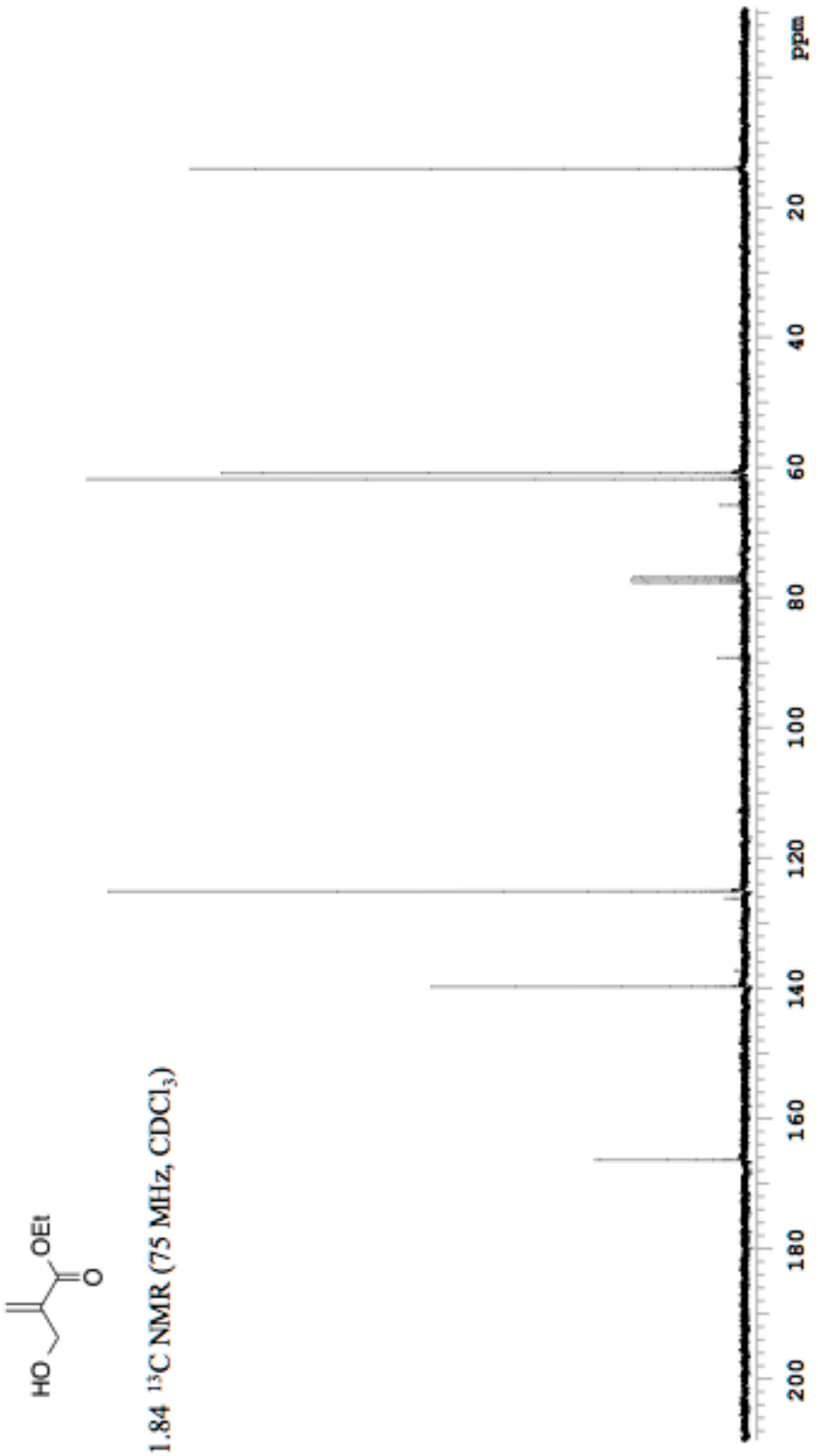

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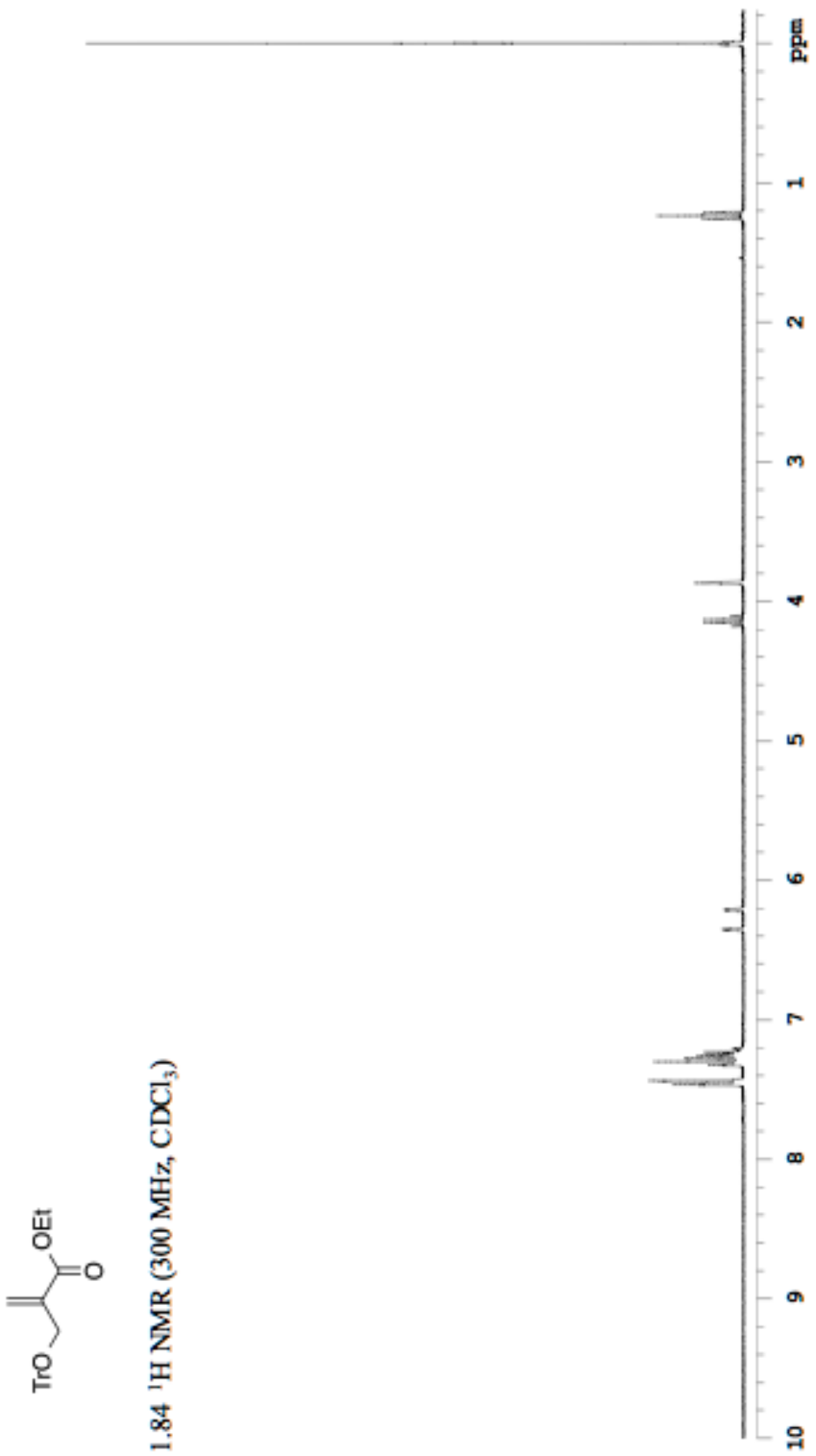
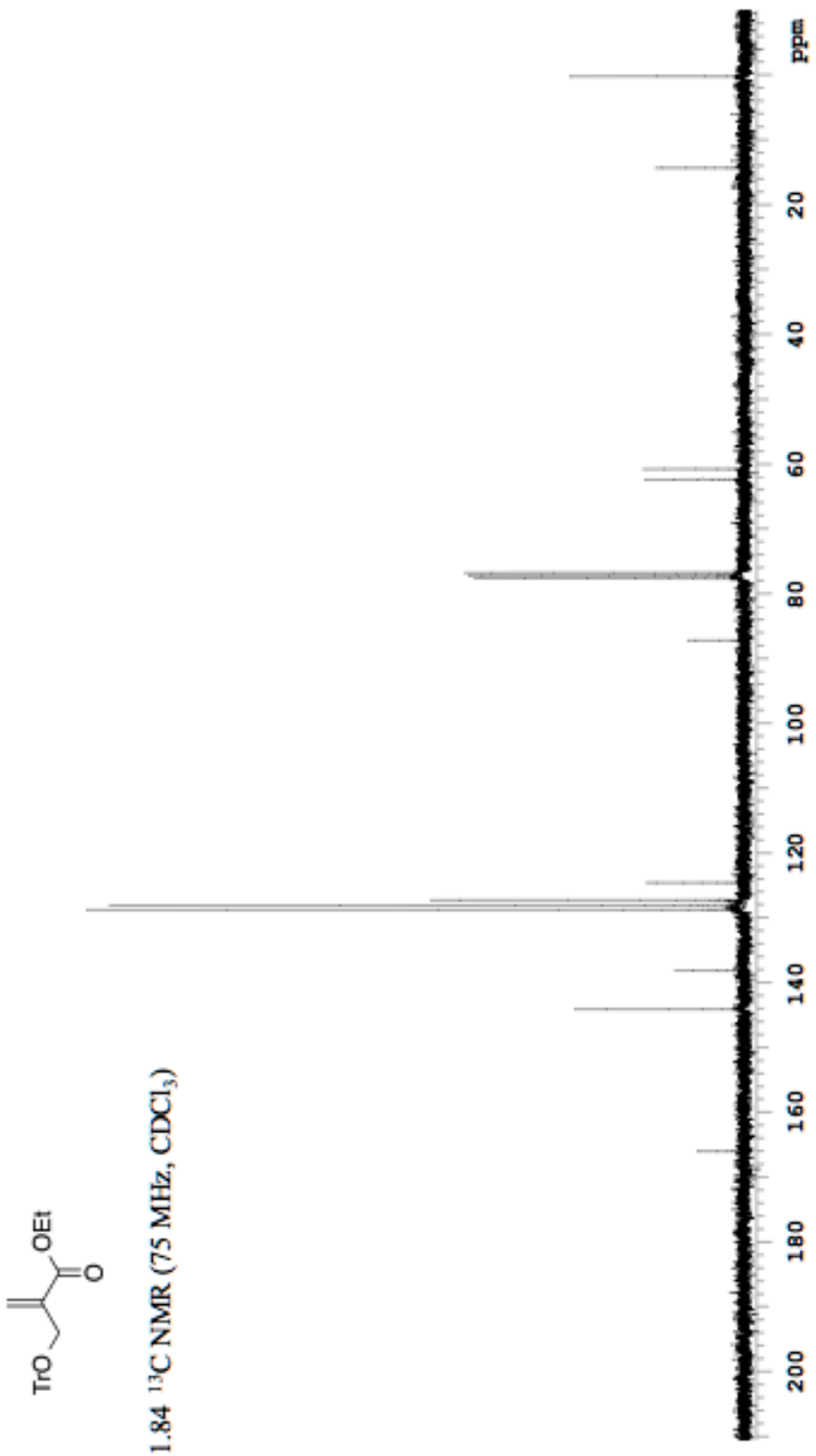
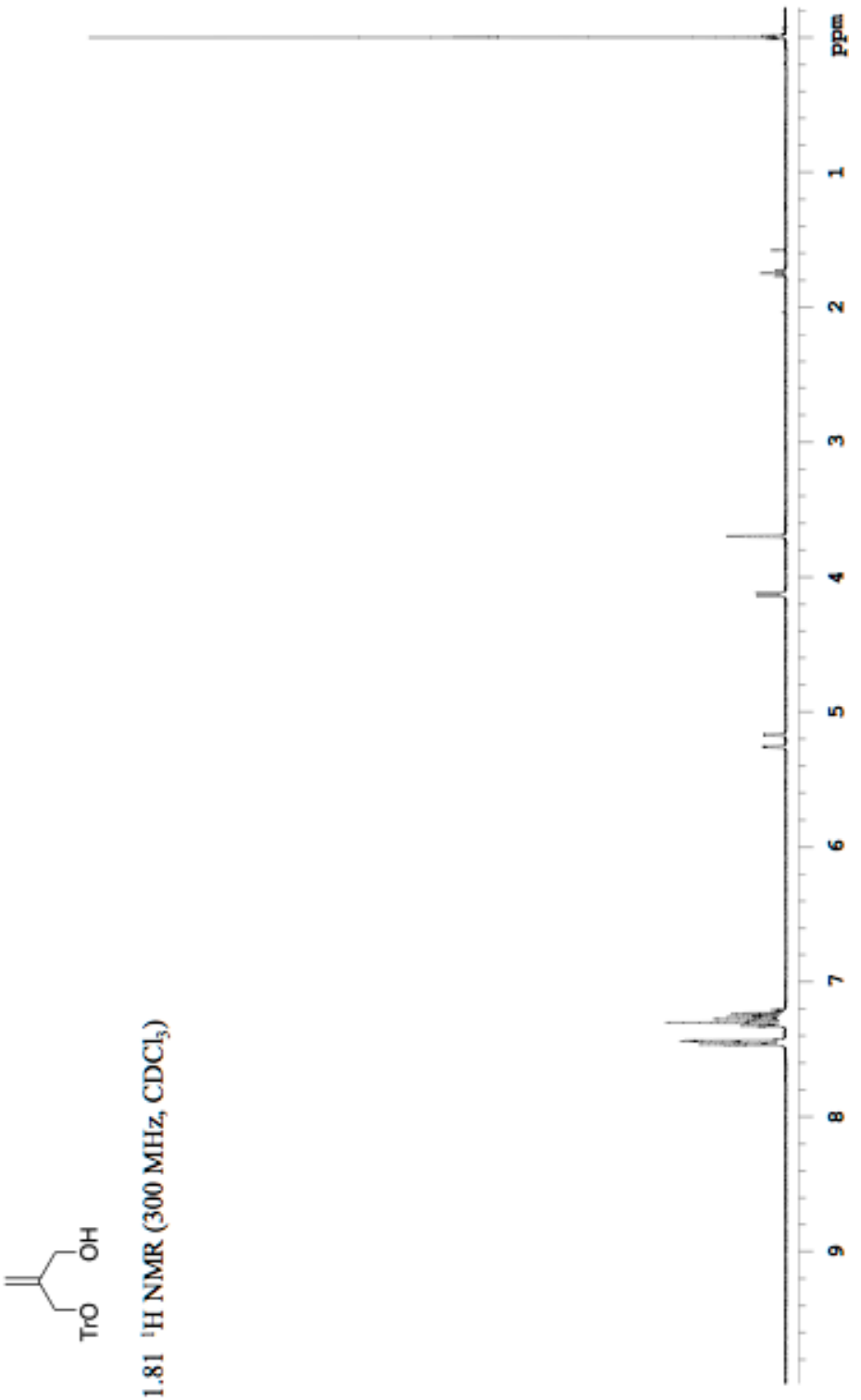


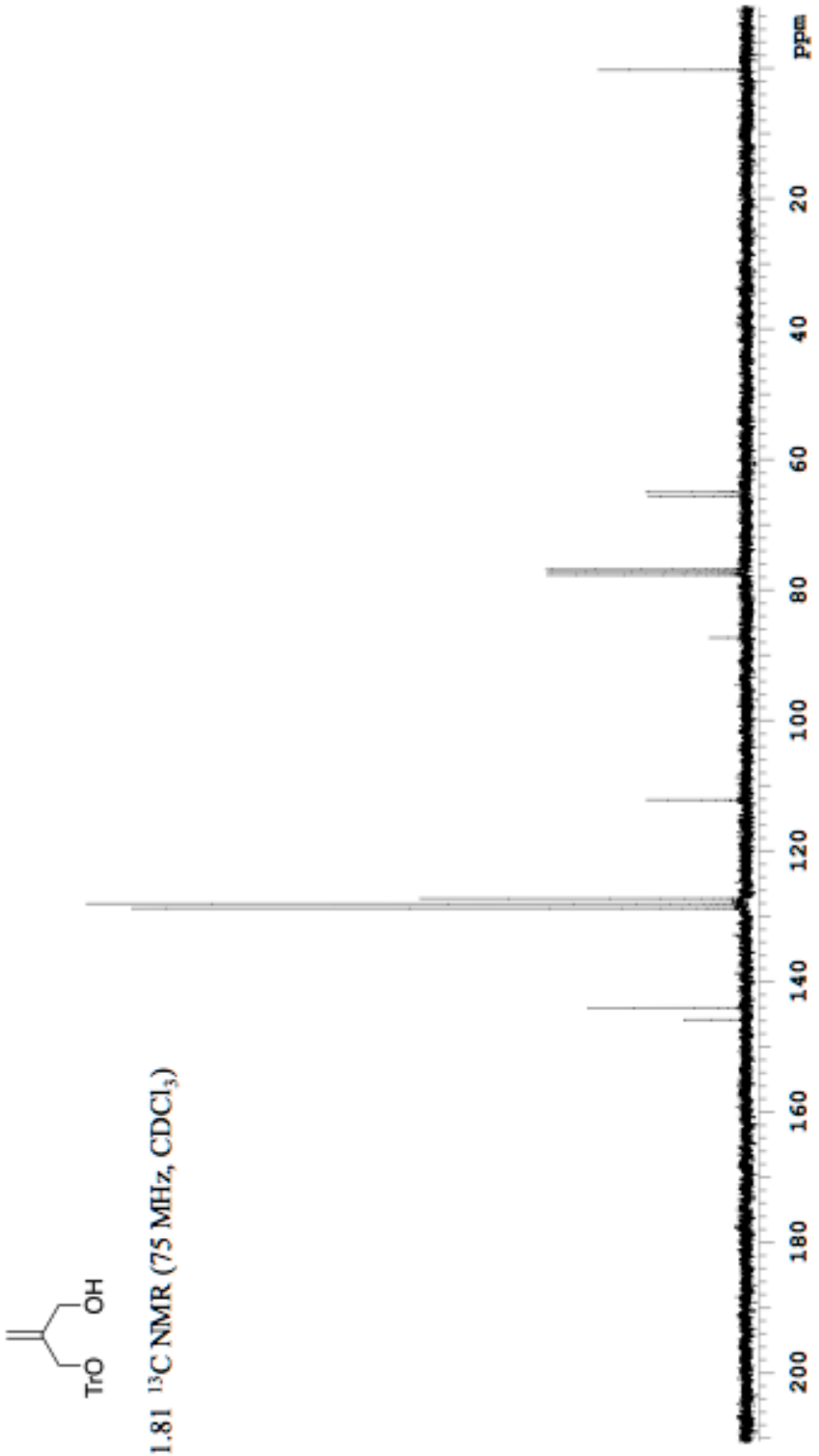
1.84 ^{1}H NMR (300 MHz, $CDCl_3$)



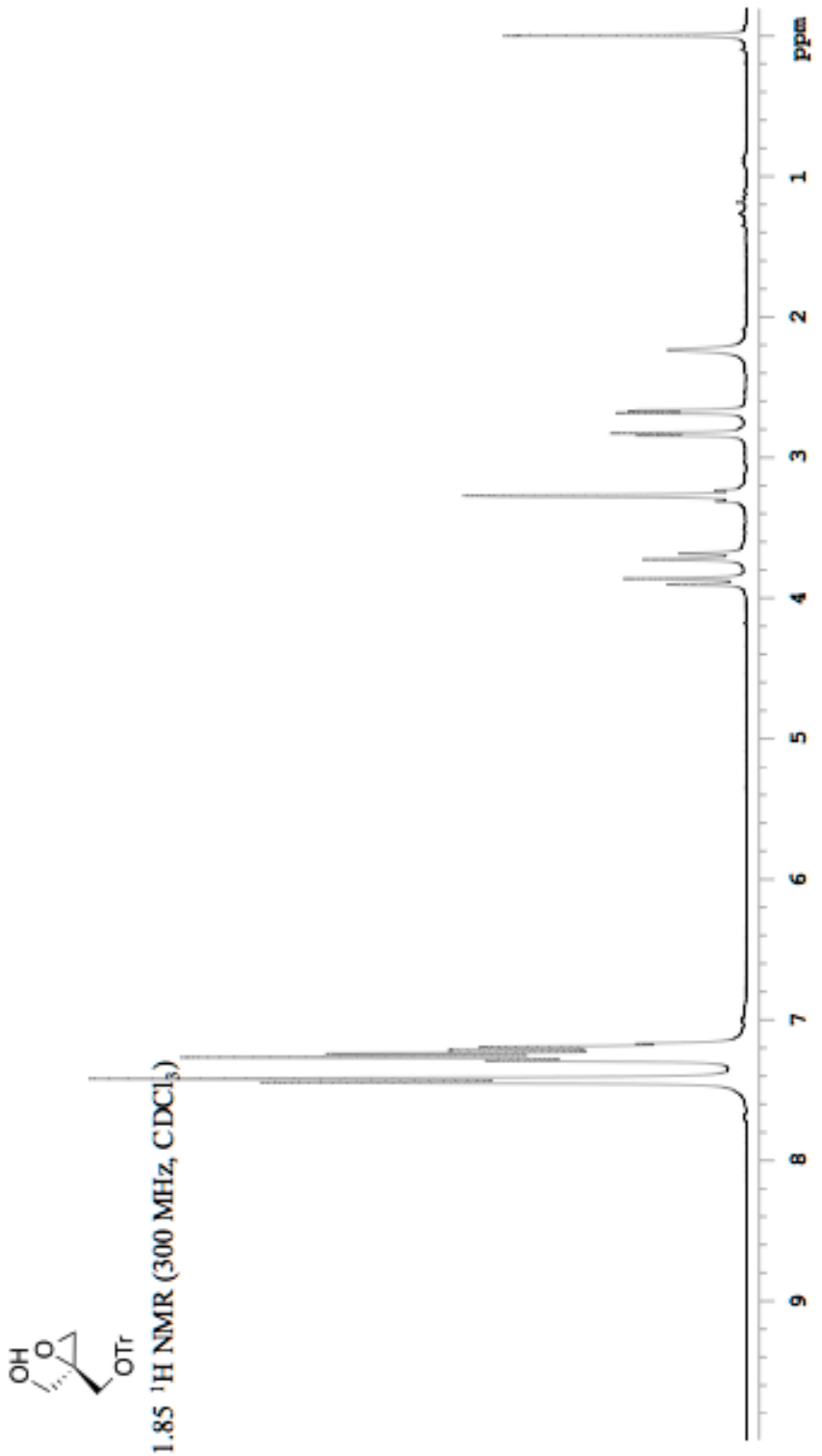
1.84 ^{13}C NMR (75 MHz, $CDCl_3$)



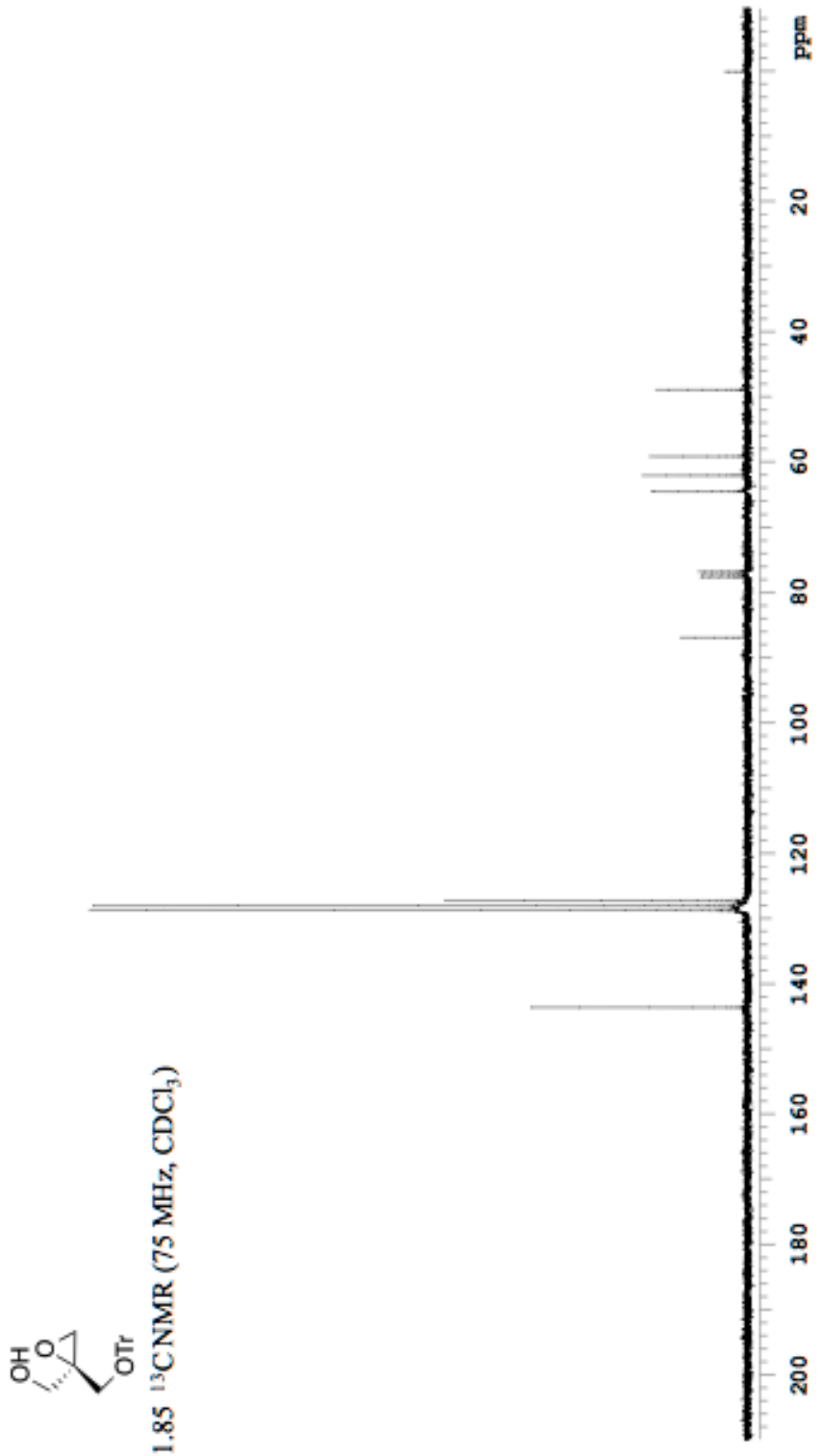
1.81 ^{1}H NMR (300 MHz, $CDCl_3$)



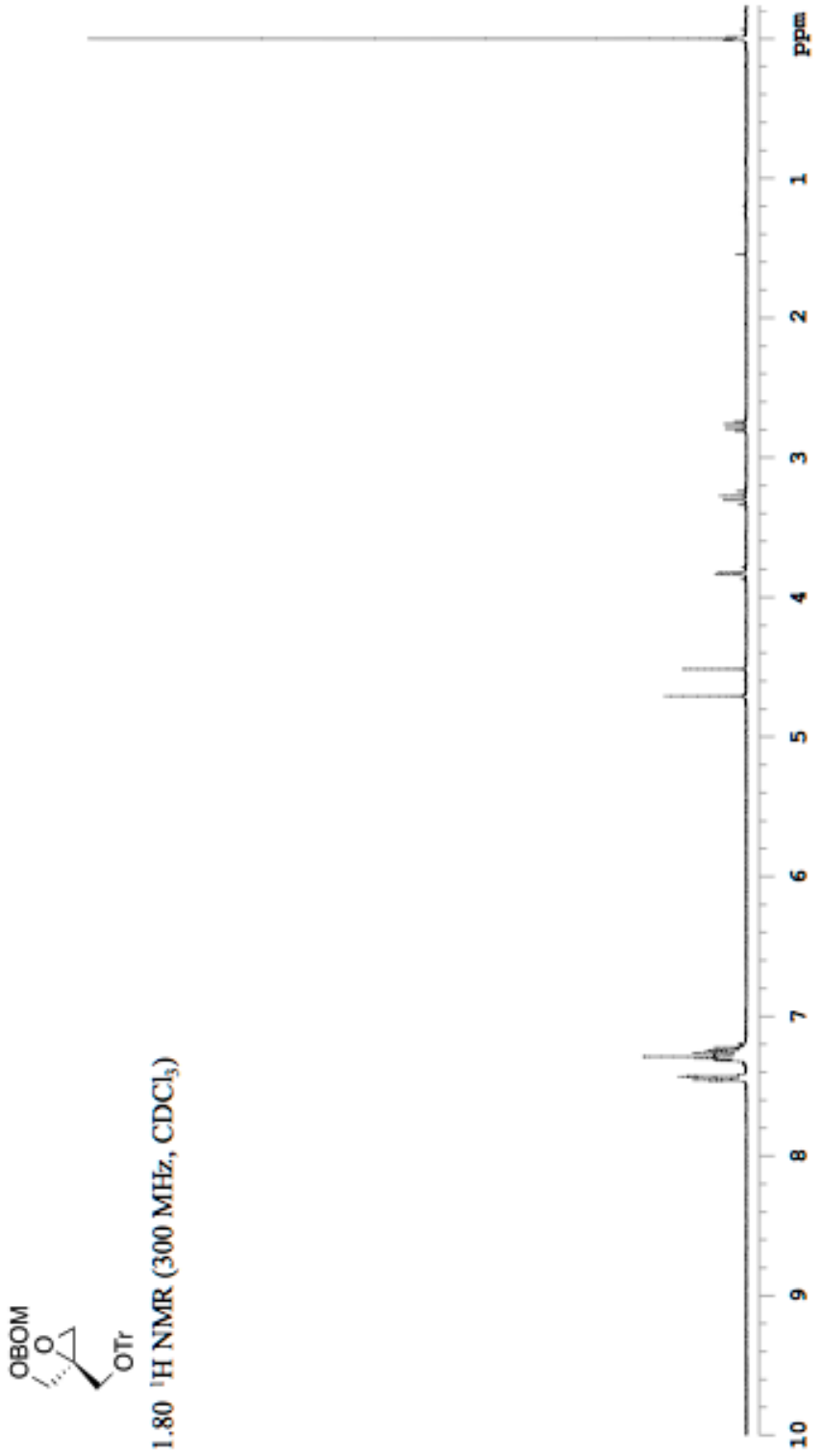
1.81 ^{13}C NMR (75 MHz, $CDCl_3$)



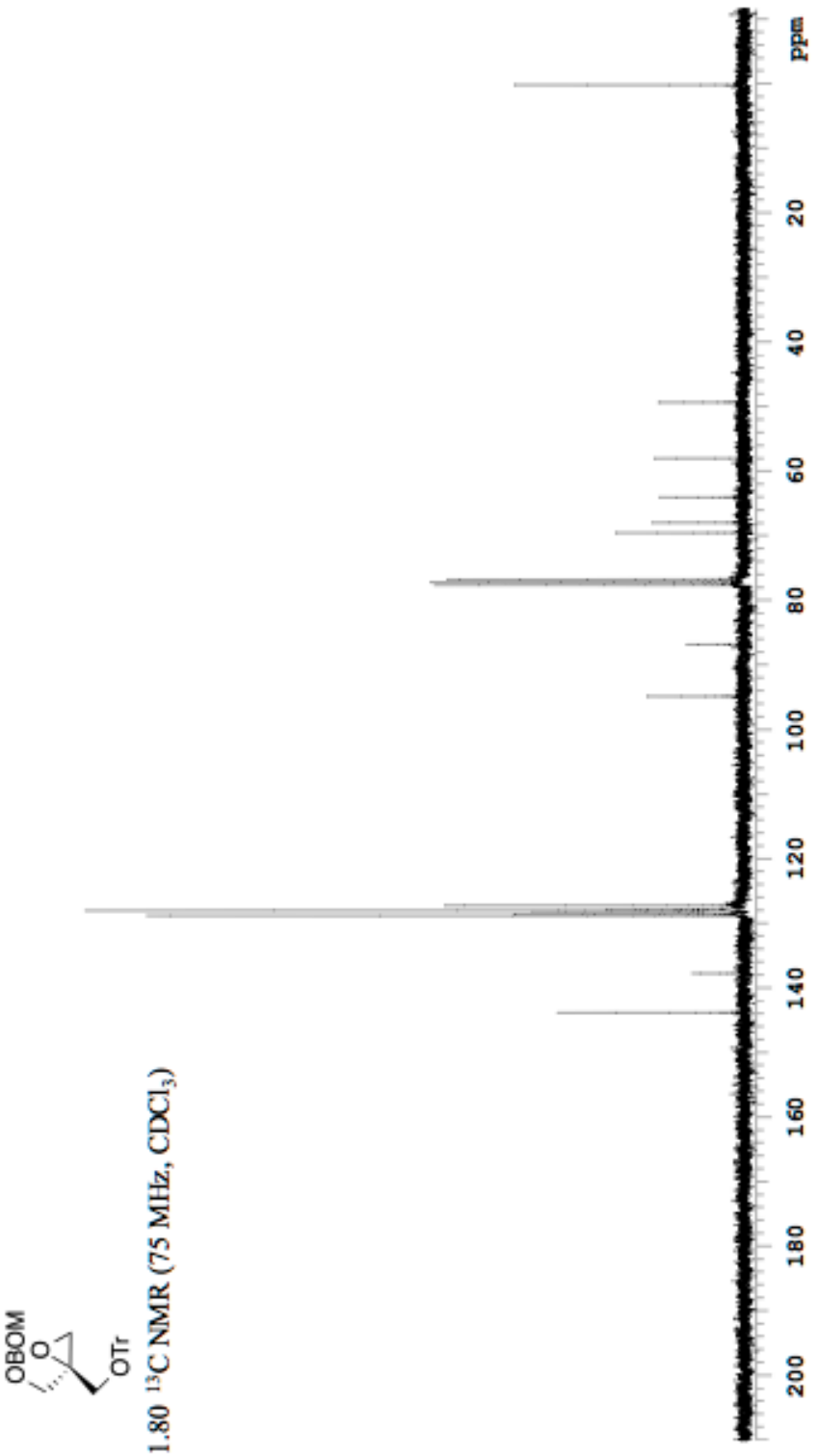
1.85 ^{1}H NMR (300 MHz, $CDCl_3$)



1.85 ^{13}C NMR (75 MHz, $CDCl_3$)

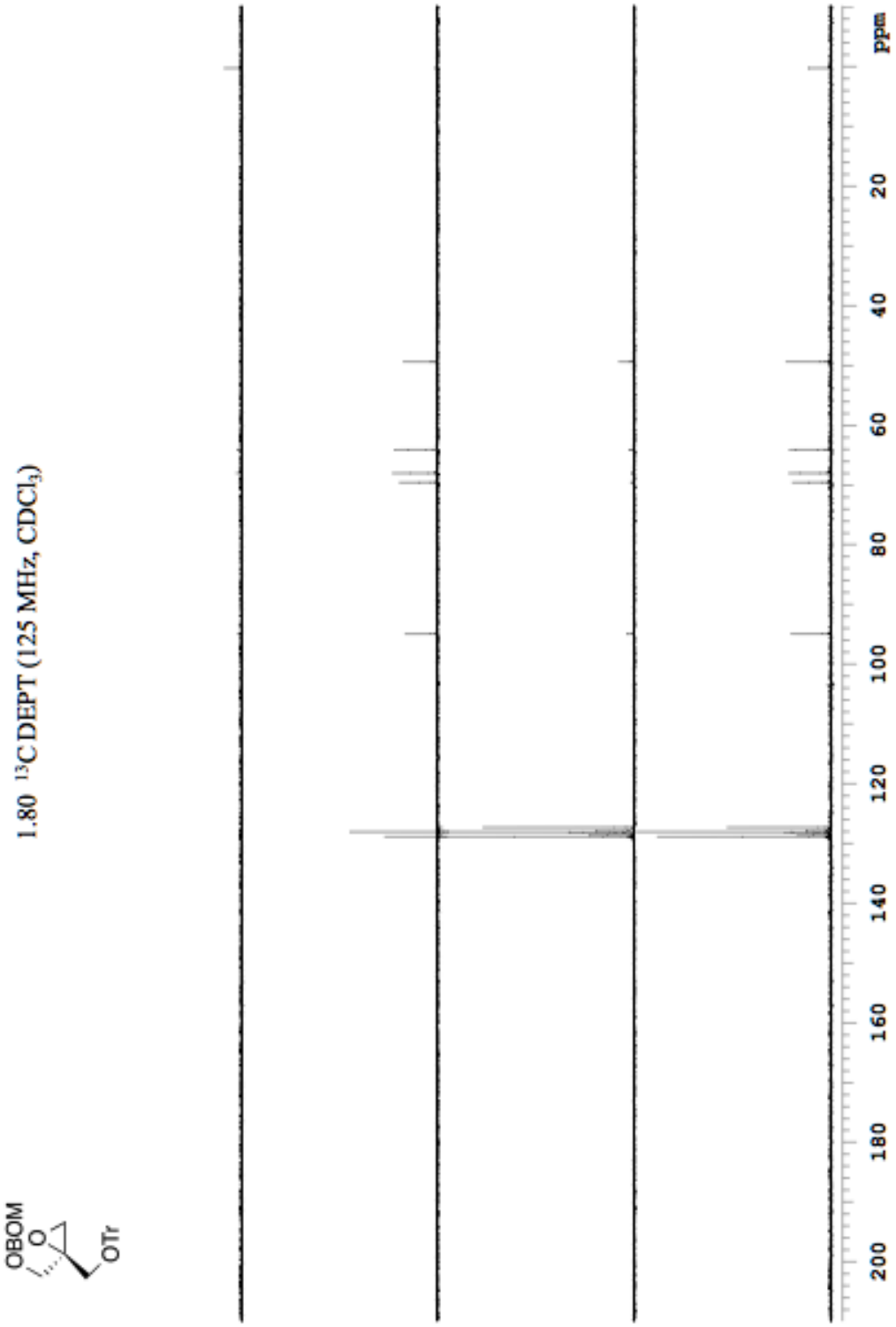


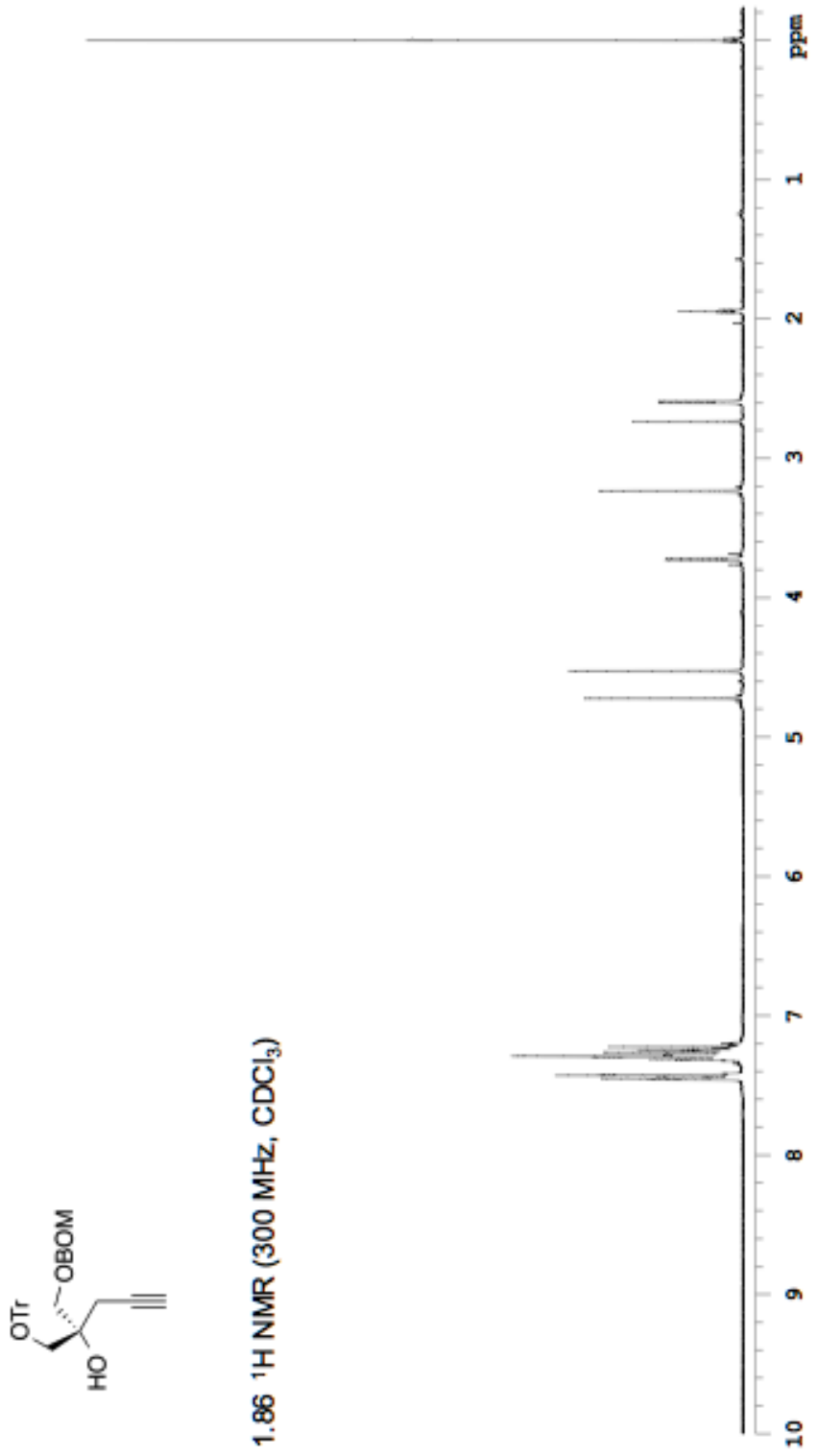
1.80 ^{1}H NMR (300 MHz, $CDCl_3$)



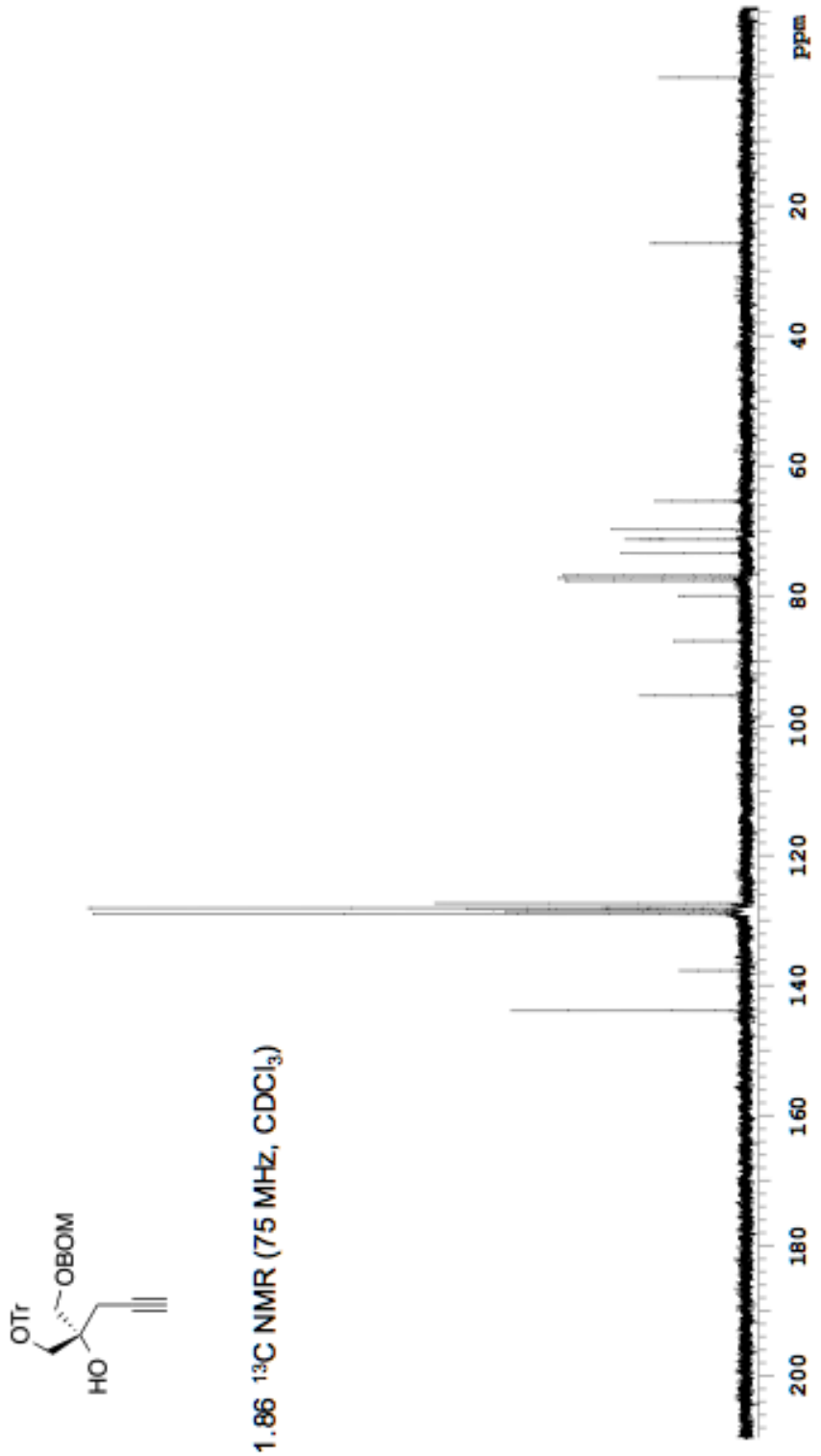
1.80 ^{13}C NMR (75 MHz, $CDCl_3$)

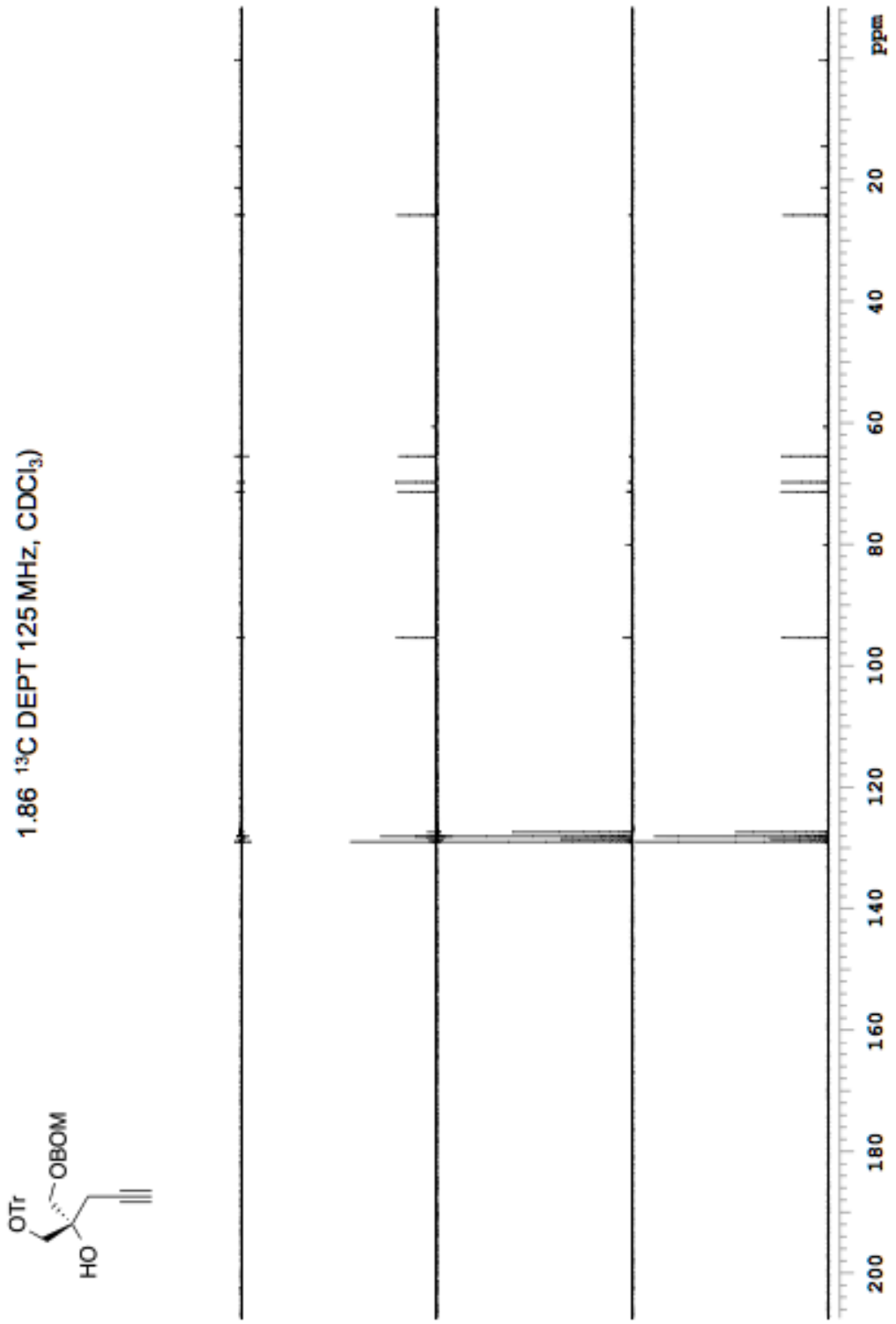
1.80 ^{13}C DEPT (125 MHz, $CDCl_3$)

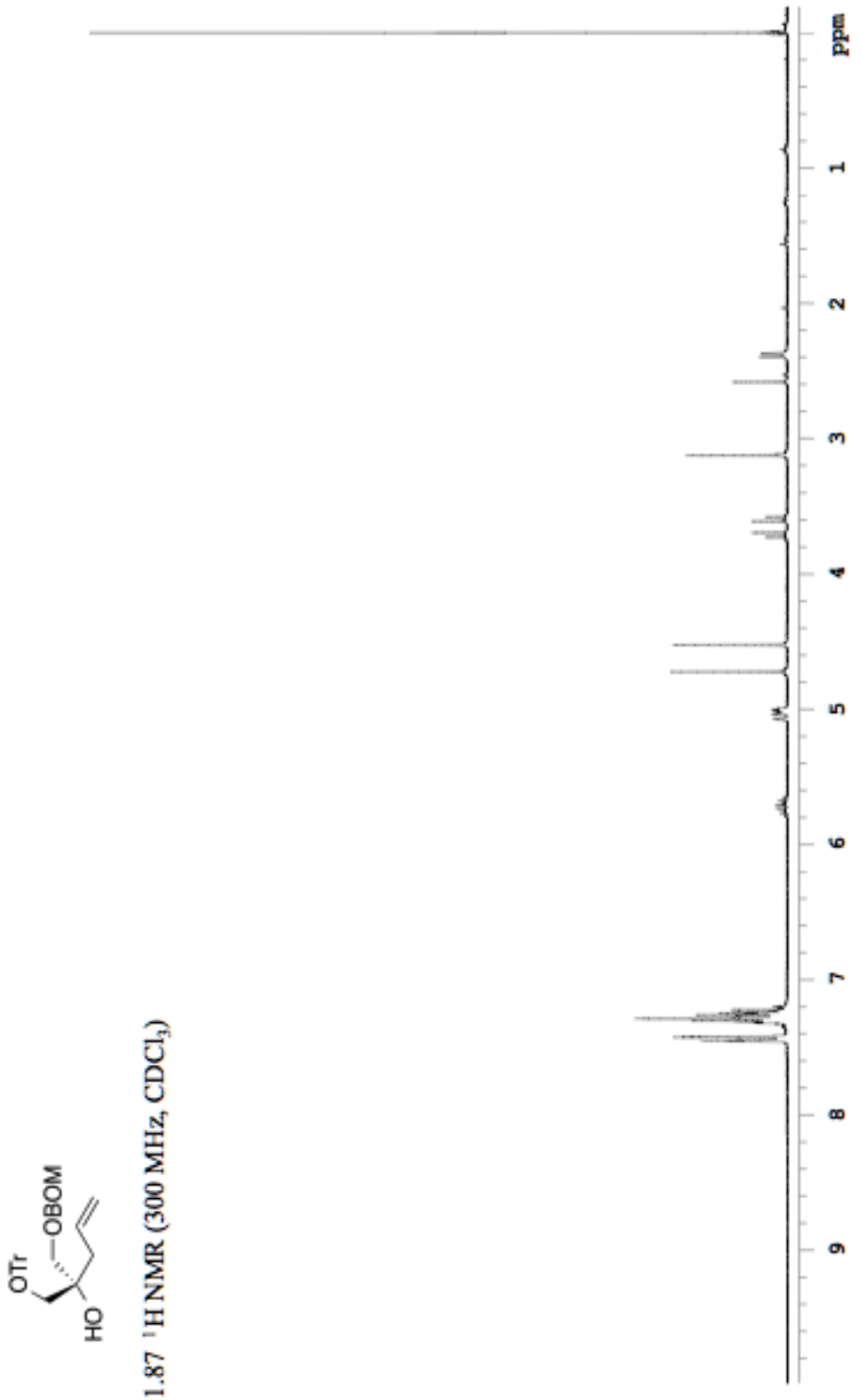




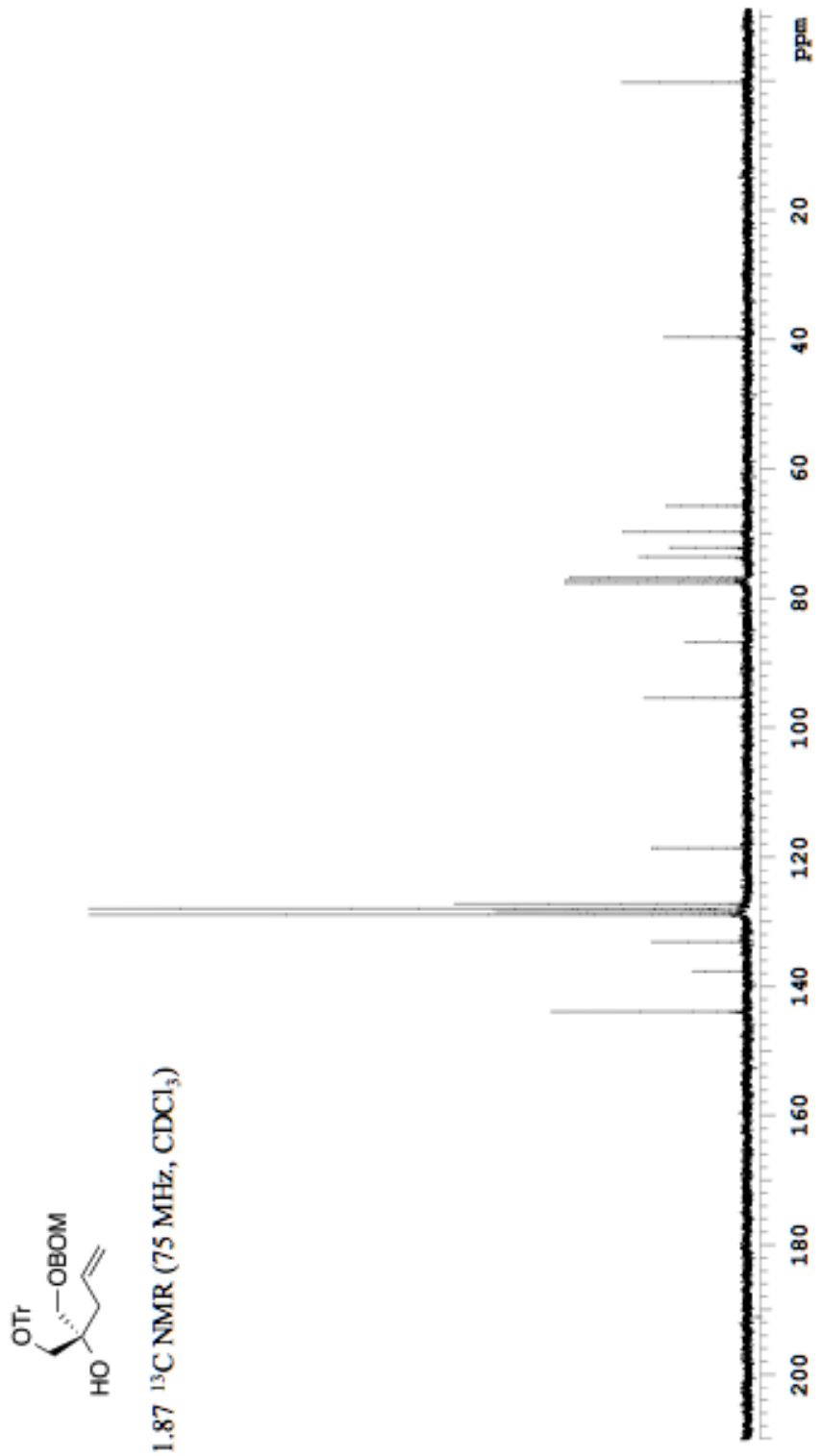
1.86 ^{1}H NMR (300 MHz, $CDCl_3$)


OTr
OBOM
HO
1.86 ^{13}C NMR (75 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20

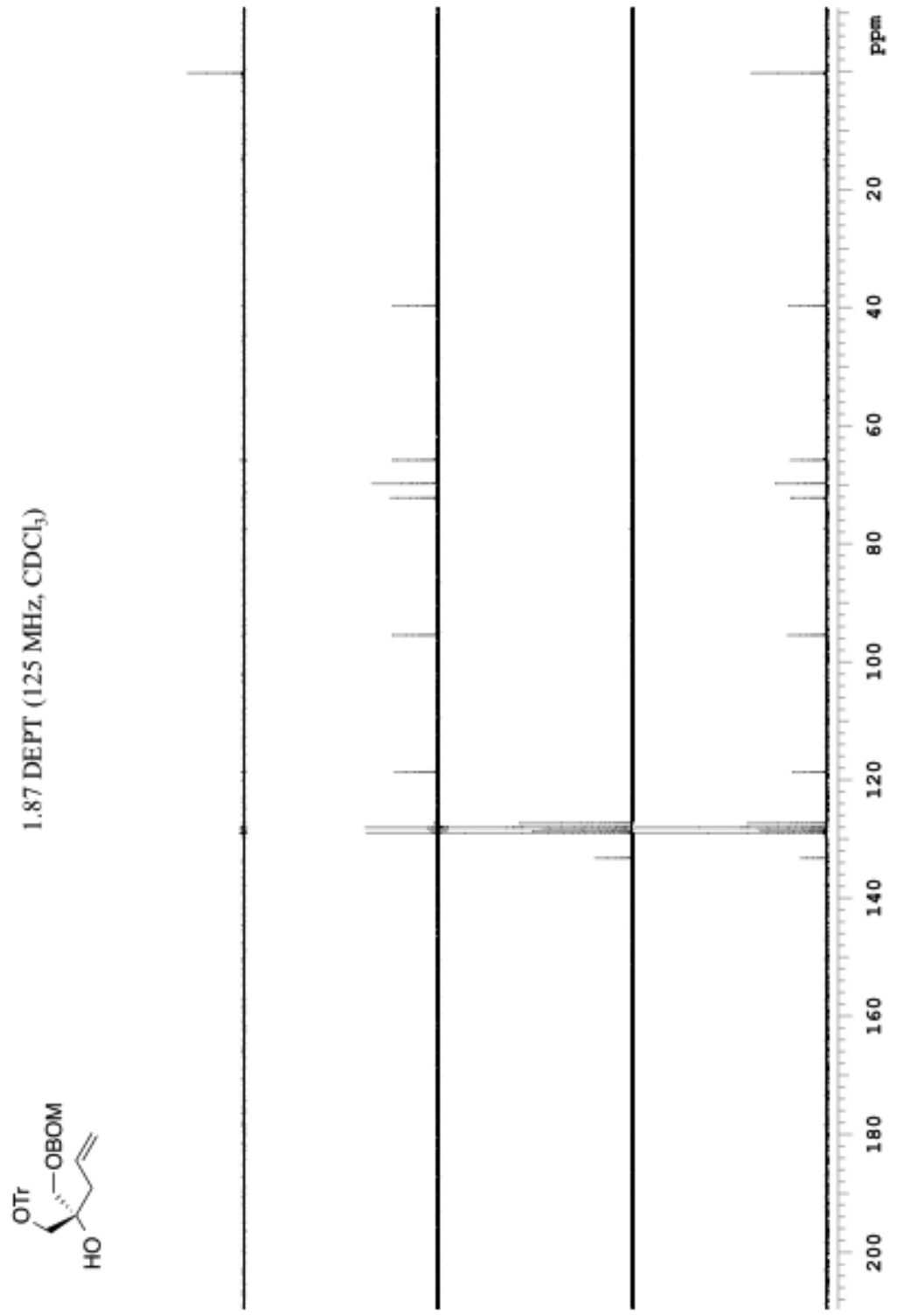

1.86 ^{13}C DEPT 125 MHz, $CDCl_3$)
OTr
OBOM
HO
200
180
160
140
120
100
80
60
40
20
ppm



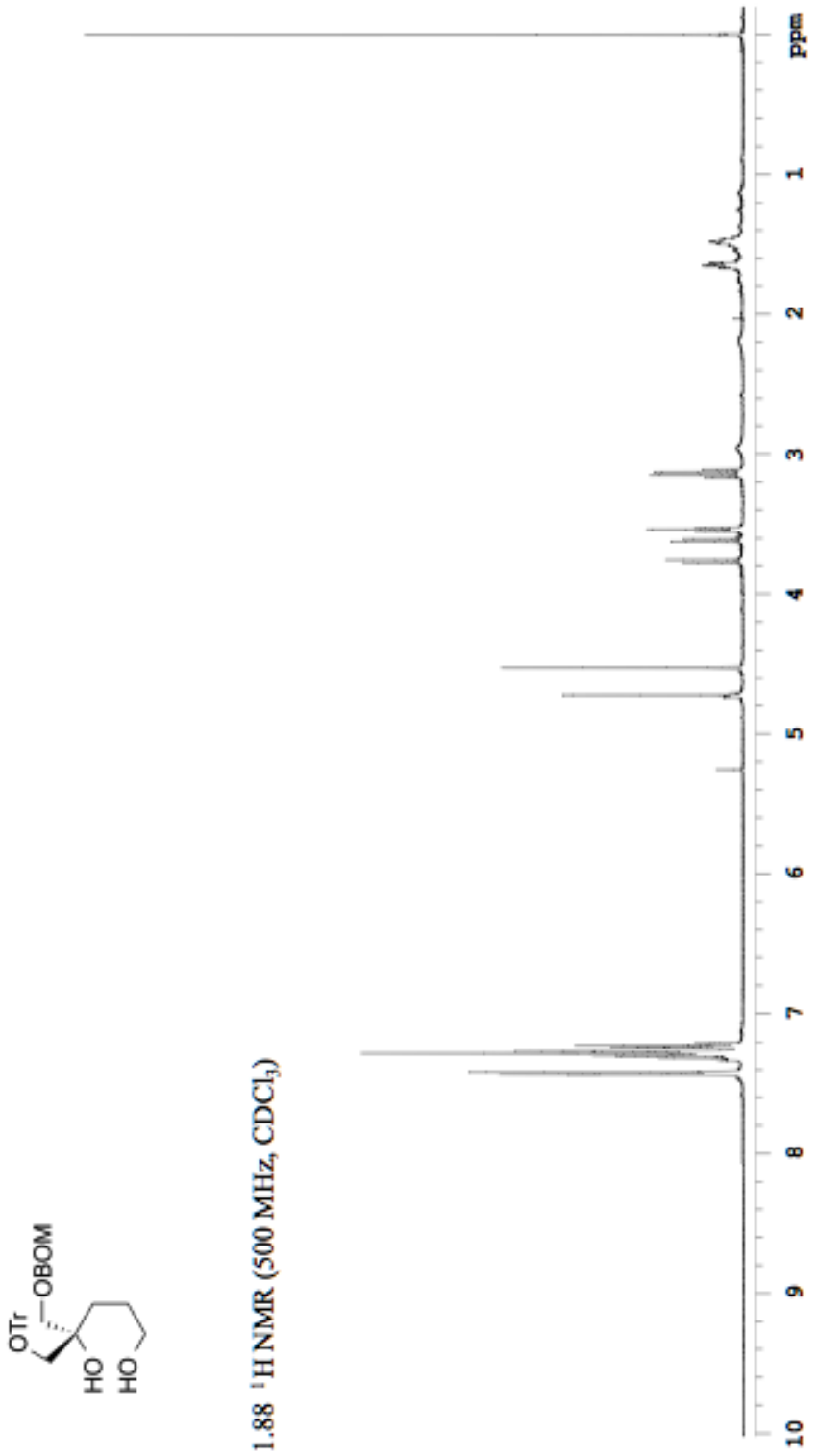
1.87 ^{1}H NMR (300 MHz, $CDCl_3$)



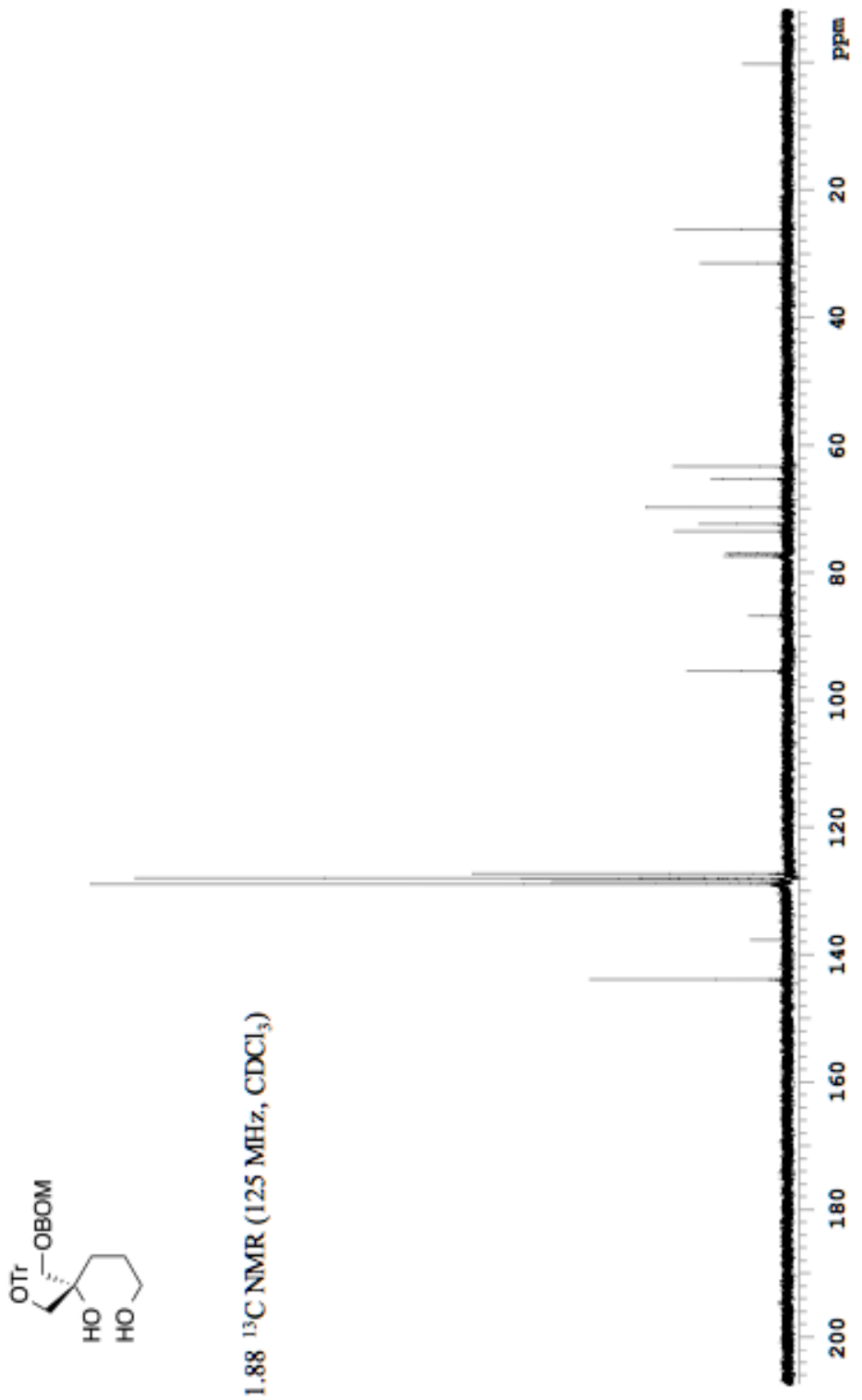
1.87 ^{13}C NMR (75 MHz, $CDCl_3$)



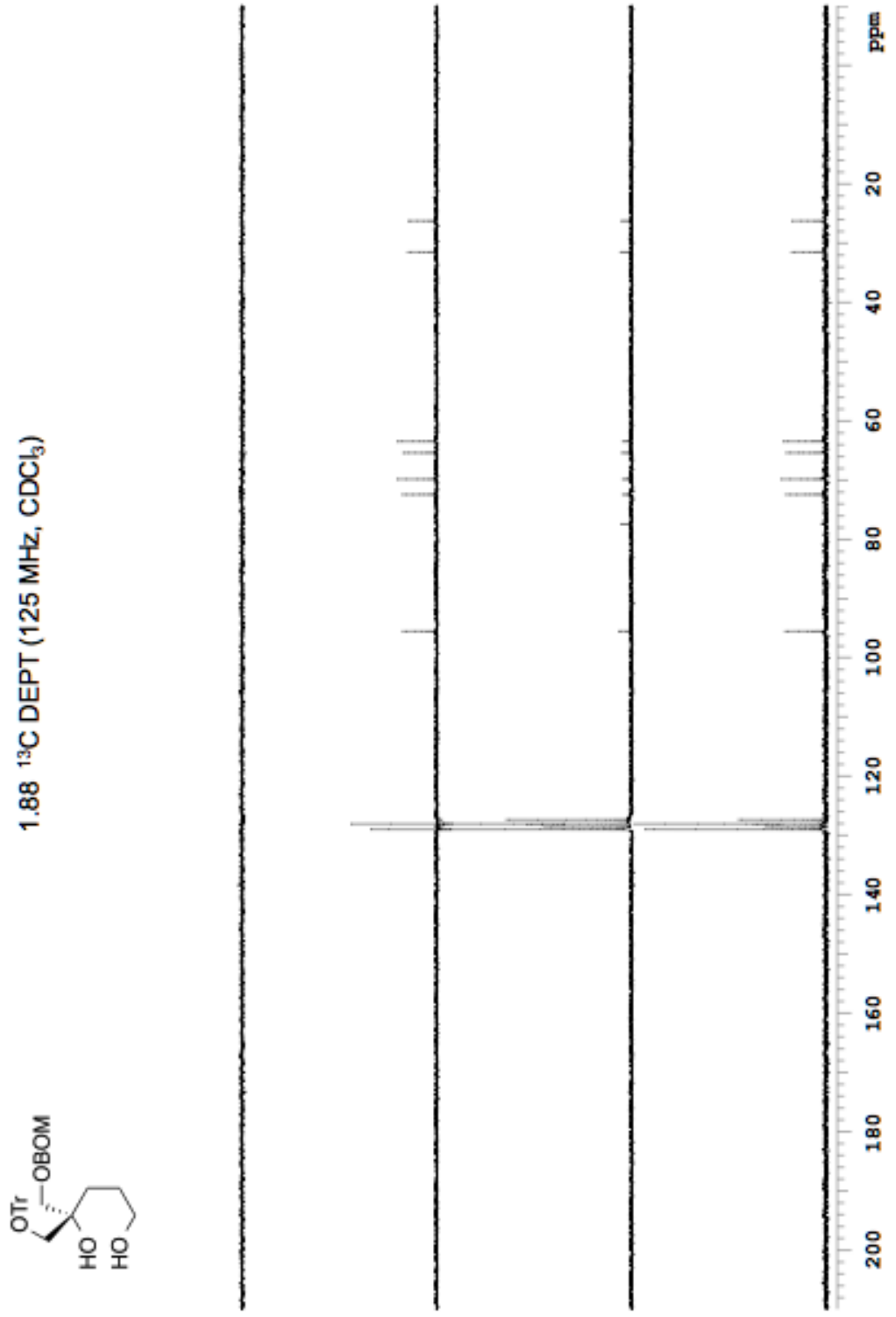
1.87 DEPT (125 MHz, $CDCl_3$)

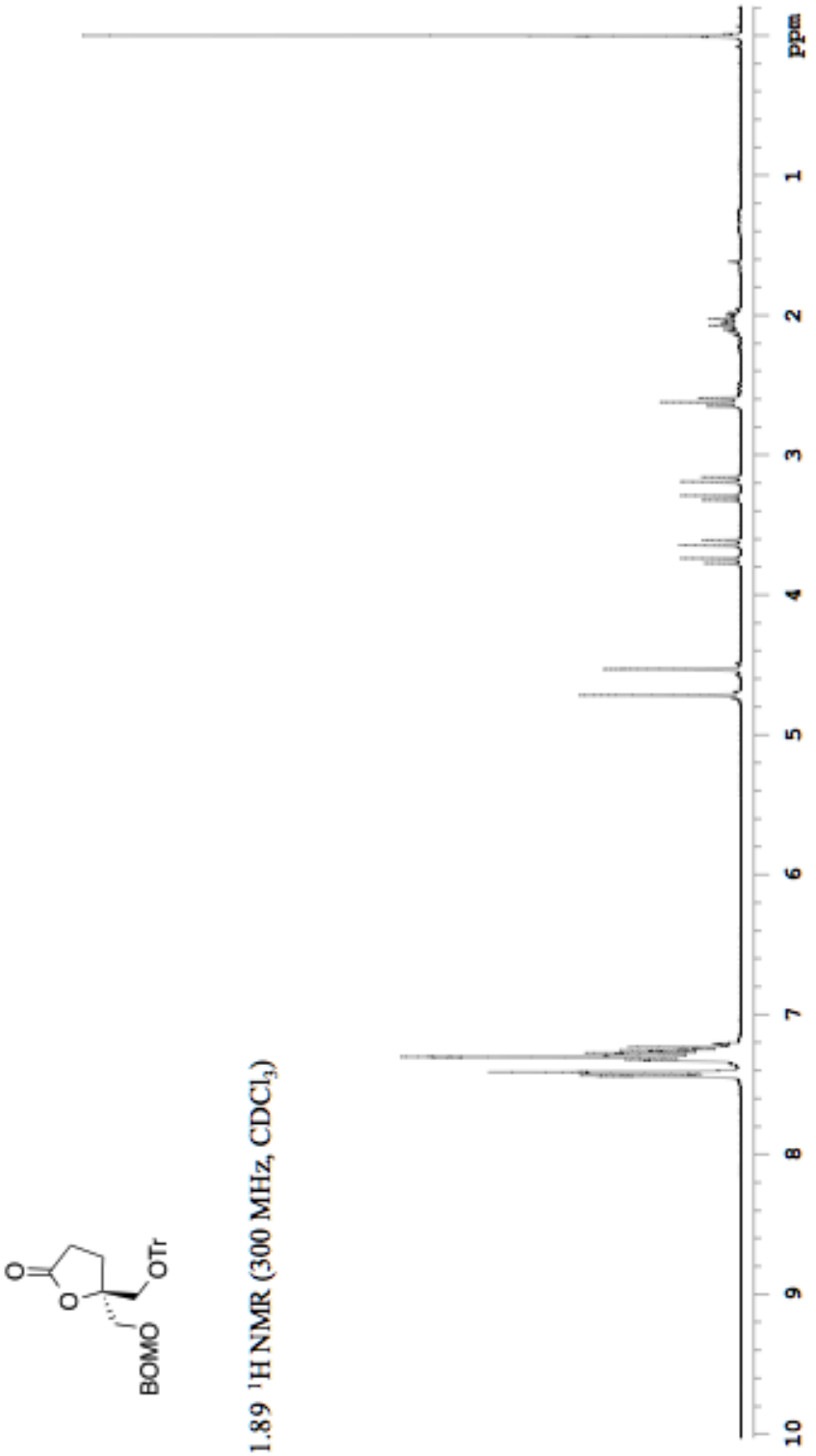


1.88 ^{1}H NMR (500 MHz, $CDCl_3$)

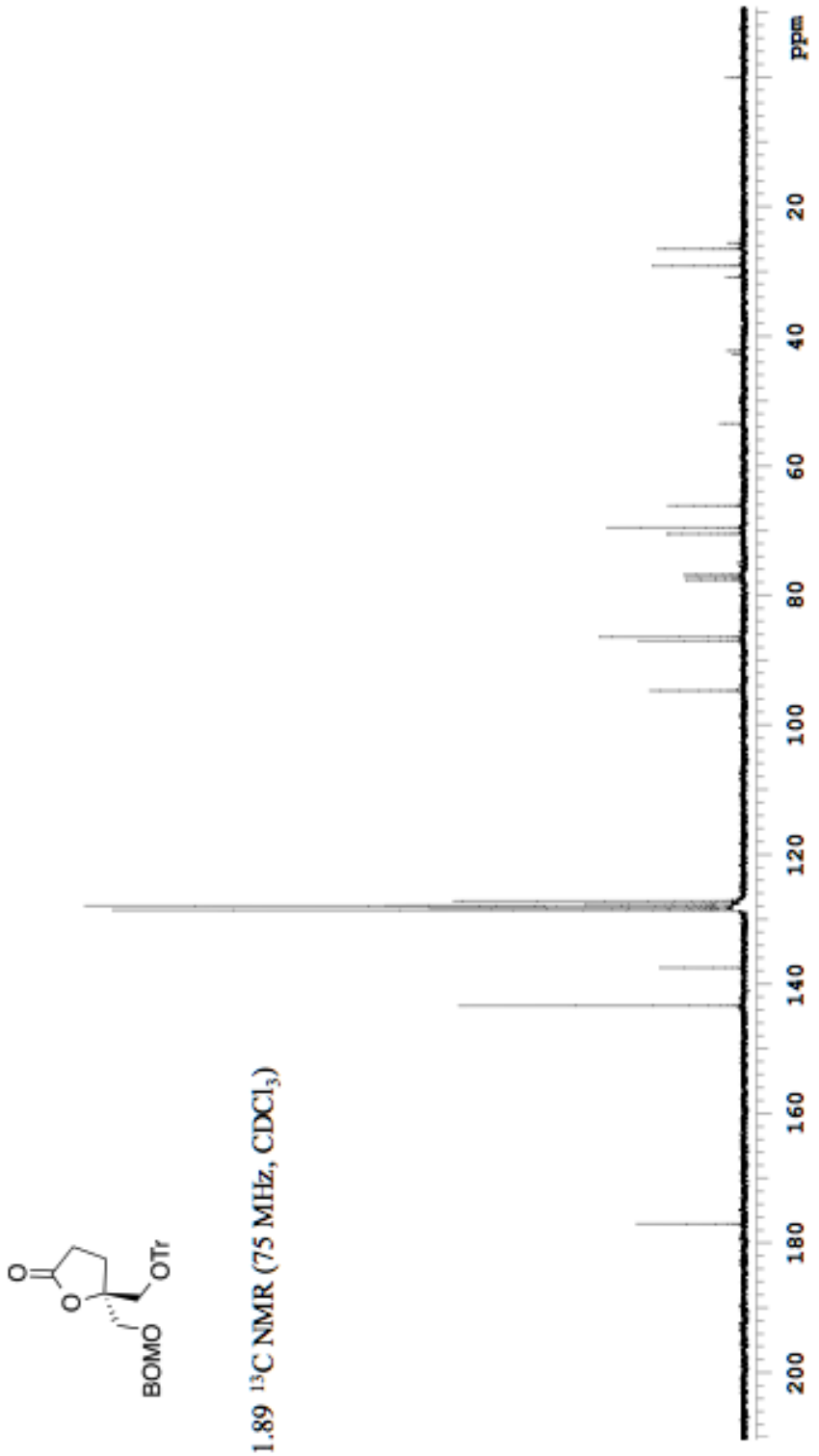


1.88 ^{13}C NMR (125 MHz, $CDCl_3$)

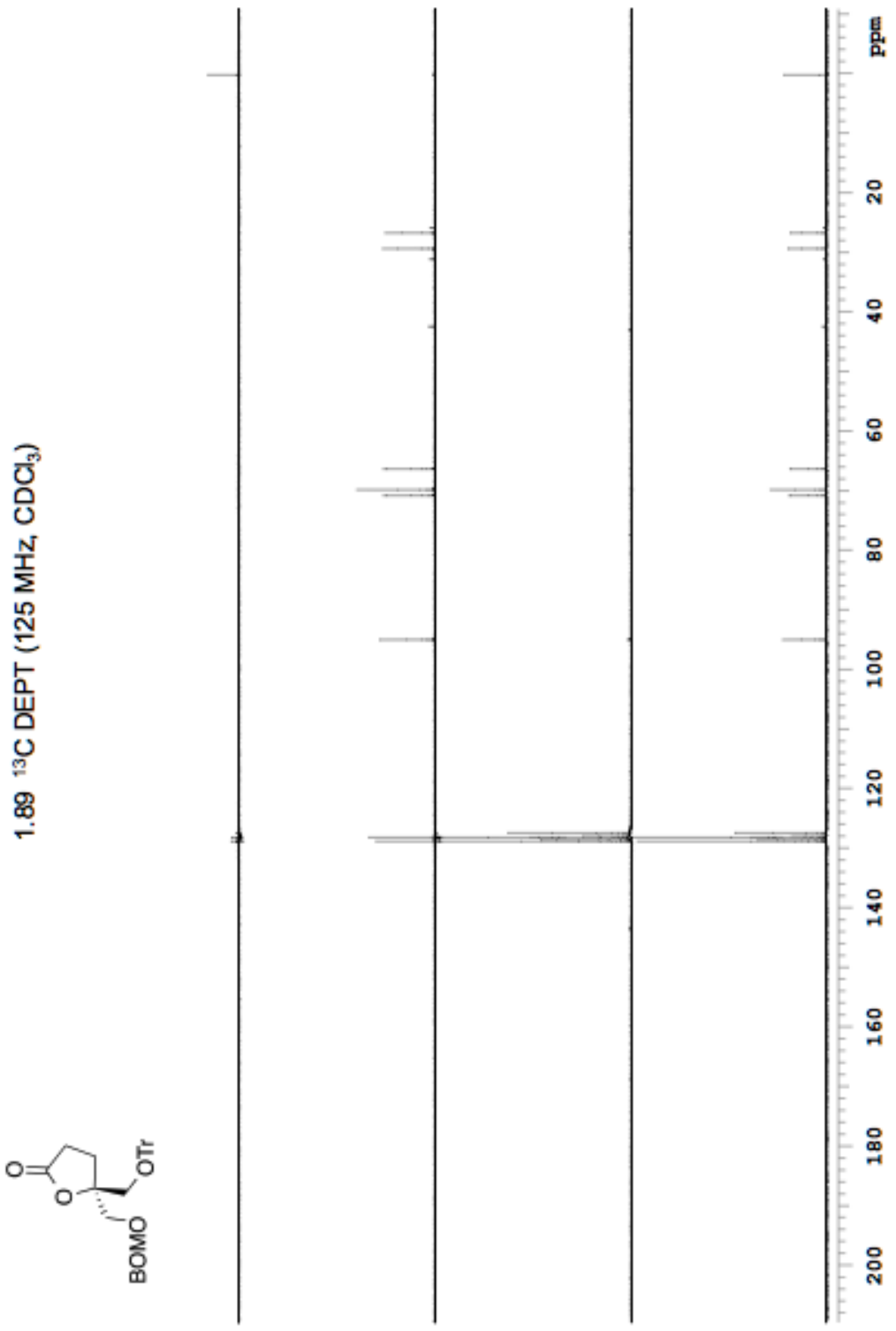

1.88 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTr
OBOM
HO
HO
ppm
200
180
160
140
120
100
80
60
40
20

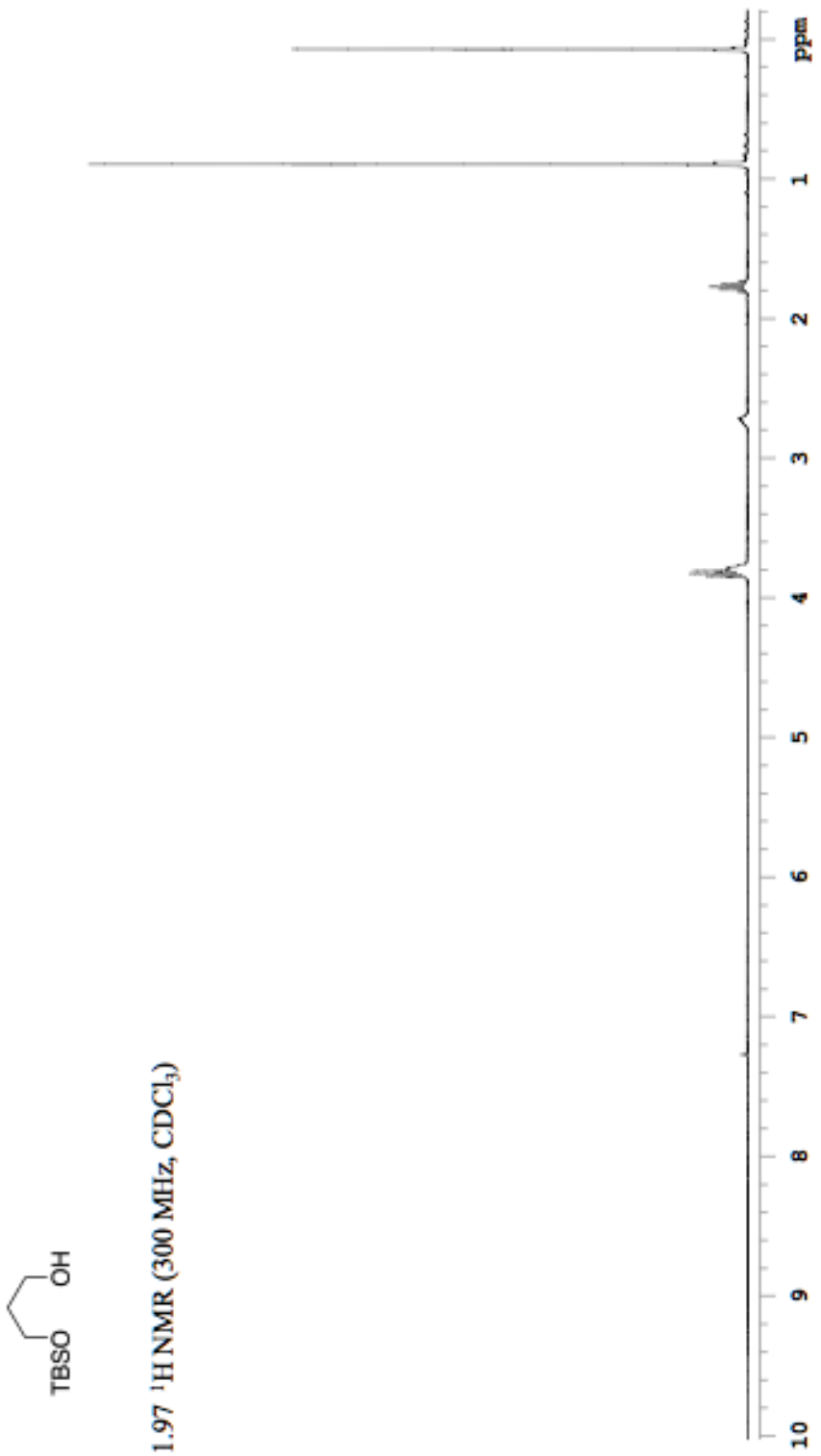


1.89 ^{1}H NMR (300 MHz, $CDCl_3$)

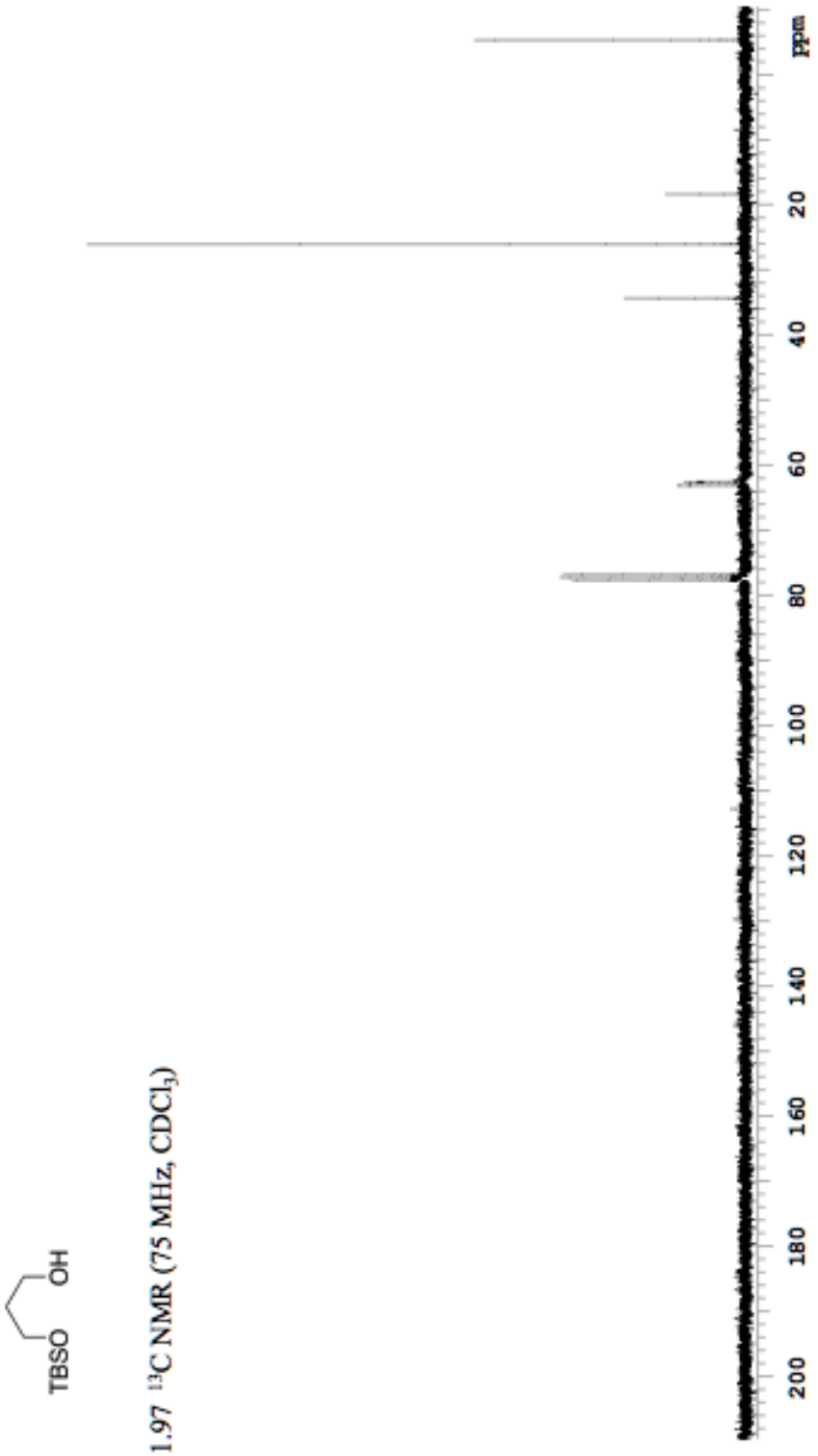


1.89 ^{13}C NMR (75 MHz, $CDCl_3$)

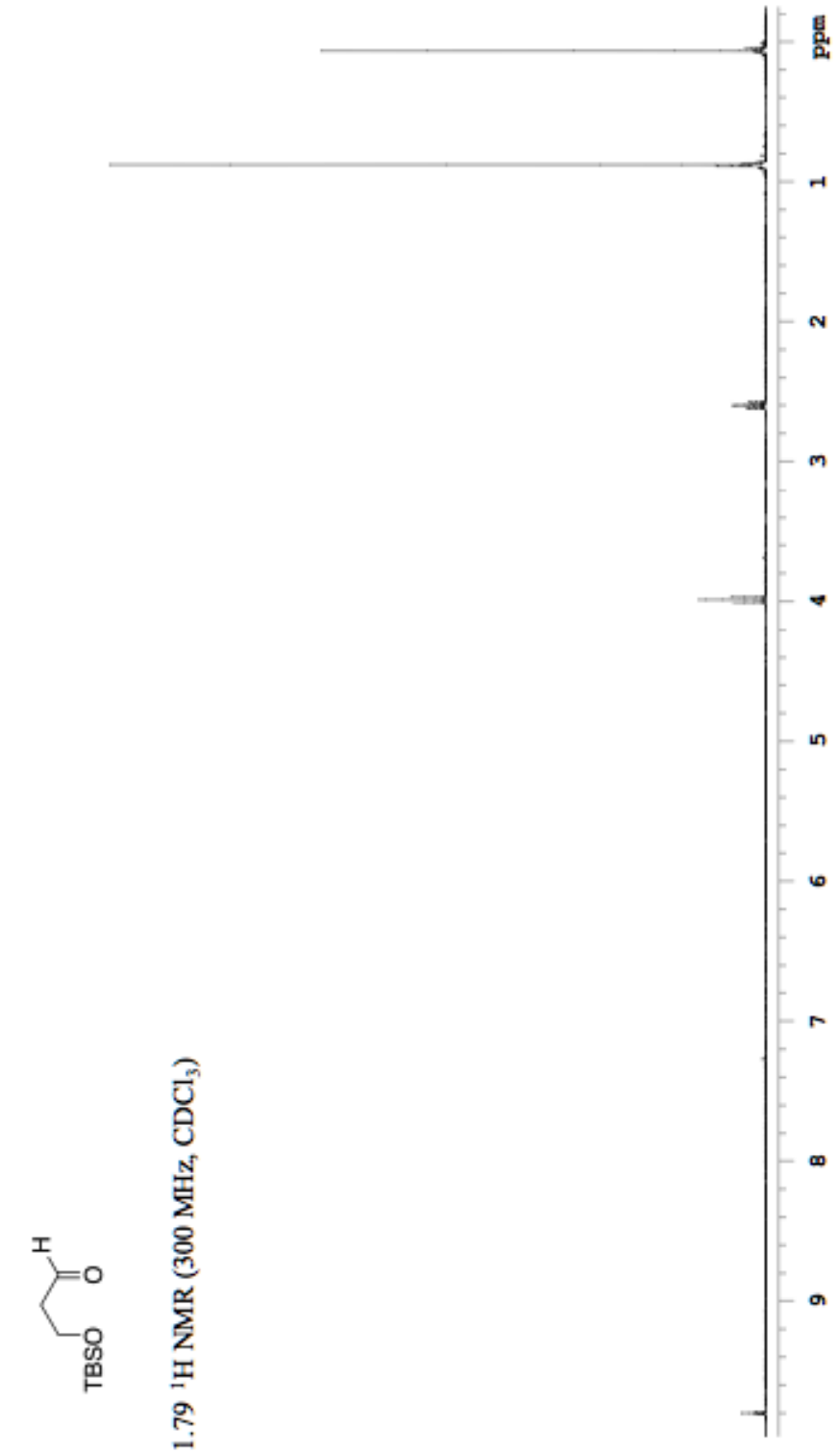

1.89 ^{13}C DEPT (125 MHz, $CDCl_3$)
BOMO
OTr
ppm
200
180
160
140
120
100
80
60
40
20



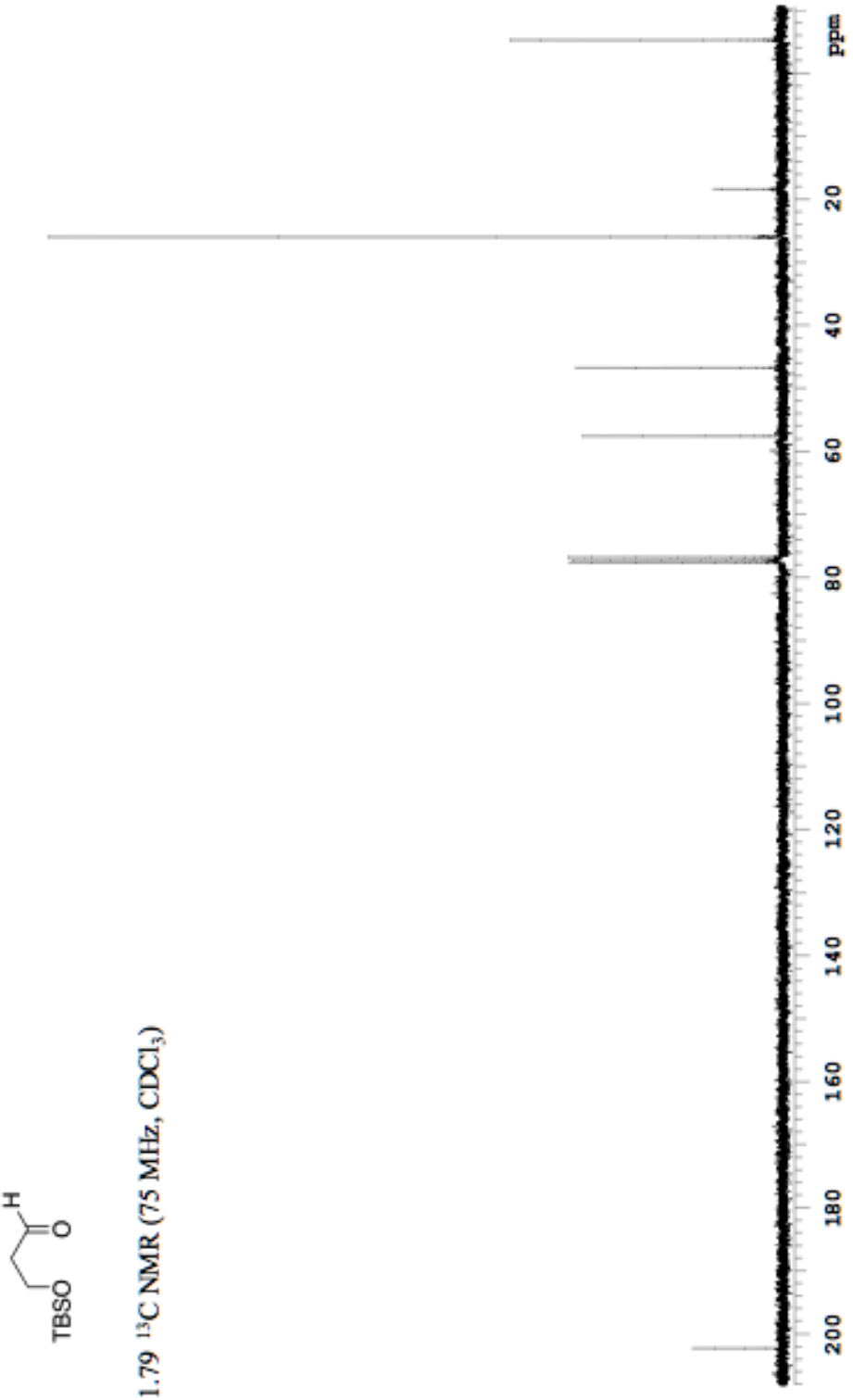
1.97 ^{1}H NMR (300 MHz, $CDCl_3$)



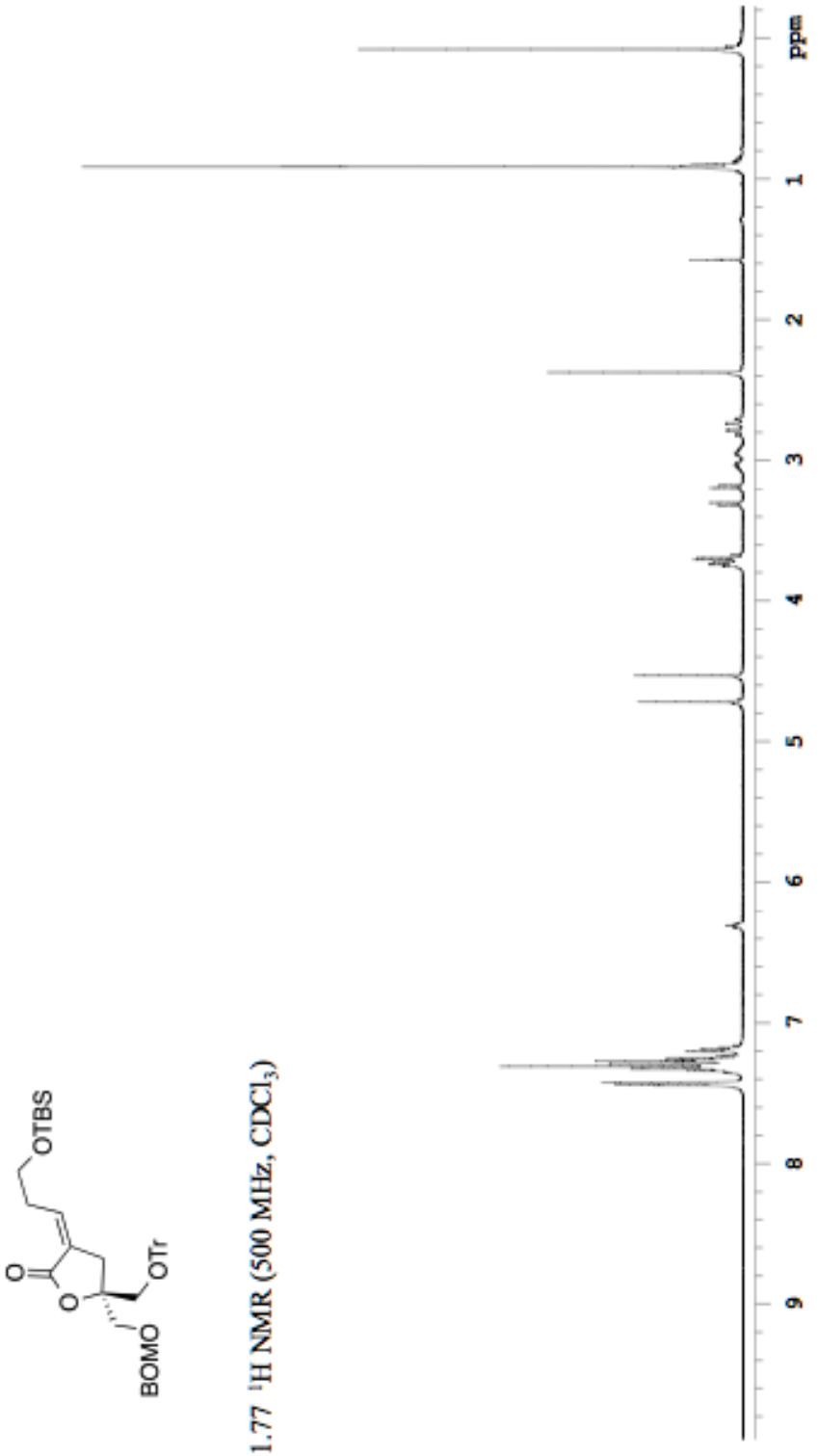
1.97 ^{13}C NMR (75 MHz, $CDCl_3$)



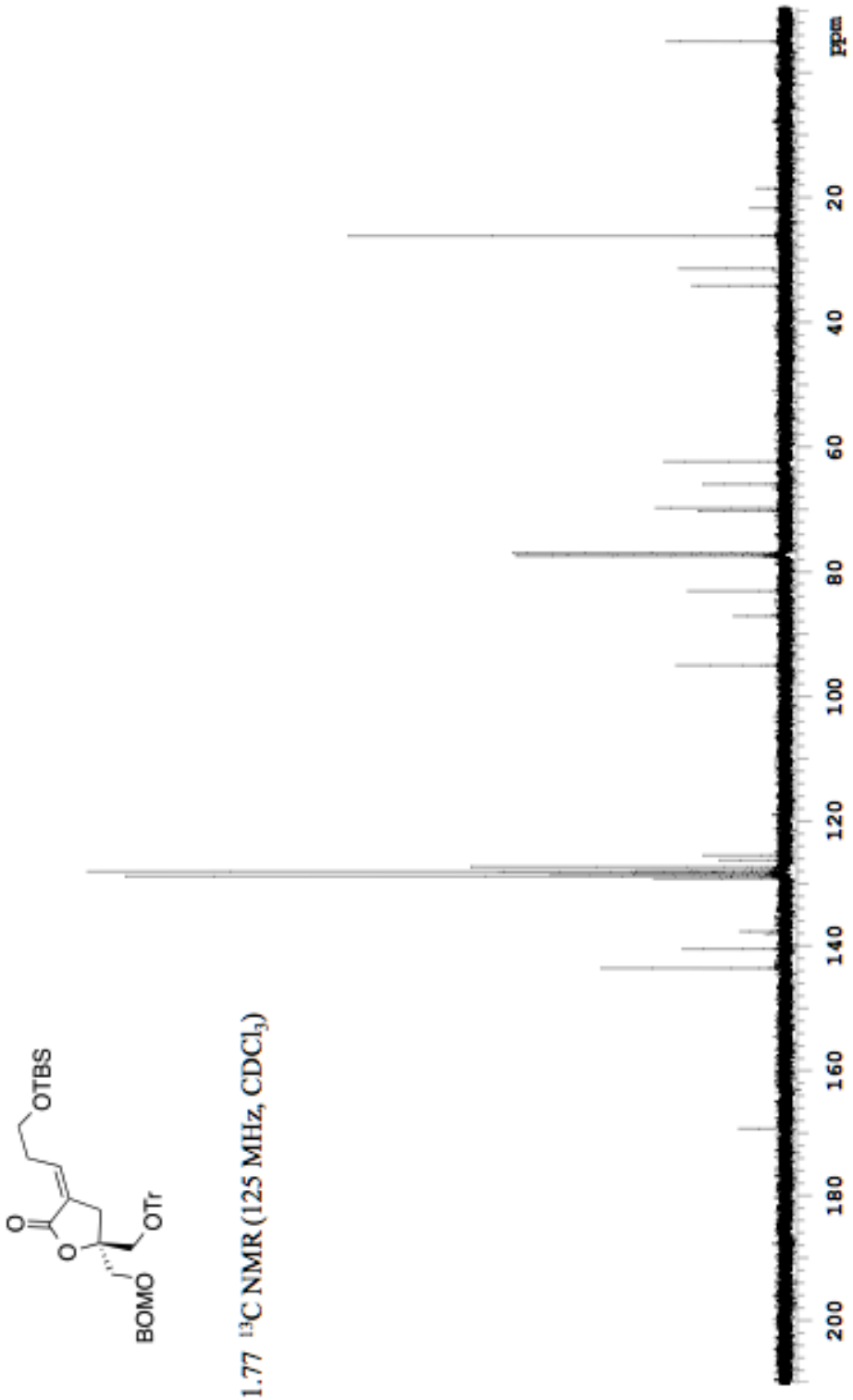
1.79 ^{1}H NMR (300 MHz, $CDCl_3$)



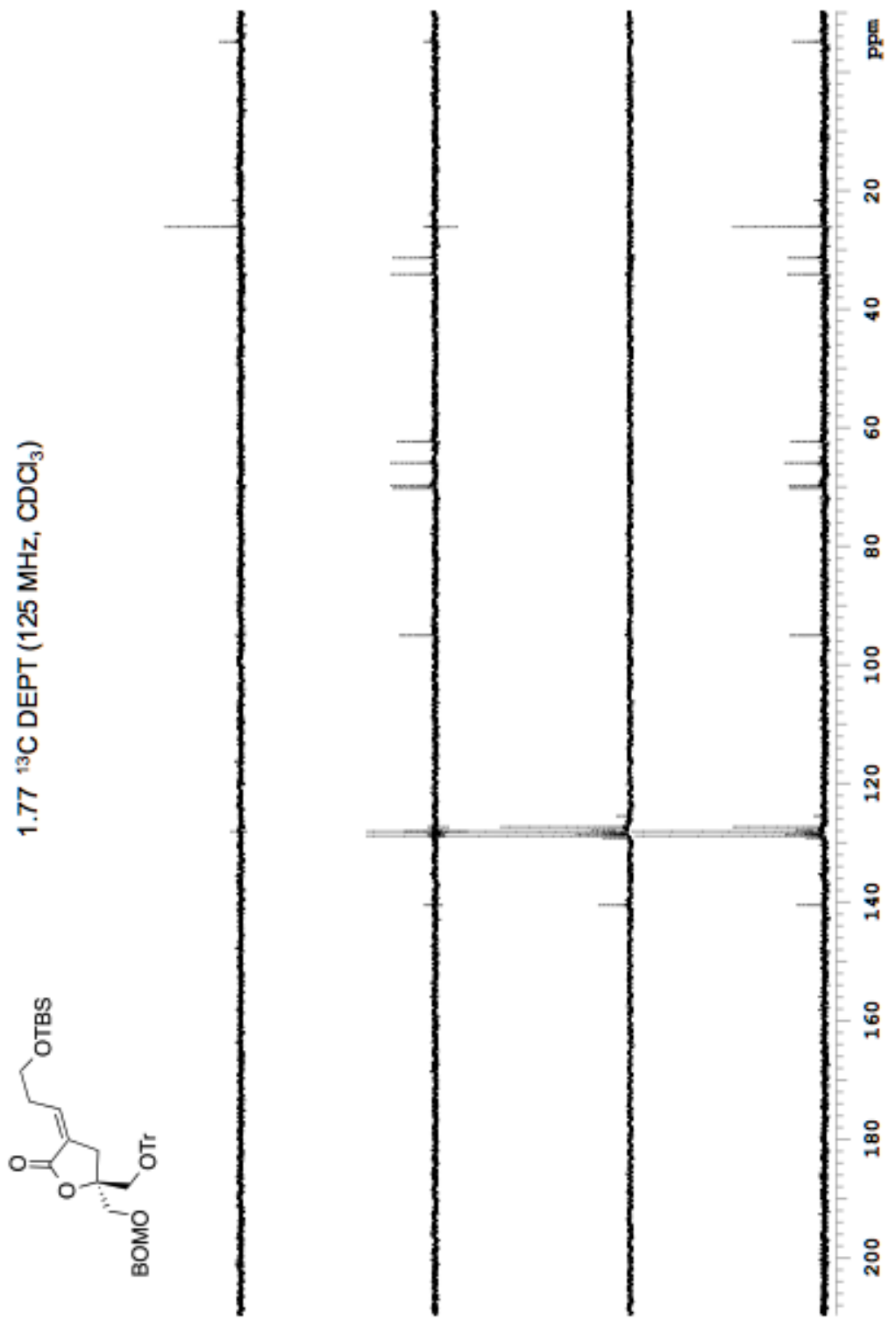
1.79 ^{13}C NMR (75 MHz, $CDCl_3$)

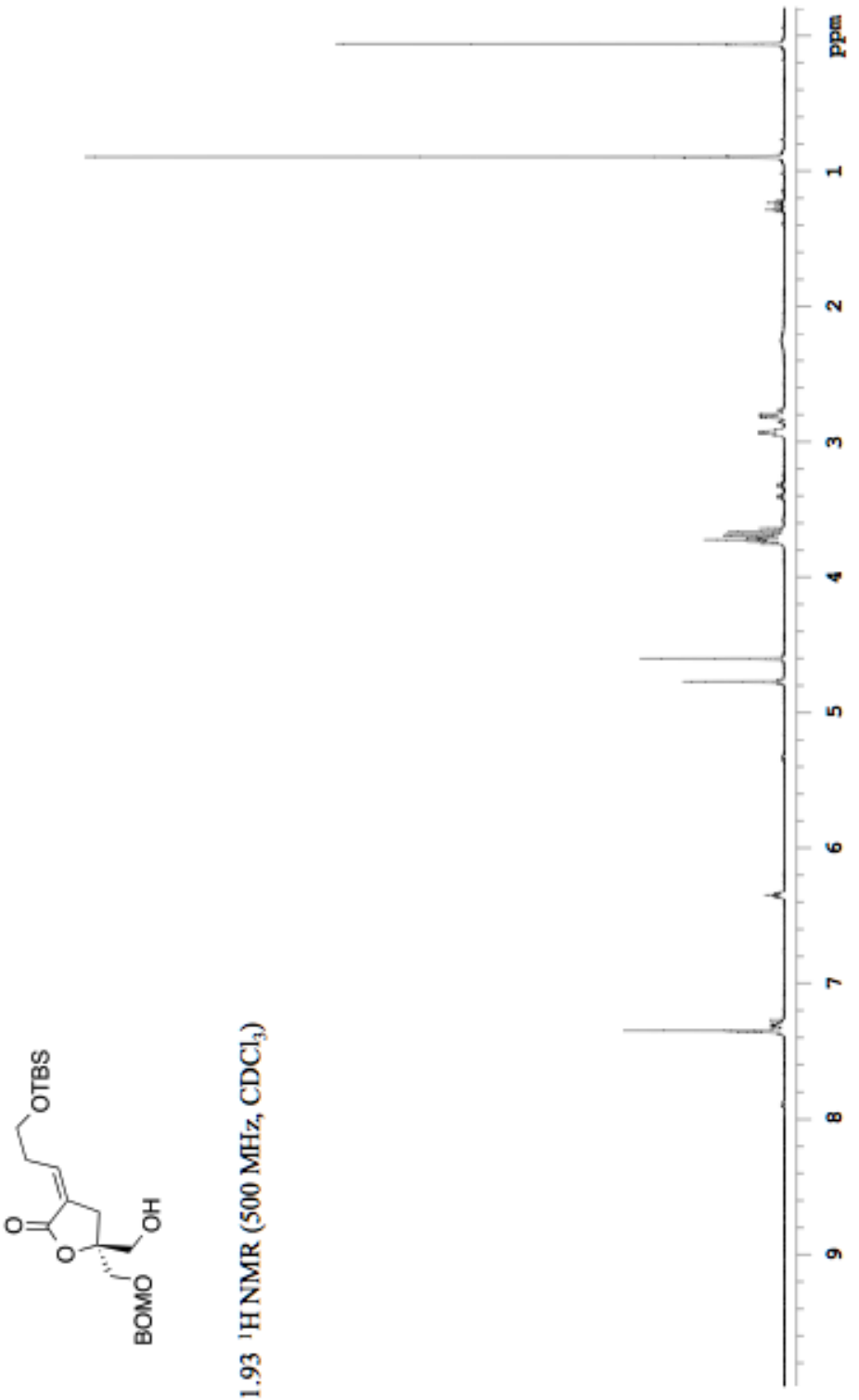


1.77 ^{1}H NMR (500 MHz, $CDCl_3$)

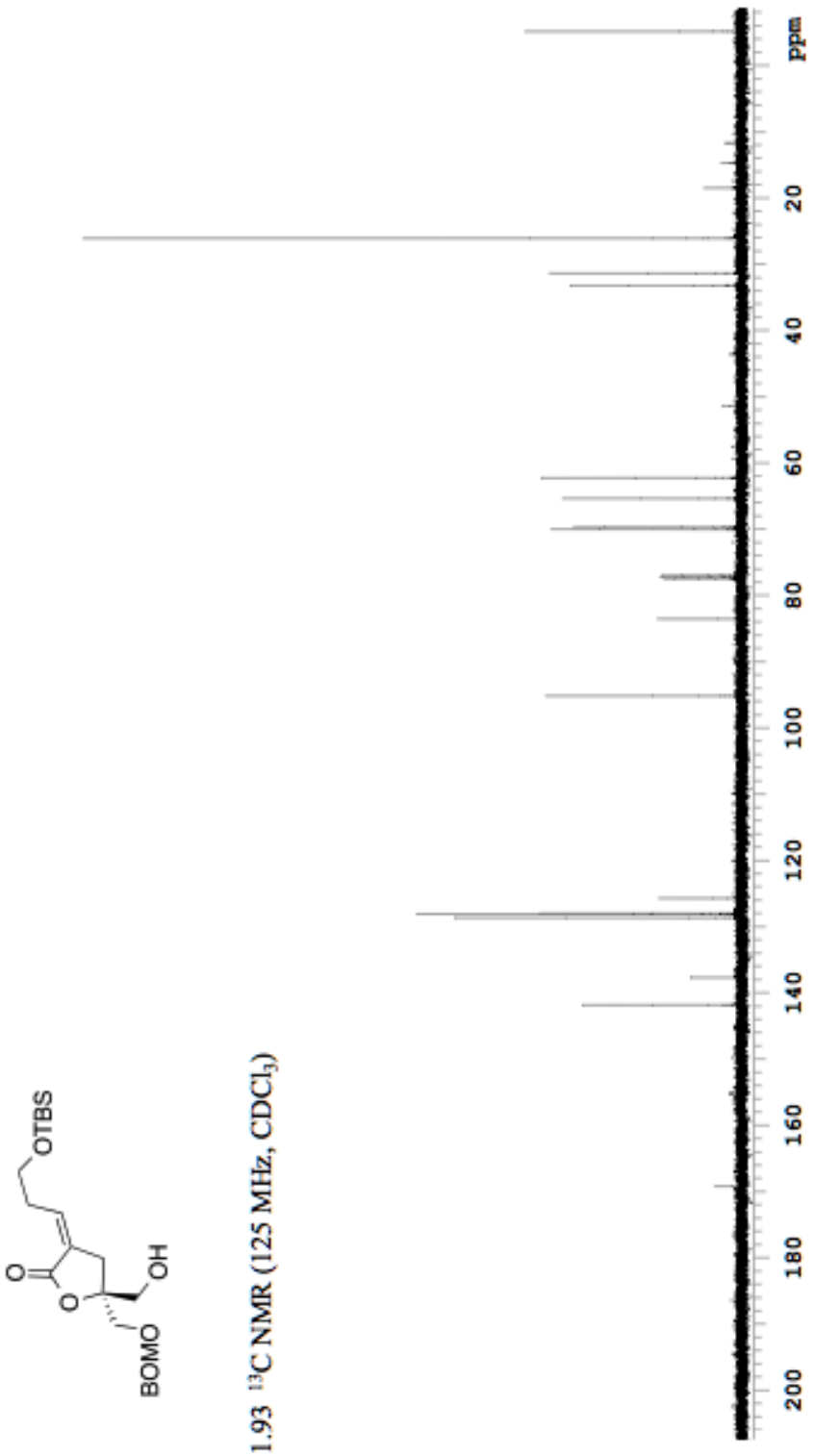


1.77 ^{13}C NMR (125 MHz, $CDCl_3$)

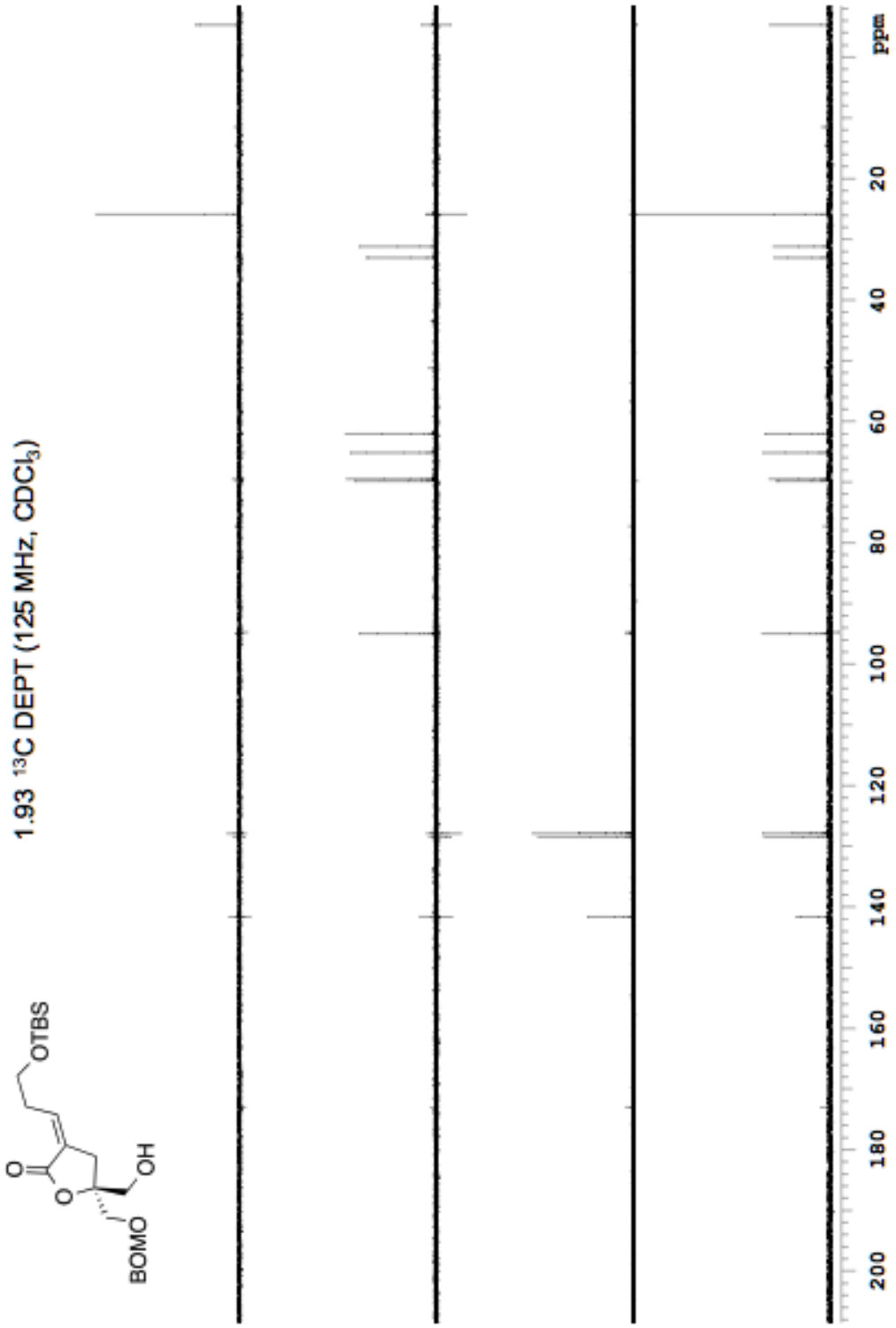

1.77 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTBS
O
O
OTr
BOMO
200
180
160
140
120
100
80
60
40
20
ppm



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

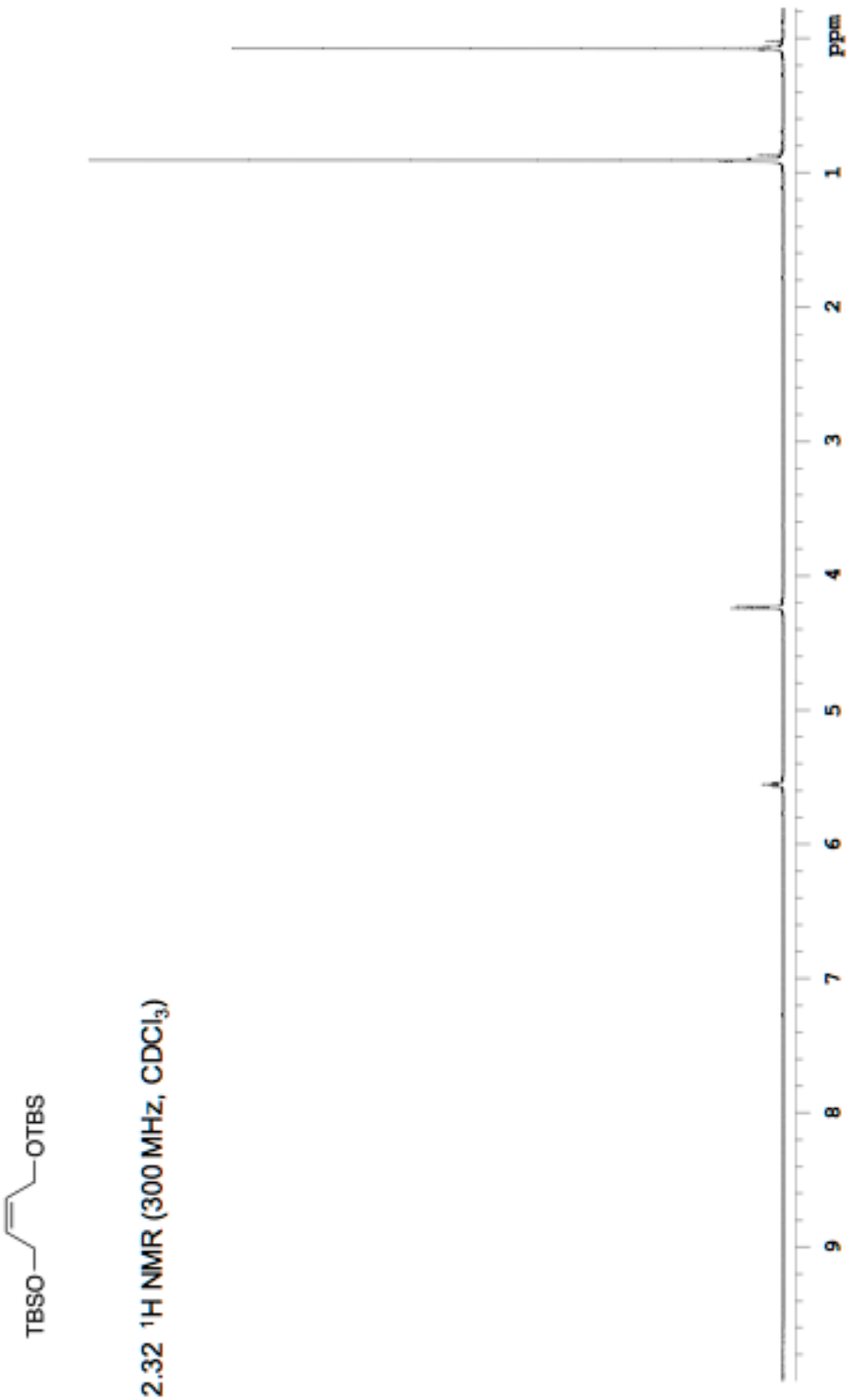


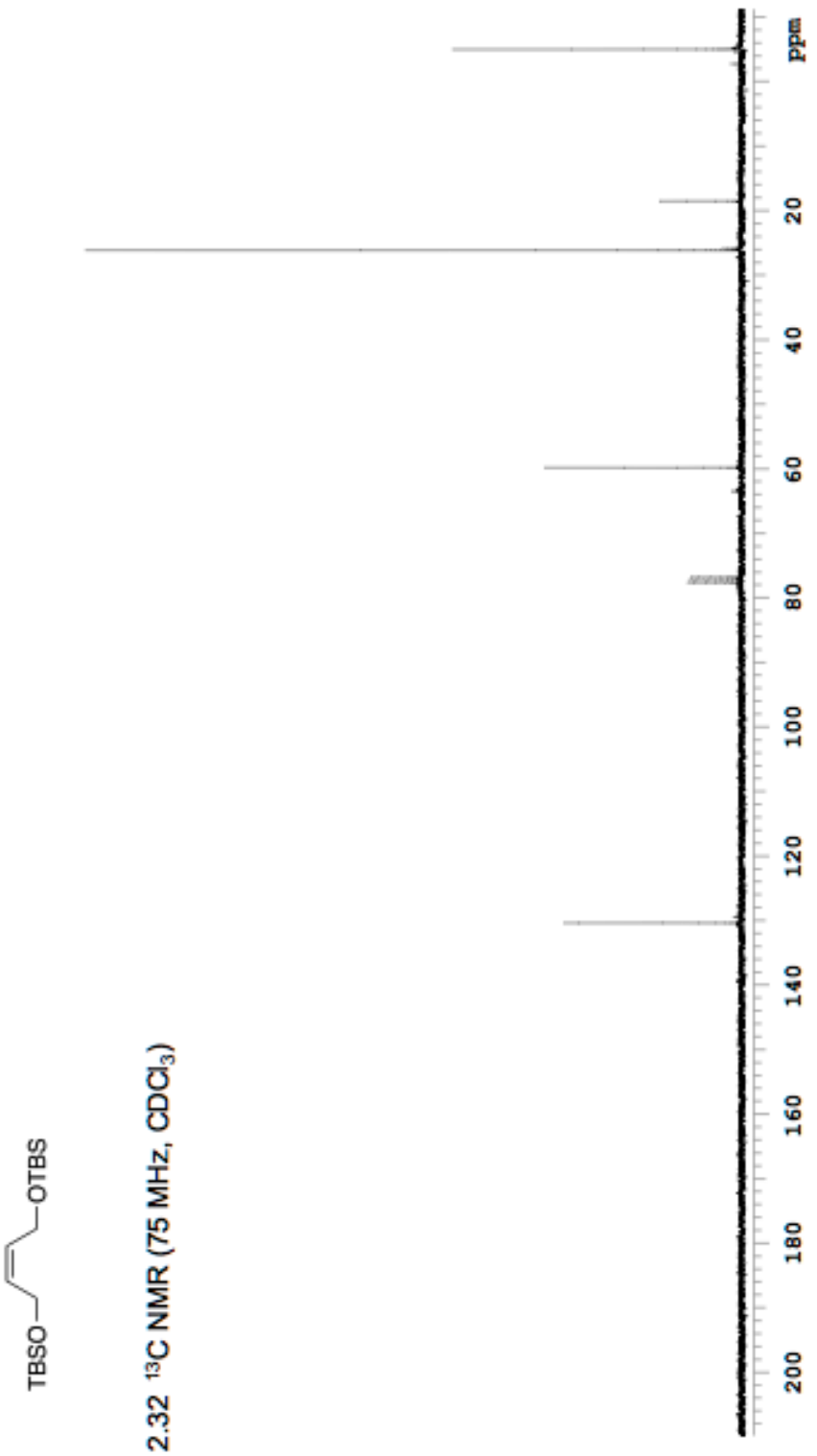
1.93 ^{13}C NMR (125 MHz, $CDCl_3$)

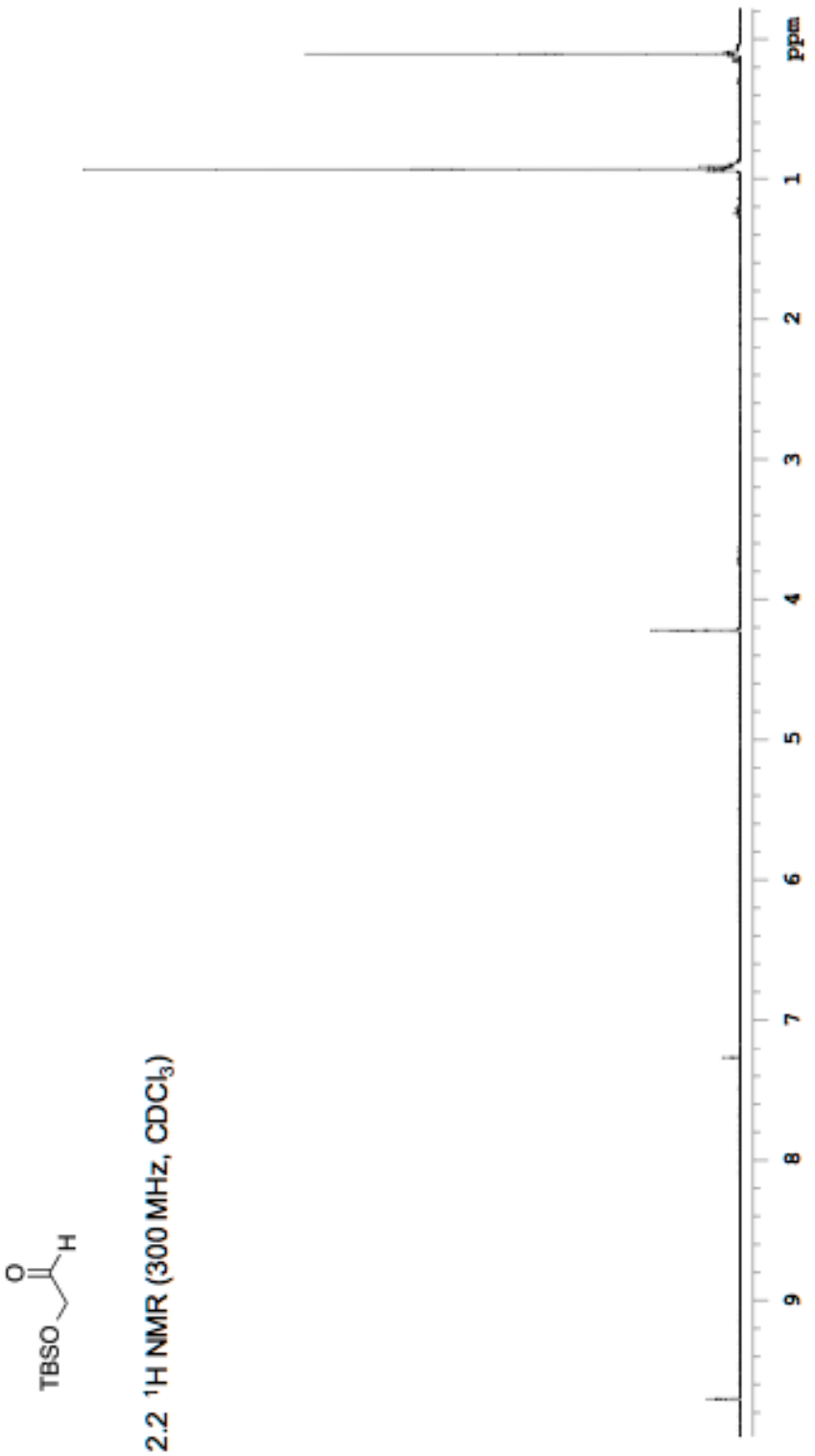

1.93 13C DEPT (125 MHz, CDCl3)
OTBS
O
O
OH
BOMO
200
180
160
140
120
100
80
60
40
20
ppm

APPENDIX B

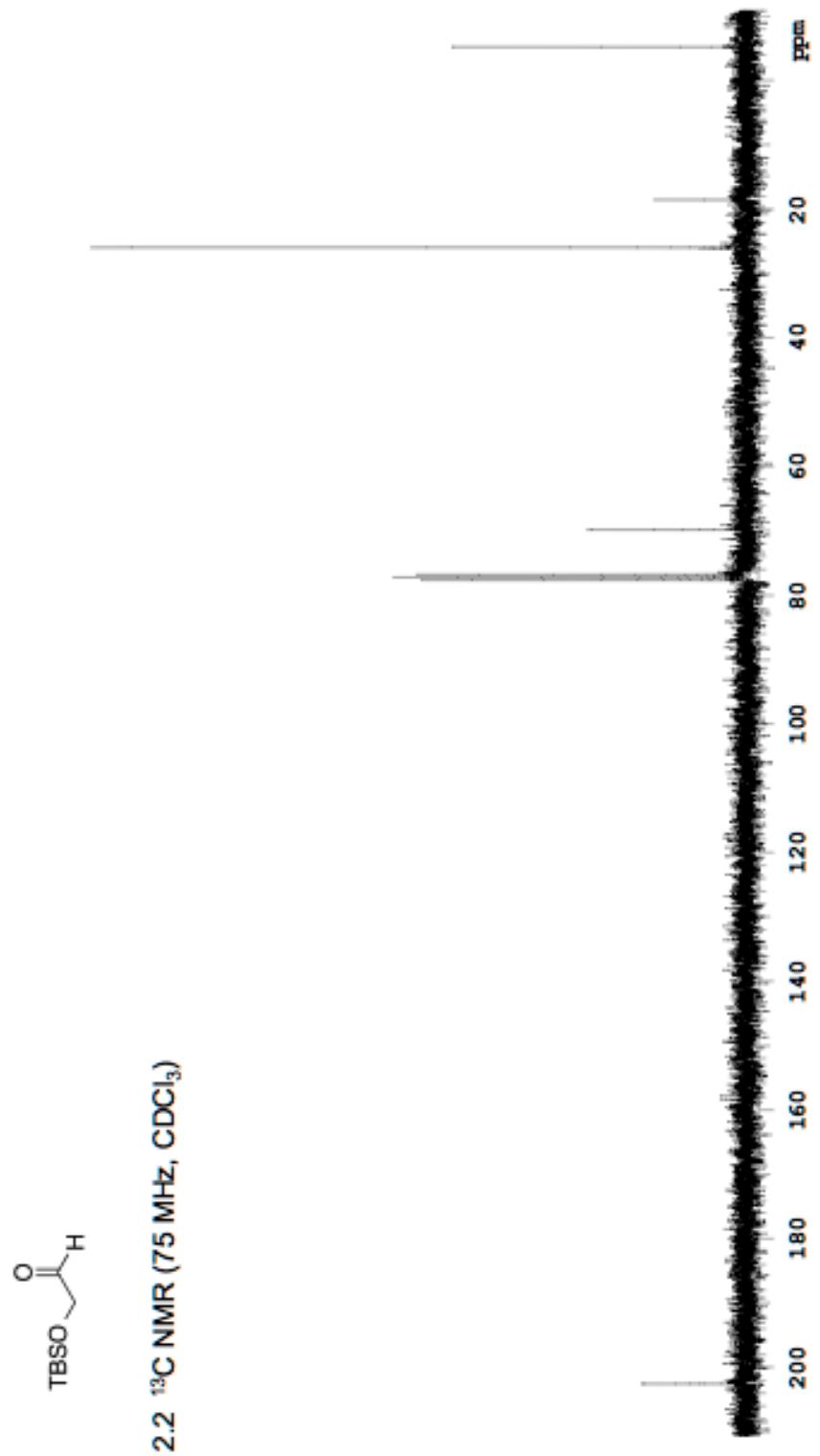
^{1}H AND ^{13}C SPECTRA FOR CHAPTER 2


TBSO
OTBS
2.32 ¹H NMR (300 MHz, CDCl₃)
ppm
9
8
7
6
5
4
3
2
1

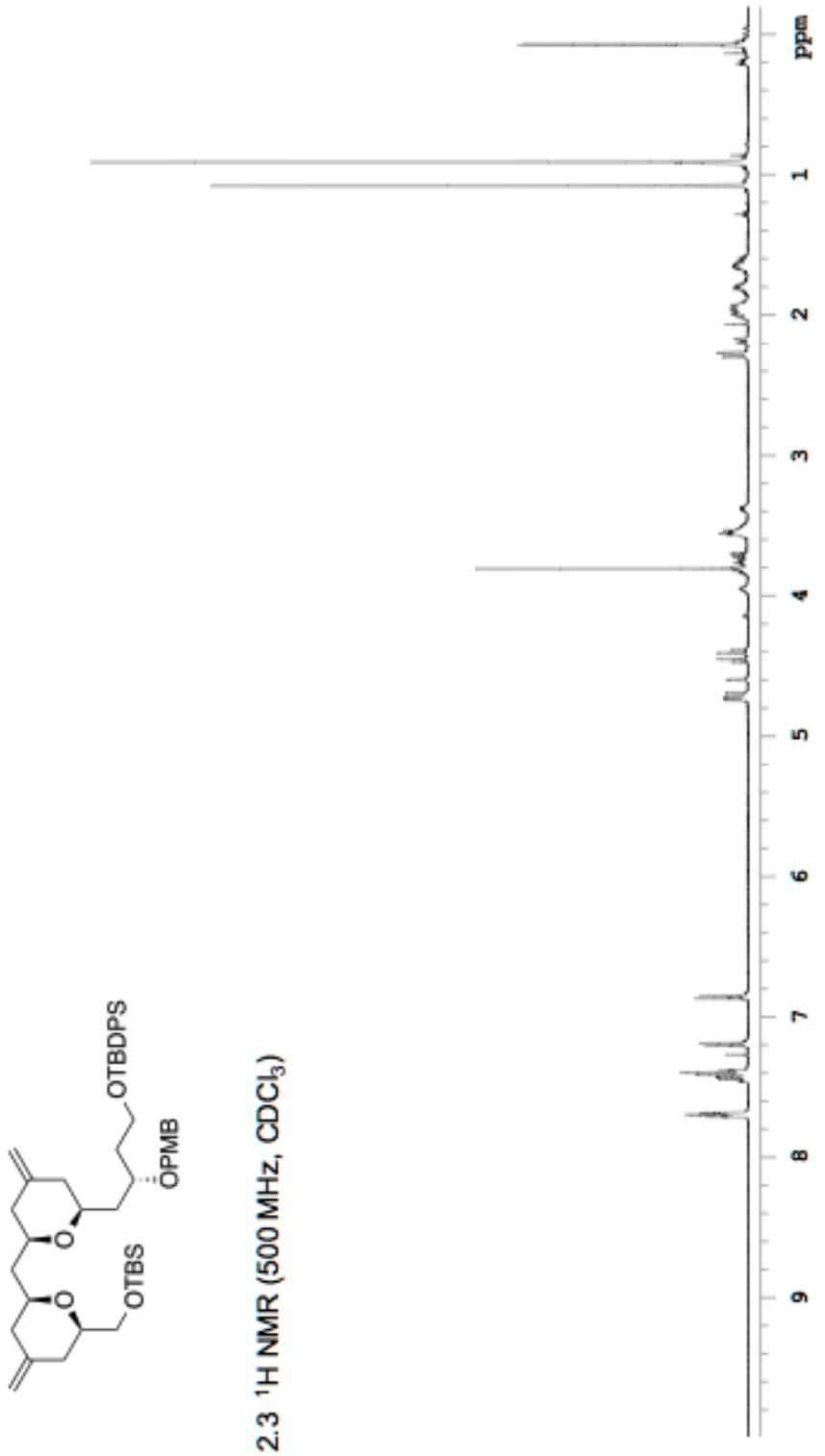

TBSO
OTBS
2.32 ^{13}C NMR (75 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20



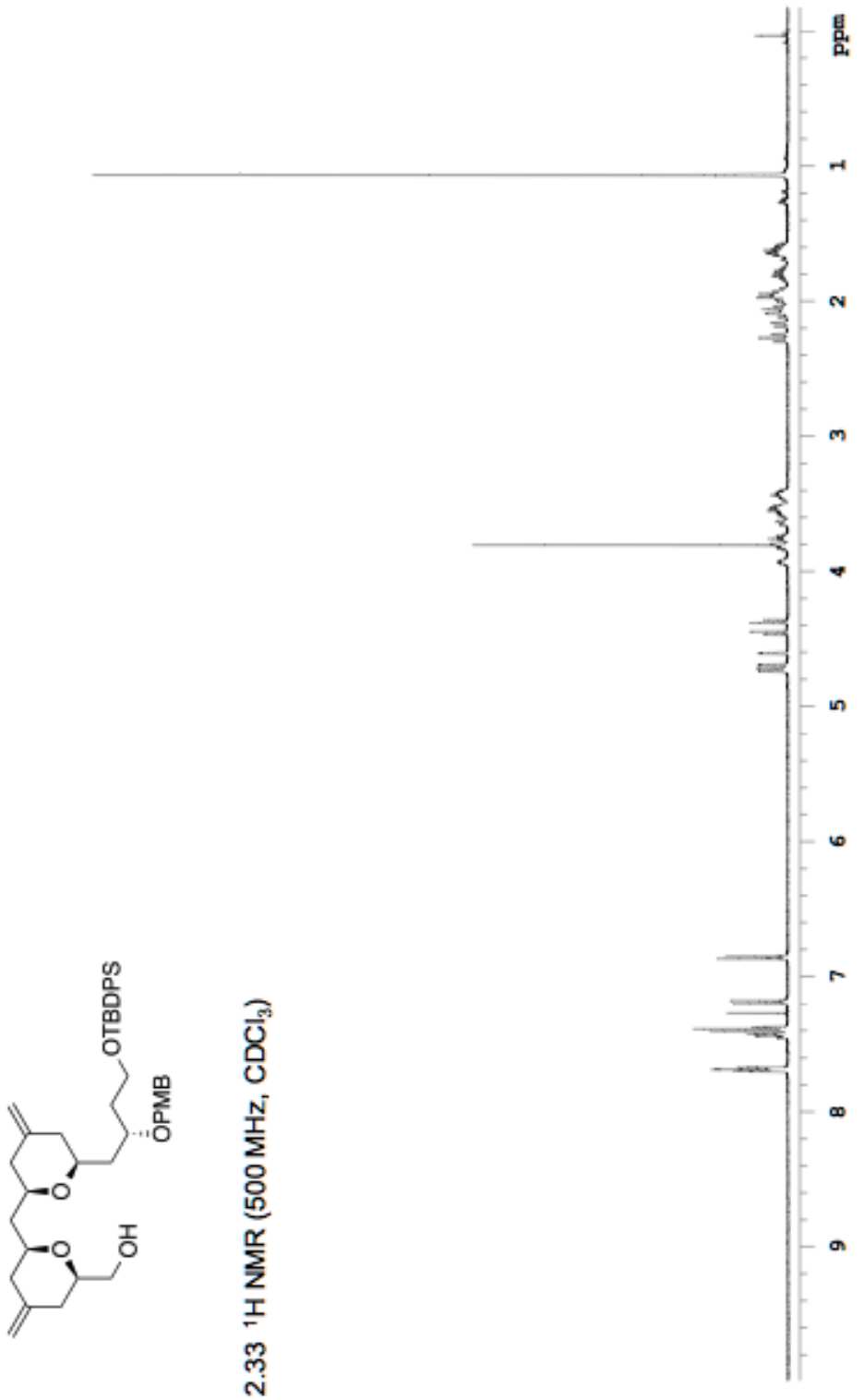
2.2 ^{1}H NMR (300 MHz, $CDCl_3$)



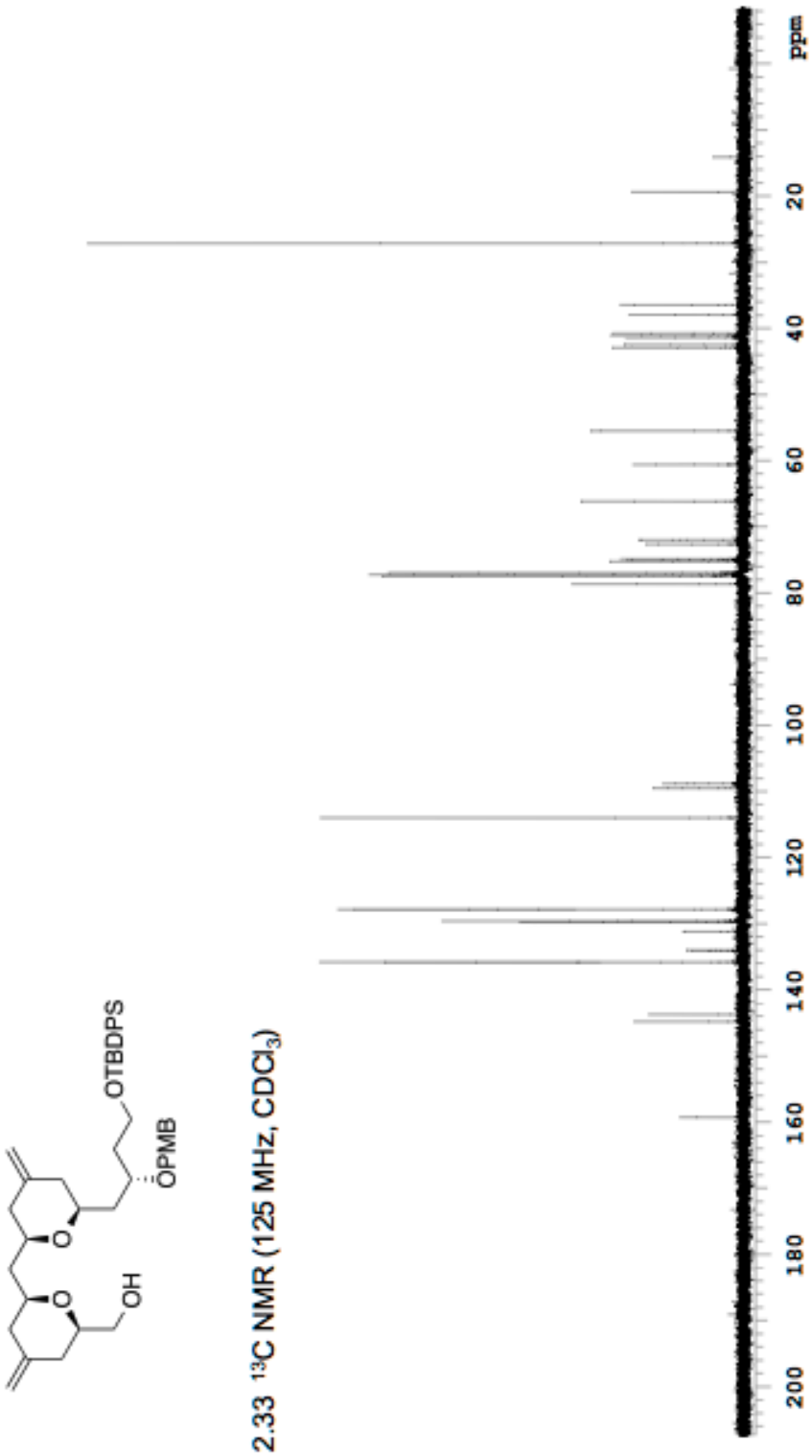
2.2 ^{13}C NMR (75 MHz, $CDCl_3$)



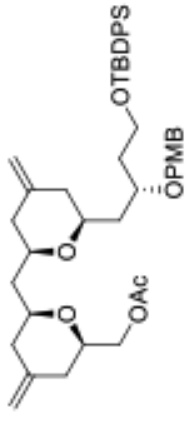
2.3 ^{1}H NMR (500 MHz, $CDCl_3$)



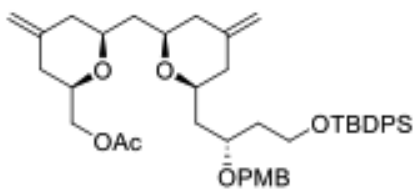
2.33 ^{1}H NMR (500 MHz, $CDCl_3$)



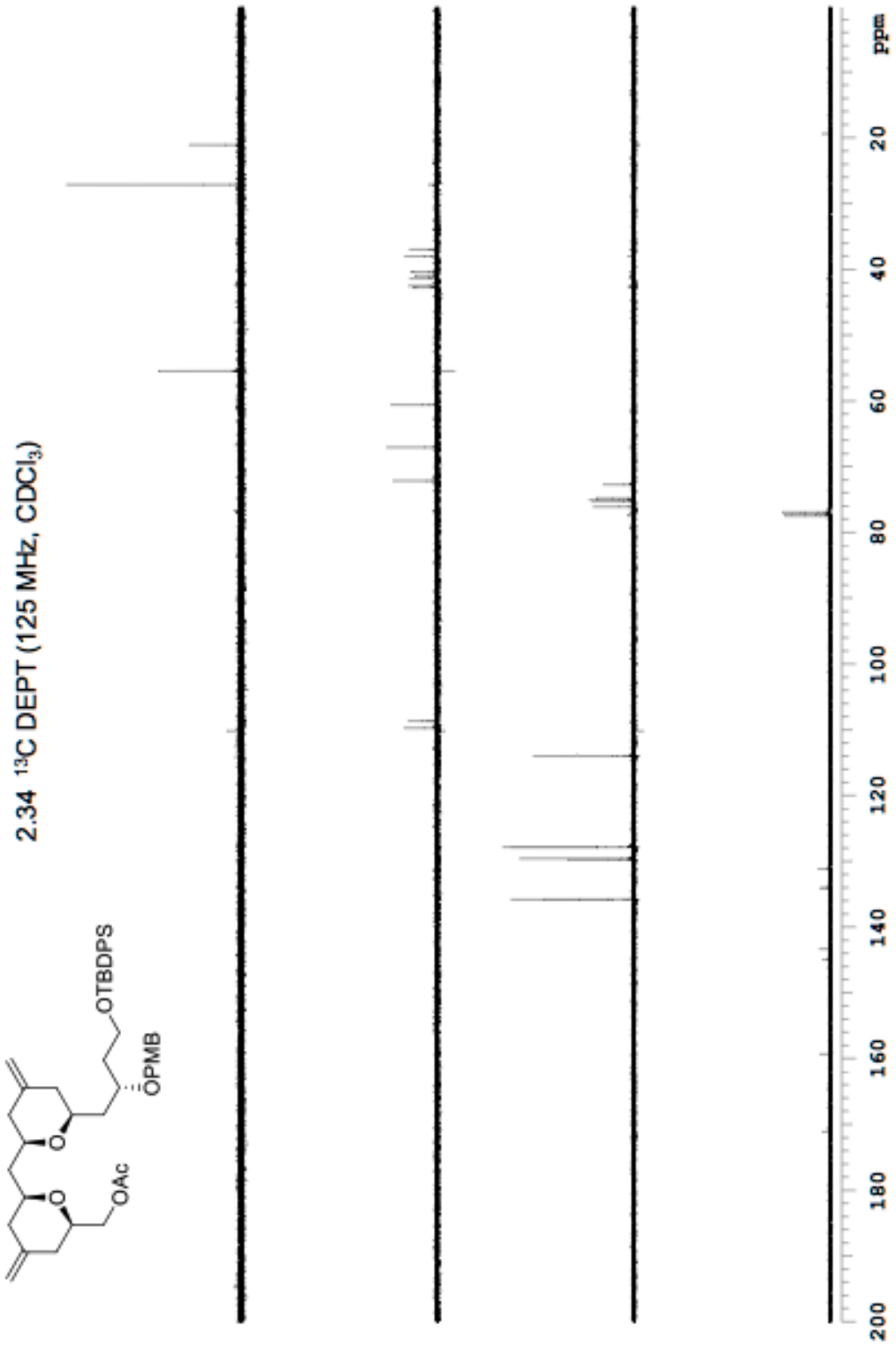
2.33 ^{13}C NMR (125 MHz, $CDCl_3$)

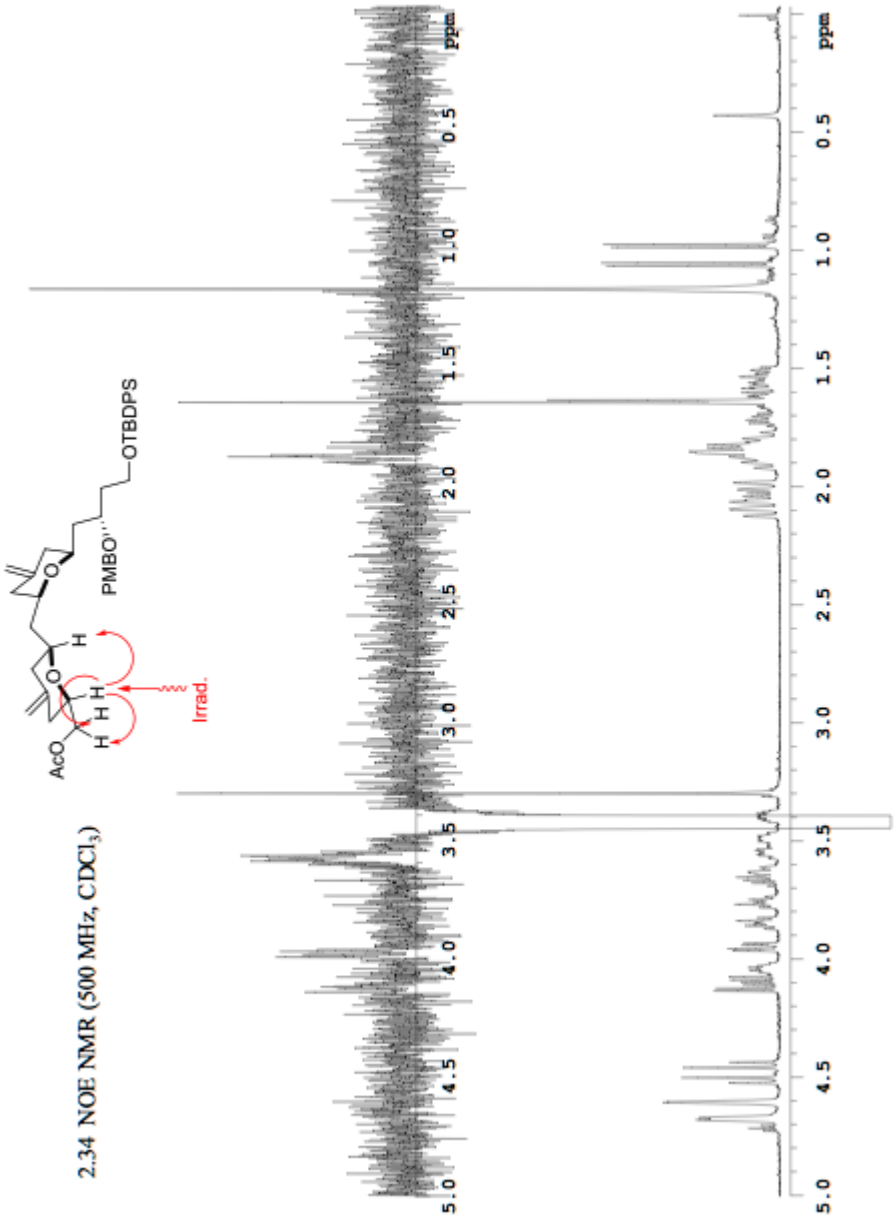


2.34 ^{1}H NMR (500 MHz, $CDCl_3$)

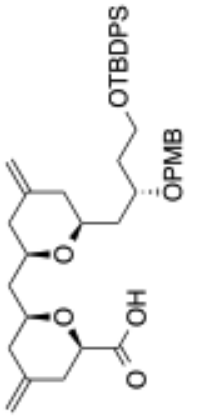


2.34 ^{13}C NMR (125 MHz, $CDCl_3$)

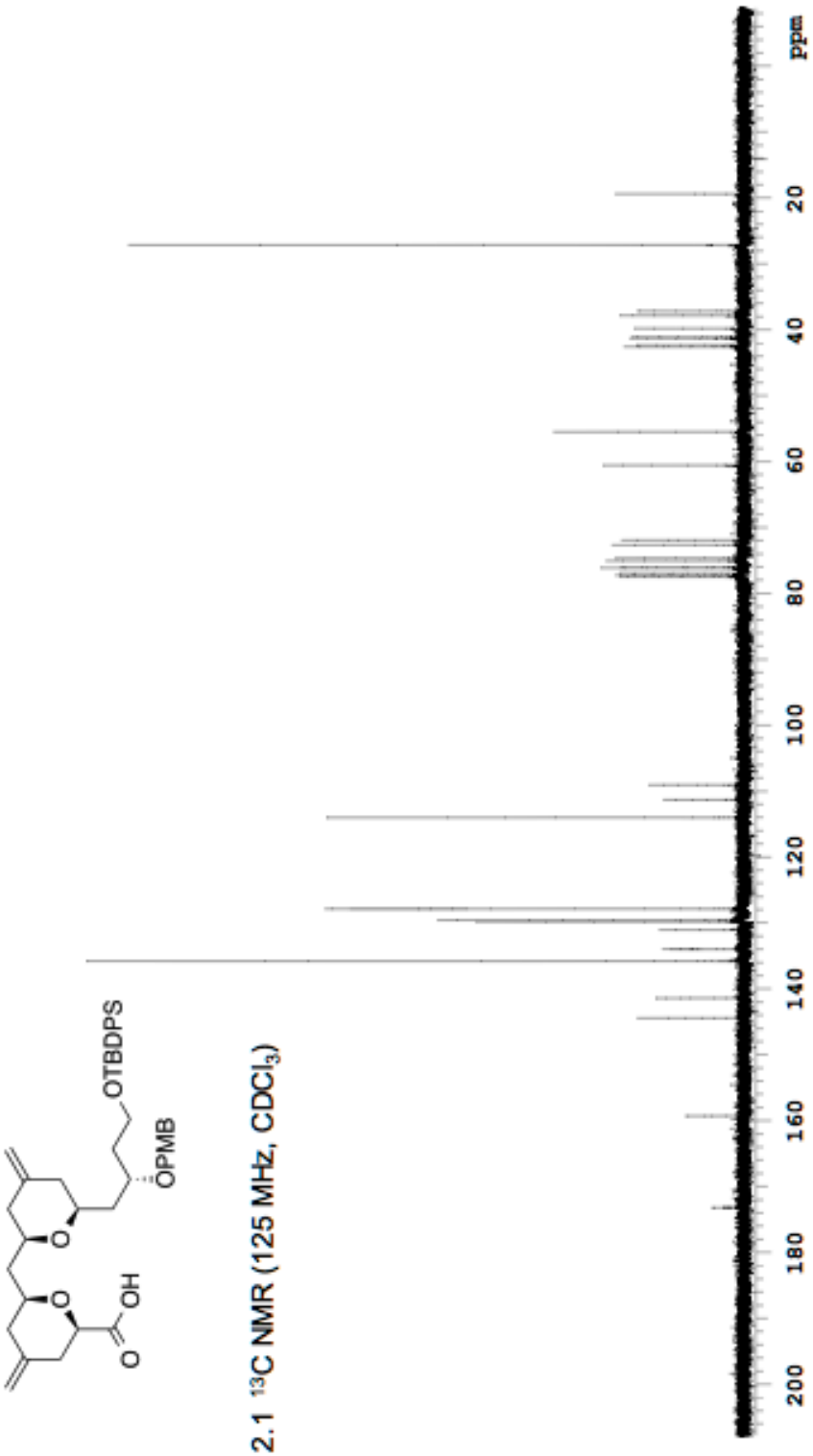

2.34 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTBDPS
OPMB
OAc
O
O
ppm
200
180
160
140
120
100
80
60
40
20



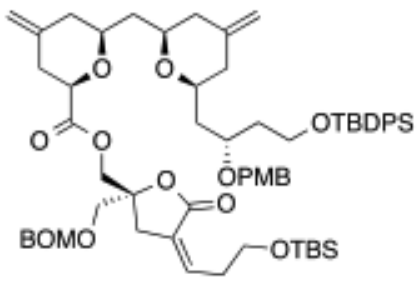
2.34 NOE NMR (500 MHz, $CDCl_3$)



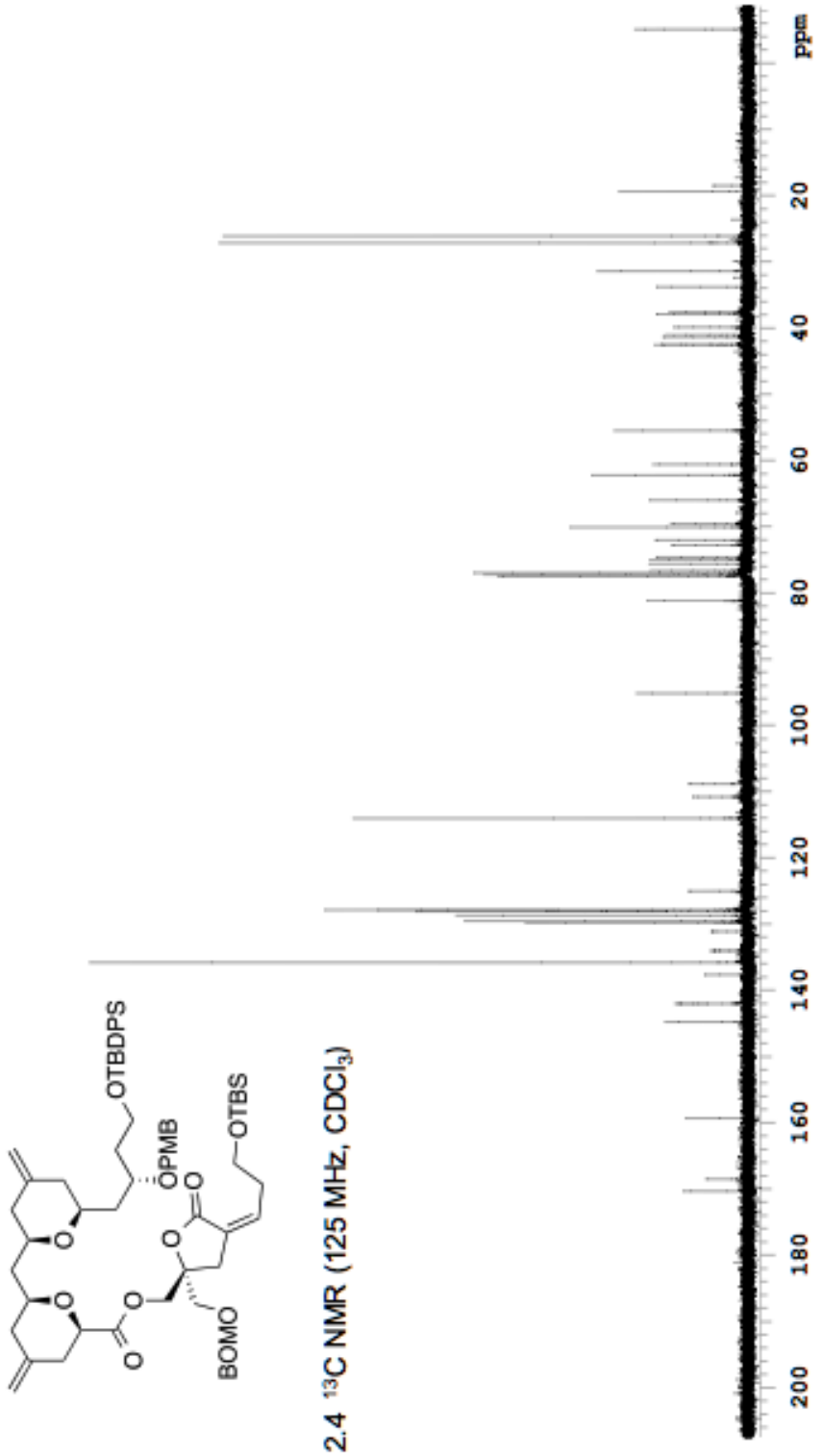
2.1 ^{1}H NMR (500 MHz, $CDCl_3$)



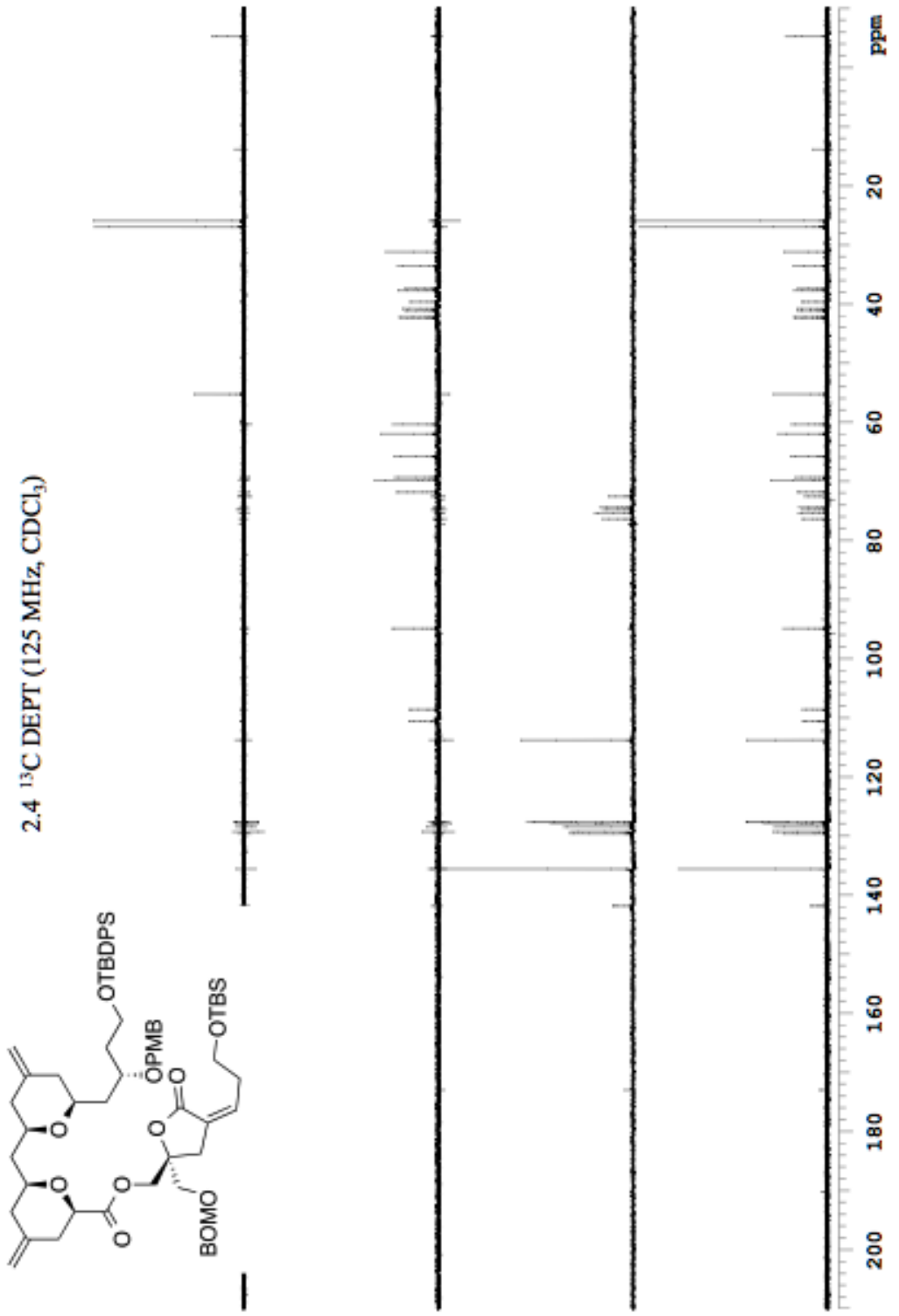
2.1 ^{13}C NMR (125 MHz, $CDCl_3$)

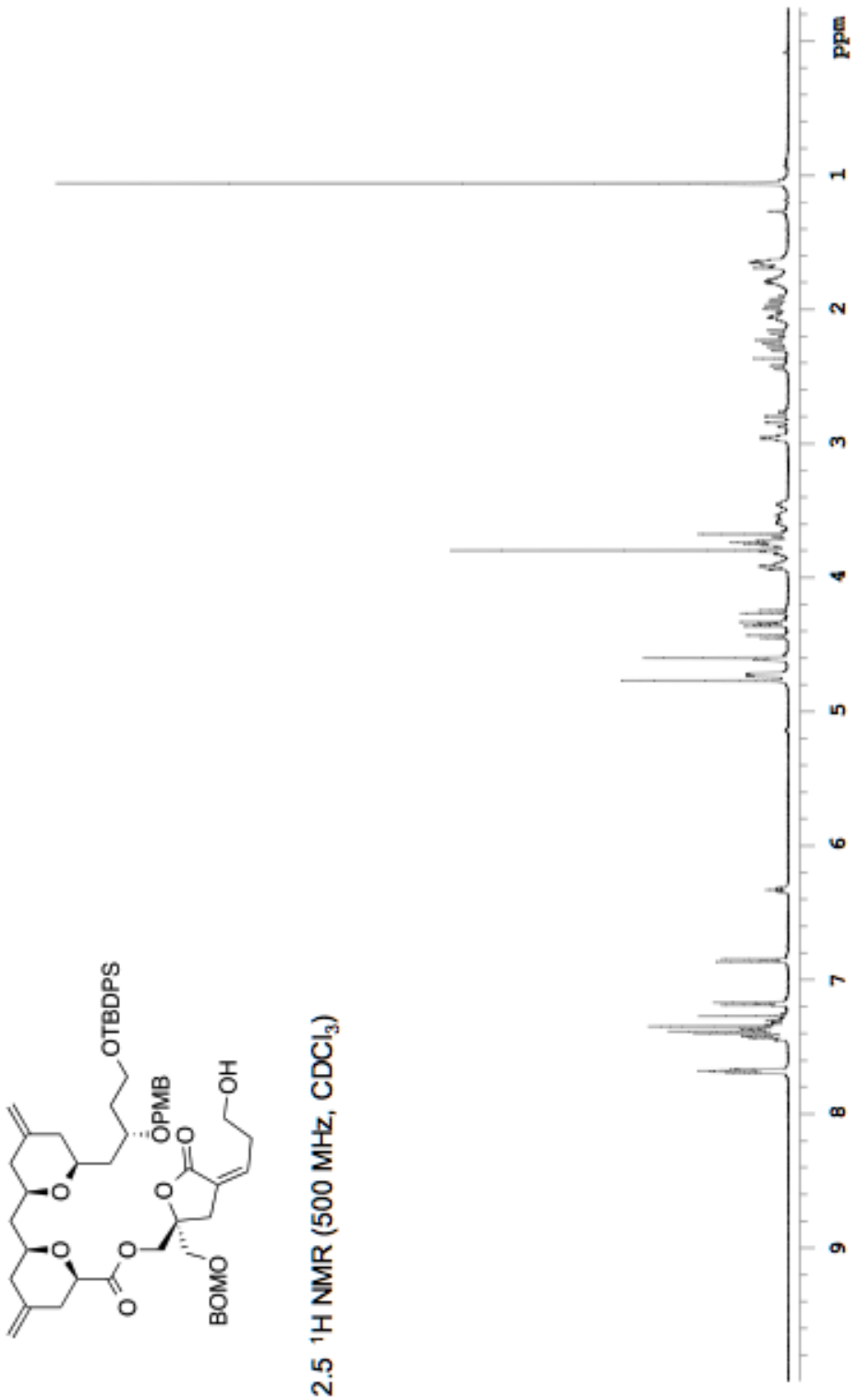


2.4 ^{1}H NMR (500 MHz, $CDCl_3$)

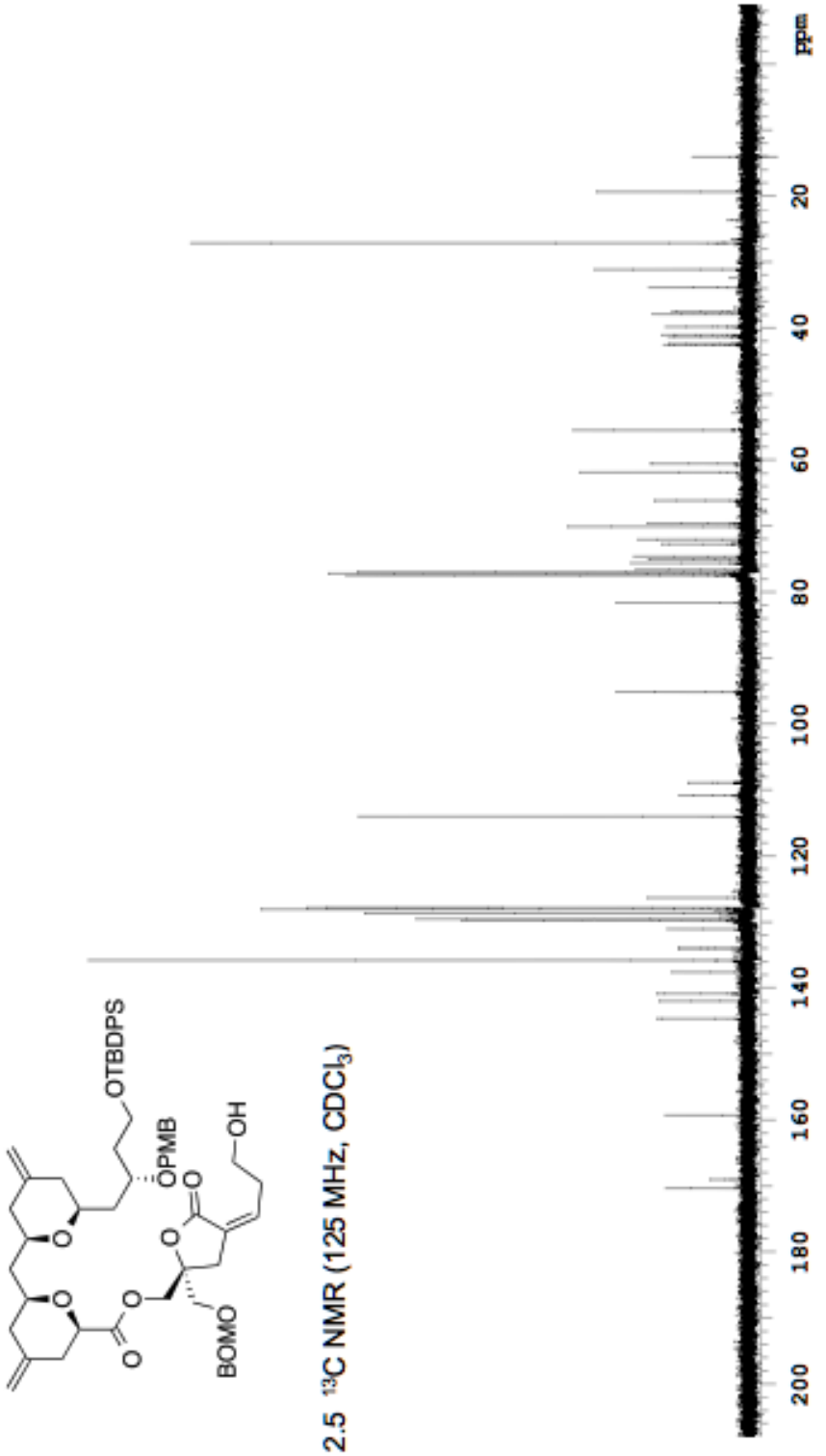


2.4 ^{13}C NMR (125 MHz, $CDCl_3$)


2.4 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTBDPS
OPMB
OTBS
BOMO
O
O
O
O
O
O
O
ppm
200
180
160
140
120
100
80
60
40
20

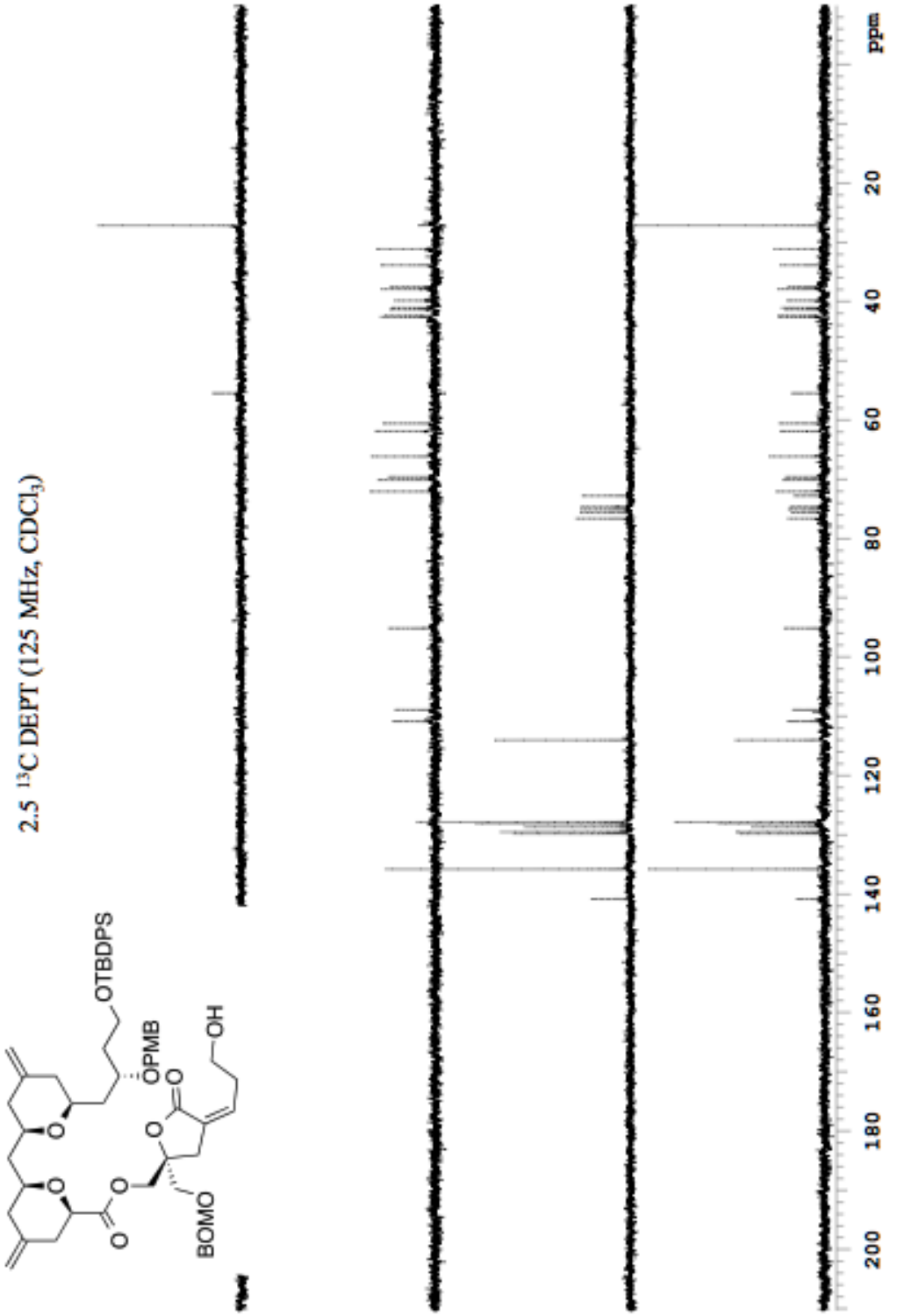


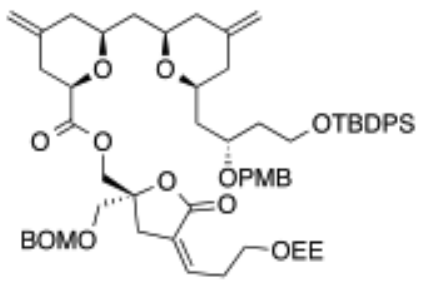
2.5 ^{1}H NMR (500 MHz, $CDCl_3$)



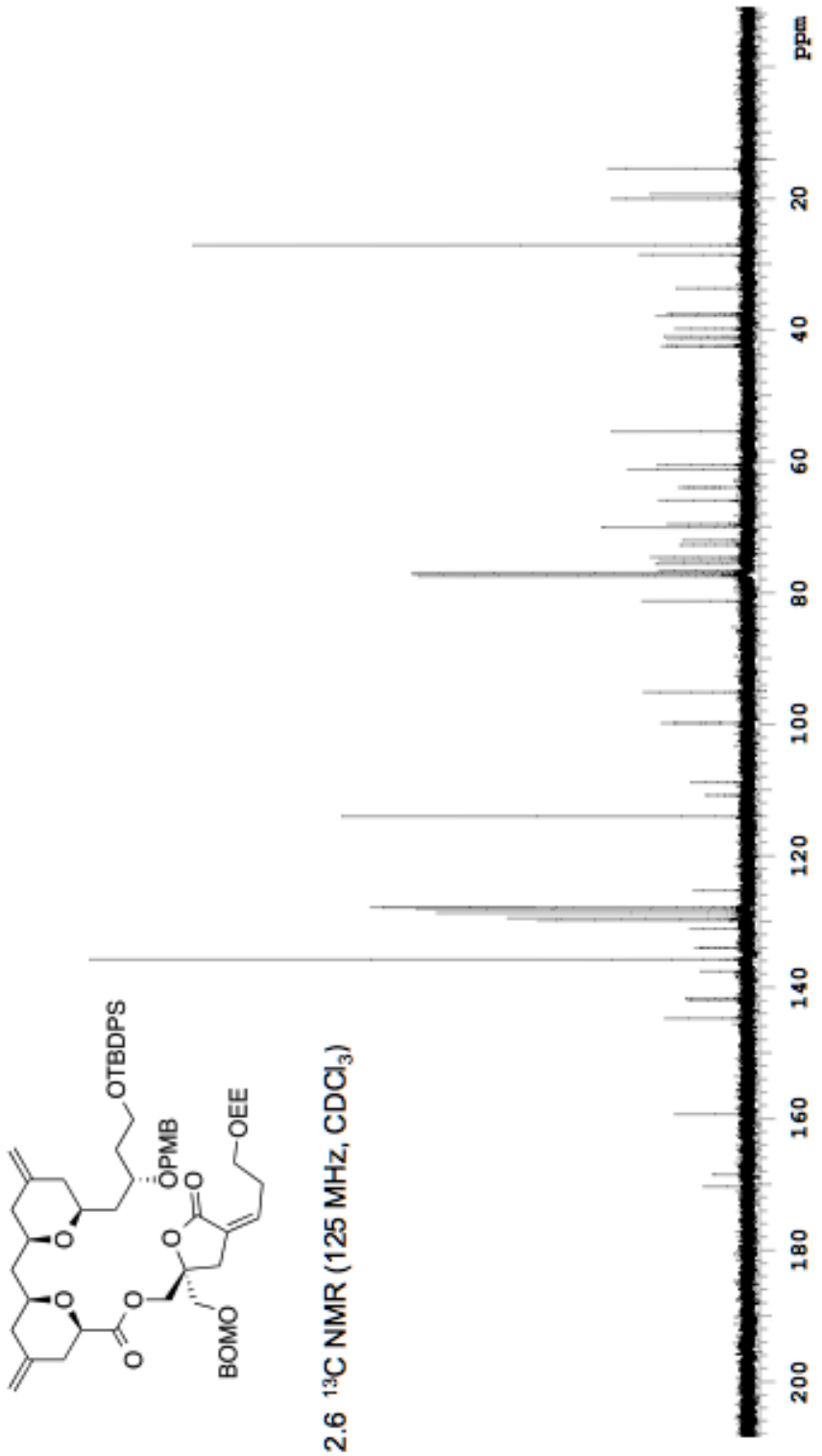
2.5 ^{13}C NMR (125 MHz, $CDCl_3$)

2.5 ^{13}C DEPT (125 MHz, $CDCl_3$)

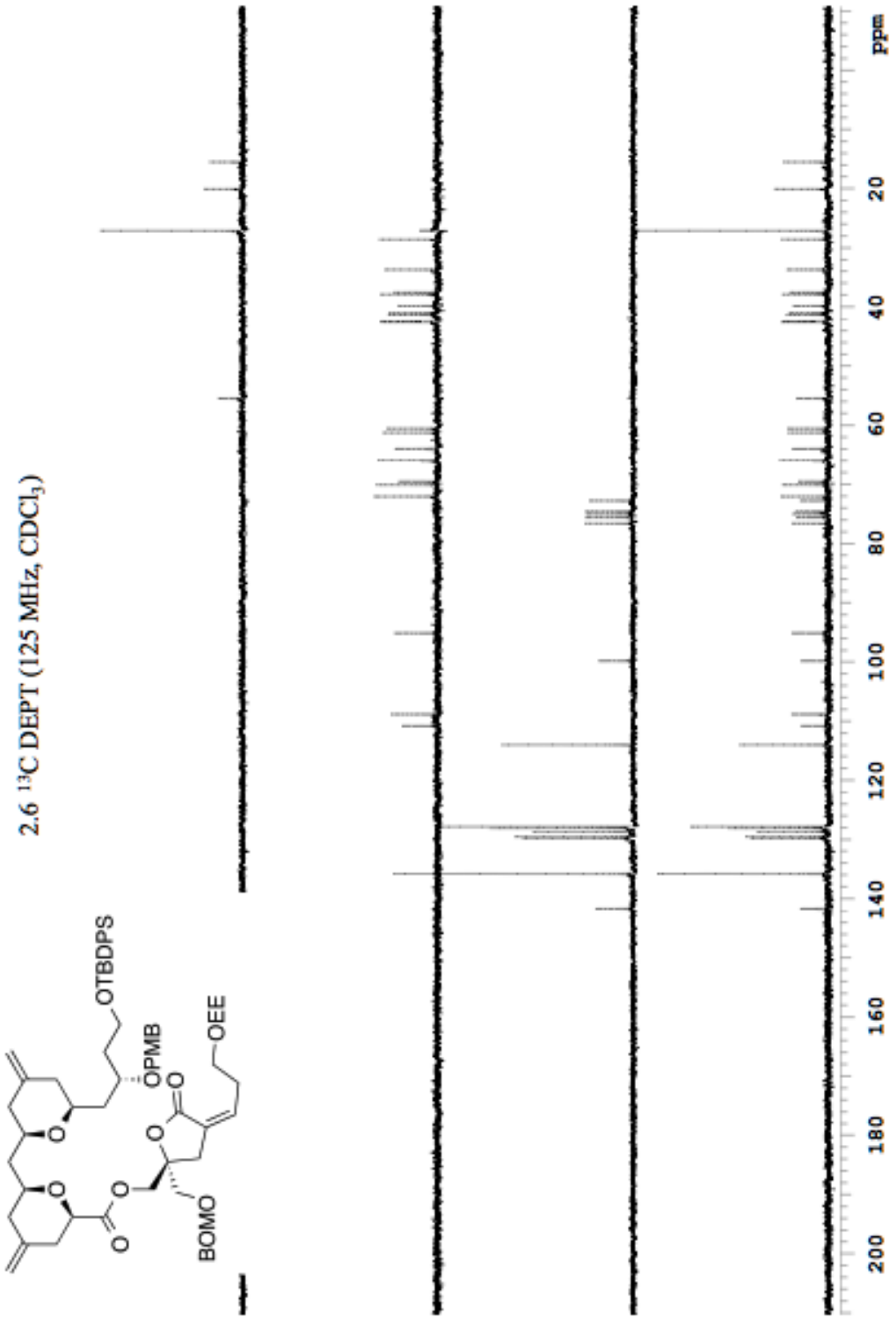


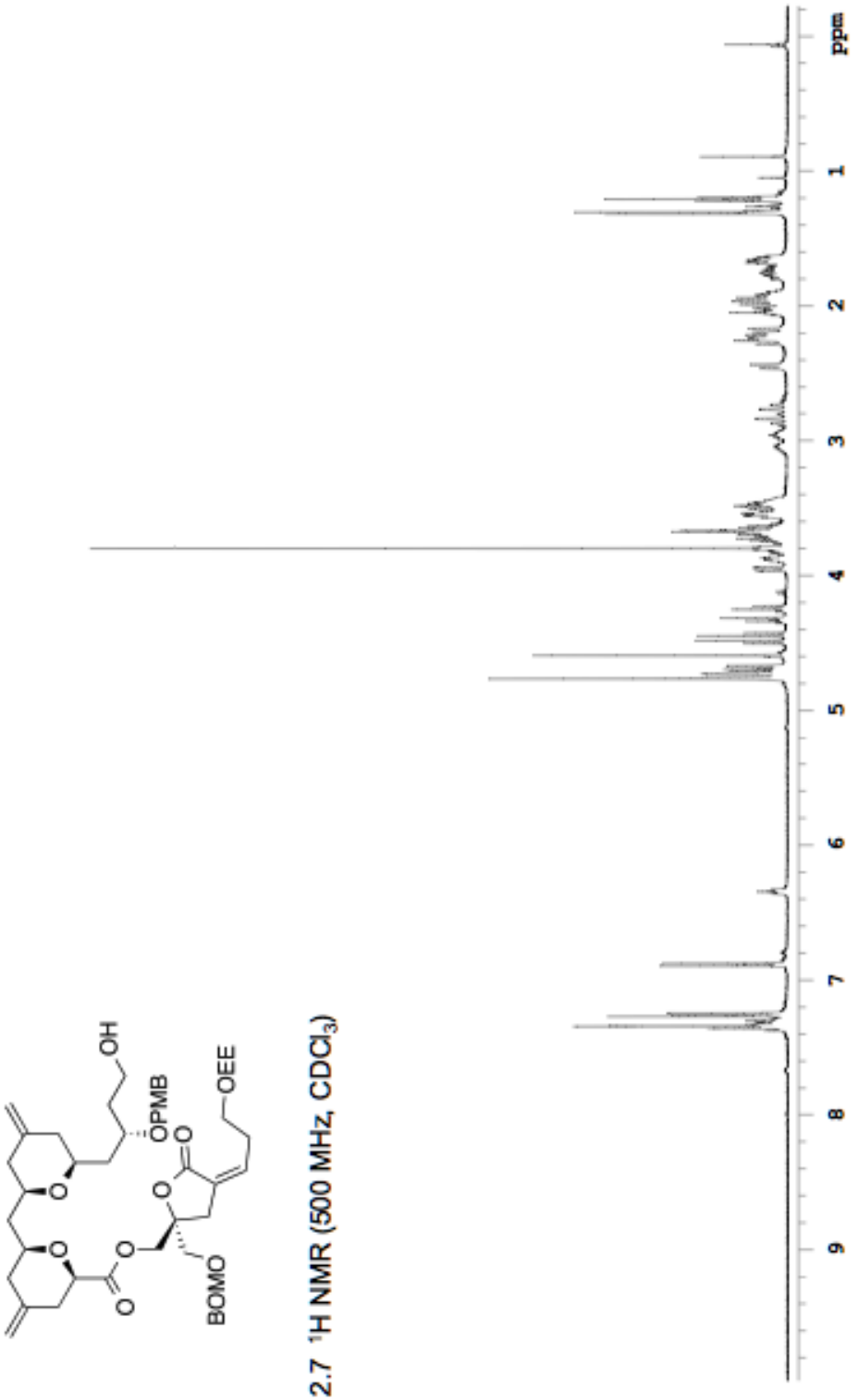


2.6 ^{1}H NMR (500 MHz, $CDCl_3$)

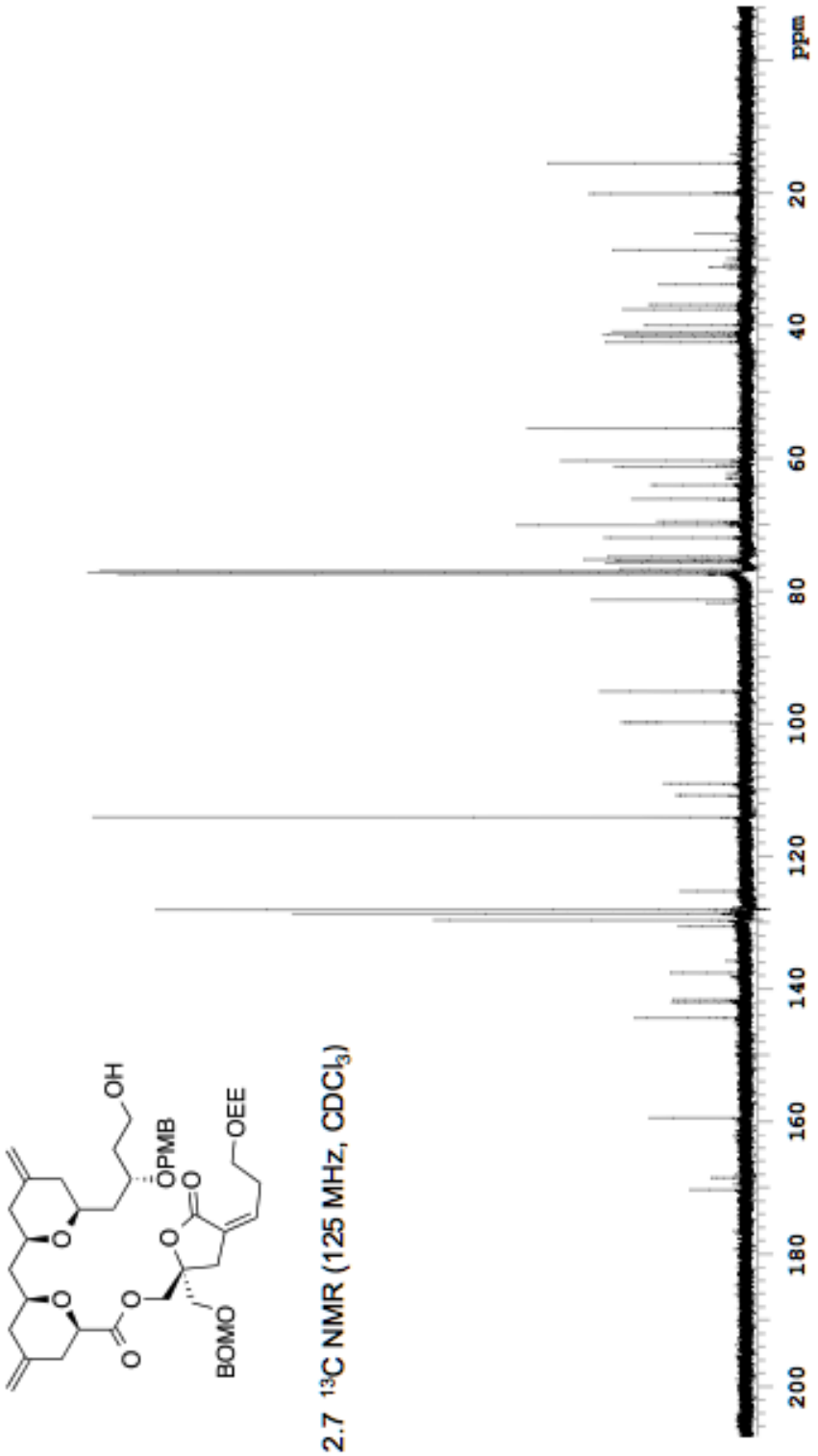


2.6 ^{13}C NMR (125 MHz, $CDCl_3$)


2.6 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTBDPS
OPMB
OEE
BOMO
O
ppm
200
180
160
140
120
100
80
60
40
20

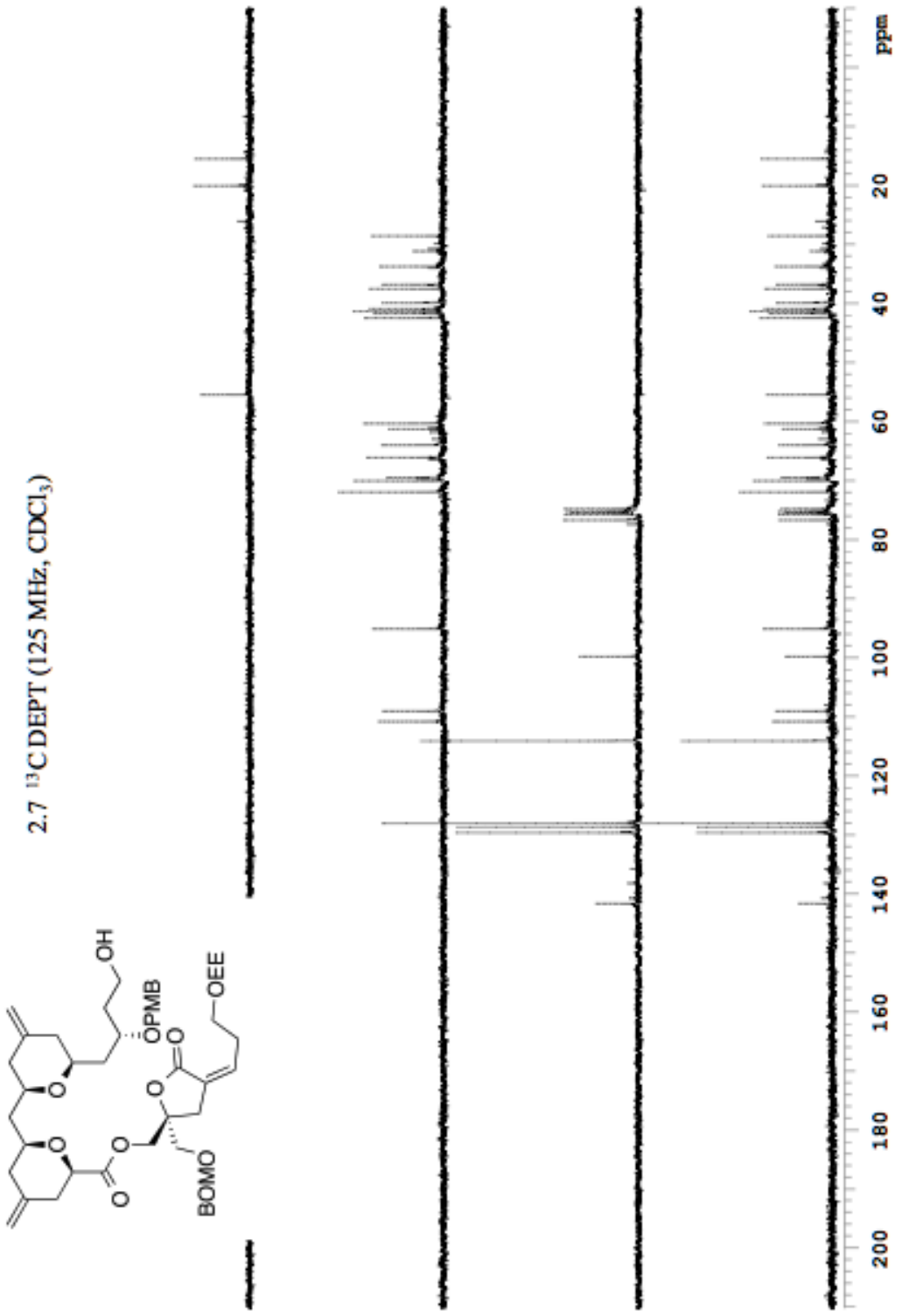


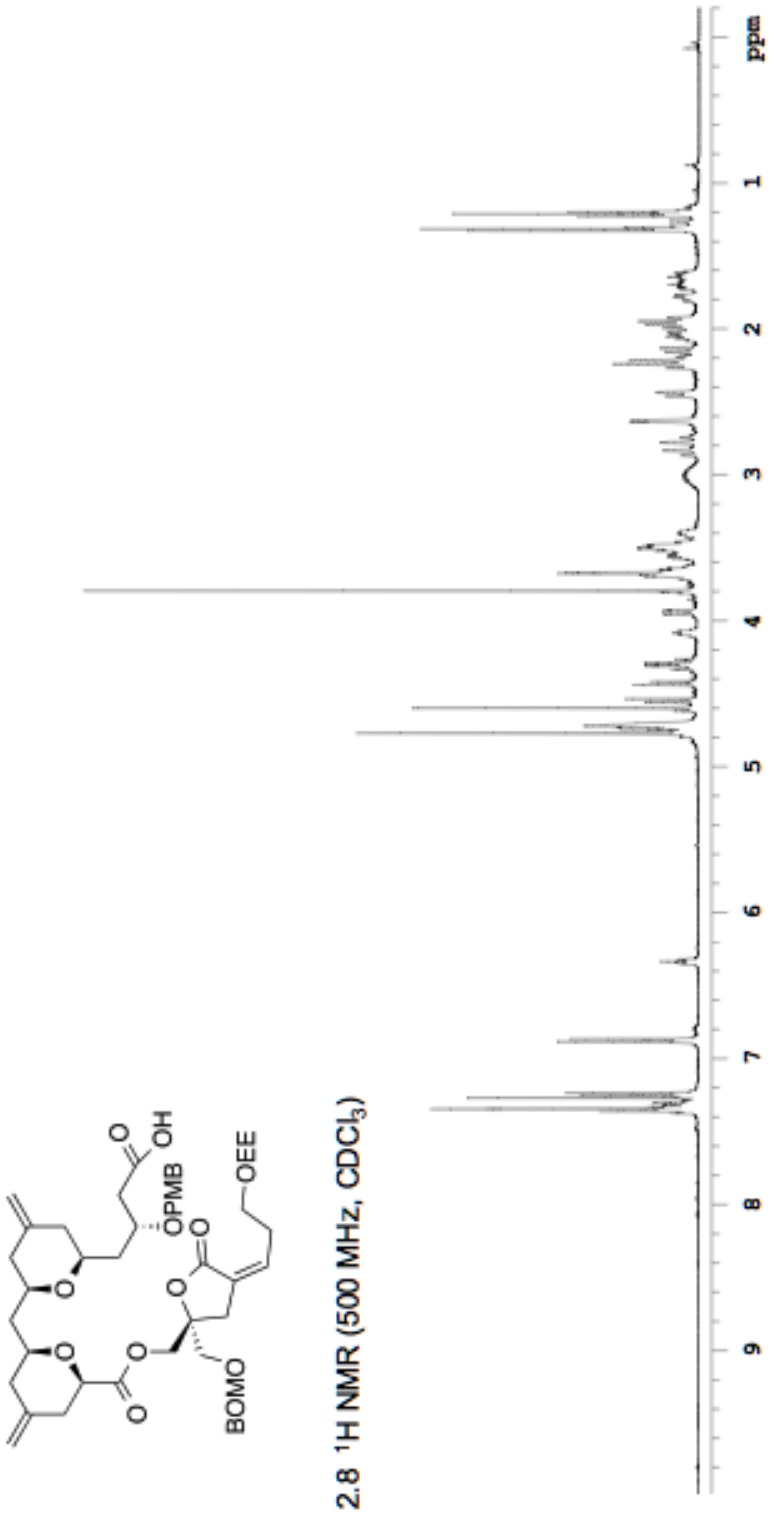
2.7 ^{1}H NMR (500 MHz, $CDCl_3$)



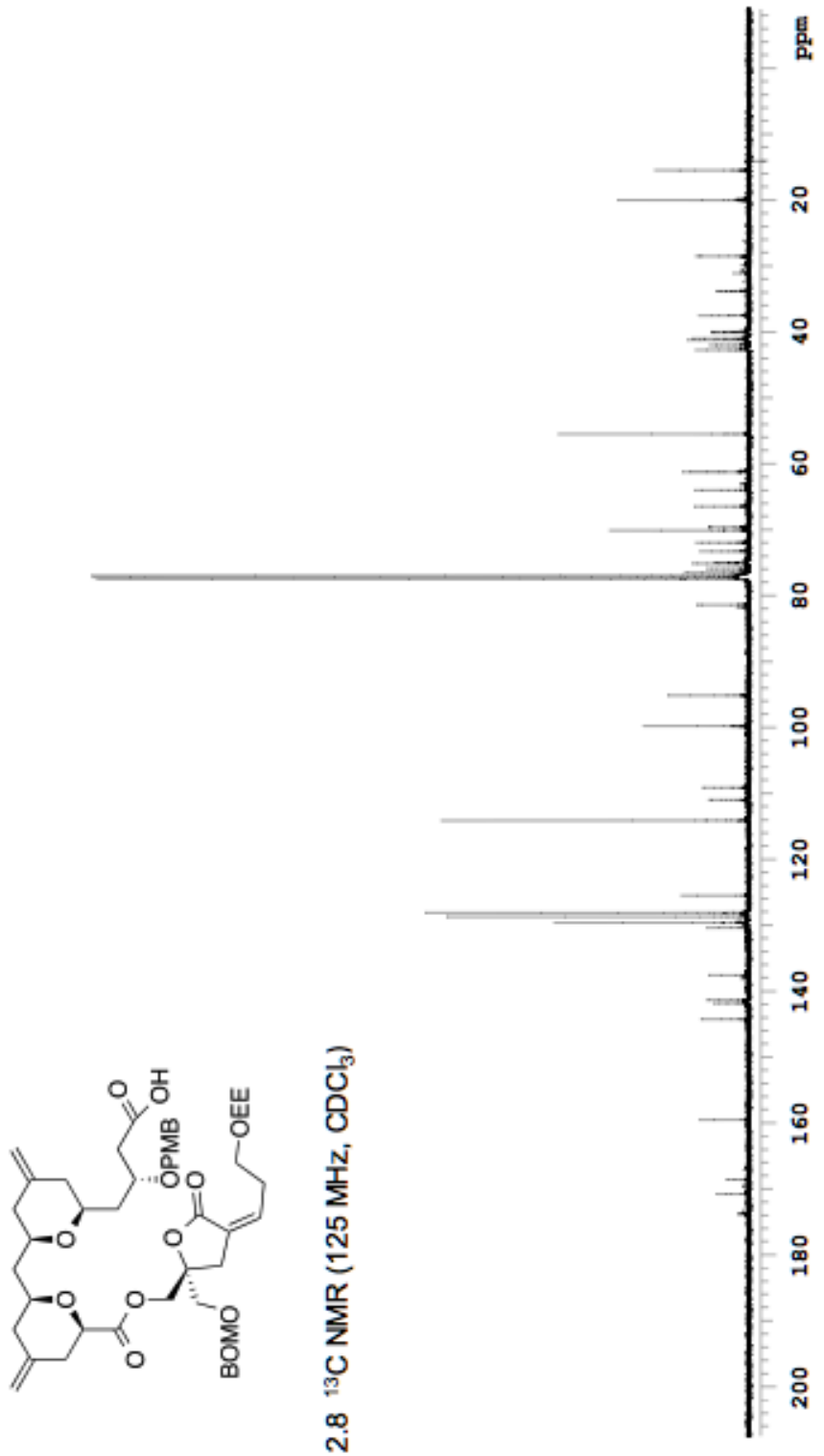
2.7 ^{13}C NMR (125 MHz, $CDCl_3$)

2.7 ^{13}C DEPT (125 MHz, $CDCl_3$)



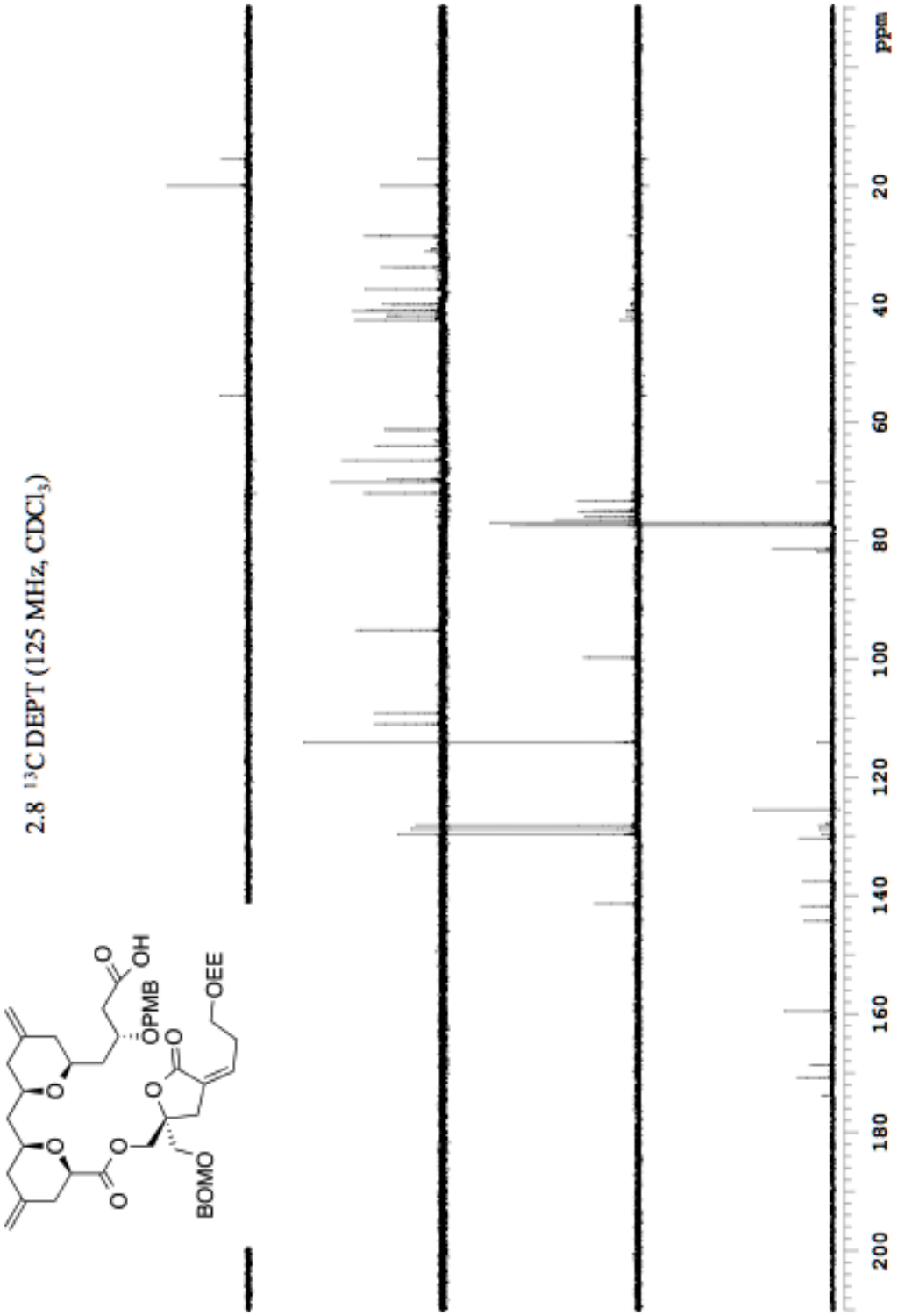


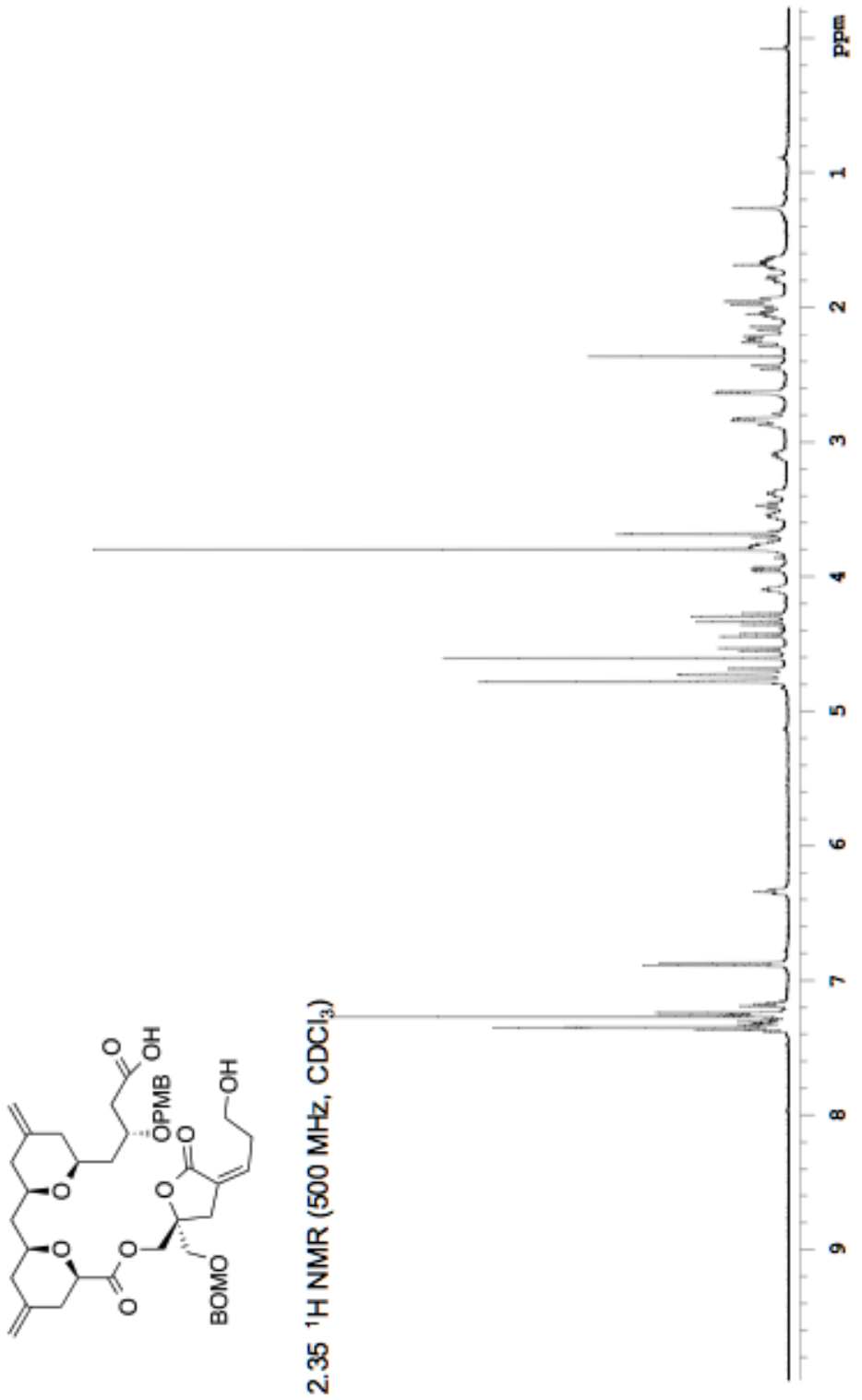
2.8 ^{1}H NMR (500 MHz, $CDCl_3$)



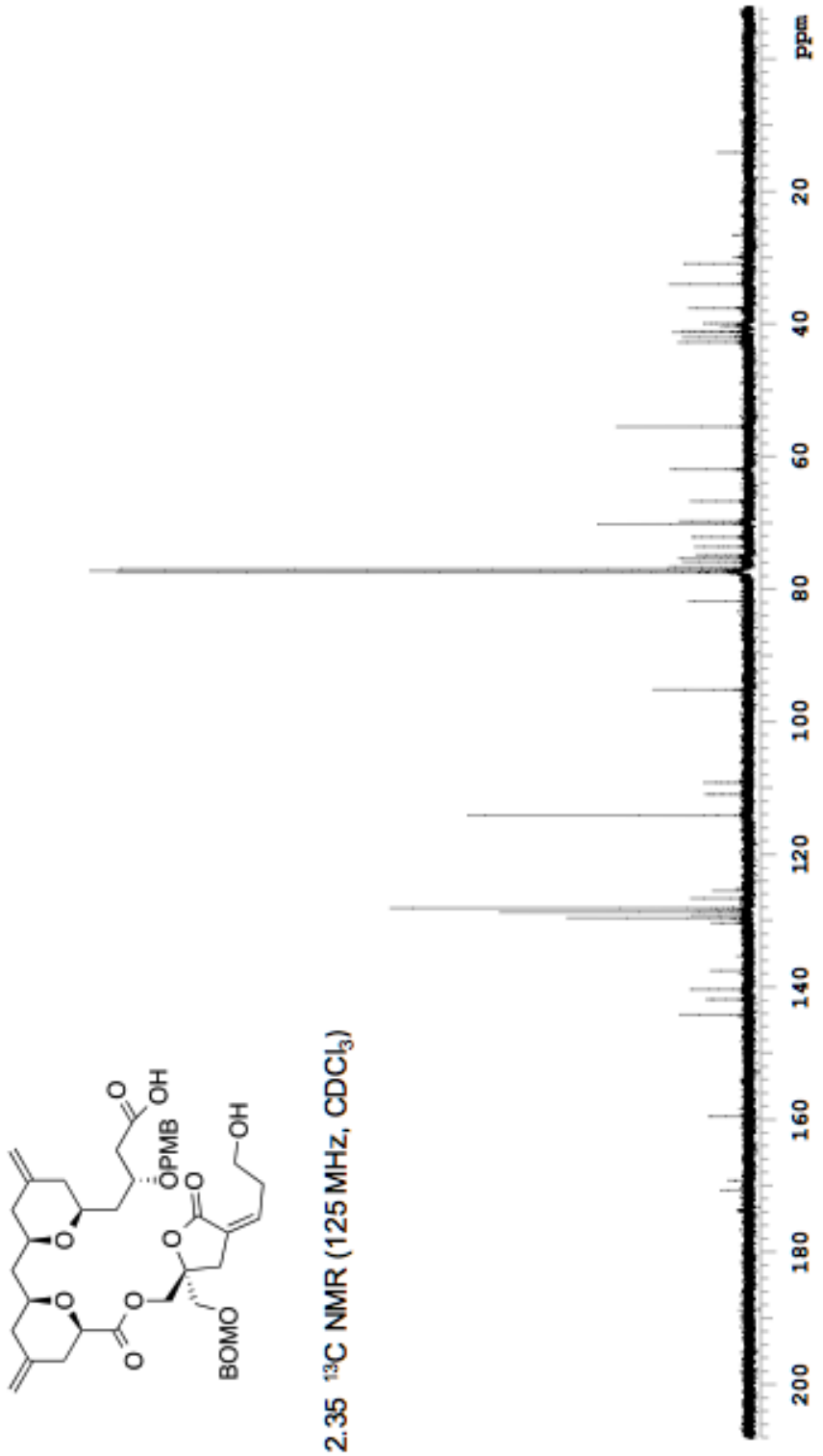
2.8 ^{13}C NMR (125 MHz, $CDCl_3$)

2.8 ^{13}C DEPT (125 MHz, $CDCl_3$)



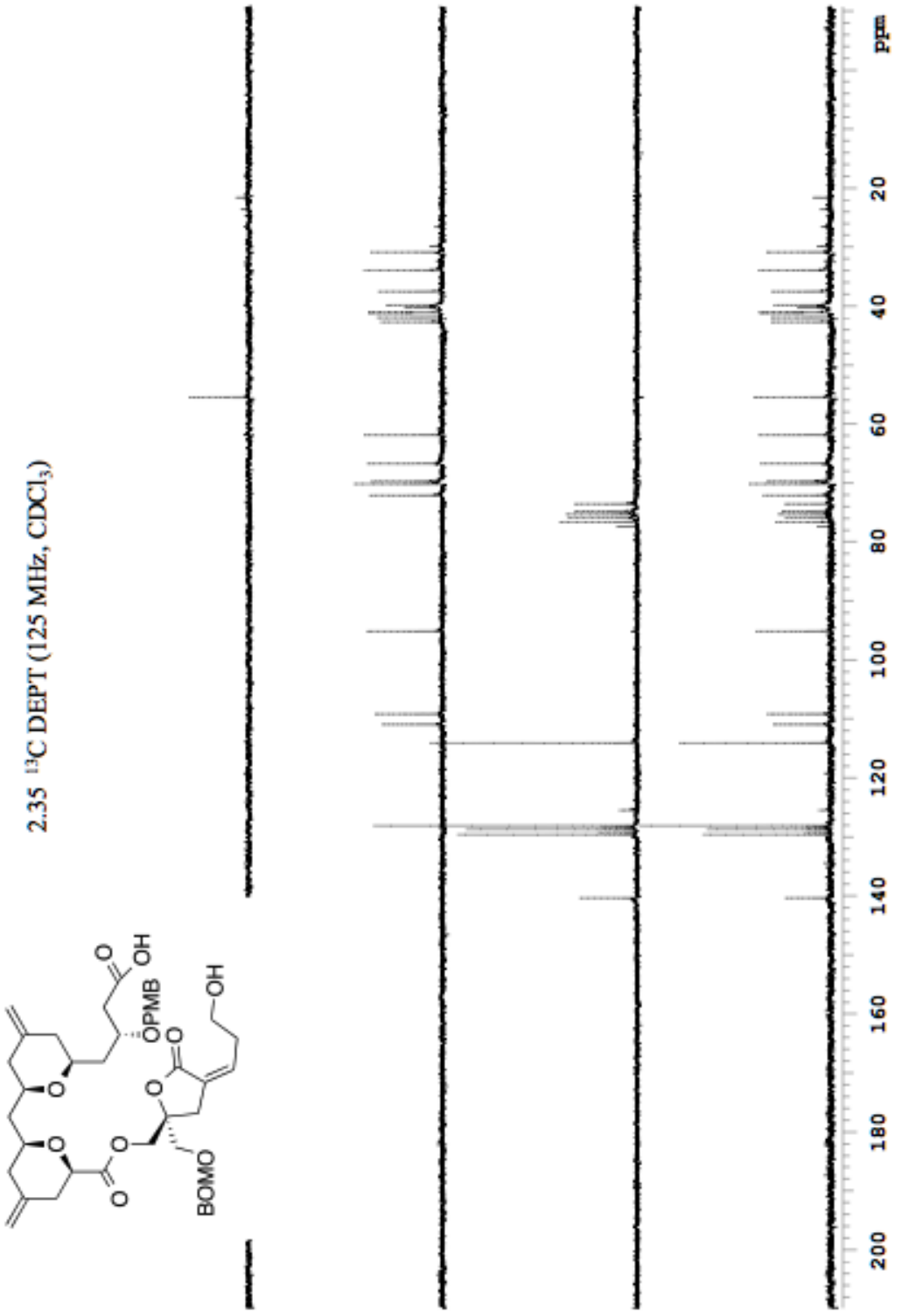


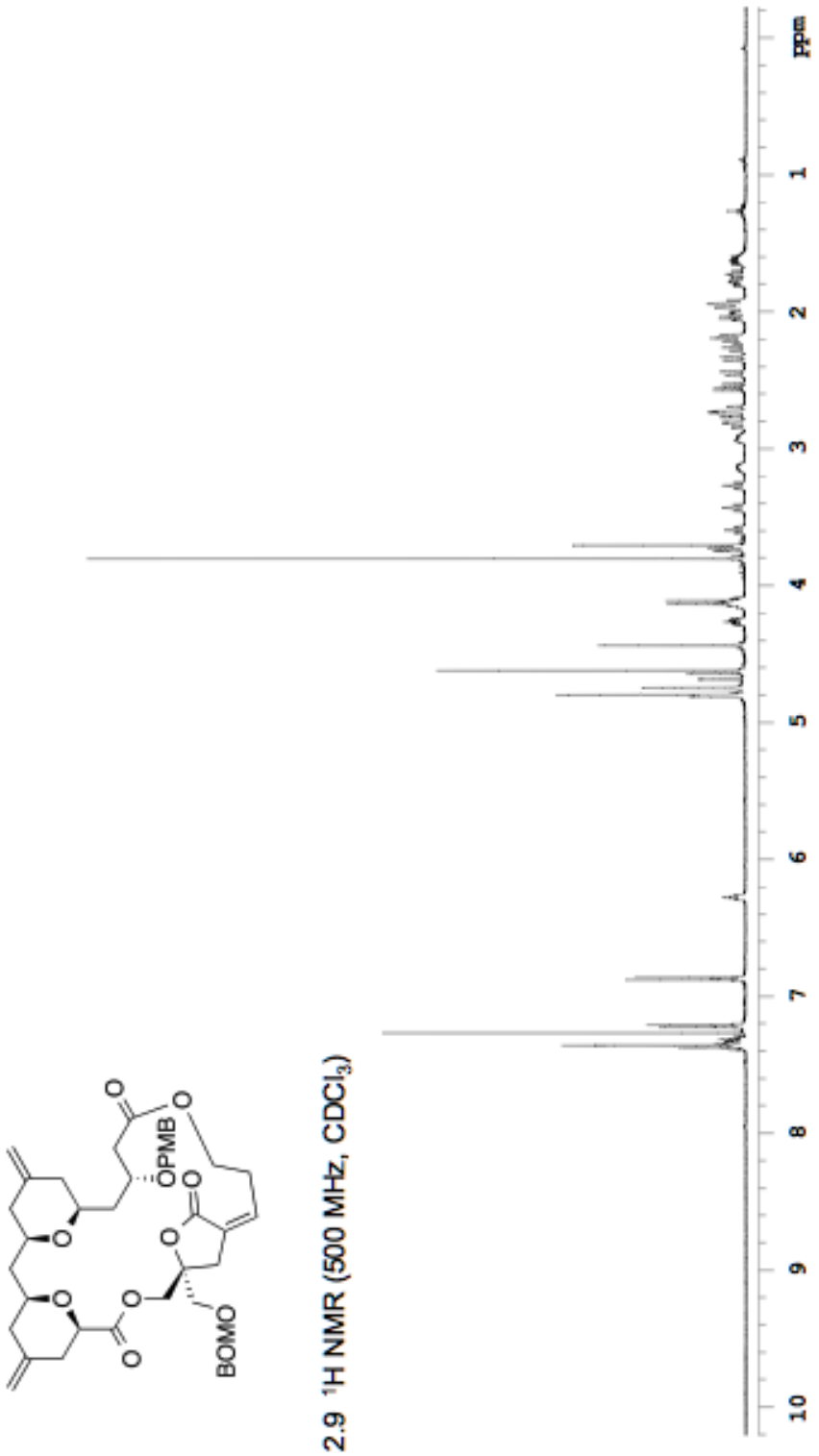
2.35 ^{1}H NMR (500 MHz, $CDCl_3$)



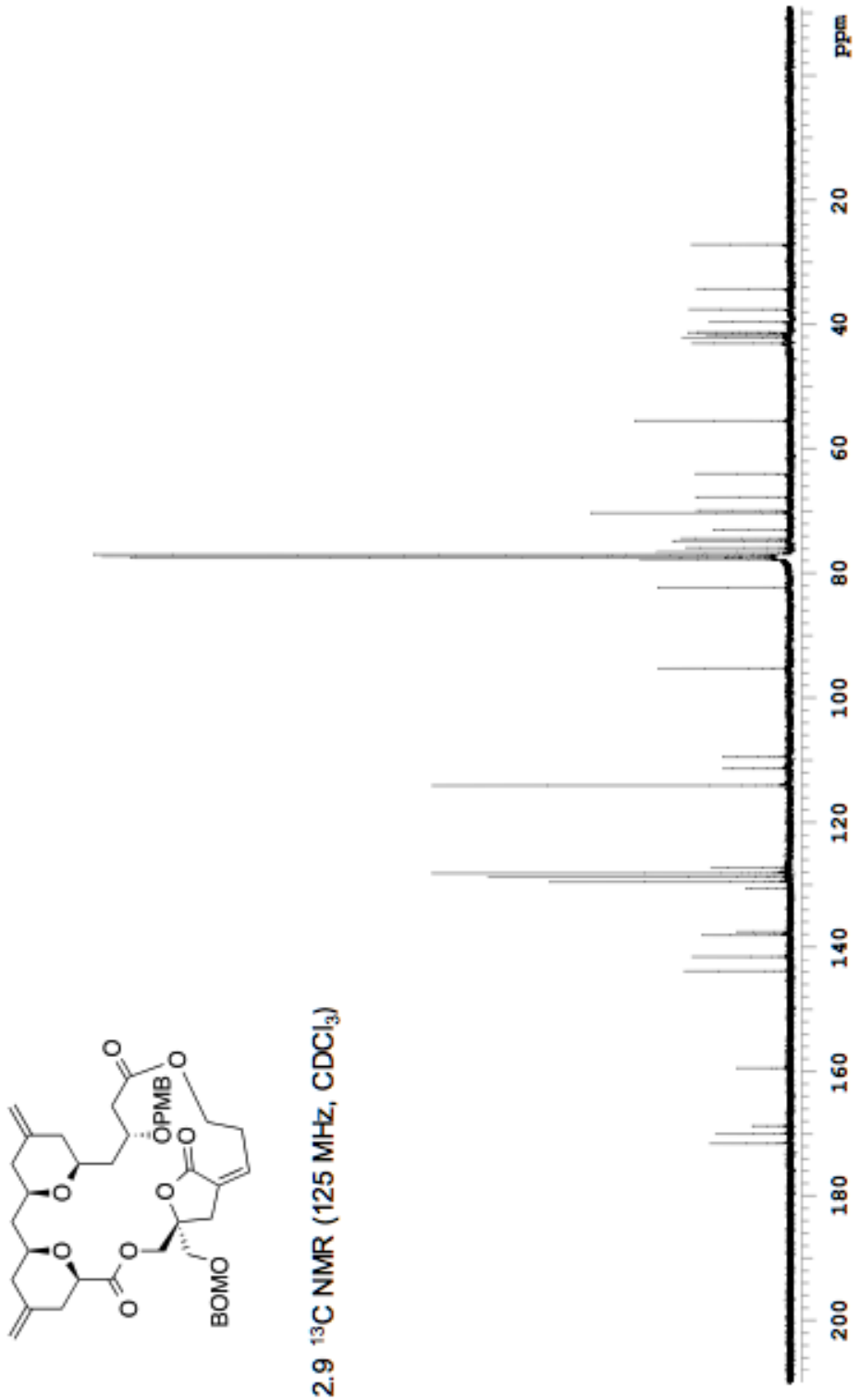
2.35 ^{13}C NMR (125 MHz, $CDCl_3$)

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

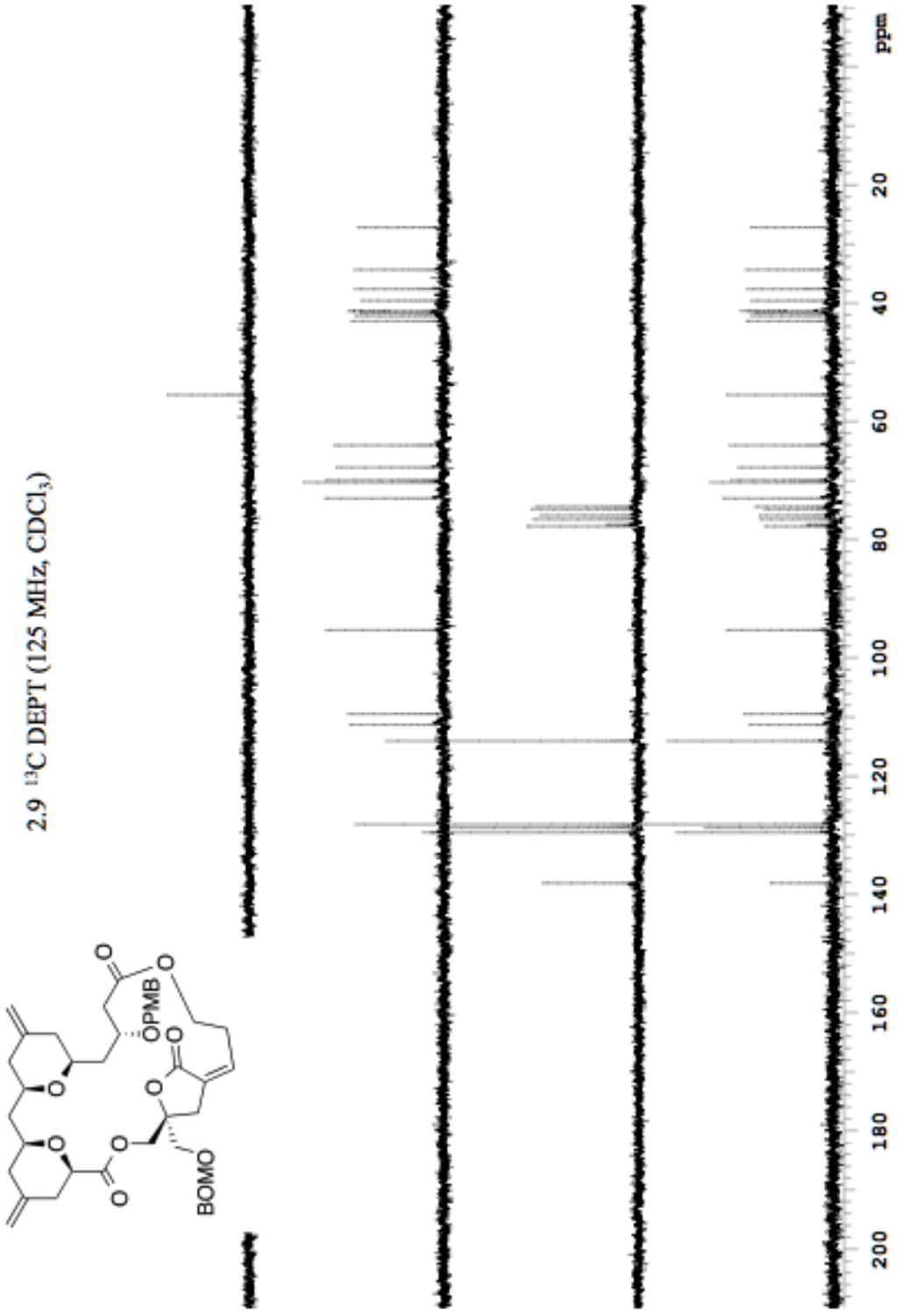




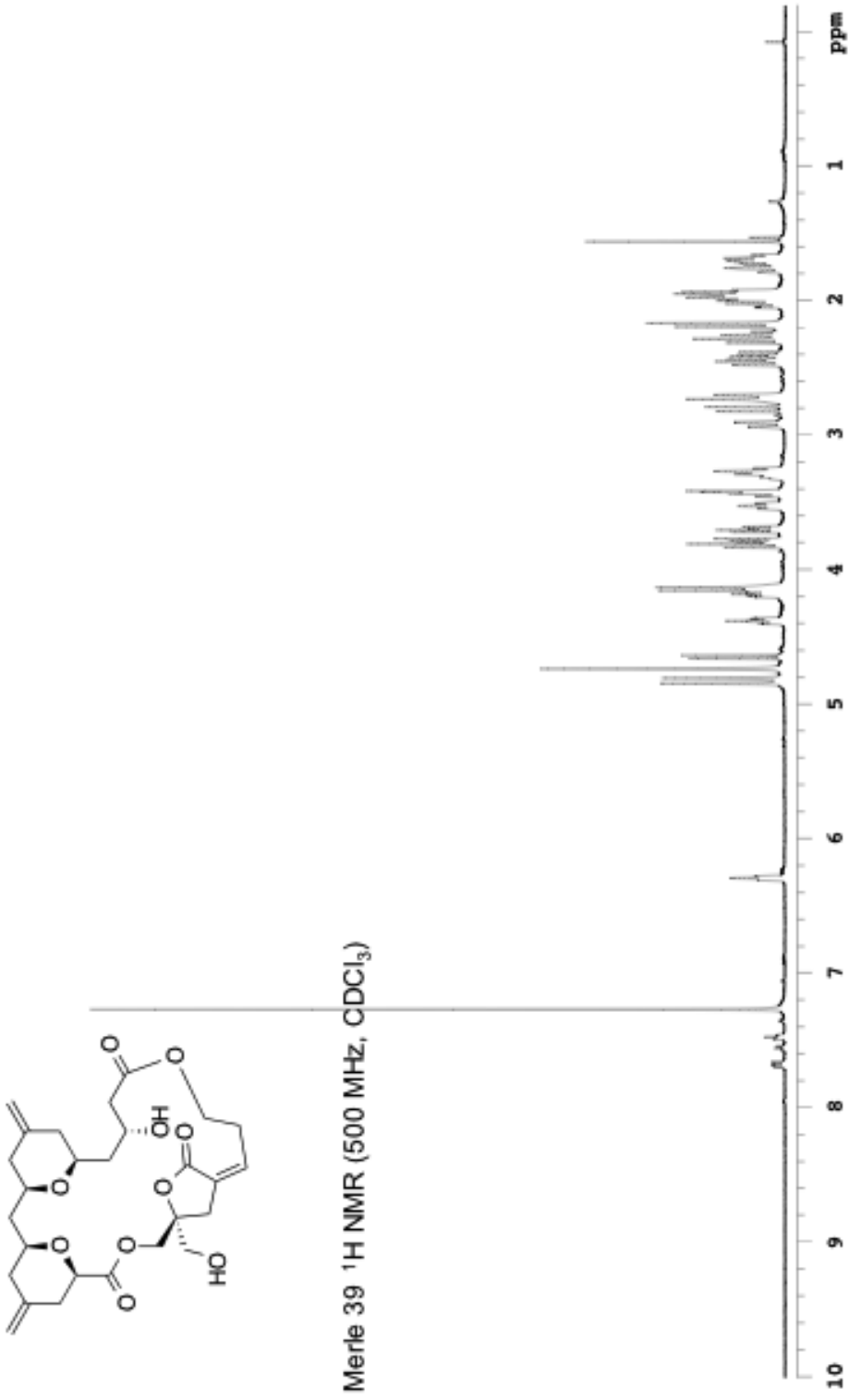
2.9 ^{1}H NMR (500 MHz, $CDCl_3$)

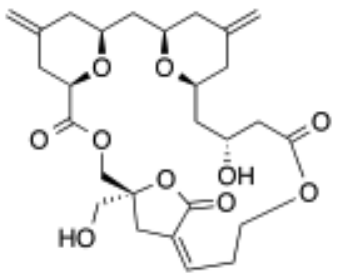


2.9 ^{13}C NMR (125 MHz, $CDCl_3$)

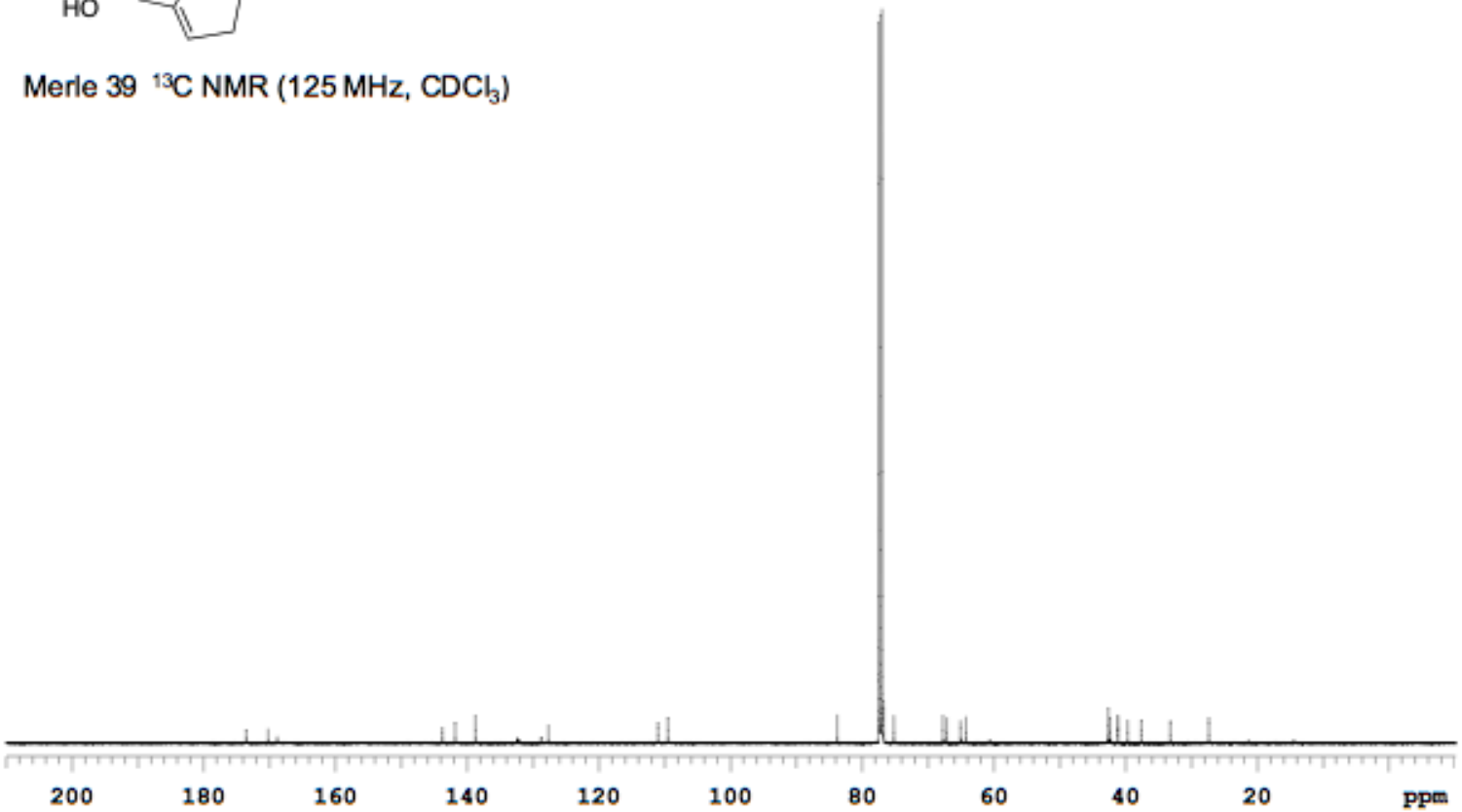


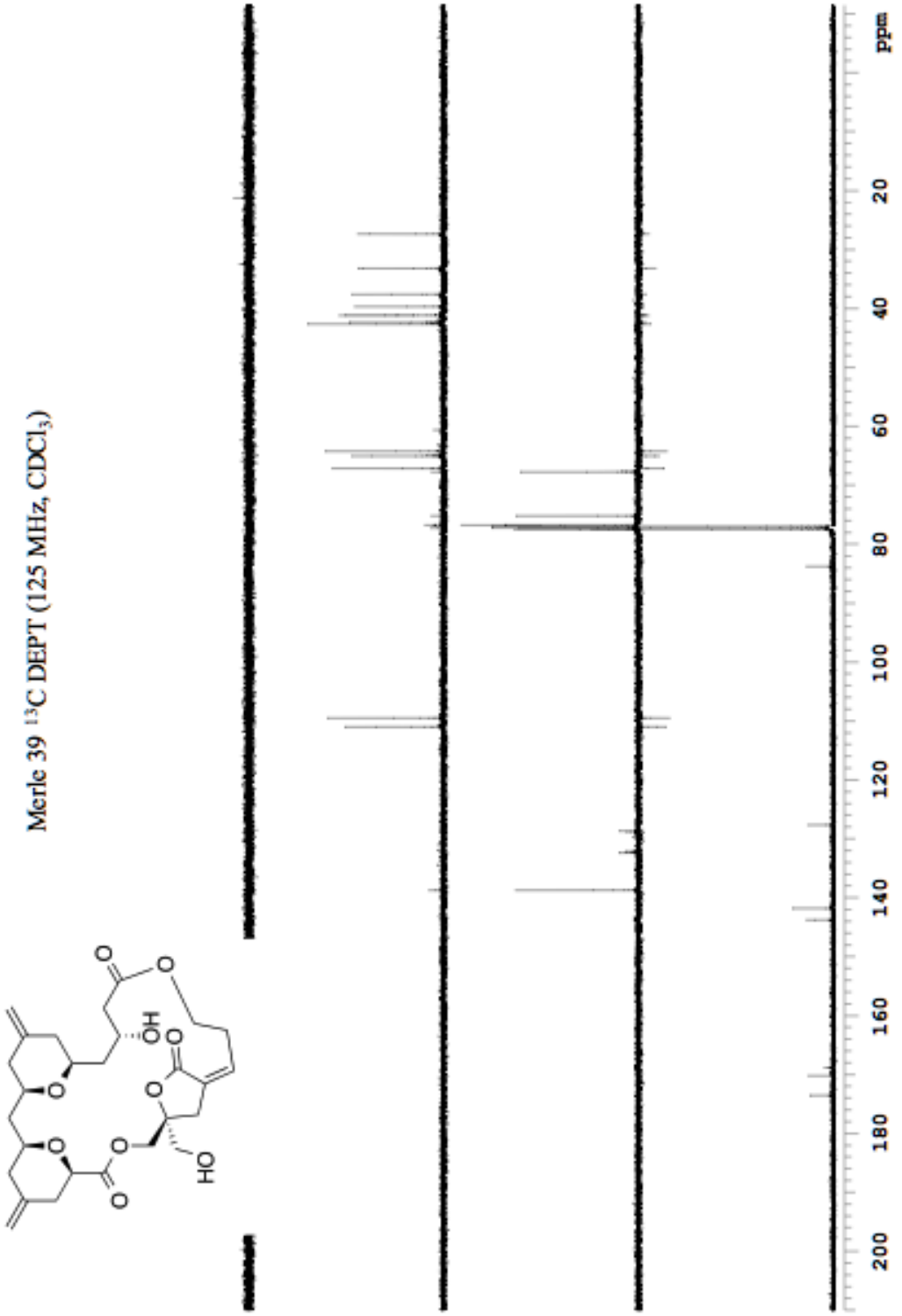
2.9 ^{13}C DEPT (125 MHz, $CDCl_3$)

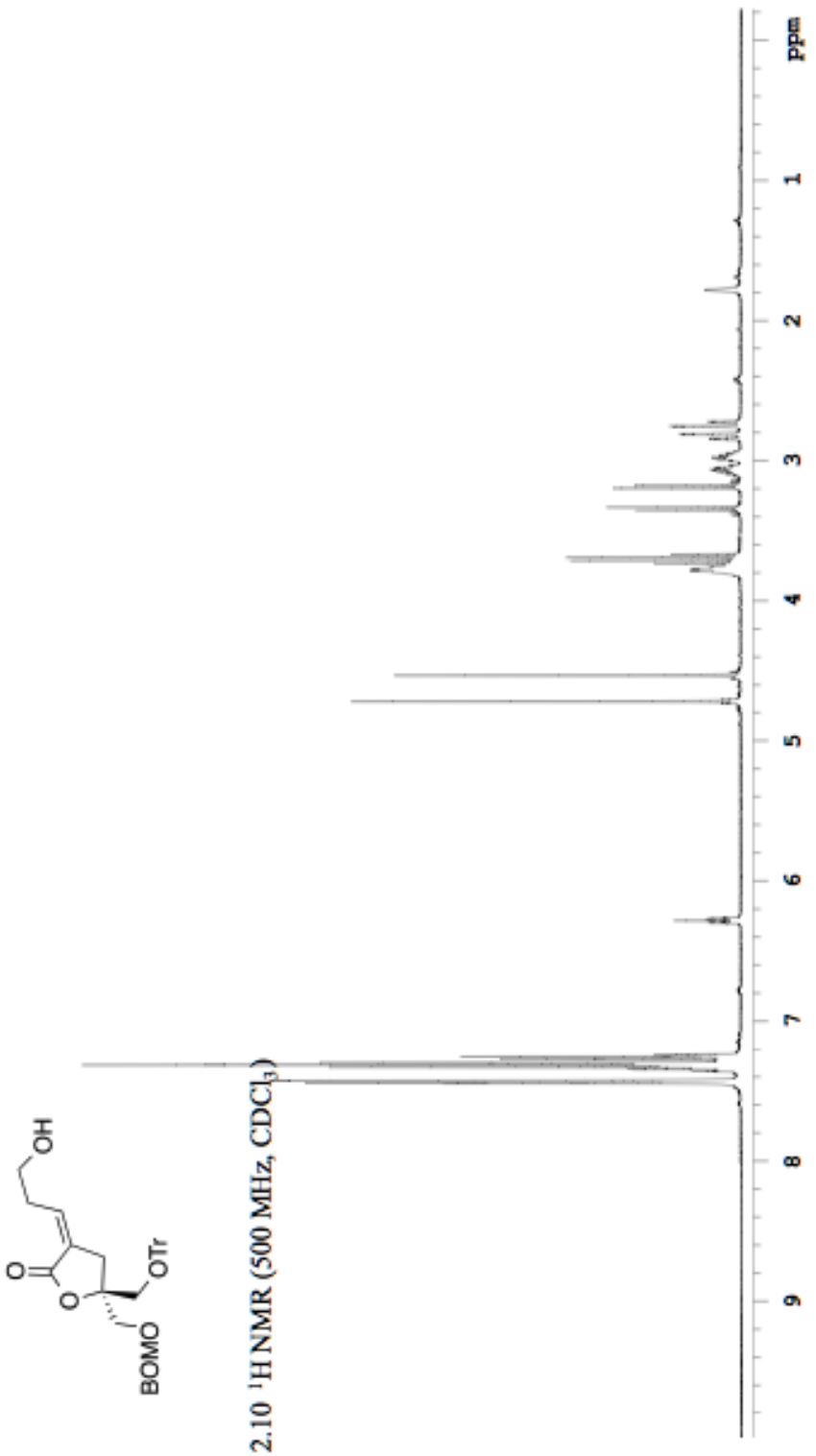

Merle 39 ^{1}H NMR (500 MHz, $CDCl_3$)
ppm
1
2
3
4
5
6
7
8
9
10
O
O
O
O
O
O
O
O
O
OH
OH



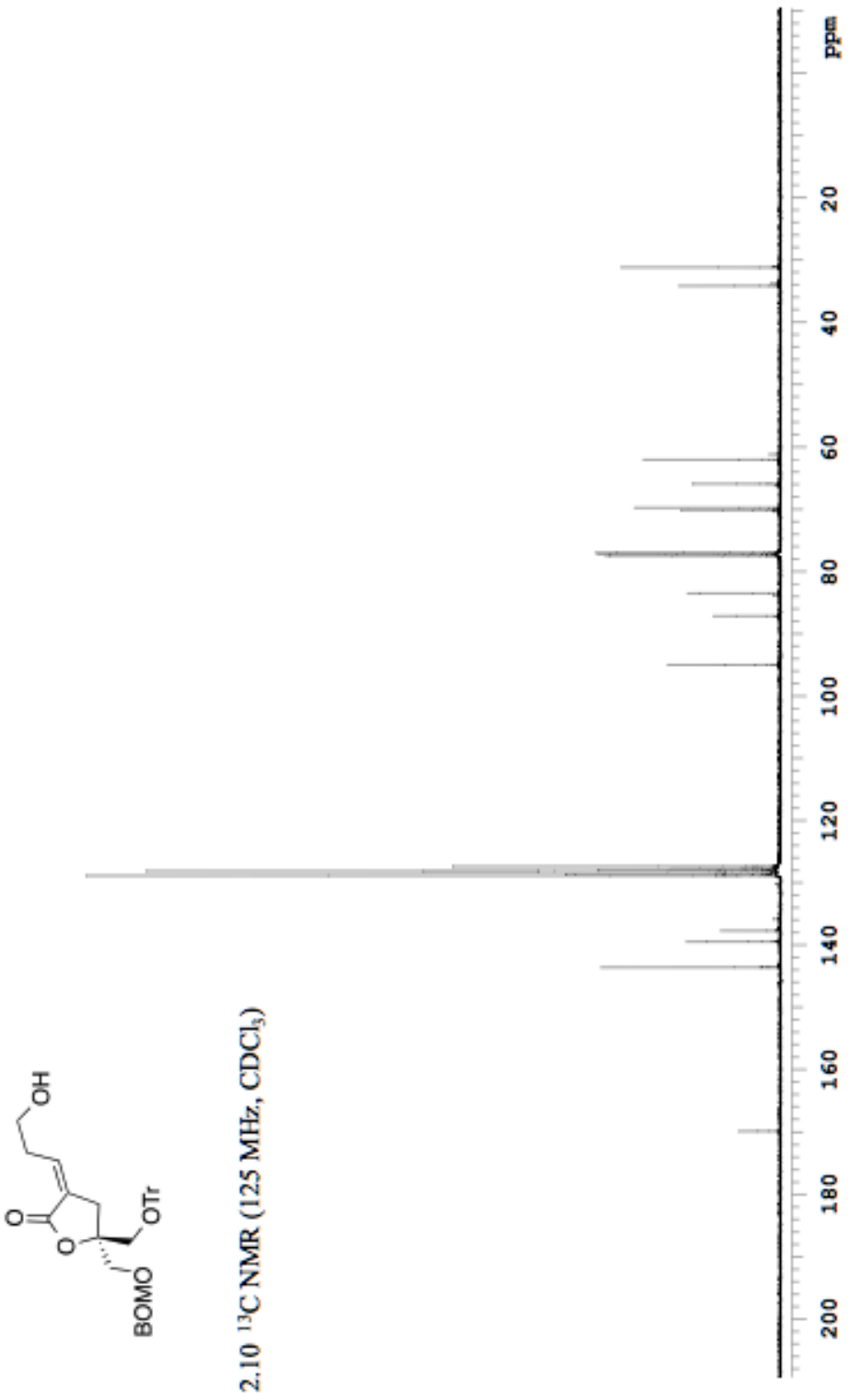
Merle 39 ^{13}C NMR (125 MHz, $CDCl_3$)



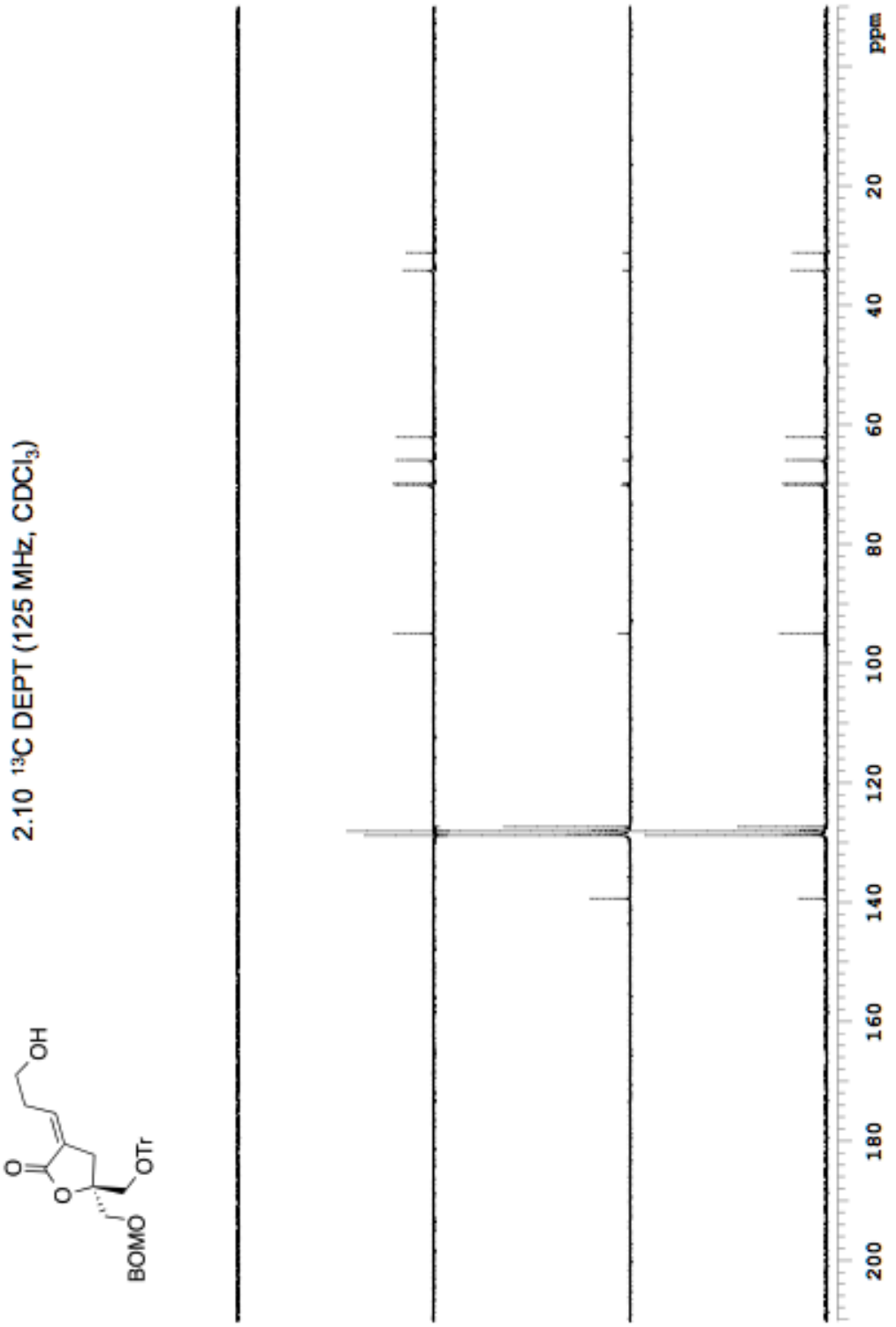

Merle 39 ^{13}C DEPT (125 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20
OH
HO
O

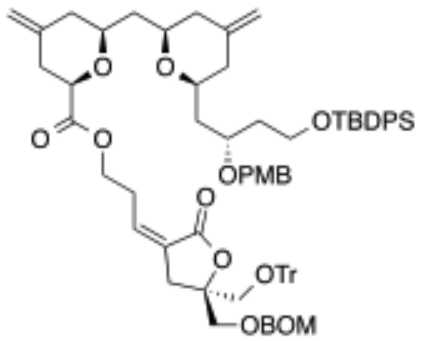


2.10 ^{1}H NMR (500 MHz, $CDCl_3$)

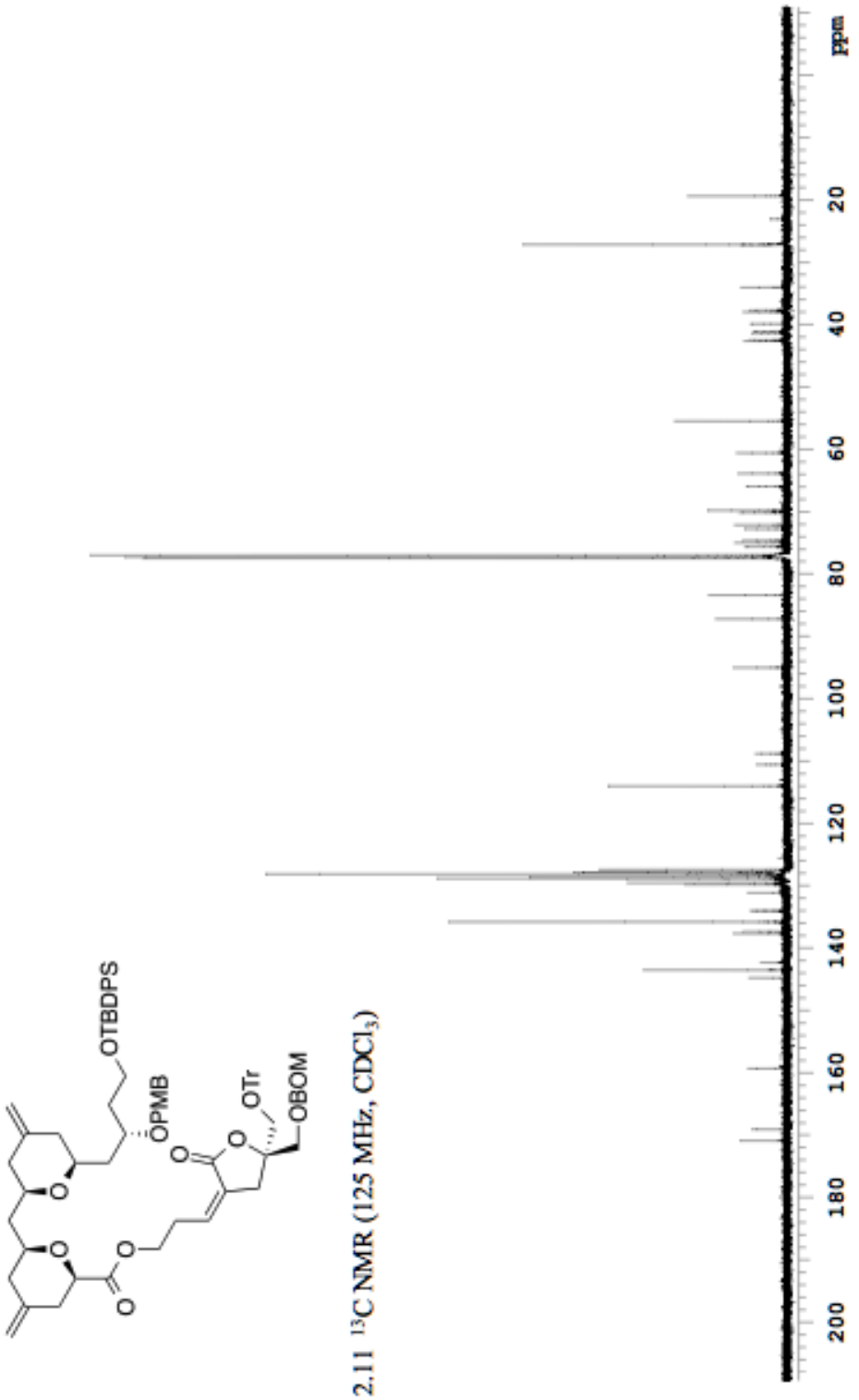


2.10 ^{13}C NMR (125 MHz, $CDCl_3$)

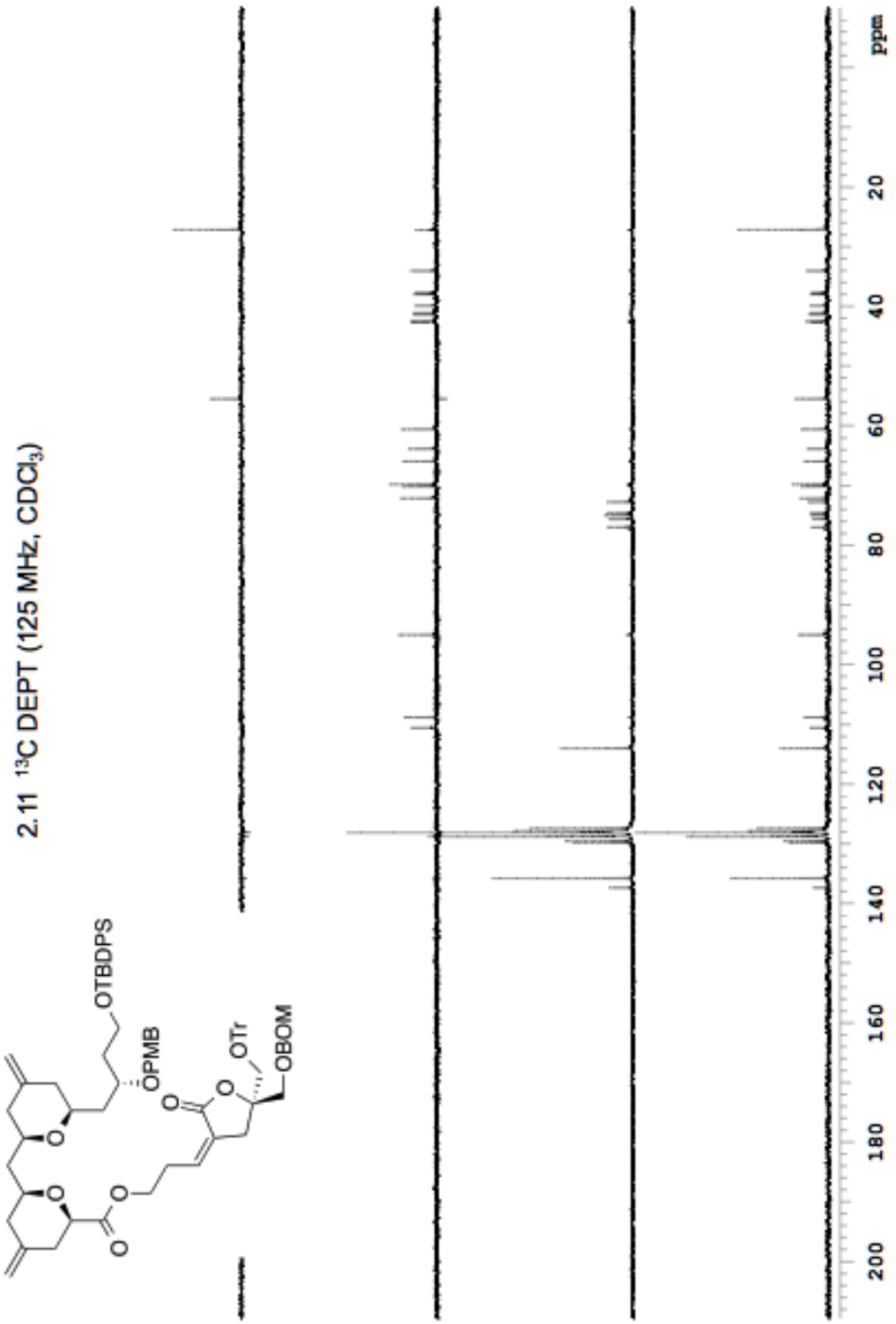

2.10 ^{13}C DEPT (125 MHz, $CDCl_3$)
OH
O
O
OTr
BOMO
200
180
160
140
120
100
80
60
40
20
ppm

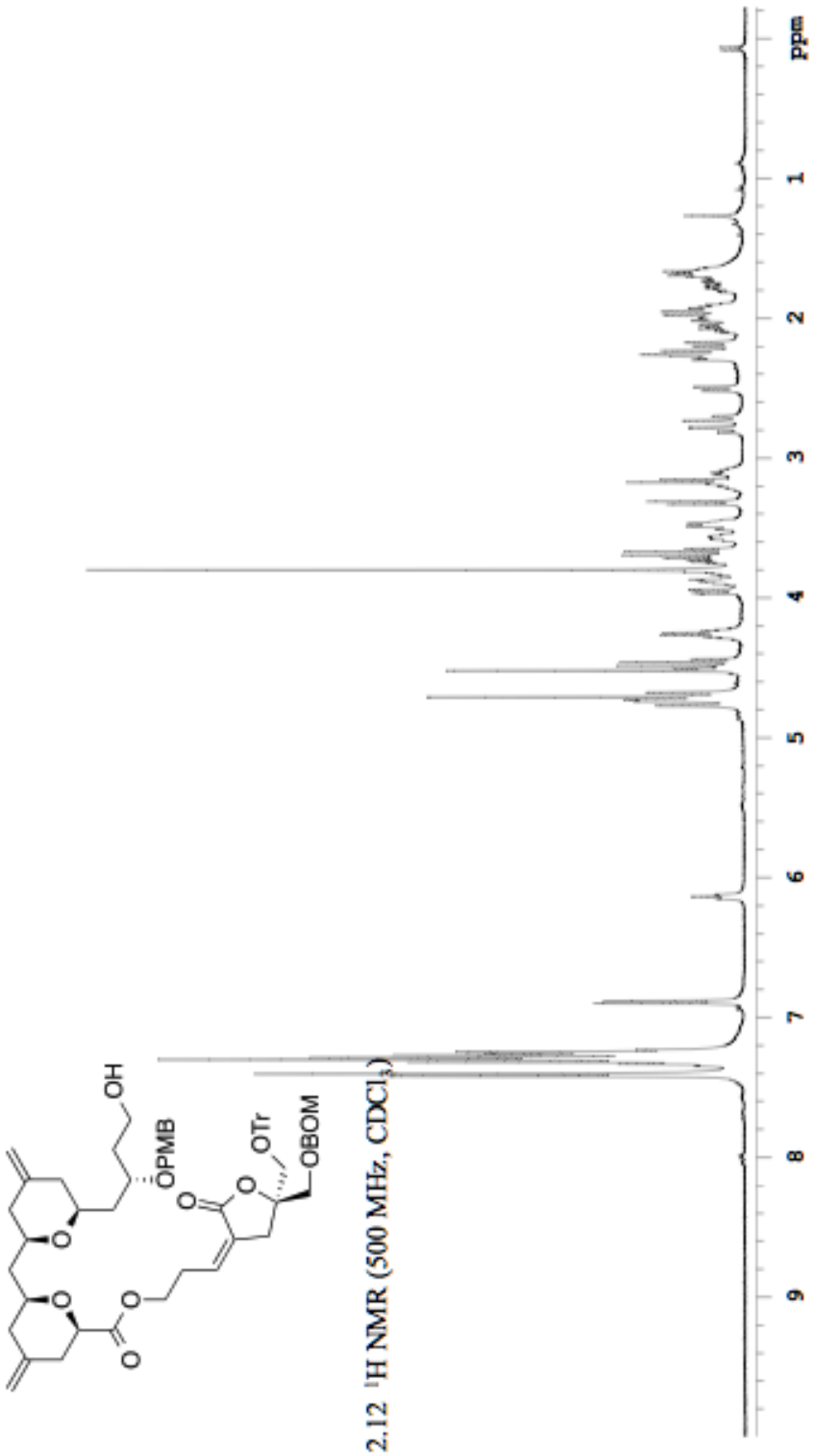


2.11 ^{1}H NMR (500 MHz, $CDCl_3$)

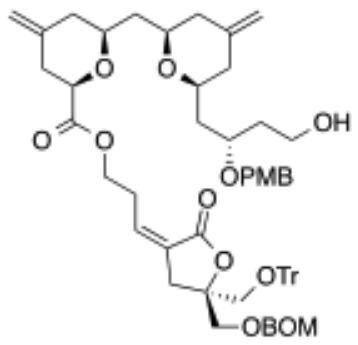


2.11 ^{13}C NMR (125 MHz, $CDCl_3$)

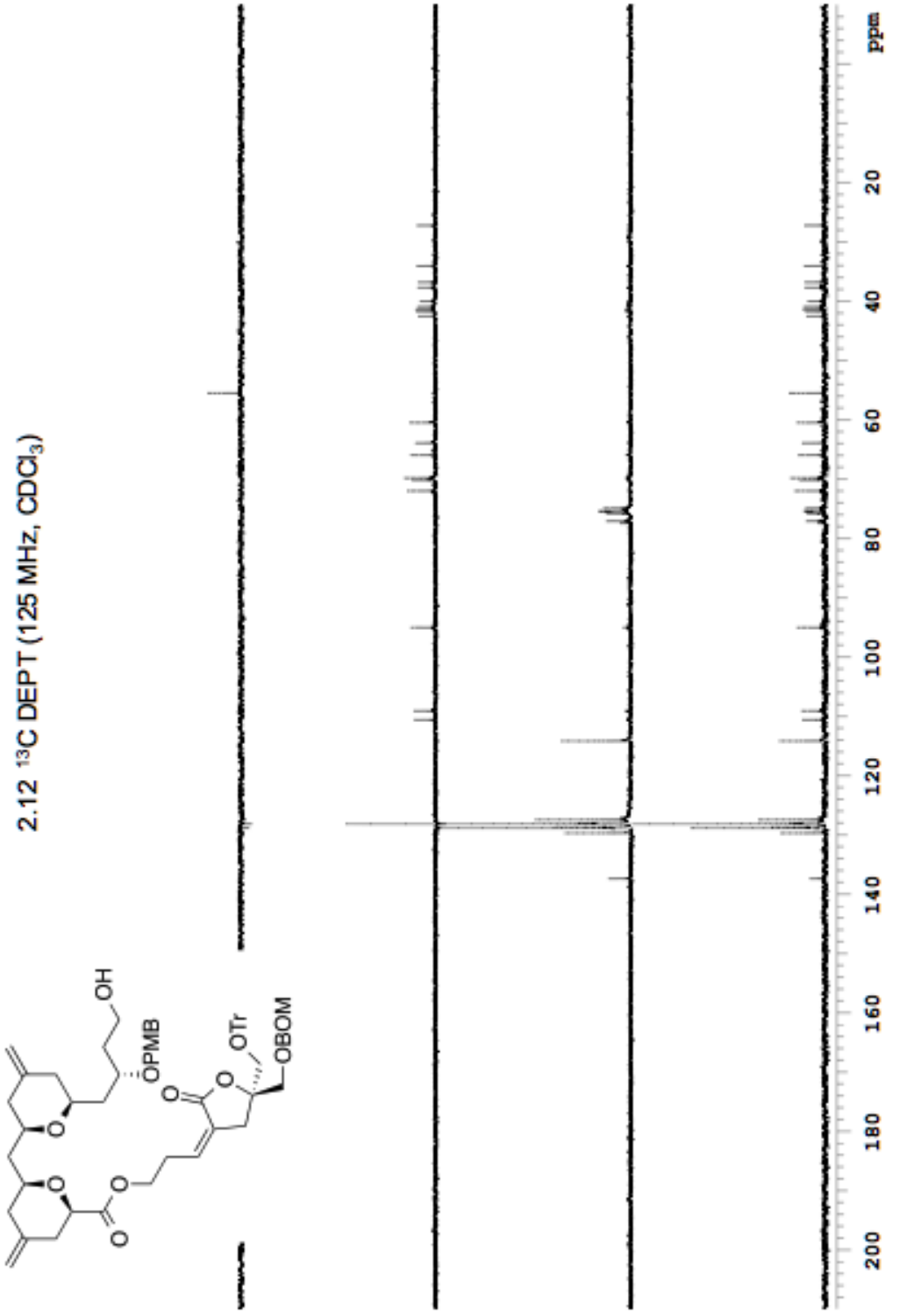

2.11 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTBDPS
OPMB
OTr
OBOM
O
O
O
O
O
O
200
180
160
140
120
100
80
60
40
20
ppm

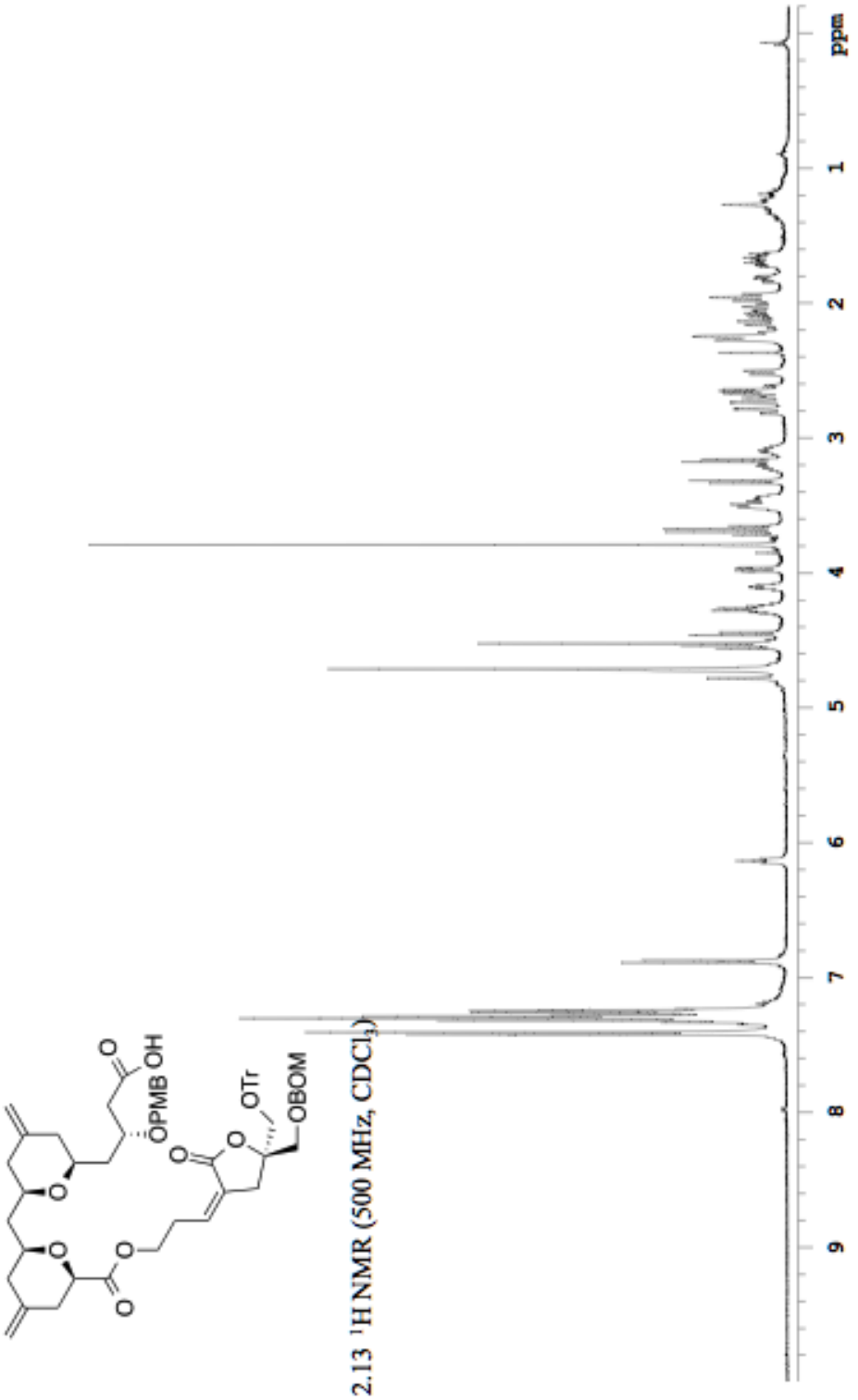


2.12 ^{1}H NMR (500 MHz, $CDCl_3$)

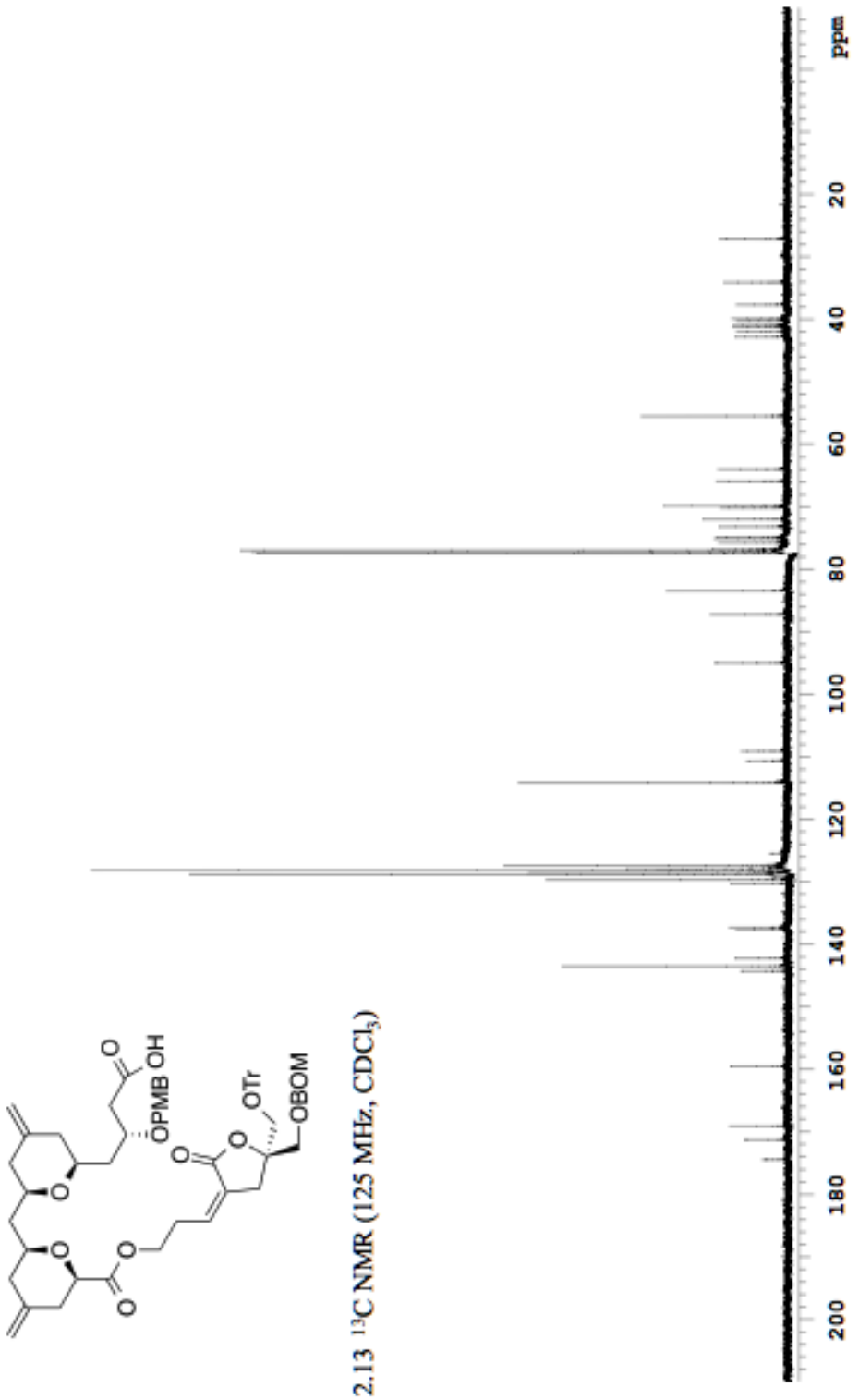


2.12 ^{13}C NMR (125 MHz, $CDCl_3$)

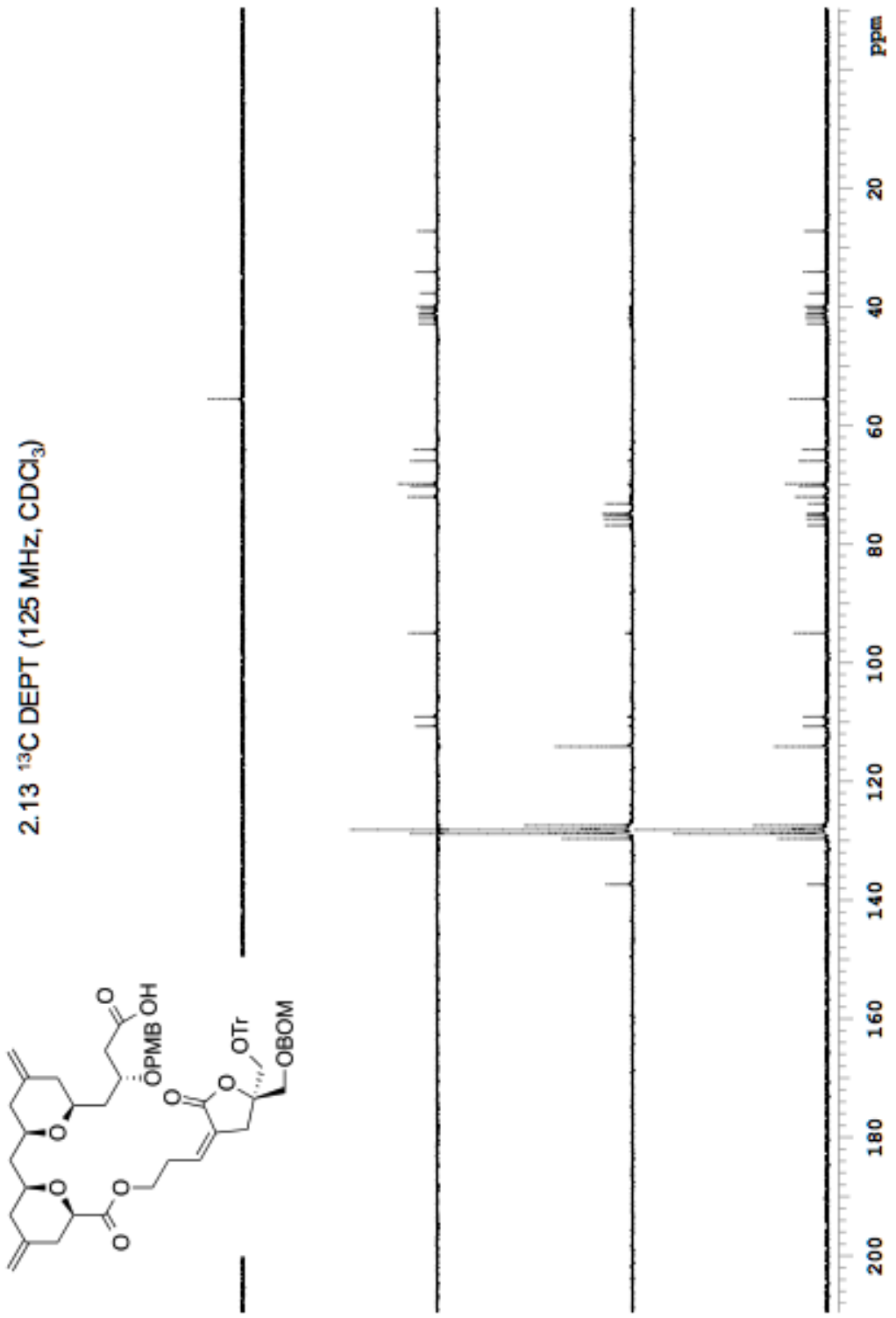

2.12 ^{13}C DEPT (125 MHz, $CDCl_3$)
OH
OPMB
OTr
OBOM
O
O
O
O
O
O
200
180
160
140
120
100
80
60
40
20
ppm

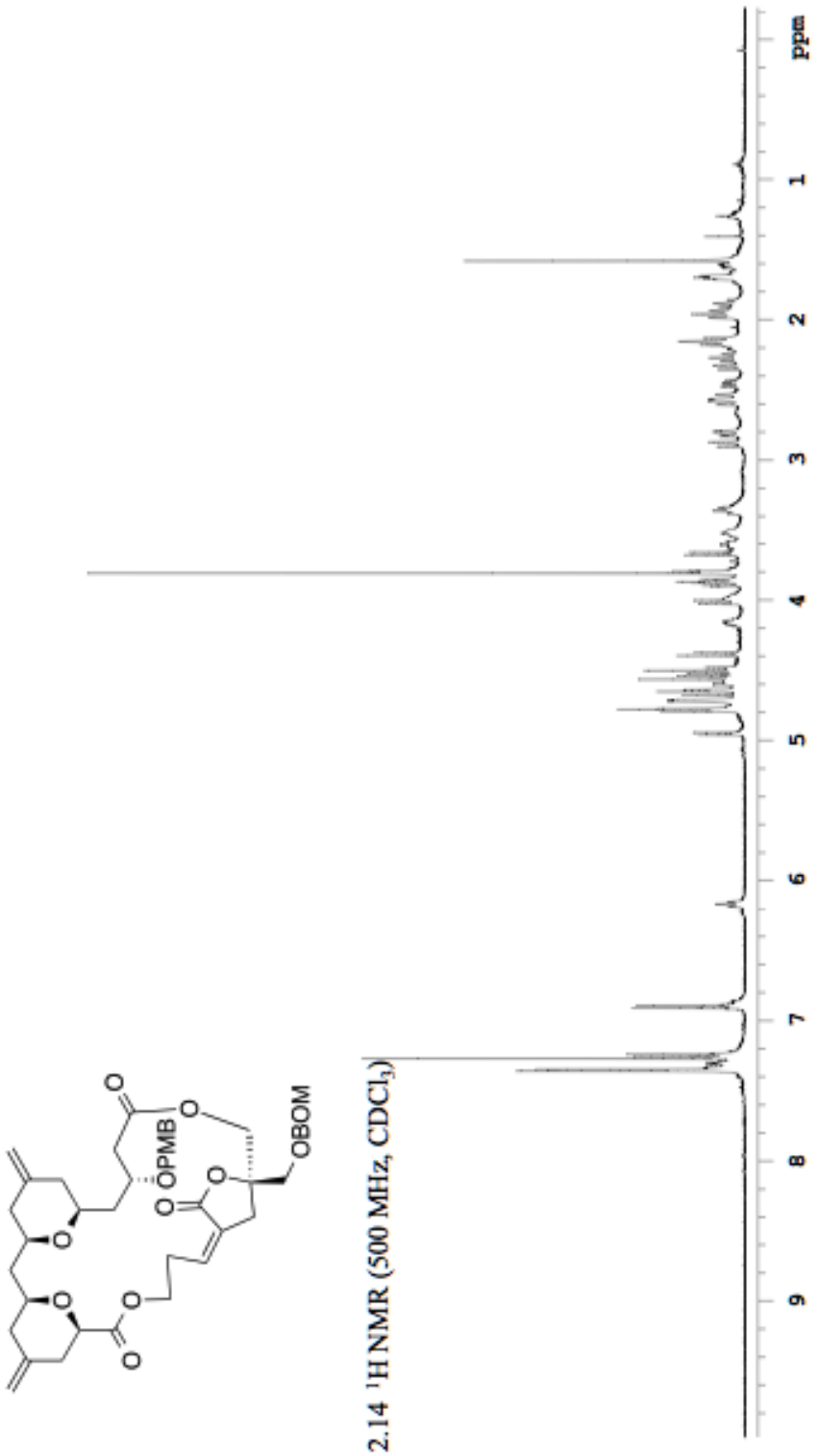


2.13 ^{1}H NMR (500 MHz, $CDCl_3$)

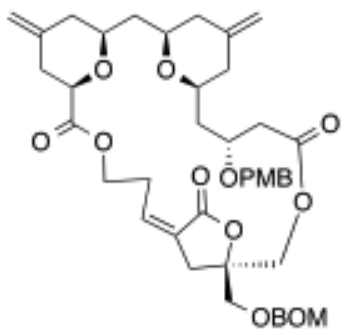


2.13 ^{13}C NMR (125 MHz, $CDCl_3$)

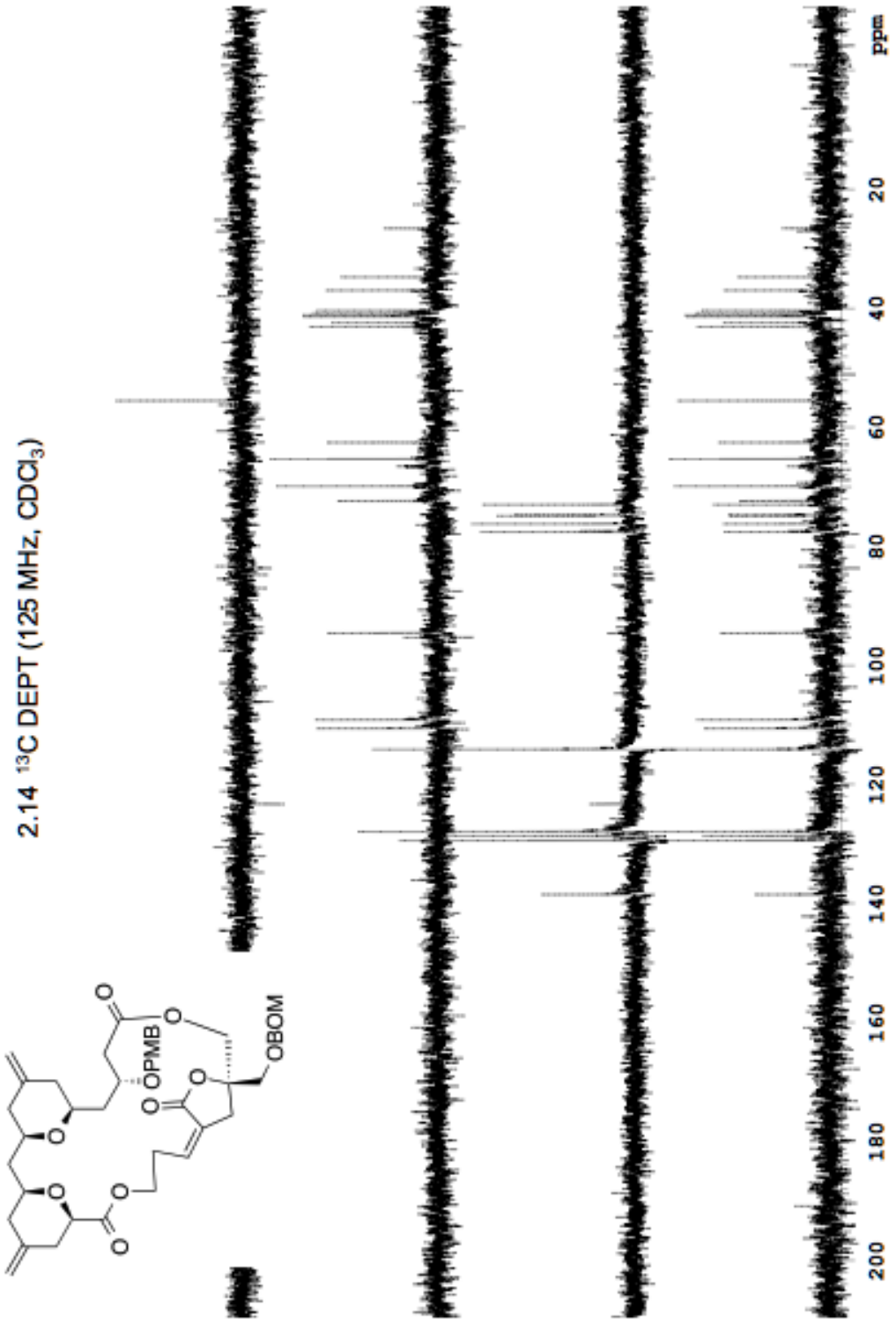

2.13 ^{13}C DEPT (125 MHz, $CDCl_3$)
OPMB OH
OTr
OBOM
O
O
O
O
O
O
O
O
O
ppm
200
180
160
140
120
100
80
60
40
20

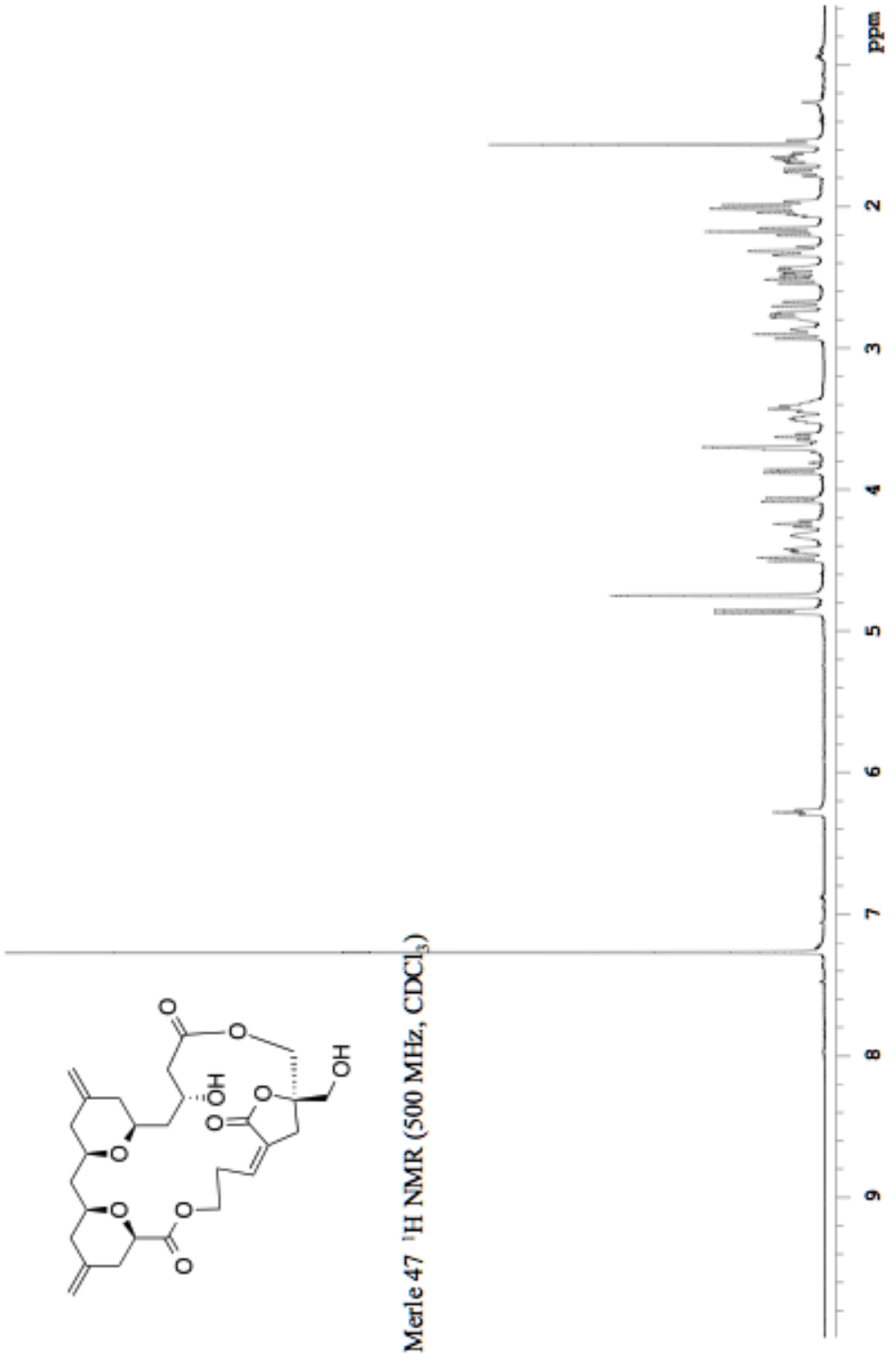


2.14 ^{1}H NMR (500 MHz, $CDCl_3$)

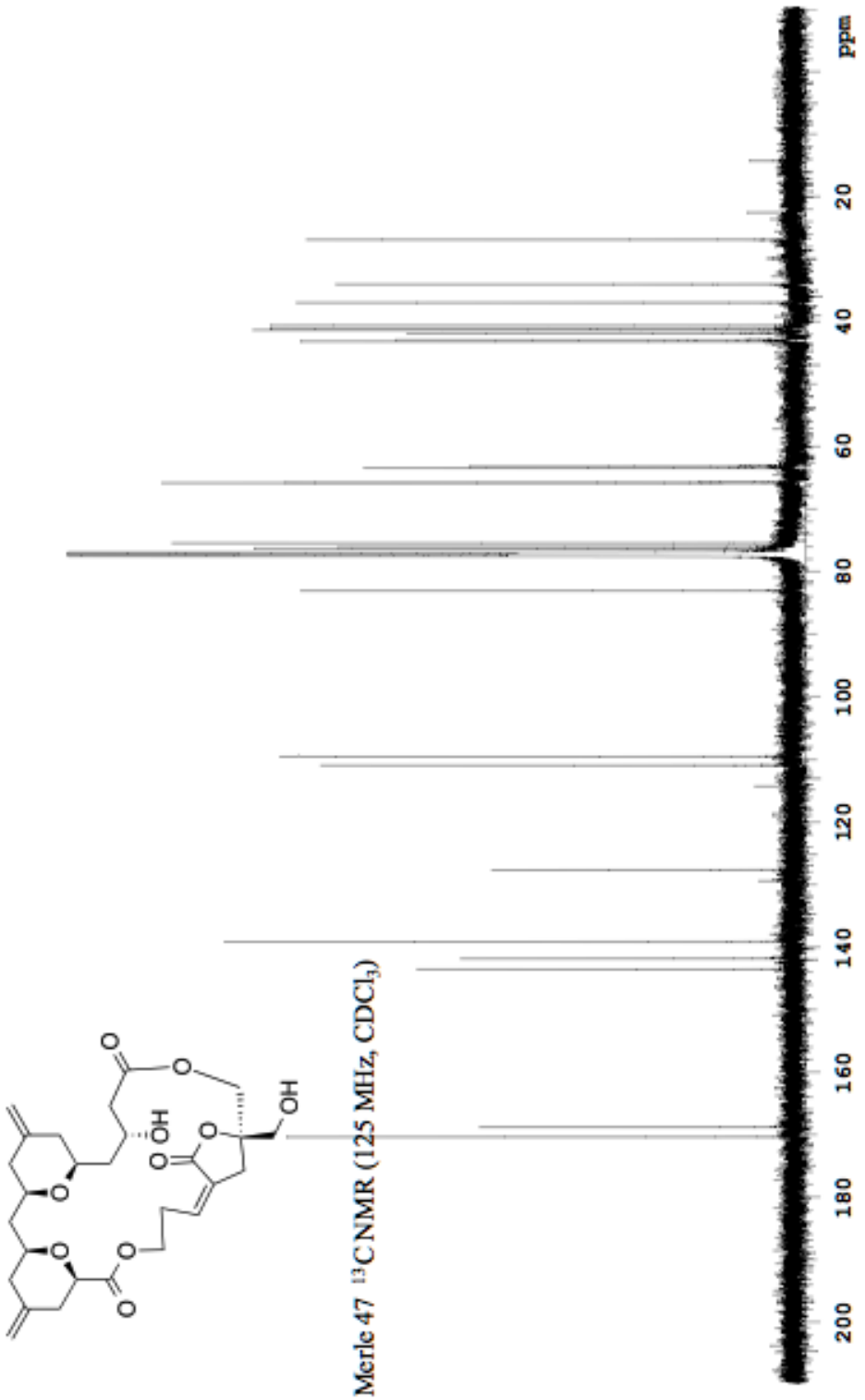


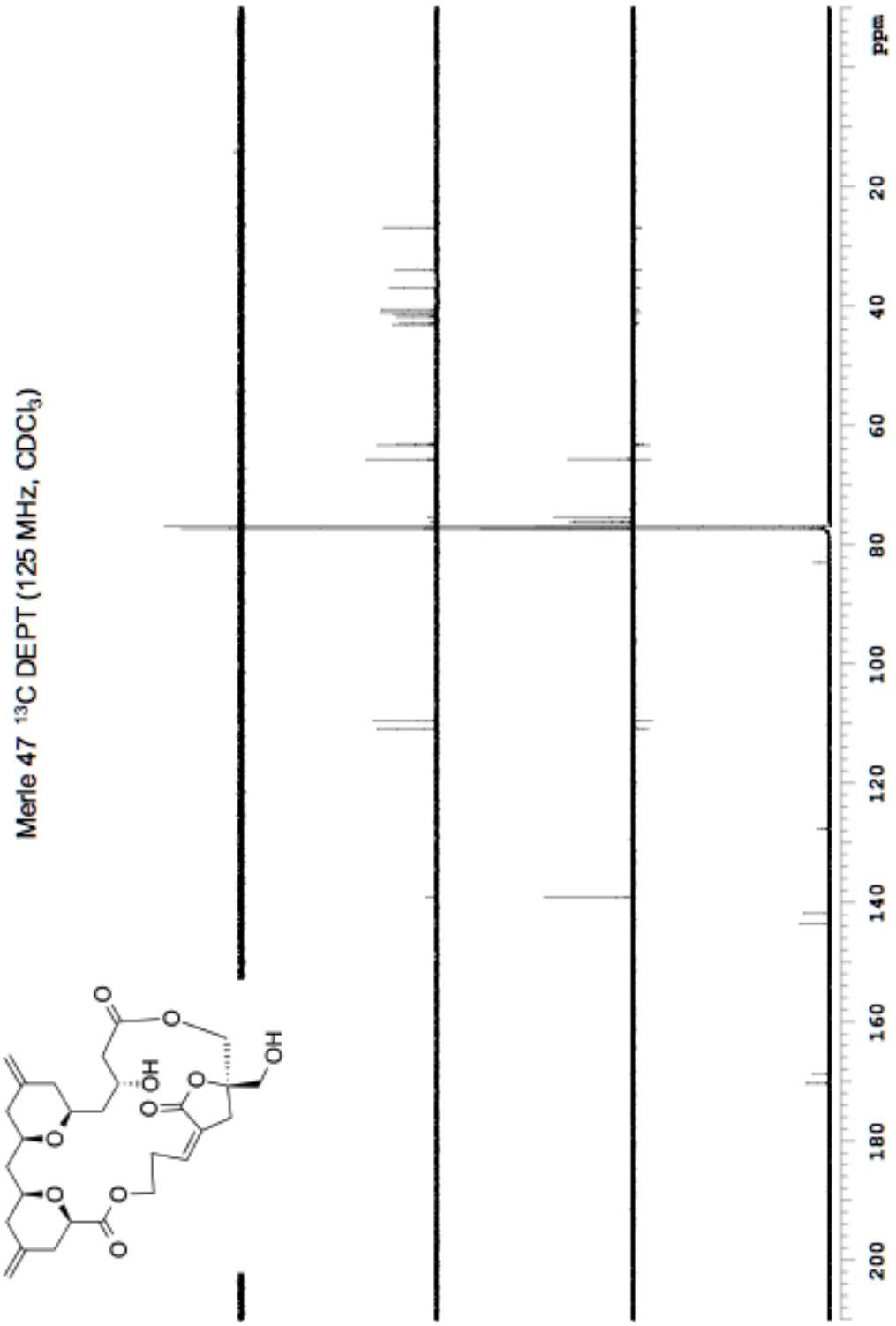
2.14 ^{13}C NMR (125 MHz, $CDCl_3$)

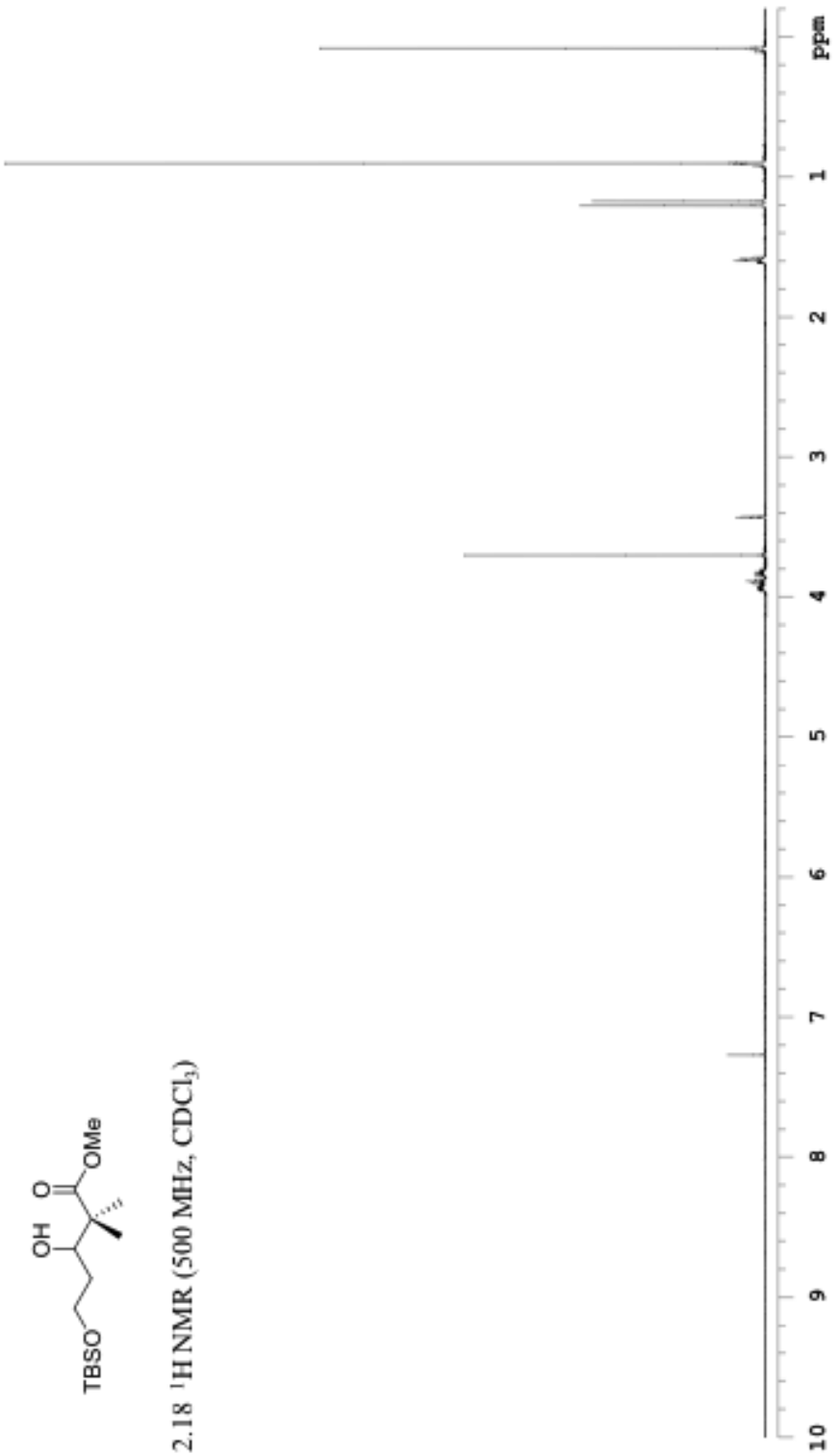

2.14 ^{13}C DEPT (125 MHz, $CDCl_3$)
OPMB
OBOM
ppm
200
180
160
140
120
100
80
60
40
20



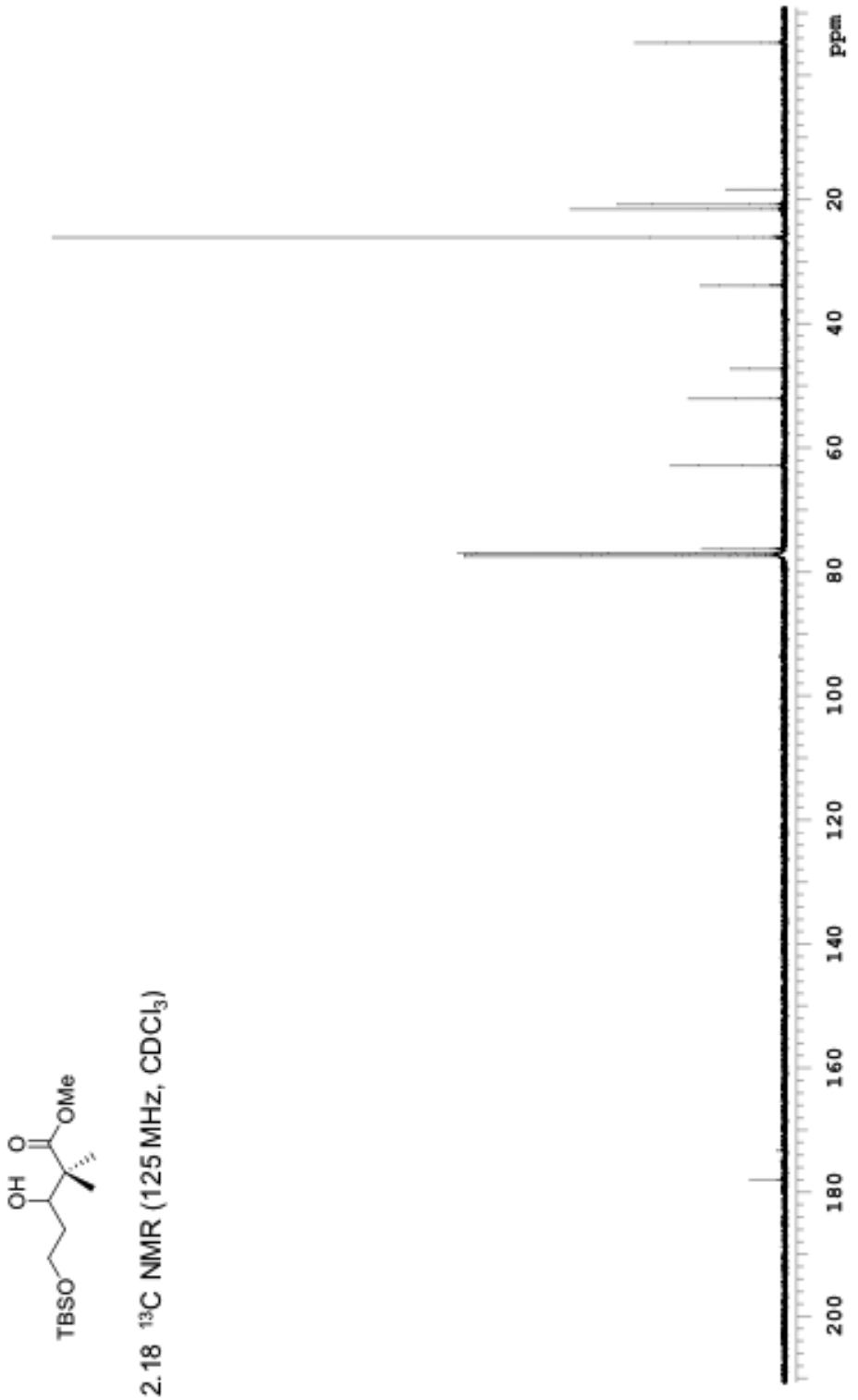
Merle 47 ^{1}H NMR (500 MHz, $CDCl_3$)


Merle 47 ^{13}CNMR (125 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20
OH
OH
O
O
O
O
O
O
O
O

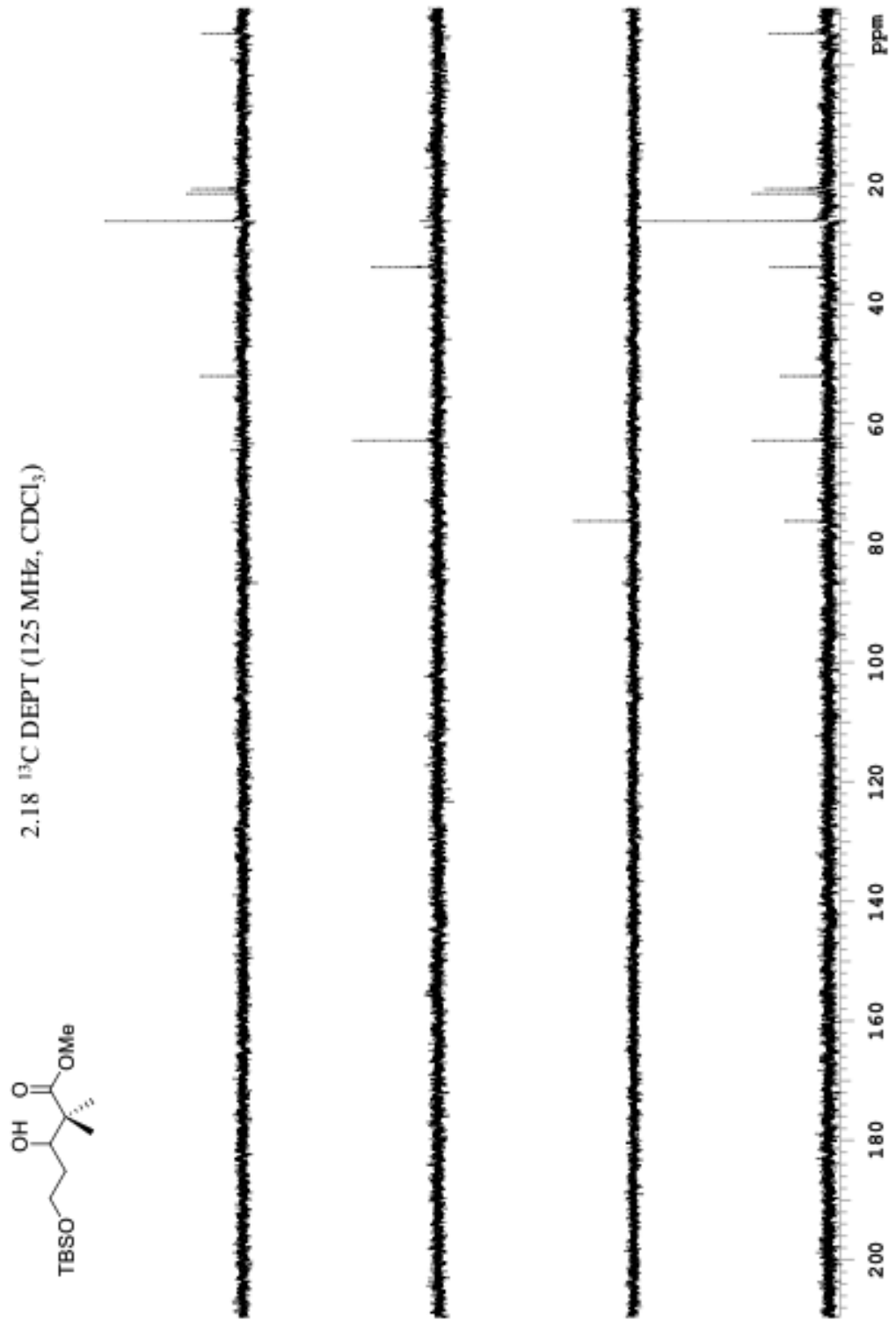

Merle 47 ^{13}C DEPT (125 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20
OH
OH
O
O
O
O
O
O
O
O



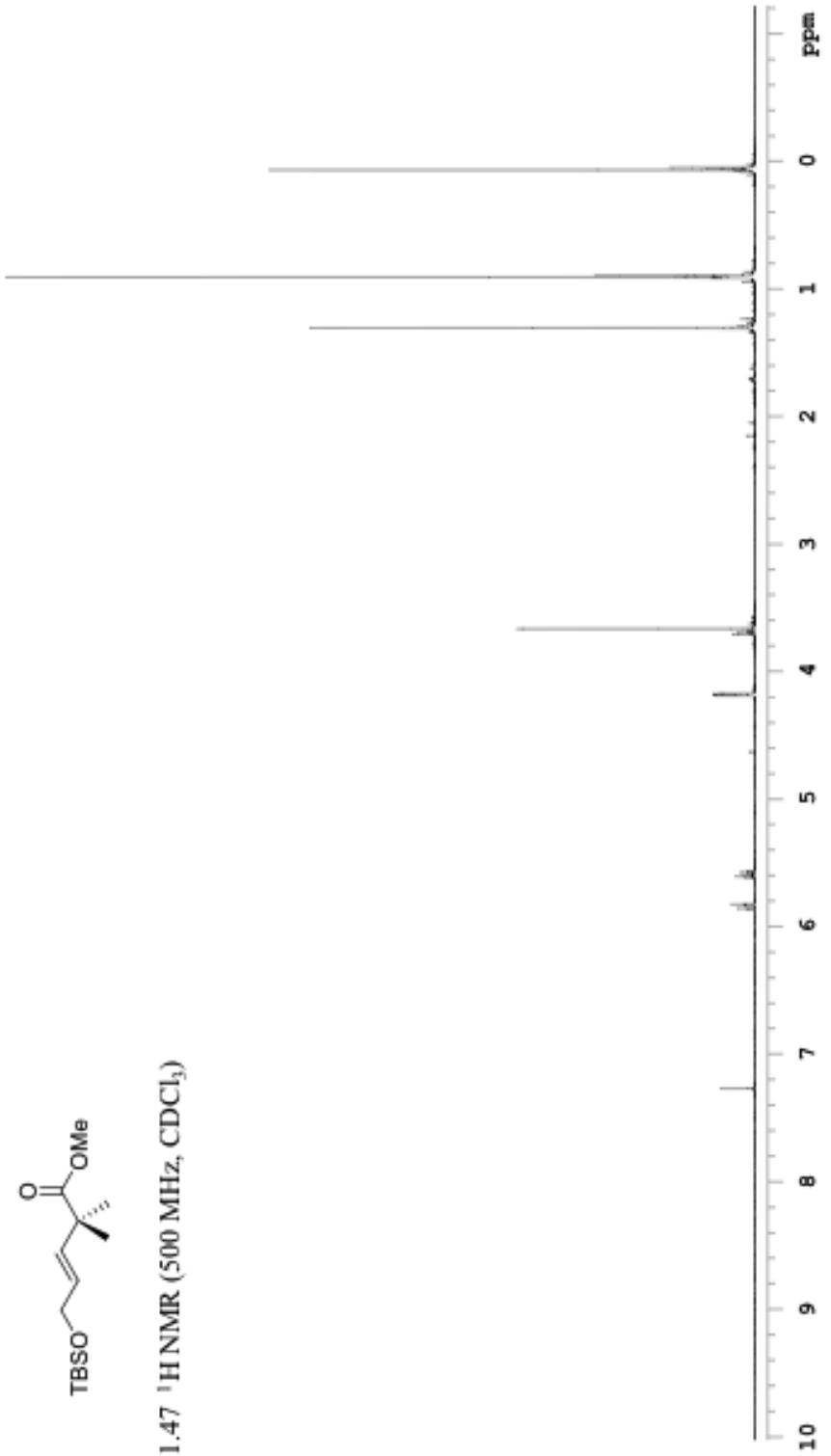
2.18 ^{1}H NMR (500 MHz, $CDCl_3$)



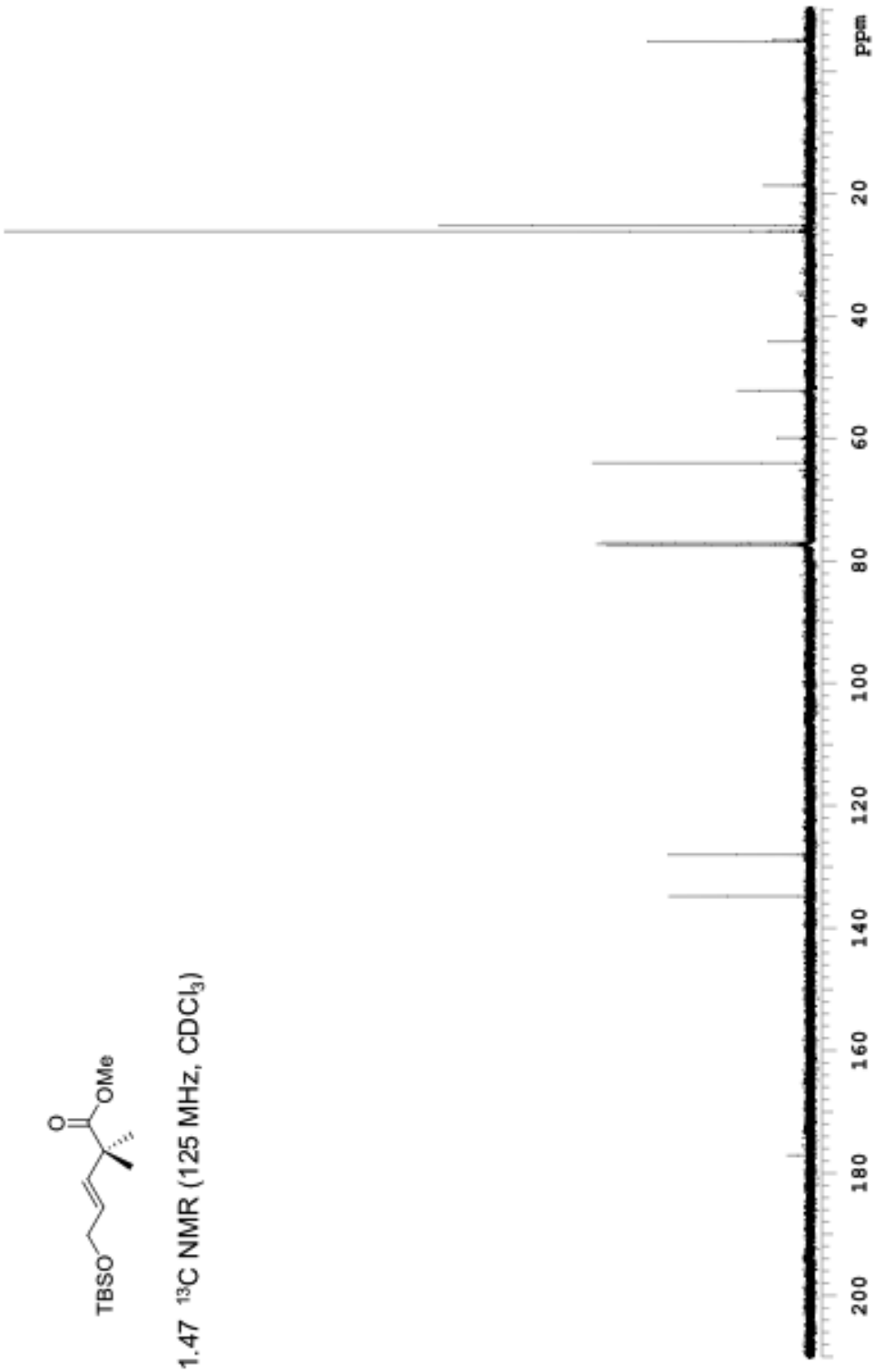
2.18 ^{13}C NMR (125 MHz, $CDCl_3$)



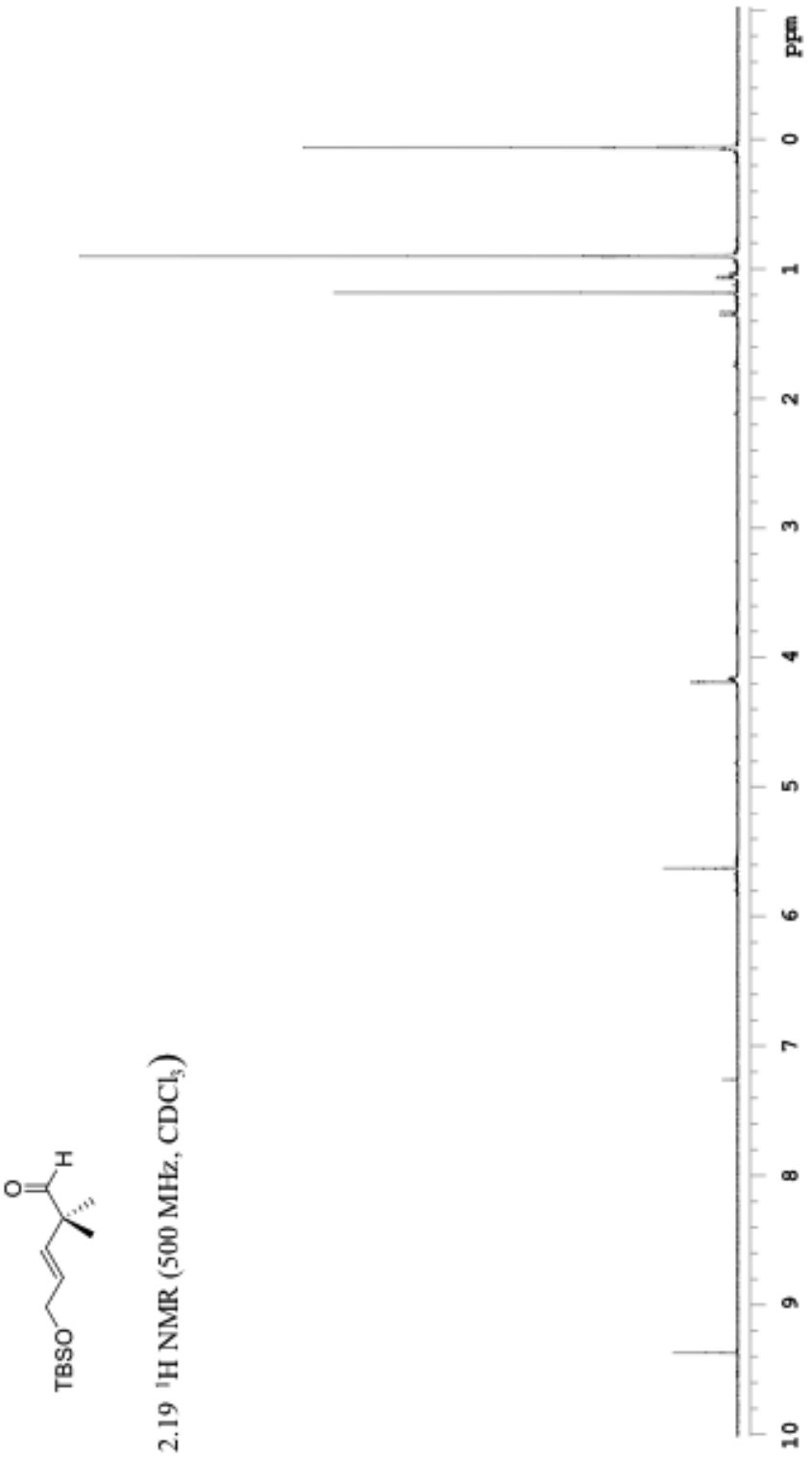
2.18 ^{13}C DEPT (125 MHz, $CDCl_3$)



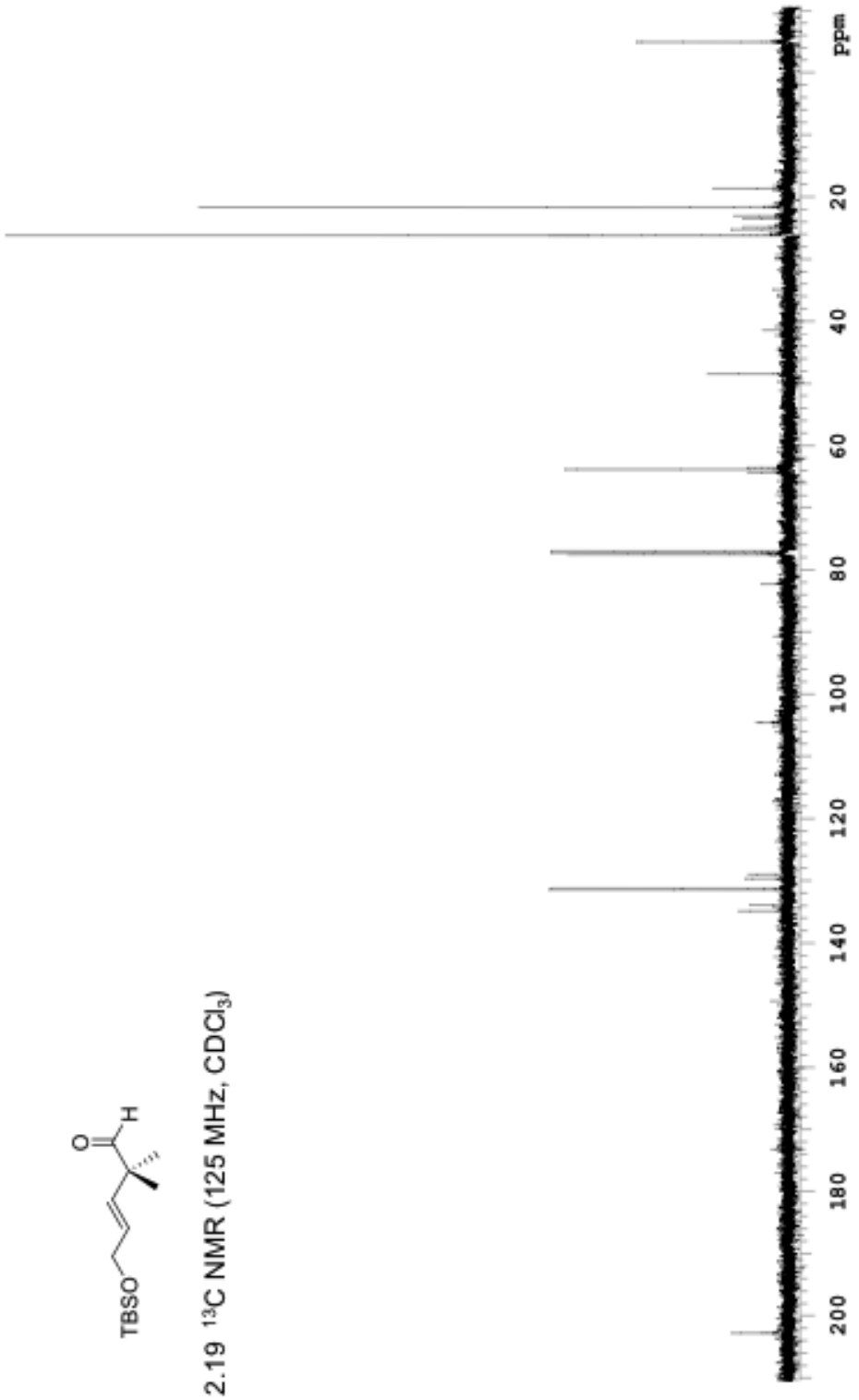
1.47 ^{1}H NMR (500 MHz, $CDCl_3$)



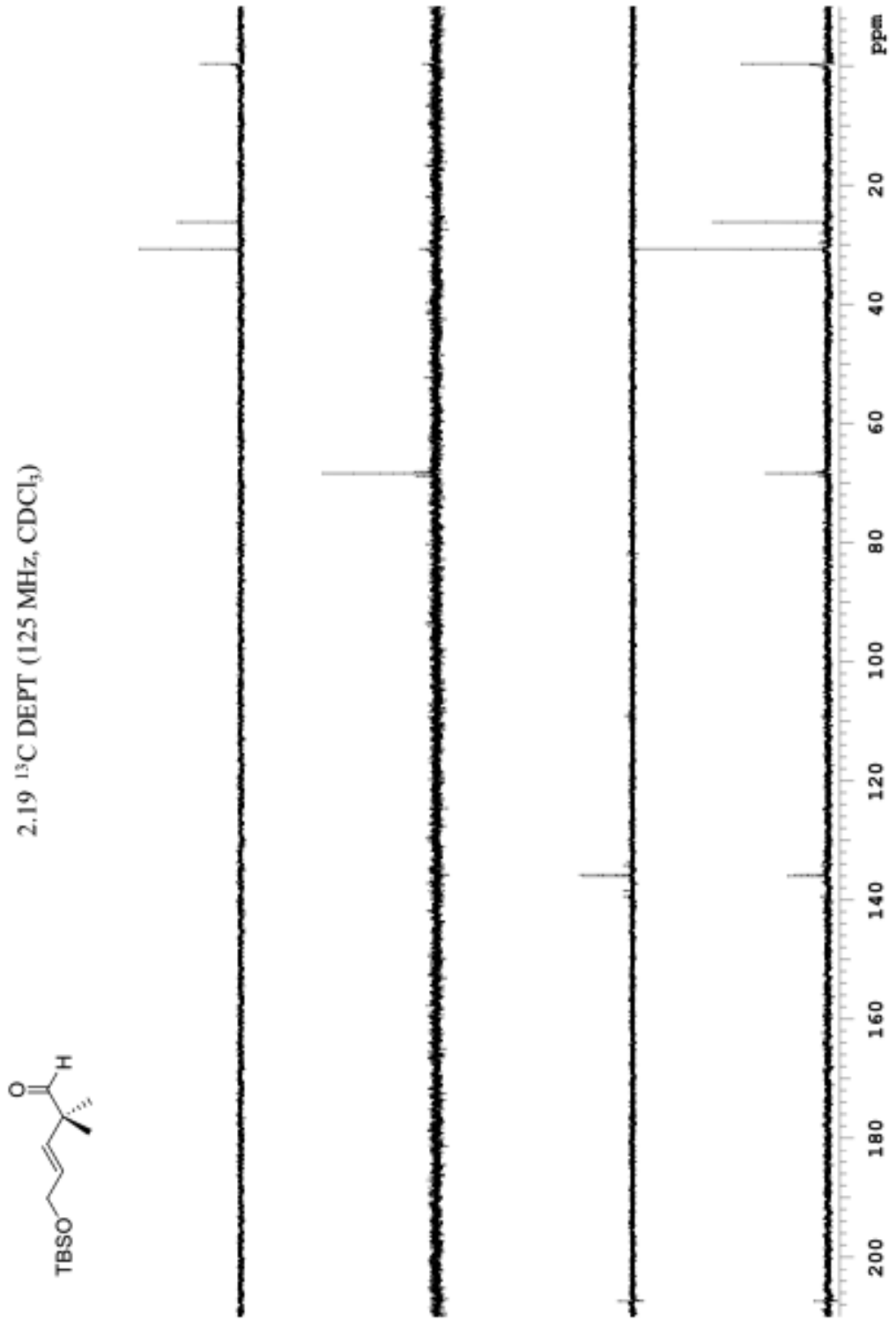
1.47 ^{13}C NMR (125 MHz, $CDCl_3$)

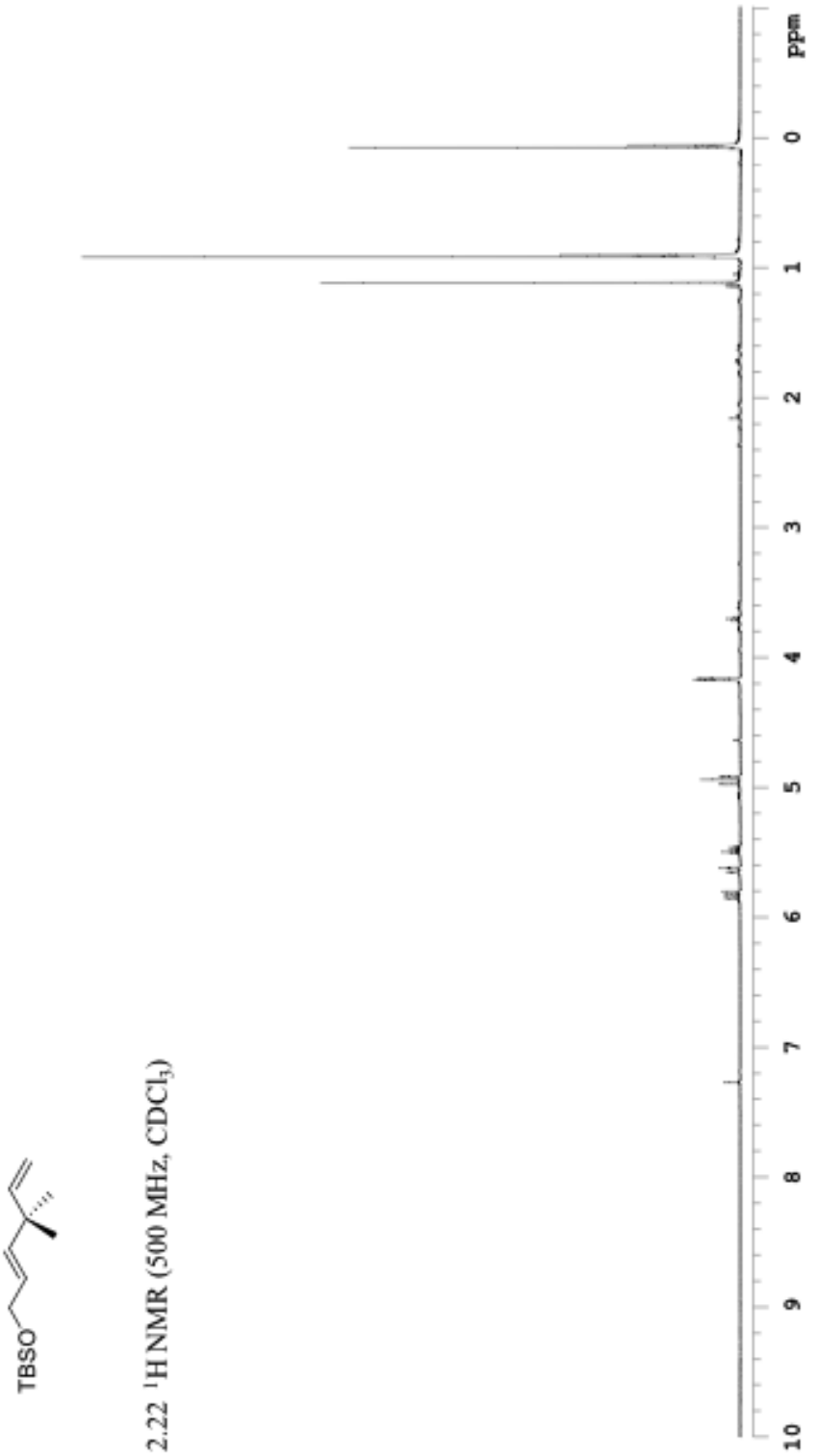


2.19 ^{1}H NMR (500 MHz, $CDCl_3$)

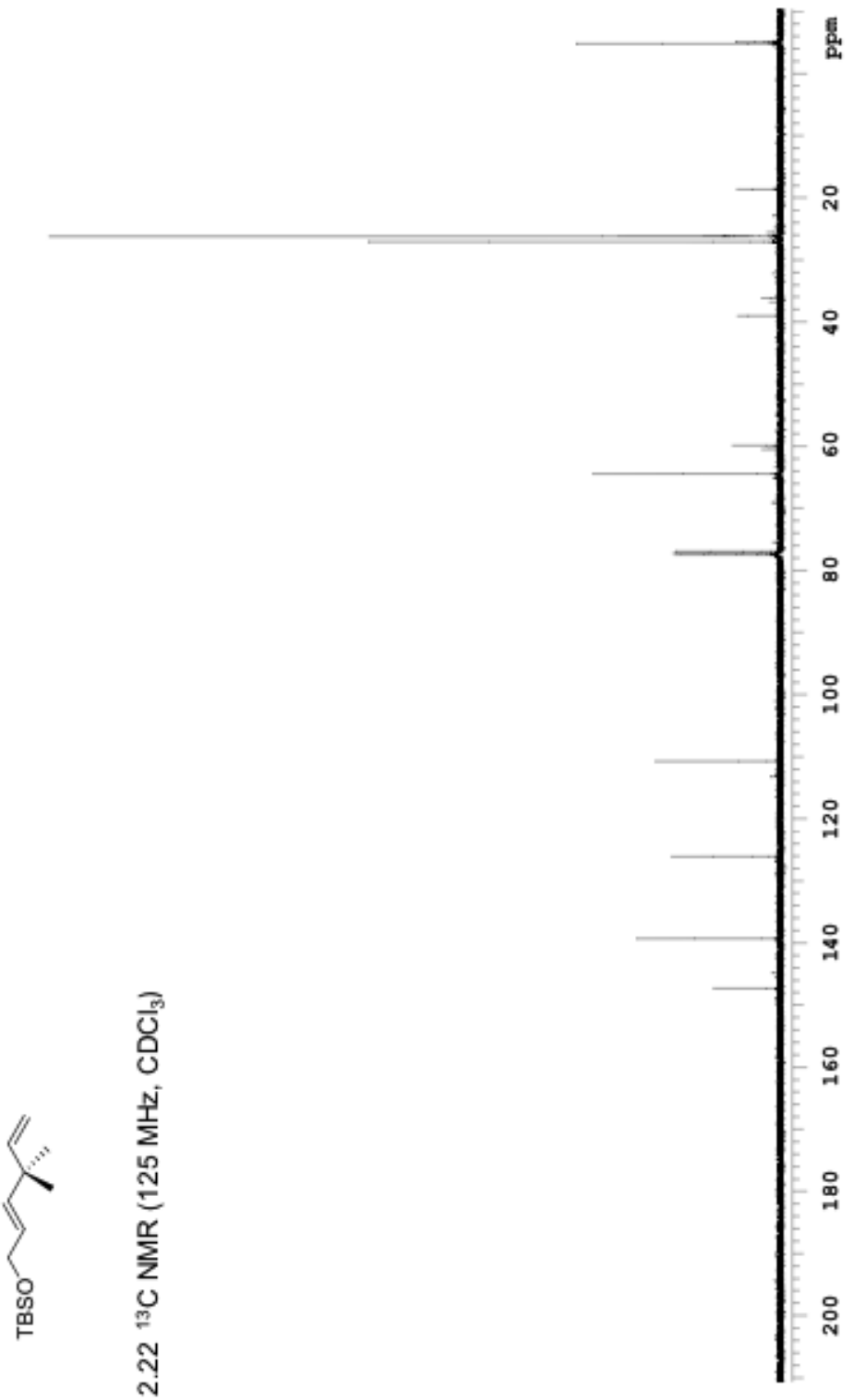


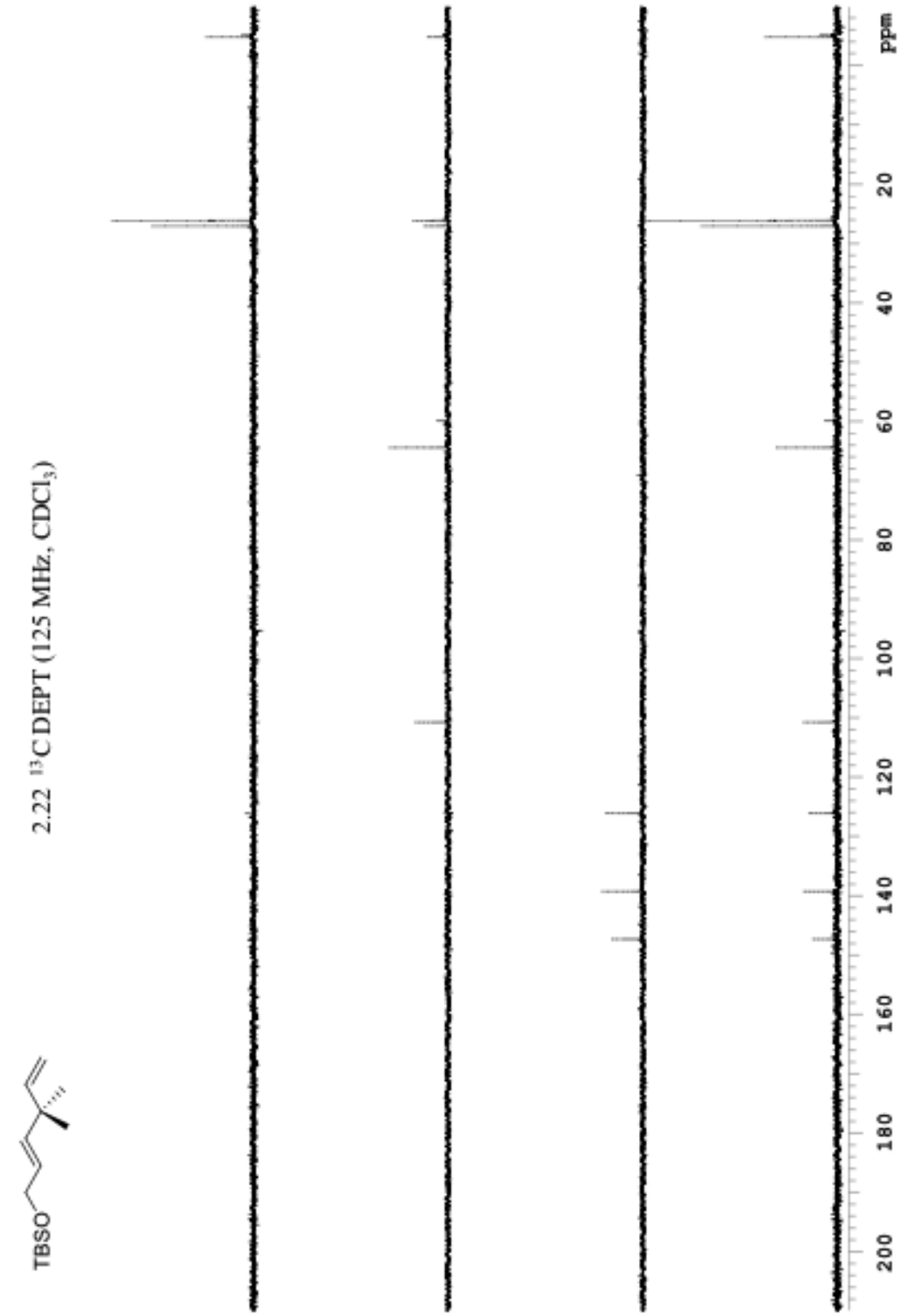
2.19 ^{13}C NMR (125 MHz, $CDCl_3$)

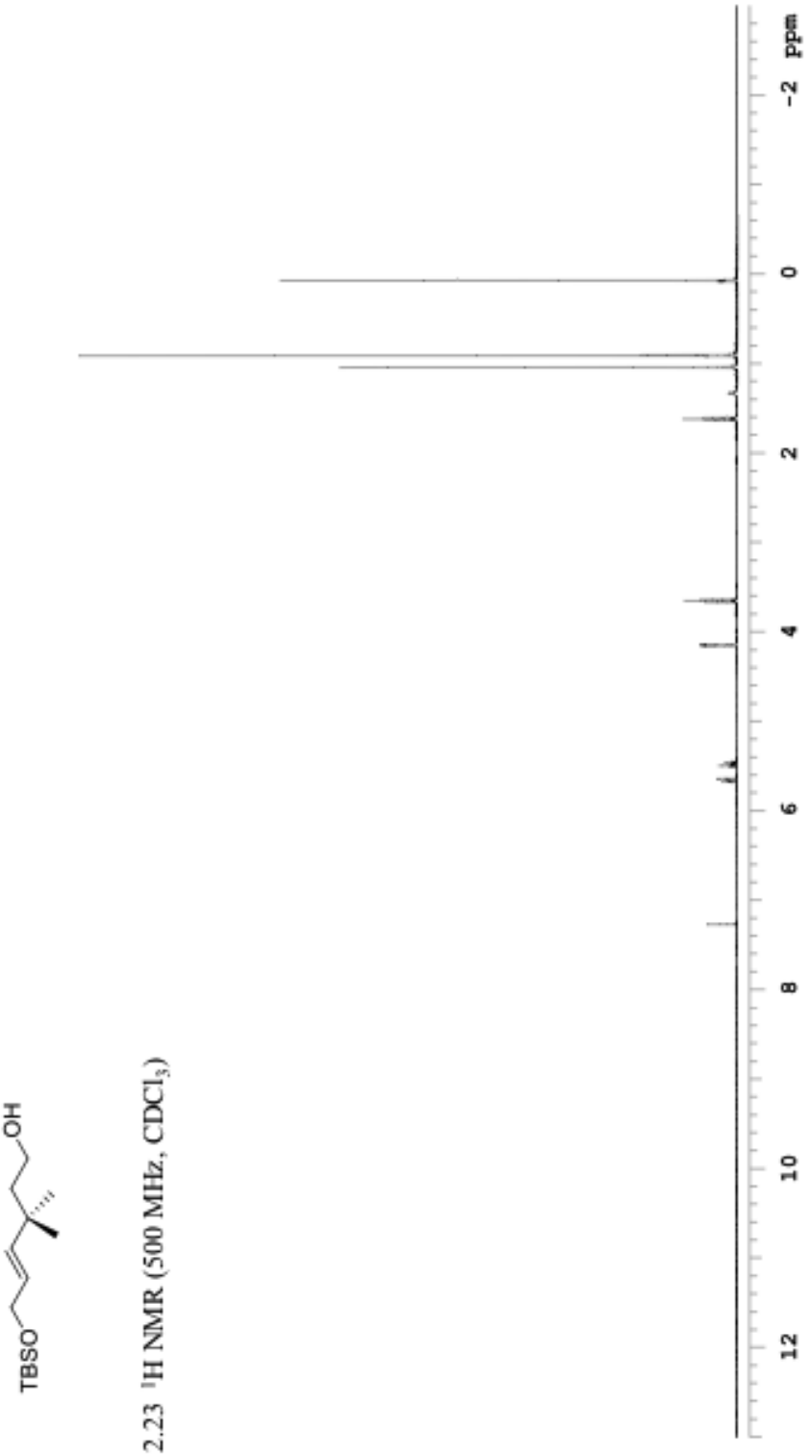

2.19 ^{13}C DEPT (125 MHz, $CDCl_3$)
TBSO
O
H
200
180
160
140
120
100
80
60
40
20
ppm



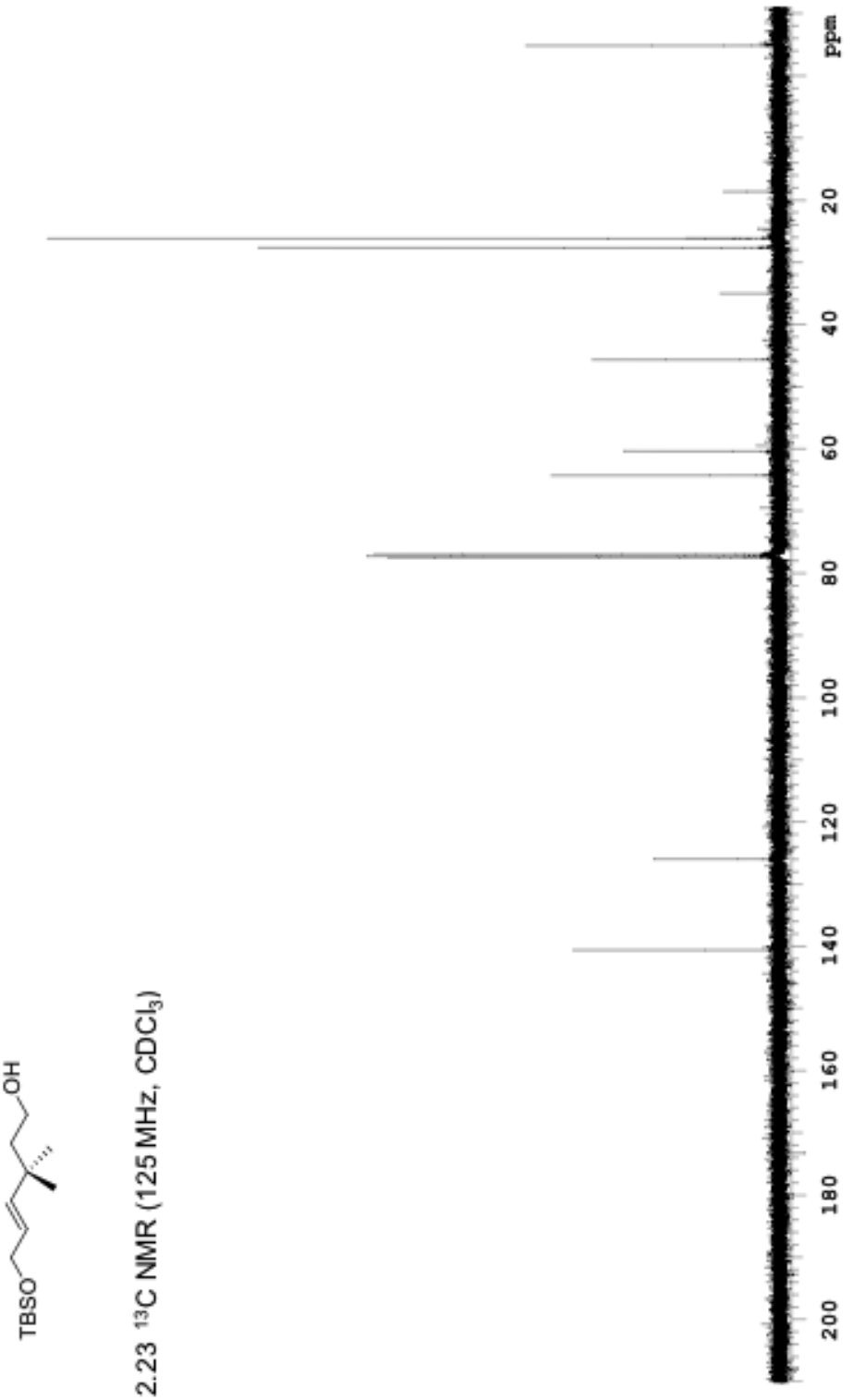
2.22 ^{1}H NMR (500 MHz, $CDCl_3$)


TBSO
2.22 ^{13}C NMR (125 MHz, $CDCl_3$)
200
180
160
140
120
100
80
60
40
20
ppm

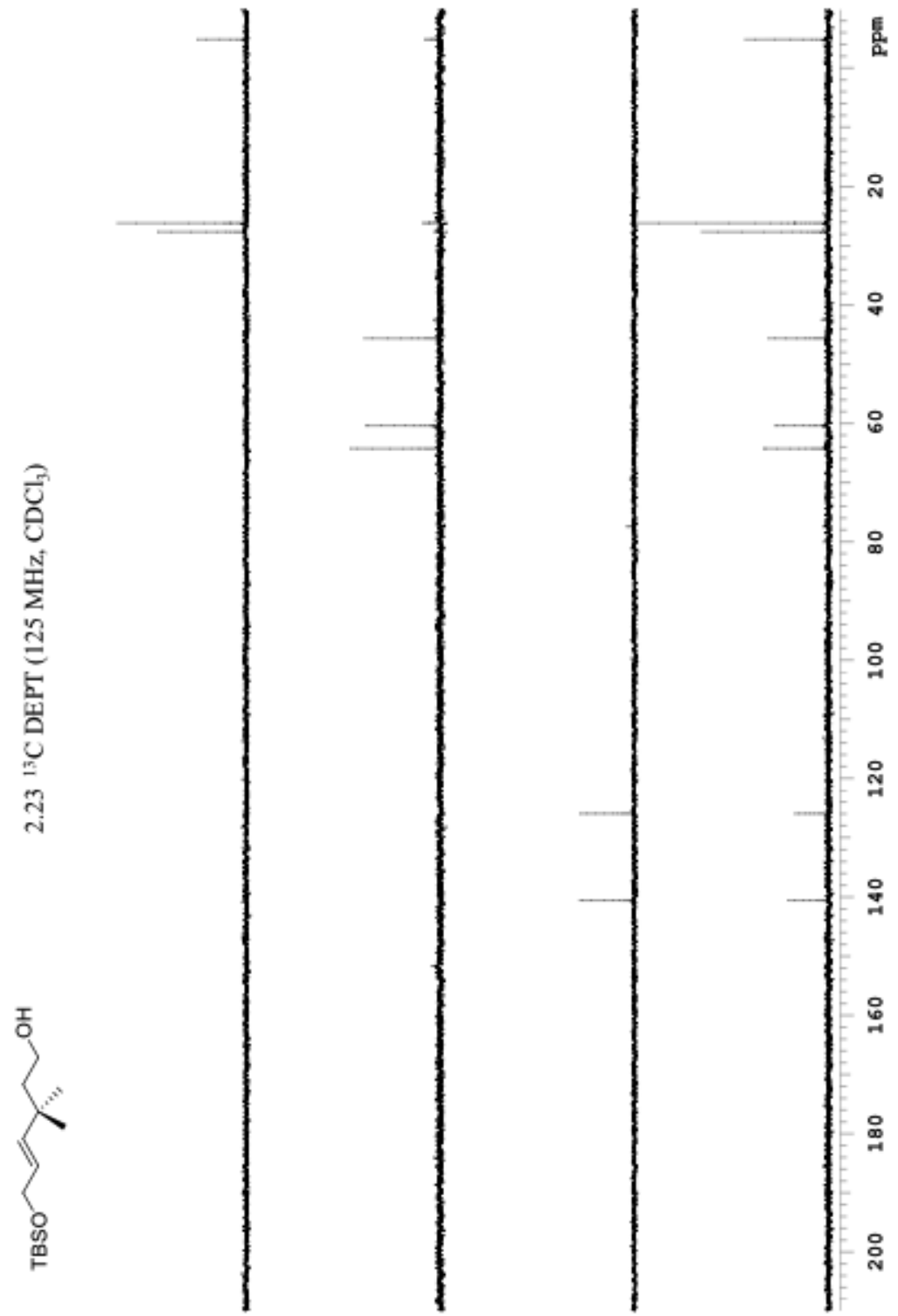

TBSO
2.22 ^{13}C DEPT (125 MHz, $CDCl_3$)
200
180
160
140
120
100
80
60
40
20
ppm

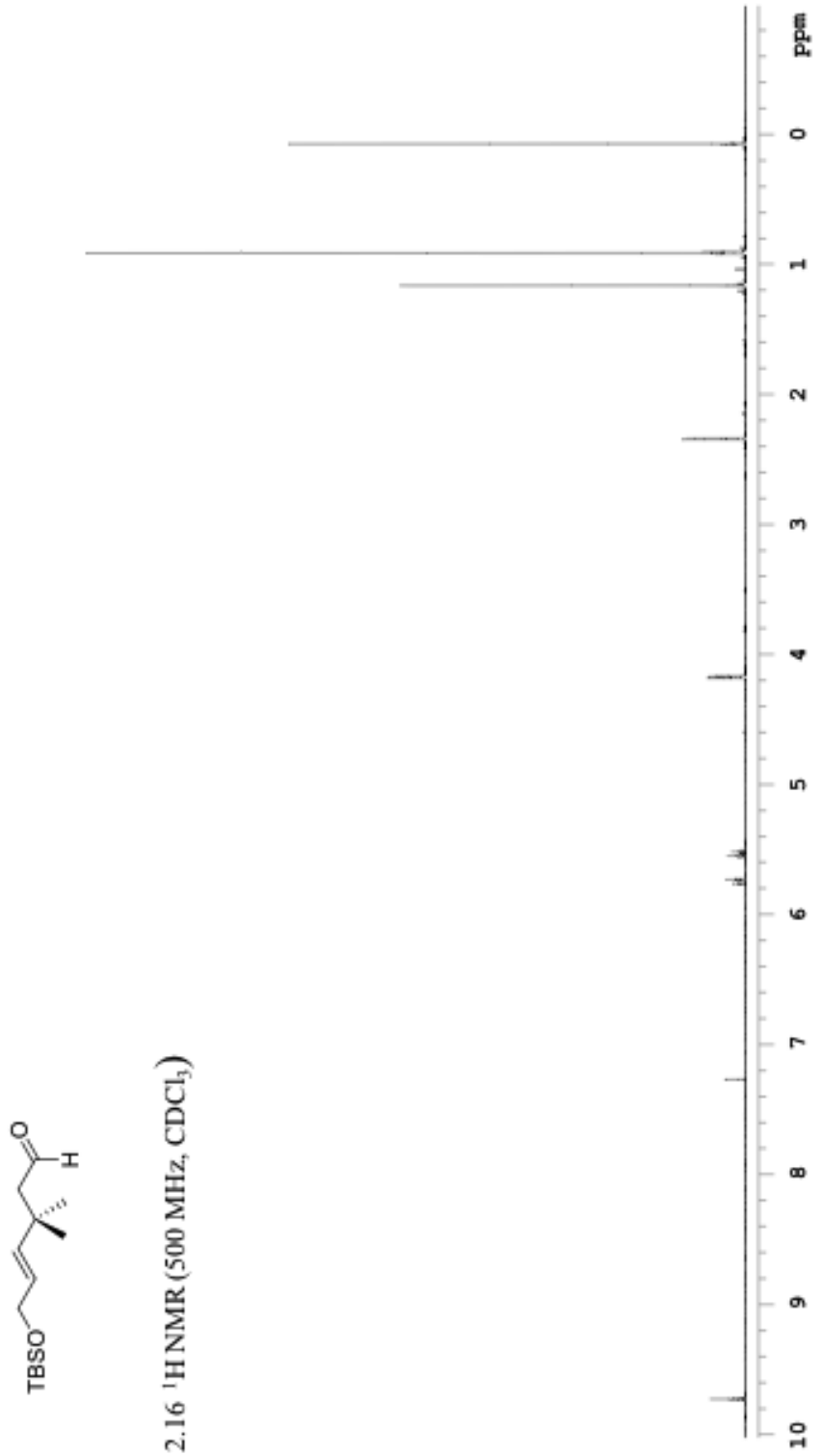


2.23 ^{1}H NMR (500 MHz, $CDCl_3$)

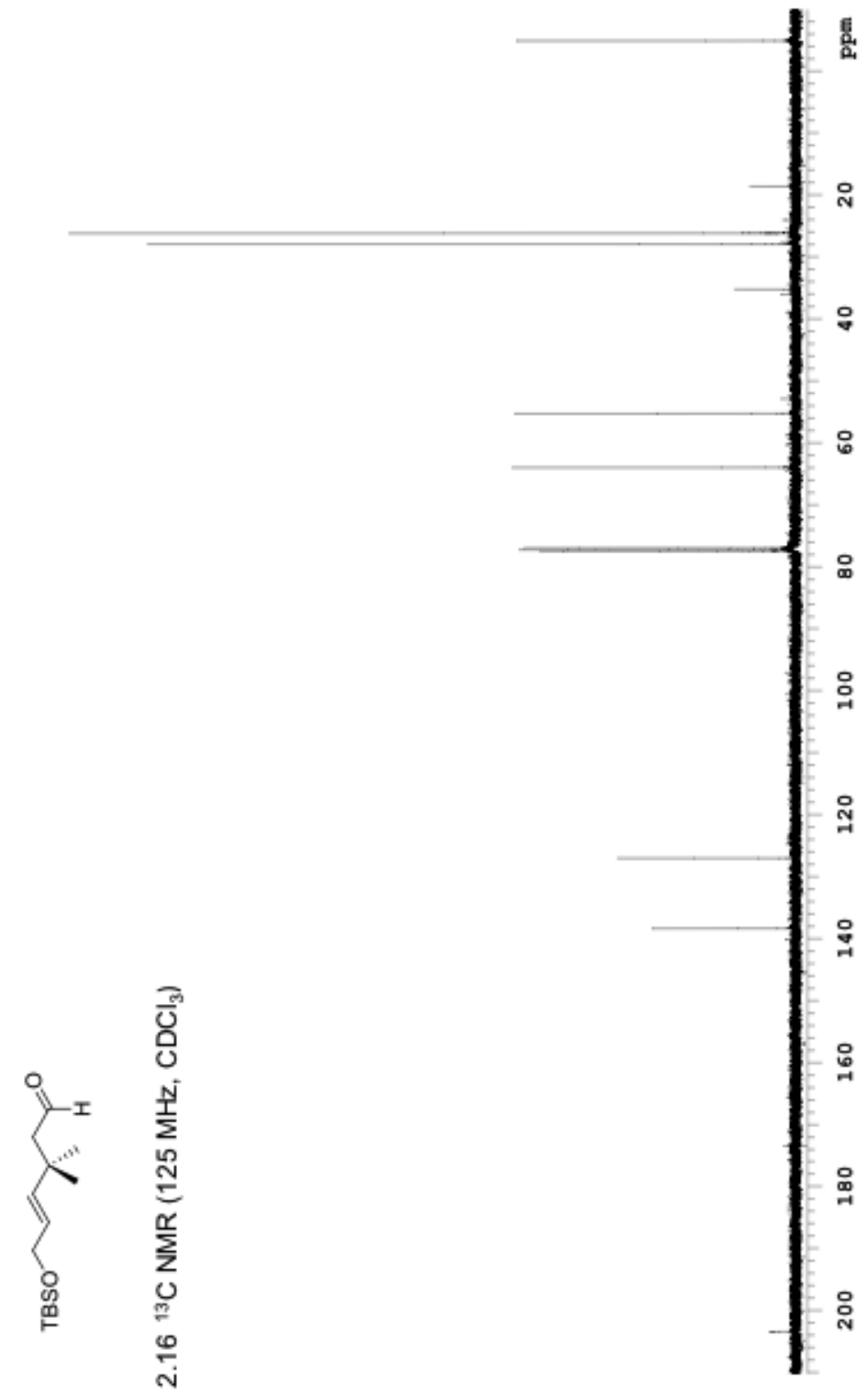


2.23 ^{13}C NMR (125 MHz, $CDCl_3$)

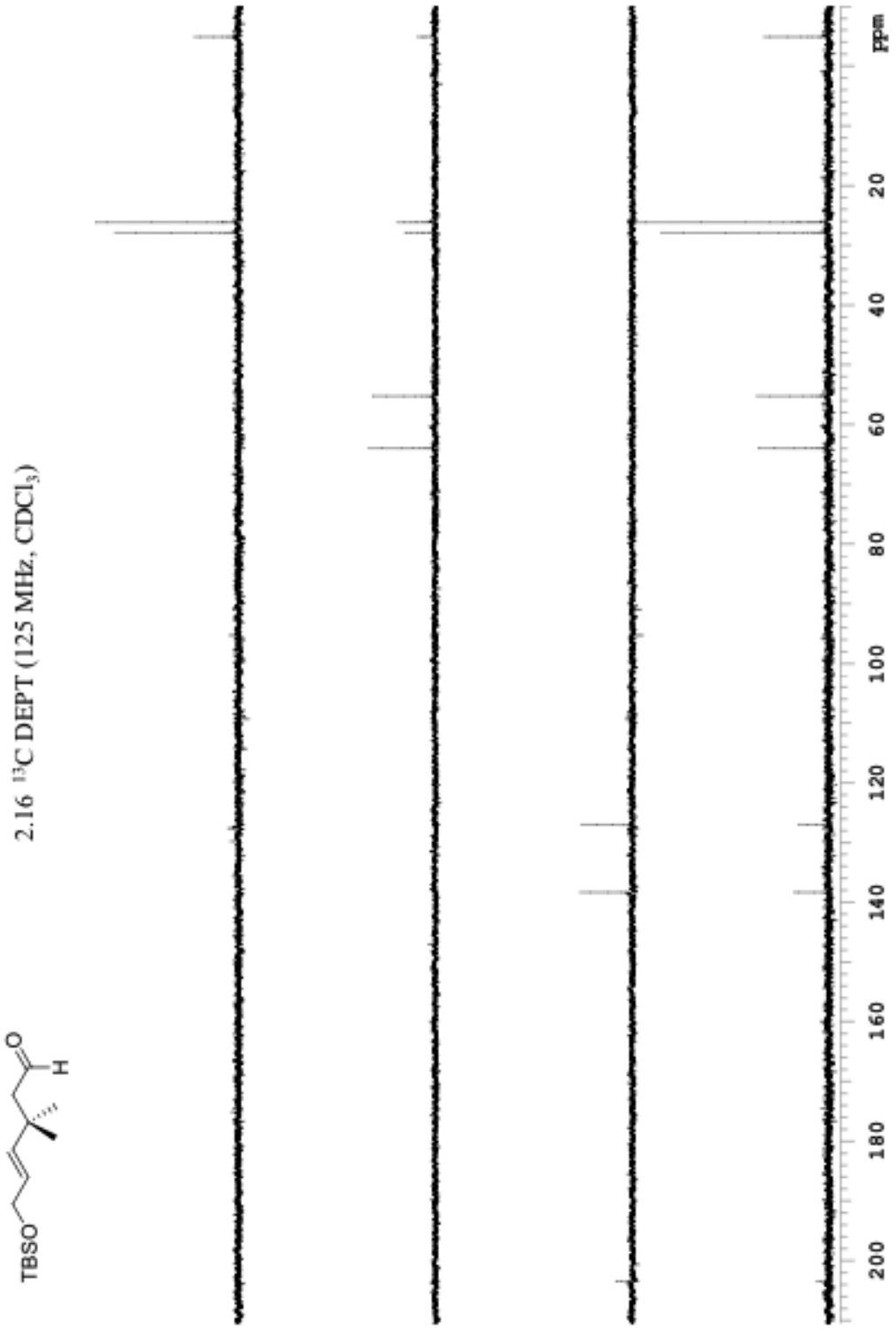

TBSO
OH
2.23 ^{13}C DEPT (125 MHz, $CDCl_3$)
200
180
160
140
120
100
80
60
40
20
ppm



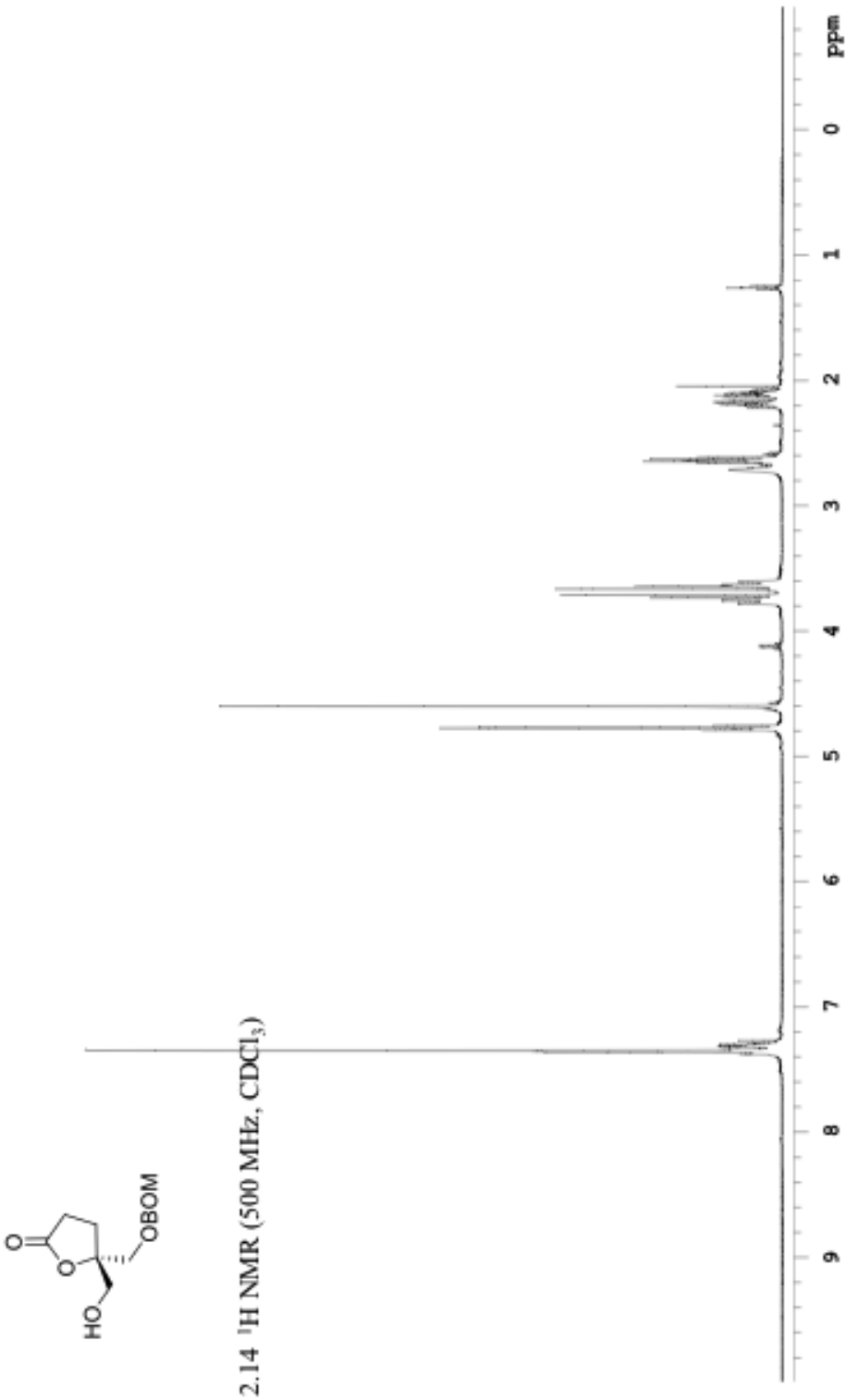
2.16 ^{1}H NMR (500 MHz, $CDCl_3$)



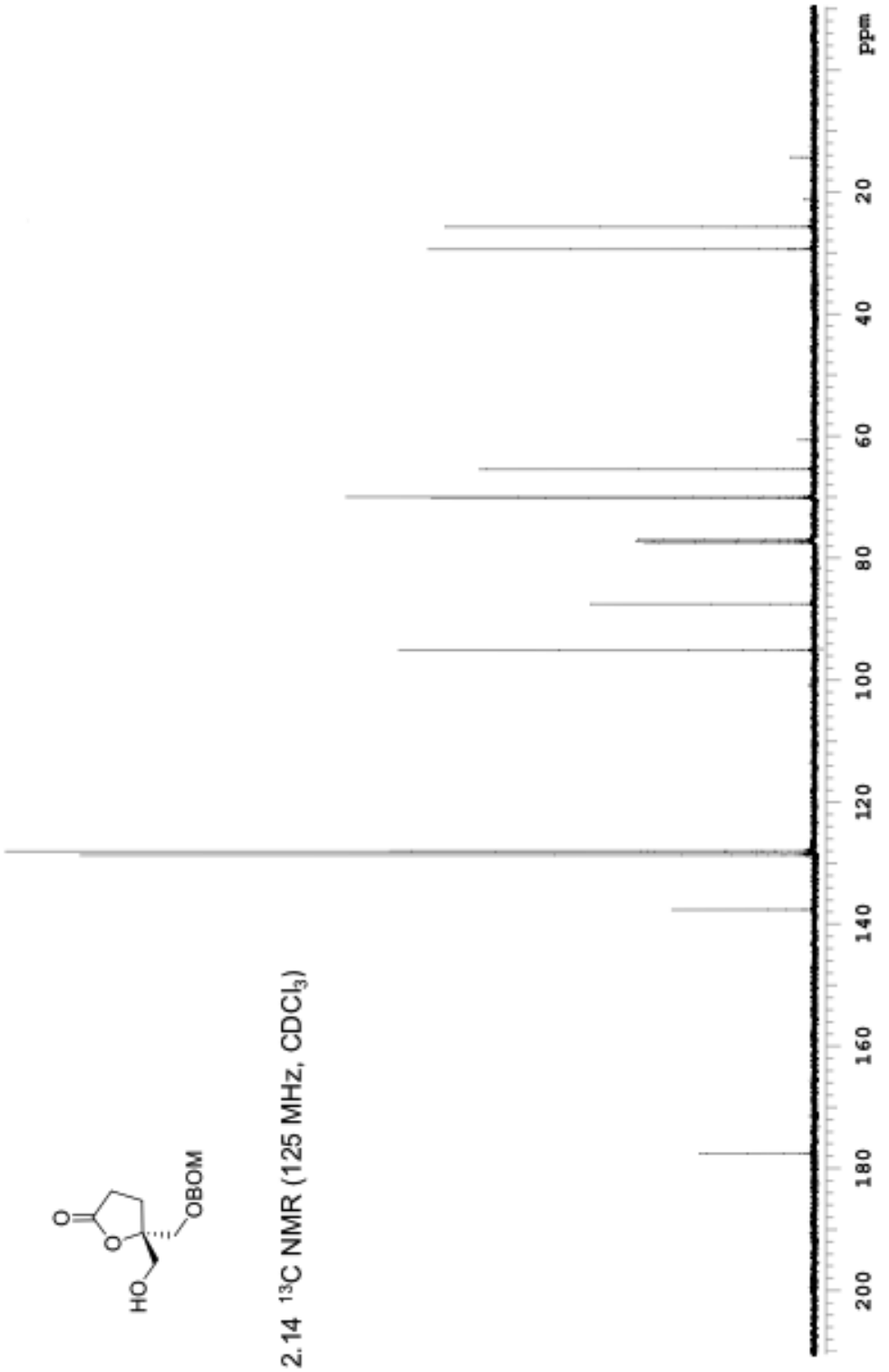
2.16 ^{13}C NMR (125 MHz, $CDCl_3$)



2.16 ^{13}C DEPT (125 MHz, $CDCl_3$)

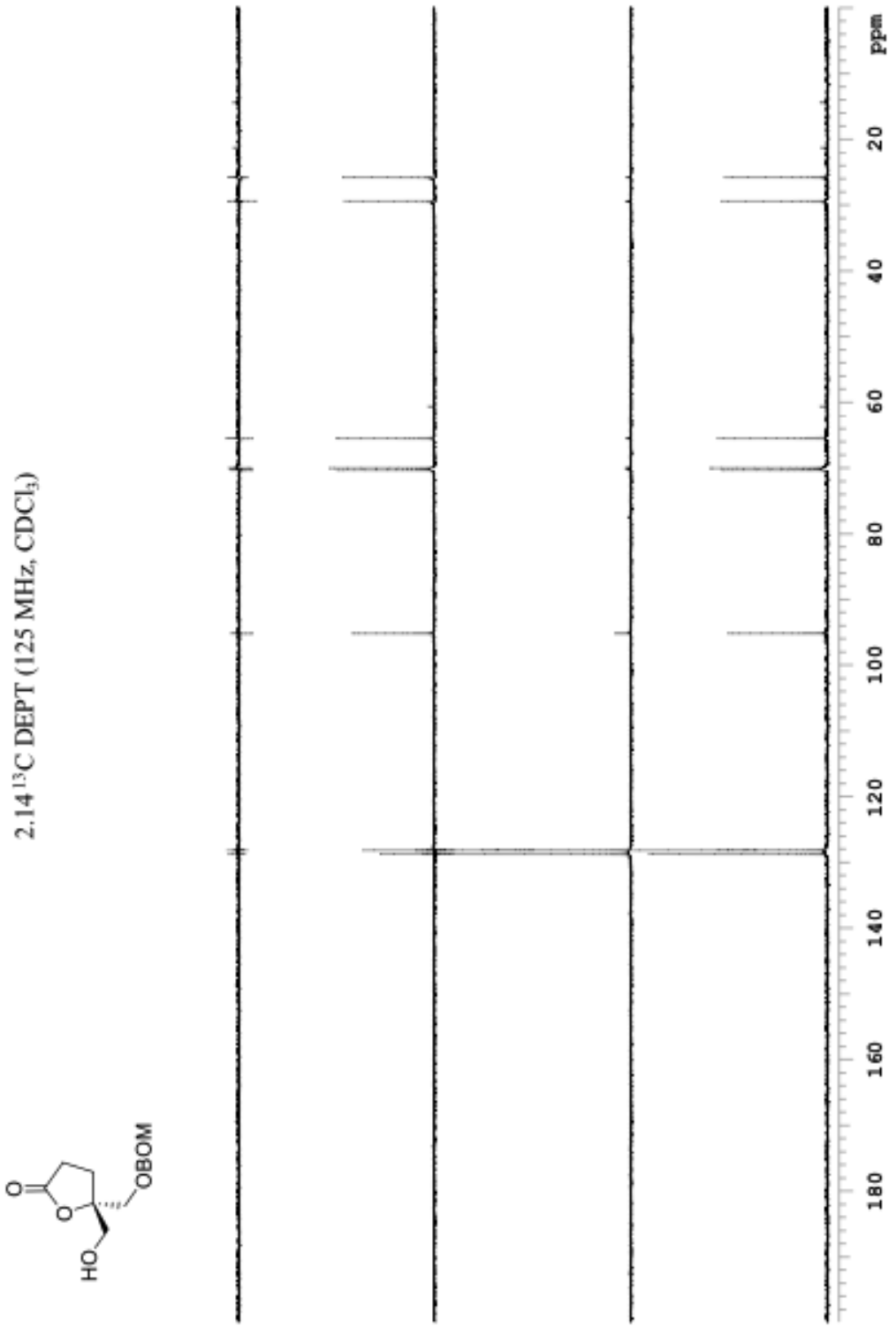


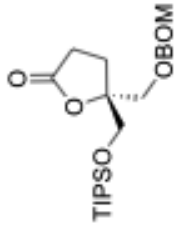
2.14 ^{1}H NMR (500 MHz, $CDCl_3$)



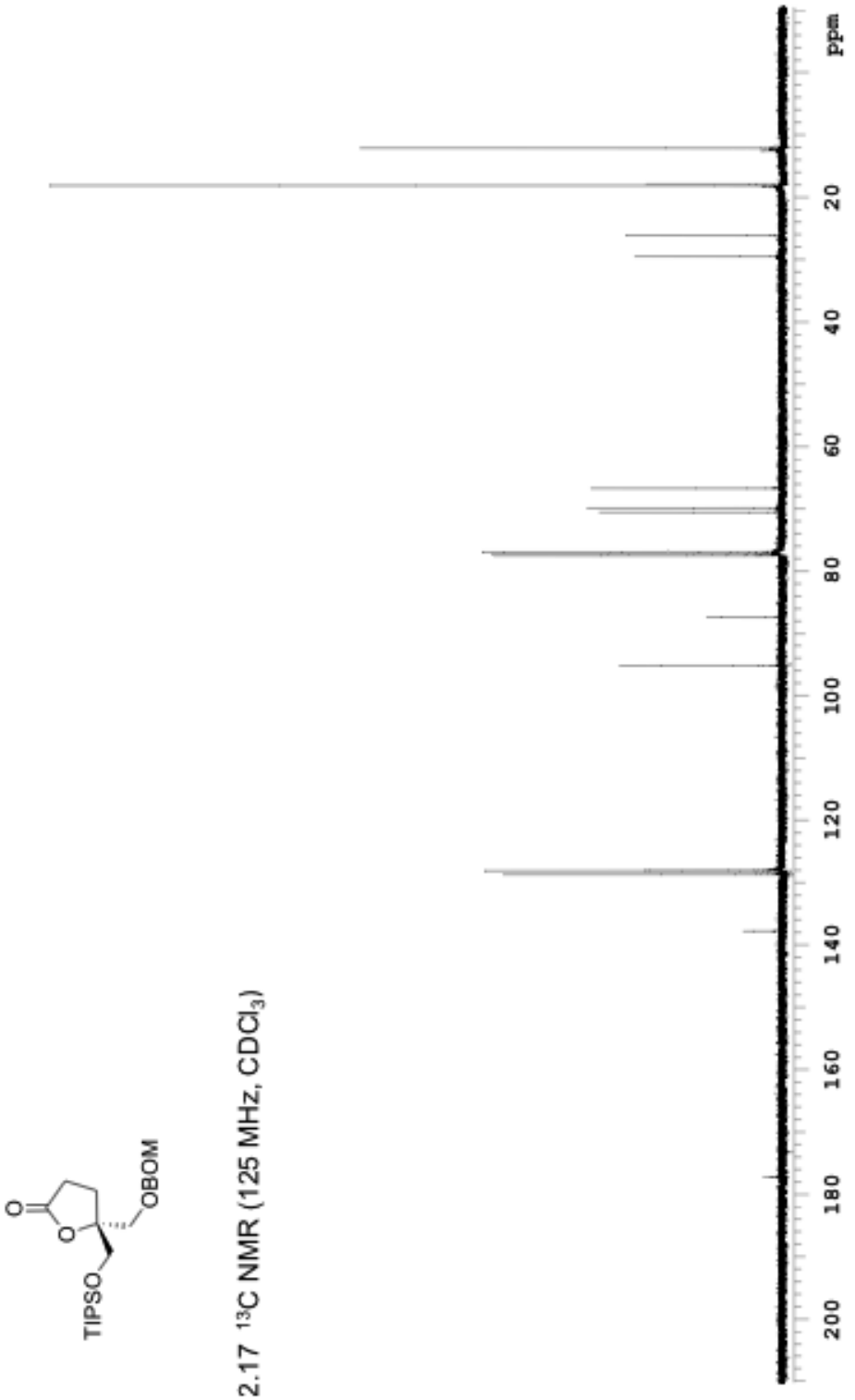
2.14 ^{13}C NMR (125 MHz, $CDCl_3$)

2.14 ^{13}C DEPT (125 MHz, $CDCl_3$)

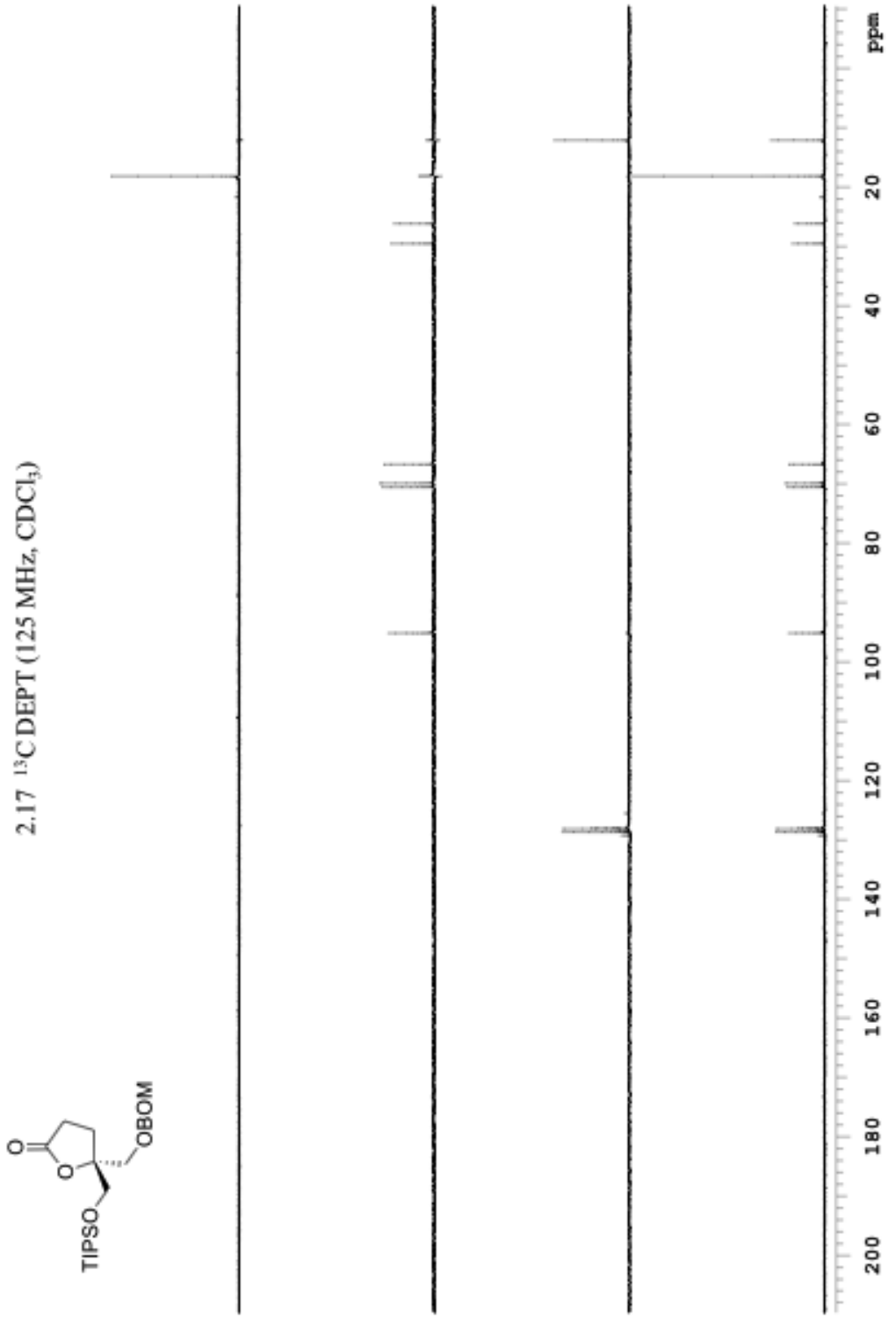


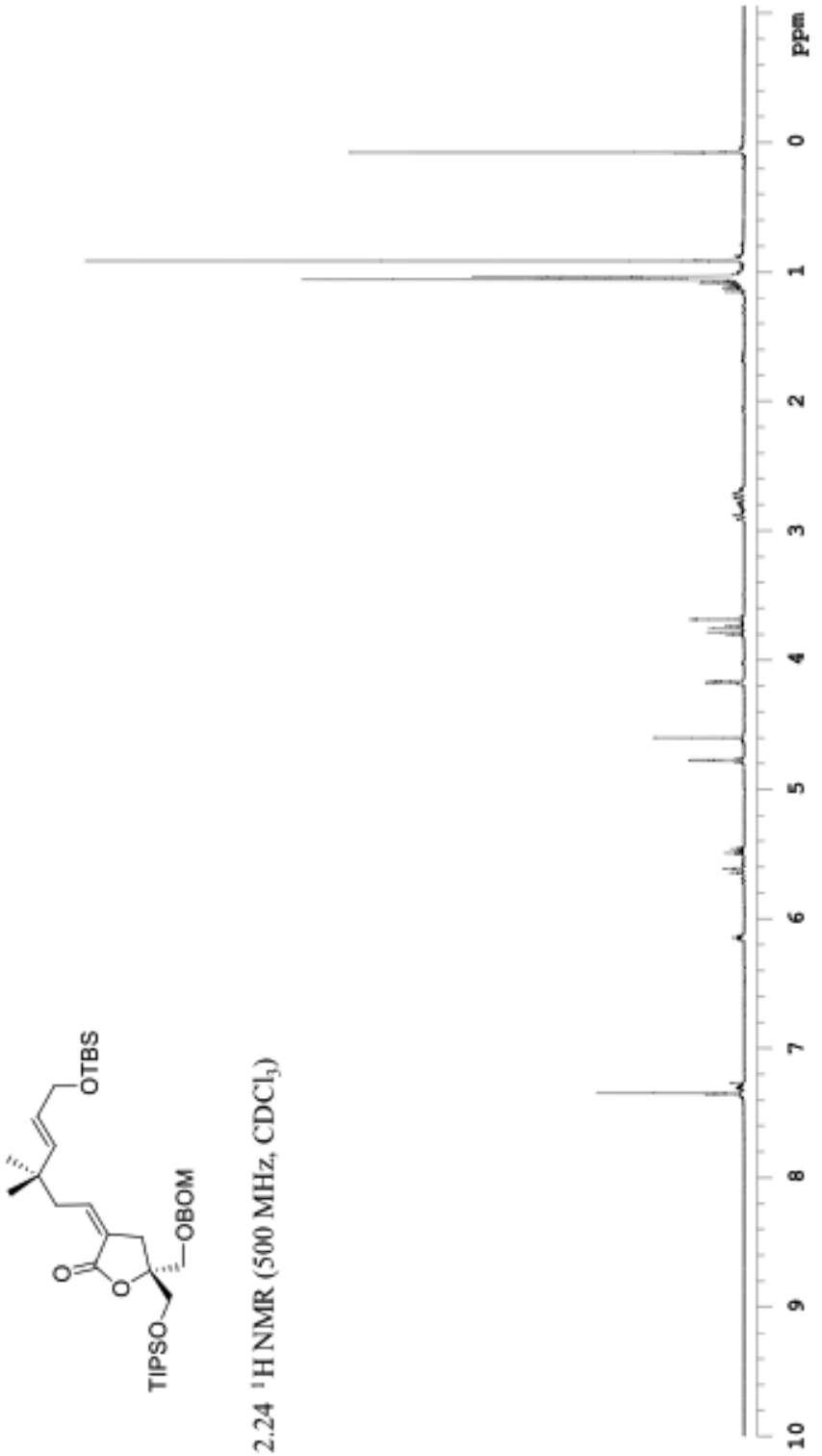


2.17 ^{1}H NMR (500 MHz, $CDCl_3$)

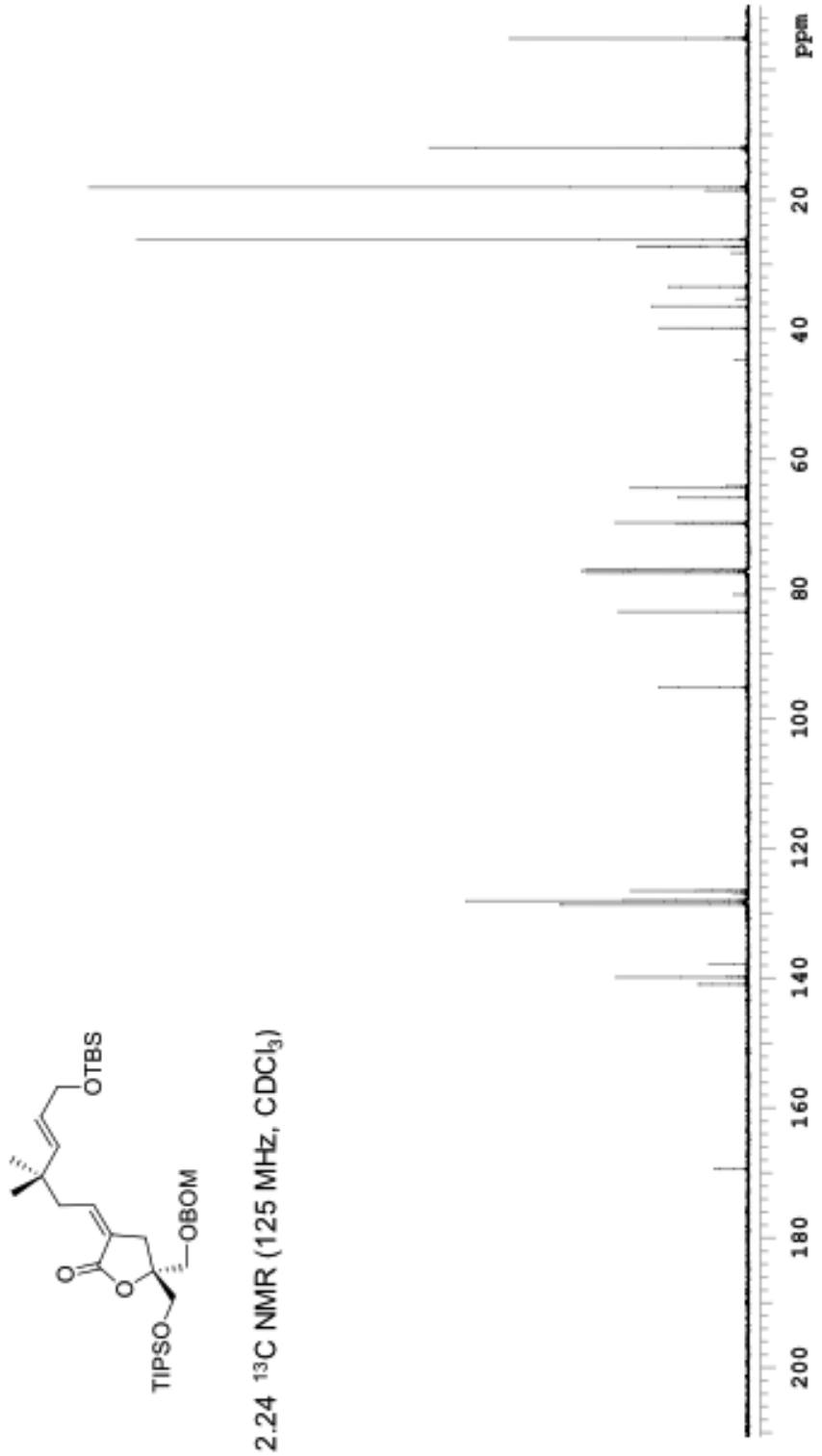


2.17 ^{13}C NMR (125 MHz, $CDCl_3$)


2.17 ^{13}C DEPT (125 MHz, $CDCl_3$)
O
O
OBOM
TIPSO
200
180
160
140
120
100
80
60
40
20
ppm

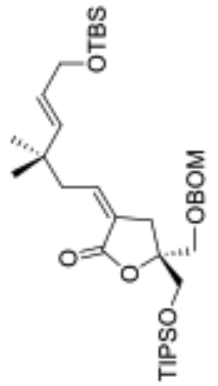


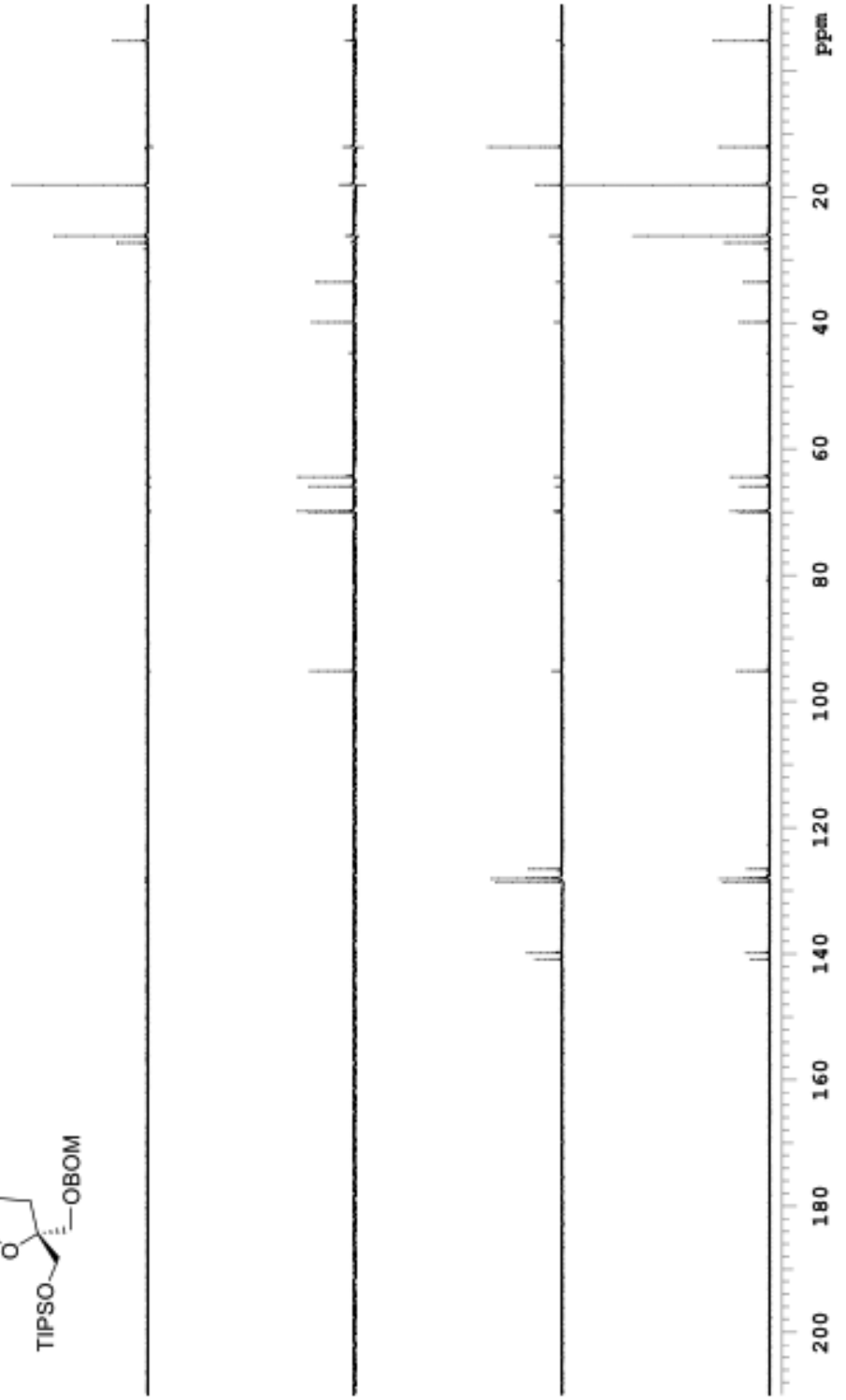
2.24 ^{1}H NMR (500 MHz, $CDCl_3$)

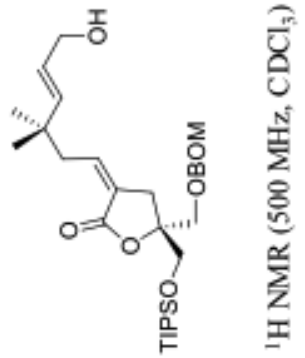


2.24 ^{13}C NMR (125 MHz, $CDCl_3$)

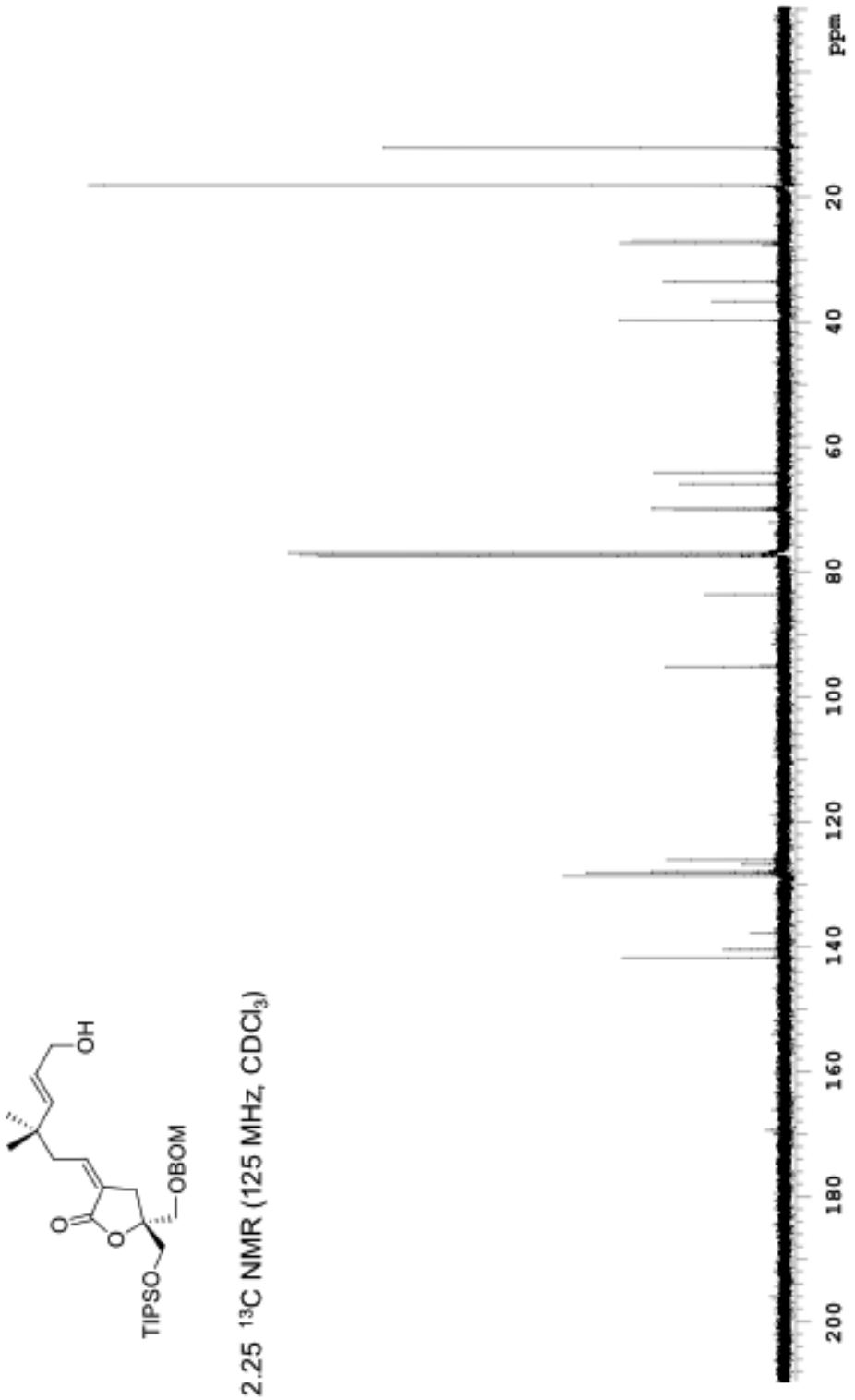
2.24 ^{13}C DEPT (125 MHz, $CDCl_3$)



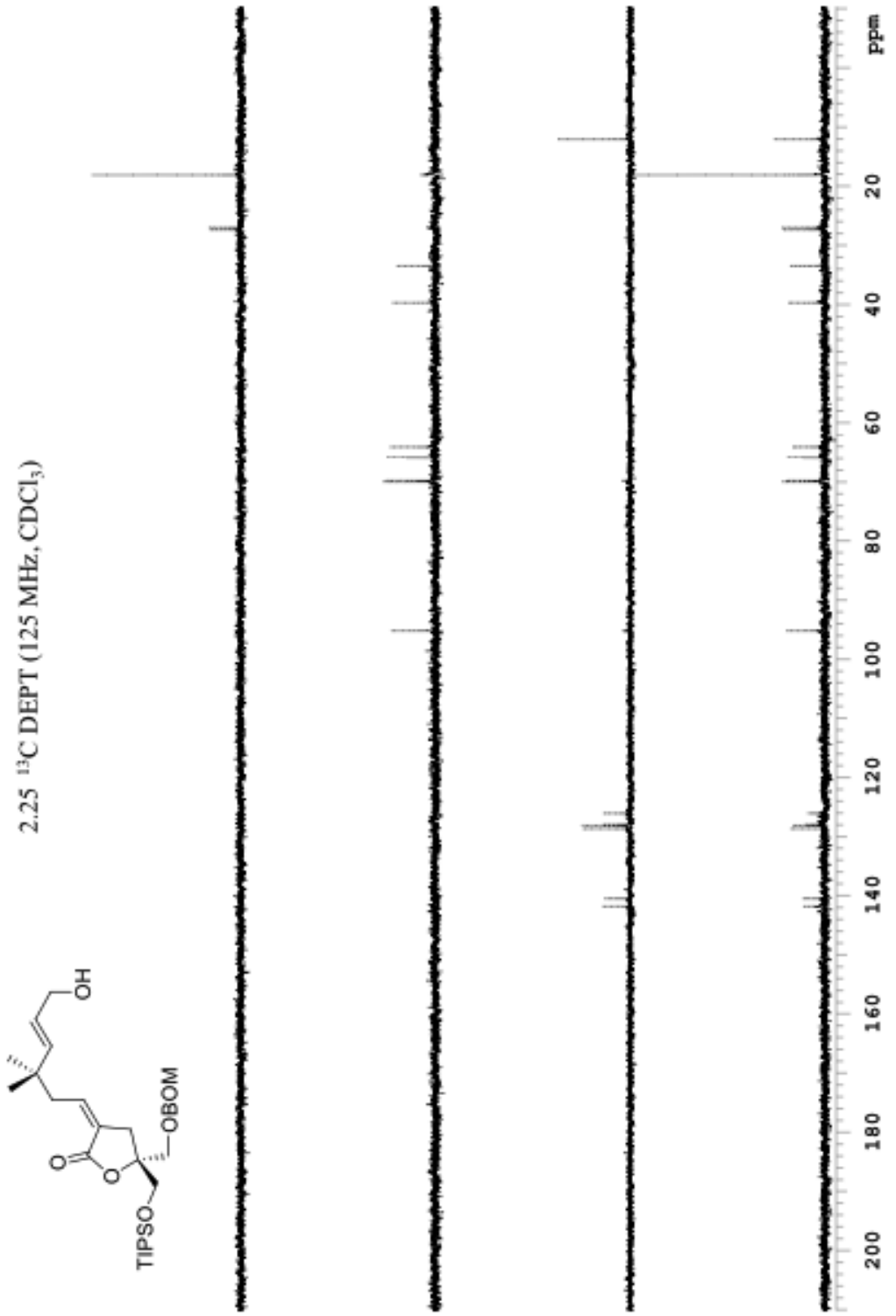


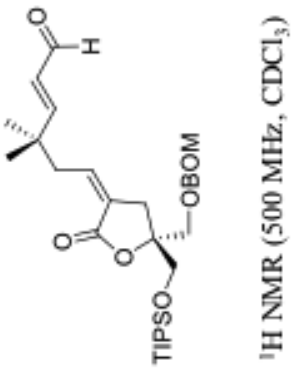


2.25 ^{1}H NMR (500 MHz, $CDCl_3$)

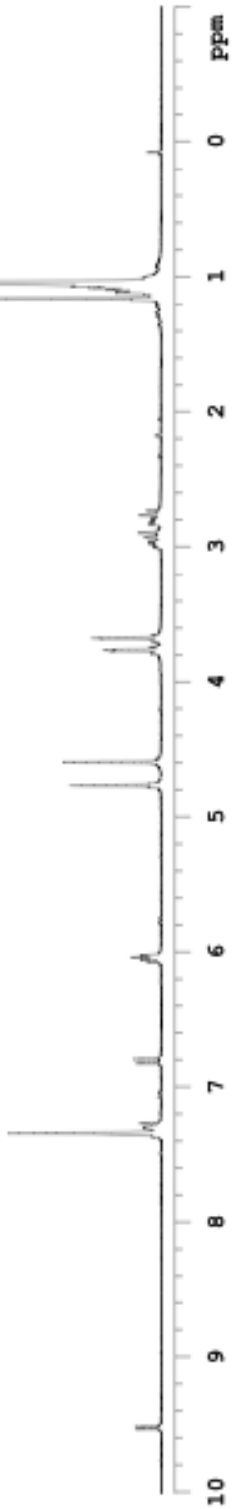


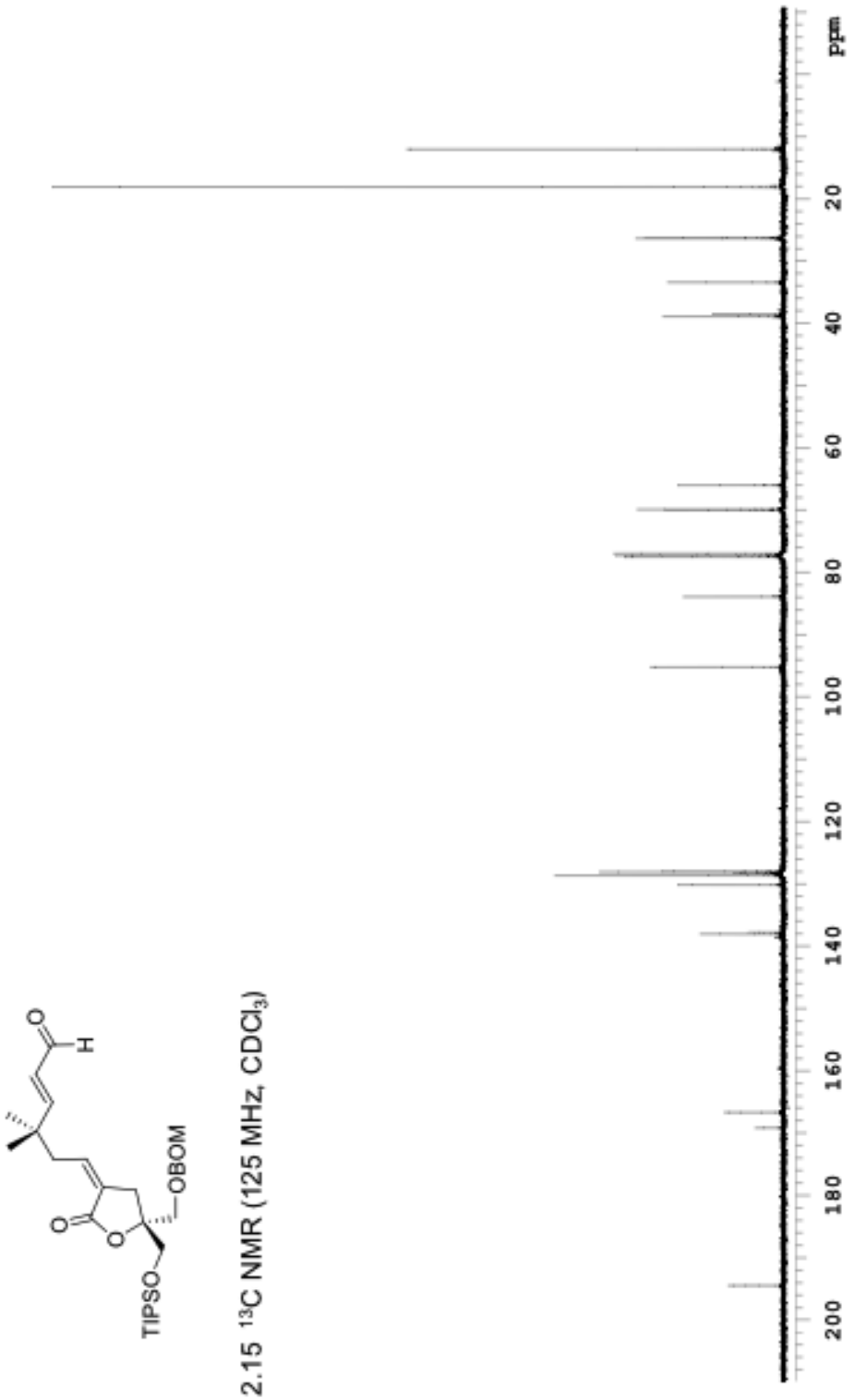
2.25 ^{13}C NMR (125 MHz, $CDCl_3$)


2.25 ^{13}C DEPT (125 MHz, $CDCl_3$)
OH
OBOM
TIPSO
O
O
ppm
200
180
160
140
120
100
80
60
40
20



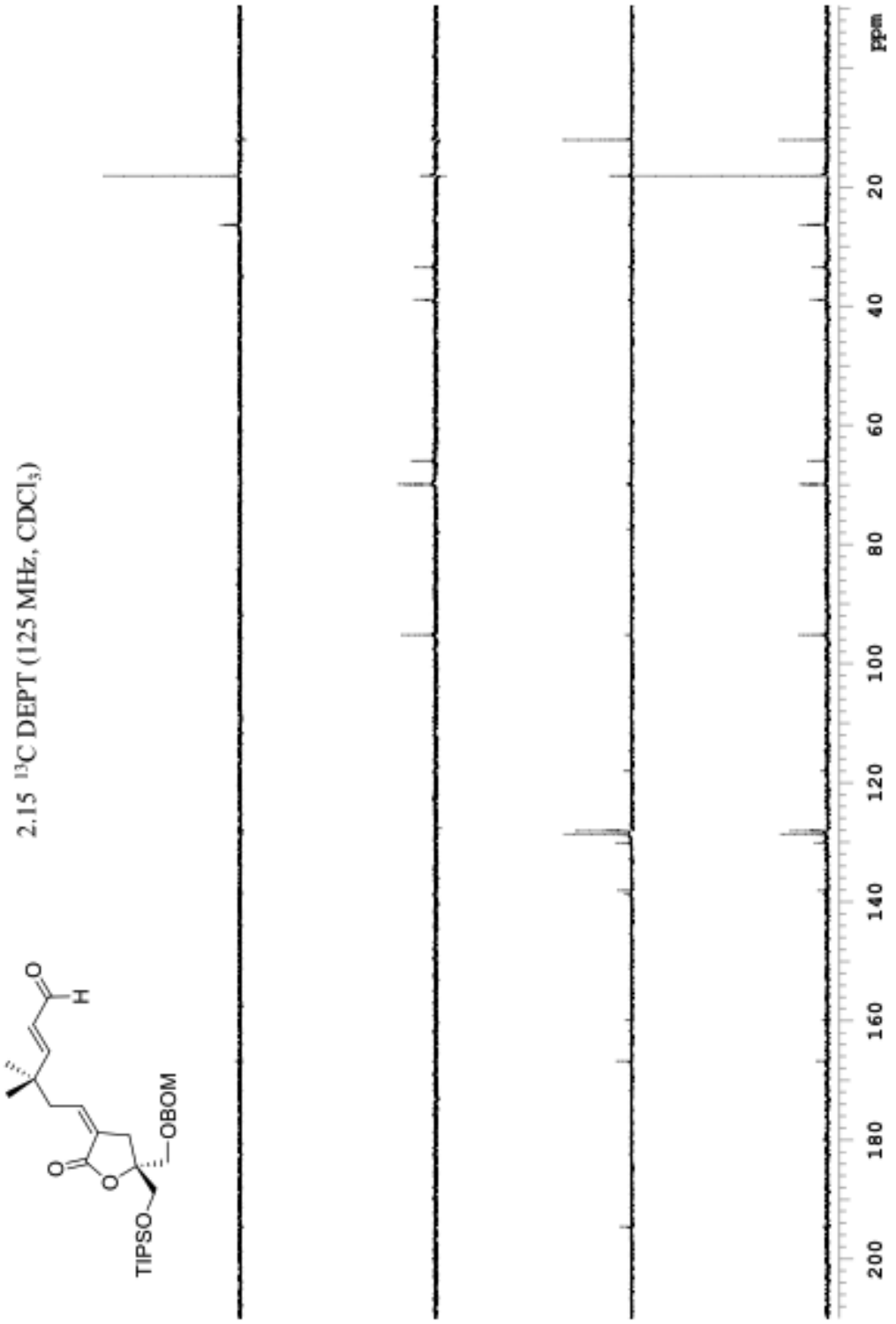
2.15 ^{1}H NMR (500 MHz, $CDCl_3$)

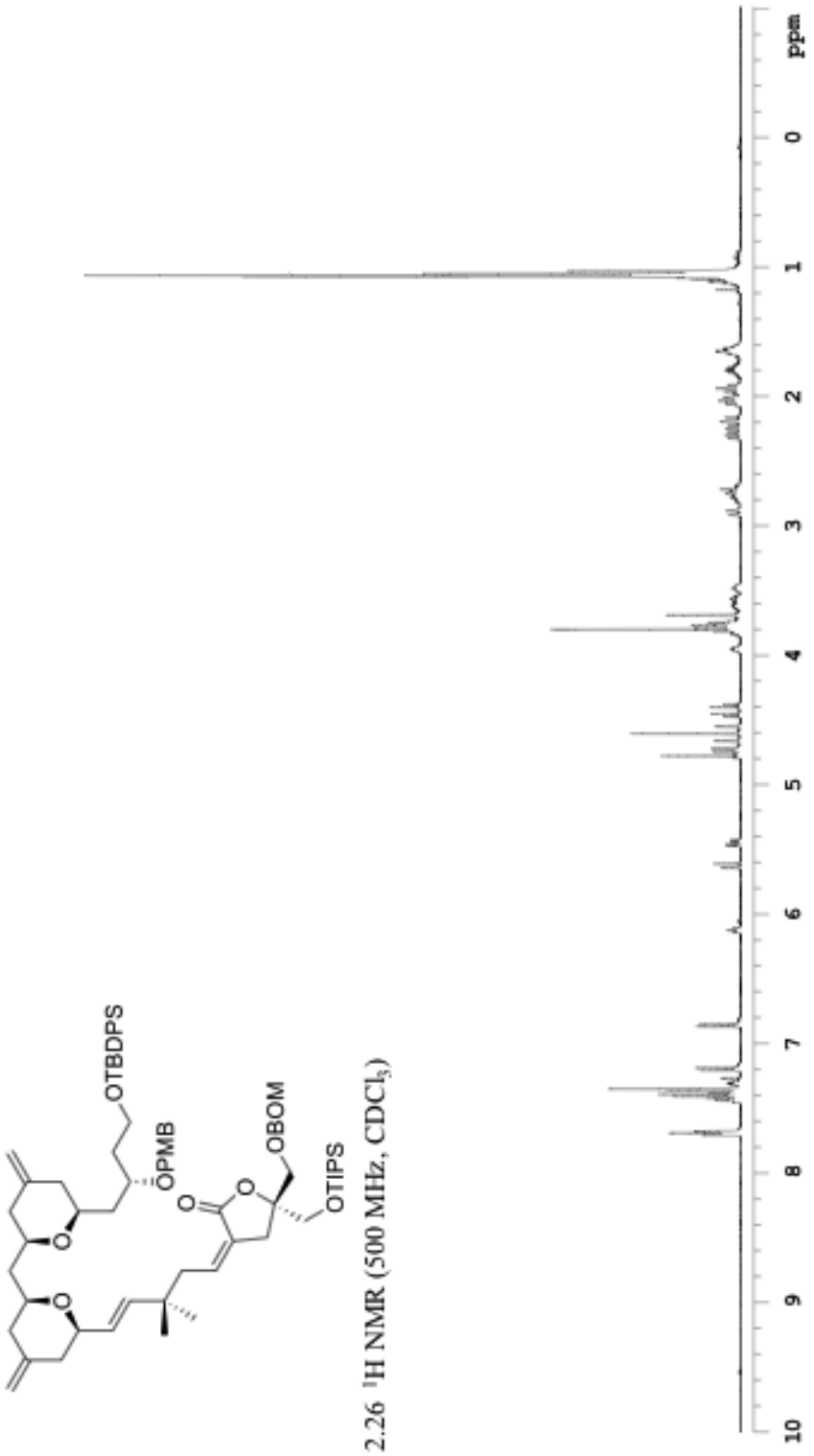




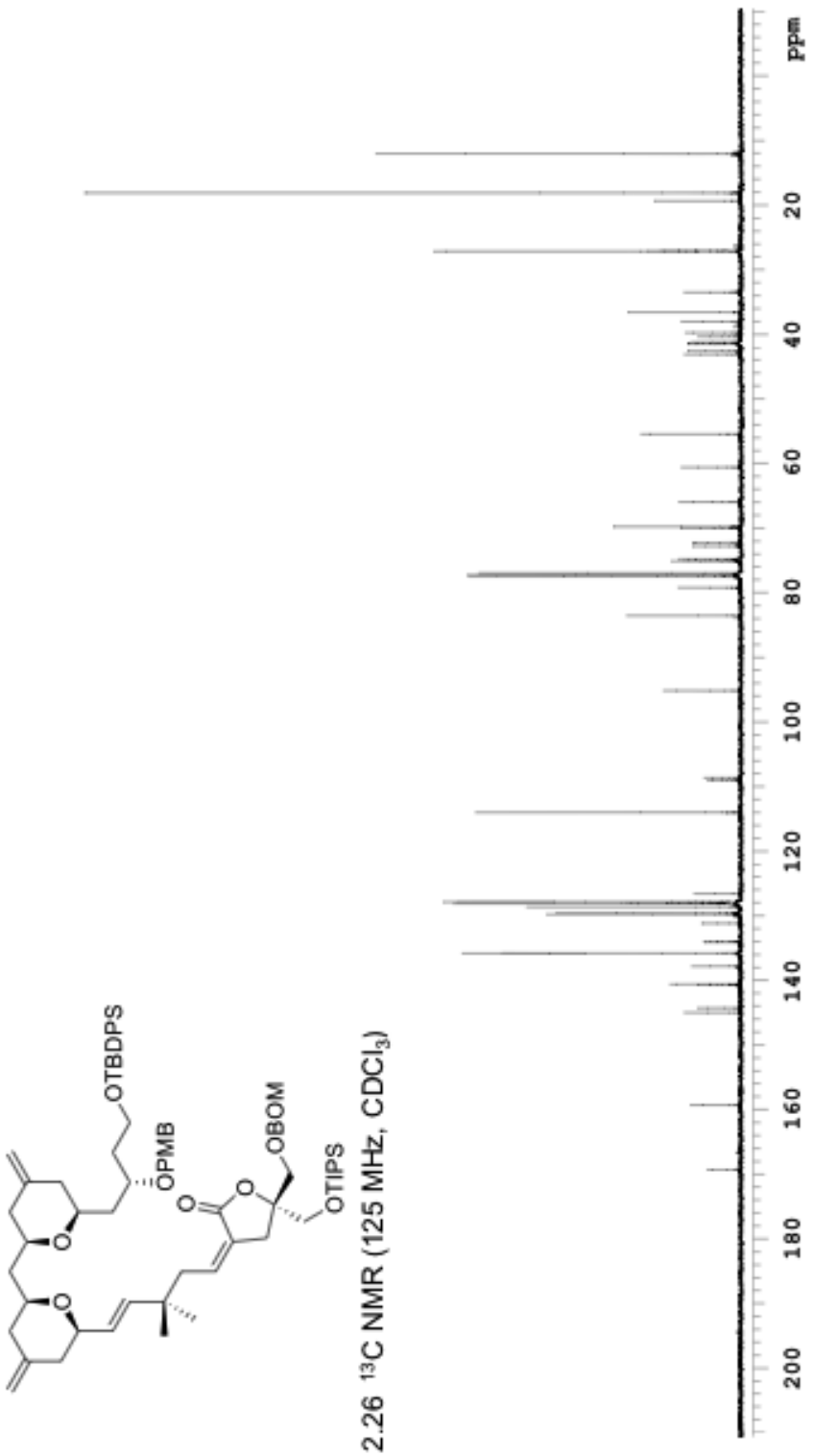
2.15 ^{13}C NMR (125 MHz, $CDCl_3$)

2.15 ^{13}C DEPT (125 MHz, $CDCl_3$)

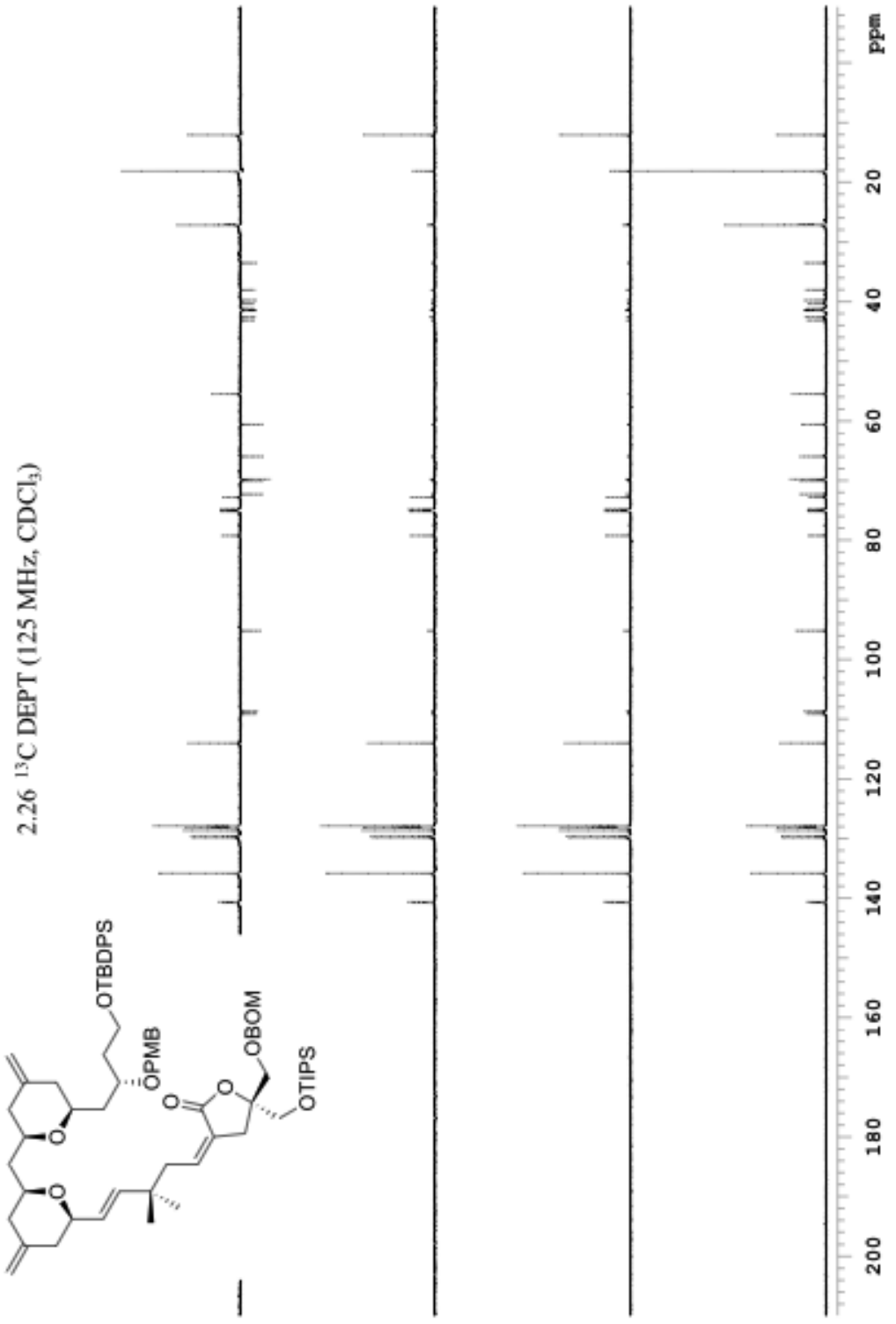


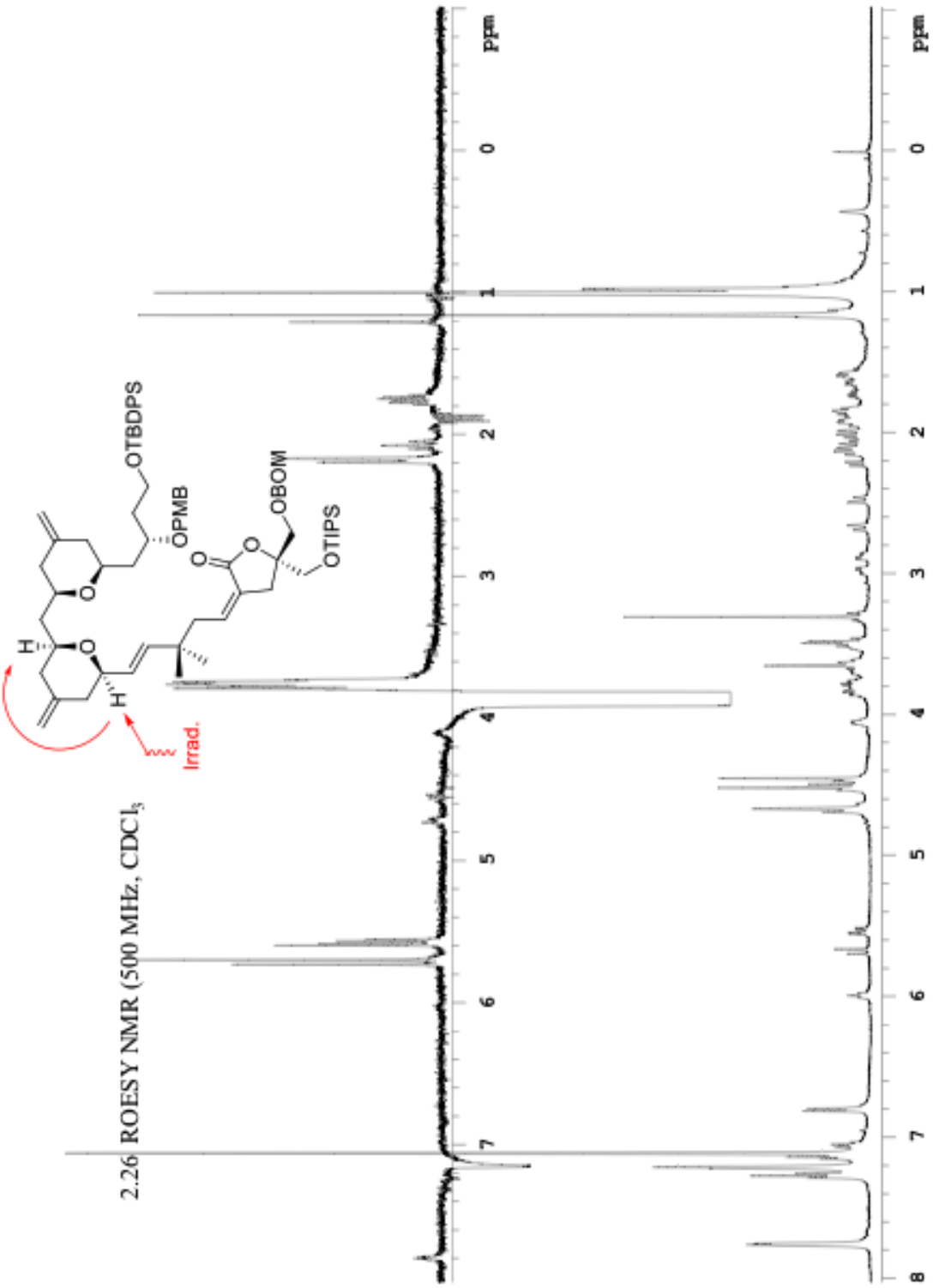


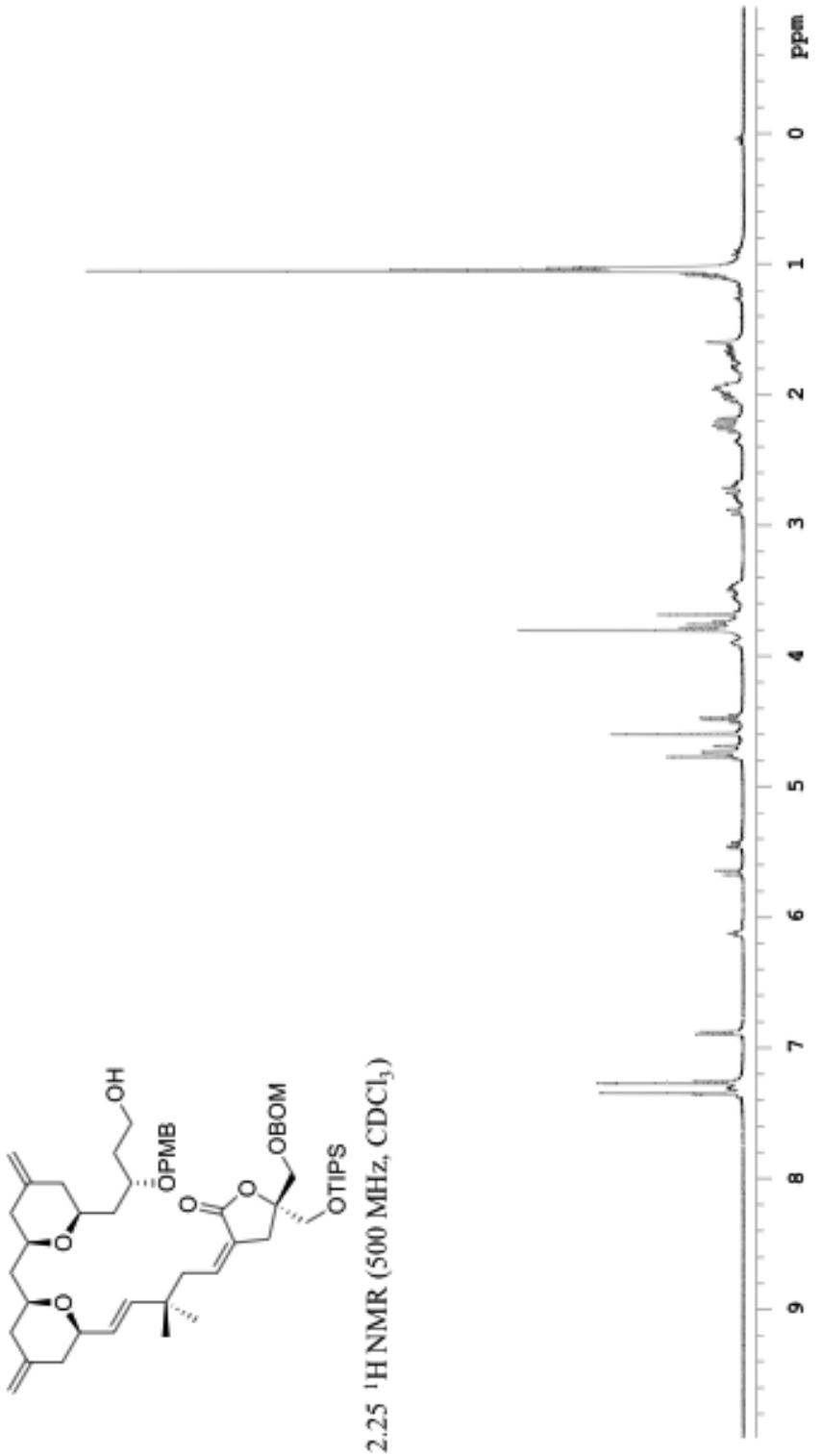
2.26 ^{1}H NMR (500 MHz, $CDCl_3$)



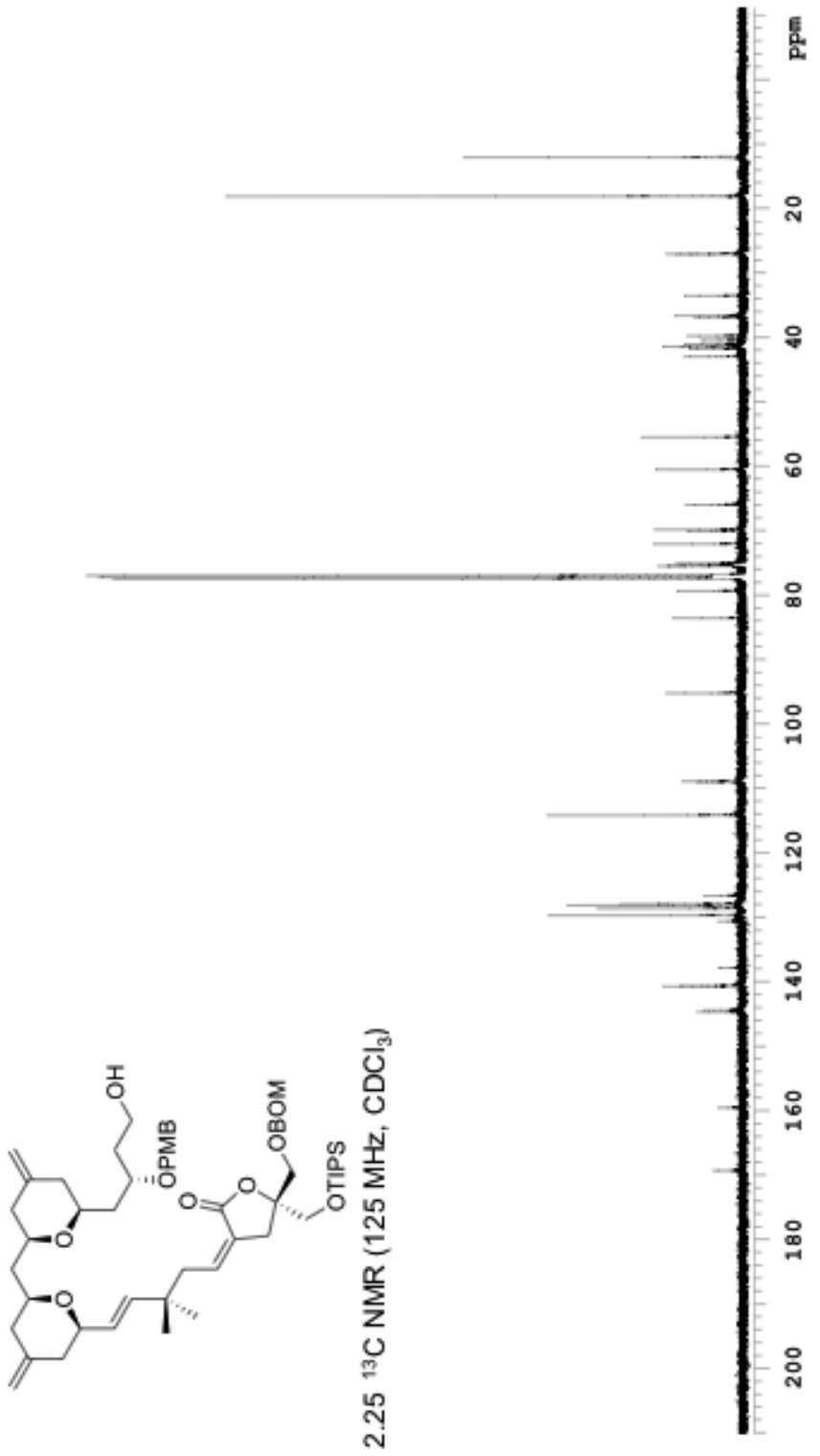
2.26 ^{13}C NMR (125 MHz, $CDCl_3$)


2.26 ^{13}C DEPT (125 MHz, $CDCl_3$)
OTBDPS
OPMB
OBOM
OTIPS
O
O
O
O
200
180
160
140
120
100
80
60
40
20
ppm


2.26 ROESY NMR (500 MHz, $CDCl_3$)
OTBDPS
OPMB
OBOM
OTIPS
H
H
Irrad.
ppm
ppm

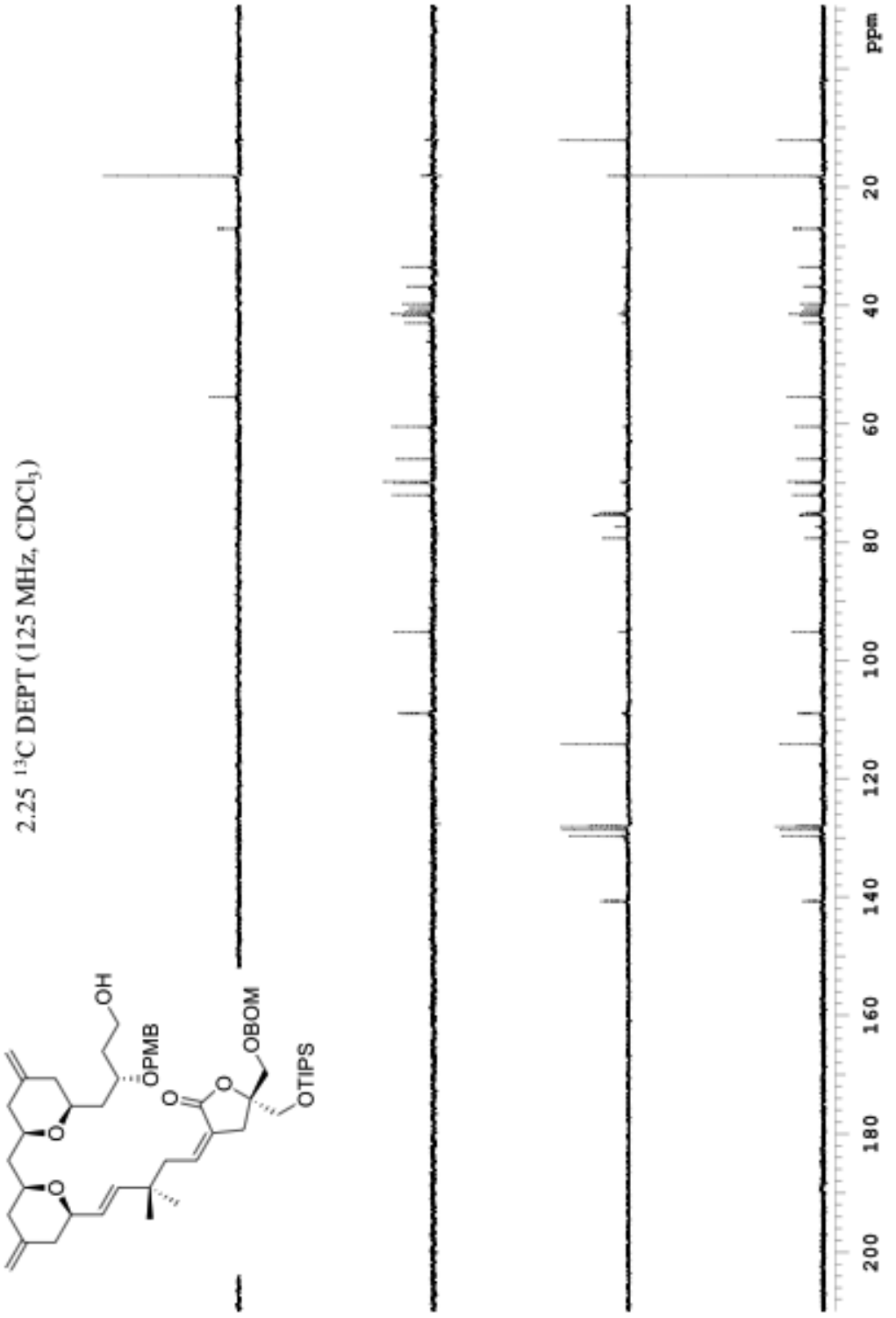


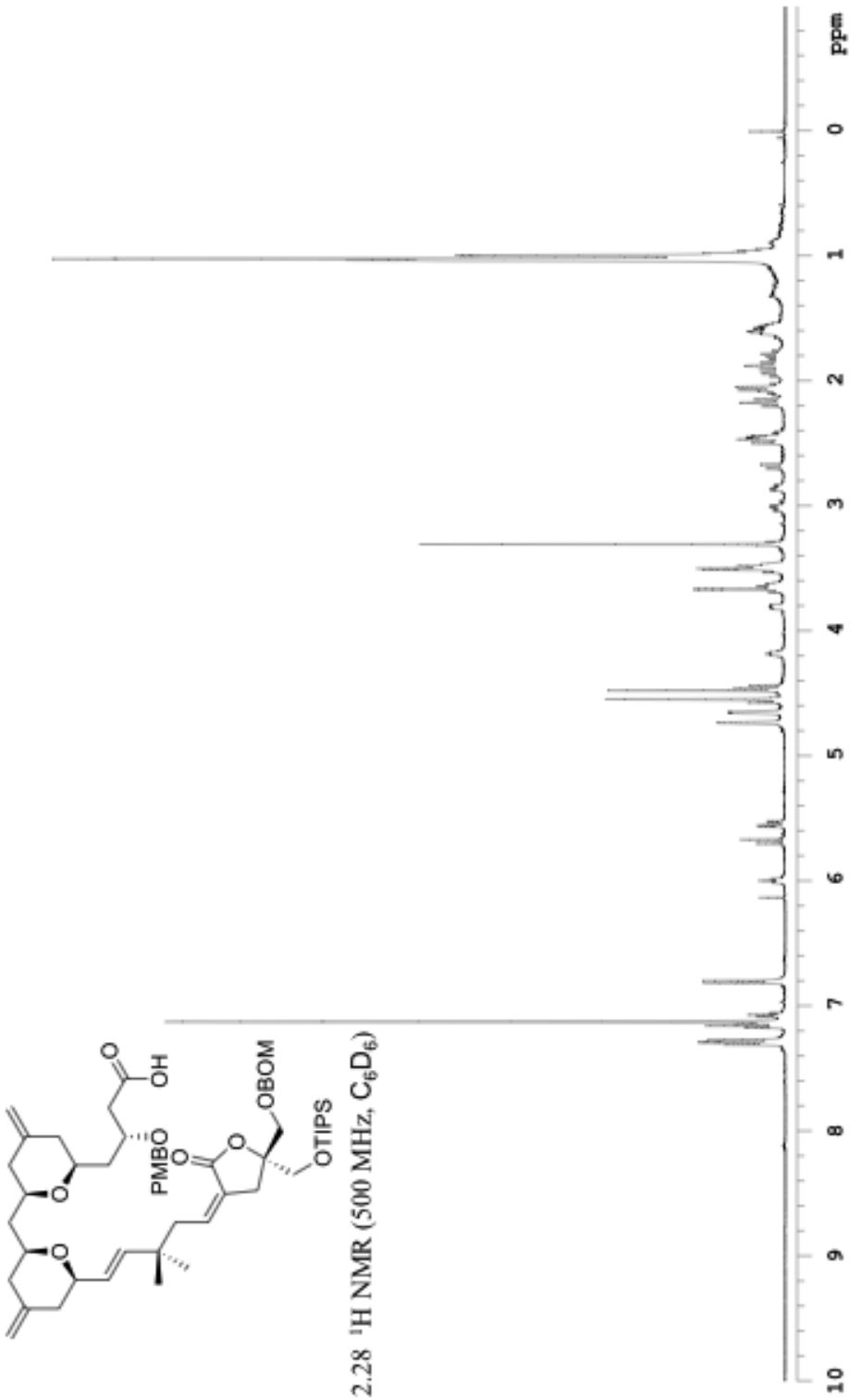
2.25 ^{1}H NMR (500 MHz, $CDCl_3$)



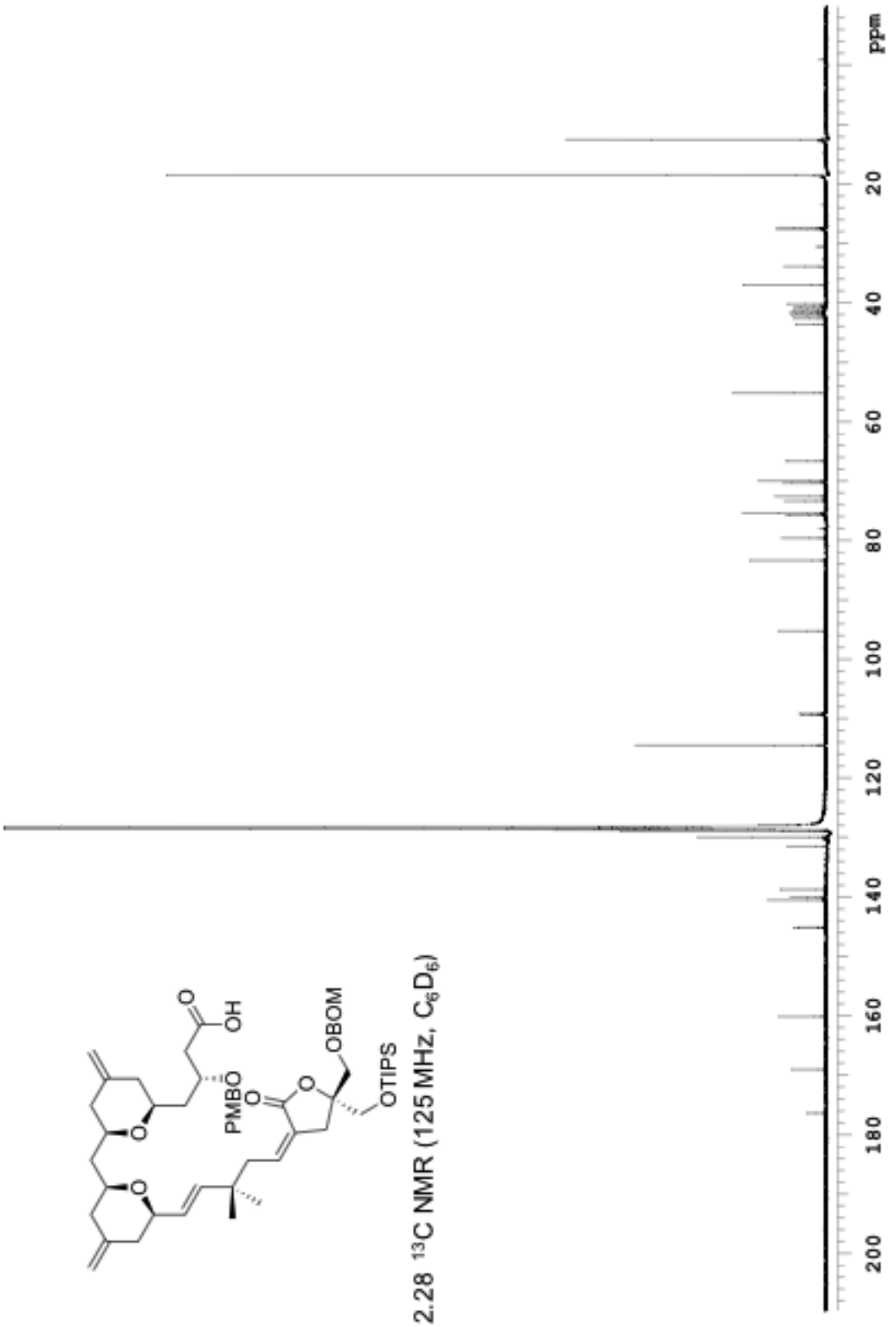
2.25 ^{13}C NMR (125 MHz, $CDCl_3$)

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

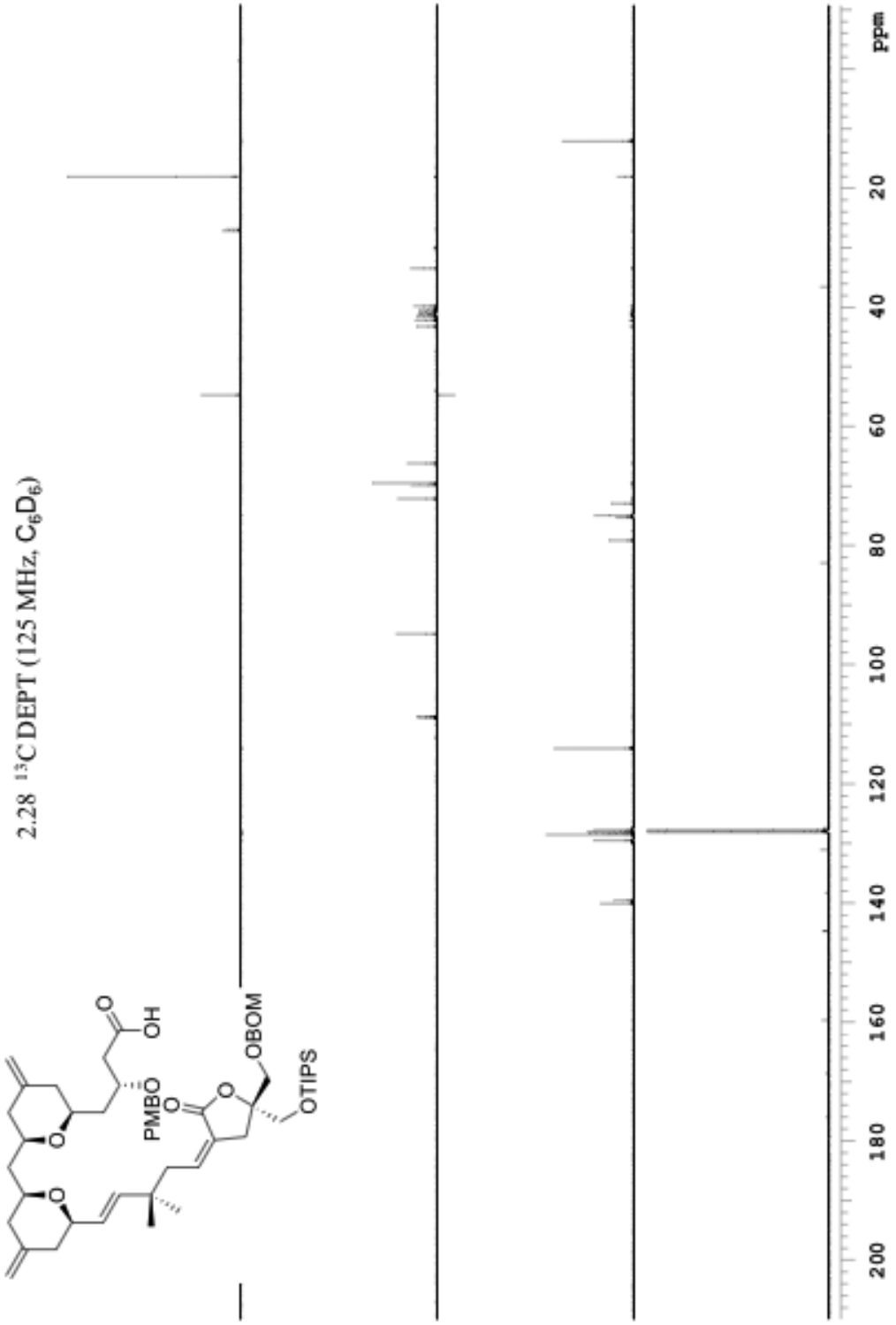


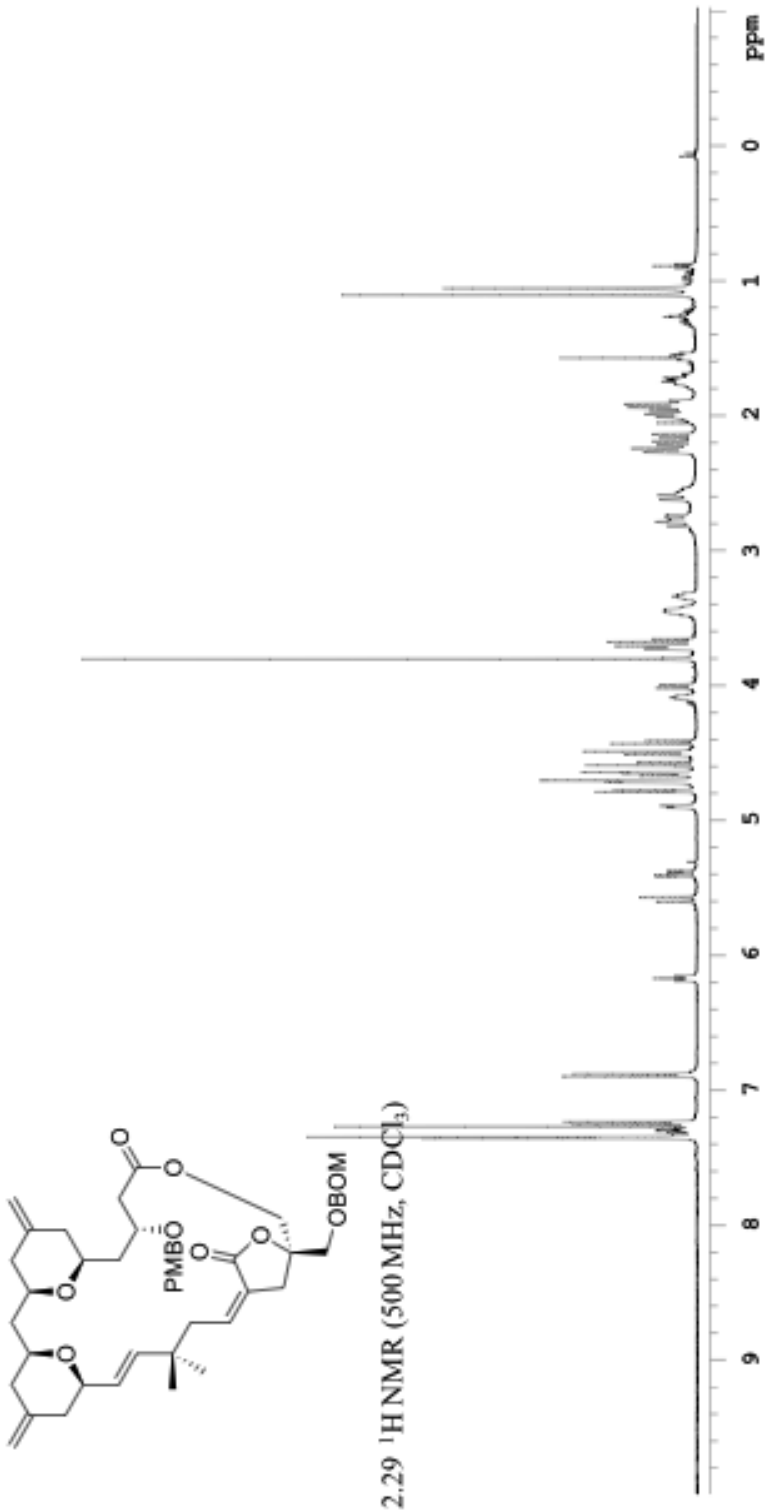


2.28 ^{1}H NMR (500 MHz, C_6D_6)

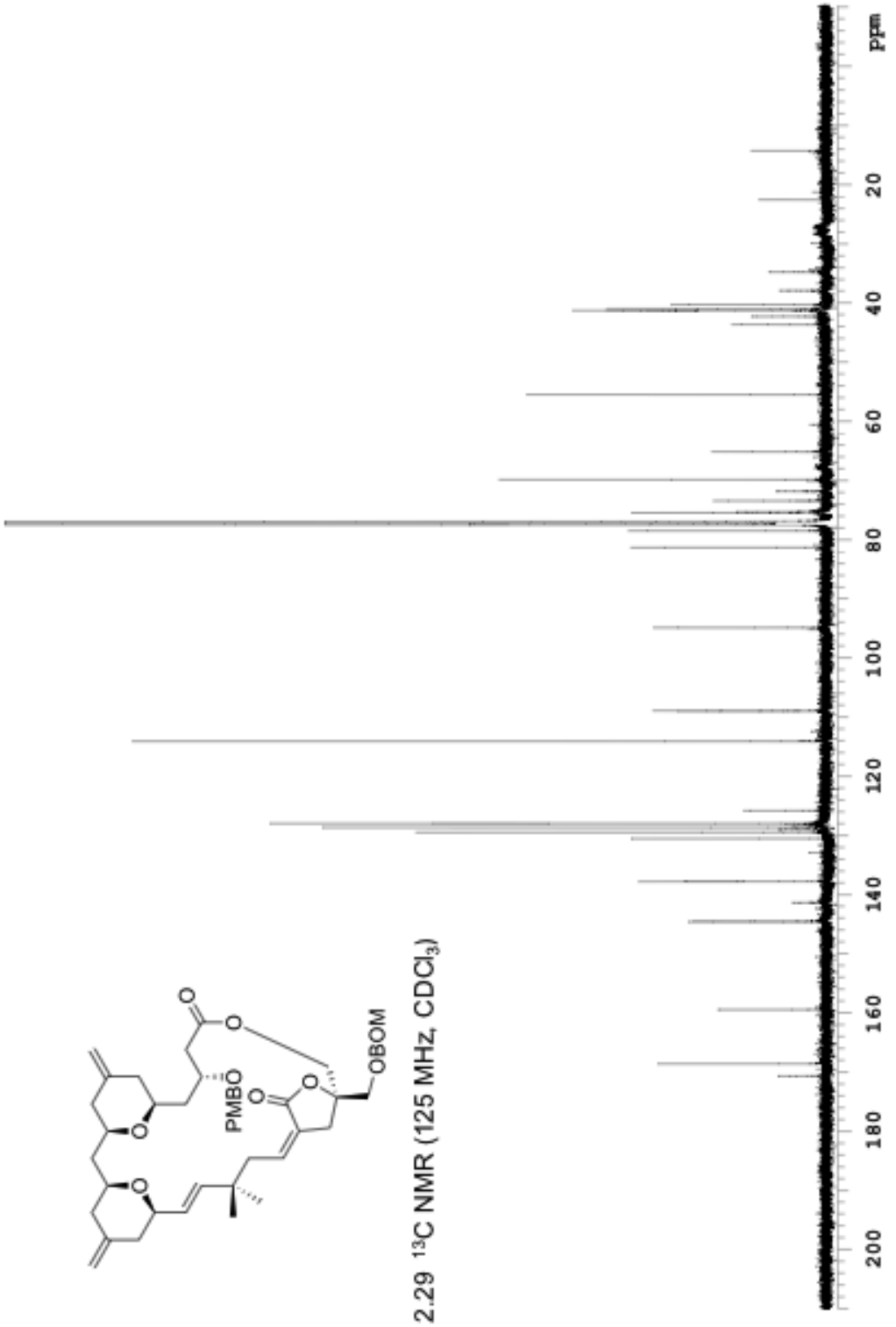


2.28 ^{13}C NMR (125 MHz, C_6D_6)


2.28 ^{13}C DEPT (125 MHz, C_6D_6)
OH
PMBO
OBOM
OTIPS
ppm
200
180
160
140
120
100
80
60
40
20

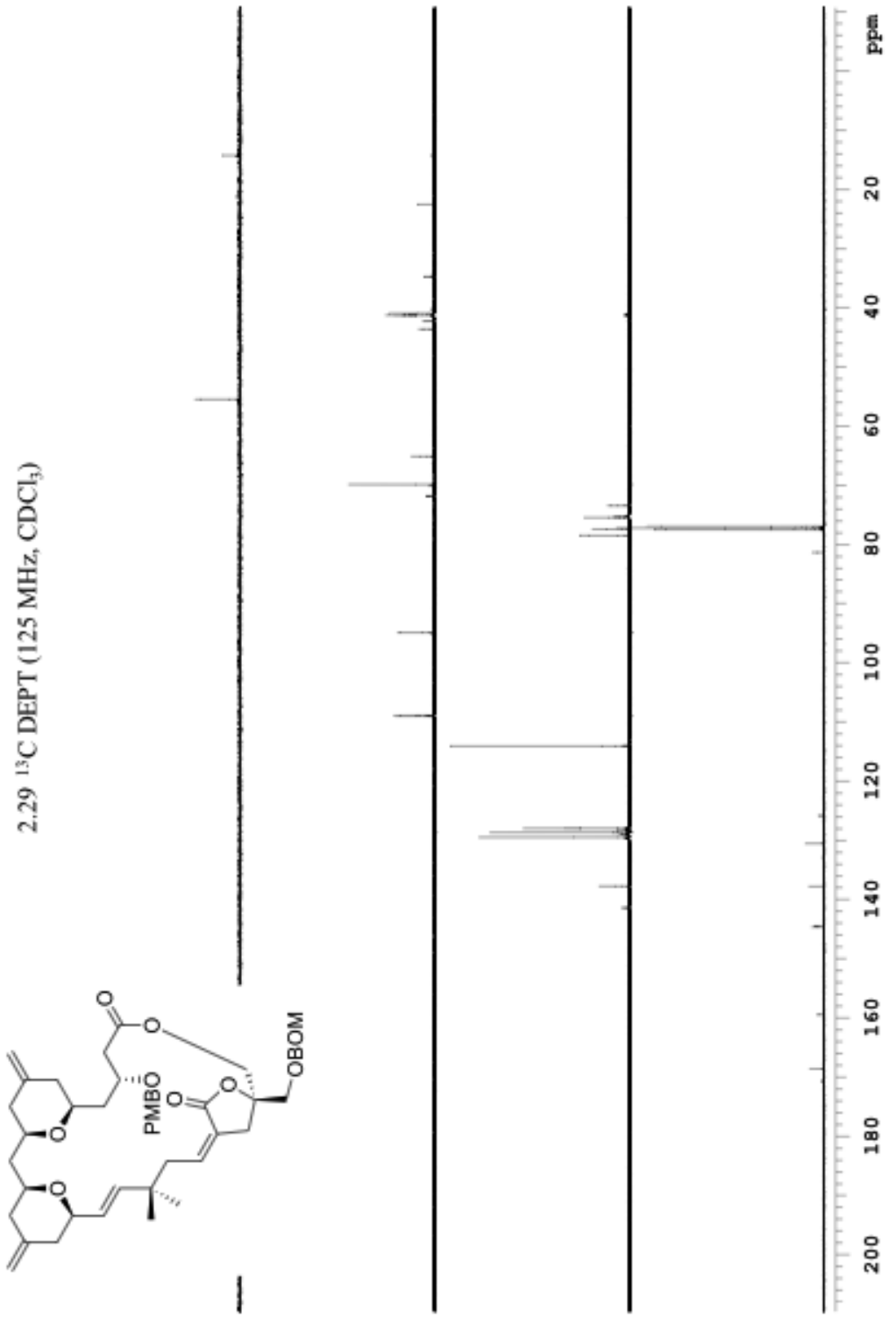


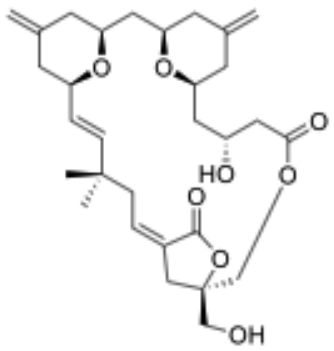
2.29 ^{1}H NMR (500 MHz, $CDCl_3$)



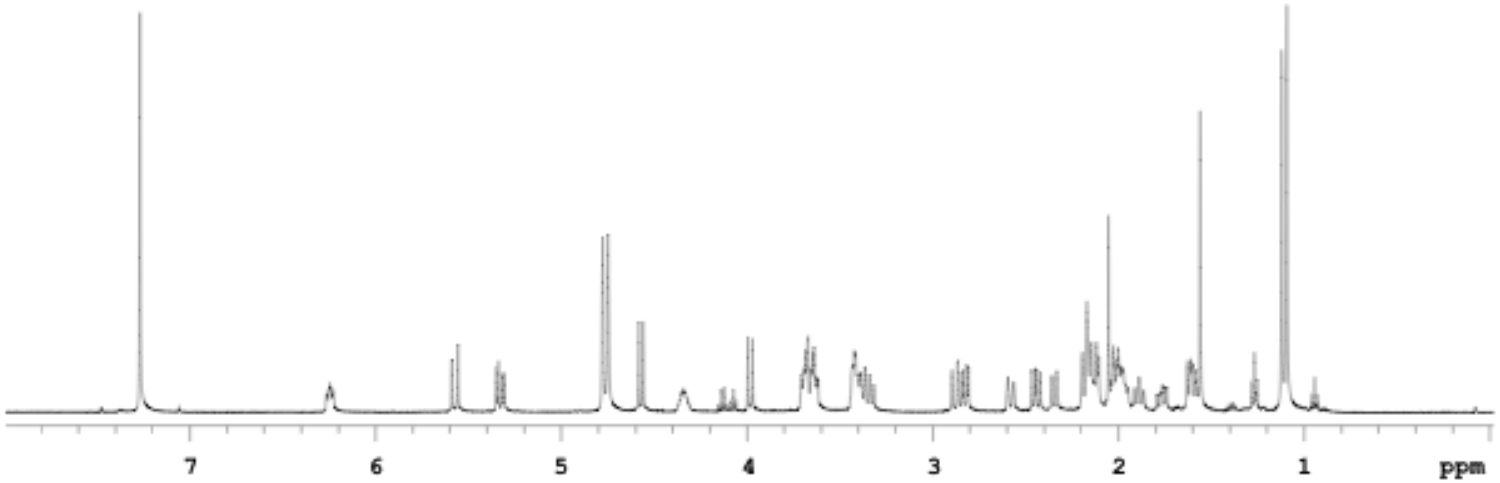
2.29 ^{13}C NMR (125 MHz, $CDCl_3$)

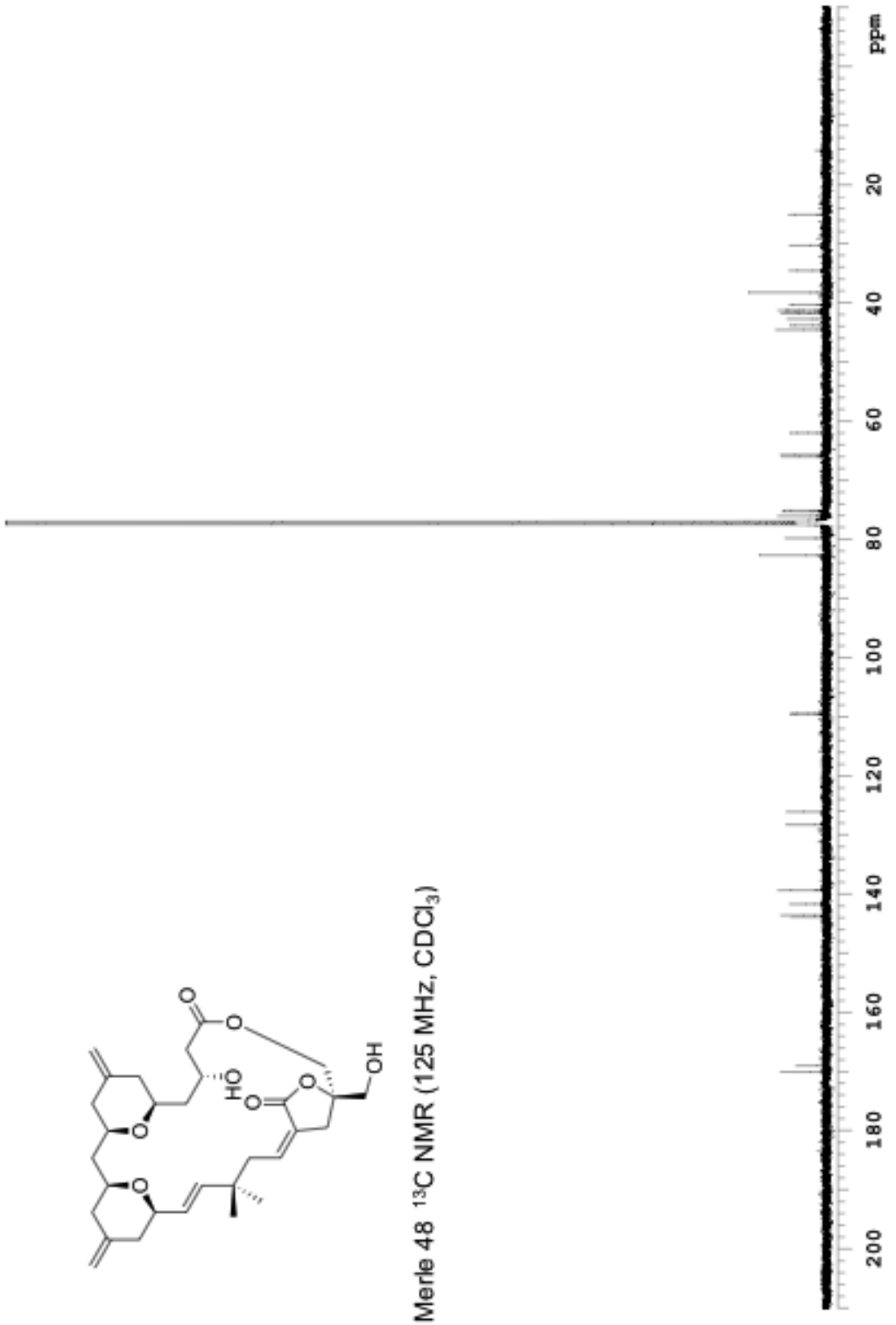
2.29 ^{13}C DEPT (125 MHz, $CDCl_3$)

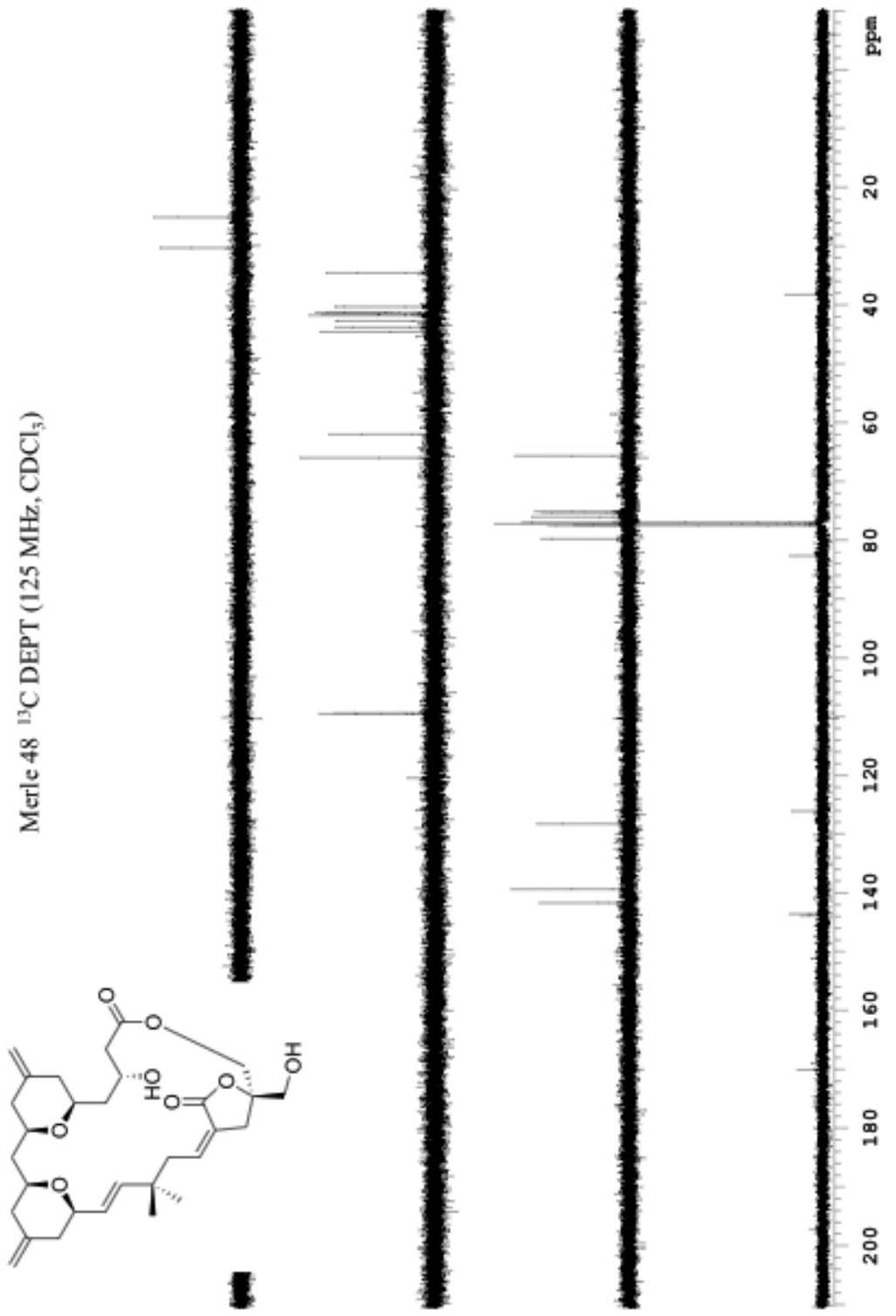




Merle 48 ^{1}H NMR (500 MHz, $CDCl_3$)




Merle 48 ^{13}C NMR (125 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20
HO
O
O
O
O
O
OH


Merle 48 ^{13}C DEPT (125 MHz, $CDCl_3$)
ppm
200
180
160
140
120
100
80
60
40
20
HO
O
O
O
O
O
OH