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Toward the Synthesis of CAY-1, an Antifungal Steroidal Saponin

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Toward the Synthesis of CAY-1,
an Antifungal Steroidal Saponin

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ABBREVIATIONS

Ac	Acetyl
Bn	Benzyl
Bz	Benzoyl
CSA	Camphorsulfonic acid
DIBAO^{i}Pr	*Al*-Isopropoxydiisobutylalane
DME	Ethylene glycol dimethyl ether
DMF	*N,N*-Dimethylforamide
DMSO	Dimethylsulfoxide
HMDS	1,1,1,3,3,3-hexamethyldisilazane
LDA	Lithium diisopropylamide
MBz	4-Methoxybenzoyl
m-CPBA	*m*-Chloroperoxybenzoic acid
Ms	Mesylate
9-BBN	9-Borabicyclo[3.3.1]nonane
NIS	*N*-Iodosuccinimide
PE	Petroleum ether
RCF	Relative centrifugal force
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyltriflate

ABSTRACT

Invasive fungal infections are prevalent and often deadly in immunocompromised patients. There continues to be a pressing need for the development of novel antifungal compounds since there are currently only 13 compounds licensed for the treatment of invasive fungal infections and antibiotic-resistant strains have been emerging. CAY-1 is an antifungal steroidal saponin which was isolated from the fruit of the cayenne pepper plant in 0.1% yield. *In Vitro* studies of CAY-1 have shown it to be an effective antifungal agent against sixteen pathogenic fungal strains and it showed no cytotoxicity toward mammalian cells up to 100 μg/mL. The development of a practical synthesis of CAY-1 will potentially allow for further exploration of its medicinal utility and provide the opportunity to synthesize derivatives of CAY-1 which could be investigated in structure-activity relationship studies. To this end, methods for the preparation of they CAY-1 aglycone and pentasaccharide moieties have been investigated.

Through this work, several partially protected stereoisomers of the CAY-1 aglycone have been prepared which can be used for the synthesis of saponin derivatives of CAY-1 for structure-activity relationship studies. Definitive characterization of one of these isomers, 3α-hydroxy-(22*S*,25*R*)-5α-spirostan-2β-yl acetate, was achieved by X-ray crystallography. Furthermore, a quantitative inversion of the C-3 stereochemical configuration of this compound was achieved via an acetate group migration of the corresponding mesylate. The possibility of competition between the acetate migration and substitution mechanisms with various nucleophiles was explored. The results, however, indicate that this inversion only occurs via the acetate migration.

Additionally, the CAY-1 pentasaccharide synthesis poses two significant challenges. First, these results demonstrate that the central 2,3-branched portion can be synthesized efficiently from a partially protected glucopyranosyl acceptor since the C-2 and C-3 alcohols differ in their reactivity in glycosylation reactions. The second challenge is the β-(1→4) linkage to the galactosyl acceptor which significantly increases the complexity of the synthesis as compared to literature reported syntheses of other branched oligosaccharides. Nonetheless, this β-(1→4) linkage was achieved using a disarmed trichloroacetimidate glucosyl donor.

INTRODUCTION

Pharmacological Need for Novel Antifungal Compounds

Pathogenic fungi cause a wide variety of infections in humans and most of these, including skin, mucosal, and allergic infections, are not typically life-threatening. However, although rare in individuals with healthy immune systems, invasive fungal infections, which affect the major internal organ systems of the body, are prevalent and often deadly in immunocompromised patients such as bone marrow transplant recipients, AIDS patients, and cancer patients receiving antineoplastic chemotherapy. The most important risk factors associated with the development of invasive fungal infections are neutropenia, which is characterized by an abnormally low number of neutrophilic leukocytes in the blood, exposure to broad-spectrum antibiotics, and exposure to an environment with high levels of pathogenic fungi.

Invasive fungal infections are most commonly caused by members of the genera *Candida* and *Aspergillus*. For example, the incidences of invasive *Candida* and *Aspergillus* infections in bone marrow transplant recipients are 10-15% and 3-7% respectively and the attributable mortality rate is 60-95%.[1] Overall, *Candida* infections are the fourth most common type of nosocomial bloodstream infection in the United States and have a higher mortality rate (33-47%) than any other bloodstream infection.[2] Furthermore, there has been a fourteen fold increase in the frequency of opportunistic invasive fungal infections from 1980-1992 in the United States and Europe.[3]

This increase in incidence of invasive fungal infections in immunocompromised individuals is largely attributed to medical advancements in the treatment of these

patients, especially in the management of bacterial infections. In fact, it has been estimated that the development of antibiotics has increased the life expectancy of humans by ten years.[4] Although physicians have had success in increasing the life expectancy of immunocompromised patients by treating bacterial infections, they are now struggling to treat the resulting opportunistic invasive fungal infections. Just as antibiotic resistant bacteria appeared soon after the introduction of the first antibiotics, the problem of antifungal resistance has been emerging as the use of existing antifungal agents has increased in response to increasing incidences of life-threatening fungal infections.

In order to avert the problem of antimicrobial resistance, the successful management of fungal infections requires a large number of antifungal agents belonging to a wide variety of classes with different mechanisms of action. Additionally, the patients most at risk for invasive fungal infections are typically taking several medications to treat the underlying diseases causing their neutropenia. Therefore, it is also important that physicians have access to a large number of antifungal medications to improve their chances of finding one that is both effective for treating the infection and will not interact with other medications the patient may be taking. Unfortunately, there is still a dire need for the development of new antifungal compounds. As of 2000, there were only ten medications approved by the U.S. Food and Drug Administration (FDA) for use in the treatment of invasive fungal infections and these drugs belong to only three classes: polyenes, pyrimidines, and azoles.

The polyene class includes the classic amphotericin B (**1**), which was approved by the FDA in 1958 but is still considered the "gold standard" for the treatment of invasive fungal infections because it has the broadest spectrum of effectiveness. Polyenes act by

binding to ergosterol, the primary sterol component in fungal cell membranes, and

thereby altering the permeability of the cell membrane and causing cell death. However,

it is generally not as effective against *Aspergillus* infections as it is towards other fungal

genera and its dosage is limited by significant toxicity, especially nephrotoxicity. From

1995-1997 the FDA approved three lipid formulations of amphotericin B which are less

toxic than the original formulation, but they are more expensive and are not more

effective.[5]
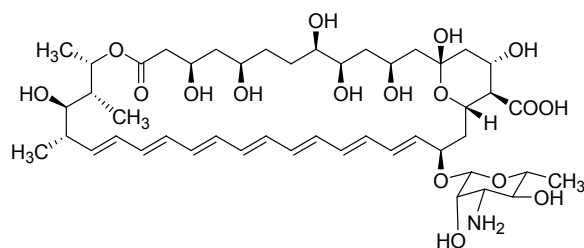
1 (Amphotericin B)

Flucytosine (**2**) is the only systemic antifungal agent in the pyrimidine class. It

was originally synthesized as an anticancer compound but unlike similar compounds in

this class, it failed to show antineoplastic activity. Subsequently, its antifungal

capabilities towards *Candida* species were discovered and the FDA approved flucytosine

as a systemic antifungal medication in 1972. Flucytosine enters fungal cells and disrupts

DNA synthesis by incorporating itself into the fungal RNA. Due to its toxicity and the

appearance of drug resistance in *Candida* species, flucytosine is primarily only used

today in combination with amphotericin B to treat *Cryptococcus neoformans* which is

one causative agent of meningitis.[6]
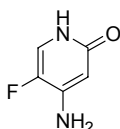
2 (Flucytosine)

Three azoles, ketoconazole (**3**), fluconazole (**4**), and itraconazole (**5**) gained widespread use in the treatment of invasive fungal infections in the 1980's and 1990's due to their broad spectrum of activity and low toxicity. Azoles act by inhibiting the synthesis of ergosterol, thereby resulting in abnormalities in the fungal cell membranes. While drug resistance has emerged in *Candida* species, the major limitation in the use of azoles has been problems with widespread drug interactions.[7] Even so, azoles remain as important systemic antifungal agents and recent research has led to the development and FDA approval of voriconazole (**6**) in 2002 which has proven to be a highly effective broad spectrum treatment for invasive fungal infections and is less toxic than amphotericin B. Indeed, one study has shown voriconazole to be a better choice over amphotericin B for the prevention of fungal infections in immunocompromised patients.[8]
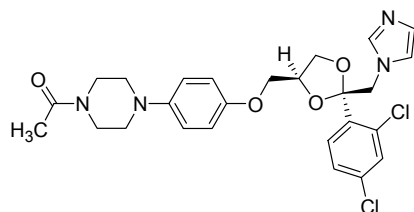
3 (Ketoconazole)
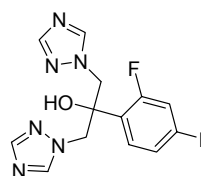
4 (Fluconazole)
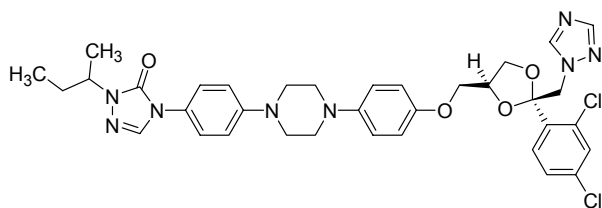
5 (Itraconazole)
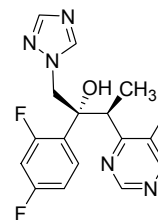
6 (Voriconazole)

A fifth azole, posaconazole (**7**), gained FDA approval in 2006 for the prevention of invasive fungal infections caused by *Candida* and *Aspergillus* species. Like the other azoles, posaconazole acts by inhibiting the synthesis of ergosterol but it appears to be a more potent inhibitor of sterol biosynthesis than other azoles especially in the *Aspergillus* species. Recent clinical trials have shown posaconazole to be more effective than fluconazole or itraconazole in the prevention of invasive fungal infections in immunosuppressed patients. Additionally, it was shown to be effective against systemic fungal infections that did not respond to treatment with amphotericin B. However, posaconazole also appears to be more hepatotoxic than fluconazole and itraconazole.[9]
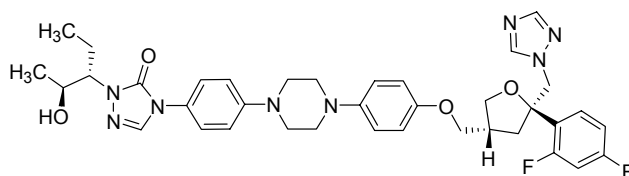
7 (Posaconazole)

The FDA approval of caspofungin acetate (**8**) in 2001 brought, for the first time in fifteen years, an antifungal compound to the market that belongs to a new class, echinocandins. These compounds disrupt the synthesis of β-(1,3)-D-glucan, a vital component of *Candida* and *Aspergillus* fungal cell membranes. This mechanism of action limits the effectiveness of echinocandins to infections caused by these genres, but the majority of invasive fungal infections do result from *Candida* and *Aspergillus* species.[10] A second echinocandin, anidulafungin (**9**), gained FDA approval in 2006 and has been shown in clinical trials to be more effective than fluconazole in the treatment of

invasive candidiasis.[11] Anidulafungin shows low toxicity and, unlike azoles, has not

shown significant adverse drug-drug interactions.[12]

8 (Caspofungin acetate)

9 (Anidulafungin)
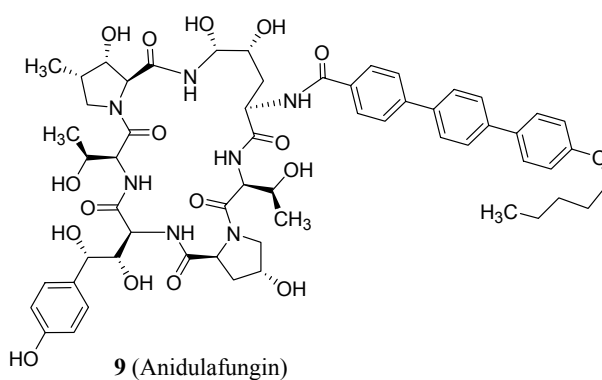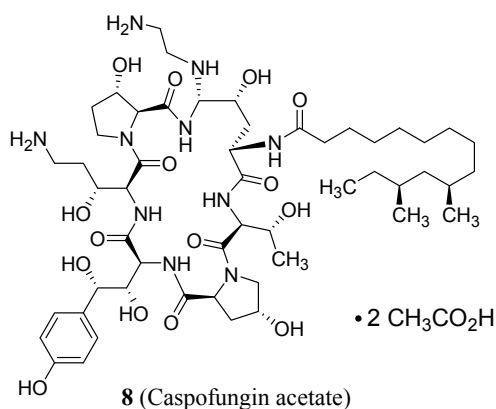
Even with these recent advances, there are currently only thirteen medications

approved by the FDA for the treatment of invasive fungal infections and they belong to

only four different classes of compounds. Additionally, of these thirteen medications,

four of them are lipid formulations of the same compound, amphotericin B. In contrast,

even the hundreds of antibacterial medications on the market which mostly belong to

eight classes are deemed insufficient due to the rapid emergence of antibiotic resistant

bacteria. Therefore, there continues to be a pressing need for the development of novel

antifungal compounds.

Natural Products Drug Discovery

Recently there has been a renewed interest in research on natural products as leads and targets for drug discovery. In fact, they have been the traditional source for medicines since the times of ancient civilizations, and even today half of the drugs on the market are either natural products or were inspired by them. Despite this record of success, American pharmaceutical companies largely abandoned natural product research in favor of combinatorial chemistry in the 1980's and 1990's in an effort to reduce the amount of time and money required for drug development. The development of high-throughput screening allowed for the automated testing of thousands of compounds for biological activity per day. While traditional stepwise organic synthesis could not meet the demand for new compounds, combinatorial chemistry increased the productivity of synthesis labs from one or two hundred new compounds per year to tens of thousands.

Unfortunately, these modern drug discovery techniques have failed to identify large numbers of ultimately successful leads. Whereas combinatorial chemistry alone has only provided a few candidates that have reached clinical trials, 61% of the new small-molecule drugs introduced from 1981-2002, and 23 of the compounds that gained FDA approval from 2000-2005, originated from natural product chemistry. Natural products are even more promising in the identification of new antibiotic agents. For example, 79% of the antibacterial compounds that were introduced from 1982-2002 were inspired by natural products.[13,14]

Additionally, while pharmaceutical companies emphasized the use of high-throughput screening for drug discovery, at the same time there has also been trend from 1996-2006 toward a decrease in the total number of new molecular entities approved by

the FDA.[15] However, in the future, as advances in combinatorial chemistry are making it possible to synthesize libraries of compounds that are increasingly complex, the combination of natural product and combinatorial chemistry promises to provide a powerful research and development tool for the pharmaceutical industry.

Saponins

One family of natural product compounds that shows promise in the identification of novel antifungal compounds is the saponin family. Saponins are divided based on the structure of their aglycone moiety into three classes: the steroid class, the triterpene class, and the steroid alkaloid class (Figure 1). In addition, saponins have an oligosaccharide moiety of up to five sugar molecules usually attached to the C-3 position of the aglycone and some saponins also have a C-26 or C-28 sugar moiety. The oligosaccharide typically consists of a combination of glucose, arabinose, glucuronic acid, rhamnose, or xylose.[16]

Figure 1. Classes of saponins.

Steroid class   Triterpene class   Steroid alkaloid class
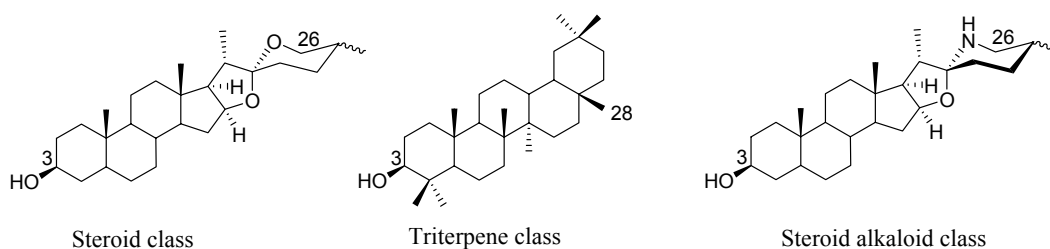
Saponins are named after one of their most notable characteristics, their amphiphilicity due to the lipophilic steroidal or triterpene moiety and the hydrophilic oligosaccharide moiety. [17] Many plants produce saponins as secondary metabolites that primarily serve as antimicrobial agents and traditionally it was believed that plants were the only natural source of saponins. More recently, however, biologists have discovered

that saponins are also abundant in many marine animals, most notably in echinoderms, but they also appear occasionally in higher animals such as fish.[18]

Medicinally, saponins have a wide range of biological activity that goes beyond their antimicrobial roles to include hypocholesterolemic, anti-inflammatory, anti-diabetic, anti-allergenic, immunomodulatory, anti-hepatotoxic, and anti-cancer capabilities.[19] It has been shown that both the aglycone and oligosaccharide moieties of saponins are important for their biological activity.[20-24] While the effectiveness of saponins has been recognized for centuries in herbal medicine, they were not typically viewed as potential drug targets in the past due to their structural complexity. One exception is digoxin (**10**), a cardiac glycoside that has been isolated from the leaves of the foxglove plant *Digitalis lanata* and marketed for over 200 years to treat congestive heart failure. Digoxin binds to Na^+/K^+ ATPase pumps in cardiac cell membranes. This alters the membrane potential by changing the concentrations of ions and ultimately leads to an increase in the strength of the contraction of the cardiac muscles.[19]
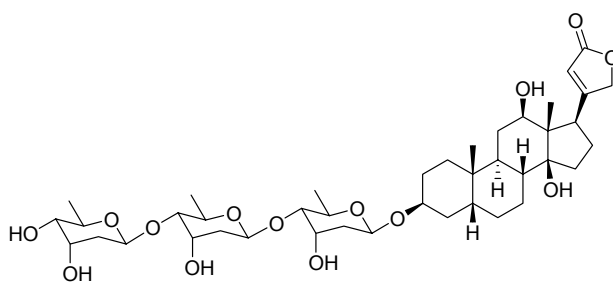
10 (Digoxin)

Unfortunately, digoxin is an unusual saponin in its availability by isolation from natural sources in sufficient quantities for distribution. Even so, recent advances in the synthesis of glycoconjugates allowed further exploration of additional medicinal uses for

saponins. In fact, saponins have drawn much attention in recent years owing in large part

to OSW-1 (**11**), a steroidal saponin isolated from bulbs of the lily plant *Ornithogalum*

saudersiae. OSW-1 shows *in vitro* anti-tumor activity levels that are 10-100 times more

effective than many well-known anticancer compounds including taxol and cisplatin.[25]

Furthermore, this saponin has the potential to be effective against cancers that are

resistant to currently available chemotherapy because it has an unusual mechanism of

action. Upon entering a cancer cell, OSW-1 causes damage to the mitochondrial

membranes, which in turn disrupts the calcium levels in the cytoplasm of the cell and

ultimately causes cell death by apoptosis.[26]
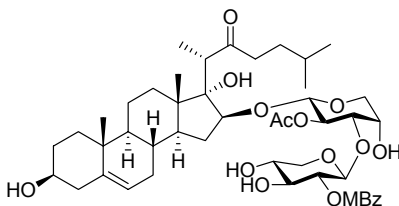
11 (OSW-1)

Many saponins have also been well documented to possess potent *in vitro*

antifungal properties. For example, saponin **12** is a derivative of the triterpene

medicagenic acid which was isolated from alfalfa root extracts. Initial testing revealed

that **12** was effective against fungal skin infections in guinea pigs and did not show

dermal toxicity in rhesus monkeys. It also showed promise toward the treatment of

invasive fungal infections by successfully curing *Cryptococcus neoformans* infections in

mice with a minimal fungal concentration of 4 µg/mL. Saponin **12** was also effective

against amphotericin B-resistant strains of *Candida tropicalis*. It was synthesized in

three steps from medicagenic acid and acetobromomaltose in 49% overall yield.[27]

12
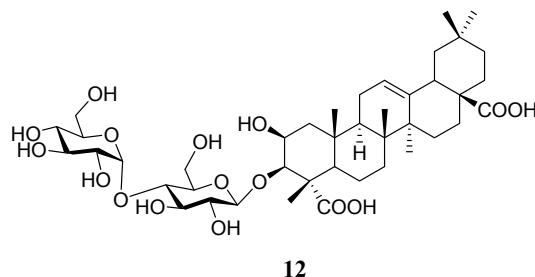
Isolation and Characterization of CAY-1

CAY-1 (**13**) is an important newly identified saponin that has the potential to be

useful in the treatment of invasive fungal infections. It was isolated in 0.1 % yield from

the fruit of the cayenne pepper plant *Capsicum frutescens* (Scheme 1). Each fraction

obtained from the solid phase extraction stage of the isolation procedure was tested for

fungicidal activity. CAY-1 is not detectable by UV so its presence was determined by

mass spectrometry and bioassays. Activity was found only in the 75% methanol fraction

which was then finally purified by high-pressure liquid chromatography (HPLC). The

structure of CAY-1 was determined by nuclear magnetic resonance including total

correlation spectroscopy (TOCSY), heteronuclear multiple-quantum coherence (HMQC),

and heteronuclear multiple-bond correlation (HMBC).[28]

13 (CAY-1)
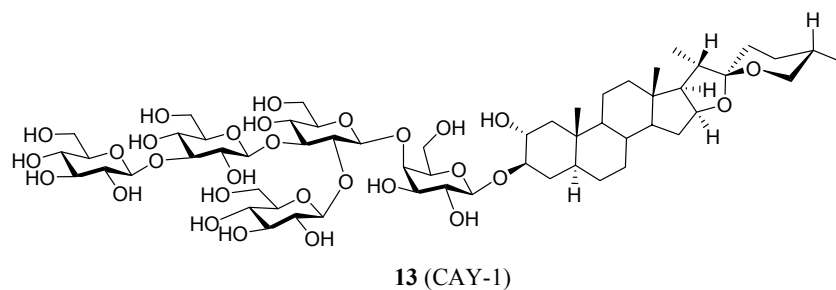
Scheme 1. Isolation of CAY-1.
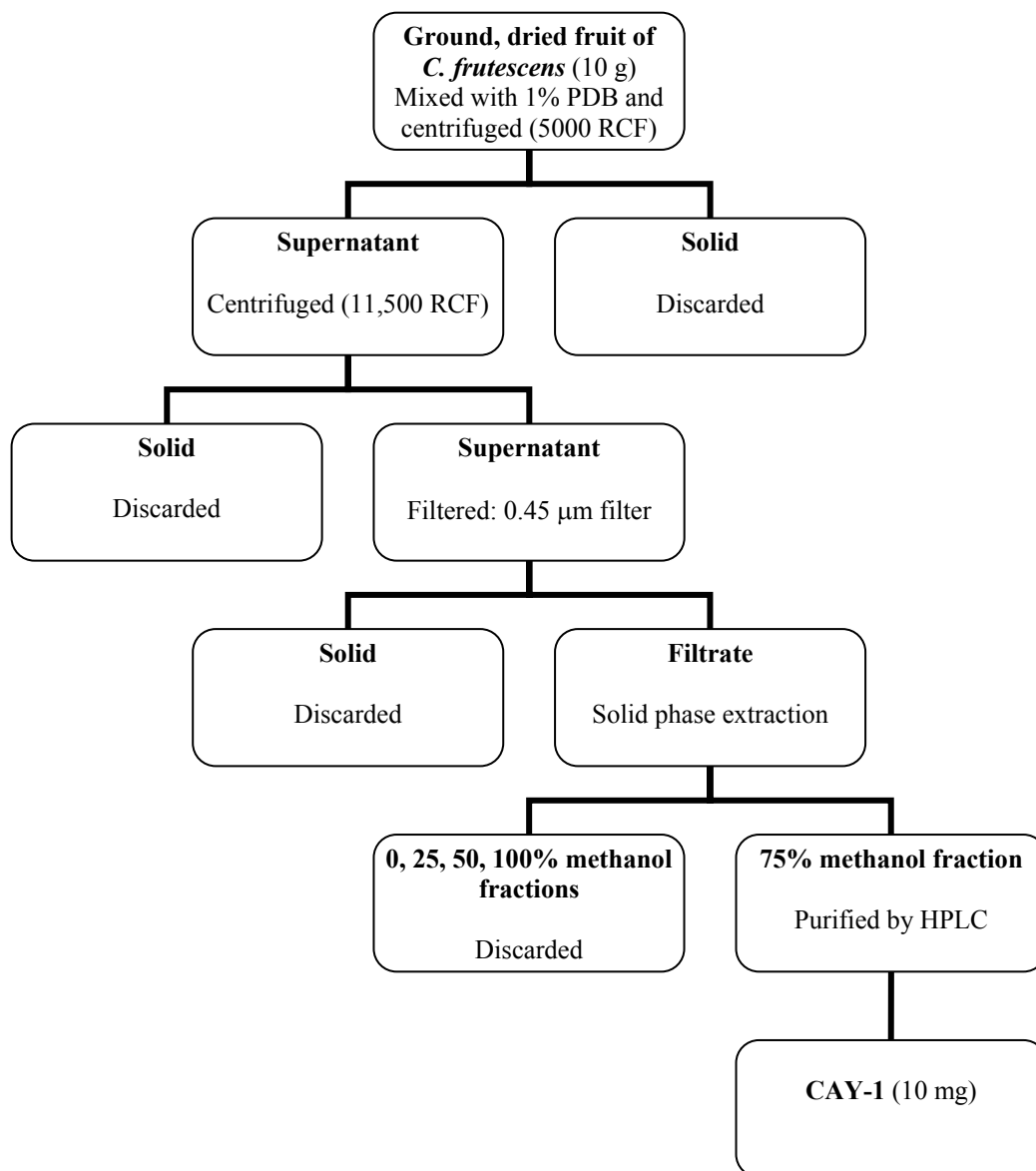
Biological Activity of CAY-1

Sufficient quantities of CAY-1 were available by isolation from cayenne peppers to perform preliminary testing for antifungal activity and cytotoxicity toward mammalian cells. It was shown to be an effective antifungal agent against sixteen fungal strains of *Candida* species, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Neurospora crassa* (Table 1). All of these strains are human pathogens known to cause invasive fungal infections in immunocompromised patients. The most promising result was the activity of CAY-1 against *C. neoformans* (IC_{90} = 1µg/mL) which causes cryptococcal meningitis, a fatal disease if left untreated.[28,29]

Table 1. CAY-1 whole cell activity.[28]

Microorganism	American Type Culture Collection no.	Inoculum (cells mL^{-1})	IC_{90} (µg/mL)	IC_{50} (µg/mL)
Candida albicans	90028	1 x 10^4	8-16	8-16
	10231	1 x 10^5	31	16
	44203	1 x 10^4	4-8	4-8
	32354	1 x 10^5	16	8
	44806	1 x 10^5	8-16	8-16
	76485	1 x 10^5	16	16
	90234	1 x 10^4	16	8
	#5 (not an ATCC strain)	1 x 10^4	16	8
	90873	1 x 10^5	8-16	8-16
C. glabrata	48435	5 x 10^4	8-16	4
C. tropicalis	90874	1 x 10^5	63	32
C. krusei	34135	1 x 10^5	> 125	32
C. parapsilosis	90018	1 x 10^5	16	8
Aspergillus fumigatus	16424	1 x 10^5	31	16
Cryptococcus neoformans	36556	5 x 10^5	1	1
Neurospora crassa	(not an ATCC strain)	1 x 10^5	4	4

CAY-1 was also tested for synergistic activity with three known antifungal agents, amphotericin B, miconazole, and cilofungin against *C. albicans* and *A. fumigatus*.

Drug synergism reduces the inhibitory concentration of the drugs and is therefore especially important for compounds that have toxic side effects at higher concentrations. In order to determine if two compounds are synergistic, the combined fractional inhibitory concentration (FIC) for each is calculated by dividing the minimal inhibitory concentration (MIC) of the drug in combination by the MIC of the drug alone. If the sum of the FIC's for the two drugs is less than 0.5, the compounds are considered synergistic. Using this methodology, it was determined that CAY-1 acts synergistically with amphotericin B against both *C. albicans* and *A. fumigatus*, a promising result considering the known toxicity of amphotericin B.[29,30]

Finally, CAY-1 was tested *in vitro* for cytotoxicity against 55 mammalian cell lines. The cells tested showed no inhibition of growth upon exposure to up to 100 μg/mL of CAY-1.[24] Further testing is needed, however, to determine the lethal dose for CAY-1 and to obtain *in vivo* results for cytotoxicity against mammalian and fungal cells.

CAY-1 Mechanism of Action

It is known that saponins alter the permeability of fungal cell membranes, which allows intracellular components to leak out of the cell and ultimately causes cell death. This was demonstrated with CAY-1 by treating *N. crassa* spores with [^{14}C]-alpha-amino-isobutyric acid. The radioactivity remaining in the cells after treatment with 25 μg/mL of CAY-1 was measured using a liquid scintillation counter and compared to controls which were dosed with dimethylsulfoxide (DMSO). The results show that the radioactivity in the treated cells decreased significantly after five minutes, suggesting that the integrity of the cellular membranes had been compromised (Figure 2).

Figure 2. The effect of CAY-1 (20 µM) compared to DMSO controls on fungal cell permeability.[29]
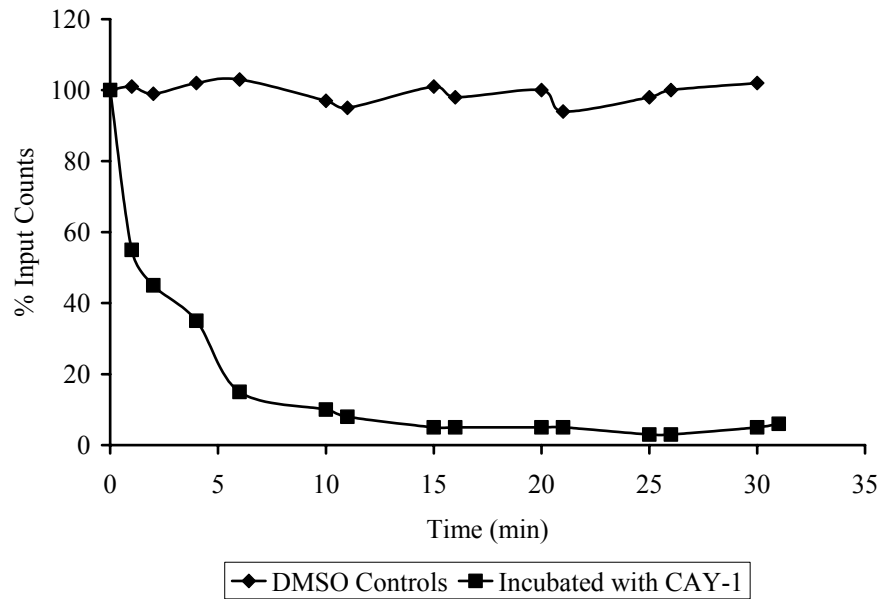
The exact mechanism by which saponins alter the permeability of fungal cell membranes is not clear. The tendency of saponins to bind to steroids suggests that antifungal saponins which are not toxic toward mammalian cells may bind preferentially to ergosterol, the major sterol component in fungal cell membranes, rather than cholesterol, the major sterol component in mammalian cell membranes. Once bound, the sterol-saponin complexes may aggregate to create pores in the membrane or to extract the sterol components from the membrane (Figure 3). Evidence to support the formation of pores in fungal membranes upon exposure to saponins has been provided by electron microscopic analysis.[16]

15

Figure 3. Cell membrane disruption by saponins.[16]
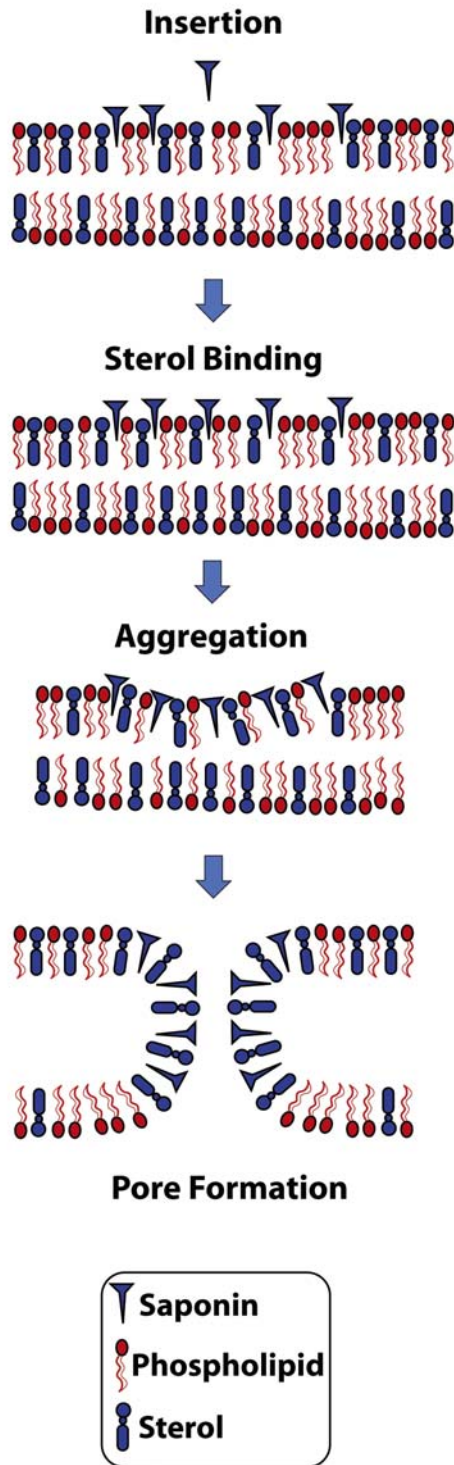
Specific Aims for the Synthesis of CAY-1

The rationale for the research towards the synthesis of CAY-1 is threefold. First, due to the difficulty in characterizing the structure of saponins isolated from nature,[31,32] the synthesis of this compound will provide verification for the precise structure and stereochemistry of this complex glycoconjugate. Of particular concern is the stereochemistry of the five glycosidic bonds contained in the CAY-1 structure.

Second, the development of a practical synthesis of CAY-1 will potentially provide a sample that is of sufficient quantity and purity to further explore its biological activity and medicinal utility as well as its mechanism of action. While the 0.1% isolated yield from the *Capsicum frutescens* plant is not exceptionally low, the laborious isolation process has limited the availability of CAY-1 in sufficient quantity and purity for additional biological testing. Consequently, the biological results obtained thus far are severely limited in their ability to predict the utility of CAY-1 in the treatment of invasive fungal infections.

Finally, a practical synthetic strategy would also allow for the opportunity to synthesize derivatives of CAY-1 which could be investigated in the future in structure-activity relationship studies. Since CAY-1 is only one of many biologically active saponins contained in the epithelial cells of the *Capsicum frutescens* plant, it is likely that the synthesis of structurally similar saponins will yield other biologically active compounds.

RESULTS AND DISCUSSION
CAY-1 AGLYCONE MOIETY

Retrosynthetic Analysis of CAY-1

The structure of CAY-1 consists of a sterol aglycone moiety and a pentasaccharide moiety attached to the C-3 position of the aglycone. Due to the complexity of each moiety, the most efficient approach to the synthesis of CAY-1 was deemed to be a convergent synthesis in which aglycone **14** and pentasaccharide **15** are synthesized separately and then joined via a glycosidic bond in the penultimate stage of the project.

The retrosynthesis of CAY-1 (Scheme 2) illustrates how hecogenin acetate (**16**) could be used as the starting material for the aglycone. This would require two synthetic transformations. First, the C-12 ketone would need to be reduced to a methylene to form tigogenin (**17**). The second transformation, the addition of an α-hydroxy substituent at C-2, would be significantly more complicated. Not only is the conversion of an alcohol into a *trans*-1,2-diol not straightforward for steroids, in this case it is also necessary to protect the C-2 alcohol in preparation for the reaction of the C-3 alcohol with the pentasaccharide moiety.

Additionally, the pentasaccharide would be synthesized by the appropriate assembly of four glucose units and one galactose unit. The challenge for the synthesis of this moiety stems from the inclusion of a 2,3-branched glucose unit. The galactose unit also complicates the synthesis since the required β-(1→4) linkage between the 2,3-

branched glucosyl donor and the galactosyl acceptor is disfavored as compared to an α-(1→4) linkage.

Scheme 2.[a] Retrosynthesis of CAY-1.
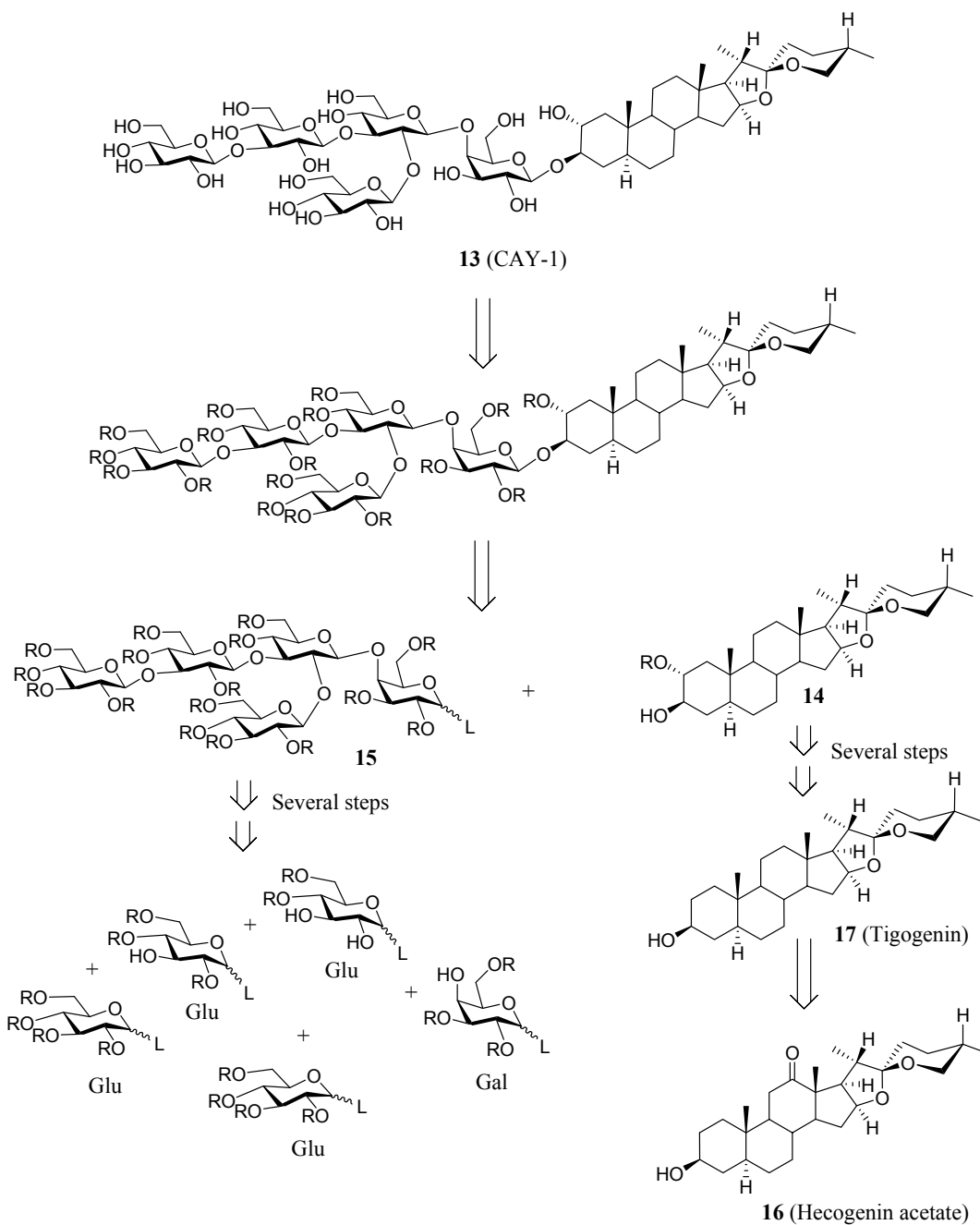
13 (CAY-1)

15

14

Glu

Glu

Glu

Glu

Gal

17 (Tigogenin)

16 (Hecogenin acetate)

[a]R = appropriate alcohol protecting groups. L = appropriate leaving groups for glycosidic bond formation.

19

Overall, there are several key challenges to the preparation of CAY-1. The first is the transformation of the 3β alcohol of hecogenin acetate into the corresponding spirostane-2α,3β-diol. Furthermore, since these are both equatorial alcohols, it is crucial for them to be differentiated by protecting groups in order for the aglycone to be useful in subsequent glycosylation reactions. Even more complex is the synthesis of the CAY-1 pentasaccharide owing to its 2,3-branched glucose unit which is bound to the C-4 alcohol of the galactose unit by a β-glycosidic bond. The development of practical methods for the accomplishment of these synthetic transformations will make it possible not only to synthesize CAY-1 but also saponin derivatives that could be tested for biological activity.

Initial Retrosynthetic Analysis of the CAY-1 Aglycone

The non-protected CAY-1 aglycone (**14,** R = H) is a known compound derived from the hydrolysis of the saponin gitonin (**18**). This saponin is one of the minor saponin components obtained during the isolation of the saponin digitonin (**19**) from *Digitalis purpurea*.[33] Digitonin is a toxic saponin which precipitates cholesterol and is therefore useful in the permeabilization of cellular and nuclear membranes.

While this saponin mixture is commercially available from a number of sources, it is impractical as a starting material for the preparation of CAY-1. This is due to the fact that gitonin is only a minor component of the commercially available digitonin mixture. Consequently, obtaining **14** from commercial sources would require separation of a minor saponin component from a saponin mixture followed by hydrolysis of the saponin to obtain non-protected aglycone component **14**. Furthermore, selective protection and isolation of one of two neighboring equatorial secondary alcohols would need to be accomplished before a glycosylation with an oligosaccharide moiety could be performed.

18 (Gitonin)

19 (Digitonin)
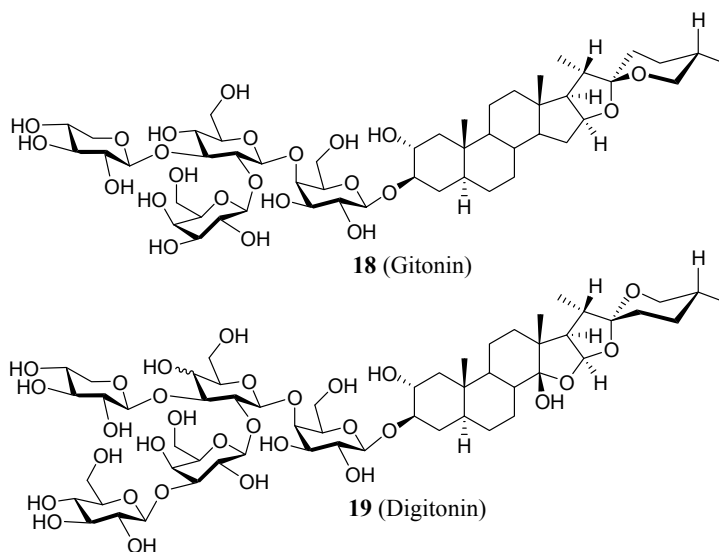
Therefore, a strategy for the synthesis of aglycone **14** from more abundant sterols was investigated. A literature search revealed a synthesis by Sondheimer *et al.* of the CAY-1 aglycone in 5 steps from diosgenin (Scheme 3).[33-35] In this synthesis, diosgenin (**20**) was oxidized to *α,β*-unsaturated ketone **21** by an Oppenauer oxidation which was then subjected to an allylic bromination to give **22**. The key step in this approach, involving an acetolysis of **22** to form *α*-acetoxy ketone **23**, proceeded in only 25% yield, severely limiting the usefulness of this strategy in a more complicated synthesis. To complete the synthesis, hydrogenation of the alkenyl functionality of **23** was followed by reduction of both the C-2 acetate protecting group and the C-3 ketone of **24** by LiAlH$_4$ to give unprotected *trans*-1,2 diol **25** in 5% overall yield from diosgenin.

There were two concerns about the utility of this synthesis for the preparation of CAY-1. The first concern was the low yield since aglycone **14** would be an intermediate in the saponin synthesis. More importantly, the lack of a protecting group on either the C-2 or C-3 alcohol in **24** renders it unusable in a glycoconjugate preparation. Since both of these secondary alcohols are equatorial, it would not be feasible to selectively protect

21

one over the other. The development of a new synthetic strategy was therefore considered necessary in the hopes of discovering a practical synthesis of CAY-1.

Scheme 3. Literature reported synthesis of CAY-1 aglycone by Herran *et al.*[33-35]
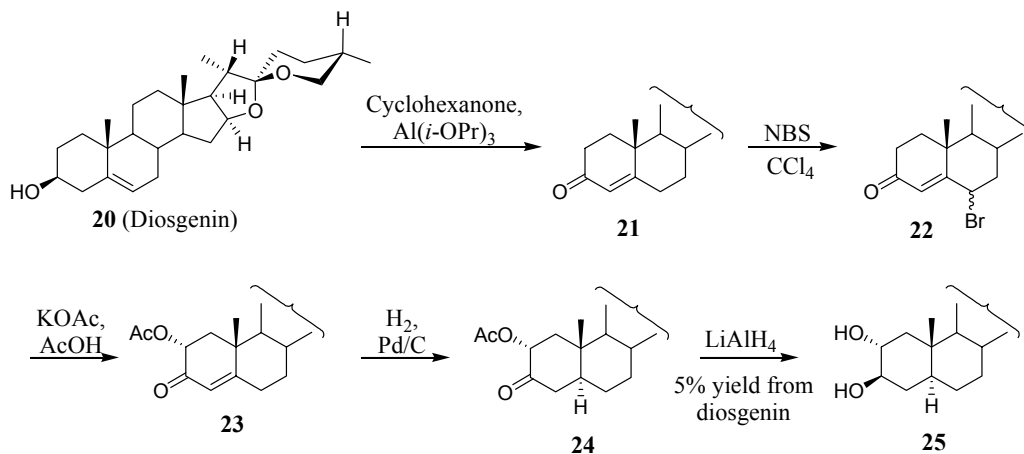
The synthesis of the steroid moiety of CAY-1 (**14**) from commercially available hecogenin acetate (**16**) was originally planned utilizing a partially protected *trans*-1,2 diol formation via an epoxide ring opening of **26β** with acetic acid. The epoxide could be formed from spirost-2-ene (**27**) which would be synthesized from tigogenin (**18**) by the elimination of the C-3 xanthate esters **28** or **29**. Tigogenin (**18**), in turn, would be available from hecogenin acetate (**16**) by the reduction of the C-12 ketone to a methylene (Scheme 4). Tigogenin is also commercially available although it is considerably more expensive than hecogenin acetate and so it was not chosen as the starting material for this synthesis.

Scheme 4. Initial retrosynthesis of the CAY-1 aglycone.

17 (Tigogenin)　　　　16 (Hecogenin acetate)
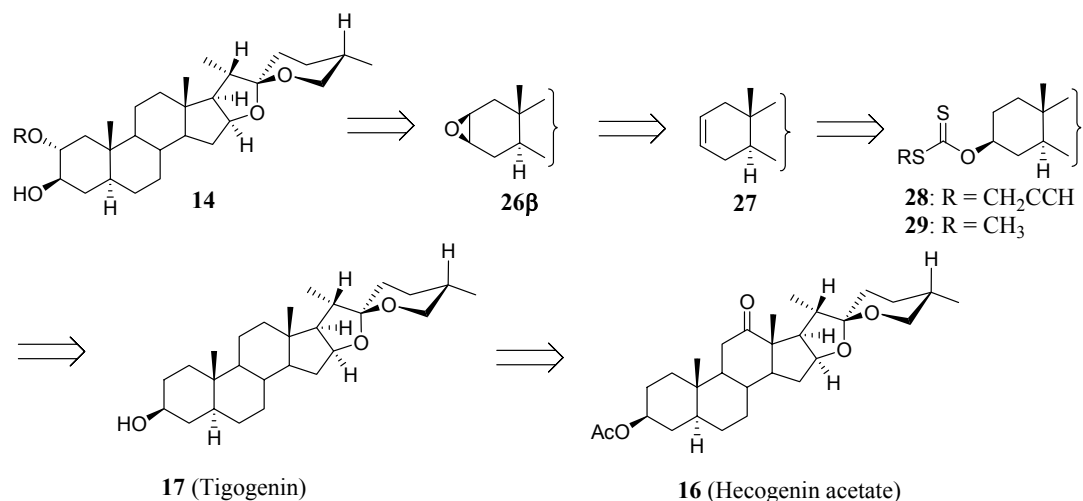
First Attempt to Synthesize the CAY-1 Aglycone

Spirost-2-ene (**27**) was prepared from commercially available hecogenin acetate

(**16**) in 87% yield over three steps following literature procedures with minor

modifications (Scheme 5).[36-38] In the first step, hecogenin acetate was reduced to

tigogenin (**17**) by a Huang-Minlon modified Wolff-Kishner reduction. The C-3 alcohol

was simultaneously deprotected in these reaction conditions. The C-3 alcohol of **17** was

then converted to its propargyl xanthate ester (**28**) which rearranged to form spirost-2-ene

in refluxing toluene with a collidinium trifluoromethanesulfonate catalyst. Alternatively,

27 was prepared by a Chugaev elimination via the pyrolysis of methyl xanthate ester

29.[39-41]

Scheme 5.[a] Synthesis of spirost-2-ene from hecogenin acetate.

16 (Hecogenin acetate)

17 (Tigogenin)

28

29

27
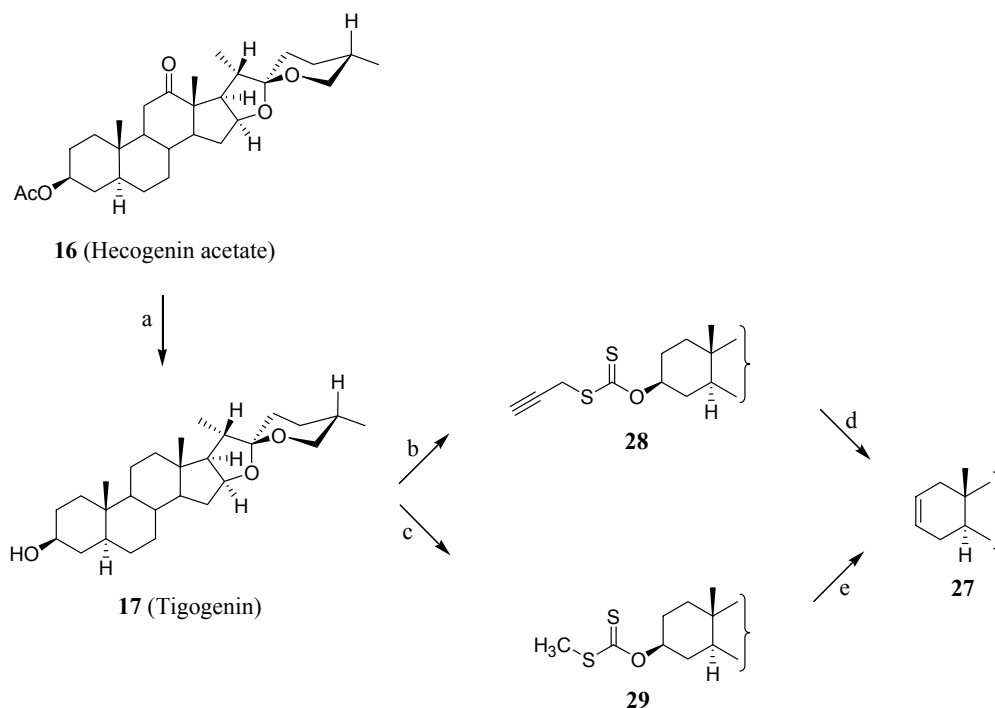
[a]Reaction conditions: (a) Hydrazine hydrate, 2-ethoxyethanol, KOH, 136°C, 98%. (b) NaH, CS$_2$, propargyl bromide, THF. (c) NaH, CS$_2$, CH$_3$I, THF. (d) Collidinium trifluoromethanesulfonate (0.1 eq.), toluene, reflux, 89% (2 steps from **17**). (e) 200°C, 94% (2 steps from **17**).

The difference in temperature requirements for these two eliminations can be explained by their mechanisms (Scheme 6). In the elimination of the propargyl xanthate ester, the collidinium trifluoromethanesulfonate salt catalyzes the rearrangement so that it proceeds at lower temperatures (110°C vs. 200°C). Without the catalyst, the elimination proceeds by the same mechanism as the methyl xanthate ester, known as the Chugaev reaction, and requires the same high temperature. Because of the thermal stability of this steroid, there was essentially no real advantage of one elimination method over the other since both provide spirost-2-ene in similar yield (89% for propargyl xanthate **28** and 94% for methyl xanthate **29**).

Scheme 6. Mechanisms for the elimination of xanthate esters to spirost-2-ene.[37,41]
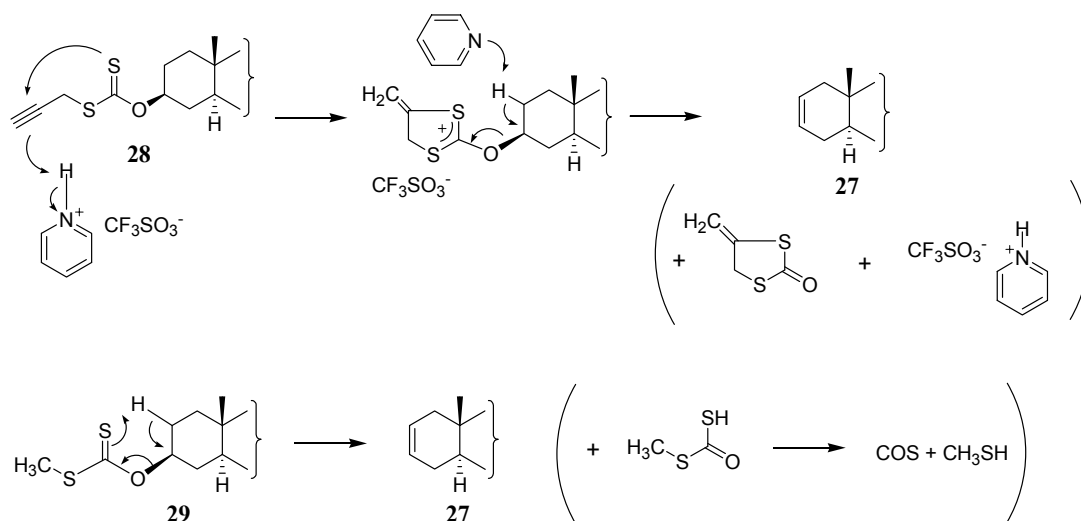
Epoxidation of **27** with *m*-CPBA proceeded in 94% yield. Unfortunately, the comparison of the ^{1}H NMR spectra of the major (91%) and minor (3%) isomers obtained in this reaction revealed that the 2α,3α-epoxide **26** was the major product. Most notably, the chemical shift for the C-19 methyl group for **26α** was 0.75 ppm as compared to 1.04 ppm for **26β** (Figure 4). The downfield shift for this signal in the case of **26β** is due to the fact that the methyl group is on the same side of the molecule as the epoxide in this isomer. This result is reasonable since epoxidations with *m*-CPBA generally occur from the least hindered side of the alkene.[42,43] In this case, the C-19 methyl renders the β-face of the molecule more hindered than the α-face and thus the α-epoxide is the major product (Scheme 7). Note that β-epoxidation does occur but only 3% of this product was obtained due to steric hinderance.

Figure 4. Comparison of ^{1}H NMR spectra (400 MHz, CDCl$_3$) for **26α** and **β**.
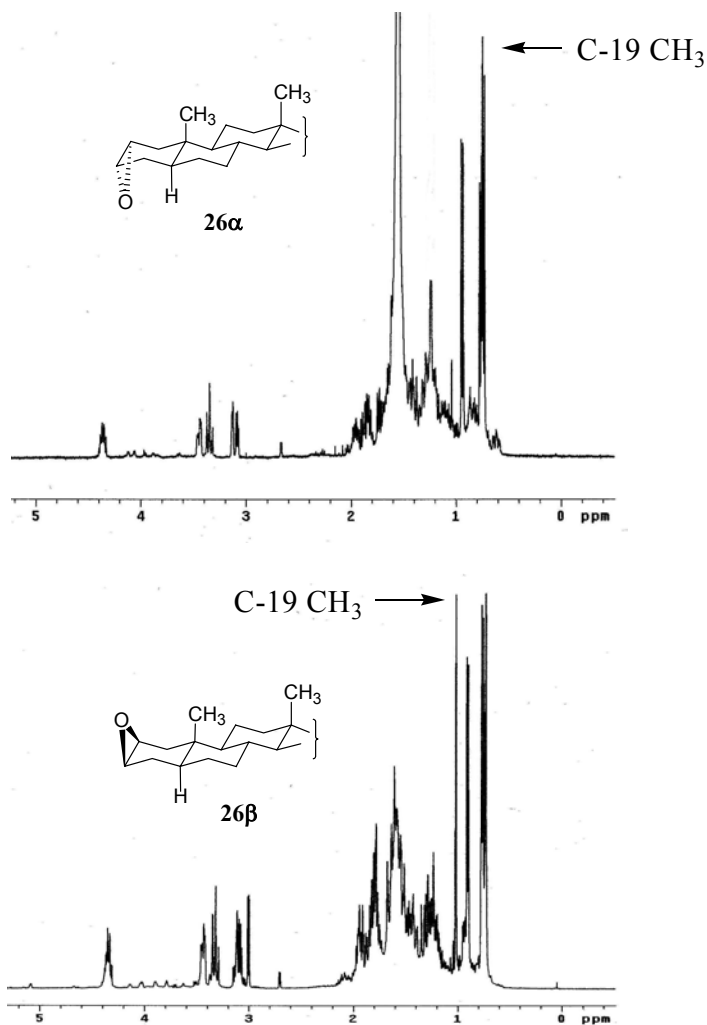
Scheme 7. Epoxidation of spirost-2-ene (**27**) with *m*-CPBA.

Top face of steroid sterically hindered by C-19 methyl group so epoxidation occurs from α-face nearly exclusively

Major (91%)
26α

Minor (3%)
26β
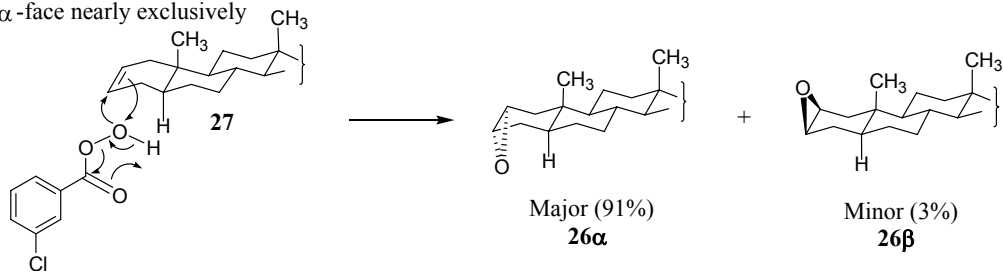
Hoping to still be able to synthesize the desired steroid via an epoxidation route, reagents favoring the **26β** product were investigated. The epoxidation of the more sterically hindered side of cholesterol 3-benzoate (**30**) and 3β-acetoxyprogest-5-en-20-one (**31**) to their 5β,6β-epoxides were successful in the literature using $KMnO_4$-$CuSO_4$.[44] However, attempts to apply these reaction conditions to spirost-2-ene (**27**) were unsuccessful.

30   **31**
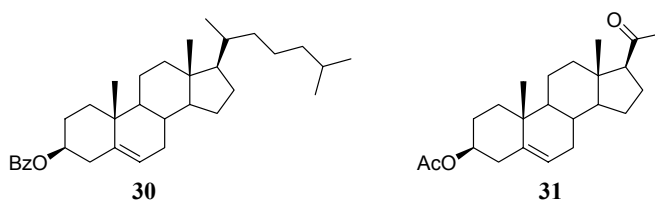
Next, the epoxide ring opening of **26α** and **26β** was explored. In both cases the acetic acid nucleophile approaches each epoxide axially to give the diaxial isomers **32** and **33** in 84% and 71% yield respectively (Scheme 8). This result is supported by the fact that in ring systems, nucleophilic attack on epoxides generally occurs from an axial approach.[45,46] Additionally, the appearance of H-2 and H-3 as broad singlets in the ^{1}H NMR of 3α-hydroxy-spirostan-2β-yl acetate (**32**) confirms their equatorial orientation (Figure 5). Axial protons, as in H-3 of tigogenin (**17**) which is also shown in Figure 5 for comparison, appear as multiplets. This is due to the difference in the coupling constants between equatorial and axial protons. The stereochemistry of **32** was further confirmed by X-ray crystallography (Figure 6). Therefore, further attempts to synthesize **26β** in good yield were abandoned considering the fact that either epoxide leads to a diaxial *trans*-1,2 diol derivative upon reaction with acetic acid.

Figure 5. Portion of ^{1}H NMR (400 MHz, CDCl$_3$) of 3α-hydroxyspirostan-2β-yl acetate (**25**) and tigogenin (**18**) showing C-2 and C-3 stereochemistry.
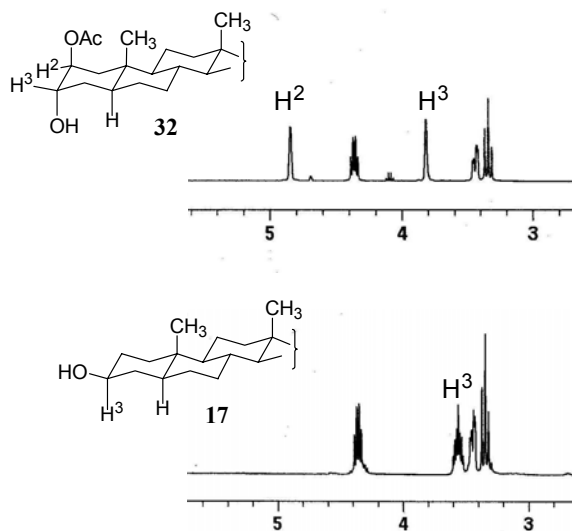
Figure 6. ORTEP drawing of 3α-hydroxyspirostan-2β-yl acetate (**32**). (Courtesy of E. D. Stevens, University of New Orleans)
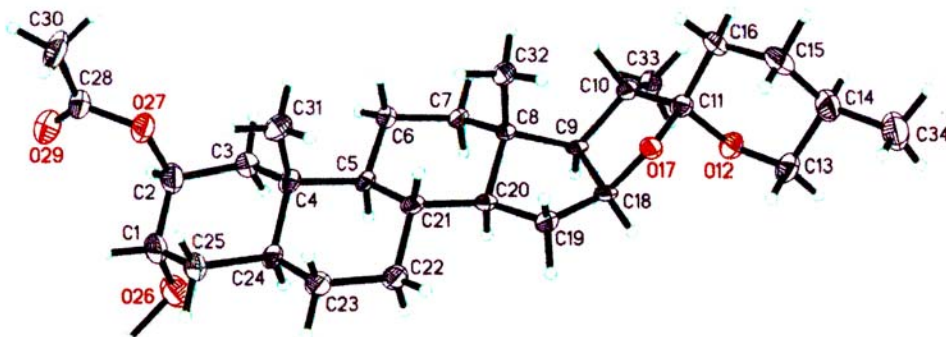
Scheme 8. Epoxide ring opening of **26α** and **β** with acetic acid.
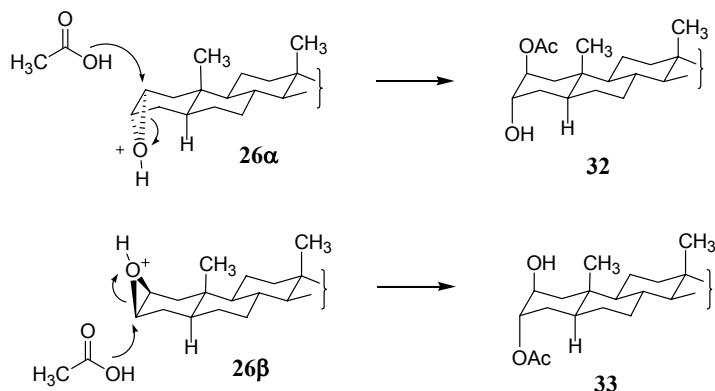
Second Retrosynthetic Analysis of the CAY-1 Aglycone

Holzapfel *et al.* reported the synthesis of a glycoconjugate containing the same steroid moiety as CAY-1 (Scheme 9). Their synthesis began with diosgenin (**20**) which provided tigogenin (**17**) by catalytic hydrogenation. Next, tigogenin was oxidized using chromic acid to spirostan-3-one (**34**) which was then converted to trimethylsilyl enol ether **35**. Enol ether **35** was oxidized with dimethyldioxirane (DMDO) and the resulting α-hydroxy ketone was protected as allyl carbonate **36**. To this point, each step in this literature report was accomplished in high yields of no less than 83%. Unfortunately, the last step, requiring a stereoselective reduction of the C-3 ketone of **36** to the β-alcohol using sodium borohydride, provided the desired stereoisomer **37** in only 63% yield.[47]

Successful syntheses of *trans*-1,2 diols from trimethylsilyl enol ethers using hydroboration-oxidation has been reported for substituted cyclohexenol derivatives.[48] Therefore, a new retrosynthesis (Scheme 10) for the steroid moiety of CAY-1 was designed using the trimethylsilyl enol ether intermediate **35** described in the synthesis by Holzapfel *et al.* Although the reported yields for the hydroboration-oxidation of trimethylsilyl enol ethers are low (~60%), this method would still provide the opportunity

for a higher yielding synthesis since it would require fewer steps. In this proposed

synthesis, the aglycone would be prepared by the hydroboration-oxidation of

trimethylsilyl enol ether **35** to give selectively protected diol **38**. This silyl enol ether **35**

would be synthesized from spirostan-3-one **35** which is available from the oxidation of

tigogenin (**17**).

Scheme 9. Literature reported synthesis of CAY-1 aglycone by Holzapfel *et al.*[47]

20 (Diosgenin)   **18** (Tigogenin)   **34**

35   **36**   **37**
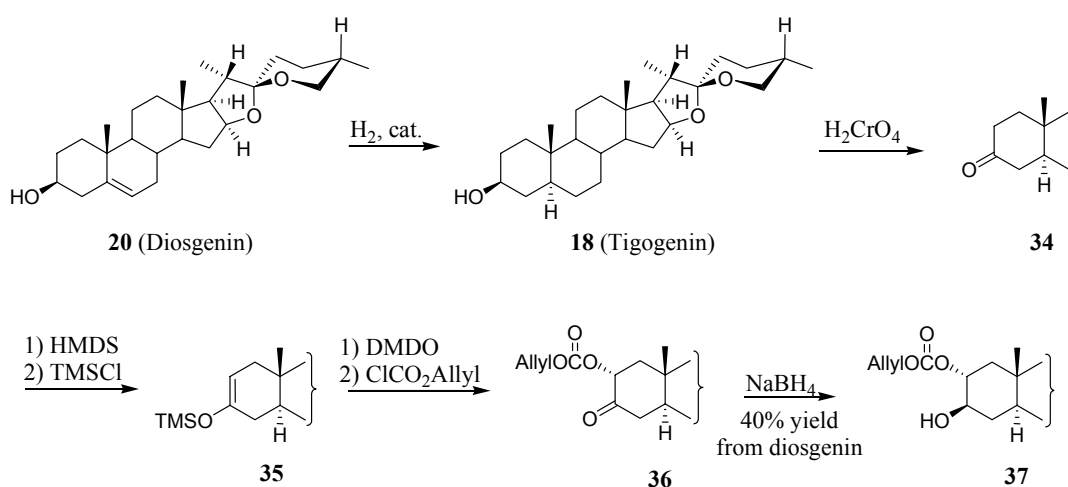
Scheme 10. Retrosynthesis of the CAY-1 aglycone via TMS enol ether intermediate **35**.

14   **38**   **35**
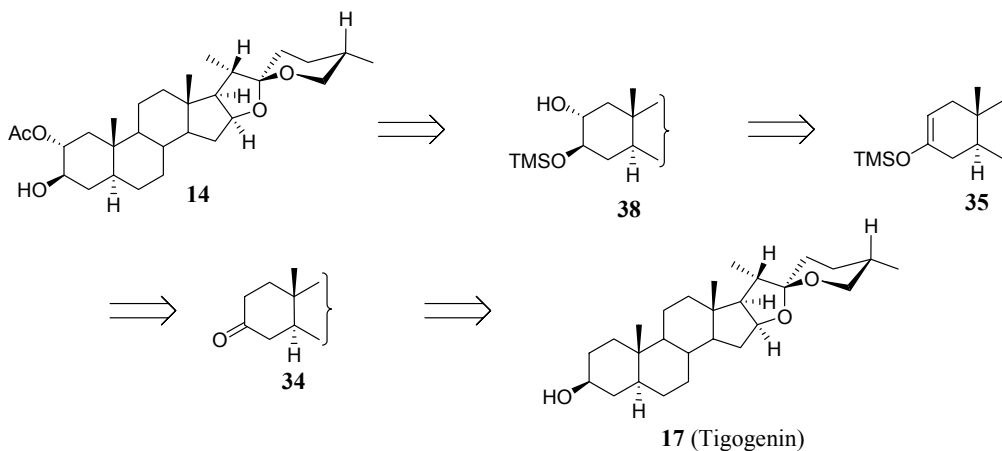
34   **17** (Tigogenin)

Second Attempt to Synthesize the CAY-1 Aglycone

Tigogenin (**17**), which had been prepared as described above from hecogenin acetate (**16**), was successfully oxidized with chromic acid to spirostan-3-one (**34**) in 93% yield (Scheme 11). Since the desired 2,3-enolate of **34** is both the kinetically and thermodynamically favored product, trimethylsilyl enol ether **35** was prepared from ketone **34** in good yield (76%) using lithium diisopropylamide and trimethylsilyl chloride. This is due to the fact that the AB ring junction of the steroid not only creates steric hinderance but it is also has increased rigidity and therefore the resulting sp^2 carbons are more strained the closer they are to a ring junction.

Unfortunately, the hydroboration-oxidation of enol **35** using 9-BBN did not provide the desired *trans*-1,2 diol. Instead, this reaction resulted in a mixture of products mostly consisting of 2α-hydroxyspirostan-3-one (**39**) and spirostan-3-one (**34**). This suggests that under these reaction conditions 9-BBN did not react with trimethylsilyl enol ether **35**. Therefore, once the solution of sodium hydroxide and hydrogen peroxide were added to the reaction mixture, **35** was still present to be oxidized to α-hydroxy ketone **39**. Since hydrogen peroxide does not generally oxidize alkenes in high yield, it is not surprising that the other major product formed in this step was the regeneration of ketone **34,** presumably from the deprotection of remaining **35** during the acidic work-up.

Scheme 11.[a] Attempted synthesis of the CAY-1 aglycone via TMS enol ether **35**.
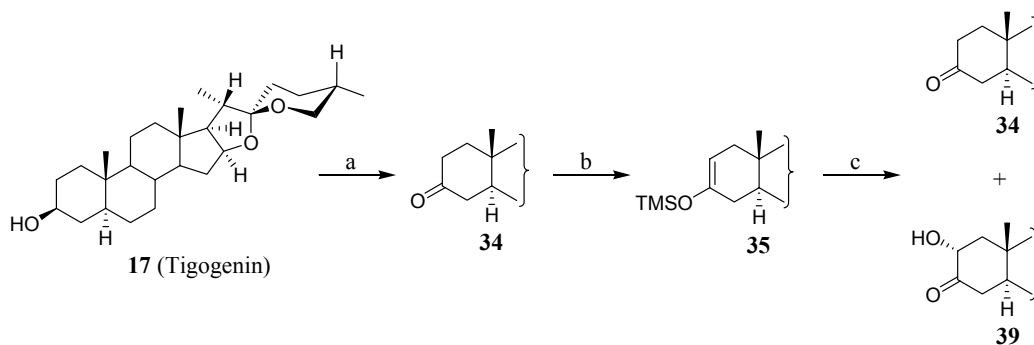
[a]Reaction conditions: (a) H$_2$CrO$_4$, acetone, 30°C, 93%. (b) LDA, TMSCl, DME, 0°C, 76%. (c) 1. 9-BBN, 2. H$_2$O$_2$, NaOH.

Third Attempt to Synthesize the CAY-1 Aglycone

The third approach toward the development of a novel synthesis of the CAY-1 aglycone involved the inversion of the C-2 and C-3 alcohols of 3α-hydroxy-spirostan-2β-yl acetate (**32**). This would require inversion of the C-3 alcohol, followed by protection of this alcohol as a benzyl ether and deprotection of the C-2 alcohol to give **40**. The C-2 alcohol of **40** would be inverted and then protected again as an acetate ester. Finally, the deprotection of the C-3 benzyl ether would provide the target sterol **14** (Scheme 12). These inversions were originally envisioned utilizing an S$_N$2 reaction for the inversion of C-3 followed by the inversion of C-2 using Mitsunobu methodology.

Although this design is less straightforward than the synthesis reported by Holzapfel *et al*., there remains the potential for the improvement in the overall yield of the synthesis. Furthermore, this route is attractive in that it provides stereoisomers of the CAY-1 aglycone that could be used in the future for the preparation of saponin derivatives of CAY-1 for structure-activity relationship studies. Such studies could not

only optimize its biological activity as an antifungal agent, but they could also help

elucidate its mechanism of action.

Scheme 12. Retrosynthesis of CAY-1 aglycone by the inversion of C-2 and C-3 of **32**.
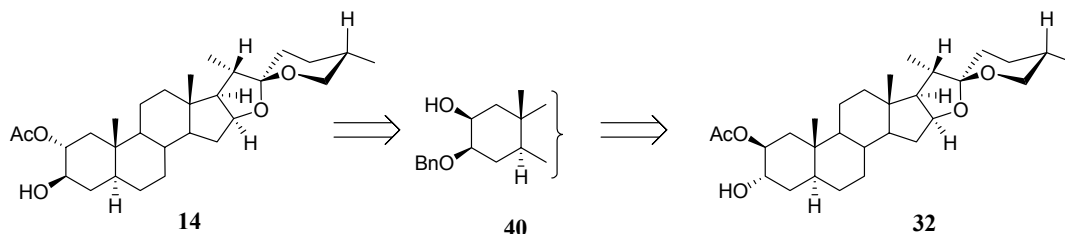
This synthesis was inspired by the literature reporting of the inversion of

secondary alcohols via an S_N2 attack on the sulfonate ester of the alcohol by the nitrate

ion. The resulting nitrate was then reduced by zinc to reform the alcohol with an overall

inversion of the stereochemical configuration (Scheme 13).[49,50]

Scheme 13. Literature reported inversion of 2° alcohols with NO_3^-.[49,50]
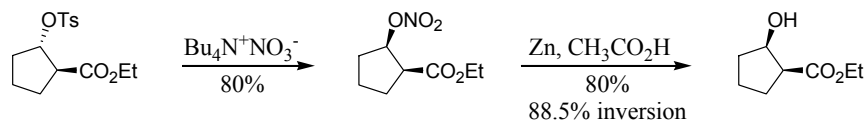
In the first step, the C-3 alcohol of spirostane **32** was converted to its mesylate in

83% yield. Unfortunately, reacting mesylate **41** with tetrabutylammonium nitrate in

refluxing toluene did not afford the expected nitrate product. This was discovered when

zinc failed to react with the product of this nitration reaction. Instead, IR spectroscopy

revealed that the functional groups present were an ester and an alcohol. After flash

column chromatography, it was determined by ^{1}H NMR that the two constitutional

isomers **42** and **43** were formed, each consisting in part of β-substituted carbons at positions 2 and 3 (Scheme 14).

Scheme 14.[a] Attempt to invert C-3 mesylate of **32** by an S_N2 reaction with NO_3^-.
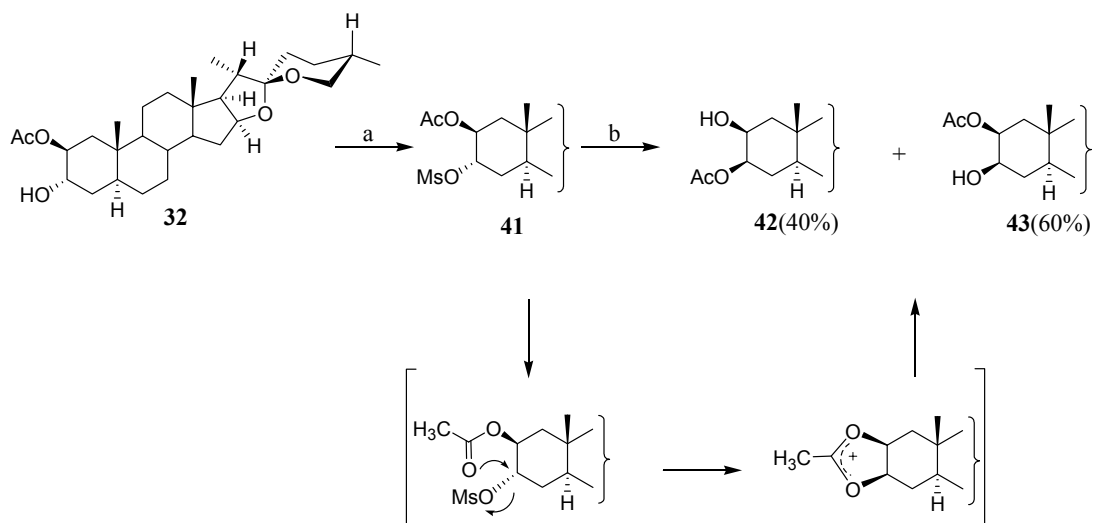
[a]Reaction conditions: (a) MsCl, Et₃N, CH₂Cl₂, 0°C, 83%. (b) N(C₄H₉)₄NO₃, toluene, reflux, 98%.

At this point, it was not clear whether spirostanes **42** and **43** were formed by the C-2 acetate migration of mesylate **41**, by the nucleophilic attack of ambient water on the C-3 mesylate of **41**, or by both of these mechanisms. To explore this, the reaction was repeated in a variety of solvent systems and temperatures (Table 2). The most practical result was obtained when **41** was refluxed in a mixture of pyridine and water. This provided a complete conversion to **42** and **43** in 7 hours as compared to over 48 hours required for toluene in the absence of tetrabutylammonium nitrate. When the reaction was attempted in acetone, only 20% conversion was achieved after 24 hours due to the lower refluxing temperature of acetone as compared to pyridine.

Table 2. Inversion of the C-3 mesylate of **41** to form **42** and **43**.

Solvent(s)	Temperature	Time (hours)	% Conversion[a]	%42[a]	%43[a]
Toluene (with $N(C_4H_9)_4NO_3$)	110°C	2	20	10	90
Toluene (with $N(C_4H_9)_4NO_3$)	110°C	20	100	40	60
Toluene (without $N(C_4H_9)_4NO_3$)	100°C	48	70	50	50
Toluene, H_2O	100°C	48	80	50	50
Acetone, H_2O	56°C	24	20	10	90
Pyridine, H_2O	100°C	7	100	10	90

[a]Yields determined by ^{1}H NMR.

While neither **42** nor **43** was prepared in greater than 80% excess, this was still a fortuitous result since overall the stereochemical configuration of C-3 of mesylate **41** was inverted in quantitative yield. Furthermore, **42** and **43** could be easily separated by column chromatography since the axially oriented alcohol of **42** created a sufficient difference in polarity over the equatorial alcohol of **43**.

Next, the acetate group migration of 3-*O*-acetyl-2β,3β-spirostanediol (**42**) was explored (Scheme 15). Although **42** was successfully converted to mesylate **44**, refluxing **44** in a mixture of water and pyridine afforded no inversion of its C-2 configuration. Instead, **44** was converted under these conditions back to a mixture of **42** and **43**. Additionally, this reaction was significantly more sluggish than the comparable reaction of mesylate **44**, requiring 12 hours for 90% conversion as compared to a quantitative conversion for **41** after 7 hours. In this way, it was determined that **42** and **43** form from mesylates **41** and **44** solely by an acetate group migration and not by a substitution reaction with water. Furthermore, since the acetate and mesylate of **44** are both on the β-face of the steroid, the acetate group migration in this case must occur via an S_N1-type mechanism.

Scheme 15.[a] Acetate group migration of **42**.
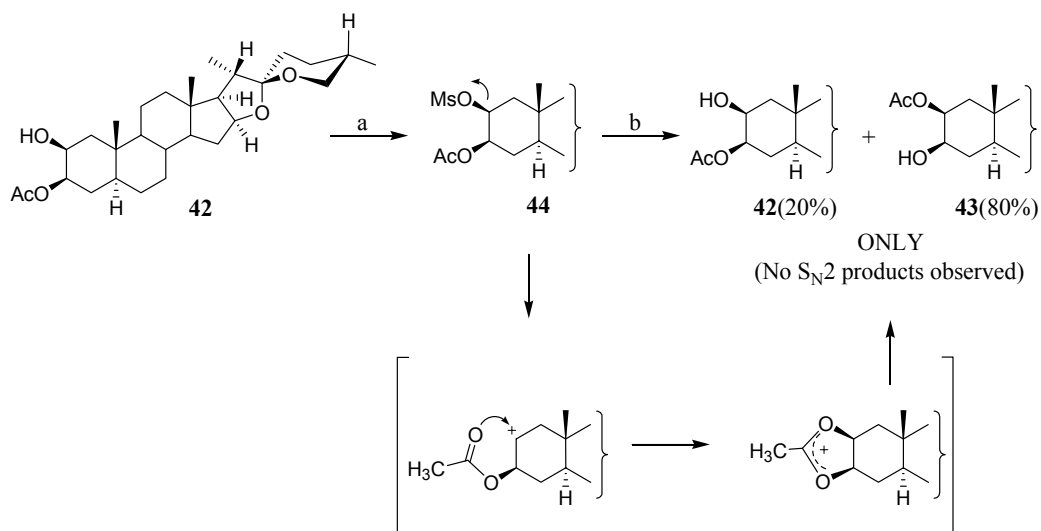
[a]Reaction conditions: (a) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0°C. (b) 1:4 H$_2$O: pyridine, reflux, 73% (two steps).

This is a reasonable explanation for the fact that this mesylate is less reactive under these conditions than **41** which is capable of reacting in an S$_N$2 fashion. It was surprising but crucial to determine that mesylate **44** was reacting only by an S$_N$1 mechanism to form the acetate group migration products, and that no S$_N$2 product formation was observed. Consequently, it was clear that an S$_N$2 reaction at C-2 was disfavored as long as there was an acetate group present at C-3.

Fourth Attempted Synthesis of the CAY-1 Aglycone

Based on the success in inverting the stereochemical configuration of the C-3 alcohol of **32** via an acetate group migration and the inability to invert the configuration at carbon 2 while the C-3 alcohol was protected as an acetate ester, a new retrosynthetic analysis was designed (Scheme 16). In this approach, the C-2 alcohol of **46** would be inverted by an S$_N$2 reaction with mesylate **45**. Sterol **46** would be prepared by the protection of the C-3 alcohol of **43** with a protecting group incapable of migration such as

a benzyl ether to give **47** which would be followed by the deprotection of the C-2 alcohol.

Scheme 16. Fourth retrosynthesis of the CAY-1 aglycone.
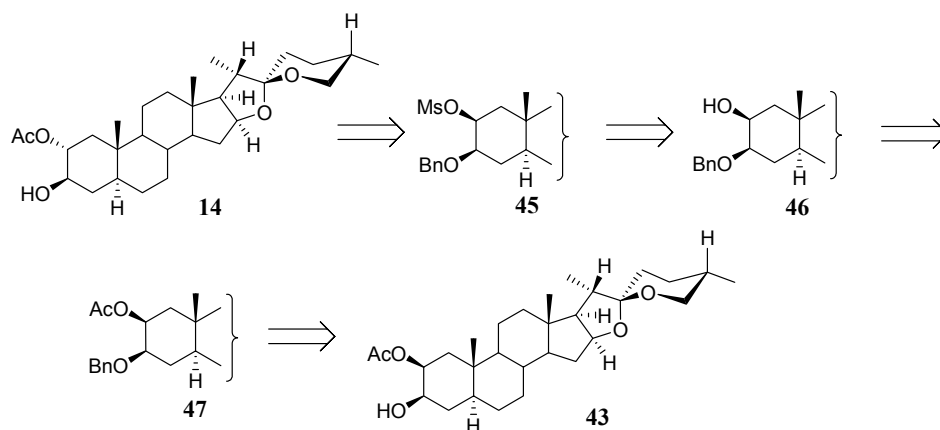
To begin this synthesis, 3β-hydroxyspirostan-2β-yl acetate (**43**) was prepared as described above from hecogenin acetate (**16**). The C-3 alcohol was then protected as benzyl ether **47**, the acetate of which was deprotected to give 3β-benzyloxyspirostan-2β-ol (**46**) in 75% yield for these two steps. **46** was then successfully converted to its mesylate in 81% yield. Next, mesylate **45** was refluxed in a 1:4 mixture of water and pyridine. These reaction conditions had seemed promising since unlike the acetate group, the neighboring benzyl ether does not migrate. However, none of the expected S_N2 product **48** was observed. Instead, **46** was regenerated in 90% yield (Scheme 17).

Scheme 17.[a] Fourth attempted synthesis of the CAY-1 aglycone.
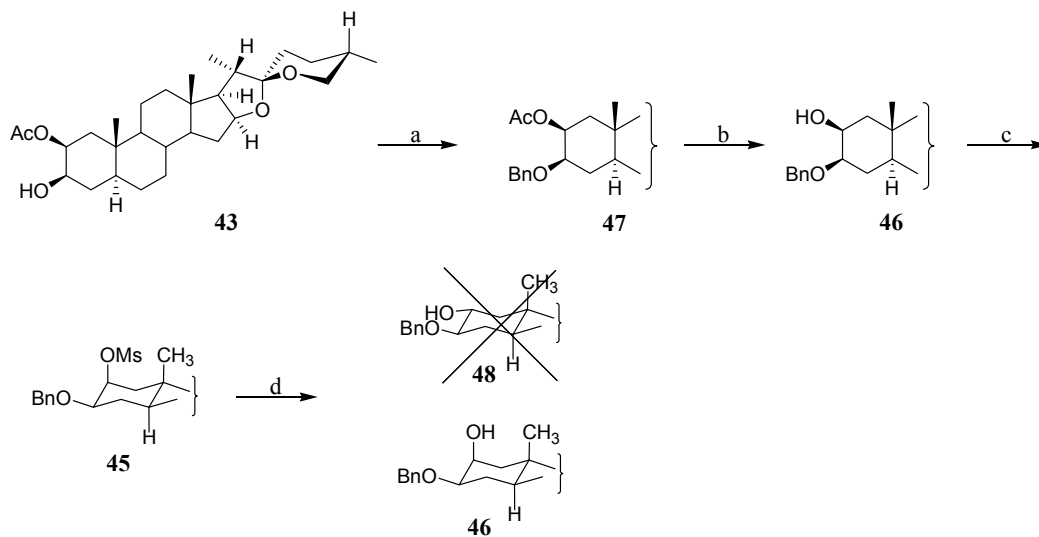
[a]Reaction conditions: (a) Benzyl trichloroacetimidate, CF_3SO_3H, dioxane, 78%. (b) NaOH, MeOH, reflux, 96%. (c) MsCl, Et_3N, CH_2Cl_2, 0°C, 81%. (b) 1:4 H_2O: pyridine, reflux, 90%.

Due to the literature report of successful inversions of secondary alcohols by the reaction of corresponding mesylates with cesium acetate,[51] the inversion of mesylate **45** was attempted using tetrabutylammonium acetate in refluxing toluene. Unfortunately, no substitution occurred under these conditions. Therefore, since the benzyl ether protecting group is stable in basic conditions, mesylate **45** was refluxed with sodium hydroxide in DMF in an attempt to favor an S_N2 mechanism. As was the case with water/pyridine, the only product observed was the S_N1 product, **46**. Considering the fact that in all substitution reactions attempted with mesylates **41**, **44**, and **45** the nucleophiles only approached from an axial orientation, even if this required following an S_N1 rather than S_N2 mechanism, it was determined that further attempts to invert the configuration of the C-2 alcohol by a substitution reaction would most likely be unsuccessful.

It should be noted that elimination was not observed for these reactions. Substitution is most likely favored over elimination in these cases because the rigidity of

38

the fused ring system limits the ability for the strain on would-be alkenyl sp^2 carbon

atoms to be alleviated.

Fifth Attempted Synthesis of the CAY-1 Aglycone

As an alternative to attempting to invert the C-2 stereochemistry by an S_N2

reaction, the inversion by oxidation of the β-C2 alcohol to a ketone followed by reduction

to the α-alcohol was explored. As described above, Holzapfel *et al.* were only able to

achieve a 63% yield of the desired stereoisomer using sodium borohydride as the

reducing agent. Therefore, it was considered that there was an opportunity to improve on

this synthetic strategy. For this strategy, the target sterol would be prepared, after a

transfer of protecting groups of **50** and **51**, from the reduction of ketone **49** which is

available from the previously synthesized **46** (Scheme 18).

Scheme 18. Fifth retrosynthesis of the CAY-1 aglycone.
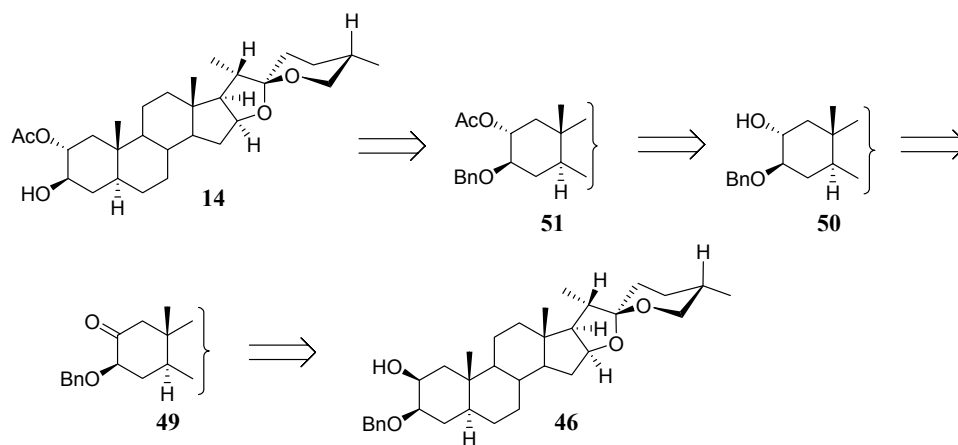
Since this synthetic strategy would require a stereoselective reduction of 3β-

benzyloxyspirostan-2-one (**49**) to an equatorial alcohol, literature methods reporting high

yields for similar transformations were investigated. Unfortunately, methods favoring

reductions to axial alcohols were much more developed with the most important breakthrough being the discovery of the bulky Selectride reducing agents.[52] In general, an axial approach to a cyclic ketone is favored for hydrides to give the equatorial alcohol as the major product. Although the equatorial product is more stable than the axial one, the main reason for this preference actually stems from the sterics of the hydride approach to the cyclic ketone (Scheme 19). Additionally, orbital effects favor an axial approach of hydrides to cyclic ketones.[53] Conversely, large hydrides are forced to approach cyclic ketones from the less sterically hindered equatorial position in spite of these orbital and torsional effects.

Scheme 19. Stereochemistry of hydride reductions of cyclic ketones.[53]

Strained intermediate:
oxygen eclipses equatorial H's as
it moves to axial position

Minor

Favorable intermediate:
oxygen moves away from equatorial
H's towards the equatorial position

Major
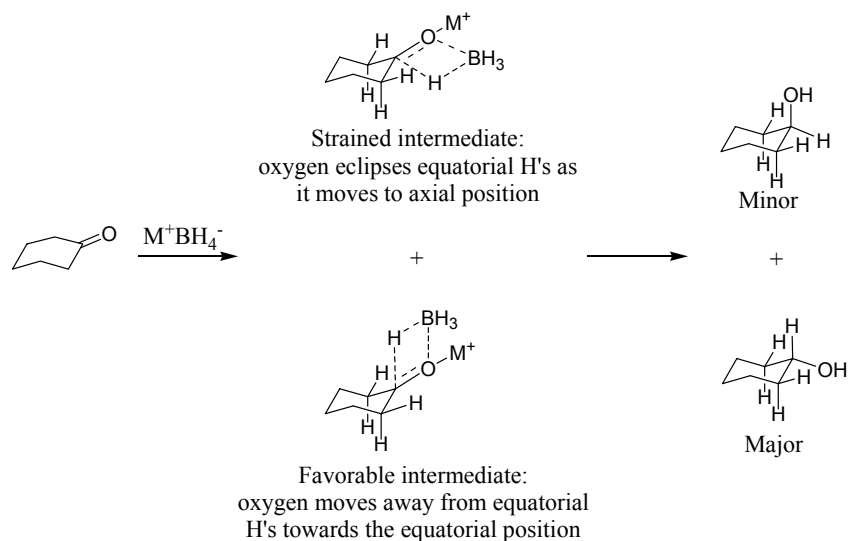
In practice, however, especially when dealing with complex substrates, bulky hydrides tend to give higher yields and are more stereoselective in favoring axial alcohol formation as compared to the ability of small hydrides, such as $NaBH_4$ and $LiAlH_4$, to favor equatorial alcohols. This was well illustrated in the synthesis of the CAY-1

aglycone reported by Holzapfel *et al.* in which the reduction of ketone **36** with $NaBH_4$ gave the equatorial alcohol **37** in only 63% yield (Scheme 20). The authors were unable to improve the yield of this reaction by varying the solvents from methanol/THF to dioxane and, finally, 2-propanol.

Scheme 20. Literature reported reduction of cyclic ketone **36** with $NaBH_4$.[47]
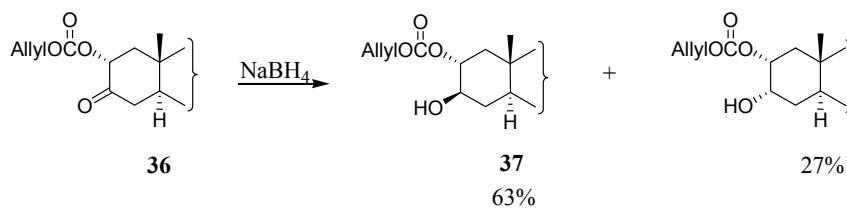
Therefore, it seemed that a more promising way to favor the formation of the required equatorial C-2 alcohol of the CAY-1 aglycone would be to utilize a reduction that proceeds via the establishment of an equilibrium since the equatorial alcohol is thermodynamically the more stable product. One such reaction is the Meerwein-Ponndorf-Verley reduction.[54] An example of the application of this reaction to a steroidal substrate was reported in a paper by Snaith *et al.* in which they describe the successful reduction of 5α-cholestan-3-one with *Al*-isopropoxydiisobutylalane (DIBAO^{i}Pr) at room temperature in toluene in 7 hours (Scheme 21).[55]

Scheme 21. Literature reported reduction of 5α-cholestan-3-one using a Meerwein-Ponndorf-Verley-type reaction.[54,55]

5α-Cholestan-3-one

98% equatorial alcohol
78% yield
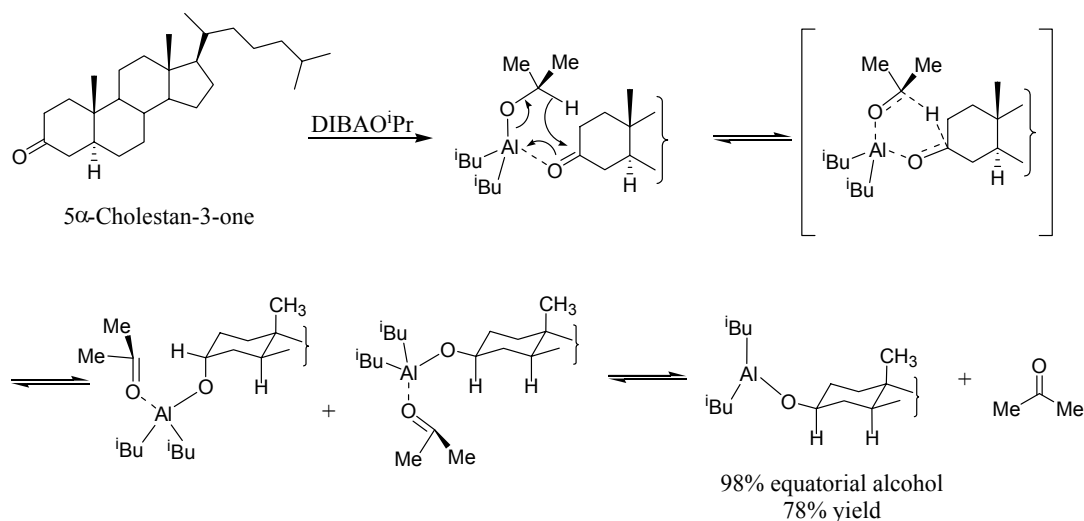
With this in mind, 3β-Benzyloxy-(22S,25R)-5α-spirostan-2β-ol (**46**) was prepared from hecogenin acetate (**16**) as described above and oxidized with chromic acid to give ketone **49** in 90% yield for this step. Next, **49** was stirred at room temperature with DIBAO^{i}Pr in toluene for 6 hours as described by Snaith *et al.* but no product formation was observed and only about 5% conversion was noted by ^{1}H NMR after 12 hours. It has been reported that reductions with DIBAO^{i}Pr in diethyl ether require 5-7 days,[56] most likely due to competition between the carbonyl of the substrate and the oxygen of the ether solvent for coordination to aluminum.[57] Consequently, it was suspected that the presence of a cyclic acetal and a neighboring benzyl ether in substrate **49**, both absent in the 5α-cholestan-3-one example, was extending the required reaction time for this ketone.

To explore the utility of Meerwein-Ponndorf-Verley-type reactions on spirostane ketones, spirostan-3-one (**34**) was stirred in toluene with 1 equivalent of DIBAO^{i}Pr. After 10 hours, the ketone of **34** was reduced to the equatorial alcohol of tigogenin (**17**) in 87% yield. Furthermore, no axial alcohol formation was observed. It was then clear

that the functional group interfering with the reduction of **49** was indeed the C-2 benzyl

ether. The reduction of **49** with DIBAO^{i}Pr in toluene was then repeated but

unfortunately, even after 5 days only 50% conversion was observed by ^{1}H NMR.

Additionally, the reduction was forming a mixture of axial and equatorial alcohols of **50**

(Scheme 22).

Scheme 22.^{a} Fifth attempted synthesis of the CAY-1 aglycone.
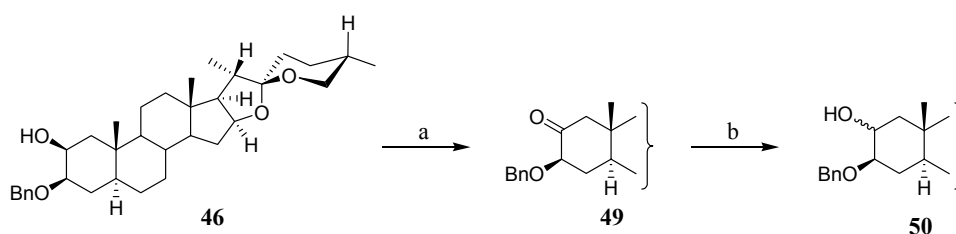
^{a}Reaction conditions: (a) H$_2$CrO$_4$, acetone, 30°C, 90%. (b) DIBAO^{i}Pr, toluene, 5 days, ~50%.

Summary of Approaches to the Synthesis of the CAY-1 Aglycone

In addition to the syntheses discussed above by Sondheimer *et al.* and by

Holzapfel *et al.*, Yu *et al.* published a 7 step synthesis of the CAY-1 aglycone from

diosgenin in 28% yield (Scheme 23).[58] Although their report is more recent than that of

Holzapfel *et al.*, it is less efficient in that it is both lower yielding and requires more

steps. Even so, it is worth highlighting the fact that these authors chose to invert the axial

C-3 alcohol of **54** by oxidizing it to ketone **55** and then reducing it to the corresponding

equatorial alcohol **56**. Yu *et al.* employed a Luche reduction and made no improvement

on the yield of this step.

Scheme 23. Literature reported synthesis of the CAY-1 aglycone by Yu *et al.*[58]
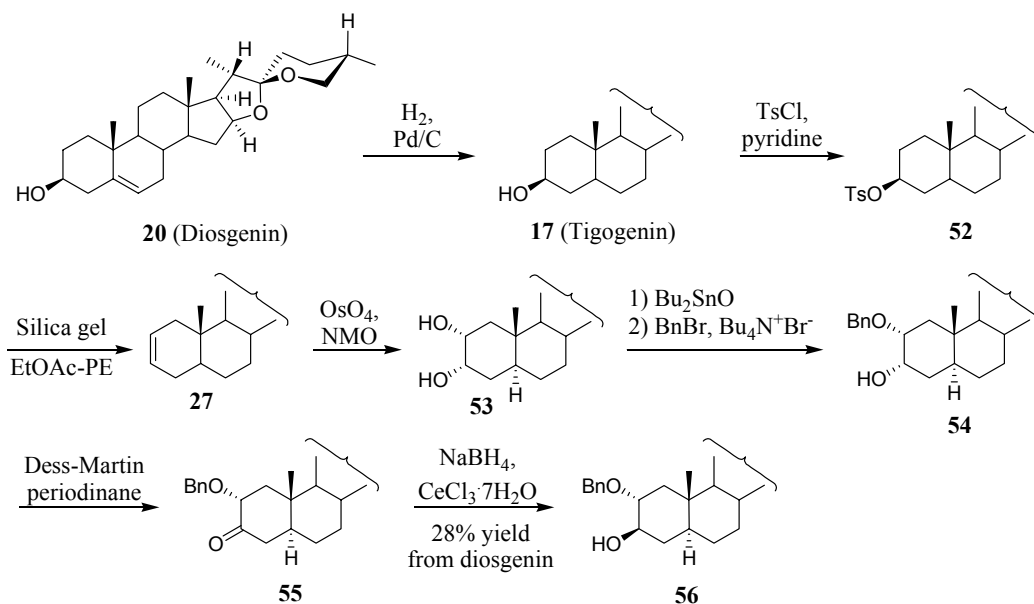
Overall, it has been determined that the synthesis of the CAY-1 aglycone from commercially available sterols is complicated by the difficulty in favoring equatorially oriented C-2 and C-3 alcohols either by substitution reactions or by reductions of corresponding ketones. In the case of substitution reactions, this is due to the preference of nucleophiles for an axial approach in cyclic systems. For reduction reactions, the complication arises from the neighboring oxygen which coordinates to the metal of hydride reducing agents and interferes with the progress and stereoselectivity of the reaction.

RESULTS AND DISCUSSION
CAY-1 PENTASACCHARIDE MOIETY

Initial Retrosynthetic Analysis of the CAY-1 Pentasaccharide

The CAY-1 pentasaccharide moiety is a 2,3-branched oligosaccharide consisting of four glucose units and one galactose unit, all linked by β-glycosidic bonds (Figure 6). Three of the glucose units are (1→3)-linked, one of which has an additional glucose unit at position 2. This branched glucose unit is also bonded to the galactose unit by a (1→4)-linkage.

Figure 7. CAY-1 Pentasaccharide Moiety.
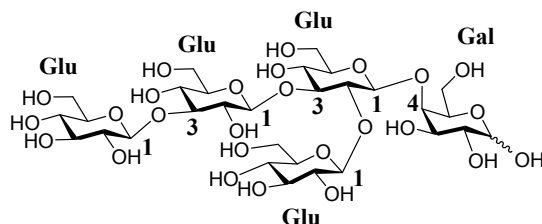
Originally, the synthesis of the CAY-1 pentasaccharide moiety was designed using a linear synthetic approach in which each of the five monosaccharide units would be added sequentially (Scheme 24). In this way, pentasaccharide **57** would be available from a coupling between branched tetrasaccharide donor **58** and position 4 of galactosyl acceptor **59**. The axial orientation of position 4 of galactose makes it less reactive in glycosidic bond formation than corresponding equatorial alcohols. Additionally, approaches toward favoring a β-glycosylation with an axial alcohol of a glycosyl acceptor would need to be explored for this step. It should be noted that since this coupling would

be performed late in the pentasaccharide synthesis, the yield for this step would be of

great concern.

Scheme 24.[a] Initial retrosynthesis of the CAY-1 pentasaccharide moiety.
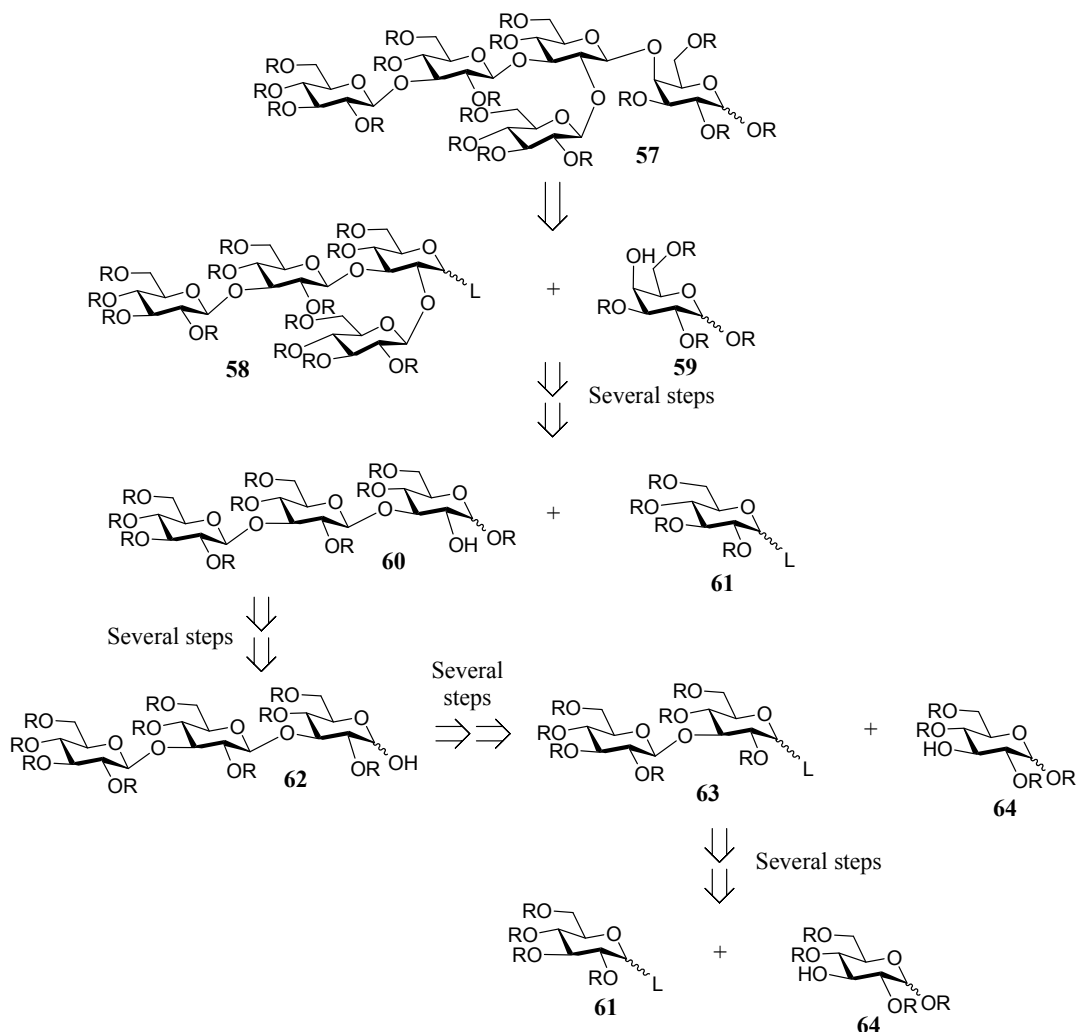
[a]R = appropriate alcohol protecting groups. L = appropriate leaving groups for glycosidic bond formation.

The most difficult aspect of this approach to the pentasaccharide synthesis is the

preparation of tetrasaccharide **58** from trisaccharide acceptor **60** and a glucosyl donor.

The synthesis of trisaccharide **60** is complicated by the requirement for a protecting

group migration from C-2 to C-1 of the reducing end of trisaccharide **62** in order to prepare tetrasaccharide **58** by this route. The linear (1→3)-linked trisaccharide **62** would be synthesized in turn from the glycosidic bond formation between disaccharide donor **63** and position 3 of glucosyl acceptor **64**. Similarly, disaccharide **63** would be prepared from the coupling between glucosyl donor **61** and position 3 of glucosyl acceptor **64**.

In addition to the aforementioned protecting group migration, the other important aspects of the pentasaccharide synthesis that needed to be considered carefully include the protecting group and glycosylation method selections. The complexity of glycosylation reactions makes it difficult, at best, to accurately predict what would be the most effective method to use for any given step in an oligosaccharide synthesis. There are, however, several trends that can be used to design and optimize a synthetic strategy.

While there are many well developed glycosylation methods for oligosaccharide synthesis, the vast majority of them follow the same general mechanism (Scheme 25).[59-63] In the presence of a promoter, the leaving group of the glycosyl donor is freed from the molecule. The structure of the oxocarbenium intermediate formed depends on the nature of the C-2 substituent of the glycosyl donor. Participating groups, such as alkyl or aryl esters and amides, can help stabilize the intermediate and tend to favor the formation of 1,2-*trans*-glycosides. It is important to note that in this case a side reaction occurs to form an orthoester. Even though orthoester formation is reversible, it can still have a deleterious effect on the glycosylation depending on the nature of the glycosyl donor and acceptor and the reaction conditions.

Glycosyl donors with non-participating groups at C-2, such as ethers, called armed donors, react faster than those with participating groups at C-2 (disarmed donors).

This is due to the fact that the C-2 substituent of an armed donor is less electron withdrawing than that of a disarmed donor and therefore the oxocarbenium intermediate forms faster. In this case, the stereoselectivity of the glycosylation is largely solvent dependent.

Scheme 25. Generalized mechanism for glycosylation reactions.[59]
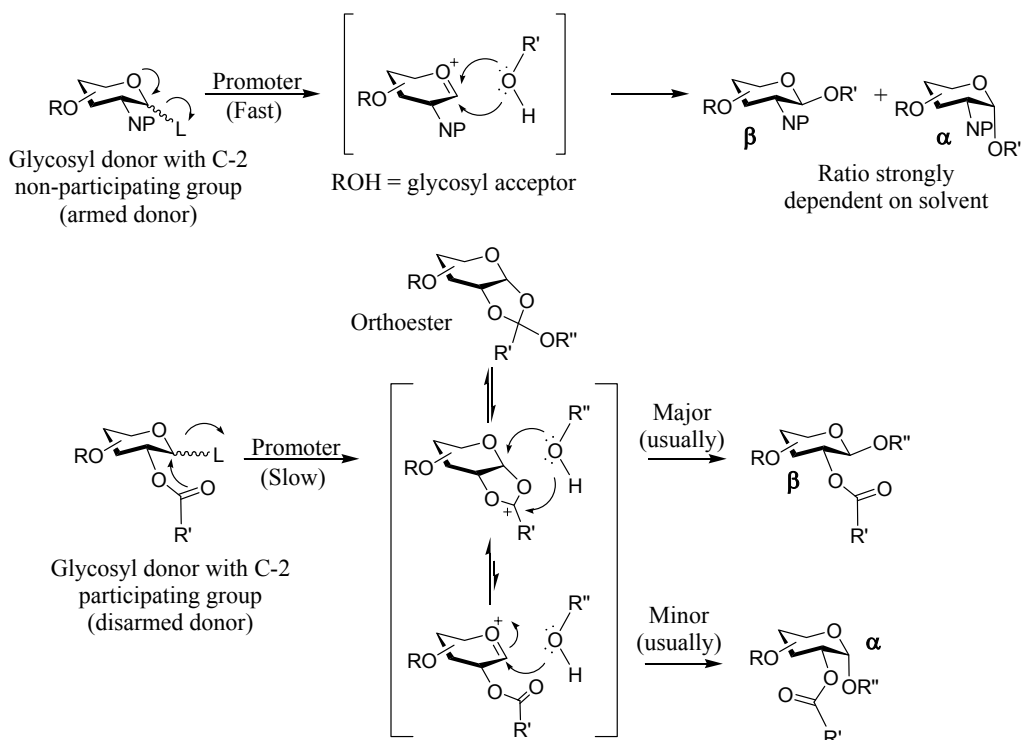
In ether solvents, armed donors generally give α-glycosides since ethers preferentially stabilize oxonium intermediates from an equatorial attack (Scheme 26).[64] The fact that the β-anomer of glucosyl ether oxonium ions is more stable than its corresponding α-anomer is in direct contrast to the anomeric effect and therefore is sometimes called the reverse anomeric effect. The anomeric effect, a special case of the gauche effect, states that there is a general preference for gauche conformations about a

C-Y bond in an X-C-Y-C system where X and Y are heteroatoms with at least one

nonbonding electron pair (Figure 7).[65]

Scheme 26. Glycosylation of an armed donor in diethyl ether.[64]
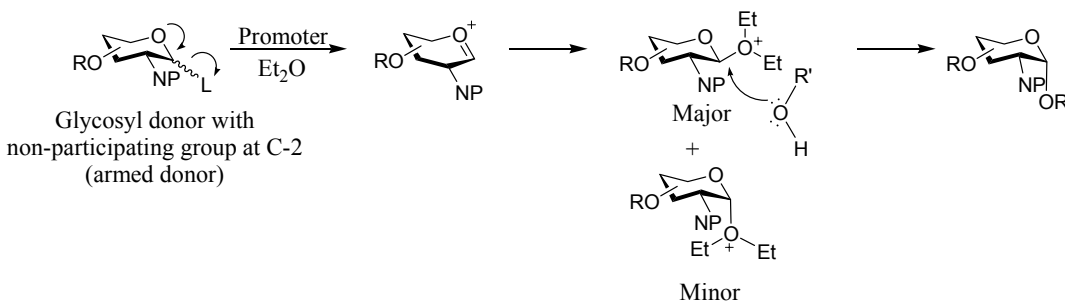
Figure 8. The anomeric effect.[65]
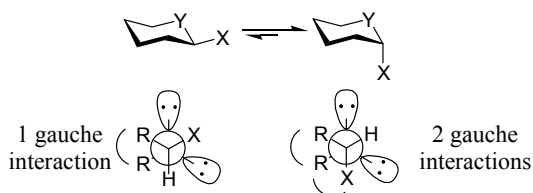
Since anti conformations are sterically preferred, the anomeric effect will only be

observed when stabilization by the anomeric effect is greater than all steric factors.[66] The

reverse anomeric effect is an observation that the axial orientation is more stable for

positively charged heteroatoms bound to the anomeric carbon. It should be noted that the

existence of a reverse anomeric effect has been debated since there are many exceptions

to this generalization.[67,68]

One explanation for the anomeric effect is that there is less lone pair interaction

between the substituent and the ring oxygen atom for gauche conformations (Figure 8).

A second explanation is that the dipoles generated by the substituent and the ring oxygen

atom are in opposing directions in the axial anomer and they are in similar directions in the equatorial anomer. Finally, the most widely accepted explanation for the anomeric effect is the stabilizing effect created by hyperconjugation between the axial lone pair on the ring oxygen atom and the σ* orbital of an axial heteroatom substituent on the anomeric carbon. This type of stabilization by hyperconjugation is not possible when the substituent is equatorial.[59]

Figure 9. Explanations for the anomeric effect.[59]
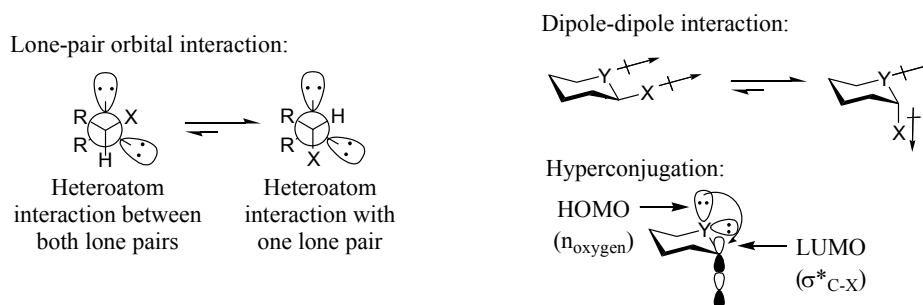
While glycosylations of armed donors in ether solvents give primarily α-glycosides as described above, using acetonitrile as the solvent favors β-glycosides under kinetic conditions.[69,70] If there is a reverse anomeric effect, the solvation of oxonium cation intermediates by acetonitrile would be an exception. In this case, acetonitrile favors solvation from the axial face and therefore glycosylations performed with armed donors in acetonitrile produce β-glycosides (Scheme 27).

Scheme 27. Glycosylation of an armed donor in acetonitrile.[69]

Glycosyl donor with
non-participating group at C-2
(armed donor)

Major

Major

+

Minor
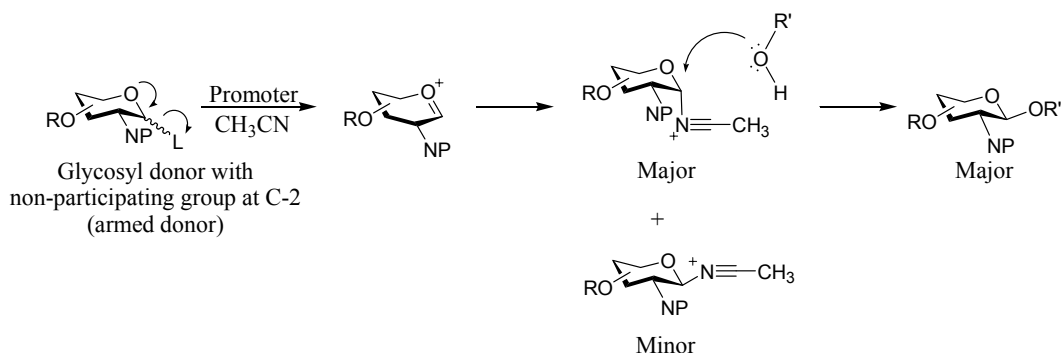
In consideration of the CAY-1 pentasaccharide, the trichloroacetimidate glycosylation method (Scheme 28) was selected due to the fact that it is generally high yielding and highly stereoselective even in complex oligosaccharide syntheses.[60,71-74] The trichloroacetimidate donors are formed by the reaction of the anomeric alcohol with trichloroacetonitrile under basic conditions. Strong bases, such as sodium hydride, and longer reaction times favor the formation of the thermodynamically more stable α-trichloroacetimidate. The stereoselectivity of the donor is important for S_N2-type glycosylations which can be favored for the trichloroacetimidate method by using weaker promoters, such as $BF_3 \cdot OEt_2$.[60]

Scheme 28. Schmidt's trichloroacetimidate method.[60]
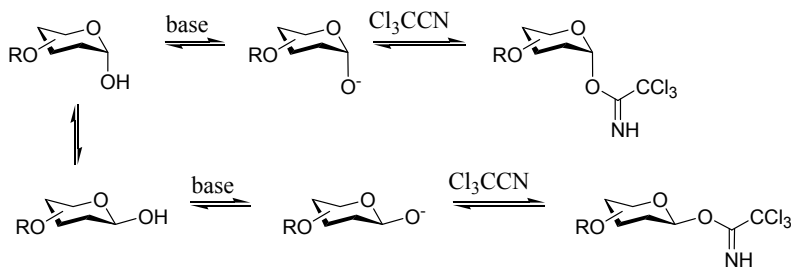
The retrosynthetic analysis of the CAY-1 pentasaccharide reveals the need for selectively protected glucosyl and galactosyl acceptors. Specifically, a glucosyl acceptor in which only the C-3 alcohol is left unprotected is required. This need is fulfilled by the commercially available saccharide 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (diacetone D-glucose). Additionally, a galactosyl acceptor in which only the C-4 alcohol is left unprotected will need to be prepared.
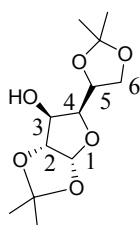
65 (Diacetone D-glucose)

Initial Approach to the Synthesis of the CAY-1 Pentasaccharide

To begin the synthesis, glucosyl trichloroacetimidate donor **69** was prepared in three steps in 33% yield following literature procedures with minor modifications (Scheme 29).[75-78] In the first step, D-glucose (**66**) was protected as pentabenzoate **67**. Next, the anomeric position of **67** was deprotected using 2-aminoethanol and then activated as trichloroacetimidate **69**. The glycosylation between diacetone D-glucose[79] and trichloroacetimidate donor **69** gave β-linked disaccharide **70** in only 53% yield. It has been reported in the literature that glycosylations using diacetone D-glucose donors tend to be low yielding.[75] This is likely due to the furanose ring which renders the C-3 alcohol less reactive, much like an axial alcohol on a pyranose ring.

Nevertheless, since this donor is commercially available, it is conceivable that it would be more efficient to proceed with the diacetone D-glucose acceptor rather than prepare an acceptor that would give higher yields in glycosylations with trichloroacetimidate donors. Therefore, the acetonide protection of disaccharide **70** was hydrolyzed under acidic conditions to give partially protected disaccharide **71** which was subsequently reprotected by reaction with benzoyl chloride to give fully protected disaccharide **72**. These steps were repeated to prepare trisaccharide **77** in eleven steps and 0.5% overall yield from D-glucose.

Scheme 29.[a] Synthesis of linear trisaccharide portion of CAY-1 pentasaccharide.
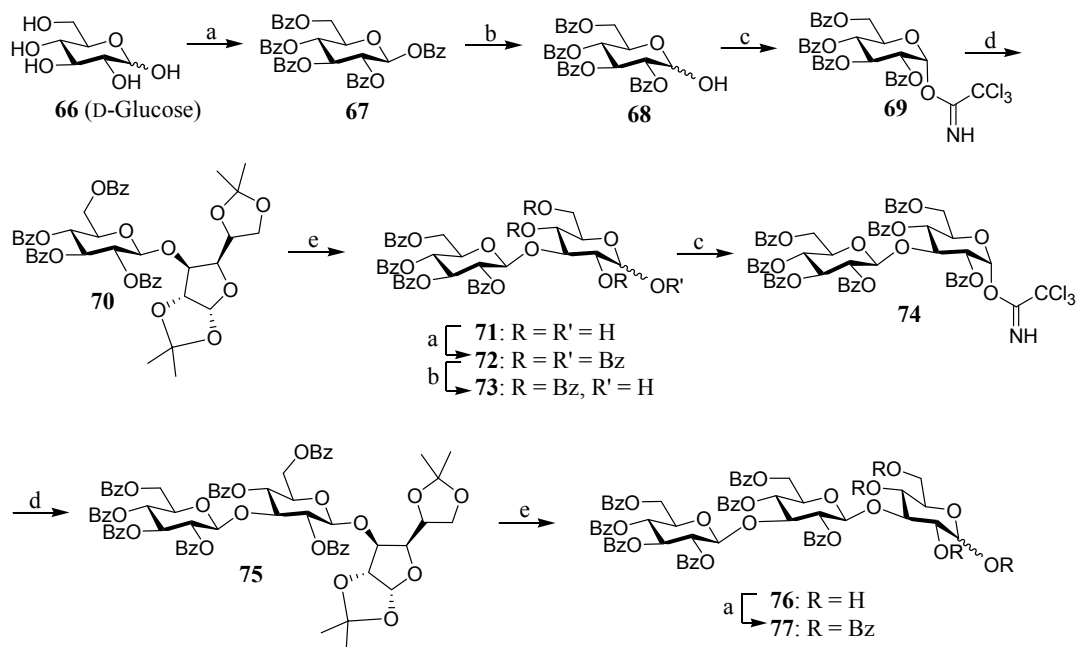
[a]Reaction conditions: (a) BzCl, Py, 56% (**67**), 78% (**72**), 49% (**77**), (b) 2-aminoethanol, DMSO, EtOAc, 70% (**68**), 40% (**73**), (c) Cl₃CCN, K₂CO₃, DCM, 48 h, 83% (**69**), 94% (**74**) (d) **65**, BF₃·OEt₂, DCM, 53% (**70**), 36% (**75**) (e) TFA, THF/H₂O, 86% (**71**) , 67% (**76**).

In order to proceed with the synthesis of the CAY-1 pentasaccharide from trisaccharide **77**, it was necessary to explore methodology that could be used to

selectively deprotect only the C-2 alcohol of the reducing sugar of the trisaccharide.

Since the preparation of trisaccharide **77** requires so many steps and is low yielding, this

conversion was explored for the monosaccharide D-glucose pentaacetate (**78**). Following

literature procedures with minor modifications, 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose

(**81**) was prepared in four steps and 16% yield from D-glucose (Scheme 30).[76,80-82]

To begin, D-glucose was protected as pentaacetate **78**. Note that **78** is

commercially available although its preparation from D-glucose using acetic anhydride in

pyridine is straightforward. Next, the anomeric alcohol of **78** was deprotected and

converted to glucosyl chloride **80** using (1-chloro-2-methylpropenyl)-dimethylamine.

Finally, the C-2 alcohol was deprotected via an acetate group migration from C-2 to C-1

of **80** to give the desired tetraacetate **81**.

Scheme 30.[a] Preparation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose.
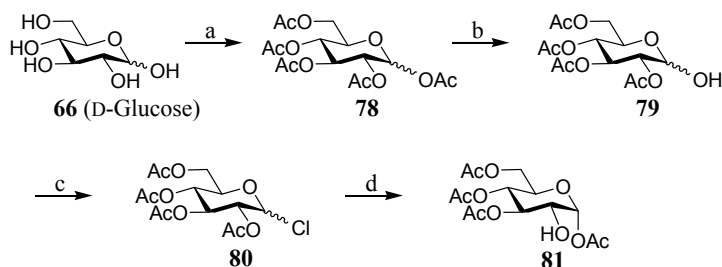
[a]Reaction conditions: (a) Ac$_2$O, Py, 83%, (b) 2-aminoethanol, DMSO, EtOAc, 70%, (c) (1-chloro-2-methylpropenyl)-dimethylamine, CHCl$_3$, (d) H$_2$O, DMF/acetone, 27% (2 steps).

At this point, there were two major concerns with this synthetic approach to the

CAY-1 pentasaccharide moiety. First, the preparation of the linear trisaccharide moiety

required 11 steps to produce trisaccharide **77** in only 0.5% yield from D-glucose.

Furthermore, based on the results of the preparation of monosaccharide tetraacetate **81**,

deprotecting the C-2 alcohol of the reducing sugar of trisaccharide **77** in preparation for

the next glycosylation would require three steps and the yield would likely be similar to

the 19% yield achieved on the monosaccharide.

In addition to yield, the second major concern was the stability of a glucosyl

acceptor like **77** during a glycosylation reaction given the migratory aptitude of the

acetate group. In fact, acetate migration would likely occur to an appreciable extent and

the result of the glycosylation would be a mixture of disaccharides, the intended product

82, and **83** from the glycosylation of **81** with itself (Scheme **31**). Therefore, a new

approach to the synthesis of the CAY-1 pentasaccharide moiety was needed.

Scheme 31. Possible acetate migration during glycosylations with acceptor **81**.
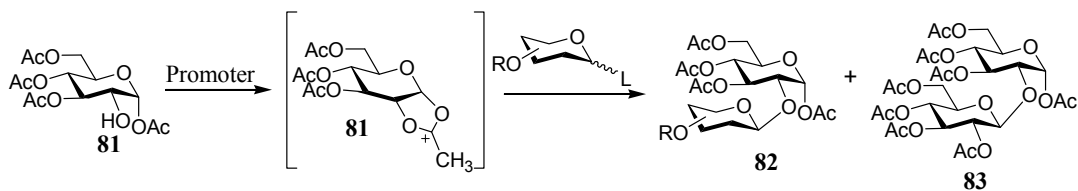
Second Retrosynthetic Analysis of the CAY-1 Pentasaccharide

A search of the literature revealed that much progress has been made in an effort

to improve the efficiency of syntheses of glycoconjugate natural products.[83-85]

Specifically, Linhardt *et al*. reported the synthesis of disaccharide **85** from partially

protected isopropyl (*R*)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**84**) and benzoyl

protected trichloroacetimidate glucosyl donor **69** in 83% yield (Scheme 32).[86] This result

suggests that the C-3 alcohol of glucosyl acceptor **84** is more reactive in glycosylation

reactions than the C-2 alcohol. Hoping to utilize this apparent difference in reactivity

between these two alcohols, the synthesis of the CAY-1 pentasaccharide was redesigned.

Scheme 32. Literature reported usage of partially protected glucosyl acceptors by Linhardt *et al*.[86]
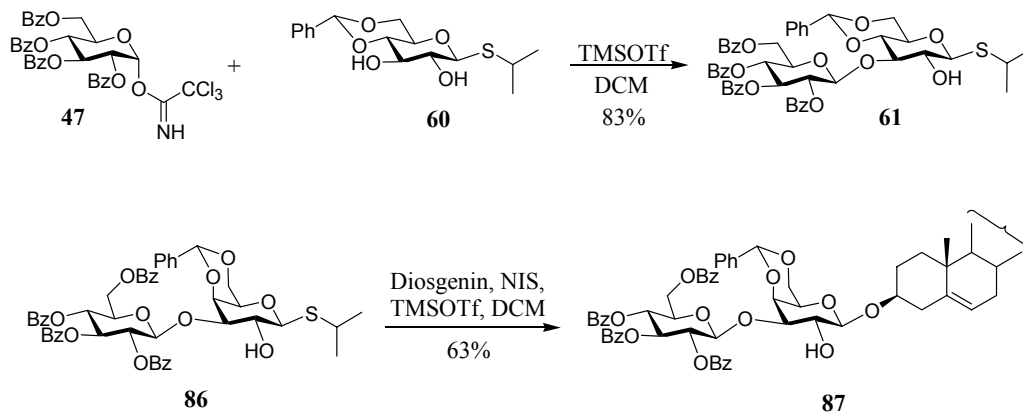
In this new design, the CAY-1 pentasaccharide moiety **88** would be prepared

from the glycosylation between thioglycoside donor **89** and galactosyl acceptor **90**

(Scheme 33). Although tetrasaccharide donor **89** does not have a C-2 participating group

to aid in favoring a (1,2)-*trans* linkage, the results reported by Linhardt *et al*. suggested

that a stereoselective glycosylation might be achieved in this step. In their case, Linhardt

et al. reported success at favoring a β-glycosylation between armed thioglycoside donor

86 and diosgenin (Scheme 32).[87]

Thioglycosides are convenient tools in oligosaccharide synthesis since they can

serve both as anomeric protecting groups and as anomeric activating groups depending

on the promoter used during glycosylations. For example, they can be used in

conjunction with trichloroacetimidate methodology since thioglycosides are stable under

trichloroacetimidate promotion conditions. When needed, they are activated using

promoters such as a combination of *N*-iodosuccinimide and trimethylsilyl triflate. In this

way, once tetrasaccharide donor **89** is prepared, it could be used immediately in the next

glycosylation reaction without the need for further deprotection and activation steps.

Scheme 33. Second retrosynthesis of the CAY-1 pentasaccharide moiety.
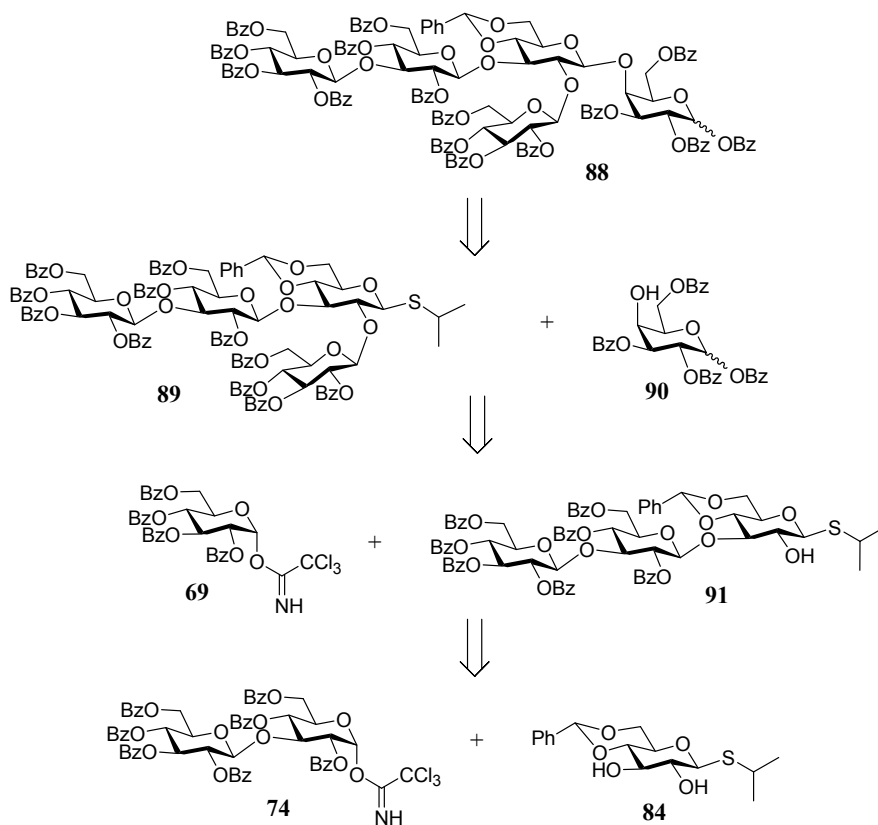
In addition to the efficiency of using thioglycosides as protecting and activating

groups, the convergent preparation of branched tetrasaccharide **89** would be a more

practical approach as compared to the previously described linear retrosynthesis. Using

partially protected glucosyl acceptor **84**, tetrasaccharide **89** could be prepared by

sequential glycosylations with trichloroacetimidate donors **74** and **69** without the need for additional deprotection or activation steps.

Second Approach to the Synthesis of the CAY-1 Pentasaccharide

Before attempting to synthesize the CAY-1 pentasaccharide moiety using the new approach, it was necessary to verify this methodology on smaller oligosaccharides. This would allow for easier characterization by ^{1}H NMR spectroscopy. Partially protected glucosyl acceptor **84** was prepared from β-D-glucose pentaacetate **78** in three steps and 51% yield using literature procedures with minor modifications (Scheme 34).[88,89] β-D-glucose pentaacetate **78** reacts with 2-propanethiol in the presence of $BF_3 \cdot OEt_2$ to give the β-anomer of thioglucopyranoside **92**.

Scheme 34.[a] Synthesis of 2,3-branched trisaccharide **94**.
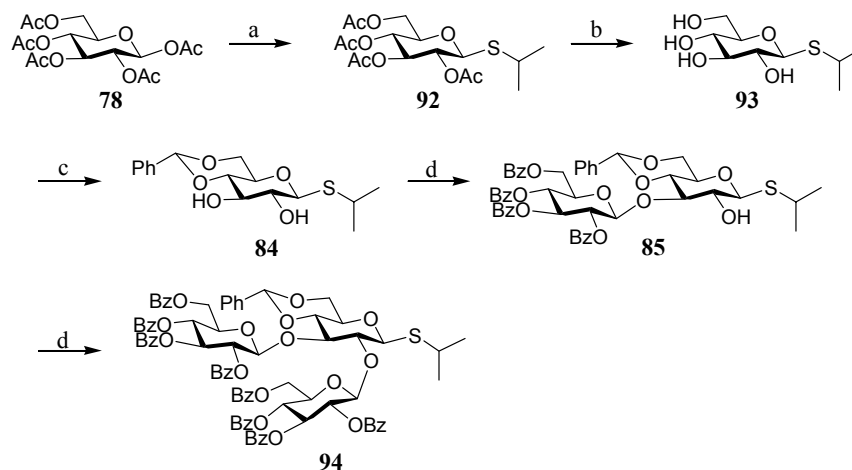
[a]Reaction conditions: (a) propane-2-thiol, $BF_3 \cdot OEt_2$, DCM, (b) NaOH, MeOH/DCM, (c) dimethoxymethylbenzene, CSA, DMF, 51% (3 steps), (d) **69**, TMSOTf, DCM, 23% (2 steps).

It is important to proceed at this point with only one anomer in order to make it possible to characterize the products of the glycosylations by ^{1}H NMR since α- and β-

anomers give different spectra. The acetate protecting groups of **92** were then hydrolyzed with NaOH in MeOH/DCM and the C-4 and C-6 alcohols of the resulting thioglycoside **93** were then protected as a benzylidene acetal to give thioglycoside donor **84**. Trichloroacetimidate donor **69** was prepared from D-glucose as described above and then reacted twice with donor **84** using trimethylsilyl triflate as the promoter to give 2,3-branched trisaccharide **94** in 23% yield for the two glycosylations.

Disaccharide **85** was isolated and characterized before the second glycosylation reaction was performed in order to verify the difference in reactivity for the C-2 and C-3 alcohols of **84**. The yield for these glycosylations was not of concern since it was reduced by the isolation of intermediate **85** for characterization. For the pentasaccharide synthesis, the two glycosylations with donor **84** would be performed in one pot to prepare 2,3-branched tetrasaccharide **89**.

The next step was to verify the stereoselectivity of a glycosylation between trisaccharide donor **94** and the C-4 alcohol of a galactosyl acceptor **90**. Due to the axial orientation of the C-4 alcohol of galactose, it is possible to prepare 1,2,3,6-tetra-*O*-benzoyl-α-D-galactopyranose (**90**) in one step by the slow addition of four equivalents of benzoyl chloride to an ice-cold solution of D-galactose in pyridine.[90,91] Trisaccharide donor **94** was then reacted with galactosyl acceptor **90** under NIS-TMSOTf promotion. Unlike trichloroacetimidates, in order to activate thioglycosides, a thiophilic promoter such as NIS is needed. Unfortunately, the glycosylation was not stereoselective and both α- and β-glycosides of tetrasacchcaride **95** were obtained.

Scheme 35. Glycosylation of trisaccharide donor **94** with galactosyl acceptor **90**.
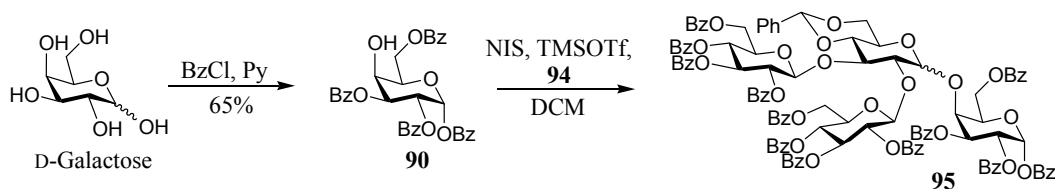
At this point, it was suspected that the glycosylation between **94** and **90** was not stereoselective due to the lack of a participating group at C-2 of glycosyl donor **94**.[92-94] Therefore, isopropyl tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**92**) was reacted with galactosyl acceptor **90** under NIS-TMSOTf promotion with the expectation that this disarmed donor would furnish the desired 1,2-*trans* glycoside by neighboring group participation (Scheme 36). However, this glycosylation afforded the α-glycoside **96** as the major product in 74% yield.

In order to finally favor a β-glycosylation with galactosyl acceptor **90** using the trichloroacetimidate method, imidate **97** was prepared from 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**79**) and reacted with **90** under TMSOTf promotion. This reaction furnished β-glycoside **98** in 37% yield.

Scheme 36. Glycosylations of galactosyl acceptor **90** with thioglycoside **92** and trichloroacetimidate **97**.
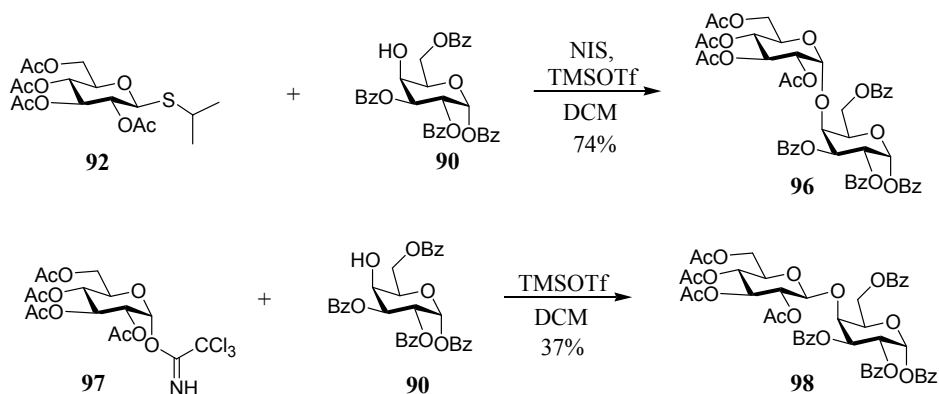
The characterization of **96** and **98** by ^{1}H NMR spectroscopy is straightforward due to the difference in coupling constants for the H-1' doublets of the non-reducing sugar unit of the disaccharides (Figure 9). For α-linked **96**, the J value for H-1' is smaller (2.4 Hz) than the J value for the H-1' doublet of β-linked **98** (3.8 Hz).

Figure 10. ^{1}H NMR (400 MHz, CDCl$_3$) comparison of **96** and **98**.
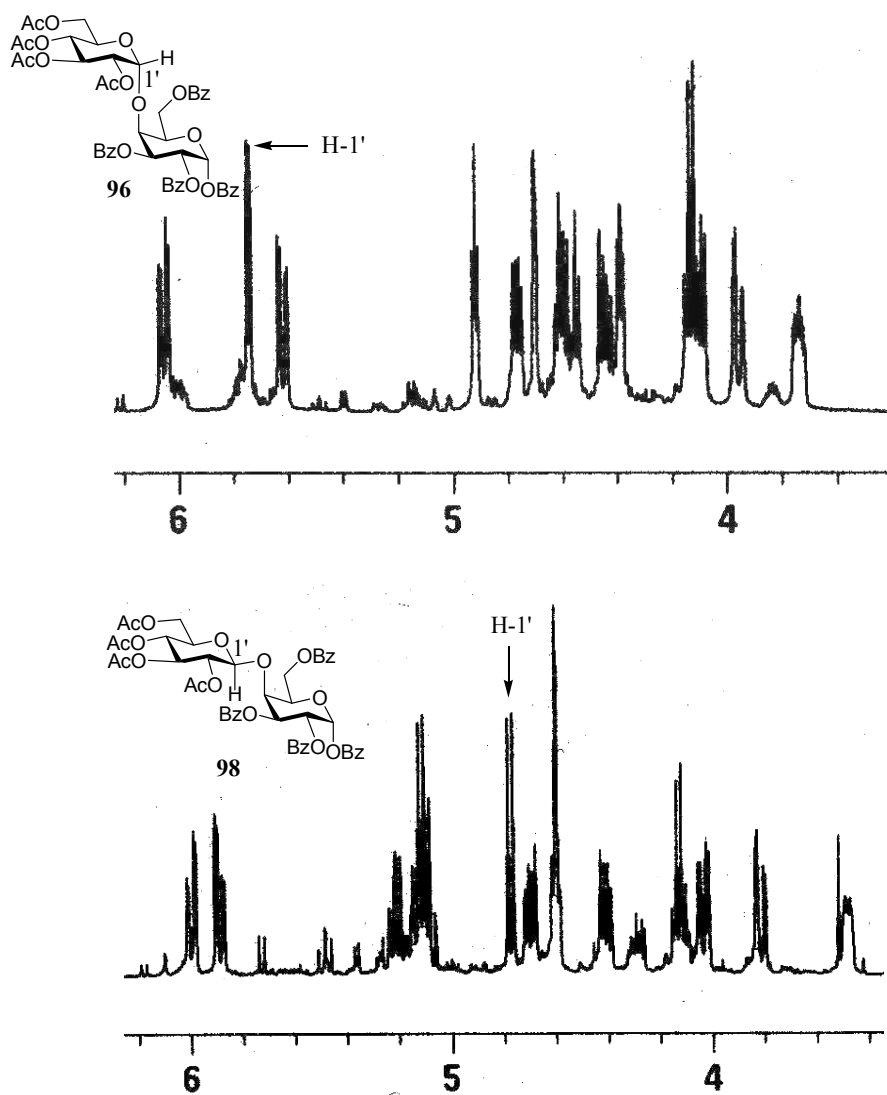
Summary of Approaches toward the CAY-1 Pentasaccharide Synthesis

While progress toward the synthesis of glycoconjugates containing 2,3-branched oligosaccharides has been reported in the literature, the reported syntheses describe glycoconjugates in which the aglycone is attached to the 2,3-branched unit of the oligosaccharide.[95-97] The CAY-1 pentasaccharide moiety, however, has a galactose unit between the 2,3-branched unit and the aglycone, complicating the synthesis.

Nonetheless, isopropyl (*R*)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**84**) has been shown to be effective in the efficient synthesis of the 2,3-branched moiety of the target pentasaccharide.

Additionally, it has been determined that a β-glycosylation with 1,2,3,6-tetra-*O*-acetyl-α-D-galactopyranose (**90**) can be achieved using a disarmed trichloroacetimidate donor despite the fact that α-glycosylations are strongly favored for this acceptor. This is likely due to the fact that the C-4 alcohol of the galactosyl acceptor is less reactive because it is in an axial orientation. Finally, the glycosylation with galactose ought to be performed early in the synthesis of the pentasaccharide moiety because it is low-yielding.

CONCLUSION

The syntheses of the aglycone and pentasaccharide moieties of CAY-1 are both challenging endeavors requiring many stereoselective steps. It has been determined that the most efficient approach to the synthesis of the CAY-1 aglycone is via the epoxidation of the trimethylsilyl enol ether of spirostan-3-one. However, further research is required to develop a highly stereoselective method for the reduction of C-2 and C-3 ketones with neighboring ether groups.

Through this work, several partially protected stereoisomers of the CAY-1 aglycone have been prepared which can be used for the synthesis of saponin derivatives of CAY-1 for structure-activity relationship studies. Definitive characterization of one of these isomers, 3α-hydroxy-(22S,25R)-5α-spirostan-2β-yl acetate (**32**), was achieved by X-ray crystallography. Such derivatives would be important not only for optimization of biological activity, but also for elucidation of the mechanism of action of CAY-1.

Furthermore, a quantitative inversion of the C-3 stereochemical configuration of **32** was achieved via an acetate group migration of the corresponding mesylate. The possibility of competition between the acetate migration and substitution mechanisms with various nucleophiles was explored. The results, however, indicate that this inversion only occurs via the acetate migration. The successful inversion of this stereocenter has not been previously reported by any method other than through an oxidation-reduction sequence with yields not exceeding 63%.[33,47,58]

Additionally, the CAY-1 pentasaccharide moiety poses two significant challenges. The first is the inclusion of a central 2,3-branched moiety. It has been

determined that this can be synthesized efficiently from the partially protected isopropyl (*R*)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside acceptor since the C-2 and C-3 alcohols differ in their reactivity in glycosylation reactions. Utilization of this partially protected glycosyl acceptor intermediate is crucial since it allows for a convergent, and therefore more efficient, approach to the synthesis of this 2,3-branched oligosaccharide.

The second challenge is the β-(1→4) linkage to the galactosyl acceptor. This significantly increases the complexity of the synthesis of the CAY-1 pentasaccharide as compared to literature reported syntheses of other branched oligosaccharides. It has been determined that this is due to the fact that a β-linkage is disfavored for the C-4 alcohol of galactose since it is axial and therefore reacts slowly. This creates a tendency for the thermodynamically more stable α-glycoside to form. Nonetheless, this β-(1→4) linkage was achieved using a disarmed trichloroacetimidate glucosyl donor.

EXPERIMENTAL

General Information

All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received unless otherwise noted. Anhydrous CH_2Cl_2, 1,2-dimethoxyethane, 3-bromopropyne, 1.5 M solution of lithium diisopropylamide in toluene, and chlorotrimethylsilane were purchased in sure-seal bottles from Aldrich Chemical Co. Chromatography refers to column chromatography on silica gel (Sorbent Technologies Silica Gel, 60 Å, 32 – 63 μm Standard Grade). ^{1}H and ^{13}C NMR spectra were recorded on a Varian-Gemini 400 MHz spectrometer or, as noted, on a Varian-Gemini 300 or 500 MHz multiprobe spectrometer. Deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (d_6-DMSO) were purchased from Cambridge Isotope Laboratories, Inc. Mass spectra were recorded using a Micromass Quattro-II triple quadropole mass spectrometer.

(22S,25R)-5α-Spirostan-3β-ol (17, **tigogenin)**[36]: Hecogenin acetate (1.0 g, 2.1 mmol) and hydrazine hydrate (1.0 mL, 20 mmol) were stirred in 2-ethoxyethanol (10 mL) under an atmosphere of nitrogen for 20 minutes at room temperature and then refluxed for 1 hour. The mixture was cooled to room temperature before KOH (1.0g, 18 mmol) was added. The mixture was heated to 136°C, refluxed for 3 hours and then cooled to room temperature. Concentrated HCl was added to neutralize the mixture and the resulting white solid was filtered and washed with water to give spirostan **17** (860 mg, 98 % yield). Selected ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.38 (q, J = 11.2 Hz, 1H), 3.58 (m, 1H), 3.47 (m, 1H), 3.37 (t, J = 10.8 Hz, 1H), 0.96 (d, J = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, J = 3.2 Hz, 3H), 0.76 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 109.5, 81.0, 71.5, 67.0, 62.3, 56.5,

54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7, 12.5.

(22*S*,25*R*)-5α-Spirost-2-ene (27)[37-39]: NaH (5.8 mg, 0.24 mmol) was stirred in THF (10 mL). Tigogenin (**17**) (100 mg, 0.24 mmol) was added and stirred at room temperature 30 minutes. Carbon disulfide (130 mg, 1.7 mmol) was added and stirred another 2 hours. 3-Bromopropyne (140 mg, 1.2 mmol) was added and the mixture was stirred an additional 3 hours at room temperature. The reaction was quenched with saturated NH$_4$Cl (40 mL), extracted with CH$_2$Cl$_2$ (3 x 50 mL), and the organic layers were combined and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (1:1 ethyl acetate: hexanes) to give propargyl xanthate ester **28** which was used immediately in the following reaction. Selected ^{1}H NMR of **28** (500 MHz, CDCl$_3$): δ 5.50 (m, 1H), 4.40 (q, *J* = 11.3 Hz, 1H), 3.85 (s, 1H), 3.82 (s, 1H), 3.48 (m, 1H), 3.38 (t, *J* = 11.0 Hz, 1H), 0.97 (d, *J* = 3.5 Hz, 3H), 0.88 (s, 3H), 0.80 (d, *J* = 3.0 Hz, 3H), 0.77 (s, 3H). Trifluoromethanesulfonic acid (1.0 mL, 11 mmol) and 2,4,6-trimethylpyridine (1.5 mL, 11 mL) were stirred at 0°C in diethyl ether (5 mL). A portion of the resulting sulfonate salt (7.0 mg, 0.02 mmol) was transferred to a solution of propargyl xanthate ester **28** (0.24 mmol) in toluene (10 mL). The reaction was refluxed 30 hours, cooled to room temperature, and the solvent was removed under reduced pressure. The resulting solid was purified by flash column chromatography (1:10 ethyl acetate: hexanes) to give **27** as a white solid (86 mg, 89 % yield over 2 steps from **18**). Selected ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.56 (m, 2 H), 4.37 (q, *J* = 11.2 Hz, 1H), 3.45 (m, 1H), 3.35 (t, *J* = 10.4 Hz, 1H), 0.94 (d, *J* = 3.8 Hz, 3H), 0.78 (s, 3H), 0.75 (s, 3H), 0.75 (d, *J* = 3.2 Hz, 3H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 126.0, 109.4, 81.0,

67.0, 62.4, 56.5, 54.2, 41.8, 41.6, 40.6, 40.2, 39.9, 35.4, 34.9, 32.2, 32.1, 31.9, 31.6, 30.5, 29.9, 29.0, 28.8, 20.9, 17.3, 16.6, 14.7, 11.9. Alternatively, **27** was prepared from **17** via methyl xanthate ester **29**. Sodium hydride (10 mg, 0.42 mmol) was suspended in THF (10 mL). Tigogenin (**17**) (100 mg, 0.24 mmol) was added and stirred at room temperature 30 minutes. Carbon disulfide (130 mg, 1.7 mmol) was added and stirred another 2 hours. Methyl iodide (170 mg, 1.2 mmol) was added and the mixture was stirred an additional 3 hours at room temperature. The reaction was quenched with saturated NH_4Cl (40 mL), extracted with CH_2Cl_2 (3 x 50 mL), and the organic layers were combined and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (1:15 ethyl acetate: hexanes) to give methyl xanthate ester **29** which was used immediately in the following reaction. Selected ^1H NMR of **29** (400 MHz, $CDCl_3$): δ 5.47 (m, 1H), 4.37 (q, J = 11.4 Hz, 1H), 3.45 (m, 1H), 3.35 (t, J = 10.8 Hz, 1H), 2.52 (s, 3H), 0.94 (d, J = 3.6 Hz, 3H), 0.85 (s, 3H), 0.77 (d, J = 3.2 Hz, 3H), 0.74 (s, 3H). Methyl xanthate ester **29** was heated at 200°C for 2 hours under reduced pressure. The resulting solid was purified by flash column chromatography (1:10 ethyl acetate: hexanes) to give **27** as a white solid (90 mg, 94 % yield).

2,3-Epoxy-(22S,25R)-5α-spirostan (26): Spirostene **27** (4.4 g, 11 mmol) was dissolved in anhydrous CH_2Cl_2 (90 mL) under an atmosphere of nitrogen. To this solution, *m*-CPBA (2.1 g, 12 mmol) was added and the mixture was stirred 30 minutes at room temperature. 5 % Na_2SO_3 (100 mL) was added and the mixture was stirred an additional 15 minutes. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The organic phases were combined and washed with saturated

NaHCO$_3$ (100 mL), followed by brine (100 mL), and dried over sodium sulfate. The

solvent was removed under reduced pressure and the resulting solid was purified by flash

column chromatography (1:5 ethyl acetate: hexanes) to give **26α** as a white solid (4.2 g,

91 % yield). Selected ^{1}H NMR of **26α** (400 MHz, CDCl$_3$): δ 4.36 (q, J = 11.2 Hz, 1H),

3.45 (m, 1H), 3.35 (t, J = 10.8 Hz, 1H), 3.13 (br s, 1H), 3.09 (m, 1H), 0.94 (d, J = 3.6 Hz,

3H), 0.77 (d, J = 3.4 Hz, 3H), 0.75 (s, 3H), 0.73 (s, 3H). ^{13}C NMR of **26α** (400 MHz,

CDCl$_3$): δ 109.4, 80.9, 67.0, 62.3, 56.3, 53.8, 52.5, 51.1, 41.8, 40.5, 40.1, 38.4, 36.4,

35.4, 33.9, 32.0, 31.9, 31.5, 30.4, 29.2, 29.0, 28.5, 20.8, 17.3, 16.5, 14.7, 13.1. ^{1}H NMR

26β (300 MHz, CDCl$_3$): δ 4.37 (q, J = 11.2 Hz, 1H), 3.46 (m, 1H), 3.35 (t, J = 10.8 Hz,

1H), 3.14 (m, 1H), 3.03 (d, J = 2.9 Hz, 1H), 1.04 (s, 3H), 0.93 (d, J = 3.3 Hz, 3H), 0.79

(d, J = 3.2 Hz, 3H), 0.76 (s, 3H).

3α-Hydroxy-(22S,25R)-5α-spirostan-2β-yl acetate (32): Epoxide **26α** (100 mg, 0.24

mmol) was refluxed in acetic acid (10 mL) for 2 hours. The solution was co-evaporated

with toluene (3 x 5 mL) and the resulting solid was purified by gravity column

chromatography (1:4 ethyl acetate: hexanes) to give **32** as a white solid (77 mg, 84 %

yield). Selected ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.87 (br s, 1H), 4.38 (q, J = 11.2 Hz,

1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4

Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), 0.75 (s, 3H). ^{13}C NMR (400 MHz,

CDCl$_3$): δ 170.5, 109.5, 81.0, 73.3, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7,

37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7, 13.9.

(22S,25R)-5α-Spirostan-3-one (34)[47]: Tigogenin (**17**) (1 g, 2.40 mmol) was dissolved in

acetone (50 mL) at 30°C. 8N Chromic acid in 40% H$_2$SO$_4$ (2.5 mL) was added and the

mixture was stirred for 30 minutes. Water (100 mL) was added and the resulting solid

was filtered and washed with water and purified by flash column chromatography (1:2 ethyl acetate: hexanes) to give **34** as a white solid (930 mg, 93 % yield) which was used immediately in the next reaction. Selected ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.39 (q, J = 11.2 Hz, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 1.02 (s, 3H), 0.96 (d, J = 3.6 Hz, 3H), 0.78 (s, 3H), 0.78 (d, J = 2.0 Hz, 3H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 212.1, 109.4, 80.9, 67.0, 62.4, 56.3, 54.0, 46.8, 44.8, 41.8, 40.7, 40.1, 38.7, 38.3, 35.9, 35.2, 32.0, 31.9, 31.5, 30.4, 29.0, 29.0, 21.4, 17.3, 16.6, 14.7, 11.7.

[(22S,25R)-5α-Spirost-2-enyl-3-oxy]-trimethylsilane (35)[47]**:** Ketone **34** (410 mg, 1.0 mmol) was dissolved in anhydrous 1,2-dimethoxyethane (10 mL) under an atmosphere of nitrogen. The mixture was cooled to 0°C and LDA (1.5 M solution in toluene, 1.3 mL, 2.0 mmol) was added and stirred 10 minutes. Chlorotrimethylsilane (0.66 mL, 5.0 mmol) was added and the mixture was stirred 2 hours at room temperature. Triethylamine (4 mL) and saturated NaHCO$_3$ solution (10 mL) were added and the mixture was extracted with CH$_2$Cl$_2$ (3 x 20 mL). The organic fractions were combined, dried over sodium sulfate, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (20:1 hexanes: ethyl acetate) to give trimethylsilyl enol ether **35** as a white solid (370 mg, 76 % yield). Selected ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.76 (d, J = 2.8 Hz, 1H), 4.40 (q, J = 11.6 Hz, 1H), 3.48 (m, 1H), 3.38 (t, J = 10.8 Hz, 1H), 0.97 (d, J = 3.6 Hz, 3H), 0.79 (d, J = 3.2 Hz, 3H), 0.77 (s, 3H), 0.77 (s, 3H), 0.18 (s, 9H).

2β-Hydroxy-(22S,25R)-5α-spirostan-3β-yl acetate (42) and 3β-Hydroxy-(22S,25R)-5α-spirostan-2β-yl acetate (43): Spirostan **32** (470 mg, 1.0 mmol), methanesulfonyl chloride (0.15 mL, 1.5 mmol) and triethylamine (0.25 mL, 1.8 mmol) were stirred at 0°C

in anhydrous CH_2Cl_2 (15 mL) under an atmosphere of nitrogen for 2 hours. The reaction

was quenched with ice-cold HCl (1 M, 50 mL) and extracted with CH_2Cl_2 (3 x 50 mL).

The organic layers were combined, washed with 50 mL water, and dried over sodium

sulfate. The solvent was removed under reduced pressure to give mesylate **41** as a white

solid which was used immediately in the next reaction without further purification (460

mg, 83 % yield). Selected ^{1}H NMR of **41** (300 MHz, CDCl$_3$): δ 4.99 (br s, 1H), 4.68 (br

s, 1H), 4.40 (q, J = 11.6 Hz, 1H), 3.48 (m, 1H), 3.37 (t, J = 11.1 Hz, 1H), 3.08 (s, 3H),

0.96 (d, J = 3.5 Hz, 3H), 0.94 (s, 3H), 0.79 (d, J = 3.2 Hz, 3H), 0.76 (s, 3H). Meslyate **41**

(50 mg, 0.09 mmol) and water (5 mL) were refluxed in pyridine (20 mL) for 7 hours.

The solvent was removed under reduced pressure and the product was co-evaporated with

toluene (3 x 5 mL) and purified by flash column chromatography (1:4 → 1:1 ethyl

acetate: hexanes) to give **42** and **43** as a white solid (10 % **42**, 90% **43,** 42 mg, 97 %

yield). Selected ^{1}H NMR of **42** (400 MHz, CDCl$_3$): δ 4.78 (m, 1H), 4.39 (q, J = 11.2 Hz,

1H), 4.10 (br s, 1H), 3.66 (m, 1H), 3.38 (t, J = 10.8 Hz, 1H), 2.09 (s, 3H), 1.06 (s, 3H),

0.96 (d, J = 3.4 Hz, 3H), 0.79 (d, J = 3.0 Hz, 3H), 0.77 (s, 3H). ^{13}C NMR of **42** (400

MHz, CDCl$_3$): δ 170.3, 109.4, 81.0, 75.7, 68.8, 67.0, 62.3, 56.3, 55.3, 45.6, 43.1, 41.7,

40.8, 40.1, 35.6, 34.6, 32.2, 31.9, 31.5, 30.4, 28.9, 28.7, 28.2, 21.5, 21.2, 17.3, 16.7, 14.7,

14.6. Selected ^{1}H NMR of **43** (400 MHz, CDCl$_3$): δ 5.14 (br s, 1H), 4.39 (q, J = 11.4 Hz,

1H), 3.69 (m, 1H), 3.47 (m, 1H), 3.37 (t, J = 10.8 Hz, 1H), 2.10 (s, 3H), 0.96 (d, J = 3.4

Hz, 3H), 0.93 (s, 3H), 0.79 (d, J = 3.0 Hz, 3H), 0.76 (s, 3H). ^{13}C NMR of **43** (400 MHz,

CDCl$_3$): δ 171.5, 109.5, 81.0, 73.5, 71.4, 67.0, 62.3, 56.3, 55.1, 45.4, 41.8, 41.2, 40.8,

40.1, 35.6, 34.7, 33.2, 32.2, 31.9, 31.5, 30.4, 28.9, 28.3, 21.6, 21.3, 17.3, 16.7, 14.6, 14.5.

Under an atmosphere of nitrogen, steroid **42** (30 mg, 0.063 mmol) was dissolved in

anhydrous CH_2Cl_2 (3 mL) and cooled to 0°C before triethylamine (16 μL, 0.095 mmol) and methanesulfonyl chloride (10 μL, 0.095 mmol) were added and stirred for 2 hours. Ice-cold HCl (1 M, 10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic fractions were dried over sodium sulfate and the solvents were removed under reduced pressure to give mesylate **44** as a white solid which was used immediately in the next reaction without further purification. Mesylate **44** and water (5 mL) were refluxed overnight in pyridine (20 mL). The solvents were removed under reduced pressure and the resulting yellow solid was purified by flash column chromatography (1:4 → 1:1 ethyl acetate: hexanes) to give **42** and **43** as a white solid (20 % **42**, 80 % **43,** 22 mg, 73 % yield in 2 steps from **42**).

3β-Benzyloxy-(22*S*,25*R*)-5α-spirostan-2β-ol (46): Steroid **43** (90 mg, 0.19 mmol) was dissolved in dioxane (10 mL) and stirred 1 hour with 4Å molecular sieves. Benzyl trichloroacetimidate (120 μL, 0.63 mmol) and trifluoromethanesulfonic acid (13 μL, 0.15 mmol) were added and the reaction was stirred in an icebath for 1 minute and then at room temperature for 5 minutes. Saturated $NaHCO_3$ (10 mL) was added and the mixture was extracted with ethyl acetate (3 x 15 mL) and the combined organic fractions were dried over sodium sulfate. The solvents were removed under reduced pressure and the resulting residue was purified by flash column chromatography (ethyl acetate: hexanes 1:4 to 1:2) to give 3β-Benzyloxy-(22*S*,25*R*)-5α-spirostan-2β-yl acetate (**47**) as a white solid which was used immediately in the next reaction (83 mg, 78 % yield). Selected ^{1}H NMR of **47** (400 MHz, $CDCl_3$): δ 7.38 − 7.32 (m, 5H), 5.42 (br s, 1H), 4.60 (d, *J* = 6.0 Hz, 1H), 4.48 (d, *J* = 6.0 Hz, 1H), 4.39 (q, *J* = 11.2 Hz, 1H), 3.47 (m, 1H), 3.37 (t, *J* = 11.2 Hz, 1H), 3.37 (m, 1H), 0.93 (d, *J* = 3.4 Hz, 3 H), 0.93 (s, 3H), 0.76 (d, *J* = 3.2 Hz,

3H), 0.73 (s, 3H). Steroid **47** (83 mg, 0.15 mmol) was dissolved in methanol (15 mL).

NaOH (1 N, 1 mL) was added and the mixture was refluxed overnight. The mixture was

neutralized with acidic Dowex resin and the resin was filtered and washed with additional

methanol. The solvents were removed under reduced pressure and the resulting solid was

co-evaporated with toluene (3 x 5 mL) to give **46** as a white solid (73 mg, 96 % yield).

Under an atmosphere of nitrogen, steroid **46** (60 mg, 0.12 mmol) was dissolved in

anhydrous CH_2Cl_2 (10 mL) and cooled to 0°C before triethylamine (38 μL, 0.230 mmol)

and methanesulfonyl chloride (23 μL, 0.230 mmol) were added and stirred for 2 hours.

Ice-cold HCl (1 M, 50 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 50

mL). The combined organic fractions were dried over sodium sulfate and the solvents

were removed under reduced pressure. The residue was purified by flash column

chromatography (1:4 ethyl acetate: hexanes) to give mesylate **45** as a white solid (55 mg,

81 % yield) which was used immediately in the next reaction. Mesylate **45** and water (5

mL) were refluxed overnight in pyridine (20 mL). The solvents were removed under

reduced pressure and the resulting yellow solid was purified by flash column

chromatography (1:4 ethyl acetate: hexanes) to give **46** as a white solid (43 mg, 90 %

yield). Selected ^{1}H NMR of **46** (400 MHz, CDCl$_3$): δ 7.37 – 7.30 (m, 5H), 4.57 (d, J =

2.6 Hz, 2H), 4.36 (q, J = 11.2 Hz, 1H), 4.12 (br s, 1H), 3.45 (m, 1H), 3.38 (m, 1H), 3.35

(t, J = 11.2 Hz, 1H), 1.01 (s, 3H), 0.93 (d, J = 3.4 Hz, 3H), 0.77 (d, J = 3.2 Hz, 3H), 0.74

(s, 3H).

3β-Benzyloxy-(22S,25R)-5α-spirostan-2-one (49): Steroid **36** (50 mg, 0.096 mmol) was

dissolved in acetone (10 mL) at 30°C. 8N Chromic acid in 40% H_2SO_4 (1.0 mL) was

added and the mixture was stirred for 30 minutes. Water (20 mL) was added and the

resulting solid was filtered and washed with water and purified by flash column chromatography (1:2 ethyl acetate: hexanes) to give **49** as a white solid (44 mg, 90 % yield). Selected ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.37 – 7.28 (m, 5H), 4.86 (d, J = 6.0 Hz, 1H), 4.52 (d, J = 6.0 Hz, 1H), 4.40 (q, J = 11.0 Hz, 1H), 3.95 (m, 1H), 3.46 (m, 1H), 3.37 (t, J = 10.8 Hz, 1H), 0.96 (d, 3.4 Hz, 3H), 0.80 (d, J = 3.2 Hz, 3H), 0.79 (s, 3H), 0.76 (s, 3H).

Penta-*O*-benzoyl-β-D-glucopyranose (67)[75]**:** D-glucose (5.0 g, 28 mmol) was refluxed in pyridine (100 mL) for 1 hour. The solution was then added to benzoyl chloride (20 mL) which had been heated to 65°C. After the mixture was cooled to room temperature, water (400 mL) was added and stirred until the product solidified, approximately 30 minutes. The solid was filtered and washed with water and recrystallized from ethyl acetate to give **67** as a white solid (11 g, 56 % yield). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.01 (d, J = 4.0 Hz, 4H), 7.89 (d, J = 3.8 Hz, 4H), 7.84 (d, J = 3.9 Hz, 2H), 7.53 – 7.21 (m, 15 H), 6.28 (d, J=3.9 Hz, 1H), 6.02 (t, J = 9.3 Hz, 1H), 5.83 (q, J=12.9 Hz, 2H), 4.64 (dd, J_1 = 6.0 Hz, J_2 = 1.4 Hz, 1H), 4.49 (dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz, 1H), 4.394 (m, 1H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 166.3, 165.8, 165.30, 165.28, 164.8, 134.0, 133.7, 133.6, 133.5, 133.3, 130.4, 130.0, 129.99, 129.96, 129.7, 128.9, 128.84, 128.82, 128.7, 128.62, 128.59, 128.5, 92.9, 73.3, 73.0, 71.0, 69.2, 62.8.

2,3,4,6-Tetra-*O*-benzoyl-D-glucopyranose (68)[76]: Pentabenzoyl-δ-D-glucopyranose **67** (10 g, 14 mmol) and 2-aminoethanol (2.0 g, 36 mmol) were stirred at room temperature in ethyl acetate (150 mL) and DMSO (2 mL) overnight, during which time the initial suspension formed a solution. The resulting solution was washed with water (3 x 150 mL) and the organic layer was dried over sodium sulfate and concentrated. The residue

was purified by flash column chromatography (CH_2Cl_2 followed by Et_2O) to give **68** as a white solid (6.0 g, 70 % yield). 100 mg of the product was recrystallized from diethyl ether/hexanes for ^1H and ^{13}C NMR analysis. ^1H NMR (300 MHz, $CDCl_3$) **68β**: δ 8.19-7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, J=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, J_1=6.45 Hz, J_2=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, J_1=8.6 Hz, J_2=2.4 Hz, 1H), 3.12 (d, J=1.3 Hz, 1H). ^{13}C NMR of **68β** ($CDCl_3$): δ 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.46, 90.6, 72.5, 70.4, 69.6, 67.8, 63.0.

***O*–(Tetra-*O*-benzoyl-α-D-glucopyranosyl)-trichloroacetimidate (69)**[77]: Tetrabenzoyl-D-glucopyranose **68** (2.0 g, 3.3 mmol), K_2CO_3 (1.1 g, 8.2 mmol), and trichloroacetonitrile (5 mL) were stirred at room temperature in dichloromethane (20 mL) for 48 hours. The resulting suspension was filtered through silica gel using a 1:1 mixture of CH_2Cl_2 and Et_2O (150 mL). The filtrate was concentrated to give **69** as a pale yellow solid (2.0 g, 83 % yield). ^1H NMR (300 MHz, $CDCl_3$): δ 8.62 (s, 1H) 8.02 (d, J = 3.9 Hz, 2H), 7.93 (br d, J = 5.7 Hz, 4H), 7.85 (d, J = 4.2 Hz, 2H), 7.56 – 7.25 (m, 12H), 6.82 (d, J = 2.0 Hz, 1H), 6.25 (t, J = 9.9 Hz, 1H), 5.79 (t, J=9.9 Hz, 1H), 5.60 (dd, J_1 = 5.1 Hz, J_2 = 1.8 Hz, 1H), 4.62 (br d, J = 5.1 Hz, 2H), 4.46 (dd, J_1 = 6.5 Hz, J_2 = 3.0 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 166.2, 165.8, 165.6, 165.4, 160.7, 133.7, 133.5, 133.4, 130.1, 129.91, 129.86, 129.7, 129.0, 128.73, 128.66, 128.60, 128.55, 128.51, 93.3, 90.9, 70.9, 70.3, 68.8, 62.6.

Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (70)[75]: Trichloroacetimidate **69** (2.0 g, 2.7 mmol) and diacetone D-glucose (650 mg, 2.5 mmol) were stirred under an atmosphere of nitrogen in anhydrous

dichloromethane (20 mL) with 3Å molecular sieves for 1 hour. The solution was cooled

in an icebath for 30 minutes before BF$_3$·OEt$_2$ (2 mL) was added and stirred for 1 hour in

the icebath followed by 1 hour at room temperature. The solution was added to an ice-

cold saturated aqueous sodium bicarbonate solution (100 mL) with vigorous stirring and

then extracted with ether (2 x 75 mL). The organic layers were combined and dried over

sodium sulfate. The solvent was removed under reduced pressure and the residue was

purified by flash column chromatography (CH$_2$Cl$_2$:THF 9:1 to 0:1) to give **70** as a white

solid (1.1 g, 53 % yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.03 (d, J = 4.4 Hz, 2H), 7.91

(t, J = 9.8 Hz, 4H), 7.80 (d, J = 4.4 Hz, 2H), 7.57 – 7.26 (m, 12H), 5.89 (m, 2H), 5.66 (t,

J = 8.0 Hz, 1H), 5.51 (t, J = 8.8 Hz, 1 H), 4.90 (d, J = 3.8 Hz, 1H), 4.73 (dd, J_1 = 6.2 Hz,

J_2 = 1.4 Hz, 1H), 4.42 (m, 2H), 4.28 (d, J = 1.4 Hz, 1H), 4.16 (m, 1H), 4.07 (m, 2H),

4.01 (dd, J_1 = 3.4 Hz, J_2 = 1.2 Hz, 1H), 3.84 (q, J = 9.2 Hz, 1H), 1.36 (s, 6H), 1.26 (s,

6H). ^{13}C NMR (400 MHZ, CDCl$_3$): δ 166.5, 166.0, 165.6, 165.4, 133.8, 133.7, 133.6,

130.1, 130.0, 129.6, 129.2, 128.9, 128.7, 128.6, 112.0, 105.3, 102.0, 85.2, 79.7, 75.8,

72.84, 72.77, 72.5, 72.2, 69.5, 69.4, 62.8, 27.0, 26.4.

Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-D-glucopyranose (71): Disaccharide **70**

(3.2 g, 3.8 mmol) was stirred at 40ºC in THF (10 mL) and a 1:1 mixture of trifluoroacetic

acid and water (40 mL) for 24 hours. The solution was then added slowly to water (400

mL) with stirring. The resulting solid was filtered and washed with water to give **71** as a

white solid (2.5 g, 86 % yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.99 – 7.86 (m, 6H), 7.80

(d, J = 3.8 Hz, 2H), 7.47 – 7.21 (m, 12H), 5.90 (t, J = 9.6 Hz, 1H), 5.71 (t, J = 9.6 Hz,

1H), 5.52 (t, J = 9.2 Hz, 1H), 5.05 (d, J = 1.6 Hz, 1H), 4.94 (d, J = 4.0 Hz, 1H), 4.69 (t, J

= 12.8 Hz, 1H), 4.41 (m, 1H), 4.14 (m, 2H), 3.94 (m, 1H), 3.80 (m, 1H), 3.69 (t, J = 9.6

Hz, 1H), 3.41 (m, 1H), 3.31 (m, 1H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 166.6, 166.0, 165.7, 165.3, 133.6, 133.5, 133.4, 130.0, 129.9, 129.5, 129.2, 129.2, 129.2, 129.0, 129.0, 128.6, 128.5, 128.4, 128.4, 102.0, 101.9, 96.6, 92.5, 77.4, 76.4, 75.2, 74.7, 73.6, 73.1, 72.3, 72.2, 72.1, 70.8, 70.6, 70.4, 70.1, 69.9, 69.7, 63.1

Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-tetra-*O*-benzoyl-D-glucopyranose (72): Disaccharide **71** (2.5 g, 3.3 mmol) was refluxed in pyridine (50 mL) for 30 minutes. The solution was added to benzoyl chloride (10 mL) that had been heated to 65°C. After the mixture was cooled to room temperature, water (200 mL) was added. The resulting syrup was separated from the aqueous layer and dissolved in CH$_2$Cl$_2$ (50 mL). The solution was washed with water (3 x 50 mL), dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was crystallized from CH$_2$Cl$_2$/hexanes to give **72** as a white solid (3.0 g, 78 % yield). ^{1}H NMR of **72α** and **β** (400 MHz, CDCl$_3$): δ 8.12 – 7.77 (m, 32H), 7.62 – 7.15 (m, 48H), 6.69 (d, *J* = 1.8 Hz, 1H), 6.19 (t, *J* = 10.0 Hz, 1H), 6.14 (d, *J* = 4.2 Hz, 1H), 5.92 (m, 2H), 5.71 – 5.41 (m, 7H), 5.08 (d, *J* = 3.8 Hz, 1H), 5.00 (d, *J* = 4.0 Hz, 1H), 4.58 (dd, J_1 = 6.0 Hz, J_2 = 1.6 Hz, 1H), 4.51 – 4.37 (m, 5H), 4.23 (m, 1H), 4.18 – 3.99 (m, 5H), 3.94 (dd, J_1 = 6.2 Hz, J_2 = 3.0 Hz, 1H), 3.86 (dd, J_1 = 6.4 Hz, J_2 = 3.0 Hz, 1H). ^{13}C NMR of **72α** and **β** (400 MHz, CDCl$_3$): δ 172.0, 166.3, 166.0, 165.8, 165.4, 164.9, 134.0, 133.6, 133.5, 133.4, 133.3, 130.4, 130.22, 130.16, 130.1, 130.0, 129.8, 129.5, 129.4, 129.0, 128.9, 128.8, 128.7, 128.6, 128.51, 128.48, 128.4, 101.1, 100.5, 92.9, 90.1, 75.4, 73.2, 73.0, 72.9, 72.4, 72.1, 71.9, 71.0, 70.6, 70.4, 69.9, 69.4, 69.1, 67.5, 67.1, 63.2.

Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-D-glucopyranose (73): Disaccharide **72** (3.4 g, 2.9 mmol) was stirred with 2-aminoethanol (500 mg, 8.2

mmol) and DMSO (0.5 mL) in ethyl acetate (50 mL) for 24 hours at room temperature. The mixture was washed with water (3 x 50 mL), dried over sodium sulfate, and concentrated. The resulting syrup was purified by flash column chromatography (CH_2Cl_2 followed by Et_2O) to give **73** as a white solid (1.2 g, 40 % yield). ^{1}H NMR of **73α** (400 MHz, $CDCl_3$): δ 8.08 – 7.77 (m, 14 H), 7.53 – 7.26 (m, 21 H), 6.11 (t, $J = 10.0$ Hz, 1H), 5.90 (t, $J = 9.6$ Hz, 1H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J_1 = 5.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.71 (dd, $J_1 = 6.2$ Hz, $J_2 = 1.4$ Hz, 1H), 4.62 (m, 1H), 4.49 (m, 1H), 4.39 (dd, $J_1 = 6.0$ Hz, $J_2 = 2.2$ Hz, 1H), 4.13 (m, 2H). ^{13}C NMR of **73α** (400 MHz, $CDCl_3$): δ 166.5, 166.0, 165.9, 165.7, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.1, 130.0, 130.0, 130.0, 129.8, 129.7, 129.6, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 102.5, 90.4, 77.4, 72.8, 72.6, 72.4, 72.3, 70.2, 69.9, 69.5, 69.0, 63.0.

Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-D-glucopyranose (76): Disaccharide **73** (1.2 g, 1.2 mmol), potassium carbonate (0.42 g, 3.0 mmol), and trichloroacetonitrile (4 mL) were stirred 48 hours in CH_2Cl_2 (20 mL) at room temperature. The resulting suspension was filtered through silica gel using a 1:1 mixture of CH_2Cl_2 and Et_2O (150 mL). The filtrate was concentrated to give *O*-(Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-tri-*O*-benzoyl-α-D-glucopyranosyl)-trichloroacetimdate **(74)** as a pale yellow solid (1.3 g, 94 % yield) which was used immediately in the next reaction without further purification. ^{1}H NMR of **74** (300 MHz, $CDCl_3$): 8.36 (s, 1H), 7.98 – 7.73 (m, 14H), 7.51 – 7.26 (m, 21H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.14 (t, $J = 9.8$ Hz, 1H), 5.88 (t, $J = 10.1$ Hz, 1H), 5.63 – 5.32 (m, 4H), 4.98 (d, $J = 4.1$ Hz, 1H), 4.40 – 4.61 (m, 3H), 4.13 (m, 2H), 3.84 (m, 1H). Trichloroacetimidate **74**

(0.66 g, 0.54 mmol) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (0.12 g, 0.45

mmol) were stirred under an atmosphere of nitrogen in CH_2Cl_2 (20 mL) with 3Å

molecular sieves for 1 hour. The solution was cooled in an icebath for 30 minutes before

$BF_3 \cdot OEt_2$ (1 mL) was added and stirred for 1 hour in the icebath followed by 1 hour at

room temperature. The solution was added to ice-cold saturated aqueous sodium

bicarbonate solution (50 mL) with vigorous stirring and then extracted with ether (3 x 50

mL). The organic layers were combined and dried over sodium sulfate. The solvent was

removed under reduced pressure and the residue was purified by flash column

chromatography (CH_2Cl_2: THF 9:1 to 0:1) to give tetra-*O*-benzoyl-β-D-glucopyranosyl-

(1→3)-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-1,2:5,6-di-*O*-isopropylidene-α-D-

glucofuranose **(75)** as a white solid (0.21 g, 36 % yield) which was used immediately in

the next reaction. ^{1}H NMR of **75** (400 MHz, CDCl$_3$): δ 8.04 (d, *J* = 4.5 Hz, 2H), 7.93 (d,

J = 4.3 Hz, 2H), 7.88 – 7.71 (m, 8H), 7.53 – 7.19 (m, 21 H), 5.95 (d, *J* = 3.2 Hz, 1H),

5.87 (t, *J* = 9.6 Hz, 1H), 5.78 (t, *J* = 9.5 Hz, 1H), 5.63 (t, *J* = 9.7 Hz, 1H), 5.52 (dd, *J$_1$* =

4.9 Hz, *J$_2$* = 4.0 Hz, 1H), 5.36 (dd, *J$_1$* = 4.8 Hz, *J$_2$* = 4.0 Hz, 1H), 5.26 (t, *J* = 9.4 Hz, 1H),

4.99 (d, *J* = 3.8 Hz, 1H), 4.67 (d, *J* = 3.9 Hz, 1H), 4.64 (dd, *J$_1$* = 6.1 Hz, *J$_2$* = 1.2 Hz, 1H),

4.49 (d, *J* = 1.6 Hz, 1H), 4.44 (dd, *J$_1$* = 6.1 Hz, *J$_2$* = 2.6 Hz, 1H), 4.33 (br s, 1H), 4.13 (m,

1H), 4.12 (m, 3H), 3.92 (m, 2H), 3.83 (m, 2H), 1.39 (s, 6H), 1.24 (s, 6H). Trisaccharide

75 (21 mg, 0.16 mmol) was stirred in THF (2 mL) and a 1:1 mixture of water and

trifluoroacetic acid (10 mL) at 40°C for 24 hours. The solution was cooled and added to

water (100 mL). Once the resulting oil solidified, it was filtered and washed with water

to give **76** as a white solid (130 mg, 67 % yield). ^{1}H NMR of **76** (400 MHz, CDCl$_3$): δ

8.04 (d, *J* = 3.6 Hz, 2H), 7.92 – 7.74 (m, 12H), 7.52 – 7.23 (m, 21H), 5.92 (t, *J* = 9.6 Hz,

1H), 5.78 (t, J = 9.6 Hz, 1H), 5.66 (t, J = 9.6 Hz, 1H), 5.53 (dd, J_1 = 4.9 Hz, J_2 = 4.2 Hz,

1H), 5.39 (m, 2H), 5.28 (m, 2H), 4.65 (d, J = 4.0 Hz, 1H), 4.64 (d, J = 4.8 Hz, 1H), 4.47

(dd, J_1 = 6.1 Hz, J_2 = 2.4 Hz, 1H), 4.17 (m, 1H), 4.04 – 3.92 (m, 4H), 3.84 – 3.80 (m,

3H), 3.63 – 3.56 (m, 2H). ^{13}C NMR of **76** (400 MHz, CDCl$_3$): δ 166.8, 166.5, 165.9,

165.7, 165.5, 165.4, 133.8, 133.7, 133.5, 130.1, 130.0, 129.9, 129.7, 129.4, 128.9, 128.7,

128.6, 128.5, 101.6, 101.2, 92.9, 77.4, 75.1, 74.7, 73.4, 72.6, 72.5, 72.0, 71.9, 70.4, 70.2,

69.9, 69.8, 69.1, 67.9, 63.2.

Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-tetra-*O*-benzoyl-D-glucopyranose (77): Trisaccharide **76** (130 mg, 0.11 mmol)

was refluxed in pyridine (5 mL) for 30 minutes. The resulting solution was added to

benzoyl chloride (2 mL) that had previously been heated to 65°C. The mixture was

cooled to room temperature and then water (50 mL) was added. The resulting oil was

separated from the aqueous layer and dissolved in CH$_2$Cl$_2$ (50 mL). The solution was

washed with water (3 x 50 mL), dried over sodium sulfate and concentrated. The residue

was purified by flash column chromatography (5:1 CH$_2$Cl$_2$:Et$_2$O) to give **77** as a white

solid (86 mg, 49 % yield). ^{1}H NMR (400 MHz, CDCl$_3$): 8.08 – 7.70 (m, 22H), 7.54 –

7.15 (m, 33H), 6.20 (t, J = 10.0 Hz, 1H), 6.15 (t, J = 10.0 Hz, 1H), 5.96 (d, J = 4.2 Hz,

1H), 5.77 (t, J = 11.2 Hz, 1H), 5.77 (m, 1H), 5.64 (t, J = 9.6 Hz, 1H), 5.61 (t, J = 9.6 Hz,

1H), 5.47 (m, 1H), 5.22 (d, J = 4.0 Hz, 1H), 5.19 (d, J = 3.8 Hz, 1H), 5.04 (m, 1H), 4.90

(t, J = 9.2 Hz, 1H), 4.66 – 4.57 (m, 2H), 4.48 – 4.41 (m, 2H), 4.26 (dt, J_1 = 5.0 Hz, J_2 =

4.0 Hz, 1H), 4.03 – 3.76 (m, 3H), 3.46 (m, 1H). ^{13}C NMR of **77α** (400 MHz, CDCl$_3$): δ

166.4, 166.1, 166.0, 166.0, 165.8, 165.6, 165.5, 165.4, 165.2, 165.0, 164.7, 134.0, 133.9,

133.7, 133.5, 133.4, 133.3, 133.2, 133.1, 130.5, 130.3, 130.1, 130.0, 129.9, 129.6, 129.5,

129.4, 129.3, 129.2, 129.1, 129.1, 128.9, 128.8, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 101.6, 93.1, 90.3, 74.8, 74.6, 73.9, 73.0, 71.9, 71.7, 71.2, 70.8, 70.6, 70.4, 70.3, 69.9, 69.8, 68.6, 68.1. ESI m/z = 1671.7 $(M+Na)^+$.

Penta-*O*-acetyl-D-glucopyranose (78)[80]: D-glucose (20 g, 110 mmol) was refluxed in pyridine (150 mL) for 1 hour. Acetic anhydride (100 mL) was added and the mixture was refluxed an additional 2 hours. The resulting solution was cooled to room temperature, poured over ice (300 mL) and stirred until the product solidified, about 2 hours. The solid was filtered, washed with water, and recrystallized from ethyl acetate/hexanes to give **78** as a white solid (36 g, 83 % yield). **78β**: D-glucose (5.0 g, 28 mmol), sodium acetate (4.0 g, 50 mmol), and acetic anhydride (30 mL) were stirred at 100°C for 3 hours. The mixture was cooled to room temperature, poured over ice (150 mL) and stirred for 2 hours. The resulting white solid was filtered and washed with water and recrystallized from methanol to give **78β** as a white solid (3.7 g, 38 % yield). ^{1}H NMR of **78β** (400 MHZ, CDCl$_3$): δ 5.72 (d, J = 4.0 Hz, 1H), 5.26 (t, J = 9.2 Hz, 1H), 5.14 (t, J = 8.8 Hz, 1H), 5.13 (t, J = 10.0 Hz, 1H), 4.30 (dd, J_1 = 6.2 Hz, J_2 = 2.2 Hz, 1H), 4.12 (dd, J_1 = 6.3 Hz, J_2 = 1.0 Hz, 1H), 3.84 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H). ^{13}C NMR of **78β** (400 MHz, CDCl$_3$): δ 170.7, 170.3, 169.8, 169.5, 168.9, 89.1, 69.9, 69.2, 67.9, 61.5, 20.9, 20.8, 20.7, 20.6, 20.5.

2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (79)[76]: D-glucose pentaacetate **78** (10 g, 26 mmol) and 2-aminoethanol (3.9 g, 64 mmol) were dissolved in ethyl acetate (250 mL) and DMSO (2.5 mL) and stirred at room temperature overnight. The mixture was washed with water (3 x 150 mL), dried over sodium sulfate and concentrated to give **79** as colorless syrup (6.2 g, 70% yield). This product was used immediately in the further

reactions without further purification. ^{1}H NMR of **79α** (300 MHz, CDCl$_3$): δ 5.51 (t, J = 9.9 Hz, 1H), 5.45 (d, J = 1.5 Hz, 1H), 5.06 (t, J = 9.9 Hz, 1H), 4.89 (dd, J_1 = 5.03 Hz, J_2 = 1.8 Hz, 1H), 4.26 – 4.06 (m, 3H), 2.07 (s, 3H), 2.06, (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H). ^{13}C NMR of **79α** (400 MHz, CDCl$_3$): δ 171.2, 170.5, 170.4, 169.9, 95.3, 73.0, 72.5, 71.9, 68.4, 68.2, 20.8, 20.7, 20.6, 20.6. ^{13}C NMR of **79β** (400 MHz, CDCl$_3$): δ 171.2, 170.5, 170.4, 169.9, 90.0, 71.2, 70.0, 68.6, 67.0, 62.0, 20.8, 20.7, 20.6, 20.6.

1,3,4,6-Tetra-O-acetyl-α-D-glucopyranose (81)[82]: Tetraacetate **79** (3.5 g, 10 mmol) was stirred in 80 mL chloroform with 3 Å molecular sieves under an atmosphere of nitrogen for 1 hour. (1-Chloro-2-methylpropenyl)-dimethylamine (2.0 mL, 11 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was filtered and the solvent was removed under reduced pressure to give tetra-*O*-acetyl-D-glucopyranosyl chloride **80** as a colorless syrup which was used immediately in the following reaction without further purification. ^{1}H NMR of **80α** (300 MHz, CDCl$_3$): δ 5.26 (br d, J = 4.1 Hz, 1H), 5.18 – 5.03 (m, 2H), 4.22 (dd, J_1 = 6.3 Hz, J_2 = 2.1 Hz, 1H), 4.13 (d, J = 0.8 Hz, 1H), 4.05 (m, 1H), 3.78 (m, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H). Glucosyl chloride **80** was dissolved in acetone (50 mL). DMF (2 mL) and water (5 mL) were added and the solution was stirred 5 hours at room temperature. Pyridine (2 mL) was added and the solvents were removed under reduced pressure. The resulting syrup was dissolved in ethyl acetate (100 mL), washed with water (2 x 100 mL), dried over sodium sulfate and concentrated. The product was crystallized from ether/hexanes to give **59** as colorless needles (0.96 g, 27 % yield in 2 steps from **79**). ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.22 (d, J = 1.5 Hz, 1H), 5.25 (t, J = 7.2 Hz, 1H), 5.09 (t, J = 7.2 Hz, 1H), 4.26 (dd, J_1 = 4.8 Hz, J_2 = 1.7 Hz, 1H), 4.05 – 3.99 (m, 2H), 3.88, (dd, J_1 = 3.9 Hz, J_2 = 1.5

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Hz, 1H), 2.18 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (300 MHz, CDCl$_3$): δ 171.7, 170.9, 169.7, 169.4, 91.5, 73.3, 70.0, 69.8, 67.6, 61.8, 21.2, 21.0, 20.9, 20.8.

Isopropyl tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (92): Penta-*O*-acetyl-β-D-glucopyranose **78β** (3.7 g, 9.4 mmol) and propane-2-thiol (1.0 mL, 13 mmol) were stirred at room temperature in CH$_2$Cl$_2$ with 3 Å molecular sieves for 30 minutes under an atmosphere of nitrogen. BF$_3$·OEt$_2$ (3.5 mL) was added and the mixture was stirred an additional 2 hours. The molecular sieves were filtered and the filtrate was added to 5 % NaHCO$_3$ (50 mL) with vigorous stirring. The organic layer was separated, washed with 5 % NaHCO$_3$ (3 x 50 mL), and dried over sodium sulfate. The solvent was removed under reduced pressure and the resulting solid was used immediately in the next reaction without further purification. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.11 (t, *J* = 9.6 Hz, 1H), 4.94 (t, *J* = 9.6 Hz, 1H), 4.86 (t, *J* = 9.6 Hz, 1H), 4.49 (d, *J* = 5.2 Hz, 1H), 4.11 (dd, *J$_1$* = 6.2 Hz, *J$_2$* = 2.8 Hz, 1H), 4.00 (dd, *J$_1$* = 6.0 Hz, *J$_2$* = 1.2 Hz, 1H), 3.61 (m, 1H), 3.05 (m, 1H), 1.94 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H), 1.19 (d, *J* = 2.2 Hz, 3H), 1.17 (d, *J* = 2.2 Hz, 3H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 170.6, 170.2, 169.4, 169.3, 83.3, 75.6, 73.9, 70.1, 68.4, 62.2, 35.6, 24.0, 23.7, 20.7, 20.7, 20.5.

Isopropyl 1-thio-β-D-glucopyranoside (93): Thioglucopyranoside **92** was dissolved in 2:1 MeOH:CH$_2$Cl$_2$ (45 mL). Sodium hydroxide (1N) was added to a pH ~ 9-10. The solution was stirred at room temperature 6 hours, neutralized with acidic Dowex resin, and filtered. The solvents were removed under reduced pressure and the resulting solid was co-evaporated with toluene (3 x 10 mL) and used immediately in the following reaction without further purification. ^{1}H NMR (300 MHz, DMSO- D$_6$): δ 4.31 (d, *J* = 4.8

Hz, 1H), 3.65 (dd, J_1 = 5.9 Hz, J_2 = 0.6 Hz, 1H), 3.40 (dd, J_1 = 5.9 Hz, J_2 = 2.9 Hz, 1H), 3.18 – 2.93 (m, 5H), 1.22 (dd, J_1 = 3.4 Hz, J_2 = 2.1 Hz, 6H). ^{13}C NMR (400 MHz, DMSO-D$_6$): δ 84.5, 80.9, 78.3, 73.3, 70.1, 61.3, 33.4, 23.9, 23.8.

Isopropyl (*R*)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (84)[86]:

Thioglucopyranoside **93** and dimethoxymethylbenzene (1.7 g, 11 mmol) were dissolved in DMF (15 mL). The solution was cooled to 0°C and CSA was added to a pH ~ 2-3. After the mixture was stirred at room temperature overnight, it was neutralized with triethylamine. Ethyl acetate (100 mL) was added and the resulting solution was washed with brine (2 x 100 mL), dried over sodium sulfate. The solvents were removed under reduced pressure and the product was purified by flash column chromatography (1:1 ethyl acetate: petroleum ether) to give **84** as a white solid (1.6 g, 51 % yield in 3 steps from **78β**). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.55 – 7.36 (m, 5H), 5.55 (s, 2H), 4.54 (d, *J* = 5.0 Hz, 1H), 4.35 (dd, J_1 = 5.2 Hz, J_2 = 2.3 Hz, 1H), 3.88 – 3.75 (m, 2H), 3.62 – 3.45 (m, 3H), 3.24 (m, 1H), 1.36 (d, *J* = 3.3 Hz, 6H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 129.4, 128.5, 126.6, 126.5, 102.1, 86.5, 81.2, 72.3, 72.3, 68.9, 63.6, 36.5, 24.4, 23.8.

Isopropyl tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→2)]-(*R*)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (94):

Trichloroacetimidate donor **69** (3.7 g, 5.0 mmol) and thioglucopyranoside **84** (1.5 g, 4.5 mmol) were co-evaporated with toluene (3 x 10 mL), dried under reduced pressure overnight, and then stirred under an atmosphere of nitrogen in anhydrous CH$_2$Cl$_2$ (50 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0°C before TMSOTf (91 μL, 0.5 mmol) was added. The reaction was stirred at 0°C for 5 hours, neutralized with triethylamine (0.3 mL), and filtered. The solvents were removed under

reduced pressure and the product was purified by gravity column chromatography (2:3 ethyl acetate: petroleum ether) to give isopropyl tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-(R)-4,6-O-benzylidene-1-thio-β-D-glucopyranoside **(85)** which was used immediately in the following reaction. ^{1}H NMR of **85** (400 MHz, CDCl$_3$): δ 8.14 – 7.82 (m, 10H), 7.23 – 7.57 (m, 15 H), 5.92 (t, J = 9.2 Hz, 1H), 5.71 (t, J = 9.2 Hz, 1H), 5.57 (s, 2H), 5.56 (dd, J_1 = 3.6 Hz, J_2 = 1.0 Hz, 1H), 5.22 (d, J = 4.0 Hz, 1H), 4.49 (dd, J_1 = 5.9 Hz, J_2 = 1.8 Hz, 1H), 4.44 (d, J = 5.0 Hz, 1H), 4.33 (t, J = 12.0 Hz, 1H), 4.32 (t, J = 12.4 Hz, 1H), 3.94 (m, 1H), 3.90 (t, J = 8.4 Hz, 1H), 3.77 (t, J = 10.8 Hz, 1H), 3.71, (t, J = 9.6 Hz, 1H), 3.49 – 3.44 (m, 2H), 3.14 (m, 1H), 1.24 (d, J = 2.9 Hz, 6H). Disaccharide **85** and trichloroacetimidate donor **69** (3.0 g, 4.0 mmol) were co-evaporated with toluene (3 x 10 mL), dried under reduced pressure overnight, and then stirred under an atmosphere of nitrogen in anhydrous CH$_2$Cl$_2$ (50 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0°C before TMSOTf (91 μL, 0.5 mmol) was added. The reaction was stirred at 0°C for 3 hours, neutralized with triethylamine (0.5 mL), and filtered. The solvents were removed under reduced pressure and the product was purified by gravity column chromatography (2:3 ethyl acetate: petroleum ether) and then recrystallized from methanol to give **94** (1.6 g, 23 % yield in 2 steps from **84**). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.29 – 7.21 (m, 45H), 5.83 (t, J = 9.2 Hz, 1H), 5.79 (t, J = 9.6 Hz, 1H), 5.61 – 5.52 (m, 3H), 5.51 (s, 2H), 5.43 (t, J = 10.0 Hz, 1H), 4.97 (d, J = 3.8 Hz, 1H), 4.80 (d, J = 4.2 Hz, 1H), 4.49 (d, J = 4.8 Hz, 1H), 4.37 (dd, J_1 = 5.9 Hz, J_2 = 1.6 Hz, 1H), 4.28 – 4.18 (m, 4H), 4.00 (t, J = 8.8 Hz, 1H), 3.90 (t, J = 9.6 Hz, 1H), 3.69 (t, J = 10.8 Hz, 1H), 3.62 (t, J = 9.2 Hz, 1H), 3.30 (m, 1H), 3.07 (m, 1H), 2.79 (m, 1H), 2.67 (m, 1H), 1.22 (d, J = 3.2 Hz, 3H), 1.09, (d, J = 3.4 Hz, 3H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 166.5, 166.1, 166.0,

165.5, 165.4, 165.3, 163.9, 133.8, 133.7, 133.6, 133.5, 133.3, 130.3, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.7, 129.6, 129.6, 129.5, 129.4, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.9, 128.7, 128.6, 128.5, 128.5, 128.3, 128.3, 126.2, 101.4, 100.0, 99.6, 96.1, 92.1, 84.0, 80.9, 78.6, 77.4, 76.7, 73.1, 72.5, 71.5, 71.3, 70.8, 69.2, 68.7, 63.6, 62.2, 34.3, 23.8, 23.6.

1,2,3,6-Tetra-*O*-benzoyl-α-D-galactopyranose (90)[90]: Under an atmosphere of nitrogen, benzoyl chloride (16 g, 90 mmol) in pyridine (5 mL) was added over 30 minutes to a suspension of D-galactose (5.0 g, 28 mmol) in pyridine (100 mL) at 0°C. The mixture was stirred 1 hour before ice (200 mL) was added and extracted with CH_2Cl_2 (3 x 100 mL). The organic layers were combined and washed with 2M H_2SO_4 (100 mL), followed by saturated aqueous $NaHCO_3$ (100 mL), and finally water (100 mL), and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue was purified by gravity column chromatography (4:1 toluene: ethyl acetate) to give **90** as a colorless syrup (11 g, 65 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 – 7.25 (m, 20H), 6.81 (d, *J* = 2.0 Hz, 1H), 6.05 (dd, J_1 = 5.4 Hz, J_2 = 1.8 Hz, 1H), 5.86 (dd, J_1 = 5.3 Hz, J_2 = 1.5 Hz, 1H), 4.76 (dd, J_1 = 5.4 Hz, J_2 = 3.3 Hz, 1H), 4.55 – 4.44 (m, 2H), 4.49 (br s, 1H), 2.79 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 166.8, 166.2, 165.8, 164.8, 133.9, 133.7, 133.5, 130.1, 130.0, 130.0, 129.9, 129.8, 129.4, 129.3, 129.1, 129.0, 128.8, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 91.0, 71.1, 70.9, 67.7, 67.4, 63.0.

Tetra-*O*-acetyl-α-D-glucopyranosyl-(1→4)-tetra-*O*-benzoyl-α-D-galactopyranose (96): Isopropyl thioglucopyranoside donor **92** (1.0 g, 2.5 mmol) and galactopyranosyl acceptor **90** were co-evaporated with toluene (3 x 10 mL), dried under reduced pressure overnight, and then stirred under an atmosphere of nitrogen in anhydrous CH_2Cl_2 (50

mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to -42°C and *N*-iodosuccinimide (620 mg, 2.7 mmol) and TMSOTf (55 μL, 0.30 mmol). The reaction was stirred at -42°C for 2 hours, neutralized with triethylamine, and filtered. The solvents were removed under reduced pressure and the resulting syrup was purified by gravity column chromatography (3:2 petroleum ether: ethyl acetate) to give **96** as a white solid (1.7 g, 74 % yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.17 – 7.23 (m, 20H), 6.80 (d, *J* = 2.0 Hz, 1H), 6.05 (dd, *J$_1$* = 5.4 Hz, *J$_2$* = 2.0 Hz, 1H), 5.74 (d, *J* = 2.4 Hz, 1H), 5.62 (dd, *J$_1$* = 5.4 Hz, *J$_2$* = 1.6 Hz, 1H), 4.92 (t, *J* = 3.6 Hz, 1H), 4.77, (dd, *J$_1$* = 4.4 Hz, *J$_2$* = 2.2 Hz, 1H), 4.70 (br d, *J* = 1.4 Hz, 1H), 4.62 – 4.54 (m, 2H), 4.46 – 4.38 (m, 2H), 4.10 (m, 1H), 3.96 (dd, *J$_1$* = 6.2 Hz, *J$_2$* = 1.2 Hz, 1H), 3.74 (m, 1H), 2.08 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H).

Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (97)[77]: 2,3,4,6-Tetra-*O*-acetyl –D-glucopyranose **79** (2.0 g, 3.3 mmol), K$_2$CO$_3$ (1.1 g, 8.2 mmol), and trichloroacetonitrile (5 mL) were stirred at room temperature in dichloromethane (20 mL) for 48 hours. The resulting suspension was filtered through silica gel using a 1:1 mixture of CH$_2$Cl$_2$ and Et$_2$O (150 mL). The filtrate was concentrated to give **97** as a pale yellow solid (2.0 g, 83 % yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.67 (s, 1H), 6.52 (d, *J* = 1.8 Hz, 1H), 5.53 (t, *J* = 9.6 Hz, 1H), 5.15 (t, *J* = 10.0 Hz, 1H), 5.10 (dd, *J$_1$* = 6.8 Hz, *J$_2$* = 2.0 Hz, 1H), 4.24 (dd, *J$_1$* = 6.2 Hz, *J$_2$* = 2.0 Hz, 1H), 4.18 (m, 1H), 4.10 (dd, *J$_1$* = 6.0 Hz, *J$_2$* = 1.0 Hz, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 170.9, 170.3, 170.2, 169.8, 161.0, 93.0, 70.2, 70.0, 69.9, 67.9, 61.5, 20.8, 20.8, 20.6.

Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-tetra-*O*-benzoyl-α-D-galactopyranose

(98): Trichloroacetimidate glucosyl donor **97** (900 mg, 1.8 mmol) and galactopyranosyl

acceptor **90** (1.0 g, 1.8 mmol) were co-evaporated with toluene (3 x 10 mL), dried under

reduced pressure overnight, and then stirred under an atmosphere of nitrogen in

anhydrous CH_2Cl_2 (50 mL), with 3Å molecular sieves for 1 hour. The mixture was

cooled to 0°C before TMSOTf (41 μL, 0.18 mmol) was added. The reaction was stirred

for 3 hours at 0°C, quenched with triethylamine, and filtered. The solvents were removed

under reduced pressure and the resulting syrup was purified by gravity column

chromatography (2:3 ethyl acetate: petroleum ether) to give **98** as a colorless syrup (620

mg, 37 % yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.11 (d, *J* = 3.6 Hz, 2H), 8.06 (d, *J* =

3.4 Hz, 2H), 7.95 (d, *J* = 3.6 Hz, 2H), 7.59 (d, *J* = 3.6 Hz, 2H), 7.51 – 7.22 (m, 12 H),

6.72 (d, *J* = 2.0 Hz, 1H), 5.98 (dd, J_1 = 5.4 Hz, J_2 = 1.2 Hz, 1H), 5.86 (dd, J_1 = 5.2 Hz, J_2

= 1.8 Hz, 1H), 5.19 (t, *J* = 8.0 Hz, 1H), 5.15 – 5.04 (m, 2H), 4.76 (d, *J* = 3.8 Hz, 1H),

4.67 (dd, J_1 = 5.8 Hz, J_2 = 2.8 Hz, 1H), 4.58 (br s, 1H), 4.38 (dd, J_1 = 5.8 Hz, J_2 = 3.0 Hz,

1H), 4.28 (m, 1H), 4.00 (dd, J_1 = 4.0 Hz, J_2 = 2.2 Hz, 1H), 3.78 (dd, J_1 = 6.0 Hz, J_2 = 1.2

Hz, 1H), 3.46 (m, 1H). ^{13}C NMR (400 MHz, CDCl$_3$): δ 170.8, 170.5, 169.7, 169.3,

166.0, 165.9, 165.3, 164.6, 134.2, 133.9, 133.5, 133.3, 130.0, 129.9, 129.8, 129.7, 129.3,

128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 100.8, 90.9, 74.0, 72.7, 71.9, 71.3, 70.8, 70.5,

68.1, 67.3, 63.4, 61.4, 20.8, 20.7, 20.6, 20.6.

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APPENDIX

^{1}H and ^{13}C NMR Spectra

Figure 11. ^{1}H NMR of **17** (400 MHz, CDCl$_3$).

17 (Tigogenin)
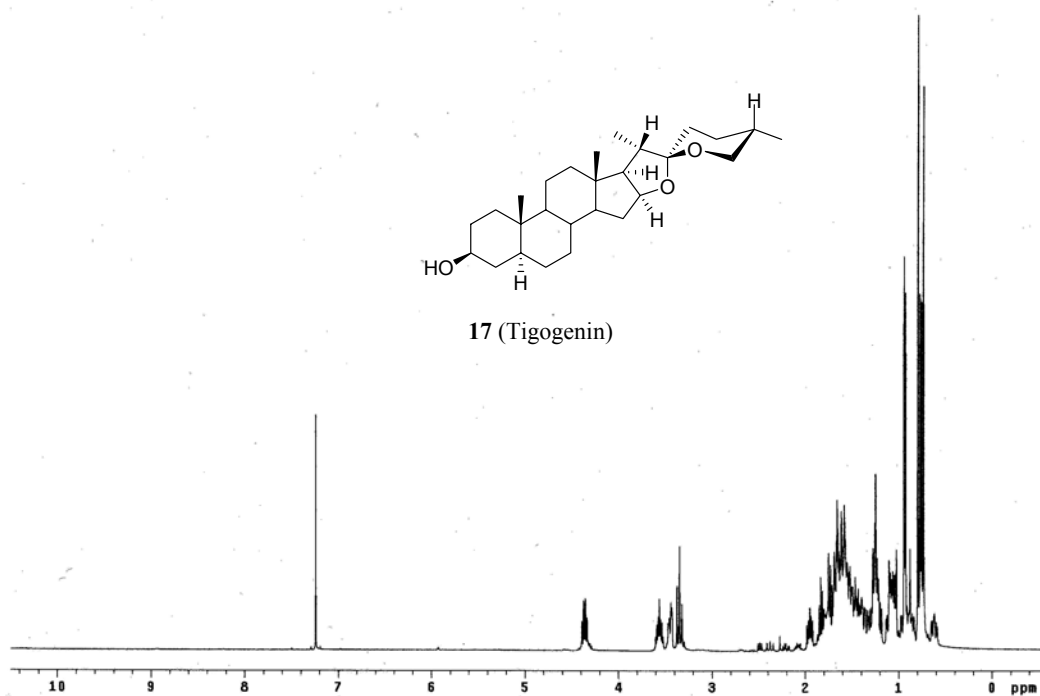
Figure 12. ^{13}C NMR of **17** (400 MHz, CDCl$_3$).

17 (Tigogenin)
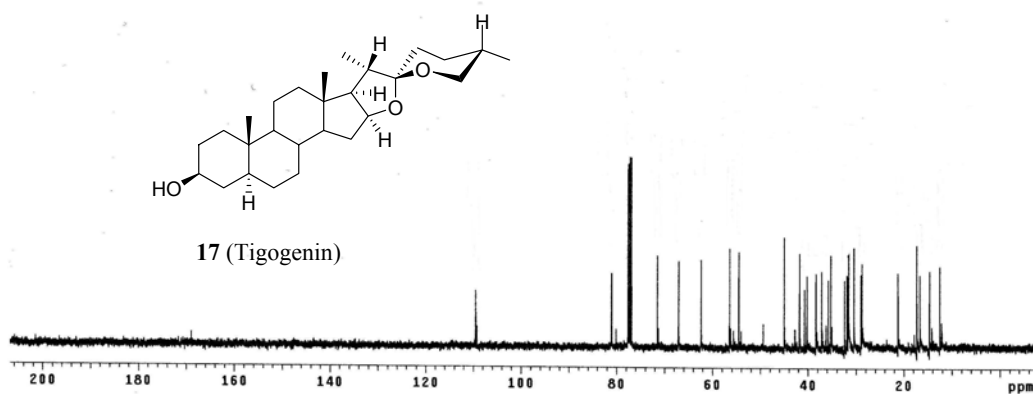
Figure 13. ^{1}H NMR of **26α** (400 MHz, CDCl$_3$).
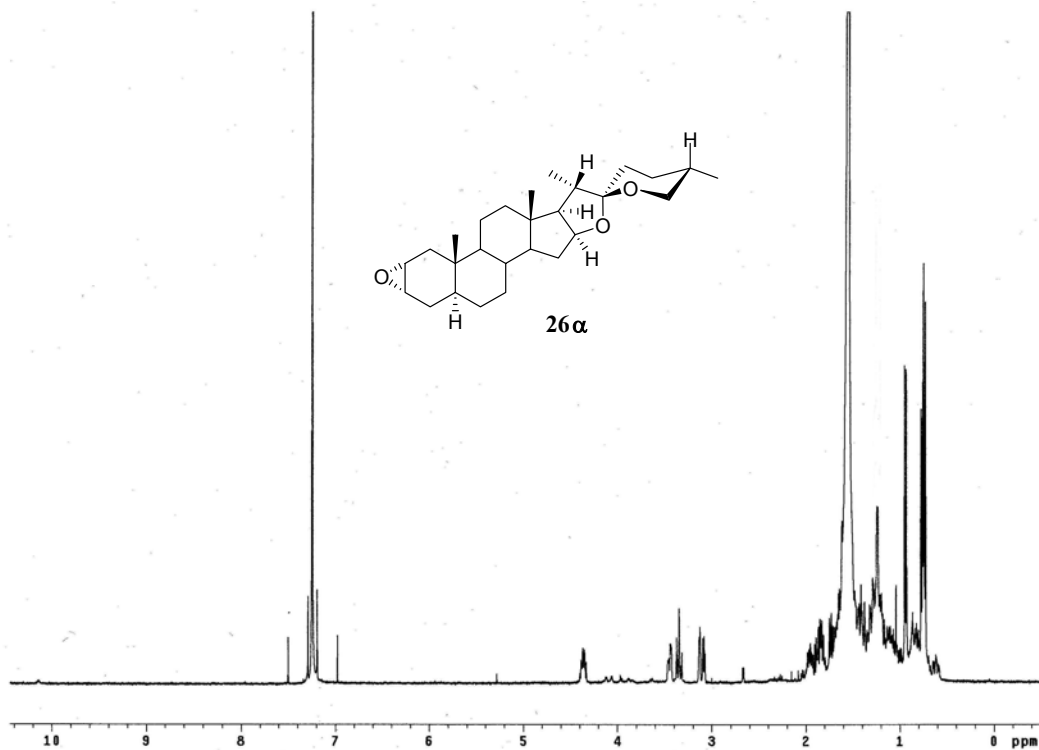
Figure 14. ^{13}C NMR of **26α** (400 MHz, CDCl$_3$).
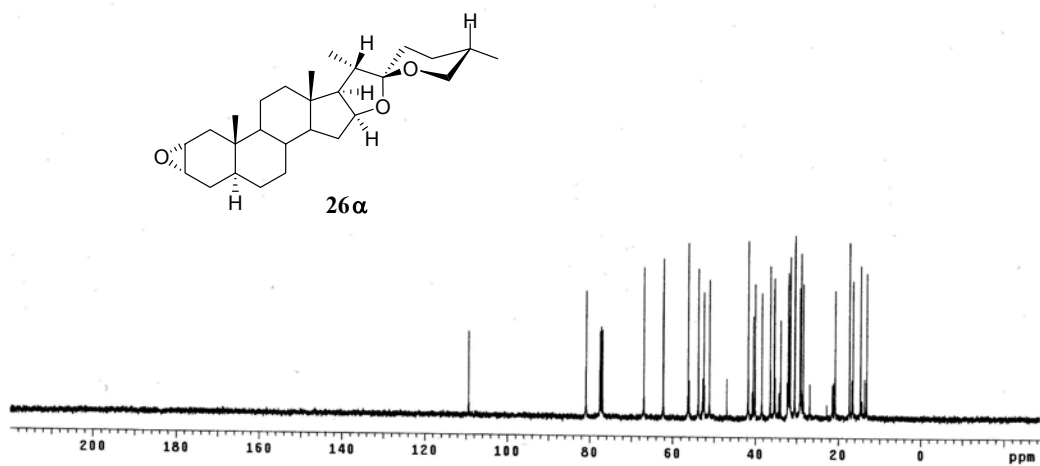
Figure 15. ^{1}H NMR of **26β** (400 MHz, CDCl$_3$).
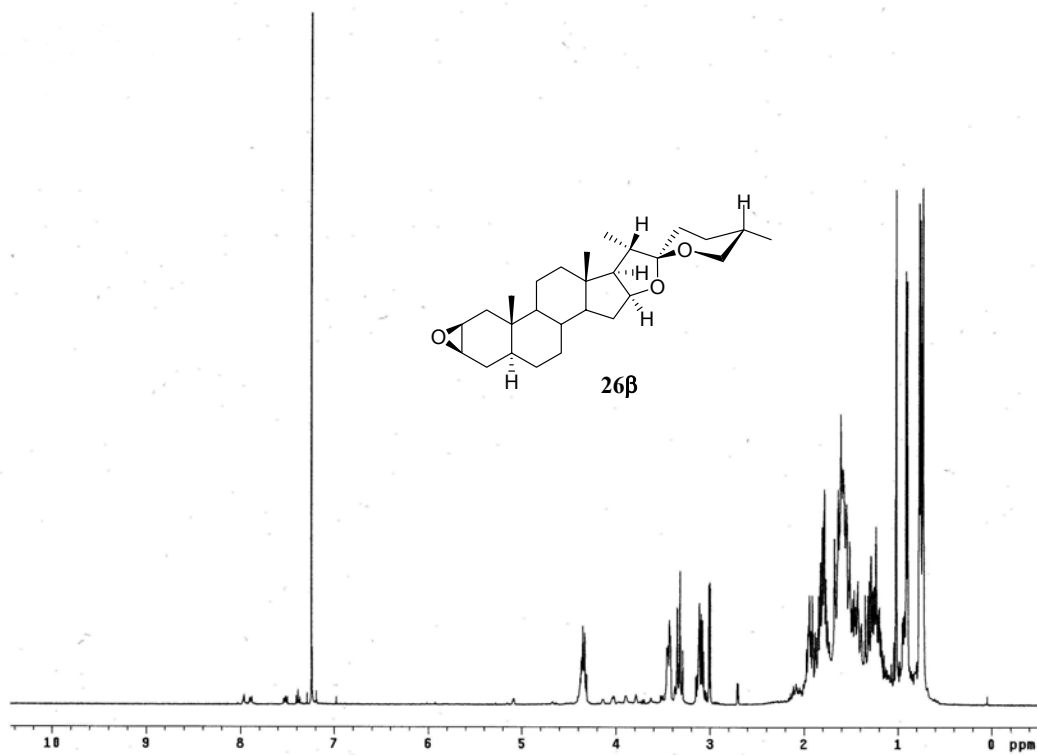
Figure 16. ^{1}H NMR of **27** (400 MHz, CDCl$_3$).
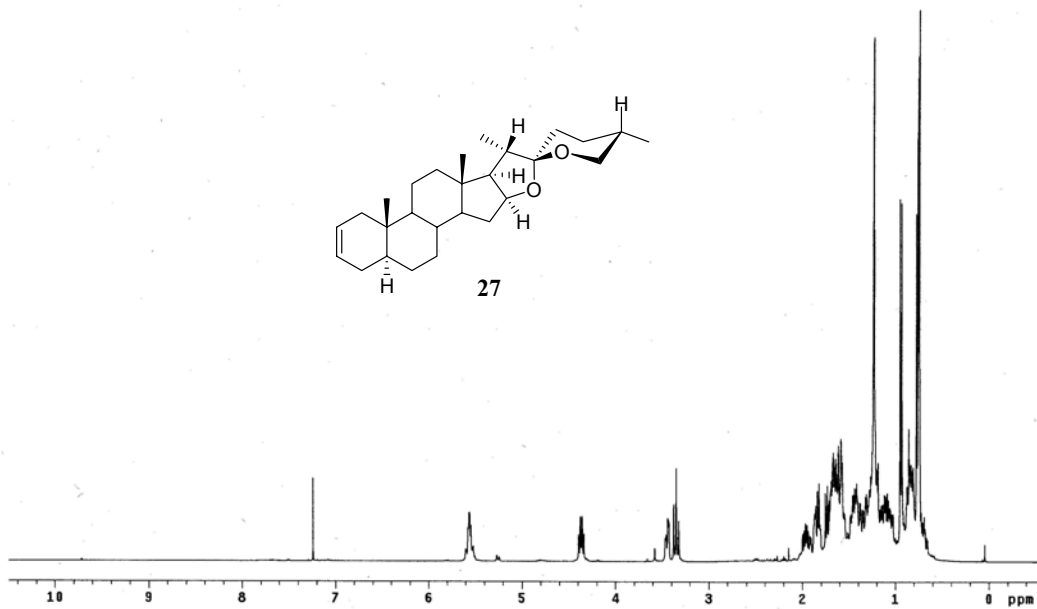
Figure 17. ^{13}C NMR of **27** (400 MHz, CDCl$_3$).
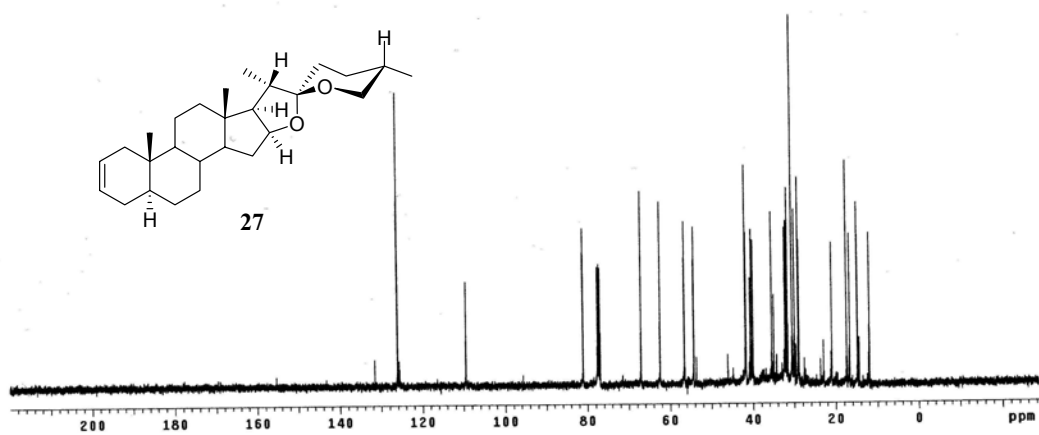
Figure 18. ^{1}H NMR of **28** (500 MHz, CDCl$_3$).
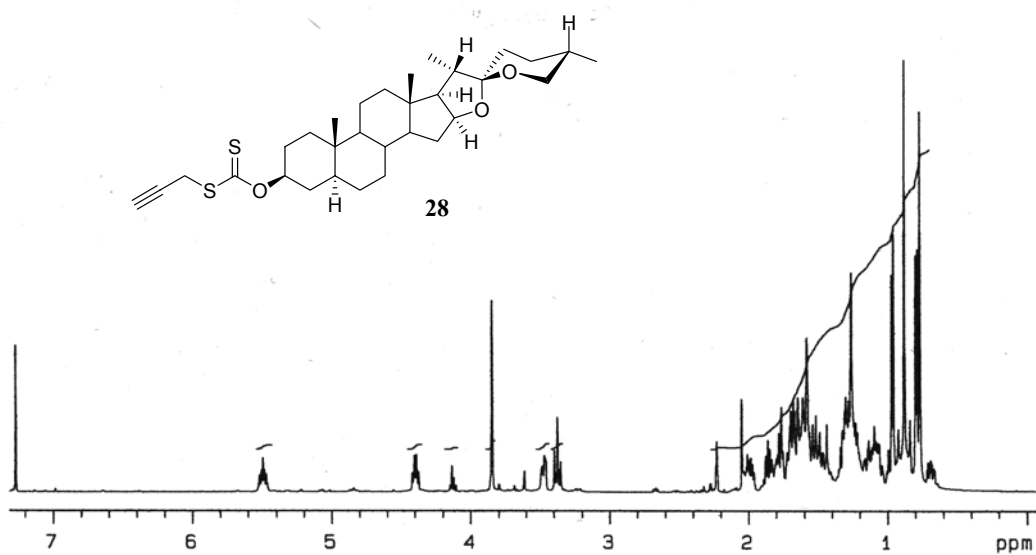
Figure 19. ^{1}H NMR of **29** (400 MHz, CDCl$_3$).
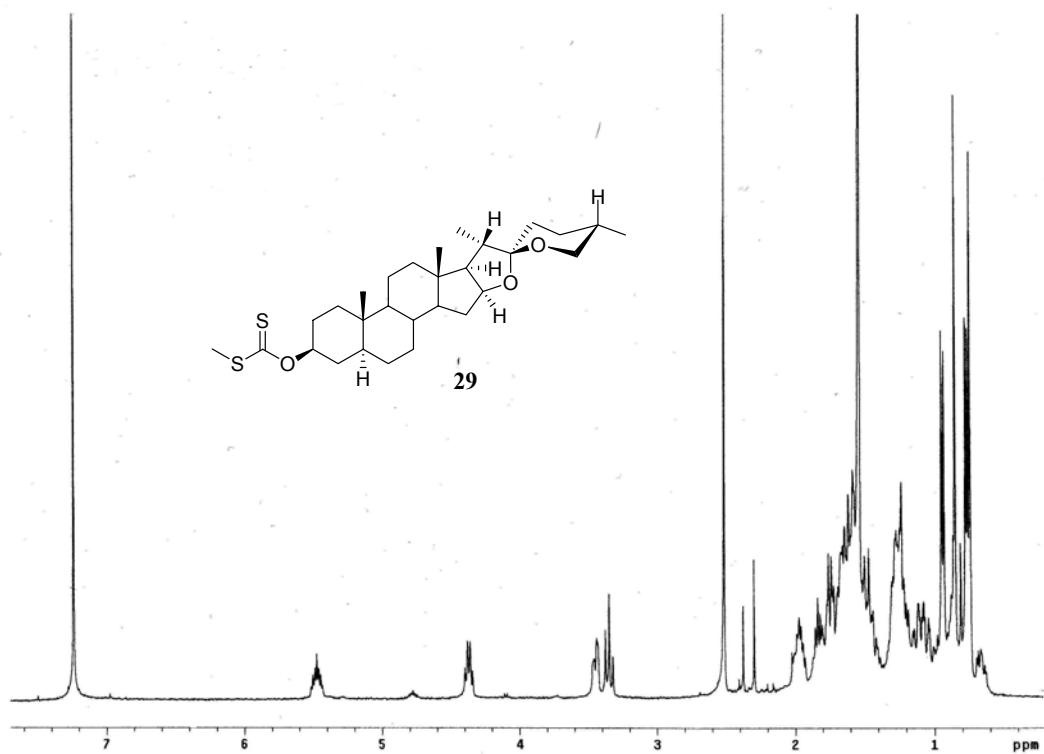
Figure 20. ^{1}H NMR of **32** (400 MHz, CDCl$_3$).
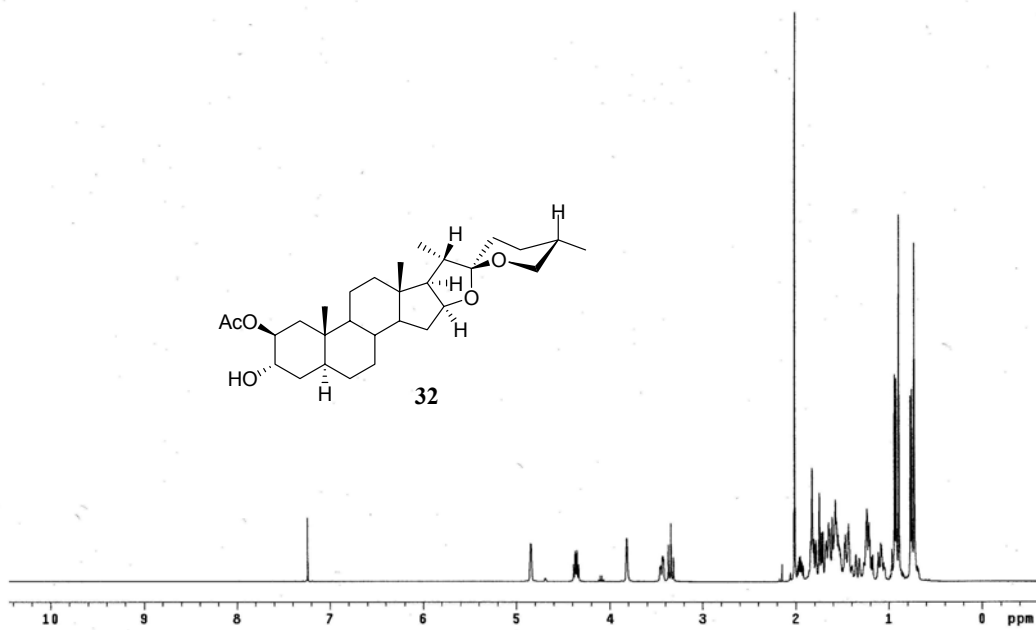
Figure 21. ^{13}C NMR of **32** (400 MHz, CDCl$_3$).
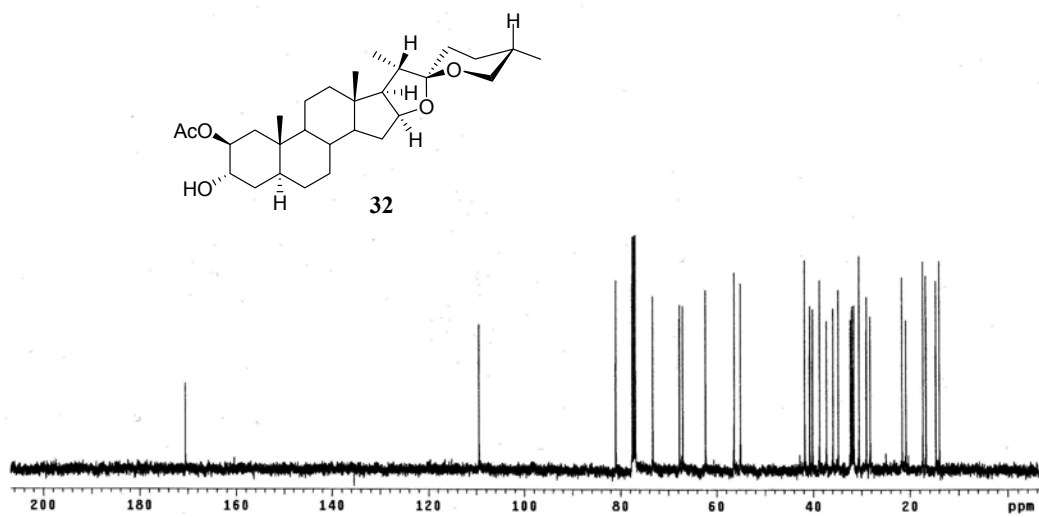
Figure 22. ^{1}H NMR of **34** (400 MHz, CDCl$_3$).
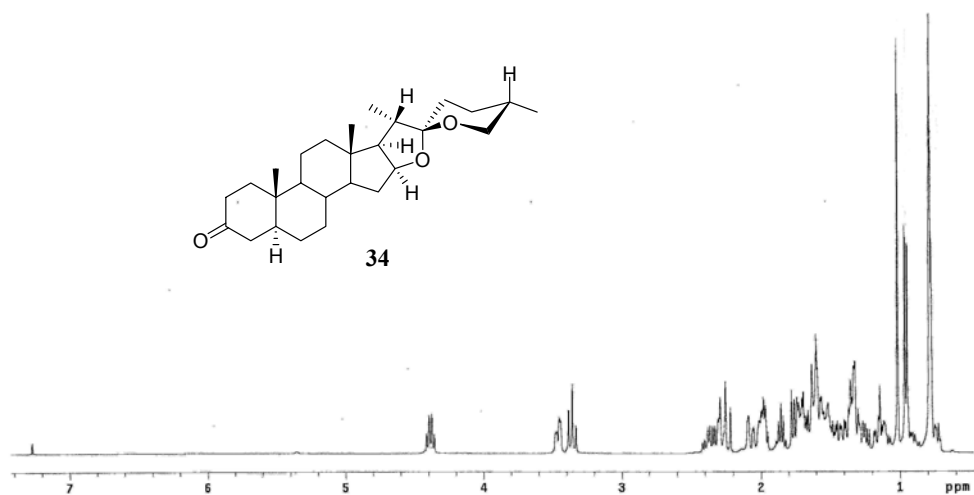
Figure 23. ^{13}C NMR of **34** (400 MHz, CDCl$_3$).
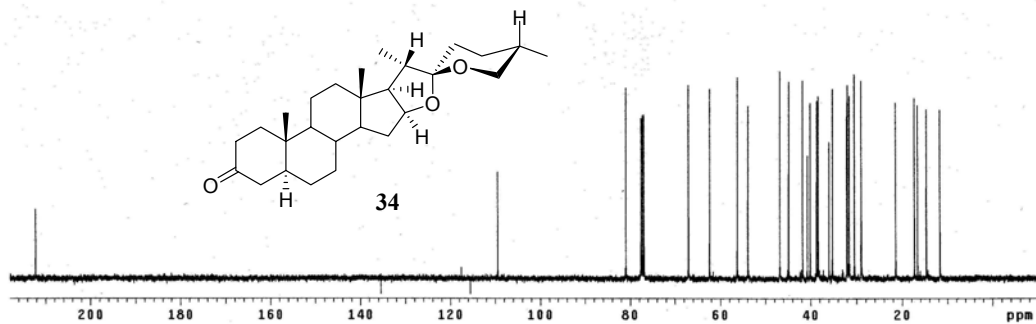
Figure 24. ^1H NMR of **35** (400 MHz, CDCl$_3$).
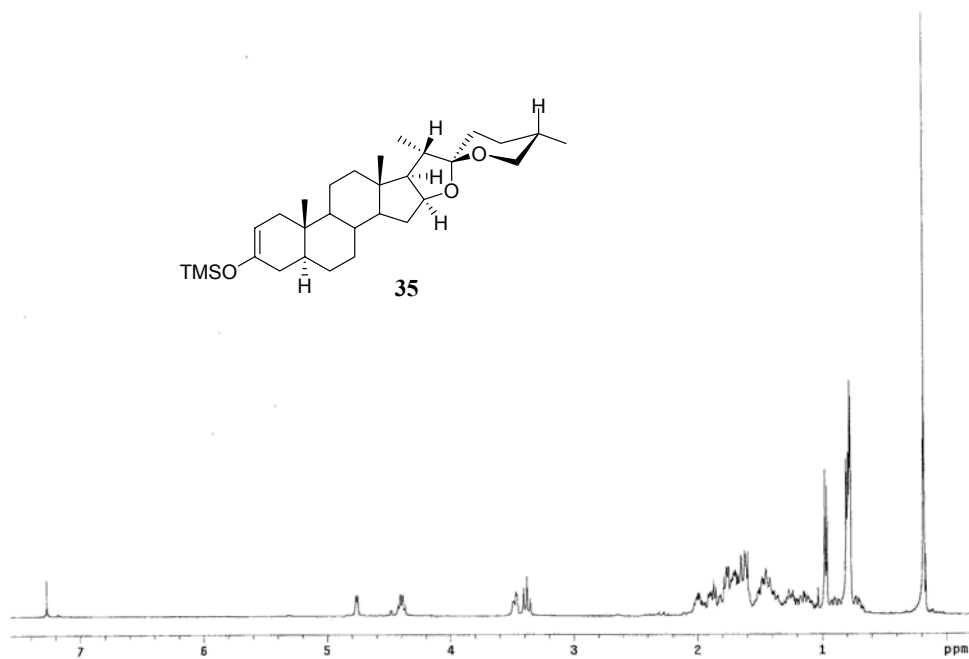
Figure 25. ^{1}H NMR of **41** (400 MHz, CDCl$_3$).
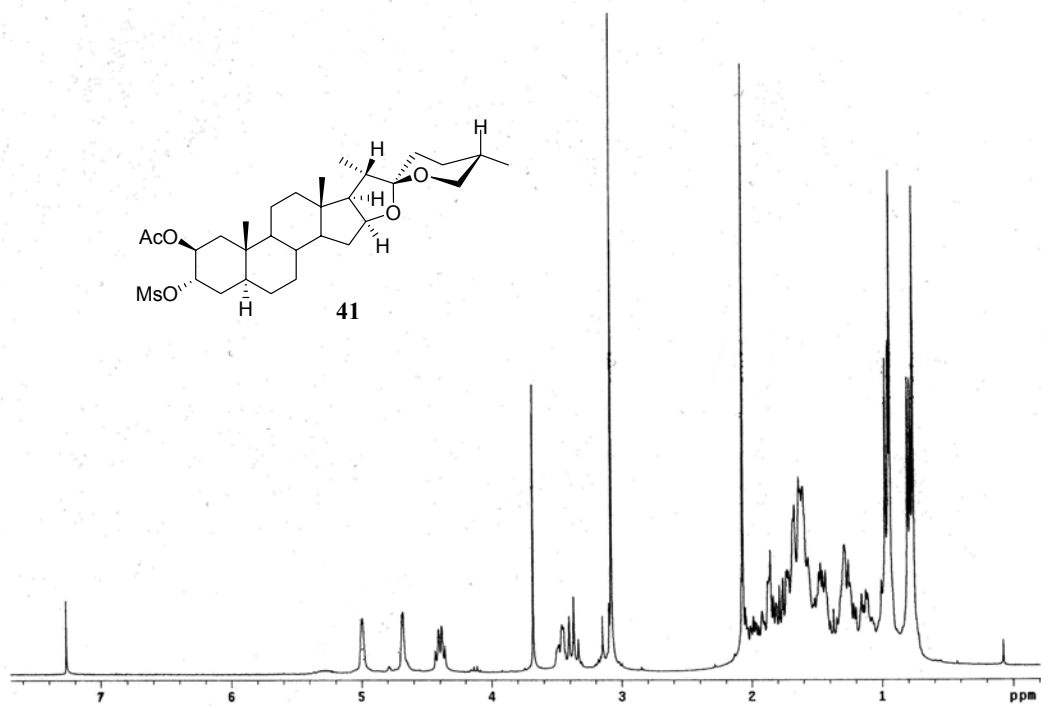
Figure 26. ^{1}H NMR of **42** (400 MHz, CDCl₃).
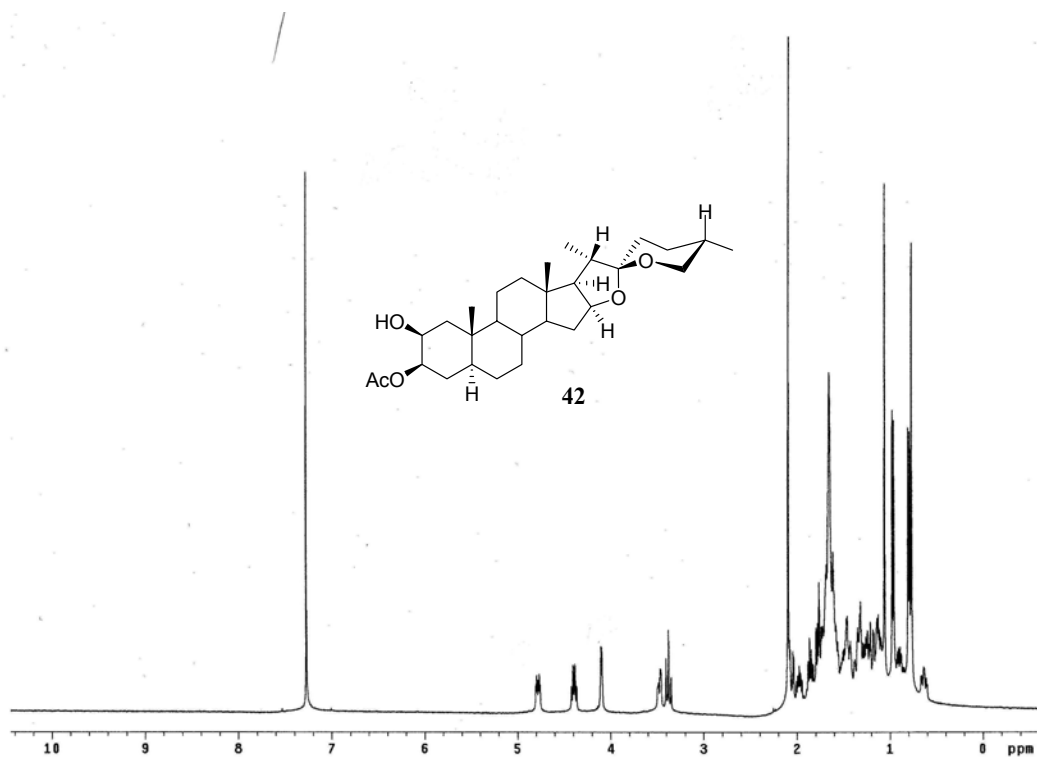
Figure 27. ^{13}C NMR of **42** (400 MHz, CDCl₃).
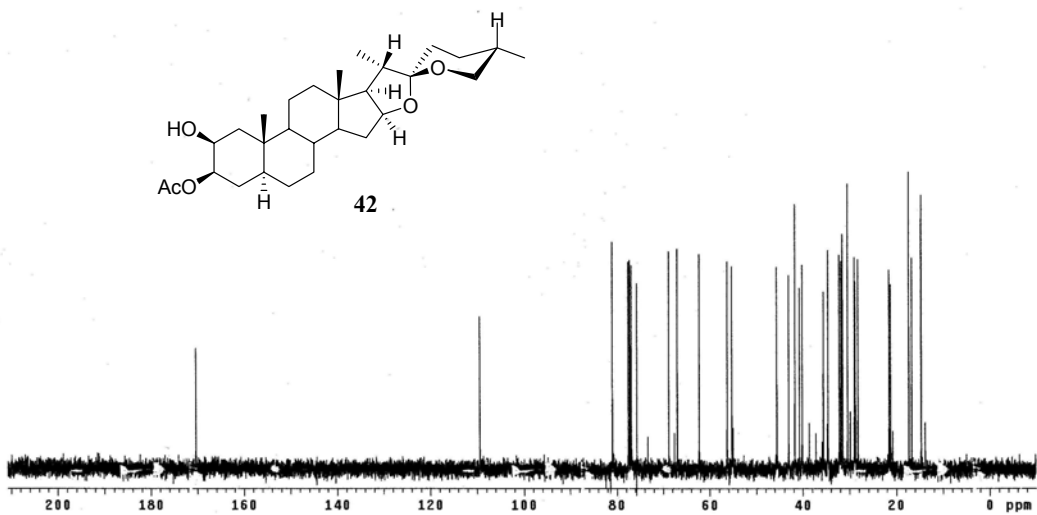
Figure 28. ^{1}H NMR of **43** (400 MHz, CDCl$_3$).
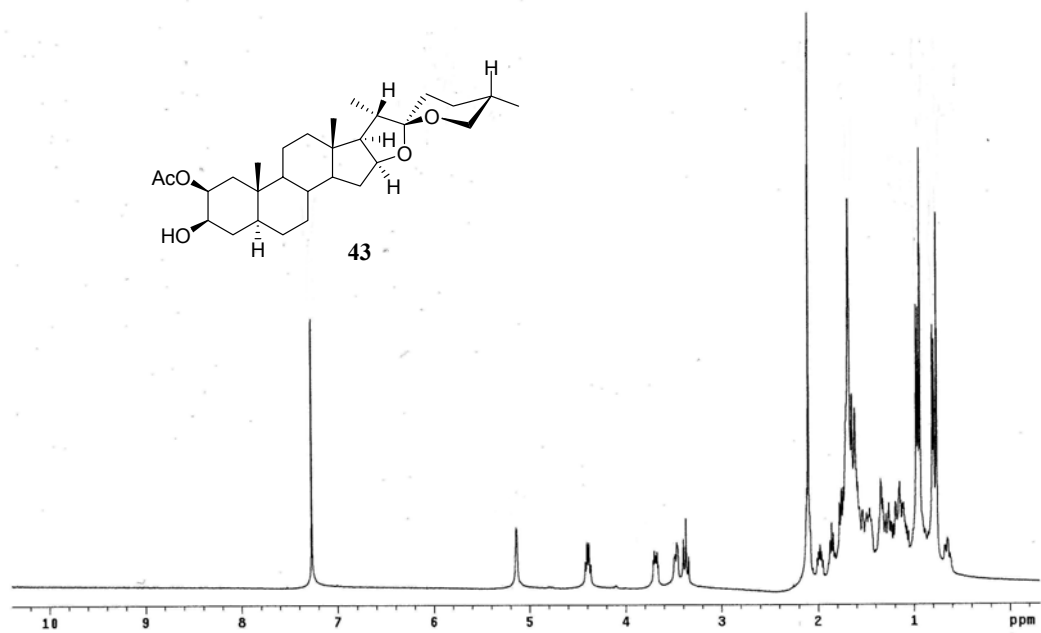
Figure 29. ^{13}C NMR of **43** (400 MHz, CDCl$_3$).
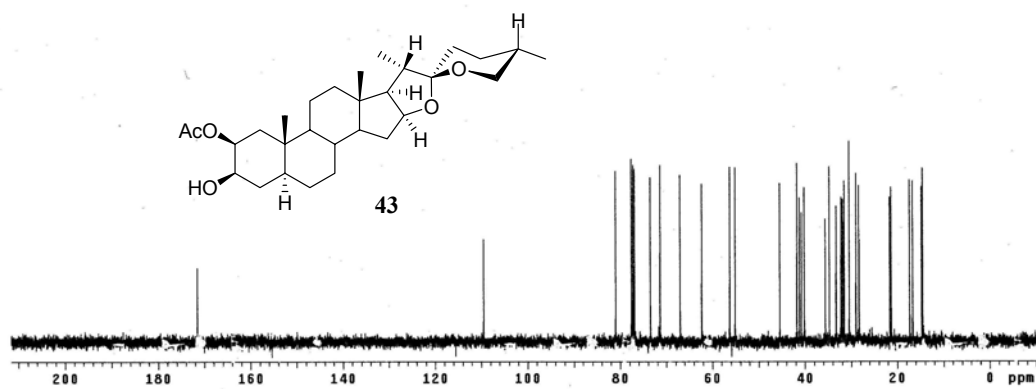
Figure 30 ^{1}H NMR of **46** (400 MHz, CDCl$_3$).
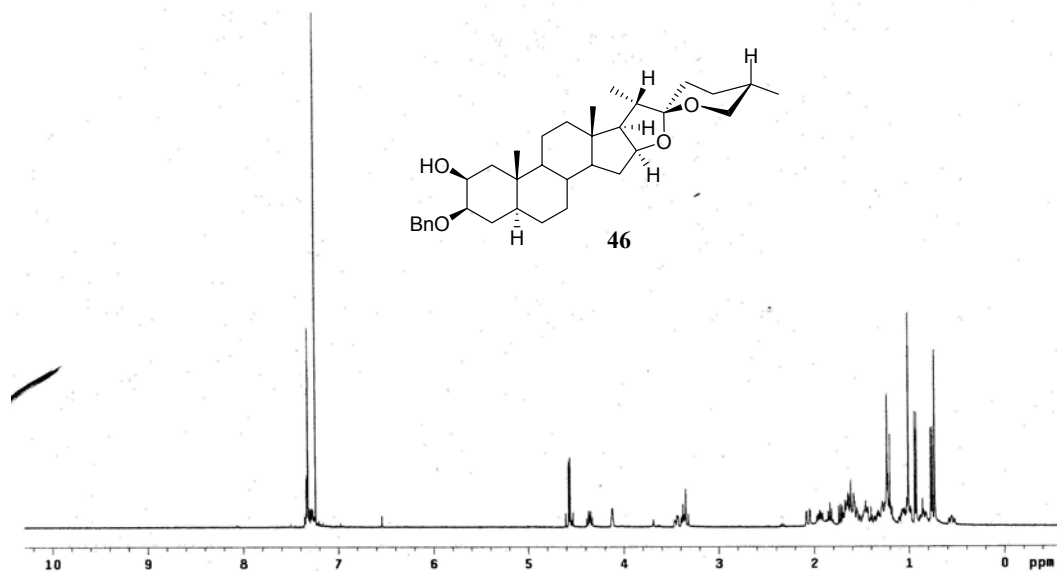
Figure 31. ^{1}H NMR of **47** (400 MHz, CDCl$_3$).
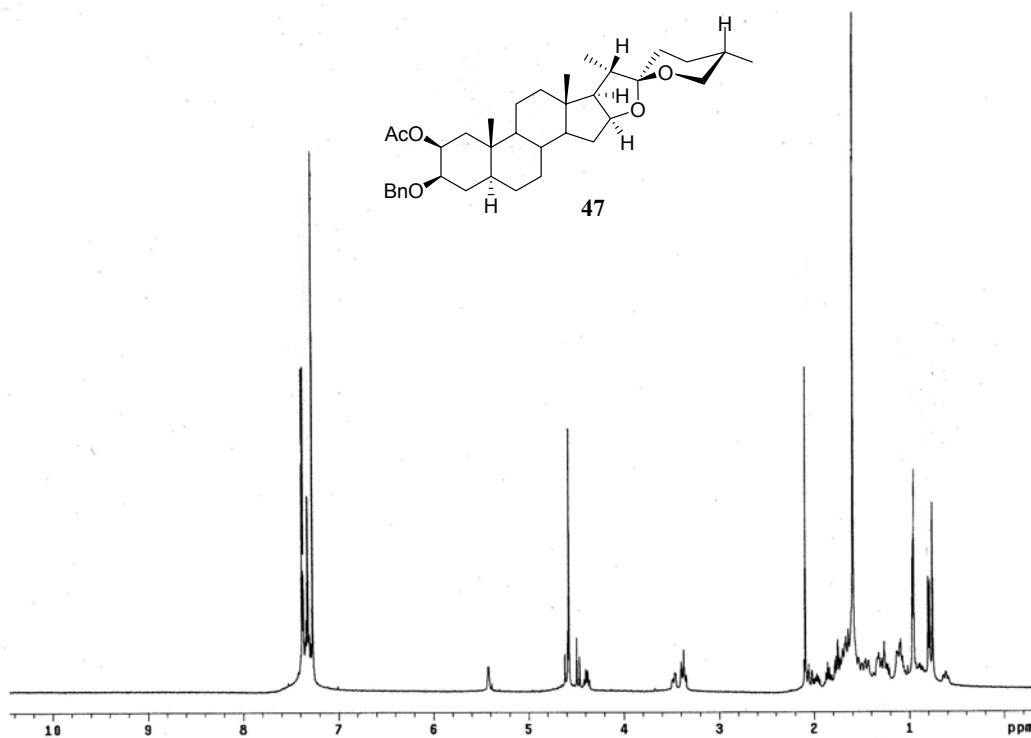
Figure 32. ^{1}H NMR of **49** (400 MHz, CDCl$_3$).
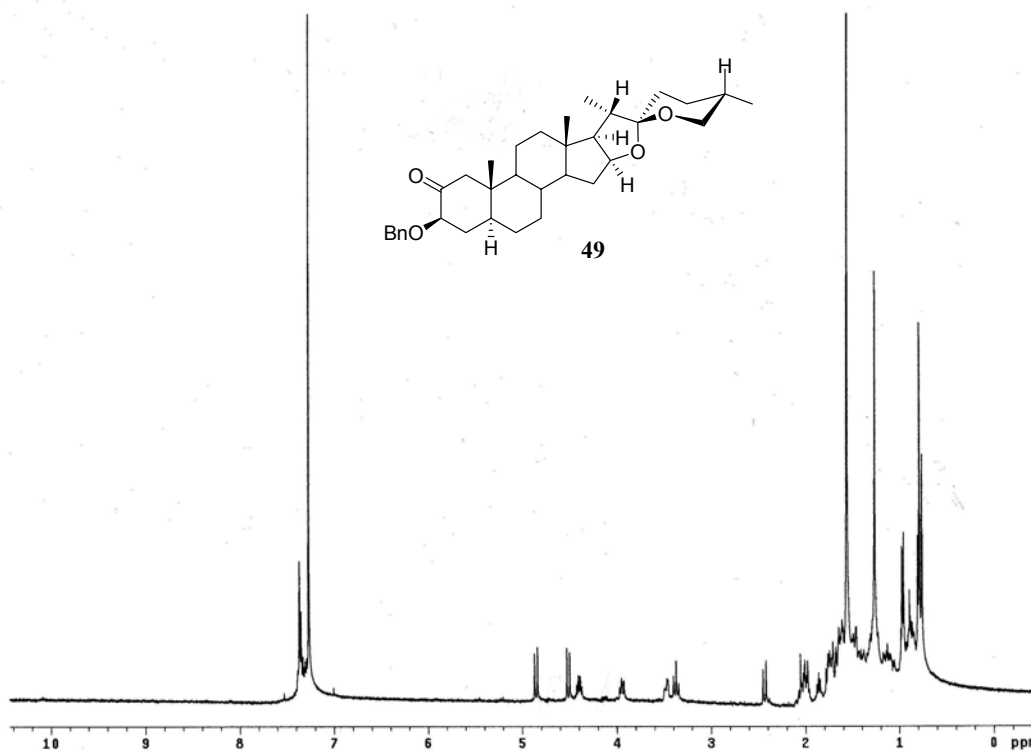
Figure 33. ^{1}H NMR of **67** (400 MHz, CDCl$_3$).
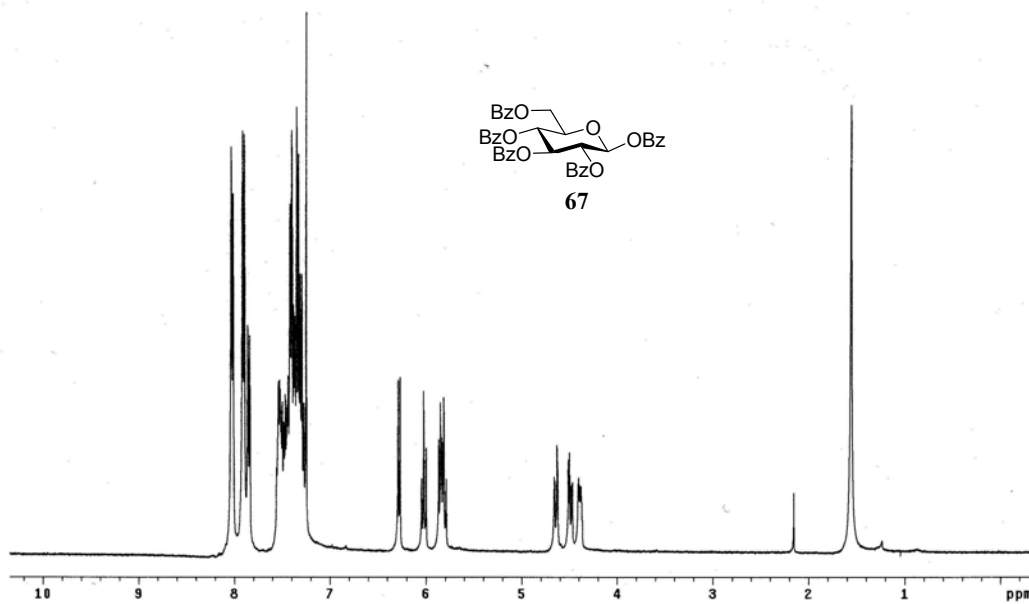
Figure 34. ^{13}C NMR of **67** (400 MHz, CDCl$_3$).
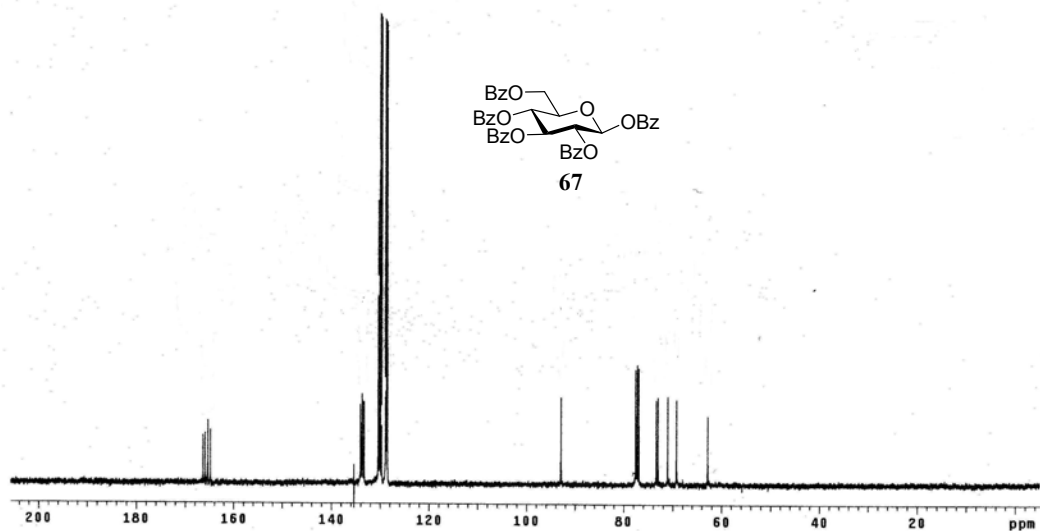
Figure 35. ^1H NMR of **68** (400 MHz, CDCl$_3$).
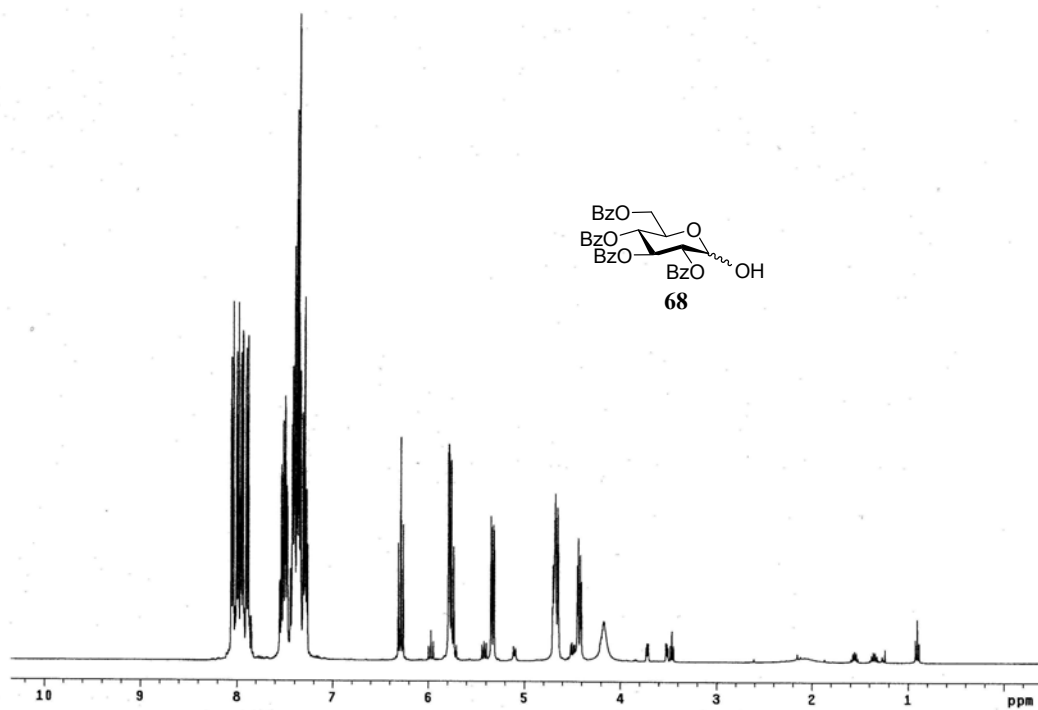
Figure 36. ^{13}C NMR of **68** (400 MHz, CDCl$_3$).
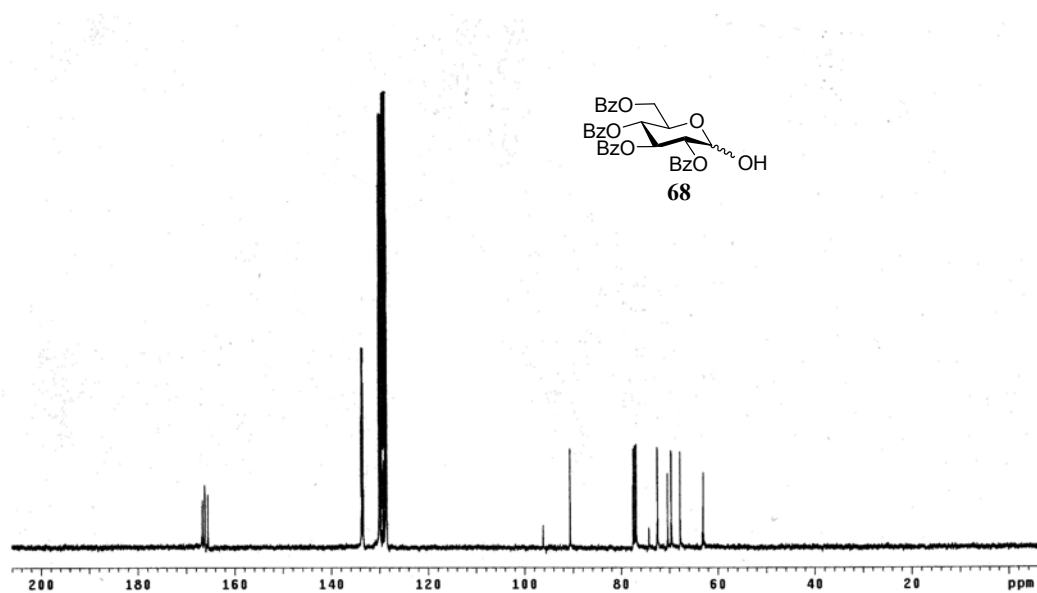
Figure 37. ^{1}H NMR of **69** (400 MHz, CDCl$_3$).
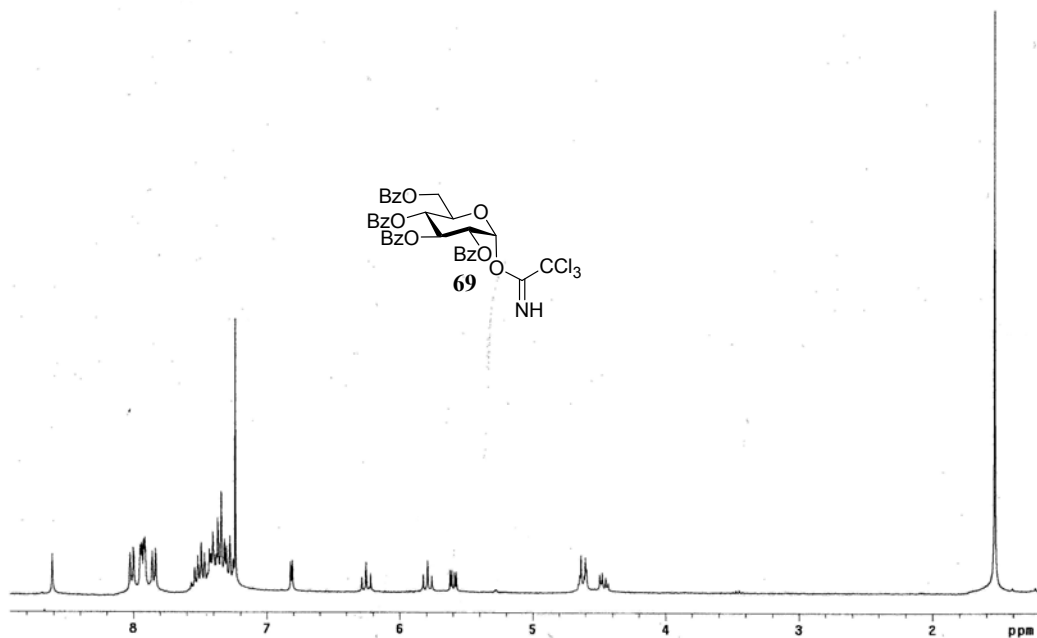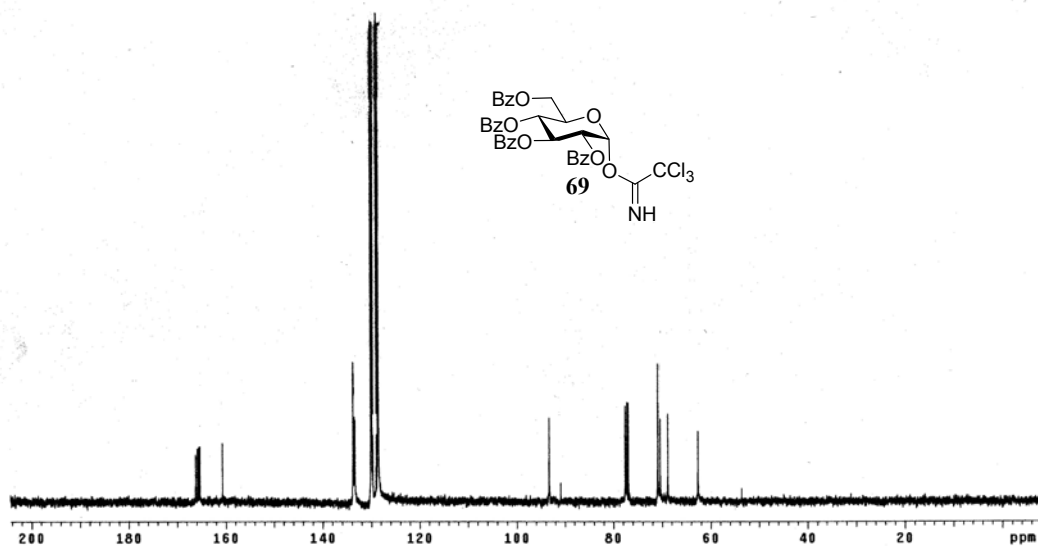
Figure 38. ^{13}C NMR of **69** (400 MHz, CDCl$_3$).
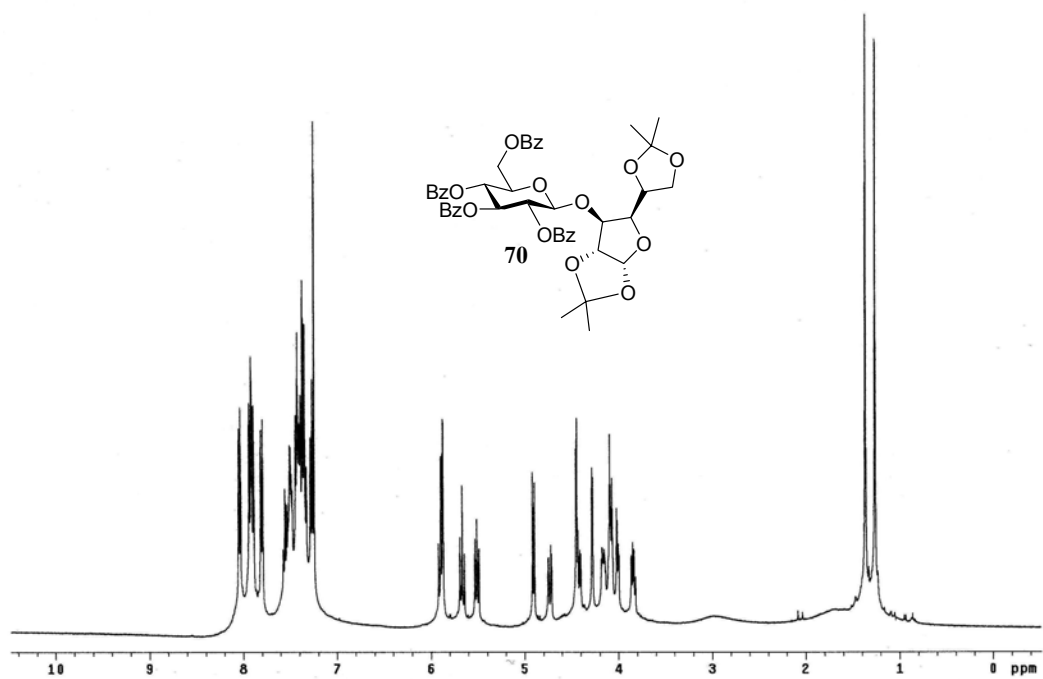

Figure 39. ^{1}H NMR of **70** (400 MHz, CDCl$_3$).

Figure 40. ^{13}C NMR of **70** (400 MHz, CDCl$_3$).
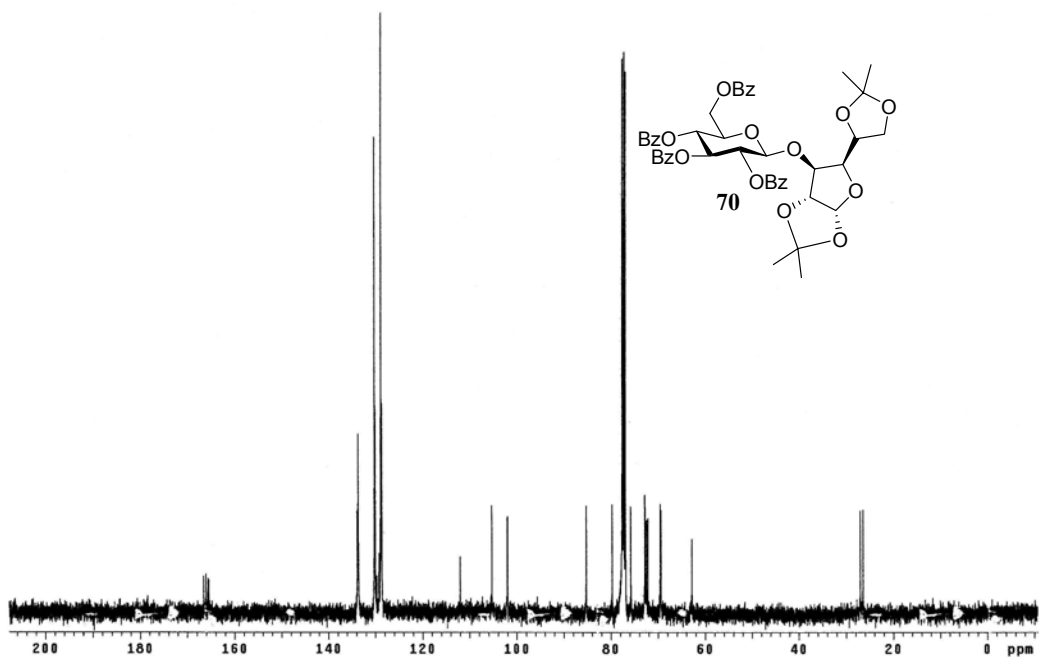

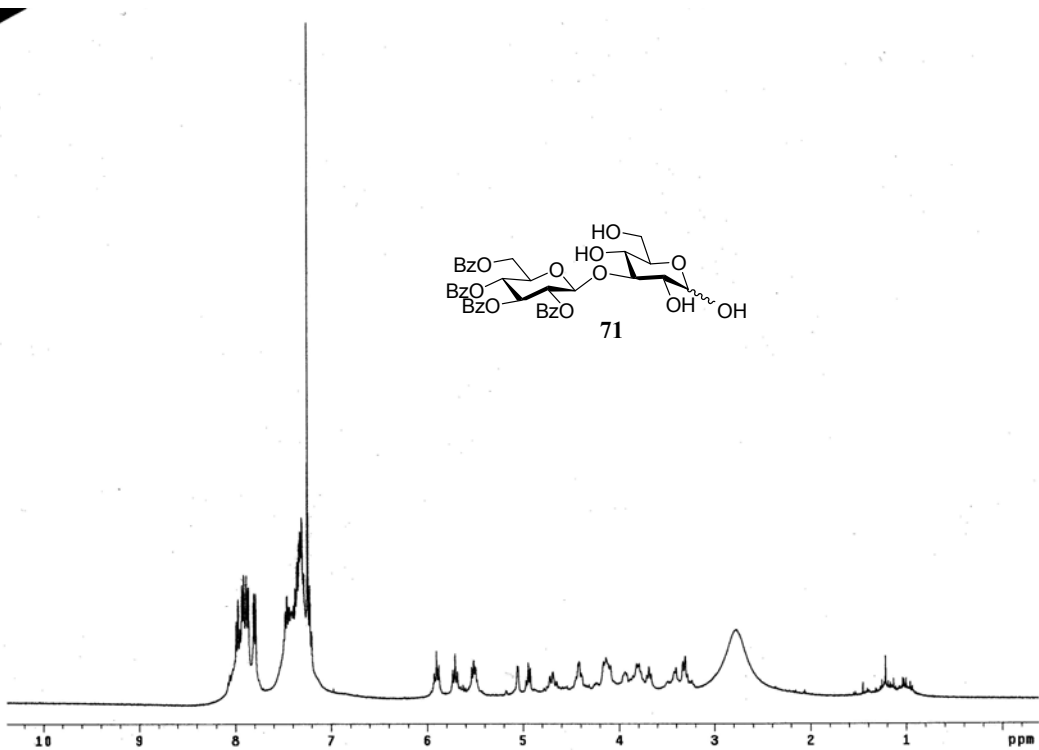**Figure 41**. ^{1}H NMR of **71** (400 MHz, CDCl$_3$).

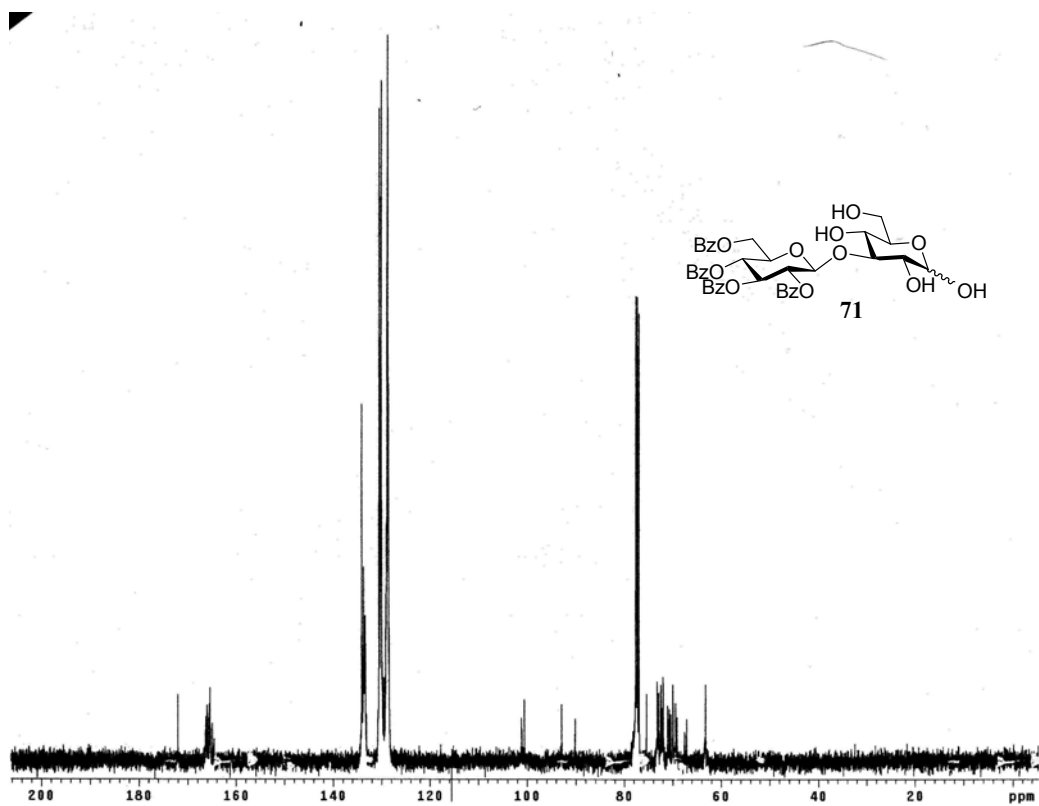**Figure 42**. ^{13}C NMR of **71** (400 MHz, CDCl$_3$).

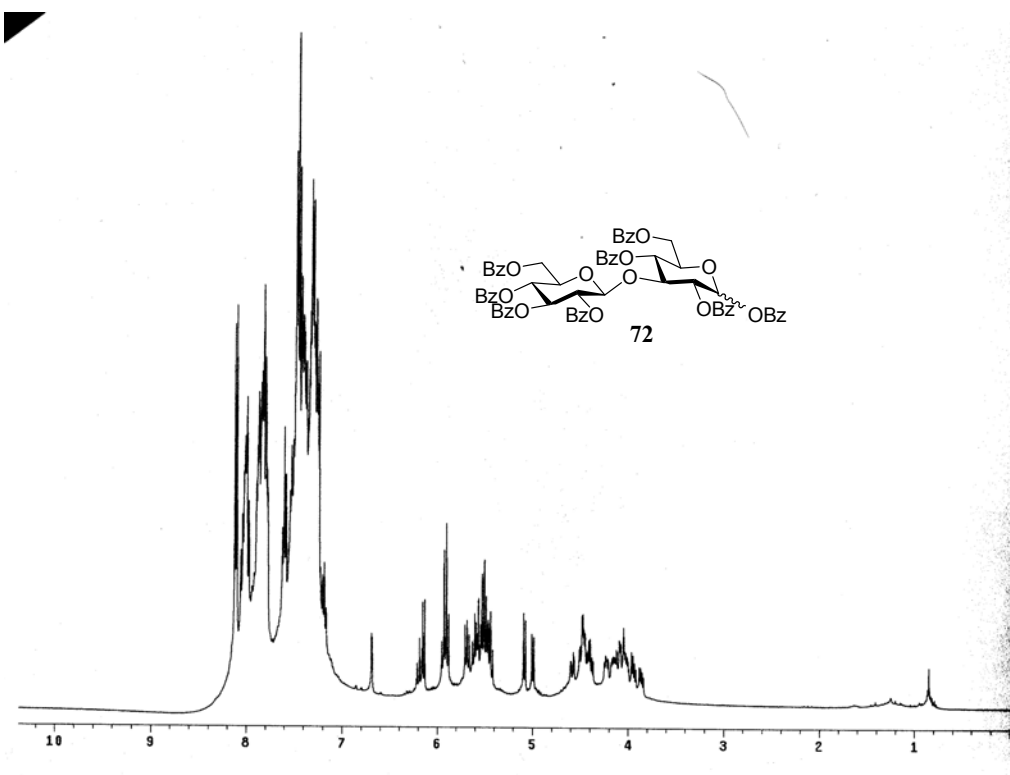**Figure 43**. ^{1}H NMR of **72** (400 MHz, CDCl$_3$).

Figure 44. ^{13}C NMR of **72** (400 MHz, CDCl$_3$).

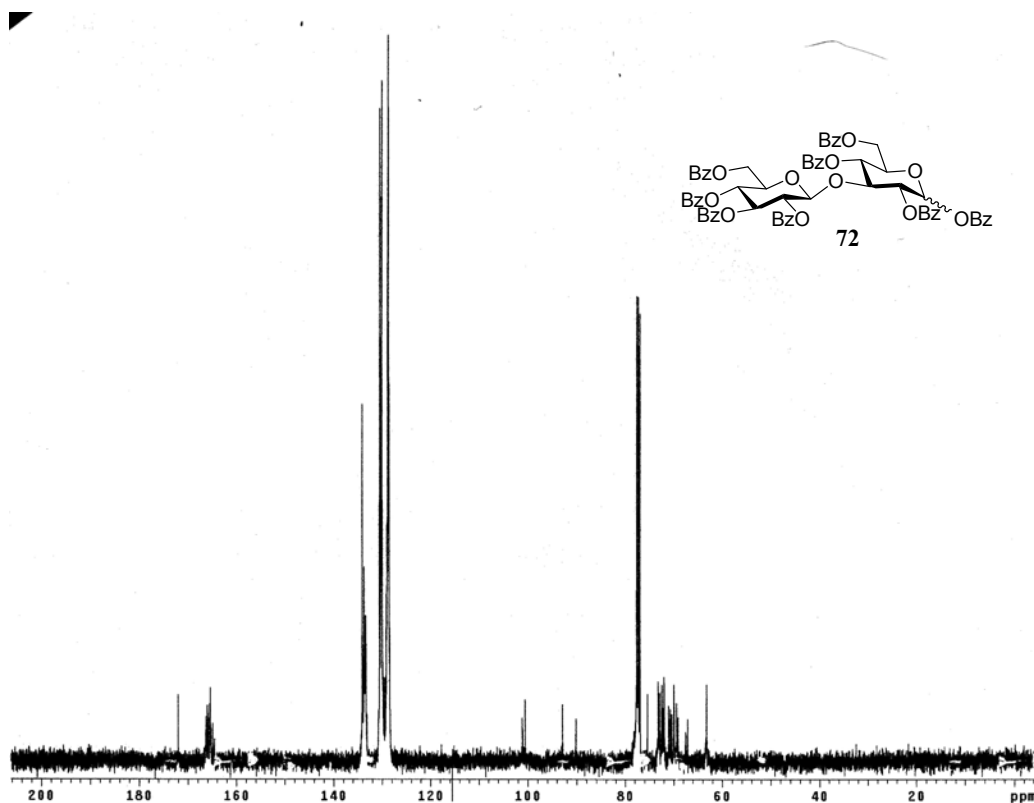**Figure 45**. ^1H NMR of **75** (300 MHz, CDCl$_3$).
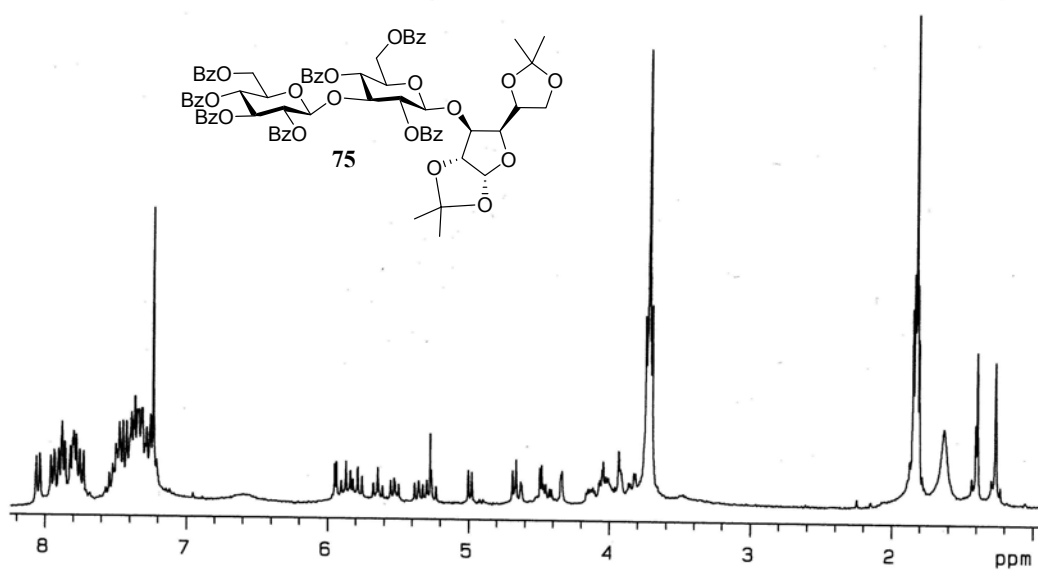
Figure 46. ^{1}H NMR of **76** (300 MHz, CDCl$_3$).
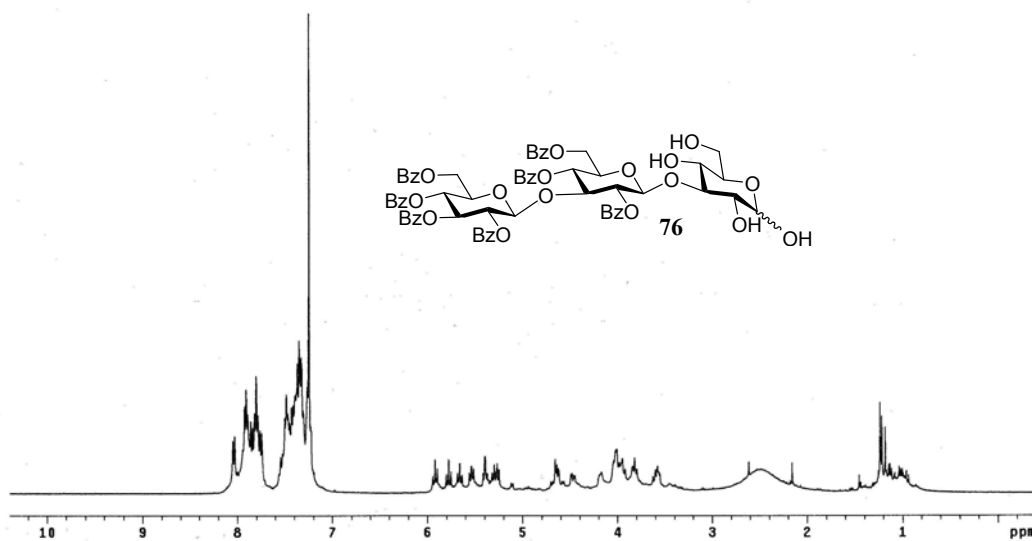
Figure 47. ^{13}C NMR of **76** (400 MHz, CDCl$_3$).

116
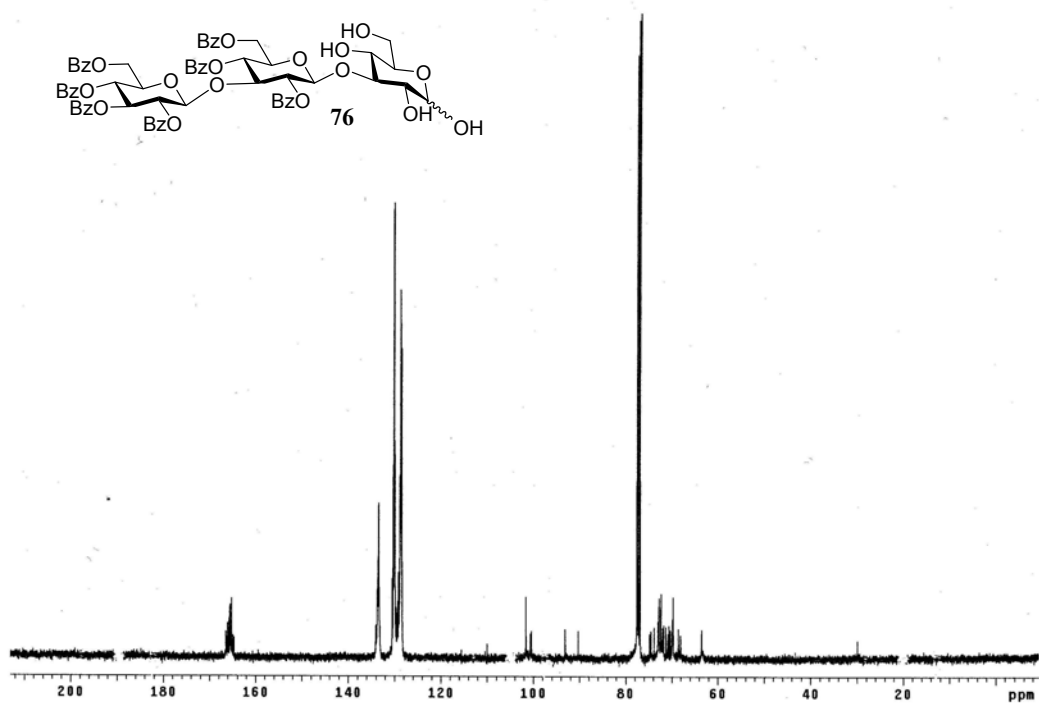
Figure 48. ^{1}H NMR of **77** (400 MHz, CDCl3).
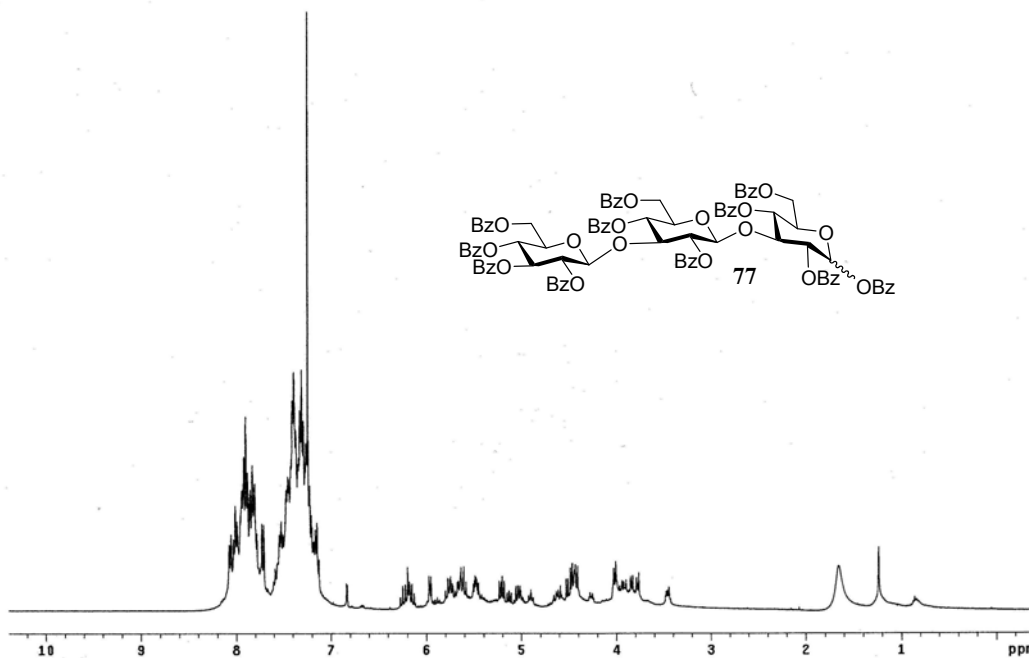
Figure 49. ^{13}C NMR of **77** (400 MHz, CDCl3).

117
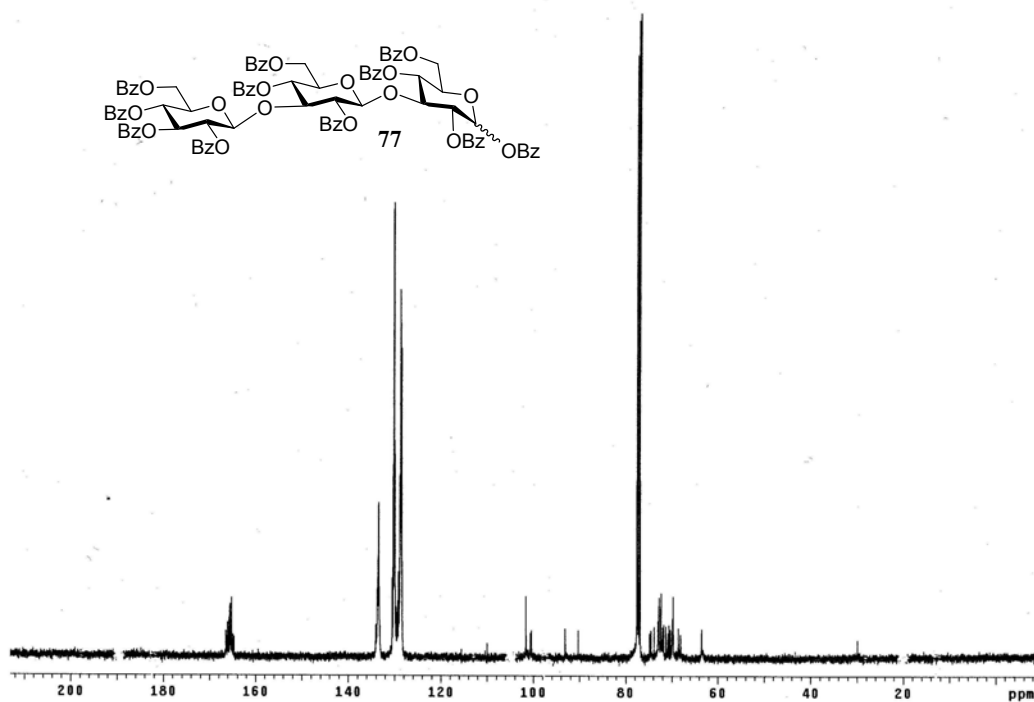
Figure 50. ^{1}H NMR of **78** (400 MHz, CDCl$_3$).
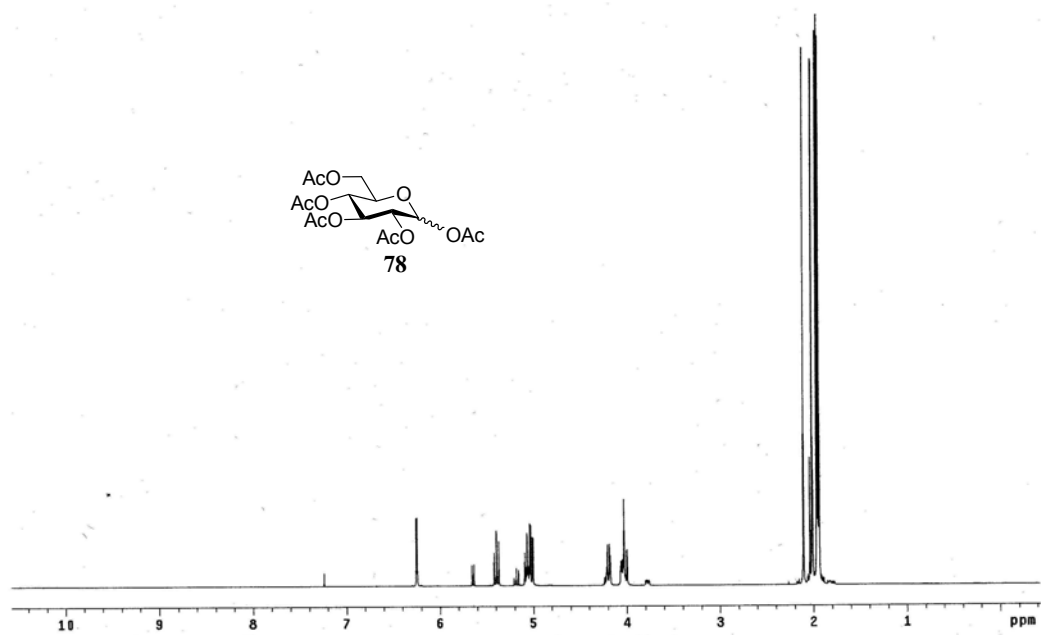
Figure 51. ^{13}C NMR of **78** (400 MHz, CDCl$_3$).
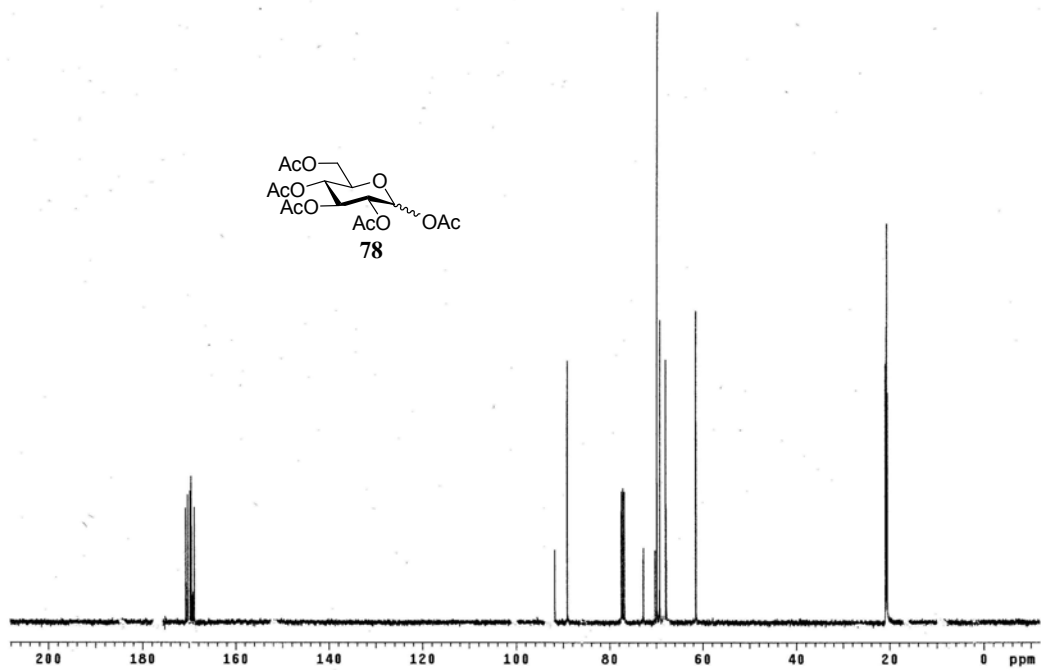
Figure 52. ^{1}H NMR of **79** (400 MHz, CDCl$_3$).
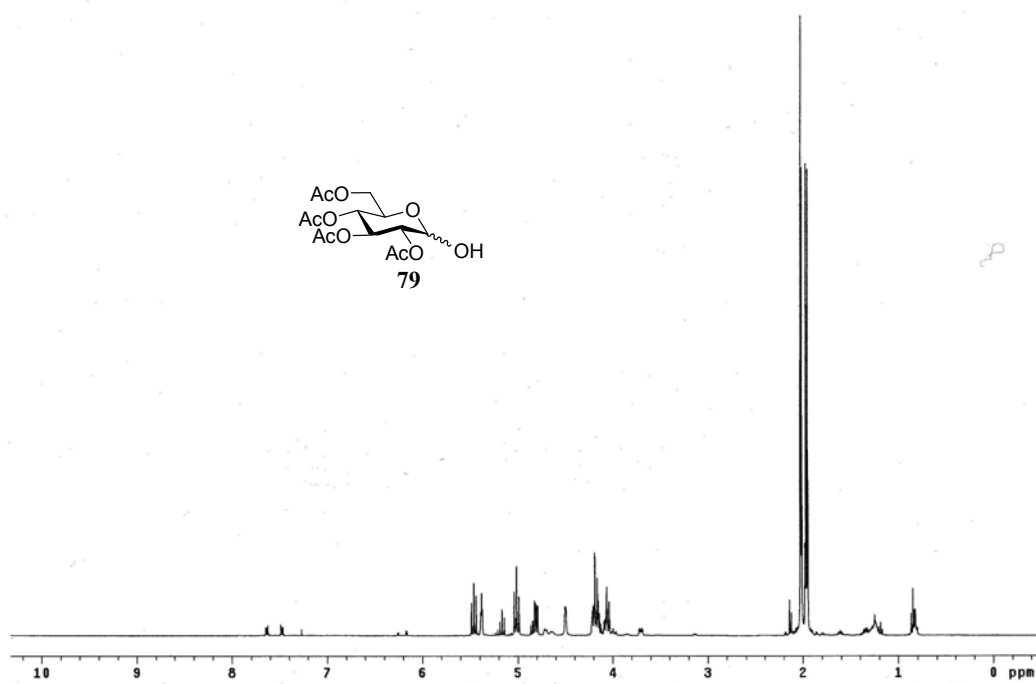
Figure 53. ^{13}C NMR of **79** (400 MHz, CDCl$_3$).
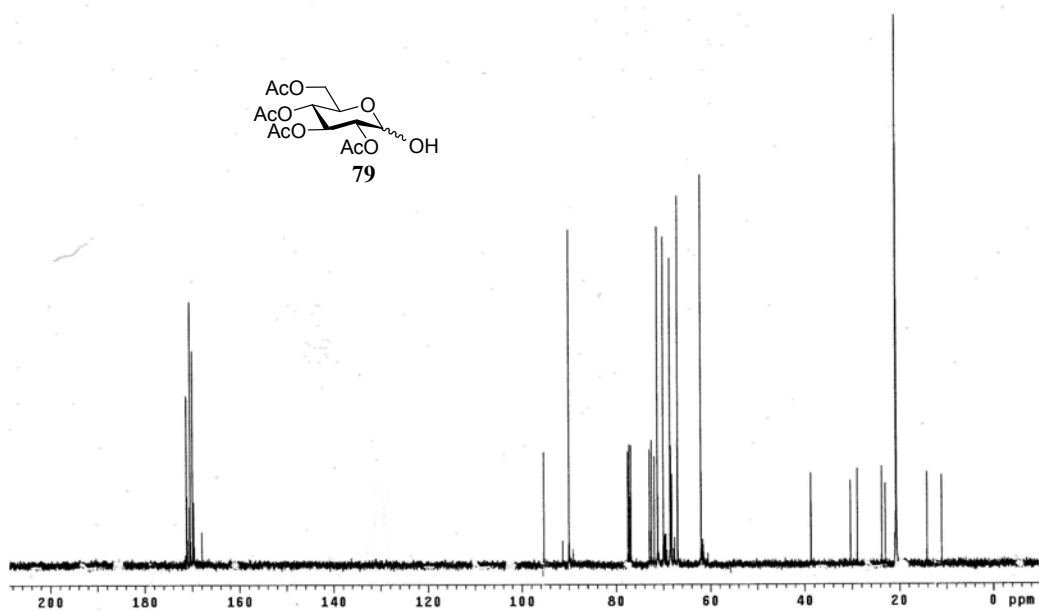
Figure 54. ^{1}H NMR of **81** (400 MHz, CDCl$_3$).
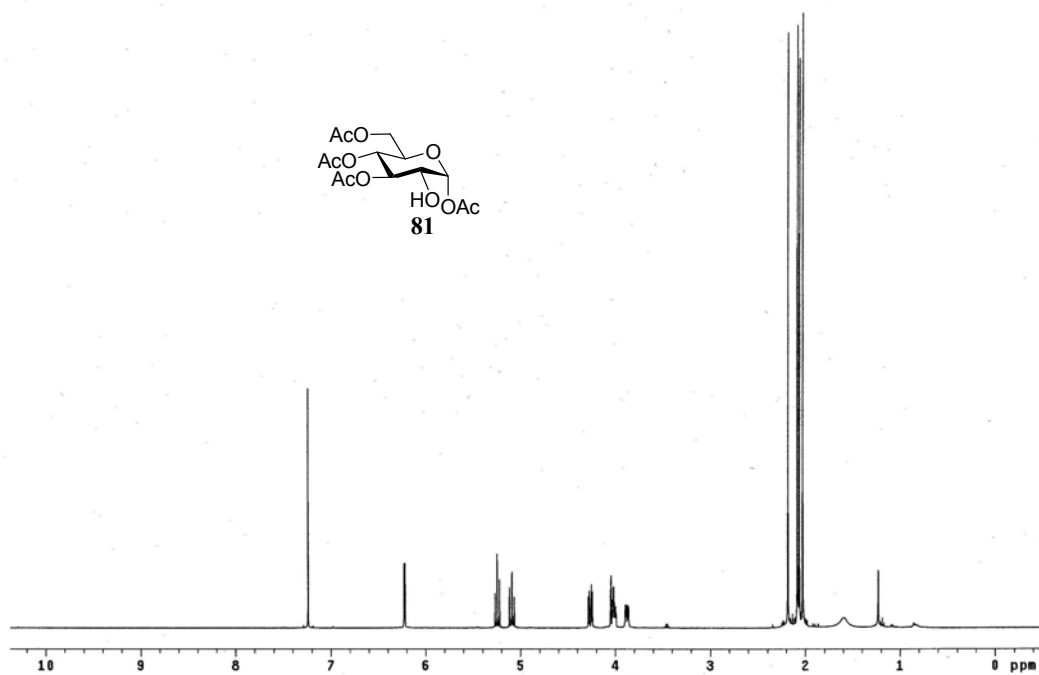
Figure 55. ^{13}C NMR of **81** (400 MHz, CDCl$_3$).
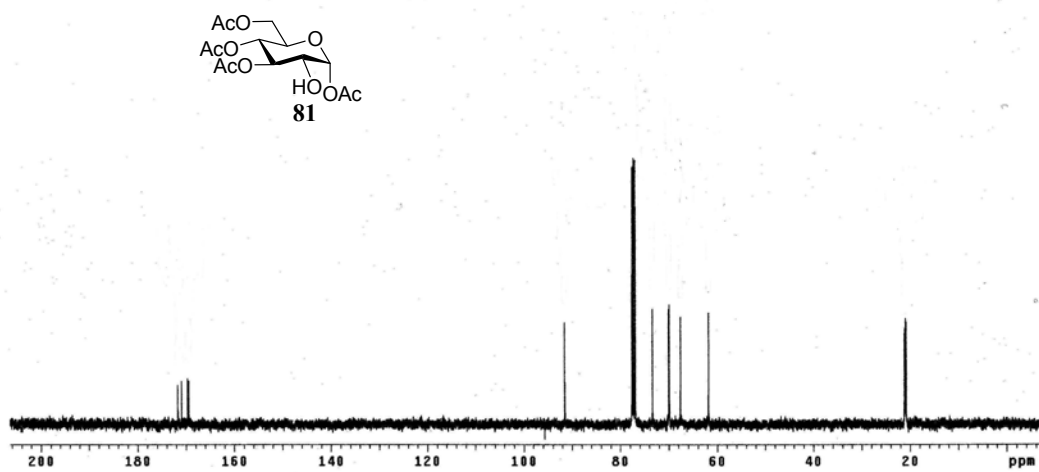
Figure 56. ^{1}H NMR of **84** (400 MHz, CDCl$_3$).
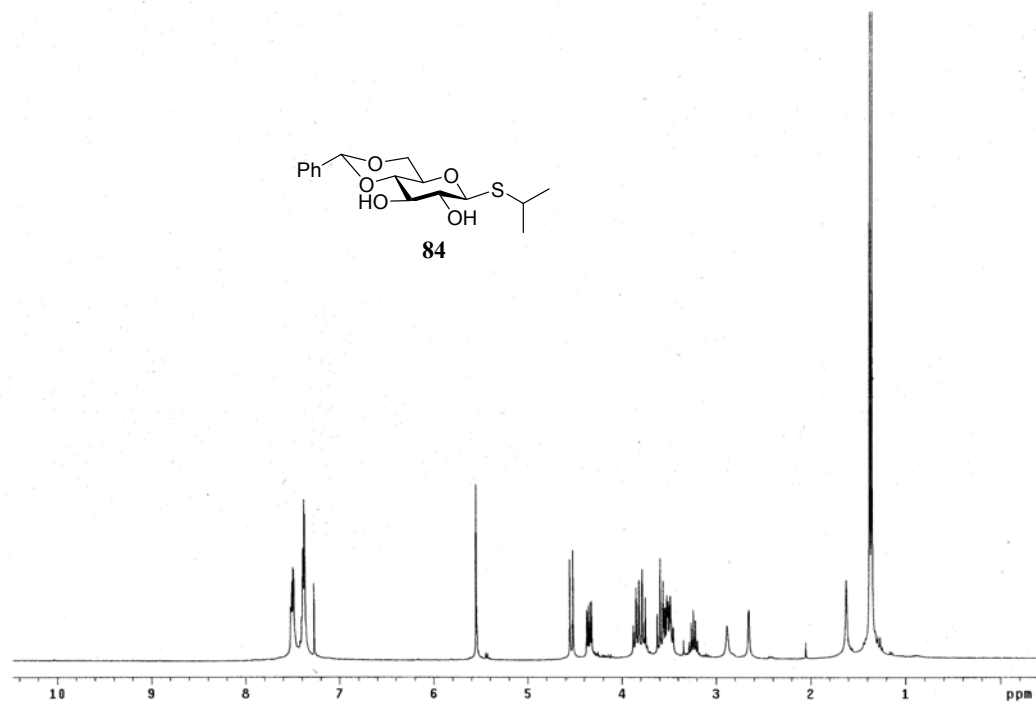
Figure 57. ^{13}C NMR of **84** (400 MHz, CDCl$_3$).
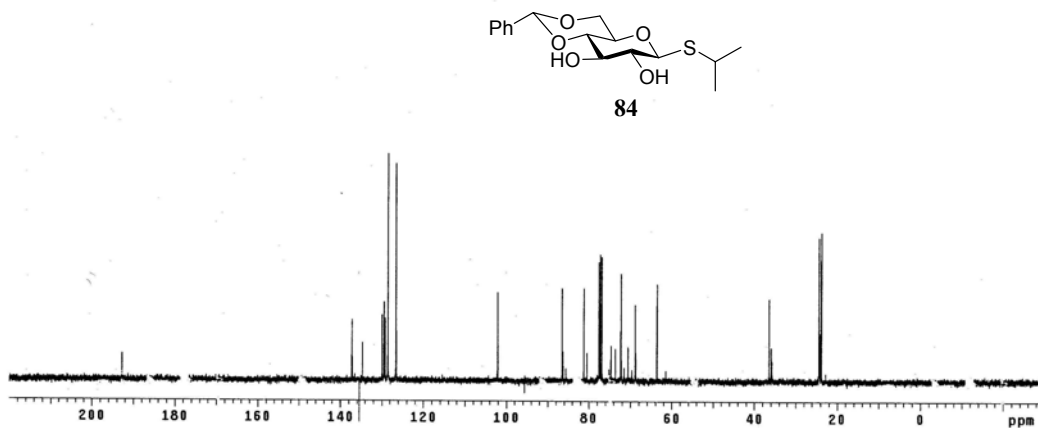
Figure 58. ^{1}H NMR of **85** (400 MHz, CDCl$_3$).
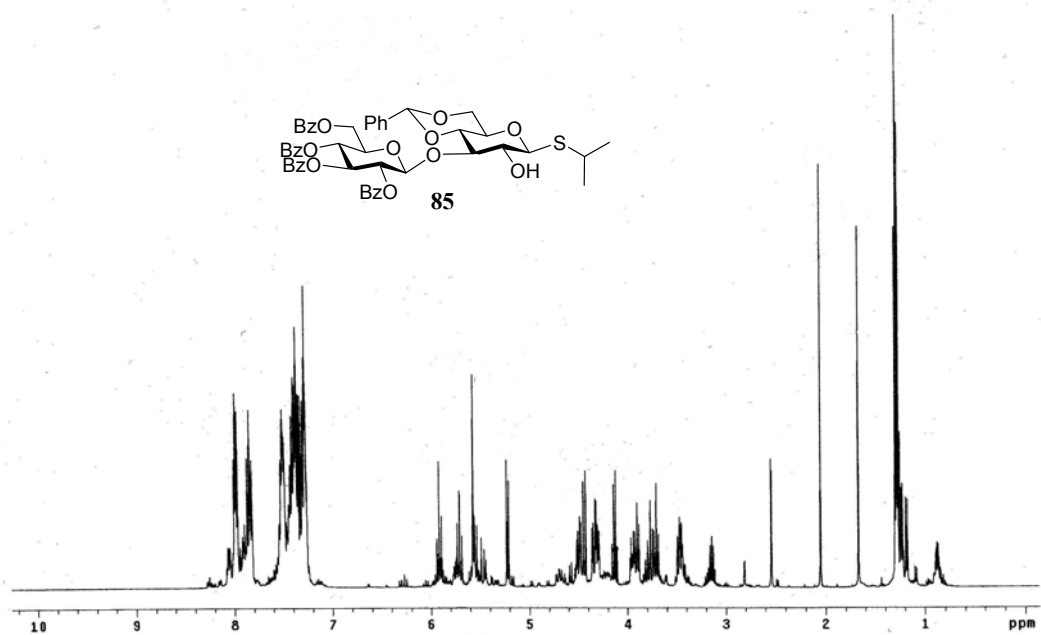
Figure 59. ^{1}H NMR of **90** (400 MHz, CDCl$_3$).
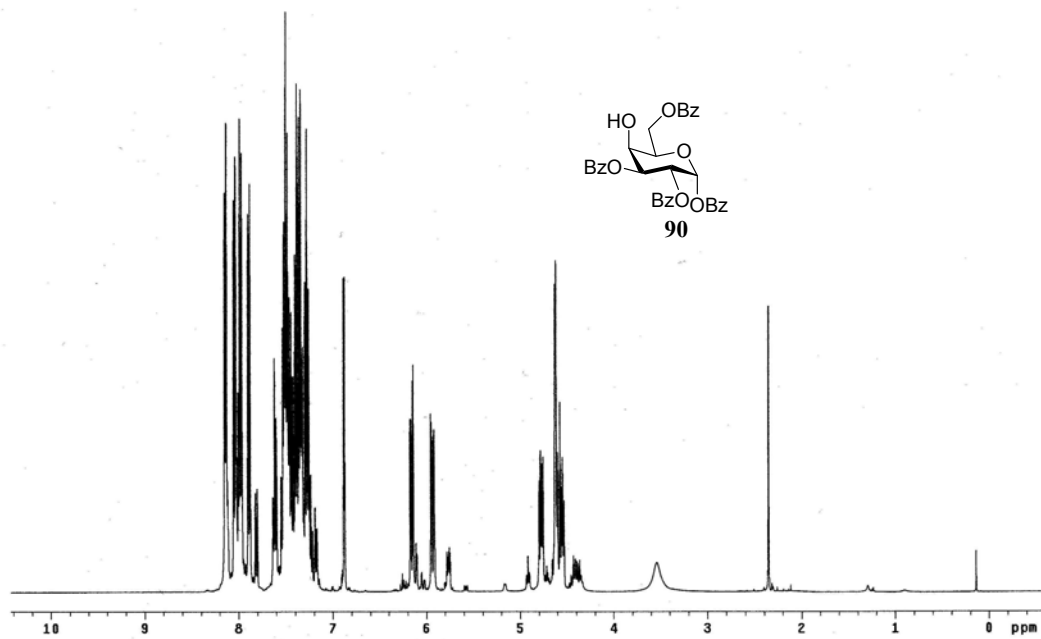
Figure 60. ^{13}C NMR of **90** (400 MHz, CDCl$_3$).
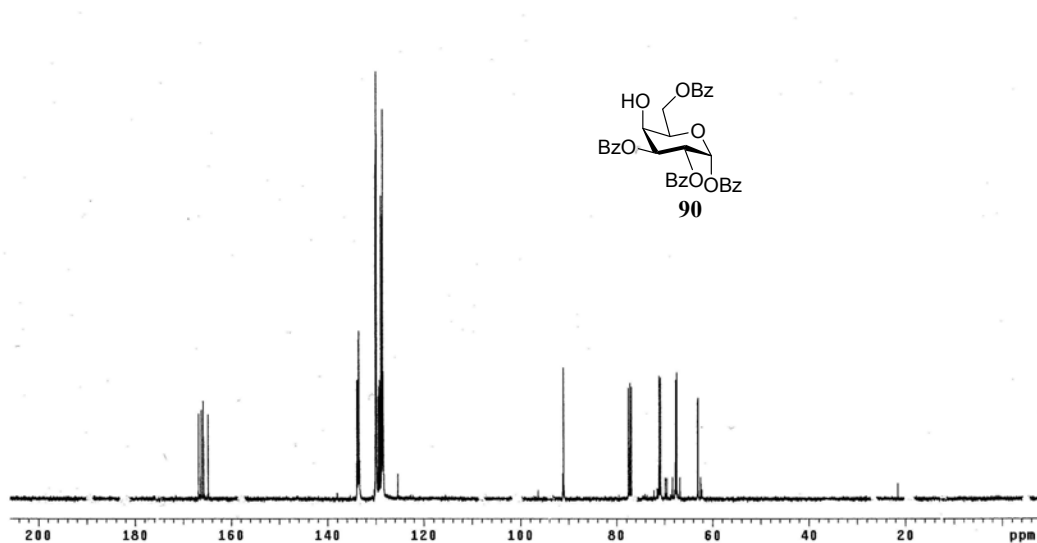
Figure 61. ^{1}H NMR of **92** (400 MHz, CDCl$_3$).
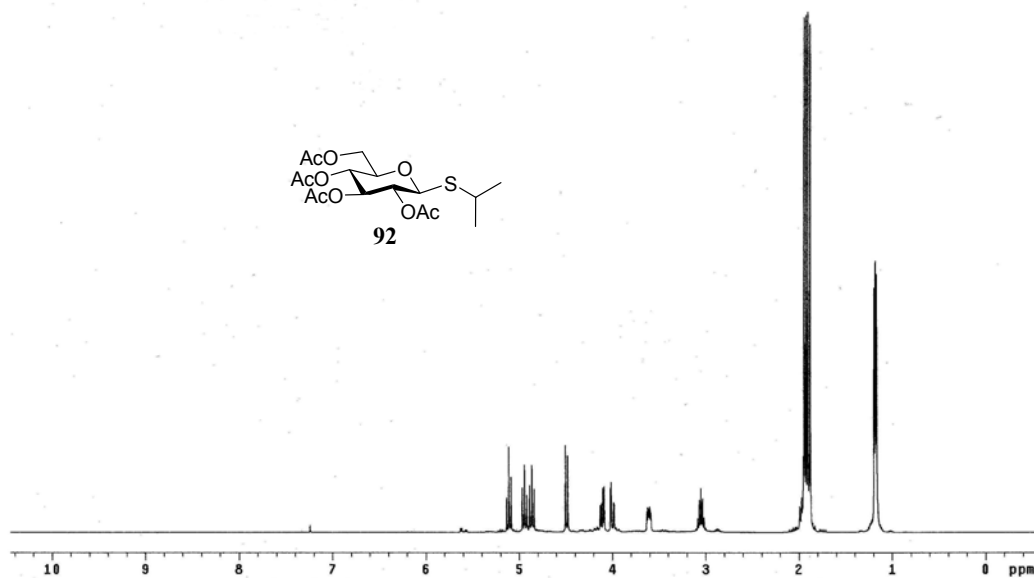
Figure 62. ^{13}C NMR of **92** (400 MHz, CDCl$_3$).
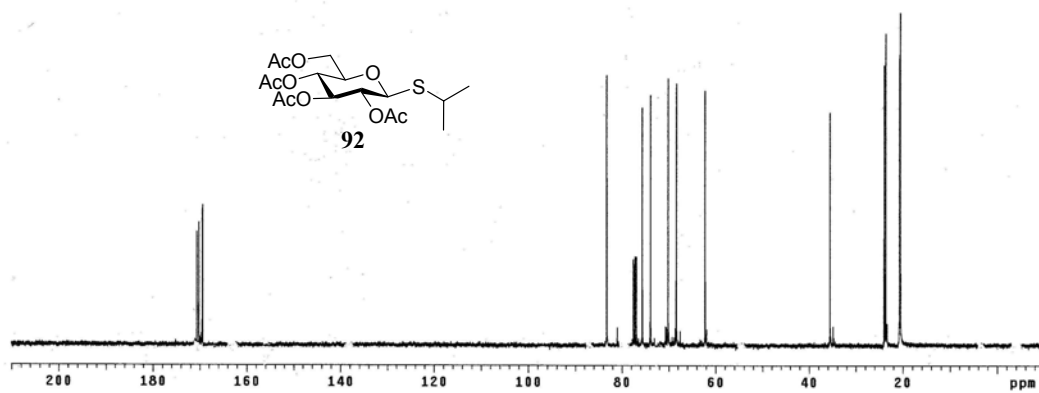
Figure 63. ^{1}H NMR of **93** (400 MHz, CDCl$_3$).
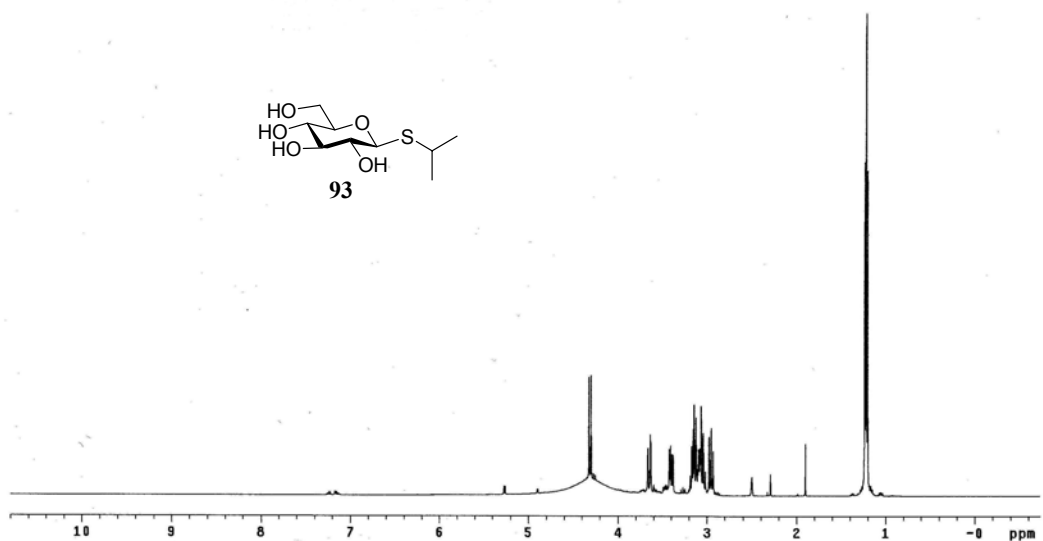
Figure 64. ^{13}C NMR of **93** (400 MHz, CDCl[3]).
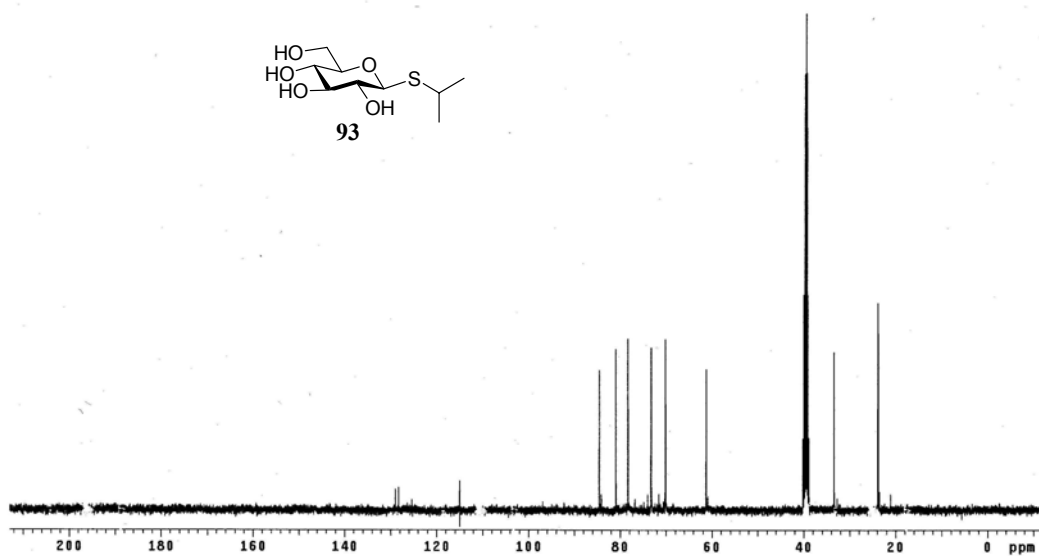
Figure 65. ^{1}H NMR of **94** (400 MHz, CDCl[3]).
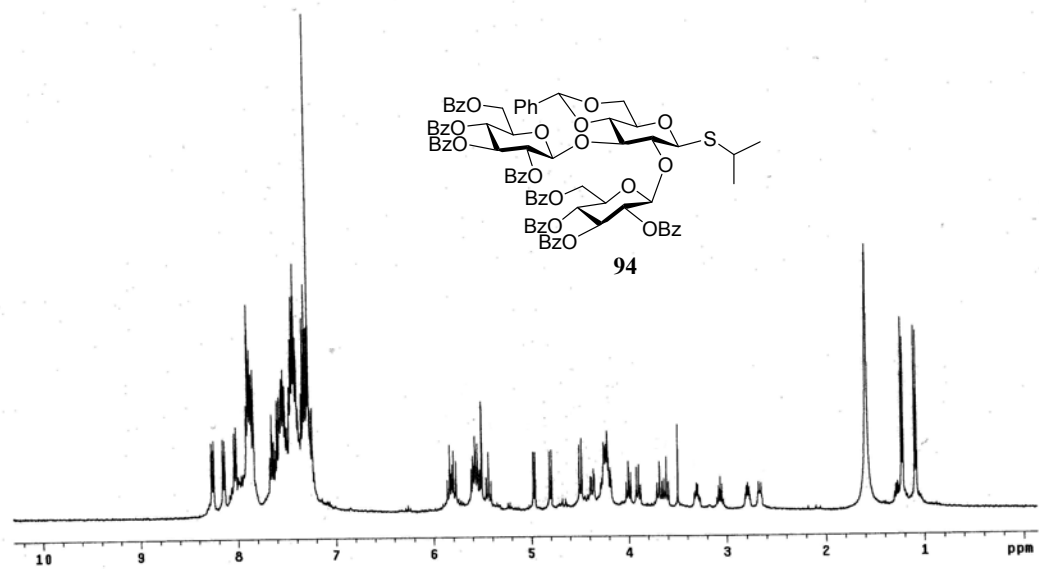
Figure 66. ^{1}H NMR of **96** (400 MHz, CDCl$_3$).
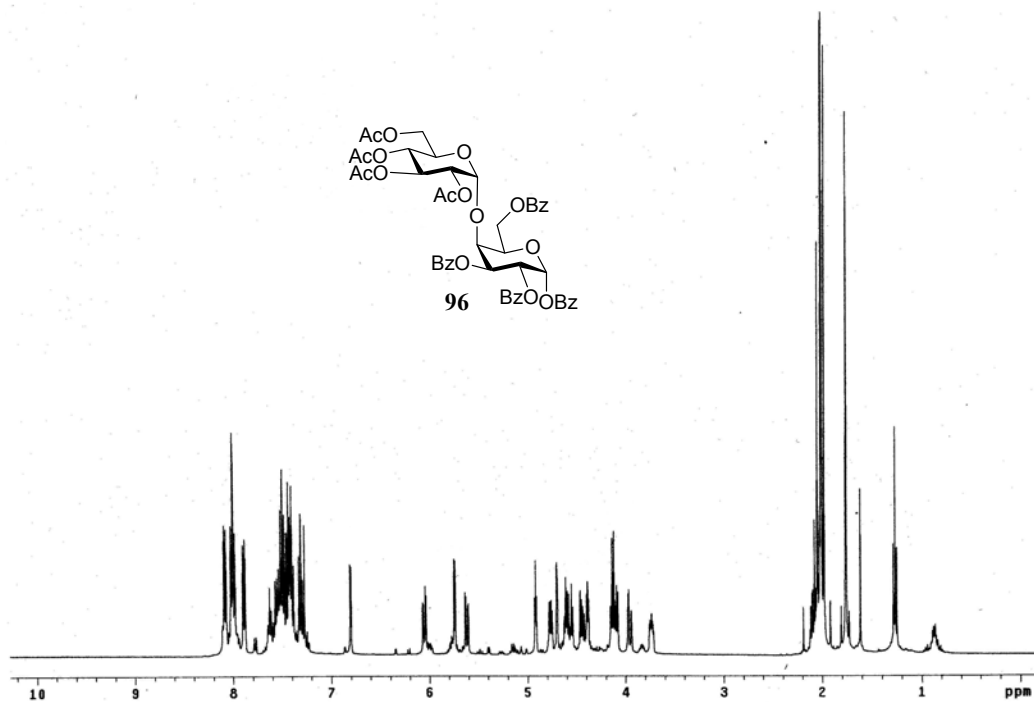
Figure 67. ^{1}H NMR of **97** (400 MHz, CDCl$_3$).
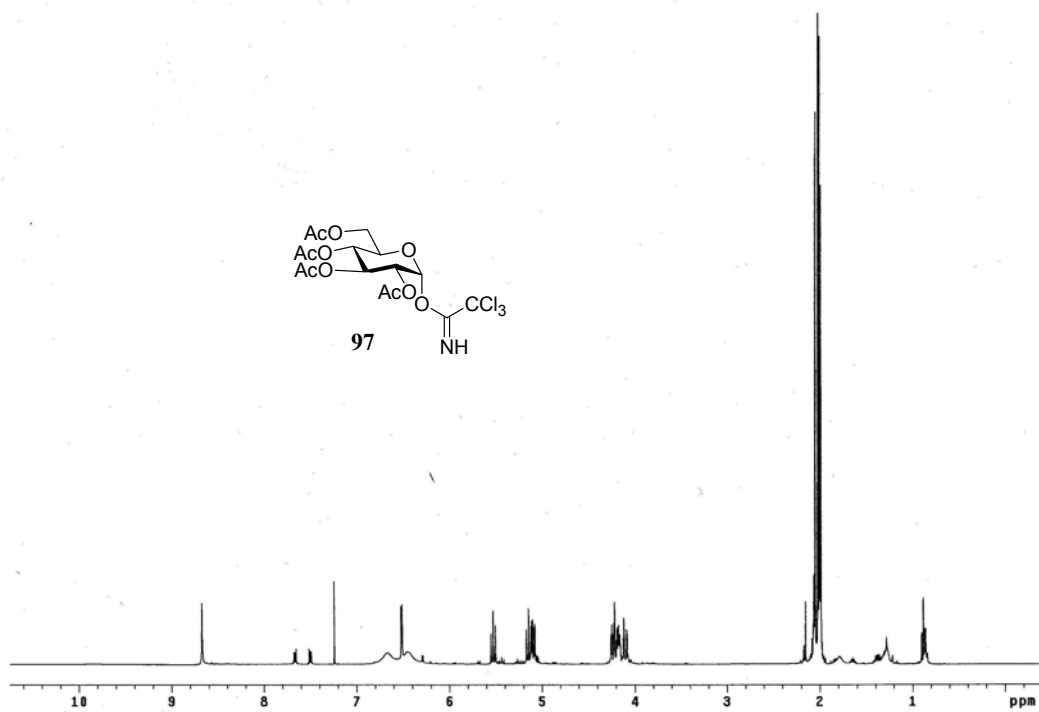
Figure 68. ^{1}H NMR of **98** (400 MHz, CDCl₃).
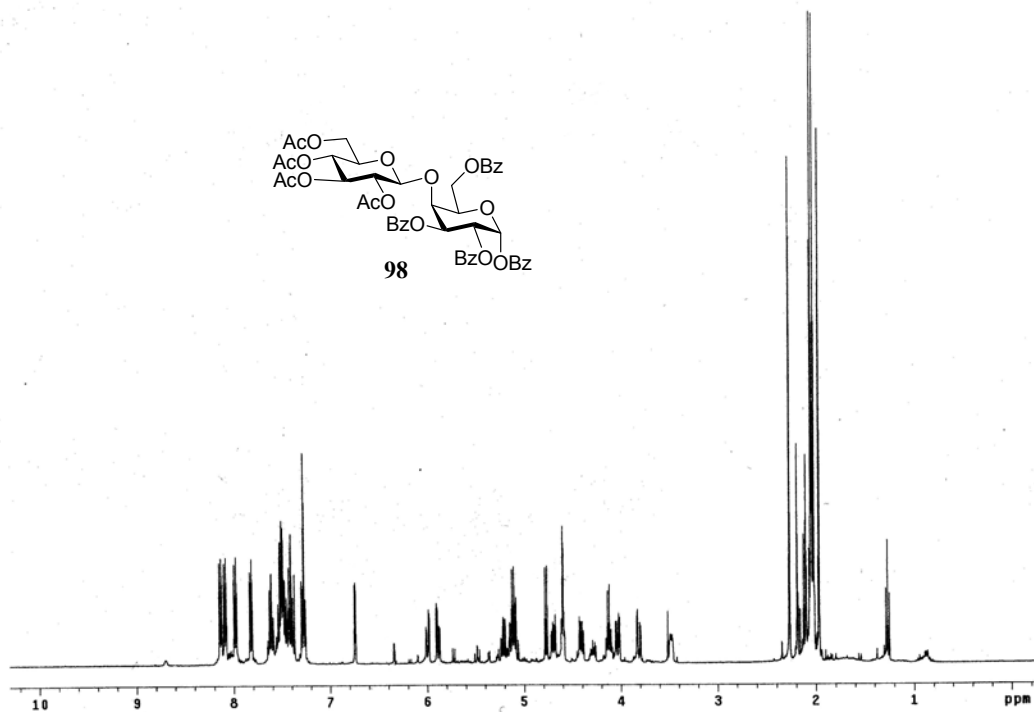
X-Ray Crystallography Data for **32**

Table 3. Crystal data and structure refinement for **32**.

Empirical formula	$C_{29}H_{46}O_5$
Formula weight	474.66
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1$
Unit cell dimensions	a = 8.247(2) Å α = 90 deg.
	b = 10.773(3) Å β = 91.932(5) deg.
	c = 14.884(4) Å γ = 90 deg.
Volume	1321.5(6) $Å_3$
Z, Calculated density	2, 1.193 Mg/m^3
Absorption coefficient	0.079 mm^{-1}
F(000)	520
Crystal size	0.60 x 0.20 x 0.03 mm
Theta range for data collection	2.33 to 22.49 deg.
Limiting indices	$-8 \le h \le 8$, $-11 \le k \le 11$, $-15 \le l \le 16$
Reflections collected / unique	9729 / 3357 [R(int) = 0.0569]
Completeness to theta = 22.49	98.4 %
Absorption correction	Empirical
Max. and min. transmission	0.925116 and 0.747318
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3357 / 338 / 329
Goodness-of-fit on F^2	1.043
Final R indices [I>2σ(I)]	R1 = 0.0598, wR2 = 0.1653
R indices (all data)	R1 = 0.0637, wR2 = 0.1698
Extinction coefficient	0.005(3)
Largest diff. peak and hole	0.335 and -0.309 $e.Å^{-3}$

Table 4. Atomic coordinates (x10^4) and equivalent isotropic displacement parameters (Å$_2$ x 10^3) for **32**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	2453(5)	0(4)	12722(2)	26(1)
O(1)	182(4)	-1446(3)	14869(2)	41(1)
C(2)	1301(5)	-67(4)	13491(3)	30(1)
O(2)	336(3)	-1200(3)	13390(2)	31(1)
C(3)	164(5)	1037(4)	13526(3)	34(1)
O(3)	1157(4)	2078(3)	13795(2)	45(1)
C(4)	-666(5)	1265(4)	12630(3)	33(1)
C(5)	560(5)	1430(4)	11897(3)	24(1)
C(6)	-232(5)	1811(4)	10996(3)	32(1)
C(7)	1030(4)	2054(4)	10288(3)	28(1)
C(8)	2208(4)	982(3)	10197(3)	19(1)
C(9)	2987(4)	632(3)	11116(2)	17(1)
C(10)	1659(4)	275(3)	11799(2)	19(1)
C(11)	4339(4)	-332(4)	11051(2)	20(1)
C(12)	5597(4)	24(3)	10348(2)	20(1)
C(13)	4770(4)	250(3)	9429(2)	18(1)
C(14)	3513(4)	1296(3)	9533(3)	18(1)
C(15)	3024(4)	1651(4)	8581(3)	26(1)
O(16)	4471(3)	758(2)	7318(2)	22(1)
C(16)	4628(4)	1534(3)	8086(3)	22(1)
C(17)	5862(4)	855(3)	8723(3)	21(1)
C(18)	3974(5)	-945(3)	9066(3)	22(1)
C(19)	723(5)	-878(4)	11462(3)	29(1)
C(20)	6810(4)	36(4)	8089(3)	22(1)
C(21)	6072(4)	315(3)	7157(3)	22(1)
O(21)	6987(3)	1283(3)	6779(2)	27(1)
C(22)	5921(5)	-792(4)	6535(3)	32(1)
C(23)	5339(6)	-427(4)	5598(3)	38(1)
C(24)	6356(5)	643(4)	5249(3)	36(1)
C(25)	6360(5)	1670(4)	5920(3)	30(1)
C(26)	8645(5)	197(4)	8169(3)	29(1)
C(27)	5795(6)	1085(5)	4315(3)	47(1)
C(28)	-122(5)	-1796(4)	14118(3)	29(1)
C(29)	-1034(6)	-2962(5)	13886(3)	46(1)

Table 5. Bond lengths [Å] and angles [deg] for **32**.

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C(1)-C(2)	1.514(5)
C(1)-C(10)	1.531(5)
O(1)-C(28)	1.198(5)
C(2)-O(2)	1.462(5)
C(2)-C(3)	1.516(6)
O(2)-C(28)	1.326(5)
C(3)-O(3)	1.438(5)
C(3)-C(4)	1.498(6)
C(4)-C(5)	1.524(5)
C(5)-C(6)	1.528(6)
C(5)-C(10)	1.549(5)
C(6)-C(7)	1.528(5)
C(7)-C(8)	1.518(5)
C(8)-C(14)	1.524(5)
C(8)-C(9)	1.539(5)
C(9)-C(11)	1.529(5)
C(9)-C(10)	1.567(5)
C(10)-C(19)	1.537(5)
C(11)-C(12)	1.546(5)
C(12)-C(13)	1.528(5)
C(13)-C(18)	1.535(5)
C(13)-C(14)	1.542(5)
C(13)-C(17)	1.550(5)
C(14)-C(15)	1.509(5)
C(15)-C(16)	1.540(5)
O(16)-C(16)	1.419(5)
O(16)-C(21)	1.431(4)
C(16)-C(17)	1.550(5)
C(17)-C(20)	1.526(5)
C(20)-C(26)	1.524(5)
C(20)-C(21)	1.526(5)
C(21)-O(21)	1.414(5)
C(21)-C(22)	1.512(6)
O(21)-C(25)	1.426(5)
C(22)-C(23)	1.511(6)
C(23)-C(24)	1.527(7)
C(24)-C(25)	1.490(6)
C(24)-C(27)	1.527(6)
C(28)-C(29)	1.499(6)
C(2)-C(1)-C(10)	115.3(3)
O(2)-C(2)-C(1)	108.4(3)
O(2)-C(2)-C(3)	108.9(3)
C(1)-C(2)-C(3)	113.2(3)

C(28)-O(2)-C(2)	119.3(3)
O(3)-C(3)-C(4)	111.1(3)
O(3)-C(3)-C(2)	105.9(3)
C(4)-C(3)-C(2)	111.3(3)
C(3)-C(4)-C(5)	111.3(3)
C(4)-C(5)-C(6)	112.7(3)
C(4)-C(5)-C(10)	112.2(3)
C(6)-C(5)-C(10)	111.6(3)
C(5)-C(6)-C(7)	111.7(3)
C(8)-C(7)-C(6)	112.6(3)
C(7)-C(8)-C(14)	110.9(3)
C(7)-C(8)-C(9)	110.9(3)
C(14)-C(8)-C(9)	110.4(3)
C(11)-C(9)-C(8)	113.2(3)
C(11)-C(9)-C(10)	113.7(3)
C(8)-C(9)-C(10)	110.9(3)
C(1)-C(10)-C(19)	109.4(3)
C(1)-C(10)-C(5)	107.8(3)
C(19)-C(10)-C(5)	113.0(3)
C(1)-C(10)-C(9)	110.0(3)
C(19)-C(10)-C(9)	109.9(3)
C(5)-C(10)-C(9)	106.7(3)
C(9)-C(11)-C(12)	112.4(3)
C(13)-C(12)-C(11)	110.9(3)
C(12)-C(13)-C(18)	110.7(3)
C(12)-C(13)-C(14)	107.9(3)
C(18)-C(13)-C(14)	111.5(3)
C(12)-C(13)-C(17)	115.0(3)
C(18)-C(13)-C(17)	111.4(3)
C(14)-C(13)-C(17)	99.7(3)
C(15)-C(14)-C(8)	119.6(3)
C(15)-C(14)-C(13)	104.5(3)
C(8)-C(14)-C(13)	113.2(3)
C(14)-C(15)-C(16)	102.8(3)
C(16)-O(16)-C(21)	105.8(3)
O(16)-C(16)-C(15)	112.1(3)
O(16)-C(16)-C(17)	104.7(3)
C(15)-C(16)-C(17)	107.6(3)
C(20)-C(17)-C(16)	103.6(3)
C(20)-C(17)-C(13)	119.8(3)
C(16)-C(17)-C(13)	103.2(3)
C(26)-C(20)-C(21)	114.4(3)
C(26)-C(20)-C(17)	114.5(3)
C(21)-C(20)-C(17)	104.5(3)
O(21)-C(21)-O(16)	109.2(3)
O(21)-C(21)-C(22)	111.8(3)

O(16)-C(21)-C(22)		107.9(3)	
O(21)-C(21)-C(20)		107.8(3)	
O(16)-C(21)-C(20)		104.8(3)	
C(22)-C(21)-C(20)		115.0(3)	
C(21)-O(21)-C(25)		113.0(3)	
C(23)-C(22)-C(21)		112.2(4)	
C(22)-C(23)-C(24)		110.4(4)	
C(25)-C(24)-C(23)		108.7(3)	
C(25)-C(24)-C(27)		111.8(4)	
C(23)-C(24)-C(27)		113.1(4)	
O(21)-C(25)-C(24)		112.1(3)	
O(1)-C(28)-O(2)		123.7(4)	
O(1)-C(28)-C(29)		124.5(4)	
O(2)-C(28)-C(29)		111.8(4)	

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Symmetry transformations used to generate equivalent atoms:

Table 6. Anisotropic displacement parameters (Å^2 x10^3) for **32**. The anisotropic displacement factor exponent takes the form: $-2pi^2[h^2 a^{*2} U11 + ... + 2 h k a^* b^* U12]$

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	U11	U22	U33	U23	U13	U12
C(1)	28(2)	25(2)	25(2)	1(2)	7(2)	-1(2)
O(1)	46(2)	44(2)	34(2)	7(2)	13(1)	-7(2)
C(2)	26(2)	32(2)	32(2)	1(2)	4(2)	-6(2)
O(2)	31(2)	30(2)	32(2)	5(1)	6(1)	-8(1)
C(3)	34(2)	30(2)	38(2)	-3(2)	14(2)	-8(2)
O(3)	48(2)	37(2)	51(2)	16(2)	18(2)	-7(2)
C(4)	26(2)	28(2)	45(2)	5(2)	17(2)	4(2)
C(5)	20(2)	20(2)	34(2)	3(2)	10(2)	1(2)
C(6)	23(2)	33(2)	40(2)	7(2)	12(2)	11(2)
C(7)	22(2)	24(2)	37(2)	9(2)	5(2)	9(2)
C(8)	14(2)	15(2)	29(2)	3(2)	6(1)	-2(1)
C(9)	14(2)	13(2)	24(2)	1(2)	4(1)	-4(1)
C(10)	17(2)	14(2)	26(2)	0(2)	6(1)	-4(1)
C(11)	18(2)	22(2)	22(2)	3(2)	3(1)	1(2)
C(12)	19(2)	16(2)	25(2)	-1(2)	5(1)	3(2)
C(13)	16(2)	14(2)	23(2)	0(2)	2(1)	-3(1)
C(14)	17(2)	10(2)	26(2)	2(2)	4(1)	-2(2)
C(15)	22(2)	22(2)	35(2)	4(2)	7(2)	7(2)
O(16)	22(1)	19(1)	26(1)	0(1)	5(1)	1(1)

C(16)	22(2)	14(2)	32(2)	1(2)	5(2)	-1(1)
C(17)	21(2)	13(2)	30(2)	0(2)	1(1)	-3(1)
C(18)	27(2)	15(2)	25(2)	1(2)	5(2)	-4(2)
C(19)	27(2)	19(2)	40(2)	-1(2)	4(2)	-10(2)
C(20)	25(2)	13(2)	29(2)	2(2)	8(2)	-4(2)
C(21)	19(2)	17(2)	30(2)	1(2)	7(1)	3(1)
O(21)	25(1)	28(1)	29(1)	5(1)	4(1)	-5(1)
C(22)	41(2)	25(2)	30(2)	-8(2)	7(2)	4(2)
C(23)	46(2)	35(2)	33(2)	-18(2)	4(2)	1(2)
C(24)	31(2)	50(3)	29(2)	-1(2)	6(2)	13(2)
C(25)	25(2)	34(2)	30(2)	9(2)	6(2)	-8(2)
C(26)	23(2)	32(2)	33(2)	0(2)	2(2)	7(2)
C(27)	43(3)	66(3)	31(2)	5(2)	4(2)	8(3)
C(28)	25(2)	32(2)	31(2)	11(2)	5(2)	-1(2)
C(29)	49(3)	48(3)	40(3)	8(2)	10(2)	-17(2)

Table 7. Hydrogen coordinates (x10^4) and isotropic displacement parameters (Å^2 x10^3) for **32**.

	x	y	z	U(eq)
H(1A)	3037	-801	12687	31
H(1B)	3270	652	12860	31
H(2)	1950	-112	14070	36
H(3A)	-668	885	13987	41
H(3B)	260(70)	2650(70)	14180(40)	79(19)
H(4A)	-1346	2019	12664	39
H(4B)	-1386	555	12476	39
H(5)	1287	2130	12091	29
H(6A)	-884	2571	11080	38
H(6B)	-975	1144	10781	38
H(7A)	466	2203	9700	33
H(7B)	1645	2815	10452	33
H(8)	1630(50)	200(40)	9930(20)	19(9)
H(9)	3515	1405	11356	21
H(11A)	3855	-1145	10887	24
H(11B)	4898	-420	11647	24
H(12A)	6180	785	10547	24
H(12B)	6402	-652	10300	24
H(14)	4200(50)	2000(50)	9700(30)	36(12)
H(15A)	2602	2511	8551	31

H(15B)	2191	1078	8325	31
H(16)	5046	2371	7919	27
H(17)	6607	1472	9022	25
H(18A)	3512	-795	8461	33
H(18B)	3110	-1199	9463	33
H(18C)	4791	-1603	9043	33
H(19A)	-154	-1061	11869	43
H(19B)	1464	-1588	11444	43
H(19C)	264	-719	10857	43
H(20)	6590(40)	-780(40)	8230(20)	16(10)
H(22A)	6991	-1204	6504	38
H(22B)	5151	-1395	6785	38
H(23A)	4186	-174	5607	46
H(23B)	5422	-1149	5191	46
H(24)	7497	339	5208	43
H(25A)	7026	2364	5701	36
H(25B)	5238	1978	5980	36
H(26A)	8927	1058	8029	44
H(26B)	9163	-362	7746	44
H(26C)	9025	-1	8784	44
H(27A)	6452	1798	4140	70
H(27B)	4652	1330	4324	70
H(27C)	5923	411	3881	70
H(29A)	-1210	-3438	14435	68
H(29B)	-405	-3461	13472	68
H(29C)	-2084	-2748	13598	68

VITA

Katharine L. Bowdy was born in New York City. She received her B.S. degree from Washington and Lee University in 1998, majoring in both Natural Sciences and Mathematics and French. She came to the University of New Orleans as a graduate student to study organic synthesis under the advisement of Professor Branko Jursic.