Université de Montréal

Synthesis of Ring A of (+)-Ambruticin S and Bicyclic Nucleosides for Antisense Drug Technology

Par

Bin Chen

Département de chimie

Faculté des Arts et Sciences

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Synthesis of Ring A of (+)-Ambruticin S and Bicyclic Nucleosides for Antisense Drug Technology

Présenté par:
Bin Chen

A été évalué par un jury composé des personnes suivantes:

Prof: André B. Charette
Président du jury: Professeur

Prof: Shawn K Collins
Membre du jury: professeur

Prof: Stephen Hanessian
Directeur de recherche

Mémoire accepté le:

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Chapter one: Synthesis of Ring A of (+)-Ambruticin S

Summary

An enantioselective synthesis of the antifungal natural product (+)-ambruticin S has been accomplished in our group. For the synthesis of a ring A lactone fragment, three approaches were developed. They all started from commercially available and inexpensive methyl α-D-glycopyranoside, which already possesses the required diol unit. A deletion of the hydroxyl group at C-4 and a one-carbon homologation of the C-6 side chain furnished the ring A lactone.

The second project is an ongoing collaboration between the Hanessian group and ISIS pharmaceuticals to develop new antisense oligonucleosides. The *cis*- and *trans*-[4.3.0]bicyclic antisense nucleosides were successfully synthesized from a common natural monosaccharide, L-arabinose, which bears the required stereocenters. A key Sakurai allylation led to the *cis*- and *trans* diastereomers under Felkin-Ahn and chelation-controlled conditions respectively. The final bicyclic targets were achieved by a practical proline-catalyzed intramolecular aldol reaction and ring-closing metathesis (RCM) strategy, and application of the Vorbrüggen method for nucleoside synthesis.

Keywords: (+)-Ambruticin S, methyl α-D-glycopyranoside, [4.3.0]bicyclic antisense nucleoside, L-arabinose, Sakurai allylation, intramolecular aldol reaction, Ring-Closing Metathesis (RCM), Vorbrüggen glycosylation.

Résumé

La synthèse énantiosélective de la (+)-ambruticine S, un produit naturel antifongique a été effectuée au sein de notre groupe. Trois approches ont été développées pour la synthèse du fragment lactone (cycle A). Ces trois voies d'accès au cycle A ont pour intermédiaire commun le methyl α-D-glycopyranoside déjà porteur du diol requis et disponible commercialement à bon prix. Une désoxygénation de l'hydroxyle en C-4 et l'homologation d'un carbone de la chaine latérale en C-6 ont permis l'obtention du cycle lactonique A.

Le deuxième projet est une collaboration entre le groupe Hanessian et ISIS Pharmaceuticals afin de développer de nouveaux oligonucléosides antisens. Les nucléosides antisens [4.3.0]-bicycliques *cis* et *trans* ont été synthétisés avec succès à partir d'un monosaccharide naturel commun, L-arabinose, porteur des stéréocentres requis. Un réaction clé d'allylation de Sakurai a permis d'obtenir les diastéréoisomères *cis* et *trans* dans des conditions de contrôle de type Felkin-Ahn et de contrôle par chélation respectivement. Les composés bicycliques finaux cibles ont été obtenus par une réaction d'aldol intramoléculaire catalyzéé par la proline, par métathèse de fermeture de cycle et par l'application de la méthode de Vorbrüggen pour la synthèse de nucléosides.

Mots clés: (+)-Ambruticine S, methyl α-D-glycopyranoside, nucléosides antisens [4.3.0]-bicycliques, L-arabinose, allylation de Sakurai, réaction d'aldol intramoléculaire, métathèse de fermeture de cycle, glycosylation de Vorbrüggen.

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Last but not least I am extremely grateful to my parents for their unconditional love and understanding throughout my life, and I also would like to thank my aunt for her utmost support. I would not have been able to do this without all of you.

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Abbreviations

$[\alpha]_D$	optical rotation
Ac	acetyl
ACCN	1, 1'-azobis(cyclohexane-1-carbonitrile)
AIBN	2, 2'-azo *bis*isobutyronitrile
9-BBN	9-borabicyclo(3.3.1)nonane
Bn	benzyl
BSA	*bis*(trimethylsilyl)acetamide
Bt	benzotriazole
Bz	benzoyl
calcd	calculated
CSA	camphorsulfonic acid
d	doublet
DBU	1,8-diazabicycloundec-7-ene
DCM	dichloromethane
dd	doublet of doublets
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
d.e.	diastereomeric excess
DIBAL-H	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine

DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
DMTr	4,4'-dimethoxytrityl
d.r.	diastereomeric ratio
e.e.	enantiomeric excess
Et	ethyl
eq (*equiv*.)	equivalent
ESI	electrospray ionization
FAB	fast atom bombardment
g	gram
HRMS	high resolution mass spectroscopy
Hz	Hertz
IR	infrared spectroscopy
J	coupling constant
LD_{50}	the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals
LiDBB	lithium di-*tert*-butylbiphenyl
LiHMDS	lithium *bis*(trimethylsilyl)amide
m	multiplet

Me	methyl
Men	menthyl
MHz	megahertz
MIC	minimum inhibitory concentration
min	minute
mL	milliliter
mmol	millimole
Ms	methanesulfonyl
MS	mass spectrometry
NapBr	2-(bromomethyl) naphthalene
NMO	*N*-methylmorpholine-*N*-oxide
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
Ph	phenyl
PhMe	toluene
pK_a	acid dissociation constant
ppm	parts per million
py	pyridine
q	quartet
Red-Al	sodium *bis*(2-methoxyethoxy)aluminumhydride

r.t.	room temperature
s	singlet
SDS	solvent delivery system
t	triplet
TBAF	tetra-*N*-butylammonium fluoride
TBAI	tetra-*N*-butylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TES	triethylsilyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tr	trityl (triphenylmethyl)
Ts	tosyl
UV	ultraviolet

Chapter One

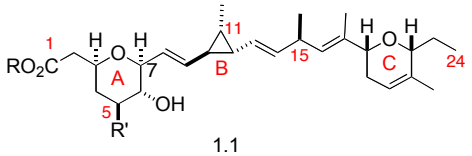
Synthesis of Ring A of (+)-Ambruticin S

1.1 Introduction

1.1.1 The Ambruticin Family

In 1977, researchers at Warner-Lambert reported the isolation of a novel antifungal agent, (+)-ambruticin S (**1.1a**), from fermentation extracts of the myxobacterium *Polyangium cellulosum var. fulvum*.[1,2] One year later, this group also isolated and characterized ambruticin F (**1.1b**),[3] which is the 5-*epi*-isomer of **1.1a**, from the same fermentation extracts of the myxobacterium. A series of ambruticin analogues (**1.1c-h**) were later reported in the 1990s by scientists at GBF from a closely related myxobacteria strain *Sorangium cellulosum* Soce10.[4] This was known as the VS series, bearing an amino group at the C-5 position, and expanded the ambruticin family to include these eight members (Table 1.)[5,6]

Table 1: The ambruticin family.



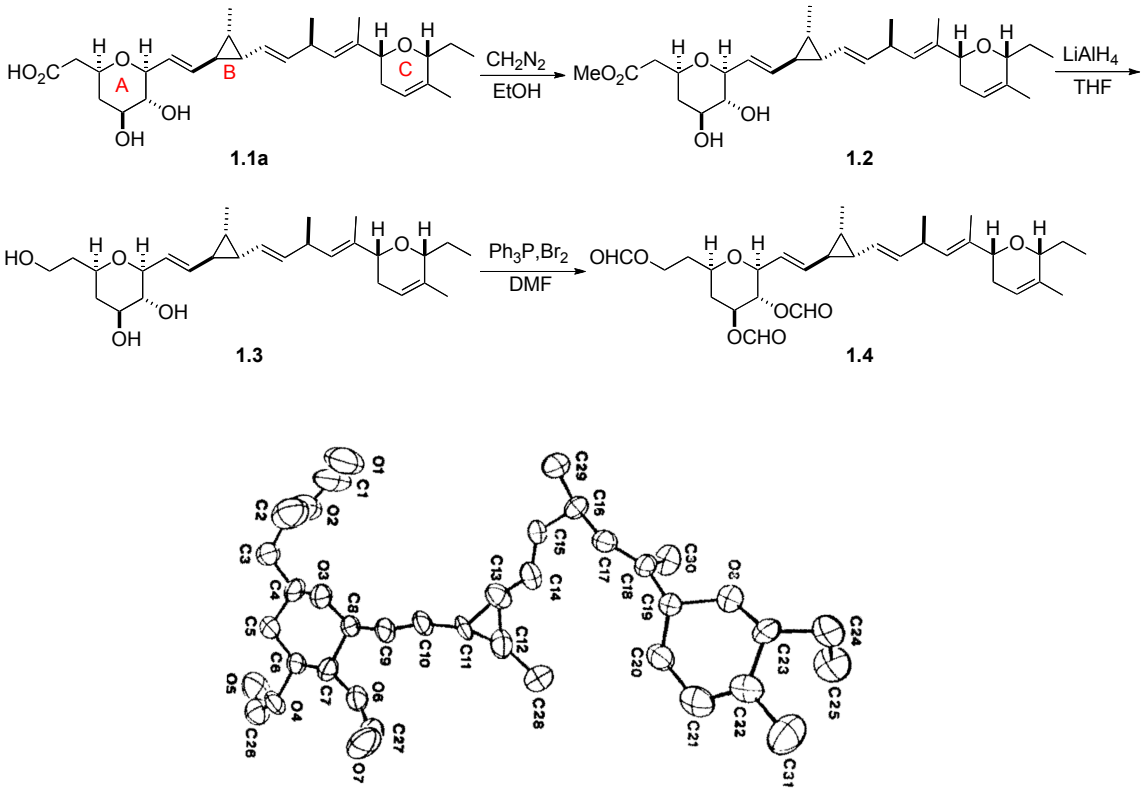
1.1

	R	R'	Ambruticin
1.1a	H	OH	S
1.1b	H	α-OH	F
1.1c	H	$(NH_3)^+$	VS-5
1.1d	H	$(NH_2Me)^+$	VS-4
1.1e	H	$(NHMe_2)^+$	VS-3
1.1f	H	$(NMe_3)^+$	VS-1
1.1g	H	$(NOHMe_2)^+$	VS-3 N-oxide
1.1h	Me	$(NMe_3)^+$	VS-2

1.1.2 Structure and Biological Activity

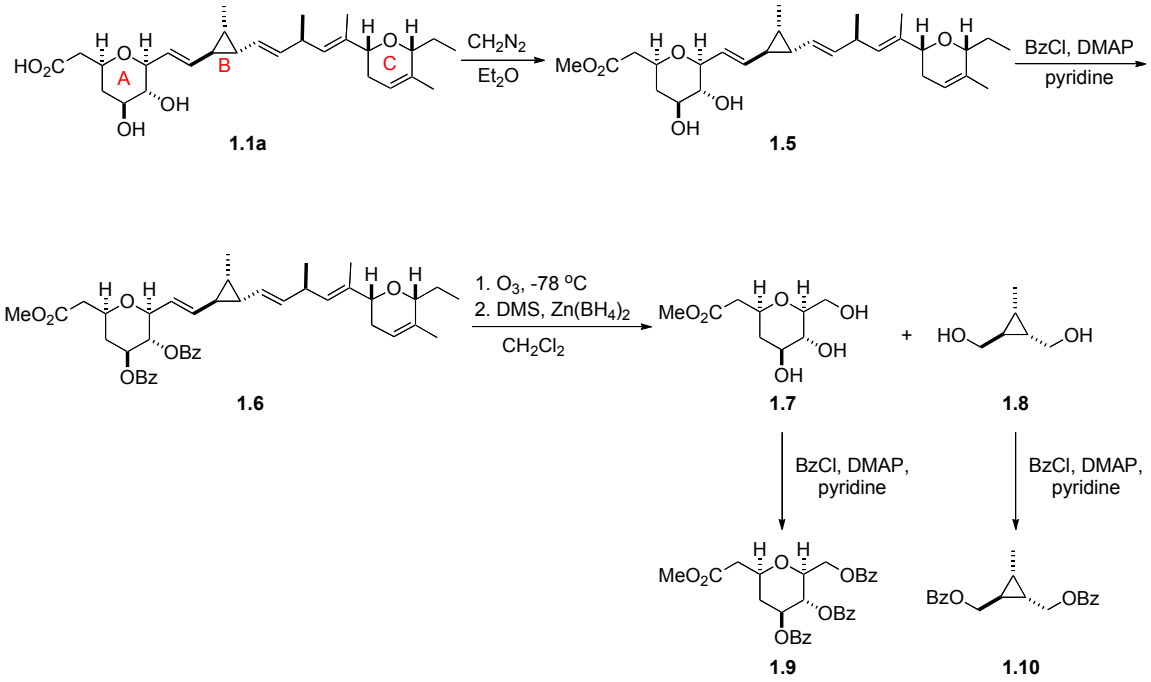
The structure of ambruticin S (**1.1a**) was elucidated from systematic degradative

studies and spectroscopic analysis. A single crystal X-ray analysis of the triformate ester of alcohol **1.3**, which was prepared from ambruticin S (**1.1a**) enabled the determination of the relative stereochemistry of the natural product (Scheme 1).[1] Further degradation studies of ozonolysis fragments of the tetrahydropyran ring A **1.9** and the cyclopropane ring **1.10**, established the absolute configuration of ambruticin S (Scheme 2).[7] The natural product features a *cis*-2,6-tetrasubstituted tetrahydropyran ring (ring A), a trisubstituted cyclopropane ring (ring B), a trisubstituted dihydropyran ring (ring C), and has a total of 10 stereogenic centers, making it a challenging target for total synthesis.



Scheme 1: Synthesis of triformate ester (**1.4**) and X-ray crystal structure.

(Picture is taken from reference 1)



Scheme 2: Degradative study to determine the absolute stereochemistry of ambruticin S.

Biological studies have demonstrated that ambruticin S has low toxicity (LD_{50} values for the sodium salt of ambruticin in mice were: intravenous 315 mg/kg, oral > 1,000 mg/kg) and exhibits potent antifungal activity *in vitro* and *in vivo* against a variety of biological pathogens.[2] The natural product is highly active against *Coccidioides immitis, Histoplasma capsulatum,* and *Blastomyces dermatitidis* as well as dermatophytic filamentous fungi, and demonstrates moderate antibacterial activity against *Streptococcus pyogenes*, with an MIC (Minimum Inhibitory Concentration) of 12.5 μg/mL, *Diplococcus pneumoniae* (MIC 12.5 μg/mL), and *Bacillus cereus* (MIC 12.5 μg/mL).[2] It also shows potent activity against *Hansenula anomala* (MIC 0.03-1.6 μg/mL).[2] The ambruticin VS series also show low toxicity and antifungal activity.[4,8,9]

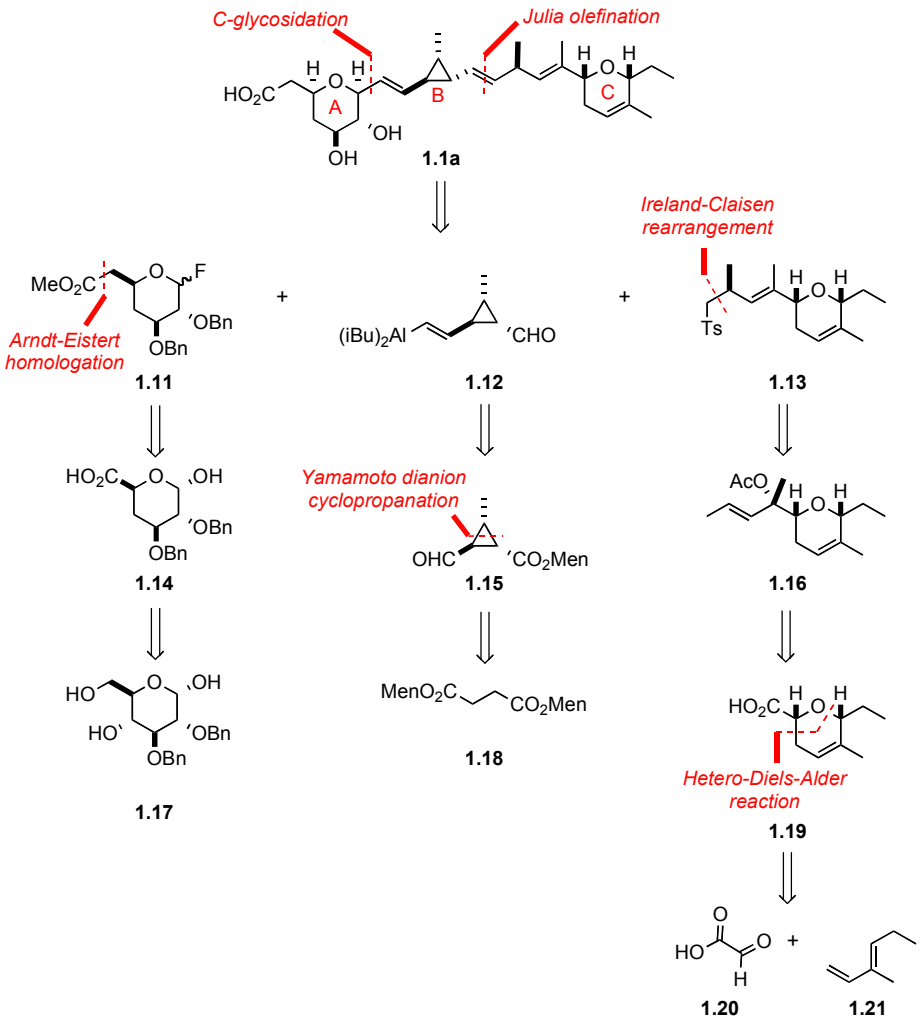
1.1.3 Total Synthesis of Ambruticin S - Ring A Strategy

The unique structural features mentioned above, and the notable biological activities of ambruticin S (**1.1a**) have attracted the attention of many research groups to pursue the target for total synthesis. There have been several methodology based studies for constructing the ring A,[10,11] ring B,[10,12] and ring C[13] fragments, and four total syntheses of ambruticin S have been reported to date. The first total synthesis of ambruticin S was accomplished by Kende and co-workers in 1990.[14,15] Three other total syntheses have been achieved by Jacobsen[16] and Martin[17,18] in 2001, followed by Lee[19] in 2002. Each of these syntheses features a different strategy for the construction of the natural product.

1.1.3.1 The Kende Approach

In Kende's retrosynthetic analysis of ambruticin S (**1.1a**) the natural product could be disconnected and simplified to give three separate ring fragments **1.11** (ring A), **1.12** (ring B), and **1.13** (ring C) using *C*-glycosidation and Julia olefination transformation to link the subunits (Scheme 3). The ring A synthon **1.11** could be generated from a protected methyl α-glucopyranoside (**1.17**)[20] by employing deoxygenation, Arndt-Eistert homologation,[21] and fluorination in the forward direction. The cyclopropane **1.12** can be synthesized using Yamamoto dianion chemistry from dimenthyl succinate (**1.18**).[22] The side chain of the ring C precursor **1.13** could be installed using an Ireland-Claisen rearrangement strategy, and the dihydropyran ring could be assembled via a thermal hetero-Diels-Alder reaction between glyoxylic acid (**1.20**) and (*E*)-3-methyl-1,3-hexadiene (**1.21**) followed by a resolution using (+)-α-

methylbenzylamine.

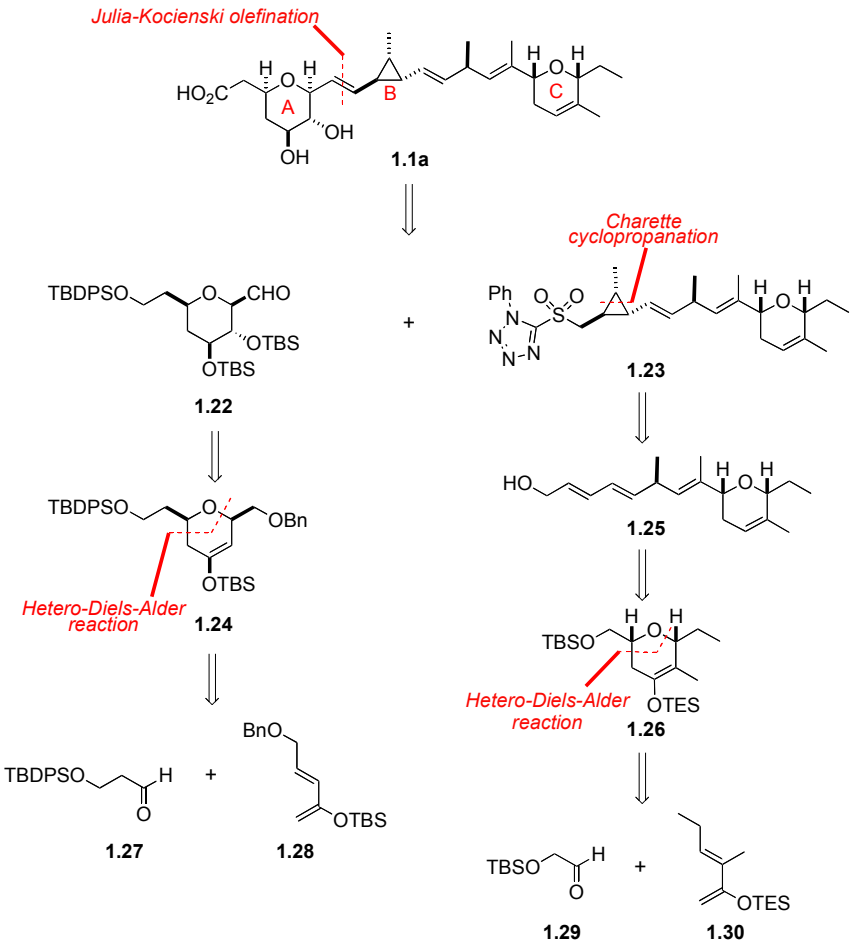


Scheme 3: Kende's retrosynthetic analysis of ambruticin S.

1.1.3.2 The Jacobsen Approach

Jacobsen envisioned that ambruticin S (**1.1a**) could be simplified to fragments **1.22** and **1.23** by disconnection of the *trans*-configured olefin between rings A and B (Scheme 4). The precursor **1.24** for the ring A fragment **1.22** could be generated from aldehyde **1.27** and diene **1.28** using an asymmetric hetero-Diels-Alder reaction. The

dihydropyran ring C in **1.26** can be also constructed through an asymmetric hetero-Diels-Alder strategy involving aldehyde **1.29** and diene **1.30** in conjunction with a suitable olefination method, and a Charette cylcopropanation[23,24] to furnish the cyclopropane ring B.

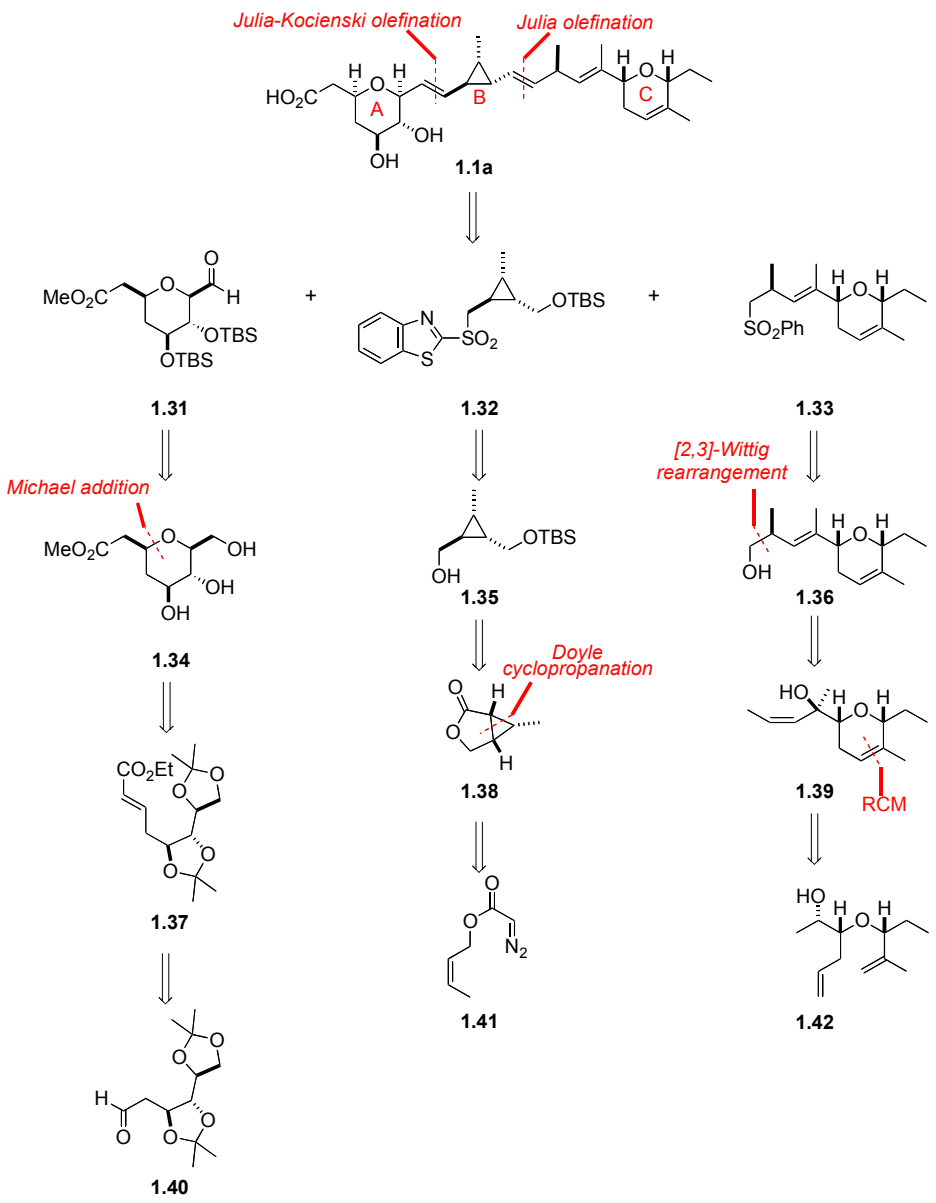


Scheme 4: Jacobsen's retrosynthetic analysis of ambruticin S.

1.1.3.3 The Martin Approach

Martin's retrosynthetic analysis of ambruticin S commenced with the disconnection the two disubstituted olefins via Julia-Kociensky and Julia olefination

transformations to give three subunits **1.31**, **1.32**, and **1.33** (Scheme 5). Ring A fragment **1.31** can be taken back to triol **1.34**, whose synthesis could be envisaged through an intramolecular Michael addition of the intermediate acetonide **1.37**. Acetonide **1.37** could be further reduced to the known aldehyde **1.40**.[25] Ring B subunit **1.32** could be simplified to the cyclopropane intermediate **1.35**, which could be taken back to the lactone **1.38**. Martin anticipated that lactone **1.38** could be prepared via a Doyle cylcopropanation reaction from diazoester **1.41**.[26,27] Ring C fragment **1.33** could be retrosynthetically reduced to homoallylic alcohol **1.36**, which could be accessed through a [2,3]-Wittig rearrangement in the forward direction from allylic alcohol **1.39**. This allylic alohol moiety of **1.39** could be made using a chelate controlled Grignard addition to a methyl ketone, and the dihydropyran ring could be disconnected using a ring-closing metathesis (RCM) reaction, simplifying its synthesis and further reducing to diene **1.42**.

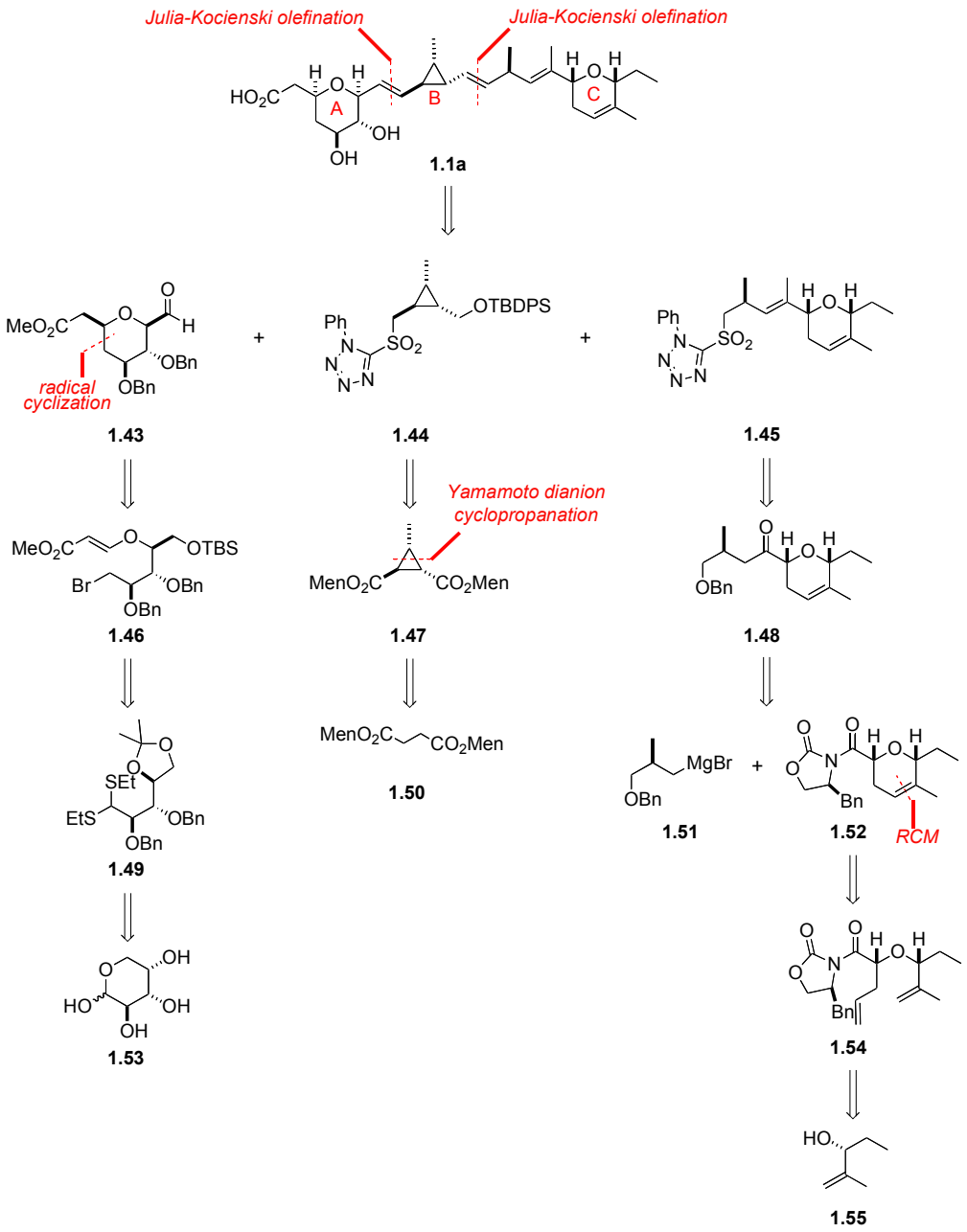


Scheme 5: Martin's retrosynthetic analysis of ambruticin S.

1.1.3.4 The Lee Approach

The key disconnections in Lee's retrosynthetic analysis of ambruticin S (**1.1a**) are similar to that of Martin's plan, where scission of the two disubstituted olefins simplifies the synthesis to three subunits **1.43**, **1.44**, and **1.45** (Scheme 6). The ring A aldehyde subunit **1.43** could be retrosynthetically reduced to intermediate **1.46** by employing a

radical cyclization. Furthermore, **1.46** could be traced back to commercially available L-arabinose (**1.53**). The ring B cyclopropane subunit **1.44** could be brought back to intermediate **1.47**, and the synthesis of this cyclopropane can be envisaged via Yamamoto's dianion chemisty to give **1.50**,[22] which mirrors the Kende approach to this ring B fragment. Ring C subunit **1.45** could be taken back to ketone **1.48**, which could be further reduced to Grignard reagent **1.51** and oxazolidinone **1.52**. From here, it was envisaged that the dihydropyran ring could be disconnected via a RCM reaction to furnish the diene **1.54**, which could ultimately be brought back to the chiral allylic alcohol **1.55**.

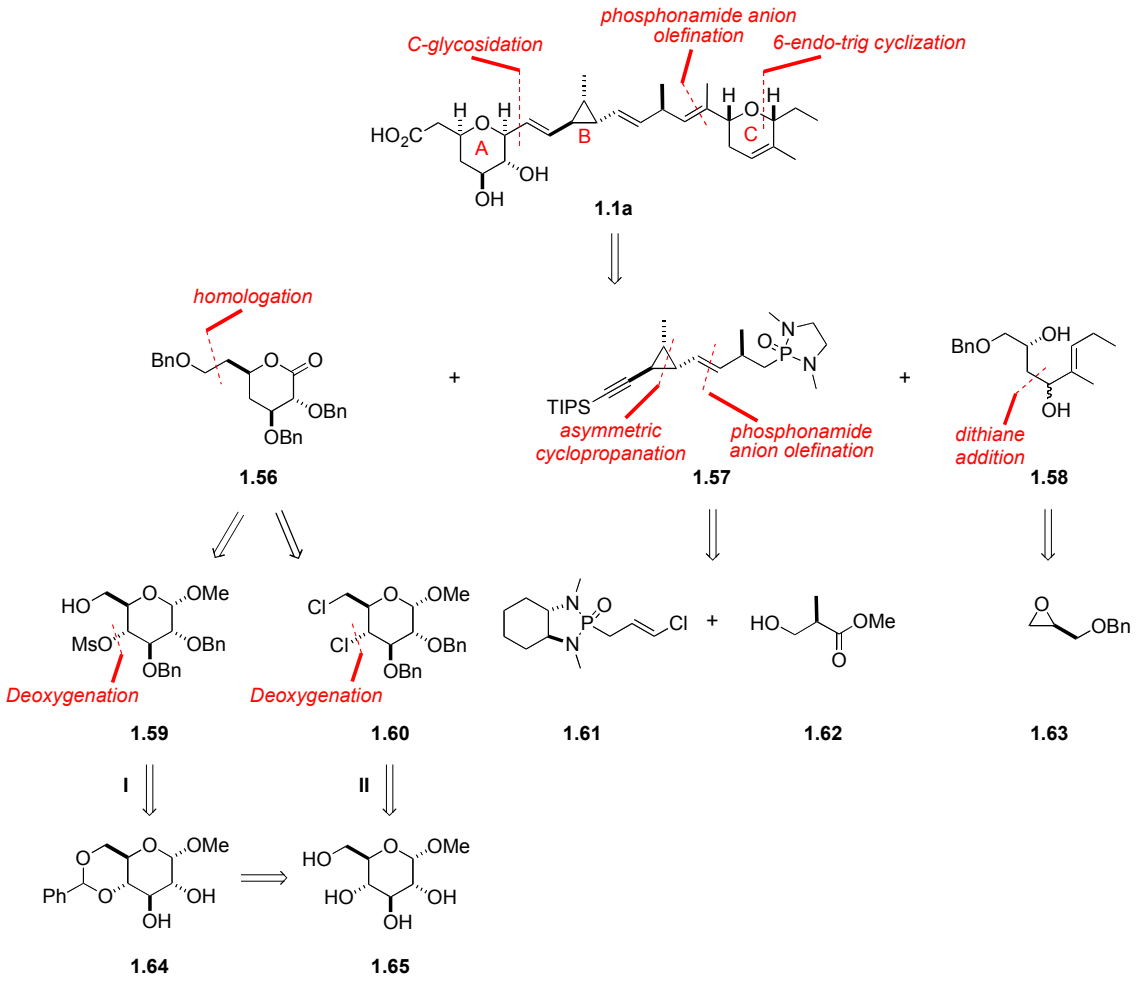


Scheme 6: Lee's retrosynthetic analysis of ambruticin S.

1.1.4 Retrosynthetic Analysis of Ambruticin S – the Ring A Strategy

The retrosynthetic analysis outlined in Scheme 7 along with the synthetic details discussed below, are new results discovered during the course of this dissertation. It was

envisioned that ambruticin S could be disconnected to furnish three subunits, which could be assembled in the forward sense using a phosphonamide anion based olefination and a *C*-glycosidation reaction respectively. The lactone **1.56,** which is readily available from methyl α-D-glucopyranoside (**1.65**), contains three of the stereogenic centers present in ring A of ambruticin S. Use of **1.65** as a starting material in the synthesis of the ring A subunit of ambruticin S will require deoxygenation at C-4 and a one carbon homologation of the C-6 side chain. The trisubstituted cyclopropane subunit **1.57** could be simplified to compounds **1.61** and **1.62**, which could be coupled in the forward direction using phosphonamide anion chemistry developed in the Hanessian laboratory to construct both the olefin[28] and cyclopropane ring[29,30,31] in **1.57**. The *C*-methyl stereogenic center of the acyclic chain of ambruticin S would come from the (*R*)-Roche ester (**1.62**). Synthesis of the ring C subunit of ambruticin S would employ a highly diastereoselective Lewis acid catalyzed 6-*endo*-*trig* cyclization[32] also developed in the Hanessian laboratory. The third and final subunit, diol **1.58**, would be provided from a dithiane anion addition to (*R*)-glycidol benzyl ether (**1.63**). For the synthesis of ring A, two possible routes that involved the use of **1.65** were envisaged.



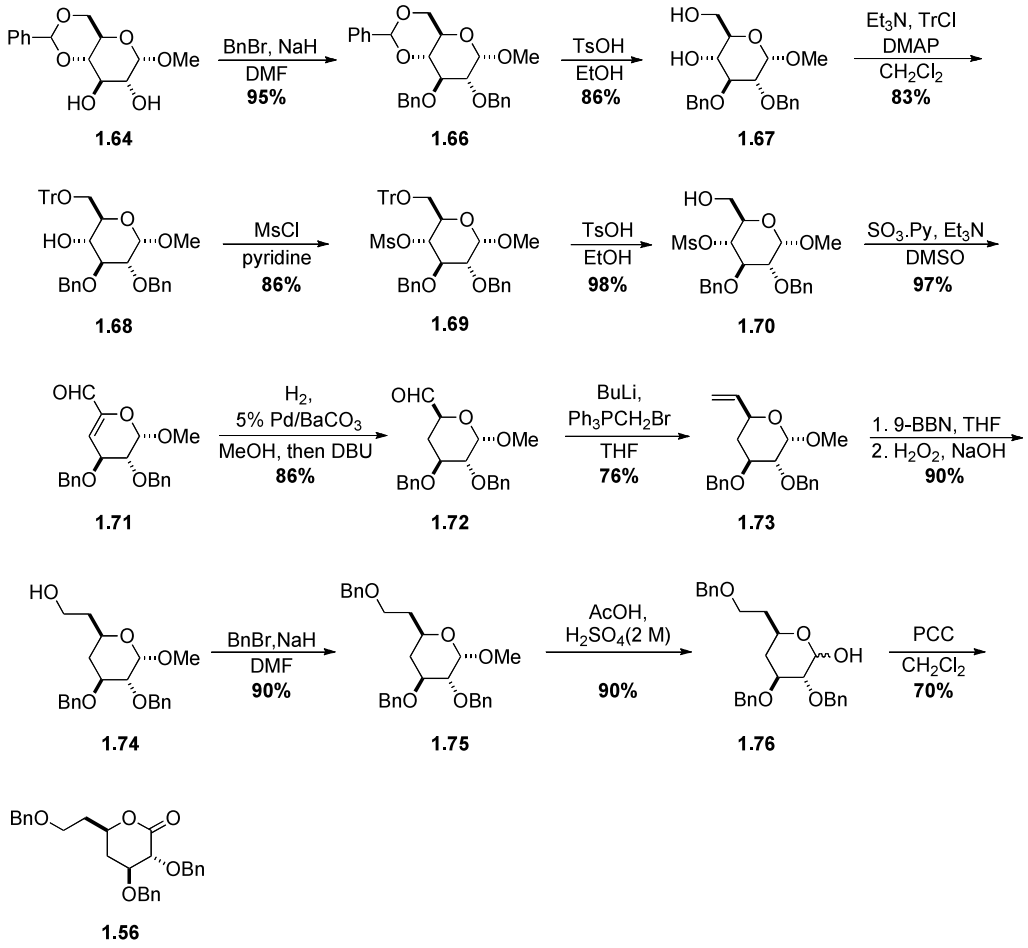
Scheme 7: Retrosynthetic analysis of ambruticin S.

1.2 Results and Discussion

1.2.1 Synthesis of Ring A Lactone 1.54 - Route I

Modification of Guiliano and Buzby's procedure,[33] enabled the synthesis of aldehyde **1.71** in six steps and 55% overall yield (Scheme 8). Starting from the commercial available methyl 4,6-*O*-benzylidene α-D-glucopyranoside (**1.64**), the diol was protected as bis-benzyl ether **1.66**. Removal of the benzylidene acetal upon treatment with TsOH in ethanol, gave the diol **1.67**. Selective protection of the primary

alcohol as the trityl ether, followed by mesylation of the secondary alcohol furnished **1.69** in 71% yield over two steps. Acid cleavage of the trityl ether gave primary alcohol **1.70** in near quantitative yield. Oxidation of **1.70** under Parikh-Doering conditions was accompanied by elimination of the methanesulfonate group to give the α,β-unsaturated aldehyde **1.71** in 97% yield. Catalytic hydrogenation of **1.71** by using $Pd/BaCO_3$ as a catalyst, and subsequent epimerization in the presence of DBU, afforded **1.72**. The one carbon homologation of the C-6 appendage was achieved upon treatment of aldehyde **1.72** with the ylide generated from methyl triphenylphosphonium bromide to give vinyl tetrahydropyran **1.73**. Hydroboration of the olefin with 9-BBN and oxidation produced the primary alcohol **1.74** in 90% yield. After protection of the free hydroxyl group as the benzyl ether, hydrolysis of the methyl glycoside under acidic conditions, and subsequent PCC oxidation of the lactol gave the desired lactone **1.56** in 57% yield over three steps. Thus, the synthesis of **1.56** was accomplished in 12 steps from commercially available and inexpensive methyl 4,6-*O*-benzylidene α-D-glucopyranoside (**1.64**) in 18% overall yield.

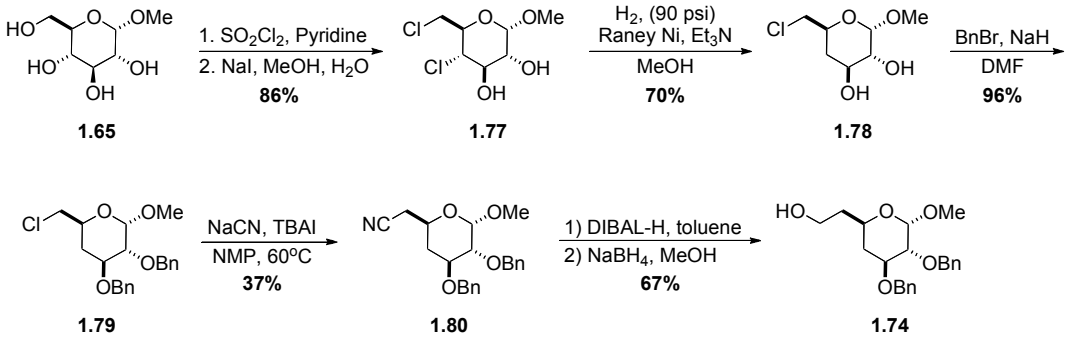


Scheme 8: Route I for the synthesis of ring A lactone

1.2.2 Route II for the Synthesis of the Ring A Lactone

Treatment of methyl α-D-glucopyranoside (**1.65**) with sulfuryl chloride in the presence of pyridine, followed by hydrolysis with sodium iodide in aqueous methanol, afforded **1.77** in 86% yield[34,35] (Scheme 9). Chemoselective dehalogenation of the secondary chloride in **1.77** was achieved using hydrogen gas in the presence of Raney nickel to furnish the diol **1.78**,[36] which was converted to the bis-benzyl ether **1.79** in 67% yield over two steps. Displacement of the primary chloride in **1.79** with NaCN was sluggish and resulted in only 37% yield (with recovery of 41% of the starting material)

for the homologated nitrile **1.80**.[37] Using different solvents, like DMSO, DMF, HMPA, and using KCN instead of NaCN, with different equivalents, all gave low yields. Nevertheless, reduction with DIBAL-H to the aldehyde, followed by treatment with $NaBH_4$ gave the intermediate primary alcohol **1.72** in 67% yield over two steps. Application of the identical three step sequence described in Scheme 8 furnished **1.56**. The synthesis of **1.56** was accomplished in 10 steps from methyl α-D-glucopyranoside (**1.65**) in 15% overall yield.

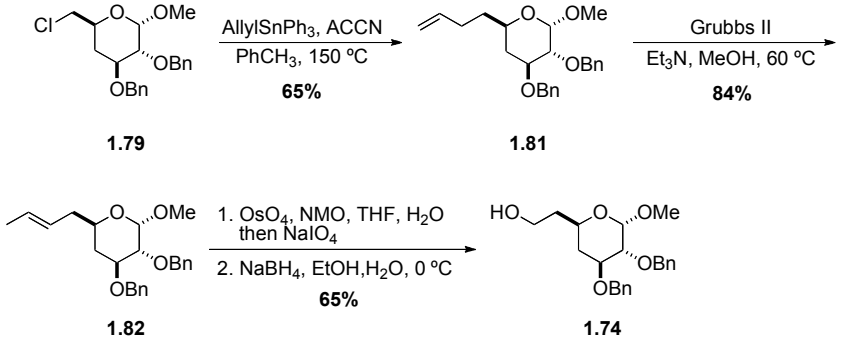


Scheme 9: Route II for the synthesis of intermediate **1.74**

1.2.3 Route III for the Synthesis of the Ring A Lactone

A third route (Route III) to the lactone **1.74** (Scheme 10), which started from intermediate **1.79**, was also devised during the course of the total synthesis of ambruticin S. The four-step route involved a radical-mediated allylation reaction of **1.79** to afford **1.81**, which was subsequently subjected to an olefin isomerization reaction using Grubbs' second generation catalyst according to a method reported by Hanessian and co-workers.[38] Dihydroxylation of the olefin, and oxidative cleavage of the resulting diol, followed by $NaBH_4$ reduction of the aldehyde gave intermediate alcohol **1.74** in seven

steps and 16% overall yield.



Scheme 10: Route III for the synthesis of intermediate **1.74**

1.2.4 Discussion for the Synthesis of Ring A

Comparing all three routes for the synthesis of ring A of ambruticin S in Table 2, Route I gave the highest yield even though it required 12 steps from methyl 4,6-*O*-benzylidene α-D-glucopyranoside (**1.64**). Route II was achieved in 9% overall yield over nine steps due to the modest yield (37%) of homologation step. Route III used a homologation and isomerization in the presence of Grubbs' second generation catalyst strategy developed in Hanessian group[38] furnished the ring A lactone in 10 steps and 12% yield. In all these approaches, Route I was the highest yielding and avoided using the highly toxic organostannane and was thus considered as the most practical and efficient way to access the desired ring A lactone.

Kende,[11,12] Martin,[13,14] and Lee[15] all start from carbohydrate derivatives, which possessed the diol with the required stereochemistry on ring A. Jacobsen[16] utilized a catalytic asymmetric hetero-Diels-Alder reaction to rapidly construct the ring A

framework and finally achieved the shortest synthesis of ambruticin S.

Table 2. Comparison of the three routes for ring A synthesis

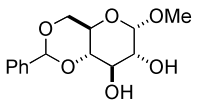
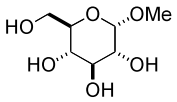
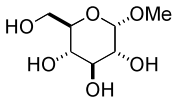
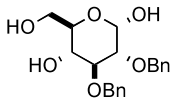
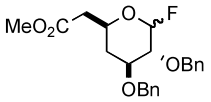
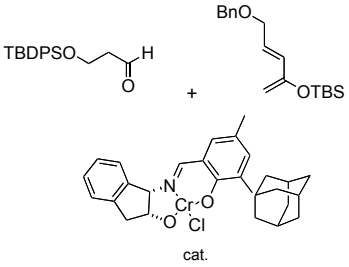
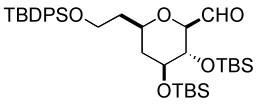
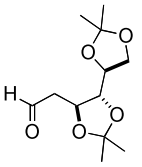
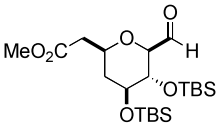
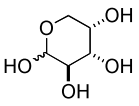
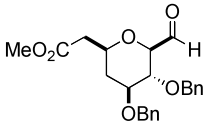
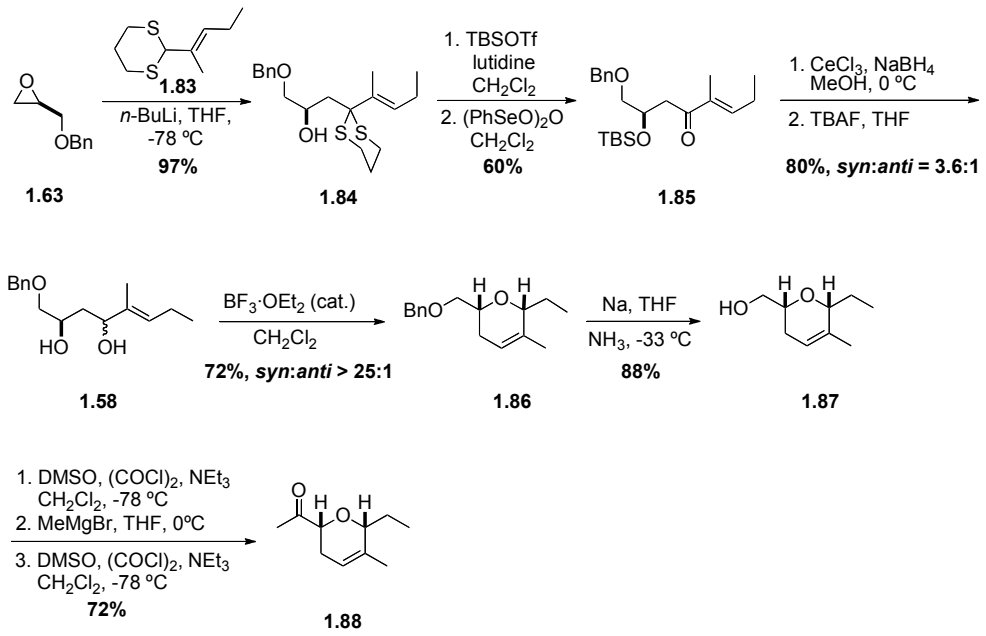
	Start material	To intermediate **1.74**	To lactone **1.56**
Route I		9 steps, 32% yield.	12 steps, 19% yield.
Route II		6 steps, 14% yield.	9 steps, 8% yield.
Route III		7 steps, 21% yield.	10 steps, 12% yield.

Table 3. Comparison of ring A syntheses in previous total syntheses

	Start material	Ring A synthons
Kende		 26% yield, 9 steps
Jacobsen		 53% yield, 5 steps
Martin		 46% yield, 5 steps
Lee		 25% yield, 11 steps

1.2.5 Synthesis of Ring C

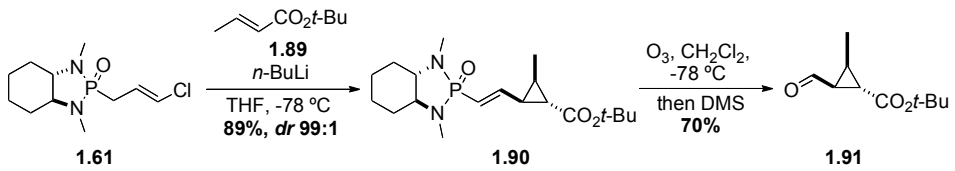
The construction of ring C of ambruticin S was accomplished by other members of our group (Scheme 11).[6] It started from (*R*)-glycidol benzyl ether (**1.63**) and dithiane **1.83**.[39] Deprotonation of the *E*-dithiane with butyllithium and treatment of the resulting anion with epoxide **1.63** afforded intermediate **1.84** in 97%. Protection of the secondary hydroxyl group in **1.84** as the TBS ether proved to be essential in order to successfully cleave the dithiane moiety and unmask the α,*β*-unsaturated system. Cleavage of the dithiane was achieved in the presence of benzeneseleninic acid anhydride to give the relatively unstable enone **1.85** in 60% overall yield.[40] Luche reduction, followed by TBS deprotection with TBAF furnished diol **1.58** as a 3.6:1 mixture of diastereomers. Treatment of the diol with a catalytic amount of $BF_3 \cdot OEt_2$ proceeded smoothly via 6-*endo*-trig cyclization to furnish the desired dihydropyran **1.86** in 81% yield on a multi-gram scale.[32] The benzyl group was then removed under Birch conditions to give alcohol **1.87** in 88% yield. Swern Oxidation, followed by addition of a methyl Grignard reagent to the resulting aldehyde gave a secondary alcohol which was then oxidized again under Swern conditions to furnish desired ketone **1.88** in 72% yield over three steps.[13]



Scheme 11: Synthesis of ring C

1.2.6 Synthesis of Ring B

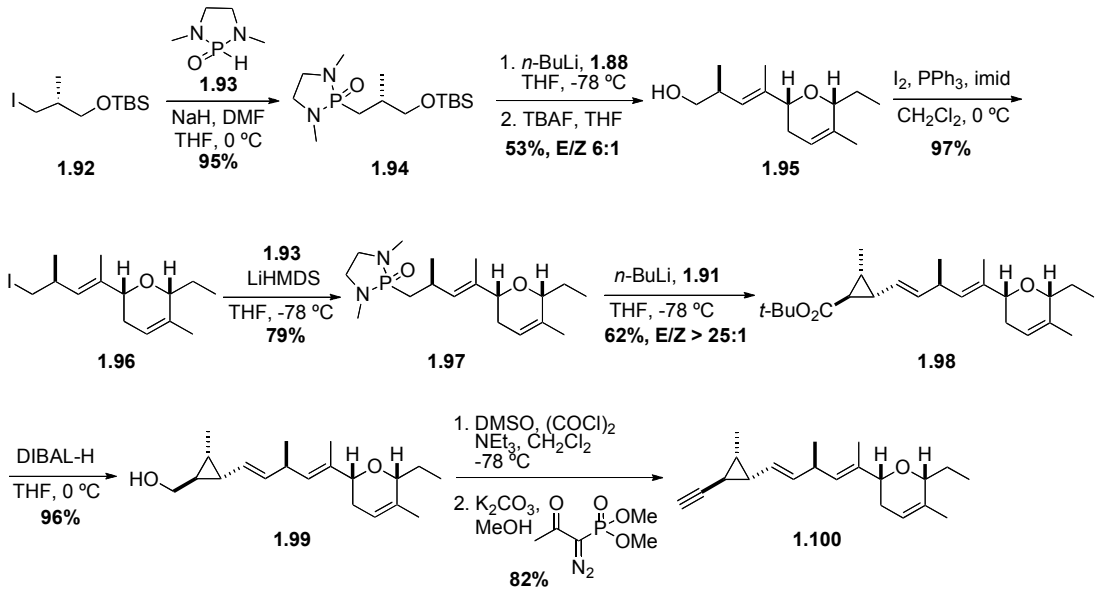
Synthesis of the cyclopropane unit, and ring B of ambruticin S was achieved by other members of our group (Scheme 12).[6] Thus, deprotonation of *trans*-chloro-allyl phosphonamide **1.61**[29] with *n*-butyllithium at low temperature, followed by addition of *tert*-butyl crotonate (**1.89**) afforded the desired cyclopropane **1.90** as a single diastereomer in 89% yield. The phosphonamide chiral auxiliary was cleaved upon ozonolysis of **1.90** to give the crystalline aldehyde **1.91** in 70% yield.[6]



Scheme 12: Synthesis of ring B

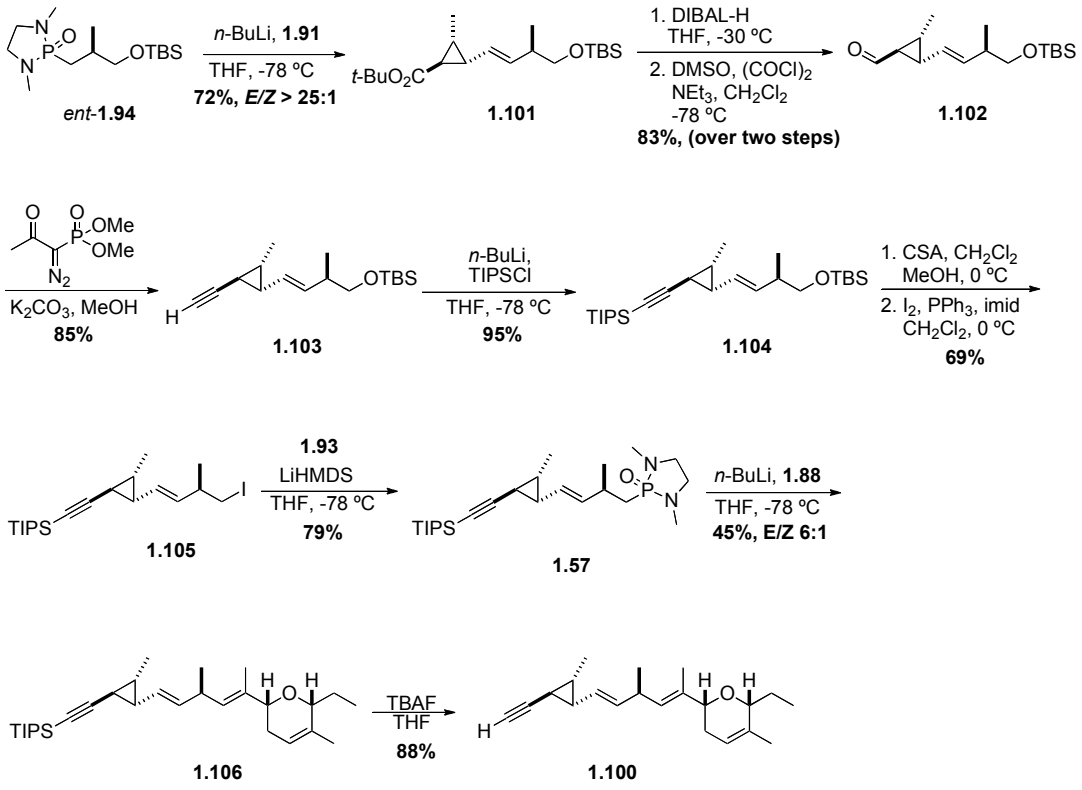
1.2.7 Synthesis of Ring B and Ring C Union Fragment 1.100

With the ring C dihydropyran **1.88** and the ring B cyclopropane **1.91** in hand, two approaches were developed to achieve the union fragment **1.100**.[6] The first approach was realized in a linear fashion starting from dihydropyran **1.88** (Scheme 13). Thus, **1.88** was reacted with phosphonamide **1.94**, which is derived from alkylation of 1,3-dimethyl-2-oxo-1,3,2-diazaphospholidine (**1.93**) with (*R*)-3-*tert*-butyldimethylsilyloxy-2-methylpropyl iodide (**1.92**).[41,42] Deprotonation of **1.94** at low temperature followed by addition of ketone **1.88** and quenching with AcOH afforded a separable 6:1 mixture of *E/Z* isomers, with the desired compound as the major one.[32] Then cleavage of the TBS ether with TBAF gave alcohol **1.95** in 58% yield (two steps). The alcohol **1.95** was then converted into the corresponding iodide in the presence of iodine and PPh_3, followed by displacement of the iodide with the anion of phosphorous acid diamide **1.93** to give phosphonamide **1.97** (76% yield, two steps). Olefination of cyclopropyl aldehyde **1.91** with the lithium anion of **1.97** proceeded with excellent selectivity (*E/Z*>25:1) to give diene **1.98**. Reduction of the *tert*-butyl ester by DIBAL-H gave alcohol **1.99** in 96% yield, followed by Swern oxidation to the corresponding aldehyde, and treatment with the Ohira-Bestmann reagent[43,44] then provided alkyne **1.100** in 82% over the final two steps.



Scheme 13: Synthesis of ring B and ring C union fragment **1.100**, first approach

In a second approach to **1.100** (Scheme 14), reaction of the crystalline aldehyde **1.91** with the anion prepared from phosphonamide *ent*-**1.94** afforded **1.101** with excellent *E/Z* selectivity (>25:1). DIBAL-H reduction of ester **1.101** to an alcohol, then Swern oxidation gave aldehyde **1.100** in 83% yield (two steps). Treatment of **1.102** with the Ohira-Bestmann reagent affored homologation product alkyne **1.103** (71% overall yield). The alkyne moiety in **1.103** was then transformed to its TIPS derivative **1.104**, followed by selective cleavage of the silyl ether. Treatment of the resulting alcohol with iodine and PPh_3 gave iodide **1.105**. The iodide was the converted to **1.57** in 79% yield in the presence of the lithium anion of 1,3-dimethyl-2-oxo-1,3,2-diazaphospholidine (**1.93**) at low temperature. Phosphonamide **1.57** was then deprotonated with *n*-butyllithium, and coupled with ketone **1.88** to give **1.106** as a separable 6:1 mixture of *E/Z* isomers in 45% yield, with the desired *E*-olefin as major isomer. Finally, treatment of **1.106** with TBAF furnished alkyne **1.100** in 88% yield.



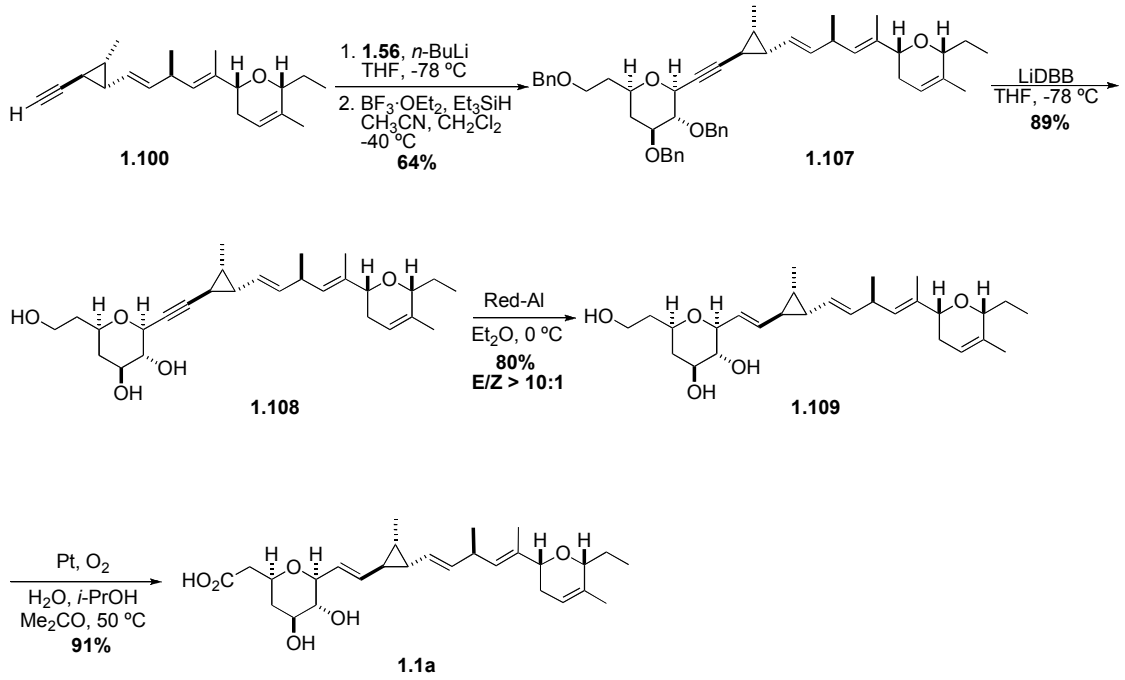
Scheme 14: Synthesis of ring B and ring C union fragment **1.100**, second approach

Comparing these two approaches to fragment **1.100**, the first one (Scheme 13) commenced from the ring C ketone **1.88**, and used sequential phosphonamide anion olefinations to successfully furnish the ring B and ring C containing fragment **1.100**. The second approach resulted in a more convergent synthesis of alkyne **1.100**, and used a reverse sequence, starting from the ring B aldehyde **1.91**. It also employed the reliable phosphonamide anion methodology to produce the *trans*-olefins with good selectivity (*E/Z* >25:1 for **1.101**; *E/Z* >6:1 for **1.106**).

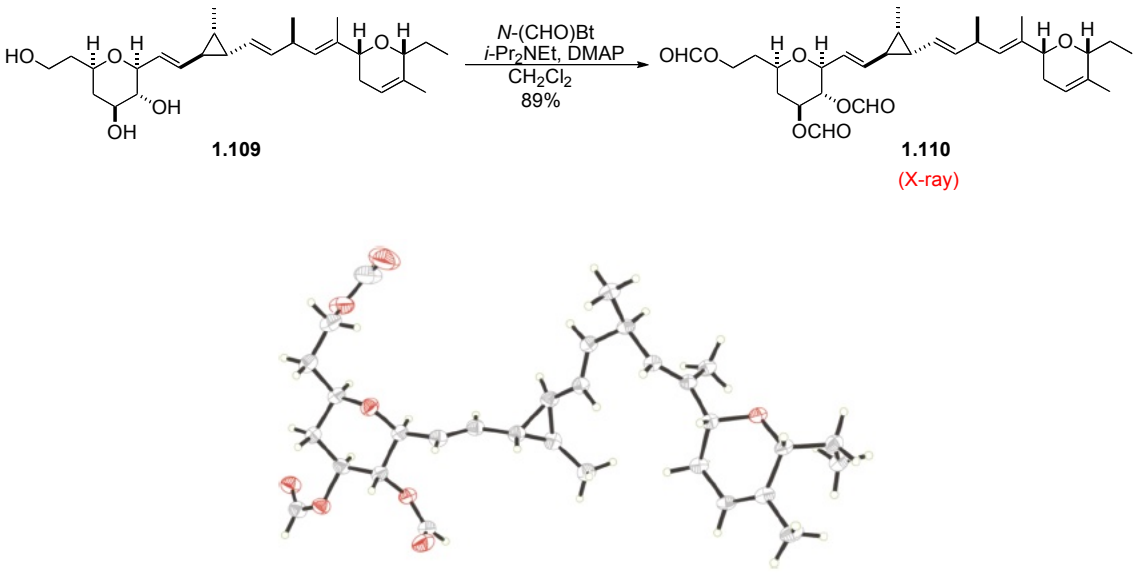
1.2.8 Completion of Synthesis of (+)-Ambruticin S (1.1a)

With alkyne **1.100** and the lactone **1.56** in hand now, the final synthesis of (+)-

ambruticin S (**1.1a**) was accomplished by Dr. Thilo Focken in our group (Scheme 15).[6] Deprotonation of **1.100** with n-butyllithium, followed by addition of lactone **1.56**, led to a 1:1 diastereomeric mixture of the desired products. The anomeric hydroxyl group of the intermidiate was then removed by treatment with triethylsilane and $BF_3 \cdot OEt_2$ at low temperature[45] and afforded *C*-glycoside **1.107** in 66% yield as a single diastereomer with the desired *syn*-stereochemistry of the newly formed tetrahydropyran. Selective removal of the benzyl groups in the presence of lithium 4,4-di-*tert*-butylbiphenylide (LiDBB)[46] provided alkyne **1.108** in 89% yield. Reduction of alkyne **1.108** with sodium bis(2-methoxyethoxy) aluminum hydride (Red-Al) in diethyl ether furnished the known[16] *trans*-triol **1.109** (80% yield, *E/Z*>10:1). Final oxidation of the primary hydroxyl group of triol **1.109** in the presence of the two secondary hydroxyls was accomplished using a method already employed by Liu and Jacobsen.[16] Thus, platinum-catalyzed oxidation of **1.109** with oxygen in aqueous solution at 50 ºC provided (+)-ambruticin S (**1.1a**) in 91% yield. The spectral data (^{1}H and ^{13}C NMR) of synthetic (+)-ambruticin S (**1.1a**) thus obtained were identical with those reported for the natural product. The absolute configuration was also confirmed from an X-ray analysis of the crystal structure of the triformate ester **1.110** corresponding to the reduced triol **1.109** (Scheme 16).[6]



Scheme 15: Completion of synthesis of (+)-ambruticin S (**1.1a**)



Scheme 16: ORTEP drawing of X-ray crystal structure of triformate ester **1.110**

1.2.9 Discussion

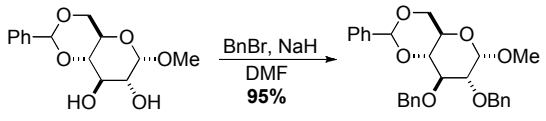
In our strategy for the synthesis of ring A, we started from commercially available and inexpensive methyl α-D-glucopyranoside (**1.65**), and taking advantage of inherent resident chirality, we developed three practical routes for the synthesis of the ring A lactone. In conjunction with other efforts in our group, we then successfully synthesized ambruticin S (**1.1a**). A highly steroselective cyclopropanation(**1.61→1.90**; Scheme 12) (89%, *dr* 99:1) led to the efficient construction of the B ring in only two steps. For the synthesis of ring C, a 6-*endo*-*trig* Lewis acid mediated cycloetherification was used which was adaptable to the synthesis of a variety of substituted analogues.

In summary, we have accomplished highly enantioselective total synthesis of (+)-ambruticin S (**1.1a**) in 17 steps and 5% overall yield (15% based on recovered starting material) in the longest linear sequence starting from glycidol **1.63**. Our approach for the total synthesis of (+)-ambruticin S (**1.1a**) provides a convenient way to a variety of ambruticin derivatives.[6]

1.3 Experimental Procedures

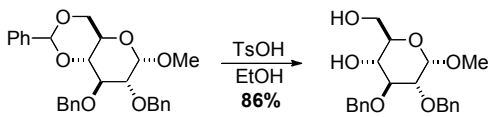
General information: All non-aqueous reactions were run in flame-dried glassware under a positive pressure of argon. Anhydrous solvents were distilled under positive pressure of dry argon before use and dried by standard methods. THF, ether, DCM and toluene were dried by the SDS (Solvent Delivery System), commercial grade reagents were used without further purification. Reactions were monitored by *S-2* analytical thin-layer chromatography (TLC) performed on pre-coated, glass-backed silica gel plates.

Visualization of the developed chromatogram was performed by UV absorbance (wave length: 254 nm), and staining TLC plates with aqueous cerium ammonium molybdate, iodine, or aqueous potassium permanganate solution. Flash chromatography was performed on 230-400 mesh silica gel with the indicated solvent systems. Melting points were recorded on BÜCHI Melting Point B-540. Infrared spectra were recorded on a Perkin-Elmer FTIR Paragon 1000 and were reported in reciprocal centimeters (cm^{-1}). Nuclear magnetic resonance spectra (NMR) were recorded either on Bruker AV-300 MHz and AV-400 MHz spectrometers. Chemical shifts for ^{1}H NMR spectra are recorded in parts per million from tetramethylsilane with the solvent resonance as the internal standard ($CDCl_3$ δ 7.27 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, and br = broad), coupling constants were reported Hz, and integration. Chemical shifts for ^{13}C NMR spectra are recorded in parts per million from tetramethylsilane using the central peak of the solvent resonance as the internal standard ($CDCl_3$, δ 77.00 ppm). All spectra were obtained with complete proton decoupling. Optical rotations were determined in a 1 dm cell at 589 nm at 20 ºC (Perkin Elmer 343). Data are reported as follows: $[\alpha]_D$, concentration (c in g/100 mL), and solvent. High-resolution mass spectra were performed using fast atom bombardment (FAB) or electrospray (ESI) techniques. Low-resolution mass spectra were obtained using electrospray ionisation technique (ESI).



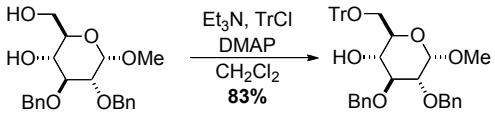
1.64 **1.66**

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**1.66**): Sodium hydride (1.72 g, 43.0 mmol) as a 60% dispersion in mineral oil was added to a stirred 0ºC DMF (90 mL) solution of **1.64** (5.0g, 17.2 mmol) and benzyl bromide (10.45 mL, 43.0 mmol). The resulting suspension was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then diluted with ether (50 mL), followed by the addition of a saturated solution of NH_4Cl (25 mL). The organic layer was separated, washed with a saturated NH_4Cl solution (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 10:1 to 4:1) to give the product **1.66** as a white solid (7.55 g, 95%), mp 90-93 ºC, (hexanes/EtOAc), lit.[47] 95.5-96 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.24 (m, 15H), 5.55 (s 1H), 4.90 (d, *J* = 14.8 Hz, 1H), 4.84 (d, *J* = 12.0 Hz, 1H), 4.81 (d, *J* = 15.2 Hz, 1H), 4.68 (d, *J* = 16.4 Hz, 1H), 4.57 (d, *J* = 4.8 Hz, 1H), 4.25 (dd, *J* = 13.2, 6.0 Hz, 1H), 4.03 (d, *J* = 12.4 Hz, 1H), 3.82-3.77 (m, 1H), 3.68 (t, *J* = 13.6 Hz, 1H), 3.58 (t, *J* = 12.4 Hz, 1H), 3.54 (dd, *J* = 12.4, 4.8 Hz, 1H), 3.38 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 138.6, 138.1, 137.3, 128.8, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.5, 125.9, 101.2, 99.1, 82.0, 79.0, 78.5, 75.3, 73.7, 69.0, 62.2, 55.3 IR (film, NaCl) 3064, 3032, 2917, 2867, 1497, 1452, 1368, 1328, 1212, 1176, 1109, 1088, 1052, 1028, 1001, 921, 738, 695 cm^{-1}. $[\alpha]_D$: (–) 26.8° (c 1.02, $CHCl_3$), lit.[47] $[\alpha]_D$: (–) 30.9° (c 2.0, $CHCl_3$). LRMS (ESI) calcd for $C_{28}H_{31}O_6$ $(M+H)^+$ 463.2, found 463.2.



1.66 **1.67**

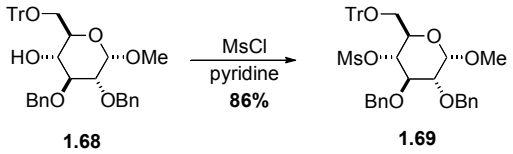
Methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (1.67): *p*-Toluenesulfonic acid monohydrate (44 mg, 0.23 mmol) was added to a stirred ethanol (140 mL) solution of dibenzyl ether methyl 2,3-di-*O*-benzyl-α-D-gycopyranoside **1.66** (8.29 g, 17.9 mmol) at room temperature. The resulting mixture then was heated and refluxed for 18 hours. After cooling to room temperature, the mixture was diluted with chloroform (100 mL) and washed with a saturated solution of $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford a colorless syrup. Colorless crystals **1.67** (5.73 g, 86%) were obtained upon treatment with (hexanes/EtOAc, 10:1), mp 76-78 ºC, (hexanes/EtOAc), lit.[33] 78-79 ºC, (petroleum ether/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.30 (m, 10H), 5.02 (d, *J* = 11.2 Hz, 1H), 4.77 (d, *J* = 12 Hz, 1H), 4.72 (d, *J* = 11.6 Hz, 1H), 4.66 (d, *J* = 12 Hz, 1H), 4.60 (d, *J* = 3.6 Hz, 1H), 3.82-3.72 (m, 3H), 3.63-3.59 (m, 1H), 3.55-3.48 (m, 2H), 3.38 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 138.6, 137.9, 128.5, 128.4, 128.0, 127.9, 98.1, 81.2, 79.7, 75.3, 73.0, 70.6, 70.3, 62.3, 55.2 IR (film, NaCl) 3430, 3063, 3030, 2917, 1496, 1454, 1360, 1155, 1091, 1053, 1028, 909, 737, 697 cm^{-1}. $[\alpha]_D$: (+) 14.0° (c 1.40, $CHCl_3$), lit.[33] $[\alpha]_D$: (+) 16.5° (c 0.279, $CHCl_3$). LRMS (ESI) calcd for $C_{21}H_{26}NaO_6$ $(M+Na)^+$ 397.2, found 397.1.



1.67 **1.68**

Methyl 2,3-di-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (1.68): Triphenylmethyl chloride (4.68 g, 16.8 mmol) was added to a 0 ºC dichloromethane (30

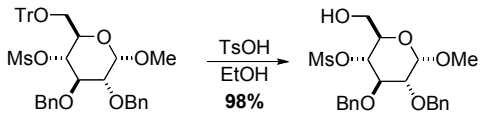
mL) solution of **1.67** (5.73 g, 15.3 mmol), followed by addition of triethylamine (3.2 mL 23.0 mmol), and a catalytic amount of *N*,*N*-dimethylaminopyridine. The reaction mixture was stirred overnight at room temperature, then quenched with a saturated solution of NH_4Cl (10 mL). After phase separation, the aqueous layer was extracted with EtOAc (50 mL) three times. The combined organic phase was washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatograpy (hexanes/EtOAc, 10:1) to give **1.68** as a light yellow viscous syrup (7.85g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.25 (m, 25H), 5.03 (d, *J* = 11.6 Hz, 1H), 4.85-4.80 (m, 2H), 4.75-4.72 (m, 2H), 3.84 (t, *J* = 9.2 Hz, 1H), 3.79-3.75 (m, 1H), 3.63-3.57 (m, 2H), 3.48 (S, 3H), 3.42 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.35 (dd, *J* = 10.0, 5.6 Hz, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ 143.7, 138.7, 138.1, 128.6, 128.4, 127.9, 127.7, 126.9, 97.9, 86.7, 81.5, 79.6, 75.5, 73.0, 71.5, 69.9, 63.8, 55.0. $[\alpha]_D$: (+) 13.0° (c 0.16, $CHCl_3$), lit.[33] $[\alpha]_D$: (+) 4.66° (c 0.3, $CHCl_3$). LRMS (ESI) calcd for $C_{40}H_{40}NaO_6$ $(M+Na)^+$ 639.3, found 639.3.



1.68 **1.69**

Methyl 2,3-di-*O*-benzyl-4-*O*-methanesulfonyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (1.69): A solution of methanesulfonyl chloride (3.00 mL, 38.65 mmol) in chloroform (25 mL) was added slowly to a 0 ºC pyridine (100 mL) solution of **1.68** (6.99 g, 11.3 mmol). The reaction mixture was stirred overnight at room temperature, then poured into water (50 mL) and extracted with diethyl ether (50 mL) three times. The

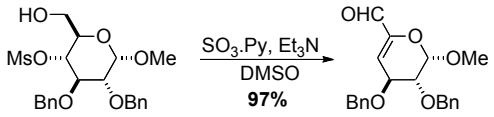
combined organic phase was washed with 5% aqueous HCl solution (100 mL), saturated solution of $NaHCO_3$ (100 mL), water (50 mL), and brine (50 mL), then dried over Na_2SO_4, filtered and concentrated under reduce pressure. The residue was purified by flash chromatograpy (hexanes/EtOAc, 10:1) to give **1.69** as amorphous solid (6.74g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.24 (m, 25H), 5.00 (d, *J* = 10.4 Hz, 1H), 4.84-4.69 (m, 4H), 4.48 (t, *J* = 9.6 Hz, 1H), 3.98-3.94 (m, 2H), 3.66 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.56 (s, 3H), 3.48 (dd, *J* = 10.8, 2.0 Hz, 1H), 3.31 (dd, *J* = 10.4, 6.8 Hz, 1H), 2.55 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 143.5, 137.8, 137.6, 128.7, 128.4, 128.3, 128.0, 127.7, 127.0, 97.4, 86.7, 80.1, 78.9, 78.0, 75.6, 73.3, 68.7, 62.7, 55.2, 38.4. $[\alpha]_D$: (+) 24.60° (c 0.20, $CHCl_3$), lit.[33] $[\alpha]_D$: (+) 20.0° (c 0.195, $CHCl_3$). LRMS (ESI) calcd for $C_{41}H_{42}NaO_8S$ $(M+Na)^+$ 717.2, found 717.2.



1.69 **1.70**

Methyl 2,3-di-*O*-benzyl-4-*O*-methanesulfonyl-α-D-glucopyranoside (1.70): *p*-Toluenesulfonic acid (72 mg, 0.38mmol) was added to a ethanol (200 mL) solution of **1.69** (6.74 g, 9.71 mmol). The resulting mixture was heated to reflux overnight. The reaction mixture was diluted with chloroform (100 mL), washed with a saturated solution of $NaHCO_3$ (100 mL), water (100 mL), and brine (100 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduce pressure. The residue was purified by flash chromatograpy (hexanes/EtOAc, 2:1) to give **1.70** as a colorless oil (4.31g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.28 (m, 10H), 5.12-5.07 (d, *J* = 12.8 Hz, 1H),

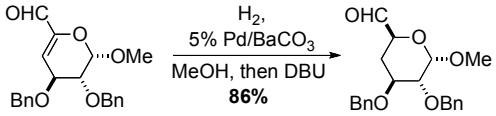
4.75 (d, J = 12.0 Hz, 2H), 4.69-4-62 (d, J = 11.2 Hz, 2H), 4.48 (t, J = 9.6 Hz, 1H), 4.05 (t, J = 9.6 Hz, 1H), 3.92 (dd, J = 12.8, 3.2 Hz, 1H), 3.78 (dd, J = 13.2, 2.0 Hz, 1H), 3.73 (dt, J = 10.0, 2.4 Hz, 1H), 3.60 (dd, J = 9.6, 3.6 Hz, 1H), 3.39 (s, 3H), 2.80 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 137.7, 137.3, 128.5, 128.4, 128.1, 127.8, 127.6, 97.6, 80.0, 78.4, 77.5, 75.6, 73.2, 69.5, 60.2, 55.4, 38.1. $[\alpha]_D$: (+) 36.1° (c 0.24, $CHCl_3$), lit.[33] $[\alpha]_D$: (+) 40.9° (c 0.235, $CHCl_3$). LRMS (ESI) calcd for $C_{22}H_{28}NaO_8S$ $(M+Na)^+$ 475.1, found 475.1.



1.70 **1.71**

Methyl 2,3-di-*O*-benzyl-4-deoxy-*β*-*L*-threo-hex-4-ene-dialdopyranoside (1.71): A solution of triethylamine (66 mL, 475 mmol) and sulfur trioxide pyridine complex (13.6 g, 85.5 mmol) in dimethylsulfoxide (38 mL) was added dropwise to a 0 ºC dichloromethane (75 mL) solution of **1.70** (4.30 g, 9.5 mmol). After 15 min the reaction mixture was allowed to warm to room temperature and stirred for three hours. Subsequently, the mixture was partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The organic phase was washed with 1 N HCl (50 mL), saturated solution of $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL), then dried over Na_2SO_4, filtered and concentrated under reduce pressure. The residue was purified by flash chromatograpy (hexanes/EtOAc, 5:1) to give **1.71** as a yellowish, viscous syrup (3.29g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.39-7.32 (m, 10H), 5.89 (d, J = 2.8 Hz, 1H), 4.96 (d, J = 2.4 Hz, 1H), 4.84 (d, J = 12.4 Hz, 1H), 4.79 (d, J = 12.2 Hz, 2H), 4.74 (d, J = 11.6 Hz,

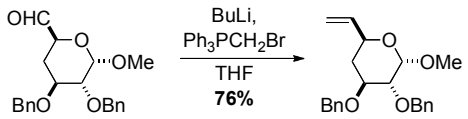
2H), 4.51 (dd, J = 8.0, 2.8 Hz, 1H), 3.83 (dd, J = 8.0, 2.4 Hz, 1H), 3.48 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 185.9, 148.2, 137.7, 137.6, 128.4, 128.0, 127.9, 127.8, 127.7, 120.4, 99.6, 76.2, 73.3, 73.1, 72.4, 56.8. $[\alpha]_D$: (+) 179.9° (c 0.68, $CHCl_3$), lit.[33] $[\alpha]_D$: (+) 147.7° (c 0.627, $CHCl_3$). LRMS (ESI) calcd for $C_{21}H_{26}NO_5$ $(M+NH_4)^+$ 372.2, found 372.1.



1.71 **1.72**

Methyl 2,3-di-*O*-benzyl-4-deoxy-α-D-xylo-hexodialdopyranoside (1.72). 5% palladium on barium carbonate (540 mg) was added to a methanol (16 mL) solution of **1.71** (500 mg, 1.41 mmol). The resulting mixture was stirred under an atmosphere of hydrogen (14.5 psi) for 12 hours. Palladium was then removed by filteration through a pad of Celite, the filtrate was concentrated under reduced pressure to a volume of approximately 8 mL, and 1,8-diazabicycloundec-7-ene (1.2 mL, 8.5 mmol) was added to it. The reaction mixture was stirred for additional 12 hours. Then it was concentrated to dryness under reduced pressure. The residue was dissolved in dichloromethane (25 mL), washed with 2 M aqueous HCl solution (10 mL), saturated solution of $NaHCO_3$ (10 mL), and brine (25 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 4:1 to 3:1) to give **1.72** as a colorless oil. (432 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.34-7.29 (m, 10 H), 4.87 (d, J = 12.4 Hz, 1H), 4.77-4.74 (m, 3H), 4.69 (d, J = 2.0 Hz, 1H), 4.18 (dd, J = 12.4, 2.8 Hz, 1H), 3.98 (ddd, J = 10.8, 9.2, 5.2 Hz, 1H), 3.48 (dd, J = 9.2, 3.2 Hz, 1H), 3.41 (s, 3H), 2.36 (ddd, J = 13.2, 5.2, 2.8 Hz, 1H), 1.48 (q, J = 12.4 Hz, 1H); ^{13}C NMR

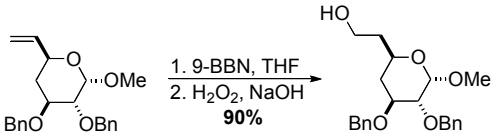
(100 MHz, $CDCl_3$) δ 199.5, 138.3, 138.1, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 99.3, 79.7, 74.5, 73.5, 72.4, 72.2, 55.6, 31.6; $[\alpha]_D$: (+) 35.0° (c 1.05, $CHCl_3$), lit.[33] $[\alpha]_D$: (+) 23.2° (c 2.95, $CHCl_3$). LRMS (ESI) calcd for $C_{21}H_{25}O_5$ $(M+H)^+$ 357.2, found 357.1.



1.72 **1.73**

Methyl 6-*C*-vinyl-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-glucopyranoside (1.73). n-BuLi (10.5 mL of a 1.6M solution in hexanes, 16.9 mmol) was added slowly to a stirred suspension of methyltriphenylphosphonium bromide (6.0 g, 16.9 mmol) in THF (60 mL) at -78 ºC. After 10 min, the resulting solution was allowed to warm to 0 ºC and stirred for 1 hour. A solution of **1.72** (2.0 g, 5.6 mmol) in THF (60 mL) was then added slowly to the ylide at 0 ºC. The reaction mixture was allowed to warm to temperature, stirred overnight, and subsequently quenched by addition of saturated NH_4Cl solution (10 mL). After extraction of the aqueous layer with diethyl ether (50 mL) three times, the combined organic phase was washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 10:1 to 8:1) to give **1.73** as a colorless oil, (1.51 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.26 (m, 10H), 5.82 (ddd, *J* = 17.6, 9.6, 6.0 Hz, 1H), 5.76 (dt, *J* = 17.2, 1.6 Hz, 1H), 5.14 (dt, *J* = 10.4, 1.2 Hz, 1H), 4.86 (d, *J* = 12.0 Hz, 1H), 4.78 (d, *J* = 12.4 Hz, 1H), 4.72 (d, *J* = 11.6 Hz, 1H), 4.70 (d, *J* = 11.4 Hz, 1H),

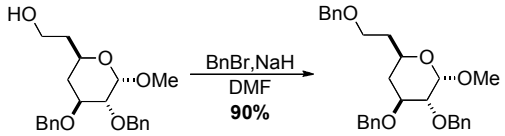
4.69 (d, J = 3.6 Hz, 1H), 4.21 (dd, J = 11.6, 5.6 Hz, 1H), 3.97 (ddd, J = 11.2, 9.2, 4.8 Hz, 1H), 3.49 (dd, J = 5.6, 3.6 Hz, 1H), 3.39 (s, 3H), 2.14 (ddd, J = 12.8, 4.8, 2.0 Hz, 1H), 1.49 (q, J = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7, 138.4, 138.3, 128.2, 127.9, 127.6, 128.5, 127.4, 115.7, 99.0, 80.3, 75.1, 73.2, 72.4, 67.6, 55.1, 37.1; $[\alpha]_D$: (+) 34.1° (c 0.50, $CHCl_3$); IR (film, NaCl) 3063, 3030, 2922, 2854, 1683, 1650, 1604, 1496, 1454, 1358, 1259, 1192, 1099, 1044, 924, 813, 733, 696 cm^{-1}; HRMS (ESI) calcd for $C_{22}H_{26}NaO_4$ $(M+Na)^+$ 377.1723, found 377.1722.



1.73 **1.74**

Methyl 6-C-(hydroxymethyl)-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-glucopyranoside (1.74). 9-BBN (24.6 mL of a 0.5 M solution in THF, 12.3 mmol) was added to a THF (10 mL) solution of **1.73** (545 mg, 1.54 mmol). The reaction mixture was stirred overnight at room temperature. Hydrogen peroxide (3.2 mL of a 30% aqueous solution, 30.8 mmol) and 3 M NaOH solution (5.1 mL, 15.4 mmol) were then added, and the resulting mixture was heated for 2 hours at 55 ºC. After phase separation, the aqueous phase was extracted with EtOAc (25 mL) three times. The combined organic phase was washed with water (25 mL), brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes: EtOAc, 2:1) to give **1.74** as a colorless oil, (516 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (m, 10H), 4.86 (d, J = 12.0 Hz, 1H), 4.78 (d, J = 11.6 Hz, 1H), 4.72 (d, J = 11.6 Hz, 1H), 4.70

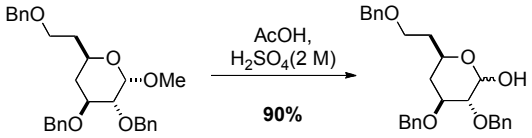
(d, *J* = 12.0 Hz, 1H), 4.66 (d, *J* = 2.4 Hz, 1H), 4.00-3.91 (m, 2H), 3.79-3.76 (m, 2H), 3.48 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.38 (s, 3H), 2.07 (ddd, J = 12.8, 4.8, 2.0 Hz, 1H), 1.78-1.72 (m, 2H), 1.48 (q, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.1, 128.0, 128.0, 127.6, 127.4, 127.3, 127.1, 98.6, 80.1, 74.8, 73.0, 72.2, 66.7, 60.5, 54.8, 37.7, 36.9; IR (film, NaCl) 3435, 3062, 3030, 2923, 1604, 1496, 1454, 1356, 1200, 1186, 1099, 1048, 996, 911, 736, 698 cm-1; $[\alpha]_D$: (+) 39.1° (c 3.00, $CHCl_3$); HRMS (ESI) calcd for $C_{22}H_{28}NaO_5$ $(M+Na)^+$ 395.1829, found 395.1820.



1.74 **1.75**

Methyl 6-*C*-(benzyloxymethyl)-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-glucopyranoside (1.75). Sodium hydride (86 mg, 2.15 mmol) as a 60% dispersion in mineral oil was added to a stirred 0 ºC DMF (7 mL) solution of alcohol **1.74** (320 mg, 0.86 mmol) and benzyl bromide (0.52 mL, 4.3 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. After diluting the mixture with diethyl ether (10 mL), it was quenched by careful addition of saturated NH_4Cl solution (2 mL). The organic layer was separated, washed with saturated solution of NH_4Cl (10 mL), brine (25 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc, 4:1) to give tribenzyl ether **1.75** as a colorless oil, (364 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.28 (m, 15H), 4.89 (d, *J* = 12.2 Hz, 1H), 4.80 (d, *J* = 11.7 Hz, 1H), 4.74-4.68 (m, 2H), 4.68 (d, *J* = 3.6 Hz, 1H),

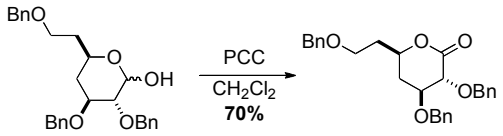
4.53 (br s, 2H), 3.97-3.91 (m, 2H), 3.66-3.57 (m, 2H), 3.51 (dd, *J* = 9.4, 3.6 Hz, 1H), 3.38 (s, 3H), 2.11 (ddd, *J* = 12.8, 5.0, 2.1 Hz, 1H), 1.84-1.78 (m, 2H), 1.43 (q, *J* = 12.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.3, 138.0, 128.0, 128.0, 127.6, 127.3, 127.3, 127.2, 127.2, 127.1, 98.5, 80.3, 75.1, 72.9, 72.7, 72.1, 66.3, 63.8, 54.5, 37.3, 35.1; IR (film, NaCl) 3062, 3030, 2922, 2861, 1604, 1586, 1496, 1454, 1357, 1245, 1204, 1188, 1099, 1048, 913, 783, 735, 698 cm^{-1}; $[\alpha]_D$: (+) 40.0° (c 1.00, $CHCl_3$); HRMS (ESI) calcd for $C_{29}H_{34}O_5Na$ $(M+Na)^+$ 485.2299, found 485.2275.



1.75 **1.76**

6-*C*-(Benzyloxymethyl)-4,6-dideoxy-2,3-di-*O*-benzyl-α,*β*-D-glucopyranoside (1.76). A diluted sulfuric acid (2 M, 34 mL) was added to a solution of tribenzyl ether **1.75** (600 mg, 1.3 mmol) in acetic acid (130 mL). The resulting solution was heated for 18 hours at 80 ºC. After removal of all volatiles under reduced pressure, the residue was dissolved in dichloromethane (150 mL). The organic phase was washed with saturated solution of $NaHCO_3$ (50 mL), brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc, 7:3) to give the hemiacetal **1.76** as a colorless oil (524 mg, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.30 (m, 15H), 5.23 (d, *J* = 3.6 Hz, 1H), 4.95-4.85 (m, 2H), 4.76-4.69 (m, 2H), 4.61-4.58 (m, 1H), 4.52-4.50 (m, 2H), 3.66-3.56 (m, 3H), 3.52-3.47 (m, 1H), 2.16-2.06 (m, 1H), 1.89-1.77 (m, 2H), 1.49-1.38 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ

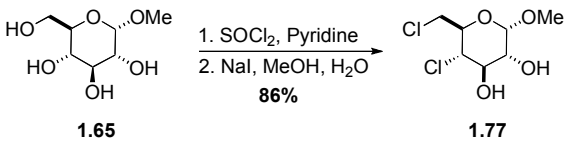
138.4, 138.3, 138.2, 138.1, 138.1, 137.9, 128.1, 128.0, 127.7, 127.6, 127.4, 127.4, 127.4, 127.3, 127.2, 127.2, 127.2, 97.0, 91.7, 83.6, 80.2, 78.2, 74.9, 74.5, 72.9, 72.6, 71.7, 71.7, 68.4, 66.2, 65.8, 64.6, 53.0, 36.6, 35.0; IR (film, NaCl) 3392, 3062, 3030, 2921, 2862, 1496, 1454, 1362, 1207, 1096, 911, 736, 697 cm^{-1}; $[\alpha]_D$: (+) 23.1° (c 2.00, $CHCl_3$); LRMS (ESI) calcd for $C_{28}H_{32}NaO_5$ $(M+Na)^+$ 471.2, found 471.2.



1.76 **1.56**

(3*R*,4*S*,6*R*)-3,4-Bis(benzyloxy)-6-(2-(benzyloxy)ethyl)-tetrahydropyran-2-one (1.56). 4 Å Molecular sieves (1.0 g) were added to a stirred CH_2Cl_2 (10 mL) solution of the hemiacetal **1.76** (400 mg, 0.89 mmol). After 15 min, the mixture was cooled to 0 ºC, PCC (880 mg, 4.02 mmol) was then added, and the reaction mixture was stirred for 2 hours at 0 ºC. After dilution with diethyl ether (10 mL) and pentane (10 mL), the mixture was filtered over Celite. The filter cake was washed with Et_2O/hexane (1:1, 50 mL), and the combined filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc, 4:1) to give lactone **1.76** as a colorless oil, (280 mg, 70%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.45-7.31 (m, 15H), 5.07 (d, *J* = 11.6 Hz, 1H), 4.77 (d, *J* = 11.6 Hz, 1H), 4.69-4.60 (m, 3H), 4.56 (d *J* = 11.8 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 4.07 (d, *J* = 6.8 Hz, 1H), 3.96-3.90 (m, 1H), 3.74-3.67 (m, 1H), 3.65-3.60 (m, 1H), 2.33 (ddd, *J* = 14.0, 5.6, 3.2 Hz, 1H), 2.06-1.89 (m, 2H), 1.77 (q, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 137.8, 137.4, 136.9, 128.1, 128.07, 128.0, 127.6, 127.4, 127.3, 127.3, 79.0, 75.3, 73.7, 73.3, 72.8, 71.6, 65.3, 35.5, 34.6; IR (film, NaCl)

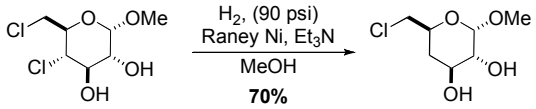
3063, 3030, 2924, 2865, 1745, 1496, 1454, 1392, 1212, 1179, 1101, 1028, 911, 737, 698 cm^{-1}; $[\alpha]_D$: (+) 69.4° (c 2.00, $CHCl_3$); HRMS (ESI) calcd for $C_{28}H_{31}O_5$ $(M+H)^+$ 447.2166, found 447.2155.



1.65 **1.77**

Methyl 4,6-dichloro-4,6-dideoxy-α-D-glucopyranoside (1.77). Sulfuryl chloride (2.6 mL, 32.4 mmol) was added dropwise to a vigorous stirred chloroform (10 mL) suspension of methyl α-D-glucopyranoside **1.65** (1.0 g, 5.15 mmol) and pyridine (4.4 mL, 54.4 mmol) at -40 ºC. The reaction mixture was stirred for 3 hours and then it was allowed to warm to room temperature and stirred overnight. The mixture was diluted with chloroform (25 mL) and washed with 10% aqueous H_2SO_4 solution (5 mL), followed by saturated solution of $NaHCO_3$ (5 mL) water (15 mL), and brine (15 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield an oily residue, which was dissolved in methanol (15 mL). To this mixture was added a solution of sodium iodide (1.12 g, 7.5 mmol) in MeOH/water (1:1, 2 mL). The resulting solution was left to stand for 8 hours and then neutralized with $NaHCO_3$. Evaporation of all volatiles gave a residue, which was extracted with hot chloroform (25 mL) and hot ethyl acetate (25 mL). The combined organic layers were dried over $MgSO_4$ and recrystallized (EtOAc/hexanes) to give dichloride **1.77** as colorless needles (1.02 g, 86%): mp 156-157 ºC (EtOAc/hexanes), lit.[48] 119-121 ºC (petroleum ether/$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.73 (d, J = 5.2 Hz, 1H), 4.45 (d, J = 4.8 Hz, 1H), 4.15 (t, J =

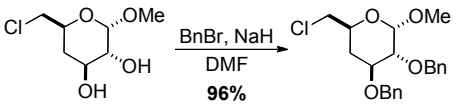
4.86 Hz, 1H), 3.99 (dd, *J* = 13.2, 4.8 Hz, 1H), 3.78 (dd, *J* = 13.2, 5.2 Hz, 1H), 3.68 (dd, *J* = 7.6, 2.8 Hz, 2H), 3.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.6, 71.0, 70.0, 69.7, 65.0, 55.9, 44.5; IR (film, NaCl) 3429, 2067, 1641, 1363, 1261, 1196, 1135, 1076, 1045, 1032, 986 cm^{-1}; $[\alpha]_D$: (+) 187.8° (c 2.00, H_2O), lit.[48] $[\alpha]_D$: (+) 121.0° (c 1.8, H_2O). LRMS (ESI) calcd for $C_7H_{13}Cl_2O_4$ $(M+H)^+$ 231.0, 233.0, found 231.1, 233.1.



1.77 **1.78**

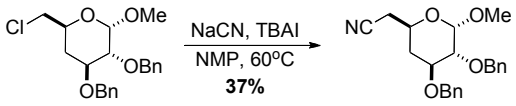
Methyl 6-chloro-4,6-dideoxy-α-D-glucopyranoside (1.78). A solution of dichloride **1.77** (1.00 g, 4.35 mmol) in MeOH (30 mL) containing triethylamine (1.3 mL, 9.3 mmol) and Raney-nickel (Raney2800, 2.00 g) was subjected to a hydrogen pressure of 90 psi for 24 hours. Raney-nickel was filtered off, and the filtrate was concentrated under reduced pressure. Brine (10 mL) was added to the residue and the aqueous layer was extracted with warm ethyl acetate (15 mL) three times. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1) to give **1.78** as a white solid (596 mg, 70%): mp 106-107 ºC, (hexanes/EtOAc), lit.[49] 110-111 ºC, (petroleum ether/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (d, *J* = 4.0 Hz, 1H), 3.99 (ddt, *J* = 17.2, 5.6, 2.0 Hz, 1H), 3.89 (ddd, *J* = 11.4, 9.2, 4.8 Hz, 1H), 3.58 (d, *J* = 5.2 Hz, 2H), 3.47 (s, 3H), 3.43 (dd, *J* = 9.2, 3.6 Hz, 1H), 2.10 (ddd, *J* = 12.4, 4.8, 2.0 Hz, 1H), 1.53 (q, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 99.3, 73.9, 68.3, 67.6, 55.1, 46.2, 35.1; IR (film, NaCl) 3306, 2955, 2920, 1645, 1468, 1451, 1382, 1355, 1339, 1190, 1130, 1081, 1047, 906, 775,

729cm-1; $[\alpha]_D$: (+) 159.0° (c 0.50, CH_3OH), lit.[49] $[\alpha]_D$: (+) 165.0° (c 1.02, CH_3OH). LRMS (ESI) calcd for $C_7H_{14}ClO_4$ $(M+H)^+$ 196.1, 198.0, found 196.2, 198.1.



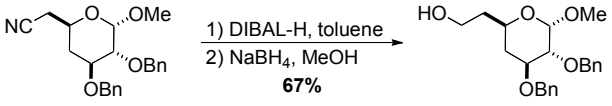
1.78 **1.79**

Methyl 6-chloro-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-glucopyranoside (1.79). Sodium hydride (500 mg, 12.5 mmol) as a 60% dispersion in mineral oil was added portionwise to a stirred DMF (12.5 mL) solution of diol **1.78** (980 mg, 5.0 mmol) and benzyl bromide (1.50 mL, 12.5mmol) at 0 ºC. The resulting suspension was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then diluted with ether (25 mL), and saturated solution of NH_4Cl (25 mL) was added carefully. The organic layer was separated and washed with saturated solution of NH_4Cl (25 mL), brine (25 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure gave a yellowish oil, which was purified by column chromatography (hexanes/EtOAc, 4:1) to give dibenzyl ether **1.79** as a colorless oil, **(**1.80 g, 96%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.41-7.30 (m, 10H), 4.87 (d, J = 12.0 Hz, 1H), 4.79 (d, J = 11.6 Hz, 1H), 4.73-4.69 (m, 3H), 4.00-3.94 (m, 2H), 3.53 (d, J = 5.2 Hz, 2H), 3.50 (dd, J = 9.2, 3.6 Hz, 1H), 3.42 (s, 3H), 2.16 (ddd, J = 12.8, 4.8, 2.0 Hz, 1H), 1.52 (q, J = 12.0 Hz, 1H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 138.4, 138.1, 128.0, 128.0, 127.7, 127.4, 127.3, 127.2, 98.7, 79.9, 74.5, 73.0, 72.3, 67.0, 54.9, 46.3, 34.5; IR (film, NaCl) 3030, 2919, 1496, 1454, 1372, 1354, 1199, 1182, 1111, 1047, 999, 914, 737, 697 cm^{-1}; $[\alpha]_D$: (+) 159.0° (c 2.00, $CHCl_3$); HRMS (ESI) calcd for $C_{21}H_{25}ClNaO_4$ $(M+Na)^+$ 399.1334, found 399.1330.



1.79 **1.80**

Methyl 6-cyano-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-glucopyranoside (1.80). NaCN (490 mg, 10.0 mmol) and n-Bu_4NI (3.70 g, 10.0 mmol) were added to a solution of chloride **1.79** (376 mg, 1.0 mmol) in N-methylpyrrolidone (10 mL). The reaction mixture was heated to 60 ºC for 48 hours, after which it was diluted with ether (25 mL) and saturated solution of $NaHCO_3$ (25 mL) was added. The organic layer was separated, washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 4:1) to give **1.80** as a colorless oil, (136 mg, 37%); starting material **1.79** was recovered (154 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.26 (m, 10H), 4.85 (d, *J* = 12.4Hz, 1H), 4.77 (d, *J* = 11.6Hz, 1H), 4.69 (d, *J* = 12.4 Hz, 1H), 4.68 (d, *J* = 11.6 Hz, 1H), 4.66 (d, *J* = 3.6 Hz, 1H), 4.03-3.90 (m, 2H), 3.48 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.40 (s, 3H), 2.52-2.50 (m, 2H), 2.16 (ddd, *J* = 12.8, 5.2, 2.4 Hz, 1H), 1.50 (q, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 138.1, 128.3, 128.28, 127.9, 127.7, 127.5, 116.7, 99.0, 79.8, 74.3, 73.3, 72.6, 63.1, 55.4, 36.5, 23.7; IR (film, NaCl) 3063, 3031, 2925, 2252, 1722, 1602, 1496, 1454, 1356, 1275, 1190, 1107, 1044, 919, 804, 739, 714, 699 cm-1; $[\alpha]_D$: (+) 23.2° (c 1.75, $CHCl_3$); HRMS (ESI) calcd for $C_{22}H_{26}NO_4$ $(M+H)^+$ 368.1856, found 368.1838.



1.80 **1.74**

Methyl 6-*C*-(hydroxymethyl)-4,6-dideoxy-2,3-di-*O*-benzyl-*α*-D-glucopyranoside (1.74). DIBAL-H (0.6 mL of a 1 M in hexanes, 0.6 mmol) was added to a stirred 0 ºC toluene (5 mL) solution of nitrile **1.80** (184 mg, 0.5 mmol). After stirring for 30 min at that temperature, the reaction mixture was quenched by addition of methanol (1 mL), followed by addition of aqueous HCl solution (2 N, 1.0 mL). The mixture was stirred for 45 min and filtered. After phase separation of the filtrate, the aqueous layer was extracted with diethyl ether (10 mL) three times. The combined organic phase was washed with 2N HCl solution (10 mL), saturated solution of $NaHCO_3$ (10 mL), and water (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford a colorless syrup. This residue was dissolved in methanol (5 mL), and sodium borohydride (38 mg, 1.0 mmol) was added to it. The mixture was stirred at room temperature for 30 min, before excess sodium borohydride was destroyed by the addition of acetone (1 mL). Evaporation under reduced pressure gave a residue, which was co-evaporated with methanol (5 mL) three times to remove boron by-products. The residue was dissolved in ethyl acetate (10 mL), washed with 2 N HCl solution (5 mL), saturated $NaHCO_3$ solution (5 mL), and water (5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 1:1) to give alcohol **1.74** as a colorless oil (125 mg, 67%).

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Chapter Two

Synthesis of Antisense *cis*- and *trans*-[4.3.0]Bicyclic Nucleosides

2.1 Introduction

2.1.1 Antisense Therapies

Disease symptoms are sometimes caused by the abnormal production of certain proteins, for example, Duchenne muscular dystrophy is caused by the absence of dystrophin, which is responsible for the growth of muscle fiber. If the abnormal production of a specific protein can be prevented, then the corresponding diseases can be treated. Traditional drug therapies are usually based on using rationally designed compounds to block or inhibit the disease-causing proteins (Figure 1). In recent years, non-small molecule therapy has attracted the attention of many research groups and pharmaceutical companies and has been widely studied. There are several approaches to this type of therapy including: antisense,[1,2] antigen,[3] ribozymes,[3] micro RNA (miRNA),[4] short interfering RNA (siRNA),[5] and aptamers.[3] Targeting the disease-causing gene, the nucleic acid-based therapies aim to disturb undesired protein production at an early stage compared with traditional drug therapies, thus, representing a new and promising trend in drug discovery (Figure 2).

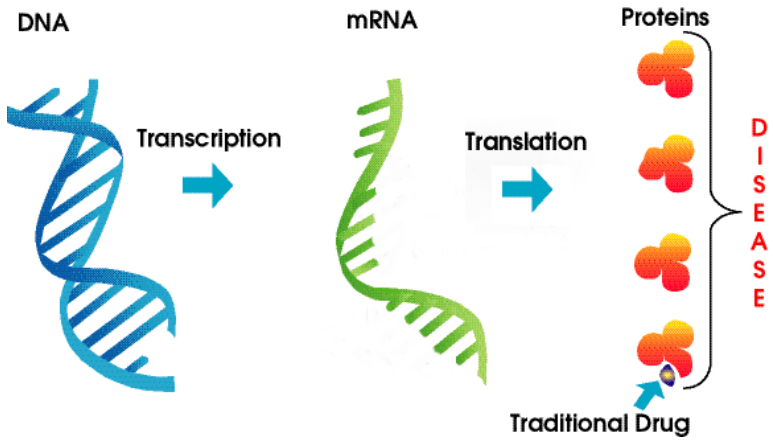


Figure 1: Traditional drug therapy.

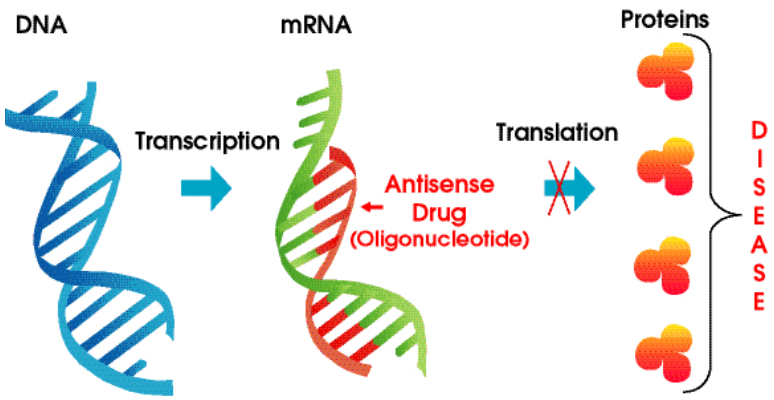


Figure 2: Antisense therapy.

Production of proteins *in vivo* involves two stages: 1) transcription – the process of converting DNA to messenger RNA (mRNA) and 2) translation – the process of converting mRNA into protein. During transcription double stranded DNA is separated into two single strands of DNA, via the action of a helicase enzyme. The unwinding and separation of the strands involves breaking hydrogen bonds between complementary base pairs. Once separated, an enzyme known as RNA polymerase reads the single stranded DNA and creates a complementary RNA sequence known as messenger RNA. The mRNA strand created during transcription is decoded in the ribosome to create a specific amino acid sequence that is eventually synthesized into a protein. If this transcription / translation process is carried out on a disease gene, unwanted proteins will be synthesized that can cause debilitating symptoms.

The basis of antisense therapy is to stop the translation of diseased mRNA. If the DNA sequence of a disease gene is known, then a synthetic strand (oligonucleotide) of DNA or RNA can be created to bind to the mRNA. If the binding event is successful,

mRNA cannot be transcribed, since it needs to be single stranded, and thus the production of the disease protein is shut off. This binding mRNA can activate an enzyme called RNase H, which will ultimately degrade the mRNA complex before protein synthesis occurs (Figure 2).

Antisense therapy has shown promise in combating different forms of cancer, HIV/AIDS, and other diseases.[1] One antisense drug, Vitravene (fomivirsen) (Figure 3),[6] discovered at ISIS pharmaceuticals has been used in the treatment of cytomegalovirus retinitis (CMV), and was approved by the FDA in 1998. Although this is the only licensed antisense drug, other antisense drug candidates are currently in the late-phase clinical trials.[7]

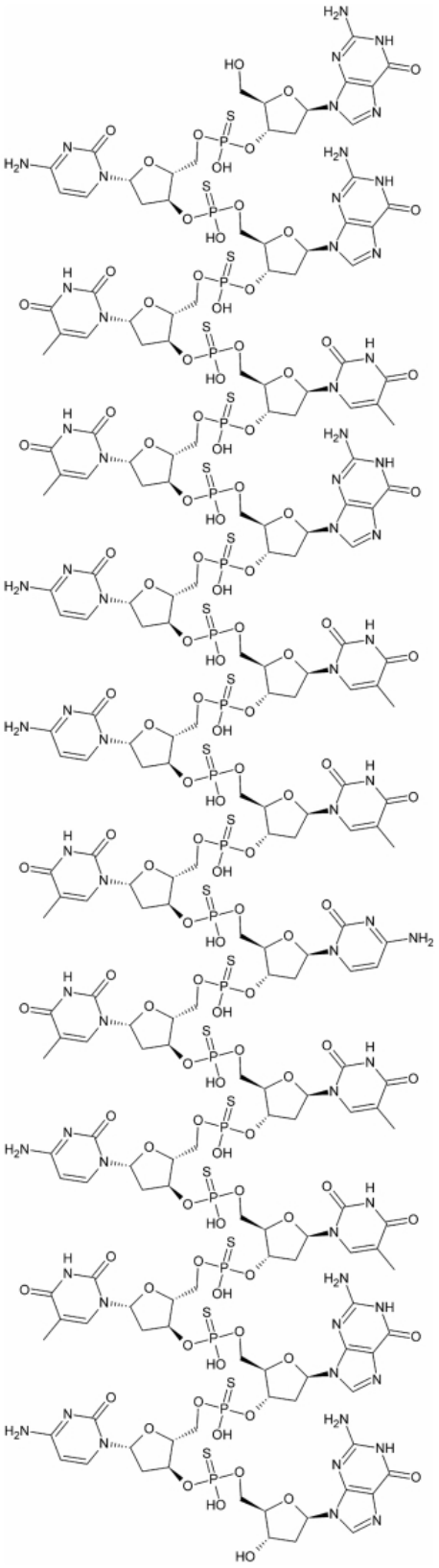


Figure 3: Stucture of Vitravene (fomivirsen).

2.1.2 Antisense Oligonucleotides

The short nucleic acid strand used to bind to mRNA and terminate its translation into disease proteins was termed an antisense oligonucleotide.[1] Due to the complementary base sequence of these oligonucleotides to the gene's mRNA sequence, which is known as a "sense" sequence, these nucleotides bear the name antisense. In general, an antisense nucleotides consists of 12–21 nucleotides, and its composition can include both naturally occurring or chemically modified single-stranded nucleic acids. Due to the poor binding affinity to mRNA and insufficient stability and pharmacokinetics in biological systems, naturally occurring nucleotides are limited in their use in antisense technology and often, chemically modified nucleotides are required for the synthesis of functional antisense oligonucleotides as potential drug candidates.[1] There are usually four potential ways to modify oligonucleotides (Figure 4).

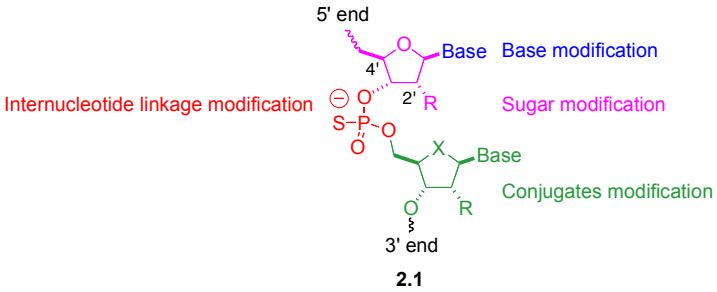


Figure 4: Modification of an oligonucleotide.[1]

For the internucleotide linkage, there are usually two types: phosphate and nonphosphate linkages. Phosphate containing oligonucleotides have been most widely studied, such as phosphodiesters (PO) **2.2**, methylphosphonates **2.3**, boranophosphates **2.4**, and phosphorothioates (PS) **2.5** (Figure 5). Phosphorothioate (PS) **2.5** is the most

widely used phosphate linkage in present antisense drug candidates. It increases the stability towards nucleolytic degradation, and PS oligodeoxynucleotides can efficiently promote cleavage of the target RNA by RNase H.[1]

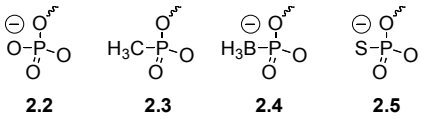


Figure 5: Examples of phosphate linkages.

Base modification can also play an important role in nucleoside design. Purine and pyrimidine bases are important in hydrogen bonding in nucleic acids. The physical properties of oligonucleotides are highly sensitive to modification of the bases and thus modifying these bases can have a dramatic effect on the size, conformation, pK_a and stereoelectronics of the molecules.[8] For example, 5-(1-propynyl)-2′-deoxyuridine (**2.6)** can enhance the stability of a triple-helix complex relative to thymidine **2.7** (Figure 6).[9] This suggests that an SAR study on modified base analogs could be very useful to provide important information for optimum base design.

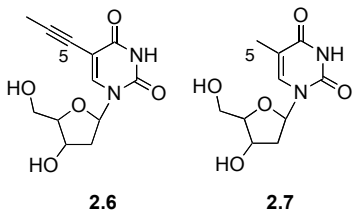
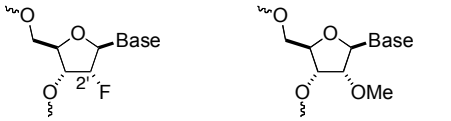


Figure 6: C-5 Propynyl-modified nucleosides.

Conjugation of oligonucleotides with diverse moieties in various positions (such as 3′ position of sugar moiety) could alter the pharmacokinetic properties of oligonucleotide drugs (Figure 4). Many conjugation strategies have been studied. Cholesterol conjugates are one of the most widely studied conjugates. For example, a 3′,5′-bis-cholesteryl-conjugated phosphorothioate oligodeoxynucleotide ISIS-9389 reported by Leiden/Amsterdam Center for Drug Research and ISIS Pharmaceuticals was found to result in almost complete hepatic uptake of the oligonucleotide in the rat.[10]

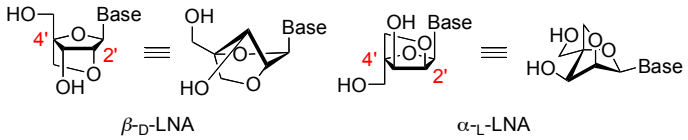
In terms of the sugar modification, modification of the 2′-position of a sugar moiety has been one of the most successful strategies to increase the drug potential of oligonucleotides. Substitution at 2′-position with electron withdrawing group has been shown to increase the binding affinity (such as the 2′-fluoro modification, **2.8**) and stability (such as the 2′-*O*-methyl and methoxyethyl modifications, **2.9**) (Figure 7). An alternate strategy exists in restricting the conformation of nucleosides most often through the synthesis of bicyclic sugars. These conformationally restricted sugars can increase the stability of resulting oligonucleotides, and 2′,4′-bicyclic nucleic acids (2′,4′-BNAs) are prime examples of these. Within this class of (2′,4′-BNAs), "locked nucleic acids" (LNAs) which contain a 2′-*O* atom and 4′-*C* 2-atom bridge (Figure 8), are even more conformationally restricted. Wengel[11] and Imanishi[12] have independently reported on the thermal stability of bridged nucleic acids (**2.10** and **2.11**, Figure 8).



2.8 2′-fluoro modification

2.9 2′-O-methyl modification

Figure 7: Modification of the 2′ position of the sugar portion of nucleosides.



β-$_D$-LNA **2.10** α-$_L$-LNA **2.11**

Figure 8: Locked nucleic acids (LNAs).

In general, because of the flexibility of the furanose ring, it is inherently nonplanar. Most of nucleic acids exist in two types conformations: the 3′-*endo* conformation or the 2′-*endo* conformation (Figure 9). When the major deviation is on the same side as the base and C4′–C5′ bond, the atom involved is termed *endo*, if it is on the opposite side, the atom will be termed *exo*. The 3′-*endo* conformation is sometimes termed as N (north) type conformation, and the 2′-*endo* conformation is termed as S (south) type conformation.[13] The N and S type conformations are usually in fast equilibrium due to the low energy barrier between these two forms. The *N*-type conformation often exists in *A*-type duplexes, and the stereoisomeric *S*-type conformation often exists in *B*-type duplexes (Figure 9).[13]

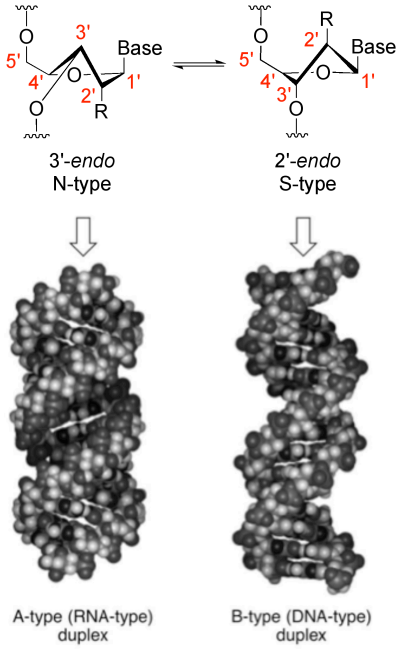


Figure 9: Conformation of nucleoside sugar moieties and structures of *A*- and *B*-type duplexes.[15]

For the *β*-D-LNA, the ribose sugar is locked by the bridge into a near perfect *N* type pucker conformation, while for the enantio-LNA (α-L-LNA), it is locked into an *S* type conformation (Figure 8).[14] The *N*-type LNAs can form more stable duplexes with complementary single-stranded RNA, and *S*-type LNAs will form stable duplexes with single-stranded DNA.[15]

Other than the 2′,4′-bicyclic nucleic acids (2′,4′-BNAs) that have already been discussed, different bi- and tricyclic nucleoside systems have been synthesized by Leumann,[16,17,18] Imanishi,[19] and Nielsen,[20] all of which contain locked furanose rings (Figure 10) and have shown moderate improvements in stability and binding affinity.

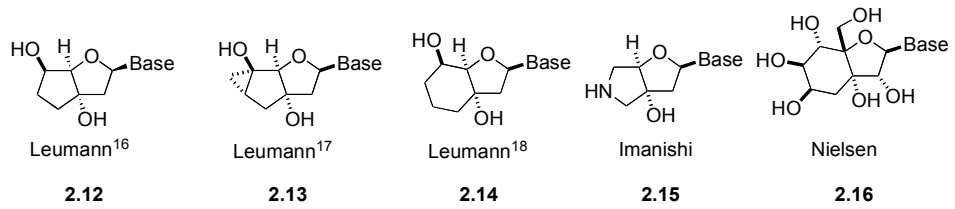


Figure 10: Examples of bi- and tricyclic LNAs.

The work described in this chapter is a part of an ongoing collaboration that the Hanessian group has with ISIS pharmaceuticals to develop new antisense oligonucleotide candidates. The *cis*-[4.3.0]bicyclic antisense nucleoside **2.17** and the diastereomeric *trans*-[4.3.0]bicyclic compound **2.18** were selected as designed molecule targets for this project (Figure 11). Leumann has reported a D-[4.3.0]bicyclic nucleoside **2.14** (Figure 10)[21] which demonstrated, in his system, that the cyclohexane ring does not obviously alter the nucleic acid binding affinity when compared with D-[3.3.0]bicyclic nucleoside **2.12**. It is the O-5′ substituent of the cyclohexane ring, which is oriented in either an axial or equatorial position in solution, that has an effect on the binding affinity to the target mRNA.[18] The major focus of the work described herein, is to synthesize and study diastereomeric [4.3.0]bicyclic nucleosides **2.17** and **2.18** (Figure 11).

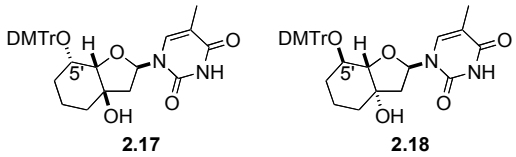
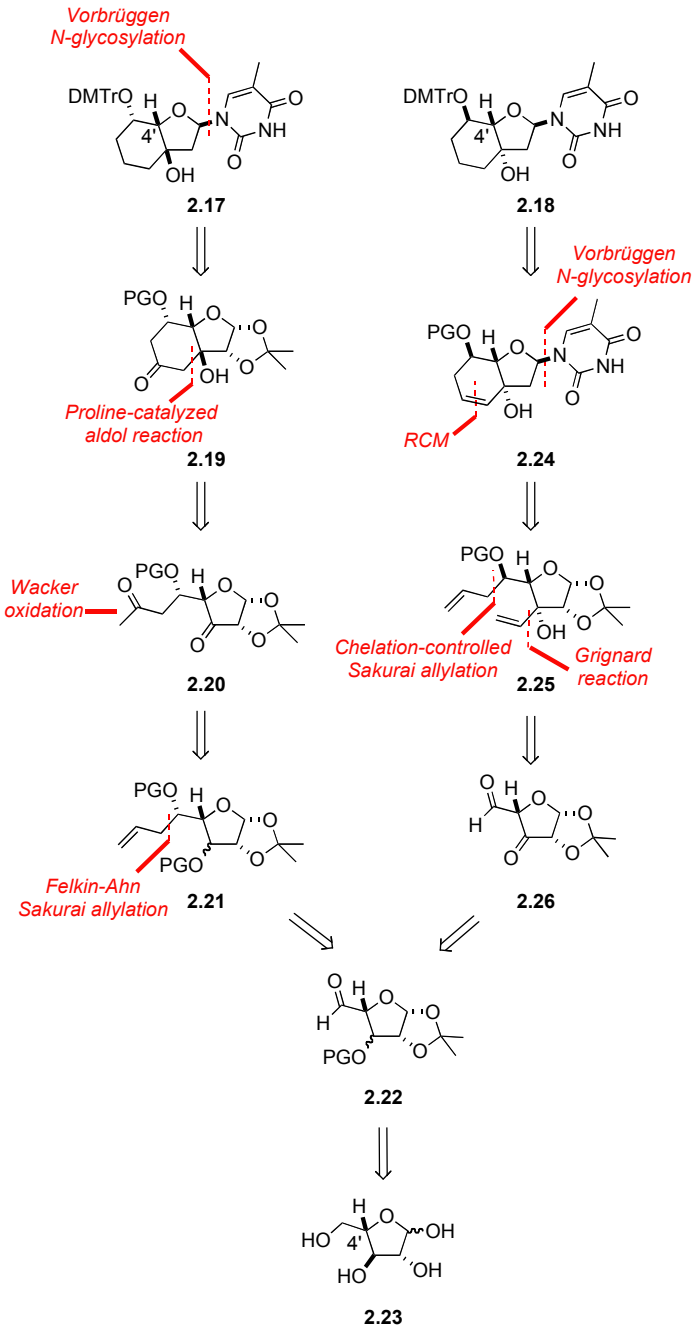


Figure 11: *cis*- (**2.17**) and *trans* -[4.3.0]bicyclic nucleosides (**2.18**).

2.1.3 Retrosynthetic Analysis of Target Nucleosides 2.17 and 2.18

It was envisaged that both bicyclic nucleosides **2.17** and **2.18** could be assembled from bicyclic carbohydrate derived building blocks, where the nucleobases of each target could be installed via a Vorbrüggen glycosylation (Scheme 17). As such, both nucleoside targets could be simplified to sub-targets **2.19** and **2.24**. In the case of the *cis*-bicyclic nucleoside analogue **2.17**, specifically intermediate **2.19**, retrosynthetic simplification to the bicyclic *β*-hydroxy ketone presented the opportunity to employ an L-proline-catalyzed intramolecular aldol reaction in the forward direction and simplified the synthesis of **2.17** to diketone **2.20**. Diketone **2.20** could be taken back to the homoallylic alcohol **2.21**, which could be further simplified to a key aldehyde intermediate **2.22** via a stereoselective Felkin-Anh controlled Sakurai allylation. The *trans*-bicyclic nucleoside **2.18** could be prepared from **2.24** via a Vorbrüggen glycosylation and a RCM reaction. The synthesis of **2.24** is possible from diene **2.25**, where it was envisaged that diastereoselective Sakurai allylation could be used to generate the homoallylic alcohol portion of **2.25**, and a diastereoselective Grignard addition could be employed to furnish the allylic alcohol portion. Due to the desire to have the opposite configuration at the tertiary alcohol bearing stereogenic center, it was reasoned that Grignard addition to a bicyclic ketone such as **2.26** should take place primarily from the less hindered *exo*-side of the bicyclic system. Both syntheses can be derived from the common aldehyde intermediate **2.22**, which could could be obtained from commercially available L-arabinose (**2.23**).

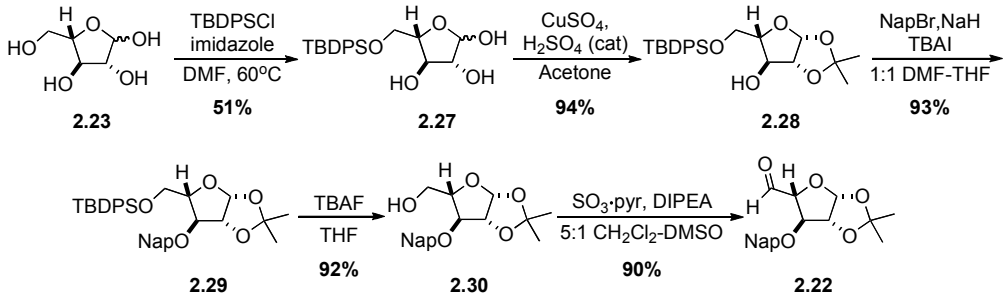


Scheme 17: Retrosynthetic analysis of *cis*- and *trans*-[4.3.0]bicyclic nucleosides.

2.2 Results and Discussion

2.2.1 Synthesis of Key Aldehyde Intermediate 2.22

As outlined in the retrosynthetic analysis above (Scheme 17), intermediate aldehyde **2.22** will play a pivotal role in the planned syntheses of both nucleoside targets. The synthetic plan hopes to capitalize on the matching stereochemistry of L-arabinose at C-4′ with **2.17** and **2.18**, while the stereochemistry at C-3′ will come from diastereoselective reactions, such as the L-proline catalyzed aldol condensation and Grignard additions respectively. The synthesis of key intermediate **2.23** commenced with the selective protection of the primary alcohol as the TBDPS ether **2.27** (Scheme 18). The C-1–C-2 diol system was selectively protected as an isopropylidene acetal to give the known acetonide **2.28**,[22] and the remaining secondary hydroxyl group was converted to the 2-methyl naphthyl ether to give **2.29** in 93% yield. Cleavage of the silyl ether protecting group using TBAF afforded the free primary alcohol **2.30**, which was subjected to a Parikh-Doering oxidation to give the key aldehyde intermediate **2.22**.

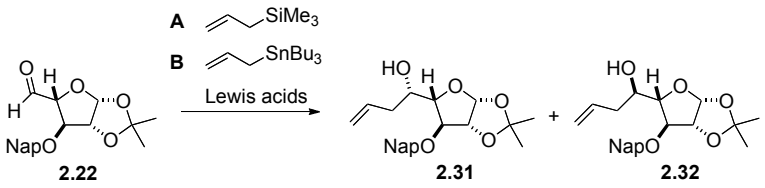


Scheme 18: Synthesis of intermediate aldehyde **2.22**.

2.2.2 Key allylation

With aldehyde intermediate **2.22** in hand, the next step was to test the key allylation reaction. In order to generate both diastereomeric homoallylic alcohols with high selectivity and yield, several Lewis acids were screened (Table 4). Analysis of the Felkin-Ahn model suggested that the (*S*)-configured alcohol would be favored when a Lewis acid such as $BF_3{\cdot}Et_2O$ were used. Thus it was pleasing to find that treatment of aldehyde **2.22** with $BF_3{\cdot}OEt_2$ and allyl trimethylsilane under standard Sakurai allylation conditions[23] afforded the desired homoallylic alcohol **2.31** as virtually a single diastereomer (*d.r.* >20:1) in 89% yield. The (*R*)-configured homoallylic alochol **2.32** could be prepared under chelation-controlled conditions. Using allyl tributyltin as nucleophile and $TiCl_4$ as a Lewis acid, the designed product was obtained in 1:1 *d.r.* and 50% yield. The lower yield of this reaction is attributed to $TiCl_4$ being a much stronger Lewis acid, which resulted in cleaveage of the acetonide group. With ZnI_2 an 82% chemical yield of allyl addition product was obtained, but the major diastereomer was **2.31**. Switching to $MgBr_2{\cdot}OEt_2$,[24] resulted in a 1:1 *d.r.* and 86% yield. Futher optimization found that 3.0 equivalents of $MgBr_2{\cdot}OEt_2$, with 2 equivalents of allyl tributyltin at –78 ºC gave **2.32** in 93% overall yield with 1:4 *d.r.* (74 % isolated yield, entry 5). Notably, using allyl trimethylsilane instead of allyl tributyltin afforded a reversal in diastereoselectivity (*d.r.* 5:1). Increasing the reaction temperature to 25 ºC favored the formation of **2.31** regardless of the choice of nucleophile.

Table 4: Screening of Lewis acids for Sakurai allylation.



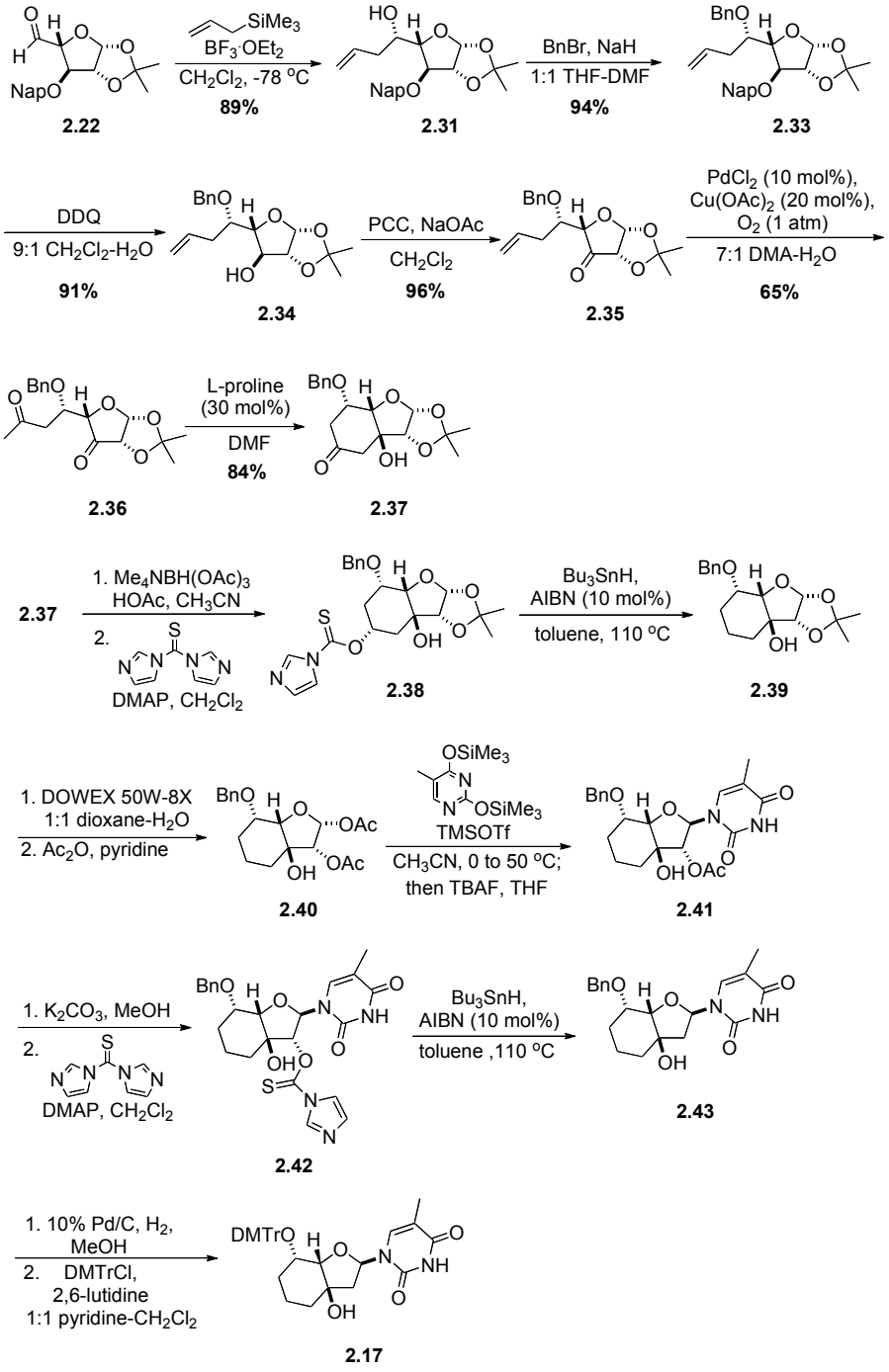
entry	Nucleophile (*equiv.*)	Lewis acids (*equiv.*)	T (°C)	*d.r.* (**2.31**:**2.32**)[a]	Yield(%)[b]
1	**A** (1.75)	$BF_3{\cdot}OEt_2$ (2.0)	-78	>20:1	89 [c]
2	**B** (2.0)	$TiCl_4$ (1.1)	-78	1:1	50
3	**B** (2.0)	ZnI_2 (1.1)	-78	5:1	82
4	**B** (2.0)	$MgBr_2{\cdot}OEt_2$ (1.1)	-78	1:1	86
5	**B** (2.0)	$MgBr_2{\cdot}OEt_2$ (3.0)	-78	1:4	93 [d]
6	**B** (2.0)	$MgBr_2{\cdot}OEt_2$ (5.0)	-78	1:4	90
7	**A** (2.0)	$MgBr_2{\cdot}OEt_2$ (3.0)	-78	5:1	77
8	**A** (2.0)	$MgBr_2{\cdot}OEt_2$ (3.0)	25	5:1	84
9	**B** (2.0)	$MgBr_2{\cdot}OEt_2$ (3.0)	25	2:1	81

[a] d.r. was determined by ^{1}H NMR of isolated mixture **2.31** and **2.32**. [b] Isolated overall yield of **2.31** and **2.32**. [c] Isolated yield of **2.31**. [d] 74% yield of **2.32**.

2.2.3 Completion of the Synthesis of *cis*-[4.3.0]Bicyclic Nucleoside 2.17

With an expedient and diastereoselective route to alcohol **2.31** in place, the desired secondary alcohol was protected as the benzyl ether **2.33**. Selective removal the naphthyl ether was achieved by DDQ oxidation and the resulting secondary alcohol was then oxidized to ketone **2.35** with PCC in 96% yield. Wacker oxidation[25] of the terminal olefin followed by a very mild and efficient L-proline-catalyzed intramolecular aldol reaction furnished the bicyclic ketol **2.37**. With **2.37** in hand, the final synthesis of *cis*-[4.3.0]bicyclic nucleoside **2.17** was achieved by Dr. Benjamin R. Schroeder. Complete reduction of the ketone group of **2.37** to a methylene group required a three-step process that involved reduction to the alcohol, thiocarbamate formation, and finally a Barton-McCombie deoxygenation[26] to give **2.39**. Removal of the acetonide under mild acidic conditions and subsequent acetylation provided the Vorbrüggen glycosylation[27] precursor **2.40**, which was treated with persilylated thymine and TMSOTf, followed by TBAF to

give afford nucleoside **2.41**. Removal of the acetyl under basic conditions gave the *trans*-diol **2.42**, Selective deoxygenation at the C-2′ position with a second Barton-McCombie deoxygenation[26] afforded **2.43**. Cleavage of the benzyl ether of **2.43** and subsequent protected as a dimethoxytrityl ether furnished the *cis*-nucleoside target **2.17**. The compound will be used later in an oligonucleotide synthesis at ISIS Pharmaceuticals.



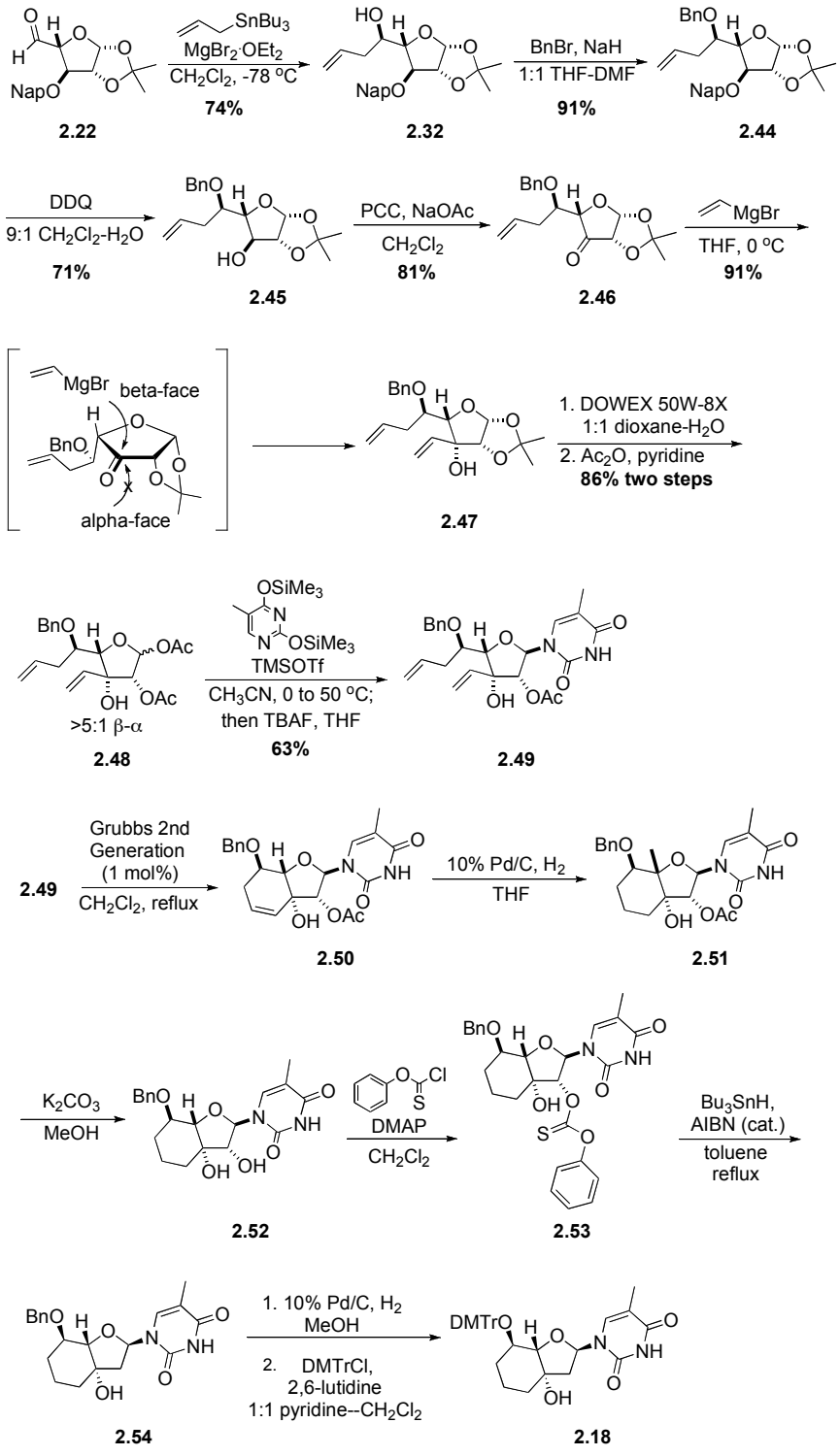
Compounds from **2.38** to **2.17** were synthesized by Dr. Benjamin R. Schroeder

Scheme 19: Completion of the synthesis of cis-[4.3.0]bicyclic nucleoside **2.17**.

2.2.4 Completion of the Synthesis of *trans*-[4.3.0]Bicyclic Nucleoside2.18

Under chelation-controlled conditions, with 3 equiv. $MgBr_2{\cdot}OEt_2$ and 2 equiv. allyl tributyltin at –78 ºC, the Sakurai allylation gave the desired homoallylic alcohol **2.32** in 74% isolated yield. Protection of the secondary hydroxyl group as a benzyl ether **2.44**, subsequent naphthyl ether cleavage by DDQ oxidation afforded **2.45** and oxidation the resulting secondary alcohol by PCC provided C 3′ ketone **2.46**. A vinyl Grignard reagent addition at the ketone **2.46** gave the desired tertiary alcohol **2.47** in 91% yield, which agreed with the prediction that vinyl nucleophiles would attack from the beta-face of the furanose ring, the alpha-face being blocked by the acetonide at C 1′ and C 2′ position. Removal of the acetonide under the mild acidic conditions, followed by selective acetylation of the C 1′ and C 2′ diol gave **2.48** (>5:1, *β*-α isomer). Treatment of **2.48** with persilylated thymine and TMSOTf, followed by TBAF afforded the Vorbrüggen glycosylation[27] product **2.49**. With **2.49** in hand, the final synthesis of the *trans*-[4.3.0]bicyclic nucleoside **2.18** was accomplished by Dr. Benjamin R. Schroeder. Ring-Closing Metathesis of **2.49** using Grubbs' 2nd generation catalyst successfully furnished the bicyclic skeleton **2.50**. Hydrogenation the resulting alkene followed by the acetyl cleavage under basic condition afforded the *cis*-diol **2.52**, which need be deoxygenated at C 2′ position. Treatment of **2.52** under the same conditions used in the previous *cis*-oligonucleoside **2.17** synthesis, in the presence of 1,1′-thiocarbonyldiimidazole (TCDI) and imidazole, however, gave C 2′-C 3′ thiocarbonate ester instead of C 2′ thiocarbonyl imidazole. When the C 2′ secondary alcohol reacted with TCDI, it could further react with the C 3′ tertiary alcohol gave a thiocarbonate ester due to the *cis* stereochemistry of the diol. Finally we found that using the phenyl carbonothioate group could minimize the

formation of the thiocarbonate ester. Barton-McCombie deoxygenation was then effective in selectively deoxygenating the C 2′ alcohol of **2.53** to afford **2.54**. Although it's not routine, we were pleased to find that using of excess Bu_3SnH in dilute solutions of phenyl carbothioate could afford up to 83% yield of the desired deoxynucleoside. Removal of benzyl group of **2.54** and subsequent protection of the resulting hydroxyl group as a dimethoxytrityl ether again afforded the *trans*-[4.3.0] bicyclic nucleoside **2.18**.

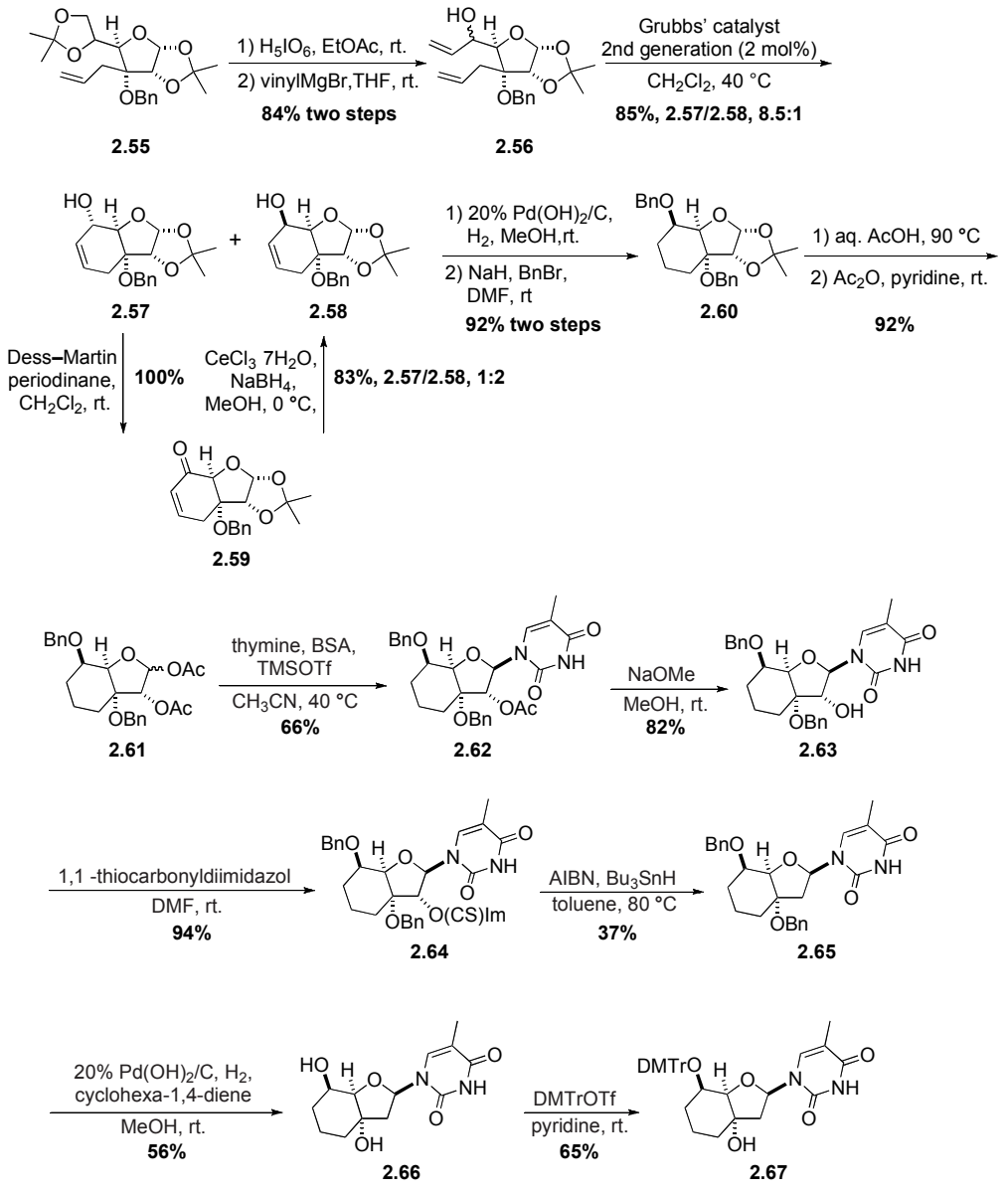


Compounds from **2.50** to **2.18** were synthesized by Dr. Benjamin R. Schroeder

Scheme 20: Completion of the synthesis of *trans*-[4.3.0]bicyclic nucleoside **2.18**.

2.2.5 Discussion

As mentioned previously, a *cis*-D-[4.3.0]bicyclic nucleoside has been reported by Leumann (Scheme 21)[18] in which a RCM strategy was used to synthesize a *cis*-sugar-scaffold. However, this route gave a mixture of products **2.57** and **2.58**, where the required one (**2.58**) is the minor isomer. We utilized an alternate route (Schemes 19 & 20) employing optimized Sakurai additions (2.22→2.31 and 2.22→2.32) to successfully to avoid this diastereoselectivity problem.



Scheme 21: Leumann's synthesis of *cis*-D-[4.3.0]bicyclic nucleoside.

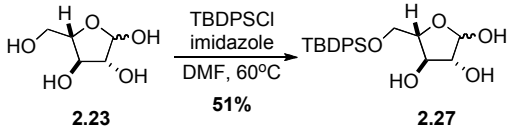
In conclusion, starting from the cheap and commercially available L-arabinose, *cis- and trans*-L-[4.3.0]bicyclic nucleosides were successfully synthesized. We used a diastereoselective Sakurai addition and a novel proline catalyzed intramolecular aldol reaction to access the *cis*-fused compound (**2.17**) and a diastereoselective Sakurai addition then ring-closing metathesis (RCM) strategy to access the *trans*-fused compound

(**2.18**). Both nucleosides are assembled to furnish the antisense oligonucleotides for further testing.

2.3 Experimental Procedures

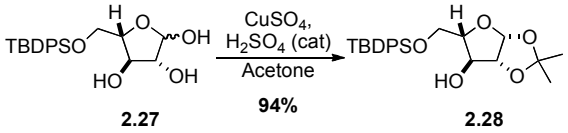
General information: All non-aqueous reactions were run in flame-dried glassware under a positive pressure of argon. Anhydrous solvents were distilled under positive pressure of dry argon before use and dried by standard methods. THF, ether, DCM and toluene were dried by the SDS (Solvent Delivery System), commercial grade reagents were used without further purification. Reactions were monitored by *S-2* analytical thin-layer chromatography (TLC) performed on pre-coated, glass-backed silica gel plates. Visualization of the developed chromatogram was performed by UV absorbance (wave length: 254 nm), and staining TLC plates with aqueous cerium ammonium molybdate, iodine, or aqueous potassium permanganate solution. Flash chromatography was performed on 230-400 mesh silica gel with the indicated solvent systems. Melting points were recorded on BÜCHI Melting Point B-540. Infrared spectra were recorded on a Perkin-Elmer FTIR Paragon 1000 and were reported in reciprocal centimeters (cm^{-1}). Nuclear magnetic resonance spectra (NMR) were recorded either on Bruker AV-300 MHz and AV-400 MHz spectrometers. Chemical shifts for ^{1}H NMR spectra are recorded in parts per million from tetramethylsilane with the solvent resonance as the internal standard ($CDCl_3$ δ 7.27 ppm, CD_3OD, δ 3.31 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, and br = broad), coupling constants were reported Hz, and integration. Chemical shifts for ^{13}C NMR spectra are recorded in parts per million from

tetramethylsilane using the central peak of the solvent resonance as the internal standard ($CDCl_3$, δ 77.00 ppm, CD_3OD, δ 49.15 ppm). All spectra were obtained with complete proton decoupling. Optical rotations were determined in a 1 dm cell at 589 nm at 20 ºC (Perkin-Elmer 343). Data are reported as follows: $[\alpha]_D$, concentration (c in g/100 mL), and solvent. High-resolution mass spectra were performed using fast atom bombardment (FAB) or electrospray (ESI) techniques. Low-resolution mass spectra were obtained using electrospray ionisation technique (ESI).

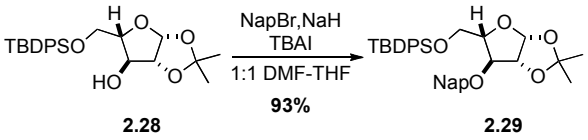


5-*O*-*t*-Butyldiphenylsilyl-α,*β*-L-arabinofuranose (2.27). Imidazole (9.1g, 133.2 mmol) and *t*-butyldiphenylchlorosilane (17.2 mL, 66.6 mmol) were added sequentially to a DMF (132 mL) solution of L-arabinose **2.23** (10.0 g, 66.6 mmol). The resulting mixture was heated to 60 ºC and was stirred for 3 hours. After cooling to room temperature, the solution was poured into 1 M HCl (170 mL), extracted with diethyl ether (80 mL) three times. The combined organic phase was washed with water (160 mL), saturated soulution of $NaHCO_3$ (160 mL) and brine (160 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 2:1 to 3:7) to give the product **2.27** as a colorless oil (13.2 g, 51%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.75-7.70 (m, 4H), 7.45–7.37 (m, 6H), 5.22-5.17 [1H total sum. 5.22 (d, H-1*β*, *J* = 4.4 Hz), 5.17 (d, H-1α, *J* = 2.8 Hz), α,*β*-ratio ~ 2.1:1], 4.12-4.04 (m, 2H), 3.94-3.89 (m, 1H), 3.87-3.75 (m, 2H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) α isomer δ 136.9, 136.9, 131.0, 128.9, 103.5, 85.0, 83.9, 77.9, 65.3, 27.4, 20.2;

β isomer δ 134.7, 134.7, 131.0, 128.9, 97.7, 84.1, 78.8, 77.1, 67.1, 27.4, 20.2; HRMS (ESI) calcd for $C_{21}H_{32}NO_5Si$ $(M+NH_4)^+$ 406.2044, found 406.2031.

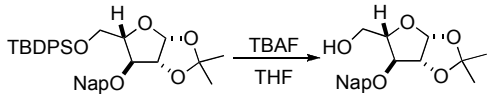


5-*O*-*t*-Butyldiphenylsilyl-1,2-isopropylidene-β-L-arabinofuranose (2.28). Anhydrous copper (II) sulfate (3.78 g, 23.7 mmol) and two drops of concentrated sulfuric acid were added sequentially to a acetone (75mL) solution of **2.27** (3.35 g, 8.6 mmol). The resulting mixture was stirred overnight and filtered through a pad of celite. The filtrate was concentrated under reduced pressure to a volume of ~ 10 mL and diluted with EtOAc (60 mL). The solution was washed with saturated solution of $NaHCO_3$ (25 mL), the aqueous phase was back-extracted with EtOAc (25 mL) three times. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 6:1 to 3:1) to give the product **2.28** as a colorless oil (3.48 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69-7.66 (m, 4H), 7.44-7.37 (m, 6H), 5.90 (d, J = 4.0 Hz, 1H), 4.56 (d, J = 4.0 Hz, 1H), 4.46–4.45 (m, 1H), 4.09–4.05 (m, 1H), 3.84-3.82 (m, 2H), 2.06 (s, 1H), 1.34 (s, 3H), 1.30 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 135.5, 133.2, 133.2, 129.8, 129.8, 127.8, 112.5, 105.5, 82.4, 87.0, 69.2, 76.4, 63.7, 26.8, 26.1, 19.2; $[\alpha]_D$: -3.2° (c 1.2, $CHCl_3$); HRMS (ESI) calcd for $C_{24}H_{36}NO_5Si$ $(M+NH_4)^+$ 446.2357, found 446.2370.



2.28 **2.29**

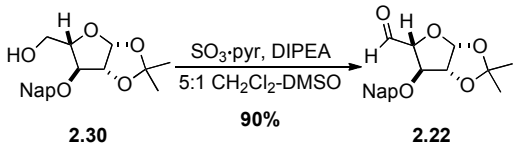
5-*O*-*t*-Butyldiphenylsilyl-1,2-isopropylidene-3-*O*-(2'-naphthylmethyl)-β-L-arabinofuranose (2.29). 2.28 (12.84 g, 30.0 mmol) as a solution in THF-DMF (1:1, 18 mL), and 2-(Bromomethyl) naphthalene (7.96 g, 36 mmol) were added sequentially to a 0°C THF-DMF (1:1, 165 mL) mixture of Sodium hydride (1.44g, 36.0 mmol) as a 60% dispersion in mineral oil and tetrabutyl ammonium iodide (1.11 g, 3.0 mmol). The resulting suspension was allowed to warm up to room temperature and was stirred until the starting material was completely consumed by TLC analysis. The reaction solution was cooled to 0 °C, quenched with MeOH (4.0 mL), and diluted with diethyl ether (500 mL). The solution was washed with water (200 mL) two times, and the aqueous phase was separated and back-extracted with diethyl ether (100 mL) three times. The combined organic extracts were washed with saturated solution of $NaHCO_3$ (200 mL) and brine (200 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 9:1) to give the product **2.29** as a colorless oil (15.86 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86-7.81 (m, 4H), 7.74-7.66 (m, 4H), 7.51-7.33 (m, 9H), 5.95 (d, J = 4.0 Hz, 1H), 4.83 (d, J = 12.2 Hz, 1H), 4.80 (d, J = 12.2 Hz, 1H), 4.75 (d, J = 4.0 Hz, 1H), 4.32-4-30 (m, 2H), 3.89-3.81 (m, 2H), 1.37 (s, 3H), 1.33 (s, 3H), 1.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6; 134.9, 134.8, 133.2, 133.2, 133.1, 129.7, 129.7, 128.3, 127.9, 127.7, 127.7, 126.5, 126.1, 126.0, 125.6, 112.4, 105.7, 85.3, 85.1, 82.8, 71.7, 63.4, 26.9, 26.7, 26.1, 19.1; $[\alpha]_D$: +5.7° (*c* 0.87, $CHCl_3$); HRMS (ESI) calcd for $C_{35}H_{44}NO_5Si$ $(M+NH_4)^+$ 586.2983, found 586.2995.



2.29 **2.30**

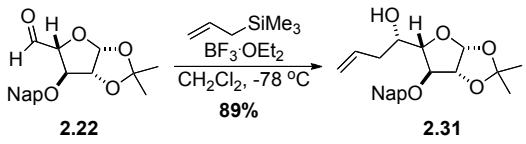
1,2-Isopropylidene-3-*O*-(2'-naphthylmethyl)-β-L-arabinofuranose (2.30).

Tetrabutylammonium fluoride (0.48 mL, 1 M solution in THF, 0.48 mmol) was added to a THF (3.3 mL) solution of **2.29** (247 mg, 0.434 mmol) at room temperature. The resulting solution was stirred until the starting material was completely consumed by TLC analysis. The solution was quenched by dropwise addition of saturated solution of $NaHCO_3$ (1 mL) and diluted with EtOAc (15 mL). The solution was washed sequentially with saturated solution of $NaHCO_3$ (3 mL) and brine (3 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 2:1) to give the product **2.30** as a white solid, (131 mg, 92%). When scale up to **2.29** (15.86g, 27.9 mmol) in THF (212 mL), with tetrabutylammonium fluoride (1M solution in THF) (30.7 mL, 30.7 mmol), 88% yield was obtained. mp: 83.1-85.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86-7.81 (m, 4H); 7.51-7.46 (m, 3H), 5.98 (d, J = 4.0 Hz, 1H), 4.82-4.72 (m, 3H), 4.32-4.28 (m, 1H), 4.08 (dd, J = 2.8, 0.4 Hz, 1H), 3.84-3.75 (m, 2H), 2.94 (br s, 1H), 1.57 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.5, 133.0, 132.8, 128.1, 127.7, 127.5, 126.4, 126.0, 125.8, 125.4, 112.6, 105.4, 85.4, 85.0, 82.6, 71.6, 62.4, 26.9, 26.1; IR (film) 3469, 2936, 1375, 1080 cm^{-1}; $[\alpha]_D$: −8.0° (*c* 1.65, $CHCl_3$); HRMS (ESI) calcd for $C_{19}H_{22}O_5Na$ $(M+Na)^+$ 353.1359, found 353.1368.



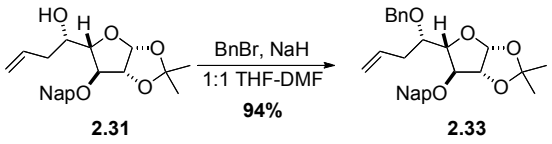
2.30 **2.22**

1,2-Isopropylidene-3-*O*-(2'-naphthylmethyl)-5-*β*-L-arabino-pentodialdo-1,4-furanose (2.22). Hunig's base (4.74 mL, 27.74 mmol) and SO_3·pyridine (98%) (4.42 g, 27.24 mmol) as a solution in DMSO (22.5 mL) were added sequentially to a –20 °C CH_2Cl_2-DMSO (9:1, 82.0 mL) solution of **2.30** (3.00 g, 9.08 mmol). The solution was stirred for 10 min and then partitioned between diethyl ether (150 mL) and ice-cold brine (300 mL). The aqueous layer was separated and back-extracted with diethyl ether (150 mL) three times. The combined extracts were washed sequentially with 0.5 M HCl (150 mL) three times, water (150 mL) and saturated solution of $NaHCO_3$ (150 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was reconstituted in toluene three times and concentrated under reduced pressure to remove any residual pyridine. The residue was dried under hi-vacuum and utilized without further purification as a colorless oil, (2.68 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.92-7.83 (m, 4H), 7.57-7.50 (m, 3H), 6.18 (d, J = 3.6 Hz, 1H), 4.80 (d, J = 11.8 Hz, 1H), 4.75 (d, J = 11.8 Hz, 1H), 4.72 (d, J = 3.6 Hz, 1H), 4.65 (s, 1H), 4.46 (s, 1H), 1.52 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.5, 134.0, 133.1, 133.0, 128.3, 127.8, 127.6, 126.7, 126.2, 126.1, 125.2, 112.0, 106.5, 88.6, 84.8, 82.8, 71.9, 26.0, 25.4; IR (film) 2984, 1730, 1098 cm^{-1}; $[\alpha]_D$: (+) 13.0° (*c* 0.37, $CHCl_3$); HRMS (ESI) calcd for $C_{19}H_{20}O_5Na$ $(M+Na)^+$ 351.1203, found 351.1205.

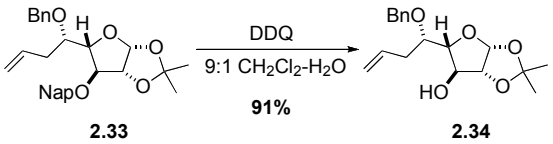


2.22 2.31

Homoallylic alcohol 2.31. $BF_3{\cdot}OEt_2$ (1.16 mL, 9.24 mmol) was added to a stirred –78 °C anhydrous CH_2Cl_2 (75 mL) solution of aldehyde **2.22** (1.52 g, 4.62 mmol). After 5 min, allyl trimethylsilane (1.29 mL, 8.09 mmol) was added to this solution. The resulting solution was stirred for 3 hours and quenched by dropwise addition of saturated solution of $NaHCO_3$ (5 mL). The solution was warmed to room temperature and partitioned between saturated solution of $NaHCO_3$ (85 mL) and CH_2Cl_2 (85 mL). The aqueous layer was separated and back-extracted with CH_2Cl_2 (50 mL) three times. The combined extracts were washed with brine (50 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 5:1) to give the product **2.31** as a colorless solid which crystallized upon standing, (3.63 g, 89%); mp = 74.0-78.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87-7.83 (m, 4H), 7.52-7.48 (m, 3H), 5.97 (d, *J* = 3.9 Hz, 1H), 5.91-5.84 (m, 1H), 5.22-5.15 (m, 2H), 4.82 (d, *J* = 11.6 Hz, 1H), 4.76 (d, *J* = 11.6 Hz, 1H), 4.73 (d, *J* = 3.9 Hz, 1H), 4.31 (d, *J* = 2.4 Hz, 1H), 4.11-4.04 (dd, *J* = 4.2, 2.7 Hz, 1H), 3.94-3.89 (m, 1H), 2.56-2.49 (m, 1H), 2.35 (br s, 1H), 2.28-2.20 (m, 1H), 1.56 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.6, 134.1, 133.1, 132.9, 128.1, 127.8, 127.6, 126.6, 126.0, 125.9, 125.6, 118.2, 112.6, 105.5, 87.6, 84.7, 82.3, 71.6, 70.0, 37.5, 27.0, 26.0; IR (film) 3488, 3057, 2984, 2936, 1383, 1375 cm^{-1}; $[\alpha]_D$: (–) 3.4° (*c* 1.67, $CHCl_3$); HRMS (ESI) calcd for $C_{22}H_{26}O_5Na$ $(M+Na)^+$ 393.1673, found 393.1679.

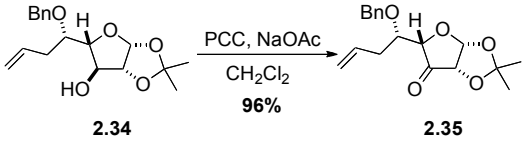


Alkene 2.33. Sodium hydride (0.420 g, 10.5 mmol) as 60% dispersion in mineral oil and benzyl bromide (2.0 mL, 16.8 mmol) were added to a stirred 0 °C THF-DMF (1:1, 28 mL) solution of homoallylic alcohol **2.31** (3.10 g, 8.4 mmol). The resulting mixture was allowed to warm to room temperature and stirred for 6 hours. The solution was cooled to 0 °C and quenched with saturated solution of NH_4Cl (5 mL) and partitioned between diethyl ether (100 mL) and water (100 mL). The aqueous portion was separated and back-extracted with diethyl ether (100 mL) three times. The combined extracts were washed sequentially saturated solution of $NaHCO_3$ (100 mL), brine (100 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 9:1) to give the product **2.33** as a colorless oil, (3.61 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90-7.81 (m, 4H), 7.53-7.47 (m, 3H), 7.32-7.28 (m, 5H), 6.02 (d, J = 3.7 Hz, 1H), 6.07-5.97 (m, 1H), 5.29-5.16 (m, 2H), 4.82-4.70 (m, 4H), 4.55 (m, 1H), 4.25-4.19 (m, 2H), 3.83-3.78 (m, 1H), 2.71-2.65 (m, 1H), 2.56-2.49 (m, 1H), 1.51 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 134.9, 133.9, 133.1, 132.9, 128.2, 128.1, 127.8, 127.8, 127.6, 127.5, 126.5, 126.0, 125.8, 125.6, 117.8, 112.1, 105.8, 85.8, 84.9, 82.6, 77.4, 71.8, 71.3, 34.6, 26.8, 26.0; IR (film) 3060, 3030, 1639, 746 cm^{-1}; $[\alpha]_D$: (+) 22.8° (*c* 1.18, $CHCl_3$); HRMS (ESI) calcd for $C_{29}H_{36}O_5N$ $(M+NH_4)^+$ 478.2588, found 478.2599.



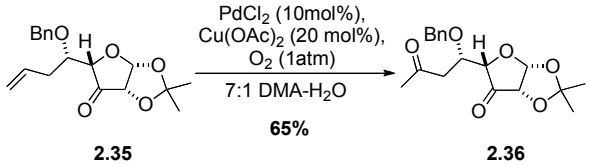
2.33 **2.34**

Alcohol 2.34. DDQ (1.82 g, 8.0 mmol) was added to a stirred CH_2Cl_2-H_2O (9:1, 110 mL) solution of naphthyl ether **2.33** (1.84 g, 4.0 mmol). The resulting mixture was vigorously stirred until the starting material was complete consumed by TLC analysis. The mixture was quenched by addition of a 10% solution of $NaHSO_3$ (100 mL), and the aqueous portion was back-extracted with CH_2Cl_2 (50 mL) three times. The combined extracts were washed sequentially with saturated solution of $NaHCO_3$ (100 mL), brine (100 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 3:1) to give the product **2.34** as a colorless oil, (1.17 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.27 (m, 5H); 5.97-5.88 (m, 1H), 5.87 (d, *J* = 4.0 Hz, 1H), 5.22-5.12 (m, 2H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.51 (d, *J* = 4.0 Hz, 1H), 4.48 (d, *J* = 11.2 Hz, 1H), 4.31 (t, *J* = 2.8 Hz, 1H), 3.85-3.82 (dd, *J* = 6.4, 2.8 Hz, 1H), 3.77-3.73 (m, 1H), 2.81 (br s, 1H), 2.63-2.57 (m, 1H), 2.45-2.37 (m, 1H), 1.44 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 133.6, 128.4, 128.1, 127.8, 118.0, 112.3, 105.5, 87.6, 86.7, 77.9, 76.3, 71.9, 34.5, 26.8, 26.0; IR (film) 3452, 3067, 3031, 2982, 2939, 1640, 744 cm^{-1}; $[\alpha]_D$: (+) 43.0° (*c* 1.41, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{24}O_5Na$ $(M+Na)^+$ 343.1516, found 343.1529.



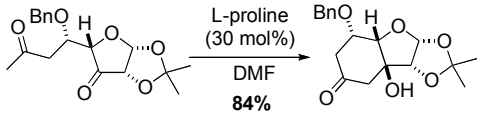
2.34 **2.35**

Ketone 2.35. A CH_2Cl_2 (40 mL) solution of secondary alcohol **2.34** (1.94 g, 6.1 mmol) was added to a stirred 0 °C CH_2Cl_2 (160 mL) mixture of PCC (3.94 g, 18.3 mmol), anhydrous sodium acetate (1.53 g, 18.6 mmol) and activated 4 Å molecular sieves. The resulting mixture was allowed to warm to room temperature and stirred for 3 hours. The solution was quenched with diethyl ether (200 mL) and was added silica gel (~ 4 g). After the mixture was stirred for 30 min, the mixture was filtered through a short pad of silica gel and eluted with diethyl ether (50 mL) three times. The combined filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 4:1) to give the product **2.35** as a colorless oil, (1.85 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.25 (m, 5H); 6.01 (d, *J* = 4.0 Hz, 1H), 5.92-5.82 (m, 1H), 5.21-5.11 (m, 2H), 4.68 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.40 (d, *J* = 4.0 Hz, 1H), 4.21 (d, *J* = 5.6 Hz, 1H), 3.87-3.83 (m, 1H), 2.55-2.50 (m, 2H), 1.49 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7, 137.5, 133.5, 127.9, 127.7, 127.3, 117.3, 114.5, 102.2, 80.8, 78.2, 76.5, 72.0, 34.6, 26.5, 26.2; IR (film) 2988, 2938, 1770, 1090, 1067 cm^{-1}; $[\alpha]_D$: (+) 10.9° (*c* 0.42, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{22}O_5Na$ $(M+Na)^+$ 341.1359, found 341.1364.



Diketone 2.36. $PdCl_2$ (0.007 g, 0.041 mmol) and $Cu(OAc)_2$ (0.015 g, 0.082 mmol) were

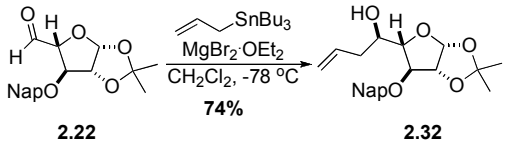
sequentially added to a stirred DMA-H_2O (7:1, 0.7 mL) solution of ketone **2.35** (0.13 g, 0.41 mmol). The resulting mixture was purged with O_2 and maintained under an atmosphere of O_2. The mixture was vigorously stirred for 2 days and filtered through a pad of Celite. The filtrate was partitioned between Et_2O (10 mL) and H_2O (10 mL). The aqueous portion was separated and back-extracted with Et_2O (10 mL) three times. The combined extracts were washed with saturated solution of $NaHCO_3$ (10 mL) and brine (10 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 7:3) to give the product **2.36** as a colorless oil, (0.088 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.25 (m, 5H), 5.99 (d, 1H, *J* = 4.0 Hz), 4.69 (d, *J* = 11.2 Hz, 1H), 4.58 (d, *J* = 11.2 Hz, 1H), 4.40 (d, *J* = 4.4 Hz, 1H), 4.38-4.33 (m, 1H), 4.20 (d, *J* = 5.6 Hz, 1H), 2.96-2.88 (m, 1H), 2.87-2.77 (m, 1H), 2.15 (s, 3H), 1.51 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.8, 206.1, 137.7, 128.1, 128.0, 127.6, 114.9, 102.4, 81.4, 76.8, 75.1, 73.4, 45.1, 30.8, 26.8, 26.4; IR (film) 2990, 2940, 1770, 1716, 1069 cm^{-1}; $[\alpha]_D$: (–) 18.9° (c 1.11, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{22}O_6Na$ $(M+Na)^+$ 357.1309, found 357.1322.



2.36 **2.37**

Ketol 2.37. L-proline (0.009 g, 0.076 mmol) was added to a stirred DMF (20 mL) solution of diketone **2.36** (0.085 g, 0.254 mmol). The resulting solution was stirred for three days and diluted with Et_2O (30 mL). The solution was washed with H_2O (10 mL) and the aqueous layer was separated and back-extracted with Et_2O (10 mL) three times.

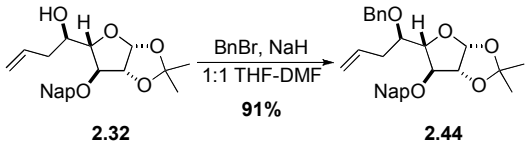
The combined organic extracts were washed with saturated solution of $NaHCO_3$ (10 mL), brine (10 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 3:2) to give the product **2.37** as a colorless oil, (0.072 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.24 (m, 5H); 5.89 (d, *J* = 3.2 Hz, 1H), 4.69 (d, *J* = 12.4 Hz, 1H), 4.59 (d, *J* = 12.4 Hz, 1H), 4.38 (d, *J* = 3.2 Hz, 1H), 4.14 (d, *J* = 4.0 Hz, 1H), 4.11-4.05 (m, 1H), 3.68 (br s, 1H), 3.21 (d, *J* = 16.4 Hz, 1H), 2.70-2.64 (m, 1H), 2.38 (d, *J* = 16.4 Hz, 1H), 2.42-2.37 (m, 1H), 1.47 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.3, 137.7, 128.3, 127.6, 127.6, 115.1, 104.4, 89.2, 84.6, 77.9, 71.9, 71.4, 46.8, 39.6, 27.2, 26.8; IR (film) 3430, 2986, 2935, 1715, 1373, 1098 cm^{-1}; $[\alpha]_D$: (–) 68.3° (*c* 0.87, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{22}O_6Na$ $(M+Na)^+$ 357.1309, found 357.1325.



2.22 **2.32**

Homoallylic alcohol 2.32. $MgBr_2{\cdot}OEt_2$ (0.155 g, 0.60 mmol) and allyltributyltin (0.129 mL, 0.40 mmol) were added sequentially to a stirred –78 °C CH_2Cl_2 (1.8 mL) solution of aldehyde **2.22** (0.065 g, 0.20 mmol). The resulting solution was stirred for two hours and quenched with saturated solution of $NaHCO_3$ (0.5 mL). The mixture was warmed to room temperature and partitioned between CH_2Cl_2 (5 mL) and saturated aq $NaHCO_3$ (5 mL). The aqueous portion was separated and back-extracted with CH_2Cl_2 (5 mL) three times. The combined extracts were washed with brine (5 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash

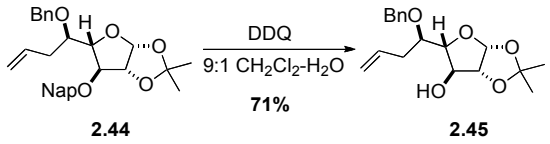
chromatography (hexane/EtOAc, 4:1) to give the product **2.32** as a colorless oil. (0.055g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89-7.74 (m, 4H), 7.51-7.44 (m, 3H), 5.95 (d, *J* = 4.0 Hz, 1H), 5.93-5.80 (m, 1H), 5.14-5.08 (m, 1H), 5.06 (s, 1H), 4.81 (d, *J* = 12.0 Hz, 1H), 4.73 (d, *J* = 4.0 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.06-3.99 (m, 2H), 3.75 (q, *J* = 6.4 Hz, 1H), 2.54 (br s, 1H), 2.26 (t, *J* = 6.8 Hz, 2H), 1.54 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.4, 134.1, 133.2, 133.1, 128.4, 127.8, 127.7, 126.7, 126.2, 126.1, 125.6, 117.6, 112.9, 105.4, 87.7, 85.2, 83.2, 71.9, 70.2, 38.1, 27.0, 26.3; IR (film) 3497, 2983, 2936, 1374, 1073 cm^{-1}; $[\alpha]_D$: −16.3° (*c* 1.32, $CHCl_3$); HRMS (ESI) calcd for $C_{22}H_{26}O_5Na$ $(M+Na)^+$ 393.1673, found 393.1678.



2.32 **2.44**

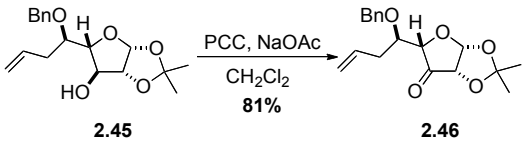
Alkene 2.44. Sodium hydride (0.062g, 1.55 mmol) as a 60% dispersion in mineral oil and benzyl bromide (0.295 mL, 2.48 mmol) were added sequentially to a stirred 0 °C DMF−THF (1:1, 4.2 mL) solution of homoallylic alcohol **2.32** (0.458 g, 1.24 mmol). The mixture was allowed to warm to room temperature and was stirred for three hours. The solution was cooled to 0 °C and quenched with saturated solution of NH_4Cl (1 mL) and partitioned between diethyl ether (20 mL) and H_2O (20 mL). The aqueous portion was separated and back-extracted with diethyl ether (10 mL) three times. The combined extracts were washed sequentially with saturated solution of $NaHCO_3$ (10 mL) three tomes and brine (10 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc,

9:1) to give the product **2.44** as a colorless oil, (0.518 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85-7.75 (m, 4H), 7.51-7.43 (m, 3H), 7.29-7.24 (m, 5H), 5.97-5.81 (m, 2H), 5.15-5.04 (m, 2H), 4.81 (d, *J* = 11.6 Hz, 1H), 4.72 (dd, *J* = 3.2, 1.2 Hz, 1H), 4.59 (d, *J* = 11.6 Hz, 2H), 4.573(d, *J* = 11.6 Hz, 1H), 4.05-4.01 (m, 2H), 3.63-3.58 (m, 1H), 2.41-2.37 (m, 2H), 1.52 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 134.6, 134.5, 133.1, 133.1, 128.3, 128.2, 127.9, 127.9, 127.7, 127.4, 126.9, 126.1, 126.1, 125.8, 117.4, 113.6, 104.8, 85.9, 84.9, 82.6, 77.8, 72.7, 72.0, 35.7, 27.4, 27.0; IR (film) 2936, 1373, 1212, 1073 cm^{-1}; $[\alpha]_D$: −30.4° (*c* 0.89, $CHCl_3$); HRMS (ESI) calcd for $C_{29}H_{32}O_5Na$ $(M+Na)^+$ 483.2142, found 483.2142.



Alcohol 2.45. DDQ (0.050 g, 0.22 mmol) was added to a stirred CH_2Cl_2-H_2O (9:1, 3.1 mL) solution of **2.44** (0.052 g, 0.11 mmol). The mixture was vigorously stirred until complete consumption of the starting material by TLC analysis. The mixture was quenched by addition of a 10% solution of $NaHSO_3$ (2 mL), and the aqueous portion was back-extracted with three CH_2Cl_2 (5 mL) three times. The combined extracts were washed sequentially with saturated aq $NaHCO_3$ (5 mL) and brine (5 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 7:3) to give the product **2.45** as a colorless oil: yield (0.026 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.24 (m, 5H), 5.98-5.90 (m, 1H), 5.86 (d, *J* = 4.2 Hz, 1H), 5.21-5.10 (m, 2H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* =

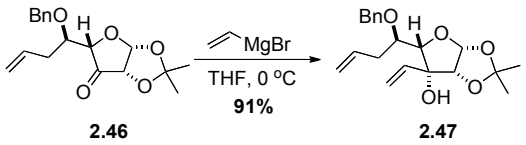
11.7 Hz, 1H), 4.54 (dd, *J* = 3.0, 1.4 Hz, 1H), 4.24 (dd, *J* = 5.0, 1.4 Hz, 1H), 3.92-3.90 (m, 1H), 3.75–3.71 (m, 1H), 2.53–2.36 (m, 2H), 2.03 (br s, 1H), 1.53 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 134.6, 128.4, 128.0, 127.6, 117.5, 113.4, 104.6, 87.8, 87.0, 77.9, 75.9, 73.0, 35.5, 27.4, 26.8; IR (film) 3436, 2985, 2938, 1384, 1374, 1211, 1067 cm^{-1}; $[\alpha]_D$: −9.2° (*c* 0.61, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{24}O_5Na$ $(M+Na)^+$ 343.1516, found 343.1525.



2.45 **2.46**

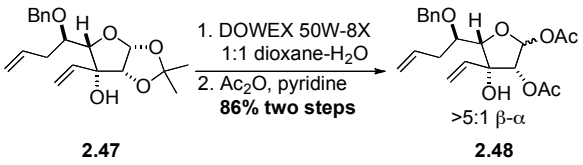
Ketone 2.46. A CH_2Cl_2 (4.4 mL) solution of alcohol **2.45** (0.205 g, 0.64 mmol) was added to a stirred 0 °C CH_2Cl_2 (17.6 mL) mixture of PCC (0.414 g, 1.92 mmol) and anhydrous NaOAc (0.160 g, 1.95 mmol). The resulting mixture was allowed to warm to room temperature and was stirred for three hours. The mixture was quenched upon addition of silica gel (0.1g), and was diluted with ethyl ether (30 mL). After the mixture was stirred for 30 min, it was filtered through a short plug of silica gel. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography (hexane/EtOAc, 3:1) to give the product **2.46** as a colorless oil (0.164 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5H); 6.07 (d, *J* = 4.5 Hz, 1H), 5.85–5.78 (m, 1H), 5.21 (m, 1H), 5.13 (m, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.42 (d, *J* = 4.2 Hz, 1H), 4.20 (d, *J* = 4.5 Hz, 1H), 3.87-3.83 (m, 1H), 2.57-2.47 (m, 2H), 1.45 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.7, 137.9, 133.7, 128.2, 128.0, 127.6, 118.3, 115.4, 102.5, 83.0, 77.8, 73.2, 35.1, 27.0, 26.9; IR

(film) 2987, 2939, 1769, 1375, 1218, 1160, 1089 cm^{-1}; $[\alpha]_D$: −13.6° (*c* 0.73, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{22}O_5Na$ $(M+Na)^+$ 341.1359, found 341.1359.



2.46 **2.47**

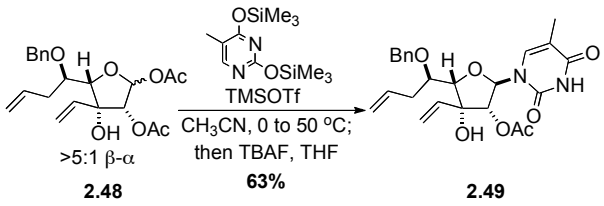
Diene 2.47. Vinyl magnesium bromide (4.02 mL, 1.0 M solution in THF, 4.02 mmol) was added dropwise to a stirred 0 °C anhydrous THF (11 mL) solution of ketone **2.46** (0.160 g, 0.50 mmol). The resulting solution was stirred at 0 °C for 10 min and quenched with saturated solution of NH_4Cl (0.1 mL). The mixture was partitioned between saturated solution of NH_4Cl (5 mL) and EtOAc (5 mL). The aqueous portion was separated and back-extracted with EtOAc (5 mL) three times. The combined extracts were washed with brine (5 mL), then dried by $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 9:1) to give the product **2.47** as a colorless oil, (0.158 g, 91%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.41-7.25 (m, 5H), 5.94-5.83 (m, 2H), 5.74 (d, *J* = 4.2 Hz, 1H), 5.48 (dd, *J* = 15.6, 1.2 Hz, 1H), 5.23 (dd, *J* = 9.3, 1.2 Hz, 1H), 5.14-5.06 (m, 2H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.63 (d, *J* = 11.4 Hz, 1H), 4.39 (d, *J* = 4.2 Hz, 1H), 3.89-3.75 (m, 2H), 3.33 (s, 1H), 2.57-2.50 (m, 1H), 2.27-2.20 (m, 1H), 1.67 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4, 138.7, 134.6, 128.2, 128.1, 127.4, 117.3, 115.7, 114.7, 104.1, 87.2, 87.1, 78.1, 77.2, 73.1, 35.4, 27.6, 27.4; IR (film) 3501, 2987, 2934, 1384, 1373, 1218, 1161, 1106, 914, 873 cm^{-1}; $[\alpha]_D$: +1.2° (*c* 0.26, MeOH); HRMS (ESI) calcd for $C_{20}H_{26}O_5Na$ $(M+Na)^+$ 369.1673, found 369.1671.



2.47 **2.48**

Diacetate 2.48. DOWEX 50W-8X ionic exchange resin (0.094 g) was added to a dioxane-H_2O (1:1, 5 mL) solution of diene **2.47** (0.094 g, 0.27 mmol). The resulting mixture was stirred for two days and filtered through a ground-glass frit. The filtrate was partitioned between H_2O (5 mL) and EtOAc (5 mL). The aqueous portion was separated and back-extracted with EtOAc (10 mL) three times. The combined organic portions were washed with saturated solution of $NaHCO_3$ (5 mL) and brine (5 mL), then dried over $MgSO_4$ and concentrated under reduced pressure. The resultant colorless oil was reconstituted in pyridine (1 mL) and cooled to 0 °C. To the solution was added acetic anhydride (0.076 mL, 0.81 mmol). The solution was allowed to warm to room temperature and was stirred overnight under Argon. The solution was diluted with EtOAc (5 mL), poured into ice water (5 mL), and extracted with EtOAc (10 mL) three times. The combined organic extract was washed sequentially with saturated solution of $NaHCO_3$ (5 mL) and brine (5 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 3:1) to give the product **2.48** as a colorless oil, (0.091 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.27 (m, 5H), 6.27 (d, J = 3.6 Hz, 1H), 5.85-5.74 (m, 2H), 5.49 (dd, J = 15.6, 1.6 Hz, 1H), 5.42-5.10 (m, 4H), 4.71 (d, J = 11.2 Hz, 1H), 4.66 (s, 1H), 4.48 (d, J = 11.2 Hz, 1H), 4.08 (d, J = 2.8 Hz, 1H), 3.79 (ddd, app. dt, J = 6.8, 2.8, 2.8 Hz, 1H), 2.53 (t, J = 7.2 Hz, 2H), 2.08 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 169.5, 136.8, 136.6, 133.3, 128.5, 128.4, 128.1, 118.5, 117.1, 98.8, 82.7, 81.2,

80.7, 76.3, 71.8, 34.7, 21.1, 20.6; IR (film) 3435, 2980, 2920, 1751, 1372, 1221, 1071, 1009, 954, 926 cm^{-1}; $[\alpha]_D$: (–) 109° (*c* 1.35, $CHCl_3$); HRMS (ESI) calcd for $C_{21}H_{26}O_7Na$ $(M+Na)^+$ 413.1571, found 413.1563.



Nucleoside 2.49. *N,O*-bis-trimethylsilyl acetamide (0.055 mL, 0.078 g, 0.385 mmol) was added to a stirred anhydrous acetonitrile (1.2 mL) mixture of thymine (0.020 g, 0.154 mmol). The mixture was heated to 85 °C for one hour and then cooled to 0 °C. To the solution was added diene **2.48** (0.030 g, 0.077 mmol) as a solution in anhydrous acetonitrile (1.2 mL) and TMSOTf (0.028 mL, 0.034 g, 0.154 mmol). The solution was heated to 45 °C for 18 hours and then cooled to 0 °C and quenched with saturated solution of $NaHCO_3$ (0.5 mL). The mixture was partitioned between (5 mL) ethyl acetate (5 mL) and saturated solution of $NaHCO_3$ (5 mL). The aqueous layer was separated and back-extracted with ethyl acetate (5 mL) three times. The combined organic extracts were washed with brine (5 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dried under hi-vacuum for 30 min and reconstituted in THF (1 mL). To the solution was added TBAF (0.077 mL, 1M solution in THF, 0.077 mmol). The solution was stirred for 30 min and partitioned between EtOAc (5 mL) and saturated solution of $NaHCO_3$ (5 mL). The aqueous portion was separated and back-extracted with EtOAc (5 mL) three times. The combined extracts were washed with

brine (5 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/EtOAc, 1:2) to give the product **2.49** as a colorless foam, (0.022 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (br s, 1H), 7.36-7.28 (m, 5H), 7.09 (d, *J* = 1.2 Hz, 1H), 5.82 (d, *J* = 5.6 Hz, 1H), 5.36 (d, *J* = 6.0 Hz, 1H), 4.67 (d, *J* = 12.4 Hz, 1H), 4.59 (d, *J* = 12.4 Hz, 1H), 4.56 (d, *J* = 3.2 Hz, 1H), 3.87 (br, 1H), 3.70-3.64 (dt, *J* = 10.8, 3.6 Hz, 1H), 2.13 (s, 3H), 3.14 (s, 3H), 1.88-1.85 (m, 1H), 1.75-1.49 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 163.6, 138.2, 126.7, 128.4, 127.7, 127.7, 111.6, 89.5, 85.2, 81.4, 78.2, 74.9, 70.8, 29.1, 25.4, 20.5, 19.0, 12.6; IR (film) 3386, 3066, 1750, 1693, 1467, 1372, 1229, 1052 cm^{-1}; $[\alpha]_D$: (–) 64.0° (*c* 0.85, $CHCl_3$); HRMS (ESI) calcd for $C_{24}H_{32}N_3O_7$ $(M+NH_4)^+$ 474.2235, found 474.2240.

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