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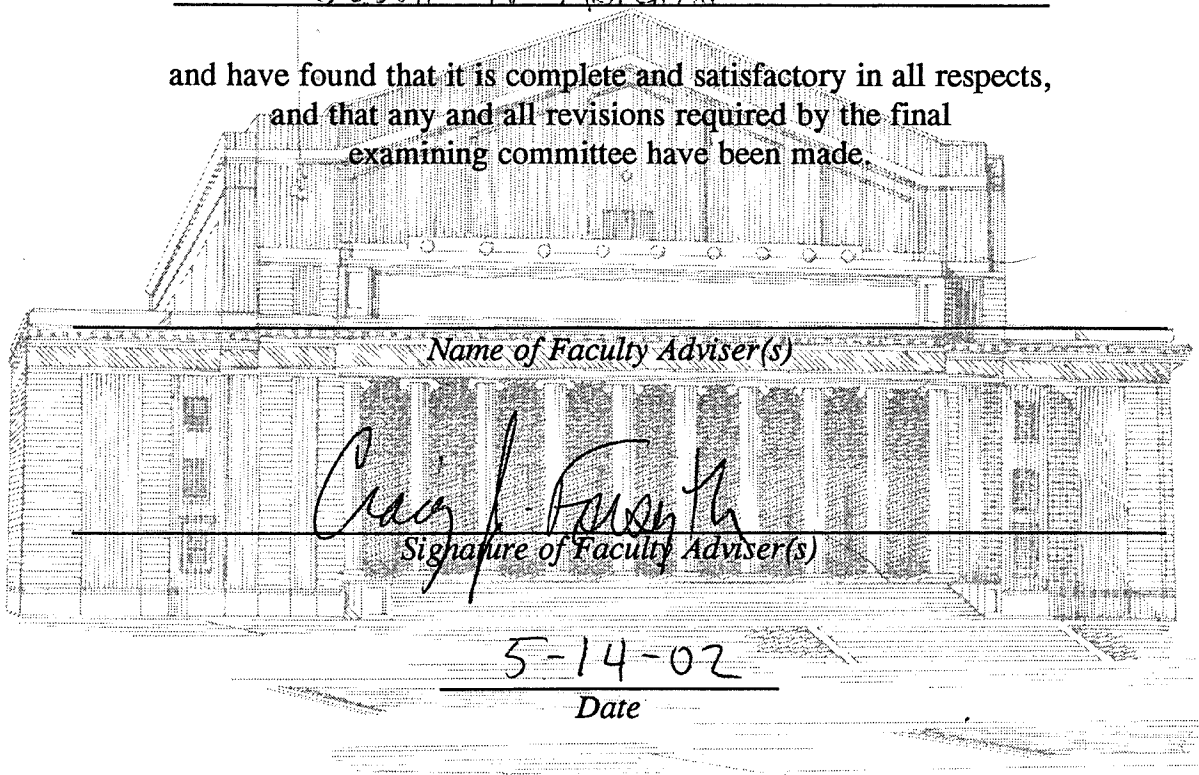

GRADUATE SCHOOL

Large-Scale Syntheses of Two Novel Bicyclic Ring Systems: Applications Toward the Construction of Swainsonine and Okadaic Acid

A Thesis
Submitted to the Faculty of the Graduate School
Of the University of Minnesota
By

Jason N. Abrams

In Partial Fulfillment of the Requirements
For the Degree of
Master of Science

Craig J. Forsyth, Advisor

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Abstract

The thesis is composed of two parts. The first focuses on synthetic efforts toward the total synthesis of swainsonine, an iminosugar, and was performed in the research laboratories of Professor George A. O'Doherty. Novel methodology was applied toward construction of multiple grams of advanced intermediates. The synthetic design also utilized high atom economy. The synthesis starts from furfural and proceeds through a novel palladium-mediated intramolecular cyclization as a key step. Potentially 3 steps remain for completion of the total synthesis. This work will compliment recent non-carbohydrate swainsonine routes.

In the second part of this thesis, the synthesis of the C15-C27 domain of okadaic acid is accomplished. Several key process improvements were realized which made this route scaleable. A large-scale synthesis was therefore undertaken, and multiple grams of advanced intermediates were produced. This work will aid in construction of novel okadaic acid analogs with improved selectivity/ potency for serine-threonine specific protein phosphatases.

Table of Contents

Part 1: Applications Toward the Total Synthesis of Swainsonine

Part 2: Synthesis of a Novel C15-C27 Domain of Okadaic Acid

Table of Contents

Part 2: Synthesis of a Novel C15-C27 Domain of Okadaic Acid

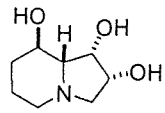
^{1}H NMR Spectra

List of Abbreviations

$[\alpha]$	specific rotation
Bn	benzyl
t-Bu	*tert*-butyl
°C	degrees Celsius
calcd	calculated
δ	chemical shift in ppm downfield from tetramethylsilane
d	doublet
DMAP	4-(dimethylamino)-pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
g	gram(s)
hr	hour(s)
Hz	hertz
IC_{50}	concentration at which 50% of enzyme is inhibited
FTIR	fourier transform infrared
J	coupling constant
m	multiplet
MHz	megahertz
mL	milliliter(s)
mol	mole(s)
mmol	millimole(s)
MS	mass spectrometry
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
Ph	phenyl
PMP	*p*-methoxy phenyl
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
q	quartet
R_f	retention factor
rt	room temperature
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
TBABr	tetrabutylammonium bromide
TBS	*tert*-butyldimethylsilyl
TBSOTf	*tert*-butyldimethylsilyl trifluoromethane sulfonate
TLC	thin layer chromatography
THF	tetrahydrofuran
TPAP	tetrapropylammoniumperruthenate
Ts	*p*-toluenesulfonyl, tosyl
N-TsIm	*N*-toluenesulfonyl imidazole
p-TsOH	*p*-toluenesulfonic acid

Background

Figure 1.

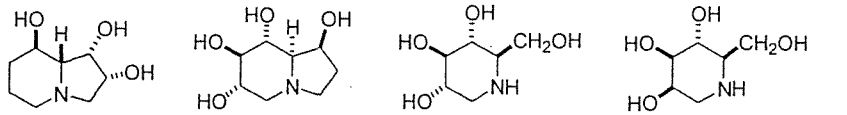


(1)

(-) swainsonine

Swainsonine (**1**) (Figure 1) is a bicyclic polyhydroxylated alkaloid with four asymmetric centers was first reported in a publication in 1973, as an isolated product from the fungal culture *Rhizoctonia leguminicola.*[1] Additionally, swainsonine has been found in other natural sources including the toxic Australian plant *Swainsona canescens,*[2] locoweed (i.e. *Astragalus* species) found in the southeastern US,[3] as well in the fungus *Metarhizium anisopliae.*[4] Swainsonine is further classified as an azasugar or iminosugar. Iminosugars are widespread in plants and microorganisms. These sugar mimics are characterized by replacement of the ring oxygen by nitrogen. Naturally occurring sugar mimics are classified into five structural classes: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines, and nortropanes.[5] Swainsonine is classified as an indolizidine, along with other iminosugars such as castanospermine **2**, while deoxynojirimycin **3** and deoxymannojirimycin **4** are categorized as piperidine type iminosugars (Figure 2). Castanospermine, deoxynojirimcyin, and deoxymannojirimycin are all 2-deoxy iminosugars, while swainsonine is a 2,3-deoxy iminosugar.

Figure 2.



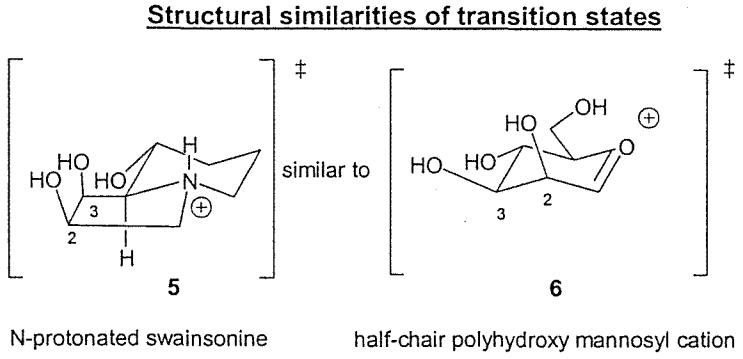
1 Swainsonine | 2 Castanospermine | 3 Deoxynojirimycin | 4 Deoxymannojirimycin

Biology

Swainsonine first gained scientific interest when it was found to be a toxic agent to livestock which ingested *Swainsona canescens* in Australia.[6] This gave rise to a great interest in these and other iminosugar compounds. Iminosugars are biologically active as glycosidase enzyme inhibitors.[7] Glycosidase enzymes cleave specific glycosidic bonds in poly- and oligosaccharides. The inhibition of glycosidase enzymes has potential for use in the regulation of a variety of cellular functions.[5]

At physiological pH, the protonated amino group of iminosugars may mimic the developing charge of the pyranyl or furanyl transition state of glycosidase enzymes.[8, 9] The configuration of hydroxyl substitutents on the ring of swainsonine like other bicyclic iminogsugars have less obvious structural relationships to sugars, but nonetheless can be compared.

Figure 3.



Protonated swainsonine **5** resembles the five-membered ring of a mannosyl cation **6** (Figure 3).[10] This iminosugar therefore acts as a potent inhibitor of α-D-mannosidase.[11, 12] This type of enzyme selectively hydrolyze a variety of α-mannopyranosyl residues. For (-)-swainsonine an IC_{50} range between 1.75 μM-8 μM has been reported for the inhibition of α-D-mannosidase (jack bean).[13] The high activity of swainsonine has been attributed to its bicyclic character, which may impose rigidity on a structure which mimics a transient species in the hydrolytic pathway of glycosidic linkages.[14]

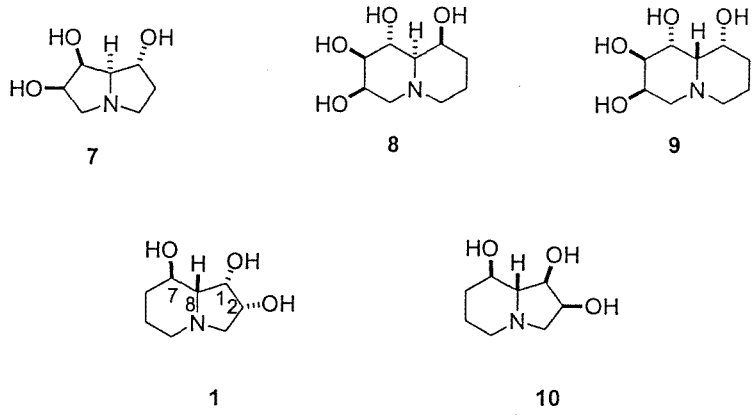
Glycoproteins are hybrid molecules made in the cell which link sugar building blocks to protein backbones. Cancer [15, 16] and HIV[17] infected cells have been shown to exhibit increased levels of glycoprotein processing enzymes. This feature may be taken advantage of by molecules such as swainsonine in cancer cell recognition and the targeting of diseases such as HIV by binding to the active site of specific glycosidases of infected cells.[18] A review outlines the therapeutic potential of swainsonine.[19]

Swainsonine has reached phase I clinical trials as a potent anticancer drug due to its antimetastatic activities.[20] Its activity has been shown to reduce the growth of human melanoma cells (MeWo) by 50 %.[21] It appears that significant quantities of swainsonine are well tolerated and produce minimal side-effects. During investigative research swainsonine was established to have the maximum tolerated oral dose as 300 μg/kg per day and has been shown to have a protective effect against high-dose chemotherapy.[22] The bioavailability data for swainsonine has not been reported, however it is assumed that increased lipid solubility of some "prodrug-like" esters of swainsonine would increase their intestinal and cellular absorption.[23]

As swainsonine is a potent inhibitor of lysosomal α-mannosidase,[24] it may have therapeutic prospects in the study and treatment of lysosomal storage diseases. These are genetic diseases in which there is a deficiency of a certain collection of enzymes that are capable of degrading molecules down to smaller building blocks. [5]

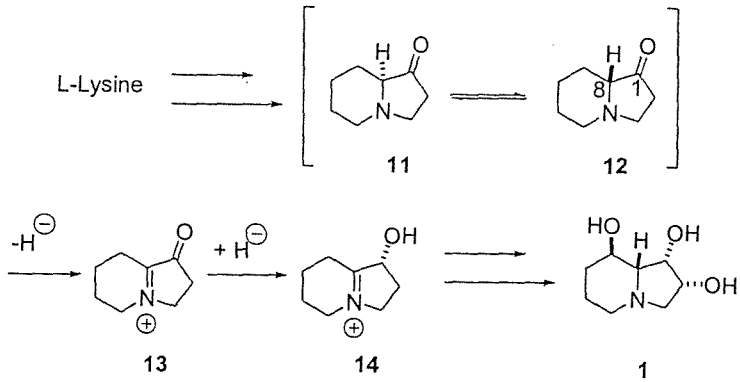
Understandably, there has been increasing demand for imingosugars due to their potential as therapeutic agents. Numerous iminosugars have been tested for activity against glycoprotein processing enzymes. From SAR studies a general understanding of iminosugar activity has been developed. However, there is still a need for greater understanding of their mechanism of action in order to design more specific inhibitors. Synthetic analogs of swainsonine could help in this role.

Figure 4.



In the vein of trying to develop more specific inhibitors, several groups have reported the synthesis of ring-size derivatives of swainsonine and its deoxy analogs. For example, a [5.5] fused *O*-pyrrolizidine analog **7** of swainsonine has been reported by the Fleet group,[25] and [6.6] *O*-quinolizidine analogs **8** and **9** have been reported by the Pearson group (Figure 4).[26] Epimers of swainsonine have been produced with significant biological activities. For instance 1,2-*di-epi*-swainsonine **10** has been shown to have a Ki of 6 μM against α-D-mannosidase.[13] Analog construction can be fostered by a general and flexible route toward iminosugars that would allow access to a variety of stereoisomers and ring-size derivatives.

Scheme 1.



Information on the probable biomimetic pathway of natural products is helpful toward the rational design of improved synthetic routes. The biosynthesis of swainsonine starts with L-lysine, similar to other indolizidine alkaloids (Scheme 1). Here a hydride migration from the 8 to 1 position occurs for reduction of ketone **13**.[27] This is followed by a reductive amination of **14** to install the correct stereochemistry at the[6, 5] ring juncture of **1**.

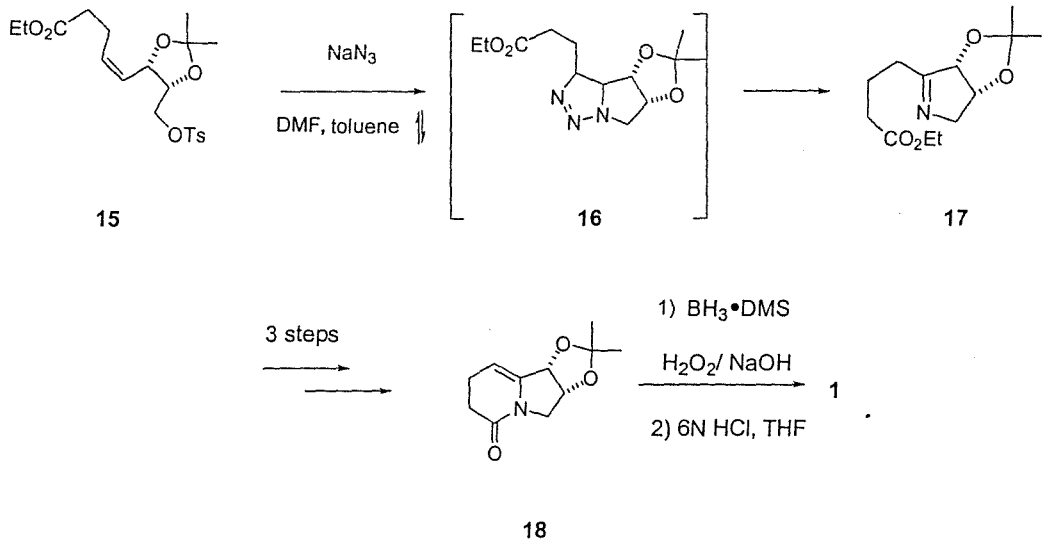
Atom economical syntheses are ideal.[28] However, the interpretation of the most practical synthesis is a subjective one which also necessitates the evaluation of industrial concerns (such as cost assessment, waste stream evolution, throughput capacity, turnover, temperature and pressure limitations, toxicity issues, to name but a few). Since many of the above issues are not addressed readily in academic settings, critical comparison of syntheses becomes a difficult challenge. Keeping subjectivity in mind, our group has endeavored to devise a highly atom economical construction of swainsonine relative to previous work. Highlights from previous syntheses are therefore provided below for background information.

A number of approaches to swainsonine have made use of a carbohydrate source as the chiral precursor. This is understandable as four contiguous chiral centers of swainsonine resemble those of manno- and glucosugars. Making use of these preexisting chiral centers can have limitations

on analog development and the necessity for orthogonal protecting group manipulations. Additionally many of these strategies are not atom economical, as they introduce one or more carbinol centers at a later stage in their syntheses. Such examples of these carbohydrate-based methods are highlighted below through the works of the Cha, Pearson, Fleet, Chamberlin, and Kibiyashi groups.[29, 26, 25, 30, 31]

Previous work

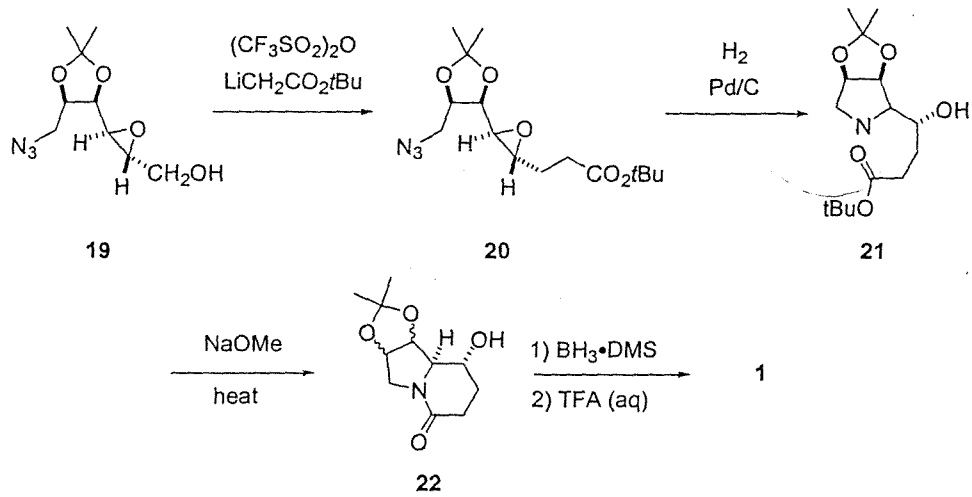
Scheme 2.



One of the most efficient carbohydrate based syntheses of swainsonine is represented by the Cha group and depicted in Scheme 2. [29] The Cha group synthesized swainsonine in 7 steps by utilizing an intramolecular 1,3-dipolar cycloaddition of an olefinic azide generated from tosylate **15** (Scheme 2). Starting from 2,3-*O*-isopropylidene-D-erthrose, the tosylate **15** was prepared in 3

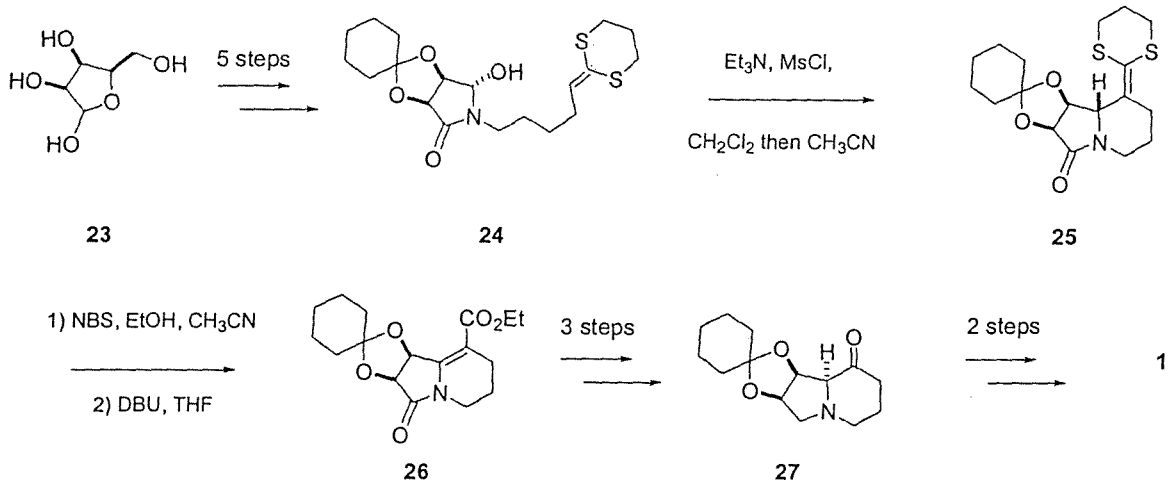
steps, which undergoes a 1,3-dipolar cycloaddition to give intermediate **16** that decomposes to the imine ester **17**. Imine ester **17** is transformed to enamide intermediate **18** within three steps. Treatment of enamide **18** with a hydroboration, oxidation protocol affords the target molecule swainsonine (**1**). A nearly identical approach to swainsonine has been developed by Pearson. [26]

Scheme 3.



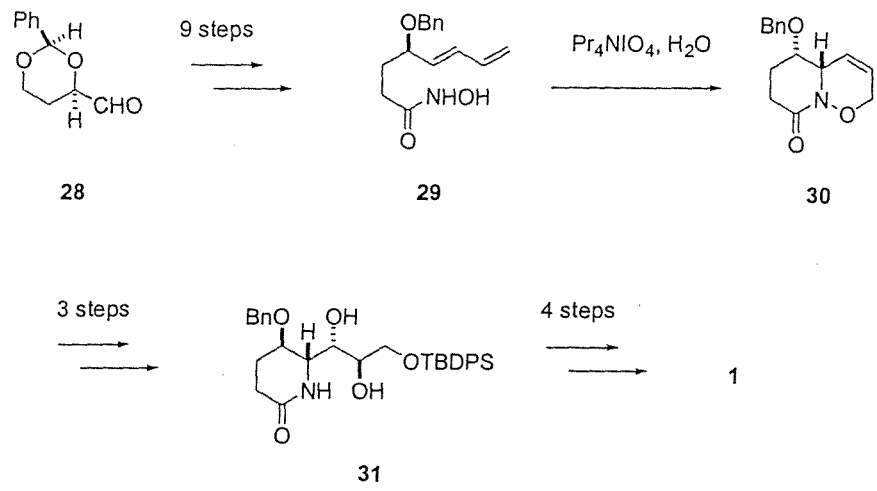
Fleet's synthesis (Scheme 3) starts from protected mannitol and yields the azido epoxide **19** in two steps.[25] The azido epoxide undergoes a two carbon extension by initial esterification of the primary alcohol to produce an intermediate triflate, followed by reaction with the lithium anion of *tert*-butyl acetate to give the chain extended ester **20**. Hydrogenation of the azido ester then affords the pyrrolidine **21**, which under basic conditions cyclizes to the δ-lactam **22**. The δ-lactam **22** is reduced with borane dimethyl sulfide complex. The acetonide group is hydrolyzed under acidic conditions to yield (**1**) in 8 steps from the protected mannitol.

Scheme 4.



Chamberlin's 14 step approach to swainsonine starts from D-(-)-lyxose **23** (Scheme 4). A transient iminium species which is generated from precursor **24**, is attacked by a tethered ketene dithiane to form lactam **25**.[30] The ring fusion of **25** needs to be corrected. This is furnished by formation of enamide intermediate **26** which can then undergo substrate controlled hydride reduction to form **27**. The natural product (**1**) is furnished in an additional 2 steps.

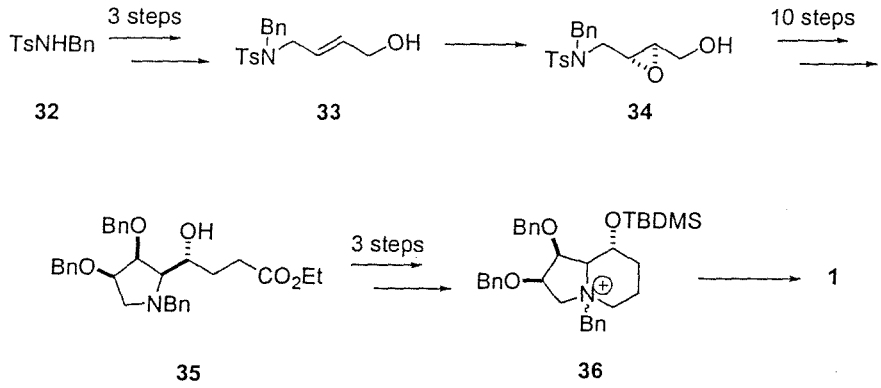
Scheme 5.



Kibayashi's 19 step approach to (-) swainsonine starts from D-malic acid and utilizes an acylnitroso cycloaddition approach (scheme 5).[31] After formation of aldehyde **28** in 3 steps from D-malic acid, hydroxamic acid **29** is produced in an additional 9 steps. Hydroxamic acid **29** is then oxidized to a transient acyl nitroso diene, which undergoes a [4 + 2] cycloaddition to give a 1:4 mixture of the trans-: cis-1,2-oxazinolactams (cis-1,2-oxazinolactam **30**, Scheme 5). The pivotal intramolecular hetero-Diels-Alder reaction occurs to yield the desired trans 1,2 oxazinolactam **30** by employing aqueous conditions. The oxazinolactam **30** is transformed into the diol **31** in 3 steps, which then yields the natural product (**1**) after an additional 4 steps. The Keck group employs a very similar hetero Diels-Alder reaction strategy for their formation of swainsonine.[32]

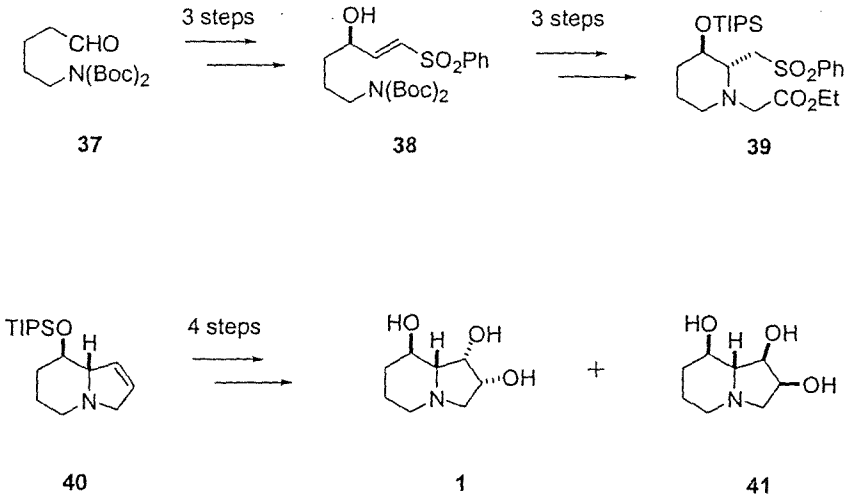
The above syntheses are representative examples which utilize chiral pool starting materials. The following examples use non-carbohydrate sources for syntheses of swainsonine. Major drawbacks to many of the following routes are their poor stereoselectivity.

Scheme 6.



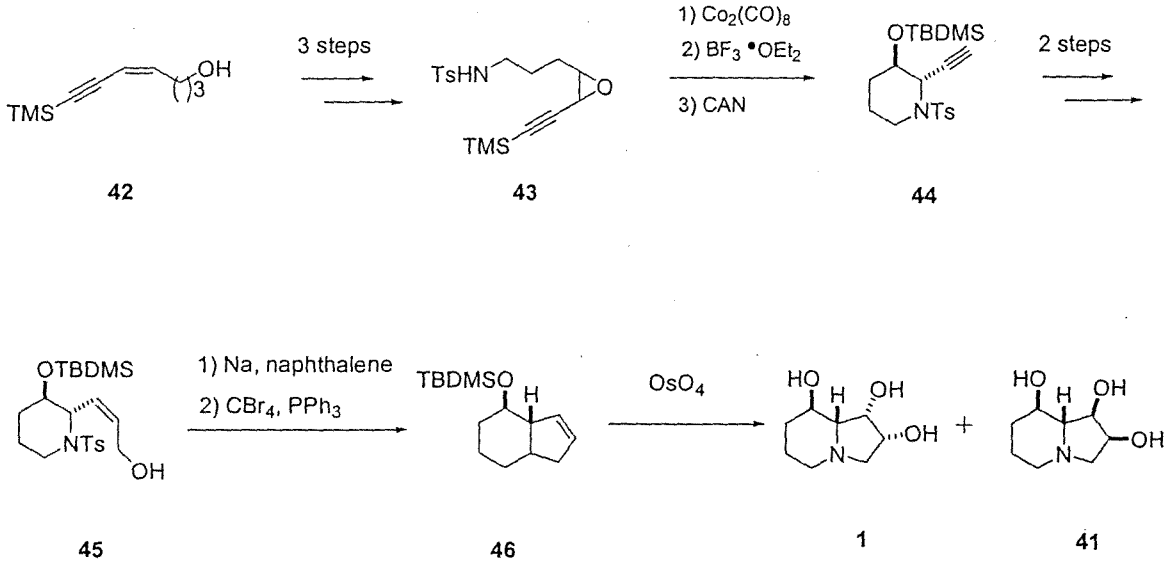
Sharpless's route (Scheme 6) [33] utilizes asymmetric catalysis to install the stereochemistry of swainsonine. The route begins from *N*-benzyl-*p*-toluenesulfonamide **32** and is converted to allylic alcohol **33** in 3 steps. Intermediate **33** with the suitably protected nitrogen is subjected to asymmetric Masamune/ Sharpless epoxidation conditions [25] to give **34**. Epoxy alcohol **34** is transformed into epoxy ester **35** in 10 steps. Epoxy ester **35** is converted to pyrrolidine hydroxy ester **36** after tosyl deprotection with sodium naphthalide. This labile intermediate is subsequently converted to a mixture of cis and trans fused bicyclic quaternary ammonium salts **36** in 3 steps, which after deprotection yields (**1**) in 19 steps. While this route allows for stereodivergent synthesis and therefore access to all 16 epimers and/or enantiomers of swainsonine, it suffers in its inefficiency.

Scheme 7.



An approach by the Carretero group (Scheme 7) utilizes an α-hydroxy α, β-unsaturated sulfone and proceeds in 10 steps from **37**.[13] The sulfone **38** is formed from the di-Boc protected derivative of 5-aminopentanal **37** in 1 step followed by a lipase mediated resolution. Ester **39** is formed from sulfone **38** in three steps which then forms the unsaturated indolizidine **40** in an additional three step sequence. The natural product is finally furnished in another three steps. Drawbacks toward this route include a resolution of the intermediate racemic sulfone. Additionally formation of a 40:60 ratio of 1,2 di-epi-swainsonine: (-) swainsonine necessitates a peracetylation/ chromatographic separation/ deacetylation sequence for their final steps.

Scheme 8.



Recently, the Hanaoka group attained (+/-) swainsonine via a key cobalt mediated 6-endo-tet mode cyclization of (cis)-epoxy alkyne **43** to afford a stereoselective constructed (trans)-2-ethynyl-3-hydroxypiperidine skeleton **44** (Scheme 8).[34] The (cis)-epoxy alkyne **43** is obtained in 5 steps from enyne **42**, which is obtained from 4-hydroxy-butyraldehyde in 4 steps. Following formation of cis alkene **45**, closure to the indolazidine core **46** is accomplished in a similar manner to Kibiyashi's route, by going through an intramolecular cyclodehydration. This synthesis is novel, but suffers in a similar fashion to Carretero's route which also forms both swainsonine and 1,2 bis-*epi*-swainsonine with the dihydroxylation step. Similarly to Carretero's work, this approach produces a racemic mixture of 1,2 di-epi-swainsonine: (-) swainsonine that necessitates peracetylation of the mixture followed by chromatographic separation and subsequent removal of acetyl groups.

O'Doherty-Abrams Synthetic Strategy to Swainsonine: Retrosynthetic Analysis

Many of the groups' syntheses suffer from poor stereoselectivity and inefficient protecting group strategies. Keeping the ultimate goal of large-scale synthesis in mind, we started from a commodity chemical, furfural. Starting from this inexpensive source could allow for a stereodivergent synthesis of swainsonine and analogs.

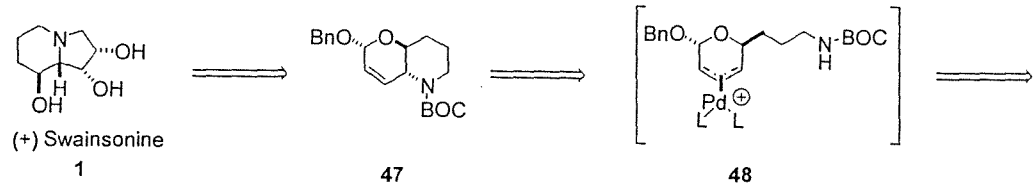
In general our group has been interested in developing practical approaches to stereochemically enriched structures with asymmetry that has been derived from asymmetric catalysis. We believe that the development of these routes will not only provide either enantiomer of the target molecule but ultimately result in synthetic approaches that are highly atom economical.

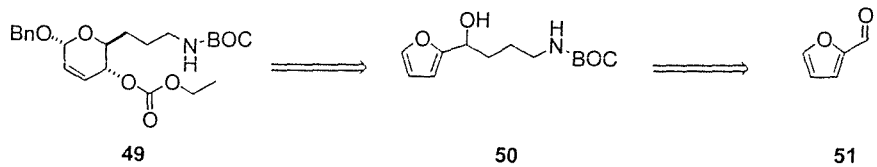
We believe our approach toward swainsonine has novelty, such as an unprecedented palladium catalyzed ring cyclization. The relative stereochemistry for our synthesis of swainsonine will be completely substrate-controlled. Our strategy is highly atom economical, as all of the carbons of swainsonine are introduced after the first reaction step. Our designed 14 step synthetic sequence, may be reduced to a highly efficient 9 steps. Importantly our route will allow for the synthesis of analogs because the hydroxyl groups can be installed stereoselectively in a step-wise fashion. Our synthesis also addresses the possibility of creating novel unnatural iminosugar terminated oligosaccharides which may bind to glycoprocessing enzymes with improved selectivity for tumor cells. The synthesis of analogs should enhance the general understanding of iminosugar glycosidase inhibition through SAR studies and discovery of important binding interactions.

Outlined in Scheme 9 is our retrosynthetic plan for the construction of swainsonine (shown as the (+) enantiomer). Proposed herein is a retrosynthetic strategy, utilizing an atom-economical route

that would yield swainsonine (**1**) exclusively. It is envisioned that both enantiomers of swainsonine can be accomplished by this route.

Scheme 9:

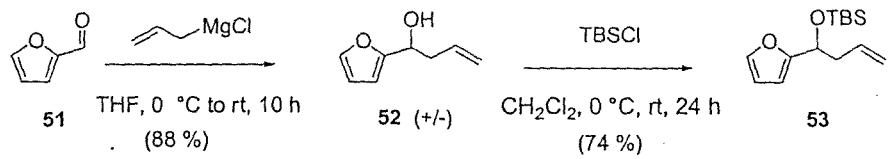




We envision (**1**) coming from perhydro-pyrano-piperidine **47** via a Sharpless asymmetric dihydroxylation followed by a reductive amination.[35] A novel catalytic palladium mediated intramolecular coupling between a tethered nitrogen nucleophile and a $\dot{\pi}$-allyl palladium (II) species on substrate **48** occurs to yield the perhydro-pyrano-piperidine. The π-allyl palladium species originates from an induced ionization of carbonate **49**. The palladium (0) source generated from $Pd_2(dba)_3 \cdot CHCl_3$ adds to the opposite face of the carbonate of **49**. Therefore, proceeding from **49** to **47** formally represents a double inversion event, providing net retention of configuration at the stereocenter of ring closure. The carbonate **49** originates from an Achmatowicz ring expansion of a 2-substituted furan **50**.[36] In turn, **50** comes from furfural **51**.

Synthesis

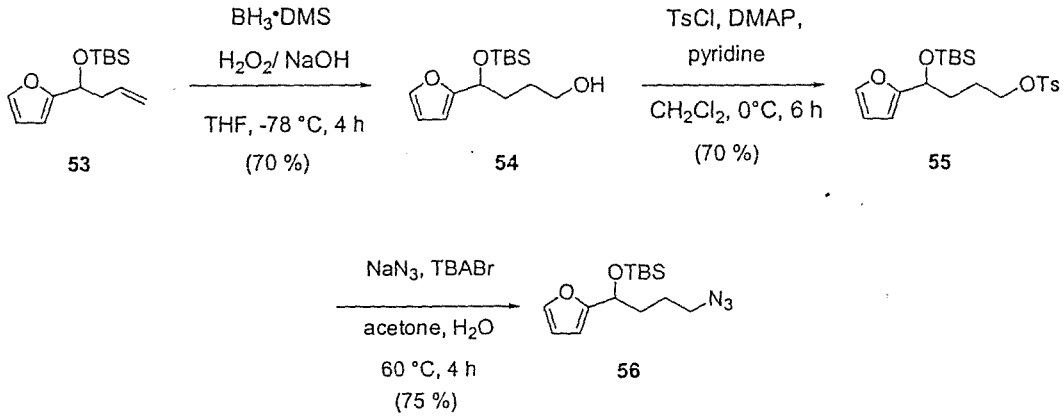
Scheme 10.



51 52 (+/-) 53

Our synthesis begins with addition of allyl magnesium chloride to furfural (**51**) producing racemic homoallylic alcohol **52** (Scheme 10). This intermediate was subsequently protected as a silyl ether with TBSCl to afford **53** (88 % yield).

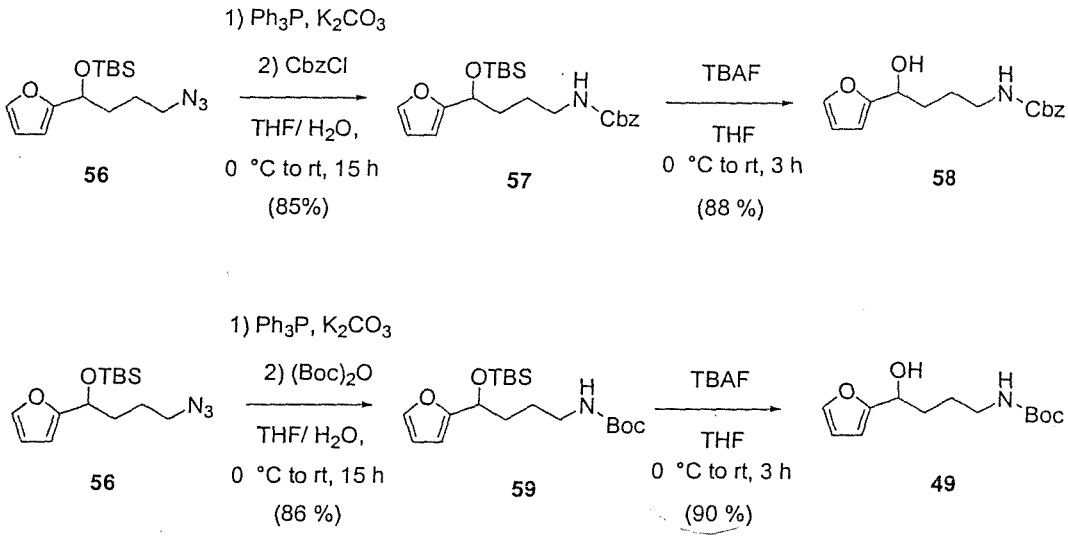
Scheme 11.



53 54 55 56

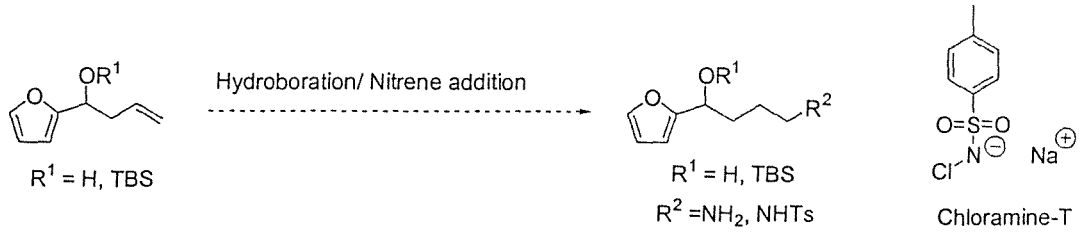
The silyl ether **53** is converted into alcohol **54** via a hydroboration/oxidation sequence (Scheme 11). Over 25 grams of alcohol 37 has been produced for future work. Alcohol **54** was then tosylated to provide tolsylate **55**. Tosylate 55 was then displaced by sodium azide to afford the azide species **56**.

Scheme 12.



Azide **56** is converted to differentially N-protected amino alcohols furans **58** and **48** respectively (Scheme 12). Azide **56** is reduced with triphenylphospine, and protected in-situ to yield Cbz-protected amine **57** or Boc-protected amine **59**. The silyl groups of amines **57** and **59** are then removed using TBAF to give alcohols **58** and **49**, both of which have been synthesized in over 30 gram quantities each in preparation for future work.

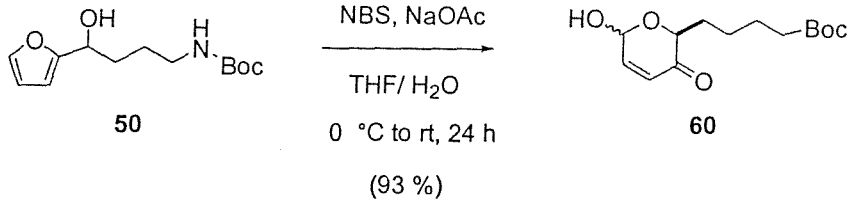
Table 1.



Entry	Substrate	Boration Source	Nitrene Source	Results
1	R^1= H (**52**)	9-BBN	Chloramine-T	Starting material
2	R^1= H (**52**)	BH_3•DMS	Chloramine-T	Starting material
3	R^1= H (**52**)	BH_3•DMS	hydroxylamine-*O*-sulfonic acid	Multiple products
4	R^1= H (**52**)	BH_3•DMS	hydroxylamine-*O*-sulfonic acid	Multiple products
5	R^1= TBS (**53**)	BH_3•DMS	hydroxylamine-*O*-sulfonic acid	Multiple products

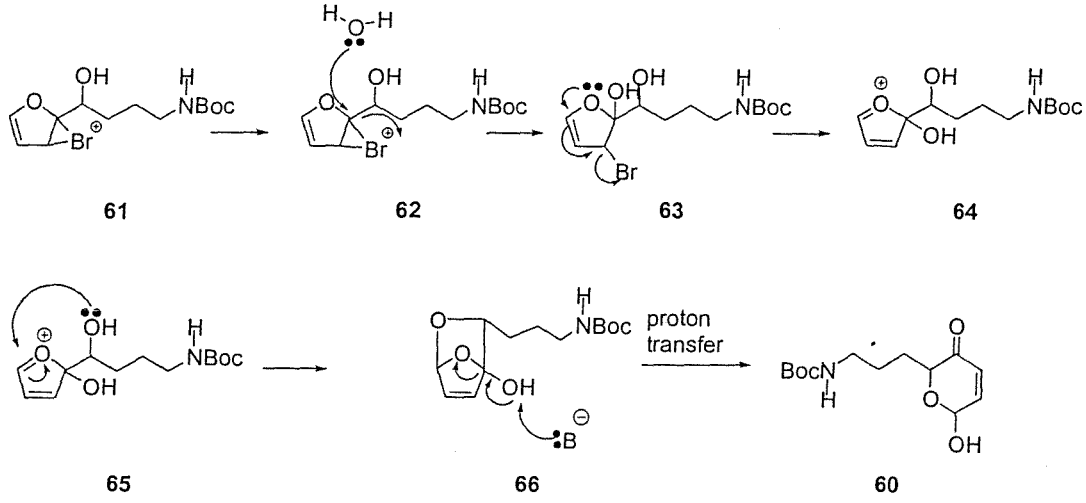
It was envisioned that amines could be synthesized by employing a hydroboration/ nitrene addition protocol. This would reduce the five step sequence for making these amines to two steps. Attempts to efficiently produce the amine products are outlined in Table 1. A hydroboration of the alkene utilized a borane source of either 9-BBN (entry 1) or BH_3•DMS (entries 2-5). This was followed by the use of Chloramine-T (entries 1-2) or hydroxylamine-*O*-sulfonic acid (entries 3-5) as the nitrene source. All entries were unsuccessful however.

Scheme 13.



Amino alcohol **50** next underwent oxidative Achmatowicz ring expansion to provide a racemic mixture of alcohols **60** (Scheme 13). The mechanism for this ring expansion using NBS/H_2O conditions is shown in Scheme 14.

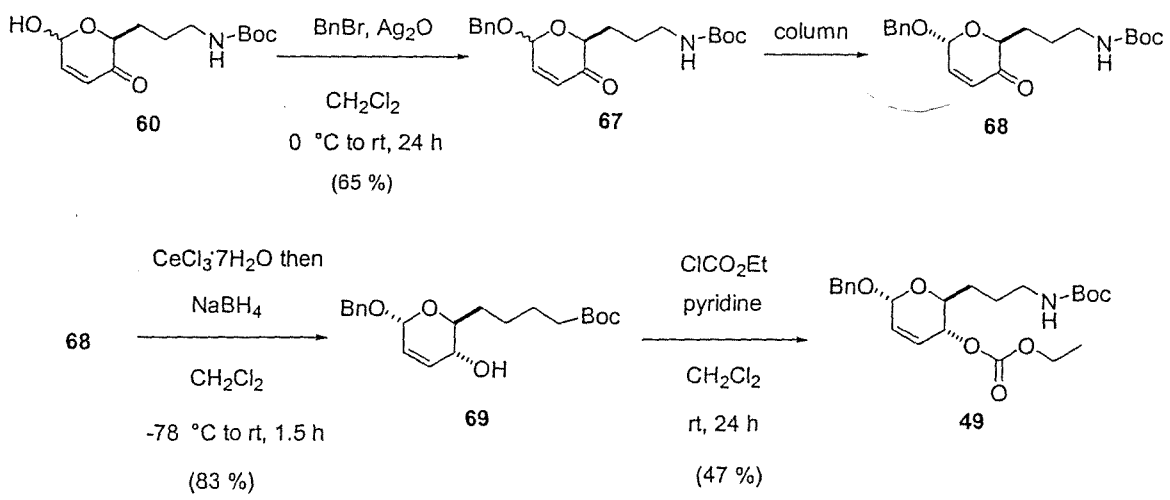
Scheme 14.



In the Achmatowicz ring expansion (Scheme 14), the bromine source *N*-bromo succinimide (NBS), first adds across the double bond of a furan derivative giving bromonium ion **61**. Water then adds to the bromonium ion as shown in **62**, providing hemiacetal **63**. Bromine is eliminated after formation of the oxonium species **64**. A nucleophilic attack by an oxygen lone pair onto the

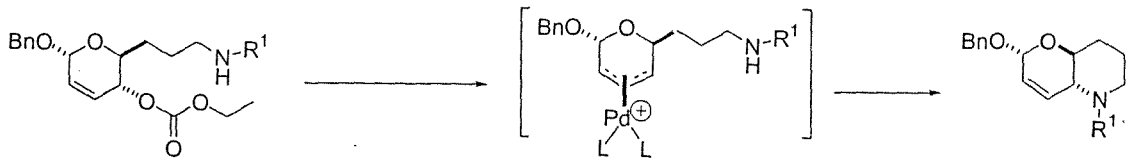
oxonium carbon as drawn in **65** produces species **66**. After proton transfer and ring fragmentation, the ring expanded product **66** is obtained. The Achmatowicz ring expanded product consists of a mixture of α and β anomers. Other conditions for this ring expansion include *m*-CPBA/ H_2O.[36]

Scheme 15.



After formation of the Achmatowicz cyclized product **60**, a protection of the racemic mixture of anomeric alcohols is accomplished with benzyl bromide in the presence of silver oxide (Scheme 15). The racemic mixture of axial and equatorial anomers **67** are separated efficiently via column chromatography. The axial anomer **68** is diastereoselectively reduced by employing Luche conditions to yield **69**. The equatorial anomer was reduced under Luche conditions with no selectivity (not shown). Presumably the axial benzyl protected anomer is more conformationally rigid than the equatorial counterpart. This allows for better selectivity in the reduction step. The α, β unsaturated alcohol **69** was then protected with ethyl chloroformate to yield carbonate **48**.

Scheme 16.

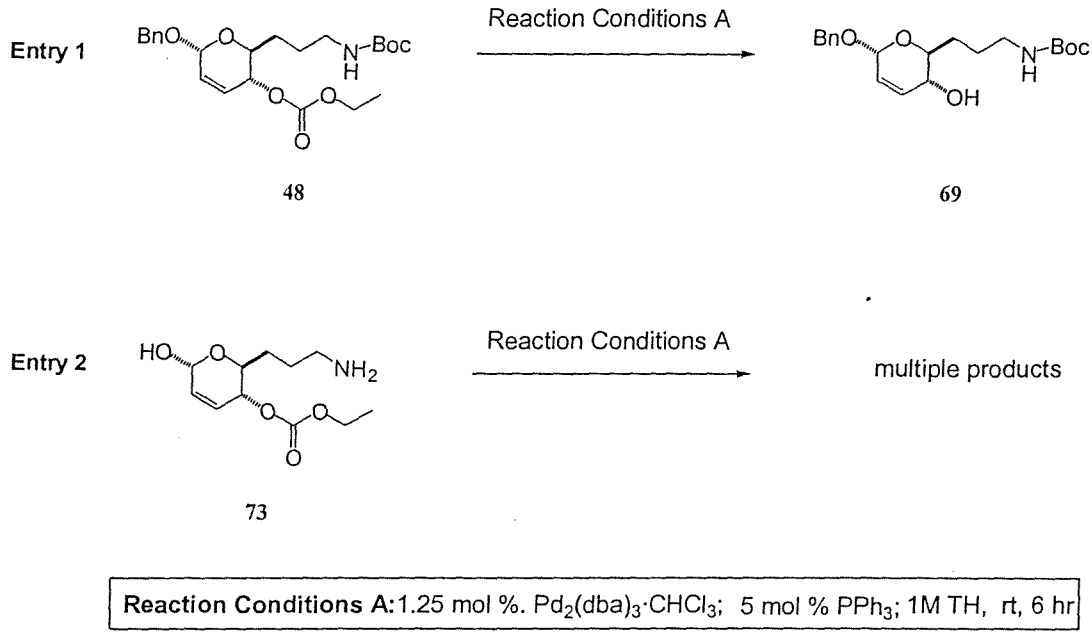


48 R^1=(a) Boc **47** R^1=(a) Boc **46** R^1=(a) Boc

70 R^1=(a) Cbz **71** R^1=(a) Cbz **72** R^1=(a) Cbz

With carbonate **48** in hand, conditions to obtain cyclized adducts **46** and **72** are realized using novel methodology (Scheme 16). The initial reaction conditions employed $Pd_2(dba)_3 \cdot CHCl_3$ with PPh_3 to generate a palladium (0) source *in situ*.

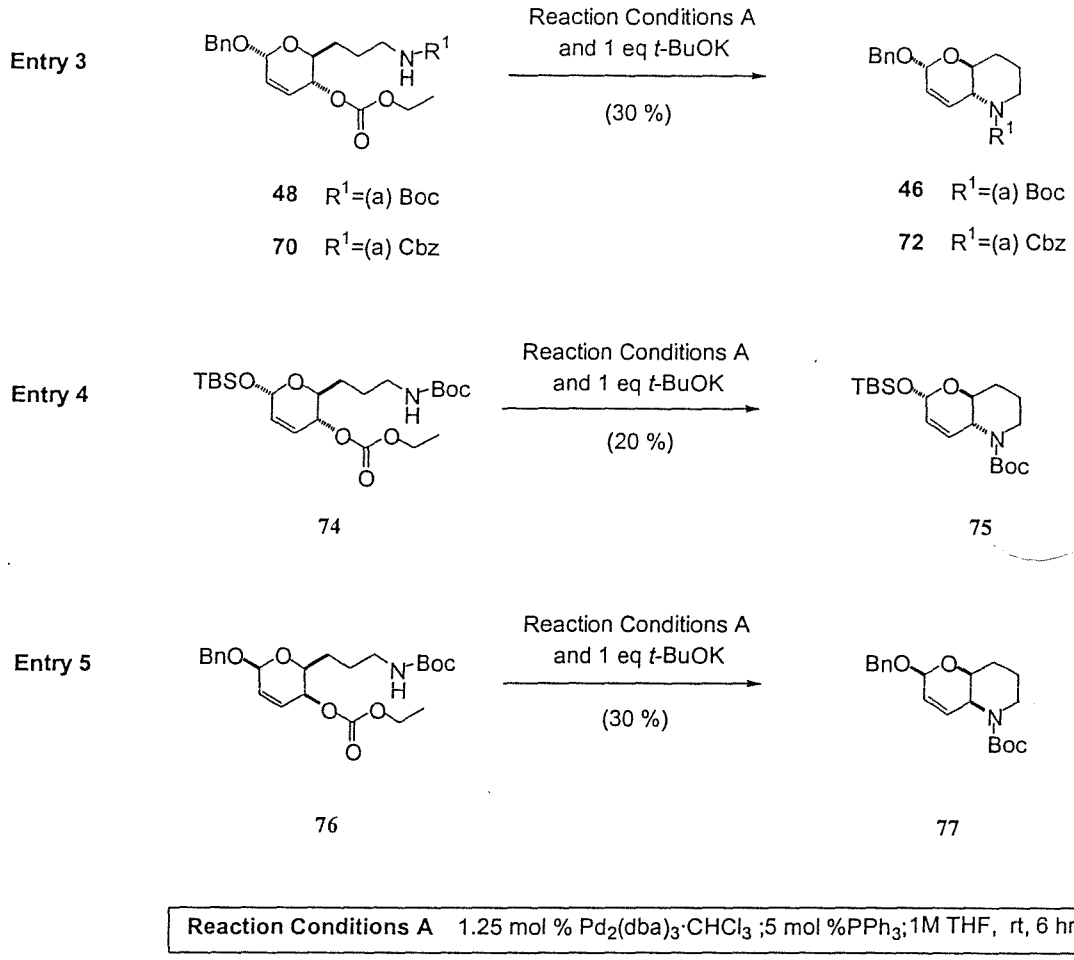
Scheme 17.



The reaction in Entry 1 was run in THF at room temperature for 6 hours, but attempts with the nitrogen protected with a Boc or Cbz group did not afford desired products **46** or **72** respectively

(Scheme 17). Instead alcohol **69** was obtained. This was encouraging news, as it appeared that ionization of the ethyl formate had occurred by the palladium (0) source. However, it was believed that the protected nitrogen of **48** was a poor nucleophile. In this vein a Boc-deprotection of amine **48** was undertaken. This gave compound **73**, in which the benzyl group was hydrolyzed (Entry 2, Scheme 17). Attempts to cyclize this adduct were unsuccessful.

Scheme 18.



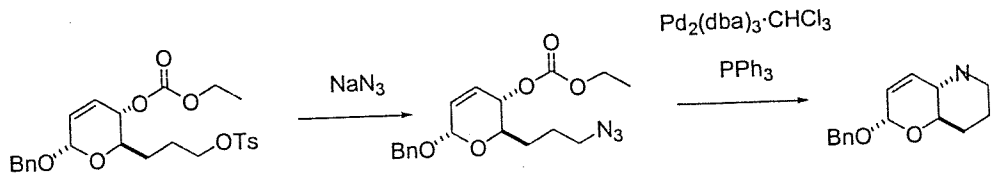
Thus we turned to base treatment. The use of the base potassium *t*-butoxide in conjunction with palladium (0) conditions and employing THF at room temperature on the *N*-Boc and *N*-Cbz substrates of cyclization precursors **48** and **70** afforded an apparent trans-fused ring closed products **46** and **72** (Entry 3, Scheme 18). Continued spectral analysis is imperitive for conclusive confirmation of these structures. Furthermore, these reaction conditions still necessitate optimization as reflected in modest yields of 30 % and 20 % respectively for **46** and **72**. A similar trans-fused bicyclic compound **75** was apparently obtained using the same reaction

conditions on carbonate **74** (Entry 4, Scheme 18). While not useful toward completion of theswainsonine synthesis it is still noteworthy that it appears a cis-fused ring closed product **77** (Entry 5, Scheme 18) was obtained from **76**.

Future Work

It is critical to improve the yield for these palladium catalyzed cyclizations. Should process optimizations fail to garner these results, other more conservative alternatives could be employed.

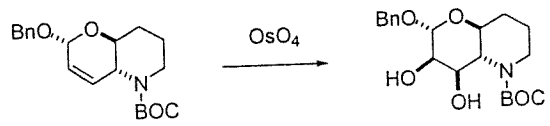
Scheme 19.



78 **79** **80**

The sequence in Scheme 19 has similar precedents and should provide the desired trans-fused perhydro-pyrano piperidine.[37] Tosylate **78** should be transformed into azide **79** with sodium azide, which can be reduced and cyclized in one-pot to **80**.

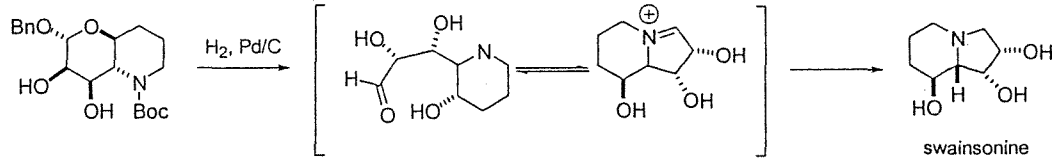
Scheme 20.



46 **81**

With encouraging results in hand all that remains to synthesize swainsonine is to find conditions for the stereoselective dihydroxylation and deprotective reductive amination (Scheme 20). We believe the dihydroxylation step with osmium tetroxide (OsO_4) to install two of the four stereocenters in swainsonine will occur from the more open β face of **46** to yield **81**.

Scheme 21.

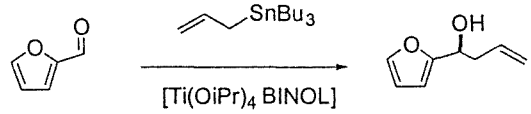


81 **82** **83** **1**

The completion of the synthesis should be accomplished via a one-pot deprotective reductive amination of **81** (Scheme 21). The benzyl group will first be cleaved leading to a hemiacetal. This can then be ring opened, as seen in the left-hand transition state drawing **82**. The amine can then attack the aldehyde leading to a transient iminium species **83**. After subsequent reduction of imminium substrate **83**, the natural product swainsonine (**1**) should be obtained.

An asymmetric variant of the route described could be employed toward the enantioselective synthesis of swainsonine. It is noteworthy that only a few other approaches to (-) swainsonine have used an achiral , racemic compound as starting material.

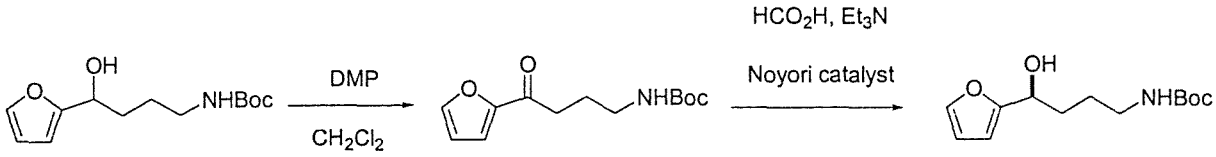
Scheme 22.



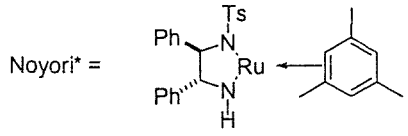
51 **85**

The asymmetry of a stereodivergent swainsonine synthesis could be derived from an enantioselective allylation of furfural **51** by employing Keck's procedure (Scheme 22).[38] This procedure uses the addition of an allyl tin species transmetallated with catalytic amounts of a chiral Lewis acid generated from a Ti (IV) species such as titanium tetraisopropoxide and *R* or *S* Binol to generate enantioselectively a homoallylic alcohol such as **85**.

Scheme 23.



50 **86** **87**



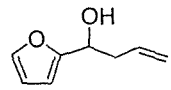
88

Another asymmetric approach may be realized by an oxidation of alcohol **50** followed by a selective reduction of furan ketone **86**, by employing the Noyori catalyst (Scheme 23).[39] As there was literature precedent by the O'Doherty group for a similar amino alcohol, [40] this oxidation/

selective Noyori reduction sequence was recently employed to the substrate below with good success to provide **87** with a 98 % enantioselectivity.[41]

Experimentals

Unless otherwise noted, all experiments were carried out under an argon atmosphere in oven-dried glassware using standard chemical reagent addition techniques including syringe, cannula, and addition funnels. The THF, diethyl ether, and benzene were distilled from Na/benzophenone ketyl under N_2 prior to use. The CH_2Cl_2, CH_3CN, triethylamine, diisopropyl amine, and toluene were distilled from CaH_2 under N_2. Purifications for intermediates **18-21** utilized recrystalization techniques with the solvent systems indicated. All other intermediates were purified using flash chromatography on ICN SiliTech 32-63D 60 Å silica gel or Baker Flash silica gel 60 (40 μm) with the indicated mobile phase systems. Analytical TLC was performed on 0.25 mm EM silica gel 60 F_{254} plates and visualized with UV (254 nm) and anisaldehyde stain (450 mL 95 % EtOH, 25 mL concentrated H_2SO_4, 15 mL of acetic acid, and 25 mL anisaldehyde). All NMR spectra were obtained using 200, 300 and 500 MHz Varian Inova instruments. All NMR spectra were obtained in $CDCl_3$ and referenced to residual $CHCl_3$ at 7.24 (^1H) and 77.0 (^{13}C) ppm. IR spectra were obtained using a Perkin-Elmer 1600 series FTIR. High resolution mass spectrometric (HRMS) data were obtained by the University of Minnesota Mass Spectrometry Laboratory using CI and FAB techniques, which utilized both Finnigan MAT 95 spectrometer and NH_3 and VG 7070E-HF spectrometer respectively.

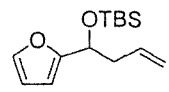


52

Homoallyl alcohol (52)

Under a nitrogen atmosphere, a 2.0 M solution of allyl magnesium chloride (78 mL, 156 mmol) was added to a 1 M THF solution of furfural (15 g, 156 mmol) at 0 °C and allowed to slowly

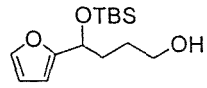
warm to room temperature. After 10 hrs the reaction was complete and diluted with diethyl ether (200 mL) followed by a sodium bisulfate quench (100 mL) and then a sodium bicarbonate quench (300 mL) The reaction mixture was extracted with diethyl ether (2 x 400 mL). The combined ether layers were washed with sodium bicarbonate (200 mL), followed by brine (200 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography afforded homoallyl alcohol **52** as a clear oil (18.93 g, 137 mmol, 88% yield). R_f 0.30 (30 % ether in hexanes). FTIR (thin film, cm-^{1}) 3366, 1638. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35 (d, J = 0.8 Hz, 1H), 6.32 (dd, J = 3.2, 1.6 Hz, 1H), 6.18 (d, J = 2.8 Hz, 1H), 5.85 (m, 1H), 5.00-5.17 (m, 2H), 3.74 (t, J = 6.6 Hz, 1H), 2.48 (bs, 1H). ^{13}C (75 MHz, $CDCl_3$) 156.0, 141.9, 133.6, 118.3, 110, 106, 66.8, 40. HRCI *m/z* calcd for $[(C_8H_{10}O_2)\text{-}OH]^+$: 121.0653; Found 121.0652.



53

TBS ether (53)

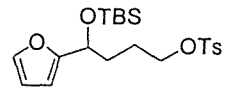
Homoallyl alcohol **52** (19.48 g, 141 mmol, 1 equiv), TBSCl (25.53 g, 169 mmol) in methylene chloride (141 mL,1 M) along with Et_3N (34.22 g, 338 mmol) and DMAP (861 mg, 7.05 mmol) were charged to a single neck 500 mL flask under a nitrogen atmosphere at ambient temperature. Reaction completion occurred within 24 hours. The reaction mixture was diluted with diethyl ether (100 mL) and quenched with 1 N HCl (100 mL), and the resulting mixture was extracted with diethyl ether (2 x 300 mL). The combined ether layers were washed with sodium bicarbonate (200 mL), followed by brine (200 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (50 g of SiO_2; eluent, 1% EtOAc: hexanes then 2 % EtOAc: hexanes then 3 % EtOAc: hexanes) afforded TBS ether **53** (26.34 g, 104 mmol, 74 % yield). R_f 0.75 (30 % ether in hexanes). FTIR (thin film, cm-^{1}) 3080 2911, 2826, 2874, 2710, 2350, 1670, 1567, 1454, 1347, 1245, 1089, 925, 832, 792. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (d, J = 0.8 Hz, 1H), 6.30 (dd, J = 3.2, 1.6 Hz, 1H), 6.18 (d, J = 2.8 Hz, 1H), 5.85 (m, 1H), 5.05 (m, 2 H), 4.71 (t, J = 6.6 Hz, 1H), 2.55 (t, 6 Hz, 2 H), 0.89 (s, 9H), 0.05 (s, 3H), -0.06 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) 156.8, 141.3, 134.6, 117.2, 109.9, 105.8, 68.4, 41.5, 25.8, 18.2, -5.0. FAB HRMS *m/z* calcd for $[(C_{14}H_{24}O_2Si)+NH_4]^+$: 270.1889; Found, 270.1900.



54

Primary alcohol (54)

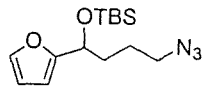
A 5M solution of BH_3•DMS (27.62 mL, 166 mmol) was added dropwise to alkene **53** (104.5 g, 414 mmol) in a THF solution (414 mL, 1 M) -78 °C. After 4 hours, a basic solution of H_2O_2 comprised of a 1.4 N NaOH solution (21.88 g NaOH, 390 mL DI H_2O) in combination with a 30 % v/v H_2O_2 solution (58.91g, 572 mmol) was added slowly over 15 minutes to the reaction mixture at 0 °C. Moderate off gassing occurred upon initial introduction of the H_2O_2. The reaction was allowed to slowly warm to ambient temperature and went to completion within 4 hours before dilution with diethyl ether (200 mL). The resulting mixture was extracted with diethyl ether (2 x 400 mL). The combined ether layers were washed with sodium bicarbonate (200 mL), followed by brine (200 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (400 g of SiO_2; gradient eluent, 5:95 diethyl ether: hexanes, 10:90 diethyl ether: hexanes, 15:85 diethyl ether: hexanes, 20:80 diethyl ether: hexanes). Purified primary alcohol **54** was obtained (79.13 g, 292 mmol, 70.4 % yield). R_f 0.20 (30 % ether in hexanes).FTIR (thin film, cm-^{1}) 3300, 2929, 2858, 1504, 1472, 1463, 1346, 1256, 1151, 1060, 1009, 938, 837. 837. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33 (d, J = 0.8 Hz, 1H), 6.30 (dd, J = 3.2, 2 Hz, 1H), 6.14 (d, J = 3.2 Hz, 1H), 4.74 (t, J = 5.8 Hz, 1H), 3.63 (t, J = 6.2 Hz, 2 H), 1.88 (m, 2H), 1.616 (m, 2H), 0.87 (s, 9H), 0.03(s, 3H), -0.06 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.5, 141.3, 110.0, 105.9, 68.5, 62.9, 33.5, 28.6, 25.8, 18.2, -5.6, -5.0. FAB HRMS *m/z* calcd for [($C_{14}H_{26}O_3Si$)+Na]+: 293.1549; Found 293.1565.



55

Tosylate (55)

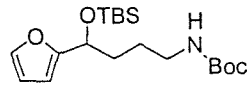
To the alcohol **54** (14.1 g, 51.99 mmol) in a 1 M solution of methylene chloride (104 mL) stirring in a single neck 500 mL round bottom flask charged dimethyl ammonium pyridine (DMAP) (644 mg, 5.28 mmol) along with Et_3N (26.3 g, 260 mmol) and tosyl chloride (12.88 g, 67.59 mmol). The reaction proceeded at 0 °C under a nitrogen atmosphere. After 6 hours the reaction was quenched with NH_4Cl (200 mL). The resulting mixture was extracted with diethyl ether (2 x 400 mL). The combined ether layers were washed with sodium bicarbonate (200 mL), followed by brine (200 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (60 g of SiO_2; gradient eluent, 3:97 ethyl acetate:hexanes, 4:96 ethyl acetate: hexanes, 5:95 ethyl acetate: hexanes) afforded tosyl ether **55** (15.44 g, 36.30 mmol, 70 % yield) as a clear oil. R_f 0.80 (30 % ether in hexanes). FTIR (thin film, cm-^{1}) 2953, 2929, 2856, 1598, 1468, 1360, 1253, 1177, 1149, 1097, 1008, 963, 923, 837, 815, 778, 742, 664. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 (d, J = 8.4 Hz, 2H), 7.33 (m, 3H), 6.29 (m, 1H), 6.11 (dd, J = 3.2, 0.8 Hz, 1H), 4.62 (m, 1 H), 4.05 (t, J = 10.5 Hz, 2H) 2.44 (s, 3H), 1.74 (m, 2H), 1.48 (m, 2H) 0.83 (s, 9H), -0.01 (s, 3H), -0.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.5, 144.6, 141.4, 133.2, 129.8, 127.9, 110.0, 106.0, 70.5, 67.7, 32.7, 25.7, 24.8, 21.6, 18.1, -5.0, -5.2. FAB HRMS *m/z* calcd for $[(C_{21}H_{32}O_5SiS)+NH_4]^+$: 442.2; Found 442.3.



56

Azide (56)

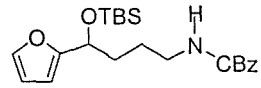
The tosyl ether **55** (108 g, 254 mmol) was added to an acetone/H_2O solution (3:1 v/v ratio, 260 mL) along with NaN_3 (49.51 g, 762 mmol) and the mixture was heated to reflux (~60°C). The phase transfer catalyst tetra butyl ammonium bromide (TBABr) was added (4.09 g, 12.70 mmol) to significantly speeds up the reaction. After 3-4 hours the reaction was diluted with diethyl ether (250 mL) and sodium bicarbonate (250 mL). The resulting mixture was extracted with diethyl ether (2 x 500 mL). The combined ether layers were washed with sodium bicarbonate (300 mL), followed by brine (300 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil with significant precipitates. Compound was then diluted with hexanes (500 mL), filtered, and concentrated again *in vacuo* to a light colored oil without precipitation. Azide **56** was obtained (64.32 g, 217 mmol, 75 % yield). R_f 0.80 (30 % ether in hexanes). FTIR (thin film, cm-[1]) 2954, 2932, 2887, 2859, 2357, 2097, 1504, 1468, 1390, 1349, 1255, 1176, 1147, 1105, 1007, 938, 836, 812, 777, 735, 665. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30 (s, 1H), 6.304 (dd, J = 3.2, 2 Hz, 1H), 6.17 (d, J = 3.2 Hz, 1H), 4.71 (t, J = 5.8 Hz, 1H), 3.27 (t, J = 7.2 Hz, 2H), 1.86 (m, 2H), 1.66 (m, 2H), 0.89 (s, 9H), 0.05 (s, 3H), -0.08 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.7, 141.4, 110.0, 105.9, 68.0, 51.4, 34.0, 25.8, 24.8, 18.1, -5.0. FAB HRMS *m/z* calcd for $[(C_{14}H_{25}O_2N_3Si) + NH_4]^+$: 313.2060; Found 313.2067.



49

Boc-amine (49)

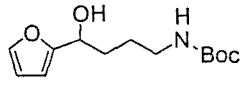
To the azide **56** (21.44 g, 72.39 mmol) at 0 °C in a THF/H_2O (145 mL, 0.5 M) solution was added triphenyl phosphine (21.84 g, 83.26 mmol). After 10 hours, the reaction was quenched with potassium carbonate in H_2O (2 g, 100 mL respectively). This was followed by the addition of *t*-butyl dicarbonate (17.6 g, 80.64 mmol). After 12 hours sodium bicarbonate (100 mL) and diethyl ether (100 mL) were added. The aqueous layer was extracted twice with 300 mL of diethyl ether. The combined ether layers were washed with sodium bicarbonate (200 mL), followed by brine (200 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a clear oil. Flash chromatography (100 g of SiO_2; gradient eluent, 5:95 diethyl ether: hexanes, 10:90 diethyl ether: h, 2945, exanes). Purified Boc-amine **49** was obtained (24.59 g, 66.52 mmol, 92% yield). R_f 0.50 (30 % ether in hexanes). FTIR (thin film, cm^{-1}) 3361, 2875, 2351, 1705, 1517, 1377, 1256, 1166, 1095, 845, 789. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 0.8 Hz, 1H), 6.31 (dd, J = 3.3, 1.8 Hz, 1H), 6.17 (d, J = 3.3 Hz,1H), 4.71 (m, 1H), 4.55 (bs, 1H), 3.13 (m,2 1H), 1.77 (m, 2H), 1.57 (m, 2H), 1.49 (s, 9H), 0.88 (s, 9H), 0.06 (s, 3H), 0.06 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 155.9, 141.3, 110.0, 105.8, 68.2, 40.3, 34.1, 30.9, 28.4, 25.8, 18.2, -5.1, -5.0. FAB HRMS *m/z* calcd for [($C_{19}H_{35}O_4NSi$)+Na]+ : 392.2233; Found 392.2231.



57

Cbz-amine (57)

To the azide **56** (21.44 g, 72.39 mmol) at 0 °C in a THF solution (73 mL, 1 M) with a trace of water (1 mL) was added triphenyl phosphine (21.84 g, 83.25 mmol). After 10 hours, the reaction was quenched with potassium carbonate/H_2O (2 g, 100 mL respectively). This was followed by the addition of benzyl chloroformate at 0°C (25.6 mL, 76.0 mmol). After 12 hours sodium bicarbonate (100 mL) and diethyl ether (100 mL) was added. The resulting mixture was extracted with diethyl ether (2 x 400 mL). The combined ether layers were washed with sodium bicarbonate (200 mL), followed by brine (200 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (100 g of SiO_2;. gradient eluent, 5:95 diethyl ether: hexanes, 10:90 diethyl ether: hexanes) providing compound **57** (25.3 g, 62.6 mmol, 87 % yield). R_f 0.50 (30 % ether in hexanes). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (s, 5H) 6.29 (dd, J = 3.2, 2.0 Hz, 1H), 6.14 (d, J = 3.19 Hz,1H), 5.09 (s, 2H), 4.69 (t, J = 6.4 Hz, 1H), 3.19 (dd, J = 13.2, 7 Hz, 2H), 1.80 (m, 2H), 1.55 (m, 2H), 1.80 (m, 2H), 0.87 (s, 9H), 0.03 (s, 3H), -0.09 (s, 3H).

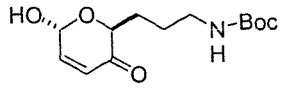


58

Hydroxyl amine (58)

To the *N*-Boc protected substrate **49** (1 g, 2.71 mmol) in a THF solution (2.7 ml, 1 M) at 0 °C and under a nitrogen atmosphere was added tetra-butyl ammonium fluoride (2.97 ml, 2.98 mmol) and

the reaction was allowed to proceed until all starting material had disappeared (within 2.5-3 hours), followed by an addition of diethyl ether (3 mL) and sodium bicarbonate (3 mL). The resulting mixture was extracted with diethyl ether (2 x 20 mL). The combined ether layers were washed with sodium bicarbonate (20 mL), followed by brine (20 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (5 g of SiO_2;. gradient eluent, 40:60 diethyl ether: hexanes, 50:50 diethyl ether: hexanes) providing hydroxyl amine **58** (610 mg, 2.39 mmol, 88% yield). R_f 0.30 (30 % ether in hexanes). FTIR (thin film, cm-^{1}) 3334, 2975, 2932, 2869, 1683, 1538, 1520, 1456, 1393, 1366, 1276, 1252, 1171, 1102. ^{1}H NMR δ 7.35 (dd, J = 0.6, 1.8 Hz, 1H), 6.31 (dd, J = 3, 1.5 Hz, 1H), 6.17 (d, J = 3.3 Hz, 1H), 4.73 (t, J = 6.6 Hz, 1H), 4.60 (bs, 1H), 3.13 (m, 2H), 2.65 (bs, 1H), 1.86 (m, 2H), 1.80 (m, 2H), 1.41 (s, 9H). ^{13}C (300 MHz, $CDCl_3$) δ 156.7, 156.1, 141.9, 110.1, 105.8, 79.2, 67.4, 65.9, 40.2, 32.5, 28.4, 26.2, 15.3. FAB HRMS *m/z* calcd for $[(C_{13}H_{21}O_4N) + H]^+$: 256.1549; Found 256.1559.

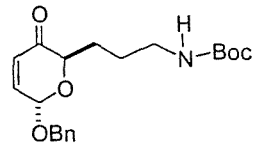


60

Anomeric alcohol (60)

To the deprotected alcohol **58** (3 g, 11.75 mmol) at 0 °C in a 3:1 (v/v) THF/water solution (39 mL, 0.3 M) was added sodium acetate trihydrate (1.60g, 11.75 mmol), sodium bicarbonate (1.97g, 23.5 mmol), and finally NBS (2.07 g, 11.63 mmol). After completion in 1.5 hours, the reaction was quenched with potassium bicarbonate (30 mL), and diluted with diethyl ether

(30 mL). The resulting mixture was extracted with diethyl ether (2 x 100 mL). The combined ether layers were washed with sodium bicarbonate (100 mL), followed by brine (100 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a clear oil. A diastereomeric mixture of anomeric alcohols **60** were obtained in 93 %. Purification was avoided. R_f 0.20 (75 % ether in hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26-6.88 (m, 1H), 6.12 (dd, J = 10.5, 1.8 Hz, 1H), 6.07 (dd, J = 10.2, 0.6Hz, 1H), 5.66 (d, J = 1.5 Hz, 1H), 5.62 (d, J = 3.3 Hz, 1H), 4.76 (bs, 1H), 4.60 (dd, J = 7.8, 3.6 Hz, 1H), 3.76-3.71 (m, 1H), 3.15 (bs, 2H), 2.037- 1.93 (m, 1H), 1.87- 1.82 (m, 1H), 1.71- 1.55 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.5, 145.0, 128.4, 127.3, 90.8, 87.6, 79.5, 78.5, 73.2, 68.0, 40.8, 40.0, 29.6, 28.4, 27.6, 26.3, 25.8, 25.7, 25.6.

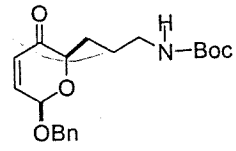


68

Benzyl ether (68)

To alcohol **60** (2.97 g, 10.95 mmol, 1 eq) in a methylene chloride solution (32.8 ml, 0.3 M) at 0 °C under nitrogen was added silver oxide (5.07 g, 21.89 mmol, 2 eq) and benzyl bromide (2.81 g, 16.42 mmol, 1.5 eq). After 24 hours the reaction was diluted with potassium carbonate (50 ml) and diethyl ether (50 ml). The resulting mixture was extracted with diethyl ether (2 x 100 mL). The combined ether layers were washed with sodium bicarbonate (100 mL), followed by brine (100 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (10 g of SiO_2;. gradient eluent, 40:60 diethyl ether: hexanes, 50:50 diethyl ether: hexanes) provided benzyl ether anomer **68** (519 mg, 1.44 mmol, 20 % yield). R_f 0.50 (50 %

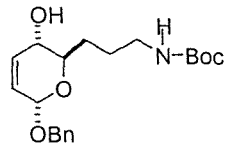
ether in hexanes). FTIR (thin film, cm-[1]) 3374, 2976, 2932, 2871, 1695, 1519, 1454, 1392, 1366, 1250, 1170, 1096, 1037, 1025. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.90 (dd, J = 10.4, 3.6 Hz, 1H), 6.09 (d, J = 10.2,1H), 5.39 (d, J = 3.6 Hz, 1H), 4.83 (d, J = 11.6 Hz, 1H), 4.66 (d, J = 10.8 Hz, 1H), 4.58 (bs, 1H), 4.44 (7.0, 3.4 Hz, 1H), 3.16 (m, 2H), 1.44 (s, 9H), 1.96- 1.57 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.9, 127.7, 92.0, 73.5, 70.2, 40.1, 28.2, 26.6, 25.7. FAB HRMS *m/z* calcd for $[(C_{13}H_{21}O_4N) + H]^+$: 362.1980. Found 362.1987.



89

Benzyl ether (89)

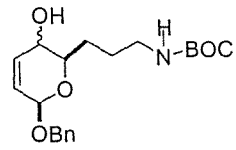
R_f 0.50 (50 % ether in hexanes). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.31 (m, 5H), 6.90 (dd, J = 10.6, 2 Hz, 1H), 6.14 (dd, J = 10.4, 1.8 Hz, 1H), 5.39 (s, 1H), 4.94 (d, J = 11.8 Hz, 1H), 4.71 (d, J = 12.0 Hz, 1H), 4.60 (bs, 1H), 4.07 (dd, J = 7.4, 4 Hz, 1H), 3.16 (dd, J = 13.6, 6.6 Hz, 2H), 1.99-1.57 (m, 4H), 1.44 (s, 9H).



69

α, β-unsaturated alcohol (69)

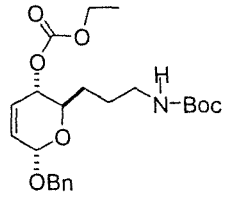
To the enone **68** (130 mg, 0.36 mmol) in a methylene chloride solution (0.36 ml, 1 M) at –78 °C and under a nitrogen atmosphere was added a 0.4 M solution of cerium trichloride heptahydrate in methanol ($CeCl_3 \cdot 7H_2O$, 0.90 mL, 0.36 mmol) followed by sodium borohydride (13.6 mg, 0.36 mmol). The reaction was complete in 1.5 hours, and potassium bicarbonate (2 ml) was added along with diethyl ether (2 ml). The resulting mixture was extracted with diethyl ether (2 x 20 mL). The combined ether layers were washed with sodium bicarbonate (10 mL), followed by brine (10 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to afford **69,** a clear oil (109 mg, 0.30 mmol, 83 % yield). Purification was not necessary for the subsequent ethyl formate protection. R_f 0.30 (50 % ether in hexanes). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.10 (s, 5H), 5.58 (dd, J = 37.6, 9.8 Hz, 2H), 4.87 (s, 1H), 4.47 (d, J = 12 Hz, 1H), 4.36 (d, J = 12 Hz, 1H), 4.25 (d, J = 6 Hz, 2H), 4.04 (bs, 1H), 3.68 (bs, 1H), δ 1.40 Hz, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.3, 140.9, 137.8, 134.0, 128.5, 128.0, 127.8, 127.6, 127.0, 126.2, 93.4, 79.3, 71.3, 70.1, 67.8, 65.3, 40.2, 30.3, 28.8, 28.5, 26.2. FAB HRMS *m/z* calcd for $[(C_{20}H_{29}NO_5) + Na]^+$: 386.50186; Found 386.1939.



90

α,β-unsaturated alcohol (90)

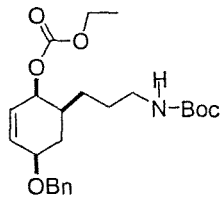
To the enone **89** (628 mg, 1.74 mmol) in a methylene chloride solution (3.5 mL, 0.5 M) at –78 °C and under a nitrogen atmosphere was added a 0.4 M solution of cerium trichloride heptahydrate in methanol ($CeCl_3 \cdot 7H_2O$) (4.34 mL, 1.74 mmol) followed by sodium borohydride (65.7 mg, 1.74 mmol). The reaction was complete in 1.5 hours, and potassium bicarbonate (2 ml) was added along with diethyl ether (2 ml). The resulting mixture was extracted with diethyl ether (2 x 20 mL). The combined ether layers were washed with sodium bicarbonate (10 mL), followed by brine (10 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to afford **90**, a clear oil (582 mg, 1.60 mmol, 92 % yield). Purification was avoided until the subsequent ethyl formate protection.



48

Carbonate (48)

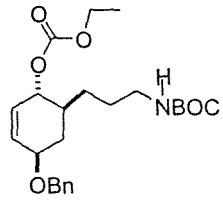
To the α, β unsaturated alcohol **69** (144 mg, 0.40 mmol) in a methylene chloride solution (0.8 ml, 0.5 M) was added pyridine (0.063 g, 0.79 mmol), dimethyl ammonium pyridine (4.83 mg, 0.040 mmol) and ethyl chloroformate (47.29 mg, 0.44 mmol). The reaction proceeded at room temperature and was complete within 24 hour at which time potassium bicarbonate (10 ml) and diethyl ether (10 ml) were added. The resulting mixture was extracted with diethyl ether (2 x 100 mL). The combined ether layers were washed with sodium bicarbonate (100 mL), followed by brine (100 mL), and then dried (Na_2SO_4) and concentrated *in vacuo* to a light colored oil. Flash chromatography (13 g of SiO_2;. eluent, 25:75 diethyl ether: hexanes) provided compound **48** (80 mg, 0.18 mmol, 47 % yield). R_f 0.60 (50 % ether in hexanes). FTIR (thin film, cm-^{1}) 3380, 2961, 2355, 1727, 1516, 1382, 1261, 1165, 1024. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (m,5H), 5.94 (d, J = 10.0 Hz, 1H), 5.84 (d, J = 10.0 Hz, 1H), 5.06 (s, 1H), 4.94 (d, J = 9.5 Hz, 1H), 4.80 (d, J = 12 Hz, 1H), 4.61 (bs, 1H), 4.57 (d, J = 11.5 Hz, 1H), 4.21 (dd, J = 14.0, 7.0 Hz, 2H), 3.909 (t, J = 9 Hz, 1H), 3.15 (m, 2H), 1.75 (m, 2H), 1.52 (m, 2H), 1.44 (m, 9H), 1.32 (t, J = 7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.9, 154.8, 137.7, 129.3, 128.5, 128.1, 127.9, 127.8, 93.4, 79.1, 72.7, 70.2, 68.3, 64.4, 40.5, 29.0, 28.4, 25.9, 14.2. FAB HRMS *m/z* calcd for $[(C_{23}H_{34}NO_7) + H]^+$: 436.2335; Found 436.2346.



76

Carbonate (76)

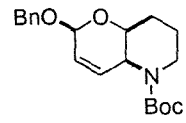
To the α, β unsaturated alcohol **90** (40 mg, 0.11 mmol) in a methylene chloride solution (0.5 M) was added pyridine (0.0175 g, 0.22 mmol), dimethyl ammonium pyridine (1 mg, 0.01 mmol), and ethyl chloroformate (13 mg, 0.11 mmol). The reaction proceeded at room temperature and was complete in 4 hours, and then worked up with potassium bicarbonate and diluted with ether. The aqueous layer was extracted twice with 100 mL of diethyl ether. The combined ether layers were washed with sodium bicarbonate, followed by brine, and then dried over sodium sulfate before concentration to a yellow oil. The compound was purified by column chromatography (mobile phase solution 25: 75 diethyl ether: hexanes) providing compound **76** and **91** (30 mg, 0.09 mmol, 45 % yield). R_f 0.60 (50 % ether in hexanes). FTIR (thin film, cm-^{1}) 3420, 2975, 1739, 1705, 1516, 1370, 1259, 1169, 1059, 1007. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (m, 5H), 6.13 (ddd, J= 10.1, 4.8, 1.4 Hz, 1H), 6.00 (d, J = 10.2 Hz, 1H), 5.13 (s, 1H), 4.84 (dd, J = 7, 2.2 Hz, 1H), 4.87 (d, J = 12 Hz, 1H), 4.66 (d, J = 11.8, 1H), 4.60 (m, 1H), 4.20 (dd, J = 14, 7 Hz, 2H), 3.74-3.68 (m, 1H), 3.18 (m, 2H), 1.81-1.59 (m, 4 H), 1.44 (s, 9H), 1.31 (t, J = 7Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 156.2, 155.3, 137.9, 133.7, 128.6, 128.4, 128.0, 126.7, 110.7, 96.7, 73.6, 69.7, 69.1, 64.4, 40.4, 28.7, 27.8, 26.4, 14.43. FAB HRMS *m/z* calcd for $[(C_{23}H_{34}NO_7) + H]^+$: 436.2335; Found 436.2333.



91

Carbonate (91)

R_f 0.60 (50 % ether in hexanes). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35 (m, 5H), 6.01-5.85 (m, 2H), 5.19 (s, 1H), 4.99 (d, J = 6.6 Hz, 1H), 4.86 (d, J = 12.0 Hz, 1H), 4.64 (d, J = 12 Hz, 1H), 4.54 (m, 1H), 4.21 (dd, J = 14.4, 7 Hz, 2H), 3.711-3.677 (m, 1H), 3.15 (d, J = 5.6 Hz, 2H), 1.74-1.58 (m, 4H), 1.44 (s, 9H), 1.32 (t, J = 7.2 Hz, 2H).

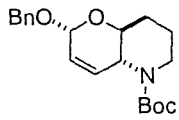


77

***Cis*-fused Perhydro-pyrano-piperidine (77)**

To carbonate **76** (40 mg, 0.09 mmol) in a 1M THF solution under a nitrogen atmosphere at room temperature was added $Pd(dba)_2$•$CHCl_3$ (4mg, 0.004 mmol), and triphenylphosphine (6 mg, 0.012 mmol). This was followed by the addition of potassium *t*-butoxide (7 mg, 0.09 mmol). The reaction went from an orange-brown color immediately to a black color. After 6 hours the starting material had disappeared, and the reaction was worked up by concentration. The compound was purified by column chromatography (mobile phase solution 10:90 diethyl ether: hexanes) providing compound **77** (16 mg, 0.051 mmol, 20 % yield). R_f 0.75 (50 % ether in hexanes). FTIR (thin film, cm-^{1}) 2964, 2927, 2859, 1694, 1454, 1414, 1393, 1377, 1365, 1343, 1301, 1274, 1253, 1176, 1152, 1121, 1105, 1048, 1027, 996, 570. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J = 4.5 Hz, 5H), 5.86 (dt, J = 11, 2.5 Hz, 1H), 5.72 (d, J = 10 Hz, 1H), 5.06 (s, 1H), 4.87

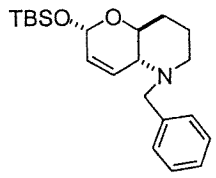
(d, J = 11.5 Hz, 1H), 4.54 (d, J = 11.5 Hz, 1H), 3.94 (s, J= 5.5 Hz, 1H), 2.29 (ddd, J = 13, 12.5, 4.5 Hz, 1H), 1.90-1.86 (m, 1H), 1.65- 1.59 (m, 1H), 1.47 (s, 9H), 1.40- 1.23 (m, 3H), 0.90-0.87 (m, 1H). ^{13}C NMR (75 MHz, CDCl3) δ 154.9, 137.9, 130.1, 128.4, 128.0, 127.6, 127.6, 92.34, 80.0, 69.7, 67.9, 30.3, 29.7, 28.4, 26.3, 24.2, 12.2. FAB HRMS *m/z* calcd for $[(C_{23}H_{34}NO_7) + H]^+$: 346.2018; Found 346.2033.



46

***Trans*-fused Perhydro-pyrano-piperidine (46)**

To carbonate **48** (110 mg, 0.25 mmol) in methylene chloride (3 ml, 1 M) under a nitrogen atmosphere at room temperature was added $Pd(dba)_2 \cdot CHCl_3$ (13 mg, 0.013 mmol), and triphenylphosphine (16.5 mg, 0.063 mmol). This was followed by the addition of potassium *t*-butoxide (28.1 mg, 0.25 mmol). The reaction went from an orange-brown color immediately to a black color. After 6 hours the starting material had disappeared and the reaction was worked up by concentration. The compound was purified by column chromatography (mobile phase solution 10:90 diethyl ether: hexanes) providing compound **46** (25 mg, 0.076 mmol, 30 % yield). R_f 0.75 (50 % ether in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.30 (m, 5H), 5.96 (s, 2H), 5.16 (s, 1H), 4.79 (d, J = 11.5 Hz, 1H), 4.61 (d, J = 11.5 Hz, 1H), 4.33-4.30 (m, 2H), 3.80-3.85 (m, 1H), 2.95-2.99 (m, 1H), 1.6-1.85 (m, 4H), 1.48 (s, 9H). FAB HRMS *m/z* calcd for $[(C_{23}H_{34}NO_7) + Na]^+$: 368.1838; Found 368.1849.



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.30 (m, 5H), 5.96 (dd, J = 9.9, 5.1 Hz, 2H), 5.81 (d, J = 10.2 Hz, 1H), 5.16 (s, 1H), 5.03 (m, 3H), 4.83 (m, 1H), 3.66 (m, 1H), 3.50 (m, 1H), 3.27 (m, 1H), 1.61-1.82 (m, 4H), 0.90 (s, 9H), 0.15 (s, 6H).

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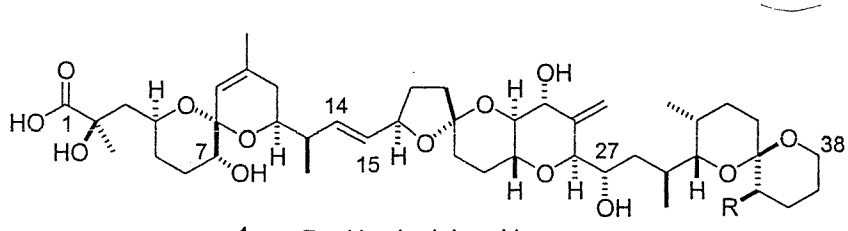
41 Dr. Michael Haukaas applied this oxidation/ selective Noyori reduction sequence to the substrate and obtained 98 % ee.

Background

Okadaic Acid (**1a**) (Figure 1) is a marine natural product produced by dinoflagellates such as *Prorocentrum lima.*[1] It is found among marine filter feeders such as black sponges of the genus *Halichondria* where it was first isolated by Tachibana *et al..*[2] It possesses a formidable structure with seventeen stereogenic centers, three types of double bonds, three spiroketals, thirteen oxygen atoms attached to a thirty eight carbon backbone, as well as a tertiary α-hydroxy acid group.

Figure 1.



1a R = H, okadaic acid
1b R = CH_3, dinophysistoxin
1c R = H, 9, 10-alpha-episulfide

The basic structure of okadaic acid can be found among other analogs, such as dinophysistoxin **1b** (35-methyl okadaic acid) [3] as well as acanthifolicin **1c** (9,10-episulfide of okadaic acid) [4] as shown (Figure 1).

Biology

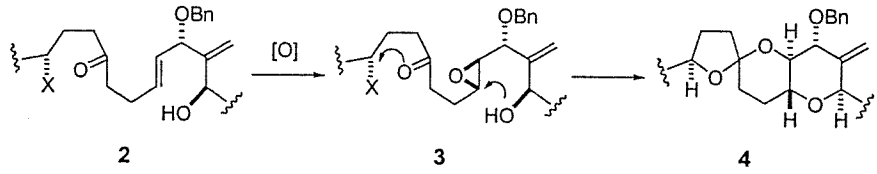
Okadaic acid and the mentioned congeners gained scientific attention when these marine toxins were determined to act as causative agents of diarrhetic shellfish poisoning (DSP) through their accumulation in edible shellfish.[4, 5] Okadaic acid has since been characterized as a non-phorbol

ester type tumor promoter, and as a potent inhibitor of protein phosphatases 1 and 2A at nanomolar concentrations.[6]

The Forsyth group has produced inhibitory activity data for the protein phosphatases 1 and 2A using a malachite green assay system with a phosphopeptide substrate KRpTIRR.[7] Against PP1, okadaic acid has an IC_{50} value of 126 nM. Against PP2A, okadaic acid has an IC_{50} value of 7 nM.[7] The Takai group has also determined inhibitory activity data for the protein phosphatases 1 and 2A. Against PP1, okadaic acid has a K_i value of 145 nM, while against PP2A, okadaic acid has a K_i value of 30 pm.[8]

Norte and coworkers have shown that all the carbons of okadaic acid are derived from acetate. It has been demonstrated that acetate labeling patterns of okadaic acid is probably not due to involvement of citric acid pathway biosynthetic intermediates. Instead the ^{13}C labeling patterns from [1,2-^{13}C]acetate suggest 3-hydroxy-3-methylglutarate and other carboxylates generated via the tricarboxylic acid pathway may be involved.[4, 5] The origins of some of the oxygen atoms may be derived from acetate and glycolate.

Scheme 1.

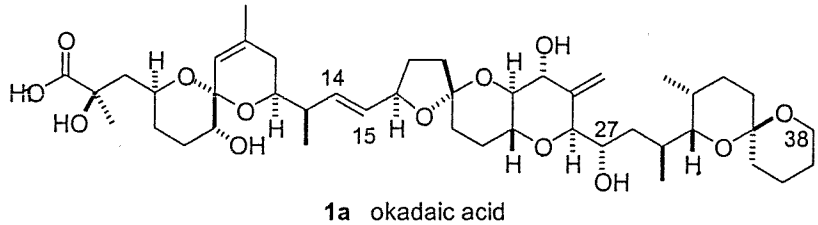


2 3 4

The biosynthesis of okadaic acid's middle core **4**, the focus of this thesis, is proposed to occur through a cyclization of a β-epoxide **3**, derived via oxidation of a disubstituted olefin **2** as outlined in Scheme 1. It is interesting that none of the total syntheses of okadaic acid make use of this biomimetic process.

Previous Work

Figure 2.

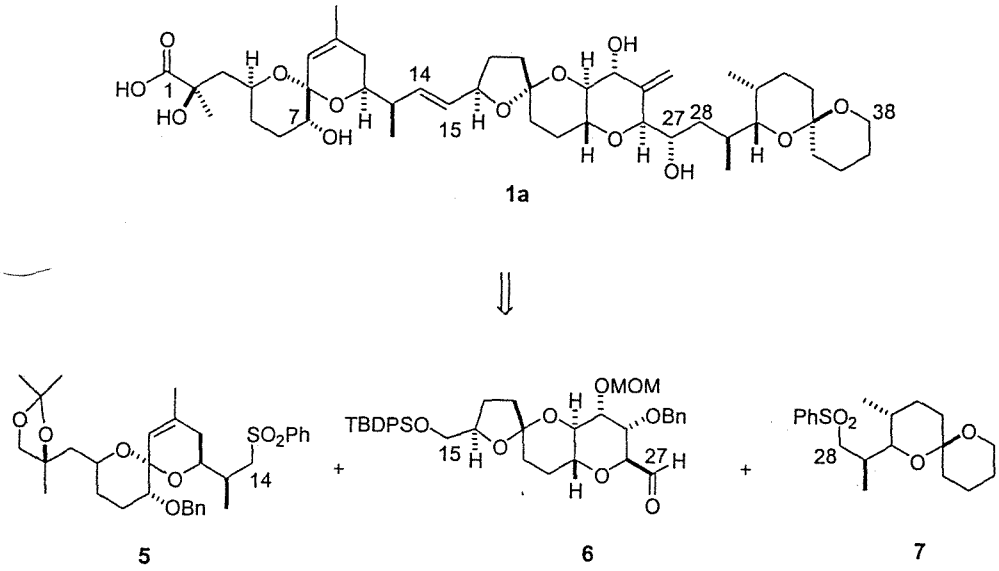


1a okadaic acid

There has been significant scientific effort toward the synthesis of okadiac acid. Three total syntheses have been accomplished by the Isobe, Forsyth, and Ley groups to date.[10] Additionally the Schlessenger and Marko groups have synthesized core fragments of okadaic acid.[11, 12]

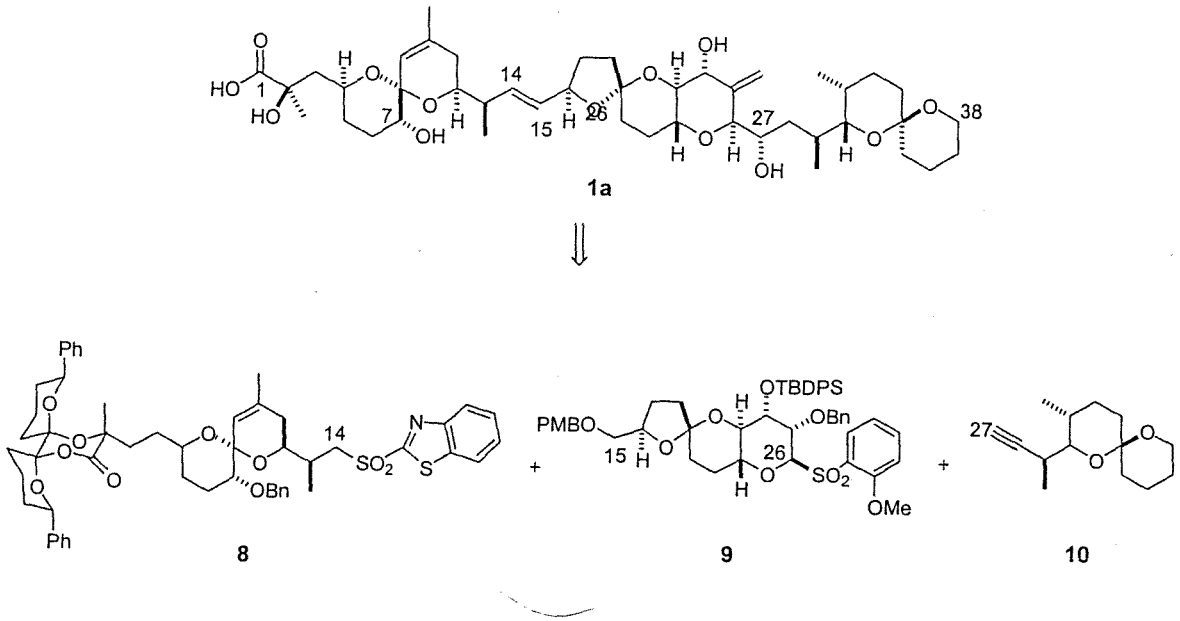
The different syntheses of okadaic acid are unique. An examination of the retrosynthetic designs of okadaic acid by other groups will provide background information for our work.

Scheme 2.



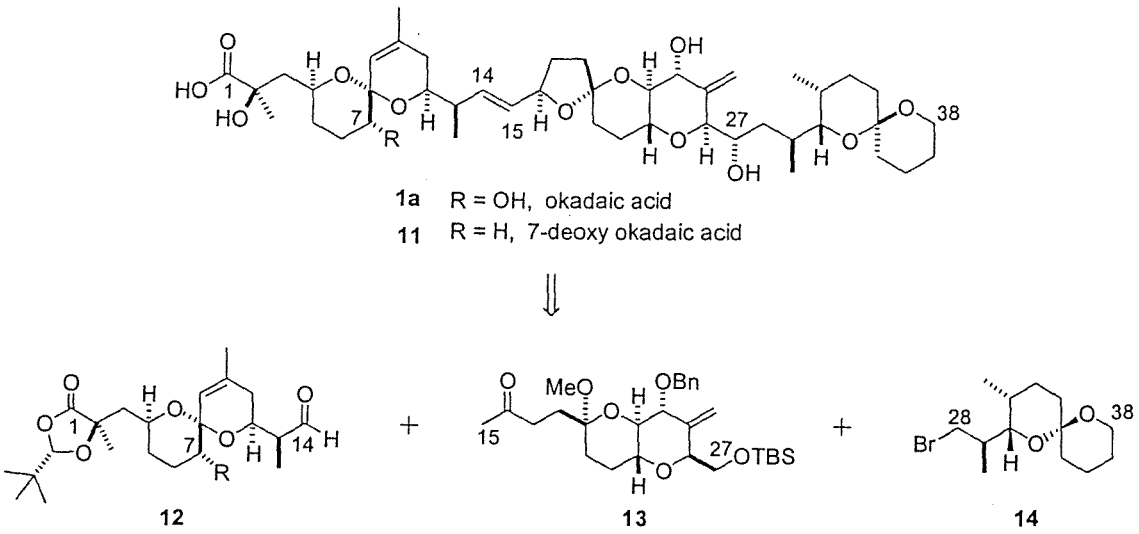
The retrosynthetic disconnection of Isobe's synthesis is shown (Scheme 2).[10a,b] The C14-C15 *E*-alkene is installed via a Julia olefination between the C14 sulfone anion of domain **5** and a C15 aldehyde of domain **6** (masked presently as a silyl protected alcohol). Likewise the C27-C28 bond comes from the addition of the C28 sulfone anion of domain **7** added to C27 aldehyde of **6**. All three domains derive their chirality from D-glucose. The stereochemistry of the α-hydroxy carboxylic acid of domain **5** stems from a stereoselective oxymercuration of an olefin followed by a reductive work-up. The Isobe group utilizes 106 steps for the total synthesis of okadaic acid.

Scheme 3.



For construction of domain **8** the Ley group utilizes methods developed in their laboratories for the asymmetric synthesis of α-hydroxy acids by employing a chiral dispiroketal (Scheme 3).[10d] A Julia olefination occurs between the C14 sulfone anion of domain **8** and the C15 aldehyde of domain **9** (masked above as a protected alcohol) to form the *E*-configured alkene. Domain **9** incorporates a sulfone functionality at the C26 anomeric center which is activated toward nucleophilic displacement by the C27 acetylide anion of domain **10**. The presence of the ortho-methoxy group on the aryl ring of **9** greatly enhances its chelation of the sulfone oxygen with lewis acids, thus increasing its reactivity. After acetylide anion addition, a regio-selective hydroboration/oxidation of the alkyne forms a single ketone that is then reduced and protected. Further elaboration forms okadaic acid. The Ley group utilizes 68 steps for its synthesis.

Scheme 4.



In our group's revised retrosynthetic direction,[10c] the *E*-configured C14-C15 alkene comes from an aldol coupling between the C15 methyl ketone of domain **13** and the C14 aldehyde of domain **12** (Scheme 4). This deviates from the original coupling strategy which utilizes the C15 carbanion of a β-keto-phosphonate coupling to the C14 aldehyde via a Masamune-Roush protocol.[13] The modified coupling strategy has the advantages of avoiding premature spiroketalization of the C16 oxygen onto the C19 ketal center that would form the 1,3-dioxaspiro[3.4] octane system irreversibly.[14]

Likewise the C27-C28 bond is seen coming from a cerium-mediated coupling of the C28 anion of lipophilic domain **14** with the C27 aldehyde **13** (masked above as an *O*-silyl ether).

Post-coupling transformations to furnish the natural product are minimal. The enone product of the aldol coupling reaction serves as a substrate for a diastereoselective ketone reduction using Corey's CBS-borane reagent.[15] This is followed by intramolecular ketalization under

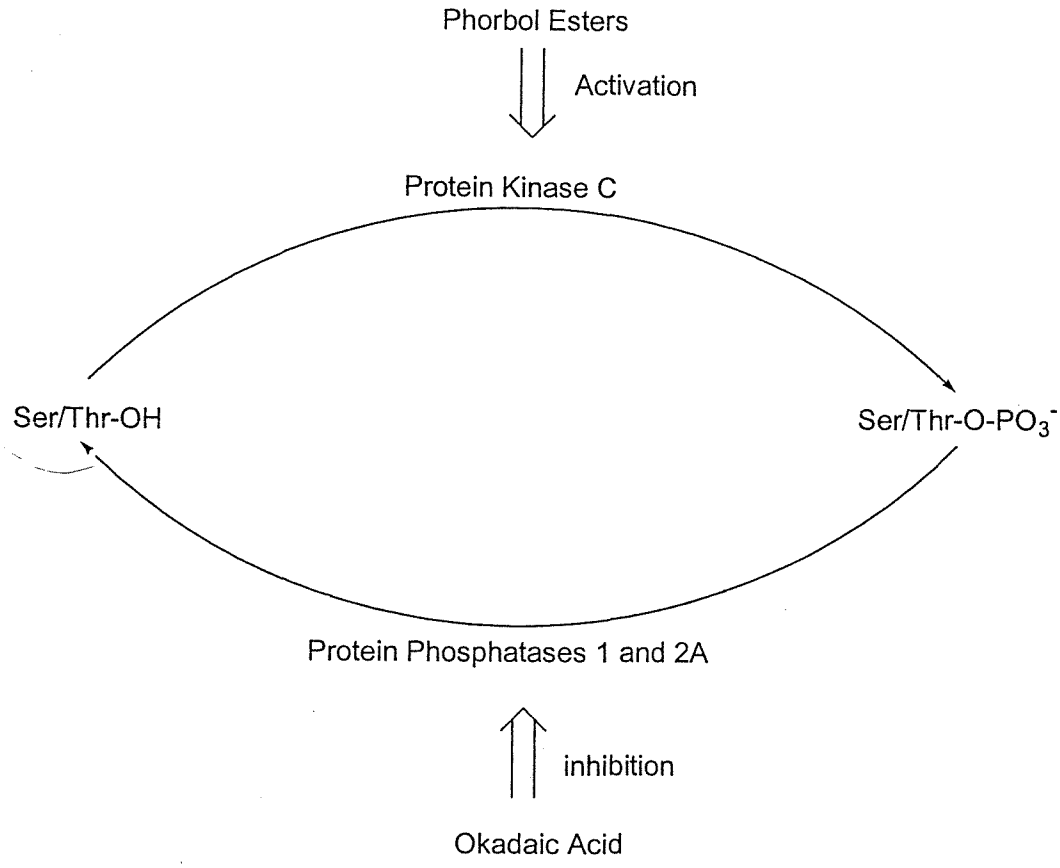
equilibrating conditions to afford the 1,3-dioxaspiro[3.4]octane ring system with the thermodynamically desired stereochemistry. [16] Saponification of the *t*-butyl group from domain **12** simultaneously releases both the C1 carboxylate and C2 hydroxyl groups to provide the α-hydroxy acid. Finally cleavage of the C27 and C24 benzyl ethers is affected with the use of lithium di-tertbutylbiphenylide (LiDBBP).[17]

The major goal of this project is the large-scale preparation of the polyether domain **13**, for use toward analog construction. It is envisioned that the syntheses of analogs would allow for the systematic probing of the okadaic acid pharmacaphore and create a better understanding of the functional groups which are necessary for maintaining the molecule's biological activity. These analogs may be used in studying the mechanism of phosphatase regulation.

Protein Phosphatase Background

Many cellular processes are regulated by the phosphorylation state of proteins. These cellular processes include glycogen synthesis, cell division, gene expression, neurotransmission, and muscle contraction.[18]

Figure 3.



Protein kinases phosphorylate proteins and are complimented by protein phosphatases which dephosphorylate proteins (Figure 3). Protein phosphatases and kinases are therefore jointly responsible for maintaining the phosphorylation of proteins in homeostasis. Both are implicated as important in cytokine signal transduction where changing this balance ultimately generates intracellular regulatory signals.[19]

Over 100 protein phosphatases have been discovered to date, but there may be greater than 1000. Understanding the roles of this vast number of phosphatases along with complimentary kinases is a significant challenge.

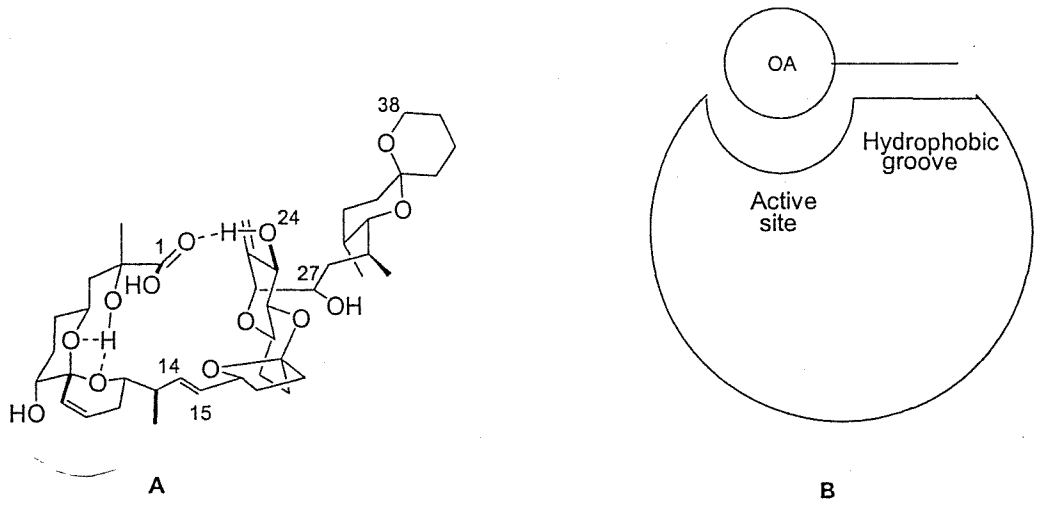
A focus in our group has been to synthesize natural products and unnatural analogs which act as specific protein phosphatase inhibitors. Protein phosphatase 1 and 2A represent two of the four principle serine-threonine specific phosphatases in the cystol of eukariotic cells. This molecular cycle is partly responsible for inducing physicochemical changes in proteins, and thus serves an important role in the regulation of their action. Other molecules in the okadaic acid class of PP1 and PP2A Ser/Thr phosphatase inhibitors include microcystin-LR, nodularin-Y, calyculin, tautomycin, cantharidin, thyrsiferyl-23-acetate, and fostriecin. All of these molecules exhibit inhibitory activity at a low or submicromolar levels consistently (IC_{50} values).[18] Additionally, all of these molecules appear to bind to the same site on the phosphatases. Therefore, a major challenge exists in understanding which phosphatase is responsible for a particular set of cellular processes. Making the challenge difficult is that the serine-threonine specific protein phosphatase family exhibits broad and overlapping substrate specificity. In fact the structural homology of the catalytic subunit of each of these enzymes show remarkable similarity.[18]

The Forsyth group would like to investigate the role of PP1 vs. PP2A in cellular regulation by synthesizing molecules that will bind more specifically to either PP1 or PP2A. The okadaic acid class of inhibitors serves as an excellent scaffold from which to create these novel molecules. This should allow for a better understanding of the role each of these enzymes plays in protein regulation.

It is noteworthy that despite all the challenges encountered in discerning the roles of PP1 vs. PP2A, there have been some recent encouraging breakthroughs in understanding how these enzymes effect intracellular signaling pathways. For instance okadaic acid was key in understanding that the peripheral hormone atrial natriuretic peptide, which controls blood pressure in the heart, is dephosphorylated by PP2A.[20]

Our group would like to gain a better understanding of the okadaic acid pharmacophore. Some limited SAR (structure activity relationship) studies using synthetic and semi-synthetic analogs have been informative in understanding okadaic acid's pharmacaphore. We would like to further elaborate these studies through a continuation of analog development and testing of these analogs for binding to PP1 and PP2A. These studies may be helpful in designing okadaic acid analogs which exhibit specificity for PP1 or PP2A.

Figure 4.

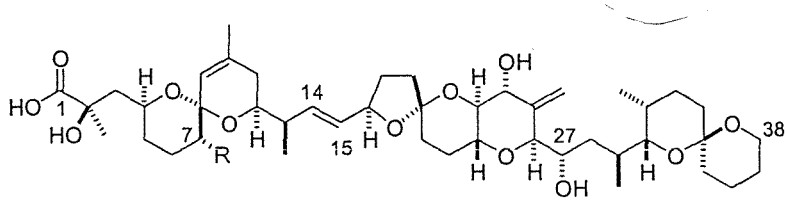


Rational design of okadaic acid analogs starts with an understanding of its solution phase conformation. Over twenty years ago, solid and solution phase studies showed that the C1-C26 portion of okadaic acid adopts a pseudo-macrolide conformation via intramolecular hydrogen bonding (Figure 4, A) and the C27-C38 domain of okadaic acid acts as a lipophilic domain.[2] A recent study that examined the crystal structure of PP1 co-crystallized with okadaic acid confirmed okadaic acid's active conformation, to a resolution of 1.9Å. This study showed that the inhibitor bound enzyme shows little conformational change whencompared with two other PP1 structures. [21] Both the pseudo-macrolide and lipophilic portions of the okadaic acid molecule appear to be important for reversible binding to PP1 and 2A (Figure 4, B). Previous studies, in our group and others, have shown that specific functional groups of okadaic acid are critical in maintaining bioactivity.[22] The C1 carboxylate appears to be a mimic of phosphoryl moieties of normal substrates and is important for the solution phase conformation of the natural product.[6] In this light, isolation of C1 esters of DSP toxin-producing strains of *Prorocentrum* show no activity

toward protein phosphatases.[23] The C2 hydroxyl, C14-15 *E*-alkene, and C24 hydroxyl groups are also important for maintaining the molecule's pseudo-macrolide conformation.[22]

Binding studies performed by Dr. Valerie Frydrychowski on truncated analogs of okadaic acid suggest that the pseudo-macrolide portion of okadaic is most substantial for inhibitory activity of PP2A, and that the lipophilic domain of PP1 plays a more significant role relative to PP2A.[7]

Figure 5.



1a R = OH, okadaic acid
11 R = H, 7-deoxy okadaic acid

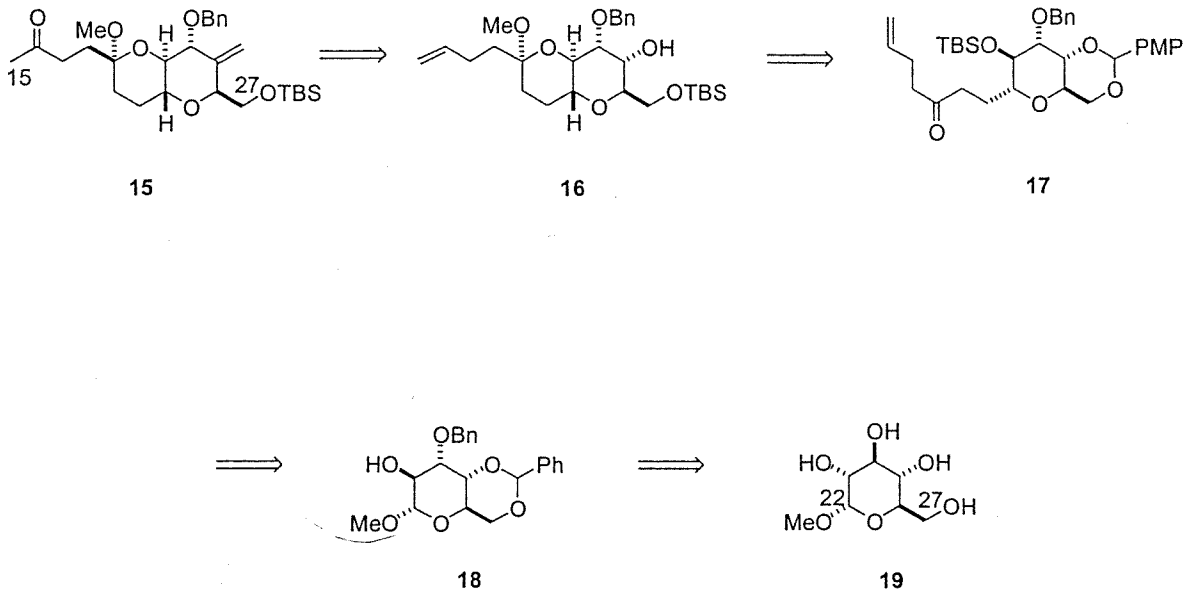
The naturally occurring analog, 7-deoxy okadaic acid **11** was first isolated from the dinoflagellate *P. Lima* (Figure 5).[24] Our group chose to use this congener of okadaic acid as an analogue scaffold as it has a less complicated structure and is nearly equipotent to okadaic acid.[7] Takai and co-workers have determined inhibitory activity data for 7-deoxy okadaic acid against PP1 and PP2A. Against PP2A, 7-deoxy okadaic acid has a K_i value of 69 pM, relative to a K_i of 30 pm for okadaic acid. Against PP1, 7-deoxy okadaic acid has a K_i value of 215 nM, relative to a K_i of 145 nM for okadaic acid.[8] The presence of the free C7 hydroxy group of okadaic lies outside the pseudo-macrolide conformation and does not appear to be a requirement for binding to PP1 or PP2A.

Our group would like to probe both the pseudo-macrolide and lipophilic portions of 7-deoxy okadaic acid. The most significant changes would involve the incorporation of variable C28-C38 lipophilic domains.

Forsyth-Abrams Synthesis

One responsibility for construction of okadaic acid involves making process changes for the large-scale construction of the C15-C27 domain. As described, this domain is one of three major fragments of okadaic acid and will remain largely unchanged for analog construction. This domain is comprised of the C, D, and E rings of okadaic acid. It contains a bis-dioxydecalin ring system from C19-C26, as well as a 1,3-dioxaspiro[3.4]octane ring system from C16-C23. The C15-C27 is further characterized as having 5 chiral centers and an *exo*-methylene unit at C25. Among the chiral pool of carbohydrates, α-methyl-D-glucopyranoside was chosen as a building block for installation of the stereochemistry of this domain.

Scheme 5.

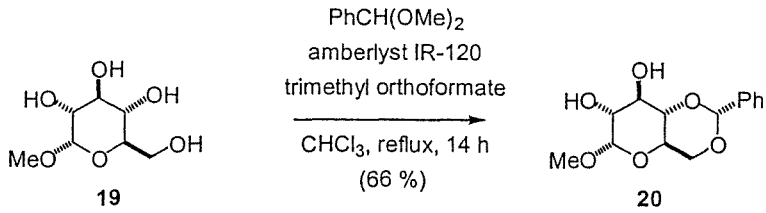


15 **16** **17** **18** **19**

The retrosynthetic design for the C15-C27 domain (Scheme 5) follows largely from the original Forsyth route.[9c] Domain **15** was envisioned to arise from *trans*-configured bis-pyran **16** via secondary alcohol oxidation, olefination, and selective Wacker oxidation.[25] The bis-pyran would then come from *C*-glycoside **17** via a one-pot acid-catalyzed double-deprotection, cyclization, and ketalization. The *C*-glycoside in turn was seen coming from altrose-configured pyran **18**, which could come from α-methyl-D-glucopyranoside **19** in just three steps.

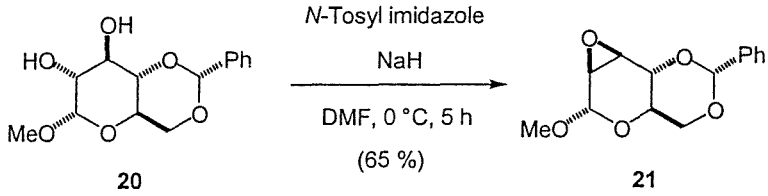
Process changes from the original procedures were essential in making the C15-C27 domain synthesis amenable for a large-scale effort. These included a change or reduction in reagent stoichiometry, minimization or reduction of work-up conditions, and exploration of more efficient routes.

Scheme 6.



Starting from α-methyl-D-glucopyranoside **19**, benzaldehyde dimethyl acetal was used to form the 4,6-di-*O*-benzylidene acetal of α-methyl-D-glucopyranoside **20** (Scheme 6).[26] The acid catalyst source of *p*-TsOH from the orginal procedure was exchanged for amberlyst IR-120 resins in order to completely eliminate the need for an aqueous work-up. This would have been a sizeable effort, as this reaction was run on as large as a one kilo scale. Methanol generated from this protection step was azeotropically removed with $CHCl_3$, the reaction solvent medium.

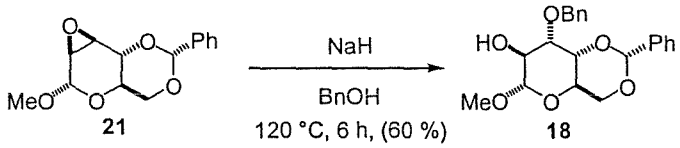
Scheme 7.



Next acetal intermediate **20** was transformed into manno epoxide **21** according to the original procedure (Scheme 7).[27] After formation of the dialkoxide of **20** with 2.1 eq of sodium hydride, one equivalent of *n*-tosyl imidazole was suitable for regioselective tosylation. This was followed by ring-closure by the remaining nucleophilic alkoxide and concomitant tosylate displacement.

Presumably, C2 of **2** is more accessible for tosylation than C3 when using the bulkier *n*-tosyl imidazole. However, the selectivity is completely diminished when using *p*-tosyl chloride. [27] Excess *n*-tosyl imidazole was avoided as it often gave a di-tosylate bi-product and lower overall yield.

Scheme 8.

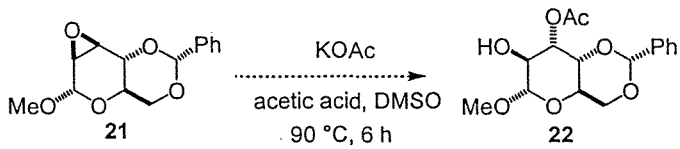


The manno epoxide **21** was next opened regio- and stereoselectively with the sodium salt of benzyl alcohol to yield 3-*O*-benzyl-α-D-altropyranoside **18** (Scheme 8).[28] In this transformation benzyl alcohol also serves as the reaction solvent. Our concerns for this modest yielding step concentrated in the improvement of product isolation. We therefore addressed the current synthetic work-up.

The work-up for this reaction requires heated vacuum distillation of excess benzyl alcohol. As the altropyranoside product **18** is readily soluble in benzyl alcohol, we believed the difficulty in removing this solvent was negatively effecting product yield. It was envisioned that reducing the neat benzyl alcohol to 10 equivalents would be a possible solution. Toluene as well as DMF were each employed as reaction solvents with the use of 10 equivalents of benzyl alcohol. Unfortunately appreciable product did not form under these conditions.

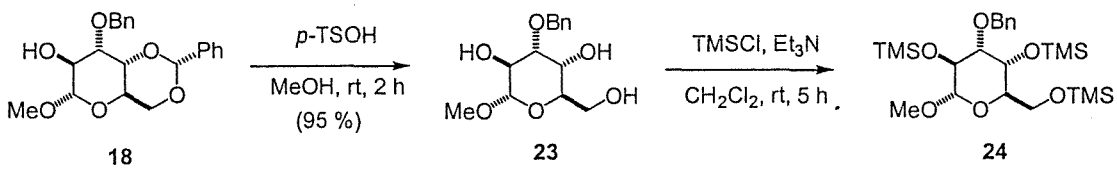
In place of using neat benzyl alcohol, an alternative approach to the opening of the manno epoxide **21** utilized potassium acetate in a solvent medium of acetic acid and DMSO. (Scheme 9).

Scheme 9.



It was envisioned an acetic acid/ DMSO solvent medium could yield **22** and then be removed more easily than benzyl alcohol (Scheme 9). A survey of experiments was undertaken using potassium acetate in ranges of 3 to 10 equivalents. DMF was also employed as a solvent medium. These experiments did not yield desired products.

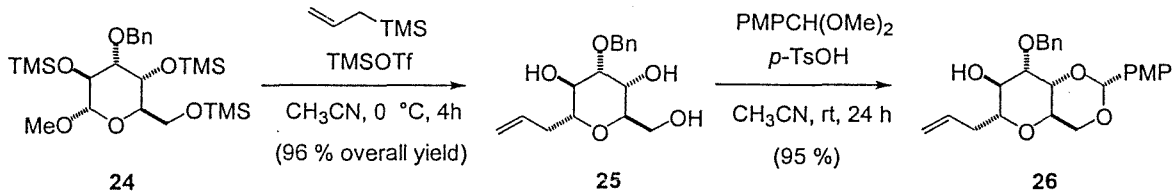
Scheme 10.



Removal of the 4,6-di-*O*-benzylidene acetal of **18** with *p*-TsOH in methanol resulted in formation of the altrose-configured triol species **23**, as in the original procedure (Scheme 10). Over 50 grams of this product was cumulatively produced.

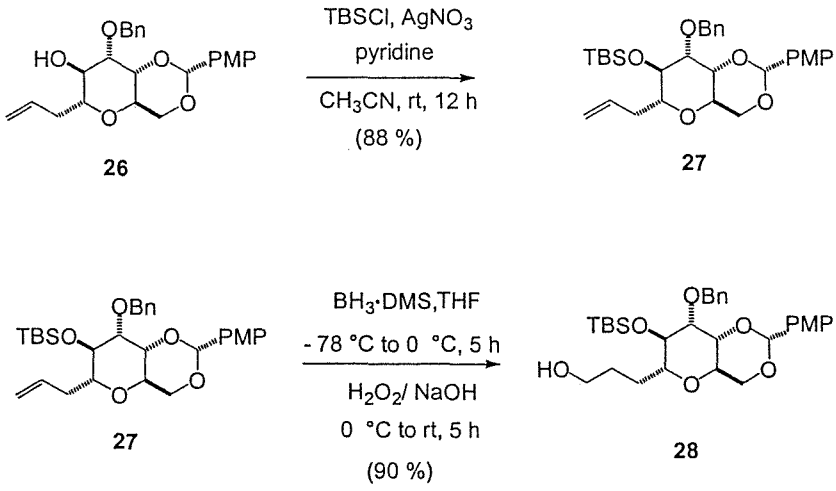
Triol species **23** was then persilylated using TMSCl to yield **24**. It was found that this source of mono-silylating agent was cheaper than the bis-silylating agent *N,O*-bistrimethylsilyl trifluoroacetamide (BSTFA) utilized in the original process,[29] and therefore more practical for a large-scale effort. The crude persilylated product **24** was next subjected to *C*-glycosidation conditions.

Scheme 11.



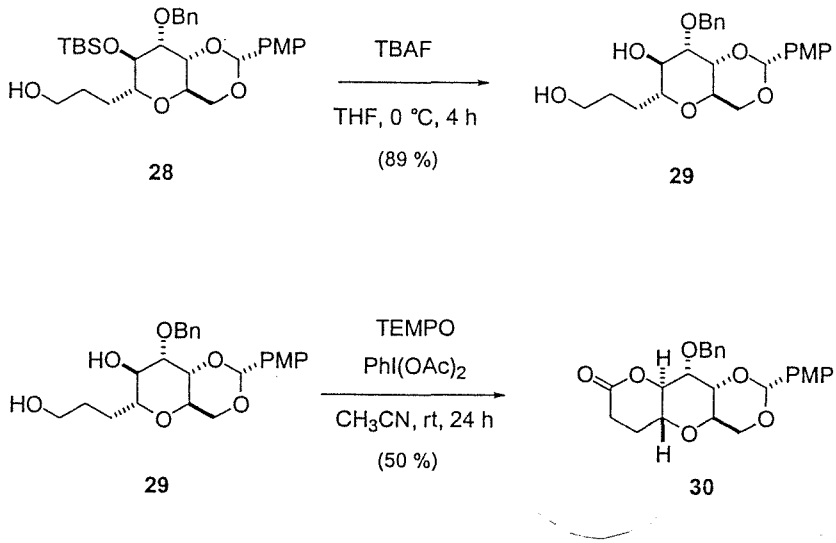
The *C*-glycosidation of persilylated altropyranoside **24** used Gray's one-pot procedure, involving a Sakurai type reaction, to afford product **25** (Scheme 11).[30] The original procedure makes use of 5 equivalents of TMSOTf. Attempts to improve this reaction were undertaken by employing $BF_3{\cdot}OEt_2$ as the Lewis acid source, but have met with little success. However, some economical improvements for this reaction were made by reducing TMSOTf from 5 equivalents to 1.5 equivalents and trimethyl allylsilane from 5 eq. to 2 eq. Following the Sakurai reaction, the 4,6 diol of **25** was reprotected as the 4, 6-*O*-anisylidene dimethyl acetal **26**. Over 40 grams of **26** was successfully produced.

Scheme 12.



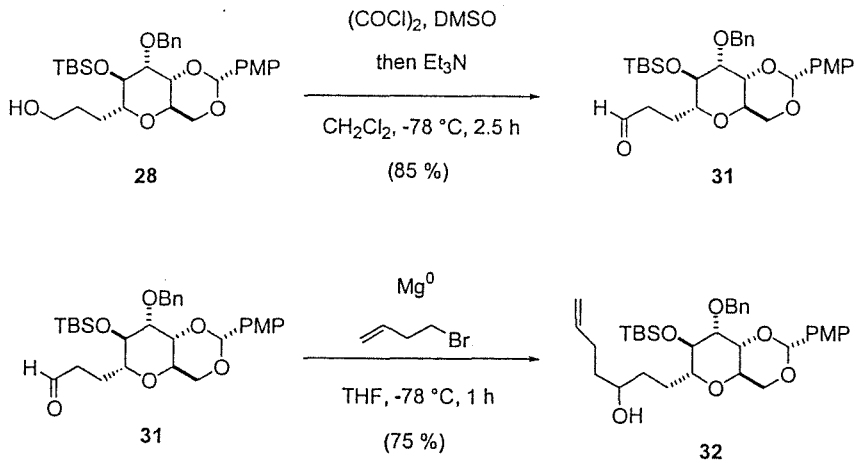
A survey of various silyl protection reactions for the remaining secondary alcohol of **26** was undertaken (Scheme 12), as the original synthesis utilized 5 eq. of the expensive reagent TBSOTf. The initial survey of reactions utilizing a combination of TBSCl and DMAP were unsuccessful at producing significant quantities of the desired secondary protected alcohol. It was finally discovered that the *in situ* formation of $TBSNO_3$ from TBSCl and $AgNO_3$ was effective at formation of the protected alcohol **27**.[31] A hydroboration/ oxidation sequence to form **28** followed. This reaction sequence was also improved. In place of 9-BBN which required a stoichiometric equivalent for every equivalent of alkene, the use of BH_3·DMS only required one third of one equivalent. The regioselectivity of the hydroboration/ oxidation with BH_3·DMS was equal to the original procedure. Over 10 grams each of **27** and **28** remains.

Scheme 13.



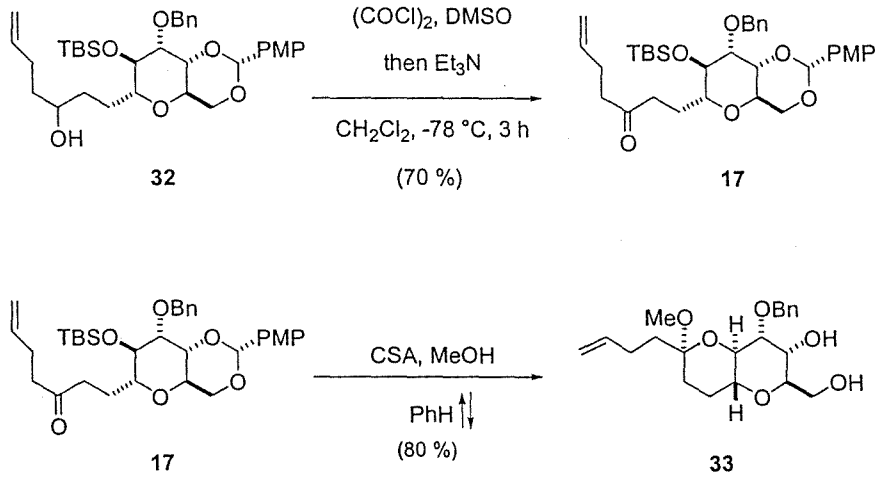
Following its formation, a small amount of the primary alcohol **28** was subjected to TBAF deprotection conditions to produce diol species **29** (Scheme 13). This was in preparation for the investigation of the following oxidation reaction. It was envisioned that an extra oxidation step required in the original synthetic sequence could be eliminated by performing a one-pot double oxidation of diol **29** to lactone **30**, going through a lactol intermediate. Several double oxidation conditions were attempted including the use of pyridinium chlorochromate (PCC),[32] and tetrapropylammonium perruthenate/ *N*-methyl-morphiline *N*-oxide (TPAP-NMO). [33] However the best conditions used a combination of TEMPO and iodobenzene diacetate (BAIB).[34] This reaction was successful on small-scale but was problematic in producing high yields on reaction scales larger than 1 gram. Therefore, synthesis of the lactone intermediate was discontinued in lue of reverting to the original process.

Scheme 14.



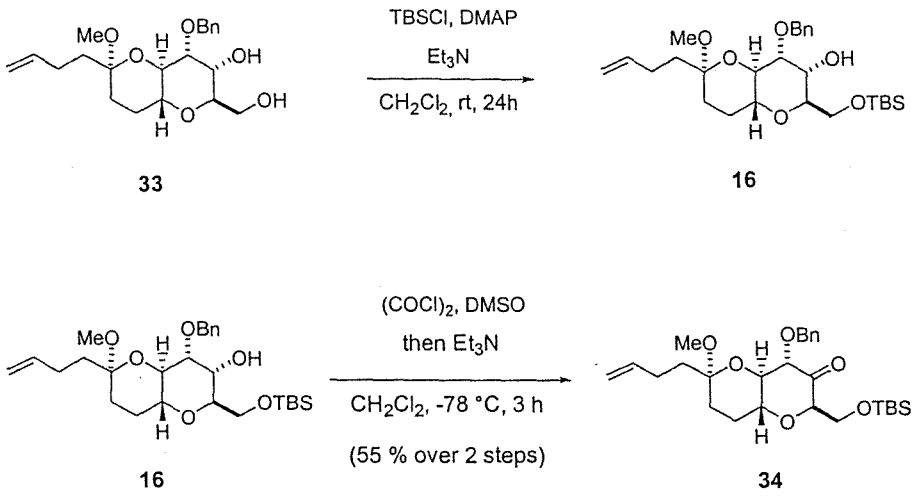
In this vein, the primary alcohol **28** was subsequently oxidized to aldehyde **31** under Swern conditions (Scheme 14).[19] It was found that either a butenyl Grignard species or butenyl lithiated species was equally effective in formation of the 1:1 mixture of diastereomeric secondary alcohols **32**. Over 10 grams of **32** remains for future work.

Scheme 15.



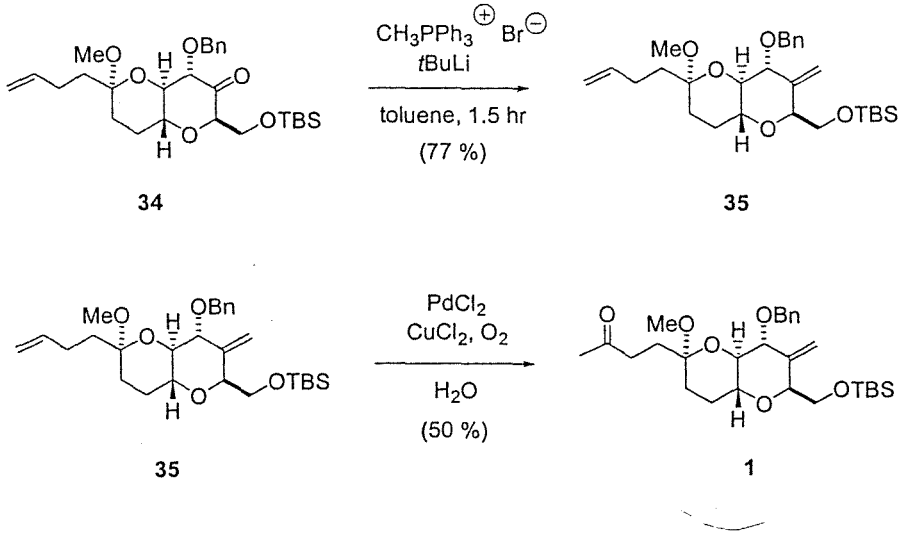
The secondary alcohol of **32** was oxidized to ketone **17** again under Swern conditions (Scheme 15). Then an acid-catalyzed one-pot reaction to form **33** followed. Catalytic amounts of camphorsulfonic acid (CSA) induced the double-deprotection, ring-cyclization and then ketalization events all in one-pot.

Scheme 16.



Selective primary alcohol protection of diol **33** was performed with TBSCl to afford TBS ether **16** (Scheme 16). The remaining secondary alcohol was then oxidized to ketone **34** under Swern conditions. After purification, the overall yield from **33** to **34** was 55 %.

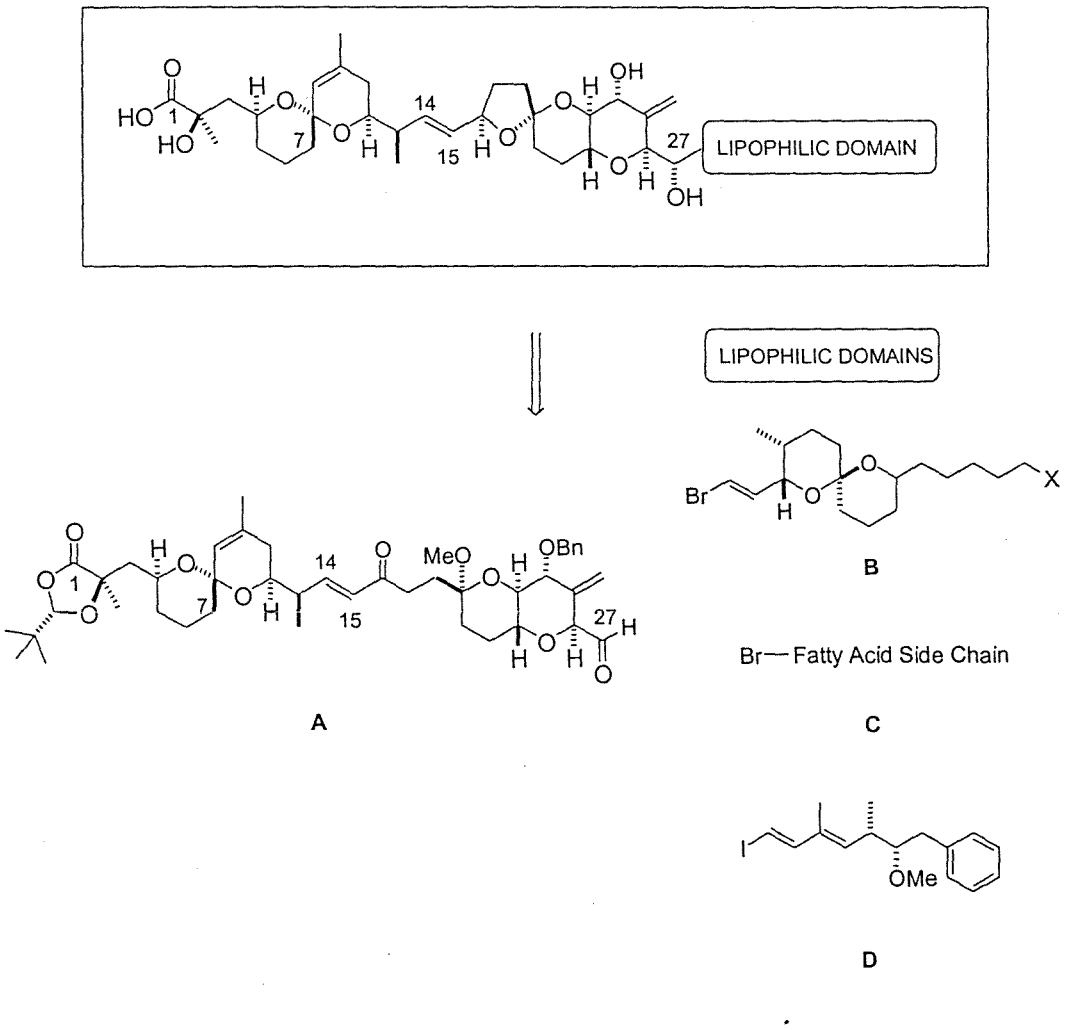
Scheme 17.



Ketone **34** was next subjected to Wittig olefination conditions to provide the sensitive exocyclic alkene **35** (Scheme 17). This di-alkene was then selectively oxidized under standard Wacker conditions to provide what appears to be the methyl ketone **1**. Further spectral evidence is necessary for definitive confirmation of this product. It is envisioned that future scale-up work of advanced intermediates will continue to provide significant quantities of the C15-C27 okadaic acid domain, which will support the creation of novel analogs of okadaic acid.

Future Goals for the Project

Figure 6.



Once significant quantities of both the C1-C15 aldehyde and C16-C27 bis-pyran domains have been synthesized, they will be coupled together. The C27 *O*-silyl ether will be cleaved and oxidized to the C27 aldehyde. One of the goals will be to add variable lipophilic domains to the C27 aldehyde to create novel okadaic acid analogs (Figure 6). Our group has begun to investigate the significance of the lipophilic domain of okadaic acid. Enzymatic assays performed in our group have shown this portion of the molecule to be important for inhibition of

PP1 and PP2A.[9] Moreover, it appears that this liphophilic portion has greater significance for inhibition of PP1 relative to PP2A. The IC_{50} values for the C1-C27 truncated analog **A** (Figure 6) verses okadaic acid shows an 800-fold decrease in activity against PP1, while showing only a 50-fold decrease in activity against PP2A. More work on gaining a clearer picture of the lipophilic domain's significance in binding to the lipophilic groove of either PP1 or PP2A needs to be accomplished.[9]

Lipophilic fragments which will be used to investigate the significance of this domain include a 5 carbon extension of the original intact spiroketal **B** (where X is a leaving group that could be attacked by amino acid residues of PP1 or PP2A, Figure 6). Our group would also like to employ a simple fatty acid side chain **C**. Additionally we would like to try the Adda side chain of microsystin **D**, which is in the okadaic acid class of molecules. Studies have shown that this side chain, a unique 20-carbon β-amir acid, is important in phosphatase inhibition. For instance, hydrogenation or ozonolysis of the alkenoic positions of the Adda side chain of microsystin attenuates its activity.[12] We would like to continue to explore the structural evidence that this side-chain is a peptidomimetic of the hydrophobic sequence (AMLF) of amino acids 36-39 of DARPP.[17]

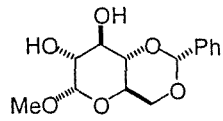
Conclusion

Significant process improvements for many of the steps in the C15-C27 domain have been accomplished. These process changes have been put to test through a large-scale effort. Novel okadaic acid analogs can now be synthesized and tested to create a more empirical set of structure activity relationships, that will help define the okadaic acid pharmacophore. Furthermore, future

analog syntheses will aid in understanding the separate role of both PP1 and PP2A in protein phosphatase regulation.

Experimentals

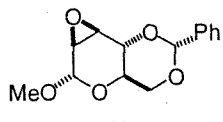
Unless otherwise noted, all experiments were carried out under an argon atmosphere in oven-dried glassware using standard chemical reagent addition techniques including syringe, cannula, and addition funnels. The THF, diethyl ether, and benzene were distilled from Na/benzophenone ketyl under N_2 prior to use. The CH_2Cl_2, CH_3CN, triethylamine, diisopropyl amine, and toluene were distilled from CaH_2 under N_2. Purifications for intermediates **18-21** utilized recrystalization techniques with the solvent systems indicated. All other intermediates were purified using flash chromatography on ICN SiliTech 32-63D 60 Å silica gel or Baker Flash silica gel 60 (40 μm) with the indicated mobile phase systems. Analytical TLC was performed on 0.25 mm EM silica gel 60 F_{254} plates and visualized with UV (254 nm) and anisaldehyde stain (450 mL 95 % EtOH, 25 mL concentrated H_2SO_4, 15 mL of acetic acid, and 25 mL anisaldehyde). All NMR spectra were obtained using 200, 300 and 500 MHz Varian Inova instruments. All NMR spectra were obtained in $CDCl_3$ and referenced to residual $CHCl_3$ at 7.24 (^1H) and 77.0 (^{13}C) ppm. IR spectra were obtained using a Perkin-Elmer 1600 series FTIR. High resolution mass spectrometric (HRMS) data were obtained by the University of Minnesota Mass Spectrometry Laboratory using CI and FAB techniques, which utilized both Finnigan MAT 95 spectrometer and NH_3 and VG 7070E-HF spectrometer respectively.



20

4,6-di-*O*-benzylidene acetal of α-methyl-D-glucopyranoside (20) [26]

To a 0 °C solution of α-methyl-D-glucopyranoside **19** (1000 g, 5.15 mol) in $CHCl_3$ (2.5 L) was added dimethyl acetal of benzaldehyde (745 g, 4.89 mol) and Amberlyst 15 I-E (10 g, 1 mol wt %). The mixture was heated to reflux and an azeotropic distillation took place at 53 °C. The $CHCl_3$ level was continually replenished during the azeotropic distillation. After 6 hours, the reaction was cooled to room temperature, filtered, and concentration to a wet yellow solid. The crude product was dissolved in ethyl acetate (1 L) and triturated with hexanes (5 L) to produce 4,6-di-*O*-benzylidene acetal of α-methyl-D-glucopyranoside **20** as a white solid (712g, 2.52 mol, 60 % yield). R_f 0.5 (100 % ethyl acetate) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45-7.52 (m, 2H), 7.33-7.39 (m, 3H), 5.51 (s, 1H), 4.78 (d, J = 4 Hz, 1H), 4.29 (dd, J = 8.9, 3.4 Hz, 1H), 3.93 (dd, J = 9 Hz, 1H), 3.73-3.82 (m, 2H), 3.64 (ddd, J = 9.1, 4.6, 4.4 Hz, 1H), 3.49-3.59 (m, 1H), 3.46 (s, 3H).

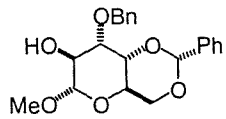


21

Manno epoxide pyran (21) [27]

To a solution of 4,6-di-*O*-benzylidene acetal of α-methyl-D-glucopyranoside **20** (31.0 g, 109 mmol) in 230 ml DMF was added sodium hydride (8.15 g, 340 mmol) at room temperature and under an argon atmosphere. Within a few minutes the reaction thickened to a paste. While

manually slurrying the reaction mixture at 0 °C, a solution of *N*-tosyl imidazole (24.5 g, 110 mmol) in DMF (130 mL) was added dropwise over 40 minutes via addition funnel. The reaction was allowed to warm to rt over 4 hours, at which time the starting material had been consumed. The reaction mixture was slowly decanted into a magnetically stirred ice-water solution (2.5 L), and immediately an off-white precipitate appeared. This was filtered to give a crude product which was washed several times with water (3 x 100 mL) to remove excess DMF. The crude cake was purified by a series of washes with 5 % ethyl acetate in hexanes (3 x 200 mL) to remove ditosylate bi-products and provide manno epoxide **21** (17.4 g, 66 mmol, 60 % yield). R_f 0.6 (20 % ethyl acetate in hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45-7.52 (m, 2H), 7.26-7.42 (m, 3H), 5.57 (s, 1H), 4.90 (s, 1H), 4.26 (d, J = 6.2 Hz, 2H), 3.66-3.77 (m, 2H), 3.48 (d, J = 3.8 Hz, 1H), 3.47 (s, 3H), 3.17 (d, J = 3.6 Hz, 1H).

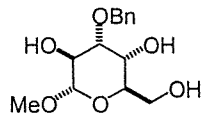


18

3-*O*-benzyl-α-D-altropyranoside (18) [28]

To benzyl alcohol (250 mL) in a 1L 3 neck flask, cooled to 0 °C and equipped with overhead stirring, was added NaH (9.08 g, 378 mmol, 10 eq.). Vigorous bubbling occurred. This was followed by addition of manno epoxide **21** (10 g, 38 mmol, 1 eq.). A heating mantle was used to heat the reaction to a temperature between 110 – 120 °C for 7 h, at which time starting material was consumed. The reaction was cooled to room temperature, followed by the addition of methanol (100 mL) and $NaHCO_3$ (20 mL). Without extractive work-up, the reaction mixture was concentrated under vacuum at 85-90 °C. Benzyl alcohol distillates were collected. Following

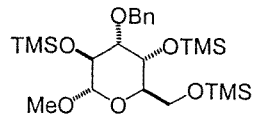
significant removal of benzyl alcohol (ca 95 %), the concentrated solution was cooled and a crude product was afforded as a white solid product was precipitated by titration with hexanes (5-10 ml). Dissolving the crude product in $CHCl_3$ (10 mL) followed by titration with hexanes (10 mL) afforded 3-*O*-benzyl-α-D-altropyranoside **18** as a purified white solid (8.5g, 23 mmol, 60 %). R_f 0.25 (2:1, hexanes: ethyl acetate). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.46-7.52 (m, 2H), 7.25-7.40 (m, 8H), 5.57 (s, 1H), 4.88 (d, J = 12.8 Hz, 1H), 4.75 (d, J = 12.8 Hz, 1H), 4.56 (s, 1H), 4.29-4.48 (m, 2H), 3.97-4.03 (m, 2H), 3.82-3.89 (m, 1H), 3.73 (dd, J = 9 Hz, 1H), 3.43 (s, 3H).



23

Triol (23) [35]

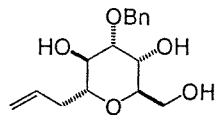
To the 3-*O*-benzyl-α-D-altropyranoside **18** (8.30 g, 16.7 mmol) in methanol (18 mL) at rt was added *p*-TsOH (212 mg, 0.13 mmol). After 1 hour, Et_3N (0.2 mL) was added, and the mixture was concentrated to an oil. The crude triol **23** was purified via flash column chromatography (1:1 diethyl ether: hexanes to 50 % ethyl acetate in hexanes) to afford a clear oil (6.00 g, 21 mmol, 95 % yield). R_f 0.25 (100 % EtOAc). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.25-7.39 (m, 5H), 4.79 (d, J = 11.2 Hz, 1H), 4.64 (d, J = 1.2 Hz, 1H), 4.53 (d, J = 11.2 Hz, 1H), 3.93-4.02 (m, 1H), 3.75-3.97 (m, 5H), 3.42 (d, J = 1.4 Hz, 1H), 3.41 (s, 3H), 2.70 (d, J = 5.6 Hz, 1H), 2.45 (bs, 1H).



24

Trisilyl ether (24)

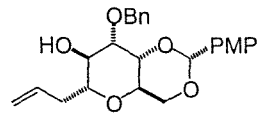
To the triol **23** (20.43 g, 71.86 mmol) in CH_2Cl_2 (500 mL) at rt was added Et_3N (60.1 mL, 431.2 mmol) followed by TMSCl (54.7 mL, 431.2 mmol). After 12 hours, $NaHCO_3$ (150 mL) was added. The separated aqueous phase was extracted with CH_2Cl_2 (3 x 200 mL), and the combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, and concentrated to an oil. The crude trisilyl ether **24** was used in the next step without further purification. R_f 0.6 (20 % ethyl acetate in hexanes).



25

***C*-glycoside triol (25)**

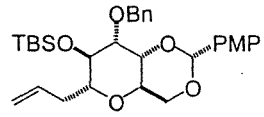
To the persylilated pyran **24** (25.47 g, 50.9 mmol, 1 eq) in acetonitrile (20 mL) was added at 0 °C allyl trimethlysilane (8.72 g, 76.3 mmol) followed by TMSOTf (16.96 g, 76.3 mmol). The reaction was complete after 2.5 hours and $NaHCO_3$ (30 mL) followed by ethyl acetate (100 mL) was added. The separated aqueous layer was extracted with ethyl acetate (3 x 75 mL) and the combined organic washed with brine (50 mL), dried over Na_2SO_4, and concentrated to an oil. The crude *C*-glycoside triol **25** was used in the next step without further purification. R_f 0.4 (100 % ethyl acetate).



26

4,6-di-*O*-*p*-anisylidene *C*-glycoside (26) [36]

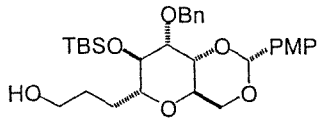
To the *C*-glycoside triol **25** (35g, 0.12 mol) in acetonitrile (250 mL) at room temperature was added *p*-TsOH (340 mg, 1.78 mmol) and benzaldehyde dimethyl acetal (39 g, 0.21 mol). After 10 h Et_3N (0.4 mL), ethyl acetate (200 mL), and $NaHCO_3$ (150 mL) were added. The separated aqueous layer was extracted with ethyl acetate (3 x 200 mL) and the combined organic layers washed with $NaHCO_3$ (100 mL) followed by brine (100 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified via flash column chromatography to afford the 4,6-di-*O*-*p*-anisylidene *C*-glyocside **26** (30 % ethyl acetate in hexanes) as a light tan solid (47.2 g, 0.11 mol, 95 % yield). R_f 0.20 (30 % ethyl acetate in hexanes). ^1H NMR δ 7.51-7.55 (m, 2H), 7.32-7.42 (m, 5H), 6.95 (d, J = 9 Hz, 2H), 5.81 (m, 1H), 5.60 (s, 1H), 5.17 (m, 2 H), 4.94 (d, J = 12.5 Hz, 1H), 4.66 (d, J = 12 Hz, 1H), 4.29 (dd, J = 10, 5.5 Hz, 1H), 4.22 (ddd J = 10, 10, 5 Hz, 1H), 4.06 (dd, J = 16, 4 Hz, 1H), 3.95 (dd, J = 12, 10.5 Hz, 2H) 3.85 (m, 4H) 3.75 (t, J = 10.5 Hz, 1H) 2.88 (ddd, J = Hz, 1H) 2.62 (ddd, J = Hz, 1H), 2.36 (bs, 1H), 1.94 (s, 1H).



27

TBS ether *C*-glycoside (27) [37]

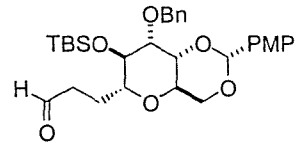
To a solution of 4,6-*O*-anisylidene *C*-glycoside triol **26** (16.03 g, 38.76 mmol), in DMF (160 mL) at 0 °C was added pyridine (14.1 mL, 174 mmol) followed by $AgNO_3$ (16.46 g, 96.90 mmol) and then TBSCl (14.61 g, 96.90 mmol). After 7 hours the reaction was complete and $NaHCO_3$ (150 mL) along with CH_2Cl_2 (150 mL) were added. The separated aqueous layer was extracted with CH_2Cl_2 (2 x 150 mL) and the combined organic layers washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated to an oil. The crude product was purified via flash column chromatography (30 % ethyl acetate in hexanes) to afford TBS ether *C*-glycoside **27** as a clear oil (17.9 g, 34.0 mmol, 88 % yield). R_f 0.75 (20 % ethyl acetate in hexanes). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.45 (d, J = 7.5 Hz, 2H), 7.27-7.39 (m, 5H), 6.91 (d, J = 7.5 Hz, 2H), 5.77-5.80 (m, 1H), 5.56 (s, 1H), 5.09-5.13 (m, 2H), 4.91 (d, J = 12 Hz, 1H), 4.63 (d, J = 12 Hz, 1H), 4.27 (dd, J = 10.25, 4.5 Hz, 1H), 4.16 (ddd, J = 11, 7.75, 5.5 Hz, 1H), 4.03 (dd, J = 9.25, 1.5 Hz, 1H), 3.86 (d, J = 3 Hz, 1H), 3.82 (s, 3H), 3.77 (m, 1H), 3.74 (dd, J = 10, 7 Hz, 2H), 2.77 (dddd, J = 14.5, 7.5, 7.5, 1H), 2.65 (ddd, J = 14.40, 8, 7.75 Hz, 1H), 0.88 (s, 9H), -0.01 (s, 3H), -0.04 (s, 3H).



28

Primary alcohol (28) [38]

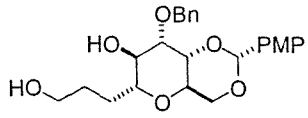
To a solution of the alkene **27** (4.82 g, 9.15 mmol, 1 eq) in THF (12.3 mL) stirring at -78 °C, was added via syringe a 1.2 M THF solution of BH_3•DMS (0.35 mL, 3.66 mmol, 0.8 eq). After 2 hours the reaction was warmed to 0 °C, and then after 3 hours a solution of H_2O_2 (3.9 mL, 25.6 mL) and NaOH (1.77 g, 29.3 mmol) was slowly added. Some moderate foaming occurred upon initial addition. After an additional 1 h of stirring at 0 °C, $NaHCO_3$ (15 mL) followed by ethyl acetate (25 ml) was added. The crude product was purified via flash column chromatography (20 % ethyl acetate in hexanes) to afford alcohol **28** as a clear oil (4.0 g, 7.3 mmol, 80 % yield). R_f 0.3 (50 % ethyl acetate in hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45 (d, J = 6 Hz, 2H), 7.32-7.37 (m, 5H), 6.92 (d, J = 6 Hz, 2H), 5.57 (s, 1H), 4.91 (d, J = 12.3 Hz, 1H), 4.63 (d, J = 12.3 Hz, 1H), 4.25 (dd, J = 10.5, 5.5 Hz, 1H), 4.17 (ddd, J = 10, 9.8, 5.5 Hz, 1H), 4.05 (dd, J = 8, 5 Hz, 1H), 3.83-3.78 (m, 1H), 3.83 (s, 3H), 3.75-3.70 (m, 2H), 3.68-3.60 (m, 2H), 3.48 (dd, J = 12, 5.5 Hz, 1H), 2.30 (m, 1H), 1.64-1.70 (m, 2H), 1.45-1.40 (m, 2H), 0.87 (s, 9H), 0.03 (s, 3H), -0.02 (s, 3H).



31

Aldehyde (31) [39]

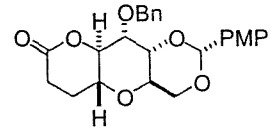
To a – 78 °C solution of $(COCl)_2$ (1.60 mL, 2.33 g, 18.4 mmol) in CH_2Cl_2 (27.0 mL) was added a solution of DMSO (2.6 mL, 2.87 g, 36.7 mmol) in CH_2Cl_2 (5.2 mL). After 15 min. of stirring, a solution of the alcohol **28** (5.0 g, 9.2 mmol) in CH_2Cl_2 (10 mL) was added slowly via syringe. The resulting reaction mixture was stirred for 1 h at – 78 °C before the addition of Et_3N (8.37 mL, 6.08 g, 59.7 mmol). After 30 min., the reaction was allowed to warm to 0 °C and stirred for an additional 1 h. At this time CH_2Cl_2 (50 mL) and $NaHCO_3$ (50 mL) were added to the reaction. The separated aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL) and the combined organic layers washed with brine (50 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified using flash column chromatography (25 % ethyl acetate in hexanes) to afford aldehyde **31** as a clear oil (1.61 g, 2.9 mmol, 85 % yield). R_f 0.8 (40 % ethyl acetate in hexanes). ^1H NMR ($CDCl_3$, 300 MHZ), δ 9.82 (s, 1H), 7.45 (dd, J = 6.45, 2.1 Hz, 2H), 7.27-7.37 (m, 5H), 6.92 (dd, J = 6.6, 1.8 Hz, 2H), 5.56 (s, 1H), 4.92 (d, J = 12.3 Hz, 1H), 4.63 (d, J = 12.3 Hz, 1H), 4.23 (dd, J = 10.2, 4.8 Hz, 1H), 4.05-4.15 (m, 2H), 3.82 (s, 3H), 3.62-3.81 (m, 4H), 2.60-2.75 (m, 1H), 2.52-2.57 (m, 2H), 1.75 (m, 1H), 0.88 (s, 9H), 0.01 (s, 3H), -0.39 (s, 3H).



29

Diol (29)

To a solution of the alcohol **27** (1.84 g, 3.39 mmol) in THF (9.2 mL) at 0 °C was added a 1 M solution of TBAF (10.2 mL, 10.16 mmol). After 1 hour at room temperature K_2CO_3 (0.20 g) and H_2O (10 mL), followed by $NaHCO_3$ (10 mL) and diethyl ether (20 mL) were added. The separated aqueous layer was extracted with diethyl ether (2 x 25 mL) and the combined organic layers washed with brine (20 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified using flash column chromatography (40 % ethyl acetate in hexanes to 100 % ethyl acetate in hexanes) to afford diol **29** as a clear oil (1.05g, 2.4 mmol, 72 % yield). R_f 0.3 (100 % ethyl acetate). $[\alpha]^{25} = +19.6$ (c2.25, $CHCl_3$). FTIR (thin film, cm^{-1}) 3401, 2954, 2926, 2871, 1615, 1589, 1518, 1463, 1455, 1382, 1303, 1251, 1173, 1124, 1098, 1070, 1054, 1029, 832. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.28-7.44 (m, 7H), 6.90 (d, J = 8.1 Hz, 2H), 5.52 (s, 1H), 4.91 (d, J = 12 Hz, 1H), 4.64 (12.3 Hz, 1H), 4.00-4.27 (m, 4H), 3.63-3.93 (m, 7H), 3.81 (s, 3H), 2.29-2.38 (m, 3H), 1.53-1.70 (m, 4H). ^{13}C (75 MHz) δ 160.1, 138.7, 130.3, 128.3, 127.5, 127.4, 113.6, 102.3, 79.5, 73.6, 71.3, 69.6, 62.3, 60.1, 55.3, 31.0, 29.7, 29.6, 25.7, 25.6. EI HRMS *m/z* calcd for $[(C_{24}H_{30}O_7) + H]^+$: 431.20; Found 431.2062.

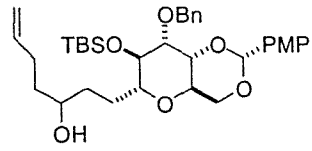


30

Lactone (30)

To a solution of diol **29** (68 mg, 0.16 mmol) in CH_3CN (0.30 mL) at 0 °C was added iodobenzene diacetate (BAIB) (152 mg, 0.473 mmol) followed by TEMPO (4.92 mg, 0.031

mmol). The reaction was allowed to warm to room temperature, crushed 4Å molecular sieves were added (7 mg, 10 wt %), and continual stirring occurred for an additional 24 hours. To the reaction mixture was added $NaHCO_3$ (0.5 mL) and ethyl acetate (2 mL). The separated aqueous later was extracted with ethyl acetate (2 x 2 mL)and the combined organic layers washed with brine (1 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified via flash column chromatography (10 % ethyl acetate in hexanes to 50 % ethyl acetate in hexanes) to afford lactone **30** as a clear oil (33 mg, 0.077 mmol, 49 % yield). R_f 0.7 (60 % ethyl acetate in hexanes). $[\alpha]^{25}$ = -24.3 (c 0.90, $CHCl_3$). FTIR (cm^{-1}) 3425.3, 2936.6, 1708.5, 1614.6, 1518.4, 1452.7, 1371.1, 1251.6, 1173.3, 1103.5, 1027.8, 831.9. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.27-7.46 (m, 7H), 6.92 (d, *J* = 7.5 Hz, 2H), 5.52 (s, 1 H), 4.95 (d, *J* = 11.4 Hz, 1H), 4.72 (d, *J* = 11.4 Hz, 1H), 4.52 (dd, *J* = 11.4, 4.2 Hz, 1H), 4.36 (dd, *J* = 10.4, 5.1 Hz, 1H), 4.29 (dd, *J* = 10.5, 5.1 Hz, 1H), 4.13 -4.23 (m, 2H), 3.89-3.96 (m, 1H), 3.83 (s, 3H), 3.69 (dd, *J* = 10 Hz, 1H), 2.75 (ddd, 1H), 2.59 (dd, *J* = 17.1, 8 Hz, 1H), 2.16-2.20 (m, 1H), 1.80-1.88 (m, 1H). ^{13}C (75 MHz) δ 169.7, 160.2, 138.0, 129.7, 128.4, 128.0, 127.8, 127.6, 113.7, 101.8, 83.2, 76.4, 76.0, 74.1, 70.2, 65.6, 62.9, 55.3, 27.5, 25.8. EI HRMS *m/z* calcd for $[(C_{24}H_{26}O_7) + Na]^+$: 449.16; Found 449.1557.

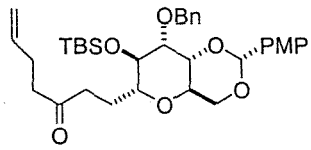


32

Secondary alcohol (32)

To a solution of butenyl bromide (770 mg, 0.579 mL) in diethyl ether (28 mL) at -78 °C was added a 1.9 M solution of *t*-BuLi in hexanes (5.81 mL, 11.05 mmol). After stirring this mixture

for 30 minutes it turned light yellow. A solution of aldehyde **31** (774 mg, 1.43 mmol, 1 eq) in diethyl ether (28 mL) at -78 °C was added drop-wise via cannula. The reaction mixture was allowed to stir for an additional 1.5 hours before $NaHCO_3$ (15 mL) and diethyl ether (50 mL) were added. The separated aqueous layer was extracted with diethyl ether (2 x 50 mL) and the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified using flash column chromatography (10 % ethyl acetate in hexanes to 25 % ethyl acetate in hexanes) to afford alcohol **32** as a clear oil as a mixture of alcohols (0.588 g, 0.98 mmol, 69 % yield). R_f 0.6 (30 % ethyl acetate in hexanes). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.44 (d, J = 9Hz, 2H), 7.35 (dd, J = 16.75, 11, 4H), 7.27 (dd, J = 15.75, 7 Hz, 2H), 6.90 (dd, J = Hz, 2H), 6.81- 6.84 (m, 1H), 5.55 (s, 1H), 5.05 (d, J = 17 Hz, 1H), 4.98 (d, J = 10 Hz, 1H), 4.90 (dd, J = 6.5 Hz, 1H), 4.61 (dd, J = 12.25, 5 Hz, 1H), 4.25 (dd, J = 10, 5 Hz, 1H), 4.13 (m, 1H), 4.03 (d, J = 9 Hz, 1H), 3.63- 3.82 (m, 7H), 2.05-2.21 (m, 2 H), 1.53-1.69 (m, 6 H), 1.42-1.45 (m, 1H), 1.25 (dd, J = 16.25, 10.5 Hz, 1H), 0.89 (s, 9 H), -0.05 (s, 3H), -0.06 (s, 3H).

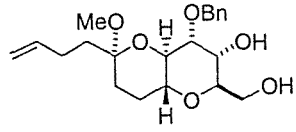


17

Ketone (17) [40]

To a – 78 °C solution of $(COCl)_2$ (0.64 mL, 92.6 mg, 0.729 mmol) in CH_2Cl_2 (1.5 mL) was added a solution of DMSO (0.10 mL, 110 mg, 1.5 mmol) in CH_2Cl_2 (0.27 mL). After 15 min. of stirring, a solution of the secondary alcohol **32** (291 mg, 0.486 mmol) in CH_2Cl_2 (0.6 mL) was added slowly via syringe. The resulting reaction mixture was stirred for 1 h at – 78 °C before the

addition of Et_3N (0.446 mL, 322 mg, 3.16 mmol). After 30 minutes, the reaction was allowed to warm to 0 °C and stirred for an additional hour. At this time CH_2Cl_2 (5 mL) and $NaHCO_3$ (5 mL) was added to the reaction. The separated aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL) and the combined organic layers washed with brine (5 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified using flash column chromatography (25 % ethyl acetate in hexanes) to afford ketone **17** as a clear oil (1.61 g, 2.68 mmol, 70 % yield). R_f 0.75 (50 % diethyl ether in hexanes). ^1H NMR ($CDCl_3$, 300 MHz). δ 7.25-7.47 (m, 7H), 6.90 (d, J = 3.2 Hz, 2H), 5.76-5.84 (m, 1H), 5.54 (s, 1H), 5.03 (ddd, J = 17.5, 3.0,1,5 Hz, 1H), 4.98 (d, J = 10.5 Hz, 1H), 4.89 (d, J = 12.5 Hz, 1H), 4.61 (d, J = 12 Hz, 1H), 4.21 (dd, J = 10, 5 Hz, 1H), 4.06-4.11 (m, 1H) 4.01 (dd, J = 10, 2.5 Hz, 1H) 3.82 (s, 3H), 3.77 (dd, J = 16.5, 3.5 Hz, 2H), 3.71 (d, J = 10.5 Hz, 1H), 3.64 (d, J = 11 Hz, 1H), 2.44-2.60 (m, 4H), 2.30-2.34 (m, 2H), 1.74-1.79 (m, 1H), (0.86 (s, 9H), -0.00 (s, 3H), -0.04 (s, 3H).

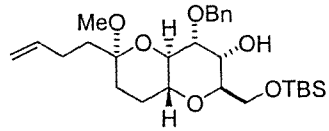


33

Bis-pyran (33)

To a solution of ketone **17** (39 mg, 0.065 mmol) in a 2:1 mixture of benzene:methanol (1, 0.5 mL respectively) was added camphor sulfonic acid (3 mg, 0.013 mmol). The mixture was heated to reflux (65 °C). After 5 h the mixture was cooled to rt and $NaHCO_3$ (1 mL) followed by ethyl acetate (2 mL) were added. The separated aqueous layer was extracted with ethyl acetate (2 x 2 mL) and the combined organic layers washed with brine (1 mL), dried over Na_2SO_4, filtered and concentrated to an oil. The crude product was purified using flash column chromatography (65 %

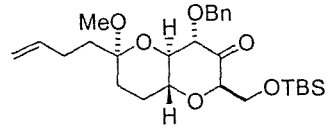
ethyl acetate in hexanes) to afford bis-pyran **33** as a clear oil (20 mg, 0.053 mmol, 60% yield). R_f 0.25 (80 % ethyl acetate in hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27-7.37 (m, 5H) 5.82-5.89 (m, 1H) 5.06 (d, *J* = 17 Hz, 1H) 4.99 (d, *J* = 10.5 Hz, 1H) 4.88 (d, *J* = 11.5 Hz, 1H) 4.73 (d, J = 12.5 Hz, 1H) 4.12 (m, 1H) 3.97 (s, 1H) 3.23 (s, 3H) 2.82 (s, 1H) 1.92-2.10 (m, 3H) 1.85-1.90 (m, 4H) 1.79-1.80 (m, 1H) 1.53-1.62 (m, 3H).



16

Alcohol (16) [41]

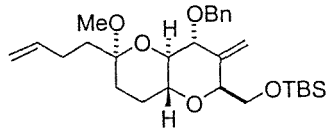
To a solution of bis-pyran **33** (34 mg, 0.071 mmol) in CH_2Cl_2 (0.5 mL) was added Et_3N (0.06 mL, 0.42 mmol) followed by TBSCl (31 mg, 0.21 mmol) and finally *N*, *N*-dimethyl amino pyridine (DMAP) (1 mg, 1 mol %) at room temperature. The reaction was run for 24 hours and then aqueous $NaHCO_3$ (0.5 mL) and CH_2Cl_2 (1 mL) was added. The separated aqueous layer was extracted with CH_2Cl_2 (2 x 1 mL) and the combined organic layers washed with brine (0.5 mL), dried over Na_2SO_4, and concentrated to an oil. The crude alcohol **16** was taken forward to the next step without further purification. R_f 0.5 (60 % ethyl acetate in hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31-7.44 (m, 5H), 5.84-5.97 (m, 1H), 5.02-5.14 (m, 2H), 4.90 (d, *J* = 12 Hz, 1H), 4.80 (d, J = 12 Hz, 1H), 4.21 (d, *J* = 1.8 Hz, 1H) 4.024 (dd, *J* = 0.9, 4.8 Hz, 1H), 3.81-3.94 (m, 4H), 3.56 (ddd, *J* = 15.68, 10.05, 5.4 Hz, 1H), 3.28 (s, 3H), 2.84 (bs, 1H), 2.11 (dd, J = 15.9, 7.5 Hz, 2H), 1.80-1.99 (m, 4H), 1.54-1.69 (m, 2H), 0.91 (s, 9H), 0.06 (s, 3H), -0.03 (s, 3H).



33

Ketone (33) [42]

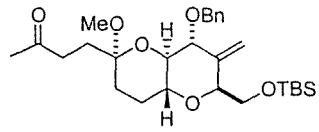
To a – 78 °C solution of $(COCl)_2$ (13 mg, 0.01 mL) in CH_2Cl_2 (0.2 mL) was added a solution of DMSO (15 mg, 0.015 mL, 0.20 mmol) in CH_2Cl_2 (0.05 mL). After 15 min. of stirring, a solution of alcohol **16** (40 mg, 0.067 mmol) in CH_2Cl_2 (0.1 mL) was added slowly via syringe. The resulting reaction mixture was stirred for 1 hour at – 78 °C before the addition of Et_3N (44 mg, 0.06 mL, 0.44 mmol, 6.5 eq). After 30 minutes, the reaction was allowed to warm to 0 °C and stirred for an additional h. At this time CH_2Cl_2 and $NaHCO_3$ were added to the reaction. The separated aqueous layer was extracted with CH_2Cl_2 and the combined organic layers washed with $NaHCO_3$ followed by brine, dried over Na_2SO_4, and concentrated to an oil. The crude product was purified using flash column chromatography (25 % ethyl acetate in hexanes) to afford ketone **33** as a clear oil (20 mg, 1.33 mmol, 55 % yield over two steps). R_f 0.5 (20 % ethyl acetate in hexanes). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.49 (d, J = 7 Hz, 2H), 7.28-7.37 (m, 3H), 5.87-5.96 (m, 1H), 5.03-5.14 (m, 2H) 5.12 (d, J = 14.7 Hz, 1H), 4.86 (d, J = 12.6 Hz, 1H), 4.16-4.27 (m, 1H), 4.15 (dd, J = 10.5, 10.5 Hz, 2H), 4.01 (s, 2H) 3.79 (dd, J = 10.2, 10.2 Hz, 1H), 3.28 (s, 3H), 2.11 (dd, J = 14.7, 8 Hz, 2H), 1.83-2.00 (m, 4H), 1.56-1.72 (m, 2H), 0.86 (s, 9H), 0.08 (s, 3H), -0.03 (s, 3H).



34

Di-alkene (34) [43]

To a rt solution of methyltriphenylphosphonium bromide (46 mg, 0.13 mmol) in toluene (0.88 mL) under argon was added potassium bis(trimethylsilyl)amide (0.5M, 0.20 mL). The yellow mixture was heated to 80-90 °C, and then the resulting solution was cooled to rt before the addition of the ketone **33** (25 mg, 0.051 mmol) in toluene was added via syringe. The resulting mixture was heated for 1.5 h at 80-90 °C, then cooled to rt before sodium bicarbonate (2mL) and diethyl ether (2 mL) were added. The separated aqueous layer was extracted with diethyl ether (3 mL) and the combined organic layers washed with brine (1 mL), dried over Na_2SO_4, and concentrated to an oil. The crude product was purified using flash column chromatography (8 % ethyl acetate in hexanes) to afford bis-alkene **34** as a clear oil (19 mg, 0.061 mmol, 77 % yield). R_f 0.5 (15 % ethyl acetate in hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26-7.42 (m, 5H), 5.79-5.90 (m, 1H), 5.43 (s, 1H) 5.06 (s, 1H) 4.97-5.03 (m, 2H), 4.91 (d, J = 12 Hz, 1H), 4.80 (d, J = 12 Hz, 1H), 4.30 (dd, J = 4.8 Hz, 4.8 Hz, 1H), 4.20 (d, J = 9 Hz, 1H), 3.77-3.88 (m, 2H), 3.63-3.71 (m, 1H), 3.41 (dd, J = 9.6 Hz, 1H), 3.22 (s, 3H), 2.05-2.08 (m, 2H), 1.80-1.92 (m, 4H), 1.54-1.61(m, 2H), 0.88(s, 9H), 0.03 (s, 3H) 0.02 (s, 3H).



1

Methyl ketone (1)

To a room temperature solution of DMF/ H_2O (7:1 v/v ratio, 0.02 mL) was added CuCl (0.8 mg, 0.008 mmol) followed by $PdCl_2$ (0.145 mg, 0.0008 mmol) followed by olefin **34** (2 mg, 50 % yield) in 0.1 mL DMF. The reaction was run under an O_2 atmosphere and was worked up after 6 hours. The crude product was purified using flash column chromatography to afford methyl ketone **1** (1 mg, 0.002 mmol, 45 % yield) (20 % ethyl acetate in hexanes). R_f 0.25 (15 % ethyl acetate in hexanes). FTIR (cm^{-1}) 2957, 2924, 2857, 2357, 2338, 1713, 1629, 1463, 1359, 1260, 1127, 1090, 1033, 839. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.32-7.46 (m, 5H), 5.48 (s, 1H), 5.12 (s, 1H), 4.93 (d, J = 12 Hz, 1H), 4.84 (d, J = 12 Hz, 1H), 4.33 (dd, J = 5.1 Hz, 1H) 4.26 (d, J = 9.6 Hz, 1H), 3.83 (dd, J = 5.4, 2.1 Hz, 2H), 3.70- 3.78 (m, 2H), 3.44 (dd, J = 9.6 Hz, 2H), 3.25 (s, 3H), 2.22 (s, 3H), 1.94-2.14 (m, 2H), 1.83-1.94 (m, 2H), 1.53-1.61 (m, 2H), 0.93 (s, 9H), 0.03 (s, 3H) 0.02 (s, 3H). EI HRMS *m/z* calcd for $[(C_{28}H_{44}O_6Si) + Na]^+$: 527.2805; Found 527.2798.

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36 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 61.

37 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 63.

38 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 64.

39 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 65.

40 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 116.

41 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 118.

Literature Cited

42 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 119.

43 Urbanek, R. A. (1998). *An Efficient Synthesis of Okadaic Acid. Synthesis of the C16-C27 Central Core of the Natural Product to Study Protein Phosphatase Regulation.* Ph.D. Thesis. University of Minnesota, U. S. A., 120.

1-jna-108-1

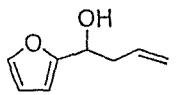
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Jul. 13, 2000
Solvent: CDCl3
File: 000713v2_0801
Starting Time: 11:51:11
Completion Time: 11:58:37
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1209122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



52

1-jha-24-1

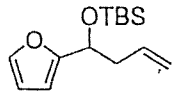
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Jan. 16, 2000
Solvent: CDCl3
File: 000118v2_0101
Starting Time: 15:58:07
Completion Time: 16:06:52
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.998 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1208122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



53

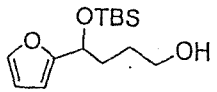
1-jna-114-1

University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul
User: cfojna
Date: Jul. 20, 2000
Solvent: CDCl3
File: 000720v2_0201
Starting Time: 09:53:16
Completion Time: 10:01:24
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1209122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



54

1-jna-54-5

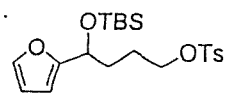
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Mar. 16, 2000
Solvent: CDCl3
File: 000318v2_3101
Starting Time: 12:37:58
Completion Time: 12:47:47
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.898 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1208122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



55

1-jna-117-1

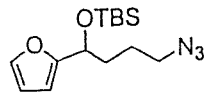
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Jul. 24, 2000
Solvent: CDCl3
File: 000724v2_2801
Starting Time: 15:42:38
Completion Time: 15:50:38
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.988 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1201122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



56

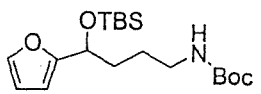
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: May. 31, 2000
Solvent: CDCl3
File: 000531v2_2401
Starting Time: 17:55:35
Completion Time: 18:03:47
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1209122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



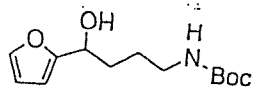
49

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
User: cfojna
Date: May. 25, 2000
Solvent: CDCl3
File: jna-66-2p
Starting Time: 18:56:22
Completion Time: 19:00:15
Total acq. time 3 minutes

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 6003.3 Hz
64 repetitions
OBSERVE H1, 300.1883385 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



58

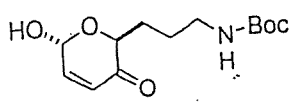
STANDARD 1H OBSERVE

Pulse Sequence: s2pul

User: cfojna
Date: May. 19, 2000
Solvent: CDCl3
File: 1-jna-68-1h
Starting Time: 17:18:45
Total acq. time 3 minutes

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.998 sec
Width 6003.3 Hz
64 repetitions
OBSERVE H1, 300.1663396 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



60

1-jna-105 32-38

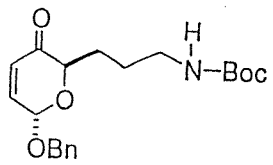
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Jul. 11, 2000
Solvent: CDCl3
File: D00711v2_1201
Starting Time: 13:54:24
Completion Time: 14:02:41
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 30.0 degrees
Acq. time 1.858 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1208122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



68

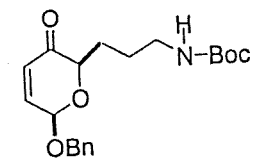
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Sep. 9, 2000
Solvent: CDCl3
File: 000909v2_0101
Starting Time: 13:02:23
Completion Time: 13:10:49
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1209122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



89

1-jna-72-2a

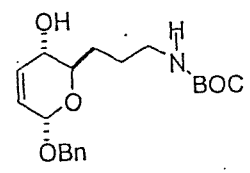
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

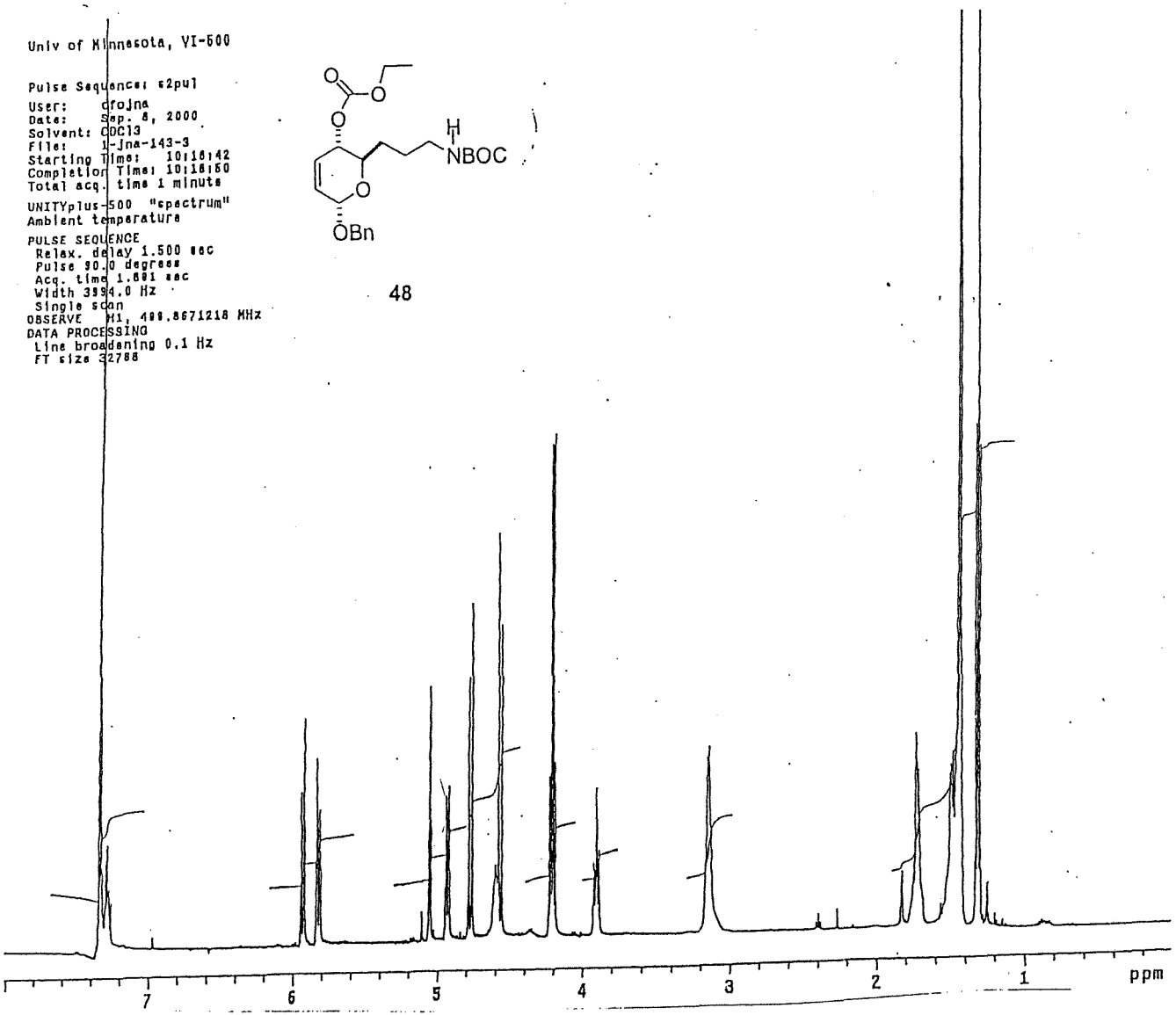
User: cfojna
Date: May. 25, 2000
Solvent: CDCl3
File: 000525v2_2001
Starting Time: 16:54:45
Completion Time: 17:02:57
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1209122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768

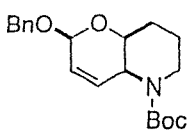


69


Univ of Minnesota, VI-500
Pulse Sequence: s2pul
User: drojna
Date: Sep. 8, 2000
Solvent: CDCl3
File: 1-jna-143-3
Starting Time: 10:16:42
Completion Time: 10:18:50
Total acq. time 1 minute
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.881 sec
Width 3994.0 Hz
Single scan
OBSERVE H1, 499.8671218 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
O
O
O
H
NBOC
O
OBn
48
7
6
5
4
3
2
1
ppm

Univ of Minnesota, VI-500

Pulse Sequence: s2pul
User: cfojna
Date: Nov. 30, 2000
Solvent: CDCl3
File: 1-jna-178-2H
Starting Time: 22:18:08
Completion Time: 22:23:07
Total acq. time 3 minutes
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.882 sec
Width 8000.0 Hz
64 repetitions
OBSERVE H1, 499.8671218 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



77

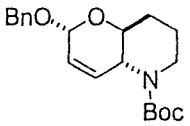
University of Minnesota
Department of Chemistry
VAC-300

Pulse Sequence: s2pul

User: cfojna
Date: Nov. 10, 2000
Solvent: CDCl3
File: 001110v3_2602
Starting Time: 19:05:48
Completion Time: 19:20:34
Total acq. time 7 minutes

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 5998.8 Hz
128 repetitions
OBSERVE H1, 299.9533609 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 65536



46

1-jna-8-1 benzylidene

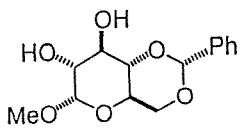
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Mar. 22, 2001
Solvent: CDCl3
File: 010322v2_1#01
Starting Time: 16:47:38
Completion Time: 16:65:27
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.998 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.120#122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



20

1-jna-16-2

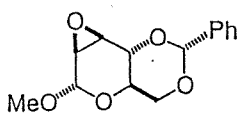
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: Mar. 28, 2001
Solvent: CDCl3
File: 010328v2_1601
Starting Time: 15:43:31
Completion Time: 15:51:24
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 60.0 degrees
Acq. time 1.859 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1208122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



21

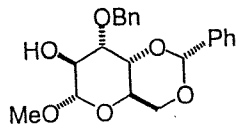
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

User: cfojna
Date: May. 25, 2001
Solvent: CDCl3
File: 010525v2_0301
Starting Time: 13:21:35
Completion Time: 13:23:28
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1209122 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



18

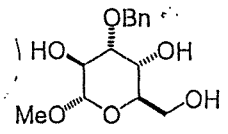
University of Minnesota, VXR-300

Pulse Sequence: s2pul

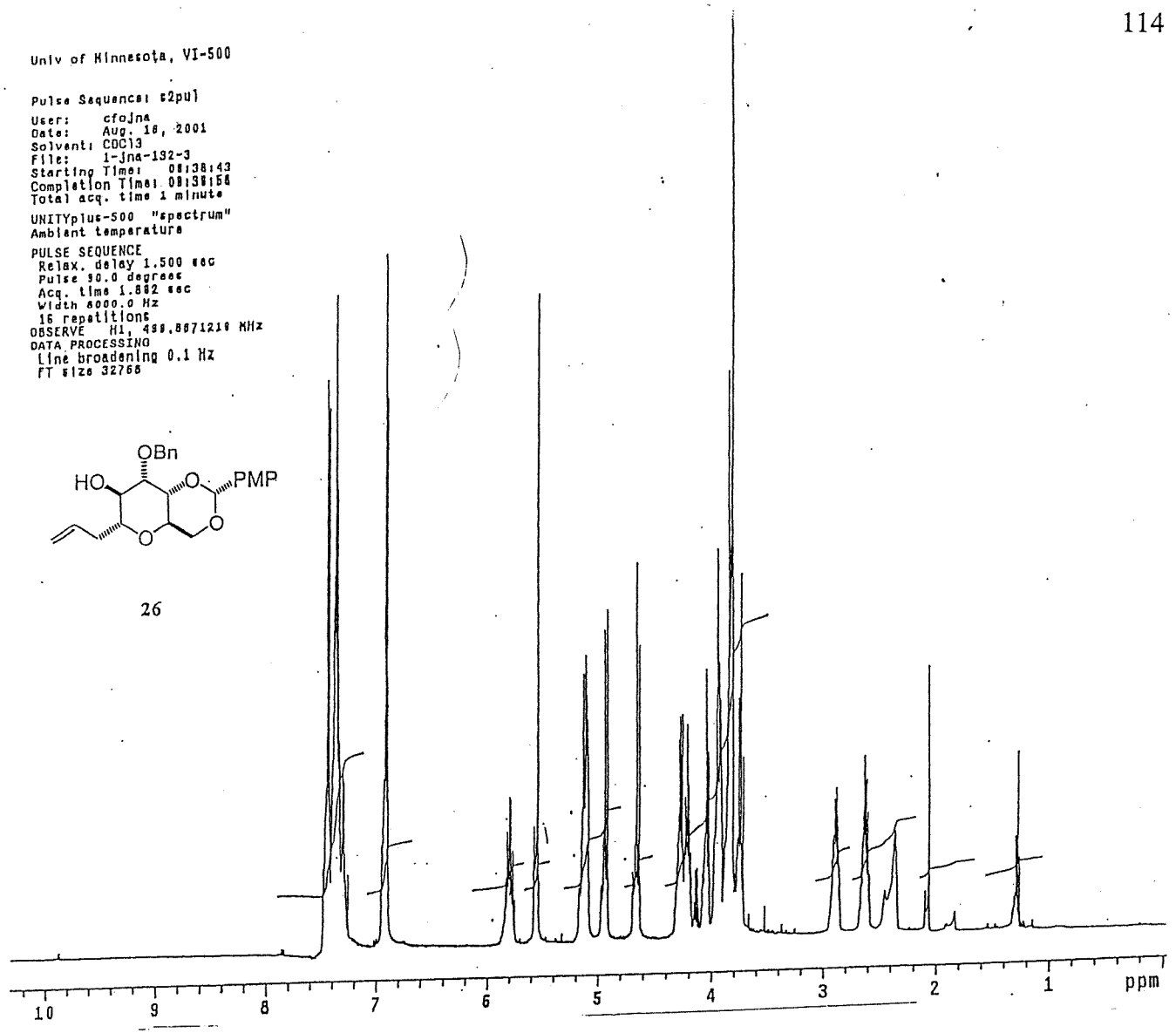
User: cfojna
Date: Jun. 23, 2001
Solvent: CDCl3
File: 1-jna-95-3
Starting Time: 17:12:32
Completion Time: 17:13:47
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

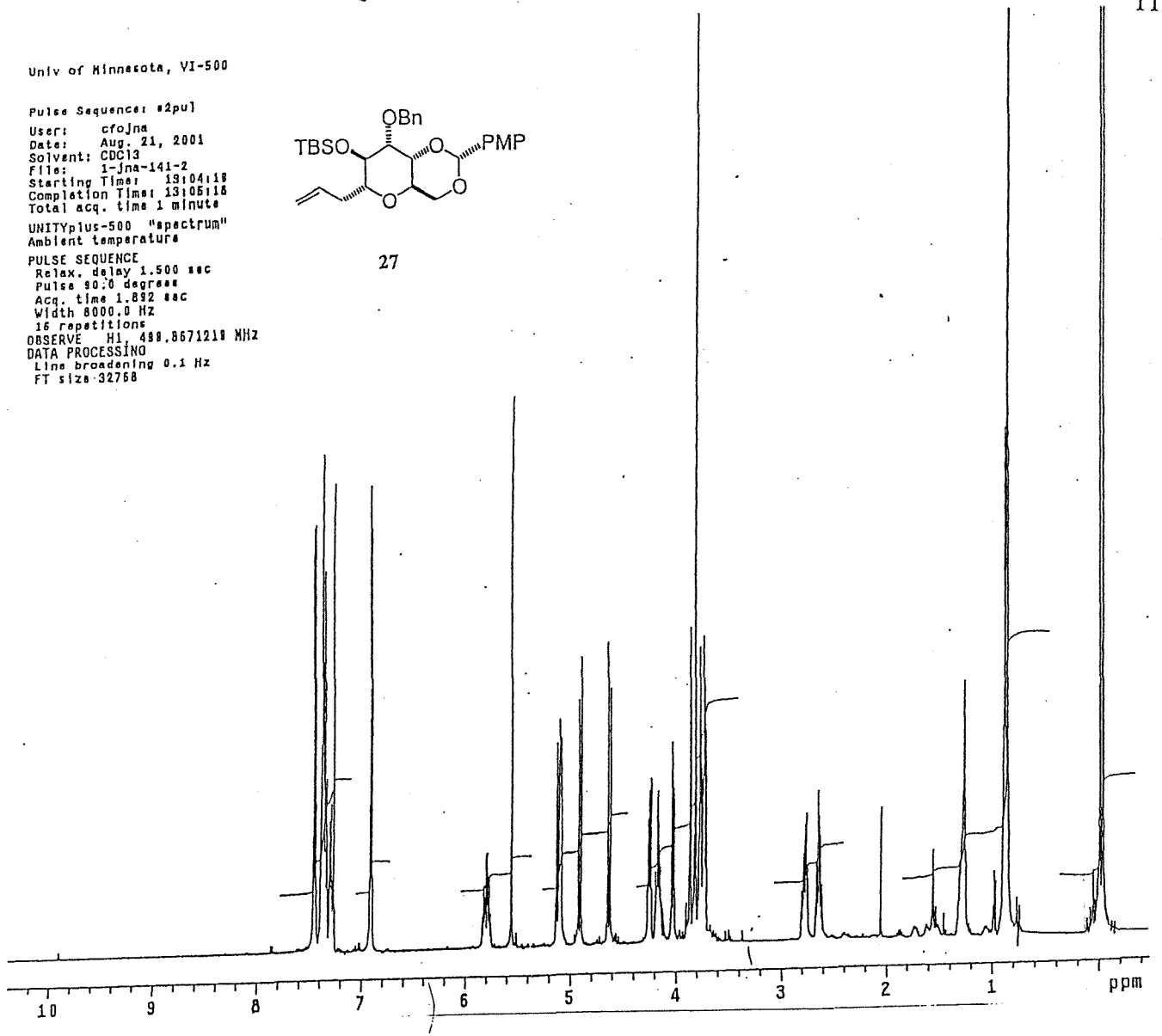
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 30.0 degrees
Acq. time 2.000 sec
Width 5999.7 Hz
16 repetitions
OBSERVE H1, 299.8589067 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



23


Univ of Minnesota, VI-500
Pulse Sequence: s2pul
User: cfojna
Date: Aug. 16, 2001
Solvent: CDCl3
File: 1-jna-132-3
Starting Time: 08:38:43
Completion Time: 08:39:56
Total acq. time 1 minute
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.882 sec
Width 5000.0 Hz
16 repetitions
OBSERVE H1, 499.8671219 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
OBn
HO
O
PMP
O
O
10
9
8
7
6
5
4
3
2
1
ppm

26


Univ of Minnesota, VI-500
Pulse Sequence: s2pul
User: cfojna
Date: Aug. 21, 2001
Solvent: CDCl3
File: 1-jna-141-2
Starting Time: 13:04:18
Completion Time: 13:05:18
Total acq. time 1 minute
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.8671218 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
OBn
TBSO
O
PMP
O
O
27
10
9
8
7
6
5
4
3
2
1
ppm

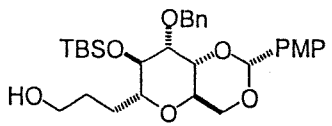
University of Minnesota
Department of Chemistry
VAC-300

Pulse Sequence: s2pul

User: cfojna
Date: Sep. 14, 2001
Solvent: CDCl3
File: 010914v3_0902
Starting Time: 13:05:04
Completion Time: 13:15:42
Total acq. time 3 minutes

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 5998.8 Hz
64 repetitions
OBSERVE H1, 299.9533609 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 65536



28

1-jna-214

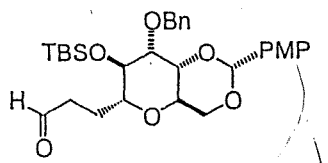
University of Minnesota
Department of Chemistry
VAC-300

Pulse Sequence: s2pul

User: cfojna
Date: Dec. 30, 2001
Solvent: CDCl3
File: 011230v3_0102
Starting Time: 16:54:07
Completion Time: 17:03:14
Total acq. time 1 minutes

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 5998.8 Hz
32 repetitions
OBSERVE H1, 299.9593609 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 65536



31

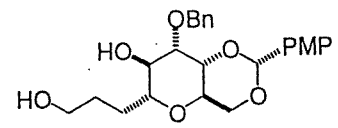
University of Minnesota
Department of Chemistry
VAC-300

Pulse Sequence: s2pul

User: cfojna
Date: Sep. 19, 2001
Solvent: CDCl3
File: 010919v3_0702
Starting Time: 15:59:23
Completion Time: 16:11:47
Total acq. time 3 minutes

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 5998.8 Hz
64 repetitions
OBSERVE H1, 299.9533609 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 65536



29

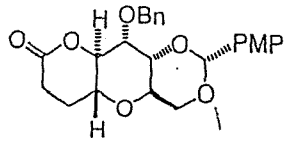
University of Minnesota, VXR-300

Pulse Sequence: s2pul

User: cfojna
Date: Sep. 21, 2001
Solvent: CDCl3
File: 1-jna-166-2
Starting Time: 17:24:49
Completion Time: 17:25:52
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

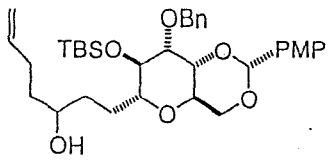
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 5999.7 Hz
16 repetitions
OBSERVE H1, 299.8588067 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



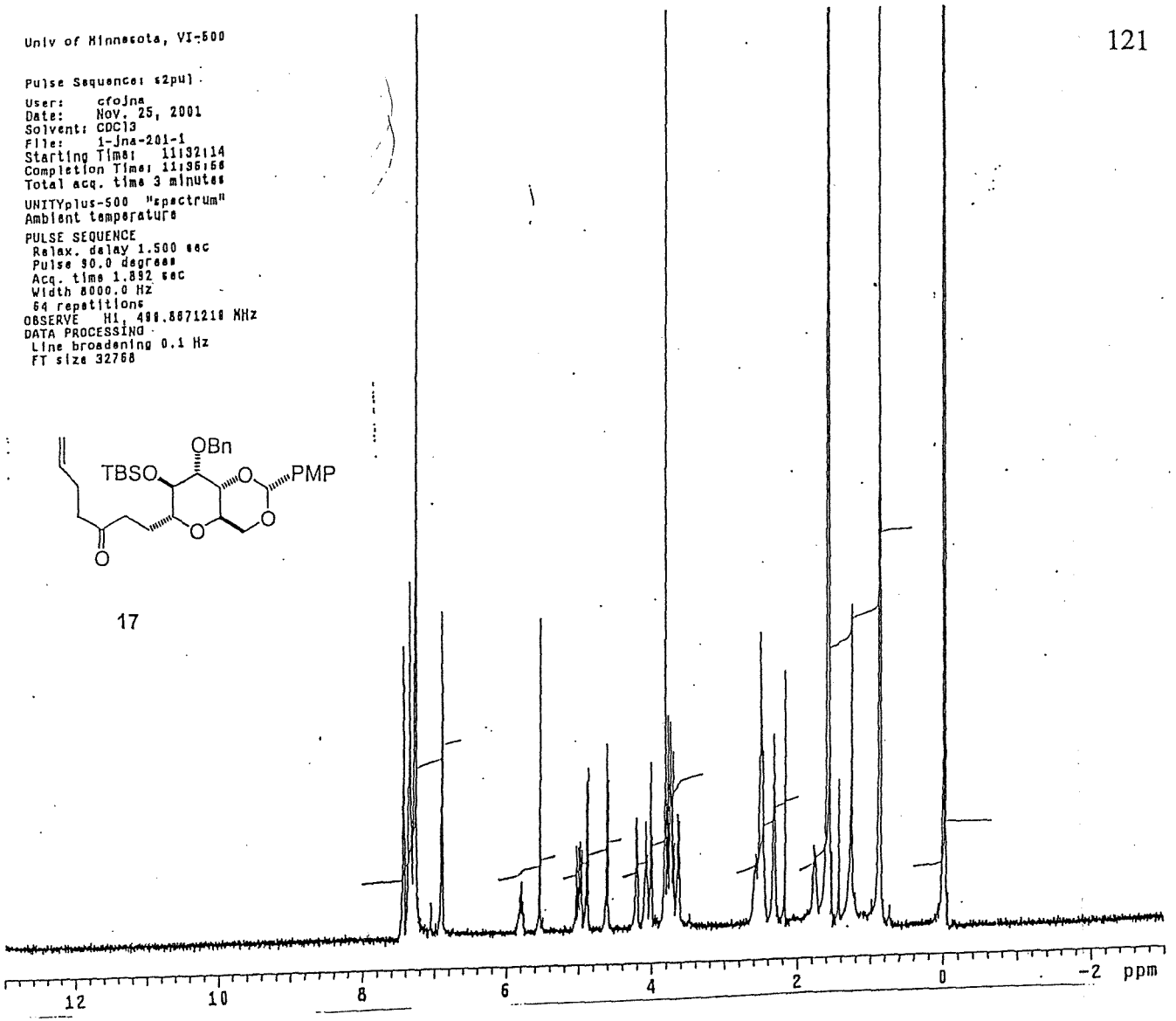
30

Univ of Minnesota, VI-500

Pulse Sequence: s2pul
User: cfojna
Date: Nov. 13, 2001
Solvent: CDCl3
File: 1-jna-189-3
Starting Time: 14:15:01
Completion Time: 14:16:00
Total acq. time 1 minute
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 488.8871218 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



32

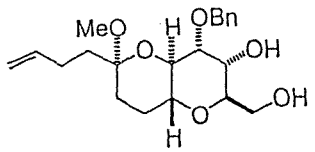

Univ of Minnesota, VI-500
Pulse Sequence: s2pul
User: cfojna
Date: Nov. 25, 2001
Solvent: CDCl3
File: 1-jna-201-1
Starting Time: 11:32:14
Completion Time: 11:35:56
Total acq. time 3 minutes
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
64 repetitions
OBSERVE H1, 499.8671218 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
OBn
TBSO
O
PMP
O
O
O
17
12
10
8
6
4
2
0
-2
ppm

Univ of Minnesota, VI-500

Pulse Sequence: s2pul
User: cfojna
Date: Nov. 28, 2001
Solvent: CDCl3
File: 1-jna-205-2
Starting Time: 19:19:48
Completion Time: 19:20:52
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.882 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.8671218 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



33

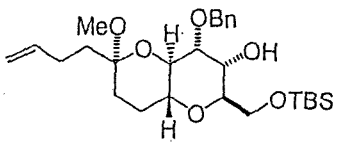
University of Minnesota,/VXR-300

Pulse Sequence: s2pul

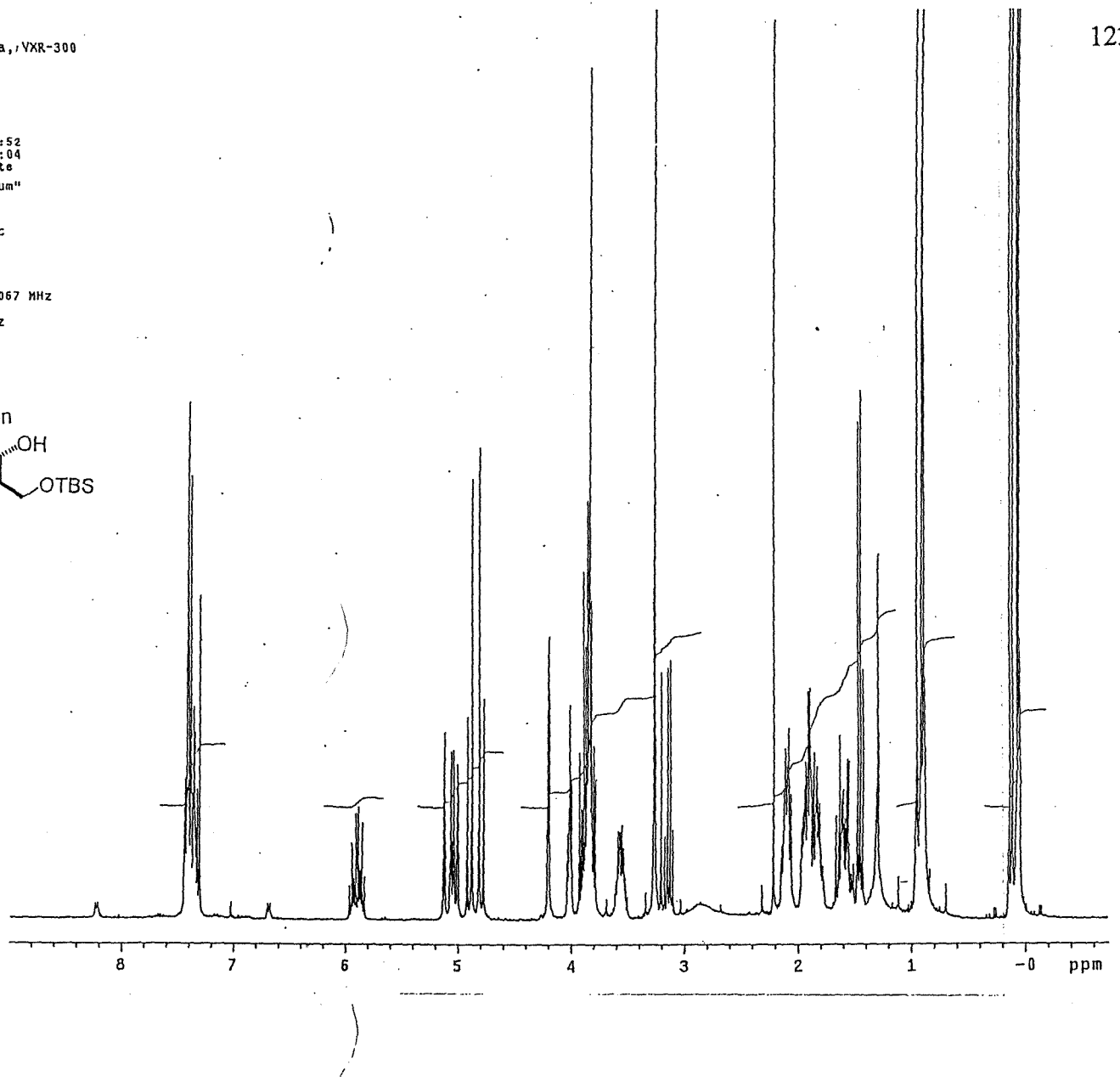
User: cfojna
Date: Feb. 11, 2002
Solvent: CDCl3
File: 1-jna-228-1
Starting Time: 10:32:52
Completion Time: 10:34:04
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 2.000 sec
Width 5999.7 Hz
16 repetitions
OBSERVE H1, 299.8589067 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



33



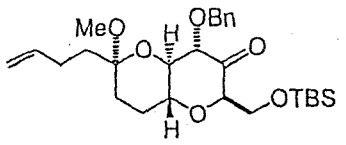
STANDARD 1H OBSERVE

Pulse Sequence: s2pul

User: cfojna
Date: Mar. 17, 2002
Solvent: CDCl3
File: 1-jna-234-2
Starting Time: 13:58:37
Completion Time: 13:59:42
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 6003.3 Hz
16 repetitions
OBSERVE H1, 300.1683397 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



16

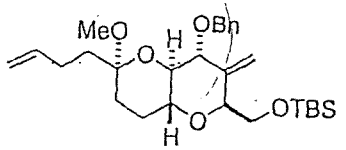
STANDARD 1H OBSERVE

Pulse Sequence: s2pul

User: cfojna
Date: Feb. 18, 2002
Solvent: CDCl3
File: 1-jna-230-2b
Starting Time: 16:19:17
Completion Time: 16:20:19
Total acq. time 1 minute

UNITYplus-500 "spectrum"
Ambient temperature

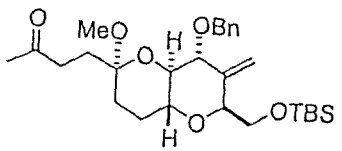
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 6003.3 Hz
16 repetitions
OBSERVE H1, 300.1683396 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



34

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
User: cfojna
Date: Mar. 4, 2002
Solvent: CDCl3
File: 1-jna-231-3
Starting Time: 12:37:01
Completion Time: 12:45:55
Total acq. time 8 minutes
UNITYplus-500 "spectrum"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 90.0 degrees
Acq. time 1.999 sec
Width 6003.3 Hz
150 repetitions
OBSERVE H1, 300.1683397 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072



1