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

Katharine Bowdy
University of New Orleans

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

A Dissertation

Submitted to the Graduate Faculty of the University of New Orleans in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Chemistry

by

Katharine L. Bowdy

B.S. Washington and Lee University, 1998

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<tbody>
<tr>
<td>Ac</td>
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</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzoyl</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphorsulfonic acid</td>
</tr>
<tr>
<td>DIBAO\textsuperscript{i}Pr</td>
<td>Di-isopropoxy-diisobutylalane</td>
</tr>
<tr>
<td>DME</td>
<td>Ethylene glycol dimethyl ether</td>
</tr>
<tr>
<td>DMF</td>
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</tr>
<tr>
<td>DMSO</td>
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</tr>
<tr>
<td>HMDS</td>
<td>1,1,3,3,3-hexamethyldisilazane</td>
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<tr>
<td>LDA</td>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
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<td>Tetrahydrofuran</td>
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<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
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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 the 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-(22S,25R)-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.

Keywords:
Antifungal compound, CAY-1, invasive fungal infections, glycoconjugate, natural product synthesis, saponins, spirostane, 2,3-branched oligosaccharide synthesis.
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%. 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. 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. This increase in incidence of invasive fungal infections in immunocompromised individuals is largely attributed to medical advancements in the treatment of these conditions.
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.\textsuperscript{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.\(^5\)

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.\(^6\)
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. 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.
A fifthazole, 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

![Chemical structure of posaconazole](7(Posaconazole).png)

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. Anidulafungin shows low toxicity and, unlike azoles, has not shown significant adverse drug-drug interactions.

![Chemical structure of Caspofungin acetate](image1)

![Chemical structure of Anidulafungin](image2)

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.\textsuperscript{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. 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.

**Figure 1.** Classes of saponins.

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. 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.\textsuperscript{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.\textsuperscript{19} It has been shown that both the aglycone and oligosaccharide moieties of saponins are important for their biological activity.\textsuperscript{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 \textit{Digitalis lanata} and marketed for over 200 years to treat congestive heart failure. Digoxin binds to Na\textsuperscript{+}/K\textsuperscript{+} 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.\textsuperscript{19}

\begin{center}
\includegraphics[width=0.5\textwidth]{digoxin.png}
\end{center}

\textbf{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 saundersiae*. 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.\(^2^5\)

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.\(^2^6\)

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

![OSW-1 (11)](image_url)
against amphotericin B-resistant strains of *Candida tropicalis*. It was synthesized in three steps from medicagenic acid and acetobromomaltose in 49% overall yield.\textsuperscript{27}

\[\text{HO} \quad \text{O} \quad \text{COOH} \quad \text{COOH} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{HO} \quad \text{HO} \quad \text{HO} \quad \text{HO} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{COOH}\]

**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).\textsuperscript{28}
Scheme 1. Isolation of CAY-1.

Ground, dried fruit of *C. frutescens* (10 g) mixed with 1% PDB and centrifuged (5000 RCF)

- **Supernatant**
  - Centrifuged (11,500 RCF)

- **Solid**
  - Discarded

- **Solid**
  - Discarded

- **Supernatant**
  - Filtered: 0.45 µm filter

- **Solid**
  - Discarded

- **Filtrate**
  - Solid phase extraction

- **0, 25, 50, 100% methanol fractions**
  - Discarded

- **75% methanol fraction**
  - Purified by HPLC

- **CAY-1** (10 mg)
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.$^{28}$

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

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

13
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 [14C]-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.\textsuperscript{29}

![Graph showing the effect of CAY-1 on fungal cell permeability]

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.\textsuperscript{16}
Figure 3. Cell membrane disruption by saponins.\textsuperscript{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, 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 $\alpha$-(1→4) linkage.

**Scheme 2.** Retrosynthesis of CAY-1.

\[ \text{R} = \text{appropriate alcohol protecting groups. } L = \text{appropriate leaving groups for glycosidic bond formation.} \]
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*. 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.
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). In this synthesis, diosgenin (20) was oxidized to \( \alpha,\beta \)-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 acetylation of 22 to form \( \alpha \)-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 LiAlH4 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
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.\textsuperscript{33-35}

![Scheme 3](image)

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 26\(\beta\) 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.
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). 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.
**Scheme 5.** Synthesis of spirost-2-ene from hecogenin acetate.

*a* Reaction conditions: (a) Hydrazine hydrate, 2-ethoxyethanol, KOH, 136°C, 98%. (b) NaH, CS₂, propargyl bromide, THF. (c) NaH, CS₂, CH₃I, 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.\textsuperscript{37,41}

Epoxidation of 27 with \textit{m}-CPBA proceeded in 94\% yield. Unfortunately, the comparison of the \textsuperscript{1}H NMR spectra of the major (91\%) and minor (3\%) isomers obtained in this reaction revealed that the 2\(\alpha\),3\(\alpha\)-epoxide 26 was the major product. Most notably, the chemical shift for the C-19 methyl group for 26\(\alpha\) was 0.75 ppm as compared to 1.04 ppm for 26\(\beta\) (Figure 4). The downfield shift for this signal in the case of 26\(\beta\) 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 \textit{m}-CPBA generally occur from the least hindered side of the alkene.\textsuperscript{42,43} In this case, the C-19 methyl renders the \(\beta\)-face of the molecule more hindered than the \(\alpha\)-face and thus the \(\alpha\)-epoxide is the major product (Scheme 7). Note that \(\beta\)-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 26$\alpha$ and 26$\beta$.

**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 $\alpha$-face nearly exclusively.

Major (91%) 26$\alpha$ + Minor (3%) 26$\beta$
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-acetoxypregest-5-en-20-one (31) to their 5β,6β-epoxides were successful in the literature using KMnO4-CuSO4. However, attempts to apply these reaction conditions to spirost-2-ene (27) were unsuccessful.

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. Additionally, the appearance of H-2 and H-3 as broad singlets in the 1H 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.*

![Scheme 9](image)

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

![Scheme 10](image)
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. Attempted synthesis of the CAY-1 aglycone via TMS enol ether 35.

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).

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.

![Scheme 12](image)

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).

**Scheme 13.** Literature reported inversion of 2° alcohols with NO_3^-.

![Scheme 13](image)

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.** Attempt to invert C-3 mesylate of 32 by an SN2 reaction with NO₃⁻.

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.

<table>
<thead>
<tr>
<th>Solvent(s)</th>
<th>Temperature</th>
<th>Time (hours)</th>
<th>% Conversion&lt;sup&gt;a&lt;/sup&gt;</th>
<th>%42&lt;sup&gt;a&lt;/sup&gt;</th>
<th>%43&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene (with N(C₄H₉)₄NO₃)</td>
<td>110°C</td>
<td>2</td>
<td>20</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Toluene (with N(C₄H₉)₄NO₃)</td>
<td>110°C</td>
<td>20</td>
<td>100</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Toluene (without N(C₄H₉)₄NO₃)</td>
<td>100°C</td>
<td>48</td>
<td>70</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Toluene, H₂O</td>
<td>100°C</td>
<td>48</td>
<td>80</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Acetone, H₂O</td>
<td>56°C</td>
<td>24</td>
<td>20</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Pyridine, H₂O</td>
<td>100°C</td>
<td>7</td>
<td>100</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

<sup>a</sup>Yields determined by <sup>1</sup>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<sub>N</sub>1-type mechanism.
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 \( S_N2 \) fashion. It was surprising but crucial to determine that mesylate 44 was reacting only by an \( S_N1 \) mechanism to form the acetate group migration products, and that no \( S_N2 \) product formation was observed. Consequently, it was clear that an \( S_N2 \) 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 \( S_N2 \) 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 SN2 product 48 was observed. Instead, 46 was regenerated in 90% yield (Scheme 17).
Scheme 17.\textsuperscript{a} Fourth attempted synthesis of the CAY-1 aglycone.

\textsuperscript{a}Reaction conditions: (a) Benzyl trichloroacetimidate, CF₃SO₃H, dioxane, 78%. (b) NaOH, MeOH, reflux, 96%. (c) MsCl, Et₃N, CH₂Cl₂, 0°C, 81%. (b) 1:4 H₂O: pyridine, reflux, 90%.

Due to the literature report of successful inversions of secondary alcohols by the reaction of corresponding mesylates with cesium acetate,\textsuperscript{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\textsubscript{N}2 mechanism. As was the case with water/pyridine, the only product observed was the S\textsubscript{N}1 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\textsubscript{N}1 rather than S\textsubscript{N}2 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
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 $\beta$-C2 alcohol to a ketone followed by reduction to the $\alpha$-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 3$\beta$-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. 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. 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.

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 NaBH₄ and LiAlH₄, 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$_4$.\textsuperscript{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.\textsuperscript{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_iPr) at room temperature in toluene in 7 hours (Scheme 21).\textsuperscript{55}
Scheme 21. Literature reported reduction of 5α-cholestan-3-one using a Meerwein-Ponndorf-Verley-type reaction.54,55

With this in mind, 3β-Benzylxy-(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 DIBAOiPr 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 1H NMR after 12 hours. It has been reported that reductions with DIBAOiPr 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 DIBAOiPr. 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\textsubscript{Pr} in toluene was then repeated but unfortunately, even after 5 days only 50% conversion was observed by \textsuperscript{1}H NMR. Additionally, the reduction was forming a mixture of axial and equatorial alcohols of 50 (Scheme 22).

**Scheme 22.**\textsuperscript{a} Fifth attempted synthesis of the CAY-1 aglycone.

\begin{center}
\includegraphics[width=0.7\textwidth]{Scheme22.png}
\end{center}

\textsuperscript{a}Reaction conditions: (a) H\textsubscript{2}CrO\textsubscript{4}, acetone, 30°C, 90%. (b) DIBAO\textsubscript{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).\textsuperscript{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.\textsuperscript{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.** Initial retrosynthesis of the CAY-1 pentasaccharide moiety.

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.
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). 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.\(^5^9\)

In ether solvents, armed donors generally give \( \alpha \)-glycosides since ethers preferentially stabilize oxonium intermediates from an equatorial attack (Scheme 26).\(^6^4\) The fact that the \( \beta \)-anomer of glucosyl ether oxonium ions is more stable than its corresponding \( \alpha \)-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).  

**Scheme 26.** Glycosylation of an armed donor in diethyl ether.  

![Glycosylation Scheme](image)

**Figure 8.** The anomeric effect.  

![Anomeric Effect](image)

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.  

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.  

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 $\sigma^*$ 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 $\alpha$-glycosides as described above, using acetonitrile as the solvent favors $\beta$-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 $\beta$-glycosides (Scheme 27).
Scheme 27. Glycosylation of an armed donor in acetonitrile.\(^69\)

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 \(\alpha\)-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\(_3\)·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.

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).\textsuperscript{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\textsuperscript{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.\textsuperscript{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**. Synthesis of linear trisaccharide portion of CAY-1 pentasaccharide.

\[ \text{66 (D-Glucose)} \rightarrow \text{67} \rightarrow \text{68} \rightarrow \text{69} \rightarrow \text{70} \rightarrow \text{71, 72, 73} \rightarrow \text{74} \rightarrow \text{75} \rightarrow \text{76, 77} \]

\[ \text{a} \quad \text{b} \quad \text{c} \quad \text{d} \quad \text{e} \]

\[ \text{Reaction conditions: (a) BzCl, Py, 56\% (67), 78\% (72), 49\% (77), (b) 2-aminoethanol, DMSO, EtOAc, 70\% (68), 40\% (73), (c) Cl}_3\text{CCN, K}_2\text{CO}_3, \text{DCM, 48 h, 83\% (69), 94\% (74), (d) 65, BF}_3\text{OEt}_2, \text{DCM, 53\% (70), 36\% (75), (e) TFA, THF/H}_2\text{O, 86\% (71), 67\% (76).} \]

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. Preparation of 1,3,4,6-tetra-O-acetyl-α-D-glucopyranose.

![Scheme 30](image)

*Reaction conditions: (a) Ac₂O, Py, 83%, (b) 2-aminoethanol, DMSO, EtOAc, 70%, (c) (1-chloro-2-methylpropenyl)-dimethylamine, CHCl₃, (d) H₂O, 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 moity 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. 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). 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 \textit{et al.}\textsuperscript{86}

In this new design, the CAY-1 pentasaccharide moiety \textbf{88} would be prepared from the glycosylation between thioglycoside donor \textbf{89} and galactosyl acceptor \textbf{90} (Scheme 33). Although tetrasaccharide donor \textbf{89} does not have a C-2 participating group to aid in favoring a (1,2)-\textit{trans} linkage, the results reported by Linhardt \textit{et al.} suggested that a stereoselective glycosylation might be achieved in this step. In their case, Linhardt \textit{et al.} reported success at favoring a $\beta$-glycosylation between armed thioglycoside donor \textbf{86} and diosgenin (Scheme 32).\textsuperscript{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 tetrasccharide 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 tetrasccharide 89 would be a more practical approach as compared to the previously described linear retrosynthesis. Using partially protected glucosyl acceptor 84, tetrasccharide 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 $\beta$-D-glucose pentaacetate 78 in three steps and 51% yield using literature procedures with minor modifications (Scheme 34). $^{88,89}$ $\beta$-D-glucose pentaacetate 78 reacts with 2-propanethiol in the presence of BF$_3$·OEt$_2$ to give the $\beta$-anomer of thioglucopyranoside 92.

**Scheme 34.** Synthesis of 2,3-branched trisaccharide 94.

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 $\alpha$- and $\beta$-
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 tetrasaccharide 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. 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).
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.\textsuperscript{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$_2$Cl$_2$, 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 (1.0 g, 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), 3.37 (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,
(22S,25R)-5α-Spirost-2-ene (27)\textsuperscript{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 NH₄Cl (40 mL), extracted with CH₂Cl₂ (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 \textsuperscript{1}H NMR of 28 (500 MHz, CDCl₃): \(\delta\) 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 \textsuperscript{1}H NMR (300 MHz, CDCl₃): \(\delta\) 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). \textsuperscript{13}C NMR (400 MHz, CDCl₃): \(\delta\) 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 NH₄Cl (40 mL), extracted with CH₂Cl₂ (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, CDCl₃): 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 CH₂Cl₂ (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 % Na₂SO₃ (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 CH₂Cl₂ (3 x 100 mL). The organic phases were combined and washed with saturated
NaHCO₃ (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 \( \text{26} \alpha \) as a white solid (4.2 g, 91 % yield). Selected \(^1\text{H NMR}\) of \( \text{26} \alpha \) (400 MHz, CDCl\(_3\)): \( \delta \) 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). \(^{13}\text{C NMR}\) of \( \text{26} \alpha \) (400 MHz, CDCl\(_3\)): \( \delta \) 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. \(^1\text{H NMR}\) \( \text{26} \beta \) (300 MHz, CDCl\(_3\)): \( \delta \) 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).

3\(\alpha\)-Hydroxy-(22S,25R)-5\(\alpha\)-spirostan-2\(\beta\)-yl acetate (32): Epoxide \( \text{26} \alpha \) (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 \( \text{32} \) as a white solid (77 mg, 84 % yield). Selected \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)): \( \delta \) 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). \(^{13}\text{C NMR}\) (400 MHz, CDCl\(_3\)): \( \delta \) 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)-5\(\alpha\)-Spirostan-3-one (34): Tigogenin (17) (1 g, 2.40 mmol) was dissolved in acetone (50 mL) at 30°C. 8N Chromic acid in 40% \( \text{H}_2\text{SO}_4 \) (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 1H NMR (400 MHz, CDCl3): 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). 13C NMR (400 MHz, CDCl3): 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): 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 NaHCO3 solution (10 mL) were added and the mixture was extracted with CH2Cl2 (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 1H NMR (400 MHz, CDCl3): 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 \( \text{CH}_2\text{Cl}_2 \) (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 \( \text{CH}_2\text{Cl}_2 \) (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\(_3\)): \( \delta \) 4.99 (br s, 1H), 4.68 (br s, 1H), 4.40 (q, \( J = 11.6 \text{ Hz} \), 1H), 3.48 (m, 1H), 3.37 (t, \( J = 11.1 \text{ Hz} \), 1H), 3.08 (s, 3H), 0.96 (d, \( J = 3.5 \text{ Hz} \), 3H), 0.94 (s, 3H), 0.79 (d, \( J = 3.2 \text{ Hz} \), 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\(_3\)): \( \delta \) 4.78 (m, 1H), 4.39 (q, \( J = 11.2 \text{ Hz} \), 1H), 4.10 (br s, 1H), 3.66 (m, 1H), 3.38 (t, \( J = 10.8 \text{ Hz} \), 1H), 2.09 (s, 3H), 1.06 (s, 3H), 0.96 (d, \( J = 3.4 \text{ Hz} \), 3H), 0.79 (d, \( J = 3.0 \text{ Hz} \), 3H), 0.77 (s, 3H). \(^{13}\)C NMR of 42 (400 MHz, CDCl\(_3\)): \( \delta \) 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\(_3\)): \( \delta \) 5.14 (br s, 1H), 4.39 (q, \( J = 11.4 \text{ Hz} \), 1H), 3.69 (m, 1H), 3.47 (m, 1H), 3.37 (t, \( J = 10.8 \text{ Hz} \), 1H), 2.10 (s, 3H), 0.96 (d, \( J = 3.4 \text{ Hz} \), 3H), 0.93 (s, 3H), 0.79 (d, \( J = 3.0 \text{ Hz} \), 3H), 0.76 (s, 3H). \(^{13}\)C NMR of 43 (400 MHz, CDCl\(_3\)): \( \delta \) 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 CH₂Cl₂ (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 CH₂Cl₂ (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-Benzyl oxy-(22S,25R)-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 NaHCO₃ (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-Benzyl oxy-(22S,25R)-5α-spirostan-2β-yl acetate (47) as a white solid which was used immediately in the next reaction (83 mg, 78 % yield). Selected ¹H NMR of 47 (400 MHz, CDCl₃): 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,
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$_2$Cl$_2$ (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$_2$Cl$_2$ (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-Benzylxy-(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$_2$SO$_4$ (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$_3$): $\delta$ 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)**: 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$_3$): $\delta$ 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$_3$): $\delta$ 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)**: 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$_2$Cl$_2$ followed by Et$_2$O) to give $\mathbf{68}$ as a white solid (6.0 g, 70 % yield). 100 mg of the product was recrystallized from diethyl ether/hexanes for $^1$H and $^{13}$C NMR analysis. $^1$H NMR (300 MHz, CDCl$_3$) $\mathbf{68}$$\beta$: $\delta$ 8.19-7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J=9.9$ Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1=6.45$ Hz, $J_2=1.2$ Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1=8.6$ Hz, $J_2=2.4$ Hz, 1H), 3.12 (d, $J=1.3$ Hz, 1H). $^{13}$C NMR of $\mathbf{68}$$\beta$ (CDCl$_3$): $\delta$ 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-$\alpha$-$D$-glucopyranosyl)-trichloroacetimidate ($\mathbf{69}$)$^{77}$: Tetrabenzoyl-$D$-glucopyranose $\mathbf{68}$ (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 $\mathbf{69}$ as a pale yellow solid (2.0 g, 83 % yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 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$_3$): $\delta$ 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-$\beta$-$D$-glucopyranosyl-(1$\rightarrow$3)-1,2:5,6-di-$O$-isopropylidene-$\alpha$-$D$-glucofuranose ($\mathbf{70}$)$^{75}$: Trichloroacetimidate $\mathbf{69}$ (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$_3$): $\delta$ 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$_3$): $\delta$ 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$_3$): $\delta$ 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, 2H), 2.61 (t, $J$ = 3.8 Hz, 2H), 2.61 (m, 2H), 2.61 (s, 6H). $^{13}$C NMR (400 MHZ, CDCl$_3$): $\delta$ 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.
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, 129.0, 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-$\text{O}$-benzoyl-$\beta$-D-glucopyranosyl-(1→3)-tetra-$\text{O}$-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-$\text{O}$-benzoyl-$\beta$-D-glucopyranosyl-(1→3)-2,4,6-tri-$\text{O}$-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 (CH₂Cl₂ followed by Et₂O) to give 73 as a white solid (1.2 g, 40 % yield). ¹H NMR of 73α (400 MHz, CDCl₃): 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, J₁ = 5.2 Hz, J₂ = 1.8 Hz, 1H), 4.95 (d, J = 3.8 Hz, 1H), 4.71 (dd, J₁ = 6.2 Hz, J₂ = 1.4 Hz, 1H), 4.62 (m, 1H), 4.49 (m, 1H), 4.39 (dd, J₁ = 6.0 Hz, J₂ = 2.2 Hz, 1H), 4.13 (m, 2H). ¹³C NMR of 73α (400 MHz, CDCl₃): 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, 130.0, 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 CH₂Cl₂ (20 mL) at room temperature. The resulting suspension was filtered through silica gel using a 1:1 mixture of CH₂Cl₂ and Et₂O (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. ¹H NMR of 74 (300 MHz, CDCl₃): 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). Trichloroacetimdate 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 CH₂Cl₂ (20 mL) with 3 Å molecular sieves for 1 hour. The solution was cooled in an icebath for 30 minutes before BF₃·OEt₂ (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 (CH₂Cl₂: 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. ¹H NMR of 75 (400 MHz, CDCl₃): 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). ¹H NMR of 76 (400 MHz, CDCl₃): 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, J₁ = 4.9 Hz, J₂ = 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, J₁ = 6.1 Hz, J₂ = 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). ¹³C NMR of 76 (400 MHz, CDCl₃): 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 CH₂Cl₂ (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 CH₂Cl₂:Et₂O) to give 77 as a white solid (86 mg, 49 % yield). ¹H NMR (400 MHz, CDCl₃): 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, J₁ = 5.0 Hz, J₂ = 4.0 Hz, 1H), 4.03 – 3.76 (m, 3H), 3.46 (m, 1H). ¹³C NMR of 77a (400 MHz, CDCl₃): 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,
Penta-O-acetyl-D-glucopyranose (78): 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).

β: 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 78 as a white solid (3.7 g, 38% yield).

2,3,4,6-tetra-O-acetyl-D-glucopyranose (79): 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, $J_2 = 1.5$
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 CH₂Cl₂ with 3 Å molecular sieves for 30 minutes under an atmosphere of nitrogen. BF₃·OEt₂ (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% NaHCO₃ (50 mL) with vigorous stirring. The organic layer was separated, washed with 5% NaHCO₃ (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. \( ^1H \) NMR (400 MHz, CDCl₃): \( \delta \) 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, CDCl₃): \( \delta \) 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:CH₂Cl₂ (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. \( ^1H \) NMR (300 MHz, DMSO- D₆): \( \delta \) 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$_6$): $\delta$ 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)**:

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$_3$): $\delta$ 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$_3$): $\delta$ 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-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)3)-(\(R\))-4,6-\(O\)-benzylidene-1-thio-\(\beta\)-D-glucopyranoside (85) which was used immediately in the following reaction. \(^1\)H NMR of 85 (400 MHz, CDCl\(_3\)): \(\delta\) 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 \(\mu\)L, 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\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) 166.5, 166.1, 166.0,
1,2,3,6-Tetra-\(O\)-benzoyl-\(\alpha\)-D-galactopyranose (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\(\text{2}\)Cl\(\text{2}\) (3 x 100 mL). The organic layers were combined and washed with 2M H\(\text{2}\)SO\(\text{4}\) (100 mL), followed by saturated aqueous NaHCO\(\text{3}\) (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\(\text{3}\)): \(\delta\) 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\(\text{3}\)): \(\delta\) 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, 128.6, 128.5, 128.4, 128.4, 128.4, 91.0, 71.1, 70.9, 67.7, 67.4, 63.0.

Tetra-\(O\)-acetyl-\(\alpha\)-D-glucopyranosyl-(1\(\rightarrow\)4)-tetra-\(O\)-benzoyl-\(\alpha\)-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\(\text{2}\)Cl\(\text{2}\) (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). ¹H NMR (400 MHz, CDCl₃): δ 8.17 – 7.23 (m, 20H), 6.80 (d, J = 2.0 Hz, 1H), 6.05 (dd, J₁ = 5.4 Hz, J₂ = 2.0 Hz, 1H), 5.74 (d, J = 2.4 Hz, 1H), 5.62 (dd, J₁ = 5.4 Hz, J₂ = 1.6 Hz, 1H), 4.92 (t, J = 3.6 Hz, 1H), 4.77, (dd, J₁ = 4.4 Hz, J₂ = 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, J₁ = 6.2 Hz, J₂ = 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): 2,3,4,6-Tetra-O-acetyl-D-glucopyranose 79 (2.0 g, 3.3 mmol), K₂CO₃ (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 CH₂Cl₂ and Et₂O (150 mL). The filtrate was concentrated to give 97 as a pale yellow solid (2.0 g, 83 % yield). ¹H NMR (400 MHz, CDCl₃): 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, J₁ = 6.8 Hz, J₂ = 2.0 Hz, 1H), 4.24 (dd, J₁ = 6.2 Hz, J₂ = 2.0 Hz, 1H), 4.18 (m, 1H), 4.10 (dd, J₁ = 6.0 Hz, J₂ = 1.0 Hz, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H). ¹³C NMR (400 MHz, CDCl₃): 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-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)4)-tetra-\(O\)-benzoyl-\(\alpha\)-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\(_2\)Cl\(_2\) (50 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0\(^\circ\)C before TMSOTf (41 \(\mu\)L, 0.18 mmol) was added. The reaction was stirred for 3 hours at 0\(^\circ\)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\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) 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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agent for reduction of cyclic ketones to thermodynamically more stable alcohols.

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oligosaccharides- Are there alternatives to the Koenings-Knorr method? *Angew.


APPENDIX

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

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

Figure 12. $^{13}$C NMR of 17 (400 MHz, CDCl$_3$).
Figure 13. $^1$H NMR of 26α (400 MHz, CDCl₃).

Figure 14. $^{13}$C NMR of 26α (400 MHz, CDCl₃).
Figure 15. $^1$H NMR of 26β (400 MHz, CDCl₃).

Figure 16. $^1$H NMR of 27 (400 MHz, CDCl₃).
Figure 17. $^{13}$C NMR of 27 (400 MHz, CDCl$_3$).

![13C NMR spectrum of 27](image1)

Figure 18. $^1$H NMR of 28 (500 MHz, CDCl$_3$).

![1H NMR spectrum of 28](image2)
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. $^1$H 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. $^1$H 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. $^1$H 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$_3$).
Figure 48. $^1$H NMR of 77 (400 MHz, CDCl$_3$).

Figure 49. $^{13}$C NMR of 77 (400 MHz, CDCl$_3$).
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, CDCl₃).

Figure 59. $^1$H NMR of 90 (400 MHz, CDCl₃).
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$_3$).

![Chemical structure of 92]

Figure 63. $^1$H NMR of $93$ (400 MHz, CDCl$_3$).

![Chemical structure of 93]
Figure 64. $^{13}$C NMR of 93 (400 MHz, CDCl₃).

Figure 65. $^1$H NMR of 94 (400 MHz, CDCl₃).
Figure 66. $^1$H NMR of 96 (400 MHz, CDCl$_3$).

![Figure 66](image)

Figure 67. $^1$H NMR of 97 (400 MHz, CDCl$_3$).

![Figure 67](image)
Figure 68. $^1$H NMR of 98 (400 MHz, CDCl$_3$).
### Table 3. Crystal data and structure refinement for 32.

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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.

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Symmetry transformations used to generate equivalent atoms:

Table 6. Anisotropic displacement parameters (Å² x 10³) for 32. The anisotropic displacement factor exponent takes the form: -2π^2 [ h^2 a^*^2 U11 + ... + 2 h k a^* b^* U12 ]

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

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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.