A Synthetic Approach to Tetrodotoxin: via Oxidative Amidation of a Phenol

by

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Abstract

This dissertation describes synthetic efforts towards tetrodotoxin. Contact with tetrodotoxin brings upon paralytic shellfish poisoning (PSP) for the unfortunate victim. The syndrome can be fatal in extreme cases and symptoms include nausea, numbing of the lips and body, throat constriction, slurred speech, and loss of coordination. Our approach stems from the oxidative amidation of a phenol as a key step. This technology installs a necessary nitrogen-functionality of the natural product at an early stage; ultimately the route was optimized to allow the synthesis to proceed in a practical manner.

Preface

A portion of the research described in Chapter 1 appeared in Chau, J. and Ciufolini, M. A. The Chemical Synthesis of Tetrodoxin: An Ongoing Quest. *Marine Drugs* **2011**, *9*, 2046-2074.

Chau, J. is responsible for: the performance of each of the experiments reported herein; much of the tactical synthetic planning; and the writing of a complete draft of this dissertation. M.A.C. provided: the overall synthetic strategy; many helpful tactical and technical suggestions; and a thorough editing of this document.

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List of Abbreviations

Ac	acetyl
add'n	addition
Am	amyl
anh	anhydrous
aq	aqueous
Ar	aryl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bom	benzyloxymethyl
br	broad
BRSM	based on recovered starting material
BSA	bis(trimethylsilyl)acetamide
Bu	butyl
Bz	benzoyl
°C	degrees Celsius
CAN	ceric ammonium nitrate
cat.	catalytic
CBS	Corey-Bakshi-Shibata
Cbz	benzyloxycarbonyl
CC	column chromatography

CDI	1,1´-carbonyldiimidazole
cf.	*confer*
cm^{-1}	wavenumber(s)
CSA	camphorsulfonic acid
δ	chemical shift (ppm downfield from tetramethylsilane)
Cyh	cyclohexyl
d	doublet
D	dextrorotatory
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*´-dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
de	diastereomeric excess
DIB	(diacetoxyiodo)benzene
DIBAL	diisobutylaluminum hydride
DIPEA	diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martion periodinane
DMSO	dimethylsulfoxide

DMTS	dimethylthexylsilyl
DNA	deoxyribonucleic acid
DOPA	3,4-dihydroxyphenylalanine
DPPA	diphenylphosphoryl azide
dr	diastereomeric ratio
E	carboxymethyl
E	entgegen (of an alkene)
ee	enantiomeric excess
ent	enantiomeric
epi	epimeric
ESI	electrospray ionization
Et	ethyl
Fmoc	fluorenylmethyloxycarbonyl
g	gram(s)
G	guanine (DNA residue)
h	hour(s)
[H]	reduction
HMPA	hexamethylphosphoramide
HMQC	Heteronuclear Multiple Quantum Coherence
HOBt	1-hydroxybenzotriazole
HRMS	high resolution mass spectrometry

Hz	Hertz (s^{-1})
i	iso (as an alkyl group)
IBX	2-iodoxybenzoic acid
imid	1,3-imidazole
INOC	intramolecular nitrile oxide cycloaddition
i.p.	intraperitoneal
IR	infrared
J	coupling constant
L	levorotatory
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
HMDS	hexamethyldisilizide
LN2	liquid nitrogen
m	meta (as a benzene substituent)
m	multiplet
M	molarity
Me	methyl
mCPBA	*meta*-chloroperoxybenzoic acid
MIC	minimum inhibitory concentration
min	minute(s)
mol	mole(s)

MOM	methoxymethyl
m.p.	melting point
Ms	methanesulfonyl
MS	mass spectrometry or molecular sieves
MTr	*p*-methoxyphenyldiphenylmethyl
n	normal (as an alkyl group)
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMP	*N*-methylpyrrolidin-2-one
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
N-PSP	*N*-phenylselenophthalimide
NR	no reaction
[O]	oxidation
p	para (as a benzene substituent)
P	unspecified protecting group
PCC	pyridinium chlorochromate
Ph	phenyl
Phth	phthalimido
PMB	*p*-methoxybenzyl

PMP	*p*-methoxyphenyl
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
psi	pounds per square inch (lbs in^{-2})
PSP	paralytic shellfish poisoning
Pv	pivaloyl chloride
PyBrOP	bromo-tris(pyrrolidino)phosphonium hexafluorophosphate
pyr	pyridine
q	quartet
quant.	quantitative
R	*rectus*
R_f	retention factor
rt	room temperature
rxn	reaction
s	singlet
S	*sinister*
SAM	*S*-adenosyl methionine
sat.	saturated
SEM	[2-(trimethylsilyl)ethoxy]methyl
SET	single electron transfer

S_N2	bimolecular nucleophilic substitution
spp.	species
STX	saxitoxin
TBAF	tetra-*n*-butylammonium fluoride
TBAT	tetra-*n*-butylammonium difluorotriphenylsilicate
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
t	tertiary (as an alkyl group)
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy, free radical
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TPAP	tetra-*n*-propylammonium
Ts	*p*-toluenesulfonyl
TTX	tetrodotoxin

UV	ultraviolet
var.	variety
VT	variable temperature
xs	excess
Z	zusammen (as an alkene)

Acknowledgements

First and foremost, I wish to thank Prof. Ciufolini for his immense guidance and support. I am extremely thankful for his wisdom and dedication to teaching. His vast chemical knowledge and meticulous observations involving chemistry and life in general has made my time in graduate school a valuable experience.

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I would also like to thank the members of the Ciufolini group. Special thanks to Dylan for helping me proofread this manuscript and to Taka, Josh, Gloria, Veeru, Steven, David and Jimmy for their insightful discussions. Their friendship and advice have made my stay in the group enjoyable and quite entertaining.

1. Introduction

Before its identity was known, tetrodotoxin (TTX, **1)** made its presence felt by causing an untold number of fatalities. The syndrome caused by contact with **1** and related toxic agents is known as paralytic shellfish poisoning (PSP).[1] After eating poisoned shellfish, the victim's mouth and lips quickly begin to tingle, then, over about thirty minutes, they become numb. The prickling and numbness may spread to the hands and other parts of the body.[2] Following these

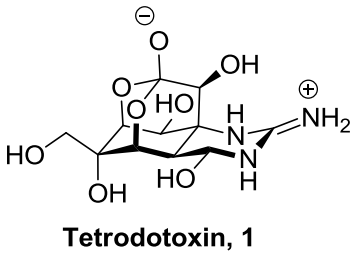


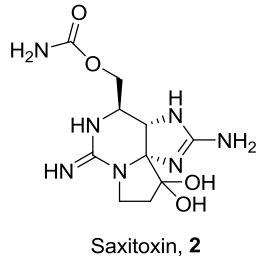
Figure 1. The marine toxin tetrodotoxin, **1**

initial symptoms, difficulty swallowing and throat constriction may appear. Limbs will become unresponsive, breathing becomes labored and speech becomes incoherent. Severe nausea may occur. In fatal cases, complete paralysis will precede death. It is believed that the unfortunate victim remains nonetheless lucid throughout. The entire process occurs over several hours, and is complete within half a day. It is estimated that as little as 2 mg can be fatal to an average person.

The pure toxin can be isolated from the ovaries of the puffer fish (*Tetraodon* and *Fugu* spp.),[3] though it has also been discovered that **1** is derived from symbiotic microorganisms.[4]

Specifically, bacteria which inhabit dinoflagellates of the genus *Alexandrium*, *Gymnodinium catenatum*, and *Pyrodinium bahamenese* var. *compressum* are thought to be a source of PSP toxins including **1**, though PSP-producing bacteria also reside in the liver of the pufferfish. Furthermore, it is likely that the toxins accumulate in larger organisms through so-called food web transfer.[5]

With an initial report by Narahashi, Moore and Scott in 1964 exploring the effect of **1** on the conductance of lobster giant axons,[6] it was established that this material derives its extraordinary toxicity from an ability to block the Na^+ channel in neurons, though, interestingly, cardiac neurons seem to be largely resistant to the effect.[7] Such a mode of action has been well established in the intervening decades.[8] The guanidinium moiety of **1** has been implicated, in that it is able to strongly bind ion channels in place of a Na^+ ion.[9] A structural analogy can be drawn to the related neurotoxin, saxitoxin (STX, **2**).

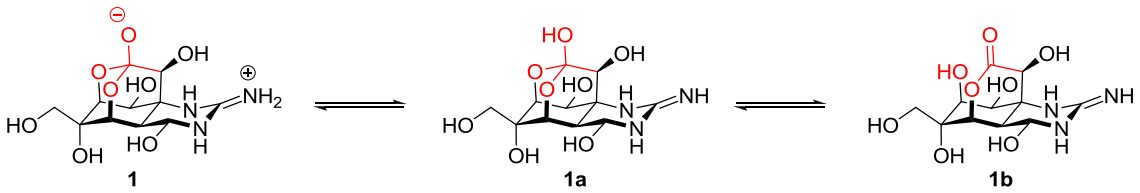


Saxitoxin, **2**

Figure 2. Saxitoxin (STX), **2**

TTX has seen wide use in medical research. For example, it was instrumental in the isolation of ion channel proteins.[10] Additionally, it has been used medicinally, for example, in the control of pain. The Na^+ channel has been implicated in pain transmission, and **1** has proven so

useful in the study of neuronal properties that the Na^+ channels themselves are classified by their sensitivity to it.[11]

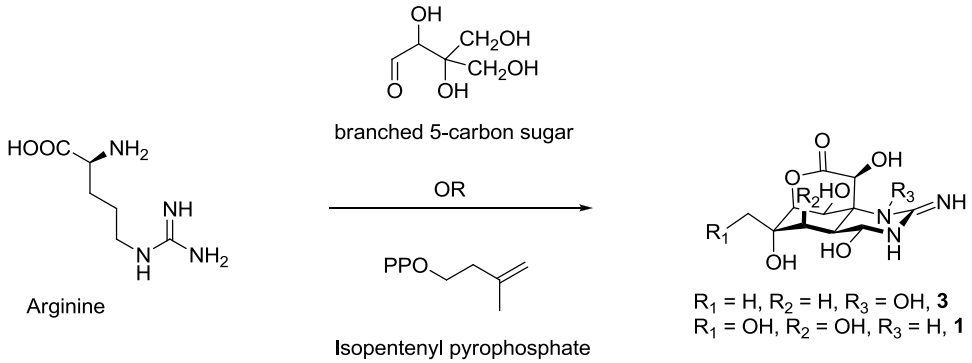


Scheme 1. Equilibria exhibited by **1**

Structurally, **1** is very unusual. Outside of the aforementioned guanidine moiety, its most remarkable feature is the incorporation of an ortholactone group. This rarely seen functional group probably is able to exist due to the highly rigid structure of the toxin.

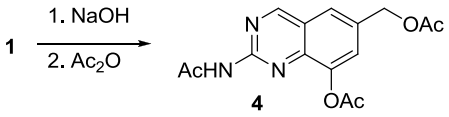
Given the dispute about which organism(s) are responsible for the production of **1**, it should not be a surprise that the biosynthetic origins of **1** also remain unclear. Some of the difficulty in the determination of the biosynthetic production of **1** arises from the greatly diminished amount of the toxin produced by pufferfish raised in captivity.[12] Similarly, cultures of the bacteria believed to be responsible for the biosynthesis of **1** produce only very small amounts of the toxin. One theory to explain the lack of toxin generated by bacterial cultures is the necessity of signaling molecules from the host organism. However, it is also possible that the bacteria that produce the bulk of the toxin cannot be cultured. A study aiming to isolate TTX from newts led to the discovery of 1-hydroxy-5,11-dideoxytetrodotoxin, **3**. Inferring that this material was a biosynthetic precursor of **1**, the authors proposed that **1** derives from the combination of arginine with isopentenylpyrophosphate, rather than a more oxidized precursor (Scheme 2). [13] However, studies attempting to generate radiolabeled **1** through feeding

experiments were not successful.[14] A detailed review of biosynthetic considerations with respect to **1** is available.[15]



Scheme 2. Proposed biosynthetic origins of **1**

A brief mention of the pioneering structural studies of **1** by Woodward,[16] Tsuda,[17] and others,[18] is in order. One early piece of structural information was the observation that treatment of TTX with aqueous NaOH, followed by Ac_2O, yields a quinazoline called the Singer acetate, **4**. (Scheme 3). However, the structural formula of the natural product was not known with certainty, and it was estimated to be in the range of $C_{10\text{-}12}H_{15\text{-}19}O_{8\text{-}10}N_3$. Compounding this uncertainty was the rudimentary nature of the analytical methods available at the time. Single-crystal x-ray



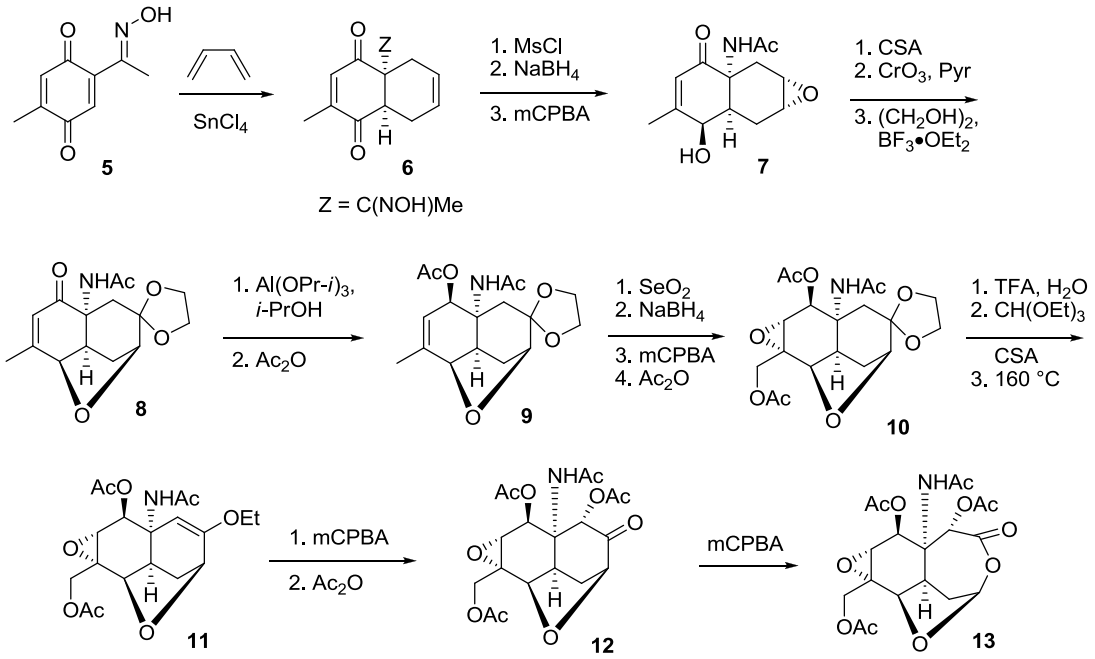
Scheme 3. Formation of the Singer acetate **4** from **1**

analysis, infrared and primitive ^1H NMR spectroscopy, additional degradation work, and isotopic exchange studies eventually yielded enough information to allow determination of the molecular

formula and arrangement of the functional groups present in **1**. A final puzzling aspect of these investigations was that all available evidence indicated the presence of a carboxy group in the molecule; yet no carbonyl absorption appeared in the IR spectrum of **1**. This led to the correct inference that TTX incorporates an ortholactone moiety (cf. Scheme 1): the first instance of such a functional group being ever detected in any natural or unnatural molecule.

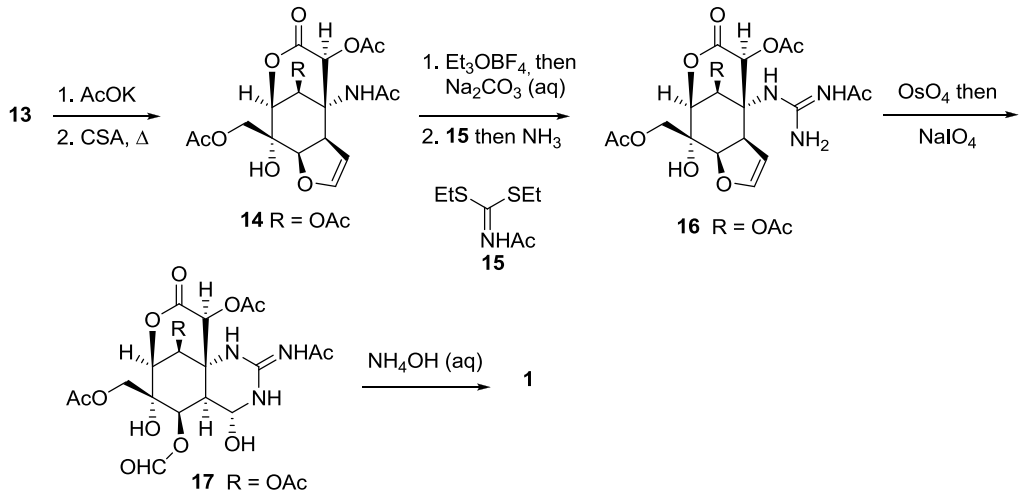
Total syntheses of TTX

The Kishi synthesis of (±)-tetrodotoxin,[19] the first synthesis of the natural product, is remarkable for its brevity. Only 28 linear steps from **5** were needed, which compares quite favorably with all later syntheses. The route began with a Diels-Alder reaction between **5** and butadiene to provide diketone **6**. This material underwent a Beckmann rearrangement in the presence of MsCl to provide the key tertiary amide at an early stage of the synthesis. Oxidation state adjustments then gave epoxide **7**. At this point, acid-catalyzed ether formation, oxidation and selective ketone protection led to **8**. This material then underwent Meerwein-Ponndorf-Verley reduction and acetylation of the emerging alcohol, giving **9**. The alkene in **9** served as a lynchpin for the ultimate introduction of three hydroxyl groups through a sequence that commenced with allylic oxidation (SeO_2), reduction ($NaBH_4$) and epoxidation (mCPBA). This was followed by release of the ethylene ketal and Baeyer-Villiger oxidation of **12** to give strained lactone **13**.



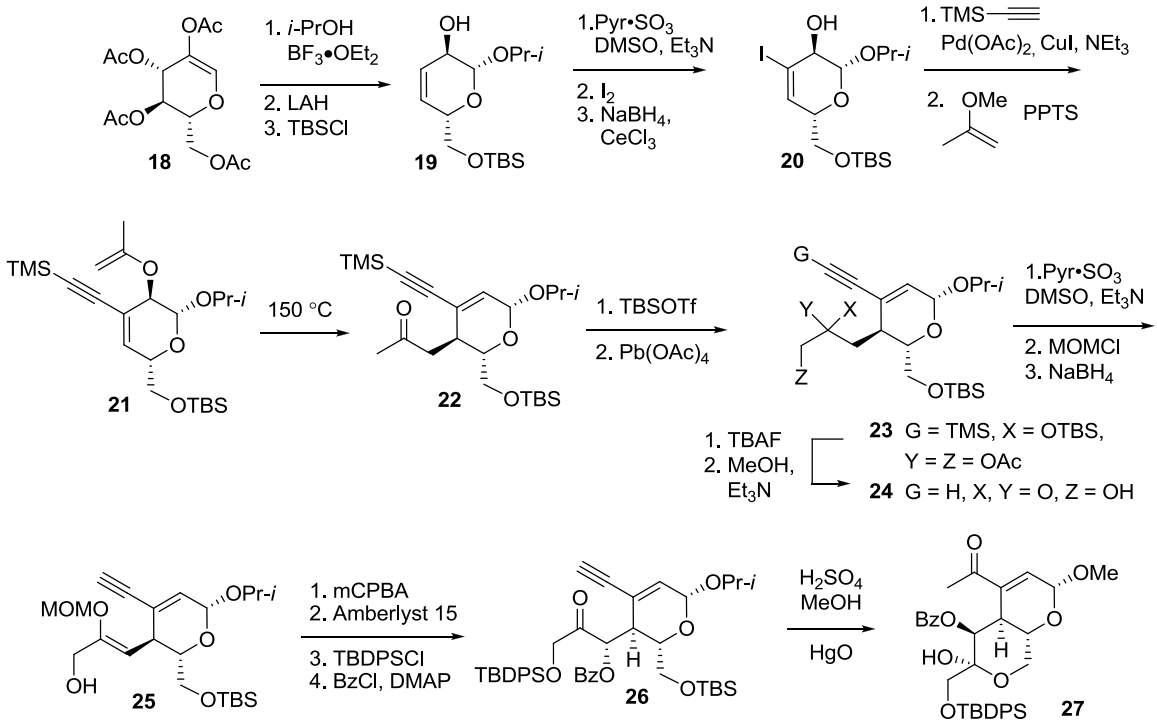
Scheme 4. Opening moves of Kishi's synthesis of **1**

Substance **13** was primed for a series of transformations which led to **1**. To this end, hydrolysis of the lactone and acidification caused transannular carboxylate attack on the epoxide, and dehydration of an intermediate hemiacetal to furnish **14**. The latter was subjected to amide cleavage using Meerwein's salt followed by alkaline aqueous conditions. The guanidine was then installed by a two-step procedure, which set the stage for Lemieux-Johnson oxidation of the vinyl ether to provide **17**. Ammonolysis of this compound removed acetate and formyl groups to complete the total synthesis of **1**. In summary, Kishi's landmark synthesis required 28 linear steps starting from **5,** producing **1** in 0.66% yield.



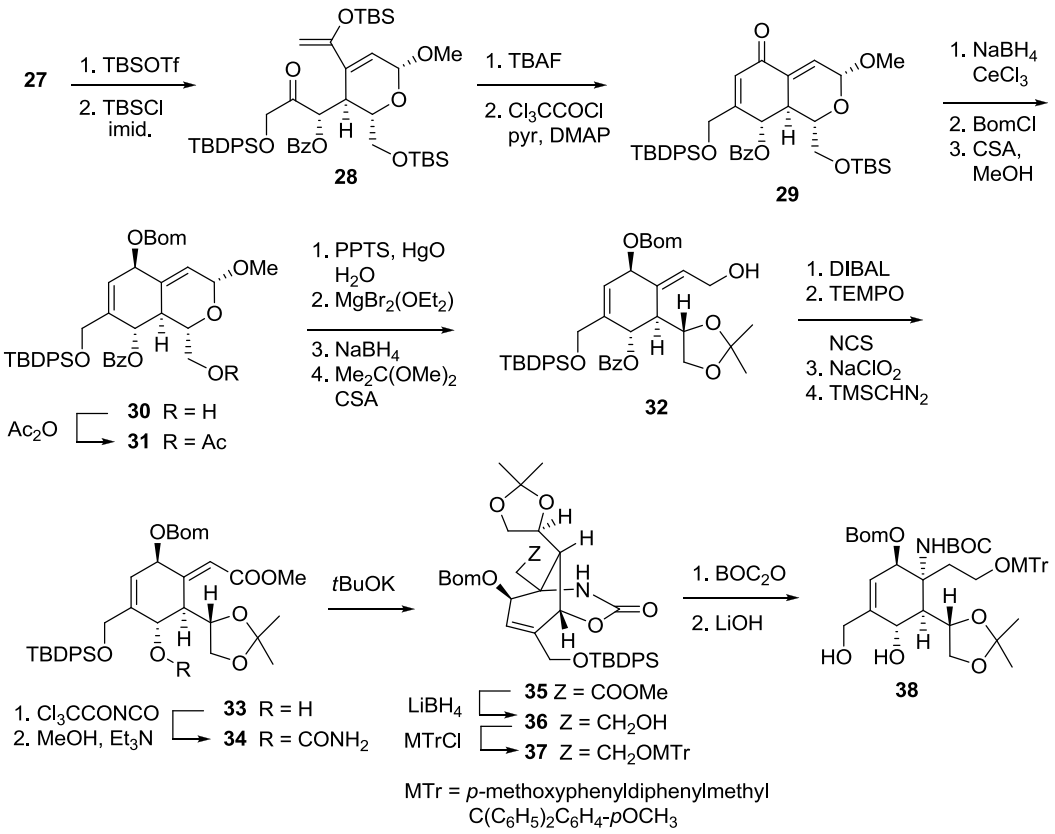
Scheme 5. Completion of Kishi's total synthesis of **1**

Thirty years later, Isobe completed a second total synthesis of **1**.[20] The route began with glucal **18**,[21] a derivative of D-glucose. The first major objective was the creation of **21**, the precursor for a key Claisen rearrangement. First, **18** was advanced to **19** by dehydration, glucoside installation, and protecting group adjustment. The resultant **19** was then oxidized, treated with iodine, and reduced to provide **20**. This material provided an appropriate substrate for coupling with TMS acetylene and vinyl ether formation. The resultant **21** underwent thermal rearrangement to **22**, and the emerging ketone **22** was advanced to the kinetic silyl enol ether derivative, in preparation for bis-acetoxylation leading to **23**. A somewhat circuitous sequence then produced MOM enol ether **25**. A Rubottom-type oxidation of the latter with mCPBA occurred diastereoselectively, and protection of the intermediate dihydroxyketone yielded **26**. Treatment of this material with H_2SO_4 and HgO in MeOH induced cleavage of the TBS ether, hemiacetal formation, and alkyne hydration.



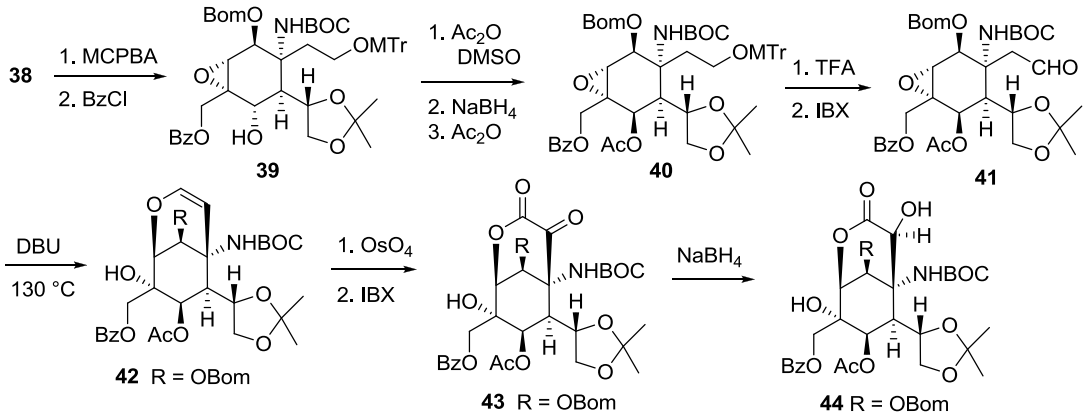
Scheme 6. Early stages of Isobe's total synthesis of **1**

Methyl ketone **27** was then converted into enol silane **28**, which underwent intramolecular aldol addition and subsequent dehydration to **29**. The next twelve steps of the synthesis involved a series of protecting group and oxidation state adjustments, resulting in primary alcohol **33**, which was advanced to the corresponding carbamate **34**. The latter was subjected to deprotonation by *tert*-BuOK, causing transannular 1,4-addition of the NH_2 subunit to the unsaturated ester and providing **35** in an efficient manner. At this point, the pendant ester was no longer needed, and it was reduced to an alcohol, which was protected to give **37**. The cyclic carbamate was activated as an *N*-BOC-derivative and cleaved under basic conditions to give **38**.



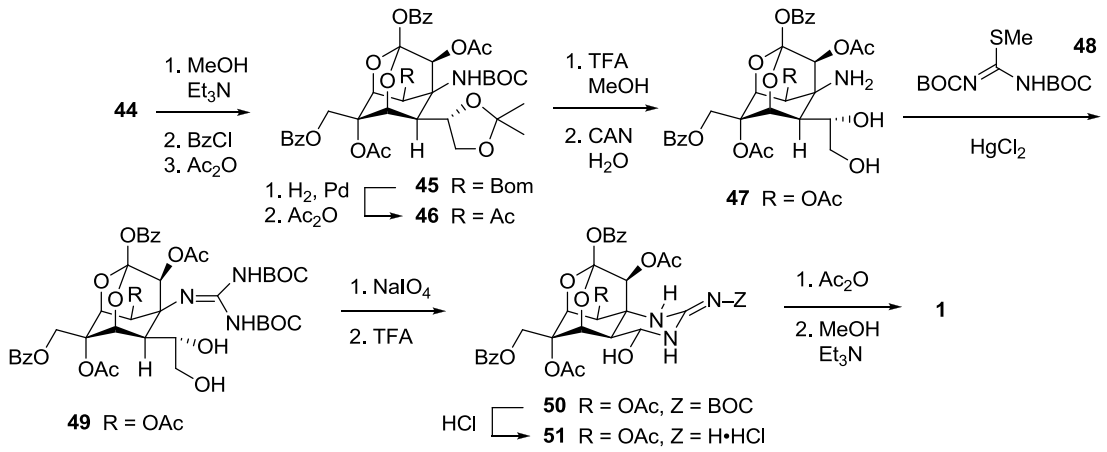
Scheme 7. Isobe's total synthesis of **1**

The next objective was the elaboration of **38** to aldehyde **41**. This material was reached upon epoxidation of the olefin using mCPBA, further protecting group manipulations, and final IBX oxidation. An unusual transannular cyclization occurred when **41** was treated with DBU at high temperature. Thus, the presumed enolate of the aldehyde reacted at the *O*-terminus with the epoxide to return vinyl ether **42**. Osmylation of the latter occurred with the incorrect facial selectivity, necessitating a redox sequence to produce the desired diastereomer **44** of the ultimate α-hydroxylactone.



Scheme 8. Isobe's total synthesis of **1**

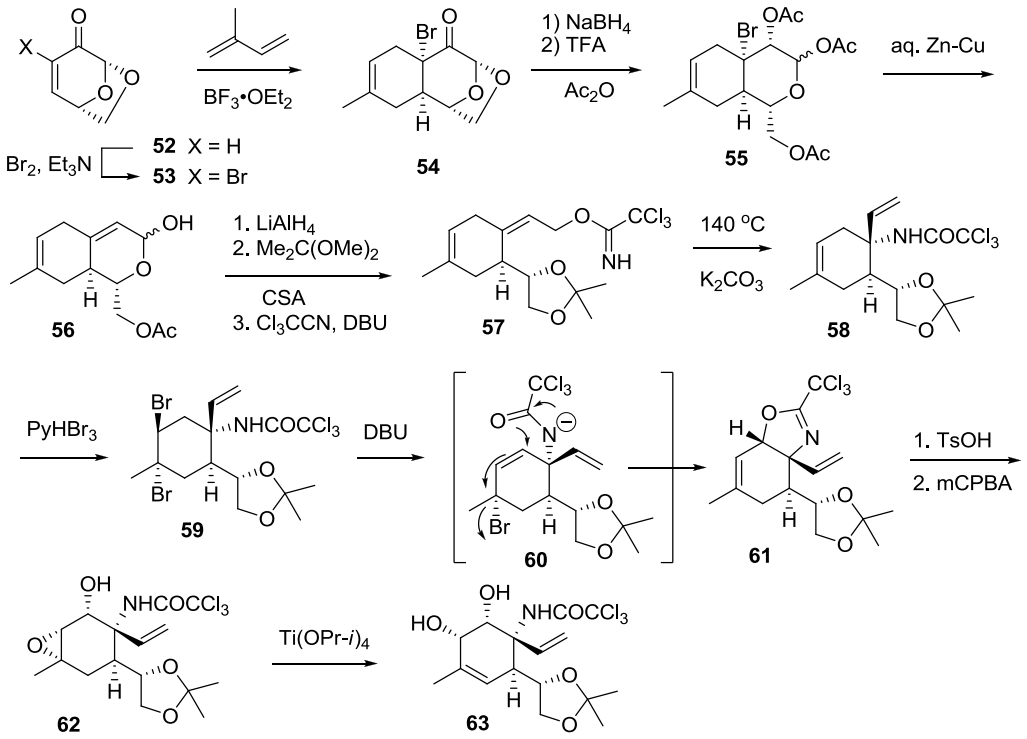
The final stages of the synthesis entailed removal of the acetyl protecting groups, which triggered closure of the ortholactone, followed by a series of protecting group manipulations to yield **46**. Finally, after acetonide and *N*-BOC cleavage using TFA, the emerging **47** was treated with guanylating reagent **48** and $HgCl_2$. The diol moiety was then cleaved using $NaIO_4$. Subsequent BOC, acetyl, and benzoyl protecting group cleavage completed the first asymmetric total synthesis of **1** in 67 steps, producing **1** in 1.22% yield.



Scheme 9. Completion of Isobe's total synthesis of **1**

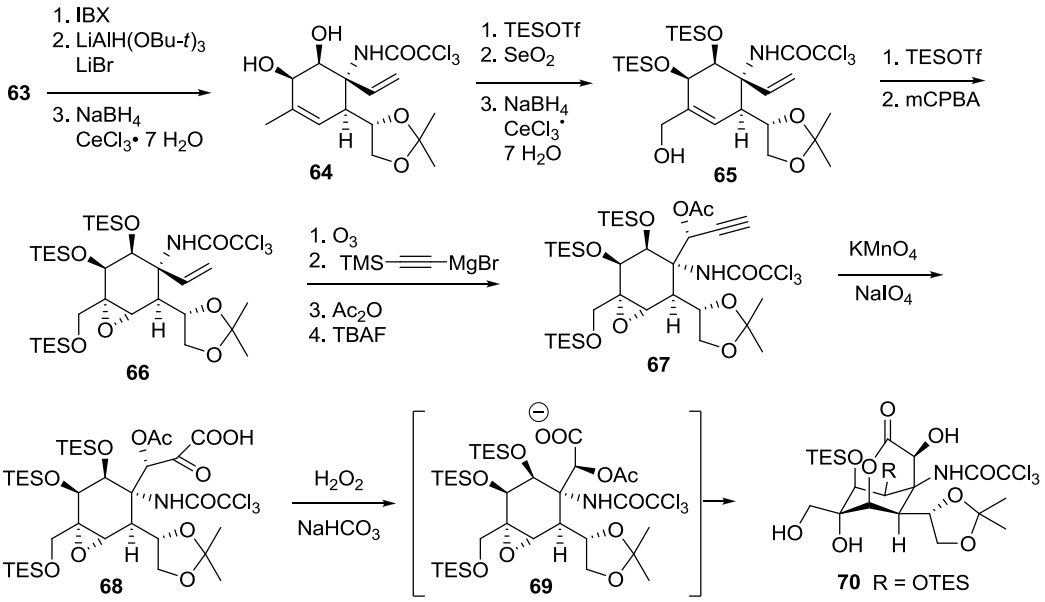
Isobe sought to improve his synthesis, publishing a second route the following year. The second effort rectified certain weaknesses, in particular, the relatively high step count. The successful incorportation of an Overman rearrangement to install the nitrogen functionality was beneficial in this regard. The trichloroacetamide thus introduced at an early stage of the synthesis conveniently survived all subsequent operations.

In the early phases of the synthesis, ketone **53**[22] underwent Diels-Alder cycloaddition with 2-methylbutadiene in the presence of BF_3•OEt_2. The product was advanced to **55**, which upon Boord olefination using Zn-Cu couple afforded **56**. Three more steps delivered Overman precursor **57**, rearrangement of which at 140 ^oC in the presence of K_2CO_3 yielded product **58**. Bromination of the olefin and treatment of **59** with DBU promoted a tandem E2 reaction – S_N2' displacement, whereby the trichloroacetamide cyclized to oxazoline **61**. Hydrolysis, selective epoxidation, and acid-catalyzed epoxide opening provided tetraol **63**.



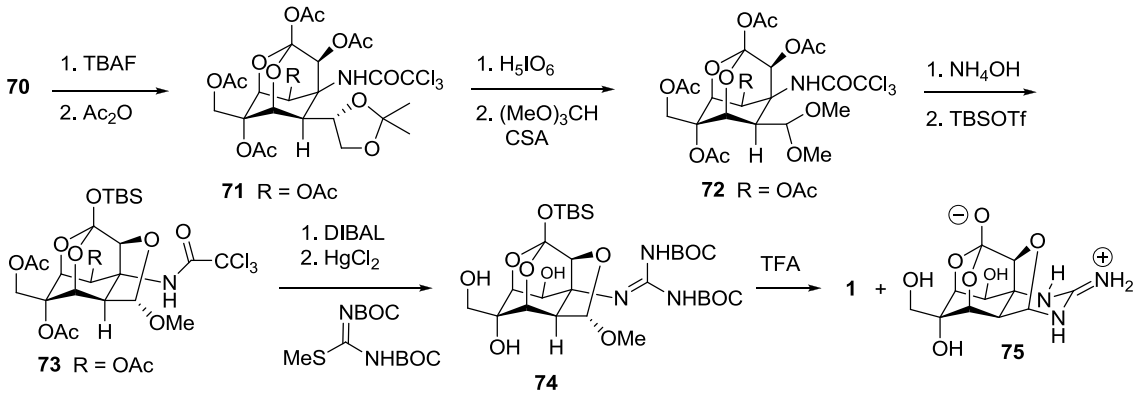
Scheme 10. Second Isobe synthesis of **1**

Unfortunately, both free alcohols of **63** require inversion of configuration to match that of **1**. Accordingly, **63** was treated with IBX, followed by a two-step reduction to give **64**. Protection and further oxidation (SeO_2 followed by $NaBH_4$) provided **65**. The next several steps were necessary to convert the olefin of this latter material into the ortholactone carbon and accompanying hydroxyl group of **1**. This proved to be a non-trivial proposition. Eventually a route was charted to α-ketoacid **68**, which, upon treatment with H_2O_2, underwent oxidative cleavage and concomitant epoxide opening by the free carboxylate to give bridged lactone **70**.



Scheme 11. Second Isobe synthesis of **1**

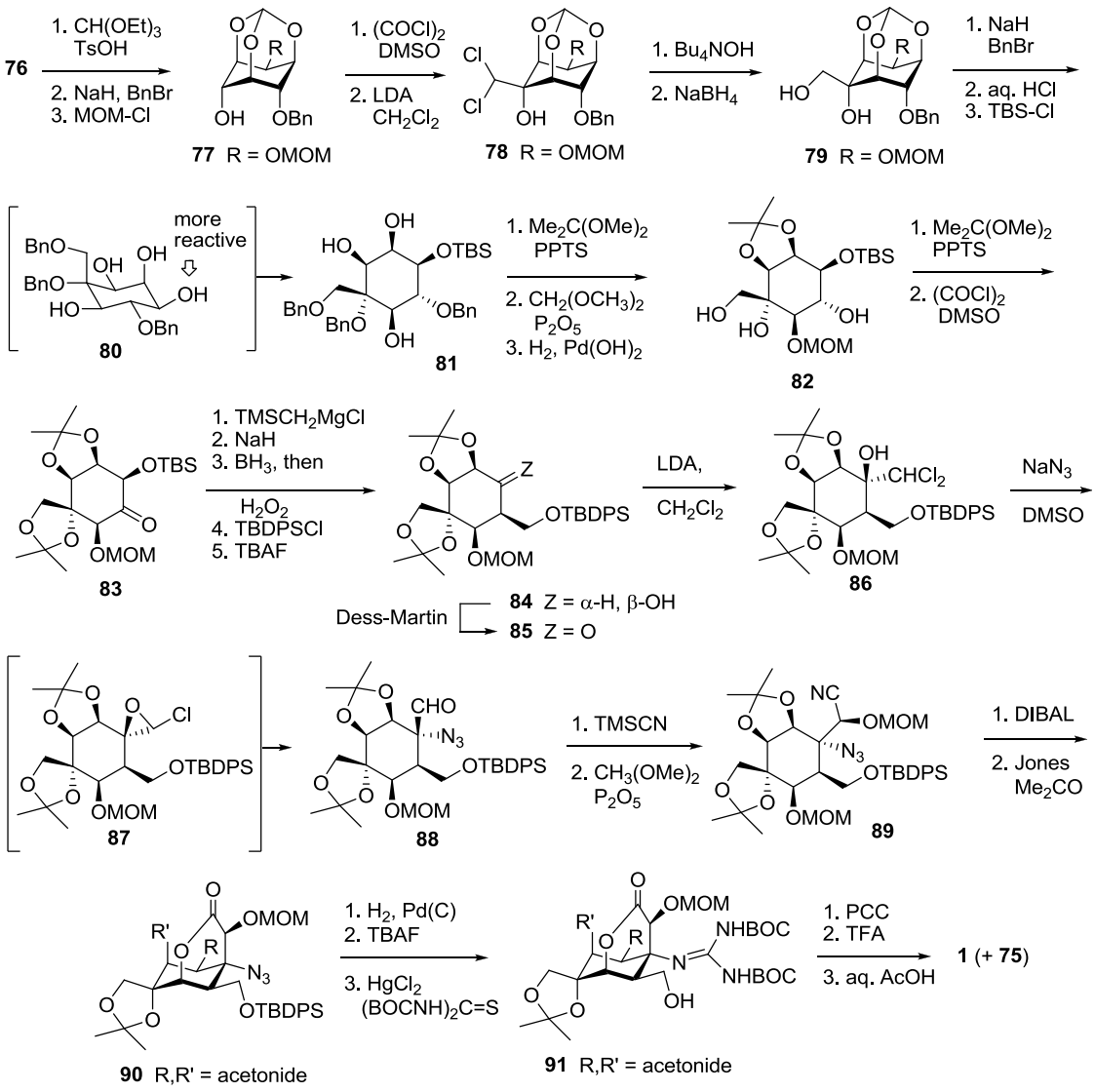
The total synthesis was completed as shown in Scheme **12**. First, TES cleavage using TBAF and exhaustive acetylation gave ortholactone **71**. Next, oxidative cleavage of the acetonide diol proceeded using periodic acid, and this operation was followed by a few corrective protection steps. Reductive deprotection of the trichloroacetamide and acetyl groups, followed by guanylation, and TFA treatment gave **1**. The final compound was obtained as a mixture incorporating a small amount of dehydrated material **75**.



Scheme 12. Second Isobe synthesis of **1**

Sato and collaborators were able to complete another synthesis of this challenging target starting from *myo*-inositol, **76**.[23] A series of protection steps produced **77**, which served as a substrate for the installation of hydroxymethyl unit, by way of dichloromethyllithium addition to a ketone obtained by Swern oxidation of **77** (cf. **77** to **78**). The action of base on the resultant **78** produced an α-hydroxyaldehyde, which was reduced ($NaBH_4$) to give **79**. Benzylation of the alcohols and release of the orthoester protection preceded an interesting, selective TBS protection to provide **81**. This selectivity may be rationalized by invoking preferential reaction from conformer **80**, which undergoes silylation at the least hindered equatorial alcohol. Protecting group manipulations and Swern oxidation of **82** then surrendered ketone **83**, Peterson reaction of which produced a methylene derivative. Hydroboration-oxidation of the latter, protection of the resulting primary alcohol, and selective excision of the TBS group returned **84**. Dess-Martin oxidation set the stage for a second round of dichloromethyllithium addition to provide **86**. An interesting transformation developed earlier by the authors ensued upon treatment of **86** with NaN_3 in DMSO: first, epoxide formation occurred to generate transient species **87**, which was attacked by azide ion at the tertiary center to give aldehyde **88**.

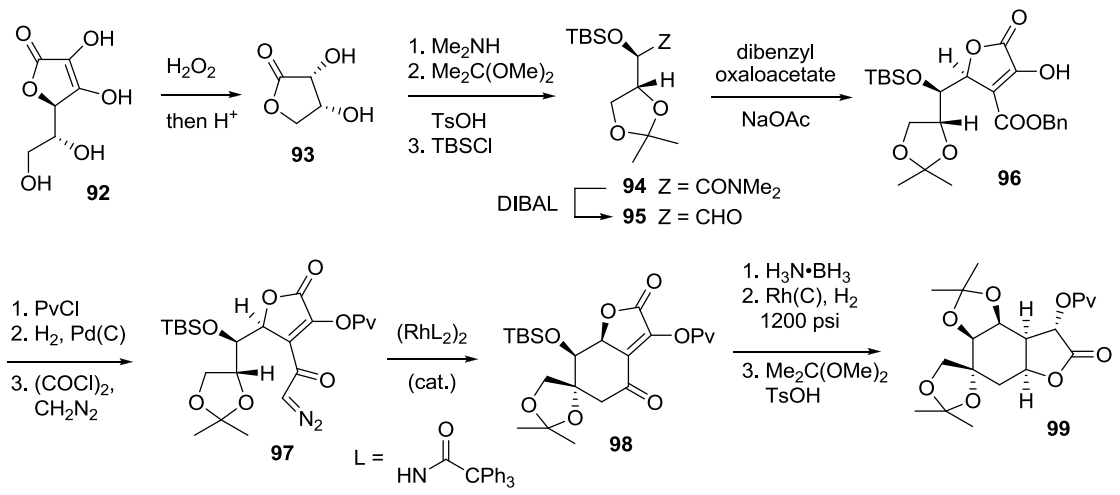
Cyanohydrin formation took place diastereoselectively to form **89**. Nitrile reduction with DIBAL and oxidation of the emerging aldehyde with chromic acid provided lactone **90** directly. A few final steps completed the 33 step synthesis of **1**. The same group later disclosed two alternative routes to enantiopure ketone **85**.[24]



Scheme 13. Sato's total synthesis of **1**

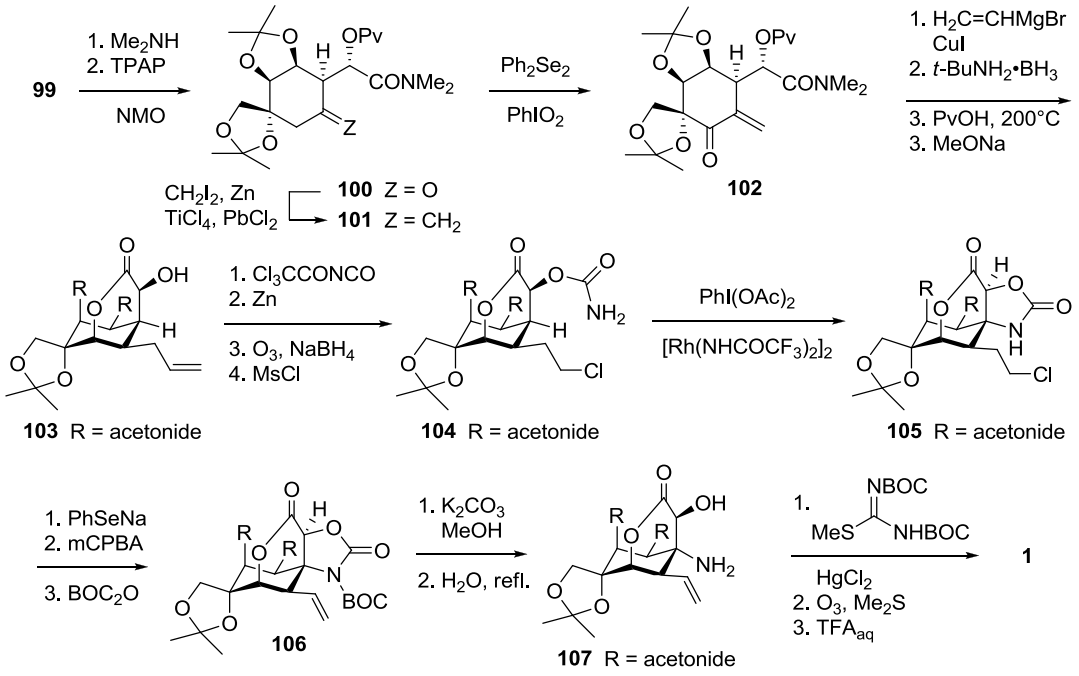
Following Sato's publication, DuBois described a conceptually novel synthesis of TTX[25] that hinged upon a Rh(II) catalyzed nitrene insertion into a C-H bond (technology developed earlier by the same group)[26] as a way to install the nitrogen functionality. The planned late-stage execution of this step demonstrates a notable confidence in the reaction.

DuBois' synthesis began with the conversion of D-isoascorbic acid derivative **92** into aldehyde **95** through well-documented chemical transformations. Aldehyde **95** was then combined with dibenzyloxaloacetate to give **96**. The latter compound was converted into diazo derivative **97** in three steps. Treatment of this material with a Rh(II) catalyst promoted carbene C-H insertion to give **98** in a completely diastereoselective manner. Reduction and protecting group adjustment furnished **99**.



Scheme 14. DuBois' total synthesis of **1**

The next subgoal was the synthesis of carbamate **104**, which would serve as a precursor for the critical nitrene insertion. Lactone **99** was thus opened using dimethylamine, and the liberated alcohol was oxidized to a ketone. Tebbe methylenation and allylic oxidation produced enone **102**. An eight-step sequence, key phases of which included a conjugate addition of a vinylcopper reagent and a stereoselective carbonyl reduction with borane-*tert*-butylamine complex, afforded **103**, and thence key intermediate **104**. This carbamate was treated with Rh(II) trifluoroacetamide and (diacetoxyiodo)benzene ("DIB") to form oxazolidinone **105**, displaying the crucial C-N bond of **1**. Straightforward manipulations advanced **106** to **107**, from which (−)-**1** was reached by established methods. The 31 step linear synthesis produced (-)-**1**



Scheme 15. Completion of DuBois' total synthesis of **1**

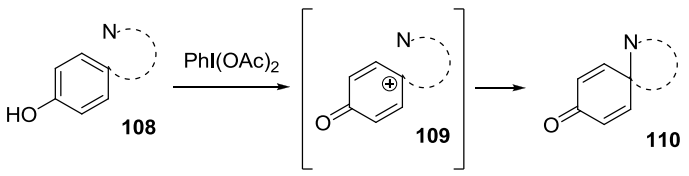
In addition to the foregoing total syntheses, the literature records numerous synthetic studies toward TTX. Space limitations do not allow a thorough illustration of these important investigations, for which the reader is referred to a recent, comprehensive review on the subject.[27] It is clear from this article, as well as from the preceding discussion, that a significant body of work has arisen over the past few decades in pursuit of an efficient laboratory synthesis of **1**. Though significant strides have been made in this area, the current state of the art – even DuBois' brilliant synthesis – is unable to outdo Kishi's route. A more concise avenue to **1** would benefit medical research, in that it might enable the conduct of medicinal chemistry studies that could lead to valuable therapeutic resources. It would be difficult to carry out such investigations using the natural product as the starting point, given its reactivity and its extremely hazardous nature.

Synthetic methodology developed in our group appears to offer interesting opportunities in the tetrodotoxin area. The following paragraphs provide an outline of the new technology and illustrate how the latter could be meaningfully harnessed to achieve a concise synthesis of **1**.

2. Retrosynthetic Considerations

The Oxidative Amidation of Phenols

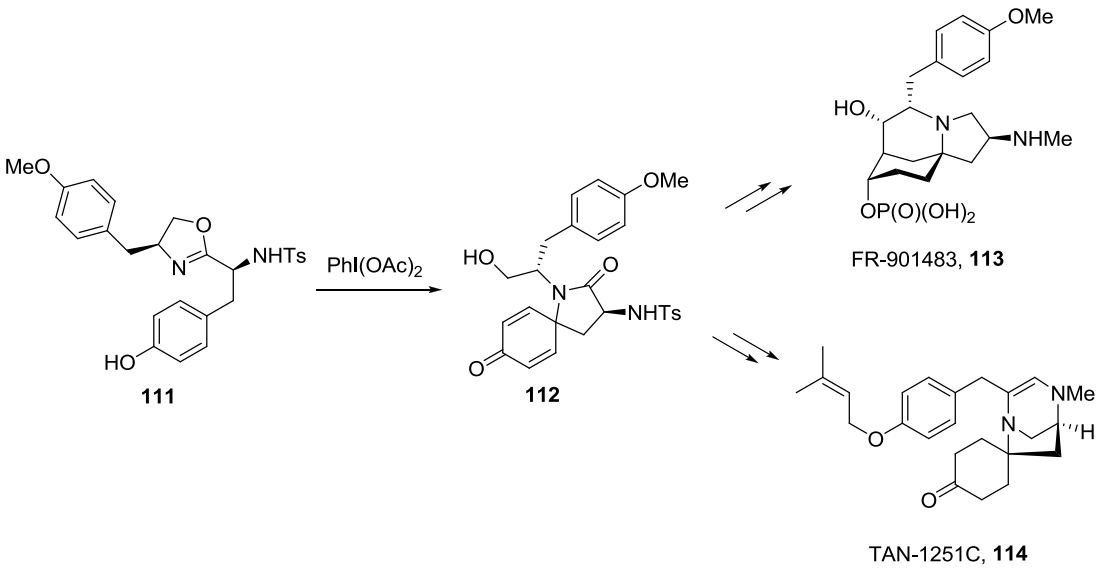
The oxidative amidation of phenols[28] is a process whereby a generic phenol, typically a 4-substituted one such as **108**, is converted into a spirodienone, **110** (Scheme 16). Group N in **108** represents a suitable nitrogen nucleophile, while the dashed curve indicates that "N" may be tethered to the aromatic nucleus or it may be independent. In the first case, the reaction will occur in the intramolecular regime; in the second, in the bimolecular mode. The transformation is induced by a hypervalent iodine reagent such as DIB, the action of which upon the substrate produces an electrophilic intermediate, naively rendered in Scheme 16 as cation **109**. Capture of **109** by the N functionality produces the ultimate **110**, wherein N always emerges in the form of an amide; hence the terminology "oxidative amidation." This transformation appears to be quite useful in the synthesis of nitrogenous natural products, in that simple phenols may be rapidly converted into products of significant molecular complexity.



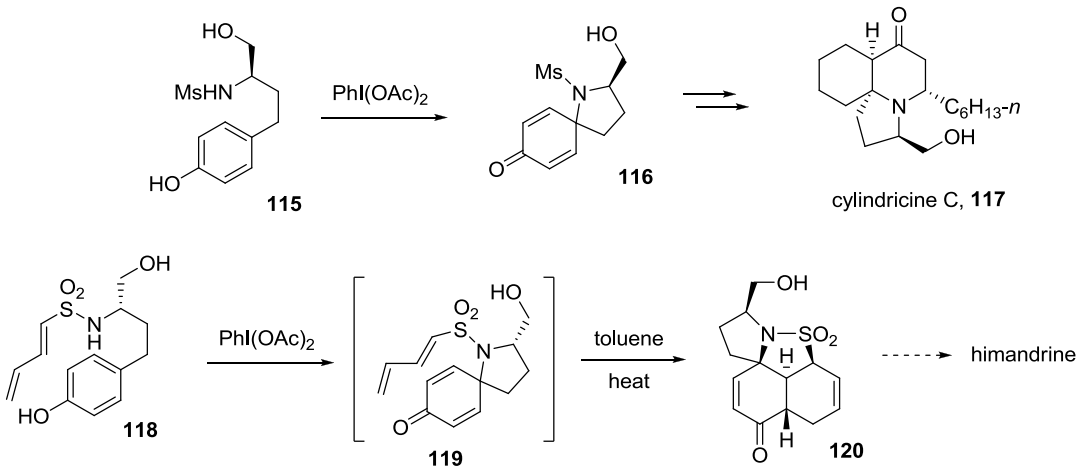
Scheme 16. Oxidative amidation of phenols

An early variant of the reaction used an appended oxazoline as the nitrogen nucleophile. This methodology constituted the centerpiece of a synthesis of the natural products FR-901483 and TAN-1251C (Scheme 17). Later work revealed that sulfonamides were also competent

trapping agents.[29] Such a reaction was used in the total syntheses of cylindricine C[30] and putative lepadiformine (Scheme 18).[31] The latter mode of reaction also proved useful for the assembly of the core ring system of himandrine.[32]

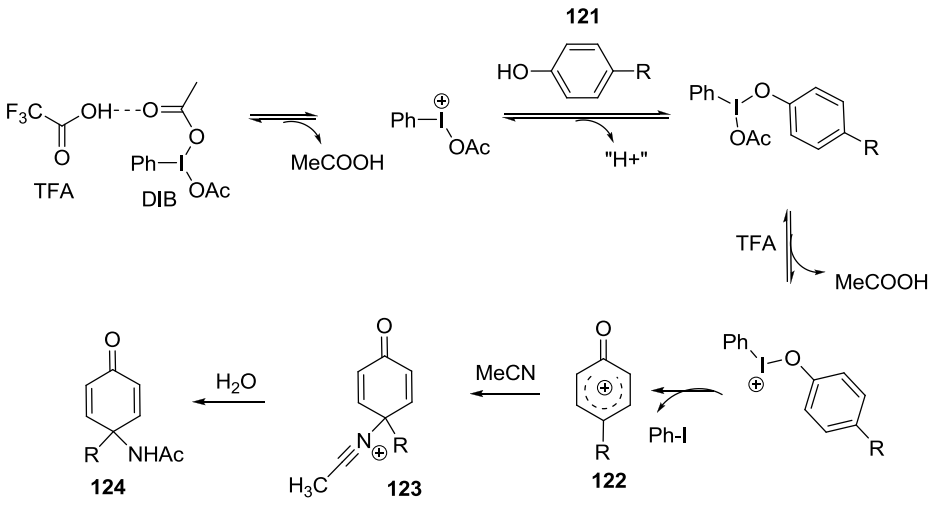


Scheme 17. The oxidative amidation of phenols using oxazolines as trapping agents



Scheme 18. The oxidative amidation of phenols using sulfonamides as trapping agents

The bimolecular variant of the reaction resorts to acetonitrile as an external nitrogen nucleophile, which presumably captures a cation such as **122** in a Ritter-like fashion (Scheme 19).[33] The process was found to occur most efficiently upon *slow* addition of a CH_3CN solution of phenol to a *dilute* CH_3CN solution of DIB and TFA.[34] Best results were obtained when the final concentration of phenol was around 110 mmol/L. It is likely that, at higher concentration, reactive species **122** is captured by the starting phenol **121**, leading to undesired polymeric products, while lowering the yield. A slight molar excess of TFA relative to DIB is necessary for rapid and complete dissolution of the hypervalent iodine reagent and efficient conversion. However, larger amounts of TFA lower the yield and complicate isolation procedures. In its current form, the protocol allows practical large-scale preparation of products **124** in an economical and expedient manner.



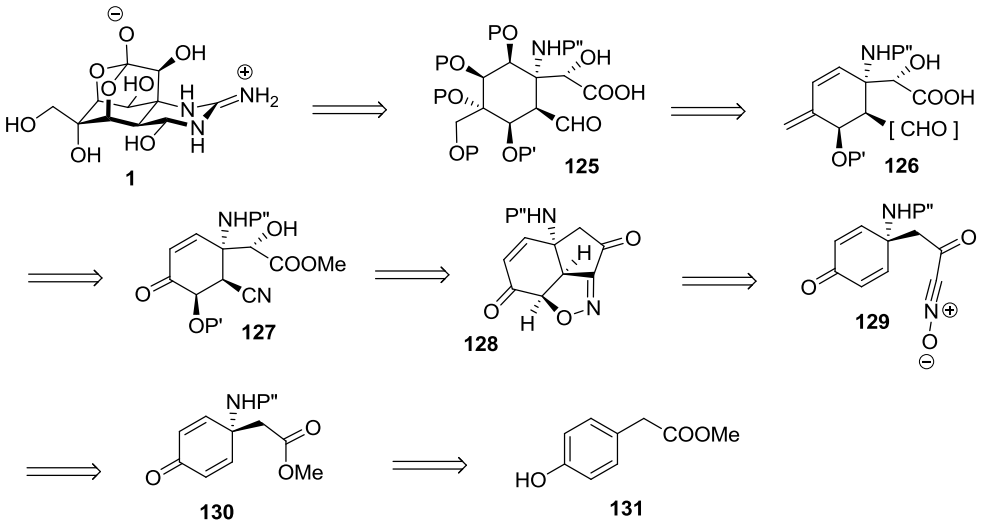
Scheme 19. Possible mechanism for the bimolecular oxidative amidation of phenols

The approach to tetrodotoxin detailed in this thesis is based on a bimolecular oxidative amidation as a key step, according to the retrosynthetic logic developed in the next paragraph.

Retrosynthetic Analysis

Excision of the guanyl segment in **1** and release of the ortholactone reveal a highly oxygenated cyclohexane precursor, rendered in Scheme 20 as protected polyol **125** (P, P', P" = suitable blocking groups). Recognizing that, in principle, substituents OP could be installed through a stereoselective bis-dihydroxylation of a diene, we recognized compound **126** as a forerunner of **125**. The CHO group in brackets indicates that the formyl substituent in **126** may be expressed, or protected, or latent. In particular, if one were to conceive a nitrile as a precursor of the aldehyde, and a ketone as that of the exomethylene segment, compound **126** may be further simplified to structure **127**. An interesting opportunity materializes at this juncture: substance **127** appears to be available through nucleophilic fragmentation of isoxazoline **128**,

which is the result of an intramolecular nitrile oxide cycloaddition ("INOC") of **129**. Furthermore, the carbonyl group in the cyclopentane segment of **128** should enable the subsequent introduction of the OH functionality found at the α-position of the ester in **127**, further simplifying the molecule. A sensible starting point for the generation of **129** would be dienone **130**, or an opportune derivative thereof, easily recognized as the product of bimolecular oxidative amidation of phenol **131**.

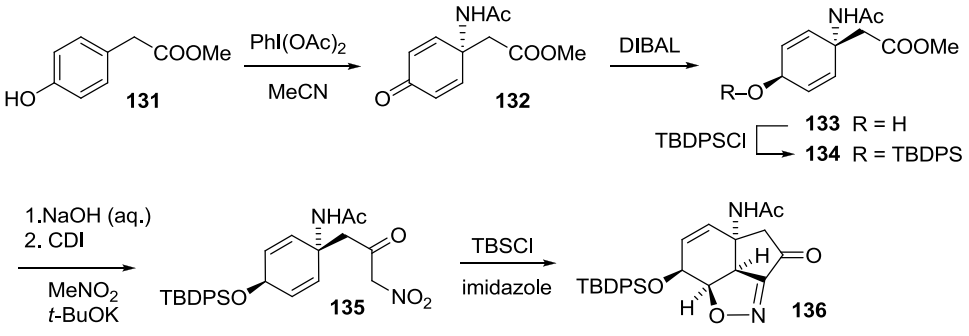


Scheme 20. Retrosynthetic analysis

On paper, this strategy could translate into a total synthesis of **1** in about 25 steps from commercial **131**: a significant improvement over all known routes. Although a total synthesis has yet to be achieved, the approach has brought us close to the ultimate goal, as detailed in the following chapter.

3. Synthetic Work

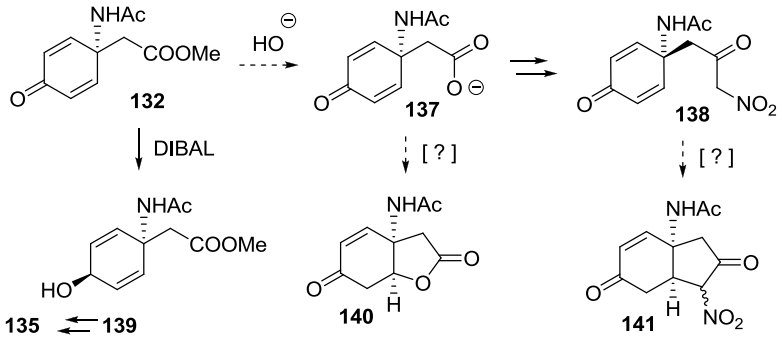
Full execution of the above strategy necessitated significant amounts of exploratory work. Former group members, Drs. B. Mendelsohn and C. Benhaim, had determined that ester **132**, prepared by them in low yield by an oxidative amidation procedure subsequently shown to be inappropriate, could be elaborated into dienic alcohol **134** in modest yield according to the method of Scheme 21.[35] Nitro compound **135** was amenable to conversion into a reactive dipole (not necessarily a nitrile oxide; *vide infra*) by the method of Torssell (TBS-Cl), resulting in the ultimate formation of isoxazoline **136** in moderate and variable yield. Finally, the nucleophilic cleavage of **136** was problematic, generally proceeding in no more than ca. 40% yield. Clearly, the procedure was entirely unsuitable for a synthetic attack on **1**. Consequently, effort was focused on developing a reliable method to readily synthesize **136** in large scale: an objective that demanded the optimization of every single step from **131** to the subgoal isoxazoline.



Scheme 21. Previous preparation of isoxazoline **136** via INOC

The first issue to be addressed was the scale-up of the oxidative amidation of **131**. The quantities of **132** required for the synthesis ruled against any protocol involving chromatography. Compound **132** also happens to be quite polar and water soluble, suggesting that a non-aqueous reaction workup may advantageously minimize losses. Fortunately, **132** is a nicely crystalline material which, arguably, could be retrieved from crude reaction mixtures merely by crystallization. A robust procedure for its preparation ultimately emerged as follows. A solution of **131** was added over 3 h (syringe pump) to a solution of DIB in MeCN containing 1.3 equiv. of trifluoroacetic acid (TFA). TFA serves both as a protonic catalyst and as an agent that transforms MeCN-insoluble DIB into a soluble, more reactive oxidant; probably $PhI(OAc)OCOCF_3$. The oxidation of the phenol to **132** is essentially instantaneous under these conditions. The reaction mixture is then evaporated and the residue is twice filtered through a short plug of silica gel to remove nonpolar byproducts and impurities. Evaporation of the percolate, dissolution of the residue in EtOAc, and cooling to -20 ^{o}C result in deposition of crystals of analytically pure **132**. This procedure minimizes the use of solvents, it avoids chromatography and aqueous washes, and it performs well on scales of up to ca. 10 g of material.

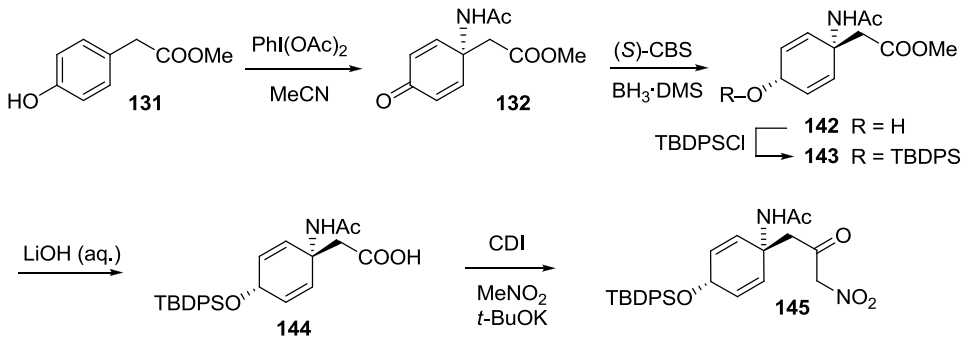
The successful conclusion of this initial investigation induced us to refocus our efforts on the elaboration of **132** to **136**. The previously devised route to **136** necessitates a reduction of the dienone to prevent undesirable Michael cyclization of either acid **137** or of **138** itself (Scheme 22). Such a reduction was traditionally carried out with DIBAL, resulting in selective formation (ca. 4:1) of that diastereomer of the alcohol, wherein the OH and NHAc groups are *trans* (Scheme 22; confirmed by X-ray crystallography of a derivative).[36] This step is fraught with



Scheme 22 Undesirable Michael cyclization of synthetic intermediates **137** and **138**

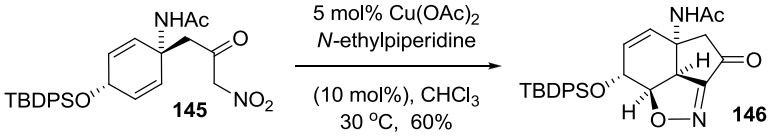
numerous technical difficulties, due to the very polar nature of alcohol **139**, its aqueous solubility, and its ability to coordinate Al(III) species. These properties conspire to severely diminish the overall yield of **139**. The search for an improved reductive method revealed that the Corey-Bakshi-Shibata (CBS) procedure suppressed all of the above problems, while selectively producing the *cis* diastereomer of the alcohol (ca. 8:2; Scheme 23). The observation that DIBAL and CBS reductions produce opposite stereochemical outcomes was initially made by a former group member, Dr. H. Liang.[37] A cogent rationale for this phenomenon remains elusive, but the selective formation of the *cis* stereoisomer was welcome news, in that it subsequently transpired that this diastereomer was more compatible with later steps (*vide infra*).

Nitroketone **145** was obtained from **132** by a series of steps similar to those employed earlier for the synthesis of **135**. The *cis* series was used in all subsequent operations.



Scheme 23 Preparation of *cis*-nitroketone, **145**

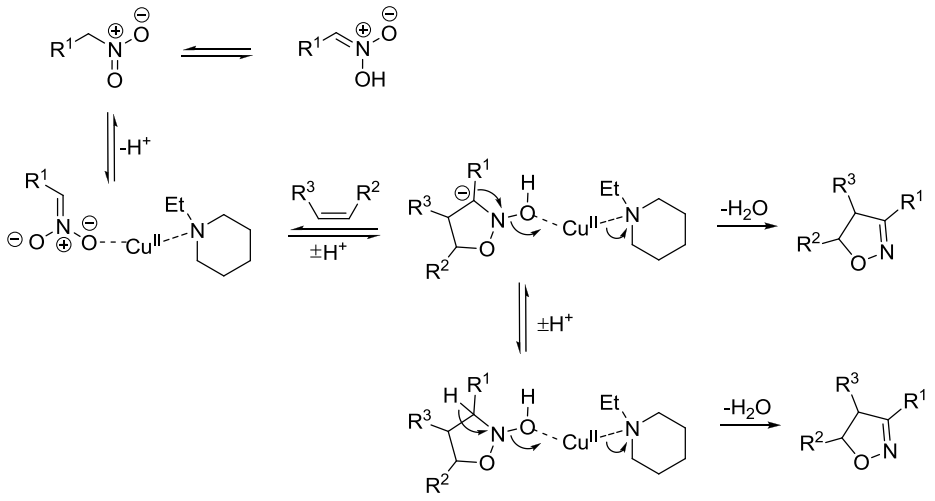
As indicated earlier, the nitrile oxide cycloaddition sequence required considerable improvement. In order to advance the work of previous group members, optimization of the INOC was necessary. A remedy was identified in the form of a DeSarlo reaction.[38] Pleasingly, treatment of **145** with catalytic amounts of copper (II) acetate and *N*-ethylpiperidine in freshly distilled chloroform at 30 °C induced slow cyclization to **146** in 60% yield. The use of purified solvent was essential to the success of this step (see experimental section for details), which was reliably operable on scales of up to six grams. Operation at low substrate concentration improved the yield of the cycloaddition by minimizing polymer formation: best results were obtained when the concentration of the substrate was around 200 mmol/L.



Scheme 24. Large scale preparation of isoxazoline **146**

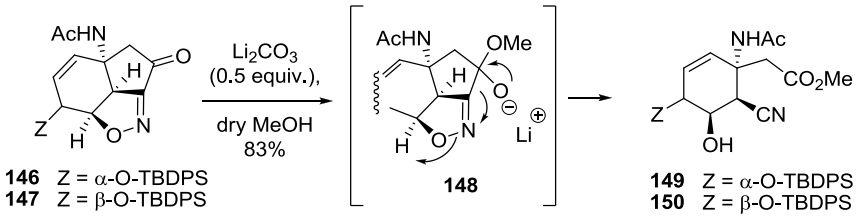
Temperature control was essential to ensure a satisfactory yield. Indeed, heating the reaction to 60 °C (original DeSarlo procedure) resulted in mostly polymeric material, with only a

trace amount of **146**. Maintaining the reaction at 30 ^{o}C maximized the formation of the desired cycloadduct, even though the cycloaddition was slow, requiring around 6.5 days for completion (some starting **145** remained even after this time). On a positive note, isoxazoline **146** was the sole reaction product. Equally important was the choice of base: the use of imidazole (11% yield of **146**), DABCO (no product detected), *N*-methylmorpholine (20% yield of **146**) in lieu of *N*-ethylpiperidine gave inferior results. A plausible mechanism for the process is presented in Scheme 25. DeSarlo postulated that the addition of Cu(II) reduced the induction time for cycloaddition. Moreover, he suggested that Cu(II) catalyzed the cycloaddition pre-equilibrium and that the rate-determining step was the irreversible dehydration step which led to isoxazoline products. It is interesting to note that *trans* stereoisomer **135** (arising from DIBAL reduction of **132**) tolerated higher reaction temperatures when subjected to DeSarlo cyclization, advancing to **136** in 40% yield after only 2.5 days at 60 °C, with marginal formation of polymeric material.



Scheme 25. INOC catalyzed by base and copper(II)

With quantities of **146** in hand, we turned to the fragmentation of the tricyclic oxazoline in basic methanol. It was rapidly determined that the reason for the poor results obtained by former group members were squarely attributable to the presence of moisture in the solvent. Indeed, when the reaction was performed with methanol freshly distilled from Mg turnings, the desired **149** was obtained in 83% yield (Scheme 26). The diastereomer **147** reacted equally efficiently. By way of mechanism, we presume that the action of Li_2CO_3 on MeOH induces the formation of some MeOLi, which then adds nucleophilically to the strained cyclopentanone carbonyl. This would produce hemiketal **148**, which on the basis of precedent[35] may be anticipated to fragment rapidly as shown.



146 Z = α-O-TBDPS
147 Z = β-O-TBDPS

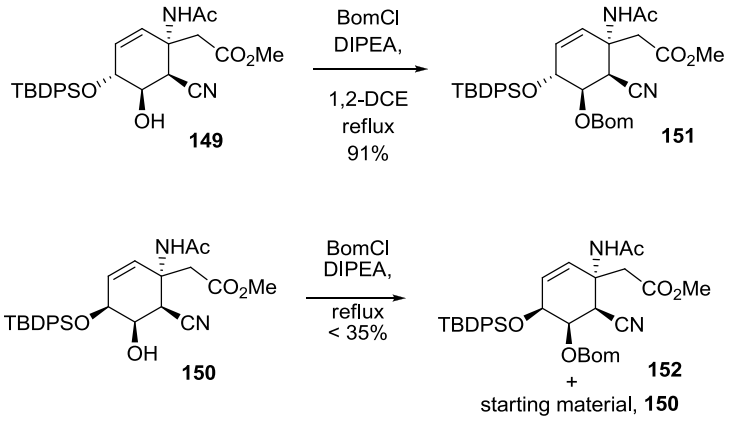
148

149 Z = α-O-TBDPS
150 Z = β-O-TBDPS

Scheme 26. Synthesis of **149** and **150**

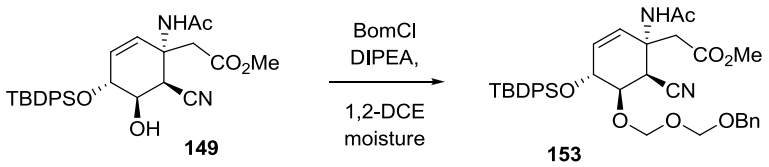
Fragmentation product **149** was sparingly soluble in common organic solvents, and the majority of it was conveniently purified by way of trituration with Et_2O. Such a treatment caused dissolution of some **149** in the ether phase. Therefore, the ether extract was concentrated and the residue was subjected to flash column chromatography to retrieve an additional 10-20% of **149**. In this manner, chromatography of the bulk of **149** was avoided. It is worthy of mention that contrary to **149**, the *trans*-diastereomer (the isomer originating from DIBAL reduction product **133**) is readily soluble in common organic solvents, necessitating a full chromatographic purification. This is one of the respects in which **149** offered advantages over its diastereomer.

Even more dramatic was the fact that whereas a subsequent Bom protection of the OH group in **149** proceeded efficiently to afford **151** in 91% yield after 48 h, the analogous protection of **150** was problematic, surrendering the requisite **152** in no more than 35% yield. Furthermore, for reasons that remain unclear, the blocking of **150** stalled at about 30-35% conversion and none of the remedies thus far examined succeeded in forcing the reaction to completion. This included the use of excess Bom-Cl, of tetrabutylammonium iodide as a promoter, and operation at higher temperatures. A final complication was that chromatographic purification of **152** was difficult as its R_f value was very similar to that of the starting material.



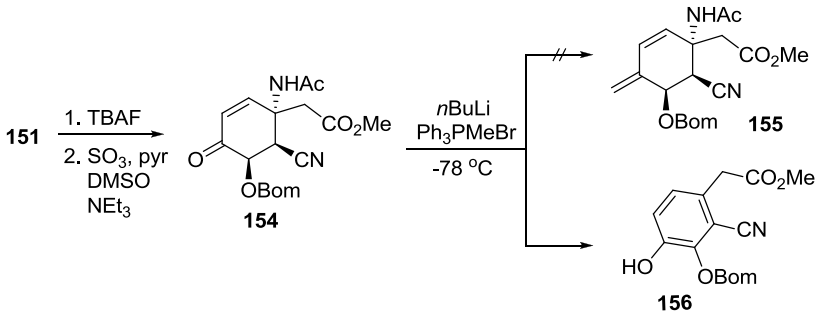
Scheme 27. Bom protection of **149** and **150**

On a final note, the foregoing protection step required carefully dried 1,2-DCE (freshly distilled from $CaSO_4$) and DIPEA (freshly distilled from CaH_2). A reaction carried out with commercial materials afforded the unanticipated product **153**, which we surmise to ensue through *in situ* hydrolysis of Bom-Cl, addition of **149** to the resultant formaldehyde, and Bom protection of a transient hemiacetal (Scheme 28)



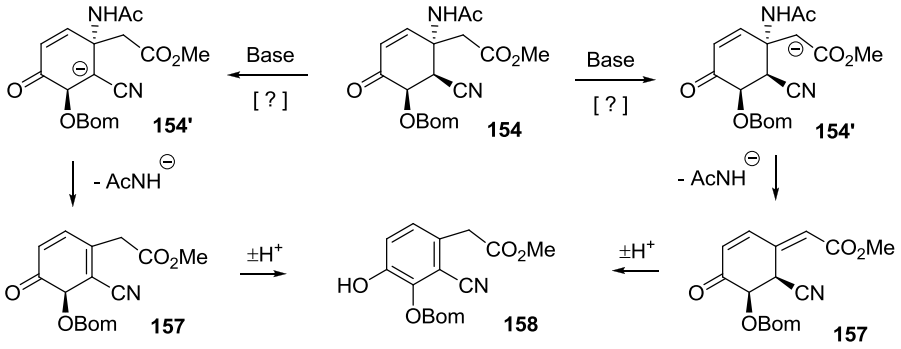
Scheme 28. Product of Bom protection, **153**, using commercial materials

The successful conclusion of this initial phase of the research had secured a robust avenue to key intermediate **151**. This enabled the launching of an investigation aiming to advance that substance to a more highly oxygenated TTX precursor. It will be recalled that the synthetic plan called for transforming **154** into **155**, which then would undergo two dihydroxylation reactions. The next subgoal of the work was thus diene **155**, which we intended to reach through Wittig reaction of **154**. Thus, **151** was treated with TBAF to release the silyl protecting group and subsequently oxidized to the enone using the Parikh-Doering procedure. Attempts to carry out a Wittig methylenation of **154** met with failure. It transpired that contact of **154** with the phosphonium ylide at -78 °C triggered aromatization to **156** in less than five minutes (Scheme 29).



Scheme 29. Hypothetical route to diene **155**

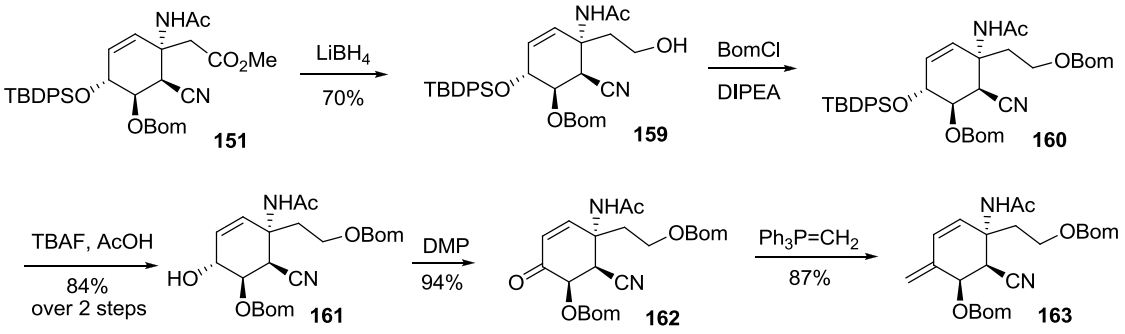
The mechanism of this pernicious side reaction presumably involves deprotonation of either the ester or the nitrile, followed by expulsion of the acetamide and prototropic isomerization of the resultant **157** (Scheme 30). A corrective measure would have to suppress enolization, an objective that might be attainable by reduction to non-deprotonatable entity. However, it was impossible at this stage to determine which functionality, ester or nitrile, was responsible for aromatization. It was technically easier to reduce the ester: we thus chose to convert **151** into **159** prior to methylenation. We were not to regret this course of action.



Scheme 30. Aromatic product, **158**, arising from Wittig reaction of **154**

Reduction of **151** with $LiBH_4$ provided **159** (Scheme 31). The reaction proceeded uneventfully, but it was sluggish and required occasional addition of borohydride over 24 hours. The emerging primary alcohol was blocked with a second Bom group, at which juncture removal of the TBDPS protection was attempted. Significant decomposition was observed when **160** was treated with either HF•pyridine or TBAF (the product was obtained in about 20 % yield). However, TBAF buffered with acetic acid gave **161** in good yield (84 % over 2 steps). The

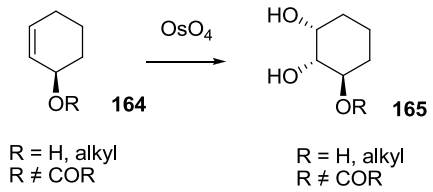
oxidation of **161** to the corresponding ketone was less than straightforward and required further experimentation. Surprisingly, **161** was immune to the action of the Parikh-Doering reagent, even though the method had performed well with the alcohol arising from deprotection of **151.** Activated MnO_2 was moderately successful, giving partial conversion with some loss of material. On the other hand, Dess-Martin periodinane (DMP) cleanly delivered **162** in 94% yield in only 10 minutes. It should be stressed that although crude **162** appeared very clean by proton nmr spectroscopy, purification is required before DMP oxidation. Failure to do so causes significant losses to decomposition. Delightfully, ketone **162** smoothly underwent Wittig olefination giving diene **163** as the sole product. This suggests that the aromatization problem was imputable to the ester functionality.



Scheme 31. Synthesis of diene, **163**

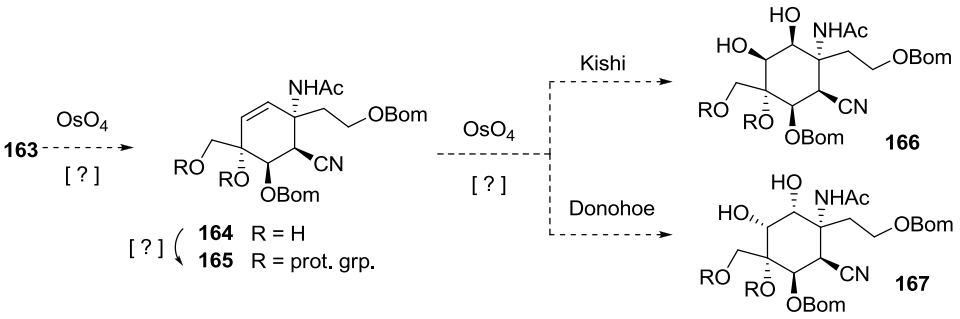
The final stage of the present investigation focused on the hydroxylation of **163**. Central to our planning was the important observation by Kishi and collaborators that allylic hydroxyl or alkoxy – *but not acyloxy* – groups exhibit a significant *anti*-directing effect in osmylation reactions (Kishi effect).[39] For instance, cyclohexenol **164** is selectively dihydroxylated to diol

165 (Scheme 28, dr = 10:1). Although several hypotheses have been advanced to rationalize this phenomenon, no universal agreement has yet been reached.[40]



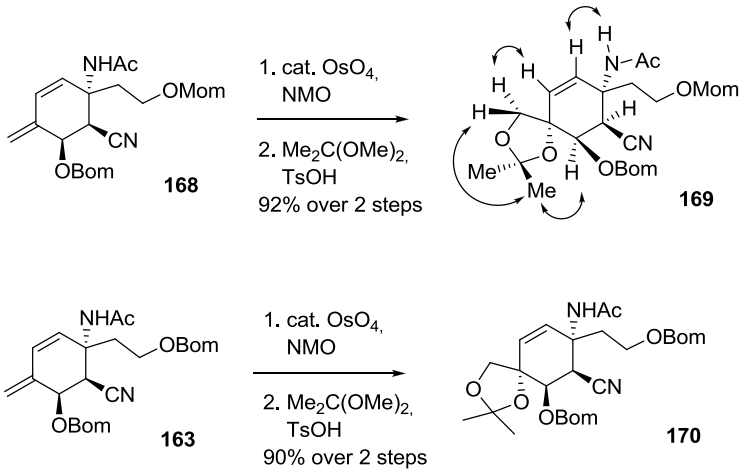
Scheme 32. Osmylation directed by the Kishi effect

We anticipated that the first osmylation of **163** would take place at the exo-methylene unit, and this for steric reasons.[41] The Kishi effect of the neighboring OBom group would direct reaction from the bottom face face of the π bond (as drawn in Scheme 33). This would set up the correct configuration of the tertiary alcohol. The stereochemistry of the osmylation of the internal olefin is a more complex issue. The Kishi effect of the tertiary oxygen substituent (may that be an OH or a protected form thereof) should guide the incoming OsO_4 to the top face of the π bond, producing compound **166** with the correct configuration of all oxygenated centers, as required for **1**. On the other hand, the work of Donohoe suggests that acetamides can exert a *syn*-directing effect in osmylation reactions.[42] At the onset of the work detailed below, there was no indication available to us regarding the relative strength of the two opposing effects. Only experiment could provide a sensible answer.



Scheme 33. Osmylation directed by the Kishi effect

Parallel research had afforded us with some compound **168**, which is an analog of **163** displaying a primary OMom group in lieu of OBom. This material constituted a valuable test bed for the osmylation reaction, and indeed, it proved to be a competent substrate for dihydroxylation under Upjohn conditions [43] (cat. OsO_4, NMO). As predicted, only the exomethylene segment reacted, providing a diol, which was protected as an acetonide (2,2-dimethoxypropane, catalytic *p*TsOH) to give **169**. No evidence could be garnered for the presence of a diastereomeric diol. Extensive 1D and 2D NMR spectroscopic analysis of **169** confirmed that the Kishi effect had controlled the stereochemical outcome of the reaction. The nuclear Overhauser correlations (2D-NOESY) shown in Scheme 34 with double-headed arrows were particularly diagnostic, and permitted an accurate assignment of the chemical shifts of all protons in the molecule.

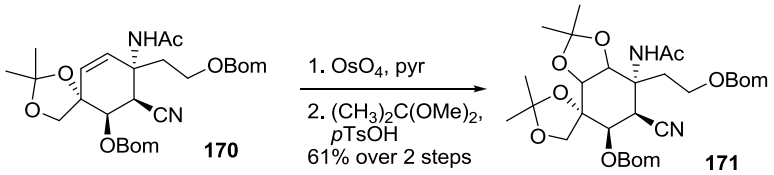


Scheme 34. Osmylation directed by the Kishi effect

In a like manner, the Bom substrate **163** underwent Upjohn osmylation and acetonide formation to furnish **170** as the sole product. Compound **170** was not analyzed as thoroughly as **169**; however, the chemical shifts and coupling constants of all ring protons were virtually identical to those of **169**, strongly suggesting that the dihydroxylation had occurred in an *anti*-sense directed by the Kishi effect. In any event, corroboration of this assignment would be forthcoming at the stage of an even more advanced intermediate.

Attempts to dihydroxylate the remaining π bond in **170** using the Upjohn method failed (no reaction), but the traditional stoichiometric osmylation in pyridine as the solvent was successful. Perhaps due to steric hindrance, the reaction was slow, requiring at least four days for full conversion. Moreover, the osmate ester underwent cleavage with difficulty. Multiple treatments with aqueous sodium thiosulfate solution were necessary to induce full release of the osmium. The emerging diol was then converted to its acetonide, **171**, again using 2,2-dimethoxypropane and *p*TsOH. While the acetonide protection of the diol arising from

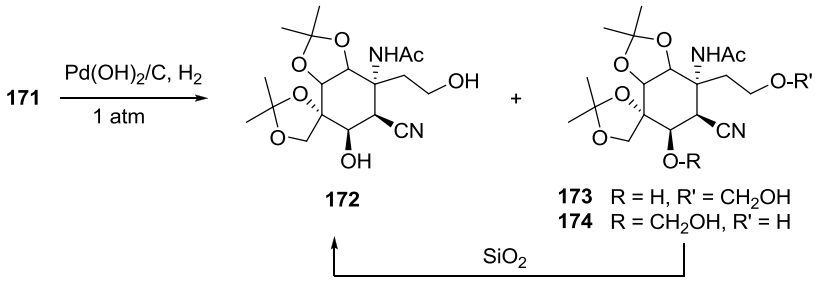
dihydroxylation of the exo-methylene proceeded efficiently, acetonide protection of the internal diol proved problematic. Conversion to **171** was poor when the reaction was not performed in thoroughly anhydrous acetone. Best results were obtained when acetone was distilled over calcium sulfate and added *immediately* to the reaction. Substituting PPTS for *p*TsOH resulted in no conversion to **171**. Furthermore, using a less hygroscopic solvent such as dichloromethane with *p*TsOH resulted in decomposition of **171**. In any event, the ultimate acetonide emerged as a single stereoisomer. The configuration of **171** was unknown at this stage. Consequently, the compound is rendered in Scheme 35 without the configuration of the newly installed oxygen atoms.



Scheme 35. Synthesis of **171**

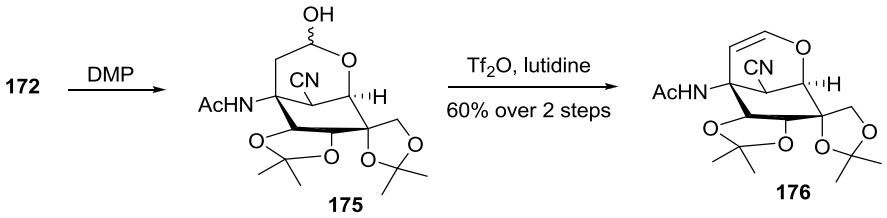
The next steps of the synthesis targeted formation of vinyl ether **176** (Scheme 36), which appeared to be an ideal substrate for the introduction of the remaining oxygen functionalities. The compound was envisaged to ensue through release of the Bom groups in **171**, followed by selective oxidation of the primary alcohol, cyclization of the resultant aldehyde to a hemiacetal, and dehydration of the latter. It should be noted that a related vinyl ether was a valuable intermediate in the Isobe synthesis of **1** (cf. Scheme 8). In accord with the foregoing, concurrent hydrogenolysis of the Bom groups of **171** in the presence of Pearlman's catalyst afforded **172**

(Scheme 36). Interestingly, conduct of this reaction on scales above 100 mg gave what appeared to be a metastable formaldehyde hemiacetal such as **173** or **174**, which could be restored to the expected diol by stirring with silica gel in EtOAc.



Scheme 36. Bom deprotection via hydrogenation

The deprotection product was oxidized selectively at the primary alcohol using DMP to give hemiacetal **175**. Dehydration of this compound could be accomplished at -78 ^{o}C with Tf_2O using 2,6-lutidine base. Weaker dehydrating agents such as MsCl and TFAA did not undergo fruitful reaction with **175**.



Scheme 37. Synthesis of vinyl ether

The rigid structure of **176** lent itself nicely to a thorough NMR analysis aiming to determine its precise configuration, thereby ascertaining the stereochemical course of the two

prior osmylation steps. This exercise led to the conclusion that whereas the first reaction had indeed occurred in a Kishi mode, the second one had taken place with Donohoe selectivity. Indeed, a diagnostic W-type coupling was detected between protons H_a and H_b (Figure 3. ^4J = 1.6 Hz), confirming that no isomerization of the nitrile had occurred at any prior stage. More

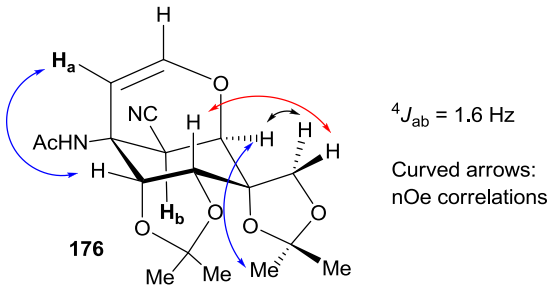


Figure 3. Spectral properties of compound **176**

significantly, the diagnostic NOESY signals rendered in Figure 3 with double-headed curved arrows are consistent only with the shown configuration. Evidently, hydrogen bonding of the acidic NH in the acetamide segment to the osmium tetroxide-pyridine complex, as invoked by Donohoe,[44] and as rendered in Figure 4 (formal charges omitted for clarity), constitutes a stronger directing force than Kishi's stereoelectronic effect. Therefore, compounds **171-176** are more accurately represented as seen in Scheme 38.

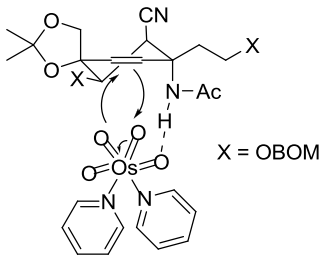
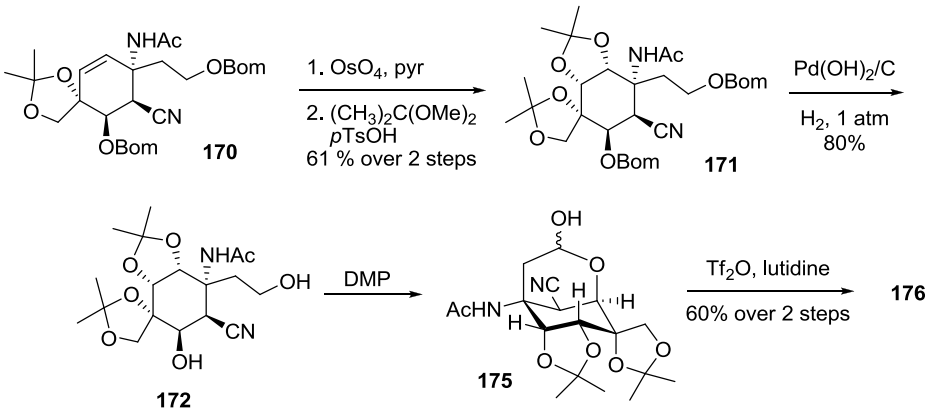


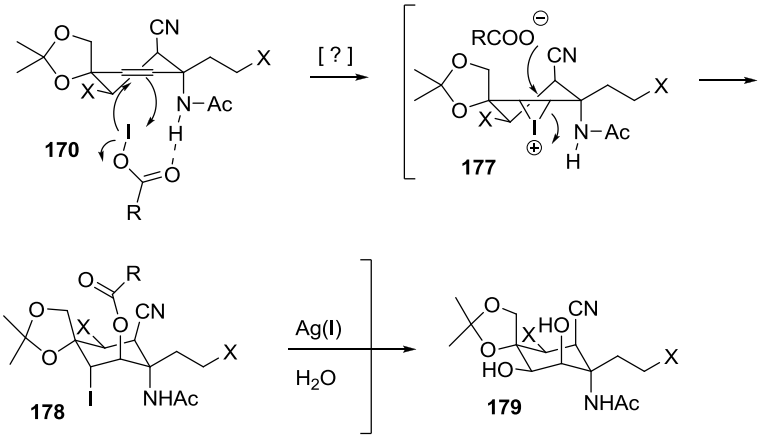
Figure 4. Possible hydrogen bonding between osmium salt and the acidic NH

Future work toward tetrodotoxin will have to circumvent the syn selectivity of the second osmylation step. Possible remedies include the exchange of an *N*-acetyl group with an alternative blocking unit that lacks H-bond donor ability (e.g., a phthalimide), or temporary *N*-



Scheme 38. Actual configuration of compounds **171-176**

alkylation of the acetamide. Alternatively, a different *syn*-dihydroxylation technique, such as the Prevost reaction, [45] might be employed to take advantage of the hydrogen-bonding ability of the amide.



Scheme 39. Possible hydrogen bonding between osmium salt and the acidic NH

3. Conclusion

In the preceding pages, we have demonstrated a robust synthesis of key intermediate **170**, and have outlined a route by which it might be advanced to the natural product tetrodotoxin. The elaboration of **170** to **176** allowed examination of the configuration resulting from osmium-promoted dihydroxylation of the internal olefin of **170**, which proceeded with Donohoe selectivity. Furthermore, we have established the bimolecular oxidative amidation technology as a key step in our synthesis, installing the nitrogen at an early stage in our synthesis. We believe the work disclosed herein will prove useful to future research efforts in the synthetic field.

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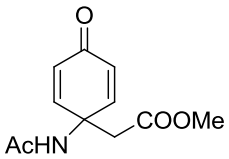
Appendix A: Experimental Protocols

Unless otherwise indicated, ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained from $CDCl_3$ solutions at room temperature on a Bruker Avance II 300 instrument. Chemical shifts are reported in parts per million (ppm) on the δ scale and coupling constants, *J*, are in hertz (Hz). Multiplicities are reported as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "dd" (doublet of doublets), "m" (multiplet), "c" (complex), "br" (broad), "ABq" (AB quartet), "app" (apparent). 2D NMR spectra were recorded at 400 MHz (^{1}H).

Infrared (IR) spectra (cm^{-1}) were recorded on a Perkin-Elmer Frontier instrument. High-resolution mass spectra (m/z) were obtained in the electrospray (ESI) mode or atmospheric pressure chemical ionization (APCI) mode on a Micromass LCT mass spectrometer by the UBC Mass Spectrometry laboratory.

Melting points were measured on a Mel-Temp apparatus and are uncorrected.

All reagents and solvents were commercial products and used without further purification except THF (freshly distilled from Na/benzophenone under N_2), CH_2Cl_2 (freshly distilled from CaH_2 under N_2), $CHCl_3$ (washed with H_2O, treated with $K_2CO_{3\,(s)}$, freshly distilled from Na_2SO_4 under Ar), MeOH (freshly distilled from magnesium and iodine under Ar), 1,2-dichloroethane (freshly distilled from $CaSO_4$ under Ar), diisopropylethylamine (distilled from CaH_2 under Ar), and acetone (freshly distilled from $CaSO_4$ under Ar). Commercial n-BuLi was titrated against diphenylacetic acid until a yellow color persisted. Flash chromatography was performed on Silicycle 230 – 400 mesh silica gel. All reactions were performed under argon atmosphere in oven dried flasks equipped with Teflon™ stirbars. All flasks were fitted with rubber septa for the introduction of substrates, reagents, and solvents via syringe.



A.1 Preparation of **132**

A MeCN (20.0 mL) solution of **131** (9.1 g, 55.0 mmol, 1.0 equiv.) was added over 3 h (syringe pump) to a solution of DIB (23.9 g, 74.0 mmol, 1.3 equiv.) and TFA (6.4 mL, 82.5 mmol, 1.5 equiv. vs. DIB) in MeCN (480 mL), at room temperature under argon with good stirring. At the end of the addition, the mixture was concentrated under reduced pressure and the residue was diluted with toluene (10.0 mL). The suspension was concentrated and the procedure was repeated twice to azeotropically remove all residual TFA. The brown residue was filtered through a silica pad (55.0 g) using first 300 mL of Et_2O (removal of brown tar and iodobenzene) and then 300 mL of Et_2O/ MeCN (2.5:1, elution of the product). Concentration afforded a brown solid, which was re-filtered through fresh silica gel using the same procedure. The solid residue was taken up with 20.0 mL of EtOAc and kept at −20 °C for 5 h. The resulting precipitate was essentially pure product **132**. A total of 7.44 g (33.3 mmol, 61%) of **132** was obtained as an off-white solid. A recrystallized sample (EtOAc/Hex = 2:1) of **132** had m.p. 100-102 °C.

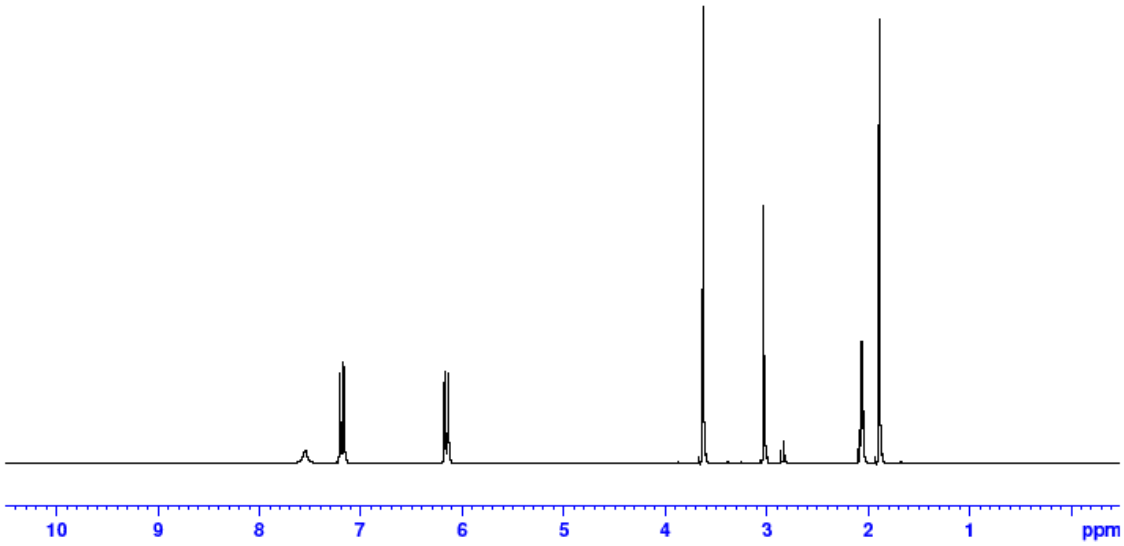
^{1}H NMR (acetone-d_6): δ 7.54 (br s, 1H); 7.17 (d, *J* = 10.3, 2H); 6.15 (d, *J* = 10.3, 2H); 3.62 (s, 3H); 3.02 (s, 2H); 1.88 (s, 3H).

^{13}C NMR (acetone-d_6): δ 184.3,169.4, 169.0, 148.8, 128.1, 53.3, 51.1, 41.7, 22.5.

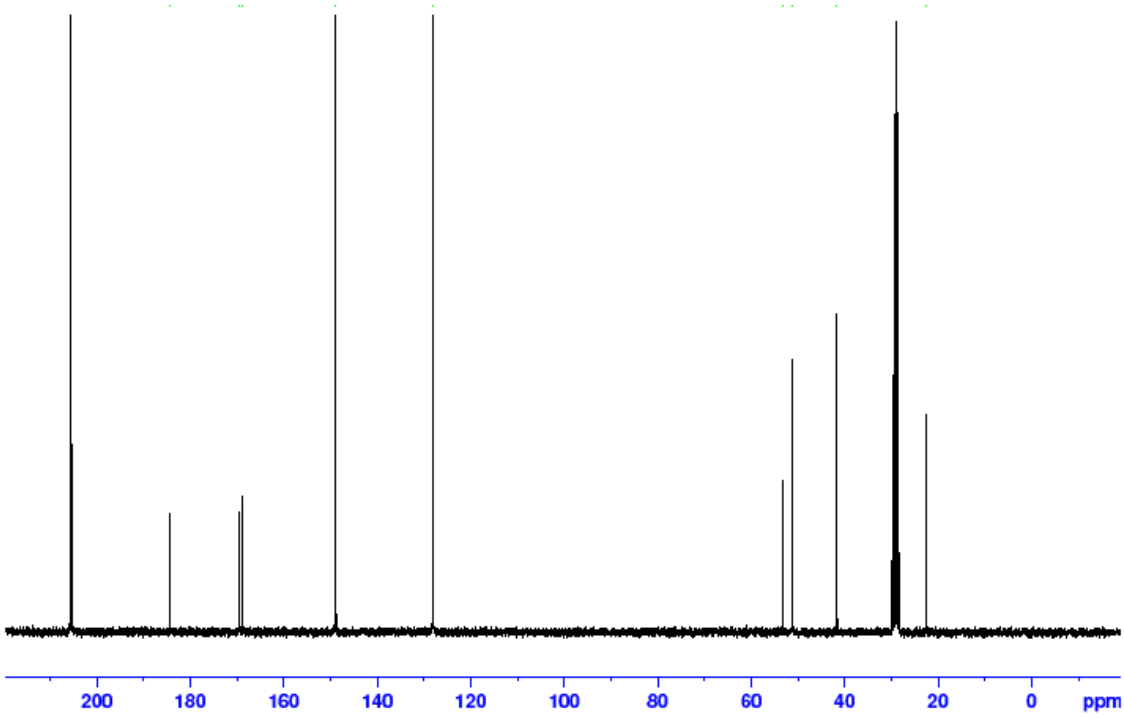
IR (film, cm^{-1}): ν 3200; 1738; 1666.

HRMS: calc. for $C_{11}H_{13}NO_4Na$ $[M + Na]^+$ 246.0737; found 246.0742.

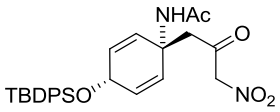
m.p. 100-102 °C.



Scheme 40. ^{1}H NMR spectrum of **132**



Scheme 41. ^{13}C NMR spectrum of **132**



A.2. Preparation of **145**

To a dry THF (135 mL) solution of **132** (6.0 g, 27 mmol, 1.0 equiv.) under argon in a 500 mL roundbottom flask immersed in a cold-water bath, was added the (*S*)-CBS catalys (0.075 g, 0.27 mmol, 0.01 equiv.). BH_3 (~10 M in methyl sulfide, 2.7 mL, 27 mmol, 1.0 equiv.) was *carefully* added dropwise via syringe to the rapidly stirring solution. The mixture was stirred for 45 minutes at room temperature. Following, the septum was removed and MeOH (3.3 mL, 81 mmol, 3.0 equiv.) was *carefully* added dropwise via syringe at 0 ^{o}C. The reaction was warmed to room temperature and the solvent was removed under reduced pressure by rotary evaporation, followed by drying under the high vacuum pump until all residual solvent was removed. The residue was taken up in dry dichloromethane (100 mL) and to this solution was added imidazole (1.83 g, 27 mmol, 1.0 equiv.) followed by TBDPSCl (7.0 mL, 27 mmol, 1.0 equiv.) at room temperature under argon. The mixture was stirred for 12 hours (overnight) and 0.05N HCl (100 mL) was added. The layers were separated and the aqueous layer was extracted with dichloromethane (2x100 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was dissolved in THF (100 mL), followed by the addition of H_2O (100 mL) and LiOH monohydrate (3.4 g, 81 mmol, 3.0 equiv.). The reaction was stirred for 1.5 hours at room temperature and then most of the THF was removed by rotary evaporation. Diethyl ether (100 mL) was added to the aqueous suspension and the layers were separated (to remove organic byproducts from previous steps since our product should be in the aqueous layer as the carboxylate). The aqueous layer was acidified with a 1:1 (vol/vol) mixture of AcOH/H_2O (30 mL) and extracted with EtOAc (3x100 mL, check pH of

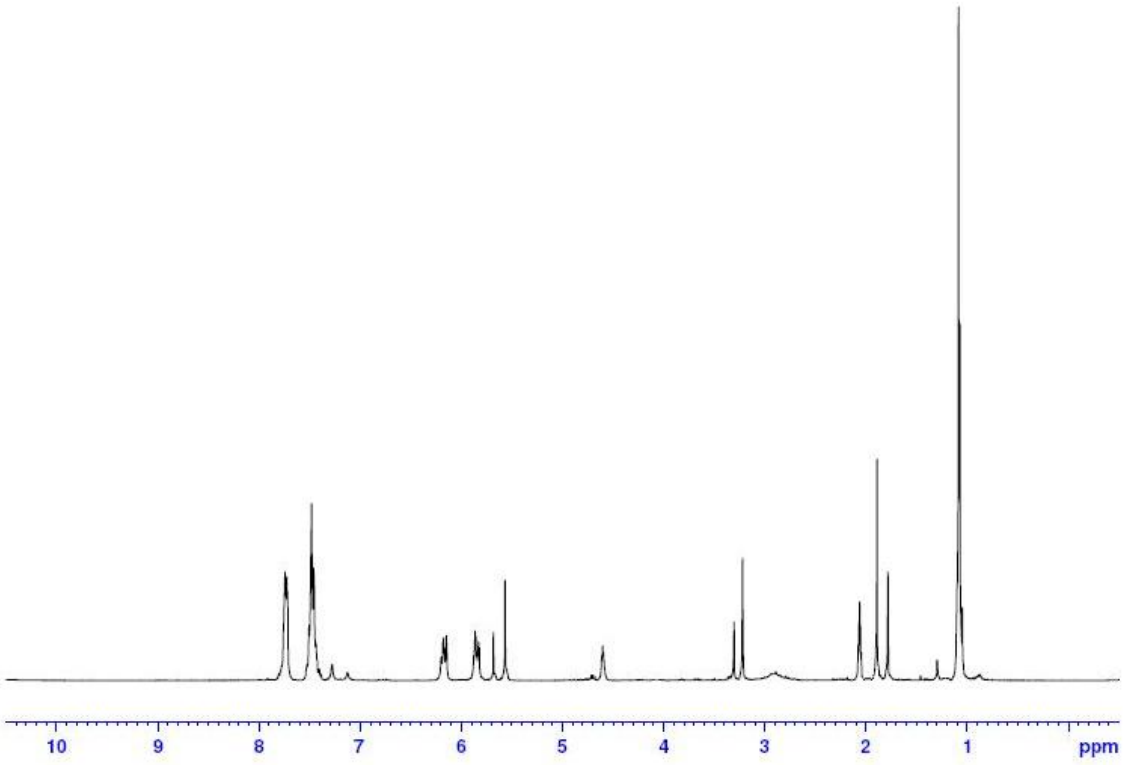
aqueous layer). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Coevaporation with toluene was carried out multiple times (3-4 times) to remove all residual acetic acid. The residue was taken up in dry THF(70 mL) and to this was added carbonyl diimidazole (CDI) (4.36 g, 27 mmol, 1.0 equiv.) at room temperature under argon and the mixture was stirred for 1 hour. Nitromethane (8.5 mL, 0.16 mol, 6.0 equiv.) and potassium *t*-butoxide (12.0 g, 0.11 mol, 4.0 equiv.) were added at room temperature and the resulting mixture was stirred for 2 hours. Following, a 1:1 (vol/vol) mixture of AcOH/H_2O (30 mL) was added and the THF was removed under reduced pressure. H_2O (70 mL) and dichloromethane (100 mL) were added to the suspension and the layers were separated. The aqueous layer was extracted with dichloromethane (2x100 mL) and the organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by gradient column chromatography (1:1 EtOAc/Hexanes-7:3 EtOAc/Hexanes) to obtain pure 7.9 g (16 mmol, 56% over 4 steps) of **145** (dr 4:1) as a light yellow oil.

^{1}H NMR (acetone-d_6): δ 7.75-7.72 (m, 4H); 7.53-7.37 (m, 6H); 6.20-6.14 (m, 2H); 5.88-5.82 (m, 2H); 5.57 (s, 2H); 4.61-4.59 (m, 1H); 3.22 (s, 2H); 1.88 (s, 3H); 1.07 (s, 9H).

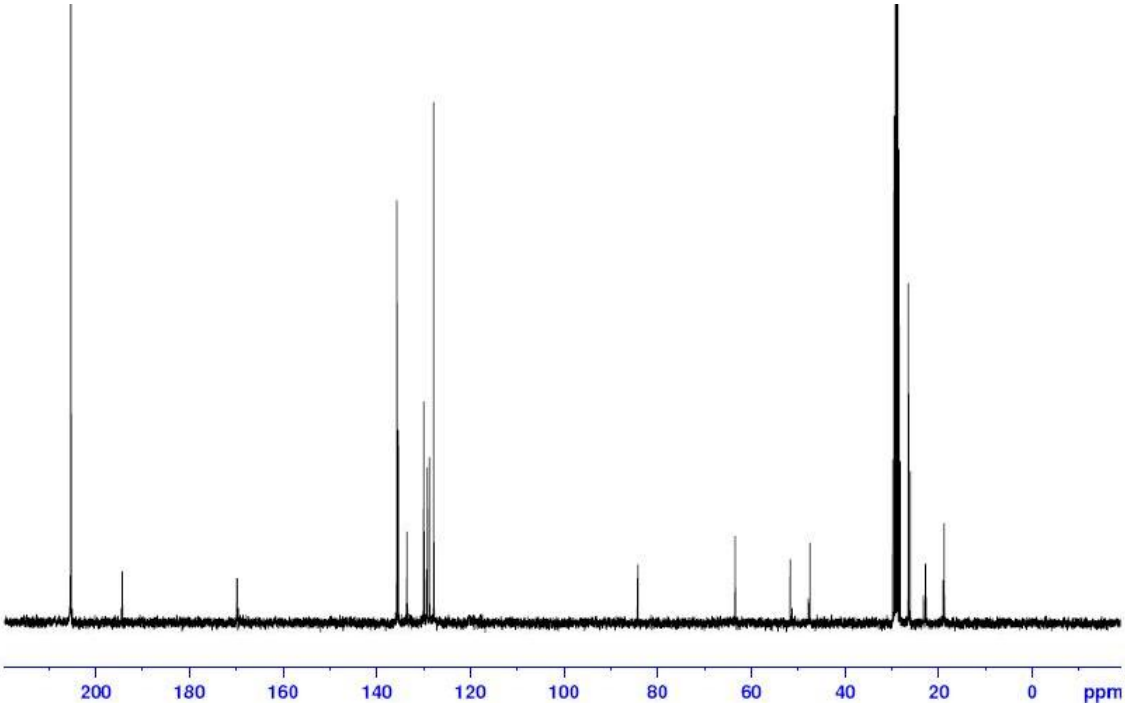
^{13}C NMR (acetone-d_6): δ 195.30, 170.78, 136.62, 134.52, 130.90, 130.16, 129.71, 128.76, 85.15, 64.37, 52.63, 48.39, 27.39, 23.75, 19.77.

IR (film, cm^{-1}): ν 1736, 1700, 1656, 1558.

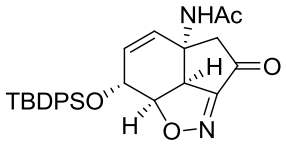
HRMS: calc. for $C_{27}H_{32}N_2O_5SiNa$ $[M + Na]^+$ 515.1978; found 515.1962.



Scheme 42. ^{1}H NMR spectrum of **145**



Scheme 43. ^{13}C NMR spectrum of **145**



A.3 Preparation of **146**

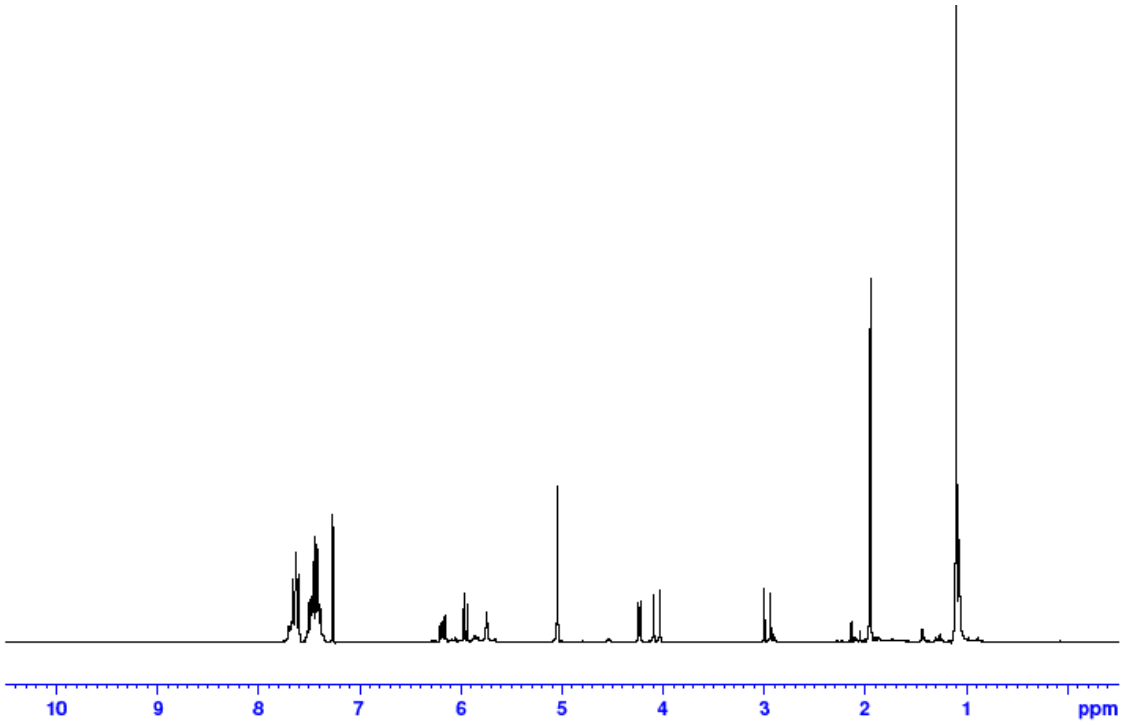
To a dry chloroform solution (25 mL) of **145** (3.5 g, 7.1 mmol, 1.0 equiv.) was added solid $Cu(OAc)_2 \bullet H_2O$ (0.072 g, 0.36 mmol, 0.05 equiv.) followed by *N*-ethylpiperidine (0.10 mL, 0.71 mmol, 0.10 equiv.) at room temperature under argon. The reaction mixture was stirred for 168 hours at 30 °C and then concentrated under vacuum. The crude residue was dissolved in minimal ethyl acetate and filtered through a short plug of silica gel, rinsing with ethyl acetate. The solvent was removed under vacuum and the reside was purified by flash column chromatography on silica gel (EtOAc/Et_2O 1:4) to give 2.0 g (4.3 mmol, 60%) of **146** as a pale yellow foam.

^{1}H NMR: δ 7.71-7.60 (m, 4H); 7.53-7.37 (m, 6H); 6.18 (dd, *J* = 10.0, 5.9, 1H); 5.60 (d, *J* = 10.0, 1H); 5.75 (br, 1H); 5.05 (app s, 2H); 4.24 (d, *J* = 5.9, 1H); 4.06 (d, *J* = 18.4, 1H); 2.97 (d, *J* = 18.4, 1H); 1.95 (s, 3H); 1.10 (s, 9H).

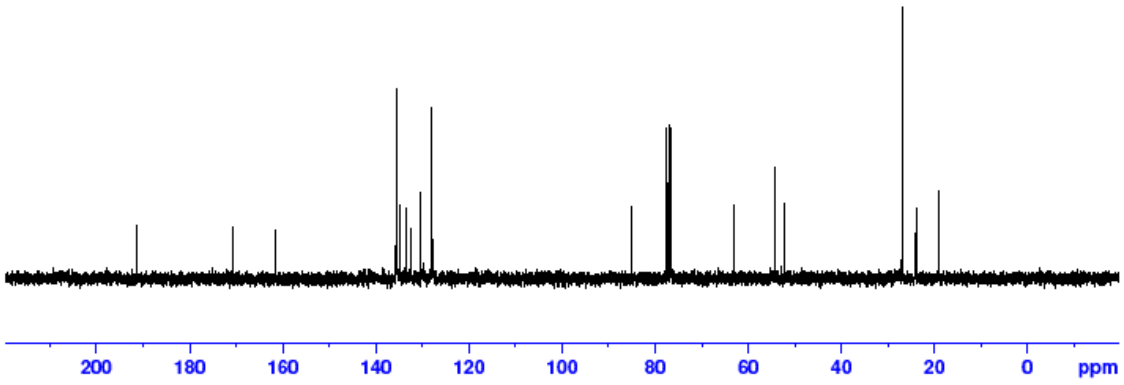
^{13}C NMR: δ 191.42, 170.84, 161.68, 135.85, 135.60, 135.55, 134.90, 133.40, 132.61, 132.45, 130.47, 130.38, 128.14, 128.02, 85.15, 63.17, 54.23, 52.32, 26.98, 23.98, 19.10.

IR (film, cm^{-1}): *v* 1747, 1660, 1599, 1538.

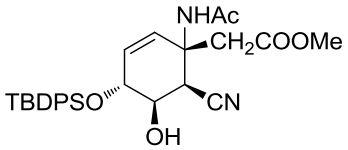
HRMS: calc. for $C_{27}H_{31}N_2O_4Si$ $[M + H]^+$ 475.2053; found 475.2059.



Scheme 44. ^{1}H NMR spectrum of **146**



Scheme 45. ^{13}C NMR spectrum of **146**



A.4 Preparation of **149**

To a dry methanol solution (63.0 mL) of **146** (1.50 g, 3.16 mmol, 1.0 equiv.) at room temperature under argon was added solid Li_2CO_3 (0.117 g, 1.58 mmol, 0.5 equiv.) in one portion. The reaction was stirred for 1.5 h and the solvent was removed under vacuum to afford a light yellow oil. The residue was purified by column chromatography on silica gel (EtOAc/Hex 1:1) to give 1.32 g (2.60 mmol, 83%) of **149** as an off-white solid.

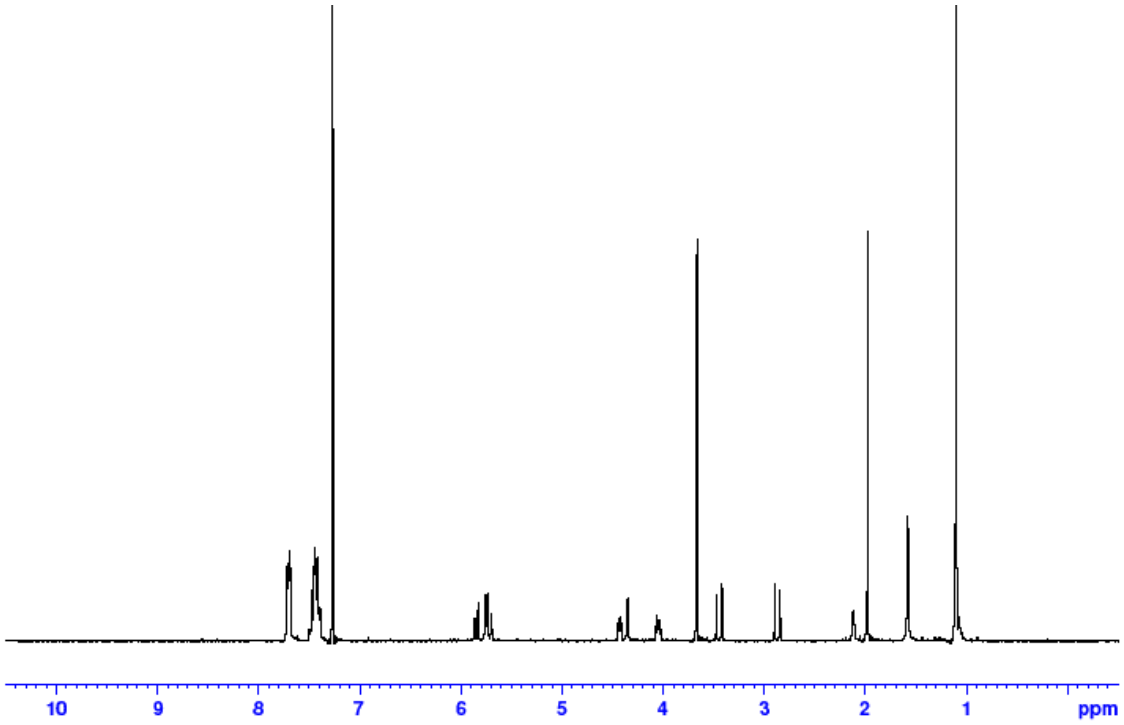
^{1}H NMR: δ 7.72-7.68 (m, 4H); 7.50-7.39 (m, 6H); 5.84 (br d, *J* = 10.3, 1H); 5.76 (br s, 1H); 5.72 (dd, *J* = 10.2, 2.6, 1H); 4.45-4.41 (m, 1H); 4.35 (d, *J* = 3.1, 1H); 4.07-4.03 (m, 1H); 3.67 (s, 3H); 3.45 (d, *J* = 16.1, 1 H); 2.87 (d, *J* = 16.1, 1 H); 1.98 (s, 3H); 1.10 (s, 9H).

^{13}C NMR: δ 170.61, 170.28, 135.91, 135.69, 133.13, 132.00, 130.23, 130.22, 128.05, 127.90, 127.83, 117.43, 71.59, 70.22, 55.09, 51.87, 39.17, 38.67, 26.93, 24.07, 19.25.

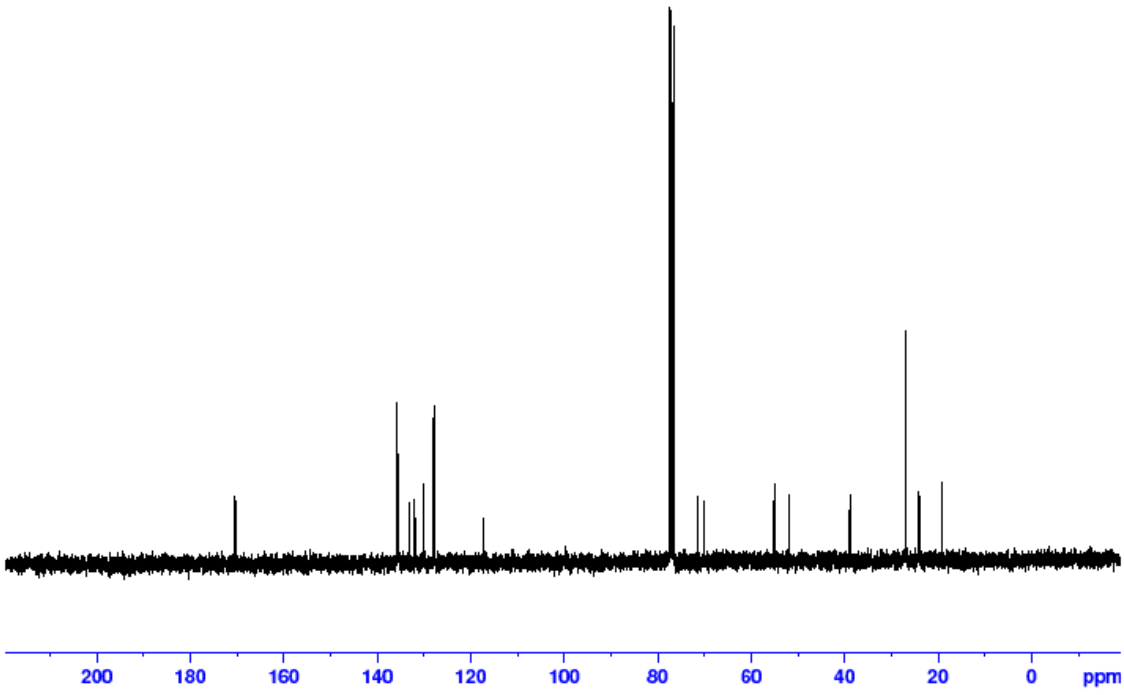
IR (film, cm^{-1}): ν 2247, 1719, 1639.

HRMS: calc. for $C_{28}H_{34}N_2O_5SiNa$ $[M + Na]^+$ 529.2135; found 529.2122.

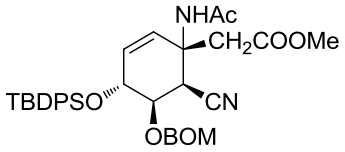
m.p. 166-168 °C.



Scheme 46. ^{1}H NMR spectrum of **149**



Scheme 47. ^{13}CNMR spectrum of **149**



A.5 Preparation of **151**

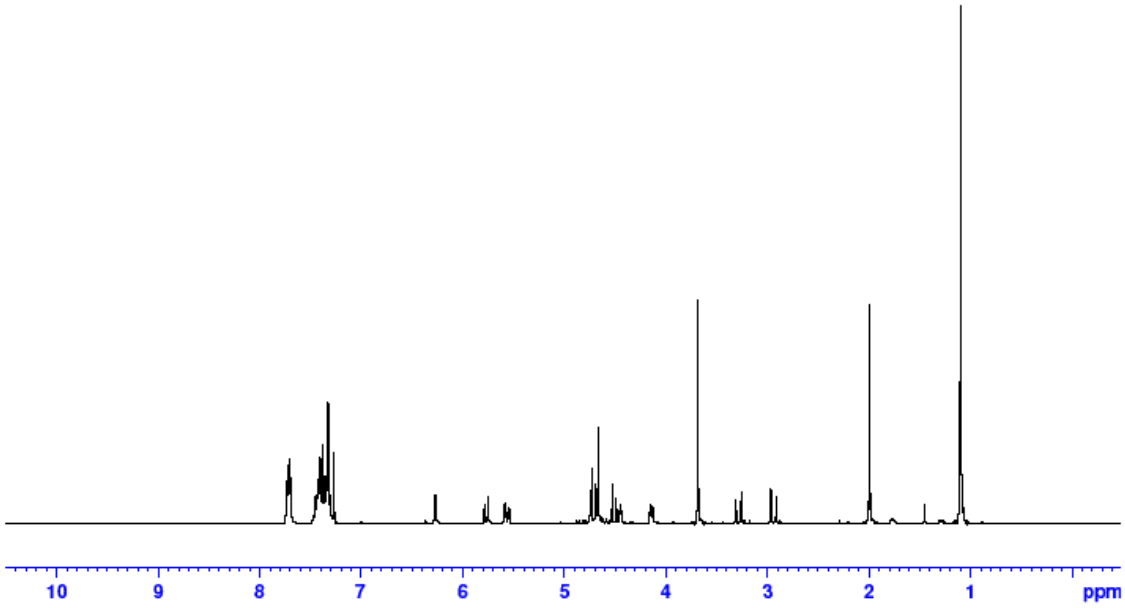
To a 1,2-dichloroethane (DCE) solution (3.0 mL) of **149** (0.303 g, 0.60 mmol, 1.0 equiv.) at room temperature under argon was added BOMCl (0.12 mL, 0.90 mmol, 1.5 equiv.) followed by DIPEA (0.19 mL, 1.08 mmol, 1.8 equiv.). The reaction mixture was heated to 75 °C and stirred for 48 h. After cooling to room temperature, the mixture was diluted with DCM (40 mL) and poured into a saturated solution of NH_4Cl (50 mL). The organic layer was separated and the aqueous layer was extracted with DCM (2x30 mL). The organic layers were combined, washed with H_2O (150 mL), dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (Et_2O/Hex 7:3) to give 0.341 g (0.55 mmol, 91%) of **151** as a colorless oil.

^{1}H NMR: δ 7.73-7.69 (m, 4H); 7.48-7.30 (m, 11H); 6.27 (br s, 1H); 5.77 (d, *J* = 10.3, 1H); 5.56 (dd, *J* = 10.3, 3.42, 1H); 4.74-4.67 (m, 4H); 4.53-4.49 (m, 1H); 4.46-4.43 (m, 1H); 4.16-4.13 (m, 1H); 3.68 (s, 3H); 3.28 (d, *J* = 15.6, 1H); 2.94 (d, *J* = 15.6, 1H); 1.99 (s, 3H); 1.10 (s, 9H).

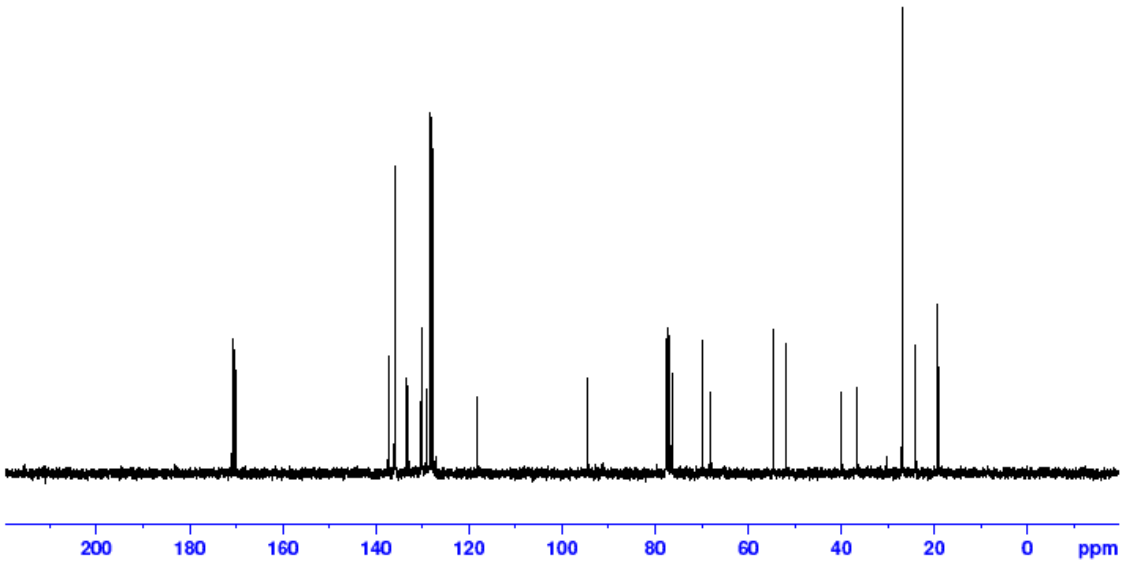
^{13}C NMR: δ 170.85, 170.23, 137.28, 135.99, 135.88, 133.62, 133.03, 130.36, 130.07, 130.00, 129.05, 128.48, 128.05, 127.89, 127.85, 127.80, 118.14, 94.47, 77.46, 76.43, 69.95, 68.25, 54.70, 54.52, 51.95, 39.98, 36.72, 30.39, 26.99, 24.05, 19.30.

IR (film, cm^{-1}): ν 2247, 1737, 1665.

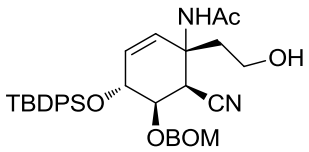
HRMS: calc. for $C_{36}H_{42}N_2O_6SiNa$ $[M + Na]^+$ 649.2710; found 649.2710.



Scheme 48. ^{1}H HNMR spectrum of **151**



Scheme 49. ^{13}C NMR spectrum of **151**



A.6 Preparation of **159**

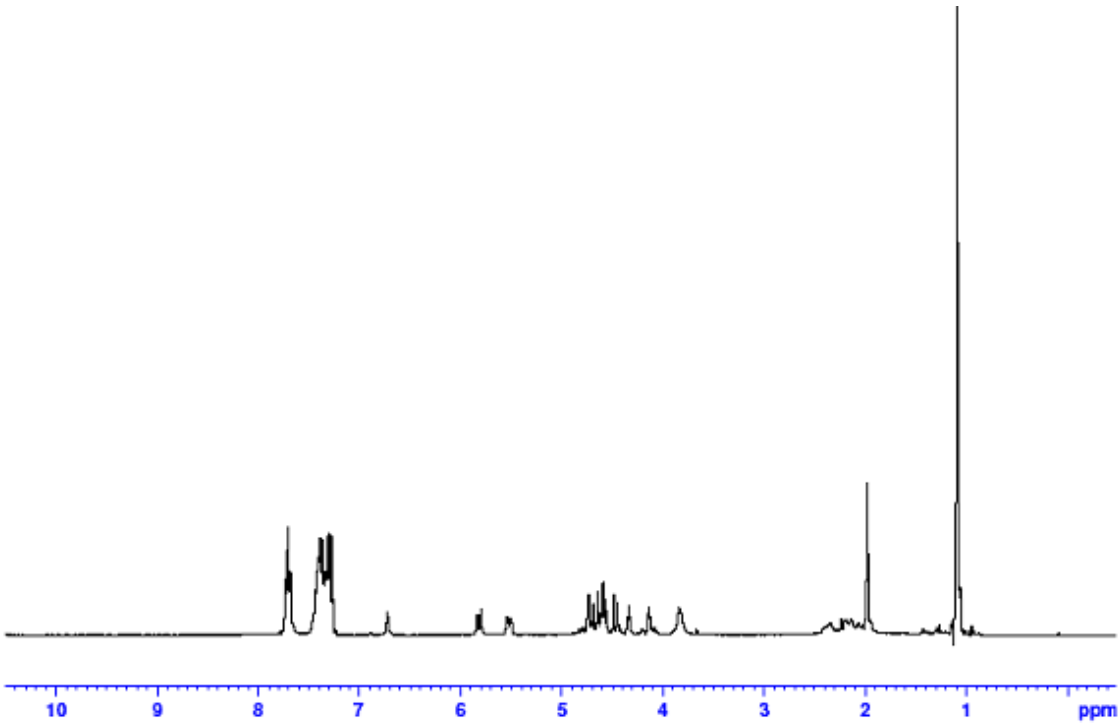
To a THF (3.0 mL) solution of **151** (0.227, 0.36 mmol, 1.0 equiv.) at room temperature under argon was added solid $LiBH_4$ (0.079 g, 3.6 mmol, 10.0 equiv.) and the reaction was stirred for 24 h. The reaction mixture was cooled to 0 °C and a saturated solution of NH_4Cl (2.0 mL) was carefully added (GAS EVOLUTION!). The reaction was stirred at room temperature for a further 30 min and then diluted with EtOAc (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2x20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (EtOAc) to give 0.152 g (0.25 mmol, 70%) of **159** as a colorless oil.

^{1}H NMR: δ 7.73-7.68 (m, 5H); 7.44-7.24 (m, 10H); 6.73 (br s, 1H); 5.82 (d, *J* = 10.4, 1H); 5.52 (dd, *J* = 10.4, 3.5, 1H); 4.74-4.45 (m, 5H); 4.35-4.33 (br m, 1H); 4.15-4.12 (br m, 1H); 3.90-3.68 (br m, 2H); 2.42-2.33 (m, 1H); 2.20-2.12 (m, 1H); 1.97 (s, 3H); 1.09 (s, 9H).

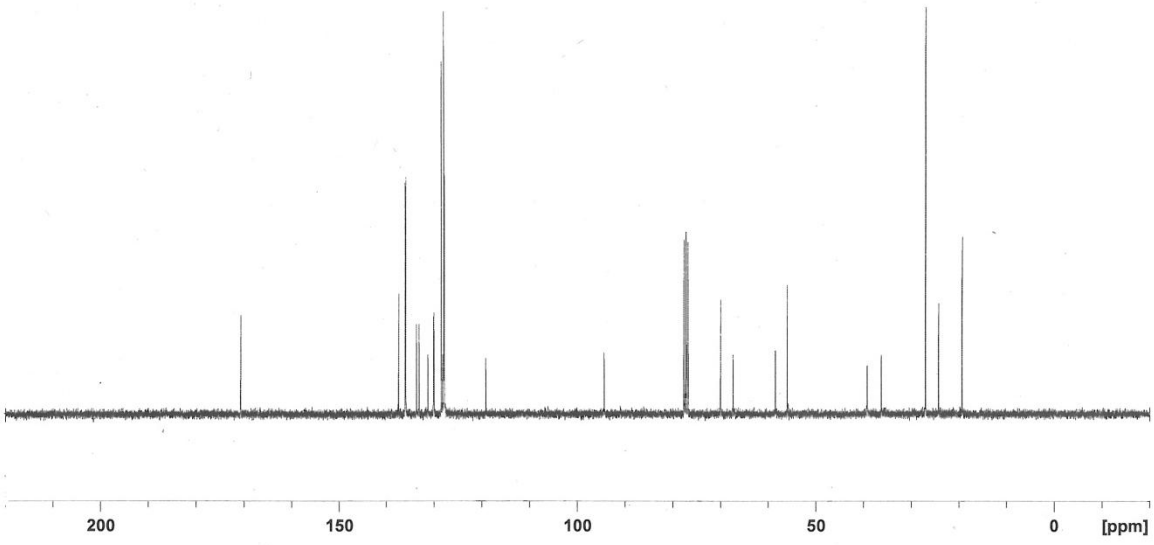
^{13}C NMR: δ 170.59, 137.35, 136.02, 135.90, 133.63, 133.09, 131.23, 130.06, 129.96, 128.46, 128.17, 127.94, 127.91, 127.82, 127.77, 119.13, 94.32, 77.43, 77.13, 69.93, 67.32, 58.40, 55.89, 39.21, 36.26, 26.99, 24.23, 19.28.

IR (film, cm^{-1}): ν 2248, 1662.

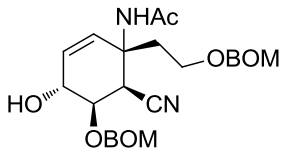
HRMS: calc. for $C_{35}H_{42}N_2O_5SiNa$ $[M + Na]^+$ 621.2761; found 621.2759.



Scheme 50. ^{1}H NMR spectrum of **159**



Scheme 51. ^{13}C NMR spectrum of **159**



A.7 Preparation of **161**

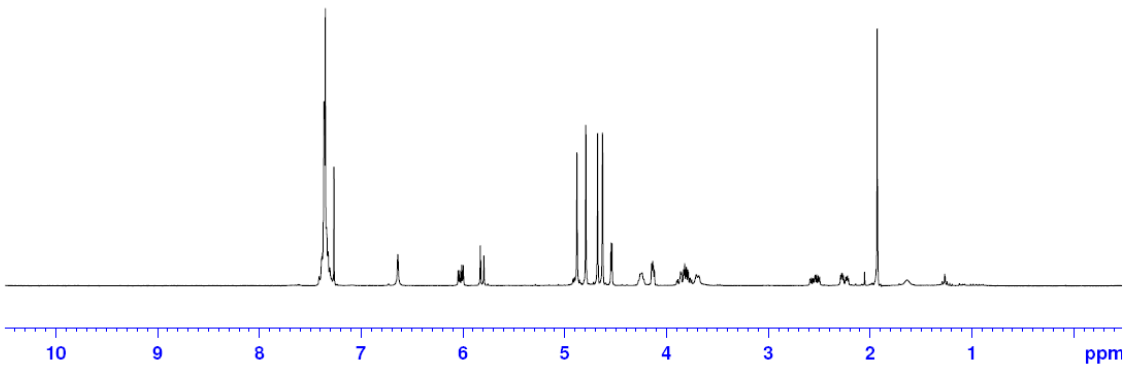
To a 1,2-dichloroethane solution (7.0 mL) of **159** (0.473 g, 0.79 mmol, 1.0 equiv.) under argon at room temperature was added DIPEA (0.34 mL, 1.98 mmol, 2.5 equiv.) followed by BOMCl (0.22 mL, 1.58 mmol, 2.0 equiv.). The reaction was stirred at 50 °C for 24 hours and then cooled to room temperature, diluted with DCM (20 mL), and poured into a saturated solution of NH_4Cl (15 mL). The organic layer was separated and the aqueous layer was extracted with DCM (2x20 mL). The organic layers were combined, washed with H_2O (50 mL), dried over Na_2SO_4, and concentrated under vacuum to afford a pale yellow oil. The crude mixture was taken up in THF (5.0 mL) at room temperature under argon and to it was added a 1 M solution of AcOH in THF (2.0 mL, 1.98 mmol, 2.5 equiv.) followed by a 1 M solution of TBAF in THF (2.0 mL, 1.98 mmol, 2.5 equiv.). The reaction mixture was stirred for 15 hours, and a saturated solution of $NaHCO_3$ (10 mL) was then added. The mixture was diluted with EtOAc (20 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (2x20 mL) and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford a pale yellow oil. The residue was purified by column chromatography on silica gel (EtOAc) to give 0.329 g (0.66 mmol, 84% over 2 steps) of **161** as a colorless oil.

^{1}H NMR: δ 7.40-7.28 (m, 10H); 6.64 (brs, 1H); 6.00 (dd, *J* = 10.2, 4.4, 1H); 5.81 (d, *J* = 10.2, 1H); 4.87 (brs, 2H); 4.78 (brs, 2H); 4.67 (brs, 2H); 4.62 (brs, 2H); 4.53 (d, *J* = 3.0, 1H); 4.28-4.18 (m, 1H); 4.14-4.11 (m, 1H); 3.89-3.63 (m, 3H); 2.58-2.49 (m, 1H); 2.28-2.20 (m, 1H); 1.92 (s, 3H).

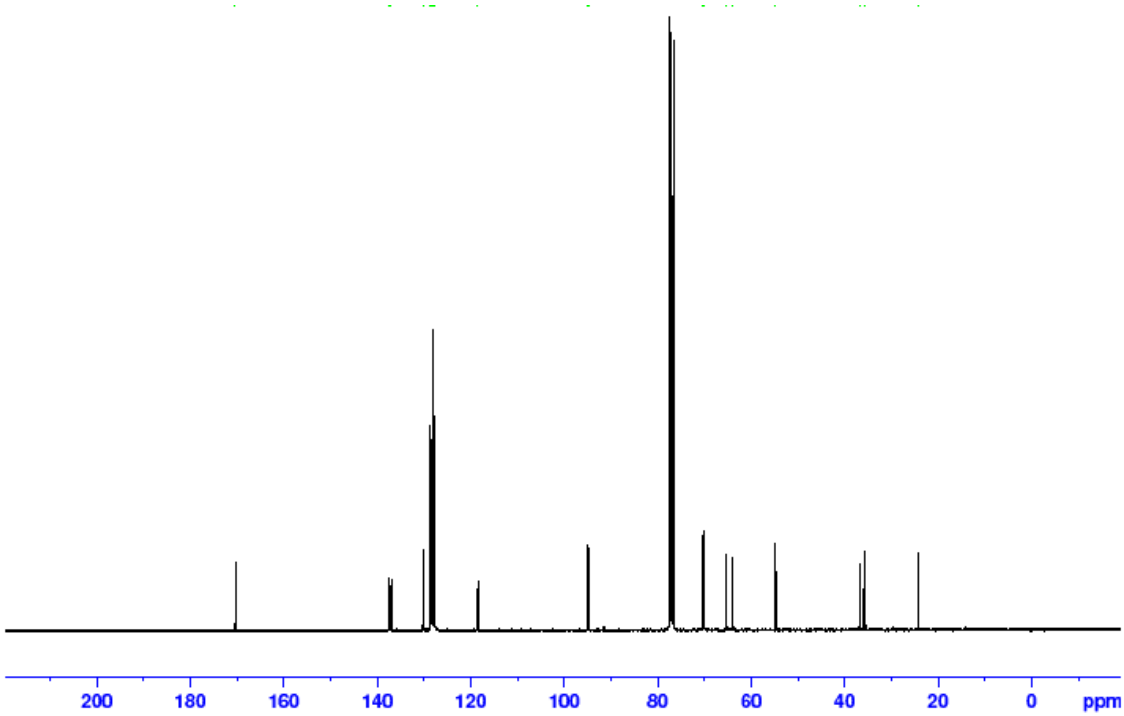
^{13}C NMR: δ 170.37, 137.38, 136.99, 130.23, 128.60, 128.54, 128.31, 128.02, 127.99, 127.75, 118.49, 94.97, 94.76, 70.28, 70.05, 65.38, 63.95, 54.78, 36.76, 35.85, 24.15.

IR (film, cm^{-1}): ν 2244, 1658.

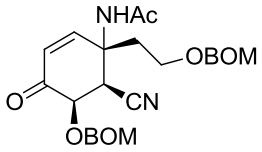
HRMS: calc. for $C_{27}H_{32}N_2O_6Na$ $[M + Na]^+$ 503.2158; found 503.2155.



Scheme 52. ^{1}H NMR spectrum of **161**



Scheme 53. ^{13}C NMR spectrum of **161**



A.8 Preparation of **162**

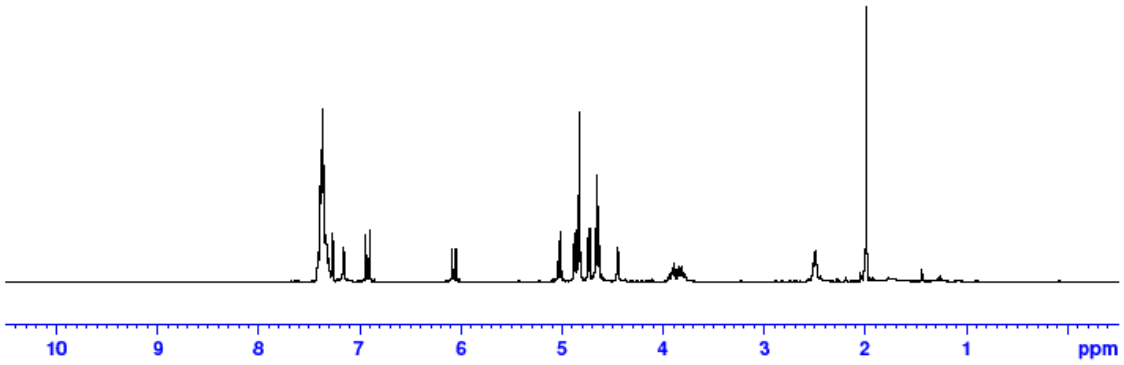
To a DCM solution (10 mL) of **161** (0.660 g, 1.37 mmol, 1.0 equiv.) at room temperature was added solid Dess-Martin periodinane (0.874 g, 2.06 mmol, 1.5 equiv.) and the mixture was stirred for 30 minutes. An aqueous solution of $Na_2S_2O_3$/$NaHCO_3$ (7:1, 20 mL) was added and the mixture was diluted with Et_2O (20 mL) and stirred until the layers turned clear (around 15 minutes). The layers were separated and the aqueous layer was extracted with Et_2O (2x20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford an off-white oil. The residue was purified by column chromatography on silica gel (EtOAc/Hex 7:3) to give 0.618 g (1.29 mmol, 94 %) of **162** as a colorless oil.

^{1}H NMR: δ 7.42-7.30 (m, 10H); 7.16 (br s, 1H); 6.92 (d, *J* = 10.6, 1H); 6.07 (d, *J* = 10.6, 1H); 5.03 (d, *J* = 7.0, 1H); 4.88-4.83 (m, 4H); 4.73 (d, *J* = 4.3, 1H); 4.67-4.63 (m, 3H); 4.45 (d, *J* = 4.3, 1H), 3.93-3.78 (m, 2H); 2.53-2.48 (m, 2H); 1.95 (s, 3H).

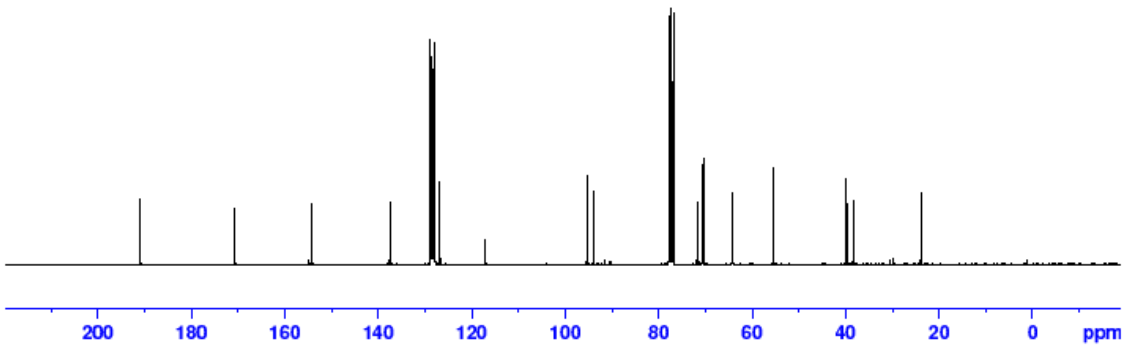
^{13}C NMR: δ 190.99, 170.67, 154.32, 137.51, 137.35, 128.82, 128.64, 128.40, 128.26, 128.09, 127.91, 126.80, 117.10, 95.32, 93.95, 71.53, 70.69, 70.43, 64.14, 55.53, 39.78, 38.30, 23.85.

IR (film, cm^{-1}): ν 2246, 1678.

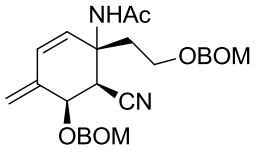
HRMS: calc. for $C_{27}H_{30}N_2O_6Na$ $[M + Na]^+$ 501.2002; found 501.1989.



Scheme 54. ^{1}H NMR spectrum of **162**



Scheme 55. ^{13}C NMR spectrum of **162**



A.9 Preparation of **163**

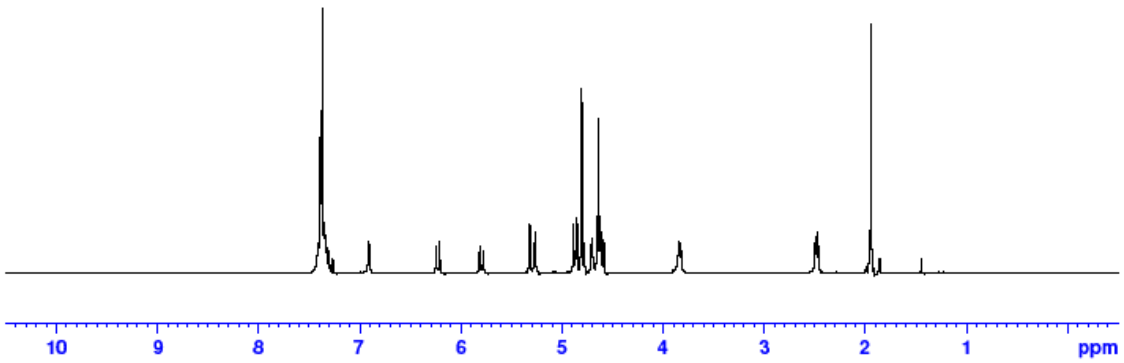
To a THF solution (3.0 mL) of methyltriphenylphosphonium bromide (0.747 g, 2.09 mmol, 5.0 equiv.) under argon at room temperature was carefully added *n*-BuLi (2.5 M in hexanes) dropwise (0.50 mL, 1.25 mmol, 3.0 equiv.) and the mixture was stirred for 1 hour. A THF solution (3.0 mL) of **162** (0.200 g, 0.42 mmol, 1.0 equiv.) was added dropwise to the reaction mixture at −78 °C. Upon completion of the addition, the reaction was stirred at −78 °C for 5 min and then warmed to 0 °C and further stirred for 2.5 h. A saturated solution (20 mL) of NH_4Cl was added and the reaction was warmed to room temperature. The mixture was diluted with Et_2O (20 mL) and the layers were separated. The aqueous layer was extracted with Et_2O (2x20 mL) and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford a pale yellow oil. The residue was purified by column chromatography on silica gel (Et_2O) to give 0.173 g (0.36 mmol, 87%) of **163** as a colourless oil.

^{1}H NMR: δ 7.41-7.28 (m, 10H); 6.91 (brs, 1H); 6.23 (d, *J* = 10.3, 1H); 5.80 (d, *J* = 10.3, 1H); 5.32 (brs, 1H); 5.27 (brs, 1H); 4.89-4.78 (m, 5H); 4.71-4.59 (m, 5H); 3.86-3.82 (m, 2H); 2.50-2.47 (m, 2H); 1.95 (s, 3H);.

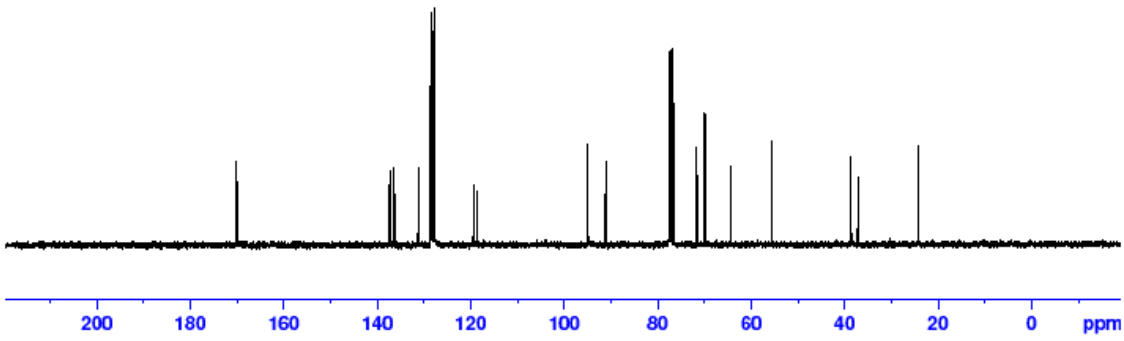
^{13}C NMR: δ 170.09, 137.43, 137.32, 136.39, 131.26, 128.60, 128.49, 128.22, 127.99, 127.89, 127.84, 127.78, 119.45, 118.57, 94.92, 91.15, 71.64, 69.95, 69.93, 64.31, 55.64, 38.61, 37.19, 24.18.

IR (film, cm^{-1}): ν 2243, 1659.

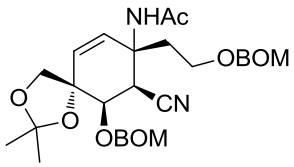
HRMS: calc. for $C_{28}H_{32}N_2O_5Na$ $[M + Na]^+$499.2209; found 499.2189.



Scheme 56. ^{1}H NMR spectrum of **163**



Scheme 57. ^{13}C NMR spectrum of **163**



A.10 Preparation of **170**

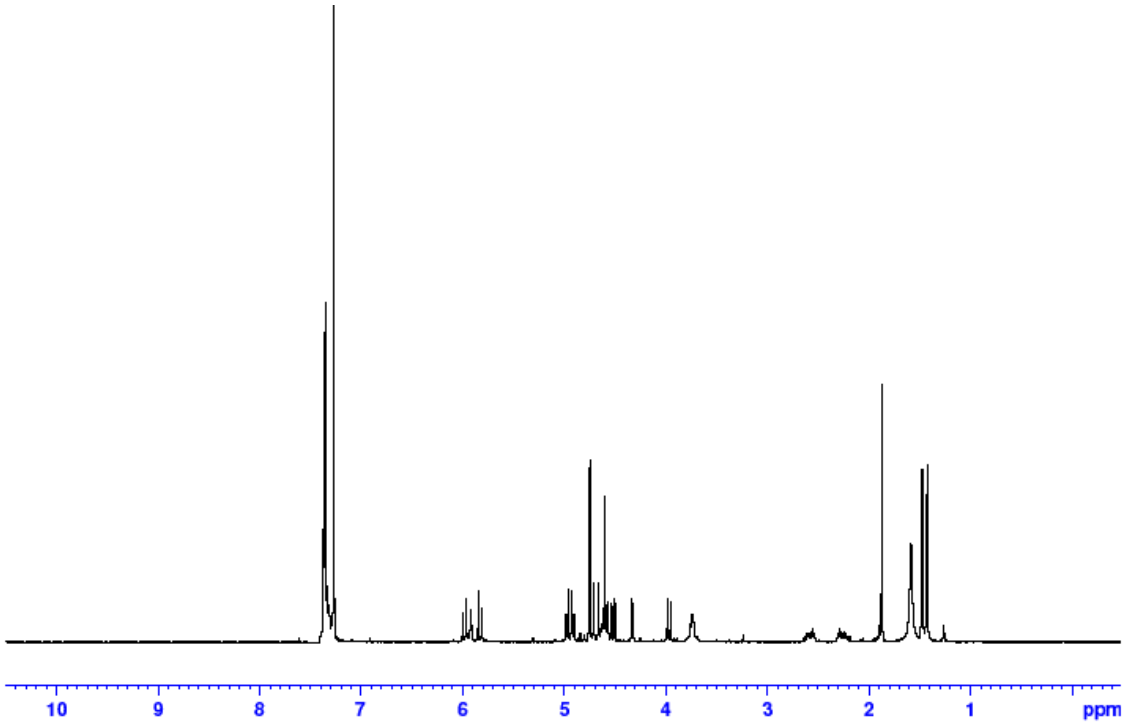
To an acetone solution (1.0 mL) of **163** (0.054 g, 0.11 mmol, 1.0 equiv.) at room temperature was added solid NMO (0.027 g, 0.22 mmol, 2.0 equiv.) and OsO_4 (4 wt. % in H_2O) (0.03 mL, 4.7 µmol, 0.05 equiv.) via syringe. The same syringe was used to draw up H_2O (0.2 mL) which was added to the reaction mixture and the mixture was stirred for 12 hours. Solid sodium bisulfite (0.011 g, 0.11 mmol, 1.0 equiv.) was added and the mixture was stirred for 30 min, filtered over celite, and the filter cake was washed with acetone. The filtrate was concentrated under vacuum to afford an off-white oil. To a dry acetone solution (1.0 mL) of the crude residue was added solid *p*-toluenesulfonic acid monohydrate (2 µg, 0.01 mmol, 0.1 equiv.) and 2,2-dimethoxypropane (0.08 mL, 0.66 mmol, 6.0 equiv.) at room temperature under argon. The reaction was stirred for 2 h and quenched with a saturated solution of $NaHCO_3$ (15 mL). The mixture was diluted with DCM (20 mL) and the layers were separated. The aqueous layer was extracted with DCM (2x10 mL) and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford a colorless oil. The residue was purified by column chromatography on silica gel (EtOAc/Hex 7:3) to give 0.056 g (0.10 mmol, 90% over 2 steps) of **170** as a colorless oil.

^{1}H NMR: δ 7.40-7.30 (m, 10H); 5.99 (d, *J* = 10.2, 1H); 5.92 (brs, 1H); 5.83 (d, *J* = 10.2, 1H); 4.98 (d, *J* = 6.9, 1H); 4.91 (d, *J* = 6.9, 1H); 4.75-4.60 (m, 6H); 4.57 (d, *J* = 3.4, 1H); 4.52 (d, *J* = 9.3, 1H); 4.33 (d, *J* = 3.4, 1H); 3.97 (d, *J* = 9.3, 1H); 3.80-3.68 (m, 2H); 2.64-2.49 (m, 1H); 2.31-2.18 (m, 1H); 1.87 (s, 3H); 1.47 (s, 3H); 1.43 (s, 3H).

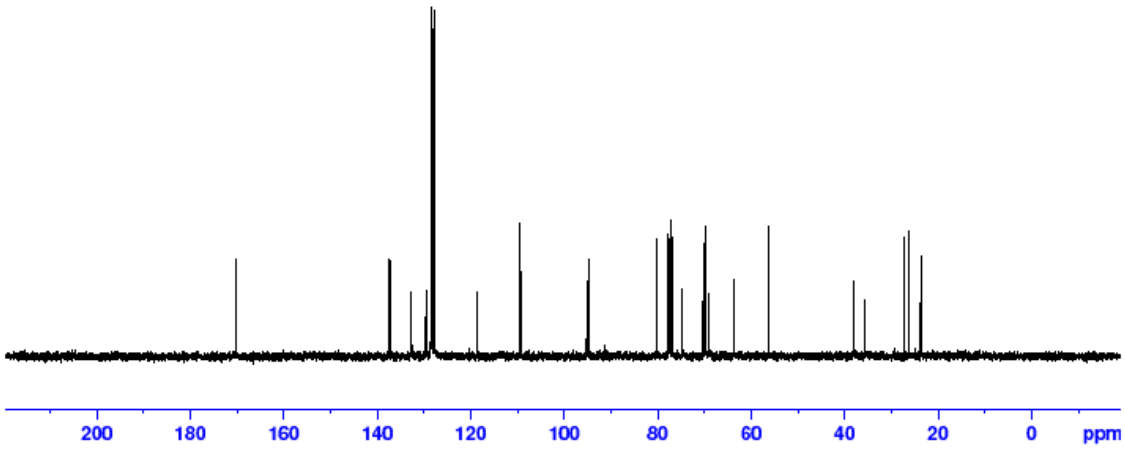
^{13}C NMR: δ 170.22, 137.64, 137.20, 132.70, 129.59, 128.53, 128.49, 127.99, 127.92, 127.88, 127.77, 118.58, 109.41, 95.19, 94.74, 80.22, 74.75, 70.24, 69.69, 69.00, 63.75, 56.77, 38.08, 35.63, 27.37, 26.23, 23.71.

IR (film, cm^{-1}): ν 2243, 1659.

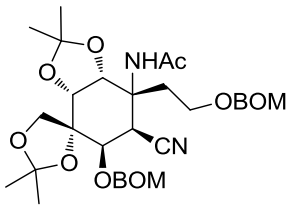
HRMS: calc. for $C_{31}H_{38}N_2O_7Na$ $[M + Na]^+$ 573.2577; found 573.2573.



Scheme 58. ^{1}H NMR spectrum of **170**



Scheme 59. ^{13}C NMR spectrum of **170**



A.11 Preparation of **171**

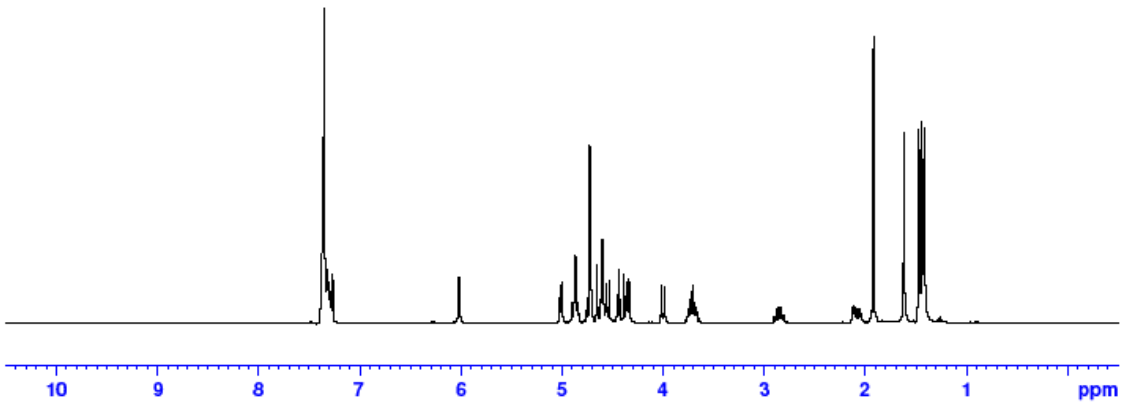
To a pyridine solution (2.0 mL) of **170** (0.320 g, 0.58 mmol, 1.0 equiv.) was added solid OsO_4 (0.221 g, 0.87 mmol, 1.5 equiv.) and the reaction was stirred for 96 h at room temperature. A saturated sodium bisulfite solution (5 mL) and acetone (5 mL) were added to the reaction and the mixture was stirred for 4 h. The reaction mixture was diluted with EtOAc (10 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (2x10 mL) and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford a light yellow oil. To a dry acetone solution (2.0 mL) of the crude residue was added solid *p*-toluenesulfonic acid monohydrate (0.011 g, 58 μmol, 0.1 equiv.) and 2,2-dimethoxypropane (0.43 mL, 3.48 mmol, 6.0 equiv.) at room temperature under argon. The reaction was stirred for 2 h and quenched with a saturated solution of $NaHCO_3$ (30 mL). The mixture was diluted with DCM (30 mL) and the layers were separated. The aqueous layer was extracted with DCM (2x20 mL) and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford a light, tan oil. The residue was purified by column chromatography on silica gel (EtOAc/Hex 1:1) to give 0.056g (61 % over 2 steps) of **171** as a clear, colourless oil.

^{1}H NMR: δ 7.39-7.29 (m, 10H); 6.02 (brs, 1H); 5.01 (d, *J* = 5.4, 1H); 4.90-4.84 (m, 2H); 4.76-4.54 (m, 7H); 4.44 (d, *J* = 6.5, 1H); 4.38 (d, *J* = 6.5, 1H); 4.34 (d, *J* = 5.4, 1H); 4.00 (d, *J* = 9.2, 1H); 3.78-3.65 (m, 2H); 2.90-2.77 (m, 1H); 2.20-2.04 (m, 1H); 1.92 (s, 3H); 1.62 (s, 3H); 1.47 (s, 3H); 1.44 (s, 3H); 1.42 (s, 3H).

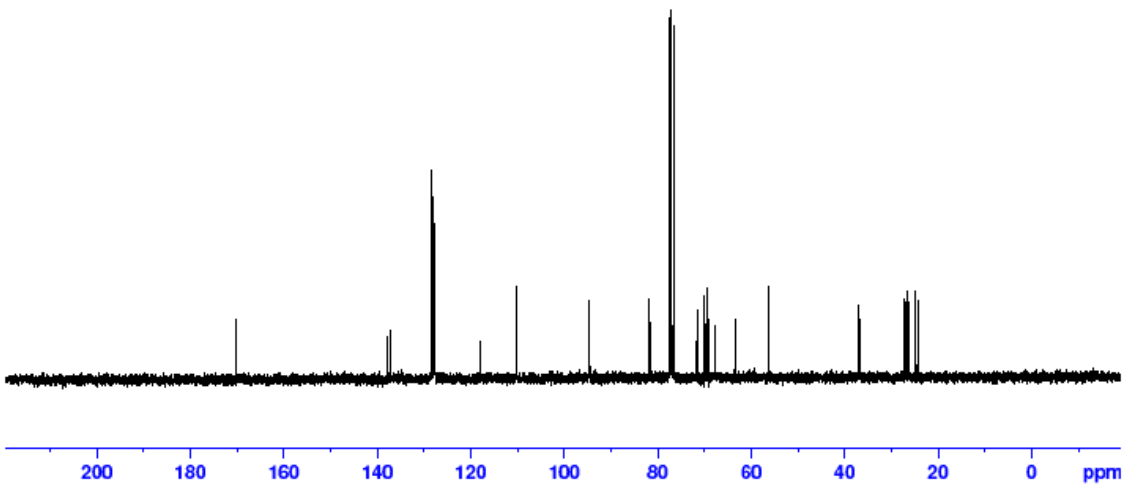
^{13}C NMR: δ 170.20, 137.84, 137.31, 128.45, 128.39, 128.03, 127.94, 127.74, 118.01, 110.23, 110.18, 94.70, 94.59, 81.75, 77.56, 77.13, 71.60, 69.95, 69.28, 67.77, 63.37, 56.32, 37.06, 36.82, 27.11, 26.50, 26.36, 24.79, 24.21.

IR (film, cm^{-1}): ν 2243, 1679.

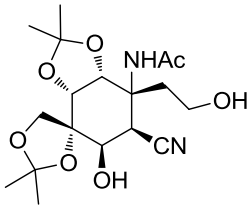
HRMS: calc. for $C_{34}H_{44}N_2O_9Na$ $[M + Na]^+$ 647.2945; found 647.2946.



Scheme 60. ^{1}H NMR spectrum of **171**



Scheme 61. ^{13}C NMR spectrum of **171**



A.12 Preparation of **172**

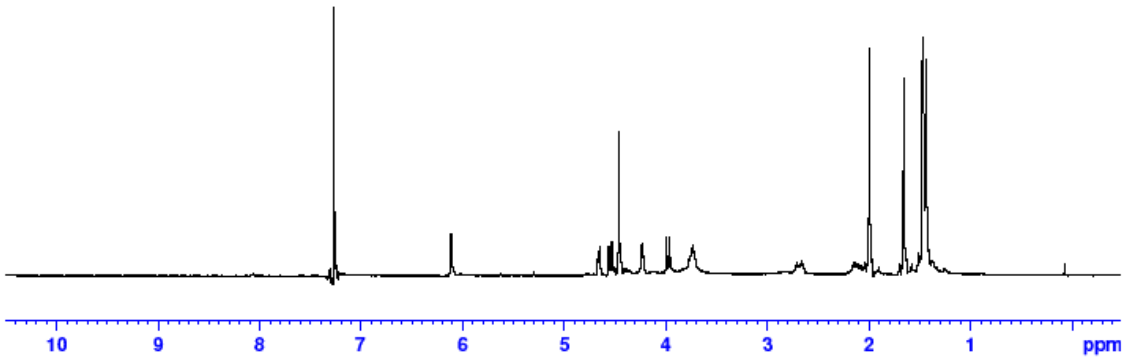
To a solution of **171** (0.180 g, 0.29 mmol, 1.0 equiv.) in EtOH at room temperature was added $Pd(OH)_2/C$ (10 %, 0.647 g, 0.46 mmol, 1.6 equiv.) and the reaction vessel was flushed with H_2. The reaction was stirred for 12 h and the mixture was filtered over celite. The filtrate was concentrated under vacuum to give an off-white oil. The residue was purified by column chromatography on silica gel (MeOH/EtOAc 1:9) to give 0.089g (80 %) of **172** as a clear, colourless oil.

^{1}H NMR: δ 6.12 (brs, 1H); 4.66 (d, *J* = 4.9, 1H); 4.54 (d, *J* = 9.2, 1H); 4.46 (apps, 2H); 4.23 (d, *J* = 4.9, 1H); 3.98 (d, *J* = 9.2, 1H); 3.77-3.71 (m, 3H); 2.73-2.64 (m, 1H); 2.16-2.10 (m, 1H); 2.00 (s, 3H); 1.66 (s, 3H); 1.47 (s, 3H); 1.46 (s, 3H); 1.44 (s, 3H).

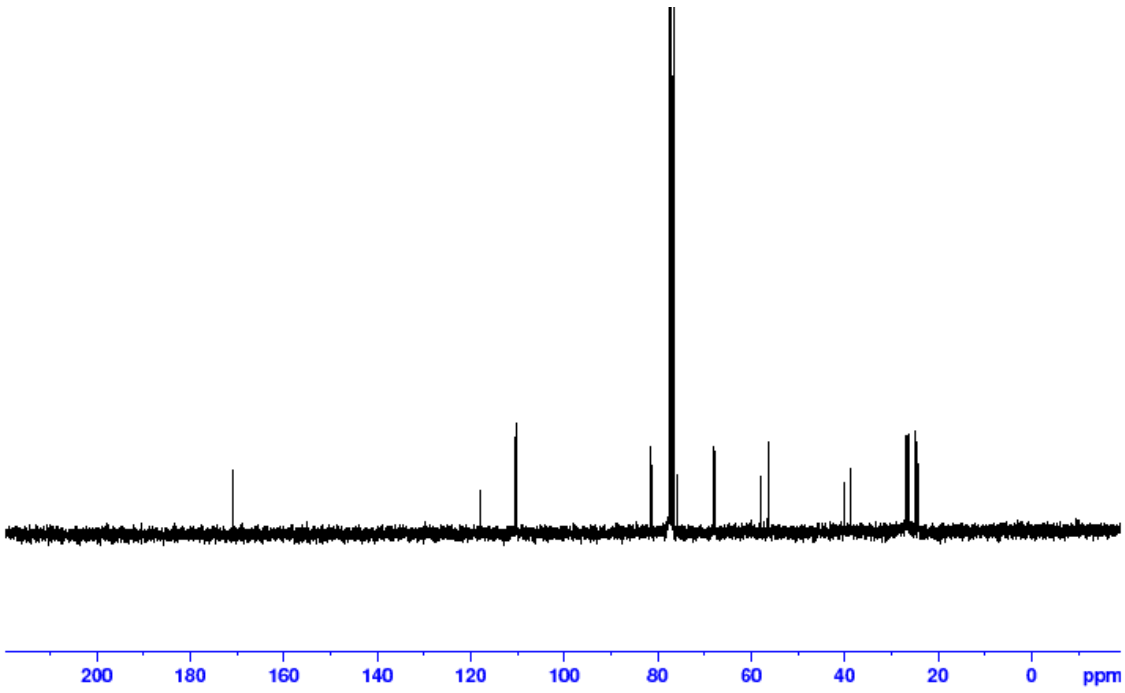
^{13}C NMR: δ 170.95, 118.05, 110.47, 110.31, 81.41, 77.60, 75.81, 67.92, 58.00, 46.40, 40.01, 38.77, 26.82, 26.62, 26.42, 24.75, 24.22.

IR (film, cm^{-1}): ν 2245, 1658.

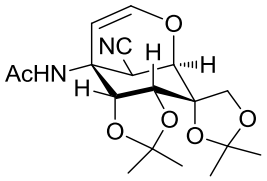
HRMS: calc. for $C_{18}H_{28}N_2O_7Na$ $[M + Na]^+$ 407.1794; found 407.1797.



Scheme 62. ^{1}H NMR spectrum of **172**



Scheme 63. ^{13}C NMR spectrum of **172**



A.13 Preparation of **176**

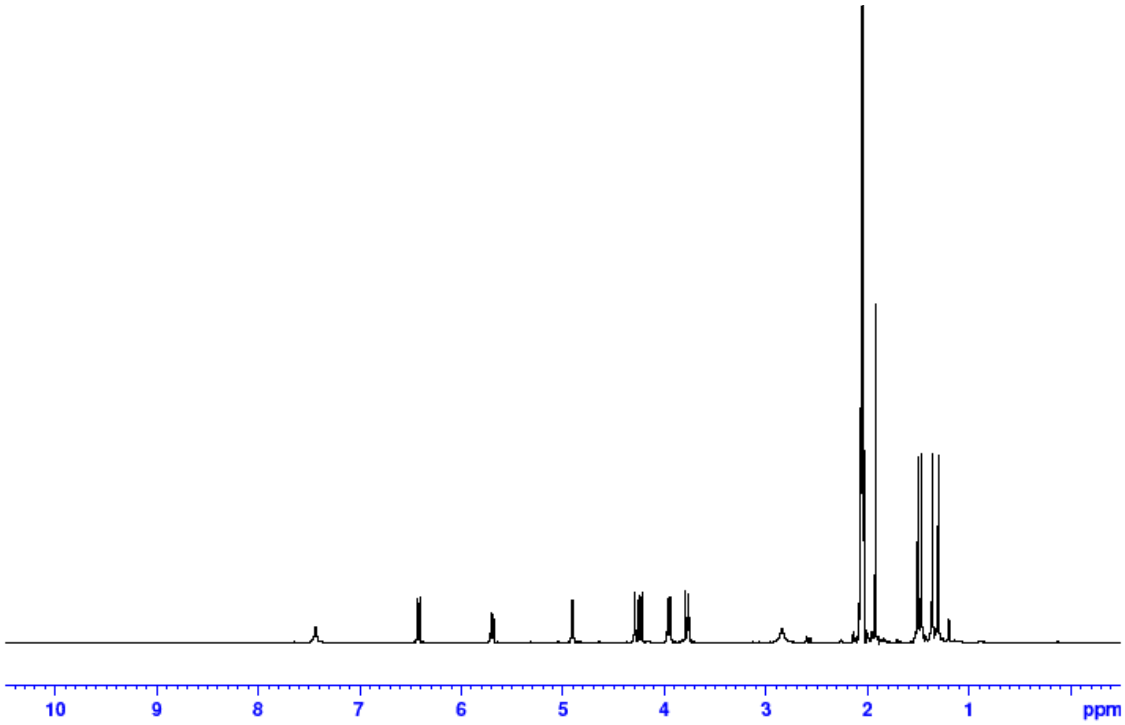
To a DCM solution (1.5 mL) of **172** (0.084 g, 0.22 mmol, 1.0 equiv.) was added solid Dess-Martin periodinane (0.139 g, 0.33 mmol, 1.5 equiv.) at room temperature and the reaction was stirred for 10 minutes. A solution of 7:1 (vol/vol) aqueous $Na_2S_2O_3$:Na_2HCO_3 (2 mL) was added and the mixture was diluted with Et_2O (5 mL). The cloudy mixture was stirred rapidly until it turned clear (~15 min.). The layers were separated and the aqueous layer was extracted with Et_2O (2x5 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford an off-white oil. The residue was taken up in DCM (1.5 mL) and to it was added 2,6-lutidine (0.37 mL, 3.30 mmol, 15.0 equiv.) and Tf_2O (1.0 M in DCM, 1.32 mL, 1.32 mmol, 6.0 equiv.), respectively, at -78 °C under argon. The mixture was stirred for 2 hours followed by the addition of a solution of saturated $NaHCO_3$ (aq.) (1.0 mL) and the reaction mixture was warmed to room temperature. The mixture was diluted with water (3.0 mL) and DCM (7.0 mL) and the layers were separated. The aqueous layer was extracted with DCM (2x5 mL) and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to afford pale, brown oil. The residue was purified by column chromatography on silica gel (EtOAc/Hex gradient 1:1-7:3) to give 0.48g (60 % over 2 steps) of **176** as a clear, colorless oil.

^{1}H NMR (acetone-d_6): δ 6.37 (d, *J* = 6.2, 1H); 5.67-5.65 (m, 2H); 4.94 (d, *J* = 5.3, 1H); 4.30-4.27 (m, 2H); 3.89 (d, *J* = 5.3, 1H); 3.82-3.79 (m, 2H); 2.06 (s, 3H); 1.54 (s, 3H); 1.48 (s, 3H); 1.39 (s, 3H); 1.38 (s, 3H).

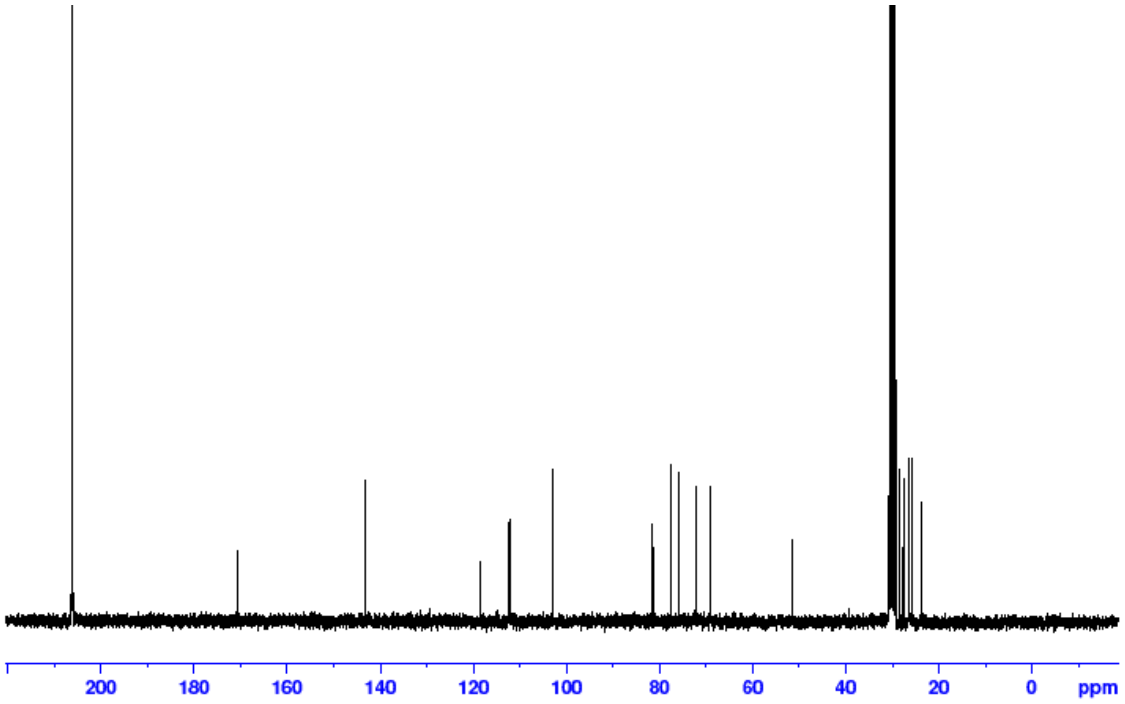
^{13}C NMR (acetone-d_6): δ 170.30, 142.56, 117.39, 112.22, 112.07, 101.69, 99.48, 80.36, 76.37, 74.92, 71.40, 68.28, 50.85, 28.30, 27.06, 26.23, 25.93, 25.25.

IR (film, cm^{-1}): ν 2246, 1668.

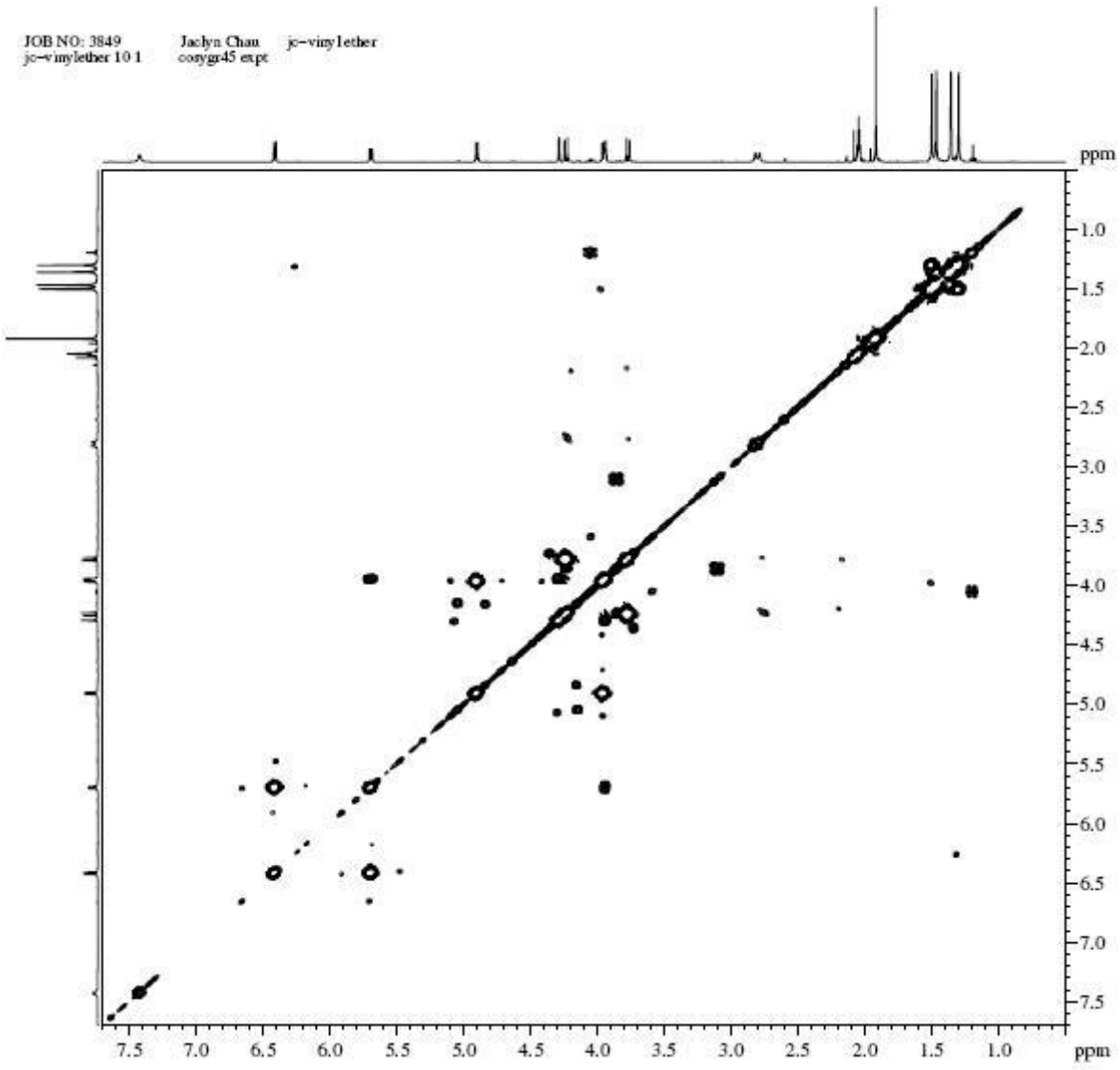
HRMS: calc. for $C_{18}H_{24}N_2O_6Na$ $[M + Na]^+$ 387.1532; found 387.1535.



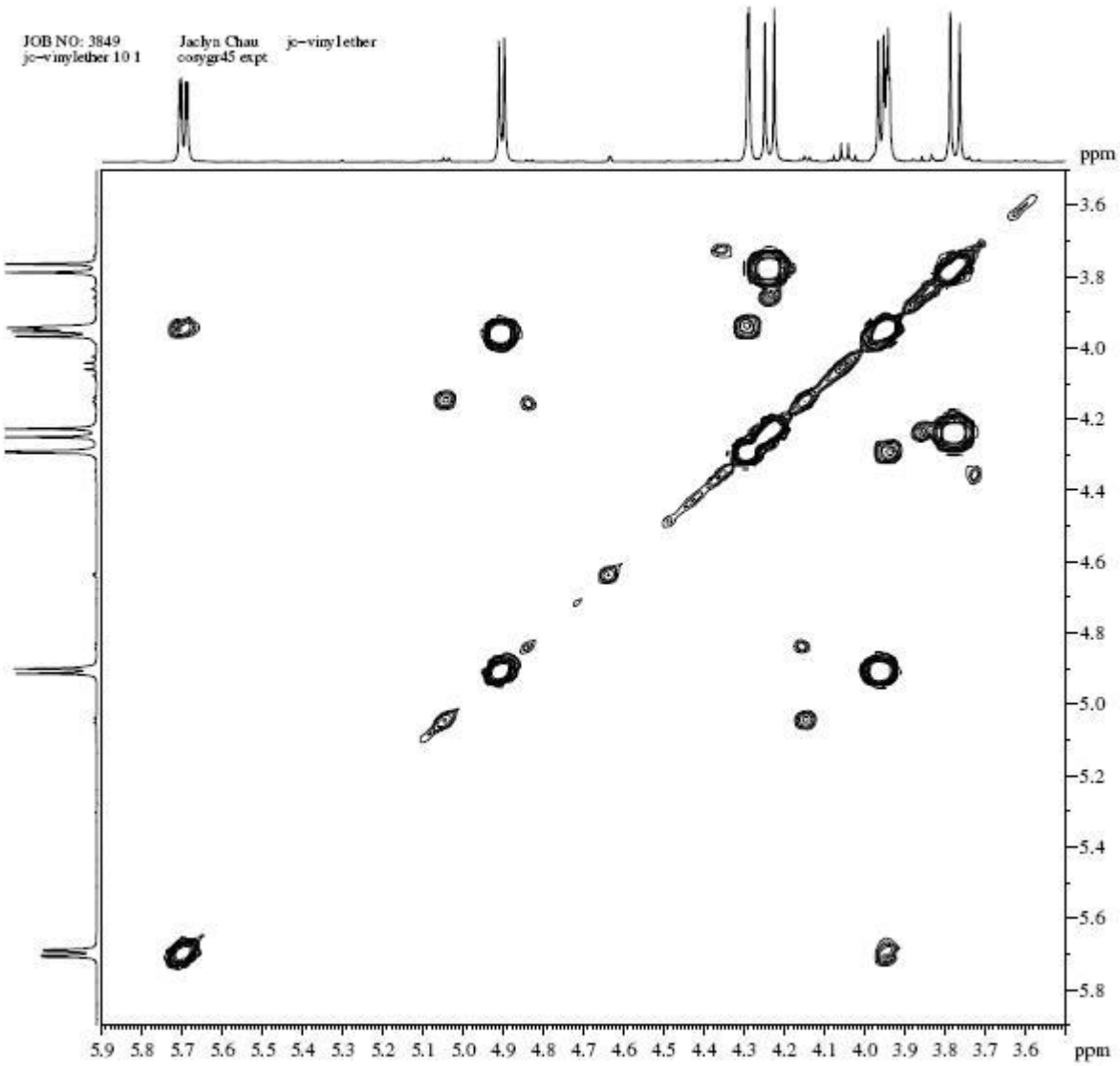
Scheme 64. ^{1}H NMR spectrum of **176**



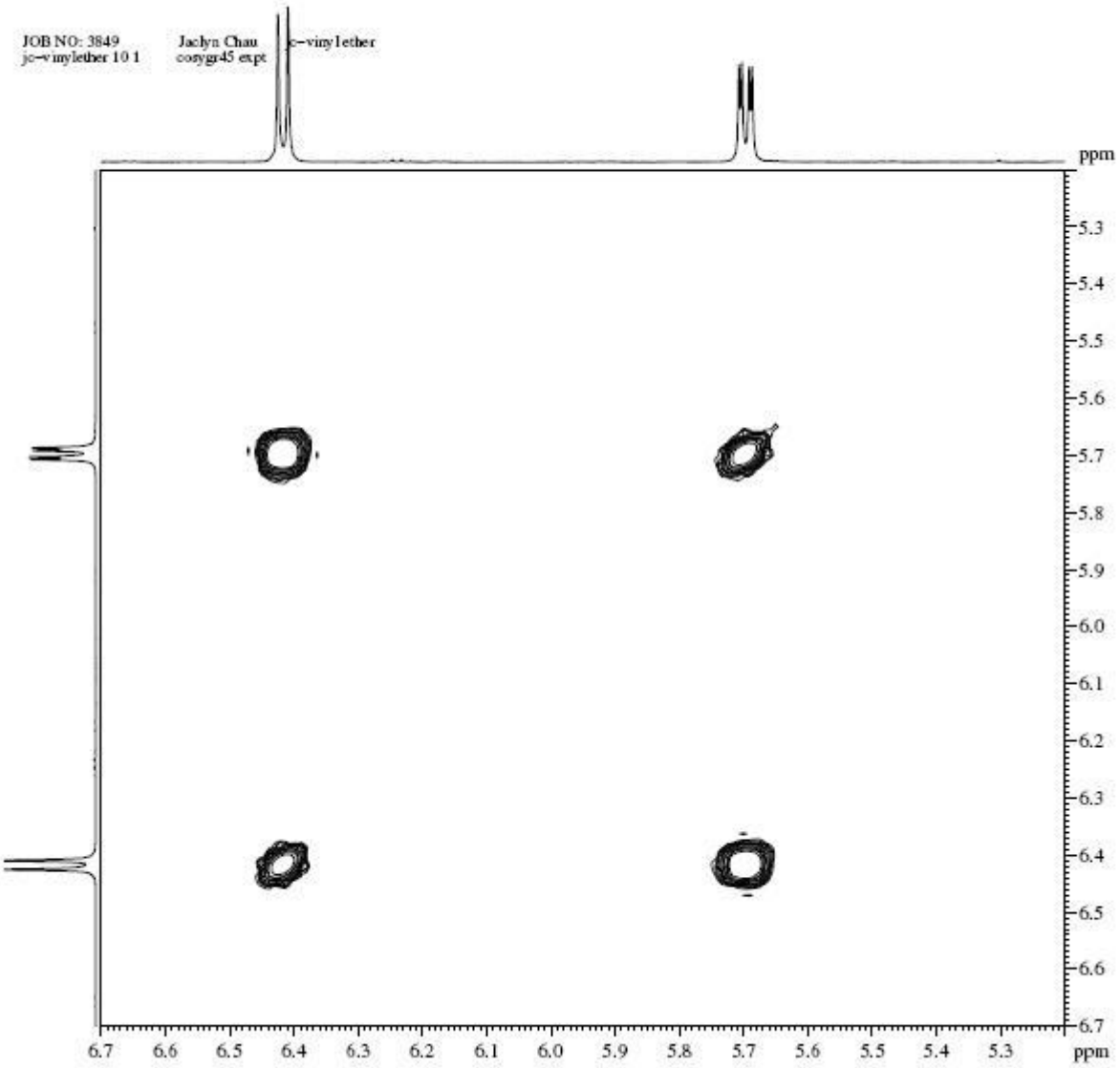
Scheme 65. ^{13}C NMR spectrum of **176**



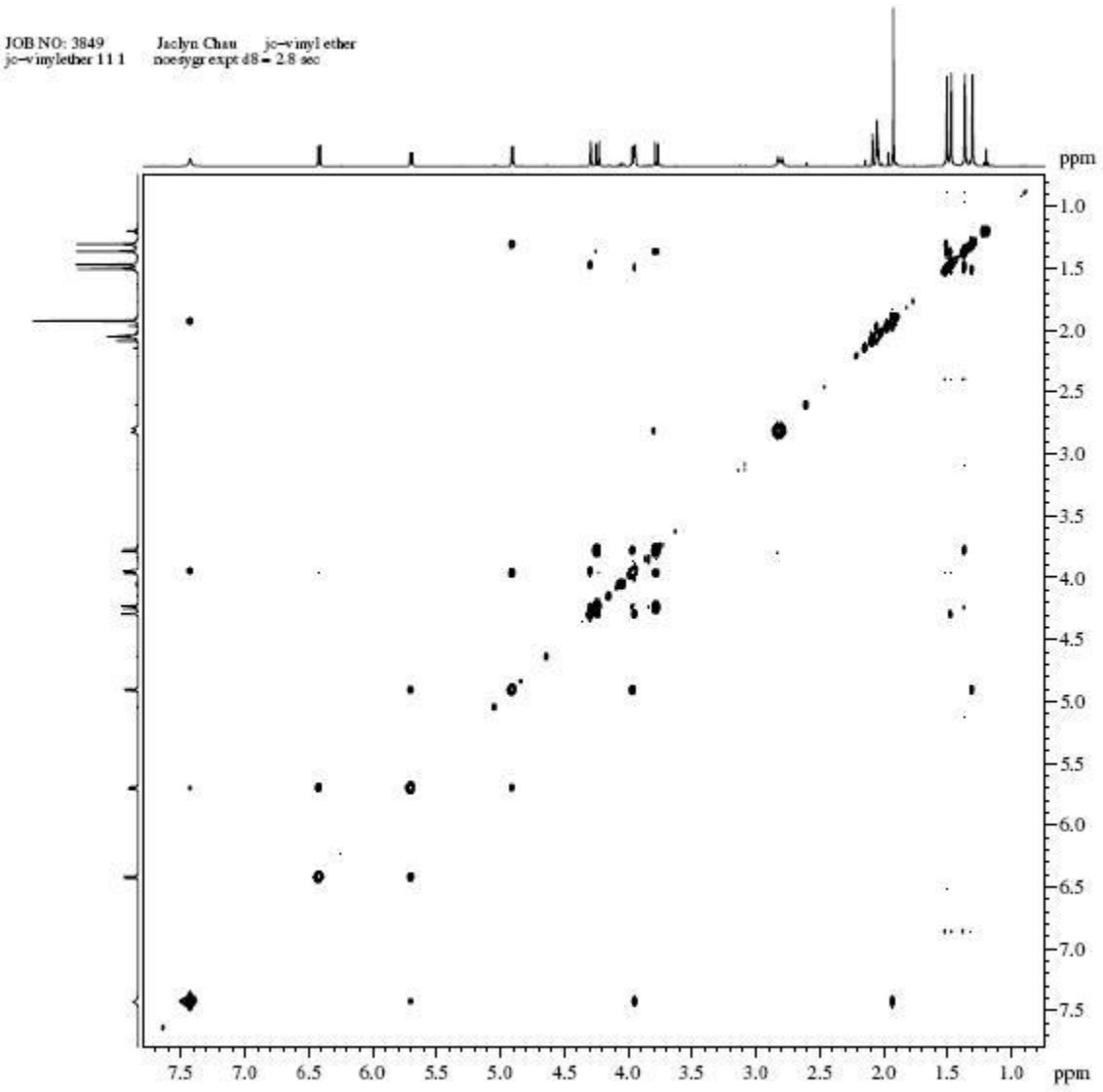
Scheme 66. COSY spectrum of **176** (expansion)



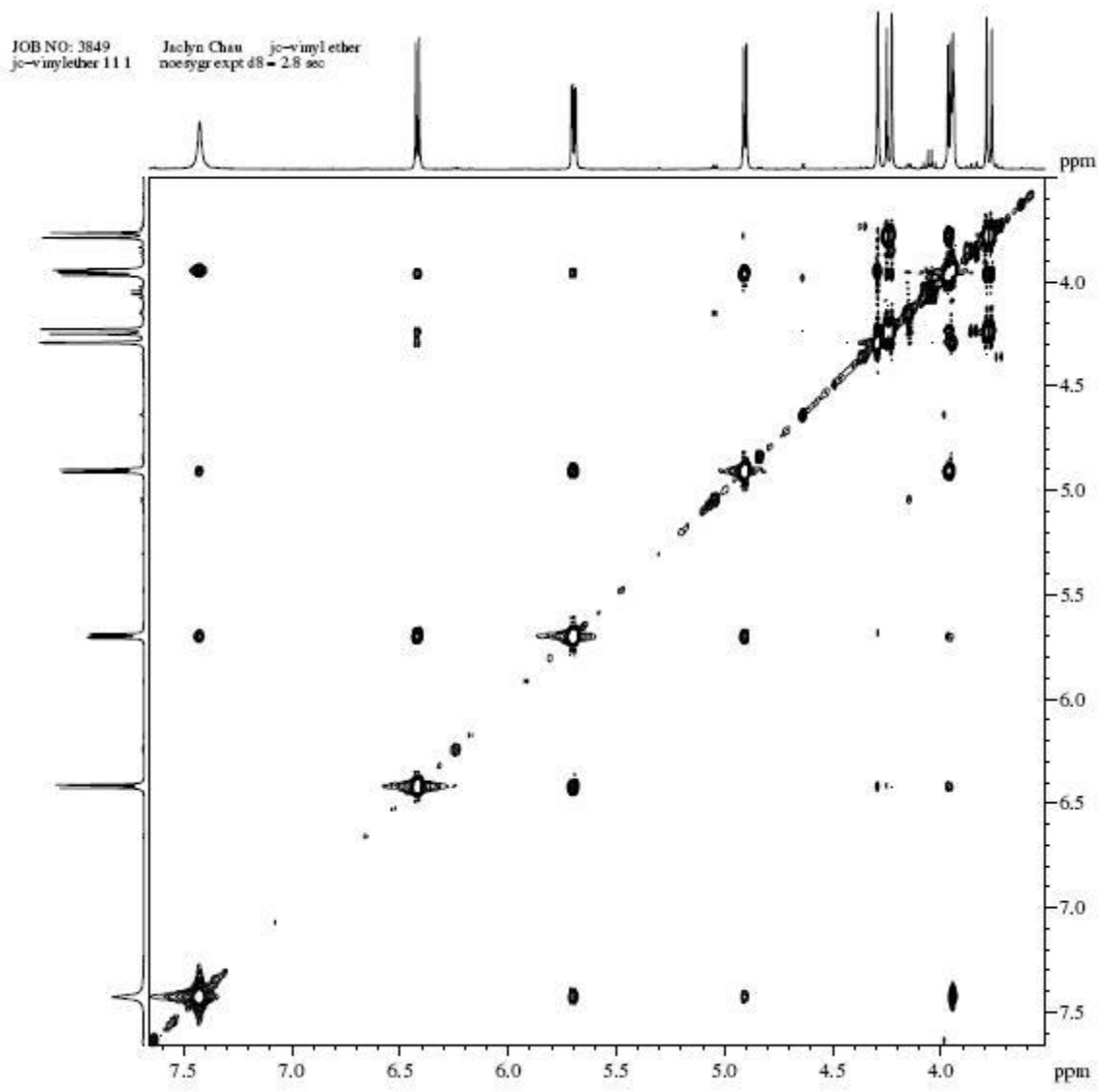
Scheme 67. COSY spectrum of **176** (expansion)



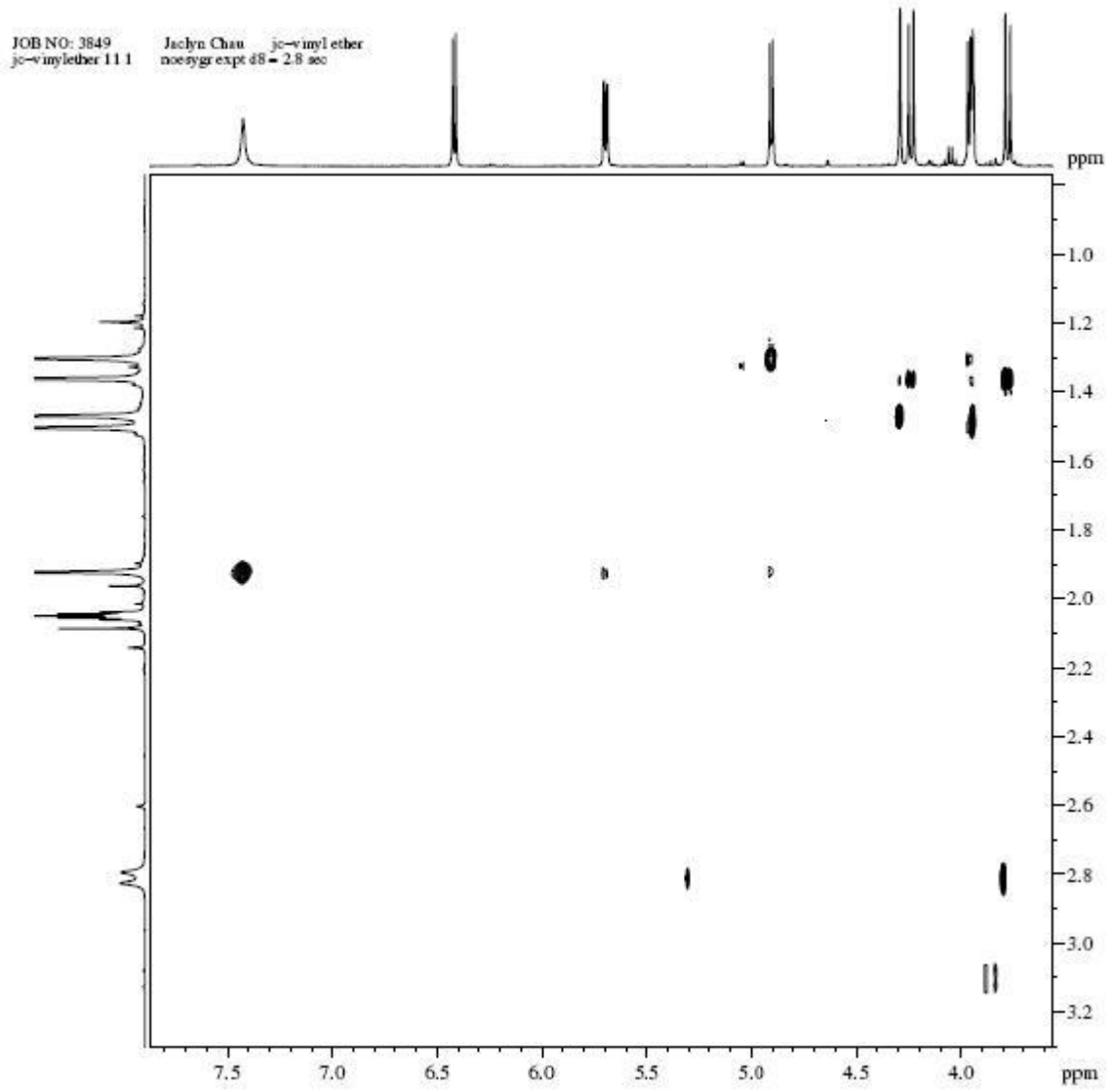
Scheme 68. COSY spectrum of **176** (expansion)



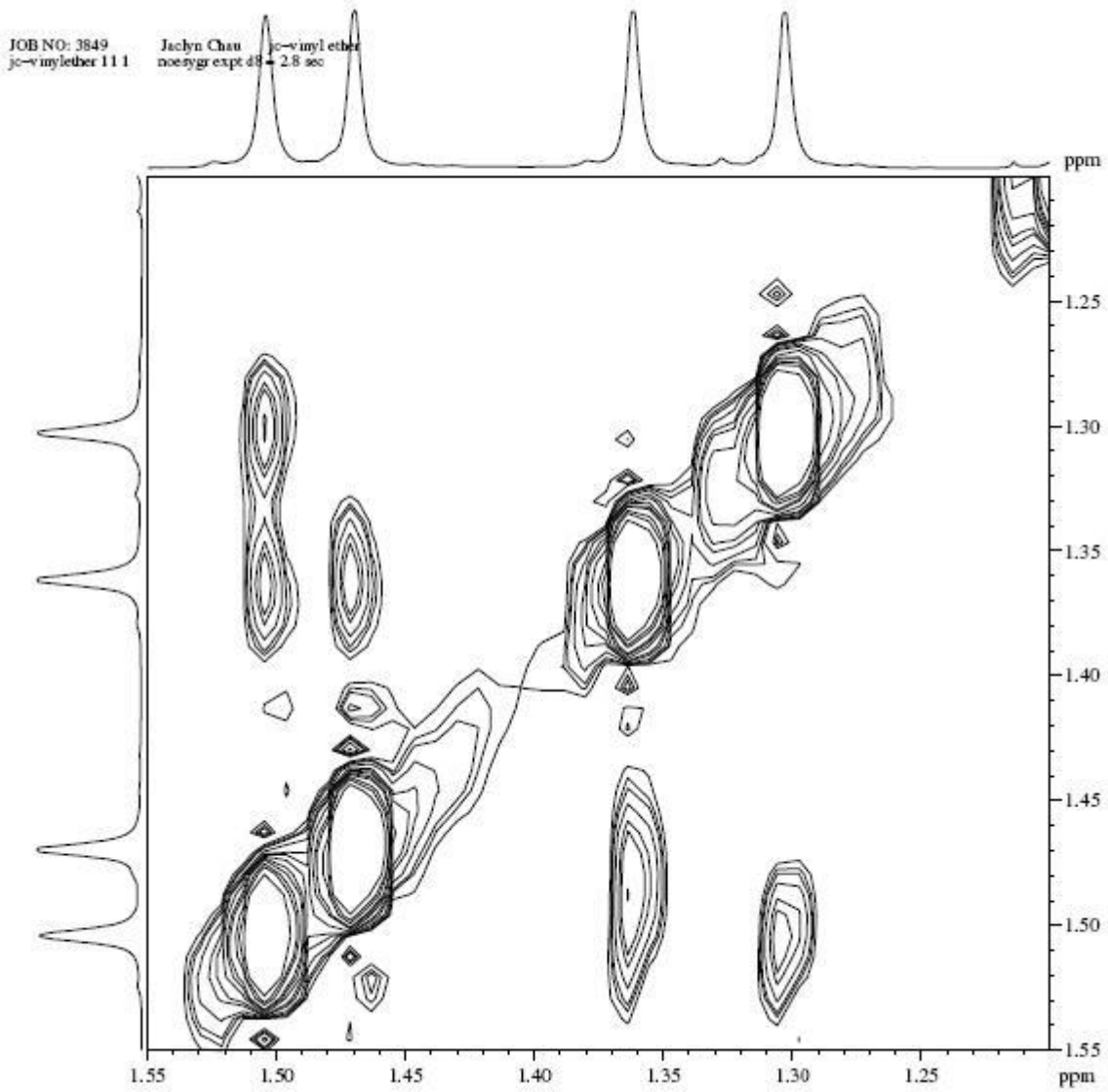
Scheme 69. NOESY spectrum of **176**



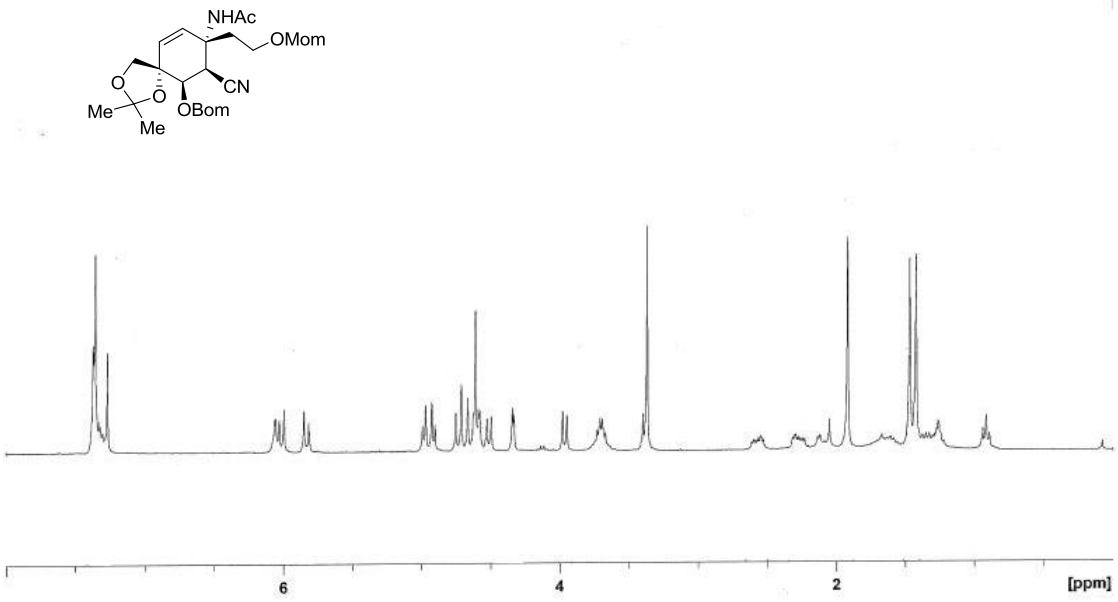
Scheme 70. NOESY spectrum of **176** (expansion)



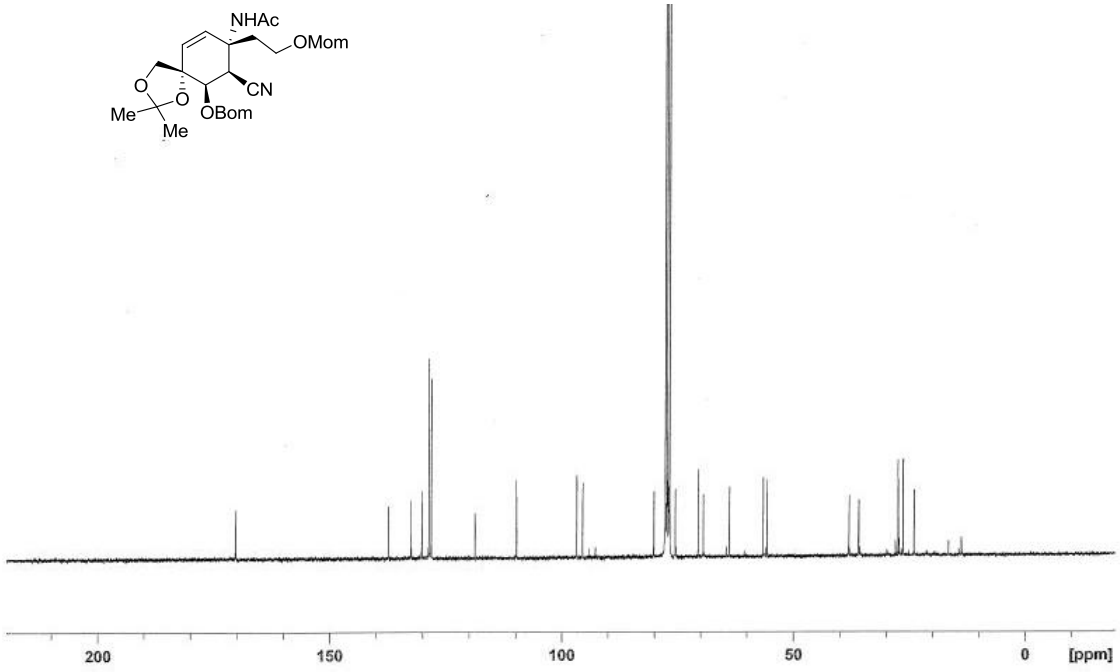
Scheme 71. NOESY spectrum of **176** (expansion)



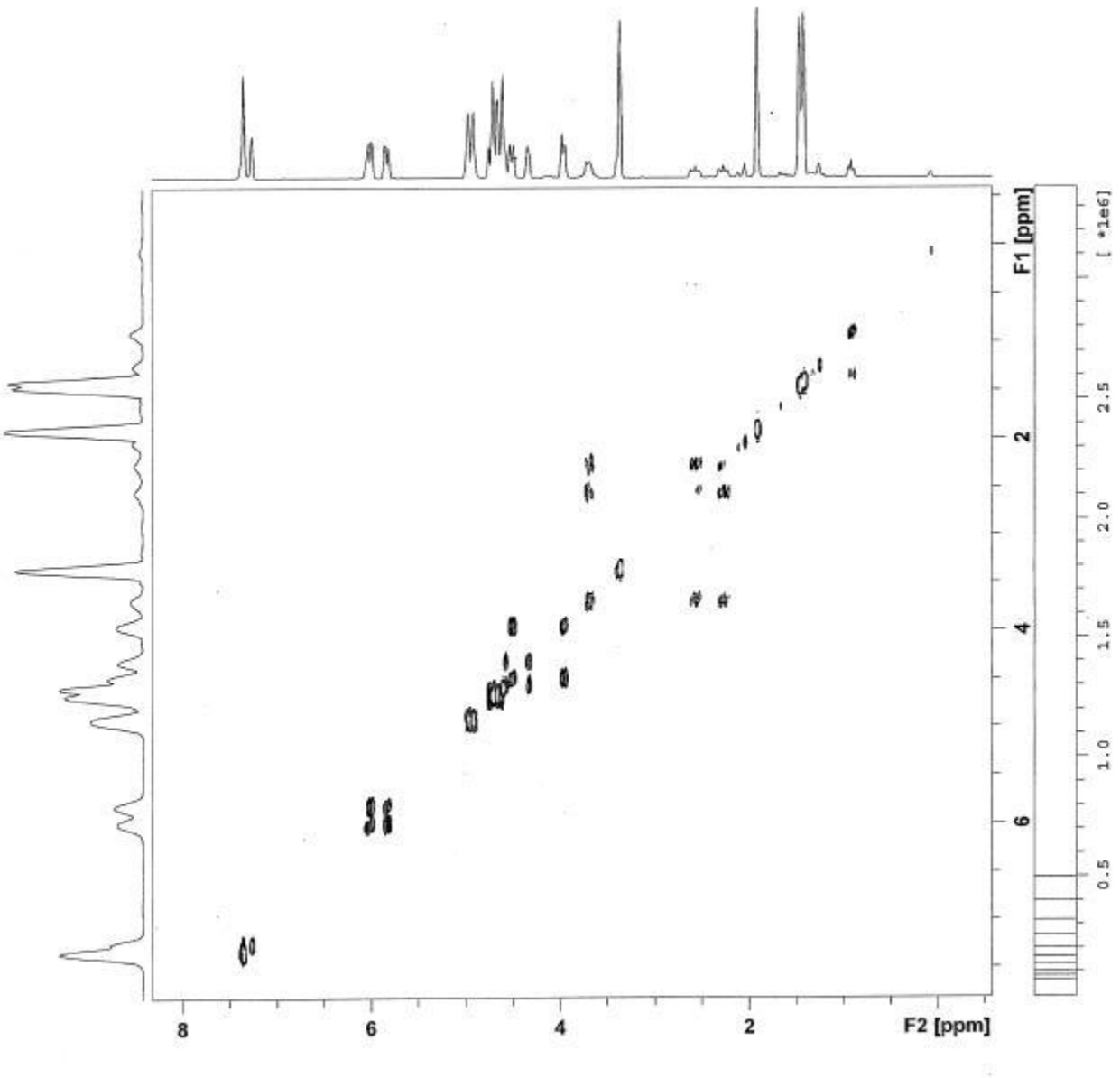
Scheme 72. NOESY spectrum of **176** (expansion)



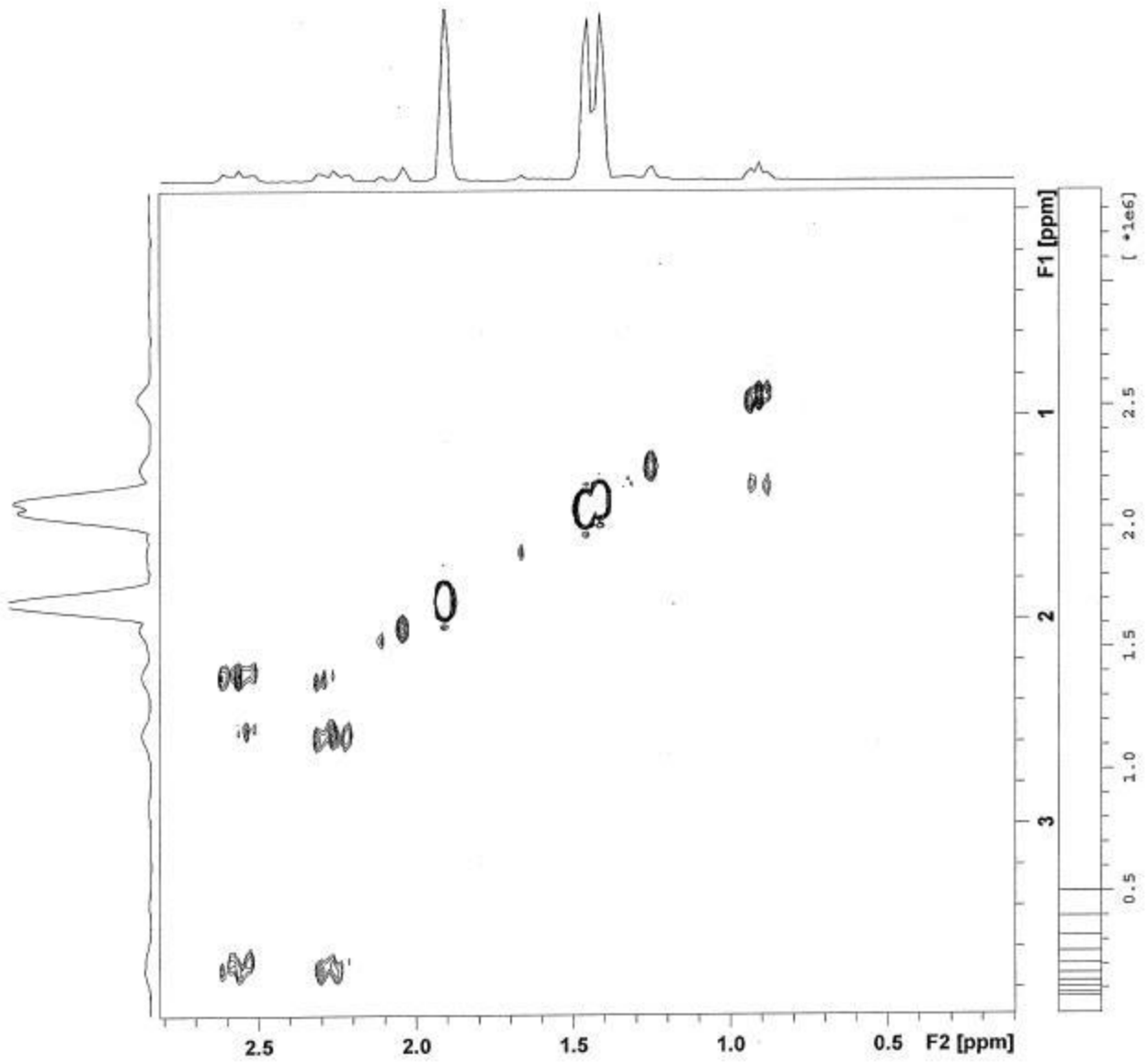
Scheme 73. ^{1}H NMR spectrum of **169**



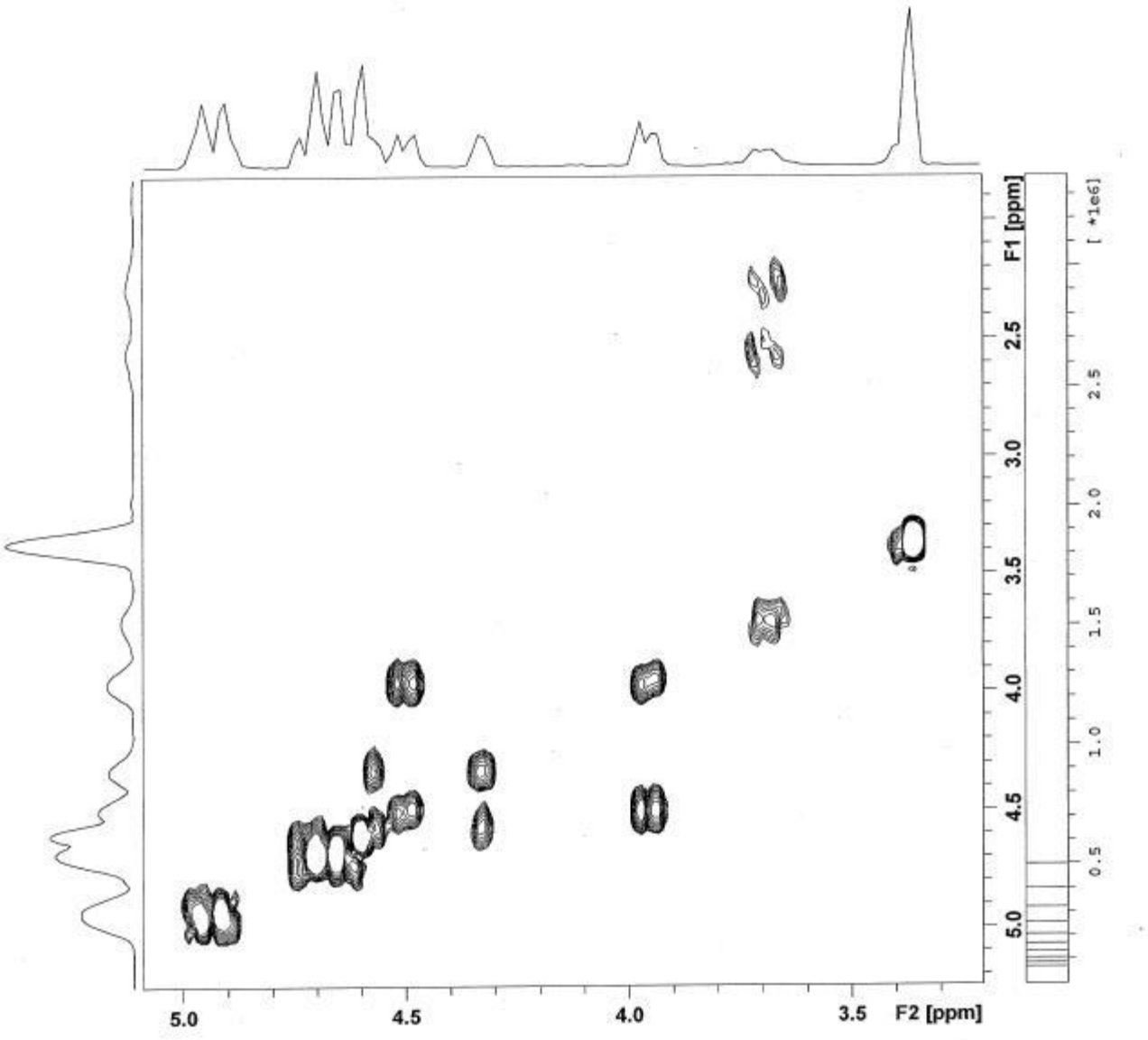
Scheme 74. ^{13}C NMR spectrum of **169**



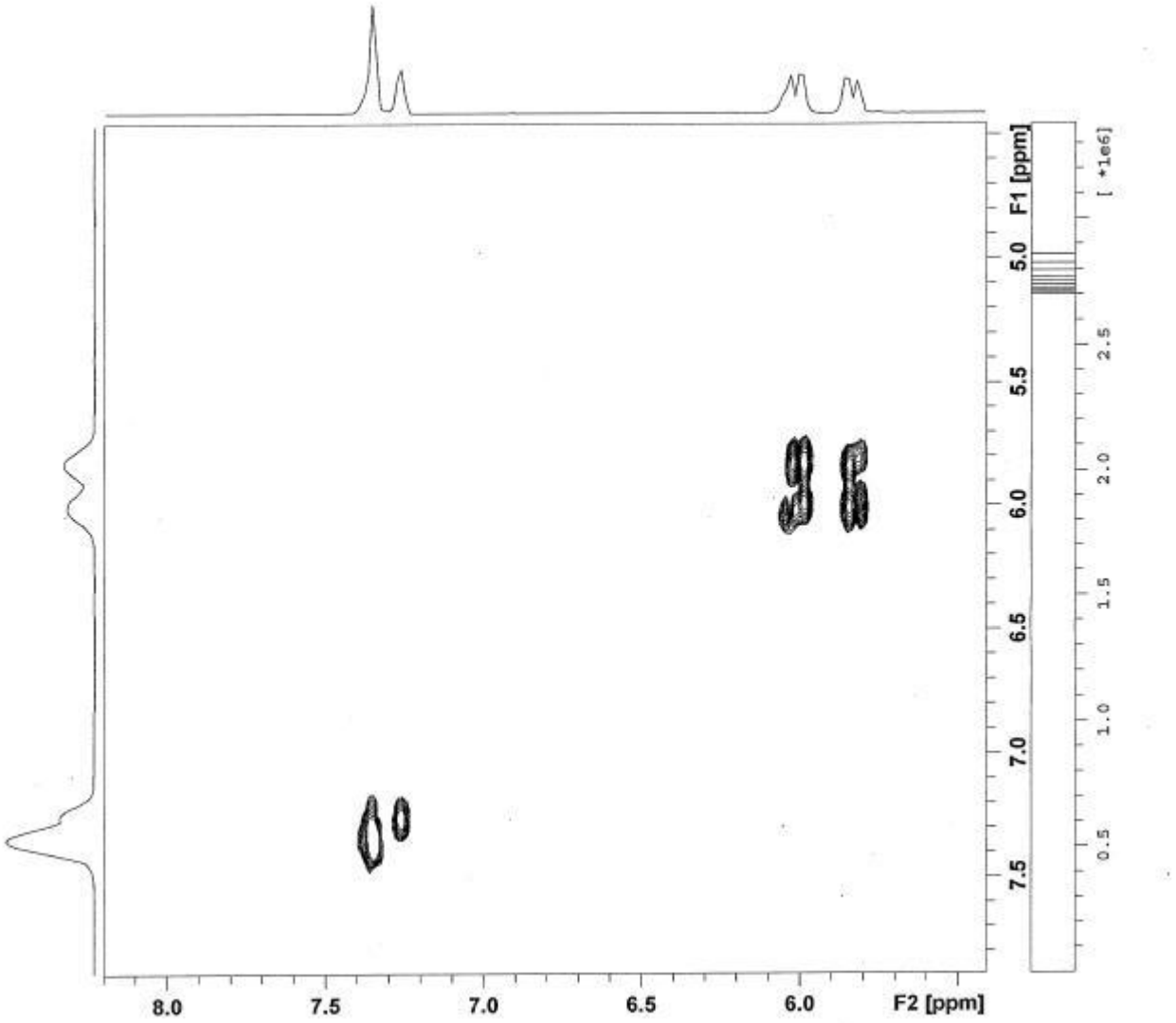
Scheme 75. COSY spectrum of **169**



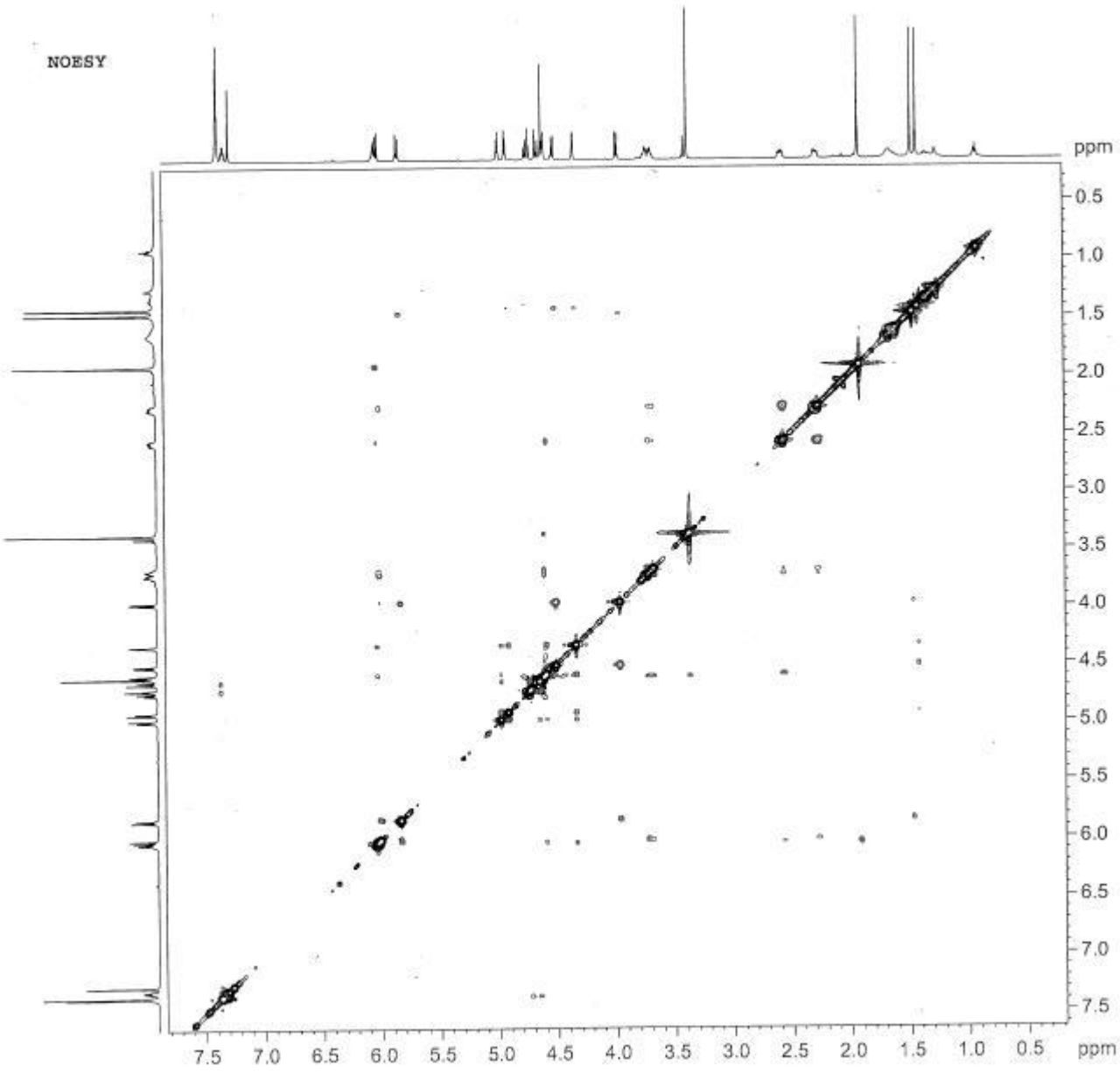
Scheme 76. COSY spectrum of **169** (expansion)



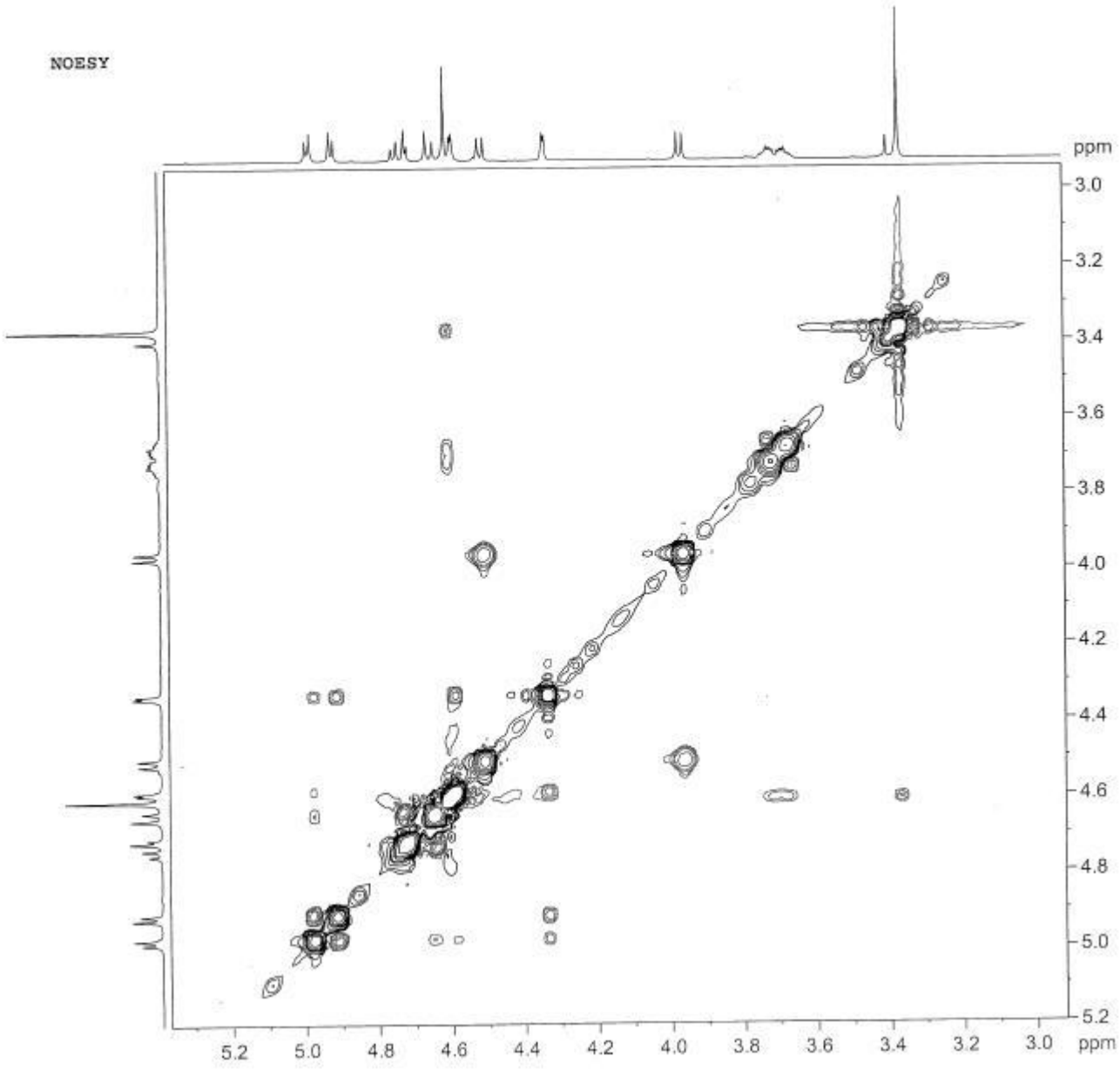
Scheme 77. COSY spectrum of **169** (expansion)



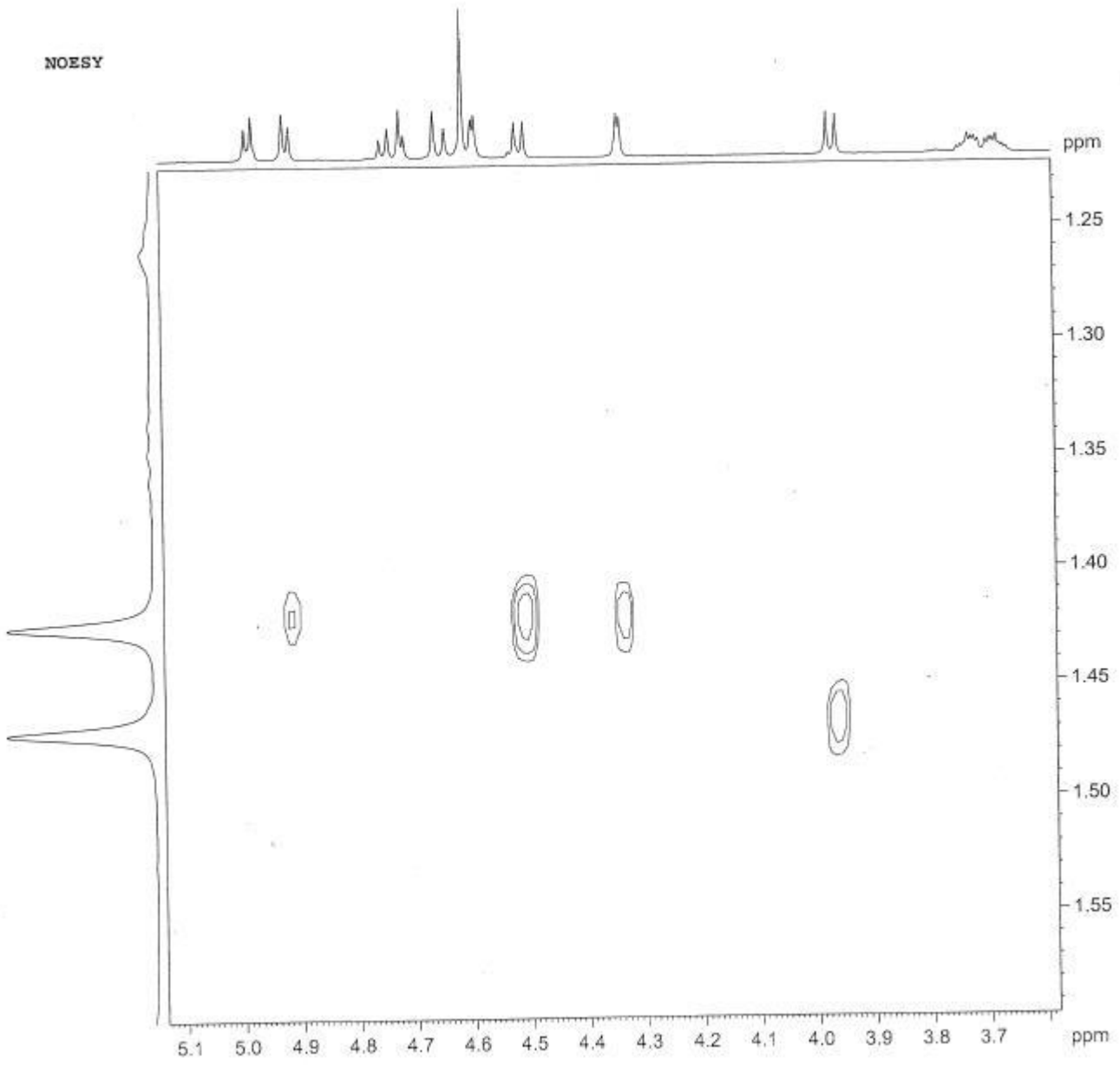
Scheme 78. COSY spectrum of **169** (expansion)



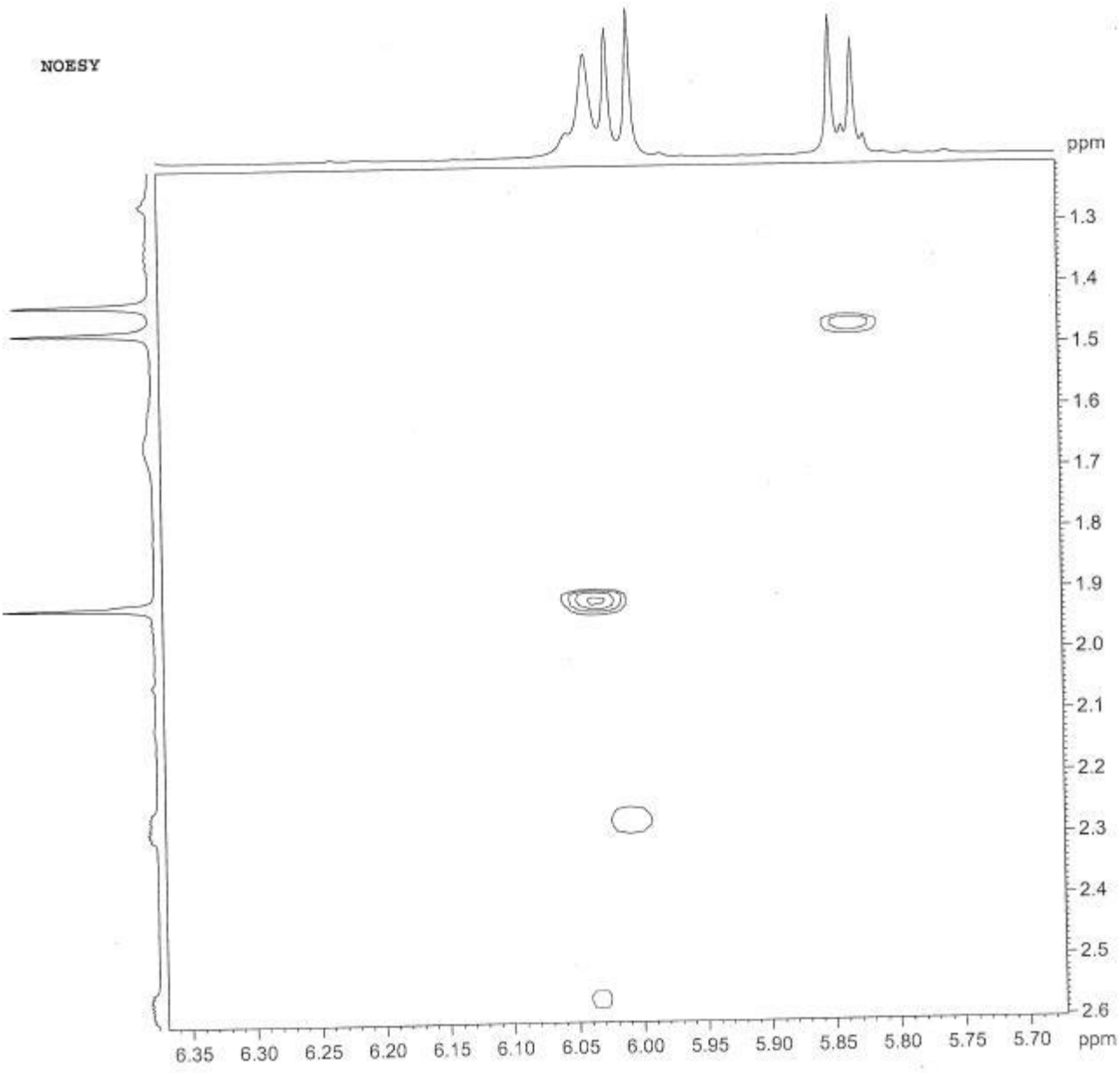
Scheme 79. NOESY spectrum of **169**



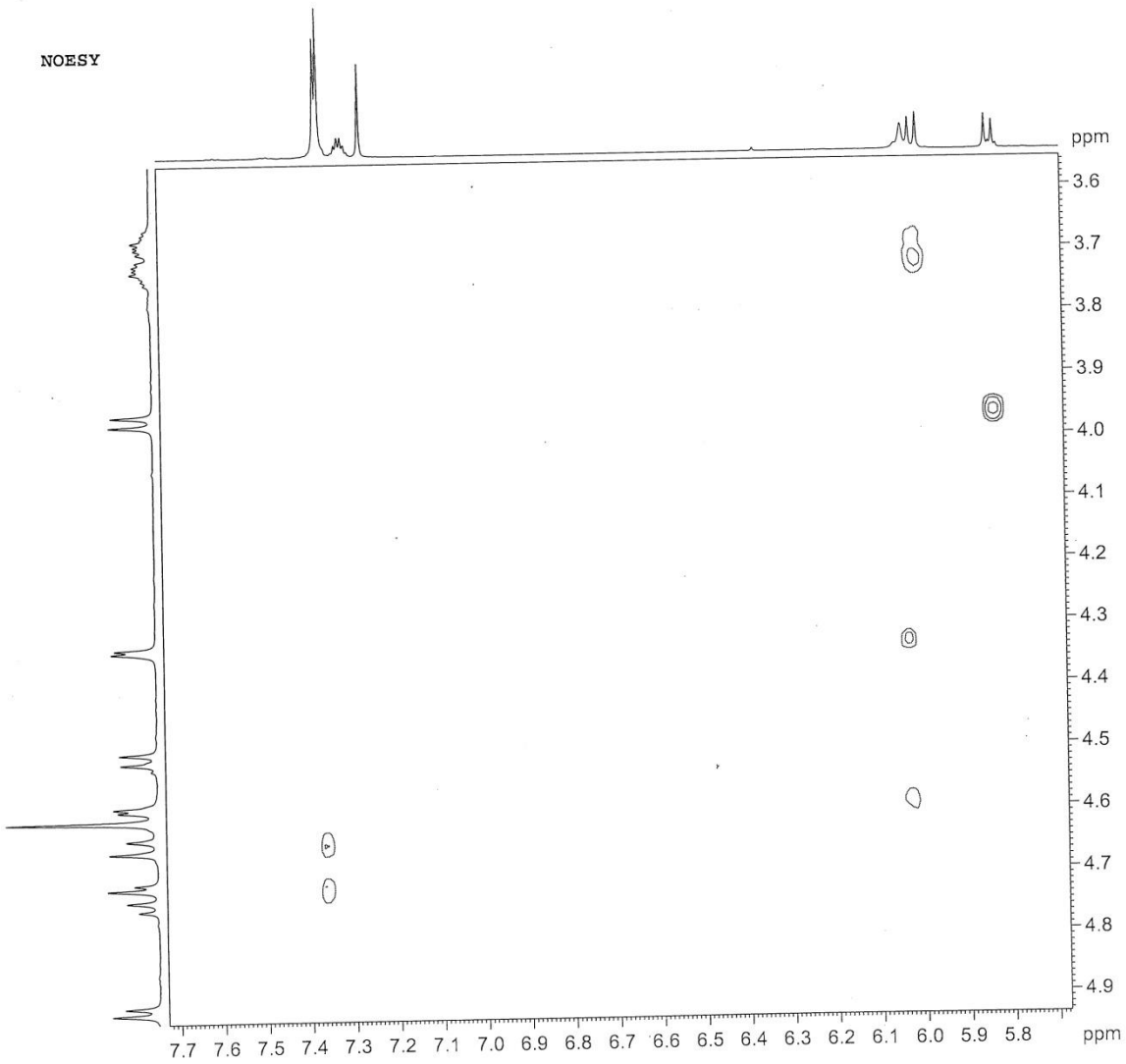
Scheme 80. NOESY spectrum of **169** (expansion)



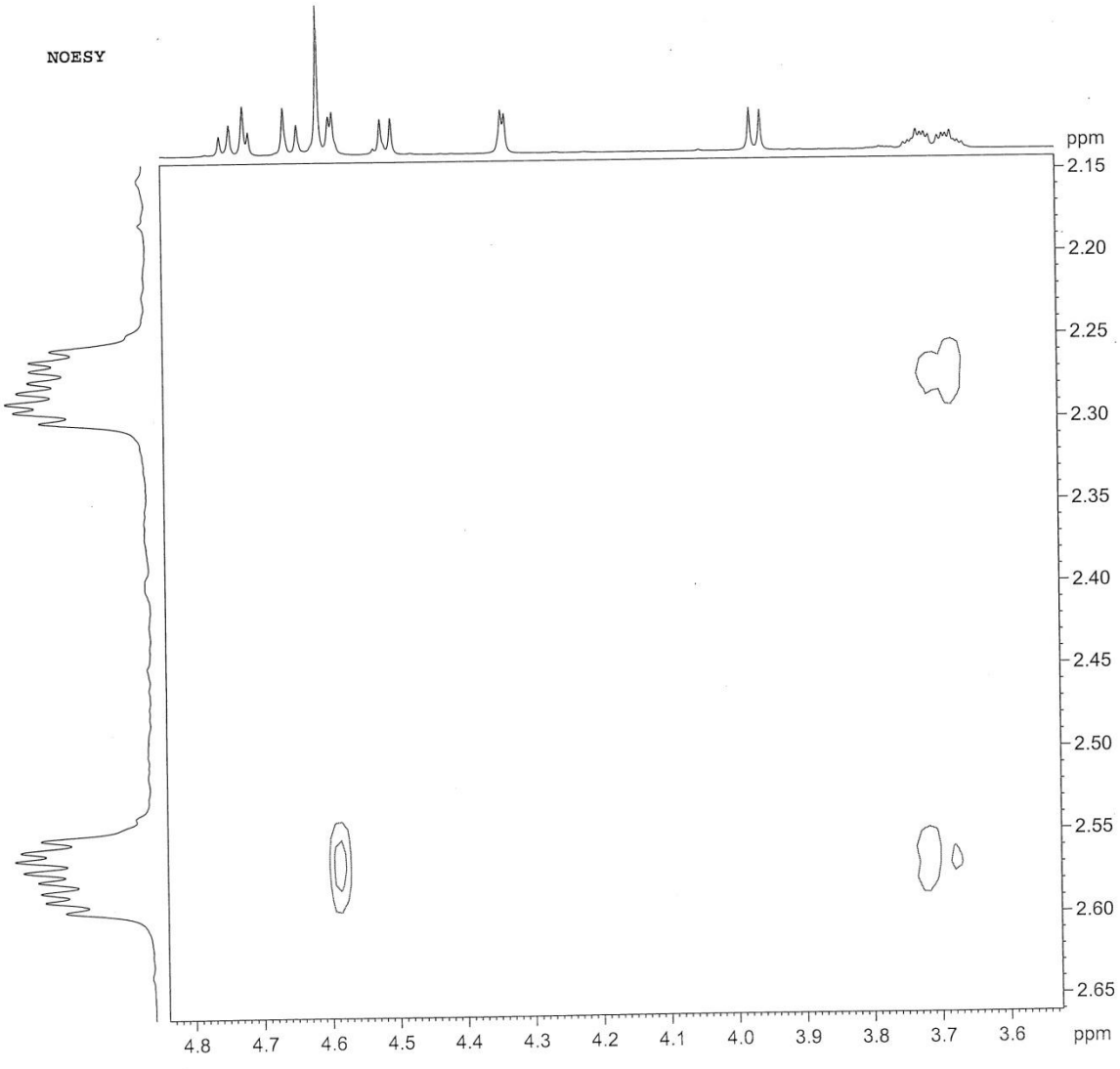
Scheme 81. NOESY spectrum of **169** (expansion)



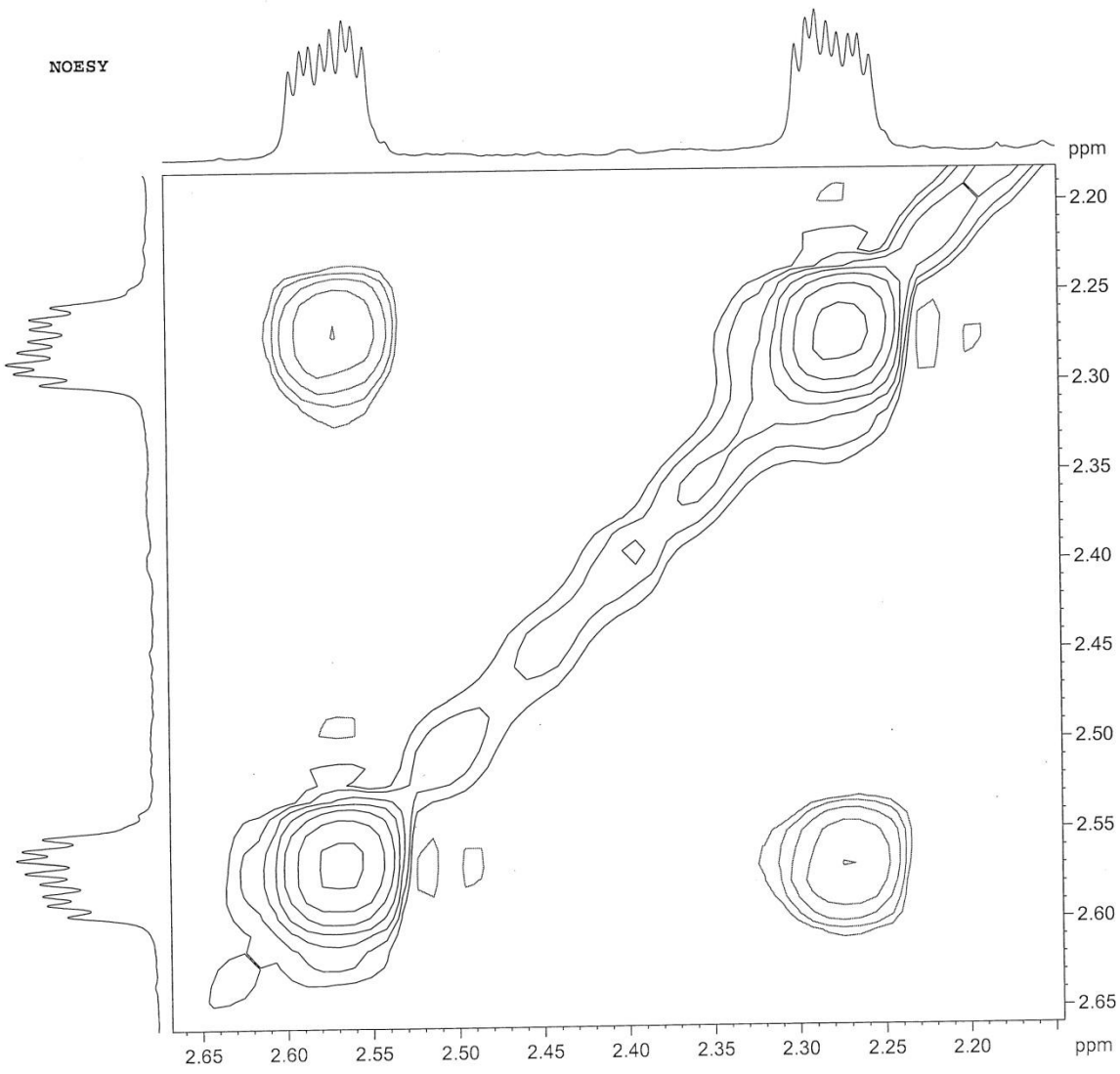
Scheme 82. NOESY spectrum of **169** (expansion)



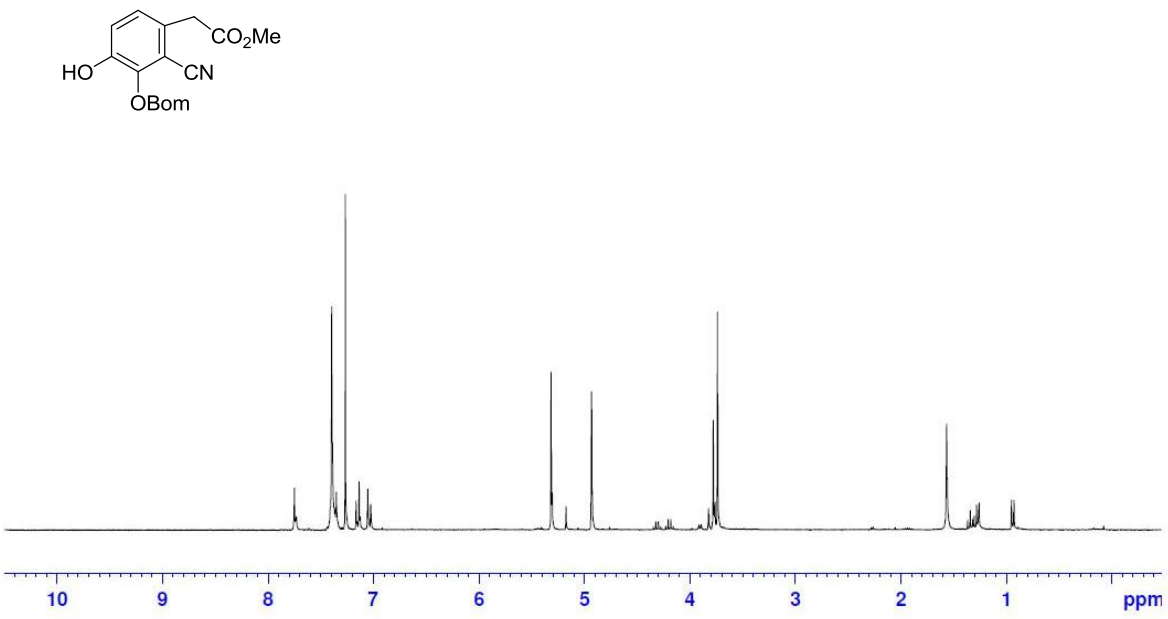
Scheme 83. NOESY spectrum of **169** (expansion)



Scheme 84. NOESY spectrum of **169** (expansion)



Scheme 85. NOESY spectrum of **169** (expansion)



Scheme 86. Crude ^{1}H NMR of **158**

LRMS: found for $C_{18}H_{17}NO_5Na$ $[M + Na]^+$ 350.2.