University of Alberta

Synthesis of Donor-based Analogues as Inhibitors of Mycobacterial Glycosyltransferases

by

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Dedicated to my husband Fu Chen.

Abstract

Tuberculosis (TB) is the disease arising from infection by *Mycobacterial tuberculosis* and kills millions of people every year. Difficulties in the treatment of TB and the emergence of multiple-drug resistant and extreme-drug resistant *M. tuberculosis* strains have increased interest in finding new antimycobacterial agents. The cell wall of mycobacteria is essential for the survival of these bacteria and enzymes involved in its assembly are key targets for anti-mycobacterial chemotherapy. One of the largest components of the cell wall is the arabinogalactan, which is composed of arabinofuranose (Ara*f*) and galactofuranose (Gal*f*) residues. These monosaccharides are incorporated into the polysaccharide by arabinosyltransferases and galactosyltransferases that employ decaprenolphophoarabinose (DPA) and uridine diphospho-galactofuranose (UDP-Gal*f*) as the donor substrate, respectively. The synthesis of analogues of DPA and UDP-Gal*f* as potential inhibitors of mycobacterial glycosyltransferases is presented in the thesis.

Carbohydrate mimics of Ara*f* and Gal*f* that have a bicyclo[3.1.0]hexane at the core were prepared. Key steps involved the formation of bicyclo[3.1.0]hexane system via an intramolecular displacement reaction followed by a separation by converting a mixture of enantiomers into diastereomers. The absolute configuration of these species was confirmed by

X-ray analysis of a crystalline derivative of the Ara*f* analogue. The bicyclo[3.1.0]hexane based mimics were then alkylated with various aldehydes through reductive amination to form the DPA and UDP-Gal*f* analogues.

The synthesis of the sulfonium ion analogs of Gal*f* was also carried out. The precursor of these compounds, a cyclic sulfide, was synthesized in nine steps from D-arabinitol. The key step is a conversion of an olefin into hydroxymethyl group thus establishing a stereogenic centre that is essential in forming a molecule that is a mimic of the galactofuranose ring. This sulfide was then coupled with alkyl halides to form sulfonium ion compounds in good yields.

All of the DPA and UDP-Gal*f* analogues were tested for their ability to inhibit GlfT2, a key galactofuranosyltransferase involved in the synthesis of the galactan portion of the mycobacterial arabinogalactan. Most of the compounds showed weak inhibition of the enzyme; however, a few were moderately active and are the mode of inhibition of these analogues is currently being studied.

Acknowledgement

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Finally, I want to thank my husband Fu Chen for being with me whenever I need him. This thesis cannot be finished without his support over the years. He gave me a wonderful life during my research.

Table of Contents

Pages

List of Figures

List of Schemes

List of Abbreviations

[α]	specific rotation
μM	micromolar
Ac	acetyl
Ac_2O	acetic anhydride
AG	arabinogalactan
AIBN	2,2’-azobis(2-methylpropionitrile)
AM	arabinomannan
aq.	aqueous
Ara*f*	arabinofuranose
Bn	benzyl
BOM	benzyloxymethyl
br	broad
Bu	butyl
Bz	benzoyl
calcd	calculated
COSY	correlation spectroscopy
d	doublet (NMR spectra)
DCC	*N*,*N*’-dicyclohexylcarbodiimide

DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*'-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPA	decaprenophosphoarabinofuranose
equiv.	equivalent
Et	ethyl
Et_3N	triethylamine
Gal*f*	galactofuranose
h	hour(s)
HOAc	acetic acid
Hz	hertz
J	coupling constant
LAM	lipoarabinomannan
m	multiplet (NMR spectra)
M	molar
m/z	mass to charge ratio (mass spectrometry)
m-CPBA	meta-chloroperoxybenzoic acid
MDR	multi-drug resistant

Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
mL	milliliter(s)
mM	millimole(s)
MOM	methoxylmethyl
MQ water	Milli-Q (deionized) distilled water
Ms	methanesulfonyl (mesyl)
MS	mass spectrometry
NaHMDS	sodium bis(trimethylsilyl)amide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
ºC	degree Celsius
Ph	phenyl
ppm	parts per million (NMR spectra)
p-TsOH	*p*-toluenesulfonic acid
Py	pyridine
q	quartet (NMR spectra)
R_f	retention factor

RNA	ribonucleic acid
rt	room temperature
s	singlet (NMR spectra)
satd.	saturated
t	triplet (NMR spectra)
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TEA	triethylamine
TESI	triethylsilyl iodide
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	tosyl, *p*-toluenesulfonyl
TS	transition state
UDP-Gal*f*	UDP-galactofuranose
UDP-Gal*p*	UDP-galactopyranose
WHO	World Health Organization
XDR	extreme-drug resistant

Chapter 1

Introduction

1.1 Biological Background

1.1.1 Tuberculosis

Tuberculosis (TB) is an infectious disease caused by mycobacteria of the "tuberculosis complex", most notably *Mycobacterium tuberculosis*. This infection is commonly transmitted when an individual inhales droplet nuclei containing *M. tuberculosis* that result from coughing by an infected person. Any organ system in the body can be infected, but the lung is the primary infection site. According to the World Health Organization (WHO), TB kills nearly two million people worldwide each year, and in adults nearly 20% of all deaths are individuals between 15 and 59.[1-5] Recent studies have shown that the global incidence of TB has continued to increase over the past 30 years.[1] Of particular note, one third of this increase can be attributed to co-infection with HIV.[1, 6-9] Another important issue is the emergence of multi-drug resistant (MDR) TB throughout the world since the early 1990s.[10-14] More recently, extreme-drug resistant (XDR) TB has become a concern.[1, 15, 16]

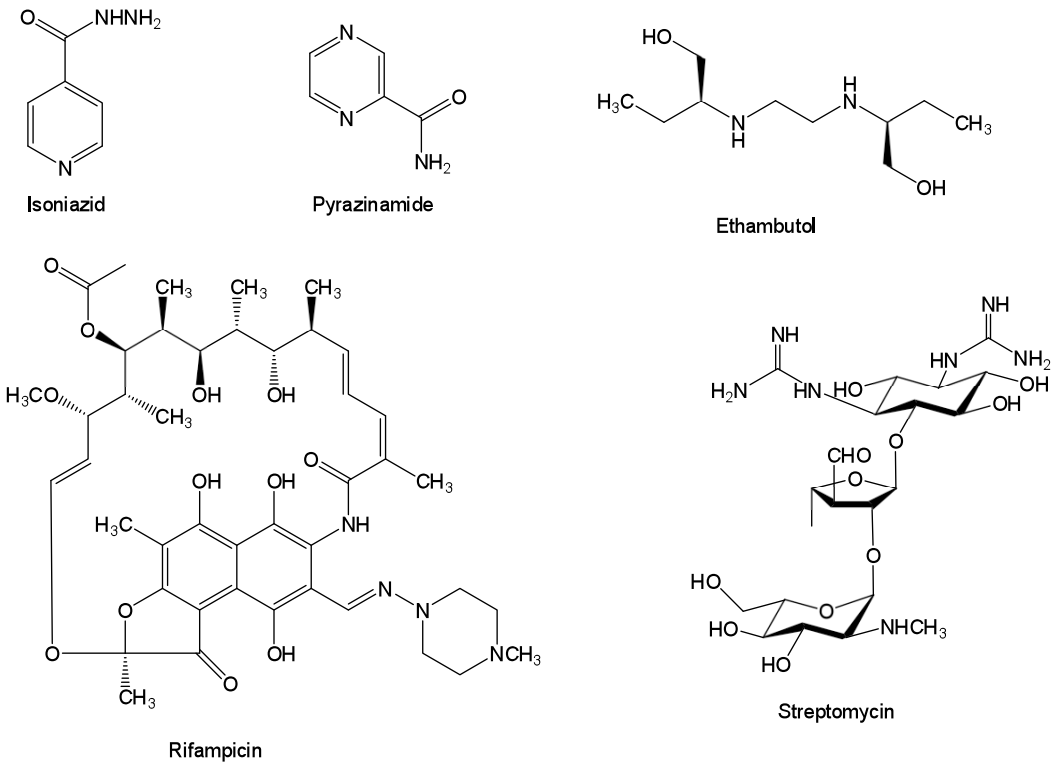


Figure 1-1. Structures of selected anti-TB drugs

The chemotherapy of tuberculosis has changed much since the introduction of the first effective antibiotic, streptomycin, in 1946. Pyrazinamide and isoniazid have been used for the treatment of the disease since 1955, and ethambutol and rifampin since 1960s and 1970s, respectively (see Figure 1-1 for structures).[17] To date, more than 30 different anti-TB drugs have been identified. However, many of these new compounds are analogues or prodrugs of the first line drugs mentioned above. The treatment of TB is very lengthy. According to standard treatment guidelines, short-course anti-TB chemotherapy normally

takes for six or eight months and requires multiple antibiotics.[18, 19] However, the emergence of multi-drug resistant (MDR) and extreme drug-resistant (XDR) strains of *M. tuberculosis* make this treatment less effective and require that new, sometimes less effective, drugs be used.[14-16] The major causes of drug resistance are the low potency of drugs, side effects, less than full treatment observation and long duration of therapy, which results in noncompliance.[20, 21] In the past few years, much effort has been put into developing new anti-TB drugs, and also to determinate the biochemical processes that could, in turn, guide the design of such compounds.[22-24]

It is known that the difficulty in the treatment of TB, and the long drug regimens that are required, are related to the unique structure of the mycobacterial cell wall. This complicated structure has very low permeability and prevents the efficient passage of antibiotics into the organism. Therefore, it is necessary to treat the patient for a long period with antibiotics.[25, 26] In the following sections, I will describe more about the structure of the mycobacterial cell wall.

1.1.2 Structure of the Mycobacterial Cell wall

M. tuberculosis can be classified as a Gram-positive bacteria, and is resistant to harsh conditions such as drying and chemical disinfectants. The longevity of the *M. tuberculosis,* like its resistance to antibiotics, can be ascribed to its unique cell wall structure, which works as a permeability barrier. The cell wall of

mycobacteria is essential for growth and survival in the infected host.[25, 27-30] A cartoon illustration of the general structure of the mycobacterial cell wall is shown in Figure 1-2. The cell wall is comprised of four major components: peptidoglycan, the mycolyl-arabinogalactan (mAG) complex, lipoarabinomannan (LAM) and extractable glycolipids.[25, 26, 29, 31]

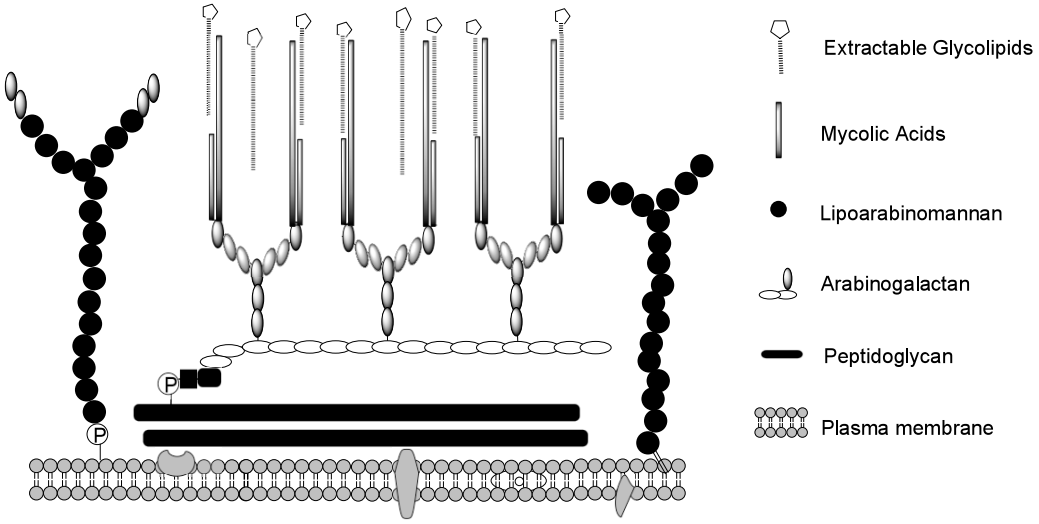


Figure 1-2. Schematic depiction of mycobacterial cell wall

1.1.2.1 Peptidoglycan

Just outside of the plasma membrane is the peptidoglycan. This component consists of chains of polysaccharide chains formed from alternating units of *N*-acetylglucosamine and *N*-glycolylmuramic acid residues. The *N*-glycolylmuramic acid units in the polysaccharide are esterified at O3 with

tetrapeptide motifs consisting diaminopimelic acid, D-glutamic acid, L-alanine and D-alanine residues. These peptides on two chains are cross-linked either between two diaminopimelic acid groups or between diaminopimelic acid and D-alanine (Figure 1-3).[28, 32-34]

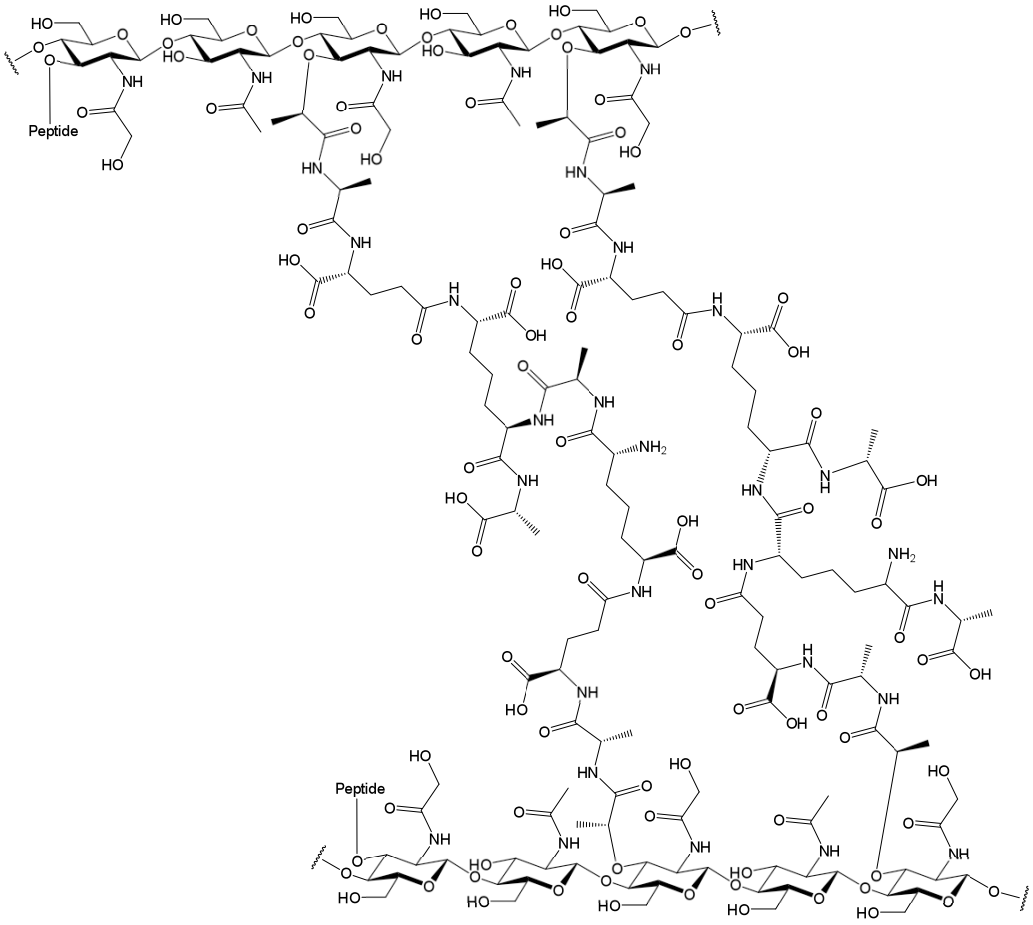


Figure 1-3. Structural motifs present in mycobacterial peptidoglycan

1.1.2.2 The mAG Complex

The mAG complex (see structure in Figure 1-4) is composed of long-chain lipids called mycolic acids, as well as D-arabinofuranosyl (Ara*f*) and D-galactofuranosyl (Gal*f*) carbohydrate residues which are connected to cell wall peptidoglycan via an α-L-rhamnopyranosyl-(1→3)-α-D-2-acetamido-2-deoxy-D-glucopyranosyl phosphate disaccharide (often call the "linker disaccharide").[35, 36] The main polysaccharide portion of the mAG complex contains at its core a galactan domain that contains approximately 30 Gal*f* residues attached via alternating β-(1→5) and β-(1→6) linkages.[37] There are three branch points along this galactan to which are attached arabinan chains made up of roughly 70 D-Ara*f* residues, which are connected via α-(1→5), α-(1→3) and β-(1→2) linkages.[38] The non-reducing end of the arabinan chains are capped with a branched hexasaccharide, which is esterified at the terminal primary hydroxyl groups with mycolic acids.[39] Mycolic acids are large (C_{70}–C_{90}), α-alkyl branched β-hydroxylated fatty acids. These lipid form a tightly packed assembly that gives the cell wall the permeability described above.[39, 40]

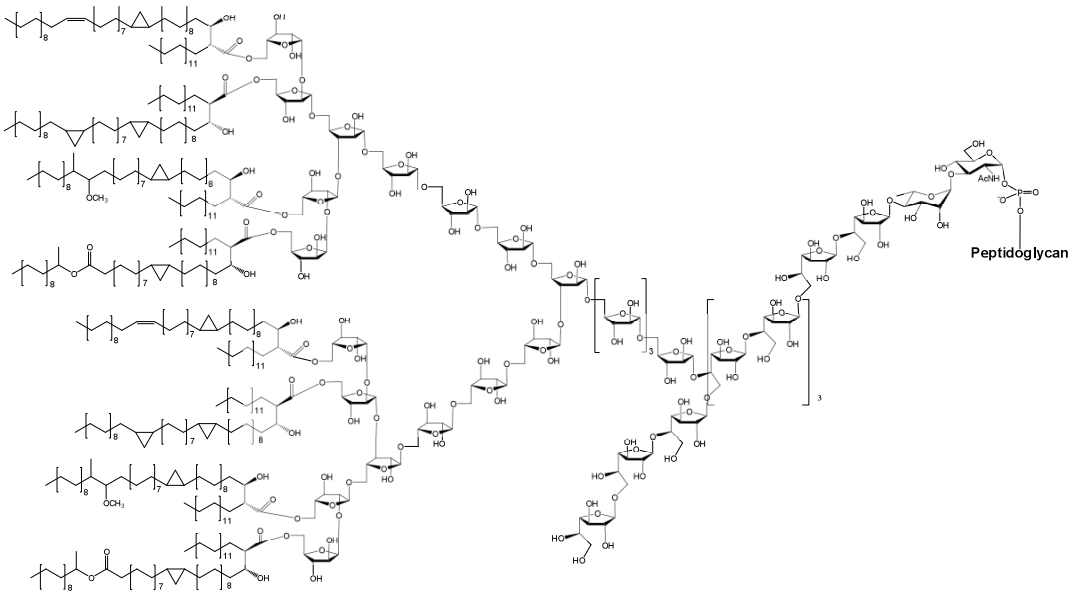


Figure 1-4. Structure of the mAG complex

1.1.2.3 Lipoarabinomannan

Lipoarabinomannan (LAM) is non-covalently bound to the cell wall and may be anchored in the plasma membrane and/or in the mycolic acid layer via the diacylglycerol of the phosphatidyl-*myo*-inositol (PI) moiety that is found at the nonreducing end of the molecule (Figure 1-5).[26] In the structure of LAM a mannan core comprised of 20−25 α-(1→6)-linked mannopyranosyl residues extends from the inositol residue.[41, 42] The polysaccharide is further modified by the presence of α-(1→2)-linked mannopyranosyl residues on approximately half of the sugar residues of the backbone glycan. Attached at the non-reducing end of this mannan is an arabinan made of 50−80 Ara*f* residues, which has a structure

similar to the arabinan of the mAG complex; that is, it has α-(1→5), α-(1→3) and β-(1→2) linkages.[42] The chains of the arabinan are capped at the non-reducing end with a hexasaccharide that is either unsubstituted or further glycosylated with α-(1→2) linked mannopyranosyl oligosaccharides to give a structure called ManLAM.[43, 44]

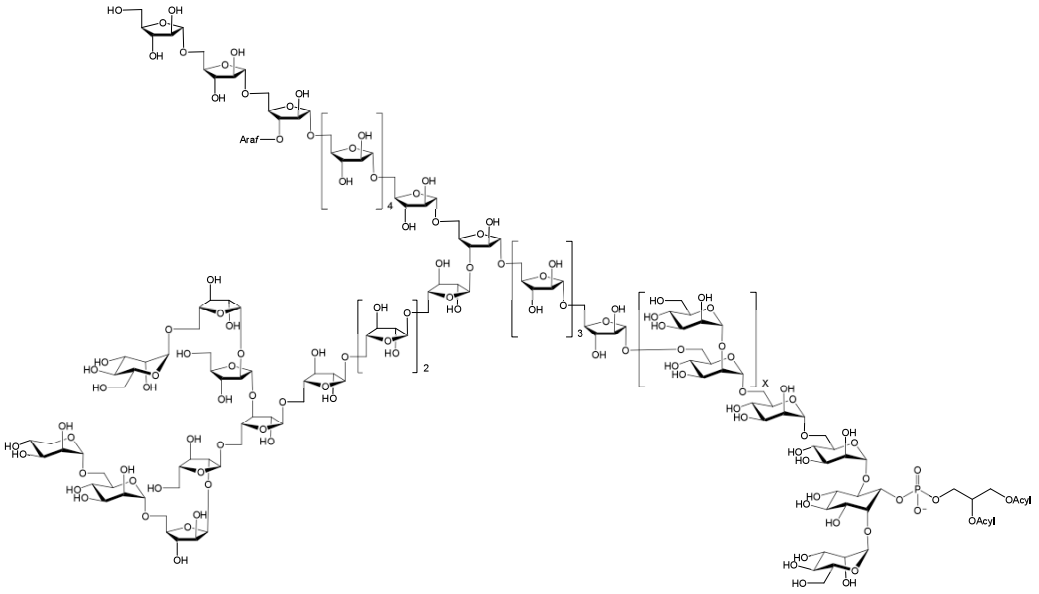


Figure 1-5. Structure of LAM

1.1.2.4 Extractable glycolipids

In addition to the large molecules described above, smaller glycolipids are noncovalently associated with mycolic acids located at the periphery of the cell wall complex. The lipid portion anchors the molecule into the mycolic acid and the oligosaccharide residue is presented at the outer part of the cell wall.[26, 39]

The structures of these molecules are diverse and are species specific. Some examples are provided in Figure 1-6.[45, 46]

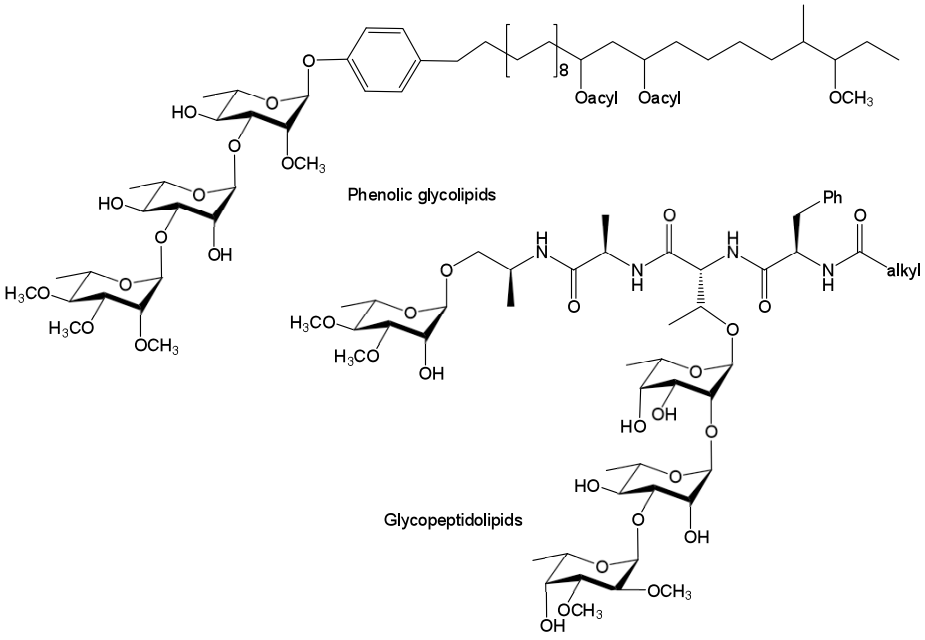


Figure 1-6. Examples of extractable glycolipids

1.1.3 Role of AG in the progression of mycobacterial disease

The largest structural component of the mycobacterial cell wall is the mycolyl-arabinogalactan (mAG) complex. The arabinosyl and galactosyl residues in this glycoconjugate adopt the furanose conformation instead of the thermodynamically stable pyranose conformation.[22] It has been proposed that the presence of these carbohydrates in this ring conformation increases the flexibility of the polysaccharide, which allows the mycolic acids to pack tightly in

an optimal orientation through van de Waals interaction. Thus, the bacteria present to the environment a structure with extremely low permeability and this, in turn, provides the organism with great protection from its environment.[26, 47]

Given its importance to the life cycle of the organisms, mycobacteria must produce an intact mAG complex. Therefore, one strategy for developing new anti-TB drugs is to identify compounds that interfere with the biochemical pathways involved in mAG assembly.[48-54] Indeed, two of the standard antibiotics used in the treatment of TB, isoniazid and ethambutol, target mAG biosynthesis. Isoniazid blocks mycolic acid biosynthesis and ethambutol inhibits arabinosyltransferases involved in the biosynthesis of the arabinan portion of the polysaccharide.[11, 55-57] Furanose oligo- and polysaccharides are not found in mammalian glycoconjugates and therefore inhibitors of the enzymes that process and introduce these residues into the mAG are particularly attractive drug candidates.[58] As mentioned above, ethambutol targets arabinan biosynthesis. Although none of the anti-TB drugs in clinical use are known to target the galactan assembly, its production is essential for the viability of the organism.

1.1.4 Biosynthesis of AG

The pathway by which the mAGP is assembled has been slowly unraveled over the last several years.[49, 59-65] The route proposed several years ago by Brennan[34] (Figure 1-7) has been shown to be generally correct and the genes and

enzymes involved in each step are now being identified.[49, 66] The process involves the sequential addition of sugar residues to a polyprenol bound intermediate. This completed polysaccharide is then transferred to peptidoglycan and then the mycolic acids are added. My thesis project is related to the arabinan and galactan portions of the cell wall and therefore the discussion below is focused on the biosynthesis of these parts of the mAG complex.

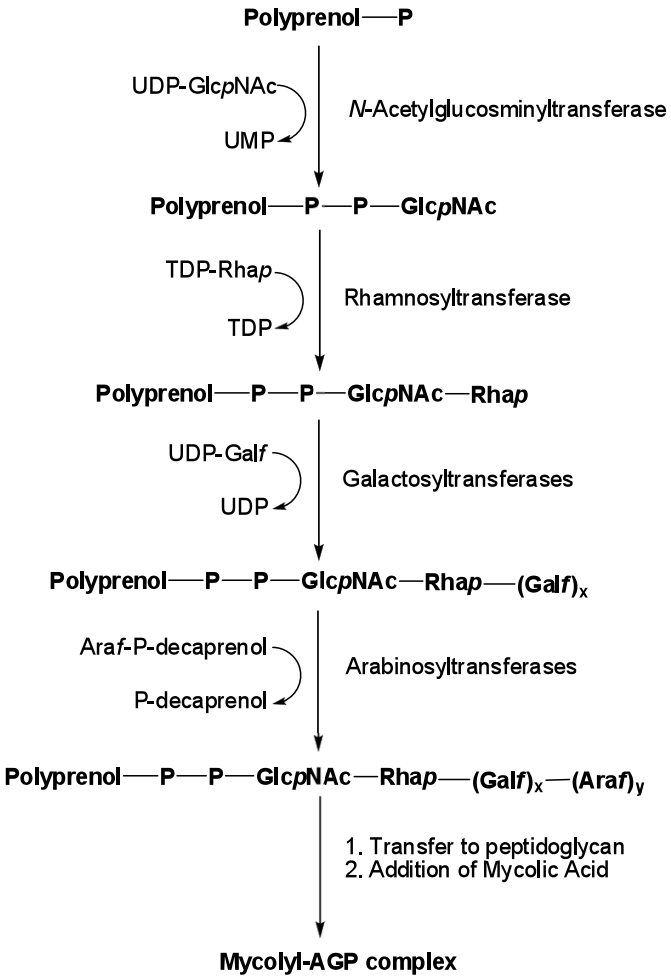


Figure 1-7. Biosynthetic pathway for the assembly of the mAGP complex

Based on current knowledge, galactan synthesis appears to involve two galactofuranosyltransferases.[63, 64, 67] The transfer of the first and second Gal*f* residues to the rhamnopyranose moiety are performed by GlfT1, a galactofuranosyltransferase encoded in *M. tuberculosis* by the Rv3782 gene. This enzyme is bifunctional, and can make both Gal*f*-β-(1→5)-Gal*f* and Gal*f*-β-(1→4)-Rha*p* linkages,[68] as illustrated in Figure 1-8. The remaining Gal*f* residues in the galactan, which are linked via β-(1→5) and β-(1→6) linkages, are introduced by another bifunctional enzyme, GlfT2, encoded in *M. tuberculosis* by Rv3808c gene. [63, 64, 67]

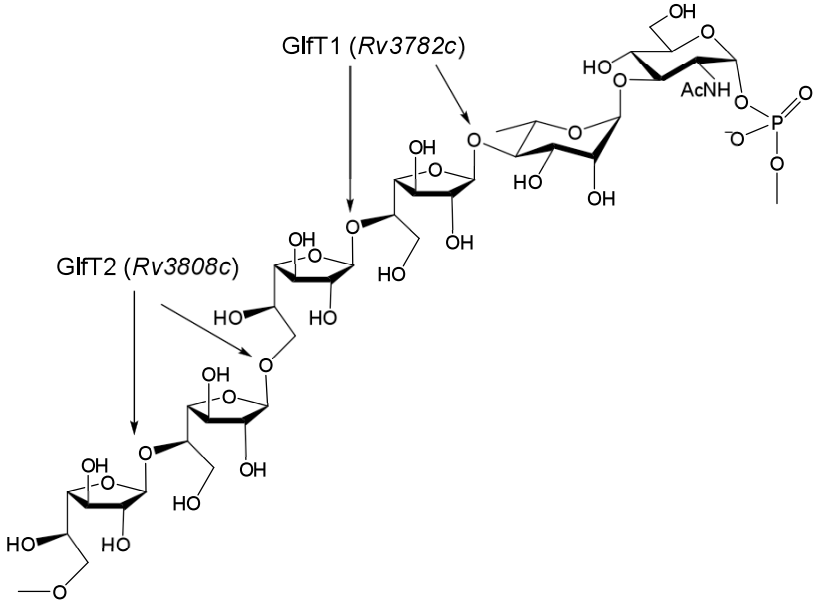


Figure 1-8. Galactofuranosyltransferases involved in mycobacterial galactan assembly.

Both GlfT1 and GlfT2 use UDP-galactofuranose (**1-1**, UDP-Gal*f*, Figure 1-9), as the donor. This sugar nucleotide is produced from UDP-Gal*p* by the action of UDP-Gal*p* mutase.[69] Both GlfT1 and GlfT2 transfer galactofuranose from UDP-Gal*f* to an acceptor oligosaccharide as illustrated in Figure 1-9.[70, 71] During this process uridine diphosphate (UDP) is liberated.[72]

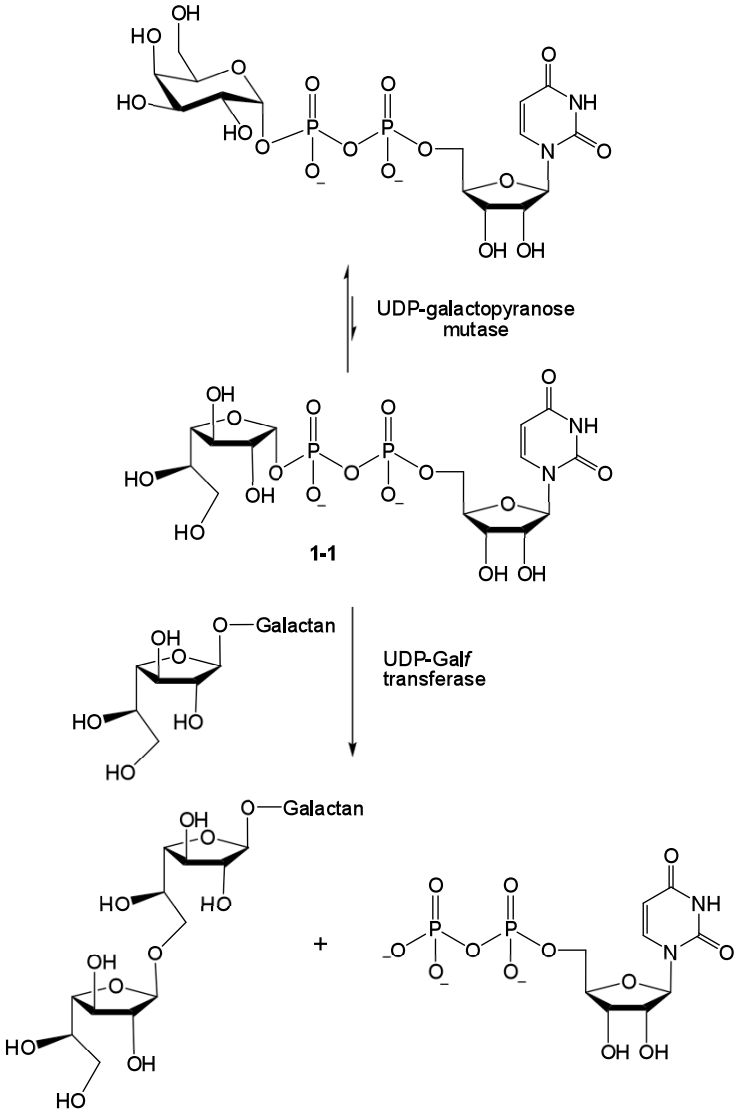


Figure 1-9. Enzymatic reactions of the formation of galactan

Several arabinosyltransferases (AraT's) are involved the biosynthesis of the arabinan portions of mAG. However, to date, isolation of these AraT's have not been successful, although assays for the enzymes in crude membrane fractions have been developed.[73-75] Most work in this area has focused on two AraT's, EmbA (Rv3794) and EmbB (Rv3795), which play important but not well understood roles in the synthesis of mycobacterial arabinan.[76] These enzymes are inhibited by ethambutol and it is therefore believed that they are the target for this anti-TB drug. More recent studies have uncovered three other AraTs involved in the assembly of the AG, which are distinct from EmbA and EmbB. These enzymes, AftA, AtfB and AftC are not inhibited by ethambutol.[66] AftA (encoded by the Rv3792 gene in *M. tuberculosis*) is an α-(1→5)-AraT that catalyzes the addition of the first Ara*f* residue to the galactan domain.[59] In contrast, AftB has been shown to be a β-(1→2)-AraT that installs the β-(1→2)-Ara*f* residues present in the hexasaccharide motifs at the terminus of the polysaccharide.[65] Finally, AftC is an α-(1→3)-AraT involved in the branching of the arabinan domain.[77] The donor substrate used by all of these enzymes is the glycophospholipid decaprenophosphoarabinofuranose (**1-2**, DPA, Figure 1-10).[78] A representative example of the reaction catalyzed by these enzymes is shown in Figure 1-10.

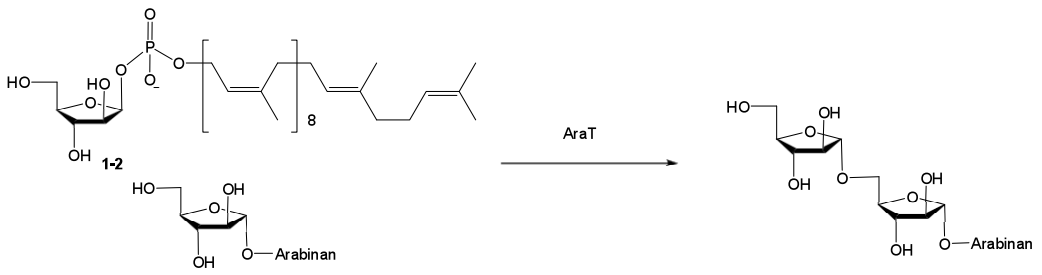


Figure 1-10. Representative reaction catalyzed by AraTs.

1.2. Background of AG glycosyltransferases inhibitors

1.2.1 General information on inhibitor

The AG serves as the linker of the mycolic acid to the peptidoglycan and is important for survival of the mycobacteria. Inhibiting the biosynthesis of the carbohydrate portions of the cell wall therefore represents an attractive approach for the development of new anti-TB drugs. One class of molecules of interest are compounds that inhibit mycobacterial glycosyltransferase activity. Ethambutol, one of the drugs currently used to treat TB, targets the Emb proteins involved in arabinan biosynthesis.[22, 79] This compound was identified through a screen of small molecules for anti-TB activity and its mode of action was not determined for many years after its clinical use was started. Another approach to identifying glycosyltransferase inhibitors is rational design. However, the rational design of inhibitors of these enzymes is difficult due to the complexity of the reactions catalyzed by these enzymes via a transition state involving a

glycosyl donor, a glycosyl acceptor and a metal ion (Mn^{2+} or Mg^{2+}). Further complications are the weak binding of glycosyltransferases with their natural substrates and a lack of structural data on these enzymes.[80]

Despite these challenges, significant efforts have been made to identify new glycosyltransferase inhibitors over the past several years. This work has led to the identification of some potent inhibitors, and an increased number of 3D structures of several glycosyltransferases has facilitated this task, by providing important mechanistic information.[70, 81-83] Different strategies have been used in synthesizing new inhibitors of glycosyltrasferases.[80, 84-88] The most important ones are the design of acceptor analogues, donor analogues (i.e. unreactive sugar nucleotides), or transition state mimetics including bisubstrate or trisubstrate analogues.[80]

As mentioned earlier, the galactose and arabinose residues in the mAG adopt the furanose conformation. Furanose oligosaccharides are not found in mammalian glycoconjugates and thus compounds that interfere with the biosynthesis of these parts of the polysaccharide should not have a significant deleterious effect on humans.[58] In the design of inhibitors of mycobacterial glycosyltransferases, donor analogues that have a furanose fragment have drawn significant attention. Amongst the furanose mimics synthesized, the most common structural modification is the replacement of the ring oxygen atom by a heteroatom (e.g., nitrogen) or by a carbon atom, for example iminosugars and

carbasugars. These compounds are suggested to be mimics of the transition state of the enzymatic reaction, rather than the natural carbohydrate substrate.[80]

1.2.2 Donor analogues of galactofuranosyltransferases.

The pathway by which galactofuranose residues are incorporated into mycobacterial galactan is now understood. The glycosyltransferases GlfT1 and GlfT2 transfer D-galactose from uridine-5'-diphospho-α-D-galactofuranose (**1-1**, UDP-Gal*f*) to an acceptor leading to the elongated product (Figure 1-9). Both GlfT1 and GlfT2 form glycosidic linkages with inversion of the anomeric configuration (Figure 1-11). Although no detailed mechanistic studies on these enzymes have been reported, it is possible to propose a mechanism as shown in Figure 1-11. In this mechanism the C–O bond in the donor is weakened with the assistance of an acidic residue in the enzyme active site. Next, the acceptor alcohol attacks the donor via an S_N2-type reaction via a transition state with a significant amount of oxonium ion character. Finally, a basic group in the enzyme active site removes the proton on the acceptor alcohol providing the elongated galactan.[81] It should also be noted that a metal ion is involved in binding the sugar nucleotide to the enzyme. Potential donor-based inhibitor analogues are generally designed considering the three different moieties found in the natural substrates: the carbohydrate, the diphosphate linkage, and the nucleoside moiety, or combinations of these.

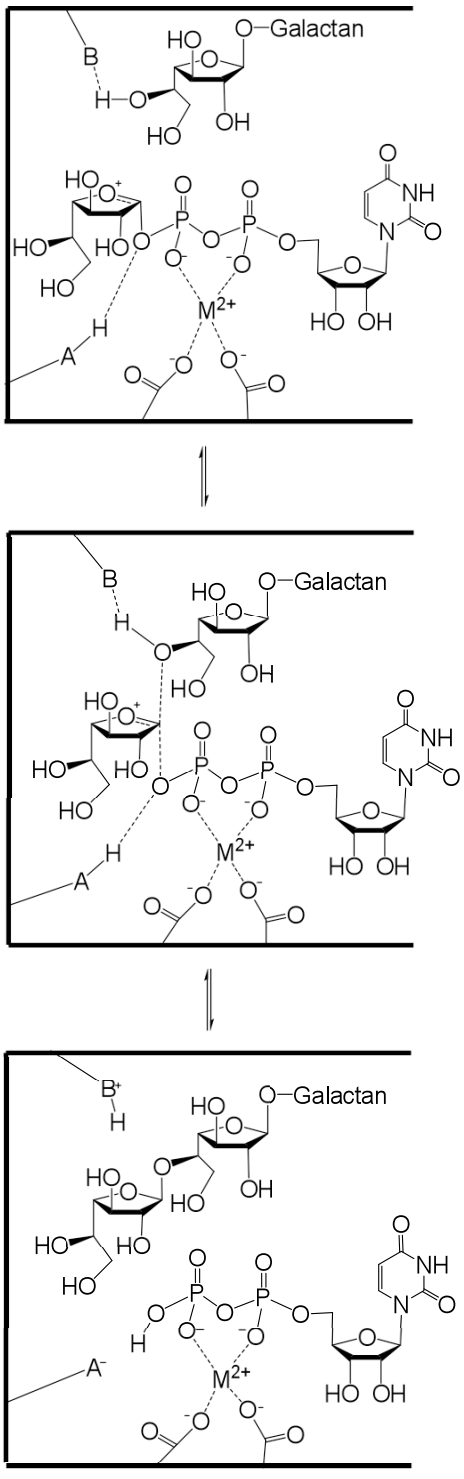


Figure 1-11. Proposed mechanism for inverting GlfT1 and GlfT2

The carbohydrate part is designed to simulate the shape and most functionalities of the natural substrate. Three types analogues are have been prepared as listed below and the examples are shown in Figure 1-12.

1) Iminosugars, analogues that contain a nitrogen in the ring. At physiological pH the nitrogen is protonated to give a positively-charged group that interacts strongly with an anionic group at the enzyme active site.[89] Fleet and coworkers[90] reported the first iminosugar based analogues of Gal*f*, for example compound **1-3**. Later on, more analogues of this class have been investigated. Thomas' group synthesized compound **1-4** mimicking the Gal*f* as the inhibitor of galactofuranosyltransferases.[91] Martin and co-workers have studied iminosugar mimics as inhibitors of glycosyltransferases such as compound **1-5**.[92-94] Wong and co-workers have synthesized UDP-sugar analogues (**1-6**) by using tartaric esters as isosteric replacement for the pyrophosphate group.[95 96]

2) Carbasugars, analogs in which the ring oxygen is replaced by a methylene group. They are attractive because of their chemical stability as well their demonstrated biological properties mainly as glycosidase inhibitors.[97-99] For example, UDP-galactose analogues **1-7** was found to be an inhibitor of β-(1→4)-galactosyltransferase by Yuasa et. al.[100] However, with regard to galactofuranosyltransferase inhibitors, no compounds based on carbasugars have been reported.

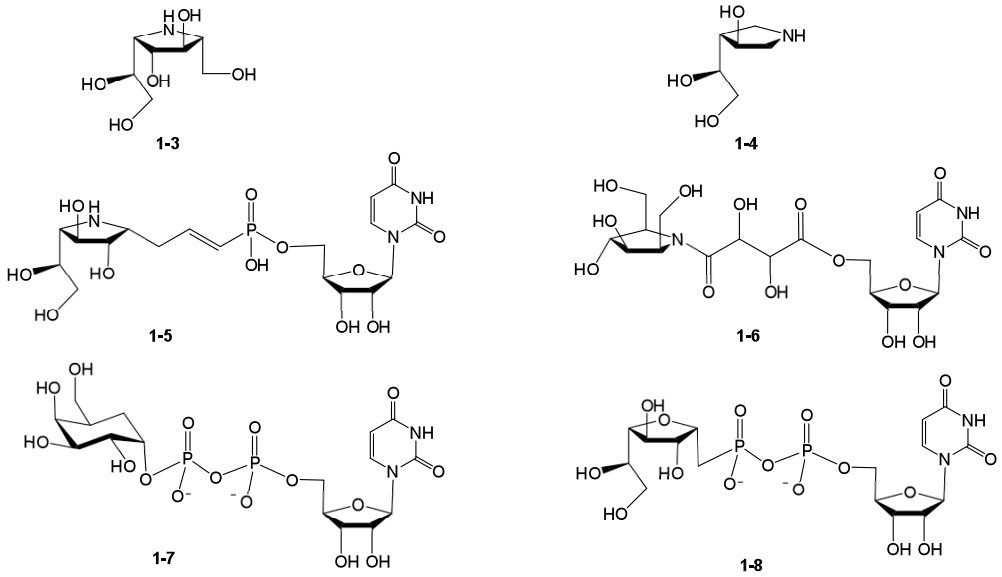


Figure 1-12. Structures of GT inhibitors

3) C-glycosides, analogs in which the glycosidic oxygen replaced with a carbon. These compounds are of interest in the design of stable analogues of sugar nucleotides which can fit the requirement of the enzyme active site without undergoing enzymatic cleavage.[22, 101-103] For example, C-glycoside donor analogue **1-8** was synthesized and studied the inhibition against glycosyltransferases.[104]

In addition to the carbohydrate part, the diphosphate moiety plays an important role in the enzymatic reaction by interacting via its dianionic charge with metallic ions, for example Mn^{2+}, which also coordinate with aspartate residues within the active site.[70, 95, 105] However, the pyrophosphate group is

labile and its negative charge is disfavored in terms of cell permeability, and therefore stable pyrophosphate mimics have been created.[90, 95, 104, 106] Various linkages that mimic the diphosphate moiety have been designed in the synthesis of potential glycosyltransferase inhibitors. As we can see from the above examples, the linkage can be a peptide-like tether,[90] malonic acid, tartaric esters,[95, 96] or modified pyrophosphate.[92-94]

1.2.3 Donor analogues as AraT inhibitors.

Particular attention has been focused on designing AraT inhibitors that are analogues of the donor substrate for these enzymes, decaprenolphosphoarabinose (DPA) (Figure 1-10).[75, 107-115] Because DPA is a substrate for more than one AraT, analogues of this compound would be expected to block a number of biosynthesis steps and in turn, be especially potent anti-TB agents.[114]

Given the hydrolytic lability of DPA, metabolically stable analogues are desired as potential AraT inhibitors. Three factors need to be considered in the design of these compounds. First, replacement of the labile glycosyl phosphate with a stable isostere is desirable.[107] Second, chelating moieties should be incorporated into the molecule to substitute for the phosphate, to coordinate with the metal ion that is usually present in glycosyltransferase active sites.[108, 116] Third, an alkyl chain, or other hydrophobic group would be expected to be required to mimic the large hydrophobic decaprenyl moiety of DPA.[107, 117]

To date, various isosteric replacements for the glycosyl phosphate (Figure 1-13) have been investigated, including the β-C-phosphonates (**1-9**),[114, 118] β-C-glycosyl sulfones (**1-10**),[119] and β-glycosyl triazoles (**1-11**).[107] Carbohydrate mimics such as the α-imino-sugar **1-12** [120] and **1-13** [108] as well as substituted DPA-Ara*f* analogues **1-14**, [113] have also been synthesized, but not all have been tested as inhibitors of AraT. Some of these compounds have, however, been tested as anti-TB agents with some (e.g., **1-9**, R = $(CH_2)_4O(CH_2)_{15}CH_3$) showing modest levels of activity.

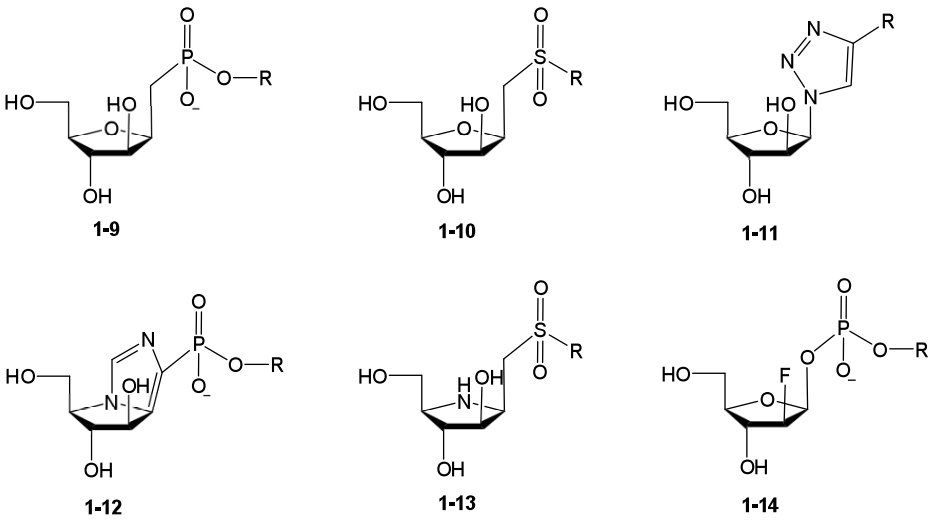


Figure 1-13. Structures of DPA-Ara*f* analogues synthesized to date.

1.3 Project Aims

1.3.1 Design of inhibitors of galactofuranosyltransferases and arabinosyltrasferases

As part of larger program on the identification of mycobacterial glycosyltransferase inhibitors, my project was aimed at synthesizing UDP-Gal*f* and DPA analogues, and the evaluation of these compounds as inhibitors of GlfT2 in a previously developed assay. Two types of carbohydrate ring mimics were targeted for synthesis as listed below.

First, we wanted to prepare both Gal*f* and Ara*f* mimics in which the five-membered ring was locked into a single conformation to "pre-organize" the molecule for enzyme recognition, which could thus lead to enhanced affinity. For this we selected the bicyclo[3.1.0]hexane derivatives **1-15** and **1-16** (Figure 1-14) as targets. In these molecules, the fusion of the cyclopropane moiety onto the five-membered ring lock the conformation of the cyclopentane ring into the conformation favored by UDP-Gal*f* and DPA.[121] More discussion on the use of bicyclo[3.1.0]hexane scaffold in inhibitor design is provided in a later section .

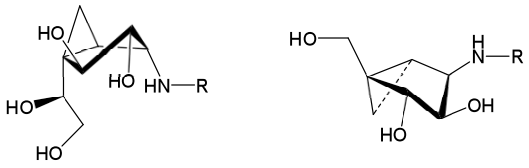
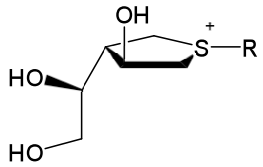


Figure 1-14. General structure of bicyco[3.1.0]hexane derivatives **1-15** and **1-16**

The amino group was included in the targets for two reasons. First, its presence would facilitate the preparation of additional analogues through, for example, reductive amination. Second, this group would be protonated at physiological pH and thus would be able to form strong ionic interactions with the anionic (carboxylate) groups that are typically present in glycosyltransferase active sites. This charge would also mimic the oxocarbenium ion-like character that develops in the glycosylation transition state.

Secondly, we wanted to synthesize Gal*f* analogues containing a sulfonium ion in the ring (**1-17**, Figure 1-15). Sulfonium ions carry a permanent positive charge, which might be advantageous in providing the necessary electrostatic interactions with the active site of the enzyme. These compounds are expected to mimic the oxocarbenim-like transition state that the donor reaches during the enzymatic reaction. More discussion on sulfonium ion inhibitors of carbohydrate-processing enzymes is provided below.



1-17

Figure 1-15. General structure of sulfonium ion target **1-17**

1.3.2 Conformational analysis of furanose rings and the bicyclo[3.1.0]hexane ring system

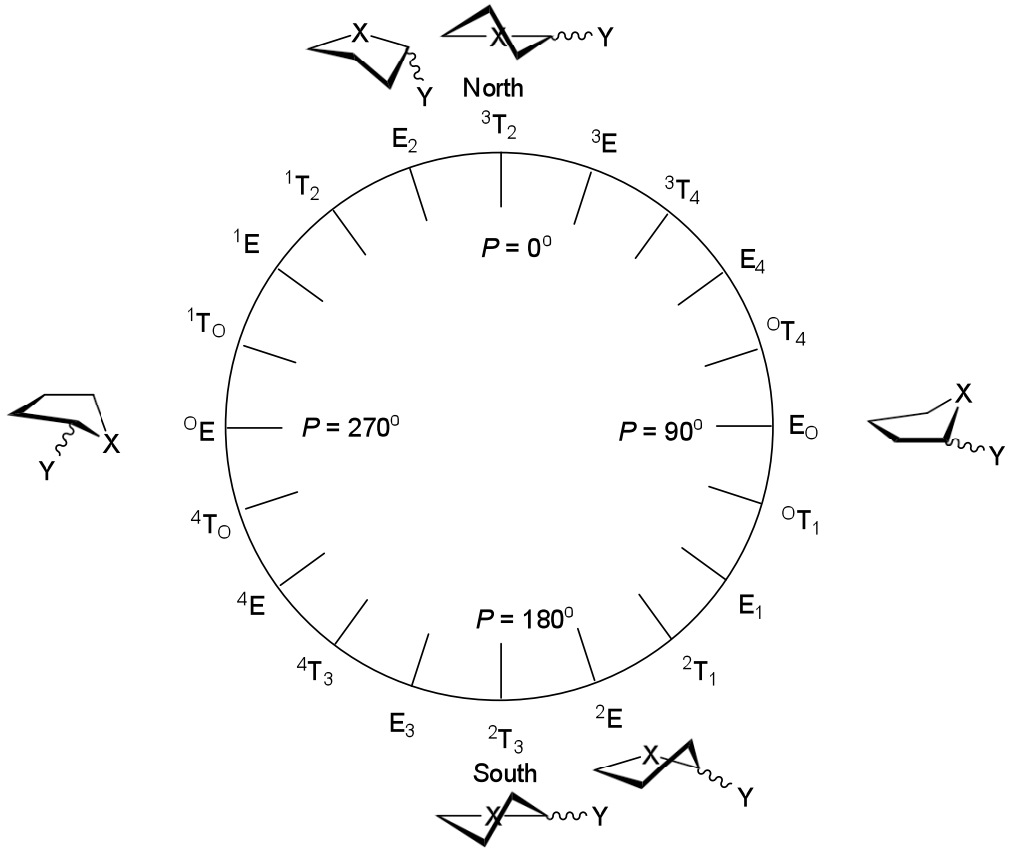
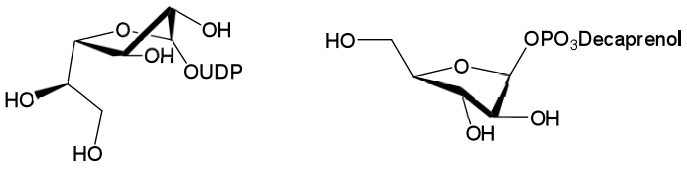


Figure 1-16. Pseudorotational wheel for a furanose ring

The standard model used to in the conformational analysis of furanose rings is one developed by Altona and Sundaralingam.[122] Normally, a conformationally unrestricted furanose ring can adopt a number of envelope (E) or twist (T) forms, as shown in the pseudorotational wheel (Figure 1-16), where *P* is the

pseudorotational phase angle. The superscripted and subscripted numbers correspond to the atoms that are, respectively, above and below the plane of the other atoms. For example, the $^{2}T_{3}$ conformer is a twist conformer with carbon 2 above, and carbon 3 below, the plane formed the ring oxygen and carbons 1 and 4. Rather than interconverting via a planar ring structure, most furanose rings equilibrate via pseudorotation through a series of intermediate E and T conformations.[123] The substituents on the ring, and their relative orientation, determine the conformers populated at equilibrium.[122]

Given the complete lack of structural data for the glycosyltransferases of interest to us, the exact conformation of the donor substrate when bound to the enzyme remains unknown. However, previous computational and NMR studies[121] have suggested that in solution, the furanose ring in UDP-Gal*f* (**1-1**) and DPA (**1-2**) adopt an envelope conformer in which C2 is either above or below the plane formed by the other ring atoms (**1-17** and **1-18**, respectively, Figure 1-17).



1-17 **1-18**

Figure 1-17. Proposed conformation of the furanose ring in UDP-Gal*f* and DPA.

It is well known that the conformational preference of furanose rings significantly influences the biological activity of molecules containing them. We wanted to prepare mimics of **1-1** and **1-2** in which the five-membered ring was locked into the low energy conformation determined from previous investigations. To do this, we chose to make analogs in which a cyclopropane ring was fused to a cyclopentane (Figure 1-18), which is the bicyclo[3.1.0]hexane system. Compounds of this predictably adopt a conformation in which the cyclopentane carbon forming the flap of the envelope is on the same side of the ring as the cyclopropane methylene group.[124]

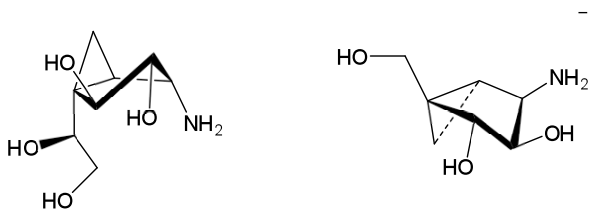


Figure 1-18. Target molecules

The approach of using locked furanose rings has found widespread application in the development of inhibitors of nucleotide processing enzymes,[125, 126] as well as in the identification of oligonucleotides that bind to their complementary nucleotide sequence with high affinity (e.g., locked nucleic acid).[127] The ribose and deoxyribose sugar of nucleotides and nucleosides adopt

two major ring forms of in solution. One is the North (N) conformation (shown in Figure 1-16) with *P* ranging between 342º and 18º ($E_2 \rightarrow {}^3T_2 \rightarrow {}^3E$) and the other one is the South (S) conformation with values of *P* between 162º and 198º ($^2E \rightarrow {}^2T_3 \rightarrow E_3$).[122, 128] However when the nucleoside binds to its target enzyme, only one of these forms is present in the active site. Thus, synthesizing locked nucleosides that favor the conformation bound by the enzyme, will lead to compounds with enhanced affinity for the protein.

Two methods have been used to obtain conformationally restricted furanose rings. One approach is the use of covalent tethers (i.e., fusion of a cyclopentane with another small ring). The other is to replace the hydroxyl group at C-2 and C-3 with other functionalities.

Since 1993, both the Marquez group and the Altmann group have focused extensively on the synthesis locked nucleosides by using the bicyclo[3.1.0]hexane system as a convenient template (Figure 1-19).[129-132] This sugar surrogate has a rigid pseudoboat conformation with the cyclopentane ring mimicking the ribose ring. The fused cyclopropane ring locks the conformation of the cyclopentane in the vicinity (± 18º) of either north or the south conformation depending on the location of the cyclopropane ring relative to the nucleobase.

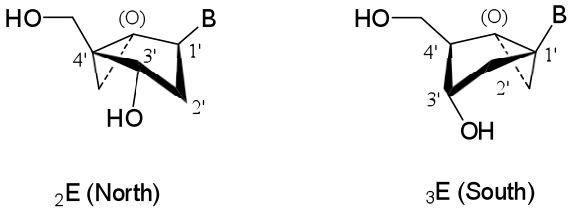
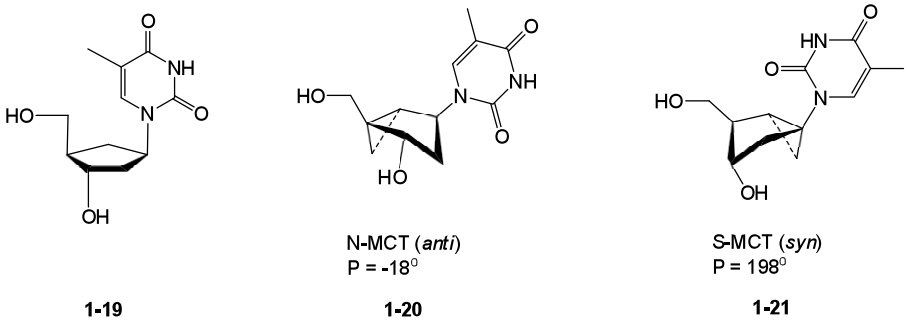


Figure 1-19. Locked nucleosides based upon the bicyclo[3.1.0]hexane ring system.

Using this template, many nucleoside monomers have been synthesized and used to study the preferred ring pucker of particular enzymes.[125, 126, 129, 131, 133-138] The most well studied compounds are the N-favoring methanocarbathymidine **1-20** (N-MCT) and the S-favoring methanocarbathymidine **1-21** (S-MCT) (Figure 1-20).[126] When the locked analogues and the flexible carbocyclic nucleoside carbathymine (**1-19**) were examined, only the **1-20** showed antiviral activity against Herpes simplex 1 and 2 viruses.[139] In other studies, the incorporation of N-MCT units into short single-stranded DNA increased the efficiency of hybridization between DNA and its complementary RNA strand.[139, 140]



1-19 1-20 1-21

Figure 1-20. Structure of thymidine and modified thymidine N-MCT and S-MCT

The same strategy was used to identify analogues of Puromycin (Figure 1-21). Puromycin (**1-22**) is an antibiotic produced by *Streptomyces alboniger* that specifically inhibits ribosomal peptide synthesis by interfering with RNA function, thus leading to premature chain termination during translation.[141, 142] Unfortunately, puromycin is cytotoxic, and efforts have been put into finding new analogues with similar properties but with lower toxicity. The Marquez group[137] has synthesized bicyclo[3.1.0]hexane-based puromycin analogues **1-23**, **1-24**, and very recently Strazewski[143] also reported similar compound **1-25**, and **1-26**. The biological activity of these compounds is currently being evaluated.

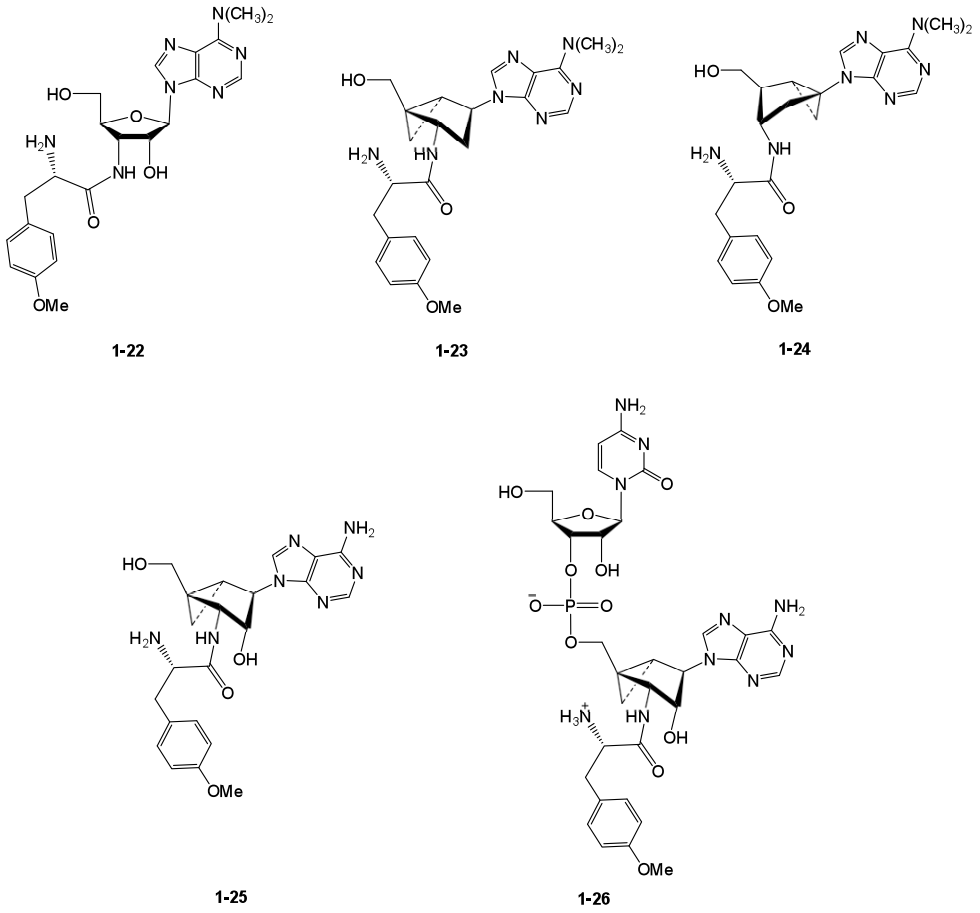
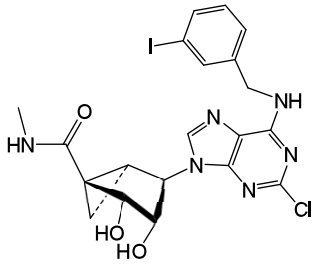


Figure 1-21. Structures of Puromycin and analogues

The bicyclo[3.1.0]hexane approach was also used by Jacobson to investigate adenosine derivatives that act as adenosine receptors and nucleotide receptor agonists.[133, 134, 144] For example, compound **1-27**[133] (Figure 1-22), in which the pseudoribose moiety adopts a N-conformation, provided favorable receptor affinity and selectivity compared to the corresponding S-conformers.



1-27

Figure 1-22. Structure of adenosine derivative **1-27**

Locked furanose ring analogs other incorporating groups different from the bicyclo[3.1.0]hexane system have also been developed and used widely in producing modified oligonucleotides.[145-148] It is well known that the B form DNA duplex possesses a C-2'-*endo* ($^2T_3$, S-form) sugar puckering and the A form RNA duplex has the C-3'-*endo* ($^3T_2$, N-form) puckering.[149, 150] By locking the five-membered ring in nucleosides or oligonucleotides into one preferred conformation, duplex RNA and DNA have higher thermal stability.[151] There are several ways of locking the conformation of the sugar moiety in a nucleoside.[138, 152-155] Wengel and coworkers synthesized the 2',4'-BNA (**1-28**, LNA)[156], which bridges the O-2'and C-4' atoms and achieves a locked C-3'-*endo* conformation (Figure 1-23). This modification dramatically improved the triple-forming oligonucleotide affinity for the target duplex, and formed a stable RNA triplex at neutral pH. A locked nucleic acid derivative **1-29**,[157] with the S type

conformation was synthesized using a different linkage, between O-2' and C-4'. The Imanishi group reported this type nucleic acid, for example **1-30**.[158]

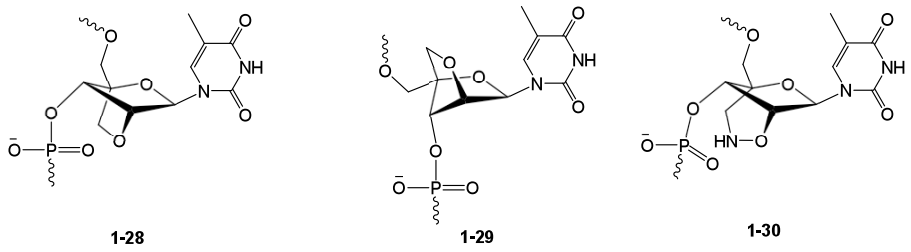


Figure 1-23. Examples of locked oligonucleotides with a O-2', C-4' bridge

In other work in this area, Nielsen and coworkers have reported bicyclic nucleoside derivatives in which bridges between C-2' and C-3' (**1-31, 1-32**),[155, 159] or between C-3' and C-4' (**1-33**)[160, 161] that have been used to achieve a locked S type conformation (Figure 1-24).

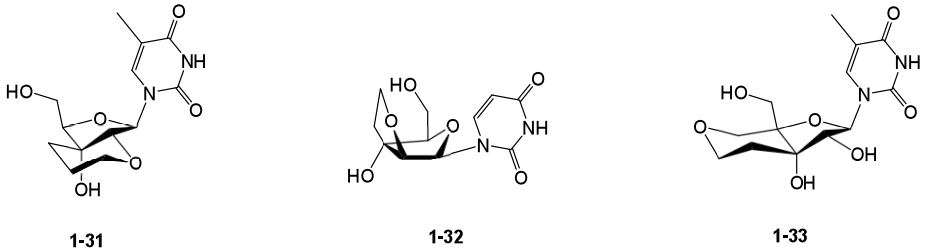


Figure 1-24. Examples of locked oligonucleotides with C-2'and C-3', C-3'and C-4' bridges

Outside the nucleotide field, Houseknecht and Lowary have synthesized oligosaccharides containing furanose rings locked into a single conformation. These molecules were synthesized to probe the conformation of the furanose rings in oligosaccharides containing arabinofuranose residues (Figure 1-25).[162]

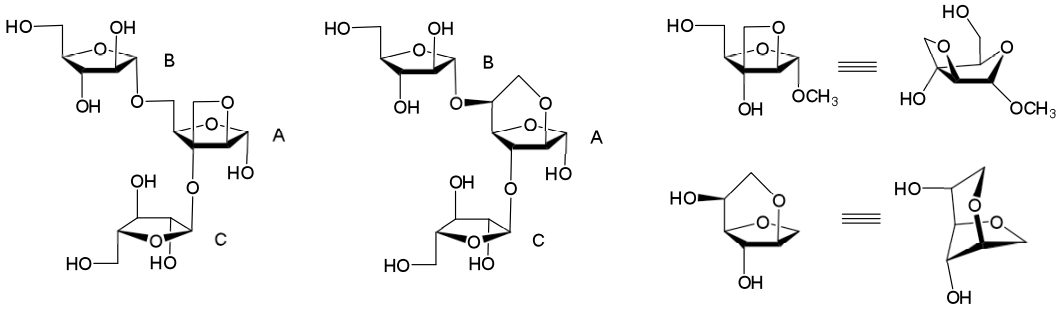


Figure 1-25. Structure of locked oligosaccharides synthesized by Houseknecht and Lowary.

The other way to lock the furanose ring is to add branches. As one example, 3'-C-branched nucleoside building blocks, e.g., **1-32**[163] and **1-33**[164, 165](Figure 1-26), have been made to improve either enzymatic stability against ODNs or to enhance the binding affinity toward complementary targets.

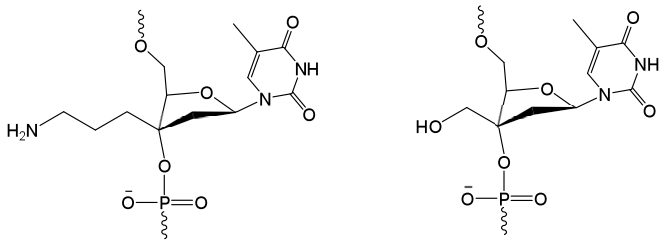


Figure 1-26. Examples of branched nucleosides

1.3.3 Sulfonium ion mimics

The inhibitors of the general structure **1-17** were designed in consideration of mimicking the transition state (TS) of the enzymatic glycosylation reaction. TS-based analogue inhibitors of glycosyltransferases are less studied than glycosidases; however, it is postulated that the enzymatic reactions catalyzed by glycosyltransferases and glycosidases proceed in a similar way, via a TS structure that has significant oxocarbenium-ion character (Figure 1-27).[81, 83, 166]

While designing a synthetic inhibitor, a means of providing the required charge state is to include an atom that carries a permanent positive charge at a suitable position. Thus, stable electrostatic interactions may be established between the charged atom and an active-site amino acid on the enzyme.

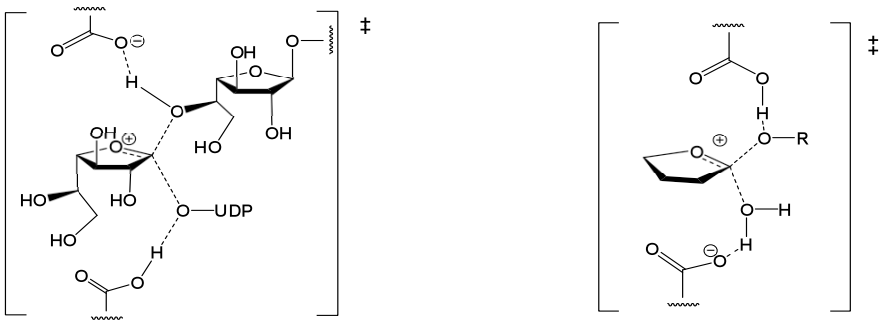


Figure 1-27. Proposed transition states of glycosyltrasferases and glycosidases.

One effective way of introduce the positive charge is to use nitrogen instead of the ring oxygen. These amine-based inhibitors are usually referred as azasugars (or iminosugars) and carry a positive charge at physiological pH and thus mimic the oxocarbenium-ion-like intermediate of the transition state.[167] The first inhibitor of this class was 1-deoxynojirimycin (Figure 1-28) an effective inhibitor of α-glycosidases. Other examples of this class were shown in Figure 1-12.

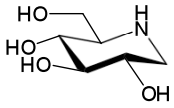


Figure 1-28. Structure of Deoxynojirimycin

The second strategy is to include a positively charged sulfur atom to establish this electrostatic paring. Sulfonium salts are known to be stable, as opposed to their highly unstable oxocarbenium ion counterpart. Yuasa and

coworkers developed the first sulfonium-ion compound **1-34** (Figure 1-29)[168], a β-glycosidase inhibitor that mimics the transition state structure. In designing the compound it was thought that the long C–S bond length (1.8 Å) and small C–S–C angle (~ 95º) would place the sulfur atom near the anomeric carbon in the TS. Since then, more sulfonium-ion were synthesized such as analogues of swainsonine (**1-35**)[169] and castanospermine (**1-36**)[170-172] (Figure 1-29), which have been studied as inhibitors of various glycosidase enzymes.

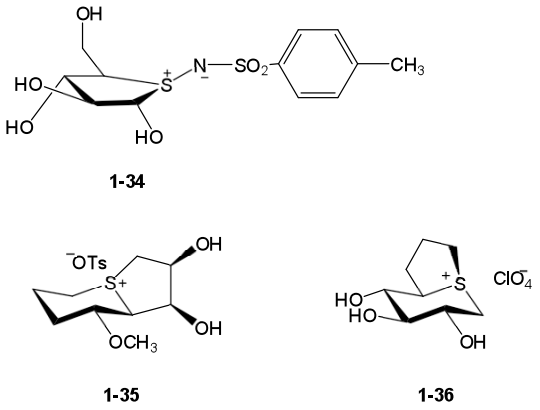


Figure 1-29. Structures of **1-34, 1-35** and **1-36**

Just over 10 years ago, in 1997, these synthetic studies were validated when two naturally-occurring sulfonium ion glycosidase inhibitors were reported. Yoshikawa et al. isolated salacinol[173] and kotalanol[174] (Figure 1-30) from *Salacia reticulate*, known as Kotalahimbutu in Singhalese, a large woody climbing plant

widespread in Sri Lanka and South India.[175] They were found to have extraordinary inhibitory activity toward α-glucosidases.[176]

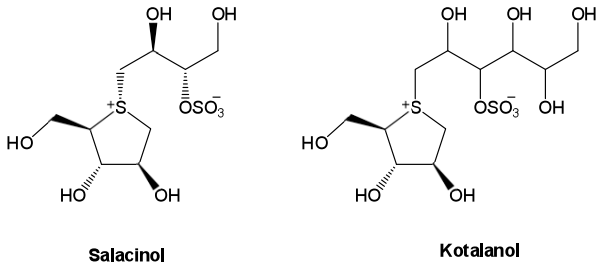


Figure 1-30. Structures of Salacinol and Kotalanol

The mechanism of inhibition of salacinol and kotalanol has not been determined yet. However, it is proposed that the sulfonium ion mimics the TS of the hydrolysis reaction and interacts with the anionic binding site of the glucosidases (Figure 1-31).[177, 178] Due to its unique structural features and its potential to become a lead drug candidate in the treatment of type II diabetes, a great deal of attention has been focus on salacinol and its analogues since its isolation.[178]

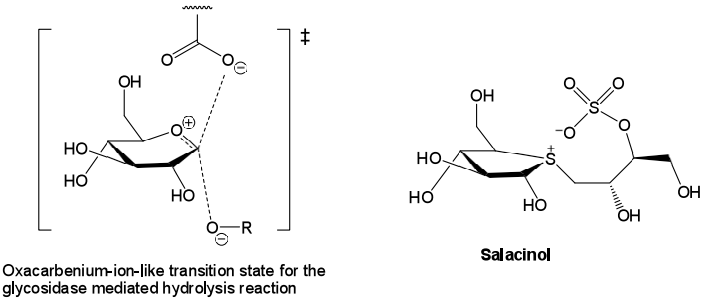


Figure 1-31. Proposed glycosidase transition state and structure of salacinol

In 2000[179] and 2001,[180] the Yuasa group and Pinto group reported the synthesis of salacinol, respectively. Since then, other carbohydrate-based cyclic sulfonium compounds have been investigated, such as the methyl sulfonium compound **1-37** by the Yuasa group[181] and sulfoxide **1-38** by the Bols group.[182] The Pinto group also reported heteroatom analogues of salacinol having nitrogen (ghavamiol, **1-39**)[183] or selenium (blintol, **1-40**)[184] instead of sulfur, and a series of *S*-alkylated sulfonium ions, e.g., **1-41**.[185] Furthermore, Pinto and coworkers have also synthesized a series of six-membered ring analogues to study the effect of the size of the heterocyclic ring on glycosidase inhibitory activity, for example **1-42**, **1-43**.[186] Recently, the Lemaire group extended this work to include analogs with larger rings, for example **1-44**.[187] Although none of these compounds have been shown to inhibit glycosyltransferases, the mechanistic similarities between glycosidases and glycosyltransferases suggests this these compounds may inhibit enzymes that make glycosidic linkages. (Figure 1-32)

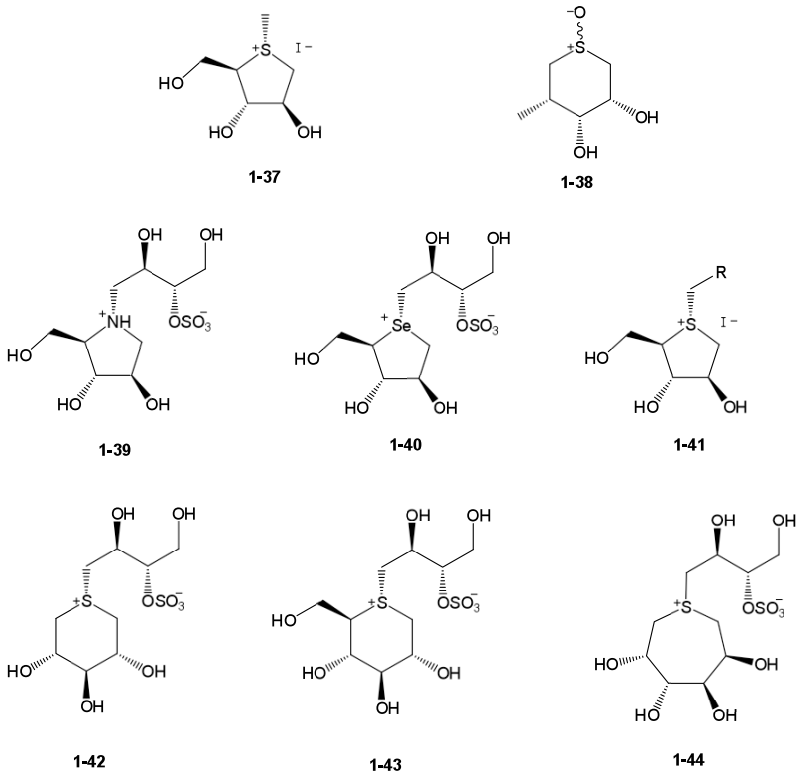


Figure 1-32. Examples of Salacinol analogues

With this background provided, the remainder of this thesis provides details of my work on the synthesis of potential inhibitors of mycobacterial AraTs and GlfT1/GlfT2. In Chapter 2, I describe the work done to prepare the bicyclo[3.1.0]hexane-based inhibitor cores and Chapter 3 then covers the work done in the preparation of small libraries of these compounds. Chapter 4 addresses synthesis of the sulfonium ion analogs. The biochemical testing of these compounds is described in Chapter 5 and the summary and future work is provided in Chapter 6.

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Chapter 2[1]

Synthesis of Bicyclo[3.1.0]hexane Mimics of Ara*f* and Gal*f* rings

2.1 Introduction

This chapter describes the synthesis of bicyclo[3.1.0]hexane derivatives **2-1** and **2-2** (Figure 2-1) as analogues of Gal*f* and Ara*f* residues, respectively. The key step in the synthesis of these compounds was the generation of the bicyclo[3.1.0] hexane core structure that could be appropriately functionalized. Previously, the construction of this ring system for the preparation of furanose ring analogues has been done using carbohydrate starting materials. This approach has allowed the inclusion of a maximum number of functional groups from an earlier stage. A summary of these previously-reported methods is provided below.

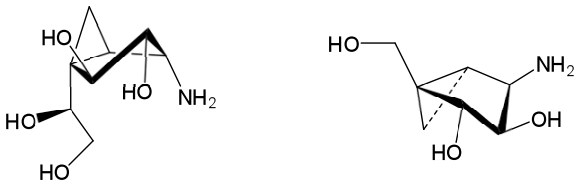
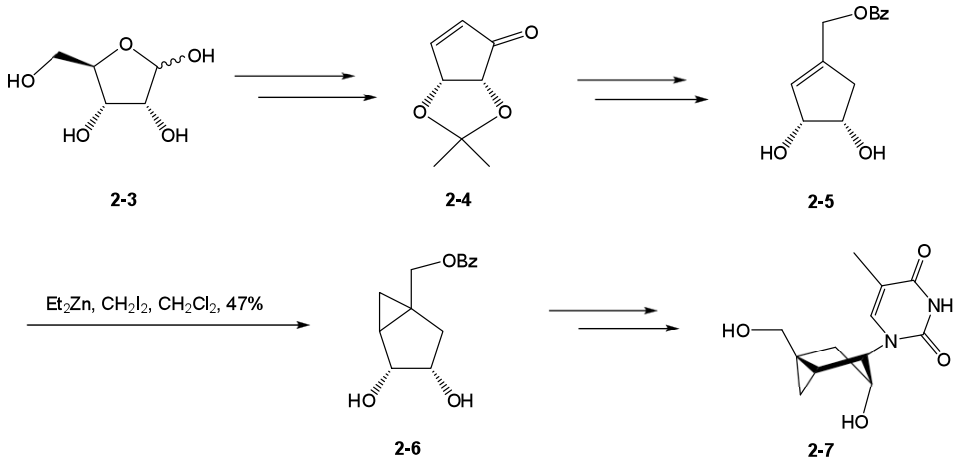


Figure 2-1. Target analogues **2-1** and **2-2**

[1] A version of this chaper has been published. Li, J.; Lowary, T. L. *Org. Lett*. **2008**, *10*, 881-884

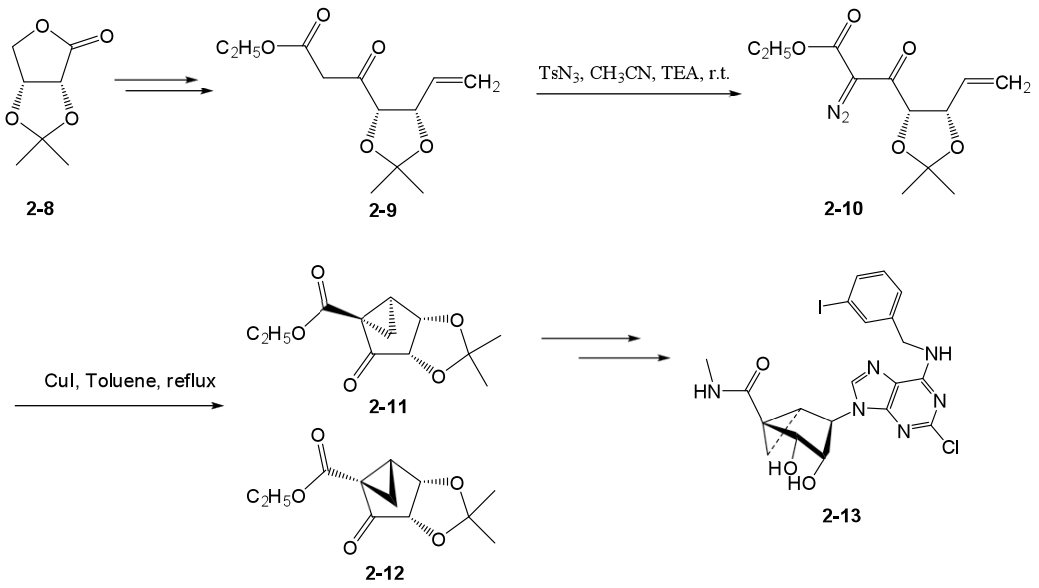
One of the most common strategies to these ring systems is to use Simmons–Smith cyclopropanation of a cyclopentene derivative.[1-3] For example, Marquez and coworkers,[1] have reported the conversion of D-ribose (**2-3**) into cyclopentenone **2-4** and then into cyclpentene diol **2-5**. Treatment under Simmons–Smith cyclopropanation conditions afforded the bicyclo[3.1.0]hexane skeleton **2-6**, which is an intermediate for the nucleotide analogue **2-7** (Scheme 2-1).



Scheme 2-1. Example of Simmons-Smith cyclopropanation in the preparation of bicyclo[3.1.0]hexane-based nucleoside analogs

A second method is intramolecular cyclopropanation of sugar templates using an unsaturated diazocarbonyl compound, which acts as a carbenoid precursor.[4-8] This approach has been used by Joshi et. al. in the synthesis of N-methanocarba nucleoside **2-13** (Scheme 2-2)[4] starting with commercially available 2,3-*O*-isopropylidene-D-erythronolactone (**2-8**). The β-keto ester **2-9**

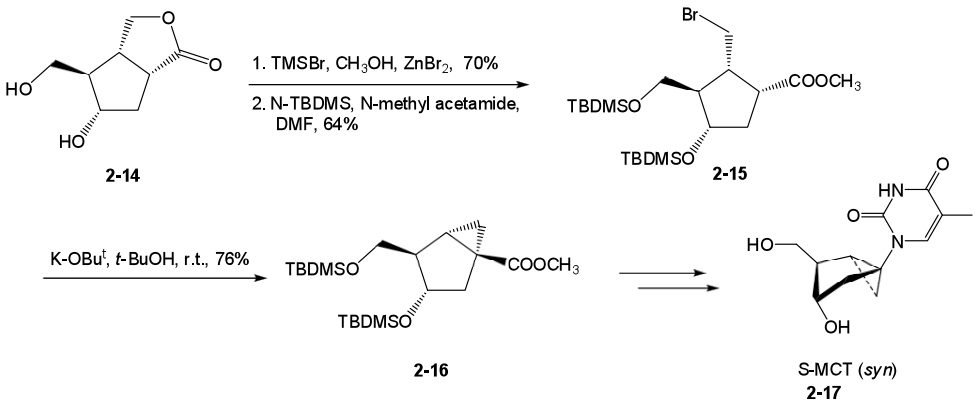
was achieved from **2-8** after a few reaction steps. Treatment of this compound with TsN_3 in CH_3CN and TEA gave the diazo compound **2-10**, which underwent a thermally-induced intramolecular cyclopropanation to give the bicyclo[3.1.0]hexan-2-one isomers **2-11** and **2-12**, which could then be transformed into **2-13** after several steps.



Scheme 2-2. Example of intramolecular cyclopropanation via diazo intermediate in the preparation of bicyclo[3.1.0]hexane-based nucleoside analogs

A third way to make the bicyclo[3.1.0]hexane system is through the intramolecular anionic displacement reactions. For example, Altmann et. al. have reported the synthesis of the thymidine analogue South-MCT (**2-17**) as shown in Scheme 2-3. The synthesis relies on the known homochiral bicyclic

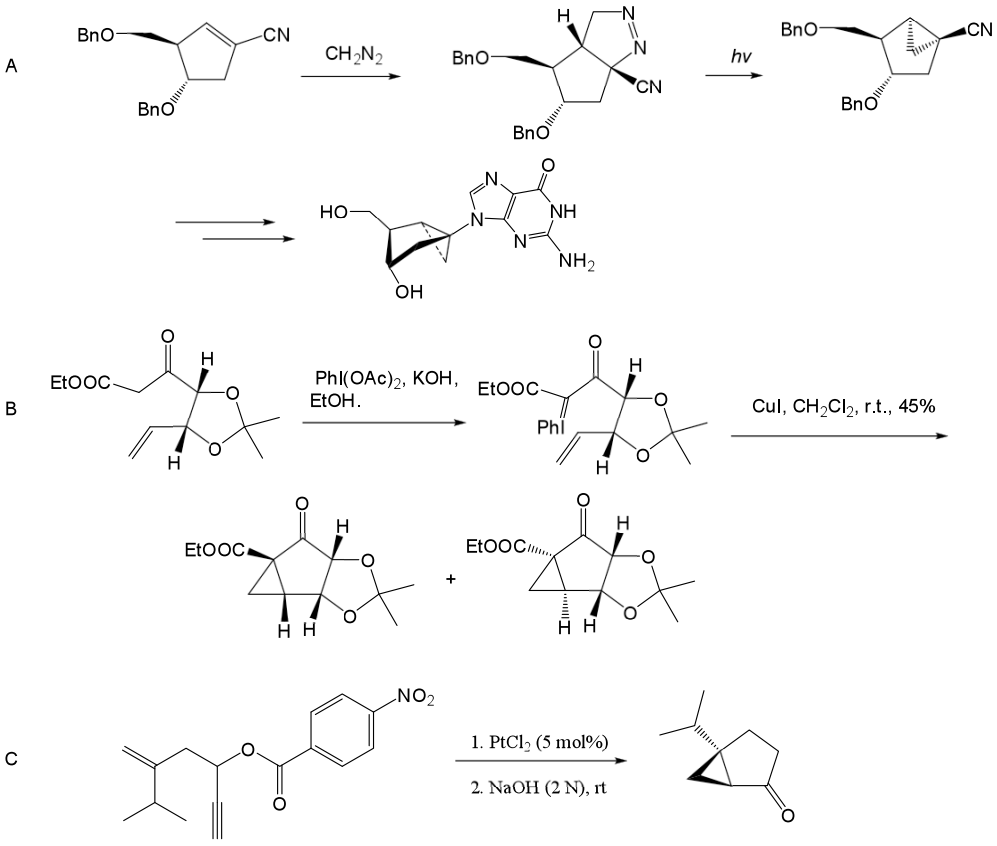
lactone **2-14** as the central intermediate. Opening of the lactone ring with TMSBr in methanol gave the γ-bromo ester, which was converted to the stable TBDMS ether **2-15**. Subsequently, formation of the three-membered ring proceeded very smoothly after treatment of bromoester with potassium *t*-butoxide in *t*-butyl alcohol at 0 ºC. The bicyclic ester **2-16** was obtained in 76% yield, and this compound could then be converted in several steps into **2-17**.[9]



Scheme 2-3. Example of forming the bicyclo[3.1.0]hexane system via anionic displacement

A number of other methods have also been reported for the assembly of this ring system. For example, as shown in Scheme 2-4A, Marquez described the preparation of bicyclo[3.1.0]hexanes via 1,3-dipolar cycloaddition of diazomethane, and the subsequent nitrogen extrusion by photolysis.[10] Another route, reported by Gallos et al., is shown in Scheme 2-4B in which ring system is

produced via iodonium ylide insertion into an alkene.[6] Finally, the Pt catalyzed synthesis of bicyclo[3.1.0]hexanes has been reported,[11, 12] i.e., the conversion of enynes to bicyclo[3.1.0] hexan-2-one (Scheme 2-4C), but these methods have not been widely adapted in target synthesis.



Scheme 2-4. Access to bicyclo[3.1.0]hexanes via (A) nitrogen extrusion (B) iodo-ylide insertion and (C) platinum-catalyzed cyclization of enynes.

Before developing the synthetic route to the two targets, we noted that compounds **2-1** and **2-2** are stereochemically related. Compound **2-2** can be

obtained from the enantiomer of **2-1** by oxidative cleavage of the acyclic diol moiety, as illustrated in Figure 2-2. Thus, we hoped to develop an approach to **2-1** and **2-2** that took advantage of this relationship, and which would allow the preparation of the targets in a highly convergent manner from a common intermediate.

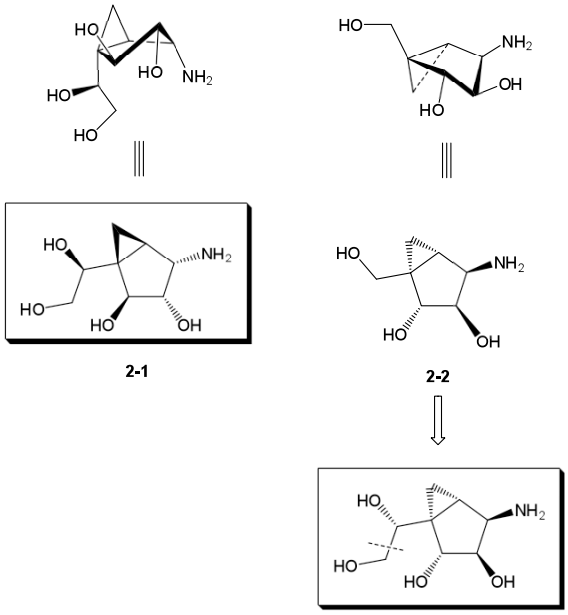


Figure 2-2. Stereochemical relationship between **2-1** and **2-2**

In designing a route to the targets, a paper from Nelson and coworkers drew our attention.[13] In this paper, the authors reported that the treatment of cyclohexanones such as **2-18**, in which a leaving group is present in C-4, with strong base can theoretically lead to numerous products as illustrated in Figure 2-3. β-Elimination of the leaving group would give either the cyclohex-3-en-one or cyclohex-2-en-one. Alternatively, intermolecular an S_N2 displacement

reaction would give the 4-substituted cyclohexanone. However, if the strength of the base is appropriate so that the enolization of the ketone can occur, the resulting enolate anion leads to the formation of the bicyclo[3.1.0]-2-hexanone by an intramolecular anionic displacement reaction. This seemed to be an excellent way of forming a racemic mixture of bicyclo[3.1.0]-2-hexanones, which could be functionalized and then the enantiomeric products separated at a later stage in the route by resolution.

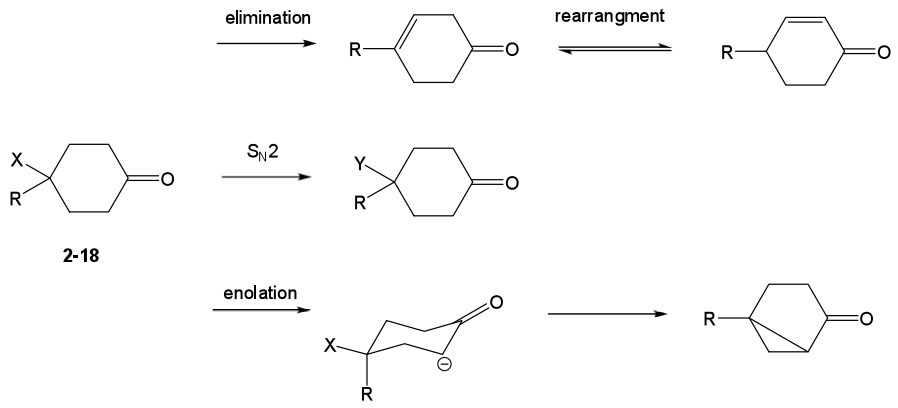
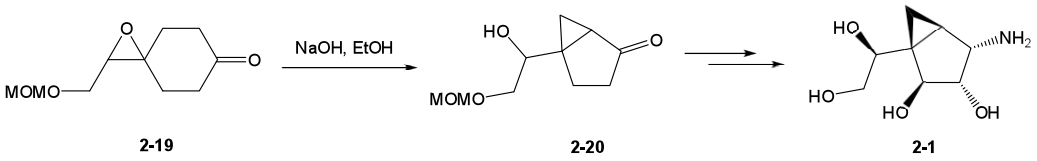


Figure 2-3. Conversion of **2-18** into different products as reported by Nelson and coworkers[13]

Based on this work, Hamon and Shirley demonstrated[14] that treatment of **2-19** with sodium hydroxide in ethanol afforded a ~1:1 mixture of bicyclo[3.1.0]hexanone **2-20** (Scheme 2-5). This molecule has the carbon skeleton required for the Gal*f* targets. We envisioned that with few more steps,

three additional stereogenic centres could be added to the cyclopentane ring thus producing the desired Gal*f* analogue (2-1).

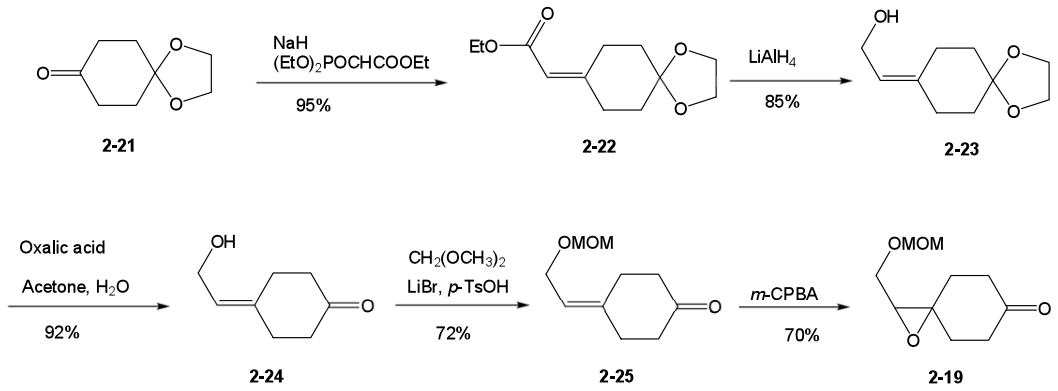


2-19 2-20 2-1

Scheme 2-5. Ring contraction in basic conditions

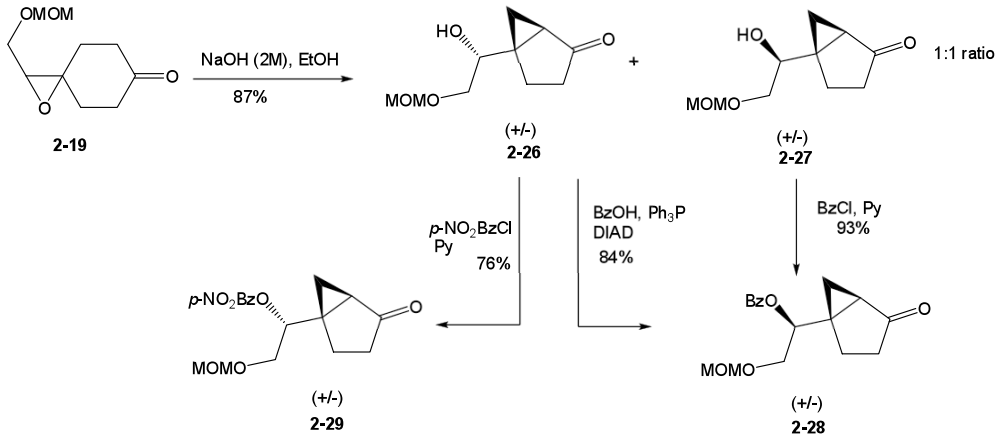
2.2 Results and discussion

As illustrated in Scheme 2-6, the synthesis of the targets started with the known 1,4-cyclohexanedione monoethylene[15] acetal (**2-21**), which was treated with NaH and triethyl phosphonoacetate to form the acetate **2-22** in 95% yield. The acetate was then reduced by $LiAlH_4$ to produce the allylic alcohol **2-23** in 85% yield.[16] Reaction of the allylic alcohol with oxalic acid in aqueous acetone led to ketal hydrolysis in 92% yield, thus affording ketone **2-24**. The hydroxyl group was next protected as a MOM ether under acidic conditions; the product of this reaction, **2-25**, was obtained in 72% yield. Epoxidation of the alkene with *m*-CPBA afforded a 70% yield of **2-19**, which was the substrate for the key ring contraction reaction.



Scheme 2-6. Synthesis of **2-19**

Treatment of **2-19** with sodium hydroxide in ethanol, as described previously,[14] afforded an 1:1 mixture of bicyclo[3.1.0]hexane derivatives **2-26** and **2-27**, which were readily separable by chromatography. The combined yield of **2-26** and **2-27** was 87% (Scheme 2-7).



Scheme 2-7. Ring contraction and synthesis of **2-28** and **2-29**

Determining the relative stereochemistry between the cyclopropane ring and carbon bearing the hydroxyl group was not possible using NMR spectroscopy. Fortunately, the *p*-nitrobenzoate ester derivative of **2-26**, compound **2-29** (prepared as illustrated in Scheme 2-7), was crystalline. Single crystal X-ray analysis enabled us to establish the structure of **2-29** as that shown in Figure 2-4.[17] With this information it was possible to determine the structure of **2-26** and, in turn **2-27**. With this stereochemistry issue addressed, **2-27** was converted into **2-28** by reaction with benzoyl chloride and pyridine. The other isomer, **2-26**, could also be converted into **2-28** through a Mitsunobu reaction with benzoic acid. In both reactions, yields over 84% were obtained and gram quantities of **2-28** could be obtained by this sequence (Scheme 2-7).

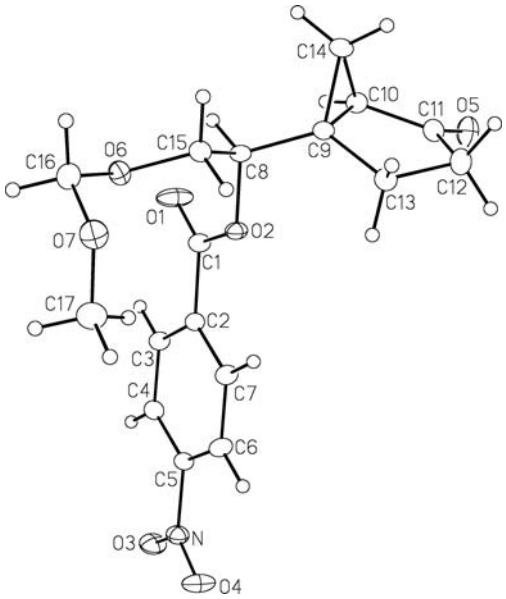
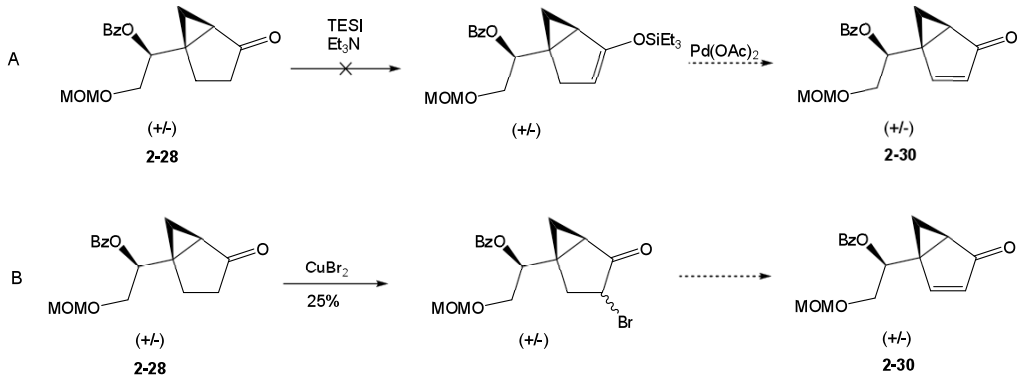


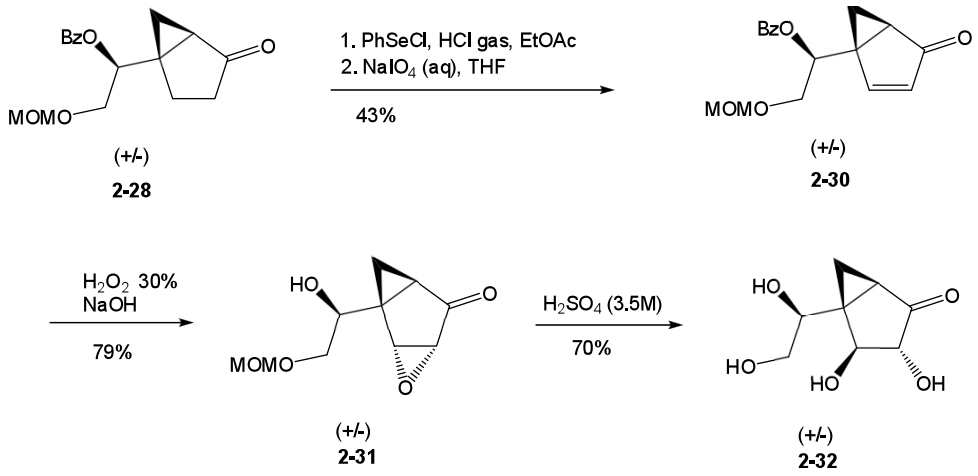
Figure 2-4. Crystal structure of **2-29**

The protected ketone **2-28** was then converted into enone **2-30** in 43% overall yield by α-selenation under acidic conditions, oxidation to the selenoxide, and in situ elimination (Scheme 2-9). Compounds **2-28** and **2-30** are readily separable by chromatography, and the former can therefore be recycled through the sequence. We attempted to improve the product yield by changing the reaction conditions including using 2 equivalents phenylselenyl chloride, changing reaction temperature, and using LDA to generate an enolate. Unfortunately, none of these attempts led to improvements in product yield. We also tried other methods for the preparation the α,β-unsaturated ketone. As shown in Scheme 2-8A, **2-28** would be expected to react with TESI in the presence of triethylamine to give the silyl enol ether, which could be oxidized by palladium acetate to form the enone **2-30**. However, TLC showed that the reaction gave a number of products and no silyl enol ether was isolated. We next treated **2-28** with cupreous bromide (Scheme 2-8B) to give an α-bromo-ketone intermediate, which would then be treated with base to form the dehydrobromination product **2-30**. Unfortunately, the yield of the bromination was only 25%; therefore, we returned to the reaction involving phenylselenyl chloride under acidic conditions.



Scheme 2-8. Unsuccessful methods for the synthesis of enone **2-30**

Reaction of **2-30** with hydrogen peroxide under basic conditions afforded epoxide **2-31** in 79% yield. The stereochemistry of this product was established on the basis of NOEs shown in Figure 2-5. In particular, NOE's were observed between the oxirane hydrogens and one of the hydrogens present on the cyclopropane methylene group. Finally, the epoxide was hydrolyzed and the MOM group was cleaved upon reaction with aqueous sulfuric acid to give **2-32** in 70% yield. Pleasantly, a single isomeric product, the one that we desired, was produced in the reaction.



Scheme 2-9. Synthesis of **2-32**

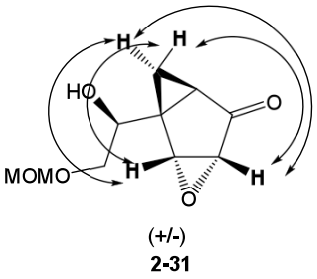
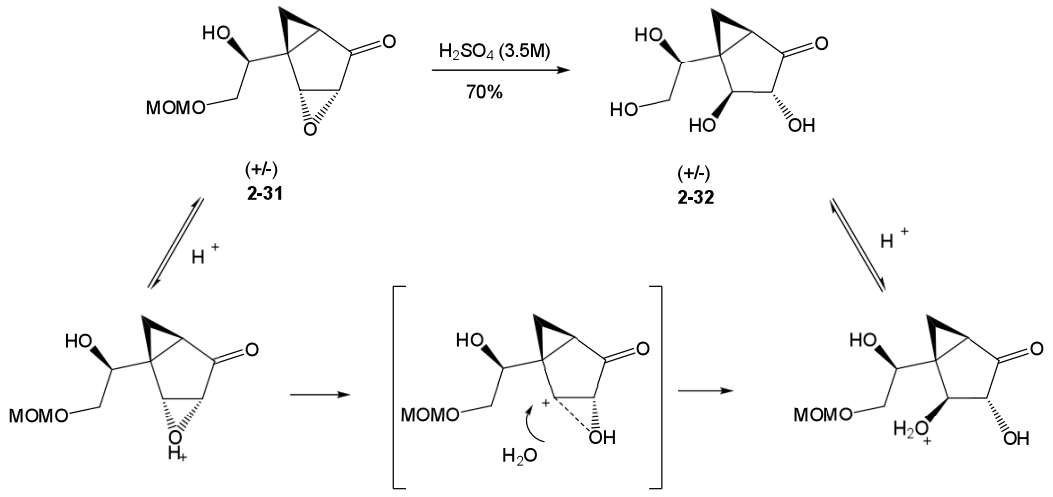


Figure 2-5. Key NOE interactions present in **2-31**

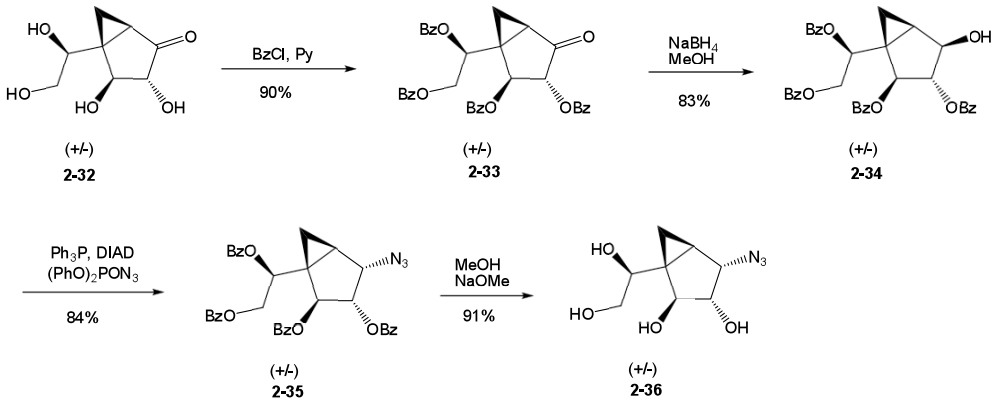
The regiochemistry of the epoxide opening was apparent from the ^{1}H NMR spectrum of **2-32**, and a crystal structure of a later intermediate (see below) also confirmed the structure. The regioselectivity of the epoxide opening presumably arises from the partial positive charge that forms during the reaction being stabilized by the cyclopropyl group, i.e., a cyclopropylcarbinyl cation[18] (Scheme 2-10). In addition, formation of the other regioisomer would proceed though a

transition state in which this charge develops on the carbon adjacent to the electropositive carbonyl carbon.



Scheme 2-10. Proposed mechanism for the synthesis of **2-32**

The continuation of the synthesis from **2-32** is shown in Scheme 2-11. Benzoylation of tetrol **2-32** gave **2-33**, and treatment of this compound with sodium borohydride reduced the ketone with high stereoselectivity affording **2-34** in 75% yield over two steps. The selectivity of the reduction presumably arises from the cyclopropane group, which hinders hydride attack from the top face of the ring. Next, the hydroxyl group was substituted by azide with inversion of configuration in 84% yield via a Mitsunobu reaction with diphenylphosphoryl azide. The fully protected azide **2-35** was then deprotected upon reaction with sodium methoxide, which afforded **2-36** in 91% yield.



Scheme 2-11. Synthesis of **2-36**

Having completed the synthesis of **2-36**, all of the functionality present in the targets was in place and what remained was the resolution of the racemic mixture of products. We decided to use the general method of separating the racemic mixture by converting the two enantiomers into diastereomers. The first attempt to do this involved the conversion of compound **2-36** into the fully protected *O*-acetyl-(*S*)-mandelic acid derivative to give diastereomers as shown in Figure 2-6, These compounds were, unfortunately, inseparable. In a second attempt, the acyclic diol in **2-36** was protected as an isopropylidene ketal under standard conditions to give the expected product **2-37** in 86% yield. Esterification of the remaining two hydroxyl groups with *O*-acetyl-(*S*)-mandelic acid led to the formation of an inseparable mixture of diastereomeric products in 95% yield. Removal of the ketal by heating in aqueous acetic acid gave diastereomers **2-40**

and **2-41**, which could be separated by careful chromatography, in 91% combined yield (Scheme 2-12).

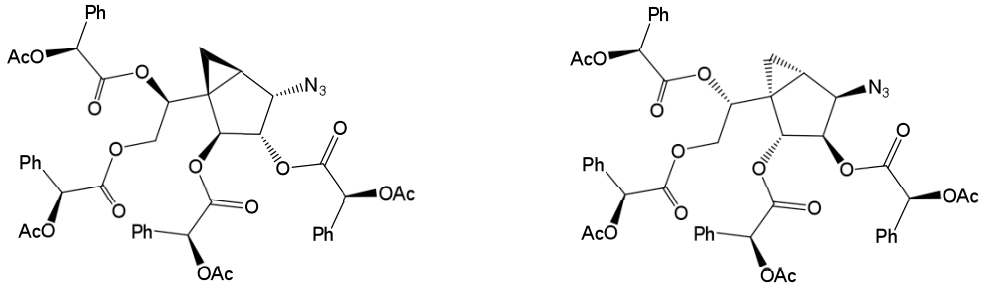
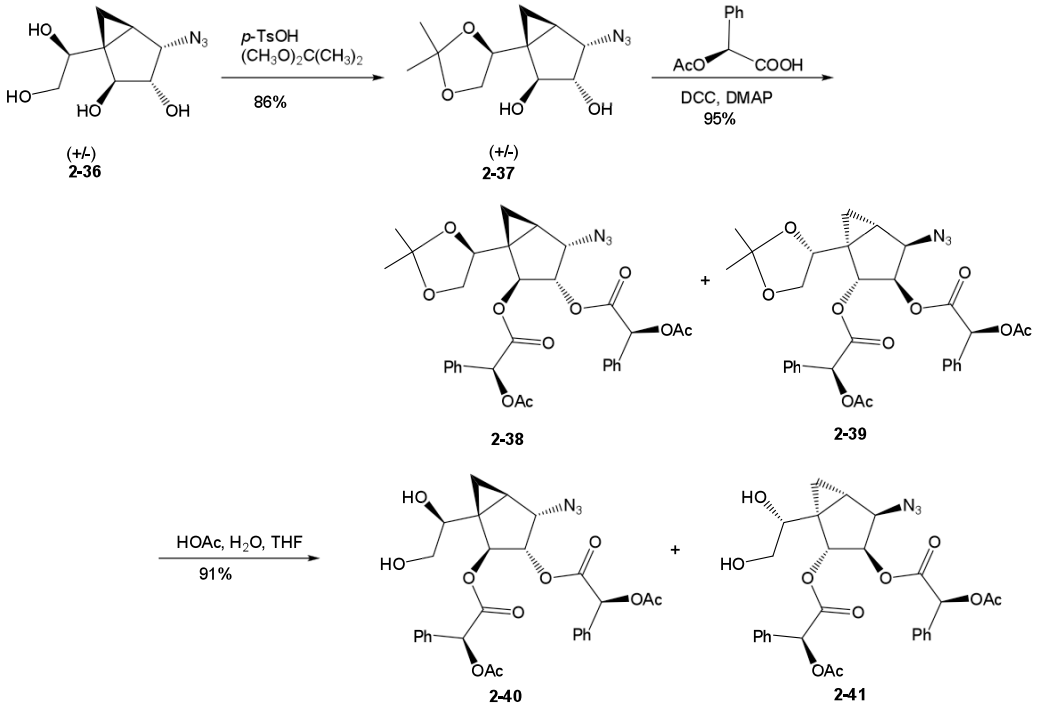


Figure 2-6. Fully protected **2-36** derivative



Scheme 2-12. Separation of the racemic mixture of **2-36**

After the diastereomers had been synthesized and separated, we next determined the structure of **2-40** and **2-41** by preparing a crystalline derivative of these compounds. Several derivatives had been made as shown in Figure 2-7; none of them formed crystals.

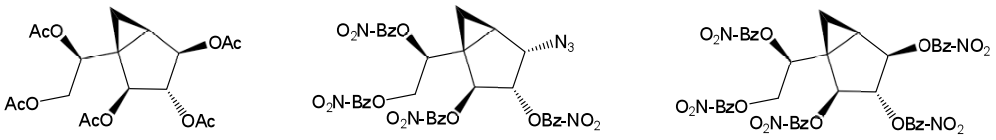
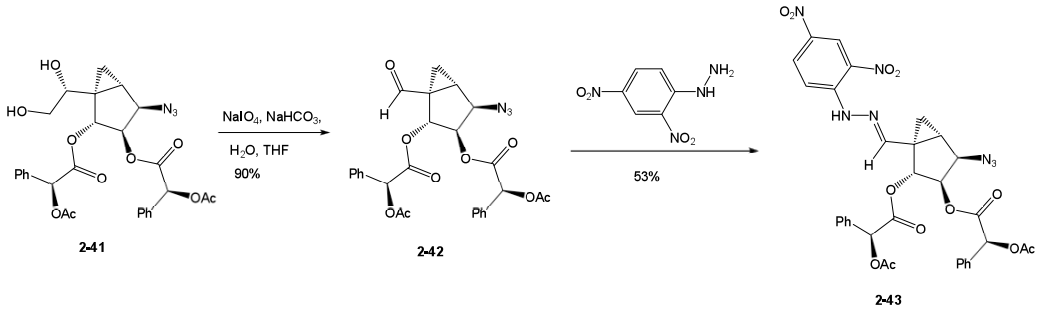


Figure 2-7. Derivatives of **2-40** synthesized to identify a crystalline derivative.

The right one was finally obtained after we remembered that 2,4-dinitrophenylhydrazone condenses with carbonyl groups to form hydrazones that are normally crystalline solids. As shown in Scheme 2-13, reaction of **2-41** with sodium periodate gave aldehyde **2-42**, which was then converted to the corresponding crystalline 2,4-dinitrophenylhydrazone derivative **2-43** in 53% yield. Single-crystal X-ray analysis of **2-43** (Figure 2-8)[19] established the absolute configuration of the molecule as that shown. This indicates that that the Ara*f* analogue **2-2** could be obtained from **2-41** and that **2-40** is the precursor to the Gal*f* analogue **2-1**.



Scheme 2-13. Synthesis of **2-43**

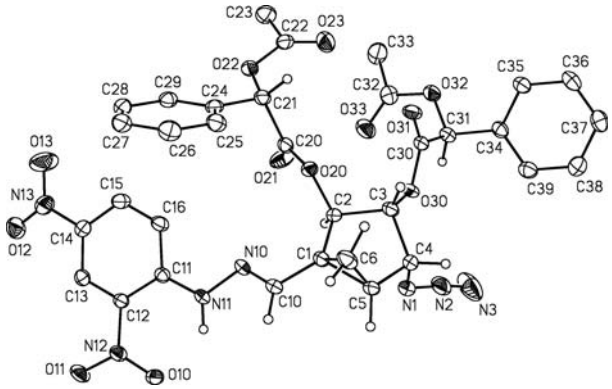
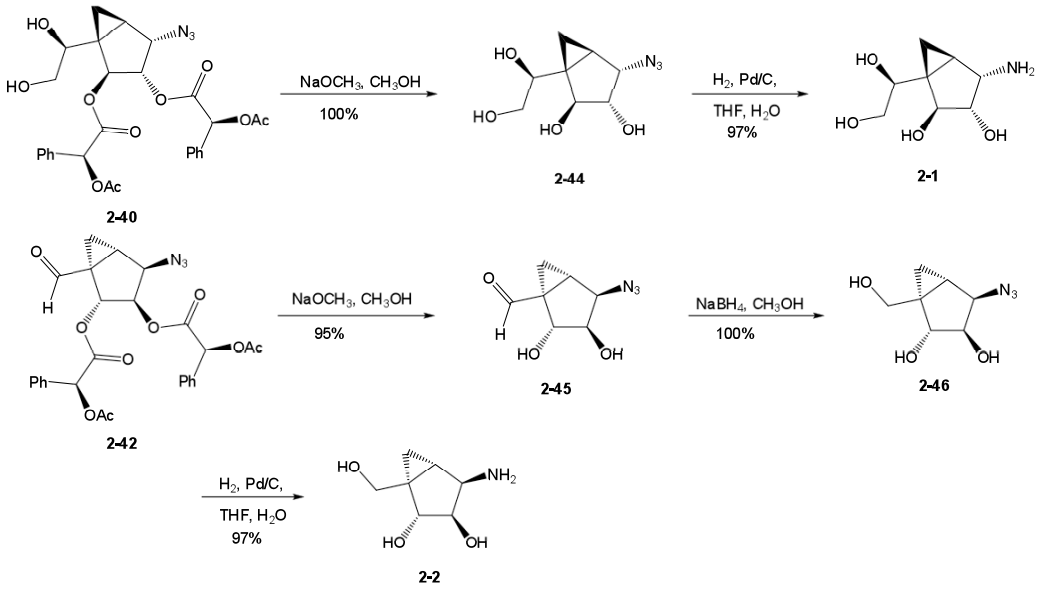


Figure 2-8. Crystal structure of **2-43**

With the absolute configurations of **2-40** and **2-41** assigned, the synthesis of the target molecules was completed in a few straightforward steps as illustrated in Scheme 2-14. The preparation of **2-1** was carried out by reaction of **2-40** with sodium methoxide to cleave the mandelate esters (giving **2-44**) and then reduction of the azide to the amine by hydrogenolysis. Target **2-1** was obtained in 97% over these two steps. To prepare **2-2**, aldehyde **2-42** was deprotected under basic

conditions affording a 95% yield of **2-45**. This compound was then reduced with sodium borohydride in quantitative yield. Finally, reaction of **2-46** with hydrogen and palladium in carbon gave **2-2** in 97% yield.



Scheme 2-14. Final steps in the synthesis of **2-1** and **2-2**

2.3 Conclusions

In this chapter, I described the synthesis of conformationally-rigid analogs of β-Ara*f* and α-Gal*f* rings based upon a bicyclo[3.1.0]hexane scaffold. The key reaction was the preparation of the core ring structure by an intramolecular substitution of an enolate onto an epoxide. This reaction provided a racemic intermediate that could be converted to both products after resolution by esterification with *O*-acetyl-(*S*)-mandelic acid. To determine the stereochemistry

of the products, single crystal X-ray analysis of key intermediates was done. The conversion of these compounds into a small library of potential transferase inhibitors is described in Chapter 3.

2.4 Experimental section

General Methods

All reagents used were purchased from commercial sources and were used without further purification. Solvents used in reactions were predried by PURESOLV-400 System from Innovative Technology Inc. Unless stated otherwise, all reactions were monitored by TLC on silica gel G-25 UV_{254} (0.25 mm, Macherey-Nagel). Spots were detected under UV light and/or by charring with acidified ethanolic anisaldehyde. Solvents were evaporated under reduced pressure and below 50 ºC (water bath). Column chromatography was performed on silica gel 60 (40–60 μm). The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). Iatrobeads refers to a beaded silica gel 6RS-8060, which was manufactured by Iatron Laboratories (Tokyo). ^{1}H NMR spectra were recorded on VARIAN INOVA-NMR spectrometers at 400, 500 MHz, and chemical shifts are referenced to $CDCl_3$ (7.26 ppm, $CDCl_3$) or CD_3OD (4.78 ppm, CD_3OD). ^{13}C NMR APT spectra[20, 21] were recorded at 100 or 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.23 ppm, $CDCl_3$) or CD_3OD (48.9 ppm, CD_3OD). ^{1}H NMR data are reported as though they are first order, and the

peak assignments are made by 2D-NMR spectroscopy (^{1}H–^{1}H COSY and HMQC). ESI-MS spectra were recorded on samples suspended in THF or CH_3OH and added NaCl. Optical rotations were measured on Perkin-Elmer 241 Polarimeter with sodium D line (589 nm) and are in units of deg·mL $(dm \cdot g)^{-1}$.

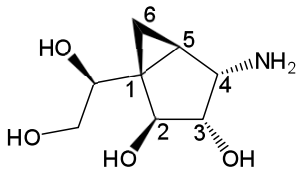
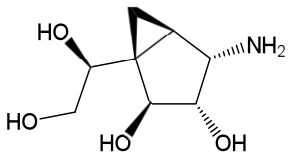


Figure 2-9. Numbering system used for NMR assignments.

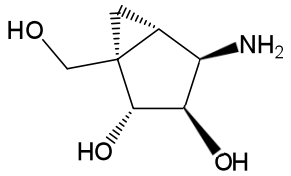


2-1

(1*S*,2*S*,3*S*,4*S*,5*R*)-4-amino-1-[(1*S*)-1,2-dihydroxyethyl]bicyclo[3.1.0]hexane-2,3-diol (2-1).

To a solution of compound **2-44** (0.013 g, 0.06 mmol) in THF (1 mL) was added 10% Pd/C (0.3 mg) and the reaction mixture was stirred under a hydrogen atmosphere for 12 h. The mixture was then filtered through Celite and concentrated. The resulting residue was purified by chromatography on Iatrobeads (16:5:1 CH_2Cl_2–CH_3OH–NH_4OH then 6:1 CH_2Cl_2–NH_4OH) to give **2-1** (0.011 g, 97%) as a foam. R_f 0.47 (6:1 CH_3OH–NH_4OH); ^{1}H NMR (400

MHz, CD_3OD, δ_H) 4.20 (dd, 1 H, *J* = 1.0, 7.2 Hz, H-2), 3.78 (dd, 1 H, *J* = 6.2, 6.2 Hz, C<u>H</u>OH), 3.64 (dd, 1 H, *J* = 6.2, 11.1 Hz, C<u>H</u>$_2$OH), 3.55–3.49 (m, 2 H, C<u>H</u>$_2$OH, H-3), 3.12 (d, 1 H, *J* = 5.4 Hz, H-4), 1.44 (dd, 1 H, *J* = 4.2, 8.4 Hz, H-5), 0.75–0.67 (m, 2 H, H-6a, H-6b); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 79.2 (C-2), 78.2 (C-3), 73.4 (<u>C</u>HOH), 66.0 (<u>C</u>H$_2$OH), 53.5 (C-4), 34.3 (C-1), 25.4 (C-5), 9.1 (C-6). HRMS (ESI) *m/z* Calcd for ($M+H^+$) $C_8H_{15}NO_4$ 190.1074. Found: 190.1072.

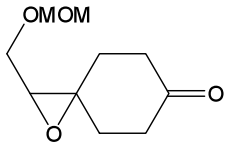


2-2

(1*R*,2*R*,3*R*,4*R*,5*S*)-4-amino-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol (2-2).

To a solution of compound **2-46** (0.006 g, 0.032 mmol) in THF (2 mL) was added 10% Pd/C (0.5 mg) and the reaction mixture was stirred under a hydrogen atmosphere for 12 h. The mixture was filtered through Celite and concentrated. The resulting residue was purified by chromatography on Iatrobeads (16:5:1 CH_2Cl_2–CH_3OH–NH_4OH then 6:1 CH_3OH–NH_4OH) to yield **2-2** (0.005 g, 97%) as a foam. R_f 0.50 (6:1 CH_3OH–NH_4OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 4.25 (d, 1 H, *J* = 7.0 Hz, H-2), 3.97 (d, 1 H, *J* = 11.6 Hz, C<u>H</u>$_2$OH), 3.57 (dd, 1 H, *J* =6.2 7.0 Hz, H-3), 3.21 (d, 1 H, *J* = 11.6 Hz, C<u>H</u>$_2$OH), 3.20 (d, 1 H, *J* = 6.2 Hz,

H-4), 1.35 (dd, 1 H, *J* = 4.0, 8.4 Hz, H-5), 0.81 (dd, 1 H, *J* = 4.0 5.8, H-6a), 0.54 (dd, 1 H, *J* = 5.8, 8.4 Hz, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 77.4 (C-2 or C-3), 77.3 (C-2 or C3), 64.4 ($\underline{C}H_2OH$), 53.4 (C-4), 33.8 (C-1), 25.6 (C-5), 10.8 (C-6). HRMS (ESI) *m/z* Calcd for ($M+H^+$) $C_7H_{13}NO_3$ 160.0968. Found: 160.0969.

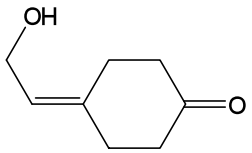


2-19

2-[(methoxymethoxy)methyl]-1-oxaspiro[2.5]octan-6-one (2-19).

A solution of *m*-CPBA (2.54 g, 11.34 mmol) in CH_2Cl_2 (10 mL) was added dropwise into an ice-cold CH_2Cl_2 (20 mL) solution of **2-25** (1.74 g, 9.45 mmol). The mixture was stirred for 2 h at room temperature before being diluted with CH_2Cl_2. The organic layer was then washed with 1 M NaOH and then water. After drying (Na_2SO_4) the organic extract was concentrated and the residue was purified by column chromatography (2:1 hexane–EtOAc) to afford compound **2-19** (1.32 g, 70%) as a light yellow oil. R_f 0.30 (2:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.63 (d, 1 H, *J* = 6.6 Hz, OC$\underline{H}_2$O), 4.60 (d, 1 H, *J* = 6.6 Hz, OC$\underline{H}_2$O) 3.68 (d, 2 H, *J* = 5.4 Hz, C$\underline{H}_2$O), 3.32 (s, 3 H, OC$\underline{H}_3$), 3.16 (t, 1 H, *J* = 5.4 Hz, MOMOCH$_2$C$\underline{H}$), 2.64–2.53 (m, 2 H, cyclohexane C$\underline{H}_2$), 2.45–2.36 (m, 2 H, cyclohexane C$\underline{H}_2$), 2.11–2.00 (m, 2 H, cyclohexane C$\underline{H}_2$), 1.88–1.82 (m, 1 H,

cyclohexane C$\underline{H}_2$), 1.79–1.72 (m, 1 H, cyclohexane C$\underline{H}_2$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 209.6 ($\underline{C}$=O), 96.7 (O$\underline{C}H_2O$), 66.0 ($\underline{C}$H), 62.0 ($\underline{C}H_2O$), 60.2 (quaternary C), 55.3 (O$\underline{C}H_3$), 38.6 (cyclohexane $\underline{C}H_2$), 38.5 (cyclohexane $\underline{C}H_2$), 33.4 (cyclohexane $\underline{C}H_2$), 27.8 (cyclohexane $\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{10}H_{16}O_4$: 223.0941. Found 223.0942.

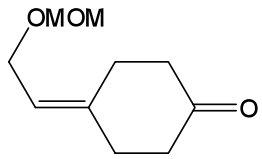


2-24

4-(2-hydroxyethylidene)cyclohexanone (2-24).

8-(2-Hydroxyethylidene)-1,4-dioxaspiro[4.5] decane (**2-23**, 2.72 g, 14.76 mmol) was dissolved in 30 mL acetone and water (1:1) and oxalic acid dihydrate (3.75 g, 29.52 mmol) was added. The reaction mixture was stirred for 3 h at room temperature and then solid $NaHCO_3$ was added to consume the excess oxalic acid. The reaction mixture was filtered and the filtered solid was washed thoroughly with ether. The ether layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (1:3 hexane–EtOAc) to afford **2-24**(1.90 g, 92%) as a yellow oil. R_f 0.25 (1:3 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.62 (t, 1 H, J = 6.9 Hz, C=C$\underline{H}$), 4.23 (d, 2 H, J = 6.9 Hz, C$\underline{H}_2$OH), 2.59–2.52 (m, 4 H, cyclohexane C$\underline{H}_2$), 2.46–2.42 (m, 4 H, cyclohexane C$\underline{H}_2$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 210.8 ($\underline{C}$=O), 138.1 ($\underline{C}$=CH), 124.1 (C=$\underline{C}$H), 58.8

($\underline{C}H_2OH$), 41.3 (cyclohexane $\underline{C}H_2$), 40.5 (cyclohexane $\underline{C}H_2$), 34.0 (cyclohexane $\underline{C}H_2$), 26.2 (cyclohexane $\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_8H_{12}O_2$: 163.0729. Found 163.0728.

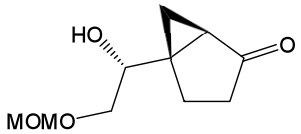


2-25

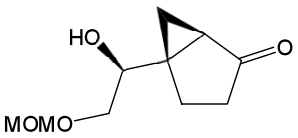
4-[2-(methoxymethoxy)ethylidene]cyclohexanone (2-25).

To a stirred solution of alcohol **2-24** (1.85 g, 13.20 mmol) in dimethoxymethane (30 mL) at room temperature, lithium bromide (0.23 g, 1.64 mmol) and *p*-toluenesulfonic acid monohydrate (0.25 g, 1.32 mmol) were added. The mixture was stirred for 1 h, while being monitored by TLC. When the reaction was done, brine was added and the solution was then neutralized with Et_3N. The aqueous solution was extracted with ether, and the organic layers were combined, dried (Na_2SO_4) and concentrated. The resulting oil was purified by column chromatography (2:1 hexane–EtOAc) to afford the product **2-25** (1.74 g, 72%) as oil. R_f 0.45 (2:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 5.51 (t, 1 H, *J* = 7.1 Hz, C=C$\underline{H}$), 4.58 (s, 2 H, OC$\underline{H}_2$O), 4.05 (d, 2 H, *J* = 7.1 Hz, C=CHC$\underline{H}_2$O), 3.31 (s, 3 H, OC$\underline{H}_3$), 2.53–2.46 (m, 4 H, cyclohexane C$\underline{H}_2$), 2.39–2.35 (m, 4 H, cyclohexane C$\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 210.6 ($\underline{C}$=O), 139.2 ($\underline{C}$=CH), 121.1 (C=$\underline{C}$H), 95.5 (O$\underline{C}H_2$O), 62.9 (=CH$\underline{C}H_2$O), 55.2 (O$\underline{C}H_3$), 41.2

(cyclohexane $\underline{C}H_2$), 40.4 (cyclohexane $\underline{C}H_2$), 33.9 (cyclohexane $\underline{C}H_2$), 26.1 (cyclohexane $\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{10}H_{16}O_3$: 207.0992. Found 207.0992.



(+/-)
2-26



(+/-)
2-27

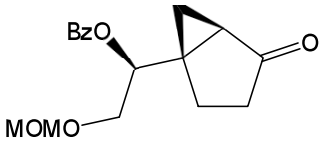
5-[1-hydroxy-2-(methoxymethoxy)ethyl]bicyclo[3.1.0]hexan-2-one (2-26/2-27).

To a solution of epoxide **2-19** (1.32 g, 6.60 mmol) in EtOH (50 mL) was added of 2 M NaOH (10 mL) and the solution was heated at reflux for 20 min. The EtOH was evaporated under reduced pressure and a satd aq NaCl solution was added to the residue, which was extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried (Na_2SO_4) and concentrated to give a yellow oil, which was purified by chromatography (6:1 Et_2O–pentane) to afford **2-26** (0.53 g, 40%) and **2-27** (0.62 g, 47%).

Data for **2-26**: R_f 0.34 (1:20 CH_3OH–CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 4.67 (s, 2 H, OC$\underline{H}_2$O), 3.76 (d, 1 H, J = 7.1 Hz, MOMOC$\underline{H}_2$), 3.59–3.56 (m, 2 H, C$\underline{H}$OH, MOMOC$\underline{H}_2$), 3.39 (s, 3 H, OC$\underline{H}_3$), 2.82 (d, 1 H, J = 2.6 Hz, O$\underline{H}$), 2.34–2.27 (m, 1 H, H-3), 2.16–2.13 (m, 2 H, H-4), 1.96–1.91 (m, 1 H, H-3), 1.81 (dd, 1 H, J = 3.4, 9.3 Hz, H-1), 1.32 (ddd, 1 H, J = 1.4, 4.9, 9.3 Hz, H-6a), 1.12

(dd, 1 H, J = 3.4, 4.9 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 213.5 (C-2), 97.1 (O<u>C</u>H$_2$O), 72.3 (<u>C</u>HOH), 71.0 (MOMO<u>C</u>H$_2$), 55.6 (O<u>C</u>H$_3$), 35.7 (C-5), 32.9 (C-3), 32.5 (C-1), 22.0 (C-4), 17.1 (C-6). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{10}H_{16}O_4$: 223.0941. Found: 223.0940.

Data for **2-27**: R_f 0.35 (1:20 CH_3OH–CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.63 (s, 2 H, OC<u>H</u>$_2$O), 3.89 (dd, 1 H, J = 3.0, 7.7 Hz, C<u>H</u>OH), 3.74 (dd, 1 H, J = 3.0, 10.4 Hz, MOMOC<u>H</u>$_2$), 3.48 (dd, 1 H, J = 7.7, 10.4 Hz, MOMOC<u>H</u>$_2$), 3.36 (s, 3 H, OC<u>H</u>$_3$), 2.85 (br, 1 H, O<u>H</u>)2.15–2.09 (m, 2 H, H-3), 2.03–1.98 (m, 2 H, H-4), 1.86 (dd, 1 H, J = 3.2, 9.2 Hz, H-1), 1.45 (dd, 1 H, J = 4.7, 9.2 Hz, H-6a), 1.02 (dd, 1 H, J = 3.2, 4.7 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 213.9 (C-2), 97.1 (O<u>C</u>H$_2$O), 71.2 (MOMO<u>C</u>H$_2$), 71.0 (<u>C</u>HOH), 55.5 (O<u>C</u>H$_3$), 35.4 (C-5), 33.0 (C-3), 31.1 (C-1), 24.5 (C-4), 16.0 (C-6). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{10}H_{16}O_4$: 223.0941. Found: 223.0941.

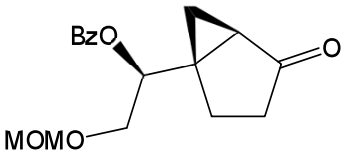


(+/-)

2-28

2-(methoxymethoxy)-1-[4-oxobicyclo[3.1.0]hex-1-yl]ethyl benzoate (2-28). Benzoyl chloride (0.44 g, 3.12 mmol) was added, with stirring, to a solution of compound **2-27** (0.52 g, 2.60 mmol) in pyridine (25 mL) at 0 ºC. The cooling bath was removed after 20 min and the mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of CH_3OH (1 mL) and diluted with EtOAc. The mixture was washed with water and 1 M HCl. The organic layer was dried (Na_2SO_4) and concentrated to afford a yellow oil, which was purified by chromatography (2:1 hexane–EtOAc) to give the product **2-28** (0.73 g, 93%) as a colorless oil. R_f 0.46 (2:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.02–8.00 (m, 2 H, Ar), 7.58–7.53 (m, 1 H, Ar), 7.46–7.43 (m, 2 H, Ar), 5.37 (dd, 1 H, *J* = 5.6, 5.6 Hz, C<u>H</u>OBz), 4.61 (s, 2 H, OC<u>H</u>$_2$O), 3.83 (d, 2 H, *J* = 5.6 Hz, MOMOC<u>H</u>$_2$), 3.31 (s, 3 H, OC<u>H</u>$_3$), 2.23–2.13 (m, 4 H, H-2, H-3), 1.90 (dd, 1 H, *J* = 3.3, 9.3 Hz, H-5), 1.58 (dd, 1 H, *J* = 5.0, 9.3 Hz, H-6a), 1.16 (dd, 1 H, *J* = 3.3, 5.0 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 212.7 (C-4), 165.6 (O–<u>C</u>=O), 133.3 (Ar), 129.8 (Ar), 129.6 (Ar × 2), 128.5 (Ar × 2), 96.5 (O<u>C</u>H$_2$O), 73.7 (<u>C</u>HOBz), 67.3 (MOMO<u>C</u>H$_2$), 55.4 (O<u>C</u>H$_3$), 33.9 (C-1), 32.8

(C-3), 31.8 (C-5), 24.1 (C-2), 17.3 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{17}H_{20}O_5$: 327.1203. Found: 327.1199.

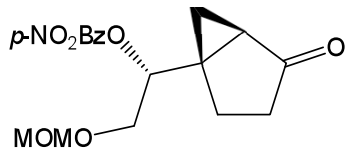


(+/-)

2-28

2-(methoxymethoxy)-1-[4-oxobicyclo[3.1.0]hex-1-yl]ethyl benzoate (2-28).

The compound **2-26** (0.15 g, 0.75 mmol), benzoic acid (0.93 g, 0.75 mmol) and triphenylphosphate (0.20 g, 0.76 mmol) was dissolved in THF (15 mL). The solution was cooled to 0 ºC and treated with DIAD (0.15 g, 0.75 mmol) dropwise. The mixture was warmed to room temperature and stirred for 1 h. The reaction was then diluted with EtOAc and washed with 1 M NaOH and water. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatographic (4:1 hexane–EtOAc) to yield compound 8 (0.19 g, 84%). The spectral data for the **2-28** produced in this manner was identical to that described above.



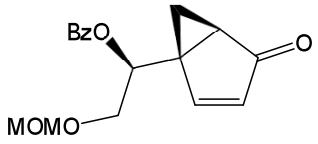
(+/-)

2-29

2-(methoxymethoxy)-1-[4-oxobicyclo[3.1.0]hex-1-yl]ethyl 4-nitrobenzoate (2-29)

To a stirred solution of **2-26** (1.23 g, 6.15 mmol) in CH_2Cl_2–pyridine (10:1, 8.8 mL) was added *p*-nitrobenzoyl chloride (1.36 g, 7.38 mmol) at 0 ºC. The mixture was then warmed to room temperature and stirred for 1 h. The reaction mixture was quenched by adding CH_3OH, and then diluted with CH_2Cl_2. The solution was washed with 1 M HCl and water. The organic layer was dried (Na_2SO_4), filtered, concentrated, and the residue was purified by chromatography (1:1 EtOAc–hexane) to provide the product **2-29** as a light yellow solid (1.60 g, 76%). This material was recrystallized from CH_3OH to give a crystalline material (m.p. = 107–109 °C). R_f 0.36 (1:1 EtOAc–Hexane); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.32–8.30 (m, 2 H, Ar), 8.23–8.21 (m, 2 H, Ar), 5.11 (dd, 1 H, *J* = 4.7, 7.0 Hz, C<u>H</u>OBz-NO_2), 4.67–4.64 (m, 2 H, OC<u>H</u>$_2$O), 3.95–3.88 (m, 2 H, MOMOC<u>H</u>$_2$), 3.35 (s, 3 H, OC<u>H</u>$_3$), 2.40–2.32 (m, 1 H, H-3), 2.16–2.13 (m, 3 H, H-2, H-3), 2.00 (dd, 1 H, *J* = 3.6, 9.4 Hz, H-5), 1.47 (dd, 1 H, *J* = 5.2, 9.4 Hz, H-6a), 1.28 (dd, 1 H, *J* = 3.6, 5.2 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 212.3 (C-4), 163.9 (O–<u>C</u>=O), 150.7 (Ar), 135.2 (Ar), 130.8 (Ar × 2), 123.6 (Ar ×

2), 96.6 (O<u>C</u>H_2O), 76.2 (<u>C</u>HOBz-NO_2), 67.3 (MOMO<u>C</u>H_2), 55.5 (O<u>C</u>H_3), 34.3 (C-1), 32.8 (C-5), 32.6 (C-3), 22.7(C-2), 17.9 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{17}H_{19}NO_7$: 372.1054. Found: 372.1055.



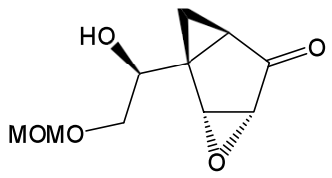
(+/-)

2-30

2-(methoxymethoxy)-1-[4-oxobicyclo[3.1.0]hex-2-en-1-yl]ethyl benzoate (2-30).

To a solution of compound **2-28** (0.65 g, 2.14 mmol) in dry EtOAc (20 mL), which had been saturated with HCl gas, was added PhSeCl (0.41 g, 2.14 mmol). After 2 h, the mixture was poured onto a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with EtOAc and the organic layers were combined, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (4:1 hexane–EtOAc) to afford the selenide isomers (0.45 g, 45%) as a yellow oil, R_f 0.68 (2:1 hexane–EtOAc). This mixture of compounds was dissolved in THF (10 mL) and $NaIO_4$ (0.42 g, 1.92 mmol) and H_2O (3 mL) were added. The mixture was stirred overnight, filtered and the filtrate was extracted with EtOAc. The organic layers were combined, dried (Na_2SO_4) and concentrated. The resulting oil was purified by column chromatography (3:1 hexane–EtOAc) to afford the enone **2-30** (0.28 g, 95% from mixture of selenides,

43% from **2-28**) as an oil. R_f 0.51 (2:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.09–8.07 (m, 2 H, Ar), 7.78 (d, 1 H, *J* = 5.7 Hz, H-2), 7.62–7.58 (m, 1 H, Ar), 7.50–7.46 (m, 2 H, Ar), 5.68 (d, 1 H, *J* = 5.7 Hz, H-3), 5.35 (dd, 1 H, *J* = 5.3, 5.3 Hz, C<u>H</u>OBz), 4.61 (s, 2 H, OC<u>H</u>$_2$O), 3.86–3.79 (m, 2 H, MOMOC<u>H</u>$_2$), 3.31 (s, 3 H, OC<u>H</u>$_3$), 2.35 (dd, 1 H, *J* = 3.9, 8.7 Hz, H-5), 1.79 (dd, 1 H, *J* = 3.9, 8.7 Hz, H-6a), 1.58 (dd, 1 H, *J* = 3.9, 3.9 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 204.8 (C-4), 165.6 (O–<u>C</u>=O), 161.4 (C-2), 133.4 (Ar), 129.8 (Ar), 129.7 (Ar × 2), 128.5 (Ar × 2), 128.4 (C-3), 96.5 (O<u>C</u>H$_2$O), 72.6 (<u>C</u>HOBz), 67.9 (MOMO<u>C</u>H$_2$), 55.4 (O<u>C</u>H$_3$), 40.1 (C-6), 36.6 (C-1), 28.9 (C-5). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{17}H_{18}O_5$: 325.1046. Found: 325.1042.

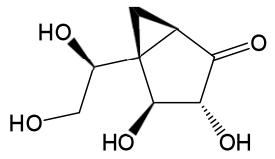


(+/-)
2-31

1-[1-hydroxy-2-(methoxymethoxy)ethyl]-3-oxatricyclo[4.1.0.0^{2,4}]heptan-5-one (2-31).

To an ice cold solution of enone **2-30** (3.07 g, 10.16 mmol) in CH_3OH (60 mL) and 30% hydrogen peroxide (20 mL) was slowly added 4 M NaOH (8 mL). After stirring for 1 h, the mixture was diluted with water and extracted repeatedly with Et_2O. The organic layers were combined, dried (Na_2SO_4) and concentrated.

The resulting yellowish oil was purified by chromatography (9:1 CH_2Cl_2–CH_3OH) to afford the epoxide **2-31** (1.72 g, 79%) as an oil. R_f 0.33 (ethyl ether); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.66 (s, 2 H, OC<u>H</u>$_2$O), 3.98 (dd, 1 H, *J* = 2.5, 2.5 Hz, H-2), 3.83–3.81 (m, 2 H, MOMOC<u>H</u>$_2$, C<u>H</u>OH), 3.72 (dd, 1 H, *J* = 8.6, 11.2 Hz, MOMOC<u>H</u>$_2$), 3.37 (s, 3 H, OC<u>H</u>$_3$), 3.19 (dd, 1 H, *J* = 1.2, 2.5 Hz, H-4), 3.13 (br, 1 H, O<u>H</u>), 1.76 (dddd, 1 H, *J* = 1.2, 2.5, 3.5, 8.5 Hz, H-6), 1.55 (dd, 1 H, *J* = 5.2, 8.5 Hz, H-7a), 1.22 (dd, 1 H, *J* = 3.5, 5.2 Hz, H-7b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 202.5 (C-5), 97.3 (O<u>C</u>H$_2$O), 71.7 (MOMO<u>C</u>H$_2$), 69.8 (<u>C</u>HOH), 57.1 (C-2), 55.6 (O<u>C</u>H$_3$), 51.0 (C-4), 38.1 (C-1), 25.6 (C-6), 21.0 (C-7). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{10}H_{14}O_5$: 237.0733. Found: 237.0735.

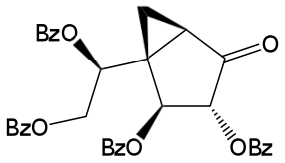


(+/-)
2-32

5-(1,2-dihydroxyethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-one (2-32).

A mixture of epoxide **2-31** (1.52 g, 7.10 mmol) and 3.5 M H_2SO_4 (10 mL) was heated with stirring at 50 ºC for 12 h whereupon a homogenous solution resulted. The solution was neutralized with solid $NaHCO_3$ to pH 7 and concentrated. The residue was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give the tetrol **2-32** (0.93 g, 70%) as a foam. R_f 0.29 (4:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 4.12 (dd, 1 H, *J* = 1.0, 6.9 Hz, H-4), 3.96–3.92 (m,

2 H, H-3, C<u>H</u>OH), 3.71 (dd, 1 H, *J* = 5.4, 11.2 Hz, C<u>H</u>$_2$OH), 3.55 (dd, 1 H, *J* = 6.4, 11.2 Hz, C<u>H</u>$_2$OH), 2.01 (ddd, 1 H, *J* = 1.0, 4.8, 8.1 Hz, H-1), 1.59–1.52 (m, 2 H, H-6a, H-6b); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 209.6 (C-2), 78.0 (C-3), 76.6 (C-4), 72.1 (<u>C</u>HOH), 66.0 (<u>C</u>H$_2$OH), 41.3 (C-5), 31.9 (C-1), 15.6 (C-6). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_8H_{12}O_5$: 211.0577. Found: 211.0577.

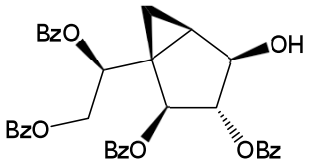


(+/-)
2-33

1-[-1,2-bis(benzoyloxy)ethyl]-4-oxobicyclo[3.1.0]hexane-2,3-diyl dibenzoate(2-33).

Benzoyl chloride (1.26 g, 9.00 mmol) was added slowly, with stirring, to a solution of **2-32** (0.38 g, 2.00 mmol) in pyridine (20 mL) at 0 ºC. The cooling bath was removed after 20 min and the mixture was kept at 10 ºC for 2 h. The reaction was quenched by the addition of CH_3OH and was then diluted with EtOAc. The mixture was washed with water and 1 M HCl. The organic layer was dried (Na_2SO_4) and concentrated to afford a yellow oil, which was purified by chromatography (3:1 hexane–EtOAc) to give **2-33** (1.08 g, 90%) as a colorless oil. R_f 0.52 (3:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.06–7.97 (m, 6 H, Ar), 7.81–7.79 (m, 2 H, Ar), 7.59–7.49 (m, 3 H, Ar), 7.45–7.32 (m, 7 H, Ar),

7.14–7.10 (m, 2 H, Ar), 6.54 (dd, 1 H, *J* = 1.3, 6.4 Hz, H-2), 5.57 (d, 1 H, *J* = 6.4 Hz, H-3), 5.40 (dd, 1 H, *J* = 5.3, 6.8 Hz, C<u>H</u>OBz), 4.94 (dd, 1 H, *J* = 5.3, 11.9 Hz, C<u>H</u>$_2$OBz), 4.84 (dd, 1 H, *J* = 6.8, 11.9 Hz, C<u>H</u>$_2$OBz), 2.48 (dd, 1 H, *J* = 4.0, 9.4 Hz, H-5), 2.03 (dd, 1 H, *J* = 4.0, 6.0 Hz, H-6a), 1.95–1.91 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 198.6 (C-4), 166.0 (O–<u>C</u>=O), 165.6 (O–<u>C</u>=O), 166.5 (O–<u>C</u>=O), 165.4 (O–<u>C</u>=O), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 130.2 (Ar × 2), 129.9 (Ar × 2), 129.8 (Ar × 2), 129.7 (Ar × 2), 129.3 (Ar), 129.1 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar × 2), 128.4 (Ar × 2), 128.3 (Ar × 2), 128.2 (Ar × 2), 76.7 (<u>C</u>HOBz), 72.9 (C-3), 72.4 (C-2), 63.5 (<u>C</u>H$_2$OBz), 34.9 (C-1), 33.4 (C-5), 18.2 (C-6). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{36}H_{28}O_9$: 627.1625. Found: 627.1624.



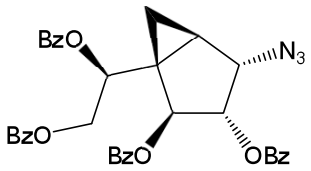
(+/-)

2-34

1-[1,2-bis(benzoyloxy)ethyl]-4-hydroxybicyclo[3.1.0]hexane-2,3-diyl dibenzoate (2-34).

Compound **2-33** (0.732 g, 1.21 mmol) was dissolved in CH_3OH at –30 ºC and $NaBH_4$ (0.092 g, 2.42 mmol) was added in one portion. The mixture was stirred for 5 min, before being warmed to room temperature and stirred for 15 min. The

reaction mixture was diluted with CH_2Cl_2 and washed 1 M HCl. The organic layer was dried (Na_2SO_4), concentrated and the residue was purified by chromatography (3:1 hexane–EtOAc) to provide the product **2-34** (0.607 g, 83%) as a foam. R_f 0.32 (1:2 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.05–8.00 (m, 6 H, Ar), 7.78–7.76 (m, 2 H, Ar), 7.61–7.34 (m, 10 H, Ar), 7.10–7.06 (m, 2 H, Ar), 6.48 (d, 1 H, *J* = 6.0 Hz, H-2), 5.30 (dd, 1 H, *J* = 5.3, 7.2 Hz, C<u>H</u>OBz), 4.93–4.88 (m, 2 H, C<u>H</u>$_2$OBz, H-3), 4.76 (dd, 1 H, *J* = 7.2, 11.7 Hz, C<u>H</u>$_2$OBz), 4.50 (dd, 1 H, *J* = 5.0, 5.4 Hz, H-4), 3.90 (br, 1 H, O<u>H</u>), 2.06 (ddd, 1 H, *J* = 4.5, 5.0, 8.5 Hz, H-5), 1.56 (dd, 1 H, *J* = 4.5, 6.3 Hz, H-6a), 1.20 (dd, 1 H, *J* = 6.3, 8.5 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 168.1 (O–<u>C</u>=O), 166.1 (O–<u>C</u>=O), 165.7 (O–<u>C</u>=O), 165.6 (O–<u>C</u>=O), 133.7 (Ar), 133.2 (Ar × 2), 133.0 (Ar), 130.1 (Ar × 2), 129.9 (Ar × 2), 129.7 (Ar × 4), 129.6 (Ar), 129.5 (Ar), 129.2 (Ar), 128.8 (Ar), 128.5 (Ar × 2), 128.4 (Ar × 2), 128.3 (Ar × 2), 128.1 (Ar × 2), 84.9 (C-3), 75.5 (C-2), 73.8 (2 C, <u>C</u>HOBz, C-4), 63.9 (<u>C</u>H$_2$OBz), 29.9 (C-1), 26.4 (C-5), 11.2 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{36}H_{30}O_9$: 629.1782. Found: 629.1776.

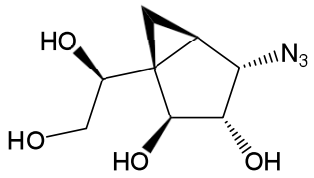


(+/-)
2-35

4-azido-1-[1,2-bis(benzoyloxy)ethyl]bicyclo[3.1.0]hexane-2,3-diyl dibenzoate (2-35).

To a solution of alcohol **2-34** (0.078 g, 0.13 mmol) and triphenyphosphine (0.034 g, 0.13 mmol) and molecular sieves in THF (5 mL) at room temperature was slowly added diisopropyl azodicarboxylate (0.023 g, 0.13 mmol) and the mixture was stirred for 5 min. Diphenylphosphoryl azide (0.036 g, 0.13 mmol) was added dropwise and the solution was stirred for another 10 min. The mixture was diluted with EtOAc and was washed with water. The organic layer was dried (Na_2SO_4), concentrated and the residue purified by chromatography (3:1 hexane–EtOAc) to give **2-35** (0.068 g, 84%) as a colorless oil. R_f 0.46 (3:1 hexane–EtOAc); IR 2100 cm^{-1} (N_3); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.09–7.97 (m, 6 H, Ar), 7.77–7.75 (m, 2 H, Ar), 7.60–7.32 (m, 10 H, Ar), 7.08–7.04 (m, 2 H, Ar), 6.52 (d, 1 H, J = 7.2 Hz, H-2), 5.28 (dd, 1 H, J = 4.7, 7.2 Hz, C<u>H</u>OBz), 5.21 (dd, 1 H, J = 5.3, 7.2 Hz, H-3), 4.96–4.88 (m, 2 H, C<u>H</u>$_2$OBz), 4.47 (d, 1 H, J = 5.3 Hz, H-4), 1.94 (dd, 1 H, J = 4.5, 8.9 Hz, H-5), 1.39 (dd, 1 H, J = 4.5, 6.6 Hz, H-6a), 1.27 (dd, 1 H, J = 6.6, 8.9 Hz, H-6b); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C)

166.2 (O–C=O), 166.0 (O–C=O), 165.7 (O–C=O), 165.6 (O–C=O), 133.6 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 130.1 (Ar × 2), 129.8 (Ar × 2), 129.7 (Ar × 2), 129.6 (Ar), 129.6 (Ar × 2), 129.5 (Ar), 129.1 (Ar), 128.5 (Ar × 3), 128.3 (Ar × 2), 128.2 (Ar × 2), 128.0 (Ar × 2), 77.3 (C-3) 75.4 (C-2), 74.2 (CHOBz), 64.3 (CH_2OBz), 60.5 (C-4), 29.6 (C-1), 25.7 (C-5), 13.4 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{36}H_{29}N_3O_8$: 654.1847. Found: 654.1842.

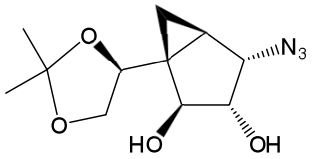


(+/-)
2-36

4-azido-1-[1,2-dihydroxyethyl]bicyclo[3.1.0]hexan-2,3-diol (2-36).

To a solution of compound **2-35** (0.063 g, 0.10 mmol) in CH_3OH (10 mL) was added 2 M $NaOCH_3$ (0.1 mL). The reaction mixture was stirred for 30 min, neutralized with HOAc and then concentrated. The resulting residue was purified by chromatrography on Iatrobeads (9:1 CH_2Cl_2–CH_3OH) to give **2-36** (0.019 g, 91%) as a colorless oil. R_f 0.25 (5:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.14 (d, 1 H, *J* = 7.3 Hz, H-2), 3.81 (d, 1 H, *J* = 5.4, H-4), 3.78 (dd, 1 H, *J* = 5.5, 6.6 Hz, CHOH), 3.72 (dd, 1 H, *J* = 5.4, 7.3 Hz, H-3), 3.66 (dd, 1 H, *J* = 5.5, 11.1 Hz, CH_2OH), 3.52 (dd, 1 H, *J* = 6.6, 11.1 Hz, CH_2OH), 1.50 (dd, 1 H, *J* = 4.2, 8.6 Hz, H-5), 0.78 (dd, 1 H, *J* = 5.7, 8.6 Hz, H-6a), 0.78 (dd, 1 H, *J* =

4.2, 5.7 Hz, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 79.3 (C-3), 79.2 (C-2), 73.2 (CHOH), 66.4 (CH_2OH), 64.8 (C-4), 34.7 (C-1), 22.8 (C-5), 8.7 (C-6). HRMS (ESI) m/z Calcd for ($M+Na^+$) $C_8H_{13}N_3O_4$: 238.0798. Found: 238.0798.

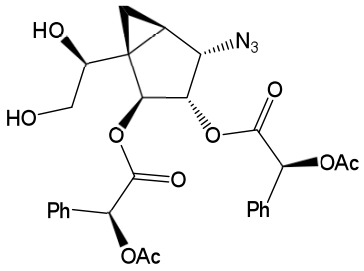


(+/-)
2-37

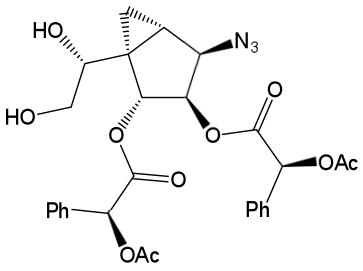
4-azido-1-[2,2-dimethyl-1,3-dioxolan-4-yl]bicyclo[3.1.0]hexane-2,3-diol (2-37).

To a solution of **2-36** (0.182 g, 0.85 mmol) and 2,2-dimethoxypropane (0.133 g, 1.27 mmol) in dry acetone (30 mL) was added *p*-toluenesulfonic acid monohydrate (0.015 g, 0.08 mmol), and the solution was stirred for 30 min at room temperature. Several drops of Et_3N were added to neutralize the acid and the solution was concentrated. The residue was purified by column chromatography (1:1 hexane–EtOAc) to give **2-37** (0.217 g, 86%). R_f 0.10 (1:1 hexane–EtOAc); ^{1}H NMR (500 MHz, CD_2Cl_2, δ_H) 4.22 (dd, 1 H, J = 3.3, 7.1 Hz, H-2), 4.07–4.01 (m, 2 H, CHO, CH_2O), 3.97 (d, 1 H, J = 5.3 Hz, H-4), 3.77 (dd, 1 H, J = 6.0, 7.2 Hz, CH_2O), 3.67 (ddd, 1 H, J = 5.3, 7.1, 8.9 Hz, H-3), 2.28 (d, 1 H, J = 3.3 Hz, 2-OH), 2.18 (d, 1 H, J = 8.9 Hz, 3-OH), 1.50 (dd, 1 H, J = 4.1, 8.6 Hz, H-5), 1.40 (s, 3 H, $(CH_3)_2C$), 1.32 (s, 3 H, $(CH_3)_2C$), 0.90 (dd, 1 H, J = 4.1, 6.0 Hz, H-6a), 0.75 (dd, 1 H, J = 6.0, 8.6 Hz, H-6b); ^{13}C NMR (100 MHz, CD_2Cl_2, δ_C)

109.4 ($(CH_3)_2\underline{C}$), 78.2 (C-2), 77.9 (<u>C</u>HO), 77.7 (C-3), 67.7 ($\underline{C}H_2O$), 63.8 (C-4), 31.9 (C-1), 26.6 ($(\underline{C}H_3)_2C$)), 25.2 ($(\underline{C}H_3)_2C$), 21.8 (C-5), 10.7 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{11}H_{17}N_3O_4$: 278.1111. Found: 278.1110.



2-40



2-41

(1*S*,2*S*,3*S*,4*S*,5*R*)-4-azido-1-[(1*S*)-1,2-dihydroxyethyl]bicyclo[3.1.0]hexane-2,3-diyl (2*S*,2'*S*)bis[(acetyloxy)(phenyl)ethanoate] (2-40) and (1*R*,2*R*,3*R*,4*R*,5*S*)-4-azido-1-[(1*R*)-1,2-dihydroxyethyl]bicyclo[3.1.0]hexane-2,3-diyl (2*S*,2'*S*)bis[(acetyloxy)(phenyl)ethanoate] (2-41).

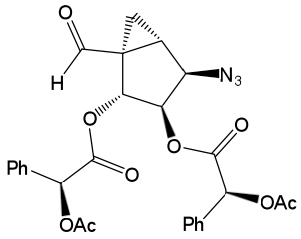
Compound **2-37** (0.255 g, 1.00 mol), *O*-acetyl-(*S*)-(+)-mandelic acid (0.427 g, 2.20 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (0.457 g, 2.20 mmol) were dissolved in CH_2Cl_2 and 4-(dimethylamino)pyridine (0.013 g, 0.11 mmol) was added. The reaction mixture was stirred for 30 min and then concentrated. The residue was purified by chromatography (4:1 hexane–EtOAc) to give an inseparable mixture of diastereomers (0.575 g, 95%) as a colorless oil. R_f 0.65 (2:1 hexane–EtOAc). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{31}H_{33}N_3O_{10}$: 630.2058. Found: 630.2058. This mixture of diastereomers was dissolved in 5:3:2 HOAc–H_2O–THF (20 mL) and was stirred at 50 ºC for 12 h. The mixture

was concentrated and the residue was purified by chromatography (1:2 hexane–EtOAc) to give compounds **2-40** and **2-41** (0.488 g, 91% combined yield).

Data for **2-40**: R_f 0.46 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +160.3 (*c* 0.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.29 (m, 10 H, Ar), 5.91 (s, 1 H, C<u>H</u>Ph), 5.81 (s, 1 H, C<u>H</u>Ph), 5.77 (d, 1 H, *J* = 7.0 Hz, H-2), 4.73 (dd, 1 H, *J* = 5.5, 7.0 Hz, H-3), 4.11 (d, 1 H, *J* = 5.5 Hz, H-4), 3.69 (dd, 1 H, *J* = 4.0, 11.2 Hz, C<u>H</u>$_2$OH), 3.64 (dd, 1 H, *J* = 4.0, 6.4 Hz, C<u>H</u>OH), 3.50 (dd, 1 H, *J* = 6.4, 11.2 Hz, C<u>H</u>$_2$OH), 2.18 (s, 3 H, acetate C<u>H</u>$_3$), 2.14 (s, 3 H, acetate C<u>H</u>$_3$), 1.74 (dd, 1 H, *J* = 4.6, 8.7 Hz, H-5), 0.96 (dd, 1 H, *J* = 6.7, 8.7 Hz, H-6a), 0.92 (dd, 1 H, *J* = 4.6, 6.7 Hz, H-6b); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.6 (O–<u>C</u>=O), 169.9 (O–<u>C</u>=O), 168.5 (O–<u>C</u>=O), 167.8 (O–<u>C</u>=O), 133.0 (Ar), 132.7 (Ar), 129.3 (Ar), 129.2 (Ar), 128.8 (Ar × 2), 128.7 (Ar × 2), 127.5 (Ar × 2), 127.4 (Ar × 2), 77.8 (<u>C</u>HPh), 77.6 (<u>C</u>HPh), 74.6 (C-2), 73.9 (C-3), 71.5 (<u>C</u>HOH), 64.1 (<u>C</u>H$_2$OH), 60.7 (C-4), 31.3 (C-1), 24.1 (C-5), 20.6 (acetate <u>C</u>H$_3$), 20.5 (acetate <u>C</u>H$_3$), 10.6 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{28}H_{29}N_3O_{10}$: 590.1745. Found: 590.1748.

Data for **2-41**: R_f 0.47 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –32.3 (*c* 0.11, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.48–7.35 (m, 10 H, Ar), 5.99 (s, 1 H, C<u>H</u>Ph), 5.95 (s, 1 H, C<u>H</u>Ph), 5.76 (d, 1 H, *J* = 7.2 Hz, H-2), 4.82 (dd, 1 H, *J* = 5.4, 7.2 Hz, H-3), 4.02 (d, 1 H, *J* = 5.4 Hz, H-4), 3.47 (dd, 1 H, *J* = 4.0, 6.7 Hz, C<u>H</u>OH), 3.37 (dd, 1 H, *J* = 4.0, 11.4 Hz, C<u>H</u>$_2$OH), 3.26 (dd, 1 H, *J* = 6.7, 11.4 Hz, C<u>H</u>$_2$OH), 2.14 (s, 3

H, acetate C<u>H</u>$_3$), 2.02 (s, 3 H, acetate C<u>H</u>$_3$), 1.67 (dd, 1 H, *J* = 7.0, 7.3 Hz, H-5), 0.79–0.78 (m, 2 H, H-6a, H-6b), ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.3 (O–<u>C</u>=O), 170.0 (O–<u>C</u>=O), 168.8 (O–<u>C</u>=O), 168.3 (O–<u>C</u>=O), 133.4 (Ar), 133.0 (Ar), 129.5 (Ar), 129.4 (Ar), 128.9 (Ar × 2), 128.8 (Ar × 2), 127.8 (Ar × 4), 77.9 (2 C, <u>C</u>HPh, <u>C</u>HPh), 74.6 (C-3), 74.1 (C-2), 71.2 (<u>C</u>HOH), 63.8 (<u>C</u>H$_2$OH), 60.4 (C-4), 30.8 (C-1), 24.0 (C-5), 20.7 (acetate <u>C</u>H$_3$), 20.2 (acetate <u>C</u>H$_3$), 10.4 (C-6). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{28}H_{29}N_3O_{10}$: 590.1745. Found: 590.1748

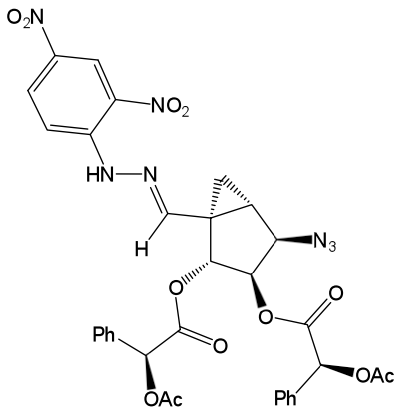


2-42

(1*S*,2*R*,3*R*,4*R*,5*S*)-4-azido-1-formylbicyclo[3.1.0]hexane-2,3-diyl (2*S*,2'*S*)bis[(acetyloxy)(phenyl)ethanoate] (2-42).

Compound **2-41** (0.026 g, 0.045 mmol) was dissolved in THF (4 mL) and $NaIO_4$ (0.020 g, 0.093 mmol) and a satd aq solution of $NaHCO_3$ (3 mL) was added. This reaction mixture was stirred for 2 h and EtOAc was added. The organic layer was dried (Na_2SO_4) and the residue was purified with chromatography (4:1 hexane–EtOAc) to give **2-42** (0.021 g, 90%) as a colorless oil. R_f 0.46 (2:1 hexane–EtOAc); $[\alpha]_D$ –16.8 (*c* 0.14, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 9.69 (s, 1 H, C<u>H</u>O), 7.52–7.37 (m, 10 H, Ar), 6.05 (s, 1 H, C<u>H</u>Ph), 5.97 (s, 1 H,

CHPh), 5.88 (dd, 1 H, *J* = 7.1 Hz, H-2), 4.79 (dd, 1 H, *J* = 5.6, 7.1 Hz, H-3), 4.07 (d, 1 H, *J* = 5.6 Hz, H-4), 2.23 (s, 3 H, acetate CH_3), 2.21 (s, 3 H, acetate CH_3), 1.96 (dd, 1 H, *J* = 5.6, 9.0 Hz, H-5), 1.53 (ddd, 1 H, *J* = 9.0, 11.4 Hz, H-6a), 0.90 (dd, 1 H, *J* = 5.6, 11.4 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 196.6 (CHO), 170.1 (O–C=O), 170.1 (O–C=O), 168.9 (O–C=O), 168.2 (O–C=O), 133.3 (Ar), 132.9 (Ar), 129.5 (Ar), 129.5 (Ar), 128.9 (Ar × 2), 128.9 (Ar × 2), 127.8 (Ar × 4), 77.5 (C-3), 76.8 (C-2), 74.2 (CHPh), 74.1 (CHPh), 59.7 (C-4), 38.8 (C-1), 34.3 (C-5), 20.6 (2 C, acetate CH_3), 14.4 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{27}H_{25}N_3O_9$: 558.1483. Found: 558.1484.

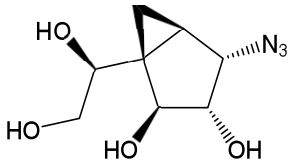


2-43

(1*R*,2*R*,3*R*,4*R*,5*S*)-4-azido-1-((*E*)-[(2,4-dinitrophenyl)hydrazono]methyl)bi-cyclo[3.1.0]hexane-2,3-diyl (2*S*,2'*S*)bis[(acetyloxy)(phenyl)ethanoate] (2-43).

Compound **2-42** (5.8 mg, 0.011 mmol) was dissolved in CH_3OH (1 mL) and 1 M 2,4-dinitrophenylhydrazine (1 mL) was added. The mixture was swirled for 1 min and the solution was concentrated and the residue was purified by

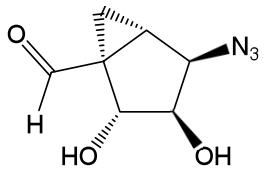
chromatography (3:1 hexane–EtOAc) to give **2-43** (4.2 mg, 53%) as yellow solid. This material was recrystallized from CH_2Cl_2 to provide a crystalline solid (m.p. 57–59 °C). R_f 0.21 (2:1 hexane–EtOAc); $[\alpha]_D$ 12.6 (*c* 0.27, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.10 (d, 1 H, *J* = 2.3 Hz, N=C<u>H</u>), 8.27 (dd, 1 H, *J* = 2.3, 9.5 Hz, Ar), 7.64 (d, 1 H, *J* = 9.5 Hz, Ar), 7.52–7.51 (m, 2 H, Ar), 7.42–7.37 (m, 5 H, Ar), 7.21–7.18 (m, 4 H, Ar), 6.10 (d, 1 H, *J* = 7.7 Hz, H-2), 6.07 (s, 1 H, C<u>H</u>Ph), 5.95 (s, 1 H, C<u>H</u>Ph), 4.89 (dd, 1 H, *J* = 5.7, 7.7 Hz, H-2), 4.18 (d, 1 H, *J* = 5.7 Hz, H-4), 2.23 (s, 3 H, acetate C<u>H</u>$_3$), 2.22 (s, 3 H, acetate C<u>H</u>$_3$), 1.94 (dd, 1 H, *J* = 5.0, 8.2 Hz, H-5), 1.33–1.27 (m, 2 H, H-6a, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.0 (O–C=O), 169.9 (O–C=O), 168.5 (O–C=O), 168.3 (O–C=O), 148.1 (Ar), 144.5 (Ar), 138.1 (Ar), 133.4 (Ar), 133.0 (Ar), 129.9 (Ar), 129.4 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar × 2), 128.5 (Ar × 2), 127.8 (Ar × 2), 127.4 (Ar × 2), 123.1 (N=<u>C</u>H), 116.7 (Ar), 76.8 (C-3), 76.3 (C-2), 74.1 (<u>C</u>HPh), 74.0 (<u>C</u>HPh), 60.1 (C-4), 31.3 (C-1), 30.2 (C-5), 20.6 (2 C, acetate <u>C</u>H$_3$), 14.3 (C-6). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{33}H_{29}N_7O_{12}$: 738.1766. Found: 738.1763.



2-44

(1*S*,2*S*,3*S*,4*S*,5*R*)-4-azido-1-[(1*S*)-1,2-dihydroxyethyl]bicyclo[3.1.0]hexane-2,3-diol (2-44).

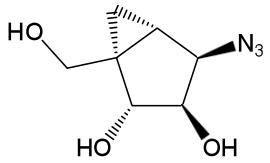
To a solution of **2-40** (0.034 g, 0.06 mmol) in CH_3OH (2 mL) was added 2 M $NaOCH_3$ (100 μL). The reaction mixture was stirred for 20 min, neutralized with HOAc and then concentrated. The resulting residue was purified by chromatography on Iatrobeads (9:1 CH_2Cl_2–CH_3OH) to give **2-44** (0.013 g, 100%) as a colorless oil. R_f 0.25 (5:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +12.3 (*c* 0.11, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.14 (d, 1 H, *J* = 7.3 Hz, H-2), 3.81 (d, 1 H, *J* = 5.4 Hz, H-4), 3.78 (dd, 1 H, *J* = 5.5, 6.6 Hz, C<u>H</u>OH), 3.72 (dd, 1 H, *J* = 5.4, 7.3 Hz, H-3), 3.66 (dd, 1 H, *J* = 5.5, 11.1 Hz, C<u>H</u>$_2$OH), 3.52 (dd, 1 H, *J* = 6.6, 11.1 Hz, C<u>H</u>$_2$OH), 1.50 (dd, 1 H, *J* = 4.2, 8.6 Hz, H-5), 0.78 (dd, 1 H, *J* = 5.7, 8.6 Hz, H-6a), 0.78 (dd, 1 H, *J* = 4.2, 5.7 Hz, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 79.3 (C-3), 79.2 (C-2), 73.2 (<u>C</u>HOH), 66.4 (<u>C</u>H$_2$OH), 64.8 (C-4), 34.6 (C-1), 22.8 (C-5), 8.7 (C-6). HRMS (ESI) m/z Calcd for ($M+Na^+$) $C_8H_{13}N_3O_4$: 238.0798. Found: 238.0798.



2-45

(1*S*,2*R*,3*R*,4*R*,5*S*)-4-azido-2,3-dihydroxybicyclo[3.1.0]hexane-1-carbaldehyde (2-45).

To a solution of **2-42** (0.019 mg, 0.035 mmol), in CH_3OH (2 mL) was added 2 M $NaOCH_3$ (100 μL). This solution was stirred for 20 min and neutralized with HOAc. The mixture was concentrated and the resulting crude product was purified by chromatography (3:1 EtOAc–hexane), to give **2-45** (0.006 g, 96% yield) as an oil. R_f 0.42 (1:9 CH_3OH–CH_2Cl_2); $[\alpha]_D$ –35.3 (*c* 0.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1 H, C<u>H</u>O), 4.70 (d, 1 H, *J* = 7.5 Hz, H-2), 4.11 (d, 1 H, *J* = 5.5 Hz, H-4), 3.79 (dd, 1 H, *J* = 5.5, 7.5 Hz, H-3), 2.51 (br s, 2 H, 2-O<u>H</u>, 3-O<u>H</u>), 2.15 (dd, 1 H, *J* = 7.1 Hz, H-5), 1.50 (d, 2 H, *J* = 7.1 Hz, H-6a, H-6b); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 198.1 (<u>C</u>HO), 76.4 (C-2), 74.3 (C-3), 61.9 (C-4), 41.8 (C-1), 27.8 (C-5), 14.1 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_7H_9N_3O_3$: 206.0536. Found: 206.0538.



2-46

(1*R*,2*R*,3*R*,4*R*,5*S*)-4-azido-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol (2-46).

To a solution of **2-45** (0.006 g, 0.034 mmol) in CH_3OH (1 mL) was added $NaBH_4$ (0.003 g, 0.075 mmol) at –30 ºC. The mixture was stirred for 5 min and then warmed steadily to room temperature and stirred for another 5 min. The reaction mixture was neutralized with 1 M HCl to pH 7 and concentrated. The residue was purified by chromatography (1:9 CH_3OH–CH_2Cl_2) to give **2-46** (0.006 g, 100%) as a foam. R_f 0.23 (1:9 CH_3OH–CH_2Cl_2); $[\alpha]_D$ –15.0 (*c* 0.06, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.24 (d, 1 H, *J* = 7.0 Hz, H-2), 3.83 (d, 1 H, *J* = 11.8 Hz, C$\underline{H}_2$OH), 3.80 (d, 1 H, *J* = 5.5 Hz, H-4), 3.69 (dd, 1 H, *J* = 5.5, 7.0 Hz, H-3), 3.36 (d, 1 H, *J* = 11.8 Hz, C$\underline{H}_2$OH), 1.39 (dd, 1 H, *J* = 3.9, 8.5 Hz, H-5), 0.80 (dd, 1 H, *J* = 3.9, 5.9 Hz, H-6a), 0.59 (dd, 1 H, *J* = 5.9, 8.5 Hz, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 78.9 (C-3), 77.7 (C-2), 64.7 (C-4), 64.2 (C$\underline{H}_2$OH), 33.8 (C-1), 23.4 (C-5), 10.6 (C-6). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_7H_{11}N_3O_3$: 208.0693. Found: 208.0695.

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Chapter 3

Synthesis of DPA and UDP-Gal*f* Mimics Based upon the Bicyclo[3.1.0]hexane Scaffold

3.1. Introduction.

Having developed a route to prepare the core bicyclo[3.1.0]hexane structures as described in Chapter 2, we next explored their conversion to a small library of analogues that were potential inhibitors of mycobacterial glycosyltransferases. The work carried out to prepare these compounds is described below, with the DPA analogues being discussed first, followed by the UDP-Gal*f* analogues.

3.2. Synthesis DPA Mimics

In previous reports,[1-7] most of the DPA analogues prepared to date have two structural features: 1) a stable isostere expected to function as a replacement of the labile glycosyl phosphate, and 2) a long lipid chain saturated or unsaturated to mimic the decaprenyl moiety of DPA. We describe here the synthesis of a panel of bicyclo[3.1.0]hexane-based DPA analogues with general structure **3-1** (Figure 3-1). The arabinose analogue was designed with an amino group, which simplifies the addition of lipid portion by reaction with aldehyde followed by reductive amination. Although these compounds do not contain a phosphate

isostere, the amino group would be protonated (and positively-charged) at physiological pH and thus would be able to form strong ionic interactions with anionic (carboxylate) amino acids, which are expected to be present in the active site of mycobacterial AraT's. At the same time, this charged ammonium group would create a positive charge close to the sugar ring, which could approximate the oxocarbenium ion that is developed in the glycosylation transition state. In selecting a group of compounds to synthesize, we chose analogues that contain a long chain alkyl group, as well as a series of polyprenol chains up to decaprenol (**3-2**–**3-8,** Figure 3-1). We envisioned that these hydrophobic groups would mimic the lipid portion of DPA.

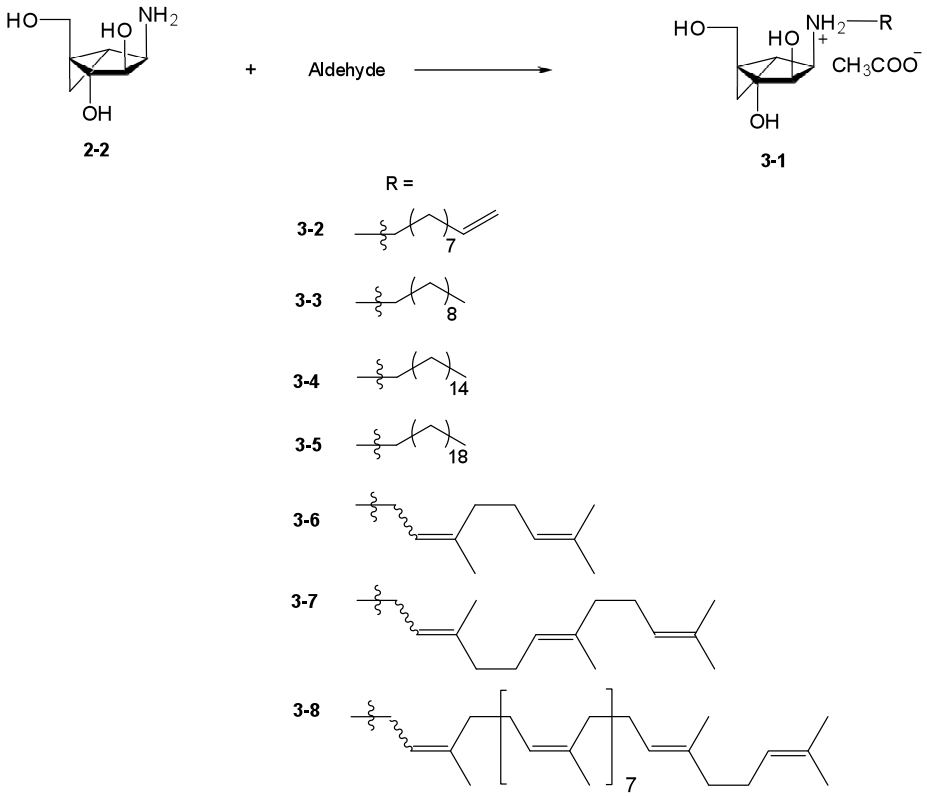
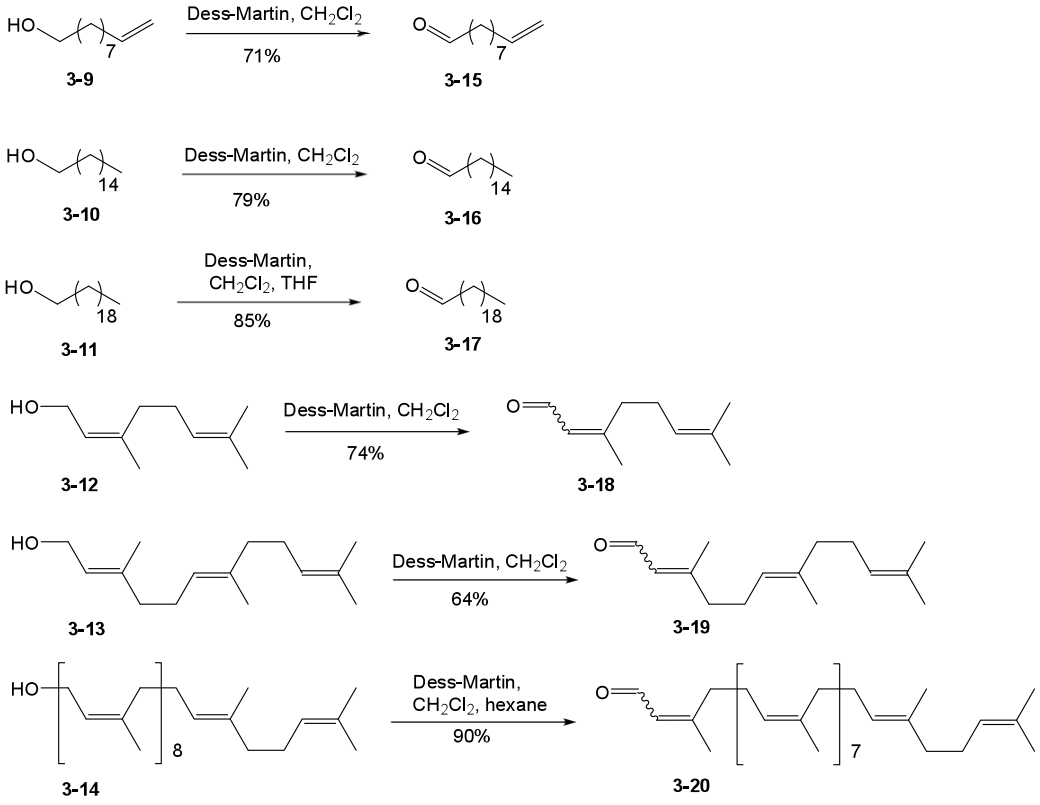


Figure 3-1. Synthesis of analogues with general structure **3-1**

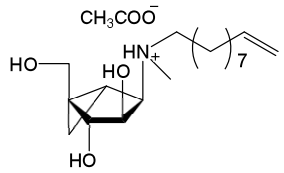
The route to the targets outlined in Figure 3-1 involves the reductive amination of amine **2-2** with a panel of hydrophobic aldehydes. Only one of these aldehydes (decenal, the precursor to **3-3**) was purchased, the remaining ones were obtained by oxidation of the corresponding commercially-available alcohols (Scheme 3-1). The oxidation was carried out by the treatment of each alcohol with the Dess–Martin periodinane reagent, and the product yields ranged from 64% to 90% (Scheme 3-1). Most of the reactions were carried out in CH_2Cl_2 with exceptions of 1-icosanol (**3-11**), which was done in a mixture of THF and CH_2Cl_2, and decaprenol (**3-14**), which was performed in hexane and CH_2Cl_2. These solvent changes were needed given the solubility of these alcohols and the product aldehydes. The α-unsaturated aldehydes (**3-18**, **3-19**, **3-20**) were formed as a mixture of two inseparable products, which resulted from isomerization during the oxidation. This is unusual because Dess-Martin oxidation of allylic alcohols does not usually give epimerized products. We do not have an explanation for the isomerization under these reaction conditions. These aldehydes were not fully characterized because new compounds were observed by TLC after exposing the aldehydes to the air for several hours. Instead, the compounds were used directly in the reductive amination step following a quick purification by column chromatography.



Scheme 3-1. Synthesis of aldehydes **3-15**–**3-20**.

The monoalkylated targets **3.2**–**3.8** were obtained by reductive amination in yields ranging from 55% to 89%, as shown in Scheme 3-2. Reaction of the appropriate aldehydes with **2-2** in freshly distilled CH_3OH or in a mixture of CH_3OH and CH_2Cl_2 afforded the corresponding imines. A longer time was needed for the reactions with compound **3-20**, which has the longest lipid chain. The imine solutions were next treated with $NaBH_4$ at –30 ºC to give the ammonium salts (**3-2**–**3-8**). The reducing agent $NaCNBH_3$ is often used in reductive amination reactions.[8] However, $NaBH_4$ is a better reducing agent, and

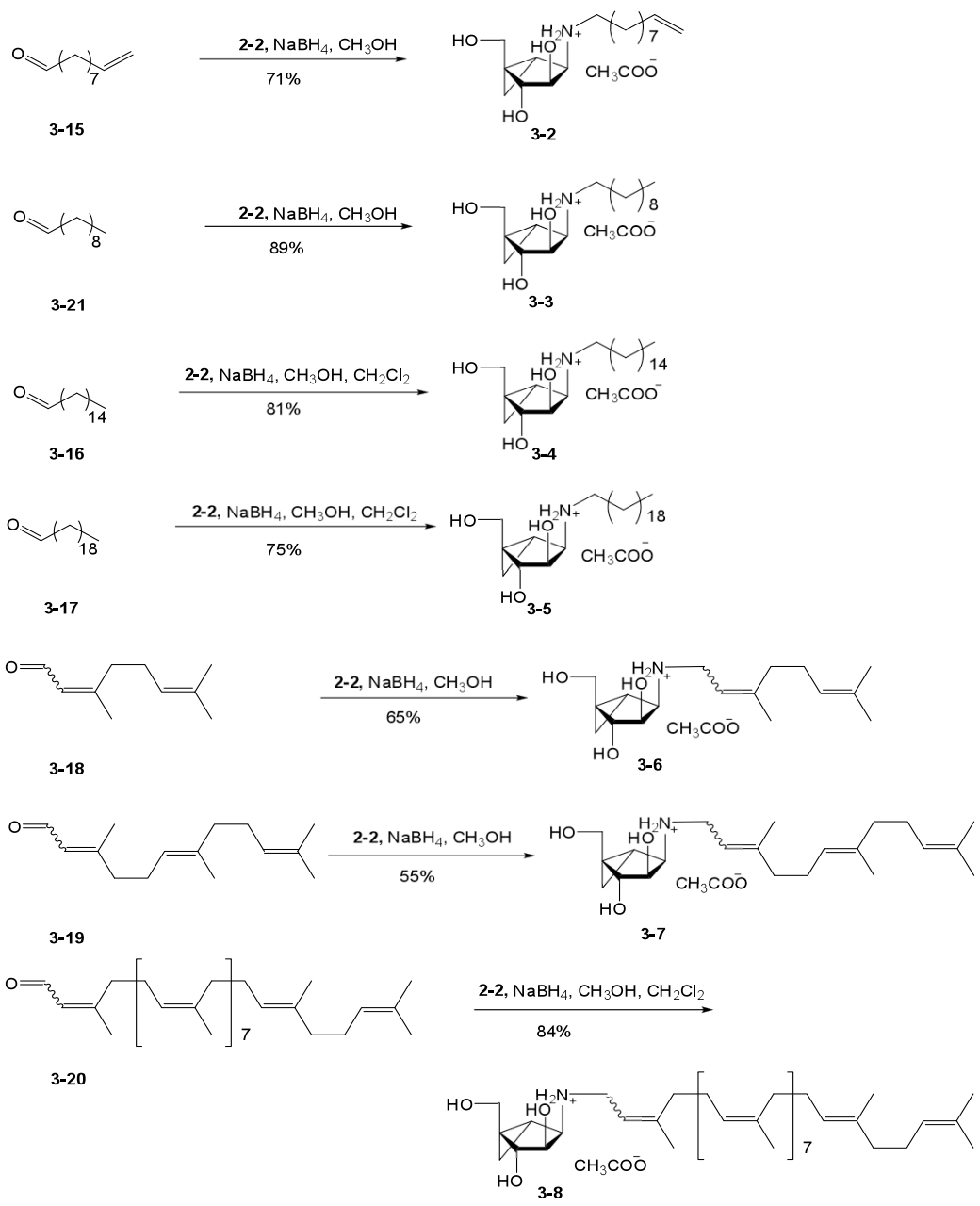
can reduce both the imines and the unreacted aldehydes in a very short time. This feature prevents dialkylation, which can result from the reaction of the monoalkylated product with unreacted aldehyde and subsequent reduction. Freshly distilled methanol was used to exclude the side product **3-22** (Figure 3-2), which can be formed by reduction of the imine product with formaldehyde, an oxidative product of methanol. The products were isolated as acetate salts, which were formed during the final workup step by quenching of the reaction with acetic acid.



3-22

Figure 3-2. *N*-methylated byproduct in the reductive alkylation of **2-2**.

Thus, we now have a small library of seven DPA analogs based upon the bicyclo[3.1.0]hexane scaffold. In the future, these compounds will be tested as inhibitors of mycobacterial AraT's by a collaborator.



Scheme 3-2. Synthesis of DPA mimics by reductive amination of **2-2** with aldehydes

3.3 Synthesis of UDP-Gal*f* mimics

With the galactofuranose analogue **2-1** in hand (see Chapter 2), our next goal was to synthesize a panel of UDP-galactofuranose (UDP-Gal*f*) analogues. As illustrated in Figure 3-3, the UDP-Gal*f* molecule can be divided into three segments: a galactofuranose residue, a pyrophosphate moiety, and a uridine residue.

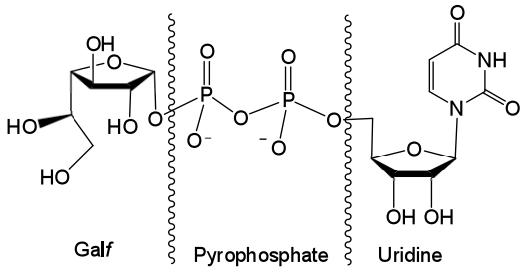


Figure 3-3. UDP-Gal*f* **1-1** and three structural segments.

As discussed in the first chapter, many donor analogues of galactosyltransferases contain a moiety that mimics the uridine diphosphate portion. Different designs for linking the uridine diphosphate domain to the sugar moiety have been explored.[9-13] Inspired by these reports, we initially chose to synthesize UDP-Gal*f* mimics with general structure **3-23** (Figure 3-4). As was true for the DPA analogues, we envisioned that the ammonium ion in the targets would allow these compounds to interact with negatively-charged amino acids in the active site of mycobacterial galactofuranosyltransferases.

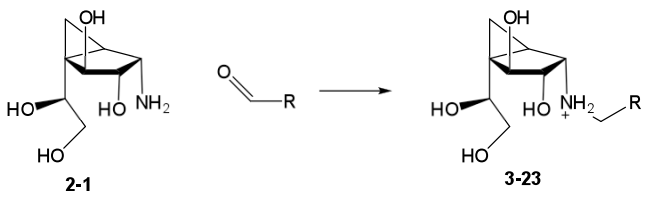
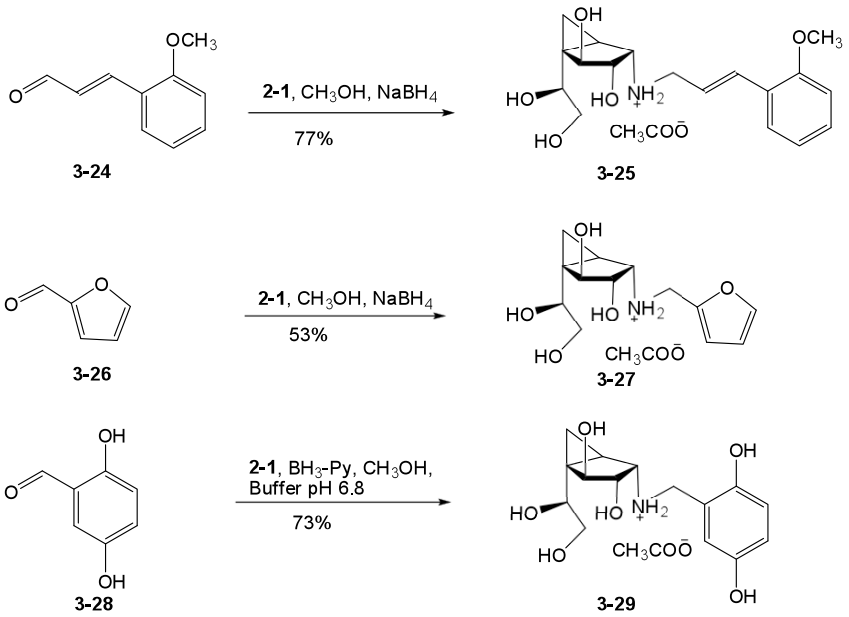


Figure 3-4. Synthesis of analogues with the general structure **3-23**

As initial targets we chose three analogues containing an aromatic domain, which could interact with amino acids in the active site either through cation–π or π–π stacking interactions.[14-17] In addition, the oxygen atoms contained as part of the aromatic domain could act as a hydrogen-bond acceptor. The synthesis of these three analogues started from the coupling of **2-1** with unsaturated or aromatic aldehydes as illustrated in Scheme 3-3.



Scheme 3-3. Synthesis of UDP-Gal*f* analogues with unsaturated groups

Commercially-available aldehydes, **3-24**, **3-26** and **3-28**, were first treated with **2-1** to form the imines, which were then reduced with either $NaBH_4$ or borane–pyridine (BH_3·Py) complex leading to ammonium salts **3-25**, **3-27**, **3-29**, respectively. The yields of these reactions were moderate ranging from 55% to 77%. In the reactions with imines derived from **3-24** and **3-26,** $NaBH_4$ was found to be a good reducing agent. Reduction of the imine formed from **3-28** could be done either by $NaBH_4$ or BH_3·Py. A disadvantage of using BH_3·Py is that it can partially reduce the double bond of the products (e.g., the alkene in **3-25**). In addition, the amount of dialkylated products increases because the reduction is slow. To prevent the formation of the methyl alkylated product, the reactions were carried out in freshly distilled methanol and under an argon atmosphere. The amine generated in the reduction step was converted to the ammonium salt by adding acetic acid to the reaction mixture.

In addition to the three targets above, we wanted prepare analogues that had more similarity to the natural substrate UDP-Gal*f*. In a previous paper by van Boom and coworkers,[18] on the synthesis of UDP-Gal*p* analogs, a target molecule was designed based on molecular modeling studies. In these studies, a five-atom linker between the uridine and sugar moiety was used as it was shown that this provided the required distance between these groups in the pyrophosphate. As illustrated in Figure 3-5, the pyrophosphate linkage of UDP-Gal*f* is also five atoms in length. We therefore chose as targets compounds **3-30**, which has

exactly five atoms between the nitrogen and oxygen, and **3-31**, which has a six atom linker, but with more hydroxyl groups that might act as the chelating sites to metal ions involved in the transferase reaction (see Figure 1-11 in Chapter 1), .

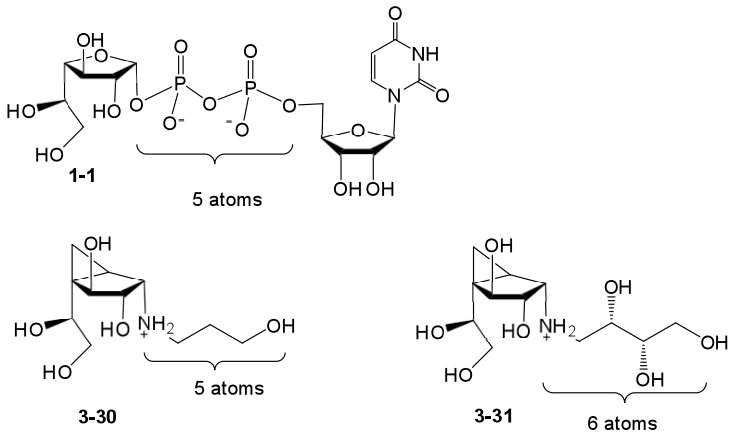
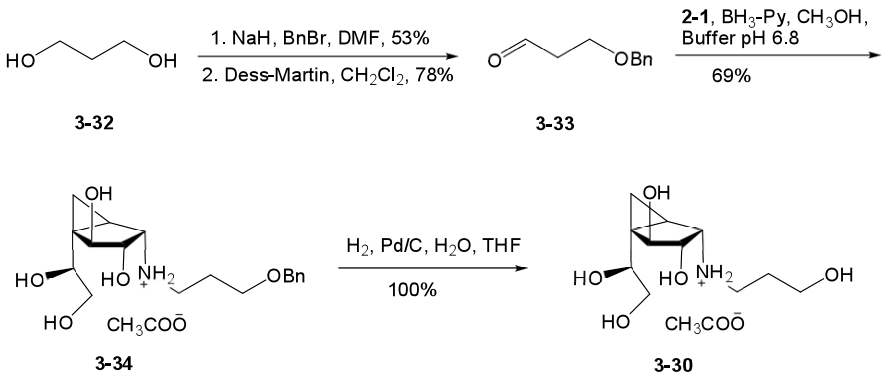


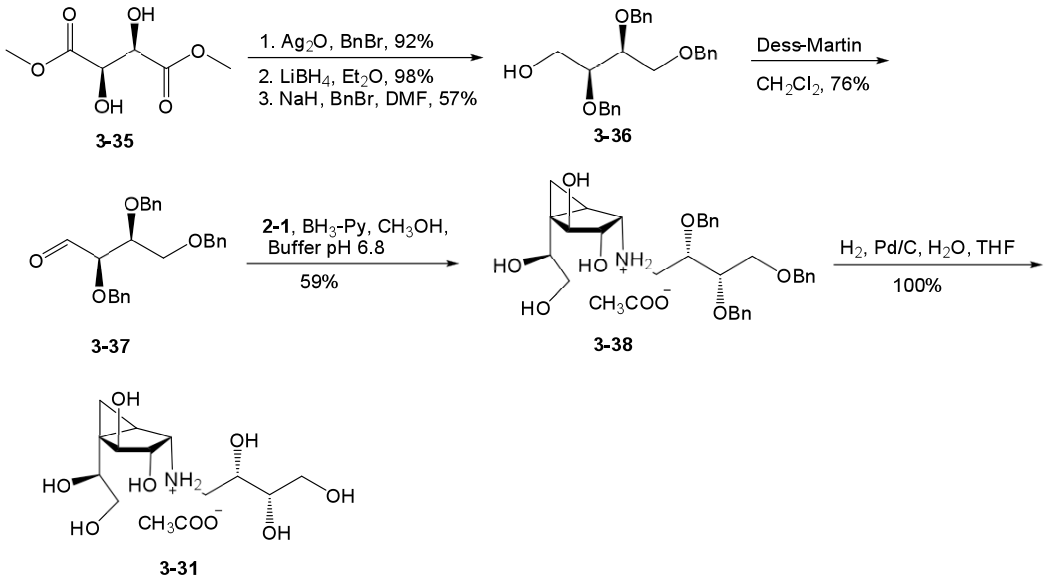
Figure 3-5. Comparison of UDP-Gal*f* with targets **3-30** and **3-31**.

The synthesis of **3-30** is shown in Scheme 3-4. Commercially available propane-1,3-diol (**3-32**) was mono benzylated in 53% yield and then was oxidized with Dess–Martin periodinane to afford the aldehyde **3-33** in 78% yield.[19] Compound **3-33** and **2-1** was mixed in freshly distilled methanol and phosphate buffer (pH 6.8) and then reacted with BH_3·Py to afford **3-34** in 69% yield. The phosphate buffer was added to increase the rate of imine reduction, as has been reported previously.[20] Hydrogenolysis of **3-34** in H_2O and THF afforded the target **3-30** in quantitative yield.



Scheme 3-4. Synthesis of **3-30**

The synthesis of **3-31** is presented in Scheme 3-5. Commercially-available, 1,4-dimethyl-L-tartrate (**3-35**) was treated with benzyl bromide and freshly prepared silver oxide to give the expected dibenzyl ether, which was reduced to the corresponding diol with $LiBH_4$ in ether; subsequent monobenzylation of the product with sodium hydride and benzyl bromide gave **3-36** in 51% over the three step sequence.[21] The primary alcohol was oxidized by Dess–Martin periodinane reagent to afford, in 76% yield, aldehyde **3-37**, which was subsequently treated with **2-1** and BH_3·Py in methanol and phosphate buffer (pH 6.8) to give a 59% yield of **3-38**. Finally, target **3-31** was obtained in quantitative yield by hydrogenolysis over Pd–C in H_2O and THF.



Scheme 3-5. Synthesis of **3-31**

In addition to **3-30** and **3-31**, we next wanted to synthesize analogues **3-39**, **3-40**, **3-41** (Figure 3-6), which include the uridine moiety. The synthesis of these compounds could be carried out using the general strategy shown in Figure 3-7. The first step is to prepare an activated uridine derivative, which is then attached a linker and finally coupled with the bicyclo[3.1.0]hexane amine **2-1**.

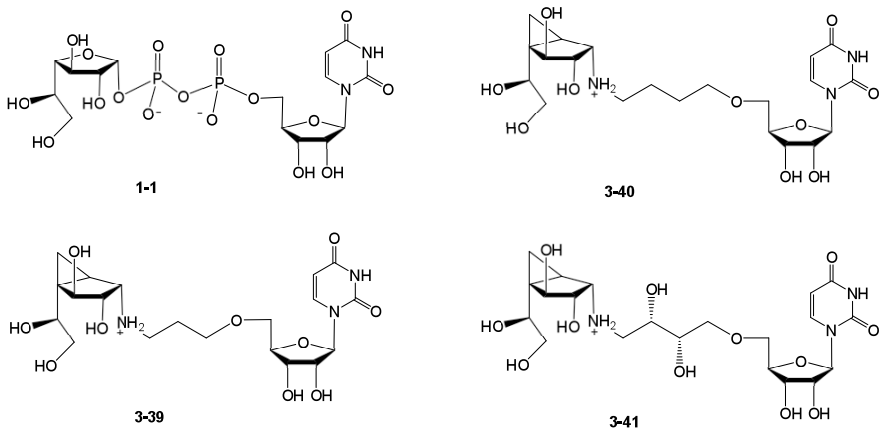


Figure 3-6. Structures of targets **3-39**, **3-40** and **3-41**

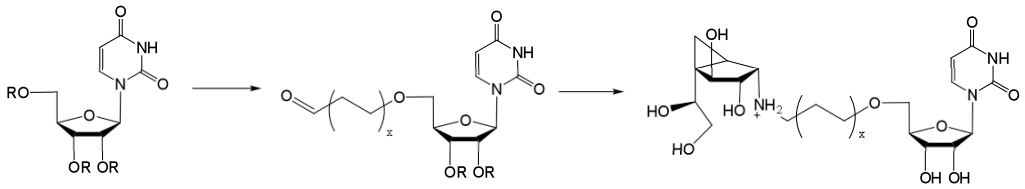
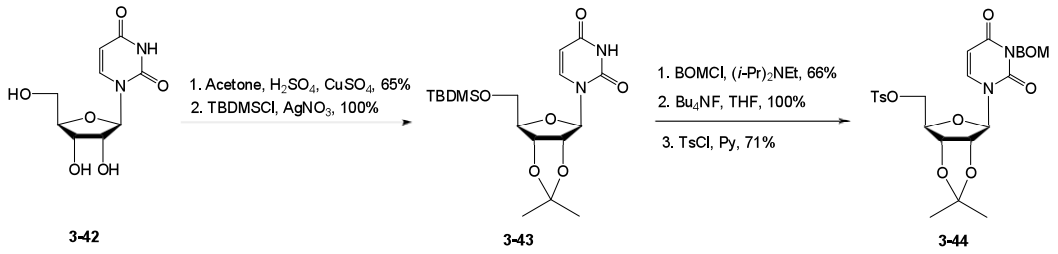


Figure 3-7. General strategy used for the synthesis of **3-39**, **3-40** and **3-41**

The synthesis of activated uridine **3-44** was performed as described previously in the literature.[13] First, treatment of uridine (**3-42**) with sulfuric acid and cupric sulfate in acetone gave the 2',3'-isopropylideneurine derivative in 65% yield after recrystallization. The product was then reacted with *tert*-butyldimethylsilyl chloride in the presence of silver nitrate to afford **3-43** in quantitative yield. Reaction of **3-43** with benzyl chloromethyl ether led to protection of amine as a benzyloxymethyl (BOM) aminal in 66% yield. The product was then desilylated by treatment with TBAF, and the product alcohol

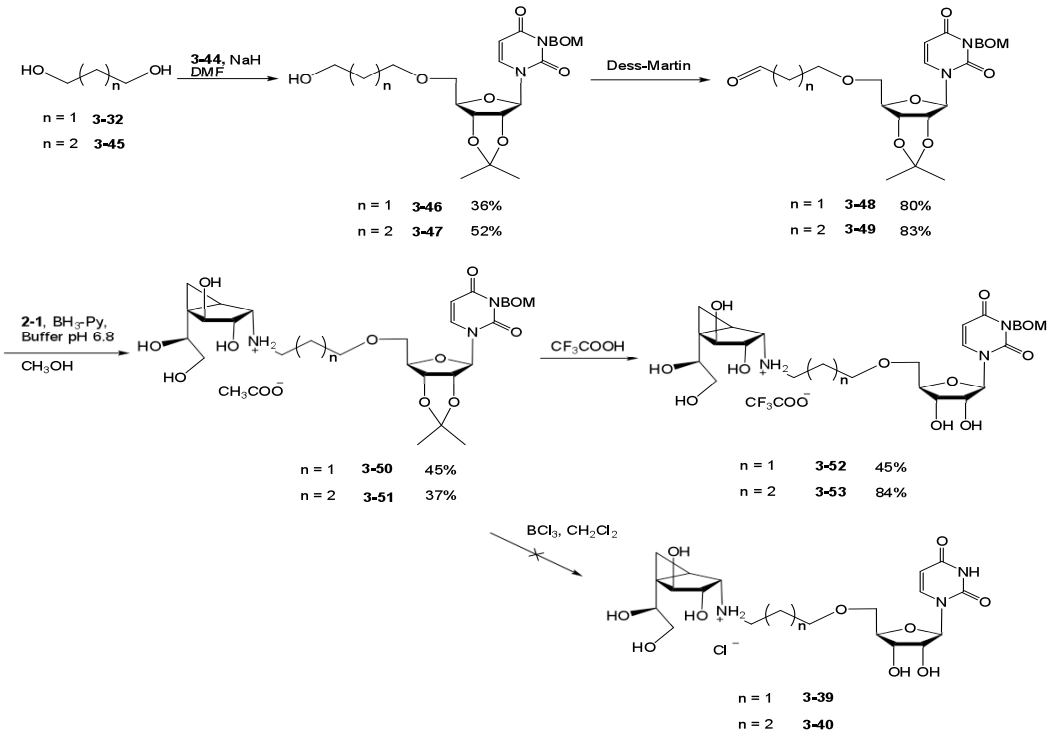
was converted to the corresponding tosylated, providing activated uridine derivative **3-44** in 71% yield over the two steps.



Scheme 3-6. Synthesis of activated uridine **3-44** as described previously.[13]

The synthesis of **3-39** and **3-40** was achieved using in the same sequence (Scheme 3-7). Reaction of tosylated uridine **3-44** with 10 equivalents of diol **3-30** or **3-45** and 1.2 equivalents of NaH in DMF afforded compounds **3-46** and **3-47** in moderate yield (36% and 52%, respectively). Attempts to improve the yield of this transformation by changing the ratio of the starting materials and the sequence of adding the reagents, were unsuccessful. The resulting primary alcohols **3-46** and **3-47** were then oxidized, in ~80% yield, into aldehydes **3-48**, **3-49**, which were treated with **2-1** and BH_3·Py in methanol and phosphate buffer (pH 6.8) to give the ammonium salts **3-50** and **3-51**, respectively. The yield of the reductive amination reaction was low and both the amine and the aldehyde were found unconsumed at the end of the reaction. However, elongation of the reaction time led to the formation of the *N*-methylated byproduct, which is

inseparable from the target compound. Therefore the decision was made to not attempt to improve the yield by increasing the reaction time.

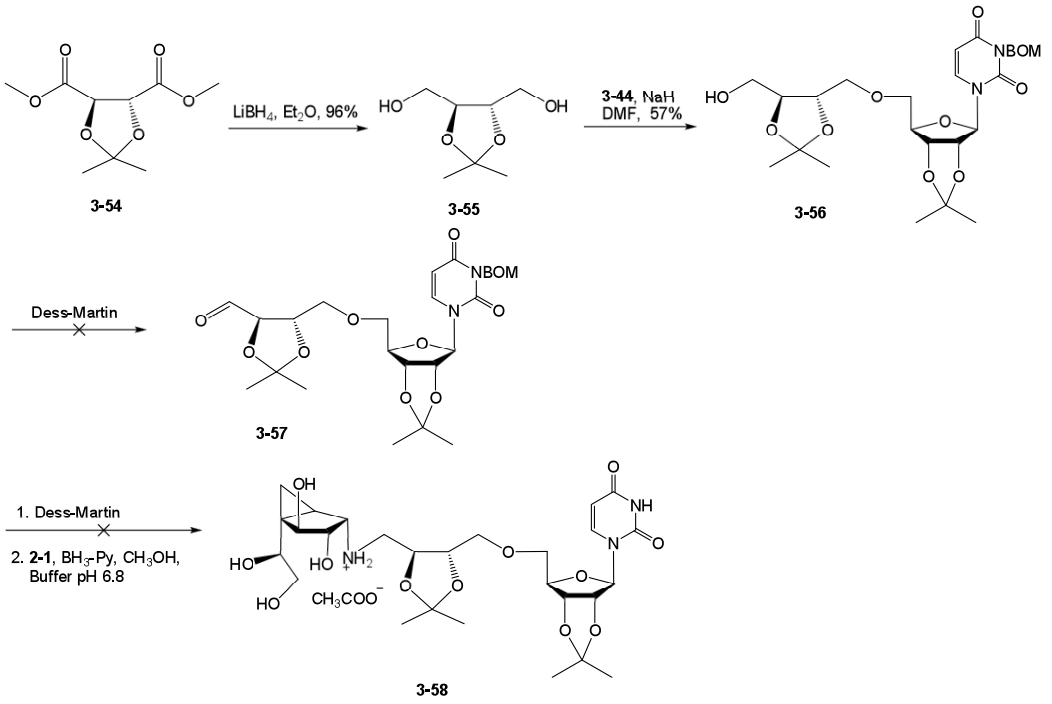


Scheme 3-7. Synthesis of **3-39** and **3-40**

It was our hope that we could obtain compounds **3-39** and **3-40** from **3-50** and **3-51**, respectively, by treatment with an acid to cleave both the isopropylidene acetal and the benzyloxymethyl (BOM) protecting group. Unfortunately, this turned out not to be successful. When the protected ammonium salts were treated with trifluoroacetic acid, only the isopropylidene acetal was cleaved and compounds **3-52** and **3-53** were obtained in moderate yield to good yield.

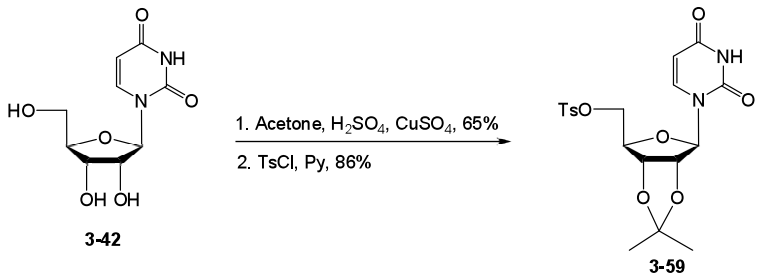
According to the literature, the BOM group on the uridine can be efficiently cleaved by treatment with boron trichloride or boron tribromide in CH_2Cl_2. However neither **3-50** nor **3-51** are soluble in CH_2Cl_2 and when reactions in other solvent systems ($CH_3CN-CH_2Cl_2$ or $THF-CH_2Cl_2$) were investigated, none of the desired product was formed. Given the problems we had in removing the BOM acetal, and the limited amount of **2-1**, we decided to not to explore the use of other approaches to **3-39** and **3-40**. Instead, we chose to test these BOM-protected compounds (**3-52** and **3-53**) against mycobacterial galactofuranosyltransferases as described in Chapter 5.

The route used for the synthesis of **3-41** is similar to that employed for **3-39**. First reduction of dimethyl 2',3'-*O*-isopropylidene-L-tartrate (**3-54**) with $LiBH_4$ in Et_2O gave diol **3-55**, which was reacted with **3-44** and NaH in DMF to afford compound **3-56** in 57% yield. Unfortunately, no aldehyde **3-57** formed by the attempted Dess–Martin oxidation. Considering the instability of the aldehyde, we also investigated if the crude oxidation mixture could be subjected to the reductive amination without purification of the intermediate aldehyde. However, when this was attempted none of the desired product was detected. (Scheme 3-8) One of the possible reasons is that the isopropylidene can migrate under the acidic Dess-Martin conditions, which gives a secondary hydroxyl group in the compound.



Scheme 3-8. Attempted synthesis of **3-41**.

Being unsuccessful in synthesizing compound **3-41**, we turned our attention to making compound **3-64** (Scheme 3-9), which has sulfur atom at the 5' position. We envisioned that this synthesis could be accomplished by reductive amination of **2-1** with the aldehyde that was derived from alcohol **3-62**. A previous paper has[22] described the synthesis of **3-62** from L-tartaric acid in six steps as described below. In addition to **3-62**, the synthesis of **3-64** required access to uridine derivative **3-59**, which is also known.[13]

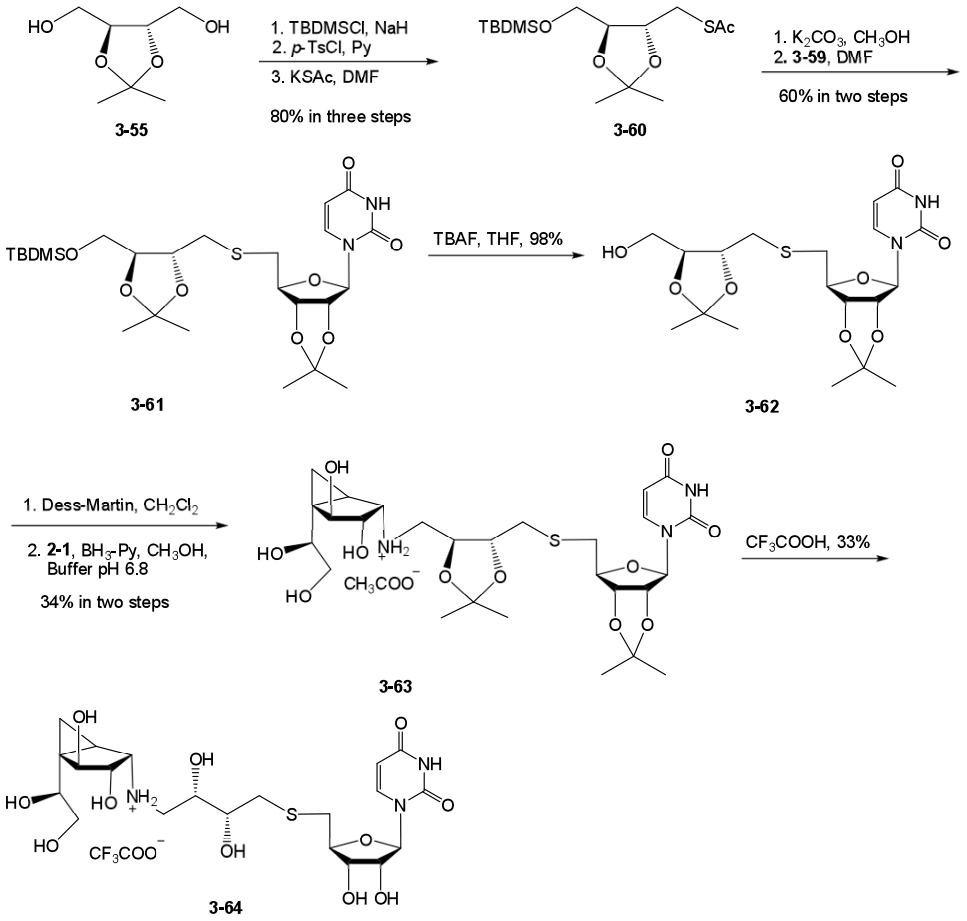


Scheme 3-9. Synthesis of tosylated uridine **3-59**[13]

The synthesis was started with the preparation 5'-*O*-tosyl derivative of the tosylated uridine derivative **3-59** (Scheme 3-9). Reaction of uridine **3-42** with acetone in the presence of sulfuric acid and cuprous sulfate afforded 2',3'-*O*-isopropylidene-uridine in 65% yield after recrystallization. The product was then reacted with *p*-toluenesulfonyl chloride in pyridine to afford **3-59** in 86% yield.

The preparation of compound **3-62** is shown in Scheme 3-10 and began with diol **3-55**, which was prepared as described above in Scheme 3-8. First, **3-55** was converted into **3-60** in 80% yield over three steps by first monosilylation, then tosylation and finally displacement with potassium thioacetate. Treatment of **3-60** with potassium carbonate in methanol gave the deacylated product, which was then treated with **3-59** in DMF to produce the intermediate **3-61** in 60% over two steps. After desilylation of **3-61**, alcohol **3-62** was obtained in 98% yield. Compound **3-62** was then oxidized with Dess–Martin periodinane in CH_2Cl_2 to give the aldehyde, which was coupled, without purification, with **2-1** and BH_3·Py

to afford a 34% yield **3-63**. The target **3-64** was obtained successfully after the cleavage of isopropylidene acetal upon treatment with trifluoroacetic acid in 33% yield.



Scheme 3-10. Synthesis of **3-64**

3.4 Conclusions

In this chapter I described the preparation of a small library of DPA and UDP-Gal*f* analogs based upon the bicyclo[3.1.0]hexane derivatives **2.1** and **2.2**. The key step in generating the products was the reductive amination between these amines and aldehydes. Although in some cases, protecting group problems led to some of the desired targets not being accessible, a small panel of compounds was nevertheless synthesized. The DPA analogs (**3-2**–**3-8**) will be tested as inhibitors of mycobacterial AraT's in the laboratory of a collaborator. The evaluation of the UDP-Gal*f* analogs (**3-25**, **3-27**, **3-29**, **3-30**, **3-31**, **3-40**, **3-41** and **3-64)** as inhibitors of mycobacterial GlfT2 is presented in Chapter 5 of this thesis.

3.5 Experimental Section

General Methods

All reagents used were purchased from commercial sources and were used without further purification. Solvents used in reactions were predried by PURESOLV-400 System from Innovative Technology Inc. Unless stated otherwise, all reactions were monitored by TLC on silica gel G-25 UV_{254} (0.25 mm, Macherey-Nagel). Spots were detected under UV light and/or by charring with acidified ethanolic anisaldehyde. Solvents were evaporated under reduced pressure and below 50 ºC (water bath). Column chromatography was performed

on silica gel 60 (40–60 μm). The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo). ^{1}H NMR spectra were recorded on VARIAN INOVA-NMR spectrometers at 400 or 500 MHz, and chemical shifts are referenced to $CDCl_3$ (7.26 ppm, $CDCl_3$) or CD_3OD (4.78 ppm, CD_3OD) or D_2O (4.78 ppm, D_2O). ^{13}C NMR APT spectra[23, 24] were recorded at 100 or 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.23 ppm, $CDCl_3$) or CD_3OD (48.9 ppm, CD_3OD). ^{1}H NMR data are reported as though they are first order, and the peak assignments were made by 2D-NMR spectroscopy (^{1}H–^{1}H COSY and HMQC). ESI-MS spectra were recorded on samples suspended in THF or CH_3OH and added NaCl. Optical rotations were measured on Perkin-Elmer 241 Polarimeter with sodium D line (589 nm) and are in units of deg·mL $(dm·g)^{-1}$. Both ^{1}H NMR and ^{13}C NMR data of the ammonium salts are reported without the signals of the acetate anion.

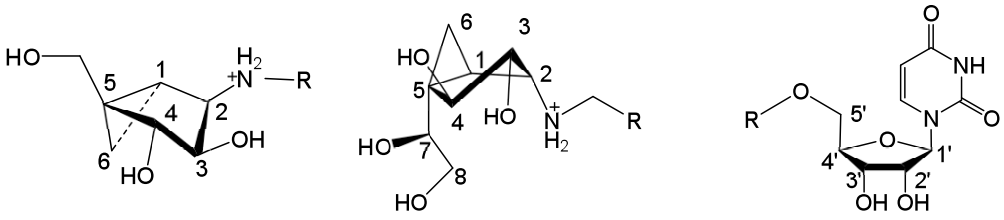
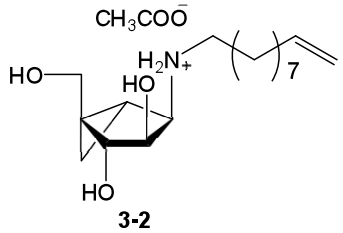


Figure 3-8. Numbering system used for NMR assignments.

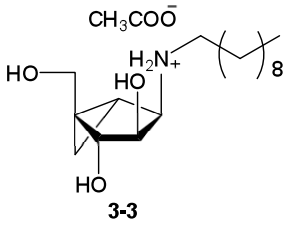


3-2

(1*S*,2*R*,3*R*,4*R*,5*R*)-*N*-(dec-9-enyl)-3,4-dihydroxy-5-(hydroxymethyl)-bicycle[3.1.0]hexan-2-ammonium acetate (3-2)

A solution of **3-15** (12 mg, 0.08 mmol) and **2-2** (12 mg, 0.07 mmol) in freshly distilled CH_3OH (2 mL) was stirred for 14 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (20 mg, 0.52 mmol) in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The resulting residue was purified by chromatography (CH_3OH–CH_2Cl_2, 1:9 → 5:1) on Iatrobeads to give **3-2** (16 mg, 59%) as a colorless oil. $[\alpha]_D$ –41.9 (*c* 1.09, CH_3OH); R_f 0.08 (CH_3OH–CH_2Cl_2 1:1); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 5.80 (ddt, 1 H, *J* = 6.7, 10.2, 17.0 Hz, C<u>H</u>=CH_2), 4.97 (ddt, 1 H, *J* = 1.6, 2.2, 17.0 Hz, CH=C<u>H</u>$_2$), 4.90 (ddt, 1 H, *J* = 1.3, 2.2, 10.2 Hz, CH=C<u>H</u>$_2$), 4.32 (d, 1 H, *J* = 7.0 Hz, H-4), 4.02 (d, 1 H, *J* = 11.7 Hz, C<u>H</u>$_2$OH), 3.82 (dd, 1 H, *J* = 6.6, 7.0 Hz, H-3), 3.48 (d, 1 H, *J* = 6.6 Hz, H-2), 3.22 (d, 1 H, *J* = 11.7 Hz, C<u>H</u>$_2$OH), 3.12–3.00 (m, 2 H, NHC<u>H</u>$_2$), 2.07–2.01 (m, 2 H, C<u>H</u>$_2$CH=CH_2), 1.76–1.64 (m, 2 H, NHCH_2C<u>H</u>$_2$), 1.59 (dd, 1 H, *J* = 3.8, 8.8 Hz, H-1), 1.42–1.28 (m, 10 H, 5 × C<u>H</u>$_2$), 0.83 (dd, 1 H, *J* = 3.8, 5.9 Hz, H-6), 0.70 (dd, 1 H, *J* = 5.9, 8.8 Hz, H-6'); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 140.1 (<u>C</u>H=CH_2),

114.8 (CH=$\underline{C}H_2$), 77.8 (C-4), 76.0 (C-3), 63.4 ($\underline{C}H_2OH$), 60.5 (C-2), 47.3 ($NH\underline{C}H_2$), 34.9 (C-5), 34.8 ($\underline{C}H_2CH=CH_2$), 30.4 ($\underline{C}H_2$), 30.2 ($\underline{C}H_2$), 30.1 (2 × C, $\underline{C}H_2$), 27.7 ($\underline{C}H_2$), 27.4 ($\underline{C}H_2$), 20.9 (C-1), 10.9 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{17}H_{32}NO_3$: 298.2377. Found: 298.2379.

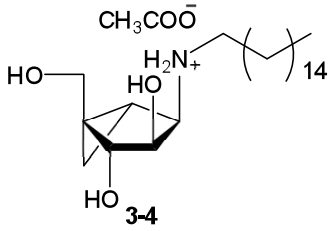


3-3

(1*S*,2*R*,3*R*,4*R*,5*R*)-*N*-decyl-3,4-dihydroxy-5-(hydroxymethyl)-bicyclo[3.1.0]hexan-2-ammonium acetate (3-3)

A solution of decanal (**3-21**, 7 mg, 0.045 mmol) and **2-2** (7.2 mg, 0.045 mmol) in freshly distilled CH_3OH (2 mL) was stirred for 14 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (20 mg, 0.52 mmol) in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The resulting residue was purified by chromatography (CH_3OH–CH_2Cl_2 from 1:9 to 5:1) on Iatrobeads to give **3-3** (12 mg, 74%) as a colorless oil. R_f 0.09 (CH_3OH–CH_2Cl_2 1:1); $[\alpha]_D$ –44.3 (*c* 0.98, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.30 (d, 1 H, *J* = 7.0 Hz, H-4), 4.00 (d, 1 H, *J* = 11.6 Hz, $C\underline{H}_2OH$), 3.78 (dd, 1 H, *J* = 6.6, 7.0 Hz, H-3), 3.39 (d, 1 H, *J* = 6.6 Hz, H-2), 3.20 (d, 1 H, *J* = 11.6 Hz, $C\underline{H}_2OH$), 3.06–2.93 (m, 2 H, $NHC\underline{H}_2$),

1.72–1.61 (m, 2 H, $NHCH_2C\underline{H}_2$), 1.56 (dd, 1 H, *J* = 3.8, 8.8 Hz, H-1), 1.42–1.28 (m, 14 H, 7 × $C\underline{H}_2$), 0.88 (t, 3 H, *J* = 7.1 Hz, $C\underline{H}_3$), 0.80 (dd, 1 H, *J* = 3.8, 5.8 Hz, H-6), 0.66 (dd, 1 H, *J* = 5.8, 8.8 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 77.8 (C-4), 76.2 (C-3), 63.6 ($\underline{C}H_2OH$), 60.5 (C-2), 47.6 ($NH\underline{C}H_2$), 34.8 (C-5), 33.0 ($\underline{C}H_2$), 30.6 (2 × C, 2 × $\underline{C}H_2$), 30.4 (2 × C, 2 × $\underline{C}H_2$), 27.8 (2 × C, 2 × $\underline{C}H_2$), 23.7 ($\underline{C}H_2$), 21.5 (C-1), 14.4 ($\underline{C}H_3$), 10.9 (C-6). HRMS (ESI) *m/z* Calcd for ($M–CH_3COO^–$) $C_{17}H_{34}NO_3$: 300.2533. Found: 300.2536.

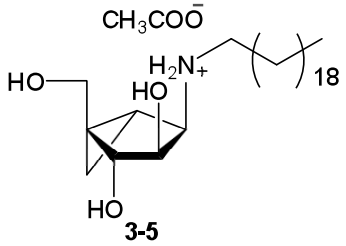


3-4

(1*S*,2*R*,3*R*,4*R*,5*R*)-*N*-hexadecyl-3,4-dihydroxy-5-(hydroxymethyl)-bicyclo[3.1.0]hexan-2-ammonium acetate (3-4)

A solution of palmitaldehyde (**3-16**, 12 mg, 0.05 mmol) and **2-2** (7.2 mg, 0.048 mmol) in CH_2Cl_2 (1.5 mL) and freshly distilled CH_3OH (1.5 mL) was stirred for 14 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (20 mg, 0.52 mmol) in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The resulting residue was purified by chromatography ($CH_3OH–CH_2Cl_2$ from 1:9 to 5:1) on Iatrobeads to give **3-4** (12 mg, 57%) as a colorless oil. $[\alpha]_D$ –28.4 (*c*

0.97, CH_3OH); R_f 0.09 (CH_3OH–CH_2Cl_2 1:1); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.31 (d, 1 H, *J* = 7.0 Hz, H-4), 4.03 (d, 1 H, *J* = 11.6 Hz, C$\underline{H}_2$OH), 3.82 (dd, 1 H, *J* = 6.6, 7.0 Hz, H-3), 3.47 (d, 1 H, *J* = 6.6 Hz, H-2), 3.23 (d, 1 H, *J* = 11.6 Hz, C$\underline{H}_2$OH), 3.12–2.97 (m, 2 H, NHC$\underline{H}_2$), 1.76–1.64 (m, 2 H, NHCH$_2$C$\underline{H}_2$), 1.59 (dd, 1 H, *J* = 3.8, 8.9 Hz, H-1), 1.42–1.28 (m, 26 H, 13 × C$\underline{H}_2$), 0.89 (t, 3 H, *J* = 6.8 Hz, C$\underline{H}_3$), 0.83 (dd, 1 H, *J* = 3.8, 5.9 Hz, H-6), 0.66 (dd, 1 H, *J* = 5.9, 8.9 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 77.8 (C-4), 76.0 (C-3), 63.4 (C$\underline{H}_2$OH), 60.5 (C-2), 47.4 (NHC$\underline{H}_2$), 34.9 (C-5), 33.1 (C$\underline{H}_2$), 30.8 (2 × C, 2 × C$\underline{H}_2$), 30.7 (5 × C, 5 × C$\underline{H}_2$), 30.6 (C$\underline{H}_2$), 30.5 (C$\underline{H}_2$), 30.3 (C$\underline{H}_2$), 27.8 (C$\underline{H}_2$), 27.4 (C$\underline{H}_2$), 23.7 (C$\underline{H}_2$), 21.0 (C-1), 14.5 (C$\underline{H}_3$), 11.0 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{23}H_{46}NO_3$: 384.3472. Found: 384.3474.

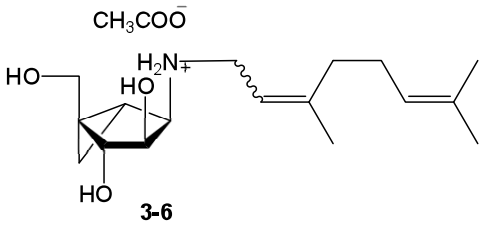


3-5

(1*S*,2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-*N*-icosyl-bicyclo[3.1.0]hexan-2-ammonium acetate (3-5)

A solution of aldehyde **3-17** (14 mg, 0.046 mmol) and **2-2** (7.4 mg, 0.046 mmol) in CH_2Cl_2 (2 mL) and CH_3OH (2 mL) was stirred for 14 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (20 mg, 0.52 mmol)

in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The resulting residue was purified by chromatography (CH_3OH–CH_2Cl_2 from 1:9 to 5:1) on Iatrobeads to give **3-5** (17.5 mg, 75%) as a colorless oil. R_f 0.08 (CH_3OH–CH_2Cl_2 1:1); $[\alpha]_D$ –23.1 (*c* 0.80, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 4.32 (d, 1 H, *J* = 7.0 Hz, H-4), 4.03 (d, 1 H, *J* = 11.6 Hz, C$\underline{H}_2$OH), 3.83 (dd, 1 H, *J* = 6.5, 7.0 Hz, H-3), 3.50 (d, 1 H, *J* = 6.5 Hz, H-2), 3.22 (d, 1 H, *J* = 11.6 Hz, C$\underline{H}_2$OH), 3.10–3.05 (m, 2 H, NHC$\underline{H}_2$), 1.75–1.68 (m, 2 H, NHCH$_2$C$\underline{H}_2$), 1.60 (dd, 1 H, *J* = 3.8, 8.8 Hz, H-1), 1.42–1.22 (m, 34 H, 17 × C$\underline{H}_2$), 0.89 (t, 3 H, *J* = 7.7, C$\underline{H}_3$), 0.84 (dd, 1 H, *J* = 3.8, 6.0 Hz, H-6), 0.71 (dd, 1 H, *J* = 6.0, 8.8 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 77.8 (C-4), 76.0 (C-3), 63.3 (C$\underline{H}_2$OH), 60.5 (C-2), 47.3 (NH$\underline{C}$H$_2$), 34.9 (C-5), 33.1 ($\underline{C}H_2$), 30.8 (10 × C, 10 × $\underline{C}H_2$), 30.6 ($\underline{C}H_2$), 30.5 ($\underline{C}H_2$), 30.4 ($\underline{C}H_2$), 30.3 ($\underline{C}H_2$), 27.7 ($\underline{C}H_2$), 27.3 ($\underline{C}H_2$), 23.7 ($\underline{C}H_2$), 21.5 (C-1), 14.4 ($\underline{C}H_3$), 10.9 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{27}H_{54}NO_3$: 440.4098. Found: 440.4090.

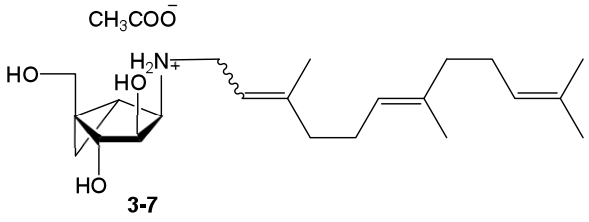


3-6

(1*S*,2*R*,3*R*,4*R*,5*R*)-*N*-(3,7-dimethylocta-2,6-dienyl)-3,4-dihydroxy-5-(hydroxymethyl)bicyclo[3.1.0]hexan-2-ammonium acetate (3-6)

A solution of aldehyde **3-18** (7.0 mg, 0.046 mmol) and **2-2** (6.7 mg, 0.042 mmol) in freshly distilled CH_3OH (2 mL) was stirred for 14 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (20 mg, 0.52 mmol) in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The residue was purified by chromatography (CH_3OH–CH_2Cl_2 1:9 → 5:1) on Iatrobeads to give **3-6** (8.9 mg, 60%) as a colorless oil (1:1 mixture of stereoisomers). R_f 0.10 (CH_3OH–CH_2Cl_2 1:1); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.31–5.28 (m, 1 H, C<u>H</u>=C), 5.14–5.09 (m, 1 H, C<u>H</u>=C), 4.31 (d, 0.5 H, *J* = 5.7 Hz, H-4a), 4.29 (d, 0.5 H, *J* = 6.1 Hz, H-4b), 4.02 (d, 0.5 H, *J* = 11.6 Hz, C<u>H</u>$_2$OHa), 4.00 (d, 0.5 H, *J* = 11.6 Hz, C<u>H</u>$_2$OHb), 3.80–3.76 (m, 1 H, H-3a, H-3b), 3.71–3.58 (m, 2 H, NHC<u>H</u>$_2$a, NHC<u>H</u>$_2$b), 3.38 (d, 0.5 H, *J* = 6.5 Hz, H-2a), 3.37 (d, 0.5 H, *J* = 6.6 Hz, H-2b), 3.22 (d, 1 H, *J* = 11.6 Hz, C<u>H</u>$_2$OHa, C<u>H</u>$_2$OHb), 2.21–2.09 (m, 4 H, CH_2CH_2), 1.81 (s, 1.5 H, CH_3a), 1.76 (s, 1.5 H, CH_3b), 1.68 (s, 1.5 H, CH_3a), 1.67 (s, 1.5 H, CH_3b), 1.62 (s, 1.5 H, CH_3a), 1.61 (s, 1.5 H, CH_3b), 1.58 (dd, 0.5 H, *J* = 3.7, 8.7 Hz, H-1a), 1.54 (dd, 0.5

H, *J* = 3.8, 8.8 Hz, H-1b), 0.83–0.80 (m, 1 H, H-6a, H-6b), 0.68–0.64 (m, 1 H, H-6a, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 145.6 (CH=<u>C</u>a), 145.3 (CH=<u>C</u>b), 133.3 (CH=<u>C</u>a), 132.9 (CH=<u>C</u>b), 124.8 (<u>C</u>H=Cb), 124.6 (<u>C</u>H=Ca), 118.0 (<u>C</u>H=Ca), 116.9 (<u>C</u>H=Cb), 77.8 (C-4a), 77.7 (C-4b), 76.2 (C-3a, C-3b), 63.6 (<u>C</u>H_2OHa, <u>C</u>H_2OHb), 59.4 (C-2a, C-2b), 44.9 (NH<u>C</u>H_2a, NH<u>C</u>H_2b), 40.7 (<u>C</u>H_2b), 34.7 (C-5a), 34.6 (C-5b), 33.1 (<u>C</u>H_2a), 27.4 (<u>C</u>H_2a), 27.2 (<u>C</u>H_2b), 25.9 (<u>C</u>H_3), 23.7 (<u>C</u>H_3), 21.9 (C-1a), 21.9 (C-1b), 17.8 (<u>C</u>H_3), 16.9 (<u>C</u>H_3), 10.9 (C-6a), 10.8 (C-6b). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{17}H_{30}NO_3$: 296.2220. Found: 296.2217.

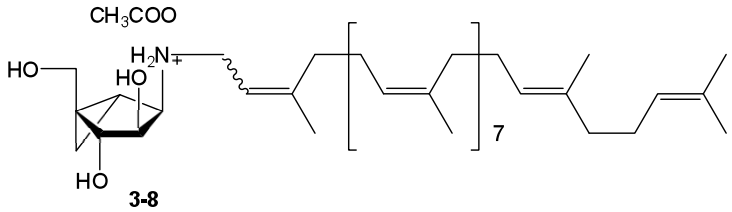


3-7

(1*S*,2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-*N*-((6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)bicyclo[3.1.0]hexan-2-ammonium acetate (3-7)

A solution of aldehyde **3-19** (16 mg, 0.07 mmol) and **2-2** (12 mg, 0.07 mmol) in freshly distilled CH_3OH (2 mL) was stirred for 14 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (20 mg, 0.52 mmol) in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The resulting residue was purified by

chromatography (CH_3OH–CH_2Cl_2 from 1:9 to 5:1) on Iatrobeads to give **3-7** (15 mg, 47%) as a colorless oil (7:3 mixture of stereoisomers). R_f 0.12 (CH_3OH–CH_2Cl_2 1:1); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.31–5.27 (m, 1 H, C<u>H</u>=C), 5.16–5.00 (m, 2 H, 2 × C<u>H</u>=C), 4.28 (d, 0.7 H, *J* = 6.0 Hz, H-4a), 4.27 (d, 0.3 H, *J* = 5.6 Hz, H-4b), 3.98 (d, 0.7 H, *J* = 11.6 Hz, C<u>H$_2$</u>OHa), 3.97 (d, 0.3 H, *J* = 11.6 Hz, C<u>H$_2$</u>OHb), 3.73–3.69 (m, 1 H, H-3a, H-3b), 3.57–3.47 (m, 2 H, NHC<u>H$_2$</u>a, NHC<u>H$_2$</u>b), 3.24–3.18 (m, 2 H, H-2a, H-2b, C<u>H$_2$</u>OHa, C<u>H$_2$</u>OHb), 2.18–1.96 (m, 8 H, 2 × CH_2CH_2), 1.79 (s, 0.9 H, CH_3b), 1.74 (s, 2.1 H, CH_3a), 1.73 (s, 0.9 H, CH_3b), 1.67 (s, 0.9 H, CH_3b), 1.66 (s, 2.1 H, CH_3a), 1.62 (s, 0.9 H, CH_3b), 1.60 (s, 2.1 H, CH_3a), 1.59 (s, 2.1 H, CH_3a), 1.52 (dd, 0.7 H, *J* = 3.9, 8.6 Hz, H-1a), 1.49 (dd, 0.3 H, *J* = 3.7, 8.6 Hz, H-1b), 0.79–0.76 (m, 1 H, H-6a, H-6b), 0.60–0.56 (m, 1 H, H-6a, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 136.8 (CH=<u>C</u>a), 136.7 (CH=<u>C</u>b), 136.5 (CH=<u>C</u>a, CH=<u>C</u>b), 133.2 (CH=<u>C</u>b), 132.1 (CH=<u>C</u>a), 125.6 (<u>C</u>H=Ca), 125.5 (<u>C</u>H=Cb), 125.4 (<u>C</u>H=Ca), 125.3 (<u>C</u>H=Cb), 124.9 (<u>C</u>H=Ca), 124.8 (<u>C</u>H=Cb), 77.9 (C-4a, C-4b), 76.5 (C-3a, C-3b), 64.1 (<u>C</u>H_2OHa, <u>C</u>H_2OHb), 59.6 (C-2a), 59.5(C-2b), 45.6 (NH<u>C</u>H_2a), 45.5 (NH<u>C</u>H_2b), 41.0 (<u>C</u>H_2b), 40.9 (<u>C</u>H_2a), 40.7 (<u>C</u>H_2b), 34.3 (C-5a), 34.2 (C-5b), 33.0 (<u>C</u>H_2a), 27.8 (<u>C</u>H_2a), 27.6, (<u>C</u>H_2b), 27.3 (<u>C</u>H_2a), 27.2 (<u>C</u>H_2b), 26.0 (<u>C</u>H_3b), 25.9 (<u>C</u>H_3a), 23.8 (<u>C</u>H_3a), 23.7 (<u>C</u>H_3b), 23.4 (C-1b), 23.1 (C-1a), 17.8 (<u>C</u>H_3b), 17.7 (<u>C</u>H_3b), 16.6 (<u>C</u>H_3a), 16.1 (<u>C</u>H_3a), 10.7 (C-6a), 10.6 (C-6b). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{22}H_{38}NO_3$: 364.2846. Found: 364.2846.

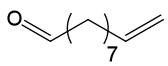


3-8

(1*S*,2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-*N*-((6*Z*, 10*Z*, 14*Z*, 18*Z*, 22*Z*, 26*Z*, 30*Z*, 34*E*)-3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenyl)bicyclo[3.1.0]hexan-2-ammonium acetate (3-8)

A solution of aldehyde **3-20** (9.2 mg, 0.013 mmol) and **2-2** (2.1 mg, 0.013 mmol) in CH_2Cl_2 (1.5 mL) and freshly distilled CH_3OH (1.5 mL) was stirred for 20 h under argon, before being cooled to –30 ºC. To this mixture was added $NaBH_4$ (10 mg, 0.26 mmol) in one portion and the solution was stirred for 5 min before warming to rt and stirring for an additional 15 min. HOAc was added to the reaction mixture until the pH was 5 and then the solution was concentrated. The residue was purified by chromatography (CH_3OH–CH_2Cl_2 1:9 → 5:1) on Iatrobeads to give **3-8** (10 mg, 84%) as a white foam (3:2 mixture of stereoisomers). R_f 0.09 (CH_3OH–CH_2Cl_2 1:1); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.32–5.27 (m, 1 H, C<u>H</u>=C), 5.18–5.05 (m, 9 H, 9 × C<u>H</u>=C), 4.29 (d, 0.6 H, *J* = 7.2 Hz, H-4a), 4.28 (d, 0.4 H, *J* = 7.0 Hz, H-4b), 4.02 (d, 0.6 H, *J* = 11.6 Hz, C<u>H</u>$_2$OHa), 4.00 (d, 0.4 H, *J* = 11.6 Hz, C<u>H</u>$_2$OHb), 3.79–3.74 (m, 1 H, H-3a, H-3b), 3.69–3.55 (m, 2 H, NHC<u>H</u>$_2$a, NHC<u>H</u>$_2$b), 3.35 (d, 0.6 H, *J* = 6.5 Hz, H-2a), 3.32 (d,

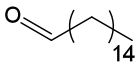
0.4 H, *J* = 6.6 Hz, H-2b), 3.21 (d, 0.6 H, *J* = 11.6 Hz, C$\underline{H}_2$OHa), 3.20 (d, 0.4 H, *J* = 11.6 Hz, C$\underline{H}_2$OHb), 2.15–1.94 (m, 36 H, 9 × $CH_2\underline{C}H_2$), 1.81–1.58 (m, 33 H, 11 × C$\underline{H}_3$), 1.57–1.50 (m, 1 H, H-1a, H-1b), 0.82–0.78 (m, 1 H, H-6a, H-6b), 0.66–0.61 (m, 1 H, H-6a, H-6b); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 137.1–135.8 (CH=$\underline{C}$), 126.1–125.4 ($\underline{C}$H=C), 77.8 (C-4), 76.2 (C-3), 63.7 ($\underline{C}H_2$OH), 59.5 (C-2), 45.1 (NH$\underline{C}H_2$), 41.1–40.8 ($\underline{C}H_2$), 34.6 (C-5a), 34.5 (C-5b), 33.4-32.9 ($\underline{C}H_2$), 27.8-27.2 ($\underline{C}H_2$), 25.9 ($\underline{C}H_3$), 23.9-23.7 ($\underline{C}H_3$, C-1), 17.8 ($\underline{C}H_3$), 16.7 ($\underline{C}H_3$), 16.6 ($\underline{C}H_3$), 10.8 (C-6a), 10.7 (C-6b). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{57}H_{94}NO_3$: 840.7228. Found: 840.7216.



3-15

Dec-9-enal (3-15)

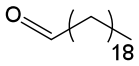
A solution of dec-9-en-1-ol (**3-9**, 32 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) was added to a solution of Dess–Martin periodinane (87 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) at 0 ºC. The mixture was stirred at 0 ºC for 2 h and then was poured into a cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to give a residue that was quickly purified by chromatography (EtOAc–Hexane 1:15) affording **3-15** (22 mg, 71%) as a colorless oil; R_f 0.43 (EtOAc–Hexane 1:10). The aldehyde was used in the next step immediately after purification.



3-16

Palmitaldehyde (3-16)

A solution of hexadecan-1-ol (**3-10**, 48 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) was added to a solution of Dess–Martin periodinane (84 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) at 0 ºC. The mixture was stirred for 2 h at 0 °C and was poured into a cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to a residue that was quickly purified by chromatography (EtOAc–Hexane 1:15) to give **3-16** (37.5 mg, 79%) as a colorless oil; R_f 0.46 (EtOAc–Hexane 1:10). The aldehyde was used in the next step immediately after purification.

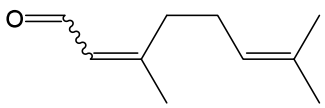


3-17

Icosanal (3-17)

A solution of icosan-1-ol (**3-11**, 48 mg, 0.16 mmol) in THF (3 mL) was added to a solution of Dess–Martin periodinane (68 mg, 0.16 mmol) in CH_2Cl_2 (4 mL) at 0 ºC. The mixture was stirred for 2 h at 0 °C and then was poured into a cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with brine and dried ($MgSO_4$) and concentrated. The resulting residue was quickly purified by chromatography (EtOAc–Hexane 1:19) to give **3-17** (40 mg, 85%) as a white

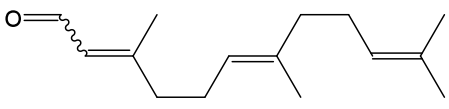
solid; R_f 0.46 (EtOAc–Hexane 1:19). The aldehyde was used in the next step immediately after purification.



3-18

3,7-dimethylocta-2,6-dienal (3-18)

A solution of nerol (**3-12**, 1:1 mixture of stereoisomers, 35 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was added to a solution of Dess–Martin periodinane (96 mg, 0.23 mmol) in CH_2Cl_2 (5 mL) at 0 ºC. The mixture was stirred for 2 h at 0 °C and then was poured into a cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with brine and dried ($MgSO_4$) and concentrated. The resulting residue was quickly purified by chromatography (EtOAc–Hexane 1:15) to give **3-18** (28 mg, 82.3%) as a colorless oil; R_f 0.36 (EtOAc–Hexane 1:10). The aldehyde was used in the next step immediately after purification.

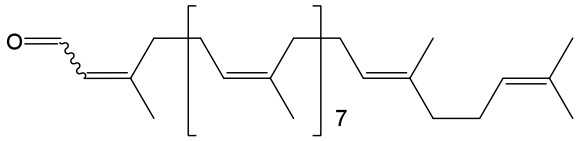


3-19

(6*E*)-3,7,11-trimethyldodeca-2,6,10-trienal (3-19)

A solution of farnesol (**3-13**, 44 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) was added to a solution of Dess–Martin periodinane (84 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) at 0 ºC. The mixture was stirred for 2 h at 0 °C and then was poured into a cold saturated

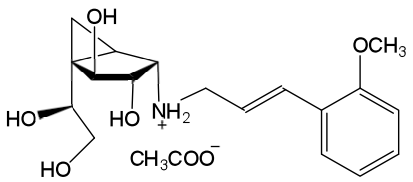
aq. $NaHCO_3$ solution. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was quickly purified by chromatography (EtOAc–Hexane 1:15) to give **3-19** (28 mg, 64%) as a colorless oil. R_f 0.39 (EtOAc–Hexane 1:10). The aldehyde was used in the next step immediately after purification.



3-20

(6*Z*, 10*Z*, 14*Z*, 18*Z*, 22*Z*, 26*Z*, 30*Z*, 34*E*)-3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenal (3-20)

A solution of decaprenol (**3-14**, 10 mg, 0.014 mmol) in hexane (0.5 mL) was added to a solution of Dess–Martin periodinane (7 mg, 0.016 mmol) in CH_2Cl_2 (5 mL) at 0 ºC. The mixture was stirred for 2 h at 0 °C and was poured into a cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with brine and dried ($MgSO_4$) and concentrated. The resulting residue was quickly purified by chromatography (EtOAc–Hexane 1:19) to give **3-20** (9.2 mg, 92%) as a white solid. R_f 0.20 (EtOAc–Hexane 1:19). The product aldehyde was used in the next step immediately after purification.

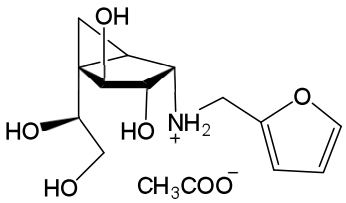


3-25

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-3,4-dihydroxy-*N*-((*E*)-3-(2-methoxyphenyl)allyl)bicyclo[3.1.0]hexan-2-ammonium acetate (3-25)

A solution of **2-1** (8.8 mg, 0.046 mmol) and (*E*)-3-(2-methoxyphenyl) acrylaldehyde (**3-24**, 7.5 mg, 0.046 mmol) in freshly distilled CH_3OH (2 mL) was stirred at rt for 1 h, before being cooled to –30 ºC. $NaBH_4$ (5.3 mg, 0.14 mmol) was added, and the solution was stirred for 5 min before being warmed to rt followed by stirring for an additional 10 min. The solution was then acidified with HOAc to pH 5 and concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:10) to give **3-25** (14 mg, 77%) as a white foam. R_f 0.36 (CH_3OH–NH_4OH 40:1); $[\alpha]_D$ +31.8 (*c* 1.41, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 7.47 (dd, 1 H, *J* = 1.3, 7.6 Hz, Ar), 7.27 (ddd, 1 H, *J* = 1.3, 7.4, 8.6 Hz, Ar), 7.09 (d, 1 H, *J* = 16.0 Hz, =C<u>H</u>Ph), 6.98 (d, 1 H, *J* = 8.4 Hz, Ar), 6.92 (dd, 1 H, *J* = 7.4, 7.6 Hz, Ar), 6.33 (ddd, 1 H, *J* = 7.0, 7.2, 16.0 Hz, CH_2C<u>H</u>=), 4.29 (d, 1 H, *J* = 6.8 Hz, H-4), 3.92 (dd, 1 H, *J* = 5.9, 6.3 Hz, H-7), 3.88–3.77 (m, 6 H, NHC<u>H</u>$_2$, OC<u>H</u>$_3$, H-3), 3.67 (dd, 1 H, *J* = 5.9, 11.1 Hz, H-8), 3.51 (dd, 1 H, *J* = 6.3, 11.2 Hz, H-8), 3.49 (d, 1 H, *J* = 6.3 Hz, H-2), 1.76 (dd, 1 H, *J* = 3.8, 8.8 Hz, H-1), 0.91 (dd, 1 H, *J* = 6.1, 8.8 Hz, H-6), 0.78 (dd, 1 H, *J* = 3.8, 6.1 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 158.5 (Ar), 133.7

(CH=), 130.8 (Ar), 128.4 (Ar), 125.9 (Ar), 121.7 (Ar), 121.5 (CH=), 112.2 (Ar), 79.7 (C-4), 76.7 (C-3), 72.2 (C-7), 66.0 (C-8), 59.9 (C-2), 56.0 (CH_3), 49.1 ($NHCH_2$), 35.7 (C-5), 20.5 (C-1), 8.9 (C-6); HRMS (ESI) *m/z* Calcd for ($M–CH_3COO^-$) $C_{18}H_{26}NO_5$: 336.1805. Found: 336.1806.

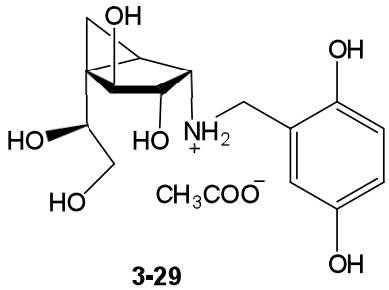


3-27

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-*N*-(furan-2-ylmethyl)-3,4-dihydroxy-bicyclo[3.1.0]hexan-2-ammonium acetate (3-27)

A solution of **2-1** (6.7 mg, 0.035 mmol) and furan-2-carbaldehyde (**3-26**, 3.4 mg, 0.035 mmol) in freshly distilled CH_3OH (2 mL) was stirred at rt for 1 h, before being cooled to –30 ºC. $NaBH_4$ (5.0 mg, 0.13 mmol) was added, and the solution was stirred for 5 min before being warmed to rt followed by stirring for an additional 10 min. The solution was then acidified with HOAc to pH 5 and concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:10) to give **3-27** (6.2 mg, 53%) as a white foam. R_f 0.27 (pure CH_3OH); $[\alpha]_D$ +55.0 (*c* 0.35, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 7.43 (d, 1 H, *J* = 1.9 Hz, furan CH), 6.34 (dd, 1 H, *J* = 1.9, 3.2 Hz, furan CH), 6.26 (d, 1 H, *J* = 3.2 Hz, furan CH), 4.19 (d, 1 H, *J* = 6.9 Hz, H-4), 3.87 (d, 1 H, *J* = 14.4 Hz, NCH_2), 3.79 (dd, 1 H, *J* = 6.1, 6.3 Hz, H-7), 3.76 (d, 1

H, *J* = 11.4 Hz, NC<u>H</u>$_2$), 3.65 (dd, 1 H, *J* = 6.1, 11.1 Hz, H-8), 3.59 (dd, 1 H, *J* = 6.2, 6.9 Hz, H-3), 3.49 (dd, 1 H, *J* = 6.3, 11.1 Hz, H-8), 2.99 (d, 1 H, *J* = 6.2 Hz, H-2), 1.50 (dd, 1 H, *J* = 5.1, 7.8 Hz, H-1), 0.68–0.66 (m, 2 H, 2 × H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 154.5 (<u>C</u>=CH), 143.3 (furan <u>C</u>H), 111.2 (furan <u>C</u>H), 108.4 (furan <u>C</u>H), 80.1 (C-4), 77.7 (C-3), 73.3 (C-7), 66.1 (C-8), 59.2 (C-2), 45.1 (N<u>C</u>H$_2$), 34.4 (C-5), 23.9 (C-1), 8.6 (C-6); HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{13}H_{20}NO_5$: 270.1336. Found: 270.1337.

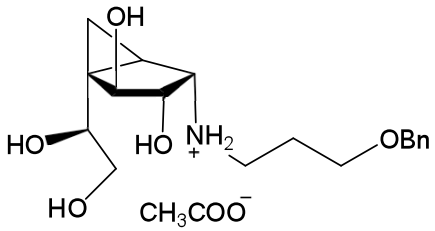


3-29

(1*R*,2*S*,3*S*,4*S*,5*S*)-*N*-(2,5-dihydroxybenzyl)-5-(1,2-dihydroxyethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium acetate (3-29)

A solution of **2-1** (11 mg, 0.053 mmol) and 2,5-dihydroxybenzaldehyde (**3-28**, 7.3 mg, 0.053 mmol) in freshly distilled CH_3OH (2 mL) was stirred at rt for 1 h. To this mixture was added phosphate buffer (0.1 M, pH 6.8, 0.2 mL) and the solution was cooled to 0 ºC before BH_3·pyridine (20 μL, 0.16 mmol) was added. The reaction mixture was then warmed to rt and stirred overnight before being acidified with HOAc to pH 5 and concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:10) to give

compound **3-29** (14.4 mg, 73%) as a white foam. R_f 0.46 (CH_3OH–NH_4OH 20:1); $[\alpha]_D$ +31.5 (*c* 0.32, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 6.77 (d, 1 H, *J* = 2.9 Hz, Ar), 6.71 (d, 1 H, *J* = 8.6 Hz, Ar), 6.66 (dd, 1 H, *J* = 2.9, 8.6 Hz, Ar), 4.29 (d, 1 H, *J* = 7.0 Hz, H-4), 4.22 (d, 1 H, *J* = 13.2 Hz, C$\underline{H}_2$N), 4.09 (d, 1 H, *J* = 13.2 Hz, C$\underline{H}_2$N), 3.97 (dd, 1 H, *J* = 5.7, 6.5 Hz, H-7), 3.76 (dd, 1 H, *J* = 6.6, 7.0 Hz, H-3), 3.66 (dd, 1 H, *J* = 5.7, 11.1 Hz, H-8), 3.66 (dd, 1 H, *J* = 6.5, 11.1 Hz, H-8), 3.29 (d, 1 H, *J* = 6.6 Hz, H-2), 1.79 (dd, 1 H, *J* = 4.0, 8.6 Hz, H-1), 0.84 (dd, 1 H, *J* = 5.9, 8.6 Hz, H-6), 0.73 (dd, 1 H, *J* = 4.0, 5.9 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 151.5 (Ar), 150.4 (Ar), 121.3 (Ar), 118.4 (Ar), 117.8 (Ar), 117.3 (Ar), 79.6 (C-4), 76.5 (C-3), 72.1 (C-7), 66.0 (C-8), 59.5 (C-2), 48.1 ($\underline{C}H_2N$), 35.5 (C-5), 20.8 (C-1), 8.6 (C-6). HRMS (ESI) *m/z* Calcd for ($M–CH_3COO^-$) $C_{15}H_{22}NO_6$: 312.1442. Found: 312.1442.

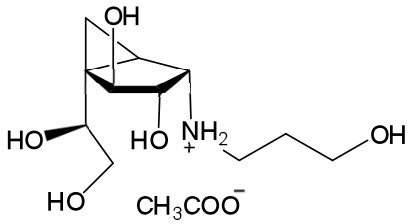


3-34

(1*R*,2*S*,3*S*,4*S*,5*S*)-*N*-(3-(benzyloxy)propyl)-5-(1,2-dihydroxyethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium acetate (3-34)

A solution of **2-1** (9.0 mg, 0.047 mmol) and **3-33** (7.8 mg, 0.047 mmol) in fresh CH_3OH (2 mL) was stirred at rt for 1 h. To this mixture was added phosphate

buffer (0.1 M, pH 6.8, 0.2 mL) and the solution was cooled to 0 ºC before BH_3·pyridine (20 μL, 0.16 mmol) was added. The reaction mixture was then warmed to rt and stirred overnight before being acidified with HOAc to pH 5 and concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH from 10:1 to 1:10) to give **3-34** (13.4 mg, 69%) as a white foam. R_f 0.48 (CH_3OH–NH_4OH 20:1); $[\alpha]_D$ +15.4 (*c* 0.47, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 7.35–7.24 (m, 5 H, Ar), 4.54 (d, 1 H, *J* = 11.9 Hz, CH_2Ph), 4.53 (d, 1 H, *J* = 11.9 Hz, CH_2Ph), 4.26 (d, 1 H, *J* = 6.8 Hz, H-4), 3.94 (dd, 1 H, *J* = 6.4, 6.2 Hz, H-7), 3.83 (dd, 1 H, *J* = 6.4, 6.8 Hz, H-3), 3.67–3.60 (m, 3 H, H-8, C$\underline{H_2}$OBn), 3.50 (dd, 1 H, *J* = 6.4, 11.1 Hz, H-8), 3.46 (d, 1 H, *J* = 6.4 Hz, H-2), 3.33–3.29 (m, 1 H, 1 × NHC$\underline{H_2}$), 3.10 (ddd, 1 H, *J* = 7.2, 7.2, 12.3 Hz, NHC$\underline{H_2}$), 2.11–1.97 (m, 4 H, $CH_2C\underline{H_2}$), 1.73 (dd, 1 H, *J* = 4.0, 8.8 Hz, H-1), 0.88 (dd, 1 H, *J* = 5.7, 8.8 Hz, H-6), 0.75 (dd, 1 H, *J* = 4.0, 5.7 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 139.3 (Ar), 129.5 (2 C, Ar × 2), 129.1 (2 C, Ar × 2), 128.8 (Ar), 79.6 (C-4), 76.5 (C-3), 74.3 (C$\underline{H_2}$Ph), 71.9 (C-7), 69.5 (C$\underline{H_2}$OBn), 66.0 (C-8), 60.9 (C-2), 46.5 (NHC$\underline{H_2}$), 35.9 (C-5), 27.6 (2 C, CH_2CH_2), 20.2 (C-1), 8.6 (C-6). HRMS (ESI) *m/z* Calcd for ($M–CH_3COO^-$) $C_{18}H_{28}NO_5$: 338.1962. Found: 338.1961.

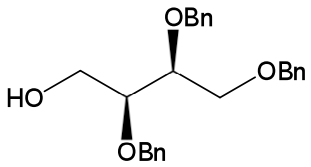


3-30

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-3,4-dihydroxy-*N*-(3-hydroxypropyl)-bicyclo[3.1.0]hexan-2-ammonium acetate (3-30)

To a solution of compound **3-34** (13.4 mg, 0.034 mmol) in THF (4 mL) and H_2O (0.5 mL) was added 10% Pd–C (4 mg), and the mixture was stirred under a H_2 atmosphere for 12 h. The mixture was then filtered through Celite and concentrated. The resulting crude residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 100% CH_3OH) to yield product **3-30** (10.3 mg, 100%) as a colorless oil. R_f 0.18 (CH_3OH–NH_4OH 20:1); $[\alpha]_D$ +23.4 (*c* 0.28, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 4.28 (d, 1 H, *J* = 6.8 Hz, H-4), 3.93 (dd, 1 H, *J* = 5.9, 6.3 Hz, H-7), 3.81 (dd, 1 H, *J* = 6.3, 6.8 Hz, H-3), 3.77–3.70 (m, 2 H, $CH_2C\underline{H_2}OH$), 3.66 (dd, 1 H, *J* = 5.9, 11.1 Hz, H-8), 3.50 (dd, 1 H, *J* = 6.3, 11.1 Hz, H-8), 3.44 (d, 1 H, *J* = 6.3 Hz, H-2), 3.33–3.29 (m, 1 H, 1 × $NHC\underline{H_2}$), 3.07 (ddd, 1 H, *J* = 7.1, 7.1, 12.3 Hz, $NHC\underline{H_2}$), 1.99–1.86 (m, 2 H, $C\underline{H_2}$), 1.73 (dd, 1 H, *J* = 4.0, 8.8 Hz, H-1), 0.89 (dd, 1 H, *J* = 5.7, 8.8 Hz, H-6), 0.76 (dd, 1 H, *J* = 4.0, 5.7 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 79.6 (C-4), 76.5 (C-3), 72.1 (C-7), 65.9 (C-8), 61.2 ($\underline{C}H_2OH$), 60.8 (C-2), 46.4 ($NH\underline{C}H_2$), 35.7 (C-5), 29.9 (2

C, CH_2CH_2), 20.5 (C-1), 8.7 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{11}H_{22}NO_5$: 248.1492. Found: 248.1493.

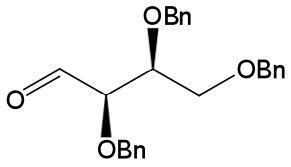


3-36

(2*S*,3*S*)-2,3,4-tris(benzyloxy)butan-1-ol (3-36)

To a solution of (2*S*,3*S*)-2,3-bis(benzyloxy)butane-1,4-diol (0.23 g, 0.76 mmol) and benzyl bromide (0.13 g, 0.76 mmol) in DMF (4 mL) at 0 ºC was added NaH (30 mg, 0.76 mmol, 60% in mineral oil). After stirring for 1 h, the reagents were quenched by the addition of H_2O. The solution was extracted with Et_2O twice and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 1:4) to give **3-36** (0.17 g, 57%) as a colorless oil. R_f 0.30 (EtOAc–Hexane 1:2); $[\alpha]_D$ +12.3 (*c* 1.31, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.28 (m, 15 H, Ar), 4.74 (d, 1 H, *J* = 11.8 Hz, CH_2Ph), 4.70–4.62 (m, 3 H, 3 × CH_2Ph), 4.54 (s, 2 H, 2 × CH_2Ph), 3.84–3.63 (m, 6 H, 2 × CHOBn, 2 × CH_2OBn, 2 × CH_2OH), 2.23 (dd, 1 H, *J* = 5.4, 7.0 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.3 (2 C, Ar × 2), 137.9 (Ar), 128.5 (2 C, Ar × 2), 128.4 (2 C, Ar × 2), 128.3 (2 C, Ar × 2), 128.0 (2 C, Ar × 2), 127.9 (2 C, Ar × 2), 127.8 (2 C, Ar × 2), 127.7 (3 C, Ar × 3), 79.2 (CHOBn), 78.5 (CHOBn), 73.5 (CH_2Ph), 72.9 (CH_2Ph), 72.8 (CH_2Ph), 69.5 (CH_2OBn), 61.5

(CH_2OH). HRMS (ESI) *m/z* Calcd for (M+Na^+) $C_{25}H_{28}O_4$: 415.1880. Found: 415.1876.

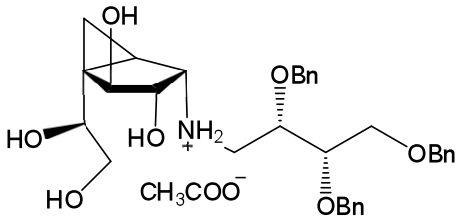


3-37

(2*R*,3*S*)-2,3,4-tris(benzyloxy)butanal (3-37)

To a solution of **3-36** (52 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) at 0 ºC was added a solution of Dess–Martin periodinane (56 mg, 0.13 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at 0 ºC for 2 h and then poured into a cold aqueous saturated $NaHCO_3$ solution. The organic layer was washed with brine, dried ($MgSO_4$), concentrated, and the residue was purified by chromatography (EtOAc–Hexane 1:6) to give **3-37** (39 mg, 76%) as a colorless oil. R_f 0.43 (EtOAc–Hexane 1:3); $[\alpha]_D$ +3.3 (*c* 0.65, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.71 (s, 1 H, C<u>H</u>O), 7.37–7.26 (m, 15 H, Ar), 4.76 (d, 1 H, *J* = 12.0 Hz, C<u>H</u>$_2$Ph), 4.64 (d, 1 H, *J* = 11.9 Hz, C<u>H</u>$_2$Ph), 4.57 (d, 1 H, *J* = 12.0 Hz, C<u>H</u>$_2$Ph), 4.56 (d, 1 H, *J* = 11.9 Hz, C<u>H</u>$_2$Ph), 4.48 (d, 1 H, *J* = 12.0 Hz, C<u>H</u>$_2$Ph), 4.46 (d, 1 H, *J* = 12.0 Hz, C<u>H</u>$_2$Ph), 4.00–3.96 (m, 2 H, 2 × C<u>H</u>OBn), 3.71–3.64 (m, 2 H, 2 × C<u>H</u>$_2$OBn); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 202.38 (<u>C</u>HO), 137.8 (Ar), 137.7 (Ar), 137.2 (Ar), 128.5 (2 C, Ar × 2), 128.4 (4 C, Ar × 4), 128.2 (2 C, Ar × 2), 128.1 (Ar), 128.0 (2 C, Ar × 2), 127.8 (Ar), 127.7 (3 C, Ar × 3), 82.8 (<u>C</u>HOBn), 77.9 (<u>C</u>HOBn), 73.4

(2 C, 2 × $\underline{C}H_2Ph$), 72.9 ($\underline{C}H_2Ph$), 68.1 ($\underline{C}H_2OBn$). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{25}H_{26}O_4$: 413.1679. Found: 413.1674.

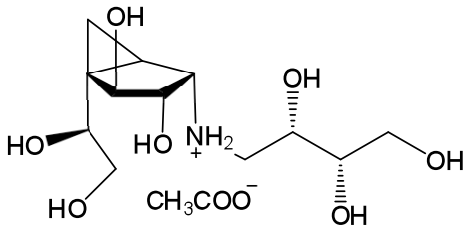


3-38

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-3,4-dihydroxy-*N*-((2*S*,3*S*)-2,3,4-tris-(benzyloxy)butyl)bicyclo[3.1.0]hexan-2-ammonium acetate (3-38)

A solution of **2-1** (11.6 mg, 0.05 mmol) and **3-37** (21 mg, 0.05 mmol) in freshly distilled CH_3OH (2 mL) was stirred at rt for 1 h. To this mixture was added phosphate buffer (0.1 M, pH 6.8, 0.2 mL) and the solution was cooled to 0 ºC, before and BH_3·pyridine (20 μL, 0.16 mmol) was added. The reaction mixture was then warmed to rt and stirred overnight before being acidified with HOAc to pH 5 and then concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 to 1:1) to give **3-38** (14.2 mg, 42%) as a white foam. R_f 0.18 (CH_3OH–CH_2Cl_2 1:1); $[\alpha]_D$ +16.5 (*c* 0.39, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 7.35–7.24 (m, 15 H, Ar), 4.68 (d, 1 H, *J* = 11.7 Hz, $C\underline{H}_2Ph$), 4.64 (d, 1 H, *J* = 11.3 Hz, $C\underline{H}_2Ph$), 4.62 (d, 1 H, *J* = 11.7 Hz, $C\underline{H}_2Ph$), 4.57 (d, 1 H, *J* = 11.7 Hz, $C\underline{H}_2Ph$), 4.51–4.50 (m, 2 H, 2 × $C\underline{H}_2Ph$), 4.19 (d, 1 H, *J* = 7.0 Hz, H-4), 3.86–3.81 (m, 2 H, 2 × $C\underline{H}OBn$), 3.78 (dd, 1 H, *J* = 6.2,

6.2 Hz, H-7), 3.71 (dd, 1 H, *J* = 3.1, 10.5 Hz, C$\underline{H}_2$OBn), 3.66–3.59 (m, 3 H, H-3, 1 × C$\underline{H}_2$OBn, 1 × H-8), 3.49 (dd, 1 H, *J* = 6.2, 11.1 Hz, H-8), 3.07 (dd, 1 H, *J* = 2.6, 12.1 Hz, NHC$\underline{H}_2$), 2.99 (d, 1 H, *J* = 6.3 Hz, H-2), 2.64 (dd, 1 H, *J* = 8.3, 12.1 Hz, NHC$\underline{H}_2$), 1.50 (dd, 1 H, *J* = 4.4, 8.6 Hz, H-1), 0.72 (dd, 1 H, *J* = 5.8, 8.6 Hz, H-6), 0.68 (dd, 1 H, *J* = 4.4, 5.8 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 139.7 (Ar), 139.5 (2 C, Ar × 2), 129.5 (2 C, Ar × 2), 129.4 (4 C, Ar × 4), 129.3 (2 C, Ar × 2), 129.2 (2 C, Ar × 2), 129.0 (2 C, Ar × 2), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 80.0 (C-4), 79.5 (C$\underline{H}$OBn), 79.2 (C-3), 77.5 (C$\underline{H}$OBn), 74.4 (C$\underline{H}_2$Ph), 74.3 (C$\underline{H}_2$Ph), 73.8 (C$\underline{H}_2$Ph), 73.1 (C-7), 70.2 (C$\underline{H}_2$OBn), 66.1 (C-8), 61.2 (C-2), 49.3 (C$\underline{H}_2$NH), 34.8 (C-5), 23.4 (C-1), 8.8 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{33}H_{42}NO_7$: 564.2956. Found: 564.2951.

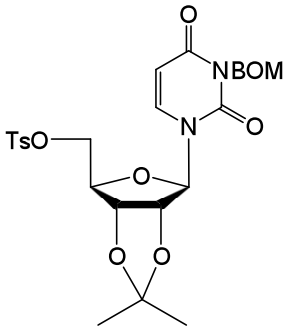


3-31

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-3,4-dihydroxy-*N*-((2*S*,3*S*)-2,3,4-tri-hydroxybutyl)bicyclo[3.1.0]hexan-2-ammonium acetate (3-31)

To a solution of **3-38** (20 mg, 0.032 mmol) in THF (4 mL) and H_2O (0.5 mL) was added 10% Pd–C (4 mg) and the reaction mixture was stirred under a H_2 atmosphere for 12 h. The mixture was then filtered through Celite and

concentrated to give pure **3-31** (11 mg, 100%) as a colorless oil. R_f 0.28 (CH_3OH–NH_4OH 20:1); $[\alpha]_D$ +18.9 (*c* 0.24, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.26 (d, 1 H, *J* = 6.8 Hz, H-4), 3.97 (ddd, 1 H, *J* = 3.3, 3.3, 8.2 Hz, $NHCH_2C\underline{H}OH$), 3.89 (dd, 1 H, *J* = 6.2, 6.2 Hz, H-7), 3.83 (dd, 1 H, *J* = 6.4, 6.8 Hz, H-3), 3.67–3.58 (m, 4 H, $C\underline{H}OHC\underline{H_2}OH$, 1 × H-8), 3.52 (dd, 1 H, *J* = 6.3, 11.1 Hz, H-8), 3.17 (d, 1 H, *J* = 6.4 Hz, H-2), 3.32 (dd, 1 H, *J* = 3.3, 12.4 Hz, $NHC\underline{H_2}$), 3.10 (dd, 1 H, *J* = 8.2, 12.4 Hz, $NHC\underline{H_2}$), 1.71 (dd, 1 H, *J* = 4.0, 8.9 Hz, H-1), 0.90 (dd, 1 H, *J* = 5.7, 8.9 Hz, H-6), 0.78 (dd, 1 H, *J* = 4.0, 5.7 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 79.7 (C-4), 76.2 (C-3), 74.4 ($\underline{C}HOH$), 72.2 ($\underline{C}HOH$), 68.8 ($\underline{C}HOH$), 65.8 ($\underline{C}H_2OH$), 63.6 ($\underline{C}H_2OH$), 61.4 (C-2), 50.6 ($\underline{C}H_2NH$), 35.7 (C-5), 20.9 (C-1), 8.9 (C-6). HRMS (ESI) *m/z* Calcd for ($M–CH_3COO^-$) $C_{12}H_{24}NO_7$: 294.1547. Found: 294.1543.

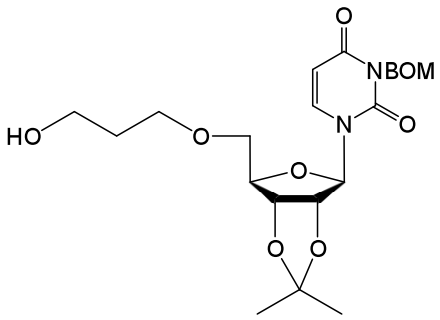


3-44

2',3'-*O*-isopropylidene-5'-*O*-tosyl-3-(benzyloxymethyl)uridine (3-44)

To a solution of 2',3'-*O*-isopropylidene-3-(benzyloxylmethyl)uridine (4.0 g, 10 mmol) in pyridine (25 mL) was added p-toluenesulfonyl chloride (2.26 g, 12

mmol) at rt. The reaction was stirred overnight and then the excess reagents were quenched by the addition of CH_3OH (3 mL). The solution was then concentrated and the residue was purified by chromatography (EtOAc–Hexane 1:2) to give **3-44** (3.94 g, 71%) as a foam. R_f 0.54 (EtOAc–Hexane 1:1); $[\alpha]_D$ +23.6 (*c* 1.33, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.77–7.75 (m, 2 H, Ar), 7.37–7.25 (m, 7 H, Ar), 7.16 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.71 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.60 (d, 1 H, *J* = 2.0 Hz, H-1), 5.46 (d, 1 H, *J* = 9.8 Hz, NC<u>H</u>$_2$OBn), 5.35 (d, 1 H, *J* = 9.8 Hz, NC<u>H</u>$_2$OBn), 4.88 (dd, 1 H, *J* = 2.0, 6.4 Hz, H-2), 4.79 (dd, 1 H, *J* = 3.7, 6.4 Hz, H-3), 4.68 (s, 2 H, C<u>H</u>$_2$Ph), 4.35 (ddd, 1 H, *J* = 3.7, 4.6, 4.6 Hz, H-4), 4.27 (d, 2 H, *J* = 4.6 Hz, 2 × H-5), 2.42 (s, 3 H, C<u>H</u>$_3$), 1.55 (s, 3 H, C<u>H</u>$_3$), 1.34 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.3 (<u>C</u>=O), 150.7 (<u>C</u>=O), 145.2 (Ar), 140.6 (<u>C</u>H=), 137.8 (Ar), 132.6 (Ar), 129.9 (2 C, Ar × 2), 128.3 (2 C, Ar × 2), 127.9 (2 C, Ar × 2), 127.7 (Ar), 127.6 (2 C, Ar × 2), 114.5 (<u>C</u>$(CH_3)_2$), 102.3 (<u>C</u>H=), 95.6 (C-1), 85.1 (C-4), 84.4 (C-2), 80.8 (C-3), 72.4 (<u>C</u>H_2Ph), 70.3 (N<u>C</u>H_2OBn), 69.2 (C-5), 27.0 (<u>C</u>H_3), 25.2 (<u>C</u>H_3), 21.6 (<u>C</u>H_3). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{27}H_{30}N_2O_9S$: 581.1562. Found: 581.1558.

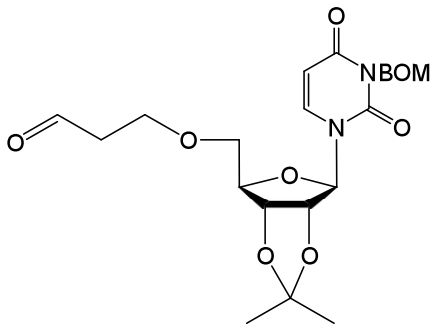


3-46

2',3'-*O*-isopropylidene-5'-*O*-(3-hydroxylpropyl)-3-(benzyloxymethyl)uridine (3-46)

To a solution of **3-44** (0.50 g, 0.9 mmol) and 1,3-propanediol (**3-32**) (0.69 g, 9.0 mmol) in DMF (4 mL) at 0 ºC was added NaH (72 mg, 1.8 mmol, 60% in mineral oil). The solution was stirred at rt for 20 h and then water H_2O (10 mL) was added and the mixture was extracted with Et_2O. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 2:1) to give **3-46** (0.15 g, 36%) as a colorless oil. R_f 0.14 (EtOAc–Hexane 2.5:1); $[\alpha]_D$ –3.3 (*c* 0.96, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.44 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 7.38–7.23 (m, 5 H, Ar), 5.77 (d, 1 H, *J* = 1.9 Hz, H-1), 5.73 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.50 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 5.47 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 4.81–4.77 (m, 2 H, H-2, H-3), 4.70 (s, 2 H, C<u>H</u>$_2$Ph), 4.37 (ddd, 1 H, *J* = 3.0, 3.0, 4.5 Hz, H-4), 3.73–3.69 (m, 3 H, H-5, C<u>H</u>$_2$OH), 3.67–3.58 (m, 3 H, H-5, C<u>H</u>$_2$O), 1.83–1.77 (m, 2 H, C<u>H</u>$_2$), 1.58 (s, 3 H, C<u>H</u>$_3$), 1.37 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.6 (<u>C</u>=O), 150.8 (<u>C</u>=O), 139.9 (<u>C</u>H=), 137.9 (Ar), 128.3 (2 C, Ar × 2), 127.6 (3 C, Ar × 3),

114.1 (<u>C</u>(CH_3)$_2$), 101.6 (<u>C</u>H=), 94.4 (C-1), 85.9 (C-2), 85.2 (C-4), 80.9 (C-3), 72.3 (<u>C</u>H_2Ph), 70.9 (N<u>C</u>H_2OBn), 70.3 (C-5), 69.6 (<u>C</u>H_2O<u>C</u>H_2), 60.6 (<u>C</u>H_2OH), 32.2 (<u>C</u>H_2), 27.2 (<u>C</u>H_3), 25.4 (<u>C</u>H_3). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{23}H_{30}N_2O_8$: 485.1894. Found: 485.1894.

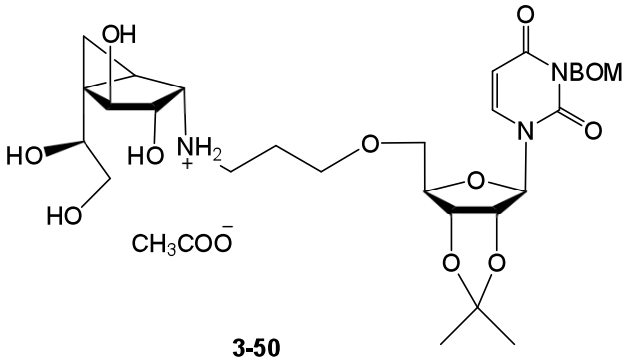


3-48

2',3'-*O*-isopropylidene-5'-*O*-(3-oxopropyl)-3-(benzyloxymethyl)uridine (3-48)

A solution of **3-46** (32 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) was added to a solution of Dess–Martin periodinane (35 mg, 0.08 mmol) in CH_2Cl_2 (4 mL) at 0 ºC. The reaction was stirred for 3 h at 0 ºC and poured into an ice-cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with H_2O, brine, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 1:1) to give **3-48** (20 mg, 62%) as a colorless oil. R_f 0.26 (EtOAc–Hexane 2:1); $[\alpha]_D$ –12.6 (*c* 0.68, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.75 (dd, 1 H, *J* = 1.6, 1.6 Hz, C<u>H</u>O), 7.38 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 7.36–7.23 (m, 5 H, Ar), 5.81 (d, 1 H, *J* = 2.3 Hz, H-1), 5.75 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.50 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 5.46 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn),

4.75 (dd, 2 H, *J* = 3.2, 6.2 Hz, H-3), 4.74 (dd, 2 H, *J* = 2.3, 6.2 Hz, H-2), 4.70 (s, 2 H, C$\underline{H_2}$Ph), 4.35 (ddd, 1 H, *J* = 2.8, 3.2, 4.2 Hz, H-4), 3.82–3.79 (m, 2 H, C$\underline{H_2}$OCH$_2$), 3.74 (dd, 1 H, *J* = 2.8, 10.6 Hz, H-5), 3.62 (dd, 1 H, *J* = 4.2, 10.6 Hz, H-5), 2.69–2.65 (m, 2 H, C$\underline{H_2}$), 1.58 (s, 3 H, C$\underline{H_3}$), 1.36 (s, 3 H, C$\underline{H_3}$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 199.9 (C$\underline{H}$O), 162.6 (C$\underline{}$=O), 150.9 (C$\underline{}$=O), 139.6 (C$\underline{H}$=), 137.9 (Ar), 128.3 (2 C, Ar × 2), 127.6 (3 C, Ar × 3), 114.1 (C$\underline{}$(CH$_3$)$_2$), 101.7 (C$\underline{H}$=), 93.9 (C-1), 85.7 (C-4), 85.2 (C-2), 80.7 (C-3), 72.3 (C$\underline{H_2}$Ph), 71.0 (C$\underline{H_2}$OBn), 70.3 (C-5), 64.8 (C$\underline{H_2}$OCH$_2$), 43.7 (C$\underline{H_2}$), 27.2 (C$\underline{H_3}$), 25.3 (C$\underline{H_3}$). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{23}H_{28}N_2O_8$: 483.1738. Found: 483.1838.

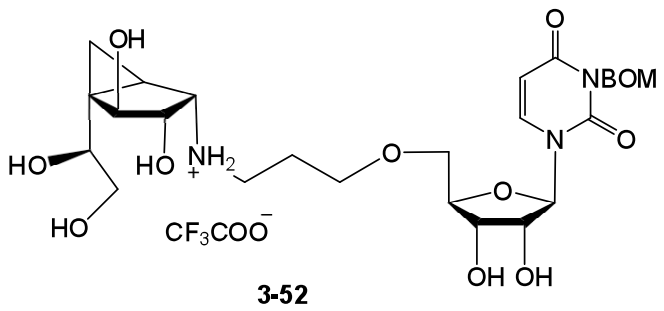


3-50

(1*R*,2*S*,3*S*,4*S*,5*S*)-*N*-(3-(2',3'-*O*-isopropylidene-3-(benzyloxymethyl)uridin)-propyl)-5-(1,2-dihydroxyethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium acetate (3-50)

To a mixture of **2-1** (8 mg, 0.043 mmol) and **3-48** (20 mg, 0.043 mmol) in freshly distilled CH_3OH (2 mL) at 0 ºC was added BH_3·pyridine (20 μL, 0.16 mmol) and phosphate buffer (0.1 M, pH 6.8, 0.4 mL). The mixture was stirred overnight at

rt and then acidified with HOAc to pH 5 before being concentrated. The resulting residue was purified by chromatography on C_{18} silica gel (H_2O–CH_3OH 10:1 → 1:10) to give **3-50** (13.3 mg, 45%) as a white foam. R_f 0.11 (pure CH_3OH; C_{18} silica gel TLC); $[\alpha]_D$ +15.4 (*c* 1.32, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD, δ_H) 7.71 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 7.37–7.21 (m, 5 H, Ar), 5.80 (d, 1 H, *J* = 1.4 Hz, H-1'), 5.73 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.45 (s, 2 H, NC<u>H$_2$</u>OBn), 4.81 (m, 2 H, H-2', H-3'), 4.66 (s, 2 H, C<u>H$_2$</u>Ph), 4.38–4.37 (m, 1 H, H-4'), 4.17 (d, 1 H, *J* = 6.9 Hz, H-4), 3.75–3.47 (m, 8 H, H-3, H-7, H-8, H-8, H-5', H-5', C<u>H$_2$</u>OC-5'), 2.91 (d, 1 H, *J* = 6.1 Hz, H-2), 2.80 (ddd, 1 H, *J* = 6.8, 7.0, 11.6 Hz, NHC<u>H$_2$</u>), 2.56 (ddd, 1 H, *J* = 7.0, 7.1, 11.6 Hz, NHC<u>H$_2$</u>), 1.79–1.71 (m, 2 H, C<u>H$_2$</u>), 1.54 (s, 3 H, C<u>H$_3$</u>), 1.49 (dd, 1 H, *J* = 6.0, 6.0 Hz, H-1), 1.36 (s, 3 H, C<u>H$_3$</u>), 0.68–0.67 (m, 2 H, 2 × H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 164.9 (<u>C</u>=O), 152.3 (<u>C</u>=O), 142.3 (<u>C</u>H=), 139.5 (Ar), 129.3 (2 C, Ar × 2), 128.7 (Ar), 128.6 (2 C, Ar × 2), 114.8 (<u>C</u>$(CH_3)_2$), 101.7 (CH=), 95.6 (C-1'), 87.6 (C-4'), 86.6 (C-2'), 82.6 (C-3'), 80.0 (C-4), 77.7 (C-3), 73.3 (C-7), 73.2 (<u>C</u>H_2Ph), 72.0 (C-5' or <u>C</u>H_2OCH_2), 71.6 (N<u>C</u>H_2OBn), 70.8 (C-5' or <u>C</u>H_2OCH_2), 66.1 (C-8), 60.7 (C-2), 46.6 (NH<u>C</u>H_2), 34.5 (C-5), 30.6 (<u>C</u>H_2), 27.5 (<u>C</u>H_3), 25.5 (<u>C</u>H_3), 24.3 (C-1), 8.9 (C-6). HRMS (ESI) *m/z* Calcd for ($M–CH_3COO^-$) $C_{31}H_{44}N_3O_{11}$: 634.2970. Found: 634.2969.

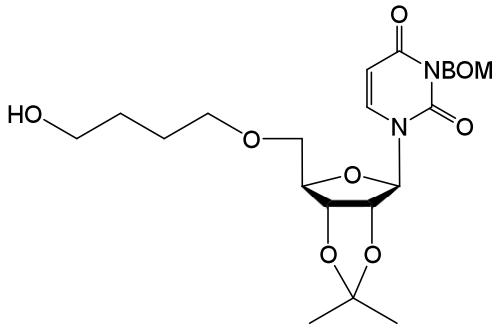


3-52

(1*R*,2*S*,3*S*,4*S*,5*S*)-*N*-(3-(3-(benzyloxymethyl)uridin)propyl)-5-(1,2-dihydroxy-ethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium 2,2,2-trifluoroacetate (3-52)

Compound **3-50** (14 mg, 0.02 mmol) was dissolved in TFA (2 mL) at 0 ºC. The solution was then stirred at rt for 12 h and concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:3) to give the **3-52** (12 mg, 84%) as a white foam. R_f 0.24 (CH_3OH–NH_4OH 40:1); $[\alpha]_D$ +14.9 (*c* 0.15, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 7.76 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 7.33–7.24 (m, 5 H, Ar), 5.78 (d, 1 H, *J* = 2.8 Hz, H-1'), 5.76 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.45 (s, 2 H, NC<u>H</u>$_2$OBn), 4.66 (s, 2 H, C<u>H</u>$_2$Ph), 4.25 (d, 1 H, *J* = 6.7 Hz, H-4), 4.15 (dd, 1 H, *J* = 2.8, 4.8 Hz, H-2'), 4.10–4.06 (m, 2 H, H-3', H-4'), 3.89–3.80 (m, 3 H, H-7, H-5', C<u>H</u>$_2$OC-5'), 3.72–3.62 (m, 3 H, H-3, H-5', C<u>H</u>$_2$OC-5'), 3.64 (dd, 1 H, *J* = 5.9, 11.1 Hz, H-8), 3.54 (d, 1 H, *J* = 6.9 Hz, H-2), 3.51 (dd, 1 H, *J* = 6.2, 11.1 Hz, H-8), 3.27–3.12 (m, 2 H, NHC<u>H</u>$_2$), 2.05–1.96 (m, 2 H, C<u>H</u>$_2$), 1. 69 (dd, 1 H, *J* = 4.0, 9.0 Hz, H-1), 0.93 (dd, 1 H, *J* = 5.8, 9.0, Hz, H-6), 0.79 (dd, 1 H, *J* = 4.0, 5.8 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 164.8 (<u>C</u>=O), 152.4 (<u>C</u>=O), 141.6 (<u>C</u>H=), 139.4 (Ar), 129.4 (2 C, Ar × 2), 128.8

(Ar), 128.7 (2 C, Ar × 2), 102.1 (CH=), 93.0 (C-1'), 84.0 (C-4'), 79.6 (C-4), 76.2 (C-3), 75.3 (C-3'), 73.2 ($\underline{C}H_2Ph$), 71.9 (C-7), 71.6 ($N\underline{C}H_2OBn$), 71.6 (C-5' or $\underline{C}H_2OCH_2$), 71.1 ($\underline{C}H$), 70.4 (C-5' or $\underline{C}H_2OCH_2$), 65.8 (C-8), 61.0 (C-2), 45.8 ($NH\underline{C}H_2$), 35.9 (C-5), 27.3 ($\underline{C}H_2$), 19.8 (C-1), 9.0 (C-6). HRMS (ESI) *m/z* Calcd for ($M–CF_3COO^-$) $C_{28}H_{40}N_3O_{11}$: 594.2657. Found: 594.2651.

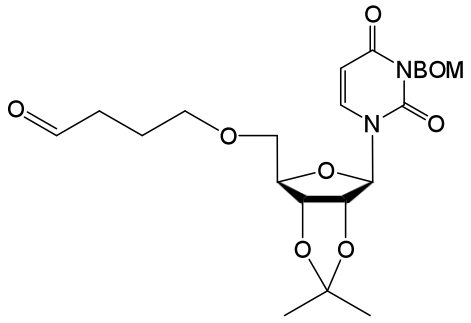


3-47

2',3'-*O*-isopropylidene-5'-*O*-(4-hydroxylbutyl)-3-(benzyloxymethyl)uridine (3-47)

To a solution of **3-44** (0.89 g, 1.6 mmol) and 1,4-butanediol (**3-45**) (1.62 g, 19.4 mmol) in DMF (4 mL) at 0 ºC was added NaH (128 mg, 3.2 mmol, 60% in mineral oil). The reaction was stirred at rt for 20 h and then H_2O (10 mL) was added. The mixture was extracted with Et_2O and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 2:1) to give **3-47** (0.40 g, 52%) as a colorless oil. R_f 0.16 (EtOAc–Hexane 2.5:1); $[\alpha]_D$ –3.7 (*c* 2.43, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.53 (d, 1 H, *J* = 8.1 Hz, $C\underline{H}$=), 7.36–7.23 (m, 5 H, Ar), 5.83 (s,

1 H, H-1), 5.71 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.59 (d, 1 H, *J* = 9.8 Hz, NC<u>H</u>$_2$OBn), 5.45 (d, 1 H, *J* = 9.8 Hz, NC<u>H</u>$_2$OBn), 4.78–4.74 (m, 2 H, H-2, H-3), 4.70 (s, 2 H, C<u>H</u>$_2$Ph), 4.38 (ddd, 1 H, *J* = 2.6, 2.6, 3.9 Hz, H-4), 3.69 (dd, 1 H, *J* = 2.6, 10.7 Hz, H-5), 3.61–3.57 (m, 3 H, H-5, C<u>H</u>$_2$OH), 3.59–3.46 (m, 2 H, C<u>H</u>$_2$), 1.64–1.55 (m, 4 H, CH_2CH_2), 1.57 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.7 (<u>C</u>=O), 150.9 (<u>C</u>=O), 139.7 (<u>C</u>H=), 137.9 (Ar), 128.3 (2 C, Ar × 2), 127.6 (3 C, Ar × 3), 114.0 (<u>C</u>$(CH_3)_2$), 101.4 (<u>C</u>H=), 94.0 (C-1), 85.9 (C-4), 85.4 (C-2), 80.9 (C-3), 72.3 (<u>C</u>H_2Ph), 71.6 (<u>C</u>H_2OCH_2), 70.7 (C-5), 70.3 (N<u>C</u>H_2OBn), 62.4 (<u>C</u>H_2OH), 29.5 (<u>C</u>H_2), 27.2 (<u>C</u>H_3), 26.2 (<u>C</u>H_2), 25.4 (<u>C</u>H_3). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_{24}H_{32}N_2O_8$: 499.2051. Found: 499.2046.

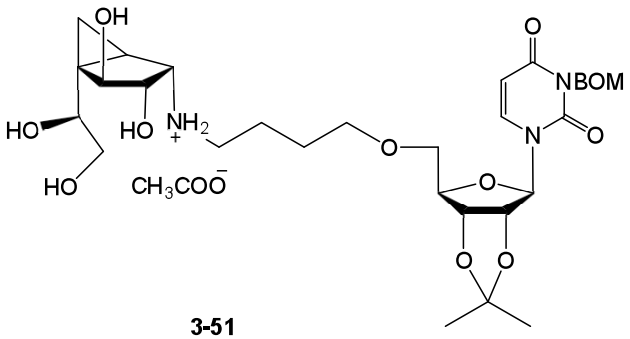


3-49

2',3'-*O*-isopropylidene-5'-*O*-(4-oxobutyl)-3-(benzyloxymethyl)uridine (3-49)

A solution of **3-47** (73 mg, 0.15 mmol) in CH_2Cl_2 (4 mL) was added to a solution of Dess–Martin periodinane (78 mg, 0.18 mmol) in CH_2Cl_2 (8 mL) at 0 ºC. The reaction was stirred for 3 h at 0 ºC and poured into an ice cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with H_2O, brine, dried

($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 1:1) to give **3-49** (60 mg, 83%) as a colorless oil. R_f 0.28 (EtOAc–Hexane 2:1); $[\alpha]_D$ +17.1 (*c* 0.70, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.72 (dd, 1 H, *J* = 1.5, 1.5 Hz, C<u>H</u>O), 7.44 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 7.43–7.24 (m, 5 H, Ar), 5.80 (d, 1 H, *J* = 2.0 Hz, H-1), 5.73 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.50 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 5.48 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 4.78–4.74 (m, 2 H, H-3, H-2), 4.71 (s, 2 H, C<u>H</u>$_2$Ph), 4.35 (ddd, 1 H, *J* = 3.0, 4.4, 4.4 Hz, H-4), 3.67 (dd, 1 H, *J* = 4.4, 10.7 Hz, H-5), 3.59 (dd, 1 H, *J* = 4.4, 10.7 Hz, H-5), 3.53–3.45 (m, 2 H, C<u>H</u>$_2$), 2.50–2.46 (m, 2 H, C<u>H</u>$_2$), 1.92–1.87 (m, 2 H, C<u>H</u>$_2$), 1.59 (s, 3 H, C<u>H</u>$_3$), 1.37 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 201.4 (<u>C</u>HO), 162.6 (<u>C</u>=O), 150.9 (<u>C</u>=O), 139.7 (<u>C</u>H=), 137.9 (Ar), 128.3 (2 C, Ar × 2), 127.6 (3 C, Ar × 3), 114.2 (<u>C</u>(CH$_3$)$_2$), 101.6 (<u>C</u>H=), 94.2 (C-1), 85.9 (C-4), 85.2 (C-2), 80.7 (C-3), 72.3 (<u>C</u>H$_2$Ph), 70.8 (<u>C</u>H$_2$OBn), 70.6 (C-5), 70.3 (<u>C</u>H$_2$OCH$_2$), 40.6 (<u>C</u>H$_2$), 27.2 (<u>C</u>H$_3$), 25.3 (<u>C</u>H$_3$), 22.2 (<u>C</u>H$_2$). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{24}H_{30}N_2O_8$: 497.1894. Found: 497.1894.

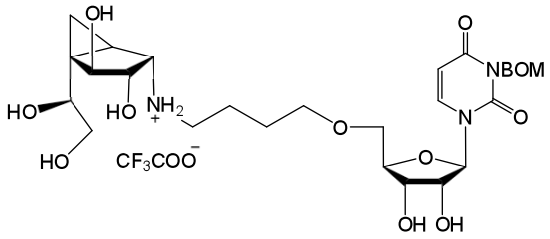


3-51

(1*R*,2*S*,3*S*,4*S*,5*S*)-*N*-(4-(2',3'-*O*-isopropylidene-3-(benzyloxymethyl)uridin)-butyl)-5-(1,2-dihydroxyethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium acetate (3-51)

To a mixture of **2-1** (7.2 mg, 0.038 mmol) and **3-49** (18 mg, 0.038 mmol) in freshly distilled CH_3OH (2 mL) at 0 ºC was added BH_3·pyridine (20 μL, 0.16 mmol) and phosphate buffer (0.1 M, pH 6.8, 0.4 mL). The mixture was stirred overnight at rt and then acidified with HOAc to pH 5 before being concentrated. The residue was purified by chromatography on C_{18} silica gel (H_2O–CH_3OH 10:1 →1:10) to give the product **3-51** (10 mg, 37%) as a white foam. R_f 0.11 (Pure CH_3OH; C_{18} silica gel TLC); $[\alpha]_D$ +10.9 (*c* 1.00, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 7.74 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 7.31–7.22 (m, 5 H, Ar), 5.80 (d, 1 H, *J* = 1.4 Hz, H-1'), 5.72 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.46 (s, 2 H, NC<u>H</u>$_2$OBn), 4.81 (m, 2 H, H-2', H-3'), 4.66 (s, 2 H, C<u>H</u>$_2$Ph), 4.39–4.37 (m, 1 H, H-4'), 4.17 (d, 1 H, *J* = 6.8 Hz, H-4), 3.74 (dd, 1 H, *J* = 6.3, 6.4 Hz, H-7), 3.69 (dd, 1 H, *J* = 3.1, 10.7 Hz, 1 × C<u>H</u>$_2$OCH$_2$), 3.65 (dd, 1 H, *J* = 6.4, 11.2 Hz, H-8), 3.61–3.57 (m, 2 H, H-3, 1 × C<u>H</u>$_2$OCH$_2$), 3.50–3.47 (m, 3 H, 2 × H-5', 1 × H-8), 2.91 (d, 1 H, *J* = 6.2 Hz,

H-2), 2.73 (ddd, 1 H, *J* = 6.2, 7.7, 11.6 Hz, NHC$\underline{H}_2$), 2.56 (ddd, 1 H, *J* = 6.4, 7.6, 11.6 Hz, NHC$\underline{H}_2$), 1.61–1.49 (m, 5 H, H-1, $CH_2\underline{C}H_2$), 1.54 (s, 3 H, C$\underline{H}_3$), 1.35 (s, 3 H, C$\underline{H}_3$), 0.68–0.67 (m, 2 H, 2 × H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 164.9 ($\underline{C}$=O), 152.3 ($\underline{C}$=O), 142.4 ($\underline{C}$H=), 139.5 (Ar), 129.4 (2 C, Ar × 2), 128.7 (Ar), 128.6 (2 C, Ar × 2), 114.8 ($\underline{C}(CH_3)_2$), 101.7 ($\underline{C}$H=), 95.7 (C-1'), 87.6 (C-4'), 86.6 (C-2'), 82.7 (C-3'), 79.9 (C-4), 77.5 (C-3), 73.3 ($\underline{C}H_2$Ph), 73.2 (C-7), 72.2 (C-5' or $\underline{C}H_2OCH_2$), 71.9 (N$\underline{C}H_2$OBn), 71.6 (C-5' or $\underline{C}H_2OCH_2$), 66.0 (C-8), 60.6 (C-2), 48.7 (NH$\underline{C}H_2$), 34.7 (C-5), 28.3 ($\underline{C}H_2$), 27.5 ($\underline{C}H_3$), 26.9 ($\underline{C}H_2$), 25.5 ($\underline{C}H_3$), 23.5 (C-1), 8.9 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{32}H_{46}N_3O_{11}$: 648.3127. Found: 648.3135.

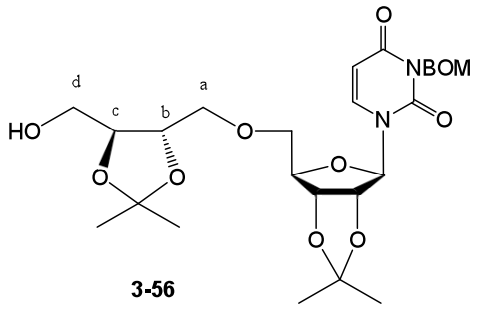


3-53

(1*R*,2*S*,3*S*,4*S*,5*S*)-*N*-(4-(3-(benzyloxymethyl)uridin)butyl)-5-(1,2-dihydroxyethyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium 2,2,2-trifluoroacetate (3-53)

Compound **3-51** (13 mg, 0.019 mmol) was dissolved in TFA (2 mL) at 0 ºC, stirred at rt for 12 h and then concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:3) to give **3-53** (12 mg,

88%) as a white foam. R_f 0.19 (CH_3OH–NH_4OH 40:1); $[\alpha]_D$ +16.0 (*c* 0.15, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 7.90 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 7.34–7.22 (m, 5 H, Ar), 5.83 (d, 1 H, *J* = 2.9 Hz, H-1'), 5.74 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.45 (s, 2 H, NC<u>H</u>$_2$OBn), 4.66 (s, 2 H, C<u>H</u>$_2$Ph), 4.27 (d, 1 H, *J* = 6.9 Hz, H-4), 4.13–4.09 (m, 3 H, H-2', H-3', H-4'), 3.90–3.80 (m, 3 H, H-7, H-3, H-5'), 3.67–3.39 (m, 6 H, H-5', 2 × H-8, H-2, C<u>H</u>$_2$OC-5'), 3.20–3.07 (m, 2 H, NHC<u>H</u>$_2$), 1.87–1.69 (m, 5 H, CH_2CH_2, H-1), 0.94 (dd, 1 H, *J* = 5.8, 8.7 Hz, H-6), 0.79 (dd, 1 H, *J* = 4.0, 5.8 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 164.9 (<u>C</u>=O), 152.5 (<u>C</u>=O), 141.3 (<u>C</u>H=), 139.4 (Ar), 129.4 (2 C, Ar × 2), 128.8 (Ar), 128.7 (2 C, Ar × 2), 101.9 (<u>C</u>H=), 92.3 (C-1'), 84.4 (C-4'), 79.6 (C-4), 76.3 (C-3), 75.8 (C-3'), 73.2 (<u>C</u>H$_2$ Ph), 71.9 (C-7), 71.7 (<u>C</u>H$_2$OC-5'), 71.6 (C-5'), 71.1 (C-2'), 70.9 (N<u>C</u>H$_2$OBn), 65.8 (C-8), 60.8 (C-2), 47.0 (NH<u>C</u>H$_2$), 35.9 (C-5), 27.8 (<u>C</u>H$_2$), 24.3 (<u>C</u>H$_2$), 19.9 (C-1), 9.1 (C-6). HRMS (ESI) *m/z* Calcd for (M–CF_3COO^-) $C_{29}H_{42}N_3O_{11}$: 608.2814. Found: 608.2809.

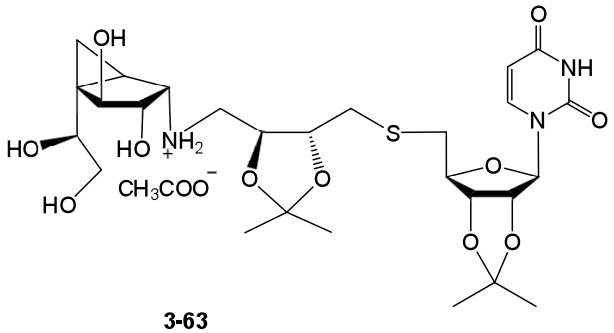


3-56

2'3'-*O*-isopropylidene-5'-*O*-((2*S*,3*S*)-2,3-*O*-isopropylidene-2,3,4-trihydroxyl-butyl)-3-(benzyloxymethyl) uridine (3-56)

To a solution of **3-44** (0.61 g, 1.09 mmol) and **3-55** (1.77 g, 10.9 mmol) in DMF (3 mL) at 0 ºC was added NaH (66 mg, 1.63 mmol, 60% in mineral oil). The reaction mixture was then warmed to rt, stirred overnight and then H_2O (10 mL) was added. The mixture was extracted with Et_2O and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 2:1) to give **3-56** (0.340 g, 57 %) as a colorless oil. R_f 0.29 (EtOAc–Hexane 2.5:1); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.45 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 7.37–7.23 (m, 5 H, Ar), 5.80 (d, 1 H, *J* = 2.1 Hz, H-1), 5.74 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.49 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 5.45 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 4.82–4.76 (m, 2 H, H-2, H-3), 4.70 (s, 2 H, C<u>H</u>$_2$Ph), 4.33 (ddd, 1 H, *J* = 3.2, 3.2, 4.4 Hz, H-4), 4.04 (ddd, 1 H, *J* = 4.9, 4.9, 8.3 Hz, H-c), 3.87 (ddd, 1 H, *J* = 4.2, 4.2, 8.3 Hz, Hb), 3.79–3.75 (m, 2 H, 2 × H-5), 3.71 (dd, 1 H, *J* = 4.5, 10.7 Hz, Ha), 3.66–3.63 (m, 3 H, Ha, 2 × Hd), 1.58 (s, 3 H, C<u>H</u>$_3$), 1.41 (s, 6 H, 2 × C<u>H</u>$_3$), 1.36 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.6 (<u>C</u>=O), 150.9 (<u>C</u>=O), 140.1 (<u>C</u>H=), 137.9 (Ar), 128.3 (2 C, Ar ×

2), 127.6 (3 C, Ar × 3), 114.0 (<u>C</u>(CH_3)$_2$), 109.5 (<u>C</u>(CH_3)$_2$), 101.9 (<u>C</u>H=), 94.0 (C-1), 85.6 (C-4), 84.8 (C-2), 80.7 (C-3), 78.6 (Cb), 76.4 (C-c), 72.3 (C-5), 71.9 (Ca), 71.4 (<u>C</u>H_2Ph), 70.3 (N<u>C</u>H_2OBn), 62.1 (C-d), 27.2 (<u>C</u>H_3), 27.1 (<u>C</u>H_3), 27.0 (<u>C</u>H_3), 25.4 (<u>C</u>H_3); HRMS (ESI) *m/z* Calcd for (M+Na^+) $C_{27}H_{36}N_2O_{10}$: 571.2262. Found: 571.2265.

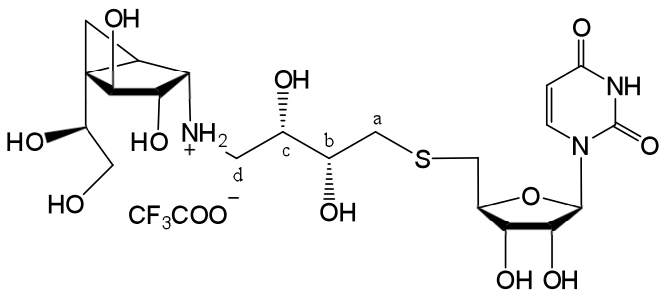


3-63

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-*N*-((2*S*,3*R*)-2,3-*O*-isopropylidene-4-(2',3'-*O*-iso- propylidene-5'-thiouridin)-2,3-dihydroxylbutyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium acetate (3-63)

A solution of **3-62** (30 mg, 0.067 mmol) in CH_2Cl_2 (4 mL) was added to a solution of Dess–Martin periodinane (29 mg, 0.067 mmol) in CH_2Cl_2 (8 mL) at 0 ºC. The solution was stirred for 3 h and then poured into an ice cold saturated aqueous $NaHCO_3$ solution. The organic layer was washed with H_2O, brine, dried ($MgSO_4$) and concentrated. The resulting aldehyde was dissolved in freshly distilled CH_3OH (2 mL), and was added to **2-1** (12.7 mg, 0.067 mmol). The mixture was stirred for 1 h, cooled to 0 ºC and then BH_3·pyridine (20 μL,

0.16 mmol) and phosphate buffer (0.1 M, pH 6.8, 0.4 mL) were added. After stirring overnight at rt, the solution was acidified with HOAc to pH 5 and then concentrated. The resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:10) to give **3-63** (10.7 mg, 37%) as a white foam. R_f 0.25 (Pure CH_3OH); $[\alpha]_D$ +13.9 (*c* 1.28, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 7.65 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.74 (d, 1 H, *J* = 2.3 Hz, H-1'), 5.69 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.05 (dd, 1 H, *J* = 2.3, 6.6 Hz, H-2'), 4.79 (dd, 1 H, *J* = 4.1, 6.6 Hz, H-3'), 4.25–4.21 (m, 2 H, H-4', H-4), 4.05–3.97 (m, 1 H, OC<u>H</u>), 3.96–3.91 (m, 1 H, OC<u>H</u>), 3.82 (dd, 1 H, *J* = 6.1, 6.3 Hz, H-7), 3.70 (dd, 1 H, *J* = 6.4, 6.7 Hz, H-3), 3.66 (dd, 1 H, *J* = 6.1, 11.1 Hz, H-8), 3.50 (dd, 1 H, *J* = 6.3, 11.1 Hz, H-8), 3.19–3.15 (m, 2 H, H-2, 1 × NHC<u>H</u>$_2$), 2.97–2.95 (m, 2 H, 2 × H-5'), 2.88–2.80 (m, 3 H, 1 × NHC<u>H</u>$_2$, C<u>H</u>$_2$S), 1.59 (dd, 1 H, *J* = 4.3, 8.6 Hz, H-1), 1.53 (s, 3 H, C<u>H</u>$_3$), 1.40 (s, 3 H, C<u>H</u>$_3$), 1.39 (s, 3 H, C<u>H</u>$_3$), 1.34 (s, 3 H, C<u>H</u>$_3$), 0.77 (dd, 1 H, *J* = 5.8, 8.6 Hz, H-6), 0.72 (dd, 1 H, *J* = 4.3, 5.8 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 166.1 (<u>C</u>=O), 151.9 (<u>C</u>=O), 144.8 (<u>C</u>H=), 115.5 (<u>C</u>$(CH_3)_2$), 110.9 (<u>C</u>$(CH_3)_2$), 103.0 (<u>C</u>H=), 95.3 (C-1'), 88.5 (C-4'), 85.5 (C-2'), 84.7 (C-3'), 80.3 (C-4), 80.2 (O<u>C</u>H), 80.0 (O<u>C</u>H), 77.4 (C-3), 72.9 (<u>C-7</u>), 66.1 (<u>C-8</u>), 60.9 (C-2), 51.4 (NH<u>C</u>H$_2$), 36.1 (S<u>C</u>H$_2$), 35.7 (S<u>C</u>H$_2$), 34.9 (C-5), 27.6 (<u>C</u>H$_3$), 27.5 (2 C, 2 × <u>C</u>H$_3$), 25.5 (<u>C</u>H$_3$), 23.0 (C-1), 8.9 (C-6). HRMS (ESI) *m/z* Calcd for (M–CH_3COO^-) $C_{27}H_{42}N_3O_{11}S$: 616.2534. Found: 616.2525.



3-64

(1*R*,2*S*,3*S*,4*S*,5*S*)-5-(1,2-dihydroxyethyl)-*N*-((2*S*,3*R*)-4-(2',3'-*O*-isopropylid-ene-5'-thiouridin)-2,3-dihydroxybutyl)-3,4-dihydroxybicyclo[3.1.0]hexan-2-ammonium 2,2,2-trifluoroacetate (3-64)

Compound **3-63** (10 mg, 0.015 mmol) was dissolved in TFA (2 mL) at 0 ºC and the solution was then stirred at rt for 12 h and concentrated. The residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 10:1 → 1:3) to give the **3-64** (3 mg, 33%) as a white foam. R_f 0.3 (CH_3OH–NH_4OH 20:1); $[\alpha]_D$ +23.2 (*c* 0.34, CH_3OH); ^1H NMR (400 MHz, D_2O, δ_H) 7.73 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.90 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.86 (d, 1 H, *J* = 4.2 Hz, H-1'), 4.40 (dd, 1 H, *J* = 4.2, 4.6 Hz, H-2'), 4.34 (d, 1 H, *J* = 7.2 Hz, H-4), 4.20-4.18 (m, 2 H, H-3', H-4'), 4.03 (ddd, 1 H, *J* = 3.1, 3.1, 9.0 Hz, H-c), 3.94-3.91 (m, 2 H, H-3, H-7), 3.81-3.75 (m, 2 H, H-b, H-8), 3.57 (d, 1 H, *J* = 6.4 Hz, H-2), 3.50 (dd, 1 H, *J* = 7.7, 11.6 Hz, H-8), 3.26 (dd, 1 H, *J* = 3.1, 12.7 Hz, H-d), 3.13-3.02 (m, 2 H, H-d, H-5'), 2.95 (dd, 1 H, *J* = 6.7, 14.2 Hz, H-5'), 2.86 (dd, 1 H, *J* = 5.0, 13.8 Hz, H-a), 2.78 (dd, 1 H, *J* = 8.2, 13.8 Hz, H-a), 1.71 (dd, 1 H, *J* = 4.1, 8.9 Hz, H-5), 0.92 (dd, 1 H, *J* = 5.6, 8.9 Hz, H-6), 0.84 (dd, 1 H, *J* = 4.1, 5.6 Hz, H-6); ^{13}C NMR

(100 MHz, D_2O, δ_C) 166.6 (C=O), 151.9 (C=O), 142.4 (CH=), 102.7 (CH=), 90.5 (C-1'), 83.1(C-4'), 77.9 (C-2'), 75.1 (C-3'), 73.3, 72.2, 71.7, 71.3, 63.9, 59.5 (C-2), 49.4 (C-d), 35.3 (C-5), 34.0 (SCH_2), 33.1 (SCH_2), 23.5 (C-1), 8.5 (C-6); HRMS (ESI) *m/z* Calcd for ($M–CF_3COO^-$) $C_{21}H_{34}N_3O_{11}S$: 536.1909. Found: 536.1906

3.6 Bibliography

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Chapter 4

Synthesis of Galactofuranose Sulfonium Ion Analogues

4.1 Introduction

This chapter describes the synthesis of a series of sulfonium ion analogues of UDP-Gal*f* (**1-1**) with the general structure **1-17** (Figure 4-1), which are potential inhibitors of mycobacterial galactofuranosyltransferases. As discussed in Chapter 1, sulfonium ion compounds have been used previously as inhibitors of carbohydrate-recognizing enzymes. This series of compounds was designed to mimic the oxacarbenium ion of the transition state of glycosylation. Retrosynthesis analysis of the target molecules suggest that the most direct route to a number of sulfonium ion analogues was through an intermolecular S_N2 reaction between an intermediate cyclic sulfide (**4-1**) with the appropriate electrophile (Figure 4-1).

In designing the cyclic scaffold to build the analogues upon, it was necessary to choose a ring system in which both the ring oxygen and the hydroxyl group on C2 were removed. Otherwise, the sulfonium ion species would not be stable. Given these structural limitations, the five-membered ring is substituted at the two carbons furthest away from the sulfur center. This arrangement would be expected to have less of a stereodirecting effect on the S_N2 reaction; thus, the

sulfonium ions should be obtained in a mixture of steroisomers at sulfur. This phenomenon has been reported previously in several papers.[1-3]

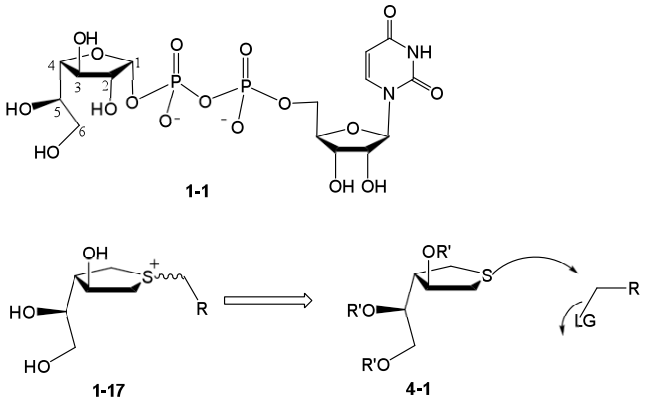


Figure 4-1. Structure of **1-1** and retrosynthetic analysis of sulfonium ion target compounds

There are a number of possibilities for the choice of alkylating agents. It has been reported previously that alkyl perchlorates[4] and triflates[5] are good electrophiles in the synthesis of sulfonium ions because the leaving groups are weakly nucleophilic anions, which reduce the possibility of decomposition of the sulfonium ion via nucleophilic substitution reactions.[1, 6, 7] In addition, alkyl iodides have been reported to be good reagents for the synthesis of *S*-alkylated sulfonium ions.[8] If these reactions are carried out in the presence of a silver salt (e.g., $AgBF_4$), the iodine ion liberated in the reaction can be precipitated as AgI and thus is unable to act as a nucleophile. Based upon these considerations,

when preparing the target molecules, we chose alkyl iodides as the alkylating agents because of their stability and easy preparation. The other partner in this coupling reaction is a cyclic sulfide of the general type **4-1**. We envisioned (Figure 4-2) that this compound could be synthesized by a ring forming reaction of a dimesylate with sodium sulfide. This dimesylate could, in turn, be obtained from D-arabinitol.

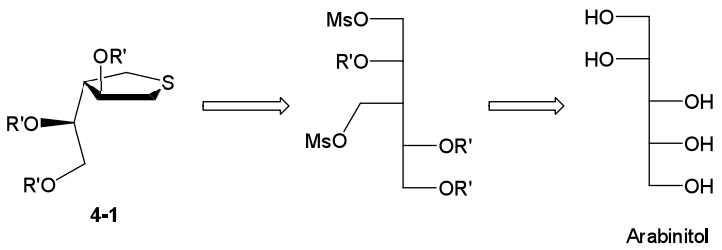


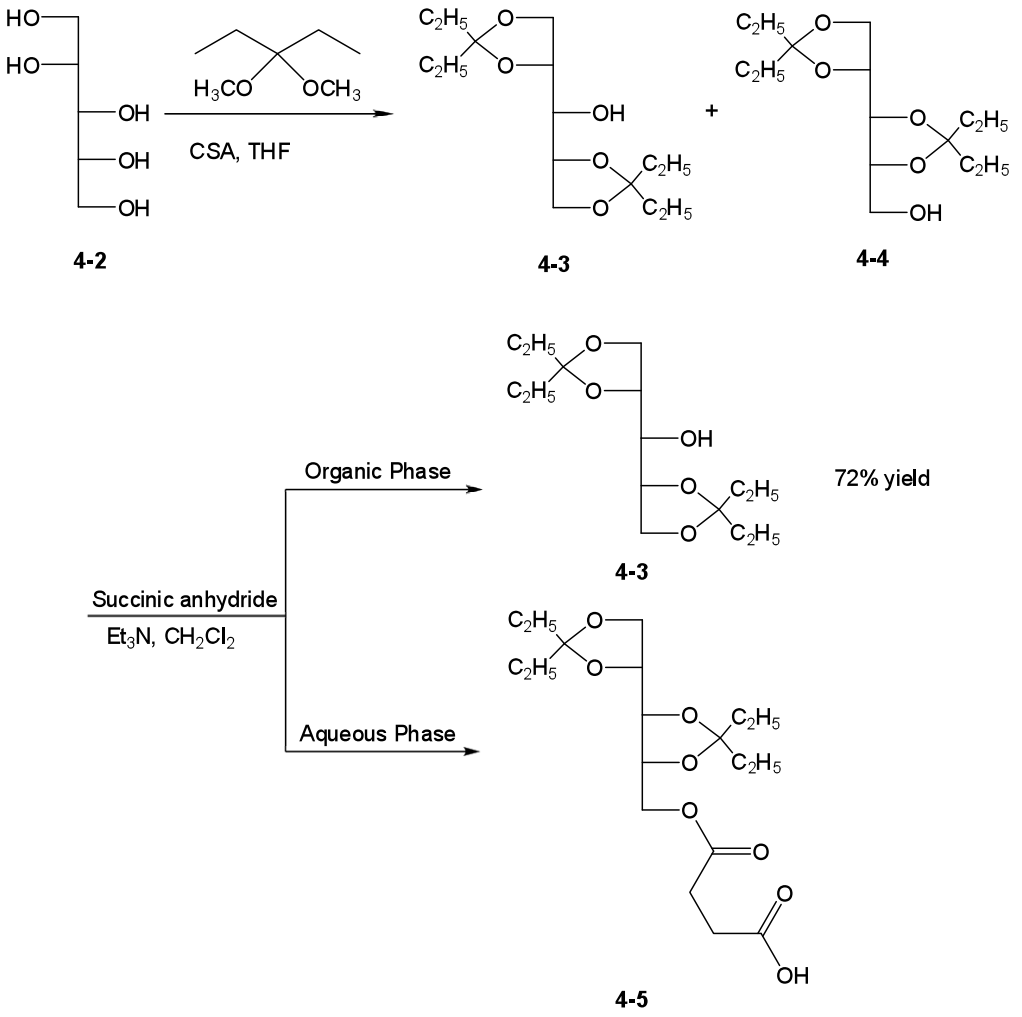
Figure 4-2. Retrosynthetic analysis of **4-1**.

4.2 Results and discussion

4.2.1 Synthesis the cyclic sulfide

The synthesis of the cyclic sulfide required for the preparation of the sulfonium ions was achieved in a nine-step sequence, starting from commercially available D-arabinitol. In order to install the carbon branch at C-3, the key point in the synthesis was to protect other four hydroxyl groups selectively. Earlier work by Linclau et al. has reported the successful conversion of L-arabinitol into

1,2:4,5-Di-*O*-(3,3-pentylidene)arabitol.[9] The same methodology was used on D-arabinitol as shown in Scheme 4-1.

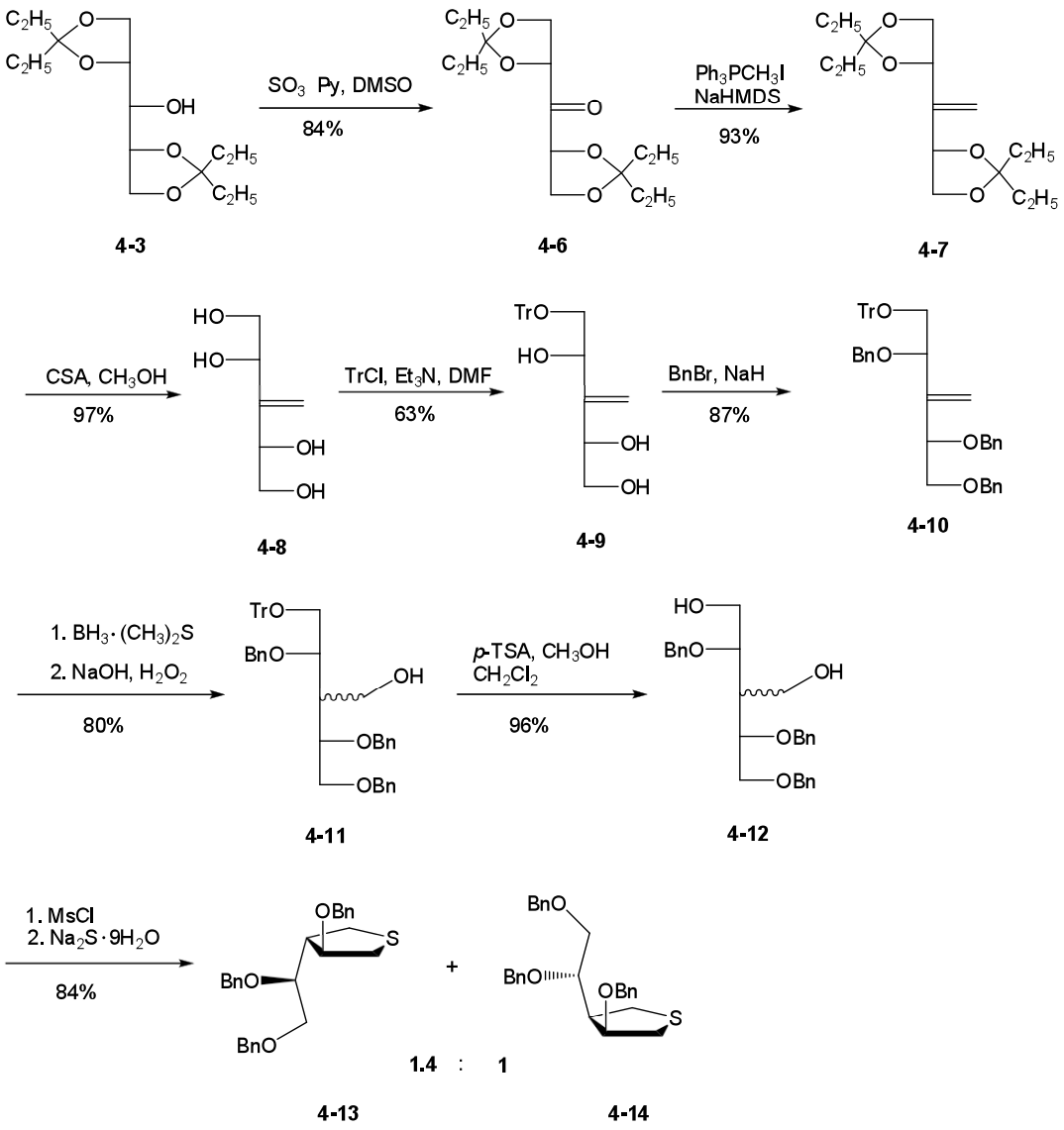


Scheme 4-1. Synthesis of **4-3**.

First, reaction of D-arabinitol (**4-2**) with 3,3-dimethoxypentane in the presence of camphorsulfonic acid afforded an inseparable mixture of the two isomeric diacetals **4-3** and **4-4**. Separation of these compounds was achieved by

treating the mixture with succinic anhydride under basic conditions, which resulted in esterification of the primary hydroxyl group in **4-4**, while leaving the secondary hydroxyl group in **4-3** untouched. Thus, compound **4-4** was converted into **4-5**, which when reacted with $NaHCO_3$ gave a water soluble compound, while **4-3** remained organic soluble. Thus pure **4-3** was obtained in 72% yield over two steps.

The synthesis of cyclic sulfide is shown in Scheme 4-2. Oxidation of **4-3** with SO_3·pyridine complex in DMSO gave the C_2-symmetrical ketone **4-6** in 84% yield. Subsequent reaction with methyl triphenylphosphonium iodide and NaHMDS proceeded without incidence, yielding olefin **4-7** in 93% yield. Cleavage of the acetal protecting groups from **4-7** was achieved upon reaction with camphorsulfonic acid, providing compound **4-8** in 97% yield. This tetrol was then reacted with one equivalent trityl chloride to produce the monoprotected compound **4-9** in 63% yield. The ditrityl ether was formed also in this reaction; however, it is easily separated from the target by chromatography. The remaining hydroxyl groups were then protected as benzyl ethers by reaction of **4-9** with benzyl bromide and NaH. The product, **4-10**, was generated in 87% yield. With a route developed for the efficient preparation of alkene **4-10**, we had the substrate required for the key reaction in the synthesis, the introduction of the branching hydroxymethyl group, as outlined below.



Scheme 4-2. Synthesis of cyclic sulfide **4-13** and **4-14**

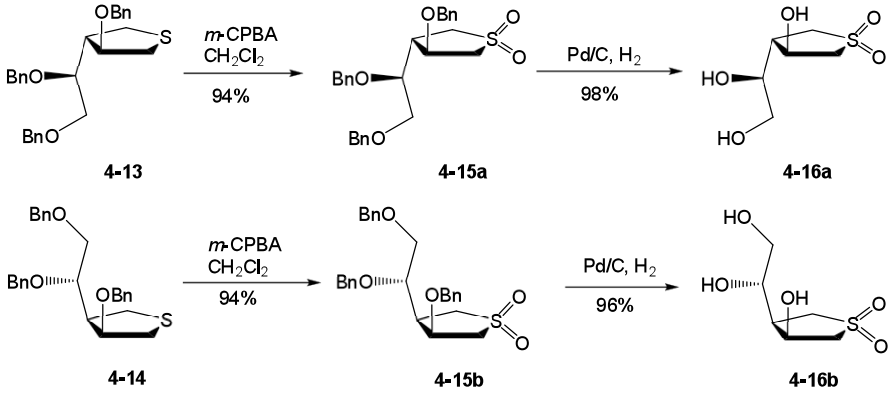
In order to convert the alkene into a hydroxymethyl group, hydroboration was used. We initially explored the use of borane–dimethylsulfide ($BH_3 \cdot S(CH_3)_2$) complex, which gave an organoborane that was subsequently oxidized to the alcohol by treatment with H_2O_2 under basic conditions.

Although the reaction proceeded to give the product in good yield (80%), an inseparable 1:1.4 mixture of stereoisomers **4-11** was produced. To try to improve the stereoselectivity, other borane reagents (BH_3·THF, BH_3·pyridine, or BH_3·NEt_3) were explored in this reaction, but none gave better results. Bulky hydroboration reagents – 9-BBN, disiamylborane and thexylborane – were also investigated to improve the stereoselectivity of the reaction. Unfortunately, with these more hindered reagents, the conversion of the olefin into the organoborane was not successful.

Having installed the hydroxymethyl group, the next challenge was the separation of the two stereoisomers and their conversion to a cyclic sulfide. First, compound **4-11** was treated with *p*-toluenesulfonic acid, which led to the cleavage of the trityl ether giving stereoisomers **4-12** in a combined 96% yield. These two isomers were also inseparable and thus treatment of the diol with methanesulfonyl chloride and triethylamine in CH_2Cl_2 was carried out. This reaction produced the expected mesylated product, which was not purified but instead was directly treated with sodium sulfide nonahydrate (Na_2S·$9H_2O$) in DMF at 100 ^{0}C to form a mixture of the cyclic sulfides **4-13** and **4-14,** which were separable. In carrying out the ring forming reaction it was necessary to warm the solution of the mesylate and Na_2S·$9H_2O$ up to 100 ^{0}C slowly. Under these conditions, the Na_2S·$9H_2O$ dissolves completely before the displacement reaction is initiated. It was found that the substituted tetrahydrofuran ring was generated

from the reaction of water and mesylate when the solution was rapidly heated to 100 °C. The combined yield of **4-13** and **4-14** was 84% and the ratio between the two compounds is 1.4:1.

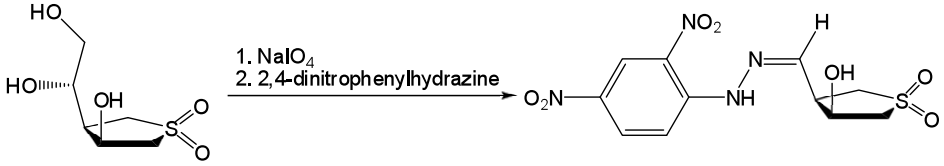
Determining the relative stereochemistry between two substituents on the cyclic thioethers **4-13** and **4-14** was not possible using NMR spectroscopy (Scheme 4-3). Therefore, further modifications were made to **4-13** and **4-14** in order to distinguish them. After oxidation to the sulfone with *m*-CPBA and hydrogenolysis by hydrogen over palladium on charcoal, compounds **4-16a** and **4-16b** were obtained in over 90% yield over the two steps.



Scheme 4-3. Synthesis of **4-16a** and **4-16b**

Neither **4-16a** nor **4-16b** was crystalline. Further modification of these compounds was therefore explored. Thus, oxidation of **4-16b** with $NaIO_4$ formed the corresponding aldehyde, which was condensed with

2,4-dinitrophenylhydrazine to give a yellow solid **4-17** (Scheme 4-4). The solid was recrystallized from methanol and acetone, and was analyzed using X-ray crystallography. From the crystal structure of **4-17** (Figure 4-3), it was possible to determine that the two substituents on the ring are *syn* to each other. Given this result, it is can be inferred that the stereochemistry of the two ring substituents in **4-13** is *anti*. Therefore, **4-13** has the correct "galactofuranose" stereochemistry, and this compound was used in the coupling reactions with alkyl iodides to produce the sulfonium ion compounds (below).



4-16b **4-17**

Scheme 4-4. Synthesis of the crystalline derivative **4-17**.

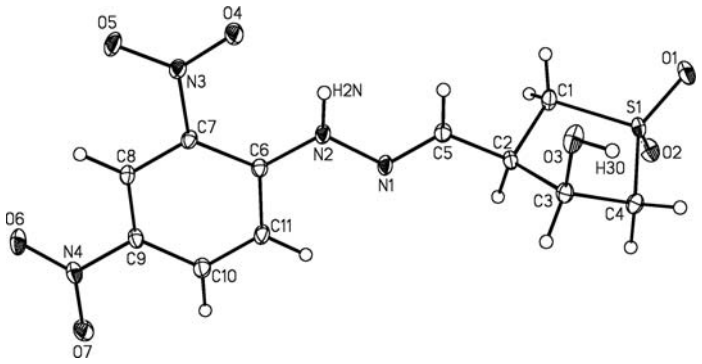


Figure 4-3. Crystal structure of **4-17**.

4.2.2 Synthesis of sulfonium ion compounds

4.2.2.1 Selection and preparation of alkyl iodides

A range of alkyl iodides and bromides were selected to prepare a diverse group of sulfonium ion analogues that could be explored as potential inhibitors of mycobacterial galactofuranosyltransferases. Alkyl iodides **4-18**, **4-19** and **4-20** and alkyl bromide **4-21** (Figure 4-4) are commercially available and their use would lead to a panel of simple sulfonium ions with different branching patterns.

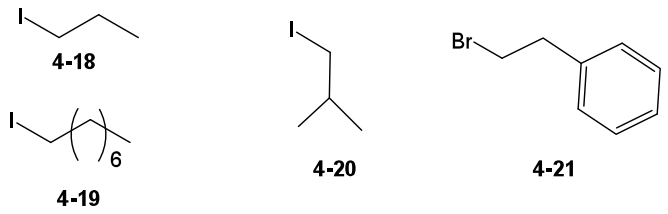
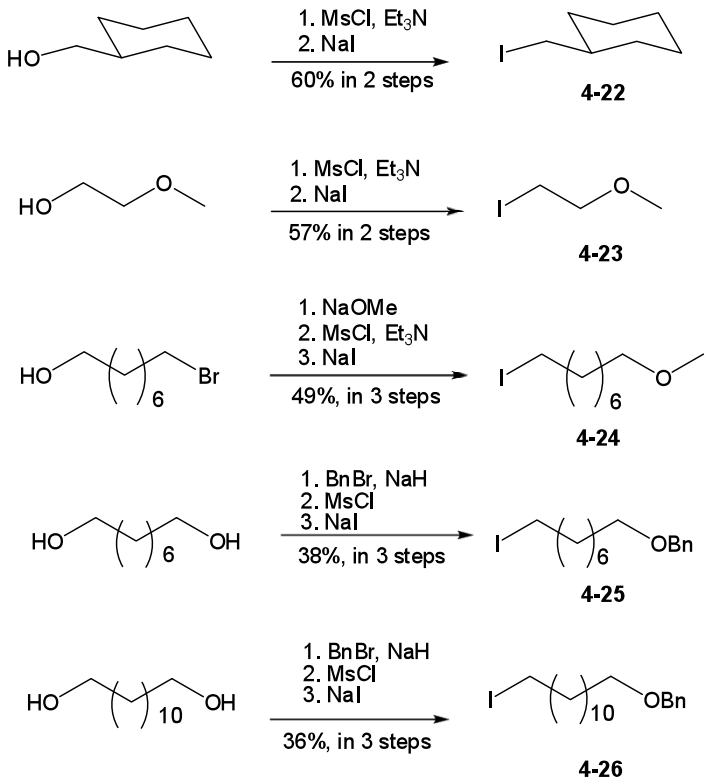


Figure 4-4. Commercial available halides

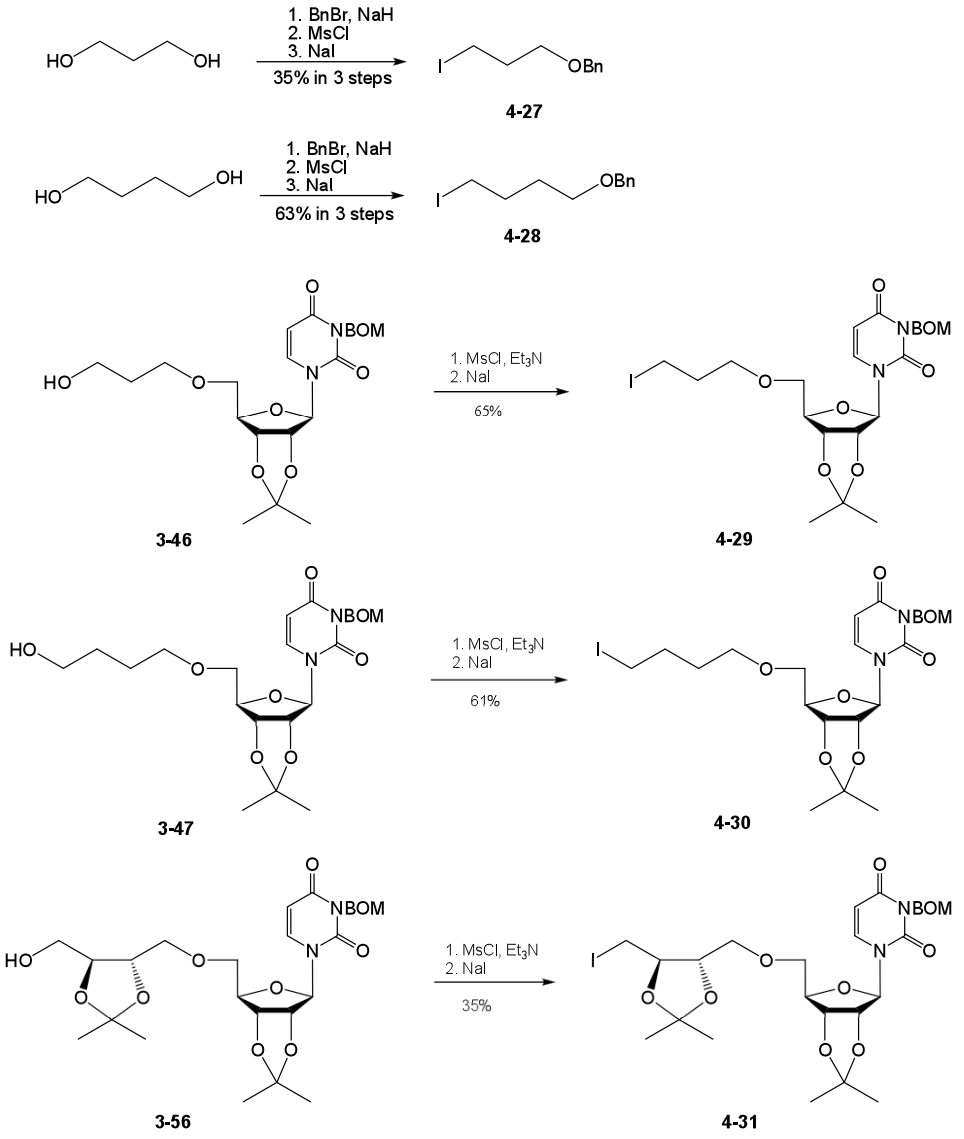
The other alkyl iodides chosen were synthesized in 2–3 steps from commercially-available materials as illustrated in Schemes 4-5, 4-6. Compound **4-22**, containing a branched alkyl group, was synthesized in two steps from cyclohexylmethanol. Reaction of the primary alcohol with methylsulfonyl chloride and triethylamine in CH_2Cl_2 afforded the mesylated product, which was then treated with freshly fused sodium iodide in acetone at 50 ºC to give **4-22** in 60% overall yield. Compounds **4-23** and **4-24** have an oxygen atom in the chain, which may form hydrogen bonding interaction with the enzymes. Iodide **4-23**

was obtained in 57% yield from 2-methoxyethanol via the mesylation–iodination sequence described above. Compound **4-24** was obtained in three steps,[8] by first heating 8-bromooctan-1-ol at reflux in sodium methoxide solution to afford the 8-methoxyoctan-1-ol, which was then mesylated and iodinated giving **4-24** in 49% yield over three steps. Iodides **4-25** and **4-26**, which will give sulfonium ion analogs with a hydroxyl group following hydrogenolysis were synthesized through mono-benzylation of the appropriate diol, mesylation and iodination. The products, **4-25** and **4-26**, were obtained in 38% and 36% yield, respectively.



Scheme 4-5. Synthesis of alkyl iodides **4-22**–**4-26**

In addition, to take advantage of the compounds prepared in Chapter 3, iodides **4-27**–**4-31** were synthesized as shown in Scheme 4-6.



Scheme 4-6. Synthesis of iodides **4-27**–**4-31**

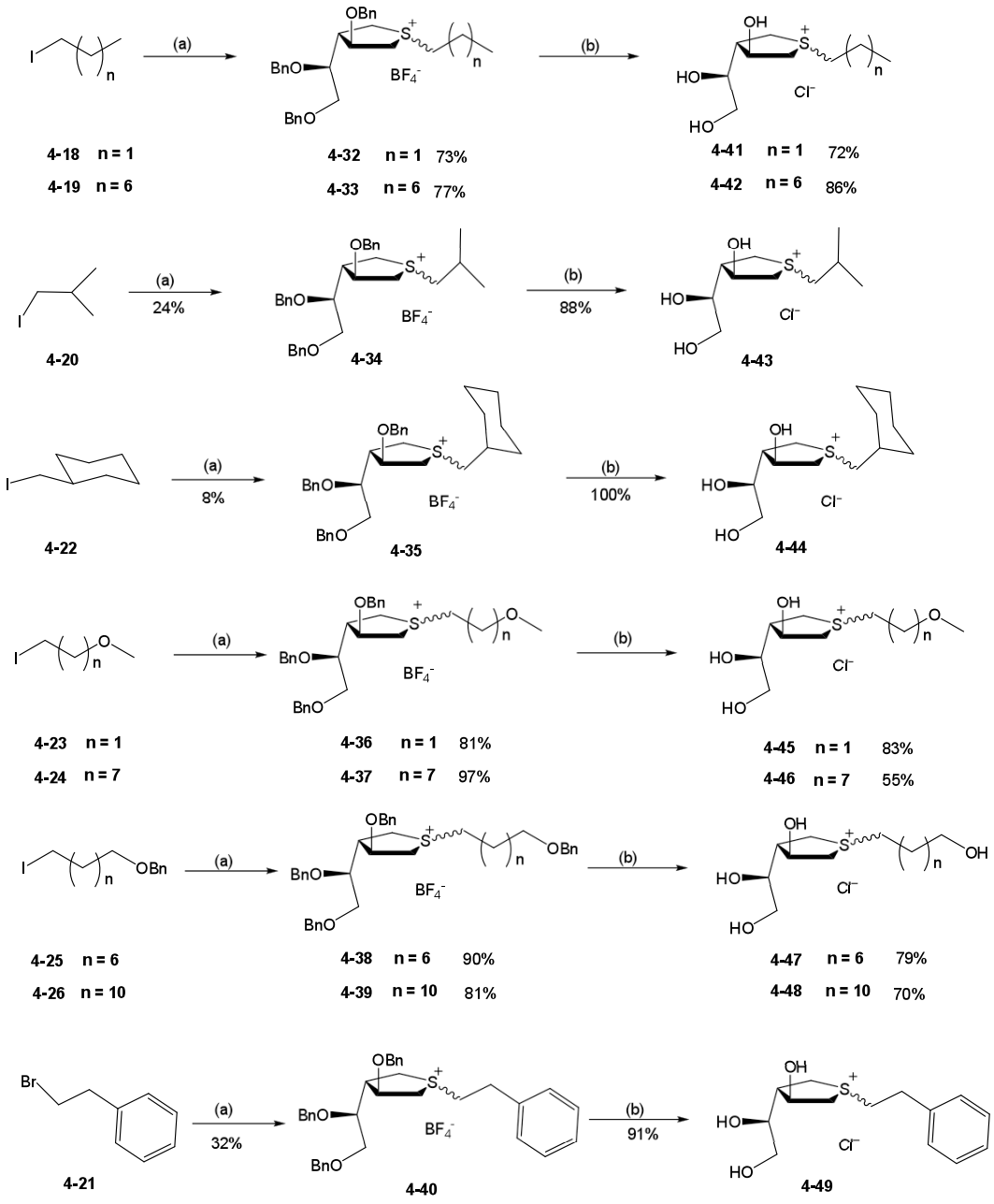
Using the method developed or the synthesis of **4-25** (above), compound **4-27** and **4-28** were obtained in 35% and 63% yield, respectively from the corresponding diols. Compounds **4-29**, **4-30** and **4-31** were synthesized by mesylation and iodination of **3-46**, **3-47** and **3-56**, respectively. The yields of **4-29** are **4-30** are moderate, about 60%, while the yield of **4-31** is low at 35%. The iodination step used for preparing **4-31** was carried out in a sealed vessel at 80 ^{o}C. The yield was much lower at 50 ^{o}C. The low yield may be due to the steric hindrance of the isopropylidene, which reduces the conversion of the intermediate mesylate to the iodide. Unreacted mesylate was observed after the reaction.

4.2.2.2 Coupling and deprotection reactions.

The coupling reactions of the alkyl iodides and the cyclic sulfide were carried out as reported by Mohan et al.,[8] by reacting the two substrates in CH_3CN in the presence of $AgBF_4$ at 65 ºC to give the sulfonium ion tetrafluoroborate salt. Subsequent, removal of the benzyl groups from the protected sulfonium ions was carried out upon treatment with boron trichloride (1 M in CH_2Cl_2) at –78 ºC. After cleavage of the benzyl ethers, the products were treated with Amberlyst resin (Cl^- form) to convert the tetrafluoroborate salt to the corresponding chloride salt. The results are presented in Scheme 4-7, 4-8 and 4-9. As shown, the coupling reactions gave mixture of stereoisomers, as anticipated. Alkylation led,

to a mixture of *R*/*S* stereoisomers on the sulfur atom, and the NMR spectrum of the products showed two sets of signals. The ratios of stereoisomers ranged from 60:40 to nearly a single products. Separation of these isomers was impossible and so they were characterized and tested as mixtures. It was also not possible to determine the structure of the major isomer by NMR spectroscopy and none of the compounds were crystalline solids.

As shown in Scheme 4-7, the coupling reaction of cyclic sulfide **4-13** and unhindered primary alkyl iodides gave the sulfonium ion products in good yield, which ranged from 73% to 97%. In contrast, the more hindered alkyl iodides, **4-20** and **4-22**, gave much poorer yield of the coupled product, 24% and 8%, respectively. Being less reactive than the corresponding iodide, 1-bromo-2-phenylethane (**4-21**) was converted into compound **4-40** in 32% yield.



Scheme 4-7. Synthesis of sulfonium ion analogues **4-41**–**4-49**. Conditions: (a) **4-13**, $AgBF_4$, CH_3CN, 65 °C; (b) BCl_3 1M in CH_2Cl_2, then Amberlyst resin (Cl^-)

In carrying out these coupling reactions, it was necessary to carry them out under an Ar atmosphere in order to minimize the formation of the sulfoxide byproduct (Figure 4-5). This byproduct, presumably resulting from air oxidation of the sulfide, was very difficult to separate from the sulfonium ion target molecules. In the purification of the products, it was found that a mixture of toluene and methanol was the best eluant system.

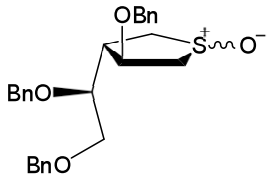
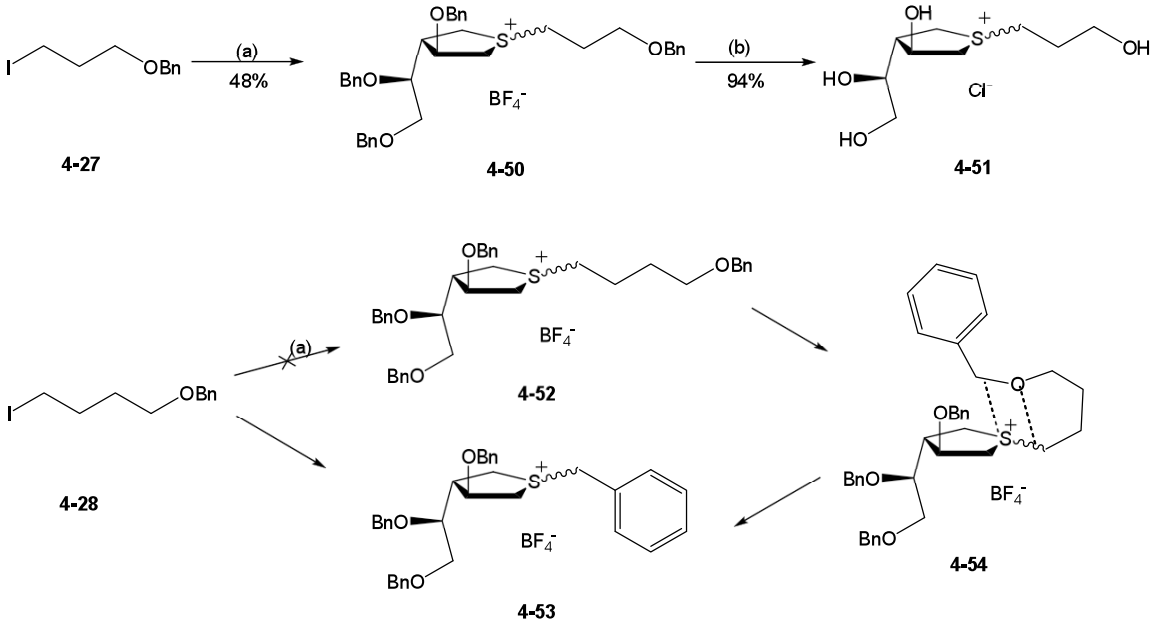


Figure 4-5. Sulfoxide byproduct formed in coupling reactions between **4-13** and alkyl iodides

The deprotection of benzyl groups proceeded smoothly upon reaction with BCl_3 in CH_2Cl_2 at –78 °C. The modest yields in some cases is due to losses during purification. It is necessary to purify these highly polar compounds to remove brown impurities that were present in the BCl_3 solution. The chromatography can be performed on Iatrobeads, while eluting with CH_2Cl_2–CH_3OH in ratios from 5:1 to 1:1.

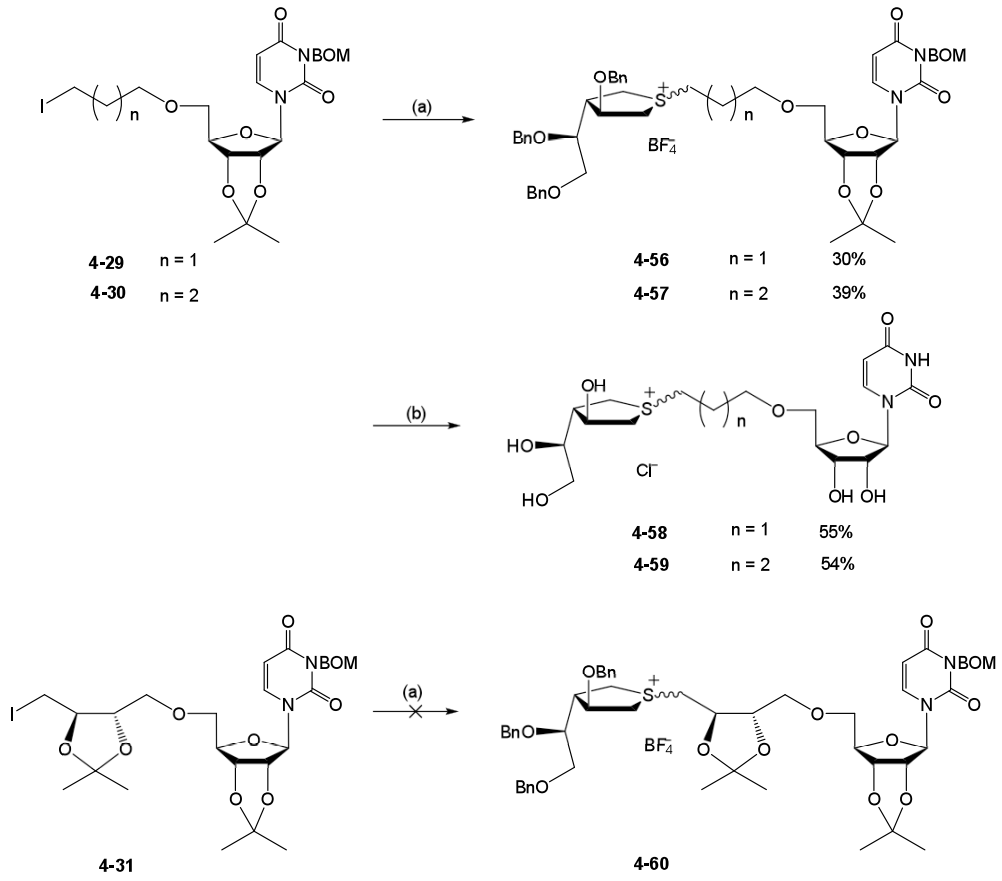
We next explored reactions of alkyl iodides **4-27** and **4-28** with cyclic sulfide **4-13** (Figure 4-8). It was found that reaction of compound **4-27** with **4-13**

proceeded as expected giving **4-50**, which was treated with BCl_3 to give the sulfonium ion compound **4-51** in 45% yield over two steps. However, when **4-28** was treated with **4-13**, the sulfonium ion compound **4-52** was not formed; instead, **4-53** was isolated. We propose that the formation of **4-53** proceeds via a transition state such as **4-54**, which gives the benzylic sulfonium ion and THF. A similar reaction was reported earlier by Pinto and co-workers.[10]



Scheme 4-8. Synthesis of sulfonium ion analogue **4-51** and attempted synthesis of **4-52**. Conditions: (a) **4-13**, $AgBF_4$, CH_3CN, 65 °C; (b) BCl_3 1 M in CH_2Cl_2, then Amberlyst resin (Cl^-)

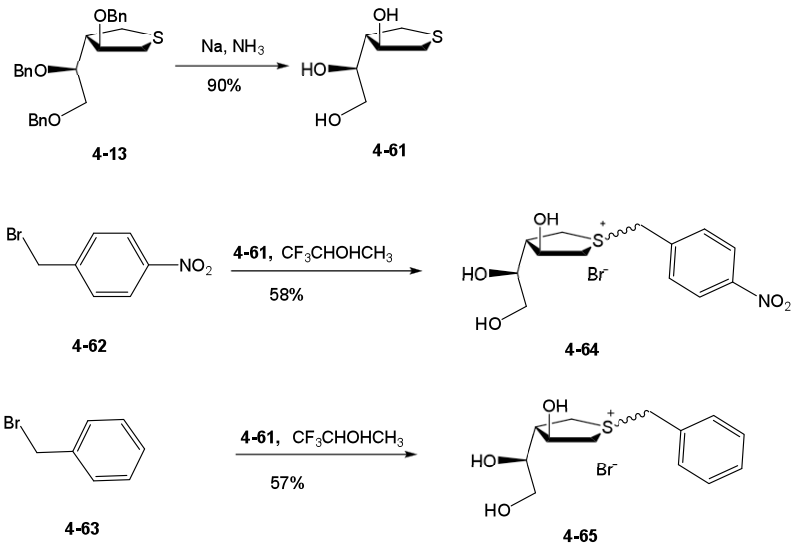
The preparation of analogues **4-58** and **4-59**, which include the uridine moiety, is shown in Figure 4-9. The uridine iodides **4-29** and **4-30** were reacted with **4-13** under the same conditions as those described above to form sulfonium ion compounds **4-56** and **4-57** in 30% and 39% yield, respectively. Attempts to increase the yield by changing reaction time and reaction temperature were unsuccessful. Compounds **4-56** and **4-57** were then debenzylated and the BOM group was cleaved upon treatment with BCl_3. The products **4-58** and **4-59** were obtained in moderate yields of 55% and 54%, respectively. When the same strategy was performed on iodide **4-31**, the coupling reaction was unsuccessful. A number of products were visible by TLC and none of the desired **4-60** was obtained, nor was starting material recovered.



Scheme 4-9. Synthesis of sulfonium ion analogues **4-58** and **4-59** and attempted synthesis of **5-60**. Conditions: (a) **4-13**, $AgBF_4$, CH_3CN, 65 °C; (b) BCl_3 1M in CH_2Cl_2, then Amberlyst resin (Cl^-)

Finally, we wanted to synthesize sulfonium ion analogues containing benzylic groups (e.g., **4-64**, Scheme 4-10). However, considering that these groups will be cleaved during deprotection of the benzyl ethers with BCl_3, an alternate approach was needed. Thus, we chose to debenzylate **4-13** and then couple the product with the appropriate alkyl halides. As illustrated in Scheme 4-10, compound **4-13** was converted into **4-61** by Birch reduction in 90% yield.

Subsequent reaction with *p*-nitrobenzyl bromide **4-62** in 1,1,1,3,3,3-hexafluoro-isopropanol at 50 ºC afforded **4-64** in 58% yield. Using the same conditions, reaction of **4-61** with benzyl bromide produced **4-65** in 57% yield.



Scheme 4-10. Synthesis of **4-64** and **4-65**.

4.2.3 Conclusions

In summary, we have synthesized a panel of sulfonium ion analogs that were intended to be inhibitors of mycobacterial galactofuranosyltransferaes. The key step in the synthesis of the targets was the reaction of a cyclic sulfide (e.g., **4-13**) with an alkyl halide (usually an iodide). The coupling reaction proceeded in modest to excellent yield for unhindered primary halides; not surprisingly, more sterically hindered derivatives gave poorer yields of the compounds. Compound **4-13** was obtained in from D-arabinintol in nine steps using a straightforward

synthesis that is amenable for scale up. The testing of these compounds as potential inhibitors of mycobacterial galactofuranosyltransferases is described in Chapter 5.

4.3 Experimental section

General Methods

All reagents used were purchased from commercial sources and were used without further purification. Solvents used in reactions were predried by PURESOLV-400 System from Innovative Technology Inc. Unless stated otherwise, all reactions were monitored by TLC on silica gel G-25 UV_{254} (0.25 mm, Macherey-Nagel). Spots were detected under UV light and/or by charring with acidified ethanolic anisaldehyde. Solvents were evaporated under reduced pressure and below 50 ºC (water bath). Column chromatography was performed on silica gel 60 (40–60 μm). The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo). ^{1}H NMR spectra were recorded on VARIAN INOVA-NMR spectrometers at 400 or 500 MHz, and chemical shifts are referenced to $CDCl_3$ (7.26 ppm, $CDCl_3$) or CD_3OD (4.78 ppm, CD_3OD). ^{13}C NMR APT spectra[11, 12] were recorded at 100 or 125 MHz, and chemical shifts are referenced to $CDCl_3$ (77.23 ppm, $CDCl_3$) or CD_3OD (48.9 ppm, CD_3OD). ^{1}H NMR data are reported as though they are first

order, and the peak assignments are made by 2D-NMR spectroscopy (^1H–^1H COSY and HMQC). ESI-MS spectra were recorded on samples suspended in THF or CH_3OH and added NaCl. Optical rotations were measured on Perkin-Elmer 241 Polarimeter with sodium D line (589 nm) and are in units of deg·mL $(dm·g)^{-1}$.

4.3.1 Experimental for synthesis of the cyclic sulfide

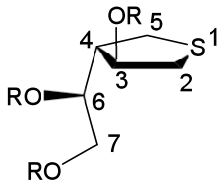
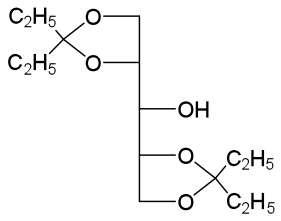


Figure 4-6. Numbering system used for NMR assignments



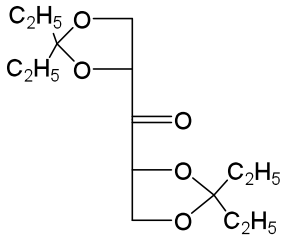
4-3

1,2:4,5-Di-*O*-pentylidene-arabinitol (4-3)

A suspension of D-arabinitol (2.97 g, 20 mmol) and 3,3-dimethoxypentane (12.21 g, 92 mmol) in THF 30 (mL) was heated at reflux for 15 min and then CSA (1.37 g, 6 mmol) was added. The reaction mixture heated at reflux for another 5 min

and the acid was quenched by the addition of 2 M NaOH (6 mL). The mixture was cooled to rt and then poured into a mixture of EtOAc (20 mL) and H_2O (5 mL). The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give a colorless oil. The crude product was then dissolved in CH_2Cl_2 (60 mL) and Et_3N (3 mL). The mixture was heated reflux and then succinic anhydride (0.55 g, 5.5 mmol) was added. The reaction mixture stirred at reflux for 1 h and then $NaHCO_3$ (5% aqueous solution, 30 mL) was added. After cooling, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated. The resulting oil was purified by column chromatography (EtOAc–Hexane 1:8) to give **4-3** (4.11 g, 72%) as a colorless oil. $[\alpha]_D$ +3.8 (*c* 2.08, CH_2Cl_2); R_f 0.31 (EtOAc–Hexane 1:6); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.20 (ddd, 1 H, *J* = 4.9, 6.6, 7.6 Hz, C<u>H</u>), 4.13 (dd, 1 H, *J* = 6.0, 8.0 Hz, C<u>H</u>$_2$O), 4.08 (dd, 1 H, *J* = 6.6, 8.3 Hz, C<u>H</u>$_2$O), 3.99 (ddd, 1 H, *J* = 6.0, 6.5, 7.9 Hz, C<u>H</u>), 3.92 (dd, 1 H, *J* = 6.5, 8.0 Hz, C<u>H</u>$_2$O), 3.85 (dd, 1 H, *J* = 7.6, 8.3 Hz, C<u>H</u>$_2$O), 3.45 (ddd, 1 H, *J* = 4.9, 5.3, 7.9 Hz C<u>H</u>OH), 2.40 (d, 1 H, *J* = 5.3 Hz, O<u>H</u>), 1.71–1.57 (m, 8 H, 4 × C<u>H</u>$_2$CH$_3$), 0.93–0.86 (m, 12 H, 4 × CH$_2$C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 113.3 (quaternary C), 112.9 (quaternary C), 76.7 (<u>C</u>H), 76.4 (<u>C</u>H), 73.0 (<u>C</u>HOH), 66.9 (<u>C</u>H$_2$O), 66.5 (<u>C</u>H$_2$O), 29.6 (<u>C</u>H$_2$CH$_3$), 29.5 (<u>C</u>H$_2$CH$_3$), 29.0 (<u>C</u>H$_2$CH$_3$), 28.9 (<u>C</u>H$_2$CH$_3$), 8.2 (CH$_2$<u>C</u>H$_3$), 8.1 (CH$_2$<u>C</u>H$_3$), 8.0 (2

× $CH_2\underline{C}H_3$). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{15}H_{28}O_5$: 311.1829. Found: 311.1828.

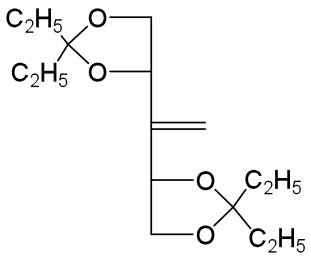


4-6

(2R,4*R*)-1,2:4,5-Bis-(3,3-pentylidenedioxy)-3-pentanone (4-6)

To a mixture of DMSO (40 mL) and Et_3N (7.2 mL, 52 mmol) was added a suspension of SO_3·Pyridine (6.8 g, 43.2 mmol) in CH_2Cl_2 (20 mL). The resulting clear solution was added dropwise to a stirred solution of **4-3** (4.11 g, 14.3 mmol) in CH_2Cl_2 (50 mL) and DMSO (100 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC for 6 h, and then poured into a mixture of saturated NH_4Cl–H_2O–Et_2O (1:1:2, 200 mL). The aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated, and the resulting residue was purified by chromatography (EtOAc–Hexane 1:9) to give **4-6** (3.86 g, 94%) as a colorless oil. $[\alpha]_D$ +74.0 (*c* 2.04, CH_2Cl_2); R_f 0.46 (EtOAc–Hexane 1:6); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.80 (dd, 2 H, *J* = 6.9, 8.0 Hz, 2 × C$\underline{H}$O), 4.29 (dd, 2 H, *J* = 8.0, 8.4 Hz, C$\underline{H_2}$O), 3.96 (dd, 2 H, *J* = 6.9, 8.4 Hz, C$\underline{H_2}$O), 1.72–1.64 (m, 8 H, 4 × $C\underline{H_2}CH_3$), 0.95–0.89 (m, 12 H, 4 × $CH_2C\underline{H_3}$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C)

205.5 (C=O), 114.9 (2 C, 2 × quaternary C), 78.7 (2 C, 2 × CH), 66.2 (2 C, 2 × CH_2O), 29.1 (2 C, 2 × CH_2CH_3), 28.2 (2 C, 2 × CH_2CH_3), 8.1 (2 C, 2 × CH_2CH_3), 8.0 (2 C, 2 × CH_2CH_3). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{15}H_{26}O_5$: 309.1672. Found: 309.1671.

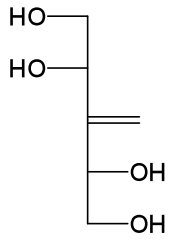


4-7

(4*S*,4'*S*)-4,4'-(ethene-1,1-diyl)bis(2,2-diethyl-1,3-dioxolane) (4-7)

To a solution of methyl triphenylphosphonium iodide (1.60 g, 3.96 mmol) in THF (30 mL) at 0 ºC was added NaHMDS (0.73 g, 3.96 mmol) and then the mixture was stirred for 30 min at rt. The mixture was then cooled to 0 ºC again and a solution of ketone **4-6** (0.95 g, 3.32 mmol) in THF (15 mL) was added slowly. The reaction mixture was stirred for 30 min and then poured into ice cold H_2O and stirred vigorously. The solution was extracted with EtOAc, and the organic phase was washed with brine, dried ($MgSO_4$) and concentrated. The resulting oil was purified by chromatography (EtOAc–Hexane 1:9) to give **4-7** (0.84 g, 93%) as a colorless oil. $[\alpha]_D$ +69.3 (*c* 1.51, CH_2Cl_2); R_f 0.58 (EtOAc–Hexane 1:6); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.32 (dd, 2 H, *J* =1.1, 1.1 Hz, C=CH_2), 4.54 (dd, 2 H, *J* = 6.1, 8.7 Hz, 2 × CHO), 4.20 (dd, 2 H, *J* = 6.1, 7.9 Hz, CH_2O), 3.58

(dd, 2 H, J = 7.9, 8.7 Hz, C<u>H</u>$_2$O), 1.73–1.64 (m, 8 H, 4 × C<u>H</u>$_2$CH$_3$), 0.95–0.91 (m, 12 H, 4 × CH$_2$C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 144.7 (<u>C</u>=CH$_2$), 112.8 (C=<u>C</u>H$_2$), 110.7 (2 C, 2 × quaternary C), 76.2 (2 C, 2 × <u>C</u>HO), 70.0 (2 C, 2 × <u>C</u>H$_2$O), 29.8 (2 C, 2 × <u>C</u>H$_2$CH$_3$), 29.4 (2 C, 2 × <u>C</u>H$_2$CH$_3$), 8.1 (2 C, 2 × CH$_2$<u>C</u>H$_3$), 8.0 (2 C, 2 × CH$_2$<u>C</u>H$_3$). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{16}H_{28}O_4$: 307.1880. Found: 307.1881.

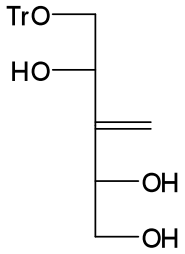


4-8

(2*S*,4*S*)-3-methylenepentane-1,2,4,5-tetraol (4-8)

A solution of **4-7** (1.88 g, 6.62 mmol) and CSA (0.14 g, 0.6 mmol) in CH_3OH (12 mL) and CH_2Cl_2 (6 mL) was heated at reflux for 16 h, cooled to rt, neutralized with Et_3N and then concentrated. The resulting residue was purified by chromatography (CH_2Cl_2–CH_3OH 19:1 → 7:1) to give **4-8** (0.95 g, 97%) as a colorless oil. $[\alpha]_D$ +26.7 (*c* 1.05, CH_3OH); R_f 0.33 (CH_2Cl_2–CH_3OH 5:1); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 5.28 (s, 2 H, C=C<u>H</u>$_2$), 4.14 (dd, 2 H, J = 4.1, 7.3 Hz, 2 × C<u>H</u>OH), 3.60 (dd, 2 H, J = 4.1, 11.4 Hz, 2 × C<u>H</u>$_2$OH), 3.48 (dd, 2 H, J = 7.3, 11.4 Hz, 2 × C<u>H</u>$_2$OH); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 151.1 (<u>C</u>=CH$_2$),

113.0 (C=$\underline{C}H_2$), 74.4 (2 C, 2 × $\underline{C}$HOH), 67.1 (2 C, 2 × $\underline{C}H_2$OH). HRMS (ESI) *m/z* Calcd for ($M+Na^+$) $C_6H_{12}O_4$: 171.0628. Found: 171.0628.

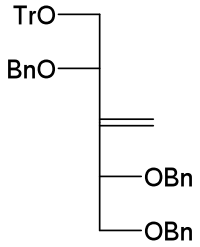


4-9

(2*S*,4*S*)-3-methylene-5-(trityloxy)pentane-1,2,4-triol (4-9)

Compound **4-8** (0.96 g, 6.49 mmol) and DMAP (0.08 g, 0.65 mmol) were dissolved in DMF (30 mL) and Et_3N (1 mL). To this mixture was added a solution of trityl chloride (1.80 g, 6.49 mmol) in DMF (20 mL) dropwise. The reaction mixture was stirred overnight to give a cloudy solution to which CH_3OH (5 mL) was added. The solution was concentrated and the resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 30:1 → 9:1 → 5:1) to give **4-9** (1.60 g, 63%) as an oil. $[\alpha]_D$ +9.4 (*c* 1.86, CH_2Cl_2); R_f 0.63 (CH_2Cl_2–CH_3OH 9:1); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 7.47–7.44 (m, 6 H, Ar), 7.31–7.26 (m, 6 H, Ar), 7.23–7.19 (m, 3 H, Ar), 5.24 (s, 1 H, C=C$\underline{H}_2$), 5.20 (s, 1 H, C=C$\underline{H}_2$), 4.24 (dd, 1 H, *J* = 5.4, 5.7 Hz, C$\underline{H}$OH), 4.03 (dd, 1 H, *J* = 3.5, 7.2 Hz, C$\underline{H}$OH), 3.52 (dd, 1 H, *J* = 3.5, 11.4 Hz, C$\underline{H}_2$OTr), 3.35 (dd, 1 H, *J* = 7.2, 11.4 Hz, C$\underline{H}_2$OTr), 3.21–3.14 (m, 2 H, C$\underline{H}_2$OH), 2.95 (s, 1 H, O$\underline{H}$), 2.84 (s, 2 H, 2 × O$\underline{H}$); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 151.1 ($\underline{C}$=CH_2), 145.5 (Ar × 3), 130.0 (Ar × 6),

128.8 (Ar × 6), 128.1 (Ar × 3), 112.7 (C=$\underline{C}H_2$), 88.2 ($Ph_3\underline{C}$), 74.3 ($\underline{C}$HOH), 72.8 ($\underline{C}$HOH), 69.2 ($\underline{C}H_2$OTr), 67.2 ($\underline{C}H_2$OH). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{25}H_{26}O_4$: 413.1723. Found: 413.1724.

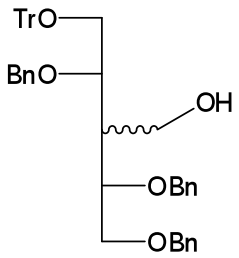


4-10

(2*S*,4*S*)-3-methylene-5-(trityloxy)-1,2,4-tribenzyloxypentane (4-10)

Compound **4-9** (0.094 g, 0.24 mmol) and benzyl bromide (0.15 g, 0.88 mmol) were dissolved in DMF (3 mL) and cooled to 0 ºC. To this mixture was added NaH (36 mg, 0.88 mmol, 60% in mineral oil) slowly and the solution was stirred for 1 h at 0 °C. The excess reagents were then quenched by the addition of H_2O and the solution was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4) and concentrated, and the resulting residue was purified by chromatography (EtOAc–Hexane 1:15) to give **4-10** (0.14 g, 87%) as an oil. $[\alpha]_D$ +27.5 (*c* 0.72, CH_2Cl_2); R_f 0.56 (EtOAc–Hexane 1:4); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.47–7.21 (m, 30 H, Ar), 5.44 (s, 1 H, C=C$\underline{H}_2$), 5.42 (s, 1 H, C=C$\underline{H}_2$), 4.66 (d, 1 H, *J* = 12.1 Hz, PhC$\underline{H}_2$), 4.51–4.35 (m, 4 H, 2 × PhC$\underline{H}_2$), 4.21 (d, 1 H, *J* = 12.1 Hz, PhC$\underline{H}_2$), 3.92 (dd, 1 H, *J* = 3.1, 7.3 Hz, C$\underline{H}$OBn), 3.84 (dd, 1 H, *J* = 3.5, 7.3 Hz, C$\underline{H}$OBn), 3.45 (dd, 1 H, *J* = 7.3, 10.6 Hz, C$\underline{H}_2$OTr), 3.38 (dd, 1 H, *J* = 7.2, 10.4 Hz, C$\underline{H}_2$OBn), 3.34 (dd, 1 H, *J* = 3.5, 10.6 Hz, C$\underline{H}_2$OTr), 3.10 (dd, 1 H, *J* =

3.1, 10.4 Hz, C$\underline{H}_2$OBn); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 144.2 ($\underline{C}$=CH_2), 144.1 (Ar × 3), 138.7 (Ar), 138.4 (Ar), 138.3 (Ar), 128.8 (Ar × 6), 128.4 (Ar × 2), 128.3 (Ar × 2), 128.2 (Ar × 2), 127.7 (Ar × 6), 128.1 (Ar × 2), 127.6 (Ar × 2), 127.5 (Ar × 3), 127.4 (Ar), 127.3 (Ar), 126.9 (Ar × 3), 115.3 (C=$\underline{C}H_2$), 86.8 ($Ph_3\underline{C}$), 79.1 ($\underline{C}$HOBn), 78.6 ($\underline{C}$HOBn), 73.3 ($\underline{C}H_2$), 73.1 ($\underline{C}H_2$), 70.9 ($\underline{C}H_2$), 70.6 ($\underline{C}H_2$), 66.9 ($\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{46}H_{44}O_4$: 683.3131. Found: 683.3131.



4-11

(2*S*,3*S*)-3,4-bis(benzyloxy)-2-((*S*)-1-(benzyloxy)-2-(trityloxy)ethyl)butan-1-ol

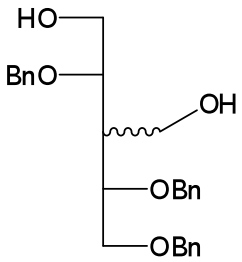
and

(2*R*,3*S*)-3,4-bis(benzyloxy)-2-((*S*)-1-(benzyloxy)-2-(trityloxy)ethyl)butan-1-ol

(4-11)

To a solution of **4-10** (4.33 g, 6.56 mmol) in THF (30 mL) at 0 ºC was added $BH_3{\cdot}SMe_2$ (10.0 mL, 19.8 mmol, 2 M in THF), and then the solution was warmed to rt and stirred for 16 h under argon. The solution was then added dropwise to a mixture of H_2O–THF–2M NaOH–30% H_2O_2 (1:1:3:1.5, 20 mL) at 0 ºC and stirred for 1 h. The mixture was extracted with EtOAc, and the organic layer

was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 1:6) to give **4-11** (3.60 g, 80 %) as a 60:40 inseparable mixture of stereoisomers as a colorless oil. R_f 0.34 (EtOAc–Hexane 1:4). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{46}H_{46}O_5$: 701.3238. Found: 701.3240.

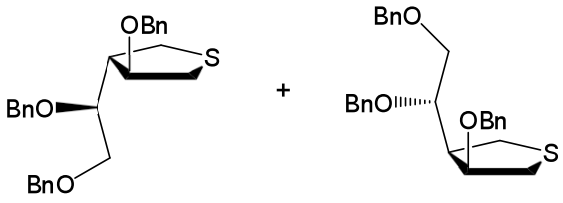


4-12

(2*S*,3*R*)-2-(benzyloxy)-3-((*S*)-1,2-bis(benzyloxy)ethyl)butane-1,4-diol and **(2*S*,3*S*)-2-(benzyloxy)-3-((*S*)-1,2-bis(benzyloxy)ethyl)butane-1,4-diol (4-12)**

To a solution of **4-11** (3.51 g, 5.18 mmol) in CH_2Cl_2 (10 mL) and CH_3OH (10 mL) was added *p*-TsOH (0.35 g, 1.84 mmol). The mixture was stirred for 3 h and was then neutralized with Et_3N before being concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:2) to give **4-12** (1.98 g, 89%) as an inseparable mixture of stereoisomers as a colorless oil. R_f 0.29 (EtOAc–Hexane 1:1); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.22 (m, 15 H, Ar), 4.71 (d, 0.6 H, *J* = 11.5 Hz, C$\underline{H}_2$Ph$_a$), 4.68 (d, 0.4 H, *J* = 11.5 Hz, C$\underline{H}_2$Ph$_b$), 4.63–4.52 (m, 3 H, C$\underline{H}_2$Ph), 4.42 (d, 0.8 H, *J* = 11.5 Hz, C$\underline{H}_2$Ph$_b$), 4.37 (d, 0.6 H, *J* = 11.6 Hz, C$\underline{H}_2$Ph$_a$), 4.34 (d, 0.6 H, *J* = 11.6 Hz, C$\underline{H}_2$Ph$_a$), 4.12 (ddd, 0.6 H, *J* =

2.8, 5.3, 5.3 Hz, C<u>H</u>OBn$_a$), 3.98 (dd, 0.4 H, *J* = 4.8, 8.6 Hz, C<u>H</u>OBn$_b$), 3.95–3.64 (m, 7 H), 2.62 (br, 2 H, O<u>H</u>), 2.12 (dddd, 0.4 H, *J* = 3.6, 3.6, 5.2, 7.2 Hz, CH$_b$), 2.04 (dddd, 0.6 H, *J* = 2.9, 2.9, 4.2, 5.9, CH$_a$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.3 (Ar$_b$), 138.1 (3 C, Ar$_a$ × 2, Ar$_b$), 138.0 (Ar$_b$), 137.9 (Ar$_a$), 128.6 (2 C, Ar$_b$ × 2), 128.5 (4 C, Ar$_a$ × 4), 128.4 (6 C, Ar$_a$ × 2, Ar$_b$ × 4), 128.0 (4 C, Ar$_a$ × 2, Ar$_b$ × 2), 127.9 (Ar × 5), 127.8 (Ar × 5), 127.7 (Ar), 127.6 (Ar × 3), 79.2 (<u>C</u>HOBn$_b$), 78.0 (<u>C</u>HOBn$_a$), 77.8 (<u>C</u>HOBn$_a$), 76.9 (<u>C</u>HOBn$_a$), 73.6 (<u>C</u>H$_2$Ph$_b$), 73.4 (<u>C</u>H$_2$Ph$_a$), 73.0 (<u>C</u>H$_2$Ph$_a$), 72.9 (<u>C</u>H$_2$Ph$_b$), 72.0 (<u>C</u>H$_2$Ph$_b$), 71.7 (<u>C</u>H$_2$Ph$_a$), 71.6 (<u>C</u>H$_2$OBn$_a$), 69.7 (<u>C</u>H$_2$OBn$_b$), 63.4 (<u>C</u>H$_2$OH$_b$), 60.8 (<u>C</u>H$_2$OH$_a$), 60.5 (<u>C</u>H$_2$OH$_b$), 60.1 (<u>C</u>H$_2$OH$_a$), 44.5 (<u>C</u>H$_b$), 43.8 (<u>C</u>H$_a$). HRMS (ESI) *m/z* Calcd for (M + Na$^+$) $C_{27}H_{32}O_5$: 459.2142. Found: 459.2140.



4-13 **4-14**

To a solution of isomers **4-12** (1.85 g, 4.24 mmol) and Et_3N (2 mL) in dry CH_2Cl_2 (40 mL) at –30 ºC was added methanesulfonyl chloride (0.90 mL, 10.32 mmol). The reaction mixture was warmed to 0 ºC steadily and stirred for 1 h and the excess reagent was quenched by the addition of ice. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution (3 × 10 mL), followed by

brine, and then dried (Na_2SO_4) and concentrated. The residue was coevaporated twice with toluene and dissolved in dry DMF (30 mL). To this solution was added $Na_2S·9H_2O$ (1.25 g, 5.21 mmol) and the mixture was heated at 100 ºC for 2 h. After being cooled to rt, the mixture was diluted with Et_2O and the organic layer was washed with H_2O (3 × 10 mL), followed by brine and then dried (Na_2SO_4) and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:10) to give **4-13** (0.76 g) and **4-14** (0.56 g) (1.4:1, 73% in total) both as yellowish oils.

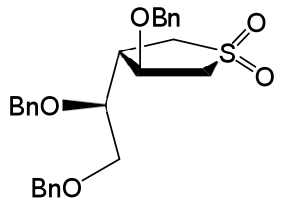
(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)tetrahydrothiophene (4-13)

$[\alpha]_D$ –95.2 (*c* 1.58, CH_2Cl_2); R_f 0.40 (EtOAc–Hexane 1:6); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H); 7.37–7.26 (m, 15 H, Ar), 4.74 (d, 1 H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.55–4.49 (m, 3 H, C$\underline{H}_2$Ph), 4.45 (d, 1 H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.38 (d, 1 H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.07 (ddd, 1 H, *J* = 6.3, 7.9, 7.9 Hz, H-3), 3.86 (ddd, 1 H, *J* = 5.0, 5.0, 5.0 Hz, H-6), 3.60 (dd, 1 H, *J* = 5.0, 10.1 Hz, H-7), 3.56 (dd, 1 H, *J* = 5.0, 10.1 Hz, H-7), 3.04–3.96 (m, 2 H, C$\underline{H}_2$S), 2.84–2.74 (m, 2 H, C$\underline{H}_2$S), 2.42 (dddd, 1 H, *J* = 3.7, 7.9, 8.3, 9.2 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.7 (Ar), 138.1 (Ar × 2), 128.4 (Ar × 6), 128.3 (Ar × 2), 127.8 (Ar × 2), 127.8 (Ar), 127.7 (Ar × 2), 127.6 (Ar), 127.6 (Ar × 2), 127.5 (Ar), 82.0 (C-3), 76.0 (C-6), 73.7(C$\underline{H}_2$Ph), 73.2 (C$\underline{H}_2$Ph), 72.3 (C$\underline{H}_2$Ph), 72.2 (C-7), 50.2 (C-4), 33.8 (C$\underline{H}_2$S),

26.9 ($\underline{C}H_2S$). HRMS (ESI) *m/z* Calcd for ($M + Na^+$) $C_{27}H_{30}O_3S$: 457.1808. Found: 457.1805.

(3*R*,4*R*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)tetrahydrothiophene (4-14)

$[\alpha]_D$ –76.2 (*c* 0.37, CH_2Cl_2); R_f 0.33 (EtOAc–Hexane 1:6); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.37–7.26 (m, 15 H, Ar), 4.71 (d, 1 H, *J* = 11.3 Hz, $C\underline{H}_2Ph$), 4.62 (d, 1 H, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 4.59 (d, 1 H, *J* = 11.8 Hz, $C\underline{H}_2Ph$), 4.55–4.53 (m, 2 H, $C\underline{H}_2Ph$, H-3), 4.45 (d, 1 H, *J* = 11.3 Hz, $C\underline{H}_2Ph$), 4.38 (d, 1 H, *J* = 11.6 Hz, $C\underline{H}_2Ph$), 3.92 (ddd, 1 H, *J* = 2.9, 4.2, 10.0 Hz, H-6), 3.73 (dd, 1 H, *J* = 2.9, 10.6 Hz, H-7), 3.48 (dd, 1 H, *J* = 4.2, 10.6 Hz, H-7), 3.14–3.08 (m, 1 H, $C\underline{H}_2S$), 2.94–2.90 (m, 1 H, $C\underline{H}_2S$), 2.81–2.74 (m, 2 H, $C\underline{H}_2S$), 2.45 (dddd, 1 H, *J* = 3.0, 7.3, 10.0, 12.4 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.7 (Ar), 138.4 (Ar), 138.1 (Ar), 128.4 (Ar × 2), 128.3 (Ar × 4), 127.7 (Ar × 4), 127.6 (Ar), 127.5 (Ar × 4), 81.1 (C-3), 77.4 (C-6), 73.5 ($\underline{C}H_2Ph$), 72.3 ($\underline{C}H_2Ph$), 70.9 ($\underline{C}H_2Ph$), 70.7 (C-7), 51.9 (C-4), 34.8 ($\underline{C}H_2S$), 30.4 ($\underline{C}H_2S$). HRMS (ESI) *m/z* Calcd for ($M + Na^+$) $C_{27}H_{30}O_3S$: 457.1808. Found: 457.1810.

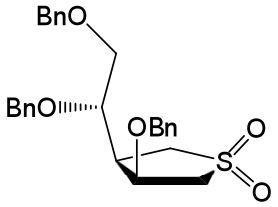


4-15a

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)tetrahydrothiophene 1,1-dioxide (4-15a)

To a solution of **4-13** (0.50 g, 1.15 mmol) in CH_2Cl_2 (15 mL) was added *m*-CPBA, (77%, 0.62 g, 2.76 mmol) at rt and the mixture was stirred for 20 min. The solution was diluted with CH_2Cl_2, and washed with NaOH (1 M). The organic layer was then washed with brine, dried (Na_2SO_4) and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:4) to give **4-15a** (0.50 g, 94 %) as a colorless oil. $[\alpha]_D$ –62.7 (*c* 0.21, CH_2Cl_2); R_f 0.29 (EtOAc–Hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H); 7.39–7.22 (m, 15 H, Ar), 4.66 (d, 1 H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.56–4.48 (m, 2 H, C$\underline{H}_2$Ph), 4.38–4.32 (m, 2 H, C$\underline{H}_2$Ph), 4.26 (d, 1 H, *J* = 11.7 Hz, C$\underline{H}_2$Ph), 4.20 (ddd, 1 H, *J* = 7.8, 7.8, 7.8 Hz, H-3), 3.95 (ddd, 1 H, *J* = 2.5, 5.0, 5.0 Hz, H-6), 3.56–3.47 (m, 2 H, H-7, H-7), 3.39 (dd, 1 H, *J* = 7.8, 13.1 Hz, H-2), 3.25 (m, 2 H, 2 × H-5), 2.99 (dd, 1 H, *J* = 7.8, 13.1 Hz, H-2), 2.70 (dddd, 1 H, *J* = 2.5, 7.8, 9.4, 9.4 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 137.9 (Ar), 137.6 (Ar), 136.9 (Ar), 128.6 (Ar × 2), 128.5 (Ar × 2), 128.4 (Ar × 2), 128.3 (Ar), 128.1 (Ar × 2), 128.0 (Ar × 2), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar × 2), 74.4 (C-3), 73.5 (C$\underline{H}_2$Ph), 73.4 (C-6), 72.6 (C$\underline{H}_2$Ph), 72.4

($\underline{C}H_2Ph$), 70.6 (C-7), 56.1 (C-2), 51.4 (C-5), 45.5 (C-4). HRMS (ESI) *m/z* Calcd for ($M + Na^+$) $C_{27}H_{30}O_5S$: 489.1706. Found: 489.1704.

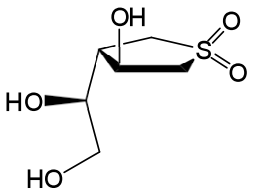


4-15b

(3*R*,4*R*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)tetrahydrothiophene 1,1-dioxide (4-15b)

To a solution of tetrahedrothiophene **4-14** (25.3 mg, 0.061 mmol) in CH_2Cl_2 (3 mL) was added *m*-CPBA (77%, 34.1 mg, 1.53 mmol) at rt and the mixture was stirred for 20 min. The solution was then diluted with CH_2Cl_2, and washed with NaOH (1 M). The organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 1:4) to give **4-15b** (26.6 mg, 94%) as a colorless oil. $[\alpha]_D$ –66.4 (*c* 0.62, CH_2Cl_2); R_f 0.22 (EtOAc–Hexane 1:3); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H); 7.38–7.24 (m, 15 H, Ar), 4.66 (d, 1 H, *J* = 11.4 Hz, $C\underline{H}_2Ph$), 4.59–4.56 (m, 3 H, 2 × $C\underline{H}_2Ph$, H-3), 4.49 (d, 1 H, *J* = 12.1 Hz, $C\underline{H}_2Ph$), 4.38 (d, 1 H, *J* = 11.4 Hz, $C\underline{H}_2Ph$), 4.29 (d 1 H, *J* = 11.4 Hz, $C\underline{H}_2Ph$), 3.88 (ddd, 1 H, *J* = 3.7, 3.7, 9.7 Hz, H-6), 3.65 (dd, 1 H, *J* = 3.7, 10.7 Hz, H-7), 3.47 (d, 1 H, *J* = 14.0 Hz, $C\underline{H}_2SO_2$), 3.39 (dd, 1 H, *J* = 3.7, 10.7 Hz, H-7), 3.14–3.04 (m, 3 H, 3 × $C\underline{H}_2SO_2$), 2.87 (dddd, 1 H, *J* = 2.9, 7.2, 9.7, 13.0 Hz,

H-4); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.1 (Ar), 137.6 (Ar), 136.9 (Ar), 128.6 (Ar × 2), 128.5 (Ar × 4), 127.7 (Ar × 4), 127.6 (Ar), 127.5 (Ar × 4), 76.0 (C-3), 75.2 (C-6), 73.5 ($\underline{C}H_2Ph$), 72.2 ($\underline{C}H_2Ph$), 71.1 ($\underline{C}H_2Ph$), 69.5 (C-7), 59.9 ($\underline{C}H_2SO_2$), 51.6 ($\underline{C}H_2SO_2$), 44.8 (C-4). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{27}H_{30}O_5S$: 489.1706. Found: 489.1702.

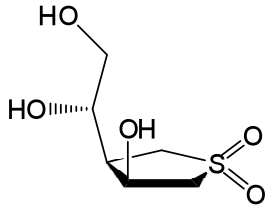


4-16a

(3*R*,4*S*)-3-(hydroxyl)-4-((*S*)-1,2-dihydroxyethyl)tetrahydrothiophene 1,1-dioxide (4-16a)

To a solution of **4-15a** (0.11 g, 0.2 mmol) in CH_3OH (5 mL) and CH_2Cl_2 (1 mL) was added HOAc (10 μL) and Pd–C (10%, 0.01 g). The reaction mixture was stirred under a H_2 atmosphere for 48 h at rt. The solution was filtered and the filtrate concentrated to give **4-16a** (0.048 g, 98%) as a colorless oil. $[\alpha]_D$ –56.9 (*c* 2.60, CH_3OH); R_f 0.22 (CH_2Cl_2–CH_3OH 9:1); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.42 (ddd, 1 H, *J* = 7.9, 7.9, 7.9 Hz, H-3), 3.97 (ddd, 1 H, *J* = 3.2, 6.0, 6.0 Hz, H-6), 3.52–3.31 (m, 3 H, H-2, 2 × H-7), 3.26 (dd, 1 H, *J* = 8.0, 13.3 Hz, H-5), 3.14 (dd, 1 H, *J* = 11.2, 13.3 Hz, H-5), 2.99 (dd, 1 H, *J* = 7.9, 13.3 Hz, H-2), 2.47 (dddd, 1 H, *J* = 3.2, 7.9, 8.0, 11.2 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD, δ_C)

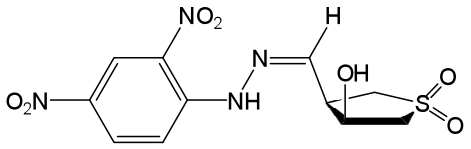
69.7 (C-6), 69.1 (C-3), 66.0 (C-7), 59.3 (C-2), 52.6 (C-5), 48.3 (C-4). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_6H_{12}O_5S$: 219.0298. Found: 219.0298.



4-16b

(3*R*,4*R*)-3-(hydroxyl)-4-((*S*)-1,2-dihydroxyethyl)tetrahydrothiophene 1,1-dioxide (4-16b)

To a solution of **4-15b** (0.39 g, 0.8 mmol) in CH_3OH (15 mL) and CH_2Cl_2 (3 mL) was added HOAc (50 μL) and Pd–C (10%, 0.04 g). The solution was stirred under a H_2 atmosphere for 20 h at rt. The solution was filtered and the filtrate concentrated to give **4-16b** (0.16 g, 96%) as a colorless oil. $[\alpha]_D$ –31.6 (*c* 1.31, CH_3OH); R_f 0.20 (CH_2Cl_2–CH_3OH 9:1); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.77 (dd, 1 H, *J* = 3.3, 3.5 Hz, H-3), 3.82 (m, 1 H, H-6), 3.62 (dd, 1 H, *J* = 4.0, 11.5 Hz, H-7), 3.51 (dd, 1 H, *J* = 5.1, 11.5 Hz, H-7), 3.39–3.19 (m, 2 H, 2 × H-2), 3.12 (d, 2 H, *J* = 10.3 Hz, 2 × H-5), 2.53 (dddd, 1 H, *J* = 3.3, 10.3, 10.3, 10.3 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 71.3 (C-6), 69.3 (C-3), 66.0 (C-7), 62.7 (C-2), 52.1 (C-5), 46.1 (C-4). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_6H_{12}O_5S$: 219.0298. Found: 219.0298.



4-17

(3*R*,4*R*)-3-(hydroxyl)-4-((2-(2,4-dinitrophenyl)hydrazinylidene)methyl)tetra-hydrothiophene 1,1-dioxide (4-17)

To a solution of **4-16b** (0.10 g, 0.51 mmol) in THF (3 mL) and H_2O (0.5 mL) was added $NaIO_4$ (0.16 g, 0.76 mmol) and $NaHCO_3$ (0.09 g, 1.0 mmol). The mixture was stirred for 30 min and then the precipitate was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (CH_2Cl_2–CH_3OH 20:1). The product (R_f 0.5 (CH_2Cl_2–CH_3OH 9:1)) was dissolved in CH_3OH and a solution of (2,4-dinitrophenyl)hydrazine (1 M, 1 mL) was added. After stirring for 10 min, the mixture was concentrated and the resulting residue was purified by chromatography (CH_2Cl_2–CH_3OH 19:1) to give **4-17** (51 mg, 30%) as a yellow solid. This material was recrystallized from CH_3OH and acetone. $[\alpha]_D$ +4.9 (*c* 0.07, acetone); R_f 0.23 (CH_2Cl_2–CH_3OH 19:1); ^{1}H NMR (500 MHz, CD_3COCD_3, δ_H) 8.97 (d, 1 H, *J* = 2.7 Hz, Ar), 8.39–8.36 (m, 1 H, Ar), 8.09–8.06 (m, 2 H, Ar, H-6), 4.98 (m, 1 H, H-4), 3.64–3.59 (m, 2 H, H-3, H-2), 3.44 (dd, 1 H, *J* = 5.0, 13.8 Hz, H-5), 3.33 (m, 1 H, H-2), 3.25 (dd, 1 H, *J* = 1.6, 13.8, Hz, H-5); ^{13}C NMR (125 MHz, DMSO, δ_C) 150.7 (N=CH), 144.5 (Ar), 136.8 (Ar), 129.6 (Ar), 129.0 (Ar), 122.8 (Ar), 116.4 (Ar), 68.7 (C-3), 60.7 (C-2),

50.6 (C-5), 44.9 (C-4). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{11}H_{12}N_4O_7S$: 367.0319. Found: 367.0321.

4.3.2 Experimental for synthesis of sulfonium ions

General procedure for the mesylation and iodination

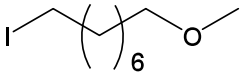
The alcohol (1.0 equiv.) was dissolved in CH_2Cl_2 (10 mL) at 0 ºC before Et_3N (1 mL) and MsCl (1.2 equiv.) were added. The mixture was stirred for 30 min at rt and then treated with H_2O (10 mL). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to give the crude mesylate, which was dissolved in acetone (10 mL) and then fused NaI (1.5 equiv.) was added. The reaction mixture was heated at 50 ºC for 4 h, before being cooled and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:40) to give the corresponding iodide.

General procedure for the preparation of sulfonium ions

To a solution of the cyclic sulfide **4-13** (1.0 equiv.) in dry CH_3CN (3 mL) was added the alkyl halide (1.0 equiv.) and $AgBF_4$ (1.0 equiv.). The reaction mixture was covered with aluminum foil and stirred at 65 ºC for 24 h. The mixture was then cooled to rt, concentrated and the resulting residue was purified by chromatography (toluene–CH_3OH 30:1) to give the sulfonium ion as mixture of isomers, which were inseparable.

General procedure for the deprotection benzyl groups

The protected sulfonium ion (1.0 equiv.) was dissolved in CH_2Cl_2 (2 mL), cooled to –78 ºC under an Ar atmosphere and then BCl_3 (1M solution in CH_2Cl_2, 4 mL) was added. The mixture was stirred at –78 ºC for 2 h and then warmed slowly to 0 ºC and stirred for another 30 min. Air was bubbled through the solution to remove the excess BCl_3 and then CH_3OH (3 mL) was added. The solution was concentrated and the residue was coevaporated with CH_3OH (2 × 5 mL). The deprotected product was then dissolved in CH_3OH (5 mL) and stirred with ion exchange resin Amberlyst (Cl^- form) for 2 h and filtered. The filtrate was concentrated and the resulting oil was passed through a short Iatrobead column using CH_2Cl_2–CH_3OH 5:1 → 1:1 as the eluant to give the target products.

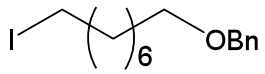


4-24

1-Iodo-8-methoxyoctane (4-24)

Sodium metal (0.5 g, 22 mmol) was added to dry CH_3OH (20 mL) at 0 ºC and the reaction mixture was stirred for ~30 min until all the metal disappeared. Then, 8-bromooctan-1-ol (1.0 g, 4.8 mmol), was added and the solution was heated at reflux for 12 h before being cooled to rt, neutralized with HOAc and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:9) to

give the intermediate methoxy alcohol as a yellowish oil (0.72 g, 94%); R_f 0.25 (EtOAc–Hexane 1:3). Using the general procedure for mesylation and iodination, the alcohol this intermediate (0.72 g, 4.4 mmol) was reacted with MsCl (0.60 g, 5.3 mmol) to give the mesylate, which was then treated with NaI (0.99 g, 6.6 mmol) to give **4-24** (0.67 g, 52%) as a colorless oil. R_f 0.38 (EtOAc–Hexane 1:19); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H); 3.35 (t, 2 H, J = 6.6 Hz, C$\underline{H_2}$O), 3.32 (s, 3 H, OC$\underline{H_3}$), 3.17 (t, 2 H, J = 7.1 Hz, IC$\underline{H_2}$), 1.88–1.78 (m, 2 H, C$\underline{H_2}$C$\underline{H_2}$I), 1.58–1.52 (m, 2 H, C$\underline{H_2}$OC$\underline{H_2}$), 1.39–1.29 (m, 8 H, 4 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 72.8 (C$\underline{H_2}$O), 58.5 (C$\underline{H_3}$O), 33.5 (C$\underline{H_2}$), 30.4 (C$\underline{H_2}$), 29.5 (C$\underline{H_2}$), 29.2 (C$\underline{H_2}$), 28.5 (C$\underline{H_2}$), 26.0 (C$\underline{H_2}$), 7.2 (C$\underline{H_2}$I). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_9H_{19}OI$: 293.0344. Found: 293.0342.

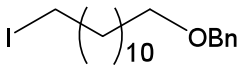


4-25

1-Iodo-8-benzyloxyoctane (4-25)

To a solution of 1,8-octanediol (1.55 g, 10.6 mmol) and benzyl bromide (1.82 g, 10.6 mmol) in DMF (20 mL) at 0 ºC was added NaH (0.42 g, 10.6 mmol, 60% in mineral oil) slowly. The reaction mixture stirred at 0 ºC for 1 h and then the excess reagents were quenched by the addition of H_2O. The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The resulting residue was purified by chromatography

(EtOAc–Hexane 1:4) to give the mono-benzylated intermediate (1.18 g, 47%) as a colorless oil, R_f 0.37 (EtOAc–Hexane 1:3). Using the general procedure for mesylation and iodination, this mono-benzylated intermediate (0.79 g, 3.3 mmol) was reacted with MsCl (0.45 g, 4.0 mmol) to give the mesylate, which was treated with NaI (0.75 g, 5.0 mmol) to yielding **4-25** (0.92 g, 80%) as a yellowish oil. R_f 0.54 (EtOAc–Hexane 1:19); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.37–7.26 (m, 5 H, Ar), 4.51 (s, 2 H, C$\underline{H_2}$Ph), 3.47 (t, 2 H, C$\underline{H_2}$OBn, J = 6.6 Hz), 3.19 (t, 2 H, IC$\underline{H_2}$, J = 7.0 Hz), 1.85–1.79 (m, 2 H, ICH$_2$C$\underline{H_2}$), 1.65–1.57 (m, 2 H, C$\underline{H_2}$CH$_2$OBn), 1.40–1.33 (m, 8 H, 4 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.7 (Ar), 128.3 (Ar × 2), 127.6 (Ar × 2), 127.5 (Ar), 72.9 (C$\underline{H_2}$Ph), 70.4 (C$\underline{H_2}$OBn), 33.5 (C$\underline{H_2}$), 30.4 (C$\underline{H_2}$), 29.7 (C$\underline{H_2}$), 29.2 (C$\underline{H_2}$), 28.5 (C$\underline{H_2}$), 26.1 (C$\underline{H_2}$), 7.2 (IC$\underline{H_2}$). HRMS (ESI) *m/z* Calcd for (M + Na$^+$) $C_{15}H_{23}OI$: 369.0686. Found: 369.0684.

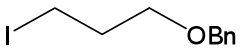


4-26

1-Iodo-12-benzyloxydodecane (4-26)

To a solution of dodecane-1,12-diol (1.04 g, 5.1 mmol) and benzyl bromide (0.88 g, 5.1 mmol) in DMF (20 mL) at 0 ºC was added NaH (0.21 g, 5.1 mmol, 60% in mineral oil) slowly. The reaction mixture stirred at 0 ºC for 1 h and then the excess reagents were quenched by the addition of H_2O. The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried ($MgSO_4$)

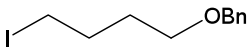
and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:4) to give the mono-benzylated intermediate (0.67 g, 45%); R_f 0.35 (EtOAc–Hexane 1:3) as a yellowish oil. Using the general procedure for mesylation and iodination, the mono-benzylated intermediate (0.60 g, 2.1 mmol) was reacted with MsCl (0.28 g, 2.5 mmol) to give the mesylate, which was reacted with NaI (0.48 g, 3.2 mmol) to give **4-26** (0.71 g, 84%) as a colorless oil. R_f 0.28 (EtOAc–Hexane 1:19); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.35–7.27 (m, 5 H, Ar), 4.50 (s, 2 H, C$\underline{H_2}$Ph), 3.47 (t, 2 H, J = 6.6 Hz, C$\underline{H_2}$OBn), 3.19 (t, 2 H, J = 7.0 Hz, IC$\underline{H_2}$), 1.85–1.79 (m, 2 H, ICH$_2$C$\underline{H_2}$), 1.65–1.59 (m, 2 H, C$\underline{H_2}$CH$_2$OBn), 1.40–1.27 (m, 16 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.7 (Ar), 128.3 (Ar × 2), 127.6 (Ar × 2), 127.4 (Ar), 72.8 (C$\underline{H_2}$Ph), 70.5 (C$\underline{H_2}$OBn), 33.6 (C$\underline{H_2}$), 30.5 (C$\underline{H_2}$), 29.8 (C$\underline{H_2}$), 29.5 (3 C, 3 × C$\underline{H_2}$), 29.4 (2 C, 2 × C$\underline{H_2}$), 28.5 (C$\underline{H_2}$), 26.1 (C$\underline{H_2}$), 7.3 (IC$\underline{H_2}$). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{19}H_{31}OI$: 425. 1312. Found: 425.1308.



4-27

1-Iodo-3-benzyloxypropane (4-27)

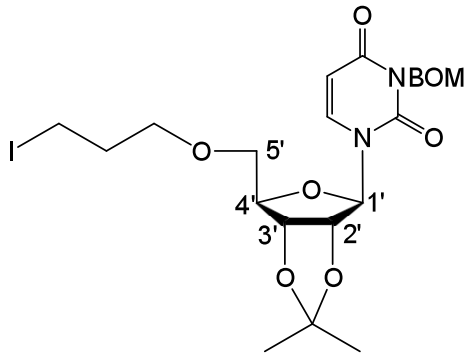
To a solution of 1,3-propanediol (2.11 g, 0.028 mmol) and benzyl bromide (4.75 g, 0.028 mmol) in DMF (20 mL) at 0 ºC was added NaH (1.11 g, 0.028 mmol, 60% in mineral oil) slowly. The reaction mixture was stirred at 0 ºC for 1 h and then the excess reagents were quenched by the addition of H_2O. The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (EtOAc–Hexane 1:2) to give the mono-benzylated intermediate (1.92 g, 42%); R_f 0.43 (EtOAc–Hexane 1:1) as a yellowish oil. Using the general procedure for mesylation and iodination, the mono-benzylated intermediate (0.50 g, 3.0 mmol) was reacted with MsCl (0.41 g, 3.6 mmol) and the resulting mesylate was treated with NaI (0.70 g, 4.6 mmol) to give **4-27** (0.71 g, 84%) as a colorless oil. R_f 0.24 (EtOAc–Hexane 1:30); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.28 (m, 5 H, Ar), 4.54 (s, 2 H, C$\underline{H_2}$Ph), 3.56 (t, 2 H, J = 5.8 Hz, C$\underline{H_2}$OBn), 3.32 (t, 2 H, J = 6.8 Hz, C$\underline{H_2}$I,), 2.11 (tt, 2 H, J = 5.8, 6.8 Hz, C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.2 (Ar), 128.4 (Ar × 2), 127.7 (Ar × 3), 73.1 (C$\underline{H_2}$Ph), 69.6 (C$\underline{H_2}$OBn), 33.5(C$\underline{H_2}$), 3.5 (C$\underline{H_2}$I). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{10}H_{13}OI$: 298.9903. Found: 298.9900.



4-28

1-Iodo-4-benzyloxybutane (4-28)

A solution of 1,4-butanediol (2.00 g, 22 mmol) and benzyl bromide (3.80 g, 22 mmol) in DMF (20 mL) at 0 ºC was added NaH (0.89 g, 22 mmol, 60% in mineral oil) slowly. The reaction mixture was stirred at 0 ºC for 1 h and the excess reagents were quenched by the addition of H_2O. The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The resulting residue was purified by chromatography (EtOAc–Hexane 1:2) to give the mono-benzylated intermediate (1.92 g, 42%); R_f 0.50 (EtOAc–Hexane 1:1) as a yellowish oil. Using the general procedure for mesylation and iodination, the mono-benzylated intermediate (0.52 g, 2.9 mmol) was reacted with MsCl (0.39 g, 3.4 mmol) to give the mesylate, which was treated with NaI (0.65 g, 4.3 mmol) yielding **4-28** (0.72 g, 86%) as a colorless oil. R_f 0.24 (EtOAc–Hexane 1:30); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.28 (m, 5 H, Ar), 4.51 (s, 2 H, C$\underline{H_2}$Ph), 3.51 (t, 2 H, J = 6.2 Hz, C$\underline{H_2}$OBn), 3.22 (t, 2 H, J = 7.0 Hz, C$\underline{H_2}$I), 1.98–1.93 (m, 2 H, ICH$_2$C$\underline{H_2}$), 1.76–1.71 (m, 2 H, C$\underline{H_2}$CH$_2$OBn); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.4 (Ar), 128.4 (Ar × 2), 127.6 (Ar × 2), 127.5 (Ar), 72.9 (C$\underline{H_2}$Ph), 69.0 (C$\underline{H_2}$OBn), 30.6 (2 C, CH$_2$CH$_2$), 6.8 (IC$\underline{H_2}$). HRMS (ESI) *m/z* Calcd for (M+Na$^+$) $C_{11}H_{15}OI$: 313.0060. Found: 313.0060.

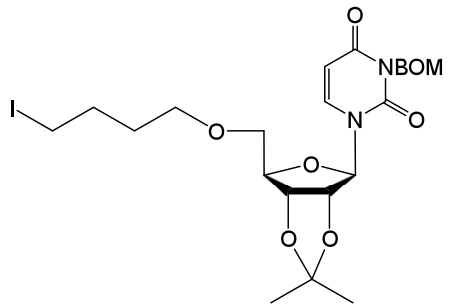


4-29

2'3'-*O*-isopropylidene-5'-*O*-(3-iodopropyl)-3-(benzyloxymethyl) uridine (4-29)

Using the general procedure for mesylation and iodination, compound **3-46** (150 mg, 0.32 mmol) was reacted with MsCl (43 mg, 0.38 mmol) to give the corresponding mesylate, which was then reacted with NaI (70 mg, 0.47 mmol). The crude product was purified by chromatography (EtOAc–Hexane 1:2) to give **4-29** (121 mg, 65%) as a colorless oil. R_f 0.11 (EtOAc–Hexane 1:2); $[\alpha]_D$ –6.0 (*c* 1.55, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.47 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 7.40–7.28 (m, 5 H, Ar), 5.83 (d, 1 H, *J* = 2.2 Hz, H-1), 5.76 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.52 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 5.49 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 4.82 (dd, 1 H, *J* = 3.0, 6.2 Hz, H-3), 4.78 (dd, 1 H, *J* = 2.2, 6.2 Hz, H-2), 4.73 (s, 2 H, C<u>H</u>$_2$Ph), 4.41 (ddd, 1 H, *J* = 3.0, 3.1, 4.4 Hz, H-4), 3.76 (dd, 1 H, *J* = 3.0, 10.5 Hz, H-5), 3.65 (dd, 1 H, *J* = 4.4, 10.5 Hz, H-5), 3.59–3.56 (m, 2 H, C<u>H</u>$_2$OC<u>H</u>$_2$), 3.22 (dd, 2 H, *J* = 6.8, 6.8 Hz, C<u>H</u>$_2$I), 2.07–2.02 (m, 2 H, C<u>H</u>$_2$), 1.62 (s, 3 H, C<u>H</u>$_3$), 1.39 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.6 (<u>C</u>=O), 150.8 (<u>C</u>=O),

139.6 (CH=), 137.9 (Ar), 128.2 (Ar × 2), 127.6 (Ar × 3), 114.1 ($\underline{C}(CH_3)_2$), 101.5 (CH=), 94.2 (C-1), 86.0 (C-2), 85.4 (C-4), 80.9 (C-3), 72.3 ($\underline{C}H_2Ph$), 71.0 (C-5), 70.9 ($N\underline{C}H_2OBn$), 70.3 ($\underline{C}H_2O$), 32.8 ($\underline{C}H_2$), 27.2 ($\underline{C}H_3$), 25.4 ($\underline{C}H_3$), 2.5 ($\underline{C}H_2I$). HRMS (ESI) *m/z* Calcd for ($M+H^+$) $C_{23}H_{29}N_2O_7I$: 595.0912. Found: 595.0912.

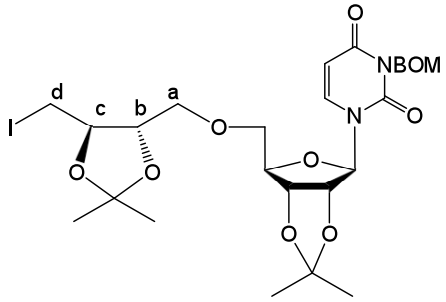


4-30

2'3'-*O*-isopropylidene-5'-*O* -(4-iodobutyl)-3-(benzyloxymethyl) uridine (4-30)

Using the general procedure for mesylation and iodination, compound **3-47** (140 mg, 0.29 mmol) was treated with MsCl (40 mg, 0.35 mmol) to give the mesylate, which was then reacted with NaI (65 mg, 0.44 mmol). The crude product was purified by chromatography (EtOAc–Hexane 1:2) to give **4-30** (105 mg, 61%) as a colorless oil. R_f 0.17 (EtOAc–Hexane 1:2); $[\alpha]_D$ –7.4 (*c* 0.57, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.48 (d, 1 H, *J* = 8.1 Hz, C$\underline{H}$=), 7.36–7.23 (m, 5 H, Ar), 5.82 (d, 1 H, *J* = 1.9 Hz, H-1), 5.72 (d, 1 H, *J* = 8.1 Hz, C$\underline{H}$=), 5.49 (d, 1 H, *J* = 9.8 Hz, $NC\underline{H}_2OBn$), 5.45 (d, 1 H, *J* = 9.8 Hz, $NC\underline{H}_2OBn$), 4.77–4.73 (m, 2 H, H-2, H-3), 4.69 (s, 2 H, $C\underline{H}_2Ph$), 4.37 (ddd, 1 H, *J* = 2.3, 3.1, 3.9 Hz, H-4), 3.68 (dd, 1 H, *J* = 2.3, 10.6 Hz, H-5'), 3.58 (dd, 1 H, *J* = 3.9, 10.6 Hz, H-5), 3.51–3.43 (m, 2 H, $C\underline{H}_2OCH_2$), 3.16 (dd, 2 H, *J* = 6.7, 6.7 Hz, $C\underline{H}_2I$), 1.86–1.80 (m, 2 H,

C$\underline{H}_2$), 1.68–1.62 (m, 2 H, C$\underline{H}_2$), 1.58 (s, 3 H, C$\underline{H}_3$), 1.36 (s, 3 H, C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.6 (C=O), 150.9 (C=O), 139.6 (CH=), 137.9 (Ar), 128.3 (Ar × 2), 127.6 (Ar × 3), 114.0 ($\underline{C}(CH_3)_2$), 101.5 (CH=), 94.0 (C-1), 85.9 (C-4), 85.4 (C-2), 80.9 (C-3), 72.3 ($\underline{C}H_2$Ph), 70.8 (C-5), 70.5 (N$\underline{C}H_2$OBn), 71.4 ($\underline{C}H_2$O), 30.4 ($\underline{C}H_2$), 30.1 ($\underline{C}H_2$), 27.2 ($\underline{C}H_3$), 25.4 ($\underline{C}H_3$), 6.3 ($\underline{C}H_2$I). HRMS (ESI) *m/z* Calcd for (M + H^+) $C_{24}H_{31}N_2O_7I$: 587.1249. Found: 587.1260.

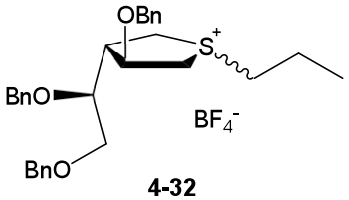


4-31

2’3’-*O*-isopropylidene-5’-*O*-((2*S*,3*R*)-4-iodo-2,3-*O*-isopropylidene-2,3-dihydr-oxyl-butyl)-3-(benzyloxymethyl) uridine (4-31)

Using the general procedure for mesylation and iodination, compound **3-56** (340 mg, 0.62 mmol) was reacted with MsCl (85 mg, 0.74 mmol) to give the mesylate, which was treated with NaI (140 mg, 0.93 mmol). The iodination was done in a sealed vessel at 80 ^{0}C. The iodide was purified by chromatography (EtOAc–Hexane 1:2) to give **4-31** (141 mg, 35%) as a colorless oil. R_f 0.43 (EtOAc–Hexane 1:1); $[\alpha]_D$ –11.6 (*c* 2.03, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.48 (d, 1 H, *J* = 8.2 Hz, CH=), 7.38–7.24 (m, 5 H, Ar), 5.84 (d, 1 H, *J* = 2.6

Hz, H-1), 5.75 (d, 1 H, *J* = 8.2 Hz, C<u>H</u>=), 5.50 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 5.46 (d, 1 H, *J* = 9.7 Hz, NC<u>H</u>$_2$OBn), 4.82 (dd, 1 H, *J* = 3.3, 6.3 Hz, H-3), 4.79 (dd, 1 H, *J* = 2.5, 6.3 Hz, H-2), 4.70 (s, 2 H, C<u>H</u>$_2$Ph), 4.35 (ddd, 1 H, *J* = 3.1, 3.3, 4.4 Hz, H-4), 3.93 (ddd, 1 H, *J* = 3.9, 5.6, 7.3 Hz, H_b), 3.85 (ddd, 1 H, *J* = 5.5, 5.5, 7.3 Hz, H_c), 3.80 (dd, 1 H, *J* = 3.1, 10.7 Hz, H-5), 3.75 (dd, 1 H, *J* = 3.9, 10.6 Hz, H_a), 3.73 (dd, 1 H, *J* = 4.4, 10.7 Hz, H-5), 3.67 (dd, 1 H, *J* = 5.6, 10.6 Hz, H_a), 3.29 (d, 2 H, *J* = 5.5 Hz, 2 × H_d), 1.59 (s, 3 H, C<u>H</u>$_3$), 1.44 (s, 3 H, C<u>H</u>$_3$), 1.40 (s, 3 H, C<u>H</u>$_3$), 1.36 (s, 3 H, C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 162.6 (<u>C</u>=O), 150.9 (<u>C</u>=O), 140.0 (<u>C</u>H=), 137.9 (Ar), 128.3 (Ar × 2), 127.6 (Ar × 3), 114.3 (<u>C</u>$(CH_3)_2$), 109.9 (<u>C</u>$(CH_3)_2$), 102.0 (<u>C</u>H=), 93.8 (C-1), 85.6 (C-4), 84.8 (C-2), 80.8 (C-3), 80.3 (C_b), 76.9 (C_c), 72.3 (<u>C</u>H_2Ph), 72.1 (C_a), 71.5 (N<u>C</u>H_2OBn), 70.3 (C-5), 27.4 (<u>C</u>H_3), 27.3 (<u>C</u>H_3), 27.2 (<u>C</u>H_3), 25.4 (<u>C</u>H_3), 5.8 (C_d). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_{27}H_{35}N_2O_9I$: 681.1280. Found: 681.1285.

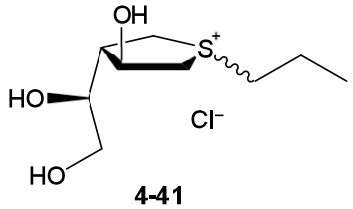


4-32

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-propyl-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-32)

Using the general procedure for preparing the protected sulfonium ions, compound **4-13** (69 mg, 0.16 mmol) was treated with **4-18** (27.2 mg, 0.16 mmol)

and $AgBF_4$ (32 mg, 0.16 mmol) to give **4-32** (65 mg, 73%) as a colorless oil; R_f 0.69 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^–$) $C_{30}H_{37}O_3S$: 477.2458. Found: 477.2459. The compound was not further characterized but rather was directly deprotected.

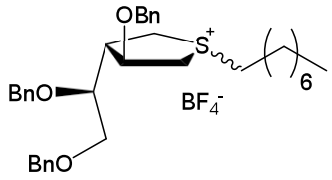


4-41

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-propyl-tetrahydro-1*H*-thiophenium chloride (4-41)

Using the general procedure for deprotection of the sulfonium ions, **4-32** (44 mg, 0.078 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst ($Cl^–$ form) to give **4-41** (13 mg, 72%) as a 7:3 mixture of inseparable stereoisomers as a colorless oil. R_f 0.06 (CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.74 (dd, 0.7 H, *J* = 2.0, 2.0 Hz, H-3_a), 4.67 (ddd, 0.3 H, *J* = 5.4, 5.6, 5.6 Hz, H-3_b), 3.89–3.75 (m, 2 H), 3.66–3.61 (m, 1 H), 3.57–3.34 (m, 6 H), 3.05 (dddd, 0.7 H, *J* = 1.6, 2.1, 4.8, 6.6, H-4_a), 2.66 (dddd, 0.3 H, *J* = 2.6, 6.0, 6.0, 8.6 Hz, H-4_b), 1.90–1.82 (m, 2 H, C$\underline{H}_2$), 0.90 (t, 3 H, *J* = 7.4 Hz, C$\underline{H}_3$); ^{13}C NMR (125 MHz, CD_3OD δ_C) 80.0 (C-3_a), 77.2 (C-3_b), 70.9 (C-6_a), 70.0 (C-6_b), 66.1 (C-7_b), 65.3 (C-7_a), 53.8 (C-4_a), 52.9 (C-4_b), 50.3 (S$\underline{C}H_{2a}$), 49.4 (S$\underline{C}H_{2b}$), 48.1 (S$\underline{C}H_{2a}$), 45.5 (S$\underline{C}H_{2b}$), 42.9

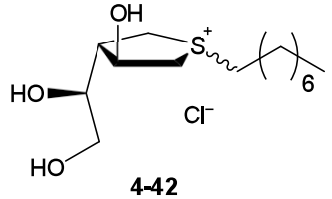
($S\underline{C}H_{2a}$), 40.2 ($S\underline{C}H_{2b}$), 20.5 ($\underline{C}H_{2b}$), 20.2 ($\underline{C}H_{2a}$), 13.1 ($\underline{C}H_{3b}$), 13.0 ($\underline{C}H_{3a}$). HRMS (ESI) *m/z* Calcd for ($M–Cl^–$) $C_9H_{19}O_3S$: 207.1049. Found: 207.1050.



4-33

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-propyl-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-33)

Using the general procedure for preparing the protected sulfonium ions, **4-13** (69 mg, 0.16 mmol) was treated with **4-19** (38.2 mg, 0.16 mmol) and $AgBF_4$ (31 mg, 0.16 mmol) to give **4-33** (76 mg, 77%) as a colorless oil. R_f 0.70 (CH_2Cl_2–CH_3OH 9:1); HRMS (ESI) *m/z* Calcd for ($M–BF_4^–$) $C_{35}H_{47}O_3S$: 547.3240. Found: 547.3245. The compound was not further characterized but rather was directly deprotected.

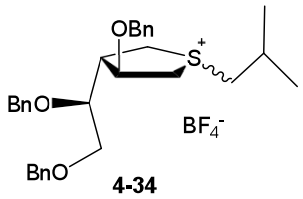


4-42

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-octyl-tetrahydro-1*H*-thiophenium chloride (4-42)

Using the general procedure for deprotection of the sulfonium ions, **4-33** (76 mg, 0.12 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst ($Cl^–$ form)

to give **4-42** (32 mg, 86%) as a 7:3 mixture of inseparable stereoisomers as a colorless oil.. R_f 0.06 (CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 4.74 (dd, 0.7 H, J = 1.9, 1.9 Hz, H-3$_a$), 4.67 (ddd, 0.3 H, J = 5.5, 5.7, 5.7 Hz, H-3$_b$), 3.89–3.74 (m, 2 H), 3.66–3.61 (m, 1 H), 3.57–3.30 (m, 6 H), 2.97 (dddd, 0.7 H, J = 1.7, 3.3, 4.8, 6.6, H-4$_a$), 2.66 (m, 0.3 H, J = 2.3, 5.9, 5.9, 8.6 Hz, H-4$_b$), 1.87–1.77 (m, 2 H), 1.52–1.45 (m, 2 H), 1.49–1.35 (m, 8 H), 0.90 (t, 3 H, J = 7.1 Hz, C$\underline{H}_3$); ^{13}C NMR (125 MHz, CD_3OD δ_C) 80.0 (C-3$_a$), 77.2 (C-3$_b$), 70.9 (C-6$_a$), 70.0 (C-6$_b$), 66.1 (C-7$_b$), 65.3 (C-7$_a$), 53.8 (C-4$_a$), 51.6 (C-4$_b$), 50.3 (S$\underline{C}$H$_{2a}$), 48.1 (S$\underline{C}$H$_{2b}$), 46.9 (S$\underline{C}$H$_{2a}$), 43.7 (S$\underline{C}$H$_{2b}$), 42.9 (S$\underline{C}$H$_{2a}$), 40.2 (S$\underline{C}$H$_{2a}$), 32.9 ($\underline{C}$H$_2$), 30.1 ($\underline{C}$H$_2$), 30.0 ($\underline{C}$H$_2$), 29.5 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 26.8 ($\underline{C}$H$_{2a}$), 26.5 ($\underline{C}$H$_{2b}$), 14.4 ($\underline{C}$H$_3$); HRMS (ESI) *m/z* Calcd for (M–Cl$^-$) $C_{14}H_{29}O_3S$: 277.1832. Found: 277.1832.

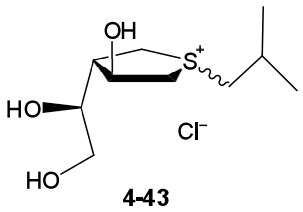


4-34

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-isobutyl-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-34)

Using the general procedure for preparing the protected sulfonium ions, **4-13** (77 mg, 0.18 mmol) was reacted with **4-20** (34 mg, 0.18 mmol) and $AgBF_4$ (35 mg, 0.18 mmol) to give **4-34** (24.3 mg, 24%) as a colorless oil. R_f 0.70 (CH_2Cl_2–CH_3OH 9:1); HRMS (ESI) *m/z* Calcd for (M–BF_4^-) $C_{31}H_{39}O_3S$:

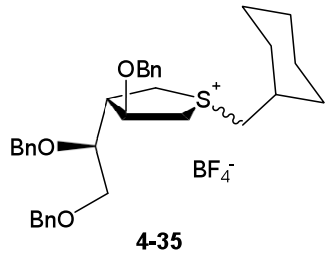
491.2614. Found: 491.2617. The compound was not further characterized but rather was directly deprotected.



4-43

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-isobutyl-tetrahydro-1*H*-thiophenium chloride (4-43)

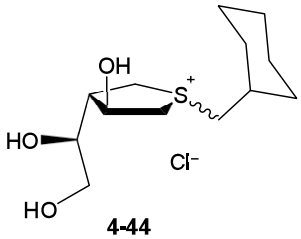
Using the general procedure for deprotection of the sulfonium ions, **4-34** (45 mg, 0.078 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-43** (18 mg, 88%) as a 7:3 mixture of inseparable stereoisomers as a colorless foam. R_f 0.06 (pure CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 4.74 (dd, 0.7 H, J = 2.0, 2.0 Hz, H-3_a), 4.68 (ddd, 0.3 H, J = 5.2, 5.2, 5.2 Hz, H-3_b), 3.92 (dd, 0.7 H, J = 1.8, 12.2 Hz), 3.86–3.78 (m, 1.3 H), 3.66–3.33 (m, 7 H), 3.06 (dddd, 0.7 H, J = 1.7, 3.2, 4.9, 6.6, H-4_a), 2.69 (dddd, 0.3 H, J = 2.4, 5.6, 5.6, 8.4 Hz, H-4_b), 2.20–2.08 (m, 1 H), 1.13 (d, 6 H, J = 6.7 Hz, C$\underline{H_3}$); ^{13}C NMR (125 MHz, CD_3OD δ_C) 80.0 (C-3_a), 77.4 (C-3_b), 70.9 (C-6_a), 70.0 (C-6_b), 66.0 (C-7_b), 65.3 (C-7_a), 55.3 (S$\underline{C}H_{2a}$), 53.9 (C-4_a), 52.1 (S$\underline{C}H_{2b}$), 51.6 (C-4_b), 50.9 (S$\underline{C}H_{2a}$), 48.9 (S$\underline{C}H_{2b}$), 43.5 (S$\underline{C}H_{2a}$), 40.9 (S$\underline{C}H_{2a}$), 27.7 ($\underline{C}H_a$), 27.6 ($\underline{C}H_b$), 21.9 ($\underline{C}H_{3a}$), 21.7 ($\underline{C}H_{3b}$). HRMS (ESI) *m/z* Calcd for ($M–Cl^-$) $C_{10}H_{21}O_3S$: 221.1206. Found:221.1207.



4-35

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-cyclohexylmethyl-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-35)

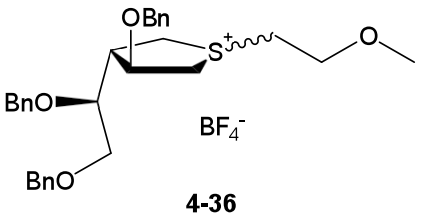
Using the general procedure for preparing the protected sulfonium ions, **4-13** (86 mg, 0.20 mmol) was reacted with iodide **4-22** (45 mg, 0.20 mmol) and $AgBF_4$ (38 mg, 0.20 mmol) to give **4-35** (10 mg, 8%) as a colorless oil. R_f 0.34 (CH_2Cl_2–CH_3OH 19:1); HRMS (ESI) *m/z* Calcd for ($M–BF_4^-$) $C_{34}H_{43}O_3S$: 531.2927. Found: 531.2925. The compound was not further characterized but rather was directly deprotected.



4-44

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-cyclohexylmethyl-tetra-hydro-1*H*-thiophenium chloride (4-44)

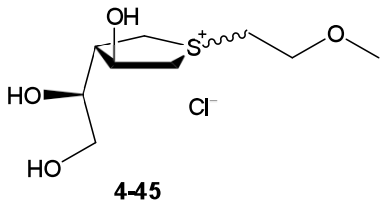
Using the general procedure for deprotection of the sulfonium ions, **4-35** (8 mg, 0.13 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-4** (3.3 mg, 100%) as a colorless foam as a 91:9 ratio of isomers . R_f 0.06 (pure CH_3OH); ^{1}H NMR (600 MHz, CD_3OD, δ_H) 4.73 (dd, 1 H, *J* = 2.0, 2.0 Hz, H-3), 3.90 (dd, 1 H, *J* = 1.8, 12.2 Hz, H-6), 3.78 (dd, 1 H, *J* = 3.9, 12.5 Hz), 3.62 (ddd, 1 H, *J* = 1.4, 5.6, 6.9 Hz), 3.46 (dd, 1 H, *J* = 6.8, 12.2 Hz), 3.42 (dd, 1 H, *J* = 6.7, 11.2 Hz), 3.38 (dd, 1 H, *J* = 7.1, 12.6 Hz), 3.34–3.31 (m, 3 H), 3.05 (dddd, 1 H, *J* = 1.9, 3.4, 3.4, 6.8 Hz, H-4), 1.91–1.77 (m, 4 H), 1.72–1.69 (m, 1 H), 1.40–1.32 (m, 2 H), 1.28–1.18 (m, 2 H), 1.17–1.11 (m, 2 H); ^{13}C NMR (100 MHz, CD_3OD δ_C) 80.0 (C-3), 70.9 (C-6), 65.3 (C-7), 54.1 (S$\underline{C}H_2$), 53.9 (C-4), 50.9 (S$\underline{C}H_2$), 43.5 (S$\underline{C}H_2$), 36.5 ($\underline{C}$H), 33.2 ($\underline{C}H_2$), 33.0 ($\underline{C}H_2$), 26.8 ($\underline{C}H_2$), 26.7 (2 C, 2 × $\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for ($M–Cl^-$) $C_{13}H_{25}O_3S$: 261.1519. Found: 261.1517.



4-36

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(2-methoxyethyl)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-36)

Using the general procedure for preparing the protected sulfonium ions, **4-13** (61 mg, 0.14 mmol) was reacted with **4-23** (28.7 mg, 0.15 mmol) and $AgBF_4$ (30 mg, 0.15 mmol) to give **4-36** (66 mg, 81%) as a colorless oil. R_f 0.70 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^–$) $C_{30}H_{37}O_4S$: 493.2407. Found: 493.2402. The compound was not further characterized but rather was directly deprotected.

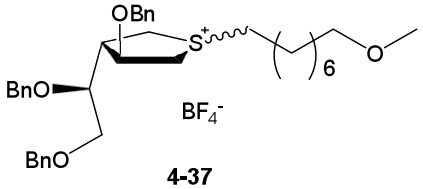


4-45

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(2-methoxyethyl)-tetrahydro-1*H*-thiophenium chloride (4-45)

Using the general procedure for deprotection of the sulfonium ions, **4-36** (38 mg, 0.065 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst ($Cl^–$ form) to give **4-45** (14 mg, 83%) as a 7:3 mixture of inseparable stereoisomers as a colorless foam. R_f 0.06 (CH_3OH); ^1H NMR (600 MHz, CD_3OD, δ_H) 4.73 (dd,

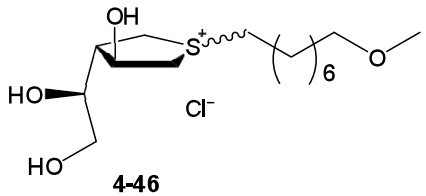
0.7 H, J = 2.0, 2.0 Hz, H-3_a), 4.67 (ddd, 0.3 H, J = 5.0, 5.0, 5.0 Hz, H-3_b), 3.90–3.61 (m, 7 H), 3.57–3.47 (m, 3 H), 3.44–3.41 (m, 4 H), 3.03 (dddd, 0.7 H, J = 1.7, 3.3, 5.0, 7.0 Hz, H-4_a), 2.68 (dddd, 0.3 H, J = 2.3, 5.3, 5.3, 8.2 Hz, H-4_b); ^{13}C NMR (125 MHz, CD_3OD δ_C) 80.0 (C-3_a), 77.4 (C-3_b), 70.8 (C-6_a), 70.0 (C-6_b), 68.7 ($\underline{C}H_2OCH_{3a}$), 68.4($\underline{C}H_2OCH_{3b}$), 66.0 (C-7_b), 65.3 (C-7_a), 59.4 ($CH_2O\underline{C}H_3$), 53.7 (C-4_a), 51.5 (C-4_b), 51.2 ($S\underline{C}H_{2a}$), 49.4 ($S\underline{C}H_{2b}$), 47.8 ($S\underline{C}H_{2a}$), 44.7 ($S\underline{C}H_{2b}$), 43.9 ($S\underline{C}H_{2a}$), 41.1 ($S\underline{C}H_{2b}$). HRMS (ESI) *m/z* Calcd for ($M–Cl^–$) $C_9H_{19}O_4S$: 223.0999. Found: 223.0997.



4-37

((3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(8-methoxyoctyl)-tetraydro-1*H*-thiophenium tetrafluoroborate (4-37)

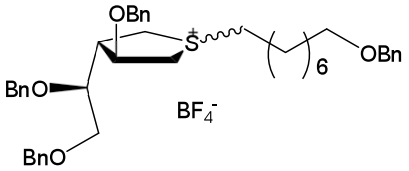
Using the general procedure for preparing the protected sulfonium ions, **4-13** (103 mg, 0.24 mmol) was reacted with **4-24** (80 mg, 0.29 mmol) and $AgBF_4$ (46 mg, 0.24 mmol) to give **4-37** (152 mg, 97%) as a colorless oil. R_f 0.70 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^–$) $C_{36}H_{49}O_4S$: 577.3346. Found: 577.3345. The compound was not further characterized but rather was directly deprotected.



4-46

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(8-methoxyoctyl)-tetrahydro-1*H*-thiophenium chloride (4-46)

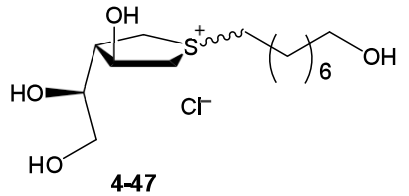
Using the general procedure for deprotection of the sulfonium ions, **4-37** (100 mg, 0.15 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give product **4-46** (32 mg, 55%) as a 7:3 mixture of inseparable stereoisomers as a colorless foam. R_f 0.06 (CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.74 (dd, 0.7 H, J = 2.0, 2.0 Hz, H-3_a), 4.67 (ddd, 0.3 H, J = 5.7, 5.7, 5.7 Hz, H-3_b), 3.89–3.75 (m, 2 H), 3.66–3.61 (m, 1 H), 3.57–3.36 (m, 8 H), 3.30 (s, 3 H, C$\underline{H}_3$), 2.97 (dddd, 0.7 H, J = 1.6, 3.1, 5.6, 6.5 Hz H-4_a), 2.66 (dddd, 0.3 H, J = 2.3, 5.9, 5.9, 8.5 Hz, H-4_b), 1.87–1.78 (m, 2 H), 1.58–1.46 (m, 4 H), 1.40–1.35 (m, 6 H); ^{13}C NMR (100 MHz, CD_3OD δ_C) 80.0 (C-3_a), 77.2 (C-3_b), 73.8 ($\underline{C}H_2OCH_3$), 70.9 (C-6_a), 70.0 (C-6_b), 66.1 (C-7_b), 65.3 (C-7_a), 58.8 ($\underline{C}H_2OCH_3$), 53.8 (C-4_a), 51.6 (C-4_b), 50.3 (S$\underline{C}H_{2a}$), 48.1 (S$\underline{C}H_{2b}$), 46.9 (S$\underline{C}H_{2a}$), 43.7 (S$\underline{C}H_{2b}$), 42.9 (S$\underline{C}H_{2a}$), 40.2 (S$\underline{C}H_{2a}$), 30.5 ($\underline{C}H_2$), 30.0 ($\underline{C}H_2$), 29.9 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 27.0 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 26.5 ($\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for ($M–Cl^-$) $C_{15}H_{31}O_4S$: 307.1937. Found: 307.1934.



4-38

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(8-benzyloxyoctyl)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-38)

Using the general procedure for preparing the protected sulfonium ions, **4-13** (80 mg, 0.18 mmol) was reacted with **4-25** (75 mg, 0.22 mmol) and $AgBF_4$ (42 mg, 0.22 mmol) to give **4-38** (123 mg, 90%) as a colorless oil. R_f 0.38 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^-$) $C_{42}H_{53}O_4S$: 653.3659 Found: 653.3659. The compound was not further characterized but rather was directly deprotected.

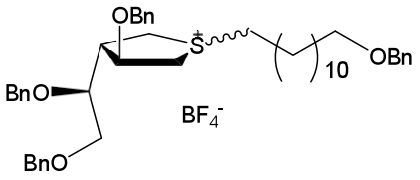


4-47

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(8-hydroxyoctyl)-tetrahydro-1*H*-thiophenium chloride (4-47)

Using the general procedure for deprotection of the sulfonium ions, **4-38** (123 mg, 0.17 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-47** (43 mg, 79%) as a 3:1 mixture of inseparable stereoisomers as a colorless foam. R_f 0.06 (CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.74 (dd, 0.75 H, J = 2.0, 2.0 Hz, H-3_a), 4.67 (ddd, 0.25 H, J = 5.6, 5.6, 5.6 Hz, H-3_b),

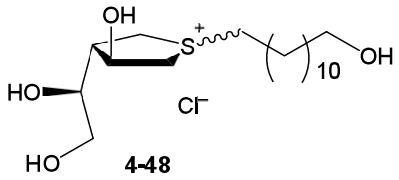
3.89–3.84 (m, 1 H), 3.81–3.74 (m, 1 H), 3.65–3.61 (m, 1 H), 3.50–3.35 (m, 8 H), 3.05 (dddd, 0.75 H, J = 1.6, 3.1, 5.6, 6.5, H-4_a), 2.68 (dddd, 0.25 H, J = 2.4, 5.9, 5.9, 8.5 Hz, H-4_b), 1.87–1.78 (m, 2 H), 1.55–1.47 (m, 4 H), 1.43–1.35 (m, 6 H); ^{13}C NMR (125 MHz, CD_3OD δ_C) 80.0 (C-3_a), 77.2 (C-3_b), 70.9 (C-6_a), 70.0 (C-6_b), 66.1 (C-7_b), 65.3 (C-7_a), 62.9 ($\underline{C}H_2OH$), 53.8 (C-4_a), 51.6 (C-4_b), 50.3 ($S\underline{C}H_{2a}$), 48.1 ($S\underline{C}H_{2b}$), 46.9 ($S\underline{C}H_{2a}$), 43.8 ($S\underline{C}H_{2b}$), 42.9 ($S\underline{C}H_{2a}$), 40.2 ($S\underline{C}H_{2a}$), 33.7 ($\underline{C}H_2$), 30.2 ($\underline{C}H_2$), 30.0 ($\underline{C}H_2$), 29.9 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.8 ($\underline{C}H_2$), 26.5 ($\underline{C}H_2$). HRMS (ESI) *m/z* Calcd for (M–Cl^-) $C_{14}H_{29}O_4S$: 293.1781. Found: 193.1783.



4-39

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(12-benzyloxy-dodecyl)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-39)

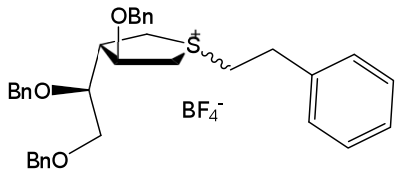
Using the general procedure for preparing the protected sulfonium ions, compound **4-13** (80 mg, 0.18 mmol) was reacted with **4-26** (89 mg, 0.22 mmol) and $AgBF_4$ (42 mg, 0.22 mmol) to give **4-39** (116 mg, 81%) as a colorless oil. R_f 0.38 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for (M–BF_4^-) $C_{46}H_{61}O_4S$: 709.4285. Found: 709.4288. The compound was not further characterized but rather was directly deprotected.



4-48

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(12-hydroxydodecyl)-tetrahydro-1*H*-thiophenium chloride (4-48)

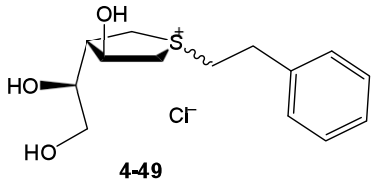
Using the general procedure for deprotection of the sulfonium ions, **4-39** (116 mg, 0.15 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-48** (39 mg, 70%) as a 6:4 ratio of inseparable stereoisomers as a colorless foam; R_f 0.06 (CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.74 (dd, 0.6 H, J = 2.0, 2.0 Hz, H-3$_a$), 4.67 (ddd, 0.4 H, J = 5.6, 5.6, 5.6 Hz, H-3b), 3.89–3.84 (m, 1 H), 3.82–3.74 (m, 1 H), 3.66–3.61 (m, 1 H), 3.54–3.34 (m, 8 H), 3.05 (dddd, 0.6 H, J = 1.9, 3.3, 4.7, 6.8, H-4$_a$), 2.68 (dddd, 0.4 H, J = 2.4, 5.0, 5.0, 8.5 Hz, H-4b), 1.86–1.77 (m, 2 H), 1.55–1.45 (m, 4 H), 1.40–1.28 (m, 14 H); ^{13}C NMR (100 MHz, CD_3OD δ_C) 80.0 (C-3$_a$), 77.2 (C-3$_b$), 70.9 (C-6$_a$), 70.0 (C-6$_b$), 66.1 (C-7$_b$), 65.3 (C-7$_a$), 63.0 ($\underline{C}H_2OH$), 53.8 (C-4$_a$), 51.6 (C-4$_b$), 50.3 (S$\underline{C}H_{2a}$), 48.1 (S$\underline{C}H_{2b}$), 46.9 (S$\underline{C}H_{2a}$), 43.8 (S$\underline{C}H_{2b}$), 42.9 (S$\underline{C}H_{2a}$), 40.2 (S$\underline{C}H_{2a}$), 33.7 (CH2), 30.7 ($\underline{C}H_2$), 30.6 ($\underline{C}H_2$), 30.4 ($\underline{C}H_2$), 30.1 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 26.9 ($\underline{C}H_2$), 26.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$); HRMS (ESI) *m/z* Calcd for (M–Cl^-) $C_{18}H_{37}O_4S$ 349.2407. Found: 349.2407.



4-40

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(2-phenethyl)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-40)

Using the general procedure for preparing the protected sulfonium ions, **4-13** (75 mg, 0.17 mmol) was reacted with **4-21** (32 mg, 0.18 mmol) and $AgBF_4$ (34 mg, 0.18 mmol) to give **4-40** (34 mg, 32%) as an colorless oil. R_f 0.70 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^-$) $C_{35}H_{39}O_3S$: 539.2614. Found: 539.2615. The compound was not further characterized but rather was directly deprotected.

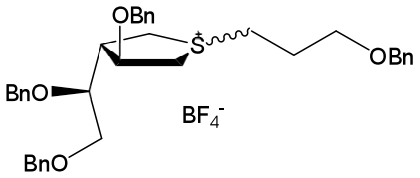


4-49

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(2-phenethyl)-tetrahydro-1*H*-thiophenium chloride (4-49)

Using the general procedure for deprotection of the sulfonium ions, **4-40** (34 mg, 0.054 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-49** (15 mg, 91%) as a 7:3 mixture of inseparable stereoisomers as a colorless foam. R_f 0.06 (pure CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 7.39–7.27 (m, 5 H, Ar), 4.71 (dd, 0.7 H, J = 1.9, 1.9 Hz, H-3_a), 4.65 (ddd, 0.3 H, J

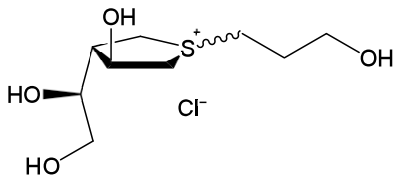
= 5.1, 5.1, 5.2 Hz, H-3_b), 3.84–3.34 (m, 9.6 H), 3.18 (dd, 1.4 H, *J* = 7.4, 7.4 Hz, C$\underline{H}_{2a}$), 2.97 (m, 0.7 H, H-4_a), 2.66 (dddd, 0.3 H, *J* = 2.0, 5.2, 5.2, 7.5 Hz, H-4_b); ^{13}C NMR (125 MHz, CD_3OD δ_C) 138.3 (Ar), 130.2 (Ar × 2), 129.8 (Ar × 2), 128.6 (Ar), 80.0 (C-3_a), 77.5 (C-3_b), 70.8 (C-6_a), 70.0 (C-6_b), 65.9 (C-7_b), 65.3 (C-7_a), 53.8 (C-4_a), 51.6 (C-4_b), 50.5 (S$\underline{C}H_{2a}$), 48.4 (S$\underline{C}H_{2b}$), 45.4 (S$\underline{C}H_{2a}$), 43.7 (S$\underline{C}H_{2b}$), 43.1 (S$\underline{C}H_{2a}$), 40.4 (S$\underline{C}H_{2a}$), 32.8 ($\underline{C}H_{2a}$), 32.6 ($\underline{C}H_{2b}$). HRMS (ESI) *m/z* Calcd for (M–Cl^-) $C_{14}H_{21}O_3S$ 269.1206. Found: 269.1202.



4-50

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(3-benzyloxypropyl)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-50)

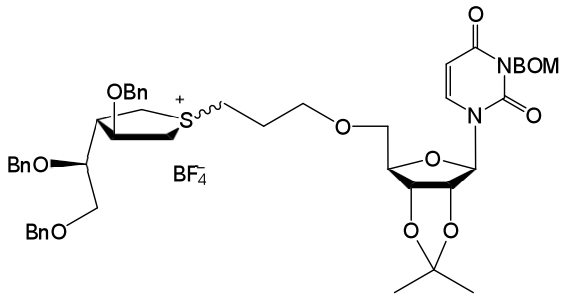
Using the general procedure for preparing the protected sulfonium ions, compound **4-13** (70 mg, 0.16 mmol) was reacted with **4-27** (50 mg, 0.18 mmol) and $AgBF_4$ (35 mg, 0.18 mmol) to give **4-50** (52 mg, 48%) as a colorless oil. R_f 0.67 (CH_2Cl_2–CH_3OH 9:1). HRMS (ESI) *m/z* Calcd for (M–BF_4^-) $C_{37}H_{43}O_4S$: 583.2877. Found: 583.2881. The compound was not further characterized but rather was directly deprotected.



4-51

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(3-hydroxpropyl)-tetra-hydro-1*H*-thiophenium chloride (4-51)

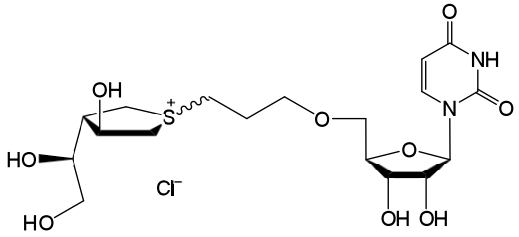
Using the general procedure for deprotection of the sulfonium ions, **4-50** (63 mg, 0.09 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-51** (23 mg, 94%) as a 7:3 mixture of stereoisomers as a colorless foam. R_f 0.05 (pure CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.73 (m, 0.7 H, H-3$_a$), 4.67 (ddd, 0.3 H, J = 5.1, 5.1, 5.1 Hz, H-3$_b$), 3.90–3.38 (m, 11 H), 3.06–3.04 (m 0.7 H, H-4$_a$), 2.66 (dddd, 0.3 H, J = 2.2, 6.1, 8.4, 11.4 Hz, H-4$_b$), 2.08–2.03 (m, 2 H, C$\underline{H}_2$); ^{13}C NMR (125 MHz, CD_3OD δ_C) 78.2 ($\underline{C}H_b$), 74.5 ($\underline{C}H_a$), 70.2 ($\underline{C}H_b$), 69.3 ($\underline{C}H_a$), 65.1 ($\underline{C}H_{2a}$), 64.7 ($\underline{C}H_{2b}$), 64.1 ($\underline{C}H_{2a}$), 60.2 ($\underline{C}H_{2b}$), 52.0 ($\underline{C}H_b$), 49.9 ($\underline{C}H_a$), 49.1 (S$\underline{C}H_{2b}$), 45.6 (S$\underline{C}H_{2a}$), 43.71 (S$\underline{C}H_{2b}$), 42.8 (S$\underline{C}H_{2b}$), 41.8 (S$\underline{C}H_{2a}$), 39.9 (S$\underline{C}H_{2a}$), 28.6 ($\underline{C}H_{2b}$), 28.3 ($\underline{C}H_{2a}$). HRMS (ESI) *m/z* Calcd for ($M-Cl^-$) $C_9H_{19}O_4S$ 223.0999. Found: 223.0999.



4-56

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(3-(2'3'-*O*-isopropylidene-3-(benzyloxymethyl)uridin-5-yl)propyl)-3-(benzyloxy)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-56)

Using the general procedure for preparing the protected sulfonium ions, **4-13** (80 mg, 0.18 mmol) was reacted with **4-29** (105 mg, 0.18 mmol) and $AgBF_4$ (35 mg, 0.18 mmol) to afford the product **4-56** (53 mg, 30%) as a colorless oil. R_f 0.45 (CH_3OH–CH_2Cl_2 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^-$) $C_{50}H_{59}N_2O_{10}S$: 879.3885. Found: 879.3883. The compound was not further characterized but rather was directly deprotected.

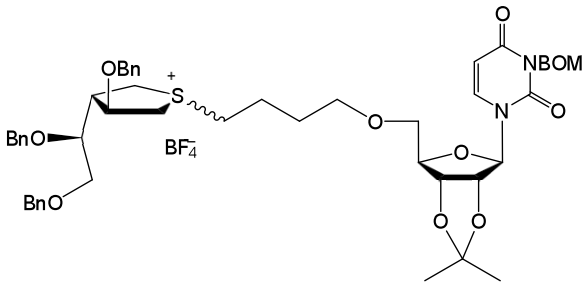


4-58

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(3-(uridin-5-yl-)propyl))-tetrahydro-1*H*-thiophenium chloride (4-58)

Using the general procedure for deprotection of the sulfonium ions, **4-56** (53 mg, 0.05 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-58** (14 mg, 55%) as a 4:1 inseparable mixture of stereoisomers as a colorless foam. R_f 0.05 (pure CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 7.87 (d, 0.8 H, J = 8.1 Hz, C<u>H</u>=$_a$) 7.76 (d, 0.2 H, J = 8.1 Hz, C<u>H</u>=$_b$), 5.83 (d, 0.8 H, J = 4.1 Hz, H-1'$_a$), 5.82 (d, 0.2 H, J = 4.1 Hz, H-1'$_b$), 5.76 (d, 0.8 H, J = 8.1 Hz, C<u>H</u>=$_a$), 5.74 (d, 0.2 H, J = 8.1 Hz, C<u>H</u>=$_b$), 4.73 (dd, 0.8 H, J = 1.8, 2.0 Hz, H-3$_a$), 4.66 (dd, 0.2 H, J = 5.4, 5.4 Hz, H-3$_b$), 4.20–4.07 (m, 3 H) , 3.91–3.37 (m, 13 H), 3.03 (m, 0.8 H, H-4$_a$), 2.58 (dddd, 0.2 H, J = 2.4, 5.6, 5.6, 11.1 Hz, H-4$_b$), 2.20–2.13 (m, 2 H, C<u>H</u>$_2$); ^{13}C NMR (125 MHz, CD_3OD δ_C) 166.1 (<u>C</u>=O), 152.3 (<u>C</u>=O), 142.5 (<u>C</u>H=), 102.9 (<u>C</u>H=), 91.5 (C-1'), 84.3 (<u>C</u>H), 80.0 (<u>C</u>H), 75.3 (<u>C</u>H), 71.6 (<u>C</u>H$_2$O), 71.3 (<u>C</u>H), 70.9 (<u>C</u>H$_a$), 70.3 (<u>C</u>H$_2$O), 70.0 (<u>C</u>H$_b$), 66.1 (C-7$_b$), 65.3 (C-7$_a$), 53.8 (C-4$_a$), 51.6 (C-4$_b$), 50.9 (S<u>C</u>H$_{2a}$), 50.4 (S<u>C</u>H$_{2b}$), 45.2 (S<u>C</u>H$_{2a}$), 43.5

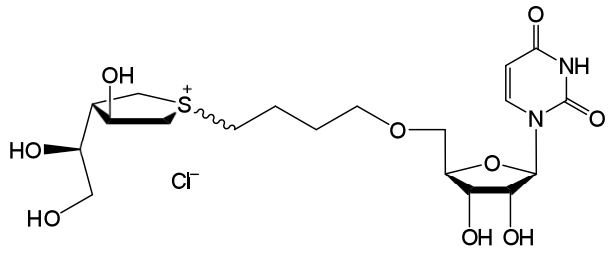
($S\underline{C}H_{2a}$), 42.3 ($S\underline{C}H_{2b}$), 40.8 ($S\underline{C}H_{2b}$), 27.3 ($\underline{C}H_{2a}$), 27.1 ($\underline{C}H_{2b}$). HRMS (ESI) *m/z* Calcd for ($M–Cl^-$) $C_{18}H_{29}N_2O_9S$ 449.1588. Found:449.1588.



4-57

(3*R*,4*S*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)-1-(3-(2'3'-*O*-isopropylidene-3-(benzyloxymethyl)uridin-5-yl)butyl)-3-(benzyloxy)-tetrahydro-1*H*-thiophenium tetrafluoroborate (4-57)

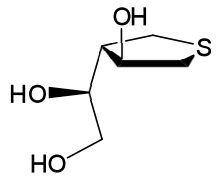
Using the general procedure for preparing the protected sulfonium ions, **4-13** (70 mg, 0.16 mmol) was reacted with **4-30** (95 mg, 0.16 mmol) and $AgBF_4$ (32 mg, 0.16 mmol to give the **4-57** (62 mg, 39%) as a colorless oil. R_f 0.45 ($CH_3OH–CH_2Cl_2$ 9:1). HRMS (ESI) *m/z* Calcd for ($M–BF_4^-$) $C_{51}H_{61}N_2O_{10}S$: 893.4041. Found: 893.4042. The compound was not further characterized but rather was directly deprotected.



4-59

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(3-(uridin-5-yl-)butyl))-tetrahydro-1*H*-thiophenium chloride (4-59)

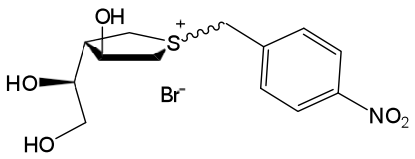
Using the general procedure for deprotection of the sulfonium ions, **4-56** (51 mg, 0.05 mmol) was treated with BCl_3 (1 M in CH_2Cl_2) and then Amberlyst (Cl^- form) to give **4-59** (14 mg, 54%) as a colorless foam as an 88:12 ratio of isomers. R_f 0.05 (pure CH_3OH); ^{1}H NMR (300 MHz, CD_3OD, δ_H) 7.90 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 5.86 (d, 1 H, *J* = 3.6 Hz, H-1'), 5.70 (d, 1 H, *J* = 8.1 Hz, C<u>H</u>=), 4.73 (dd, 1 H, *J* = 2.0, 2.3 Hz, H-3), 4.17–4.07 (m, 3 H, H-2', H-3', H-4') , 3.88 (dd, 1 H, *J* = 1.8, 12.3 Hz, H-5), 3.80–3.73 (m, 2 H, H-2, H5'), 3.66–3.35 (m, 10 H), 3.05 (m, 1 H, H-4), 1.97–1.86 (m, 2 H, C<u>H</u>$_2$), 1.83–1.75 (m, 2 H, C<u>H</u>$_2$); ^{13}C NMR (100 MHz, CD_3OD δ_C) 166.2 (<u>C</u>=O), 152.3 (<u>C</u>=O), 142.5 (<u>C</u>H=), 102.6 (<u>C</u>H=), 91.2 (C-1'), 84.6 (<u>C</u>H), 80.1 (<u>C</u>H), 75.7 (<u>C</u>H), 71.4 (<u>C</u>H$_2$O), 71.3 (<u>C</u>H), 71.1 (<u>C</u>H$_2$O), 70.9 (<u>C</u>H), 65.3 (C-7), 53.9 (C-4), 50.4 (S<u>C</u>H$_2$), 46.7 (S<u>C</u>H$_2$), 42.9 (S-<u>5</u>), 29.3 (<u>C</u>H$_2$), 24.0 (<u>C</u>H$_2$); HRMS (ESI) *m/z* Calcd for (M–Cl^-) $C_{19}H_{31}N_2O_9S$ 463.1745. Found: 463.1742.



4-61

(*S*)-1-((3*S*,4*R*)-4-hydroxytetrahydrothiophen-3-yl)ethane-1,2-diol

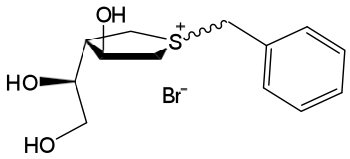
Ammonia (20 mL) was condensed at –78 ºC and a solution of **4-13** (0.16 g, 0.37 mmol) in THF (6 mL) was added. To this mixture was then added sodium metal (0.5 g, 22 mmol) and CH_3OH (30 μL). The reaction mixture was stirred for 1 h –78 ºC and then CH_3OH (10 mL) was added. The solution was then warmed to rt, neutralized with HOAc and concentrated. The resulting residue was purified by chromatography (CH_2Cl_2–CH_3OH 9:1) to give **4-61** (54.0 mg, 90%) as a colorless oil. R_f 0.27 (CH_2Cl_2–CH_3OH 9:1); $[\alpha]_D$ –9.2 (*c* 1.73, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 4.22 (ddd, 1 H, *J* = 6.0, 7.7, 7.7 Hz, H-3), 3.72 (ddd, 1 H, *J* = 5.0, 5.0, 6.7 Hz, H-6), 3.56 (dd, 1 H, *J* = 5.0, 11.3 Hz, H-7), 3.51 (dd, 1 H, *J* = 6.7, 11.3 Hz, H-7), 2.94 (dd, 1 H, *J* = 6.0, 10.5 Hz, H-2), 2.88 (dd, 1 H, *J* = 8.8, 10.6 Hz, H-5), 2.82 (dd, 1 H, *J* = 7.6, 10.6 Hz, H-5), 2.68 (dd, 1 H, *J* = 7.7, 10.5 Hz, H-2), 2.87 (dddd, 1 H, *J* = 5.0, 7.6, 7.7, 8.8 Hz, H-4); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 76.2 (C-3), 71.6 (C-6), 66.4 (C-7), 52.6 (C-4), 37.3 (C-2), 27.9 (C-5). HRMS (ESI) *m/z* Calcd for (M + Na^+) $C_6H_{12}O_3S$: 187.0399. Found: 187.0400.



4-64

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(4-nitrobenzyl)-tetrahydro-1*H*-thiophenium bromide (4-64)

To a solution of **4-61** (10 mg, 0.061 mmol) in 1,1,1,3,3,3-hexafluoroisopropanol (1 mL) was added 4-nitrobenzyl bromide (**4-62**, 18 mg, 0.09 mmol). The mixture was stirred at 50 ºC for 18 h, cooled to rt and then concentrated. The resulting residue was purified by chromatography (CH_2Cl_2–CH_3OH 2:1) on Iatrobeads to give **4-64** (13.5 mg, 58%) as a yellowish oil as a 7:3 mixture of inseparable stereoisomers. R_f 0.06 (pure CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 8.34–8.32 (m, 2 H, Ar), 7.82–7.79 (m, 2 H, Ar), 4.93–4.88 (m, 2 H, C$\underline{H_2}$Ph), 4.79 (dd, 0.7 H, J = 2.0, 2.0 Hz, H-3$_a$), 4.67 (ddd, 0.3 H, J = 5.4, 5.4, 5.4 Hz, H-3$_b$), 3.91–3.87 (m, 1 H), 3.79–3.71 (m, 1.4 H), 3.67–3.63 (m, 1.3 H), 3.60–3.48 (m, 2 H), 3.43–3.39 (m, 1.3 H), 3.11 (dddd, 0.7 H, J = 1.6,.3.2, 4.8, 6.5 Hz, H-4$_a$), 2.74 (dddd, 0.3 H, J = 2.1, 5.6, 5.6, 7.8 Hz, H-4$_b$); ^{13}C NMR (125 MHz, CD_3OD δ_C) 138.3 (Ar), 137.7 (Ar), 132.8 (Ar × 2), 125.6 (Ar), 80.3 (C-3$_a$), 77.5 (C-3$_b$), 70.8 (C-6$_a$), 70.0 (C-6$_b$), 66.2 (C-7$_b$), 65.3 (C-7$_a$), 54.1 (C-4$_a$), 51.8 (C-4$_b$), 50.1 (S$\underline{C}$H$_{2a}$), 49.9 (S$\underline{C}$H$_{2a}$), 48.8 (S$\underline{C}$H$_{2b}$), 46.2 (S$\underline{C}$H$_{2b}$), 43.4 (S$\underline{C}$H$_{2a}$), 40.0 (S$\underline{C}$H$_{2b}$). HRMS (ESI) *m/z* Calcd for (M–Cl$^-$) $C_{13}H_{18}NO_5S$: 300.0900. Found: 300.0904



4-65

(3*R*,4*S*)-3-((*S*)-1,2-dihydroxyethyl)-4-hydroxy-1-(4-benzyl)-tetrahydro-1*H*-thiophenium bromide (4-65)

To a solution of **4-61** (11 mg, 0.07 mmol) in 1,1,1,3,3,3-hexafluoroisopropanol (0.5 mL) was added benzyl bromide **4-63** (14 mg, 0.08 mmol). The mixture was stirred at 50 ºC for 5 h, cooled to rt and then concentrated. The residue was purified by chromatography (CH_2Cl_2–CH_3OH 2:1) on Iatrobeads to give **4-65** (12.6 mg, 57%) as a colorless oil as a 7:3 mixture of inseparable stereoisomers. R_f 0.06 (pure CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 7.55–7.46 (m, 5 H, Ar), 4.79 (dd, 0.7 H, *J* = 2.0, 2.0 Hz, H-3_a), 4.77 (s, 1.4 H, C$\underline{H_2}$-Ph_a), 4.74 (s, 0.6 H, C$\underline{H_2}$-Ph_b), 4.65 (ddd, 0.3 H, *J* = 5.7, 5.7, 5.7 Hz, H-3_b), 3.88 (ddd, 0.3 H, *J* = 2.3, 5.7, 5.7 Hz), 3.78 (dd, 0.7 H, *J* = 1.9, 12.4 Hz), 3.72–3.34 (m, 6 H), 3.11 (dddd, 0.7 H, *J* = 1.9,.3.4, 3.4, 6.8 Hz, H-4_a), 2.70 (dddd, 0.3 H, *J* = 2.4, 5.9, 5.9, 8.4 Hz, H-4_b); ^{13}C NMR (100 MHz, CD_3OD δ_C) 131.6 (2 C, Ar_b × 2), 131.4 (2 C, Ar_a × 2), 131.3 (Ar_b), 131.1 (Ar_a), 131.0 (Ar_a), 130.9 (2 C, Ar_b × 2), 130.8 (2 C, Ar_a × 2), 130.4 (Ar), 80.2 (C-3_a), 77.2 (C-3_b), 70.8 (C-6_a), 70.0 (C-6_b), 66.1 (C-7_b), 65.3 (C-7_a), 53.9 (C-4_a), 51.7 (C-4_b), 50.9 (S$\underline{C}H_{2a}$), 49.4 (S$\underline{C}H_{2a}$), 47.8 (S$\underline{C}H_{2b}$), 47.4 (S$\underline{C}H_{2b}$), 42.7 (S$\underline{C}H_{2a}$), 39.4 (S$\underline{C}H_{2b}$). HRMS (ESI) *m/z* Calcd for (M–Cl^-) $C_{13}H_{19}O_3S$: 255.1049. Found: 255.1047.

4.4 Bibliography

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Chapter 5

Inhibition Activity of Target Analogues as Inhibitors of Mycobacterial GlfT2

5.1 Introduction

This chapter describes the testing of the UDP-Gal*f* analogues prepared in Chapters 3 and 4 against mycobacterial galactofuranosyltransferase 2 (GlfT2). As outlined in Chapter 1, this enzyme is essential for the biosynthesis of the mycobacterial cell wall, and our group has been studying this enzyme for several years.[1-4] The assay we used to test these compounds against GltT2 is a coupled spectrophotometric assay developed previously in the group,[3] which is outlined in Figure 5-1.

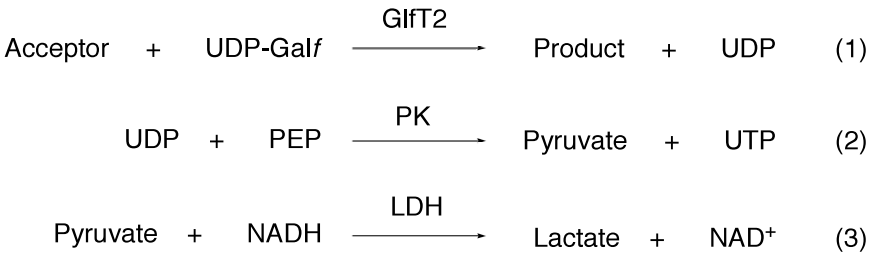
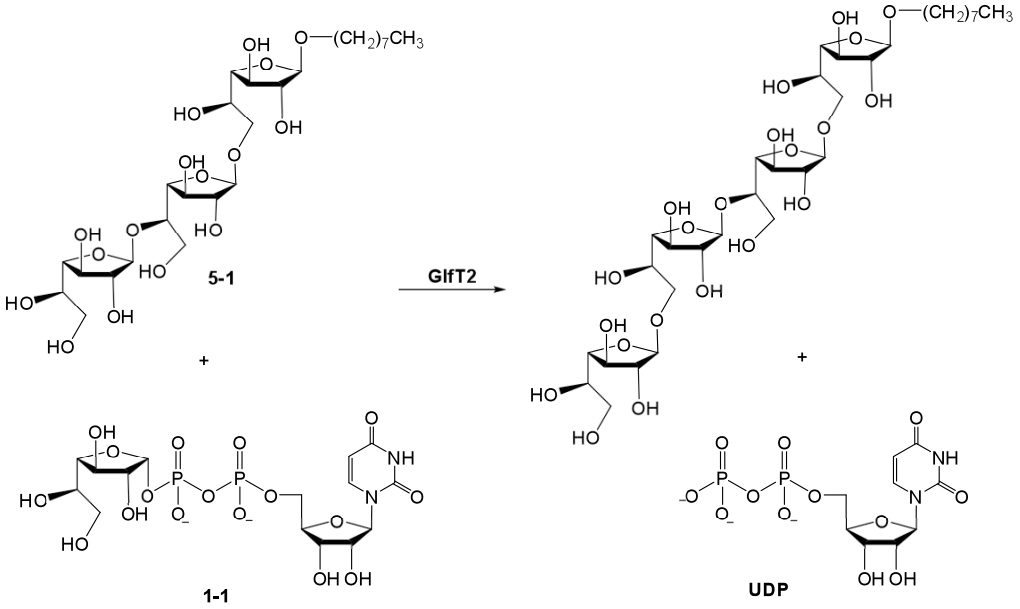


Figure 5-1. Coupled spectrophotometric assay for measuring GlfT2 activity; Phosphoenopyruvate (PEP), Pyruvate kinase (PK); Lactate Dehydrogenase (LDH), Nicotinamide adenine dinucleotide (NADH)

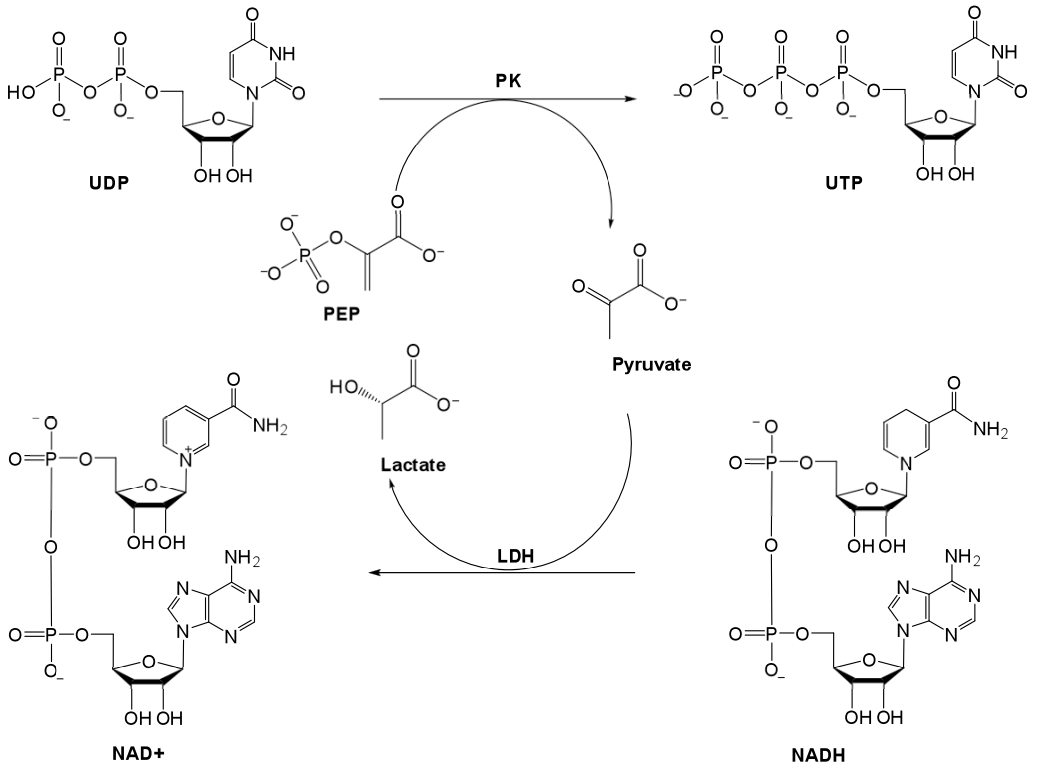
This assay is based on three coupled reactions (Figure 5-1). The acceptor used in the assay is trisaccharide **5-1**,[1] which is known to be a substrate of GlfT2. Catalyzed by GlfT2, the trisaccharide reacts with the native donor substrate UDP-Gal*f* (**1-1**) to form the elongated tetrasaccharide; at the same time one molecule of UDP is generated (Scheme 5-1).



Scheme 5-1. Reaction catalyzed by GlfT2

The rate of the GlfT2 reaction can be measured spectrophotometrically when linked with two enzymatically-catalyzed reactions. As shown in Scheme 5-2, UDP is phosphorylated with phosphoenopyruvate (PEP) catalyzed by pyruvate kinase (PK) to form UTP and pyruvate. The resulting pyruvate undergoes a

redox reaction with NADH. Catalyzed by lactate dehydrogenase (LDH), pyruvate is reduced into lactate, while NADH is oxidized into NAD^+. NADH has absorption at 340 nm and the concentration of NADH will decrease when all three reactions proceed, and the absorbance of the solution will decrease correspondingly. The first step is the rate determining step, and because the units of PK and LDH activity are present in about 8,000 and 18,000 fold excess of GlfT2 units, respectively. Therefore, the rate of the absorbance decrease is proportional to the rate of the GlfT2 reaction.



Scheme 5-2. Reactions catalyzed by PK and LDH

5.2 Results and discussion

The analogues, described in Chapters 3 and 4, shown in Figure 5-2–5-4, were investigated as potential inhibitors of GlfT2 using a coupled spectrophotometric assay as reported. In these assays, a potential inhibitor is added to the incubation mixture together with trisaccharide **5-1** and UDP-Gal*f* (**1-1**). The assays were performed in 384-array microplate wells incubated at 37 ºC and monitored using a Spectra Max 340PC microplate reader. The experimental data were obtained in the kinetic read mode and analyzed using SoftMax®Pro Version 4.6. All other data were graphed using Microsoft Excel.

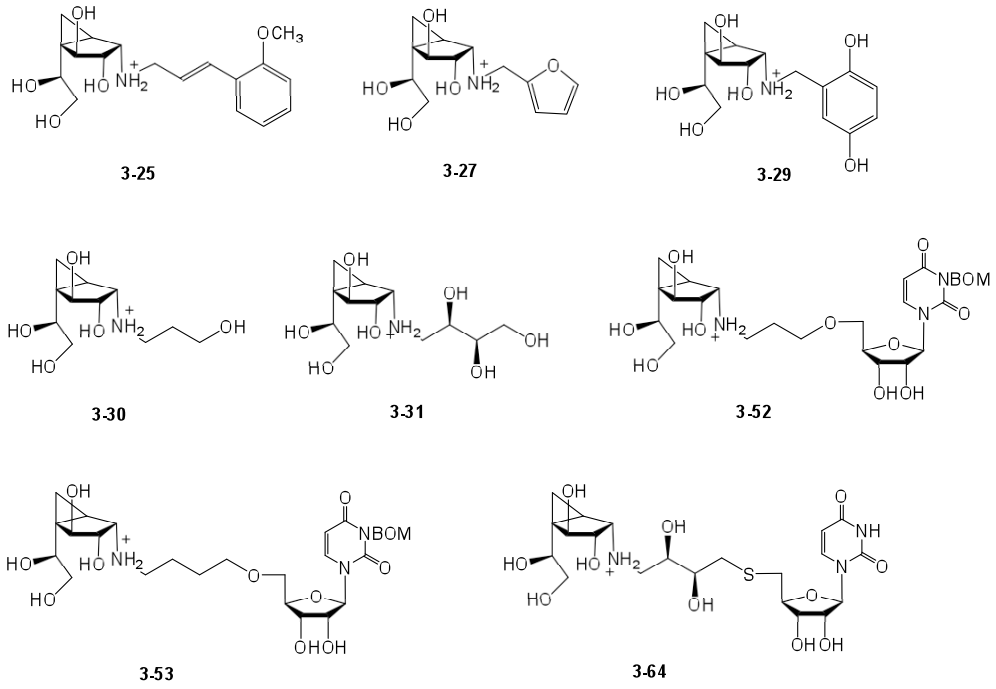


Figure 5-2. Structures of the bicyclo[3.1.0]hexane-based Gal*f* analogues

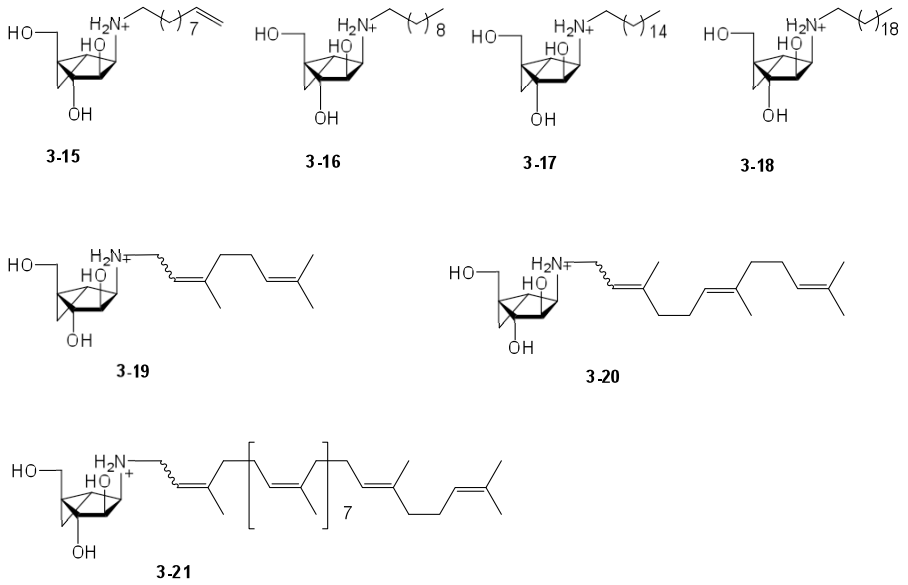


Figure 5-3. Structures of the bicyclo[3.1.0]hexane-based Ara*f* analogues

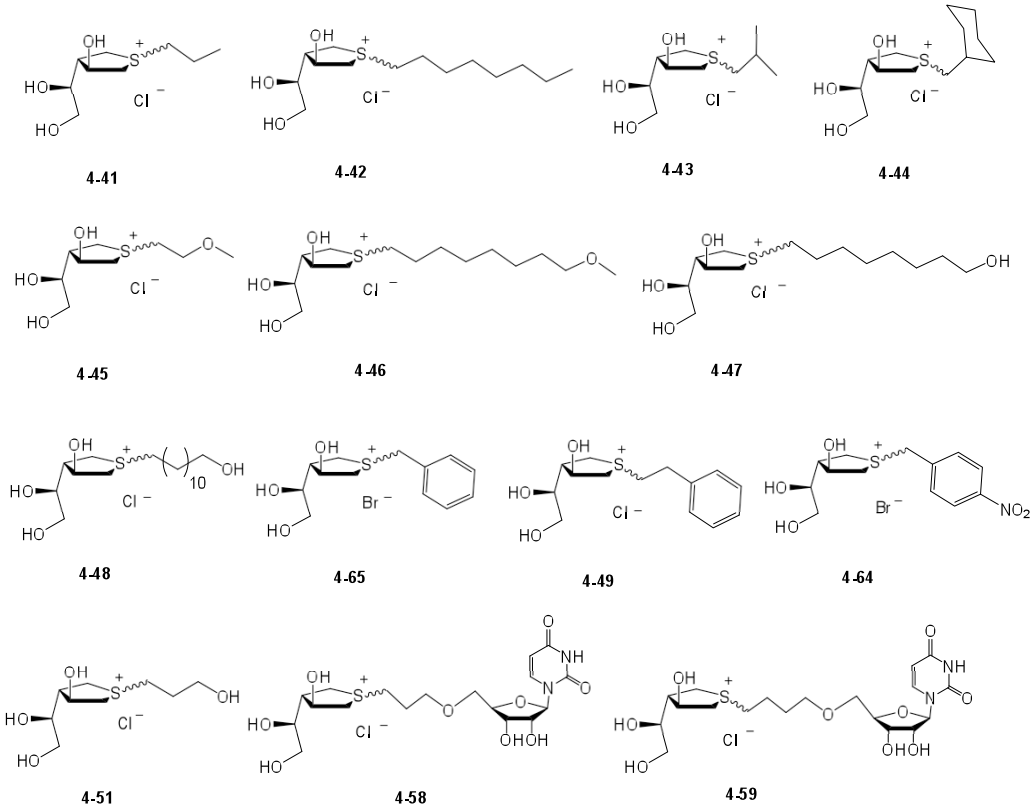


Figure 5-4. Structures of sulfonium ion analogues Gal*f* analogues

Most of the analogues were screened at a final concentration of 4 mM; compounds **3-17**−**3-21** were screened at 2 mM, because of their low solubility in the assay medium. First, they were tested with all components except UDP-Gal*f* and GlfT2 to detect the impurities that could be shuttled directly by linking enzymes and give GlfT2-independent velocities. The experiments showed that none of the analogues caused GlfT2-independent shuttling. Compounds were then tested for donor activity against GlfT2 and, not unsurprisingly, all of them had less than 10% of control activity compared to 750 μM UDP-Gal*f* (the K_M of UDP-Gal*f* for GlfT2 is ~400 μM). The analogues were then screened for inhibition activity using 750 μM UDP-Gal*f*; the percentage activities compared to the no-inhibitor control are shown in Figures 5-5–5-8.

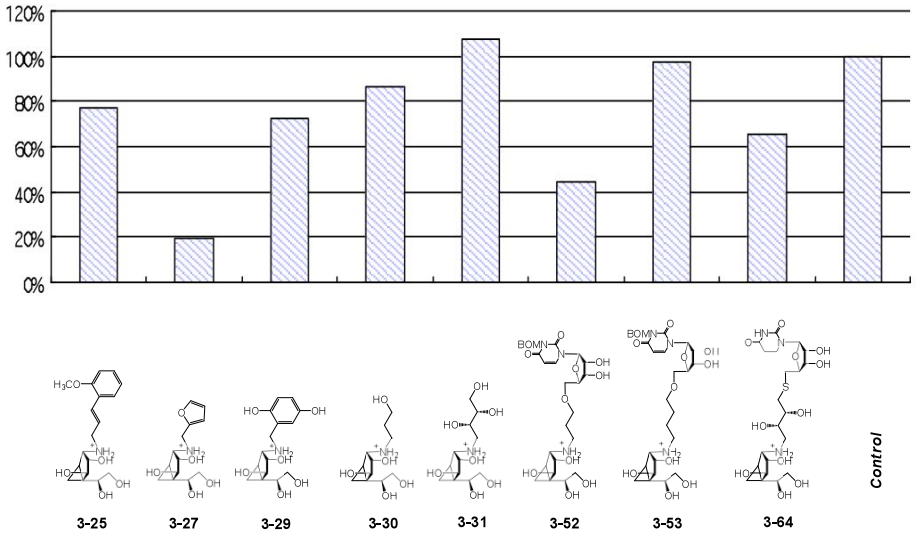


Figure 5-5. Inhibition activity of the bicyclo[3.1.0]hexane-based Gal*f* analogues against GlfT2.

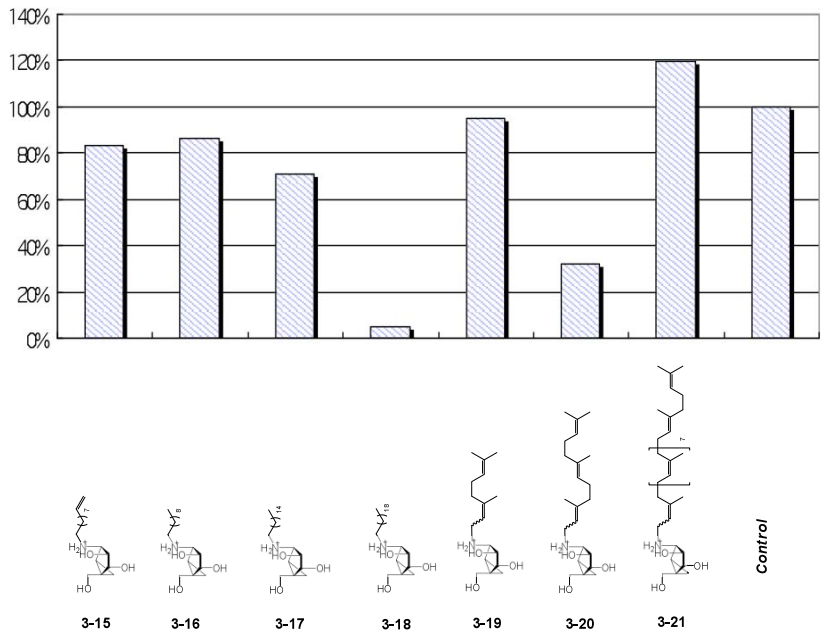


Figure 5-6. Inhibition activity of the bicyclo[3.1.0]hexane-based Ara*f* analogues against GlfT2.

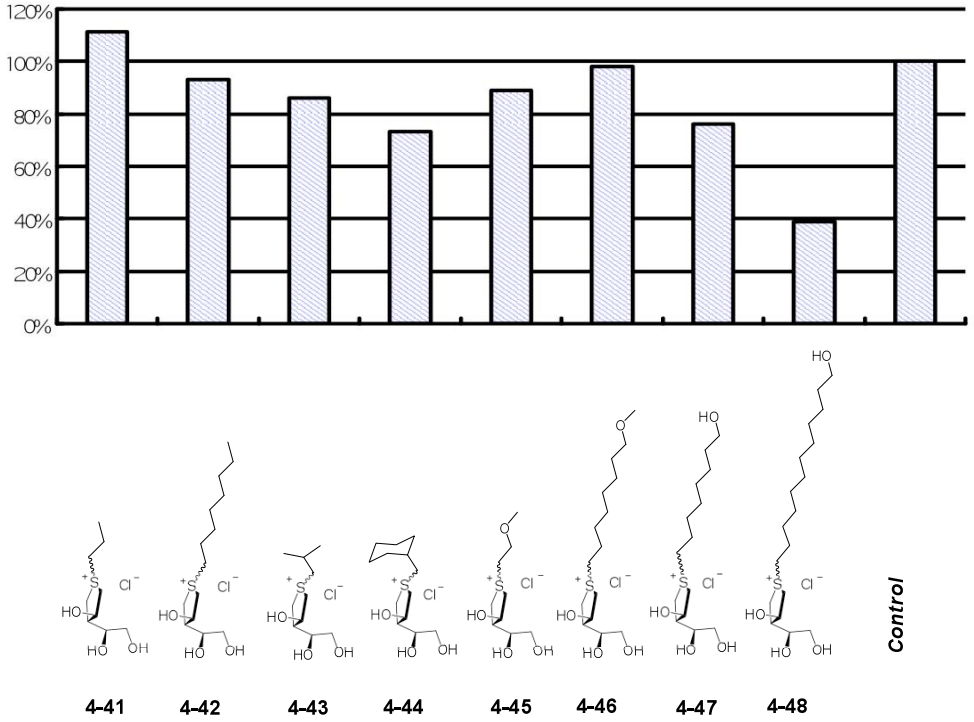


Figure 5-7. Inhibition activity of the sulfonium ion analogues against GlfT2.

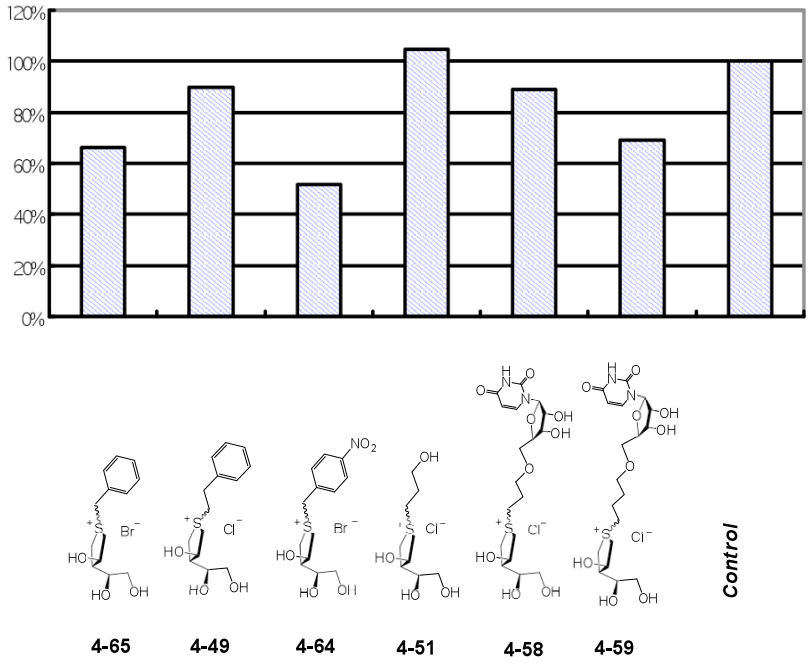


Figure 5-8. Inhibition activity of the sulfonium ion analogues against GlfT2.

The results suggest that most of the analogues are weak inhibitors of GlfT2 and that the inclusion of the uridine moiety did not enhance inhibitory binding. It is interesting to note that two of the more active compounds, **3-18** and **3-20**, were designed as Ara*f* mimics and therefore were not expected to inhibit the enzyme. There is one other feature worth mentioning. Compounds **4-48**, **3-18** and **3-20**, which have the longer lipid tails showed better inhibition. Although compound **3-21** has a long lipid tail, it does not show any inhibition partially because of its insolubility under the assay conditions. In a recent report by Kiessling,[5] a new acceptor binding model for the GlfT2 catalyzed reaction was proposed. In this model, it was suggested that a lipid on the acceptor can bind to

a site remote from the active site of the enzyme. They further suggested that the lipid chain plays an important role in the length of the polymerized product. It is possible that inhibition by donor analogues **4-48**, **3-18** and **3-20** reported in this study could reflect binding to both donor and "lipid binding" sites.

5.3 Conclusions

In summary, testing of the panel of analogues synthesized in Chapters 3 and 4 against GlfT2 revealed that most of the compounds were not significant inhibitors of the enzyme. Four of the compounds, **3-18**, **3-27**, **3-52** and **4-48**, showed the most activity and further studies directed at understanding their mode of inhibition are underway.

5.4 Experimental

General Methods.

Solutions of 2M KCl, 1 M $MgCl_2$, and 1 M MOPS (pH 7.6) were prepared in de-ionized distilled (MilliQ, MQ) water, filtered and stored at 4 ^{o}C. Recombinant GlfT2, prepared and stored as previously reported were used in the assay.[3] On the day of experiment, donor analogues were reconstituted in filtered MQ water to give a 32 mM stock. Due to limited solubility in water, compound **3-15** − **3-21** were dissolved in methanol to give a 32 mM stock solution.

Solutions of 15 mM NADH, 5 U/mg PK, 16.8 U/mg LDH, and 40 mM UDP-Gal*f* were prepared in 50 mM MOPS (pH 7.6); 100 mM PEP was prepared in 250 mM MOPS (pH 7.6); 40 mM trisaccharide **5-1** was prepared in filtered MQ water. All solutions were stored on ice during use.

Evaluation of ability of compounds to inhibit linking enzymes

To check the analogues against the linking enzymes (PK and LDH), the assay components were mixed in 384-array mictotitre plate wells to give a mixture of 50 mM MOPS (pH 7.6), 50 mM KCl, 20 mM $MgCl_2$, 1.1 mM NADH, 3.5 mM PEP, 7.5 U PK, 16.8 U LDH, 2 mM **5-1** and 4 mM analogues (2 mM for **3-17**−**3-21**). Reactions were incubated at 37 ºC and monitored at 340 nm at 10–15 sec intervals for 20 min using a Spectra Max 340PC microplate reader as reported previously.[3]

Evaluation for ability of compounds to serve as donors substrates for GlfT2.

Reactions were initiated with the addition of GlfT2 (0.5 μg) to assays to give a final volume of 40 μL containing 50 mM MOPS (pH 7.6), 50 mM KCl, 20 mM $MgCl_2$, 1.1 mM NADH, 3.5 mM PEP, 7.5 U PK, 16.8 U LDH, 2 mM **5-1** and 4 mM analogues (2 mM for **3-17**−**3-21**). Reactions were incubated at 37 ºC and monitored at 340 nm at 10-15 sec intervals for 20 min using a Spectra Max 340PC microplate reader in the kinetic read mode.

Evaluation for ability of compounds to serve as inhibitors of GlfT2.

Reactions to screen the ability of analogues to inhibit GlfT2 were initiated with the addition of GlfT2 (0.5 ug) to assays to give a final volume of 40 μL containing 50 mM MOPS (pH 7.6), 50 mM KCl, 20 mM $MgCl_2$, 1.1 mM NADH, 3.5 mM PEP, 7.5 U PK, 16.8 U LDH, 2 mM **5-1**, 4 mM analogues (2 mM for **3-17**−**3-21**) and 0.75 mM donor UDP-Gal*f*. Reactions were incubated at 37 ºC and monitored at 340 nm at 10–15 sec intervals for 20 min using a Spectra Max 340PC microplate reader. The inhibition screening assays were repeated at two-times linking enzyme levels (15 U PK and 33.6 U LDH), to rule out inhibition of the linking enzymes by the analogues.

5.5 Bibliography

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Chapter 6

Conclusions and Future Work

Conclusions

In summary, this thesis describes the synthesis of two types of UDP-Gal*f* analogues and one type of DPA analogues. These compounds were then investigated as inhibitors for galactofuranosyltransferase 2 (GlfT2) from mycobacteria.

In Chapter 2, we have described the synthesis of conformationally-restricted analogues of β-arabinofuranosyl and α-galactofuranosyl rings starting from an achiral starting material. A key step is a base-promoted ring contraction of epoxy ketone to form the bicyclo[3.1.0]hexane system. A second key feature is a late stage resolution of enantiomers via partial derivatization with *O*-acetyl-(*S*)-mandelic acid. The α-Gal*f* mimic was obtained in 2.8% yield over 20 steps and β-Ara*f* mimic was synthesized in 2.4% yield over 22 steps. The bicyclo[3.1.0]hexane amine analogues of α-Gal*f* and β-Ara*f* were then derivatized into *N*-alkylated compounds, which are mimics of UDP-Gal*f* and DPA, respectively.

The synthesis of *N*-alkylated compounds was described in Chapter 3. The alkylation was accomplished through a reductive amination reaction. The

bicyclo[3.1.0]hexane amine intended to mimic the α-Gal*f* ring was derivatized into eight UDP-Gal*f* analogues; and the β-Ara*f* mimic was derivatized into seven DPA analogues. In both cases, reductive amination proceeded successfully. A key point was to use freshly distilled methanol, which could stop the formation of a side product, a methylated analog. Most of the aldehydes used in these reactions were synthesized and were used immediately after the purification.

A second type of UDP-Gal*f* analogues was synthesized in Chapter 4. In these compounds, the sugar mimicking moiety is a sulfonium ion. The key intermediate was a cyclic sulfide, which was synthesized in 11.2% yield in 10 steps starting from D-arabinitol. The important step in the sequence is the formation of an organoborane followed by oxidation to convert an alkene to a hydroxymethyl group. With the cyclic sulfide in hand, 14 sulfonium ion compounds were obtained via a coupling reaction between the sulfide and different alkyl iodides. The reactions were performed under an argon atmosphere in the presence of $AgBF_4$ in CH_3CN at 65 ºC.

Finally, all of the analogues were tested as potential inhibitors of GlfT2, which is described in Chapter 5. The compounds were, at best, modest inhibitors of the enzyme and further studies with the most active compounds are ongoing.

Future work

To design and synthesize efficient inhibitors remains a difficult task. In the mimics of UDP-Gal*f* obtained in the thesis, there is one aspect that needs to be considered – the pyrophosphate moiety. As discussed in Chapter 1, the pyrophosphate plays an important role in the enzymatic reaction. To complete this series of work, compounds such as **6-1** and **6-2**, could be synthesized and tested for their inhibition abilities.

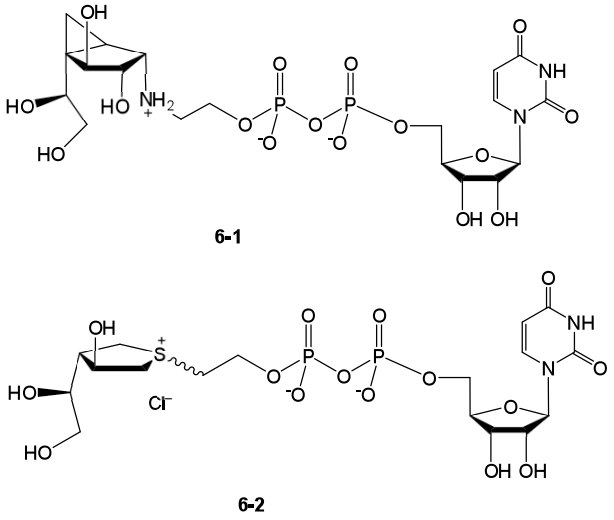

Figure 6-1. Example of analogues with pyrophosphate

From the inhibition result, compound **3-18** has a fairly good inhibition for GlfT2. But the molecule is designed to be a mimic of the Ara*f* ring system. The corresponding alkylated galactofuranose mimics **6-3** and **6-4** could also be prepared consideration. In addition, to improve the solubility of these analogues

in water, we could synthesize compound **6-5**, which have an oxygen-containing lipid chain.

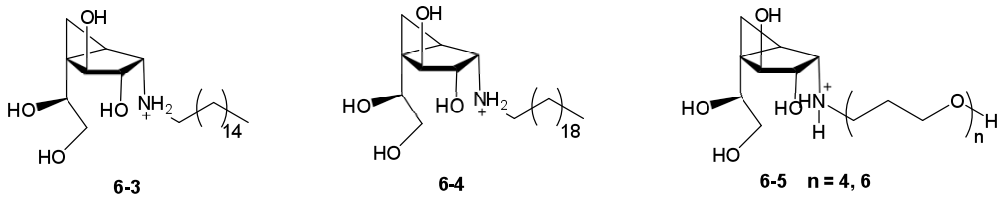

Figure 6-2. Targets with long lipid chain