PROGRESS TOWARD THE TOTAL SYNTHESIS OF MARINEOSINS A & B; TOTAL SYNTHESIS OF TAMBJAMINE K AND UNNATURAL ANALOGS WITH IMPROVED ANTICANCER ACTIVITY, AND DISCOVERY OF SELECTIVE M_1 ANTAGONISTS.

By

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For Jon,

My soul mate and best friend

"The man in black fled across the desert, and the gunslinger followed"

The Gunslinger (The Dark Tower, Book 1)

Stephen King

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Ac	acetyl
AC	adenylyl cyclase
AcH	acetylcholine
AdoMet	*S*-adenosylmethionine
ATP	adenosine triphosphate
AcOH	acetic acid
Ar	aromatic
9-BBN	9-borabicyclo [3.3.1]nonane
BF_3OEt_2	boron trifluoride diethyl ether
Bn	benzyl
BnBr	benzyl bromide
Boc	*t*-butyloxycarbonyl
BOM	benzyloxymethyl
BTMSE	1,2-Bis(trimethoxysilyl)ethane
Bz	benzoyl
^oC	degrees Celsius
cAMP	cyclic adenosine monophophate
CsA	cyclosporine A
CAN	ceric ammonium nitrate
CBS	Corey-Bakshi-Shibata

cat.	catalytic
$CDCl_3$	deuterated chloroform
CH_2Cl_2	dichloromethane
CH_3CN	acetonitrile
CD	circular dichroism
conc	concentration
CM	cross metathesis
CNS	central nervous system
COSY	correlation spectroscopy
CRC	concentration response curve
CSA	10-camphorsulfonic acid
Cs_2CO_3	cesium carbonate
δ	chemical shift in ppm
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DBS	deep brain stimulation
dd	doublet of doublet
ddd	doublet of doublet of doublet
dddd	doublet of doublet of doublet of doublet
dt	doublet of triplet
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DDG	2-dehydro-3-deoxygalactarate

DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DET	diethyltartrate
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPEA	*N,N*-diisopropylethylamine
DIEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
DME	Dimethoxyethane
DMF	N, N-dimethylformamide
DMP	Dess-Martin periodinane
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)pyrimidinone
DMS	dimethylsulfide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTBMP	di-*tert*-butyl-4-methylpyridine
DPPE	1,2-Bis(diphenylphosphino)ethane
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq.	equivalent
Et	ethyl
Et_3N	triethylamine
Et_2O	diethyl ether

EtOAc	ethyl acetate
EtOH	ethanol
EC_{50}	half maximal effective concentration
FAD	flavin adenine dinucleotide
FVP	flash vacuum pyrolysis
FMO	frontier molecular orbital
GABA	*gamma*-amino butyric acid
GPCR	G-protein coupled receptor
h	hour
HBC	4-hydroxy-2,2'-bipyrrole-carbaldehyde
HBM	4-hydroxy-2,2'-bipyrrole-5-methanol
HCl	hydrogen chloride
HMPA	hexamethylphosphoramide
HOMO	highest occupied molecular orbital
HOBt	hydroxybenzotriazole
HPLC	high pressure liquid chromatography
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum coherence
HWE	Horner-Wadsworth-Emmons
HTS	high throughput screen
IC_{50}	half maximal inhibitory concentration
IL	interleukin
IMDA	intramolecular Diels Alder

ImH	imidazole
IEDHDA	inverse electron demand hetero Diels Alder
IP_3	inositol triphosphate
*i*PrOH	isopropanol
JNK	c-Jun-*N*-terminal kinase
K_2CO_3	potassium carbonate
KHMDS	potassium hexamethyldisilazide
L	liter(s)
LDA	lithium diisopropylamide
$LiAlH_4$	lithium aluminum hydride
LTMP	lithium 2,2,6,6-tetramethylpiperidine
LUMO	lowest unoccupied molecular orbital
MAOS	microwave-assisted organic synthesis
MAP	2-methyl-*N*-amyl-pyrrole
MBC	4-methoxy-2,2'-bipyrrole-5-carbaldehyde
*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MHz	megahertz
min	minute(s)
mL	milliliters
mol	mole(s)
MeI	iodomethane
MeOH	methanol

MeCN	acetonitrile
MOM	methoxymethyl
MPS	macrophomate synthase
MP	macroporous
mTOR	mammalian target of rapamycin
mRNA	messenger ribonucleic acid
MS	molecular sieves
Ms	methanesulfonyl
MSN	medium spiny neuron
mw	microwave
mAChR	muscarinic acetylcholine receptor
M_1-M_5	muscarinic acethylcholine receptor subtypes 1-5
NAD(P)H	nicotinamide adenine dinucleotide phosphate oxidase
NAD(P)	nicotinimide adenine dinucleotide phosphate
NaOH	sodium hydroxide
NBS	*N*-bromosuccinimide
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser enhancement spectroscopy
NF	nuclear factor
NMO	*N*-methylmorpholine N-oxide
OBX	obatoclax
Pd/C	palladium on carbon

PLP	pyridoxal phosphate
PNS	peripheral nervous system
PG	prodigiosin
PKA	protein kinase A
Piv	trimethylacetyl (pivaloyl)
PLC	phospholipase C
PMB	4-methoxybenzyl
PMP	*p*-methoxyphenol
Ph	phenyl
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
pyr	pyridine
SAE	Sharpless asymmetric epoxidation
SAR	structure-activity relationship
SEM	2- trimethylsilylethyoxymethyl
S_NAr	nucleophilic aromatic substitution
RCM	ring closing metathesis
RED-Al	bis(2-methoxyethoxy)aluminum hydride
RNA	ribonucleic acid
rt	room temperature
TBHP	*t*-butylhydrogen peroxide
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide

TPAP	tetrapropylammounium perruthenate
TBS	*t*-butyldimethylsilyl
TES	triethylsilyl
TBDPS	*t*-butyldiphenylsilyl
TMS	trimethylsilyl
TIPS	triisopropylsilyl
TPP	thiamine pyrophosphate
Tf	trifluoromethanesulfonyl
Tf_2O	triflic anhydride
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	trimethylethylenediamine
Ts	*p*-toluenesulfonyl
TsOH	*p*-toluenesulfonic
*p*TSA	*p*-toluenesulfonic acid
TsCl	*p*-toluenesulfonyl chloride
UV	ultraviolet
$VO(acac)_2$	vanadyl acetoacetonate

CHAPTER 1

INTRODUCTION

1.1. The Colorful History of the Prodigiosin Alkaloids.

The prodigiosin pigments are known for their bright colors and various biological activities.[1,2] These bright red natural products have a long and 'colorful' history. The deep red color of these compounds is due to the pyrrolylpyrromethene core, in which three pyrrole units make up a system with substantial extended conjugation (Figure 1.1).[1,3]

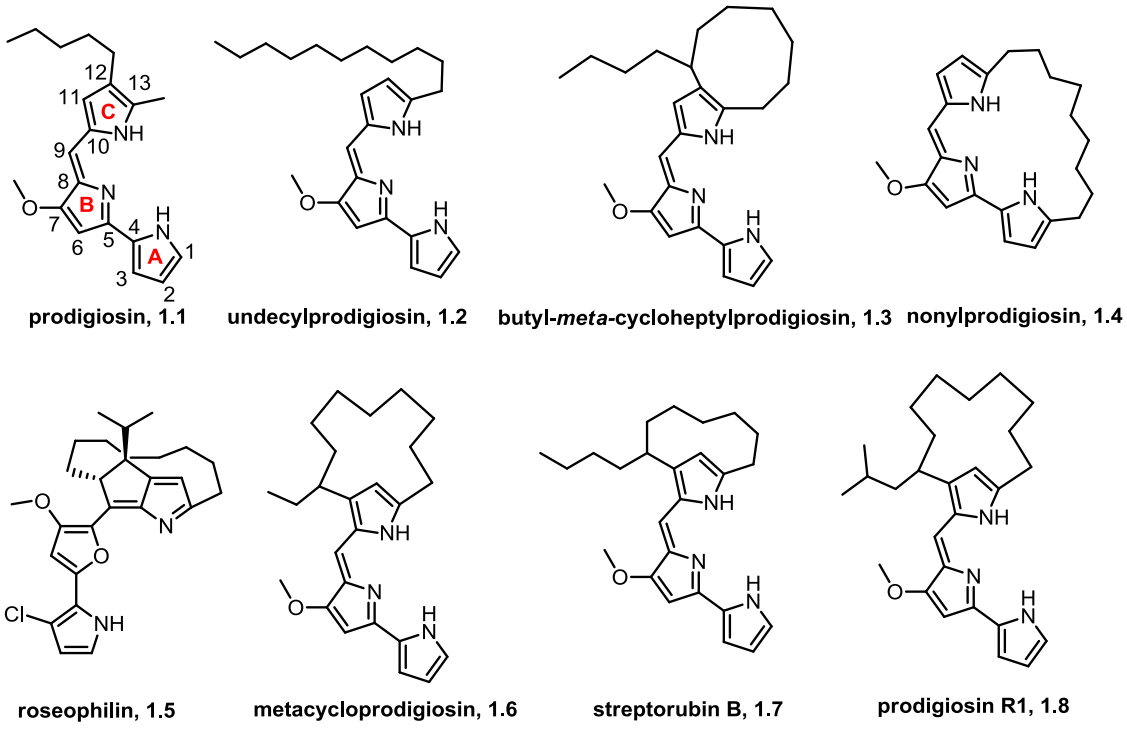


Figure 1.1. Structures of the prodigiosin alkaloids.

Various acyclic and cyclic substitutions can be incorporated onto the C-ring by different bacterial species. Nonylprodigiosin is completely cyclic, with an alkyl chain that

extends from the C-ring to connect to the A-ring. Roseophilin is another prodiginine alkaloid that incorporates a methoxyfuran in place of the methoxypyrrole of the prodigiosins and incorporates a strained macrocyclic motif that contains the azafulvene moiety. This strained macrocyclic azafulvene has been the focus of numerous total and formal syntheses.[1]

The prodigiosin alkaloids are secondary metabolites produced by a number of Gram-negative and Gram-positive bacteria, including *Serratia* spp., actinomycetes (such as *Streptomyces coelicolor* A(3) 2), and various marine bacteria, including *Hahella chejuensis*, *Pseudoalteromonas dentrificans*, and KCTC 2396 (Figure 1.2, Panel A).[4,5] Since the bacteria strains producing the prodigiosin closely resembled drops of blood (Figure 2A), it was hypothesized that this pigment may have been responsible for the many miraculous events recorded throughout history.[1,4] The compound was named prodigiosin for this observation[1,6] Prodigious is an adjective used to describe a noun that is extraordinary in size, amount, extent, degree, or force. It can also be used to describe something which is wonderful, marvelous, or miraculous. The etymology is the Latin prodigiosis from prodigium, which means omen. For the Romans, a prodigium was a literally a sign about the future that came from the gods.[7]

This 'prodigious' pigment has been proposed to be responsible for the miraculous phenomenon of the bleeding host in the European Middle Ages.[1,4,8] The Mass of Bolsena was one of the most famous cases which was commemorated as the festival of Corpus Christi and was immortalized by Raphael in 1508, when he was commissioned to decorate the papal apartments of Julius II in the Vatican (Figure 1.2, Panel C). Even before the Middle Ages, prodigiosin alkaloids had been implicated by historians as early

as 332 BC. At the Siege of Tyre, Alexander the Great predicted victory based on the 'bloody' bread served throughout the camp.[1,4] Since the 'blood' was inside the bread, not on the outside, Alexander's seer predicted that the omen meant the besieged city would bleed from the inside. Tyre was considered an impregnable island fortress off the coast of modern Lebanon, situated about one half mile off the mainland, surrounded by 18 feet deep water, and with walls 150 feet high. Alexander's army started to build a 200 yard wide land bridge from the mainland to the island in January of 332 BC. These endeavors took seven long months, but the ensuing struggle of the Tyrians was short-lived. Today, Tyre is still connected to mainland by this manmade causeway (Figure 1.2, Panel D). [9]

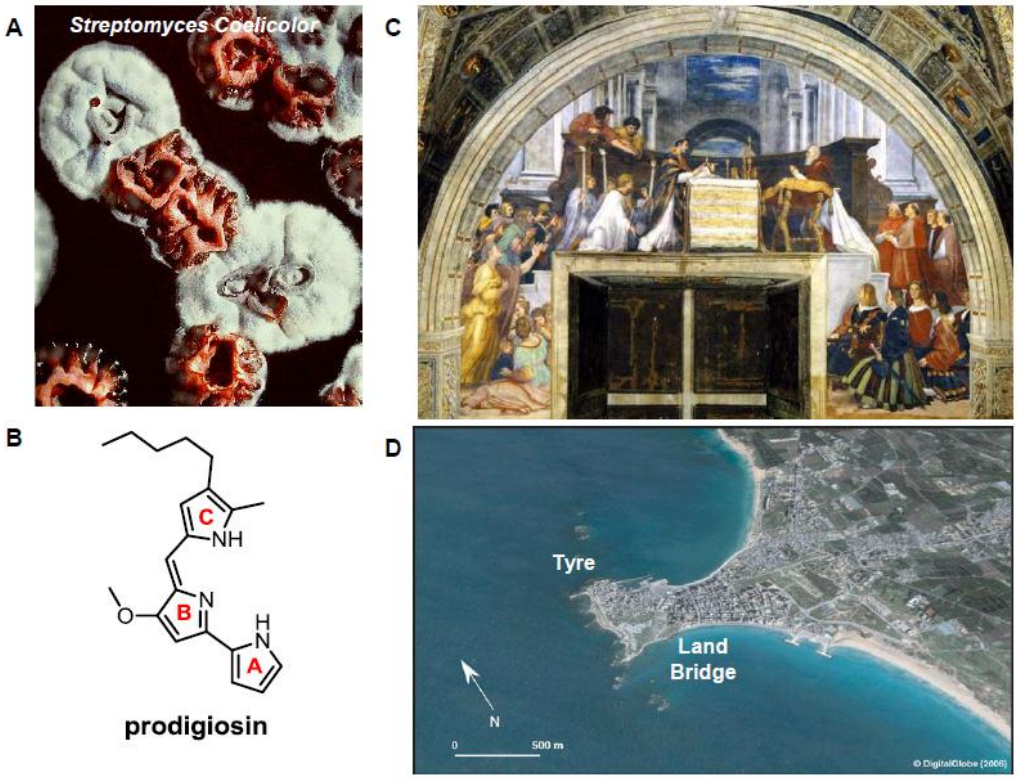


Figure 1.2. Panel A: *Streptomyces coelicolor* producing the blood red prodigiosin pigment. Panel B: Structure of prodigiosin. Panel C: Raphael's "Mass of Bolsena" fresco in the Vatican.[1] Panel D: Present-day Google Earth image of the island of Tyre and Alexander the Great's land bridge that now connects the island to the mainland and completely altered the coastal currents.

While the prodigious prodigiosins produced by bacteria were the subject of miracles and signs from God, until the late 1900s, the true nature of their ability to perform 'miracles' would be unknown. During this time, the biological properties of the prodigiosins and their potential to become life-saving therapeutics would begin to be realized.

1.2. Biosynthetic Origins of Prodigiosin Alkaloids.

In 1960, the structure of prodigiosin was confirmed through chemical synthesis by Rapoport and Holden.[10] Over the next 50 years, biosynthetic studies to identify the gene clusters responsible for prodigiosin production would prove challenging yet rewarding. The first major milestone was the confirmation that the biosynthesis is a bifurcated pathway that combines an alkyl pyrrole (MAP, 2-*M*ethyl-3-n-*A*myl-*P*yrrole in prodigiosin) with the 4-*M*ethoxy-2,2'-*B*ipyrrole-5-*C*arbaldehyde (MBC) through an enzymatic condensation.[11] Feeding studies revealed that proline, serine, acetate, glycine, and *S*-adenosylmethionine were the required components necessary for the biosynthesis of prodiginines.[4,12] Once the genomes of prodigiosin-producing bacteria were sequenced, the gene clusters for pigment production were identified by inactivation and measurement of accumulated biosynthetic intermediates. It became evident that gene clusters responsible for production of MBC were highly conserved throughout the prodiginine-producing bacteria. However, gene clusters responsible for MAP or other alkyl pyrrole formation, were completely different. This difference may be explained by the need to incorporate different starter units to construct slightly different monopyrroles. Undecylprodigiosin biosynthetic cluster components were abbreviated *red*, whereas the

prodigiosin biosynthetic gene clusters of *Serratia* sp. ATCC 39006 and *S. marcescens* ATCC274 were abbreviated *pig*. A summary of our current knowledge of the biosynthetic pathways of prodiginine natural products is described in Figure 1.3.[4]

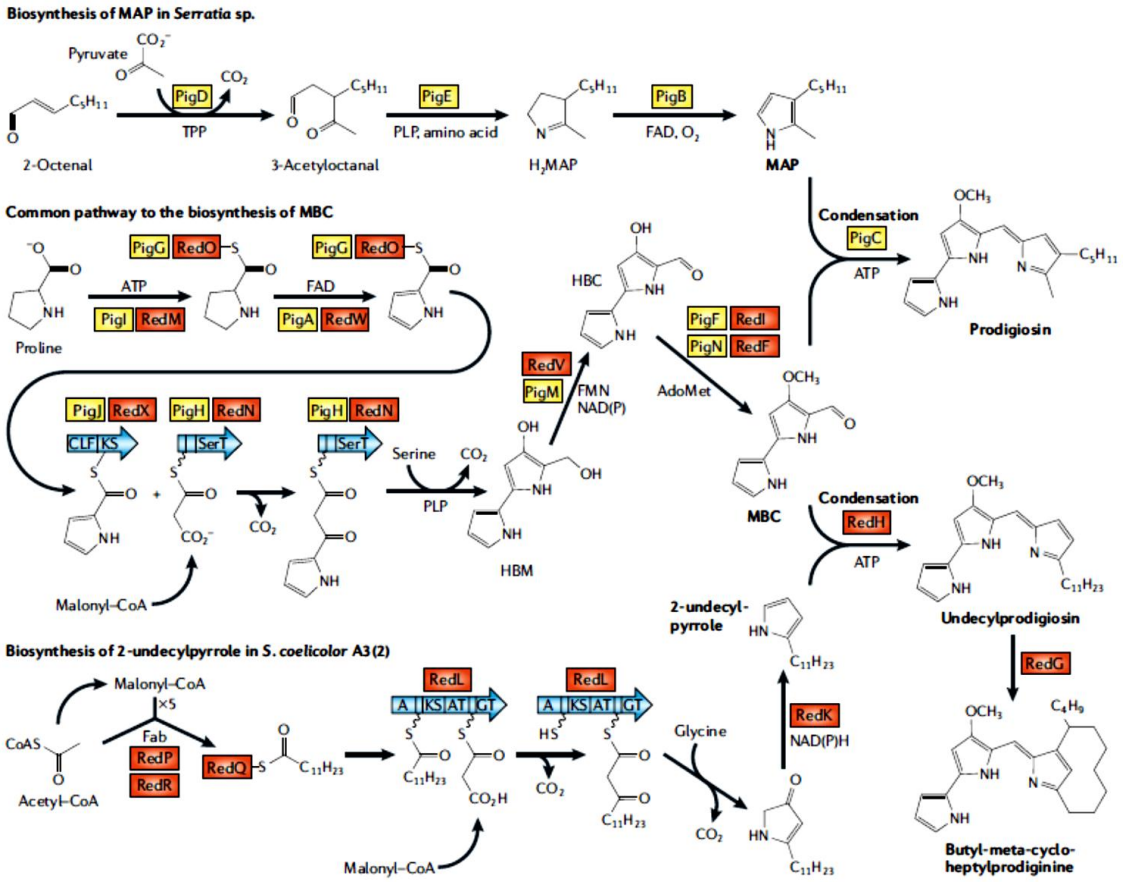
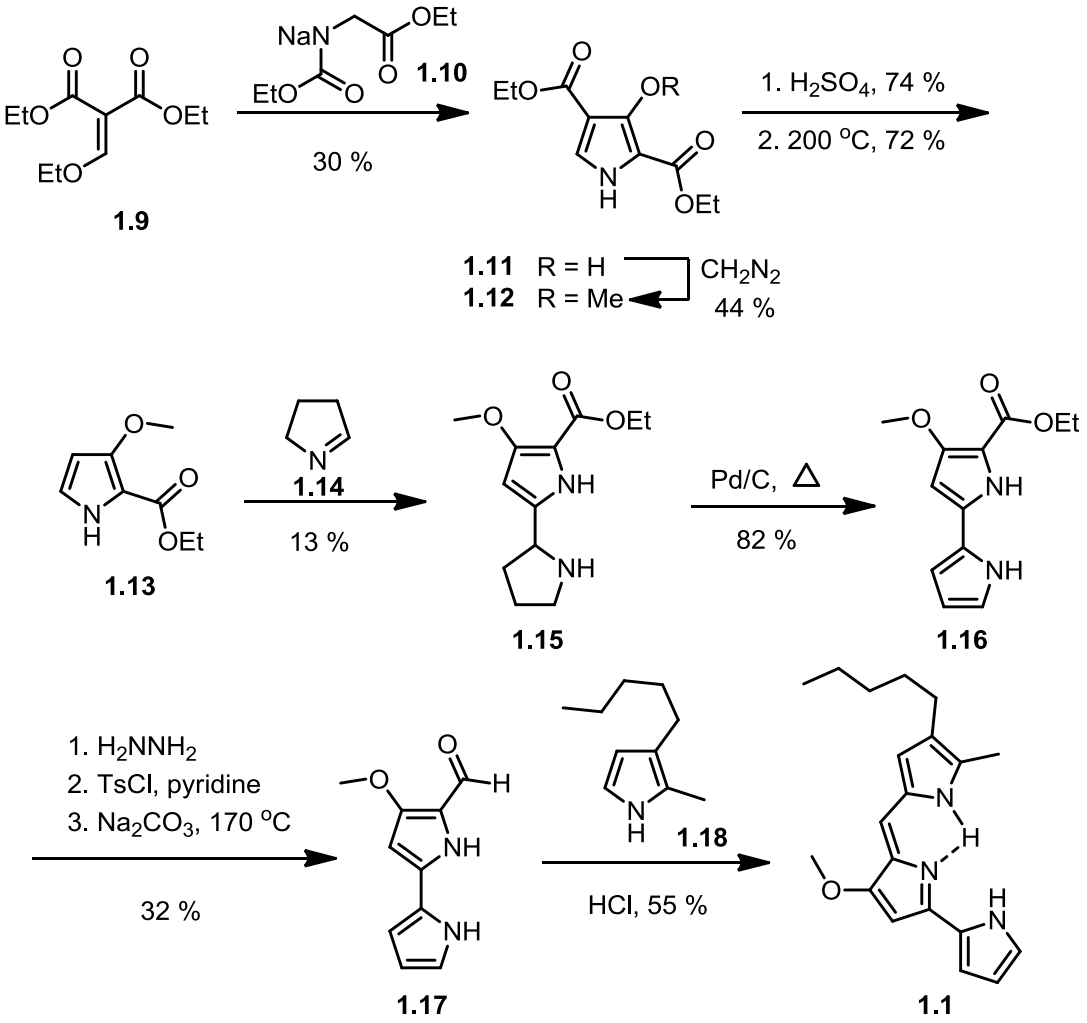


Figure 1.3. Biosynthesis of Prodigiosin, Undecylprodigiosin, and Butyl-*meta*-cycloheptylprodigiosin.[4] (adapted from Williamson 2006).

1.3. Biomimetic Syntheses of Prodigiosin Alkaloids.

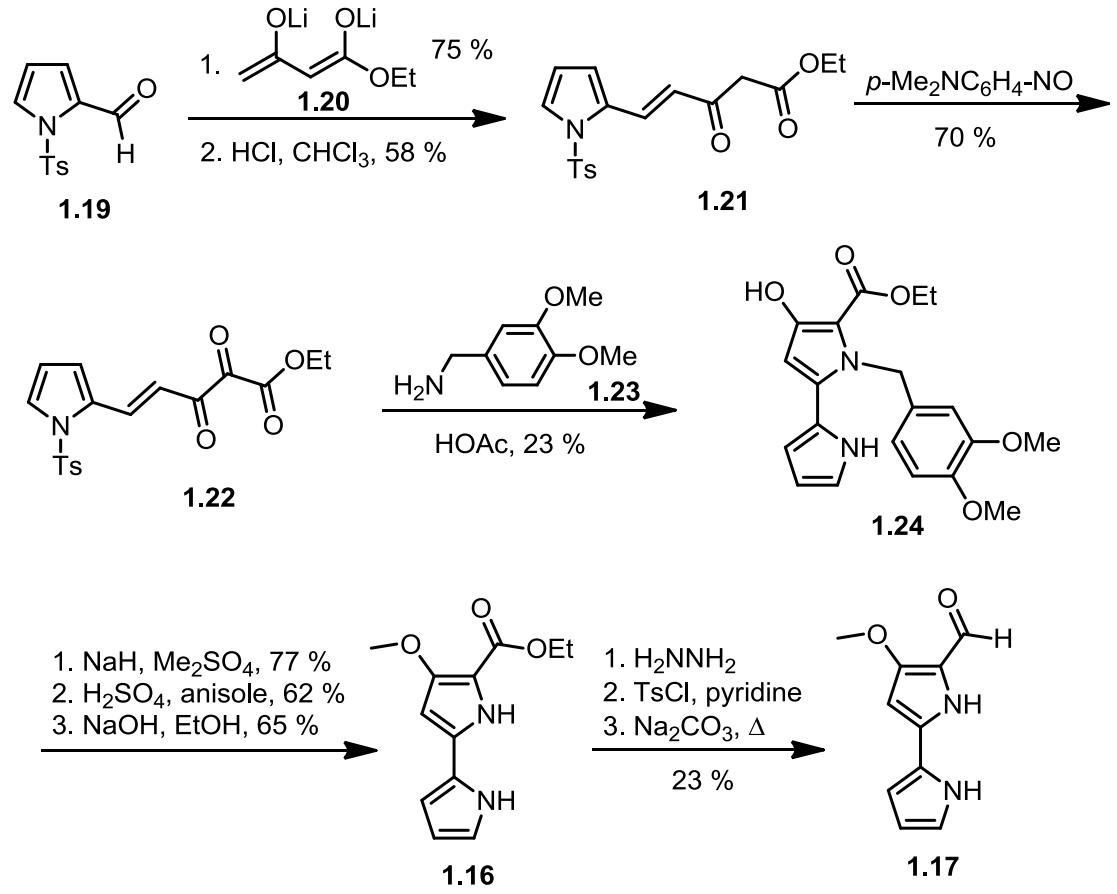
At the time of the first total synthesis of prodigiosin in 1962, the bifurcate pathway had been identified and a biomimetic approach, which involved synthesizing MBC and subsequent condensation with MAP, was the route chosen for the early endeavors in prodiginine chemical synthesis. [4,13]



Scheme 1.1. First total synthesis of prodigiosin **1.1** by Rapoprt and Holden.[10]

The 1962 Rapoport Holden synthesis began with the condensation of the sodium salt of diethyl *N*-ethoxycarbonyl glycinate **1.9** with diethyl ethoxymethylenemalonate **1.10** to give diethyl 3-hydroxypyrrole-2,4-dicarboxylate **1.11**.[10] *O*-alkylation with diazomethane and subsequent selective hydrolysis of the more reactive ester at C4 followed by decarboxylation of the resulting acid afforded pyrrole **1.13** which was condensed with imine **1.14** to provide pyrrole-pyrrolidine **1.15** in very low yield. Dehydrogenation of the pyrrolodine ring was achieved with Pd/C to give ester **1.16**,

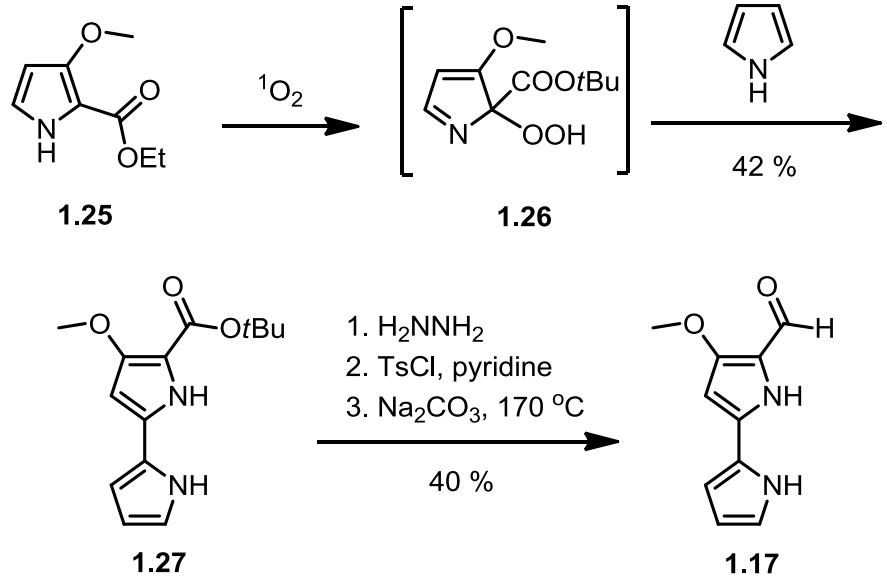
which was transformed to aldehyde **1.17** through McFayden-Stevens reduction. Condensation of aldehyde **1.17** with alkyl pyrrole **1.18** completed the first total synthesis of prodigiosin (**1.1**). This route highlighted the difficulty of pyrrole synthesis and the methods available at the time, namely, condensation reactions and thermal decarboxylations and fragmentations with variable yields. In the last 50 years, subsequent routes to access the tripyrrole prodiginine cores have led to considerable advances in heterocyclic chemistry.



Scheme 1.2. First generation synthesis of MBC **1.17** by Wasserman and co-workers.[14]

Wasserman and co-workers developed a method for the synthesis of MBC **1.17** based on cyclization of a vicinal tricarbonyl intermediate (**1.22**) (Scheme 1.2).[14]

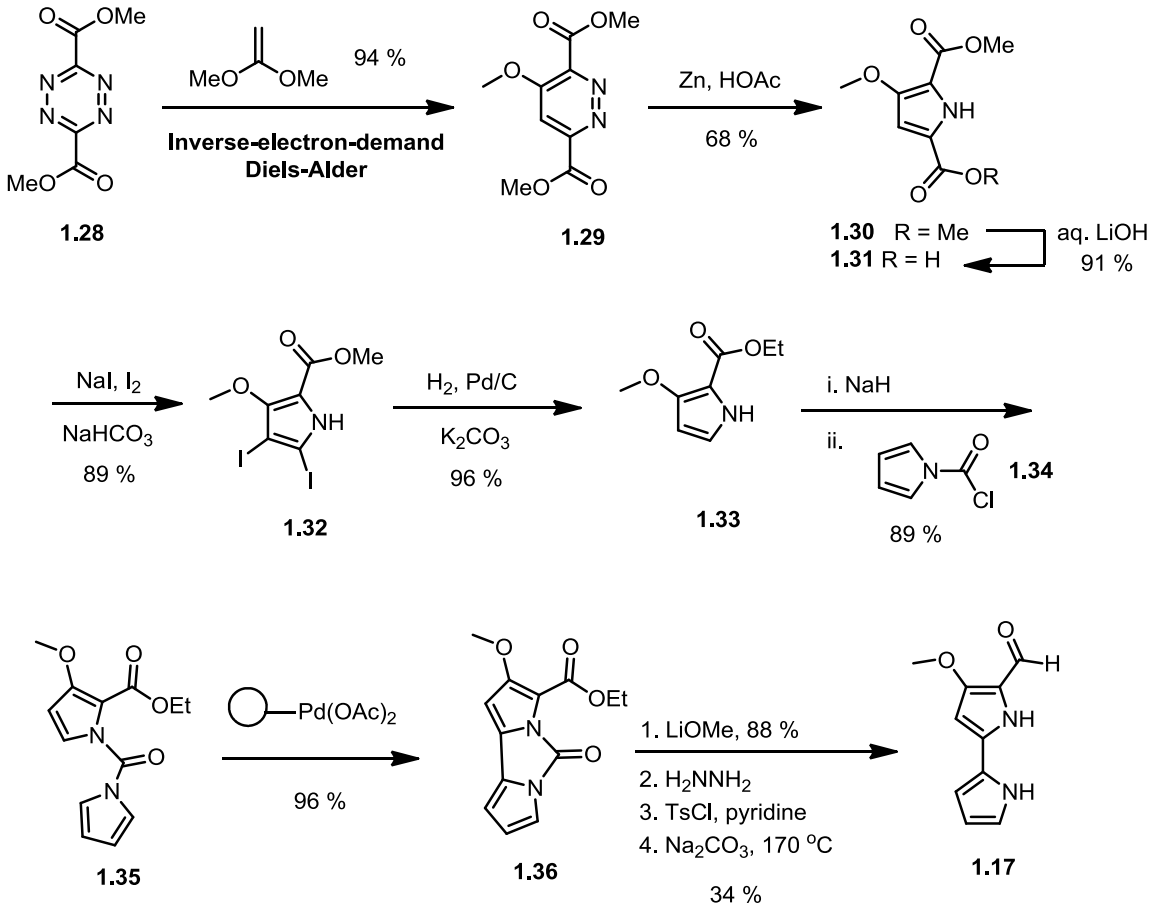
Aldeyhde **1.19** was treated with dianion **1.20**. The most basic, terminal anion reacts with the aldehyde and subsequent acidic work-up quenched the second anion and provided the elimination product of the addition, the β-keto ester **1.21**. Oxidation of the activated methylene with N,N-dimethyl-*p*-nitrosoaniline provided tricarbonyl **1.22**. Reaction with benzylamine **1.23** proceeds through a 1,2-addition to the highly electrophilic center carbonyl and subsequent intramolecular 1,4-addition. Acidic dehydration provides the aromatic bipyrrole **1.24**. O-alkylation and removal of the dimethoxy benzyl protecting group gave ester **1.16**. McFayden-Stevens reduction provided the desired aldehyde **1.17**



Scheme 1.3. Second generation synthesis of MBC **1.17** by Wasserman and co-workers.[15]

In a second-generation synthesis by Wasserman and co-workers, an oxidative method was used to combine the two pyrrole units (Scheme 1.3).[15] Treatment of ester **1.25** with methylene blue and irradiation with a tungsten halogen lamp under an oxygen-rich atmosphere provided peroxide **1.26**. Addition of pyrrole to the reaction mixture traps the intermediate peroxide generating the *t*-butyl ester bipyrrole **1.27**. McFayden-Steven's

reduction afforded aldehyde **1.17**. Both of these routes relied upon the inefficient, McFayden-Stevens reduction, resulting in low overall yields. Nevertheless, Wasserman and co-workers were able to use their routes to achieve the synthesis of prodigiosin, undecylprodigiosin, and metacycloprodigiosin in addition to several unnatural analogues.[15-19]

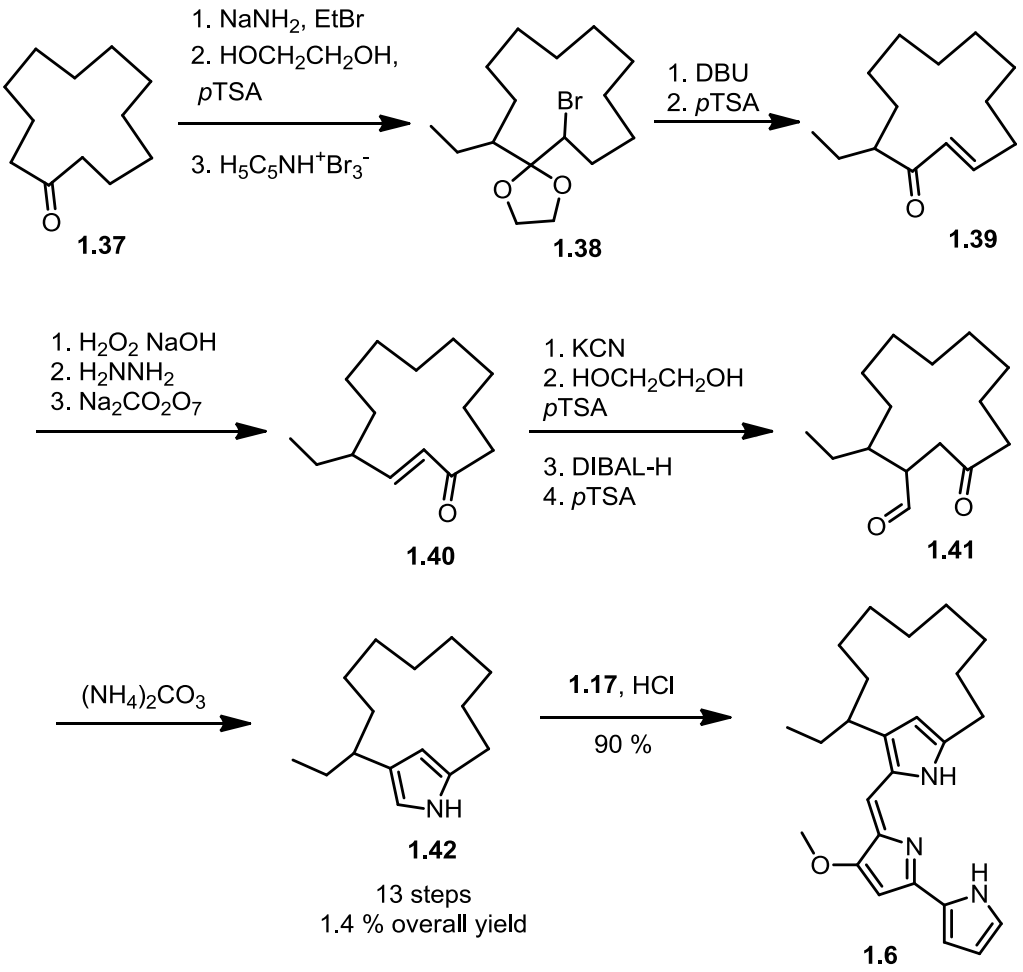


Scheme 1.4. Synthesis of MBC **1.17** by Boger and co-workers featuring a key inverse-electron-demand Diels-Alder reaction.[20]

Boger and co-workers prepared the MBC core through a key inverse-electron-demand Diels-Alder reaction between 1,2,4,5 tetrazine **30** and 1,1-dimethoxy ethane to give the resulting pyridazine **1.29** in excellent yield (94%) (Scheme 1.4).[20] Ring contraction with zinc dust and acetic acid provided the 2,5-dimethylcarboxylate pyrrole

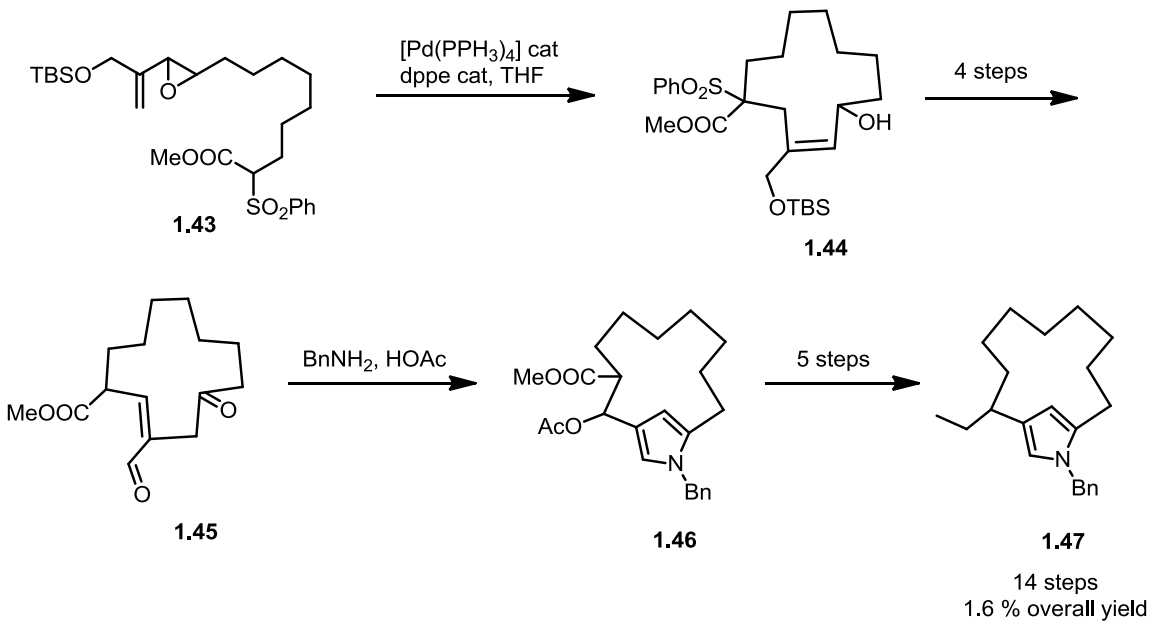
1.30 in good yield. Hydrolysis of the more sterically-accessible and electrophilic C5 ester and iodine-mediated decarboxylation provided the diiodo intermediate **1.32**. Hydrogenolysis provided pyrrole **1.33** and acylation gave urea **1.35**, which was subjected to oxidative coupling with stoichiometric polymer-supported palladium acetate. Hydrolysis of the urea tether and subsequent conventional, yet low-yielding, McFayden-Stevens reduction provided MBC **1.17** in 34 % yield. Boger and co-workers used the MBC **1.17** in various condensation reactions to probe the structure-activity relationship (SAR) of this class of compounds and the parameters responsible for cytotoxic and antibacterial activities.

While routes to achieve the total synthesis of prodigiosin heavily focused on the generation of MBC **1.17**, the synthesis of variously substituted alkyl mono-pyrroles, especially the cyclic pyrrole congeners, proved a considerable synthetic challenge. Wasserman and co-workers were the first to access the strained macrocyclic pyrrolophanes for the total synthesis of metacycloprodigiosin (Scheme 1.5).[19] Cyclododecanone was transformed into the brominated derivative **1.38** through routine manipulations. Elimination and deprotection provided enone, **1.39**, which then underwent several reaction to achieve transposition and provide enone **1.40**. Conjugate addition of cyanide anion to transposed enone **1.40**, protection of the carbonyl, reduction of the cyano moiety to the aldehyde, and subsequent deprotection provided 1,4-dicarbonyl **1.41**. Paal-Knorr condensation completed the synthesis of the desired 12-membered pyrrolophane macrocycle (**1.42**) in 1.4 % yield over 13 steps. Biomimetic condensation with MBC **1.17** provided metacycloprodigiosin (**1.6**).



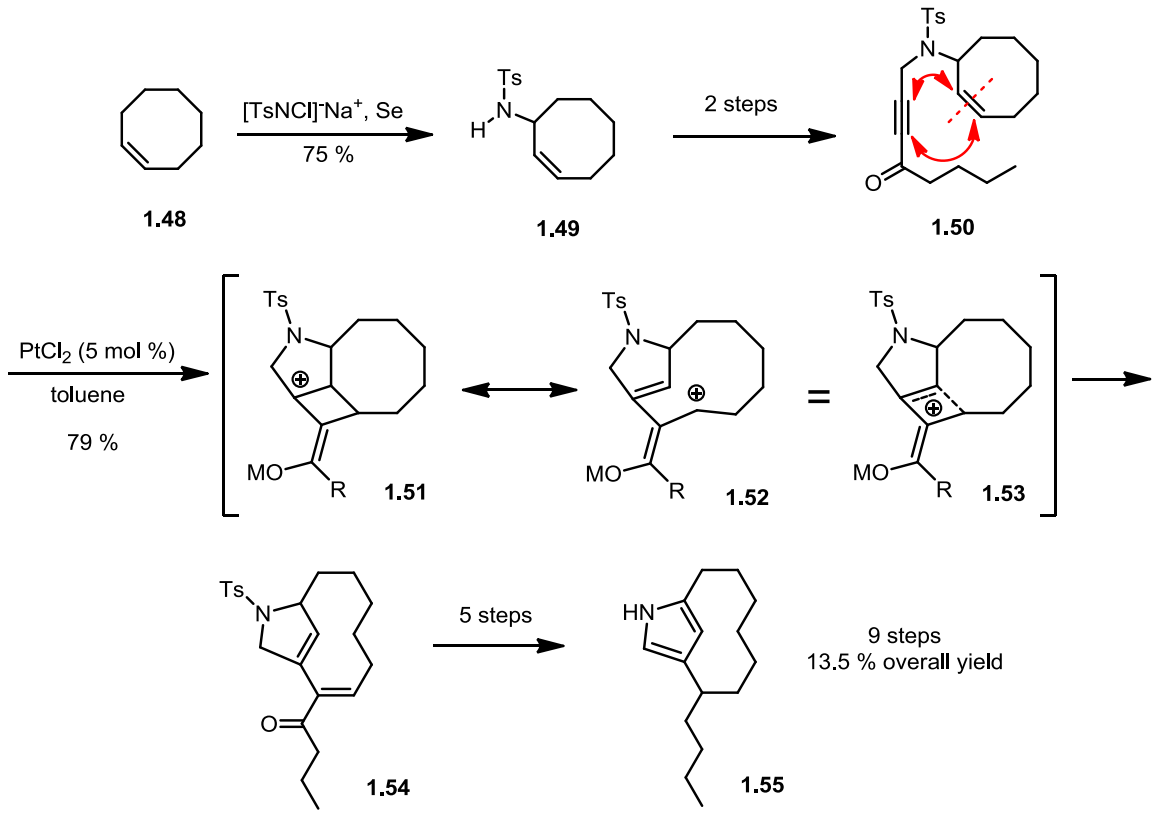
Scheme 1.5. Synthesis of metacycloprodigiosin by Wasserman and co-workers.[19]

Fürstner and co-workers achieved the synthesis of the 12-memebered pyrrolophane core of metacycloprodigiosin by utilizing an intramolecular Tsuji-Trost reaction of the vinyl epoxide **1.43** to provide the highly-functionalized 12-membered ring (**1.44**) (Scheme 1.6).[21] Functional group manipulations and base-induced elimination of the sulfone provided 1,4-dicarbonyl **1.45**. Paal-Knorr condensation with benzyl amine gave the protected pyrrole **(1.46**). Additional steps led to the formation of the benzyl-protected pyrrolophane moiety in 1.6 % yield over 14 steps.



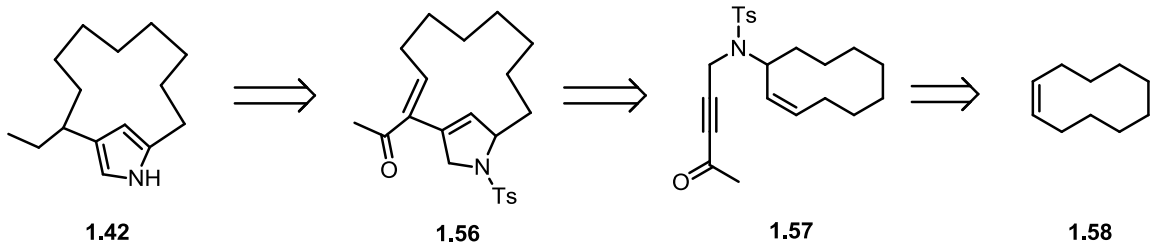
Scheme 1.6. Synthesis of the metacycloprodigiosin pyrrolophane core by Fürstner and co-workers.[21]

The Paal-Knorr condensation has proven a very effective method of generating the alkyl-substituted pyrrolophane cores of prodigiosin alkaloids. However, in Fürstner and co-workers' synthesis of streptorubin B, a novel ring expansion reaction was utilized to generate the 10-membered ring pyrrolophane core (**1.55**) (Scheme 1.7).[22] Allylic amination of cyclooctene **1.48** provided amine **1.49**, which underwent alkylation with propargyl bromide and subsequent acylation of the acetylide with butanoyl chloride to provide enyne **1.50**. The ring expansion reaction is a *formal* "enyne metathesis" reaction catalyzed by platinum (II) chloride that is hypothesized to occur through a nonclassical carbocation intermediate (**1.51-1.53**), based on observed byproducts, to yield macrocycle **1.54**. Subsequent transformations provided the alkyl pyrrole component (**1.55**) of streptorubin B (**1.7**) in 9 steps from cyclooctene and 13.5 % overall yield.



Scheme 1.7. Fürstner and co-workers' 'Enyne metathesis" route to prepare the C-ring pyrrole of streptorubin B (**1.7**). [22]

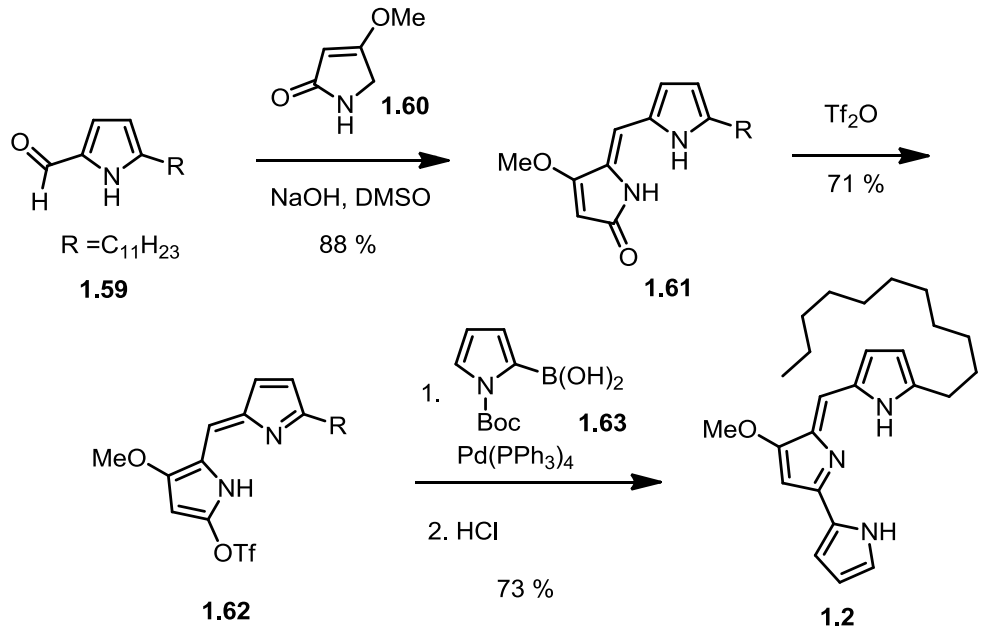
This ring expansion reaction also proved highly successful at accessing the 12-membered pyrrolophane core of metacycloprodigiosin (**1.6**) from cyclodecene in 9 steps and 3.4 % yield (Scheme 1.8). [22]



Scheme 1.8. Synthesis of the C-ring pyrrole of metacycloprodigiosin (**1.6**) by Fürstner and co-workers utilizing their developed ring-expansion reaction.[22]

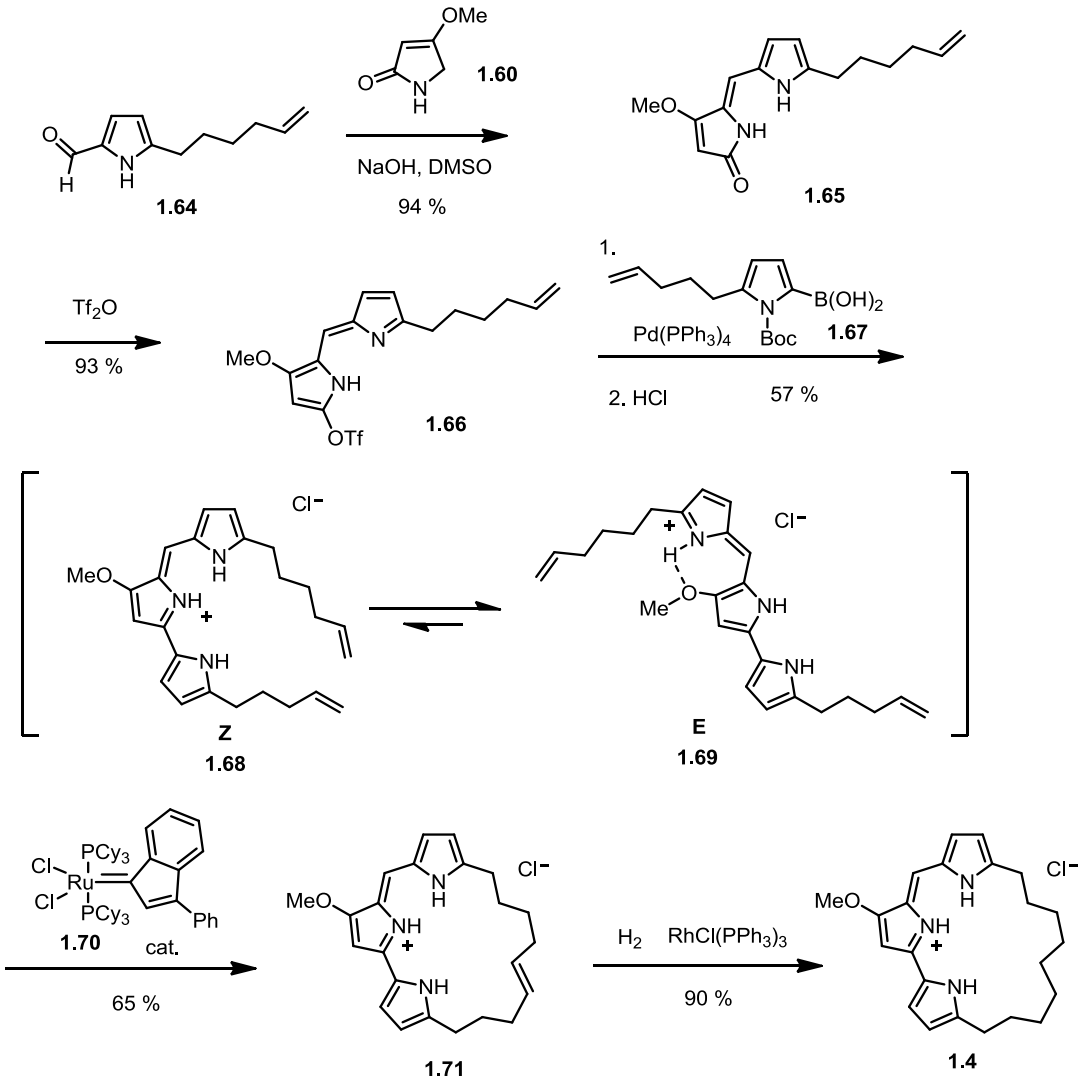
1.4. Cross-Coupling Reactions in the Synthesis of Prodigiosin Alkaloids.

D'Alessio and co-workers were the first to move away from the biomimetic condensation of MBC **1.17** and an alkyl pyrrole. Instead of combining the A and B rings to form MBC **1.17** and incorporating the C-ring in the condensation reaction, D'Alessio and co-workers condense the B and C ring and incorporate the A ring through a Suzuki cross-coupling reaction (Scheme 1.9).[23,24] Aldehyde **1.59** underwent base-mediated condensation with commercially available pyrrolinone **1.60** to give enelactam **1.61**. Treatment with triflic anhydride provided triflate **1.62**, which was used in a Suzuki cross-coupling reaction with 1-Boc-pyrrole-2-boronic acid (**1.60**) to provide undecylprodigiosin (**1.2**) in 3 steps and 45.6 % yield from aldehyde **1.59**. This great improvement yield and the requirement for significantly fewer steps highlights the importance and impact of the development of cross-coupling reactions as a means of forming C-C bonds, especially in heterocyclic, aromatic systems.



Scheme 1.9. D'Alessio *et al.* total synthesis of undecylprodigiosin utilizing a key Suzuki cross-coupling reaction for the generation of the bipyrrole moiety.[23,24]

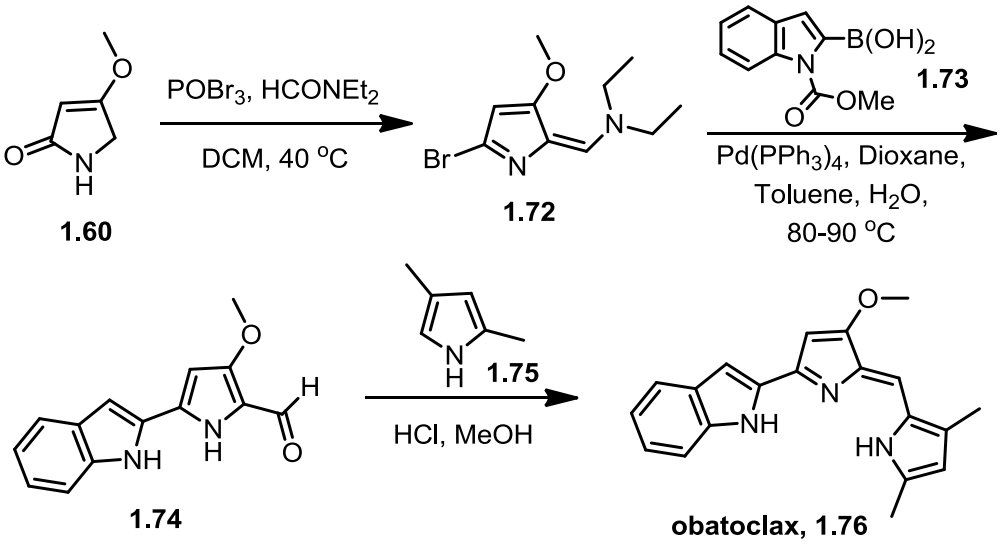
The methods developed by D-Alessio and co-workers were incorporated into the first total synthesis of nonylprodigiosin by Fürstner and co-workers (Scheme 1.10).[25] By employing the basic condensation of formylated alkyl pyrrole **1.64** and pyrrolinone **1.60**, triflate formation, and Suzuki cross-coupling, a mixture of *E* (**1.68**) and *Z* (**1.69**) isomers about the methylene bond was obtained. Ring-closing metathesis (RCM) with ruthenium-based catalyst **1.70** could only occur with the *Z*-isomer (**1.68**). As the *Z*-isomer reacts to form the macrocyclic product, the preequilibrium is constantly shifting to form more of the minor *Z*-isomer in solution, which is subsequently intercepted in the metathesis reaction. Hydrogenation of the presence of Wilkinson's cationic rhodium catalyst completed the first total synthesis of nonylprodigiosin (**1.4**). The convergent nature of this route has enabled the synthesis of many unnatural analogs with heterocyclic variants for SAR studies.[26,27]



Scheme 1.10. Fürstner and co-worker's synthesis of nonylprodigiosin (**1.4**) utilizing a key RCM reaction.[25]

Diari and co-workers developed a highly scalable route for the synthesis of an unnatural prodigiosin analog, obatoclax, for clinical trials (Scheme 1.11).[28] Diari's route is based on D'Alessio's route, which incorporates a key Suzuki cross-coupling reaction.[25] However, Diari's route utilizes the Suzuki reaction to form the C-C bond between the A-ring and B-ring and includes the biomimetic acid-mediated condensation reaction to

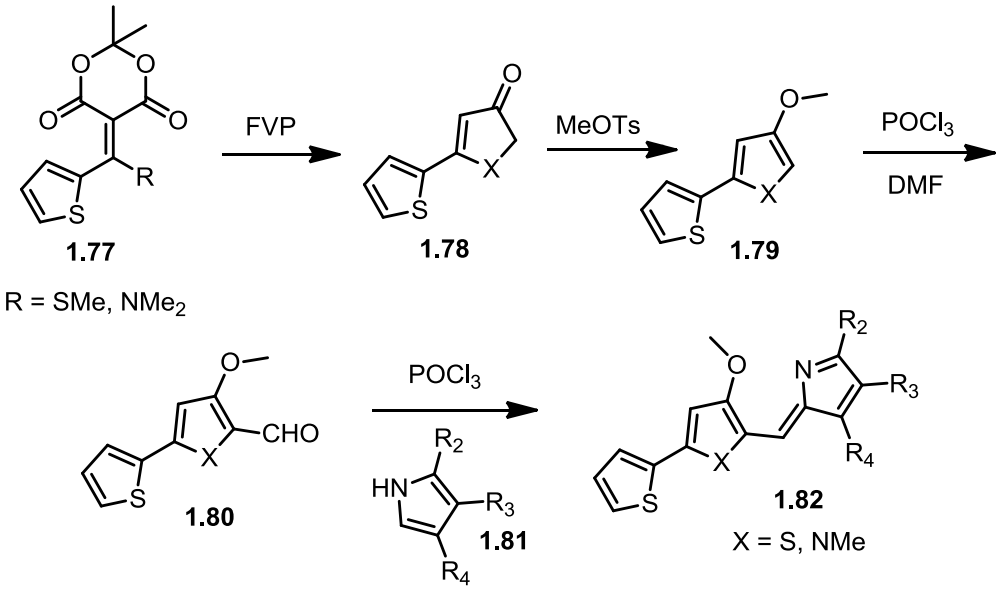
incorporate the C-ring pyrrole. A Vilsmeier-Haack haloformylation is utilized to generate bromoenamine **1.72**, and subsequent Suzuki cross-coupling with the N-protected indole boronic acid **1.73** provides the MBC analog **1.74**. Acidic condensation with 2,4-dimethylpyrrole (**1.75**) completes the synthesis of obatoclax (**1.76**) in 3 steps from commercially available starting material.



Scheme 1.11. Diari and co-workers route for the large-scale synthesis of obatoclax (**1.76**) for clinical trials. [28]

Recently, a Meldrum's acid route that allows easy access to prodigiosin analogues with variations at the A, B, and C ring has been developed by McNab and co-workers (Scheme 1.12).[29] Starting from known **1.77** analogs, flash vacuum pyrolysis (FVP) is employed to generate ketone **1.78**, which was immediately methylated to provide methyl ether **1.79**. Vilsmeier formylation conditions were utilized to provide the aldehyde **1.80**. Phosphoryl chloride catalyzed condensation of aldehyde **1.80** with 2-unsubstituted pyrrole **1.84** provided prodigiosin unnatural analogs **1.85**. By altering the 3 core rings to incorporate various heterocycles, they hope to develop detailed structure-activity relationships to better understand the prodigiosin bioactivity. The Meldrum's acid route is

unique in the fact that it enables variation at all 3 pyrrole rings, not just at the C-ring, which is available from the condensation routes, or the A-ring, with is available from the cross-coupling routes.

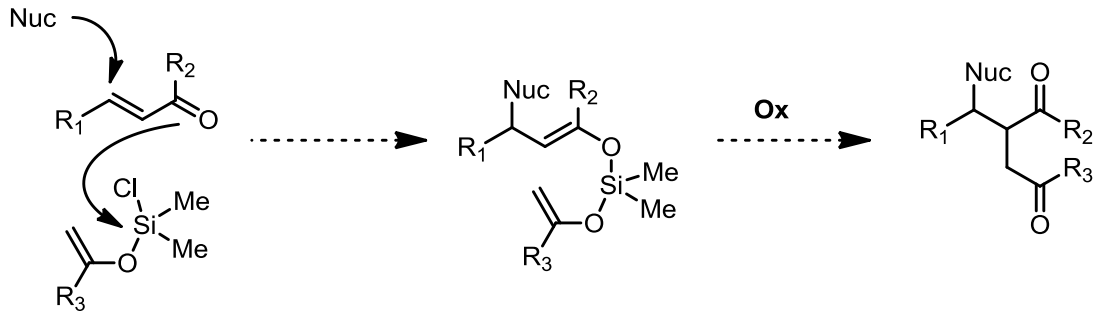


Scheme 1.12 Meldrum's acid route to prodigiosin analogues developed by McNab and co-workers.[29]

1.5. Enantioselective Syntheses of Prodigiosin Alkaloids.

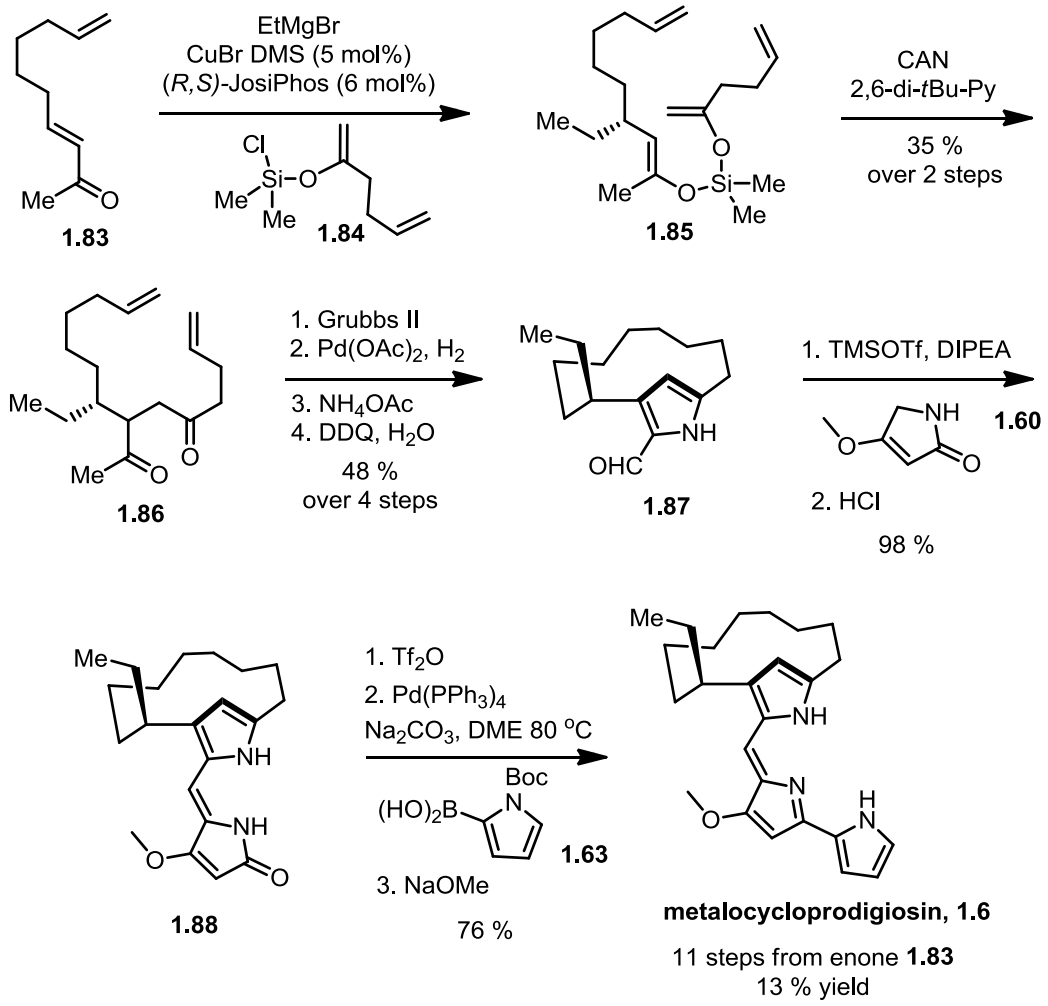
While there have been numerous efforts to achieve the total synthesis of prodigiosin alkaloids, it was not until 2009 that the first enantioselective total syntheses of a tripyrrole prodiginine natural product wwas achieved by Thomson and co-workers.[30]

Thomson and Clift utilized a stereoselective, merged 1,4-addition/oxidative coupling process to achieve the total synthesis of metalocycloprodigiosin (**1.6**) and the recently isolated prodigiosin R1 (**1.8**) (Scheme 1.13).[3]

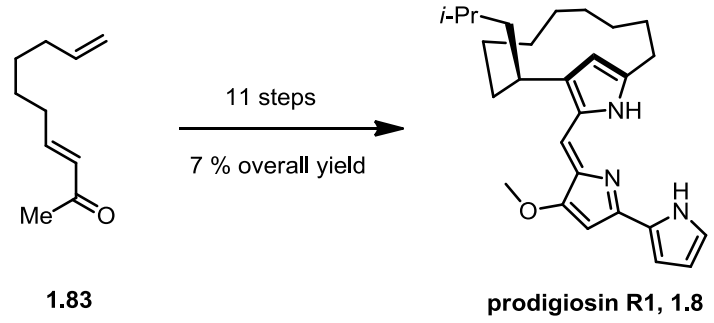


Scheme 1.13. Merged 1,4 addition/oxidative coupling for the synthesis of metacycloprodigiosin (**1.6**) and prodigiosin R1 (**1.8**) by Thomson and Clift[30]

Using conditions developed by Feringa and co-workers, Thomson and Clift began the synthesis of metalocycloprodigiosin (**1.6**) by regio- and stereoselective copper-catalyzed 1,4-addition of ethylmagnesium bromide to enone **1.83** followed by enolate trapping with chlorosilane **1.84** to give silyl bis-enol ether **1.85** as a mixture of enol isomers (Scheme 1.14). Using conditions developed in Thomson's lab for oxidative bond formation, dione **1.86** was generated in 35% yield over two steps from enone **1.83**. RCM, hydrogenation, and Paal-Knorr condensation provided the chiral, saturated, C-ring pyrrole, and methyl oxidation with DDQ provided aldehyde **1.87**. Aldehyde **1.87** was then utilized in a trimethylsilyltriflate-mediated aldol condensation reaction with pyrrolinone **1.60** to give conjugated lactam **1.88**. Triflation, Suzuki cross-coupling with boronic acid **1.63**, and Boc-deprotection afforded metalocycloprodigiosin (**1.6**) in 11 steps from enone **1.83** and proceeded in 13% overall yield. This enantioselective strategy was also applied to the first total synthesis of prodigiosin R1 (**1.8**) in 11 steps and 7% overall yield (Scheme 1.15).[30] These two syntheses represent the first time that any members of the tripyrrole prodigiosin alkaloids have been prepared in enantioenriched form.

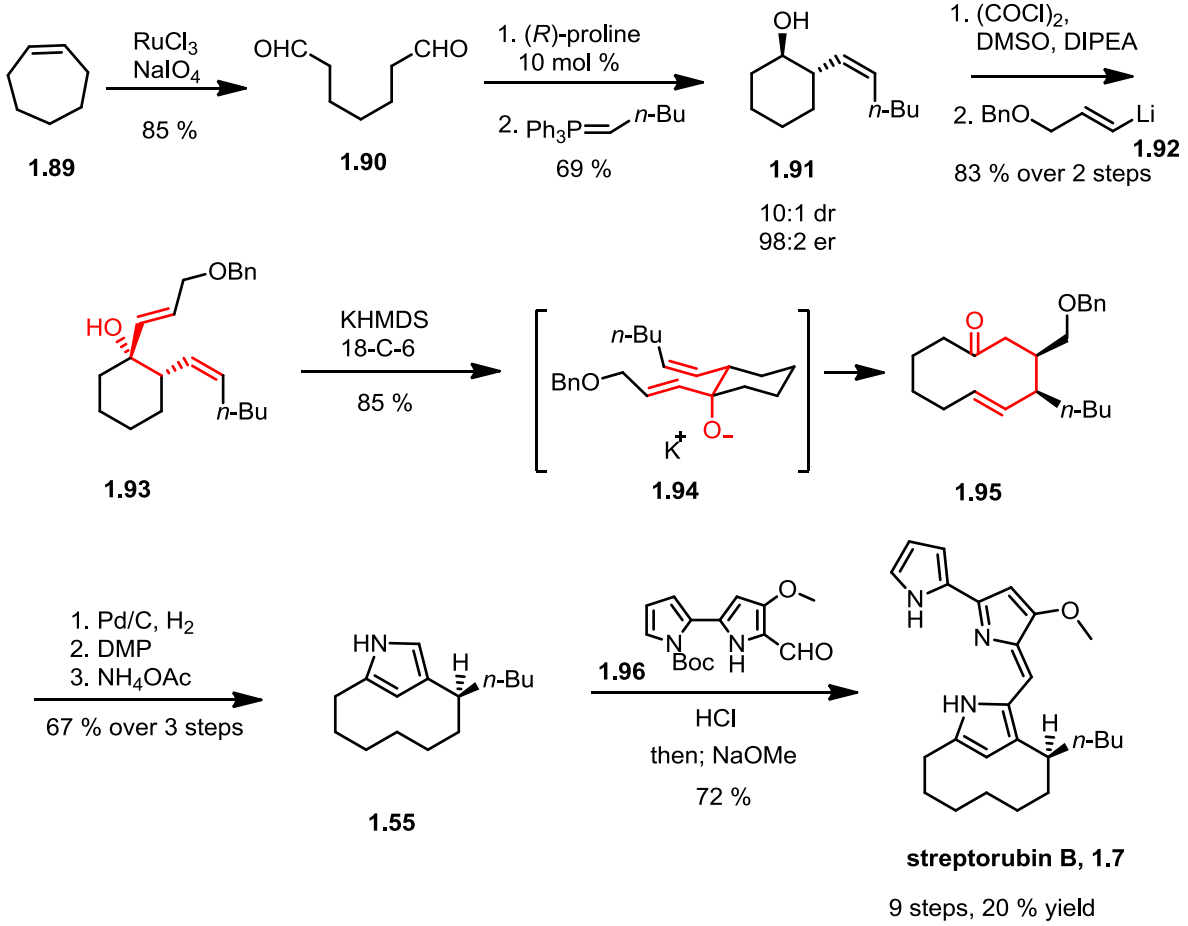


Scheme 1.14. Enantioselective total synthesis of metalocycloprodigiosin (**1.6**) by Thomson and Clift.[30]



Scheme 1.15. Enatioselective total synthesis of prodigiosin R1 (**1.8**) by Thomson and Clift.[30]

In 2011, Thomson and coworkers once again designed an enatioselective route for the preparation of a prodigiosin analogue, streptorubin B (**1.7**) (Scheme 1.16).[31] After attempts to incorporate their previously developed methodology, they found that the RCM used to form the 12-memebred ring of metalocycloprodigiosin (**1.6**) and prodigiosin R1 (**1.8**) was not a viable option for the formation of the 10-membered ring in streptorubin B (**1.7**). In light of this discovery, they investigated alternative routes to achieve the enantioselective synthesis of this highly strained prodigiosin alkaloid. The synthesis began with the oxidative cleavage of cycloheptene **1.89** to form dialdehyde **1.90**, which underwent a one-pot enantioselective aldol/Wittig reaction to provide alcohol **1.91** in 10:1 dr and 98:2 er. Swern oxidation followed by addition of vinyl anion **1.92** gave alcohol **1.93**, the precursor to an anionic oxy-Cope rearrangement in 97:3 er. Exposure of alcohol **1.93** to KHMDS and 18-crown-6 produced the desired 10-memebred ring (**1.95**) in 85% yield. Alkene reduction and concomitant benzyl ether cleavage, oxidation of the liberated alcohol to the aldehyde, and Paal-Knorr condensation provided pyrrole **1.55**. Using chemistry developed in our lab based on the work of Diari and co-workers,[29,33] Thomson and co-workers completed the first enantioselective synthesis of streptorubin B (**1.7**) in 9 steps and 20% overall yield.[31] After comparing the Circular-Dichroism (CD) spectra of the natural and synthetic streptorubin B (**1.7**), it was determined that the *S*-enantiomer is the naturally-occurring stereoisomer, which is unique from the 12-membered pyrrolophane, metalocycloprodigiosin (**1.6**), which was determined to naturally occur as the *R*-enantiomer.



Scheme 1.16. Enantioselective total synthesis of streptorubin B (**1.7**) by Thomson and co-workers, incorporating a tandem aldol/Wittig sequence and a key anionic oxy-Cope rearrangement.[31]

The evolution of synthetic, heterocyclic chemistry is clearly evident in the preparation prodigiosin alkaloids. From the condensations and decarboxylations of Rapoport and Holden in 1960, to RCM, Suzuki cross-coupling, and enantioselective 1,4 conjugate additions of the twenty-first century; alkaloid synthesis has come a long way.

1.6. Biological Activity of Prodigiosin Alkaloids and Unnatural Analogs.

The interest in prodiginine natural products is not simply due to the synthetic challenges offered by the unique structures. This class of natural products exhibits many interesting biological activities, from immunosuppression, to anticancer activity, to antimicrobial and antimalarial activity.[1,4] In many cases, these activities occur at nanomolar concentrations with a considerable therapeutic window.

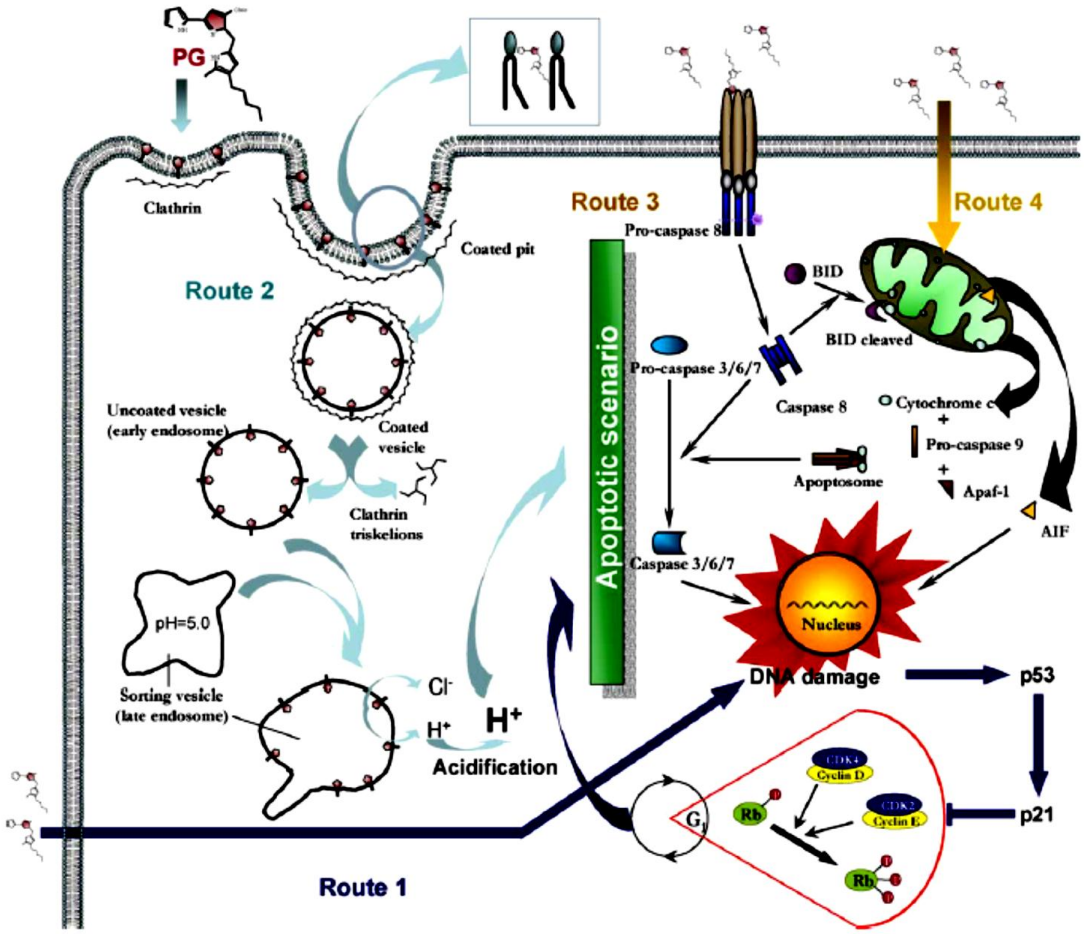


Figure 1.4. Numerous actions of the prodigiosin by different pathways. The prodigiosin alkaloids have been shown to facilitate an apoptotic scenario by 4 alternative routes.[33](adapted from Pérez-Tomás 2003).

The prodigiosin alkaloids have been implicated to exert biological activities through numerous possible mechanisms of action in various cell types and disease states (Figure 1.4).[33] Prodigiosin alkaloids possess considerable hydrophobic components and are highly unstable in aqueous solutions. These compounds may diffuse rapidly through membranes and interact with DNA with a preference for the AT sites at the minor groove promoting copper-mediated dsDNA cleavage. Cells respond to this DNA damage by activating cell-cycle arrest, DNA repair, or triggering apoptosis (Route 1).[34-36] Prodiginine compounds may be incorporated into the lipid bilayer of the plasmatic membrane, where by endocytosis they reach the endosome compartment and uncouple vacuolar H^+-ATPase (V-ATPase) through promotion of the H^+/ Cl^- symporter. This causes neutralization of acidic compartments within the cells, thus inducing intracellular acidification and triggering autophagy-induced cell death and eventually apoptotic cell death (Route 2). [37-40] A prodiginine compound may activate an unidentified prodigiosin receptor or a known death receptor, inducing caspase 8 activation and consequently, apoptosis (Route 3). Prodigiosin alkaloids may diffuse freely through membranes and interact with the mitochondrial outer membrane, uncoupling F_o-F_1-ATPase and triggering apoptosis (Route 4).[41,42] The pathway followed by a particular prodiginine compound would depend very much on the cell type studied, the drug concentration within the cell, the uncharacterized hierarchy of the prodiginine targets, and the interaction between distinct pathways.[33]

In human neuroblastoma cell lines prodigiosin (**1.1**) acts as a proton sequestering agent that destroys the intracellular pH gradient, and it has been proposed that the main cytotoxic effect could be related to action on mitochondria, where it exerts an uncoupling

effect on the electronic chain transport of protons to mitochondrial ATP synthase.[43]

Nonylprodigiosin-HCl has been shown to inhibit apoptosis in PC12-RasN17 cells through Ras and the activation of the Ras-PI3K-Akt pathway.[44] Acidification of cytoplasm, DNA damage, suppression of NF-kappa beta, and sustained JNK activity could all activate Ras and initiate a protective effect in this cell line; however, to date, this is the first example of prodigiosins exhibiting an antiapoptotitc effect in any cancer cell line.

Prodigiosins have also been shown to uncouple lysosomal vacuolar-type ATPase through promotion of H^+/ Cl^- symport. Prodigiosin (**1.1**), metacycloprodigiosin (**1.6**), and undecylprodigiosin (**1.2**) all raise intralysosomal pH through inhibition of lysosomal acidification driven by vacuolar (V-)-ATPase without inhibiting ATP hydrolysis in a dose-dependent manner with IC_{50} values of 30-120 pmol/mg of protein.[45]

Prodigiosin (**1.1**) has also demonstrated induction of p21, a negative cell-cycle regulator, in a p-53 independent manner as prodigiosin induced p21 in MCF-7 cells with both mutated and dominant negative p53.[46] The p53 protein is mutated in most human cancers and this mutation prevents cancer cells from suffering the cytostatic and/or cytotoxic effects of anticancer drugs.[47] Since prodigiosin (**1.1**) does not require the presence of this protein to exert its effect on cancer cells, this could make prodiginine compounds a promising lead for treatment of p53-mutant cancer cell lines.

In vitro, prodiginine alkaloids have been shown to intercalate DNA with a preference for the AT sites in the minor groove.[34] Prodiginine compounds display oxidative copper-promoted dsDNA-cleavage and the superior copper-nuclease activity correlates to superior anticancer properties in leukemia (HL-60) cell line. Through SAR

studies, it has been found that a nitrogen-containing A-ring and the B-ring methoxy substituent are required for potent cytotoxicity.[20,23,48,49]

X-ray crystallography has revealed that prodigiosin binds to copper in a 1:1 Cu (I) complex (**1.98**) and that all 3 nitrogens coordinate to the metal atom (Scheme 1.5).[48] The C-pyrrole ring is oxidized and contains an OH group attached to C1 to generate an sp^3-hybridized carbon atom and a new double bond between N1 and C4. Coordination with Zn forms a 1:2 $Zn(I)_2$ complex (**1.97**) where prodigiosin has not undergone oxidation. Also, the A-ring does not participate in Zn (II) binding. These results indicate that all 3 nitrogens of the tripyrrole core of the prodigiosins is required for oxidative dsDNA cleavage.

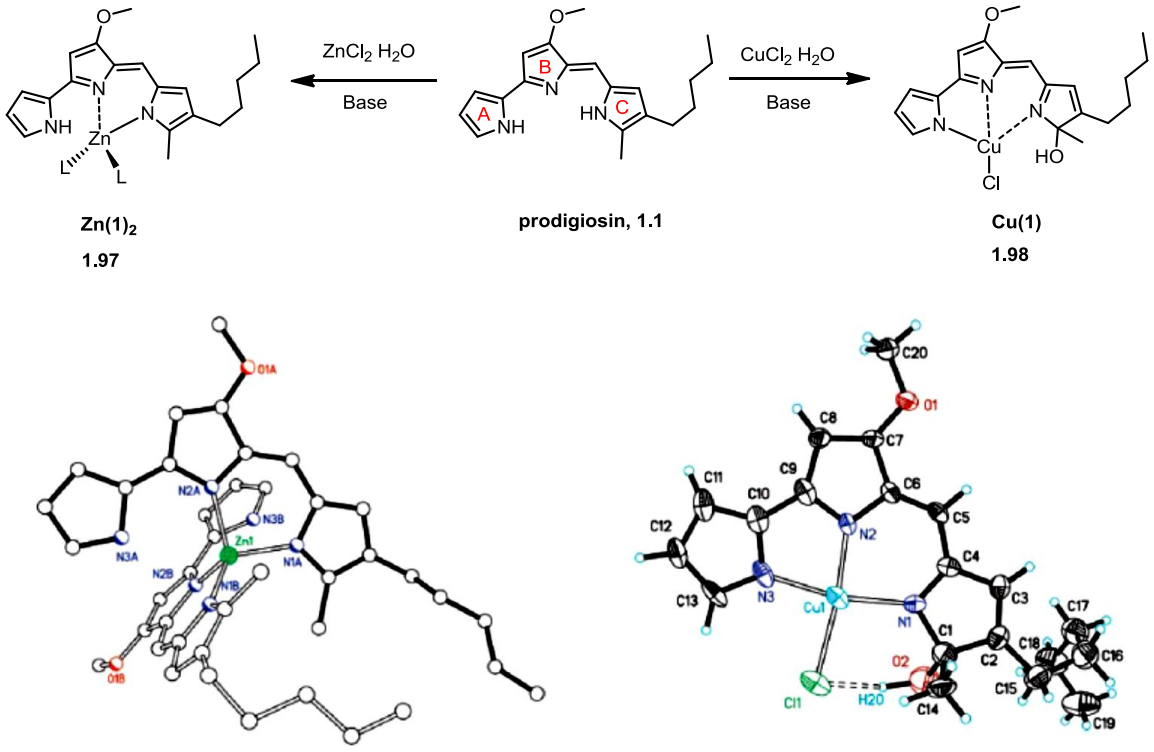


Figure 1.5. Crystal structures of prodigiosin (**1.1**) bound to zinc as a dimeric species (left) and copper as a monomeric species (right).[48]

Replacement of the A-ring with substituted indoles and unnatural alkyl pyrrole substituents has produced new antiproliferative prodigiosin analogs.[49] However, substitution of the A-ring with thiophene, furan, or phenyl decreases nuclease activity and abolishes anticancer activity. One of the most important and successful unnatural prodiginine alkaloids is a synthetic compound named obatoclax (**1.76**), which was developed by GeminX Pharmaceuticals and was described as a BH3 mimetic drug.[50] However, the molecular target of the majority of prodiginine derivatives remains unidentified and the pathways involved in observed biological activities uncharacterized.

In 2012, Pérez-Tomás, R. and co-workers discovered a new molecular target for prodigiosin (**1.1**) and obatoclax (**1.76**), the mammalian target of rapamycin (mTOR) (Figure 1.6).[51] mTOR is an evolutionarily conserved serine/threonine protein kinase which is constituted of two signaling complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Both complexes have specific effects on distinct cellular functions, such as controlling mRNA translation, ribosome biogenesis, autophagy, and metabolism.[52-54] It was discovered that the two prodiginine alkaloids inhibit both mTORC1 and mTORC2 and thus counteract S6K-1/IRS-1 negative feedback loop in melanoma.[51] Melanoma is notoriously resistant to cytotoxic reagents, and the development of this resistance has been related to the presence of different feedback loops that link both P13K/AKT/mTOR and mitogen activated protein kinase (MAPK) pathways. These pathways are critical to melanoma progression and both are deregulated in melanoma, but not in normal cells. [55,56]

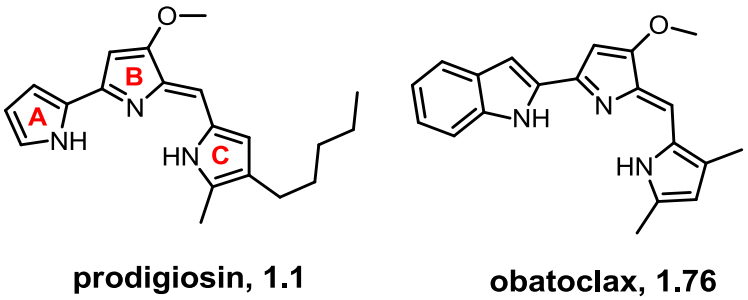


Figure 1.6. Structures of prodigiosin (**1.1**) and obatoclax (**1.76**).

Pérez-Tomás, R. and co-workers discovered that prodigiosin (**1.1**) and obatoclax (**1.76**) both induced activation of autophagic-dependent and apoptotic cell death and that induction of autophagy actually occurred before apoptosis.[51] Kinetics and affinity evaluation revealed high-affinity binding between mTOR and the two prodigiosin alkaloids. *In silico* modeling was performed to identify key interactions between each of the prodiginine compounds and mTOR. In the induced fit prodigiosin-mTOR complex, the hydrophonic environment around the pentyl side chain includes Ile2500, Ile2559, and Val2504 (Figure 1.7). Stacking interactions were observed between His2340 and PG pyrrole rings. The "H-bond ring" created by the alcohol chain of Ser2342 and the two extreme pyrrole nitrogens in the A-ring and C-ring appeared to be a key interaction. Obatoclax-mTOR complex has a key H-bond interaction between the C-ring pyrrole and a glutamine residue (Figure 1.7). The indole moiety appears to be involved in hydrophobic interactions between I2559 and I2500. The lack of a key H-bonding interaction between the indole ring and residues within the mTOR binding site is surprising since it has been shown that a nitrogen containing A-ring is necessary for activity.[20,23,48,49] The role of the B-ring in mTOR binding is not evident from the *in* silico models. Clearly, prodiginine research could benefit from biological testing of additional

analog libraries to elucidate the molecular mechanisms and targets of these interesting molecules. With both of these compounds in Phase I/II clinical trials, it would be highly desirable to generate compounds with known mechanisms of action and improved chemical properties to facilitate bioavailability and the development of oral formulations.[51]

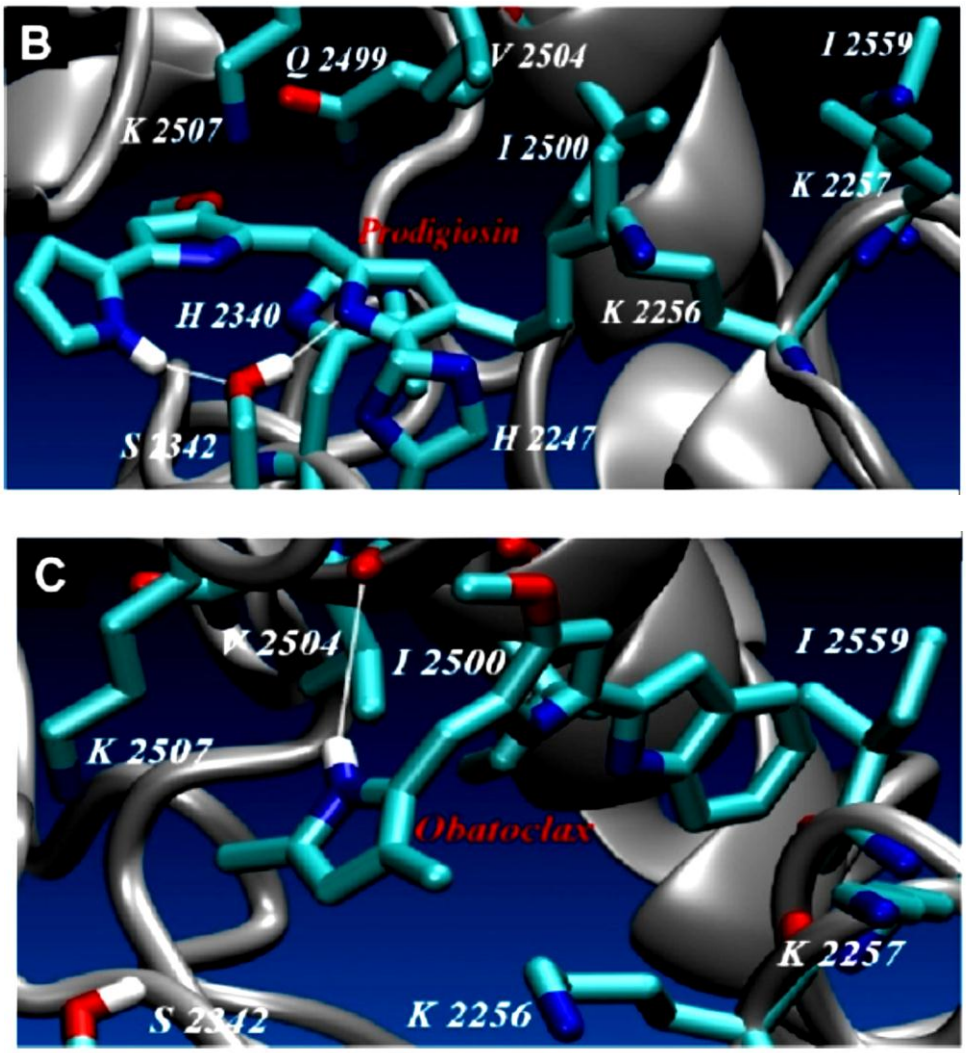
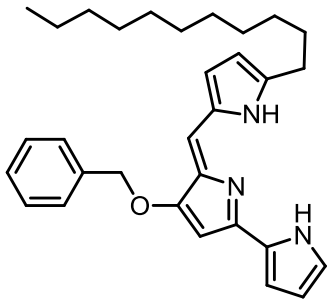


Figure 1.7. *In silico* induced fit models of prodigiosin-mTOR complex (top) and obatoclax-mTOR complex (bottom).[51] (adapted from Pérez-Tomás 2012).

The anticancer activity of the prodigiosin alkaloids alone makes them intriguing synthetic targets; however, the immunosuppressive properties of prodiginine compounds

have been receiving a large amount of attention due to the need of compounds with this activity and novel mechanisms of action. Cyclosporin A (CsA), FK 506, and rapamycin are currently the immunosuppressive drugs utilized to prevent allograft rejection in organ and tissue transplants and to treat autoimmune diseases.[2,57] Prodigisin alkaloids have been shown to suppress cytotoxic T-cells without affecting B cell-mediated immune functions, which is unique from the traditional immunosuppressants.[57] In another sturdy, co-treatment with prodigiosin and CsA in mice that have undergone bone marrow transplants provided a more effective treatment than either drug dosed alone. Prodigiosin **(1.1**) inhibited only IL-2Rα expression, but not IL-2 expression; whereas CsA inhibited both.[58] Exogenously added IL-2 reversed the suppressive activity of CsA, but not that of prodigiosin (**1.1**). These results indicated that prodigiosin **(1.1**) and CsA have similar inhibitory potencies but different modes of action that warrant additional investigation. It is of great interest to generate prodiginine analogs that 'dial out' anticancer activity and increase immunosuppressant activity in order to investigate their potential to treat autoimmune diseases and to prevent allograft rejection. D'Alessio and coworkers identified PNU-156804, a compound which prevented heart allograft rejection in mice in a dose-dependent fashion (Figure 1.8).[23]



1.99 (PNU-156804)

Figure 1.8. Structure of PNU-156804 (**1.99**) synthesized by D'Alessio and co-workers.[23]

The ability to uncouple the anticancer and immunosuppressive activity of these compounds is highly desirable in the search for clinically relevant drug candidates. In the last decade, numerous diseases have been identified to result from immune system deregulation, such as lupus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, Psoriasis, and Diabetes mellitus type 1.[59] These diseases are included in a class known as autoimmune diseases, and could potentially be treated by selective immunosuppressant agents.

If immunosuppression and anticancer activity are able to be optimized or 'dialed-out', then one could also imagine optimizing the observed antimalarial activity over other biological activities to obtain effective therapeutics for eradication of the *Plasmodium falciparum* parasite. While synthetic prodigiosin analogs have shown potent antimalarial activity, dose-limiting toxicity is still a problem and additional compounds will need to synthesized and further SAR developed.[61] Reynolds and co-workers have synthesized extensive libraries of unnatural prodigiosin analogs and have found that the A-ring pyrrole is critical for antimalarial activity, as has been observed in numerous other SAR studies of various biological activities. Variation of the C-ring by incorporating hydrophobic groups of various size and length proved integral to activity enhancement *in vitro* and in mouse models. [60]

1.7. Summary.

The prodigiosin alkaloids possess a rich history, and have been the focus of numerous synthetic endeavors.[10-31] These natural products are produced by various bacteria species, and have been shown to possess antibacterial, antimalarial, anticancer,

and immunosuppressant activities.[33-60] The interesting and numerous biological activities of the prodiginine alkaloids are the main reason these natural products have been the subject of numerous synthetic endeavors and SAR studies over the past 50 years and will continue to intrigue chemists and biologists for many years to come.

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CHAPTER 2

PROGRESS TOWARD THE TOTAL SYNTHESIS OF MARINEOSINS A & B

2.1. Isolation and Structure Elucidation of Marineosins A & B.

Marineosins A and B (**2.1** and **2.2**) were isolated by Fenical and co-workers in 2008 from cultures of a marine sediment-derived actinomycete related to the genus *Streptomyces*.[1] The key structural features common to the marineosins are the spiroiminal center, two pyrrole moieties, and a 12-membered macrocyclic ring (Figure 2.1).

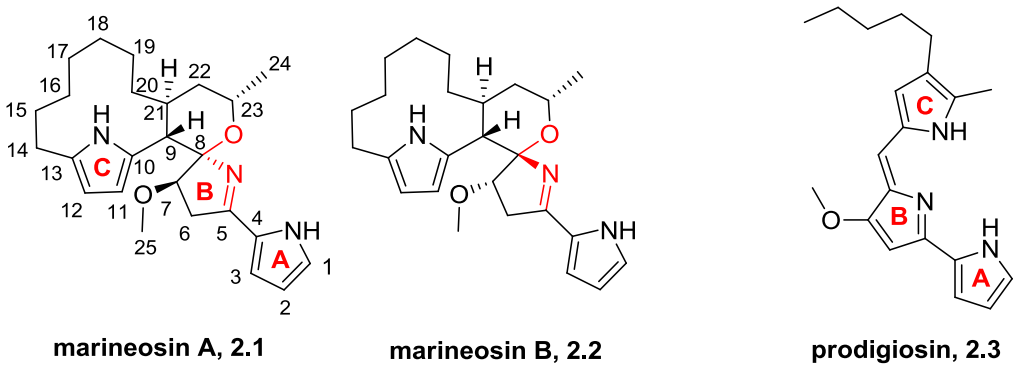
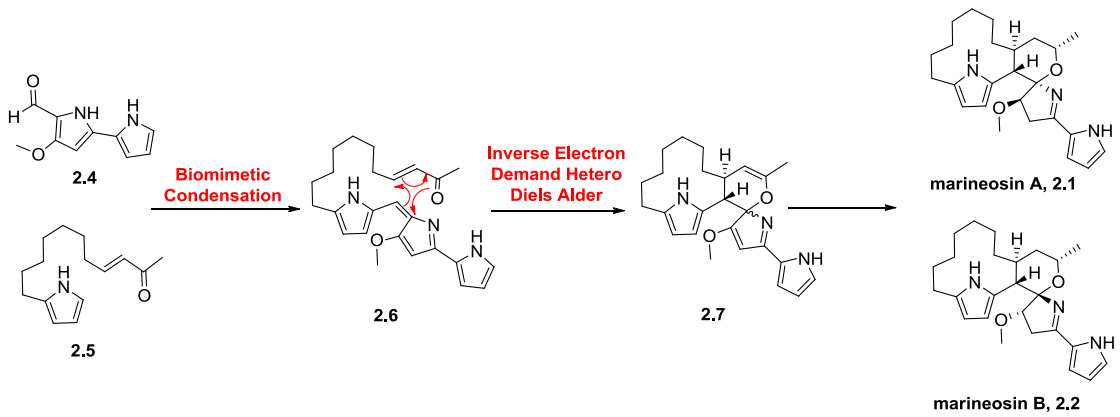


Figure 2.1. Structures of marineosins A & B (**2.1** & **2.2**) and prodigiosin **2.3**.[1]

The marineosins appear to be derived from unknown modifications of prodigiosin-like bacterial pigment pathways. A biosynthetic route suggested by Fenical and co-workers (Figure 2.2),[1] begins with the condensation of **2.4**, the known primary precursor bipyrrole aldehyde (MBC) of the prodigiosin alkaloids, with a novel 2-keto-undec-3-enylpyrrole **2.5** to give **2.6**. Enone **2.6** could then undergo an intramolecular, inverse-electron-demand hetero-Diels-Alder cyclization to form the tetrahydropyran ring and spiroiminal simultaneously.



Scheme 2.1. Possible biosynthesis of **2.1** and **2.2** via an inverse-electron-demand hetero-Diels-Alder cyclization.

2.2. Biological Activity of Marineosins A & B.

Following isolation and structure elucidation through 1D and 2D NMR techniques, the marineosins were tested for their ability to arrest cell division in the HCT-116 human colon tumor cell line.[1] Marineosin A exhibited an IC_{50} value of 0.5 μM in the HCT-116 cytotoxicity assay. In contrast, marineosin B showed considerably weaker cytotoxicity with an IC_{50} value of 46 μM. Testing of marineosin A in the National Cancer Institute (NCI) 60 cell line panel showed considerable selectivity against melanoma and leukemia cell lines. Due to the unprecedented spiroiminal core and the potential of marineosin A as an anticancer therapeutic, these new members of the prodiginine family of natural products are intriguing synthetic targets.

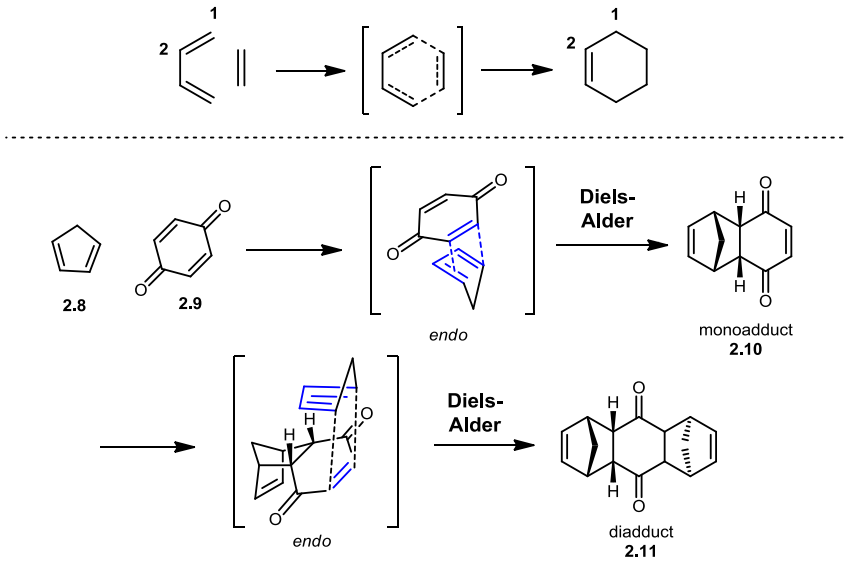
By synthesizing marineosin analogs and performing biological assays for anticancer activity, immunosuppression, and antimalarial activity, we hope to identify the minimal pharmacophore necessary for each of these activities, to prepare simplified analogs based on the knowledge of the key structural feature required for activity, and to optimize compounds that display one activity preferentially over the other activities.

Once we have obtained these selective compounds, gene-expression profiling can be performed to reveal candidate pathways involved in the observed activities and RNAi modifier studies can be utilized to identify which proteins in a signaling pathway are putative targets for our compounds. We can also perform affinity chromatography experiments to identify protein targets and determine specificity. This knowledge would prove invaluable to prodiginine research, as well as cancer, immunology, and malaria research.

2.3. The Diels-Alder Reaction in Organic Synthesis.

2.3.1. Discovery of the Diels-Alder Reaction

In 1928 Otto Diels and Kurt Alder published their landmark paper describing the [4+2] cycloaddition of cyclopentadiene and quinone (Scheme 2.2).[2] After several key theoretical breakthroughs, such as Alder's *endo* rule and Woodward's prediction of regiochemistry based on the electronic nature of diene and dienophile sbstituents, the synthetic utility of this reaction, which effectively couples a conjugated diene and an olefin (dienophile), became evident.[3-5] The ability to generate complex cyclohexene-containing products with excellent regio- and stereoselectivity at multiple stereocenters makes this reaction one of the most valuable tools available to synthetic chemists.



Scheme 2.2. The Diels-Alder reaction is a concerted [4+2] pericyclic reaction. Otto Diels and Kurt Alder discovered the reaction when they identified the products formed from reaction of cyclopentadiene (**2.8**) and quinone (**2.9**) in 1928. They received the Nobel Prize in chemistry in 1950 for this reaction.[2,3]

2.3.2 Theoretical Framework for Regio- and Stereochemical Outcomes of the Diels-Alder Reaction.

The Diels-Alder reaction is a pericyclic [4+2] reaction which is characterized by a cyclic transition state where three π bonds are broken concomitantly forming two σ bonds and a π bond in a single concerted step (Scheme 2.2). Frontier molecular orbital theory (FMO) and symmetry considerations predict that the key orbital interactions in the Diels-Alder reaction are the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO) (Figure 2.2).[6,7] Decreasing the energy gap between the HOMO and LUMO orbitals will cause the reactivity of the system to increase. Orbital coefficients derived from FMO theory predict the regiochemistry of the reaction, which can be exploited by incorporating electron-donating and electron-withdrawing substituents into the reactants.[7,8] Incorporation of these substituents changes the energy of the system and creates geometric asymmetries in the key frontier molecular orbitals, which affects the

synchronicity of bond formation in the transition state, thus affecting the regio- and stereoselectivity of the reaction.[9] The favored transition state will be the one that arises from complementing orbital coefficients of the diene-dienophile pair, which leads to the formation of pseudo-*ortho* substituted products and precludes the formation of possible pseudo-*meta* substituted products with C1 substituted dienes. By the same reasoning, C2 substituted dienes will preferentially form pseudo-*para* adducts over the corresponding pseudo-*meta* adducts.[8,9]

Formation of the kinetically favored *endo* transition state is rationalized by invoking secondary orbital bonding interactions between the diene and dienophile to form the *syn* product (Figure 2.2).[3] The thermodynamically favored *anti* product, obtained from the *exo* transition state, may also be isolated in some systems when steric interactions become increasingly important. However, by incorporating suitable conjugating substituents into the dienophile, the *endo* transition state is considerably more energetically favorable.[3,8]

The Diels-Alder reaction is classified by the flow of electrons. A normal-electron-demand Diels-Alder reaction is characterized by a reaction between an electron-rich diene and an electron-poor dienophile, where the HOMO of the diene and the LUMO of the dienophile are the key frontier molecular orbital interactions in this class of pericyclic reaction. The opposite polarization, an electron-rich dienophile and electron-poor diene, is also possible and the reaction is classified as an inverse electron-demand Diels-Alder reaction (Figure 2.2).[9,10]

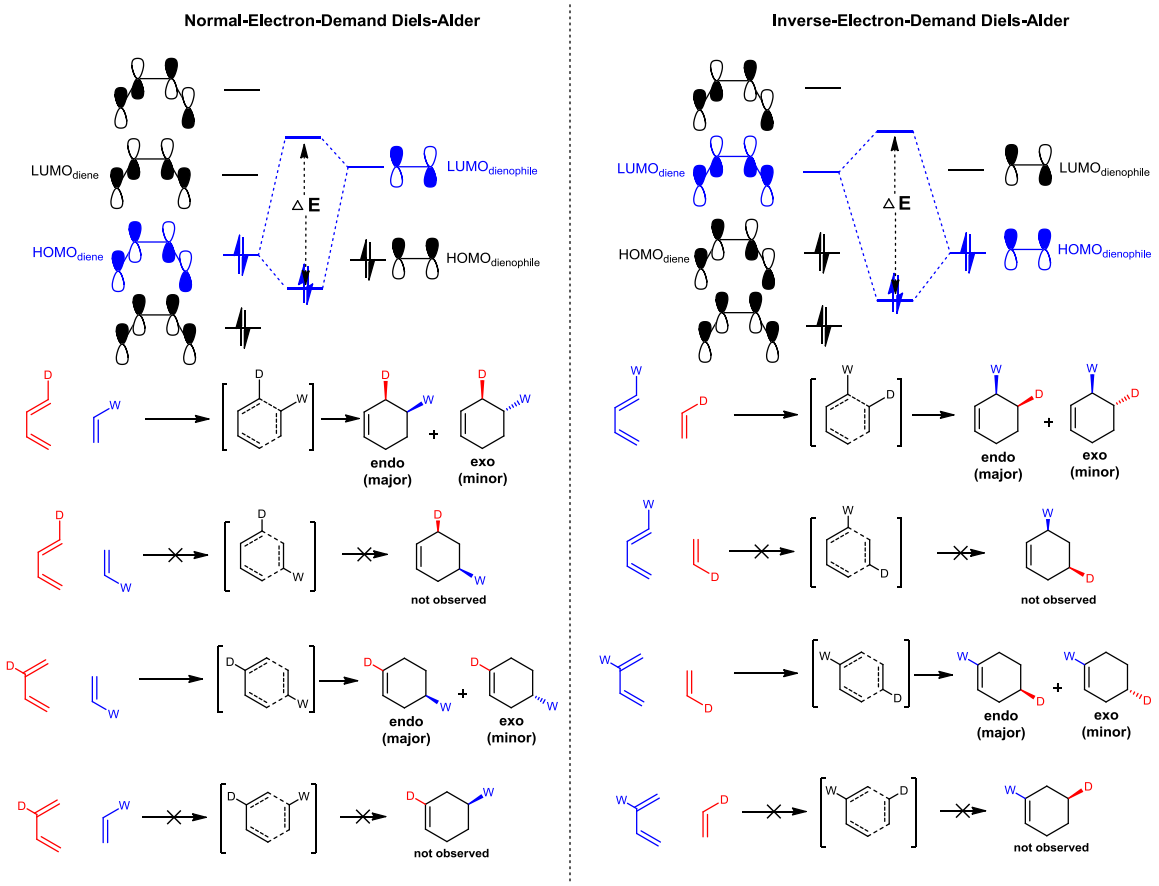


Figure 2.2. Frontier molecular orbitals involved in the normal-electron-demand and inverse-electron demand Diels-Alder reaction. Regio- and stereochemical outcomes of the Diels-Alder reaction with 1- and 2-sunstituted dienes. (D =electron-donating substituent, W = electron-withdrawing substituent).[3, 8-10]

The formation of stereogenic chiral elements is predictable in a relative sense since the Diels-Alder reaction is regioselective, stereospecific and diastereoselective due to the orbital coefficients of the reactants from FMO theory and Alder's *endo* rule.[3] The high level of control and predictability makes the Diels-Alder reaction a powerful transformation in modern organic synthesis; however, the ability to achieve absolute stereocontrol and not just relative control of stereogenic outcomes will continue to be an important topic of future Diels-Alder research. The development of reagent-controlled methods of stereoselectivity could provide access to Diels-Alder adducts that could not

be achieved through substrate-controlled methods and will make the Diels-Alder reaction an even more powerful tool for modern organic synthesis.

2.3.3. The Elusive "Diels-Alderase".

The existence of "Diels-Alderases", enzymes that catalyze Diels-Alder reactions in nature, is a subject of great speculation.[11] There are certainly numerous cases of Diels-Alder retrons in natural products that could indeed arise from enzyme catalysis and have been predicted to involve a Diels-Alder reaction in proposed biosynthetic routes (Figure 2.3).[3,11] However, despite numerous biosynthetic proposals that incorporate the Diels-Alder reaction, the isolation of an enzyme that performs this reaction or identification of a gene cluster with a Diels-Alderase domain has yet to occur.[11]

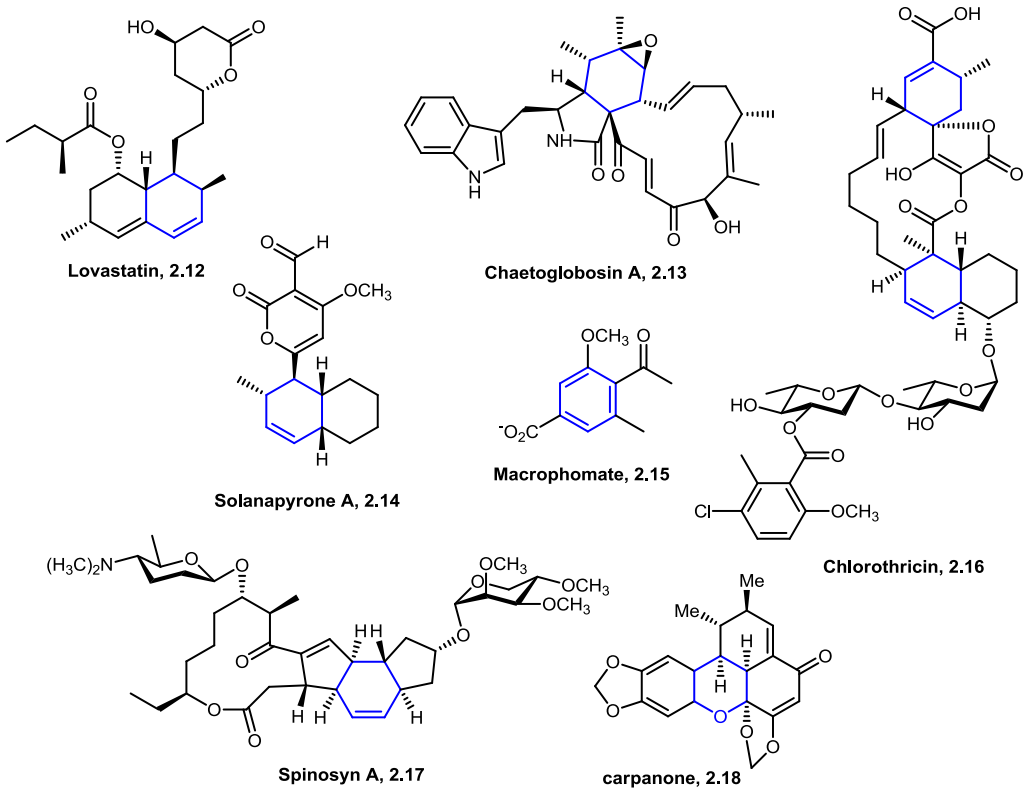
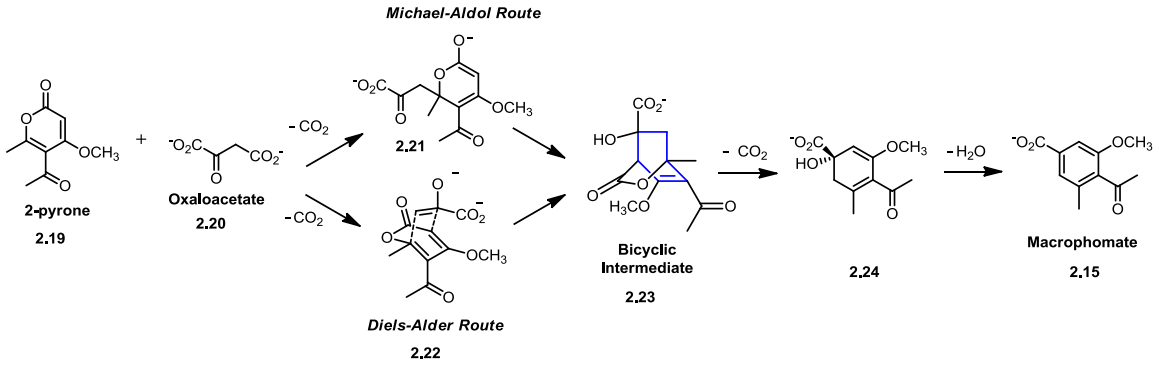


Figure 2.3. Examples of natural metabolites for which biosynthetic Diels-Alder transformations have been proposed. [3,11]

Macrophomate Synthase (MPS) is the most extensively studied of all the putative Diels-Alderases.[11,14,15] However, quantum and molecular mechanics support a stepwise sequence of a Michael-addition followed by an aldol reaction (Scheme 2.3).[16] Solution of the crystal structure of MPS revealed a similar tertiary to 2-dehydro-3-deoxygalactarate (DDG) aldolase, despite weak sequence identity (20%). Both enzymes generate pyruvate enolate in the active site; MPS by decarboxylation of oxaloacetate **2.20** and DDG aldolase by deprotonation of pyruvate.[15,17,18] It was initially proposed that MPS utilizes the pyruvate enolate to bicyclic intermediate **2.23** *en route* to macrophomic acid **2.15** by either an inverse-electron-demand Diels-Alder reaction or a sequential Michael-aldol mechanism (Scheme 2.3).[19,12,14] Hilvert and co-workers demonstrated that, like DDG aldolase, once MPS generates the pyruvate enolate, it efficiently mediates an aldol reaction with a variety of aldehyde substrates.[20] These results certainly support the stepwise Michael-aldol pathway as the most likely mechanism employed by this enzyme.

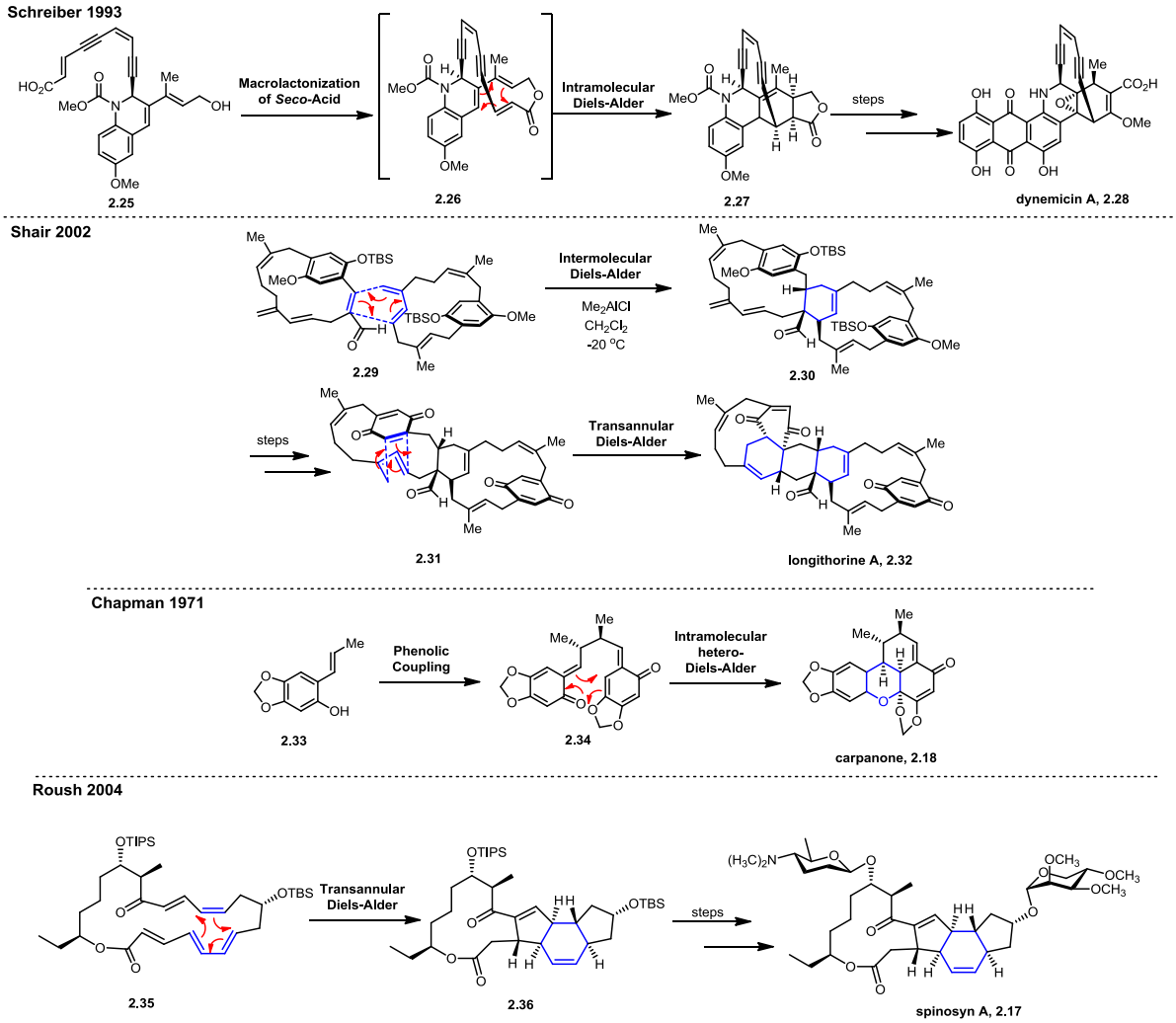


Scheme 2.3. Proposed biosynthetic mechanisms for macrophomate acid synthase: The Michael-Aldol mechanism (top) and the Diels-Alder Mechanism (bottom).[11-14,19] (adapted from Kelly 2008).

2.3.4. The Diels-Alder Reaction in Complex Natural Product Synthesis.

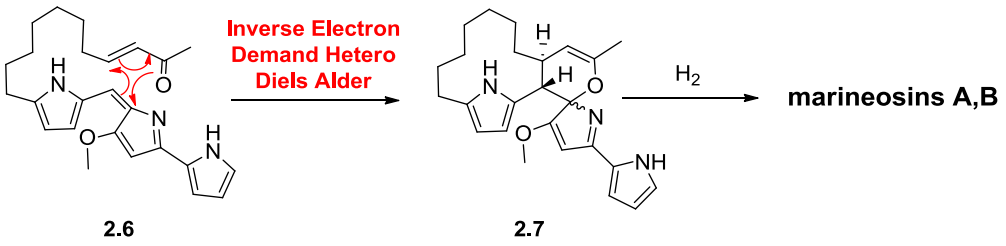
There have been numerous synthetic endeavors that incorporate a 'biomimetic' intramolecular Diels-Alder reaction to impart stereoselectivity and stereospecificity to

highly-substituted cyclic cores.[3,11,21-24] Shown in Scheme 2.4 are a few relevant examples where a Diels-Alder cycloaddition is a key transformation in the synthetic route to access a complex natural product.[3,11,] These transformations are examples of intramolecular Diels-Alder reactions (IMDA) and/or transannular Diels-Alder reactions, common synthetic strategies increasingly associated with the biosynthetic origins of various secondary metabolites.[3,11] Many of these routes exemplify creativity in organic synthesis and have proven highly successful in practice.[21-24]



Scheme 2.4. Incorporation of biomimetic Diels-Alder reactions into the syntheses of complex natural products.[21-24]

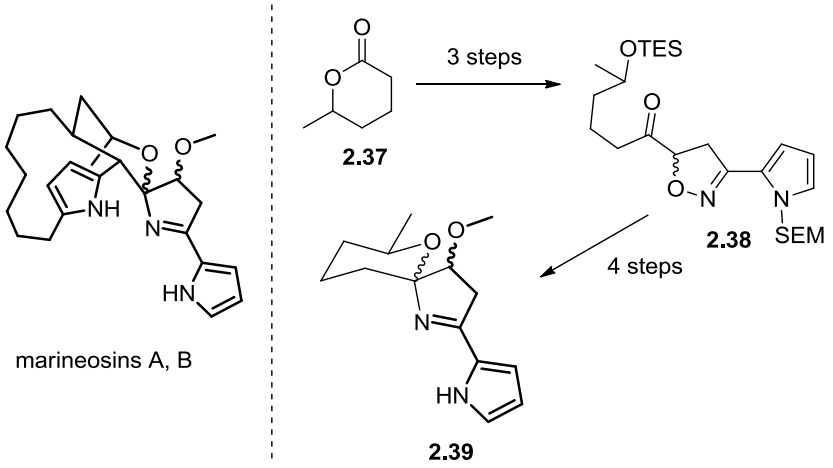
The possibility of the existence of a Diels-Alderase in the thousand-year-old prodigiosin biosynthetic pathway is very exciting and we eagerly anticipate the development of routes to synthesize compounds to test this biosynthetic hypothesis for the synthesis of marineosins A and B (Scheme 2.5).



Scheme 2.5. Fenical's biosynthetic proposal of marineosins A and B.

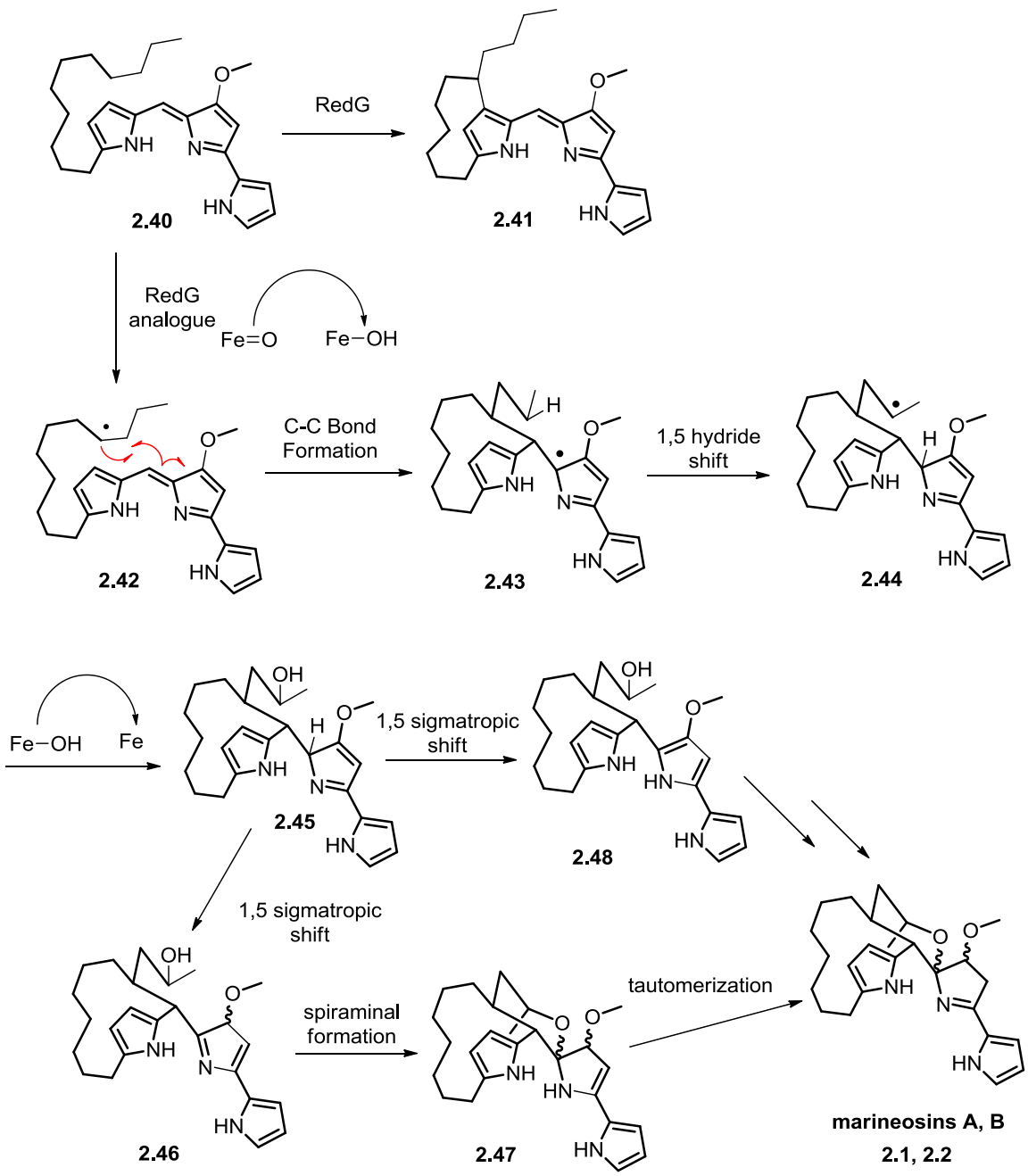
2.4. Snider's Synthesis of the Spiroiminal Core of the Marineosins and Proposal of an Alternative Biosynthetic Route.

In 2010, Snider and co-workers published a model synthesis of the novel spiroiminal core (**2.39**) of the marineosins in 7 steps from lactone **2.37**(Scheme 2.6).[25]



Scheme 2.6. Snider and co-workers route to the novel spiroiminal core of marineosins A and B.[25]

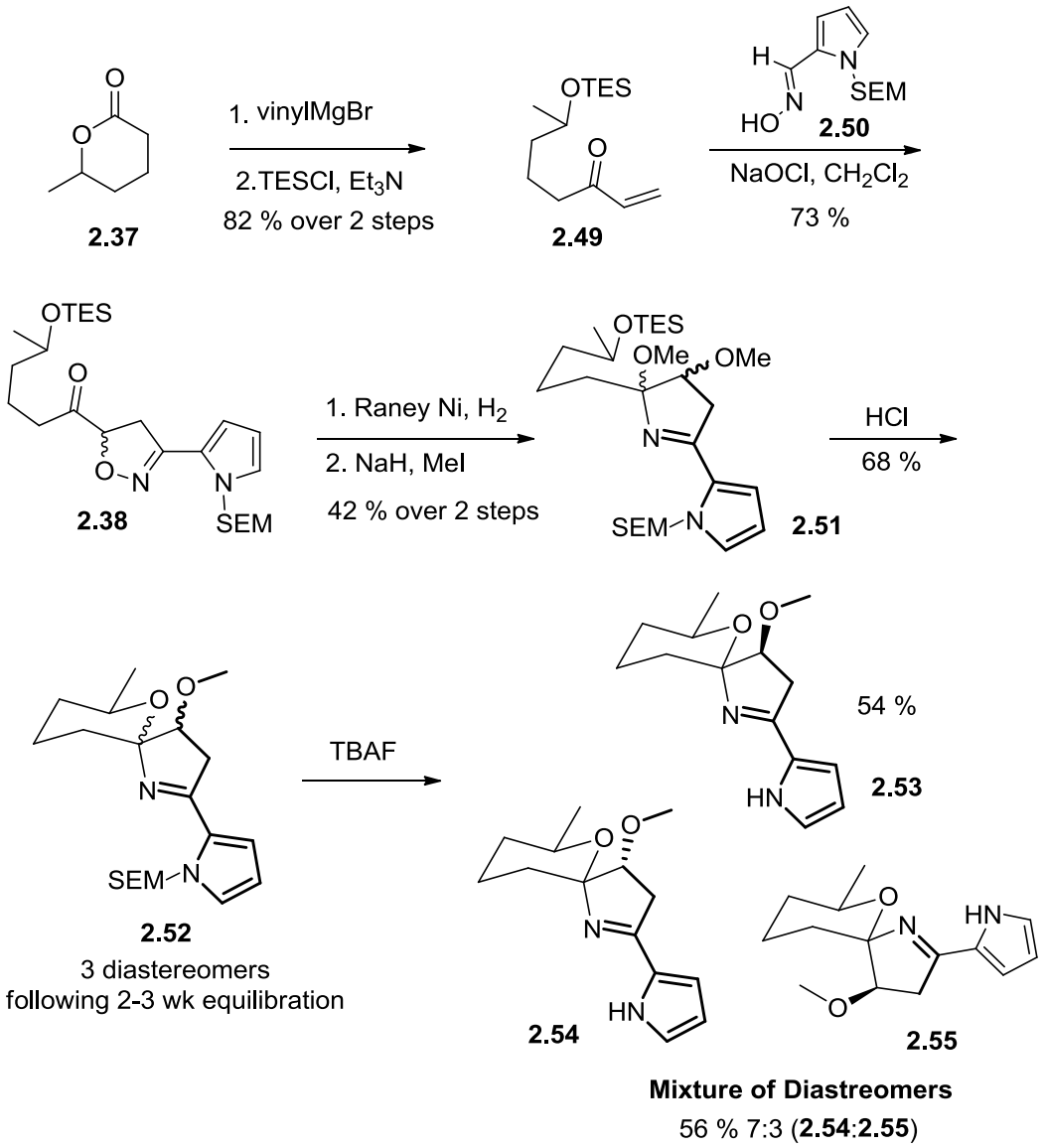
In addition to preparing the spiroiminal core of the marineosins through a non-Diels-Alder-based route, Snider suggests an alternative to the Diels-Alder biosynthetic proposal (Scheme 2.7).[25] Undecylprodigiosin (**2.40**), a likely precursor in *Streptomyces* sp., could undergo oxidation of the methylene group to a radical or cation by a RedG homologue that is a nonheme iron-dependent dioxygenase. This radical or cation **2.42** could then add to the exocyclic double bond to give macrocyclic radical **2.43**. RedG is responsible for conversion of undecylprodigiosin (**2.40**) to butyl-meta-cycloheptylprodigiosin (**2.42**) by C-H activation/oxidation to a radical or cation and subsequent intramolecular Friedel-Crafts reaction to pyrrole to form **2.42**.[26-28] In the case of marineosins, radical **2.43** could then undergo 1,5 hydride shift would provide macrocycle **2.44**, with the radical in close proximity to the enzyme active site where the molecule was first oxidized. A second one-electron oxidation would provide alcohol **2.45**. 2H- pyrrole **2.45** could then undergo a 1,5 sigmatropic hydride shift to generate 3*H*-pyrrole **2.46**, which would rapidly undergo cyclization to give enamine **2.47**. Isomerization to the imine should provide marineosins A and B. A 1,5 sigmatropic shift could also provide the 1*H*-pyrrole **2.48**, but for cyclization occur, the aromaticity of the resulting pyrrole would have to be broken, which would be a very energetically unfavorable process. For this reason, it is hypothesized that the unstable 3*H*-pyrrole **2.46** would lead to product formation.



Scheme 2.7. Snider and co-workers' alternative biosynthetic proposal.[25] (adapted from Snider 2010).

Snider's synthesis began with the addition of vinyl magnesium bromide to lactone **2.37** and quenching with TESCl to form enone **2.49**. Reaction of **2.49** with oxime **2.50** in aqueous sodium hypochlorite provided isoxazoline **2.38**. Hydrogenolysis of the

isoxazoline over Raney Nickel and subsequent methylation gave iminal **2.51**. Exposure of the iminal to acidic conditions provided various amounts of all possible marineosin diastereomers **2.52** as described in Scheme 2.8. Removal of the SEM protecting groups from the three diastereomers obtained after 2-3 weeks of equilibration proceeded smoothly to provide the deprotected analogs **2.53-2.55**.



Scheme 2.8. Snider and co-workers' synthesis of the spiroiminal core of marineosin A and B.[25]

Snider and co-workers conclude that incorporation of the fused macrocycle should force the equatorial methyl group to the proper axial conformation and that interaction between the macrocyclic ring and the methoxy substiuent should favor the formation of marineosin A and B isomers over all other isomers.

2.5. Marineosins A & B as Intriguing Synthetic Targets.

Despite the debated biosynthetic route, the marineosins appear to belong to the family of prodigiosin pigments, and the evolution of synthetic, heterocyclic chemistry is evident in the rich history of these alkaloids. From the condensations and decarboxylations of Rapoport and Holden in 1962, to ring-closing metathesis, Suzuki cross-coupling, and enantioselective 1,4 conjugate additions of the twenty-first century; alkaloid synthesis has come a long way. It is obvious why the marineosins, prodigiosin-like alkaloids containing an unprecedented number of stereogenic centers (5), would provide an intriguing framework to explore current methods of asymmetric synthesis and to catalyze the development of novel methods for the challenging synthesis of alkaloids containing electron-rich pyrrole moieties.

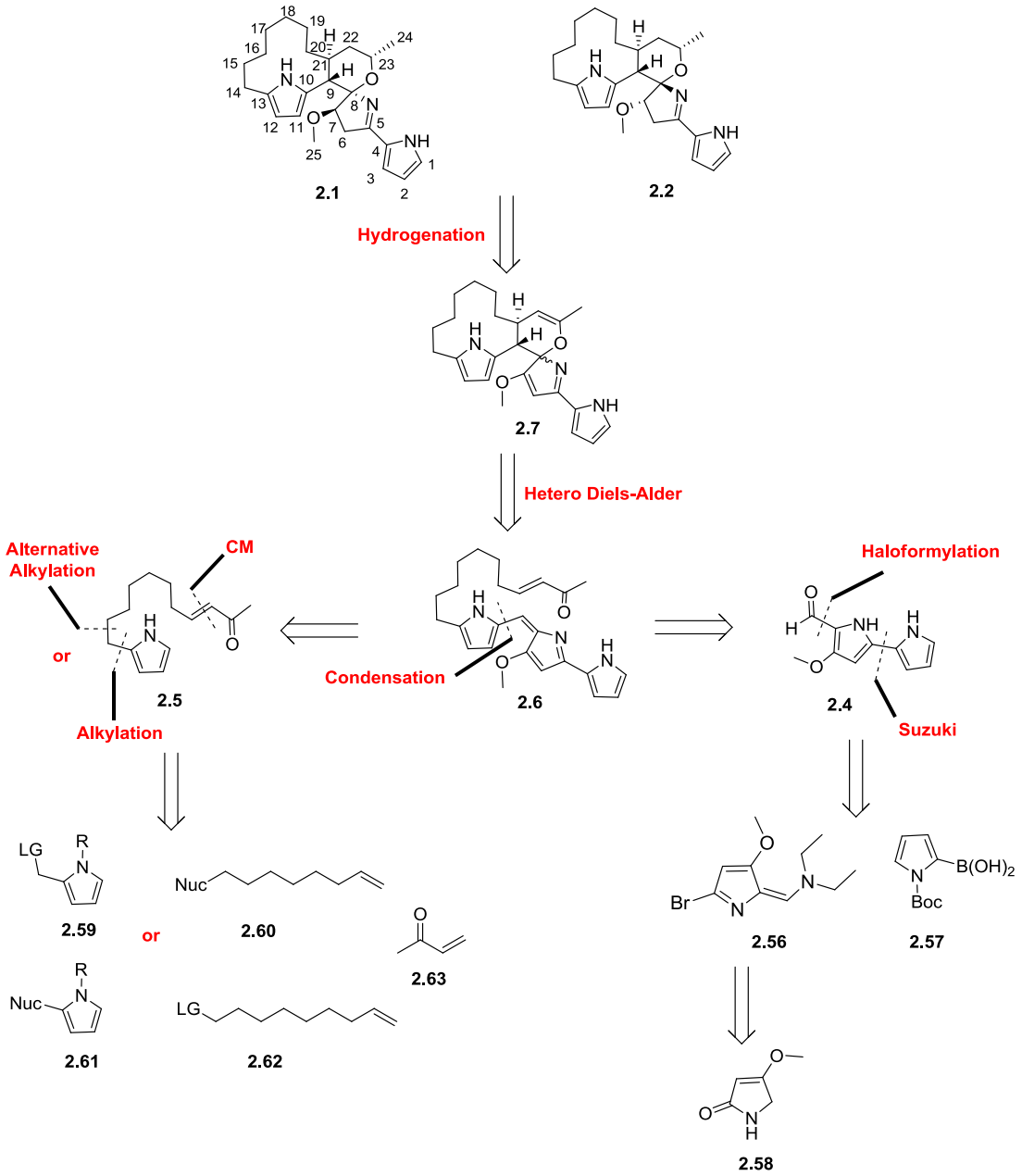
The prodigiosin alkaloids possess a rich-array of biological activities. Anticancer, antibacterial, antimalarial, and immunosuppressive properties have been observed in many prodiginine natural products and unnatural analogs. The ability to fine-tune one particular activity over another makes this group of natural products an intriguing study in structure-activity relationships. Synthesis of the marineosins, preparation of unnatural analogs and subsequent biological testing will add to the SAR knowledge of the prodigiosin alkaloids. Of particular interest will be the role of the transformed B-ring of

the spiroiminal center. While a nitrogen-containing A-ring is required for biological activity and variations in the C-ring are well-tolerated in most cases, the role of the azafulvene B-ring in the biological activity of the prodigiosin alkaloids has yet to be investigated in detail.[29] In addition, the prodigiosin alkaloids have been shown to possess a wide variety of 'tunable' biological activities, but the mechanisms of action behind many of these activities have yet to be discovered and will be a future goal within our laboratory.

Although the existence of a naturally occurring Diels-Alderase is still in question, a stepwise process for marineosin formation in the prodigiosin biological pathway could explain the Diels-Alder retron. A RedG homologue could be responsible for C-H activation in the alkyl chain, followed by addition to the pyrrolomethene core of the tripyrrole pigment. This mechanism would be consistent with the biosynthesis of other cyclic prodigiosin natural products, such as metalocycloprodigiosin, prodigiosin R1, and streptorubin B, and the fact that RedG has been predicted to catalyze oxidative cyclization of undecylprodigiosin (**1.2**) to form butyl-*meta*-cycloheptylprodigiosin (**1.3**) in *S. coelicolor* A3 (2).[25-28] With these possibilities in mind, we set out to achieve the total synthesis of marineosins A and B by incorporating a key intramolecular inverse-electron-demand hetero-Diels-Alder reaction.

2.6. Synthesis of the Prodiginine Substrate and the Intramolecular Diels-Alder Reaction.

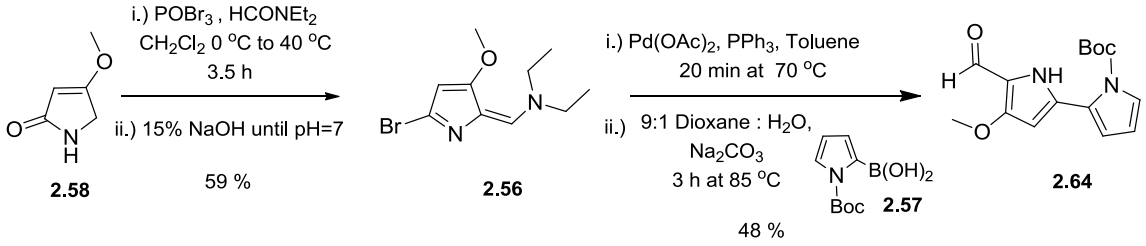
As outlined in Scheme 2.9, the retrosynthetic analysis of marineosins A and B begins with chemoselective hydrogenation of the electron-rich carbon-carbon double bonds in the Diels-Alder adduct **2.7**.



Scheme 2.9. Retrosynthetic analysis of marineosins A and B (**2.1** and **2.2**).(Nuc = nucleophile, LG = leaving group, R = protecting group or H).

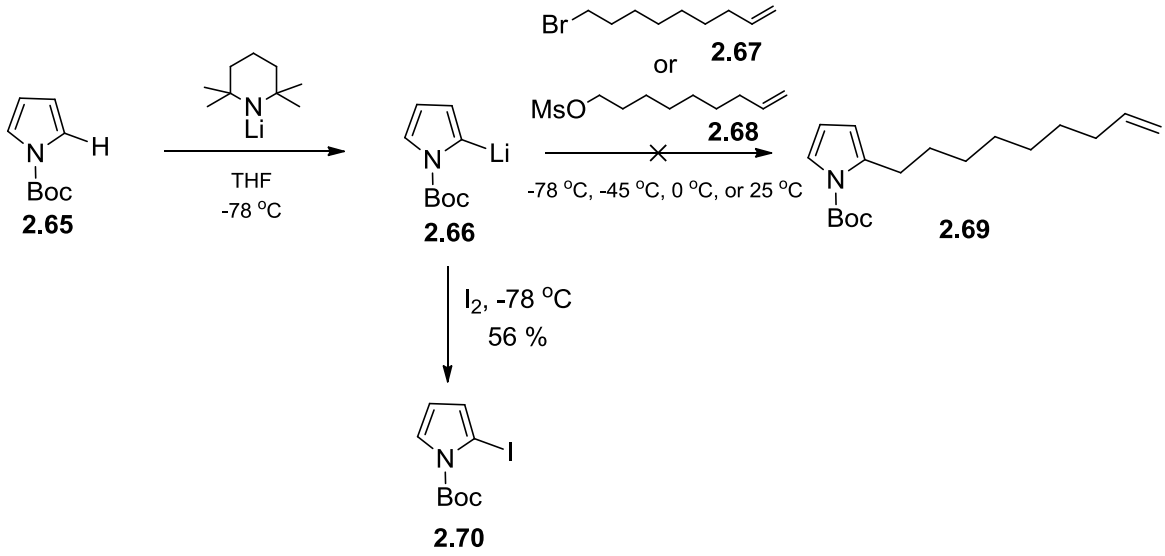
Acid-mediated condensation between aldehyde **2.4** and enone **2.5** gives the Diels-Alder substrate **2.6**. Aldehyde **2.4** is synthesized via Vilsmeier-Haack haloformylation of 4-methoxy-3-pyrrolin-2-one **2.58** and subsequent Suzuki coupling of bromoenamine **2.56** with *N*-Boc-pyrrole-2-boronic acid **2.57**. The key reactions that provide the carbon framework of enone **2.5** are a cross metathesis (CM) and an alkylation to form the C13-C14 bond or the C14-C15 bond.

The synthesis of aldehyde **2.4** was very straightforward following known methods utilized in the synthesis of the structurally similar BH3 mimetic obatoclax by Diari and co-workers.[30] The Vilsmeier-Haack haloformylation of 4-methoxy-3-pyrrolin-2-one **2.58** was carried out with phosphorous oxybromide and *N*,*N*-diethyl formamide to yield bromoenamine **2.56** in 59% yield (Scheme 2.10). Suzuki cross-coupling of bromoenamine **2.56** with *N*-Boc-pyrrole-2-boronic acid gave Boc-protected aldehyde **2.64** in 48% yield. According to the literature procedures, the deprotected aldehyde (**2.4**) was expected to be the major product; however, it was only isolated in 10% yield. Isolated **2.4** proved to be relatively unstable and polymerized even when stored at 0 ^{o}C under argon, so it was decided to use the Boc-protected **2.64** in the acid-mediated condensation and allow deprotection to occur *in situ*.



Scheme 2.10. Synthesis of aldehyde **2.64**.

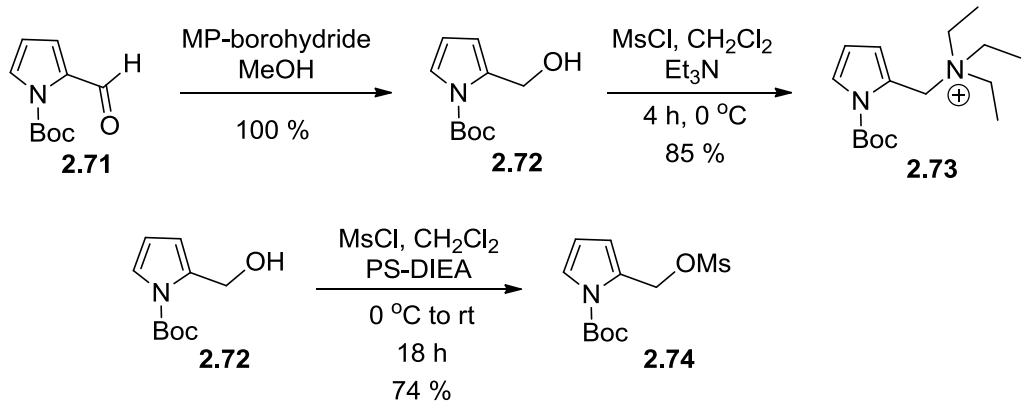
The synthesis of enone **2.5** proved to be significantly more challenging and began with attempts to form the C13-C14 bond to combine the pyrrole and alkyl chain moieties. Deprotonation at the 2-position of pyrrole **2.65** was achieved using lithium 2,2,6,6-tetramethylpiperidide (LTMP) at -78 ^{o}C (Scheme 2.11). Unfortunately, the nucleophilicity of the pyrrolyl anion **2.66** was too low to react with alkyl bromide **2.67**.[31] Displacement also failed to occur with the alkyl mesylate **2.68**. Deprotonation was not the problem, since the anion could be quenched by adding iodine, a much hotter electrophile, to the reaction mixture.



Scheme 2.11. Attempted alkylation of N-Boc pyrrole **2.65** to form alkyl pyrrole **2.69**.

At this point, we decided to form the C14-C15 bond to provide alkyl pyrrole **2.69**. In this alkylation route, the pyrrole fragment would now be the electrophilic partner and the alkyl chain the nucleophile. Polymer-supported borohydride reduction of N-Boc-pyrrole-2-carboxaldehyde **2.71** resulted in quantitative conversion to alcohol **2.72** with required no purification (Scheme 2.12). The polymer-bound reagent was simply removed

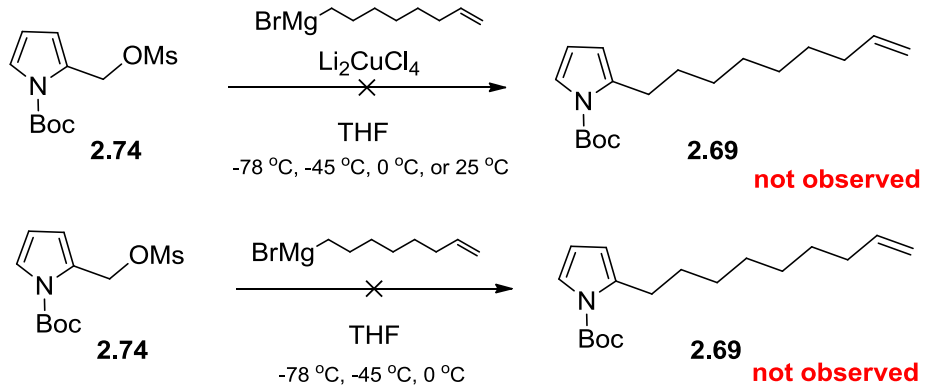
by filtration and the solvent was removed *in vacuo* to give alcohol **2.72**. Mesylation of the alcohol was carried out at 0 ^{o}C with methanesulfonylchloride and triethylamine. Unfortunately, the major product obtained was quaternary ammonium salt **2.73**, which resulted from displacement of the mesylate by triethylamine. In order to overcome this problem, polymer-supported diisopropylethylamine was used instead. Under these conditions, formation of mesylate **2.74** was successful (74% yield).



Scheme 2.12. Formation of mesylate **2.74** from N-Boc-pyrrole-2-carboxaldehyde **2.71**.

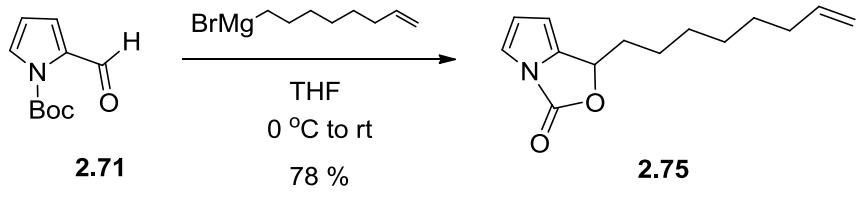
To perform the cuprate addition, 8-bromo-1-octene (**2.67**) was first converted to the Grignard reagent and then added to a solution of the mesylate **2.74** and catalytic Li_2CuCl_4 (Scheme 2.13).[32] The cuprate addition proved unsuccessful, and only starting material was recovered. Upon warming to room temperature, the starting material decomposed and no discernible product was observed upon work-up and purification. The displacement was then attempted with the Grignard reagent alone, but this method gave no desired product and no starting material could be recovered. This was most likely a result of addition to the *t*-butyl carbamate protecting group, followed by decomposition of a highly unstable, deprotected pyrrolylmesylate. In light of these observations, it was concluded that the Boc-protected pyrrole may not be the best electrophilic substrate for

this type of addition.



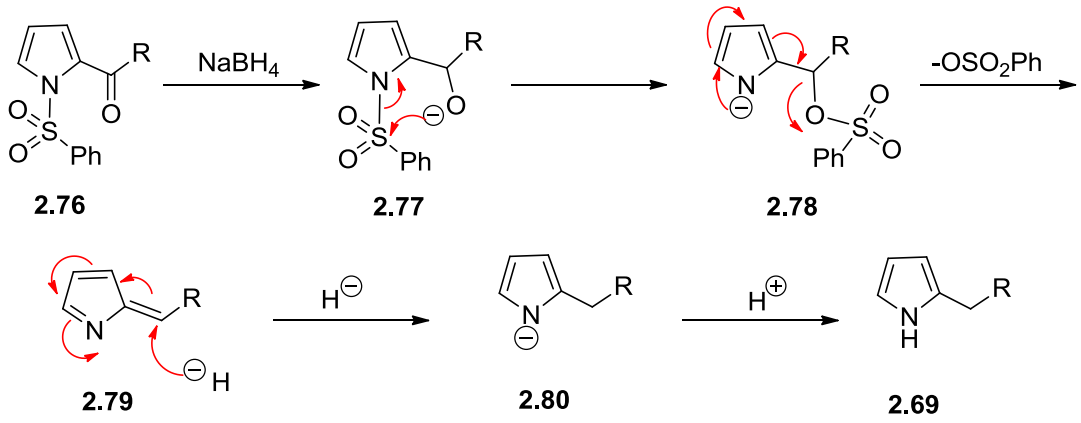
Scheme 2.13. Unsuccessful S_N2 displacement of mesylate to form alkylpyrrole **2.69**.

In order to explore the reactivity of differentially substituted pyrrole systems, the 8-bromo-1-octene Grignard reagent was simply added to N-Boc-pyrrole-2-carboxaldehyde (**2.71**). Incorporation of aldehyde **2.71** as the electrophilic reagent, should take advantage of the Grignard reagent's preference for 1,2-addition to the most electrophilic center. As expected, the desired 1,2-addition was observed; however, the only isolable product was oxazolidinone **2.75**, which resulted from transesterification of the Boc-carbamate *t*-butyl ester by the alkoxide generated upon 1,2-addition of the Grignard reagent (Scheme 2.14). Since opening of the oxazolidinone would require additional steps, a different protecting group was explored.



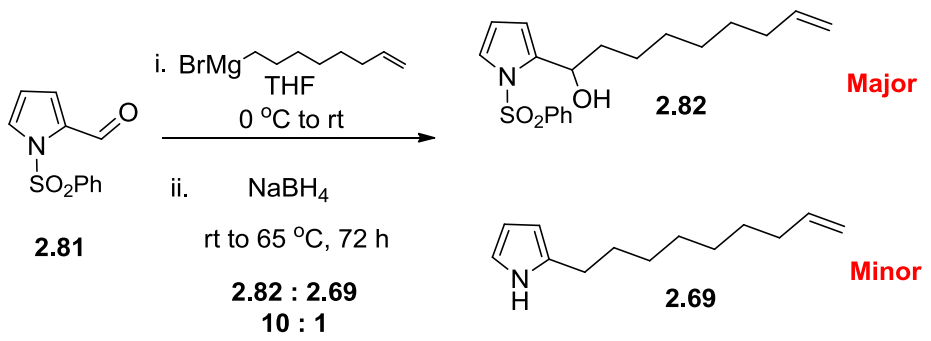
Scheme 2.14. Unexpected cyclization of Grignard addition product to form oxazolidinone **2.75**.

The commercially available 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde was chosen as a desirable protecting group. It was hypothesized, based on the observation that the Boc protecting group could be converted to a cyclic carbamate, that the sulfonate protecting group of **2.76** could be transferred to the alkoxide **2.77** and eliminated upon treatment with sodium borohydride and heat to give intermediate **2.79** (Scheme 2.15). Addition of a second equivalent of hydride should provide anion **2.80** and mildly acidic work-up generate alkylpyrrole **2.69** in one step. There is also literature precedence for this type of reaction. In 1985 Muchowski *et. al* showed that alkylpyrroles could be synthesized by the reduction of acylpyrroles with sodium borohydride in refluxing isopropanol.[33]



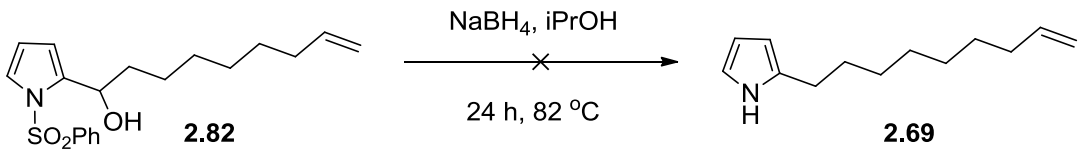
Scheme 2.15. Hypothesized one-pot addition, rearrangment, deoxygenation, deprotection to form an alkylpyrrole from a sulfonamide-protected acylpyrrole.

Following addition of the 8-bromo-1-octene Grignard reagent at 0 ^{o}C to 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde (**2.81**), the reaction mixture was stirred 4 h at room temperature. Sodium borohydride in methanol was then added and the solution was heated to reflux. After 72 h, minimal formation of alkyl pyrrole **2.69** had occurred. The major product was the secondary alcohol **2.82**, not the alkylpyrrole **2.69** (Scheme 2.16).



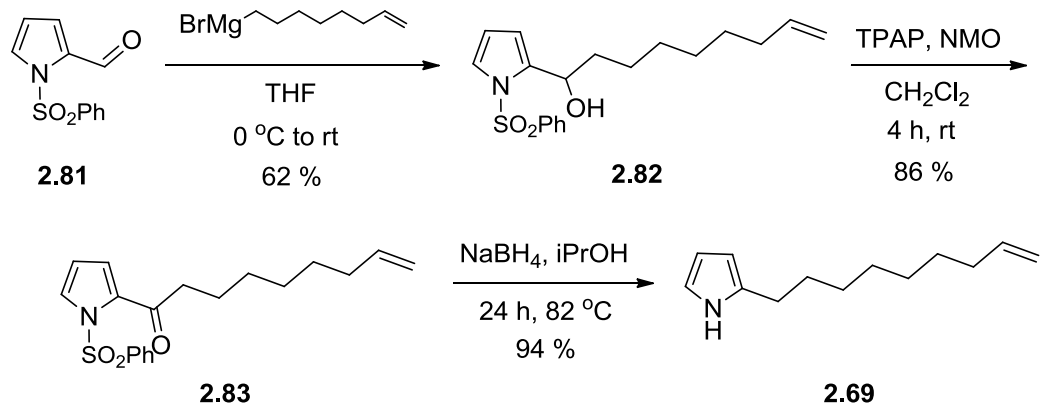
Scheme 2.16. Unsuccessful One-pot addition, rearrangment, deoxygenation, and deprotection to give alkyl pyrrole **2.69**.

After isolating alcohol **2.82**, we treated it with sodium borohydride in refluxing isopropanol to determine if the rearrangement could occur with the already reduced alcohol, or if the ketone had to be reduced and *in situ* rearrangement occur to facilitate elimination (Scheme 2.17).



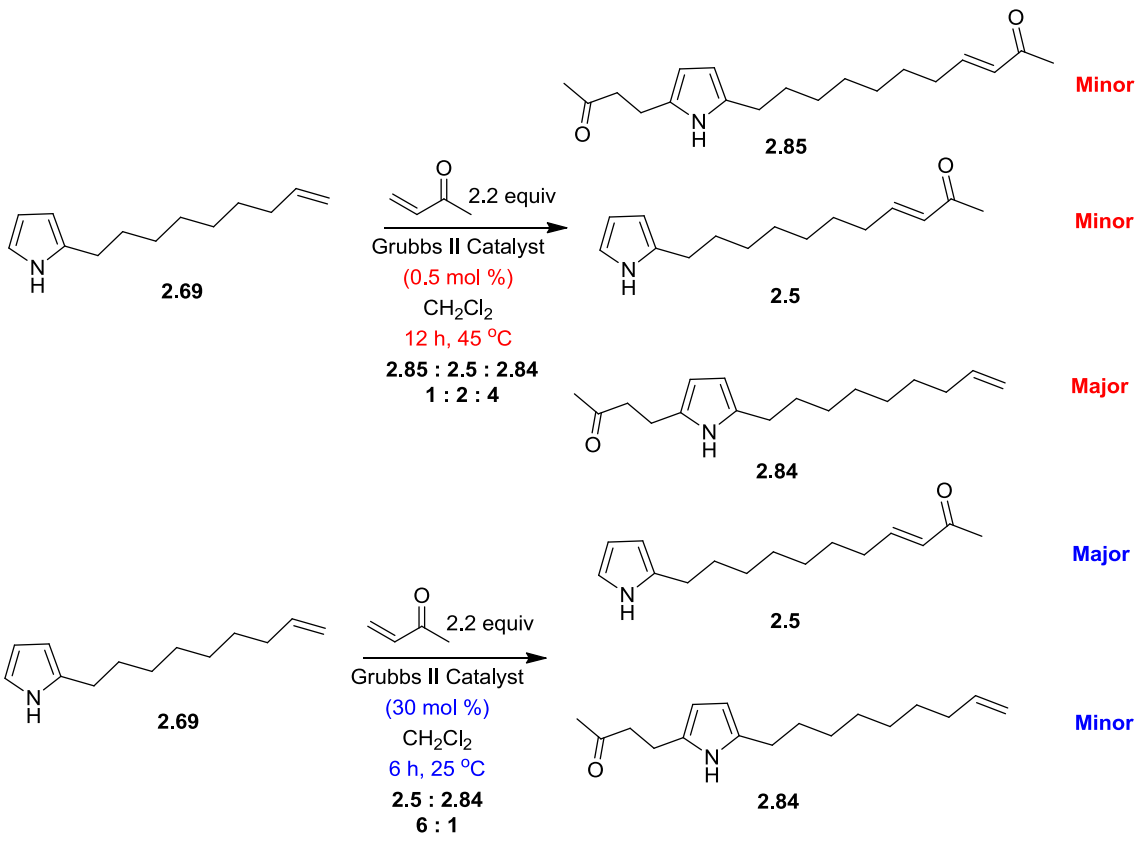
Scheme 2.17. Attempted sodium borohydride-mediated deoxygenation/deprotection of alcohol **2.82** to give alkylpyrrole **2.69**.

Unfortunately, no product formation was observed, so a Ley oxidation was performed to oxidize alcohol **2.82**.[34] After purification to remove any ruthenium, acylpyrrole **2.83** was obtained in 86% yield. Acylpyrrole **2.83** was suspended in isopropanol, treated with excess sodium borohydride, and refluxed for 24 hours. At this time, the reaction had reached completion, and after purification, the desired alkylpyrrole **2.69** was obtained in 94% yield. Scheme 2.18 describes the current synthesis of alkylpyrrole **2.69**.



Scheme 2.18. Current synthesis of alkylpyrrole **2.69**.

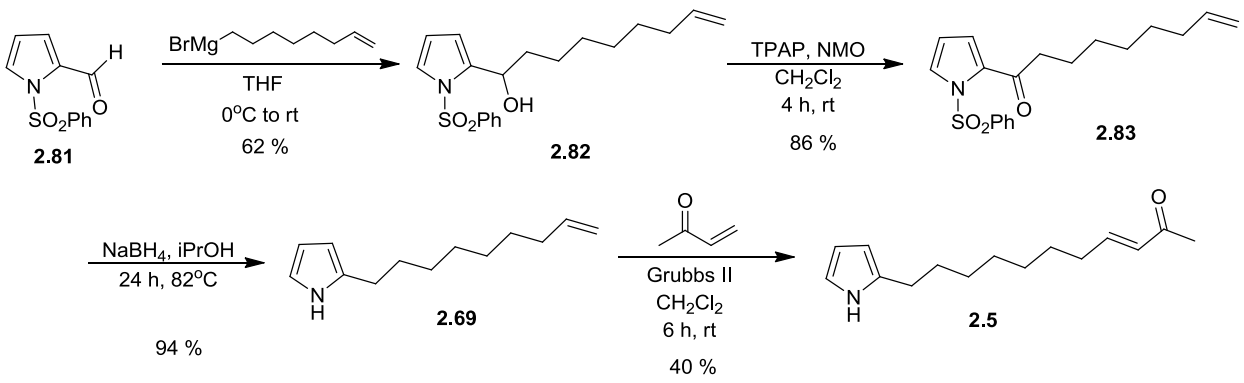
The next step in the current synthesis is the cross metathesis of alkylpyrrole **2.69** with 3-buten-2-one using the Grubbs' second generation catalyst.[35] When the reaction was refluxed in dichloromethane for 12 hours, the major product was not the expected enone **2.5**, but a pyrrole alkylated at the 2-position by the 3-buten-2-one Michael acceptor (**2.84**) (Scheme 2.19).



Scheme 2.19. Minimization of the unexpected Michael adducts **2.84** and **2.85** in the cross metathesis of alkenylpyrrole **2.69** and 3-buten-2-one to form enone **2.5**.

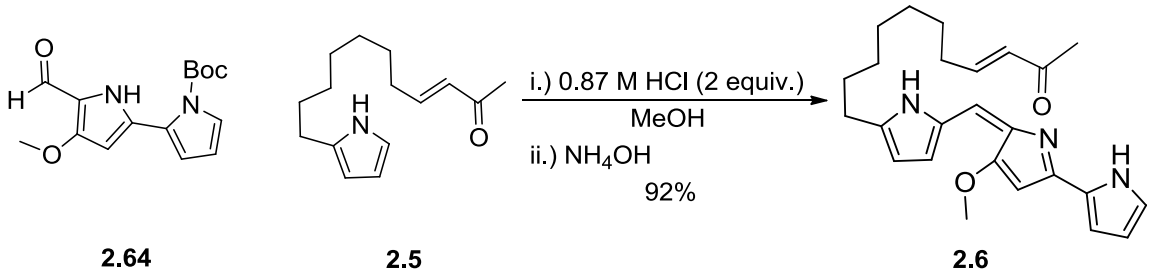
The presence of this unexpected by-product reveals that the Grubb's catalyst may act as a Lewis acid that activates the Michael acceptor to attack by the electron-rich pyrrole. In order to minimize this side reaction, a higher catalyst loading was used (30 mol %) and the reaction was carried out at room temperature. After 6 h, the side product had begun to form, so the reaction was stopped although conversion was only 70 %. After purification, enone **2.5** was isolated in 40% yield.

Although the yield was low, the reaction sequence was simply performed on a 10 gram scale in order to produce a large quantity of the Diels-Alder substrate for catalyst screening. The complete synthetic route to access enone **2.5** is described in Scheme 2.20.



Scheme 2.20. Current synthesis of enone **2.5.**

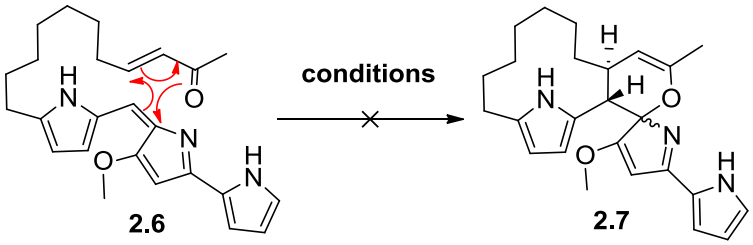
The acid-mediated condensation of enone **2.5** with aldehyde **2.64** was carried out using 2 equivalents of HCl at a 0.87 M concentration in methanol according to procedure used in the synthesis of Obatoclax by Dairi *et. al.*[30] After 10 minutes the reaction had reached completion and the conversion was quantitative. The reaction was quenched with excess ammonium hydroxide, which readily removed the carbamate protecting group, giving Diels-Alder substrate **2.6** (Scheme 2.21)**.**



Scheme 2.21. Acid-mediated condensation of enone **2.5** with aldehyde **2.64** to give Diels-Alder substrate **2.6**.

With the Diels-Alder substrate in hand, it was time to screen conditions to catalyze the [4+2] cycloaddition. The various conditions for the catalyst screen are outlined in Table 2.1. Unfortunately, none of the Lewis acids that are commonly used for inverse electron-demand hetero Diels-Alder reactions gave any product formation.[36]

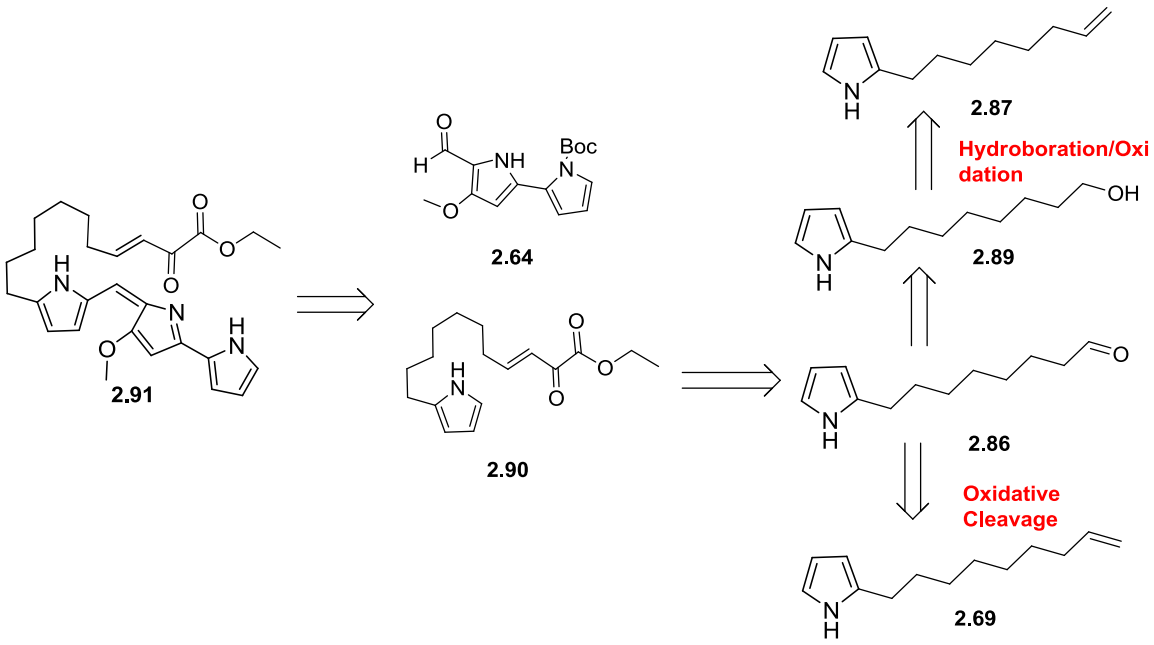
Table 2.1. Lewis Acids and conditions used to catalyze the intramolecular hetero Diels-Alder reaction.



Catalyst	Equiv. (mol%)	Solvent	Temperature (^{o}C)	Time (h)	Result
$Eu(FOD)_3$	20	Cylcohexane	80	72	No rxn
$Eu(FOD)_3$	20	Cyclohexane	25	96	No rxn
$Yb(OTf)_3$	20	CH_2Cl_2	25	96	No rxn
$Sc(OTf)_3$	20	CH_2Cl_2	25	96	No rxn
$SnCl_4$	100	CH_2Cl_2	25	4, 12	Decomposed
Me_2AlCl	100	CH_2Cl_2	25	4, 12	Decomposed
AcOH	xs	EtOH (1:1)	120 (mw)	0.25, 0.5	No rxn
AcOH	xs	EtOH (1:1)	150 (mw)	0.5	No rxn
AcOH	xs	Neat	160 (mw)	0.5	No rxn
HCl	xs	Dioxane (1:1)	120 (mw)	0.5	No rxn
TFA	xs	DCE	120 (mw)	0.25, 0.5	Decomposed
Me_2AlCl	500	CH_2Cl_2	-78	2, 4, 6, 16, 24, 48	No rxn
$SnCl_4$	500	CH_2Cl_2	-78	2, 4, 6, 16, 24, 48	No rxn
$SnCl_4$	500	CH_2Cl_2	-78 to -45	24	Decomposed
BBr_3	400	CH_2Cl_2	-78	2	Decomposed
Me_2AlCl	500	CH_2Cl_2	-20	4, 8, 16, 24, 48, 72, 96, 108	No rxn
---	---	Mesitylene	215	1	Decomposed
Methylene blue hν	100	CH_2Cl_2	25	4, 8, 12	No rxn
$ZnCl_2$	100	CH_2Cl_2	25	6, 12, 24, 48	No rxn
$ZnCl_2$	800	CH_2Cl_2	25	12, 24, 48	No rxn

Without any promising leads, it was concluded that Diels-Alder substrate **2.6** was fairly unreactive. In order to make the heterodiene more reactive as an inverse electron-demand Diels-Alder substrate, we decided to add an electron-withdrawing group to the

enone, namely, an α-keto ester. This would provide a more electron-deficient, and thus more reactive, heterodiene. In light of the conjugate addition observed during the cross metathesis of 3-buten-2-one with alkylpyrrole **2.69**, we thought that a Wittig-type reaction might prove more effective when incorporating the pyruvate moiety. This required either cleaving one carbon from the alkyl chain during the synthesis of this fragment or incorporating a carbon chain that is one carbon shorter in the Grignard reaction and then generating an aldehyde following a hydroboration/oxidation sequence. The new retrosynthesis is described in Scheme 2.22.

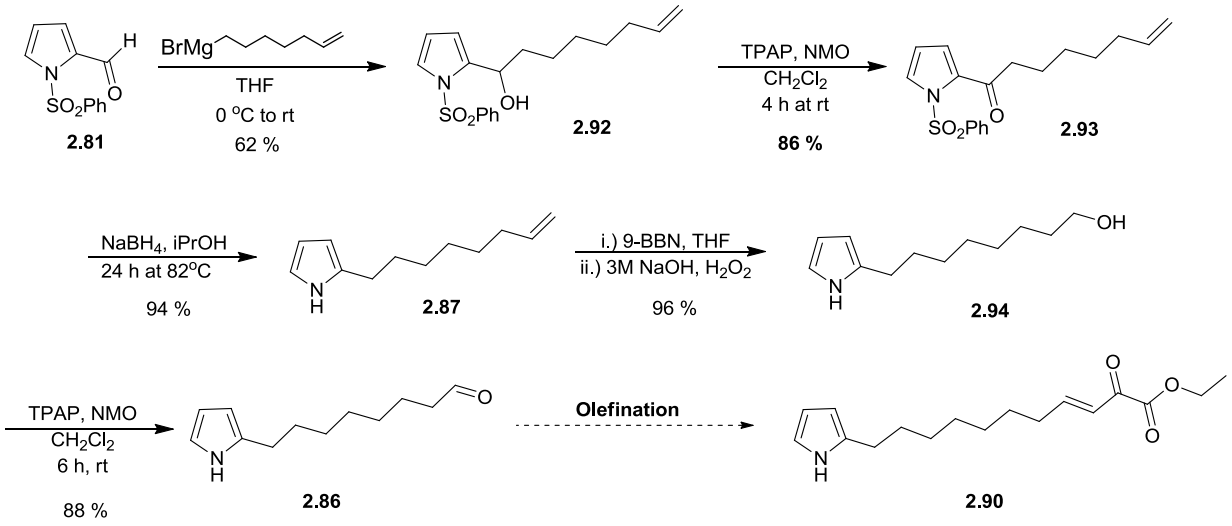


Scheme 2.22. Retrosynthesis of Diels-Alder substrate **2.91** via synthesis of aldehyde **2.86** by either an oxidative cleavage or hydroboration/oxidation route.

Ozonolysis was the first method used to synthesize aldehyde **2.86** from alkylpyrrole **2.69**. Unfortunately, these conditions also resulted in cleavage of the electron-rich pyrrole to form a terminal amide. Dihydroxylation and subsequent oxidative cleavage proved unsuccessful as well due to incomplete dihydroxylation and production

of complex reaction mixtures during oxidative cleavage.

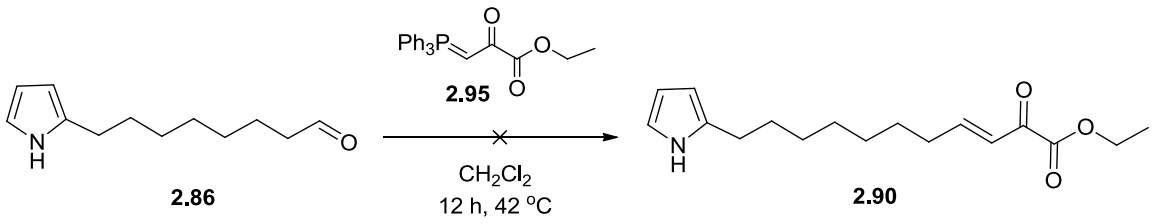
It was finally decided that 7-bromo-1-heptene would be used in the Grignard addition (Scheme 2.23). After Ley oxidation of the resulting alcohol (**2.92**), acylpyrrole **2.93** underwent sodium borohydride-mediated reduction, deoxygenation, and deprotection to give alkylpyrrole **2.87**. Hydroboration and oxidation of **2.87** gave quantitative conversion to alcohol **2.94** with a 96% yield following purification. Ley oxidation of alcohol **2.94** gave clean conversion to aldehyde **2.86**, which was purified by filtration of the reaction mixture through a celite plug and rinsing with dichloromethane. This method of aldehyde formation provided good yields (73% over two steps from alkylpyrrole **2.87**) and has become the method of choice.



Scheme 2.23. Synthesis of α-keto ester **2.90**.

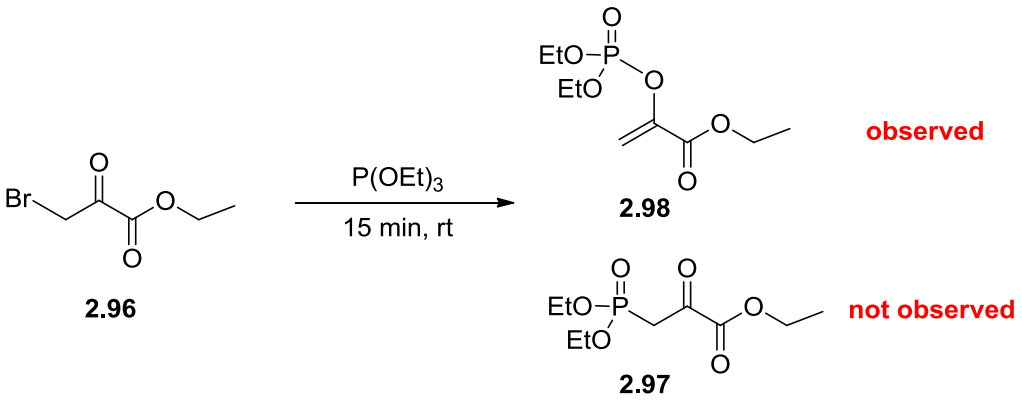
Reaction of aldehyde **2.86** with ethyl(triphenylphosphoranylidene)pyruvate (**2.95**) to give α-keto ester **2.90** proved unsuccessful. The stabilized ylide is so highly stabilized that it is fairly unreactive. After refluxing in dichloromethane overnight, the ylide was

still present and the aldehyde had decomposed, possibly as a result of polymerization through a series of condensation reactions (Scheme 2.24.).



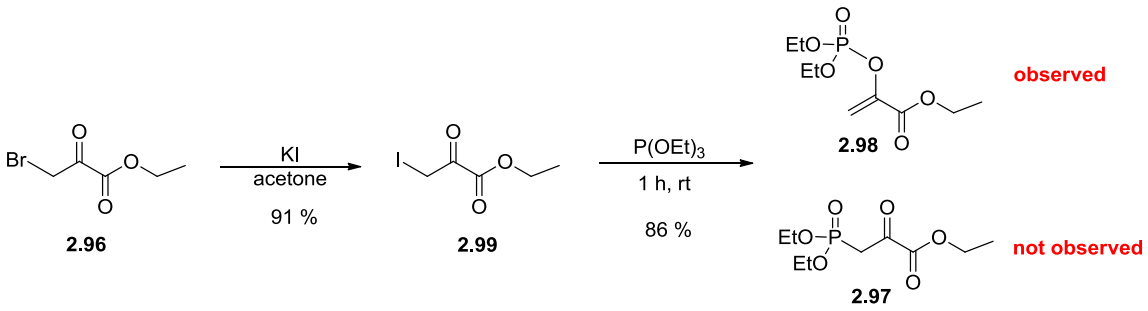
Scheme 2.24. Unsuccessful Wittig olefination of aldehyde **2.86** to generate α-keto ester **2.90**.

The next approach to generate α-ketoester **2.90** was to use phosphonate **2.97** in a Horner-Wadsworth-Emmons olefination as a hotter reagent than the phosphoranylidene. Ethyl bromopyruvate (**2.96**) was treated with triethylphosphite at room temperature, resulting in a highly exothermic reaction (Scheme 2.25). After cooling to room temperature, TLC determined that all the starting material had been consumed, and LC-MS confirmed that the product had the correct mass. However, H^1 and C^{13} NMR revealed that the product was not the desired phosphonate **2.97** but was vinyl phosphate **2.98**. According to an extensive review of the Michaelis–Arbuzov rearrangement by Bhatacharya and Thyagarman,[37] the vinylphosphate is the major product with α-chloro ketones and α-bromo ketones. With the addition of an electron-withdrawing α–ester, it is logical that the ethyl α-bromopyruvate would form the vinylphosphate product exclusively, as was observed.



Scheme 2.25. Formation of undesired vinyl phosphate **2.98**.

Bhatacharya and Thyagarman report that α-iodo ketones form the phosphonate exclusively. Ethyl iodopyruvate (**2.99**) is not commercially available, but it was easily synthesized from **2.96** in 91% yield using a Finkelstein reaction (Scheme 2.26).



Scheme 2.26. Attempted preparation of Horner-Wadsworth-Emmons reagent, phosphonate **2.97**.

Addition of triethylphosphite to ethyl iodopyruvate **2.99** should result in the desired phosphonate **2.97**. Unfortunately, iodination of bromopyruvate and subsequent Michaelis-Arbuzov reaction failed to provide phosphonate **2.97**. Once again, vinyl phosphate **2.98** was the only product observed, presumably a result of the increased acidity of the α-carbon due to the presence of the α-keto ester. This result was undesired but not entirely unexpected, since Bhatacharya and Thyagarman investigated α-iodo

ketones, not α-iodo-α-ketoesters.

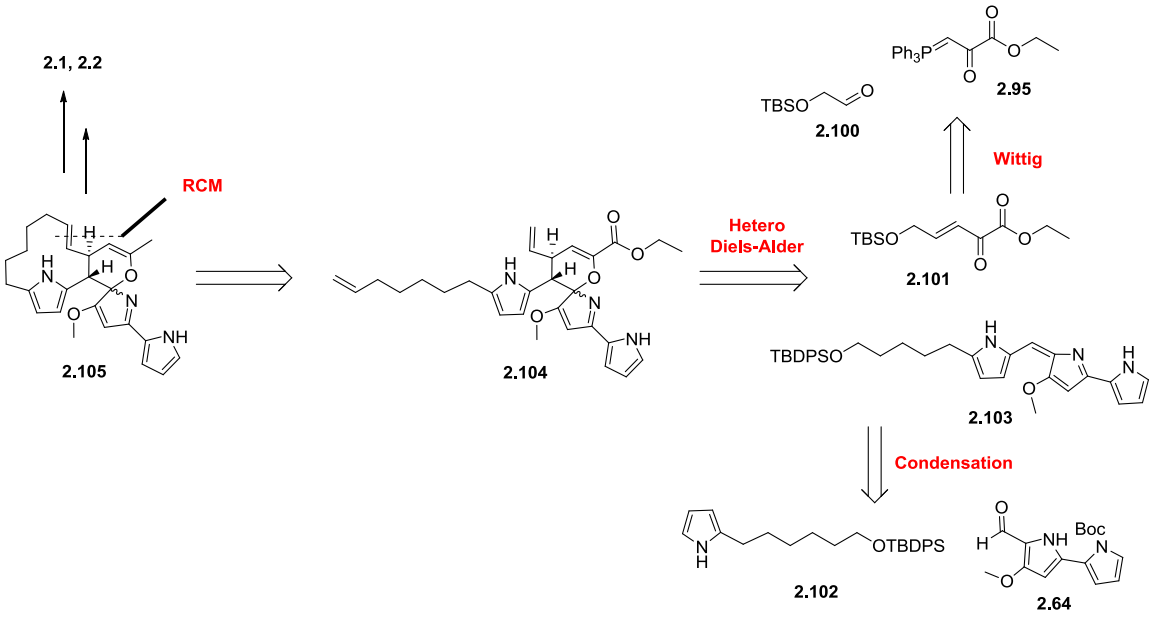
If phosphonate formation had been successful, the final step in the synthesis of α-ketoester **2.90** would have been a Horner-Wadsworth-Emmons reaction with aldehyde **2.86** performed in the presence of lithium chloride and DBU in acetonitrile, conditions developed by Roush and Masamune for base-sensitive substrates.[38] These conditions were chosen since the deprotected pyrrole would most likely be problematic if sodium hydride is used. Without α-ketoester **2.90**, further investigation of an intramolecular Diels-Alder route to produce the marineosins was not possible. Due to the failure to produce the desired phosphonate **2.97** and the inherent instability of aldehyde **2.86**, this route was abandoned in favor of one with more stable intermediates.

In summary, Diels-Alder substrate **2.6** has been successfully synthesized. However, **2.6** did not undergo a [4+2] cycloaddition in the presence of various Lewis acids. We decided to derivatize **2.6** by adding an α-ketoester to the enone in order to make substrate **2.90**, a more electron-deficient heterodiene, which would be more likely to undergo the desired cycloaddition based on reactivity. To date, the relatively unstable aldehyde **2.86** has been synthesized; however, incorporation of the pyruvate moiety through an olefination reaction proved unsuccessful, which has led to the exploration of alternate routes.

2.7. Intermolecular Diels-Alder Reaction.

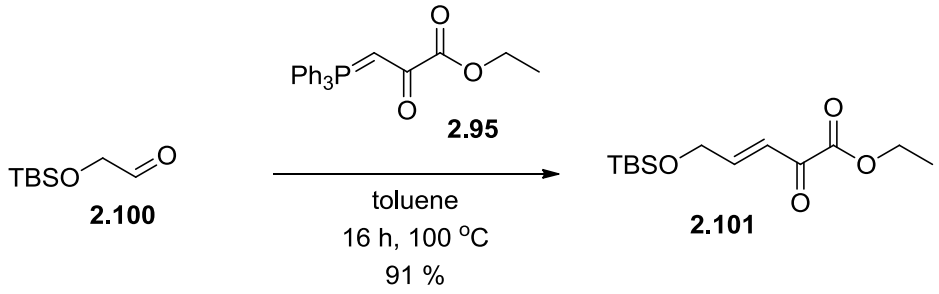
In light of the difficulty encountered with olefination reactions to produce **2.90** and the unreactive nature of Diels-Alder substrate **2.6**, an alternative synthesis has been developed that incorporates many of the key reactions from previous routes. The two

notable changes are the RCM to form macrocycle and an *inter*molecular inverse electron demand hetero Diels-Alder (Scheme 2.27).



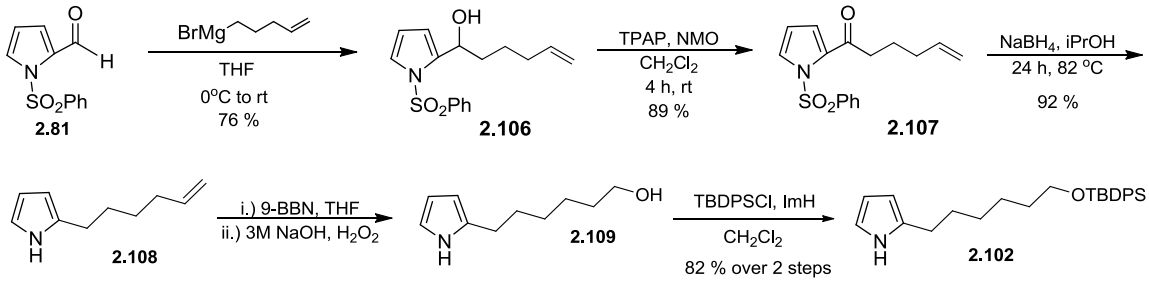
Scheme 2.27. Restrosynthetic analysis of intermolecular hetero Diels-Alder route.

The alternative route starts from commercially available, (*tert*-butyldimethylsilyloxy)-acetaldehyde (**2.100**). This compound has a high boiling point (165-167^{o}C) and no extraneous, deprotected, highly reactive functional groups, so we hypothesized that unlike aldehyde **2.86**, it should not decompose in the conditions necessary to activate the highly stabilized ethyl (triphenylphosphoranylidene) pyruvate (**2.95**). After refluxing in toluene overnight, heterodiene **2.101** was readily obtained (Scheme 2.28).



Scheme 2.28. Synthesis of heterodiene **2.101**.

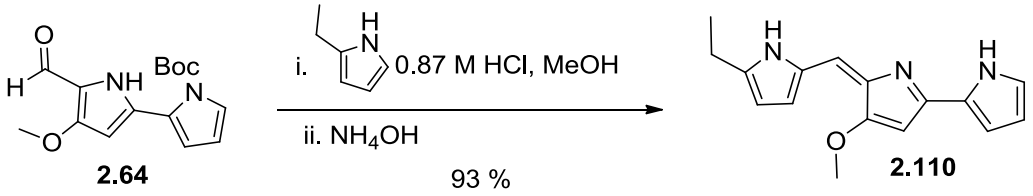
The synthesis of silylether **2.102** is very straightforward (Scheme 2.29). Most of the steps have been accomplished before in the synthesis of aldehyde **2.86**. The route begins with Grignard addition to 1-(phenylsulfonyl)-pyrrole-2-carboxaldehyde (**2.81**), the only difference is that the Grignard reagent was prepared from 5-bromo-1-pentene, to give alcohol **2.106** in 76% yield. Ley oxidation and subsequent sodium borohydride-mediated reduction, deoxygenation, and deprotection yielded alkylpyrrole **2.108** (81% over 2 steps). Hydroboration and oxidation of **2.108** gave alcohol **2.109**, which was immediately subjected to TBDPS protection yielding silylether **2.102** (82% over 2 steps).



Scheme 2.29. Synthesis of silylether **2.102**.

Instead of immediately subjecting silylether **2.102** to acid-mediated condensation with aldehyde **2.64** to give dieneophile **2.103** (Scheme 2.27), we decided to construct a simpler model system to save our starting material and simplify NMR interpretation. This

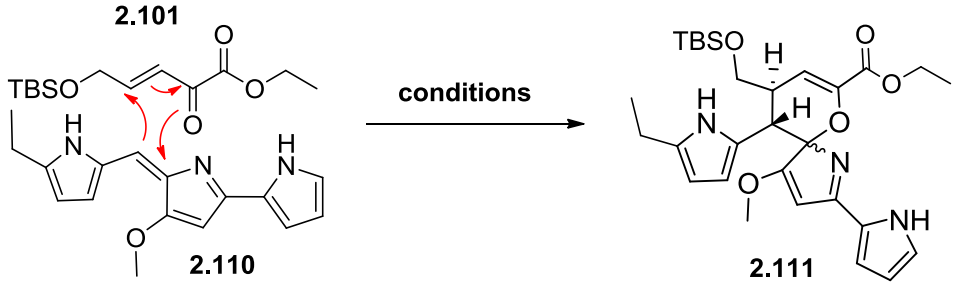
model system was synthesized by condensation of aldehyde **2.64** with commercially available 2-ethylpyrrole to yield the Diels-Alder model substrate **2.110** (Scheme 2.30).



Scheme 2.30. Condensation of 2-ethylpyrrole with aldehyde **2.2** to give model dienophile **2.110**.

At this point, we began to screen different Lewis acids for their ability to catalyze a [4+2] cycloaddition between heterodiene **2.101** and model dienophile **2.110** to generate Diels-Alder adduct **2.111** (Table 2.2).

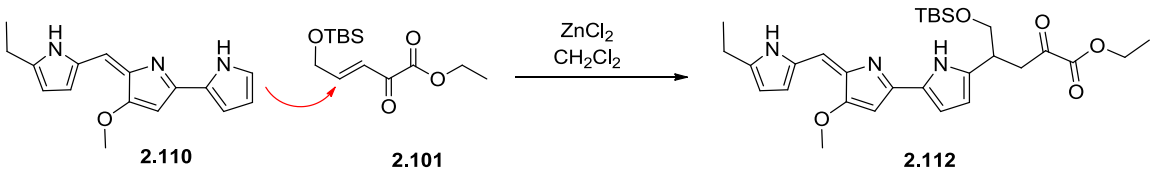
Table 2.2. Lewis Acids and conditions used to catalyze the intermolecular Diels-Alder reaction.



Catalyst	Equiv.	Solvent	Temperature (^{o}C)	Time (h)	Result
$Eu(FOD)_3$	0.2	Cyclohexane	25	72	No rxn
$Yb(OTf)_3$	0.2	Cyclohexane	25	72	No rxn
$Sc(OTf)_3$	0.2	CH_2Cl_2	25	72	No rxn
Me_2AlCl	1	CH_2Cl_2	0	12	No rxn
$SnCl_4$	1	CH_2Cl_2	0	12	Decomposed
BBr_3	1	CH_2Cl_2	-78	2	Decomposed
$ZnCl_2$	10	CH_2Cl_2	0	4	Reacted

Finally, a positive result was obtained using $ZnCl_2$ (10 equiv.) in CH_2Cl_2 at 0 ^{o}C. The mass of the resulting adduct as determined by LC-MS matched the expected mass of

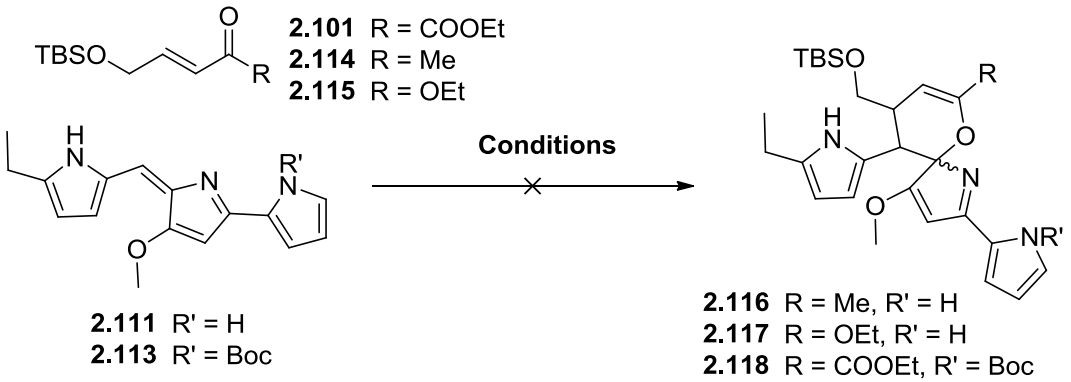
the Diels-Alder product **2.111**. Also, ^{1}HNMR and ^{13}CNMR revealed the correct number of proton and carbon atoms with similar shifts as would be expected for the Diels-Alder adduct. However, the splitting patterns in the ^{1}HNMR were slightly unexpected; therefore, HMBC and HSQC spectra were obtained for the compound. Based on HMBC correlations, it was determined that the Diels-Alder reaction had not occurred, but instead the $ZnCl_2$ had catalyzed a conjugate addition between the A-ring pyrrole of the dienophile (**2.110**) and the α,β-unsaturated α-keto ester (**2.101**) (Scheme 2.31).



Scheme 2.31. $ZnCl_2$-mediated conjugate addition of dienophile **2.110** to heterodiene **2.112**.

In light of this result with the pyruvate heterodiene **2.101**, we decided to investigate the intermolecular Diels-Alder reaction further by experimenting with different heterodienes and by incorporating a carbamate-protected dienophile **2.113** (Scheme 2.32). The methyl ketone (**2.114**) and ethyl ester (**2.115**) heterodienes were prepared from commercially available Wittig reagents and (*tert*-butyldimethylsilyloxy)-acetaldehyde **1.100** in the same manner as heterodiene **2.101** was prepared (Scheme 2.28). Unfortunately, the methyl ketone (**2.114**) and ethyl ester (**2.115**) heterodiene derivatives failed to react with dienophile **2.110** under any of the conditions previously described in Table 2.2. Not even the conjugate addition adduct was observed. When the A-ring pyrrole of dienophile **2.110** was protected as the *t*-butyl carbamate (**2.113**), no reaction occurred with pyruvate heterodiene **2.101** in the presence of $ZnCl_2$. Although

carbamate protection efficiently prevented the conjugate addition reaction, it did not facilitate the desired [4+2] cycloaddition.



Scheme 2.32. Reaction of various heterodienes and protected/deprotected dienophiles in the presence of Lewis acids from Table 2.2 in an attempt to initiate an intermolecular Diels-Alder cycloaddition.

Since none of the prepared heterodienes reacted in the desired manner, it was finally concluded that dienophile **2.110** was not a competent partner for a Diels-Alder reaction.

2.8. Molecular Modeling of the Prodigine Substrate for the Intramolecular Diels-Alder Reaction.

In light of the difficulties encounter with facilitating the Diels-Alder reaction, we enlisted molecular modeling in an attempt to understand this lack of reactivity in both the intramolecular and the intermolecular variants. Molecular mechanics sampling for the intramolecular substrate **2.6** was conducted starting from the hypothesized transition state geometry using both stochastic and systematic conformer searches and gradient energy minimization with the Merck MMFF94 forcefield as implemented in the MOE software package (Chemical Computing Group).[39] Analysis of the top 10,000

conformers with the lowest relative energies (20kcal from lowest energy conformer) indicated a failure to identify favorable Diels-Alder transition state geometries. Out of 10,000 systematic search conformers generated for **2.6**, less than 15% of the structures sampled have a folded topology, and the key atoms remained separated by almost five angstroms (Figure 2.4). Of those conformers with a folded topology, only 15% form intramolecular hydrogen bonds. Thus, as a result of intramolecular hydrogen bonds and a large degree of conformational flexibility present in the long, alkyl linker moiety, the intramolecular Diels-Alder mechanism is likely energetically disfavored. Moreover, since attempts at intermolecular variants proved equally unsuccessful, it was concluded that the key olefin of the extended poly-pyrrole π-system is not a competent dienophile. This extended conjugation removes electron density from the olefin, which needs to be electron-rich in order to raise the alkene HOMO to a state that is energetically favorable for an inverse demand Diels-Alder reaction to occur. With this information, we concluded that a Diels-Alder cycloaddition is not a viable synthetic route for the synthesis of Marineosins A & B in the laboratory.[40] However, this does not rule out an enzyme-templated process in nature that could impart the correct electronic state and conformation in order to catalyze the reaction.

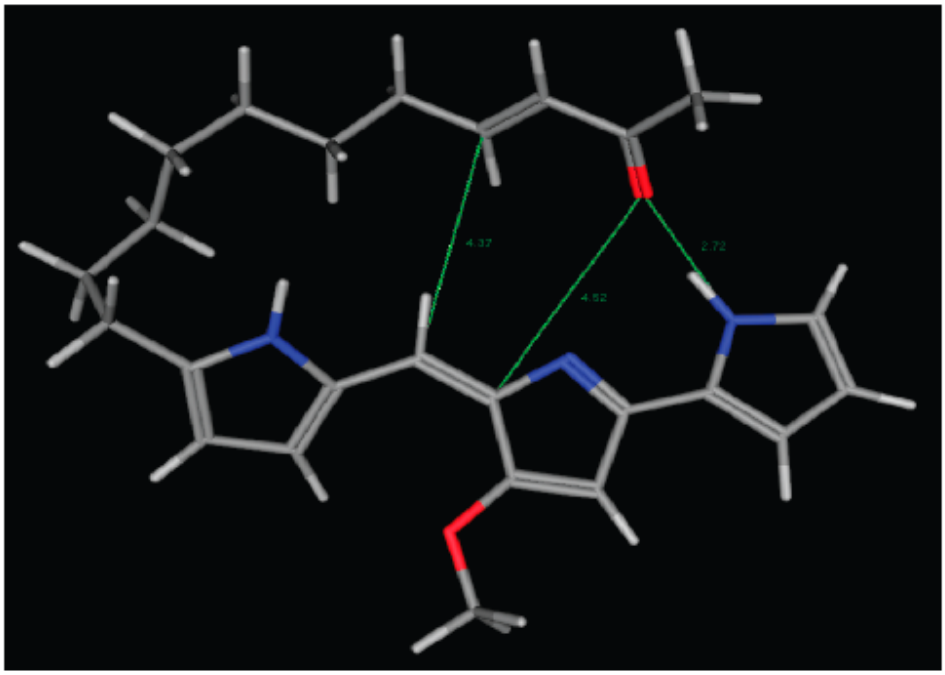
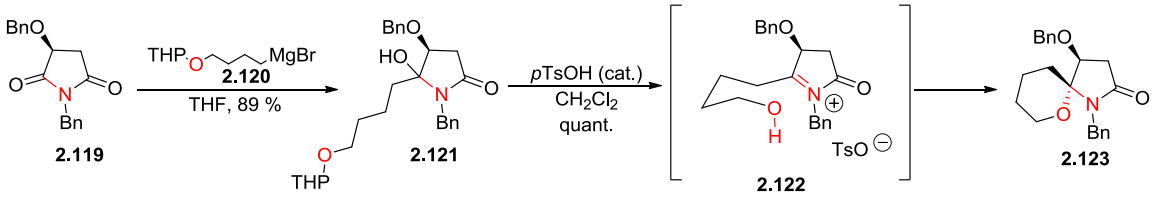


Figure 2.4. Most favored Merck MMFF94 minimized conformer **2.6**.

In summary, we constructed various heterodienes in an attempt to catalyze an intermolecular inverse electron-demand hetero-Diels-Alder reaction with model dienophile **2.111**. Unfortunately, no reaction occurred with any of the substrates except the pyruvate derivative, which underwent a conjugate addition reaction with the A-ring pyrrole of model dienophile **2.110**. Through organic synthesis and computational modeling, we have concluded that the biosynthetic proposal of an inverse electron-demand hetero-Diels-Alder reaction is not a viable method for the synthesis of Marineosins A & B. Therefore, we have decided to pursue the synthesis of these marine alkaloids by alternative routes that do not proceed through a tripyrrole prodigiosin alkaloid intermediate.

2.9. Acid-catalyzed Intramolecular N-Acyliminium Cyclization for the Synthesis of Marineosin A.

After many unsuccessful attempts to synthetically validate the proposed biomimetic route to Marineosins A & B, we decided to investigate other ways to synthesize spiroaminal centers in alkaloid natural products. One of the most straightforward and amenable routes involves Grignard addition to a chiral hydroxy-malimide, readily prepared from (*S*)-2-hydroxysuccinic acid, followed by acid-catalyzed intramolecular N-acyliminium cyclization to form the spiroaminal core (Scheme 2.33). [41]



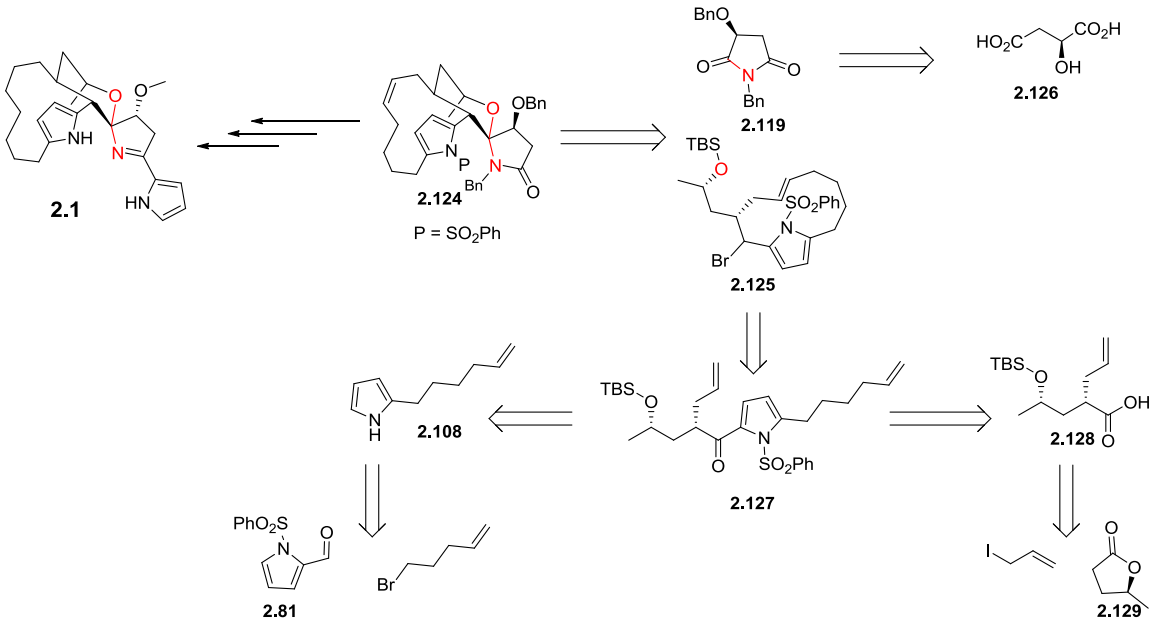
Scheme 2.33. Preparation of aza-spiropyran **2.123.** by Huang and coworkers.[41]

This key reaction could easily provide the core structure of Marineosin A as described by the retrosynthesis outlined in Scheme 2.34

2.9.1. Addition of an α-Nucleophilic Pyrrole to (*S*)-Hydroxy Malimide.

The key aspect of this new route is the formation of an α-nucleophilic pyrrole, which should add regioselectivly to the (*S*)-hydroxy malimide **2.119** to form the spriocyclic core. Marineosin A (**2.1**) is obtained from lactam spiroaminal **2.124** by deprotection/hydrogenation, stereocenter inversion, triflate formation, and a Suzuki cross coupling to install the final pyrrole moiety. Metalation and addition of bromide **2.125** to malimide **2.119**, followed by acid-catalyzed imtramolecular *N*-acyliminium cyclization

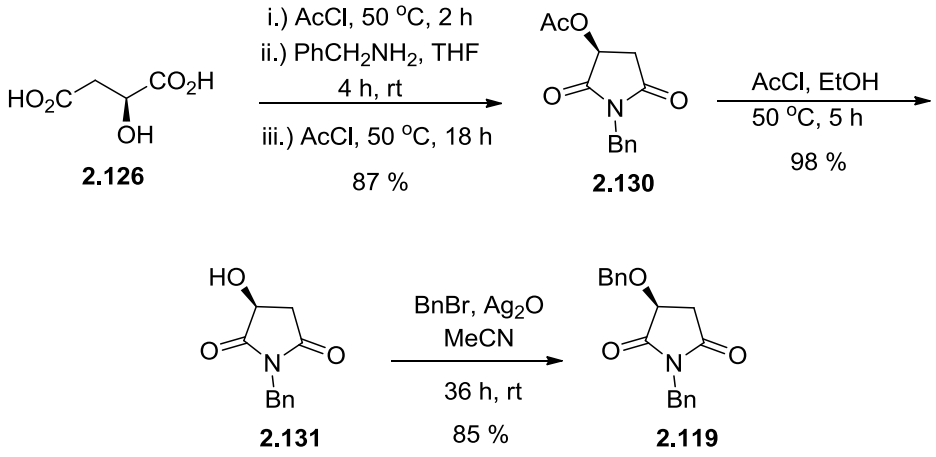
will provide lactam spiroaminal **2.124** as the thermodynamically favored product. Malimide **2.119** is readily obtained from (*S*)-2-hydroxy-succinic acid (**2.126**) by a known procedure.[42-44] Bromide **2.125** is synthesized by reduction, subsequent bromination, and ring-closing metathesis of acylpyrrole **2.127**. Addition of the pyrrolylanion of alkylpyrrole **2.130** to the pyridylthioester of acid **2.128** followed by sulfonamide protection will provide acylpyrrole **2.127**. Alkylpyrrole **2.108** is accessed through previously described transformations (Scheme 2.29), beginning with Grignard addition of 5-bromo-1-pentene to 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81**. Acid **2.128** is obtained through stereoselective alkylation of lactone **2.129**, lactone hydrolysis, and silyl protection of the resulting alcohol.



Scheme 2.34. Retrosynthesis of marineosin A utilizing *N*-acyliminium spirocyclization.

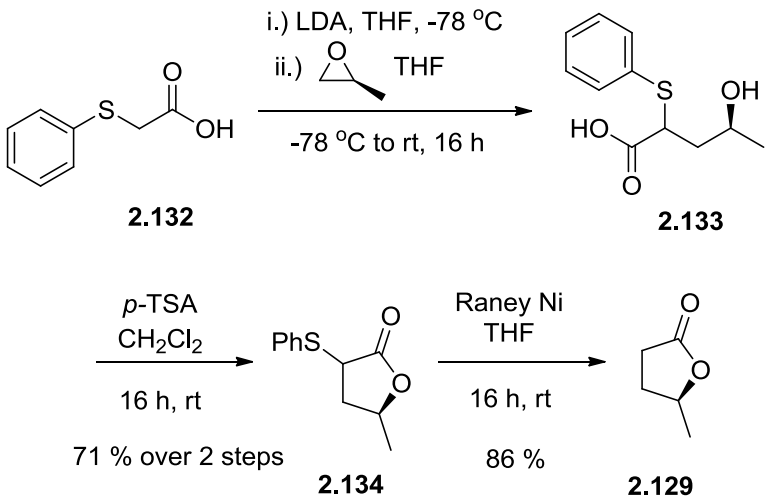
Malimide **2.119** was prepared in 73% overall yield by a known method which begins with a 3-step-1-pot sequence to give the malimide core (Scheme 2.35).[42-44] (*S*)-2-hydroxy-succinic acid (**2.126**) was suspended in excess acetylchloride and heated to

50 ^{o}C for 2 hours, then the mixture was concentrated and excess benzylamine in THF was added at the resulting oil. This mixture was stirred at room temperature for 4 hours, and then the solution was concentrated a second time. The remaining residue was resuspended in excess acetylchloride and heated at 50 ^{o}C for 18 hours. After concentrating the mixture a final time, the resulting oil was purified by flash chromatography to give acetate-protected hydroxy-malimide **2.130** in 87 % yield. Acid-catalyzed transesterification of malimide **2.130** with acetylchloride in ethanol, gave hydroxy malimide **2.131** in 98 % yield. The free hydroxyl group was subsequently protected using benzylbromide and silver (I) oxide to give benzyl ether **2.119** (85 %).



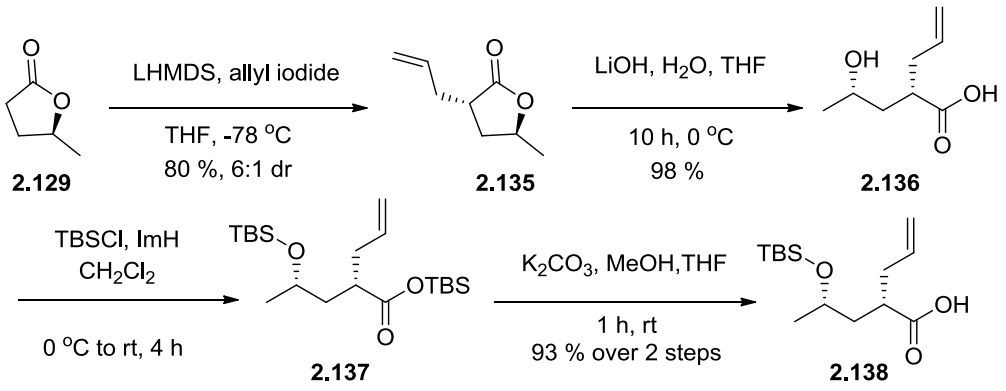
Scheme 2.35. Synthesis of malimide **2.119** from (*S*)-2-hydroxy-succinic acid.

Lactone **2.129** was readily obtained following a procedure developed by White and coworkers (Scheme 2.36).[45] Regioselective, basic epoxide-opening of commercially available (*S*)-2-methyloxiriane was achieved with the dianion of (phenylthio)acetic acid **2.132** yielding hydroxy-acid **2.133**. Acid-catalyzed lactonization of hydroxy-acid **2.133** with catalytic *p*-TSA gave α–thiophenyllactone **2.134** in 71% yield over 2 steps. Raney nickel desulfurization completed the synthesis of lactone **2.129** (86% yield).



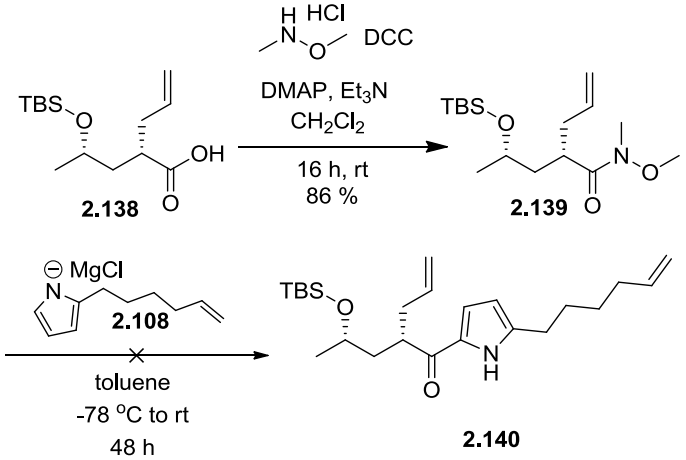
Scheme 2.36. Synthesis of lactone **2.129** from (phenylthio)acetic acid and (*S*)-2-methyloxirane.

In order to access acid **2.138**, lactone **2.129** was first treated with lithium hexamethyldisilazide then allyl iodide at -78 °C to afford α-allyl lactone **2.135** in 6:1 dr (anti:syn) and 80 % yield (Scheme 2.37). Hydrolysis of α-allyl lactone **2.135** with 1.1 equivalents of lithium hydroxide in THF/H_2O (7:1 v/v) at 0 °C for 10 hours provided hydroxy-acid **2.136** in 98% yield with essentially no racemization of the α–stereocenter. Extra equivalents of base or higher temperatures decreased the reaction time but led to considerable racemization. Hydroxy-acid **2.136** was then bis-protected as silylether/silylester **2.137** which was immediately purified and then chemoselectively deprotected to give acid **2.138** in 93 % yield over 2 steps.



Scheme 2.37. Synthesis of acid **2.138** from lactone **2.129**.

With acid **2.138** in hand and alkylpyrrole **2.108**, which was prepared as previously described in Scheme 2.29, it was time to start considering coupling conditions to yield acylpyrrole **2.140**. Since the ketone was the desired product following pyrrolylanion addition, utilization of a Weinreb amide was investigated first. Weinreb amide **2.139** was easily prepared from acid **2.138** by amide coupling with N,O-Dimethylhydroxylamine hydrochloride in 86 % yield (Scheme 2.38). Unfortunately, the amide proved to be completely unreactive to addition of the pyrrolylanion of alkylpyrrole **2.108**, even at room temperature and for extended reaction times.



Scheme 2.38. Unsuccessful addition of pyrrolylanion **2.108** to Weinreb amide **2.139** to form acylpyrrole **2.140**.

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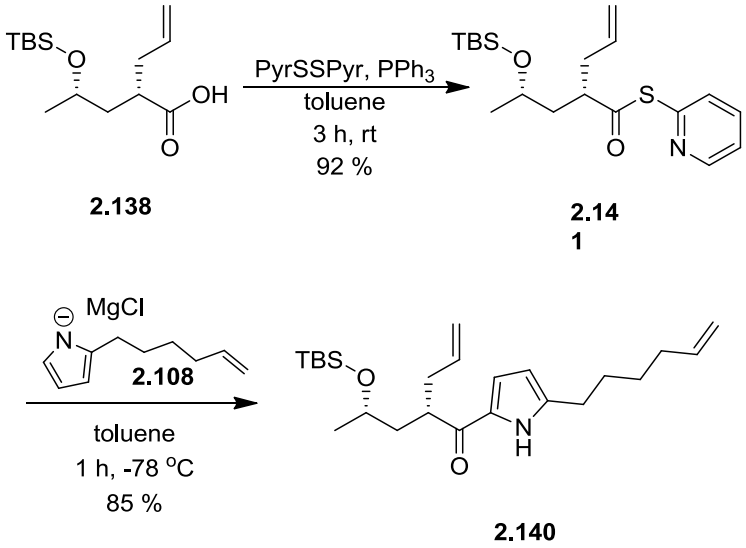
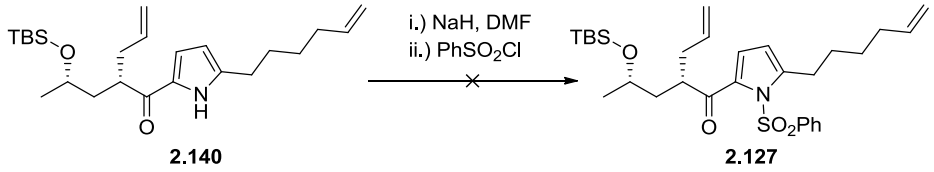
In light of this result, we decided to prepare a more reactive acylating reagent, a thioester. Thioester **2.141** was prepared from acid **2.138**, 2,2'-dipyridyldisulfide, and triphenylphosphine in 92 % yield (Scheme 2.39).[44] Thioester **2.141** was very stable; it could be purified by column chromatography and stored at 0 ^{o}C for three months without any appreciable decomposition. Addition of the pyrrolylanion **2.108** to thioester **2.141** in toluene at -78 ^{o}C gave the desired acylpyrrole **2.140** in 85 % yield. Attempts to combine the thioester formation and pyrrolylanion addition into a one-pot procedure were unsuccessful. Only unreacted thioester **2.141** and alkylpyrrole **2.108** were recovered from the reaction mixture; however, when the thioester was purified and then treated with pyrrolylanion **2.108**, addition proceeded smoothly with complete conversion.



Scheme 2.39. Synthesis of acylpyrrole **2.140** by pyrrolylanion addition to thioester **2.141**.

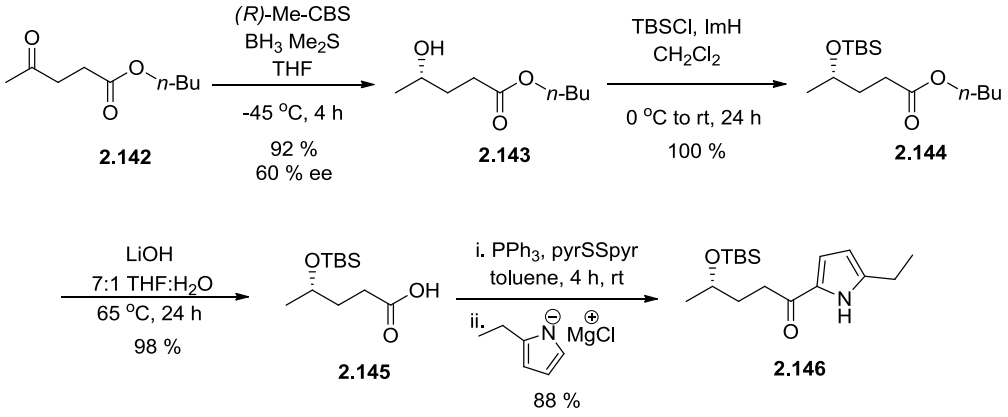
The next step was to protect the pyrrole nitrogen of acylpyrrole **2.140** with a suitable protecting group that would be compatible with organometallic reagents. Choice of a proper protecting group is critical since acylpyrrole **2.140** will become bromide

2.125 that must undergo oxidative addition to become the Grignard reagent. Since the phenylsulfonamide protecting group had proven robust in previous organometallic reactions, it was chosen as the ideal protecting group. Also, an electron-withdrawing protecting group should deactivate the electron-rich alkylpyrrole thus making it less likely to undergo undesirable side reactions. Traditional protection conditions were employed with acylpyrrole **2.140** (Scheme 2.40); however, no reaction occurred under these conditions.



Scheme 2.40. Attempt to protect acylpyrrole **2.140** using traditional protection conditions.

A model system was then designed in order to test protection conditions and to save the advanced intermediate acylpyrrole **2.140** (Scheme 2.41).

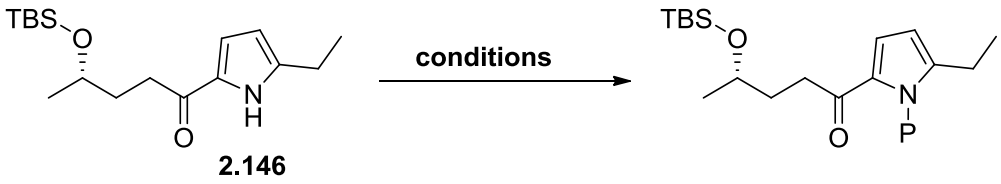


Scheme 2.41. Synthesis of model acylpyrrole **2.146** from butyl levulinate **2.142**.

Synthesis of the model system began with Corey-Bakshi-Shibata (CBS) reduction of butyl levulinate **2.142** to give alcohol **2.143** in 92 % yield but only 60 % ee (as determined by Mosher ester analysis)[47]. Since this was only a model system to determine

protection conditions, we decided to carry on with this modest enantiomeric excess. The free alcohol was protected to give silylether **2.144** (100 %) and then the ester hydrolyzed to give acid **2.145** in 98 % yield. Acid **2.145** was then treated with 2,2'-dipyridyldisulfide and triphenylphosphine to give the thioester, and the pyrrolylanion of commercially available 2-ethylpyrrole was added *in situ* giving model acylpyrrole **2.146** in 88 % yield for the one-pot procedure. With model acylpyrrole **2.146** in hand, we began investigating different protecting groups and conditions as listed in Table 2.3.

Table 2.3. Trial conditions for the protection of model acylpyrrole **2.146**.



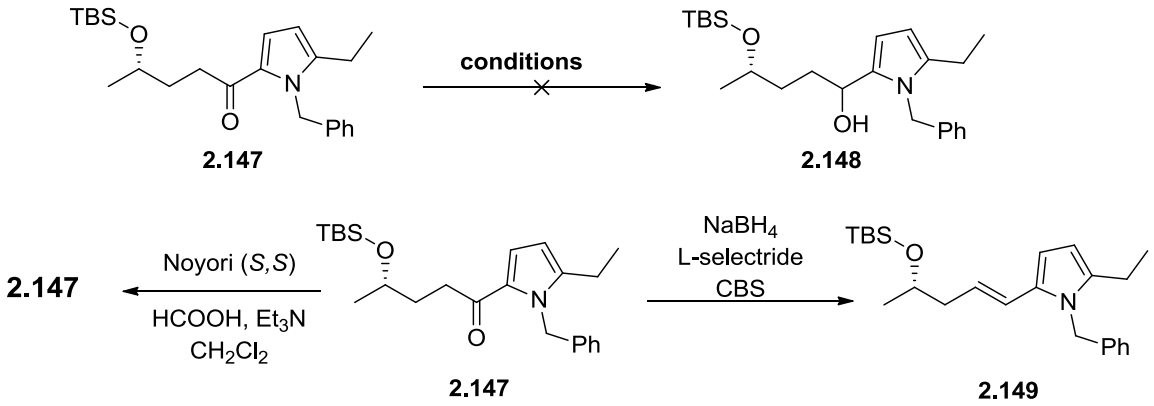
2.146

Entry	Base	Solvent	Electrophile	Additive	Method[a]	Yield (%)[b]
1	NaH	DMF	$PhSO_2Cl$	---	A	0
2	NaH	DMF	$TolSO_2Cl$	---	A	0
3	NaH	DMF	$TolSO_2OO_2STol$	---	A	0
4	NaH	DMF	$PhSO_2Cl$	---	B	0
5	NaH	THF	$PhSO_2Cl$	---	A	0
6	NaH	THF	$PhSO_2Cl$	15-crown-5	C	0
7	NaH	DMF	$MeSO_2Cl$	---	A	0
8	KOtBu	THF	$MeSO_2Cl$	18-crown-6	C	0
9	Collidine	DCM	$MeSO_2Cl$	---	A	0
10	BuLi	THF	$TolSO_2OO_2STol$	---	D	0
11	BuLi	THF	$PhSO_2Cl$	---	D	0
12	LiHMDS	THF	$TolSO_2OO_2STol$	---	D	0
13	LiHMDS	THF	$PhSO_2Cl$	---	D	0
14	DBU	DCM	$PhSO_2Cl$	---	A	0
15	Cs_2CO_3	MeCN	$PhSO_2Cl$	---	E	0
16	Et_3N	MeCN	$TolSO_2Cl$	DMAP	F	0
17	NaH	DMF	BnBr	---	A	95

[a] Method A: Base is added to solution of pyrrole at rt and stirred for 0.5 h, then electrophile is added and stirred at rt for 2 h. Method B: Same as A, except stirred 8 h after addition of electrophile. Method C: Base is added to a solution of pyrrole and additive at rt and stirred for 0.5 h, then electrohphile is added and stirred at rt for 2 h. Method D: Base is added to solution of pyrrole at 0 ^oC and stirred for 0.5 h, then electrophile is added the reaction is warmed to rt and stirred for 2 h. Method E: Same as method A, but instead of stirring at rt, the mixture is heated to 80 ^oC. Method F: Same as method B, but reaction was stirred for 16 h.
[b] isolated yield of desired protected product.

Protection of the acylpyrrole **2.146** with an electron-withdrawing sulfonamide group proved highly unsuccessful; however, Entry 17 shows that protection as the benzylamine was not only successful, but high yielding (95 %). With *N*-benzylacylpyrrole **2.147** in hand, we decided to investigate reduction conditions with this

readily available model system to provide alcohol **2.148**. Unfortunately, every reduction attempted ($NaBH_4$, L-selectride, CBS) resulted in the elimination product **2.149** as the only isolated product (Scheme 2.37). Noyori transfer hydrogentation gave no product formation even after extended reaction times (7 days).

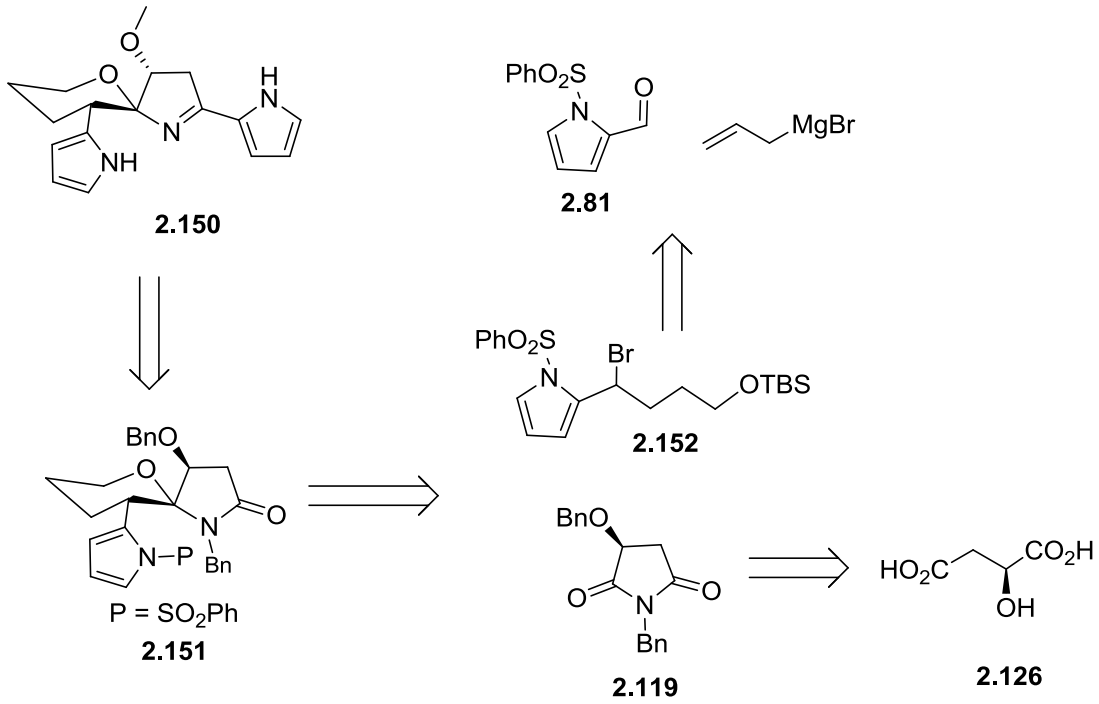


Scheme 2.42. Attempted reduction of N-benzylacylpyrrole **2.147** to alcohol **2.148**.

In light of these results, it was concluded that the benzyl protecting group was not suited for these transformations. All of the sulfonamide protection conditions (Entries 1-16) for the model system listed in Table 2.3 were then tried with the actual system (acylpyrrole **2.140**). When none of the conditions successfully protected the pyrrole, we tried reducing acylpyrrole **2.140** to the alcohol with no protecting group. This, too, only resulted in the elimination product.

Prior to adjusting the synthesis further, we decided to develop a model system to test the validity of the key core-forming addition to malimide **2.119** followed by acid-catalyzed spirocyclization. The inclusion of a sulfonamide protected-pyrrole was crucial, since a naked benzene or furan could not adequately represent the electronic and steric properties of a benzenesulfonamide-protected pyrrole (Scheme 2.43). Bromide **2.76** is a

simplified version of bromide **2.125** (Scheme 2.34). The model bromide lacks the macrocyclic ring, so it requires a much simpler synthesis. Also, bromide **2.152** contains a primary alcohol instead of a secondary alcohol, so there are no diastereomers to complicate NMR interpretation.

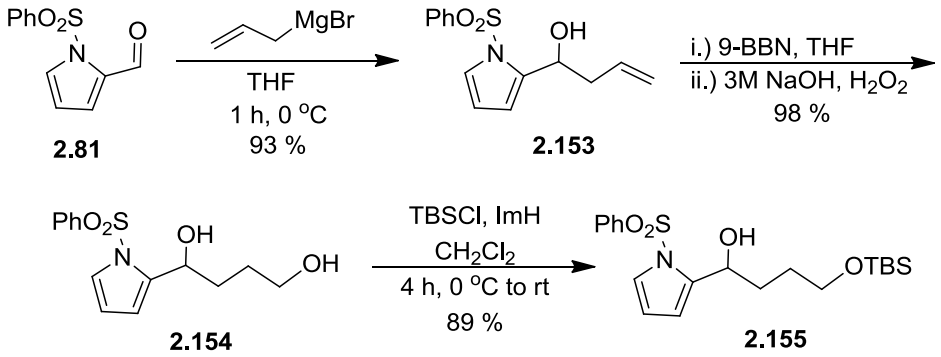


Scheme 2.43. Retrosynthetic analysis for model synthesis of the spiroiminal core **2.150** of marineosin A.

As outlined in Scheme 2.43, model marineosin A (**2.150**) is obtained from spiroaminal **2.151** through a Mitsunobu inversion, triflate formation and Suzuki cross-coupling. Spiroaminal core **2.151** is prepared by Grignard addition of bromide **2.152** to malimide **2.119** and subsequent acid-catalyzed spirocyclization of the intermediate N-acyliminium ion. Malimide **2.119** is readily available from 2-(*S*)-hydroxysuccinic acid **2.126** (Scheme 2.35). Bromide **2.152** is synthesized by addition of allylmagnesiumbromide to 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81**, followed

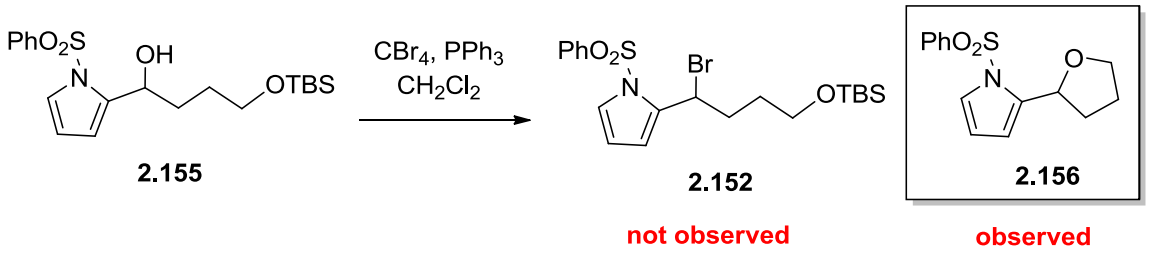
by hydroboration/oxidation, and mono-silylether protection of the resulting primary alcohol.

The seemingly straightforward synthesis of bromide **2.152** proved to be quite a challenge. Grignard addition of allylmagnesiumbromide to 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81** provided homoallylic alcohol **2.153** in 93 % yield (Scheme 2.44). Subsequent hydroboration/oxidation sequence gave diol **2.154** in excellent isolated yield (98 %). Mono-protection of the primary alcohol as the *tert*-butyldimethylsilylether yielded alcohol **2.155** as the only product (89 %).



Scheme 2.44. Synthesis of alcohol **2.155** from 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81**.

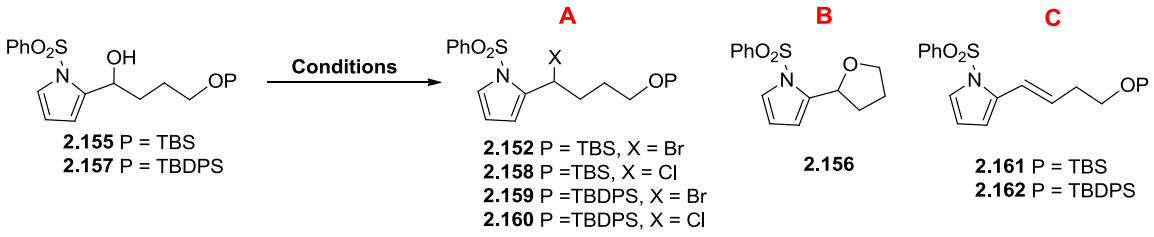
In order to prepare bromide **2.152**, alcohol **2.155** was added to a solution of carbon tetrabromide and triphenylphosphine in dichloromethane. Unfortunately, the only product obtained in quatitative yield was tetrahydrofuran **2.156** (Scheme 2.45).



Scheme 2.45. Formation of tetrahydrofuran **2.156** as an undesired side product in an attempted bromination of alcohol **2.155**.

The observed product formation is a result of silylether cleavage by hydrogen bromide formed *in situ* and subsequent cyclization by intramolecular displacement of the resulting bromide, or the phosphonium salt intermediate, by the free primary alcohol. In light of this occurrence, an investigation of bromination conditions was initiated (Table 2.4).

Table 2.4. Investigation of bromination conditions.

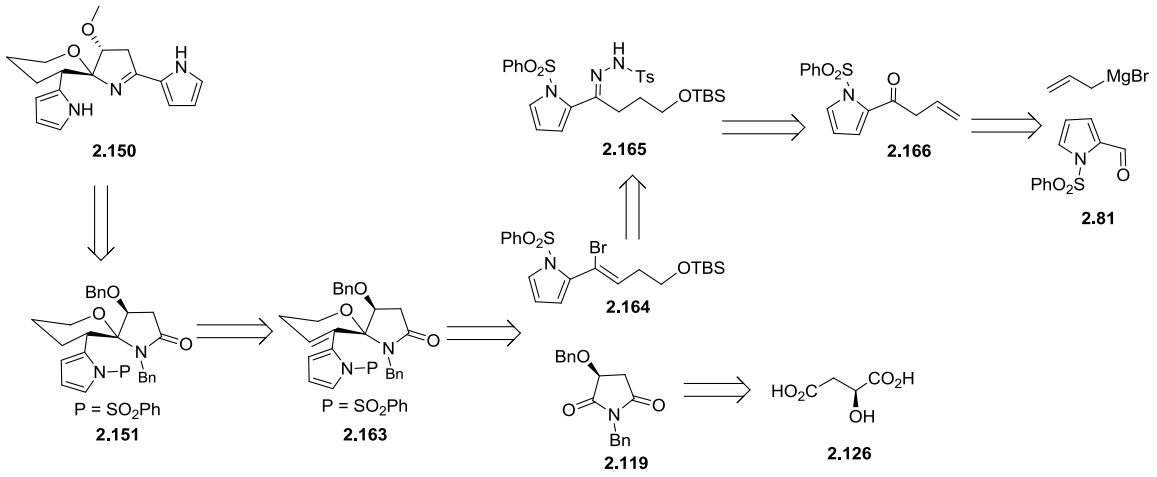


Substrate	Conditions	Product Distribution A : B : C
2.155	PPh_3, CBr_4, CH_2Cl_2	0 : 100 : 0
2.155	Ph_3PBr_2, THF:pyr (6:1)	0 : 15 : 85
2.155	DPPE, Br_2, CH_2Cl_2	0 : 0 : 100
2.155	Ph_3PCl_2, CH_2Cl_2	0 : 10 : 90
2.157	PPh_3, CBr_4, CH_2Cl_2	0 : 0 : 100
2.157	Ph_3PBr_2, THF:pyr (6:1)	0 : 0 : 100
2.157	DPPE, Br_2, CH_2Cl_2	0 : 0 : 100
2.157	Ph_3PCl_2, CH_2Cl_2	0 : 0 : 100

Attempted bromination with triphenylphosphine dibromide in the presence of pyridine as a proton sponge inhibited tetrahydrofuran formation; however, none of the desired product was formed. Instead of bromide **2.152**, alkene **2.161** was the major product with minor tetrahydrofuran formation. The next bromination conditions tested were 1,2-Bis(diphenylphosphino)ethane (DPPE) (0.5 equiv) and bromine (1 equiv) in dichloromethane. The use of DPPE instead of triphenylphosphine generally results in

simpler purification and decreased elimination side products.[48] Unfortunately, these conditions still gave alkene **2.161** as the sole product. An attempt to use neutral chlorination conditions also failed to deliver the desired halogenated product **2.158**. The *tert*-butyldimethylsilyl ether protecting group was then replaced by a *tert*-butyldiphenylsilylether (**2.157**), which is much more robust in acidic media. Unfortunately, the TBDPS group only served to prevent tetrahydrofuran formation completely and to provide the undesired elimination product, alkene **2.162** as the only product.

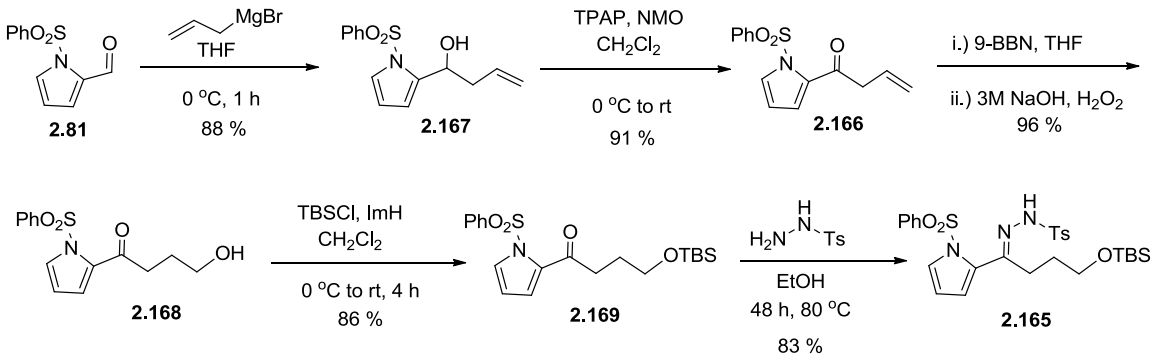
With these results, it was decided that if the α-bromide could actually be formed, it would be unstable and possess a highly thermodynamically stable elimination pathway. Also, if this sp^3 bromide was subjected to magnesium metal to undergo oxidative addition, the resulting organometallic reagent would possess a highly favorable β– elimination pathway. In light of these conclusions, it was decided to take advantage of the stability of alkene **2.161** by preparing an α-pyrrolo-vinylbromide that could later be hydrogenated to provide marineosin core **2.151**. A Shapiro reaction was chosen as the key reaction to provide the vinyl bromide since the required hydrazone could readily be synthesized using conditions that have already been optimized for this pyrrole substrate. The modified retrosynthetic route is described in Scheme 2.44.



Scheme 2.46. Retrosynthesis of model marineosin core **2.151** by Grignard addition of vinylbromide **2.164**.

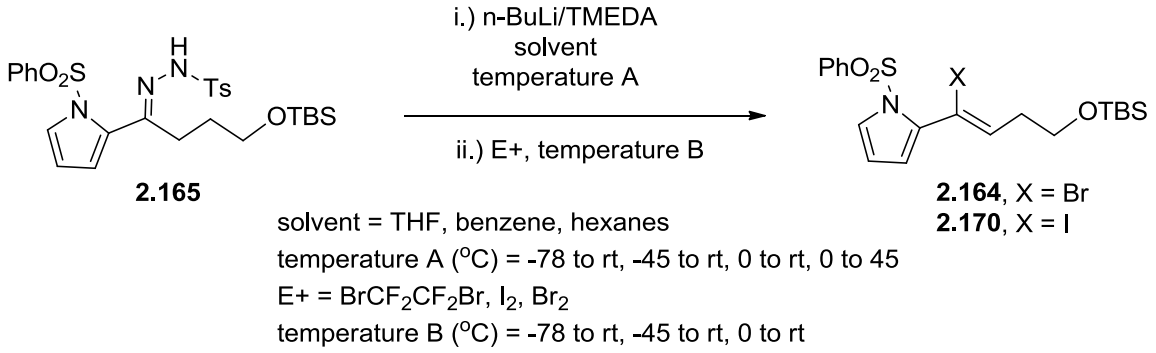
Vinylbromide **2.164** will undergo a Grignard reaction with malimide **2.119** to provide unsaturated spiroaminal **2.163**, which can be hydrogenated to the desired model marineosin core **2.151**. The substrate for the Shapiro reaction, tosylhydrazone **2.165**, is synthesized by hydroboration/oxidation of ketone **2.166**, silyl protection of the primary alcohol, and subsequent hydrazone formation. Ketone **2.166** is prepared by Grignard addition of allylmagnesium bromide to 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81** and oxidation of the resulting alcohol.

The synthesis of tosylhydrazone **2.165** proved straightforward and highly efficient (Scheme 2.47). Grignard addition of allylmagnesium bromide to 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81** gave the secondary homoallylic alcohol **2.167** in 88 % yield. Ley oxidation rapidly provided ketone **2.166** in 91 % yield, and hydroboration/oxidation of the terminal alkene gave a 96 % yield of primary alcohol **2.168**. Protection as the silylether (**2.169**) proceeded in 86 % yield and subsequent condensation with tosylhydrazide gave tosylhydrazone **2.165** in 83 % yield.



Scheme 2.47. Synthesis of tosylhydrazone **2.165** from 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde **2.81**.

With tosylhydrazone **2.165** in hand, it was time to experiment with conditions for the Shapiro reaction (Scheme 2.48).



Scheme 2.48. Shapiro reaction with tosylhydrazone **2.165** in an attempt to form vinylbromide **2.164** or vinyliodide **2.170**

Unfortunately, every temperature, solvent, and electrophile combination failed to provide the desired vinylbromide **2.164** or vinyliodide **2.170**. It was concluded that perhaps the hydrazone was too sterically hindered or simply too stable to undergo the required fragmentation reaction, since in every case, only starting material was recovered, with no evidence of the desired vinylbromide or even the alkene that would result from

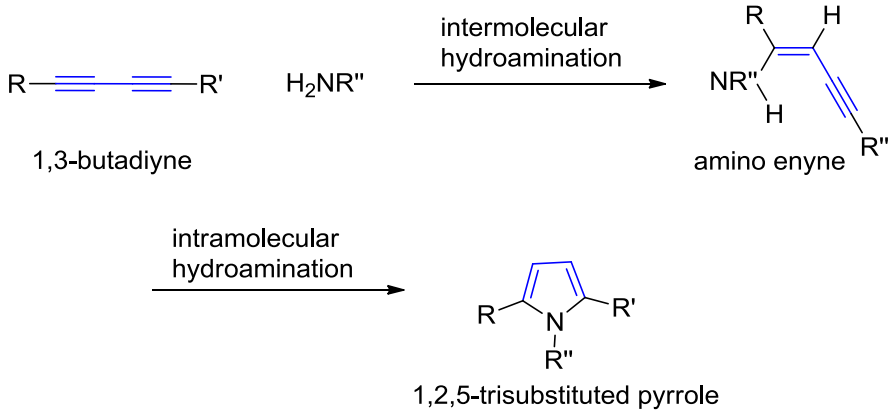
hydrogen abstraction and/or unsuccessful electrophilic attack.

At this point, we decided to rethink the use of an α-nucleophilic pyrrole for the regioselective addition to (*S*)-hydroxy malimide **2.119** to form the spriocyclic core.

2.9.2. Hydroamination for Late-Stage Pyrrole Formation.

With all the problems encountered performing simple tranformations on pyrrole-based substrates, we decided to develop a synthetic approach that called for a late-stage pyrrole installation.

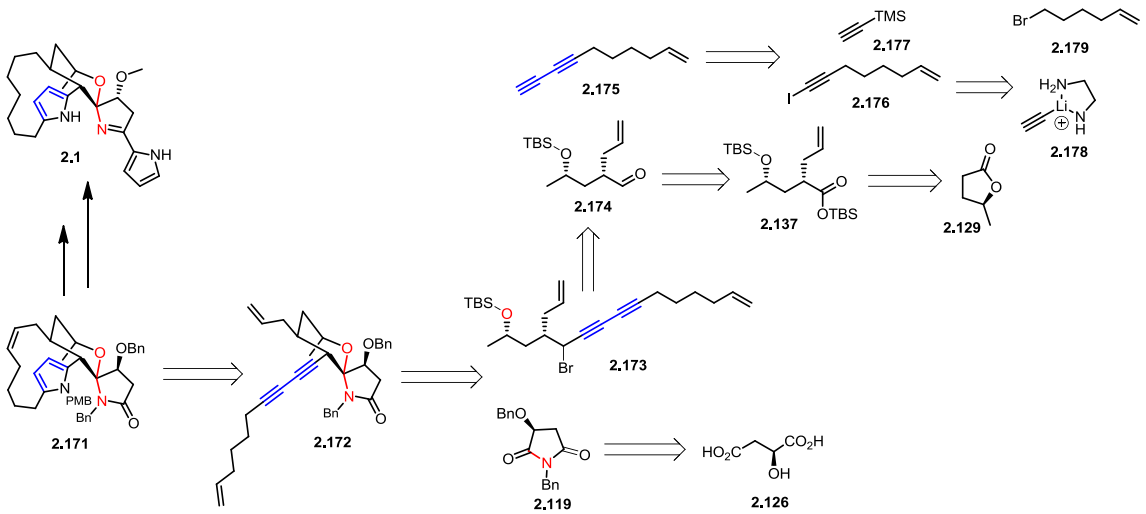
In 2010, Zheng and Hua reported conditions for the formation of 1,2,5-trisubstituted pyrroles from 1,3 diynes using cuprous chloride and a primary amine (Scheme 2.49).[49] Since this was the necessary pyrrole substitution pattern and a 1,3-diyne could easily be incorporated into a previously established route, this method was ideal.



Scheme 2.49. 1,2,5-substituted pyrrole synthesis by intermolecular hydroamination followed by intramolecular hydroamination.

2.9.3. Addition of a Diyne to (S)-Hydroxy Malimide.

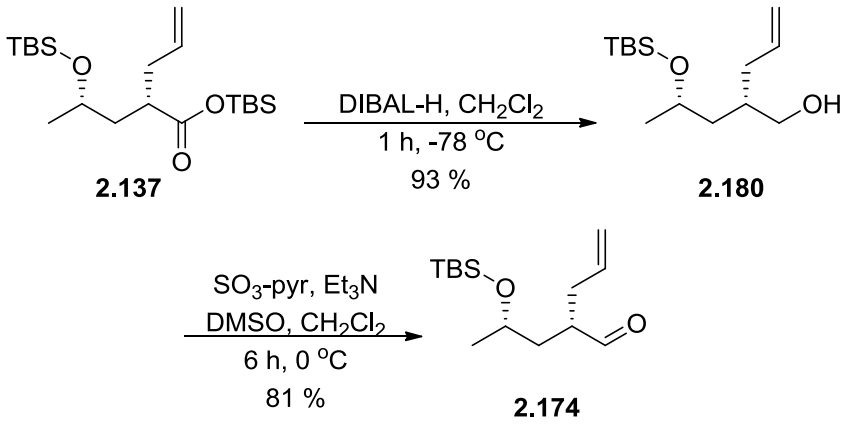
Using this hydroamination methodology, a diynyl bromide would replace the α-bromopyrrole as the Grignard reagent in this new synthesis as outlined in Scheme 2.51.



Scheme 2.51. Retrosynthesis of marineosin A (**2.1**) from a 1,3-diyne pyrrole precursor **2.173**.

Unsaturated marineosin core **2.171** could be prepared using Zheng-Hua diyne hydroamination strategy followed by a ring-closing methathesis reaction.[49] Spiroaminal **2.172** would be achieved through Grignard addition of diynyl bromide **2.173** to malimide **2.119**. diynyl bromide **2.172** could be provided by addition of the deprotonated acetylide **2.175** to aldehyde **2.174**, which is prepared from lactone **2.129**. Diyne **2.175** is prepared by a copper-catalyzed Cadiot-Chodkiewicz alkyne-alkyne cross-coupling with ethynyltrimethylsilane **2.177** and iodoacetylene **2.176** followed by silane deprotection. Lithium acetylide (**2.178**) addition to 6-bromo-1-hexene **2.179**) followed by iodination should provide iodoacetylene **2.176**.

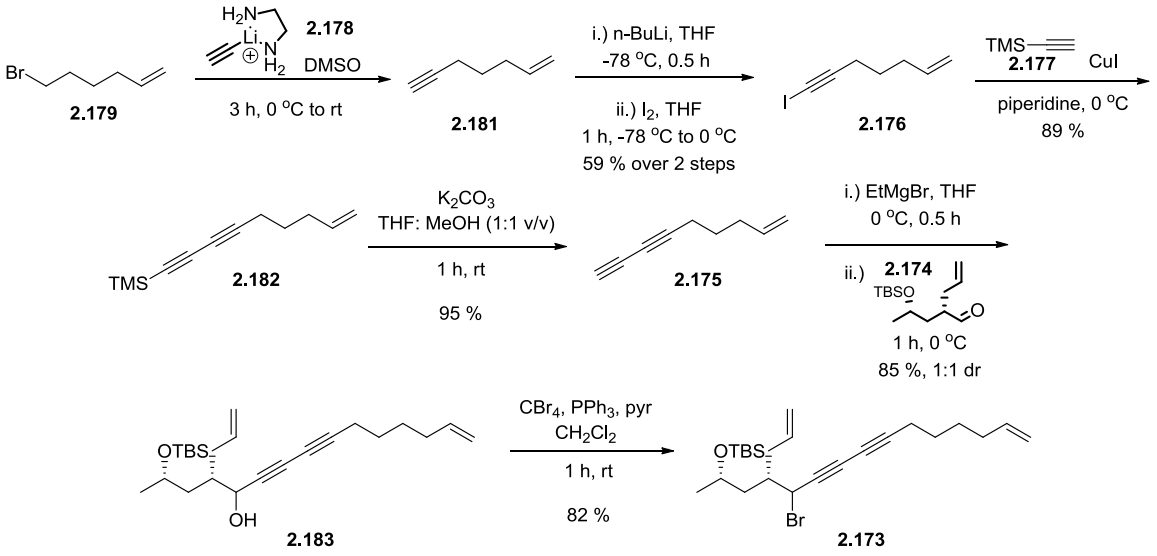
The forward synthesis began with the preparation of aldehyde **2.174** (Scheme 2.52) from previously prepared (Scheme 2.37) TBS ester **2.137.** Ester **2.137** is treated with diisobutylaluminumhydride (DIBAL-H) to provide alcohol **2.180** in 93 % yield. Parikh-Doering oxidation of alcohol **2.180** completed the synthesis of aldehyde **2.174** (81% yield).



Scheme 2.52. Preparation of aldehyde **2.174** from previously prepared ester **2.137**.

The synthesis of 1,3-diyne **2.175** proved equally as straightforward (Scheme 2.53). Nucleophilic substitution of 6-bromo-1-hexene **2.179** with commercially available lithium acetylide ethylenediamine complex **2.178** gave terminal acetylene **2.181**. Iodination of the terminal acetylene by deprotonation with *n*-butyl lithium and quenching of the resulting anion with iodine provided iodoacetylene **2.176** in 59 % yield over 2 steps. A Cadiot-Chodkiewicz cross-coupling of iodoacetlyene **2.176** and ethynyltrimethylsilane **2.177** with Alami's improved conditions gave the TMS-protected 1,3-diyne **2.182** in excellent yield (89 %).[50] Silane deprotection by basic methanolysis provided the terminal 1,3-diyne (**2.175**) in 95 % yield. Treatment of 1,3-diyne **2.175** with a ethyl magnesium bromide provided the acetylide Grignard reagent, which was then added to aldehyde **2.174** at 0 °C to provide diynyl alcohol **2.174** in 85 % isolated yield (1:1 dr). At this point in the synthesis the diastereomers from the allylation reaction are easily separated and the reported yield of 85 % is for the major diastereomers *syn, syn, anti* and *syn, syn, syn*). The configuration of the stereocenter formed in the addition of diyne **2.175** to aldehyde **2.174** is not important, since this center will become the

nucleophilic center of the Grignard reagent. Since Grignard reactions can follow anionic or radical addition pathways, it is very likely that this stereocenter will racemize. Bromination of diynyl alcohol **2.183** with carbon tetrabromide, triphenylphosphine, and pyridine in dichloromethane readily provided diynyl bromide **2.173** in 82 % isolated yield (Scheme 2.53). It is important to note that without the pyridine additive (4 equiv.), the silylether was cleaved and the cyclized tetrahydrofuran was the only product observed, as with the pyrrole substrate (Scheme 2.45).

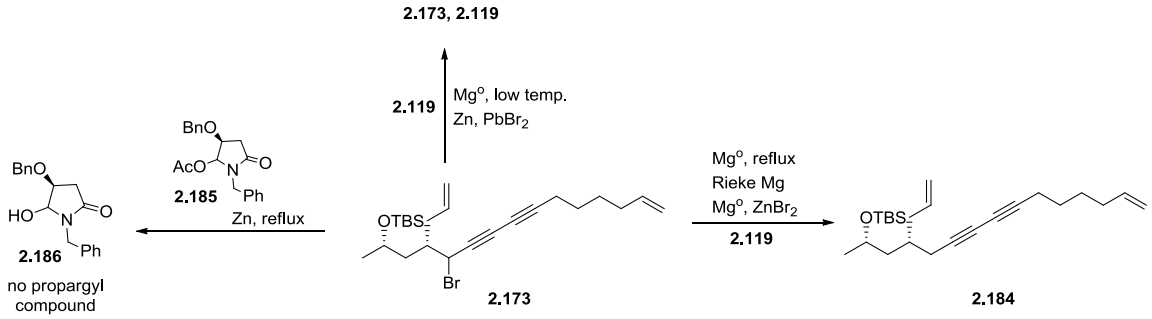


Scheme 2.53. Synthesis of 1,3 diyne **2.175**, subsequent addition to aldehyde **2.174**, and bromination of the resulting alcohol **2.183** to provide diynyl bromide **2.173**.

Diynyl bromide **2.173** was only stable at room temperature for approximately 4 days. After one week, the bromide had completely decomposed. Fortunately, if kept at -35 °C, the bromide was stable for about three weeks. With diynyl bromide **2.173** in hand, it was time to begin exploring conditions for the addition to malimide **2.119**.

Since work by Huang had shown that numerous Grignard reagents could regioselectively add into malimide **2.119**,[41] we first chose to convert diynyl bromide **2.173** to a magnesium Grignard reagent. Stirring the **2.173** with magnesium metal in THF

at -78 ^{o}C followed by addition of malimide **2.119** provided no product formation. diynyl bromide **2.173** and malimide **2.119** were the only compounds recovered (Figure 2.5). Such a low temperature was chosen to avoid β-elimination once the organometallic complex had formed. Since a highly conjugated ene-diyne compound would be formed upon β-elimination, it was considered to be a very likely side reaction. The elimination product was not observed at -78 ^{o}C, and since the reaction failed to initiate, the decision was made to raise the temperature. At 0 ^{o}C in THF and diethyl ether, the same result was observed. Since the lack of reactivity was caused by an inability to initiate, the magnesium/diynyl bromide **2.173**/THF or ether solutions were heated to reflux for 1 hour and then cooled to 0 ^{o}C and malimide **2.119** was added. Unfortunately, no addition was observed with these reactions either. However, instead of recovering diynyl bromide **2.173**, only des-bromo diyne **2.184** was obtained (Scheme 2.54). Interestingly, none of the β-elimination product was recovered, only the dihydro diynyl compound **2.184**. Since this side reaction could be due to hydrogen abstraction from the solvent, it was decided to use a Barbier method of addition. Although the Grignard reagent was forming, it was given enough time to abstract a hydrogen atom before the electrophile was added. If the two reagents were combined, then as soon as the Grignard reagent forms, it should react with the electrophile available in solution.diynyl bromide **2.173** and malimide **2.119** were combined in THF and added to magnesium metal. This solution was rapidly stirred and heated to reflux for 2 hours. Unfortunately, the same result was observed: des-bromo diyne **2.184** and malimide **2.119** were the only isolated products.



Scheme 2.54. Summary of diynyl bromide (**2.173**) additions to malimide **2.119** and reduced malimide **2.185.**

Since malimide **2.119** is much less reactive than a traditional electrophile, like an aldehyde, it was theorized that the diynyl Grignard was forming, but the hydrogen abstraction pathway proceeds much faster than addition to the sterically hindered imide. To test this theory, malimide **2.119** was replaced with *p*-methoxybenzaldehyde. Surprisingly, none of the expected alcohol was observed, only des-bromo diyne **2.184** and *p*-methoxybenzaldehyde were isolated. At this point, it was concluded that diynyl bromide **2.173** was the main problem, since it was unable to react with a simple electrophile. It was decided to investigate different types of additions besides the traditional Grignard. First, Rieke magnesium was used to generate the organometallic reagent using a Grignard addition procedure and a Barbier addition procedure, both carried out at -78 ^{o}C. Rieke magnesium is traditionally used for difficult Grignard reagents, such as secondary benzyl and allyl halides, so it was chosen for these properties. Unfortunately, the only isolated products were malimide **2.119** and des bromo diyne **2.184**. This time, the des-bromo compound **2.184** was observed at -78 ^{o}C, which was not the case with magnesium ribbon where no reaction occurred. So it was concluded that the Grignard reagent was able to initiate at a much lower temperature with Rieke magnesium, but that hydrogen abstraction was still the favored pathway. The next

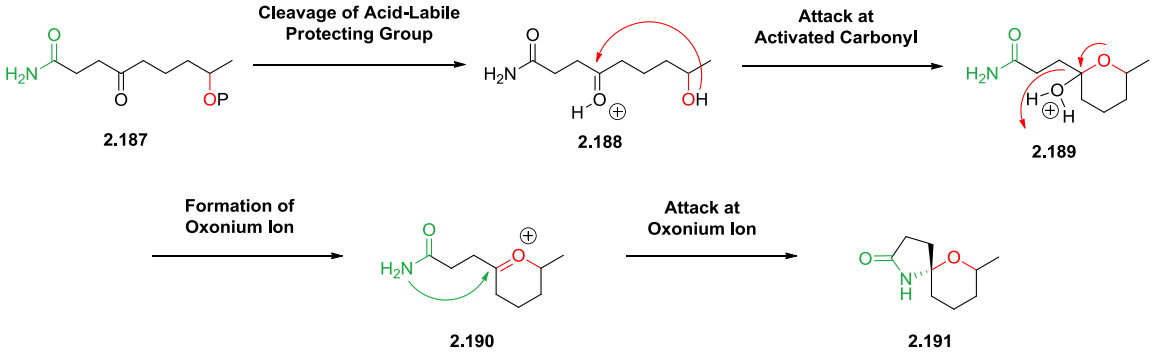
approach was described by Kobayashi where zinc dibromide is added to magnesium metal, propargyl bromide, and ether at room temperature.[51] These conditions are optimized to make a hydrogen abstraction pathway less favorable by preventing magnesium bromide salts from irreversibly crashing out of solution; however, these conditions did not work with our system. Once again, the dihydro diynyl compound **2.184** was isolated. The next conditions tested were developed by Kim and Han for use with propargyl halide additions to cyclic imides.[52] Diynyl bromide **2.173**, malimide **2.184**, zinc dust, and lead II bromide were suspended in THF and stirred at room temperature. After 48 hours, only starting material was recovered. Once it was determined that the Kim-Han procedure was unsuccessful, a method by Jiang and Tian that utilizes propargyl zincates to displace acetate from a reduced hydroxymalimde (**2.185**) was utilized.[53] The diynyl bromide **2.173** was combined with acetoxyamide **2.185** and zinc dust in THF and heated to reflux for 18 hours (Scheme 2.54). When the reaction was quenched, the only isolated product was deprotected hydroxymalimide **2.186**. No diynyl bromide was recovered, so it is highly likely this relatively unstable starting material decomposed under the reaction conditions. In light of these results, it was concluded that the instability of diynyl bromide **2.173** combined with its steric bulk were preventing it from successfully undergoing addition to the malimide **2.119**

. The addition/acid-catalyzed N-acyliminium intramolecular spirocyclization method for spiroaminal synthesis may highly successful with substrates like primary halides that do not readily eliminate, undergo rapid abstraction pathways, or have significant steric bulk. Unfortunately, our diynyl bromide **2.173** proved incompatible with the conditions necessary to achieve this addition. Since this route to access the

marineosin core structure has proven unsuccessful to date, a new synthesis that involves acyclic key intermediates and a late-stage lactamization of a hydroxyketoamide has been envisaged.

2.10 Acid-catalyzed Spirocyclization of a Hydroxyketoamide for the Synthesis of Marineosin A.

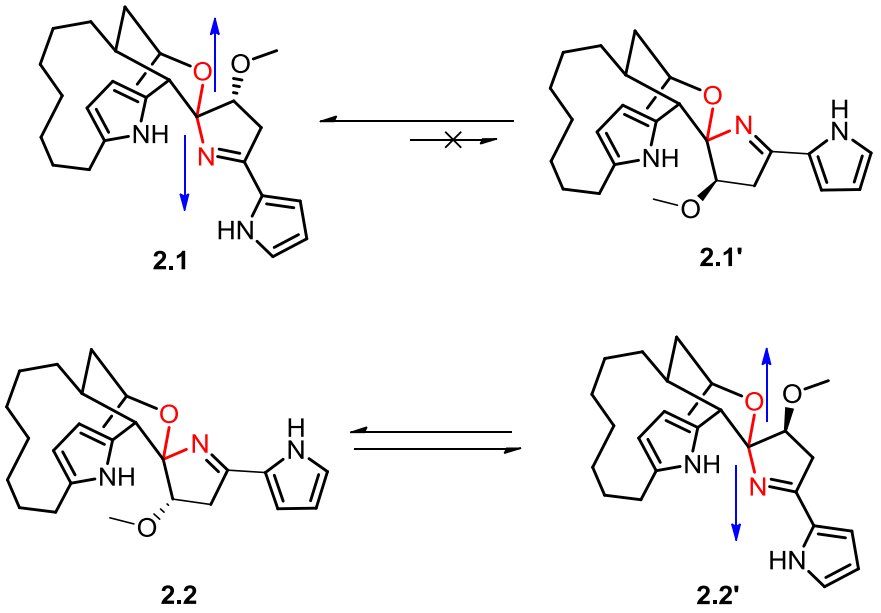
Cyclization of hydroxyketoamides to form spiroaminals containing amide moieties has proven highly successful in the synthesis of complex natural products.[54,55]. These routes generally involve concomitant removal of acid labile protecting groups to reveal the reactive functional groups and subsequent acid-catalyzed spirocyclization, which can significantly improve the efficiency of a complex synthesis.[55]



Scheme 2.55. Mechanism of the acid-catalyzed lactamization of a hydroxyketoamide.

As described in Scheme 2.55, the spirocyclization reaction begins by treating protected hydroxyketoamide **2.187** with acid to remove the acid-labile alcohol protecting group. The free alcohol then adds to the activated carbonyl to preferentially form ketal **2.189.** Subsequent loss of water generates oxonium ion **2.190**, which is quenched by intramolecular attack of the primary amide to give spiroaminal **2.191**.

One caveat of this method of spirocyclization is that the conformation of the spiroaminal center cannot be controlled; the compound will adopt the most thermodynamically stable conformation. If the natural product does not exist as the most stable conformer, then this method of spirocyclization would be ineffective. Molecular modeling studies can assist in determining the ability of this methodology to provide the desired compound by predicting the most stable conformations, and are commonly employed for this task.[55]



Scheme 2.56. Marineosins A (**2.1**) and B (**2.2**) and alternative spirocenter conformations (**2.1'** and **2.2'**).

In the case of marineosins A (**2.1**) and B (**2.2**), marineosin A should be the most thermodynamically favored product. In both marineosin diastereomers, the *trans*-fused macrocyclic ring is placed in the equatorial position in the chair conformation of the cyclohexane ring. Also, the methoxy substituent is positioned away from the macrocyclic ring in both marineosin A and B, which may favor formation of these two stereoisomers over the other two possible diastereomers (**2.1'** and **2.2'**). Finally, the nitrogen of the

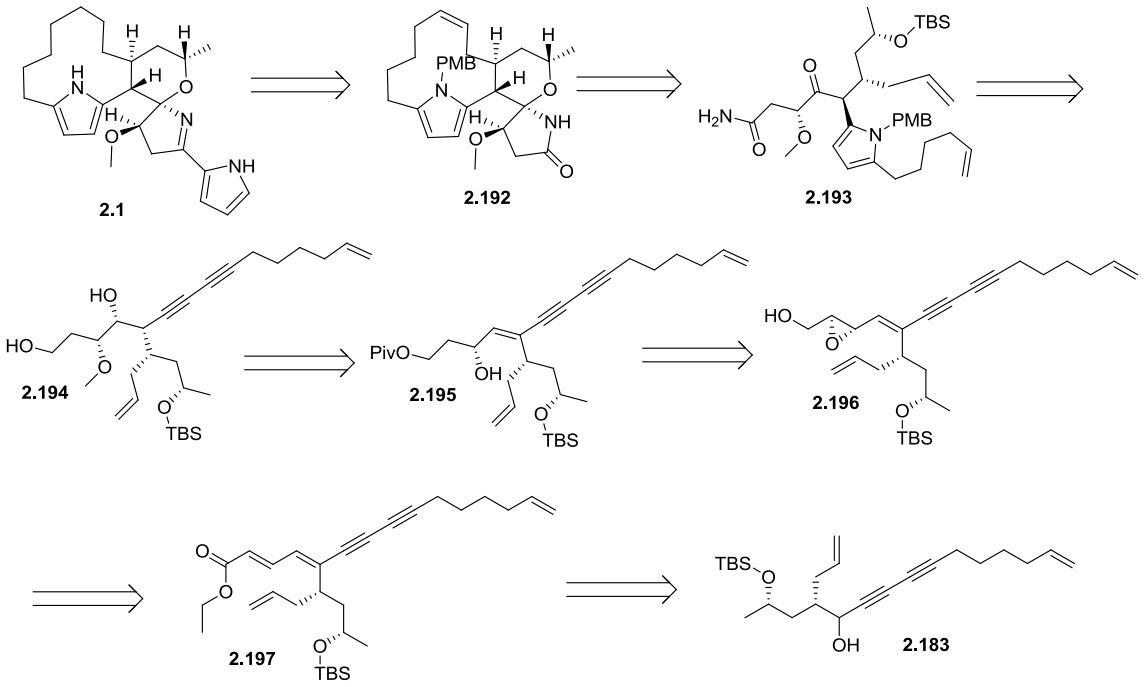
spiroiminal center of marineosin A is placed in the axial position and stabilized by the anomeric effect, making this conformation more favorable than one in which the nitrogen is in the non-anomerically stabilized equatorial position (**2.1'**). This should facilitate formation of the desired marineosin A isomer as the most thermodynamically favored product in our acid-catalyzed spirocyclization. Preference for formation **2.2** over **2.2'** may prove more difficult due to the balance between decreasing the steric hindrance by adapting a conformation that positions the methoxy group away from the macrocyclic ring (**2.2**) versus the electronic stabilization provided by of the anomeric effect (**2.2'**). By enantioselectively preparing the hydroxyketoamide for the formation of the marineosin A isomer (which differs from marineosin B at the methoxy chiral center and spirocenter), we should obtain the desired, biologically active, marineosin A stereoisomer upon acidic spirocyclization.

2.10.1. Diyne Substrates for Hydroxyketoamide Synthesis.

Based on the success of acid-catalyzed spirocyclizations of hydroxyketoamides to produce spiroaminals in the total synthesis of natural products, a route was devised which incorporates this key reaction to provide marineosin A (Scheme 2.57).

Unsaturated marineosin A core structure **2.192** can be prepared by the key acid-catalyzed spirocyclization of hydroxyketoamide **2.193** and an RCM reaction. Hydroxyketoamide **2.193** is prepared using the hydroamination methodology developed by Hua and co-workers with a 1,3 diyne,[49] which is accessed by oxidation and amide formation from diol **2.194**. Diol **2.194** is derived from allylic alcohol **2.195** through an asymmetric epoxidation, methylation, and concomitant pivolate deprotection/epoxide

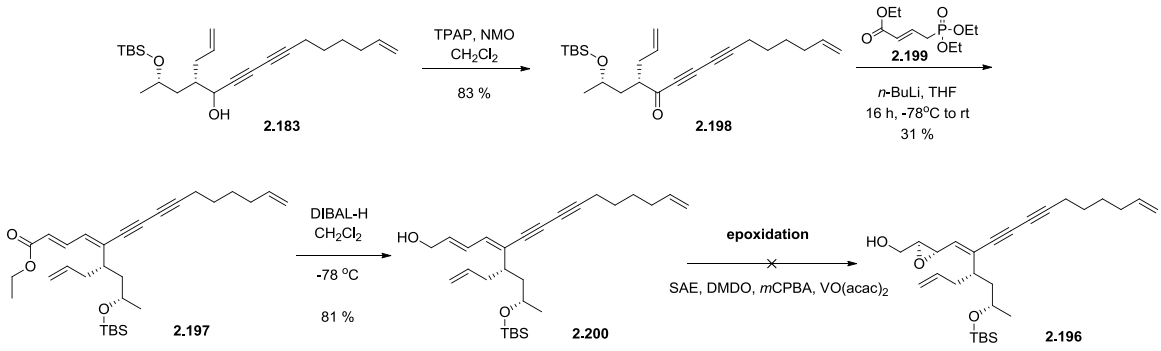
opening to the 1,2-diol with DIBAL-H.[56] Allylic alcohol **2.195** can be prepared from hydroxyepoxide **2.196** by epoxide opening with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) to give the 1,3-diol and subsequent pivolate protection of the primary alcohol.[56] Reduction of ester **2.197** and asymmetric epoxidation should provide epoxide **2.196**. Ester **2.197** could readily be obtained from previously prepared diynyl alcohol **2.183** by oxidation and olefination of the resulting ketone.



Scheme 2.57. Retrosynthesis of marineosin A (**2.1**) by spirocyclization of hydroxyketoamide **2.193**.

The forward synthesis of hydroxyketoamide **2.193** began with Ley oxidation of diynyl alcohol **2.183** to give diynyl ketone **2.198** in 83 % yield (Scheme 2.58). A Horner-Wadsworth-Emmons reaction with phosphonate **2.199** and *n*-butyllithium was employed to access ester **2.197**. Unfortunately, ester **2.197** was only obtained in 31 % yield. It was decided to continue with the route, but to simultaneously investigate alternative

olefination conditions in order to obtain improved yields. Reduction of ester **2.197** with DIBAL-H gave allylic alcohol **2.200** in 86 % yield; however, subsequent epoxidation of allylic alcohol **2.200** using modified Sharpless conditions proved unsuccessful.[57] After 1 week at -20 ^{o}C, no product formation was observed. The reaction was allowed to warm to room temperature, but after stirring for 3 days, only starting material remained. Attempts to utilize dimethyldioxirane (DMDO), *meta*-chloroperoxybenzoic acid (*m*CPBA), or vanadyl acetoacetonate, or basic methods (H_2O_2, NaOH or Triton B) resulted in decomposition of the starting material and/or non-selective olefin oxidation.

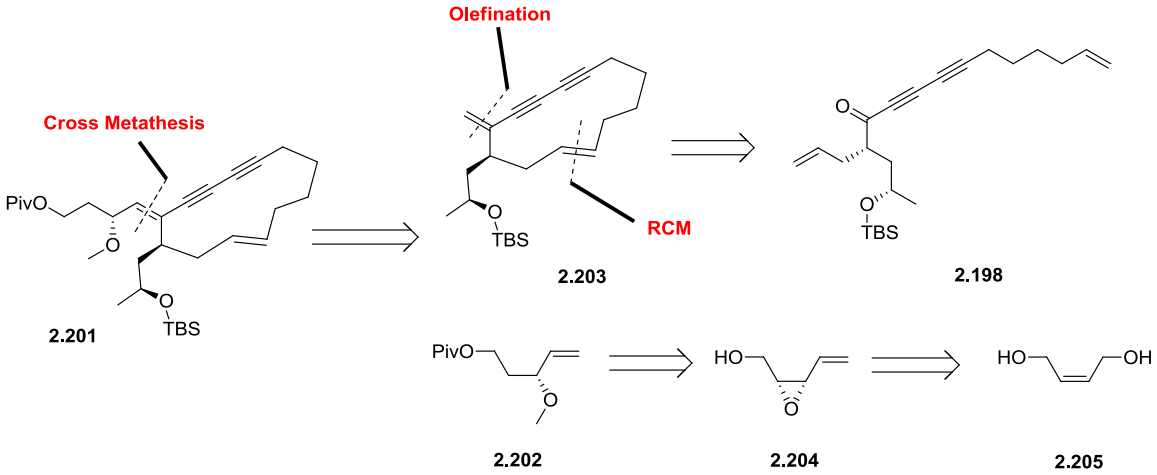


Scheme 2.58. Synthesis of epoxide **2.196** from diynyl alcohol **2.183**.

The absence of any product formation led to the conclusion that the reactivity of the alkene was being attenuated by the extended conjugation. Mechanistically, an epoxidation reaction is an electrophilic substitution of an alkene. Electron-rich alkenes react very quickly and electron-deficient alkenes react very slowly, if at all. Unfortunately, our system, with substantial delocalization of electron density through the highly conjugated diene/diyne π system, proved unreactive to epoxidation with mild, stereoselective conditions and decomposed upon exposure to more reactive conditions. In addition to this difficulty with the epoxidation, olefination of ketone **2.198** continued to

be low-yielding with a variety of conditions. Aspects of this route, such as the late-stage installation of the electron-rich pyrrole and the acid-catalyzed spirocyclization, were appealing; however other aspects were undesirable, like low-yielding olefinations, unsuccessful epoxidations, and a very long linear sequence of reactions. In light of these results and observation, this route was modified to remove the troublesome reactions and provide a more convergent synthesis of the required hydroxyketoamide **2.193**.

Excluding the sequential asymmetric epoxidation reactions by preparing terminal alkene **2.202** for cross metathesis (CM) with a methylene derivative of diynyl ketone **2.183** would not only remove a troublesome reaction sequence, but would also provide a more convergent route (Scheme 2.59).



Scheme 2.59. RCM/CM route to incorporate allylic methoxy fragment, alkene **2.202**.

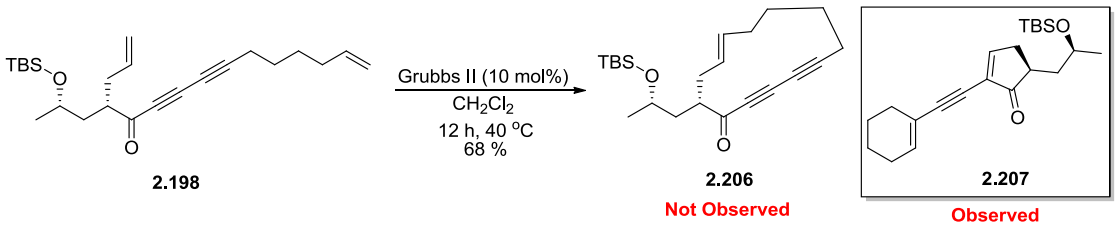
Macrocycle **2.201** could be obtained through a CM reaction between terminal alkene **2.202** and geminal alkene **2.203**, which is prepared from ketone **2.198** by a RCM reaction and a simplified olefination. Grubbs and co-workers have put an extensive amount of work into discerning the reactivity differences of alkenes in cross metathesis

reactions with the Grubb's second-generation catalyst.[58] Type 1 olefins undergo rapid homodimerization and the homodimers are consumable, whereas Type 2 alkenes undergo slow homodimerization, and the homodimers are sparingly consumable. Type 3 olefins do not homodimerize, and type 4 olefins are spectators that are inert to cross metathesis. Alkene **2.202** is a terminal alkene with a secondary allylic alcohol protected by a small group, which makes it a type 2 alkene. Geminal alkene **2.203** is a type 3 olefin since it is a 1,1-disubstituted olefin, so cross metathesis between **2.202** and **2.203** should proceed smoothly if type 2 alkene **2.202** is added slowly to minimize homodimerization. Since type 3 olefin **2.203** does not homodimerize, it will not be a problem to have a high concentration of this alkene in solution.

Alkene **2.202** would be readily prepared from vinyl epoxide **2.204** by directed epoxide-opening to the 1,3-diol, mono-protection of the primary alcohol as the pivolate, and subsequent methylation of the free secondary alcohol. Vinyl epoxide **2.204** could be synthesized from *cis*-2-butene-1,4-diol **2.205** through mono-protection, epoxidation, oxidation of the free alcohol to the aldehyde, Wittig olefination, and deprotection of the alcohol.

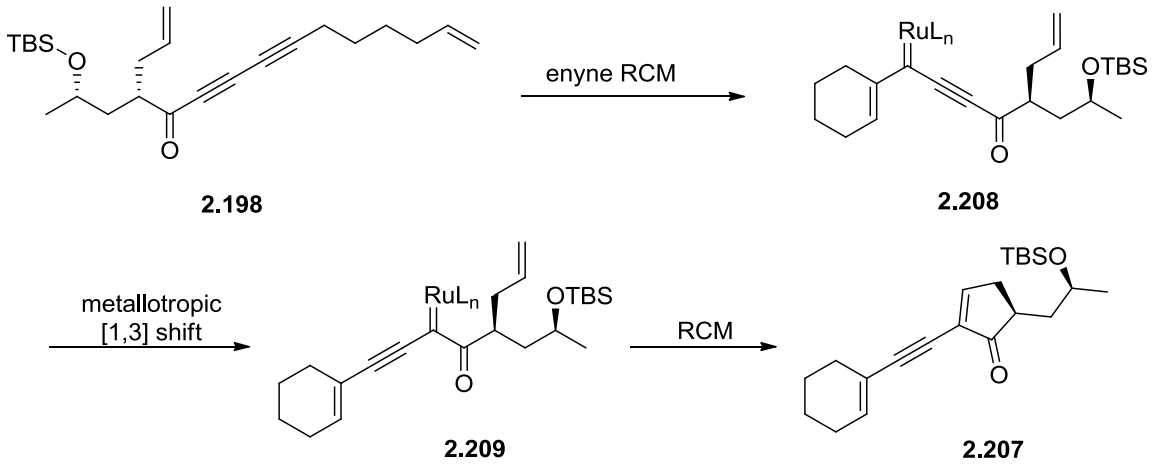
Before finalizing the changes to access diol **2.133** (Scheme 2.56), it was necessary to investigate the reactivity of the three key functional handles of d ketone **2.113**. It was crucial to determine whether pyrrole formation followed by RCM then olefination would work better than RCM, pyrrole formation, then olefination. Since olefination of diynyl ketone **2.198** was low-yielding, it was decided to approach functionalization of the other two reactive groups of **2.198** first, the terminal alkene or 1,3-diyne moieties, in order to determine if olefination would proceed more smoothly when these groups were absent.

Ring-closing metathesis of diynyl ketone **2.198** appeared to proceed smoothly. TLC showed formation of a single product spot and consumption of all starting material and LC-MS data revealed that a compound with the correct mass had indeed formed. However, ^{1}HNMR revealed a very different story. The compound that had formed was actually 1,5-diene-3-yne **2.207**.[59]



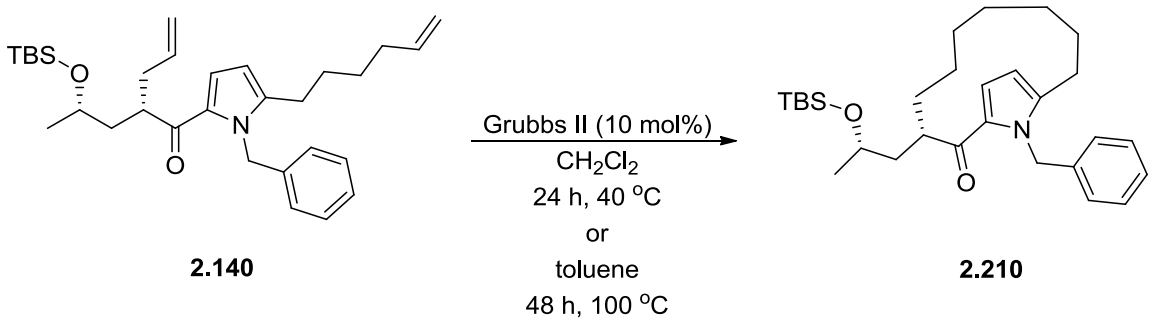
Scheme 2.60. Attempted ring-closing metathesis of diynyl ketone **2.198**.

This formation of this compound can be explained by envisioning a RCM-induced metallotropic [1,3-shift] as described by Lee and coworkers to produce a 1,5-diene-3-yne (Scheme 2.61).[59] First, enyne metathesis occurs generating alkylidene intermediate **2.208**. This intermediated can then undergo a [1,3] metallotropic shift, which can be considered a special case of enyne metathesis where there is no tether between the ene and the yne counterparts. This shift results in the rearranged alkylidene **2.209**, which can then undergo RCM with the remaining alkene to give the observed 1,5-diene-3-yne **2.207**.



Scheme 2.61. Formation of 1,5-diene-3-yne **2.107** from attempted RCM of ketone **2.198** with Grubbs II catalyst.[59]

Since RCM could not be achieved with the diyne in place, we decided to use a pyrrole derivative in the RCM reaction. Since pyrrole **2.140** had already been synthesized and could be successfully protected as *N*-benzylpyrrole **2.210** (Scheme 2.62), it was simply used for the RCM because the preparation was already optimized and the compound had been stored and was immediately available. Interestingly, after refluxing at 40 ^{o}C for 24 hours, only starting material was recovered. Changing the solvent to toluene and refluxing at 100 ^{o}C provided no improvement.



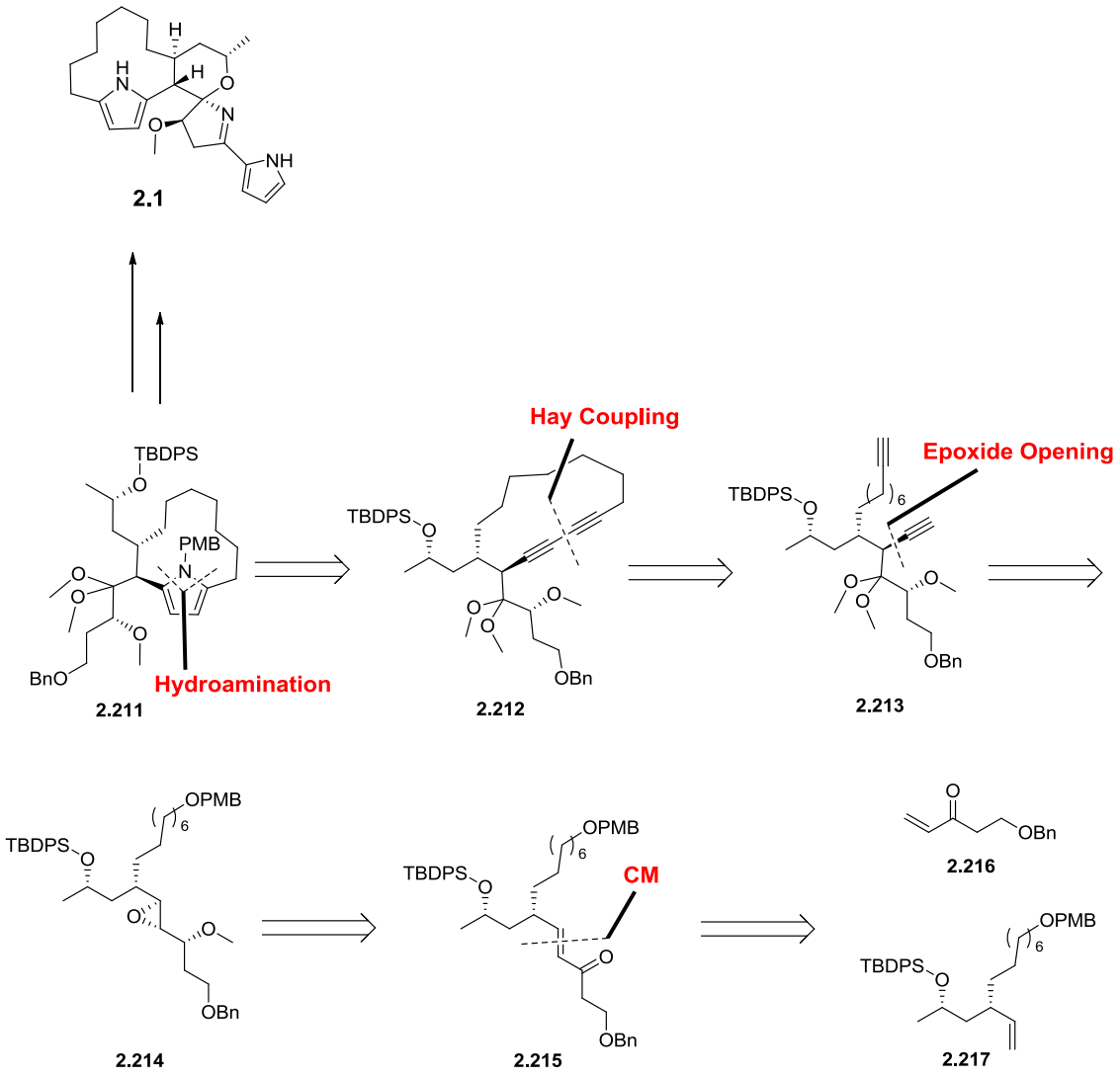
Scheme 2.62. Unsuccessful RCM with N-benzylpyrrole **2.140**.

Even with a protected pyrrole, it appeared that the catalyst was being poisoned. This was unexpected since the pyrrole nitrogen had been protected and the pyrrole was substituted at the 2-position by an electron-withdrawing acyl substituent. One possible explanation is that protection of the pyrrole nitrogen with an alkyl group (benzyl) actually increases the basicity of the amine, as is seen with simple alkyl amines. If this is the case, the more basic pyrrole would actually chelate to the catalyst more efficiently than the deprotected pyrrole. To test this theory, ring-closing metathesis was attempted with deprotected pyrrole **2.140**. Unfortunately, no reaction was observed with this substrate either. However, this does not discount the hypothesis that the benzyl-protected pyrrole nitrogen would be more basic. The deprotected pyrrole may be an efficient chelator as well, since the nitrogen is less hindered and more open for chelation.

Routes to achieve the synthesis of the required hydroxyketoamide for the acid-mediated spirocyclization have proven unsuccessful so far in our progress toward the total synthesis of marineosin A. Our major focus to achieve the synthesis of the requisite hydroxyketoamide has been on the preparation of a diyne and then incorporating it into the molecule as a single unit, followed by a ring-closing reaction to form the macrocycle. Considering the results of these endeavors, we decided to investigate the formation of the diyne for the ring-closing reaction.

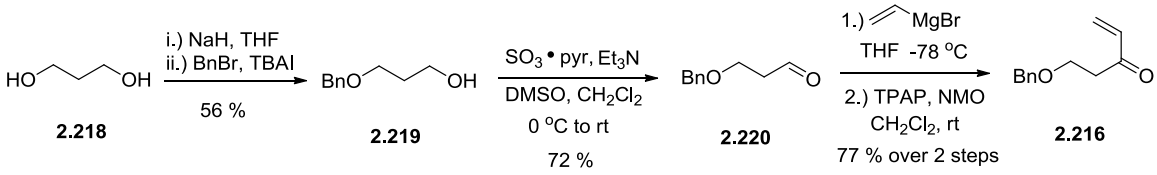
2.10.2 Intramolecular Alkyne-Alkyne Coupling for Hydroxyketoamide Synthesis.

In light of the problems encountered with electron-rich pyrrole intermediates and reactive diynes, a route to achieve the synthesis of the key hydroxyketoamide without these components was designed. We decided to investigate the possibility of forming an alkyne within a stereochemically advanced intermediate, followed by subsequent intramolecular Hay alkyne-alkyne coupling (Scheme 2.63).[60] Hydroamination of diyne **2.212** could incorporate the C-ring pyrrole into the macrocycle forming intermediate **2.211**, which can provide the hydroxyketoamide following a few functional group interconversions.[61] Intramolecular Hay coupling of two terminal alkynes would provide the requisite diyne **2.212**. Regioselective epoxide opening with an acetylide could provide the first terminal alkyne and deprotection, oxidation, and homologation could provide the second terminal alkyne of dialkyne **2.212**. Epoxide **2.214** is generated from stereoselective reduction of enone **2.215** and subsequent asymmetric epoxidation. Enone **2.215** is obtained from cross metathesis of an easily accessed enone **2.216** and a mono-substituted terminal alkene **2.217**.



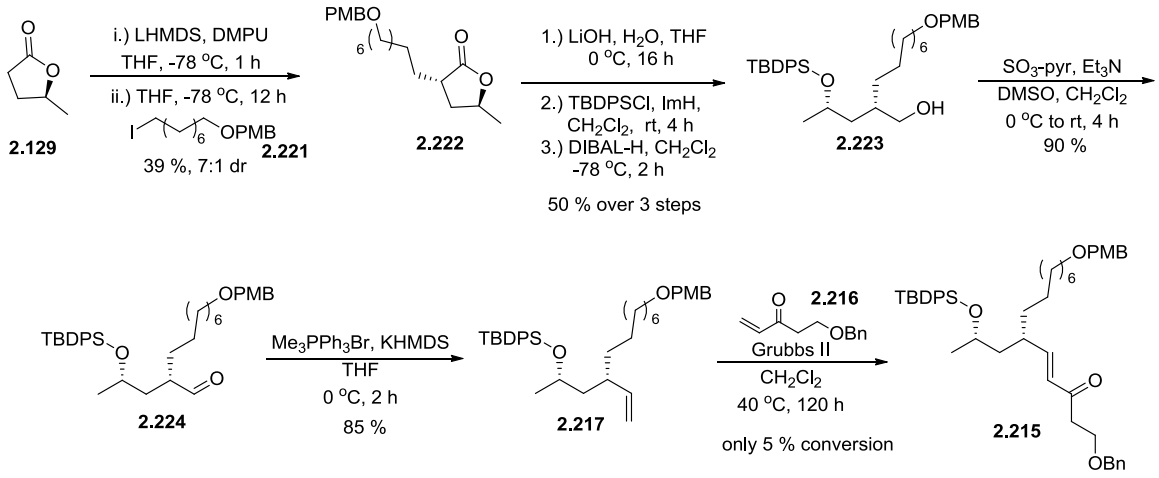
Scheme 2.63. Retrosynthesis of marineosin A (**2.1**) incorporating a key Hay coupling to form the diynyl macrocycle **2.212.**

Enone **2.216** was prepared from 1,3-propanediol (**2.218**), which was mono-protected to give benzyl ether **2.219** and oxidized to β-hydroxy aldehyde **2.220** under Parikh-Doering conditions (Scheme 2.64).[62] Addition of vinyl magnesium bromide to the aldehyde and subsequent Ley oxidation provided the desired enone **2.216** in 31 % overall yield.



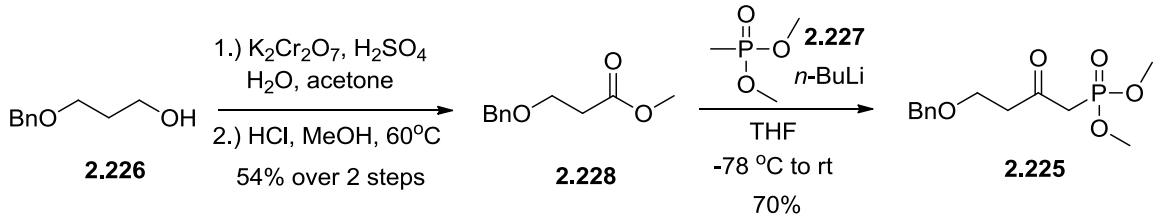
Scheme 2.64. Synthesis of enone **2.216** from 1,3-propanediol (**2.218**).

Preparation of terminal alkene **2.217**, which contains the required stereochemical elements, was not as straightforward (Scheme 2.65). Stereoselective alkylation with alkyl iodide **2.221** of lactone **2.129** proceeded slowly (12 h) and in low yield (39 %) with the addition of DMPU to breakup lithium aggregates. Without DMPU, the reaction showed no evident conversion to alkyl lactone **2.222**. Since the diastereoselectivity was good (7:1), this route was carried forward. Careful hydrolysis of lactone **2.129**, subsequent bis-protection as the TBDPS ether/ester, and finally DIBAL-H reduction of the ester provided the primary alcohol (**2.223**) in 50 % yield over 3 steps. Parikh-Doering oxidation to aldehyde **2.224** and Wittig olefination gave the required terminal olefin **2.217**.[62,63] Unfortunately cross metathesis with the prepared vinyl ketone **2.216** proved unsuccessful. Only 5 % conversion to product was observed, even after 5 days and addition of 60 mol % of catalyst. 5 days at 100 °C in toluene resulted in no noticeable increase in conversion.



Scheme 2.65. Attempted synthesis of enone **2.215** from lactone **2.129.**

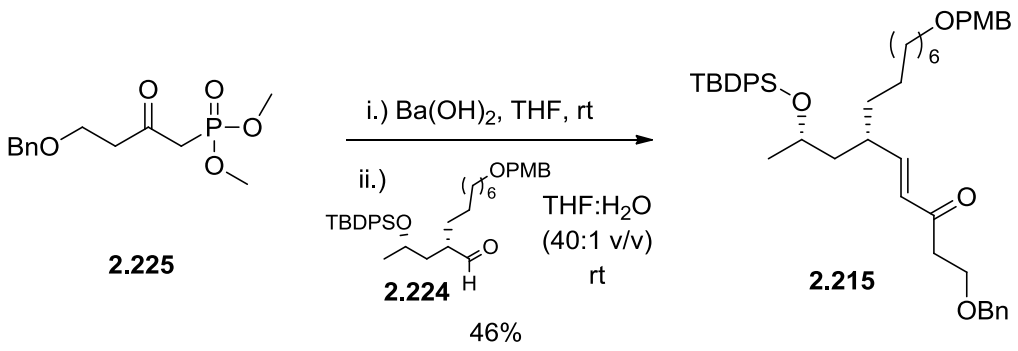
Since there are numerous methods for the synthesis of αβ-unsaturated ketones, we decided to explore the Horner-Wadsworth-Emmons (HWE) reaction to prepare this intermediate.[64] In order to use this method, we had to prepare the required α-ketophosphonate **2.225**. This was achieved in a three-step sequence (Scheme 2.66), beginning with Jones oxidation of mono-benzyl ether **2.226** and subsequent esterification of the resulting carboxylic acid. Deprotonation of methyl-dimethylphosphonate **2.227** and treatment with the prepared β-hydroxy ester **2.228** provided the necessary phosphonate **2.225**.



Scheme 2.66. Synthesis of α-ketophosphonate **2.225**.

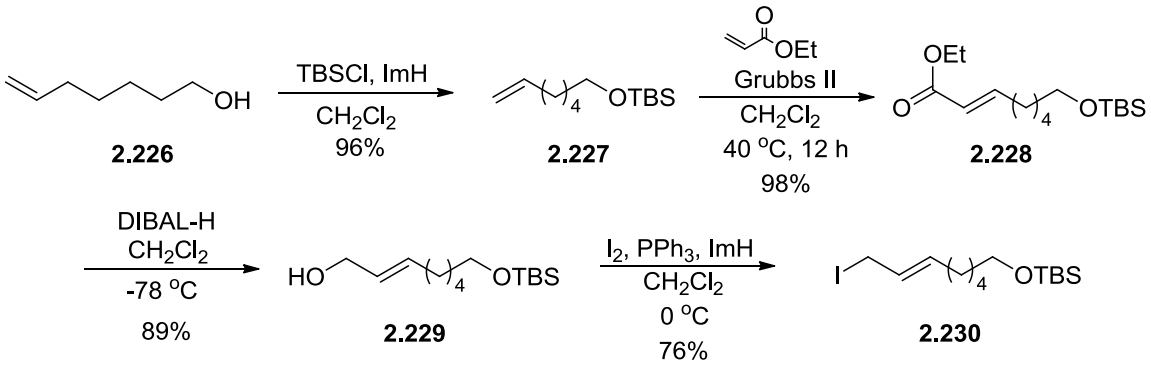
Using the conditions developed by Roush and co-workers for the HWE reaction with base-sensitive substrates, phosphonate **2.225** was treated with barium hydroxide in

THF at room temperature.[64] After 1 h, aldehyde **2.224** was added as a solution in THF:H_2O (40:1 v/v) at room temperature. The HWE reaction provided the desired enone **2.215** in 46 % yield (58 % yield brsm) with no noticeable racemization.



Scheme 2.67. HWE reaction of aldehyde **2.224** and phosphonate **2.225** to provide enone **2.215**.

While exploring conditions for the preparation of the enone **2.215**, efforts were underway to optimize the stereoselective alkylation of lactone **2.129**. Based on previous alkylation studies with allyl iodide, it was decided to use a substituted allylic iodide (**2.230**) in the alkylation reaction. Following optimization of iodination conditions, the route described in Scheme 2.68 was utilized to prepare the required allylic iodide **2.230**.



Scheme 2.68. Synthesis of alkyl iodide **2.230** from 6-hepten-1-ol (**2.226**).

6-hepten-1-ol (**2.226**) was protected as the TBS ether (**2.227**), and the resulting alkene underwent CM with ethyl acrylate to provide the αβ-unsaturated ester **2.228** which was reduced to allylic alcohol **2.229** with DIBAL-H. Subsequent iodination with iodine, triphenylphosphine, and imidazole provided allylic iodide **2.230** in excellent yield (64 % over 4 steps with purification). Allylation of lactone **2.129** with allylic iodide **2.230** proceeded in the absence of DMPU, much more rapidly (2 h), and with better yield (82%) of the allylated lactone **2.231** than alkylation with the alkyl iodide **2.221** (Scheme 2.69).

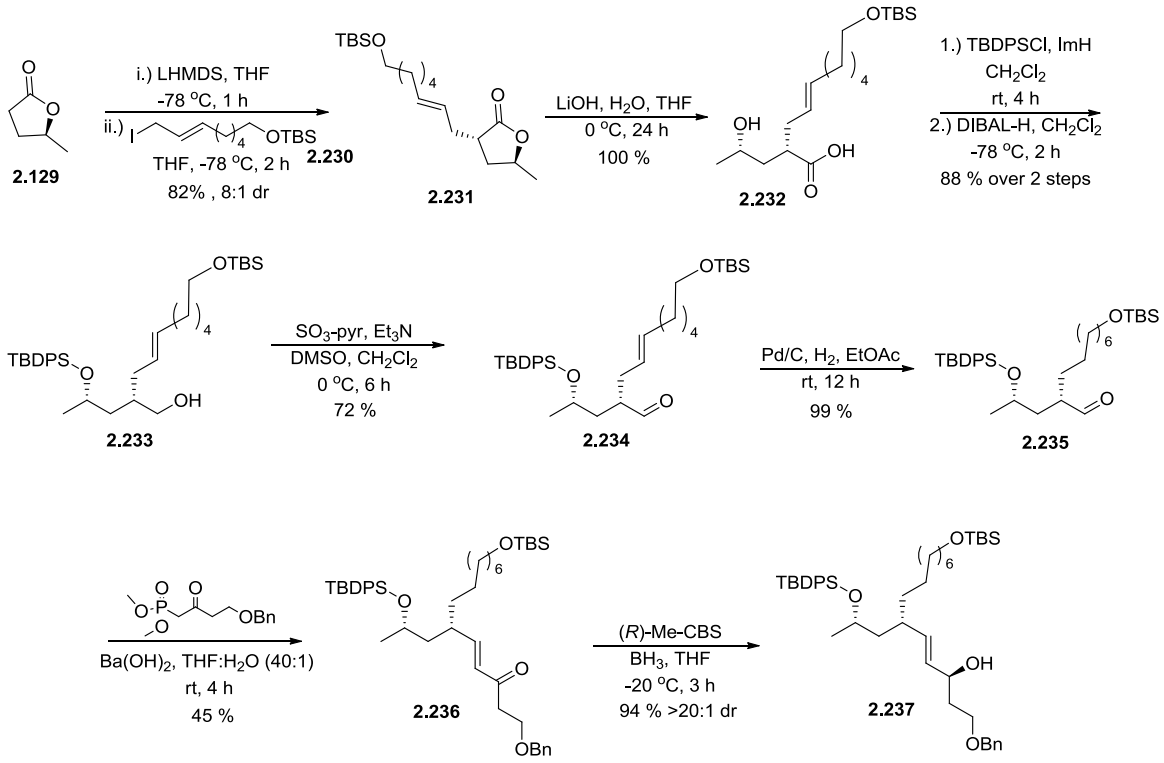
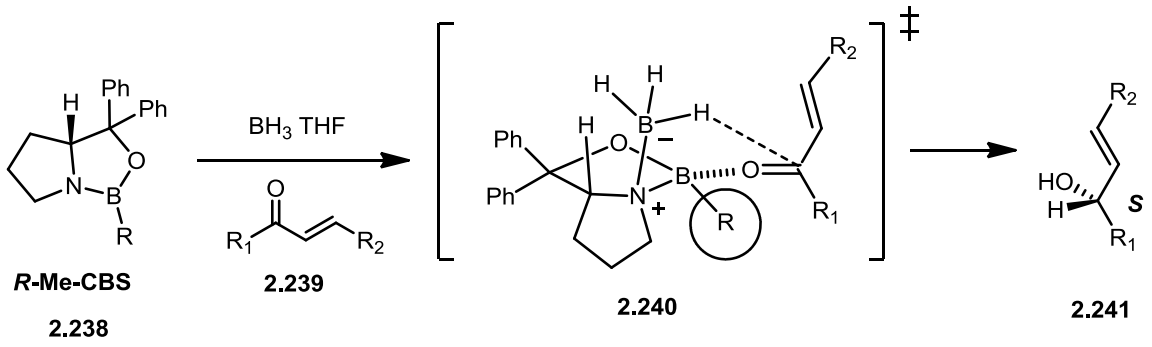


Figure 2.69. Synthesis of chiral allylic alcohol **2.237** from lactone **2.129**.

Careful hydrolysis of lactone **2.231**, TBDPS bis-protection, and subsequent reduction of the TBDPS ester gave primary alcohol **2.233** in 85 % yield over 3 steps. Parikh-Doering oxidation and hydrogenation gave the saturated aldehyde **2.235** for the

HWE reaction. The HWE reaction proceeded in 45 % yield (69 % brsm) to give enone **2.236**. Stereoselective reduction of enone **2.236** was achieved using the Corey-Bakshi-Shibata (CBS) catalyst **2.238** and a method optimized by Drummond and Sutherland to give the desired secondary allylic alcohol **2.237** in 94% yield and greater than 20:1 dr.[65,66]

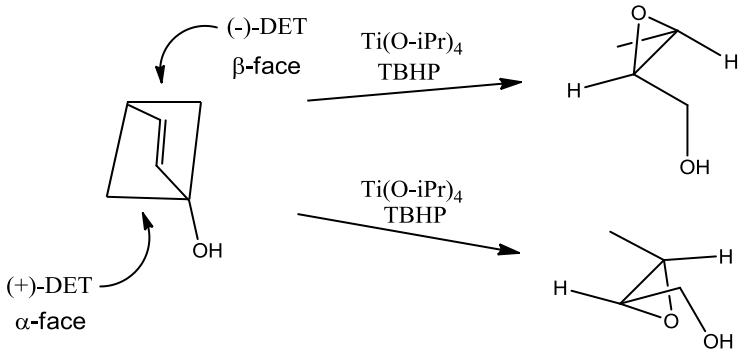
The CBS catalyst **2.238** is synthesized from proline and is utilized to enantioselectively reduce ketones to alcohol. The enantioselectivity is derived from transition state **2.240** where the larger substituent is positioned away from the large R group on the catalyst. Internal hydride delivery from borane results in formation of the *S* enantiomer **2.241** in good to excellent ee based on the size difference of R_1 and R_2.[65]



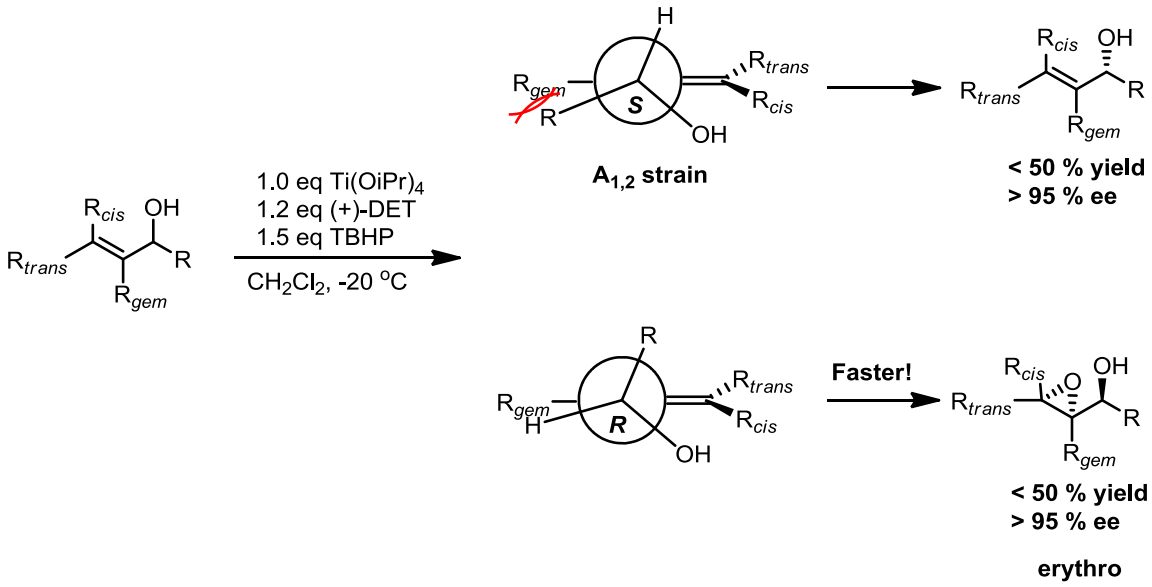
Scheme 2.70. Mechanism of the CBS reduction.

Kinetic resolution utilizing Sharpless asymmetric epoxidation (SAE) conditions (Scheme 2.71) is an effective way to generate a chiral allylic alcohol from a racemic mixture.[67-69] By utilizing the reagent control provided by the chiral catalyst, one is able to choose which enantiomer of the allylic alcohol is obtained in > 95% ee. The other enantiomer will be converted to the erythro epoxy alcohol due to its faster reaction rate. The alcohol with the fastest reaction rate is the one that minimizes the $A_{1,2}$ strain between

the R group of the secondary alcohol and the geminal substituent on the double bond (Scheme 2.72). If (+)-DET is chosen as the chiral reagent, the *R* enantiomer decreases this strain, so it is the fastest to react, leaving the *S* enantiomer as unreacted, enantiomerically-enriched allylic alcohol.[67]



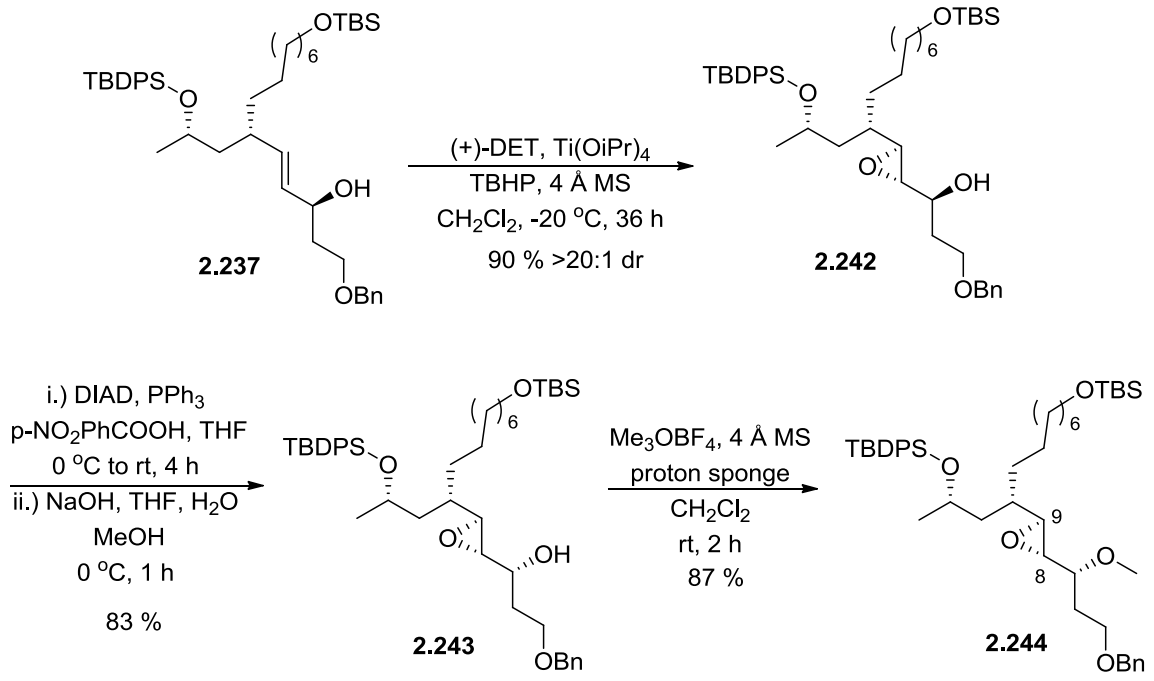
Scheme 2.71. Mnemonic for determining facial selectivity in the SAE reaction.



Scheme 2.72. Rational for observed kinetic resolution utilizing SAE.

The stereoselectivity of the CBS reduction was confirmed by Sharpless kinetic resolution to form the desired epoxide **2.242** (Scheme 2.73). (+)-DET gave complete conversion to the epoxide product, which confirmed that the reduction had indeed

occurred exclusively from the α–face to give the (*S*) stereoisomer of the alcohol (**2.237**). If considerable addition had occurred from the β–face, then unreacted (*R*)-allylic alcohol would have been recovered from the epoxidation reaction.[67]

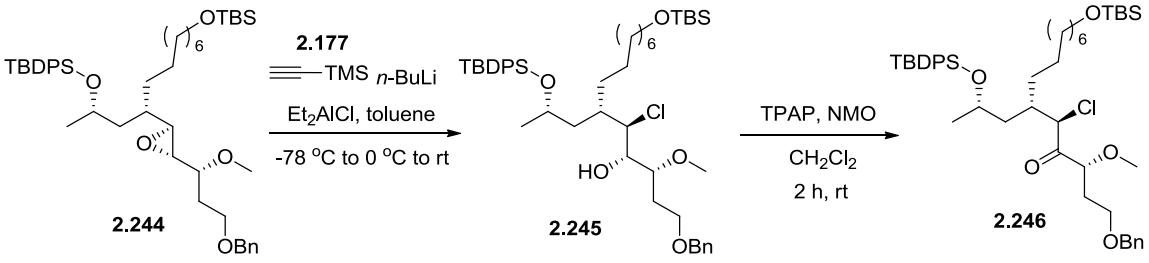


Scheme 2.73. Kinetic resolution of alcohol **2.237** and synthesis of α-methoxy epoxide **2.244**.

Since the threo isomer was required and the erythro isomer is obtained from the Sharpless asymmetric epoxidation, we inverted the alcohol stereocenter of hydroxyl epoxide **2.242** using Mitsunobu conditions to provide the desired *R*-hydroxy epoxide **2.243**. Subsequent soft methylation with trimethyloxonium tetrafluoroborate and proton sponge provided the methyl ether **2.244** in excellent yield (87%). [70]

At this point, it was time to investigate conditions to regioselectively open the epoxide by addition of mono-TMS-protected acetylene. We began by treating epoxide **2.244** with diethyl aluminum chloride, which has been shown to provide regioselective

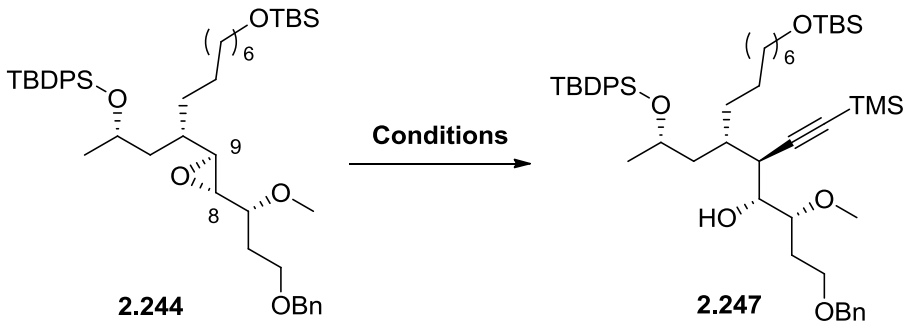
epoxide-opening of hindered substrates.[71] We hoped the methoxy substitutent would chelate to the epoxide and increase the electrophilicity of the C9 carbon to facilitate addition at this center. TMS-acetylene **2.177** was deprotonated with *n*-butyl lithium at -78 ^{o}C and the resulting solution added to the epoxide at -78 ^{o}C. After stirring for 12 h with no reaction, the solution was warmed to 0 ^{o}C and stirred for an additional 12 h. After observing no reaction, the mixture was warmed to room temperature and stirred for 24 h. At this point, there was still no evidence of conversion to product, so additional diethyl aluminum chloride was added at room temperature. After 8 h, all starting material had been consumed and TLC revealed conversion to a single product that was more polar than the starting epoxide **2.244**. Upon inspection of ^{1}H NMR and ^{13}C NMR data, it was concluded that the epoxide had indeed been opened regioselectively at the desired position. However, the nucleophile responsible was not acetylide **2.177**, it was the chloride anion from the Lewis acid additive to provide chloro-alcohol **2.245**. Subsequent oxidation to ketone **2.246** provided a cleaner spectrum with clear coupling constants and shifts, thus corroborating the identity of the nucleophile as the chloride anion.



Scheme 2.74. Undesired regioselective opening of epoxide **2.244** with chloride anion to form chloro-alcohol **2.245**.

Attempts to open the epoxide **2.244** with a relevant nucleophile proved futile. Various Lewis acids, solvents, nucleophiles, temperatures, and orders of addition were studied as outlined in Scheme 2.75. The only addition to occur successfully was the

chloride anion addition which resulted from treating the epoxide with excess diethyl aluminum chloride. At this point it was concluded that this route would be unable to provide the desired hydroxyketoamide and that substantial alterations would have to be made to incorporate the C9 stereocenter.



Conditions

Lewis Acids: BF_3OEt_2, Et_2AlCl, Et_2AlCN, $Ti(OiPr)_4$, CuBr-SMe_2
Bases for TMS acetylene deprotonation: n-BuLi, EtMgBr
Solvents: THF, Toluene, CH_2Cl_2, Et_2O
Temperatures: -78 ^oC, -45 ^oC, -20 ^oC, 0 ^oC, 25 ^oC, 40 ^oC, 55 ^oC, 100 ^oC

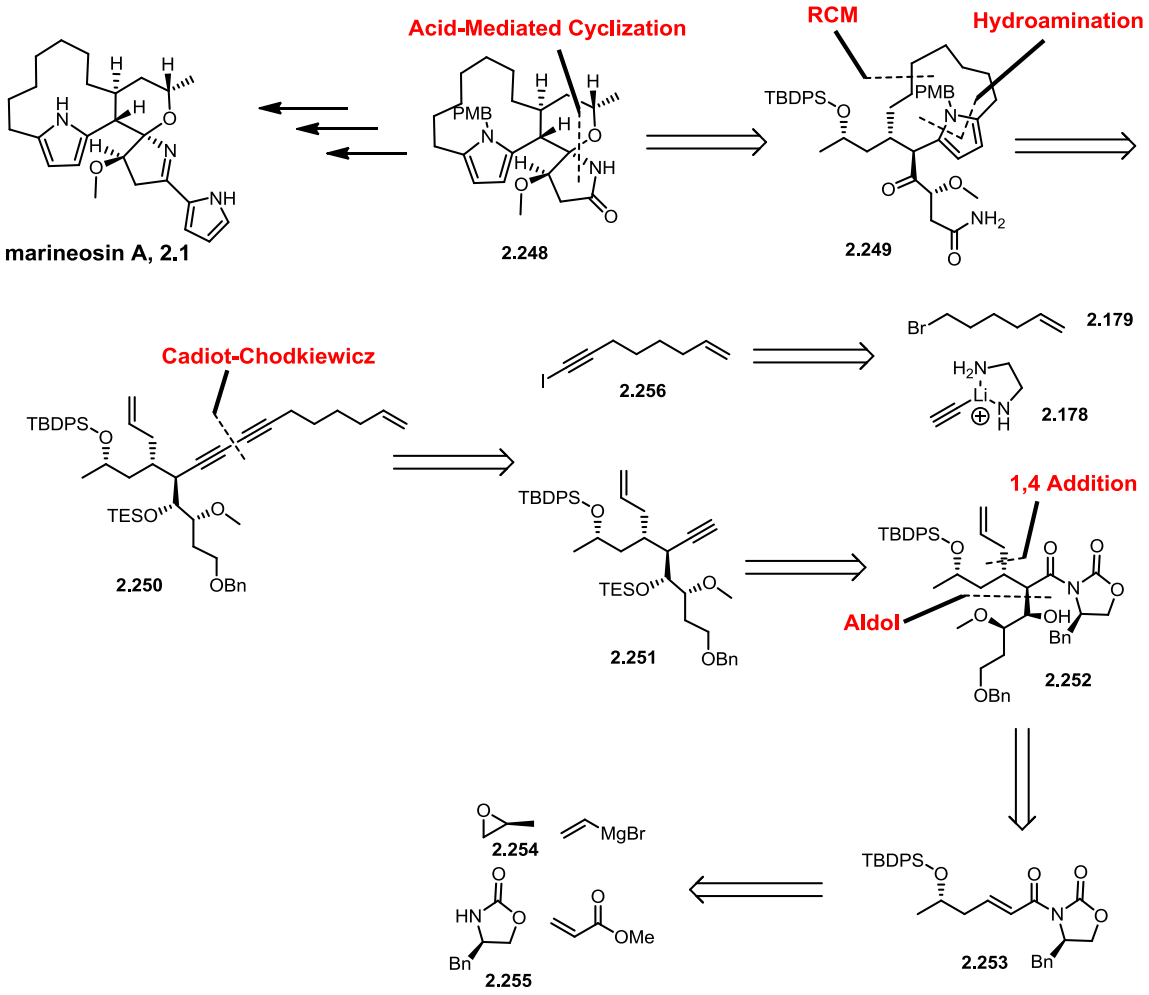
Scheme 2.75. Attempts to form alkyne **2.247** by regioselective addition to epoxide **2.244**.

2.10.3 Intermolecular Alkyne-Alkyne Coupling for Hydroxyketoamide Synthesis.

We began to consider alternative disconnections to achieve the hydroxyketoamide core, and in light of our previous success with the Cadiot-Chodkiewicz hetero alkyne-alkyne cross-coupling, we decided to incorporate an intermolecular diyne formation and still utilize an intermolecular/intramolecular hydroamination reaction to generate the pyrrole followed by an RCM reaction to form the macrocycle (Scheme 2.76).[72-75] We had experienced successful cross-metathesis reactions with an electron-rich, deprotected alkyl pyrrole in our work with the proposed biosynthetic route of the marineosins (Chapter 2, Section 2.6), and we believe that this pyrrole will display similar reactivity.[76] The spiroiminal center will still be formed by an acid-mediated cyclization of a

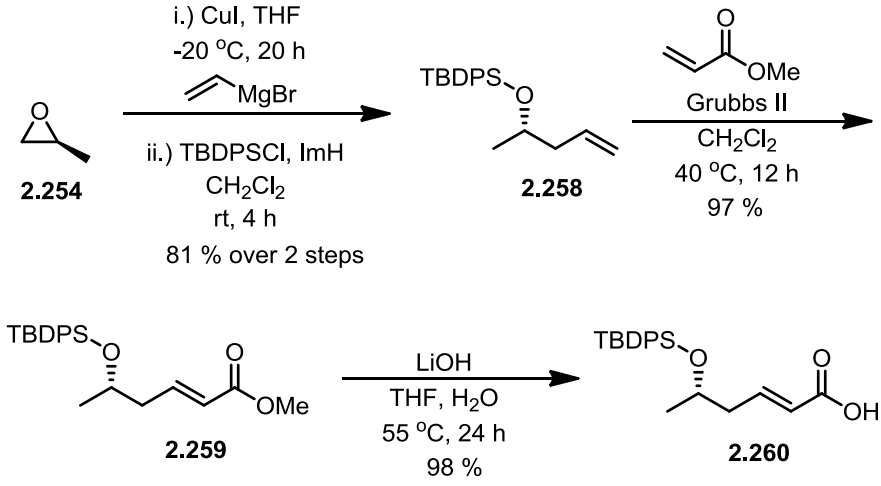
hydroxyketoamide intermediate, followed by installation of the final pyrrole moiety.

As described in Scheme 2.76, a readily available iodoalkyne **2.256** will be coupled to a highly derivatized terminal alkyne **2.251** to provide the diyne for pyrrole formation and subsequent cyclization. Alkyne **2.251** can be prepared by a stereoselective 1,4-addition, followed by an stereoselective aldol reaction directed by the same acylated oxazolidinone **2.252**. The opportunity to utilize a single auxiliary to direct the formation of three stereocenters, including the elusive C9 stereocenter, was highly appealing.[77-79]



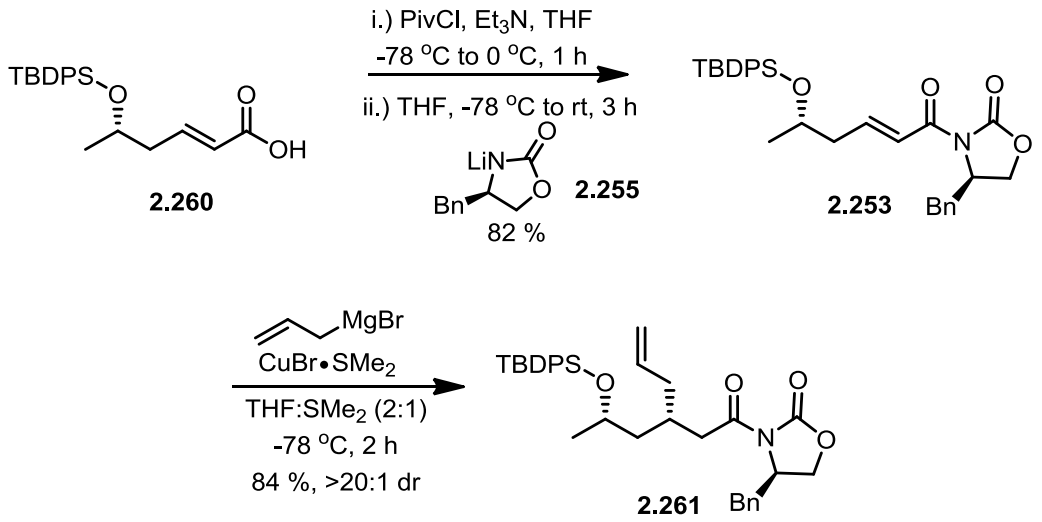
Scheme 2.76. Retrsynthesis of marineosin A **2.1** through key intermolecular Cadiot-Chodkiewwicz hetero alkyne-alkyne cross coupling and a Michael addition/aldol reaction sequence.

The acylated oxazolidinone was readily prepared starting from (*S*)-methyl oxirane **2.254** by the regioselective addition of vinyl cuprate to the least substituted position of the epoxide (Scheme 2.77). Protection of the resulting, crude alcohol **2.257** as the TBDPS ether provided the product **2.258** in 81% yield over 2 steps. Cross metathesis with methyl acrylate and subsequent hydrolysis of the methyl ester **2.259** provided the required carboxylic acid **2.260** in excellent yield.



Scheme 2.77. Synthesis of carboxylic acid **2.259** from (*S*)-methyl oxirane **2.254**.

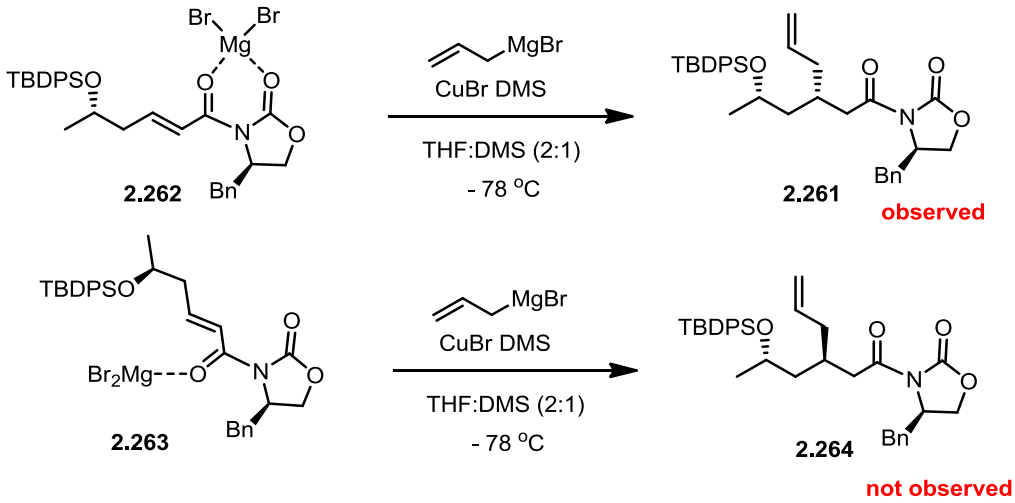
Acid **2.260** was then treated with trimethylacetyl chloride and triethylamine to form the mixed anhydride *in situ* which was added to a solution of lithiated (*R*)-4-Benzyl-2-oxazolidinone **2.255** to provide acylated oxazolidinone **2.253** for the conjugate addition (Scheme 2.78). To our delight, the 4-benzyl oxazolidinone proved to be an excellent substrate for the conjugate addition, providing a single diastereomer in 84 % yield and greater than 20:1 dr. [79]



Scheme 2.78. Acylation of oxazolidinone **2.255** with acid **2.260** and subsequent conjugate addition to provide **2.261**.

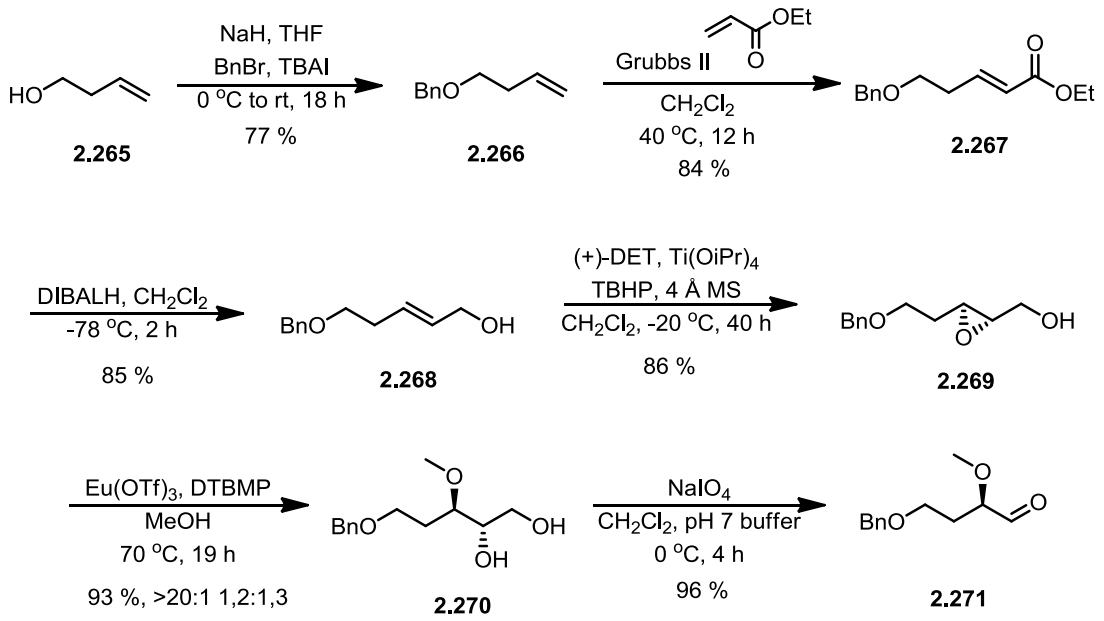
This excellent stereoselectivity is a result of the rigid, chelate transition state **2.262** described in Scheme 2.79.[79] The benzyl substituted oxazolidinone has historically shown inconsistent and undesirable results for facial selectivity in conjugate addition reactions. However, Williams and co-workers have shown that in the case of conjugate addition with allyl cuprate, the benzyl substrate displays excellent stereoselectivity.[79] The more active allyl cuprate species appears to react more quickly than other cuprates at -78 ^{o}C and with preferential formation of the adduct resulting from chelated transition state **2.262**, as opposed to open-chain transition state **2.263**, which is the common reactive chelate in aldol reactions with oxazolidinone auxiliaries (*vide infra*), and has been used to explain the typical, opposite stereoselection (**2.264**) of benzyl-substituted versus phenyl-substituted derivatives. Williams and co-workers observed that the benzyl-substituted auxiliary only showed modest selectivity in the conjugate addition reaction. However, when allyl cuprate was utilized as the nucleophile under the same reaction conditions, they observed excellent stereoselectivity for the formation of the same stereoisomer as

the phenyl-substituted auxiliary. In light of the poor stereoselectivity of the phenyl-substituted oxazolidinones in aldol reactions, we decided to use the benzyl-substituted auxiliary for the conjugate addition reaction with allyl cuprate, so that the subsequent aldol reaction could be performed with the same auxiliary. In this manner, we will effectively set three stereocenters with one chiral auxiliary.



Scheme 2.79. Possible transition states for conjugate addition reaction with an oxazolidinone auxiliary.[79]

With the completion of the highly successful conjugate addition reaction, it was time to investigate the subsequent aldol reaction. The α-methoxy aldehyde was prepared as described in Scheme 2.80. 3-buten-1-ol (**2.265**) was protected as the benzyl ether (**2.266**), and cross metathesis provided the unsaturated ester (**2.267**) which was reduced to the allylic alcohol (**2.268**) with DIBAL-H. Sharpless asymmetric epoxidation provided epoxide **2.268** in excellent yield. Regioselective, europium–mediated solvolysis of the epoxide provided an excellent yield of the 1,2 diol **2.269** (94 %, >20:1 1, 2 : 1, 3).[80] Oxidative cleavage of **2.269** provided the desired α-methoxy aldehyde **2.270** for the aldol reaction.

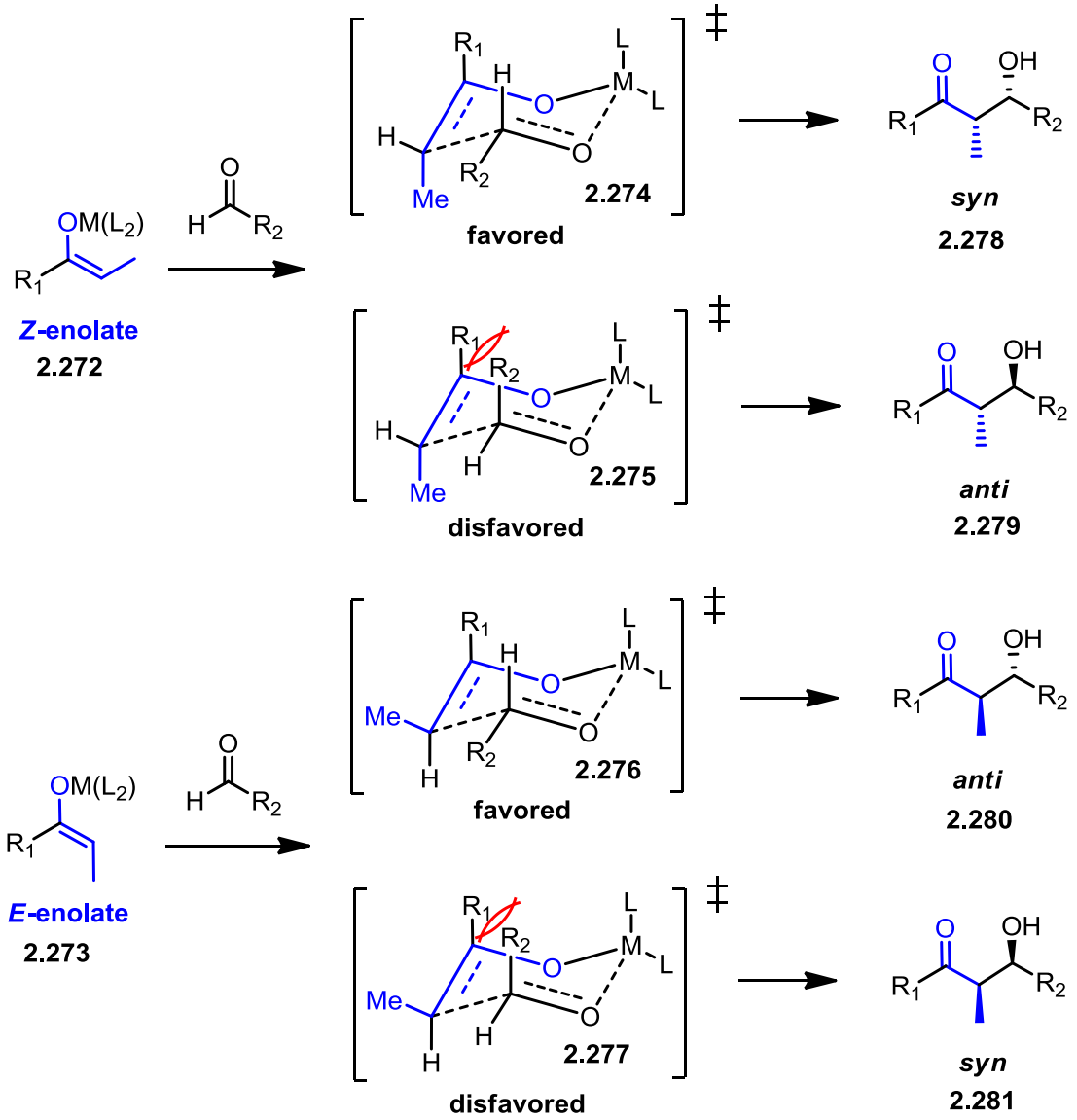


Scheme 2.80. Preparation of α-methoxy aldehyde **2.270** from 3-buten-1-ol (**2.265**).

In order to choose the correct conditions to facilitate the aldol reaction and promote formation of the desired stereoisomer, it was necessary to investigate the theory behind stereoselection in the aldol reaction. This theory is based upon the Zimmerman-Traxler transition state model which explains that the factors controlling stereoselectivity are the preference for placing substituents equatorially in six-membered ring transition states and the avoidance of 1, 3-diaxial interactions.[81,82]

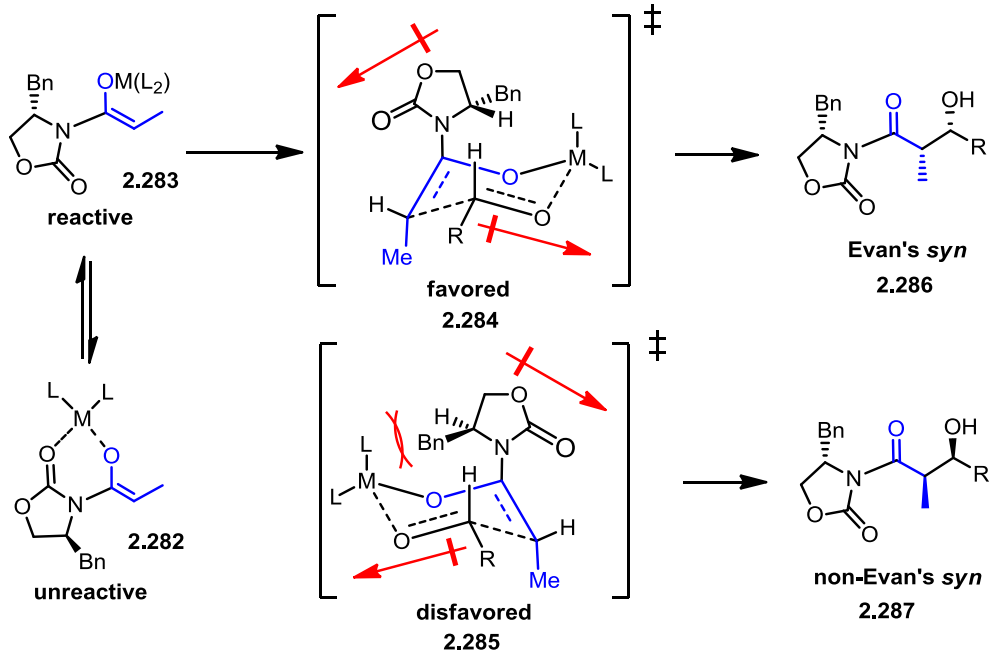
In the aldol reaction, two new stereocenters are set. To account for all combinations, there is a possibility of forming 2^x diastereomers, where x is the number of new stereocenters formed in the reaction. In the case of the aldol reaction, x = 2, so there are four possible diastereomers. Formation of the *Z*-enolate (**2.272**) and reaction with an aldehyde can form a *syn* or *anti* aldol adduct (Scheme 2.81). Formation of the *E*-enolate (**2.273)** will provide the enantiomers of the *syn* or *anti* adducts that result from the *Z*-enolate. The stereocontrol in this case is a result of the 1, 3-diaxial interaction between

the R_2 group of the aldehyde and the R_1 substituent of the enolate in the Zimmerman-Traxler transition states **2.275** and **2.277**. Minimization of this interaction places the aldehyde R_2 group in a pseudoequatorial position (**2.274** and **2.276**) and results in the formation of *syn* aldol adducts (**2.278**) from *Z*-enolates and *anti*-aldol adducts (**2.280**) from *E*-enolates.



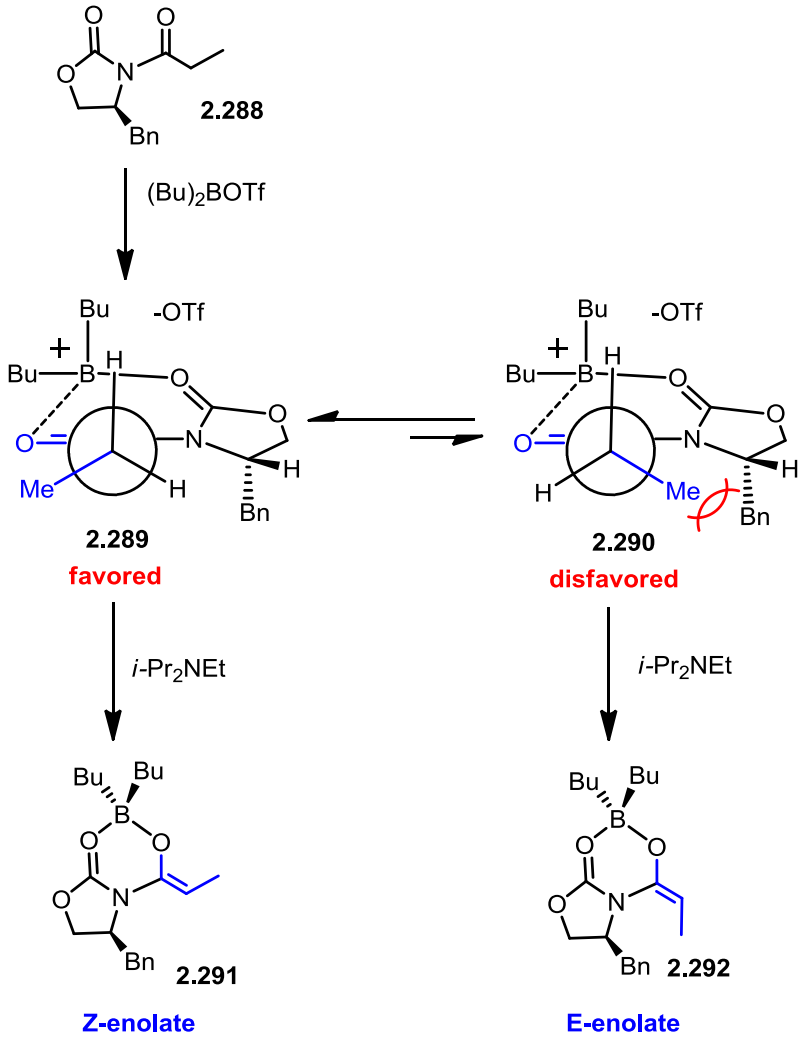
Scheme 2.81. Four diastereomers are possible in the aldol reaction. Avoidance of 1,3 diaxial interactions precludes formation of two of the diastereomers (**2.279** and **2.281**), resulting in the formation of *anti* adducts from *E*-enolates (**2.280**) and *syn* adducts (**2.278**) from *Z*-enolates.[81,82] (adapted from Li 2009).

Incorporation of the Evan's oxazolidinone forces the transition state to adopt the conformation described in Scheme 2.82, where the polar imide of the oxazolidinone points away from the acyl moiety and aldehyde coordinated to the metal, thus forming a dipole. In order to prevent the chiral benzyl substituent of the oxazolidinone from experiencing unfavorable 1,3-diaxial interactions with the metal ligands in transition state **2.285**, this substituent is oriented away from the reactive components of the aldol reaction. This removes the possibility of forming a *syn* relationship between the auxiliary substituent and the α-substituent, and controls the formation of the *anti*-relationship between the auxiliary and the α-substituent, known as the Evan's *syn* product (**2.286**). Formation of a product with the *syn* relationship between the auxiliary and the α-substituent is known as the *non*- Evan's *syn* product (**2.287**) which is achievable through alternative reagents and reaction conditions.



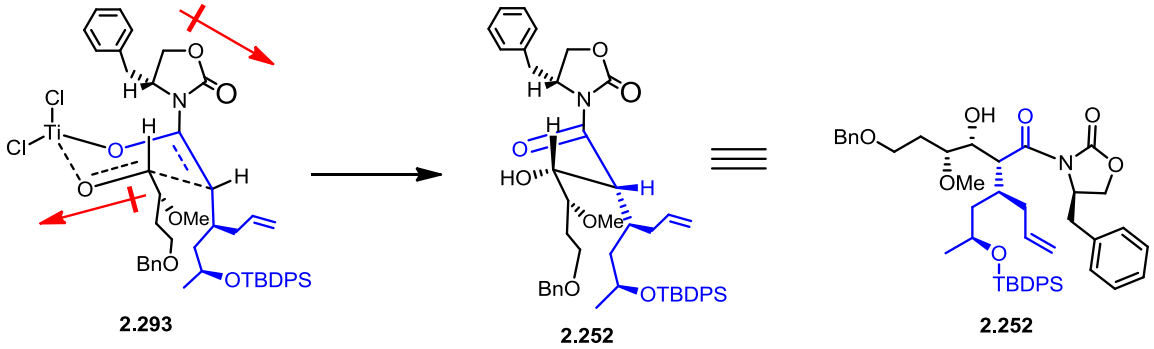
Scheme 2.82. Dipole Model of auxiliary control to provide Evan's *syn* adol products (**2.286**)[81,82] (adapted from Li 2009).

Utilization of soft enolization conditions will favor formation of the Z-enolate over the E-enolate as described in Scheme 2.83. Chelation between the Lewis acid additive and the imide and amide carbonyls occurs first, followed by deprotonation. The *Z*-enolate **2.291** is favored by removing the gauche interaction between the enolate substituent and the chiral auxiliary in transition **2.289**. As a result, the chirality of the α-carbon is set, and it is *trans* to the auxiliary substituent, as predicted by the Zimmerman-Traxler transition state model.



Scheme 2.83. Soft enolization conditions favor the formation of *Z*-enolates. [81,82] (adapted from Li 2009).

By choosing the correct chirality for the oxazolidinone and utilizing soft enolization conditions to favor formation of the *Z*-enolate **2.293**, we should be able to control the stereoselectivity of the aldol reaction to give the desired product **2.252** as highlighted in Scheme 2.84



Scheme 2.84. Zimmerman-Traxler transition state for the formation of aldol adduct **2.252.**

Aldol procedures requiring boron as the chelation metal proved unsuccessful and only facilitated removal of the TBDPS protecting group. Titanium conditions developed by Crimmins and co-workers proved to be much more substrate-tolerant in our case.[78] Unfortunately, the aldol reaction failed to occur with the α–methoxy aldehyde, and only starting materials were recovered.

In addition to stereocontrol provided by avoidance of 1,3-diaxial interactions in the transition state, enolate geometry, and auxiliary chirality, with an α-chiral aldehyde one must also consider the effect of this stereocenter on the transition state. To better understand this phenomenon we have to consider the Felkin-Ahn model of carbonyl addition (Figure 2.5).

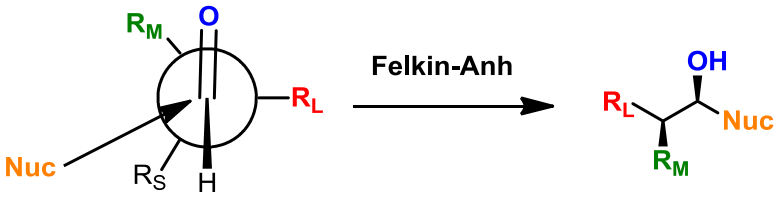


Figure 2.5. Felkin-Ahn Model for addition to α-chiral carbonyl compounds.[83]

In the Felkin-Ahn model, typically the largest substituent is placed perpendicular to the carbonyl and antiperplanar to the incoming nucleophile (Figure 2.5). The larger the nucleophile and R_L, the greater the Felkin selectivity.[83,84]

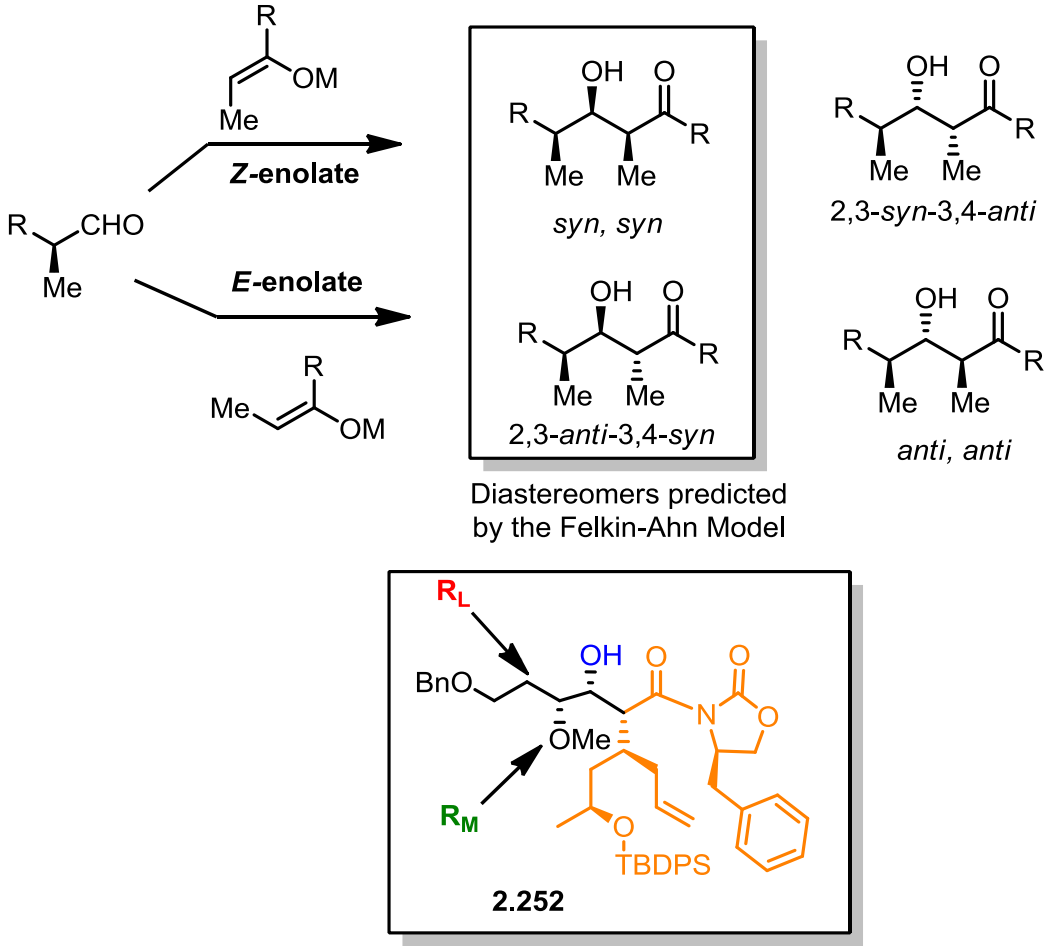


Figure 2.6. Felkin-Ahn Model predicts stereochemically distinct products based on enolate geometry (top). Desired product **2.252** could be formed through the *Z*-enolate if size-based assignments are made (bottom).[84]

As described in Figure 2.6, formation of the *Z*-enolate should provide the *syn, syn* Felkin product, which is the desired product, if we consider R = CH_2CH_2OBn the large group and R = OCH_3 the medium group. However, the incorporation of an α-polar substituent (such as an α-methoxy) affects the Felkin transition state in which R_L is always placed *anti* to the incoming nucleophile. This α-polar substituent effect is known as the antiperiplanar effect, which maximizes separation between the incoming anionic nucleophile and the electronegative α-substituent.. The effect is based on the preference to align the best acceptor σ^* orbital parallel to the π and π^* orbitals of the carbonyl and antiperiplanar to the incoming anion, thus stabilizing the incoming anion (Figure 2.7).[85]

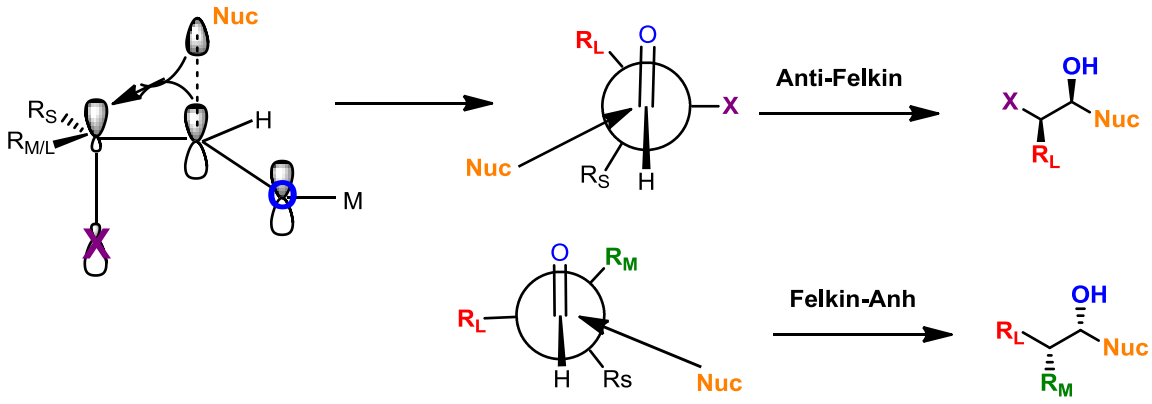


Figure 2.7. Felkin-Ahn antiperiplanar effect (left) can lead to anti-Felkin selectivity if **X** (the electronegative substituent) is not also the largest substituent (right).[85-87]

With α-methoxy aldehyde **2.271**, R_M = OCH_3 and R_L = CH_2CH_2OBn; however, the antiperiplanar effect will place the α-methoxy substituent away from the incoming nucleophile and the large R group next to the carbonyl and closer to the incoming nucleophile (Figure 2.8). In our case, the nucleophile and R_L are very large, which typically makes Felkin selectivity very favorable. However, minimization around R_L

places the electronegative α-methoxy substituent into the path of the incoming anionic nucleophile, which is electronically very unfavorable. Consideration of the electronegative α-substituent would predict the anti-Felkin product would be favored based on the antiperiplanar effect. However, this places the large R_L substituent into the path of the very large, incoming nucleophile, thus resulting in significant steric repulsion.

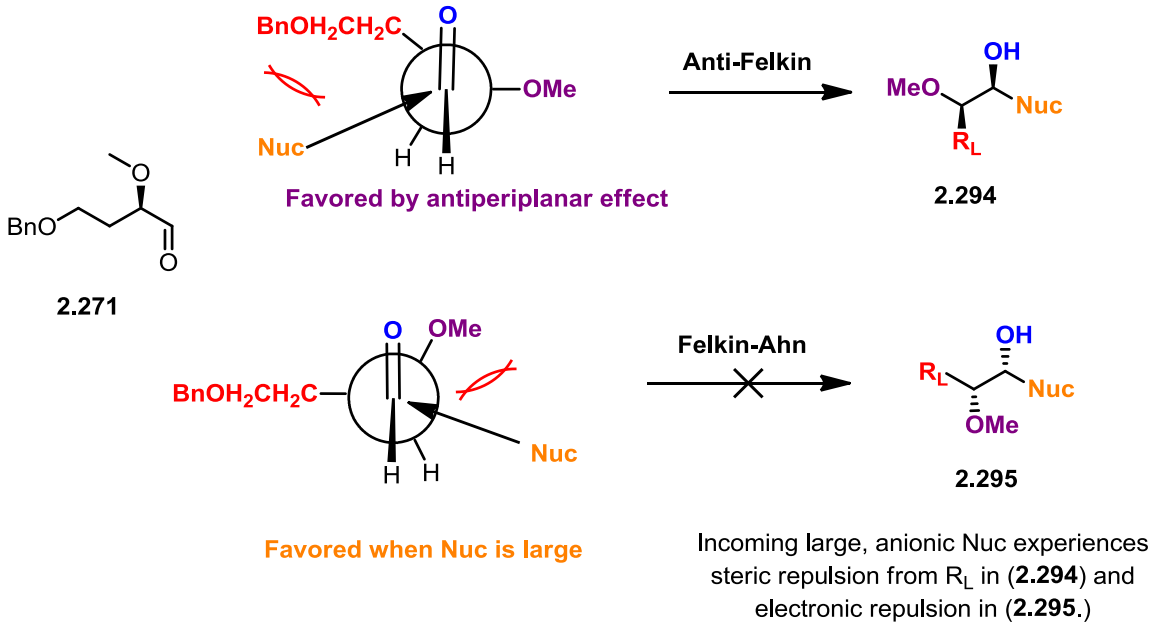


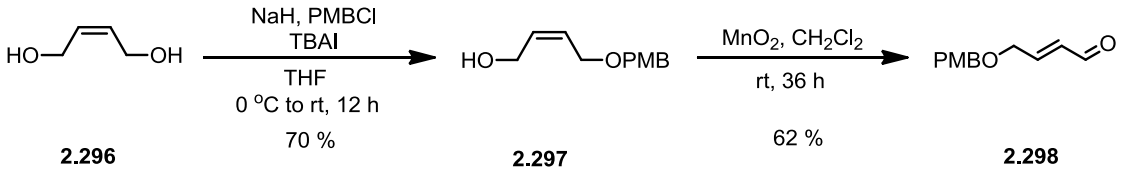
Figure 2.8. Analysis of aldehyde **2.271** based on Felkin-Ahn theory.

This analysis led to the conclusion that neither of the possible reaction pathways is particularly favorable, and this is very likely the reason for the observed lack of reactivity.

To investigate whether the combined steric and electronic liabilities of the α-methoxy aldehyde were responsible for this lack of reactivity, the aldol reaction was performed with a non-branched aldehyde, 6-hexanal. In this case, the aldol product was

readily formed, so it was concluded that the aldol reaction must be performed with a simpler substrate that does not experience opposing steric and electronic effects.

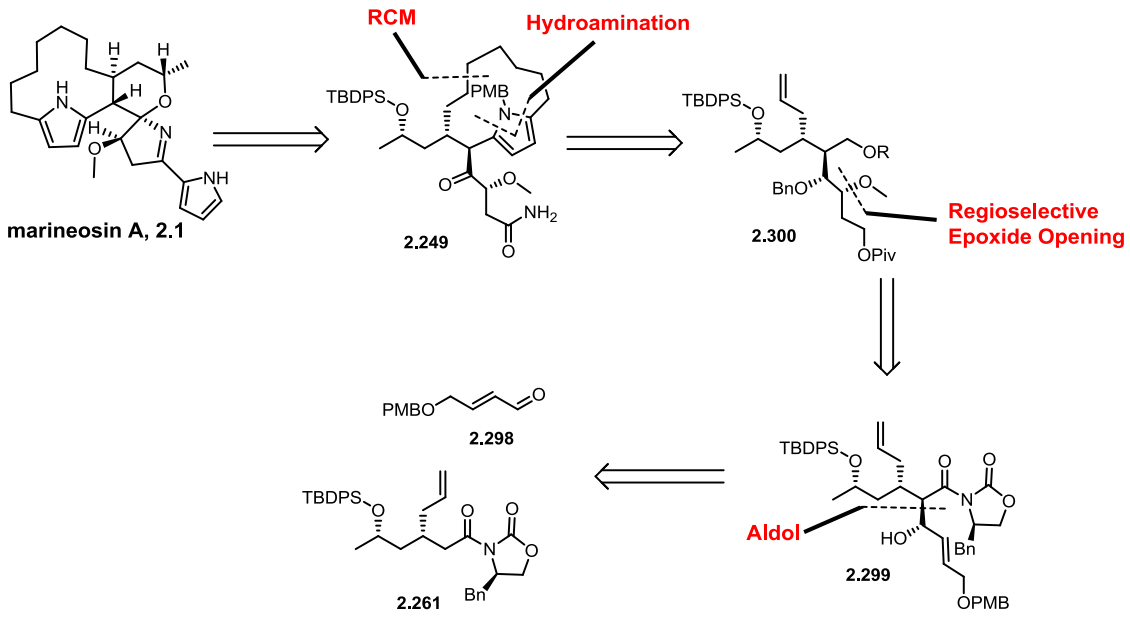
Unsaturated aldehyde **2.298**, possessing a flat and sterically undemanding surface, was chosen as a suitable substrate and was easily prepared in two steps. Mono-protection of 1,4-*cis*-buten-diol **2.296** with PMB chloride and subsequent oxididation/isomerization with excess manganese dioxide provided aldehyde **2.298** for use in the aldol reaction.



2.296 **2.297** **2.298**

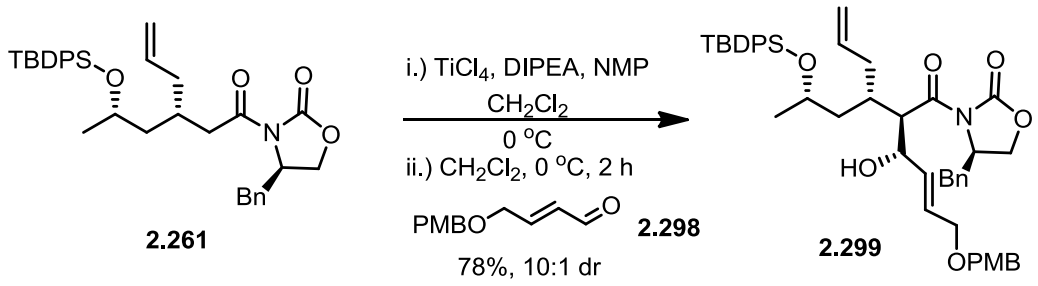
Scheme 2.84. Synthesis of sterically-undemanding aldehyde **2.296** from *cis*-butenediol **2.294**.

Aldehyde **2.298** should provide the aldol product **2.252**, and subsequent stereoselective epoxidation, regioselective epoxide opening, and functional group manipulations will provide the required hydroxyketoamide core **2.249** as described in Scheme 2.85. A key asymmetric epoxidation reaction, regioselective opening of the epoxide with Red-Al, and subsequent methylation will provide the chiral methoxy substituent of intermediate **2.300** that was unable to be directly incorporated as an α-methoxy aldehyde in the aldol reaction.



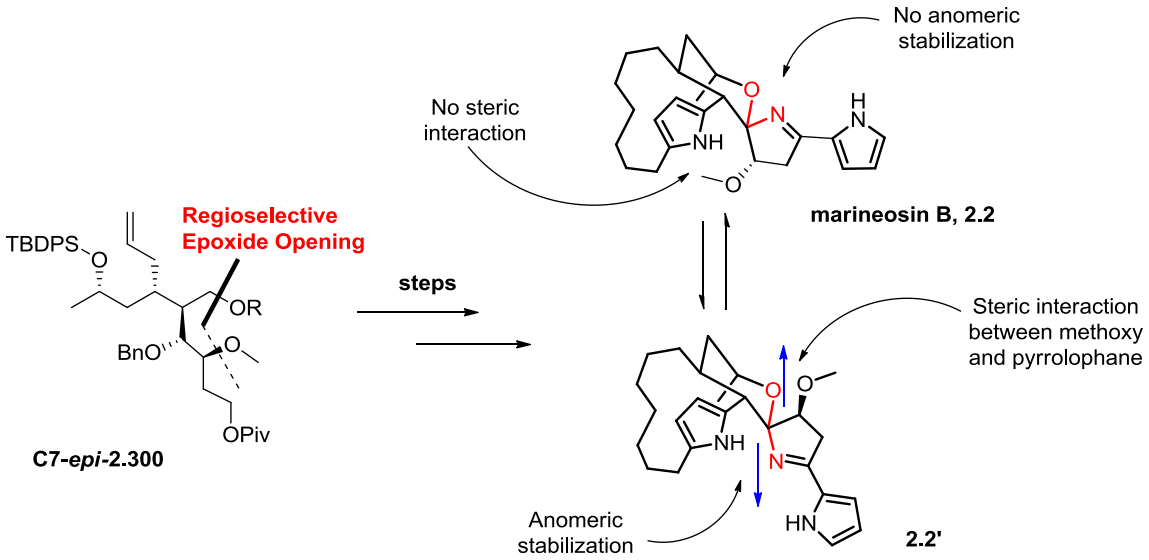
Scheme 2.85. Incorporation of aldehyde **2.298** into the retrosynthesis of marineosin A.

Fortunately, the simplified, unsaturated aldehyde **2.298** proved to be an excellent partner in the aldol reaction, providing the desired *syn* isomer **2.299** in 65% yield and 10:1 dr (Scheme 2.86). Recovered starting material (25%) was easily separated and resubmitted to improve the yield (78%, one resubmission). The success of the conjugate addition/aldol reaction sequence is an important milestome, since this is the first time we have been able to stereoselectively form the C9 stereocenter of marineosin A.



Scheme 2.86. Aldol reaction with sterically undemanding aldehyde **2.298**.

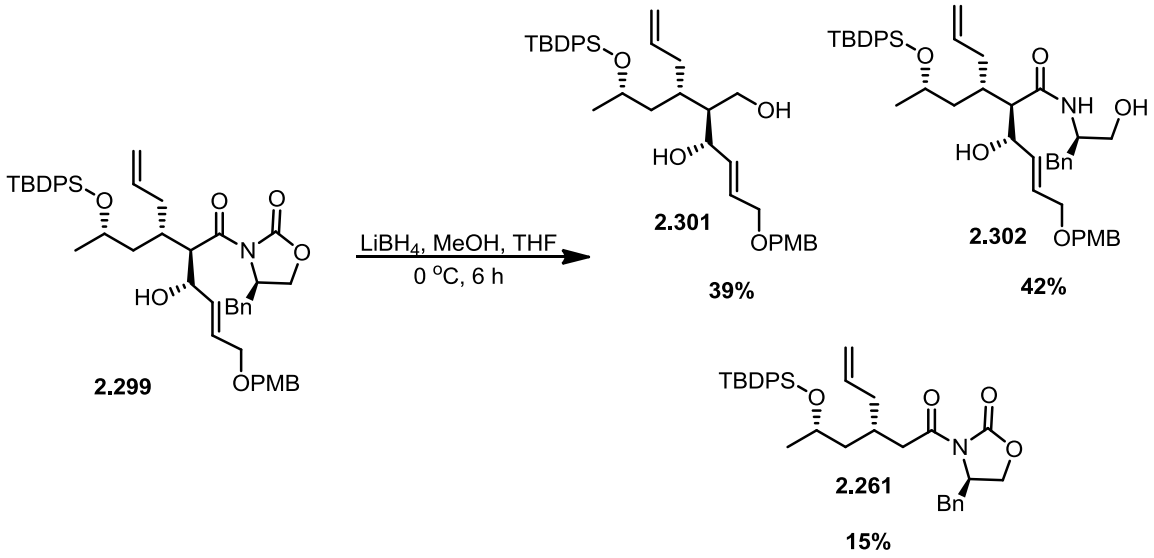
Intermediate **2.299** contains the three stereocenters that are the same in both marineosin A and marineosin B. Although our main focus is currently on the synthesis of the biologically active marineosin A diastereomer, one could envision a route to marineosin B which simply uses alternative asymmetric epoxidation conditions to prepare the other diastereomer (Scheme 2.87). Acid-catalyzed spirocyclization would not benefit from anomeric stabilization, but the inverted methoxy sbstituent could provide enough steric repulsion to orient this substituent away from the bulky, electron-rich pyrrolophane to favor formation of the non-anomerically stabilized spiroiminal.



Scheme 2.87. C7-*epi*-**2.300** could be prepared from aldol adduct **2.299** using an asymmetric epoxidation to provide marineosin B (**2.2**).

After successfully optimizing the conjugate addition/aldol sequence, auxiliary removal proved to be a daunting task. In addition to the desired diol, two by-products were formed, namely, the retro-aldol product **2.261** and the amido alcohol **2.302** resulting from hydride addition to the oxazolidinone auxiliary. After trying numerous conditions, only lithium borohydride and methanol in THF provided any of the desired diol product

2.301. It was concluded that these conditions provided the best and most consistent yield of diol **2.301** (36-42%) and that the isolated retro-aldol product (**2.261**) could be resubmitted to the aldol conditions to provide additional starting material (**2.299**)

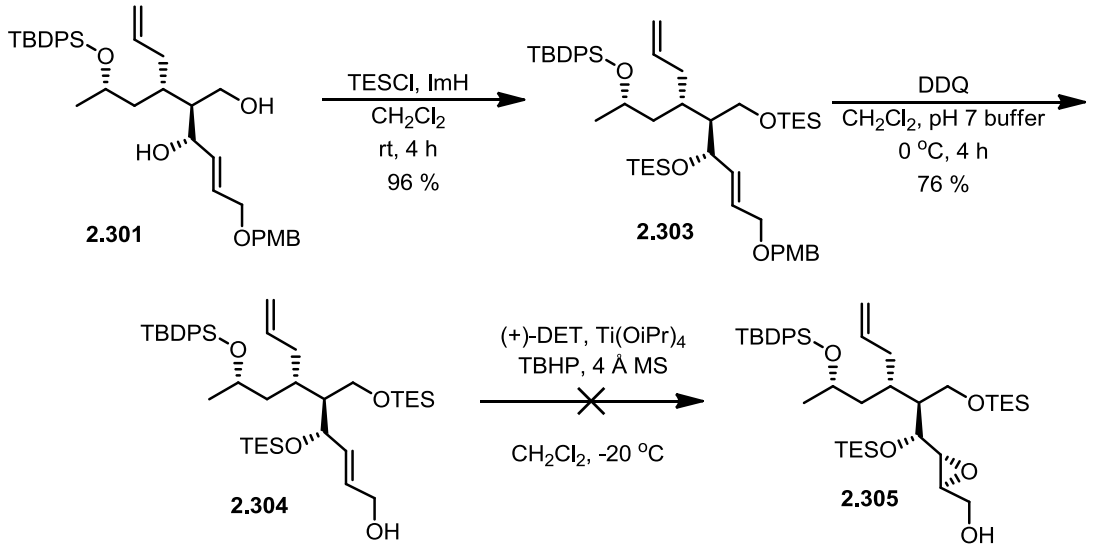


Scheme 2.88. Auxiliary removal to give diol **2.301** and by products.

The 42% of material lost as the amido alcohol **2.302** was unfortunate, so we decided to look into the other auxiliaries for the conjugate addition/aldol sequence. We tried three different auxiliaries (phenyl oxazolidinone, benzyl thiazolidinethione, and benzyl oxazolidinthione), and found the reaction sequence unsuccessful in all cases due to lack of stereoselectivity in the aldol reaction (phenyl), cuprate addition to the auxiliary (thiazolidinethione), or opening of the auxiliary to give amido alcohol **2.302** when treated with $LiBH_4$ (oxazolidinthione). At this point it was decided that a 36-42% yield of diol **2.299** was acceptable at this stage of the synthesis, and the route was continued.

Diol **2.301** was protected as the bis-TES ether (**2.303**) and the PMB protecting group was oxidatively removed by treatment with DDQ to give the primary allylic alcohol **2.304**. Surprisingly, Sharpless' asymmetric conditions for the epoxidation of

allylic alcohol **2.304** provided 0% conversion to product, even after a 7 day reaction time and the addition of stoichiometric amounts of reagents. It was concluded that the olefin was most likely too sterically crowded to undergo the desired reaction. The secondary TES ether may be blocking the α–face of the alkene and thus preventing any product formation due to the facial selectivity of the reactants.

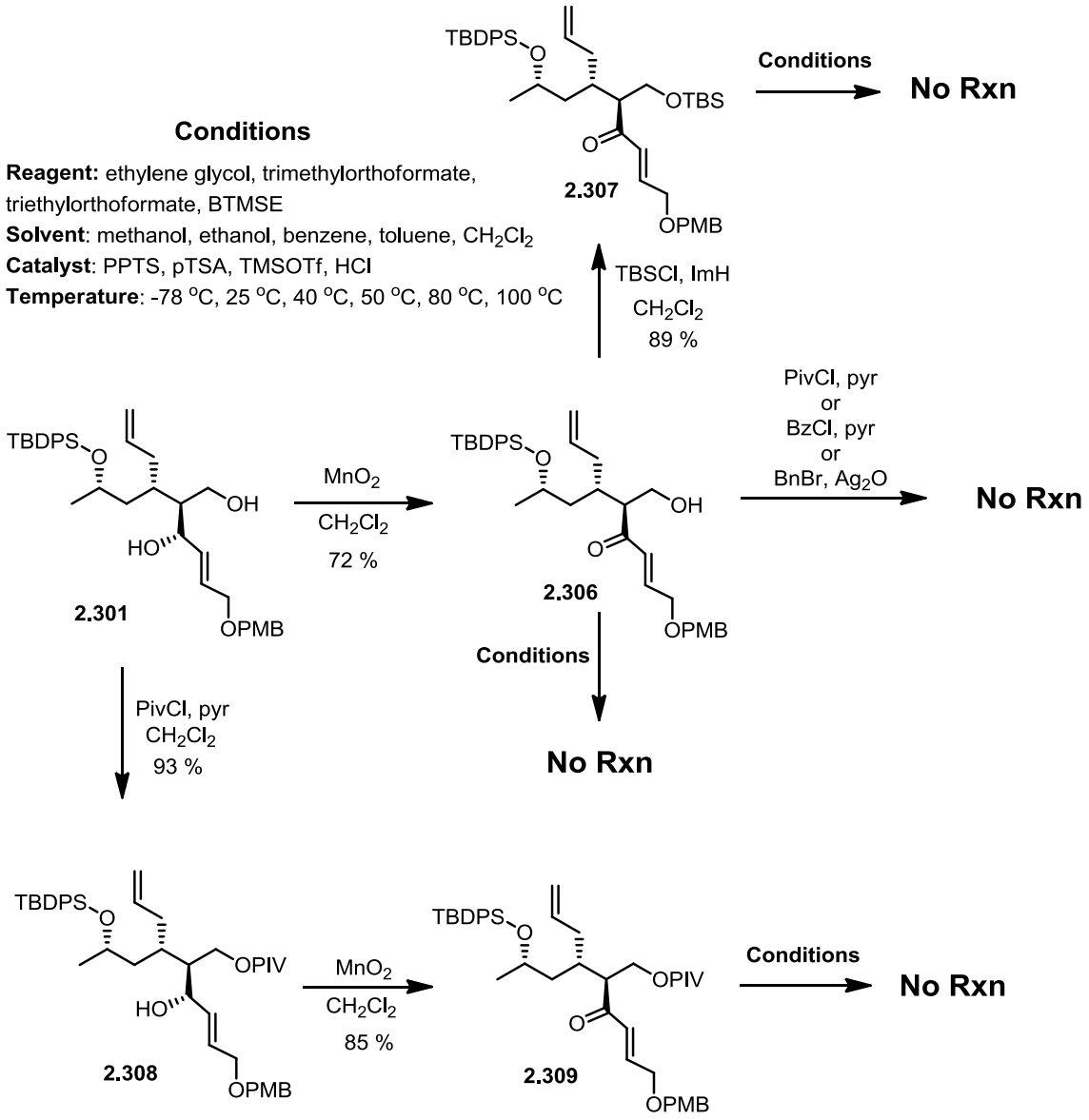


Scheme 2.89. Attempted asymmetric epoxidation of alkene **2.304**.

We decided to explore the effects of different protecting groups and oxidation states of diol **2.301** as summarized in Scheme 2.90.

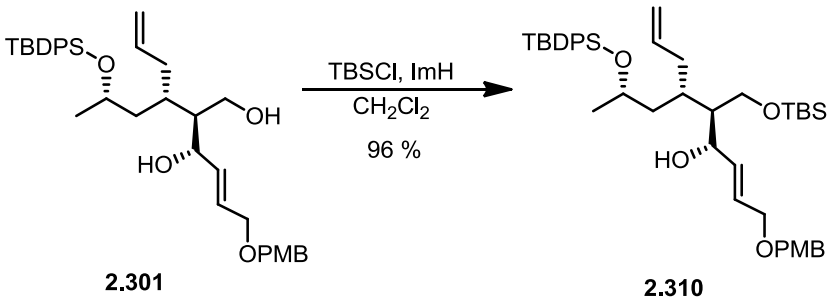
Although diol **2.01** could be selectively oxidized with manganese dioxide to provide enone **2.306**, subsequent protection of the primary alcohol as the benzyl ether, pivolate ester, and benzoate ester was unsuccessful. Dioxolane protection of the hindered, enone **2.306** was also unsuccessful. The primary alcohol of enone **3.206** was found to be efficiently reactive with TBSCl to provide the TBS ether; however, subsequent acidic conditions required for the dioxolane protection were incompatible with the primary TBS ether. By manipulating the primary alcohol of diol **2.301** first, it was found that it could

selectively be protected as the pivolate ester over the secondary alcohol. Pivolate ester **2.308** was oxidized to enone **2.309**, but subsequent reactions to form he dioxolane ketal all failed. Since we were unable to protect the hindered enone derivatives (**2.306**, **2.307**, and **2.309**) and remove the steric bias within our molecule, we decided to exploit the presence of the chiral secondary alcohol and the inherent steric bulk of alkene **2.304** by attempting a substrate-directed asymmetric epoxidation.



Scheme 2.90. Attempts to remove the steric bias of diol **2.301** by oxidation and dioxolane protection.

First, diol **2.301** had to be mono-protected to leave the secondary alcohol free for chelation. This was accomplished with TBSCl and imidazole to provide mono-TBS ether **2.310** in 96 % yield (Scheme 2.91). With chiral allylic alcohol **2.310** in hand it was time to analyze the conformation of the substrate and decide what strategy would give the desired stereoselectivity.



2.301 **2.310**

Scheme 2.91. Mono-protection of allylic alcohol **2.310** for substrate-controlled epoxidation.

Due to the reliability, chemoselectivity, and stereoselectivity associated with vanadyl acetoacetonate, this reagent was chosen for the substrate-controlled epoxidation. Typically, excellent, substrate-controlled stereoselectivity is observed in metal-catalyzed epoxidations by exploiting the $A_{1,2}$ strain within a molecule (Figure 2.9).[88]

In the case of vanadyl acetoacetonate, the role of the geminal substituent is crucial.[88,89] If the germinal substiuent is sufficiently large, it can control the epoxidation to generate solely the erythro product. However, if the geminal substituent is very small, $A_{1,2}$ strain becomes less important. If R_{cis} or R is very large, $A_{1,3}$ strain will become the controlling factor.

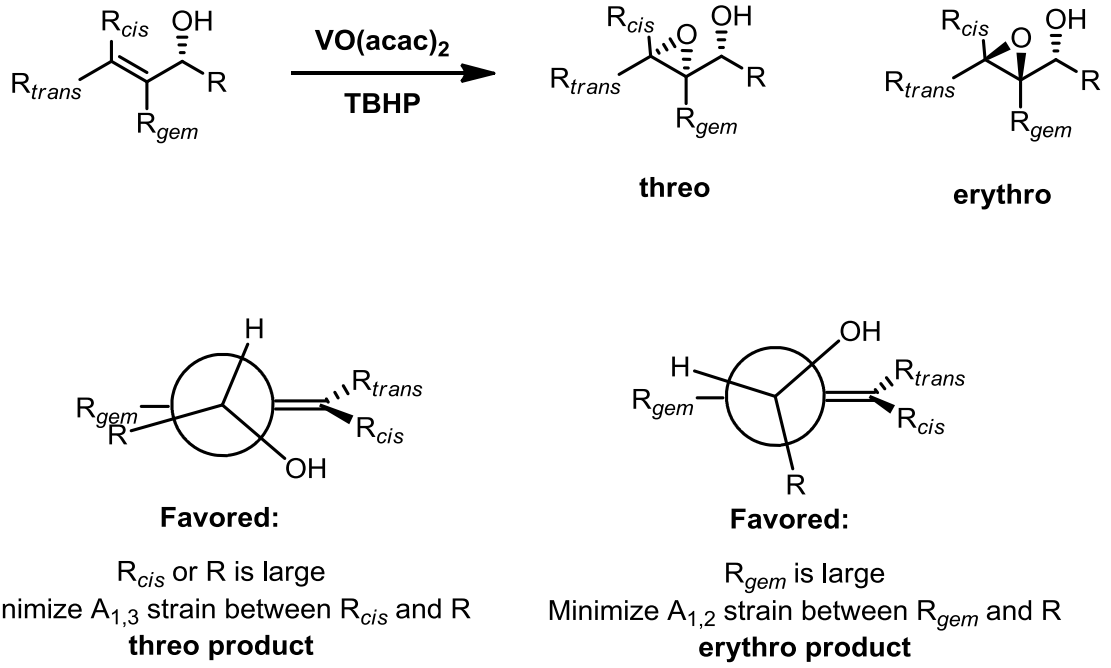


Figure 2.9. Vanadyl acetoacetonate epoxidation and rational for the observed stereochemical outcomes.[88,89]

In our case, the geminal substituent is a hydrogen atom, which is very small, and R is very large. As explained by the Newman projections in Figure 2.10, the more favorable conformation is the one in which $A_{1,3}$ strain is minimized by placing the very large R group away from the double bond and gauche with the geminal hydrogen. This conformation should provide the desired threo product. One could easily see how a methyl geminal substituent could increase $A_{1,2}$ strain and lower the stereocontrol of $A_{1,3}$ strain. It is also evident that a large *cis* substituent could improve the stereocontrol by maximizing the $A_{1,3}$ strain. In our case, we are expecting the $A_{1,3}$ strain to be the controlling factor. The only question remaining is how strong that control will be.

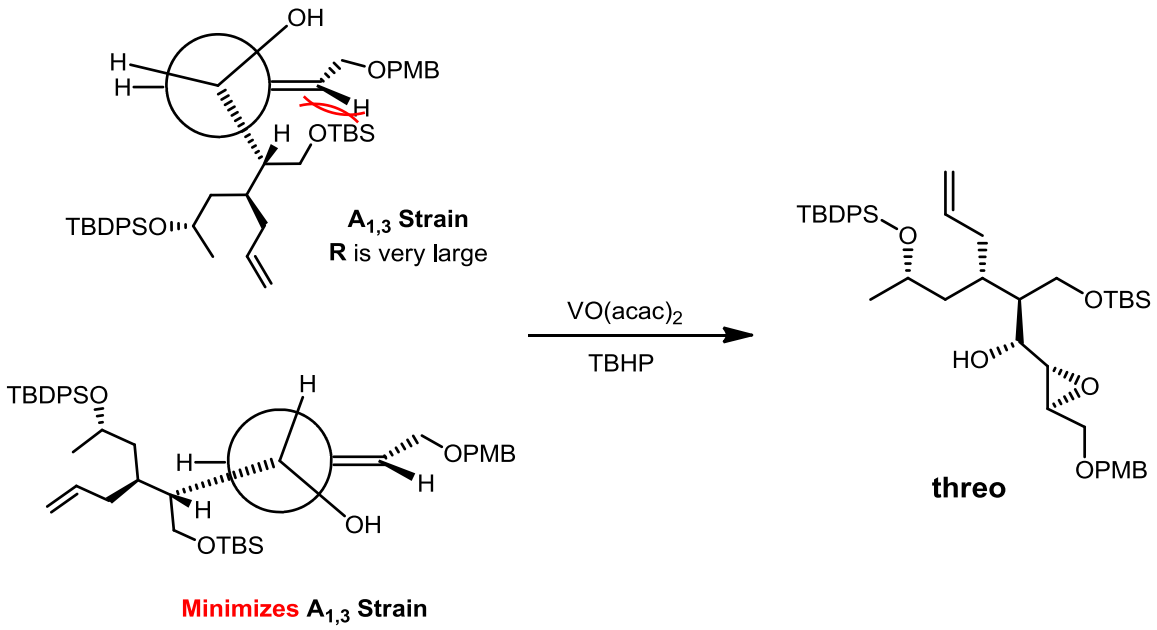
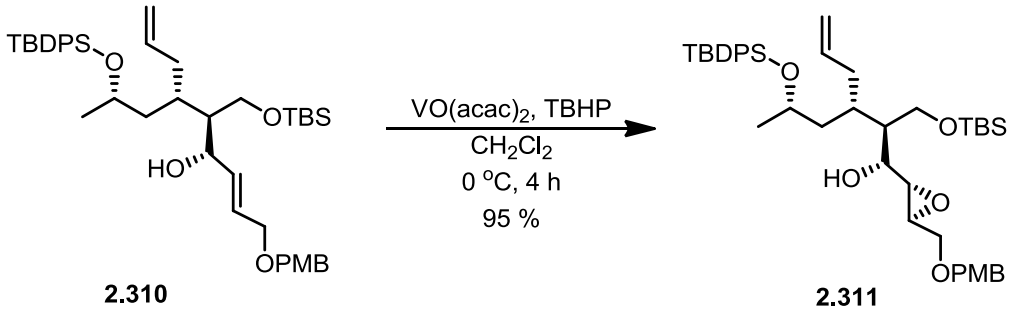


Figure 2.10. Analysis of $A_{1,3}$ strain within allylic alcohol **2.310** and predicted outcome of epoxidation reaction.

Treatment of allylic alcohol **2.310** with *tert*-butyl hydrogen peroxide (TBHP) and vanadyl acetoacetonate at 0 ^{o}C resulted in 100% conversion to a single diastereomer (>20:1 by NMR) in only 4 hours.



Scheme 2.92. Substrate-controlled stereoselective epoxidation of allylic alcohol **2.310** with vanadyl acetoacetonate.

A crystal structure was unable to be obtained from the viscous oil; however, molecule modeling agrees with the assignment of the product as threo isomer **2.311**. The energy-minimized conformation of allylic alcohol **2.310** reveals that the most favored

conformation is indeed the one that minimizes the $A_{1,3}$ strain. This leaves the α-face of the alkene open for epoxidation in the most energetically favored conformation, as predicted.

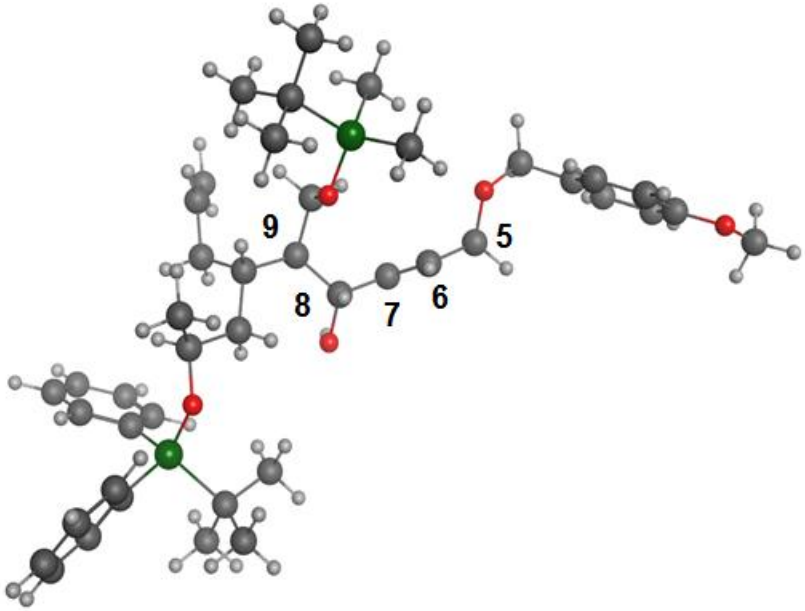


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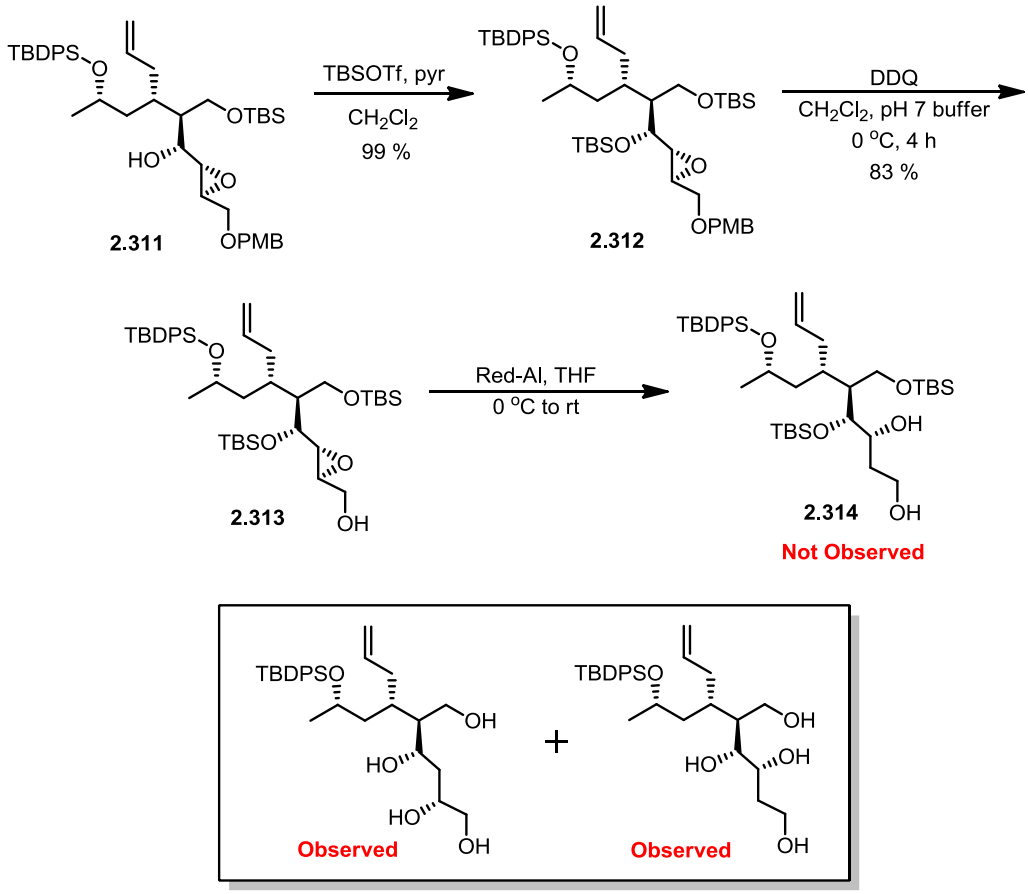
Figure 2.11. Most favored energy minimized conformation of allylic alcohol **2.310**. Lowest energy conformation reveals open bottom face of C6-C7 alkene in agreement with minimization of $A_{1,3}$ strain.

With threo epoxide **2.311** in hand, it was time to choose protecting groups and investigate the regioselective epoxide-opening. Protection of the free secondary alcohol as the TBS ether was unsuccessful with TBSCl and imidazole; however, the more reactive TBSOTf and pyridine facilitated complete conversion to silyl ether **2.312** (Scheme 2.93). The PMB protecting group was oxidatively removed using DDQ, revealing the free primary alcohol (**2.313**). The alcohol was then treated with Red-Al to regioselectively open the epoxide through chelation with the free alcohol and internal

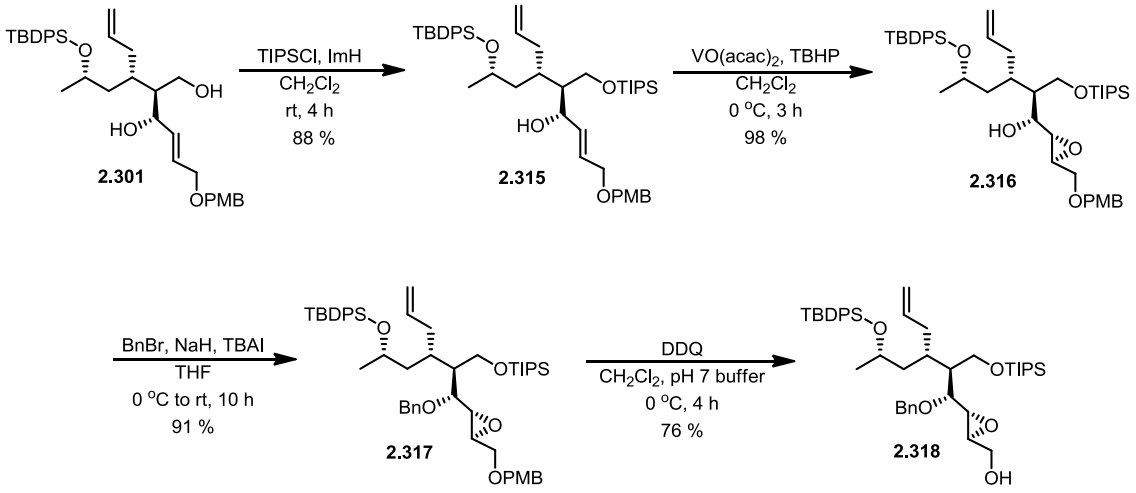
hydride delivery to provide 1,3-diol **2.314**.[56] Unfortunately, the TBS groups proved too labile under the strongly basic conditions and mixed regioisomers of tetra-ols were obtained.



Scheme 2.93. Undesired protecting group removal in regioselective epoxide-opening.

. The protecting group strategy was reassessed, and the primary alcohol of diol **2.301** was chemoselectively protected as the TIPS ether (**2.315**). Epoxidation proceeded smoothly with this highly hindered substrate to provide the threo epoxide (**3.316**) as before. The secondary alcohol of **3.316** was carefully protected as the benzyl ether by adding sodium hydride as 0 °C, immediately followed by addition of excess benzyl bromide and catalytic TBAI. The mixture was allowed to slowly warm to room

temperature once all of the reagents were combined. These precautions were taken in order to avoid an intramolecular Payne rearrangement considering the steric crowding at this position in the molecule. Fortunately, the reaction proceeded in 91 % yield to provide benzyl ether **3.317** with no evidence of a Payne rearrangement by NMR analysis.[91]

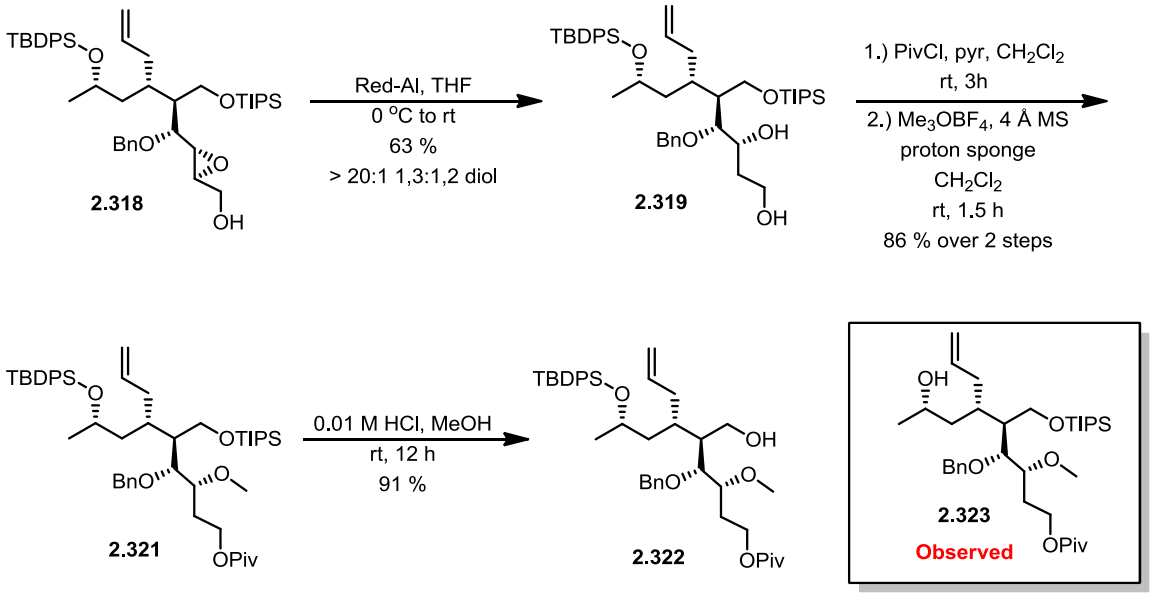


Scheme 2.94. Preparation of alcohol **2.318** for regioselective epoxide-opening.

The PMB ether of **2.317** was oxidatively cleaved to reveal the desired 2,3-epoxy alcohol (**2.318**), which was treated with Red-Al. After 4 hours, the reaction was near completion, but removal of the TIPS protecting group was becoming apparent by LCMS. The reaction was quenched, extracted, and purified to provide the desired 1,3-diol **2.319** as the major product (63%), recovered starting material **2.318** (15%), and the TIPS removal product as a minor by-product (<5%). None of the 1,2 diol was observed by NMR. The primary alcohol was selectively protected as the pivolate ester (**2.320**), and the secondary alcohol subjected to soft methylation conditions to provide the fully protected intermediate **2.321**, as a single diastereomer.

In order to incorporate the alkyne, it was necessary to identify conditions that could selectively deprotect a primary TIPS ether in the presence of a secondary TBDPS

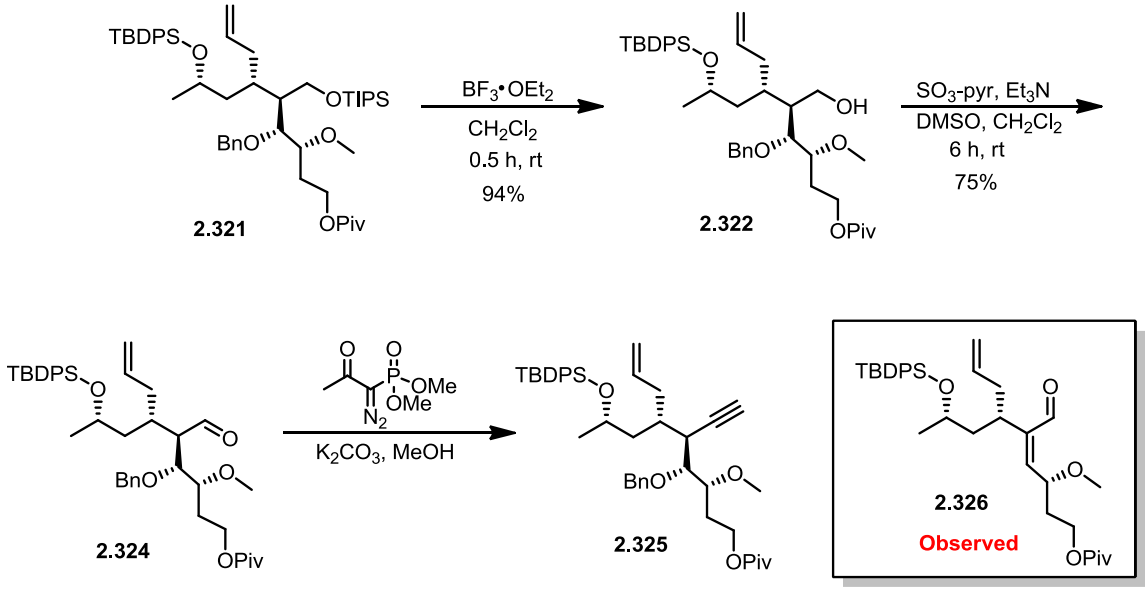
ether. The chosen conditions would need to be acidic since TIPS ethers are slightly more acid labile than a TBDPS ether. In general, it should also be easier to remove a primary acid-labile protecting group over the secondary, more acid-stable protecting group. Previous research indicated that chemoselective removal of silyl ethers was possible with dilute HCl in MeOH and other protic solvents.[91,92] In light of these successes, **2.321** was treated with 0.01 M HCl in MeOH, and after 5 hours, all starting material had been consumed. Upon purification and NMR analysis, it became evident that the secondary TBDPS ether had been selectively deprotected under these conditions to provide the free secondary alcohol **2.323** in 91% yield.



Scheme 2.95. Chemoselective deprotection of a primary TIPS ether in the presence of a secondary TBDPS ether.

These results were disheartening, but conditions were fortuitously revealed that could successfully remove the TBDPS protecting group from the protected hydroxyketoamide in the acid-catalyzed spirocyclization step. After unsuccessful experiments with several organic acids, we decided to test Lewis acids. According to rate studies performed by Mabic and Lepoittevin, TBDPS ethers are stable to BF_3OEt_2 in

CH_2Cl_2; however, TIPS ethers should be hydrolyzed within 1 h.[93] In our hands, treatment of intermediate **2.321** with BF_3OEt_2 in CH_2Cl_2 at room temperature resulted in complete conversion to the desired deprotected primary alcohol **2.322** in 0.5 hours and 94 % isolated yield. The hindered primary alcohol was readily oxidized under Parikh-Doering conditions to provide the desired aldehyde **2.324** in 79 % yield with no racemization. However, excess reagents were needed in order for the reaction to reach completion within the average 4-6 hour reaction time commonly observed for these conditions. Since aldehyde **2.324** proved sensitive to air oxidation to the carboxylic acid, it was stored at -20 ^oC under argon and used within 1 week, or stored at 0 ^oC under argon and carried on to the next reaction within 24 hours.

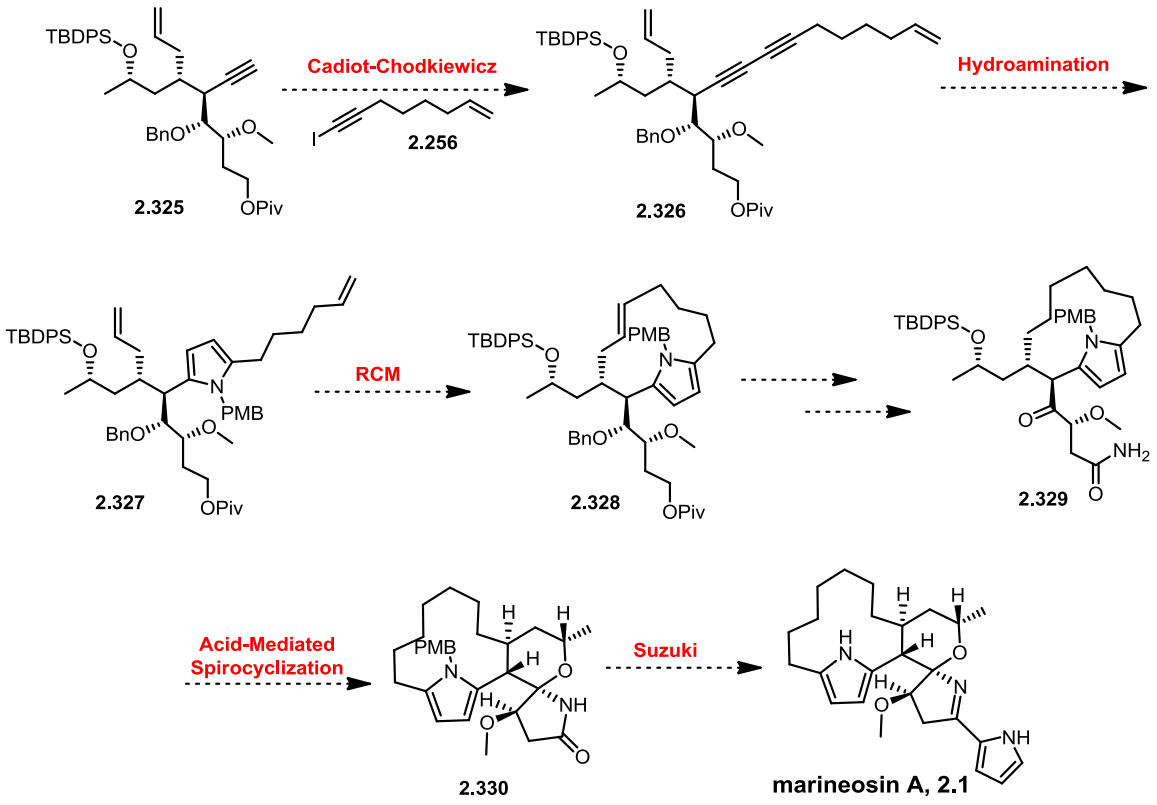


Scheme 2.96. Attempted homologation to form alkyne **2.325**

Aldehyde **2.324** was then subjected to Ohira-Bestmann conditions for homologation to alkyne **2.325**.[95] Unfortunately, the only observed product was the β-hydroxy elimination product **2.326**. Corey-Fuchs homologation proved unsuccessful as

well; formation of the geminal dibromide intermediate never occurred at room temperature and gently heating the reaction mixture resulted in decomposition of the starting material.[96]

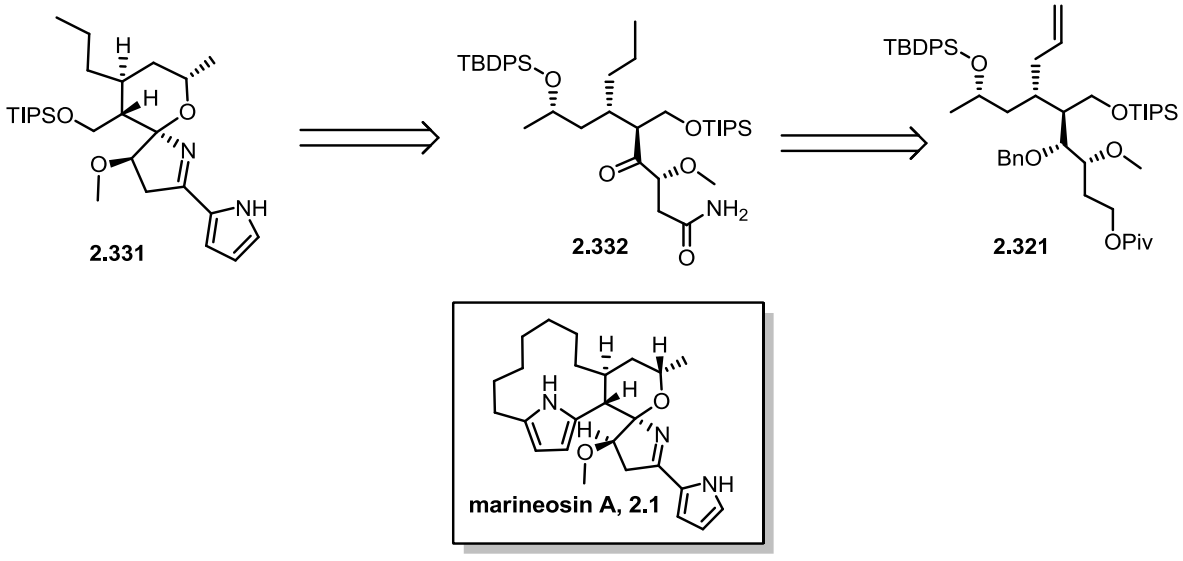
Our inability to generate the advanced alkyne intermediate **3.325** prevented further pursuit of the total synthesis of marineosin A through completion of the route described in Scheme 2.97. Since the hydroamination route for pyrrole formation was no longer a viable route, alternative methods of pyrrole formation would need to be explored.



Scheme 2.97. Summary of unsuccessful intermolecular alkyne-alkyne coupling/hydroamination route.

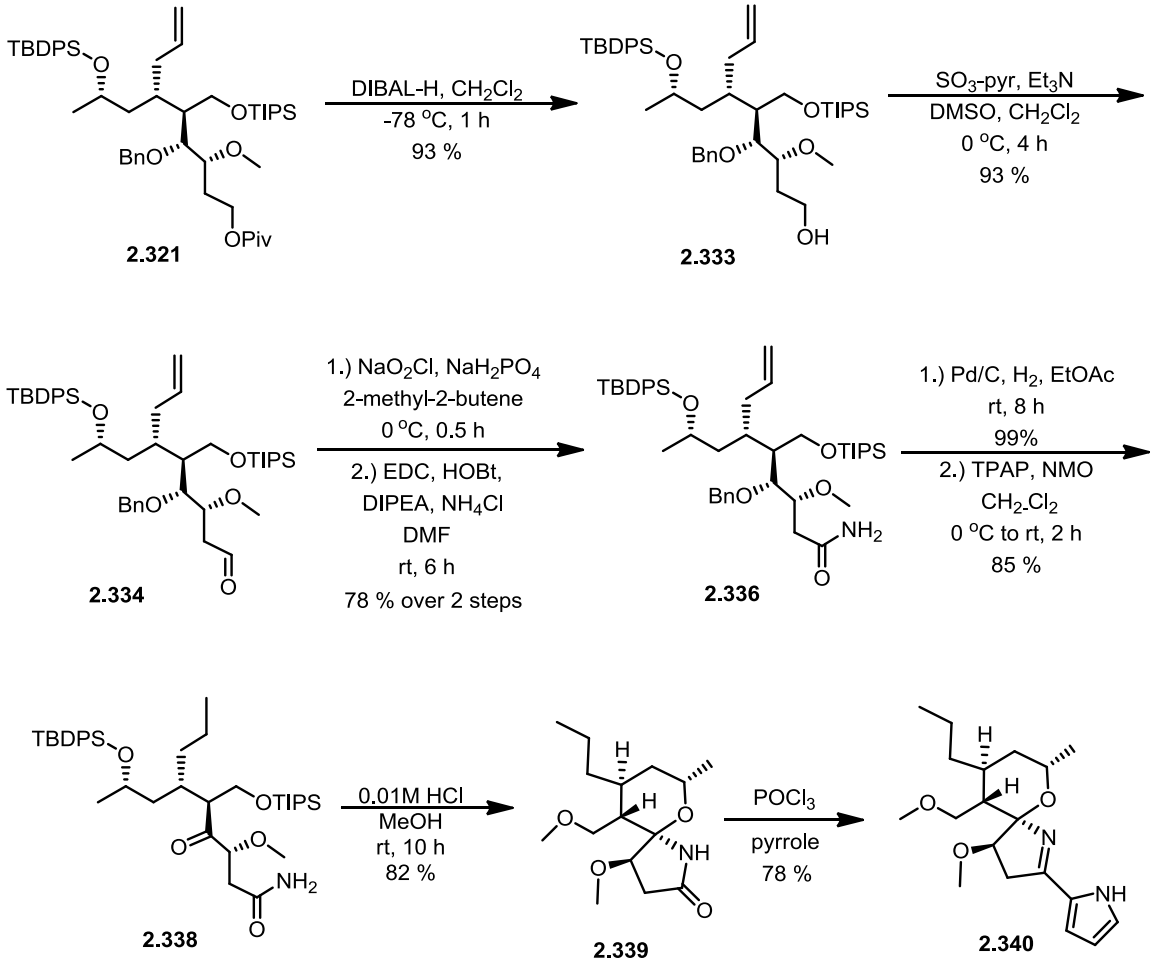
2.10.4. Model Synthesis of the Fully-Functionalized Core of Marineosin A.

Due to the challenge of installing the pyrrolophane C-ring of marineosin A, we decided to exclude this moiety and complete a model synthesis of marineosin A based on the acid-catalyzed spirocyclization strategy. Completion of a model synthesis of the fully-functionalized spiroiminal core of marineosin A will provide a chance to optimize the reaction sequence for the total synthesis of marineosin A once the pyrrolophane moiety is completed. A model system will also enable investigation of the late-stage spirocyclization in order to determine the viability of our current route to achieve the total synthesis of marineosin A. The marineosin A model (**2.331**) will not contain the macrocycle or the C-ring pyrrole, but will contain functional group handles that enable their installation. **2.331** will be prepared by the acid-mediated spirocyclization from hydroxyketoaminde **2.332**, which will be obtained from the stereocehmically advanced alcohol **2.321**.



Scheme 2.98. Retrosynthetic analysis of marineosin A model **2.331** from previously synthesized TIPS alcohol **2.321**.

The TIPS-protected alcohol **2.321** was utilized for initial model studies. Pivalate reductive removal with DIBAL-H proceeded smoothly to give the primary alcohol **2.333**, which was oxidized with Parikh-Doering conditions to give aldehyde **2.334**. Pinnick oxidation rapidly provided carboxylic acid **2.335**, which was coupled to ammonium chloride with traditional amide coupling conditions to provide the primary amide **2.336**.[96] Hydrogenolyisis of the benzyl ether and concomitant hydrogenation of the double bond provided alcohol **2.337**, which was oxidized to the hydroxyketoamide (**2.338**) with Ley conditions.



Scheme 2.99. Completion of the synthesis of the fully-functionalized sprioiminal core of marineosin A.

Keeping in mind the undesired deprotection of the TBDPS ether (Scheme 2.95), 0.01 M HCl in MeOH was chosen to facilitate the cyclization. Unfortunately, the TIPS ether was hydrolyzed under these conditions and replaced by a methyl ether, as confirmed by 1D and 2D NMR experiments. The major product was obtained as a single diastereomer in 81 % yield. HMBC, HSQC, COSY, and NOESY experiments confirmed the absolute structure of the product as the marineosin A isomer based on the stereochemistry relative to the axial methyl group within the pyran ring, which was incorporated from (*S*)-methyl oxirane in our synthetic route.

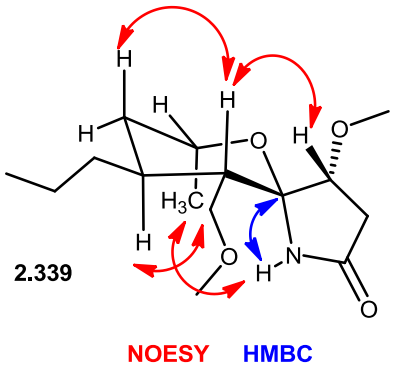
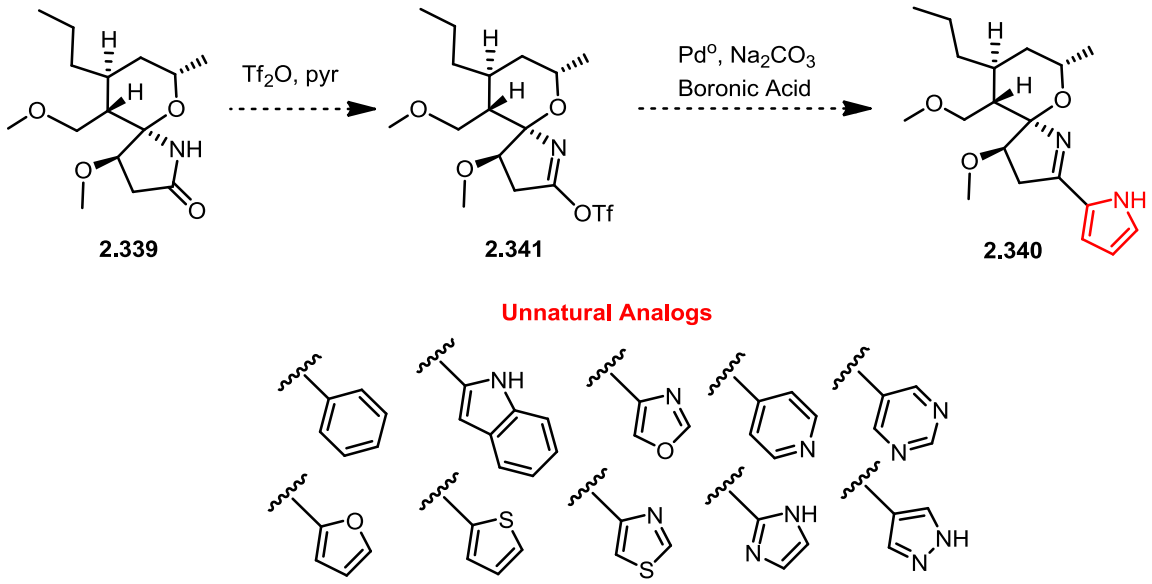


Figure 2.12. Key NOESY and HMBC correlations of marineosin model **2.340** confirming connectivity and absolute stereochemistry.

Suspension of the spirocyclic lactam **2.340** in pyrrole and treatment with phosphorous oxychoride, provided the spiroiminal core **2.240**.[97] However, the product was unable to be separated from the pyrrole solvent by chromatography due to the considerable polarity of the two components and the instability of the product to reverse-phase chromatography. Once appreciable material has been prepared, this reaction will be attempted on a larger scale (> 1.5 mg) to enable additional purification efforts and full characterization.

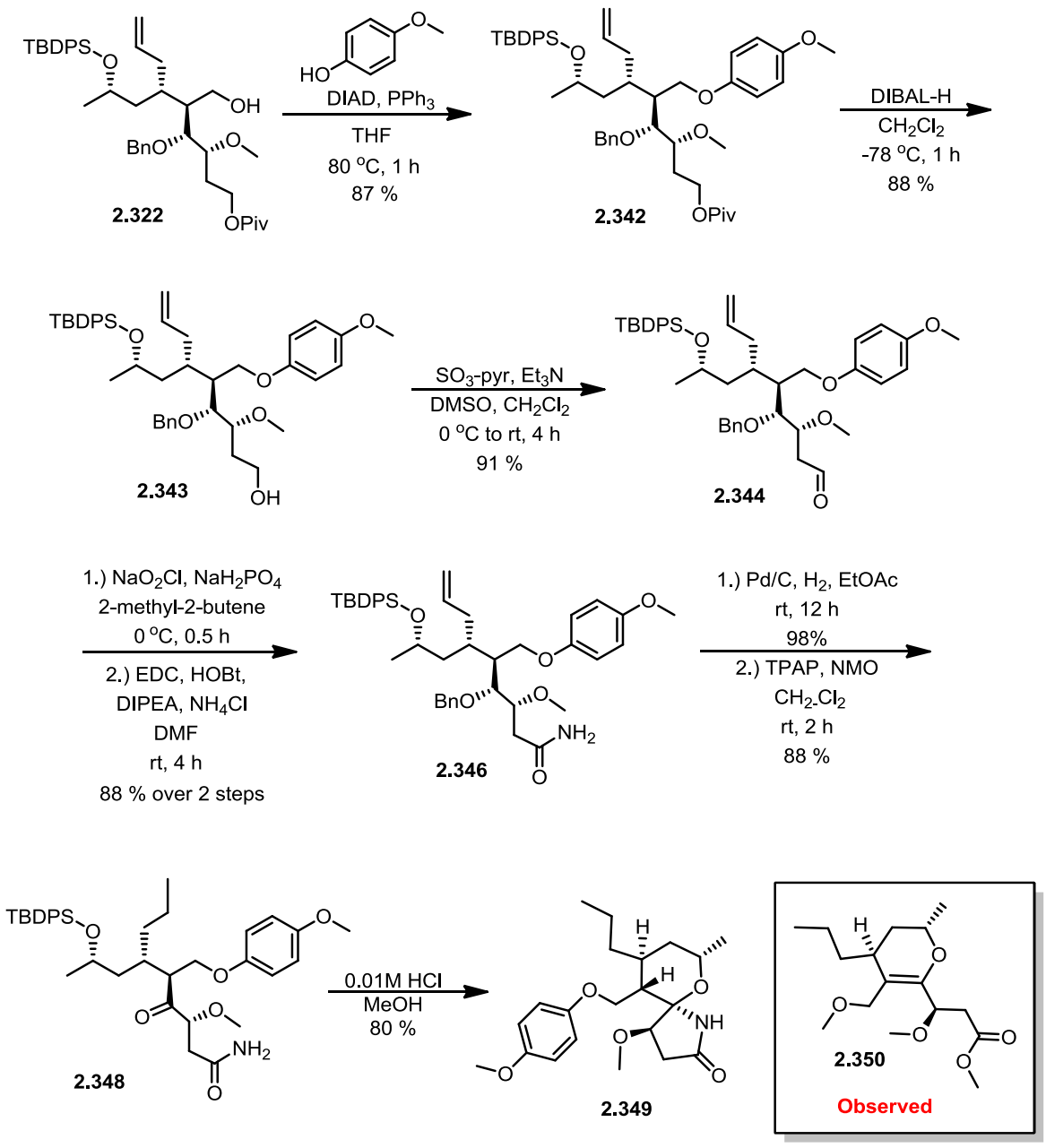
Lactam **2.339** will also be treated with triflic anhydride to provide triflate **2.341**, which can be coupled to 1-Boc-2-pyrrole boronic acid and other boronic acids to generate a library of unnatural analogs for biological testing (Scheme 2.100).



Scheme 2.100. Planned synthesis of unnatural analogs of marineosin A.

In order to prevent ionization of the TIPS ether and reaction with methanol, we decided to replace this protecting group with the more stable *p*-methoxyphenyl (PMP) ether. This way the PMP group can be selectively removed to incorporate additional derivatization (Scheme 2.101).

After optimizing conditions for the Mitsunobu reaction to incorporate the PMP protecting group, PMP ether **2.342** was carried through the same reaction sequence as TIPS ether **2.321** to successfully prepare the required hydroxyketoamide **2.348** (Scheme 2.101).



Scheme 2.101. Synthesis of the sprioiminal core of marineosin A with a PMP primary alcohol protecting group.

Exposure to acidic methanol gave surprising results. Pyran **2.350** was formed as the exclusive product. The PMP group was removed by the acidic reaction conditions, and the intermediate ion was quenched by methanol. The primary amide underwent methanolysis to provide a methyl ester and the oxonium ion was quenched by an

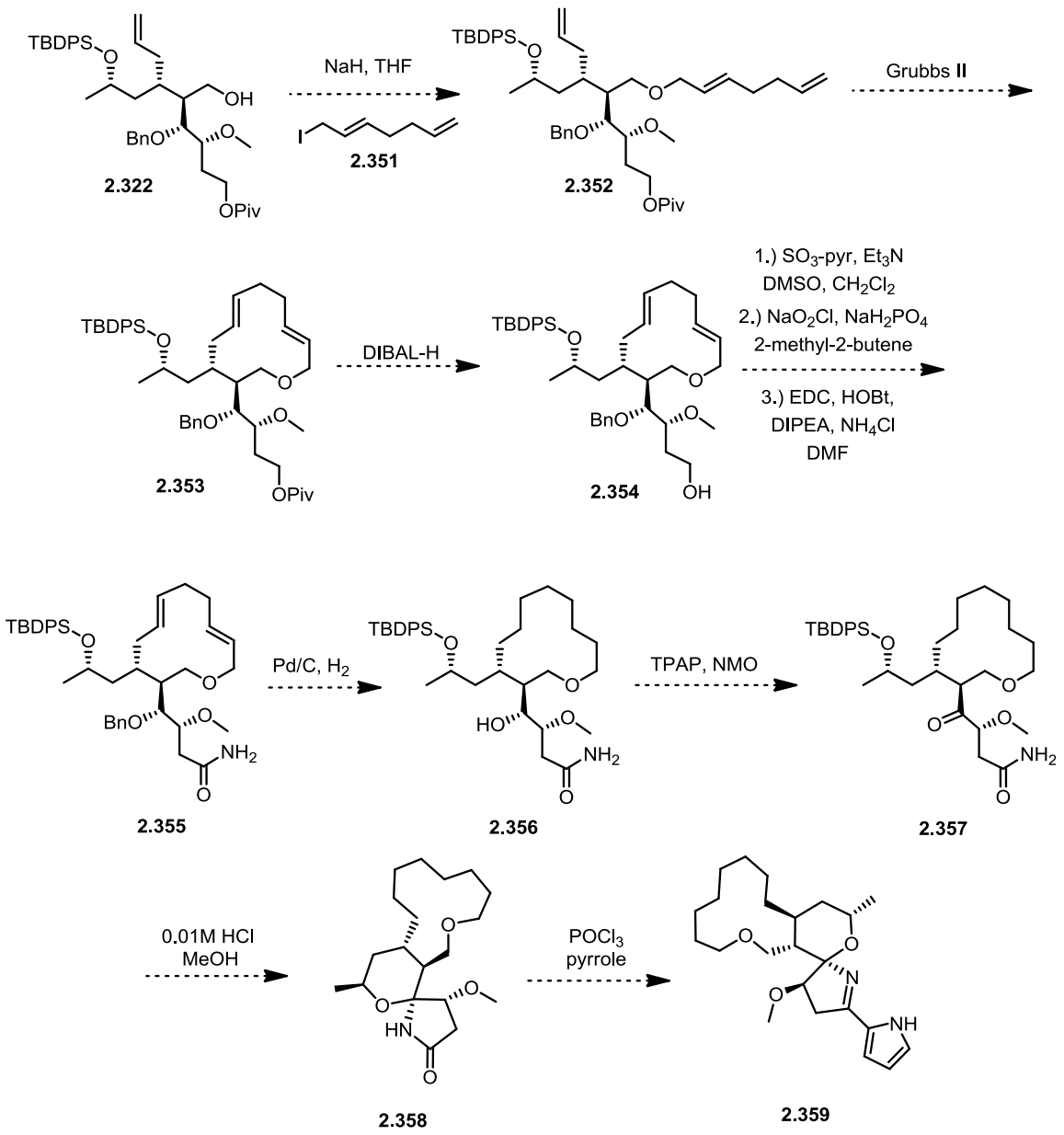
elimination reaction in the absence of a primary amide moiety to capture the electrophilic intermediate. It is unclear why the two very stable protecting groups (TIPS and PMP) are removed in such dilute acidic conditions and why these two very similar substrates produce very different products through divergent reaction pathways. Clearly, in order to generate a stable and predictable marineosin A model for derivatization and biological evaluation, we will need to incorporate an alcohol substituent that it not designed for subsequent removal as a protecting group.

2.11 Future Work.

2.11.1 Completion of Marineosin A Macrocyclic Model System.

In light of the difficulties encountered with protecting groups in the acid-mediated spirocyclization, we plan to alkylate the primary alcohol **2.322** with a substituent that is not designed for removal as a protecting group (Scheme 2.102). After preparing allylic iodide **2.351**, this electrophile will be used to alkylate primary alcohol **2.322**. Alkylation with commercially-available 7-bromo-1-heptene was unsuccessful, but the allylic iodide **2.351** should be considerably more reactive, as we have observed in previous experiments with the alkylation of lactones (Chapter 2, Scheme 2.69).

After *O*-alkylation to form ether **2.352**, a 12-membered ring containing the alkyl ether will be formed by RCM (Scheme 2.102). Macrocycle **2.353** will then undergo the series of optimized reactions to provide hydroxyketoamide **2.357** for the acid-mediated spirocyclization to provide lactam **2.358**. Acylation of lactam **2.358** with pyrrole will provide the fully derivatized model core **2.359** of marineosin A.



Scheme 2.102. Planned route for completion of the model system of marineosin A.

Hopefully, the alkyl group/macrocycle will prove more stable than the TIPS and PMP protecting groups and will provide the desired marineosin A isomer. We then plan to make unnatural analogs as previously described in Scheme 2.100 and hope to identify the minimal pharmacophore for biological activity. In this model system, the only missing piece will be the pyrrole moiety within the macrocycle. The oxygen within the

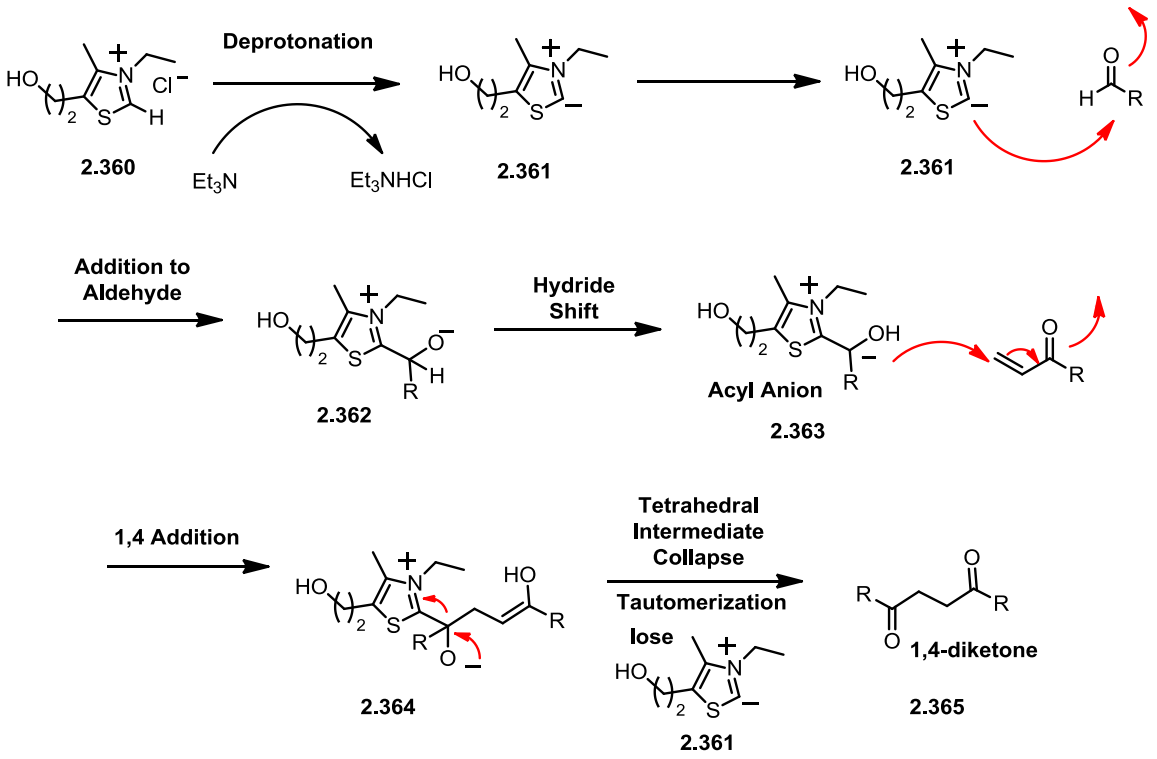
macrocyclic ring is still a hydrogen bond acceptor and could have the same role in cells as the pyrrole. Of course the pyrrole can function as a hydrogen bond donor or acceptor, so these macrocyclic ethers could highlight the role of the C-ring pyrrole in the observed biological activity. If the macrocyclic ethers prove completely inactive, this could indicate that the C-ring pyrrole serves primarily as a hydrogen bond donor. Retained activity with ether replacements could indicate the C-ring pyrrole is primarily a hydrogen bond acceptor. Either result will reveal interesting information about the SAR of these structurally-complex natural product analogs. SAR studies have implicated that the pyrrole A ring is key for activity; [29] however, numerous prodigiosins with alternative C-rings have maintained or improved biological activity and these derivatives have been used to tune the activity to favor one biological process over another.[29,98] We are hopeful that our macrocyclic etherlibrary will also possess anticancer, antimalarial, antibacterial, or immunosuppressive activity as so many of the prodigiosin alkaloids do and that we will be able to identify derivatives to modulate these potential, variable activities.

2.11.2 Total Synthesis of Marineosin A

In addition to our plans to complete a model system of the fully-derivatized spiroiminal core of marineosin A (Scheme 1.102), we also plan to complete the total synthesis of this intriguing natural product and to synthesize derivatives with various nitrogen-containing heterocycles as the A-ring. Our plan for the completion of the synthesis will include an intermolecular Stetter reaction as a key carbon-carbon bond forming reaction (Scheme 2.103).[99]

In the Stetter reaction, triazolium salt **2.360** is deprotonated to form a Wittig-type

reagent (**2.361**), which then adds into the aldehyde component to give alkoxy intermediate **2.362**. A hydride shift then occurs to give the acyl anion intermediate **2.363**.

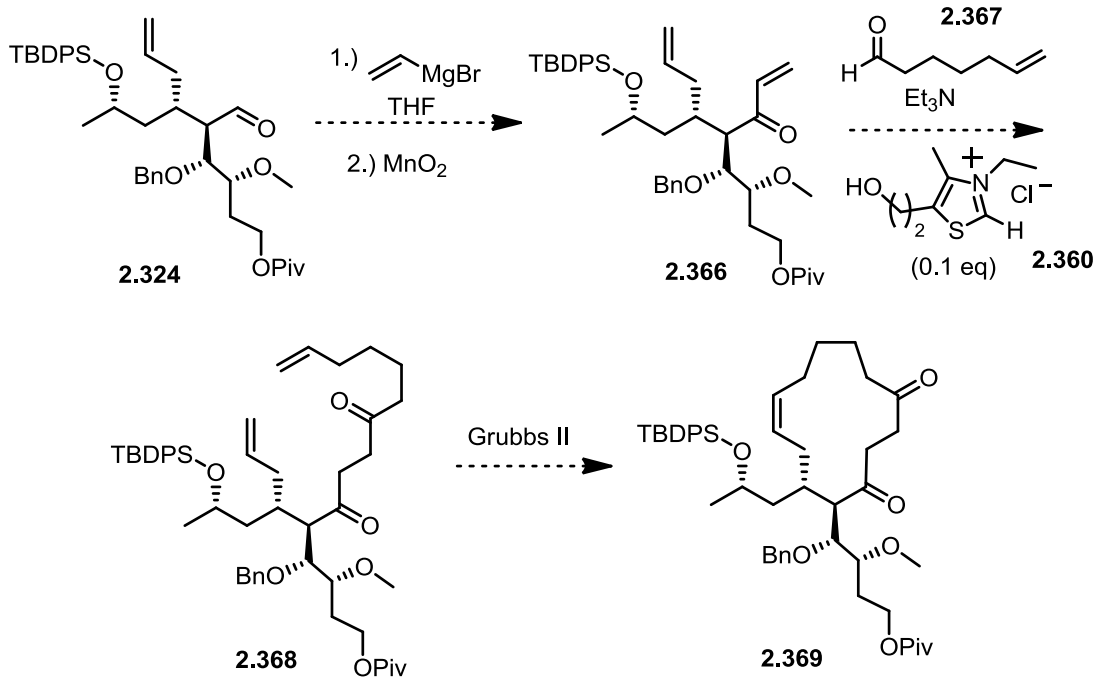


Scheme 2.103. The mechanism of the Stetter reaction.

This is an example of umpolung chemistry, where the activity of a functional group is reversed. In this case, the electrophilic aldehyde is transformed into a nucleophilic acyl anion (**2.363**), which then adds to a αβ-unsaturated carbonyl through a conjugate addition to provide the 1,4 dione (**2.365**) following collapse of the tetrahedral intermediate (**2.364**) and regeneration of the Wittig salt **2.361**.

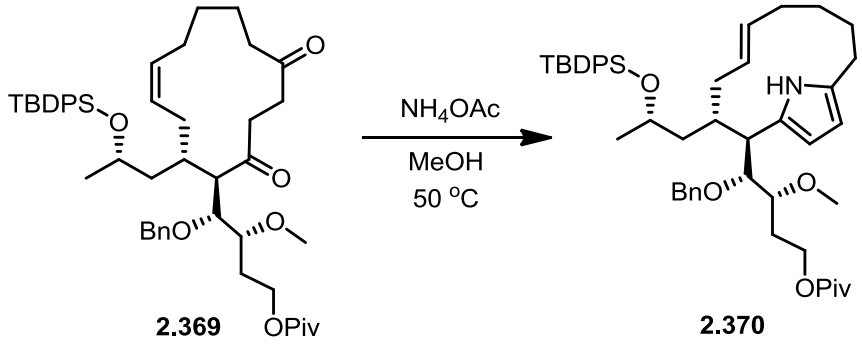
The Stetter reaction will be incorporated into the conjugate addition/aldol route (Scheme 2.85) starting from aldehyde **2.324**. Addition of vinyl magnesium bromide and subsequent oxidation of the resulting allylic alcohol with manganese dioxide will provide enone **2.366** (Scheme 2.103). Intermolecular Stetter reaction with enone **2.366** and

aldehyde **2.367** will provide 1,4-dione **2.368**, which can then undergo a ring-closing metathesis reaction to form macrocyclic 1,4-dione **2.369**.



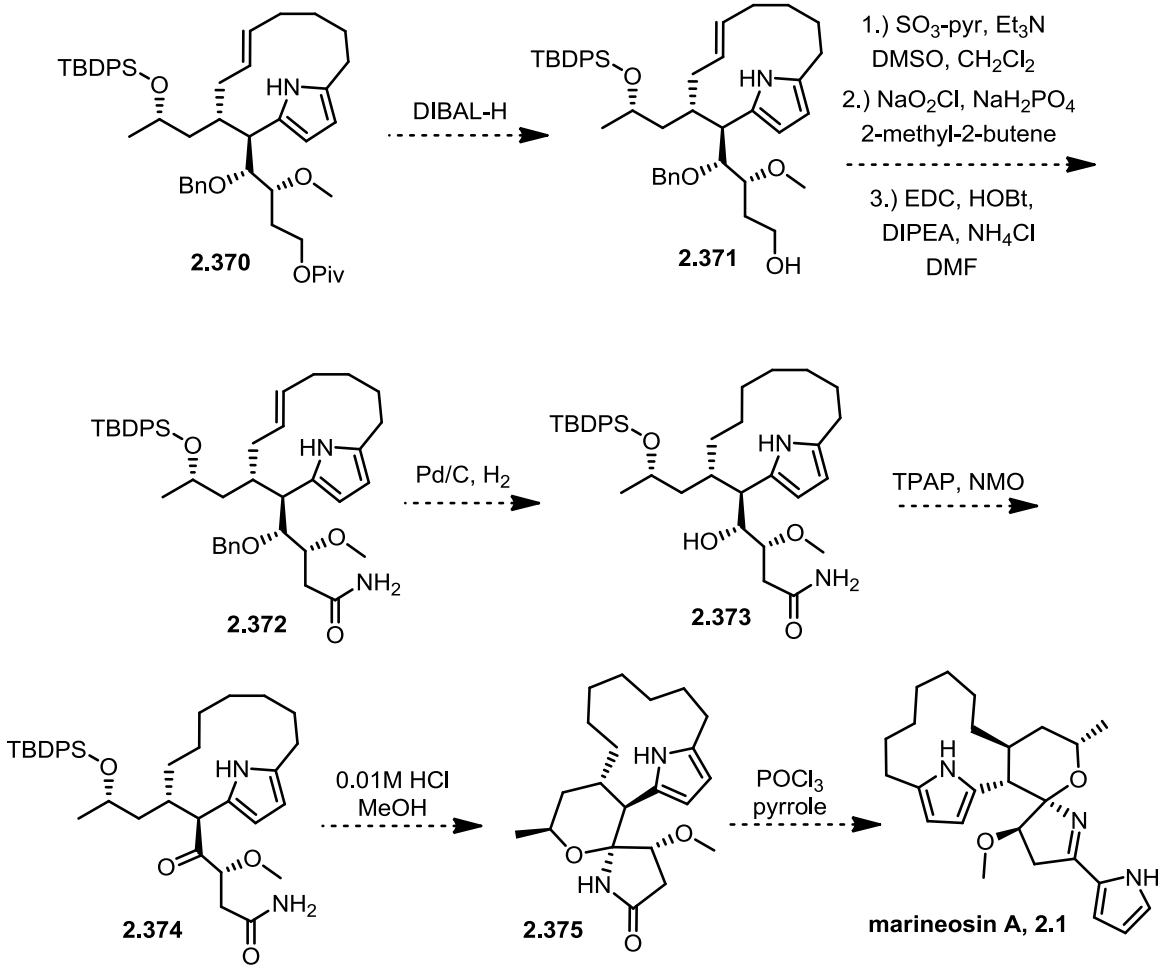
Scheme 2.103. The intermolecular Stetter reaction will be a key carbon-carbon bond forming reaction for the completion of the total synthesis of marineosin A.

Dione **2.369** can then be utilized in a Paal-Knorr condensation reaction with ammonium acetate to provide the 2,5-disubstituted pyrrole **2.370** (Scheme 2.104).[100-102]



Scheme 2.104. Paal-Knorr pyrrole synthesis to provide the 2,5-disubstituted pyrrole **2.370** of marineosin A.

Performing the RCM prior to pyrrole synthesis will circumvent any undesirable interaction between the ruthenium catalyst and the electron-rich pyrrole, which has been known to poison the metathesis catalyst and prevent the desired ring-closing reaction from occurring (Chapter 2, Scheme 2.19 and 2.62). Following pyrrole formation, the resulting pyrrolophane **2.370** will be subjected to the same series of reactions optimized in our model studies to generate marineosin A as a single diastereomer (Scheme 2.105).



Scheme 2.105. Completion of the total synthesis of marineosin A.

With marineosin A in hand, we plan to generate unnatural A-ring analogs through triflate formation and Suzuki cross-coupling with various commercially-available

heterocyclic boronic acids as outlined in Scheme 2.100 for library generation around the model system. These compounds will be tested in viability, proliferation, and invasion assays in the HCT-116 colon cancer cell line, as well as various other cell lines. We are also interested in the immunosuppressive and antimalarial properties of prodigiosin analogs and will investigate these activities through various collaborations. Hopefully, future studies will enable identification of a minimal pharmacophore and the design and optimization of a second-generation, simplified synthesis for the preparation of this pharmacophore. A simplified synthesis could provide rapid access to larger libraries of unnatural analogs for optimization of various biological properties and mechanism of action studies.

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CHAPTER 3

TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION OF TAMBJAMINE K AND A LIBRARY OF UNNATURAL ANALOGS

3.1. Tambjamine Alkaloids.

The tambjamines A–J (**3.1–3.10**) are a 2,2'-bipyrrolic class of cytotoxic alkaloids with diverse aliphatic termini isolated from marine invertebrates, such as bryozoans, nudibranchs, and ascidians, and are related to the prodigiosin **3.11** family of alkaloids (Figure 3.1).[1–8]

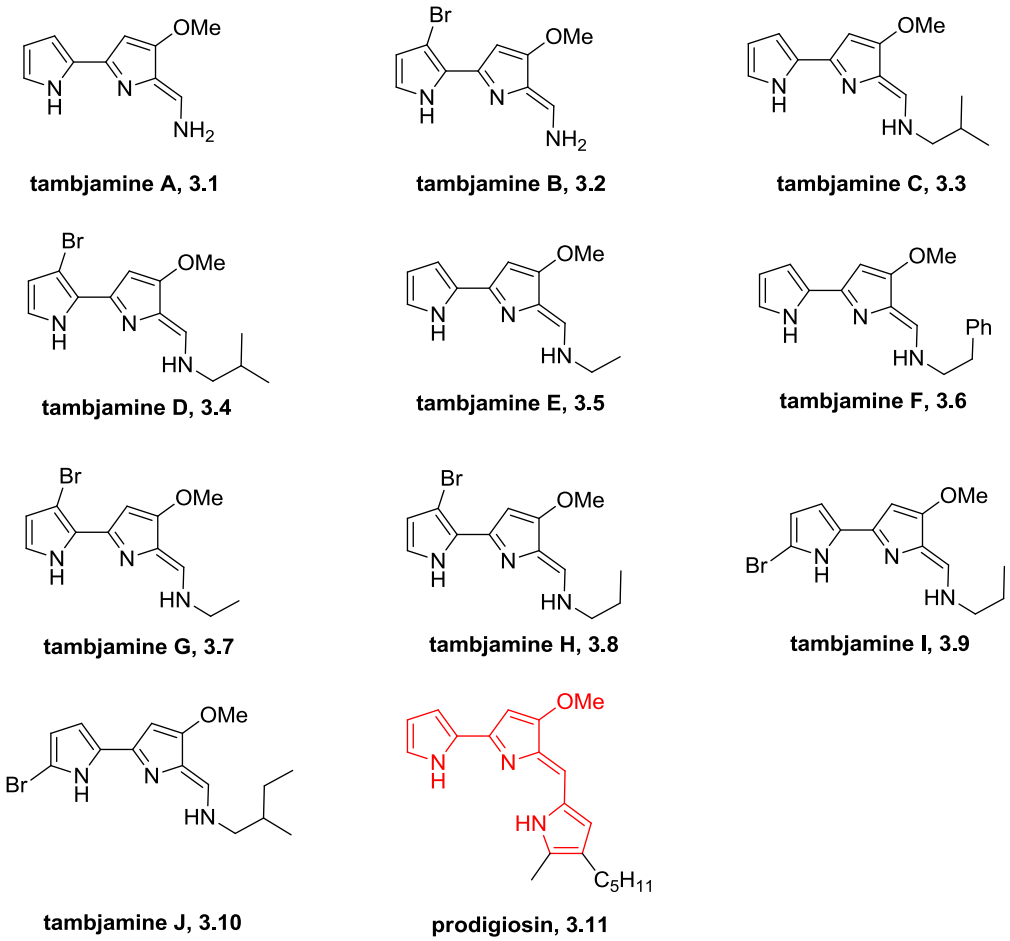


Figure 3.1. Structures of the tambjamines A–J (**3.1–3.10**), prodigiosin (**3.11**).[1-8]

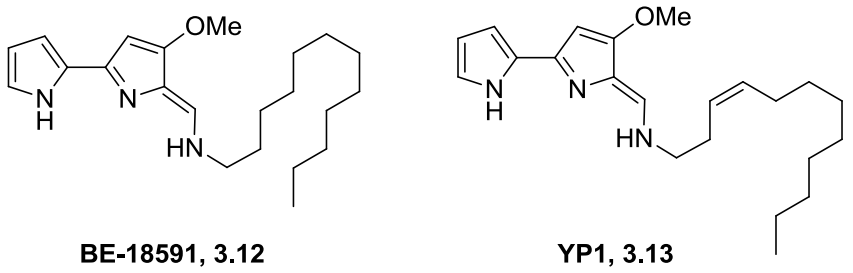
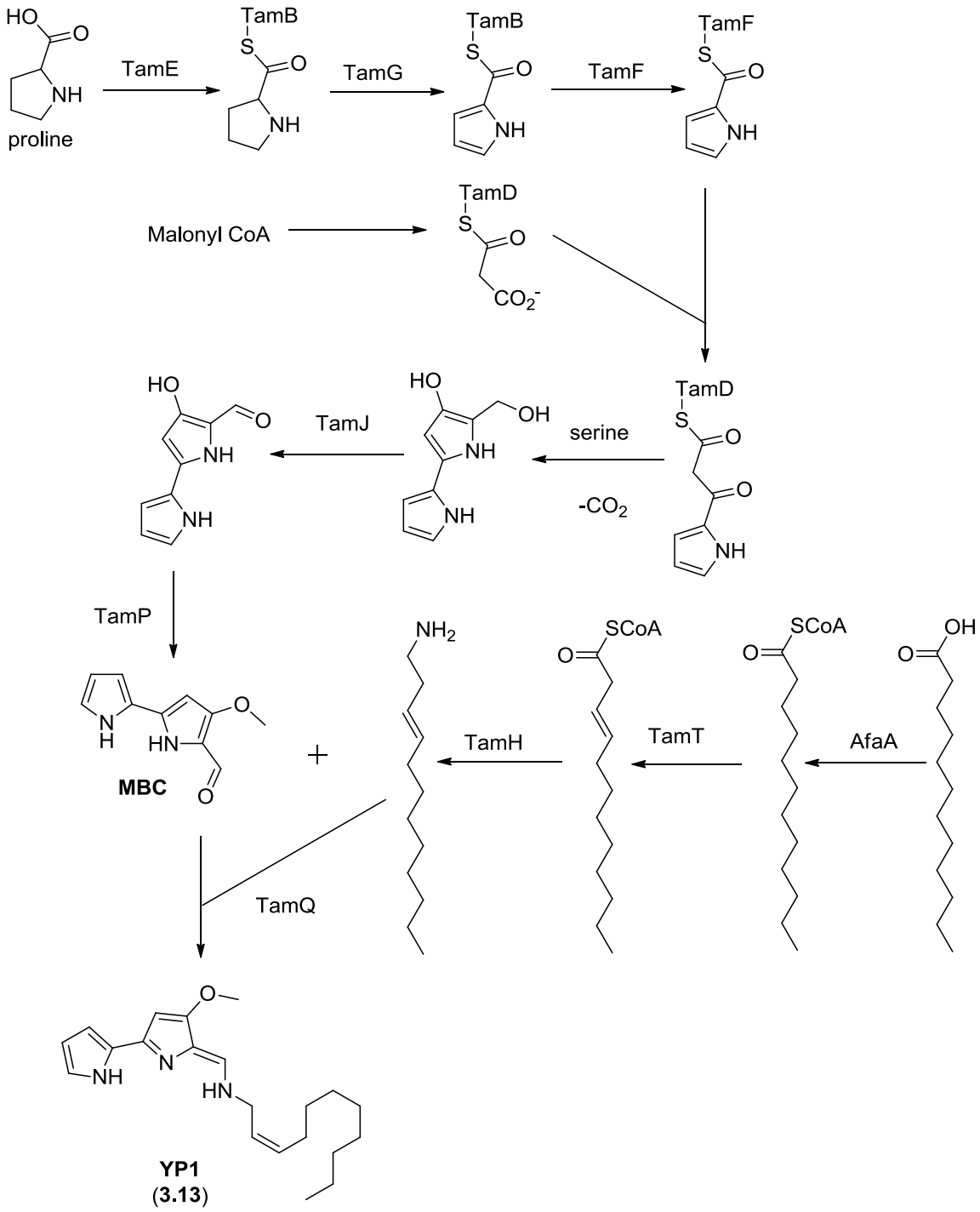


Figure 3.2. Structures of the tambjamine alkaloid relatives BE-18591 (**3.12**) and YP1 (**3.13**).

BE-18591 (**3.12)** and YP1 (**3.13)** are the only tambjamine alkaloids not isolated from marine invertebrates, but are instead produced by bacterial strains.[9] *Pseudoalteromonas tunicata*, is a marine bacterium that produces a range a biologically active products, including YP1 (**3.13)**. BE-18591 (**3.12)** is the only tambjamine alkaloid isolated from a terrestrial bacterium, *Streptomyces* BA18591.

3.2. Identification of the Tam Biosynthetic Pathway.

The gene cluster encoding for the biosynthesis of tambjamine YP1 (**3.13)** has been identified, and this knowledge proves that the prodigiosin alkaloids and tambjamine alkaloids share similar biosynthetic pathways.[9] Proline, malonyl Co-A, and serine are incorporated into the synthesis of MBC as described by Williamson and co-workers in the biosynthesis of prodigiosin.[10] AfaA activates dodecenoic acid, TamT introduces the double bond between the β and γ carbons, and TamH transfers an amino group from an amino acid.[9] The resulting dodec-3-en-1-amine is condensed with MBC by TamQ to form YP1 (**3.13)**.



Scheme 3.1. Biosynthetic pathway for the production of tambjamine alkaloid YP1 (**3.13**).[9] (adapted from Burke 2007).

Of the 19 proteins encoded in the Tam cluster, 12 were found to have high sequence homology to the Red proteins responsible for undecylprodigiosin synthesis in *Streptomyces coelicolor* A3(2) and Pig proteins involved in the biosynthesis of prodigiosin in *Serratia* sp. Table 3.1 highlights the Tam proteins with sequence similarity to the Red and Pig proteins.[9-11] The Red and Pig proteins involved in MBC biosynthesis are marked by and (M). The relationship between these two families of natural products is evident in the biosynthetic origins of these alkaloids.

Table 3.1. Tam proteins, predicted functions, and Pig and Red proteins that possess sequence homology.[9] (adapted from Burke 2007).

ORFs present in fosmid insert	Predicted protein function	Pig and Red proteins with sequence similarity
ORF1	Hypothetical protein	
ORF2	Putative phosphopantetheinyl transferase	
TamA	AMP binding protein	
TamB	Peptidyl carrier protein	RedO, PigG (M)
TamC	Putative oxidase	RedG
TamD	HBM synthetase (seryl transferase)	RedN, PigH (M)
TamE	L-prolyl-AMP ligase	RedM, PigI (M)
TamF	Pyrrolyl-β-ketoacyl ACP synthase	RedX, PigJ (M)
TamG	L-prolyl-PCP dehydrogenase	RedW, PigA (M)
TamH	Putative class III aminotransferase	PigE
TamJ	HBC dehydrogenase	RedV, PigM (M)
TamK	Hypothetical protein	
TamI	Putative permease	
TamM	Putative permease	
TamN	Putative ABC transporter, ATP binding protein	
TamO	Hypothetical protein	
TamP	HBC *O*-methyl transferase	RedI, PigF (M)
TamQ	Terminal condensing enzyme	RedH, PigC
TamR	Hypothetical protein	RedY, PigK
TamS	Phosphopantetheinyl transferase	RedU, PigL (M)
TamT	Putative dehydrogenase	

3.3. Biological Activity of the Tambjamine Alkaloids.

Members of the tambjamine class of alkaloids have demonstrated a wide range of biological activities including antitumor, antimicrobial, and immunosuppressive

properties. For tambjamines D (**3.4**) and E (**3.5**), the antitumor properties have been correlated with DNA intercalation and oxidative cleavage of single-strand DNA.[12] Much like the prodigiosin family of alkaloids, this DNA cleavage is induced in the presence of copper without activation by an external reductant. This implies that Cu (I) is formed reductively through the concomitant oxidation of the tambjamine to a π-radical cation. This species may damage DNA through an electron transfer mechanism with the DNA bases, resulting is apoptosis.[13]

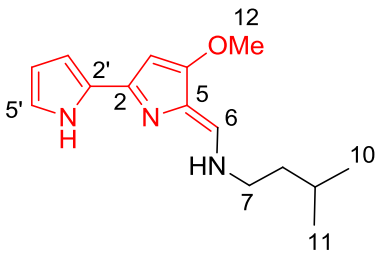
Tambjamine C (**3.3**), E-J (**3.5-3.10**), BE-18591 (**3.12**), and YP1 (**3.13**) have shown useful antimicrobial and cytotoxic effects, and SAR studies have revealed that the more lipophilic compounds show greater potency in both settings.[14] Unfortunately, none of the significantly cytotoxic compounds showed selectivity for the cancer cell lines over non-transformed cell lines. However, this limitation may be overcome by generating unnatural analogs for biological testing in an attempt to 'dial out' this undesirable lack of selectivity.

The tambjamines have also shown activity as ionophores.[15,16] Although they have been shown to induce apoptosis by oxidative DNA damage, they can effectively increase intracellular pH, which can also lead to apoptosis. BE-18591 (**3.12**) has displayed especially interesting activity.[16] This compound shows reversible inhibition of the lysosomal proton pump (V-ATPase), the mitochondrial proton pump (F-ATPase), and the ATP-dependent proton pump (P-ATPase) in rabbit gastric mucosa with IC_{50} values from 1-80 nM. Coupled to its antibacterial activity against *Heliobacter pylori*, this alkaloid could prove highly useful in the treatment of stomach ulcers. BE-18591 (**3.12**) has also shown immunosuppressive activity at nanomolar concentrations. However the selectivity

for T-cell proliferation over B-cell proliferation was less than the selectivity of prodigiosin.[16] However, the potential for developing unnatural compounds with desirable biological activity based on the structure of the tambjamine alkaloids is very promising.

3.4. Isolation of Tambjamine K.

In early 2010, Gavagnin and co-workers described the isolation and characterization of a new member of the tambjamine family, tambjamine K (**3.14**), isolated from the Azorean nudibranch *Tambja ceutae*.[17] Like other members of this family, **3.14** displayed antiproliferative and cytotoxicity against tumor (CaCo-2, IC_{50} =3.5 nM, HeLa IC_{50} = 14.6 μM, C6 IC_{50} = 14 μM, H9c2 IC_{50} = 2.7 μM, and 3T3-L1 IC_{50} = 19 μM) and non-tumor cell lines. Interestingly, **3.14** displayed differential effects across these tumor cell lines with a variance of >5000-fold (CaCo-2 vs 3T3-L1).[10] Based on these data and our own efforts in related areas, we initiated an effort for the total synthesis and biological evaluation of **3.14** along with a library of unnatural analogs with unprecedented diversity in the eastern C7 position to survey moieties other than aliphatic alkyl chains.



tambjamine K, 3.14

Figure 3.3. Structure of newly isolated tambjamine alkaloid, tambjamine K (**3.14**).[17]

3.5. Total Synthesis of Tambjamine K.

Interest in the tambjamine family originated in our evaluation[18] of Fenical's biosynthetic proposal[19] for the synthesis of marineosin A via an intramolecular inverse-electron-demand Diels–Alder reaction with prodigiosin analog **3.15** (Figure 3.4). Like **3.1–3.14**, marineosin A displayed potent cytotoxic activity against HCT116 cells (IC_{50} = 500 nM).[18,19]

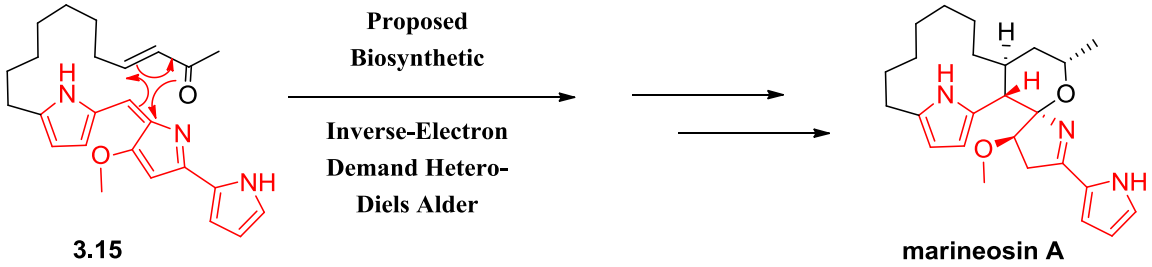
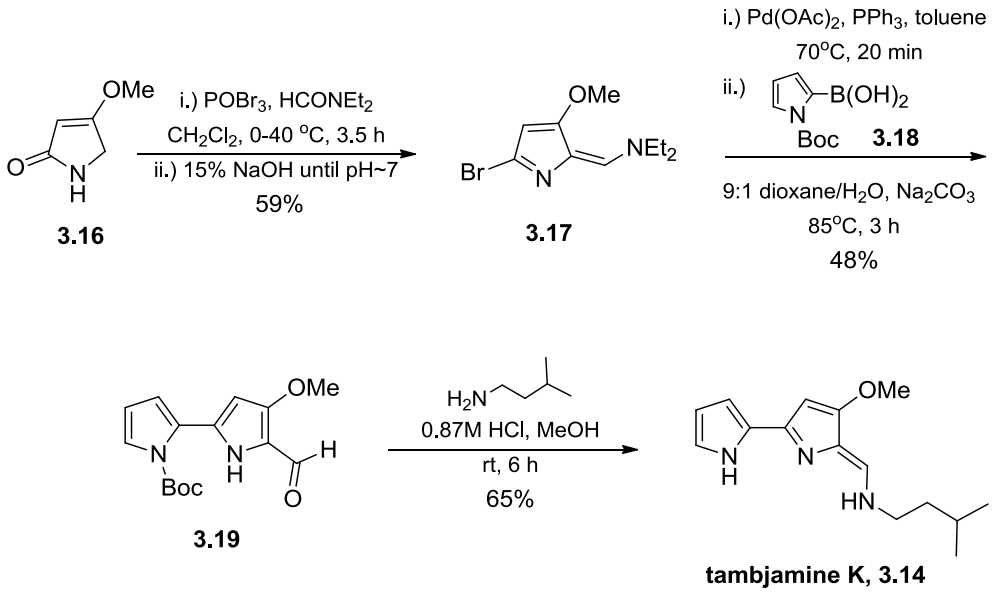


Figure 3.4. Proposed biosynthesis of Marineosin A via and inverse electron-demand Diels-Alder reaction with prodigiosin analog **3.15**.

Our synthetic approach to access **3.12** was similar to that which we employed for the synthesis of **3.13**.[18,20] As shown in Scheme 3.2, a Vilsmeier–Haack haloformylation was performed on 4-methoxy-3-pyrrolin-2-one **3.16** to provide bromoenamine **3.17** in 59% yield. Suzuki cross-coupling with Boc-1*H*-pyrrol-2yl boronic acid **3.18** delivered the Boc-protected analog **3.19** in 48% yield. Finally, an acid mediated condensation between **3.19** and isopentyl amine[16] afforded tambjamine K (**3.14**) in 65% yield and 18% overall yield for the three step sequence. Synthetic **3.14** was identical in all aspects to the natural product (Table 3.2).



Scheme 3.1. Total synthesis of tambjamine K.[20]

Table 3.2. Comparison of NMR data from natural product isolate to synthetic natural product.

Carbon	Natural δ_H (mult, J in Hz)	Synthetic δ_H (mult, J in Hz)	Natural δ_C	Synthetic δ_C
2	--	--	143.8	143.2
3	6.00 s	5.98 d (2.4)	91.5	91.5
4	--	--	164.1	164.0
5	--	--	110.8	110.6
6	7.36, d (15.0)	7.33, d (15.2)	140.1	140.0
7	3.95 s	3.93 s	58.4	58.3
8	3.49 m	3.48 q (6.8)	49.4	49.2
9	1.64 m	1.62 q (6.8)	38.9	38.7
10	1.70 m	1.71 m	25.5	25.2
11	0.96, d (6.4)	0.95, d (6.8)	22.3	22.1
12	0.96, d (6.4)	0.95, d (6.8)	22.3	22.1
NH	9.23,10.70, 12.50	9.93	--	--
2'	--	--	122.3	122.2
3'	6.77, m	6.75, m	113.7	113.6
4'	6.30, m	6.28, m	110.7	110.5
5'	7.07, m	7.09, m	124.5	124.5

3.6. Biological Activity of Tambjamine K and Prodiginine Unnatural Analogs.

While **3.14** was studied in a number of tumor cell lines, it was not evaluated in cell viability assays with HCT116 colorectal carcinoma or MDA-MB-231 breast carcinoma cell lines—tumor lines of interest to our lab.[21] Moreover, we had not yet evaluated **3.15** or another related prodigiosin analog **3.20** we employed as a template for an intermolecular inverse-electron demand Diels–Alder (IEDDA) reaction to access the marineosin A core.[18] Concentration-response curves (CRCs) were generated for viability of the HCT116 and MDA-MB231 cell lines upon treatment with compounds **3.14**, **3.15**, and **3.20** (Figure 3.5).

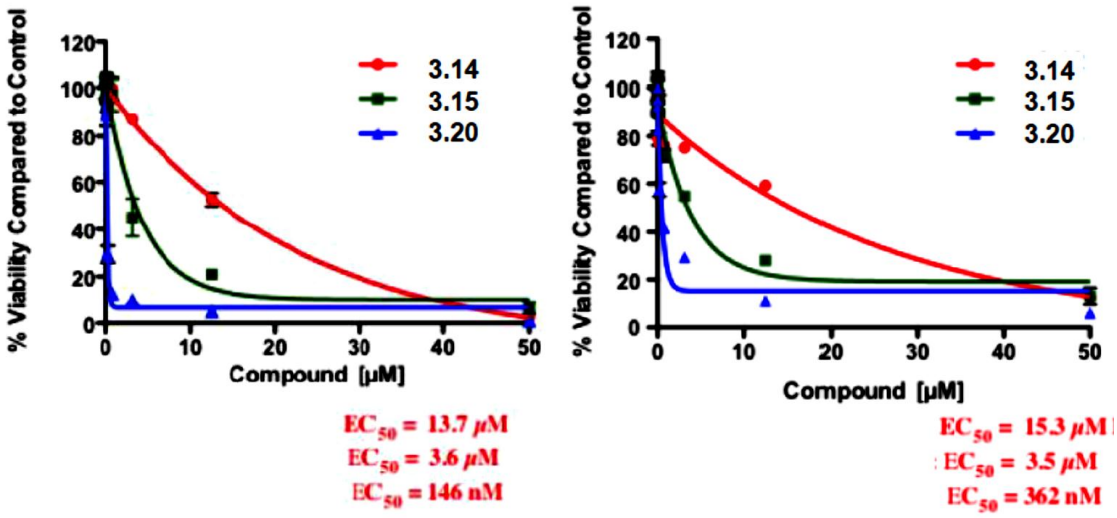
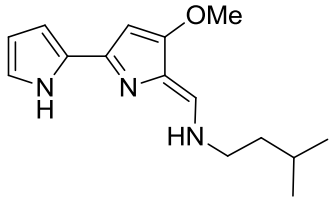
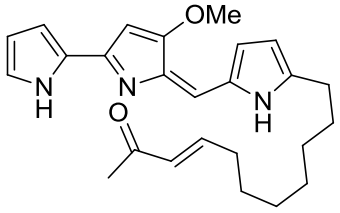
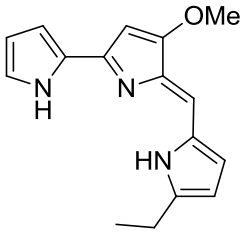


Figure 3.5. Viability concentration -response curves for tambjamine K (**3.14**) and prodigiosin alkaloids **3.15** and **3.20** in HCT116 colonrectal cancer cell line (left) and MDA-MB-231 breast cancer cell line (right).

As summarized in Table 3.3, tambjamine K (**3.14**) displayed weak cytotoxicity against HCT116 (IC_{50} =13.7 μM) and MDA-MB-231 (IC_{50} = 15.3 μM). In contrast, the Intramolecular IEDDA prodigiosin analog **3.15** was more potent with IC_{50} values of 3.5 μM for both tumor lines. The intermolecular IEDDA prodigiosin analog **3.20** was found

to be extremely potent, with IC_{50} values of 146 nM and 362 nM, for HCT116 and MDA-MB-231 cell lines, respectively.

Table 3.3. Structures and activities of tambjamine K and unnatural analogs.

Compound	Structure	HCT 116 IC_{50}[a] (μM)	MDA-MB-231 IC_{50}[a] (μM)
3.14		**13.7**	**15.3**
3.15		**3.6**	**3.5**
3.20		**0.14**	**0.36**

[a] 8000 cells/well in 96 well plate followed by 24 h for attachment. Added vehicle or compounds in RPMI 1640 plus 10% FBS + penicillin-streptomycin. Cells allowed to grow for 48 h, then viability was assessed.

Figure 3.6 shows the HCT-116 cellular populations for the vehicle, and compounds **3.14**, **3.15**, **3.20** after 48 hour treatment at two concentrations.

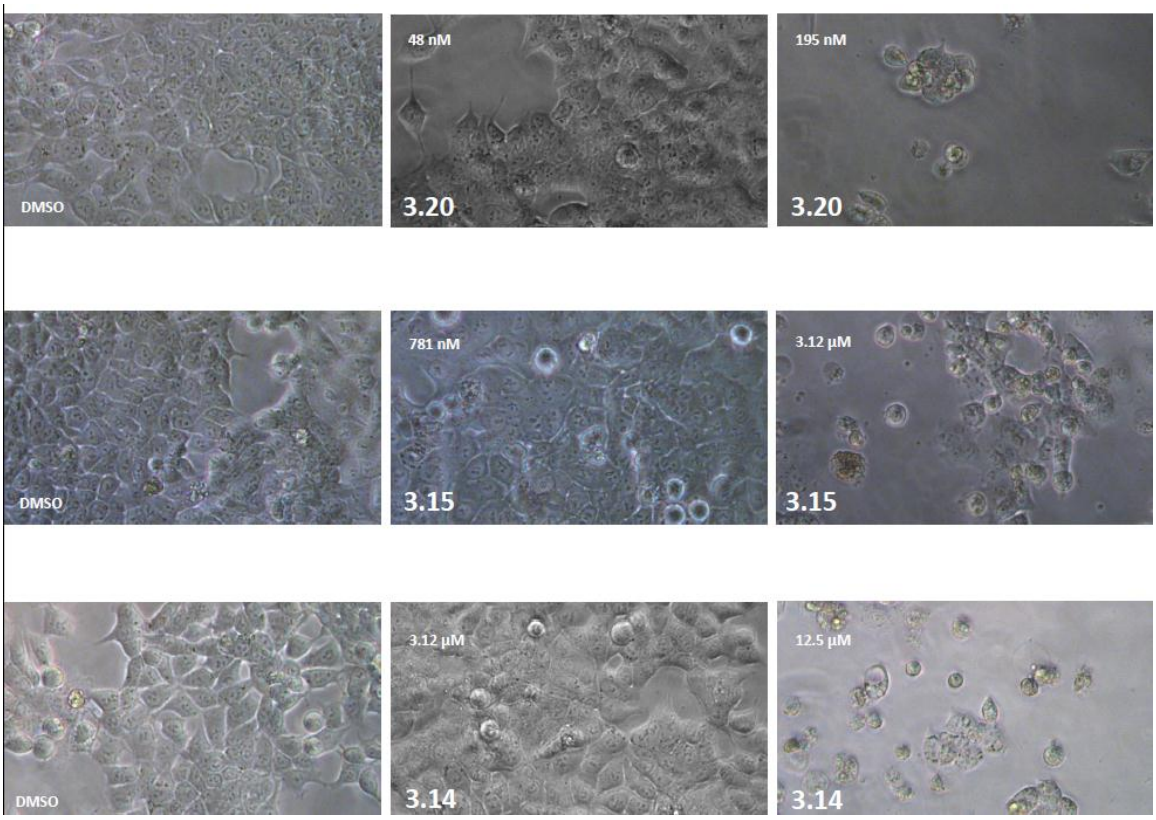


Figure 3.6. HCT116 cell populations after 48 h treatment with vehicle (left), and compounds **3.14**, **3.15** and **3.20** (center and right).

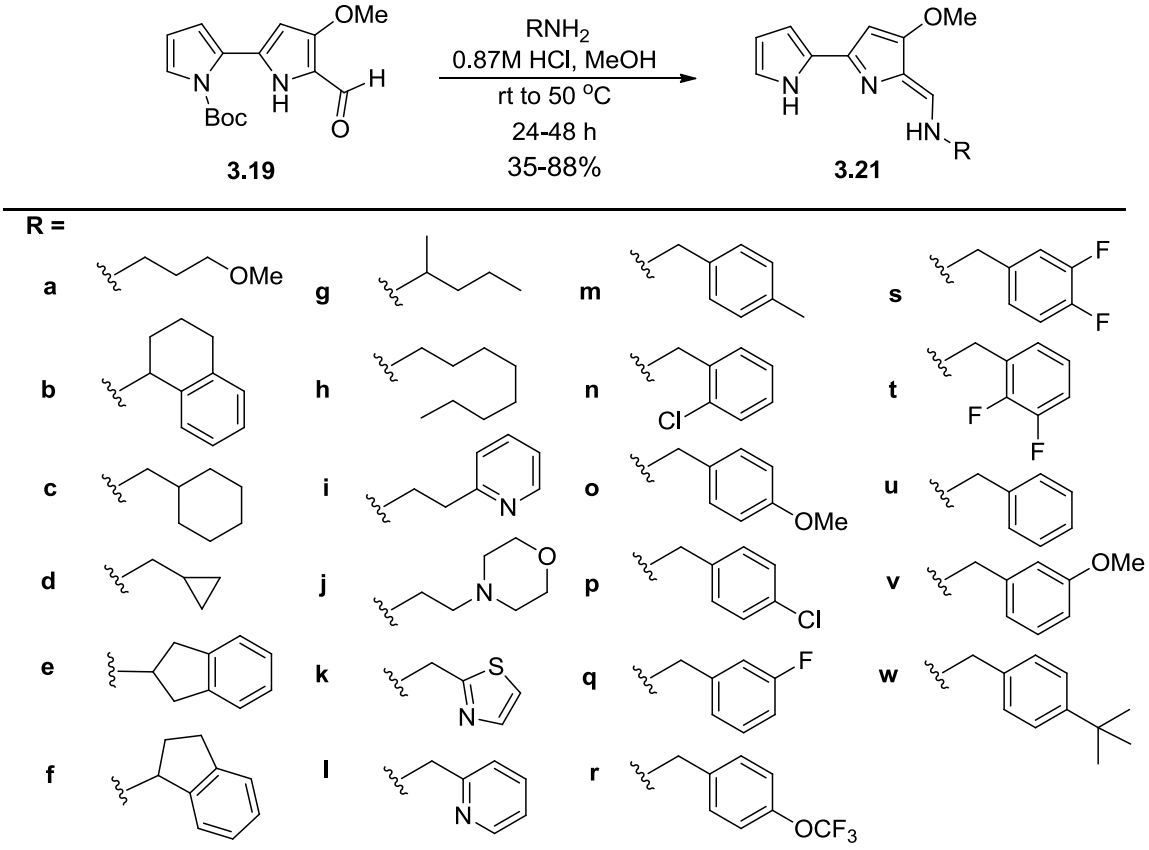
3.7. Synthesis of a Library of Unnatural Tambjamine Analogs.

These data prompted us to synthesize and evaluate a library of unnatural tambjamine analogs to capitalize on the SAR observed for this class of natural products akin to our earlier work developing unnatural analogs with activities beyond the natural product.[22-24]

Importantly, Quinn and co-workers[25] previously prepared a combinatorial library of 10 unnatural tambjamine analogs, but all possessed limited R diversity and aliphatic side chains dominated. Our library was designed to incorporate functionalized benzyl, heteroaryl moieties and other previously undescribed analogs with varying degrees of

lipophilicity and basicity to further develop SAR.

The library was prepared as shown in Scheme 3.2, and differed from the route to access **3.14** only in extended reaction time, as several amines proved sluggish in their conversion to unnatural tambjamine analogs **3.21**; however, all analogs were successfully prepared in yields ranging from 35% to 88%.



Scheme 3.2. Library synthesis of tambjamine unnatural analogs **3.21**.

3.8. Biological Activity of a Library of Unnatural Tambjamine Analogs.

We triaged the library of analogs **3.21** by a employing a 10 μM single point screen in the standard 48 hour viability assay using both HCT116 and MDA-MB-231 cells.[21] The majority of analogs, especially the benzyl congeners, **3.21m**–**3.21w**, had no

effect on viability in either tumor cell line. Figures 3.7 and 3.8 show the single point data for the analogs **3.21** in these assays, and it is important to note that differential activity was observed between the two tumor cell lines (Figure 3.7 and 3.8). The most potent analog was **3.21b**, a racemic 1,2,3,4-tetrahydronaphthalene congener, which significantly decreased HCT116 viability (<10% viability) while having only marginal effect on MB231 (40% viability). Other unnatural analogs **3.21c**, **3.21e**, **3.21f**, and **3.21g** decreased HCT116 cell viability to less than 20%, with minimal effect on MB231 viability. Clearly, constraining the benzyl amine moiety in **3.21b**, **3.21e**, and **3.21f** into a bicyclic ring system is important for activity relative to the inactive benzyl derivatives **3.21m**–**3.21w**.

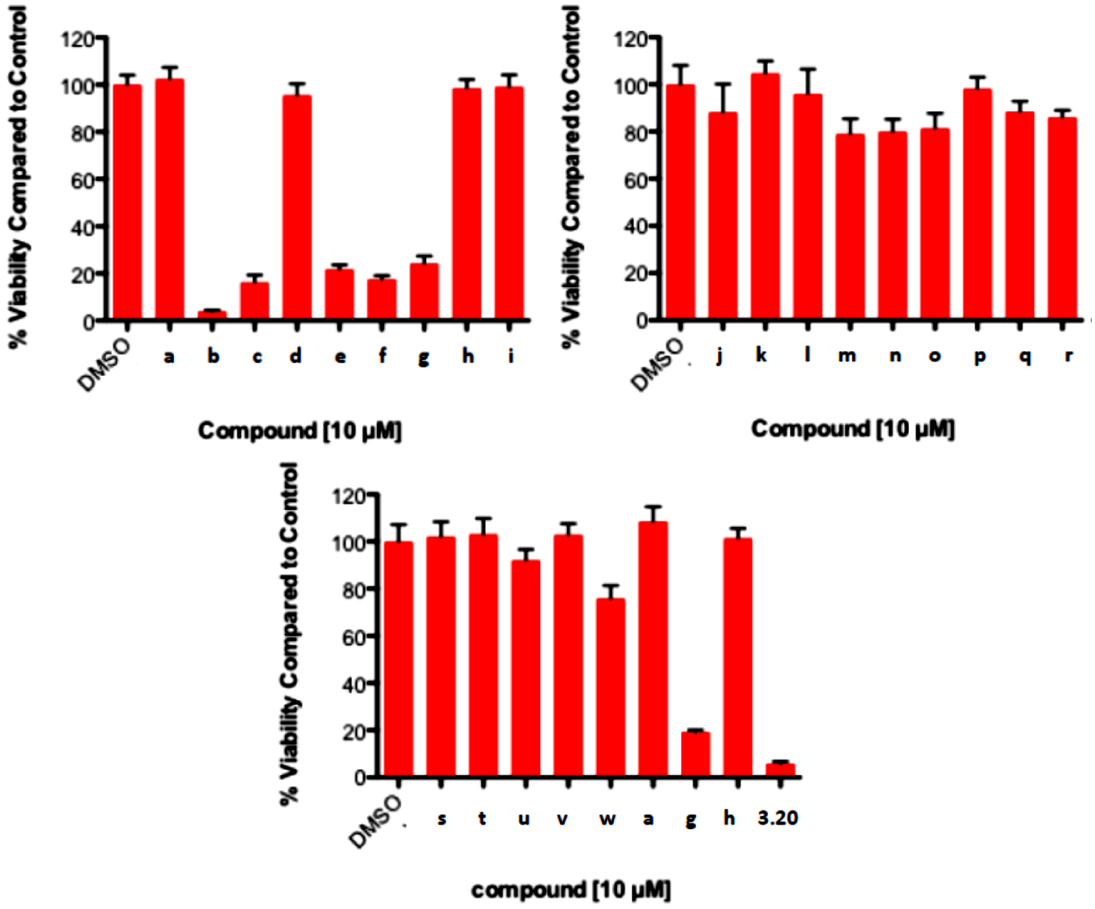


Figure 3.7. Single point (10 µM) screen of library of analogs **3.21**. 48 h cell viability assay with HCT116 colorectal carcinoma line

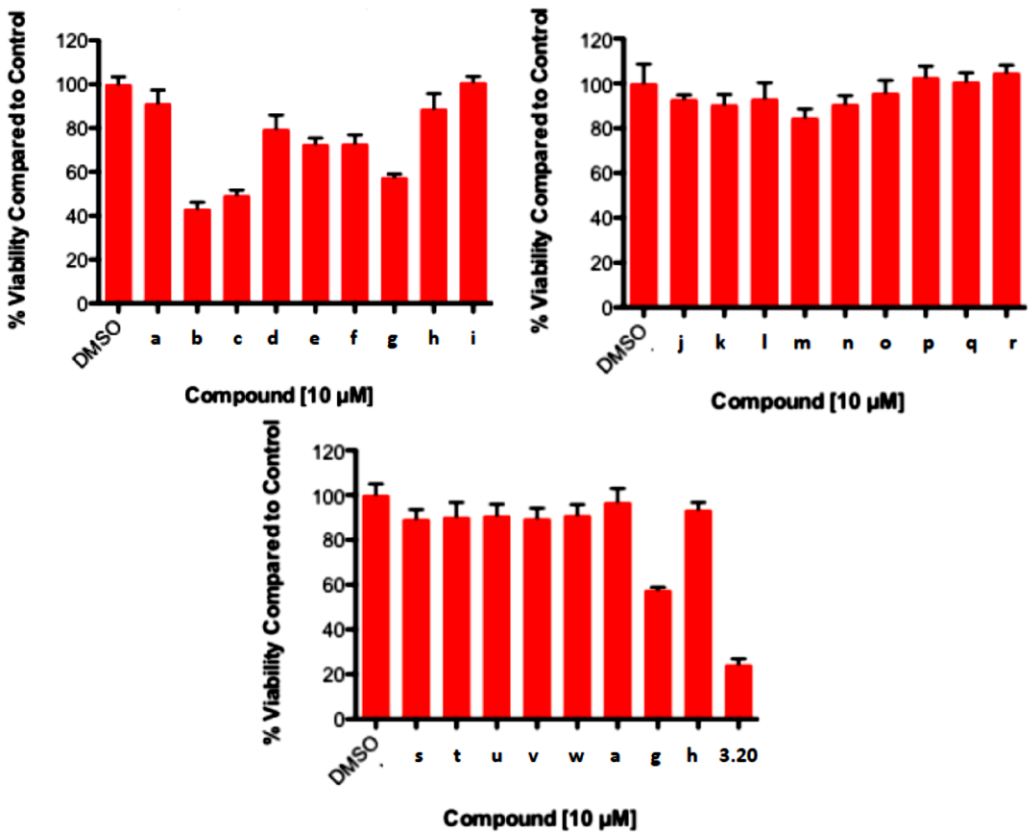


Figure 3.8. S ingle point (10 μM) screen of library of analogs **3.21**. 48 h cell viability assay with MDA-MB-231 breast carcinoma line.

Based on the promising single point data, we then determined IC_{50} values for **3.21b** in both cell lines. **3.21b** displayed moderately potent cytotoxicity against HCT116 cells (IC_{50} = 1.8 μM) with the CRC reaching baseline (0% viability at 10 μM dose) (Figure 3.9). In contrast, **3.21b** displayed weak cytotoxicity in the MDA-MB-231 cells (IC_{50} = 4.0 μM) with the CRC only achieving ~50% decrease in cell viability at the highest (10 μM) dose; therefore, the calculated IC_{50} is for a partial response. The results of the CRCs are summarized in Table 3.10. Most importantly, in our standard cytotoxicity assay in non-transformed cells, **3.21b** displayed no toxicity.

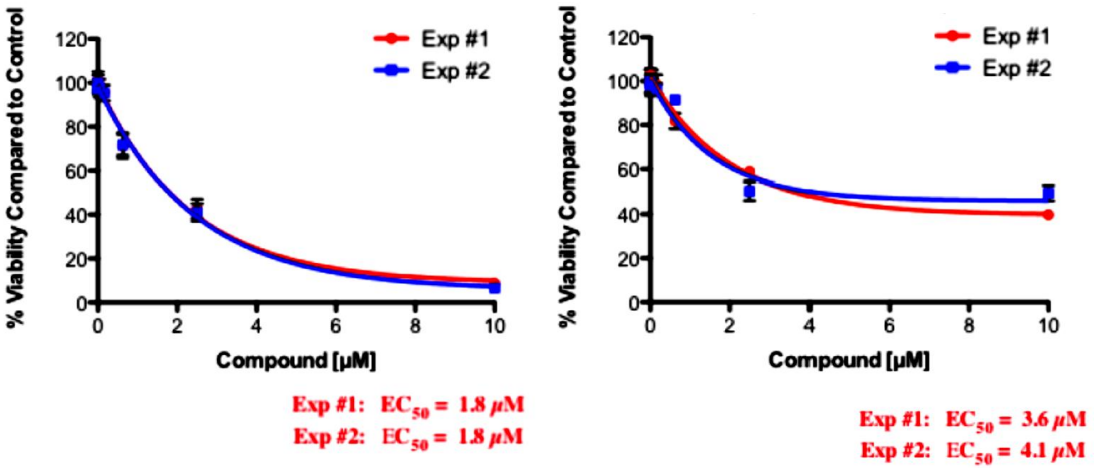
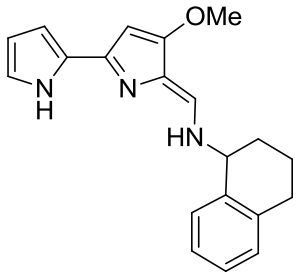


Figure 3.9. Viability concentration-response curves for tambjamine unnatural analog **3.21b** in the HCT116 cell line (left) and the MDA-MB-231 cell line (right).

Table 3.4. Structure and activity of tambjamine unnatural analog **3.21b**.

Compound	Structure	HCT 116 IC_{50}[a] (μM)	MDA-MB-231 IC_{50}[a] (μM)
3.21b		**1.8**	**3.85**

Based on these data, we then evaluated select unnatural analogs with activity in the HCT116 cell viability assay and evaluated them in standard 48 hour cell proliferation assays (Figure 3.10) using another colorectal line (SW620) and a non-small cell lung carcinoma (NSCLC) line (H520). Interestingly, **3.20**, the most potent tambjamine analog in both the HCT116 (IC_{50} = 146 nM) and MDA-MB-231 (IC_{50} = 362 nM) viability assays, had no effect on proliferation in either the SW620 or the H520 cell lines.

However, unnatural analog **3.21b**, displayed a significant effect on inhibiting proliferation in both tumor cell lines, while other analogs showed varying effects.

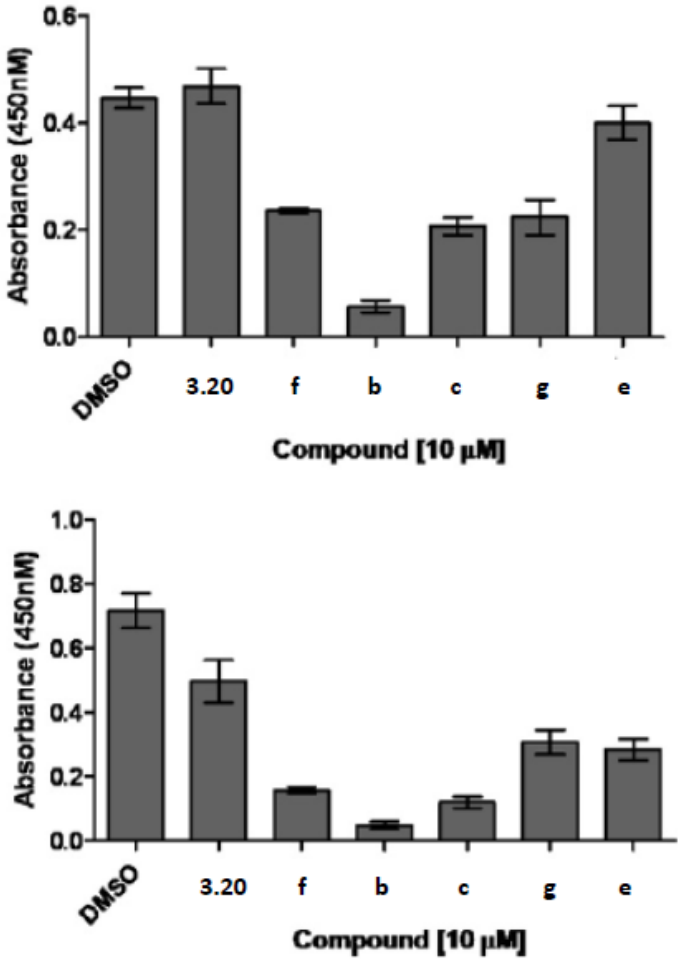


Figure 3.10. Single point (10 µM) screen of select unnatural tambjamine analogs in 48 h cell proliferation assays. (A) Proliferation assay with SW620 colorectal carcinoma line; (B) proliferation assay with H520 NSCLC line.

As both unnatural tambjamine analogs **3.20** and **3.21e** displayed minimal to no effect on proliferation (viability) in SW620 cells, we examined their ability to block invasion in this tumor line, as the ability of tumor cells to invade into the surrounding microenvironment is the defining step in tumor progression. As shown in Figure 3.11, both **3.20** and **3.21e** significantly blocked invasion, with **3.20** completely inhibiting

invasion. Moreover, both analogs displayed minimal or no cytotoxicity in this colorectal tumor cell line, further highlighting the value of unnatural analog synthesis.

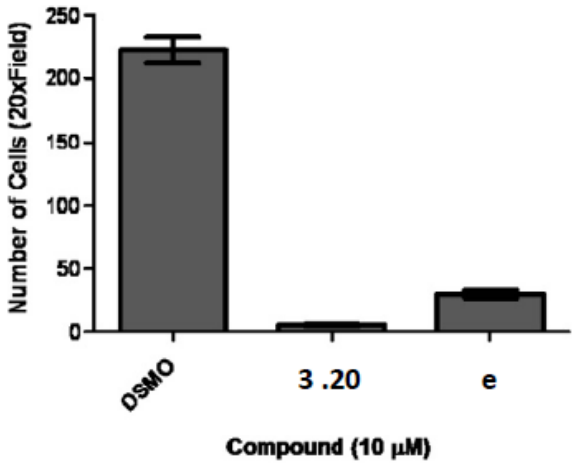


Figure 3.11. Single point (10 μM) screen of select unnatural tambjamine analogs in 24 h cell invasion assay in the SW620 colorectal carcinoma line.

3.9. Conclusions and Future Work.

In summary, we completed the first total synthesis of tambjamine K (**3.14**) in 18% overall yield coupled with evaluation in viability assays in both colon (HCT116) and breast cancer (MDA-MB-231) cell lines. We also prepared a library of unnatural tambjamine analogs with unprecedented diversity and improved biological activity against a number of tumor cell lines in viability, proliferation and invasion assays. This effort demonstrated that subtle changes to the tambjamine core afford varying degrees of selectivity against different tumor cell lines. These data argue for further exploration of the tambjamine scaffold coupled with evaluation (viability, proliferation, and invasion) in additional human tumor cell lines. Current efforts are focused on synthesizing a second-

generation library including the discrete enantiomers of **3.21b** and **3.21e**, chiral α-methyl congers of the benzylic analogs **3.21m**–**3.21w** and more focused analogs based on **3.20**. In parallel, we are working to identify the molecular target(s) for these unnatural analogs by evaluating **3.20**, **3.21b**, and **3.21e** against large panels of kinases, growth factors, and phosphatases as a primary approach. After investigating the SAR of the selective anticancer activity, we also plan to test our unnatural analogs for immunosuppressive properties, since this activity has been observed in tambjamine and prodiginine alkaloids. Resent literature has shown that prodiginine unnatural analogs possess potent antimalarial activity, but suffer from problems with general cytotoxicity.[26] We plan to test our tambjamine unnatural analogs that show insignificant general toxicity for the ability to eradicate the *Plasmodium falciparum* parasite that is responsible for nearly all malaria deaths. Hopefully, this scaffold will prove as rich in tunable biological activity as the closely-related prodiginine alkaloids.

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CHAPTER 4

TECHNOLOGY ENABLED SYNTHESIS AND BIOLOGICAL EVALUATION OF 3,6-DISUBSTITUTED-[1,24]-TRIAZOLO[4,3-*b*]PYRIDAZINES AS M_1 ANTAGONISTS FOR TREATMENT OF DYSTONIAS.

4.1. Introduction.

4.1.1. Dystonias.

4.1.1.1. Symptoms.

Dystonia is a movement disorder in which sustained muscle contractions cause twisting and repetitive movements or abnormal postures.[1] While not widely known, dystonia affects an estimated 300,000 people in North America alone.[2]

Two major categories of dystonias exist, primary and secondary.[1] Primary dystonias develop spontaneously in the absence of any associated disease or apparent cause and show no other neurological symptoms, except tremor and myoclonus.[1] These dystonias have a genetic component, and symptoms are typically irreversible once they appear. Early onset primary dystonias are rare, frequently have a genetic basis, and can progress to affect multiple body parts, which is known as generalized dystonia. Late-onset dystonias are much more common and are typically focal in nature, meaning they affect one specific body part. Hand dystonia (writer's cramp) and cervical dystonia (torticollis) are two of the most common examples of focal dystonias. These late-onset cases can also have a heritable predisposition.

Secondary dystonias occur when dystonic symptoms result from other disease states or a brain injury.[1] Many neurodegenerative diseases have dystonic symptoms,

including Huntington's disease, Parkinson's disease, and Rett syndrome. Brain lesions caused by trauma, vascular injury, viral infection or demyelination can also result in dystonic symptoms.

Since dystonias are not one disease with one cause, but multiple diseases and even symptoms of other diseases, it would prove very difficult to determine the cause of each dystonia and design treatments to address each cause. In the case of dytonias, it would be much more reasonable to develop a symptomatic treatment that addresses the dystonic symptoms and successfully alleviates these symptoms in patients. Such a therapeutic could then be utilized to ameliorate all primary dystonias and even secondary dystonias resulting from a completely unique brain injury.

4.1.1.2. Current Treatment Options.

While symptomatic treatment is the currently available method, there are many negative aspects of these treatments.[3,4] Most pharmacological treatments, such as anti-cholinergics, GABA agonists, and dopaminergic agents, have severe dose-limiting side effects due to lack of selectivity. Botulinum toxin (Botox) injection works very well for focal dystonias. However, the toxin has to be directly injected into the affected area, so this is not an effective treatment for generalized dystonia. The effects are only temporary and multiple injections will be required. Also, immunity to the toxin develops overtime, rendering it useless as a treatment over time. Some neurosurgical treatments have proven very effective. Deep Brain Stimulation (DBS) of the globus pallidus is an option for generalized dystonias, but is not effective in all cases. Also, this is a highly invasive brain surgery that places electrodes in the patient's brain to stimulate certain neurons to relieve symptoms. Ideally, an effective pharmacotherapy could be designed that is orally

available and capable of treating a wide range of dystonic symptoms with minimal side effects.

4.1.2. Muscarinic Acetylcholine Receptor (mAChR) Subtype 1 (M_1) as a target for treatment of dystonias

4.1.2.1. The Role of Acetycholine as a Neurotransmitter.

Acetylcholine (ACh) is a non-amino acid derived small molecule that plays essential roles in both the mammalian central nervous system (CNS) and peripheral nervous system (PNS) as a neurotransmitter.[5-10] Cholinergic signaling within the autonomic arm of the PNS occurs at the pre-ganglionic sympathetic and parasympathetic synapses, the post-ganglionic parasympathetic synapses, and at a subset of postganglionic sympathetic synapses in skin and sweat glands. ACh is also released at the skeletal neuromuscular junction, where it stimulates muscle contraction. In the CNS, Cholinergic neuronal signaling also plays a key role; its release controls a multitude of integral brain functions, motor control and regulation, reward behavior, sleep/wake cycles, metabolism, learning and memory, and attention.

4.1.2.2. Muscarinic Acetylcholine Receptors.

mAChRs are Type I rhodopsin-like G-protein coupled receptors (GPCRs) that exist as five distinct subtypes (M_1-M_5) and share a high degree of homology, especially among the orthosteric Ach-binding sites.[6,9,12-14] M_1, M_3, and M_5 exhibit higher homology to each other than to M_2 and M_4, which share the highest homology with each other.[6,9] Synaptic localization patterns as well as downstream signaling pathways are generally similar for M_1, M_3, and M_5 subtypes versus M_2 and M_4.[13,14] The former three are considered stimulatory or excitatory due to predominantly post-synaptic localization and

coupling to the Gαq/phospholipase-C (PLC)/inositol triphosphate (IP_3)/Ca^{2+} pathway, while the latter two are regarded as inhibitory with predominantly pre-synaptic localizations and coupling to the Gαi/adenylyl cyclase (AC)/cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) effector system.[13,14] M_1, M_4, and M_5 subtypes are predominantly localized within the CNS, while the M_2 and M_3 subtypes are expressed throughout the CNS and the periphery.

4.1.2.3. M_1 as a Potential Target for Dystonia Therapy.

M_1 has been implicated as a potential target for dystonia therapy due to its post-synaptic expression on medium spiny GABA-ergic neurons (MSNs) of the dorsal striatium (Figure 4.1).[15-17] Activation of M_1 receptors on the MSNs leads to increased excitation of these neurons, which ultimately results in increased muscle contraction. Antagonism of striatal M_1 receptors by selective small molecules would lead to decreased excitation of the MSNs, which could ultimately alleviate the motor dysfunction present in patients with dystonia. However, no subtype selective antagonists for the M_1 receptor have been reported, and therefore discovery of such ligands is clearly required in order to pharmacologically test this therapeutic hypothesis. Current pharmacological treatments for dystonia commonly display off-target activity at multiple mAChR subtypes.[3,4] If a therapeutic agent could be designed that selectively targets one subtype over all of the others, it could potentially ameliorate dystonic symptoms without complications from dose-limiting side effects.

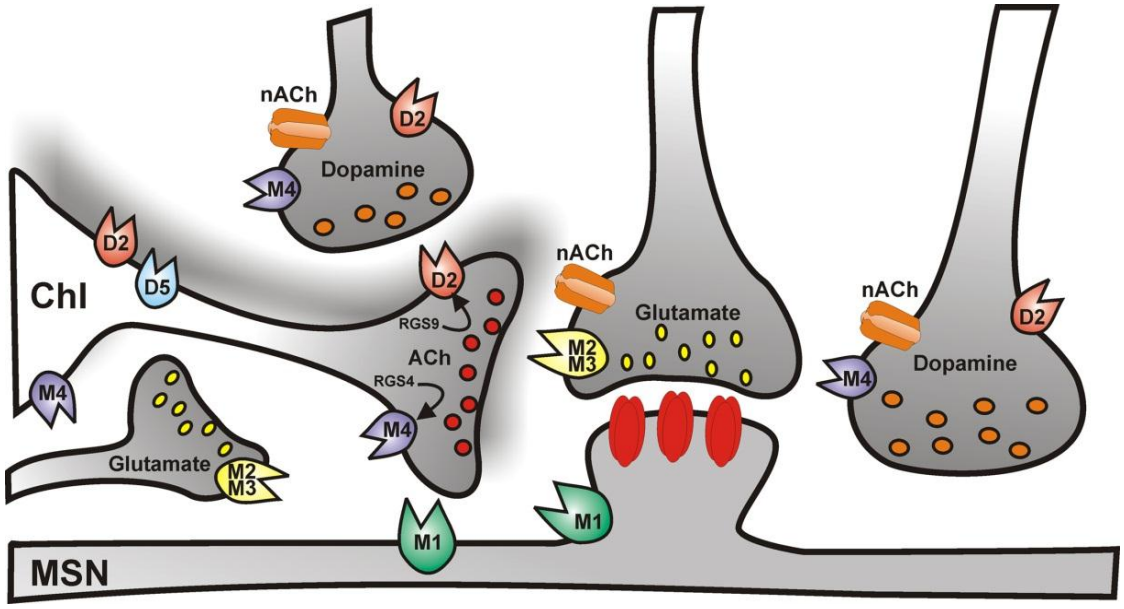


Figure 4.1. Localization of M_1 and other receptors within the CNS.[15]

4.2. Discovery of an HTS lead for M_1 Antagonism.

In the course of our program in small molecule probe development for the Molecular Library Screening Center Network (MLSCN),[18] a high-throughput screen identified the 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine scaffold **4.1** as an attractive hit for muscarinic receptor subtype 1 (M_1) antagonism (Figure 1.1). While numerous reports describe syntheses of **1.1**, yields are typically moderate (<50%) with prolonged reaction times at high temperatures (steps requiring 18 to >60 h at reflux).[19-22] In order to employ an iterative analog library synthesis approach for the lead optimization of **4.2**, a weak, but selective muscarinic acetylcholine receptor antagonist (M_1 IC_{50} = 22 μM, M_4 IC_{50} >150 μM), significant refinements were required in the synthetic protocols for delivering analogs **4.1**, with diversity at both C3 and C6.[19-22]

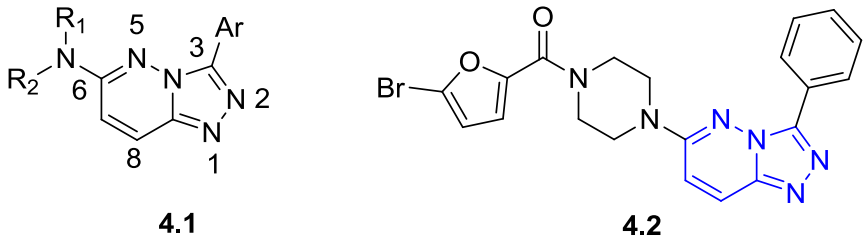


Figure 4.2. Generic structure of 3,6-disubstituted-[1,2,4]triazolo[4,3-b]pyridazine **4.1** and our M1 antagonist screening lead **4.2.**

As many of the leads identified from HTS campaigns are small heterocyclic compounds, our laboratory has devoted significant effort to develop efficient protocols for the preparation of diverse heterocyclic templates employing microwave-assisted organic synthesis (MAOS).[23-29] In recent reports, we have described general, high-yielding MAOS protocols for the expedient synthesis of 1,2,4-triazines **4.3**,[23] imidazoles **4.4**,[24] quinoxalines **4.5**,[25] pyrazinone **4.6**,[26] 5-aminooxazoles **4.7**,[27] quinoxalinones **4.8**,[28] pyrazolo[1,5-*a*]pyrimidines **4.9**,[29] and pyrazolo[3,4-*d*]pyrimidines **4.10**[30] from simple starting materials (Figure 4.3). Therefore, application of MAOS to develop a general, high-yielding, and expedient synthesis of the 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine scaffold **4.1** seems warranted (Scheme 4.1).

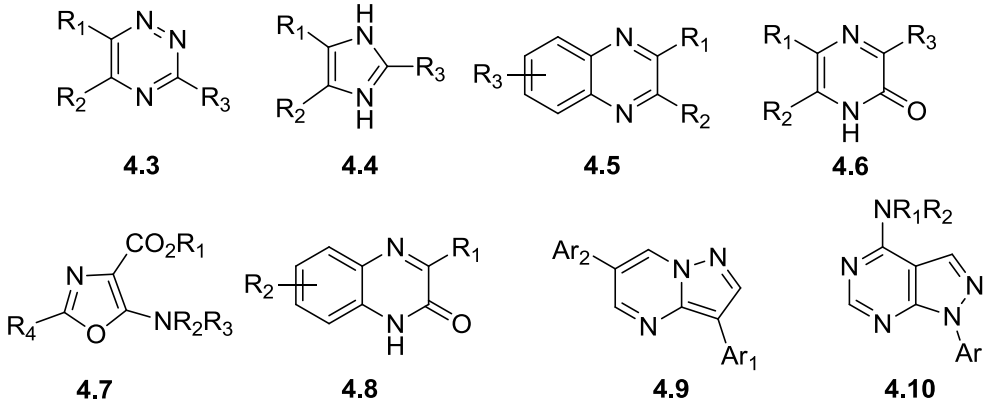
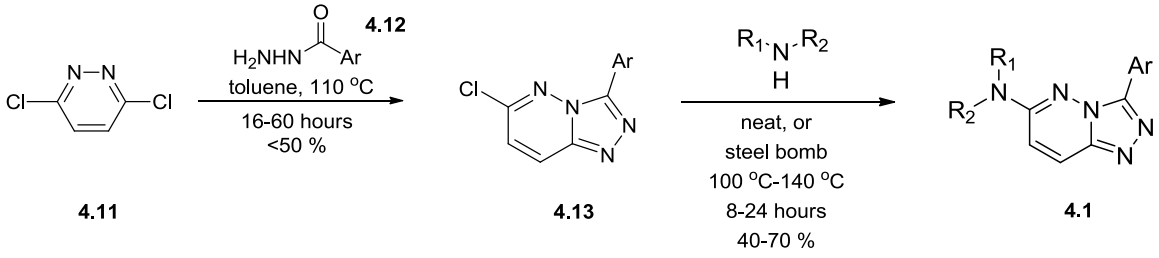


Figure 4.3. Heterocyclic templates accessed through MAOS in our laboratory.[23-30]

4.3. Methodology Development for Expedited Library Synthesis.

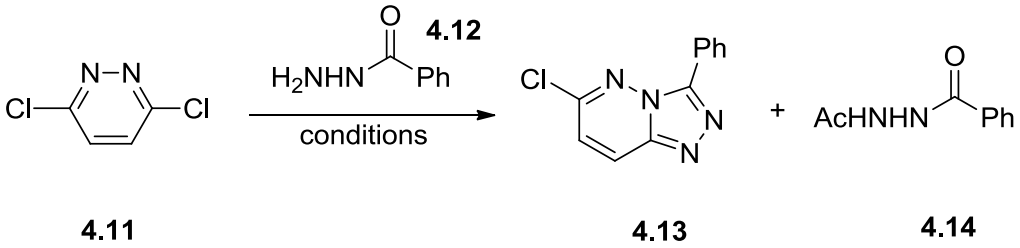
Classical conditions for the synthesis of 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*] pyridazines **4.1** involve refluxing 3,6-dichloropyridazine **4.11** with an acylhydrazide **4.12** in toluene for 16 h, or more typically for 60 h, to provide the 3-aryl-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazine **4.13** in yields less than 50% (Scheme 1.1).[20-22] Introduction of the amino moiety in the 6-position was accomplished through an S_NAr reaction employing either neat or steel bomb conditions at 100–140°C for 8–30 h to deliver analogs **4.1** in yields ranging from 40% to 70%.[21-23] Moreover, previous efforts were focused on traditional medicinal chemistry approaches and the development of structure–activity relationships (SARs), with little concern for achieving high chemical yields or reaction generality for either the heterocycle synthesis or the S_NAr reaction. Indeed, the 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine scaffold **4.1** has been an important pharmacophore for the development of $GABA_A$ receptor agonists at the α2/α3-subunit.[20-22] Interestingly, microwave-assisted organic synthesis has never before been applied to this heterocyclic system, and even more surprising when one considers a 1–6 day reaction time to deliver a single derivative of **4.1**.



Scheme 4.1. Classical synthesis of 3,6-disubstituted-[1,2,4]triazolo 4,3-*b*]pyridazines **4.1**.

By varying solvent and temperature parameters, microwave conditions were rapidly developed to accelerate and generalize the synthesis of the 3-phenyl-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazine **4.13** core employing 3,6-dichloropyridazine **4.11** and acylhydrazide **4.12** (Table 4.1). When acetic acid was employed as a solvent or catalyst, a corresponding acetylated phenyl acylhydrazide **4.14** was obtained in varying quantities. Optimal acetic acid conditions employed 5% HOAc/EtOH at 150 ^{o}C for 10 min to afford the desired **4.13**, along with **4.14** in an 85:15 ratio (Table 4.1, entry 9). Despite the side product, the conversion to **4.13**/**4.14** was quantitative and isolated yields of **4.13** exceeded 82%. Application of the same MAOS conditions, but replacement of acetic acid with 5% 4 N HCl in dioxane, afforded 100% conversion to **4.13** in 95% isolated yield without producing **4.14** (Table 1.1, entry 13). Thus, a reaction that previously required up to 60 h of conventional heating to provide <50% yield,[20-22] now afforded 95% yield of the desired product **4.13** in 10 minutes by virtue of MAOS—a 360-fold reduction in reaction time.

Table 4.1. Optimization of MAOS conditions to produce **4.13.**



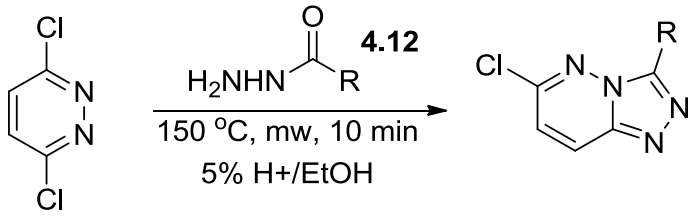
4.11 **4.13** **4.14**

Entry	T (^{o}C)	Solvent	Time (min)	4.13:4.14[a]
1	140	HOAc	10	42:58
2	160	HOAc	10	28:72
3	180	HOAc	10	24:76
4	200	HOAc	10	13:87
5	150	50% HOAc/EtOH	10	78:22
6	170	50% HOAc/EtOH	10	64:36
7	150	10% HOAc/EtOH	10	79:21
8	170	10% HOAc/EtOH	10	74:26
9	150	5% HOAc/EtOH	10	85:15
10	170	5% HOAc/EtOH	10	77:23
11	135	5% HOAc/EtOH	20	80:20
12	135	EtOH[b]	10	--
13	150	5% HCl/EtOH[c]	10	100:0

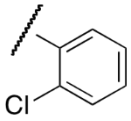
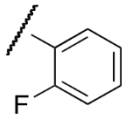
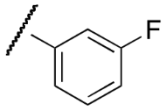
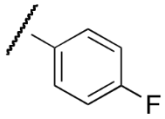
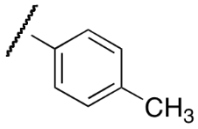
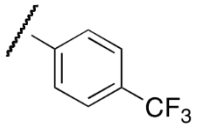
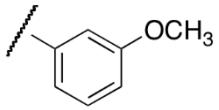
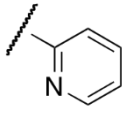
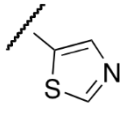
[a] Ratio determined by analytical LCMS and ^{1}H NMR; conversion >95%.
[b] No product formed without acid catalysis.
[c] 5% 4 N HCl/dioxane.

Attention was now directed at the application of these new MAOS conditions to a diverse array of acylhydrazides to ensure that this new protocol would indeed be general. As shown in Table 4.2, the MAOS protocol, employing either catalytic HOAc (Method A) or HCl (Method B), proved to be general with respect to a wide range of electron-rich (entry **4.13g**), electron-deficient (entry **4.13f**), and hindered acylhydrazides (entry **4.13a**) as well as heterocyclic congeners (entries **4.13h**, **4.13i**) affording the desired 3-aryl/heteroaryl-6-chloro-[1,2,4]-triazolo-[4,3-*b*]pyridazines **4.13** in isolated yields ranging from 74% to 97% in 10 min at 150 ^{o}C.

Table 4.2. Generality of the MAOS protocol to deliver analogs **4.13.**

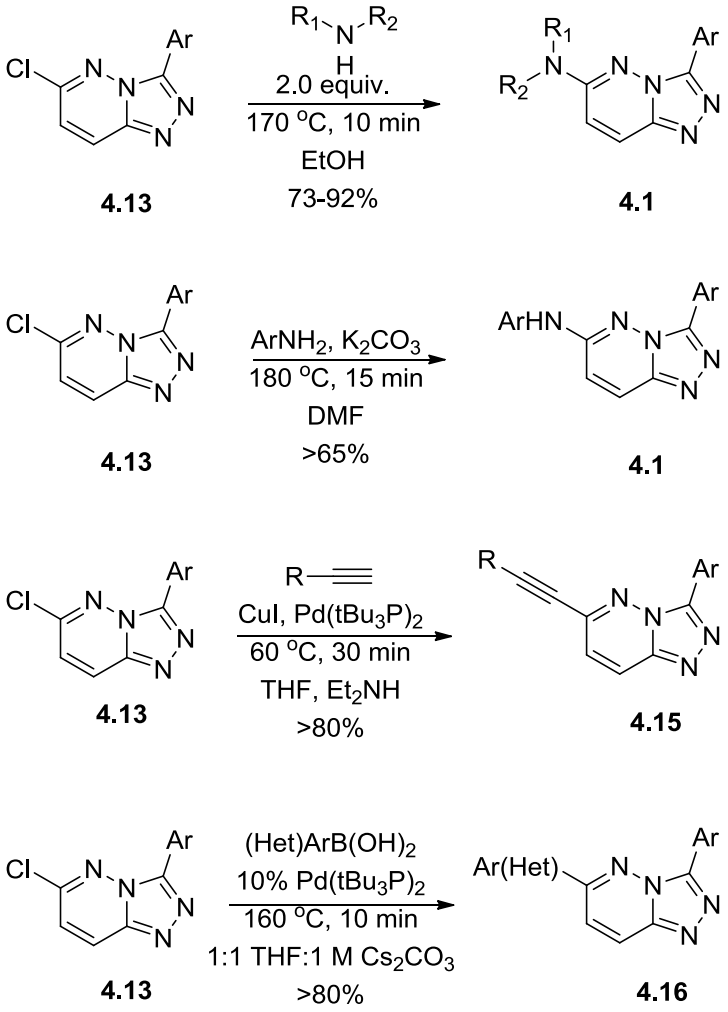


4.11 **4.13**

Compound	R	Yield[a] (%)	Yield[b] (%)
4.13a		79	92
4.13b		77	93
4.13c		81	91
4.13d		87	96
4.13e		80	95
4.13f		75	97
4.13g		74	96
4.13h		70	88
4.13i		72	86

[a] 5% HOAc/EtOH, remaining mass balance congeners or **4.14**.
[b] 5% 4 N HCI/dioxane. All yields for isolated, analytically pure materials.

Developing a general MAOS-mediated S_NAr protocol for the reaction of diverse amines with analogs **4.13** to deliver 3-aryl, 6-amino-[1,2,4]triazolo[4,3-b]pyridazines **4.1** proved more difficult. Nucleophilic amines (benzyl, aliphatic, piperidines, and piperazines) reacted smoothly in EtOH at 170 ^oC for 10 min to produce analogs **4.1** in yields ranging from 73% to 92% (Scheme 4.2). Less nucleophilic amines, such as anilines, required K_2CO_3 in DMF with microwave irradiation for 15 min at 180^oC to produce analogs **4.1** in yields exceeding 65%. Furthermore, analogs **4.13** readily participated in general microwave-assisted Sonogashira and Suzuki cross-coupling reactions to afford analogs **4.15** and **4.16** in yields exceeding 80% in every case examined (Scheme 4.2).

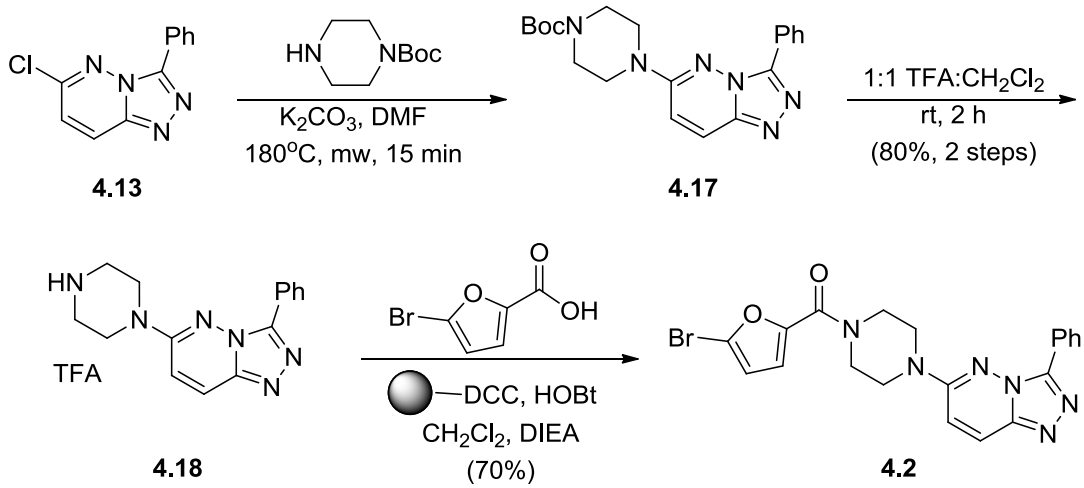


Scheme 4.2. MAOS protocols to functionalize 3-aryl-6-chloro-[1,2,4]-triazolo-[4,3-*b*]pyridazine. **4.13**.

4.4. Resynthesis of the Lead Compound and Plan for Library Synthesis

Utilizing these new MAOS protocols, we resynthesized the M_1 versus M_4 selective antagonist HTS hit **4.2** (Scheme 4.3). Beginning with **4.13**, delivered in 95% yield (Table 4.1), an S_NAr reaction with Boc-piperazine provided **4.17**, which was then deprotected using 1:1 TFA:DCM to afford **4.18** in 80% yield for the two steps. **4.18** was then acylated employing standard polymer-supported reagents and scavengers to generate

the original HTS hit **4.2** in 70% yield.[13] Evaluation of **4.2** against M_1–M_5 in a functional cell-based assay indicated that **4.2** was indeed a weak but selective M1 antagonist (M_1 IC_{50} = 22 μM, M_2–M_5 IC_{50} >> 50 μM). Prior to this discovery there was only one other M_1 selective small molecule antagonist,[30] and prior to its discovery, the only M_1 selective antagonist was MT7, a 71 amino acid peptide toxin from the green mamba snake.[31] Encouraged by this result, we employed an iterative parallel synthesis approach, employing our new MAOS protocols, to rapidly develop structure–activity relationships in an attempt to improve the M_1 antagonist potency while maintaining selectivity for M_2–M_5.



Scheme 4.3. Application of MAOS protocols for the resynthesis of the M_1 antagonist HTS hit **4.2**.

As shown in Figure 4.4, we plan to vary the substituents at the C-3 and C-6 positions, synthesizing small 12- to 24-member libraries employing the synthetic routes depicted in Scheme 4.3 and Figure 4.4. We will incorporate alternative aryl moieties into the eastern portion of the molecule by utilizing various aryl acylhydrazides in the initial condensation reaction. Alternative amides will be synthesized by acylation of the

secondary amine with acid chloride and activated carboxylic acids or anhydrides. Finally, we plan to replace the amide linkage with a non-hydrolysable bond through incorporation of different amines in the S_NAr reaction.

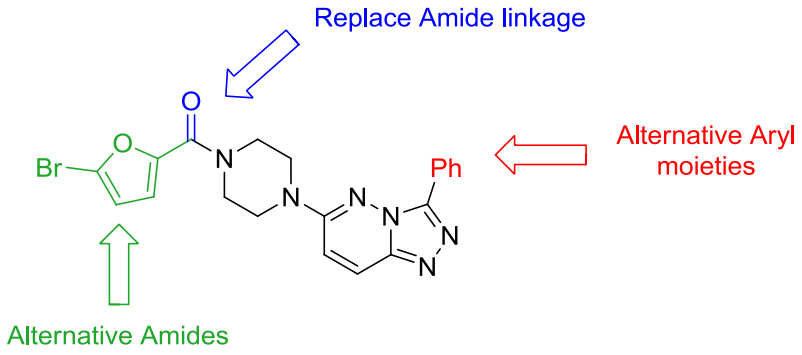


Figure 4.4. Synthetic plan to optimize **4.2** for M_1 antagonist potency, while maintaining selectivity versus M_2–M_5.

4.5. Biological Activity of Analog Libraries

The first library was prepared by variation of the eastern portion of the molecule through incorporation of various electron-rich, electron-poor, and electron-neutral aryl acylhydrazides (**4.2A-I**). Analogs of **4.2** were triaged in a cell-based functional single-point (10 μM) screen for the compounds' ability to decrease the response to an EC_{80} concentration of acetylcholine (Figure 4.5). Three compounds (**4.2C**, **4.2G, 4.2I**) were found to have greater M_1 antagonist efficacy than the original HTS lead (**4.2B**) in the single point assay.

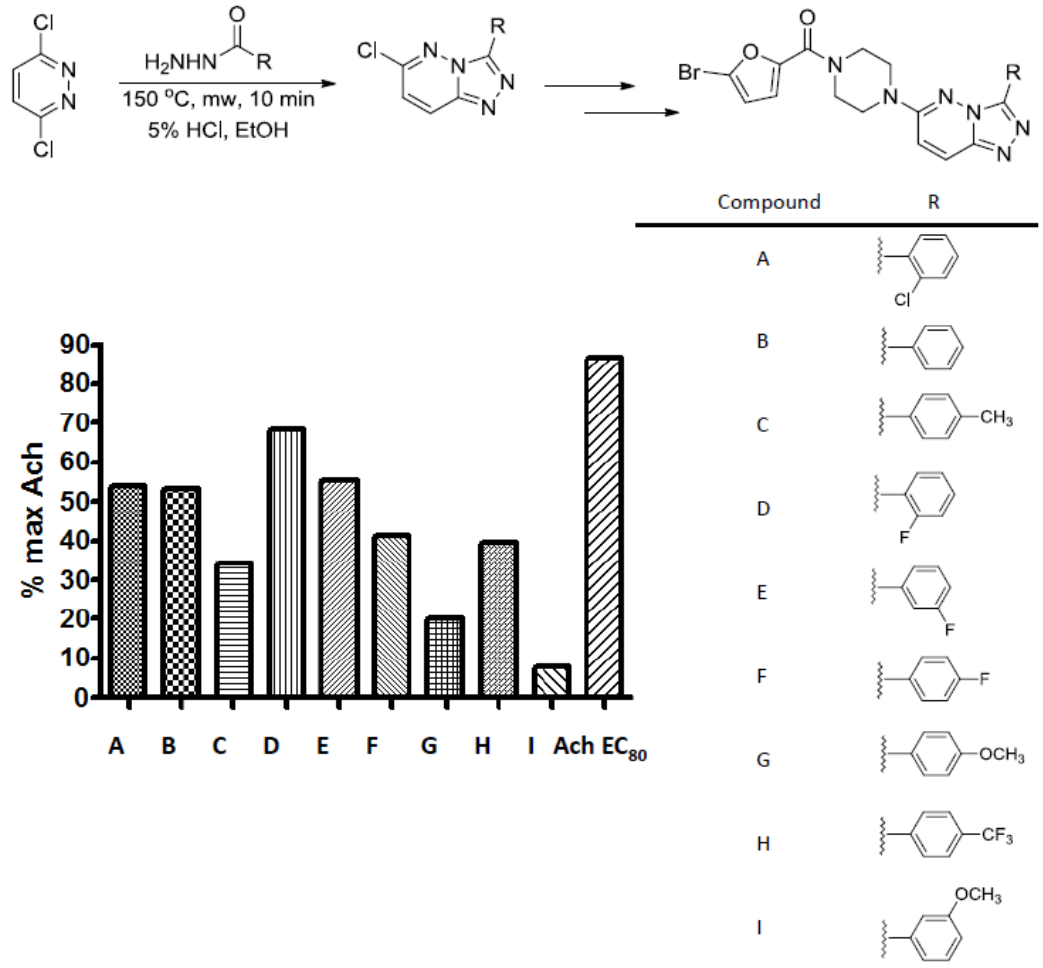


Figure 4.5. Single point 10 μM screen for M_1 antagonism.by analogs **4.2A-I.**

Concentration-response curves (CRCs) were generated for these three compounds to determine the potency of the analogs (Figure 4.6). **4.2C** and **4.2I** displayed IC_{50} values of 4.1 μM and 3.6 μM, respectively. **4.2G** was determined to have an IC_{50} of 10 μM. Further library development will be performed with the *meta*-methoxy analog **4.2I**, since SAR indicates that it is the most active compound.

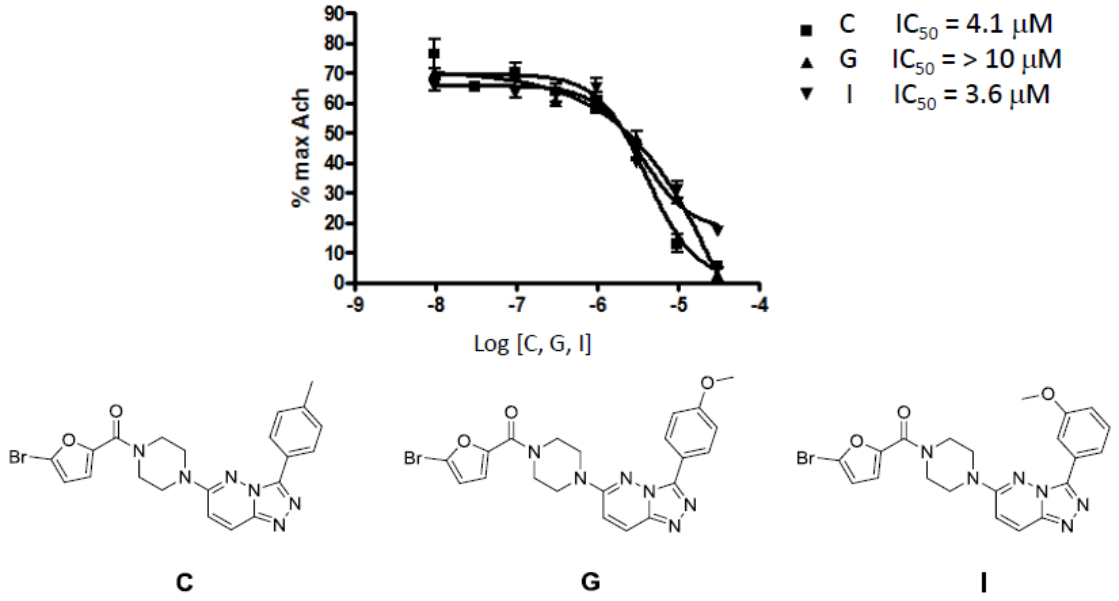


Figure 4.6. Concentration-response curves for M_1 antagonism by analogs **4.2C**, **4.2G**, and **4.2I** in a calcium mobilization mobilization assay using recombinant M_1-expressing CHO cells.

We next investigated the selectivity of our compounds for the M1 receptor over the other subtypes (M2-M5). Cell lines individually expressing M2-M5 were treated with various concentrations of compounds **4.2B**, **4.2C**, and **4.21** followed by an EC_{80} of ACh in order to generate CRCs (Figure 4.7).

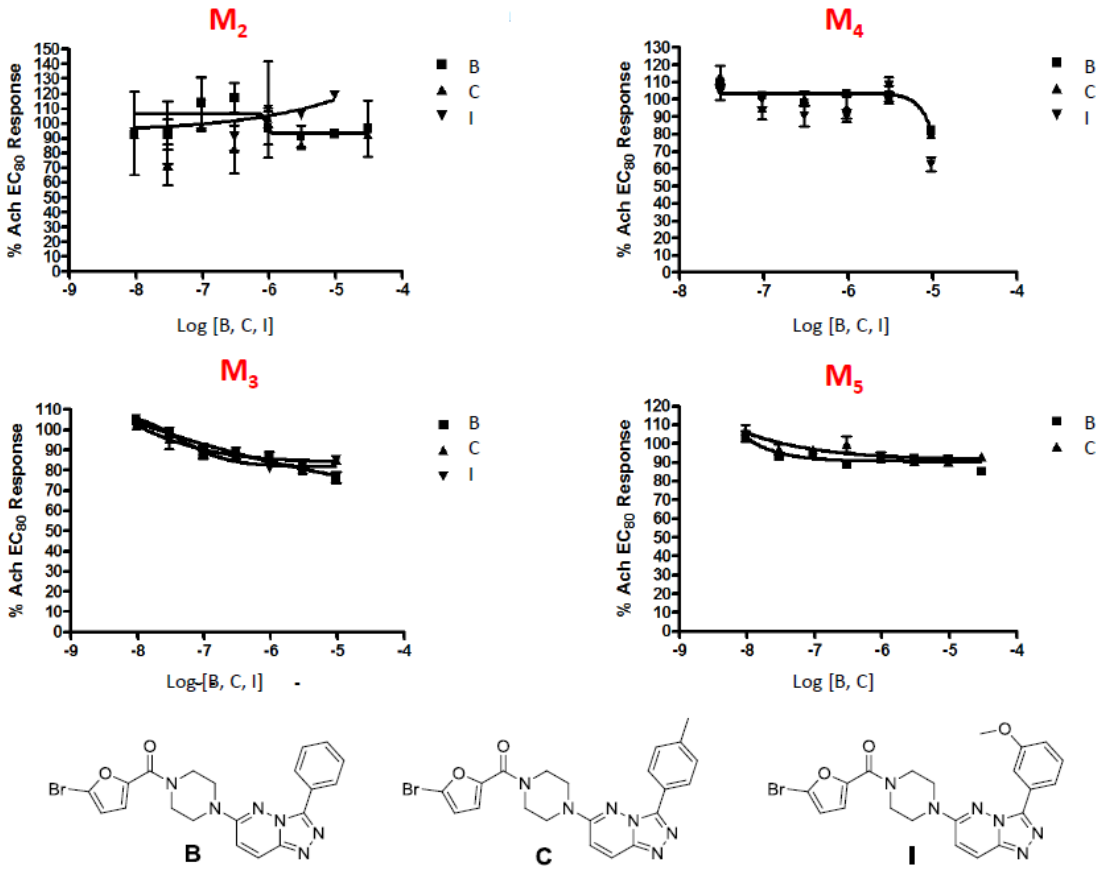
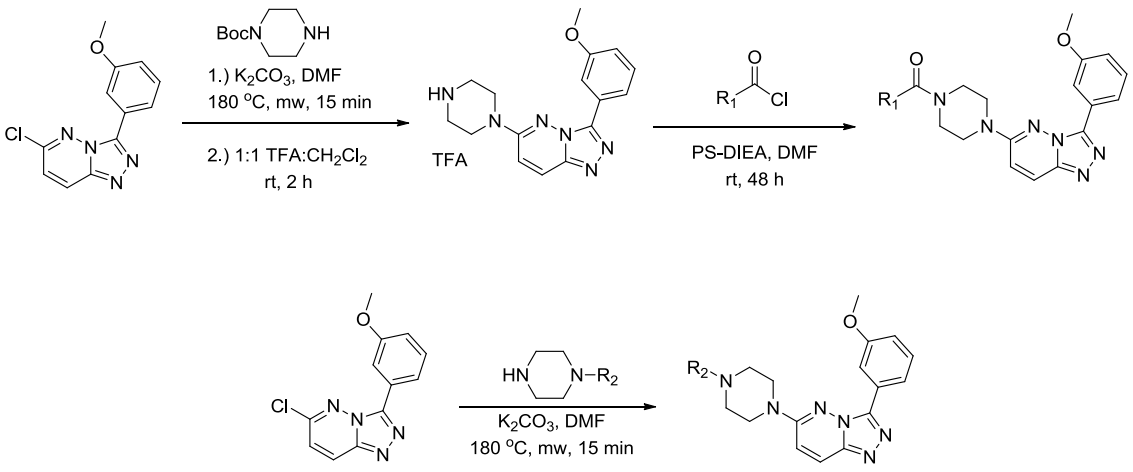
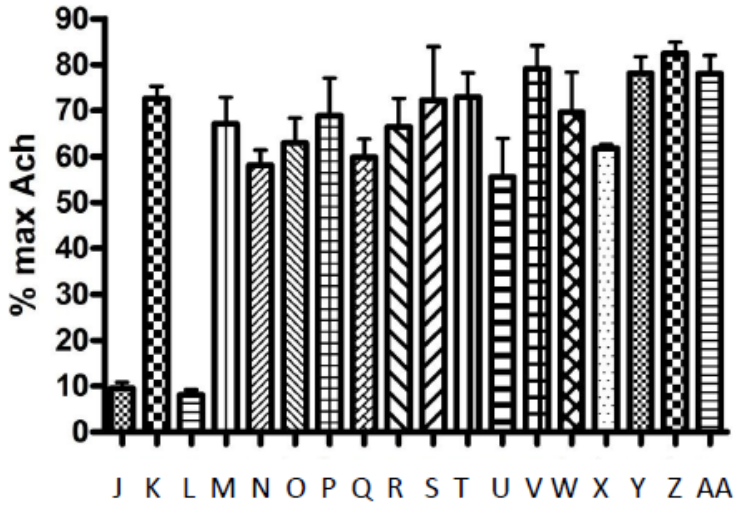


Figure 4.7. Selectivity for M_1versus M_2-M_5 for analogs **4.2B**, **4.2C**, and **4.2I**.

In light of these promising results, we began to generate additional libraries with various appendages in the western portion. Alternative amides were prepared according to Figure 4.8 by acylation with various acid chloride with diversity at R_1. We also prepared various pyridyl and pyramidyl piperazines by performing the S_NAr reaction with these piperazines.





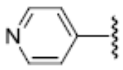
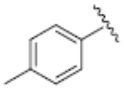
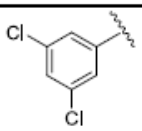
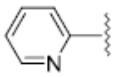
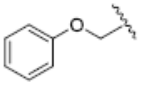
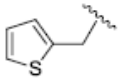
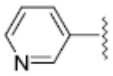
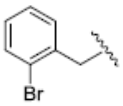
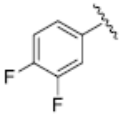
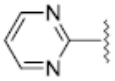
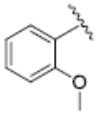
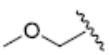
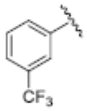
Compound	R_2	Compound	R_1	Compound	R_1	Compound	R_1
J		N		S		X	
K		O		T		Y	
L		P		U		Z	
M		Q		V		AA	
		R		W			

Figure 4.8. Single point 10 μM screen for M_1 antagonism.by analogs **4.2J-AA.**

SAR for this series was rather 'flat', with subtle changes leading to a complete loss of M_1 inhibitory activity. Out of all of the analogs, only four demonstrated significant M_1 antagonism; however, we managed to improve upon HTS hit **4.2**. As summarized in Figure 4.9, exploration of the C3 position identified both the 3-OMe phenyl derivative **4.2I** and the 4-Me phenyl congener **4.2C** as engendering more potency (M_1 IC_{50} = 3.59 μM and 4.09 μM, respectively), while maintaining selectivity (M_2–M_5 IC_{50} >> 50 μM). When holding the 3-OMe phenyl moiety constant at C3 and exploring alternatively functionalized piperazines for the bromofuranoic amide at C6, we identified two pyridyl piperazine analogs, **4.2J** and **4.2L**, which maintained M_1 antagonism (M_1 IC_{50} = 3.99 μM and 6.64 μM, respectively) and selectivity (M_2–M_5 IC_{50} >> 50 μM). Moreover, these latter analogs, with basic amines, afforded improved solubility and physiochemical characteristics.

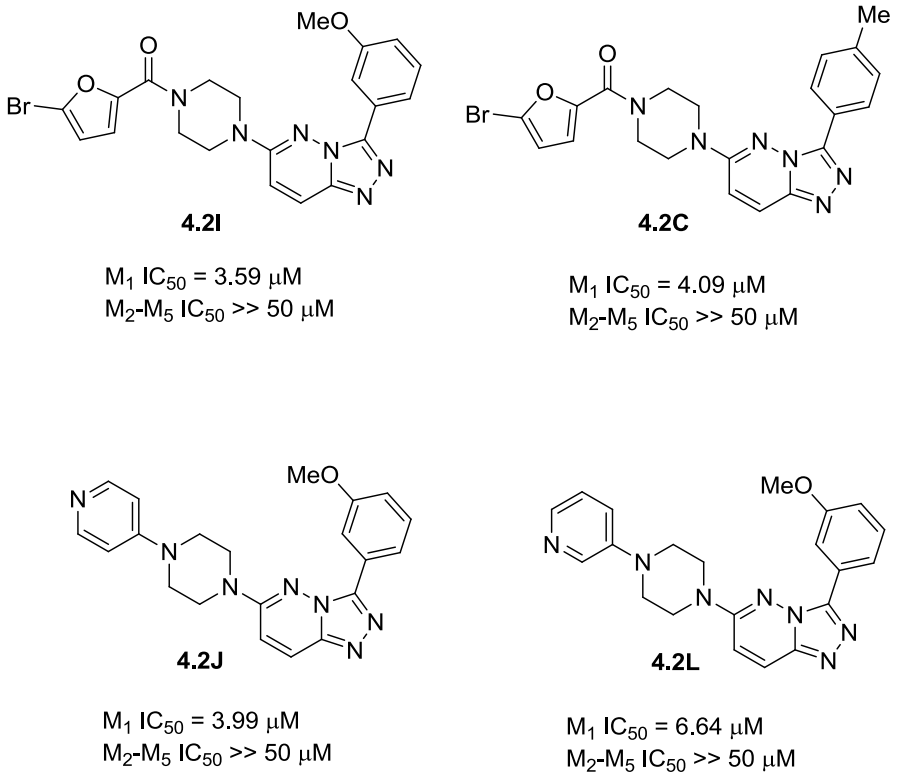
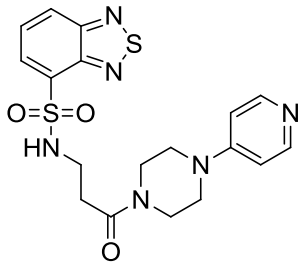


Figure 4.9 Optimized analogs of **4.2** as highly selective M_1 antagonists with improved M_1 inhibitory activity as compared to HTS hit **4.2**.

4.6. Summary.

We have applied MAOS to the preparation of 3,6-disubstituted-[1,2,4]-triazolo-[4,3-*b*]pyridazines **4.1**, and developed general and high-yielding protocols with over a 360-fold acceleration in reaction rate. For both the heterocyclic synthesis and the subsequent S_NAr steps, reaction time, yield, and overall reaction generality were dramatically improved under these MAOS protocols; more importantly, these new protocols allow for an iterative analog library synthesis approach for lead optimization to be employed for the rapid synthesis of large numbers of analogs of **4.2**. Employing these new MAOS protocols, a lead optimization campaign centered on the selective, but weak M_1 antagonist hit **4.2** (M_1 IC_{50} = 22 μM) delivered two analogs, **4.2J** and **4.2L**, with over

a 6-fold increase in M_1 inhibitory activity (M_1 IC_{50} = 3.99 μM and 6.64 μM, respectively) while maintaining selectivity versus M_2–M_5 (IC_{50} >> 50 μM). These compounds represent a novel chemotype of selective, small molecule M_1 antagonists, and hold promise as leads for potential new therapeutic agents for dystonias.[33]



VU0255035

mAChR	IC_{50} (nM)[a]	Fold selectivity	Ki (nM)[a]	Fold selectivity
M_1	133		14.9	
M_2	>10,000	>75	661.3	45
M_3	>10,000	>75	876.9	59
M_4	>10,000	>75	1177.6	79
M_5	>10,000	>75	2362.3	159

[a]IC_{50}s and K_is are the average of at least three independent determinations. K_is for atropine: M_1 (0.8 nM), M_2 (2.6 nM), M_3 (1.1 nM), M_4 (0.6 nM), M_5 (1.6 nM); average of three determinations.

Figure 4.10 Optimized analogs of **4.2** as highly selective M_1 antagonists with improved M_1 inhibitory activity as compared to HTS hit **4.2**.[34]

While further derivatization around the triazolopyridazine scaffold did not provide a significant increase in potency, the SAR mapping of our scaffold led to the development of a much more potent M_1 orthosteric anatagonist, VU0255035, (Figure 4.10) with the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD).[34] This scaffold maintains the 4-pyridylpiperazine moiety evident in one of our most potent

compounds. It also incorporates a bicyclic nitrogen-containing heterocyclic moiety reminiscent of the triazolopyridazine core. VU0255035 is highly selective for M_1 with an IC_{50} of 133 nM at M_1 and greater than 75x selectivity versus the other four muscarinic acetylcholine receptor subtypes. VU0255035 is the first highly selective M_1 antagonist with nanomolar potency and will serve as a very important tool compound for the study of selective M_1 antagonism, the role this activity plays within the nervous system, and the potential of this receptor as a therapeutic target for the treatment of dystonias and other movement disorders.

4.7. Future Work.

Future work could include the development of an *in vitro* phenotypic assay for dystonia subtypes with known genetic mutations responsible for the errant phenotype. Once an assay is developed these compounds could be tested for their ability to alleviate the cellular phenotype. *In vivo* mouse models could also be developed and could provide excellent insight into the potential of these compounds to treat dystonic symptoms in an intact nervous system.

In addition to dystonia, these selective M_1 antagonists could be utilized to study the role of M_1 in other disease states, such as Parkinson's disease. Until now, there have been no means to selectively antagonize M_1 over the other receptor subtypes, therefore, when M_1 has been implicated in disease states, its involvement has been unable to be definitively determined.[16,17] The availability of these compounds will not only aid future research within our lab and the VCNDD, but also within the greater scientific community of neurological research.

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CHAPTER 5

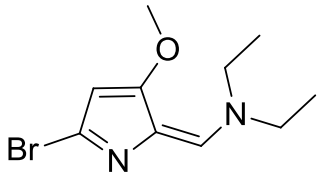
EXPERIMENTAL METHODS

5.1. General

All ^{1}H & ^{13}C NMR spectra were recorded on Bruker DRX-500 (500 MHz), Bruker AV-400 (400 MHz) or Bruker AV-NMR (600 MHz) instrument. Chemical shifts are reported in ppm relative to residual solvent peaks as an internal standard set to δH 7.26 or δC 77.0 ($CDCl_3$) and δH 3.31 or δC 49.0 (CD_3OD). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constant (Hz). IR spectra were recorded as thin films and are reported in wavenumbers (cm^{-1}). Low resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization. High resolution mass spectra were recorded on a Waters Qtof-API-US plus Acquity system. The value Δ is the error in the measurement (in ppm) given by the equation $\Delta = [(ME - MT)/ MT] \times 106$, where ME is the experimental mass and MT is the theoretical mass. The HRMS results were obtained with ES as the ion source and leucine enkephalin as the reference. Optical rotations were measured on a Perkin Elmer-341 polarimeter. Analytical thin layer chromatography was performed on 250 μM silica gel 60 F254 plates. Visualization was accomplished with UV light, and/or iodine chamber and the use of ninhydrin, anisaldehyde ceric ammonium molybdate, and potassium permanganate solutions followed by charring on a hot-plate. Chromatography on silica gel was performed using Silica Gel 60 (230-400 mesh) from

Sorbent Technologies. Analytical HPLC was performed on an Agilent 1200 analytical LCMS with UV detection at 214 nm and 254 nm along with ELSD detection. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All polymer-supported reagents were purchased from Biotage, Inc. Flame-dried (under vacuum) glassware was used for all reactions. All reagents and solvents were commercial grade and purified prior to use when necessary. Mass spectra were obtained on a Micromass Q-Tof API-US mass spectrometer was used to acquire high-resolution mass spectrometry (HRMS) data.

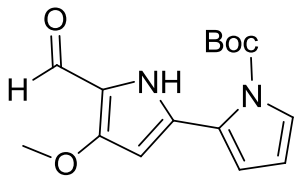
5.2. Progress Toward the Total Synthesis of Marineosins A & B.



2.56

(*E*)-*N*-((5-bromo-3-methoxy-2*H*-pyrrol-2-ylidene)methyl)-*N*-ethylethanamine (2.56). A 100 mL round bottom flask was charged with N,N-diethylformamide (2.86 g, 28.29 mmol) and 23 mL of dichloromethane. The solution cooled to 0 ^{o}C in an ice bath, and a solution of phosphorous oxybromide (10.14 g, 35.36 mmol) in dichloromethane (7 mL) was added slowly over 20 minutes. After addition was complete, the reaction mixture was stirred for an additional 20 minutes. A solution of 4-methoxy-3-pyrrolin-2-one (2.00 g, 17.68 mmol) in dichloromethane (18 mL) was added dropwise over 10 minutes, and the mixture was stirred for an additional 20 minutes. The flask was then removed from the ice bath, transferred to an oil bath, and refluxed (42^{o}C) for 3.5 hours. The reaction mixture was then transferred to a 500 mL round bottom flask and cooled to 0^{o}C in an ice bath. Water (20 mL) was added dropwise over 10 minutes, followed by 15 wt% sodium hydroxide (230 mL) over 40 minutes. The reaction was stirred for an additional 20 minutes and then transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted with dichloromethane (3 x). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The resulting yellow oil was subjected to flash chromatography (4:1 Hex:EtOAc) to give **2.56** as an oil that solidified upon standing at 20 ^{0}C to give a tan solid (2.67 g, 59%).

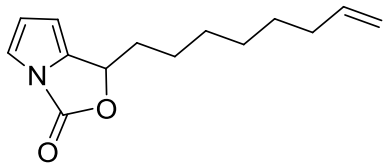
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.29 (t, *J* = 7.2 Hz), 1.30 (t, *J* = 7.2 Hz), 3.40 (q, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 5.59 (s, 1H), 6.99 (s, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.4, 14.5, 44.5, 51.0, 57.9, 96.4, 120.7, 133.6, 138.5, 165.3. **IR** (KBr) υ_{max} 2975, 2934, 1629, 1529, 1408, 1290, 1264, 1195, 1116, 1072, 906, 737 cm^{-1}. **HRMS**: $C_{10}H_{16}N_2OBr$, Calculated: [M+H]+, 259.0446, Found: [M+H]+, 259.0448.



2.64

***tert*-butyl 5'-formyl-4'-methoxy-1*H*,1'*H*-2,2'-bipyrrole-1-carboxylate (2.64).** $Pd(PPh_3)_4$ was generated *in situ* by adding triphenylphosphine (1.22 g, 4.64 mmol) to a magnetically stirred suspension of Palladium II Acetate (0.23 g, 1.03 mmol) in degassed toluene (5.0 mL) then heating the ensuing mixture at 70 ^oC for 20 minutes under at atmosphere of argon. A solution of N-Boc-pyrrole-2-boronic acid (3.26 g, 15.46 mmol) and bromoenamine **2.56** (2.67 g, 10.31 mmol) in H_2O/1,4-dioxane (1:9 v/v, 86 mL) was degassed, purged with argon gas and added to the solution of $Pd(PPh_3)_4$ in toluene. Anhydrous sodium carbonate (3.28 g, 30.93 mmol) was added and the reaction mixture stirred at 85^oC. After 3 hours, the mixture was cooled and poured into water (150 mL). The mixture was reduced to pH 7 using 2 M HCl and transferred to a separatory funnel. The solution was partitioned with dichloromethane and extracted (4 x). The organic layers were combined, dried over sodium sulfate, and condensed *in vacuo* to give a brown residue that was purified by flash chromatography (4:1 Hex:EtOAc) affording aldehyde **2.64** as an orange solid (1.44 g, 48%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.61 (s, 9H), 3.88 (s, 3H), 6.07 (d, *J* = 3.5 Hz, 1H), 6.24 (t, *J* = 3.5 Hz, 1H) 6.66 (dd, *J* = 3.5 Hz, 1.76 Hz, 1H), 7.33 (dd, *J* = 3.4, 1.80 Hz, 1H), 9.53 (s, 1H), 10.73 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 27.8, 57.8, 85.7, 94.7, 111.4, 116.8, 118.2, 124.5, 125.9, 130.2, 149.6, 157.6, 174.3. **IR** (KBr) υ_{max} 3221, 2979, 2833, 1735, 1623, 1549, 1502, 1433, 1370, 1330, 1287, 1255, 1140, 1021 cm^{-1}. **HRMS**: $C_{15}H_{19}N_2O_4$, Calculated: [M+H]+,.291.1345, Found: [M+H]+,.291.1345.

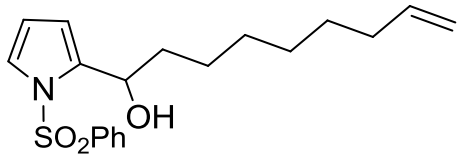


2.75

1-(oct-7-enyl)pyrrolo[1,2-*c*]oxazol-3(1*H*)-one (2.75). Magnesium ribbon (54.8 mg, 2.25 mmol) was flame-dried in a 50 mL round bottom flask fit with a reflux condenser. Anhydrous THF (10 mL) and a small crystal of iodine were then added to the flask. 8-bromo-1-octene (352.4 mg, 1.84 mmol) was added dropwise at room temperature, and then the solution was heated to reflux. After 2 hours, the mixture was cooled to 0^{o}C and a prepared solution of N-Boc-pyrrole-2-carboxaldehyde (0.1 M in THF, 10 mL) was added to the prepared Grignard reagent. The solution was stirred at room temperature for 1 hour, cooled to 0 ^{o}C, and quenched with 1M HCl until the pH was neutral. The mixture was extracted with dichloromethane (3x), washed with brine, dried over sodium sulfate, and condensed *in vacuo* to give a brown residue. The residue was purified by flash chromatography (10:1 Hex:EtOAc) and condensed under reduced pressure to give **2.75** as a clear yellow oil (185 mg, 78%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.31-1.40 (m, 6H), 1.44-1.50 (m, 2H), 1.86-1.92 (m, 2H), 2.03 (q, *J* = 7.0 Hz, 2H) 4.93 (dt, *J* = 10.2, 1.0 Hz, 1H), 4.99 (ddd, *J* = 17.1, 3.5,

1.6 Hz, 1H), 5.43 (t, *J* = 6.4 Hz, 1H), 5.74-5.84 (m, 1H), 6.00 (dd, *J* = 1.8, 1.3 Hz, 1H), 6.43 (t, *J* = 3.1 Hz, 1H), 7.04 (d, *J* = 3.0 Hz, 1H) **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 24.0, 28.6, 28.7, 28.9, 33.6, 34.5, 79.3, 102.4, 112.2, 114.3, 118.1, 136.6, 138.8, 150.0. **IR** (KBr) υ_{max} 2927, 2856, 1797, 1467, 1416, 1404, 1322, 1267, 1096, 906, 754, 712 cm^{-1}. **LRMS**: m/z = 234.1 [M+H]+.

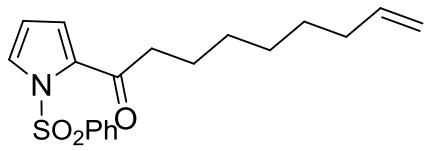


2.82

1-(1-(phenylsulfonyl)-1*H*-pyrrol-2-yl)non-8-en-1-ol (2.82). Magnesium ribbon (2.07 g, 85 mmol) was cut into small pieces, placed in a 500 mL, 2-neck schlenk flask fit with a reflux condenser, and flame-dried under vacuum. A small crystal of iodine was added and the flask was flushed with argon. The magnesium was suspended in anhydrous THF (250 mL) and vigorously stirred. 8-bromo-1-octene (11.78 g, 61.6 mmol) was added to the solution dropwise at room temperature. The resulting mixture was then heated to reflux (65 ^{o}C) for 2 hours. 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde (10.0 g, 42.5 mmol) was added to a 500 mL flame-dried, schlenk flask. Anhydrous THF (105 mL) was added and the mixture was stirred and cooled to 0 ^{o}C in an ice bath. After refluxing for 2 hours, the prepared Grignard reagent was cooled to room temperature and cannulated into the aldehyde solution over a 30 minute period. The resulting brown slurry was stirred at 0 ^{o}C for an additional 30 minutes and was then allowed to warm to room temperature. After 3 hours, 2 M HCl was slowly added to the vigorously stirred solution until it reached pH 7. The mixture was then transferred to a separatory funnel, diluted with water, partitioned with dichloromethane, and extracted (4 x). The combined organic layers were combined,

dried over sodium sulfate, and condensed under reduced pressure to give a brown residue. This residue was purified by flash chromatography (3:1 Hex:EtOAc) and dried *in vacuo* to afford alcohol **2.82** as a viscous, orange oil (9.08 g, 62%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.21-1.26 (m, 4H), 1.29-1.36 (m, 4H), 1.71-1.84 (m, 2H), 2.01 (q, *J* = 7.2 Hz, 2H), 2.72 (d, *J* = 4.0 Hz, 1H), 4.80 (td, *J* = 7.2, 4.0 Hz, 1H), 4.93 (dt, *J* = 10.4, 1.2 Hz, 1H), 4.98 (ddd, *J* = 17.2, 3.6, 1.6 Hz, 1H), 5.74-5.85 (m, 1H), 6.26 (t, *J* = 3.2 Hz, 1H), 6.28 (dd *J* = 5.2, 3.2 Hz, 1H), 7.31 (dd *J* = 3.2, 2.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 2 H), 7.61 (tt *J* = 8.0 Hz, 1.2 Hz, 1H), 7.79 (d *J* = 8.0 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 26.0, 28.8, 28.9, 29.1, 33.7, 35.0, 65.2, 111.7, 112.4, 114.2, 123.5, 126.5, 129.4, 133.9, 138.3, 139.1, 139.3. **IR** (KBr) υ_{max} 3567, 2928, 2856, 1448, 1366, 1177, 1152, 1090, 1056, 726, 685 cm^{-1}. **HRMS**: $C_{19}H_{26}NO_3S$, Calculated: [M+H]+,.348.1633, Found: [M+H]+,.348.1632.

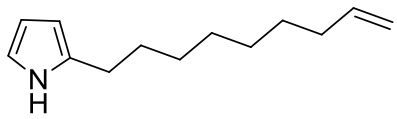


2.83

1-(1-(phenylsulfonyl)-1*H*-pyrrol-2-yl)non-8-en-1-one (2.83). TPAP (0.84 g, 2.39 mmol) was added to a 250 mL flame-dried round bottom flask containing a stirred and cooled (0^{o}C) solution of alcohol **2.82** (8.3 g, 23.9 mmol), NMO (5.6 g, 47.8 mmol), and 4 amgstrom molecular sieves (12 g) in dichloromethane (100 mL). The reaction was warmed to room temperature and stirred for 4 hours. The reaction mixture was filtered to remove the molecular sieves, diluted with dichloromethane, and partitioned with water. The mixture was washed with 1 M HCl (1 x) and water (2 x). The organic layer was dried over sodium sulfate and condensed under reduced pressure to give a black oil. The oil

was purified by flash chromatography (4:1 Hex:EtOAc) and dried *in vacuo* to afford **2.83** as a clear, colorless oil (7.08 g, 86%).

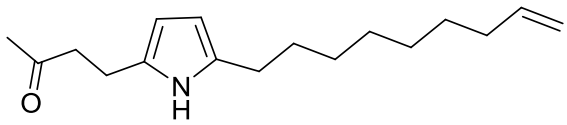
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.24-1.27 (m, 4H), 1.31-1.36 (m. 2H), 1.59 (quin. *J* = 7.2 Hz, 2H), 2.00 (q, *J* = 6.8 Hz, 2H), 2.66 (t, *J* = 7.2 Hz, 2H), 4.92 (d, *J* =10.0 Hz, 1H), 4.97 (dd, *J* =16.8, 2.0 Hz, 1H), 5.73-5.83 (m. 1H) 6.34 (t, *J* = 3.6 Hz, 1H), 7.31 (dd, *J* = 3.6, 1.6 Hz, 1H) 7.52 (t, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.2 Hz, 1H), 7.80 (dd, *J* = 3.2, 1.6 Hz, 1H), 8.00 (dd, *J* = 7.2 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 24.7, 28.6, 28.7, 28.9, 33.6, 39.3, 110.3, 114.2, 123.2, 128.0, 128.6, 130.0, 133.4, 133.5, 138.9, 139.0, 188.9. **IR** (KBr) υ_{max} 2928, 2856, 1676, 1448, 1440, 1367, 1175, 1144, 1089, 1062, 751, 726, 684 cm^{-1}. **HRMS**: $C_{19}H_{24}NO_3S$, Calculated: [M+H]+,.346.1477, Found: [M+H]+, .346.1481.



2.69

2-(non-8-enyl)-1*H*-pyrrole (2.69). Sodium borohydride (3.1 g, 81.88 mmol) was added to a flame-dried, 500 mL round bottom flask fit with a reflux condenser. The flask was purged with argon and anhydrous 2-propanol (150 mL) was added. Acylpyrrole **2.83** (5.64 g, 16.29 mmol) was suspended in 120 mL of anhydrous 2-propanol, and this solution was added dropwise to the sodium borohydride solution at room temperature. After addition was complete, the mixture was heated to reflux (82 ^oC) for 24 hours. The reaction was cooled to room temperature and the excess sodium borohydride was quenched by slowly adding water to the stirring solution. When no more hydrogen gas evolved, the reaction was partitioned with dichloromethane, and extracted (3 x). The

organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure to give a yellow oil. This oil was purified by flash chromatography (5:1 Hex:EtOAc) and concentrated *in vacuo* to afford **2.69** as a clear, yellow oil (2.94 g, 94%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 1.32-1.41 (m, 8H), 1.62 (quin, *J* = 7.6 Hz, 2H), 2.04 (dd, *J* =7.6 Hz, 6.8 Hz, 2H), 2.59 (t, *J* = 7.6 Hz, 2H), 4.93 (dt, *J* = 10.0 Hz, 1.2 Hz, 1H), 4.99 (ddd, *J* = 17.0 Hz, 4.0 Hz, 1.6 Hz, 1H), 5.76-5.86 (m, 1H), 5.91 (d, *J* = 0.8 Hz, 1H), 6.13 (dd, *J* = 5.6 Hz, 3.2 Hz, 1H), 6.66 (dd, *J* = 4.4 Hz, 2.8 Hz, 1H), 7.89 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 27.7, 28.9, 29.0, 29.2, 29.3, 29.6, 33.8, 104.8, 108.2, 114.1, 115.9, 132.8, 139.2. **IR** (KBr) υ_{max} 3391, 2927, 2855, 1640, 1569, 1465, 1438, 1095, 1025, 994, 910, 784, 712 cm^{-1}. **HRMS**: $C_{13}H_{22}N$, Calculated: [M+H]+, 192.1752, Found: [M+H]+, 192.1752.



2.84

4-(5-(non-8-enyl)-1*H*-pyrrol-2-yl)butan-2-one (2.84). 3-buten-2-one (100 mg, 1.44 mmol) was added to a flame-dried, 100 mL round bottom flask containing alkylpyrrole **2.69** (125 mg, 0.65 mmol) and dichloromethane (3.5 mL). Grubb's second-generation catalyst (28 mg, 0.033 mmol) was added in one portion at room temperature. The mixture was stirred for 12 hours at reflux, condensed *in vacuo*, and immediately purified by flash chromatography (3:1 Hex:EtOAc) affording **2.84** as a pale yellow oil (73 mg, 43%).[1] **^{1}HNMR** ($CDCl_3$, 400 MHz) δ (ppm): 1.28-1.39 (m, 8H), 1.55-1.62 (m, 2H), 2.04 (q, *J* = 6.8 Hz, 2H), 2.16 (s, 3H), 2.52 (t, *J* = 7.6 Hz, 2H), 2.76-2.83 (m, 4H), 4.03 (s, 3H), 4.93

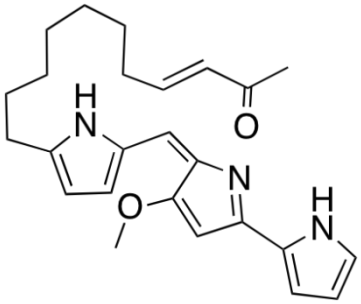
[1] A 4:2:1 mixture of **2.84**:**2.5**:**2.85** was formed by this reaction. The major isomer **2.84** and desired product **2.5** were isolated and fully characterized. The identity and ratio of **2.85** were determined by crude ^{1}HNMR of **2.84**:**2.85** mixture and LRMS.

(dt, *J* = 10.4 Hz, 1.2 Hz, 1H), 4.99 (ddd, *J* = 17.2 Hz, 3.6 Hz, 1.6 Hz 1H), 5.71-5.75 (m, 2H), 5.74-5.87 (m, 1H), 8.14 (s, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 21.4, 27.8, 28.9, 29.0, 29.2, 29.3, 29.6, 30.1, 33.8, 44.3, 104.2, 105.0, 114.1, 129.8, 132.0, 139.2, 209.7. **LRMS**: m/z = 262.2 [M+H]+.

2.5

(*E*)-11-(1*H*-pyrrol-2-yl)undec-3-en-2-one (2.5). 3-buten-2-one (1.21 g, 17.25 mmol) was added to a flame-dried, 100 mL round bottom flask containing alkylpyrrole **2.69** (1.5 g, 7.84 mmol) and dichloromethane (60 mL). Grubbs' second-generation catalyst (2 g, 2.35 mmol) was added in one portion at room temperature. The mixture was stirred for 6 hours, condensed *in vacuo*, and immediately purified by flash chromatography (3:1 Hex:EtOAc) affording **2.5** as a clear colorless oil (725 mg, 40%).

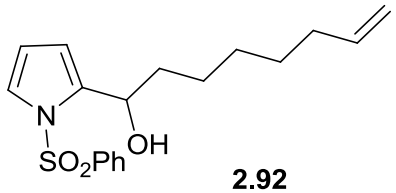
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.31-1.33 (m, 6H), 1.46 (quin, *J* =2.4 Hz, 2H), 1.62 (quin, *J* = 2.8 Hz, 2H), 2.22 (q, *J* = 7.2 Hz, 2H), 2.25 (s, 3H), 2.60 (t, *J* = 7.6 Hz, 2H), 5.91 (d, *J* = 0.8 Hz, 1H), 6.07 (dt, *J* = 16 Hz, 1.6 Hz, 1H), 6.13 (dd, *J* = 5.6 Hz, 2.8 Hz, 1H), 6.67 (dd, *J* = 4.0 Hz, 2.4 Hz, 1H), 6.80 (dt, *J* =16 Hz, 6.8 Hz, 1H), 7.95 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 26.8, 27.7, 28.0, 29.0, 29.1, 29.2, 29.6, 32.4, 104.9, 108.2, 116.0, 131.3, 132.7, 148.5, 198.8. **IR** (KBr) υ_{max} 3383, 2928, 2855, 1670, 1624, 1457, 1429, 1362, 1257, 1024, 977, 784, 713 cm^{-1}. **HRMS**: $C_{15}H_{24}NO$, Calculated: [M+H]+, 234.1858, Found: [M+H]+, 234.1856.



(*E*)-11-(5-((*E*)-(3-methoxy-5-(1*H*-pyrrol-2-yl)-2*H*-pyrrol-2-ylidene)methyl)-1*H*-pyrrol-2-yl)undec-3-en-2-one (2.6). Aldehyde **2.64** (627 mg, 2.16 mmol) was suspended in anhydrous methanol (21 mL) in a flame-dried, 100 mL round bottom flask and cooled to 16 ^{o}C in a tap water bath. Pyrrolylenone **2.5** (605 mg, 2.59 mmol) was suspended in anyhydrous methanol (26 mL) and added to the aldehyde solution, followed by 0. 87 M HCl in methanol (3.73 mL, 3.24 mmol). The reaction mixture immediately turned a deep red color, and LCMS showed that the reaction had reached completion in less than 15 minutes. Ammonium hydroxide (6 mL) was added and the reaction was stirred for 2 hours. 2 M HCl was added until the solution reached pH 7 and then the mixture was transferred to a separatory funnel, diluted with water, partitioned with dichloromethane, and extracted (3 x). The combined organic layers were then filtered through a phase separator to remove any excess water and condensed *in vacuo* to give a black residue. This residue was purified by reverse phase chromatography using acetonitrile and 0.1% TFA/water (gradient: 35:65 to 85:15) to afford **2.6** as a viscous, deep red oil (805 mg, 92%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.31-1.38 (m, 6H), 1.49 (quin, *J* = 6.8 Hz, 2H), 1.72 (quin, *J* = 7.2 Hz, 2H), 2.23 (q, *J* = 7.2 Hz, 2H), 2.25 (s, 3H), 2.81 (t, *J* = 7.8 Hz, 2H), 4.03 (s, 3H), 6.06 (d, *J* = 16 Hz, 1H), 6.13 (s, 1H), 6.22 (d, *J* = 3.6 Hz, 1H), 6.39 (t, *J* = 1.8 Hz, 1H), 6.79 (dt, *J* = 16 Hz, 7.0 Hz, 1H), 6.87 (s, 1H), 6.99 (s, 1H), 7.03 (s, 1H),

7.29 (s, 1H). **^{13}C NMR** (MeOD, 100 MHz) δ (ppm): 25.2, 27.6, 28.58, 28.61, 28.63, 28.7, 32.0, 58.6, 93.6, 111.8, 112.3, 113.8, 114.4, 116.6, 117.4, 121.7, 123.2, 126.3, 126.5, 127.6, 130.6, 149.6, 150.4, 167.7, 200.0. **IR** (KBr) υ_{max} 3250, 3127, 2929, 2856, 1674, 1630, 1604, 1549, 1517, 1457, 1411, 1374, 1288, 1254, 1201, 1184, 1138, 1043, 990, 960, 837, 800, 748, 720 cm^{-1}. **HRMS**: $C_{25}H_{32}N_3O_2$, Calculated: [M+H]+, 406.2495, Found: [M+H]+, 406.2494.

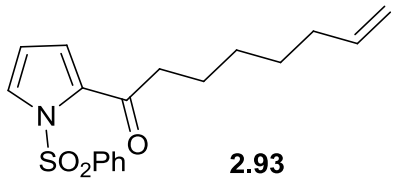


2.92

1-(1-(phenylsulfonyl)-1*H*-pyrrol-2-yl)oct-7-en-1-ol (2.92). Magnesium ribbon (0.88 g, 36.3 mmol) was cut into small pieces, placed in a 250 mL, 2-neck schlenk flask fit with a reflux condenser, and flame-dried under vaccum. A small crystal of iodine was added and the flask was flushed with argon. The magnesium was suspended in anhydrous THF (112 mL) and vigorously stirred. 7-bromo-1-heptene (5.00 g, 28.24 mmol) was added to the solution dropwise at room temperature. The resulting mixture was then heated to reflux (65 ^{o}C) for 2 hours. 1-(phenylsulfonyl)-2-pyrrolecarboxaldehyde (4.75 g, 20.17 mmol) was added to a 250 mL flame-dried, schlenk flask. Anhydrous THF (50 mL) was added and the mixture was stirred and cooled to 0 ^{o}C in an ice bath. After refluxing for 2 hours, the prepared Grignard reagent was cooled to room temperature and cannulated into the aldehyde solution over a 30 minute period. The resulting brown slurry was stirred at 0 ^{o}C for an additional 30 minutes and was then allowed to warm to room temperature. After 3 hours, 2 M HCl was slowly added to the vigorously stirred solution until it reached pH 7. The mixture was then transferred to a separatory funnel, diluted with water, partitioned

with dichloromethane, and extracted (4 x). The combined organic layers were combined, dried over sodium sulfate, and condensed under reduced pressure to give a brown residue. This residue was purified by flash chromatography (3:1 Hex:EtOAc) and dried *in vacuo* to afford alcohol **2.92** as a viscous, light orange oil (4.15 g, 62%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.20-1.36 (m, 6H), 1.74-1.83 (m, 2H), 1.99 (dd, *J* = 14.2 Hz, 6.8 Hz, 2H) 2.73 (d, *J* = 4.2 Hz, 1H), 4.80 (m, 1H), 4.92 (dt, *J* = 10.2 Hz, 1.0 Hz, 1H), 4.97 (ddd, *J* = 17.1 Hz, 3.4 Hz, 1.8 Hz, 1H), 5.72-5.83 (m, 1H), 6.26 (t, *J* = 3.4 Hz, 1H), 6.27 (dd, *J* = 5.0 Hz, 3.4 Hz, 1H), 7.30 (dd, *J* = 4.0 Hz, 1.8 Hz, 1H), 7.50 (t, *J* = 7.4 Hz, 2H), 7.60 (tt, *J* = 7.4 Hz, 1.0 Hz, 1H), 7.78 (d, *J* =7.4 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 25.8, 28.7, 33.6, 35.0, 65.1, 111.6, 112.3, 114.2, 123.4, 126.5, 129.4, 133.9, 138.3, 138.9, 139.3.

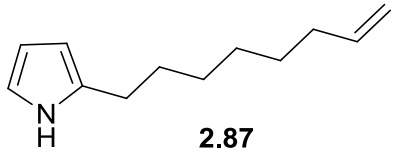


2.93

1-(1-(phenylsulfonyl)-1*H*-pyrrol-2-yl)oct-7-en-1-one (2.93). TPAP (0.42 g, 1.19 mmol) was added to a 100 mL flame-dried round bottom flask containing a stirred and cooled (0 ^oC) solution of alcohol **2.92** (3.95 g, 11.85 mmol), NMO (2.78 g, 23.69 mmol), and 4 A molecular sieves (6 g) in dichloromethane (60 mL). The reaction was warmed to room temperature and stirred for 4 hours. The reaction mixture was filtered to remove the molecular sieves, diluted with dichloromethane, and partitioned with water. The mixture was washed with 1 M HCl (1 x) and water (2 x). The organic layer was dried over sodium sulfate and condensed under reduced pressure to give a black oil. The oil was purified by flash chromatography (4:1 Hex:EtOAc) and dried *in vacuo* to afford **2.93** as a

clear, colorless oil (3.36 g, 86%).

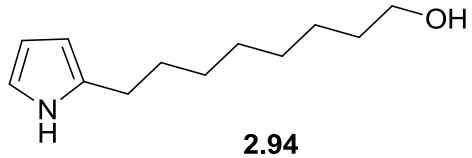
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.24-1.31 (m, 2H), 1.35 (quin, *J* = 7.2 Hz, 2H), 1.60 (quin, *J* = 7.4 Hz, 2H), 2.00 (q, *J* = 7.2 Hz, 2H), 2.66 (t, *J* = 7.6 Hz, 2H), 4.92 (dt, *J* = 10.0 Hz, 1.0 Hz, 1H), 4.97 (ddd, *J* = 7.1 Hz, 3.4 Hz, 1.6 Hz, 1H), 5.71-5.82 (m, 1H), 6.34 (t, *J* = 3.5 Hz, 1H), 7.03 (dd, *J* = 3.8 Hz, 1.7 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.59 (tt, *J* = 7.4 Hz, 1.2 Hz, 1H), 7.80 (dd, *J* = 3.2 Hz, 1.7 Hz, 1H), 7.99 (d, *J* =7.3 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 24.6, 28.50, 28.53, 33.4, 39.3, 110.3, 114.3, 123.2, 128.0, 128.6, 130.0, 133.4, 133.5, 138.8, 139.0, 188.9.



2.87

2-(oct-7-enyl)-1*H*-pyrrole (2.87). Sodium borohydride (2.95 g, 44.50 mmol) was added to a flame-dried, 250 mL round bottom flask fit with a reflux condenser. The flask was purged with argon and anhydrous 2-propanol (135 mL) was added. Acylpyrrole **2.23** (2.96 g, 8.90 mmol) was suspended in 40 mL of anhydrous 2-propanol, and this solution was added dropwise to the sodium borohydride solution at room temperature. After addition was complete, the mixture was heated to reflux (82 ^{o}C) for 24 hours. The reaction was cooled to room temperature and the excess sodium borohydride was quenched by slowly adding water to the stirring solution. When no more hydrogen gas evolved, the reaction was transferred to a separatory funnel, partitioned with dichloromethane, and extracted (3 x). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure to give a yellow oil. This oil was purified by flash chromatography (5:1 Hex:EtOAc) and concentrated *in vacuo* to afford **2.24** as a clear, yellow oil (1.48 g, 94%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.33-1.42 (m, 6H), 1.64 (quin, *J* = 7.6 Hz, 2H), 2.05 (q, *J* = 7.0 Hz, 2H), 2.60 (t, *J* = 7.6 Hz, 2H), 4.94 (dt, *J* = 10.2 Hz, 1.0 Hz, 1H), 5.00 (ddd, *J* = 17.1 Hz, 3.5 Hz, 1.6 Hz, 1H), 5.76-5.87 (m, 1H), 5.92 (d, *J* = 0.6 Hz, 1H), 6.14 (dd, *J* = 5.7 Hz, 2.8 Hz, 1H), 6.67 (dd, *J* = 4.1 Hz, 2.6 Hz, 1H), 7.90 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 27.6, 28.7, 28.8, 29.1, 29.5, 33.7, 104.8, 108.2, 114.2, 115.9, 132.7, 139.0.

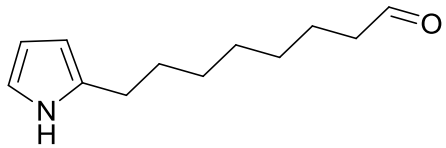


2.94

8-(1*H*-pyrrol-2-yl)octan-1-ol (2.94). To a 100 mL, flame-dried round bottom flask, was added alkylpyrrole **2.87** (600 mg, 3.39 mmol) and 34 mL of anhydrous THF. The solution was cooled to 0 ^oC in an ice bath and a solution of 9-BBN (17.6 mL, 0.5M in THF) was slowly added. After 1 hour, the ice bath was removed and the mixture stirred overnight. After 24 hours, the reaction mixture was cooled to 0^oC, and 3 M NaOH (5.5 mL) was added dropwise, followed by dropwise addition of hydrogen peroxide (5.5 mL). The reaction was slowly warmed to room temperature after 1 hour and stirred 24 hours. The reaction was transferred to a separatory funnel and slowly diluted with water. The solution was partitioned with dichloromethane, extracted (5 x), dried over sodium sulfate, and condensed under reduced pressure to give a brown oil. This oil was purified by flash chromatography (6:1 Hex:EtOAc) and condensed *in vacuo* to afford **2.94** as a pale yellow oil (635 mg, 96%).

^{1}HNMR ($CDCl_3$, 400 MHz) δ (ppm): 1.28-1.39 (m, 10H), 1.53-1.64 (m, 4H), 2.59 (t, *J* =7.6 Hz, 2H), 3.64 (t, *J* =6.6 Hz, 2H), 5.91 (d, *J* =0.6 Hz, 1H), 6.13 (dd, *J* = 5.7 Hz, 2.8

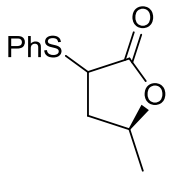
Hz, 1H), 6.66 (dd, *J* = 4.1 Hz, 2.6 Hz), 7.95 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 25.6, 27.6, 29.18, 29.22, 29.3, 29.5, 32.7, 63.0, 104.8, 108.1, 115.9, 132.7.



2.86

8-(1*H*-pyrrol-2-yl)octanal (2.86). TPAP (113 mg, 0.32 mmol) was added to a 50 mL flame-dried round bottom flask containing a stirred and cooled (0 ^{o}C) solution of alcohol **2.94** (625 mg, 3.20 mmol), NMO (750 mg, 6.40 mmol), and 4 A molecular sieves (3 g) in dichloromethane (25 mL). The reaction was warmed to room temperature and stirred for 6 hours. The reaction mixture was filtered through a silica/celite plug that was liberally rinsed with dichloromethane. The filtrate was condensed under reduced pressure to give **2.86** as a pale yellow liquid (580 mg, 88%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.24-1.29 (m, 6H), 1.49-1.52 (m, 4H), 1.80-1.86 (m, 2H), 2.39 (td, *J* = 3.2 Hz, 1.8 Hz, 2H), 2.56 (t, *J* = 8.0 Hz, 2H), 5.87 (s, 1H), 6.08 (dd, *J* = 5.6 Hz, 2.8 Hz, 1H), 6.63 (d, *J* = 1.6 Hz, 1H), 8.07 (bs, 1H), 9.73 (t, *J* = 2.0 Hz, 1H).

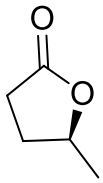


2.134

(5*S*)-5-methyl-3-(phenylthio)dihydrofuran-2(3H)-one (2.134). To a solution of diisopropylamine (21. 8 g, 215.3 mmol) in 145 mL of THF at -78 ^{o}C was added 160 mL (258.3 mmol) of a 1.6M solution of *n*-BuLi in hexanes. After stirring at -78 ^{o}C for 15 min, (phenylthio)acetic acid (14.5 g, 86.1 mmol) in 43 mL of THF was added and the mixture was stirred for an additional 15 min. To the resulting solution was added (*S*)-(-)-

propylene oxide (5 g, 86.1 mmol) in one portion. The mixture was allowed to warm to room temperature over a period of 3 h and was then stirred for 16 h. The reaction was quenched with 2M NaOH (100 mL0 and was extracted with Et_2O (2 x). The organic phase was discarded and the aqueous phase acidified to pH = 3 with 2M HCl. The acidic solution was extracted with Et_2O (3 x) and the organic phase washed with brine (1 x) and dried with $MgSO_4$. Evaporation of the solvent gave a clear oil (18.11 g, 93%) which was carried on to the next step without further purification. The oil was dissolved in benzene (80mL) and a catalytic amount of *p*-toluenesulfonic acid (0.76 g, 3.98 mmol) was added at room temperature. The solution was stirred overnight, the solvent evaporated, and the crude product chromatographed (100% CH_2Cl_2) to afford **2.134** (12.6 g, 76%) as a 2.8:1 mixture of stereoisomers.

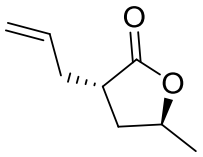
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.34 (d, *J* = 6.4 Hz, 2.2H), 1.38 (d, *J* = 6.0 Hz, 0.8H), 1.85 (ddd, *J* = 13.2 Hz, 10.8 Hz, 10.4 Hz, 0.75H), 2.26 (dt, *J* = 12.4 Hz, 8.4Hz, 0.25H), 2.38 (ddd, *J* = 13.6 Hz, 6.2 Hz, 4.0 Hz, 0.25H), 2.74 (ddd, *J* = 13.2 Hz, 9.0 Hz, 6.4 Hz, 0.75H), 3.91 (dd, *J* = 8.4 Hz, 3.6 Hz, 0.25H), 3.97 (dd, *J* = 10.8 Hz, 8.8 Hz, 0.75H), 4.48-4.60 (m, 1H), 7.31-7.36 (m, 3H), 7.53-7.57 (m, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 174.7, 174.5, 133.4, 133.2, 132.3, 131.9, 129.2, 129.1, 128.6, 128.4, 75.4, 74.8, 46.5, 45.4, 37.5, 37.3, 21.0, 20.8.



2.129

(*S*)-5-methyldihydrofuran-2(3H)-one (2.129). A solution of **2.134** (6.0 g, 28.81 mmol) in 30 mL of THF was added to a suspension of Raney nickel (30 g) in 230 mL of THF. The flask was purged with Ar (1 x), with H_2 (3 x), and then kept under an atmosphere of H_2. The mixture was vigorously stirred for 16 h at room temperature. The nickel was filtered off, the filtrate evaporated, and the crude product chromatographed (1:4 Et_2O/CH_2Cl_2) to give 2.48 g (86%) of **2.129** as a clear, colorless liquid.

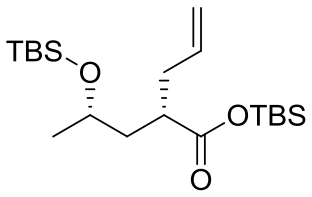
$[\alpha]^{23}_D$ = -33.0° (*c* = 10.0, CH_2Cl_2) **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 1.40 (d, *J* = 6.4 Hz, 3H), 1.77-1.87 (m, 1H), 2.31-2.39 (m, 1H), 2.52-2.56 (m, 2H), 4.59-4.67 (m, 1H).



2.135

(3*S*,5*S*)-3-allyl-5-methyldihydrofuran-2(3H)-one (2.135). LiHMDS (1.0M in THF, 7.83 mmol) was added dropwise to a 0.15 M solution of **2.129** (0.8 g, 7.99 mmol) in THF at -78 °C. The mixture was stirred for 30 min, and then allyl iodide (1.48 g, 8.79 mmol) was added dropwise. The solution was stirred for 1.5 h while maintaining the temperature at -78 °C. The reaction was quenched by addition of saturated NH_4Cl and allowed to warm to room temperature. The mixture was extracted with Et_2O (3 x), dried over $MgSO_4$, the solvent evaporated, and the crude product chromatographed (4:1 Hex:EtOAc) to give 895mg (80%) of **2.135** as a 6:1 mixture of diastereomers.

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.36 (d, *J* = 6.4 Hz, 2.6H) , 1.41 (d, *J* = 6.4 Hz, 0.4H), 1.49-1.57 (m, 0.14H), 1.98 (ddd, *J* = 17.6 Hz, 0.86H), 2.13 (dt, *J* = 13.2 Hz, 7.2 Hz, 0.86H), 2.197-2.30 (m, 1H), 2.40-2.47 (m, 0.14H), 2.52-2.59 (m, 0.84H), 2.60-2.66 (m, 0.14H), 2.71-2.78 (m, 0.84H), 4.44-4.53 (m, 0.14H), 4.64 (sex, *J* = 6.4 Hz, 0.86H), 5.1-5.14 (m, 2H), 5.72-5.82 (m, 1H).

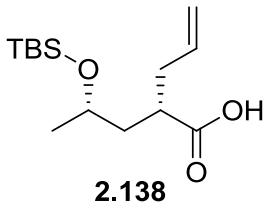


2.137

(*S*)-*tert*-butyldimethylsilyl 2-((*S*)-2-((tert-butyldimethylsilyl)oxy)propyl) pent-4-enoate (2.137). Allyl lactone **2.135** (2.02 g, 14.41 mmol) was suspended in THF (84 mL) and cooled to 0 ^oC. A solution of LiOH (0.4 g, 16.7 mmol) in H_2O (12 mL) is added dropwise. When addition was complete, the resulting solution was stirred for 10 h at 0^oC. The mixture was diluted with water (20 mL) and Et_2O (30 mL) and the layers separated. The organic layer was discarded and the aqueous layer was carefully acidified (pH = 3) with 1M HCl. The aqueous layer was then extracted with ethyl acetate (5 x), dried over $MgSO_4$, the solvent evaporated, and the crude product (**2.136**, 2.24 g, 98%) carried on to the next step without further purification. Crude **2.136** (2.24 g, 14.15 mmol) was resuspended in CH_2Cl_2 (58 mL) and the solution was cooled to 0^oC. Imidazole (4.33 g, 63.68 mmol) was added in one portion, followed by *tert*-butyldimethylsilylchloride (6.40g, 42.45 mmol). The resulting cloudy, white solution was stirred 16 h at room temperature. The reaction was quenched by addition of H_2O (20 mL), and the solution was extracted with CH_2Cl_2 (3 x). The organic layer was dried over $MgSO_4$, the solvent evaporated, and the crude product chromatographed (6:1 Hex:EtOAc) to give **2.137**

(5.45g, 100%).

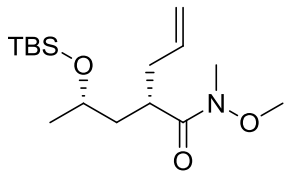
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.04 (s, 6H), 0.26 (s, 6H), 0.88 (s, 9H), 0.94 (s, 9H), 1.13 (d, *J* = 6.0 Hz, 2.6H), 1.15 (d, *J* = 6.0 Hz, 0.4H), 1.41-1.48 (m, 0.86H), 1.49-1.54 (m, 0.14H), 1.70-1.77 (m, 0.86H), 1.80-1.87 (m, 0.14H), 2.24-2.37 (m, 2H), 2.52-2.56 (m, 0.14H), 2.63-2.70 (m, 0.86H), 3.78-3.86 (m, 1H), 5.01-5.08 (m, 2H), 5.69-5.79 (m, 1H).



2.138

(*S*)-2-((*S*)-2-((tert-butyldimethylsilyl)oxy)propyl)pent-4-enoic acid (2.138). Silylester **2.137** (1.00 g, 2.59 mmol) was suspended in a solution of MeOH (30 mL), THF (10 mL), and H_2O (10 mL). K_2CO_3 (1.07 g, 7.76 mmol), was added in one portion at room temperature, and the reaction stirred for 1 h. The mixture was diluted with Et_2O (30 mL), the layers were separated and the organic layer discarded. The aqueous layer was carefully acidified (pH = 3) and extracted with EtOAc (5 x). The organic layer was dried with $MgSO_4$, the solvent evaporated, and the crude product dried *in vacuo* to give **2.138** (655 mg, 93%).

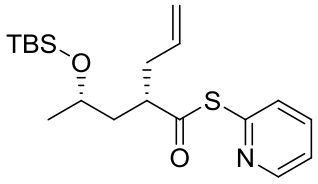
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.88 (s, 9H), 1.14 (d, *J* = 6.4 Hz, 2.6H), 1.16 (d, *J* = 6.4 Hz, 0.4H), 1.50-1.59 (m, 1H), 1.76-1.83 (m, 0.86H), 1.83-1.91 (m, 0.14H), 2.22-2.31 (m, 1H), 2.36-2.44 (m, 1H), 2.54-2.58 (m, 0.14H), 2.67-2.74 (m, 0.86H), 3.87-3.94 (m, 1H), 5.04-5.11 (m, 2H), 5.71-5.81 (m, 1H).



2.139

(*S*)-2-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)propyl)-*N*-methoxy-*N*-methylpent-4-enamide (2.139). Acid **2.138** was suspsended in CH_2Cl_2 (18 mL) and DCC (380 mg, 1.84 mmol) was added at room temperature, followed by DMAP (22.4 mg, 0.184 mmol). The solution was stirred for 15 min, then N,O-dimethylhydroxylamine HCl (179 mg, 1.84 mmol) and triethylamine (186 mg, 1.84 mmol) were added, and the resulting mixture stirred for 4 h at room temperature. The mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (3 x). The organic layer was dried over $MgSO_4$, the solvent removed, and the crude product purified (4:1 Hex:EtOAc) to give **2.139** (498 mg, 86%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.02 (s, 3H), 0.04 (s, 3H), 0.88 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H), 1.49-1.55 (m, 2H), 1.83-1.90 (m, 1H), 2.15-2.25 (m, 1H), 2.32-2.39 (m, 1H), 3.18 (s, 3H), 3.70 (s, 3H), 3.77-3.83 (m, 1H), 4.98-5.07 (m, 2H), 5.70-5.80 (m, 1H).

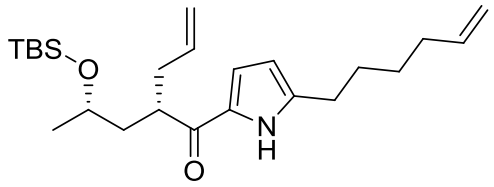


2.141

(*S*)-S-pyridin-2-yl 2-((*S*)-2-((tert-butyldimethylsilyl)oxy)propyl)pent-4-enethioate (2.141). To a solution of acid **2.138** (376 mg, 1.38 mmol) in toluene (7 mL), was added PPh_3 (435 mg, 1.66 mmol) and 2,2'-bisdipyridyldisulfide (365 mg, 1.66 mmol) at room temperature. The solution was stirred for 3 h and then diluted with water (5mL), extracted with EtOAc (3 x), and dried over $MgSO_4$. The solvent was evaporated and the crude

product purified (2:1 Hex:EtOAc) to give **2.141** (465 mg, 92%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.07 (s, 3H), 0.11 (s, 3H), 0.90 (s, 9H), 1.15 (d, *J* = 6.0 Hz, 3H). 1.54-1.61 (m, 1H), 1.87-1.98 (m, 1H), 2.30-2.37 (m, 1H), 2.49-2.56 (m, 1H), 2.98-3.05 (m, 1H), 3.87-3.94 (m, 1H), 5.07-5.14 (m, 2H), 5.73-5.84 (m, 1H), 7.25-7.28 (m, 1H), 7.61 (d, *J* =8.0 Hz, 1H), 7.70-7.75 (td, *J* = 8.0 Hz, 2.0 Hz, 1H), 8.60-8.62 (m, 1H).

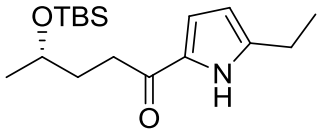


2.140

(*S*)-2-((*S*)-2-((tert-butyldimethylsilyl)oxy)propyl)-1-(5-(hex-5-en-1-yl)-1H-pyrrol-2-yl)pent-4-en-1-one (2.140). MeMgCl (3.0M in THF, 2.11 mmol) was added to a solution of alkylpyrrole **2.108** (336 mg, 2.25 mmol) in toluene (11mL) at -45 ^oC. The solution was stirred for 0.5 h and then cannulated into a solution of thioester **2.141** (350 mg, 0.957 mmol) in toluene (9.5mL) at -78 ^oC. The resulting mixture was stirred at -78 ^oC for 0.5 h and was then allowed to slowly warm to room temperature and stirred for 1 h. The reaction was quenched with saturated NH_4Cl, extracted with EtOAc (3 x), and dried over $MgSO_4$. The solvent was evaporated and the crude product purified (9:1 Hex:EtOAc) to give **2.140** (328 mg, 85%).

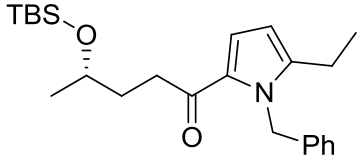
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): -0.11 (s, 3H), -0.03 (s, 3H), 0.84 (s, 9H), 1.12 (d, *J* = 6.0 Hz, 3H), 1.44 (quin, *J* = 7.6 Hz, 2H), 1.52-1.58 (m, 1H), 1.66 (quin, *J* =7.6 Hz, 2H), 1.89-1.99 (m, 1H), 2.08 (q, *J* = 7.2 Hz, 2H), 2.20-2.27 (m, 1H), 2.40-2.47 (m, 1H), 2.62 (t, *J* = 7.6 Hz, 2H), 3.28-3.34 (m, 1H), 3.75-3.79 (m, 1H), 4.93-5.04 (m, 4H), 5.69-5.83

(m, 2H), 5.98 (t, *J* = 3.2 Hz, 1H), 6.83 (t, *J* = 2.8 Hz, 1H), 9.04 (bs, 1H).



2.146

(*S*)-4-((tert-butyldimethylsilyl)oxy)-1-(5-ethyl-1H-pyrrol-2-yl)pentan-1-one (2.146). To a solution of acid **2.137** (363 mg, 1.56 mmol) in toluene (4 mL), was added PPh_3 (449 mg, 1.71 mmol) and 2,2'-bisdipyridyldisulfide (377 mg, 1.71 mmol) at room temperature. The solution was stirred for 3 h. MeMgCl (3.0M in THF, 1.79 mmol) was added to a solution of 2-ethylpyrrole (192 mg, 2.02 mmol) in toluene (7 mL) at -45^{o}C. The solution was stirred for 0.5 h and then cannulated into the of solution acid **2.137** at -78 ^{o}C. The resulting mixture was stirred at -78 ^{o}C for 0.5 h and was then allowed to slowly warm to room temperature and stirred for 1 h. The reaction was quenched with saturated NH_4Cl, extracted with EtOAc (3 x), and dried over $MgSO_4$. The solvent was evaporated and the crude product purified (9:1 Hex:EtOAc) to give **2.146** (425 mg, 88%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 1.16 (d, *J* = 6.0 Hz, 3H), 1.26 (t, *J* = 7.6 Hz, 3H), 1.72-1.89 (m, 2H), 2.66 (q, *J* =6.8 Hz, 2H), 2.68-2.75 (m, 1H), 2.81-2.89 (m, 1H), 3.86-3.93 (m, 1H), 6.00 (t, *J* = 3.6 Hz, 1H), 6.82 (dd, *J* = 3.6 Hz, 2.4 Hz, 1H), 9.05 (bs, 1H).

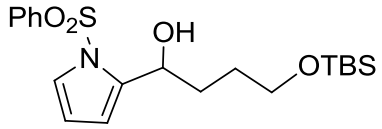


2.147

(*S*)-1-(1-benzyl-5-ethyl-1H-pyrrol-2-yl)-4-((*tert*-butyldimethylsilyl)oxy)pentan-1-one (2.147). NaH (60% in mineral oil, 2.05 mmol) was suspended in DMF (14 mL) and

acylpyrrole **2.146** (425 mg, 1.37 mmol) was added at room temperature. The mixture was stirred for 0.5 h, then benzylbromide (351 mg, 2.05 mmol) was added and the resulting solution stirred for 2 h. The reaction was quenched with H_2O, extracted with CH_2Cl_2 (3 x), washed with 5% LiCl, dried over $MgSO_4$, and the solvent evaporated. The crude product was purified to give **2.147** (520 mg, 95%).

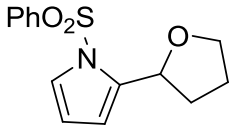
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.03 (s, 3H) 0.04 (s, 3H), 0.90 (s, 9H), 1.13 (d, *J* = 6.0 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), 1.65-1.83 (m, 2H), 2. 51 (q, *J* = 7.6 Hz, 2H), 2.72-2.79 (m, 1H), 2.86-2.94 (m, 1H), 3.80-3.88 (m, 1H), 5.68 (d, *J* = 3.6 Hz, 2H), 6.05 (d, *J* = 4.0 Hz, 1H), 7.05 (d, *J* = 4.0 Hz, 1H), 7.18-7.27 (m, 5H).



2.155

4-((*tert*-butyldimethylsilyl)oxy)-1-(1-(phenylsulfonyl)-1*H*-pyrrol-2-yl)butan-1-ol (2.155). Diol **2.154** (400 mg, 1.35 mmol) was suspended in CH_2Cl_2 (14 mL) and cooled to 0 ^oC. Imidazole (138 mg, 2.03 mmol) was added followed by *tert*-butyldimethylsilylchloride (224 mg, 1.49 mmol) and the resulting cloudy solution was stirred for 4 h at room temperature. The reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 x). The organic extract was dried over $MgSO_4$, the solvent evaporated, and the crude product purified (3:1 Hex:EtOAc) to give **2.155** (494 mg, 89%).

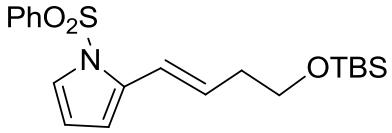
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.04 (s, 3H), 0.05 (s, 3H), 0.89 (s, 9H), 1.52-1.66 (m, 2H), 1.81-1.97 (m, 2H), 3.34 (bs, 1H), 3.62 (t, *J* = 6.0 Hz, 2H), 4.87-4.90 (m, 1H), 6.26 (t, *J* =3.2 Hz, 1H), 6.30-6.32 (m, 1H), 7.29 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.59 (t, *J* =7.6 Hz, 1H), 7.78 (d, *J* =8.0 Hz, 2H).



2.156

1-(phenylsulfonyl)-2-(tetrahydrofuran-2-yl)-1H-pyrrole (2.156).

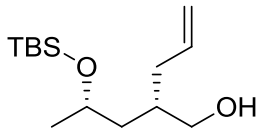
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.90 (m, 3H), 2.24-2.29 (m, 1H), 3.75-3.81 (m, 1H), 3.87-3.92 (m, 1H), 5.25-5.28 (m, 1H), 6.22-6.25 (m, 2H), 7.26-7.28 (m, 1H), 7.47 (t, *J* = 7.2 Hz, 2H), 7.57 (t. *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H).



2.161

(*E*)-2-(4-((*tert*-butyldimethylsilyl)oxy)but-1-en-1-yl)-1-(phenylsulfonyl)-1*H*-pyrrole (2.161).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.89 (s, 9H), 2.38 (q, *J* = 7.0 Hz, 2H), 3.66 (t, *J* =6.4 Hz, 2H), 5.85-5.97 (m, 1H), 6.20-6.27 (m, 1H), 6.79 (d, *J* = 15.6 Hz, 1H), 7.26-7.29 (m, 2H), 7.45-7.50 (m, 2H), 7.45-7.60 (m, 1H), 7.76-7.81 (m, 2H).

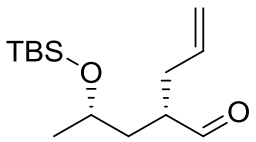


2.180

(*S*)-2-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)propyl)pent-4-en-1-ol (2.180). Silylester **2.137** (18.8 g, 48.72 mol) was suspended in CH_2Cl_2 (200 mL), cooled to -78 ^oC, and DIBAL-H (1.0M in CH_2Cl_2) was added dropwise. After addition was complete, the mixture was stirred for 1.5 h at -78 ^oC. MeOH (5 mL) was added very slowly at -78 ^oC

until H_2 evolution had ceased. The mixture was then warmed to room temperature and a saturated solution of Rochelle's salt (150 mL) was added with vigorous stirring. After 2 h, the solution had become clear, so was extracted with CH_2Cl_2 (5 x), dried over $MgSO_4$, and the solvent was evaporated to give crude product which was purified (2:1 Hex:EtOAc) to give **2.180** (11.65 g, 93%).

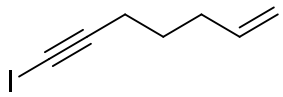
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.08 (s, 3H), 0.09 (s, 3H), 0.91 (s, 9H), 1.18 (d, *J* = 4.4 Hz, 3H), 1.43 (m, 1H), 1.60 (m, 1H), 1.88 (m, 1H), 1.96 (m, 1H), 2.08 (m, 1H), 3.41 (dd, *J* = 11.0 Hz, 7.2 Hz), 3.55-3.60 (m, 1H), 4.04-4.09 (m, 1H), 5.00-5.07 (m, 2H), 5.72-5.82 (m, 1H).



2.174

(*S*)-2-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)propyl)pent-4-enal (2.174). Alcohol **2.180** (2.00 g, 7.74 mmol) was suspended in DMSO (10 mL) and CH_2Cl_2 (30 mL) and cooled to 0 ^oC. Triethylamine (7.83 g, 77.37 mmol) was added, followed by sulfur trioxide pyridine complex (4.93 g, 30.95 mmol) and the mixture was stirred for 6 h at 0 ^oC. The solution was diluted with H_2O (20 mL), extracted with CH_2Cl_2 (3 x), and washed with 5% LiCl solution (1 x). The organic layer was dried over $MgSO_4$, the solvent was evaporated, and the crude product purified (4:1 Hex:EtOAc) to give **2.174** (1.60 g, 81%).

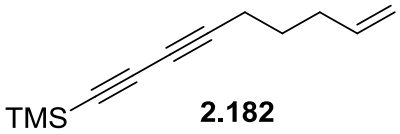
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 3H), 0.07 (s, 3H), 0.90 (s, 9H), 1.17 (d, *J* = 6.0 Hz, 3H), 1.55-1.62 (m, 1H), 1.81-1.87 (m, 1H), 2.19-2.24 (m, 1H), 2.44-2.49 (m, 1H), 2.59-2.63 (m, 1H), 3.92-3.96 (m, 1H), 5.07-5.12 (m, 2H), 5.70-5.79 (m, 1H), 9.66 (d, *J* = 2.4 Hz, 1H).



2.176

7-iodohept-1-en-6-yne (2.176). 6-bromo-1-hexene (15.0 g, 92.0 mmol) was added dropwise to a stirred slurry of lithium acetylide –ethylene-diamine complex (14.1 g, 138 mmol) in DMSO (40 mL) cooled to 0^{o}C. When addition was complete, the mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with H_2O, extracted with Et_2O (3 x), washed with 5% LiCl (2 x), and dried over $MgSO_4$. The solvent was carefully removed under reduced pressure and the crude product **2.181** carried on to the next step without further purification. Crude **2.181** was suspended in THF (115 mL), cooled to -78 ^{o}C, and *n*-BuLi (2.5M in hexanes, 87.9 mmol) was added dropwise. After addition was complete, the mixture was stirred for 0.5 h. To the stirred, cooled solution was added iodine (23.0 g, 90.4 mmol) in THF (115 mL). After addition was complete, the resulting solution was allowed to slowly warm to room temperature and stir for 1 h. The reaction was quenched with a saturated NH_4Cl solution and extracted with Et_2O (3 x). The organic layer was dried over $MgSO_4$, the solvent evaporated, and the crude product purified (silica, hexanes) to yield 12.7 g of **2.176** (59% over 2 steps).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.48-1.54 (m, 4H), 2.06 (q, *J* = 7.2 Hz, 2H), 2.37 (t, *J* = 6.8 Hz, 2H), 4.94-5.04 (m, 2H), 5.75-5.85 (m, 1H).

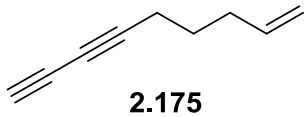


2.182

Trimethyl(nona-8-en-1,3-diyn-1-yl)silane (2.182). Alkynyl iodide **2.176** (2.0 g, 8.54 mmol) was suspended in piperidine (13 mL) and cooled to 0 ^{o}C. Ethynyltrimethylsilane (2.18 g, 22.2 mmol) was added, followed by cuprous iodide (146 mg, 0.769 mmol). The

solution was stirred at 0 °C for 2 h. The reaction was then quenched with saturated NH_4Cl and extracted with CH_2Cl_2 (3 x). The extract was washed with brine (2 x), dried over $MgSO_4$, and the solvent evaporated. The crude product was purified (silica, hexanes) to give **2.182** (1.55 g, 89%).

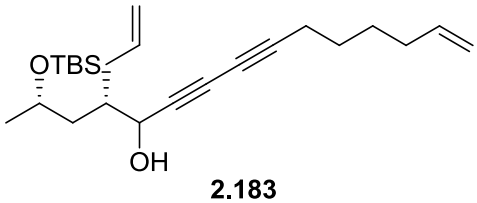
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.18 (s, 9H), 1.47-1.59 (m, 4H), 2.06 (q, *J* = 7.2 Hz, 2H), 2.28 (t, *J* = 6.8 Hz, 2H), 4.94-5.03 (m, 2H), 5.73-5.84 (m, 1H).



2.175

Nona-8-en-1,3-diyne (2.175). K_2CO_3 (10.0 g, 72.61 mmol) was added to a solution of TMS-diyne **2.182** (3.71 g, 18.15 mmol) in THF/MeOH (23 mL/23 mL) at 0 °C, and the mixture was stirred for 3 h. The reaction was diluted with H_2O (25 mL) and extracted with Et_2O (3 x). The organic extract was dried over $MgSO_4$, the solvent evaporated, and the crude product purified (silica, hexanes) to give **2.175** (2.28 g, 95%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.46-1.60 (m, 4H), 1.96 (t, *J* =1.2 Hz, 1H), 2.04-2.09 (m, 2H), 2.27 (td, *J* = 6.8 Hz, 1.2 Hz, 2H), 4.95-5.04 (m, 2H), 5.74-5.84 (m, 1H).

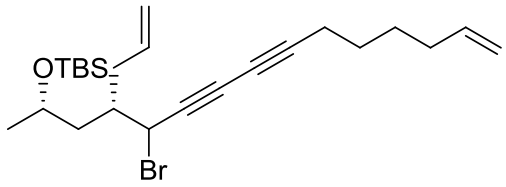


2.183

(4*S*,5*S*)-4-((*S*)-2-((tert-butyldimethylsilyl)oxy)propyl)pentadeca-1,14-dien-6,8-diyn-5-ol (2.183). Diyne **2.175** 1.33 g, 10.06 mmol) was suspended in THF (50 mL), cooled to 0 °C, and treated with EtMgBr (1.0M in THF, 9.93 mmol). The mixture was stirred for 0.5 h, and then a solution of aldehyde **2.174** (1.72 g, 6.71 mmol) was added dropwise at 0 °C. After 1 h, the reaction was quenched with saturated NH_4Cl, extracted with Et_2O (3 x),

and dried over $MgSO_4$. The solvent was evaporated and the crude product purified (silica, 19:1 Hex:EtOAc) to give **2.183** (2.22g, 85%) as a 1:1 mixture of diastereomers (*cis, cis, trans* : *cis, cis, cis*).

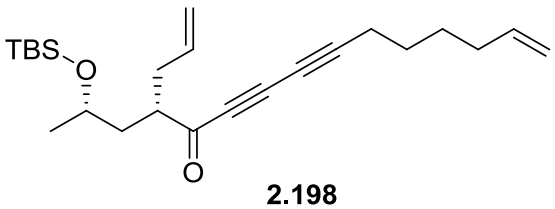
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.07 (s, 3H), 0.12 (s, 1.5H), 0.15 (s, 1.5H), 0.89 (s, 4.5H), 0.93 (s, 4.5H), 1.15 (d, *J* = 6.0 Hz, 1.5H), 1.18 (d, *J* = 6.8 Hz, 1.5H), 1.46-1.57 (m, 6H), 1.89-1.99 (m, 1.5H), 2.06 (q, *J* = 7.2 Hz, 2H), 2.11-2.16 (m, 1H), 2.28 (t, *J* = 6.8 Hz, 2H), 2.41-2.44 (m , 0.5H), 3.96-4.01 (m, 0.5H), 4.09-4.16 (m, 0.5H), 4.36-4.44 (m, 1H), 4.94-5.12 (m, 4H), 5.67-5.80 (m, 2H).



2.173

(((2*S*,4*S*)-4-allyl-5-bromopentadeca-14-en-6,8-diyn-2-yl)oxy)(tert-butyl)dimethyl silane (2.173). To a solution of alcohol **2.183** (1.50 g, 3.86 mmol) in CH_2Cl_2 (40 mL) was added CBr_4 (2.56 g, 7.72 mmol) followed by pyridine (1.22 g, 15.44 mmol). PPh_3 (2.03 g, 7.72 mmol) was then added and the solution was stirred at room temperature for 1 h. The reaction was quenched with sat. $NaHCO_3$, extracted with CH_2Cl_2 (3 x), dried over $MgSO_4$, condensed *in vacuo*, and purified by flash chromatography (19:1 Hex: EtOAc) to give **2.173** (1.43 g, 82%) as a clear, colorless, viscous oil.

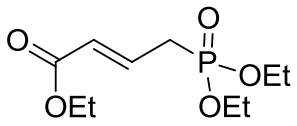
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.07 (s, 6H), 0.89 (s, 9H), 1.15 (d, *J* = 6.0 Hz, 3H), 1.48-1.57 (m, 5H), 1.58-1.66 (m, 1H), 1.72-1.81 (m, 1H) 2.05-2.09 (m, 2H), 2.15-2.27 (m, 1H), 2.96-2.33 (m, 2H), 3.39-2.46 (m, 1H), 3.88-3.97 (m, 1H), 4.84 (dd, *J* =14.6 Hz, 3.2 Hz, 1H), 4.95-5.15 (m, 4H), 5.66-5.84 (m, 2H).



2.198

(*S*)-4-((*S*)-2-((*tert*-butyldimethylsilyl)oxy)propyl)pentadeca-1,14-dien-6,8-diyn-5-one (2.198). Propargyl alcohol **2.183** (1.13 g, 2.92 mmol) was suspended in CH_2Cl_2 (20 mL) with 4 angstrom molecular sieves (3.0 g) and cooled to 0°C. NMO (683 mg, 5.83 mmol) was added in one portion, followed by TPAP (103 mg, 0.292 mmol). The mixture was warmed to room temperature and stirred for 1.5 h. The reaction was filtered, the solvent evaporated, and the resulting black residue immediately purified (19:1 Hex:EtOAc) to give pure **2.198** (938 mg, 83%).

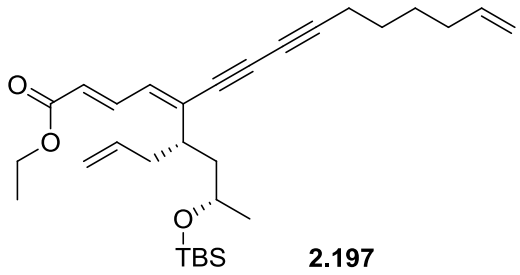
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.03 (s, 3H), 0.04 (s, 3H), 0.88 (s, 9H), 1.13 (d, *J* = 6.0 Hz, 3H), 1.48-1.64 (m, 5H), 1.86-1.93 (m, 1H), 2.08 (q, *J* = 7.2 Hz, 2H), 2.26 (quin, *J* =7.2 Hz, 1H), 2.38 (t, *J* = 7.2 Hz, 2H), 2.46 (quin, *J* = 7.2 Hz, 1H), 2.82-2.89 (m, 1H), 3.78-3.85 (m, 1H), 4.96-5.10 (m, 4H), 5.64-5.74 (m, 1H), 5.74-5.84 (m, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -4.8, -4.3, 18.0, 19.5, 24.2, 25.9, 27.2, 27.9, 33.1, 36.3, 40.0, 50.2, 64.1, 66.4, 72.0, 90.2, 114.9, 117.4, 134.7, 138.1, 190.0.



2.199

(*E*)-ethyl 4-(diethoxyphosphoryl)but-2-enoate (2.199). Triethylphosphite (3.13 g, 18.84 mmol) was added to ethyl-4-bromocrotonate (80%) (5.0 g, 20.72 mmol) at room temperature. The solution was stirred at 85 °C for 24 h, cooled to room temperature, and purified (1:2 Hex:EtOAc) to give **2.199** (5.40 g, 92%).

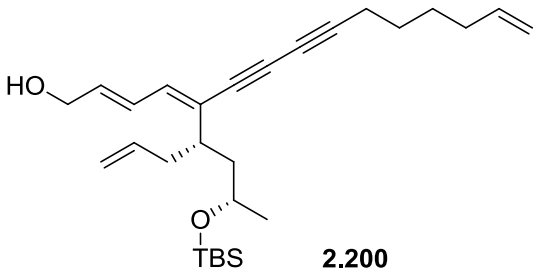
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.27 (t, *J* = 6.8 Hz, 3H), 1.31 (t, *J* = 7.2 Hz, 6H), 2.72 (ddd, *J* = 24.0 Hz, 8.0 Hz, 1.2 Hz, 2H), 4.06-4.14 (m, 4H), 4.17 (q, *J* = 7.2 Hz, 2H), 5.94 (ddd, *J* = 14.8 Hz, 5.0 Hz, 1.2 Hz, 1H), 6.85 (sex, *J* = 8.0 Hz, 1H).



2.197

(2*E*,4*E*)-ethyl-5-((4*S*,6*S*)-6-((tert-butyldimethylsilyl)oxy)hept-1-en-4-yl)pentadeca-2,4,14-trien-6,8-diynoate (2.197). To a solution of phsophonate **2.199** (974 mg, 3.89 mmol) in THF (40 mL) cooled to -78 ^oC, was added *n*-BuLi (2.5M in hexanes, 3.46 mmol) dropwise. The mixture was stirred at -78 ^oC for 10 min then warmed to 0^oC and stirred for 50 min. The mixture was re-cooled to -78 ^oC and a pre-cooled (-78^oC) solution of ketone **2.198** (836 mg, 2.16 mmol) in THF (15 mL) was added dropwise. The mixture was allowed to warm to room temperature over 1 h and stir overnight. The reaction was quenched with saturated NH_4Cl, extracted with EtOAc (3 x), washed with brine, and dried over $MgSO_4$. The solvent was removed and the crude product purified (19:1 Hex:EtOAc) to give **2.197** (324 mg, 31%).

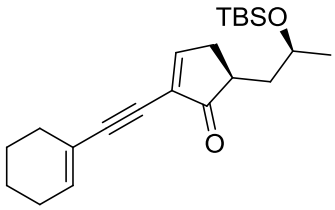
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): -0.01 (s, 3H), 0.02 (s, 3H), 0.88 (s, 9H), 1.12 (d, *J* = 6.0 Hz, 3H), 1.31 (t, *J* =6.8 Hz, 3H), 1.51-1.63 (m, 6H), 2.10 (q, *J* =6.8 Hz, 2H), 2.13-2.23 (m, 2H), 2.39 (t, *J* = 6.8 Hz, 2H), 2.47-2.54 (m, 1H), 3.66-3.72 (m, 1H), 4.22 (q, *J* = 6.8 Hz, 2H), 4.96-5.05 (m, 4H), 5.64-5.71 (m, 1H), 5.76-5.86 (m, 1H), 5.91 (d, *J* = 15.2 Hz, 1H), 6.42 (d, *J* = 11.6 Hz, 1H), 7.69 (dd, *J* = 15.2 Hz, 11.6 Hz, 1H).



2.200

(2*E*,4*E*)-5-((4*S*,6*S*)-6-((tert-butyldimethylsilyl)oxy)hept-1-en-4-yl)pentadeca-2,4,14-trien-6,8-diyn-1-ol (2.200). Ester **2.197** (225 mg, 0.466 mmol) was suspended in CH_2Cl_2 (5 mL) and cooled to -78 ^oC. DIBAL-H (1.0M in CH_2Cl_2, 1.40 mmol) was added dropwise to the cooled solution. After addition was complete, the reaction was stirred at -78 ^oC for 1.5 h. MeOH (0.5 mL) was added very slowly until H_2 evolution had ceased, then the mixture was warmed to room temperature and a saturated solution of Rochelle's salt (15 mL) was added with vigorous stirring. After 15 min the solution had become clear, so the product was extracted with CH_2Cl_2 (3 x), dried over $MgSO_4$, and the solvent evaporated to give crude product which was purified (8:1 Hex:EtOAc) to give **2.200** (162 mg, 81%).

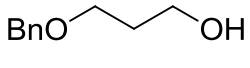
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.01 (s, 3H), 0.03 (s, 3H), 0.88 (s, 9H), 1.12 (d, *J* = 6.0 Hz, 3H), 1.50-1.61 (m, 6H), 2.08 (q, *J* = 6.8 Hz, 2H), 2.12-2.24 (m, 2H), 2.37 (t, *J* = 6.8 Hz, 2H), 2.39-2.45 (m, 1H), 3.70-3.76 (m, 1H), 4.25 (d, *J* = 5.2 Hz, 2H), 4.96-5.05 (m, 4H), 5.63-5.74 (m, 1H), 5.76-5.86 (m, 1H) 5.93 (dt, *J* = 15.2 Hz, 5.6 Hz, 1H), 6.35 (d, *J* = 11.2 Hz, 1H), 6.73 (dd, *J* = 15.2 Hz, 11.2 Hz, 1H).



2.207

(*S*)-5-((*S*)-2-((tert-butyldimethylsilyl)oxy)propyl)-2-(cyclohex-1-en-1-ylethynyl) cyclopent-2-enone (2.207). Ketone **2.198** (100 mg, 0.259 mmol) was suspended in CH_2Cl_2 (86 mL) and Grubbs second-generation catalyst (22 mg, 0.026 mmol) was added in one portion. The mixture was heated to reflux and stirred for 16 h. The solvent was evaporated and the crude product purified (15:1: Hex:EtOAc) to give **2.207** (63 mg, 68%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.06 (s, 3H), 0.07 (s, 3H), 0.88 (s, 9H), 1.14 (d, *J* = 6.0 Hz, 3H), 1.42 (dt, *J* =14.0 Hz, 8.0 Hz, 1H), 1.55-1.67 (m, 5H), 1.91 (dt, *J* = 13.6 Hz, 4.8Hz, 1H), 2.10-2.14 (m, 2H), 2.16-2.19 (m, 2H) 2.45-2.49 (m, 1H), 2.88-2.97 (m, 1H), 4.01-4.15 (m, 1H), 6.21-6.23 (m, 1H), 7.64 (t, *J* = 2.8 Hz, 1H).

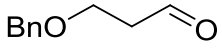


2.219

3-(benzyloxy)propan-1-ol (2.219). Sodium hydride (5.52 g, 137.98 mmol, 60% dispersion in mineral oil) was suspended in anhydrous THF (175 mL) in a flame-dried 500 mL round bottom flask. The solution was cooled to 0 ^oC and 1,3-propanediol (10.0 g, 131.41 mmol) was added dropwise. The resulting mixture was stirred for 30 min at 0 ^oC at which time benzyl bromide (23.6 g, 137.98 mmol) was added dropwise followed by addition of *tert*-butyl ammonium iodide (4.85 g, 13.14 mmol). The mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched by dropwise addition of saturated NH_4Cl solution. The resulting mixture was extracted with ethyl acetate (3 x),

dried over Na_2SO_4, and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (1:1 Hex:EtOAc) to give alcohol **2.219** as a clear, colorless oil (12.25 g, 56%).

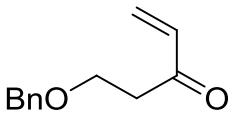
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.87 (quin, *J* = 5.8 Hz, 2H), 3.67 (t, *J* = 5.8 Hz, 2H), 3.79 (t, *J* = 5.8 Hz, 2H), 4.53 (s, 2H), 7.27-7.38 (m, 5H).



2.220

3-(benzyloxy)propanal (2.220). Alcohol **2.219** (6.0 g, 36.10 mmol) was suspended in anhydrous dichloromethane and dimethyl sulfoxide (3:1 v/v, 144 mL). The solution was cooled to 0 ^oC and triethylamine (50.2 mL, 360.97 mmol), was added followed by sulfur trioxide pyridine complex (23.0 g, 144. 39 mmol) in 4 portions. The resulting mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched with water and extracted with dichloromethane (3x), dried over sodium Na_2SO_4, and condensed under reduced pressure. The crude oil was purified by flash chromatography (2:1 Hex:EtOAc) to provide aldehyde **2.220** as a clear, colorless oil (4.21 g, 72 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.70 (td, *J* = 6.1, 1.8 Hz, 2H), 3.82 (t, *J* = 6.1 Hz, 2H), 4.54 (s, 2H), 7.27-7.38 (m, 5H), 9.80 (t, *J* = 1.8 Hz, 1H).

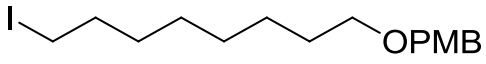


2.216

5-(benzyloxy)pent-1-en-3-one (2.216). Aldehyde **2.220** (3.50 g, 21.32 mmol) was suspended in anhydrous THF (85 mL) in a 250 mL flame-dried round bottom flask and the solution was cooled to -78 ^oC. Vinylmagnesium bromide (1.0 M in THF, 25.58 mmol,

25.6 mL) was added dropwise and the resulting solution was stirred for 1 h at -78 ^{o}C. The reaction was quenched by addition of saturated NH_4Cl solution and warmed to room temperature. The mixture was extracted with dichloromethane (2x), dried over anhydrous Na_2SO_4, and condensed *in vacuo* to provide a pale yellow residue. This residue was carried on without further purification and suspended in anhydrous dichloromethane (49 mL) with 4 angstrom molecular sieves (7.0 g) and cooled to 0 ^{o}C. *N*-methylmorpholine-*N*-oxide (3.46 g, 29.54 mmol) was added, followed by tetrapropylammonium perruthenate (260 mg, 0.74 mmol). The reaction was warmed to room temperature and stirred until complete conversion was evident by TLC. The solution was filtered through celite and condensed *in vacuo* to give a dark green residue. This residue was purified by flash chromatography (4:1 Hex:EtOAc) to provide enone **2.216** as a clear colorless oil (3.12 g, 77%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.91 (t, *J* = 6.5 Hz, 2H), 3.80 (t, *J* = 6.4 Hz, 2H), 4.53 (s, 2H), 5.87 (dd, *J* = 10.5, 1.0 Hz, 1H), 6.24 (dd, *J* = 17.6, 1.0 Hz, 1H), 6.38 (dd, *J* = 17.6, 10.5 Hz, 1H), 7.26-7.36 (m, 5H).

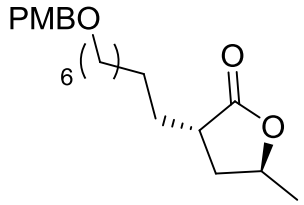


2.221

1-(((8-iodooctyl)oxy)methyl)-4-methoxybenzene (2.221). Sodium hydride (1.44 g, 35.9 mmol, 60% dispersion in mineral oil) was suspended in anhydrous THF/DMF (9:1 v/v, (120 mL) in a flame-dried 250 mL round bottom flask. The solution was cooled to 0 ^{o}C and 1,8-octanediol (5.0 g, 34.2 mmol) was added dropwise. The resulting mixture was stirred for 30 min at 0 ^{o}C at which time *p*-methoxybenzyl chloride (5.62 g, 4.87 mmol) was added dropwise followed by addition of *tert*-butyl ammonium iodide (1.26 g, 3.42

mmol). The mixture was warmed to room temperature and then heated to 65 ^{o}C and stirred for 18 h. The reaction was quenched by dropwise addition of saturated NH_4Cl solution. The resulting mixture was extracted with ethyl acetate (3 x), dried over Na_2SO_4, and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (1:1 Hex:EtOAc) to give mono-PMB alcohol as a clear, colorless oil (4.81 g, 53%). This alcohol was then carried on to the subsequent reaction. The alcohol (2.0 g, 7.51 mmol) was suspended in anhydrous THF (38 mL) and triphenylphosphine (2.96 g, 11.3 mmol), pyridine (1.84 g, 23.3 mmol), and iodine (2.67 g, 10.5 mmol) were added successively and the mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of $Na_2S_2O_3$, and extracted with Et_2O (3x). The organic layers were combined, dried over anhydrous Na_2SO_3, and condensed *in vacuo* to give an orange residue. This residue was purified by flash chromatography (5:1 Hex:EtOAc) to give iodide **2.221** a clear, colorless oil (2.16 g, 77%).

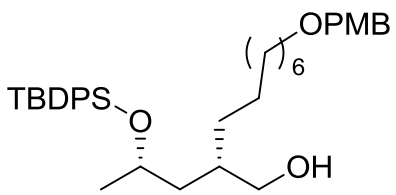
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.28-1.40 (m, 8H), 1.59 (quin, *J* = 7.2 Hz, 2H), 1.81 (quin, *J* = 7.2 Hz, 2H), 3.18 (t, *J* = 7.1 Hz, 2H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 4.43 (s, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 7.41, 26.22, 28.59, 29.34, 29.83, 30.54, 33.63, 55.39, 70.22, 72.63, 113.85, 129.32, 130.88, 159.20.



2.222

(3*S*,5*S*)-3-(8-((4-methoxybenzyl)oxy)octyl)-5-methyldihydrofuran-2(3H)-one (2.222). To a stirring solution of LDA (2.0 M in THF/heptane, 1.05 mL, 2.10 mmol) in anhydrous THF (2.0 mL) at 0 ^{o}C, was added DMPU (960 mg, 7.49 mmol) dropwise and the resulting mixture was stirred for 15 min and then cooled to -78 ^{o}C. Lactone **2.129** (200 mg, 2.00 mmol) in THF (5.0 mL) was added dropwise over a 0.5 h period. The reaction was stirred for anadditional 0.5 h at -78 ^{o}C. Iodide **2.221** (902 mg, 2.40 mmol) in THF (2.0 mL) was added dropwise and the reaction mixture was stirred at -78 ^{o}C for 18 h. The reaction was quenched by addition of saturated NH_4Cl and warmed to room temperature. The mixture was extracted with EtOAc (3x), dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (5:1 Hex/EtOAc) to give alkyl lactone **2.222** as a clear, colorless oil (272 mg, 39 %, 7:1 dr).

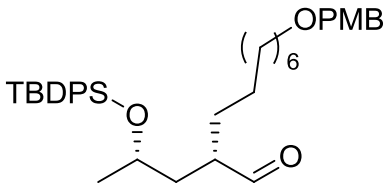
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.24-1.38 (m, 11H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.55-1.62 (m, 3H), 1.78-1.86 (m, 1H), 1.97-2.11 (m, 2H), 2.56-2.65 (m, 1H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 4.43 (s, 2H), 4.65 (sex, *J* = 6.4 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 21.43, 26.34, 27.53, 29.46, 29.54, 29.92, 30.87, 35.26, 39.51, 55.46, 70.36, 72.69, 75.11, 113.92, 129.40, 1130.97, 159.27, 179.62.



2.223

(*S*)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-10-((4-methoxybenzyl)oxy)decan-1-ol (2.223). Alkyl lactone **2.222** (220 mg, 0.63 mmol) was suspended in THF (10.5 mL) and cooled to 0 ^{o}C. Lithium hydroxide (38 mg, 1.59 mmol) was dissolved in H_2O (1.5 mL) and this solution was added dropwise to the THF solution with vigorous stirring. The resulting mixture was stirred for 16 h at 0 ^{o}C and was then carefully acidified to pH = 4. The acidified solution was extracted with EtOAc (4x), dried over Na_2SO_4, and condensed *in vacuo*. The resulting pale yellow oil was carried on without further purification. The crude acid was suspended in dichloromethane (12 mL) Imidazole (160 mg, 2.35 mmol) was added, followed by TBDPSCl (518 mg, 1.88 mmol) at room temperature. The resulting mixture was stirred at rt for 4 h at which time the reaction was quenched by addition of water and extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give the TBDPS ether/ester as a pale yellow oil that was carried on without further purification. The TBDPS ether/ester (530 mg, 0.63 mmol) was suspended in anhydrous dichloromethane (9 mL) and the resulting solution was cooled to -78 ^{o}C. DIBAL-H (1.0 M in CH_2Cl_2, 2.5 mL, 2.5 mmol) was added dropwise and the solution stirred for 2 h at -78 ^{o}C. The reaction was quenched by addition of a saturated aqueous solution of sodium potassium tartrate, warmed to room temperature, and stirred for 0.5 h. The mixture was extracted with dichloromethane (3x), dried over Na_2SO_4, condensed *in vacuo* and purified by flash chromatography (4:1 Hex/EtOAc) to provide alcohol **2.223** as a clear, colorless oil (186 mg, 50% over 3 steps).

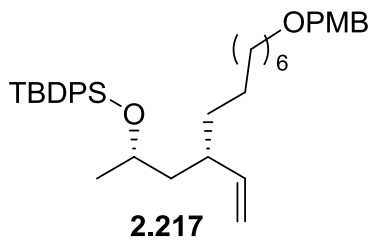
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.06 (s, 9H), 1.08 (d, *J* = 6.5 Hz, 3H), 1.17-1.28 (m, 10H), 1.30-1.38 (m, 2H), 1.40-1.53 (m, 2H), 1.59 (quin, *J* = 7.2 Hz, 2H), 1.63-1.70 (m, 1H), 3.33-3.48 (m, 2H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 4.00 (sex, *J* = 5.6 Hz, 1H), 4.43 (s, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.36-7.43 (m, 6H), 7.67-7.70 (m, 4H).



2.224

(*S*)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-10-((4-methoxybenzyl)oxy)decanal (2.224). Alcohol **2.223** (150 mg, 0.254 mmol) was suspended in DCM/DMSO (3:1 v/v, 5.0 mL) and cooled to 0 ^oC. Triethylamine (257 mg, 2.54 mmol) was added, followed by sulfur trioxide pyridine complex (162 mg, 1.02 mmol). The resulting mixture was warmed to room temperature and stirred for 4 h. The reaction was auenched by addition of water, extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a yellow oil. This oil was purified by flash chromatography (5:1 Hex/EtOAc) to provide aldehyde **2.224** as a clear, colorless oil (135 mg, 90%).

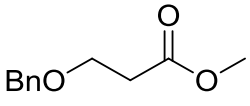
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.04 (s, 9H), 1.06 (d, *J* = 4.0 Hz, 3H), 1.19-1.28 (m, 10H), 1.28-1.35 (m, 2H), 1.47-1.55 (m, 2H), 1.55-1.60 (m, 2H), 1.79-1.90 (m, 1H) 2.39-2.46 (m 1H), 3.33-3.48 (m, 2H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 3H), 3.92 (sex, *J* = 6.0 Hz, 1H), 4.43 (s, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.35-7.44 (m, 6H), 7.64-7.69 (m, 4H). 9.44 (d, *J* = 3.0 Hz, 1H).



2.217

***tert*-butyl(((2*S*,4*S*)-12-((4-methoxybenzyl)oxy)-4-vinyldodecan-2-yl)oxy)diphenyl silane (2.217).** Methyltriphenylphosphonium bromide (100 mg, 0.28 mmol) was suspended in anhydrous THF (3.0 mL) and the solution cooled to 0 ^{o}C. KHMDS (0.5 M in toluene, 0.52 mL, 0.26 mmol) was added dropwise and the resulting mixture was stirred at 0 ^{O}C for 1h. Aldehyde **2.224** (27 mg, 0.22 mmol) was suspended in anhydrous THF (2.2 mL) and added dropwise to the solution of Wittig salt, and then the mixture was slowly warmed to room temperature and stirred 2h. The reaction was quenched by addition of saturated aqueous NH_4Cl, extracted with EtOAc (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a yellow oil. This oil was purified by flash chromatography (6:1 Hex/EtOAc) to give alkene **2.217** as a clear, colorless oil (107 mg, 85%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (d, *J* = 6.2 Hz, 3H), 1.04 (s, 9H), 1.13-1.34 (m, 13H), 1.55-1.62 (m, 4H), 2.12-2.19 (m, 1H), 3.43 (t, *J* = 6.7 Hz, 2H), 3.80 (s, 3H), 3.82-3.87 (m, 1H), 4.43 (s, 2H), 4.70-4.77 (m, 1H), 4.83 (dd, *J* = 10.4, 2.0 Hz, 1H), 5.35-5.44 (m, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 7.34-7.43 (m, 6H), 7.65-7.70 (m, 4H).

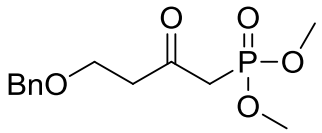


2.228

Methyl 3-(benzyloxy)propanoate (2.228). $K_2Cr_2O_7$ (2.9 g, 9.9 mmol) was dissolved in H_2O (5.0 mL). Conc. H_2SO_4 (2.0 mL) was added dropwise with vigorous stirring. The

reaction was cooled to 0 ^{o}C (15 min) and then a solution of alcohol **2.219** (1.5 g, 9.0 mmol) in acetone (18.0 mL) was added dropwise to the prepared Jone's reagent. The mixture was then stirred for 2 h. The reaction mixture was diluted with EtOAc and water and then extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, condensed *in vacuo* and carried on to the next step without further purification. The crude acid was suspended in methanol (100 mL) and conc. HCl (0.2 mL) was added. The reaction was heated to 60 ^{o}C and stirred for 12 h. The mixture was allowed to cool to room temperature and saturated aqueous $NaHCO_3$ was slowly added. The solution was diluted with ethyl acetate and water, extracted with EtOAc (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a yellow-green residue. This residue was purified by flash chromatography (2:1 Hex/EtOAc) to provide ester **2.228** as a clear, colorless oil (980 mg, 54% over 2 steps).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.63 (t, *J* = 6.4 Hz, 2H), 3.70 (s, 3H), 3.75 (t, *J* = 6.4 Hz, 2H), 4.54 (s, 2H), 7.27-7.37 (m, 5H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 35.10, 51.83, 65.69, 73.24, 127.81, 128.52, 138.18, 172.18.

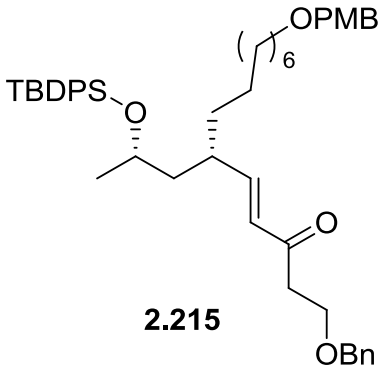


2.225

Dimethyl (4-(benzyloxy)-2-oxobutyl)phosphonate (2.225). To a solution of dimethyl methylphosphonate (2.40 g, 19.4 mmol) in anhydrous THF (16.0 mL) was added *n*-BuLi (2.5 M in hexanes, 7.65 mL, 19.1 mmol) dropwise at -78 ^{o}C. The white suspension was stirred 1 h at -78 ^{o}C at which time ester **2.228** (940 mg, 4.84 mmol) in THF (8.0 mL) was added dropwise. The reaction was stirred for an additional 1 h at -78 ^{o}C and was then

quenched with saturated NH_4Cl and warmed to room temperature. The mixture was extracted with ethyl acetate (3x), dried over Na_2SO_4, condensed *in vacuo*, and purified by flash chromatography (1:9 Hex/EtOAc) to provide phosphonate **2.225** as a clear, colorless oil (970 mg, 70%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.90 (t, *J* = 6.2 Hz, 2H), 3.12 (s, 1H), 3.17 (s, 1H), 3.75 (t, *J* = 6.2 Hz, 2H), 3.75 (s, 3H), 3.78 (s, 3H), 4.50 (s, 2H), 7.27-7.36 (m, 5H).

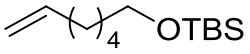


2.215

(*S*,*E*)-1-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-14-((4-methoxy benzyl)oxy)tetradec-4-en-3-one (2.215). Phosphonate **2.225** (512 mg, 1.79 mmol) was suspended in THF (3.3 mL). $Ba(OH)_2$ (262 mg, 1.53 mmol) was added at room temperature, and the resulting mixture was stirred for 1 h. Aldehyde **2.224** (902 mg, 1.53 mmol) in THF/H_2O (40:1 v/v, 5.1 mL) was added dropwsie at room temperature and the reaction was stirred 4 h. The reaction mixture was diluted with ethyl acetate and water and neutralized by addition of 1.0 M HCl. The mixture was extracted with EtOAc (3x), dried over Na_2SO_4, and condensed *in vacuo*. The crude product was purified by flash chromatography (4:1 Hex:EtOAc) to provide enone **2.215** as a clear, colorless oil (529 mg, 46%, 58% brsm).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.03 (s, 12H), 1.12-1.37 (m, 12H), 1.55-1.70 (m, 4H), 2.31-2.37 (m, 1H), 2.72 (td, *J* = 6.6, 2.4 Hz, 2H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.72-3.76

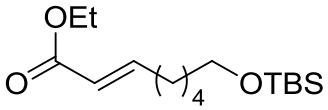
(m, 3H), 3.80 (s, 3H), 4.43 (s, 2H), 4.50 (s, 2H), 5.79 (d, *J* = 15.9 Hz, 1H), 6.44 (dd, *J* = 15.9, 9.1 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.30-7.40 (m, 11H), 7.63-7.67 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.38, 24.47, 27.16, 27.20, 29.59, 29.62, 29.64, 29.90, 35.01, 39.24, 40.32, 44.94, 55.39, 65.63, 67.98, 70.34, 72.64, 73.35, 113.88, 127.54, 127.61, 127.71, 127.76, 127.82, 128.51, 129.36, 129.63, 129.76, 130.24, 130.92, 134.21, 134.78, 136.03, 136.06, 138.35, 152.29, 159.23, 198.43.



2.227

***tert*-butyl(hept-6-en-1-yloxy)dimethylsilane (2.227).** 6-hepten-1-ol (10 g, 87.6 mmol), was suspended in anhydrous dichloromethane (175 mL) in a flame-dried 250 mL round bottom flask. Imidazole (8.94 g, 131.4 mmol) was added followed by TBSCl (15.84 g, 105.1 mmol). The reaction was stirred at room temperature for 4 h and was quenched by the addition of water, extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (6:1 Hex:EtOAc) to provide TBS ether **2.227** as a clear, colorless oil (19.24 g, 96 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.89 (s, 9H), 1.29-1.44 (m, 4H), 1.49-1.56 (m, 2H), 2.05 (q, *J* = 7.2 Hz, 2H), 3.60 (t, *J* = 6.6 Hz, 2H), 4.93 (dm, *J* = 10.2 Hz, 1H), 4.99 (ddd, *J* = 17.2, 3.6, 1.7 Hz, 1H), 5.81 (ddt, *J* = 17.2, 10.2, 6.6 Hz, 1H).

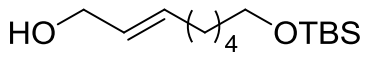


2.228

(*E*)-ethyl 8-((*tert*-butyldimethylsilyl)oxy)oct-2-enoate (2.228). TBS ether **2.227** (19.24 g, 82.82 mmol) was suspended in anhydrous CH_2Cl_2 (207 mL) in a flame-dried 500 mL

round bottom flask fit with a reflux condenser under an atmosphere of argon. Ethyl acrylate was added (33.2 g, 331.3 mmol), followed by Grubbs' second generation catalyst (703 mg, 0.83 mmol), and the resulting dark red solution was heated to 40 ^{o}C for 12 hours. At this time, the dark green solution was exposed to air, condensed under reduced pressure, purified by flash chromatography (5:1 Hex/EtOAc), and concentrated *in vacuo* to give ester **2.228** as a clear, colorless oil (24.2 g, 97%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.04 (s, 6H), 0.89 (s, 9H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.32-1.39 (m, 2H), 1.44-1.54 (m, 4H), 2.20 (q, *J* = 7.0 Hz, 2H), 3.60 (t, *J* = 6.6 Hz, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 5.81 (d, *J* = 15.6 Hz, 1H), 5.81 (dt, *J* = 15.6, 7.0 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.10, 14.46, 25.49, 26.15, 28.00, 32.30, 32.67, 60.35, 62.99, 121.64, 149.23, 166.93.

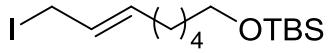


2.229

(*E*)-8-((*tert*-butyldimethylsilyl)oxy)oct-2-en-1-ol (2.229). Ester **2.228** (24.2 g, 80.70 mmol) was suspended in anhydrous dichloromethane (161 mL) in a flame dried 1 L round bottom flask and cooled to -78 ^{o}C. DIBAL-H (1.0 M in CH_2Cl_2, 201.7 mmol, 201.7 mL) was added dropwise. The resulting mixture was stirred for 2 h until complete conversion was evident by TLC. The reaction was quenched by addition of sodium potassium tartrate, warmed to room temperature, and stirred for 2 h until the organic layer was clear. The mixture was extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (4:1 Hex:EtOAc) to provide allylic alcohol **2.229** as a clear, colorless oil (18.9 g, 91%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.04 (s, 6H), 0.89 (s, 9H), 1.31-1.43 (m, 4H), 1.51

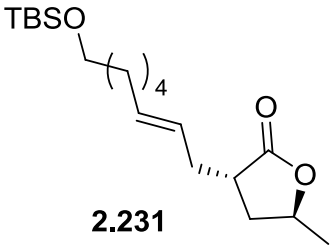
(quin, J = 7.0 Hz, 2H), 2.05 (q, J = 7.0 Hz, 2H), 3.60 (t, J = 6.6 Hz, 2H), 4.08 (d, J = 5.4 Hz, 2H), 5.60-5.73 (m, 2H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.10, 18.53, 25.51, 26.14, 29.06, 32.34, 32.83, 63.36, 63.91, 129.15, 133.43.



2.230

(*E*)-*tert*-butyl((8-iodooct-6-en-1-yl)oxy)dimethylsilane (2.230). Imidazole (2.81 g, 41.3 mmol) was suspended in dichloromethane (61 mL). Triphenylphosphine (3.97 g, 15.2 mmol) was added followed by iodine (3.85 g, 15.2 mmol) and the resulting mixture was stirred at room temperature for 0.5 h. Allylic alcohol **2.229** (3.56 g, 13.8 mmol) in dichloromethane (55 mL) was slowly added, and the resulting reaction stirred for 1.5 h at which time TLC indicated complete conversion. The reaction was quenched by addition of saturated $NaHCO_3$ extracted with DCM (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a yellow oil. This oil was purified by flash chromatography (9:1 Hex: EtOAc) to give iodide **2.230** as a clear, colorless oil (3.84 g, 76%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.04 (s, 6H), 0.89 (s, 9H), 1.29-1.42 (m, 4H), 1.51 (quin, J = 7.2 Hz, 2H), 2.03 (q, J = 5.2 Hz, 2H), 3.59 (t, J = 6.4 Hz, 2H), 3.87 (d, J = 6.4 Hz, 2H), 5.70-5.73 (m, 2H).

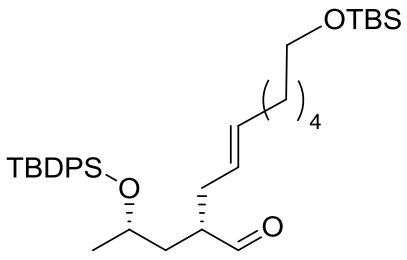


2.231

(3*S*,5*S*)-3-((*E*)-8-((*tert*-butyldimethylsilyl)oxy)oct-2-en-1-yl)-5-methyldihydrofuran-2(3H)-one (2.231). Lactone **2.129** (949 mg, 9.48 mmol) was suspended in anhydrous

THF (63.0 mL) in a flame-dried 250 mL round bottom flask and cooled to -78 °C. LiHMDS (1.0 M in THF, 9.38 mL, 9.38 mmol) was added dropwise over a 0.5 h period. The reaction was stirred for an additional 1 h at -78 °C. Iodide **2.230** (3.84 g, 10.42 mmol) in THF (17.0 mL) was added dropwise and the reaction mixture was stirred at -78 °C for 2 h. The reaction was quenched by addition of saturated NH_4Cl and warmed to room temperature. The mixture was extracted with EtOAc (3x), dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (5:1 Hex/EtOAc) to give allyl lactone **2.231** as a clear, colorless oil (2.62 g, 82 %, 8:1 dr).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.04 (s, 6H), 0.89 (s, 9H), 1.31-1.43 (m, 4H), 1.37 (d, J = 6.3 Hz, 3H), 1.51 (quin, J = 7.1 Hz, 2H), 1.92-2.03 (m, 3H), 2.08-2.16 (m, 1H), 2.18-2.25 (m, 1H), 2.45-2.51 (m, 1H), 2.64-2.74 (m, 1H), 3.60 (t, J = 6.6 Hz, 2H), 4.63 (sex, J = 6.2 Hz, 1H), 5.44 (dm, J = 67.9 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.10, 18.53, 21.50, 25.50, 26.13, 29.32, 32.65, 32.84, 33.75, 33.35, 39.70, 63.35, 75.23, 125.83, 134.29, 179.08.



2.234

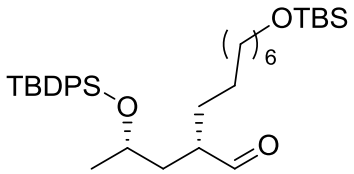
(*S*,*E*)-10-((*tert*-butyldimethylsilyl)oxy)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl) dec-4-enal (2.234). Allyl lactone **2.231** (2.52 g, 7.40 mmol) was suspended in THF (64.8 mL) and cooled to 0 °C. Lithium hydroxide (354 mg, 14.8 mmol) was dissolved in H_2O (9.3 mL) and this solution was added dropwise to the THF solution at 0 °C with vigorous stirring. The resulting mixture was stirred for 24 h at 0 °C and was then carefully

acidified to pH = 4. The acidified solution was extracted with EtOAc (4x), dried over Na_2SO_4, and condensed *in vacuo*. The resulting pale yellow oil was carried on without further purification. The crude acid was suspended in dichloromethane (87 mL) Imidazole (2.02 g, 29.6 mmol) was added, followed by TBDPSCl (6.10 g, 22.2 mmol) at room temperature. The resulting mixture was stirred at rt for 4 h at which time the reaction was quenched by addition of water and extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give the TBDPS ether/ester as a pale yellow oil that was carried on without further purification. The TBDPS ether/ester was suspended in anhydrous dichloromethane (118 mL) and the resulting solution was cooled to -78 ^oC. DIBAL-H (1.0 M in CH_2Cl_2, 29.6 mL, 29.6 mmol) was added dropwise and the solution stirred for 2 h at -78 ^oC. The reaction was quenched by addition of a saturated aqueous solution of sodium potassium tartrate, warmed to room temperature, and stirred for 0.5 h. The mixture was extracted with dichloromethane (3x), dried over Na_2SO_4, condensed *in vacuo* and purified by flash chromatography (4:1 Hex/EtOAc) to provide alcohol **2.233** as a clear, colorless oil (3.81 g, 88% over 3 steps).

Alcohol **2.233** (3.81 g, 6.54 mmol) was suspended in DCM/DMSO (3:1 v/v, 65 mL) and cooled to 0 ^oC. Triethylamine (6.61 g, 65.4 mmol) was added, followed by sulfur trioxide pyridine complex (4.16 g, 26.1 mmol). The resulting mixture was stirred for 6 h at 0 ^oC. The reaction was quenched by addition of water, extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a yellow oil. This oil was purified by flash chromatography (5:1 Hex/EtOAc) to provide aldehyde **2.234** as a clear, colorless oil (3.08 g, 72%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.90 (s, 9H), 1.04 (m, 12H), 1.26-

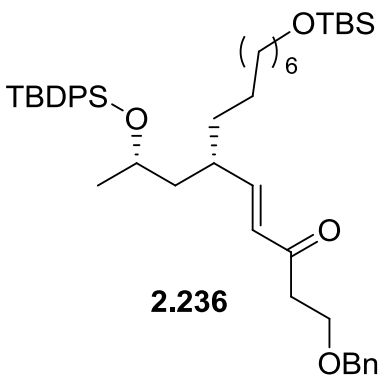
1.35 (m, 4H), 1.49-159 (m, 3H), 1.79-1.89 (m, 1H), 1.90-2.00 (m, 2H), 2.02-2.14 (m, 1H), 2.23 (quin, J = 6.8 Hz, 1H), 2.49-2.55 (m, 1H), 3.60 (t, J = 6.6 Hz, 2H), 3.95 (sex, J = 6.0 Hz, 1H), 5.33 (dm, J = 65.4 Hz, 2H), 7.35-7.45 (m, 6H), 7.65-7.70 (m, 4H), 9.47 (d, J = 2.4 Hz, 1H).



2.235

(*S*)-10-((*tert*-butyldimethylsilyl)oxy)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl) decanal (2.235). Aldehyde **2.234** (2.17 g, 3.74 mmol) was suspended in anhydrous ethyl acetate (37 mL) in a 50 mL round bottom flask. 10% palladium on activated carbon (199 mg, 0.19 mmol), was added and the flask was sealed and evacuated then filled with argon (3 x). The flask was then evacuated and refilled with hydrogen (3 x) from a balloon. The reaction was stirred for 12 h at room temperature until reaction was complete by TLC. The mixture was filtered through celite and washed through liberally with ethyl acetate. The filtrate was condensed *in vacuo* to give aldehyde **2.235** as a clear colorless oil that was carried on to the next step without further purification (2.16 g, 99%).

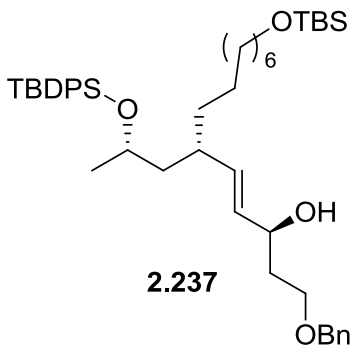
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.90 (s, 9H), 1.04 (m, 12H), 1.23-1.31 (m, 12H), 1.47-1.55 (m, 3H), 1.63-1.70 (m, 2H), 1.83 (ddd, J = 14.2, 9.0, 4.6 Hz, 1H), 2.41-2.47 (m, 1H), 3.60 (t, J = 6.6 Hz, 2H), 3.92 (sex, J = 5.8 Hz, 1H), 7.32-7.44 (m, 6H), 7.63-7.68 (m, 4H), 9.44 (d, J = 2.9 Hz, 1H).



2.236

(*S*,*E*)-1-(benzyloxy)-14-((*tert*-butyldimethylsilyl)oxy)-6-((*S*)-2-((*tert*-butyldiphenyl silyl)oxy)propyl)tetradec-4-en-3-one (2.236). Phosphonate **2.225** (1.46 g, 5.11 mmol) was suspended in THF (9.3 mL). $Ba(OH)_2$ (845 mg, 4.93 mmol) was added at room temperature, and the resulting mixture was stirred for 1 h. Aldehyde **2.235** (2.13 g, 3.65 mmol) in THF/H_2O (40:1 v/v, 12.2 mL) was added dropwsie at room temperature and the reaction was stirred 4 h. The reaction mixture was diluted with ethyl acetate and water and neutralized by addition of 1.0 M HCl. The mixture was extracted with EtOAc (3x), dried over Na_2SO_4, and condensed *in vacuo*. The crude product was purified by flash chromatography (4:1 Hex:EtOAc) to provide enone **2.236** as a clear, colorless oil (1.22 g, 45%).

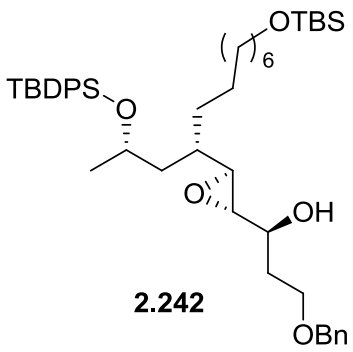
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.90 (s, 9H), 1.04 (m, 12H), 1.15-1.37 (m, 16H), 1.46-1.54 (m, 2H), 1.67 (ddd, J = 14.2, 8.2, 4.2 Hz, 1H), 2.31-2.40 (m, 1H), 2.73 (td, J = 6.6, 2.4 Hz, 2H), 3.60 (t, J = 6.6 Hz, 2H), 3.72-3.75 (m, 3H), 4.51 (s, 2H), 5.79 (d, J = 16.0 Hz, 1H), 6.44 (dd, J = 16.0, 9.2 Hz, 1H), 7.27-7.42 (m, 11H), 7.63-7.67 (m, 4H).



2.237

(3*S*,6*S*,*E*)-1-(benzyloxy)-14-((*tert*-butyldimethylsilyl)oxy)-6-((*S*)-2-((*tert*-butyl diphenylsilyl)oxy)propyl)tetradec-4-en-3-ol (2.237). Enone **2.236** (984 mg, 1.32 mmol) was suspended in anhydrous THF (13 mL) in a flame-dried 50 mL round bottom flask. The solution was cooled to -20 ^{o}C and *R*-(+)-2-methyl-CBS oxazaborolidine (367 mg, 1.32 mmol) in toluene (1.3 mL) was added, followed by dropwsie addition of BH_3 THF complex (1.0 M in THF, 4.1 mmol, 4.1 mL). The resulting mixture was stirred for 3 h and then quenched by addition of water, extracted with ethyl acetate (3x), dried over Na_2SO_4, and condensed *in vacuo*. The crude product was purified by flash chromatography (4:1 Hex:EtOAc) to give alcohol **2.237** as a clear, colorless oil (927 mg, 94%, >20:1 dr).

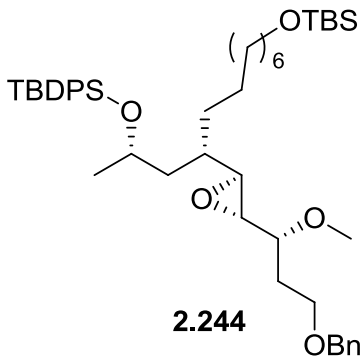
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.90 (s, 9H), 1.04 (m, 12H), 1.18-1.29 (m, 14H), 1.47-1.54 (m, 2H), 1.59 (ddd, J = 13.8, 8.0, 4.2 Hz, 1H), 1.67-1.73 (m, 2H), 2.11-2.19 (m, 1H), 3.51-3.65 (m, 2H), 3.60 (t, J = 6.6 Hz, 2H), 3.79-3.84 (m, 1H), 4.48 (d, J = 3.4 Hz, 2H), 5.13 (dd, J = 15.6, 6.0 Hz, 1H), 5.24 (dd, J = 15.6, 8.5 Hz, 1H), 7.28-7.41 (m, 11H), 7.63-7.69 (m, 4H).



2.242

(*S*)-3-(benzyloxy)-1-((2*S*,3*S*)-3-((5*S*,7*S*)-2,2,5,17,17,18,18-heptamethyl-3,3-diphenyl-4,16-dioxa-3,17-disilanonadecan-7-yl)oxiran-2-yl)propan-1-ol (2.242). Into a 100 mL flame-dried round bottom flask was placed 4 angstrom molecular sieves (1.8 g) and anhydrous dichloromethane (11.2 mL). The solution was cooled to -20 ^{o}C and (+)-diethyltartrate (276 mg, 1.34 mmol) and titanium (IV) isopropoxide (317 mg, 1.12 mmol) were added successively. After 10 min, *tert*-butyl hydrogenperoxide (5.5 M in decane, 0.41 mL, 2.23 mmol) was added dropwise. After 30 min, allylic alcohol **2.237** (831 mg, 1.12 mmol) in anhydrous dichloromethane (11.2 mL) was added dropwise. The reaction was stirred at -20 ^{o}C for 36 h. The reaction was quenched by dropwise addition of saturated sodium chloride solution and warmed to room temperature. The mixture was extracted with dichloromethane (3x), dried over Na_2SO_4, and filtered through celite. The filtrate was condensed *in vacuo* to give a yellow oil which was purified by flash chromatography (3:1 Hex:EtOAc) to provide epoxide **2.242** as a clear colorless oil (761 mg, 90%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.05 (s, 6H), 0.90 (s, 9H), 1.03 (s, 9H), 1.05 (d, J = 6.4 Hz, 3H), 1.13-1.30 (m, 14H), 1.46-1.54 (m, 4H), 1.72-1.90 (m, 2H), 2.64 (d, J = 6.0 Hz, 2H), 3.59 (t, J = 6.6 Hz, 2H), 3.68 (dm, J = 30.0 Hz, 2H), 3.75-3.78 (m, 1H), 3.99 (q, J = 6.2 Hz, 1H), 4.52 (s, 2H), 7.27-7.42 (m, 11H), 7.65-7.70 (m, 4H).



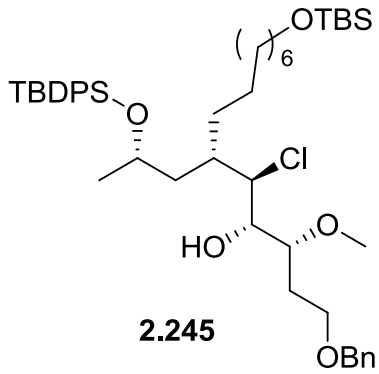
2.244

(5*S*,7*S*)-7-((2*S*,3*S*)-3-((*R*)-3-(benzyloxy)-1-methoxypropyl)oxiran-2-yl)-2,2,5,17,17, 18,18-heptamethyl-3,3-diphenyl-4,16-dioxa-3,17-disilanonadecane (2.244). Epoxide **2.242** (374 mg, 0.49 mmol) was suspended in anhydrous THF (10.0 mL). The reaction was cooled to 0 ^{o}C and triphenylphosphine (322 mg, 1.23 mmol), 4-nitrobenzoic acid (197 mg, 1.18 mmol0, and DIAD (229 mg, 1.13 mmol) were added successively. The reaction was warmed to room temperature and stirred for 4 h then quenched with water, extracted with ethyl acetate (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. This oil was suspended in THF (1.6 mL) and MeOH (2.0 mL). 2.0 M NaOH (1.98 mmol, 1.5 mL) was added, and the resulting mixture was stirred for 1 h at which time TLC indicated complete conversion to the hydrolysis product, alcohol **2.243**. The mixture was diluted with EtOAc and quenched with 1.0 M HCl then extracted with EtOAc (3x), dried over Na_2SO_4, condensed *in vacuo*, and purified by flash chromatography (3:1 Hex/EtOAc) to give alcohol **2.243** as a clear, colorless oil (311 mg, 83%)

Alcohol **2.243** (60 mg, 0.079 mmol) was suspended in anhydrous dicholoromethane (1.6 mL) and powdered 4 angstrom molecular sieves (51 mg) were added. Proton sponge (50.7 mg, 0.24 mmol) was added, followed by trimethyloxonium tetrafluoroborate (29 mg, 0.20 mmol). The resulting mixture was stirred for 2 h at room temperature and then

quenched with water, extracted with DCM (3x), dried oer Na_2SO_4, and condensed *in vacuo*. The crude product was purified by flash chromatography (4:1 Hex:EtOAc) to give ether **2.244** as a clear, colorless oil (53 mg, 87 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.06 (s, 6H), 0.91 (s, 9H), 1.04 (m, 12H), 1.09-1.32 (m, 14H), 1.47-1.58 (m, 4H), 1.76 (q, *J* = 6.1 Hz, 2H), 1.67 (ddd, *J* = 14.2, 8.2, 4.2 Hz, 1H), 2.38 (dd, *J* = 7.6, 2.1 Hz, 1H), 2.63 (dd, *J* = 6.6, 2.2 Hz, 1H), 3.05 (q, *J* = 6.6 Hz, 1H), 3.43 (s, 3H), 3.53-3.57 (m, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 4.00 (sex, *J* = 6.4 Hz, 1H), 4.49 (s, 2H), 7.27-7.41 (m, 11H), 7.67-7.72 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.05, 18.57, 19.44, 24.02, 26.00, 26.18, 27.20, 27.29, 29.66, 29.71, 30.03, 31.85, 32.78, 33.08, 37.48, 43.22, 58.22, 58.70, 63.50, 66.37, 68.20, 73.26, 78.97, 127.54, 127.59, 127.63, 127.76, 127.80, 127.88, 128.56, 129.55, 129.65, 134.53, 135.07, 136.05, 136.12, 136.15, 138.57.

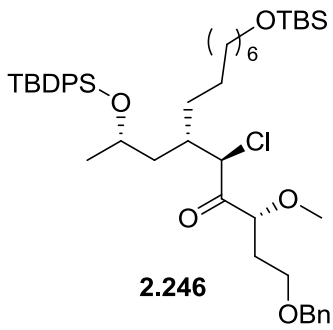


2.245

(3*R*,4*S*,5*R*,6*S*)-1-(benzyloxy)-14-((*tert*-butyldimethylsilyl)oxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-5-chloro-3-methoxytetradecan-4-ol (2.245). Epoxide **2.244** (42 mg, 0.054 mol) was suspended in anhydrous toluene (0.45 mL) and cooled to -78 ^oC. Et_2AlCl (1.0 M in toluene, 0.16 ml, 0.16 mmol) was added dropwsie and the solution was stirred for 10 min. TMS-acetylide in toluene {prepared by addition of *n*-Buli (2.5 M n hexanes, 0065 mL, 0.16 mmol) to TMS-acetylene (17.6 mg, 0.179 mmol) at -78

^{o}C in toluene (0.9 mL) with 0.5 h stirring}was added dropwise to the epoxide solution. After 6 h, the reaction was warmed to 0 ^{o}C, stirred for an additional 6 h, and then warmed to room temperature and stirred 12 h. Additional Et_2AlCl (0.54 mmol, 0.54 mL) was added and the reaction was stirred for 6 h at which time, TLC indicated that complete conversion had occurred. The reaction was quenched by addition of $NaHCO_3$, extracted with DCm (3x), dried over Na_2SO_4, and condensed *in vacuo*. The crude product was purified by flash chromatography (2:1 Hex:EtOAc) to provide alcohol **2.245** as the only isolated product.

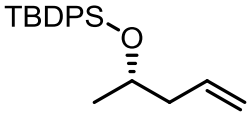
^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 0.05 (s, 6H), 0.90 (s, 9H), 1.03 (d, *J* = 3.8 Hz, 3H), 1.04 (s, 9H), 1.21-1.34 (m, 13H), 1.40-1.44 (m, 1H), 1.51 (quin, *J* = 7.0 Hz, 2H), 1.61 (ddd, *J* = 14.3, 9.3, 3.8 Hz, 1H), 1.68 (ddd, *J* = 14.3, 7.8, 3.8 Hz, 1H), 1.86-1.92 (m, 1H), 1.94-2.00 (m 1H), 2.40-2.43 (m 1H), 3.37 (s, 3H), 3.54-3.63 (m, 4H), 3.89 (t, *J* = 6.4 Hz, 1H), 3.91-3.99 (m, 1H), 4.13 (dd, *J* = 10.2, 1.7 Hz, 1H), 4.52 (d, *J* = 2.2 Hz, 2H), 7.27-7.43 (m, 11H), 7.67-7.72 (m, 4H). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): -5.05, 18.58, 19.40, 24.28, 26.03, 26.19, 27.21, 27.24, 27.67, 28.75, 29.70, 29.85, 29.89, 30.19, 31.12, 33.11, 34.77, 41.35, 58.72, 63.55, 66.40, 66.76, 67.84, 73.28, 73.51, 76.88, 127.50, 127.53, 127.71, 127.80, 127.90, 128.59, 128.62, 129.57, 129.75, 134.40, 135.18, 136.07, 136.11, 136.18, 138.42.



2.246

(3*R*,5*R*,6*S*)-1-(benzyloxy)-14-((*tert*-butyldimethylsilyl)oxy)-6-((*S*)-2-((*tert*-butyl diphenylsilyl)oxy)propyl)-5-chloro-3-methoxytetradecan-4-one (2.246). Alcohol **2.245** (10 mg, 0.0114 mmol) was suspended in anhydrous dichloromethane (1.5 mL). 4 angstrom molecular sieves (5 mg) were added, followed by 4-methylmorpholine N-oxide (2.7 mg, 0.023 mmol) and tetrapropylammonium perruthenate (0.4 mg, 0.0011 mmol) and the reaction was stirred for 2 h at rt. The mixture was filtered through celite and washed through liberally with dichloromethane. The filtrate was condensed *in vacuo* to give a dark green residue that was purified by flash chromatography (2:1 Hex:EtOAc) to give ketone **2.246** as a clear, colorless oil.

^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 0.06 (s, 6H), 0.90 (s, 9H), 1.03 (s, 9H), 1.05 (d, *J* = 6.5 Hz, 3H), 1.19-1.30 (m, 6H), 1.47-1.58 (m, 8H), 1.78-1.85 (m, 1H), 2.03-2.11 (m, 1H), 2.26-2.32 (m, 1H), 3.35 (s, 3H), 3.92 (sex, *J* = 5.8 Hz, 1H), 4.14 (dd, *J* = 8.4, 4.0 Hz, 1H), 4.50 (dd, *J* = 27.0, 12.0 Hz, 2H), 4.83 (d, *J* = 4.6 Hz, 1H), 7.28-7.43 (m, 11H), 7.62-7.69 (m, 4H).



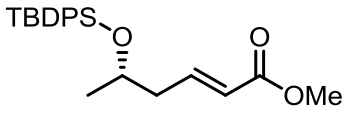
2.258

(*S*)-*tert*-butyl(pent-4-en-2-yloxy)diphenylsilane (2.258). A flame-dried 250 mL round bottom flask was charged with cuprous iodide (1.64 g, 8.61 mmol) under an atmosphere

of argon. Anhydrous THF (17.5 mL) was added and the resulting solution was cooled to -20 ^{o}C with vigorous stirring. Vinyl magnesium bromide (1.0 M in THF, 100 mL) was cannulated under a positive stream of argon into the stirring cuprous iodide over a 20 minute period. The resulting black solution was stirred for 30 minutes and a solution of (*S*)-propylene oxide (3.02 g, 43.04 mmol) in anhydrous THF (5.5 mL) was added dropwise. The reaction mixture was stirred for 20 hours at -20 ^{o}C. At this time, the reaction was quenched by dropwise addition of saturated NH_4Cl and warmed to room temperature. The mixture was extracted with diethyl ether (4 x), dried over sodium sulfate, and filtered through celite. The organic layer was carefully concentrated under reduced pressure to avoid evaporation of the volatile homoallylic alcohol **2.257**. The remaining solvent was removed by gently blowing air across the surface of the solvent. After 20 minutes, the remaining yellow liquid was carried on to the next step without further purification. The crude homoallylic alcohol was suspended in dichloromethane (108 mL) and imidazole (5.86 g, 86.1 mmol) was added followed by TBDPS-Cl (16.5 mL, 64.6 mmol) at room temperature. The reaction stirred for 2 hours at room temperature and was quenched by the addition of water. The mixture was extracted with dichloromethane (3 x), dried over sodium sulfate, and concentrated *in vacuo* to give a thick yellow oil. The crude oil was purified by flash chromatography (9:1 Hex:EtOAc) and condensed under reduced pressure to give **2.258** as a clear yellow oil (11.9 g, 85% over 2 steps).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.14 (s, 9H), 1.145 (d, *J* = 3.0 Hz, 3H), 2.22-2.33 (m, 2H), 3.98 (sex, *J* = 6.0 Hz, 1H), 5.01-5.06 (m, 2H), 5.84 (dddd, *J* = 17.0, 11.2, 10.4, 7.6 Hz, 1H), 7.41-7.49 (m, 6H), 7.75-7.78 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ

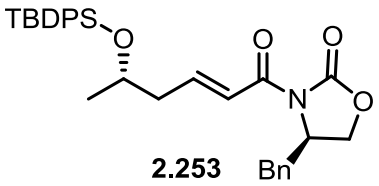
(ppm): 19.38, 22.97, 27.15, 44.08, 69.34, 116.92, 127.57, 127.62, 129.58, 129.64, 134.58, 134.86, 135.23, 136.00, 136.02. **HRMS**: $C_{21}H_{28}ONaSi$, Calculated: [M+H]+, 347.1807, Found: [M+H]+, 347.1804.



2.259

(*S*,*E*)-ethyl 5-((*tert*-butyldiphenylsilyl)oxy)hex-2-enoate (2.259). Homoallylic alcohol **2.258** (11.2 g, 34.5 mmol) was suspended in anhydrous CH_2Cl_2 (138 mL) in a flame-dried 250 mL round bottom flask fit with a reflux condenser under an atmosphere of argon. Methyl acrylate was added (10.8 mL), followed by Grubbs' second generation catalyst (580 mg, 0.69 mmol), and the resulting dark red solution was heated to 40 ^{o}C for 12 hours. At this time, the dark green solution was exposed to air, condensed under reduced pressure, purified by flash chromatography (17:3 Hex/EtOAc), and concentrated *in vacuo* to give a **2.259** as a clear, colorless oil (12.8 g, 97%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.05 (s, 9H), 1.09 (d, *J* = 6.0 Hz, 3H), 2.29-2.33 (m, 2H), 3.72 (s, 3H), 3.96 (sex, *J* = 5.9 Hz, 1H), 5.76 (ddd, *J* = 15.7, 1.3, 1.2 Hz, 1H), 6.92 (ddd, *J* = 15.7, 7.8, 7.6 Hz, 1H), 7.35-7.45 (m, 6H), 7.65-7.67 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.32, 23.30, 27.10, 42.27, 51.52, 68.59, 123.16, 127.65, 127.74, 129.73, 129.81, 134.13, 134.45, 135.99, 145.97, 166.94. **HRMS**: $C_{23}H_{30}O_3NaSi$, Calculated: [M+H]+, 405.1862, Found: [M+H]+, 405.1859.



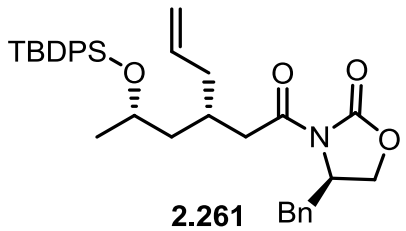
2.253

(*R*)-4-benzyl-3-((*S*,*E*)-5-((*tert*-butyldiphenylsilyl)oxy)hex-2-enoyl)oxazolidin-2-one (2.253). Ester **2.259** (11.85 g, 30.97 mmol) was suspended in THF/H_2O (7:1 v/v, 155 mL) in a 250 mL round bottom flask fit with a reflux condenser. Lithium hydroxide (7.42 g, 309.7 mmol) was added in three portions, and the resulting solution was heated to 55 ^oC for 18 hours. After hydrolysis was complete, the mixture was cooled to room temperature and acidified to pH = 2 by dropwise addition of concentrated HCl. The acidic solution was extracted with ethyl acetate (3 x), dried over sodium sulfate, and condensed *in vacuo* to give crude carboxylic acid **2.260** (11.4 g, 100%) as a viscous clear, yellow oil that was carried on without further purification.

Carboxylic acid **2.260** (14.26 g, 39.77 mmol) was suspended in anhydrous THF (200 mL) in a 500 mL flame-dried round bottom flask under an atmosphere of argon. The solution was cooled to -78 ^oC and triethylamine (6.65 mL, 47.73 mmol) was added, followed by dropwise addition of pivaloyl chloride (5.48 mL, 44.54 mmol). The resulting milky white solution was stirred at -78 ^oC for 15 min, then it was warmed to 0 ^oC and stirred for 45 min, followed by cooling to -78 ^oC. The solution was cannulated dropwise at -78 ^oC to a preformed solution of (*R*)-(-)-4-benzyl-2-oxazolidinone (7.4 g, 41.76 mmol), *n*-BuLi (2.5 M in Hexanes, 41.76 mmol), and anhydrous THF (104 mL) (*n*-BuLi added to oxazolidinone in THF at -78 ^oC and stirred for 25 min). The resulting suspension was stirred at -78 ^oC for 20 min and then warmed to room temperature and stirred for 2 hours. The reaction was quenched by addition of saturated NH_4Cl, extracted with ethyl acetate

(3 x), dried over sodium sulfate, and condensed *in vacuo* to give an orange oil. The resulting oil was purified by flash chromatography (3:1 Hex/EtOAc) to yield acyloxazolidinone **2.253** as a viscous, pale, yellow oil (17.13 g, 82%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.07 (s, 9H), 1.13 (d, *J* = 6.2 Hz, 3H), 2.43 (q, *J* = 5.6 Hz, 2H), 2.78 (dd, *J* = 13.0, 9.6 Hz, 1H), 3.33 (dd, *J* = 13.5, 3.2 Hz, 1H), 4.03 (sex, *J* = 5.9 Hz, 1H), 4.19-4.23 (m, 2H), 4.73 (ddd, *J* = 13.2, 7.2, 3.4 Hz, 1H), 7.19-7.43 (m, 14H), 7.68-7.70 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.35, 23.42, 26.49, 27.12, 38.03, 42.69, 55.42, 66.21, 68.66, 122.49, 127.45, 127.67, 127.77, 129.09, 129.60, 129.72, 129.80, 134.12, 134.46, 135.54, 135.99, 136.02, 148.22, 153.49, 164.85. **HRMS**: $C_{32}H_{37}NO_4NaSi$, Calculated: [M+H]+, 550.2390, Found: [M+H]+, 550.2393.

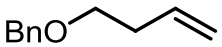


2.261

(*R*)-4-benzyl-3-((*S*)-3-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)hex-5-enoyl) oxazolidin-2-one (2.261). Recrystallized CuBr-$S(CH_3)_2$ complex (22.2 g, 108.00 mmol) was suspended in dimethylsulfide and THF (1:2 v/v, 216 mL) in a flame-dried 500 mL round bottom flask and cooled to -78 ^oC. This solution was cannulated into a cooled solution (-78 ^oC) of allylmagnesium bromide (1.0 M in Et_2O, 86.86 mmol) in a second flame-dried 500 mL round bottom flask. The resulting, thick, black solution was vigorously stirred for 1 hour at -78 ^oC and then acyloxazolidinone **2.253** (11.46 g, 21.72 mmol) in anhydrous THF (72 mL) was slowly added over 20 min. The reaction mixture was stirred for an additional 1.5 hours at -78 ^oC and was then quenched with saturated NH_4Cl and warmed to room temperature. The black mixture was extracted with diethyl

ether (4 x), dried over sodium sulfate, the solvent volume reduced to approximately 200 mL by rotary evaporation, and the resulting solid/oil mixture filtered through a celite plug with liberal diethyl ether washes. The resulting solution was condensed *in vacuo* to give a turquoise oil that was purified by flash chromatography (5:1 Hex/EtOAc) to provide the desired conjugate addition adduct **2.261** as a clear, colorless oil (10.48 g, 85%) and a single diastereomer by ^{1}HNMR (>20:1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.05 (s, 9H), 1.12 (d, *J* = 6.1 Hz, 3H), 1.41 (quin, *J* = 7.0 Hz, 1H), 1.60 (quin, *J* = 7.0 Hz, 1H), 1.91-2.03 (m, 2H), 2.22 (quin, *J* = 6.1 Hz, 1H), 2.63 (dd, *J* = 17.9, 5.8 Hz, 1H), 2.68 (dd, *J* = 13.0, 10.0 Hz, 1H), 2.87 (dd, *J* = 17.9, 7.2 Hz, 1H), 3.28 (dd, *J* = 13.4, 3.3 Hz, 1H), 3.92 (sex, *J* = 6.2 Hz, 1H), 4.10-4.20 (m, 3H), 4.65 (ddd, *J* = 13.4, 6.9, 3.2 Hz, 1H), 4.92-4.91 (m, 2H), 5.62 (dddd, *J* = 16.7, 10.2, 9.0, 7.2 Hz, 1H), 7.20-7.43 (m, 11H), 7.66-7.70 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.34, 23.57, 26.50, 27.19, 30.43, 38.08, 38.38, 39.53, 43.77, 55.29, 66.19, 67.70, 77.37, 116.95, 127.48, 127.55, 127.66, 129.04, 129.10, 129.56, 129.63, 134.40, 134.92, 135.49, 136.09, 136.11, 136.32, 153.49, 172.53. **HRMS**: $C_{35}H_{43}NO_4NaSi$, Calculated: [M+H]+, 592.2859, Found: [M+H]+, 592.2854.

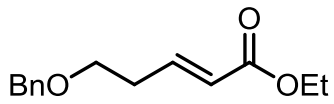


2.266

((But-3-en-1-yloxy)methyl)benzene (2.266). Sodium hydride (3.88 g, 97.07 mmol, 60% dispersion in mineral oil) was suspended in anhydrous THF (140 mL) in a flame-dried 250 mL round bottom flask. The solution was cooled to 0 ^{o}C and But-3-en-1-ol (5.0 g, 69.34 mmol) was added dropwise. The resulting mixture was stirred for 30 min at 0 ^{o}C at which time benzyl bromide (17.79 g, 104.01 mmol) was added dropwise followed by

addition of *tert*-butyl ammonium iodide (2.56 g, 6.93 mmol). The mixture was warmed to room temperature and stirred for 18 h. The reaction was quenched by dropwise addition of saturated NH_4Cl solution. The resulting mixture was extracted with ethyl acetate (3 x), dried over Na_2SO_4, and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (1:1 Hex:EtOAc) to give benzyl ether **2.266** (8.62 g, 77%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.39 (q, *J* = 6.8 Hz, 2H), 3.54 (t, *J* = 6.8 Hz, 2H), 4.53 (s, 2H), 5.06 (d, *J* = 10.2 Hz, 1H), 5.12 (ddd, *J* = 17.2, 3.5, 1.5 Hz, 1H), 5.86 (ddt, *J* = 17.2, 10.3, 6.8 Hz, 1H), 7.27-7.38 (m, 5H)..**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 34.40, 69.78, 73.06, 116.53, 127.71, 127.81, 135.44, 138.63.

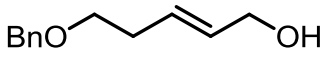


2.267

(*E*)-ethyl 5-(benzyloxy)pent-2-enoate (2.267). Benzyl ether **2.266** (8.62 g, 53.13 mmol) was suspended in anhydrous dichloromethane (106 mL) under an atmosphere of argon in a flame-dried 250 mL round bottom flask fit with a reflux condenser. Ethyl acrylate (21.28 g, 212.54 mmol) was added to the solution followed by Grubbs second generation catalyst (902 mg, 1.06 mmol) in one portion. The resulting mixture was heated to 40 ^oC and stirred for 12 h. The reaction was cooled to room temperature and concentrated under reduced pressure to provide a brown oil which was purified by flash chromatography (2:1 Hex:EtOAc) to give ester **2.267** a clear, colorless oil (10.46 g, 84 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.29 (t, *J* = 7.2 Hz, 3H), 2.51 (ddd, *J* = 13.8, 6.6, 1.5 Hz, 2H), 3.59 (t, *J* = 6.6 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 4.52 (s, 2H), 5.89 (dt, *J* =

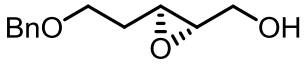
15.7, 1.5 Hz, 1H), 6.98 (dt, *J* = 15.7, 6.8 Hz, 1H), 7.28-7.37 (m, 5H)..**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 14.41, 32.77, 60.36, 68.44, 73.20, 123.09, 127.83, 128.57, 138.24, 145.70, 166.58.



2.268

(*E*)-5-(benzyloxy)pent-2-en-1-ol (2.268). Ester **2.267** (1.80 g, 7.68 mmol) was suspended in anhydrous dichloromethane (31 mL) in a 100 mL round bottom flask. The solution was cooled to -78 ^{o}C and DIBAL-H (1.0 M in CH_2Cl_2, 19.21 mL, 19.21 mmol) was added dropwise. The reaction was stirred for 1 h at -78 ^{o}C and was then quenched by addition of sodium potassium tartrate, warmed to room temperature, and stirred for 1 h until the organic layer was clear. The mixture was extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (1:1 Hex:EtOAc) to provide allylic alcohol **2.268** as a clear, colorless oil (1.25 g, 85%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.36-2.40 (m, 2H), 3.52 (t, *J* = 6.7 Hz, 2H), 4.08-4.09 (m, 2H), 4.52 (s, 2H), 5.71-5.74 (m, 2H), 7.27-7.37 (m, 5H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 32.57, 63.53, 69.54, 72.85, 127.54, 127.63, 128.32, 129.15, 130.93, 138.26.

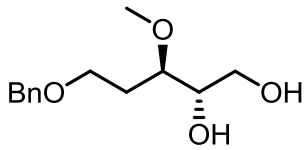


2.269

((2*S*,3*S*)-3-(2-(benzyloxy)ethyl)oxiran-2-yl)methanol (2.269). Into a 100 mL flame-dried round bottom flask was placed 4 angstrom molecular sieves (650 mg) and anhydrous dichloromethane (32.5 mL). The solution was cooled to -20 ^{o}C and (+)-

diethyltartrate (135 mg, 0.66 mmol) and titanium (IV) isopropoxide (130 mg, 0.46 mmol) were added successively. After 10 min, *tert*-butyl hydrogenperoxide (5.5 M in decane, 2.36 mL, 13.00 mmol) was added dropwise. After 30 min, allylic alcohol **2.268** (1.26 g, 6.56 mmol) in anhydrous dichloromethane (13 mL) was added dropwise. The reaction was stirred at -20 ^{o}C for 48 h. The reaction was quenched by dropwise addition of saturated sodium chloride solution and warmed to room temperature. The mixture was extracted with dichloromethane (3 x), dried over sodium sulfate, and filtered through celite. The filtrate was condensed *in vacuo* to give a yellow oil which was purified by flash chromatography (1:2 Hex:EtOAc) to provide epoxide **2.269** as a clear colorless oil (1.16 g, 86%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.78-1.86 (m, 1H), 1.89-1.98 (m, 1H), 2.97 (quin, *J* =2.4 Hz, 1H), 3.08-3.11 (m, 1H), 3.58-3.62 (m, 3H), 3.88 (dd, *J* = 12.4, 2.8 Hz, 1H), 4.52 (s, 2H), 7.27-7.37 (m, 5H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 31.98, 53.67, 58.44, 61.67, 66.78, 73.02, 127.58, 127.61, 128.35, 138.11.

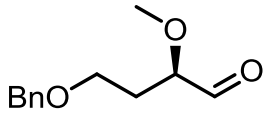


2.270

(2*S*,3*R*)-5-(benzyloxy)-3-methoxypentane-1,2-diol (2.270). Epoxide **2.269** was suspended in anhydrous methano1 (10 mL) in 25 mL round bottom flask fit with a reflux condensor. 2,6-di-*tert*-butyl-4-methylpyridine (79 mg, 0.38 mmol) was added followed by addition of europium (III) triflate (230 mg, 0.38 mmol), and the reaction was heated to 70 ^{o}C. After 19 h, the reaction mixture was diluted with water and ethyl acetate, extracted with ethyl acetate (3x), dried over Na_2SO_4, and condensed *in vacuo*. The crude oil was

purified by flash chromatography (1:1 Hex:EtOAc to 100% EtOAc) to give diol **2.270** as a clear, colorless oil (430 mg, 93 %, > 20:1 1,3:1,2 diol).

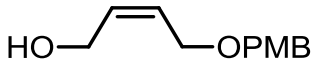
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.89 (q, *J* = 5.2 Hz, 2H), 3.36 (s, 3H), 3.39-3.44 (m, 1H), 3.54-3.59 (m, 1H), 3.63-3.73 (m, 4H), 4.52 (dd, *J* = 21.2, 11.6 Hz, 2H), 7.27-7.37 (m, 5H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 29.68, 57.82, 63.69, 66.13, 72.35, 73.27, 80.17, 127.72, 127.77, 128.42, 137.68.



2.271

(*R*)-4-(benzyloxy)-2-methoxybutanal (2.271). Diol **2.270** (100 mg, 0.416 mmol) was suspended in dichloromethane: pH 7 buffer (1:1 v/v, 5.2 mL). The biphasic mixture was vigorously stirred and cooled to 0 ^oC, then $NaIO_4$ (534 mg, 2.50 mmol) was added in 3 portions and the resulting solution was stirred at 0 ^oC for 4 h. The reaction mixture was diluted with water and dichloromethane, extracted with dichloromethane (2x), dried over Na_2SO_4, and condensed under reduced pressure. The product was purified by flash chromatography (1:3 Hex:EtOAc) to give aldehyde **2.271** as a clear, colorless oil (83 mg, 96 %).

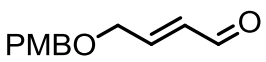
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.90-1.98 (m, 1H), 2.00-2.08 (m, 1H), 3.45 (s, 3H), 3.55-3.64 (m, 2H), 3.76-3.79 (m, 1H), 4.49 (dd, *J* = 21.0, 11.8 Hz, 2H), 7.27-7.37 (m, 5H), 9.67 (d, *J* = 1.5 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 30.80, 58.53, 65.22, 73.16, 83.18, 127.83, 128.58, 138.32, 203.35.



2.297

(*Z*)-4-((4-methoxybenzyl)oxy)but-2-en-1-ol (2.297). Sodium hydride (3.81 g, 95.34 mmol, 60% dispersion in mineral oil) was suspended in anhydrous THF (185 mL) in a flame-dried 500 mL round bottom flask. The solution was cooled to 0 ^{o}C and 1,4-*cis*-buten-1-ol (8.0 g, 90.80 mmol) was added dropwise. The resulting mixture was stirred for 30 min at 0 ^{o}C at which time *p*-methoxybenzyl chloride (15.64 g, 99.88 mmol) was added dropwise followed by addition of *tert*-butyl ammonium iodide (3.35 g, 9.08 mmol). The mixture was warmed to room temperature and stirred for 12 h. The reaction was quenched by dropwise addition of saturated NH_4Cl solution. The resulting mixture was extracted with ethyl acetate (3 x), dried over Na_2SO_4, and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (2:3 Hex:EtOAc) to give PMB ether **2.297** (13.15 g, 70%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.80 (s, 3H), 4.05 (d, *J* = 6.0 Hz, 2H), 4.15 (d, *J* = 6.3 Hz, 2H), 4.45 (s, 2H), 5.76 (dm, *J* = 35.0 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.5, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 55.20, 58.60, 65.28, 72.06, 113.78, 128.21, 129.43, 129.86, 132.28, 159.24. **HRMS**: $C_{12}H_{16}O_3Na$, Calculated: [M+H]+, 231.0997, Found: [M+H]+, 231.0995.

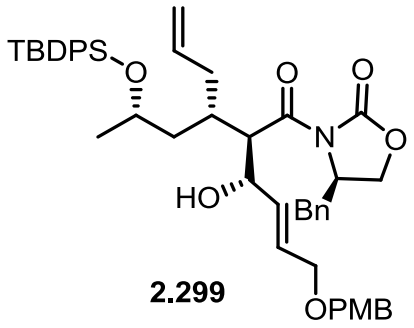


2.298

(*E*)-4-((4-methoxybenzyl)oxy)but-2-enal (2.298). PMB ether **2.297** (8.78 g, 42.16 mmol) was suspended in dichloromethane (135 mL). Manganese dioxide (73.3 g, 843.18 mmol) was added in 5 portions and the resulting heterogenous mixture was vigorously

stirred for 24 h at room temperature. Additional manganese dioxide (36.65 g, 421.59 mmol) was added in 3 portions and the reaction was stirred for 12 h. The mixture was filtered through celite and condensed *in vacuo* to give a pale yellow oil. The oil was purified by flash chromatography (3:2 Hex:EtOAc) to provide aldehyde **2.298** as a clear, colorless oil (5.36 g, 62 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.83 (s, 3H), 4.28 (dd, *J* = 4.1, 1.9 Hz, 2H), 4.54 (s, 2H), 6.41 (ddt, *J* = 15.9, 8.0, 1.9 Hz, 1H), 6.86 (dt, *J* = 15.9, 4.1 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 9.60 (d, *J* = 7.7 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 55.42, 68.40, 72.83, 114.06, 129.52, 129.58, 131.94, 153.37, 159.59, 193.48. **HRMS**: $C_{12}H_{14}O_3Na$, Calculated: [M+H]+, 229.0841, Found: [M+H]+, 229.0841.

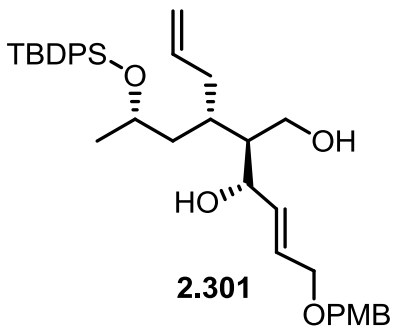


2.299

(*R*)-4-benzyl-3-((2*R*,3*S*,*E*)-2-((4*S*,6*S*)-6-((*tert*-butyldiphenylsilyl)oxy)hept-1-en-4-yl)-3-hydroxy-6-((4-methoxybenzyl)oxy)hex-4-enoyl)oxazolidin-2-one (2.299). To a flame-dried 500 mL round bottom flask was added acyloxazolidinone **2.261** (11.5 g, 20.18 mmol) and anhydrous dichloromethane (200 mL). After cooling to 0 ^{o}C, Titanium chloride (2.32 mL, 21.19 mmol) was added dropwise and the resulting yellow solution was stirred for 15 minutes. At 0 ^{o}C, diisopropylethylamine (3.87 mL, 22.20 mmol) was slowly added and the dark red mixture was stirred for 40 minutes while maintaining the

temperature at 0 ^{o}C. N-methylpyrrolidinone (1.95 mL, 20.18 mmol) was added dropwise and the solution stirred for 10 additional minutes. Aldehyde **2.298** (4.35 g, 21.09 mmol) in anhydrous dichloromethane (81 mL) was added dropwise at 0 ^{o}C. After addition was complete, the solution was stirred for 1.5 h at 0 ^{o}C, quenched with saturated NH_4Cl, and warmed to room temperature. The dark orange solution was extracted with dichloromethane (3 x) and filtered through a celite plug. The solvent was removed under reduced pressure at rt, and the resulting orange oil was purified by flash chromatography (3:2 Hex/EtOAc) to provide the *syn* aldol adduct **2.299** (10.15 g, 65%, 10:1 dr).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.05 (s, 9H), 1.07 (d, *J* = 6.0 Hz, 3H), 1.49-1.56 (m, 1H), 1.61-1.67 (m, 1H), 1.80-1.86 (m, 2H), 2.01-2.09 (m, 1H), 2.11-2.18 (m, 1H), 2.48 (dd, *J* = 13.0, 10.7 Hz, 1H), 3.30 (dd, *J* = 13.2, 3.2 Hz 1H), 3.79 (s, 3H), 3.97-4.04 (m, 5H), 4.27 (t, *J* = 7.5 Hz, 1H), 4.43 (s, 3H), 4.46-4.50 (m, 1H), 4.60-4.66 (m, 1H), 4.83 (d, *J* = 17.1 Hz, 1H), 4.90 (d, *J* = 10.0 Hz, 1H), 5.55 (dddd, *J* = 16.8, 9.9, 7.2, 5.0 Hz, 1H), 5.81-5.83 (m, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 7.17-7.43 (m, 14H), 7.66-7.71 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.33, 23.12, 27.14, 34.68, 35.21, 38.42, 40.85, 50.64, 55.40, 55.74, 65.97, 67.65, 69.83, 72.02, 113.90, 117.11, 127.43, 127.60, 127.72, 129.09, 129.43, 129.46, 129.62, 129.68, 130.17, 130.40, 131.88, 134.52, 134.81, 135.52, 136.05, 153.84, 159.33, 173.41. **HRMS**: $C_{47}H_{58}NO_7Si$, Calculated: [M+H]+, 776.3983, Found: [M+H]+, 776.3982.

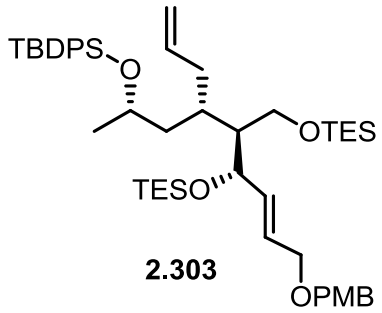


2.301

(2*S*,3*S*,*E*)-2-((4*S*,6*S*)-6-((*tert*-butyldiphenylsilyl)oxy)hept-1-en-4-yl)-6-((4-methoxy benzyl)oxy)hex-4-ene-1,3-diol (2.301). Aldol adduct **2.299** (10.4 g, 13.40 mmol) was suspended in anhydrous tetrahydrofuran (136 mL) and split into 6 separate 40 mL scintillation vials. Methanol (0.36 mL, 8.93 mmol) was added to each of the 6 vials and they were cooled to 0 ^{o}C. $LiBH_4$ was slowly added to each vial (4.53 mL, 2.0 M in THF), and the reaction mixtures were stirred for 3 h until the reaction was complete by TLC. Water was added dropwise to each vial and all 6 vials were combined and extracted with ethyl acetate (4 x), dried over Na_2SO_4, condensed *in vacuo*, and purified by flash chromatography (1:1 Hex/EtOAc) to give diol **2.299** as a clear, colorless oil (3.21 g, 40%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.05 (s, 12H), 1.37-1.44 (m, 1H), 1.55-1.64 (m, 1H), 1.68-1.74 (m, 2H), 1.90 (quin, 4.0 Hz, 1H), 2.03-2.09 (m, 1H), 2.28-2.35 (m, 1H), 3.58-3.61 (m, 1H), 3.67-3.72 (m, 1H), 3.80 (s, 3H), 3.93 (sex, *J* = 6.1 Hz, 1H), 3.99 (d, *J* = 4.2 Hz, 2H), 4.24 (bs, 1H), 4.46 (s, 2H), 4.88-4.93 (m, 2H), 5.56 (dddd, *J* = 17.0, 10.4, 9.0, 6.4 Hz, 1H), 5.82-5.85 (m, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 7.35-7.44 (m, 6H), 7.67-7.70 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.31, 23.54, 27.15, 33.30, 35.22, 41.85, 47.61, 53.39, 61.67, 68.15, 69.86, 72.19, 74.32, 113.93, 116.36, 127.56, 127.68, 129.00, 129.47, 129.51, 129.60, 129.69, 130.32, 133.39, 134.44,

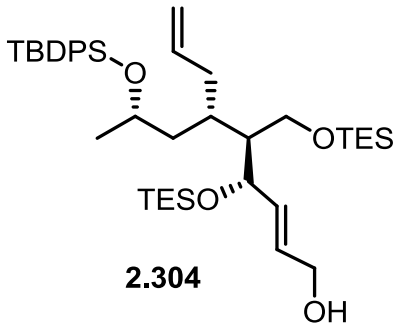
134.79, 136.03, 137.13, 159.35. **HRMS**: $C_{37}H_{50}O_5NaSi$, Calculated: [M+H]+, 625.3325, Found: [M+H]+, 625.3325.



(5*S*,7*S*,8*S*,9*S*)-7-allyl-11,11-diethyl-9-((*E*)-3-((4-methoxybenzyl)oxy)prop-1-en-1-yl)-2,2,5-trimethyl-3,3-diphenyl-8-(((triethylsilyl)oxy)methyl)-4,10-dioxa-3,11-disilatridecane (2.303). Diol **2.299** (433 mg, 0.718 mmol) was suspended in anhydrous dichloromethane (7.2 mL) in a 25 mL flame-dried round bottom flask. Imidazole (245 mg, 3.59 mmol) was added followed by TESCl (325 mg, 2.15 mmol). The resulting mixture was stirred at room temperature for 4 h and then quenched by addition of water, extracted with dichloromethane (2x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (9:1 Hex:EtOAc) to provide *bis*-TES ether **2.303** as a clear colorless oil (573 mg, 96%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.51-0.60 (m, 12H), 0.90-0.96 (m, 18H), 1.01 (d, *J* = 6.0 Hz, 3H), 1.03 (s, 9H), 1.44-1.51 (m, 1H), 1.57-1.67 (m, 2H), 1.77 (dt, *J* = 14.0, 7.8 Hz, 1H), 1.85-1.92 (m, 1H), 2.26 (dm, *J* = 14.0 Hz, 1H), 3.54 (dd, *J* = 10.2, 5.6 Hz, 1H), 3.63 (dd, *J* = 10.2, 5.6 Hz, 1H), 3.81 (s, 3H), 3.91 (sex, *J* = 6.2 Hz, 1H), 3.95 (d, *J* = 2.7 Hz, 2H), 4.32-4.35 (m, 1H), 4.42 (s, 2H), 4.82 (s, 1H), 4.86 (d, *J* = 5.6 Hz, 1H), 5.50-5.60 (m, 1H), 5.69 (d, *J* = 3.4 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.33-7.42 (m, 6H), 7.67-7.72 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 4.60, 5.30, 7.06, 7.14, 19.37, 23.46, 27.22, 32.69, 35.86, 42.78, 49.86, 55.46, 60.04,

68.85, 70.27, 71.69, 72.78, 113.93, 115.40, 127.41, 127.55, 127.63, 129.34, 129.51, 29.58, 130.78, 134.86, 135.28, 135.90, 136.07, 138.39, 159.30.



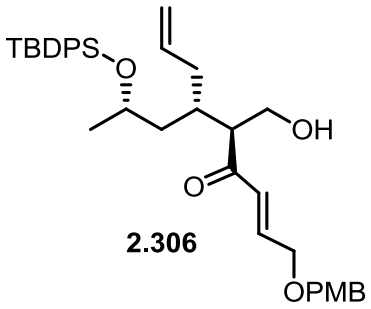
2.304

(4*S*,5*S*,6*S*,*E*)-6-((S)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-4-((triethylsilyl)oxy)-5-(((triethylsilyl)oxy)methyl)nona-2,8-dien-1-ol (2.304). *Bis*-TES ether **2.303** (533 mg, 0.641 mmol) was suspended in dichloromethane/ pH 7 phosphate buffer (7:1 v/v, 16 mL). The solution was cooled to 0 ^{o}C and DDQ (153 mg, 0.673 mmol) was added in 2 portions and the resulting mixture was stirred for 4 h at 0 ^{o}C. The reaction was quenched by addition of saturated $NaHCO_3$, extracted with dichloromethane (4x), dried over Na_2SO_4, and condesned *in vacuo* to give a yellow residue. This residue was purified by flash chromatography (6:1 Hex:EtOAc) to give allylic alcohol **2.304** as a clear, colorless oil (346 mg, 76%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.55 (quin, *J* = 7.6 Hz, 12H), 0.93 (t, *J* = 8.0 Hz, 18H), 1.01 (d, *J* = 6.2 Hz, 3H), 1.03 (s, 9H), 1.46 (quin, *J* = 6.7 Hz, 1H), 1.59-1.66 (m, 2H), 1.75 (dt, *J* = 14.1, 8.0 Hz, 1H), 1.84-1.92 (m, 1H), 2.25 (dm, *J* = 14.1 Hz, 1H), 3.54 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.62 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.91 (sex, *J* = 6.1 Hz, 1H), 4.07 (d, *J* = 4.5 Hz, 2H), 4.33 (t, *J* = 5.8 Hz, 1H), 4.42 (s, 2H), 4.83 (s, 1H), 4.86 (d, *J* = 3.8 Hz, 1H), 5.50-5.60 (m, 1H), 5.63-5.76 (m, 2H), 7.34-7.43 (m, 6H), 7.67-7.69 (m, 4H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 4.60, 5.30, 7.05, 7.12, 19.38, 23.52, 27.21, 32.63,

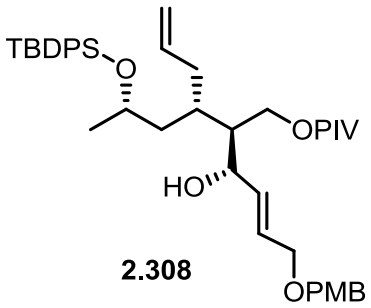
35.78, 42.68, 49.82, 59.94, 63.48, 68.83, 72.71, 115.47, 127.56, 127.63, 129.51, 129.55, 129.60, 134.79, 134.91, 135.21, 136.06, 138.57.



2.306

(5*S*,6*S*,E)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-5-(hydroxymethyl)-1-((4-methoxybenzyl)oxy)nona-2,8-dien-4-one (2.306). Diol **2.299** (643 mg, 1.06 mmol) was suspended in dichloromethane (18 mL) and manganese dioxide (1.85 g, 21.33 mmol) and the resulting heterogenous solution was stirred for 24 h at room temperature. The mixture was filtered through celite and condensed under reduced pressure to give a clear oil. This oil was purified by flash chromatography (2:1 Hex:EtOAc) to provide enone **2.306** as a clear colorless oil (460 mg, 72%).

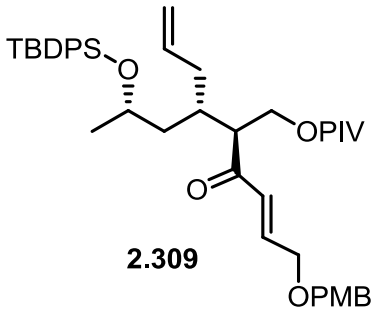
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (s, 9H), 1.06 (d, *J* = 6.1 Hz, 3H), 1.46 (dt, *J* = 14.0, 6.3 Hz, 1H), 1.58 (quin, *J* = 6.8 Hz, 1H), 1.82-1.94 (m, 2H), 2.03-2.11 (m, 1H), 2.83-2.88 (m, 1H), 3.59 (dd, *J* = 11.6, 3.2 Hz, 1H), 3.81 (s, 3H), 3.86 (dd, *J* = 11.6, 7.6 Hz, 1H), 3.93 (sex, *J* = 6.1 Hz, 1H), 4.13 (dd, *J* = 4.2, 1.8 Hz, 2H), 4.50 (s, 2H), 4.88-4.94 (m, 2H), 5.46 (ddt, *J* = 17.0, 10.0 7.1 Hz, 1H), 6.44 (d, *J* = 15.9 Hz, 1H), 6.84-6.90 (m, 3H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.34-7.43 (m, 6H), 7.66-7.69 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.35, 23.34, 27.19, 33.80, 35.10, 41.24, 54.15, 55.48, 60.35, 68.08, 68.69, 72.78, 114.09, 117.29, 127.66, 127.79, 128.96, 129.58, 129.72, 129.80, 129.84, 135.94, 136.04, 143.63, 159.58, 203.62.



2.308

(2*S*,3*S*,*E*)-2-((4*S*,6*S*)-6-((*tert*-butyldiphenylsilyl)oxy)hept-1-en-4-yl)-3-hydroxy-6-((4-methoxybenzyl)oxy)hex-4-en-1-yl Pivalate (2.308). Diol **2.299** (300 mg, 0.498 mmol) was suspended in anhydrous dichloromethane (10 mL) in a flame-dried 25 mL round bottom flask. Pyridine (71 mg, 0.897 mmol) was added, followed by pivaloyl chloride (63 mg, 0.523 mmol). The reaction was stirred at room temperature for 3 h and then quenched by the addition of water and extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. This oil was purified by flash chromatography (3:1 Hex:EtOAc) to provide pivalate **2.308** as a clear, colorless oil (318 mg, 93%).

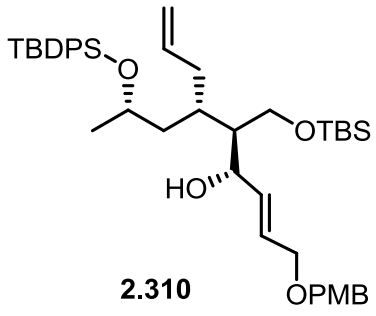
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.04 (m, 12H), 1.15 (s, 9H), 1.43 (dt, *J* = 14.0, 6.4 Hz, 1H), 1.64 (dt, *J* = 14.0, 6.2 Hz, 1H), 1.72 (dt, *J* = 14.4, 8.0 Hz, 1H), 1.84-1.89 (m, 1H), 1.95-2.01 (m, 1H), 2.22 (dm, *J* = 14.0 Hz, 1H), 3.80 (3, 3H), 3.90 (q, *J* = 6.2 Hz, 1H), 3.97 (dd, *J* = 9.2, 5.6 Hz, 2H), 3H), 4.06 (dd, *J* = 11.5, 5.6 Hz, 1H), 4.14-4.18 (m, 1H), 4.43 (s, 2H), 4.90-4.94 (m, 2H), 5.55-5.64 (m, 1H), 5.68-5.82 (m, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.34-7.42 (m, 6H), 7.66-7.70 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.36, 23.56, 27.21, 27.36, 32.93, 35.62, 38.84, 41.71, 45.70, 55.45, 62.66, 68.27, 69.90, 72.05, 72.19, 113.97, 116.27, 127.59, 127.72, 129.04, 129.52, 129.64, 129.74, 130.42, 133.96, 134.49, 134.92, 136.10, 137.81, 159.39, 178.64.



2.309

(*S*,*E*)-2-((4*S*,6*S*)-6-((*tert*-butyldiphenylsilyl)oxy)hept-1-en-4-yl)-6-((4-methoxybenzyl) oxy)-3-oxohex-4-en-1-yl pivalate (2.309). Pivalate **2.308** (110 mg, 0.160 mmol) was suspended in dichloromethane (8 mL) and manganese dioxide (348 mg, 4.00 mmol) was added in 2 portions. The resulting heterogenous solution was vigorously stirred for 24 h, then filtered through celite, condensed *in vacuo*, and purified by flash chromatography (4:1 Hex:EtOAc) to provide enone **2.309** as a clear colorless oil (93 mg, 85 %).

^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 19.34, 23.18, 27.19, 27.27, 34.31, 34.91, 38.82, 40.94, 51.17, 55.47, 62.79, 68.13, 68.68, 72.73, 113.95, 114.07, 114.51, 117.49, 127.65, 127.77, 127.89, 129.17, 129.53, 129.69, 129.77, 129.82, 129.87, 132.53, 134.33, 134.77, 135.65, 136.04, 143.12, 159.56, 178.41, 200.31.

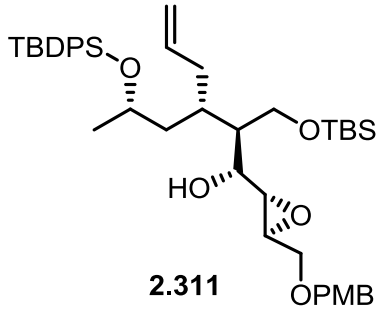


2.310

(4*S*,5*S*,6*S*,*E*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-6-((*S*)-2-((*tert*-butyldiphenyl silyl)oxy)propyl)-1-((4-methoxybenzyl)oxy)nona-2,8-dien-4-ol (2.310). Diol **2.299** (670 mg, 1.11 mmol), was suspended in anhydrous dichloromethane in a flame-dried 25 mL round bottom flask. Imidazole (114 mg, 1.67 mmol) was added followed by TBSCl

(176 mg, 1.17 mmol). The reaction was stirred at room temperature for 4 h and was quenched by the addition of water, extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (3:1 Hex:EtOAc) to provide TBS ether **2.310** as a clear, colorless oil (762 mg, 96 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.02 (d, *J* = 1.4 Hz, 6H), 0.87 (s, 9H), 1.04 (m, 12H), 1.42-1.49 (m, 1H), 1.58-1.62 (m, 1H), 1.68-1.75 (m, 1H), 1.81-1.86 (m, 1H), 2.08 (dm, *J* = 14.4 Hz, 1H), 3.13 (d, *J* = 7.0 Hz, 1H), 3.66 (d, *J* = 6.5 Hz, 2H), 3.80 (3, 3H), 3.89 (sex, *J* = 6.2 Hz, 1H), 4.01 (d, *J* = 3.8 Hz, 2H), 4.23-4.27 (m, 1H), 4.45 (s, 2H), 4.85-4.91 (m, 2H), 5.52 (ddt, *J* = 17.0, 9.9, 7.0 Hz, 1H), 5.80-5.82 (m, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.34-7.43 (m, 6H), 7.66-7.69 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.47, -5.45, 18.21, 19.36, 23.43, 26.02, 27.21, 33.32, 35.35, 41.96, 47.36, 55.45, 62.75, 68.28, 70.13, 71.85, 73.66, 113.93, 116.33, 127.61, 127.73, 128.40, 128.83, 129.43, 129.50, 129.62, 129.73, 130.64, 133.62, 134.51, 134.94, 136.08, 137.09, 159.32.

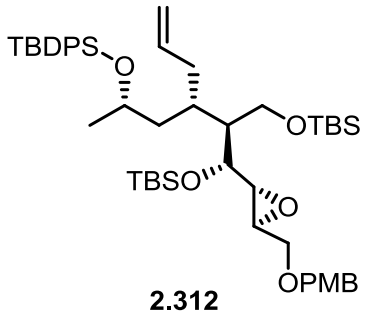


2.311

(1*R*,2*S*,3*S*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-((S)-2-((*tert*-butyldiphenyl silyl)oxy)propyl)-1-((2*S*,3*S*)-3-(((4-methoxybenzyl)oxy)methyl)oxiran-2-yl)hex-5-en-1-ol (2.311). TBS ether **2.311** (325 mg, 0.45 mmol) was suspended in anhydrous dichloromethane (4.5 mL) in a flame-dried 25 mL round bottom flask. The solution was

cooled to 0 ^{o}C and vanadyl acetylacetonate (6.0 mg, 0.023 mmol) was added in one portion followed by dropwise addition of *tert*-butyl hydrogen peroxide (0.25 mL, ~5.5 M in decane). The resulting dark red solution was stirred at 0 ^{o}C for 4 h until reaction was determined complete by TLC. The crude reaction mixture was transferred directly to a silica column that had been equilibrated with 1% Et_3N in hexane and was purified using 4:1 Hex/EtOAc to give oxirane **2.311** as a clear, colorless oil and a single diastereomer by ^{1}HNMR (315 mg, 95%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.02 (d, *J* = 2.0 Hz, 6H), 0.87 (s, 9H), 1.03 (s, 9H), 1.04 (d, *J* = 3.0 Hz, 3H), 1.42-1.48 (m, 1H), 1.59-1.65 (m, 1H), 1.68-1.75 (m, 1H), 1.80-1.86 (m, 1H), 1.88-1.92 (m, 1H), 2.22 (dm, *J* = 14.0 Hz, 1H), 2.63 (d, *J* = 4.2 Hz, 1H), 3.05 (dd, *J* = 4.2, 2.4 Hz, 1H), 3.23 (dt, *J* = 6.2, 2.4 Hz, 1H), 3.37 (dd, *J* = 11.6, 6.2 Hz, 1H), 3.69-3.73 (s, 2H), 3.76 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.80 (s, 3H), 3.85 (q, *J* = 4.2 Hz, 1H), 3.91 (sex, *J* = 6.2 Hz, 1H), 4.50 (dd, *J* = 25.4, 11.6 Hz, 2H), 4.86-4.90 (m, 2H), 5.49-5.59 (m, 1H), 6.87 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.33-7.43 (m, 6H), 7.66-7.69 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.42, 18.28, 19.35, 23.36, 25.92, 26.04, 27.21, 33.01, 35.53, 41.99, 46.81, 54.34, 55.45, 56.71, 61.99, 68.45, 69.92, 70.05, 73.05, 113.98, 116.14, 127.59, 127.70, 129.61, 129.69, 130.19, 134.53, 135.00, 136.09, 137.62, 159.45.

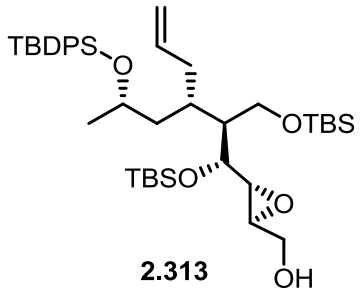


2.312

(5*R*,6*S*,7*S*,9*S*)-7-allyl-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-((2*R*,3*S*)-3-(((4-methoxybenzyl)oxy)methyl)oxiran-2-yl)-2,2,3,3,9,12,12-heptamethyl-11,11-diphenyl-4,10-dioxa-3,11-disilatridecane (2.312). Oxirane **2.311** (230 mg, 0.31 mmol) was suspended in anhydrous dichloromethane (6 mL) in a flame-dried 25 mL round bottom flask. Pyridine was added (99 mg, 1.26 mmol) followed by TBSOTf (248 mg, 94 mmol). The reaction was stirred at room temperature for 1.5 h and quenched with water once TLC indicated complete conversion. The mixture was extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. The oil was purified by flash chromatography (6:1 Hex:EtOAc) to provide *bis*-TBS ether (263 mg, 99 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.01 (d, *J* = 1.6 Hz, 6H), 0.02 (s, 3H), 0.04 (s, 3H), 0.86 (s, 9H), 0.87 (s, 9H), 1.01-1.02 (m, 12H), 1.45-1.52 (m, 1H), 1.59-1.75 (m, 3H), 1.88-1.94 (m, 1H), 2.32 (dm, *J* = 14.0 Hz, 1H), 2.80 (dd, *J* = 4.8, 2.2 Hz, 1H), 3.09 (dt, *J* = 6.4, 2.2 Hz, 1H), 3.20 (dd, *J* = 11.5, 6.4 Hz, 1H), 3.63 (d, *J* = 6.4 Hz, 2H), 3.70 (dd, *J* = 11.5, 2.2Hz, 1H), 3.80 (s, 3H), 3.90 (dd, *J* = 5.0, 3.6 Hz, 1H), 3.96 (sex, *J* = 6.2 Hz, 1H), 4.47 (dd, *J* = 30.2, 11.5 Hz, 2H), 4.84-4.88 (m, 2H), 5.48-5.58 (m, 1H), 6.86 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.32-7.41 (m, 6H), 7.66-7.71 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.20, -4.79, -4.19, 18.32, 18.38, 19.33, 23.50, 26.10, 26.11, 27.19, 32.49, 35.61, 43.23, 48.23, 55.45, 55.67, 56.72, 59.77, 68.87,

70.32, 70.57, 73.02, 113.96, 115.77, 127.62, 127.64, 129.55, 129.58, 130.31, 134.99, 135.08, 136.09, 138.05, 159.40.

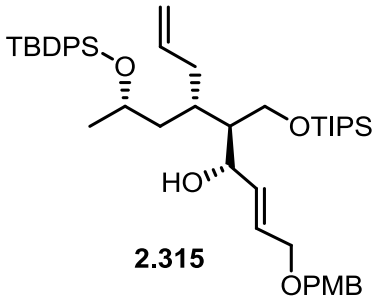


2.313

((2*S*,3*R*)-3-((5*R*,6*S*,7*S*,9*S*)-7-allyl-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2,3,3,9,12,12-heptamethyl-11,11-diphenyl-4,10-dioxa-3,11-disilatridecan-5-yl)oxiran-2-yl)methanol (2.313). *Bis*-TBS ether **2.312** (248 mg, 0.30 mmol) was suspended in dichloromethane/ pH 7 phosphate buffer (9:1 v/v, 10 mL) and the reaction was cooled to 0 ^{o}C. DDQ (75mg, 0.33 mmol) was added in one portion and the biphasic mixture was vigorously stirred for 4 h at 0 ^{o}C. The reaction was quenched by addition of saturated $NaHCO_3$ and extracted with dichloromethane (4x), dried over Na_2SO_4, and condensed under reduced pressure to provide a yellow oil. This oil was purified by flash chromatography (5:1 Hex:EtOAc) to provide alcohol **2.313** as a clear, colorless oil (180 mg, 83%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.02 (s, 6H), 0.03 (s, 3H), 0.05 (s, 3H), 0.87 (s, 9H), 1.04 (s, 9H), 1.05 (d, J = 6.1 Hz, 3H), 1.48-1.77 (m, 5H), 1.85-1.91 (m, 1H), 2.29 (dm, J = 14.0 Hz, 1H), 2.96 (dd, J = 4.6, 2.2 Hz, 1H), 3.08 (quin, J = 2.2 Hz, 1H), 3.46-3.52 (m, 1H), 3.63-3.65 (m, 2H), 3.85 (ddd, J = 12.5, 5.0, 2.4 Hz, 1H), 3.94-3.98 (m, 2H), 4.84-4.87 (m, 2H), 5.45-5.56 (m, 1H), 7.35-7.44 (m, 6H), 7.68-7.70 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): -5.21, -4.76, -4.23, 18.34, 18.38, 19.34, 23.42, 26.09, 27.18, 32.53, 35.48, 43.02, 48.26, 56.62, 57.19, 59.77, 61.83, 68.87, 70.31, 115.93,

127.62, 127.65, 129.62, 134.93, 135.03, 136.06, 137.79.

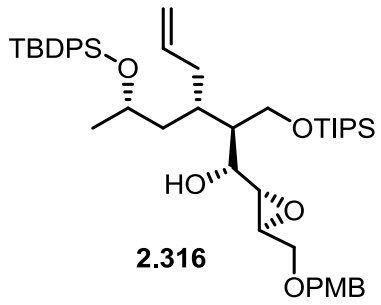


2.315

(4*S*,5*S*,6*S*,*E*)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-1-((4-methoxybenzyl)oxy-5(((triisopropylsilyl)oxy)methyl)nona-2,8-dien-4-ol (2.315). Diol **2.301** (2.16 g, 3.58 mmol) was suspended in anhydrous dichloromethane (36 mL) in a flame-dried 100 mL round bottom flask. Imidazole (1.22 g, 17.91 mmol) was added in one portion, followed by dropwise addition of chlorodiisopropylsilane (1.53 mL, 7.17 mmol) at room temperature. After 1 h, an additional 1.22 g of imidazole and 1.53 mL of chlorodiisopropylsilane were added. After 1 h, 1.53 mL of chlorodiisopropylsilane was added and the solution was stirred for an additional 1 h and quenched by addition of water after the reaction was determined to be complete by TLC. The mixture was extracted with dichloromethane (3 x), dried over Na_2SO_4, condensed *in vacuo*, and purified by flash chromatography (2:1 Hex/EtOAc) to give triisopropylsilyl ether **2.315** as clear, colorless oil (2.39 g, 88%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.04 (s, 33H), 1.44-1.49 (m, 1H), 1.55-1.63 (m, 1H), 1.71 (quin, *J* = 7.2 Hz, 1H), 1.87-1.93 (m, 1H), 2.03-2.09 (m, 1H), 3.45 (d, *J* = 7.2 Hz, 1H), 3.76 (d, *J* = 6.8 Hz, 2H), 3.80 (s, 3H), 3.90 (sex, *J* = 6.2 Hz, 1H), 4.00 (d, *J* = 3.0 Hz, 2H), 4.25-4.29 (m, 1H), 4.45 (s, 2H), 4.84-4.91 (m, 2H), 5.49 (dddd, *J* = 16.9, 9.8, 7.2, 7.0 Hz, 1H), 5.84-5.85 (m, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.34-7.43 (m, 6H), 7.66-7.69 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.86,

18.12, 19.32, 23.36, 27.16, 33.28, 35.12, 41.90, 47.45, 55.41, 63.42, 68.17, 70.11, 71.79, 73.87, 113.88, 116.43, 127.58, 127.70, 128.46, 129.37, 129.58, 129.69, 130.64, 133.35, 134.45, 134.88, 136.03, 136.70, 159.26. **HRMS**: $C_{46}H_{71}O_5Si_2$, Calculated: [M+H]+, 759.4840, Found: [M+H]+, 759.4836.

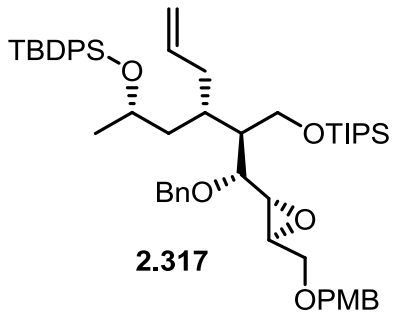


2.316

(1*R*,2*S*,3*S*)-3-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-1-((2*S*,3*S*)-3-(((4-methoxybenzyl)oxy)methyl) oxiran-2-yl)-2-(((triisopropylsilyl)oxy)methyl)hex-5-en-1-ol (2.316). Triisopropylsilyl ether **2.315** (2.35 g, 3.10 mmol) was suspended in anhydrous dichloromethane (16 mL) in a flame-dried 50 mL round bottom flask. The solution was cooled to 0 ^{o}C and vanadyl acetylacetonate (41.0 mg, 0.155 mmol) was added in one portion followed by dropwise addition of *tert*-butyl hydrogen peroxide (1.70 mL, ~5.5 M in decane). The resulting dark red solution was stirred at 0 ^{o}C for 4 h until reaction was determined complete by TLC. The crude reaction mixture was transferred directly to a silica column that had been equilibrated with 1% Et_3N in hexane and was purified using 4:1 Hex/EtOAc to give oxirane **2.316** as a clear, colorless oil and a single diastereomer by ^{1}HNMR (2.36 g, 98%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.03 (s, 33H), 1.46 (quin, *J* = 6.4 Hz, 1H), 1.64 (quin, 7.2 Hz, 1H), 1.72 (quin, *J* = 7.2 Hz, 1H), 1.86-1.89 (m, 2H), 2.13-2.18 (m, 1H), 2.85 (d, *J* = 4.7 Hz, 1H), 3.08 (dd, *J* = 4.4, 2.3 Hz, 1H), 3.20 (dt, *J* = 6.1, 2.2 Hz, 1H), 3.38 (dd, *J* = 11.6, 6.2 Hz, 1H), 3.76-3.86 (m, 4H), 3.80 (s, 3H), 3.92 (sex, *J* = 6.4 Hz,

1H), 4.50 (dd, *J* = 25.6, 11.6 Hz, 2H), 4.86-4.90 (m, 2H), 5.53 (dddd, *J* = 19.6, 9.8, 9.2, 7.4 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 7.33-7.43 (m, 6H), 7.67-7.69 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.90, 18.13, 19.30, 23.31, 27.15, 32.92, 35.39, 42.06, 46.96, 54.47, 55.39, 56.52, 62.68, 68.33, 69.86, 70.57, 72.99, 113.91, 116.18, 127.53, 127.64, 129.54, 129.63, 130.14, 134.47, 134.94, 136.02, 137.34, 159.38. **HRMS**: $C_{46}H_{71}O_6Si_2$, Calculated: [M+H]+, 775.4789, Found: [M+H]+, 775.4785.

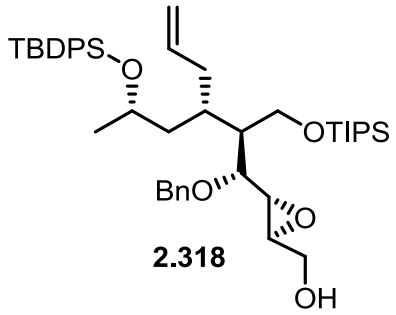


2.317

(5*S*,7*S*,8*S*)-7-allyl-8-((*R*)-(benzyloxy)((2*S*,3*S*)-3-(((4-methoxybenzyl)oxy)methyl) oxiran-2-yl)methyl)-11,11-diisopropyl-2,2,5,12-tetramethyl-3,3-diphenyl-4,10-dioxa-3,11-disilatridecane (2.317). Sodium hydride (60% dispersion in mineral oil) (248 mg, 6.19 mmol) was suspended in anhydrous tetrahydrofuran (34 mL) in a flame-dried 100 mL round bottom flask, and the solution was cooled to 0 ^{o}C. A solution of oxirane **2.316** (2.40 g, 3.10 mmol) in THF (10 mL) was slowly added to the sodium hydride solution, followed by immediate addition of benzyl bromide (1.47 mL, 12.38 mmol) and *tert*-butylammonium iodide (229 mg, 0.619 mmol). The solution was warmed to room temperature and stirred for 9 h. The reaction was quenched by the addition of saturated NH_4Cl, extracted with ethyl acetate (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a yellow oil that was purified by flash chromatography (17:3 Hex/EtOAc) to provide benzyl ether **2.317** as a clear, colorless oil (2.43 g, 91%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (d, *J* = 5.4 Hz, 33H), 1.48 (quin, *J* = 6.7 Hz,

1H), 1.61-1.68 (m, 1H), 1.68-1.74 (m, 1H), 1.84-1.91 (m, 2H), 1.96-2.02 (m, 1H), 2.22-2.29 (m, 1H), 2.91 (dd, *J* = 5.2, 2.0 Hz, 1H), 3.17 (dt, *J* = 6.1, 2.4 Hz, 1H), 3.30 (dd, *J* = 10.9, 6.5 Hz, 1H), 3.55-3.60 (m, 2H), 3.69 (dd, 10.0, 5.5 Hz, 1H), 3.73-3.78 (m, 1H), 3.80 (s, 3H), 3.81-3.81 (m, 1H), 3.95 (sex, *J* = 6.4 Hz, 1H), 4.47 (dd, *J* = 27.9, 11.9 Hz, 2H), 4.56 (dd, *J*= 85.2, 11.5 Hz, 2H), 4.82-4.86 (m, 2H), 5.54 (dddd, *J* = 17.8, 10.6, 7.8, 7.6 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 2H), 7.22-7.40 (m, 13H), 7.66-7.69 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.05, 18.21, 19.29, 23.51, 27.13, 32.83, 35.97, 42.83, 47.51, 55.40, 55.66, 56.39, 60.64, 68.65, 70.21, 73.01, 73.54, 113.91, 115.60, 127.44, 127.53, 127.59, 128.33, 129.51, 129.54, 134.81, 135.04, 136.02, 138.45, 139.07, 159.37. **HRMS**: $C_{53}H_{77}O_6Si_2$, Calculated: [M+H]+, 865.5259, Found: [M+H]+, 865.5259.

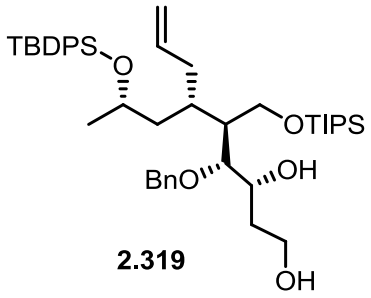


2.318

((2*S*,3*S*)-3-((1*R*,2*S*,3*S*)-1-(benzyloxy)-3-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-2-(((triisopropylsilyl) oxy)methyl)hex-5-en-1-yl)oxiran-2-yl)methanol (2.318). Benzyl ether **2.317** (1.70 g, 1.96 mmol) was suspended in dichloromethane: pH 7 phosphate buffer (7:1 v/v, 40 mL) in a 100 mL round bottom flask. The solution was cooled to 0 ^{o}C and vigorously stirred. 2,3-Dichloro-5,6-Dicyanobenzoquinone (535 mg, 2.36 mmol) was added in 3 portions and the resulting biphasic solution was vigorously stirred for 2 h at 0 ^{o}C. An additional 107 mg of DDQ was added and the solution was stirred 2 h at 0 ^{o}C. The reaction was quenched by addition of saturated $NaHCO_3$, extracted with dichloromethane (4 x), dried over Na_2SO_4, and condensed *in vacuo* to provide a yellow residue that was

purified by flash chromatography (5:1 Hex/EtOAc) to provide alcohol **2.318** as a clear, colorless oil (1.11 g, 76%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.02-1.05 (m, 33H), 1.44-1.53 (m, 2H), 1.62-1.67 (m, 1H), 1.68-1.75 (m, 1H), 1.84-1.91 (m, 1H), 1.94-2.01 (m, 1H), 2.21-2.27 (m, 1H), 3.05 (dd, *J* = 4.88, 2.2 Hz, 1H), 3.11 (quin, *J* = 2.3 Hz, 1H), 3.44 (ddd, *J* = 12.7, 7.4, 5.0 Hz, 1H), 3.63 (t, *J* = 5.1 Hz, 1H), 3.68-3.82 (m, 3H), 3.95 (sex, *J* = 6.2 Hz, 1H), 4.52 (dd, *J* = 55.6, 11.8 Hz, 2H), 4.83-4.87 (m, 2H), 5.54 (dddd, *J* = 15.3, 10.2, 7.2, 6.2 Hz, 1H), 7.25-7.42 (m, 11H), 7.68 (d, *J* = 7.2 Hz, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.07, 18.22, 19.31, 23.50, 27.14, 32.92, 35.94, 42.76, 47.47, 56.72, 56.79, 60.71, 61.77, 68.65, 73.81, 77.36, 115.73, 127.52, 127.55, 127.60, 128.39, 129.55, 129.58, 134.83, 135.02, 136.04, 138.32, 139.11. **HRMS**: $C_{45}H_{69}O_5Si_2$, Calculated: [M+H]+, 745.4684, Found: [M+H]+, 745.4681.

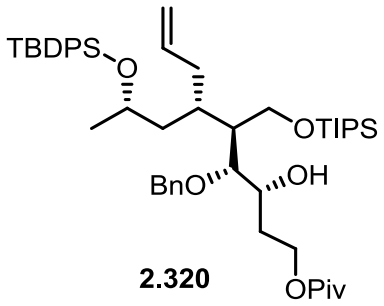


2.319

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-5-(((triisopropylsilyl)oxy) methyl)non-8-ene-1,3-diol (2.319). Alcohol **2.318** (1.07 g, 1.44 mmol) was suspended in anhydrous tetrahydrofuran (96 mL) in a 250 mL flame-dried round bottom flask and cooled to 0 ^{o}C. Sodium bis(2-methoxyethoxy)aluminum hydride (65% wt in toluene, 2.68 mL, 8.62 mmol) was added dropwise and the resulting solution was warmed to room temperature and stirred for 5 h, until the reaction neared completion and the free primary alcohol resulting from TIPS deprotection began to form. At this

time, saturated sodium potassium tartrate was added dropwise and the resulting cloudy emulsion was diluted with 50 mL of ethyl acetate and stirred for 30 min until the organic layer became clear. The product was extracted by ethyl acetate (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a thick, clear oil. This oil was purified by flash chromatography (7:3 Hex:EtOAc) to provide the desired 1,3-diol **2.319** (670 mg, 63%, 70% based on recovered starting material) as the only product (^{1}HNMR >20:1 1,3 diol:1,2 diol).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.04-1.09 (m, 33H), 1.49-1.56 (m, 1H), 1.60-1.67 (m, 1H), 1.74-1.82 (m, 3H), 1.89-1.95 (m, 1H), 2.04-2.12 (m, 1H), 2.16-2.21 (m, 1H), 2.94 (bs, 1H), 3.51 (t, *J* = 6.0 Hz, 1H), 3.74-3.89 (m, 5H), 3.95-4.02 (m, 2H), 4.52 (s, 2H), 4.83-4.87 (m, 2H), 5.37-5.47 (m, 1H), 7.26-7.44 (m, 11H), 7.68-7.71 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.04, 18.12, 18.15, 19.29, 23.17, 27.12, 31.99, 34.83, 34.95, 42.33, 46.24, 60.94, 62.56, 68.73, 73.45, 74.56, 82.31, 116.48, 127.56, 127.63, 127.75, 127.84, 128.50, 129.55, 129.60, 134.72, 134.96, 136.00, 136.79, 138.51. **HRMS**: $C_{45}H_{71}O_5Si_2$, Calculated: [M+H]+, 747.4840, Found: [M+H]+, 747.4843.

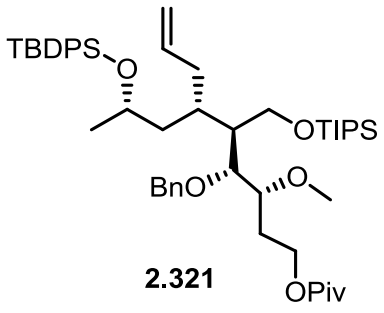


2.320

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-hydroxy-5-(((triisopropylsilyl)oxy)methyl)non-8-en-1-yl Pivalate (2.320). 1,3 diol **2.319** (540 mg, 0.723 mmol) was suspended in anhydrous dichloromethane (36 mL) in a 100 mL flame-dried round bottom flask. Pyridine was added (0.59 mL, 7.23 mmol)

followed by addition of pivaloyl chloride (0.53 mL, 4.34 mmol) at room temperature, and the resulting solution was stirred for 2 h. The reaction was quenched by addition of saturated $NaHCO_3$, extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a cloudy, white residue. This residue was purified by flash chromatography (4:1 Hex:EtOAc) to give pivalate ester **2.320** as a clear, colorless oil (596 mg, 99%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.02-1.08 (m, 33H), 1.18 (s, 9H), 1.57 (dquin, *J* = 59.8, 6.8 Hz, 2H), 1.69-1.82 (m, 3H), 1.96-2.04 (m, 1H), 2.05-2.12 (m, 1H), 2.16-2.22 (m, 1H), 3.18 (d, *J* = 4.9 Hz, 1H), 3.50 (t, *J* = 6.0 Hz, 1H), 3.73 (dd, *J* = 10.5, 6.8 Hz, 1H), 3.81 (dd, *J* = 10.6, 3.1 Hz, 1H), 3.84-3.88 (m, 1H), 3.97 (sex, *J* = 6.1 Hz, 1H), 4.16-4.26 (m, 2H), 4.53 (s, 2H), 4.81-4.85 (m, 2H), 5.38-5.48 (m, 1H), 7.24-7.43 (m, 11H), 7.67-7.70 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.07, 18.17, 18.20, 19.31, 23.27, 27.14, 27.36, 32.17, 32.70, 35.13, 38.83, 42.65, 46.20, 60.98, 62.35, 68.73, 70.44, 73.54, 77.36, 82.55, 116.24, 127.57, 127.64, 127.73, 128.47, 129.55, 129.62, 134.77, 135.03, 136.02, 137.28, 138.74, 178.73. **HRMS**: $C_{50}H_{79}O_6Si_2$, Calculated: [M+H]+, 831.5415, Found: [M+H]+, 831.5417.

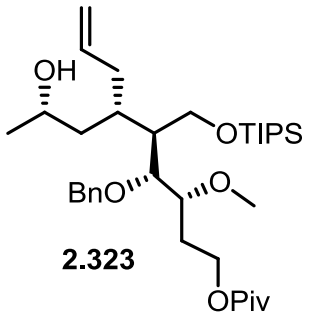


2.321

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-(((triisopropylsilyl)oxy)methyl)non-8-en-1-yl Pivalate (2.321). Pivalate ester **2.320** (440 mg, 0.53 mmol) was suspended in anhydrous dichloromethane (52 mL)

in a 100 mL flame-dried round bottom flask. 1,8-Bis(dimethylamino) naphthalene (680 mg, 3.18 mmol) was added followed by addition of trimethyloxonium tetrafluoroborate (392 mg, 2.65 mmol) in 3 portions at room temperature. The resulting solution was stirred at room temperature for 1.5 h until TLC indicated that the reaction was complete. The reaction was quenched by addition of saturated $NaHCO_3$, extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow residue. This residue was purified by flash chromatography (17:3 Hex:EtOAc) to provide the desired methyl ether **2.321** as a clear, colorless oil (430 mg, 96%).

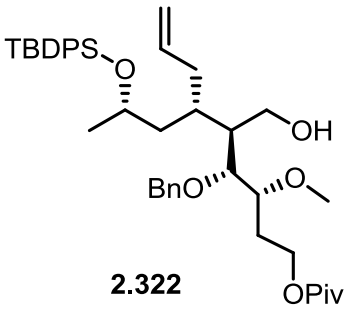
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00-1.07 (m, 33H), 1.19 (s, 9H), 1.45 (quin, *J* = 6.8 Hz, 1H), 1.61-1.74 (m, 3H), 1.82-1.94 (m, 1H), 1.95-2.05 (m, 1H), 2.07-2.13 (m, 1H), 2.14-2.21(m, 1H), 3.36 (s, 3H), 3.55 (d, *J* = 9.2 Hz, 1H), 3.60-3.68 (m, 2H), 3.80 (dd, *J* = 8.4, 1.7 Hz, 1H), 3.92 (sex, *J* = 6.1 Hz, 1H), 4.11-4.17 (m, 1H), 4.21-4.27 (m, 1H), 4.70 (dd, *J* = 143.5, 11.4 Hz, 2H), 4.78-4.82 (m, 2H), 5.42-5.52 (m, 1H) 7.22-7.41 (m, 11H), 7.65-7.67 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.09, 18.27, 19.30, 23.41, 27.13, 27.39, 28.89, 32.98, 35.82, 38.81, 43.12, 46,22, 57.49, 61.90, 62.53, 68.69, 74.02, 77.36, 78.35, 81.37, 115.44, 127.33, 127.50, 127.60, 127.82, 128.27, 129.46, 129.55, 134.67, 135.15, 136.01, 138.82, 139.21, 178.67. H**RMS**: $C_{51}H_{80}O_6NaSi_2$, Calculated: [M+H]+, 867.5391, Found: [M+H]+, 867.5389.



2.323

(3R,4R,5S,6S)-4-(benzyloxy)-6-((S)-2-hydroxypropyl)-3-methoxy-5-(((triisopropyl silyl)oxy)methyl)non-8-en-1-yl Pivalate (2.323). Ether **2.321** (35.0 mg, 0.041 mmol) was suspended in anhydrous dichloromethane (1.0 mL) and added to a solution of 0.01 M HCl in methanol (25 mL) at room temperature. The reaction was stirred for 24 h and was then diluted with dichloromethane and partitioned with water. The solution was extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed under reduced pressure to give a clear residue. This residue was purified by flash chromatography (3:1 Hex:EtOAc) to provide the free secondary alcohol **2.323** as a clear colorless oil (22.5 mg, 91 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.03-1.06 (m, 21H), 1.16 (d, *J* = 6.1 Hz, 3H), 1.20 (s, 9H), 1.25-1.36 (m, 1H), 1.49 (ddd, *J* = 13.8, 10.4, 3.2 Hz, 1H), 1.61-1.70 (m, 1H), 1.76-1.83 (m, 1H), 1.85-1.94 (m, 2H), 2.19-1.25 (m, 1H), 2.58 (dm, *J* = 13.8 Hz, 1H), 3.08 (s, 1H), 3.46 (s, 3H), 3.61 (t, *J* = 9.5 Hz, 1H), 3.69 (dd, *J* = 9.5, 4.6 Hz, 2H), 3.78 (t, *J* = 3.0 Hz, 1H), 3.89-3.97 (m, 1H), 4.12-4.23 (m, 2H), 4.65 (dd, *J* = 94.6, 11.8 Hz, 2H), 4.90-4.95 (m, 2H), 5.64-5.74 (m, 1H), 7.27-7.36 (m, 5H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.06, 18.21, 18.25, 23.25, 27.38, 29.86, 30.31, 31.85, 35.98, 38.88, 41.80, 42.13, 58.31, 61.20, 61.83, 64.47, 73.43, 79.86, 80.17, 115.61, 127.14, 127.75, 127.83, 128.31, 128.54, 138.70, 138.85, 178.59.

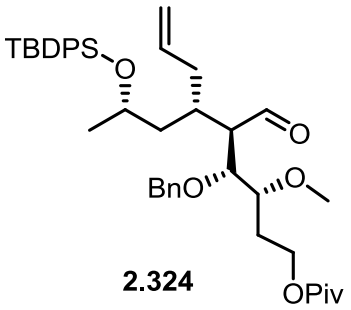


2.322

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-5-(hydroxymethyl)-3-methoxynon-8-en-1-yl Pivalate (2.322). Methyl ether **2.321** (212 mg, 0.251mmol) was suspended in anhydrous dichloromethane (25 mL) in 50 mL flame-dried round bottom flask. Boron trifluoride diethyl etherate (46.5% BF_3, 0.133 mL, 0.502 mmol) was added dropwise at room temperature and the resulting mixture was stirred at room temperature for 0.5 h until TLC showed that the reaction had reached completion. The reaction was quenched by addition of saturated $NaHCO_3$, extracted with dichloromethane (3x), dried over dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (7:3 Hex:EtOAc) to give the free primary alcohol **2.322** as a clear, colorless oil (162 mg, 94%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (s, 9H), 1.03 (d, *J* = 6.2 Hz, 3H), 1.20 (s, 9H), 1.46 (quin, *J* = 6.6 Hz, 1H), 1.61-1.71 (m, 2H), 1.77-1.83 (m, 1H), 1.85 (t, *J* = 5.3 Hz, 1H), 1.88-1.97 (m, 1H), 1.99-2.05 (m, 1H), 2.06-2.12 (m, 1H), 3.39 (s, 3H), 3.51-3.65 (m, 3H), 3.74 (dd, *J* = 8.3, 2.2 Hz, 1H), 3.93 (sex, *J* = 6.1 Hz, 1H), 4.20 (dd, *J* = 7.7, 5.5 Hz, 1H), 4.63 (dd, *J* = 115.4, 11.6 Hz, 2H), 4.84-4.87 (m, 2H), 5.40-5.50 (m, 1H), 7.25-7.42 (m, 11H), 7.65-7.68 (m, 4H).**^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 19.30, 23.15, 27.17, 27.40, 29.56, 33.07, 35.46, 38.88, 42.26, 45.96, 57.98, 60.81, 61.97, 68.79, 73.66, 77.36, 78.98, 80.56, 116.08, 127.55, 127.61, 127.65, 127.98, 128.40, 129.57, 129.65, 134.56, 134.92, 136.03, 137.92, 138.74, 178.70. **HRMS**: $C_{42}H_{60}O_6NaSi$, Calculated:

[M+H]+, 711.4057, Found: [M+H]+, 711.4055.

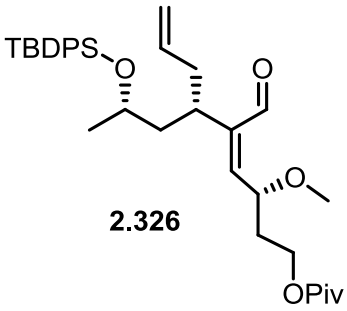


2.324

(3R,4R,5R,6S)-4-(benzyloxy)-6-((S)-2-((tert-butyldiphenylsilyl)oxy)propyl)-5-formyl-3-methoxynon-8-en-1-yl pivalate (2.324). Alcohol **2.322** (170 mg, 0.247 mmol) was suspended in CH_2Cl_2/DMSO (4:1 v/v, 12.5 mL) in a 25 mL flame-dried round bottom flask and cooled to 0 ^oC. Triethylamine (0.34 mL, 2.47 mmol) was added followed by addition of sulfur trioxide pyridine complex (157 mg, 0.987 mmol) in 2 portions. The resulting solution was allowed to warm to room temperature and stirred for 6 h. Additional portions of triethylamine (0.34 mL, 2.47 mmol) and sulfur trioxide pyridine complex (157 mg, 0.987 mmol) were added. The reaction was stirred for 10 h and then quenched by addition water, extracted with dichloromethane (3x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. This oil was purified by flash chromatography (5:1 Hex:EtOAc) to provide aldehyde **2.322** as a clear, colorless oil (127 mg, 75%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.99 (s, 9H), 1.01 (d, J = 6.4 Hz, 3H), 1.18 (s, 9H), 1.50 (ddd, J = 14.4, 7.6, 5.6 Hz, 1H), 1.68-1.78 (m, 2H), 1.80-1.85 (m, 1H), 2.26 (dm, J = 14.0 Hz, 1H), 2.34-2.41 (m, 1H), 2.71 (dt, J = 9.8, 2.8 Hz, 1H), 3.19-3.23 (m, 1H), 3.36 (s, 3H), 3.99 (sex, J = 6.0 Hz, 1H), 4.11-4.20 (m, 3H), 4.66 (dd, J = 123.6, 11.6 Hz, 2H), 4.90-4.97 (m, 2H), 5.53 (ddt, J = 23.0, 9.8, 7.2 Hz, 1H), 7.27-7.41 (m, 6H), 7.64-7.67 (m, 4H), 9.62 (d, J = 2.4 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.34,

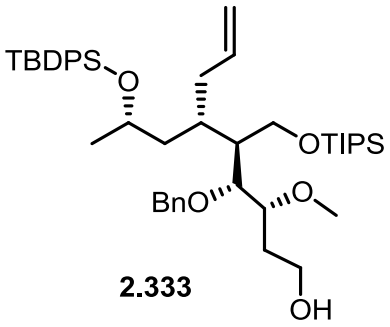
23.44, 27.17, 27.41, 29.35, 32.88, 35.63, 38.90, 41.37, 56.02, 58.04, 61.48, 68.11, 73.99, 75.54, 81.26, 117.09, 127.59, 127.75, 127.99, 128.48, 129.63, 129.76, 134.31, 134.87, 136.06, 136.08, 137.03, 138.38, 178.61, 202.94.



2.326

(3R,6S,Z)-6-((S)-2-((tert-butyldiphenylsilyl)oxy)propyl)-5-formyl-3-methoxynona-4,8-dien-1-yl Pivalate (2.326).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.03 (d, *J* = 6.2 Hz, 3H), 1.04 (s, 9H), 1.18 (s, 9H), 1.69 (quin, *J* = 7.0 Hz, 1H), 1.79-1.87 (m, 2H), 1.97-2.04 (m, 1H), 2.11-2.17 (m, 1H), 2.22-2.30 (m, 1H), 2.52-2.59 (m, 1H), 3.18 (s, 3H), 3.84 (sex, *J* = 6.2 Hz, 1H), 4.10-4.22 (m, 3H), 4.86-4.90 (m, 2H), 5.47 (ddt, *J* = 16.2, 10.6, 7.2 Hz, 1H), 6.24 (d, *J* = 8.8 Hz, 1H), 7.36-7.44 (m, 6H), 7.66-7.68 (m, 4H), 9.34 (d, *J* = 1.6 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.33, 23.64, 27.22, 27.38, 29.90, 34.20, 36.35, 37.65, 38.88, 44.03, 57.13, 60.66, 68.86, 73.94, 116.80, 127.68, 127.77, 127.92, 129.72, 129.79, 134.53, 134.91, 136.02, 136.52, 136.93, 145.12, 170.52, 178.48.

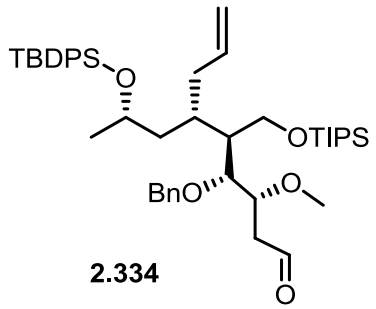


2.333

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-(((triisopropylsilyl)oxy)methyl)non-8-en-1-ol (2.333). Pivalate ester **2.321** (240 mg, 0.284 mmol) was suspended in anhydrous dichloromethane (28 mL) in a 50 mL flame-dried round bottom flask and cooled to -78 ^{o}C. Diisobutylaluminum hydride (1.0 M in dichloromethane, 0.85 mL, 0.852 mmol) was added dropwise, and the resulting solution was stirred for 1 h until TLC indicated the reaction was complete. The reaction was quenched by addition of sodium potassium tartrate, warmed to room temperature, and stirred for 0.5 h until the organic layer was clear. The mixture was extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (2:1 Hex:EtOAc) to provide the desired free primary alcohol **2.333** as a clear, colorless oil (200 mg, 93%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00 (s, 9H), 1.02 (d, *J* = 6.1 Hz, 3H), 1.05 (s, 21H), 1.43 (quin, *J* = 6.8 Hz, 1H), 1.57-1.63 (m, 1H), 1.65-1.74 (m, 2H), 1.75-1.81 (m, 1H), 1.98-2.07 (m, 1H), 2.10-2.16 (m, 2H), 2.87 (d, *J* = 6.3 Hz, 1H), 3.41 (s, 3H), 3.58 (dd, *J* = 10.5, 6.2 Hz, 1H), 3.66 (dd, *J* = 10.7, 3.6 Hz, 1H), 3.71-3.82 (m, 4H), 3.91 (sex, *J* = 6.1 Hz, 1H), 4.69 (dd, *J* = 156.8, 11.4 Hz, 2H), 4.81-4.85 (m, 2H), 5.42-5.52 (m, 1H) 7.23-7.41 (m, 11H), 7.65-7.68 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.05, 18.24, 19.29, 23.45, 27.11, 31.31, 33.03, 35.55, 42.94, 46.47, 57.01, 61.44, 62.08, 68.64,

74.19, 78.20, 84.97, 115.58, 127.46, 127.49, 127.60, 127.97, 128.31, 129.47, 129.56, 134.62, 135.14, 136.01, 138.62, 138.95.



2.334

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-(((triisopropylsilyl)oxy)methyl)non-8-enal (2.334). Alcohol **3.333** (200 mg, 0.263 mmol) was suspended in CH_2Cl_2/DMSO (4:1 v/v, 13 mL) in a 50 mL flame-dried round bottom flask and cooled to 0 ^oC. Triethylamine (0.37 mL, 2.63 mmol) was added followed by addition of sulfur trioxide pyridine complex (167 mg, 1.05 mmol) in 2 portions. The resulting solution was stirred for 4 h at 0 ^oC, quenched by addition water, extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. This oil was purified by flash chromatography (4:1 Hex:EtOAc) to provide aldehyde **2.334** as a clear, colorless oil (185 mg, 93%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.99 (s, 9H), 1.02 (d, *J* = 6.1 Hz, 3H), 1.04-1.06 (m, 21H), 1.41 (quin, *J* = 6.7 Hz, 1H), 1.57-1.71 (m, 3H), 2.04-2.11 (m, 2H), 2.54 (dd, *J* = 16.7, 1.5 Hz, 1H), 2.74 (dd, *J* = 16.7, 3.0 Hz, 1H), 3.39 (s, 3H), 3.57 (dd, *J* = 10.9, 6.8 Hz, 1H), 3.66 (dd, *J* = 10.8, 3.3 Hz, 1H), 3.82 (dd, *J* = 9.0, 1.8 Hz, 1H), 3.90 (sex, *J* = 6.2 Hz, 1H), 4.12 (ddd, *J* = 8.2, 3.7, 2.1 Hz, 1H), 4.67 (dd, *J* = 134.0, 11.4 Hz, 2H), 4.80-4.84 (m, 2H), 5.38-5.48 (m, 1H) 7.22-7.41 (m, 11H), 7.63-7.67 (m, 4H), 9.78 (dd, *J* = 2.8, 1.5 Hz, 1H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.96, 18.22, 19.28, 23.43, 27.09, 32.84, 35.33, 42.82, 43.86, 46.77, 57.19, 61.83, 68.50, 74.30, 78.85, 80.12, 115.76,

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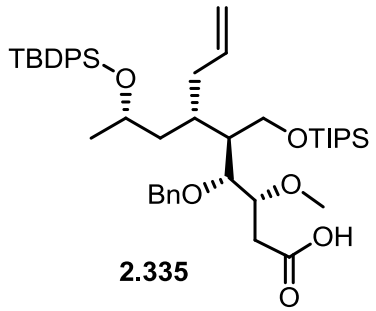
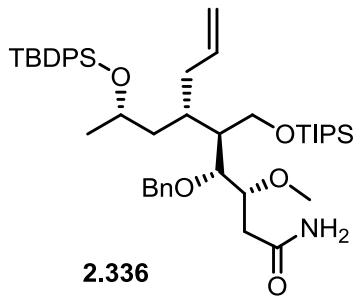


2.335

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-(((triisopropylsilyl)oxy)methyl)non-8-enoic acid (2.335). To a stirring solution of aldehyde **2.334** (182 mg, 0.240 mmol) in *tert*-butanol (9 mL) was added 2-methyl-2-butene (0.26 mL, 2.40 mmol). In a separate vial, monobasic sodium phosphate (252 mg, 1.82 mmol) was dissolved in water (9 mL) and sodium chlorite (163 mg, 1.80 mmol) was added in 2 portions. The *tert*-butanol solution was cooled to 0 ^{o}C and the chlorite/phosphate solution in water was added dropwise. The resulting yellow solution was stirred at 0 ^{o}C for 0.5 h and was then quenched with saturated sodium thiosulfate and acidified to pH = 3. The aqueous solution was then extracted with ethyl acetate (4 x), dried over Na_2SO_4, and concentrated *in vacuo* to give a clear oil. This oil was purified by flash chromatography (1:1 Hex:EtOAc) yielding carboxylic acid **2.335** as a clear, colorless oil (180 mg, 97%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00 (s, 9H), 1.02 (d, *J* = 6.3 Hz, 3H), 1.04-1.06 (m, 21H), 1.43 (quin, *J* = 6.7 Hz, 1H), 1.59-1.73 (m, 3H), 2.05-2.14 (m, 2H), 2.59 (dd, *J* = 16.5, 3.0 Hz, 1H), 2.73 (dd, *J* = 16.3, 8.7 Hz, 1H), 3.60 (s, 3H), 3.57 (dd, *J* = 10.4, 6.4 Hz, 1H), 3.67 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.83 (dd, *J* = 9.0, 1.8 Hz, 1H), 3.90 (sex, *J* = 6.1 Hz, 1H), 4.05 (dt, *J* = 8.2, 2.2 Hz, 1H), 4.68 (dd, *J* = 141.9, 11.4 Hz, 2H), 4.81-4.85 (m,

2H), 5.41-5.51 (m, 1H) 7.23-7.41 (m, 11H), 7.64-7.67 (m, 4H).[13]**C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.02, 18.22, 19.27, 23.42, 27.11, 32.94, 35.08, 35.55, 42.86, 46.51, 57.47, 61.78, 68.56, 74.49, 77.35, 78.42, 80.90, 115.73, 127.50, 127.54, 127.63, 127.99, 128.34, 129.49, 129.61, 134.55, 135.08, 135.99, 136.01, 138.43, 138.67, 176.17.

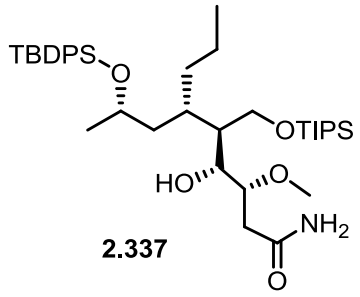


2.336

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-(((triisopropylsilyl)oxy)methyl)non-8-enamide (2.336). Carboxylic acid **2.335** (225 mg, 0.290 mmol) was suspended in N,N-dimethylformamide (1.5 mL). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (83.4 mg, 0.44 mmol) and 1-Hydroxybenzotriazole (58.8 mg, 0.44 mmol) were added subsequently at room temperature, followed by addition of diisopropylethylamine (0.20 mL, 1.16 mmol) and ammonium chloride (31 mg, 0.580 mmol). The resulting solution was stirred for 6 h at room temperature until TLC indicated complete conversion. The reaction was quenched by addition of water, extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow residue. This residue was purified by flash chromatography (1:1 Hex/EtOAc then 1:3 Hex/EtOAc) to give amide **2.336** as a clear colorless oil (180 mg, 80%).

[1]H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00 (s, 9H), 1.02 (d, *J* = 6.1 Hz, 3H), 1.04-1.07 (m, 21H), 1.44 (quin, *J* = 6.8 Hz, 1H), 1.63-1.73 (m, 3H), 2.01-2.08 (m, 1H), 2.16-2.22 (m, 1H), 2.43 (dd, *J* = 15.8, 3.0 Hz, 1H), 2.53 (dd, *J* = 15.8, 8.4 Hz, 1H), 3.41 (s, 3H),

3.62-3.71 (m, 2H), 3.86-3.95 (m, 3H), 4.67 (dd, *J* = 124.8, 11.5 Hz, 2H), 4.79-4.84 (m, 2H), 5.16 (bs, 1H), 5.42-5.52 (m, 1H), 5.98 (bs, 1H), 7.23-7.41 (m, 11H), 7.64-7.66 (d, *J* = 7.6 Hz, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 12.04, 18.28, 19.30, 23.46, 27.11, 33.06, 35.82, 36.42, 43.08, 46.05, 57.21, 61.71, 68.63, 74.32, 78.21, 81.02, 115.63, 127.48, 127.53, 127.63, 127.76, 128.36, 129.51, 129.58, 134.68, 135.03, 136.00, 138.64, 138.90, 174.12.

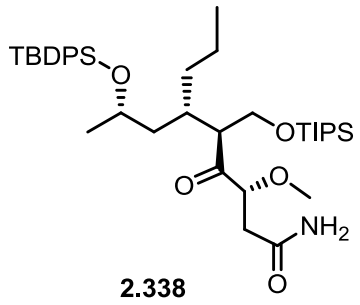


2.337

(3*R*,4*R*,5*S*,6*S*,8*S*)-8-((*tert*-butyldiphenylsilyl)oxy)-4-hydroxy-3-methoxy-6-propyl-5-(((triisopropylsilyl)oxy)methyl)nonanamide (2.337). Amide **2.336** (180 mg, 0.232 mmol) was suspended in anhydrous ethyl acetate (23 mL) in a 50 mL round bottom flask. 10% palladium on activated carbon (62 mg, 0.058 mmol), was added and the flask was sealed and evacuated then filled with argon (3 x). The flask was then evacuated and refilled with hydrogen (3 x) from a balloon. The reaction was stirred for 8 h at room temperature until reaction was complete by TLC. The mixture was filtered through celite and washed through liberally with ethyl acetate. The filtrate was condensed *in vacuo* to give alcohol **2.337** as a clear colorless oil that was carried on to the next step without further purification (158 mg, 99%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.77 (t, *J* = 7.2 Hz, 3H), 0.83-0.92 (m, 2H), 1.03-1.08 (m, 33H), 1.33-1.40 (m, 1H), 1.41-1.47 (m, 1H), 1.59-1.65 (m, 2H), 1.84-1.90 (m, 1H), 2.51 (d, *J* = 5.5 Hz, 2H), 2.68 (d, *J* = 2.7 Hz, 1H), 3.39 (s, 3H), 3.66-3.74 (m, 2H),

3.82 (dd, *J* = 10.9, 5.1 Hz, 1H), 3.88 (dd, *J* = 13.5, 6.1 Hz, 1H), 3.93-3.97 (m, 1H), 5.20 (bs, 1H), 6.01 (bs, 1H), 7.35-7.44 (m, 6H), 7.67-7.72 (m, 4H).

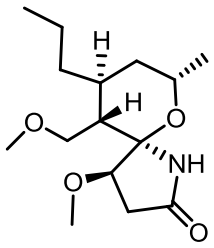


2.338

(3*R*,5*S*,6*S*,8*S*)-8-((*tert*-butyldiphenylsilyl)oxy)-3-methoxy-4-oxo-6-propyl-5-(((triisopropylsilyl)oxy)methyl)nonanamide (2.338). Alcohol **2.337** (152 mg, 0.222 mmol) was suspended in anhydrous dichloromethane (22 mL) in a flame-dried 50 mL round bottom flask with 4 angstrom molecular sieves (100 mg). The reaction was cooled to 0 ^{o}C and 4-methylmorpholine N-oxide (52 mg, 0.443 mmol) was added followed by tetrapropylammonium perruthenate (15.6 mg, 0.044 mmol). The reaction was warmed to room temperature and stirred for 2 h. Upon complete conversion as indicated by TLC, the mixture was filtered through celite and washed through liberally with dichloromethane. The filtrate was condensed *in vacuo* to give a dark green residue that was purified by flash chromatography (1:3 Hex/EtOAc) to give hydroxyketoamide **2.338** as a clear, colorless oil (129 mg, 85 %).

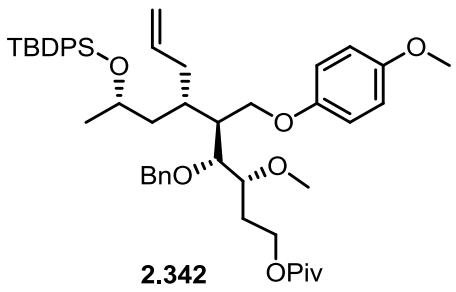
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.67 (t, *J* = 6.8 Hz, 3H), 0.79-0.93 (m, 3H), 1.02 (s, 30H), 1.09 (d, *J* = 6.1 Hz, 3H), 1.25 (ddd, *J* = 13.7, 8.1, 4.1 Hz, 2H), 1.41 (ddd, *J* = 13.7, 8.9, 4.6 Hz, 1H), 1.65-1.73 (m, 1H), 2.37 (dd, *J* = 14.8, 8.1 Hz, 1H), 2.59 (dd, *J* = 15.3, 4.1 Hz, 1H), 3.00 (ddd, *J* = 8.7, 6.8, 4.8 Hz, 1H), 3.38 (s, 3H), 3.70 (dd, *J* = 9.5, 4.7 Hz, 1H), 3.78-3.83 (m, 2H), 4.17 (dd, *J* = 8.1, 4.0 Hz, 1H), 5.27 (bs, 1H), 5.86 (bs, 1H), 7.34-7.43 (m, 6H), 7.65-7.68 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 11.97,

14.52, 18.10, 18.76, 19.28, 22.83, 27.11, 32.26, 33.98, 37.04, 42.26, 53.06, 58.29, 63.55, 68.24, 83.87, 127.67, 127.70, 129.68, 134.65, 135.98, 136.02, 171.99, 212.41. **HRMS**: $C_{39}H_{66}NO_5Si_2$, Calculated: [M+H]+, 684.4480, Found: [M+H]+, 684.4485.



2.339

(4R,5R,7S,9S,10S)-4-methoxy-10-(methoxymethyl)-7-methyl-9-propyl-6-oxa-1-azaspiro[4.5]decan-2-one (2.339). Hydroxyketoamide **2.338** (68 mg, 0.099 mmol) was suspended in anhydrous methanol (30 mL). Concentrated HCl (0.026 mL, 0.3 mmol) was added at room temperature and the reaction was allowed to stir for 10 h. After this time the reaction was quenched by dropwise addition of $NaHCO_3$ and extracted with ethyl acetate (3 x) The organic layers were combined, dried over Na_2SO_4, and condensed *in vacuo* to give a clear, colorless residue. The crude residue was purified by flash chromatograpy (1:6 Hex:EtOAc) to yield the desired marineosin model stereoisomer **2.339** as a clear, colorless oil (22 mg, 82%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 0.89-0.94 (m, 5H), 1.14-1.16 (m, 3H), 1.20 (d, *J* = 6.6 Hz, 3H), 1.93-1.96 (m, 1H), 2.34-2.37 (m, 1H) 2.52 (m, 2H) 3.22 (dd, *J* = 10.4 , 4.4 Hz, 1H), 3.28-3.31 (m, 1H), 3.31 (s, 3H), 3.44 (s, 3H), 3.74-3.79 (m, 1H), 4.15 (dd, *J* = 9.6, 8.0 Hz, 1H), 6.23 (bs, 1H).). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 14.0, 22.2, 26.0, 30.6, 35.2, 40.6, 55.9, 58.2, 62.0, 65.4, 70.4, 72.7, 79.2, 90.3, 175.3.

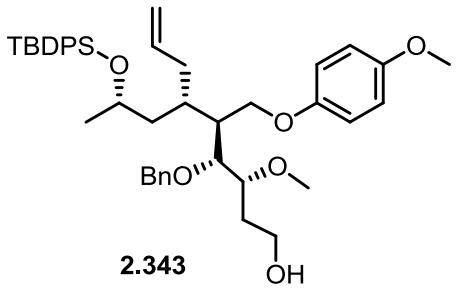


2.342

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-((4-methoxyphenoxy)methyl)non-8-en-1-yl Pivalate (2.342). A 15-20 mL microwave vial was charged with triphenylphosphine (298 mg, 1.14 mmol) in anhydrous tetrahydrofuran (18 mL) and cooled to 0 ^{o}C. 4-methoxy phenol (195 mg, 1.57 mmol) was added followed by dropwise addition of diisopropylazodicarboxylate (0.21 mL, 1.07 mmol). The resulting solution was stirred at 0 ^{o}C for 15 min and alcohol **2.322** (300 mg, 0.435 mmol) was added dropwise as a solution in tetrahydrofuran (1.8 mL). The reaction was warmed to room temperature, stirred for 15 minutes, and then transferred to a heating mantle preheated to 80 ^{o}C. After 0.5 h, the reaction was determined to be complete by TLC and was then cooled to room temperature and quenched with saturated $NaHCO_3$. The mixture was extracted with ethyl acetate (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. This oil was purified by flash chromatography (13:7 Hex:EtOAc) to provide the desired *p*-methoxyphenyl ether **2.342** as a clear, colorless oil (300 mg, 87%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.01 (s, 9H), 1.04 (d, *J* = 10.5 Hz, 3H), 1.12 (s, 9H), 1.46 (quin, *J* = 6.9 Hz, 1H), 1.85-1.94 (m, 1H), 1.95-2.02 (m, 1H), 2.04-2.08 (m, 1H), 2.18-2.29 (m, 2H), 3.35 (s, 3H), 3.49 (d, *J* = 9.4 Hz, 1H), 3.77 (s, 3H), 3.78-3.82 (m, 1H), 3.87-3.97 (m, 1H), 3.99 (d, *J* = 9.3 Hz, 1H), 4.20 (dd, *J* = 7.6, 5.4 Hz, 2H), 4.71 (dd, *J* = 143.5, 11.6 Hz, 2H), 4.84-4.90 (m, 2H), 5.46-5.56 (m, 1H), 6.78 (dd, *J* = 28.8,

9.1 Hz, 4H), 7.24-7.40 (m, 11H), 7.65-7.68 (m, 4H).**^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 19.29, 23.17, 27.16, 27.33, 28.88, 33.14, 35.71, 38.80, 42.25, 43.21, 55.92, 57.70, 62.08, 66.39, 68.80, 74.11, 77.50, 80.70, 114.81, 115.29, 115.84, 127.46, 127.52, 127.64, 127.97, 128.33, 129.52, 129.61, 134.55, 134.98, 136.05, 138.49, 139.08, 152.83, 154.04, 178.67. **HRMS**: $C_{49}H_{66}O_7NaSi_2$, Calculated: [M+H]+, 817.4476, Found: [M+H]+, 817.4479.

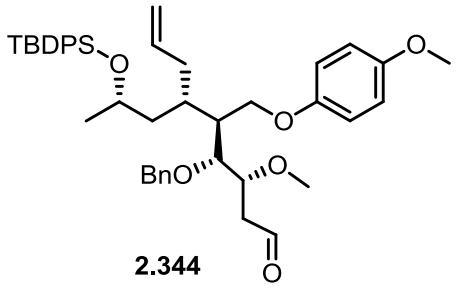


2.343

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-((4-methoxyphenoxy)methyl)non-8-en-1-ol (2.343). *p*-Methoxyphenyl ether **2.342** (290 mg, 0.365 mmol) was suspended in anhydrous dichloromethane (36.5 mL) in a 100 mL flame-dried round bottom flask and cooled to -78 ^{o}C. Diisobutylaluminum hydride (1.0 M in dichloromethane, 1.10 mL, 1.094 mmol) was added dropwise, and the resulting solution was stirred for 0.5 h until TLC indicated the reaction was complete. The reaction was quenched by addition of sodium potassium tartrate, warmed to room temperature, and stirred for 0.5 h until the organic layer was clear. The mixture was extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a clear oil. This oil was purified by flash chromatography (13:7 Hex:EtOAc) to provide the desired free primary alcohol **2.343** as a clear, colorless oil (240 mg, 93%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00 (s, 9H), 1.04 (d, *J* = 10.1 Hz, 3H), 1.45 (quin,

J = 7.0 Hz, 1H), 1.62-1.70 (m, 2H), 1.77-1.84 (m, 1H), 1.98-2.10 (m, 2H), 2.19-2.25 (m, 2H), 2.59 (dd, *J* = 7.1, 4.0 Hz, 1H), 3.38 (s, 3H), 3.61 (d, *J* = 8.6 Hz, 1H), 3.73-3.83 (m, 4H), 3.77 (s, 3H), 3.92-3.98 (m, 2H), 4.70 (dd, *J* = 146.2, 11.6 Hz, 2H), 4.85-4.90 (m, 2H), 5.46-5.56 (m, 1H), 6.79 (dd, *J* = 33.3, 9.2 Hz, 4H), 7.24-7.41 (m, 11H), 7.65-7.68 (m, 4H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.29, 23.22, 27.14, 31.59, 33.05, 35.53, 42.12, 43.35, 55.90, 57.22, 61.37, 66.59, 68.79, 74.15, 77.35, 84.28, 114.84, 115.27, 115.94, 127.52, 127.55, 127.62, 128.05, 128.36, 129.52, 129.59, 134.55, 134.96, 136.04, 138.29, 138.84, 152.70, 154.09. **HRMS**: $C_{44}H_{59}O_6Si$, Calculated: [M+H]+, 711.4081, Found: [M+H]+, 711.4080.

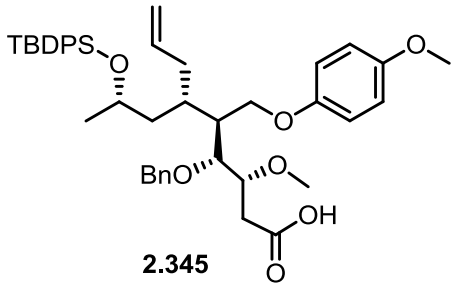


2.344

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-((4-methoxyphenoxy)methyl)non-8-enal. Alcohol **3.343** (220 mg, 0.309 mmol) was suspended in CH_2Cl_2/DMSO (4:1 v/v, 15.5 mL) in a 50 mL flame-dried round bottom flask and cooled to 0 ^oC. Triethylamine (0.43 mL, 3.09 mmol) was added followed by addition of sulfur trioxide pyridine complex (197 mg, 1.24 mmol) in 2 portions. The resulting solution was stirred for 5 h at 0 ^oC, quenched by addition water, extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. This oil was purified by flash chromatography (3:1 Hex:EtOAc) to provide aldehyde **2.344** as a clear, colorless oil (200 mg, 91%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.01 (s, 9H), 1.04 (d, *J* = 6.7 Hz, 3H), 1.45 (quin, *J*

= 6.9 Hz, 1H), 1.62-1.71 (m, 2H), 2.01-2.06 (m, 1H), 2.15-2.20 (m, 2H), 2.55-2.63 (m, 1H), 2.78 (ddd, *J* = 16.8, 8.0, 2.6 Hz, 1H), 3.35 (s, 3H), 3.73 (dd, *J* = 10.0, 6.3 Hz, 1H), 3.77 (s, 3H), 3.84 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.90-3.98 (m, 3H), 4.69 (dd, *J* = 126.4, 11.6 Hz, 2H), 4.83-4.89 (m, 2H), 5.42-5.52 (m, 1H), 6.79 (dd, *J* = 35.2, 9.0 Hz, 4H), 7.24-7.41 (m, 11H), 7.64-7.68 (m, 4H), 9.79 (t, *J* = 2.0 Hz, 1H).**^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 19.30, 23.16, 27.16, 32.85, 35.36, 41.92, 43.58, 44.24, 55.91, 57.39, 66.32, 68.75, 74.31, 78.44, 79.65, 114.85, 115.31, 116.14, 127.55, 127.64, 127.65, 128.05, 128.40, 129.57, 129.64, 134.56, 134.90, 136.04, 137.93, 138.63, 152.61, 154.13, 201.81. **HRMS**: $C_{44}H_{56}O_6NaSi$, Calculated: [M+H]+, 731.3744, Found: [M+H]+, 731.3745.

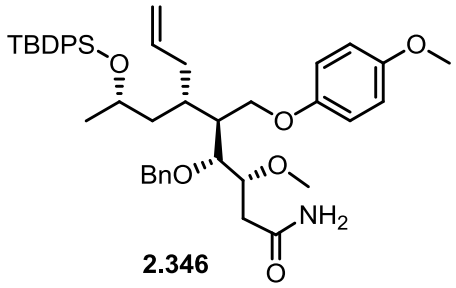


2.345

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-((4-methoxyphenoxy)methyl)non-8-enoic acid (2.345). To a stirring solution of aldehyde **2.344** (195 mg, 0.275 mmol) in *tert*-butanol (14 mL) was added 2-methyl-2-butene (0.29 mL, 2.75 mmol). In a separate vial, monobasic sodium phosphate (289 mg, 2.09 mmol) was dissolved in water (14 mL) and sodium chlorite (187 mg, 2.06 mmol) was added in 2 portions. The *tert*-butanol solution was cooled to 0 ^{o}C and the chlorite/phosphate solution in water was added dropwise. The resulting yellow solution was stirred at 0 ^{o}C for 1 h and was then quenched with saturated sodium thiosulfate and acidified to pH = 3. The aqueous solution was then extracted with ethyl acetate (4 x), dried over Na_2SO_4, and concentrated *in vacuo* to give a clear oil. This oil was purified by

flash chromatography (1:1 Hex:EtOAc) yielding carboxylic acid **2.345** as a clear, colorless oil (188 mg, 96%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00 (s, 9H), 1.02 (d, *J* = 6.2 Hz, 3H), 1.42-1.49 (m, 2H), 1.62-1.71 (m, 2H), 2.01-2.07 (m, 1H), 2.15-2.20 (m, 2H), 2.66 (dd, *J* = 16.3, 3.3 Hz, 1H), 2.78 (dd, *J* = 16.2, 8.6 Hz, 1H), 3.40 (s, 3H), 3.73 (dd, *J* = 9.8, 6.0 Hz, 1H), 3.77 (s, 3H), 3.85 (dd, *J* = 9.8, 3.1 Hz, 1H), 3.88-3.99 (m, 3H), 4.70 (dd, *J* = 131.4, 11.6 Hz, 2H), 4.85-4.90 (m, 2H), 5.42-5.53 (m, 1H), 6.79 (dd, *J* = 25.7, 9.0 Hz, 4H), 7.27-7.41 (m, 11H), 7.65 (d, *J* = 7.6 Hz, 4H). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 19.28, 23.17, 29.85, 32.86, 34.83, 35.37, 41.97, 43.49, 55.90, 57.58, 66.19, 68.70, 74.57, 77.85, 80.78, 114.83, 115.36, 116.20, 127.56, 127.67, 127.74, 128.13, 128.44, 129.58, 129.67, 134.51, 134.88, 136.03, 136.05, 137.90, 138.39, 152.59, 154.16, 173.69. **HRMS**: $C_{44}H_{57}O_7Si$, Calculated: [M+H]+, 725.3874, Found: [M+H]+, 725.3874.

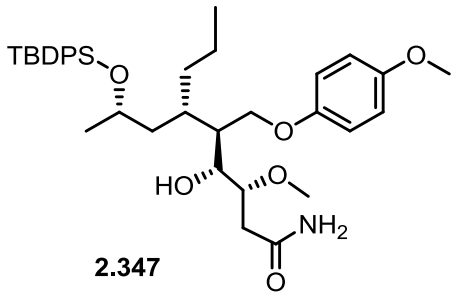


2.346

(3*R*,4*R*,5*S*,6*S*)-4-(benzyloxy)-6-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-((4-methoxyphenoxy)methyl)non-8-enamide (2.346). Carboxylic acid **2.345** (170 mg, 0.234 mmol) was suspended in N,N-dimethylformamide (2.5 mL). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (135 mg, 0.703 mmol) and 1-Hydroxybenzotriazole (95 mg, 0.703 mmol) were added subsequently at room temperature, followed by addition of diisopropylethylamine (0.33 mL, 1.875 mmol) and ammonium chloride (50 mg, 0.937 mmol). The resulting solution was stirred for 6 h at

room temperature until TLC indicated complete conversion. The reaction was quenched by addition of water, extracted with dichloromethane (3 x), dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow residue. This residue was purified by flash chromatography (1:1 Hex/EtOAc then 1:3 Hex/EtOAc) to give amide **2.346** as a clear colorless oil (156 mg, 92%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.01 (s, 9H), 1.03 (d, *J* = 6.2 Hz, 3H), 1.45 (quin, *J* = 6.7 Hz, 1H), 1.64-1.72 (m, 2H), 2.00-2.05 (m, 1H), 2.13-2.25 (m, 2H), 2.47 (dd, *J* = 15.7, 2.8 Hz, 1H), 2.56 (dd, *J* = 15.6, 8.5 Hz, 1H), 3.39 (s, 3H), 3.74-3.78 (m, 1H), 3.77 (s, 3H), 3.83-3.90 (m, 2H), 3.93 (sex, *J* = 6.1 Hz, 1H), 4.00 (dd, *J* = 8.9, 1.6 Hz, 1H), 3.88-3.99 (m, 3H), 4.70 (dd, *J* = 127.3, 11.6 Hz, 2H), 4.84-4.88 (m, 2H), 5.20 (bs, 1H), 5.45-5.55 (m, 1H), 5.90 (bs, 1H), 6.80 (dd, *J* = 18.1, 9.2 Hz, 4H), 7.26-7.41 (m, 11H), 7.64-7.66 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 19.31, 23.31, 27.17, 33.16, 35.64, 36.81, 42.38, 43.40, 55.90, 57.47, 66.39, 68.74, 74.25, 77.37, 77.72, 81.19, 114.84, 115.46, 116.02, 127.56, 127.61, 127.66, 127.96, 128.41, 129.56, 129.63, 134.63, 134.95, 136.04, 138.19, 138.77, 152.70, 154.12, 174.19. **HRMS**: $C_{44}H_{58}NO_6Si$, Calculated: [M+H]+, 724.4033, Found: [M+H]+, 724.4032.

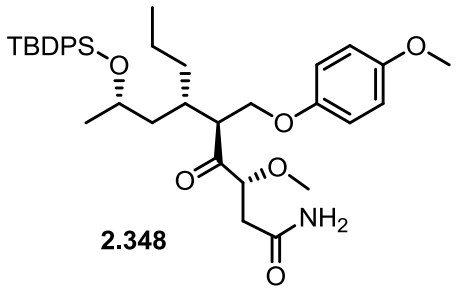


2.347

(3*R*,4*R*,5*S*,6*S*,8*S*)-8-((*tert*-butyldiphenylsilyl)oxy)-4-hydroxy-3-methoxy-5-((4-methoxyphenoxy)methyl)-6-propylnonanamide (2.347). Amide **2.346** (154 mg, 0.213 mmol) was suspended in anhydrous ethyl acetate (16 mL) in a 15-20 mL microwave vial.

10% Palladium on activated carbon was added and the vial was sealed and evacuated then filled with argon (3 x). The vial was then evacuated and refilled with hydrogen (3 x) from a balloon fit with a needle adapter and needle to pierce the septum. The reaction was stirred for 8 h at room temperature until reaction was complete by TLC. The mixture was filtered through celite and washed through liberally with ethyl acetate. The filtrate was condensed *in vacuo* to give alcohol **2.347** as a clear colorless oil that was carried on to the next step without further purification (132 mg, 98%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.83 (t, *J* = 7.32 Hz, 3H), 1.04 (s, 9H), 1.07 (d, *J* = 6.1 Hz, 3H), 1.37-1.49 (m, 2H), 1.63 (dt, *J* = 13.4, 5.6 Hz, 1H), 1.89-1.94 (m, 1H), 2.08-2.15 (m, 1H), 2.32 (d, *J* = 2.6 Hz, 1H), 2.47 (dd, *J* = 14.8, 8.8 Hz, 1H), 2.57 (dd, *J* = 15.2, 2.9 Hz, 1H), 2.38 (s, 3H), 3.69 (dd, *J* = 9.7, 7.5 Hz, 1H), 3.73-3.77 (m, 1H), 3.76 (s, 1H), 3.87 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.91 (sex, *J* = 6.2 Hz, 1H), 4.01 (dt, *J* = 9.4, 2.8 Hz, 1H), (quin, *J* = 6.7 Hz, 1H), 5.23 (bs, 1H), 5.81 (bs, 1H), 6.80 (s, 4H), 7.34-7.42 (m, 6H), 7.67-7.72 (m, 4H). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 14.64, 19.35, 21.31, 23.77, 27.17, 29.84, 32.79, 32.82, 35.61, 42.64, 43.52, 55.89, 57.31, 66.73, 68.82, 70.44, 80.45, 114.82, 115.31, 127.51, 127.63, 129.53, 129.62, 134.50, 135.05, 136.09, 136.14, 152.74, 154.10, 174.09. **HRMS**: $C_{37}H_{54}NO_6Si$, Calculated: [M+H]+, 636.3720, Found: [M+H]+, 636.3723.

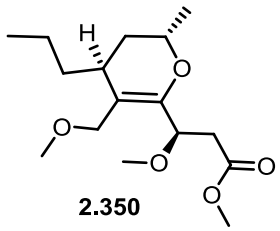


2.348

(3*R*,5*S*,6*S*,8*S*)-8-((*tert*-butyldiphenylsilyl)oxy)-3-methoxy-5-((4-methoxyphenoxy)

methyl)-4-oxo-6-propylnonanamide (2.348) Alcohol **2.347** (128 mg, 0.201 mmol) was suspended in anhydrous dichloromethane (20 mL) in a flame-dried 50 mL round bottom flask with 4 angstrom molecular sieves (150 mg). The reaction was cooled to 0 ^{o}C and 4-methylmorpholine N-oxide (48 mg, 0.403 mmol) was added followed by tetrapropylammonium perruthenate (7.1 mg, 0.0201 mmol) and the reaction was stirred for 1 h at 0 ^{o}C. After 1 h, the solution was warmed to room temperature and stirred for 3 h when complete conversion was indicated by TLC. The mixture was filtered through celite and washed through liberally with dichloromethane. The filtrate was condensed *in vacuo* to give a dark green residue that was purified by flash chromatography (1:3 Hex/EtOAc) to give hydroxyketoamide **2.348** as a clear, colorless oil (112 mg, 88 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.71 (t, *J* = 7.1 Hz, 3H), 0.85- 0.98 (m, 3H), 1.03 (s, 9H), 1.09-1.20 (m, 2H), 1.12 (d, *J* = 6.1 Hz, 3H), 1.37-1.49 (m, 2H), 1.34 (ddd, *J* = 13.7, 7.8, 5.2 Hz, 1H), 1.49 (ddd, *J* = 13.4, 8.3, 5.2 Hz, 1H), 1.82-1.90 (m, 1H), 2.45 (dd, *J* = 14.7, 7.8 Hz, 1H), 2.62 (dd, *J* = 15.0, 4.6 Hz, 1H), 3.35- 3.43 (m, 1H), 3.38 (s, 3H), 3.75 (s, 1H), 3.85-3.91 (m, 2H), 4.06 (t, *J* = 8.8 Hz, 1H), 4.24 (dd, *J* = 7.5, 4.7 Hz, 1H), 5.25 (bs, 1H), 5.80 (bs, 1H), 6.76 (dd, *J* = 23.0, 8.4 Hz, 4H), 7.34-7.42 (m, 6H), 7.66-7.68 (m, 4H). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 14.50, 19.03, 19.26, 22.84, 27.10, 27.14, 32.37, 34.11, 37.00, 42.06, 49.94, 55.84, 58.28, 68.18, 68.30, 83.50, 114.78, 115.59, 127.66, 127.71, 129.70, 129.71, 134.55, 135.98, 136.00, 152.61, 154.26, 171.86, 211.75. **HRMS**: $C_{37}H_{52}NO_6Si$, Calculated: [M+H]+, 634.3564, Found: [M+H]+, 634.3560.

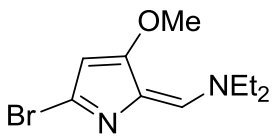


2.350

(R)-methyl 3-methoxy-3-((2S,4S)-5-(methoxymethyl)-2-methyl-4-propyl-3,4-dihydro-2H-pyran-6-yl)propanoate (2.350). Hydroxyketoamide **2.348** was suspended (53 mg, 0.0836 mmol) in anhydrous methanol (25 mL) in a 100 mL round bottom flask. Concentrated hydrochloric acid (0.041 mL, 0.502 mmol) was added dropwise at room temperature and the resulting solution was stirred for 16 h until TLC confirmed complete conversion. The mixture was quenched with $NaHCO_3$ and extracted with dichloromethane (3 x). The organic layers were combined, dried over Na_2SO_4, and condensed *in vacuo* to give a pale yellow oil. The crude oil was purified by flash chromatograpy (1:2 Hex:EtOAc) to yield the undesired elimination product **2.250** as a clear, colorless oil (19.3 mg, 77%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.91 (t, *J* = 7.2 Hz, 3H), 1.09-1.25 (m, 2H), 1.29 (d, *J* = 6.0 Hz, 3H), 1.37-1.49 (m, 2H), 1.51-1.58 (m, 1H), 1.71 (dt, *J* = 13.7, 2.0 Hz, 1H), 2.14-2.19 (m, 1H), 2.57 (dd, *J* = 15.1, 5.5 Hz, 1H), 2.78 (dd, *J* = 15.1, 8.0 Hz, 1H) 3.30 (s, 3H), 3.32 (s, 3H), 3.66 (s, 3H), 3.84-3.90 (m, 1H), 3.95 (dd, *J* = 33.1, 11.5 Hz, 2H), 4.54 (dd, *J* = 8.0, 5.7 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ (ppm): 14.33, 20.46, 21.32, 32.76, 33.29, 36.58, 38.86, 51.75, 56.38, 57.95, 67.83, 69.69, 73.60, 112.64, 149.07, 171.88.

5.3. Total Synthesis of Tambjamine K and a Library of Unnatural Analogs.

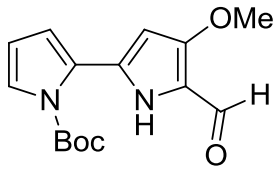


3.17

(*E*)-*N*-((5-bromo-3-methoxy-2*H*-pyrrol-2-ylidene)methyl)-*N*-ethylethanamine (3.17). A 100 mL round bottom flask was charged with N,N-diethylformamide (2.86 g, 28.29 mmol) and 23 mL of dichloromethane. The mixture was cooled to 0 ^{o}C, and a solution of phosphorous oxybromide (10.14 g, 35.36 mmol) in dichloromethane (7 mL) was added slowly over 20 minutes. After addition was complete, the reaction mixture was stirred for an additional 20 minutes. A solution of 4-methoxy-3-pyrrolin-2-one (**7**) (2.00 g, 17.68 mmol) in dichloromethane (18 mL) was added dropwise over 10 minutes, and the mixture was stirred for an additional 20 minutes. The flask was then removed from the ice bath, transferred to an oil bath, and refluxed (42 ^{o}C) for 3.5 hours. The reaction mixture was then transferred to a 500 mL round bottom flask, cooled to 0 ^{o}C, and quenched by dropwise addition of water (20 mL). Sodium hydroxide (3.0 M in H_2O, 230 mL) was slowly added and the mixture was stirred for an additional 20 minutes. The layers were separated and the aqueous layer was extracted with dichloromethane (3 x). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The resulting yellow oil was subjected to flash chromatography (4:1 Hex:EtOAc) to give **3.16** as a tan solid (2.67 g, 59%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.29 (t, *J* = 7.2 Hz, 3H), 1.30 (t, *J* = 7.2 Hz, 3H), 3.40 (q, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 5.59 (s, 1H), 6.99 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 12.4, 14.5, 44.5, 51.0, 57.9, 96.4, 120.7, 133.6, 138.5, 165.3. **IR** (KBr) υ_{max} 2975, 2934, 1629, 1529, 1408, 1290, 1264, 1195, 1116, 1072, 906, 737 cm^{-1}. **HRMS**: $C_{10}H_{16}N_2OBr$, Calculated: [M+H]+, 259.0446, Found: [M+H]+, 259.0448.

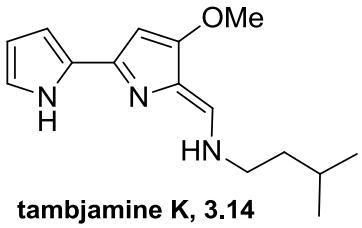


3.19

***tert*-butyl 5'-formyl-4'-methoxy-1*H*,1'*H*-2,2'-bipyrrole-1-carboxylate (3.19).** $Pd(PPh_3)_4$ was generated *in situ* by adding triphenylphosphine (1.22 g, 4.64 mmol) to a magnetically stirred suspension of Palladium II Acetate (0.23 g, 1.03 mmol) in degassed toluene (5.0 mL) then heating the ensuing mixture at 70 ^oC for 20 minutes under an atmosphere of argon. A solution of N-Boc-pyrrole-2-boronic acid (3.26 g, 15.46 mmol) and bromoenamine **3.17** (2.67 g, 10.31 mmol) in H_2O/1,4-dioxane (1:9 v/v, 86 mL) was degassed, purged with argon gas and added to the solution of $Pd(PPh_3)_4$ in toluene. Anhydrous sodium carbonate (3.28 g, 30.93 mmol) was added and the reaction mixture stirred at 85 ^oC. After 3.5 hours, the mixture was cooled and poured into water (150 mL). The solution was reduced to pH 7 using 2 M HCl, partitioned with dichloromethane, and extracted (4 x). The organic layers were combined, dried over sodium sulfate, and condensed *in vacuo* to give a brown residue that was purified by flash chromatography (4:1 Hex:EtOAc) affording aldehyde **3.19** as an orange solid (1.44 g, 48%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.61 (s, 9H), 3.88 (s, 3H), 6.07 (d, *J* = 3.5 Hz, 1H), 6.24 (t, *J* = 3.5 Hz, 1H) 6.66 (dd, *J* = 3.5 Hz, 1.76 Hz, 1H), 7.33 (dd, *J* = 3.4, 1.80 Hz, 1H), 9.53 (s, 1H), 10.73 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 27.8, 57.8, 85.7,

94.7, 111.4, 116.8, 118.2, 124.5, 125.9, 130.2, 149.6, 157.6, 174.3. **IR** (KBr) υ_{max} 3221, 2979, 2833, 1735, 1623, 1549, 1502, 1433, 1370, 1330, 1287, 1255, 1140, 1021 cm^{-1}. **HRMS**: $C_{15}H_{19}N_2O_4$, Calculated: [M+H]+ 291.1345, Found: [M+H]+ 291.1345.

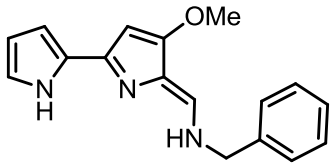


tambjamine K, 3.14

Tambjamine K (3.14). Isopentylamine (36.0 mg, 0.414 mmol) was added to a stirred suspension of aldehyde **3.19** (100 mg, 0.345 mmol) in methanol (5.0 mL) at room temperature, followed by 0.87 M HCl in methanol (0.6 mL). After 6 hours, the reaction was quenched with saturated sodium bicarbonate, extracted with dichloromethane (3 x), and washed with brine (1 x). The organic layers were combined, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a dark brown residue. This residue was purified by reverse phase chromatography using acetonitrile and 0.1% TFA/water (gradient: 15:85 to 55:45) to give **Tambjamine K** as an orange oil that solidified upon standing (58 mg, 65%).

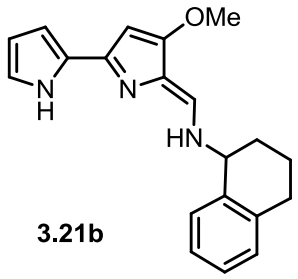
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (d, *J* = 6.8 Hz, 6H), 1.62 (q, *J* = 6.8 Hz, 2H), 1.71 (m, 1H), 3.48 (q, *J* = 6.8 Hz, 2H), 3.93 (s, 3H), 5.98 (d, *J* = 2.4 Hz, 1H), 6.28 (m, 1H), 6.75 (m, 1H), 7.09 (m, 1H), 7.33, (d, *J* = 15.2 Hz), 9.93 (s, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 22.1, 25.2, 38.7, 49.2, 58.3, 91.5, 110.5, 110.6, 113.6, 122.2, 124.5, 140.0, 143.2, 164.0. **IR** (KBr) υ_{max} 3234, 2960, 2917, 1674, 1605, 1529, 1463, 1428, 1368, 1203, 1137, 968, 728, 721 cm^{-1}. **HRMS**: $C_{15}H_{22}N_3O$, Calculated: [M+H]+, 260.1763, Found: [M+H]+, 260.1764.

General method for the synthesis of ***tambjamine unnatural analogs (3.21)****:* primary amine (0.517 mmol) was added to a stirred suspension of aldehyde 16 (30 mg, 0.103 mmol) in methanol (2.0 mL) at room temperature. 0.87 M HCl in methanol (0.6 mL) was subsequently added, and the reaction was stirred for 24 h. Reactions that had not reached completion by this time were stirred at 50 ^{o}C for an additional 24 h period. Reaction mixtures were concentrated, and crude compounds were purified by reverse phase chromatography using acetonitrile and 0.1% TFA/water to give tambjamine unnatural analogs **3.21** in yields ranging from 35–88%.



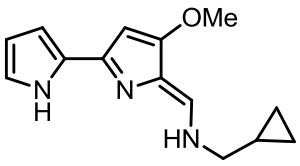
3.21u

(*Z*)-*N*-benzyl-1-(4'-methoxy-1H,5'H-[2,2'-bipyrrol]-5'-ylidene)methanamine (3.21u). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 3.91 (s, 3H), 4.61 (d, *J* = 6.0 Hz, 2H), 5.97 (d, *J* = 1.9 Hz, 1H), 6.28-6.30 (m, 1H), 6.76 (s, 1H), 7.10 (s, 1H), 7.33-7.42 (m, 6H), 10.44 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 54.11, 58.56, 91.93, 110.91, 111.39, 114.36, 122.41, 125.04, 128.18, 128.67, 129.35, 135.88, 139.83, 144.18, 164.68. **IR** (KBr) υ_{max} 3235, 3116, 3034, 2931, 1683, 1603, 1532, 1203, 1163, 1137, 1119, 749 cm^{-1}. **HRMS**: $C_{17}H_{18}N_3O$, Calculated: [M+H]+ 280.1450, Found: [M+H]+ 280.1450.



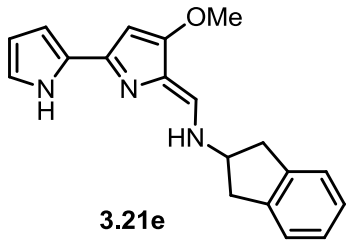
3.21b

(*Z*)-*N*-((4'-methoxy-1H,5'H-[2,2'-bipyrrol]-5'-ylidene)methyl)-1,2,3,4-tetrahydro naphthalen-1-amine (3.21b). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 1.83-1.91 (m, 2H), 1.94-2.00 (m, 2H), 2.12-2.17 (m, 2H), 2.75-2.82 (m, 1H), 2.88-2.96 (m, 1H), 3.89 (s, 3H), 4.75 (q, *J* = 5.8 Hz, 1H), 5.97 (d, *J* = 1.9 Hz, 1H), 6.26-6.28 (m, 1H), 6.74-6.75 (m, 1H), 7.09-7.10 (m, 1H), 7.18-7.30 (m, 6H), 10.09 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 18.54, 28.66, 30.56, 58.24, 91.62, 110.51, 110.73, 113.83, 122.19, 124.73, 126.46, 128.44, 129.05, 129.73, 132.28, 138.08, 138.31, 143.44, 164.16. **HRMS**: $C_{20}H_{22}N_3O$, Calculated: [M+H]+ 320.1763, Found: [M+H]+ 320.1760.



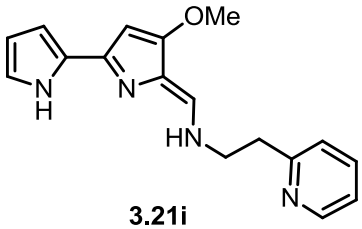
3.21d

(*Z*)-1-cyclopropyl-N-((4'-methoxy-1H,5'H-[2,2'-bipyrrol]-5'-ylidene)methyl) methanamine (3.21d). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 0.36 (q. *J* = 5.6 Hz, 2H), 0.67 (q, *J* = 5.6 Hz, 2H), 1.07-1.17 (m, 1H), 3.32 (t, *J* = 6.4 Hz, 2H), 3.93 (s, 3H), 5.98 (d, *J* = 1.8 Hz, 1H), 6.27-6.29 (m, 1H), 6.75 (s, 1H), 7.10 (s, 1H), 7.39 (d, *J* = 15.0 Hz, 1H), 10.04 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 3.90, 11.13, 55.68, 58.54, 91.80, 110.75, 110.88, 113.90, 122.51, 124.73, 139.87, 143.51, 164.34. **IR** (KBr) υ_{max} 3243, 3013, 2932, 1682, 1606, 1531, 1429, 1245, 1204, 1165, 1138, 967, 834, 720 cm^{-1}. **HRMS**: $C_{14}H_{18}N_3O$, Calculated: [M+H]+ 244.1450, Found: [M+H]+ 244.1442.



3.21e

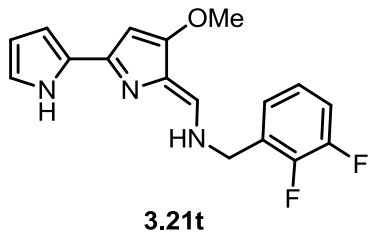
(*Z*)-*N*-((4'-methoxy-1H,5'H-[2,2'-bipyrrol]-5'-ylidene)methyl)-2,3-dihydro-1H-inden-2-amine (3.21e). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 3.21 (dd, *J* = 15.7, 7.0 Hz, 2H), 3.36 (dd, *J* =15.7, 7.0 Hz, 2H), 3.93 (s, 3H), 4.37 (sex, *J* = 7.0 Hz, 1H), 5.98 (d, *J* = 2.4 Hz, 1H), 6.27-6.29 (m, 1H), 6.76 (s, 1H), 7.10 (s, 1H), 7.20-7.26 (m, 5H), 7.42 (d, *J* = 15.0 Hz, 1H), 10.13 (bs, 1H).**^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 39.80, 58.58, 61.39, 91.92, 110.86, 111.29, 114.31, 122.40, 124.85, 125.09, 127.42, 138.57, 139.72, 144.06, 164.51. **IR** (KBr) υ_{max} 3237, 3027, 2924, 1685, 1684, 1601, 1530, 1431, 1363, 1293, 1201, 1135, 1119, 966, 752, 721 cm^{-1}. **HRMS**: $C_{19}H_{20}N_3O$, Calculated: [M+H]+306.1606, Found: [M+H]+ 306.1598.



3.21i

(*Z*)-*N*-((4'-methoxy-1H,5'H-[2,2'-bipyrrol]-5'-ylidene)methyl)-2-(pyridin-2-yl) ethanamine (3.21i) **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 3.23 (t, *J* = 7.0 Hz, 2H), 3.88 (s, 3H), 3.98 (t, *J* = 7.0 Hz, 2H), 5.91 (s, 1H), 6.26 (s, 1H), 6.72 (s, 1H), 7.05 (s, 1H), 7.15 (dd, *J* = 7.4, 5.0 Hz, 1H), 7.28 (s, 1H), 7.61 (td, *J* = 7.5, 1.6 Hz, 1H), 8.56 (d, J = 5.0 Hz, 1H), 9.63 (bs, 1H), 10.84 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 38.18, 50.07, 58.59, 91.33, 110.88, 110.99, 113.45, 122.14, 122.78, 124.38, 124.48, 136.98, 140.85, 142.76, 149.70, 157.20, 164.13. **IR** (KBr) υ_{max} 3184, 3098, 2926, 2850, 1662,

1601, 1528, 1550, 1457, 1312, 1186, 1136, 747 cm^{-1}. **HRMS**: $C_{17}H_{19}N_4O$, Calculated: [M+H]+ 295.1559, Found: [M+H]+ 295.1555.



3.21t

(*Z*)-*N*-(2,3-difluorobenzyl)-1-(4'-methoxy-1H,5'H-[2,2'-bipyrrol]-5'-ylidene) methanamine (3.21t). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 3.93 (s, 3H), 4.67 (d, *J* = 6.0 Hz, 2H), 5.98 (d, *J* = 2.2 Hz, 1H), 6.28-6.30 (m, 1H), 6.77-6.79 (m, 1H), 7.10-7.22 (m, 4H), 7.39 (d, *J* = 14.6 Hz, 1H), 10.32 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 47.45, 58.66, 92.06, 111.06, 111.66, 114.79, 117.86, 118.03, 122.31, 125.18, 125.34, 139.62, 144.78, 165.10. **IR** (KBr) υ_{max} 3236, 3117, 3017, 2933, 1683, 1601, 1533, 1494, 1202, 1138, 957, 756, 748, 721 cm^{-1}. **HRMS**: $C_{17}H_{16}N_3OF_2$, Calculated: [M+H]+ 316.1261, Found: [M+H]+ 316.1263.

Viability assay method: SW620 and H520 cells (2.5 x 10^4/100 μl) were seeded in 96-well microtiter plates prior to treatment. Cells were treated with 10 μM concentration of synthesized compound in quadruplicate for 24 h and 48 h in RPMI 1640 Supplemented media and 100 μg/ml penicillin–streptomycin. The Quick Cell Proliferation Assay Kit from BioVision (Mountain View, CA) was used to measure proliferation. The RPMI media is removed and replaced with 100 μl of the WST-1/ECS reagent diluted 1:10 in RPMI Supplemented media. The plates are incubated for 1 h at 37°C in 5% CO_2 in the

air. The change in proliferation is quantified by measuring the absorbance of the dye solution at 450 nm on a microtiter plate reader.

Invasion assay method: SW620 cells (1.0×10^6/ml) were seeded in 6 mm round dish prior to treatment. Cells were treated with 10 μM concentration of synthesized compound for 24 h in RPMI 1640 supplemented media and 100 μg/ml penicillin–streptomycin. 40 μl (2.5 mg/ml) of BD Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA) was added to the top of insert of 24-well transwell permeable support plates with polycarbonate membrane (Corning Inc, Corning, NY). Then the cells were trypsinized and 3×10^5/250 μl cells were added to the top of the chamber in serum free RPMI media, and 1 ml of RPMI media with 10% FBS was added to the bottom of the well. Then the plates were incubated for 72 h at 37°C in 5% CO_2 in the air. Then the wells were stained with 1% crystal violet in 50% methanol for 1 h and washed in PBS. The membrane was cut off, adhered to a slide with glycerol, and analyzed in 20x field via microscopy. 3–20x fields were quantified per membrane.

5.4. Technology Enabled Synthesis and Biological Evaluation of 3,6-disubstituted-[1,24]-triazolo[4,3-*b*] pyridazines as M_1 Antagonists for Treatment of Dystonias

Typical MAOS experimental for **6-chloro-3-p-tolyl-[1,2,4]-triazolo-[4,3-b] pyridazine (4.13e).** (*Method A*) To a 5 mL microwave reaction vessel were added 3,6-dichloropyridazine (100 mg, 0.671 mmol) and *p*-toluic hydrazide (111 mg, 0.738 mmol) in a 3.0 mL solution of 5% AcOH/EtOH. The vial was irradiated in a microwave synthesizer at 150 ^{o}C for 10 min. LCMS (single peak, 2.91 min, m/e, 245.1 (M+1)) indicated that all starting material had been consumed affording 131 mg (80%) of 6-chloro-3-*p*-tolyl-[1,2,4]triazolo[4,3-b]-pyridazine as a white solid following column purification. (*Method B*) To a 5 mL microwave reaction vessel were added 3,6-dichloropyridazine (100 mg, 0.671 mmol) and *p*-toluic hydrazide (111 mg, 0.738 mmol) in a 3.0 mL solution of 5% 4 N HCl/EtOH. The vial was irradiated in a microwave synthesizer at 150 ^{o}C for 10 min. LCMS (single peak, 2.91 min, m/e, 245.1 (M+1)) indicated that all starting material had been consumed affording 156 mg (95%) of 6-chloro-3-*p*-tolyl-[1,2,4]triazolo[4,3-b]pyridazine as a white solid following a silica plug and concentration *in vacuo*.

^{1}H NMR (DMSO-*d*6, 600 MHz) δ (ppm): 2.41 (s, 3H), 7.43 (d, *J* = 8 Hz, 2H), 7.53 (d, *J* = 9.7 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.52 (d, *J* = 9.7 Hz, 1H); **^{13}C NMR** (DMSO-*d*6, 150 MHz) δ (ppm): 21.1, 122.5, 122.8, 127.1, 127.3, 129.5, 140.3, 143.8, 146.8, 149.1; LC–MS: single peak, 2.91 min, m/e, 245.1 (M+1).

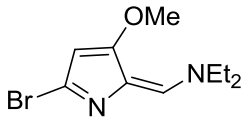
Typical MAOS experimental for ***N*-(4-methoxybenzyl)-3-p-tolyl-[1,2,4]triazolo[4,3-b]pyridazin-6-amine.** To a 5 mL microwave reaction vessel were added 6-chloro-3-p-

tolyl- [1,2,4]-triazolo-[4,3-b]pyridazine (50 mg, 0.205 mmol) and 4-methoxy-benzyl amine (35 μL 6 mmol) in 3.0 ml of ethanol. The vial was heated in a microwave synthesizer to 170 ^{o}C for 25 min. Preparative LCMS afforded 51.6 mg (73%) of N-(4-methoxybenzyl)-3-p-tolyl-[1,2,4]-triazolo-[4,3-b]pyridazin-6-amineas a white solid.

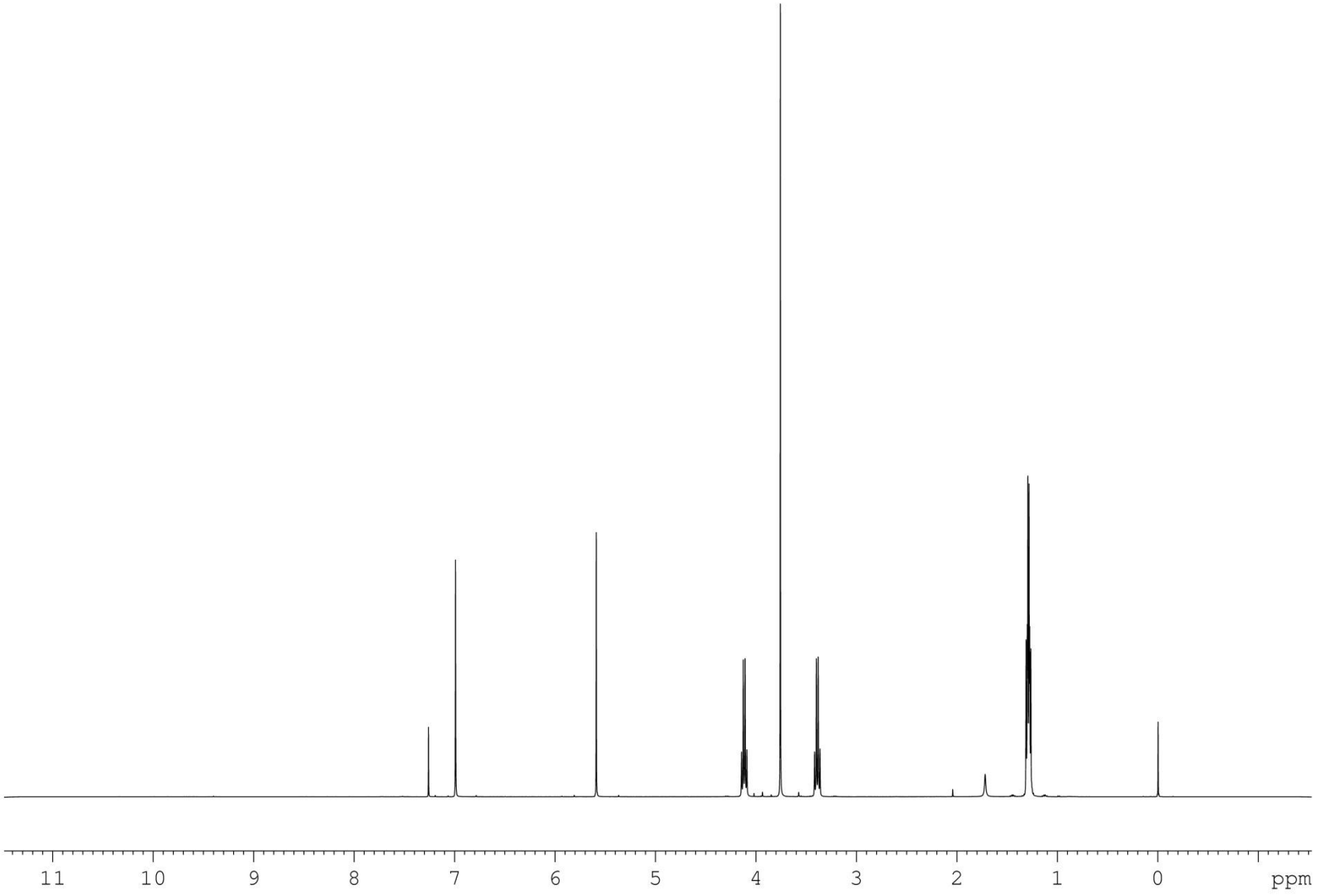
^{1}H NMR (DMSO-*d*6, 600 MHz) δ (ppm): 2.38 (s, 3H), 3.71 (s, 3H), 4.41 (d, *J* = 5.5 Hz, 2H), 6.88 (d, *J* = 9.9 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 6.7 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.97 (d, *J* = 9.8 Hz, 1H), 8.21 (d, *J* = 8.2 Hz, 2H). **^{13}C NMR** (DMSO-*d*6, 150 MHz) δ (ppm): 21.5, 44.7, 55.5, 114.2, 117.0, 124.4, 124.6, 127.0, 129.3, 129.6, 130.9, 139.4, 143.8, 146.3, 154.1, 158.8; LC–MS: single peak, 3.00 min, m/e, 346.2 (M+1).

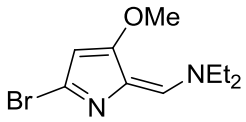
Appendix A1:

Spectra Relevant to Chapter 2



2.56





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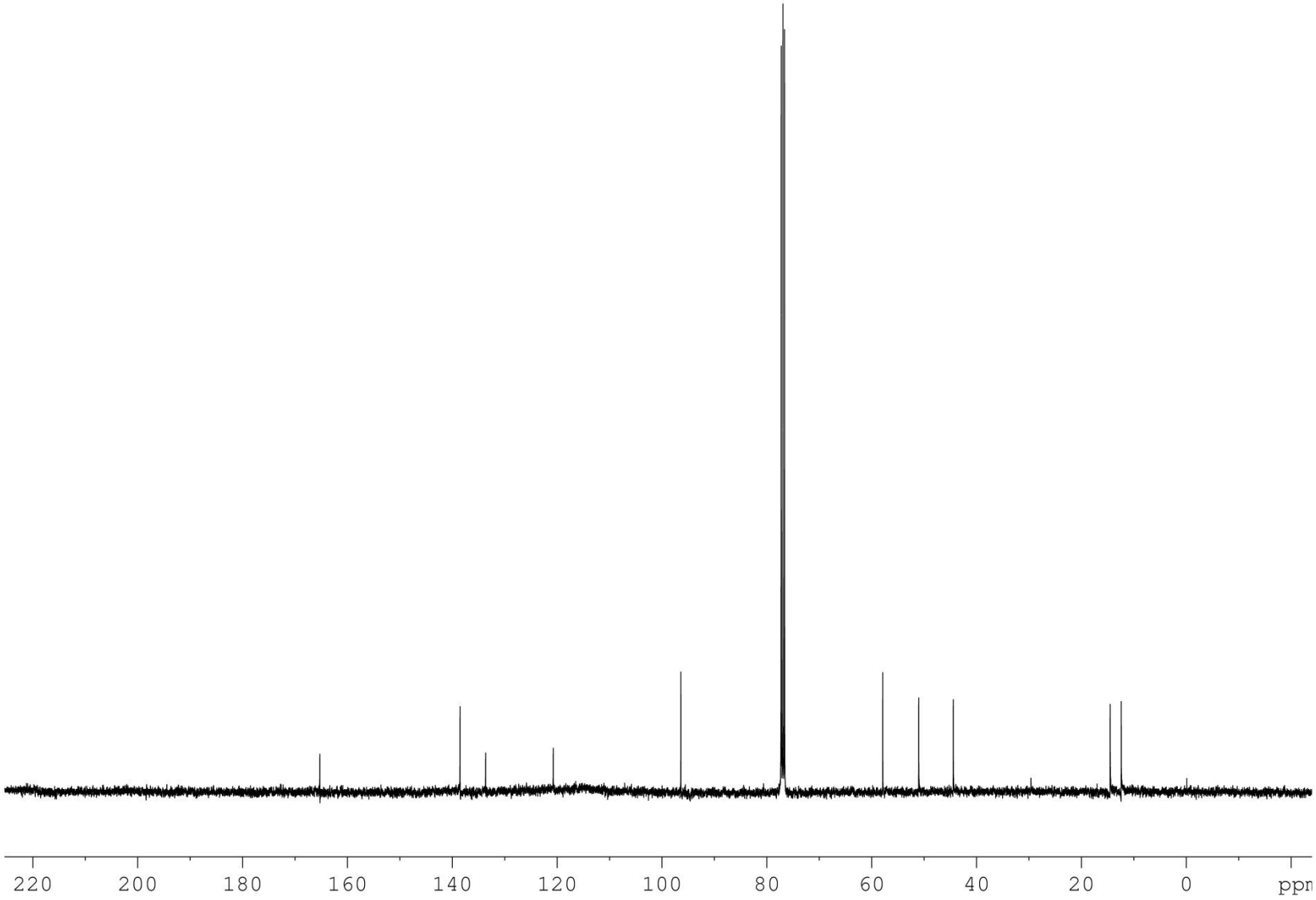

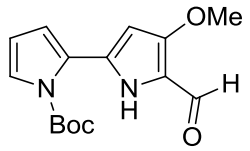

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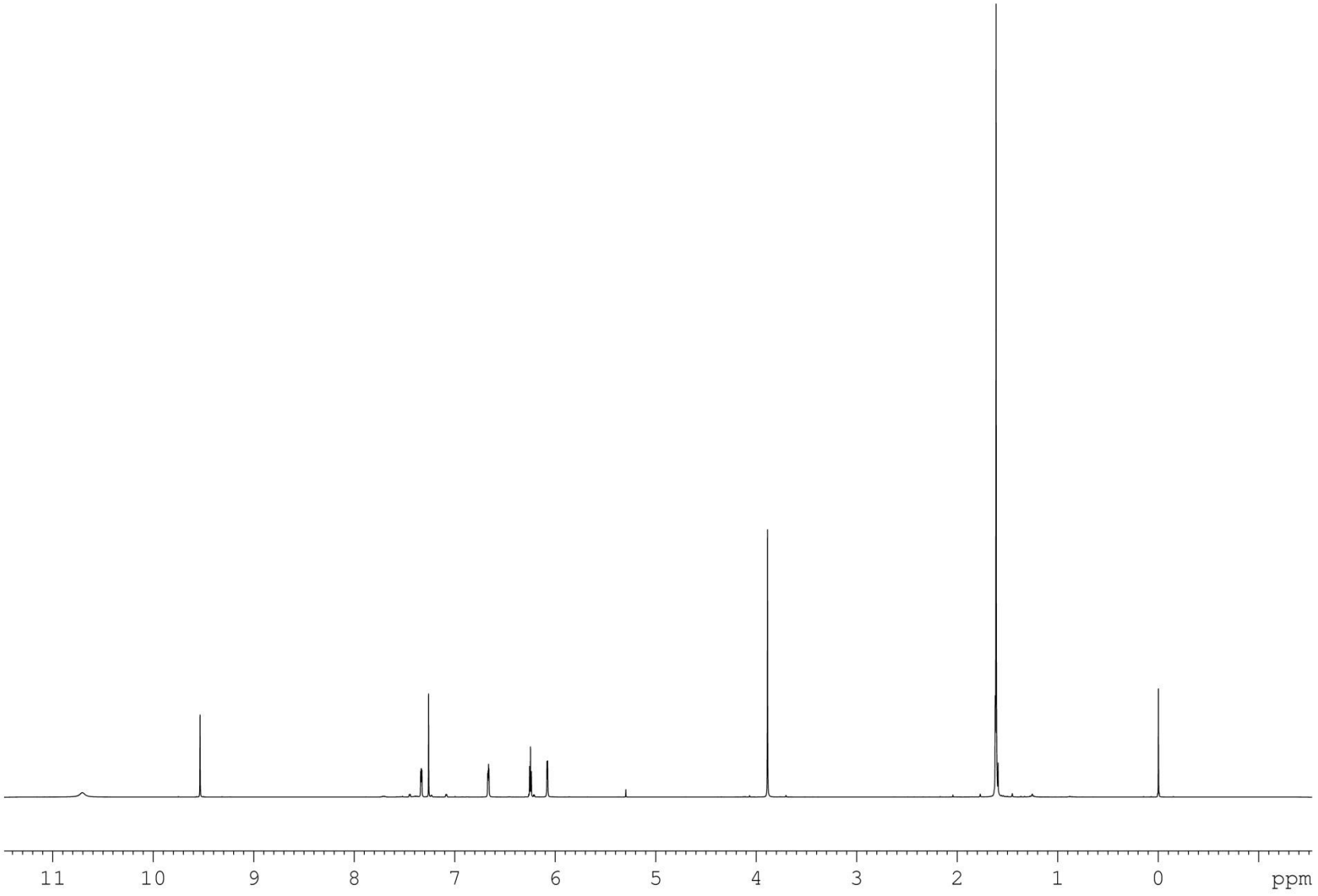

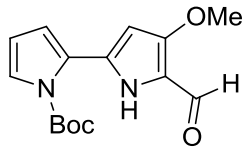

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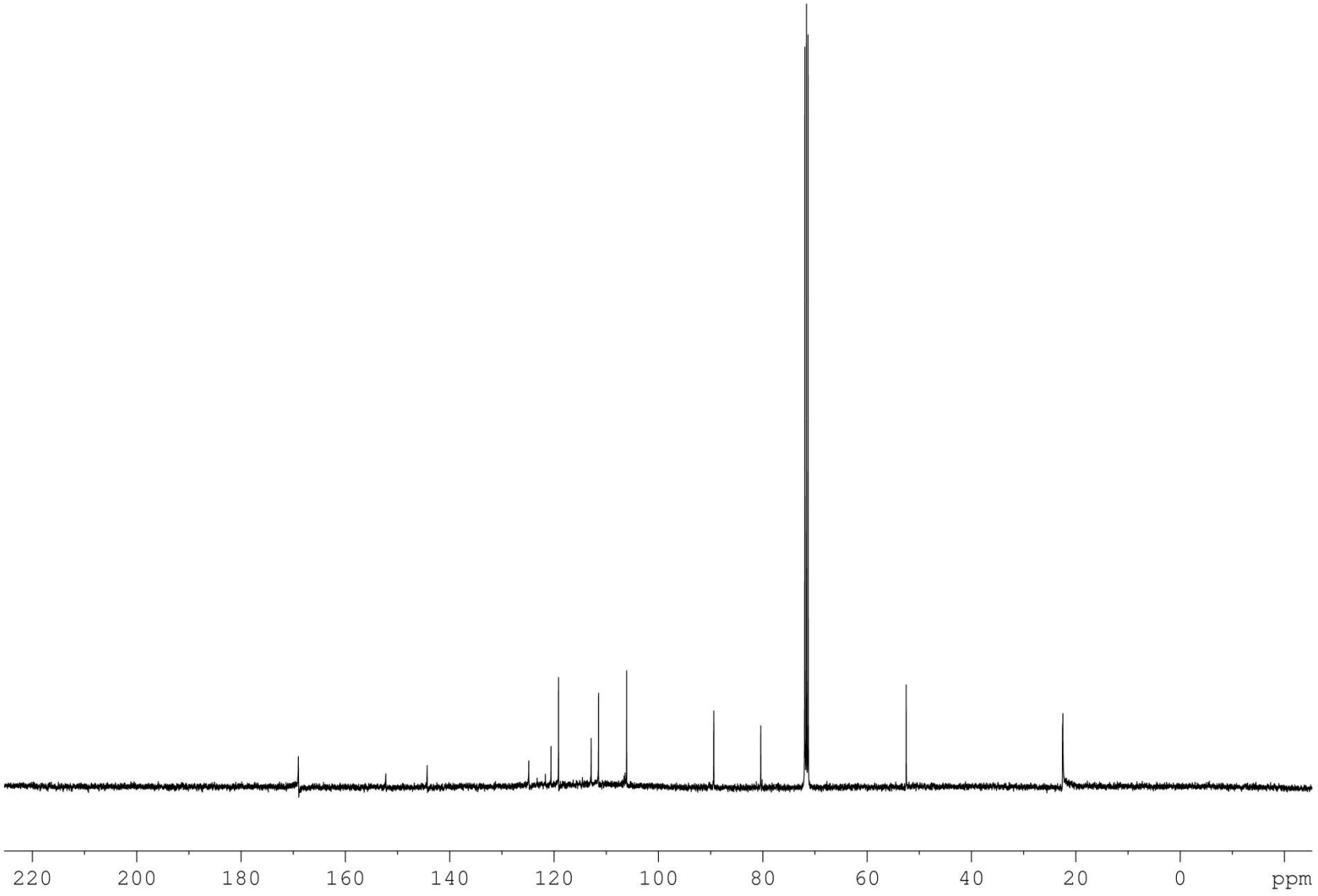

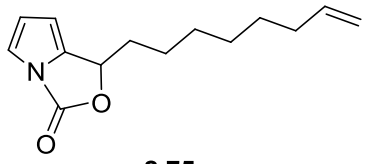

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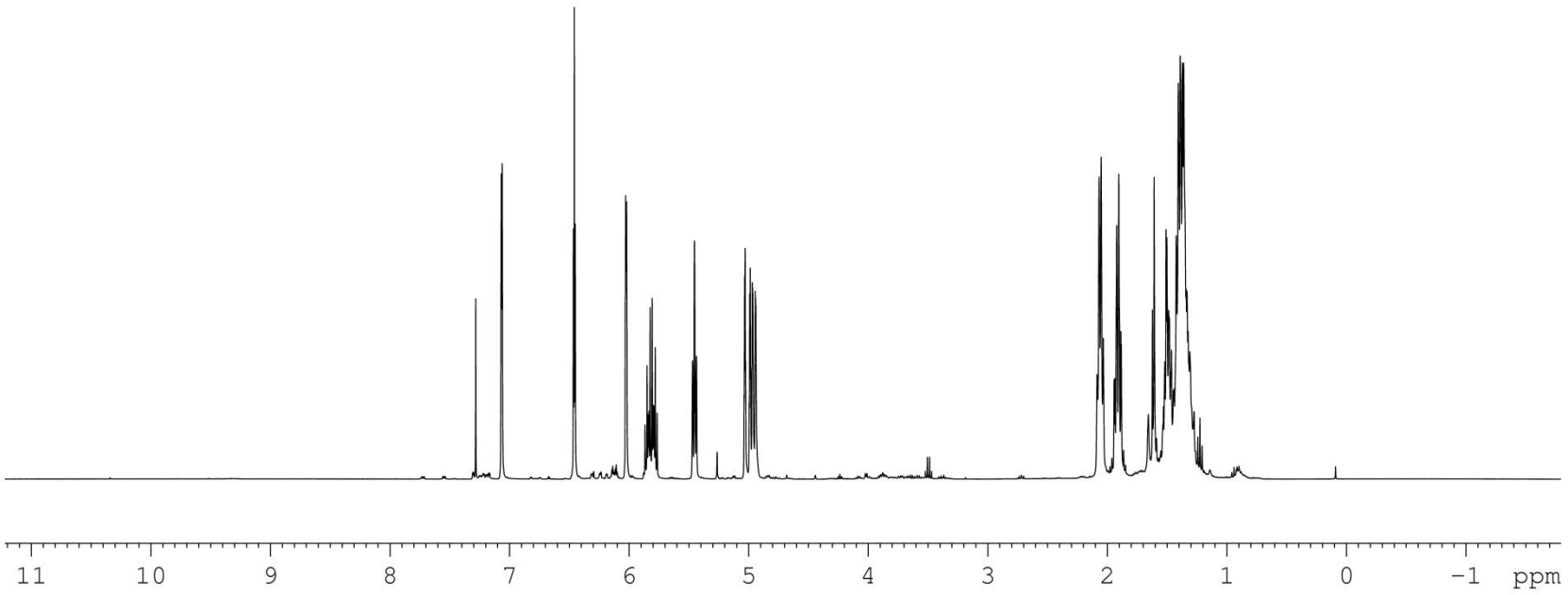

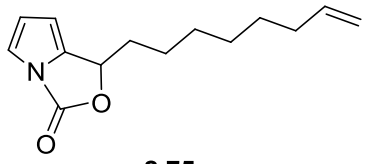

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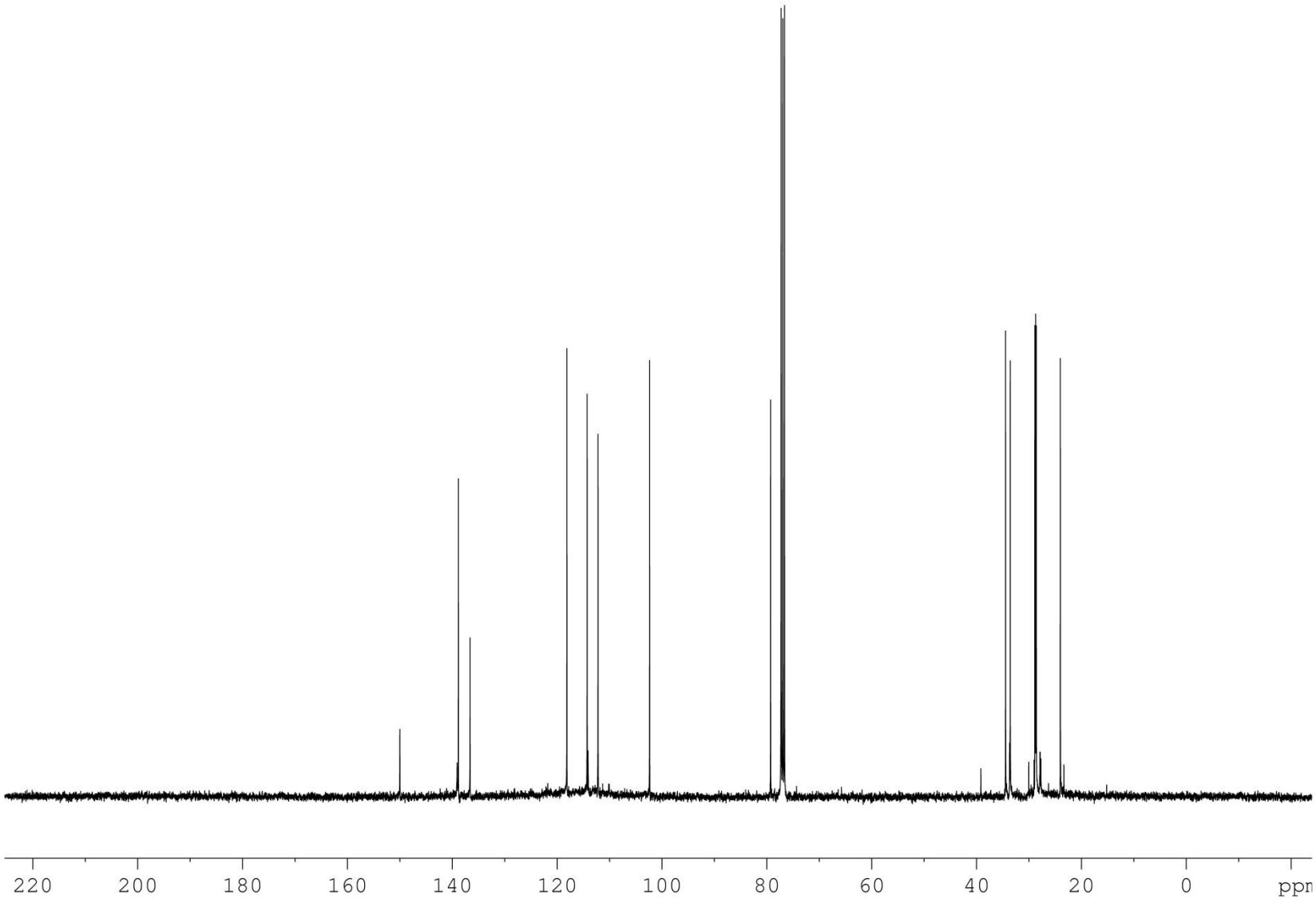

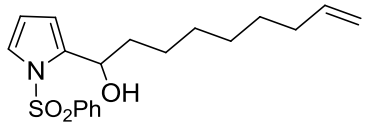

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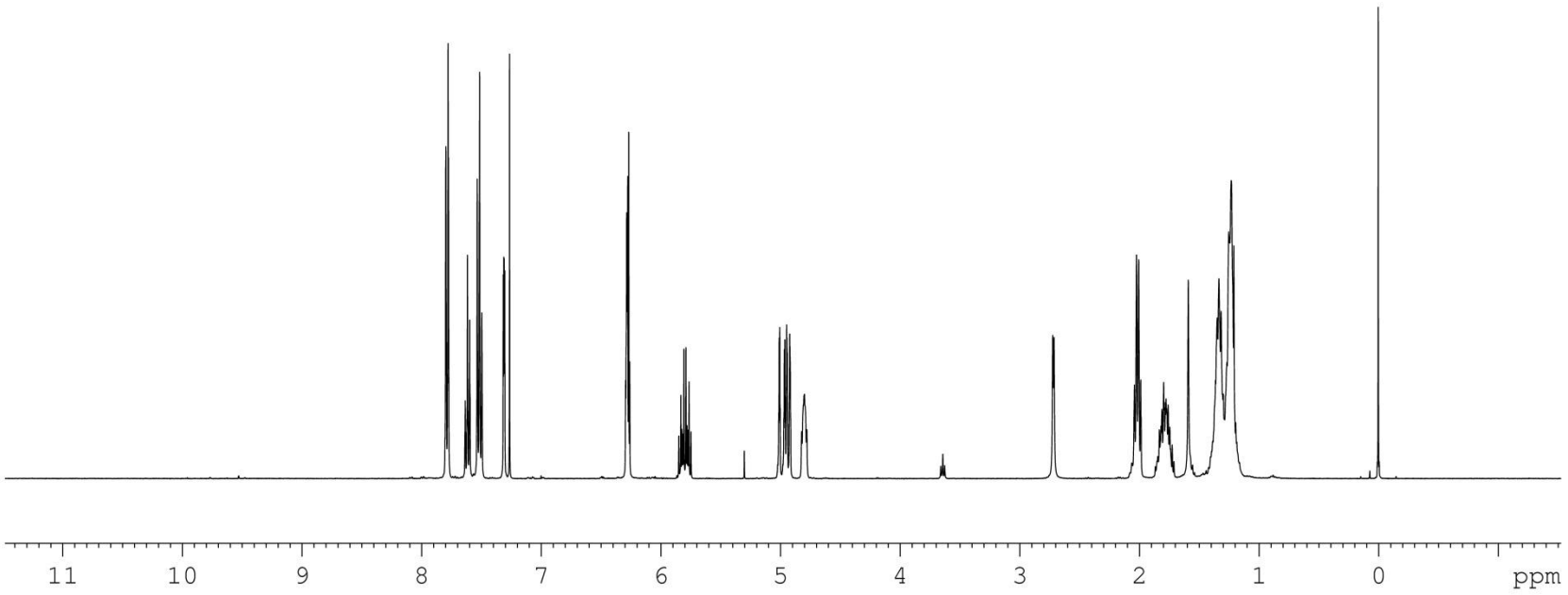

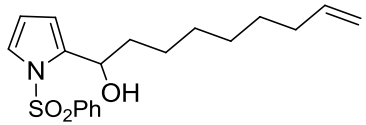

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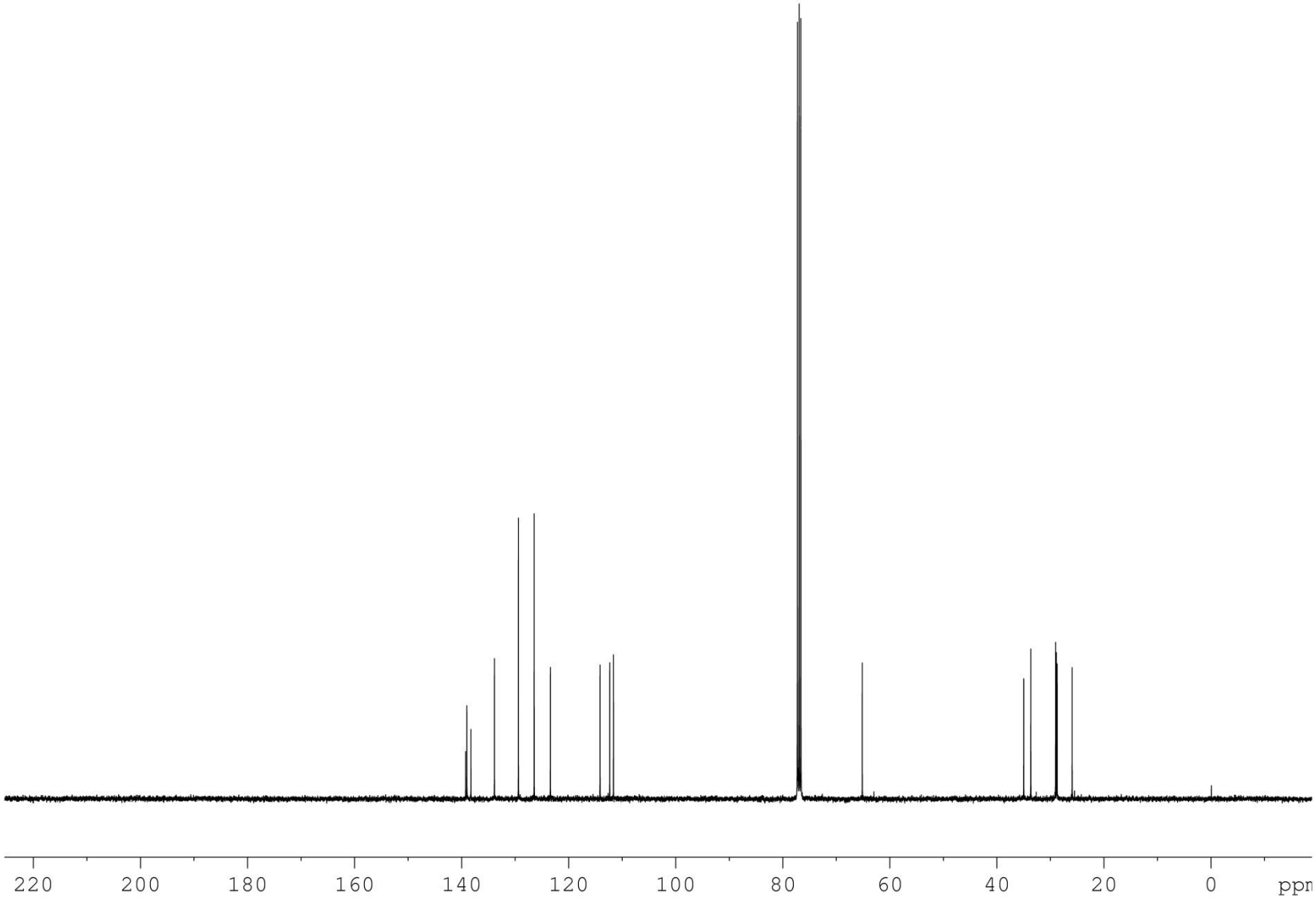

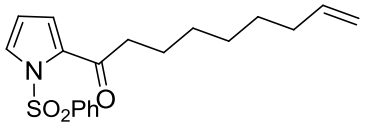

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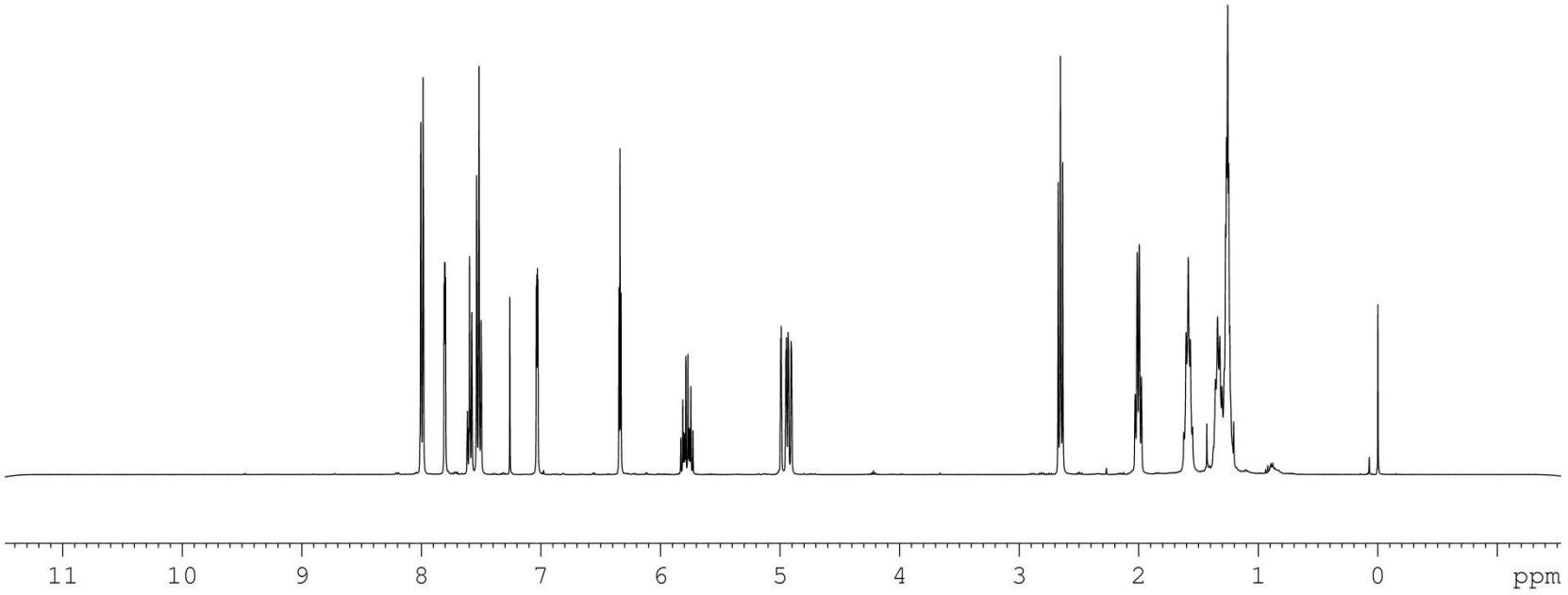

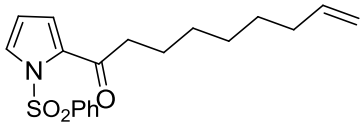

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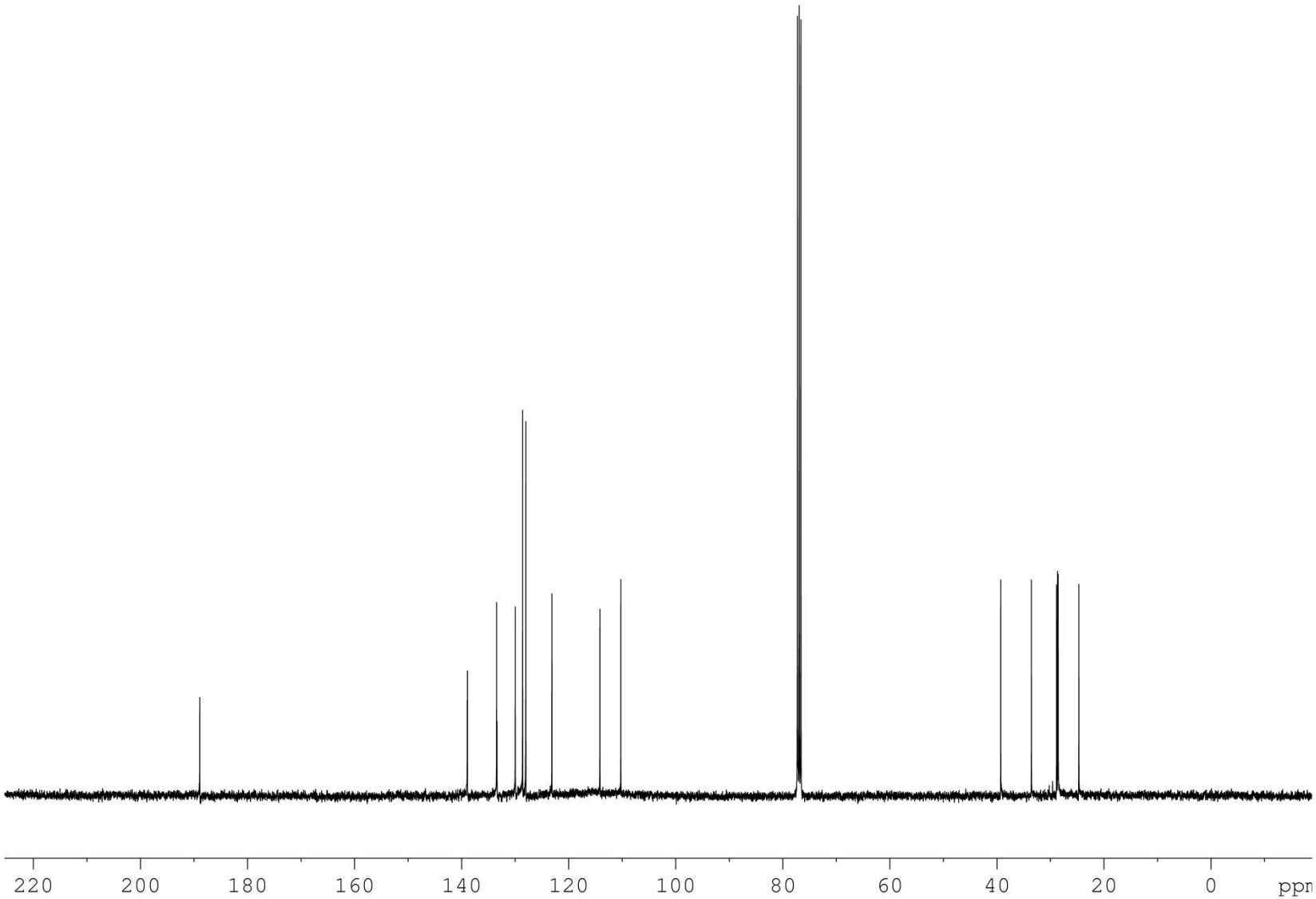

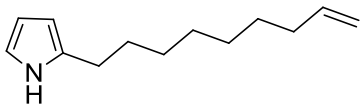

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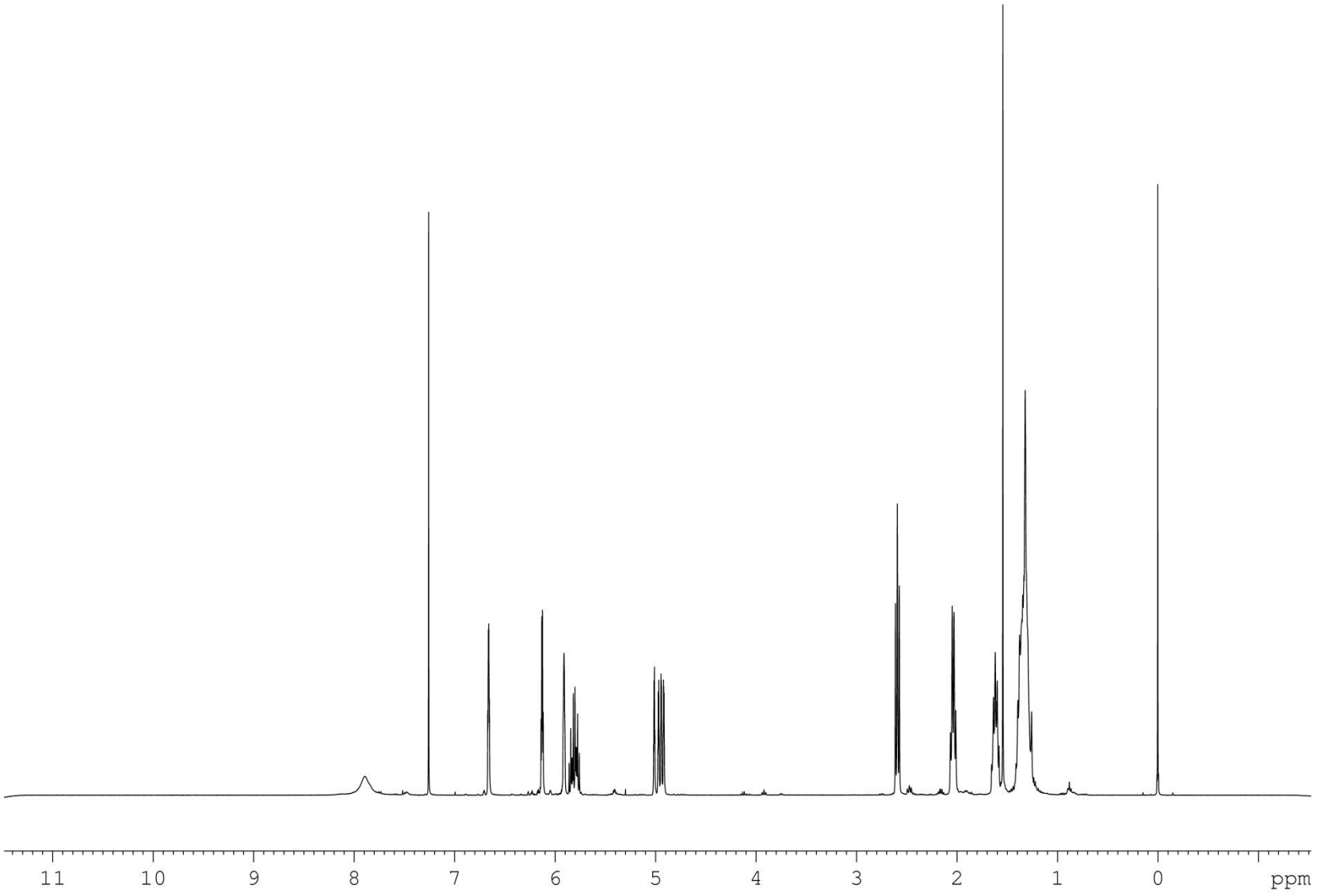

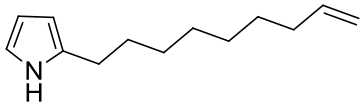

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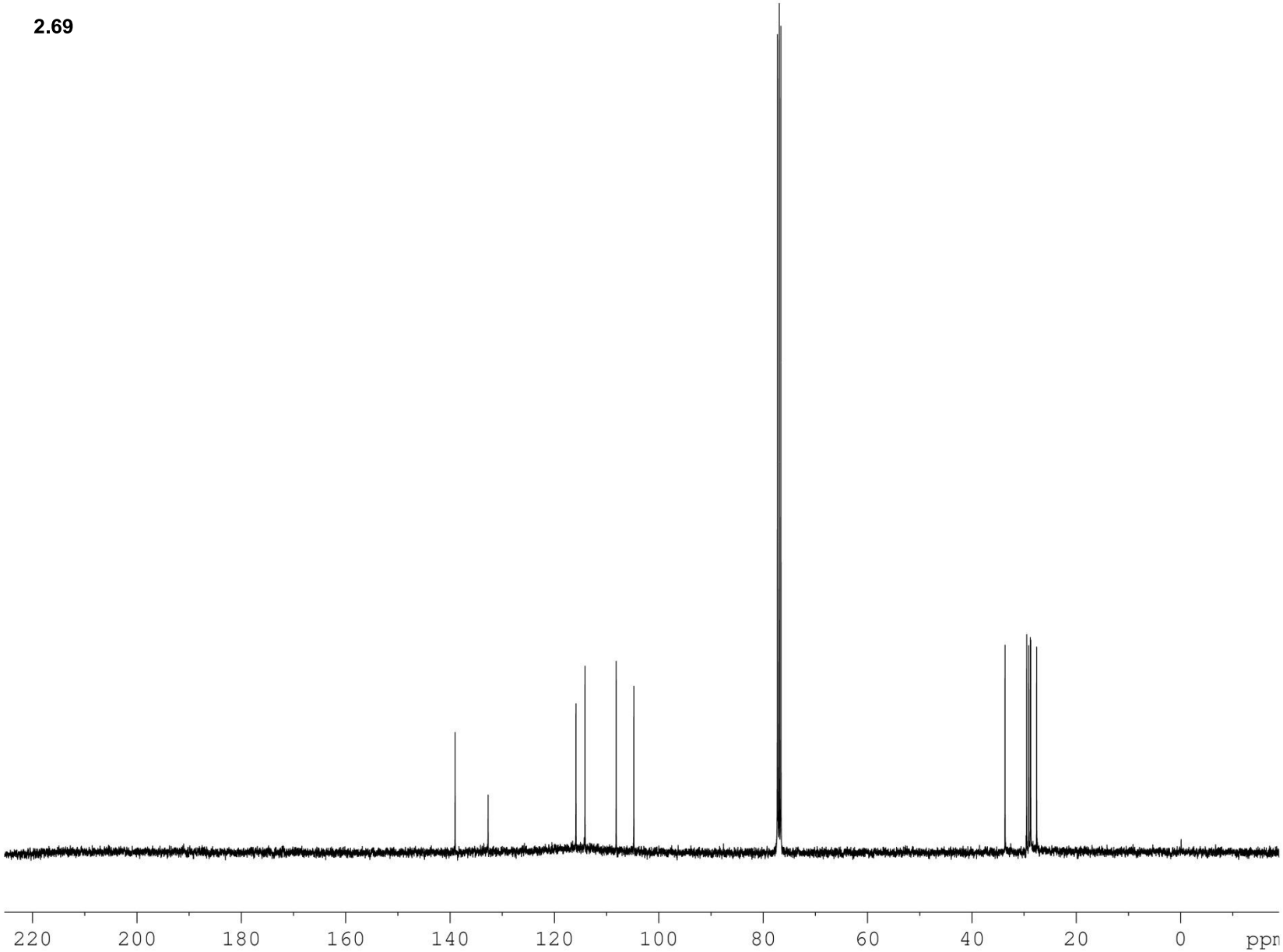

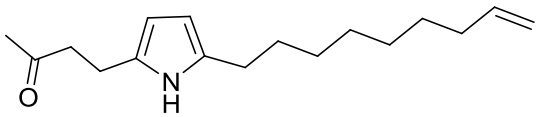

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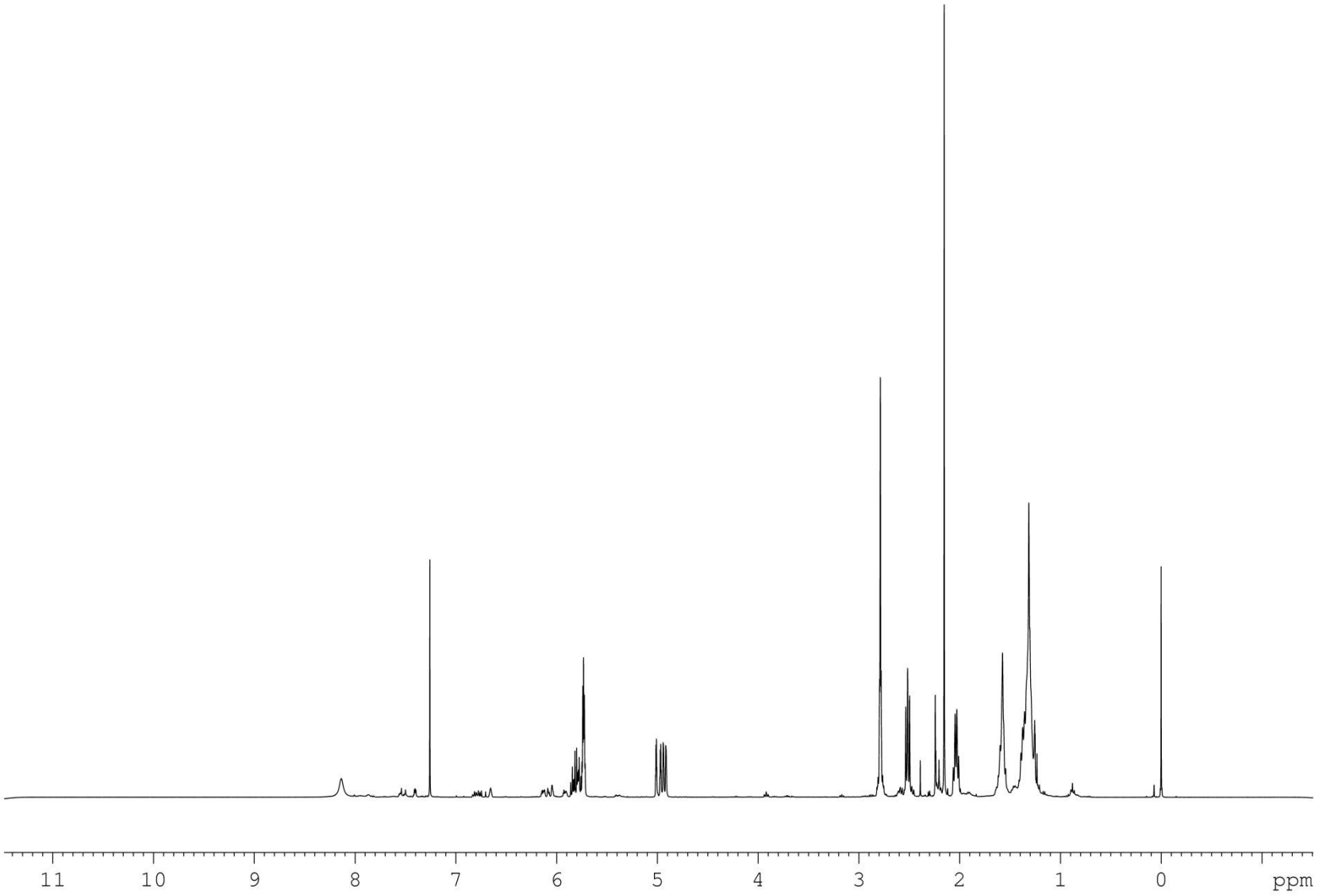

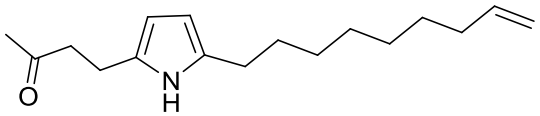

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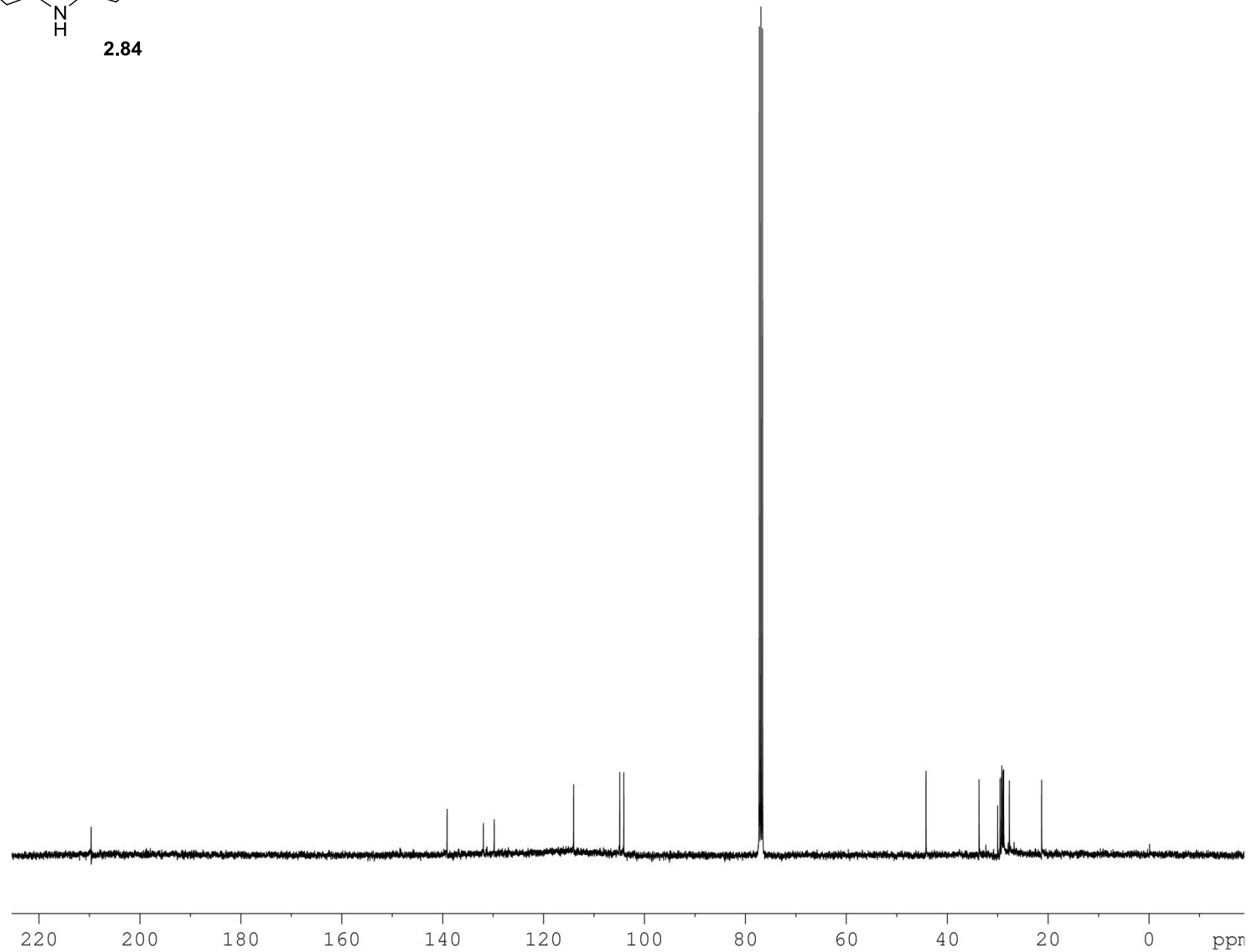

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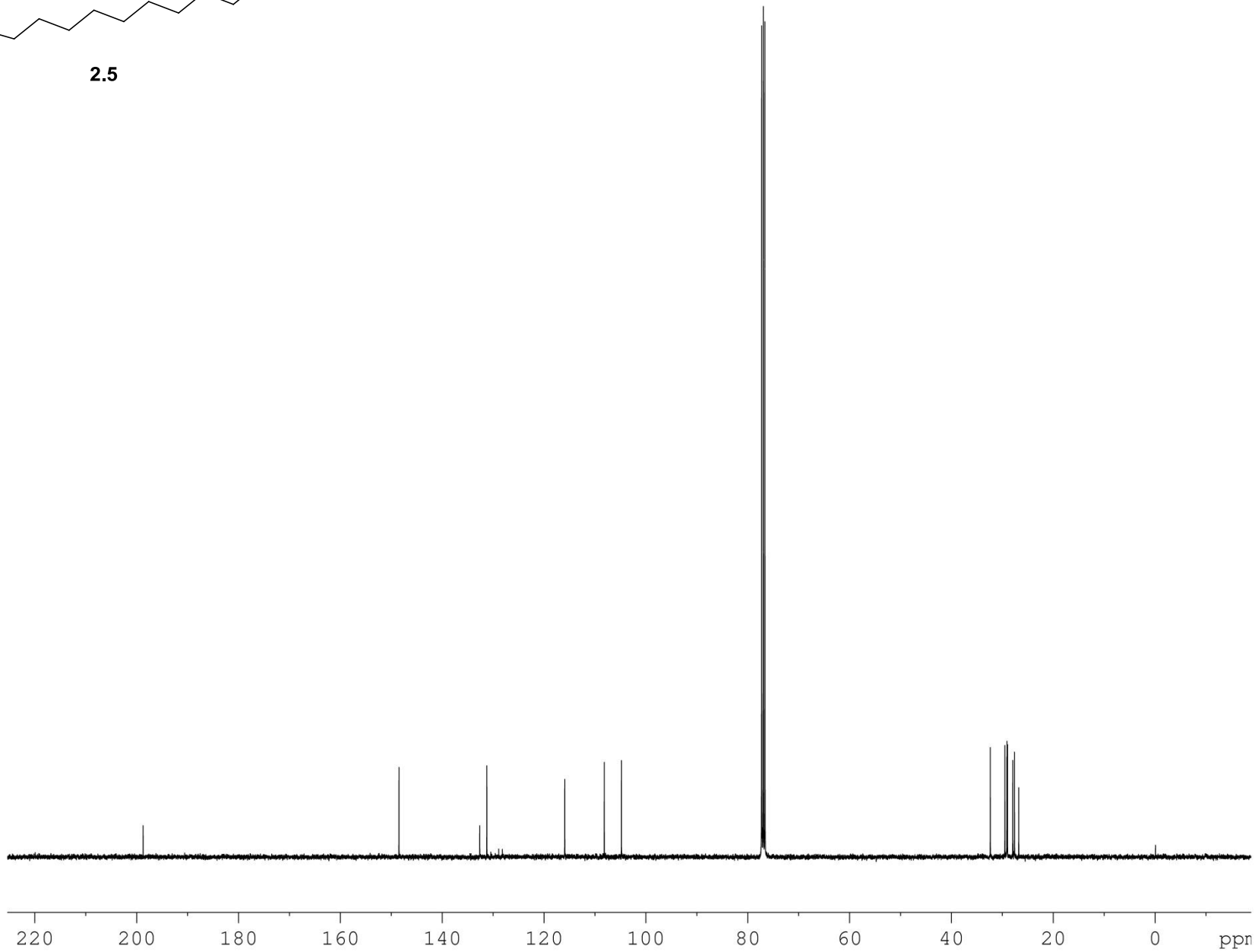

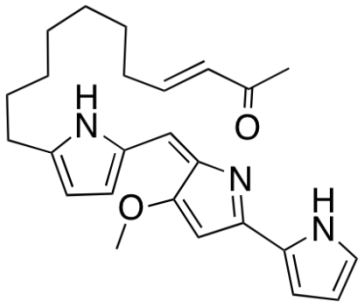
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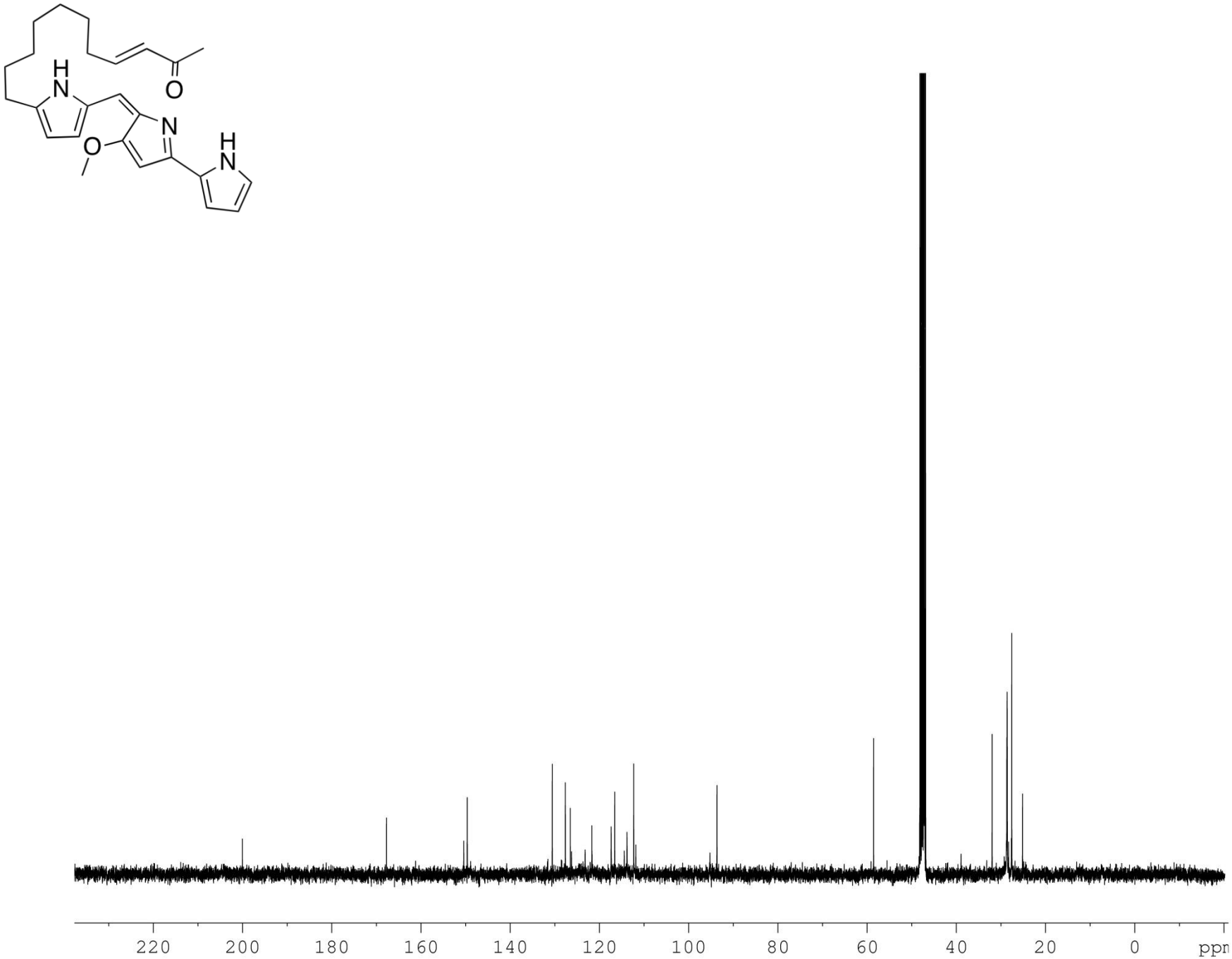
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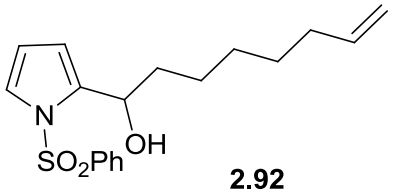

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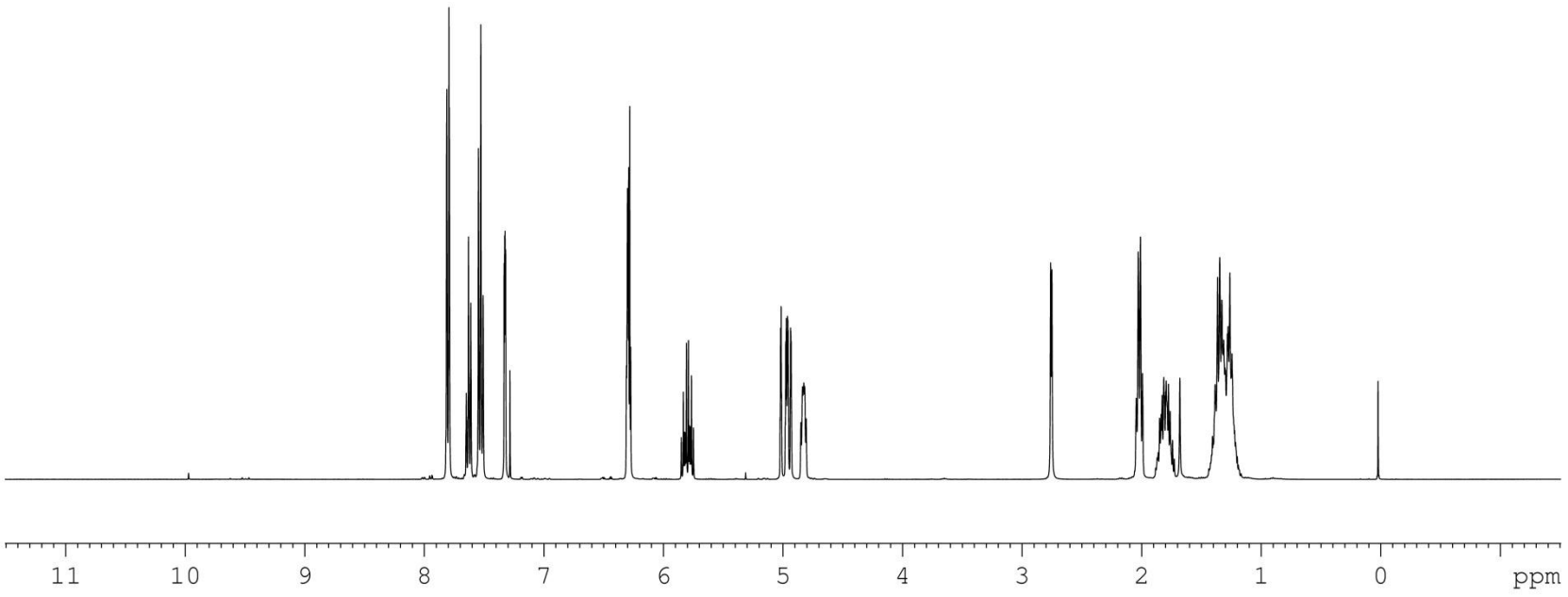

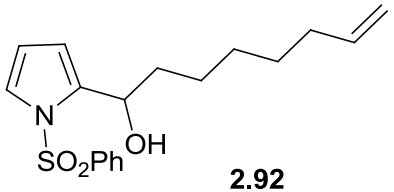

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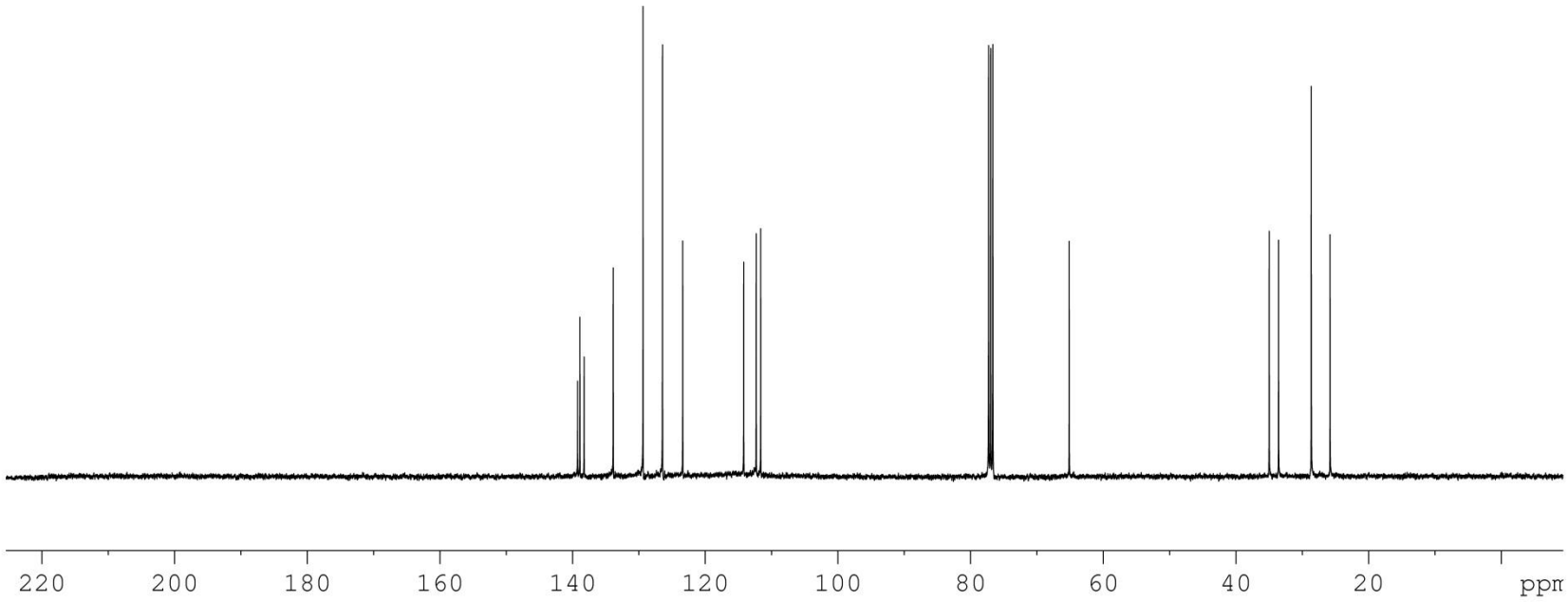

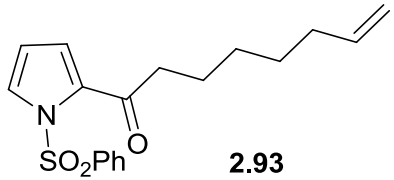

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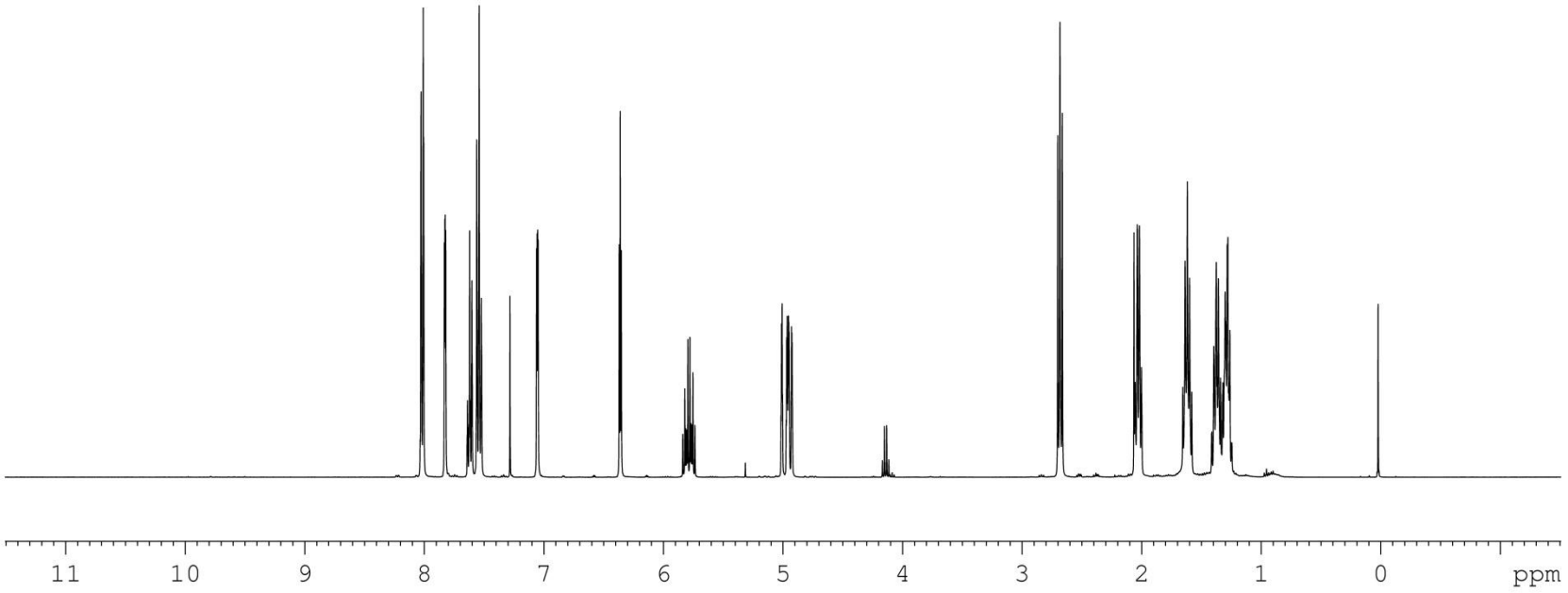

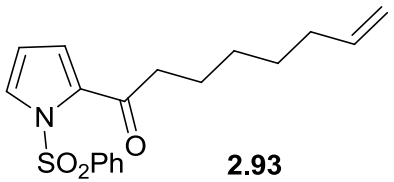

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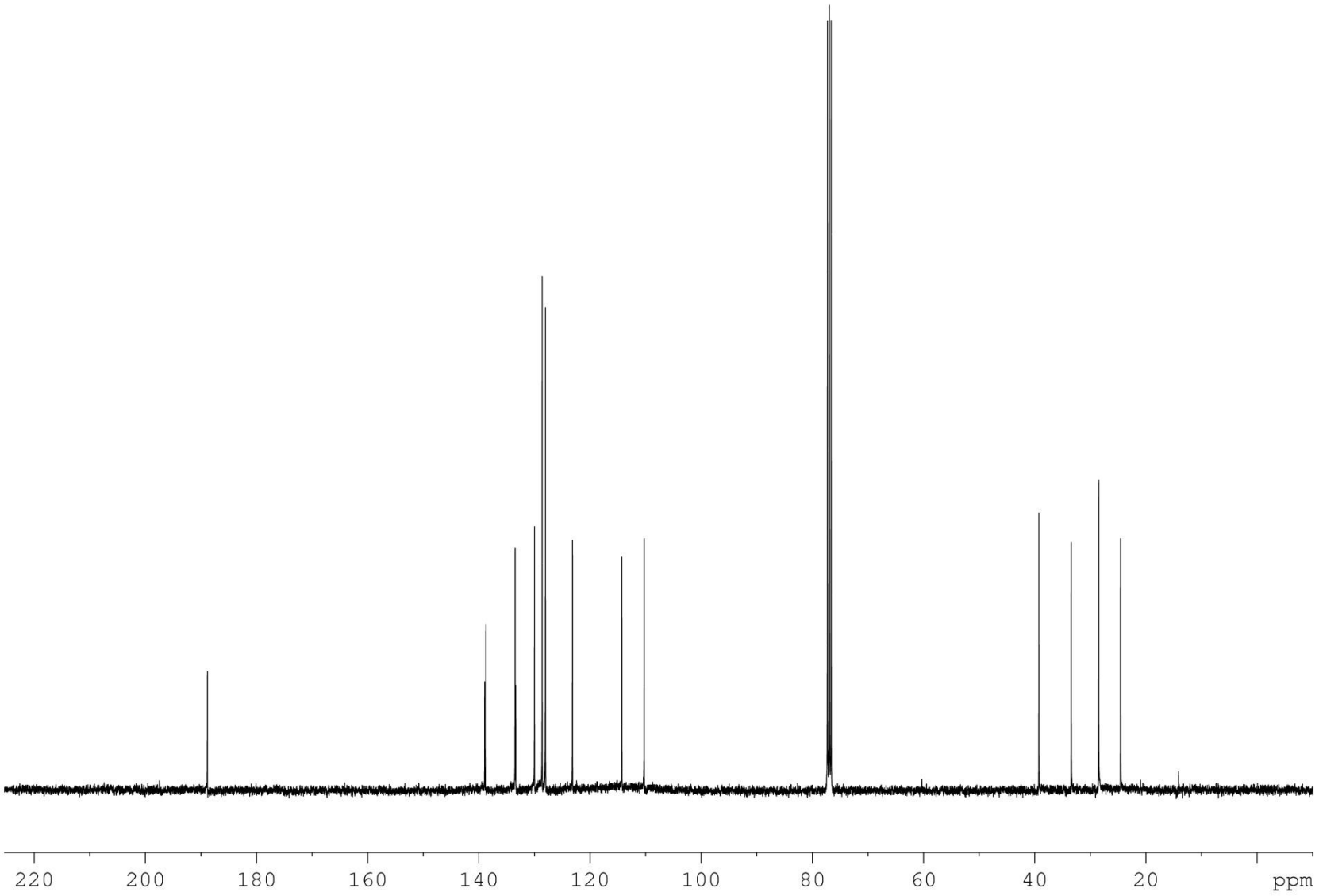

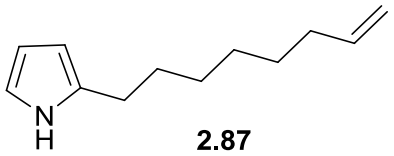

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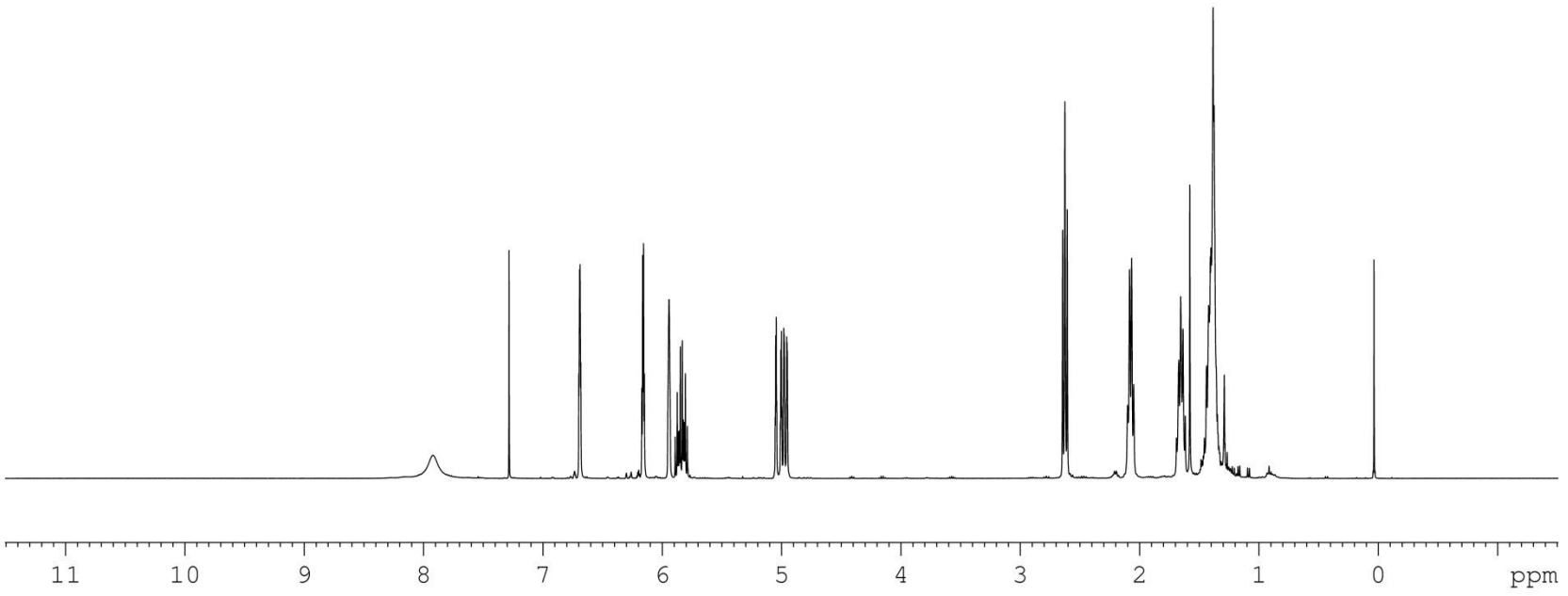

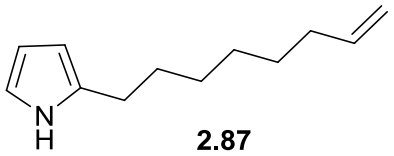

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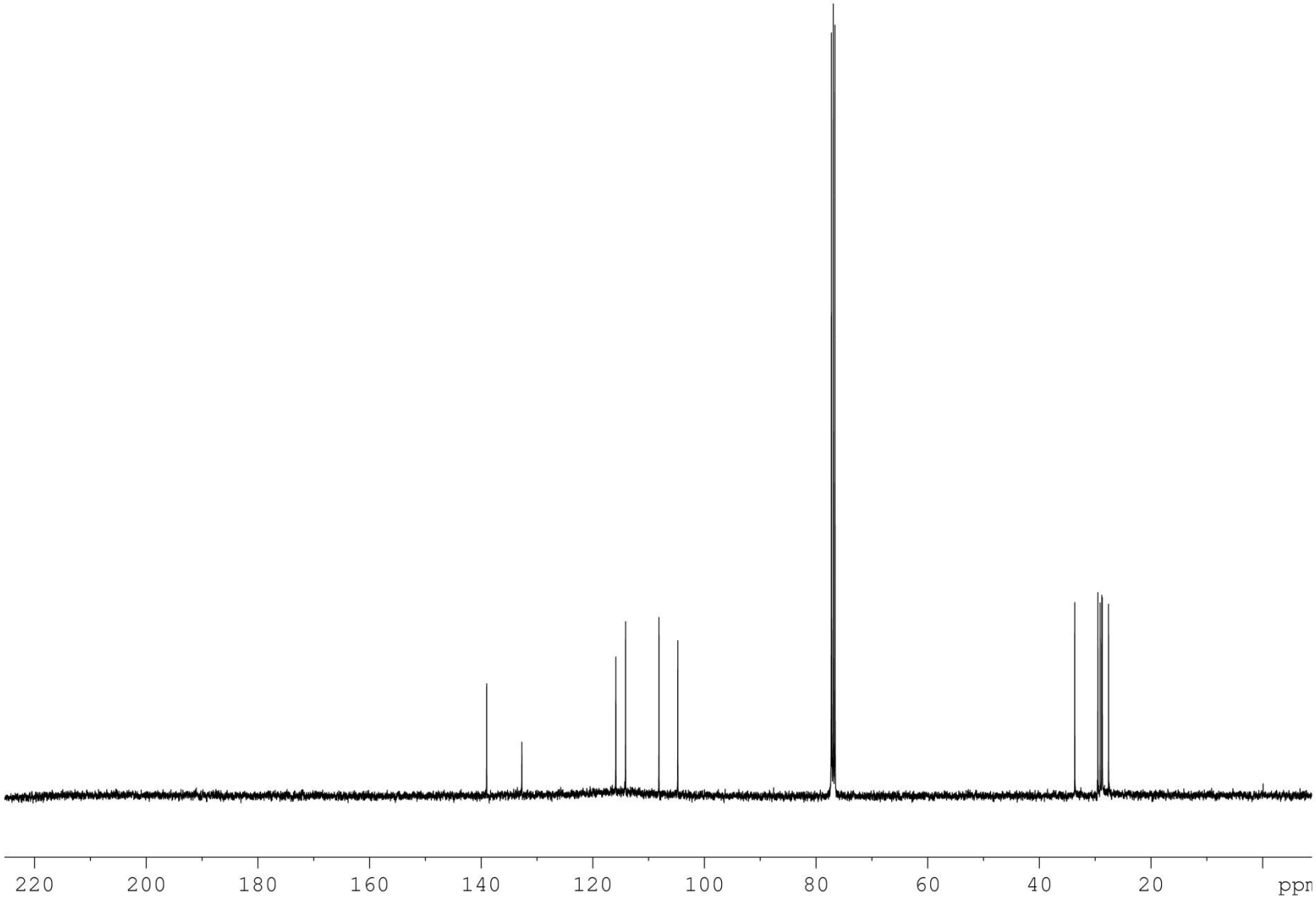

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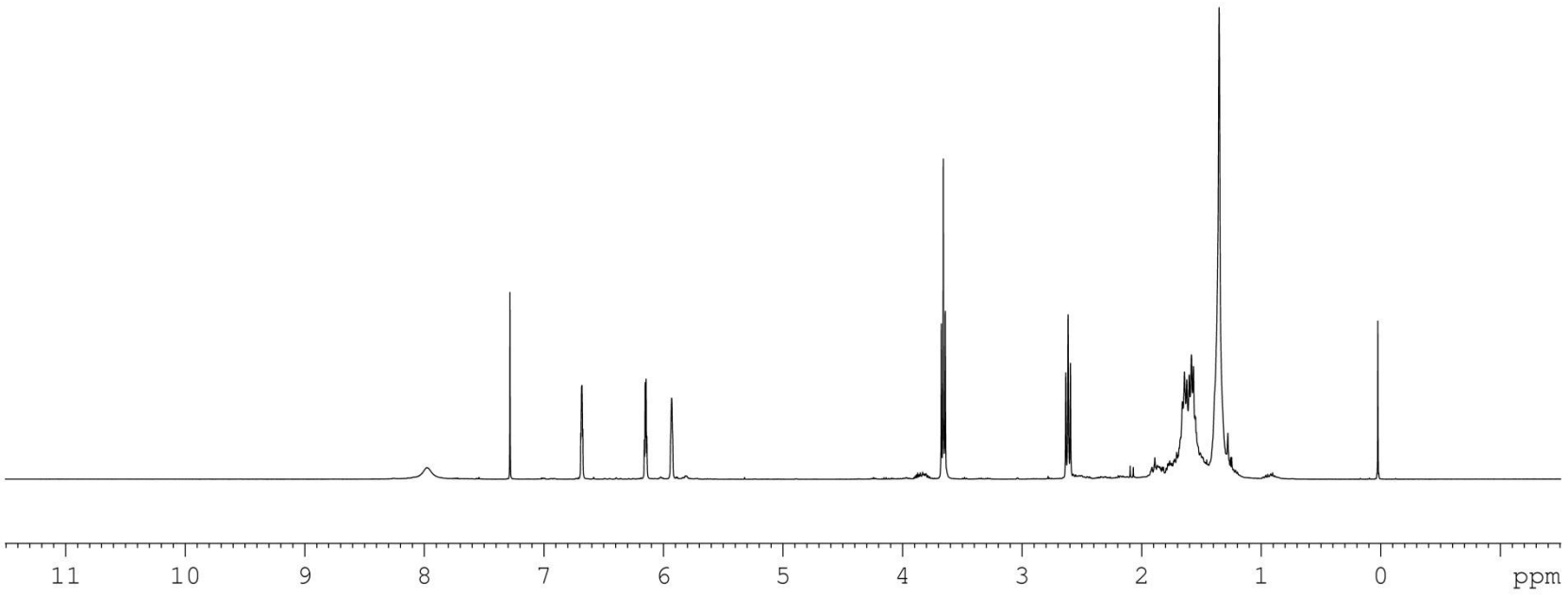

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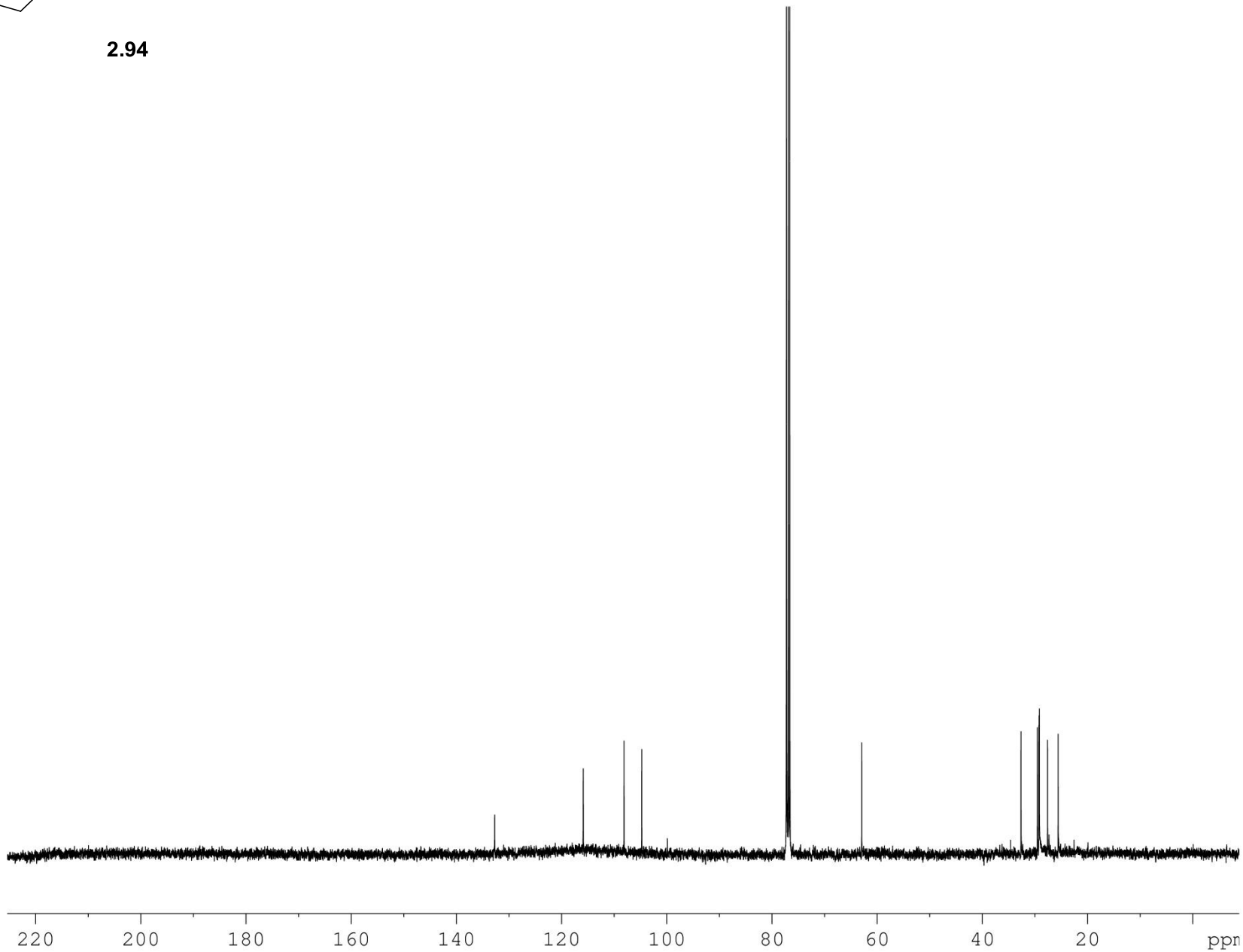

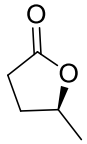

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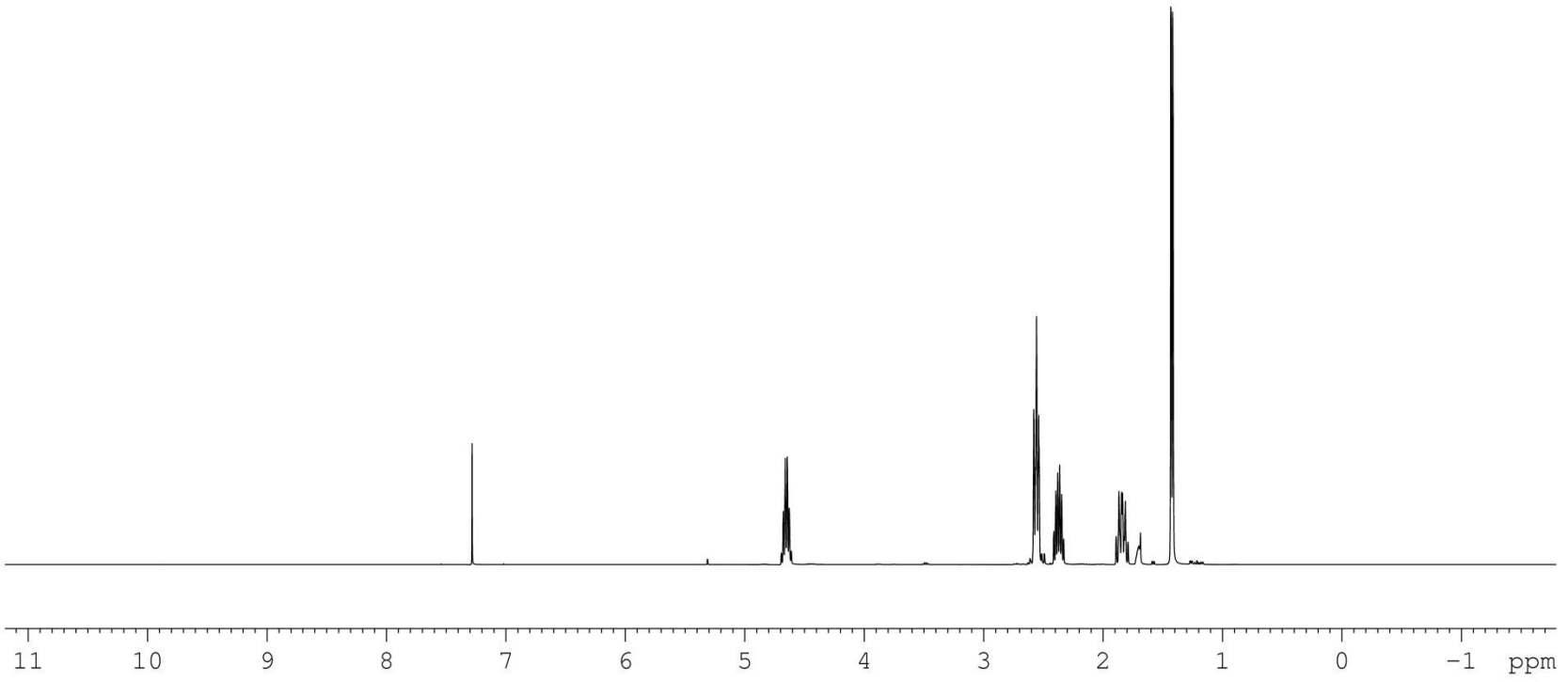

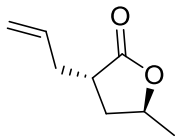

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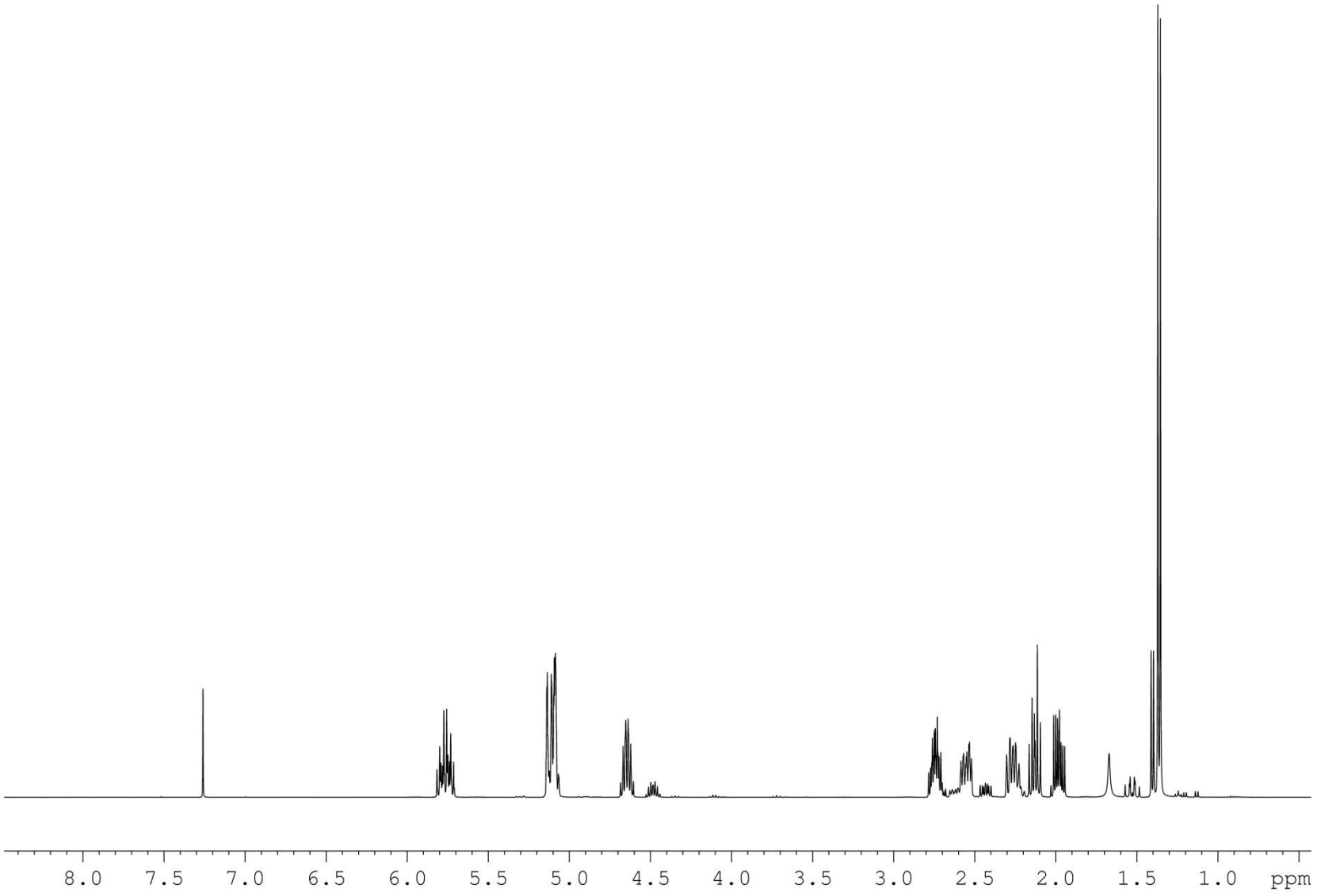

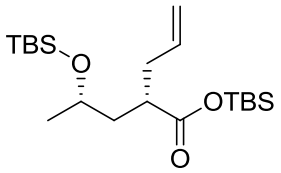

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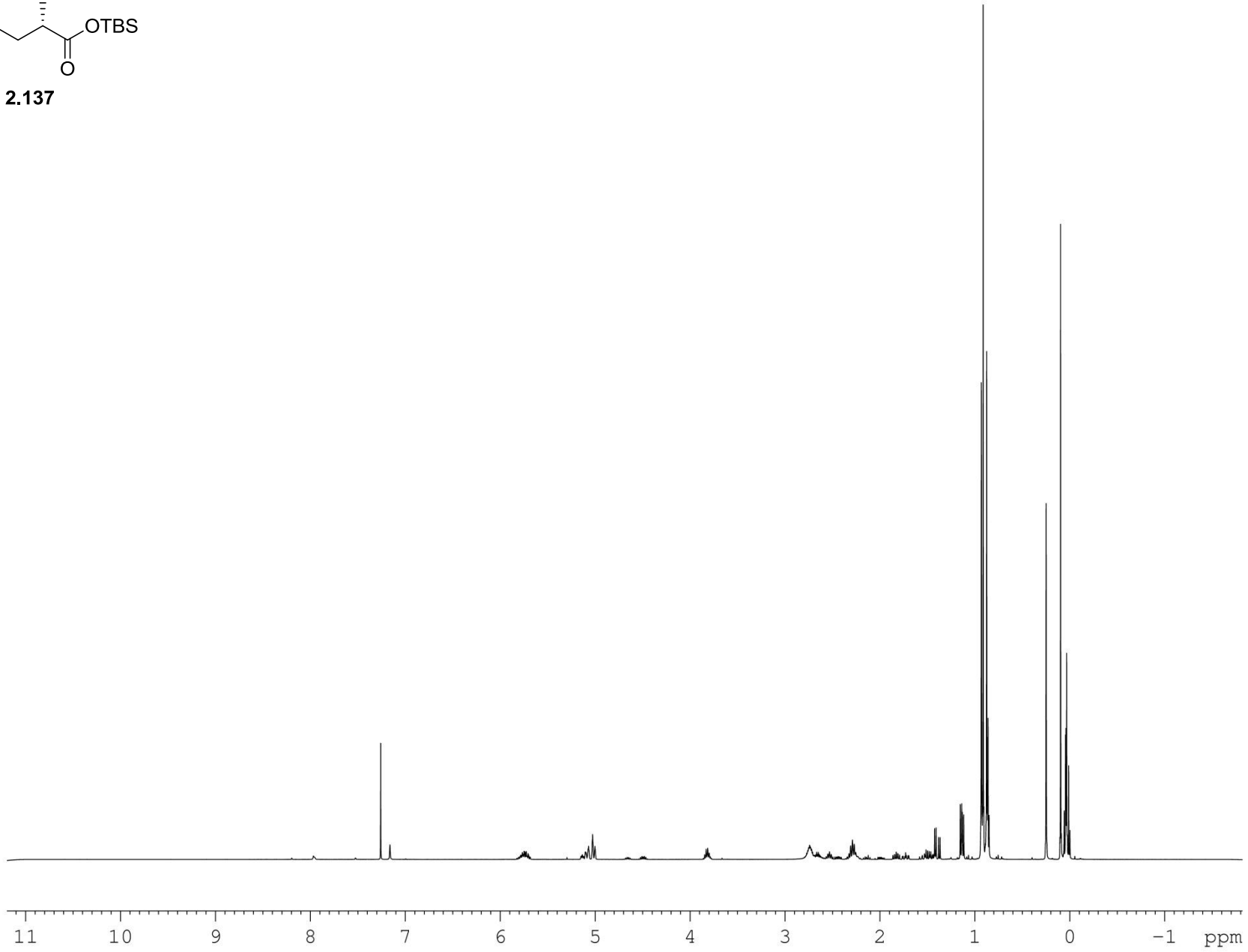

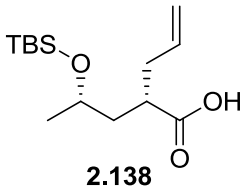

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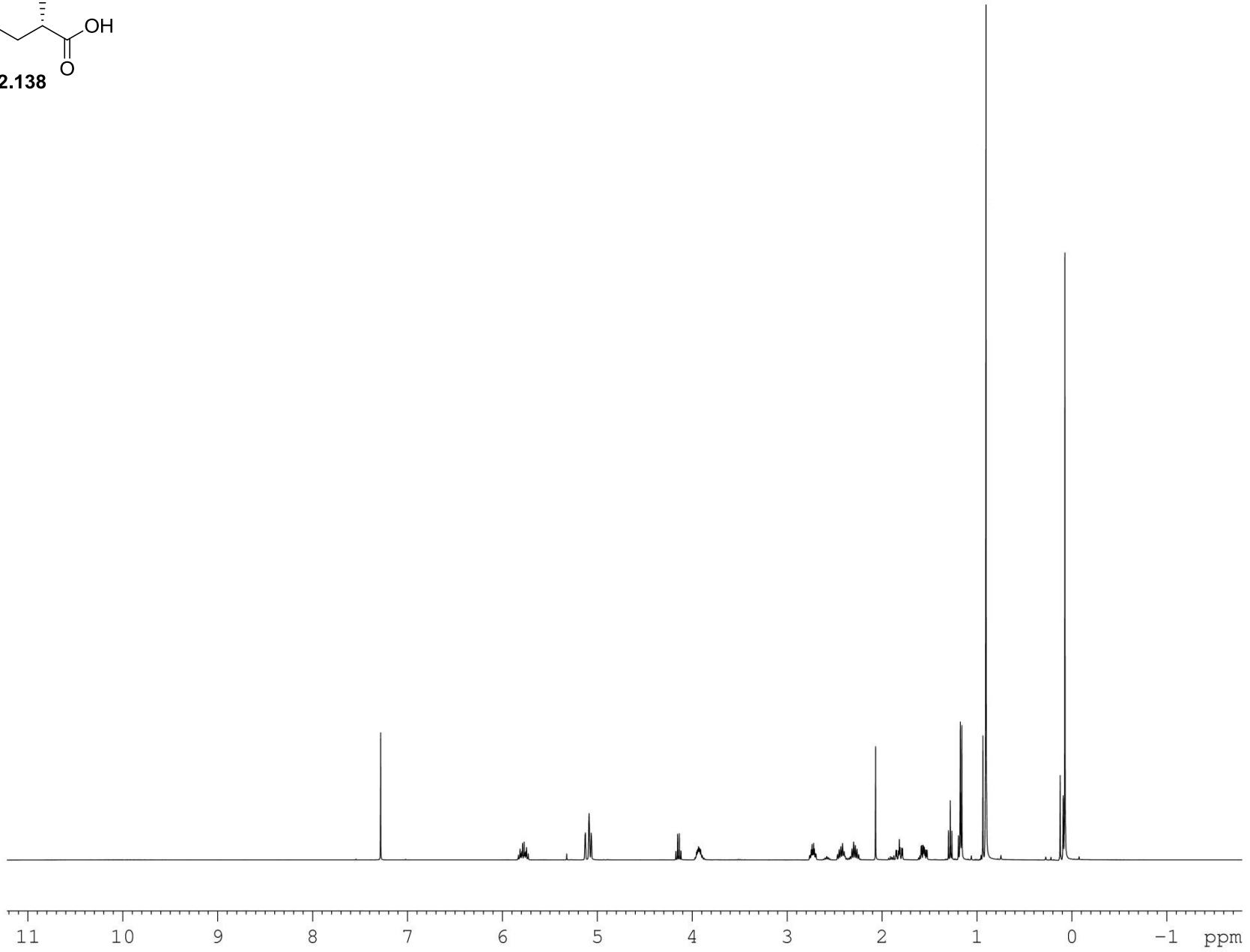

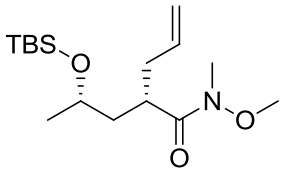

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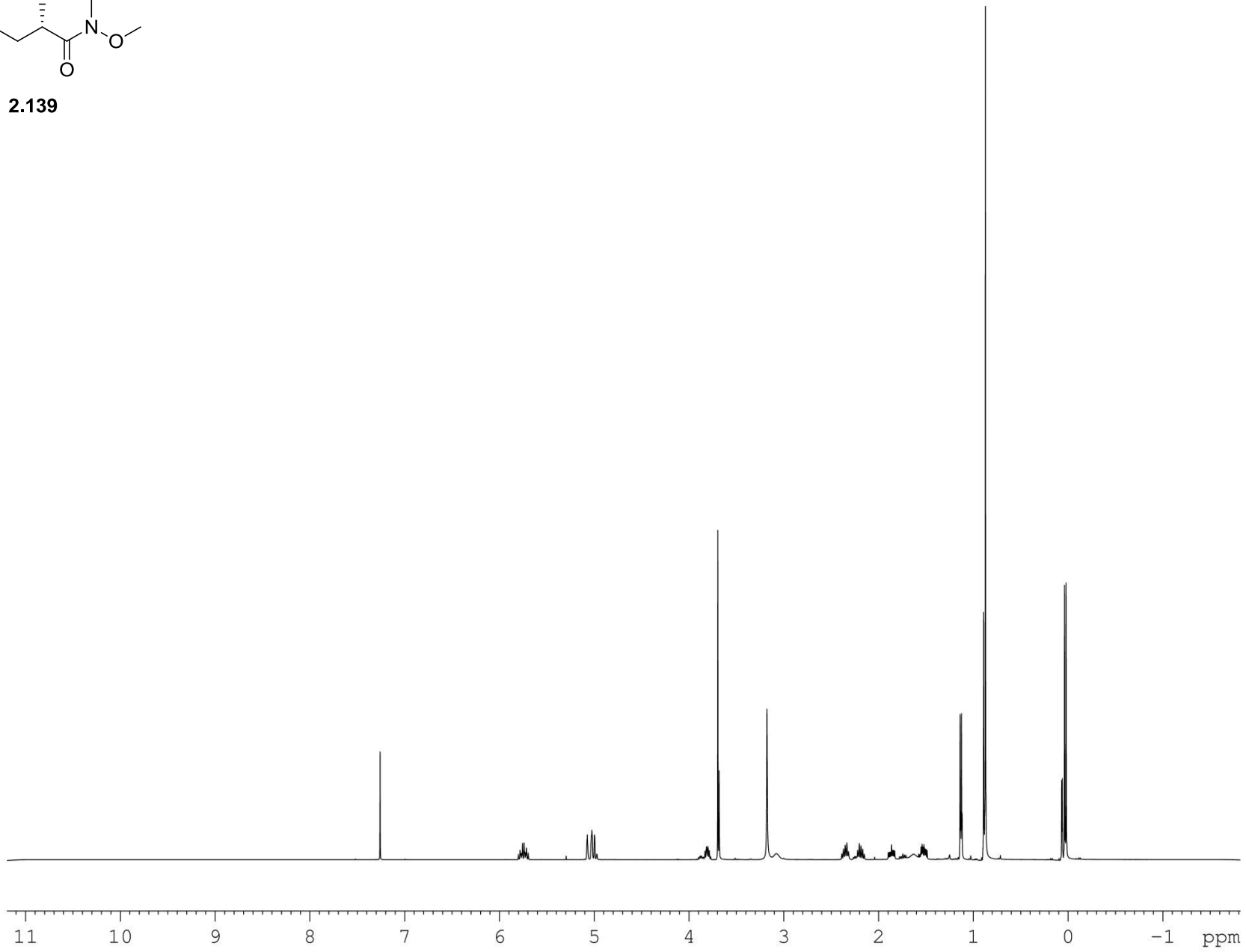

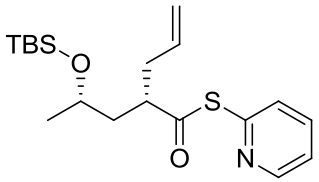

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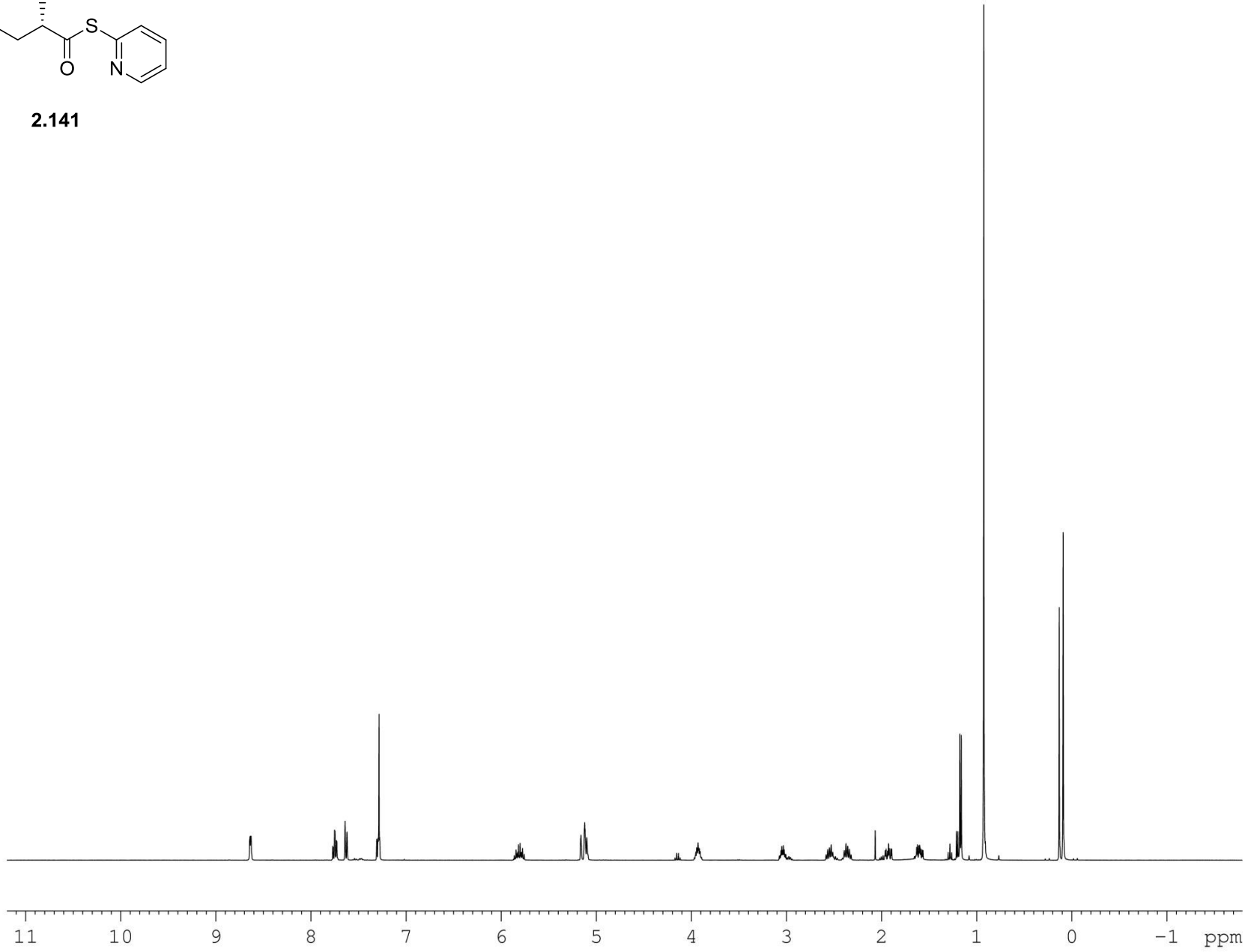

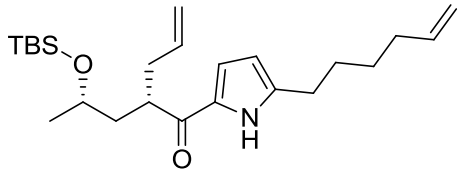

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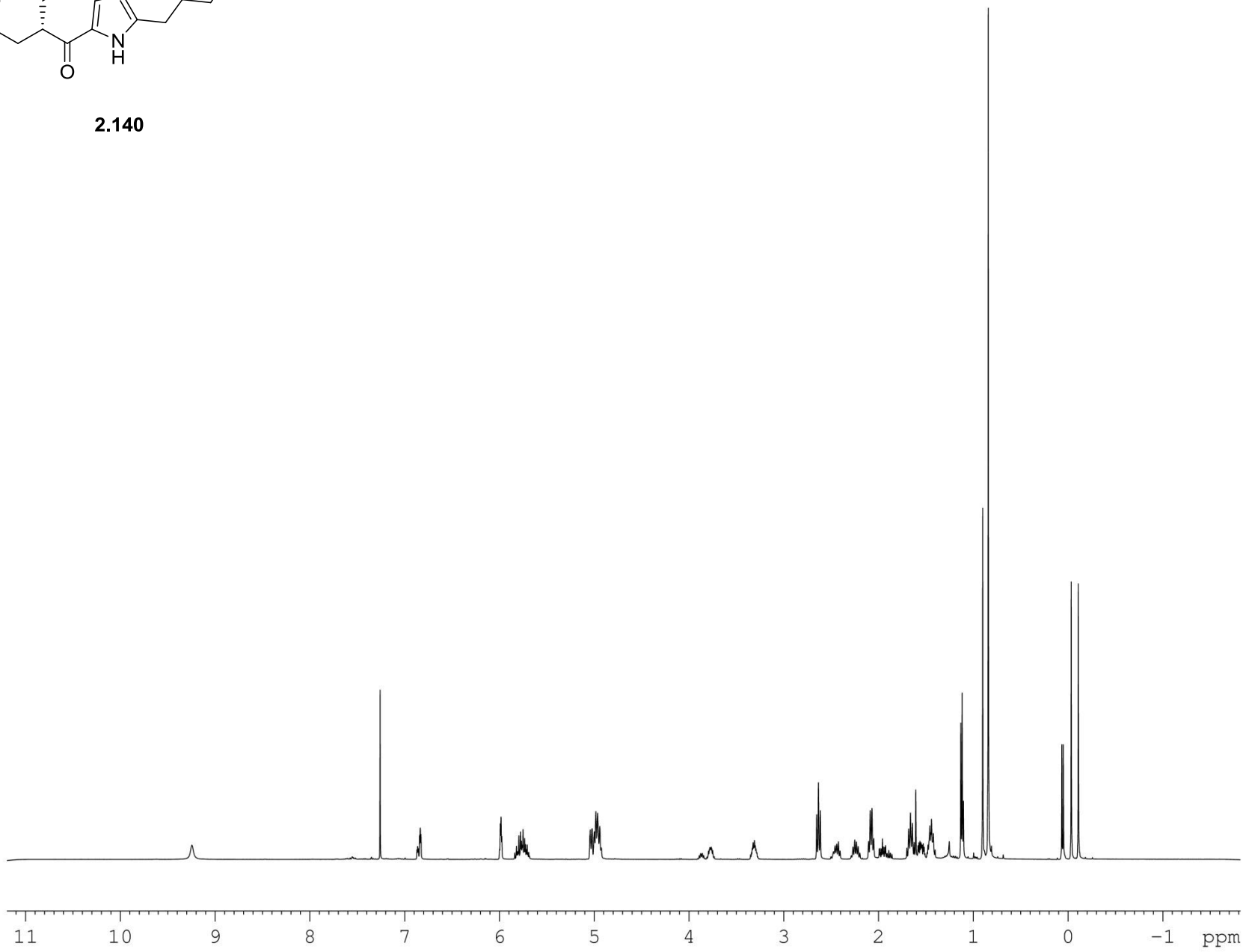

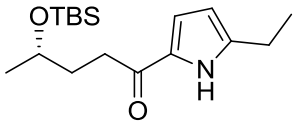

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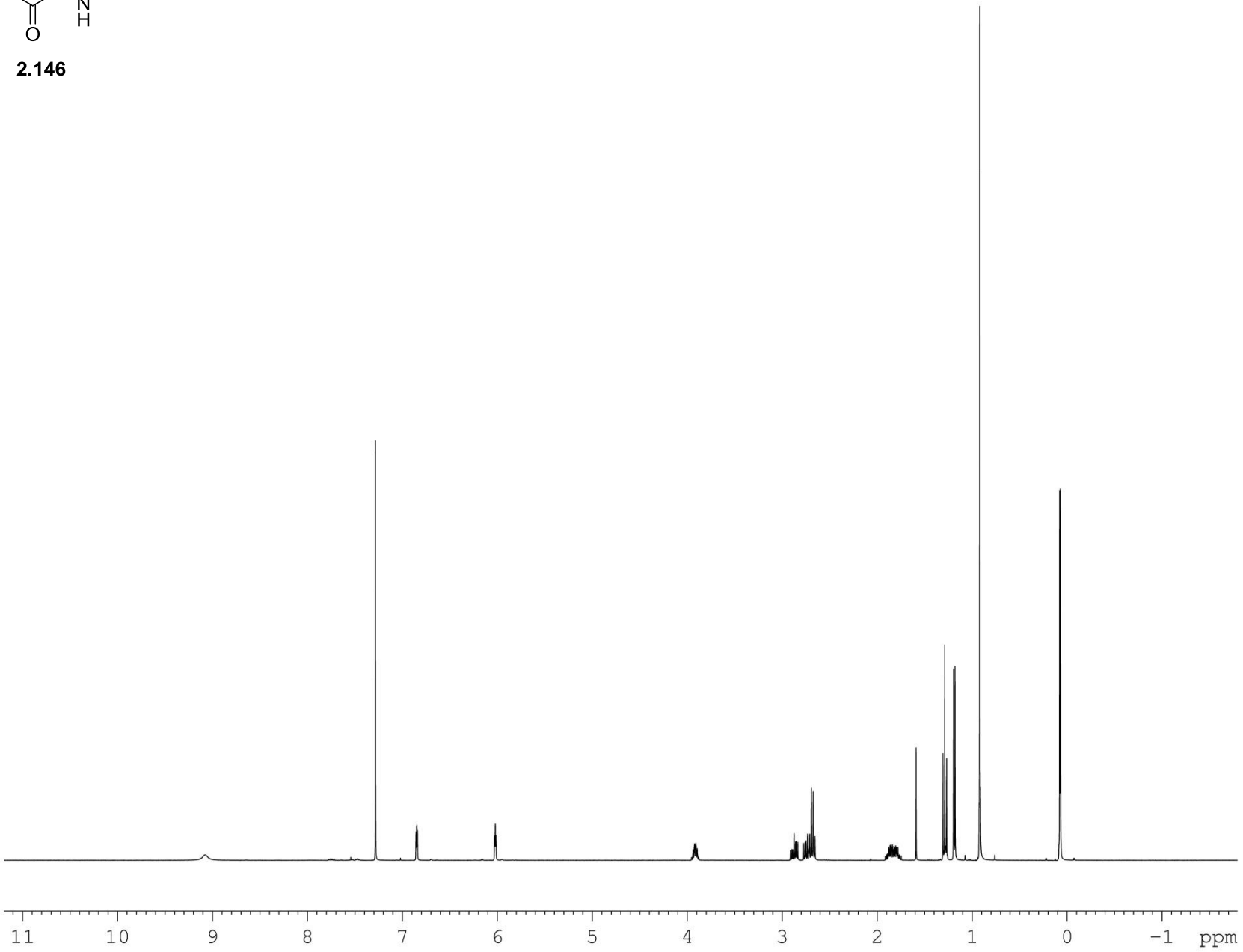

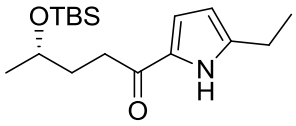

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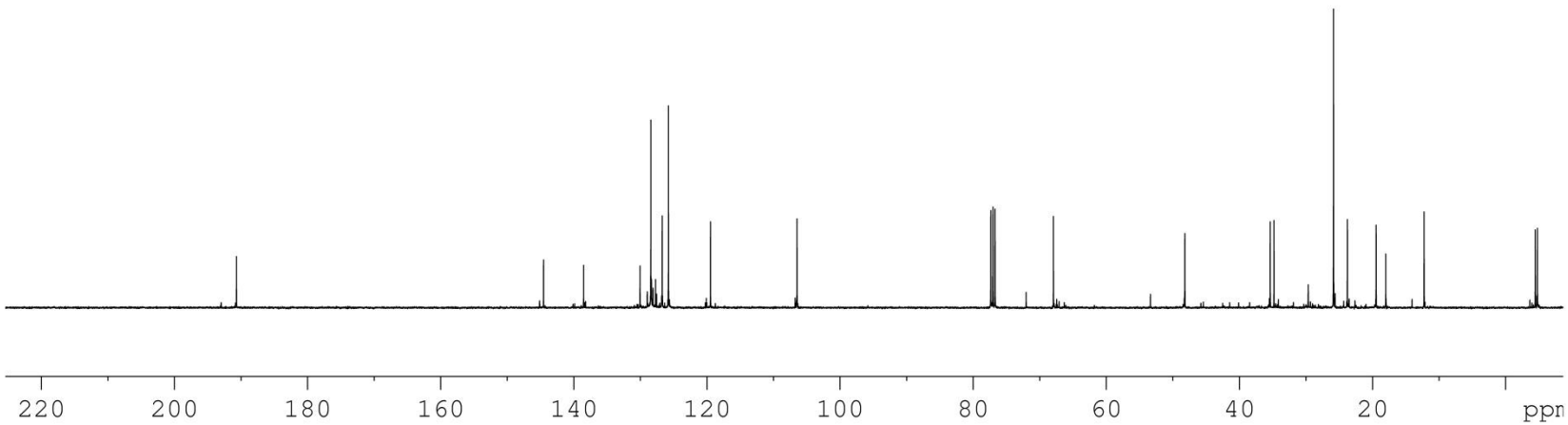

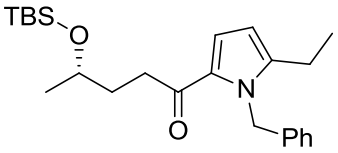

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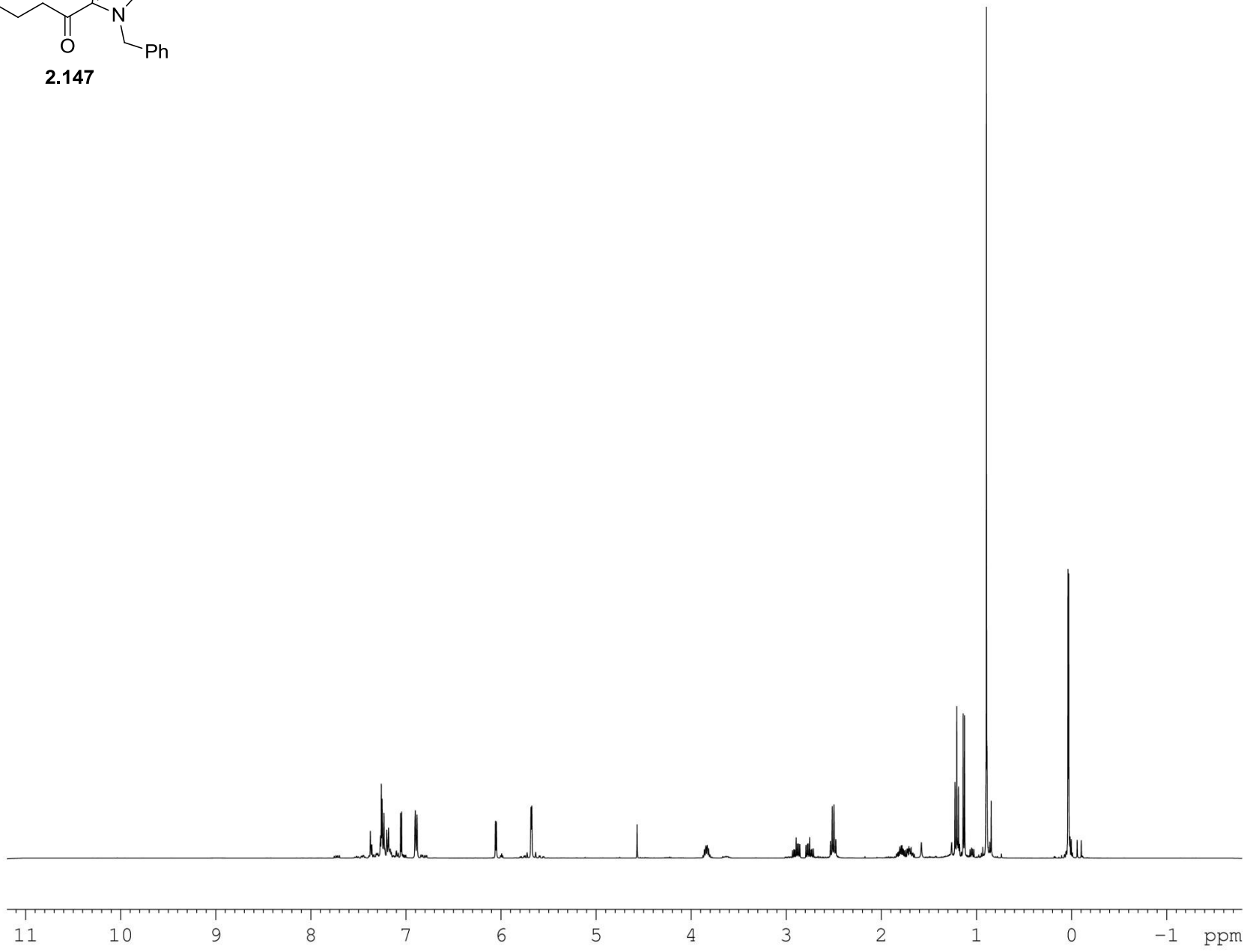

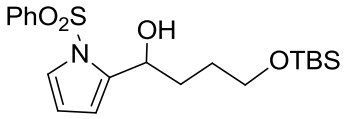

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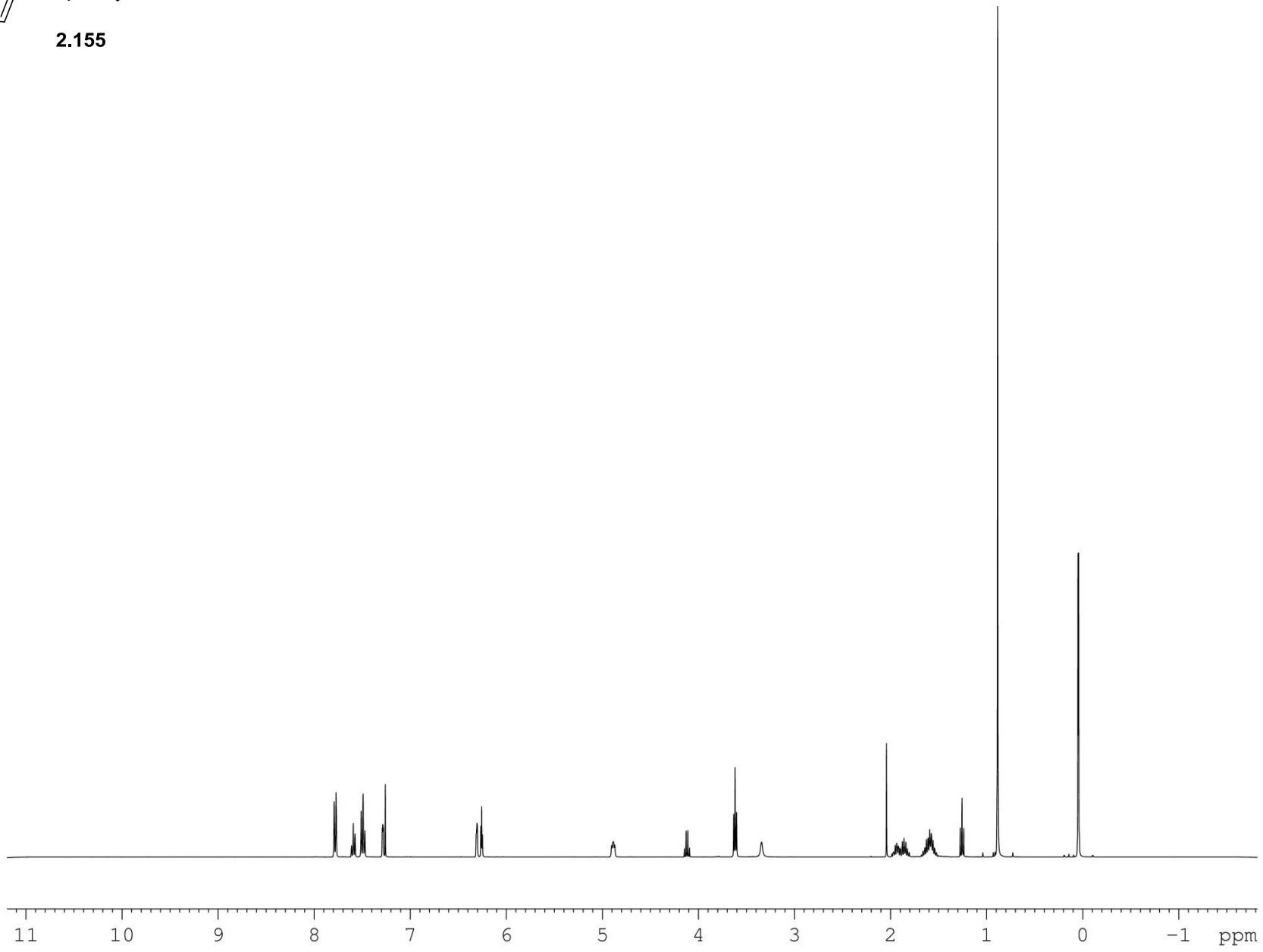

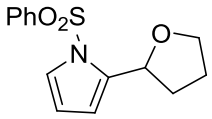

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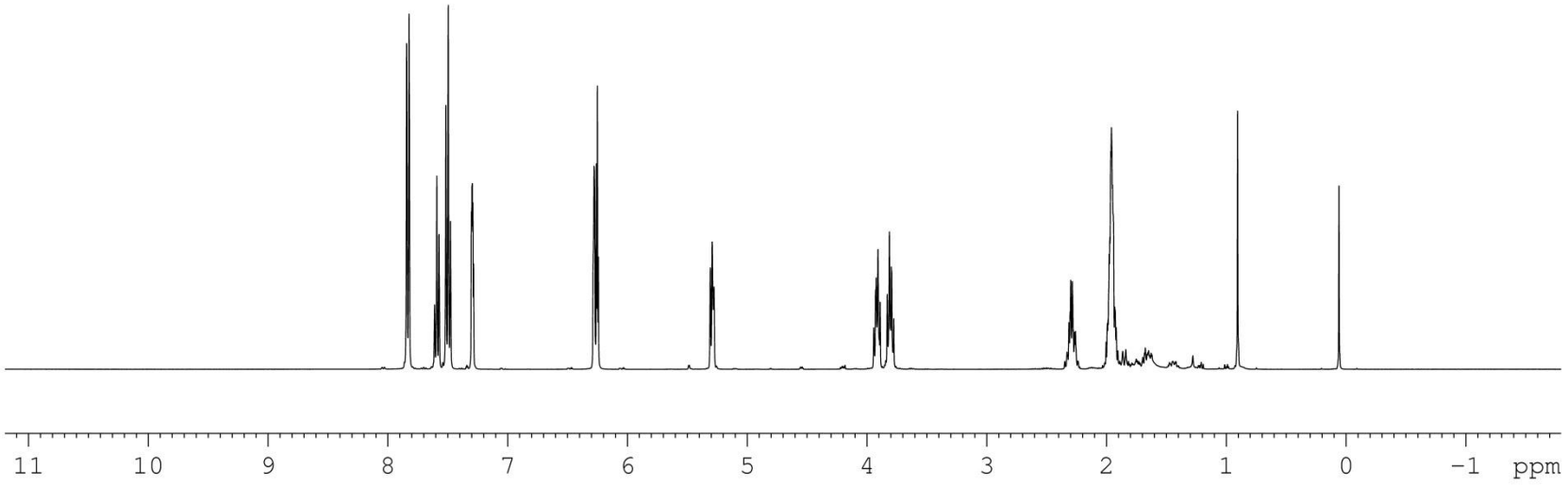

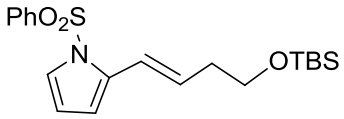

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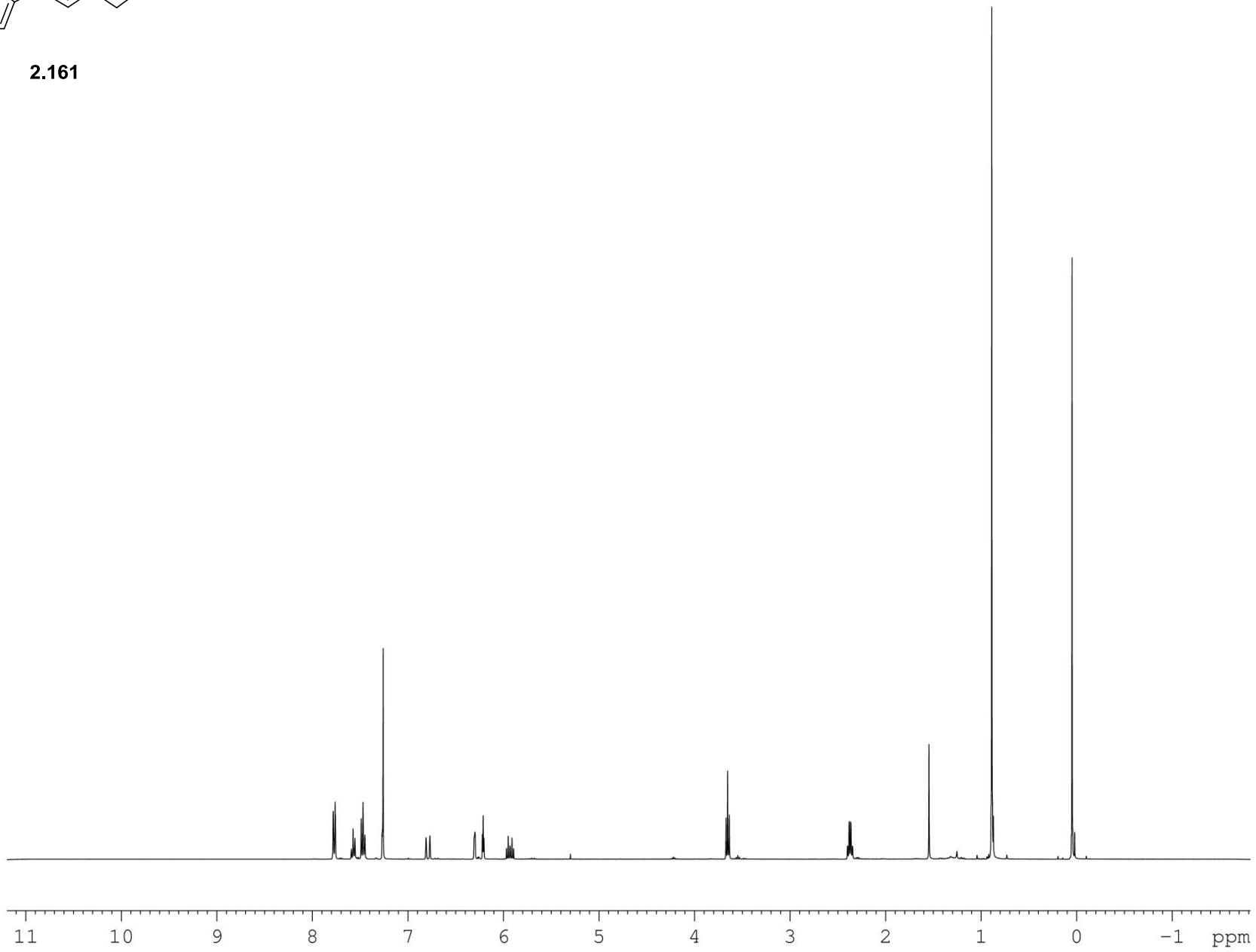

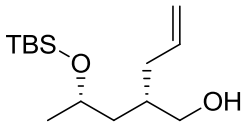

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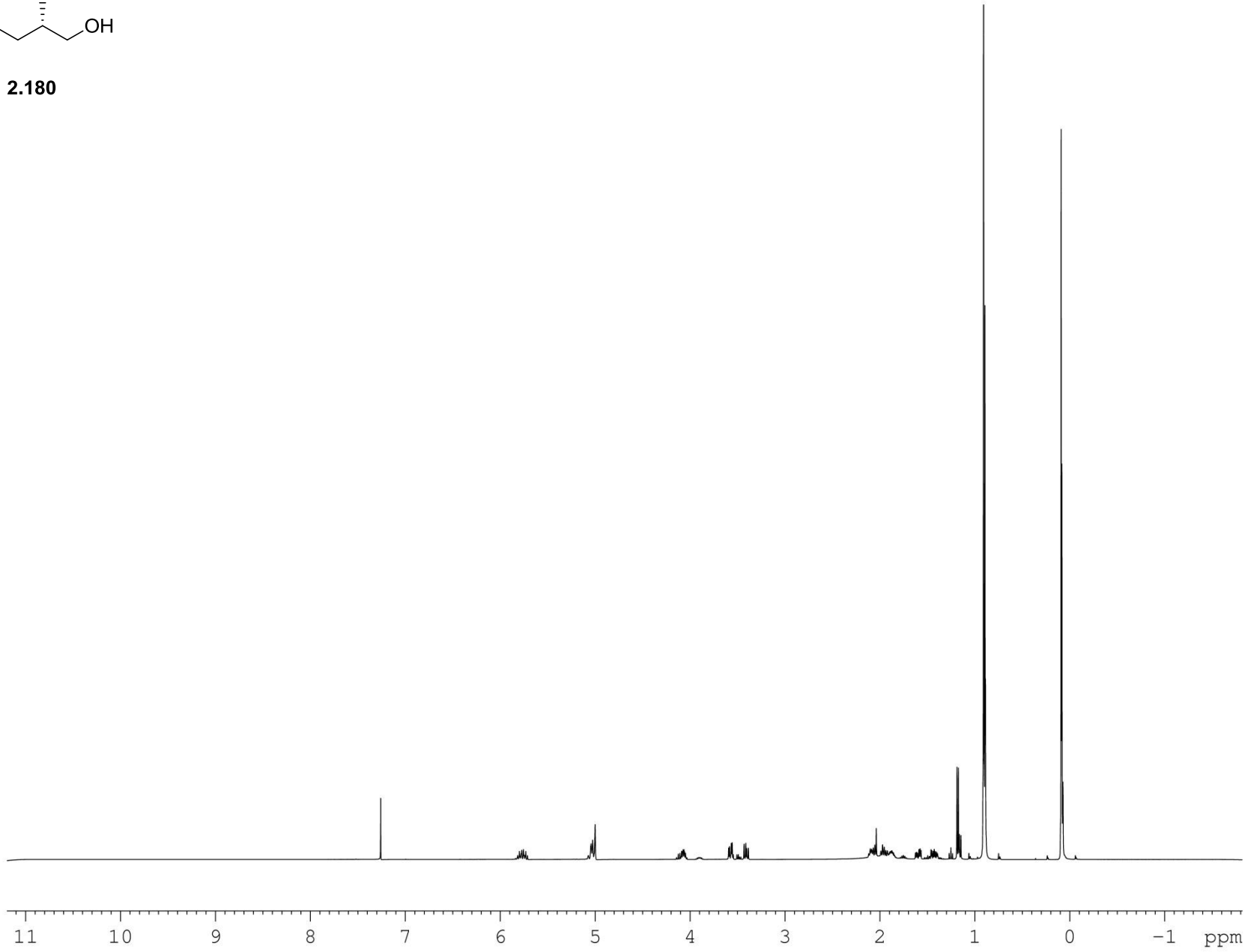

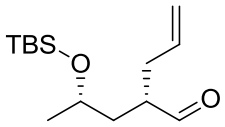

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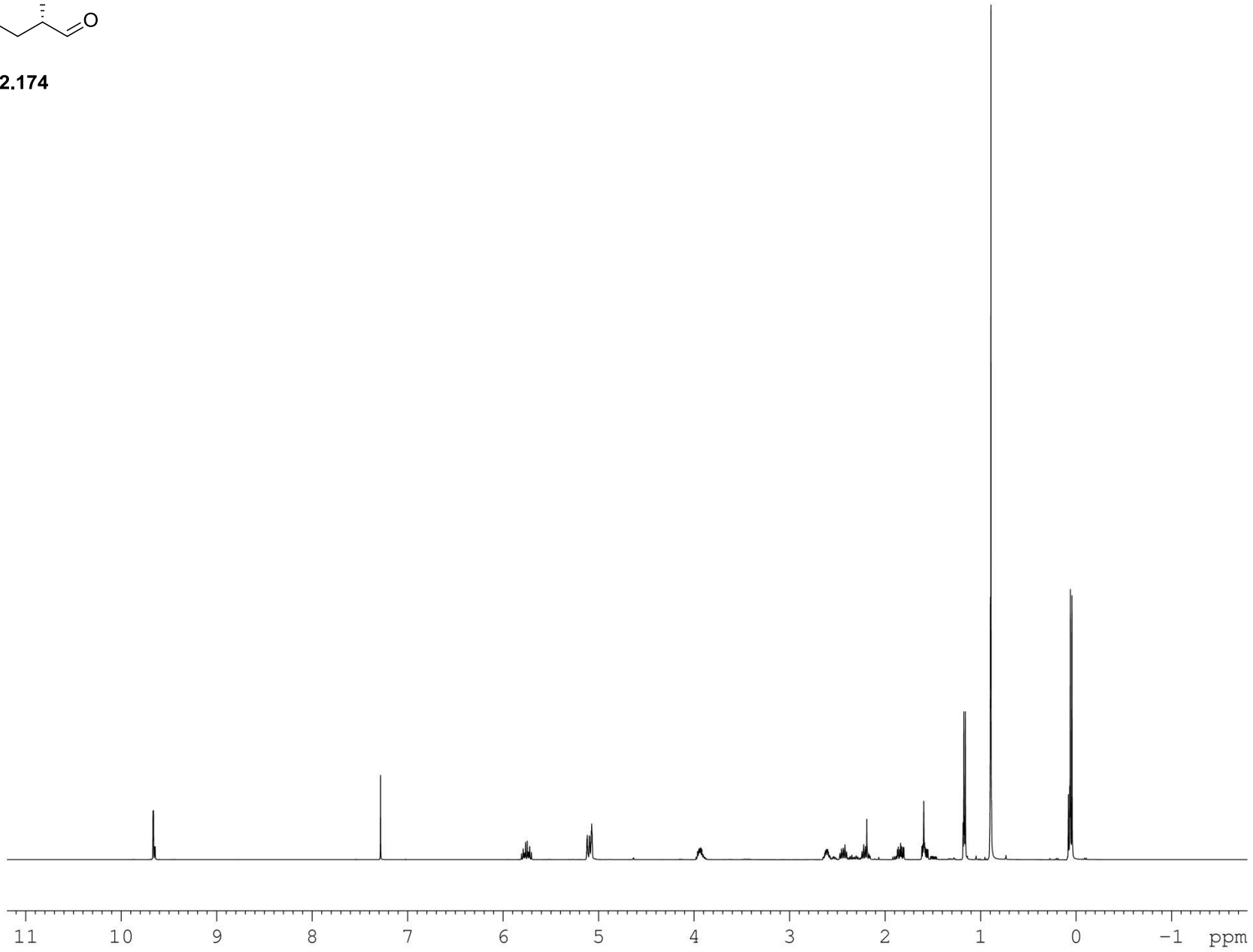

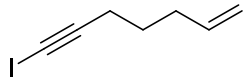

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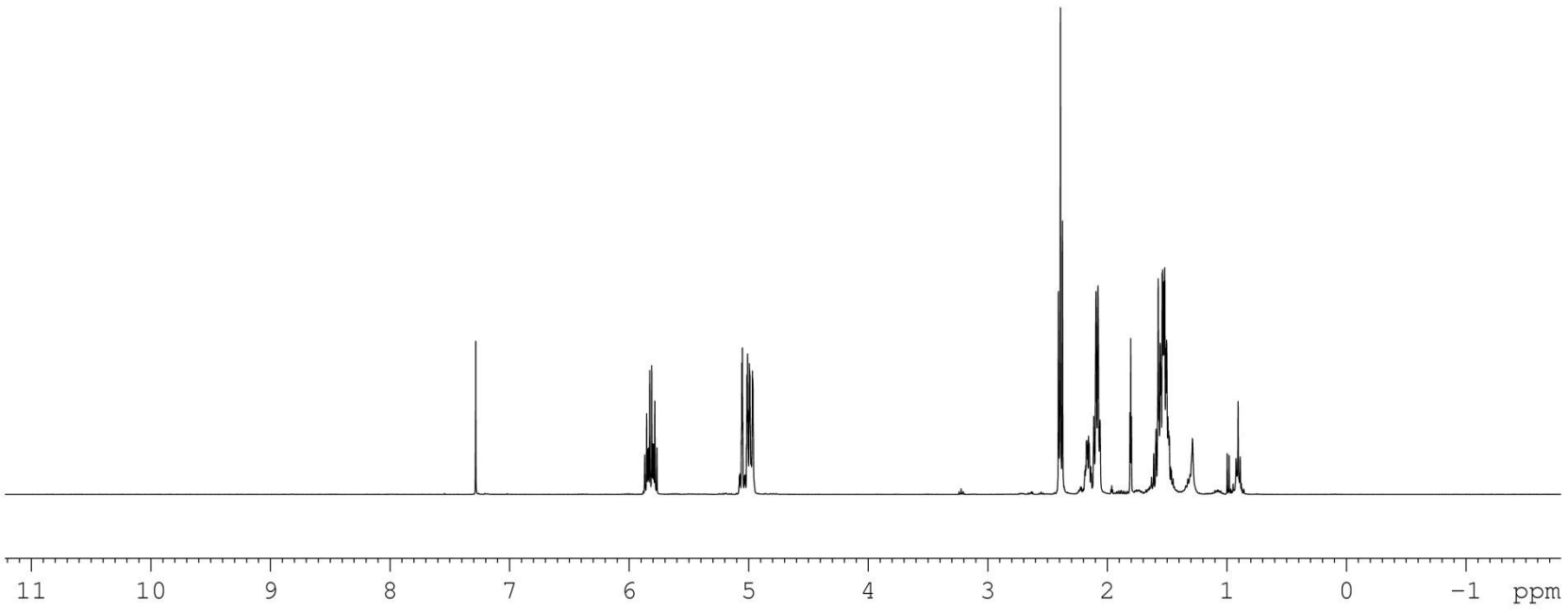

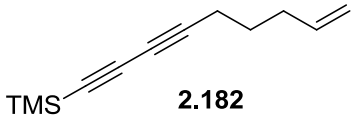

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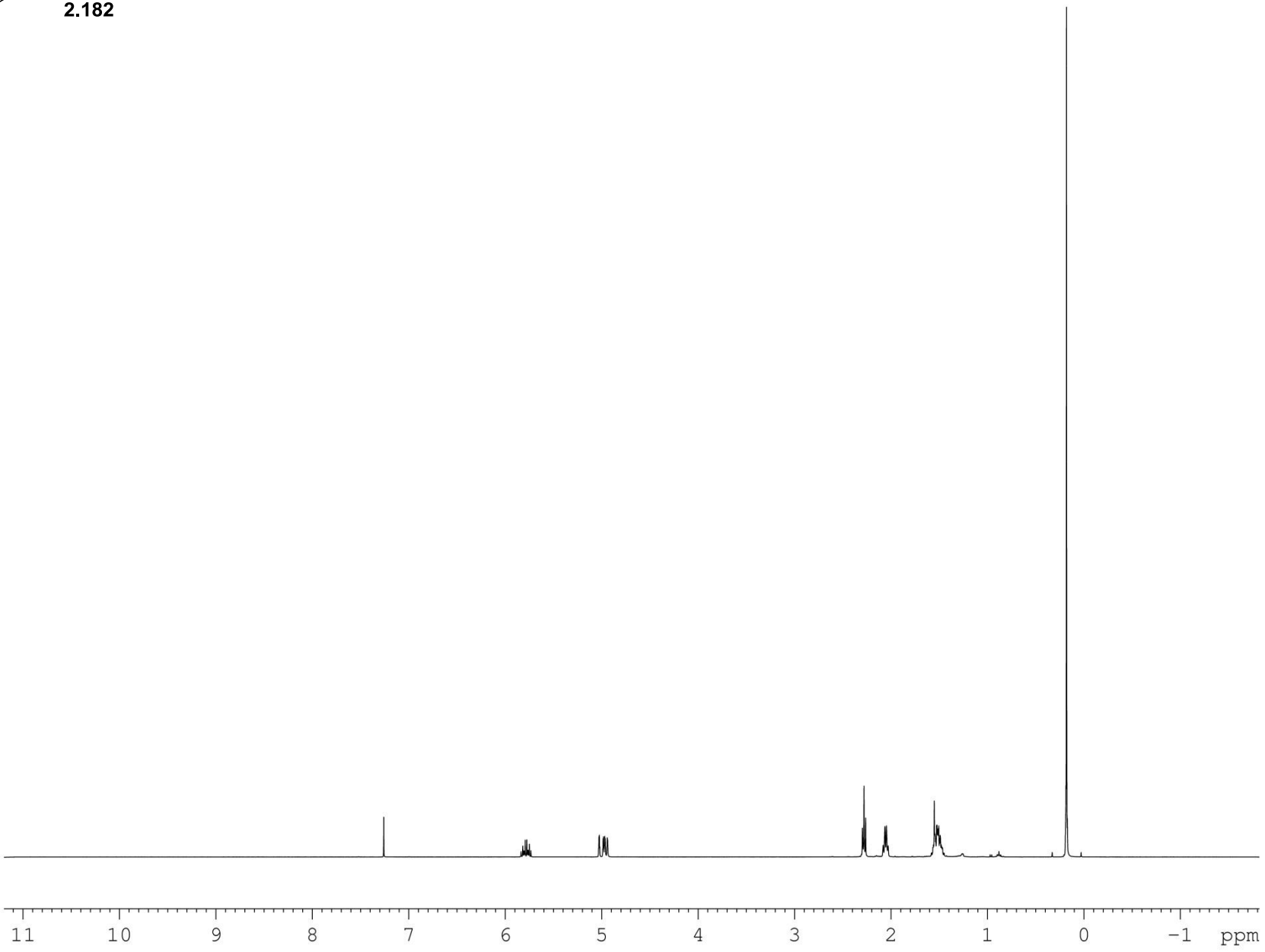

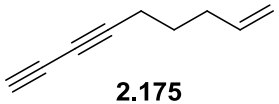

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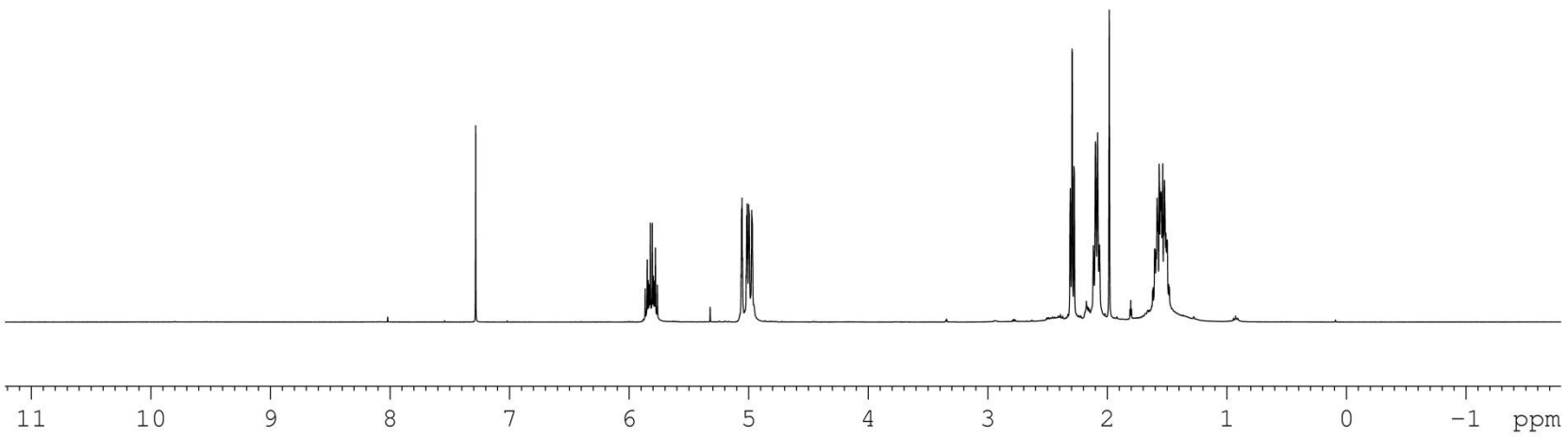

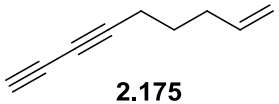

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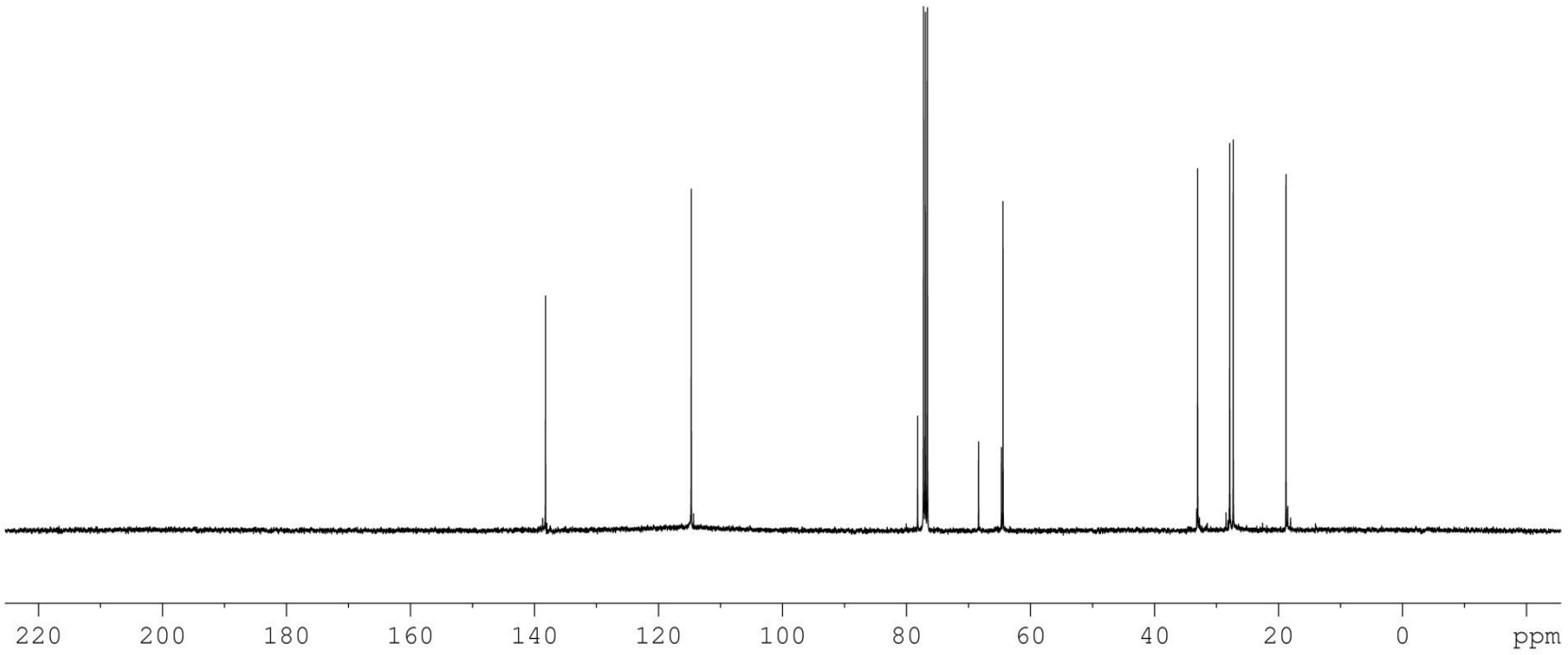

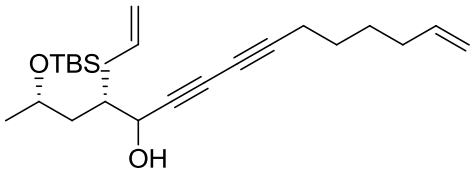

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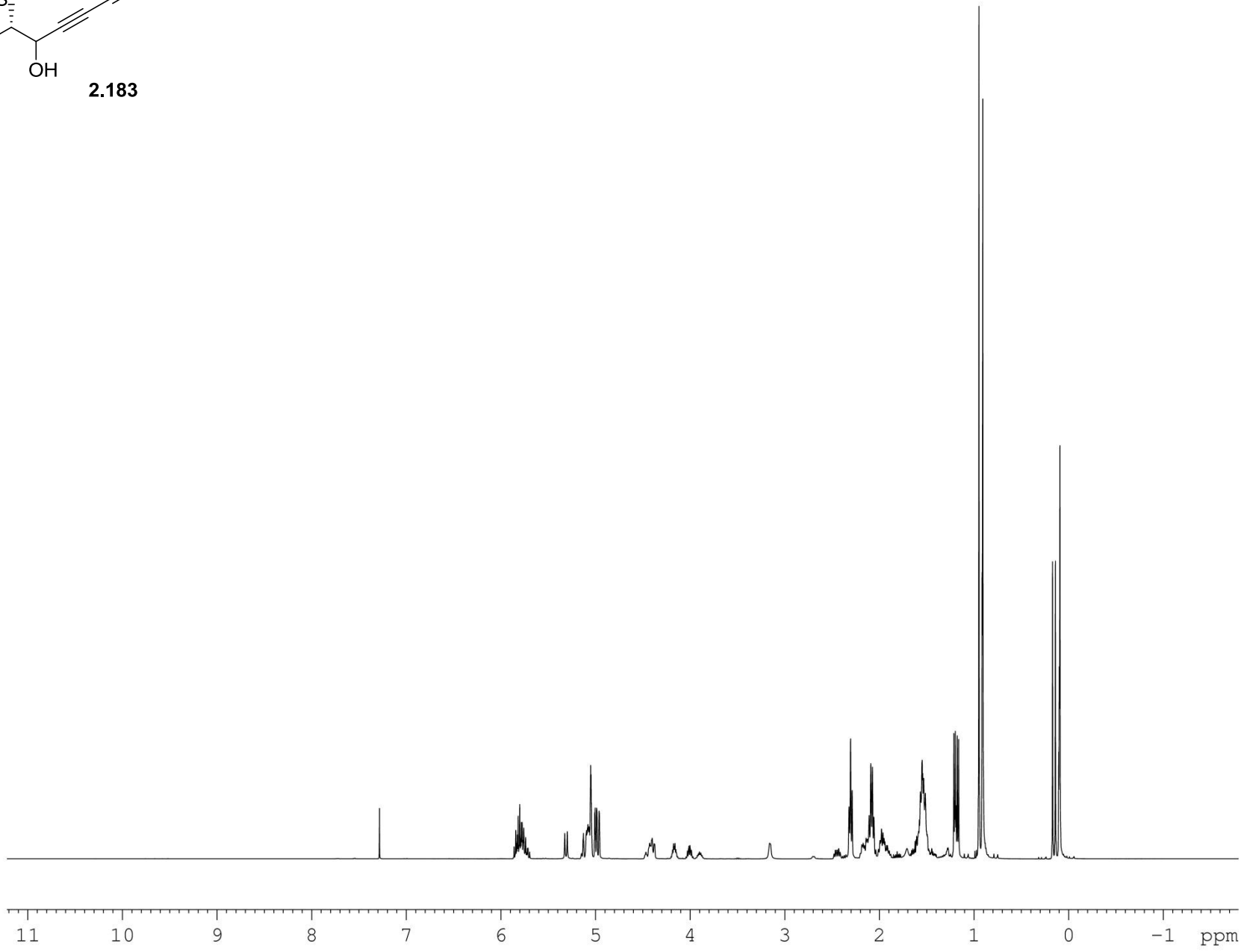

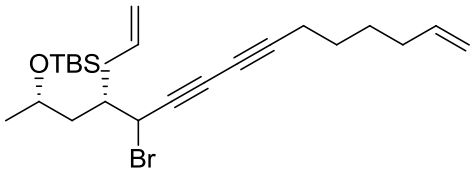

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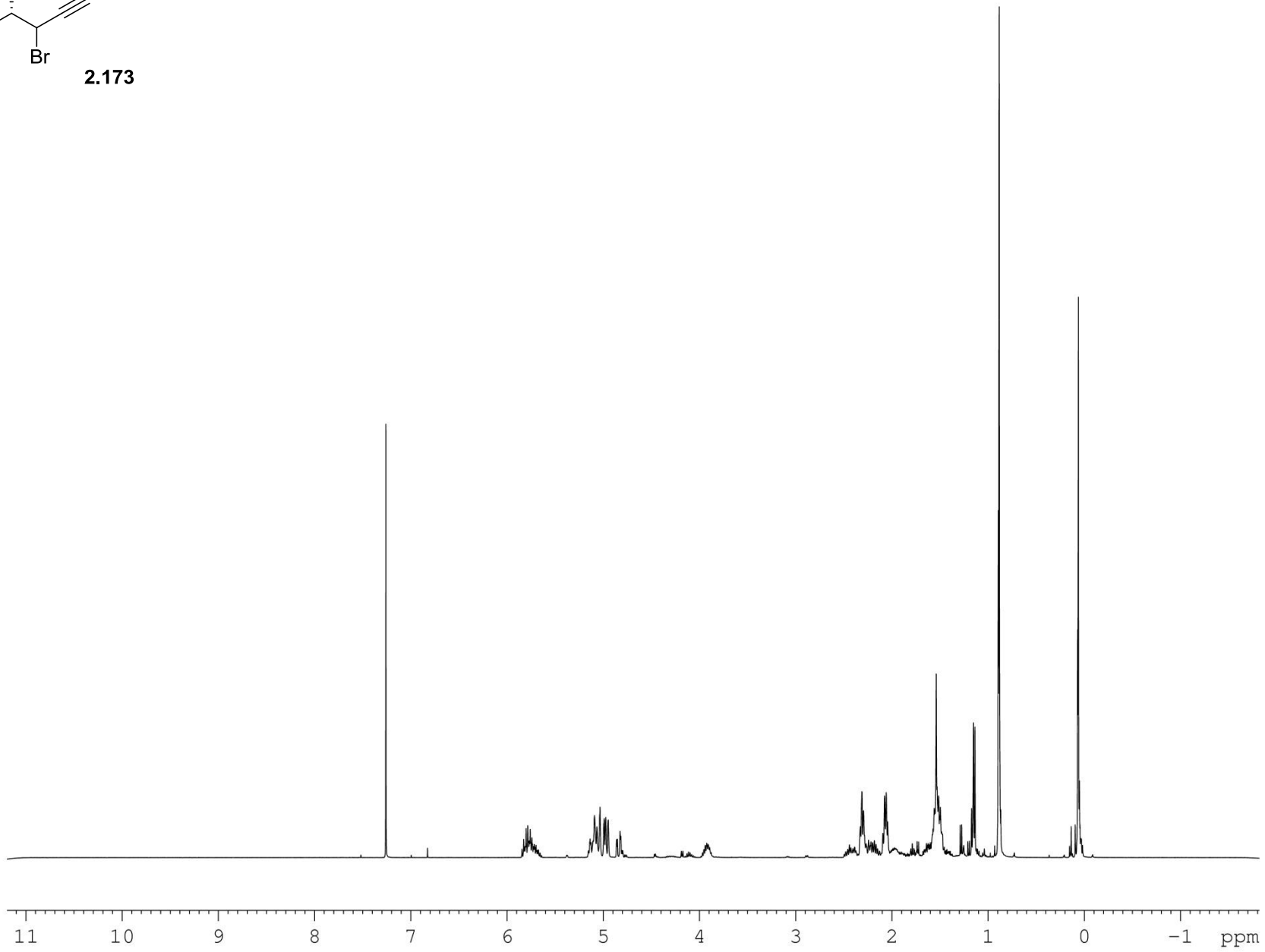

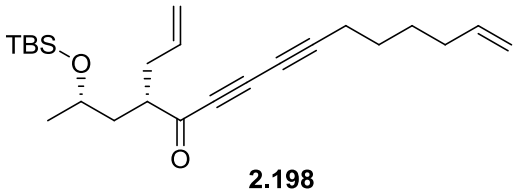

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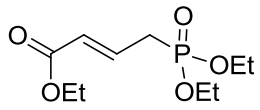

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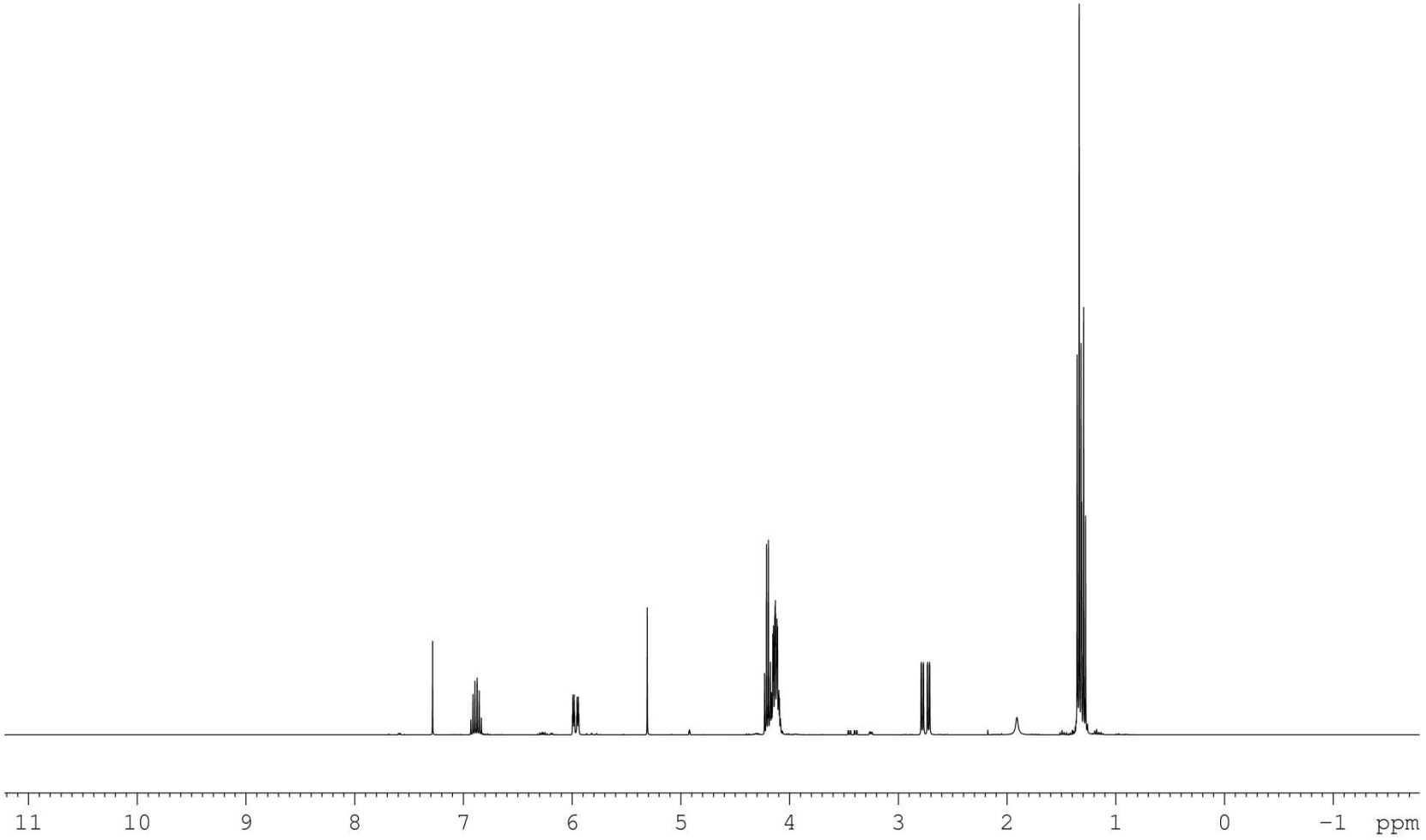

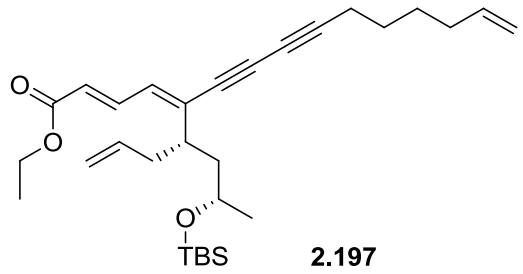

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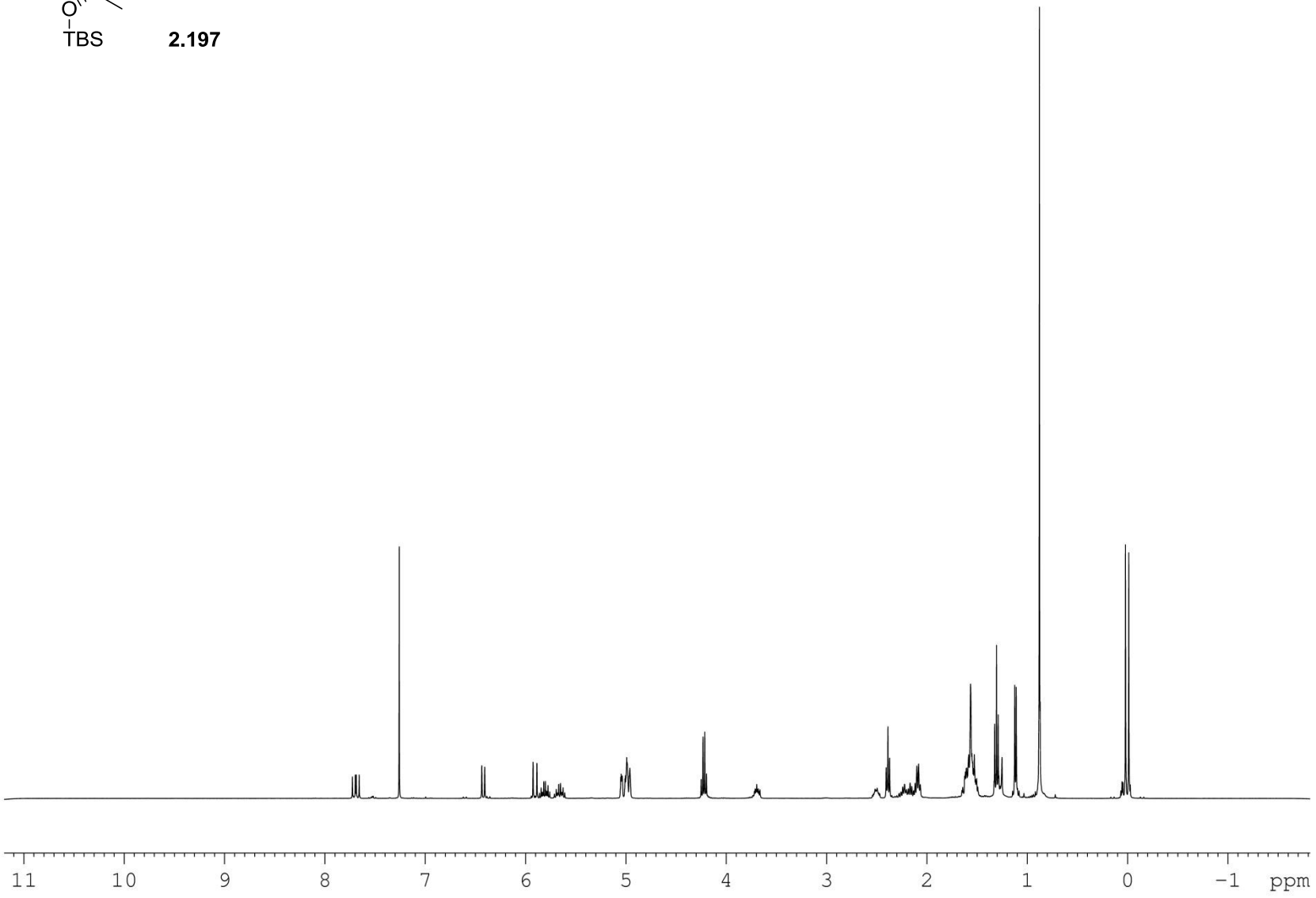

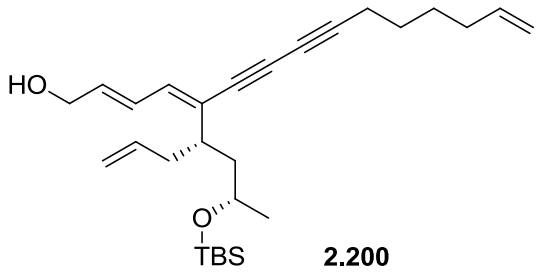

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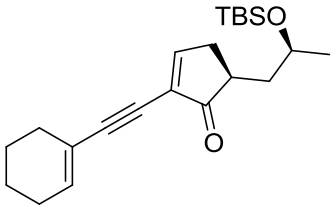

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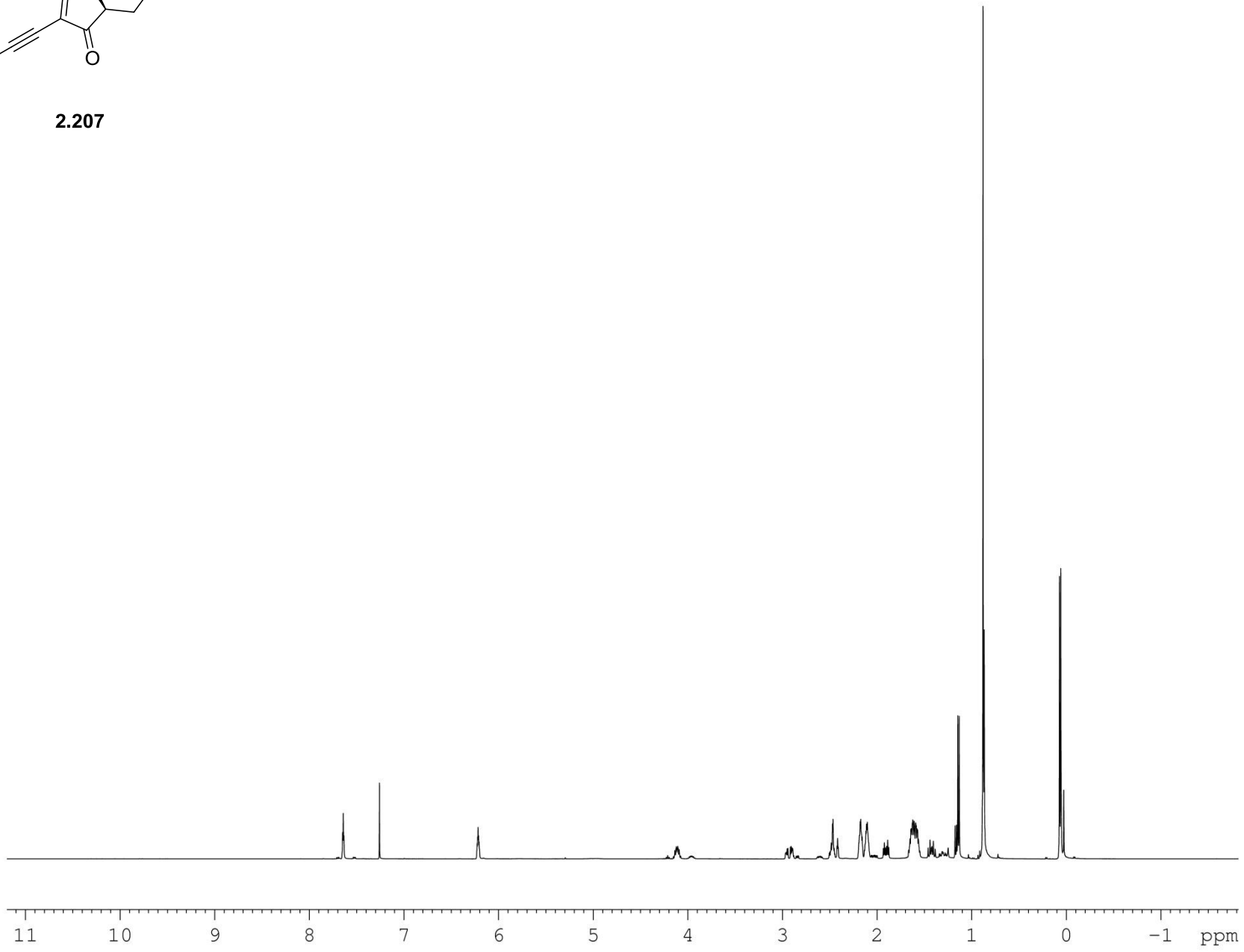

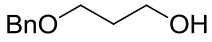

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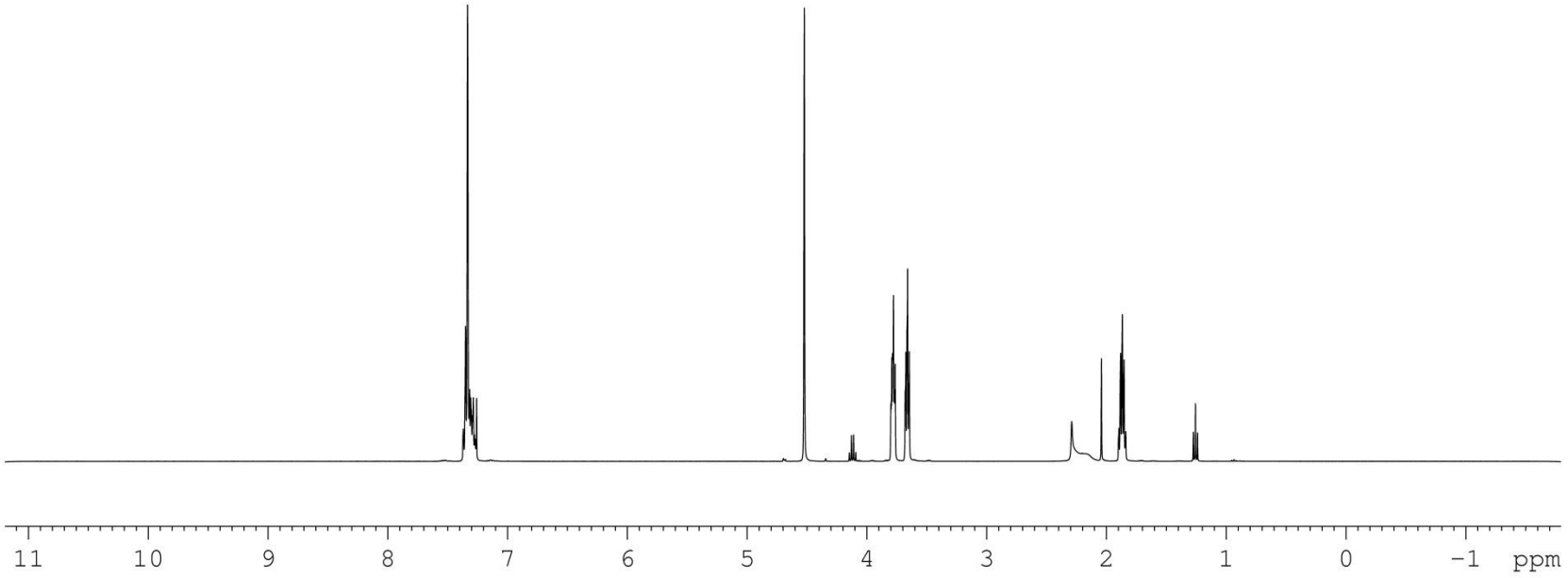

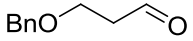

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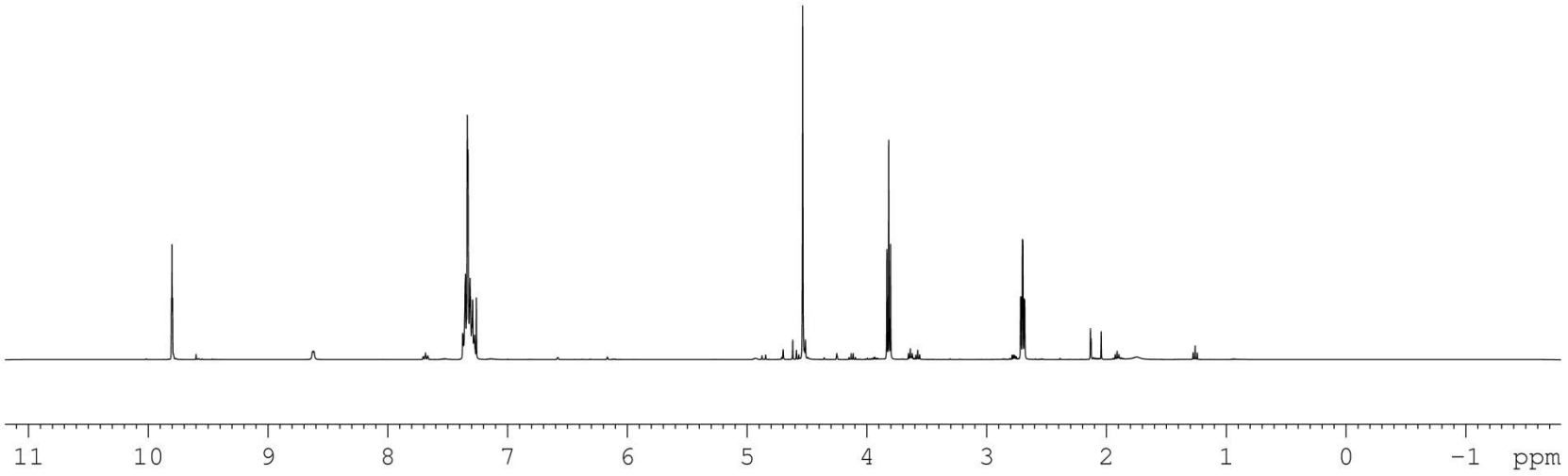

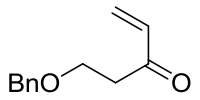

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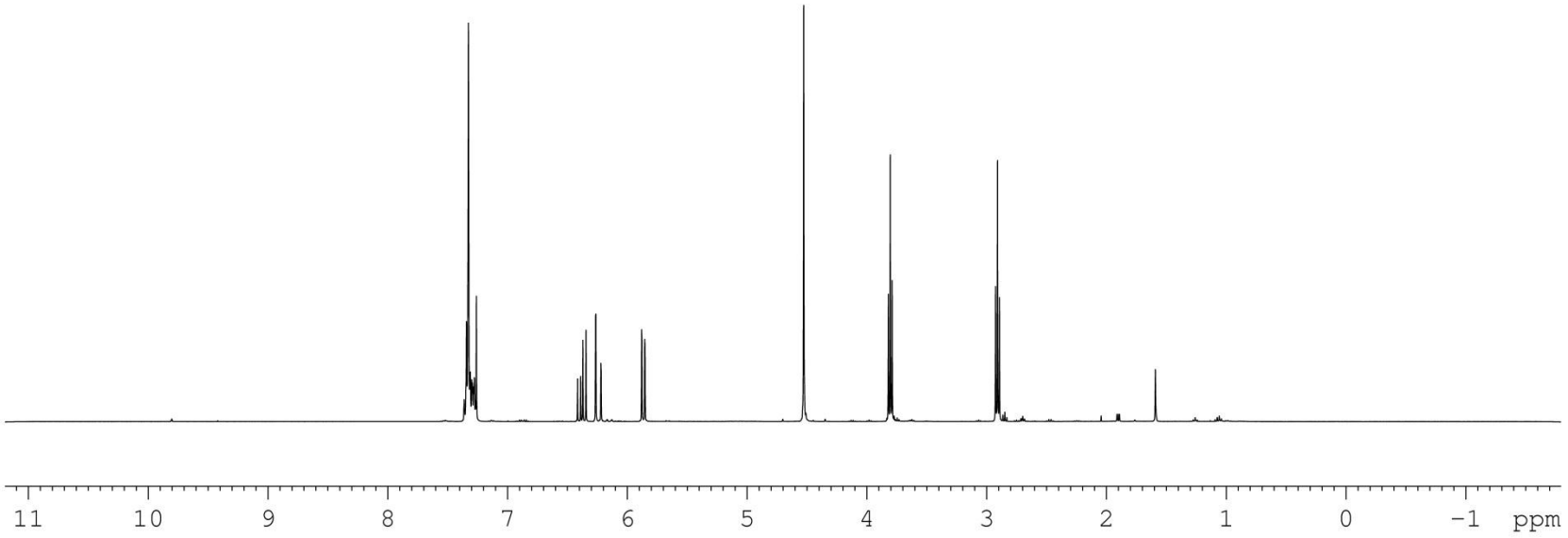

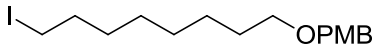

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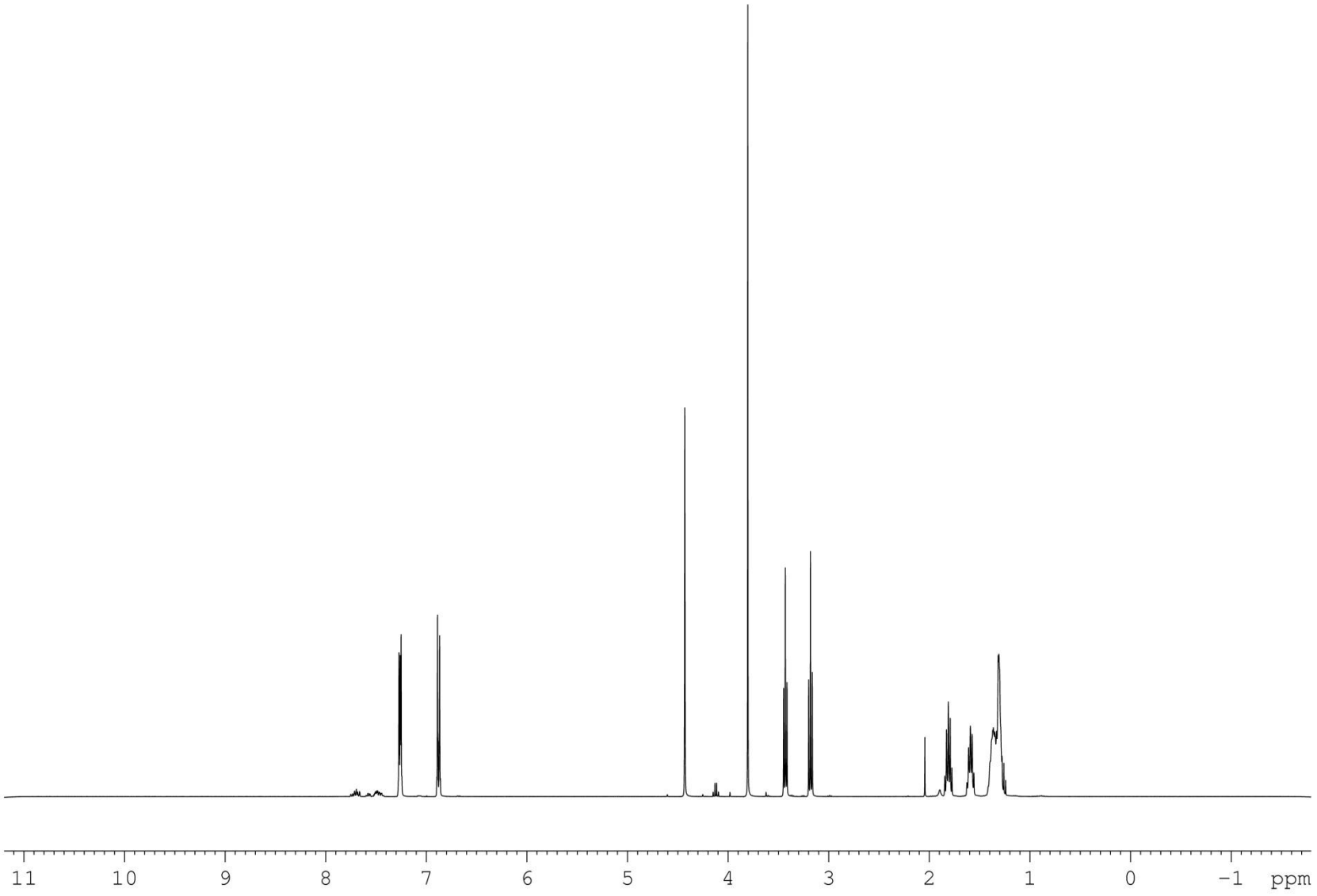

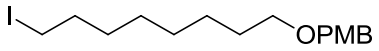

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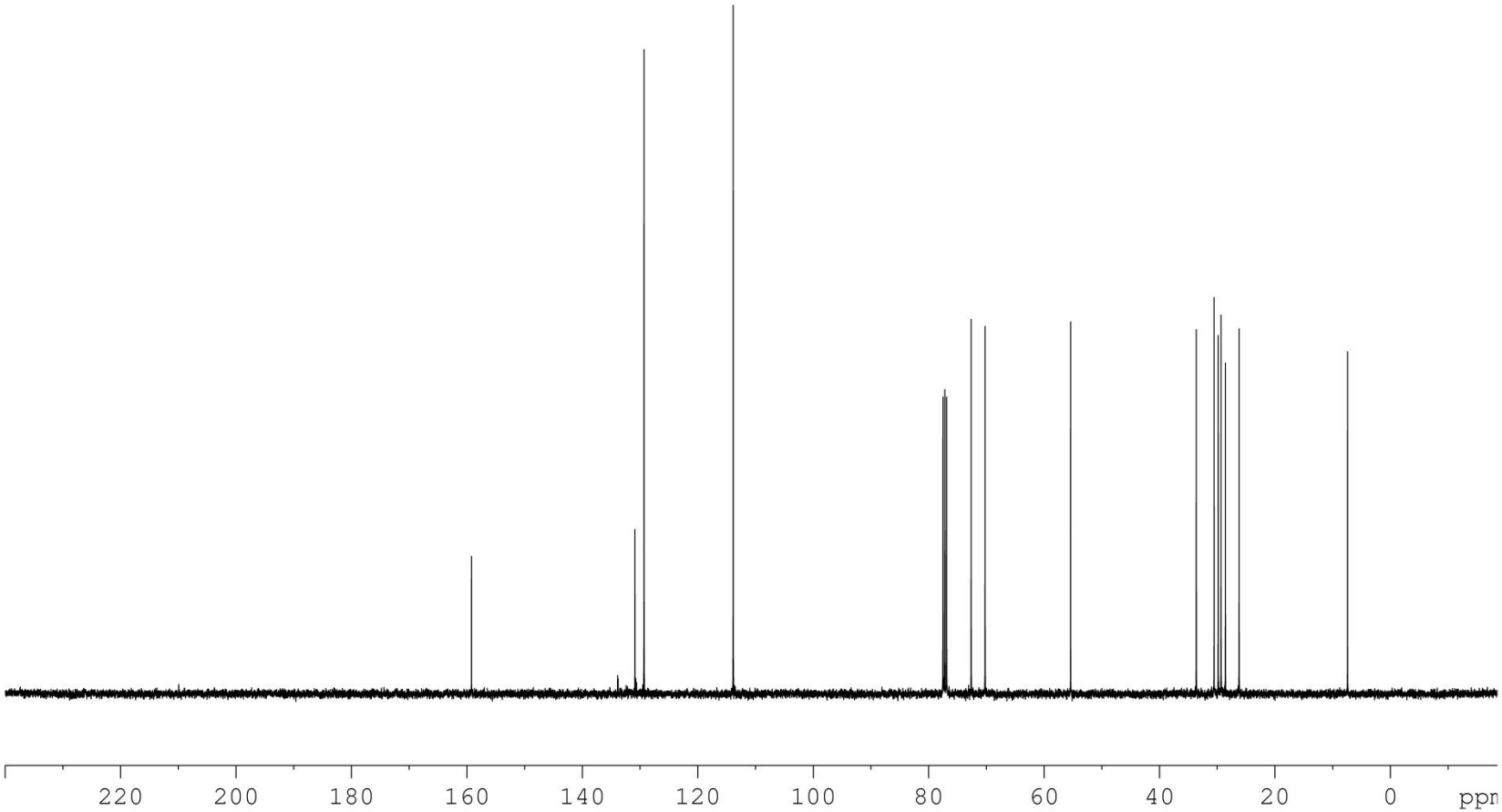

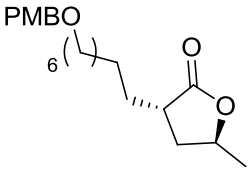

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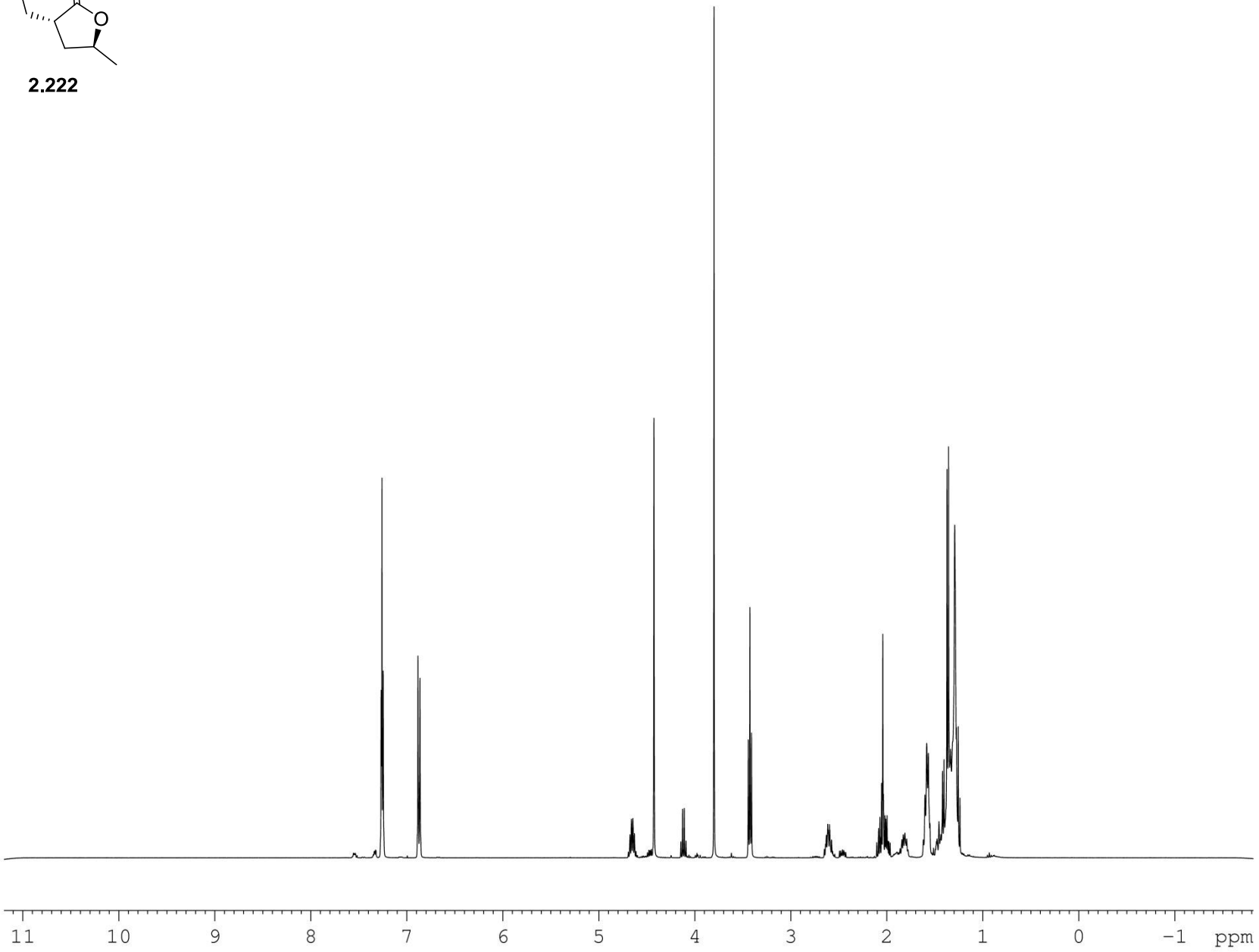

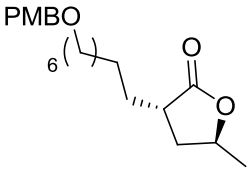

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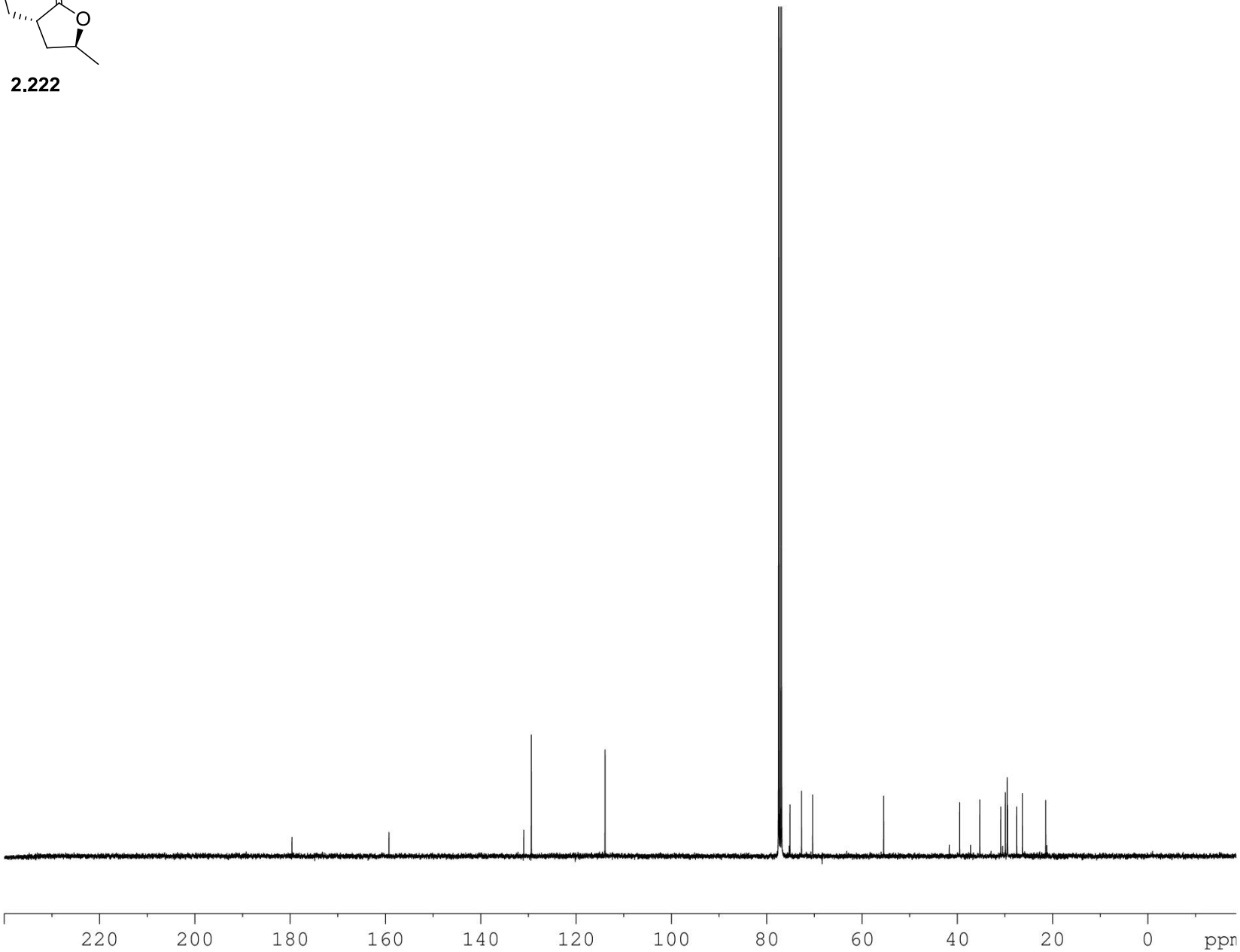

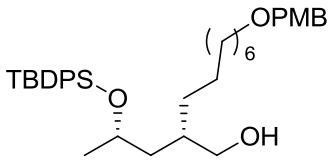

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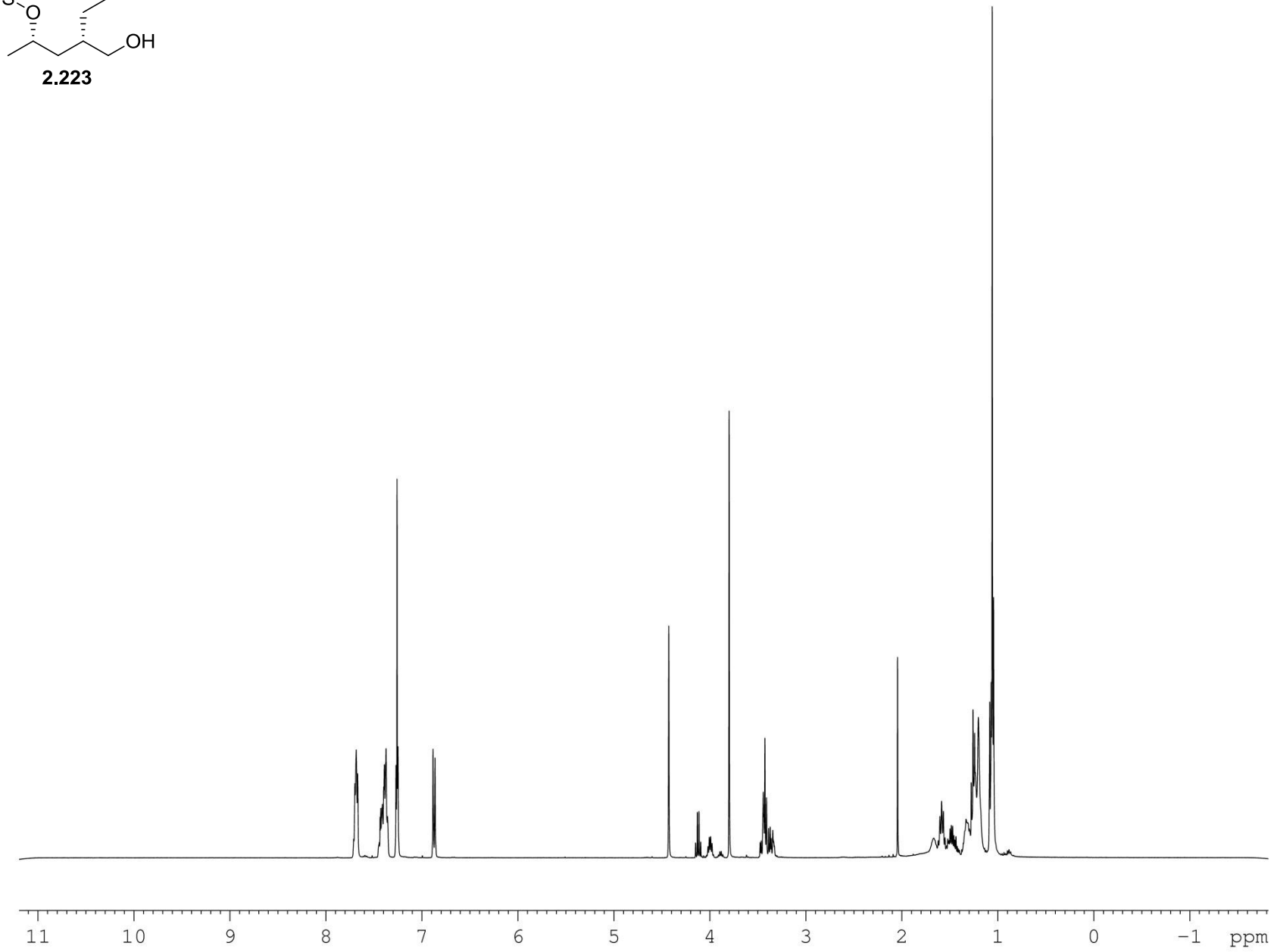

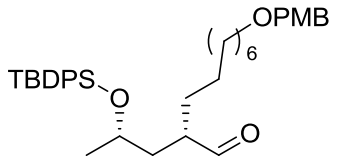

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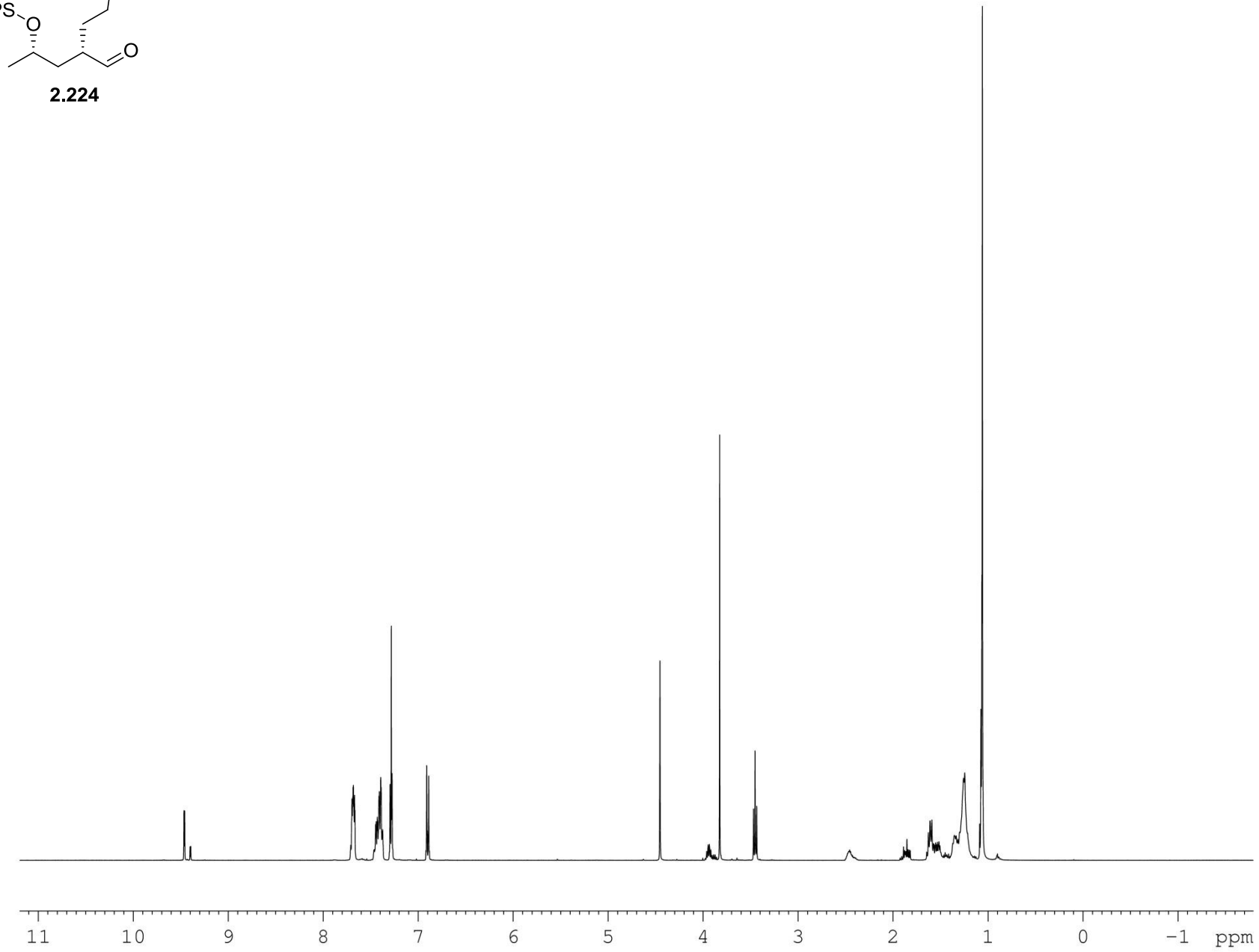

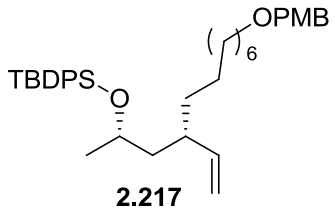

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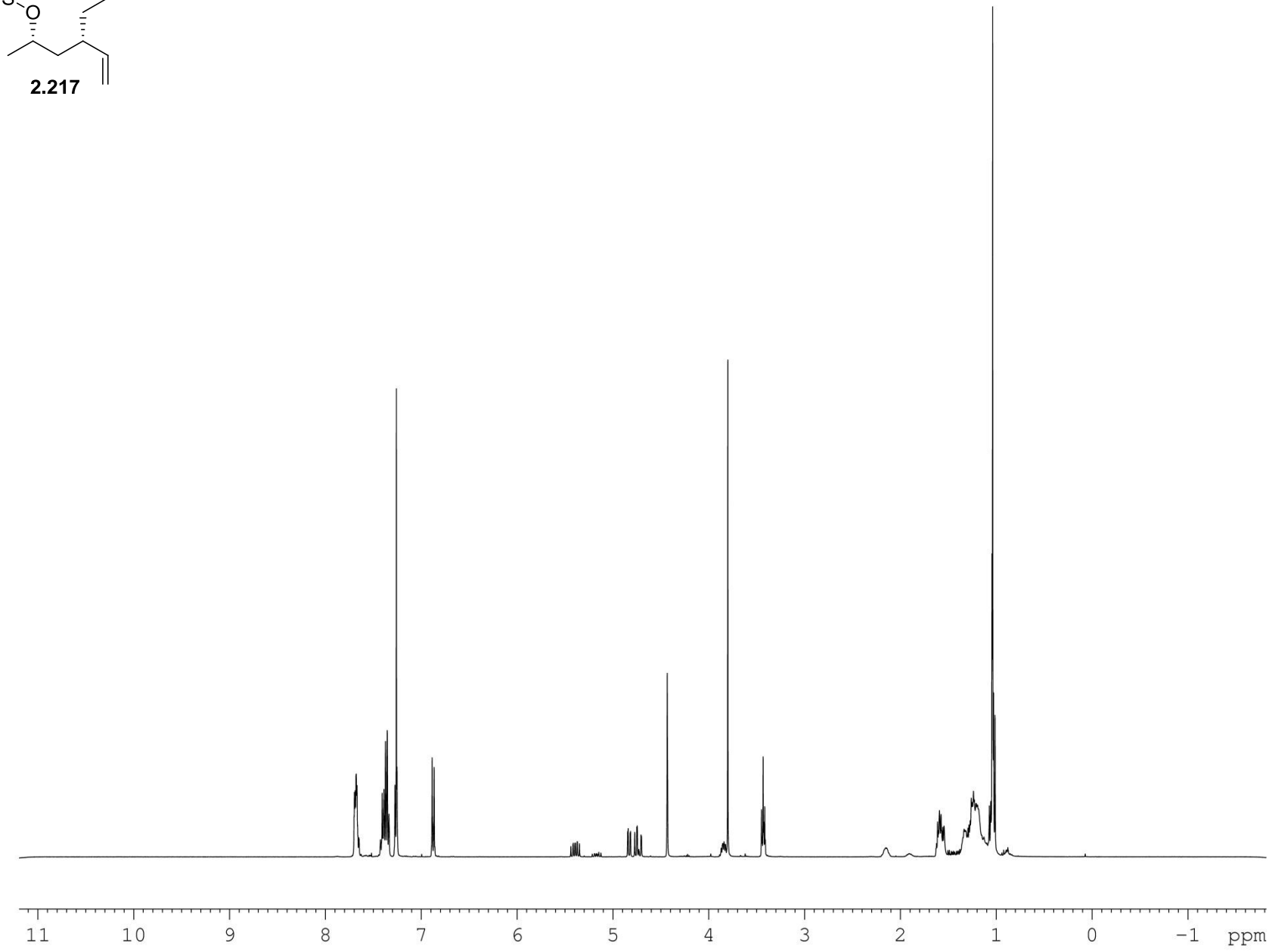

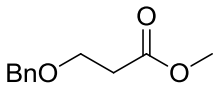

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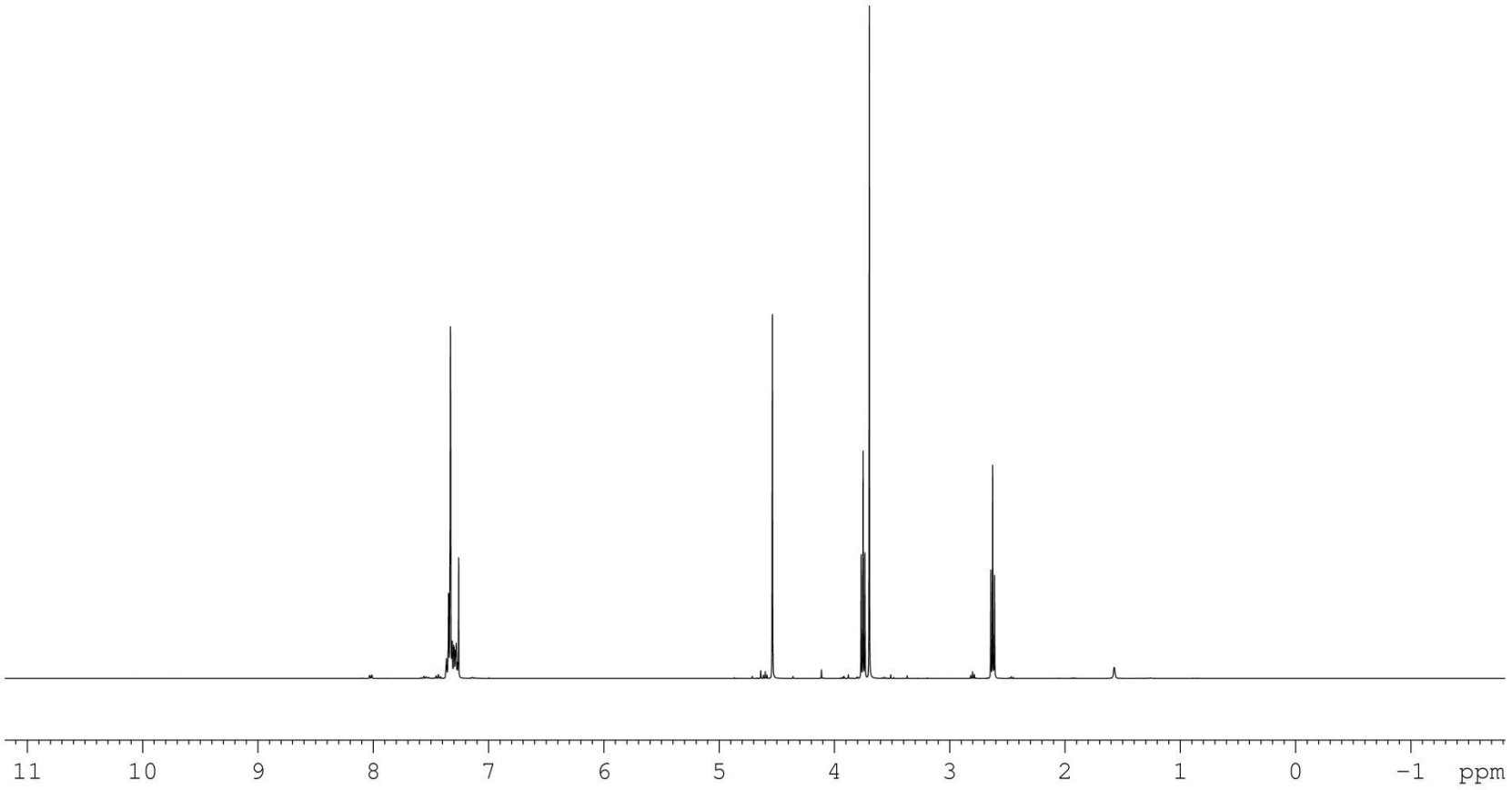

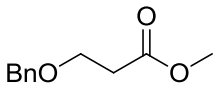

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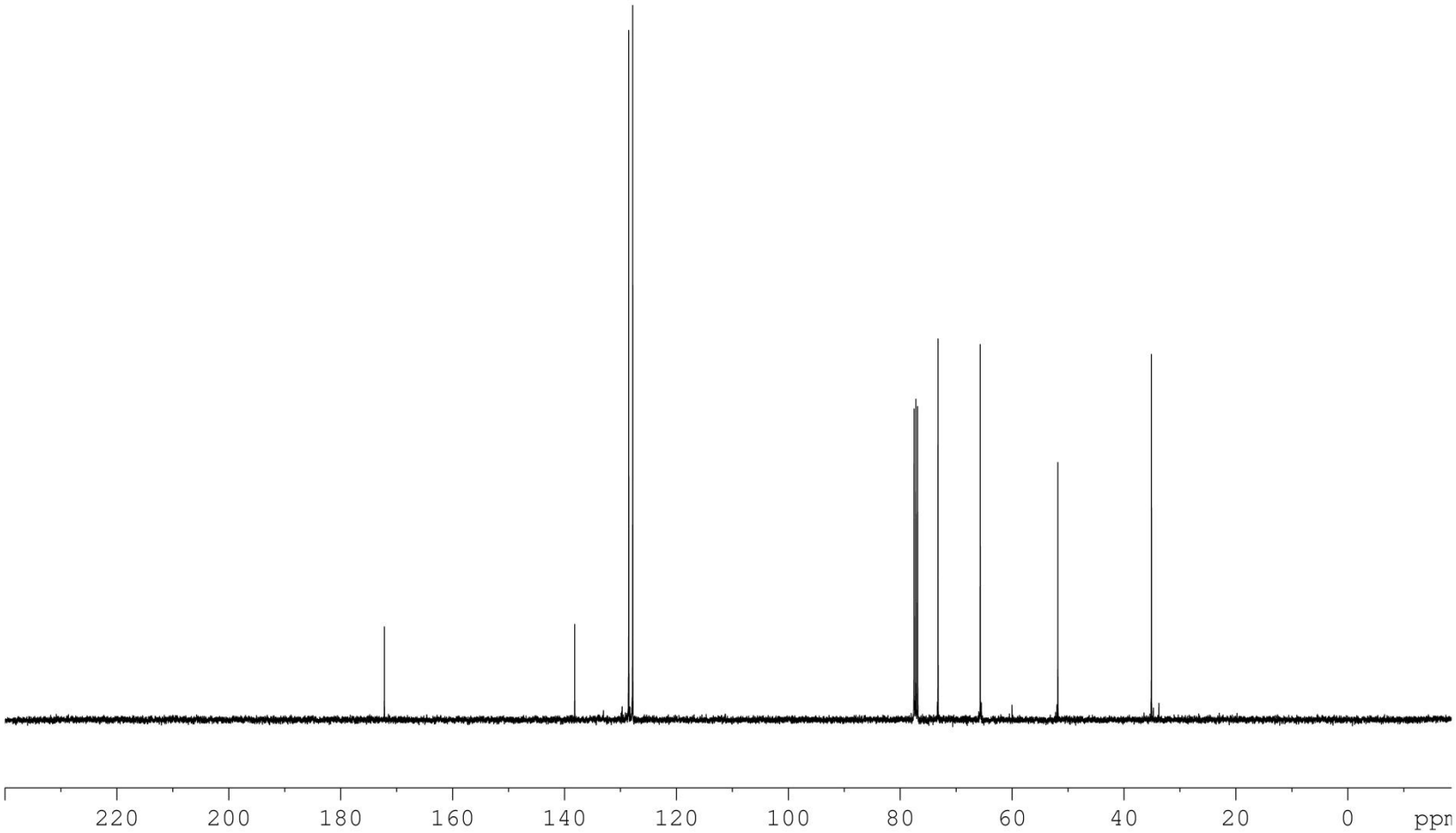

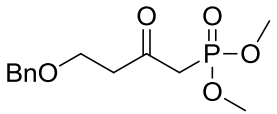

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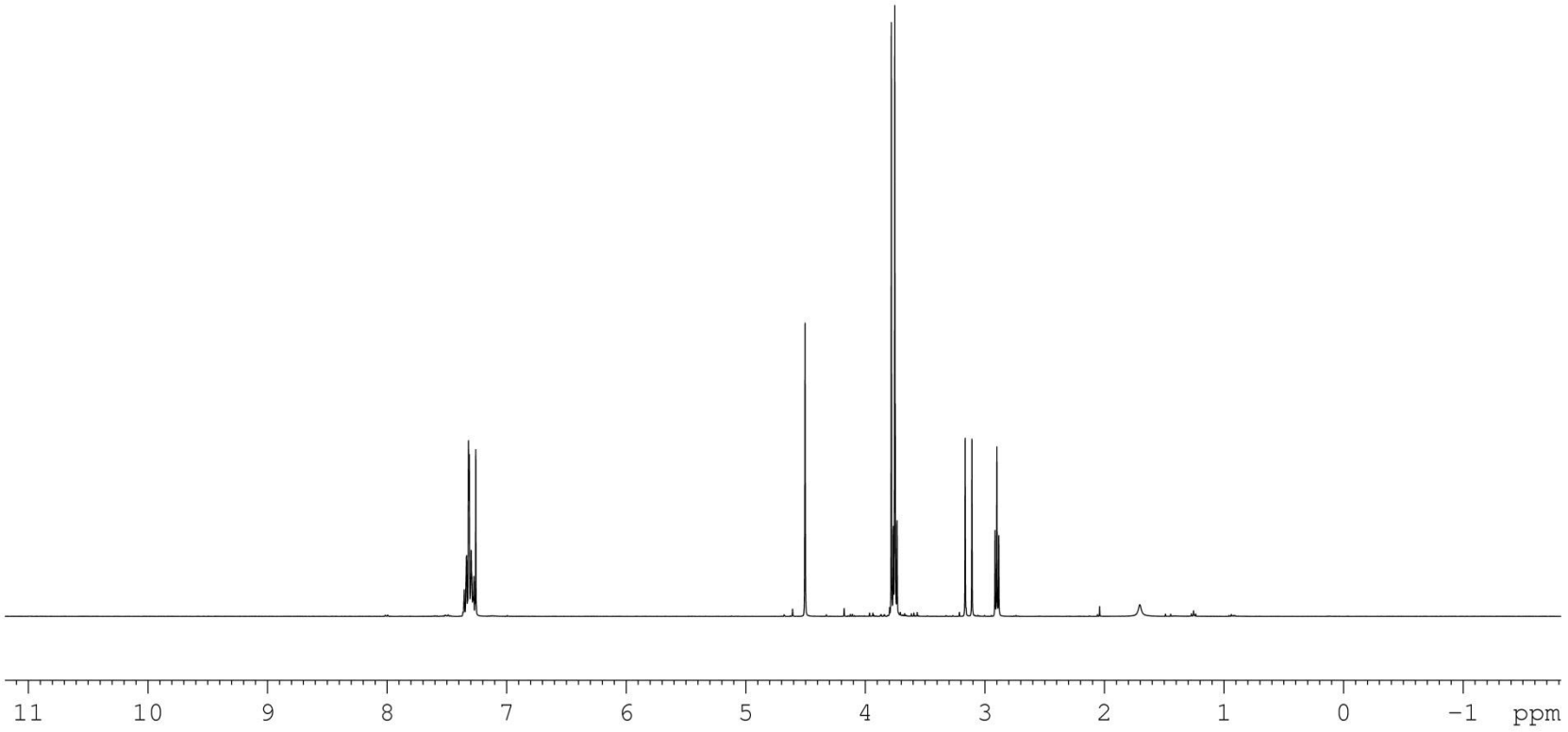

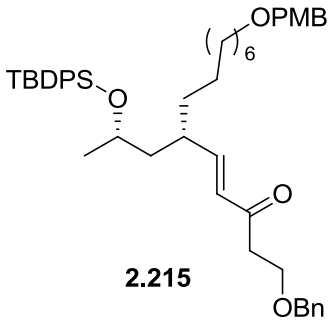

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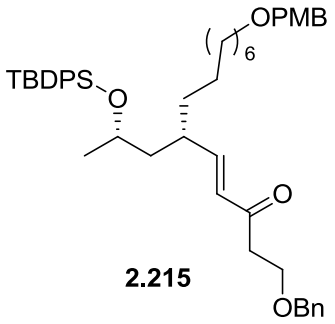

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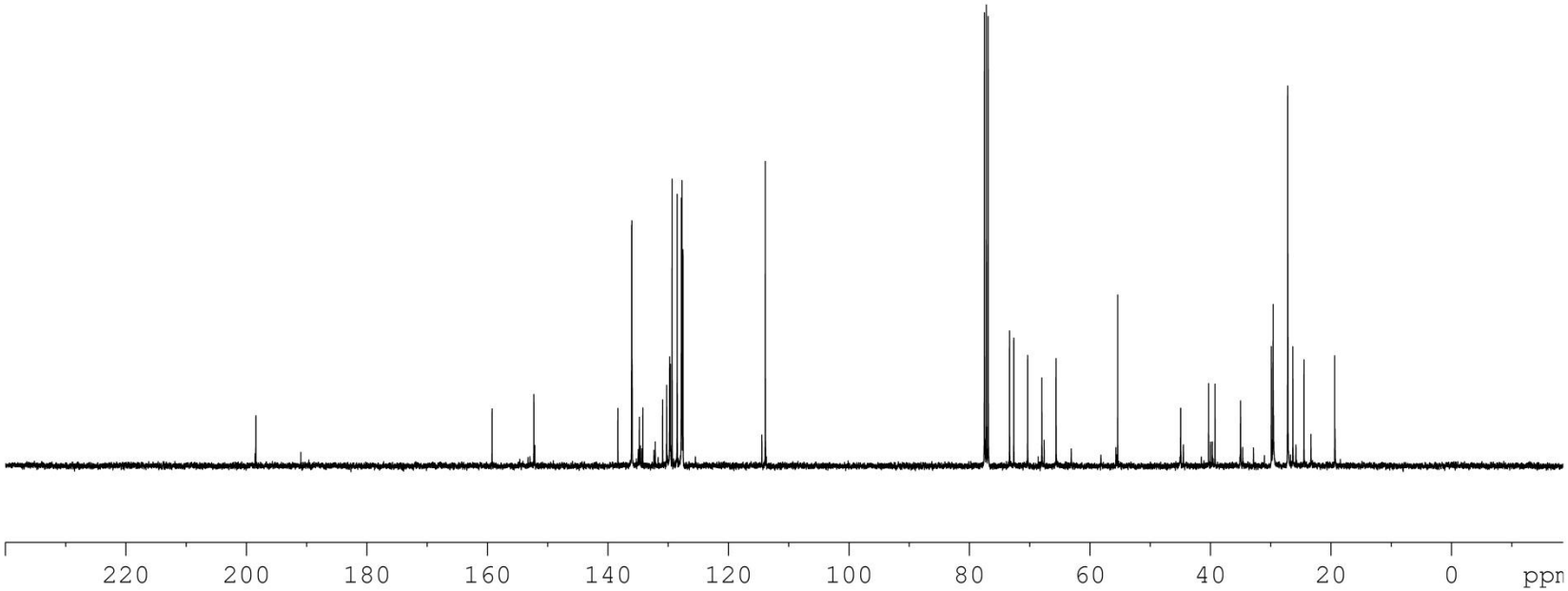

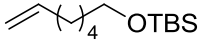

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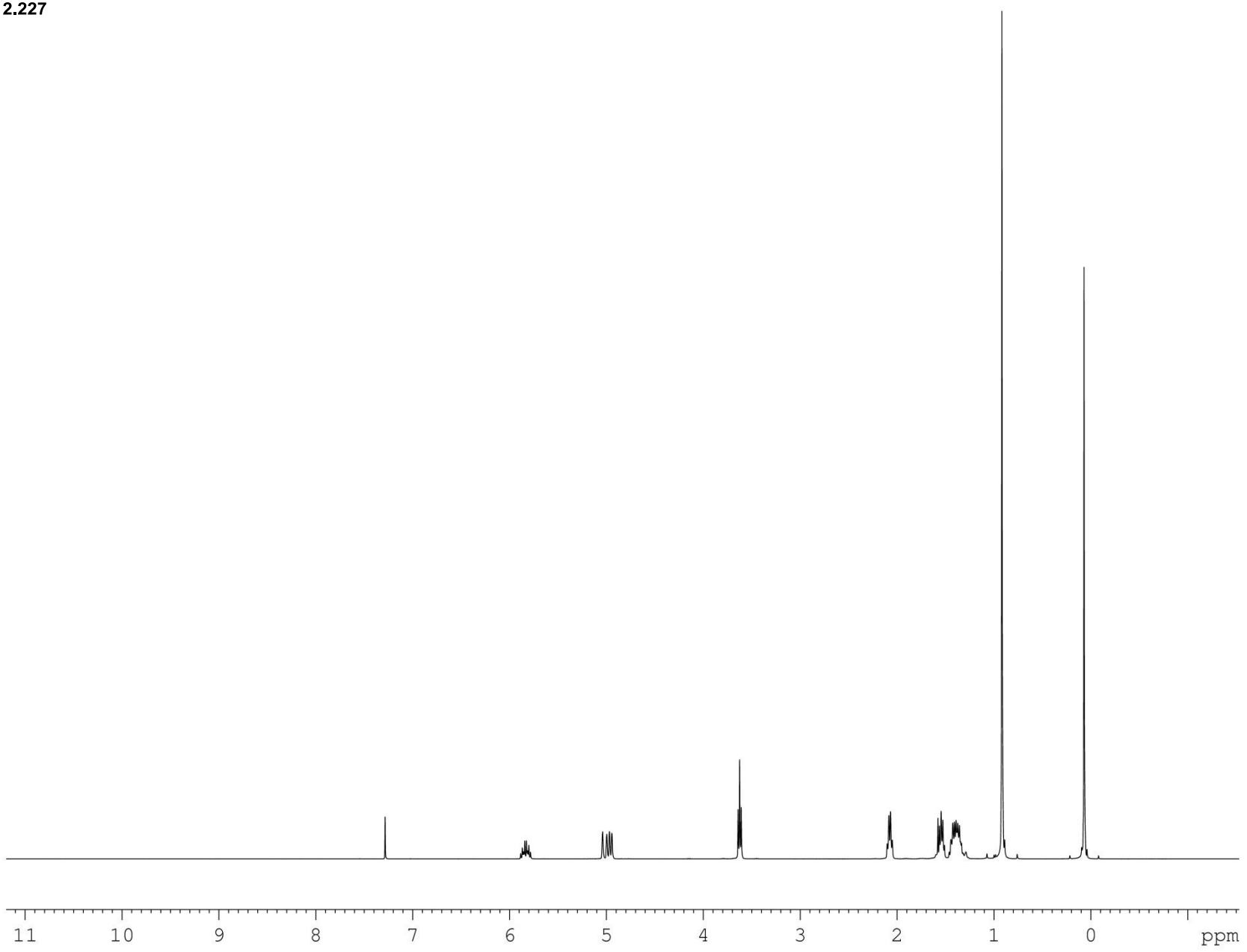

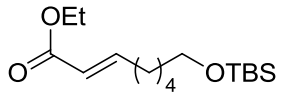

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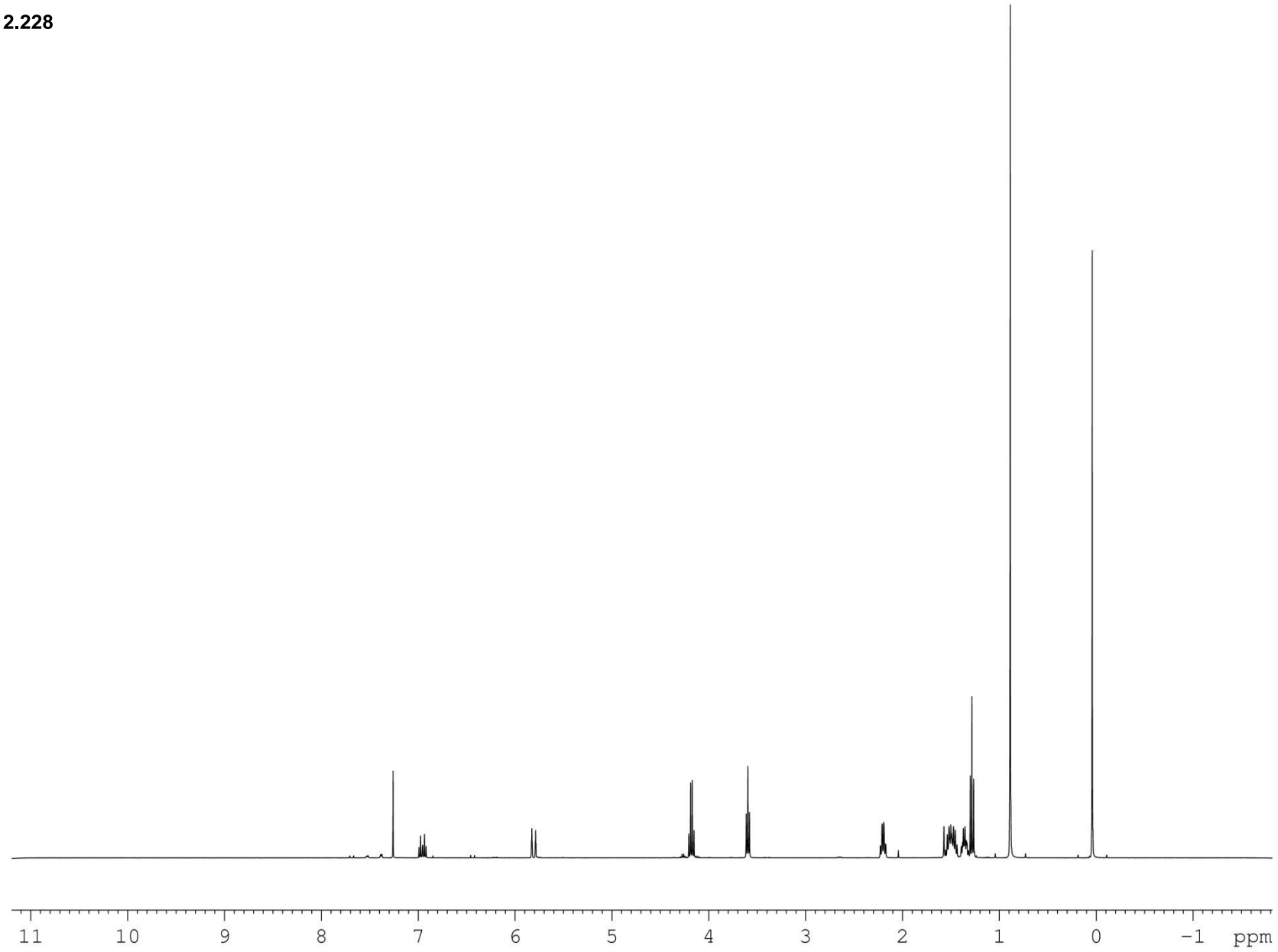

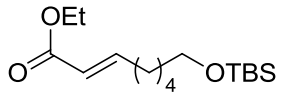

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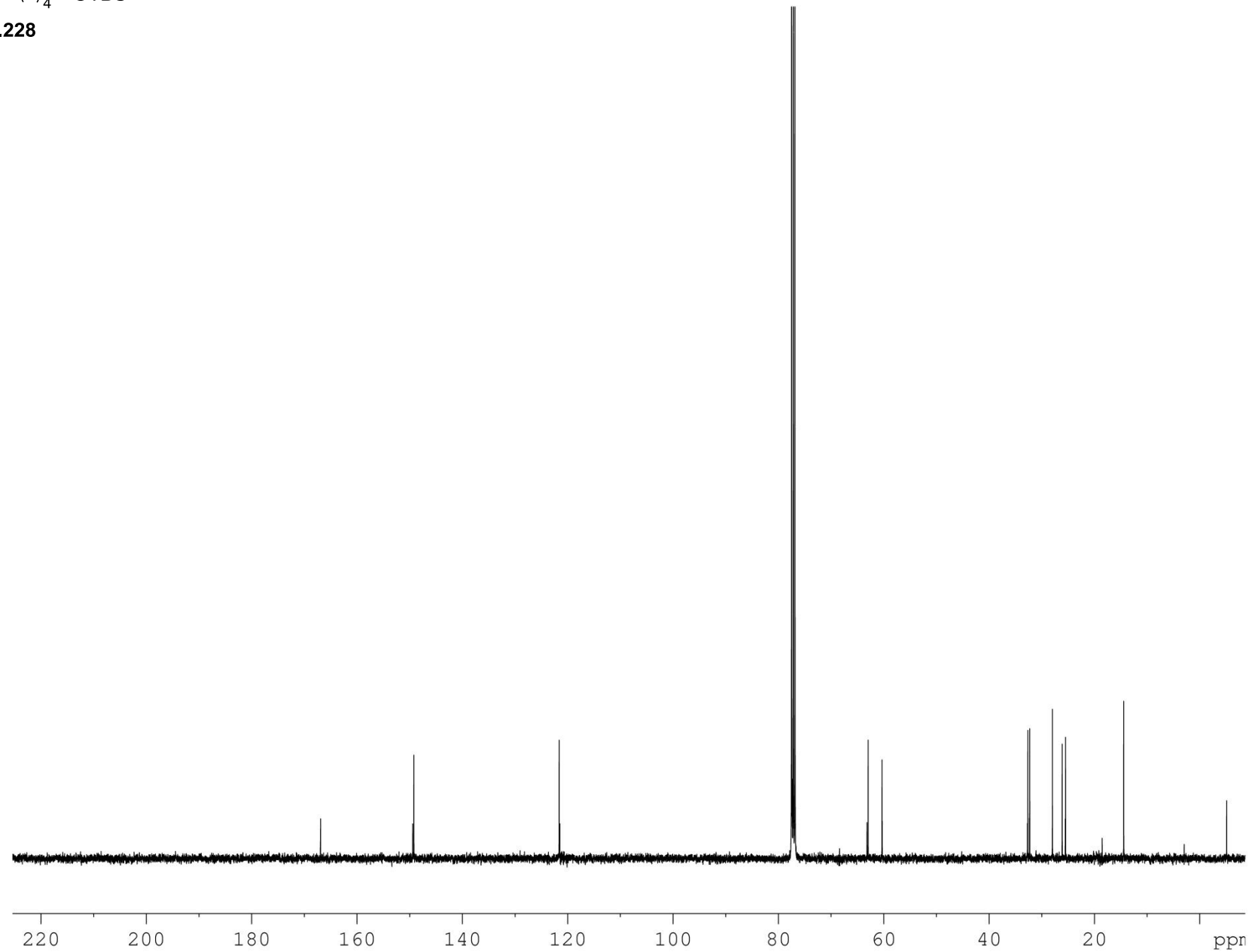

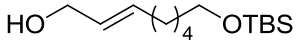

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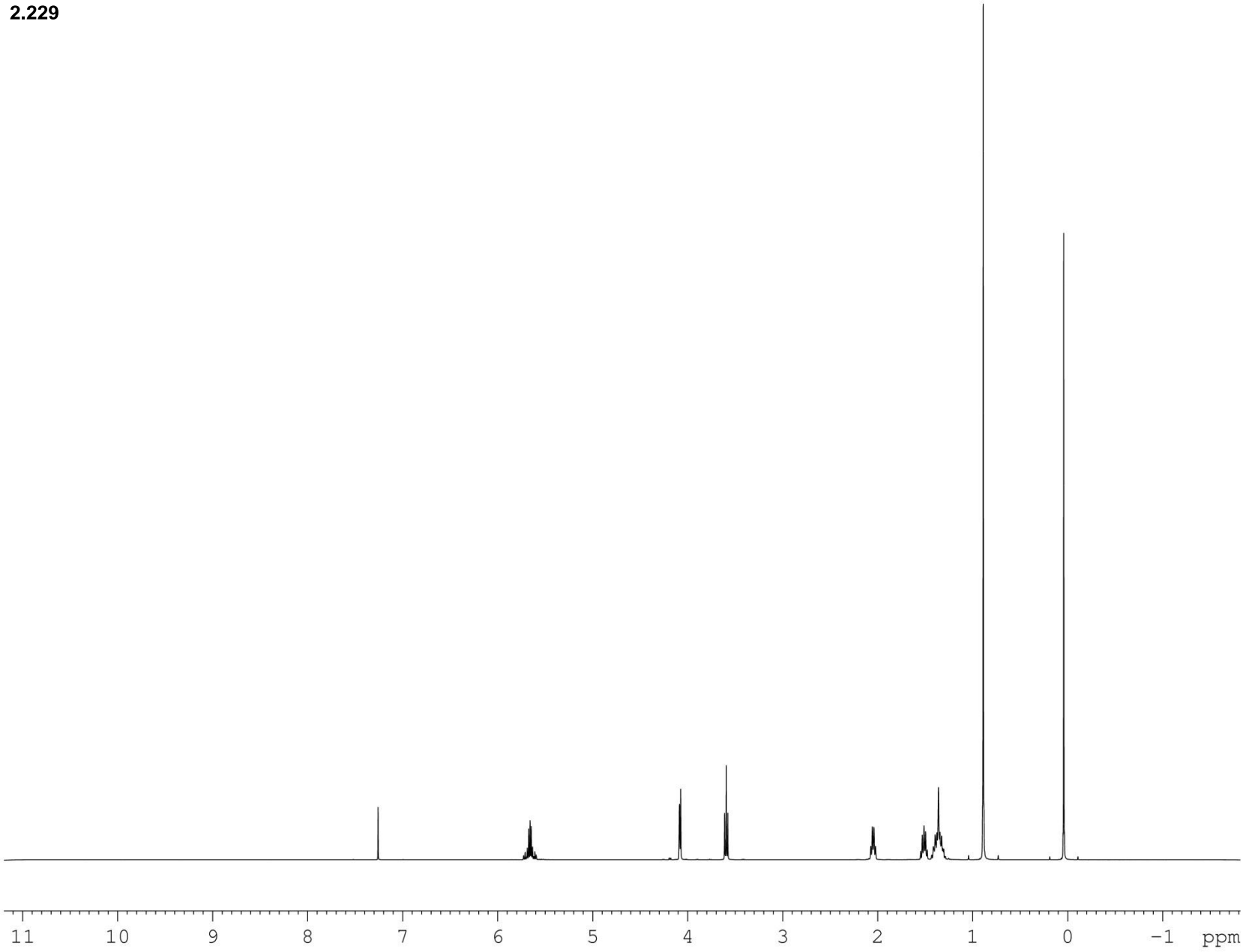

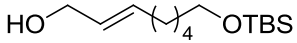

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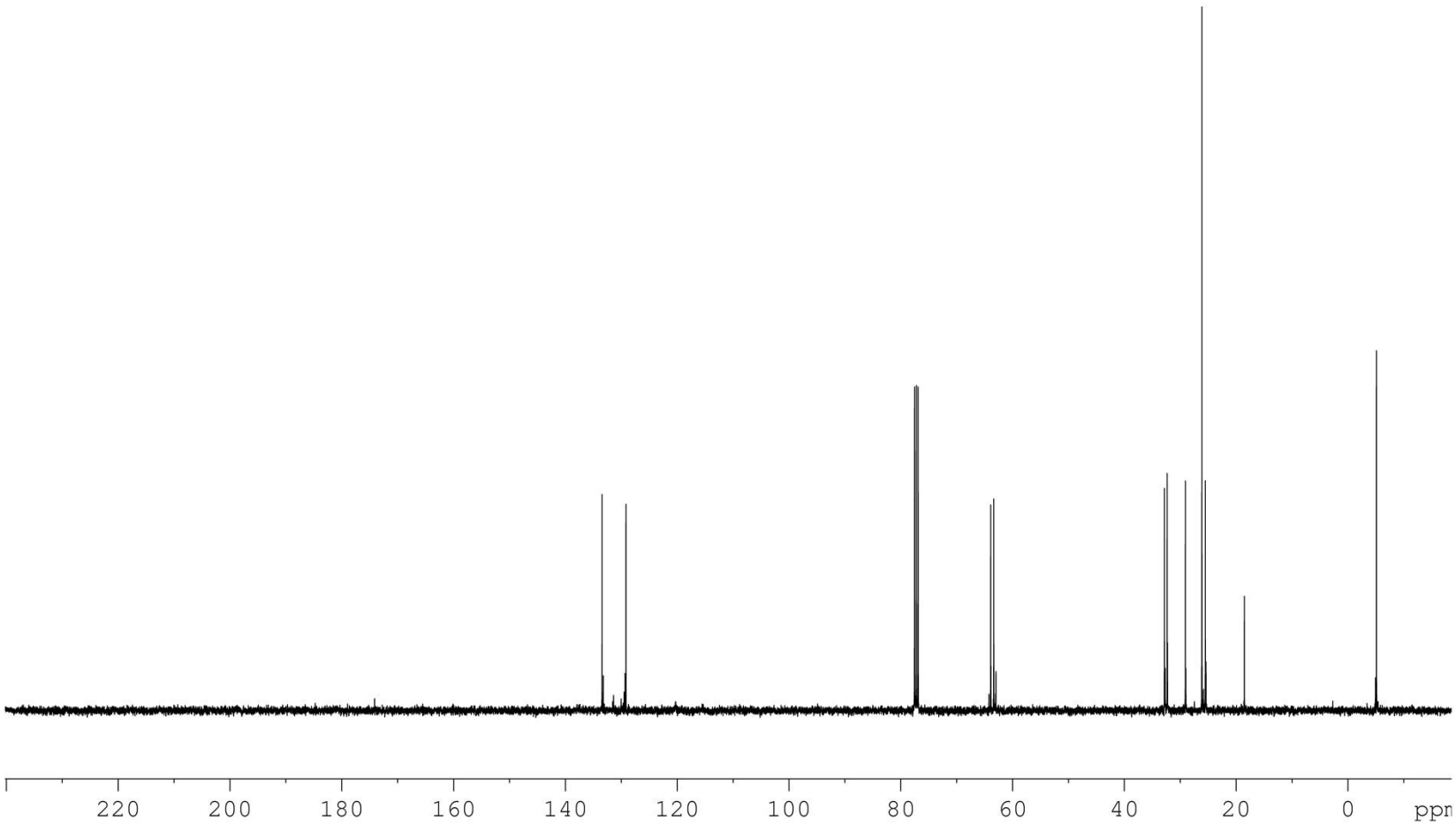

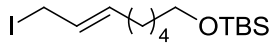

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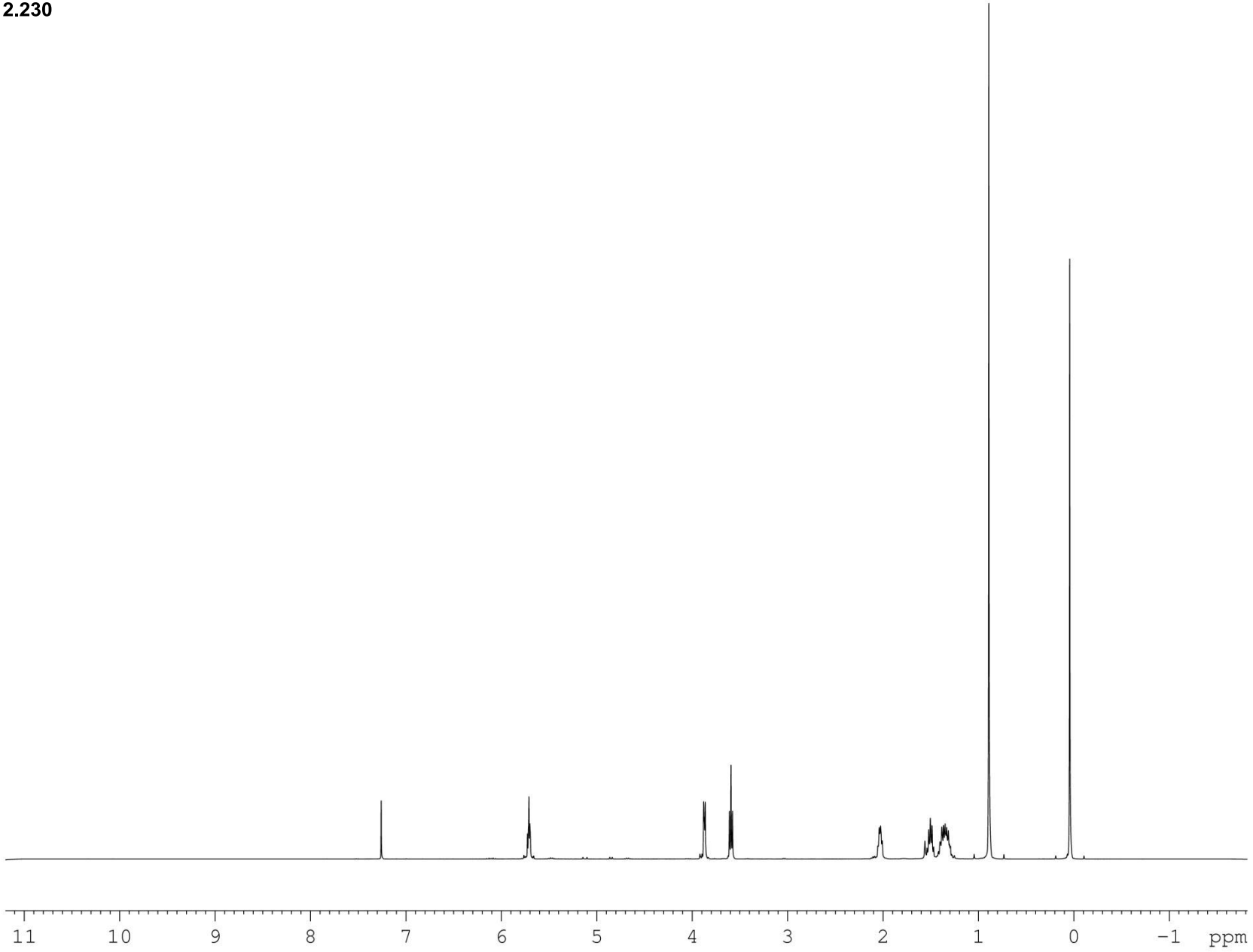

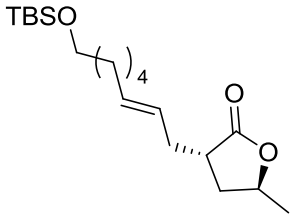

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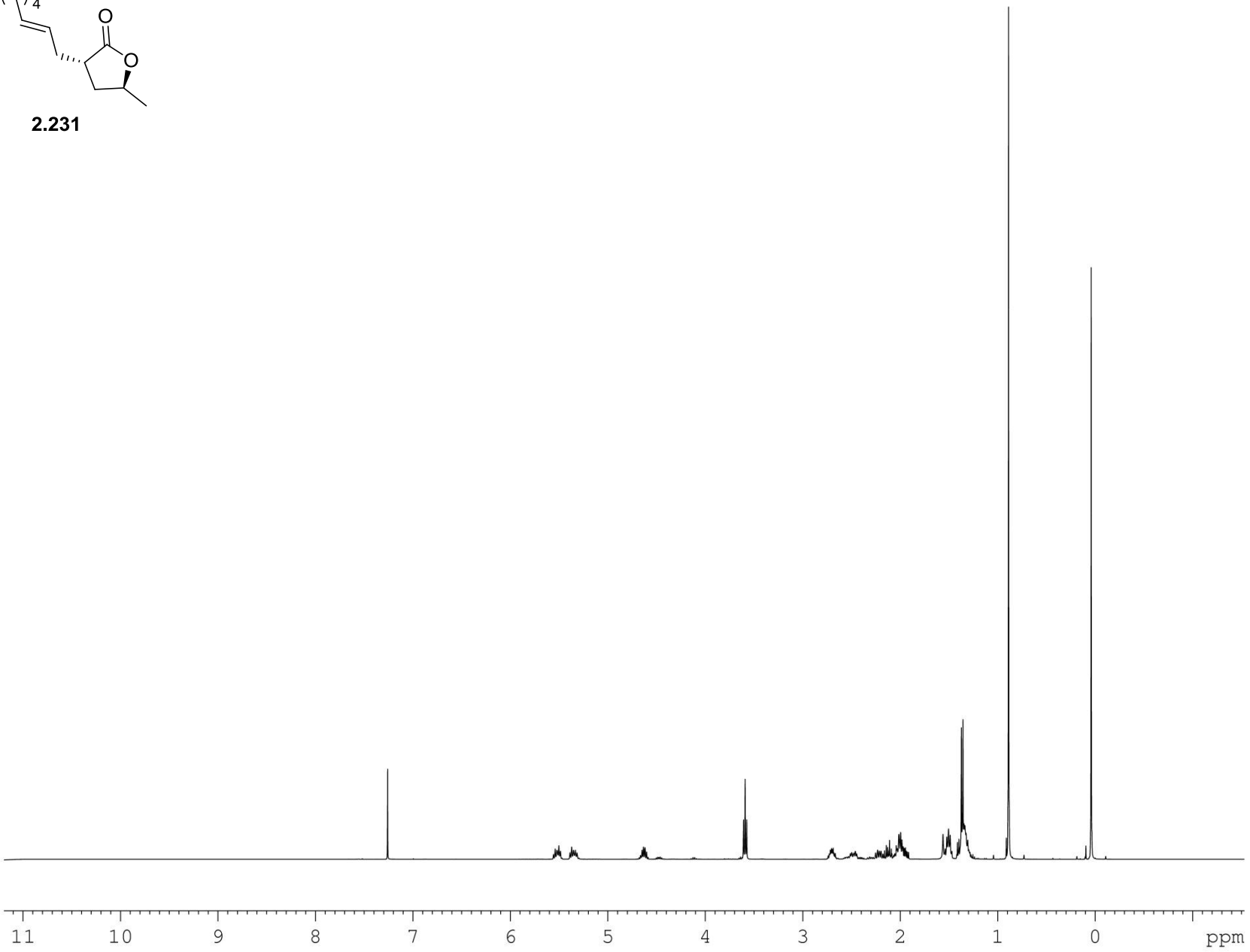

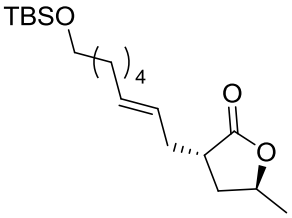

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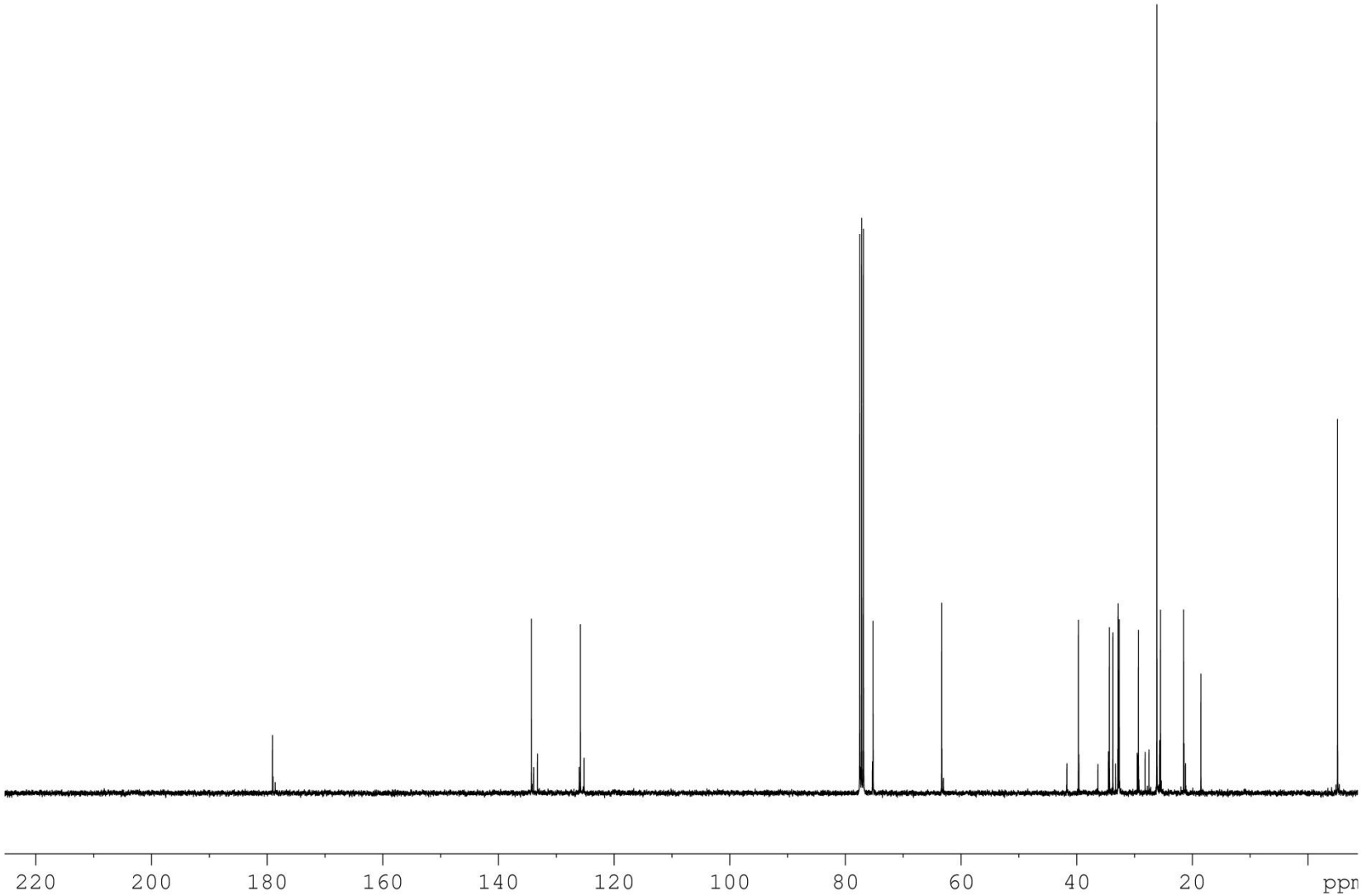

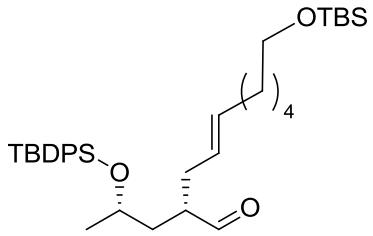

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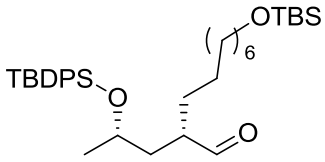

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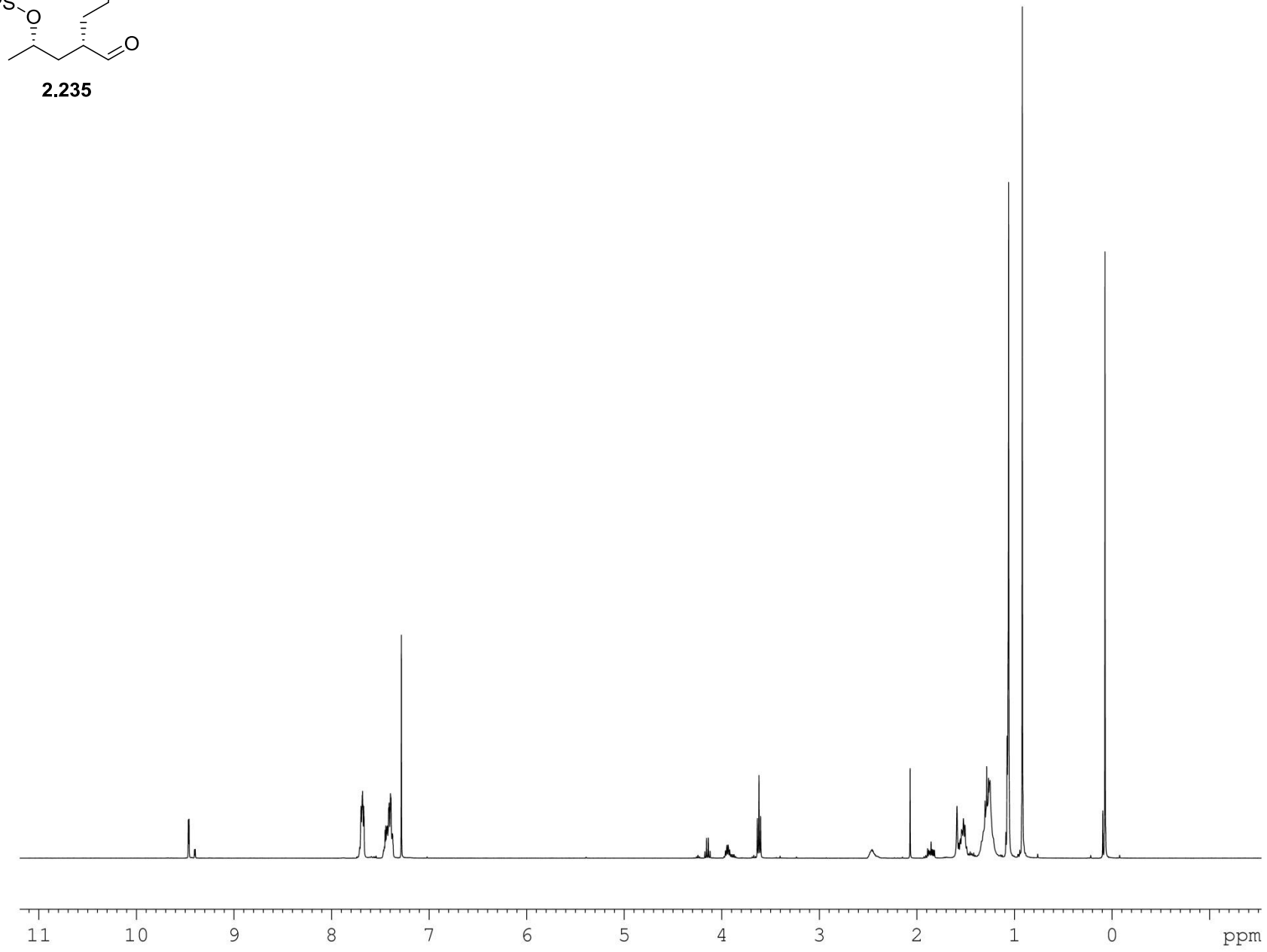

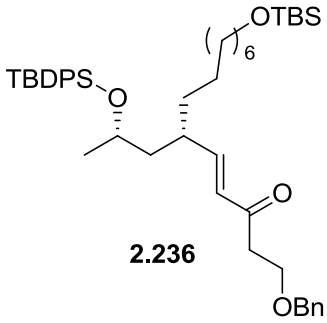

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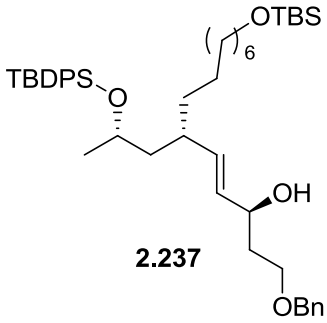

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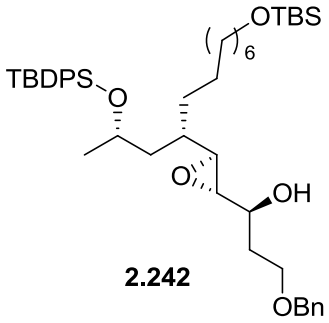

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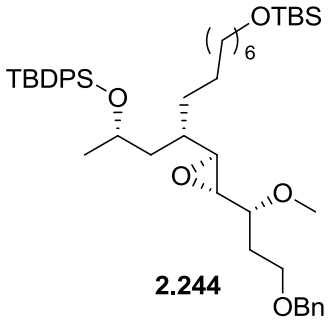

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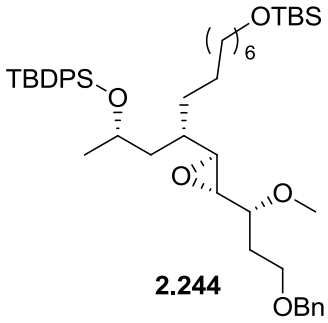

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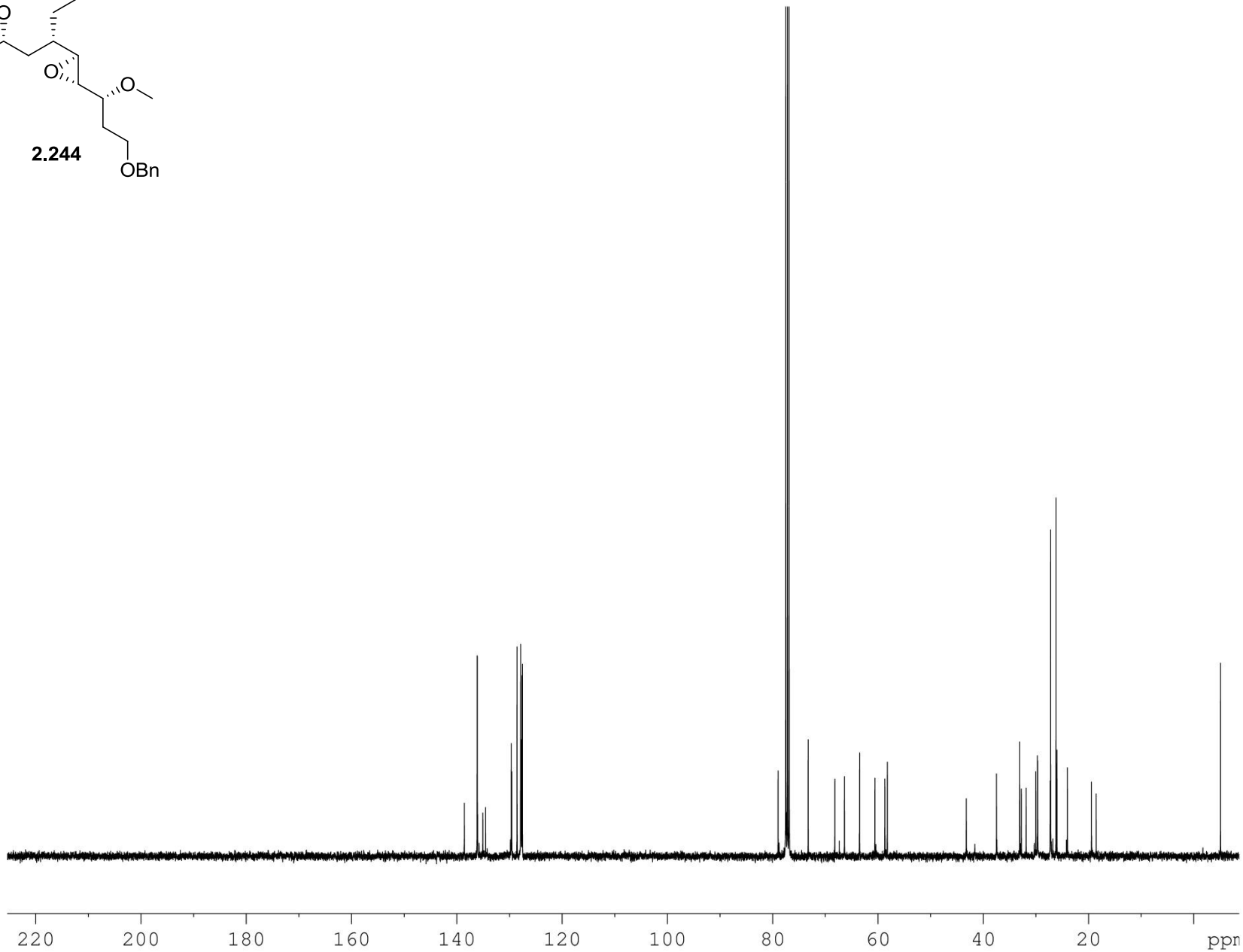

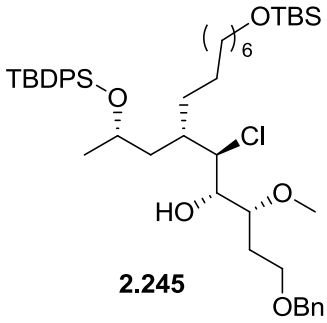

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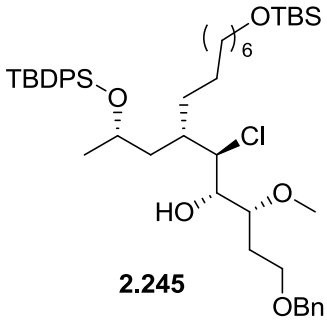
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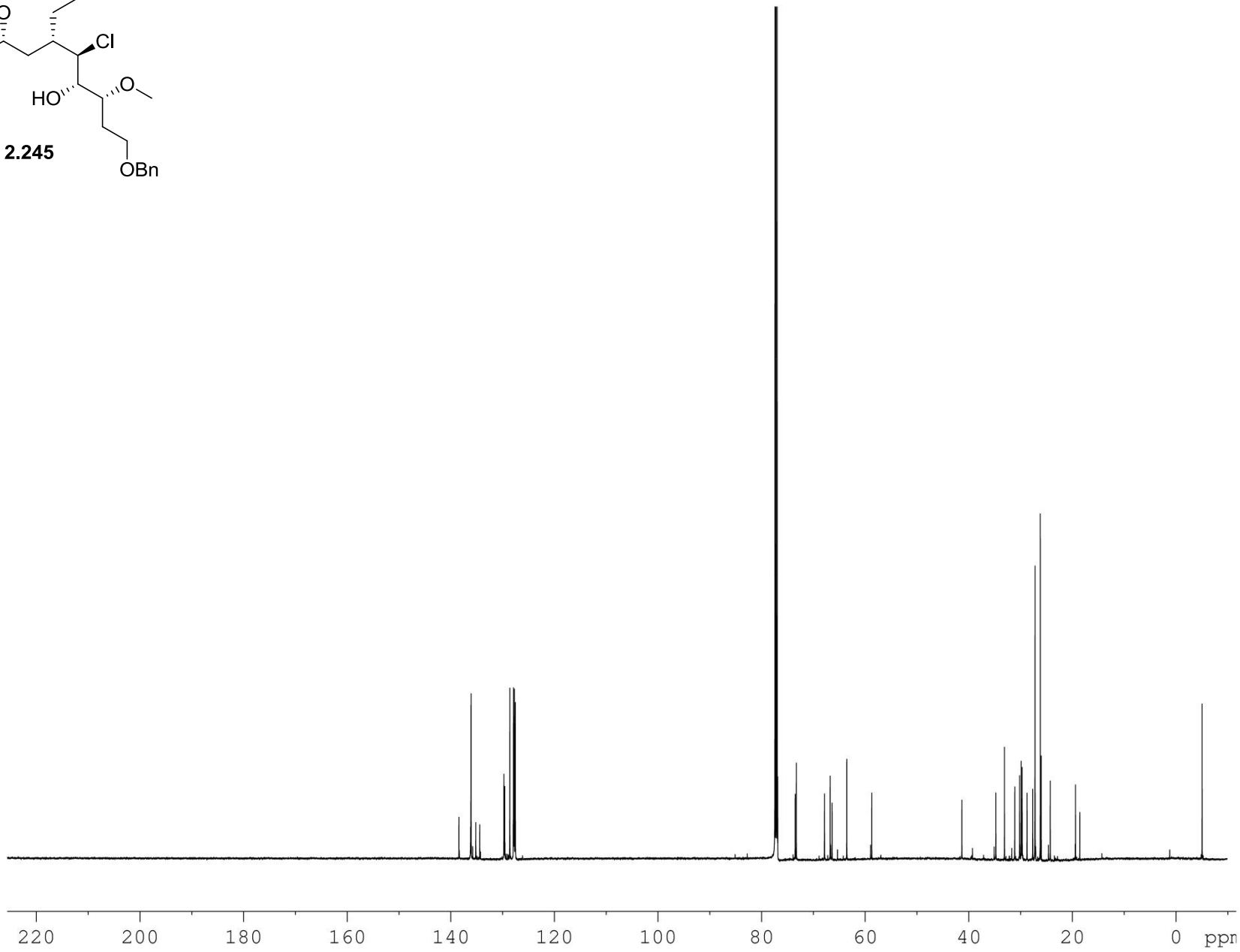
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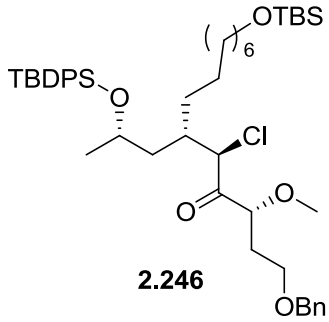

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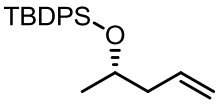

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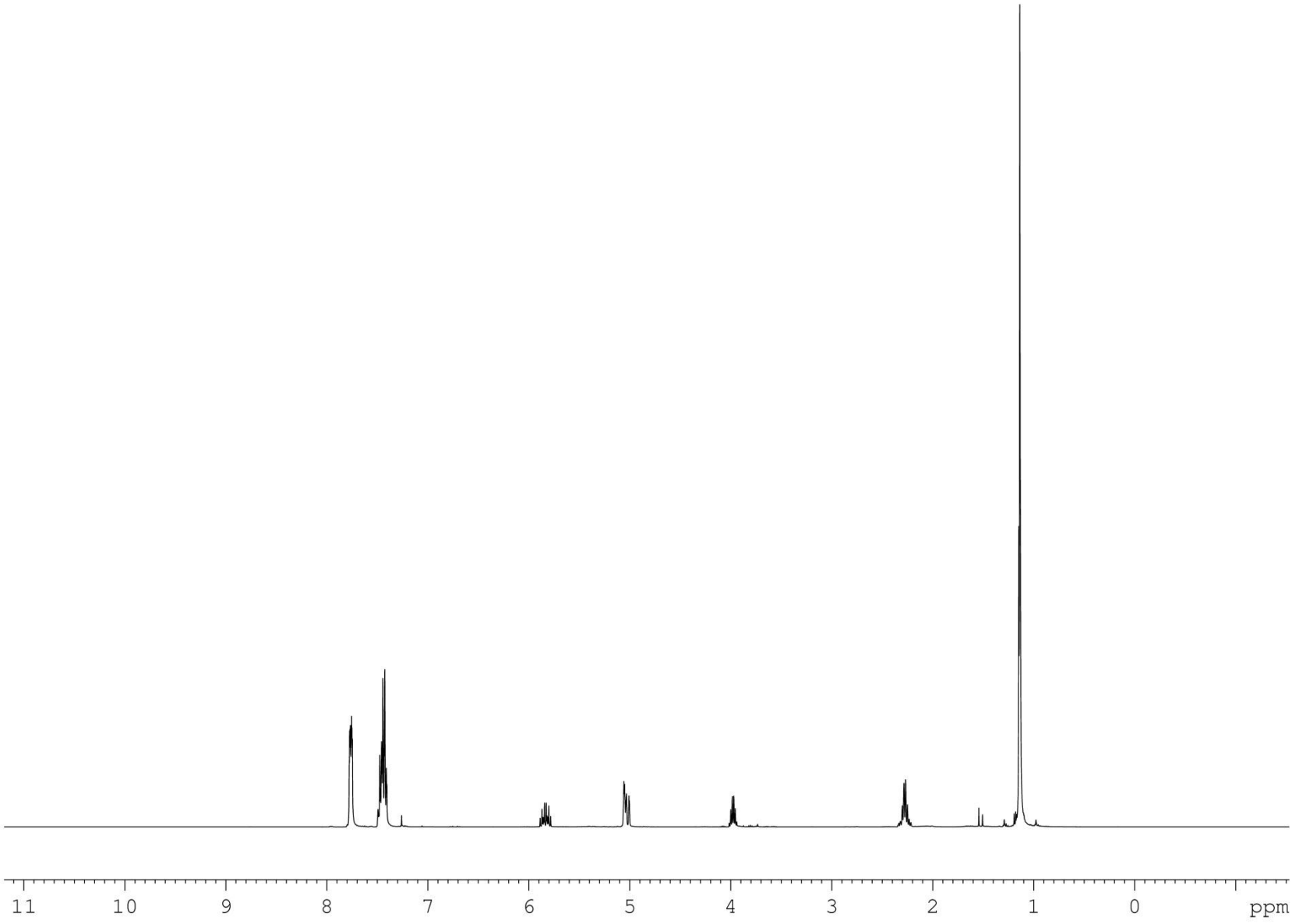

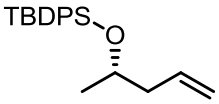

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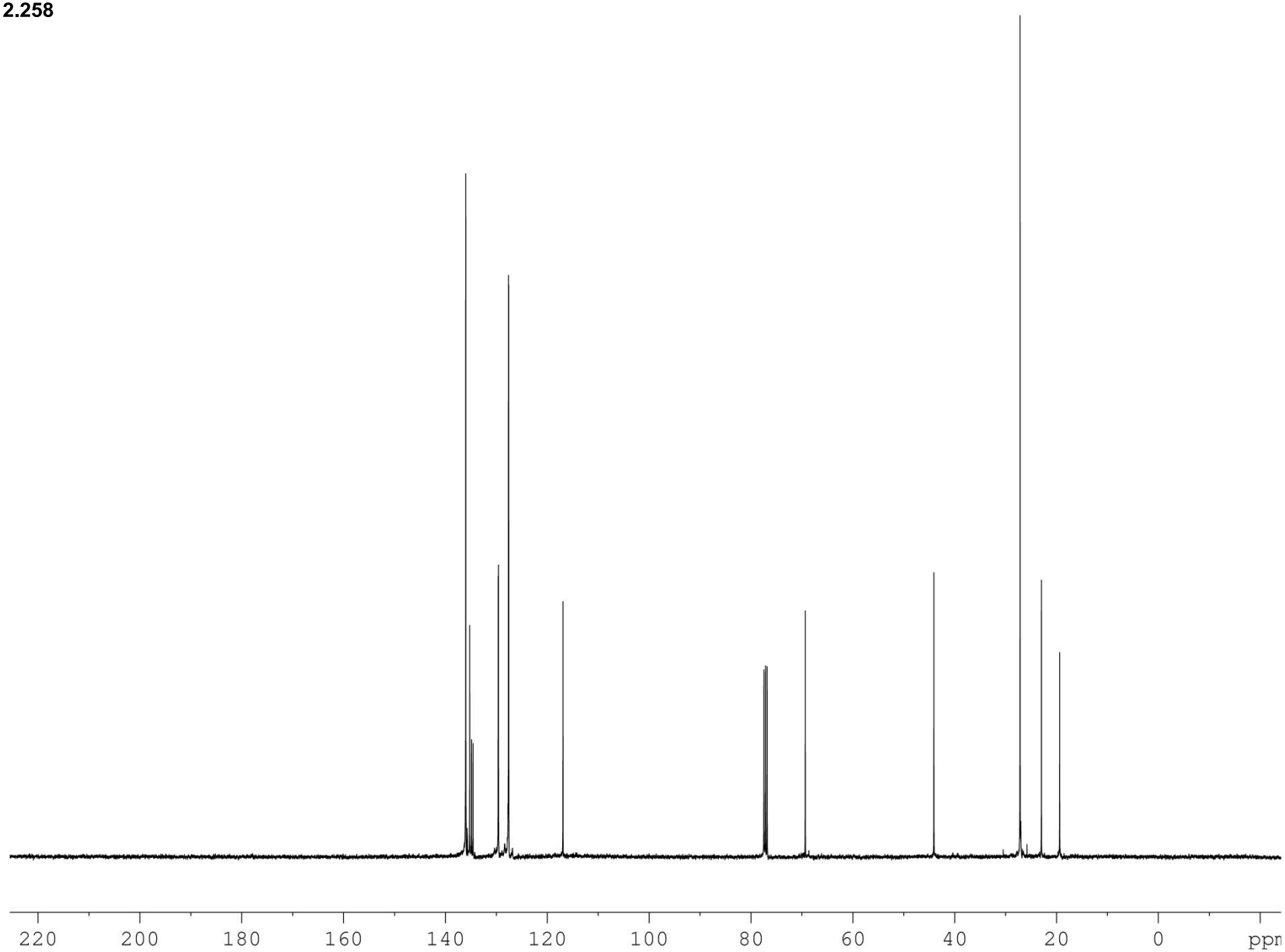

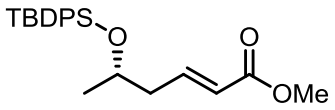

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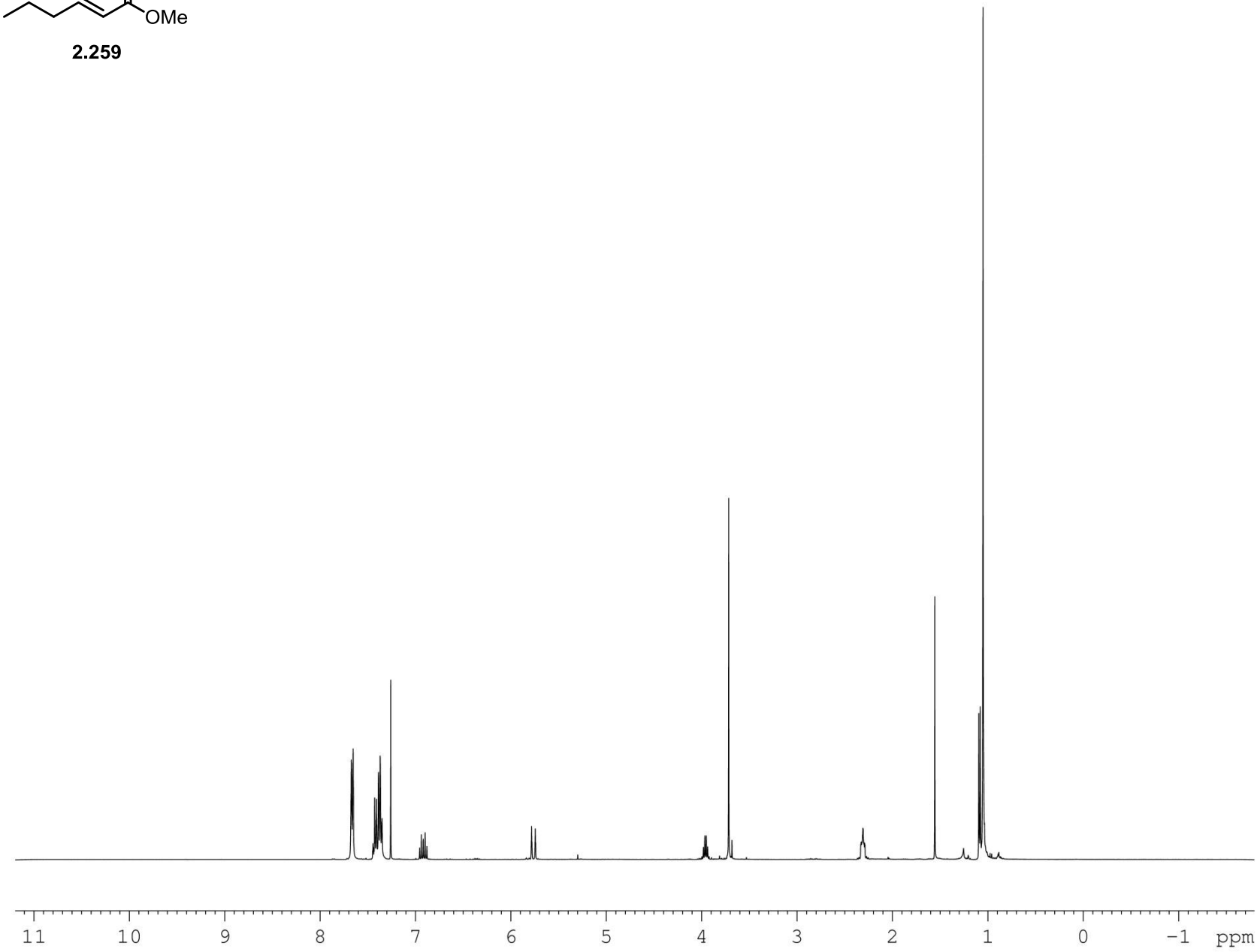

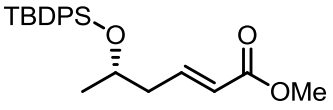

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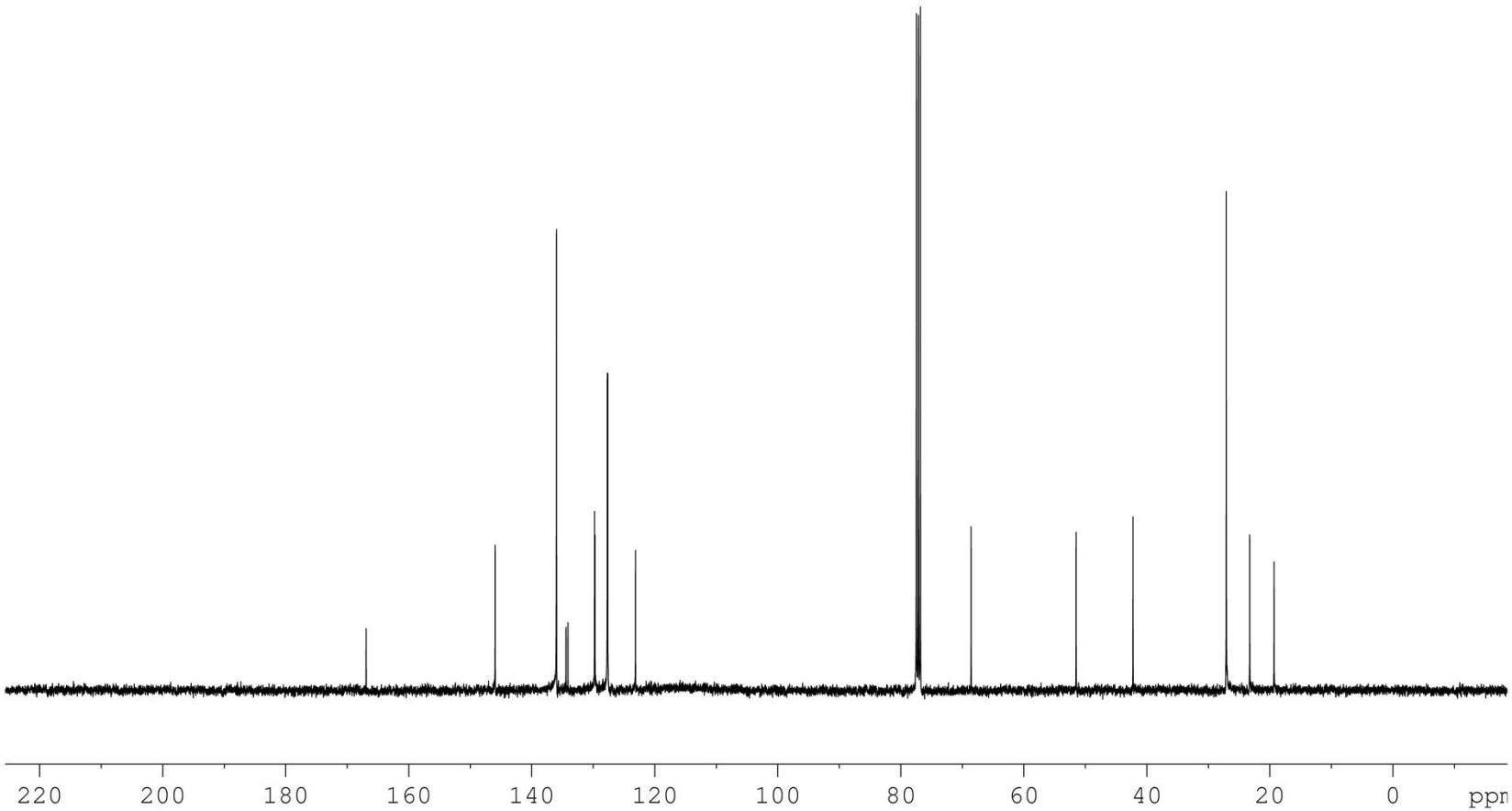

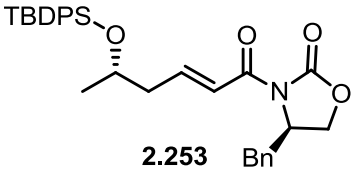

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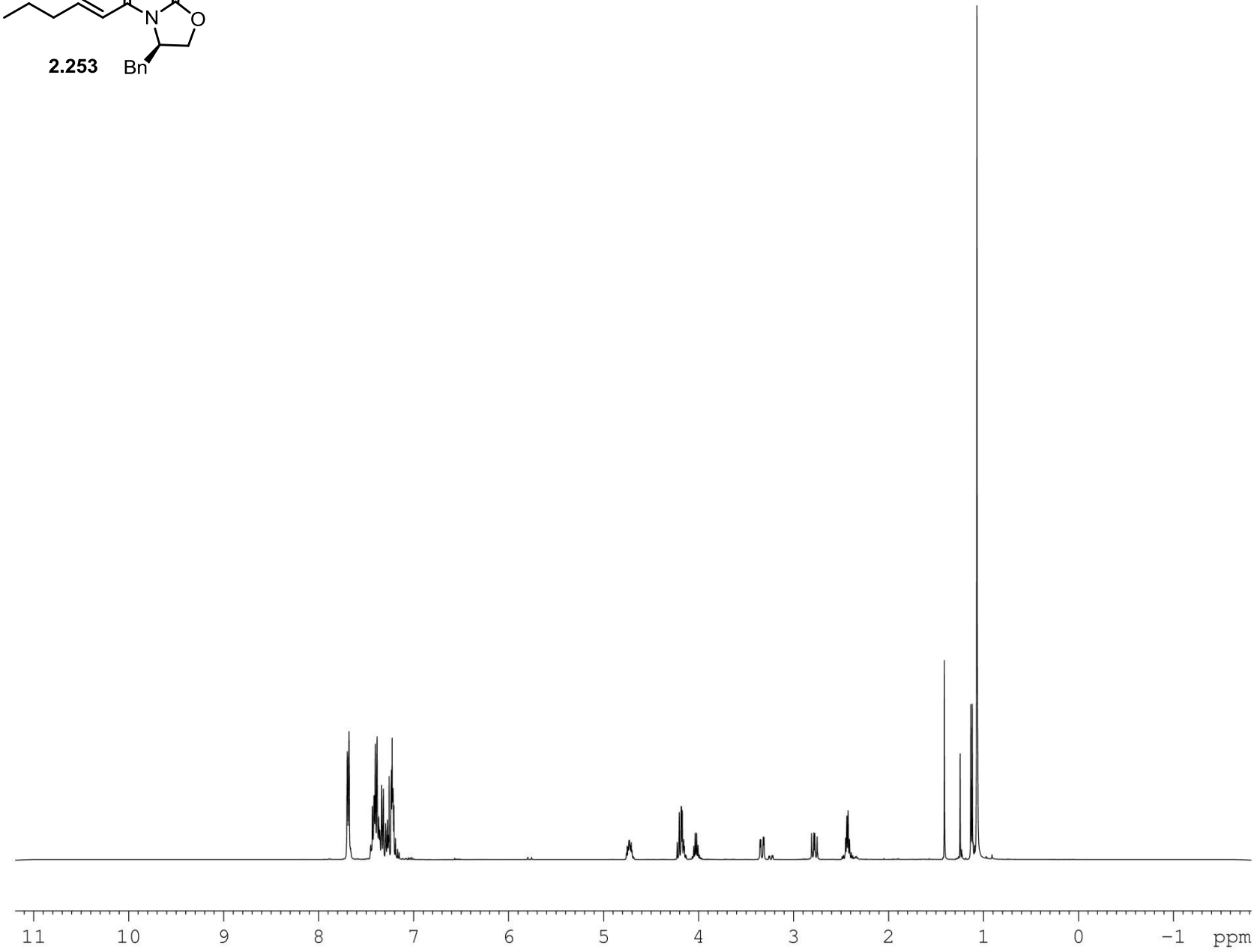

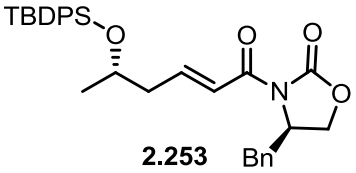

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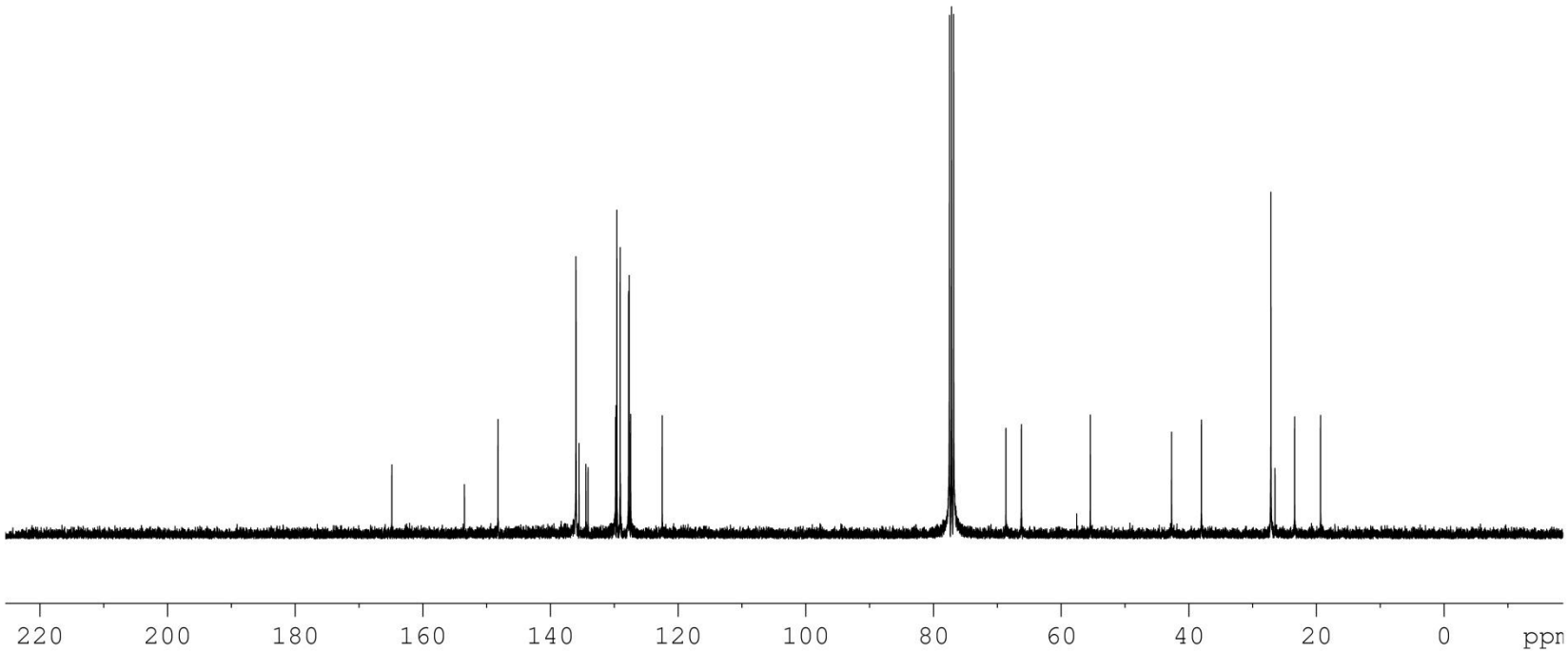

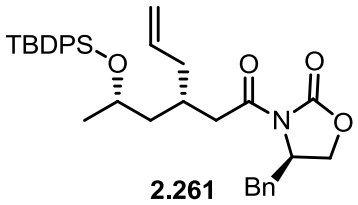

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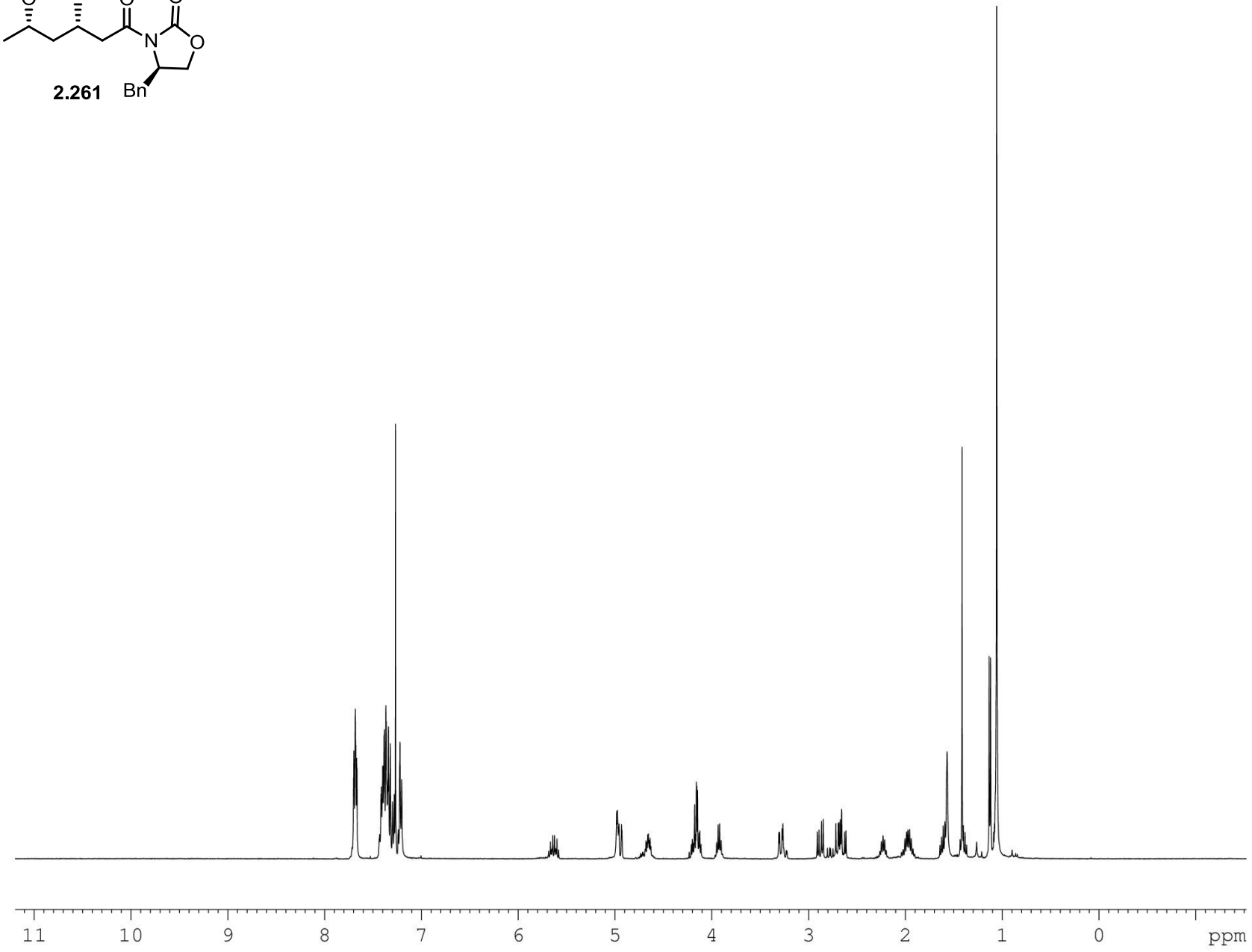

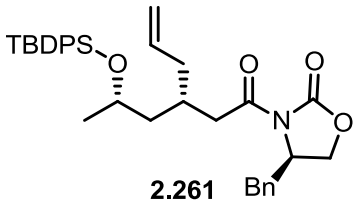

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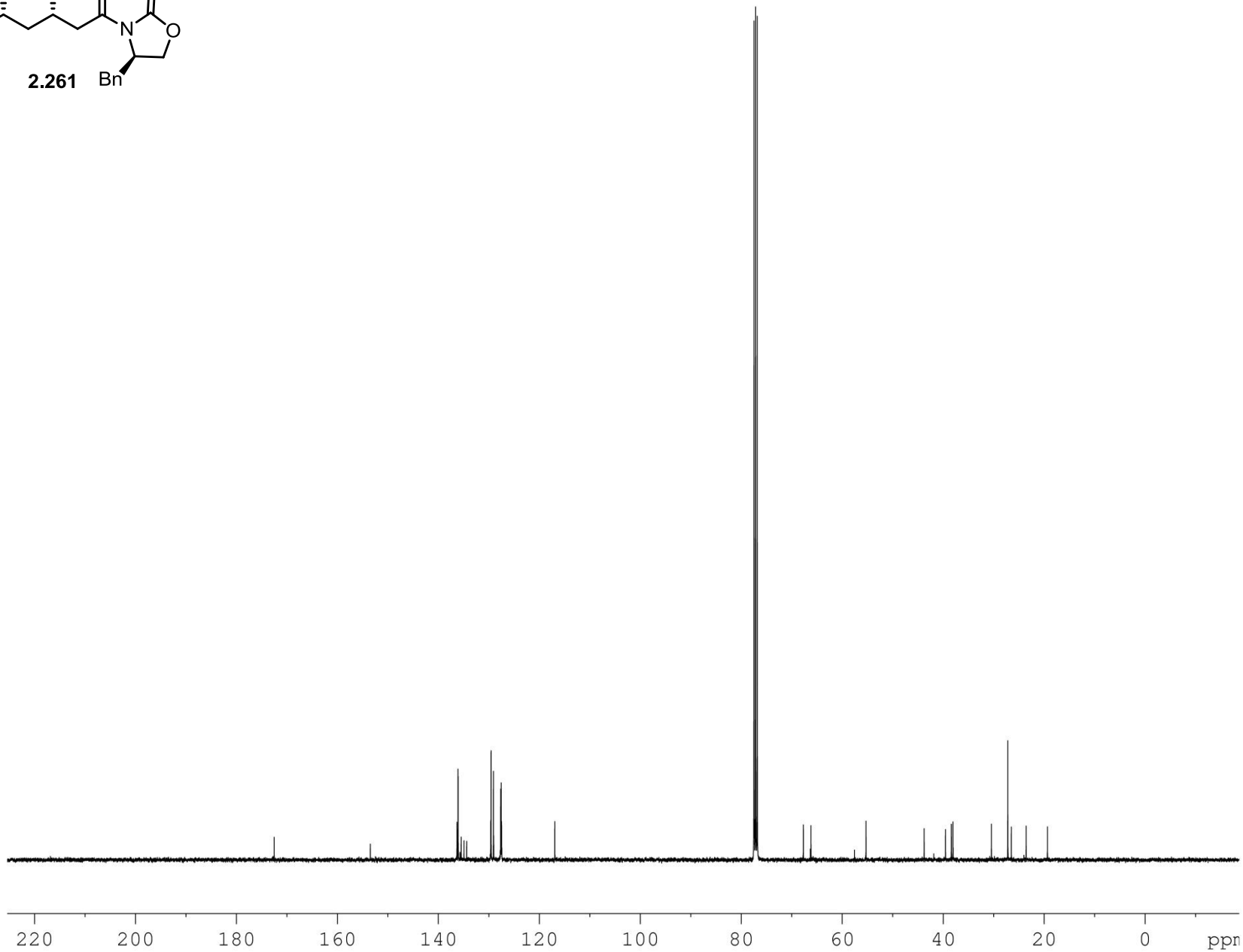

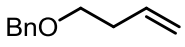

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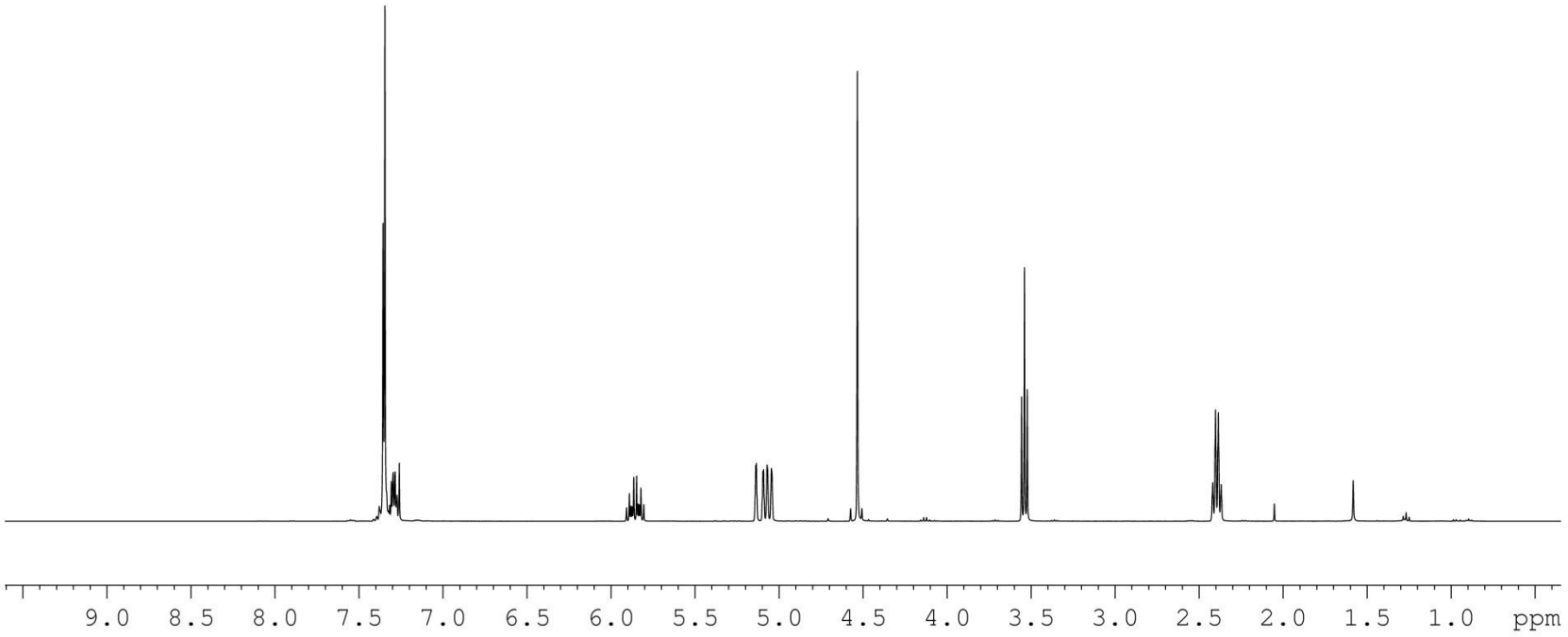

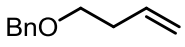

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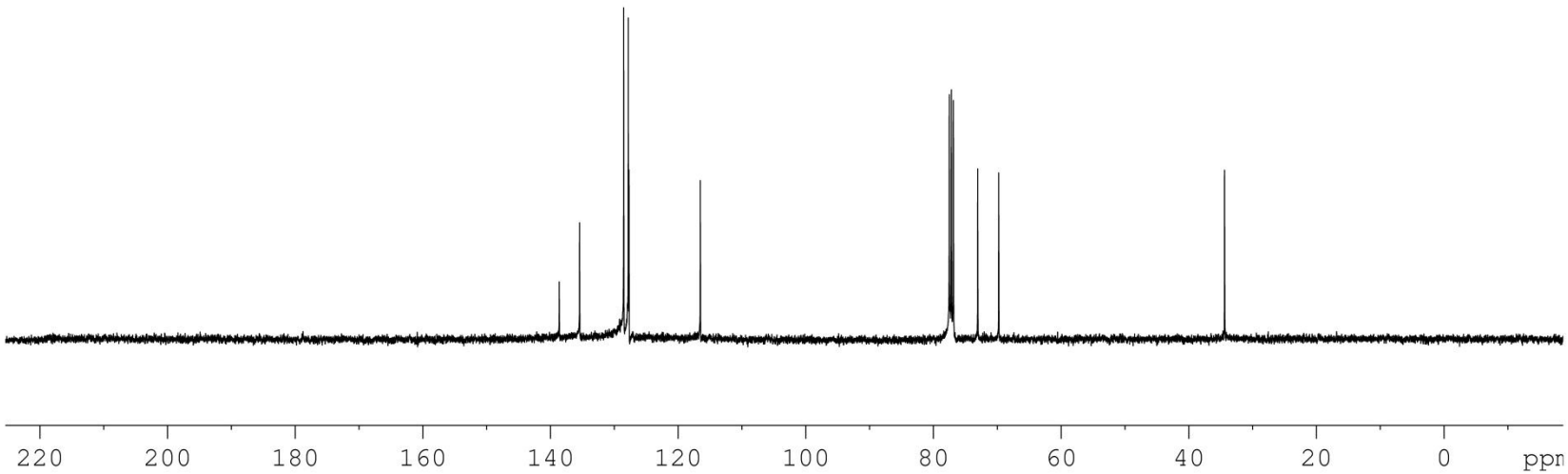

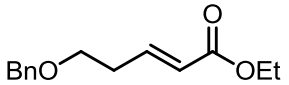

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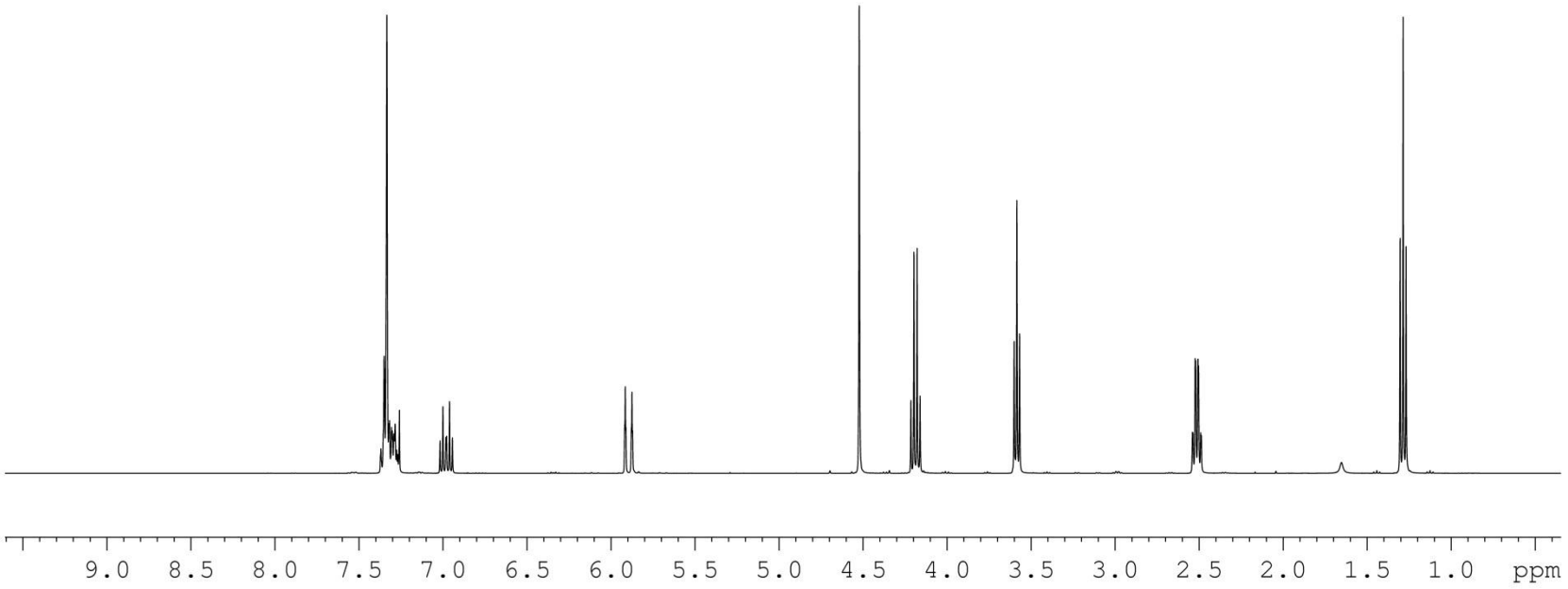

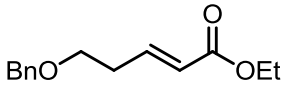

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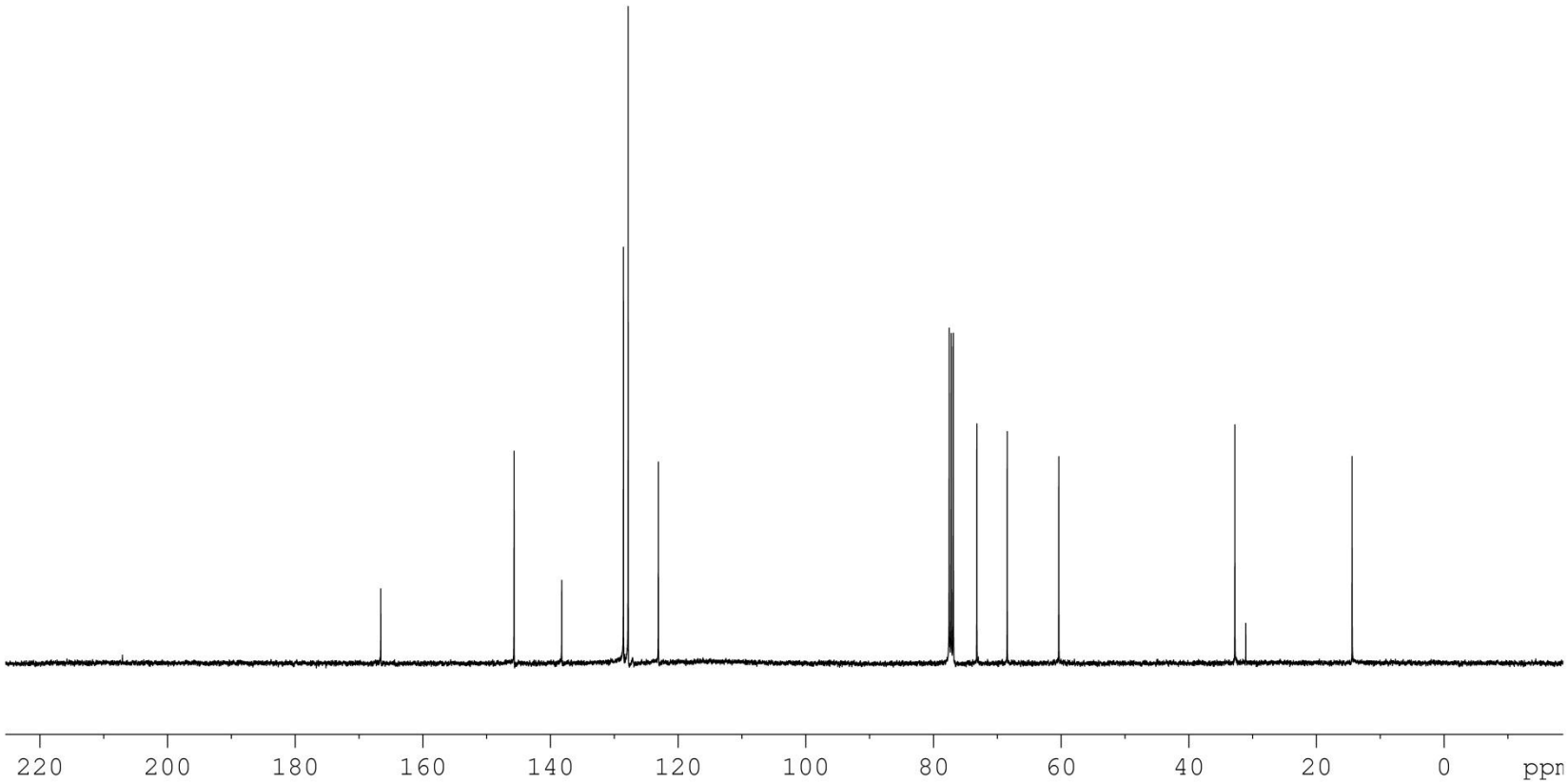

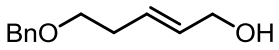

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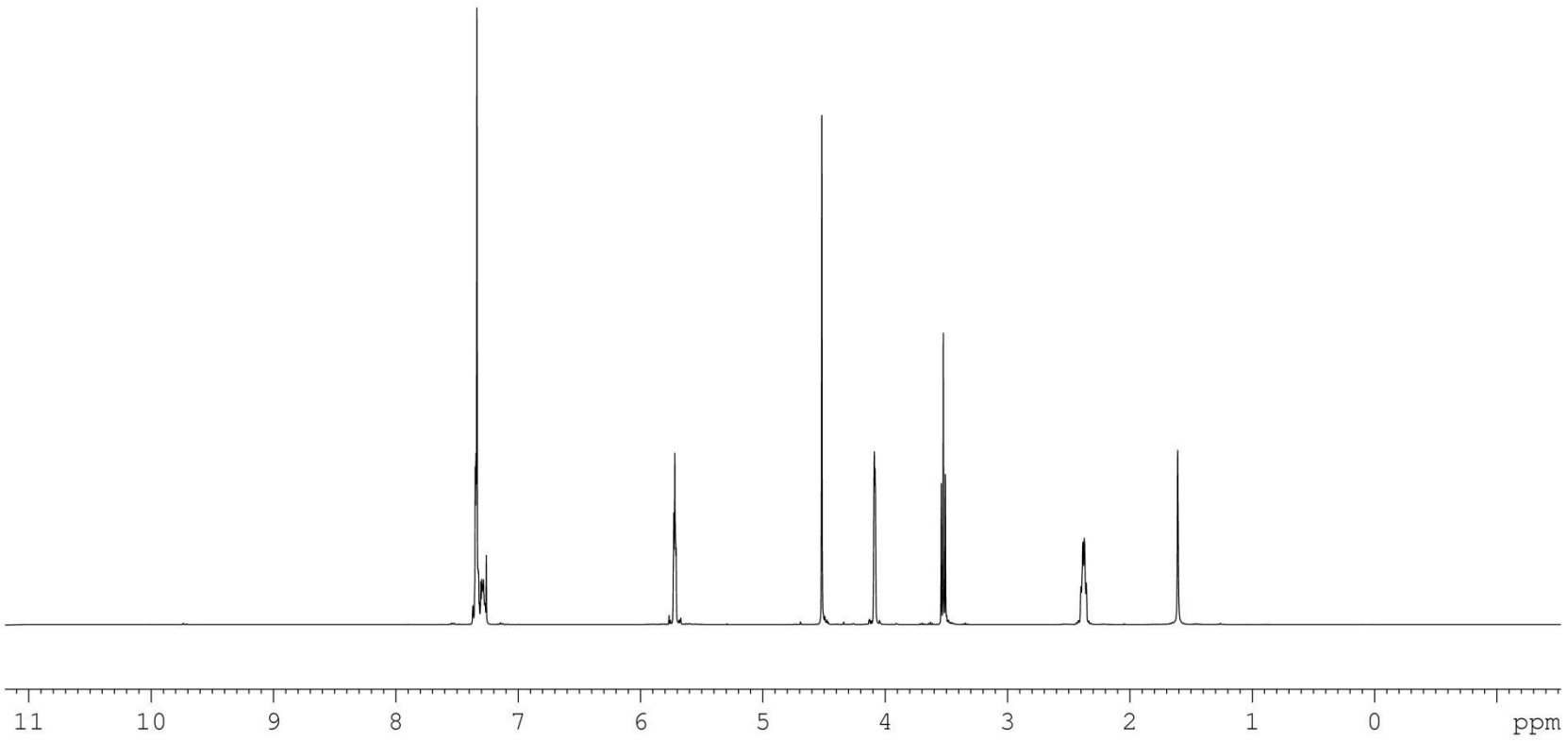

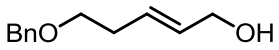

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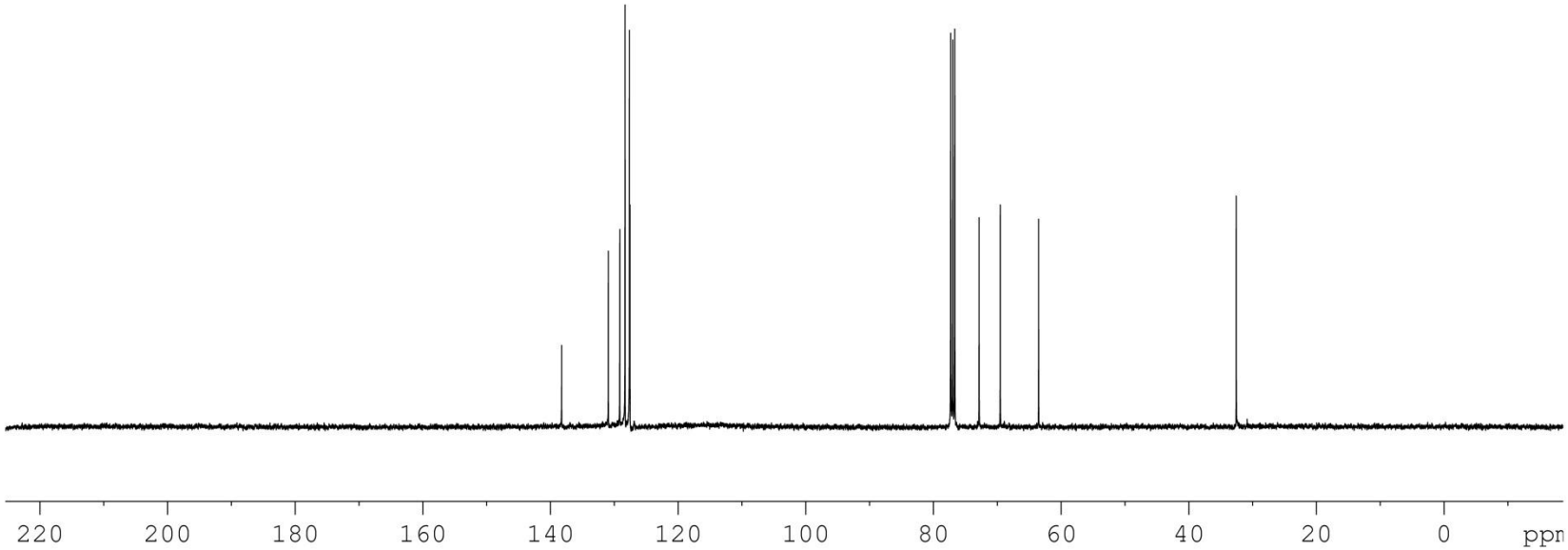

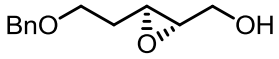

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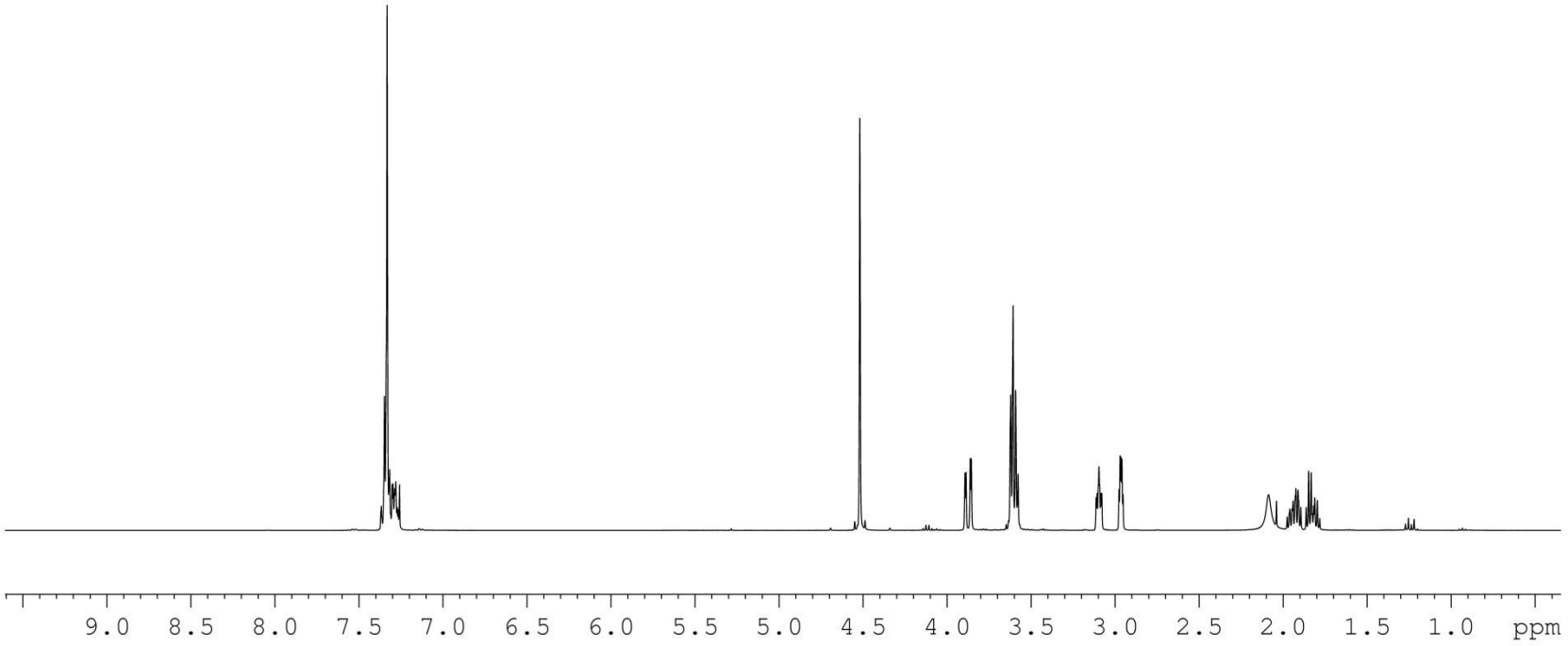

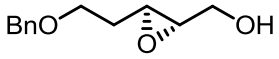

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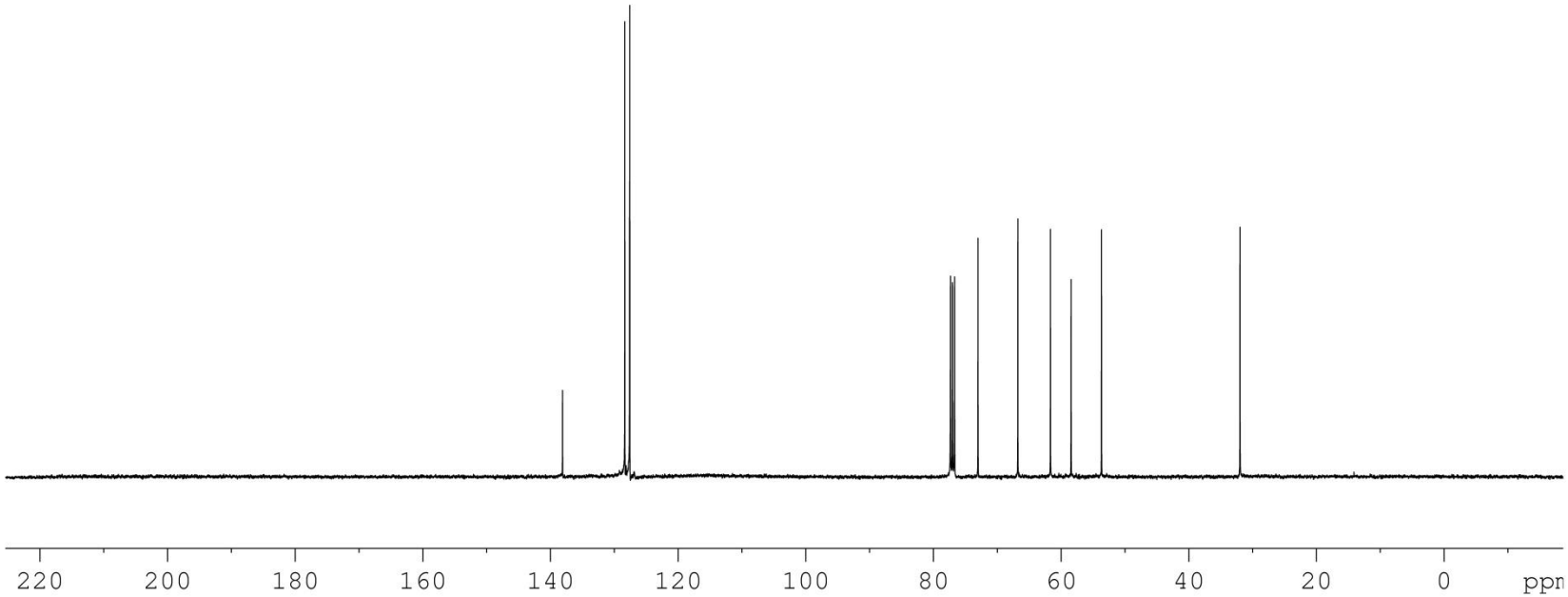

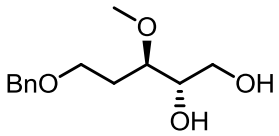

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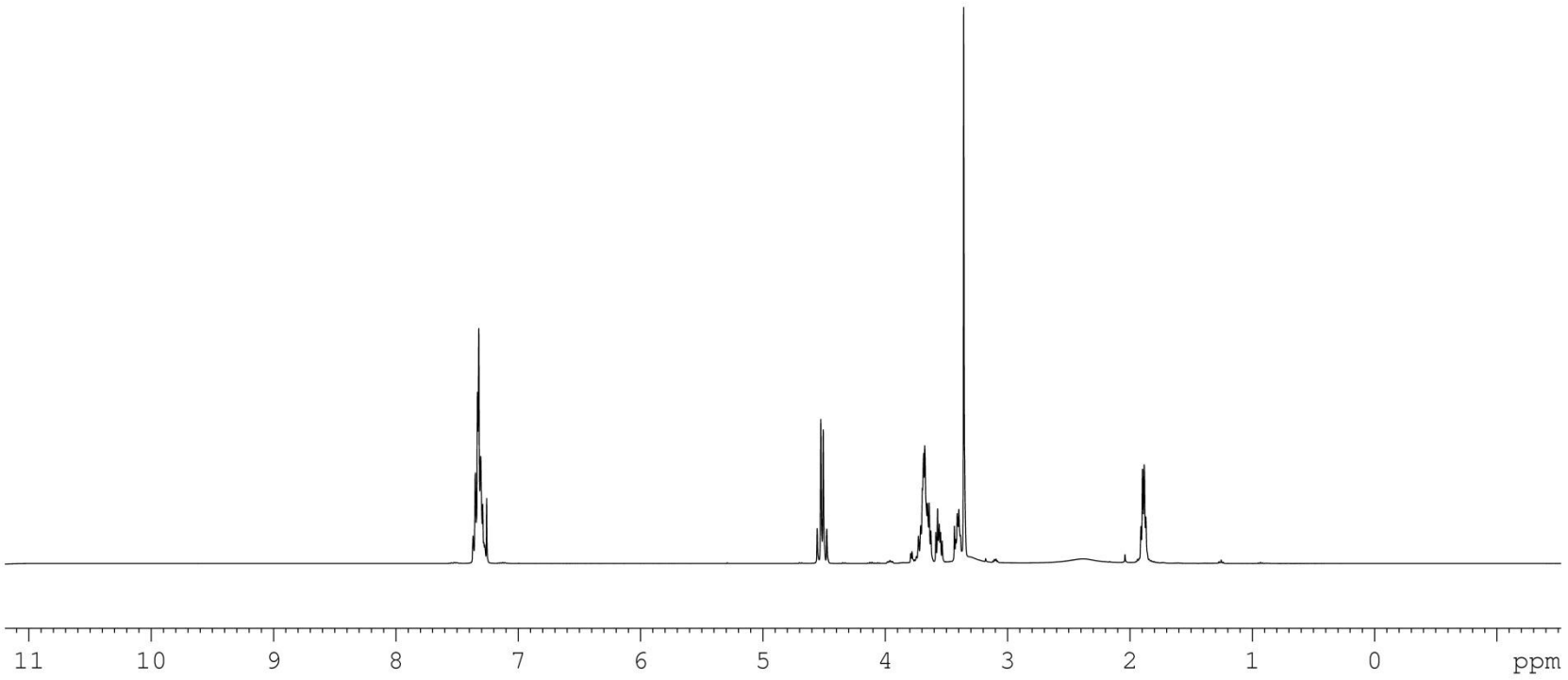

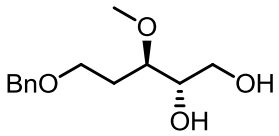

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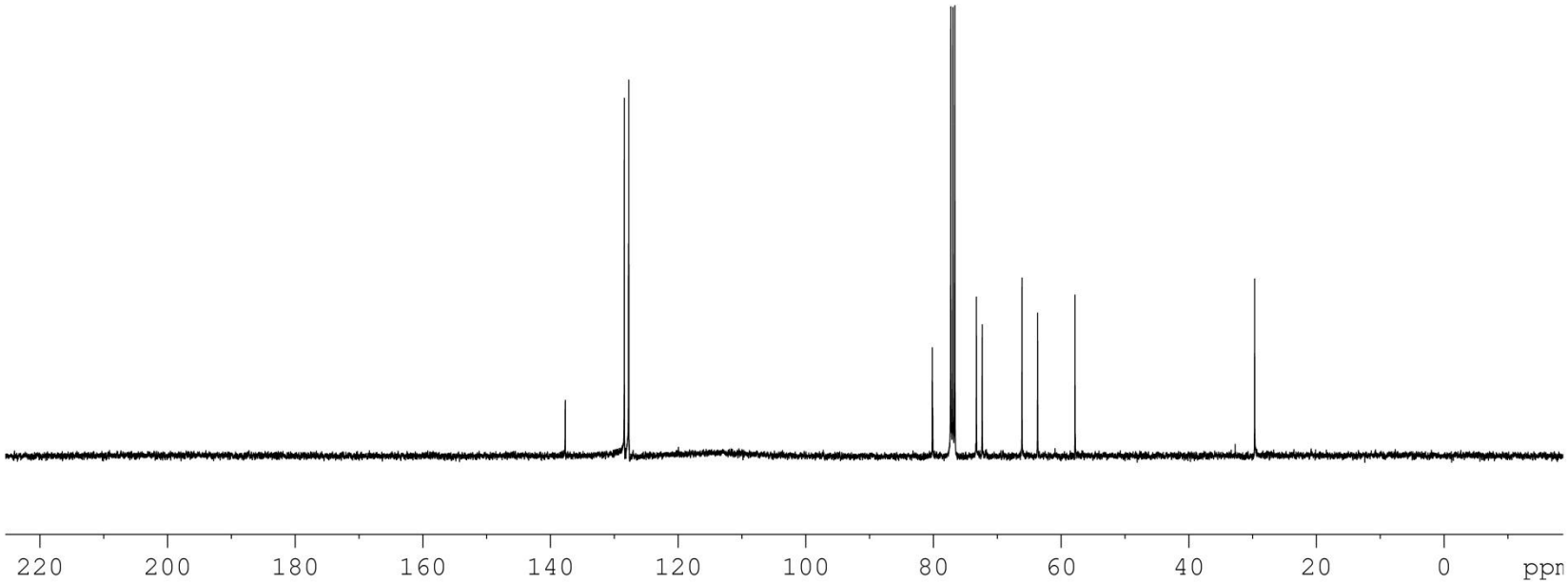

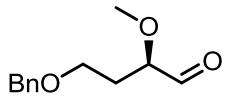

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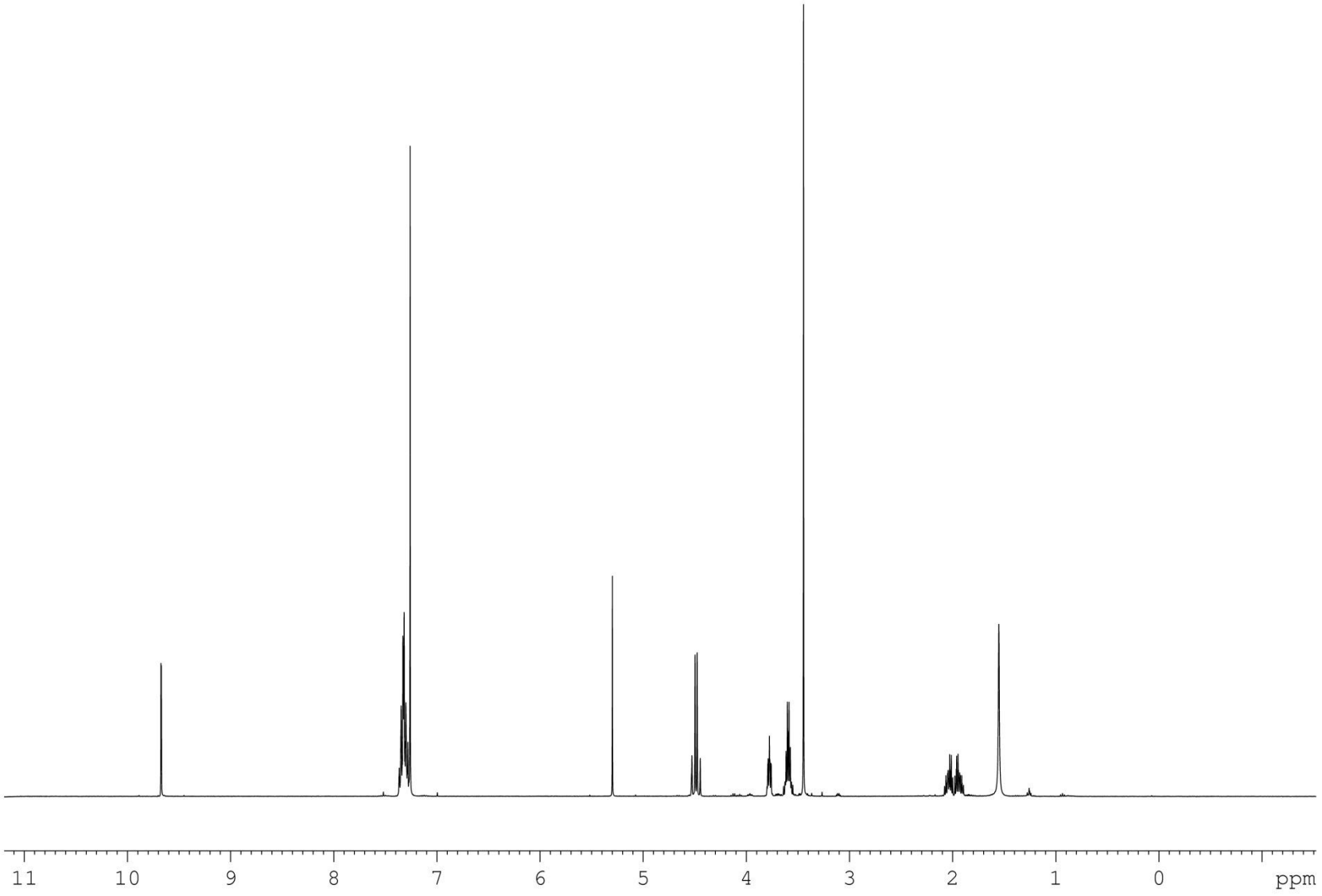

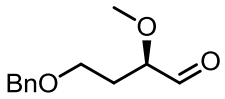

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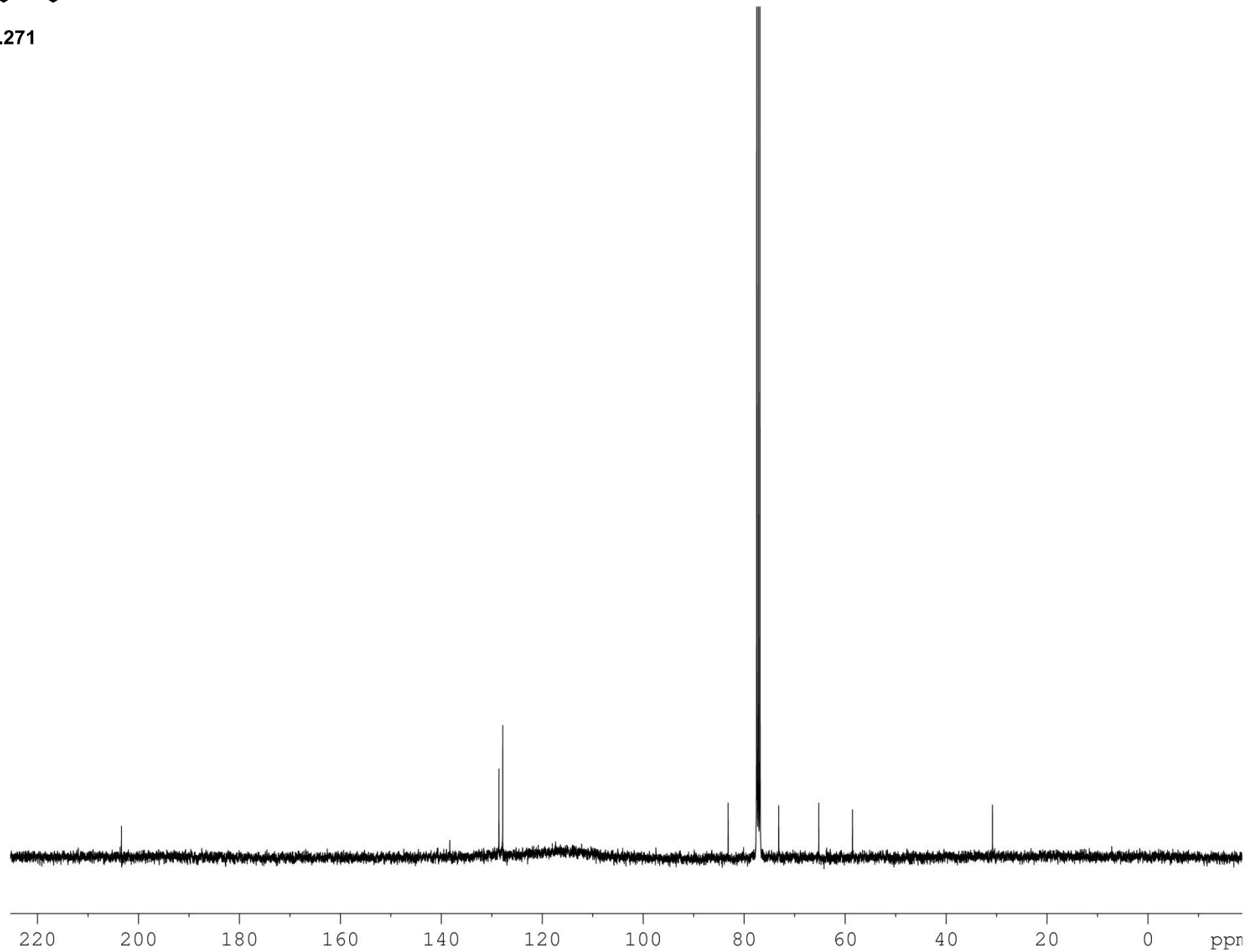

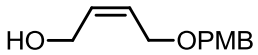

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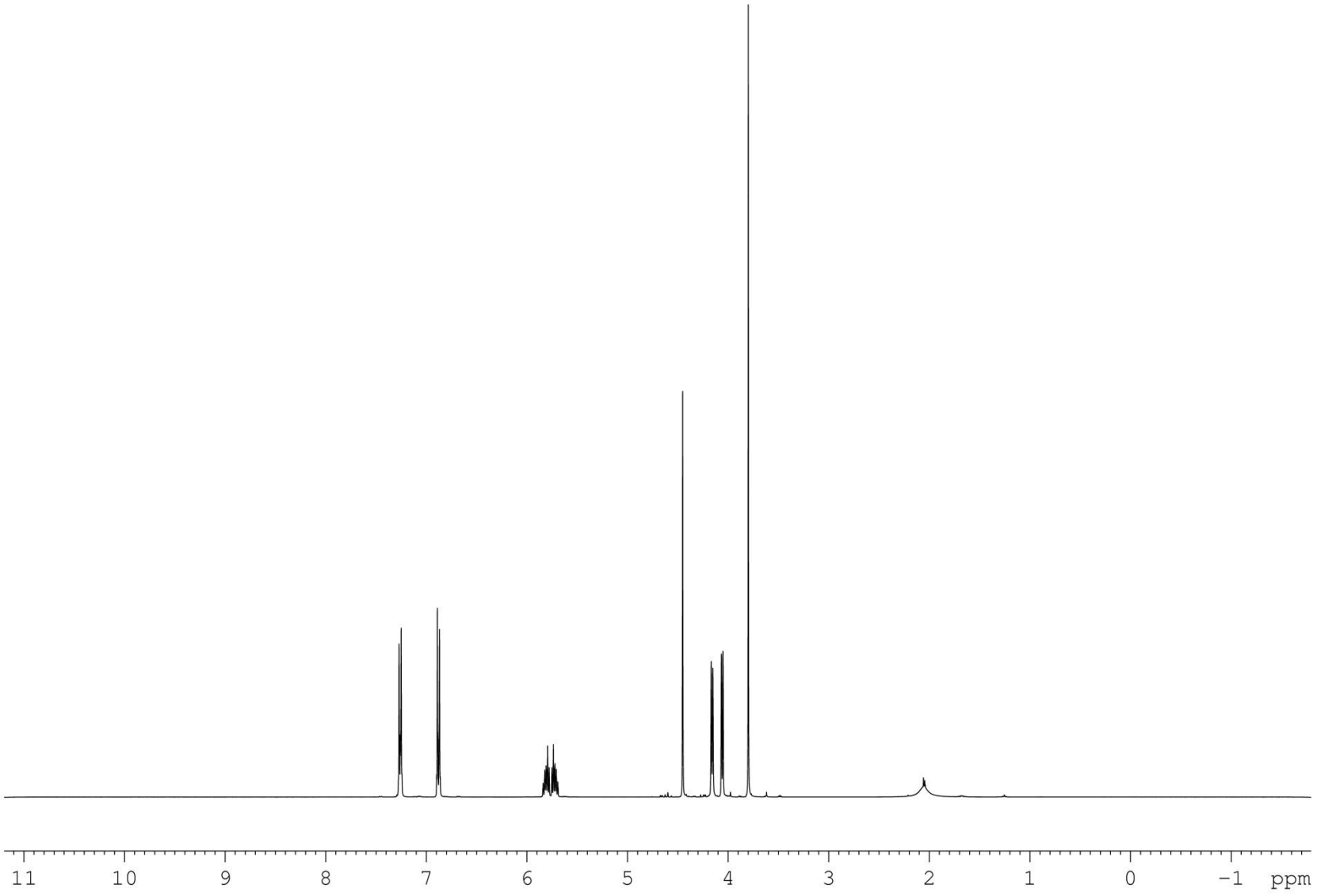

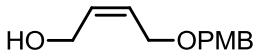

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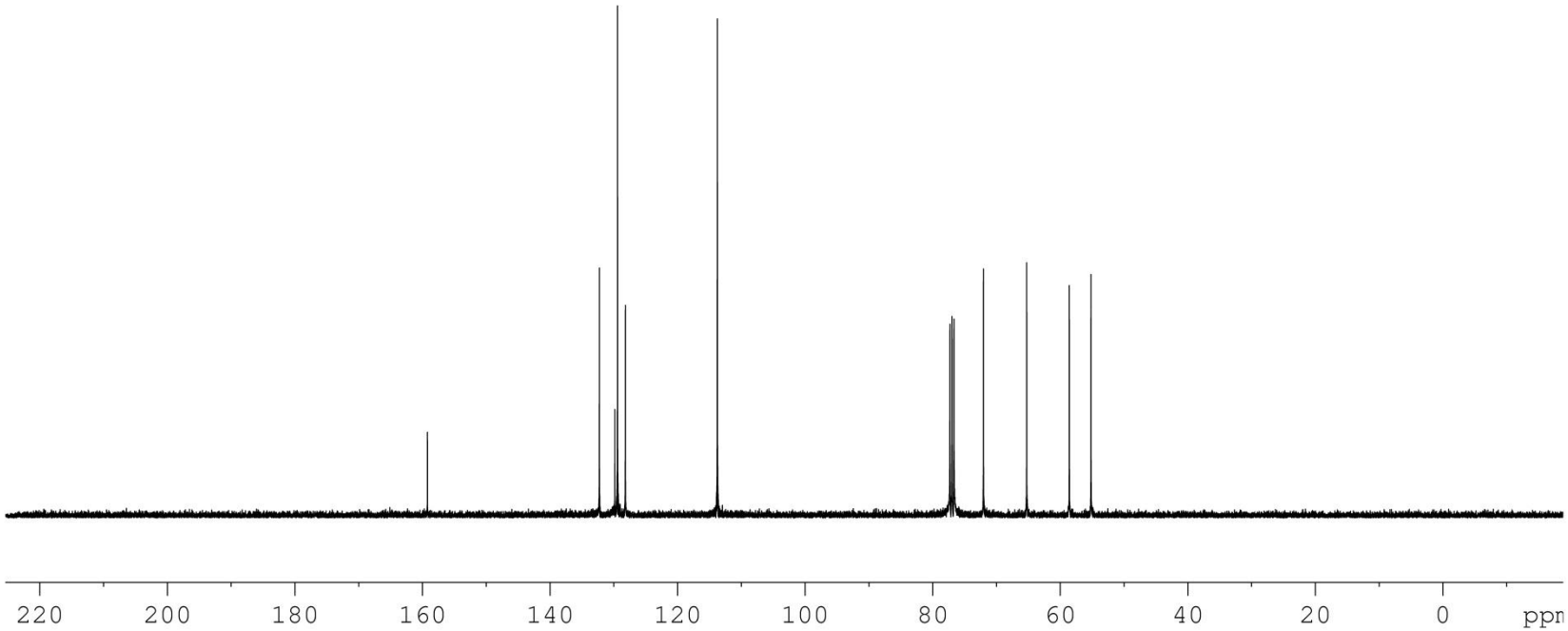

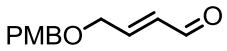

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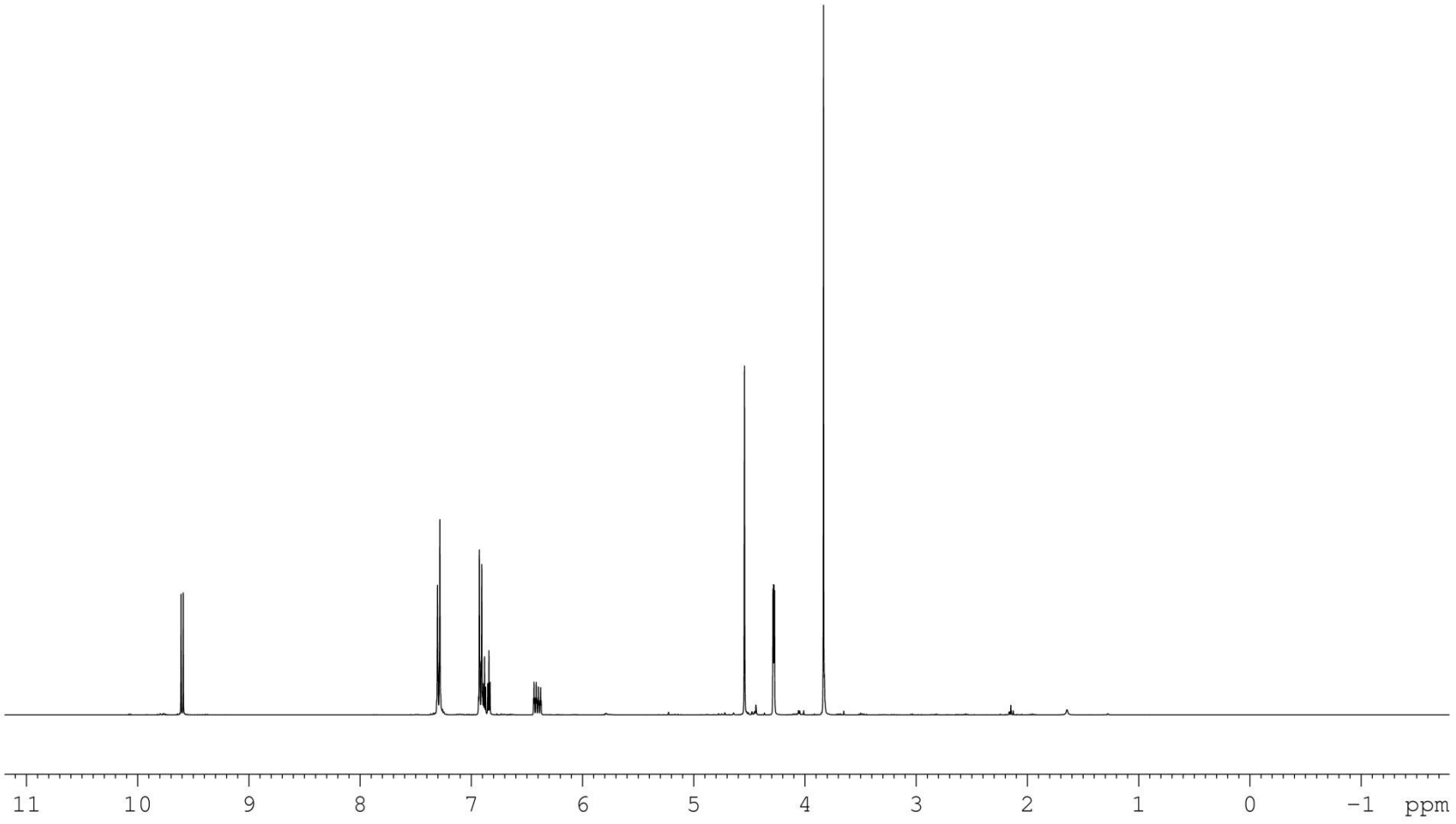

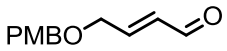

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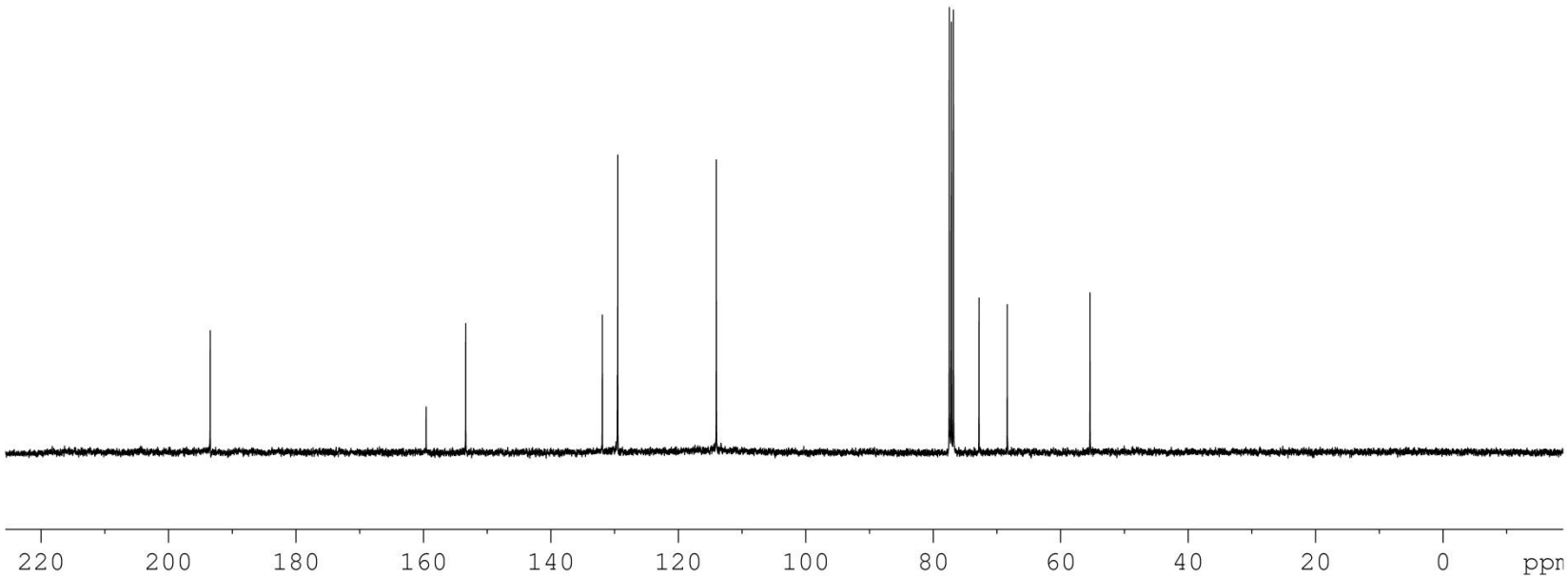

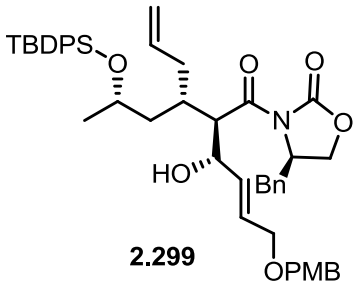

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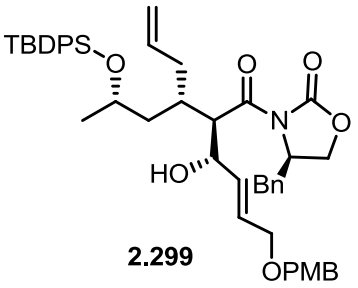
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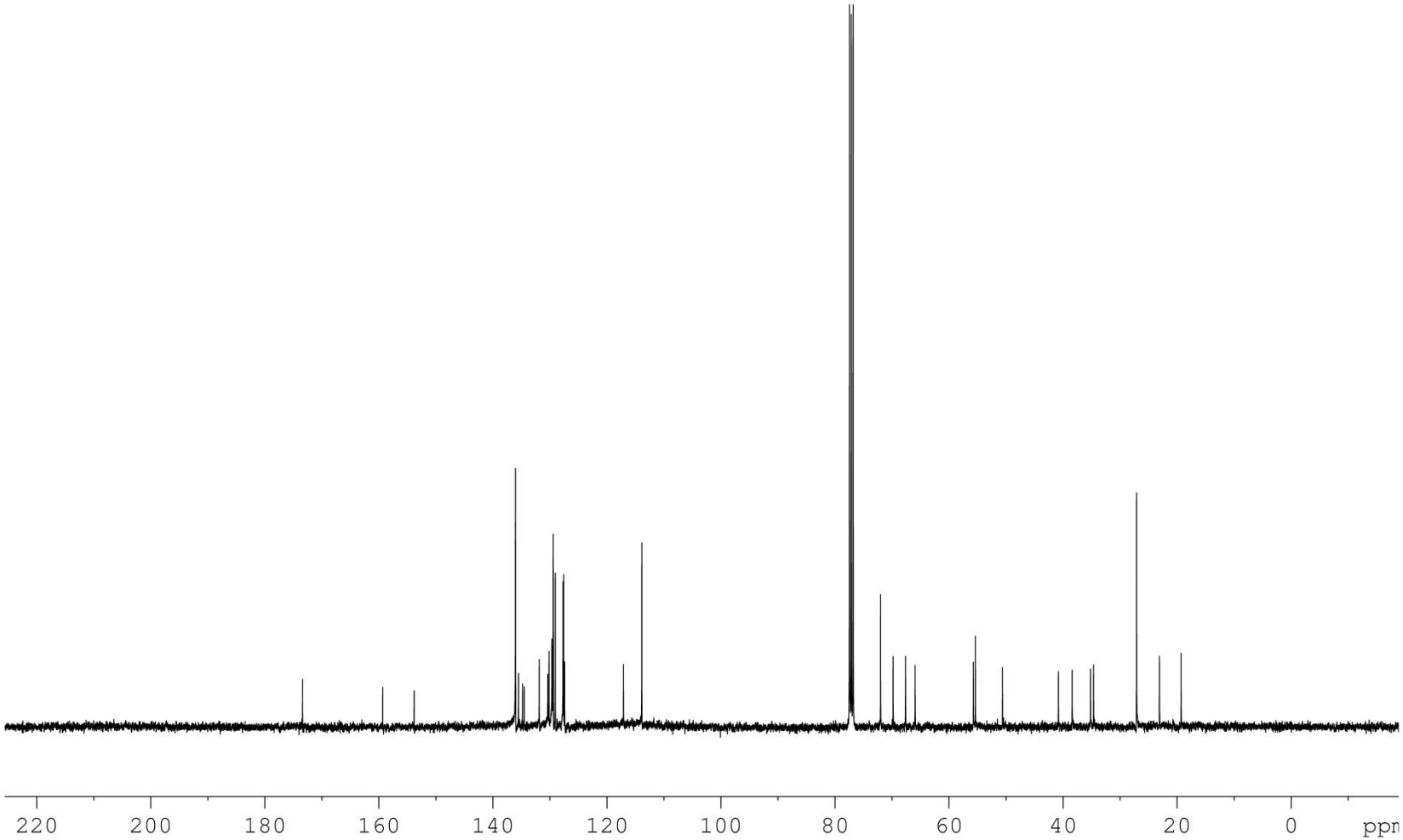
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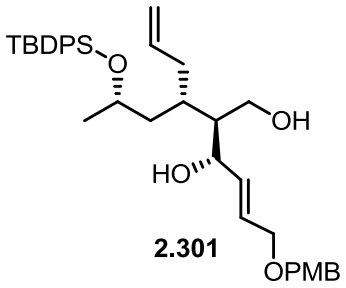

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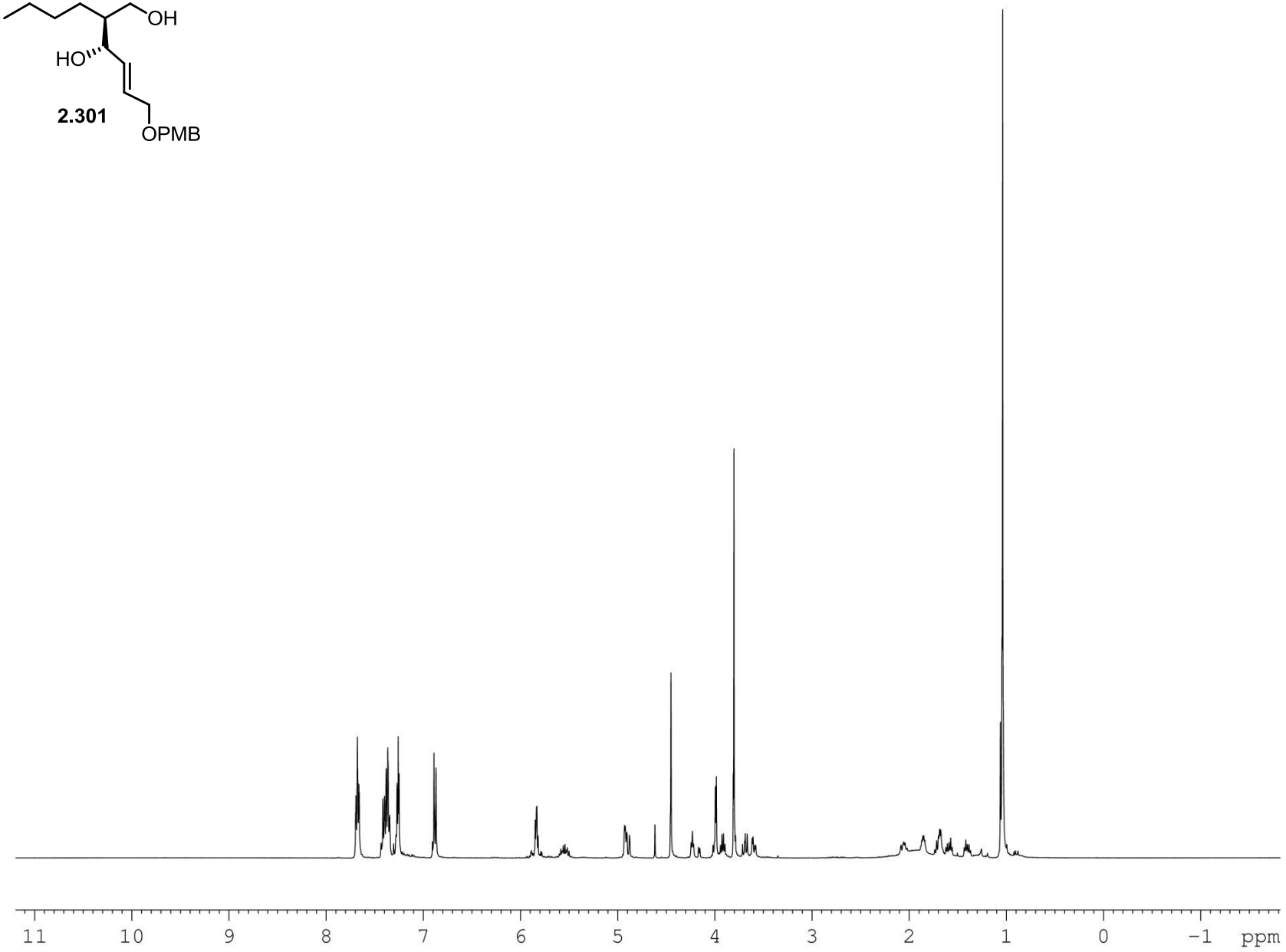

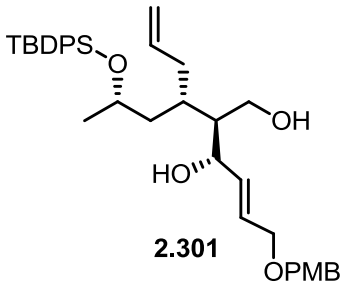

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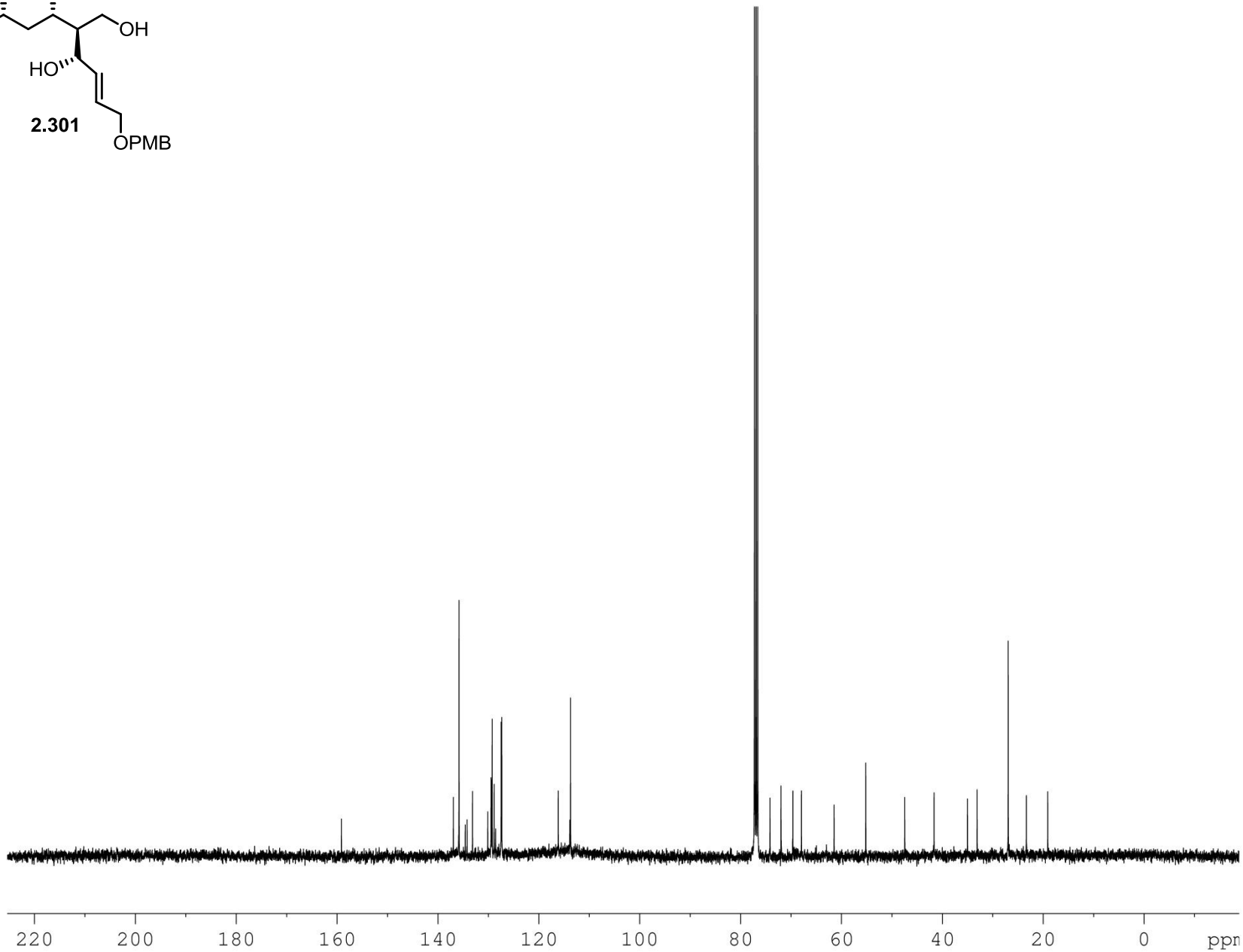

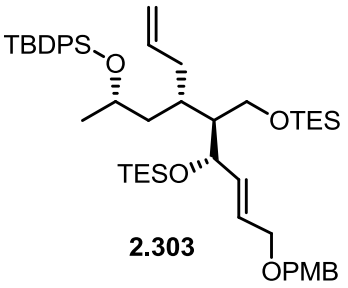

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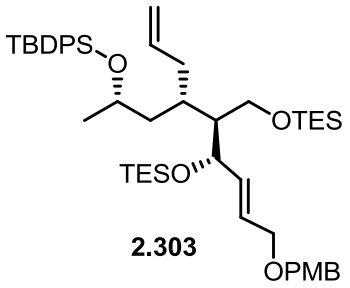
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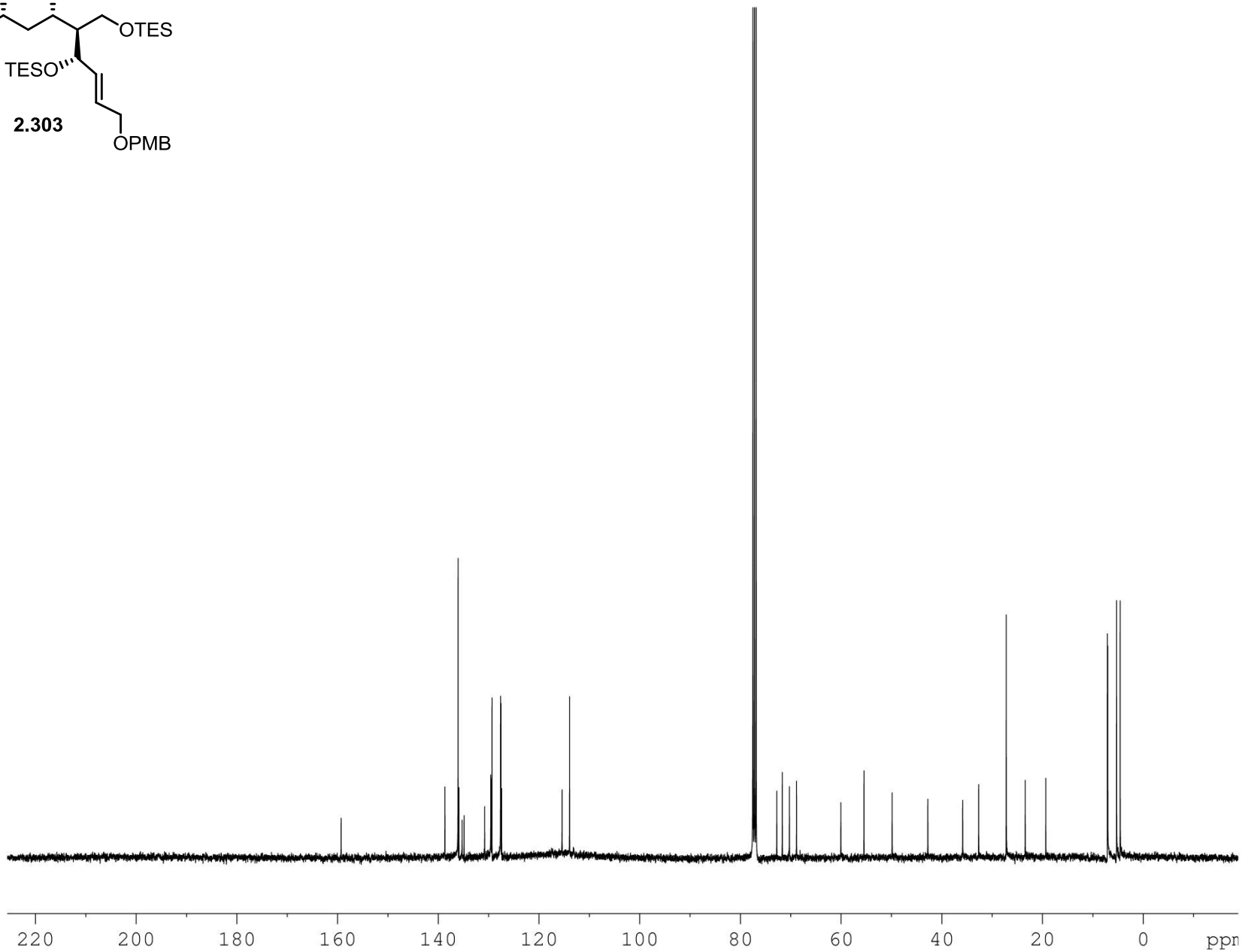
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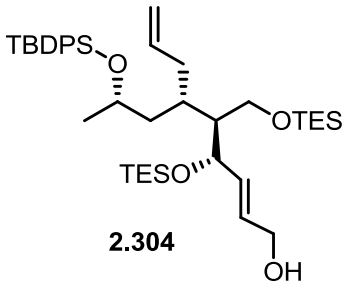

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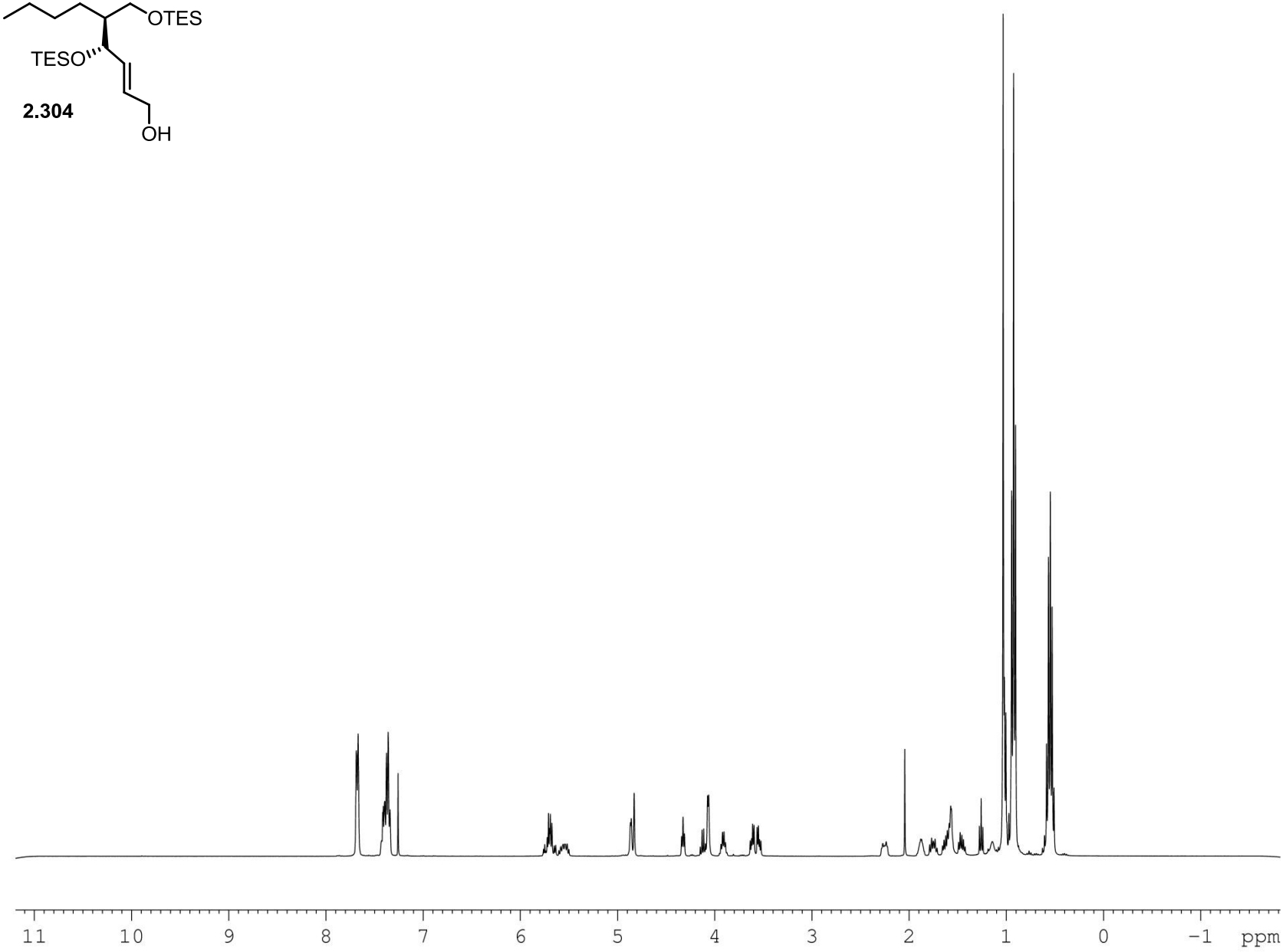

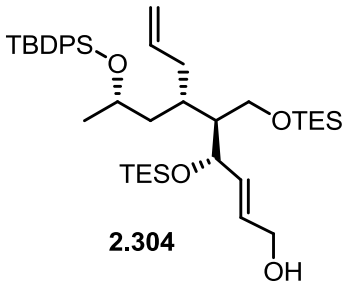

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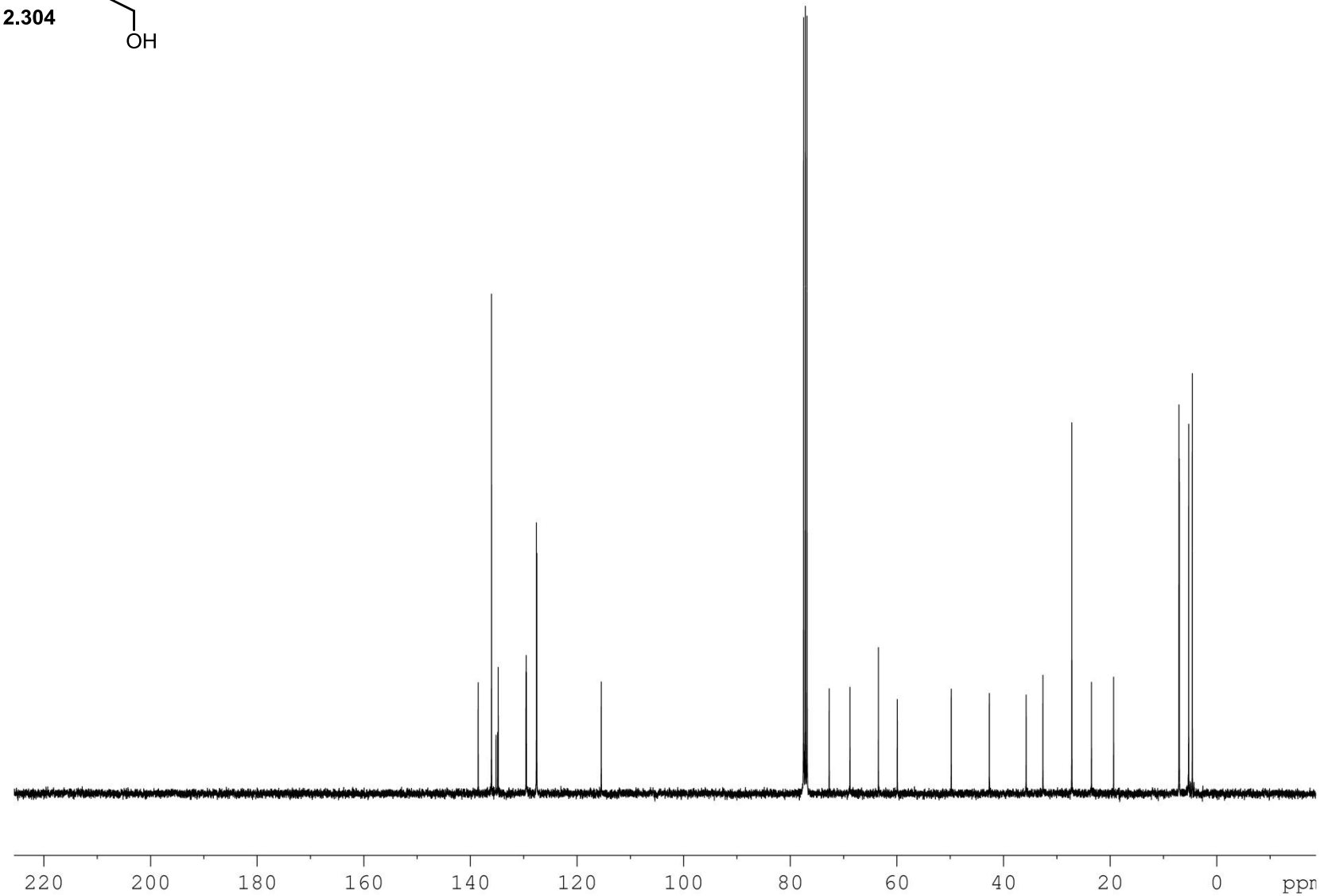

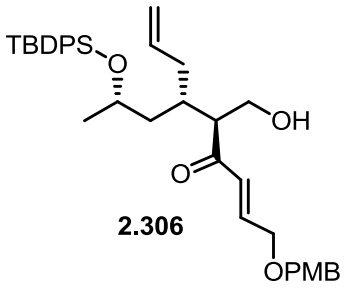

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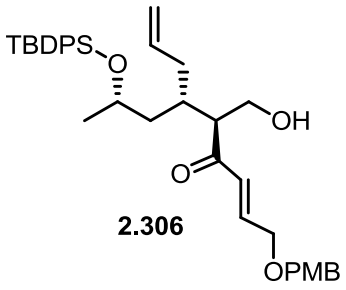

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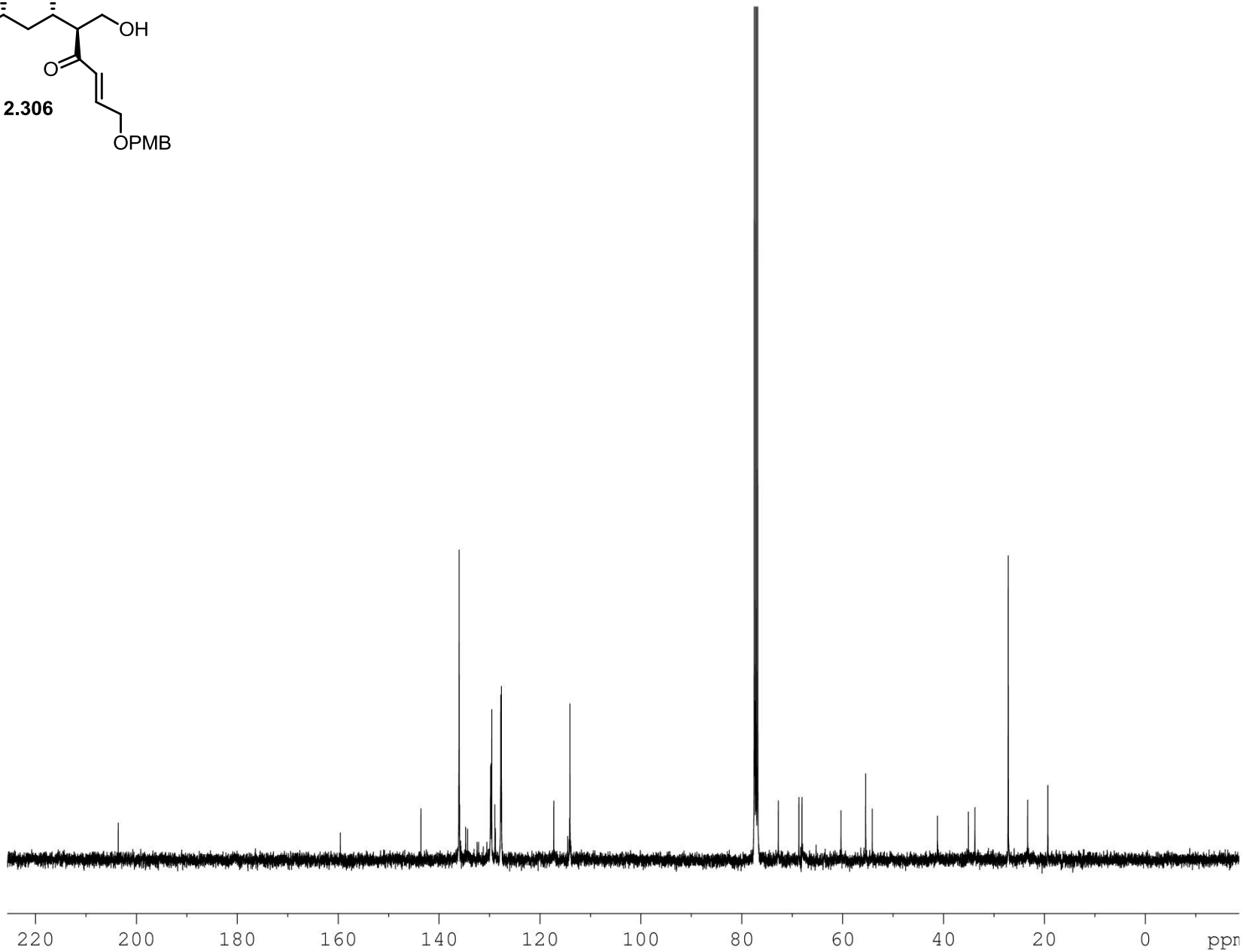

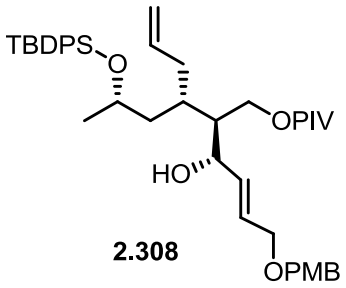

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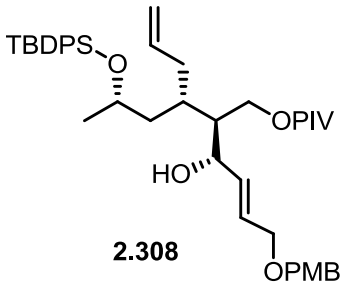

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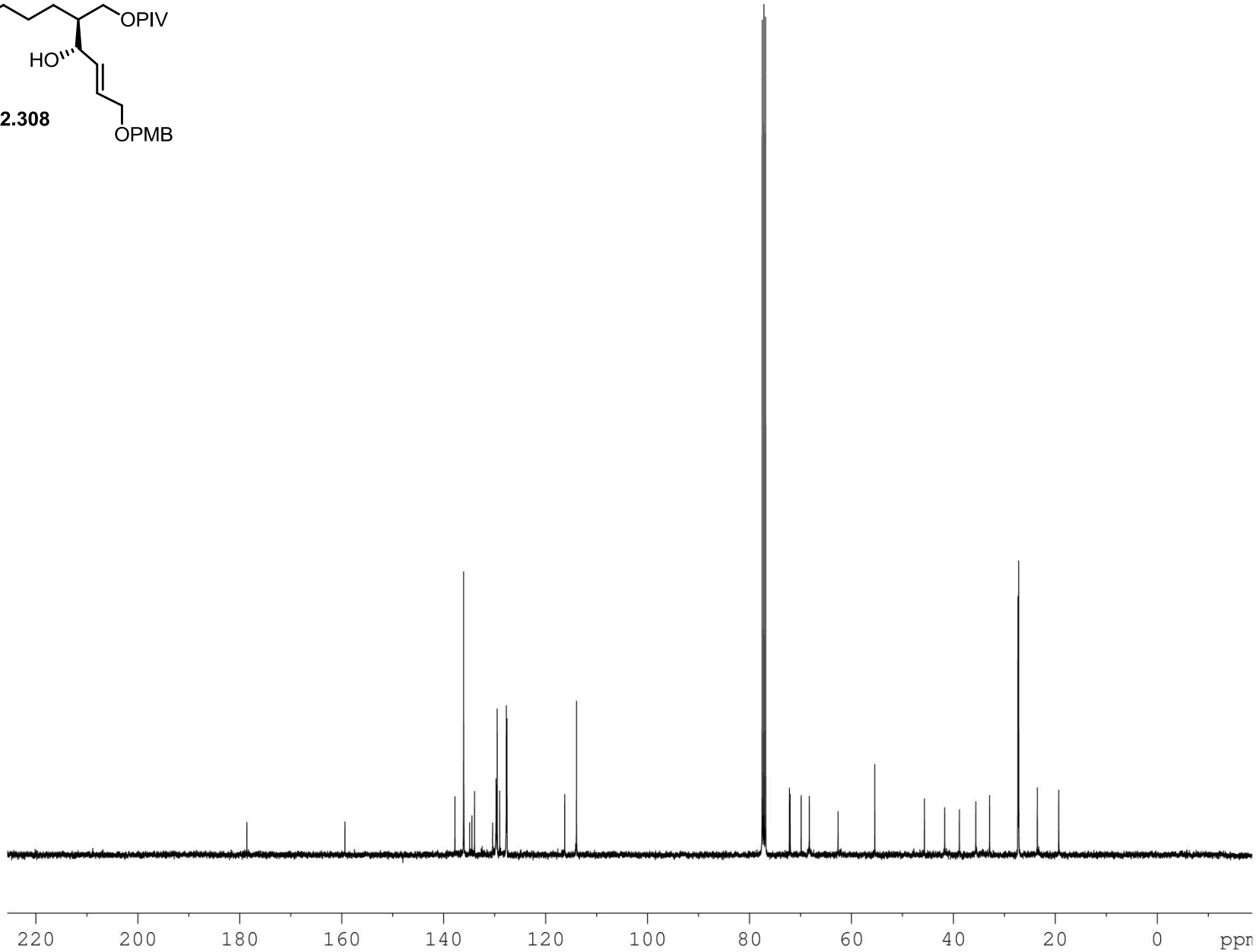

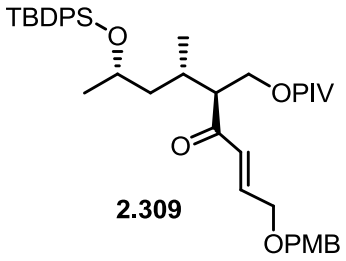

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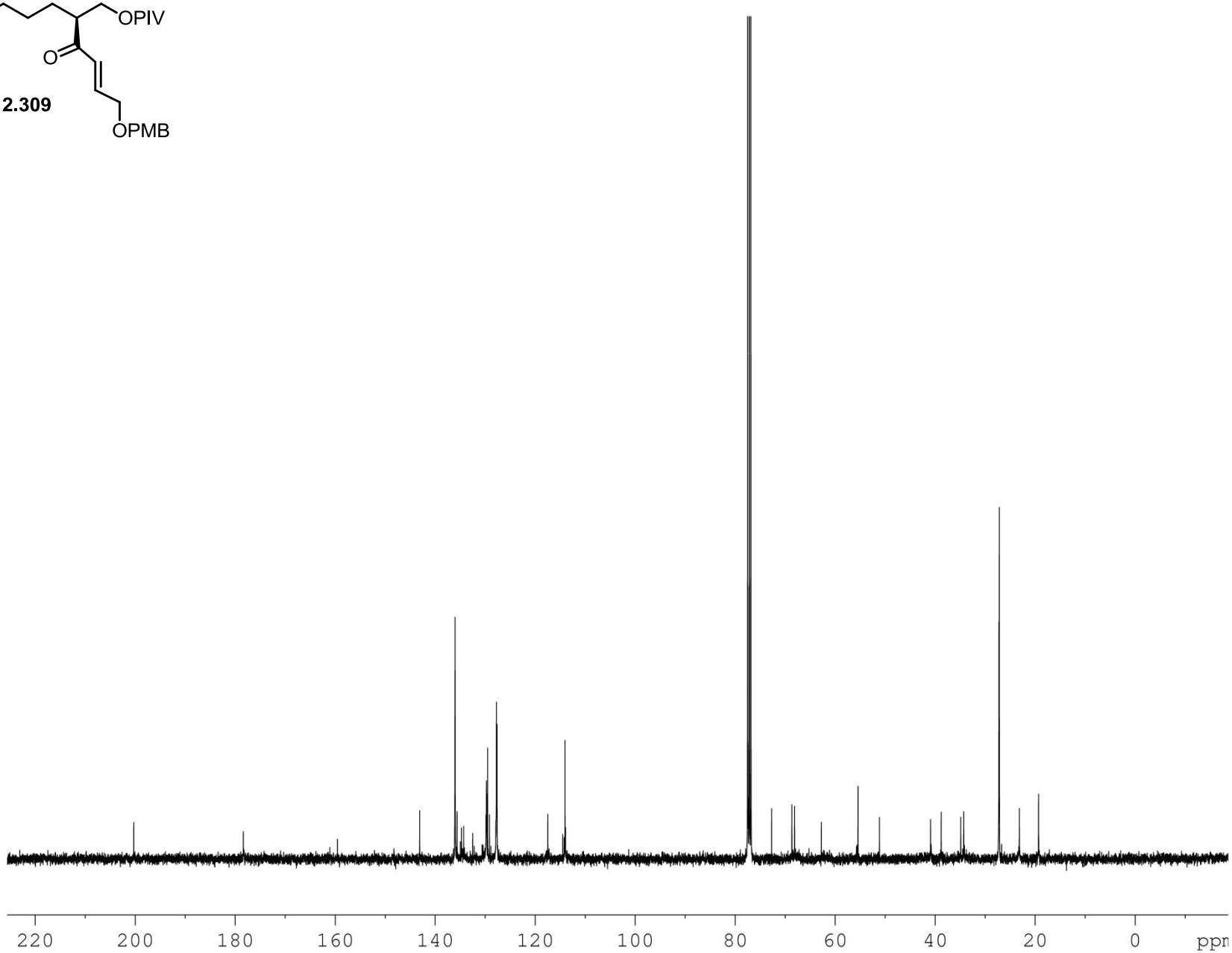

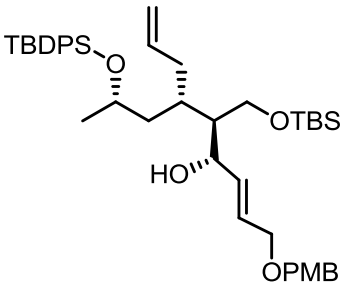

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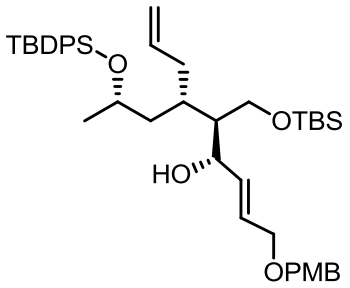

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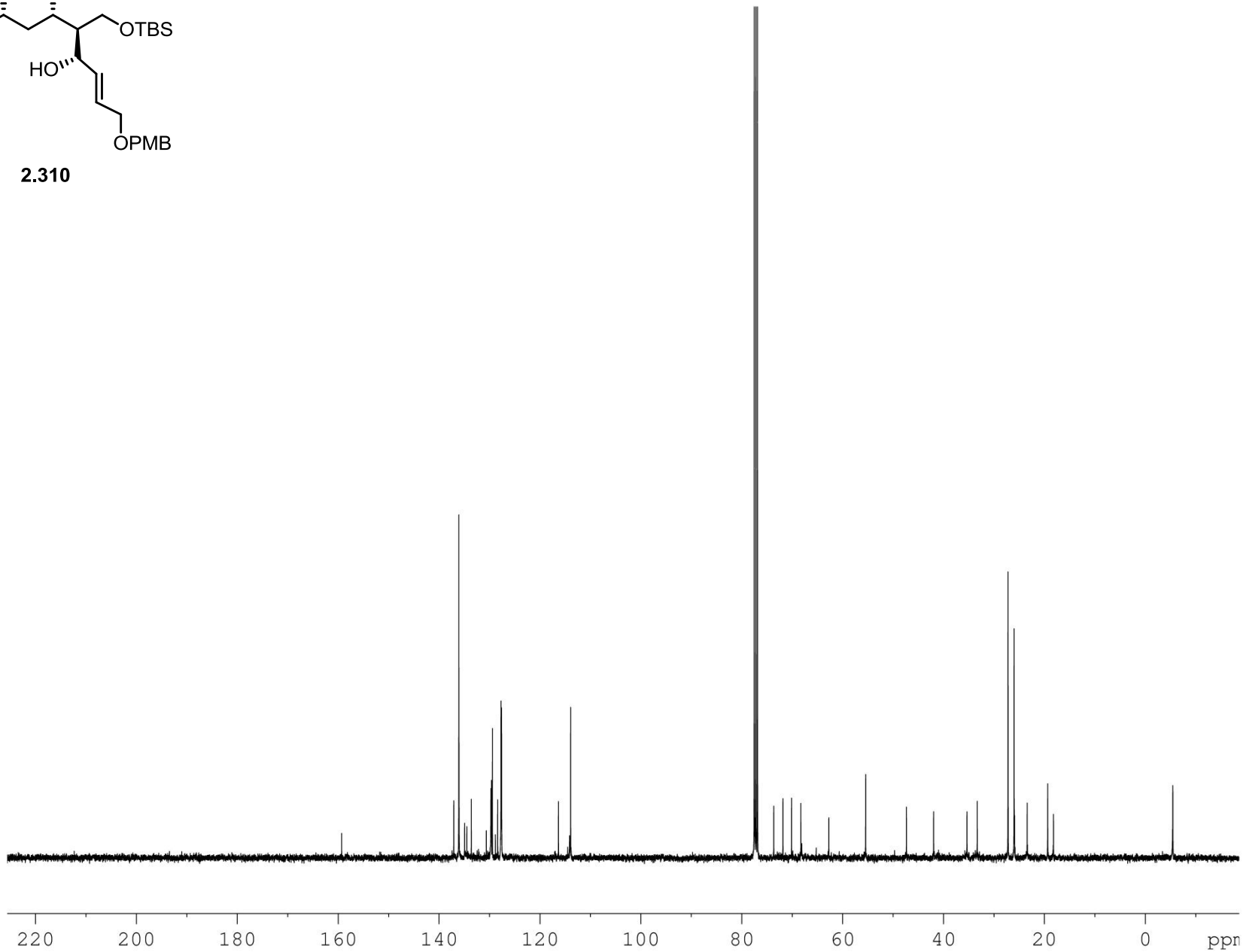

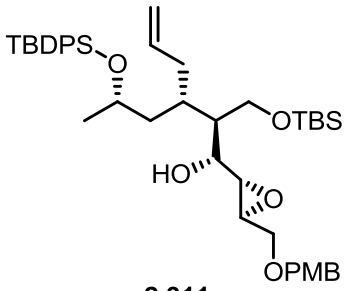

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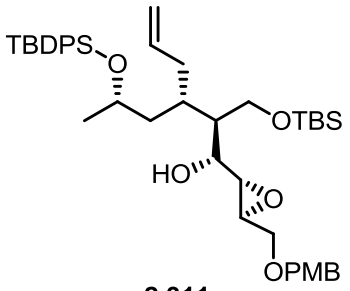
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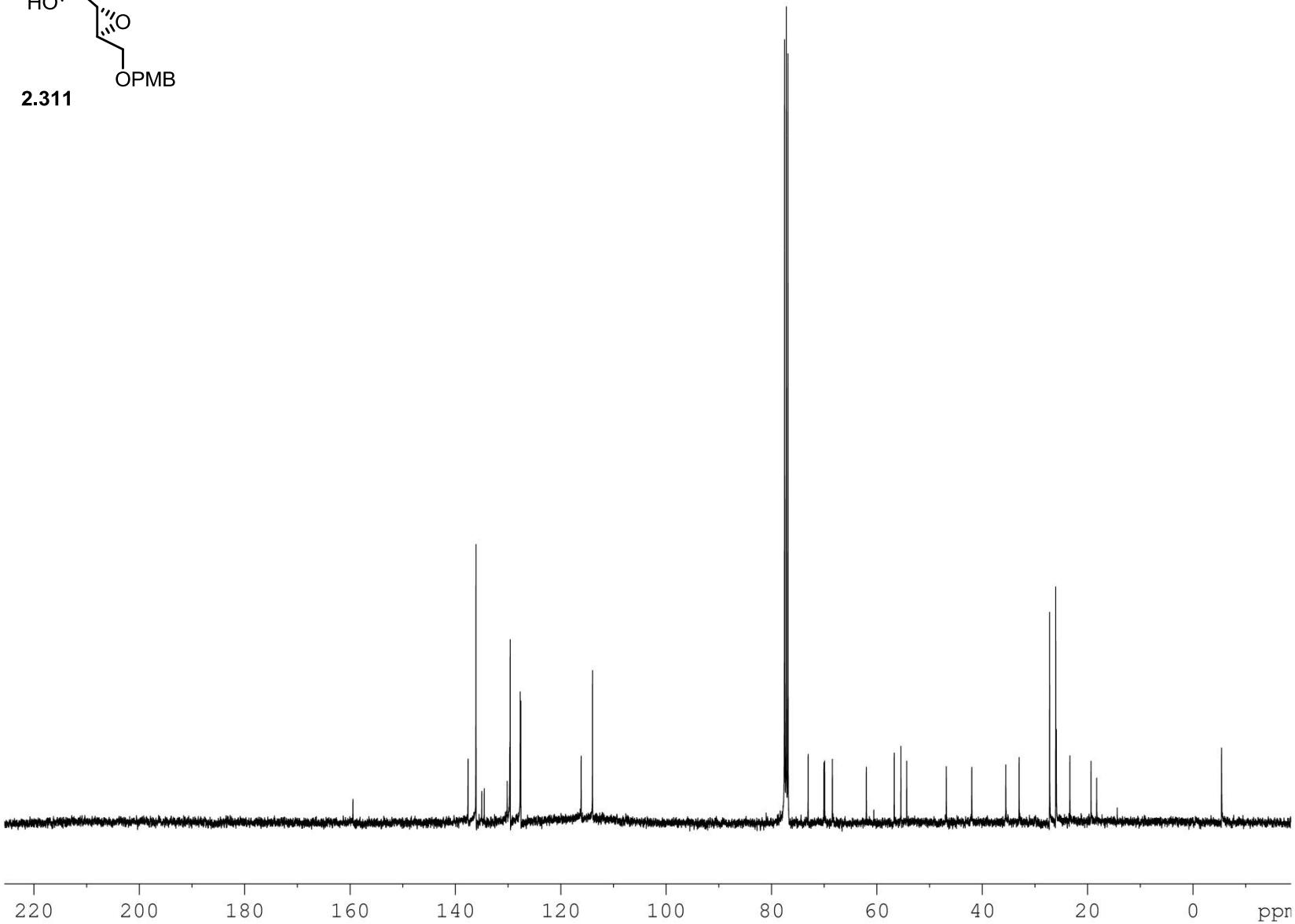
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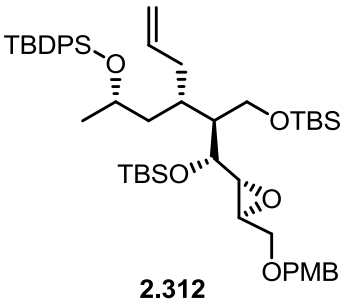

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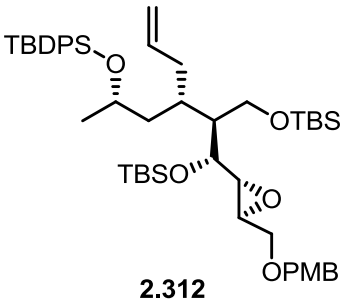
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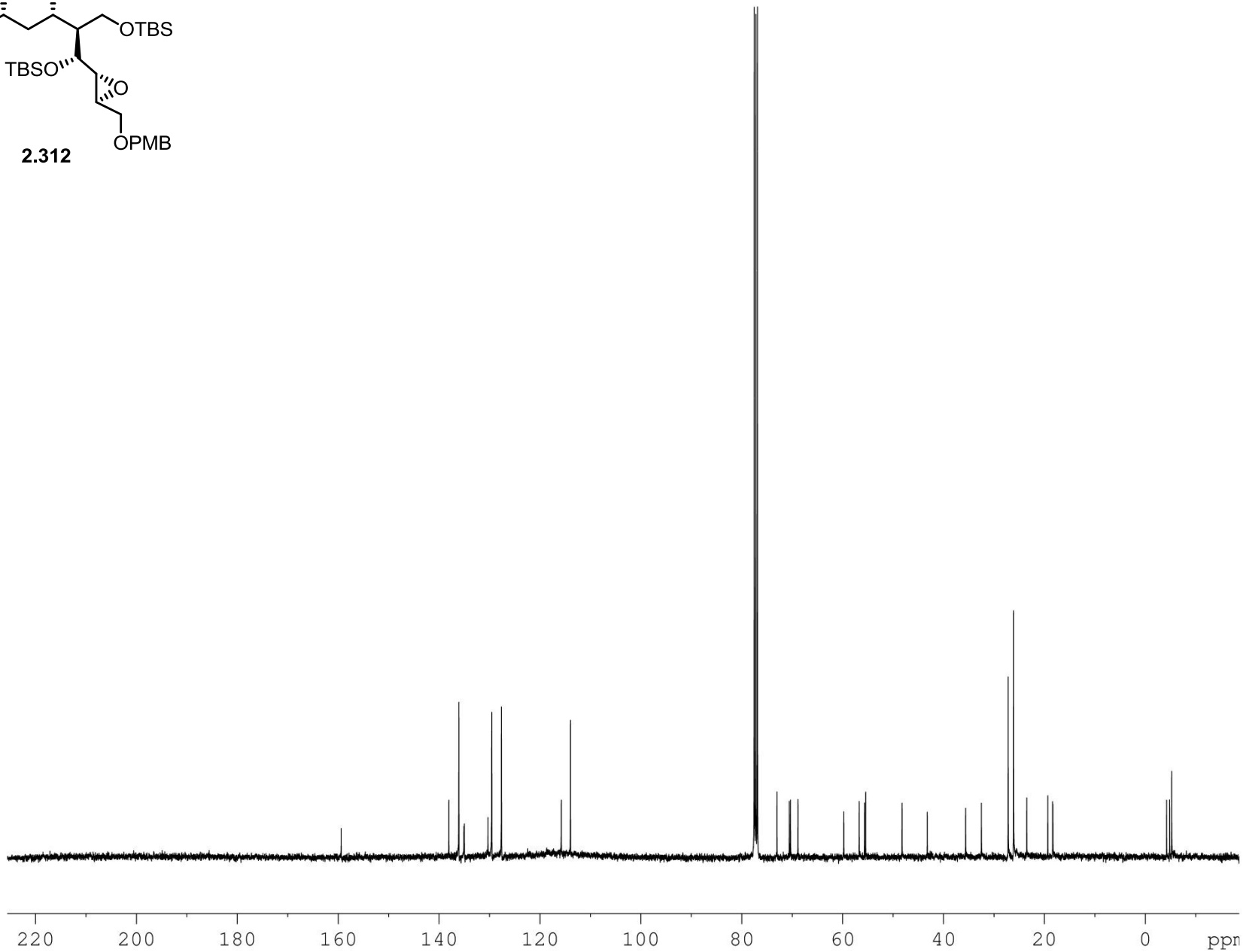
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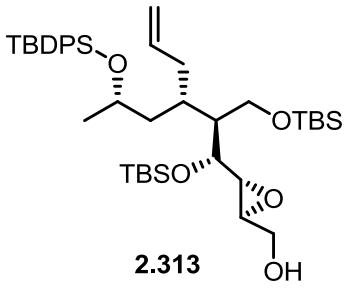

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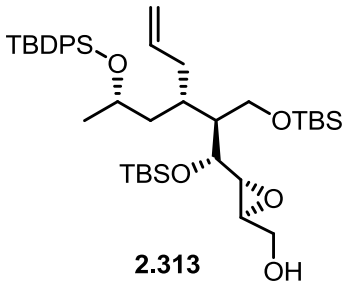
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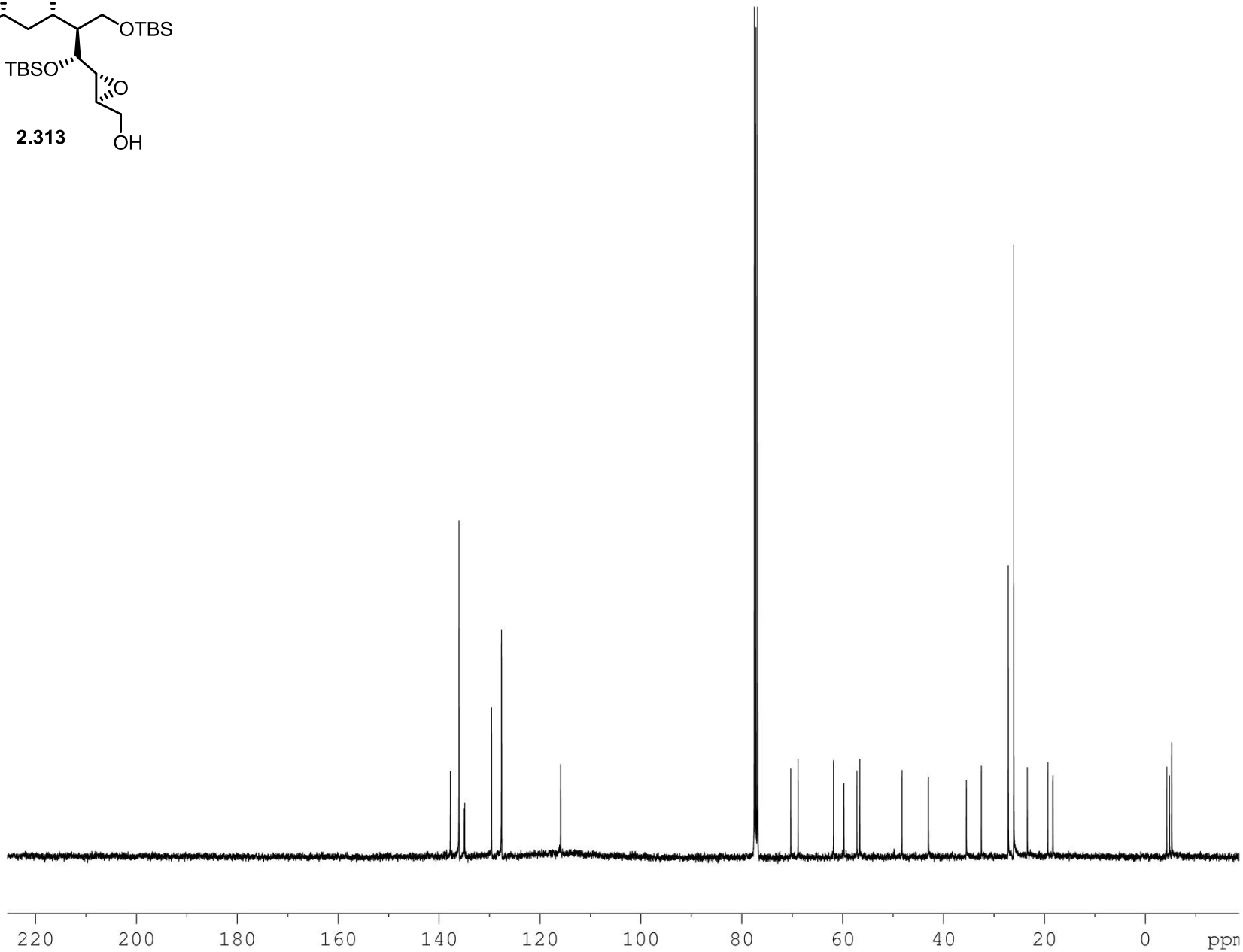
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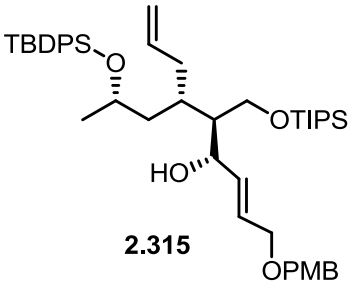

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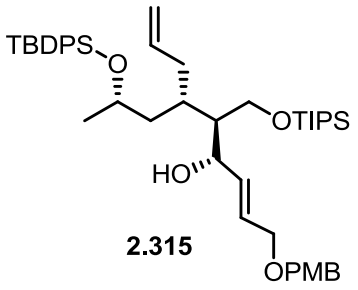
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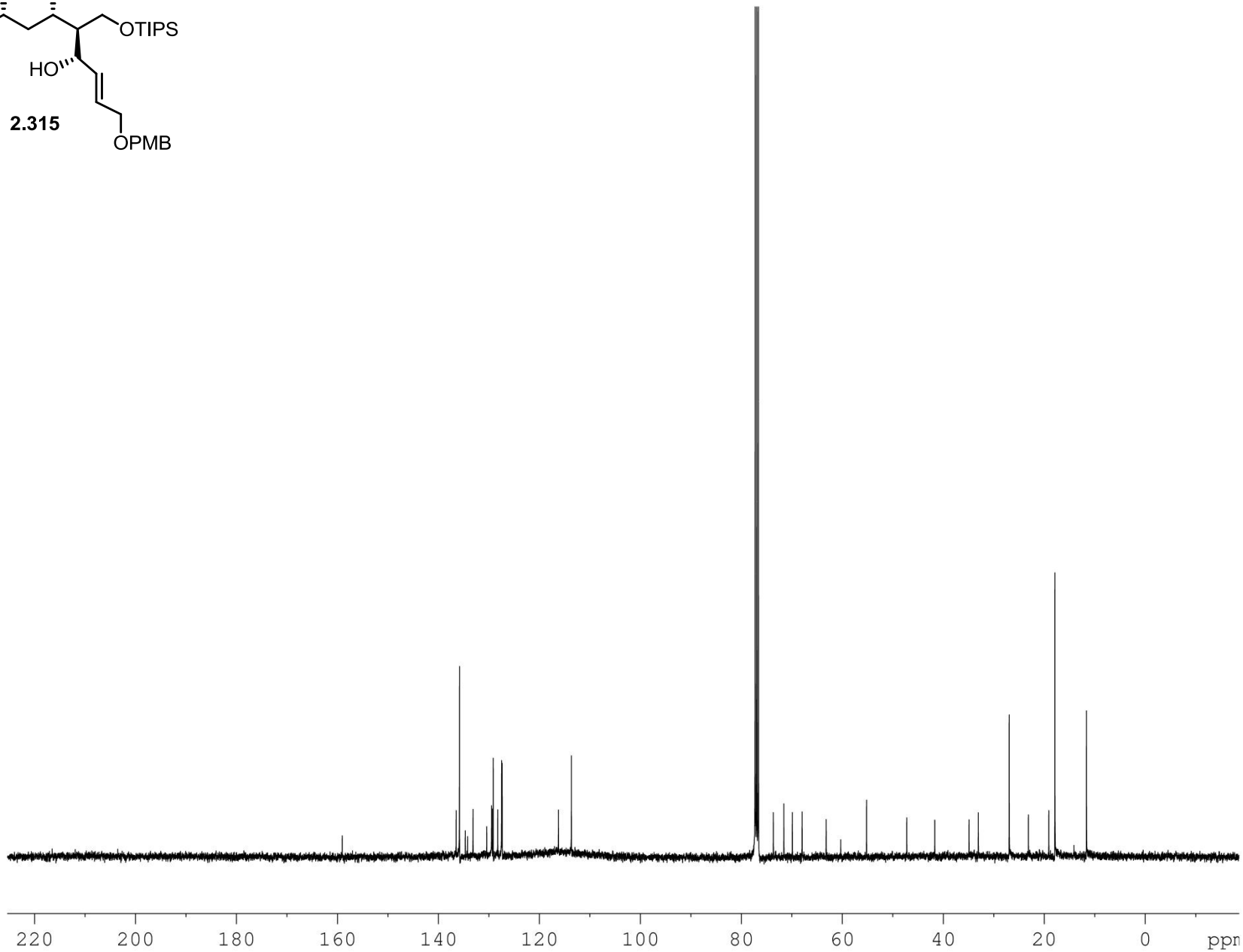
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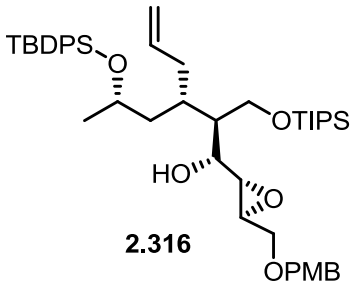

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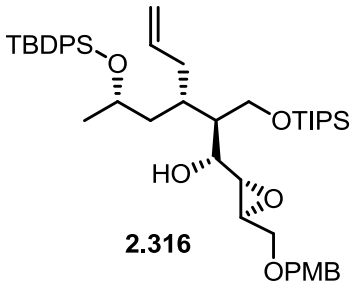
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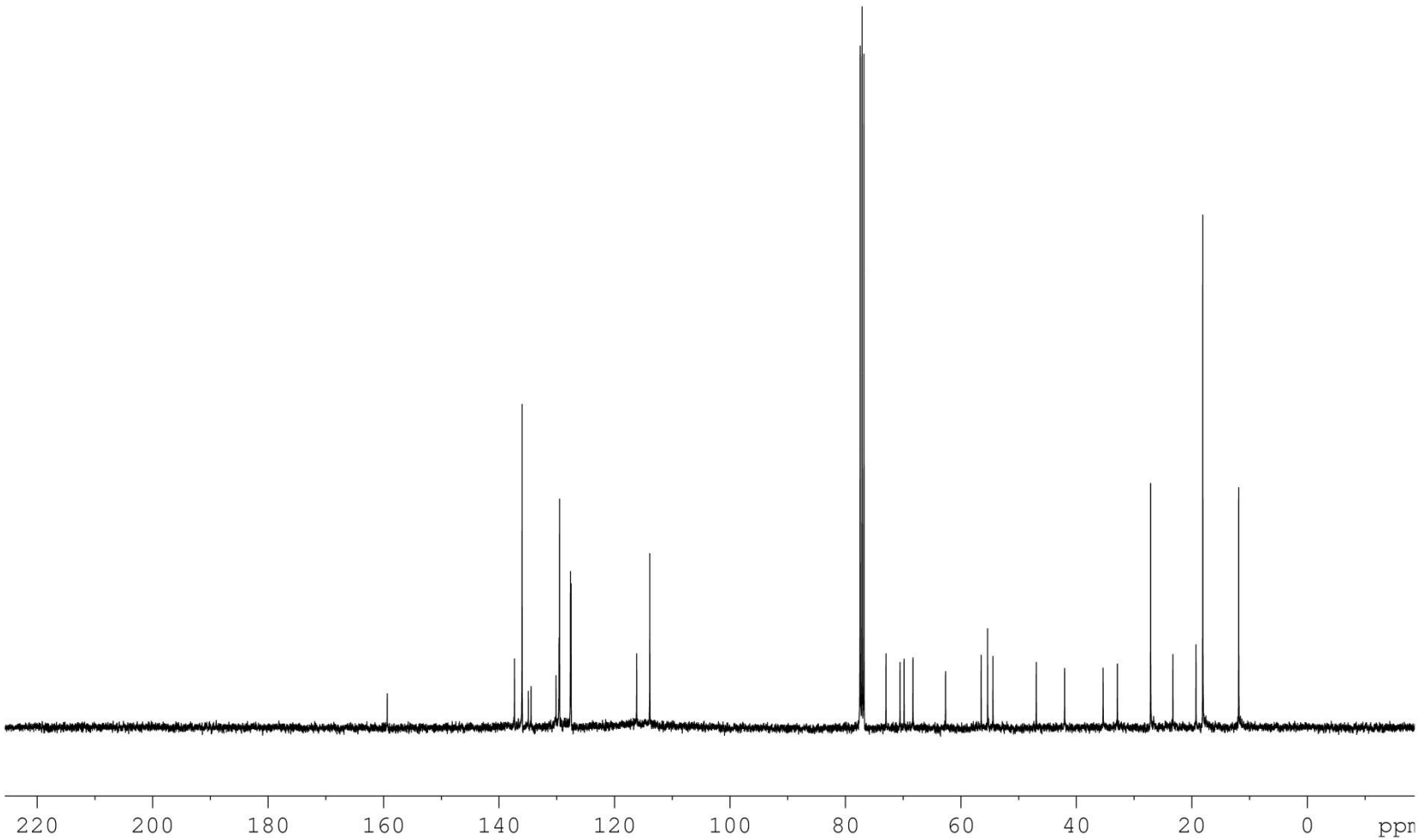
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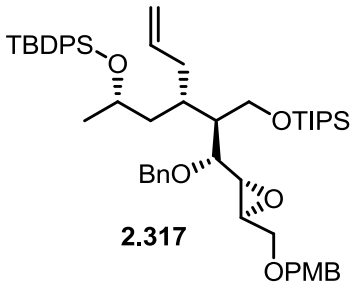
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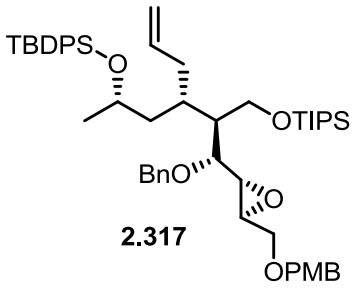
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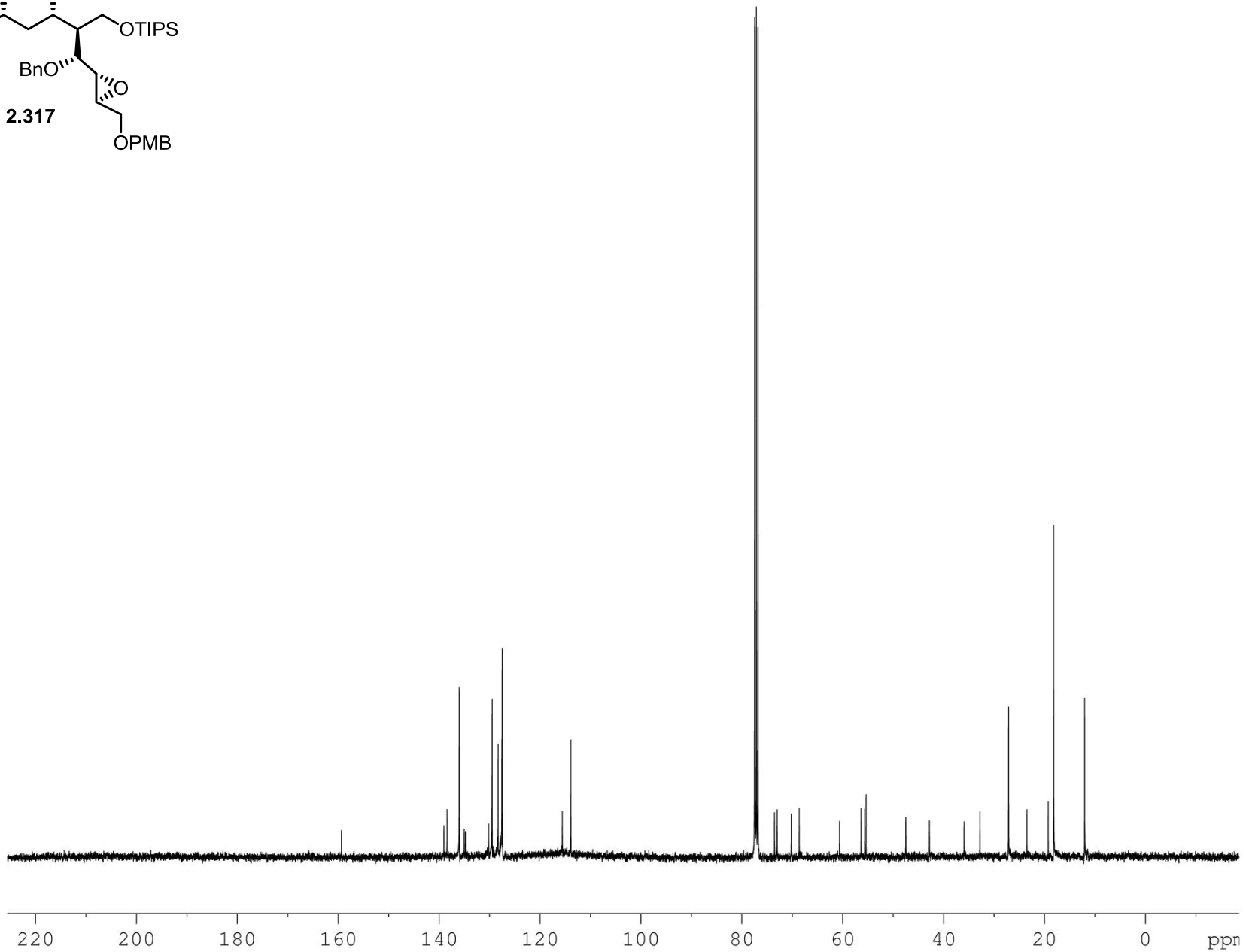
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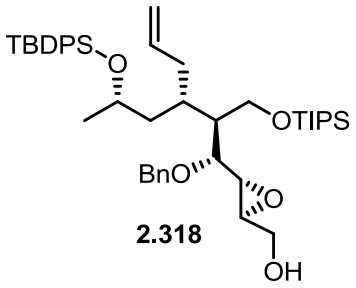

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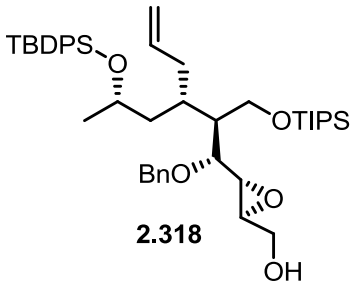
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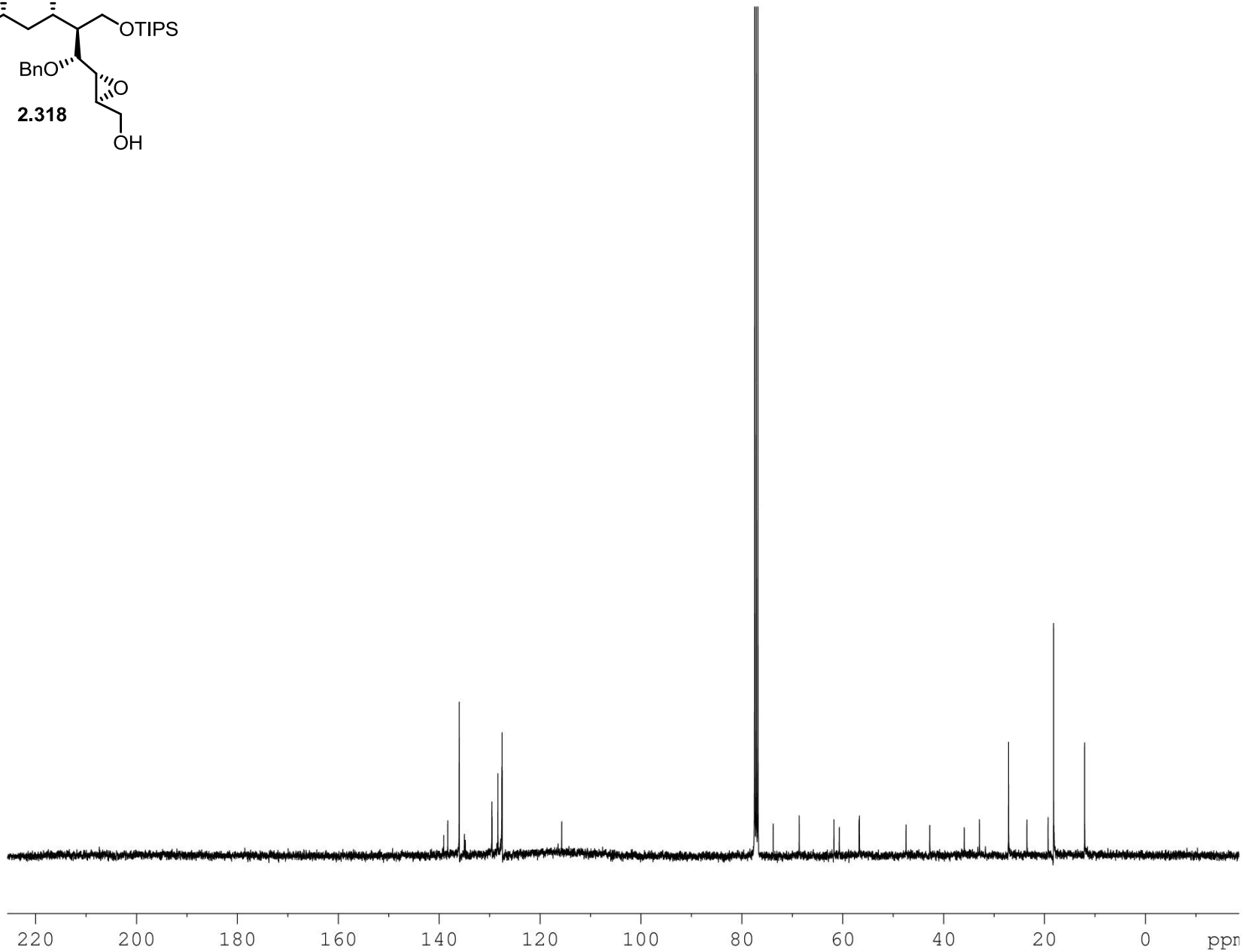
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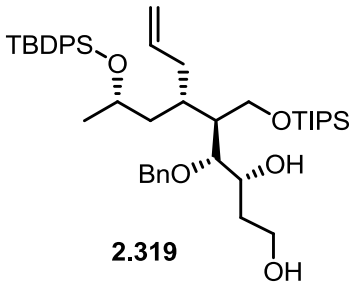

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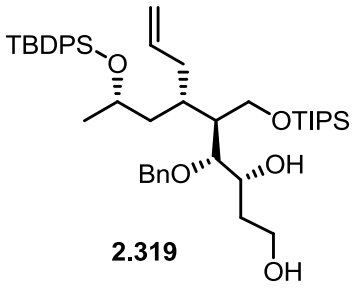
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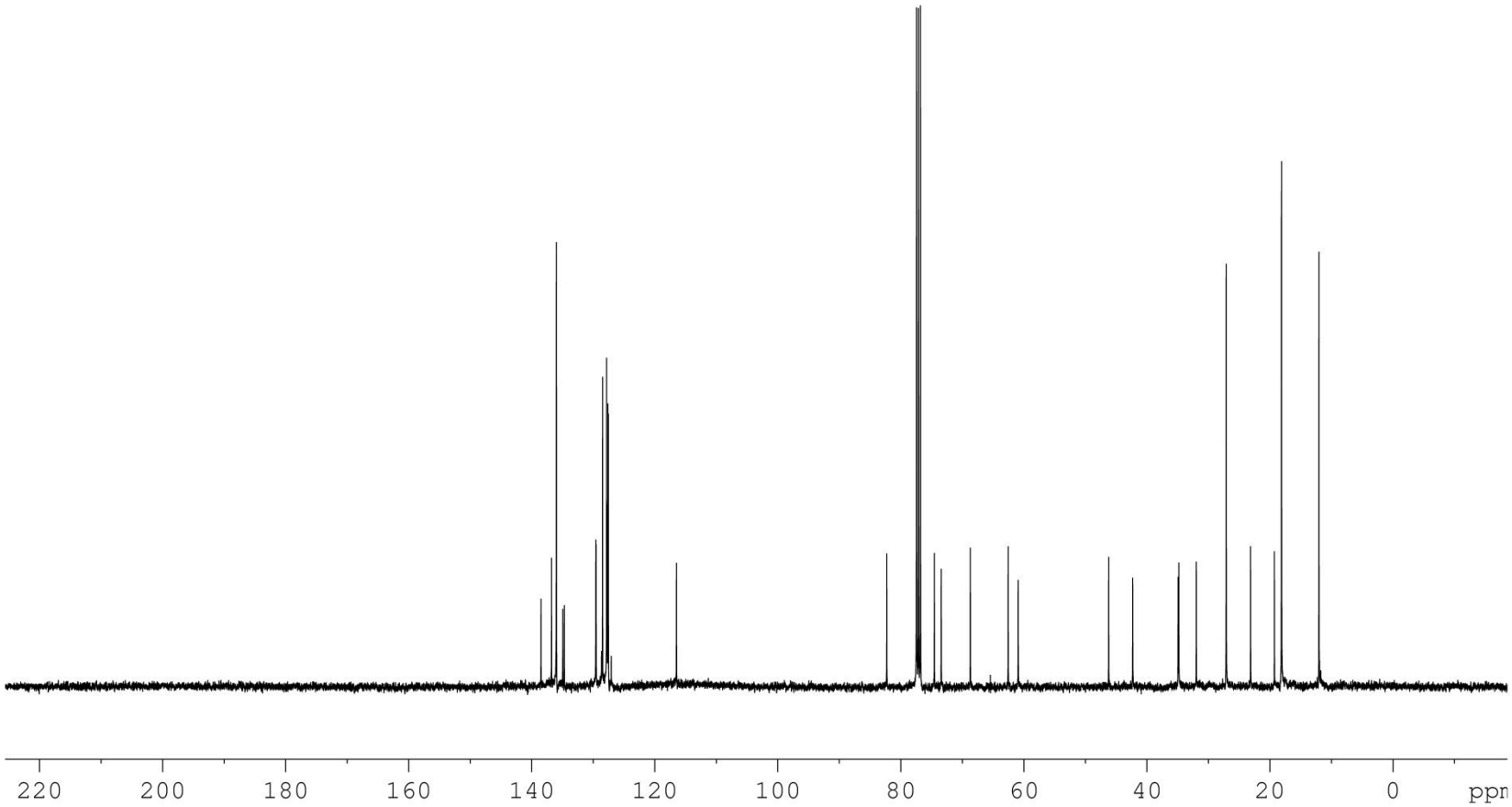
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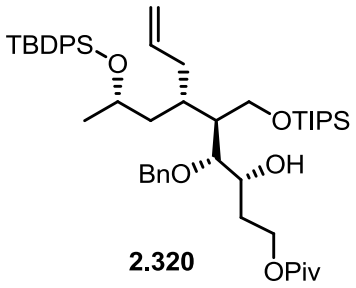

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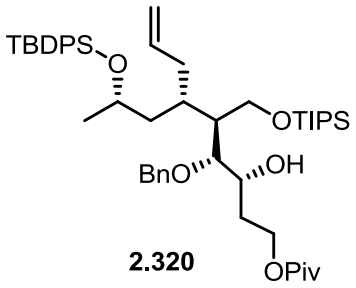
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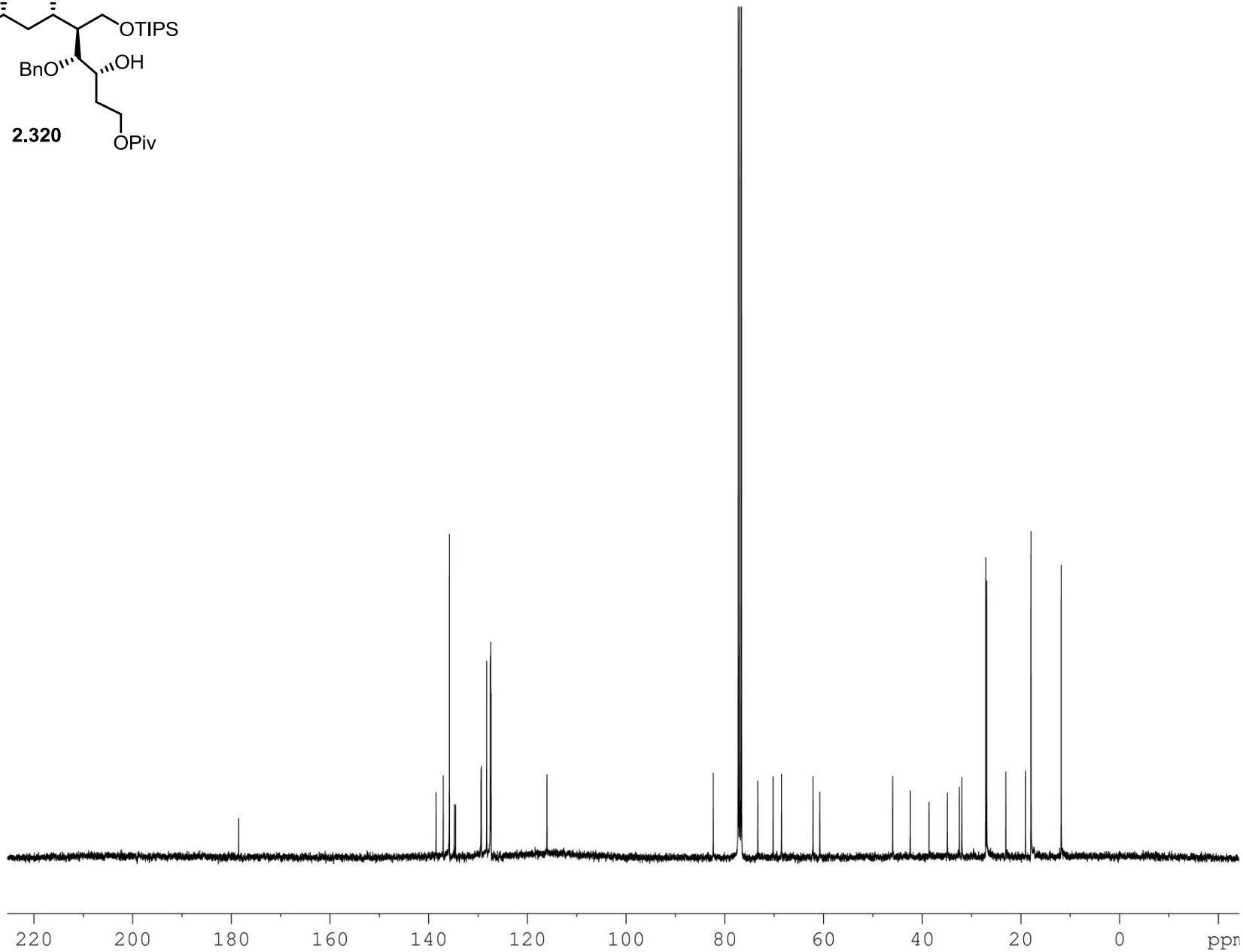
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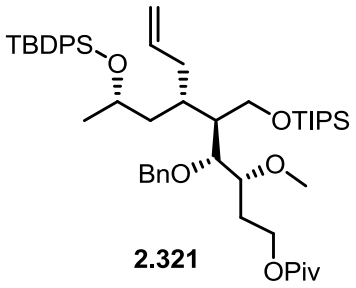

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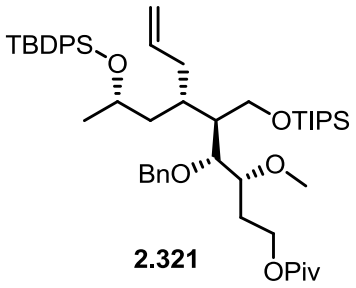
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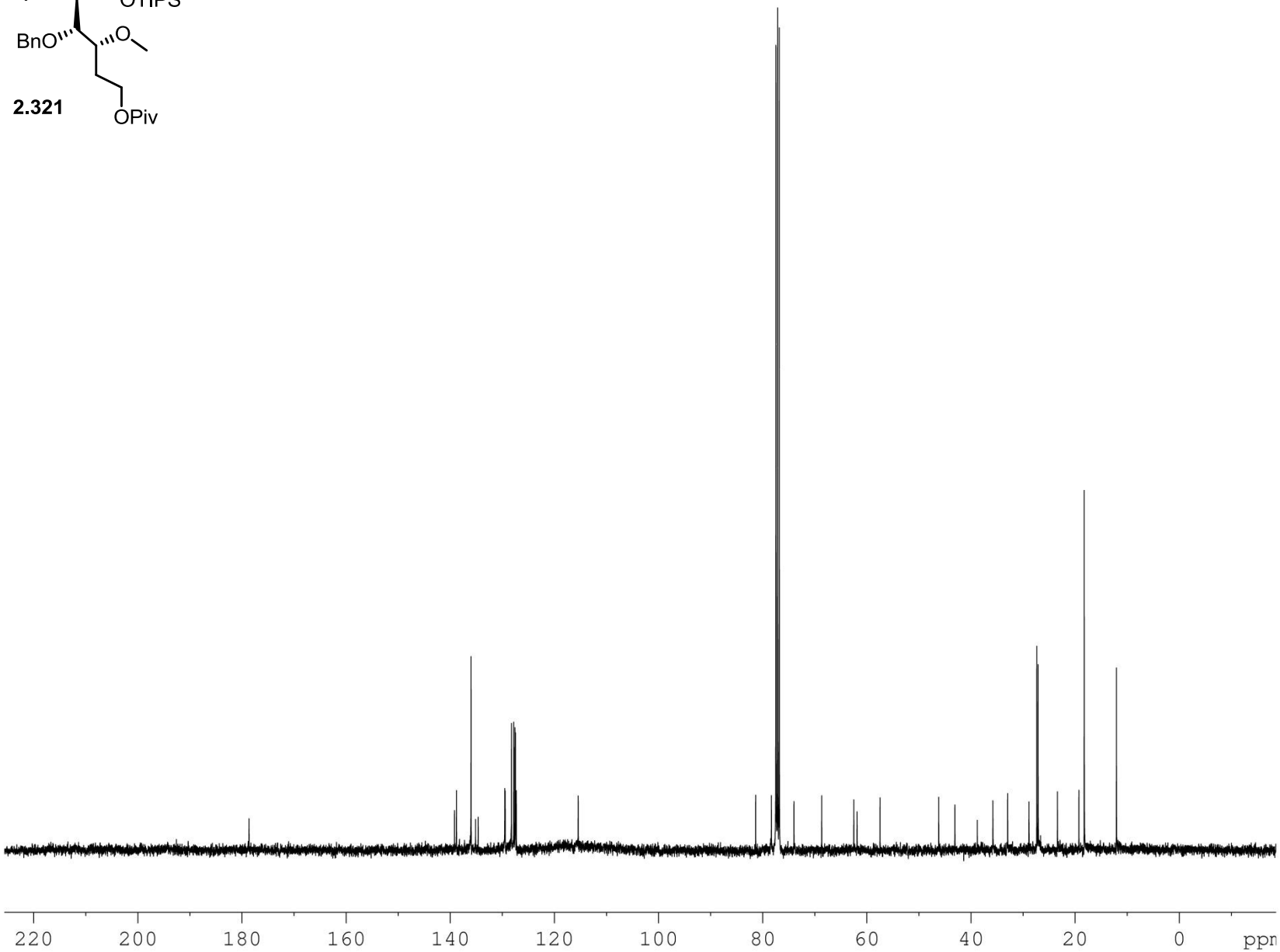
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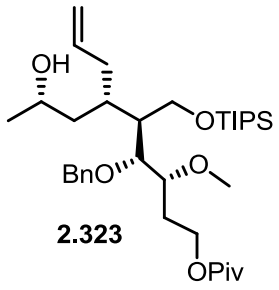

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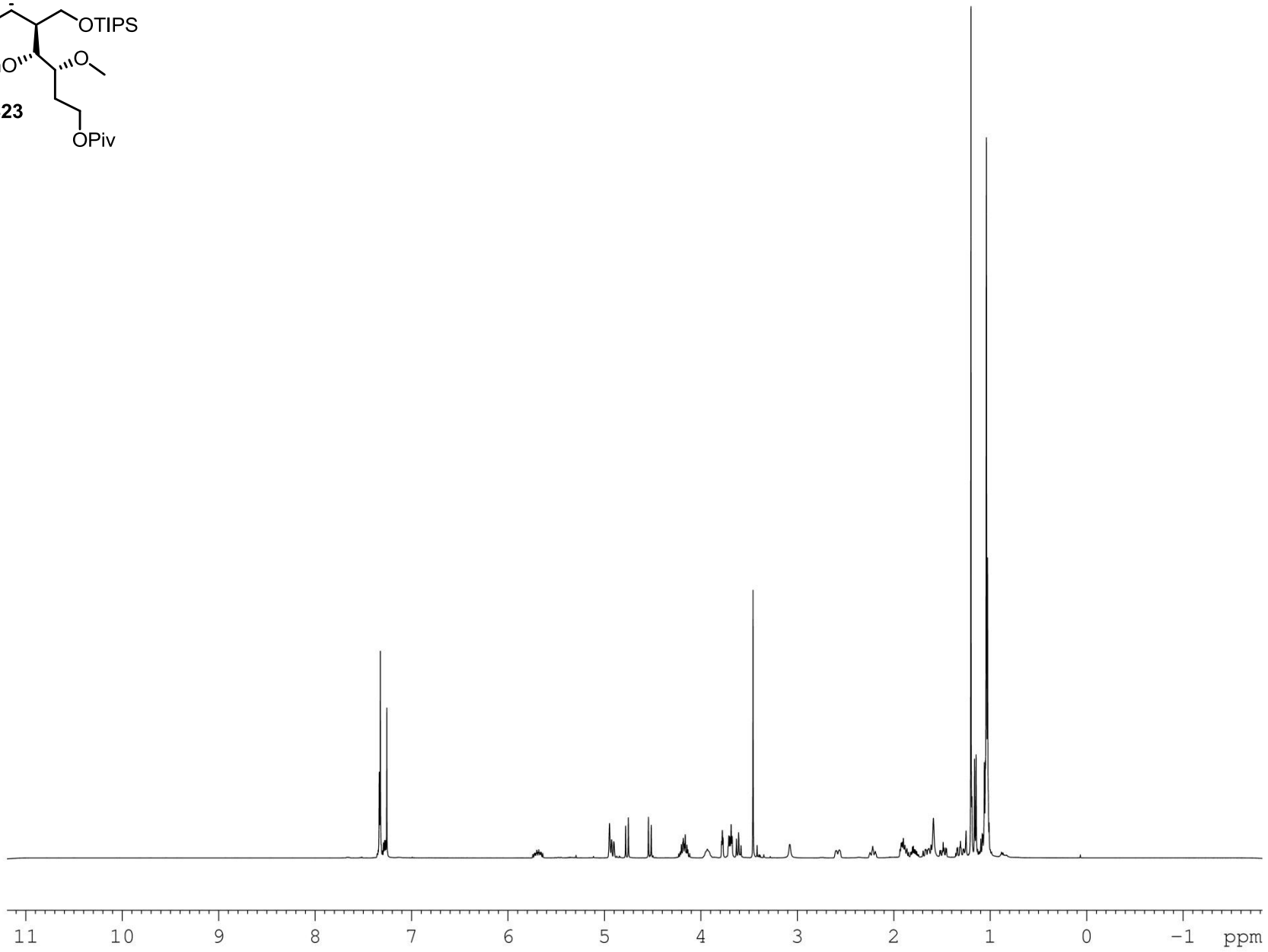

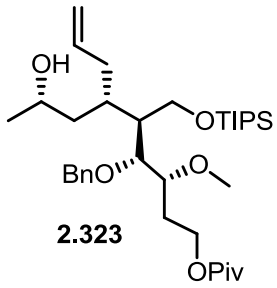

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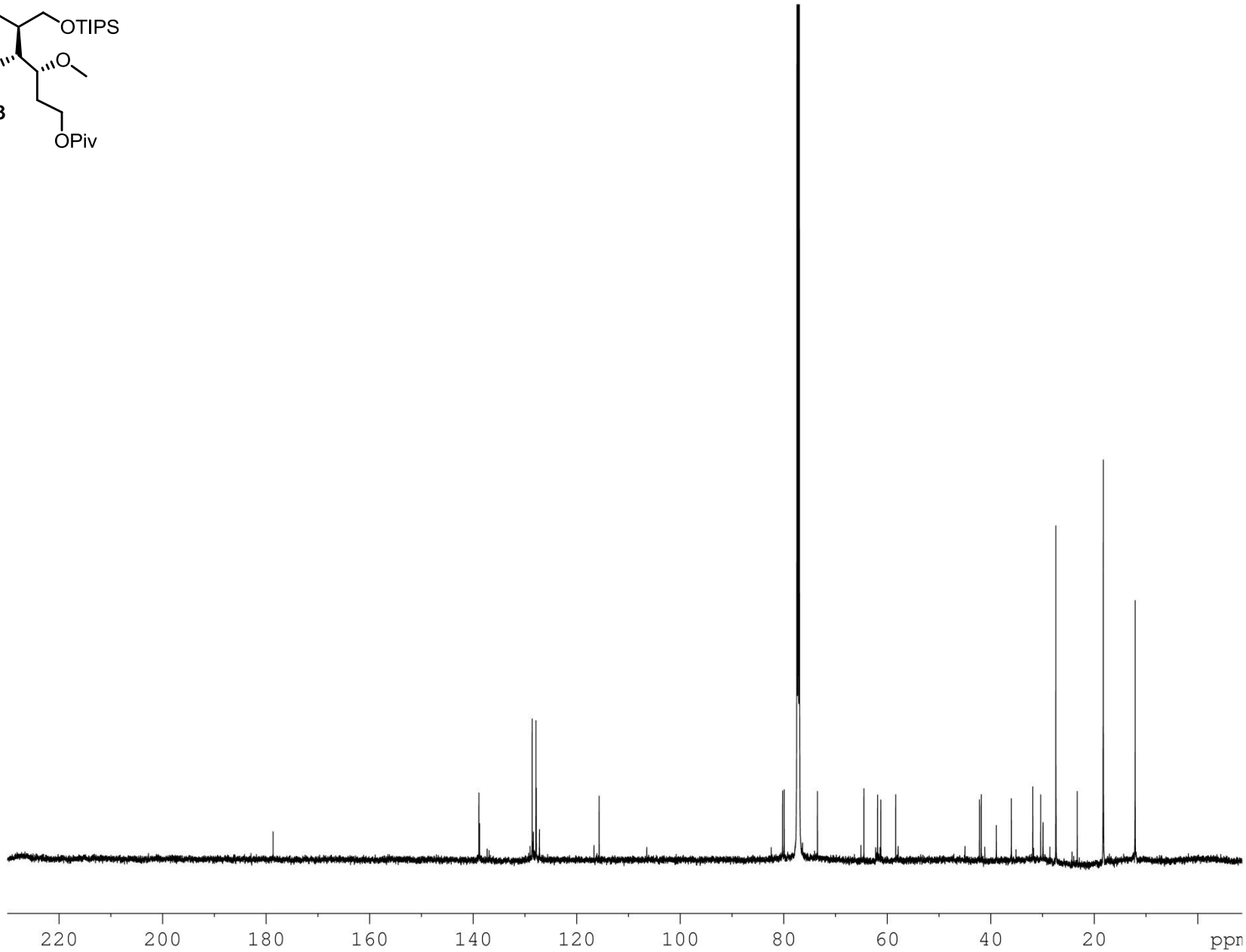

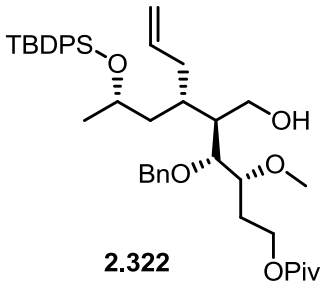

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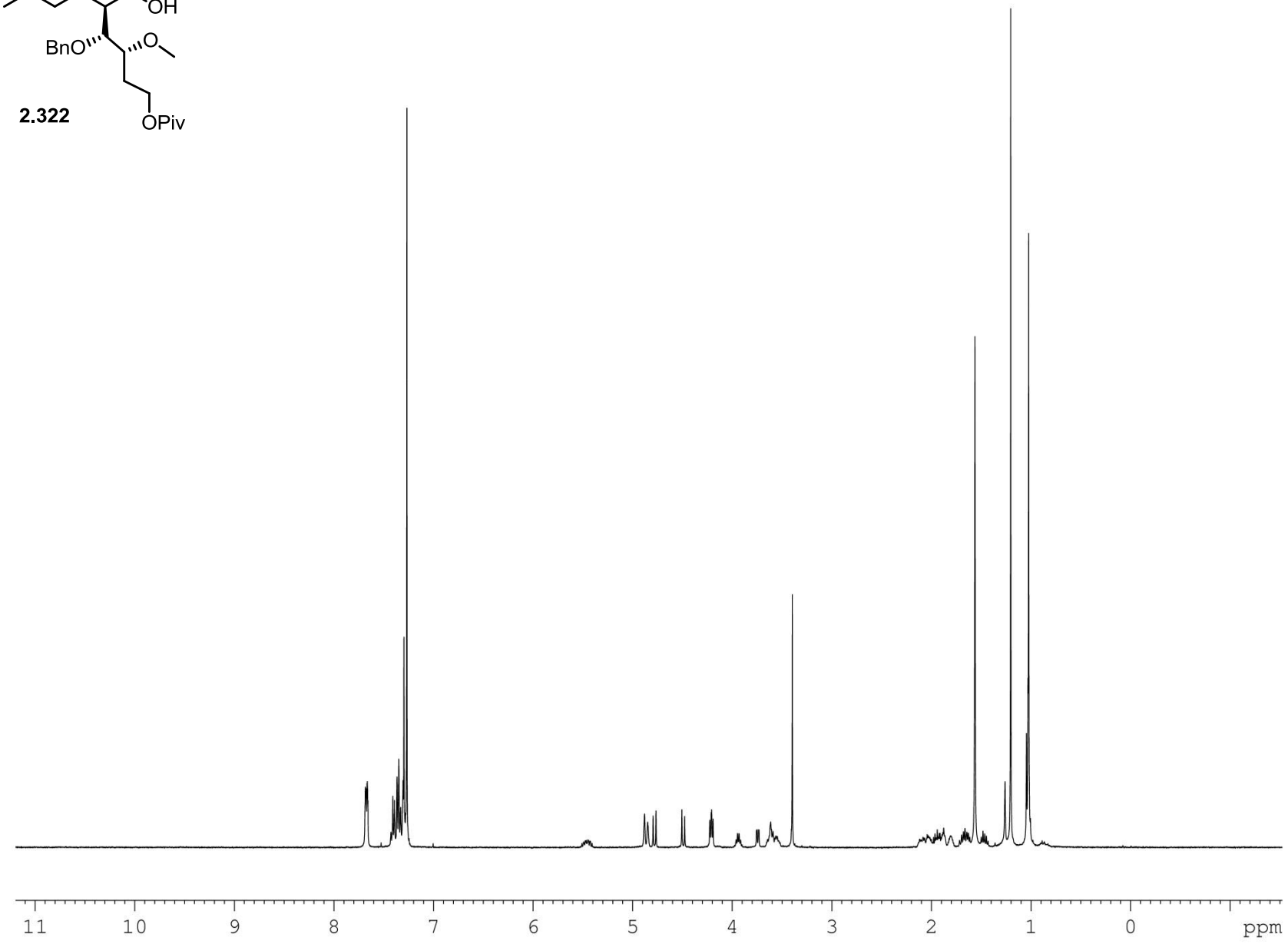

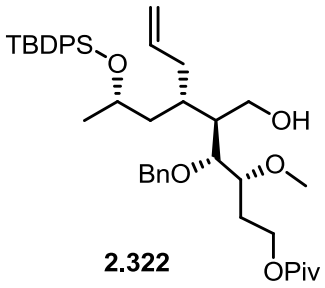

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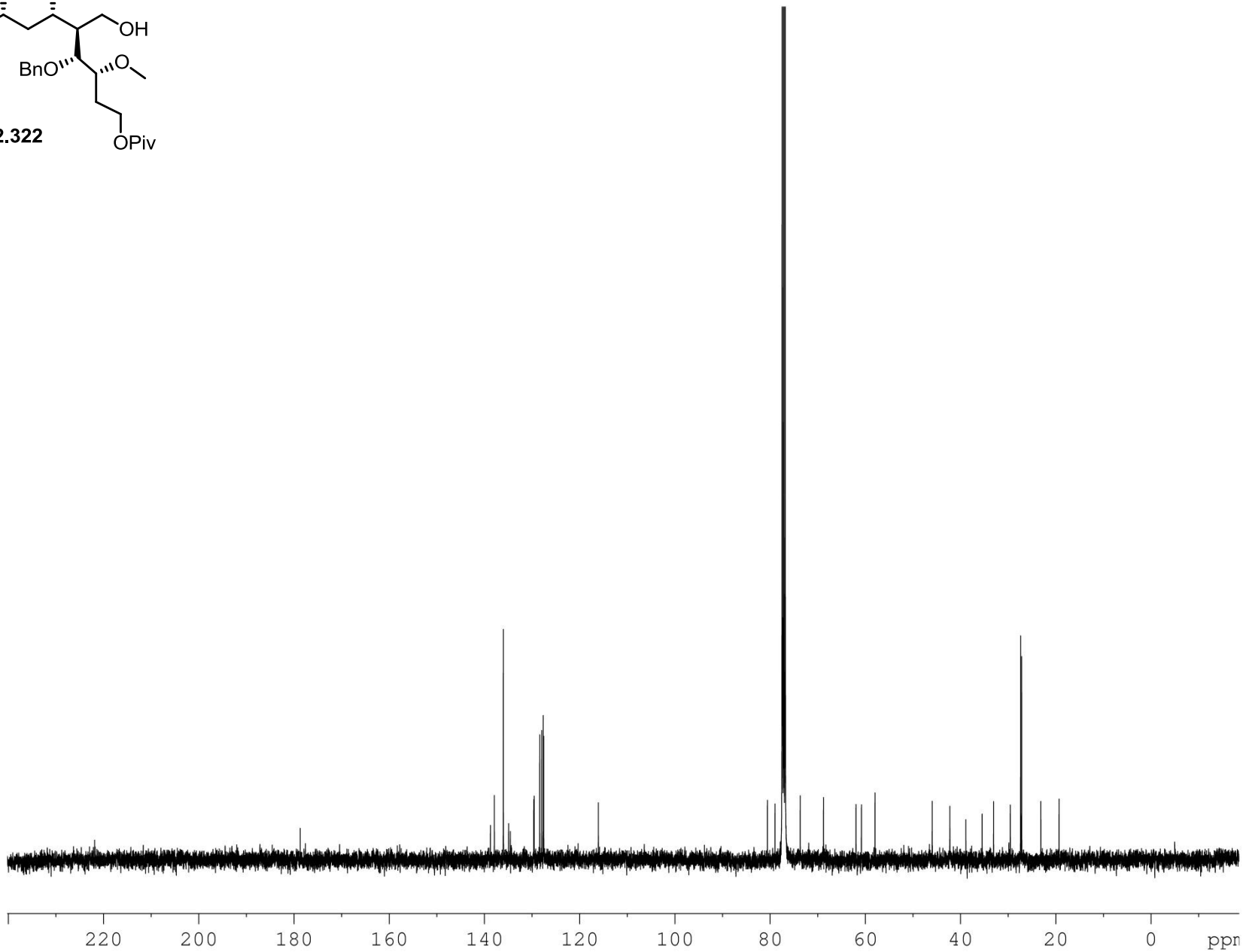

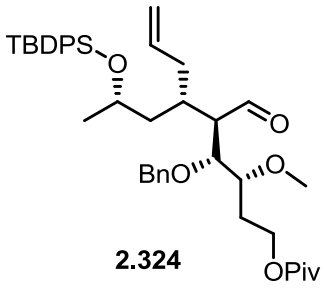

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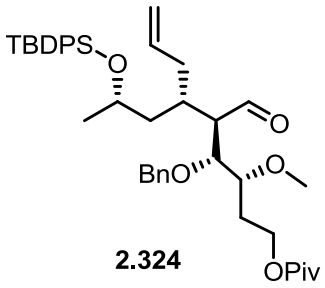

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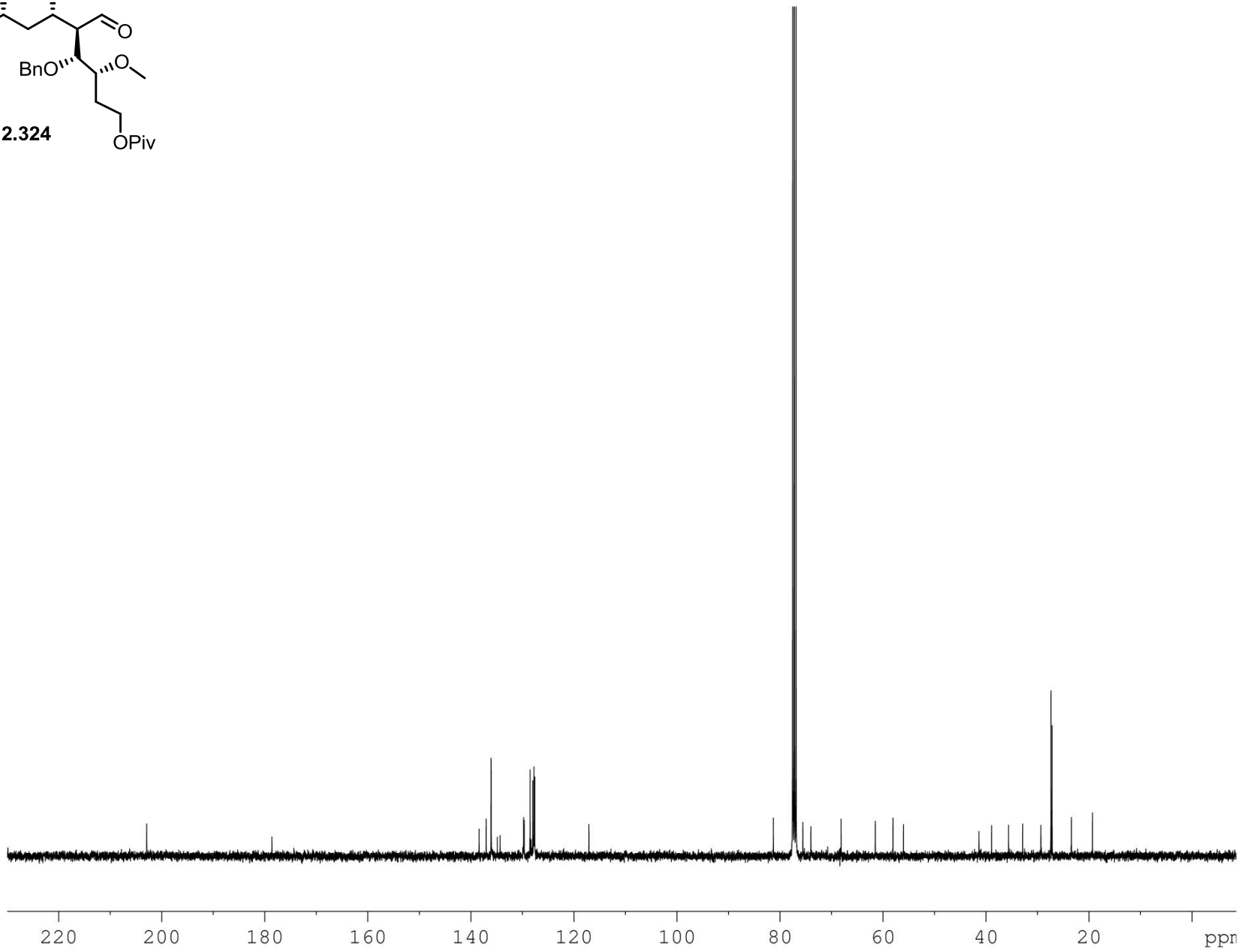

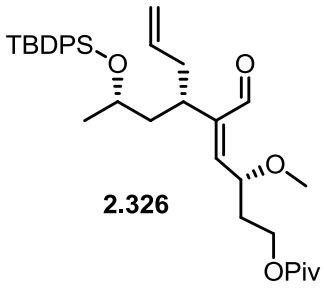

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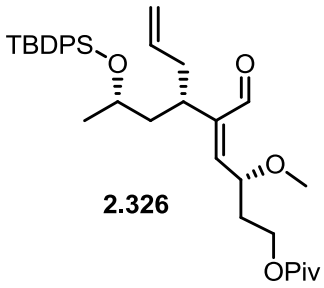

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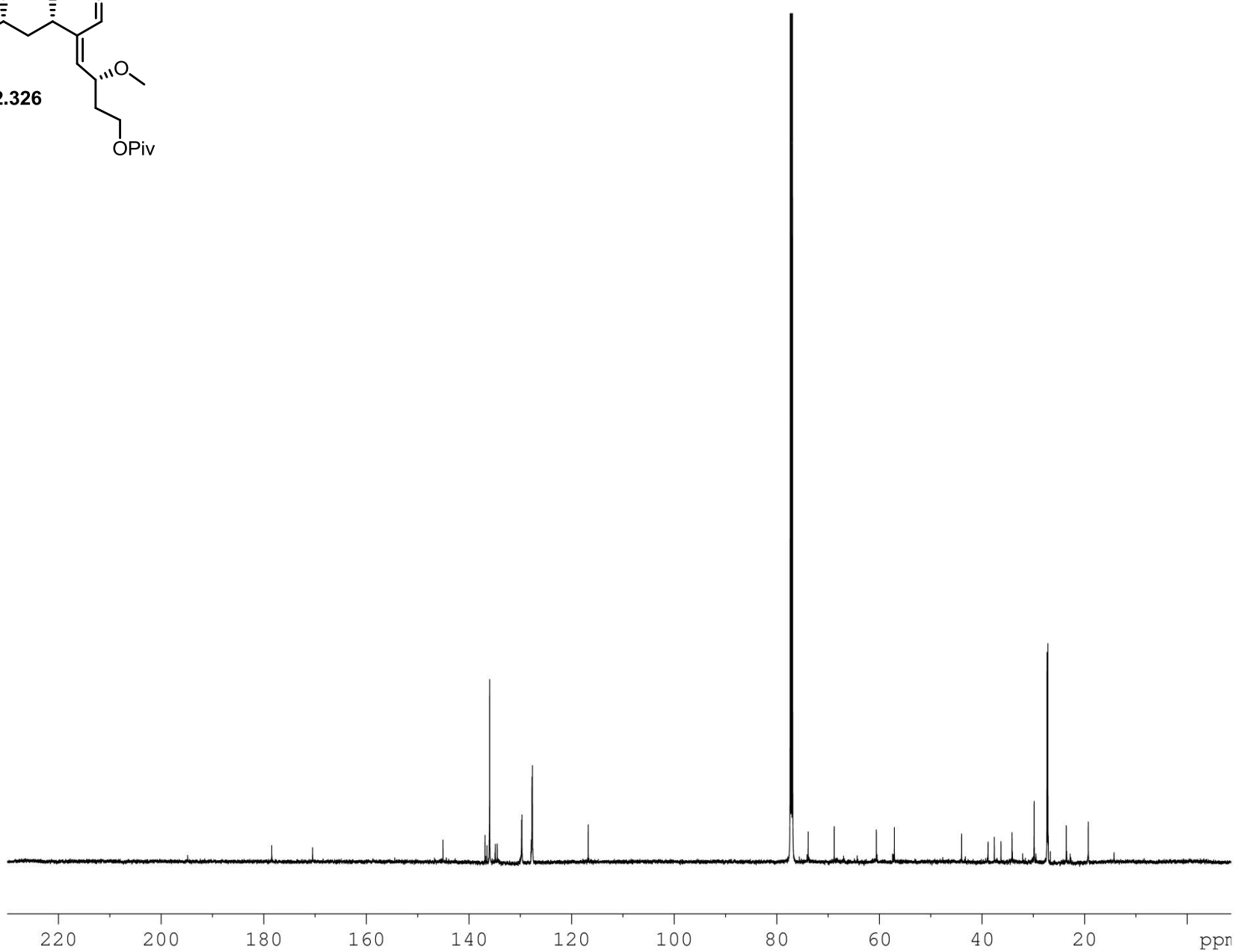

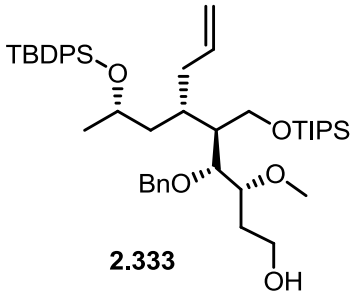

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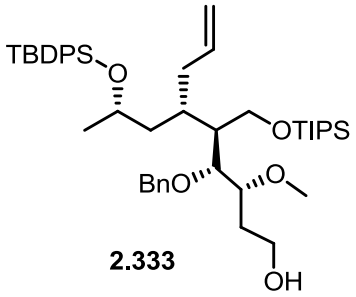
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OH

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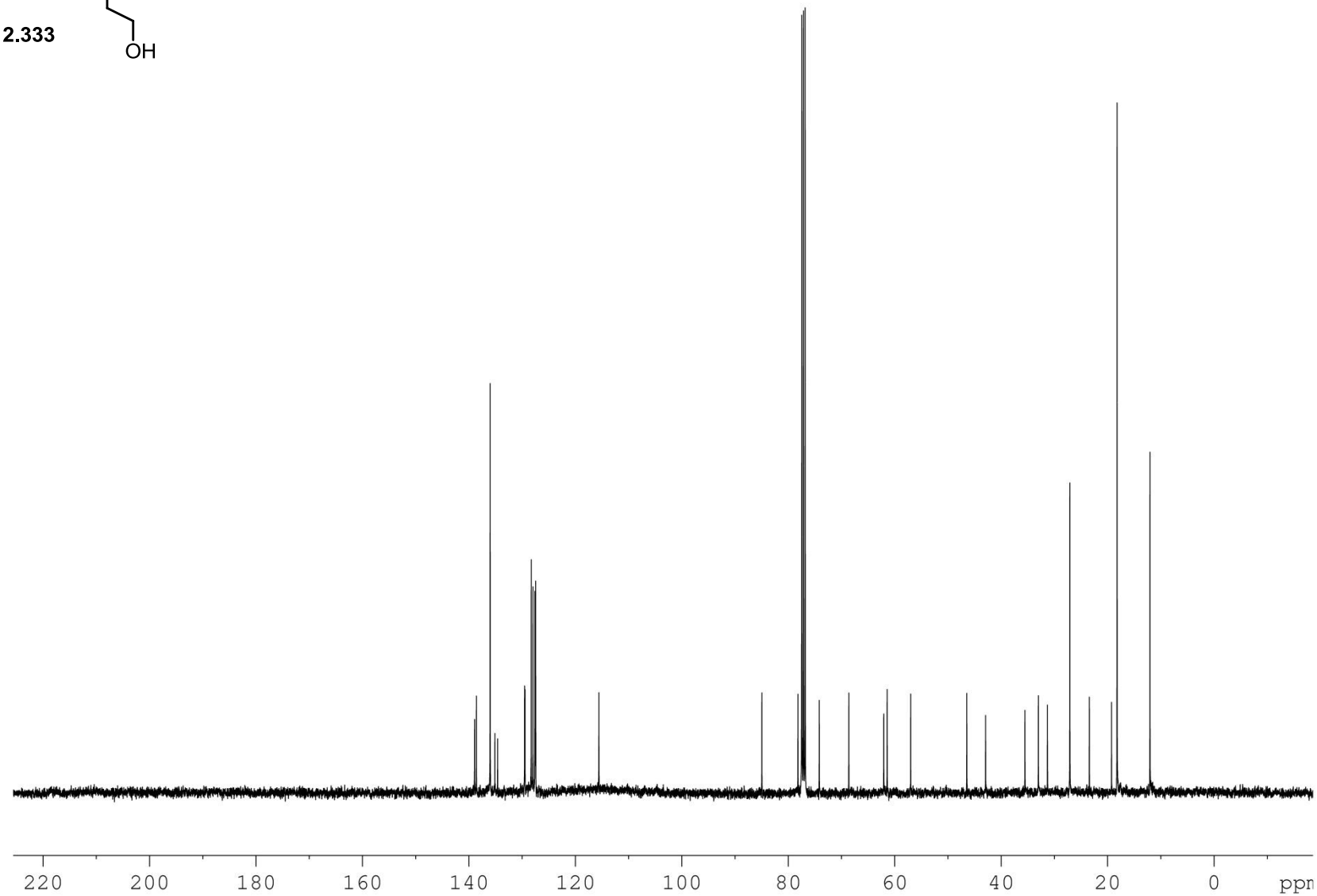
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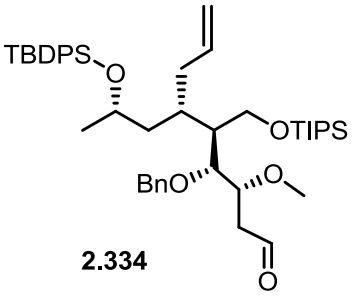

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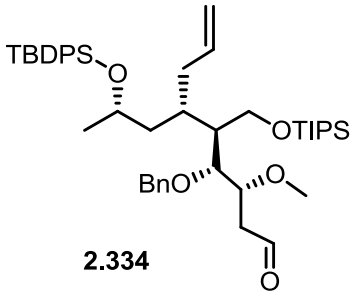

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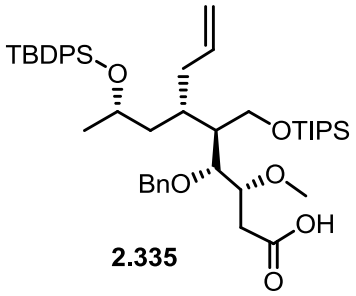

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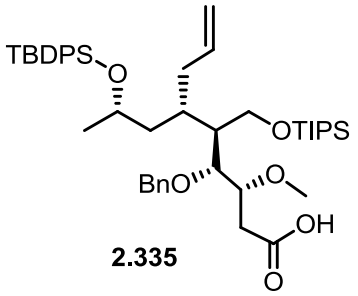
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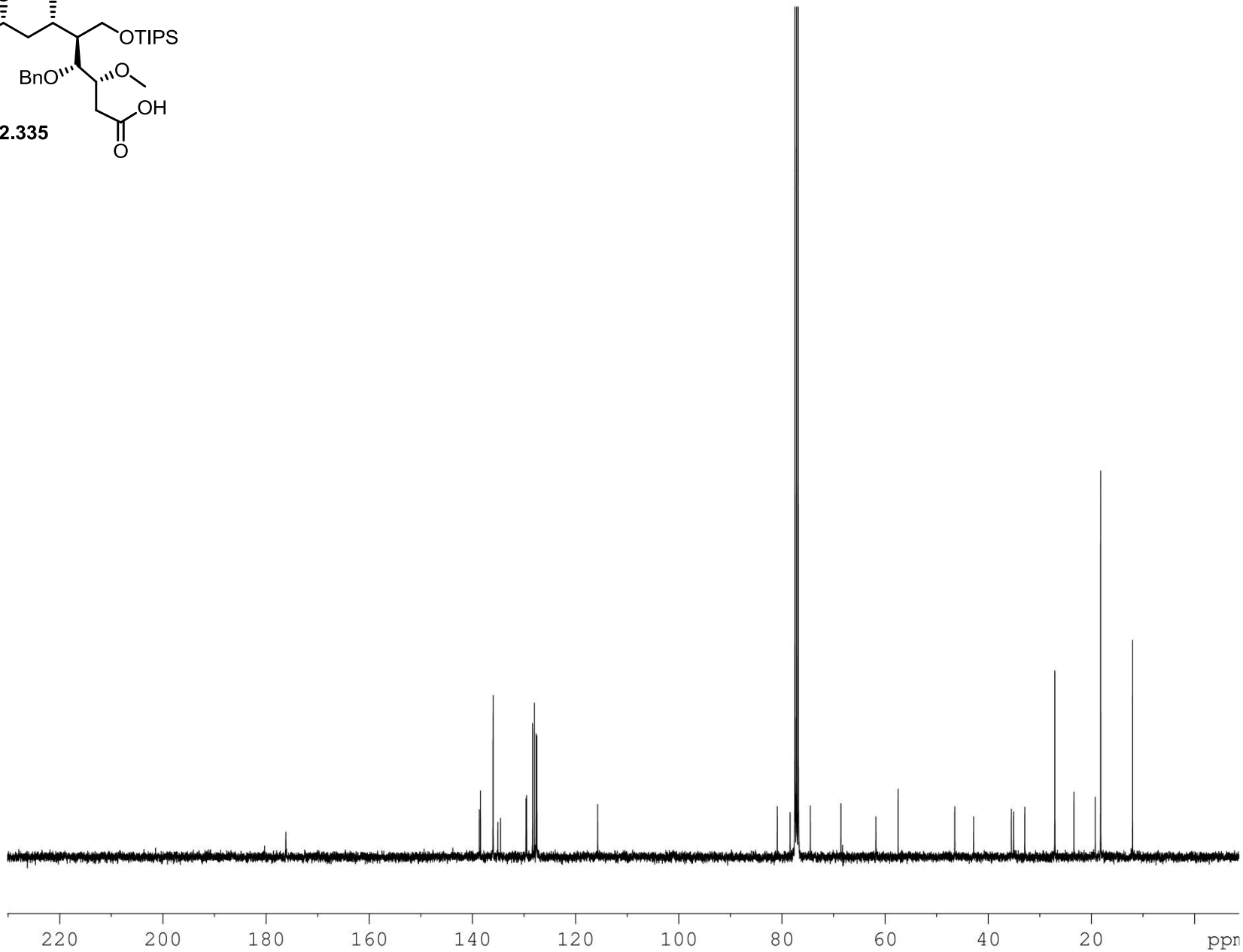
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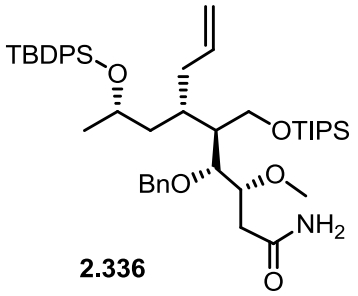

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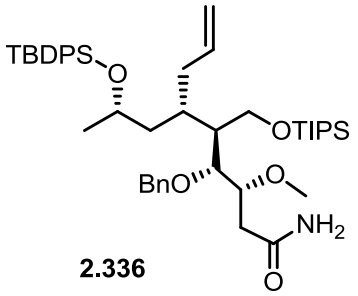
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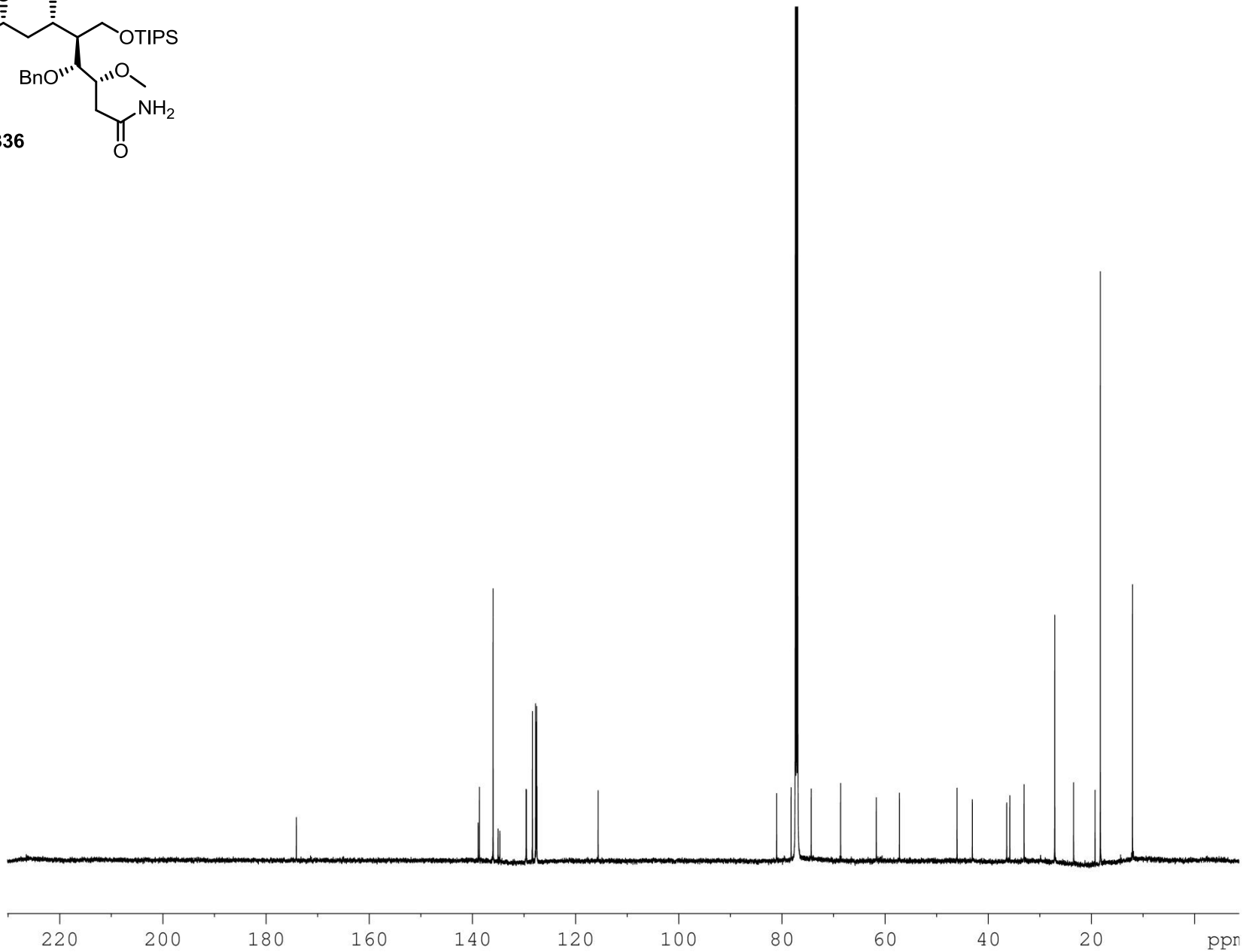
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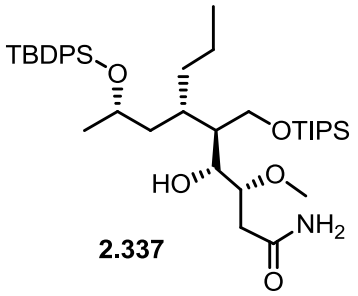

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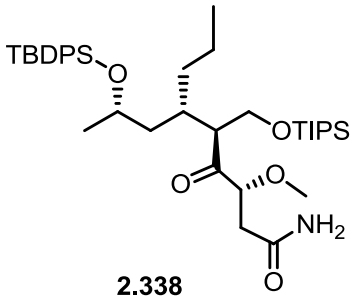

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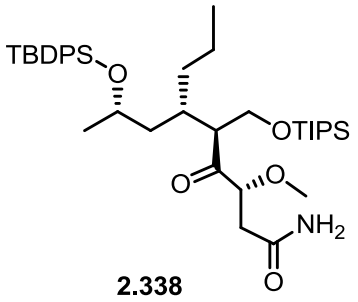

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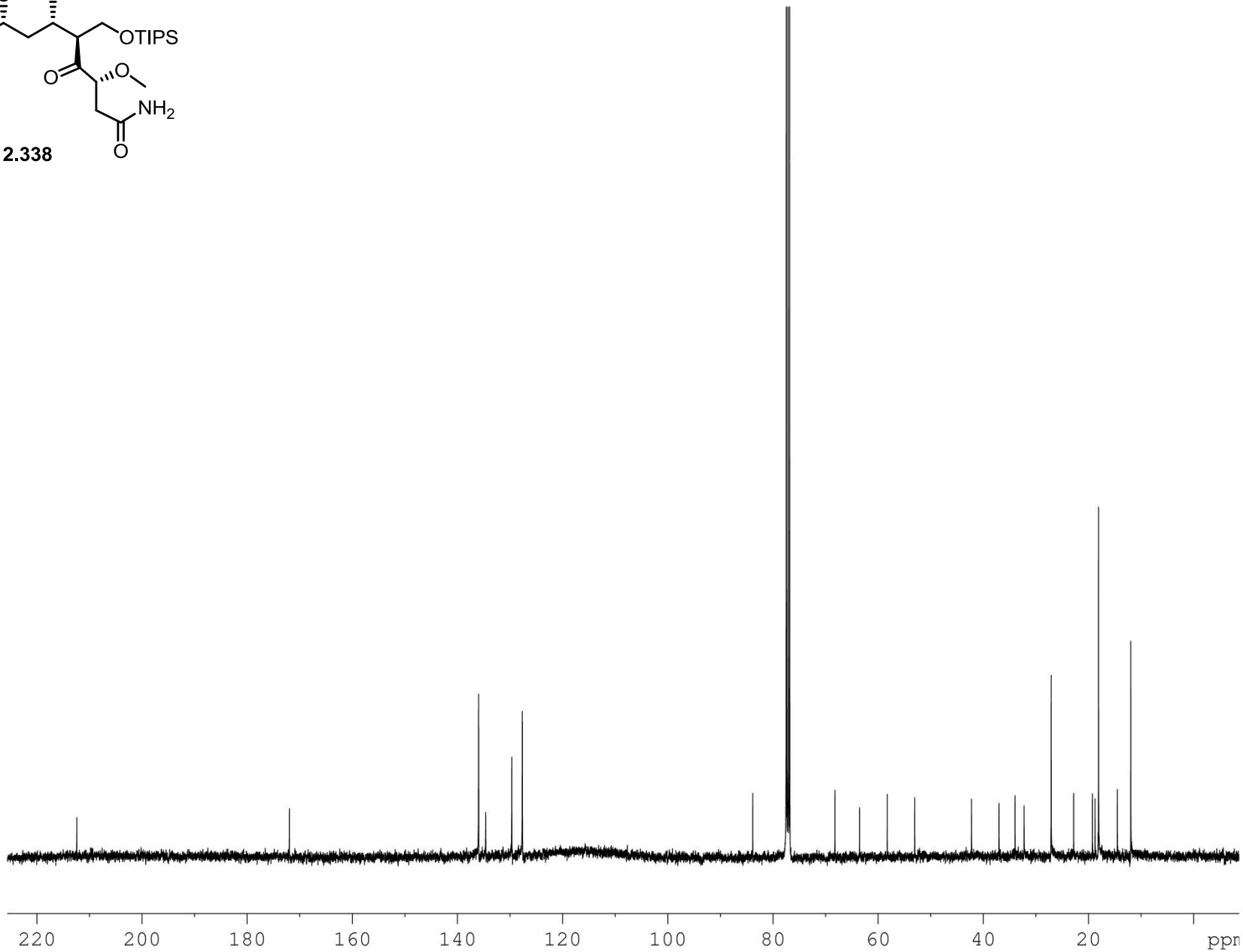

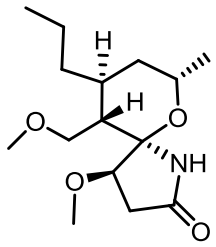

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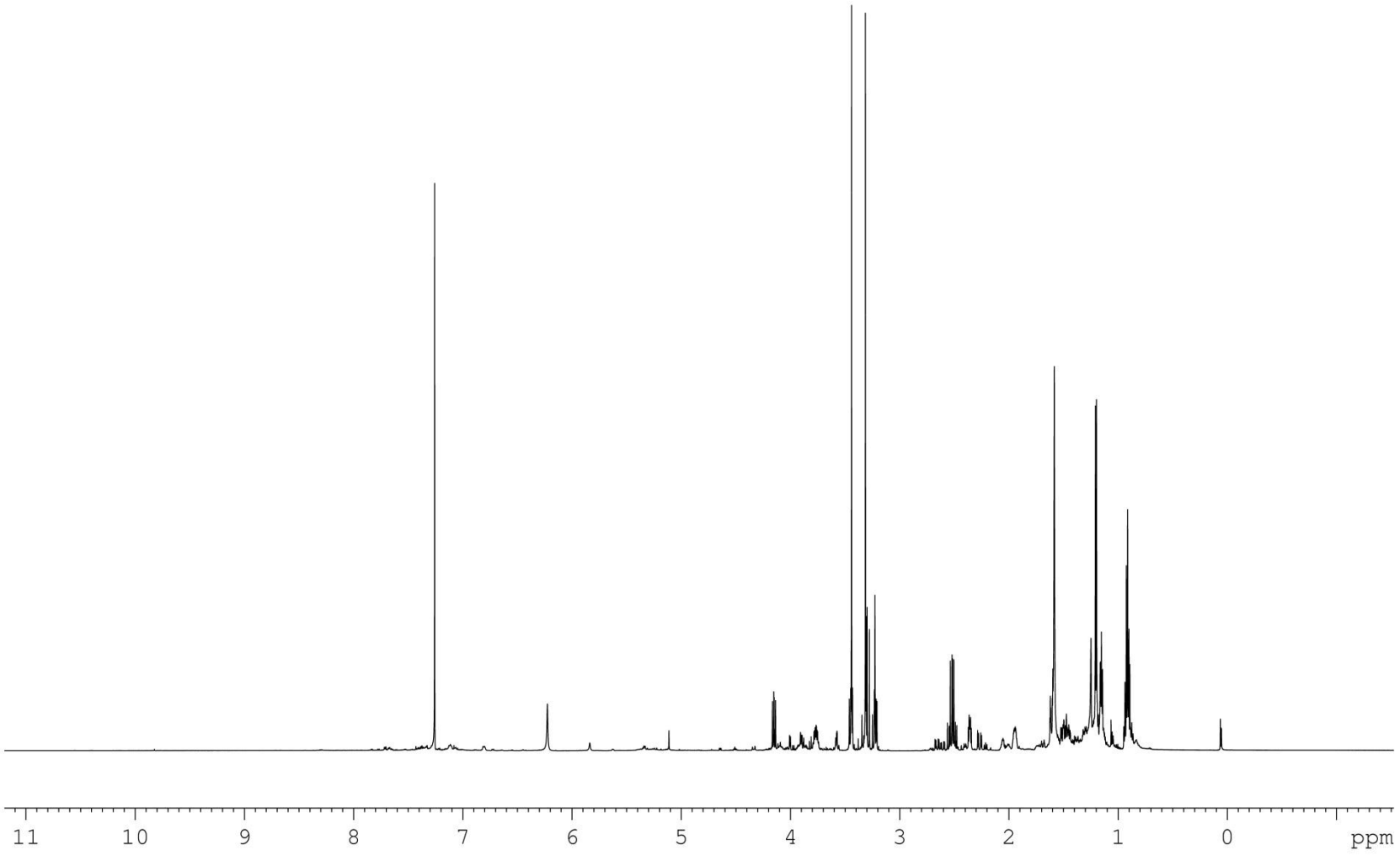

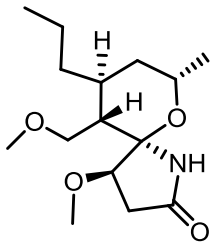

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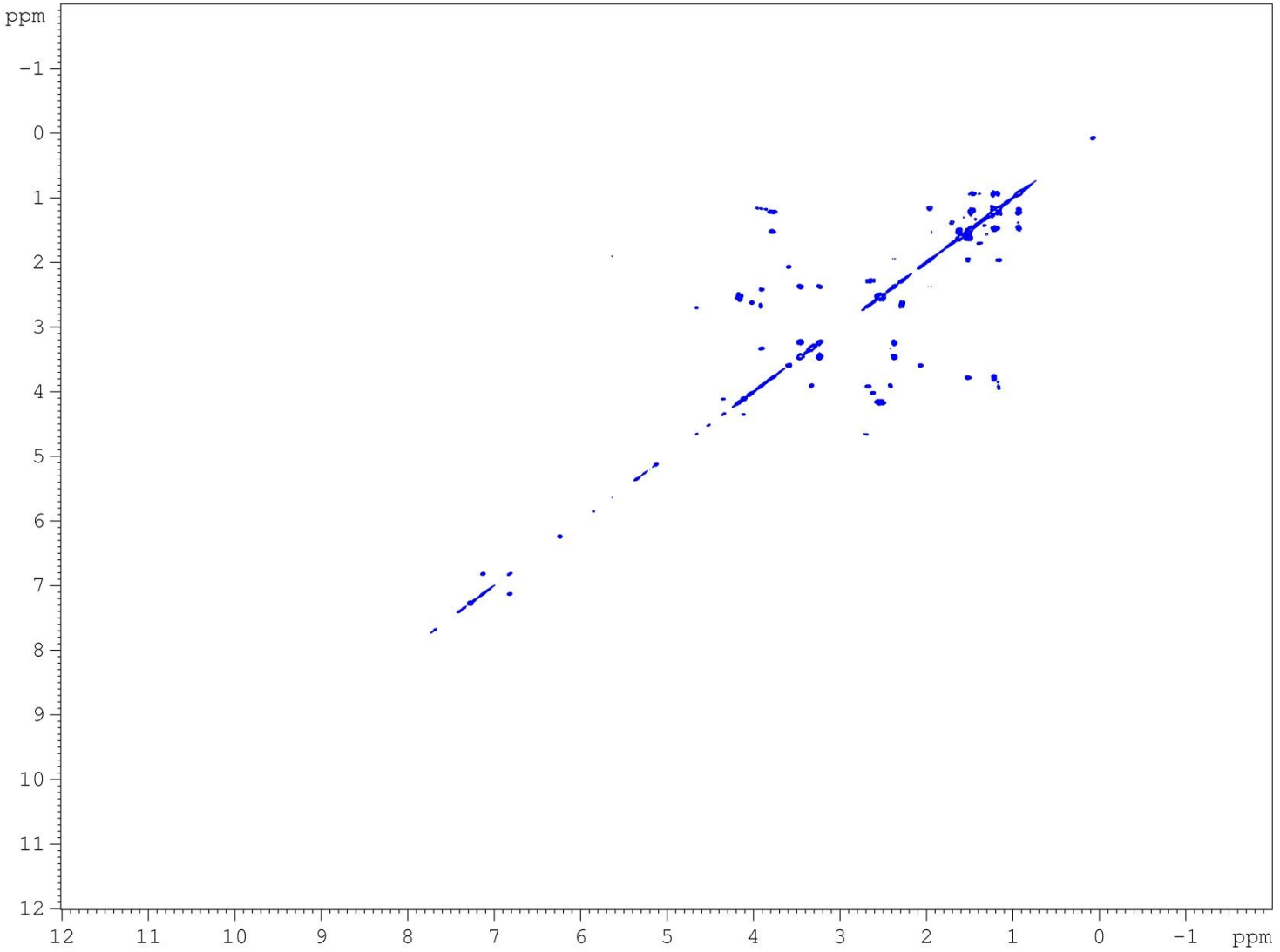

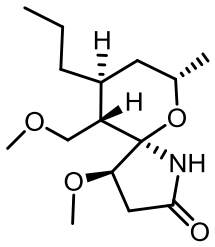

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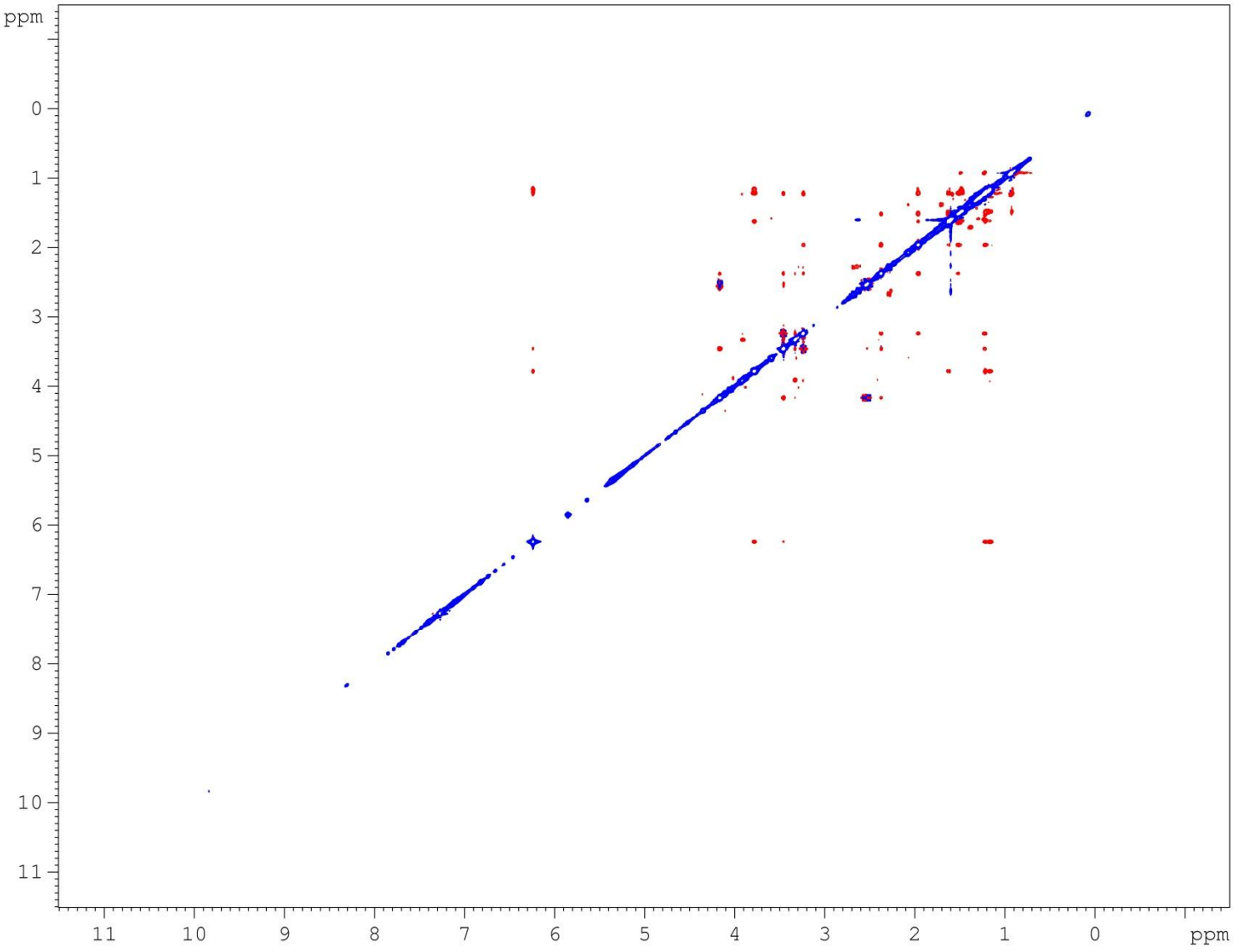

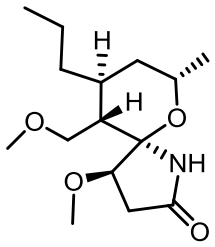
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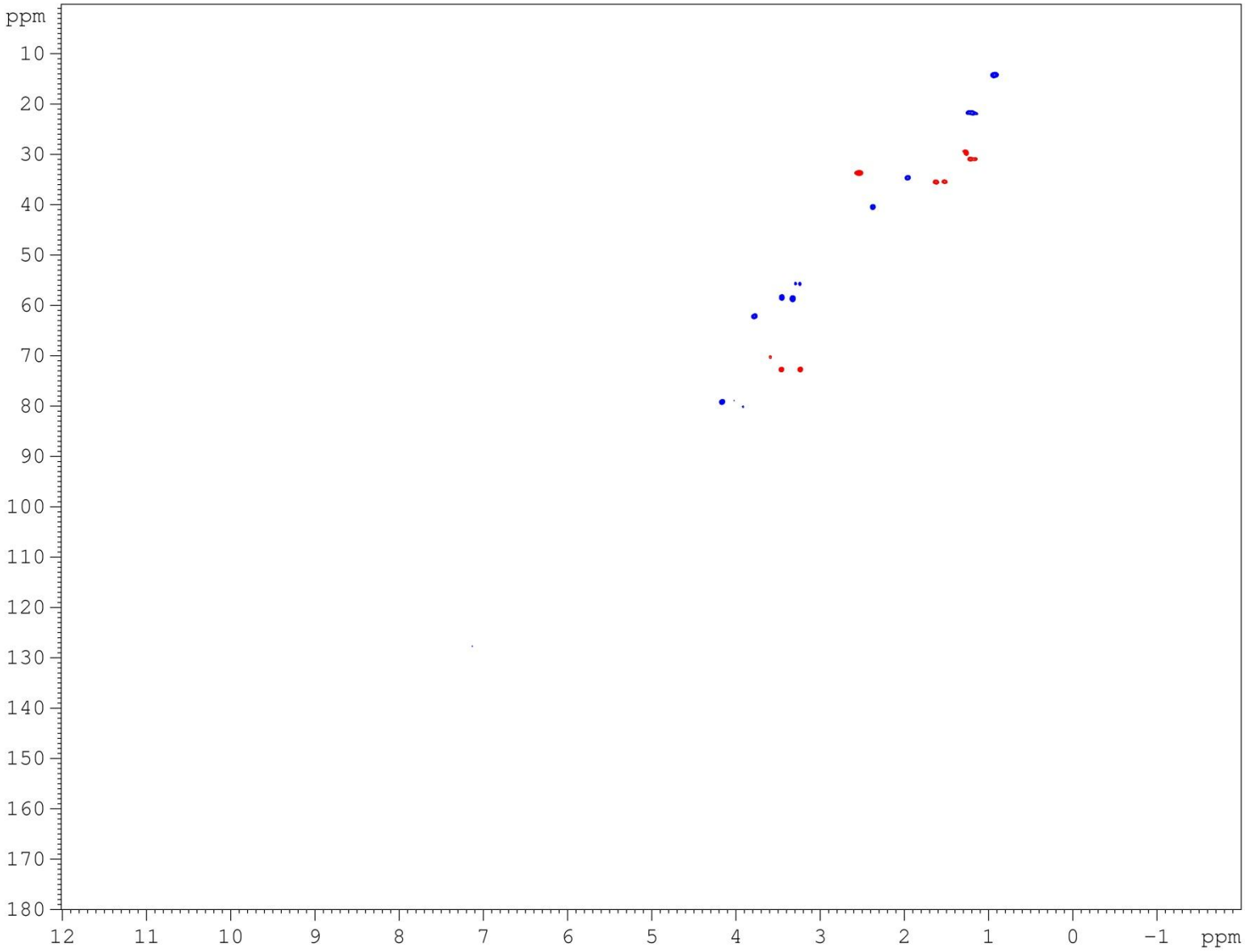
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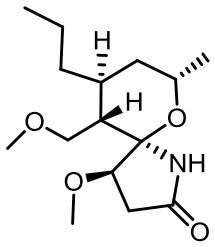
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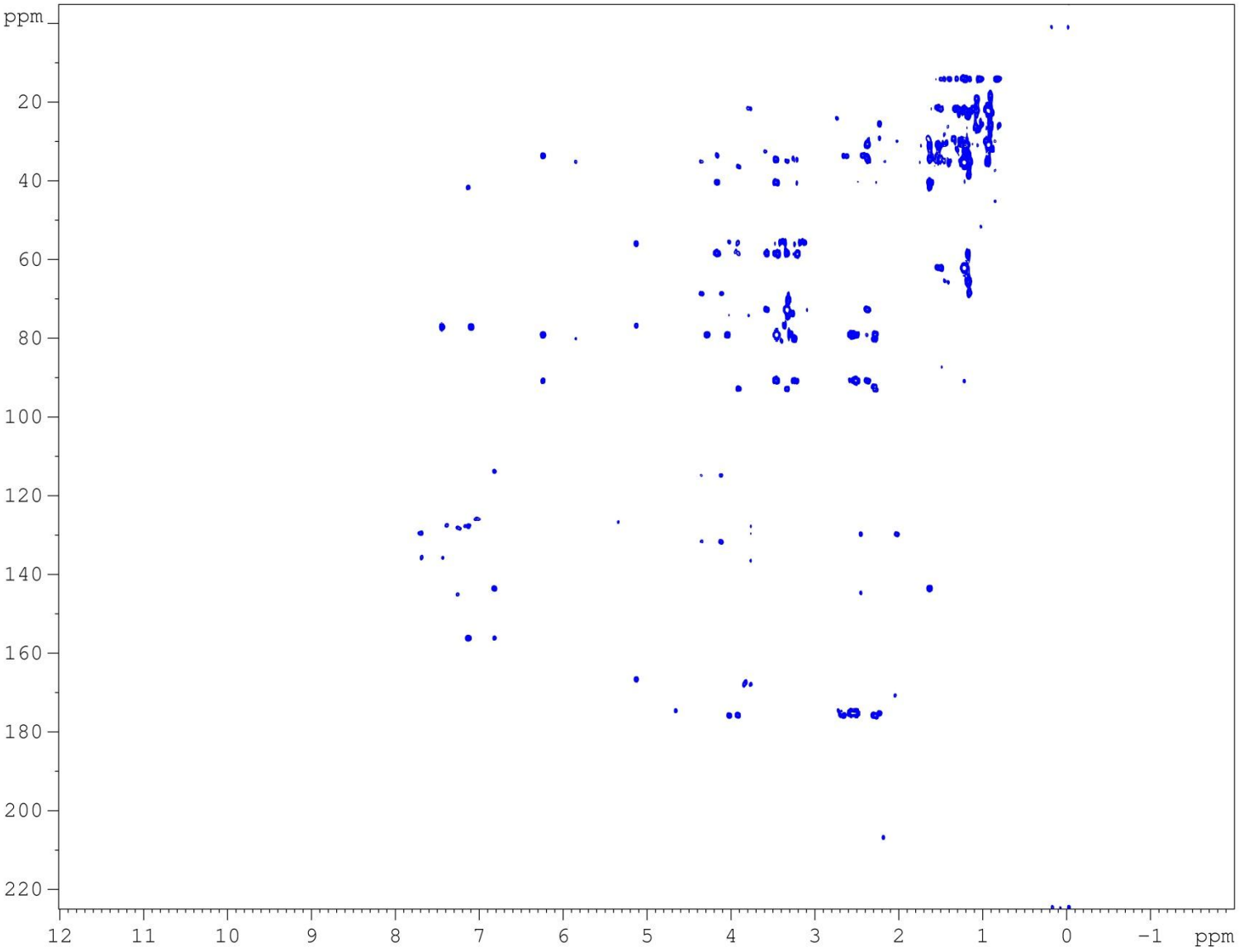
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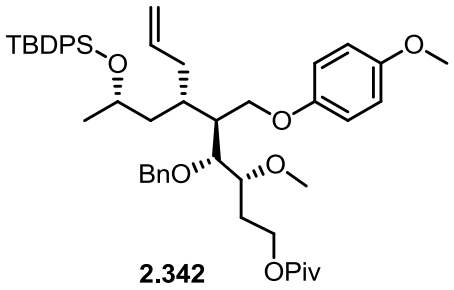

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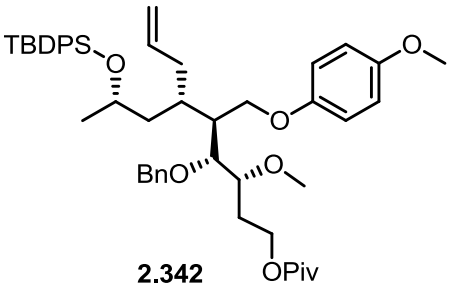

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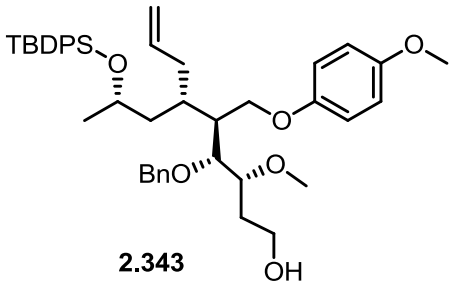

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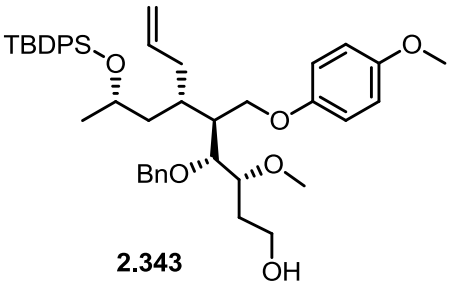

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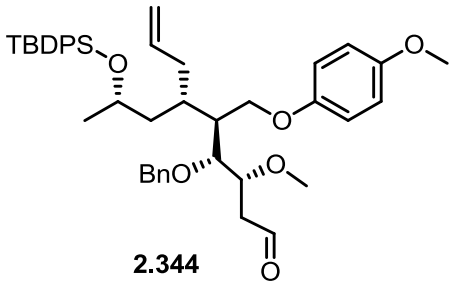

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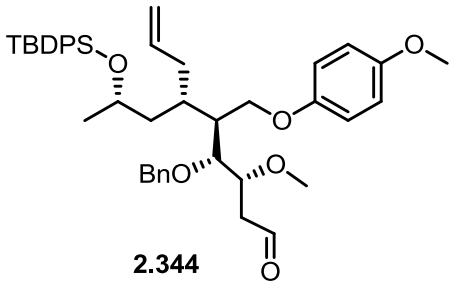

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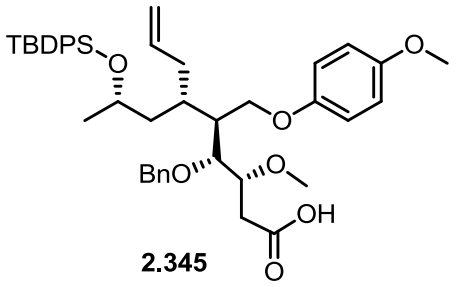

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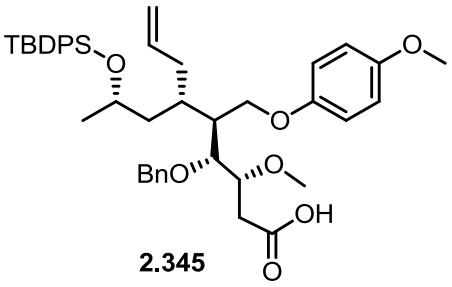

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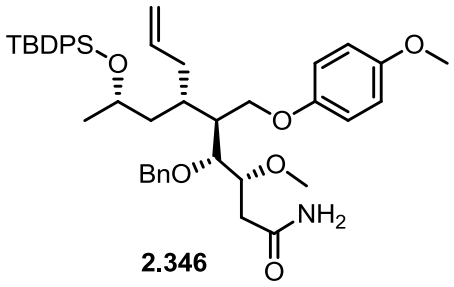

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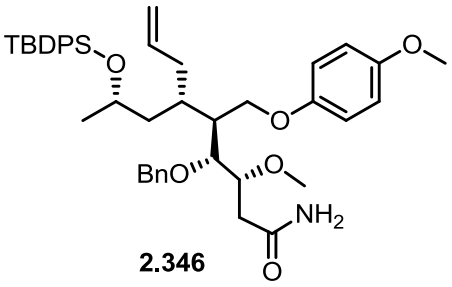

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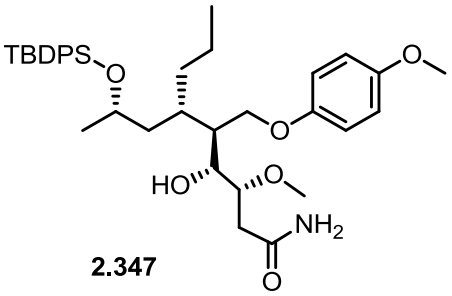

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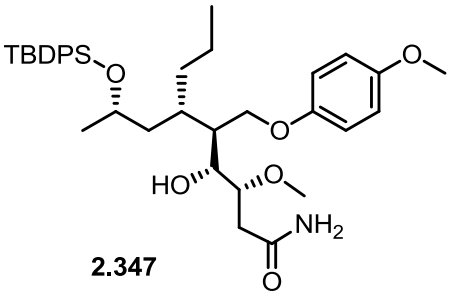

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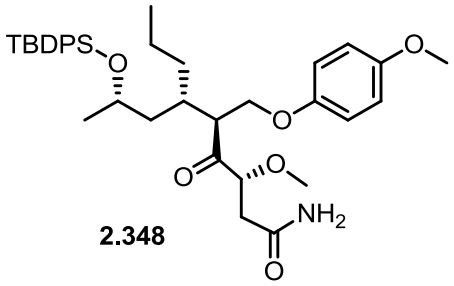

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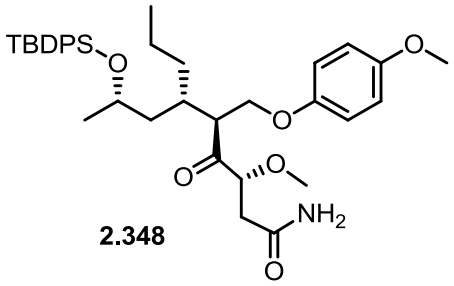

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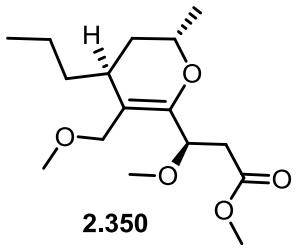

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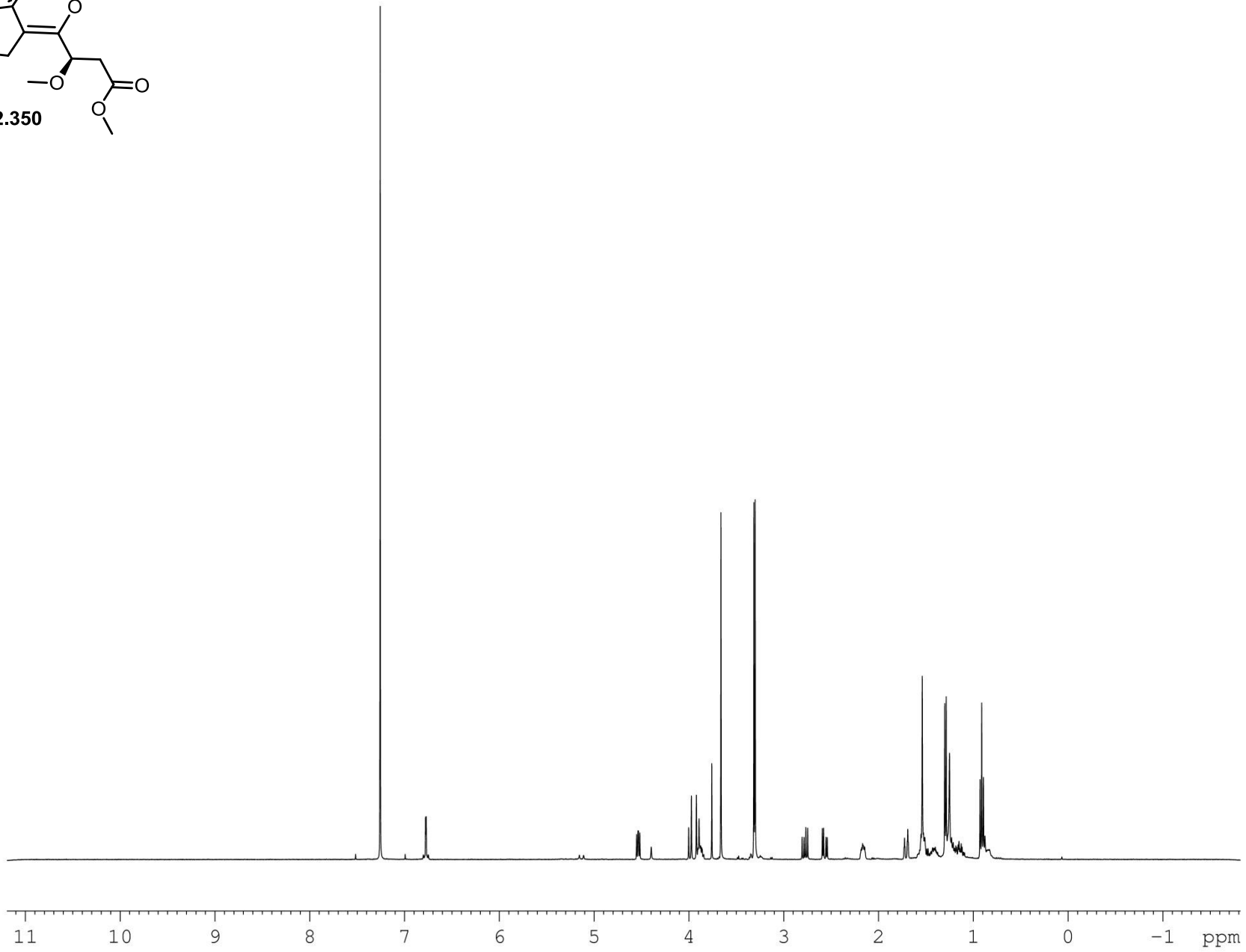

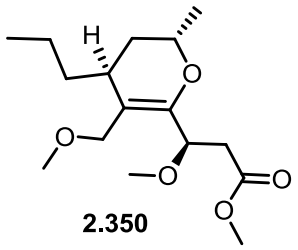

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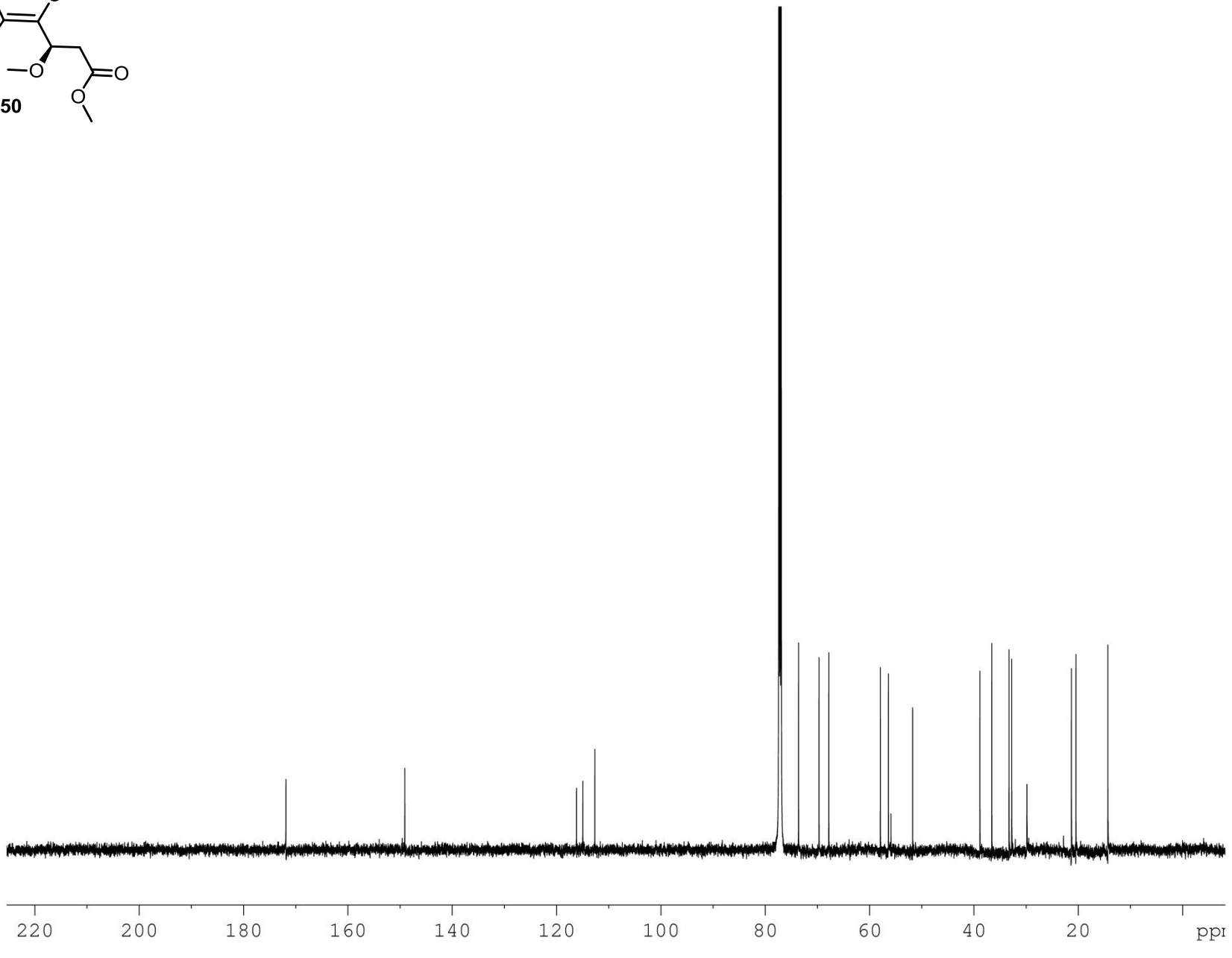

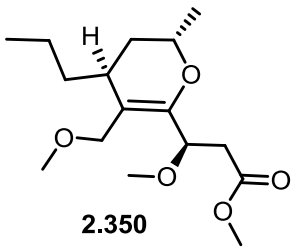

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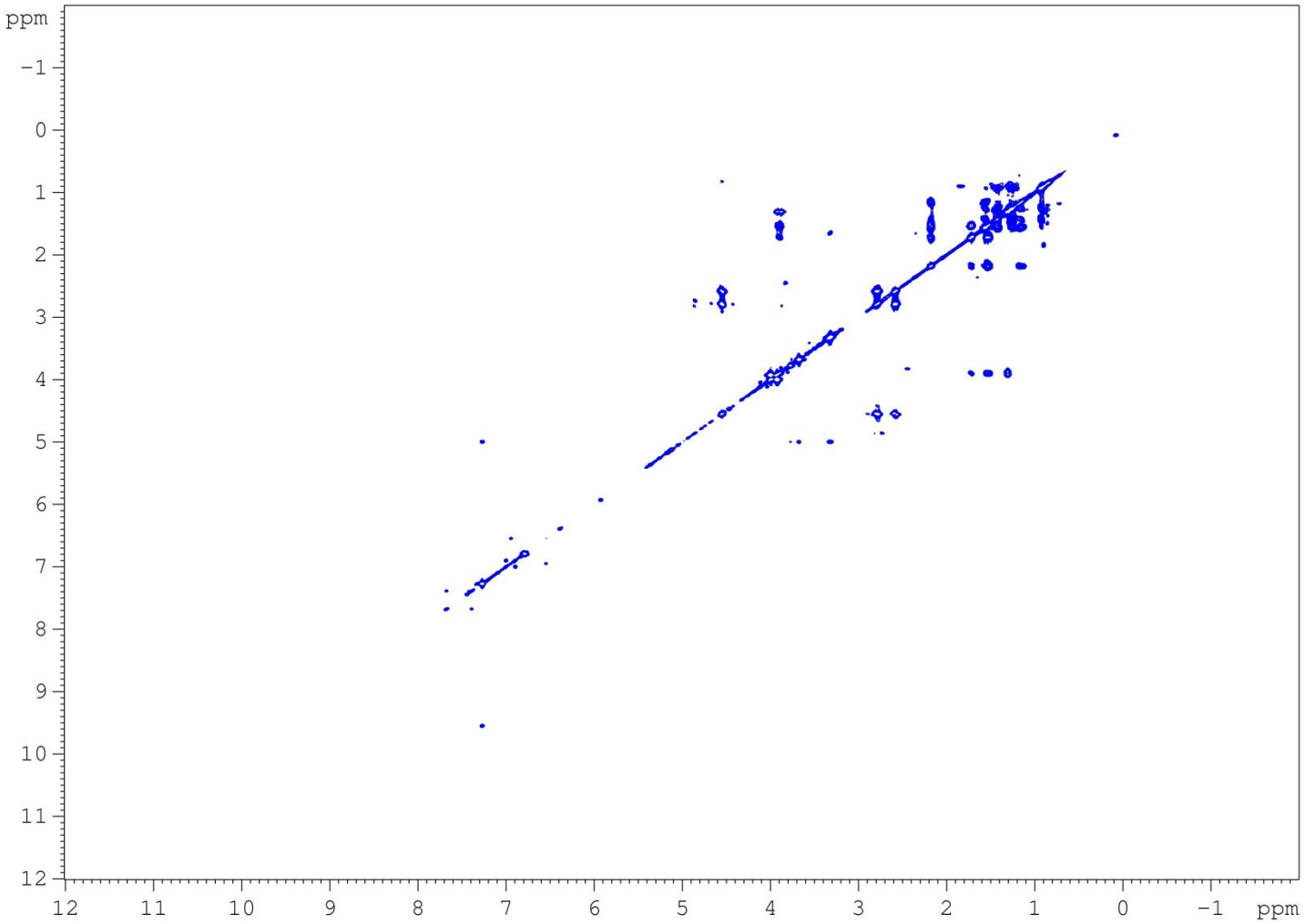

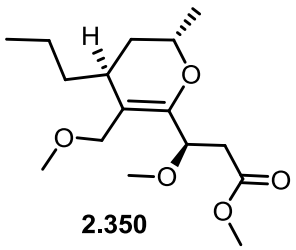

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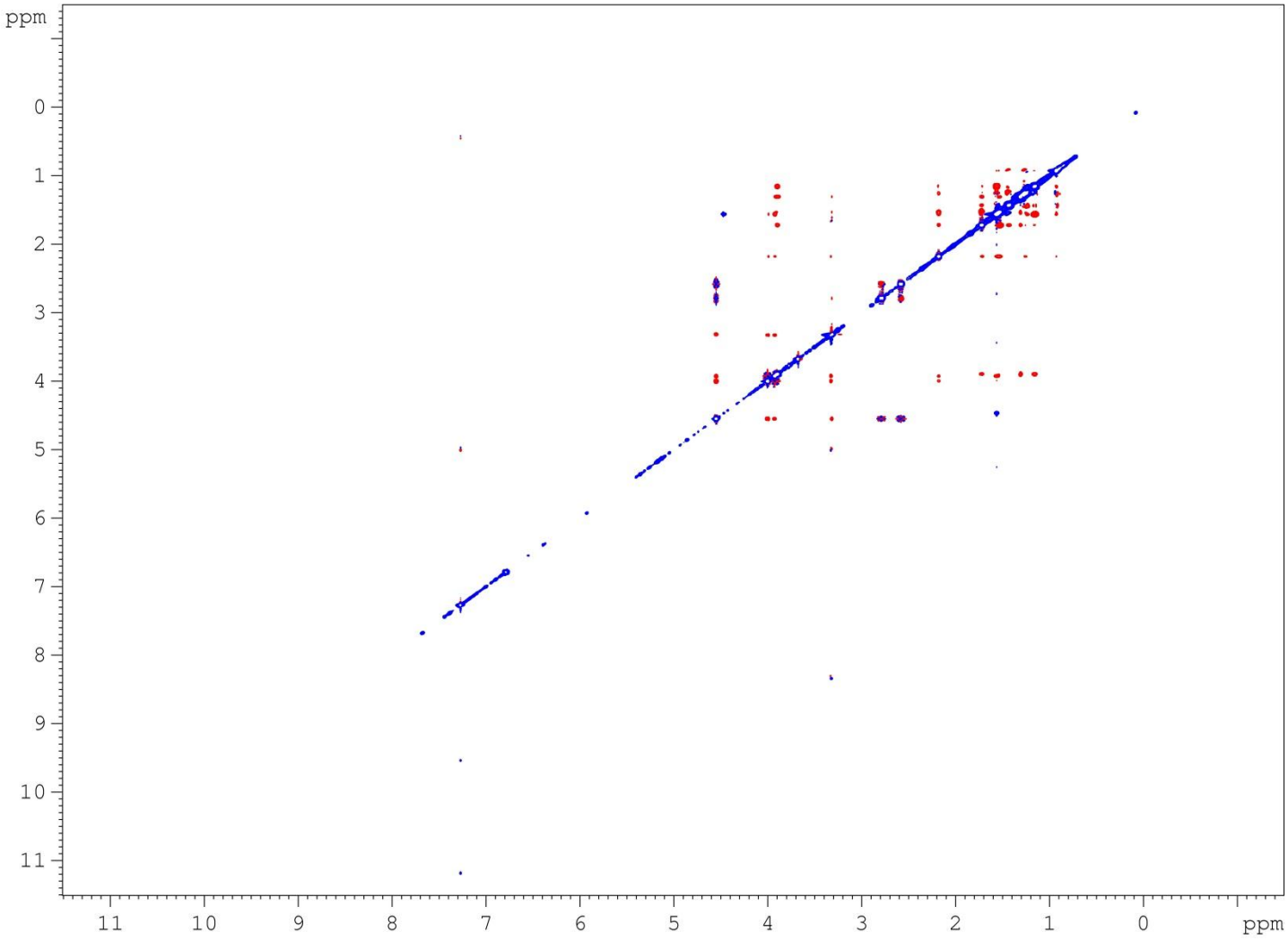

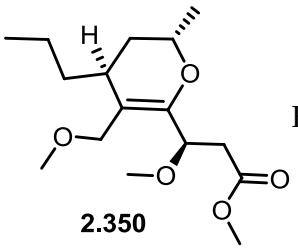
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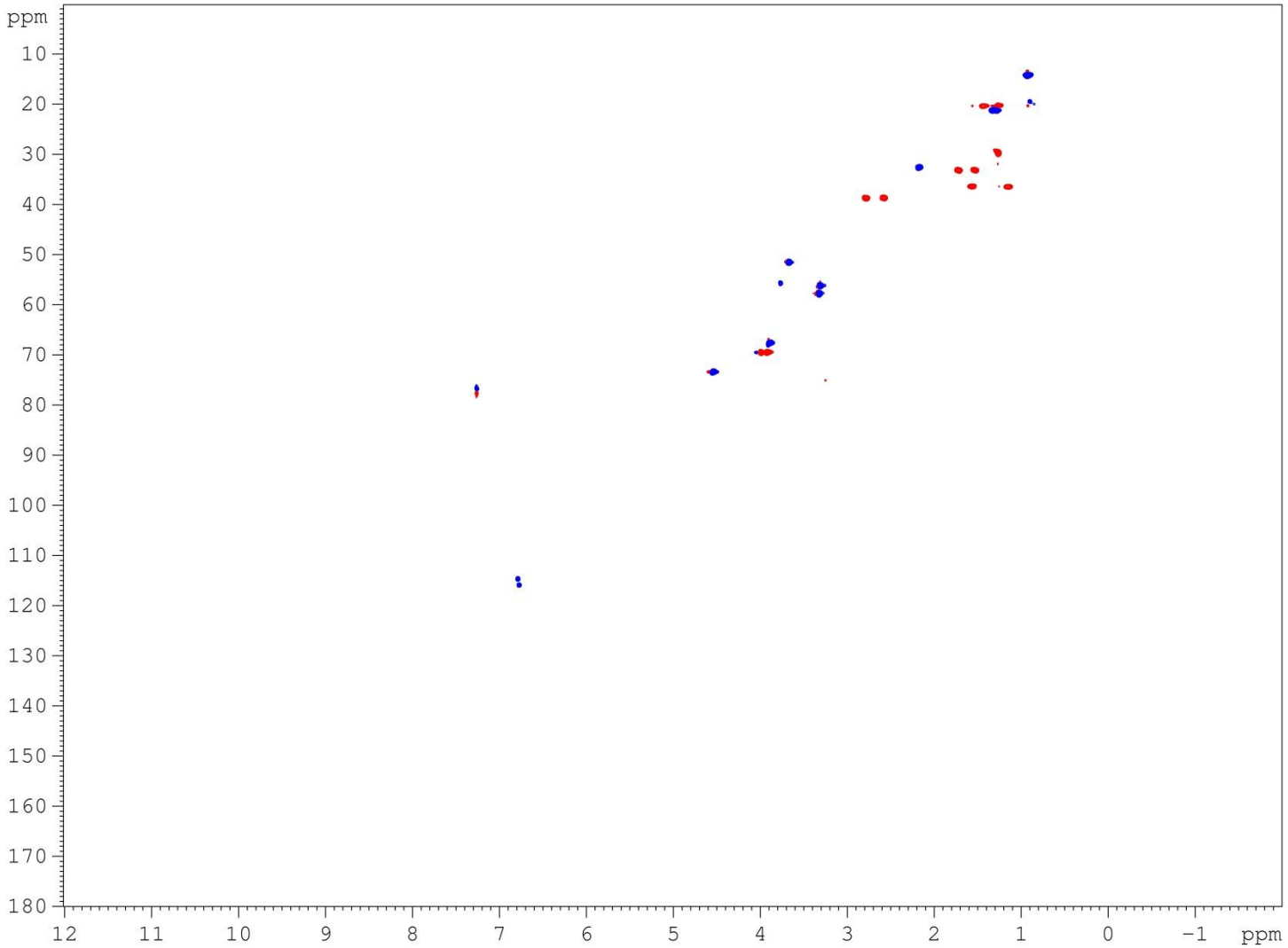
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7
6
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4
3
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1
0
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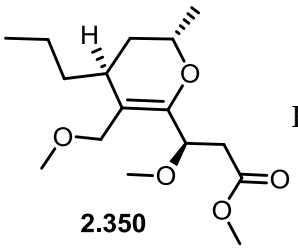
ppm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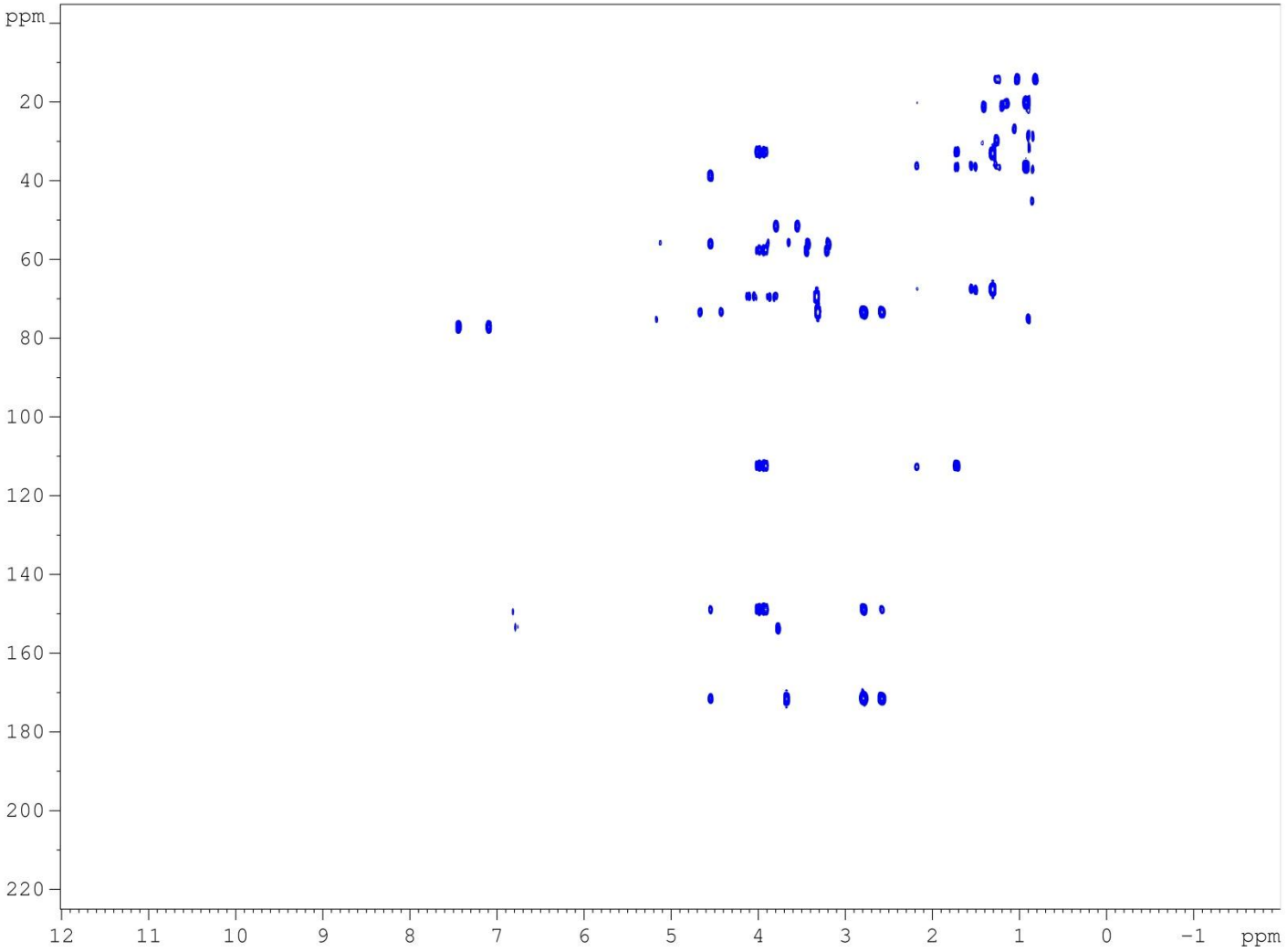
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2
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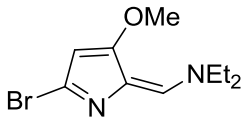
0
−1
ppm

Appendix A2:

Spectra Relevant to Chapter 3



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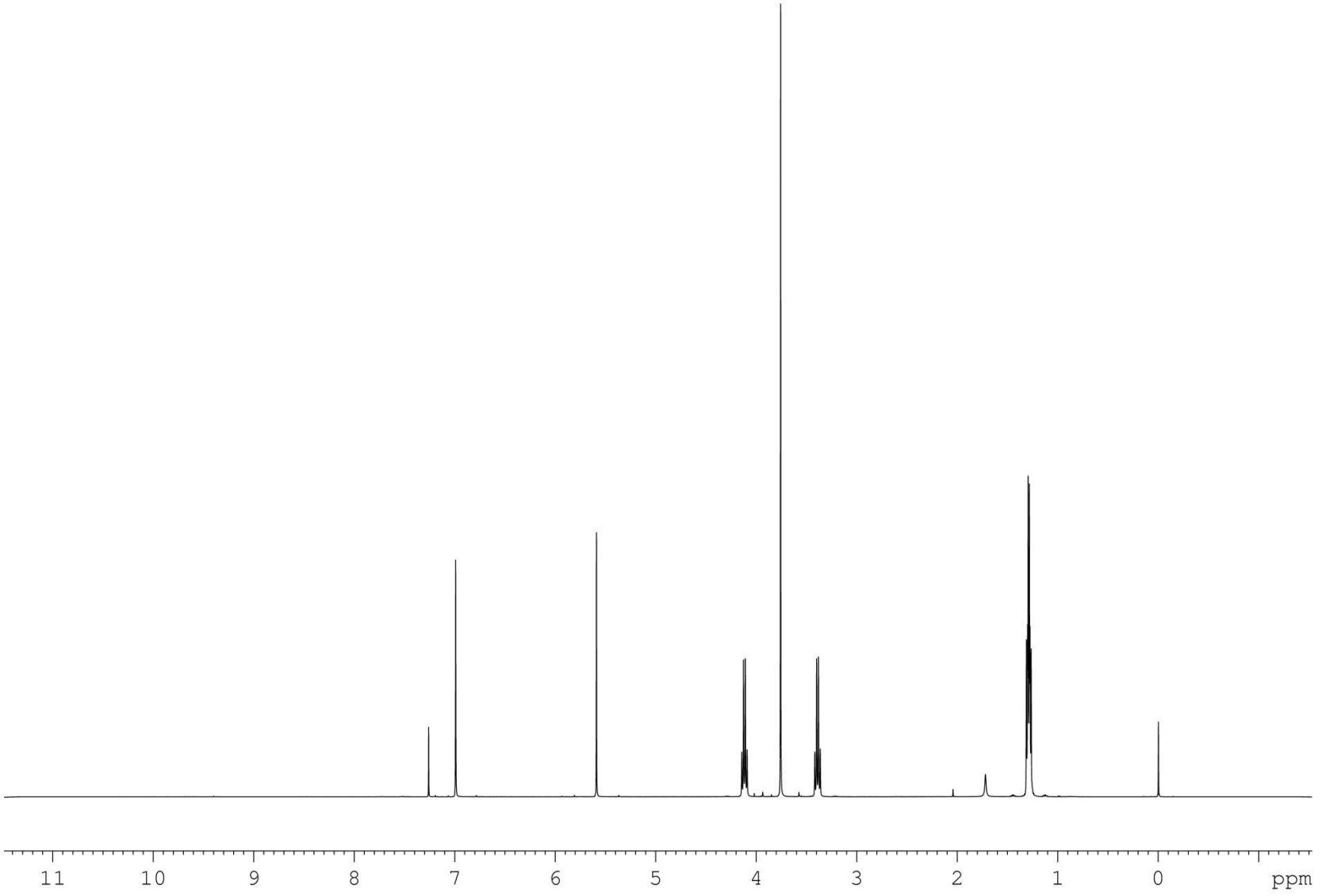

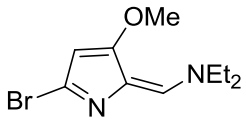

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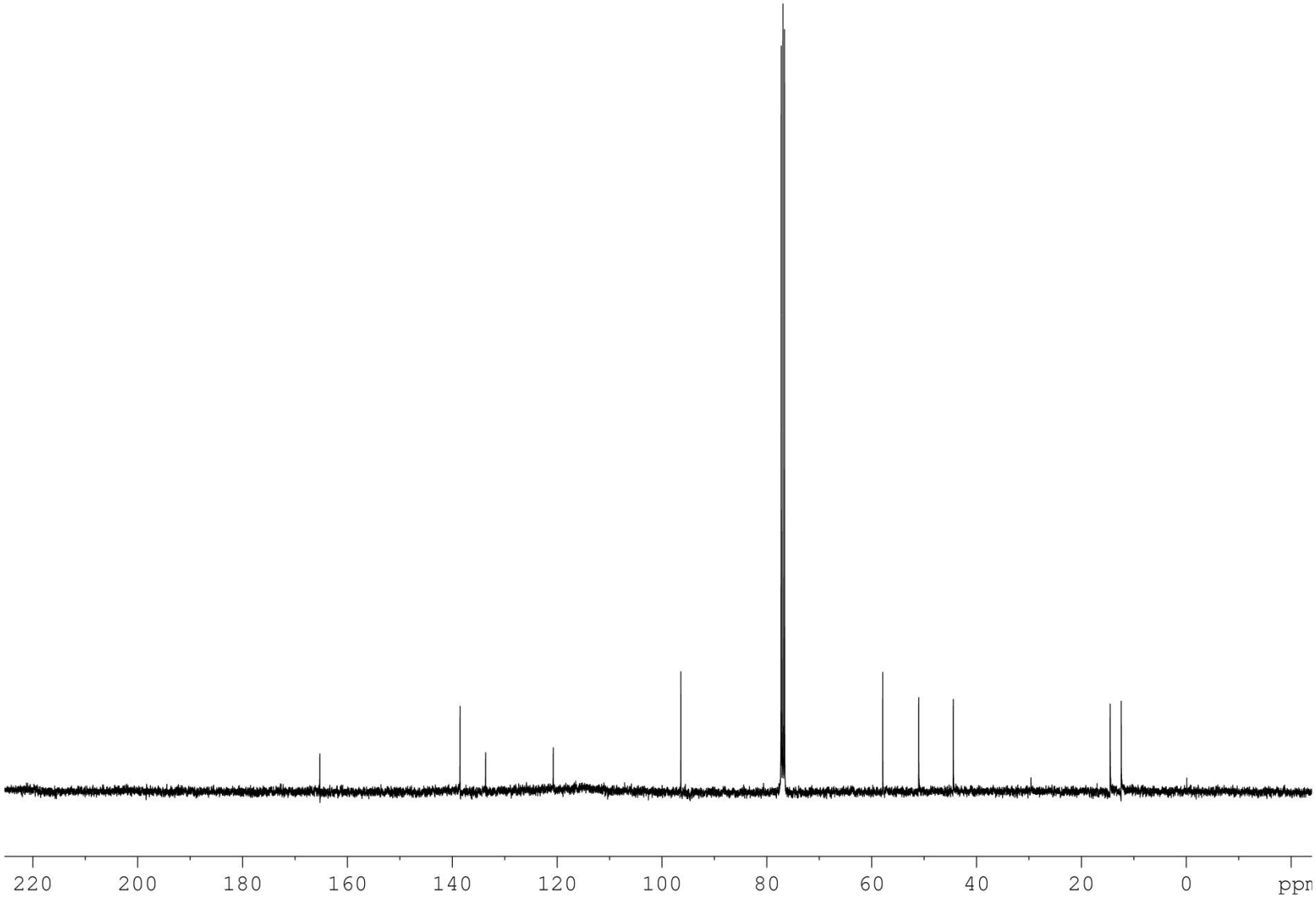

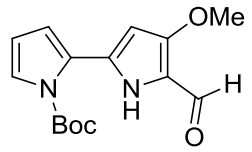

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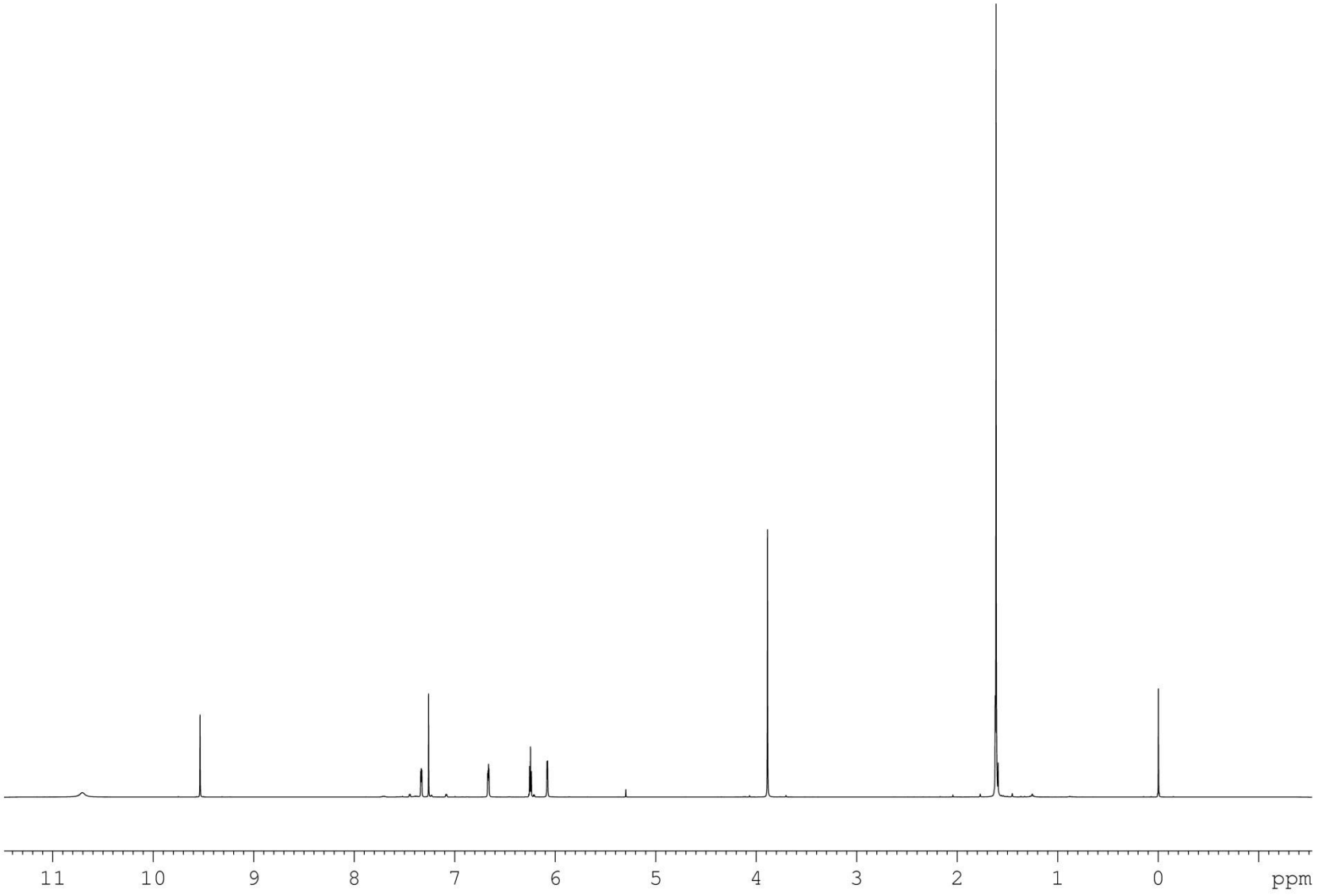

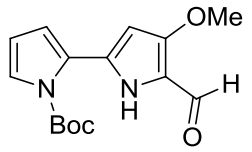

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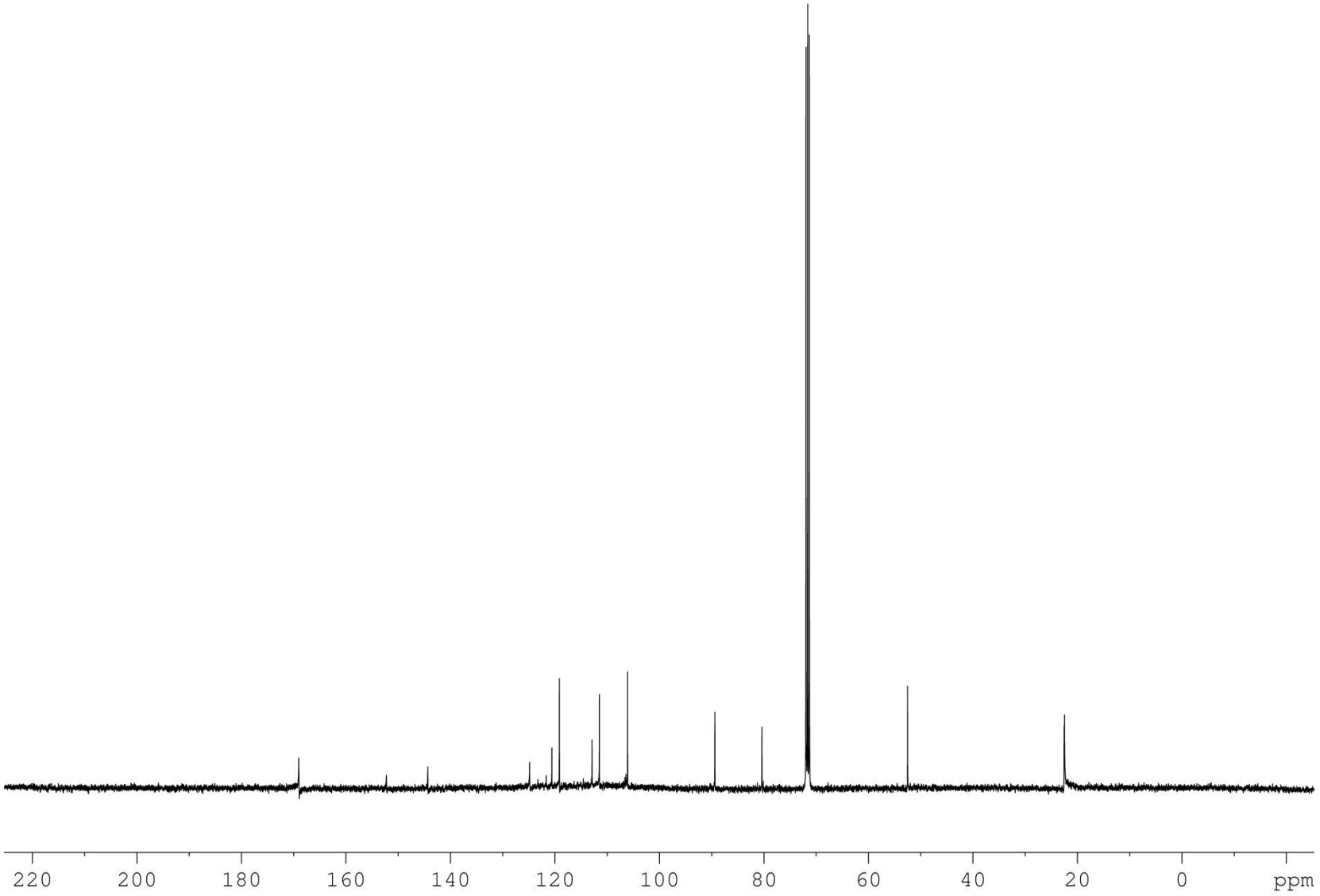

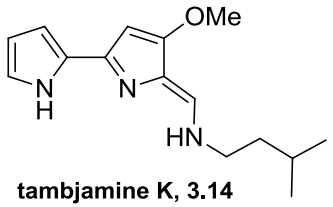

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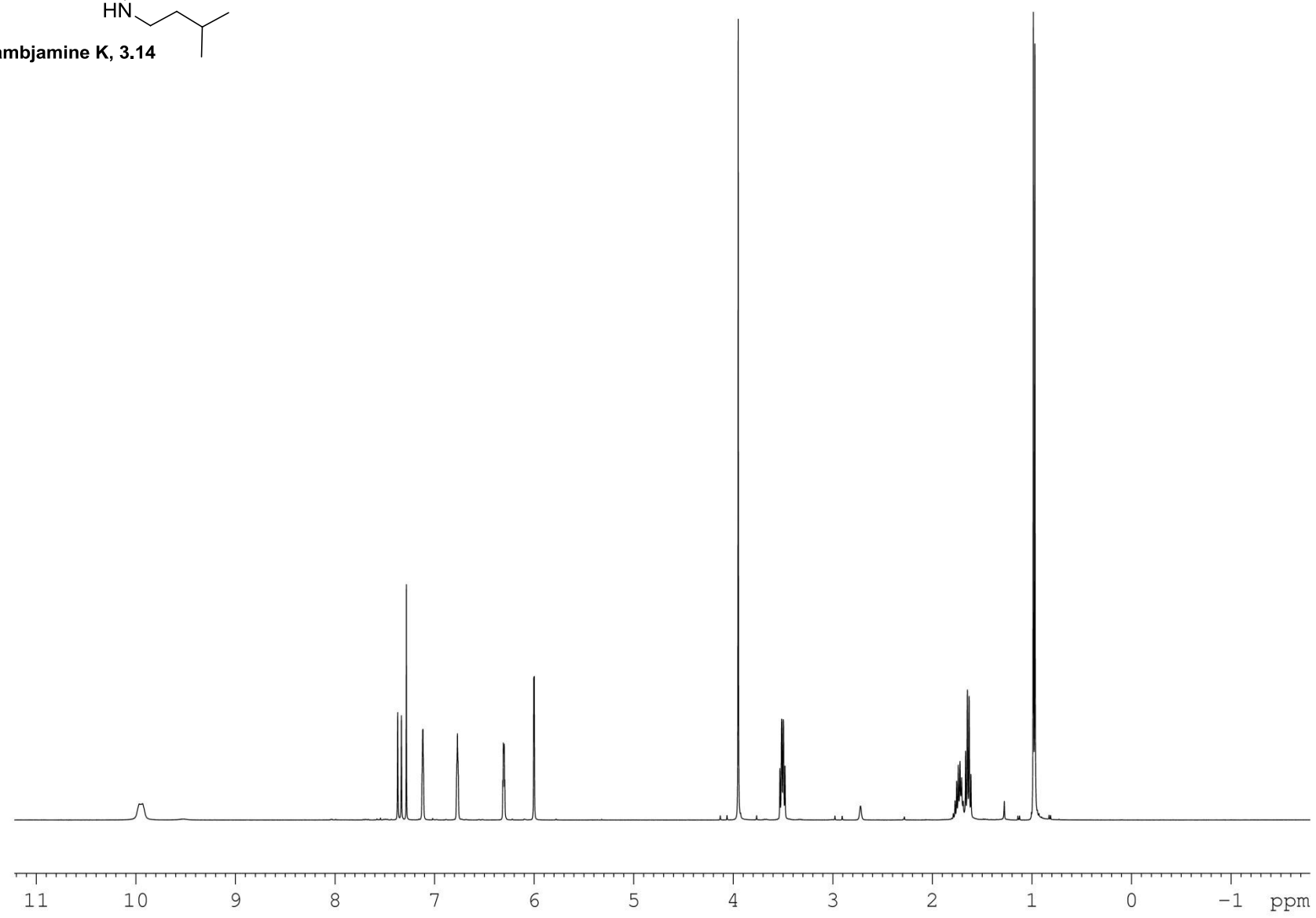

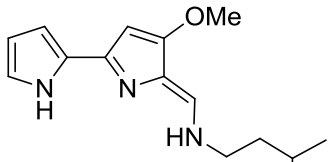

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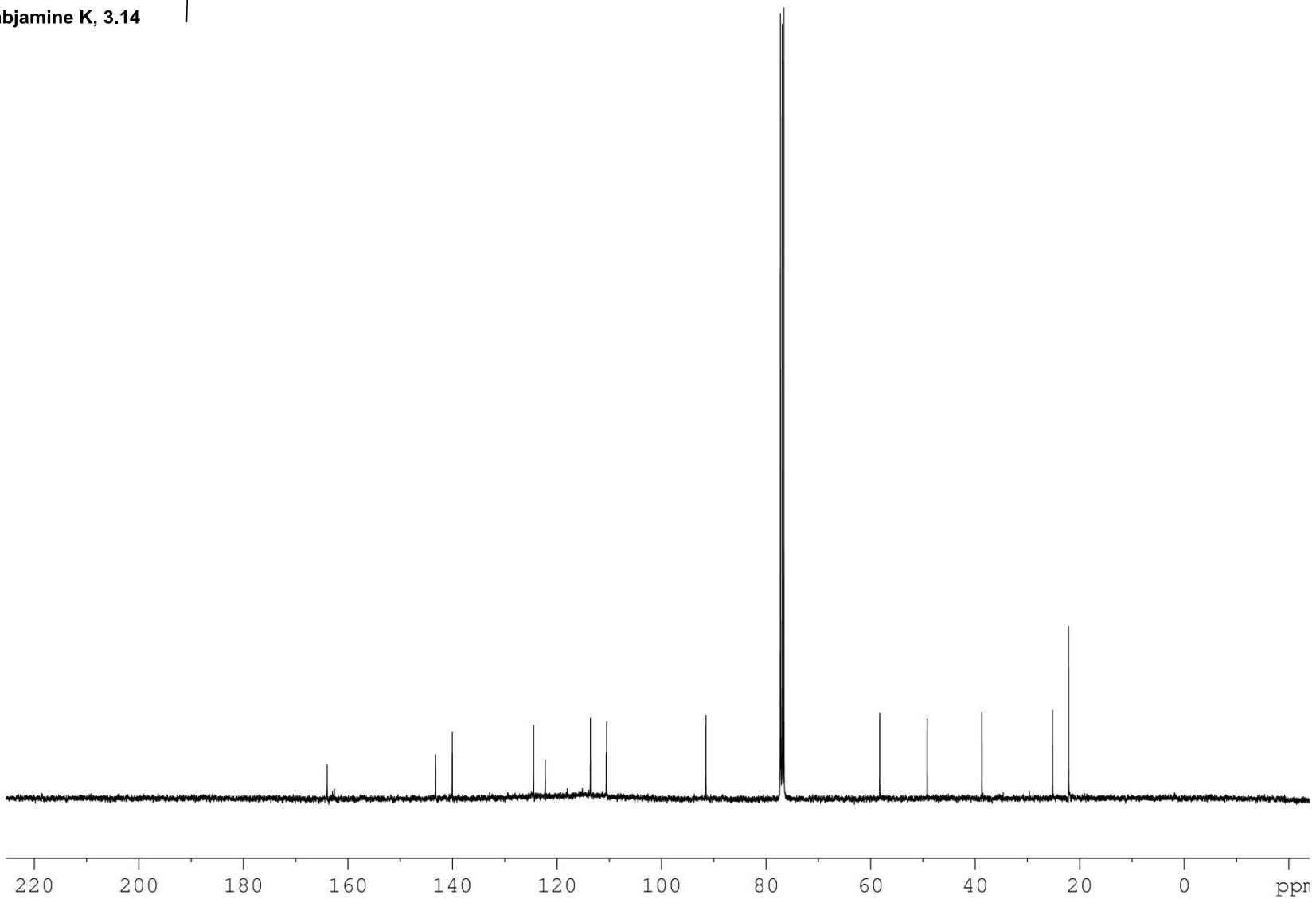

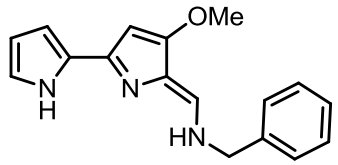

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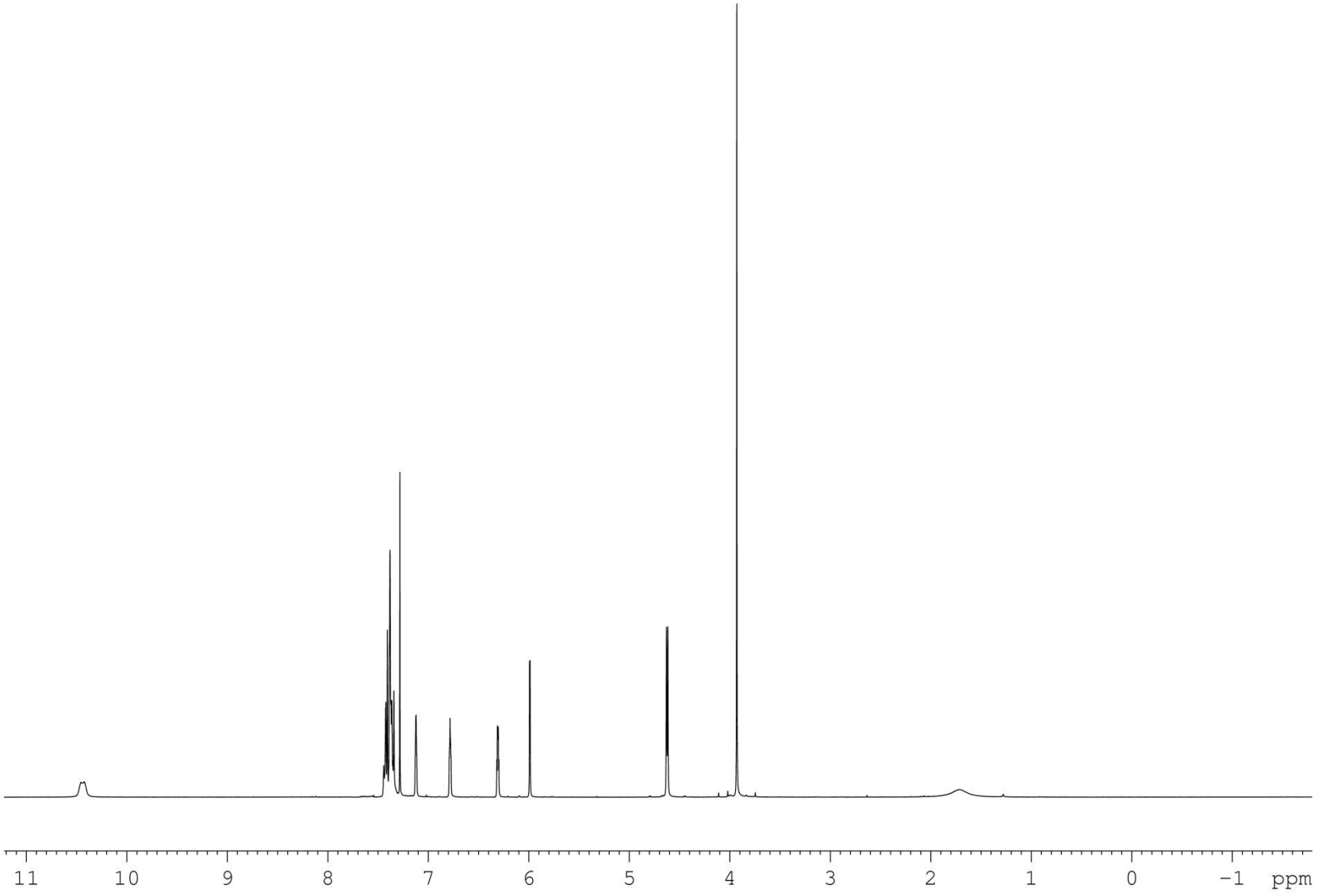

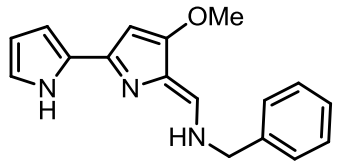

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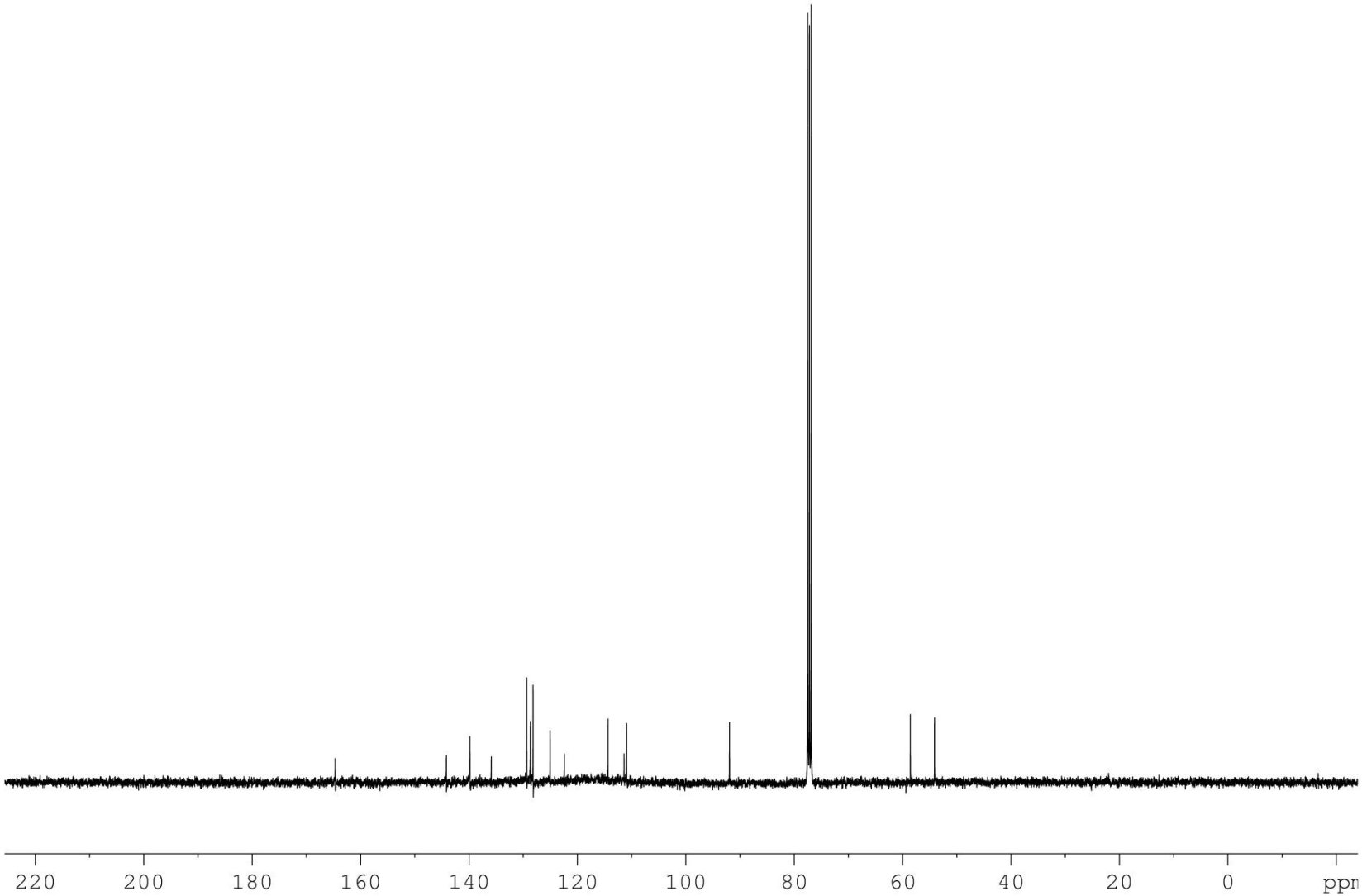

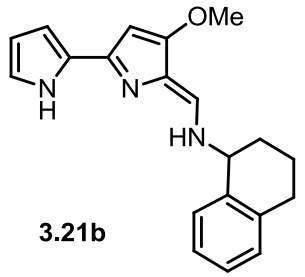

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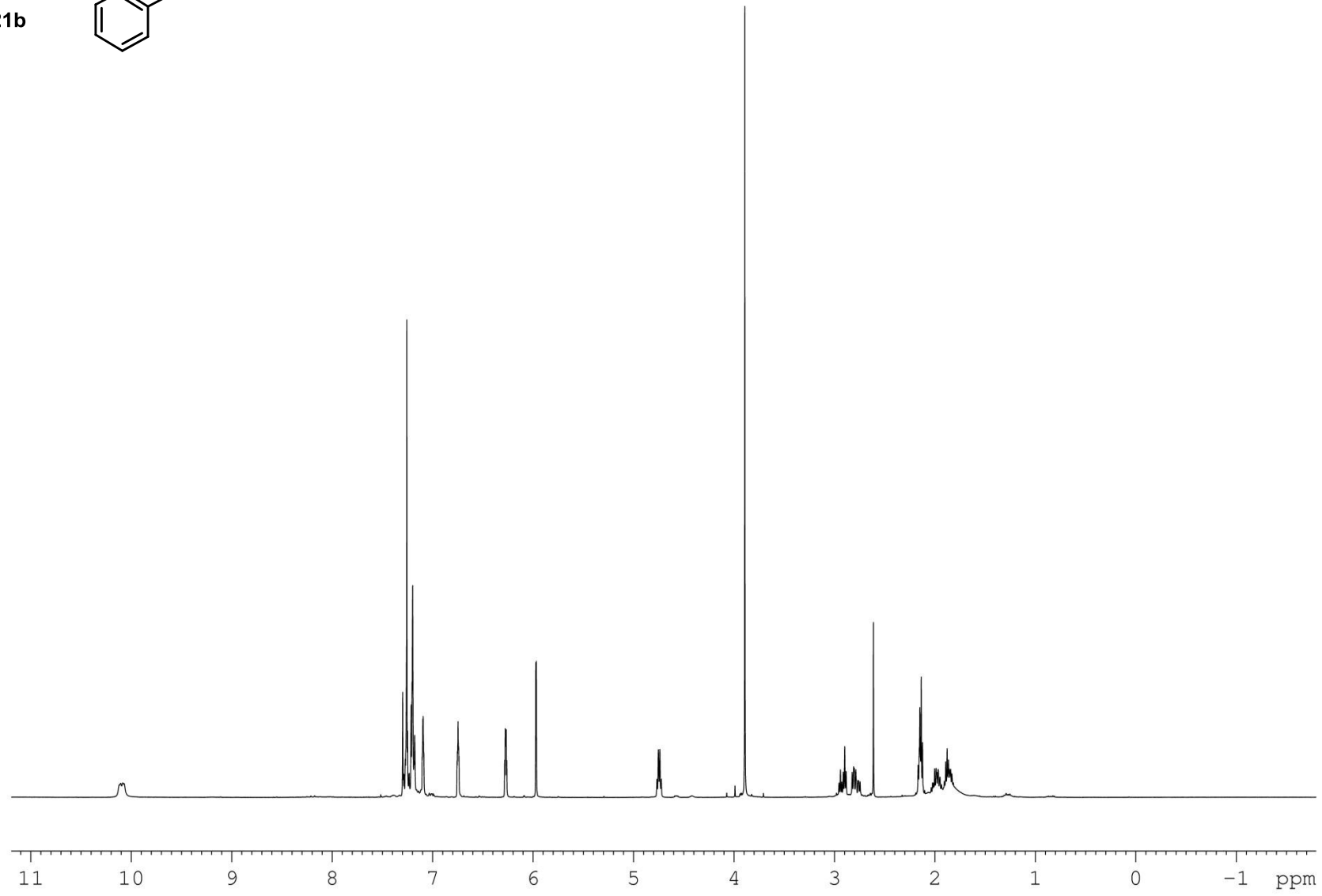

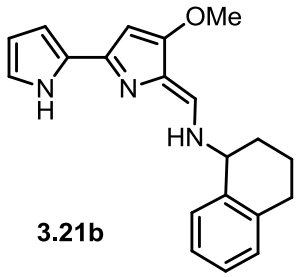

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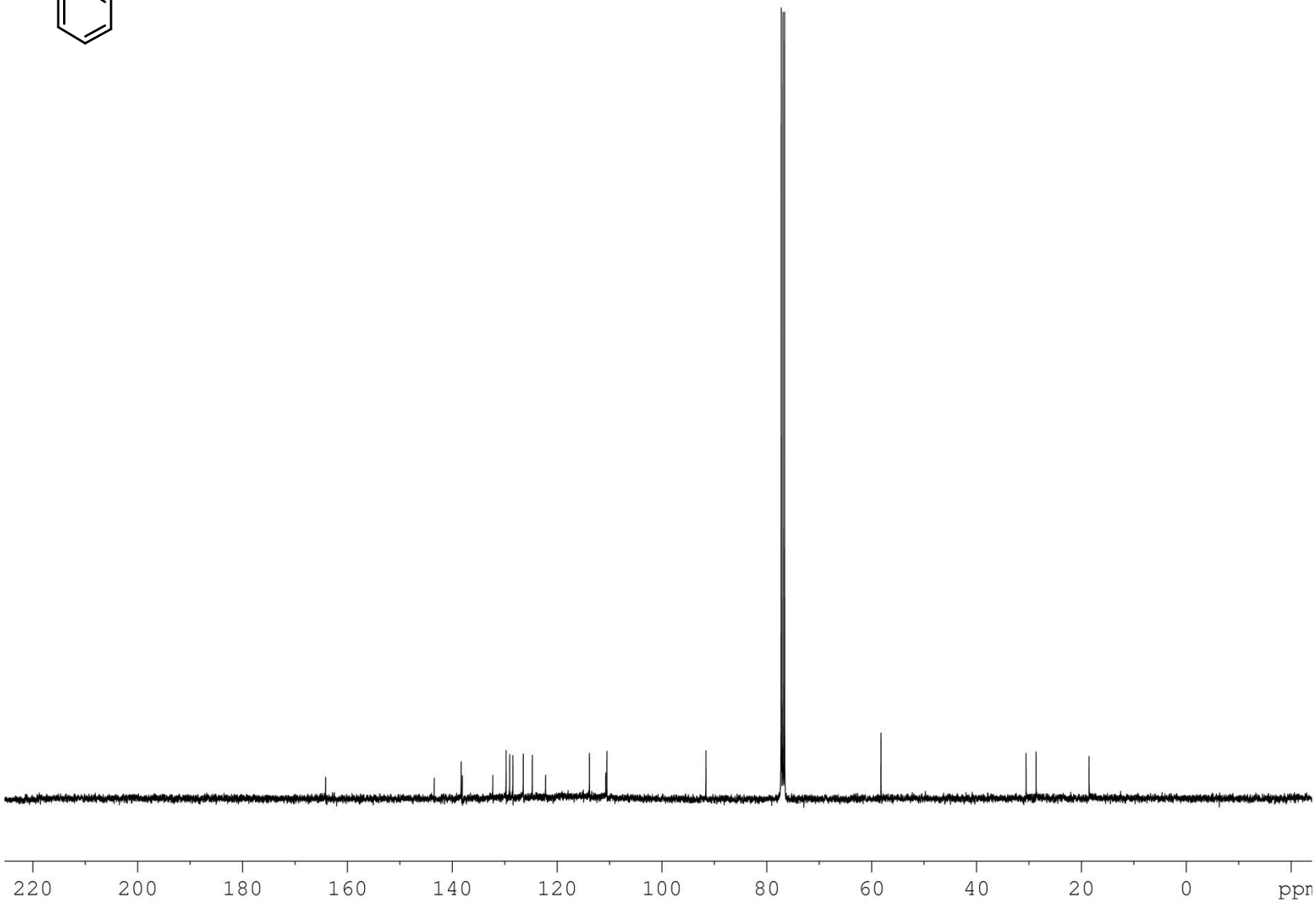

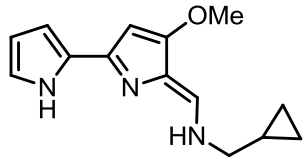

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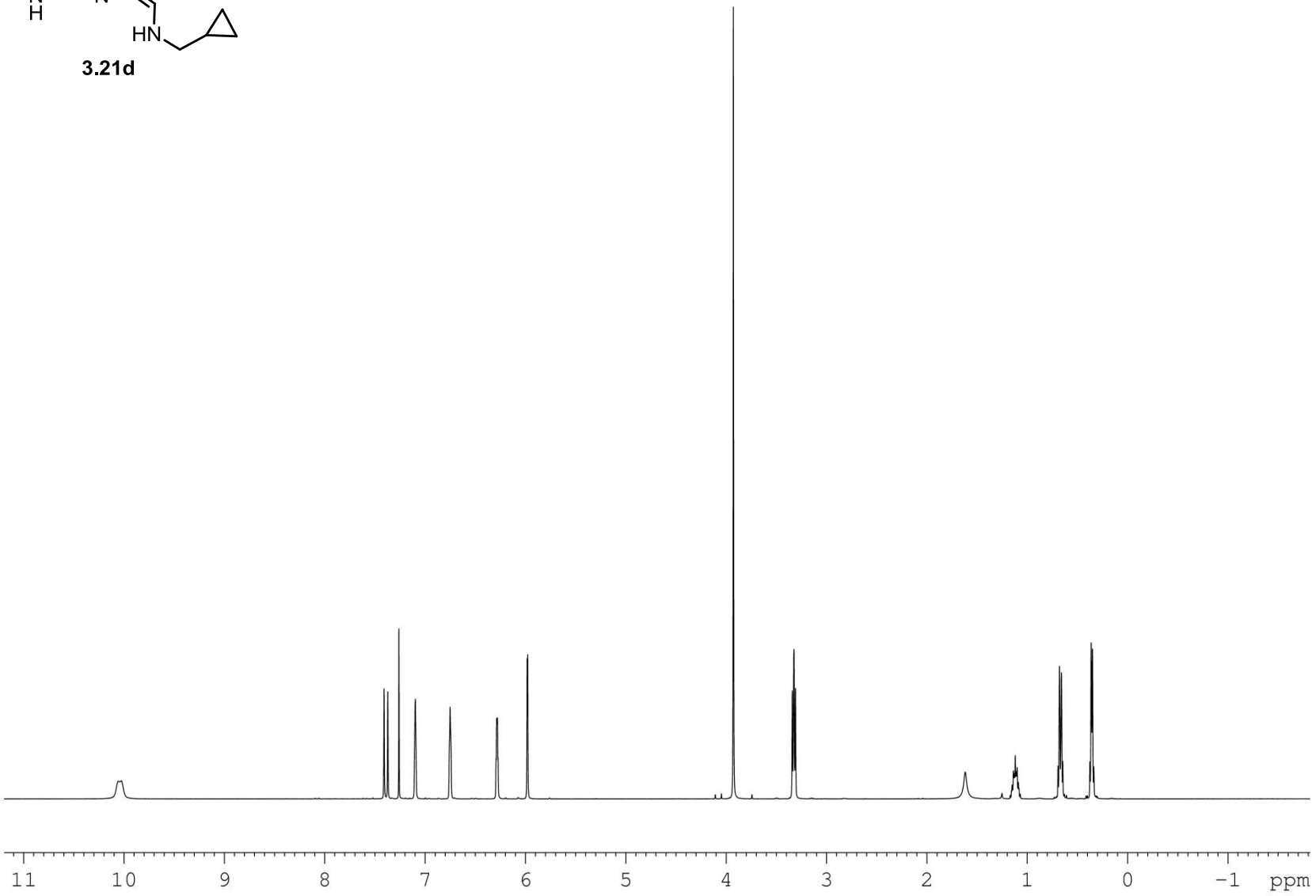

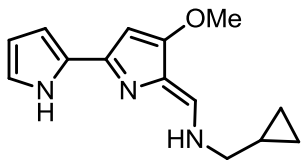

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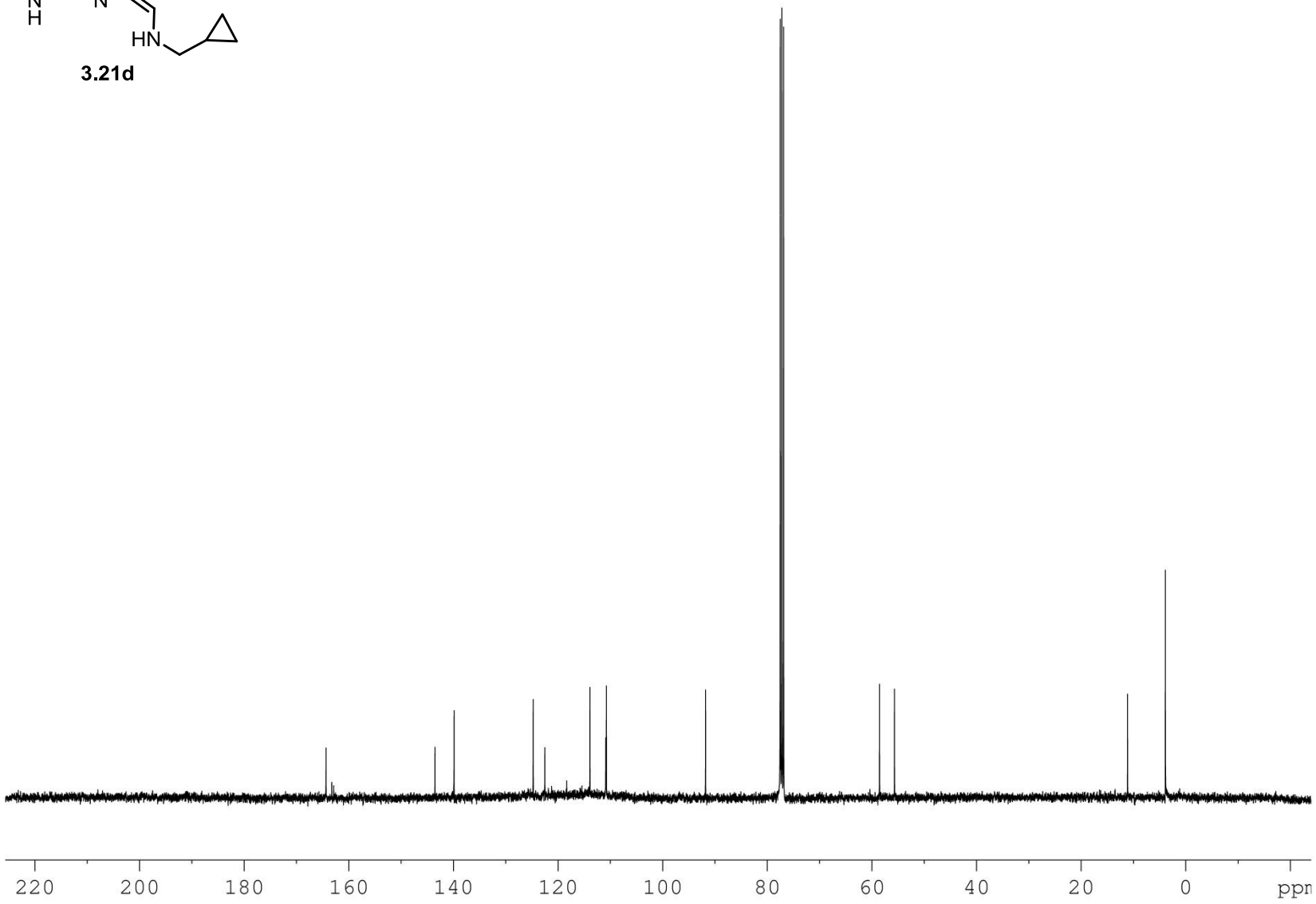

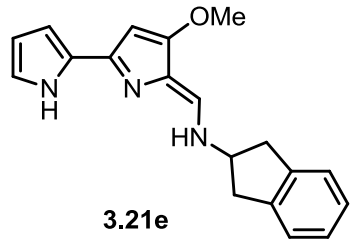

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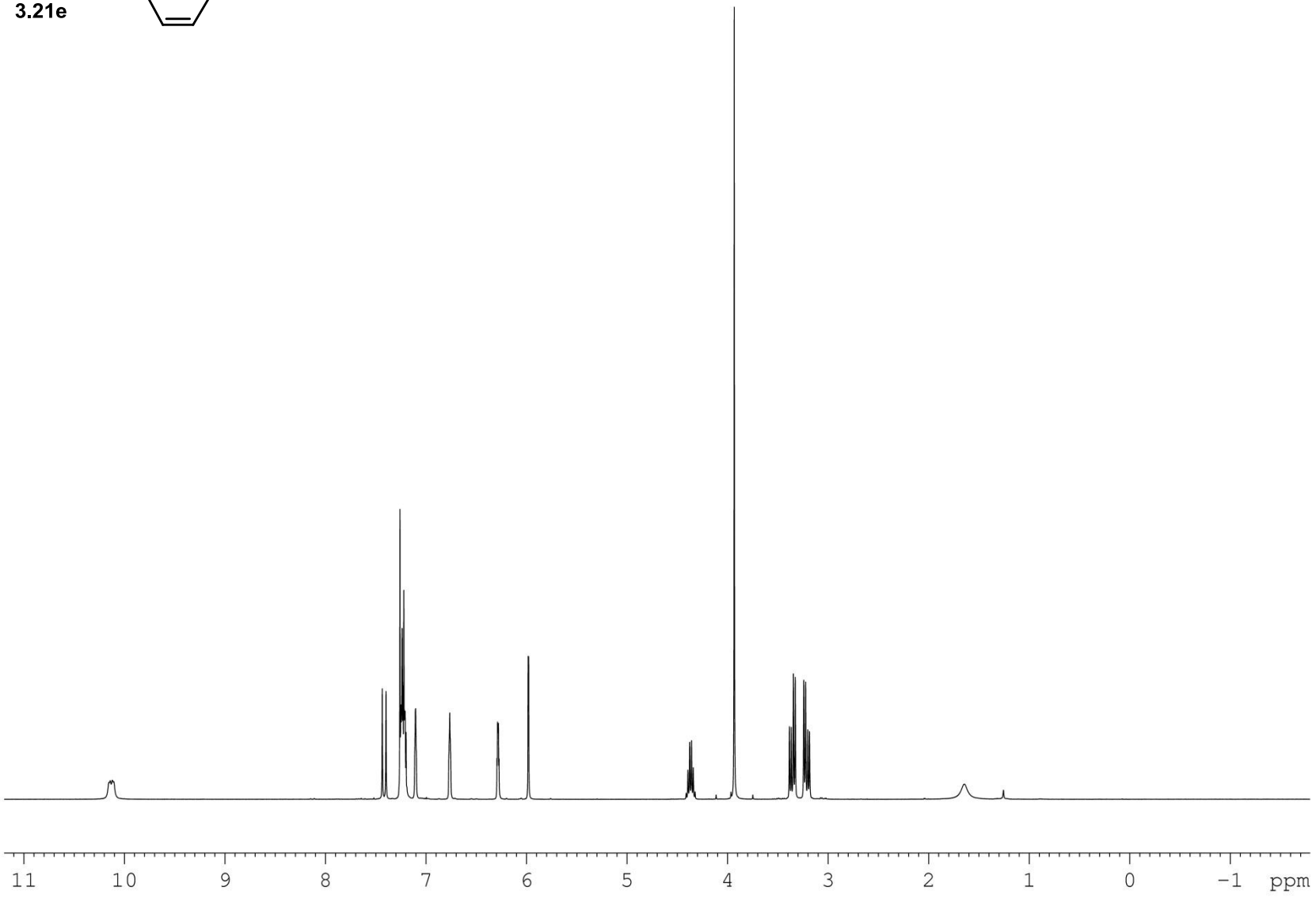

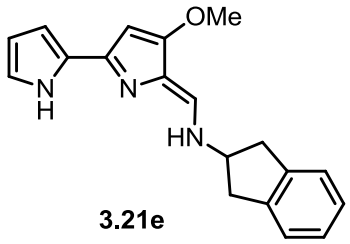

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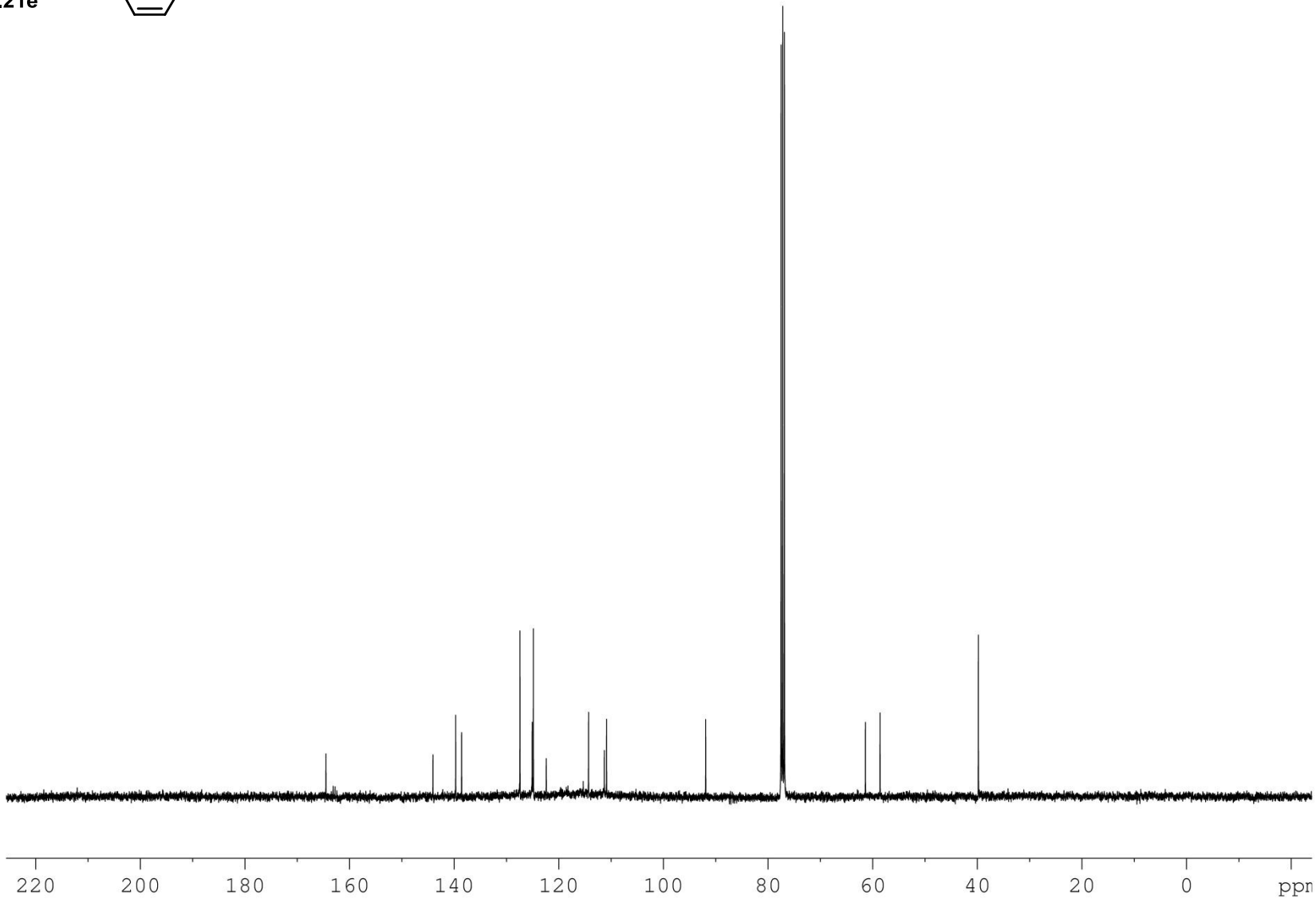

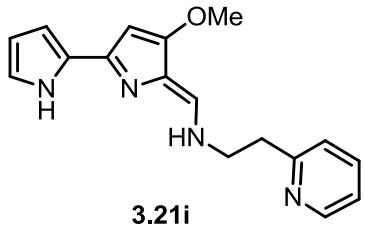

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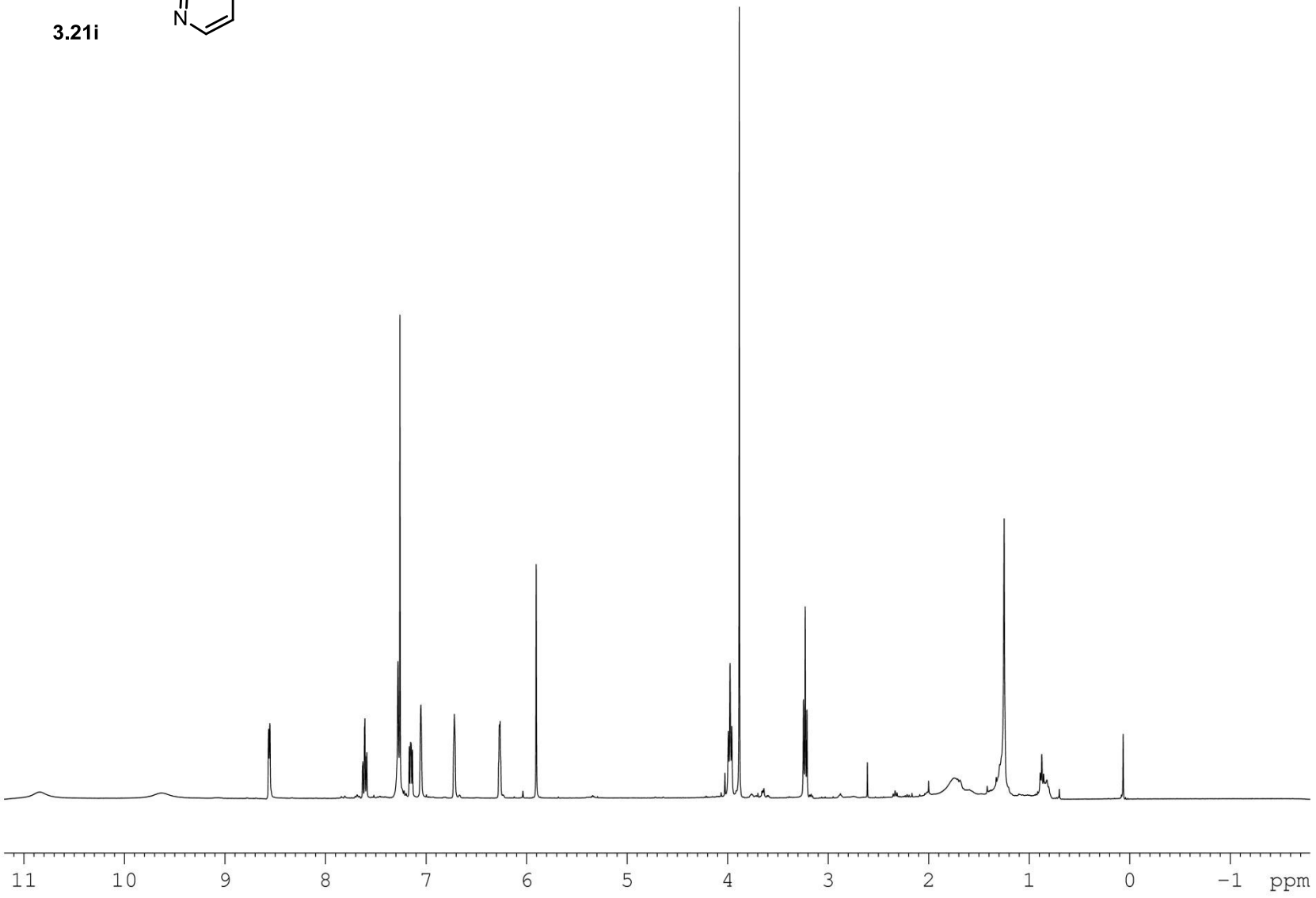

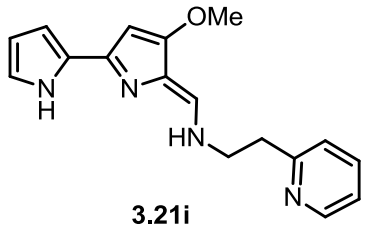

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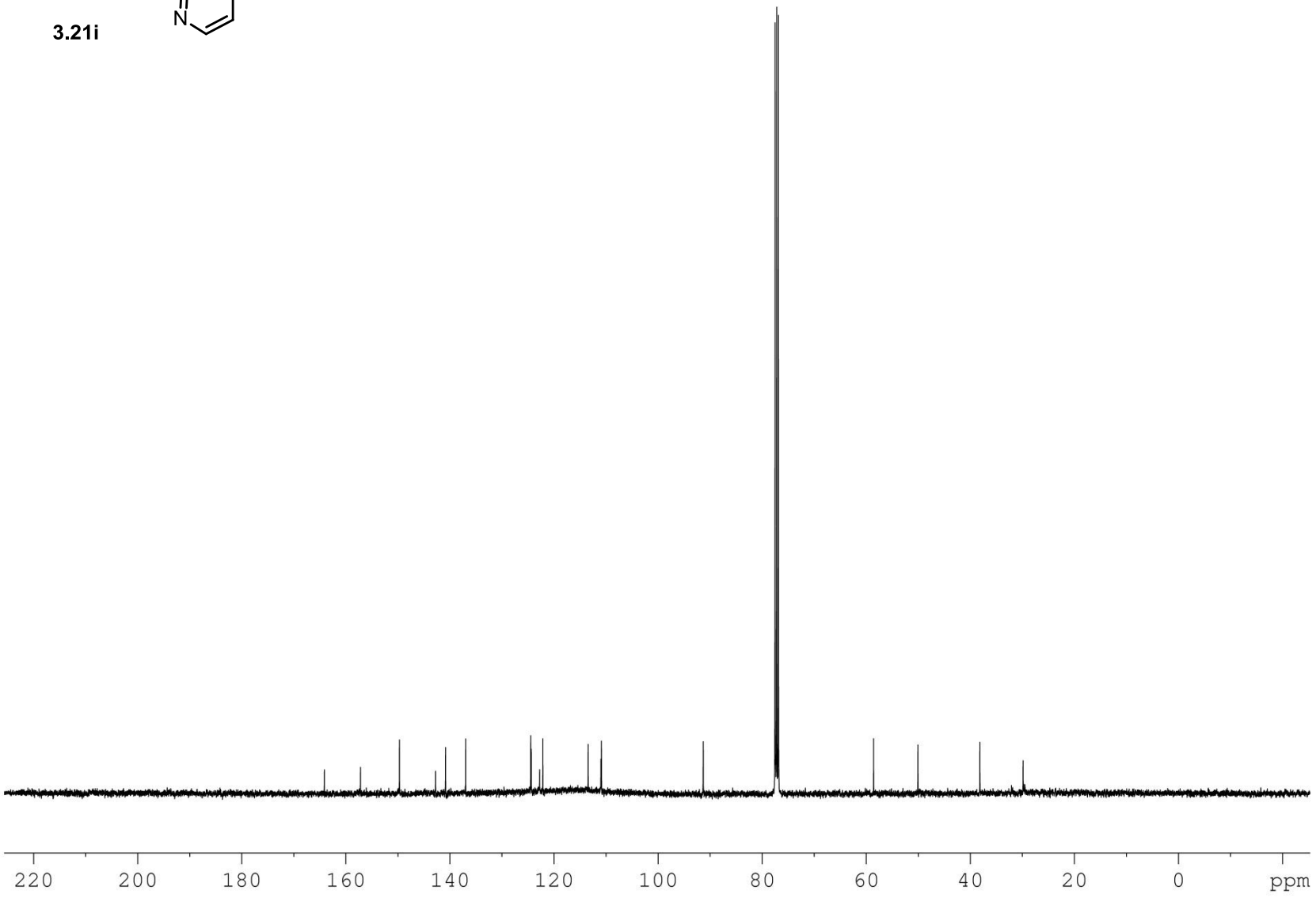

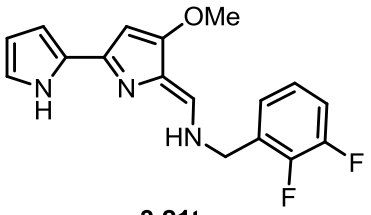

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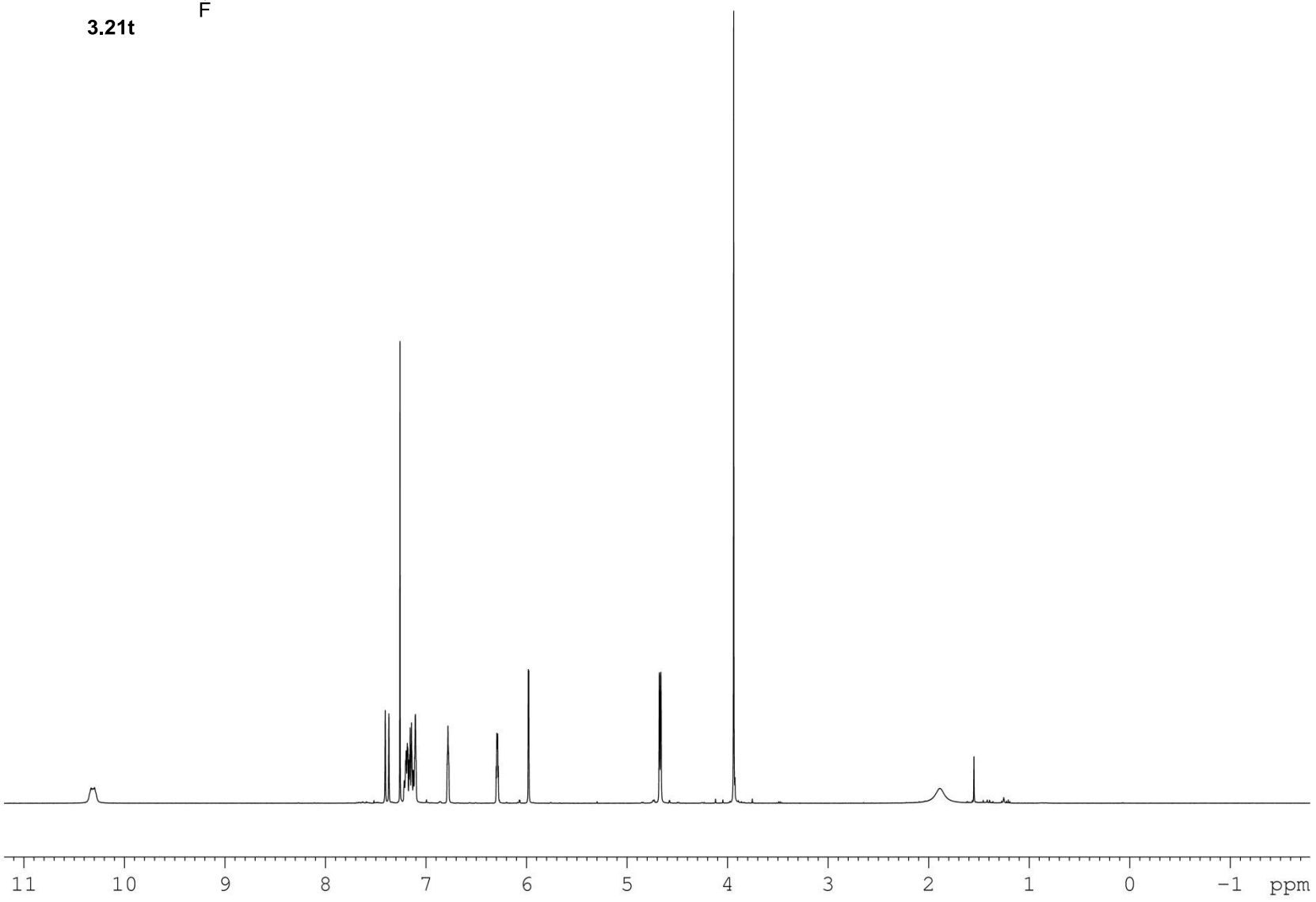

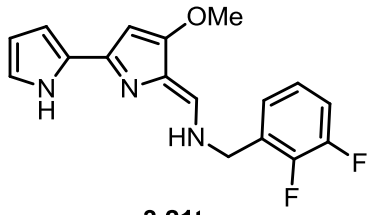

3.21t