Design and Synthesis of *Quillaja* Saponin Adjuvants and Synthesis of Lablaboside Saponins

by

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A Dissertation

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David Y. Gin, PhD and Derek S. Tan, PhD   Date 12/12/2013
Dissertation Mentors

Dedicated To Professor David Y. Gin

Alle Ding' sind Gift, und nichts ohn' Gift;

allein die Dosis macht, daß ein Ding kein Gift ist.

Paracelsus

ABSTRACT

The clinical success of conjugate anticancer and antiviral vaccines critically depends on the identification of, and access to, novel potent adjuvants with minimal toxicity. In this context, the saponin QS-21 is currently the most promising immunopotentiator in several anti-tumor and infectious disease vaccine therapies. However, the clinical use of this scarce natural product is encumbered by its chemical instability and dose-limiting toxicity. To address these liabilities, the first chemical synthesis was constructed in a modular fashion, which facilitated the creation of a substantial library of non-natural saponins. Thus far, many analogues incorporating variants of the acyl chain and central tetrasaccharide have decreased toxicity and maintained or exceeded potency of the naturally derived saponin. To explore structure-activity relationships and improve the efficiency of the triterpene-central tetrasaccharide coupling, alternatives to the natural glycosidic ester linkage were explored. Eight structural variants were synthesized and probed for adjuvanticity using a previously established vaccination schedule. Surprisingly, efficacy and toxicity varied greatly with very conservative structural modifications, thus highlighting an essential motif in the structure-activity relationships of the synthetic *Quillaja saponaria* family of saponins adjuvants.

The lablaboside saponins have been identified as promising adjuvants isolated from the Hyacinth bean. Initial studies suggested Lablaboside F in particular is both more potent than QS-21 and devoid of significant dose-dependent hemolytic toxicity. To achieve the total synthesis of Lablaboside F, stereoselective glycosylation of oleanolane-type triterpenes with a C-2 substituted glucuronic acid donor was accomplished with

Tris(pentafluorophenyl)borane. Application of the rarely-utilized benzophenone ketal protecting group for the three rhamnose moieties allowed for a successful and highly efficient global deprotection. Additionally, oxidation of the C24 methyl group present in Lablabosides B-E was effected in three steps from benzyl oleanolate culminating in a thiolate mediated diastereoselective tandem Micheal–aldol reaction.

BIOGRAPHY

William (Bill) Walkowicz was born and raised in southeastern Wisconsin. From an early age he showed an eager competitiveness across many academic disciplines and athletic endeavors. Once in secondary school, he became focused on two subjects that would remain with him indefinitely: the natural sciences and long distance running. His first chemistry class was a transformative experience, fulfilling his need for quantitative analysis but also confronting the messy reality of chemical and biological sciences. After achieving several high honors in high school, including state championships in Academic Decathlon, Cross Country (Team), and Track and Field (Relay), as well as the Wisconsin Scholar-Athlete of the Year, he matriculated to the University of Wisconsin-La Crosse where he could continue pursuing both passions.

Bill quickly became one of the top students in both chemistry and molecular biology by starting his research career in the laboratory of his *Organic Chemistry Theory I* professor, Dr. Aaron Monte, where he made valuable contributions to a variety of projects, including synthesis of a metabolic enzyme inhibitor, a novel antibiotic, and 5-HT$_{2A/C}$ receptor agonists. Throughout his time in La Crosse, Bill was a core member of the perennial powerhouse Cross Country and Track and Field Teams. Indeed, in his final year he was voted Captain of the Cross Country team, named the Wisconsin Intercollegiate Athletic Conference Scholar Athlete of the Year, and helped lead the team to the victory at the Division III Cross Country National Championships. Two weeks after graduating summa cum laude, Bill ran his final collegiate race, the 3000m Steeplechase, helping his team win the Division III Outdoor Track and Field Championship.

In the fall of 2006, Bill left beautiful La Crosse, WI for sunny San Diego, CA at the University of California-San Diego, where he earned a Masters Degree in Organic Chemistry. The laboratory of Professor Michael Burkart served as an excellent training ground for synthetic organic chemistry as well as projects in chemical biology. After two years, scores of burritos, and exactly two trips to the beach, Bill left the west coast to pursue more pressing issues, both romantic and scientific in New York City at the Gerstner Sloan-Kettering Graduate School.

Bill drove with his future wife and faithful navigator, Hillary Fry, from sea to shining sea, to begin an urban adventure with as much as the two mid-westerners could cram in a rented mini-van. After a couple brief forays into biochemistry and immunology, Bill eagerly joined the lab of Professor David Y. Gin to begin his studies of saponin immunoadjuvants. In addition to his success in the laboratory, Bill continued to improve in long-distance running, achieving lifetime bests in every distance over 5 km, as well as winning a handful races in Central Park and Brooklyn. Moreover, he led the MSKCC Corporate Challenge running team to the JP Morgan Chase Corporate Challenge World Championships in 2012, where the mixed team bested squads from around the globe to capture the championship.

In his final year as a student, Bill and Hillary were married in Wisconsin. Upon returning to New York City just as they had arrived 5 years earlier (with a car packed to the gills), Bill sat down and wrote his thesis. After four years in the Gin group, he had completed his graduate studies with the synthesis of several QS-21 analogues and two members of the lablaboside family of saponins. He will take his skills in carbohydrate chemistry across the street to the Danishefsky laboratory for his post-doctoral work.

ACKNOWLEDGEMENTS

First and foremost I would like to thank Professor David Gin for accepting me into his laboratory, having confidence in my abilities as a scientist, and illustrating the qualities of an admirable scientist with enthusiasm. He will be missed.

I would also like to thank Professor Derek Tan for serving as my advisor for the second half of my thesis work. Additionally, many thanks to my committee members Professor Sam Danishefsky, and Professor Morgan Huse, as well as the chair of my thesis committee, Professor Minkui Luo for helpful guidance throughout my graduate work.

All of the work in the lab was accomplished with helpful guidance from Dr. Eric Chea, Rashad Karimov, Dr. Bryan Cowen, Dr. Lars Nordstroem, and Dr. Alberto Fernandez-Tejada as well as with fruitful collaboration with Dr. Phillip O. Livingston, Dr. Govind Ragupathi, and George Constantine. Dr. David Y. Gin conceived of the project and provided intellectual advice until his death in 2011. Dr. Derek Tan provided guidance to complete the projects.

In mostly chronological order, I would like to say thank you to several secondary educators. Don Lamb, Tony Bralick, Linda Carlson, Lee Schmidt, Duane Stein, and Babs Merkert provided many challenging classes and academic opportunities in suburban Waukesha, providing excellent preparation for my collegiate studies.

I am indebted to Professor Aaron Monte, whose class, *Organic Chemistry Theory*, served as a major inflection point in my scientific career. Moreover, working in his laboratory with like-minded individuals, such as Dr. Dani Schultz, bolstered my interest in organic chemistry and inspired me to pursue advanced degrees in chemistry.

Additionally, Professor Kenneth Maly and Daniel T. Haumschild were essential components of my well-rounded undergraduate education.

In my time at the University of California-San Diego, I was surrounded by a plethora of brilliant and colorful mentors. Professor Michael D. Burkart facilitated an environment fostering independent growth and collaboration among all the group members. Specifically, Dr. Andrew Mercer, Professor Jordan Meier, Dr. Brian D. Jones, Dr. Timothy Foley, Dr. Andrew Worthington, Dr. Gene Hur, Dr. Alex Mandel, and Dr. Robert Haushalter were instrumental in molding my initial habits as a chemist and a scientist.

I have been very fortunate to be surrounded by many talented, hard working, and fun colleagues in the Gerstner Sloan–Kettering Graduate School. Specifically, I would like to thank my class-mate and student council co-chair Dr. Ellen Hukkelhoven, my collaborator and good friend Dr. Nicholas Gauthier, as well as the next generation of social and professional leaders of the graduate school, Marta Kovatcheva and Jenny Karo. Their efforts in cultivating a socially dynamic and engaging atmosphere have been appreciated.

Similarly, all of my colleagues in the Gin group have served as excellent mentors for a young scientist. Dr. Shelly Adams, Dr. Michael Bultman, Dr. Eric Chea, Dr. Bryan Cowen, Dr. Alberto Fernandez-Tejada, Rashad Karimov, Dr. Michael Krout, Dr. Pingfan Li, Dr. Lars Nordstroem, Nathan Park, Dr. Daniel Pla, Dr. Nicholas Perl, Dr. Sudeep Prajapati, Dr. Troy Reynolds Dr. Yuan Shi, and Dr. Matthew Volgraf. I would especially like to thank my bay-mates Dr. Nick Perl and Dr. Daniel Pla for excellent conversations as well as patient instruction for a new Gin lab chemist. Finally, I owe a

big thank you to the final member of the Gin lab, Rashad Karimov, for all the wide-ranging discussions, helpful advice, and friendship over the final two years of my thesis work.

Many other people have made significant contributions to my thesis work. I owe at least a small debt of gratitude to Albert, Cheryl, Lana Del Rey, Robyn, Rihanna, and Sasha for providing a great deal of energy and enthusiasm for experiments from before dawn until the short period of time when the City that Never Sleeps finally goes to bed.

I would also like to thank my family. My parents Ed and Patti, as well as my sisters Jen, Kate and Bec have been very supportive and were early instigators of my intellectual curiosity. Moreover, my meta-family, Dr. Raymond Biersbach, Dr. Rachel Gottschalk, Daniel T. Haumschild, David Kriebel, and Dr. Michael X. Macrae, has been a continual inspiration to be a better scientist, human being, and global citizen.

Finally, and most importantly, thank you to my wife, Hillary for her compassion, understanding, creativity, love, and support.

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LIST OF ABBREVIATIONS

Ac: Acetate

Api: Apiose

Bn: Benzyl

Bz: Benzoyl

CSA: Camphorsulfonic acid

DAMP: Danger Associated Molecular Pattern

DBU: 1,8-diazabicylcoundec-7-ene

Hmg: Hydroxymethylgulatoyl

Fuc: Fucose

Gal: Galactose

Glc: Glucose

GlcA: Glucuronic acid

KLH: Keyhole limpet hemocyannin

NMR: Nuclear magnetic resonance

OVA: Ovalbumin

PAMP: Pathogen Associated Molecular Pattern

Ph: Phenyl

PPS: Protected prosapogenin

PS: Prosapogenin

QS: *Quillaja* saponin

Rha: L-Rhamnose

RP-HPLC: Reverse Phase High Pressure Liquid Chromatography

Xyl: Xylose

SAR: Structure–Activity Relationships

TBAF: tetrabutylammonium fluoride

TBP: 2,4,6-tri-*tert*-butylpyridine

TBS: *tert*-butyldimethylsilyl

TES: Triethylsilyl

TIPS: Triisopropylsilyl

Tf: Triflate

TFA: Trifluoroacetic acid

Tr: Trityl

CHAPTER 1.

MEDICINAL SAPONINS – PROPERTIES, FUNCTIONS, AND SYNTHESIS

1   Introduction

Plant-derived natural products with soap-like properties, known as saponins, have played an important role in medicine and daily life for thousands of years.[1] When used as a food additive, saponins exhibit antifungal and antiprotozoal effects and function as preservatives. Additionally, some saponin mixtures, such as those derived from hot water extraction of the bark of the *Quillaja saponaria* tree have been used as a general tonic for any ailment in South American folk medicine. These crude mixtures contained a vast array of related structures with varying contributions to the observed biological activities. Systematic examination of the components of saponin mixtures has allowed for the discovery of the active principles, which have found myriad applications in modern medicine, including as anti-hyperlipidemics, anti-inflammatories, and antibiotics, which will be discussed in section 1.1. The most important and widely investigated use of saponins, as immunopotentiators, will be discussed at the end of section 1.2.
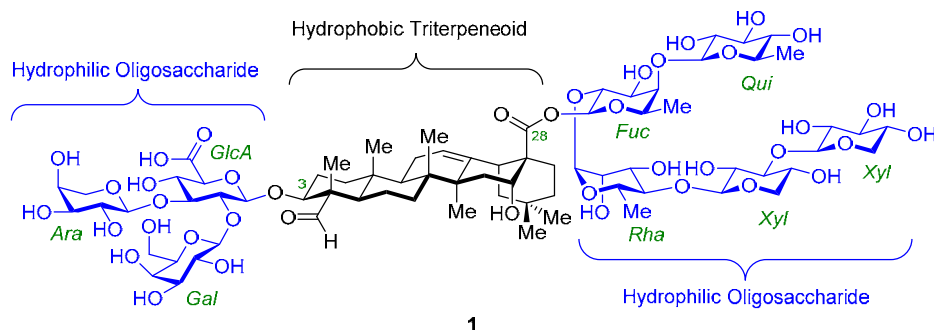
Figure 1.1. Structure of *A. gypsophiloides* saponin 1.

1.1   Features of Saponins

The defining structural feature of a saponin is a hydrophilic glycoside or oligosaccharide attached to a hydrophobic aglycone, which gives rise to the amphiphilic soap-like properties. In general, the oligosaccharide portion consists most frequently of glucose, galactose, glucuronic acid, rhamnose, xylose, and arabinose moieties, which can be either branched or linear.[2] The sugar portion is, in almost all cases, attached at C3 of a triterpenoid or steroid aglycone giving rise to a large number of monodesmosides, in which there is only one hydrophilic moiety attached to the aglycone. Additionally, bisdesmoside saponins, as seen in *A. gypsophiloides* saponin 1 (**1**), have a second hydrophilic moiety, most frequently attached to C28 of the triterpeneoid aglycone. The incredible number and diversity, both in structure and function, of saponin natural products arises from the combination of non-template driven creation of the oligosaccharide portion and a large and ever increasing number of hydrophobic triterpenoids. Indeed, a recent review catalogued nearly 600 distinct aglycones isolated and characterized in 2011 alone.[3] Thus, combining the thousands of known triterpenoids with the number of possible oligosaccharides, as well as other sugar modifications (such as acylation), gives rise to millions of potential saponins.
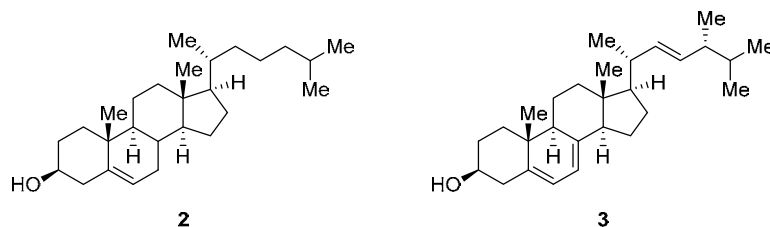
Figure 1.2. Structure of cholesterol (**2**) and ergosterol (**3**).

The biological activity of saponins arises in large part from their amphiphilic physiochemical properties, which facilitate interaction with the hydrophobic components

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of membranes, such as cholesterol (**2**), erogosterol (**3**) other sterols.[4] These interactions can take many forms, including insertion of the saponin into the membrane, abstraction of sterols from the membrane, and binding to membrane proteins. In addition to this general phenomenon, many saponins have been shown to have functions that have very little to do with their soap-like properties exerting effects more akin to ligand–receptor interactions than simple disruption of the cellular membrane.[5] Thus, when dissecting observed biological activities, one must be mindful of both general and specific intermolecular interactions.

1.1.1   General Effects of Saponins on Biological Systems

The most commonly observed effect of saponins on living cells is the formation of pores in the cellular membrane. Indeed, hemolysis of erythrocytes is a frequently employed means for detection of saponins. Pore formation is thought result from saponin–cholesterol aggregation, giving rise to large pores, 40–50 Å in diameter with micelle-like properties.[6] In support of this hypothesis is the observation that membranes rich in cholesterol are permeabilized much easier than cholesterol-free membranes.[7]

In addition to simple formation of pores, saponin interaction with membrane sterols can result in lipid abstraction from the membrane, which leads to an increase in membrane fluidity. Similarly, interaction with existing membrane pores, such as Na^+, K^+, Ca^{2+}, and Mg^{2+} ion-channels has been observed,[8] showing both agonist and antagonist activity.[9] Moreover, the presence of an acyl group on the glycoside moiety has been shown to have a strong effect on membrane permeablizing properties of saponins as seen between escin IV (**4**), which is hemolytic, and escin IIa (**5**), which is only hemolytic at high concentrations.[10] However, these effects, like the aforementioned

structure–activity relationships (SAR), can only be reliably described within a family of saponins. As such, in saponin molecules, variation of multiple structural features results in broad changes in biological activity, thus precluding generalization of SAR.
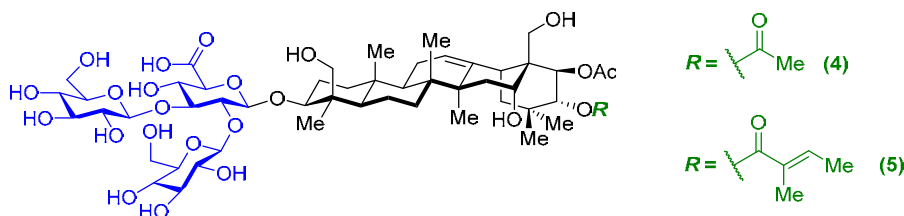
Figure 1.3. Structure of escin IV (**4**) and escin IIa (**5**).

Attempts to associate structural features broadly with specific mechanisms of action and biological activity have been generally unsuccessful, rife with contradictory and incorrect conclusions. As such, the most general statement that can be made is that saponins have been shown to interact in many ways with membrane lipids and transmembrane channels in both non-specific (e.g. lipid aggregation) and specific (e.g. ion-channel blocking) ways. The diversity of structures and functions precludes further generalization of SAR, especially with respect to the non-specific lipid aggregation/membrane permeabilizing effects.[11]

1.1.2 Anti-Hyperlipidemics

Saponins of many varieties have been shown to decrease serum cholesterol, one of the most important indicators of atherosclerosis, in both human[12] and animal subjects.[13] There are likely several modes of action responsible. The most basic mechanism involves aggregation of saponins with bile salts in the gut, thereby inhibiting cholesterol absorption into the blood and resulting in increased excretion of dietary lipids and bile salts.[14] As such, this effect is seen most prominently with a high cholesterol diet.[15] By contrast, the synthetic saponin β-tigogenin-cellobioside (**6**) was shown to

decrease non high-density lipoprotein (HDL) cholesterol levels in the serum and liver without interfering with bile salt absorption, suggesting a more specific mode of action than previously described.[16] A similar monodesmoside found in tomatoes, esculeoside A (**7**), was shown to inhibit acetylases necessary for incorporation of cholesterol into low-density lipoprotein (LDL),[17] which is partially responsible for the formation of atherosclerotic plaques.
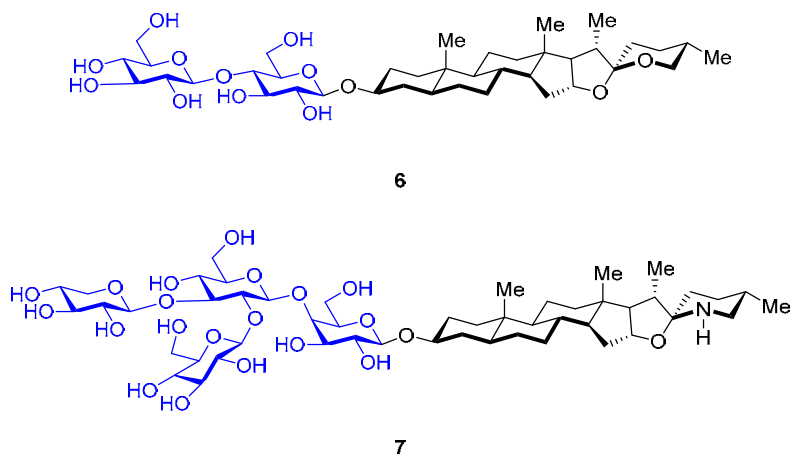
Figure 1.4. Structure of tiqueside (**6**) and tomatine (**7**).

A study comparing the efficacy of feeding several families of saponins,[18] including *Quillaja*, soybean, Karaya root, and tea saponins to rats on a high cholesterol diet found that Karaya root saponins both increased HDL and lowered LDL cholesterol, while the *Quillaja* saponins were the only group to statistically lower serum triglyceride levels. In the study all saponin supplementation decreased LDL cholesterol, but only Karaya root and *Quillaja* saponins elicited a decrease in the overall atherosclerotic index. However, the role of dietary saponins on cholesterol is complicated by the potential actions of gut microflora on the complex structure of the saponins. Indeed, supplementation of soyasaponins (including soyasaponin A_1, **8**) in the diet of Syrian hamsters gave an overall decrease in serum cholesterol.[19] However, upon closer

inspection, it was found that two distinct groups emerged, one that had a large decrease in serum cholesterol and lowering of cholesterol to HDL ratio and one group that did not. The former groups' fecal matter was found to contain much higher amounts of a saponin metabolite, the result of gut-derived microbial modification. Although the specific mechanism is unclear, these results suggest that gut microbes affect cholesterol metabolism and subsequent association with saponins which leads to eventual excretion of dietary lipids. Overall, dietary supplementation of saponins or consumption of saponin-rich foods may play an important role in reduction of serum cholesterol and thus atherosclerosis and other cardiovascular diseases.

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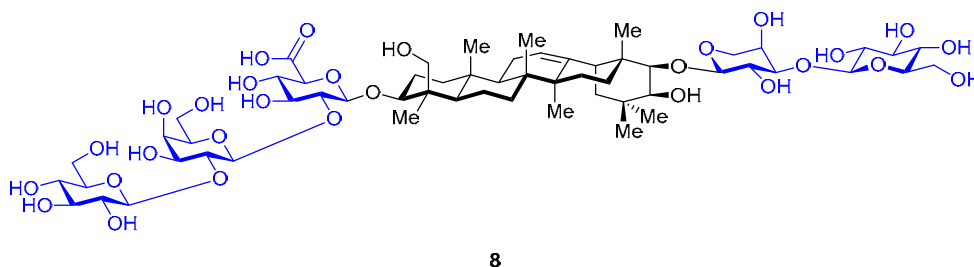
Figure 1.5. Structure of soyasaponin A$_1$ (**8**).

1.1.3 Antifungal Activity

Specific antifungal activity of saponins generally follows a similar mechanism as previously described: interaction with ergosterol (**3,** Figure 1.2) resulting in permeabilization. While oral administration or systemic distribution of saponins for this function is a difficult prospect, CAY-1, a saponin isolated from Cayenne pepper, has been shown to synergize with the extraordinarily toxic antifungal amphotericin B (**9**) and may provide a viable alternative to the conventional antifungal therapy.[20]
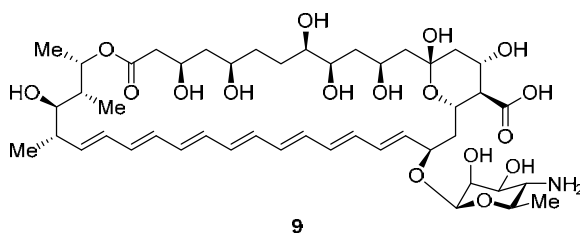
Figure 1.6. Structure of amphotericin B (**9**).

In a more straightforward application of saponins, topical antifungal drugs have also been explored but with little success.[21] However, a potentially more effective application for antifungal saponins is food preservation. Indeed, saponins from the succulent *Yucca schidigera*, such as shidigera saponin A1 (**10**, Figure 1.7), prevent growth of fungi responsible for food spoilage at levels too low for systemic effects or significant alteration of flavor.[22] The type of concentrations required to prevent food spoilage are similar to those found in many plants themselves. As such, it has been postulated that saponins evolved as secondary metabolites as a defense mechanism against deleterious fungi.[23] These suggestions are supported by two observations: plants with high concentrations of saponins are disease resistant and plants with mutant forms of saponin biosynthetic enzymes are more susceptible to fungal or microbial attack.[24]
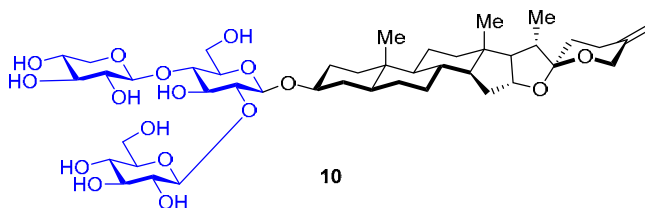
Figure 1.7. Structure of Yucca shidigera 1 (**10**).

1.1.4 Anti-Protozoal Activity

In much the same way as the saponins control growth of non-commensal fungi, saponins from the succulent *Yucca schidigera* such as (**10**) have been shown to protect humans and animals from parasitic protozoans.[25] Interestingly, this effect was uncovered

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by anecdotal reports of reduced joint pain in patients with rheumatoid arthritis. It was observed that both dried whole *Y. schidigera* root and the known anti-parasitic drug metronidazole relieved joint pain, thus it was suspected that the joint pain was caused by a parasitic protozoan. Confirmation of this mechanism was provided by the presence then clearance of joint-resident protozoa with metronidazole.[26] Furthermore, both *Yucca* and *Quillaja* saponin extracts were also shown to have antiprotozoal effects in the gut of ruminants,[27] and also had beneficial side effect of reducing methane production. Moreover, a saponin extract from *Maesa balansae* (containing , maesabalide I,[28] **11**) was shown to have *in vitro* and *in vivo* activity against intracellular *Leishmania infantum* amastigotes at low concentrations as well as to eradicate of leighmanial amastigotes in 95% of animals treated after subcutaneous injection.[29] Thus, saponins and saponin extracts have strong potential in the treatment of a variety of parasitic protozoal infections.

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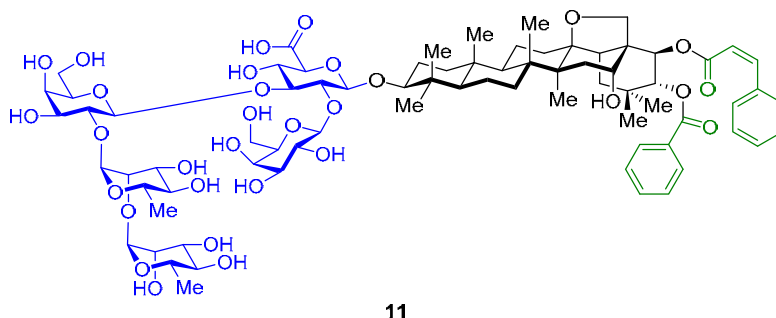
Figure 1.8. Structure of maesabalide I (**11**).

1.1.5 Immunopotentiating Activity

Saponin mixtures such as QuilA, as well as purified saponins, have been recognized as potent immunopotentiators for decades. As a component of vaccines, a saponin function as an immunoadjuvant, which is defined as any substance that acts to accelerate, prolong, or enhance antigen-specific immune responses when used in

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combination with specific vaccine antigens.[30] Incorporation of saponin adjuvants into vaccine formulations has been particularly attractive because of their unique capacity to stimulate both cell-mediated (Th1) and humoral (Th2) immune responses. A Th1 response is generally considered to be pro-inflammatory, and is desired to clear intracellular pathogens such as viruses and intracellular bacteria from the host. On the other hand, a Th2 response is generally anti-inflammatory and more appropriate for neutralizing extracellular toxins, parasites, and extracellular bacteria.[31] Judicious choice of adjuvant allows for tailoring the immune response to achieve the optimal mix of Th1 and Th2 to clear the pathogen most efficiently and safely.
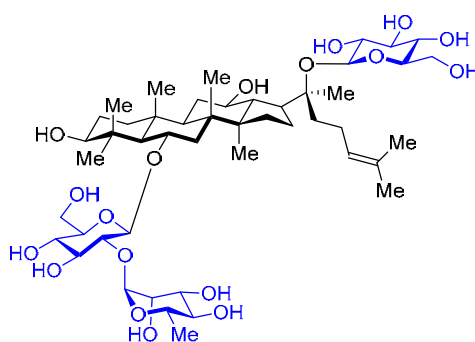
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Figure 1.9. Structure of ginsenoside Re (**12**).

1.2 Saponin Immunoadjuvants

Dozens of saponin families have been reported to have immunoadjuvant activity. Among the most promising are the ginsenosides (from the *Panax* genus), platycosides (from *Platycodon grandiflorum*), the lablaboside saponins (from *Dolichos lablab*) and the *Quillaja* saponins.[32] In addition to the traditional use of ginseng extract as a stimulant, isolated saponins, such as Rb1, have been shown to potentiate the immune response in pigs and mice against viral[33] and bacterial[34] targets. Moreover, ginsenoside Re (**12**) has

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been shown to elicit a mixed Th1 and Th2 response along with antigen-specific antibodies against OVA in mice.[35]
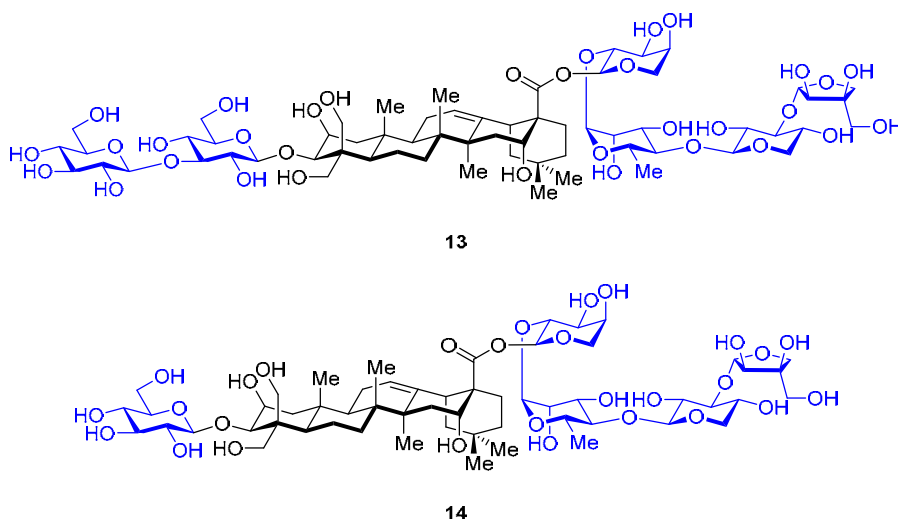
Figure 1.10. Structure of platycodins D2 (**13**) and D (**14**).

A mixture of platycosides, isolated from the 'Korean Bellflower' plant, was shown to elicit a promising antibody response against OVA in mice,[36] characterized by a mixed Th1 and Th2 response in an immunization model. Moreover, anti-OVA antibody titers were significantly enhanced relative to alum/OVA and QuilA/OVA and OVA alone. From this mixture, the most promising saponins are platycodin D2 (**13**) and platycodin D (**14**), which have shown efficacy in pre-clinical animal models against recombinant hepatitis B antigen[37] as well as the avian influenza virus[38] respectively. Interestingly, despite very similar chemical structures, platycodin D2 is much less hemolytic than platycodin D.
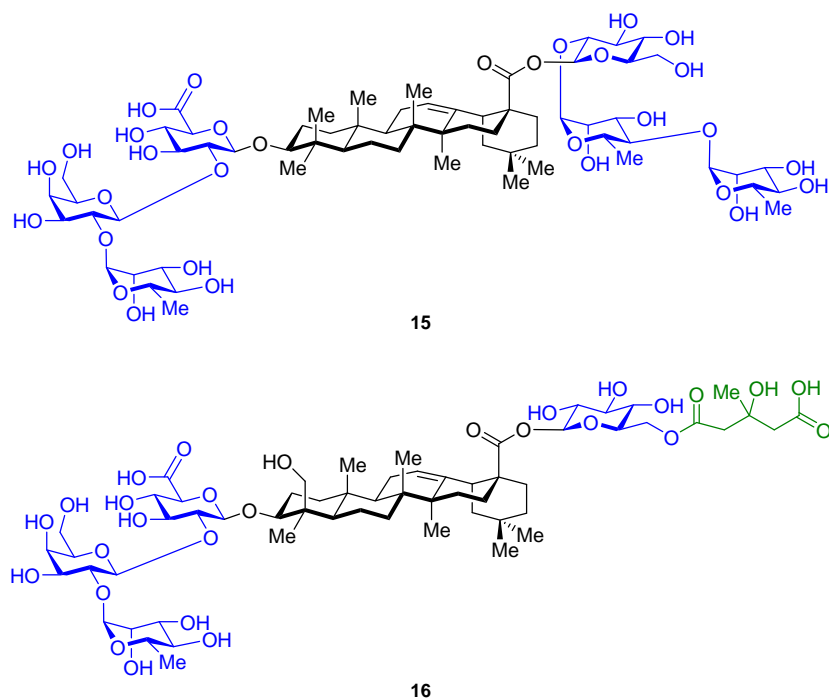
Figure 1.11. Structure of lablabosides F (**15**) and D (**16**).

The lablaboside family of saponins, derived from the edible hyacinth bean, has shown promise as an immunopotentiator.[39] In a direct comparison with QS-21, lablabosides B (**16**) and F (**15**) elicited an equivalent antibody response against OVA, with negligible hemolytic toxicity, which has been the primary liability of QS-21. However, in a pre-clinical vaccination model in mice against the causative virus for Aujeszky's disease (pseudorabies), lablaboside F induced a response strongly skewed to Th2,[39a] whereas QS-21 elicited a strong mixed Th1 and Th2 response. Such a skewed response may limit application of the lablabosides as components of vaccines. However, the aglycone component of lablaboside F is oleanolic acid, an inexpensive trieterpene derived from the American pokeweed, which may facilitate an economic synthesis of industrially useful quantities of this saponin.
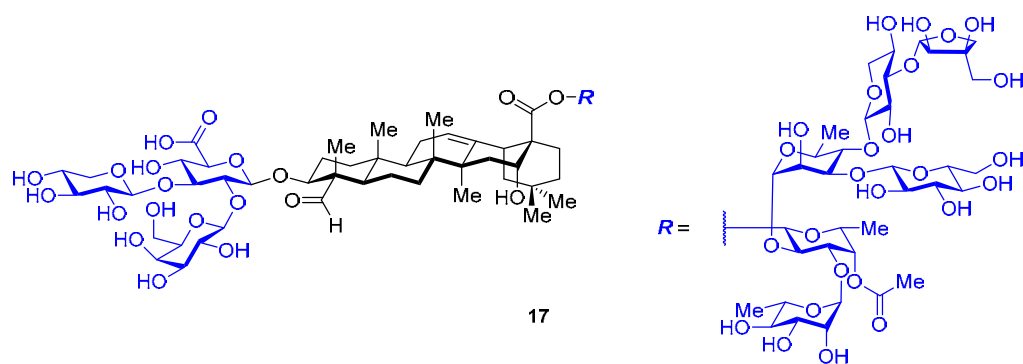
Figure 1.12. Structure of QS-7 (**17**).

Finally, the *Quillaja* saponins have been studied extensively for decades, both as a crude mixture of saponins (Quil A) as well as purified components. The semi-purified saponin extract was first shown to have adjuvant activity in a veterinary setting in 1974.[40] It was not until 17 years later that the components of the mixture were isolated, characterized, and analyzed as individual components.[41] Among the most studied are QS-7 (**17**) and QS-21 (**18**, major component), which were both isolated in small quantities via RP-HPLC. As the investigational immunopotentiator of choice, QS-21 has been administered to over 20,000 patients across dozens of clinical trials.[42] Indeed, QS-21 was a necessary component of the first successful anti-malarial vaccine as shown by the recently reported Phase III clinical trial by GlaxoSmithKline. By contrast, QS-7 has not been heavily investigated in the clinic. This is likely due to the structurally complex oligosaccharide moieties and difficulties in procuring clinically relevant quantities of the saponin. However, QS-7 has been shown to potentiate the immune response synergistically when co-administered with QS-21,[43] with negligible toxicity, thus making it a candidate for a multi-adjuvant vaccine.
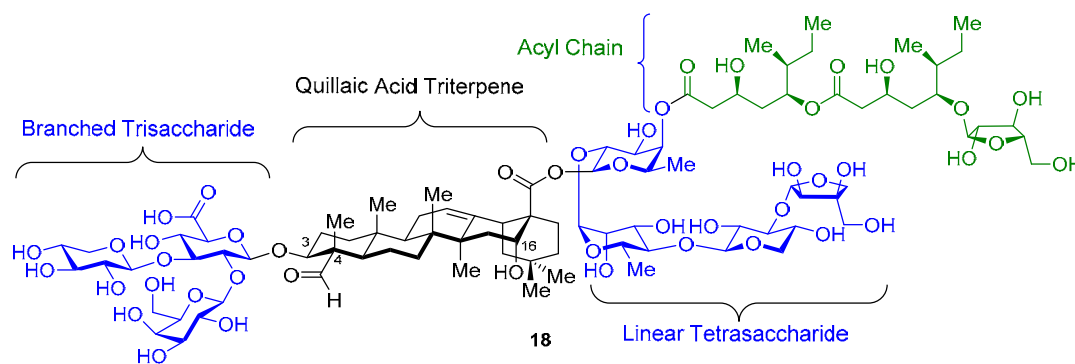
Figure 1.13. Structure of QS-21-Api (**18**).

1.2.1 Proposed Immunoadjuvant Mechanisms of Action

There are several mechanisms by which saponin adjuvants are believed to exert their immunopotentiating effects, although a dearth of experimental data precludes definitive mechanistic information. The most well studied saponin adjuvant, QS-21-Api, bears an aldehyde substituent at C4 of the quillaic acid triterpene, which has been postulated to covalently interact with lymphocyte cell surface receptors via formation of a Schiff-base.[44] This theory is bolstered by the observed abrogation of adjuvant activity after reductive amination of the C4 aldehyde substituent with a series of simple amines. However, an alternative explanation to the observed change in biological activity is based on the introduction of a positive charge, which may significantly alter electrostatic interactions of the saponin with a putative macromolecular target. Indeed, a cationic *Quillaja* analogue synthesized by the Gin group was shown to be completely devoid of adjuvant activity.[45] Moreover, recent studies from the Gin group suggest that the C4 aldehyde substituent is not an essential for adjuvant activity.[46] In the final step of the synthesis of QS-21, partial reduction of the C4 aldehyde substituent to the corresponding primary alcohol (**19**) was observed. This minor component was purified via RP-HPLC

from the major product, QS-21-Api and probed for immunoadjuvant activity. No significant difference was observed between **18** and **19**.
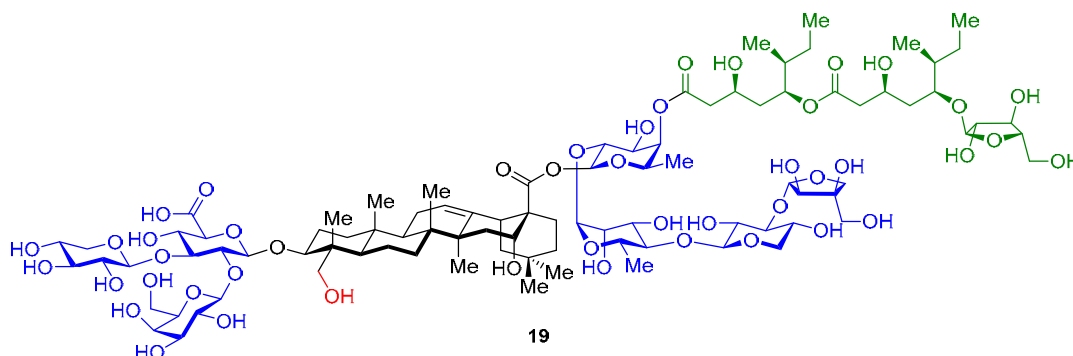
Figure 1.14. Structure of QS-21-des-aldehyde (**19**).

Although published mechanistic studies with *Quillaja* saponins have been extremely limited, *in vitro* studies of immunostimulating complexes (ISCOMs, cage-like structures resulting from mixing cholesterol, phosphatidylcholine and QuilA) suggested that bone marrow-derived dendritic cells are responsible for the observed immunopotentiation.[47] Indeed, Robson *et al* observed that dendritic cells, but not macrophages or naïve B cells were capable of priming antigen-specific CD4$^+$ T cells. Moreover, they found that dendritic dells derived from IL-12 knockout mice were incapable of priming CD4$^+$ T cells, suggesting a critical role for IL-12 in the saponin derived induction of the immune response.

Recent mechanistic postulations include activation of the NALP3 inflammasome[48] and general activation of the inflammatory response, which occurs via detection of Pathogen or Danger Associated Molecular Patterns (PAMPs or DAMPs) and seen in Figure 1.15.[49] The polysaccharide motifs present in saponins may act as PAMPs/DAMPs to activate NALP3, which has been definitively shown to contribute positively to the immunopotentiating activity of other adjuvants.[50]

14
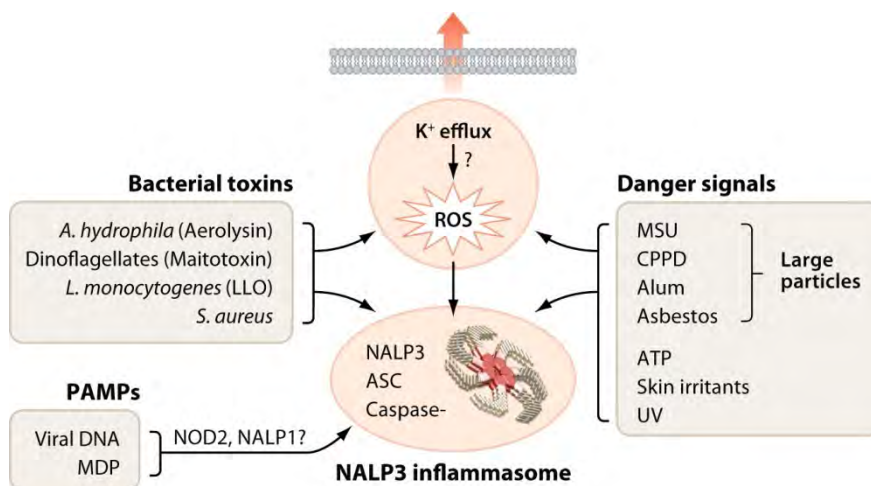
Figure 1.15. NALP3 inflammasome is the integrator of many immunostimulatory pathways. Adapted from ref 48.

To probe the mechanism of action more specifically, the Gin group has synthesized a series of *Quillaja*-like saponins as chemical tools. An immunoadjuvant-active fluorophore-labeled saponin (**20**) was readily taken up dendritic cells *in vitro*,[45] whereas an inactive but structurally similar saponin was not readily internalized, giving further support to the aforementioned necessity of dendritic cells to the mechanism of action. Moreover, a similar radiolabeled saponin[46] (**21**) was taken up by lymphocytes at the site of subcutaneous injection then trafficked to the draining lymph node. The radiolabel was retained in the lymph node for more than 24 hours, where the putative activated lymphocyte can further propagate the immune response. As with the fluorophore-labeled saponin, inactive variants did not exhibit this activity.
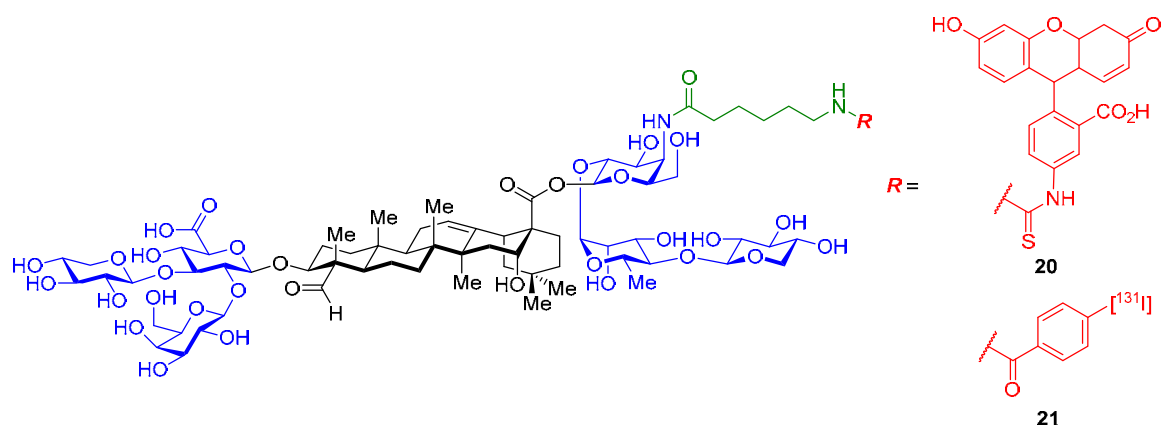
Figure 1.16. Structure of fluorescent and radioactive *Quillaja* saponin analogues.

1.2.2 Proposed Immunoadjuvant Structure–Activity Relationships

Scores of saponins have been examined for immunopotentiating activity, giving a spectrum of activity and toxicity along with many diverse structural elements. As such, there have been many attempts to make broad statements correlating structure to function.[39b] For example, in the *Quillaja* saponins, the acyl chain (as shown with QS-21, **18**) is necessary for immunopotentiating activity, but also responsible for much of the hemolysis-related toxicity.[51] However, in another class of immunopotentiators, the lablaboside saponins,[39b,52] in the two most active saponins, lablaboside F and lablaboside B (**15** and **16**, *vide supra*), the presence or absence of an acyl chain had little no discernible effect on activity or toxicity. Furthermore, another important class of saponin adjuvants, the soyasaponins (e.g. soyasaponin A[1], **8**, *vide supra*), has no acyl chain but exerts strong immunopotentiating effects.[39b] Thus, while it is possible to make conclusions within a family of natural products about saponin SAR, caution must be exercised when making broad generalizations across different saponin families. Indeed, there are simply too many variables for such generalizations to be useful.

1.2.3 Limitations of Saponin Adjuvants

While saponin adjuvants have held much promise for several decades, their lack of inclusion in many FDA-approved vaccine formulations is the result of several major drawbacks, including excessive toxicity and chemical instability.[41,53] For example, subcutaneous injection of QS-21 results in local erythema and systemic flu-like symptoms, which makes it unattractive for use in prophylactic vaccines. Toxicity likely arises from hydrolysis of the hydrolytically unstable acyl chain after injection, which undergoes spontaneous hydrolysis in neutral phosphate buffered saline over the course of several days. Additionally, isolation of saponin natural products is an expensive and resource-intensive process,[41] making isolation an economically unfeasible and environmentally unsustainable option if large quantities of saponin are required. Nonetheless, QS-21 has seen much success in a dozens of clinical trials in both veterinary and human applications across many different pathogens and diseases, including parasites,[54] viruses,[55] bacteria,[56] and cancer.[57]

1.3 Synthesis of Triterpeneoid Saponins

Studies of pure saponin molecules may initially require milligram quantities, which are generally accessible via isolation and HPLC purification. However, as more material is required for study and clinical applications, chemical synthesis may prove to be a more effective means of procuring large amounts of pure saponin. However, total synthesis of triterpenoids is not economical. Thus semi-synthesis from commercially available, inexpensive triterpenes isolated from natural sources is a viable option in many cases.

1.3.1 Biosynthesis of Triterpenoid Saponins

As mentioned previously, the staggering diversity of triterpeneoid structures has resulted in a broad spectrum of biological activities. Indeed, the nearly 600 recently cataloged triterpenoids are derived from the triterpene squalene. As shown in Scheme 1.1, biosynthesis of squalene arises from condensation of isopentenyl pyrophosphate (**23**) with dimethylallyl pyrophosphate (**22**) into geranyl pyrophosphate (**24**), which then combines with another isopentenyl pyrophosphate (**23**) to give farnesyl pyrophosphate (**25**). Condensation of these 15-carbon sesquiterpene units gives the 30-carbon triterpene squalene (**26**).

Scheme 1.1. Biosynthesis of squalene (**26**).
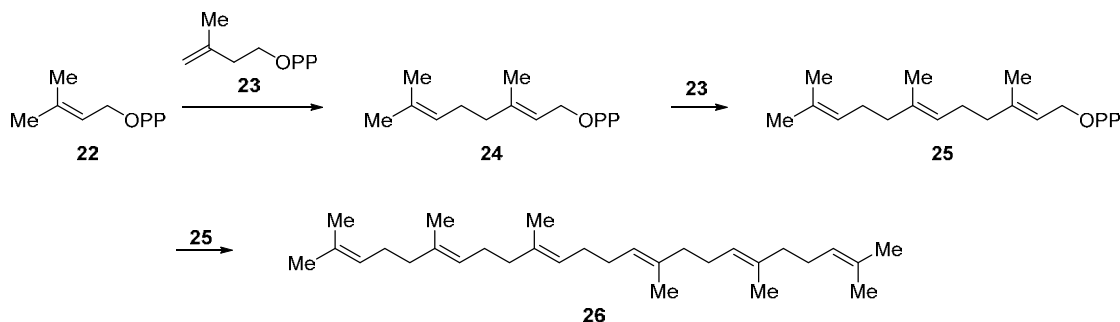
To arrive at the final common precursor of all triterpenoid saponins, squalene is oxidized to give 2,3-oxidosqualene (**27**) Scheme 1.2.[58] Adoption of a chair-chair-chair conformation and cationic cyclization of 2,3-oxidosqualene, facilitated by oxidosqualene cyclases, furnishes the first committed step to either the phytosterols or triterpeneoid saponins.[59] For the triterpeneoid saponins, the cyclase reaction forms the tetracyclic cationic dammarenyl cation (**28**, Scheme 2). Rearrangement and D-ring expansion gives the baccharenyl cation (**29**), which undergoes another cyclyzation to **30** followed by ring expansion to give the germanicyl cation (**31**), which then undergoes a 1,3 proton shift to

furnish the oleanyl cation (**32**). Finally, deprotonation gives β-amyrin (**33**), the precursor

to all oleanolane triterpenoids.[60]

Scheme 1.2. Biosynthesis of β-amyrin (**33**).
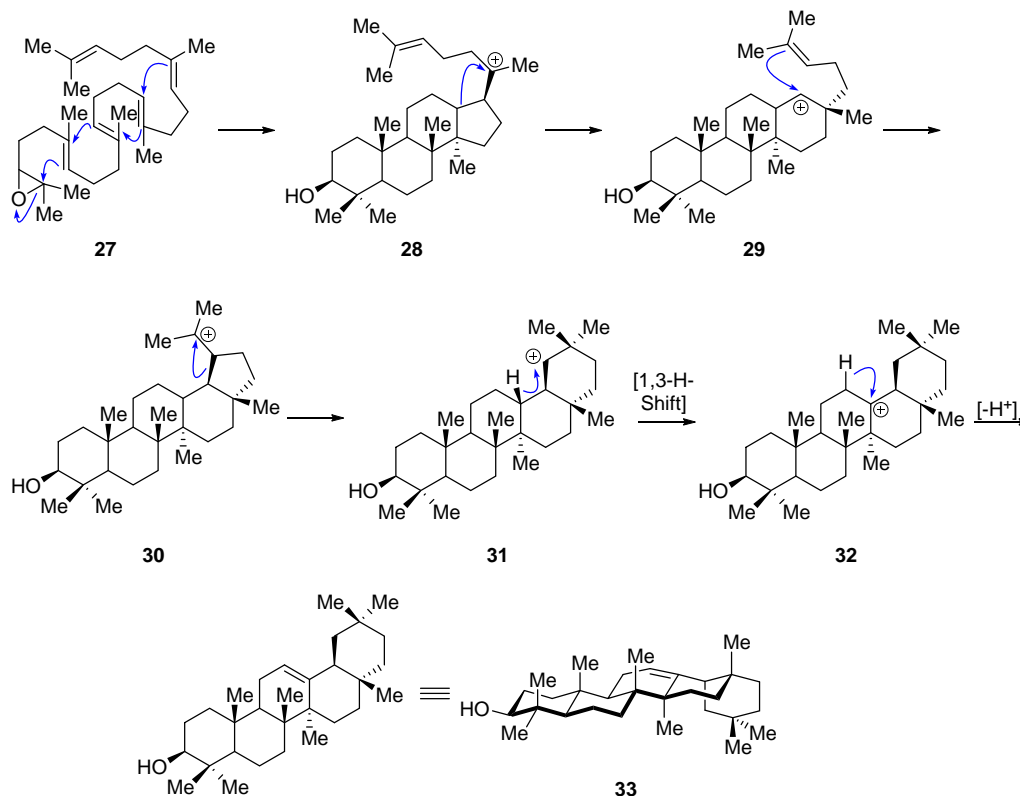
Further oxidation of the triterpene skeleton is mediated by cytochrome P450

enzymes. This large gene family (accounting for ~1% plant protein coding sequences[61])

can furnish oxidations to most non-quaternary carbons to furnish alcohol, ketone,

aldehyde, acetal, and ketal moieties. Indeed, within individual organisms, a multitude of

distinct saponins can be synthesized, complicating the isolation of significant quantities

of particular triterpenoids.   Common oxidation points, such as C28, are used as

chemoenzymatic handles for further decoration with glycosidic moieties.   As such,

glycosyltransferases add activated monosaccharide units to give linear and branched

oligosaccharides, mostly frequently two to five monosaccharides in length,[60] which can

19

be further adorned with acyl or sulfate groups. As such, sequential, non-template driven modifications result in the tremendous diversity of structure observed in saponin natural products.

1.3.2 Synthesis of QS-21

QS-21, comprising two isomeric saponins (**18** and **34**), is the most studied, and one of the most complex saponin immunoadjuvants that has been successfully synthesized. The primary component, QS-21-Api (**18**), was synthesized after a nearly 10-year effort by the Gin group in 2005,[62] with the minor component, QS-21-Xyl (**34**) completed three years later[63] with significant improvements in the overall synthesis. The primary challenges encountered in the synthesis of the large saponin natural product arose from the hydrolytically sensitive acyl functionality on the bridging fucose moiety and formation of triterpene–glucuronic acid moiety bond with the required equatorial stereochemistry. The former issue complicated the synthesis of the oligosaccharide units, which had been successfully synthesized with neighboring group participation-facilitating ester protecting groups.[64] Thus, to avoid extensive late-stage protecting group modification, alternate protecting strategies were required. Additionally, formation of the desired β-glycoside linkage between the fully formed branched trisaccharide and the poorly nucleophilic quillaic acid triterpene without the use of neighboring group participation proved extremely difficult.
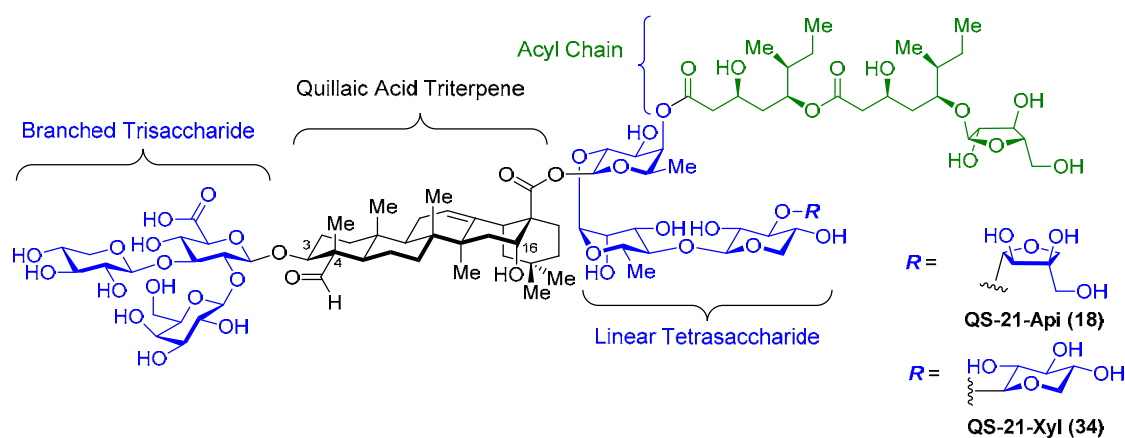
Figure 1.17. Structure of QS-21-Api (**18**) and QS-21-Xyl (**34**).

While synthesis of each component was challenging,[64] the thoughtful design of the components enabled relatively straightforward assembly into the final product. Of the three required couplings, only glycosylation of the branched trisaccharide with quillaic acid proved challenging, as mentioned previously.

Attempts to glycosylate the trisaccharide under dehydrative conditions with quillaic acid derivatives (semi-synthesized from a commercially available semi-purified saponin mixture[65]) gave exclusively α-glycoside products, despite extensive efforts from the lead author.[66] Schmidt glycosylation with $BF_3 \cdot Et_2O$,[67] employing trichloroacetimidate donor **35** gave α-glycoside products or low yields of anomeric mixtures (51%, 2:1 β:α). However, the relatively exotic Lewis acid tris-pentafluorophenyl borane[68] facilitated a rapid, β-selective coupling at room temperature of quillaic acid ester **36** with 3 mol % catalyst loading relative to the donor trichloroacetimidate, **35** to form protected prosapogenin **37**. This combination of reagents proved especially fruitful in the synthesis of the lablaboside saponins, the subject of Chapter 4.

Scheme 1.3. Coupling of branched trisaccharide to quillaic acid triterpene.
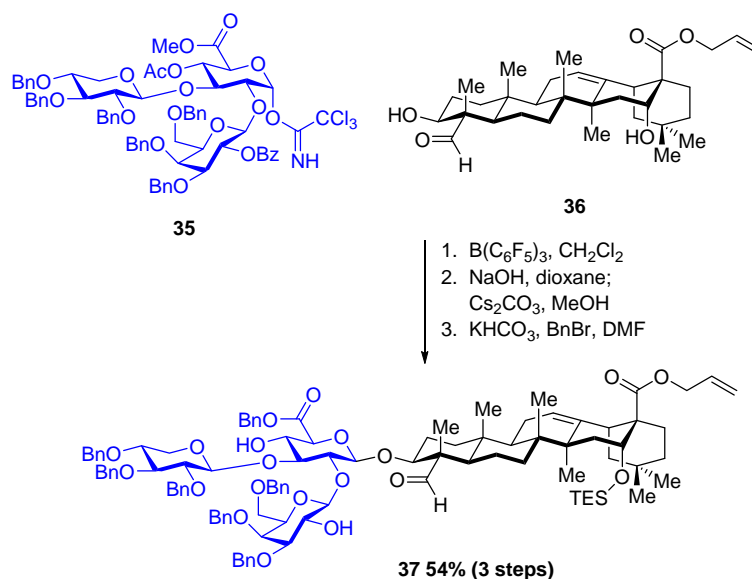
Deacylation of the fucosyl moiety (**38**) revealed competent nucleophile **39** for Yamaguchi esterification of acyl chain **40**. Next, selective removal of the anomeric triisopropylsilyl group from **41**, followed by formation of trichloroacetimidate gave donor **43**. Schmidt glycosylation, joining the prosapogenin moiety **44** with acylated tetrasaccharide furnished the fully protected product **45** with complete β-glycoside selectivity in 70% yield. Carefully controlled trifluoroacetic acid hydrolysis of acetonide and silyl groups followed by hydrogenolysis and HPLC purification furnished QS-21-Api (**18**).

Scheme 1.4. Late-stage assembly of major domains of QS-21-Api (**18**).

38 R = OAc

K₂CO₃, MeOH

39 R = OH (90%, 2 steps)

2,4,6-Cl₃C₆H₂COCl, Et₃N, DMAP

40

41 *X* = OTIPS

42 *X* = OH

43 *X*= α-OC(NH)CCl₃ (45%, 2 steps)

BF₃·Et₂O CH₂Cl₂

1. TESOTf, 2,6-lutidine, CH₂Cl₂
2. HCO₂H, Pd(OAc)₂, Et₃N, PPh₃, 1,4-dioxane

37

44, 81%, (2 steps)

45, 70%

1. TFA, H₂O, CH₂Cl₂ 0 °C
2. 150 psi H₂, Pd/C, THF, MeOH

QS-21-Api (**18**, 75%, 2 steps)
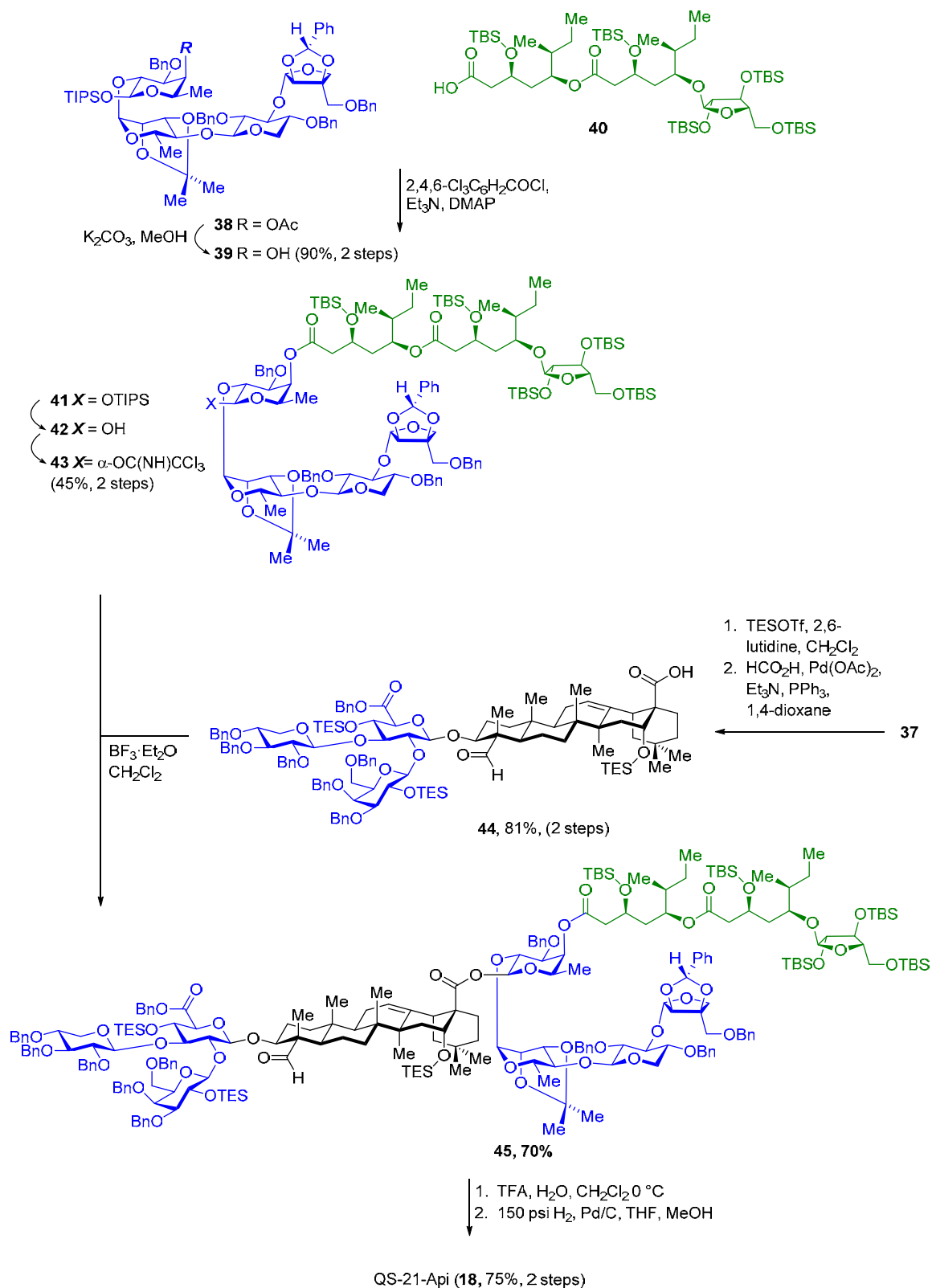
1.3.3 Semisynthesis of Protected Prosapogenin

After the initial synthesis of QS-21-Api, the Gin group made several further advances, allowing for a much higher throughput of QS-21 and analogues. The most significant advance was the three-step semisynthesis of a protected prosapogenin from a commercially available, semi-purified saponin extract.[63] Basic hydrolysis of the crude saponin mixture allows for isolation of significant quantities of the desired prosapogenin **46**. Persilylation with triethylsilyl triflate followed by methanolysis of TES esters furnished diacid, which was then selectively alkylated at the glucuronic acid carboxylate furnishing protected prosapogenin **47** in multi-gram quantities. Access to significant quantities of **47** greatly facilitated generation of a large number of analogues, the synthesis of which will be outlined in Chapter 2.

Scheme 1.5. Semi-synthesis of protected prosapogenin (**47**).
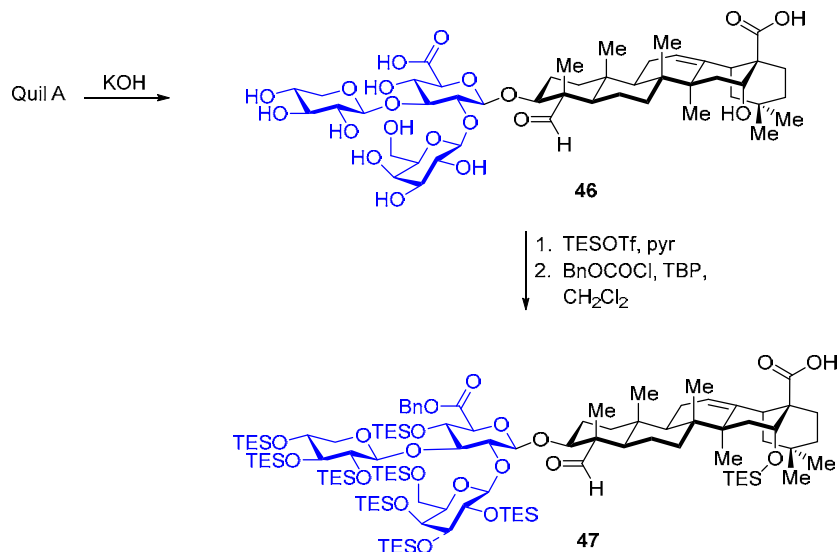
The modular strategy employed in the initial synthesis of QS-21-Api allowed for a relatively straightforward path to creating analogues of this complex saponin.

Previously studies, giving a preliminary picture of the SAR will be outlined in Chapter 2. Application of this strategy toward exploration of the SAR of the central glycosidic linkage will then be described in detail. In Chapter 3, we move away from the *Quillaja* saponins, with a methodology study toward selective oxidation of the geminal dimethyl groups present on the A-ring of oleanolic acid, which is accomplished through a three step sequence culminating in a diastereoselective tandem Michael–aldol reaction. Application of this facile oxidation strategy to the synthesis of the lablaboside family of saponins is the subject of Chapter 4. Also included is the use of rare protecting groups in the synthesis of oligosaccharides as well as application of the previously outlined, but rarely utilized, tris-pentafluorophenyl borane glycosylation catalyst.

CHAPTER 2.

STRUCTURE–ACTIVITY RELATIONSHIPS OF THE CENTRAL GLYCOSIDIC

LINKAGE IN *QUILLAJA* SAPONIN ADJUVANTS

2   Introduction

Immunological adjuvants help stimulate an immune response against co-administered antigens and have become increasingly important in recent years.[30,69] While current FDA approved adjuvants (Alum[70], AS04[56] and MF59[71]) have found many applications, more challenging applications (oncology, HIV, malaria) require a better adjuvant.   QS-21, derived from the bark of the *Quillaja saponaria* Molina tree, has showed promising results in dozens of clinical trials over the last two decades.[41-42] The primary constituent, QS-21-Api (**18**) (Figure 2.1), is composed of four domains; a branched trisaccharide, an oleanolane-type triterpene, a bridging linear tetrasaccharide, and a pseudo-dimeric acyl chain. The modular strategy employed in the first synthesis of QS-21-Api[62] has allowed for facile generation of dozens of analogues, providing a preliminary picture of SAR.[45,72]
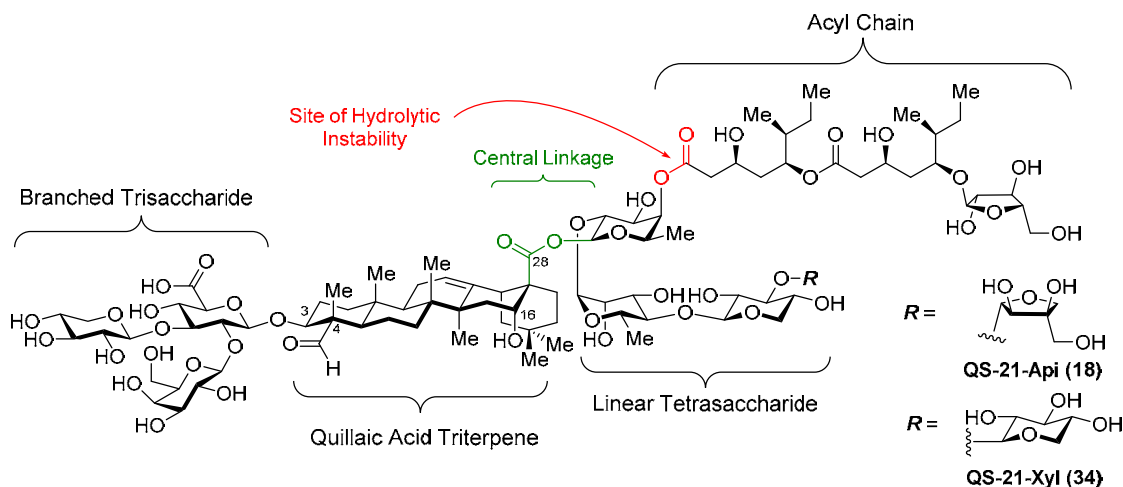
Figure 2.1. The major structural domains of the potent immunoadjuvant, QS-21.

2.1   Hydrolytic Instability

With the first generation of *Quillaja* saponin (QS) analogues, we sought to address the most prominent liability of the natural product: hydrolytic instability of the acyl chain leading to hemolysis related toxicity. Replacement of the labile ester groups with the corresponding amides emerged as the solution. Installation of the requisite nitrogen atoms required a completely new synthesis of the acyl chain as well as the bridging monosaccharide moiety. Since there are no naturally occurring C4-deoxy-aminosugars, Gin *et al* displaced an activated C4 equatorial hydroxyl from selectively protected glucal **48** to form the desired axial-disposed azide **49**, as shown in Scheme 2.1. After changing protecting groups, dihydroxylation, and selective silylation furnished aza-Gal monosaccharide **50**, a key intermediate for all future QS analogues.

Scheme 2.1. Syntheis of 4-aza-galatose glycosyl acceptor.
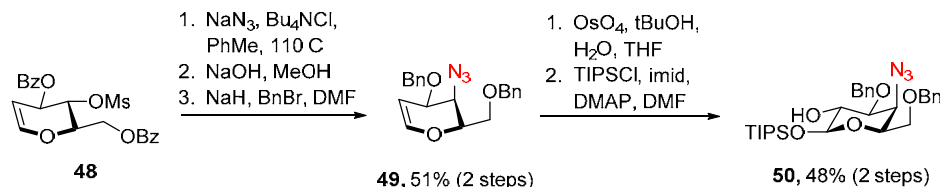
2.2   Late Stage Assembly of Major Domains

Full assembly of the second generation of analogues was achieved with Schmidt glycosylation of protected prosapogenin **51** with trichloroacetimidate **52**. Azide reduction with benzeneselenol revealed the nucleophilic amino group ready for diversification. Over several years, the Gin group made a plethora of derivatives, as shown in Scheme 2.2 via activation of carboxylic acid as a mixed anhydride followed by addition of amine **54**.
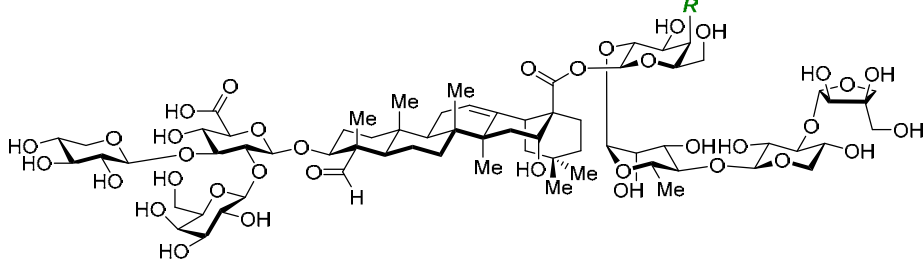
Scheme 2.2. Late stage assembly of major domains of *Quillaja* analogues.
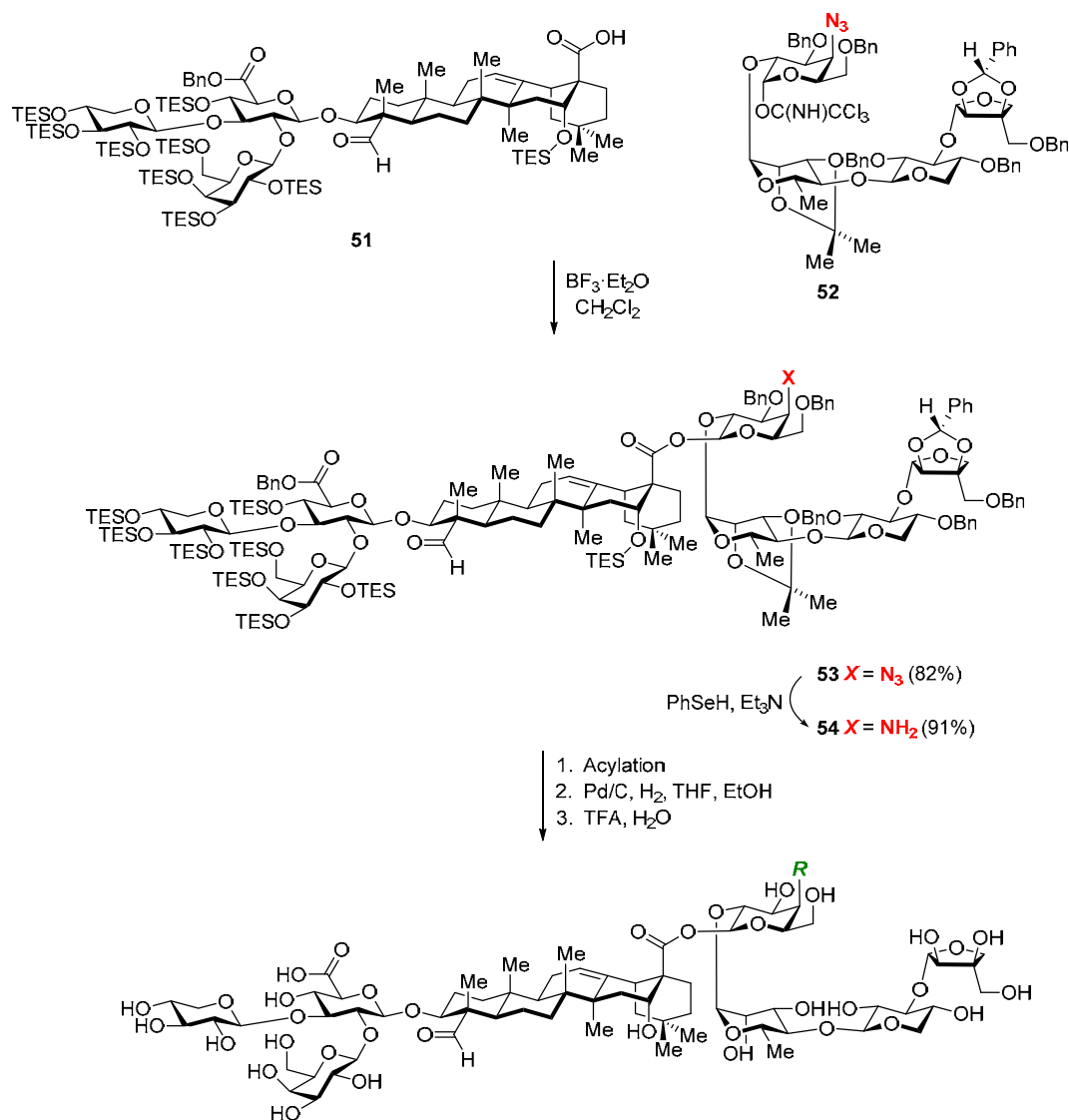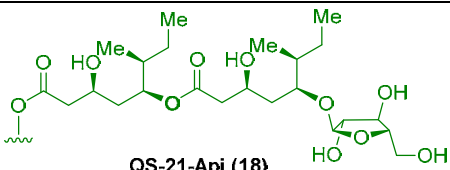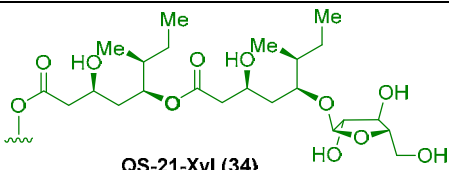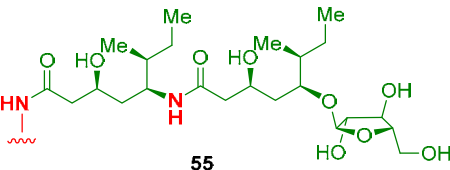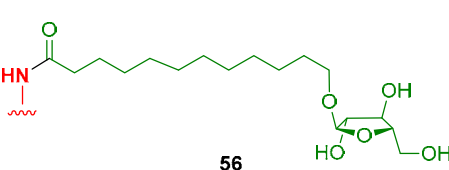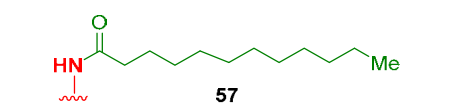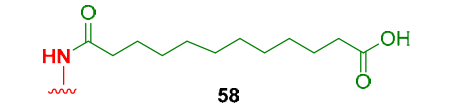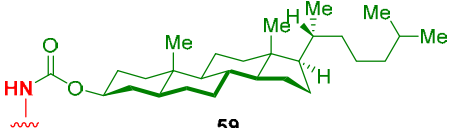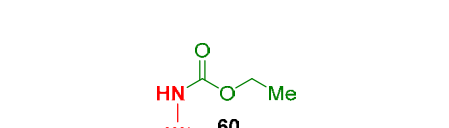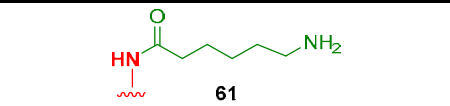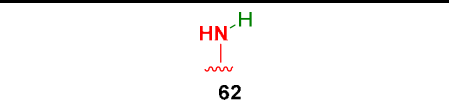
2.3 Preliminary SAR of Amide Containing QS Analogues

From this series, a number of SAR can be deduced (Figure 2.2). Incorporation of a hydrolytically stable acyl chain markedly decreased the toxicity and chemical instability compared to the QS-21-Api (**18**).[72] Moreover, antibody responses comparable to those elicited by the natural product could be achieved with a significantly simplified acyl chain (**56-59**). From this series, one of the most obvious conclusions arises from the introduction of a positive charge, which recapitulates previous variants (mentioned in

Chapter 1), whereby cationic QS analogues, such as amine **61** were shown to be devoid of immunoadjuvant activity.[53a] Moreover, analogues with very lipophilic side chains, such as cholesterol (**59**), had significantly diminished adjuvant activity. This observation is potentially complicated by the very low solubility of cholesterol derivative. However, other modifications had remarkably little effect on adjuvanticity, suggesting that the specific identity of the acyl chain was not important. Indeed, antibody sub-typing in mice subjected to the standard immunization protocol (*vide infra*) revealed a similar physiological response when given either QS-21 or an active QS analogue, suggesting a similar mode of action. Thus, dodecanoic acid acyl chain, as seen in **58**, was chosen as the optimal acyl chain component because of high efficacy, reasonable toxicity, and minimal synthetic complexity.

Activity	Toxicity	Synthesis	Activity	Toxicity	Synthesis
++++	++	+++++	++++	++	+++++

Activity	Toxicity	Synthesis	Activity	Toxicity	Synthesis
++++	++	++++++	+++	+++++	++++

Activity	Toxicity	Synthesis	Activity	Toxicity	Synthesis
++	++	++++	+++	+++	++++

Activity	Toxicity	Synthesis	Activity	Toxicity	Synthesis
+	+	++++	++	++	++++

Activity*	Toxicity	Synthesis	Activity*	Toxicity	Synthesis
n/a	+	+++	n/a	+	+++

Figure 2.2. The acyl side chain can be varied tremendously with retention adjuvant activity. Amide congener (**55**), exhibited very similar adjuvanticity as QS-21, but without toxicity, showing for the first time that toxicity is not required for adjuvanticity. A negative charge on the acyl chain (**58**) was well tolerated, but a positive charge (**61**) abrogated activity. Because these analogues were examined for adjuvant properties across several experiments, immunoadjuvant activity, toxicity, and synthetic difficulty are approximated. *These analogues feature a linear trisaccharide, which was shown to have similar immunopotentiating effects as the linear tetrassacharide present in all other analogues, as shown in Figure 2.3.

30

With an optimal acyl chain component in hand, the length and identity of the central oligosaccharide was probed for SAR. Iterative truncation from tetrasaccharide to monosaccharide demonstrated that the linear trisaccharide, present in **62**, was optimal in terms of efficacy and synthetic complexity as shown in Table 2. Compared to the full length tetrasaccharide (**58**), trisaccharide variant **62** showed no change in adjuvanticity. However, further truncation to disaccharide variant (**63**) or monosaccharide variant (**64**) resulted in a large decrease in activity.[45] Moreover, replacement of rhamnose and xylose moieties with a commercially available disaccharide, lactose, furnished analogue **65**, which showed significantly attenuated adjuvant activity. Taken together, these data highlight the importance of both the identity and length of central oligosaccharide for full adjuvant activity of these synthetic QS saponins.
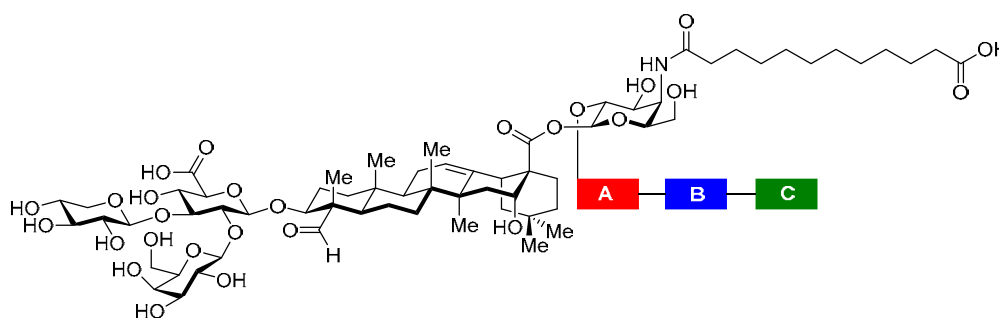
SQS-Analogue	Sugar A	Sugar B	Sugar C	Activity	Toxicity	Synthesis
SQS-0-0-4-5 (58)	α-1-4-Rha	β-1-3-Xyl	β-Api	+++	+++	+++++
SQS-0-0-5-5 (62)	α-1-4-Rha	β-1-3-Xyl	-	+++	+++	+++
SQS-0-0-6-5 (63)	α-1-4-Rha	-	-	++	++	++
SQS-0-0-9-5 (64)	-	-	-	++	+	++
SQS-1-0-11-18 (65)*	β-1-4-Glc	β-Gal	-	+	+	++

Figure 2.3. The minimal central oligosaccharide required for optimal activity is the trisaccharide shown in **62**. Further trucation, as well as replacement with another disaccharide, erodes immunoadjuvant activity.*A structurally related analogue, with no branched trisaccharide and a different acyl chain.

2.4   Central Linkage Variants

As a result of these studies, a new lead structure (**62**) (Figure 2.4) emerged, consisting of a branched trisaccharide, quillaic acid triterpene, linear trisaccharide, and dodecanoic acid acyl chain. From this established lead scaffold, we began our own studies, focusing on the most prominent unexplored structural feature of the *Quillaja* saponins, the central glycosidic linkage. Herein, we report the synthesis of a series of variants to the central glycosidic ester linkage (Figure 2.4), which exhibited a remarkable range in adjuvanticity and toxicity. Construction of these variants proved challenging, as glycosidic bond formation without the aid of neighboring group participation was compounded by the sterically demanding environment surrounding C28 of the quillaic acid triterpene. As such, it was necessary to employ unusual glycosylation promoters, such as sodium hydride, in the creation of several glycosidic linkages.
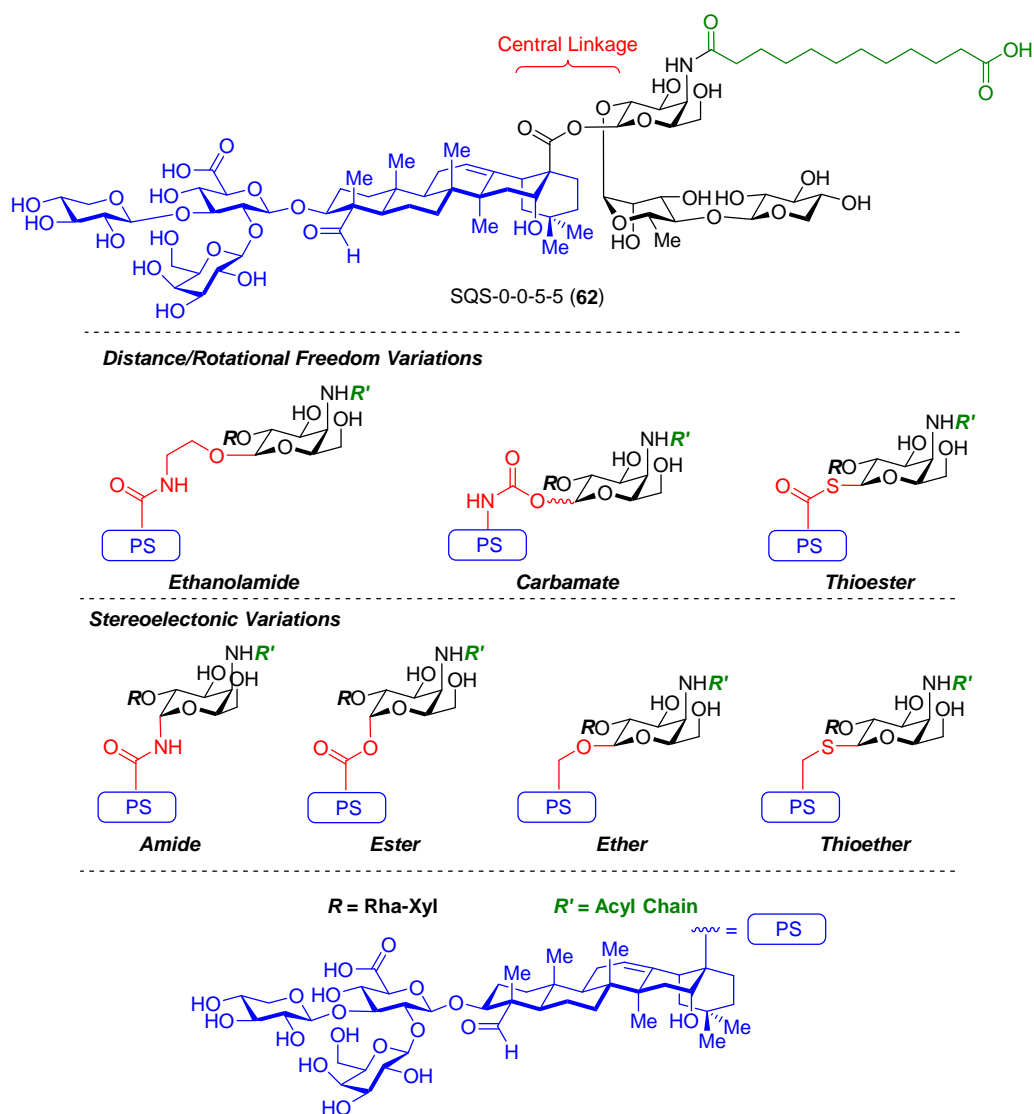
Figure 2.4. Structure of leading pre-clinical candidate and proposed central linkage variants.

2.5   Central Linkage Variants

Our efforts to systematically probe the SAR of the central glycosidic linkage were two-pronged: 1) variation in the distance/rotational freedom between the triterpene and central trisaccharide and 2) subtle variation in the stereoelectronic configuration of the central linkage. Relative to the natural glycosidic ester linkage, increasing the distance between the triterpene and trisaccharide by three bond lengths (ethanolamide), one bond length (carbamate), or one-half bond length (thioester) allows for increased rotational

freedom and the potential to form different conformations. To probe stereoelectonic effects, the three-bond distance between the triterpene and trisaccharide was maintained, while the number of H-bond donors/acceptors (amide, ether, thioether) and the anomeric configuration (β to α) of the bridging aza-galactose moiety were varied.

2.5.1   Synthesis of Modified Prosapogenin Moieties

Synthesis commenced with functionalization of the fully protected prosapogenin **65** (Scheme 2.3).[73] From this carboxylic acid, treatment with diphenylphosphoryl azide gave an acyl azide, which, upon continued heating, underwent the Curtius rearrangement to give isocyanate **67**. Alternatively, activation of **65** with thionyl chloride proceeded in quantitative yield to give acyl chloride **66**, which can be easily functionalized with a variety of nucleophiles. Addition of ammonia gave primary amide **69**, while addition of ethanolamine gave exclusive ethanolamide **68** in good yields.

Scheme 2.3. Functionalization of protected prosapogenin (PPS).

34
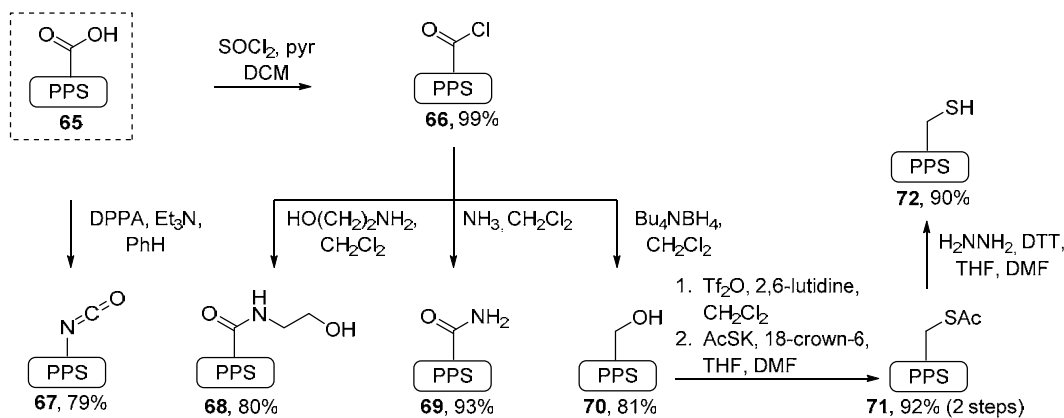
Selective reduction of acyl chloride to neo-pentyl alcohol (**70**) proved challenging. Most reducing agents, including sodium borohydride, Red-Al, DIBAL-H, and Super Hydride were unselective, resulting in C4 aldehyde reduction and benzyl ester cleavage in addition to the desired acyl chloride reduction. However, selective reduction was achieved with tetrabutylammonium borohydride to give neopentyl alcohol **70**. Conversion to the corresponding thiol proved challenging. Sluggish conversion of **70** to the corresponding tosylate or mesylate was mirrored by a complete lack of reactivity with sulfur nucleophiles. However, the aliphatic triflate, formed *in situ* with triflic anhydride, proved to be a competent electrophile for a naked thioacetate, prepared by addition of crown ether to potassium thioacetate. Treatment of thioacetate **71** with hydrazine under reducing conditions furnished the desired prosapogenin thiol **72** in excellent yield over three steps.

2.6   Challenging Glycosylations

While glycosylations to form all of the aforementioned linkages have been well documented, examples featuring a similarly demanding steric environment are limited in many cases (amide[74], thioether[75]) and without precedent in others (thioester). Initial efforts began with previously described trisaccharide hemiacetal **73**[45] which underwent dehydrative glycosylation with prosapogenin ethanolamide **69** to give exclusive β-glycoside product (**74**) in excellent yield (Scheme 2.4).

Scheme 2.4. Synthesis of variants with traditional glycosylation methods.

73

Ph₂SO, Tf₂O, TBP, CH₂Cl₂, -45 °C, add **68**

Ph₂SO, Tf₂O, TBP, CH₂Cl₂, -45 °C, add **69**

76

70, AgOTf, TBP, CH₂Cl₂, -40 °C

72, NaH, 0 °C, THF, DMF

74, 87%

75α, 70%

Y = O, **77**, 68%
S, **78**, 69%
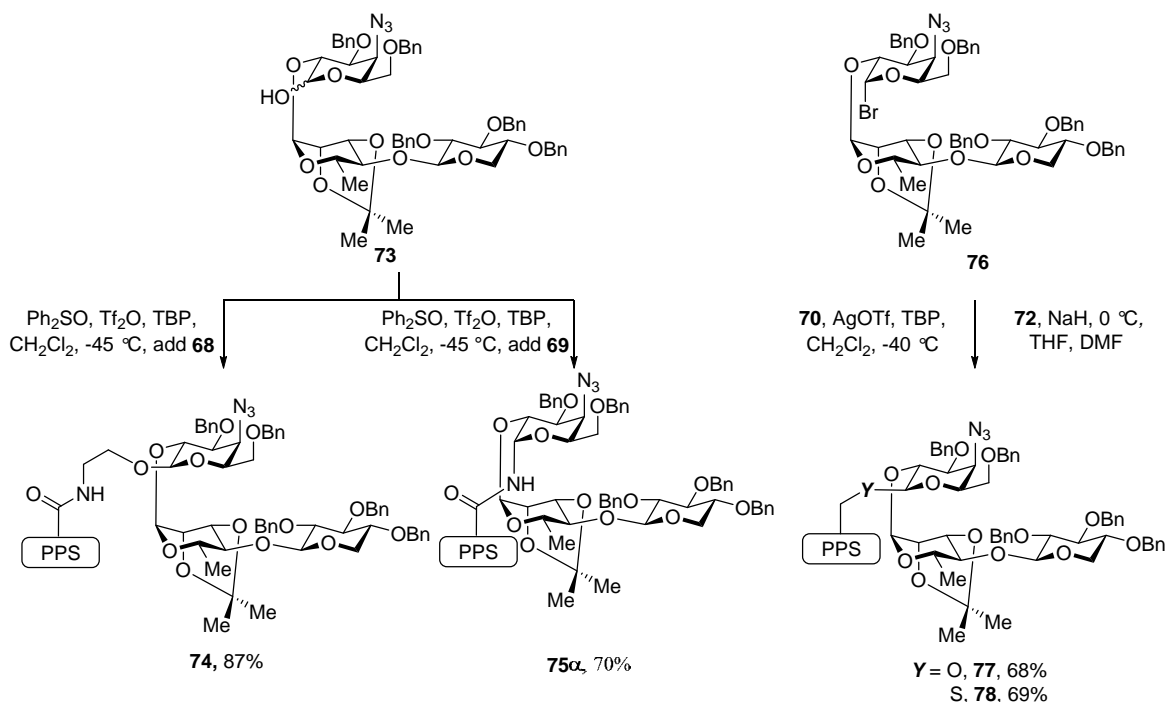
By contrast, glycosylation of primary amide **69** required much fine-tuning of reaction conditions to achieve anomeric selectivity. Utilizing two-fold excess of acceptor **73** along with very short reaction times, furnished an excess of β-anomer **75β** (60–78% yield, 2–4:1 β:α). The anomeric preference is reversed with longer reaction times and a two-fold excess of donor **73** (71%, 6:1 α:β). This reversal can be explained in part by the observed acid sensitivity of the β-anomer and facile decomposition under ambient conditions. Kinetic attack of the putative oxocarbenium/glycosyl triflate is from the equatorial disposition (Scheme 2.5) furnishing an excess of β-anomer (**75β**), as observed with short very short reactions. However, under the near-neutral reaction conditions, the newly formed β-amide can be protonated by the pyridinium present in the reaction medium, facilitating glycoside bond breakage, reforming the primary amide **69** and oxocarbenium **79**. The primary amide acceptor **69** eventually reacts to irreversibly form α-anomer **75α**.

Scheme 2.5. Hypothesized glycosylation equilibrium resulting from a sterically encumbered b-amide.
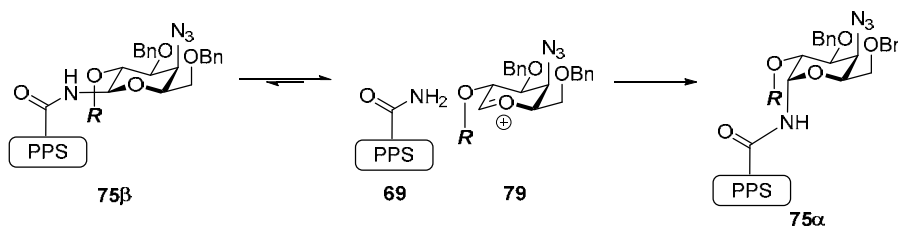
Although synthesis of the fully protected β-amide variant was successfully synthesized (analogous to α-amide variant **100** *vide infra*), global deprotection was impossible. We hypothesize that this is due to the sterically congested environment and compressed distance between the amide carbonyl and anomeric carbon of aza-galactose moiety. The newly formed β-disposed anomeric proton showed a coupling constant ($J = 10.5$ Hz) much higher than most other β-linked analogues ($J = 7.8$ Hz), which suggests a significant amount of steric strain. By contrast, the longer, axial-disposed bond present in α-anomer **75α** reduces this strain and shows no increased lability under acidic conditions.

Much to our surprise, repeated attempts at glycosylation of the same hemiacetal donor **73** with neopentyl alcohol **70** under dehydrative conditions gave no isolable glycosylation product. However, glycosylation was smoothly effected with bromide donor **76** (prepared from hemiacetal **73** with oxalyl bromide/DMF) to form ether **77** with the silver triflate promoted Koenigs–Knorr reaction to give >20:1 β-selectivity at low temperature. However, the thiophilicity of silver precluded its use in the analogous reaction to form the thioether. Instead, sodium hydride promoted formation of thiolate rapidly displaced bromide to give thioether **78** with complete β-selectivity.

Scheme 2.6. Conventional method for formation of glycosyl thioethers.
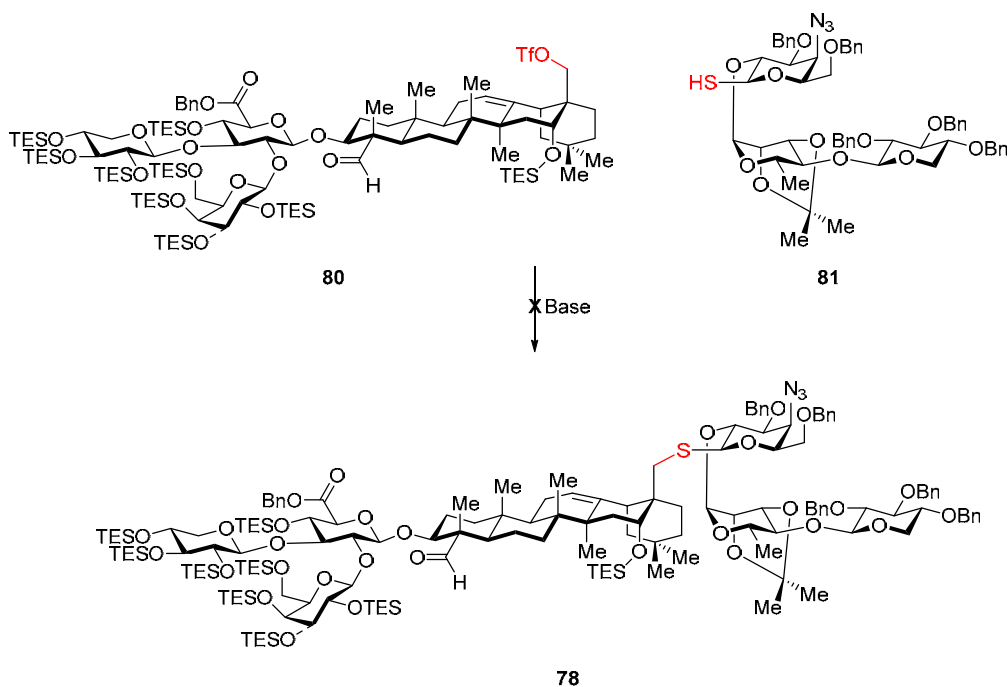
More conventional methods for creation of glycosyl thioethers involve formation of glycosyl thiohemiacetals, followed by displacement of a reactive electrophile such as an aziridine,[75a,76] halide,[77] or triflate.[78] Facile generation of β-thiohemiacetal **81** via bromide **76** was accomplished by treating with cesium thioacetate followed by deacylation under reducing conditions (Scheme 2.7). Displacement of a highly activated leaving group at the sterically encumbered neopentyl C28 proved very challenging. As shown in Scheme 2.6, attempts to displace the *in situ* generated prosapogenin triflate **80** were unsuccessful, despite trials with a litany of bases, from organic amines, inorganic salts, or strong bases, giving only trace amounts of the desired product by TLC.

Scheme 2.7. Synthesis of variants with anomeric nucleophiles.

82, 79% (2:1 β:α)   **83**, 70%   **84**, 87%

2.7   Reverse-Polarity Glycosylations

Additional analogues required a conceptual reverse of polarity to install the desired linkages. Formation of the glycosyl carbamates was effected by addition of sodium hydride to hemiacetal **73** followed by addition of isocyanate **67** to give an easily separable mixture of anomers **82α/β** in consistent yield but varying anomeric selectivity (65–79%, 2:1–1:2 β:α). By contrast, under nearly identical conditions, acylation proceeded with good and repeatable selectivity to preferentially form the α-glycosyl ester **83**. Addition of sodium hydride to a solution of acyl chloride **66** and thiohemiacetal **81** furnished β-thioester **84** in excellent yield.

With these glycosylation products in hand, advancement to final analogues proceeded in four straightforward steps as shown in Scheme 2.8. Reduction with hydrogen sulfide/triethylamine to form amines (**85–91**), acylation with dodecanedioic

acid monobenzyl ester to form fully protected analogues (**92–98**), global deprotection (hydrogenolysis, trifluoroacetic acid-mediated hydrolysis), and HPLC purification gave final analogues **99–105**.

Scheme 2.8. Synthesis of central linkage variants.
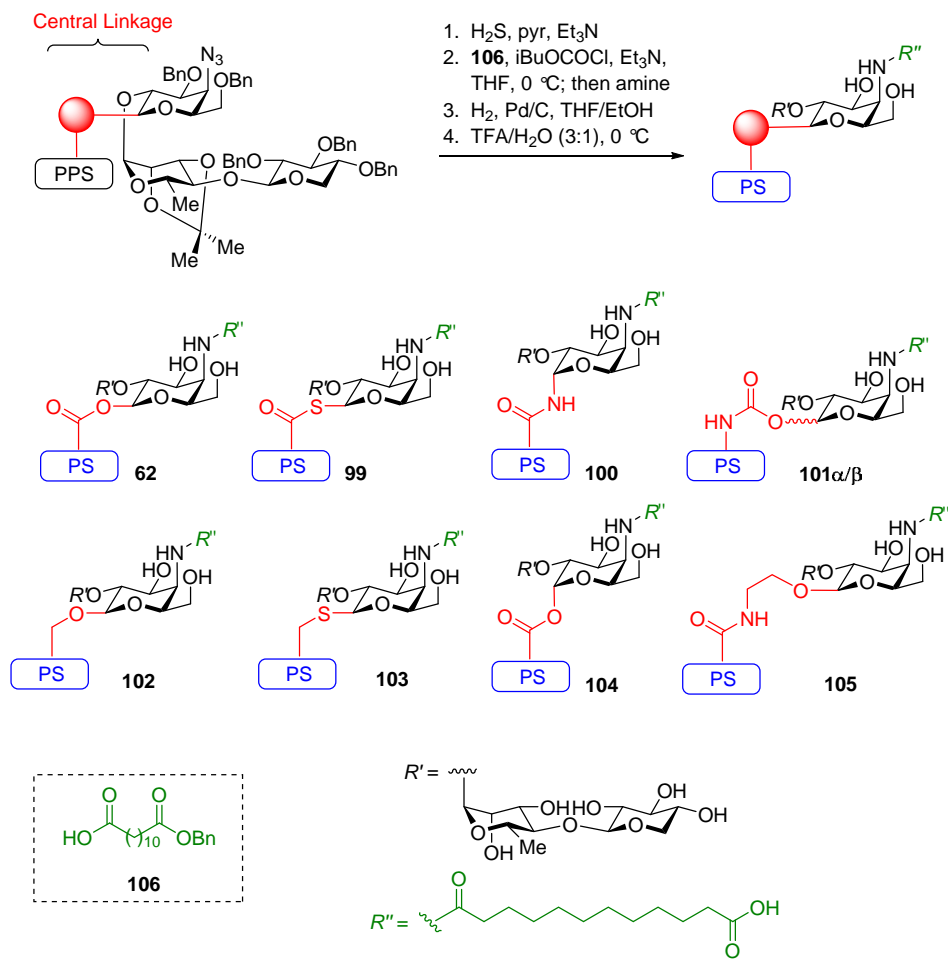
2.8   Biological Evaluation

Currently, there exists no rapid, *in vitro* method for measuring adjuvant efficacy. This is due in part to the unknown and likely multivariate mechanisms of adjuvant action. Therefore, these QS analogues were probed for adjuvant activity using an established, *in vivo* preclinical evaluation protocol in which cohorts of mice were immunized with a QS saponin analogue and a four-antigen cocktail consisting of a poorly immunogenic

ganglioside, GD3 (melanoma antigen) conjugated to a highly immunogenic carrier protein KLH (keyhole limpet hemocyanin), a glycoprotein MUC1 (prostate/breast cancer antigen) conjugated to KLH, and an immunogenic protein antigen OVA (ovalbumin). Antibody titers were used as a measure of immune response while body weight loss was used to measure general toxicity. To compare adjuvant efficacy and toxicity most accurately, antibody responses and percent weight loss over the first week after immunization were compared at the most clinically relevant dose (maximum tolerated dose) as shown in Figure 2.5. Antibody titers against all antigens at all doses and toxicity data are available in the supplementary information.[a]

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[a] Immunization experiments gave consistent data across a range of doses across all experiments performed.
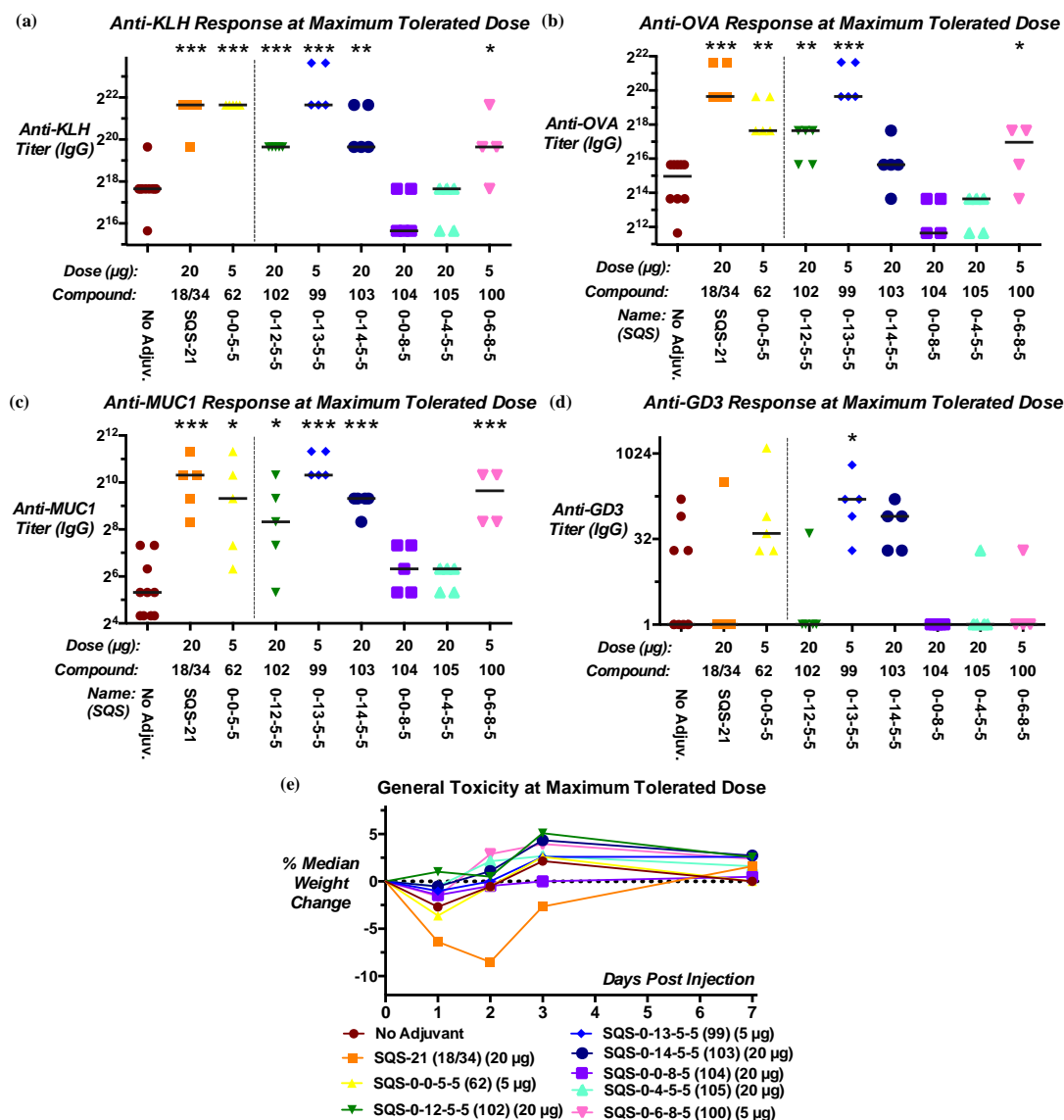
Figure 2.5. Biological Assessment at the Maximum Tolerated Dose. a) Anti-KLH titers (IgG), **(b)** anti-OVA titers (IgG) **(c)** anti-MUC1 (IgG) **(d)** anti-GD3 titers (IgG) indicating potent adjuvant activity for SQS-0-13-5-5 (**99**) and attenuated activity for SQS-0-0-8-5 (**104**). Median titer values are represented as black horizontal bars. Statistical significance is compared to the no-adjuvant control and was assessed using an unpaired Student's t-test with CI = 95%: * = $0.01 \leq p \leq 0.05$ (significant), ** = $0.001 < p < 0.01$ (very significant), *** = $p < 0.001$ (extremely significant). **(e)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for all QS analogues at maximum tolerated dose.

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Analogues with a larger distance between the triterpene and trisaccharide (ethanolamide **105** (SQS-0-4-5-5) and carbamates **101α/β**(SQS-0-5-8-5/0-5-5-5[b])) induced Ab titers against all antigens tested that were not significantly different compared to the no-adjuvant negative control, an effect more clearly demonstrated against the protein antigens (KLH, Figure 2.5a and OVA, Figure 2.5b) than glycopeptides and oligosaccharide (MUC1, Figure 2.5c and GD3, Figure 2.5d). This marked decrease in adjuvant activity suggests that the central linkage is less tolerant to modification than the acyl side chain or linear oligosaccharide.[45,72] Adjuvant activity is diminished even more with one of the most conservative modifications in α-ester **104** (SQS-0-0-8-5), which elicited, paradoxically, lower antibody titers against OVA than those of the no-adjuvant control. By contrast, other conservative modifications (α-amide **100** SQS-0-6-8-5), β-ether **102** (SQS-0-12-5-5), β-thioether **103** (SQS-0-14-5-5), β-thioester **99** (SQS-0-13-5-5)) showed comparable efficacy and reduced toxicity compared to QS-21 and our previous leading preclinical candidate **62** (SQS-0-0-5-5). Most importantly, β-thioester **99** stimulated a strong, repeatable, and consistent response against all antigens and across multiple experiments at the 5 μg dose, with negligible toxicity (<1% body weight loss).

It is especially intriguing that, relative to the natural β-ester linkage found in our leading clinical candidate **62**, two of the most conservative modifications (α-ester **104** (SQS-0-0-8-5) and β-thioester **99** (SQS-0-13-5-5), exhibit opposing extremes of adjuvant activity. Indeed the β-thioester **99** exhibited an approximate four-fold increase in potency relative to QS-21, while the α-ester **104** showed no adjuvant activity even at the

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[b] Immunological evaluation data shown in supplementary information, Figures 2.6-2.13.

highest dose tested (50 μg, Supplementary Figure 2.11). Large changes in conformation are unlikely be responsible for the increase in activity from oxo- to thio-ester, as thioesters generally adopt similar conformations to the corresponding oxo-esters.[79] However, C–S bond lengths in thioesters are ~0.4 Å longer than the corresponding C–O bonds in oxo-esters, which could impact binding to a putative cellular target. In contrast, α-ester **104** may adopt a very different conformation relative to the corresponding β-anomer present in the natural product. Taken together, these data hint at a specific macromolecular interaction that may be responsible for initiation of the immune cascade, which contrasts strongly with other immunoadjuvants that are known to act through more general processes.[71a] Moreover, this is in agreement with previous *in vitro* data from our group,[45] which showed that only active adjuvants (as opposed to attenuated but structurally similar saponins) are rapidly internalized by antigen–presenting cells and trafficked to the draining lymph nodes, where the immune response is further propagated. Investigations on the macromolecular target remain an outstanding question in our group.

2.9   Conclusion

In conclusion, we have synthesized a series of saponins to explore the SAR of the central glycosidic linkage in the *Quillaja* saponins. Although variations were conservative, we observed striking modulation of both adjuvanticity and toxicity, highlighting the triterpene–trisaccharide junction as an essential structural motif for the biological activity of the *Quillaja* saponins. Investigations to further optimize the central linkage to be more efficacious with less toxicity may come from minor variations to analogues presented here, such as an oxidized variant (sulfoxide or sulfone) of thioether **103**. Creation of such potent and non-toxic immunoadjuvants will aid in the development

of new therapeutic and prophylactic vaccines and may also aid in elucidation of the mechanism of action.

2.10   Supplemental Figures
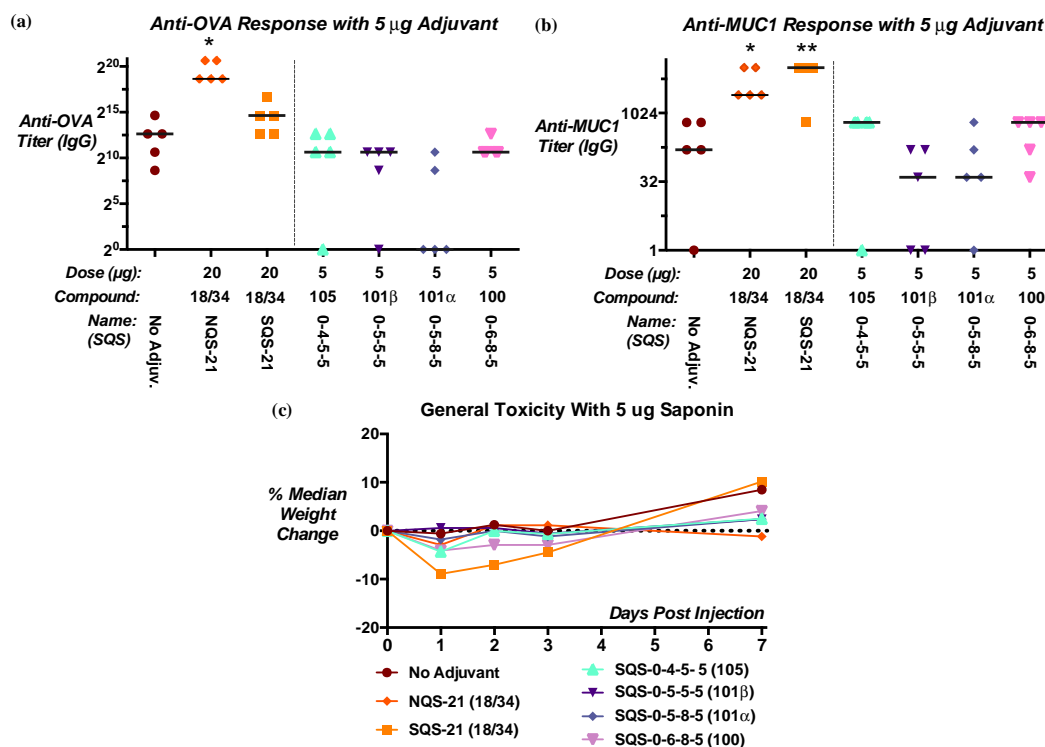
Figure 2.6. Biological Assessment with 5 μg Saponin. a) anti-OVA titers (IgG) **(b)** anti-MUC1 (IgG) indicating no adjuvant-active saponins at 5 μg dose. Median titer values are represented as black horizontal bars. Statistical significance is compared to the no-adjuvant control and was assessed using an unpaired Student's t-test with CI = 95%: * = $0.01 \leq p \leq 0.05$ (significant), ** = $0.001 < p < 0.01$ (very significant), *** = $p < 0.001$ (extremely significant). **(c)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for all QS analogues at the 5 μg dose.
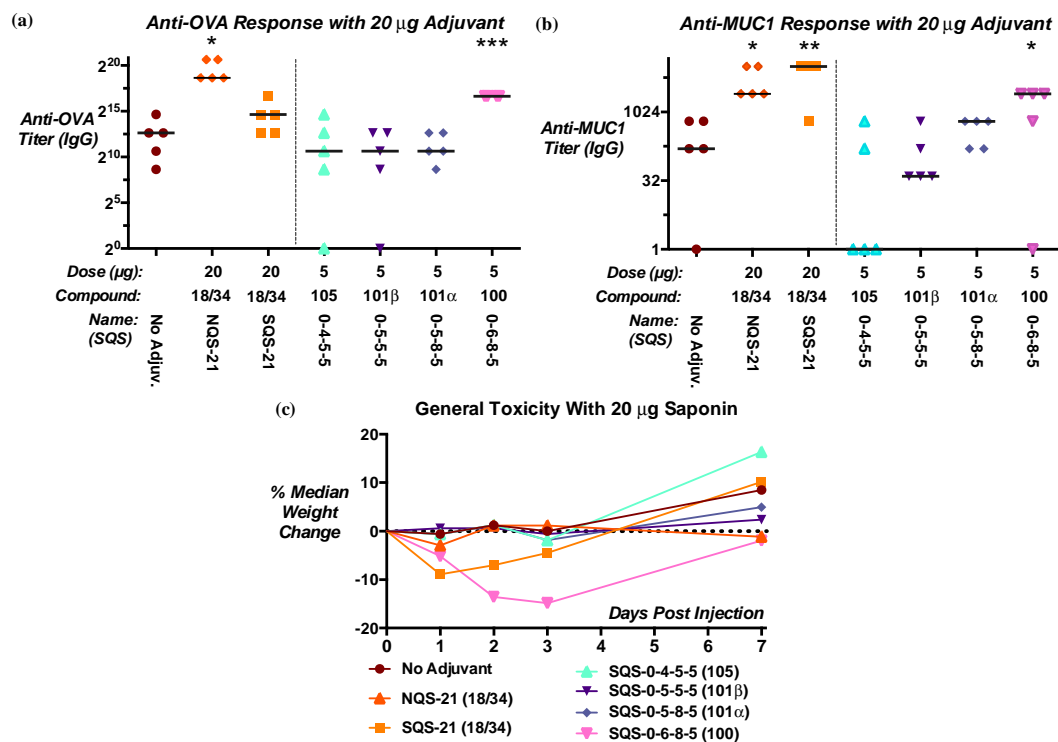
Figure 2.7. Biological Assessment with 20 μg Saponin. a) anti-OVA titers (IgG) **(b)** anti-MUC1 (IgG) indicating potent adjuvant activity for α-amide, 0-6-8-5 (**100**). Median titer values are represented as black horizontal bars. Statistical significance is compared to the no-adjuvant control and was assessed using an unpaired Student's t-test with CI = 95%: * = $0.01 \le p \le 0.05$ (significant), ** = $0.001 < p < 0.01$ (very significant), *** = $p < 0.001$ (extremely significant). **(c)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating unacceptable toxicity for the only adjuvant active saponin, 0-6-8-5 (**100**).

Figure 2.8. Biological Assessment with 50 μg Saponin. a) anti-OVA titers (IgG) **(b)** anti-MUC1 (IgG) indicating potent adjuvant activity for α-amide, 0-6-8-5 (**100**). Median titer values are represented as black horizontal bars. Statistical significance is compared to the no-adjuvant control and was assessed using an unpaired Student's t-test with CI = 95%: * = $0.01 \leq p \leq 0.05$ (significant), ** = $0.001 < p < 0.01$ (very significant), *** = $p < 0.001$ (extremely significant). **(c)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating unacceptable toxicity for the only adjuvant active saponin, 0-6-8-5 (**100**).

Figure 2.9. Biological Assessment with 5 μg Saponin. a) anti-KLH titers (IgG) **(b)** anti-MUC1 (IgG) indicating potent adjuvant activity for β-Thioester, 0-13-5-5 (**99**) and attenuated activity for α-ester 0-0-8-5 (**104**).. Median titer values are represented. **(c)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for all QS analogues at 5 μg.

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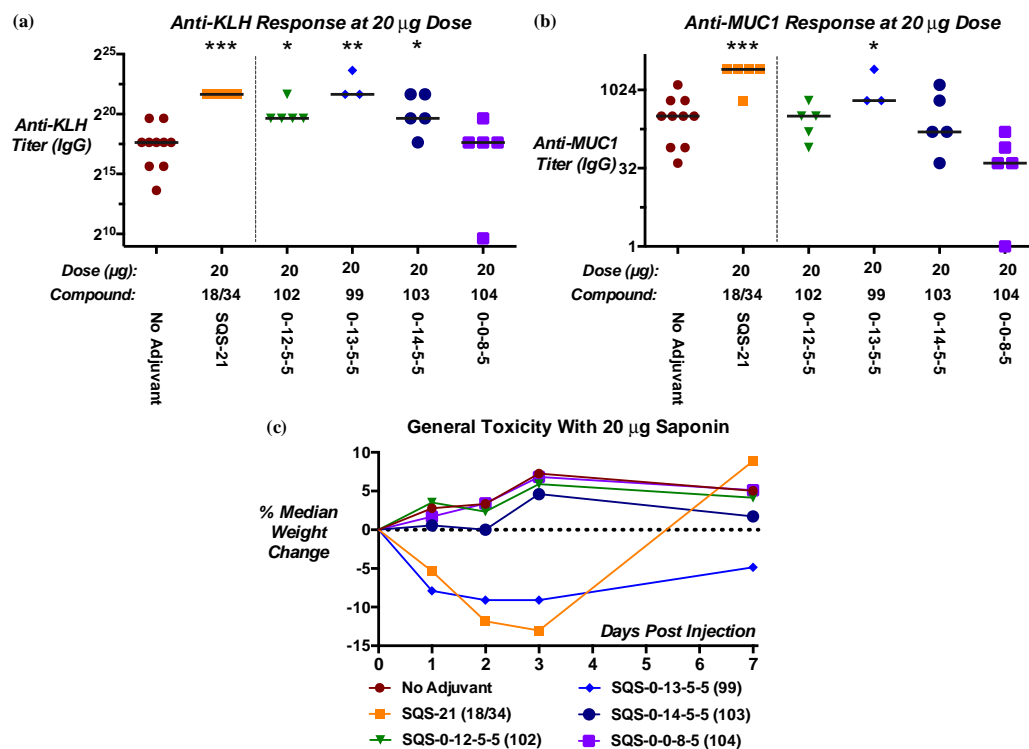
Figure 2.10. Biological Assessment with 20 μg Saponin. a) anti-KLH titers (IgG) **(b)** anti-MUC1 (IgG) indicating potent adjuvant activity for β-Thioester 0-13-5-5 (**99**) and β-ether 0-12-5-5 (**102**) and attenuated activity for α-ester 0-0-8-5 (**104**). Median titer values are represented. **(c)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for all QS analogues at 20 μg, although 2/5 mice did not survive the final boost vaccination for β-thioester 0-13-5-5 (**99**).
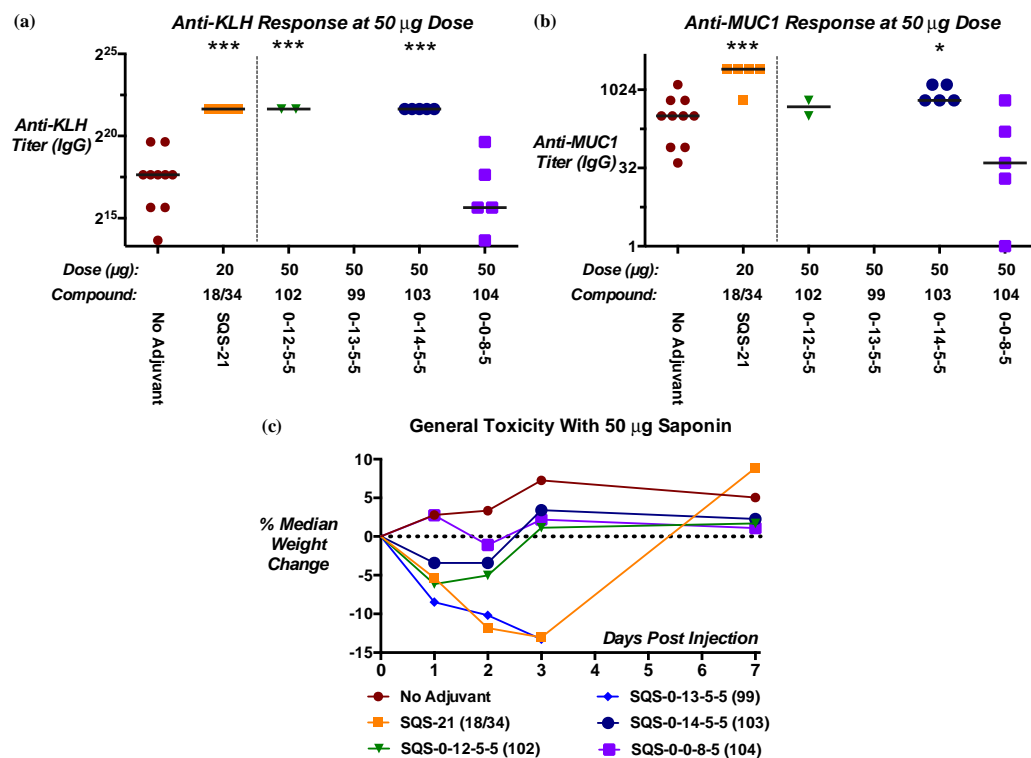
Figure 2.11. Biological Assessment with 50 μg Saponin. a) anti-KLH titers (IgG) **(b)** anti-MUC1 (IgG) indicating potent adjuvant activity for β-ether 0-12-5-5 (**102**) and β-thioether 0-14-5-5 (**103**) and attenuated activity for α-ester 0-0-8-5 (**104**). Median titer values are represented. **(c)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for all QS analogues at 20 μg except for β-thioester 0-13-5-5 (**99**), which killed all 5 mice after day 3.
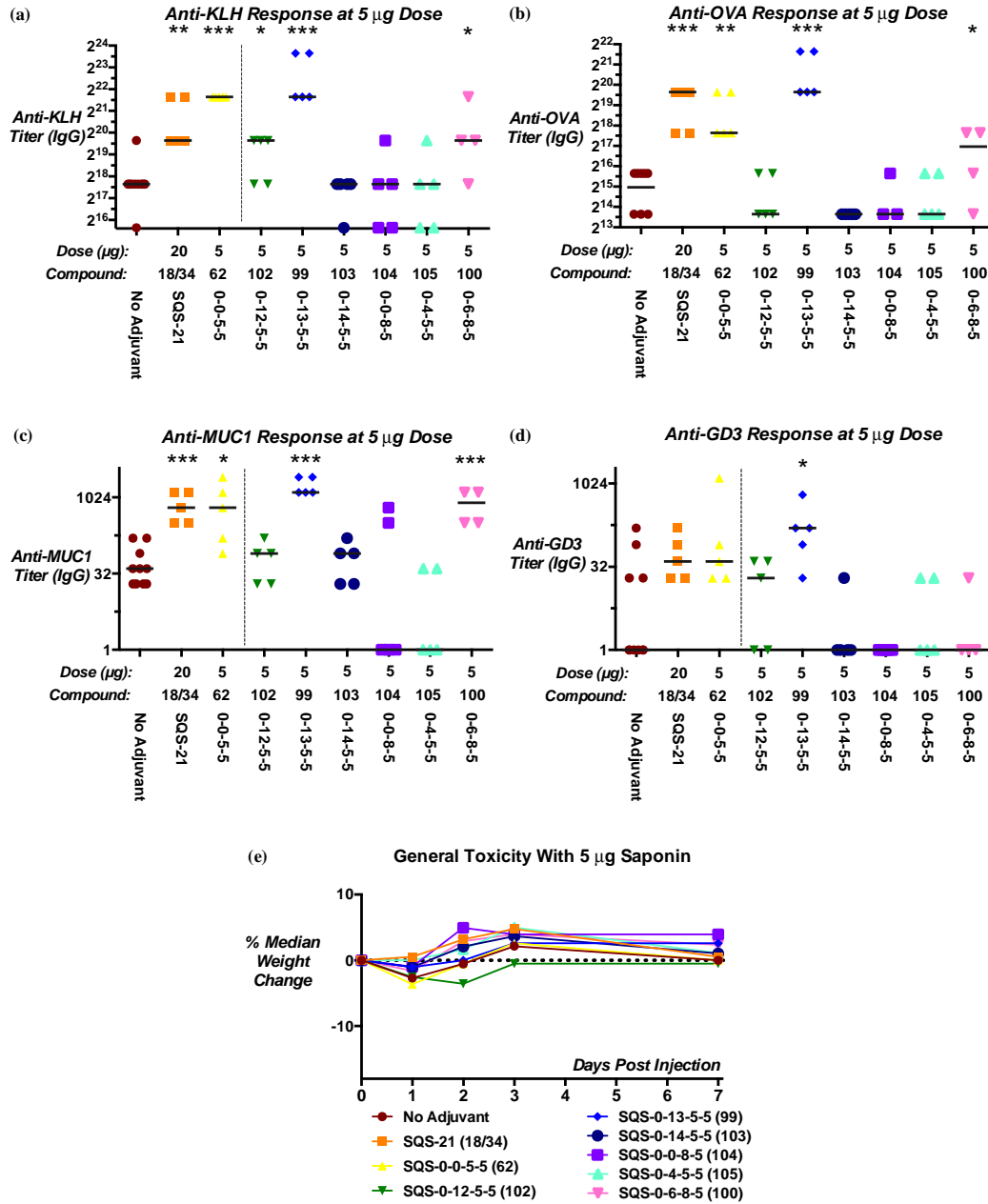
Figure 2.12. Biological Assessment with 5 μg Saponin. a) Anti-KLH titers (IgG), **(b)** anti-OVA titers (IgG) **(c)** anti-MUC1 (IgG) **(d)** anti-GD3 titers (IgG) indicating potent adjuvant activity for SQS-0-13-5-5 **(99)** and attenuated activity for SQS-0-0-8-5 **(104)**. Median titer values are represented as black horizontal bars. Statistical significance is compared to the no-adjuvant control and was assessed using an unpaired Student's t-test with CI = 95%: * = $0.01 \leq p \leq 0.05$ (significant), ** = $0.001 < p < 0.01$ (very significant), *** = $p < 0.001$ (extremely significant). **(e)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for all QS analogues at the 5 μg dose.

Figure 2.13. Biological Assessment with 20 μg Saponin. a) Anti-KLH titers (IgG), **(b)** anti-OVA titers (IgG) **(c)** anti-MUC1 (IgG) **(d)** anti-GD3 titers (IgG) indicating potent adjuvant activity for SQS-0-13-5-5 (**99**) and attenuated activity for SQS-0-0-8-5 (**104**). Median titer values are represented as black horizontal bars. Statistical significance is compared to the no-adjuvant control and was assessed using an unpaired Student's t-test with CI = 95%: * = $0.01 \leq p \leq 0.05$ (significant), ** = $0.001 < p < 0.01$ (very significant), *** = $p < 0.001$ (extremely significant). **(e)** Toxicity assessment based on median percent weight loss over one week after the first vaccine injection, indicating acceptable toxicity at indicated dose for most QS analogues at the 20 μg dose, with the lead structure β-ester 0-0-5-5 (**62**) and β-thioester 0-13-5-5 (**99**) showing signs of serious toxicity.

FACILE AND SELECTIVE OXIDATION OF C23 AND C24 METHYL GROUPS IN OLEANALANE-TYPE TRITERPENES

3 Introduction

Oleanolane-type triterpenes are among the most common triterpenes isolated from plants, arising from the common precursor β-amyrin **33**[c] (Figure 3.1). While this family of triterpenes shares a common carbon skeleton, a substantial variety of oxidation patterns exist. A recent review catalogued 95 distinct oleanalane-type triterpenes isolated in 2011 from a variety of plants with a plethora of enzymatically induced modifications including ring cleavage, cyclopropanation, peroxidation, lactonization, methyl shifts and others.[3] The most prominent modification is simple oxidation, most frequently occurring on one or more of the eight methyl groups of β-amyrin. Common oxidation points, such as C23 methyl group in the A-ring, are found in multiple oxidation states. For example, all four oxidation states of C23 have been isolated: a saturated methyl group is found in oleanolic acid (**107**, Figure 3.2), C23 is oxidized once to form a hydroxymethyl group in hederagenin (**108**), further oxidation to the aldehyde is found in gypsogenin (**109**), and oxidation to the carboxylic acid is seen in acanjapogenin G (**110**).[80]
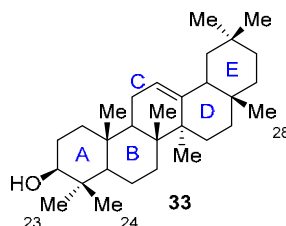
Figure 3.1. The triterpene precursor, β-amyrin.

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[c] Drawn flat to more easily show ring designations.
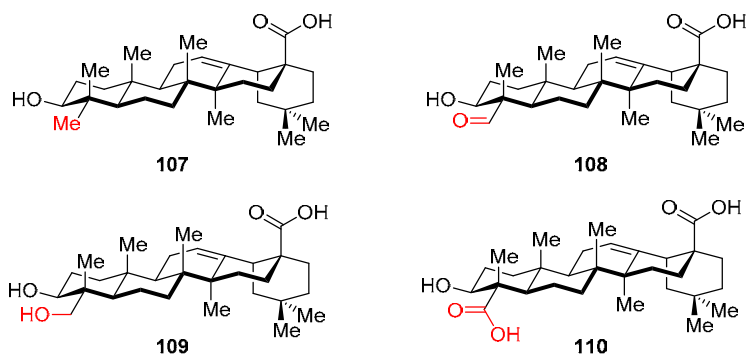
Figure 3.2. Oxidation variants at C23 of oleanolane triterpenes.

3.1   Synthesis of C24-Oxidized Oleanolane-Type Triterpenes

A closely related, but much less common β-amyrin-derived product arises from oxidation of the axial (C24) methyl group as seen in the immunoadjuvant soyasaponin and lablaboside saponins and the natural product hyptatic acid. A previous synthesis of hyptatic acid used a cumbersome sequence involving oxime directed C–H activation to effect oxidation of C24 (Scheme 3.1).[81] Recent efforts in the Gin group adapted this sequence to obtain small quantities of the desired axial neo-pentyl alcohol. However, the length of route was not amenable to throughput of adequate material to synthesize the requisite triterpene component of the soyasaponin or lablaboside saponins. Thus, we envisioned a short sequence to afford the desired oxidation from benzyl oleanolate, involving radical mediated cleavage of the A-ring,[82] allylic oxidation, and finally a thiolate promoted diastereoselective tandem Michael–aldol reaction to reform the A-ring with the desired methyl group oxidation.

Scheme 3.1. Oxime-directed C–H oxidation.

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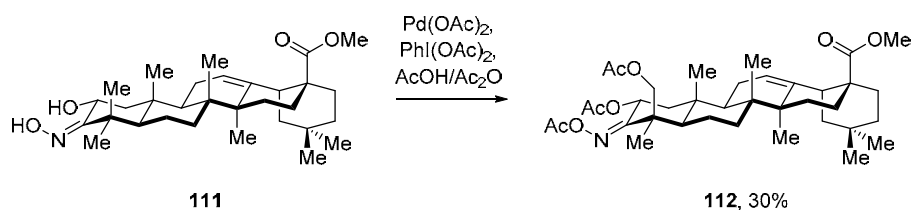
3.2 Tandem Michael–Aldol Reactions in Total Synthesis

Tandem Michael–aldol reactions employing aryl and aliphatic thiolates have been used in both inter- and intramolecular fashion for decades. After the first reports by Nozaki and colleagues in 1980[83] with the dimethyl aluminate of thiophenol, the Danishefsky group applied the reaction the total synthesis of avermectin A_1.[84] Michael addition of thiophenol dimethylaluminate to the α,β-unsaturated aldehyde **113** followed by aldol reaction with the dihydrofuranone furnished a 5,6 bicycle **114** (Scheme 3.2). Treatment with *m*-CPBA facilitated elimination of the sulfoxide, forming the α,β-unsaturated aldehyde **115**.

Scheme 3.2. Tandem Michael–aldol reaction in the total synthesis of avermectin A_1. Oxidation and elimination give enal **115**.
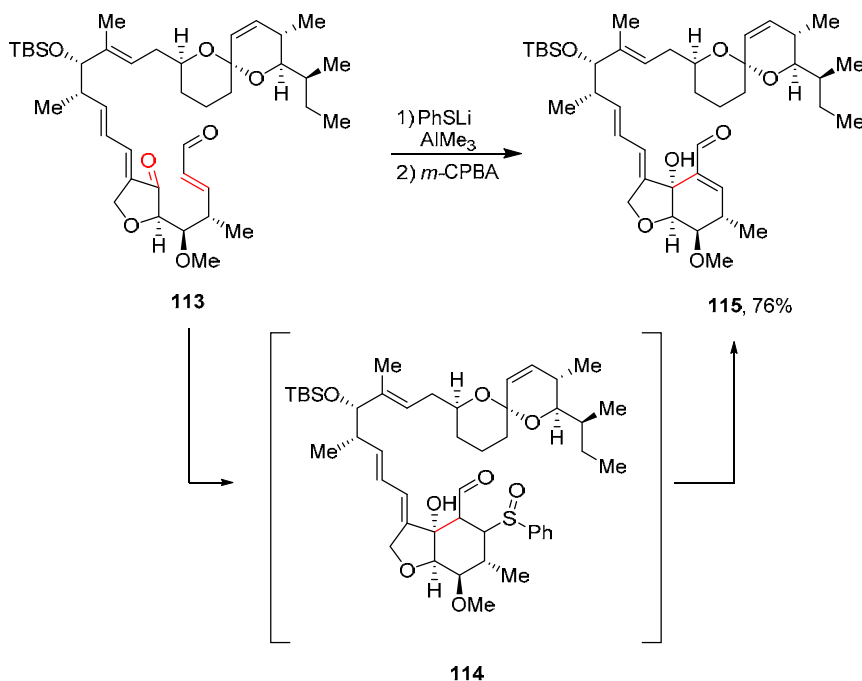
While the strategy designed by Nozaki and implemented by Danishefsky bears significant resemblance to the Baylis–Hillman reaction, further application of the conditions developed by Nozaki did not involve immediate elimination of thiolate. The

retention of two sp³-centers in the thiolate product facilitated development of diastereoselective thiol promoted tandem Michael–aldol reactions.

The total synthesis of the spiro-alkaloid nitramine by Koomen and Wanner employed a tandem Michael–aldol in a similar manner as the Danishefsky group, but without subsequent oxidation and elimination.[85] α,β-Unsaturated imide **116** was treated with PhSAlMe₂ in THF, forming the desired *syn* diastereomer spirocycle **118** in excellent yield (Scheme 3.2). Given the reversibility of the Michael–aldol reaction, a product highly enriched in one diastereomer suggests a strong thermodynamic preference for the observed product. Similar conditions employing the iodo–magnesium complex of thiophenol (PhSMgI, analogous to Grignard reagent) resulted in a mixture of *syn* and *anti* products, highlighting the importance of the lithium cation, likely through stabilization of the transition state **117** (Scheme 3.3). Desulfurization of **118** furnished alcohol **119**, which, after reduction and deprotection, furnished the natural product **120**.

Scheme 3.3. Tandem Michael–aldol in the total synthesis of nitramine.
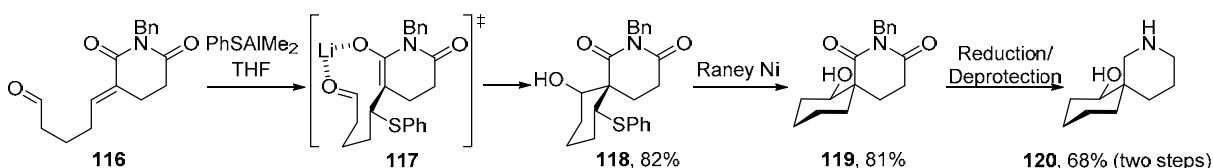
3.3   Three-Step Oxidation Sequence to Achieve Oxidation of C24

To accomplish the desired oxidation at C24 of oleanolic acid required for the synthesis of the soyasaponin and lablaboside saponins, we envisioned a diastereoselective ring-closing conjugate addition reaction to form the two contiguous stereocenters at C3 and C4 of **121** (Scheme 3.4). The requisite α,β-unsaturated aldehyde **122** would be obtained via an allylic oxidation of **123**. Finally, the aldehyde 123 would arise from a

radical mediated oxidative ring-opening of the inexpensive and abundant ester of oleanolic acid, **124.**

Scheme 3.4. Retrosynthesis plan to achieve oxidation of C24.
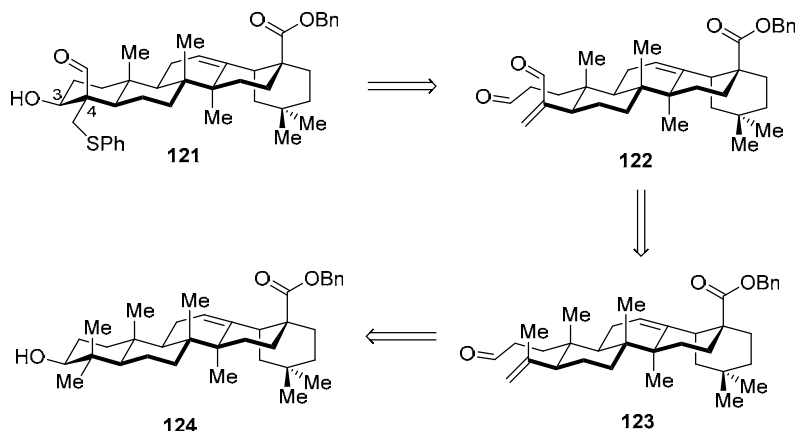
3.3.1 Optimization of A-ring Cleavage

Initiation of the Suárez cleavage occurs via radical mediated reaction of iodine with bis-acetoxyiodobenzene to form two equivalents of acetyl hypoiodite, which then forms alkyl hypoiodite **125** (Scheme 3.5).[86] Photolytic cleavage results in generation of an *O*-centered radical, **126,** which fragments to form an aldehyde and the tertiary radical species **127**. Homolytic proton abstraction results in olefin **128**. Initial small-scale studies furnished enal **123** in 60% yield. However, increasing to gram-scale resulted in a dramatic decrease in yield of the desired product, with a concomitant increase in the formation of allyl iodide byproduct **128** in significant quantities (20–34%).

Scheme 3.5. Reaction mechanism of Suárez cleavage.
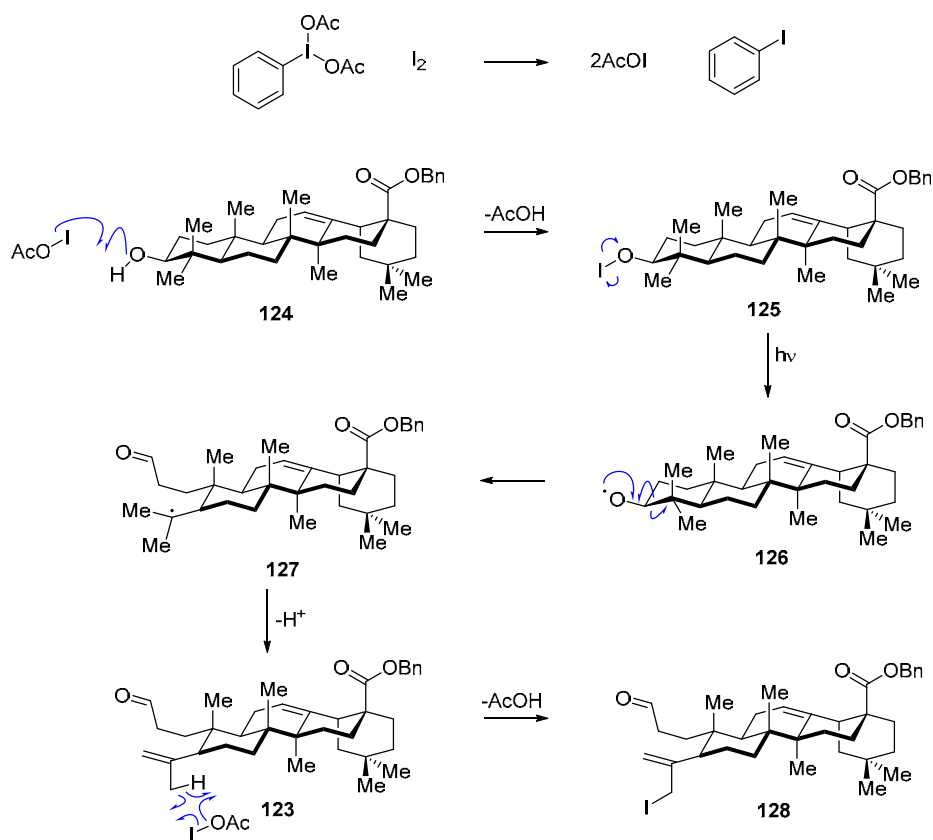
The conditions initially described by Suárez,[82a] and those implemented in our early efforts, require a full equivalent of iodine and excess PhI(OAc)$_2$. However, as can be appreciated in the mechanism (Scheme 3.5), both iodine atoms can be used to form the alkyl hypoiodite species **125**. We hypothesized that, by decreasing the amount of molecular iodine (thereby decreasing the amount of reactive acetyl hypoiodite), we could avoid formation of the allyl iodide by product **128**. Indeed, starting the reaction with 0.5 equiv iodine, then additional small aliquots until all starting material had been consumed completely suppressed formation of the undesired species. With these conditions, the Suárez cleavage of **124** could be achieved in good yield in a highly reproducible fashion on gram-scale to form the desired enal **123**.

3.3.2   Development of Tandem Michael–Aldol

As mentioned previously, thiol promoted tandem Michael–aldol reactions have been used in several instances in the synthesis of natural products. Most commonly, lithium thiophenolates have been employed as nucleophiles. As such, initial efforts using these conditions gave a mixture of undesired stereoisomers[d] (3:1, **121c**:**121d**), both with axial thioethers (Scheme 3.6). To assign the structures of the products of the Michael–aldol, the thioethers were desulfurized with Raney nickel to the corresponding 1,3-diols and compared with published NMR data.[81,87]

Scheme 3.6. Tandem Michael–aldol with thiophenol.
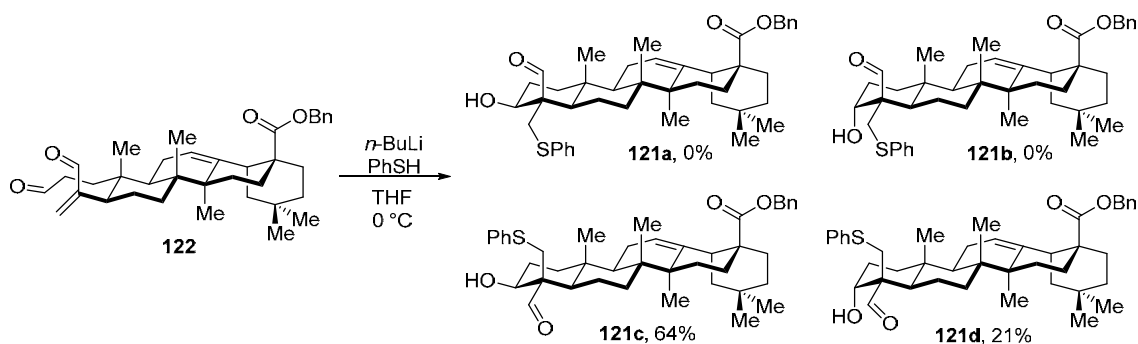
In an attempt to manipulate the diastereoselectivity of the tandem Michael–aldol reaction, we altered the sterics and electronics of the thiolate nucleophile. Employing the bulky *tert*-butyl thiol (Scheme 3.7) furnished a 10:1 mixture of desired to undesired diastereoisomers with a 71% isolated yield of the desired product, **129a**. Compared to the aromatic thiol in entry 1, we observed a strong preference for an equatorial disposition for the newly formed thioether. We suspect that this is due to the unfavorable 1,3-diaxial interactions of the *tert*-butyl group with the C25 axial methyl group

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[d] Diastereomeric configuration nomenclature is consistent with all nucleophiles, i.e. equatorial –OH at C3 with equatorial thioether is **a**, axial –OH at C3, equatorial thioether is **b**, etc.

(highlighted in red in Scheme 3.7) that occur with formation of the undesired axial-disposed thioether. With such a profound change in selectivity, we sought to determine the extent of reagent-controlled diastereoselectivity.

Scheme 3.7. Tandem Michael–aldol with *tert*-butylthiol.
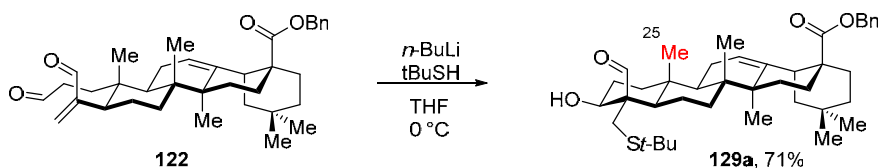
Thus, we examined several commercially available 2,6-disubstitued thiophenols to determine if the reaction outcome was more dependent on the sterics or electronics of the thiolate nucleophile. As shown in entries 3 and 4 in Table 3.1, reaction with 2,6-disubstituted thiophenols resulted in similar yields compared to unsubstitued aromatic thiol (entry 1). However, use of the bulkier aromatic thiols furnished significant amounts of the desired diastereomer, **130a** (entry 3) and **131a** (entry 4) compared to the unsubstituted thiophenol. Interestingly, reaction with the more electron-poor 2,6-dichlorothiophenolate resulted in a similar ratio of desired:undesired diastereomers compared to the more electron rich 2,6-dimethylthiophenolate. Thus, the reaction outcome is dependent mostly on sterics for determination of the diastereomeric ratio.

Table 3.1. Reagent controlled diastereoselective tandem Michael–aldol reaction.
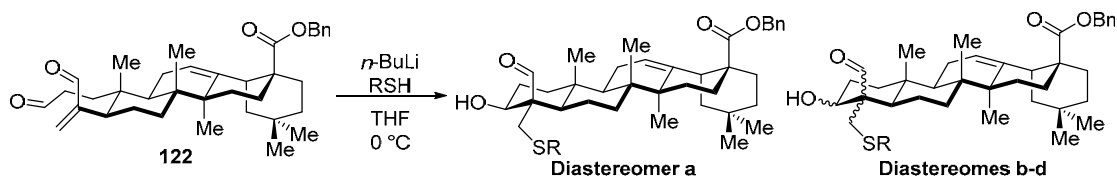
Entry	Nucleophile	Product	Ratio[a]	Yield[b]
1	PhSH	**121**	0:1	85%
2	t-BuSH	**129**	10:1	71%
3	2,6-dichlorobenzenethiol	**130**	3.7:1	87%
4	2,6-dimethylbenzenethiol	**131**	3.5:1	51%
5	Ph$_3$CSH	**132**	5:1	89%
6	EtSH	**133**	2:1	65%
7	i-Pr$_3$SiSH	**134**	1:25	45%
8	PhSeH	**135**	0:1	63%

[a]Ratio: Diastereomer a:Σ(Diastereomers b-d) [b]Combined yields of all diastereomers.

Examination of commercial aliphatic thiols confirmed our previous hypothesis, with bulky thiols having a strong equatorial preference in the product. Trityl thiol gave very good diastereoselectivity (entry 5) with excellent yield, similar to the *tert*-butyl derivative **129** (entry 2). By contrast, smaller aliphatic thiols such as ethanethiol gave reasonable yields with decreased selectivity (entry 6). Moreover, increasing the distance between the bulky group and the nucleophile, as with triisopropylsilyl thiol derivative **134** (entry 7), reversed diastereoselectivity and decreased yield. Continuing this trend,

reaction with benzeneselenolate, which has a longer C–Se bond than the corresponding C–S bond in thiophenol, decreased both yield and diastereoselectivity (entry 8).
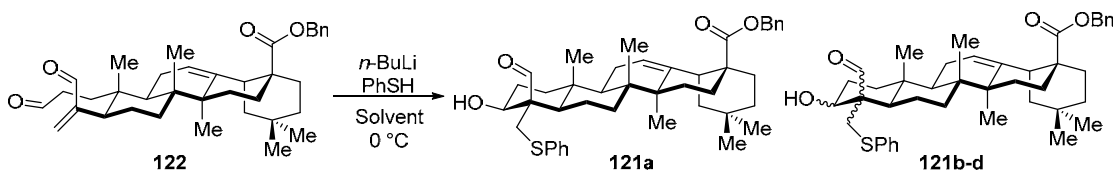
Table 3.2. Effect of solvent on diastereoselectivity on the tandem Michael–aldol reaction.

Entry	Solvent	Ratio[a]	Yield[b]
1	THF	0:1	85%
2	Ether	2:1	70%
3	CH_2Cl_2	0:1	42%
4	Toluene	1:15	66%
5	Hexanes	<1:20	60%

[a]Ratio: Diastereomer a:Σ(Diastereomers b-d) [b]Combined yields of all diastereomers.

Solvent had a strong effect on diastereoselectivity and yield (Table 3.2). In all solvents except diethyl ether, the desired equatorial-disposed thioether product (**121a**) was formed in small quantities or not at all. While the mechanistic rationale for such a profound effect it is not entirely clear, we hypothesize that the weakly coordinating diethyl ether oxygen lone-pairs may be facilitating a lithium cation-dependent stabilization of the desired product.
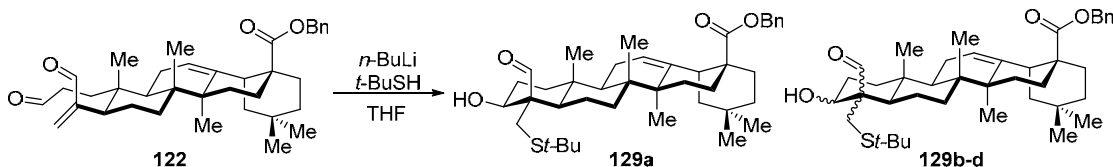
Table 3.3. Effect of temperature on diastereoselectivity of the tandem Michael–aldol reaction.

Entry	Nucleophile	Temp (°C)	Ratio[a]	Yield
1	*t*-BuSH	-40	1:7	58%
2	*t*-BuSH	0	10:1	71%
3	*t*-BuSH	23	>20:1	48%
4	(phenyl)SH	0	0:1	85%
5	(phenyl)SH	23	0:1	59%

[a]Ratio: Diastereomer a:Σ(Diastereomers b-d) [b]Combined yields of all diastereomers.

Analysis of the effect of temperature on reaction outcome suggests that the equatorial thioether is the thermodynamic product of the reaction (Table 3.3) with bulky nucleophiles. With *tert*-butyl thiol, reaction at –40 °C (entry 1) gives 1:7 ratio of the desired:undesired isomers. Increasing the temperature to 0 °C (entry 2) reverses the selectivity, while increasing to room temperature (entry 3) results in near exclusive formation of the desired diastereomer. A similar trend is not observed with thiophenol. Indeed, no desired product is formed in THF. However, yields suffered as the temperature was increased for reaction with both thiolates (entries 3 and 5), likely due to decomposition under the reaction conditions. As mentioned previously, the thermodynamic preference for an equatorial *tert*-butyl thioether likely arises from unfavorable 1,3-diaxial interactions with the axial C25 methyl group (Scheme 3.7), an effect much less pronounced with the less bulky thiophenol.

While these experiments have been performed under well-controlled conditions, preliminary experiments indicate reaction time may play an important role in determining the final ratio of diastereomers. For instance, while employing lithium thiophenolate at 0° C for 20-30 min furnishes no desired product (Table 3.1, Entry 1), extending the reaction to 12 hr furnishes almost exclusively the desired product in 40% yield (data not shown). Although the data is far from complete, our data suggests that there is a thermodynamic ratio of products for each set of reaction conditions. However, the sterics and electronics of the lithium thiolates may drastically alter the kinetics of the overall reaction. This may arise from the previously mentioned unfavorable 1,5-diaxial interactions of the thiolate alkyl or aryl group with the C25 methyl substituent of the triterpene highlighted in Scheme 3.7.

3.4   Raney Nickel Desulfurization

While desulfurization with Raney nickel is generally a straightforward, albeit harsh procedure, this particular substrate presented a major challenge. The crowded reaction center requires forcing conditions with very active Raney nickel, which is difficult to both procure and maintain. Indeed, effective desulfurization only occurs with a new bottle of Raney nickel from select manufacturers. Nonetheless, **129** was desulfurized (as well as debenzylated and reduced) to give acid diol **136** in small quantities. In the near future, protection of the carboxylate followed by selective protection of the primary alcohol will give the desired triterpenoid to be used in the synthesis of the lablaboside saponins.

Scheme 3.8. Raney nickel desulfurization.
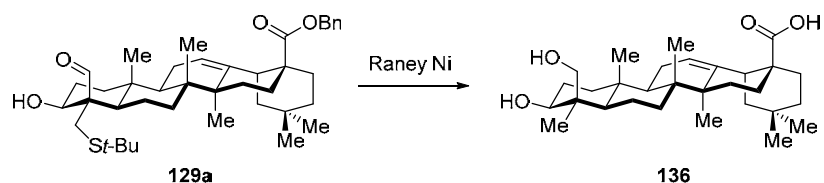
3.5 Conclusion

We have developed a short sequence to access a C24-oxidized triterpene of interest in the synthesis of several immunopotentiating saponins. The only published synthesis required more than ten steps, including several tedium purifications. Additionally, we are the first to report the scope of a thiol promoted diastereoselective tandem Michael–aldol reaction. The ring–closing reaction, under both stereo- and electronic control sets two contiguous stereocenters in a facile and repeatable fashion. After optimization of desulfurization and selective protection conditions, these triterpenoids will be advanced to the synthesis of the lablaboside saponins.

CHAPTER 4.

SYNTHESIS OF THE LABLABOSIDE SAPONINS

4   Introduction

The lablaboside saponins were isolated[52] from the edible hyacinth bean, a legume widely cultivated throughout Asia. While used mostly as a foodstuff in Japan and India, the bean has been used extensively for medicinal purposes in China. The white seeds of the *Dolichos lablab* plant have been prescribed for alimentary disorders as well as for treatment of alcoholism. However, before 1998, no systematic study of purified components had been performed. Yoshikawa and co-workers, a Japanese group with tremendous expertise[52,88] in the study of medicinal foodstuffs, isolated a series of novel saponins from the seeds of *D. lablab* that exhibited potent immunoadjuvant activity.

Lablabosides A-F are bisdesmoside saponins, featuring a linear trisaccharide attached to C3 of oleanolic acid or a C24-oxidized variant, epi-hederangenin, and an oligosaccharide attached to C28 (Table 4.1). As mentioned in Chapter 1, extensive oligosaccharide variation occurs at C28, with a mono-, di- or trisaccharide appended to the triterpenoid. Additionally, lablaboside D (**16**) is adorned with an acyl chain (hydroxy-methylglutaroyl) at C6 of glucose.

Table 4.1. Structure of the lablaboside saponins.
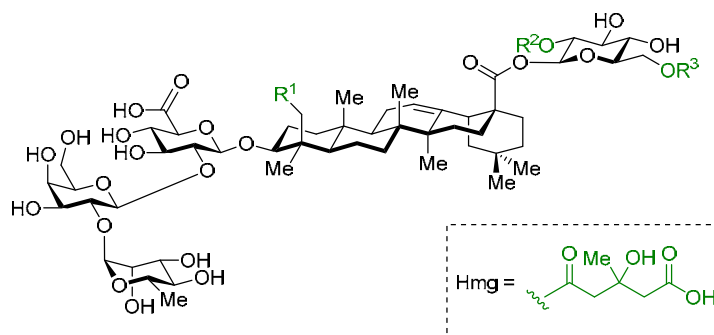
Saponin	R^1	R^2	R^3
lablaboside A (**137**)	H	H	H
lablaboside B (**138**)	OH	H	H
lablaboside C (**139**)	OH	α-Rha	H
lablaboside D (**16**)	OH	H	Hmg
lablaboside E (**140**)	OH	α-Rha-4-α-Rha	H
lablaboside F (**15**)	H	α-Rha-4-α-Rha	H

4.1 Immunoadjuvant Activity

Initial studies of the immunoadjuvant activity compared the lablaboside saponins to QS-21 and other structurally related adjuvant-active saponin molecules, such as the soyasaponins and escin saponins (shown in Chapter 1, **8** and **4/5**).[39b] Compared to QS-21, both soyasaponins and lablaboside saponins exhibited negligible hemolytic toxicity, while maintaining potent immunopotentiating properties. This is especially true for lablabosides D (**16**) and F (**15**), which were shown to induce greater passive haemaglutination titers than QS-21.

Further studies examining the immunopotentiating activity examined the efficacy of lablaboside F as an immunoadjuvant against a lethal infection of Aujeszky's disease virus in mice.[39a,39c] Aujeszky's disease, or pseudorabies, is an endemic disease of swine

and cattle in most of the world. Development of a prophylactic vaccine requires an immunoadjuvant capable of inducing a mixed Th1 and Th2 response to protect against a lethal dose of virus most effectively. In this comparative study, aluminum salts, QS-21, and two oil-in-water formulations (similar to the MF59 adjuvant) were compared for immunoadjuvant activity when co-administered with soluble and particulate antigens. Compared to QS-21, lablaboside F elicited similar levels of IgG1a, but a significantly lower IgG2 response, albeit with no toxicity. As such, the vaccine showed no statistically significant survival benefit compared to a no-adjuvant control. To our knowledge, further development of lablaboside F as an immunopotentiator has not been explored.

Despite the pre-clinical failure to show efficacy against Aujeszky's disease, the initially observed potent immunopotentiating activity and negligible toxicity makes lablaboside F an attractive saponin to explore further in other clinically relevant systems. As such, we sought to synthesize the entire family of lablaboside saponins to assess the validity of the initial studies using the pre-clinical assay developed in our group and determine if further development is warranted.

Scheme 4.1 Failed global deprotection in previous work
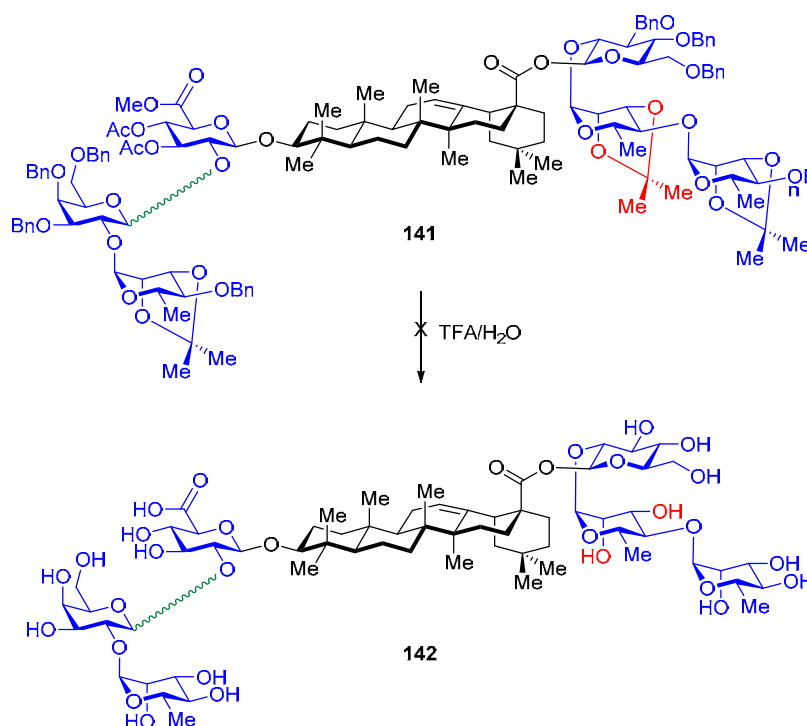
4.2   Previous Synthetic Efforts and Global Deprotection Strategy

Preliminary synthetic studies in our group resulted in the synthesis of a fully

protected epimer **141** (α-Gla instead of the natural β-Gal anomer, highlighted in green) of

lablaboside F (**142**). The most important findings of these studies resulted from the many

failed attempts to remove all protecting groups.   In the final deprotection step, an

acetonide protecting group on the rhamnose moiety highlighted in Scheme 4.1 could not

be removed, despite repeated efforts by several investigators. Indeed, the acetonide was

recalcitrant to a litany of acid-mediated hydrolysis conditions resulting in decomposition

of the saponin before hydrolysis of the protecting group. To get around this problem, we

sought to employ only hydrogenolysis-labile protecting groups to facilitate a simple, one-

step global deprotection.   To this end, we selected a benzophenone ketal, a protecting

group rarely utilized in carbohydrate chemistry.[89]

Scheme 4.2 Synthesis of rhamnose–rhamnose disaccharide.
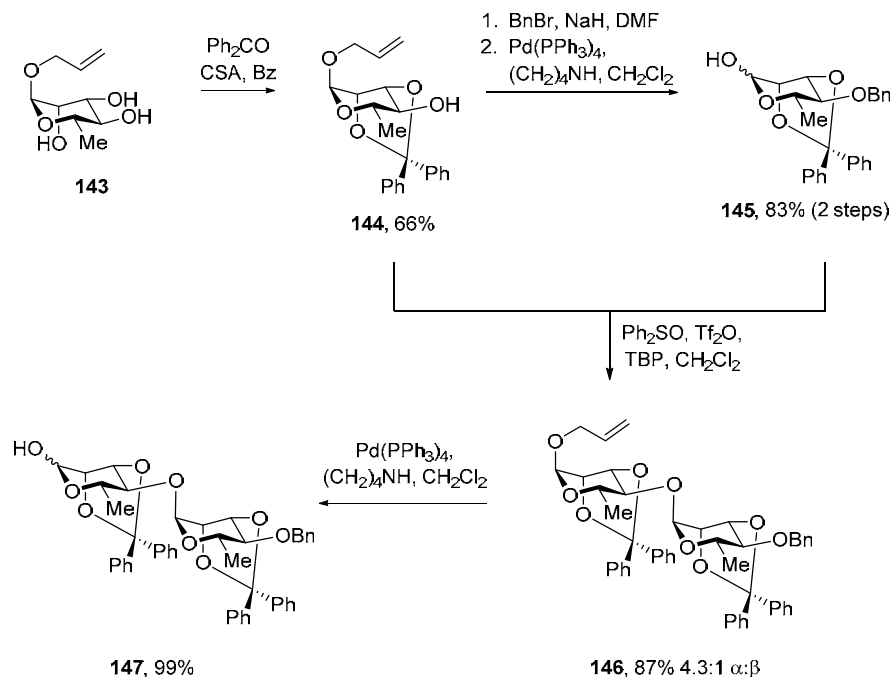
4.3 Synthesis of Eastern Trisaccharide

Synthesis commenced with of protection of allyl-L-rhamnose (**143**).[90] Ketalization with benzophenone, catalyzed by camphorsulfonic acid under reduced pressure, furnished ketal **144**. Benzylation under standard conditions followed by deallylation with tetrakis(triphenylphosphine)palladium furnished rhamnose hemiacetal **145**, which was a less reactive glycosyl relative to a similarly protected acetone ketal of rhamnose. Standard dehydrative glycosylation conditions[74a] (Ph$_2$SO, Tf$_2$O, 15 min at −78 °C, 60 min at −55 °C, then addition of acceptor) gave low yields (<30%). However, by extending activation time from 60 to 90 min, high yields of the desired α-anomer **146** were obtained. This is likely due to the electron-poor glycosyl donor hemiacetal **145**, which retards the rate of oxocarbenium/glycosyl triflate formation.[91] Deallylation furnished hemiacetal **147**.

70

Scheme 4.3. Synthesis of fully protected eastern trisaccharide
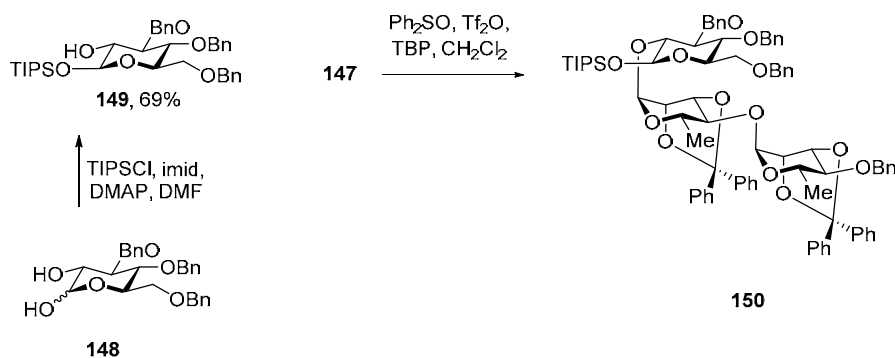
Next, the known glucose 1,2-diol **148**,[92] formed by dihydroxylation of tribenzyl glucal, was selectively silylated with triisopropylsilyl chloride to furnish 2-hydroxyglucose donor **149**, which was glycosylated under dehydrative conditions to give trisaccharide **150**. Desilylation followed by bromination with oxalyl bromide furnished α-glycosyl bromide **152**, which will serve as the glycosyl donor in the final bond-forming step in the syntheses of lablaboside E and F (Scheme 4.4).

Scheme 4.4. Synthesis of eastern trisaccharide glycosyl donor.
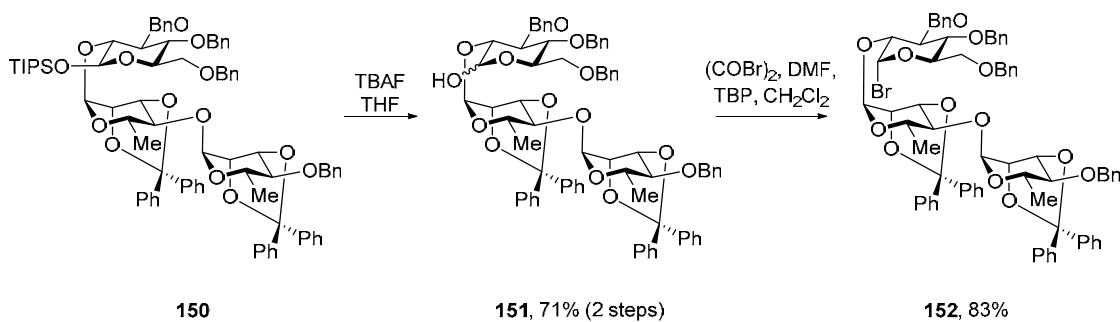
4.4 Synthesis of Western Trisaccharide

To synthesize the linear trisaccharide common to all the lablabosides, we began by epoxidation of known uronic acid glycal **153** with dimethyldioxirane.[93] After a solvent exchange, this epoxide was treated with zinc chloride, facilitated ring opening

with allyl alcohol furnishing **154**.[94] The resulting alcohol served as acceptor in the

dehydrative glycosylation of known galactose hemiacetal **155**. The benzoyl ester on C2

of galactose hemiacetal[64a,95] facilitated β-selective glycosylation, furnishing disaccharide

156 in excellent yield with 5:1 anomeric selectivity. Without a benzoyl

protecting/directing group, exclusive formation of α-anomer was observed.

Scheme 4.5. Synthesis of western trisaccharide donor.
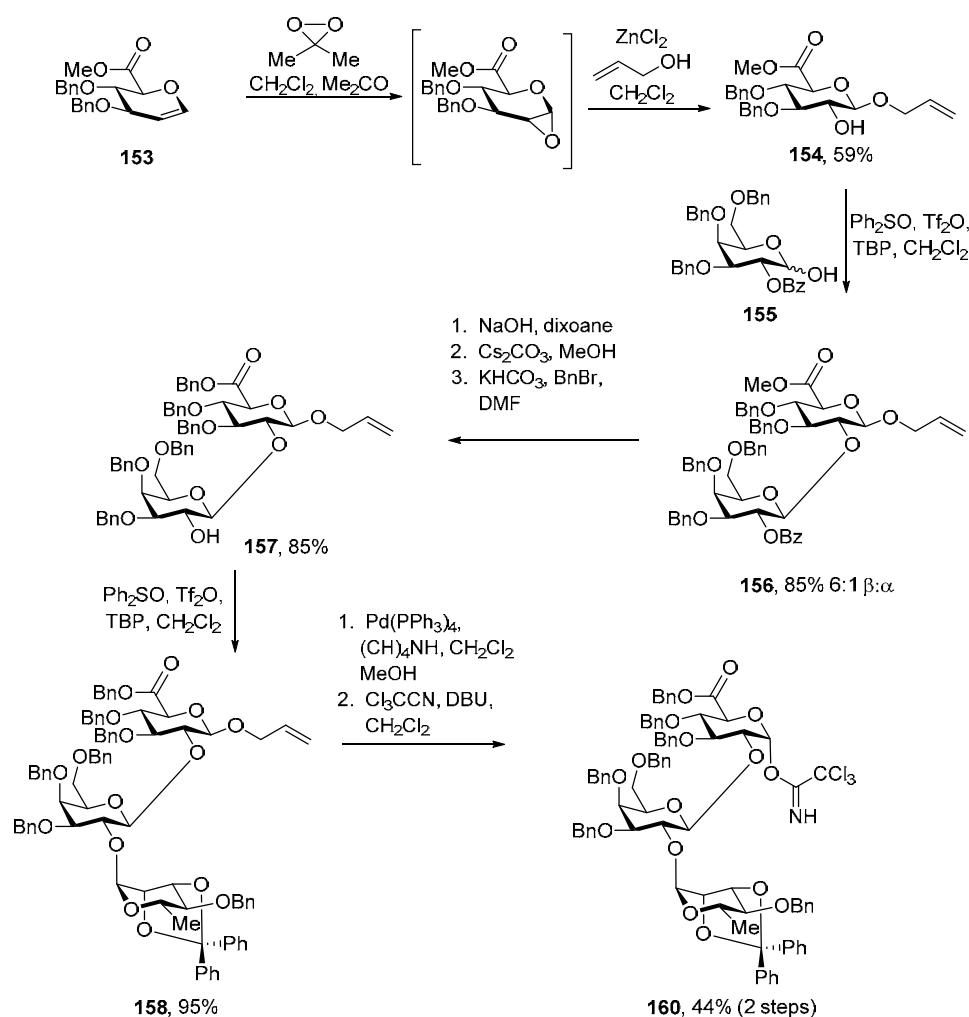
To facilitate the one-step global deprotection, a protecting group change was

necessary on the glucuronic acid moiety. A one-pot procedure removed the methyl ester

by hydrolysis in dioxane and the benzoyl group by methanolysis, followed by alkylation of glucuronate with benzyl bromide to give **157** in excellent yield over three steps.[62b]

Dehydrative glycosylation of rhamnose hemiacetal **145** proceeded in nearly quantitative yield to furnish fully protected trisaccharide **158**. As previously stated, extended reaction times were necessary to achieve high yields with benzophenone ketal protected rhamnose donors. Deallylation followed by formation of trichloroacetimidate furnished trisaccharide donor **160**.

4.5   End Game Glycosylation

With both trisaccharide donors in hand, we had to choose the appropriate order of the two late-stage glycosylations. The most important considerations revolve around the efficiency of synthesis of each oligosaccharide. The western trisaccharide donor **160** requires 14 linear steps from commercially available starting materials, whereas the eastern trisaccharide **152** requires only nine steps. Thus, it was desirable to glycosylate at the triterepene C28 carboxylate first, and then glycosylate at C3 with the more precious western trisaccharide in the penultimate step.

Scheme 4.6 Desirable end game glycosylation route.
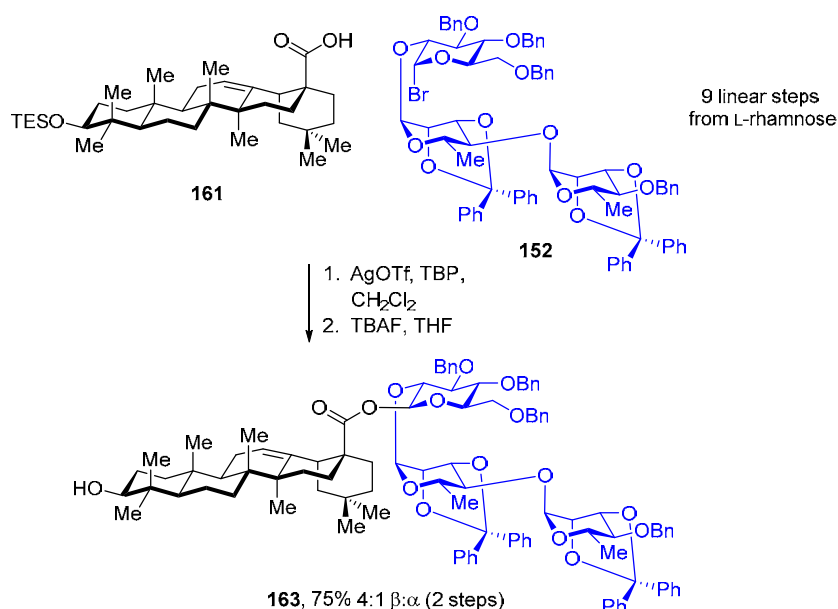
4.5.1 End game glycosylations - C28 then C3

Glycosylation of C3-protected oleanolic acid derivative **161** under Koenigs–Knorr conditions gave the β-anomer when employing an excess of the acceptor (Scheme 4.7). Desilylation required elevated temperature and extended reaction time, but was achieved cleanly with tetrabutylammonium fluoride to form **163**. Before attempting the final glycosylation with the precious substrate, we used allyl oleanolic acid **165** as glycosyl acceptor to develop optimal conditions. We found that employing the conditions identified by the Gin group for the construction of a very similar bond en route to the synthesis of QS-21 furnished almost identical results.[62] Using an excess of the cheap and abundant acceptor **165**, glycosylation, catalyzed by tris-pentafluorophenyl borane, proceeded smoothly to give the desired product in 75% yield with 20:1 anomeric selectivity favoring the β-anomer. Deallylation gave carboxylic acid **167**.

Scheme 4.7. Glycosylation of western trisaccharide with an inexpensive and abundant acceptor **165**.
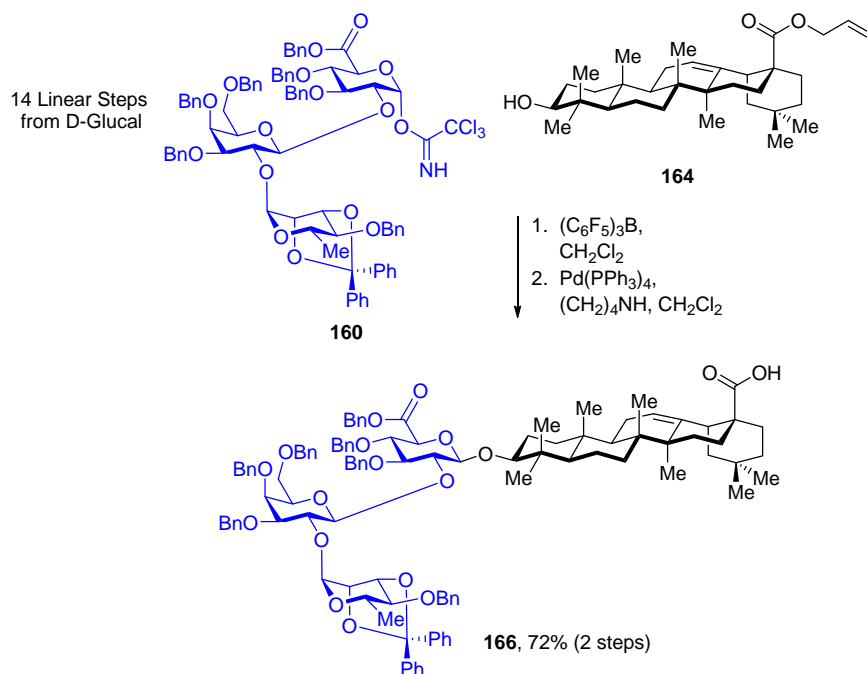
Attempts to perform an analogous glycosylation with imidate **166** with the more complex donor **163** yielded no detectable glycosylation product (Scheme 4.8). While it is surprising that protected glycosides on the distal end of the triterpene would affect exposure of the C3 alcohol to the electrophilic glycosyl donor, repeated attempts failed to deliver even trace amounts of the desired product. As such, we were forced to reverse the order of the late-stage glycosylations.

Scheme 4.8. Failed glycosylation en route to fully protected lablaboside F.
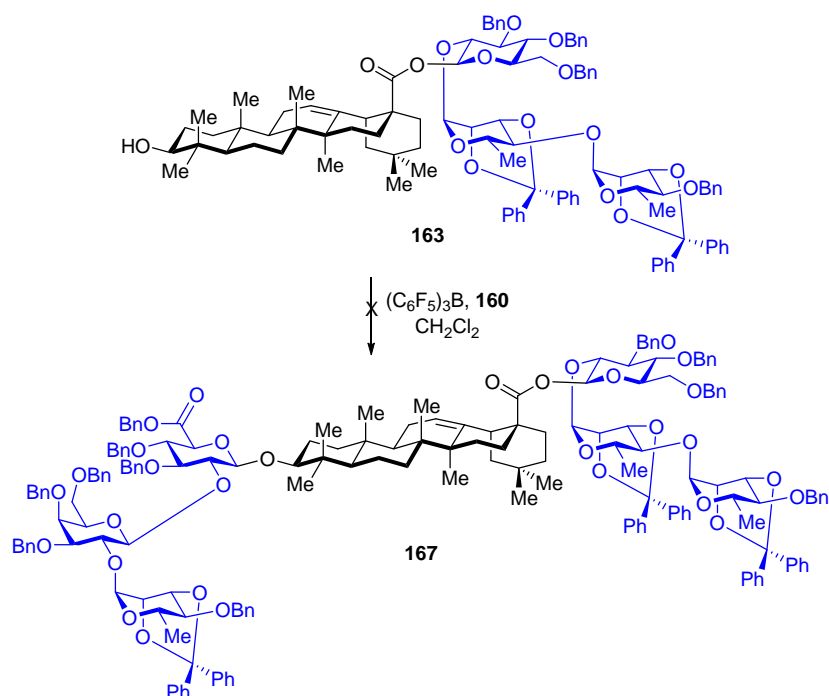
4.5.2 End game glycosylation - C3 then C28

We envisioned bringing together advanced intermediate carboxylic acid **167** and eastern trisaccharide bromide **152** using the silver triflate-promoted Koenigs–Knorr glycosylation (similar to Scheme 4.9). However, repeated attempts failed to deliver an anomerically pure product. Moreover, the mixture of anomers was inseparable on standard silica gel under panoply of conditions. Drawing on lessons learned constructing similar glycosidic linkages in Chapter 2 led to employment of phase-transfer glycosylation conditions, whereby the glycosylation would proceed through a S_N2-type displacement. As such, the desired β-anomer was achieved in good yield to form fully protected lablaboside F **164** (Scheme 4.9).

Scheme 4.9 Final glycosylation to form lablaboside F.
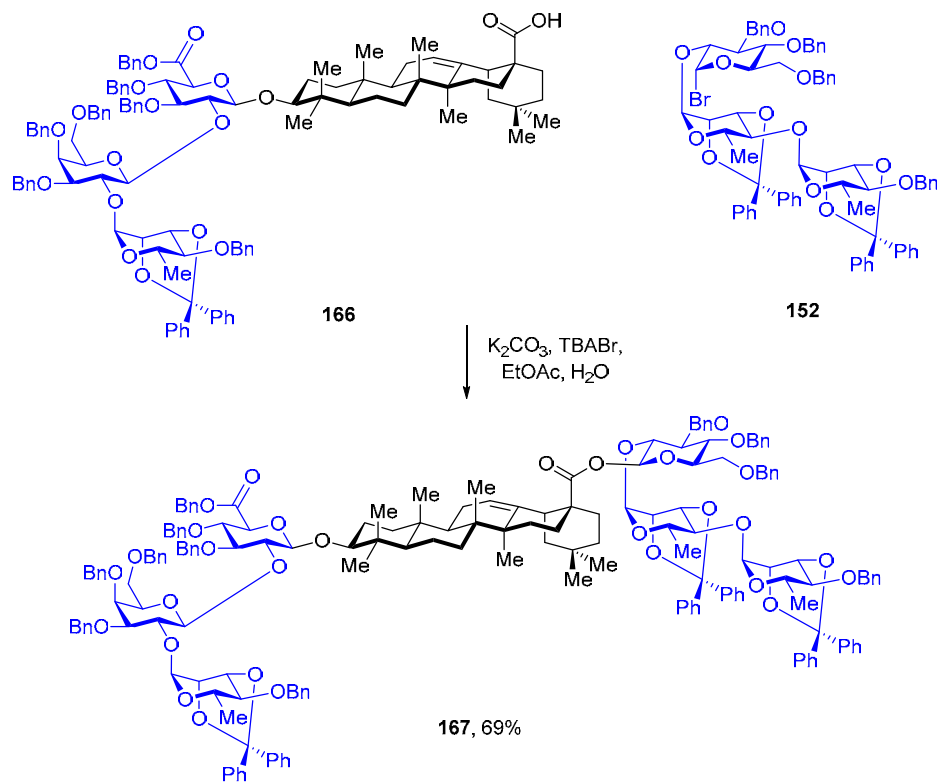
4.6   Deprotection and Comparison to Literature Data

One-step global deprotection proceeded via high pressure hydrogenolysis over 24 hours to furnish the desired saponin, **168**. Spectral comparison to the natural product was less straightforward than anticipated. In the isolation paper, NMR analyses were performed in pyridine-d_5, with all carbon chemical shifts and characteristic proton reasonances in tabular form.[52] Surprisingly, our NMR analyses performed in pyridine initially gave a 3:1 mixture of two compounds in apparent equilibrium. As shown in Figure 4.1, removal of solvent followed by NMR analysis performed in methanol-d_4 showed only one compound. Subsequent NMR analyses in pyridine-d_5 once again suggested two compounds, but in a different ratio than previously observed. We hypothesize that minor differences in the salt form of the glucuronic acid

(pyridinium/sodium/free acid) may account for the observed multiplicity in sugar peaks. Additionally, the experimentally determined chemical shifts for many of the characteristic resonances were slightly off, as shown in Table 4.2.
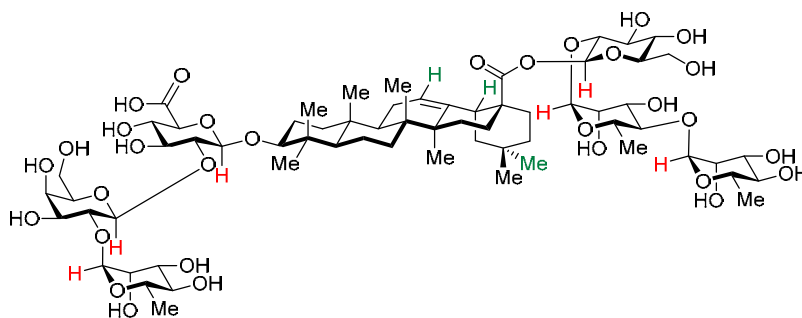
Table 4.2. Pertinent experimental and literature ^{1}H-NMR data for lablaboside F (**168**).

^{1}H-NMR Chemical Shift	Literature ppm (*J*, Hz)	Experimental ppm (*J*, Hz)
GlcA	5.01 (7.3)	5.05 (7.5)
Gal	5.62 (7.2)	5.62 (7.7)
Glc	6.11 (8.2)	6.18 (8.2)
Rha	6.20 (br s)	6.31(1.5)
Rha	6.22 (br s)	6.32 (1.5)
Rha	6.61 (br s)	6.76 (1.5)
C18 methine	3.10 (m)	3.10 (13.8, 5.0)
C12 olefin	5.43 (br s)	5.45 (t, 4.2)
C29 methyl	1.82 (s)	1.43 (s)

Indeed, all other available characterization data, including high-resolution mass spectrometry and specific rotation, matched the literature values. The authors of the isolation paper did not respond to a request for the NMR spectra or FID files.

Scheme 4.10. Global deprotection of lablaboside F.

164

Pd/C, H₂
THF, MeOH

168, 77%
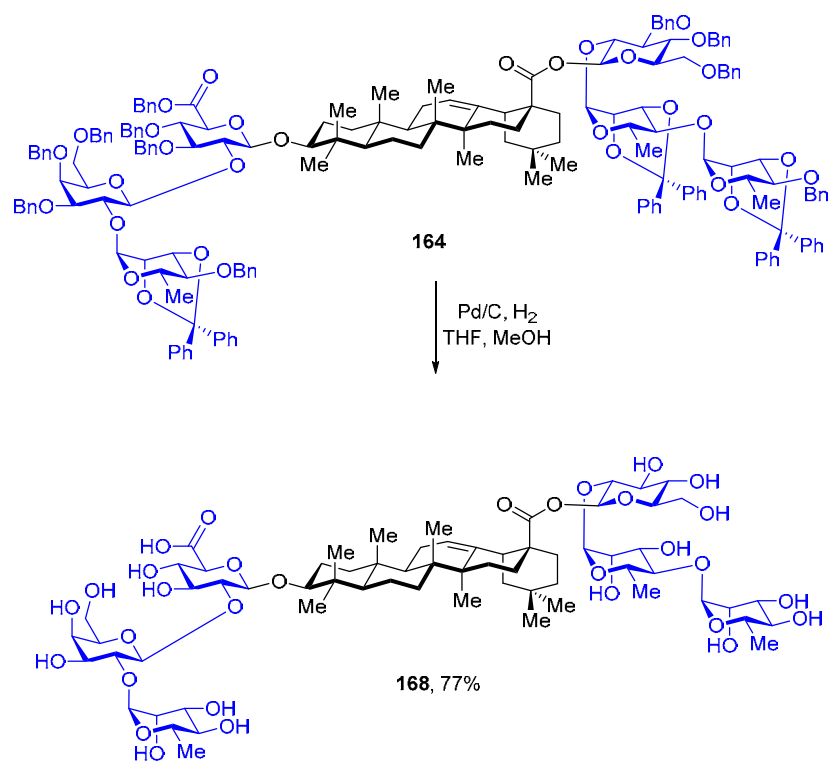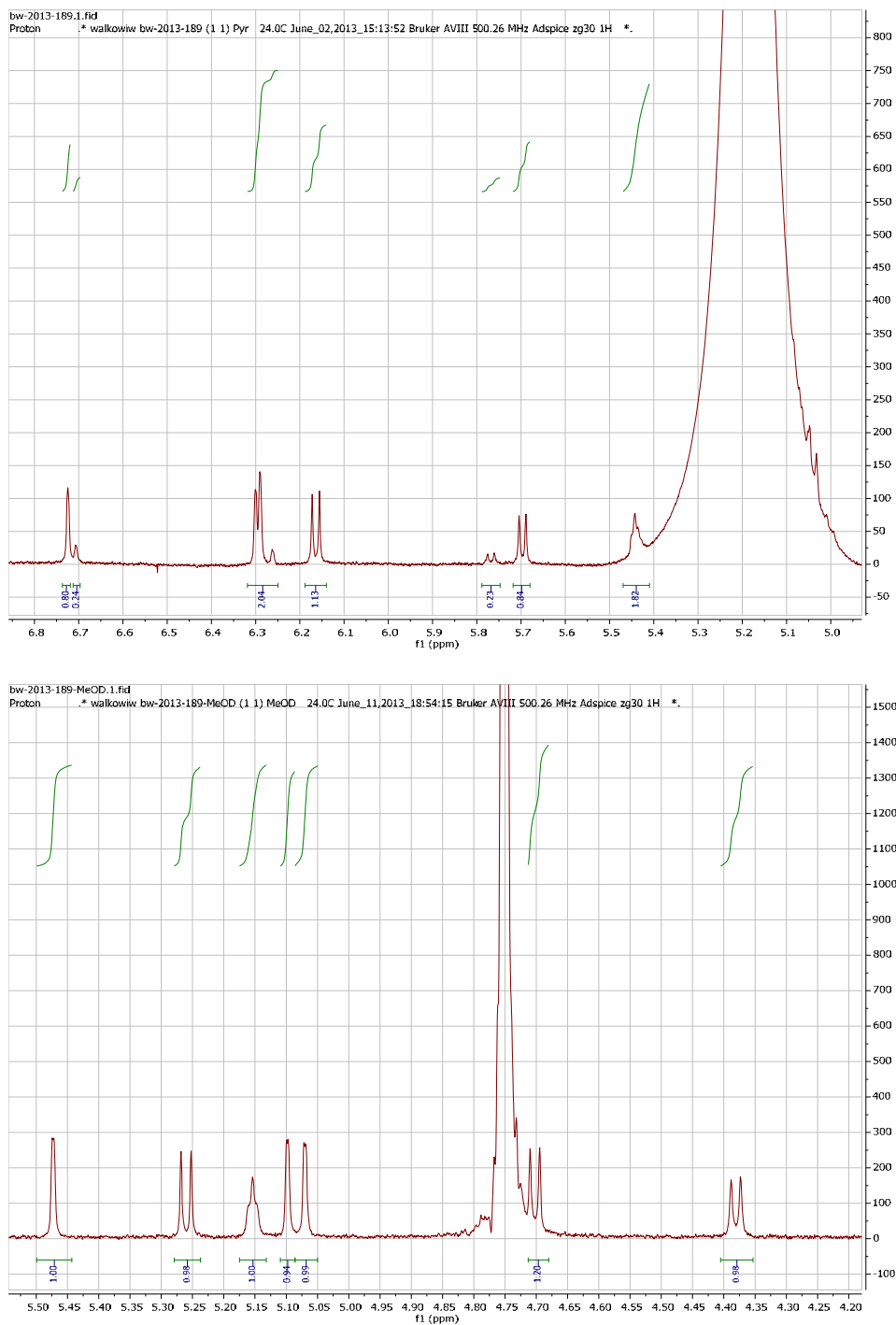
Figure 4.1. **^{1}H-NMR spectra of lablaboside F in two solvents**, one sample in pyridine-d_5 and methanol-d_4, showing anomeric and olefin peaks. Top spectrum in pyridine-d_5 shows a 4:1 mixture of two compounds. Spectrum in methanol-d_4 shows one compound.

Scheme 4.11. Synthesis of lablaboside A.
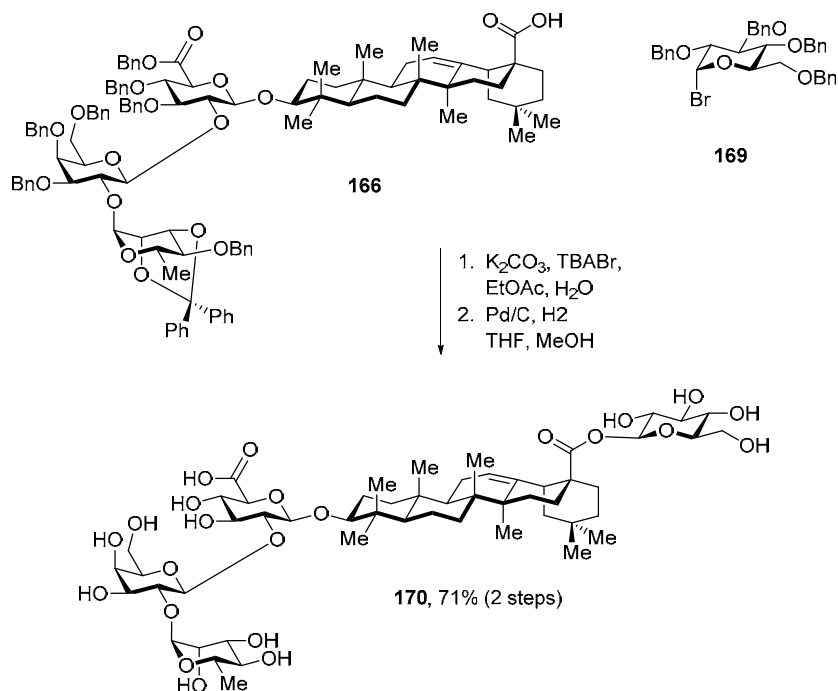
Synthesis of the remaining analogues began with lablaboside A (Scheme 4.11). Using the previous intermediate carboxylic acid **166**, phase-transfer glycosylation was achieved to form fully protected lablaboside A **170**. Once again, hydrogenolysis gave a very clean crude mixture in methanol, which after HPLC purification, furnished a fluffy white solid **171**. As with lablaboside F, two compounds in equilibrium were observed in pyridine-d_5. However, NMR analyses performed in methanol-d_4 indicated a single product. Experimental data for specific rotation matched literature values.
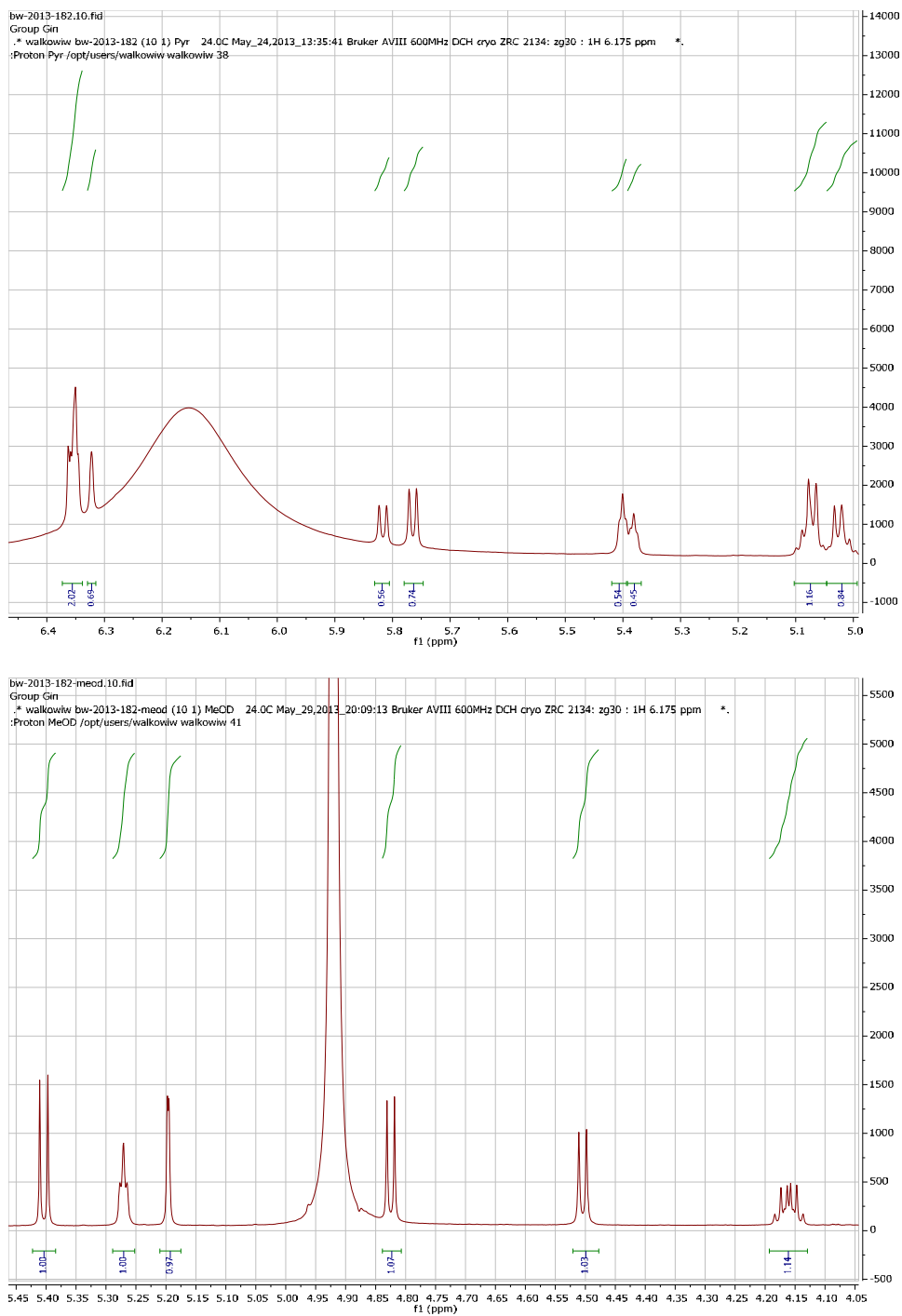
Figure 4.2. ^{1}**H-NMR spectra of lablaboside A in two solvents**, one sample in pyridine-d_5 and methanol-d_4, showing anomeric and olefin peaks. Top spectrum in pyridine-d_5 shows a 1.2:1 mixture of two compounds. Spectrum in methanol-d_4 shows one compound.

4.7   Conclusion

Efforts towards the synthesis of the remaining lablaboside saponins are currently in progress. As mentioned in the previous chapter, small amounts of the requisite epi-hederangenin triterpene have been synthesized. Current efforts are underway to procure enough material for successful synthesis of the remaining lablabosides.

We have demonstrated the efficacy of dehydrative glycosylation reactions with a variety of glycosyl donors. Moreover, we have solved an infrequently encountered, but monumentally confounding problem, of the recalcitrant acetonide protecting group, a common protecting group for *cis*-1,2 diols. Indeed, employment of the under-utilized benzophenone ketal allowed for a facile, high-yielding, one-step global deprotection of a complex saponin.

Once the entire family has been successfully synthesized, our group will examine the immunopotentiating effects of the series of saponins in our previously described immunization protocol. Examination of the specific antibody sub-types elicited by these saponins may inform the proper utilization of the lablaboside saponins in clinical applications.

CHAPTER 5.

CONCLUSIONS AND FUTURE DIRECTIONS

5   Conclusions

We have successfully synthesized and evaluated the immunostimulating properties of a series of central–linkage variants to the *Quillaja* saponins, providing valuable insight to the SAR. Additionally, two of the six lablaboside saponins have been synthesized.

Examination of the central–linkage SAR in the QS saponins highlighted the importance of the triterpene–oligosaccharide junction for the observed biological properties. These studies provided a new lead structure for further SAR studies to find an improved immunoadjuvant. The first successful synthesis of natural product saponins lablaboside F and lablaboside A, with lablabosides B-E forthcoming, will provide validation of the immunoadjuvant properties initially reported by the isolation group. Moreover, successful employment of the rarely used benzophenone ketal highlights a viable alternative for the occasionally obstinate isopropylidiene ketal protecting group often used with vicinal *syn*-diols.

5.1   Future Development of the *Quillaja* Saponins

Development of the QS saponin analogues from the toxic, very expensive, and chemically unstable natural product has been extensive since completion of the initial synthesis in 2005. However, progress toward an economical and clinically viable target immunoadjuvant has been hamstrung in two major ways; lack of demonstrated *in vivo* efficacy and no experimental evidence for the mechanism of action.

5.1.1   Demonstration of *In Vivo* Efficacy

Pre-clinical evaluation of the QS saponins has identified several promising candidate saponins for further development. However, quantitative differentiation of immunoadjuvant activity among these promising candidates remains a challenge. Indeed, the most reliable antibody responses are elicited from well-established immunogenic proteins ovalbumin and keyhole limpet heamocyannin. However, the clinically relevant co-administered carbohydrate-based tumor antigens, GD3 and MUC1, give unimpressive and unreliable antibody responses. To more effectively quantify immunopotentiating activity against relevant antigens, an experiment comparing the efficacy of the leading candidate adjuvants, among the nearly 50 synthetic QS saponins, as a component of a prophylactic or therapeutic vaccine. Immunization followed by a challenge with a disease-causing agent should be performed, which will give a preliminary assessment of the clinical utility of these non-natural saponin adjuvants. Moreover, by utilizing a variety of antigens/disease causing agents (e.g. diphtheria toxin, influenza, GD3-expressing tumor, etc) we will be able to determine the qualitative and quantitative differences in the immune response elicited by the non-natural analogues relative to the natural product.

5.1.2   Elucidation of the Mechanism of Action

The obvious SAR of the central linkage in the QS saponins shown in Chapter 2, as well as other concurrent studies in our group, suggest that the central linkage region is the site of interaction for a putative macromolecular interaction. As such, other minor modifications to the triterpenoid (e.g. epimerization of the C16 hydroxyl group) should be explored to optimize the potency of the QS saponins. A more potent saponin may

contribute an increase in binding efficiency to the putative target, which may in turn facilitate chemical cross-linking. To achieve these variants to quillaic acid, a conceptually similar strategy to the three-step oxidation sequence discussed in Chapter 3 may prove useful: rapid introduction of chemical handles to complex starting materials. For example, Hartwig *et al* achieved a selective oxidation of methyl oleanolate (**172**) at C23 to form hederangenin (**173**) in two-steps, utilizing an iridium-based catalyst, directed by the C3 secondary alcohol as shown in Scheme 5.1.[96] Similarly, unpublished work in our group utilizing oxime directed C–H activation furnished oxidation of the E-ring of oleanolic acid-derived triterpenoid **175** as shown in Scheme 5.1.[97]

Scheme 5.1. Transition-metal catalyzed C–H activation using directing groups of oleanolane triterpenoids.
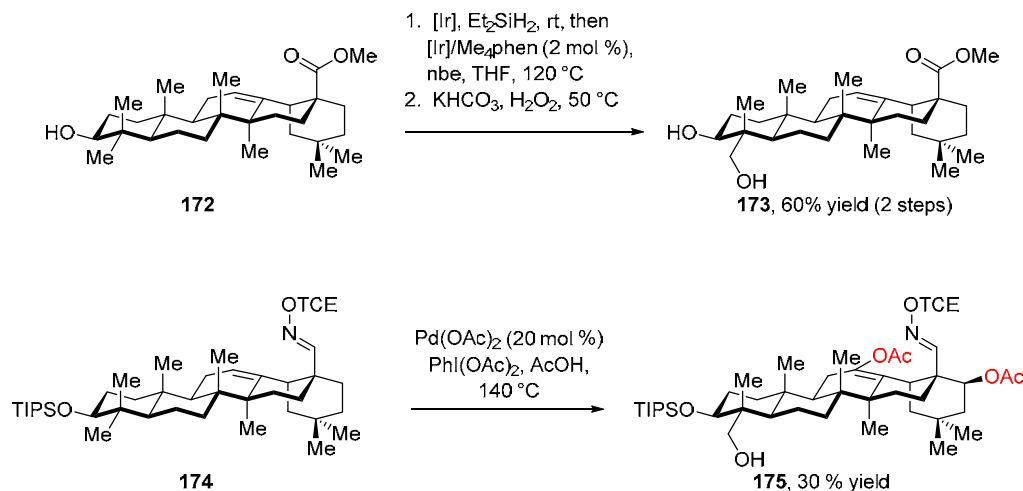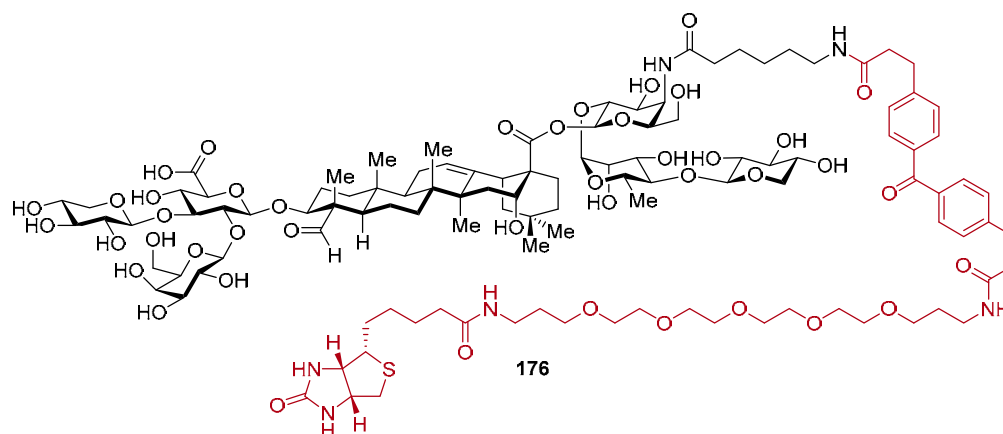
Figure 5.1. Cross-linking saponin probe with biotin tag for affinity purification.

In parallel, another challenge is to develop an appropriate strategy for chemical cross-linking. Previous work in our group utilized a benzophenone moiety linked to the acyl side chain **172**. Preliminary photo cross-linking experiments tentatively identified histone H1 as the protein target, but no further validation experiments have been performed. If our assumption about the site of interaction is correct, then the benzophenone moiety may not be in close enough proximity to the actual target. Since histone H1 is a common cellular protein, present in each histone, identification in a single assay may be a false positive. To solve this problem, an inducible cross-linking functional group, such as a diazirine must be introduced proximal to the pertinent triterpenoid–oligosaccharide junction. Functionalization of the E-ring of quillaic acid may facilitate facile introduction of such groups as shown in Scheme 5.2.

Scheme 5.2. Proposed general strategy to create photo cross-linking tools. D = directing group, P = protecting group
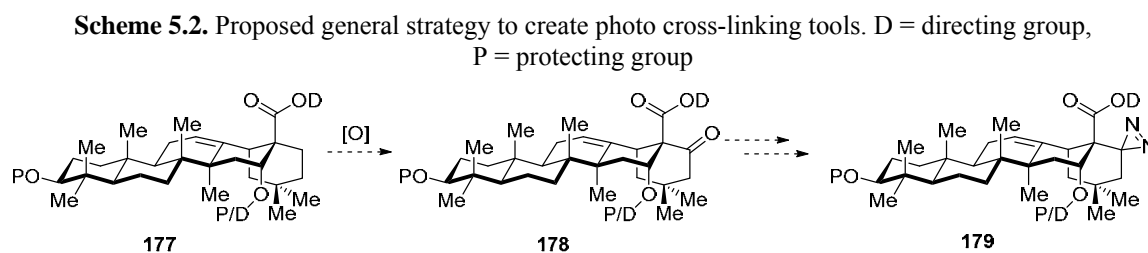
5.2 Future Development of the Lablaboside Saponins

After successful completion of the entire family of lablaboside saponins, we must validate the previously reported immunoadjuvant activity. Indeed, saponins isolated from natural sources, although appearing to be analytically pure, exhibit markedly different properties, as shown with naturally derived versus synthetic QS-21, especially with respect to toxicity.

More important than full investigation of the lablaboside saponins is utilization of the three-step sequence to obtain C24 oxidized triterpenoids outlined in Chapter 3 in two ways; application to natural product synthesis and generation of new QS saponins. Several natural products purported to have immunopotentiating properties, including the soyasaponins, feature an oxidized C24, and the short oxidation sequence would be an essential strategy towards successful synthesis. Additionally, employment of the axial neopenyl alcohol **180**, aldehyde **181**, and carboxylic acid **182** with the more well-established components of the strongly immunopotentiating *Quillaja* saponins (Figure 5.2) may furnish even more potent or less toxic QS saponins.
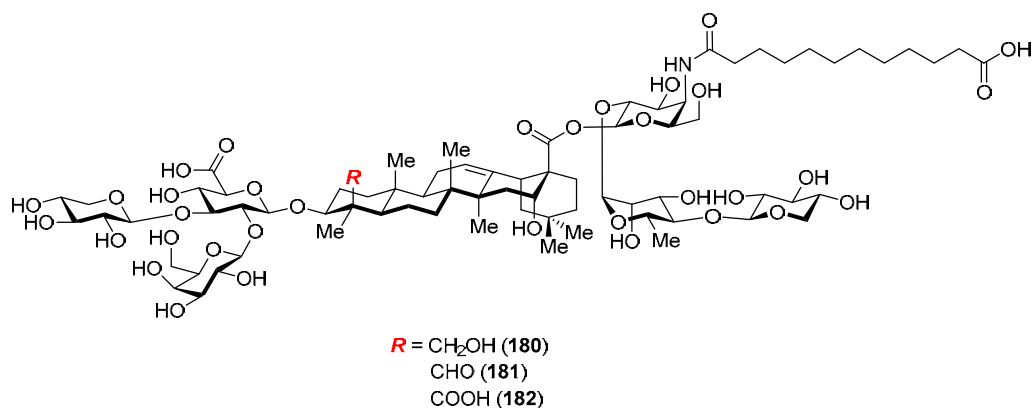
Figure 5.2. Proposed C24 oxidized QS saponins.

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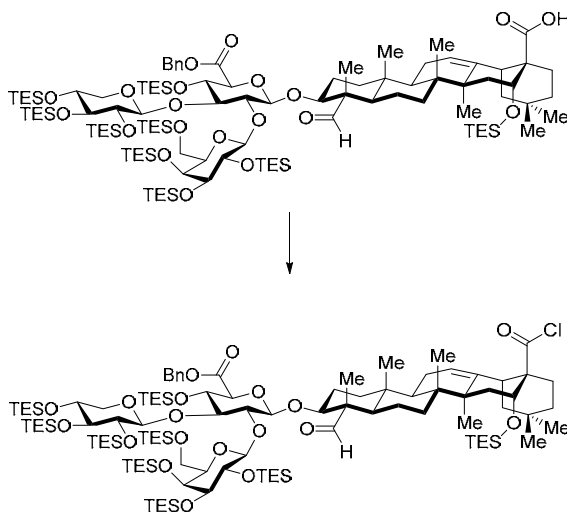
EXPERIMENTAL PROCEDURES FOR CHAPTER 2

General Procedures. Reactions were performed in flame-dried sealed-tubes or modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unlessotherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate and sulfoxide reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then flame-dried under vacuum. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed using glass plates pre-coated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm).

Materials. Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed columns of neutral alumina under an argon atmosphere. Methanol was distilled from magnesium at 760 Torr. Trifluoromethanesulfonic anhydride was distilled from phosphorus pentoxide at 760 Torr. Boron trifluoride diethyl etherate was distilled from calcium hydride at 760 Torr. All other chemicals were obtained from commercial vendors and were used without further purification unless noted otherwise.

Instrumentation. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer or a Bruker Tensor 27. Data are presented as the frequency of absorption (cm^{-1}). Proton and carbon-13 nuclear magnetic resonance (^{1}H NMR and ^{13}CNMR) spectra were recorded on a Bruker Avance III instrument; chemical

shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent (d-chloroform: δ 7.26 for ^{1}H NMR, δ 77.16 for ^{13}C NMR; d6-benzene: δ 7.16 for ^{1}H NMR, δ 128.06 for ^{13}C NMR; d4-methanol: δ 3.31 for ^{1}H NMR, δ 49.00 for ^{13}C NMR; d3-acetonitrile: δ 1.94 for ^{1}H NMR, δ 1.32 for ^{13}C NMR; deuterium oxide: δ 4.79 for ^{1}H NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment. RP-HPLC purification and analyses were carried out on a Waters 2545 binary gradient HPLC system equipped with a Waters 2996 photodiode array detector, and absorbances were monitored at wavelengths of 210–600 nm.

(66): Thionyl chloride (31 µl, 0.425 mmol, 2 equiv) was added, drop-wise, to an ice-cooled solution of **65** and pyridine (170 µl, 2.13 mmol, 10 equiv) in dichloromethane (6 ml). After two hours, a majority of the volatiles were removed under a stream of nitrogen, then high-vacuum. Residual solids were suspended in anhydrous benzene and

filtered through celite. Solvent removal *in vacuo* furnished **66** (441 mg, 99 % yield) as a white foam.

TLC R_f 0.36 (20:1 hexanes/ethyl acetate); **FTIR** (NaCl, film):) 2953, 2911, 2877, 1792 (O–CCl st), 1756, 1725, 14.58, 1414, 1378, 1240, 1171, 1102, 1007, 739 cm⁻¹; **¹H-NMR** (600 MHz, C_6D_6) δ 9.75 (s, 1H), 7.20 – 7.17 (m, 2H), 7.10 – 6.97 (m, 3H), 5.42 (t, *J* = 3.5 Hz, 1H), 5.18 (d, *J* = 12.4 Hz, 1H), 4.99 (dd, *J* = 22.3, 9.9 Hz, 2H), 4.79 – 4.71 (m, 2H), 4.59 (d, *J* = 7.0 Hz, 1H), 4.47 (t, *J* = 8.7 Hz, 1H), 4.37 (t, *J* = 9.1 Hz, 1H), 4.25 – 4.18 (m, 2H), 4.15 (d, *J* = 9.4 Hz, 1H), 4.13 – 4.08 (m, 1H), 4.05 (dd, *J* = 11.2, 5.0 Hz, 1H), 4.00 (dd, *J* = 9.3, 7.4 Hz, 1H), 3.95 (dd, *J* = 9.5, 5.5 Hz, 1H), 3.84 – 3.76 (m, 2H), 3.73 – 3.66 (m, 3H), 3.65 – 3.61 (m, 1H), 3.49 (t, *J* = 10.8 Hz, 1H), 3.16 (dd, *J* = 14.1, 4.1 Hz, 1H), 2.39 (t, *J* = 13.6 Hz, 1H), 1.97 – 1.67 (m, 8H), 1.58 (dd, *J* = 10.2, 7.4 Hz, 1H), 1.46 (m, 139H), 0.81 – 0.70 (m, 18H), 0.62 (dd, *J* = 8.0, 3.0 Hz, 7H). **¹³C-NMR** (151 MHz, C_6D_6) δ 209.67, 177.78, 168.61, 141.76, 135.75, 128.54, 128.38, 128.24, 128.19, 123.96, 102.72, 101.57, 101.40, 83.84, 79.58, 79.26, 77.85, 77.00, 76.78, 76.32, 75.76, 75.32, 73.16, 73.01, 72.28, 71.69, 66.90, 65.97, 61.41, 59.13, 54.48, 49.01, 46.61, 46.53, 42.64, 41.61, 39.92, 37.92, 35.97, 35.25, 34.90, 32.58, 32.34, 30.61, 30.40, 26.59, 25.40, 24.13, 23.46, 20.43, 16.88, 15.66, 11.87, 7.82, 7.64, 7.51, 7.48, 7.45, 7.38, 7.37, 7.35, 7.27, 7.19, 7.07, 7.06, 6.29, 6.15, 6.08, 6.00, 5.95, 5.86, 5.81, 5.76, 5.68, 5.62, 5.48, 5.42, 5.33, 5.14, 4.96, 4.94, 4.77, 4.57; **HRMS** *m/z* (ESI): For methyl ester derivative, calcd for $C_{102}H_{210}O_{25}NaSi_9$ [M+Na]⁺ 2110.2982, found 2110.2986.
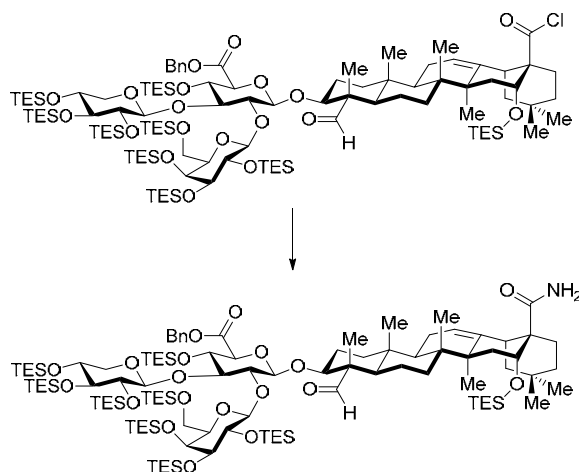
(69): A large excess of freshly condensed ammonia (~1 ml, ~900 equiv) in dichloromethane (2 ml) was added to an ice-cooled solution of **66** (110 mg, 0.525 mmol, 1 equiv) in dichloromethane (5 ml). After 20 min, reaction mixture was warmed to room temperature allowing excess ammonia to evaporate. Mixture was diluted with water and layers separated. After extraction with with dichloromethane (2 × 10 mL), organic fractions combined and washed with brine, then dried over sodium sulfate, and concentrated and the purified by silica gel chromatography (hexanes:EtoAc + 0.5% triethylamine 10:1 to 2:1) to afford **69** (100 mg, 92 % yield) as a white foam.

TLC R_f 0.26 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3454, 2953, 2911, 2877, 1753, 1725, 1674, 1602, 1456, 1414, 1377, 1239, 1104, 1005, 913, 864, 826, 740 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.72 (s, 1H), 7.39 − 7.29 (m, 5H), 6.06 (s, 1H), 5.46 (t, J = 3.6 Hz, 1H), 5.36 (s, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.56 (d, J = 7.4 Hz, 1H), 4.49 (s, 1H), 4.43 (d, J = 7.3 Hz, 1H), 4.18 (d, J = 7.4 Hz, 1H), 3.95 − 3.90 (m, 2H), 3.88 − 3.82 (m, 2H), 3.82 − 3.77 (m, 2H), 3.75 (t, J = 9.2 Hz, 1H), 3.62 − 3.53 (m, 3H), 3.48 (ddd, J = 10.5, 8.4, 5.1 Hz, 1H), 3.39 (dd, J = 9.4, 2.5 Hz, 1H), 3.35 (t, J = 8.7 Hz, 2H), 3.25 (dd, J = 8.7, 7.4 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.57 (dd, J =

13.7, 4.2 Hz, 1H), 2.36 (t, J = 13.1 Hz, 1H), 2.03 (dt, J = 14.6, 4.0 Hz, 1H), 1.92 (dd, J =

8.9, 3.6 Hz, 2H), 1.90 – 1.42 (m, 12H), 1.32 (s, 3H), 1.30 – 1.25 (m, 2H), 1.19 – 0.84 (m,

96H), 0.79 (s, 3H), 0.78 – 0.55 (m, 53H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.78, 180.71,

168.38, 145.17, 135.29, 128.47, 128.27, 128.14, 122.50, 103.71, 101.41, 100.85, 86.45,

78.81, 78.73, 76.46, 75.95, 75.91, 75.83, 75.09, 72.62, 72.53, 71.38, 71.11, 66.85, 65.34,

60.25, 53.81, 49.39, 49.19, 47.24, 45.99, 42.27, 41.95, 39.56, 37.95, 36.05, 35.39, 34.67,

34.54, 34.19, 32.57, 31.98, 31.61, 31.27, 30.54, 29.07, 26.32, 25.38, 25.29, 24.22, 23.40,

22.68, 20.71, 20.21, 16.90, 15.86, 14.14, 12.26, 11.45, 7.57, 7.47, 7.25, 7.16, 7.15, 7.14,

6.99, 6.85, 6.79, 5.93, 5.65, 5.45, 5.38, 5.34, 5.27, 5.23, 5.18, 5.01, 4.42; **HRMS** *m/z*

(ESI): Calcd for C$_{108}$H$_{205}$NO$_{19}$Si$_9$Na [M+Na] 2095.2927, found 2095.3020.
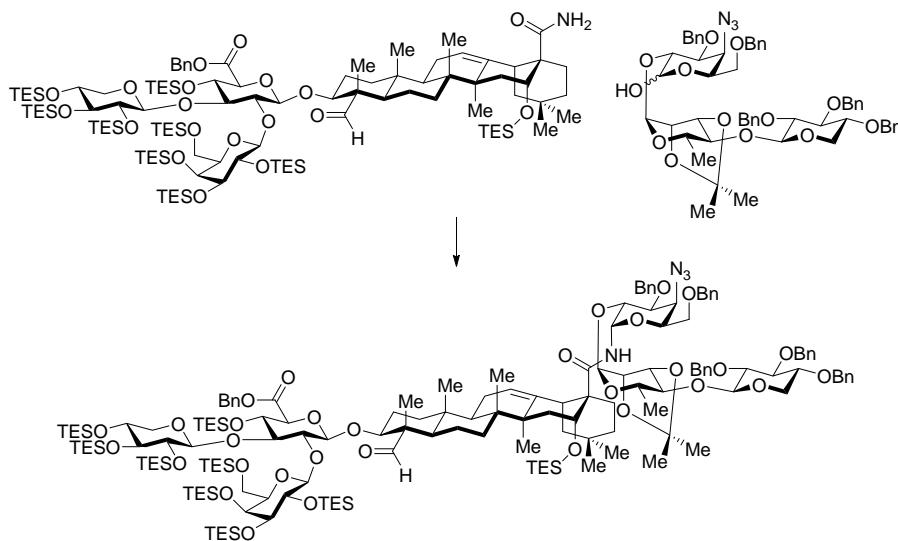
(75): Trifluoromethanesulfonic anhydride (22 μL, 0.13 mmol, 3.0 equiv) was added to a

solution of trisaccharide **73** (85 mg, 0.087 mmol, 2.00 equiv), phenyl sulfoxide (53 mg,

0.260 mmol, 6.0 equiv) and 2,4,6-tri-tertbutylpyridine (65 mg, 0.261 mmol, 6.0 equiv) in

dichloromethane (5 mL) at −78 °C. The reaction stirred in a cold bath at −78 °C for 8

min and then was transferred to a bath between -55 and -50 °C for 65 min. A solution of

amide **69** (90 mg, 0.043 mmol, 1 equiv) was added in dichloromethane (2 mL) via syringe. Bath temperature was warmed to -45 °C for 45 min then 0 °C for 15 min. Triethylamine was added, concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 10:1 to 2:1) furnishing readily separable dissacharides **75α** (80 mg) and **75β** (13 mg) as a flaky white film (6.1:1, α:β, 71% total).

TLC R_f 0.64 (2:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3420, 2953, 2911, 2876, 2105, 1751, 1675, 1496, 1457, 1413, 1375, 1240, 1160, 1098, 1005, 898, 865, 825, 732, 697 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.71 (s, 1H), 7.98 – 7.92 (m, 1H), 7.59 – 7.54 (m, 1H), 7.53 – 7.48 (m, 1H), 7.42 – 7.26 (m, 27H), 6.64 (d, J = 8.5 Hz, 1H), 5.43 (t, J = 3.6 Hz, 1H), 5.29 (d, J = 12.4 Hz, 1H), 5.19 (d, J = 4.7 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.92 (d, J = 11.0 Hz, 1H), 4.89 – 4.81 (m, 4H), 4.73 (dd, J = 11.5, 3.0 Hz, 2H), 4.68 (d, J = 11.0 Hz, 1H), 4.63 (dd, J = 11.5, 5.3 Hz, 2H), 4.56 (d, J = 7.5 Hz, 1H), 4.52 (s, 2H), 4.48 (s, 1H), 4.43 (d, J = 7.3 Hz, 1H), 4.21 – 4.15 (m, 2H), 4.09 (dd, J = 7.1, 4.7 Hz, 1H), 4.00 (dd, J = 3.0, 1.5 Hz, 1H), 3.96 – 3.90 (m, 3H), 3.88 – 3.70 (m, 8H), 3.67 – 3.51 (m, 8H), 3.51 – 3.45 (m, 2H), 3.39 (dd, J = 9.4, 2.5 Hz, 1H), 3.38 – 3.29 (m, 3H), 3.25 (dd, J = 8.6, 7.4 Hz, 1H), 3.22 – 3.16 (m, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.62 (dd, J = 13.4, 4.2 Hz, 1H), 2.33 (t, J = 13.2 Hz, 1H), 1.98 – 1.63 (m, 7H), 1.62 – 1.45 (m, 5H), 1.44 (s, 3H), 1.43 – 1.30 (m, 10H), 1.29 (s, 3H), 1.28 – 1.17 (m, 3H), 1.12 – 1.03 (m, 4H), 0.89 (s, 81H), 0.84 (s, 3H), 0.81 – 0.56 (m, 51H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.68, 178.34, 168.32, 144.58, 141.57, 138.70, 138.57, 138.19, 137.70, 137.13, 135.25, 133.16, 129.26, 128.52, 128.49, 128.46, 128.44, 128.38, 128.35, 128.31, 128.29, 128.27, 128.22, 128.14, 128.08, 128.05, 128.04, 127.99, 127.97, 127.95, 127.94, 127.91, 127.88,

127.86, 127.85, 127.81, 127.76, 127.65, 127.57, 122.60, 110.03, 103.64, 102.61, 101.39, 100.82, 97.64, 86.47, 83.82, 82.01, 81.63, 79.21, 78.99, 78.79, 78.71, 78.32, 77.92, 76.76, 76.64, 76.44, 76.15, 75.90, 75.80, 75.65, 75.06, 74.75, 73.97, 73.53, 73.25, 72.76, 72.60, 72.50, 72.06, 71.38, 71.08, 69.00, 67.78, 66.83, 65.32, 63.77, 60.24, 59.21, 53.84, 49.36, 49.20, 47.17, 46.06, 41.87, 41.25, 39.70, 37.98, 36.02, 35.39, 34.66, 34.52, 34.09, 32.56, 32.21, 31.59, 31.46, 30.52, 29.06, 27.32, 26.25, 25.39, 25.31, 25.27, 24.16, 23.39, 22.66, 20.70, 20.26, 18.77, 17.91, 17.22, 15.95, 14.14, 12.27, 11.45, 7.56, 7.46, 7.25, 7.17, 7.14, 7.13, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.37, 5.33, 5.25, 5.22, 4.95, 4.41; **HRMS** *m/z* (ESI): Calcd for $C_{163}H_{266}N_4O_{31}NaSi_9$ 3050.7182, found 3050.7034.
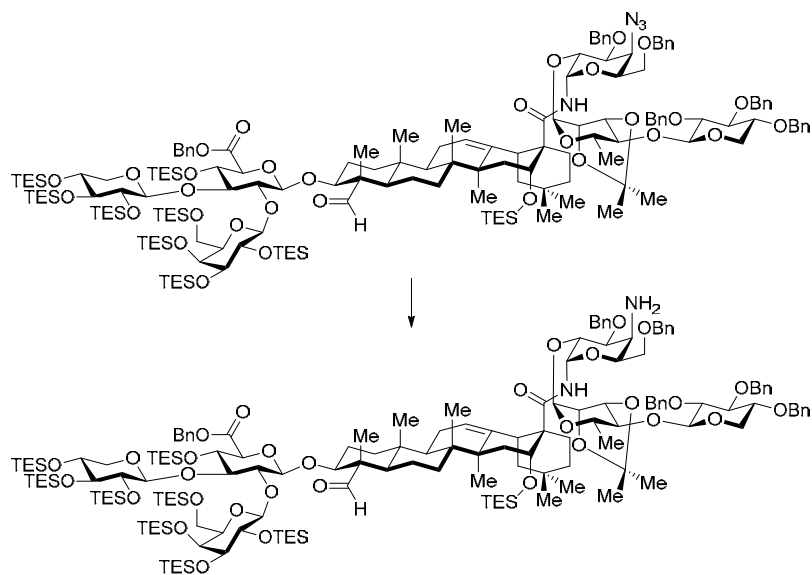
**(91):** Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **75α** (45 mg, 0.0148 mmol, equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) in a 50 mL conical vial. After two min, vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to RT and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was

concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **91** (36 mg, 81 % yield).

TLC R_f 0.35 (5:1 benzene:ethyl acetate); **FTIR** (NaCl film) 3393, 3031, 2953, 2911, 2876, 1752, 1724, 1676, 1497, 1457, 1414, 1380, 1240, 1169, 1097, 1006, 909, 864, 826, 737, 697, 666, 602 cm^{-1}; **^{1}H-NMR** (500 MHz, CDCl$_3$) δ 9.70 (s, 1H), 7.31 (s, 30H), 6.60 (d, J = 8.5 Hz, 1H), 5.49 – 5.44 (m, 0H), 5.44 – 5.39 (m, 1H), 5.33 – 5.25 (m, 2H), 5.10 (d, J = 12.4 Hz, 1H), 4.94 – 4.79 (m, 4H), 4.75 – 4.59 (m, 5H), 4.60 – 4.51 (m, 4H), 4.48 (s, 1H), 4.42 (d, J = 7.3 Hz, 1H), 4.18 (dt, J = 7.6, 3.7 Hz, 2H), 4.10 (dd, J = 6.7, 3.7 Hz, 1H), 3.96 – 3.70 (m, 10H), 3.71 – 3.51 (m, 11H), 3.48 (td, J = 9.8, 9.3, 5.0 Hz, 1H), 3.44 – 3.27 (m, 5H), 3.25 (t, J = 8.0 Hz, 1H), 3.19 (t, J = 10.3 Hz, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.62 (dd, J = 14.5, 3.9 Hz, 1H), 2.32 (t, J = 13.1 Hz, 1H), 1.99 – 1.92 (m, 1H), 1.92 – 1.85 (m, 1H), 1.83 – 1.74 (m, 2H), 1.73 – 1.47 (m, 7H), 1.40 (d, J = 11.7 Hz, 1H), 1.37 (s, 6H), 1.35 (s, 2H), 1.30 (s, 6H), 1.29 (s, 6H), 1.28 – 1.15 (m, 2H), 1.13 – 1.02 (m, 3H), 1.03 – 0.85 (m, 98H), 0.84 (s, 3H), 0.83 – 0.52 (m, 61H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 212.70, 178.33, 168.33, 144.82, 138.70, 138.55, 138.20, 138.08, 137.47, 135.24, 128.46, 128.43, 128.38, 128.31, 128.28, 128.13, 128.02, 127.96, 127.93, 127.80, 127.75, 127.70, 127.67, 127.58, 122.45, 109.77, 103.64, 102.58, 101.38, 100.82, 97.47, 86.42, 83.83, 82.56, 82.04, 79.38, 78.97, 78.79, 78.71, 78.15, 77.94, 76.59, 76.44, 76.21, 75.87, 75.80, 75.65, 75.07, 74.78, 74.46, 73.98, 73.39, 73.23, 72.60, 72.51, 71.38, 71.20, 71.08, 68.24, 68.10, 66.83, 65.32, 63.76, 60.25, 53.81, 49.31, 49.17, 48.77, 47.35, 45.93, 45.74, 41.88, 41.22, 39.73, 37.93, 36.00, 35.41, 33.97, 32.57, 32.06, 31.43, 30.50, 29.70, 27.46, 26.25, 25.57, 25.37, 24.34, 23.42, 20.22, 18.18, 17.16, 15.94, 12.27, 7.56, 7.46, 7.25, 7.17, 7.14,

7.13, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.37, 5.33, 5.25, 5.22, 4.95, 4.41; **HRMS** (ESI)

m/z: Calcd for $C_{163}H_{269}N_2O_{31}Si_9$ 3002.7458 [M+H], found 3002.7354.
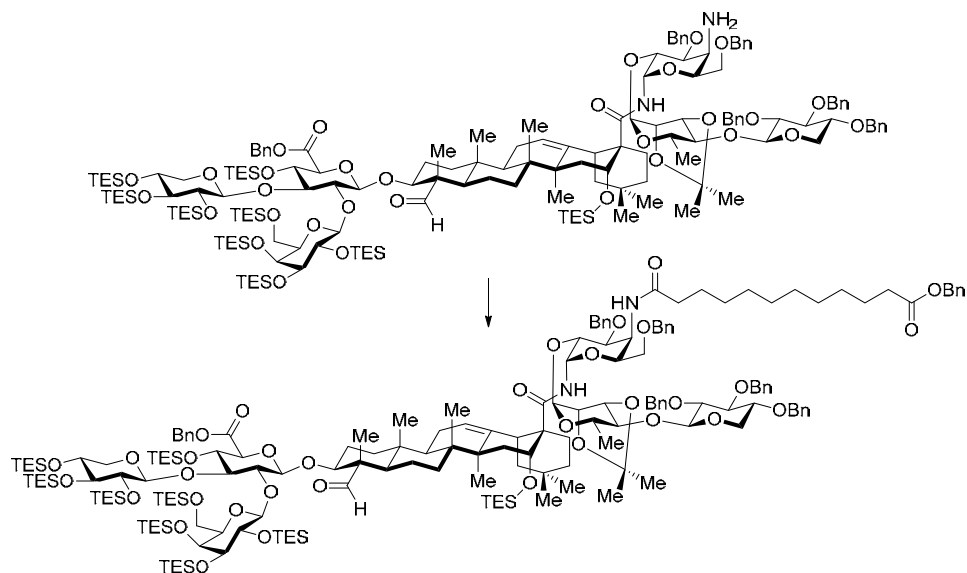
(93): Isobutyl chloroformate was added to an ice-cooled solution of carboxylic acid **106** (21 mg, 0.064 mmol, 6 equiv) and triethylamine (15 µL, 0.107 mmol, 10 equiv) in tetrahydrofuran (2 mL) and stirred for 4 hours, then transferred via cannula to an ice-cooled solution of amine **86** (32 mg, 0.011 mmol, 1.0 equiv) in tetrahydrofuran (1 mL). After 5 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give amide **93** (26 mg, 74 % yield).

TLC *R$_f$* 0.62 (2:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3610, 3584, 3032, 2954, 2878, 1745, 1725, 1680, 1549, 1499, 1457, 1415, 1381, 1242, 1168, 1099, 1009, 911, 865, 825, 733 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$-*d*) δ 9.71 (s, 1H), 7.43 − 7.20 (m, 35H),

6.54 (d, *J* = 8.1 Hz, 1H), 5.45 (s, 1H), 5.32 (d, *J* = 3.4 Hz, 1H), 5.28 (d, *J* = 12.4 Hz, 1H), 5.12 – 5.06 (m, 3H), 4.90 (d, *J* = 11.0 Hz, 1H), 4.88 – 4.80 (m, 6H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 11.0 Hz, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.56 (d, *J* = 7.4 Hz, 1H), 4.52 (d, *J* = 11.9 Hz, 1H), 4.46 – 4.39 (m, 4H), 4.20 – 4.13 (m, 2H), 4.08 (dd, *J* = 6.5, 3.3 Hz, 1H), 3.95 – 3.88 (m, 4H), 3.87 – 3.69 (m, 8H), 3.65 (dd, *J* = 9.1, 4.1 Hz, 1H), 3.63 – 3.52 (m, 7H), 3.51 – 3.45 (m, 3H), 3.43 – 3.31 (m, 5H), 3.31 – 3.27 (m, 1H), 3.25 (t, *J* = 8.1 Hz, 1H), 3.22 – 3.16 (m, 1H), 3.13 (t, *J* = 11.0 Hz, 1H), 2.66 (dd, *J* = 13.6, 4.4 Hz, 1H), 2.36 – 2.26 (m, 3H), 2.14 (t, *J* = 7.4 Hz, 2H), 1.98 – 1.85 (m, 3H), 1.85 – 1.75 (m, 3H), 1.74 – 1.50 (m, 14H), 1.49 (s, 4H), 1.42 – 1.33 (m, 6H), 1.12 – 1.04 (m, 4H), 1.04 – 0.81 (m, 110H), 0.81 – 0.54 (m, 69H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.49, 178.25, 173.61, 172.87, 168.34, 145.03, 138.68, 138.57, 138.20, 137.81, 137.44, 136.11, 135.23, 128.65, 128.55, 128.51, 128.48, 128.44, 128.43, 128.40, 128.37, 128.35, 128.31, 128.28, 128.27, 128.23, 128.16, 128.15, 128.13, 128.09, 127.97, 127.94, 127.89, 127.85, 127.82, 127.80, 127.76, 127.73, 127.67, 127.65, 127.60, 127.58, 122.15, 109.74, 103.60, 102.51, 101.38, 100.83, 97.51, 86.38, 83.84, 82.08, 80.58, 78.79, 78.71, 78.02, 77.92, 76.68, 76.46, 76.43, 76.34, 75.87, 75.82, 75.79, 75.67, 75.06, 74.79, 74.74, 73.80, 73.48, 73.23, 72.59, 72.50, 71.38, 71.10, 71.05, 68.20, 67.65, 66.85, 66.05, 66.03, 65.33, 63.78, 60.25, 53.75, 49.23, 49.10, 47.39, 46.27, 45.87, 41.94, 41.05, 39.99, 39.81, 37.89, 37.01, 36.98, 36.62, 35.97, 35.39, 34.33, 34.31, 33.92, 33.17, 32.54, 31.98, 31.30, 30.47, 29.70, 29.54, 29.52, 29.48, 29.46, 29.43, 29.39, 29.38, 29.34, 29.28, 29.25, 29.22, 29.20, 29.16, 29.14, 29.11, 28.40, 27.53, 27.49, 26.26, 25.86, 25.80, 25.75, 25.66, 25.50, 25.31, 24.97, 24.94, 24.68, 24.38, 23.46, 23.35, 20.57, 20.20, 18.17, 17.19, 15.93, 14.41, 13.13, 12.16, 7.56, 7.46, 7.42, 7.26, 7.25, 7.22, 7.16, 7.14, 7.13, 7.10, 7.08, 7.06, 7.05, 6.98, 6.94, 6.90, 6.88,

6.85, 6.78, 5.92, 5.63, 5.43, 5.36, 5.32, 5.25, 5.23, 5.18, 5.17, 4.95, 4.92, 4.43, 4.41;

HRMS (ESI) *m/z*: Calcd for $C_{182}H_{294}N_2O_{34}NaSi_9$ [M+Na]$^+$ 3326.9159, found 3326.9211.
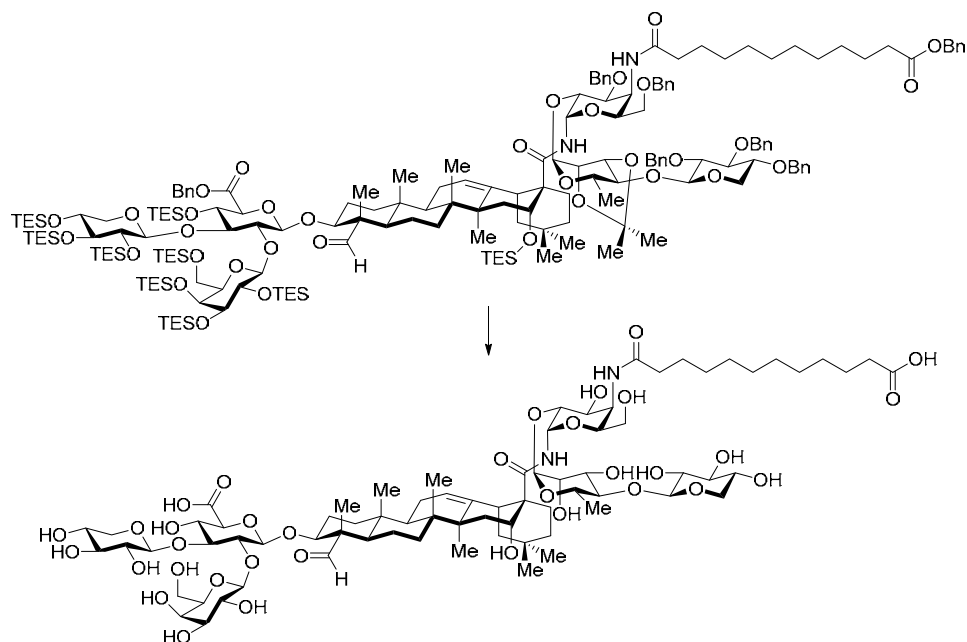
(100): A solution of fully protected amide analogue (**93**) (10.2 mg, 0.003 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (8 mg, 0.0042 mmol, 2.5 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 µm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (3.0 mL, TFA/H$_2$O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 15 · 46% acetonitrile (0.05%

TFA) in water (0.05% TFA) to 14 min followed by another linear gradient from 46% to 90 % acetonitrile (0.05% TFA) in water (0.05% TFA) to 16 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 14.72 min) was collected and lyophilized to dryness to afford SQS-0-6-8-5 (**105**) (2.5 mg, 50% yield) as a white solid.

^{1}H NMR (600 MHz, MeOD) δ 9.34 (s, 1H), 5.30 – 5.26 (m, 1H), 5.14 (s, 1H), 4.71 (d, *J* = 7.2 Hz, 3H), 4.49 (d, *J* = 7.7 Hz, 1H), 4.33 (t, *J* = 7.8 Hz, 2H), 4.21 (d, *J* = 4.3 Hz, 1H), 4.18 (s, 1H), 3.85 (s, 1H), 3.83 – 3.69 (m, 7H), 3.68 – 3.59 (m, 6H), 3.58 – 3.55 (m, 2H), 3.51 (t, *J* = 6.7 Hz, 2H), 3.48 – 3.35 (m, 10H), 2.92 (dd, *J* = 10.4, 3.4 Hz, 0H), 2.21 (t, *J* = 7.4 Hz, 2H), 2.18 (t, *J* = 7.4 Hz, 2H), 2.16 – 2.13 (m, 1H), 1.91 – 1.71 (m, 5H), 1.71 – 1.47 (m, 10H), 1.40 (d, *J* = 9.9 Hz, 2H), 1.27 (s, 6H), 1.22 (d, *J* = 3.1 Hz, 3H), 1.07 (s, 3H), 1.05 (d, *J* = 6.2 Hz, 2H), 1.03 – 0.95 (m, 3H), 0.92 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H), 0.72 (s, 3H); **HRMS** (ESI) *m/z*: Calcd for $C_{76}H_{122}N_2O_{34}Na$ [M+Na]$^+$ 1629.7777, found 1629.7731.
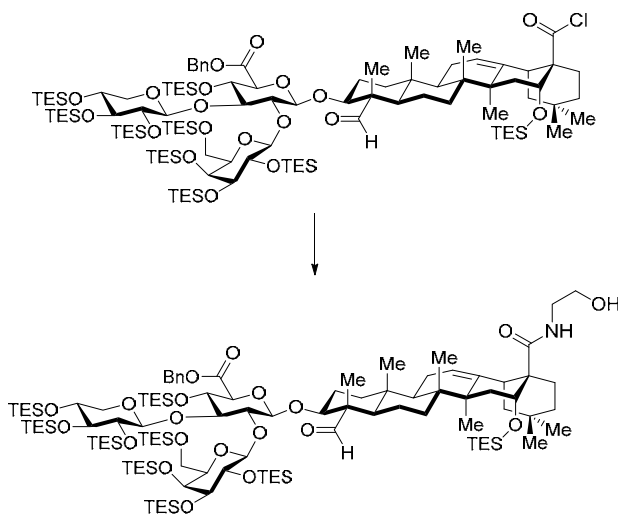
(74): Ethanolamine (29 μL, 0.478 mmol, 10 equiv) was added to an ice-cooled solution of acyl chloride **66** (100 mg, 0.0478 mmol), 1 equiv) in dichloromethane (2 mL). After 10 minutes, reaction was warmed to room temperature, concentrated, and purified by silica gel chromatography (hexanes:ethyl acetate, 4:1 to 2:1) to give ethanolamide **68** (89 mg, 88% yield).

TLC R_f 0.32 (2:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3407 (br), 2953, 2911, 2877, 1754, 1725, 1656, 1632, 1518, 1459, 1414, 1378, 1239, 1171, 1103, 1005, 971, 899, 864, 825, 799, 738, 695, 668 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.72 (s, 1H), 7.40 − 7.29 (m, 5H), 6.54 (t, J = 5.5 Hz, 1H), 5.51 − 5.46 (m, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.56 (d, J = 7.4 Hz, 1H), 4.53 − 4.50 (m, 1H), 4.43 (d, J = 7.3 Hz, 1H), 4.17 (d, J = 7.4 Hz, 1H), 3.95 − 3.89 (m, 2H), 3.88 − 3.77 (m, 4H), 3.75 (t, J = 9.3 Hz, 1H), 3.70 − 3.65 (m, 2H), 3.62 − 3.53 (m, 3H), 3.50 − 3.32 (m, 5H), 3.27 − 3.18 (m, 2H), 3.13 (t, J = 10.9 Hz, 1H), 3.03 (t, J = 4.9 Hz, 1H), 2.56 (dd, J = 13.5, 4.1 Hz, 1H), 2.37 (t, J = 13.0 Hz, 1H), 2.11 − 2.03 (m, 1H), 1.92 (dd, J = 8.9, 3.6 Hz, 2H), 1.88 − 1.76 (m, 2H), 1.74 − 1.66 (m, 2H), 1.66 − 1.49 (m, 5H), 1.46 − 1.36 (m, 6H), 1.31 (s, 3H), 1.29 − 1.24 (m, 2H), 1.17 − 1.07 (m, 5H), 1.07 − 0.88 (m, 97H), 0.81 − 0.54 (m, 61H); **^{13}C-NMR** (151 MHz, CDCl3) δ 212.72, 179.86, 168.37, 144.93, 135.26, 128.45, 128.25, 128.12, 122.65, 103.68, 101.39, 100.83, 86.42, 78.79, 78.71, 76.44, 75.93, 75.89, 75.82, 75.80, 75.07, 72.60, 72.52, 71.37, 71.09, 66.84, 65.33, 62.93, 60.23, 53.79, 49.33, 49.08, 47.30, 45.95, 43.01, 41.88, 41.84, 39.65, 37.93, 36.02, 35.38, 34.07, 32.53, 31.81, 31.40, 30.53, 26.31, 25.36, 24.25, 23.42, 20.16, 16.72, 15.84, 12.26, 7.56, 7.47, 7.25, 7.16, 7.15,

7.13, 6.98, 6.85, 6.78, 5.92, 5.65, 5.44, 5.37, 5.34, 5.26, 5.23, 5.01, 4.42; **HRMS** (ESI)

m/z: Calcd for $C_{110}H_{209}NO_{20}NaSi_9$ (M+Na)$^+$ 2139.3189, found 2139.3206.
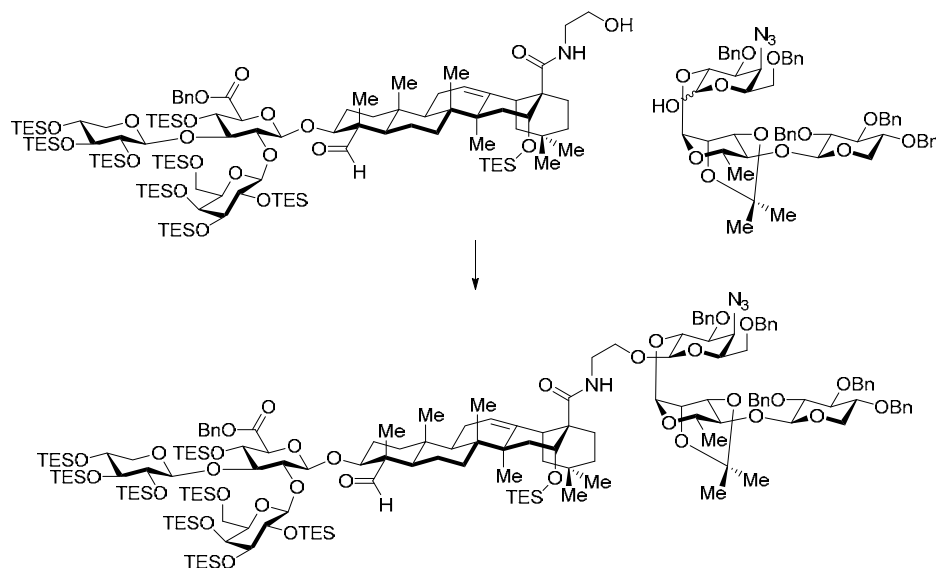
(74): Trifluoromethanesulfonic anhydride (5.2 μL, 0.041, 1.5 equiv) was added to a solution of trisaccharide **73** (20 mg, 0.021 mmol, 1.00 equiv), phenyl sulfoxide (12.5 mg, 0.061 mmol, 3.0 equiv) and 2,4,6-tri-tertbutylpyridine (18 mg, 0.074 mmol, 3.6 equiv) in dichloromethane (1 mL) at −78 °C. The reaction stirred in a cold bath at −78 °C for 5 min and then was transferred to a bath between -40 °C for 60 min. A solution of ethanolamide **68** (84 mg, 0.040 mmol, 1.95 equiv) was added in dichloromethane (1.0 ml) via syringe. After 30 min, flask was transferred to an ice-bath and stirred for 15 min. Triethylamine was added, concentrated and purified via silica gel chromatography (hexanes:ethyl acetate, 10:1 to 2:1) furnishing β-glycoside **74** (55 mg, 87% yield) as a colorless film.

TLC R_f 0.67 (5:1 benzene/ethyl acetate); **FTIR** (NaCl, film) 3430, 2953, 2911, 2877, 2105, 1751, 1724, 1653, 1497, 1457, 1414, 1379, 1240, 1172, 1098, 1006, 910, 864, 826, 799, 737, 697, 666 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.71 (s, 1H), 7.39 – 7.22 (m, 30H), 6.36 (t, J = 5.5 Hz, 1H), 5.43 (t, J = 3.8 Hz, 1H), 5.38 (s, 1H), 5.27 (d, J = 12.3 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.91 – 4.87 (m, 2H), 4.87 – 4.80 (m, 2H), 4.76 – 4.69 (m, 2H), 4.68 (d, J = 11.1 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.58 – 4.52 (m, 4H), 4.51 (d, J = 3.0 Hz, 1H), 4.43 (d, J = 7.2 Hz, 1H), 4.20 (d, J = 7.5 Hz, 2H), 4.15 (dd, J = 7.4, 5.5 Hz, 1H), 4.07 (d, J = 3.5 Hz, 1H), 4.04 (d, J = 5.6 Hz, 1H), 3.96 – 3.91 (m, 3H), 3.89 – 3.79 (m, 6H), 3.77 (t, J = 9.2 Hz, 1H), 3.69 (ddd, J = 10.8, 6.8, 4.7 Hz, 1H), 3.65 – 3.53 (m, 11H), 3.49 (ddd, J = 10.4, 8.4, 5.1 Hz, 1H), 3.40 (dd, J = 9.4, 2.5 Hz, 1H), 3.38 – 3.17 (m, 7H), 3.14 (t, J = 11.0 Hz, 1H), 2.52 (dd, J = 13.7, 4.5 Hz, 1H), 2.34 (t, J = 13.0 Hz, 1H), 2.02 – 1.51 (m, 12H), 1.42 – 1.38 (m, 1H), 1.37 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H), 1.22 (d, J = 6.0 Hz, 3H), 1.13 – 1.03 (m, 4H), 1.03 – 0.90 (m, 82H), 0.89 (s, 3H), 0.87 (s, 3H), 0.81 – 0.56 (m, 56H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.60, 177.85, 168.36, 144.49, 138.81, 138.73, 138.23, 137.41, 136.89, 135.20, 128.58, 128.55, 128.47, 128.41, 128.32, 128.26, 128.24, 128.21, 128.18, 128.16, 128.07, 127.95, 127.88, 127.82, 127.77, 127.48, 122.56, 109.12, 103.64, 102.86, 102.06, 101.38, 100.82, 98.24, 86.43, 83.87, 81.87, 81.39, 78.81, 78.71, 78.25, 78.12, 77.95, 76.45, 75.99, 75.97, 75.92, 75.81, 75.49, 75.07, 74.87, 74.62, 73.71, 73.21, 72.61, 72.51, 71.83, 71.38, 71.09, 68.76, 68.44, 66.87, 65.33, 64.56, 63.81, 60.24, 58.36, 53.82, 49.25, 49.12, 47.22, 46.06, 41.85, 41.73, 39.94, 39.57, 37.97, 36.06, 35.95, 35.39, 34.66, 34.53, 34.07, 32.59, 31.98, 31.59, 31.42, 30.48, 29.06, 27.80, 26.91, 26.49, 26.30, 25.35, 25.27, 24.38, 23.35, 22.66, 20.70, 20.21, 18.77, 17.62, 16.94, 15.75, 14.14, 12.23, 11.45, 7.56, 7.46, 7.25, 7.17, 7.16, 7.13, 7.09,

6.99, 6.89, 6.85, 6.78, 5.92, 5.64, 5.44, 5.37, 5.34, 5.26, 5.23, 5.20, 5.18, 5.14, 5.06, 5.01, 4.41; **HRMS** (ESI) *m/z*: Calcd for $C_{165}H_{270}N_4O_{32}NaSi_9$ $(M+Na)^+$ 3094.7445, found 3094.7344.
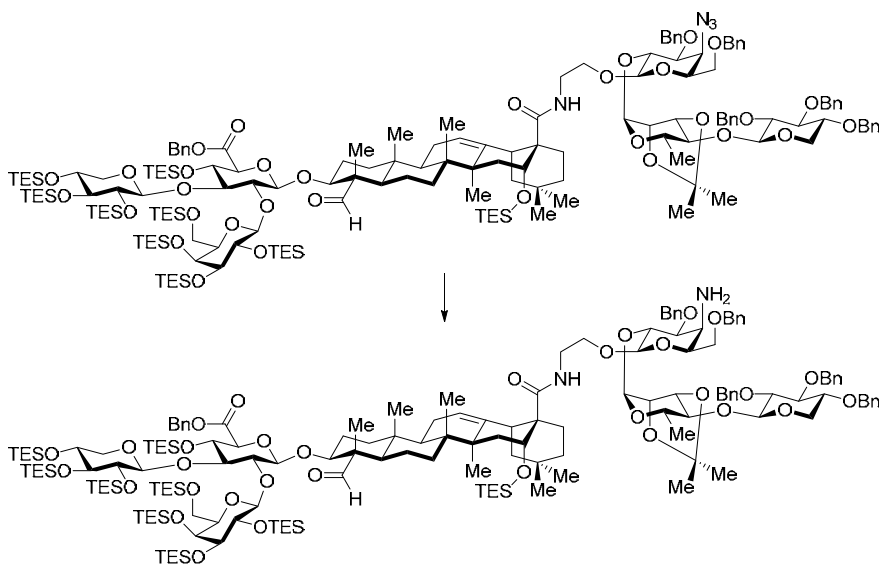
(91): An excess of hydrogen sulfide was bubbled through an ice-cooled solution of azide **74** (35 mg, 0.011, 1 equiv) in pyridine and triethylamine (3:1, 2 mL) for two min via steel needle, then needle removed from septum. After stirring for 2 min, ice-bath was removed and warmed to ambient temperature. After 4.5 hr, the dark green solution was purged of excess hydrogen sulfide, then volatiles removed with a stream of nitrogen. The resulting light-orange solid was purified by silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 8:1 to 1:1) to give amine **91** (27 mg, 78% yield).

TLC R_f 0.44 (3% methanol/dichloromethane); **FTIR** (NaCl film) 3422, 3031, 2953, 2910, 2876, 1751, 1734, 1719, 1653, 1648, 1507, 1496, 1465, 1457, 1454, 1419, 1413, 1379, 1240, 1097, 1008, 908, 863, 825, 734, 697, 668 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$)

δ 9.70 (s, 1H), 7.39 – 7.22 (m, 30H), 6.31 (t, *J* = 5.5 Hz, 1H), 5.42 – 5.36 (m, 2H), 5.27 (d, *J* = 12.4 Hz, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 4.91 – 4.87 (m, 2H), 4.83 (q, *J* = 11.1 Hz, 2H), 4.73 – 4.64 (m, 3H), 4.61 (d, *J* = 11.7 Hz, 1H), 4.58 – 4.54 (m, 3H), 4.54 – 4.51 (m, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.42 (d, *J* = 7.2 Hz, 1H), 4.23 (d, *J* = 7.7 Hz, 1H), 4.20 – 4.14 (m, 2H), 4.07 (d, *J* = 5.6 Hz, 1H), 3.96 – 3.90 (m, 3H), 3.88 – 3.78 (m, 5H), 3.78 – 3.67 (m, 4H), 3.67 – 3.63 (m, 1H), 3.63 – 3.53 (m, 8H), 3.51 – 3.43 (m, 2H), 3.43 – 3.29 (m, 6H), 3.28 – 3.23 (m, 2H), 3.22 – 3.17 (m, 1H), 3.13 (t, *J* = 10.9 Hz, 1H), 2.52 (dd, *J* = 13.3, 2.9 Hz, 1H), 2.33 (t, *J* = 13.0 Hz, 1H), 2.02 – 1.94 (m, 1H), 1.89 – 1.76 (m, 4H), 1.72 – 1.54 (m, 3H), 1.48 – 1.38 (m, 2H), 1.38 – 1.32 (m, 8H), 1.29 (s, 3H), 1.21 (d, *J* = 6.0 Hz, 3H), 1.10 – 1.02 (m, 4H), 1.02 – 0.90 (m, 83H), 0.89 – 0.86 (m, 9H), 0.81 – 0.55 (m, 58H); ^{13}C-NMR (151 MHz, CDCl$_3$) δ 212.57, 177.70, 168.37, 144.75, 138.81, 138.70, 138.23, 137.78, 137.34, 135.20, 131.04, 129.31, 128.53, 128.48, 128.46, 128.41, 128.34, 128.30, 128.26, 128.25, 128.18, 128.15, 128.13, 128.05, 128.03, 128.00, 127.97, 127.94, 127.88, 127.86, 127.84, 127.81, 127.76, 127.70, 127.51, 127.48, 124.77, 122.23, 109.10, 103.65, 103.09, 102.06, 101.37, 100.82, 98.07, 86.39, 83.86, 82.07, 81.86, 78.78, 78.70, 78.25, 78.07, 77.96, 76.44, 76.06, 75.93, 75.88, 75.82, 75.80, 75.49, 75.06, 74.65, 74.58, 73.59, 73.35, 73.21, 72.60, 72.51, 71.36, 71.10, 71.01, 69.35, 68.65, 66.88, 65.32, 64.51, 63.80, 60.23, 53.79, 49.27, 49.00, 48.74, 47.18, 45.97, 39.89, 39.56, 37.94, 35.95, 35.35, 34.03, 32.58, 31.91, 31.49, 30.49, 27.82, 26.50, 26.29, 25.34, 24.34, 23.44, 20.18, 17.53, 16.81, 15.92, 12.23, 7.55, 7.46, 7.25, 7.17, 7.16, 7.13, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.37, 5.33, 5.25, 5.22, 5.01, 4.41; **HRMS** (ESI) *m/z*: Calcd for C$_{165}$H$_{273}$N$_2$O$_{32}$Si$_9$ (M+H) 3046.7720, found 3046.7788.
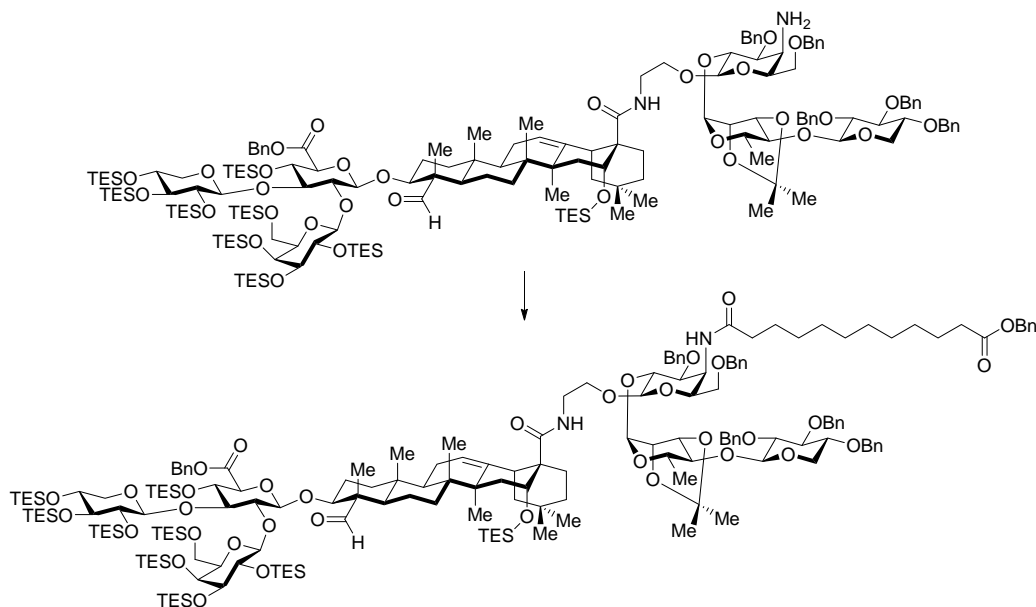
(98): Isobutyl chloroformate (6.1 μL, 0.047 mmol, 3.0 equiv) was added to an ice-cooled solution of carboxylic acid **106** (30 mg, 0.094 mmol, 6 equiv) and triethylamine (22 μL, 0.156 mmol, 10 equiv) in tetrahydrofuran (3.0 mL) and stirred for 4 hours, then transferred via cannula to an ice-cooled solution of amine **91** (42 mg, 0.013 mmol, 1 equiv) in tetrahydrofuran (2.0 mL). After 24 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give ethanolamide **98** (44 mg, 84 % yield) as a colorless film.

TLC R_f 0.37 (3:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3582, 3417, 3090, 3063, 3030, 2952, 2911, 2876, 1738, 1727, 1657, 1547, 1512, 1498, 1454, 1413, 1379, 1240, 1166, 1094, 1069, 1007, 910, 863, 823, 799, 731, 696 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.70 (s, 1H), 7.39 – 7.24 (m, 35H), 6.16 (t, J = 5.6 Hz, 1H), 5.45 (d, J = 10.0 Hz, 1H),

5.40 (s, 1H), 5.31 (t, J = 3.7 Hz, 1H), 5.25 (d, J = 12.4 Hz, 1H), 5.14 – 5.07 (m, 3H), 4.91 – 4.79 (m, 5H), 4.74 (d, J = 11.4 Hz, 1H), 4.71 (d, J = 11.7 Hz, 1H), 4.67 (d, J = 11.0 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.56 (d, J = 7.4 Hz, 1H), 4.54 – 4.46 (m, 3H), 4.45 – 4.39 (m, 2H), 4.25 (d, J = 7.5 Hz, 1H), 4.20 – 4.14 (m, 2H), 4.07 (d, J = 5.6 Hz, 1H), 3.95 – 3.89 (m, 3H), 3.88 – 3.78 (m, 5H), 3.75 (t, J = 9.3 Hz, 1H), 3.72 – 3.44 (m, 15H), 3.43 – 3.28 (m, 5H), 3.25 (t, J = 8.0 Hz, 1H), 3.23 – 3.16 (m, 2H), 3.13 (t, J = 10.9 Hz, 1H), 2.49 (dd, J = 13.1, 4.6 Hz, 1H), 2.37 – 2.29 (m, 3H), 2.17 (tt, J = 11.2, 5.7 Hz, 2H), 1.95 (dt, J = 14.5, 3.5 Hz, 1H), 1.85 – 1.75 (m, 4H), 1.69 – 1.52 (m, 8H), 1.48 – 1.42 (m, 3H), 1.38 – 1.30 (m, 8H), 1.29 (s, 3H), 1.28 – 1.23 (m, 6H), 1.21 (d, J = 6.2 Hz, 7H), 1.19 – 1.15 (m, 2H), 1.10 – 1.01 (m, 4H), 1.01 – 0.89 (m, 85H), 0.89 – 0.84 (m, 9H), 0.81 – 0.54 (m, 60H); ^{13}C-NMR (151 MHz, CDCl$_3$) δ 212.60, 177.74, 173.67, 173.33, 168.38, 144.94, 138.80, 138.65, 138.21, 137.58, 137.40, 136.12, 135.19, 128.52, 128.48, 128.43, 128.41, 128.31, 128.27, 128.25, 128.15, 128.13, 128.10, 128.03, 127.87, 127.84, 127.81, 127.77, 127.76, 127.73, 127.56, 127.48, 122.05, 109.09, 103.63, 102.64, 102.07, 101.37, 100.82, 98.08, 86.37, 83.85, 81.85, 79.51, 78.78, 78.70, 78.17, 78.04, 77.97, 76.43, 75.94, 75.92, 75.85, 75.80, 75.50, 75.06, 74.83, 74.67, 73.66, 73.21, 72.97, 72.59, 72.50, 71.36, 71.12, 70.85, 68.98, 68.15, 66.88, 66.04, 65.32, 64.56, 63.82, 60.22, 53.76, 49.25, 48.93, 47.13, 46.13, 45.94, 41.83, 41.76, 39.53, 39.49, 37.91, 36.96, 36.62, 35.96, 35.26, 34.33, 34.04, 32.56, 31.83, 31.49, 30.49, 29.44, 29.40, 29.36, 29.23, 29.14, 27.79, 26.48, 26.31, 25.92, 25.32, 24.96, 24.68, 24.41, 23.39, 23.35, 20.15, 17.42, 16.75, 15.85, 12.22, 7.56, 7.46, 7.24, 7.16, 7.16, 7.13, 6.98, 6.85, 6.79, 5.91, 5.63, 5.43, 5.37, 5.33, 5.25, 5.22, 5.01, 4.41; **HRMS** (ESI) m/z: Calcd for C$_{184}$H$_{298}$N$_2$O$_{35}$Si$_9$Na [M+23] 3379.9421, found 3370.9590.
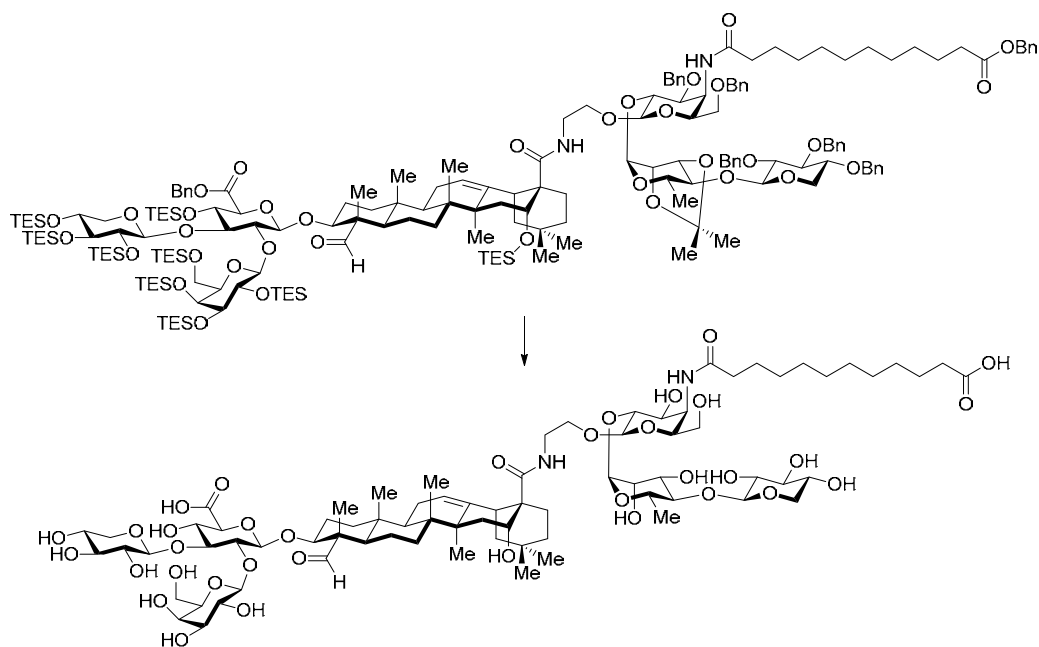
(105): A solution of fully protected β-ethanolamide analogue (**98**) (25.0 mg, 0.008 mmol, 1.0 equiv) in tetrahydrofuran (5 mL) and ethanol (5 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (17 mg, 0.016 mmol, 2.2 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (5.0 mL, TFA/H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 10・49% acetonitrile (0.05% TFA) in water (0.05% TFA) over 18 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 16.42 min) was collected and lyophilized to dryness to afford SQS-0-4-5-5 (**105**) (5.5 mg, 45% yield) as a white solid.

¹H NMR (600 MHz, D₂O/CD₃CN) δ 9.30 (s, 1H), 6.98 (d, *J* = 9.6 Hz, 1H), 6.80 (t, *J* = 5.2 Hz, 1H), 5.41 (t, *J* = 3.8 Hz, 1H), 4.86 (d, *J* = 1.8 Hz, 1H), 4.60 (d, *J* = 7.8 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 1H), 4.35 (d, *J* = 7.8 Hz, 1H), 4.26 (d, *J* = 7.8 Hz, 1H), 3.86 – 3.67 (m, 10H), 3.65 (dd, *J* = 11.1, 7.8 Hz, 2H), 3.61 – 3.50 (m, 4H), 3.50 – 3.37 (m, 8H), 3.34 – 3.18 (m, 5H), 3.18 – 3.07 (m, 5H), 2.69 (dd, *J* = 13.1, 2.6 Hz, 1H), 2.29 – 2.12 (m, 5H), 1.86 – 1.74 (m, 4H), 1.72 – 1.56 (m, 4H), 1.53 – 1.35 (m, 9H), 1.24 (s, 4H), 1.12 (d, *J* = 6.2 Hz, 3H), 1.03 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.65 (s, 3H); **HRMS** (ESI) *m/z*: Calcd for $C_{78}H_{126}N_2O_{35}Si_9Na$ [M+23] 1673.8039, found 1673.8019.
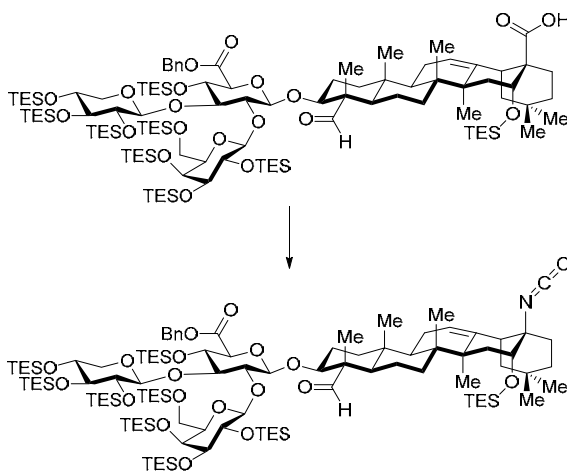
(67): Diphenylphosphoryl azide (24 μl, 0.116 mmol, 1.5 eq) was added to a solution of **65** (161 mg, 0.0776 mmol, 1 equiv) and triethylamine (19 μl, 0.136 mmol, 1.75 eq) in benzene (8 ml) in a vessel fitted with a water-cooled condenser, then submerged in a 90 C oil bath. After 30 min, additional portions of triethylamine (86 μl, 0.62 mmol, 8 equiv) and diphenylphosphoryl azide (80 μl, 0.387 mmol, 5 equiv) were added sequentially. After 20 min, reaction was cooled to room temperature, concentrated, and purified by

silica gel chromatography (hexanes/ethyl acetate, 40:1 to 10:1) to give isocyanate **67** (127 mg, 79% yield).

TLC R_f 0.41 (20:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 2953, 2912, 2876, 2248 (NCO st), 1754, 1724, 1458, 1413, 1377, 1239, 1171, 1101, 1006, 971, 908, 864, 825, 801, 736, 695 cm^{-1}; **^{1}H-NMR** (500 MHz, CDCl$_3$) δ 9.71 (s, 1H), 7.38 − 7.28 (m, 5H), 5.37 (s, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.55 (d, J = 7.4 Hz, 1H), 4.42 (d, J = 7.3 Hz, 1H), 4.18 (d, J = 7.3 Hz, 1H), 3.96 − 3.72 (m, 8H), 3.63 − 3.54 (m, 3H), 3.51 − 3.45 (m, 1H), 3.42 − 3.31 (m, 3H), 3.28 − 3.22 (m, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.51 − 2.43 (m, 1H), 2.22 (t, J = 13.6 Hz, 1H), 2.07 (m, 1H), 1.99 − 1.55 (m, 9H), 1.53 (s, 2H), 1.52 − 1.35 (m, 3H), 1.33 (s, 3H), 1.32 (s, 3H), 1.23 (m, 6H), 0.93 (m, 103H), 0.81 − 0.54 (m, 60H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.92, 168.33, 142.29, 135.26, 128.52, 128.49, 128.46, 128.34, 128.28, 128.25, 128.23, 128.21, 128.19, 128.19, 128.13, 123.76, 122.06, 103.68, 101.40, 100.83, 86.53, 78.81, 78.71, 77.45, 76.44, 75.91, 75.81, 75.08, 72.61, 72.52, 71.38, 71.08, 66.87, 66.84, 65.33, 62.11, 62.08, 60.22, 53.84, 49.44, 48.29, 47.09, 46.16, 41.34, 41.32, 39.77, 39.63, 37.91, 37.18, 37.14, 36.38, 36.11, 33.70, 32.45, 32.40, 32.38, 32.36, 30.63, 30.61, 26.51, 26.44, 25.37, 24.30, 24.27, 23.42, 20.26, 17.04, 17.01, 15.78, 12.30, 7.57, 7.47, 7.38, 7.28, 7.25, 7.23, 7.22, 7.16, 7.14, 7.11, 7.08, 7.05, 7.04, 7.03, 6.99, 6.95, 6.94, 6.91, 6.85, 6.83, 6.81, 6.79, 6.77, 5.93, 5.65, 5.48, 5.44, 5.41, 5.37, 5.35, 5.30, 5.28, 5.26, 5.23, 5.19, 4.94, 4.88, 4.86, 4.42, 4.38; **HRMS** (ESI) *m/z*: Calcd for C$_{108}$H$_{203}$NO$_{19}$NaSi$_9$ (M+Na)$^+$ 2093.2771, found 2093.2708.
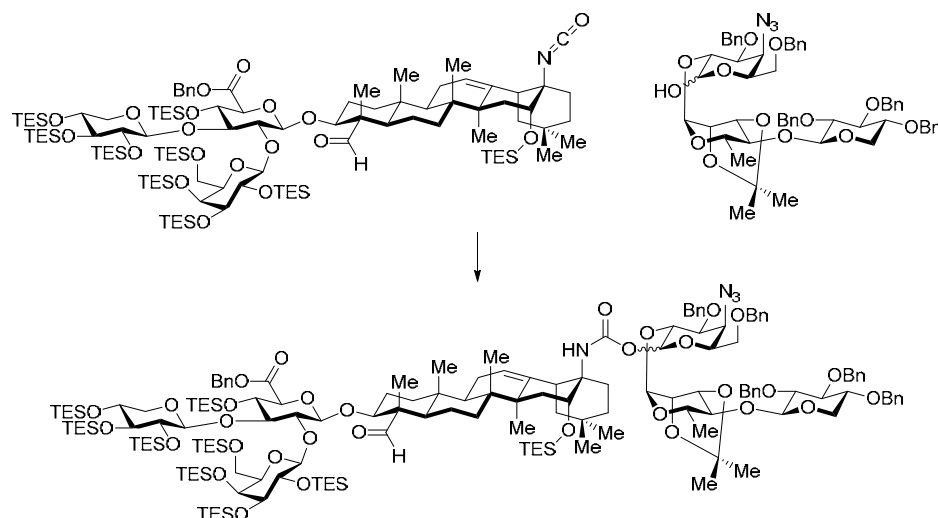
(82α/β): Sodium hydride (60% dispersion in mineral oil, 4.3 mg, 0.108 mmol, 3 equiv) was added to a solution of hemiacetal (35 mg, 0.036 mmol, 1 equiv) in tetrahydrofuran (0.5 mL). After 80 min, isocyanate was added in 0.5 mL tetrahydrofuran. After three hours, suspension was diluted with concentrated ammonium chloride, and extracted with ethyl acetate (3 × 25 mL). Combined organics were washed with brine and dried over sodium sulfate, decanted, concentrated, and purified via silica gel chromatography (hexanes/ethyl acetate, 20:1 to 4:1) to give easily separable glycosyl carbamates (57 mg β-carbamate and 29 mg α-carbamate, total yield 79%).

α-product

TLC R_f 0.60 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 2953, 2877, 2108, 1745, 1456, 1379, 1240, 1096, 1008, 733, 665 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.69 (s, 1H), 7.41 – 7.26 (m, 31H), 5.97 (d, J = 3.8 Hz, 1H), 5.37 (t, J = 3.6 Hz, 1H), 5.31 – 5.26 (m, 2H), 5.10 (d, J = 12.4 Hz, 1H), 4.88 (d, J = 11.0 Hz, 1H), 4.85 – 4.76 (m, 3H), 4.74 – 4.67 (m, 2H), 4.66 – 4.58 (m, 3H), 4.43 (d, J = 7.2 Hz, 1H), 4.36 (s, 1H), 4.28 – 4.24 (m,

1H), 4.21 – 4.15 (m, 2H), 4.15 – 4.09 (m, 2H), 4.07 (d, J = 5.8 Hz, 1H), 3.99 – 3.89 (m, 4H), 3.89 – 3.73 (m, 6H), 3.65 – 3.51 (m, 9H), 3.50 – 3.44 (m, 1H), 3.40 (dd, J = 9.4, 2.5 Hz, 1H), 3.38 – 3.33 (m, 2H), 3.30 (t, J = 7.9 Hz, 1H), 3.25 (dd, J = 8.6, 7.4 Hz, 1H), 3.19 (dd, J = 11.7, 8.9 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.53 (dd, J = 14.4, 4.4 Hz, 1H), 2.28 (t, J = 13.4 Hz, 1H), 2.21 – 2.14 (m, 1H), 1.94 – 1.48 (m, 13H), 1.39 – 1.19 (m, 15H), 1.15 – 0.82 (m, 101H), 0.82 – 0.51 (m, 61H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.17, 168.37, 151.39, 142.42, 138.80, 138.46, 138.23, 137.57, 137.47, 135.23, 128.48, 128.45, 128.42, 128.40, 128.35, 128.29, 128.26, 128.25, 128.23, 128.19, 128.15, 128.12, 128.09, 128.01, 128.00, 127.91, 127.89, 127.86, 127.74, 127.61, 127.59, 127.53, 127.49, 127.46, 124.22, 109.09, 103.52, 102.47, 101.36, 100.82, 99.01, 91.59, 86.14, 83.76, 81.63, 78.77, 78.71, 78.33, 78.22, 77.92, 77.61, 76.44, 76.33, 75.91, 75.82, 75.79, 75.48, 75.05, 74.52, 73.92, 73.83, 73.10, 72.59, 72.48, 72.18, 71.39, 71.10, 68.85, 68.29, 66.85, 65.62, 65.33, 63.63, 60.28, 60.14, 56.04, 53.74, 49.08, 46.67, 45.96, 44.57, 41.06, 39.67, 37.76, 36.31, 35.94, 33.26, 32.49, 32.14, 31.93, 31.92, 30.65, 29.70, 27.66, 26.56, 26.15, 25.28, 24.40, 23.38, 20.16, 17.45, 16.76, 15.72, 12.04, 7.56, 7.46, 7.25, 7.19, 7.16, 7.13, 7.10, 7.07, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.42, 5.36, 5.33, 5.30, 5.28, 5.25, 5.22, 4.88, 4.41; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{266}$N$_4$O$_{32}$NaSi$_9$ [M+Na] 3066.7132, found 3066.6929.

β-product

TLC R_f 0.42 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3422, 2953, 2877, 2107, 1745, 1497, 1456, 1378, 1240, 1096, 1007, 909, 863, 825, 730, 697, 666 cm^{-1}; **^{1}H NMR** (500 MHz, CDCl$_3$) δ 9.70 (s, 1H), 7.40 – 7.27 (m, 30H), 5.35 (s, 1H), 5.34 – 5.32 (m, 1H), 5.29 (d, J = 2.0 Hz, 1H), 5.27 (d, J = 6.3 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.90 –

4.81 (m, 4H), 4.77 – 4.69 (m, 3H), 4.65 – 4.60 (m, 3H), 4.57 – 4.48 (m, 4H), 4.42 (d, J = 7.2 Hz, 1H), 4.18 (d, J = 7.3 Hz, 1H), 4.11 – 4.06 (m, 2H), 4.04 (d, J = 5.7 Hz, 1H), 3.96 – 3.72 (m, 9H), 3.71 – 3.52 (m, 11H), 3.52 – 3.43 (m, 1H), 3.42 – 3.31 (m, 3H), 3.26 (q, J = 7.6 Hz, 2H), 3.22 – 3.16 (m, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.52 – 2.45 (m, 1H), 2.33 (t, J = 13.7 Hz, 1H), 2.02 (d, J = 14.2 Hz, 1H), 1.93 – 1.73 (m, 4H), 1.74 – 1.65 (m, 2H), 1.64 – 1.56 (m, 1H), 1.52 – 1.51 (m, 1H), 1.49 (s, 3H), 1.47 – 1.33 (m, 3H), 1.31 (d, J = 2.5 Hz, 6H), 1.28 – 1.23 (m, 4H), 1.10 (d, J = 13.8 Hz, 2H), 1.05 – 0.87 (m, 89H), 0.85 (s, 3H), 0.79 (s, 3H), 0.76 – 0.52 (m, 53H); ^{13}C NMR (151 MHz, CDCl$_3$) δ 212.76, 168.32, 151.95, 141.94, 138.80, 138.58, 138.23, 137.62, 136.81, 135.24, 128.55, 128.49, 128.45, 128.42, 128.29, 128.26, 128.19, 128.13, 127.98, 127.96, 127.93, 127.91, 127.77, 127.74, 127.53, 127.49, 124.99, 109.02, 103.65, 102.34, 101.39, 100.82, 98.50, 93.20, 86.46, 83.96, 82.45, 81.63, 78.80, 78.71, 78.14, 78.12, 78.00, 76.43, 75.99, 75.90, 75.80, 75.63, 75.06, 74.93, 74.09, 73.72, 73.21, 72.60, 72.50, 72.05, 71.92, 71.89, 71.37, 71.07, 68.10, 66.83, 65.32, 64.44, 63.83, 60.21, 58.27, 55.96, 53.83, 49.33, 47.32, 46.59, 46.06, 41.20, 39.63, 37.91, 36.12, 36.01, 33.17, 32.48, 32.24, 32.08, 30.54, 29.70, 27.79, 26.45, 26.41, 25.34, 24.56, 23.50, 20.20, 17.97, 16.90, 15.80, 12.26, 7.56, 7.46, 7.24, 7.16, 7.13, 7.06, 6.98, 6.85, 6.79, 5.92, 5.64, 5.43, 5.36, 5.33, 5.25, 5.22, 4.92, 4.87, 4.41; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{266}$N$_4$O$_{32}$NaSi$_9$ [M+Na] 3066.7132, found 3066.7073.
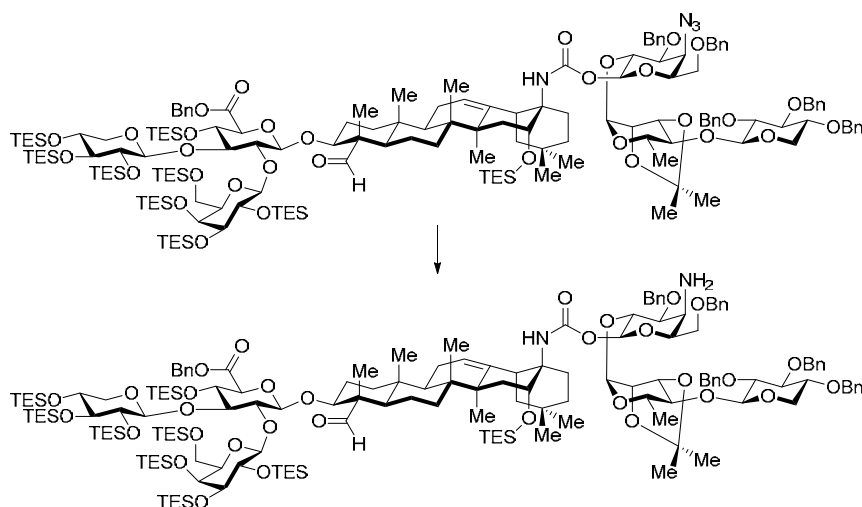
(87β): An excess of hydrogen sulfide was bubbled through an ice-cooled solution of

azide **82β** (17 mg, 0.006 mmol, 1 eq) in pyridine and triethylamine (3.5:1, 4.5 mL) for

two min via steel needle, then needle removed from septum. After stirring for 2 min, ice-

bath was removed and warmed to ambient temperature. After 7 hr, the dark green

solution was purged of excess hydrogen sulfide, then volatiles removed with a stream of

nitrogen. The resulting light-orange solid was purified by silica gel chromatography

(hexanes:ethyl acetate + 0.5% triethylamine, 8:1 to 1:1) to give amine **(87β)** (14 mg, 83%

yield).

TLC R_f 0.42 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3425,

3066, 3033, 2955, 2913, 2878, 1741, 1498, 1458, 1415, 1382, 1314, 1242, 1098, 904,

865, 827, 735, 699, 667 cm^{-1}; **^{1}H NMR** (500 MHz, CDCl$_3$) δ 9.70 (s, 1H), 7.41 − 7.27

(m, 30H), 5.39 − 5.30 (m, 3H), 5.28 (d, J = 12.4 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.92

− 4.80 (m, 4H), 4.77 (s, 1H), 4.72 (d, J = 11.7 Hz, 1H), 4.68 − 4.59 (m, 5H), 4.59 − 4.46

(m, 4H), 4.43 (d, J = 7.2 Hz, 1H), 4.18 (d, J = 7.3 Hz, 1H), 4.13 − 4.06 (m, 2H), 3.97 −

3.87 (m, 4H), 3.87 – 3.45 (m, 20H), 3.42 – 3.31 (m, 4H), 3.26 (dt, J = 11.1, 8.0 Hz, 2H), 3.22 – 3.16 (m, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.54 – 2.46 (m, 1H), 2.33 (dd, J = 15.2, 11.4 Hz, 1H), 2.02 (dd, J = 13.6, 2.4 Hz, 1H), 1.94 – 1.75 (m, 4H), 1.75 – 1.53 (m, 5H), 1.48 – 1.34 (m, 6H), 1.28 – 1.24 (m, 4H), 1.12 – 0.86 (m, 104H), 0.85 (s, 4H), 0.79 (s, 4H), 0.78 – 0.53 (m, 60H); ^{13}**C NMR** (151 MHz, CDCl$_3$) δ 212.78, 168.32, 152.02, 142.07, 138.80, 138.55, 138.24, 138.01, 137.35, 135.24, 128.49, 128.45, 128.42, 128.41, 128.30, 128.27, 128.12, 128.02, 127.98, 127.94, 127.93, 127.82, 127.76, 127.74, 127.53, 127.50, 124.90, 109.00, 103.66, 102.31, 101.38, 100.81, 98.37, 93.52, 86.48, 83.96, 82.45, 82.37, 78.80, 78.70, 78.11, 77.99, 76.43, 76.07, 75.89, 75.79, 75.63, 75.06, 74.95, 74.15, 73.99, 73.69, 73.21, 72.59, 72.50, 72.02, 71.37, 71.07, 71.03, 68.82, 66.83, 65.31, 64.37, 63.83, 60.21, 55.86, 53.81, 49.33, 48.50, 47.33, 46.58, 46.03, 41.19, 39.81, 39.62, 37.89, 36.11, 36.00, 33.16, 32.66, 32.48, 32.27, 32.02, 31.93, 30.90, 30.55, 29.70, 29.37, 27.82, 26.64, 26.46, 26.41, 25.34, 24.54, 24.45, 23.50, 22.70, 20.18, 17.97, 17.01, 16.88, 15.78, 14.14, 12.25, 7.56, 7.46, 7.24, 7.16, 7.13, 7.05, 6.98, 6.85, 6.79, 5.91, 5.63, 5.43, 5.36, 5.33, 5.25, 5.22, 4.95, 4.86, 4.40; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{269}$N$_2$O$_{32}$Si$_9$ [M+H]$^+$ 3018.7407, found 3018.7476.
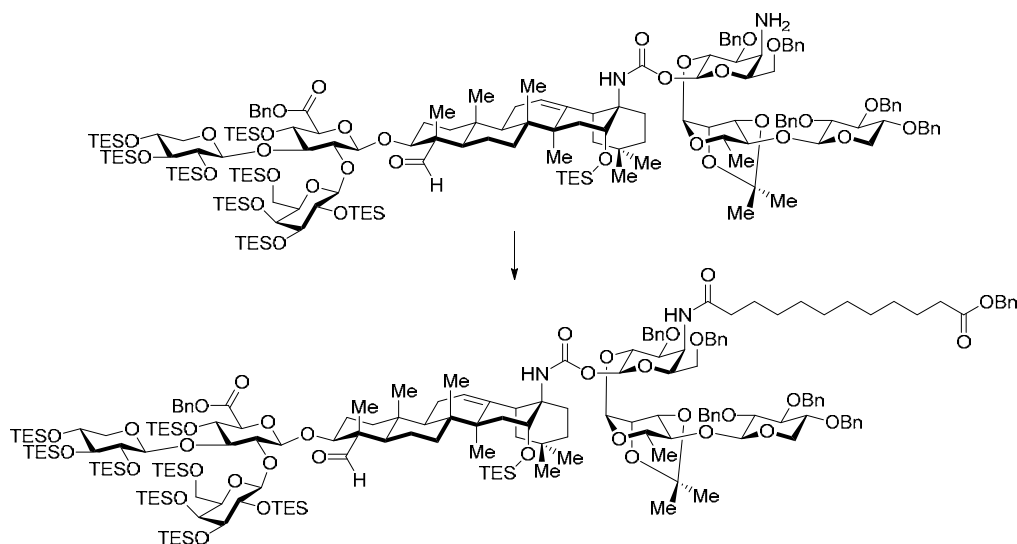
(94β): Isobutyl chloroformate (1.2 μL, 0.009 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **106** (4.8 mg, 0.015 mmol, 6 equiv) and triethylamine (2.8 μL, 0.020 mmol, 8 equiv) in tetrahydrofuran (2.5 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine **87β** (7.5 mg, 0.002 mmol, 1 equiv) in tetrahydrofuran (1.5 mL). After 1 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give amide **94β** (6.0 mg, 71 % yield) as a colorless film.

TLC R_f 0.66 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3424, 2952, 2876, 1744, 1679, 1496, 1454, 1379, 1240, 1096, 1008, 825, 733, 696, 665 cm^{-1}; **1H NMR** (500 MHz, CDCl$_3$) δ 9.70 (s, 1H), 7.38 – 7.27 (m, 35H), 5.43 – 5.32 (m, 4H), 5.28 (d, J = 12.4 Hz, 1H), 5.13 – 5.07 (m, 4H), 4.92 – 4.86 (m, 3H), 4.86 – 4.81 (m, 3H), 4.76 (s, 2H), 4.72 (d, J = 11.8 Hz, 1H), 4.64 – 4.59 (m, 3H), 4.57 – 4.52 (m, 2H), 4.47 –

4.39 (m, 3H), 4.18 (d, J = 7.3 Hz, 1H), 4.12 – 4.06 (m, 2H), 3.96 – 3.90 (m, 3H), 3.89 –

3.72 (m, 7H), 3.68 – 3.44 (m, 14H), 3.39 (dd, J = 9.5, 2.5 Hz, 1H), 3.38 – 3.32 (m, 2H),

3.26 (dt, J = 8.8, 7.3 Hz, 2H), 3.19 (dd, J = 11.6, 9.0 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H),

2.56 – 2.49 (m, 1H), 2.39 – 2.29 (m, 4H), 2.22 – 2.08 (m, 2H), 2.08 – 1.99 (m, 1H), 1.92

– 1.75 (m, 4H), 1.74 – 1.57 (m, 8H), 1.55 (s, 5H), 1.35 (s, 5H), 1.33 (s, 3H), 1.31 – 1.17

(m, 22H), 1.14 – 1.03 (m, 4H), 1.02 – 0.88 (m, 106H), 0.85 (s, 3H), 0.80 (s, 3H), 0.74 (s,

65H); ^{13}**C NMR** (151 MHz, CDCl$_3$) δ 212.63, 173.67, 173.21, 168.33, 151.68, 142.11,

138.78, 138.51, 138.24, 137.83, 137.38, 136.11, 135.24, 128.52, 128.45, 128.42, 128.39,

128.30, 128.29, 128.27, 128.15, 128.14, 128.12, 127.98, 127.94, 127.83, 127.77, 127.73,

127.54, 124.83, 109.02, 103.67, 102.43, 101.38, 100.82, 98.46, 93.36, 83.97, 82.49,

79.92, 78.78, 78.70, 78.13, 78.00, 76.43, 75.91, 75.81, 75.65, 75.04, 74.97, 74.58, 73.72,

73.23, 72.59, 72.50, 71.96, 71.37, 71.08, 68.46, 66.84, 66.05, 65.32, 64.43, 63.86, 60.20,

56.05, 53.77, 49.21, 47.34, 46.65, 46.10, 45.99, 41.23, 39.63, 37.86, 36.92, 36.11, 35.99,

34.33, 33.15, 32.47, 32.20, 31.98, 30.55, 29.70, 29.44, 29.38, 29.22, 29.14, 27.81, 26.45,

26.39, 25.85, 25.32, 24.96, 24.59, 23.50, 20.18, 18.08, 17.00, 15.79, 14.14, 12.24, 7.56,

7.46, 7.24, 7.15, 7.13, 7.05, 6.98, 6.85, 6.78, 5.92, 5.63, 5.43, 5.36, 5.33, 5.25, 5.22, 4.87,

4.40; **HRMS** (ESI) m/z: Calcd for [M+H]$^+$, found.
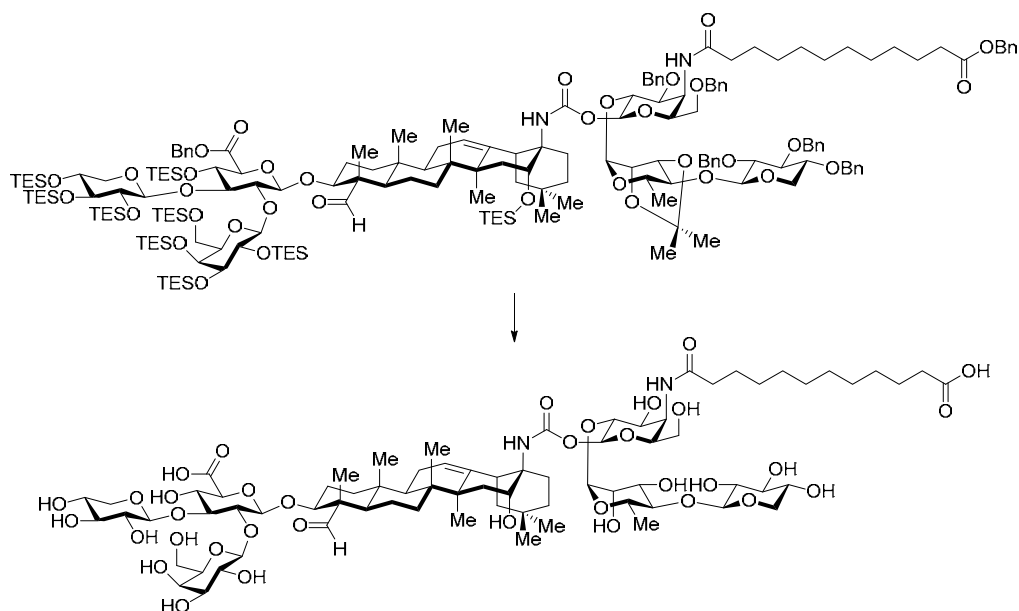
(**101β**) A solution of fully protected β-carbamate analogue (**94β**) (9 mg, 0.003 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (13 mg, 0.011 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (2.0 mL, TFA/H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 15 · 51% acetonitrile (0.05% TFA) in over 18 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 17.35 min) was collected and lyophilized to dryness to afford SQS-0-5-5-5 (**101β**) (3.3 mg, 77 % yield) as a fluffy white solid.

¹H NMR (600 MHz, D₂O/CD₃CN) δ 9.90 (s, 1H), 5.98 – 5.94 (m, 1H), 5.87 (s, 1H), 5.73 (d, *J* = 8.0 Hz, 1H), 5.44 (s, 1H), 5.21 (d, *J* = 7.8 Hz, 1H), 5.10 (d, *J* = 7.8 Hz, 1H), 5.00 (d, *J* = 7.8 Hz, 2H), 4.92 (d, *J* = 7.9 Hz, 1H), 4.44 – 4.37 (m, 4H), 4.34 – 4.19 (m, 6H), 4.17 – 4.10 (m, 5H), 4.08 – 3.93 (m, 8H), 3.93 – 3.82 (m, 4H), 3.77 – 3.66 (m, 4H), 3.63 (q, *J* = 7.2 Hz, 1H), 3.00 – 2.92 (m, 1H), 2.83 – 2.71 (m, 7H), 2.65 – 2.61 (m, 1H), 2.31 – 2.14 (m, 5H), 2.12 – 2.02 (m, 5H), 2.01 – 1.94 (m, 1H), 1.85 (s, 3H), 1.62 (s, 3H), 1.51 – 1.45 (m, 6H), 1.42 – 1.33 (m, 9H); **HRMS** (ESI) *m/z*: Calcd for C₇₆H₁₂₂N₂O₃₅Na [M+Na]⁺ 1645.7726, found 1645.7681.
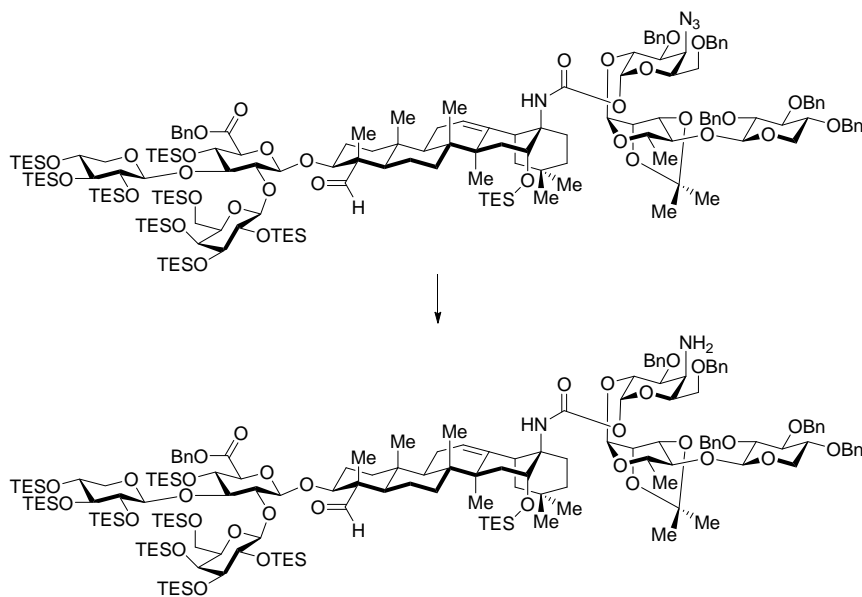

(87α): An excess of hydrogen sulfide was bubbled through an ice-cooled solution of azide **82α** (29 mg, 0.010 mmol, 1 eq) in pyridine and triethylamine (3.5:1, 4.5 mL) for two min via steel needle, then needle removed from septum. After stirring for 2 min, ice-

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bath was removed and warmed to ambient temperature. After 6 hr, the dark green solution was purged of excess hydrogen sulfide, then volatiles removed with a stream of nitrogen. The resulting light-orange solid was purified by silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 8:1 to 1:1) to give amine (87α) (22.5 mg, 78% yield).

TLC R_f 0.11 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3426, 3066, 3033, 2955, 2913, 2878, 1741, 1498, 1458, 1415, 1382, 1314, 1242, 1098, 1009, 904, 865, 827, 735, 699, 667 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.68 (s, 1H), 7.40 – 7.27 (m, 33H), 6.00 (d, J = 3.6 Hz, 1H), 5.37 (s, 1H), 5.33 – 5.25 (m, 2H), 5.09 (d, J = 12.4 Hz, 1H), 4.89 (d, J = 11.1 Hz, 1H), 4.85 (d, J = 11.0 Hz, 1H), 4.82 (d, J = 7.3 Hz, 1H), 4.79 (d, J = 11.0 Hz, 1H), 4.70 (d, J = 11.7 Hz, 1H), 4.68 – 4.49 (m, 7H), 4.42 (d, J = 7.3 Hz, 1H), 4.40 (s, 1H), 4.34 (s, 1H), 4.19 – 4.07 (m, 4H), 4.02 (t, J = 6.2 Hz, 1H), 3.95 – 3.89 (m, 3H), 3.88 – 3.77 (m, 4H), 3.75 (t, J = 9.2 Hz, 1H), 3.71 (dd, J = 10.0, 3.6 Hz, 1H), 3.67 – 3.51 (m, 9H), 3.50 – 3.44 (m, 2H), 3.42 – 3.38 (m, 1H), 3.35 (t, J = 8.5 Hz, 2H), 3.31 (t, J = 7.7 Hz, 1H), 3.27 – 3.23 (m, 1H), 3.19 (dd, J = 11.5, 9.1 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.48 (dd, J = 13.4, 2.7 Hz, 0H), 2.32 – 2.20 (m, 2H), 1.94 – 1.76 (m, 5H), 1.75 – 1.67 (m, 1H), 1.68 – 1.57 (m, 2H), 1.53 – 1.47 (m, 3H), 1.35 – 1.32 (m, 2H), 1.31 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H), 1.22 (d, J = 6.0 Hz, 3H), 1.13 – 1.07 (m, 2H), 1.03 – 0.88 (m, 105H), 0.86 (s, 4H), 0.85 (s, 4H), 0.80 (s, 3H), 0.79 – 0.53 (m, 66H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 212.18, 168.37, 151.64, 142.45, 138.81, 138.46, 138.24, 138.00, 137.81, 135.24, 128.45, 128.44, 128.39, 128.26, 128.25, 128.18, 128.12, 127.90, 127.88, 127.83, 127.74, 127.58, 127.46, 127.36, 124.25, 109.02, 103.49, 102.43, 101.35,

100.82, 98.69, 91.74, 83.75, 81.55, 78.71, 78.21, 77.91, 76.44, 75.81, 75.47, 75.05, 74.49, 73.77, 73.70, 73.10, 72.59, 72.48, 71.39, 71.20, 71.10, 70.42, 68.82, 66.85, 65.40, 65.32, 63.61, 60.29, 55.89, 53.75, 49.28, 49.10, 46.71, 45.98, 44.92, 41.08, 39.67, 37.77, 36.31, 35.94, 33.27, 32.51, 32.16, 31.99, 30.65, 29.70, 27.70, 26.57, 26.22, 25.28, 24.42, 23.40, 20.17, 17.45, 16.74, 15.70, 12.04, 7.56, 7.46, 7.25, 7.18, 7.13, 7.12, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.36, 5.33, 5.25, 5.22, 4.89, 4.41.
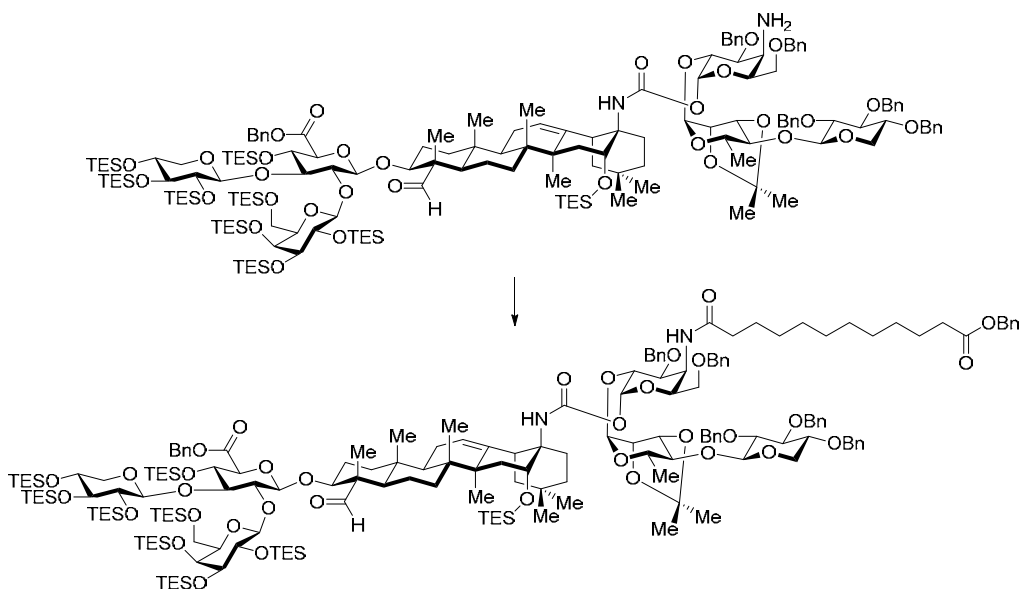

(94α): Isobutyl chloroformate (4.5 μL, 0.037 mmol, 5 equiv) was added to an ice-cooled solution of carboxylic acid **106** (14 mg, 0.045 mmol, 6 equiv) and triethylamine (8.3 μL, 0.060 mmol, 8 equiv) in tetrahydrofuran (2 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine **87α** (22.5 mg, 0.008 mmol, 1 equiv) in tetrahydrofuran (0.6 mL). After 2.5 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel

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chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give amide

94α (23 mg, 93 % yield) as a colorless film.

TLC R_f 0.73 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3421,

3089, 3064, 3031, 2953, 2913, 2876, 1740, 1678, 1655, 1607, 1587, 1456, 1413, 1380,

1312, 1240, 1165, 1097, 1008, 908, 863, 825, 735, 697, 666 cm^{-1}; **^{1}H NMR** (600 MHz,

CDCl$_3$) δ 9.69 (s, 1H), 7.38 – 7.27 (m, 35H), 5.99 (d, J = 3.6 Hz, 1H), 5.50 (d, J = 10.1

Hz, 1H), 5.36 (s, 1H), 5.31 (s, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.10 (d, J = 14.9 Hz, 3H),

4.91 – 4.87 (m, 2H), 4.86 – 4.77 (m, 4H), 4.70 (d, J = 11.7 Hz, 1H), 4.63 (d, J = 11.1 Hz,

1H), 4.61 (d, J = 11.7 Hz, 1H), 4.56 (d, J = 7.4 Hz, 1H), 4.51 (d, J = 11.6 Hz, 1H), 4.46 –

4.40 (m, 4H), 4.33 (s, 1H), 4.18 (d, J = 7.3 Hz, 1H), 4.15 – 4.09 (m, 3H), 3.95 – 3.89 (m,

3H), 3.88 – 3.73 (m, 7H), 3.65 – 3.52 (m, 8H), 3.51 – 3.45 (m, 3H), 3.40 (d, J = 9.4 Hz,

1H), 3.37 – 3.33 (m, 2H), 3.30 (t, J = 7.6 Hz, 1H), 3.25 (t, J = 8.0 Hz, 1H), 3.19 (dd, J =

13.6, 6.8 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.47 (dd, J = 13.6, 2.4 Hz, 1H), 2.34 (t, J =

7.6 Hz, 2H), 2.30 – 2.15 (m, 4H), 1.90 – 1.57 (m, 13H), 1.52 – 1.47 (m, 2H), 1.46 (s, 3H),

1.33 (s, 4H), 1.30 – 1.20 (m, 24H), 1.13 – 1.02 (m, 6H), 1.02 – 0.90 (m, 95H), 0.89 (s,

3H), 0.87 (s, 3H), 0.85 (s, 6H), 0.80 – 0.51 (m, 66H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ

212.23, 173.69, 173.41, 168.36, 151.40, 142.24, 138.78, 138.40, 138.24, 138.05, 137.56,

136.11, 135.24, 128.52, 128.45, 128.42, 128.39, 128.25, 128.19, 128.15, 128.14, 128.12,

128.00, 127.90, 127.80, 127.73, 127.63, 127.47, 124.43, 109.00, 103.51, 102.53, 101.36,

100.82, 98.90, 91.22, 86.13, 83.77, 81.49, 78.78, 78.71, 78.14, 77.90, 76.44, 76.26, 76.10,

75.92, 75.82, 75.50, 75.06, 74.48, 73.83, 73.75, 73.09, 72.59, 72.48, 71.68, 71.40, 71.10,

70.85, 69.88, 68.76, 66.84, 66.05, 65.40, 65.32, 63.62, 60.29, 56.00, 53.76, 49.12, 46.88,

46.68, 45.97, 44.85, 41.05, 39.64, 37.78, 36.96, 36.28, 35.93, 34.33, 33.25, 32.49, 32.14, 31.97, 30.63, 29.70, 29.40, 29.37, 29.31, 29.19, 29.12, 27.69, 26.57, 26.20, 25.87, 25.29, 24.94, 24.40, 23.38, 22.70, 20.15, 17.48, 16.63, 15.74, 14.14, 12.05, 7.56, 7.46, 7.25, 7.19, 7.13, 7.11, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.36, 5.33, 5.25, 5.22, 4.89, 4.41;

HRMS (ESI) *m/z*: Calcd for $C_{182}H_{294}N_2O_{35}NaSi_9$ [M+Na] 3342.9108, found 3342.9001.
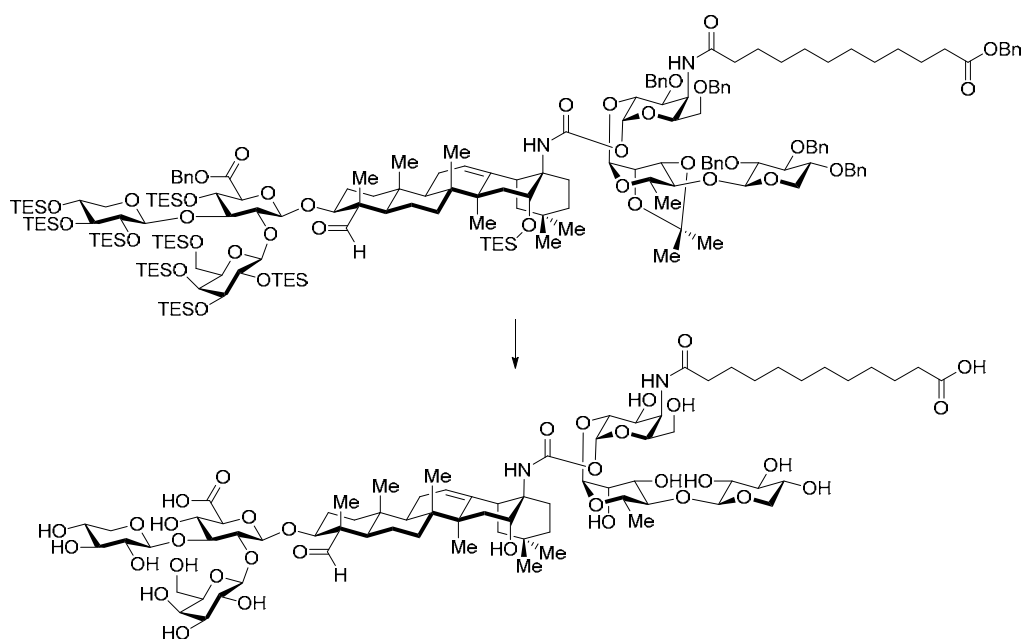
(101α) A solution of fully protected β-carbamate analogue **(94α)** (5 mg, 0.0015 mmol, 1.0 equiv) in tetrahydrofuran (1 mL) and ethanol (1 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (4 mg, 0.004 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (1.0 mL, TFA/H_2O 3:1). After vigorous

stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 15 · 51% acetonitrile (0.05% TFA) in over 18 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 17.2 min) was collected and lyophilized to dryness to afford SQS-0-5-8-5 (**101α**) (2.0 mg, 82 % yield) as a fluffy white solid.

¹H NMR (600 MHz, D₂O/CD₃CN) δ 9.90 (s, 1H), 6.45 (d, *J* = 3.7 Hz, 1H), 6.11 (s, 1H), 5.89 (s, 1H), 5.31 (s, 1H), 5.19 (d, *J* = 7.8 Hz, 1H), 5.07 (d, *J* = 7.8 Hz, 1H), 4.97 (d, *J* = 7.8 Hz, 1H), 4.93 (d, *J* = 7.8 Hz, 1H), 4.86 (s, 1H), 4.47 (t, *J* = 6.3 Hz, 1H), 4.46 − 4.36 (m, 3H), 4.35 − 4.20 (m, 5H), 4.18 − 4.11 (m, 2H), 4.11 − 3.95 (m, 5H), 3.93 − 3.82 (m, 4H), 3.77 − 3.66 (m, 3H), 2.98 (dd, *J* = 13.5, 1.7 Hz, 1H), 2.77 − 2.72 (m, 1H), 2.46 − 2.35 (m, 3H), 2.31 − 2.14 (m, 4H), 2.01 − 1.98 (m, 1H), 1.82 (s, 3H), 1.72 (d, *J* = 6.1 Hz, 3H), 1.63 (s, 2H), 1.60 − 1.53 (m, 1H), 1.48 (s, 3H), 1.37 (s, 3H), 1.36 (s, 3H).


137
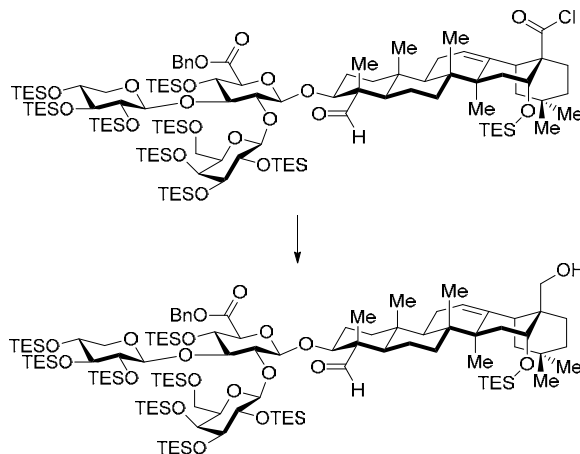
(70): Solid tetrabutylammonium borohydride (32 mg, 0.124 mmol, 2 equiv) was added to an ice-cooled solution of acyl chloride **65** (130 mg, 0.062 mmol, 1 equiv) in dichloromethane (4 mL) for 4 hr then diluted with a saturated solution of sodium bicarbonate (50 mL). Aqueous mixture was extracted with dichloromethane (3x25 mL), organic fractions combined, washed with brine, dried over sodium sulfate, filtered, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 30:1 to 10:1) to give neopentyl alcohol **70** as a white foam (99 mg, 77%).

TLC R_f 0.38 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3538 (OH st), 2952, 2877, 1754, 1722, 1459, 1413, 1377, 1239, 1171, 1103, 1005, 908, 863, 825, 774, 728, 695 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.60 (s, 1H), 7.31 – 7.17 (m, 5H), 5.17 (d, J = 12.4 Hz, 1H), 5.14 (t, J = 3.7 Hz, 1H), 4.97 (d, J = 12.4 Hz, 1H), 4.44 (d, J = 7.4 Hz, 1H), 4.31 (d, J = 7.2 Hz, 1H), 4.07 (d, J = 7.4 Hz, 1H), 3.96 (t, J = 3.3 Hz, 1H), 3.82 (s, 1H), 3.80 (d, J = 8.9 Hz, 1H), 3.77 – 3.66 (m, 4H), 3.63 (t, J = 9.2 Hz, 1H), 3.52 – 3.42 (m, 3H), 3.36 (ddd, J = 10.5, 8.4, 5.1 Hz, 1H), 3.28 (dd, J = 9.4, 2.5 Hz, 1H), 3.26 – 3.18 (m, 3H), 3.13 (t, J = 8.0 Hz, 1H), 3.01 (dt, J = 10.9, 5.4 Hz, 2H), 2.08 (t, J = 13.2 Hz, 1H), 1.87 (dd, J = 13.9, 4.3 Hz, 1H), 1.80 – 1.50 (m, 9H), 1.50 – 1.28 (m, 5H), 1.26 (s, 4H), 1.19 (s, 3H), 1.16 – 0.93 (m, 5H), 0.92 – 0.71 (m, 100H), 0.71 – 0.38 (m, 58H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.67, 168.37, 143.98, 135.27, 128.47, 128.45, 128.28, 128.14, 121.89, 103.62, 101.40, 100.84, 86.32, 78.81, 78.73, 76.45, 75.96, 75.82, 75.08, 74.40, 72.62, 72.53, 71.39, 71.31, 71.09, 66.84, 65.34, 60.26, 53.89, 49.42, 47.44, 46.13, 42.10, 41.58, 40.33, 39.90, 38.02, 36.03, 35.96, 33.34, 32.85, 32.19, 30.78, 29.60, 26.76, 25.39, 24.37, 23.39, 20.27, 16.83, 15.95, 12.24, 7.57, 7.50, 7.48, 7.26, 7.21, 7.17, 7.15, 7.08, 7.02, 7.00,

6.96, 6.86, 6.80, 5.93, 5.66, 5.46, 5.42, 5.38, 5.35, 5.32, 5.30, 5.27, 5.24, 5.21, 5.07, 4.43;

HRMS (ESI) *m/z*: Calcd for $C_{108}H_{206}O_{19}NaSi_9$ [M+Na]$^+$ 2082.2975, found 2082.2942.
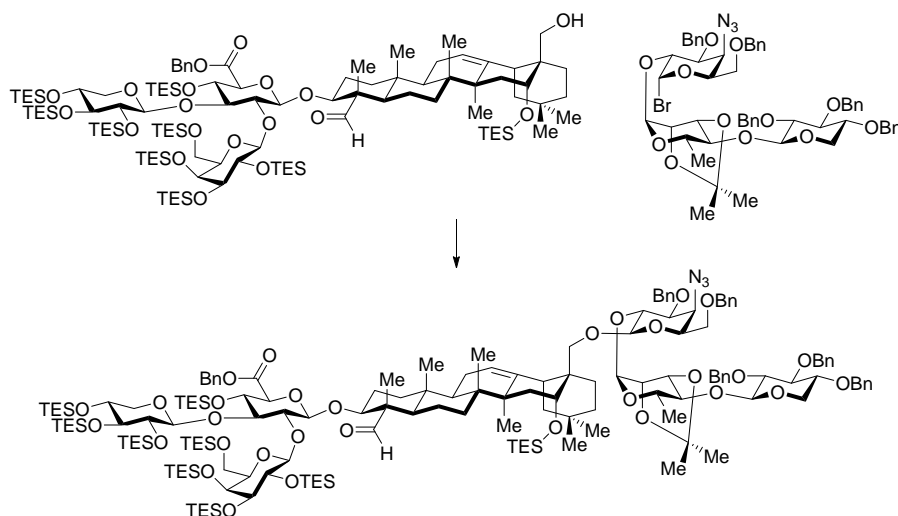
(77): To a suspension of primary alcohol acceptor **70** (58 mg, 0.0280mmol, 1.0 equiv), bromide donor **76** (29 mg, 0.0280 mmol, 1.0 equiv), 2,4,5-tritertbutylpyridine (20.8 mg, 0.084 mmol, 3.0 equiv), and ~25 mg 4 Å MS in 1 mL dichloromethane, cooled to –40 °C, was added solid AgOTf (15 mg, 0.058 mmol, 2.1 equiv). After 45 min, reaction was warmed to 0 °C, stirred for 15 min, then diluted with 5 mL dichloromethane. Crude suspension was sonicated for two min, filtered through a pad of celite, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 15:1 to 4:1) to give β-glycoside **77** (58 mg, 0.0192 mmol, 69% yield).

TLC R_f 0.45 (hexanes:ethyl acetate, 5:1); **FTIR** (NaCl film) 2953, 2911, 2876, 2106, 1753, 1725, 1456, 1413, 1379, 1240, 1170, 1097, 1006, 910, 863, 825, 800, 735 cm^{-1};

¹H-NMR (600 MHz, CDCl$_3$-*d*) δ 9.69 (s, 1H), 7.42 – 7.16 (m, 30H), 5.37 (s, 1H), 5.28

(d, J = 12.4 Hz, 1H), 5.15 (t, J = 3.6 Hz, 1H), 5.09 (d, J = 12.4 Hz, 1H), 4.89 – 4.80 (m, 4H), 4.72 – 4.66 (m, 2H), 4.64 – 4.60 (m, 2H), 4.57 – 4.51 (m, 4H), 4.42 (d, J = 7.3 Hz, 1H), 4.19 (d, J = 7.4 Hz, 1H), 4.14 – 4.11 (m, 2H), 4.09 (d, J = 7.6 Hz, 1H), 4.00 – 3.97 (m, 2H), 3.97 – 3.89 (m, 4H), 3.87 – 3.72 (m, 8H), 3.64 – 3.53 (m, 10H), 3.52 – 3.45 (m, 2H), 3.39 (dd, J = 9.5, 2.5 Hz, 1H), 3.37 – 3.27 (m, 4H), 3.27 – 3.16 (m, 4H), 3.13 (t, J = 10.9 Hz, 1H), 2.17 (t, J = 13.2 Hz, 1H), 1.98 (dd, J = 14.1, 4.5 Hz, 1H), 1.85 – 1.75 (m, 3H), 1.75 – 1.60 (m, 6H), 1.45 (s, 3H), 1.43 – 1.32 (m, 8H), 1.30 (s, 4H), 1.28 (s, 3H), 1.26 – 1.24 (m, 1H), 1.22 (d, J = 6.1 Hz, 3H), 1.19 – 1.03 (m, 4H), 1.02 – 0.88 (m, 102H), 0.88 – 0.81 (m, 11H), 0.81 – 0.52 (m, 66H); ^{13}**C-NMR** (151 MHz, CDCl$_3$) δ 212.35, 168.38, 144.17, 138.79, 138.60, 138.22, 137.52, 137.08, 135.24, 128.53, 128.52, 128.49, 128.45, 128.44, 128.41, 128.37, 128.32, 128.30, 128.27, 128.26, 128.24, 128.21, 128.14, 128.12, 128.08, 128.04, 128.01, 128.00, 127.99, 127.91, 127.90, 127.86, 127.82, 127.80, 127.76, 127.75, 127.72, 127.65, 127.51, 127.49, 121.68, 109.17, 103.48, 102.78, 102.07, 101.37, 100.83, 97.66, 86.06, 83.81, 82.06, 80.79, 78.78, 78.71, 78.25, 78.11, 78.01, 77.47, 76.42, 76.00, 75.81, 75.53, 75.49, 75.06, 74.69, 74.61, 74.36, 73.72, 73.18, 72.59, 72.51, 72.09, 71.38, 71.28, 71.06, 68.26, 66.83, 65.72, 65.33, 63.75, 60.25, 58.96, 53.90, 49.29, 47.41, 46.05, 41.98, 41.37, 39.90, 39.50, 37.96, 37.67, 35.99, 33.03, 32.85, 32.06, 31.59, 30.86, 30.79, 30.77, 30.31, 30.27, 29.70, 29.05, 27.78, 27.73, 26.63, 26.52, 26.41, 26.33, 26.27, 25.32, 25.27, 24.48, 23.36, 22.66, 20.21, 18.16, 18.13, 17.45, 16.85, 15.94, 14.14, 12.17, 11.45, 7.56, 7.48, 7.46, 7.25, 7.22, 7.20, 7.17, 7.13, 7.07, 7.00, 6.98, 6.95, 6.94, 6.91, 6.85, 6.79, 6.78, 5.91, 5.64, 5.44, 5.40, 5.36, 5.34, 5.30, 5.29, 5.27, 5.25, 5.23, 4.90, 4.41, 4.40; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{267}$N$_3$O$_{31}$Si$_9$Na [M+Na] 3037.7230, found 3037.7188.
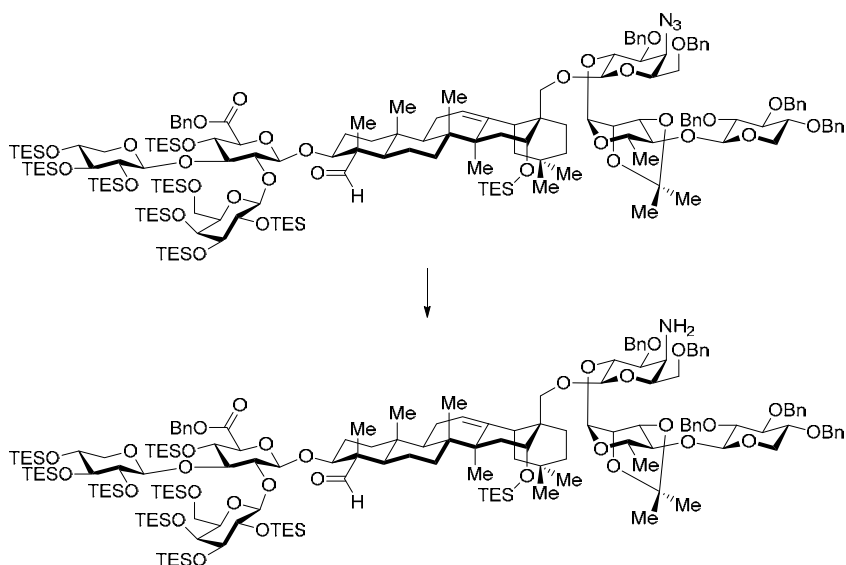
(88): Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **77** (45 mg, 0.015 mmol, 1 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, septum sealed with Teflon tape and parafilm, then warmed to RT and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen. The resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **88** (40 mg, 88 % yield).

TLC R_f 0.41 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl film) 3608, 3583, 3028, 2954, 2910, 2876, 1753, 1725, 1631, 1497, 1454, 1413, 1380, 1240, 1168, 1095, 1006, 900, 862, 825, 799, 730, 695, 665 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$-d) δ 9.70 (s, 1H), 7.38 − 7.22 (m, 31H), 5.40 (s, 1H), 5.29 (d, J = 12.3 Hz, 1H), 5.17 (d, J = 3.8 Hz, 1H), 5.10 (d, J = 12.3 Hz, 1H), 4.91 (d, J = 7.6 Hz, 1H), 4.89 − 4.81 (m, 3H), 4.71 (d, J = 11.7 Hz, 1H), 4.65 − 4.62 (m, 2H), 4.61 (d, J = 4.9 Hz, 1H), 4.58 − 4.55 (m, 3H), 4.49 (d, J = 11.5 Hz, 1H), 4.43 (d, J = 7.3 Hz, 1H), 4.20 (d, J = 7.3 Hz, 1H), 4.19 −

4.13 (m, 3H), 4.03 – 3.98 (m, 1H), 3.98 – 3.90 (m, 3H), 3.91 – 3.69 (m, 7H), 3.68 – 3.53 (m, 9H), 3.48 (ddd, J = 10.5, 8.5, 5.1 Hz, 1H), 3.42 – 3.19 (m, 10H), 3.14 (t, J = 10.9 Hz, 1H), 2.19 (t, J = 13.2 Hz, 1H), 2.02 (dd, J = 14.0, 4.2 Hz, 1H), 1.86 – 1.50 (m, 13H), 1.47 (s, 3H), 1.35 (s, 6H), 1.31 (s, 3H), 1.29 (s, 3H), 1.23 (d, J = 6.2 Hz, 3H), 1.20 – 1.12 (m, 3H), 1.11 – 1.05 (m, 2H), 1.04 – 0.90 (m, 89H), 0.89 – 0.82 (m, 10H), 0.81 – 0.55 (m, 56H). ^{13}**C-NMR** (151 MHz, CDCl$_3$) 212.40, 168.40, 144.16, 138.82, 138.60, 138.25, 138.00, 137.61, 135.27, 128.51, 128.49, 128.47, 128.44, 128.31, 128.29, 128.25, 128.15, 128.09, 127.92, 127.90, 127.81, 127.79, 127.77, 127.76, 127.55, 127.52, 121.77, 109.16, 103.50, 102.96, 102.10, 101.40, 100.86, 97.56, 86.07, 83.84, 82.12, 81.65, 78.82, 78.74, 78.27, 78.19, 78.04, 76.45, 76.04, 75.84, 75.67, 75.56, 75.09, 74.77, 74.53, 73.61, 73.28, 73.22, 72.62, 72.54, 71.42, 71.20, 71.09, 69.16, 66.86, 65.56, 65.36, 63.79, 60.29, 53.94, 49.35, 48.97, 47.44, 46.09, 42.01, 41.42, 39.96, 39.59, 38.01, 36.08, 36.02, 33.08, 32.89, 32.11, 30.82, 30.32, 27.80, 26.67, 26.36, 25.35, 24.53, 23.39, 20.24, 18.19, 16.96, 15.97, 12.19, 7.59, 7.49, 7.28, 7.26, 7.24, 7.20, 7.16, 7.03, 7.01, 6.88, 6.82, 5.94, 5.67, 5.47, 5.39, 5.37, 5.31, 5.28, 5.26, 4.95, 4.45; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{270}$NO$_{31}$Si$_9$ [M+H]$^+$ 2989.7505, found 2989.7542.
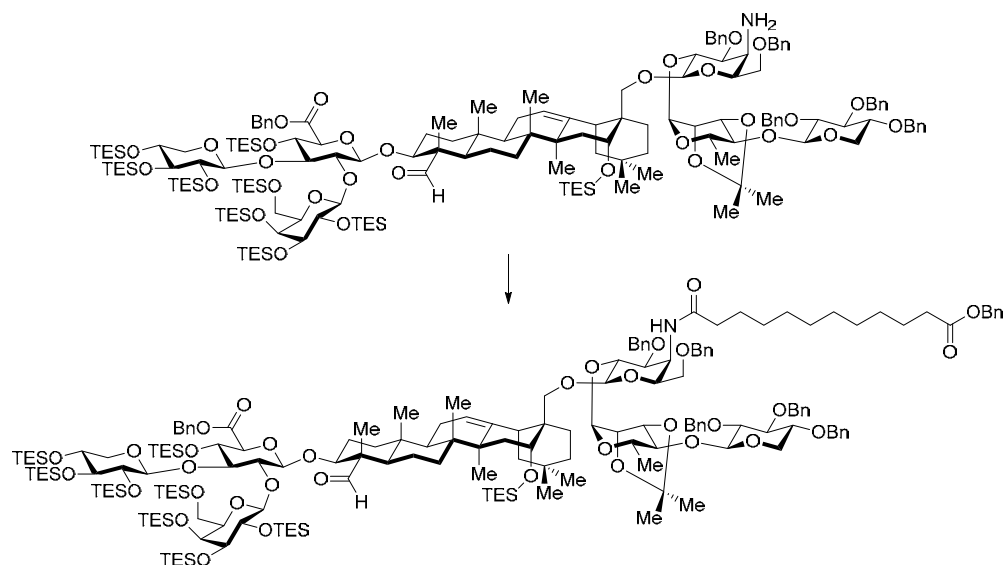
(95): Isobutyl chloroformate (7.0 µL, 0.053 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **106** (26 mg, 0.081 mmol, 6 equiv) and triethylamine (37 µL, 0.268 mmol, 20 equiv) in tetrahydrofuran (2 mL) and stirred for 2 hours, then transferred via cannula to an ice-cooled solution of amine **88** (40 mg, 0.0134 mmol, 1 equiv) in tetrahydrofuran (1.5 mL). After 4 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give glycosyl ether **95** (25 mg, 57 % yield) as a colorless film.

TLC R_f 0.24 (hexanes:ethyl acetate, 4:1); **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.70 (s, 1H), 7.40 – 7.16 (m, 35H), 5.52 (d, J = 10.1 Hz, 1H), 5.38 (s, 1H), 5.28 (d, J = 12.3 Hz, 1H), 5.15 (t, J = 3.6 Hz, 1H), 5.13 – 5.07 (m, 3H), 4.92 – 4.78 (m, 5H), 4.76 (d, J = 11.1 Hz, 1H), 4.71 (d, J = 11.6 Hz, 1H), 4.63 (t, J = 10.9 Hz, 2H), 4.56 (d, J = 7.4 Hz, 1H), 4.55 – 4.47 (m, 2H), 4.43 (d, J = 7.2 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 4.21 – 4.14 (m, 3H),

4.12 (d, J = 5.9 Hz, 1H), 3.99 (s, 1H), 3.97 – 3.90 (m, 3H), 3.89 – 3.71 (m, 6H), 3.65 –

3.42 (m, 13H), 3.40 (dd, J = 9.4, 2.5 Hz, 1H), 3.38 – 3.17 (m, 7H), 3.13 (t, J = 10.9 Hz,

1H), 2.35 (t, J = 7.5 Hz, 2H), 2.24 – 2.14 (m, 3H), 2.02 (dd, J = 14.0, 4.3 Hz, 1H), 1.86 –

1.75 (m, 3H), 1.75 – 1.49 (m, 15H), 1.44 – 1.05 (m, 33H), 1.04 – 0.82 (m, 104H), 0.82 –

0.50 (m, 60H); ^{13}**C-NMR** (151 MHz, CDCl$_3$) δ 212.46, 173.70, 173.26, 168.40, 144.02,

138.82, 138.57, 138.24, 137.73, 137.59, 136.13, 135.27, 128.55, 128.47, 128.43, 128.35,

128.31, 128.29, 128.28, 128.21, 128.18, 128.17, 128.14, 128.09, 127.91, 127.86, 127.83,

127.79, 127.77, 127.74, 127.69, 127.61, 127.52, 121.90, 109.20, 103.53, 103.16, 102.13,

101.40, 100.86, 97.60, 86.15, 83.84, 82.15, 79.25, 78.82, 78.73, 78.30, 78.15, 78.13,

78.03, 76.45, 76.01, 75.85, 75.83, 75.76, 75.57, 75.12, 75.09, 74.82, 74.51, 73.72, 73.23,

72.70, 72.62, 72.54, 71.41, 71.09, 71.00, 68.86, 66.86, 66.08, 65.74, 65.36, 63.80, 60.28,

53.90, 49.32, 47.35, 46.18, 46.06, 41.92, 41.41, 39.94, 39.66, 38.00, 36.98, 36.08, 36.00,

34.35, 33.08, 32.86, 32.09, 30.81, 30.36, 29.73, 29.46, 29.41, 29.38, 29.28, 29.25, 29.16,

27.73, 26.66, 26.23, 25.90, 25.34, 24.98, 24.47, 23.39, 20.23, 18.07, 17.00, 15.96, 12.20,

7.59, 7.51, 7.49, 7.32, 7.28, 7.25, 7.23, 7.20, 7.16, 7.09, 7.05, 7.03, 7.01, 6.97, 6.92, 6.88,

6.82, 5.94, 5.67, 5.59, 5.56, 5.46, 5.43, 5.39, 5.37, 5.33, 5.30, 5.28, 5.26, 5.19, 5.17, 5.14,

5.08, 4.99, 4.95, 4.44; **HRMS** (ESI) *m/z*: Calcd for C$_{182}$H$_{295}$N$_3$O$_{34}$Si$_9$Na [M+Na]$^+$

3133.9207, found 3133.9258.
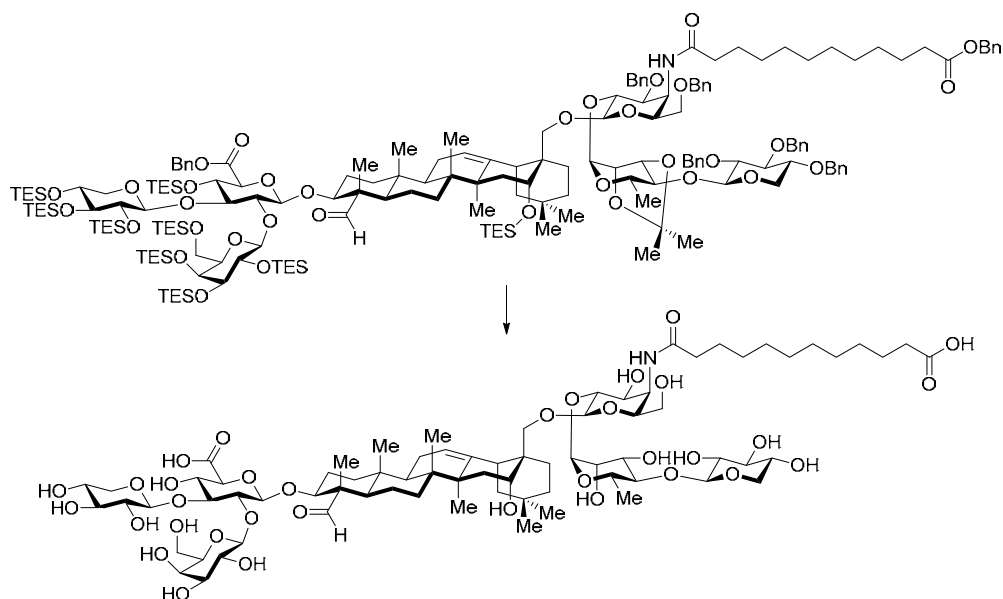
(102): A solution of fully protected ether analogue (**95**) (25 mg, 0.008 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (25 mg, 0.023 mmol, 3 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (3.0 mL, TFA/H$_2$O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (4:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 20 · 66% acetonitrile (0.05% TFA) in water (0.05% TFA) over 16 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 12.55 min) was collected and lyophilized to dryness to afford SQS-0-12-5-5 (**102**) (7.5 mg, 62 % yield) as a white solid.

¹H-NMR (600 MHz, D₂O/CD₃CN, 1:1) δ 9.97 (s, 1H), 7.73 (d, *J* = 9.7 Hz, 1H), 5.82 (t, *J* = 3.6 Hz, 1H), 5.74 (d, *J* = 1.9 Hz, 1H), 5.26 (d, *J* = 7.8 Hz, 1H), 5.13 (d, *J* = 7.8 Hz, 1H), 5.08 (d, *J* = 7.8 Hz, 1H), 5.01 (d, *J* = 7.8 Hz, 1H), 4.78 (s, 1H), 4.71 (d, *J* = 7.6 Hz, 1H), 4.61 (d, *J* = 4.0 Hz, 1H), 4.51 – 4.43 (m, 4H), 4.43 – 4.33 (m, 5H), 4.30 (t, *J* = 8.9 Hz, 2H), 4.25 – 4.18 (m, 2H), 4.17 – 4.00 (m, 9H), 3.93 (p, *J* = 8.9, 8.5 Hz, 6H), 3.86 – 3.77 (m, 4H), 3.78 – 3.72 (m, 1H), 2.90-2.77 (m, *J* = 7.5 Hz, 5H), 2.75 – 2.65 (m, 2H), 2.48 – 2.41 (m, 3H), 2.41 – 2.01 (m, 17H), 1.90 (s, 3H), 1.83 (d, *J* = 6.3 Hz, 5H), 1.74 (d, *J* = 12.2 Hz, 1H), 1.69 (s, 3H), 1.64 (t, *J* = 12.9 Hz, 1H), 1.55 (s, 3H), 1.47 (s, 3H), 1.46 (s, 3H), 2.83 – 2.77 (m, 1H), 1.43 (s, 3H); **HRMS** (ESI) *m/z*: Calcd for C₇₆H₁₂₃NO₃₄Na [M+Na]+ 1616.7824, found 1616.7848
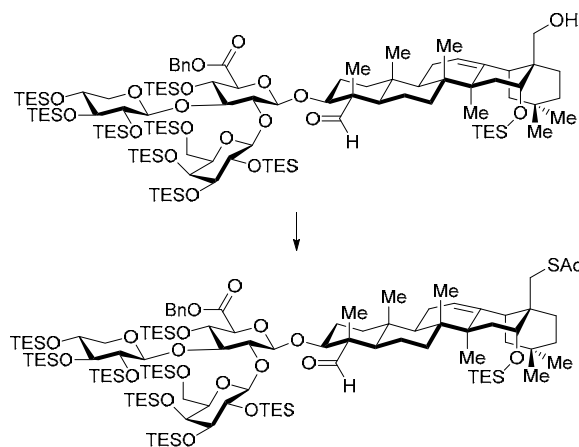

(71): Triflic anhydride (12.6 µL, 0.075 mmol, 1.5 equiv) was added to an ice-cooled solution of neopentyl alcohol **70** (103 mg, 0.05 mmol, 1 equiv) and pyridine (80 µL, 1.0 mmol, 20 equiv) and dichloromethane (4 mL). After 15 min, dichloromethane was removed with a stream of argon. Residual volatiles were removed under reduced pressure. Resulting oil was taken up in tetrahydrofuran (2 mL), cooled to 0 °C, then

treated with 4 Å MS (~100 mg), and dimethylformamide (2 mL). Suspension was treated

with potassium thioacetate (57 mg, 0.5 mmol, 10 equiv). After 2.5 hr, suspension was

decanted into a saturated solution of sodium bicarbonate and extracted with ethyl acetate

(3 × 25 mL). Combined organics were washed with brine, dried over sodium sulfate,

concentrated and purified with silica gel chromatography (hexanes: ethyl acetate, 50:1 to

25:1) to give thioaceate **71** (98 mg, 92%).

TLC R_f 0.60 (10:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 2953, 2911, 2876, 1754,

1723, 1700, 1696, 1653, 1635, 1576, 1560, 1539, 1457, 1414, 1375, 1239, 1171, 1103,

1005, 970, 898, 864, 826, 799, 736, 695, 668, 628 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ

9.65 (s, 1H), 7.31 – 7.22 (m, 5H), 5.25 – 5.19 (m, 2H), 5.02 (d, J = 12.4 Hz, 1H), 4.48 (d,

J = 7.4 Hz, 1H), 4.36 (d, J = 7.3 Hz, 1H), 4.11 (d, J = 7.4 Hz, 1H), 3.88 – 3.81 (m, 3H),

3.81 – 3.70 (m, 4H), 3.68 (t, J = 9.2 Hz, 1H), 3.56 – 3.47 (m, 3H), 3.41 (ddd, J = 10.5,

8.4, 5.1 Hz, 1H), 3.32 (dd, J = 9.4, 2.5 Hz, 1H), 3.30 – 3.24 (m, 2H), 3.18 (dd, J = 8.7,

7.4 Hz, 1H), 3.06 (t, J = 11.0 Hz, 1H), 2.86 (d, J = 13.4 Hz, 1H), 2.57 (d, J = 13.5 Hz,

1H), 2.25 (s, 3H), 2.16 (dd, J = 13.9, 12.4 Hz, 1H), 2.07 (dd, J = 14.0, 4.0 Hz, 1H), 1.88 –

1.51 (m, 10H), 1.49 – 1.44 (m, 2H), 1.43 – 1.30 (m, 3H), 1.29 (s, 3H), 1.24 (s, 3H), 1.16

– 1.10 (m, 2H), 1.07 – 0.99 (m, 2H), 0.97 – 0.81 (m, 93H), 0.79 (s, 3H), 0.75 – 0.47 (m,

56H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.83, 195.72, 168.36, 143.29, 135.26, 128.48,

128.46, 128.30, 128.15, 128.11, 122.68, 103.65, 101.42, 100.84, 86.43, 78.82, 78.73,

76.45, 76.30, 75.95, 75.83, 75.81, 75.09, 72.62, 72.53, 71.38, 71.08, 66.85, 65.34, 60.22,

53.89, 49.43, 47.57, 46.13, 45.72, 41.44, 41.02, 39.89, 38.86, 38.02, 36.34, 36.03, 33.41,

32.80, 32.13, 31.71, 30.83, 30.81, 26.82, 25.40, 24.29, 23.48, 20.24, 16.73, 15.94, 12.30,

7.58, 7.48, 7.27, 7.18, 7.17, 7.15, 7.00, 6.87, 6.81, 6.80, 5.93, 5.66, 5.46, 5.38, 5.36, 5.29, 5.27, 5.24, 5.01, 4.43; **HRMS** (ESI) *m/z*: Calcd for $C_{110}H_{208}O_{19}SSi_9Na$ [M+Na]+ 2140.2883, found 2140.2852.
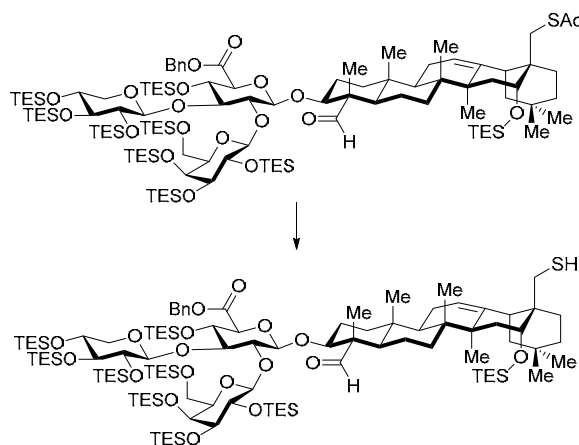
(72): Hydrazine (10 µL, 0.323 mmol, 7 equiv) was added to a solution of thioacetate **71** (98 mg, 0.046 mmol, 1 equiv) and dithiothreitol (21 mg, 0.139 mmol, 3 equiv) in tetrahydrofuran (4 mL, 1:1) for 6 hr, concentrated and purified with silica gel chromatography (hexanes: ethyl acetate) to give thiol **72** as a colorless film (86 mg, 90%).

TLC R_f 0.71 (10:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 2953, 2911, 2877, 1756, 1726, 1653, 1458, 1414, 1375, 1240, 1172, 1104, 1006, 971, 899, 865, 827, 801, 739, 695, 679, 668; **^{1}H-NMR** (600 MHz, CDCl$_3$) δ 9.72 (s, 1H), 7.34 (m, 5H), 5.29 – 5.25 (m, 2H), 5.09 (d, *J* = 12.4 Hz, 1H), 4.56 (d, *J* = 7.5 Hz, 1H), 4.43 (d, *J* = 7.3 Hz, 1H), 4.18 (d, *J* = 7.4 Hz, 1H), 4.14 – 4.09 (m, 1H), 3.94 – 3.89 (m, 2H), 3.88 – 3.77 (m, 4H), 3.75 (t, *J* = 9.3 Hz, 1H), 3.63 – 3.54 (m, 3H), 3.51 – 3.45 (m, 1H), 3.39 (dd, *J* = 9.3, 2.5 Hz, 1H), 3.37 – 3.32 (m, 2H), 3.25 (t, *J* = 8.0 Hz, 1H), 3.13 (t, *J* = 10.9 Hz, 1H), 2.38 – 2.31 (m,

1H), 2.31 – 2.22 (m, 2H), 2.15 (dd, J = 14.0, 4.1 Hz, 1H), 1.87 (dt, J = 11.1, 4.1 Hz, 2H), 1.82 – 1.73 (m, 2H), 1.72 – 1.38 (m, 10H), 1.37 (s, 3H), 1.31 (s, 2H), 1.25 (s, 1H), 1.23 – 1.15 (m, 2H), 1.16 – 1.05 (m, 3H), 1.04 – 0.89 (m, 92H), 0.87 (s, 3H), 0.87 (s, 3H), 0.81 – 0.55 (m, 57H); ^{13}**C-NMR** (151 MHz, CDCl$_3$) δ 212.71, 168.34, 143.60, 135.25, 128.50, 128.46, 128.44, 128.27, 128.15, 128.13, 128.09, 122.34, 103.63, 101.40, 100.83, 78.80, 78.72, 76.44, 75.94, 75.81, 75.71, 75.08, 72.61, 72.51, 71.38, 71.08, 66.83, 65.33, 60.23, 41.39, 39.82, 38.41, 37.99, 36.71, 36.43, 36.42, 36.01, 33.12, 32.84, 32.09, 30.96, 30.84, 26.76, 25.38, 24.23, 23.43, 20.23, 16.71, 15.92, 12.25, 7.57, 7.49, 7.47, 7.25, 7.19, 7.15, 7.14, 7.01, 6.99, 6.85, 6.79, 6.78, 5.92, 5.65, 5.45, 5.41, 5.37, 5.35, 5.30, 5.28, 5.26, 5.23, 5.04, 4.42, 4.40; **HRMS** (ESI) *m/z*: Calcd for C$_{108}$H$_{206}$O$_{18}$NaSi$_9$S [M+Na]+ 2098.2746, found 2098.2778.
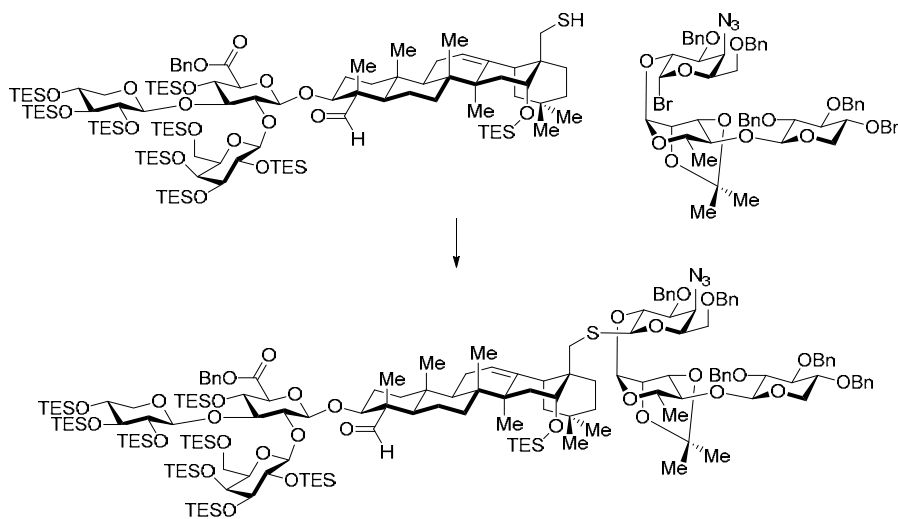

(78): A solution of bromide **76** (32 mg, 0.031 mmol, 1.5 equiv) in tetrahydrofuran (1.5 mL) was added dropwise to a suspension of thiol **72** (43 mg, 0.021 mmol, 1.0 equiv) and sodium hydride (60% dispersion in mineral oil, 2.5 mg, 0.062, 3.0 equiv) in tetrahydrofuran/dimethylformamide (2 mL, 1:1) over four min. After 20 min, a saturated

solution of ammonium chloride was added, diluted with water, and extracted with

(3 × 25 mL). Combined extracts were washed with brine, dried over sodium sulfate, and

concentrated. Before loading onto silica column, silver triflate (2 mg) was added to crude

solution in DCM to destroy excess glycosyl bromide. Mixture was purified with silica

gel chromatography (benzene:ethyl acetate, 1:0 to 30:1) to give glycosyl thioether **78** as a

colorless film (43 mg, 69% yield). Note: Extended reaction times gave lower yields, due

to formation of the trisaccharide glycal, through base-promoted elimination of the

thiolate.

TLC R_f 0.52 (benzene:ethyl acetate, 20:1); **FTIR** (NaCl film) 3032, 2953, 2912, 2876,

2107, 1752, 1724, 1701, 1497, 1457, 1413, 1380, 1240, 1169, 1094, 1006, 899, 864, 826,

736, 697, 668, 610 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$-d) δ 9.69 (s, 1H), 7.40 − 7.24 (m,

30H), 5.53 (s, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.19 (t, J = 3.7 Hz, 1H), 5.09 (d, J = 12.4

Hz, 1H), 4.91 (d, J = 5.3 Hz, 1H), 4.89 (d, J = 8.5 Hz, 1H), 4.87 − 4.79 (m, 2H), 4.72 (t, J

= 11.1 Hz, 2H), 4.64 (d, J = 10.8 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.57 − 4.52 (m, 4H),

4.42 (d, J = 7.2 Hz, 1H), 4.20 − 4.16 (m, 2H), 4.14 (d, J = 9.6 Hz, 1H), 4.10 (d, J = 3.4

Hz, 1H), 4.08 − 4.01 (m, 2H), 4.00 − 3.97 (m, 1H), 3.96 − 3.73 (m, 9H), 3.64 − 3.52 (m,

9H), 3.48 (ddd, J = 10.5, 8.4, 5.1 Hz, 1H), 3.39 (dd, J = 9.4, 2.5 Hz, 1H), 3.37 − 3.32 (m,

2H), 3.30 (dd, J = 8.9, 7.5 Hz, 1H), 3.25 (dd, J = 8.7, 7.4 Hz, 1H), 3.22 − 3.16 (m, 1H),

3.13 (t, J = 11.0 Hz, 1H), 2.49 − 2.37 (m, 2H), 2.21 − 2.05 (m, 2H), 1.84 − 1.73 (m, 3H),

1.55 − 1.46 (m, 7H), 1.39 − 1.22 (m, 16H), 1.11 − 0.83 (m, 99H), 0.83 − 0.50 (m, 63H);

^{13}C-NMR (151 MHz, CDCl$_3$) δ 212.40, 168.35, 143.40, 138.78, 138.60, 138.23, 137.48,

137.46, 136.69, 135.23, 128.55, 128.51, 128.46, 128.44, 128.41, 128.32, 128.29, 128.27,

128.26, 128.24, 128.20, 128.18, 128.13, 128.12, 128.09, 128.02, 127.93, 127.91, 127.87, 127.76, 127.75, 127.54, 127.51, 127.49, 122.30, 108.91, 103.49, 102.27, 102.24, 101.37, 100.83, 98.65, 86.14, 84.66, 83.87, 83.20, 82.12, 78.78, 78.70, 78.25, 78.07, 77.99, 77.98, 76.42, 76.32, 76.10, 75.96, 75.80, 75.58, 75.43, 75.05, 74.81, 74.03, 73.75, 73.73, 73.19, 72.59, 72.51, 71.58, 71.38, 71.05, 68.23, 66.84, 65.44, 65.32, 63.80, 60.25, 58.59, 53.87, 49.24, 47.56, 46.05, 45.40, 45.35, 42.86, 41.53, 41.27, 41.20, 39.78, 39.54, 39.02, 39.01, 38.70, 37.94, 36.40, 36.38, 35.98, 35.95, 34.00, 33.03, 32.77, 32.04, 31.93, 31.65, 30.74, 30.73, 30.38, 29.73, 29.70, 28.91, 27.77, 26.76, 26.73, 26.61, 26.40, 25.31, 24.47, 24.35, 24.34, 23.76, 23.72, 23.35, 22.99, 22.97, 22.70, 20.22, 17.56, 17.54, 16.81, 16.09, 15.88, 14.14, 14.07, 14.06, 13.15, 12.19, 10.98, 10.96, 7.56, 7.46, 7.25, 7.19, 7.16, 7.13, 6.98, 6.85, 6.79, 6.78, 5.91, 5.64, 5.43, 5.36, 5.33, 5.27, 5.25, 5.22, 4.94, 4.41; **HRMS** (ESI) *m/z*: Calcd for $C_{163}H_{267}N_3O_{30}NaSi_9S$ 3053.7001 $[M+Na]^+$, found 3053.7014.
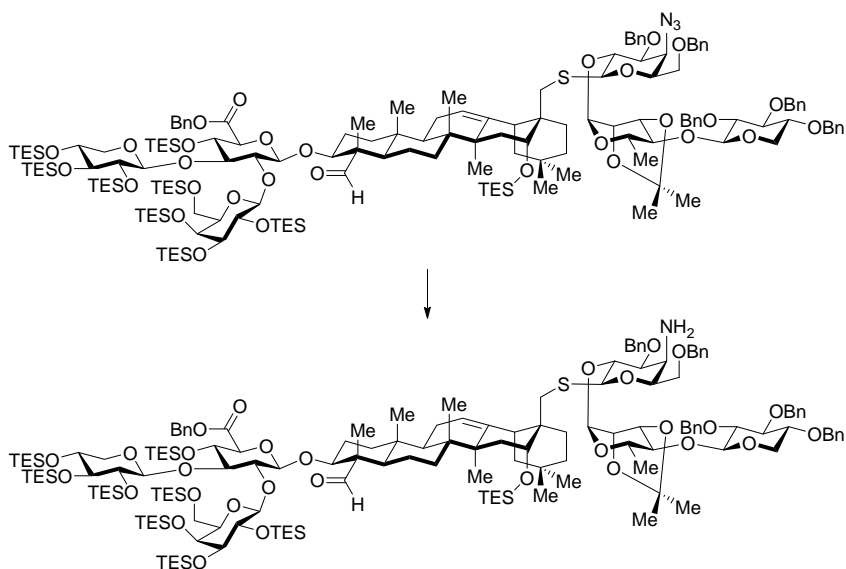
(89): Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **78** (41 mg, 0.014 mmol, 1.0 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min.

Vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to RT and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **89** (38 mg, 94 % yield).

TLC R_f 0.47 (hexanes:ethyl acetate, 2:1+0.5% triethylamine); **FTIR** (NaCl film) 3608, 2953, 2911, 2876, 1754, 1725, 1692, 1530, 1497, 1454, 1413, 1380, 1240, 1094, 1005, 825, 734, 696 cm^{-1}; **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.39, 168.36, 143.46, 138.81, 138.78, 138.57, 138.24, 137.97, 137.18, 135.23, 128.49, 128.46, 128.44, 128.42, 128.41, 128.37, 128.33, 128.28, 128.25, 128.13, 128.09, 128.00, 127.96, 127.93, 127.91, 127.87, 127.85, 127.83, 127.78, 127.75, 127.70, 127.66, 127.57, 127.52, 127.50, 122.28, 108.89, 103.50, 102.32, 102.29, 101.36, 100.83, 98.44, 86.11, 84.76, 83.97, 83.89, 82.18, 78.78, 78.71, 78.25, 78.10, 78.00, 77.98, 77.92, 76.54, 76.43, 76.40, 76.24, 75.97, 75.80, 75.60, 75.05, 74.86, 73.93, 73.65, 73.63, 73.20, 73.17, 72.59, 72.51, 71.39, 71.06, 70.64, 69.42, 66.84, 66.81, 65.34, 65.32, 63.81, 60.27, 53.87, 49.26, 49.02, 47.52, 46.03, 45.36, 42.86, 41.71, 41.31, 41.29, 41.22, 39.80, 39.56, 39.09, 39.03, 37.95, 36.61, 36.40, 36.37, 35.98, 35.96, 35.95, 33.07, 32.78, 32.01, 31.93, 31.71, 30.78, 30.75, 29.70, 29.66, 29.37, 28.40, 27.81, 27.78, 26.73, 26.71, 26.61, 26.42, 26.35, 25.31, 24.68, 24.40, 24.37, 23.34, 22.70, 22.39, 20.20, 17.56, 17.07, 16.92, 16.91, 16.09, 15.90, 15.88, 14.14, 13.13, 12.16, 7.56, 7.48, 7.46, 7.25, 7.21, 7.19, 7.17, 7.13, 7.00, 6.98, 6.95, 6.93, 6.88, 6.85, 6.79, 6.78, 5.91, 5.75, 5.64, 5.44, 5.40, 5.36, 5.33, 5.28, 5.27, 5.25, 5.23, 4.96, 4.94, 4.41, 4.40; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{270}$NO$_{30}$Si$_9$S 3005.7277 [M+H]$^+$, found 3005.7317.
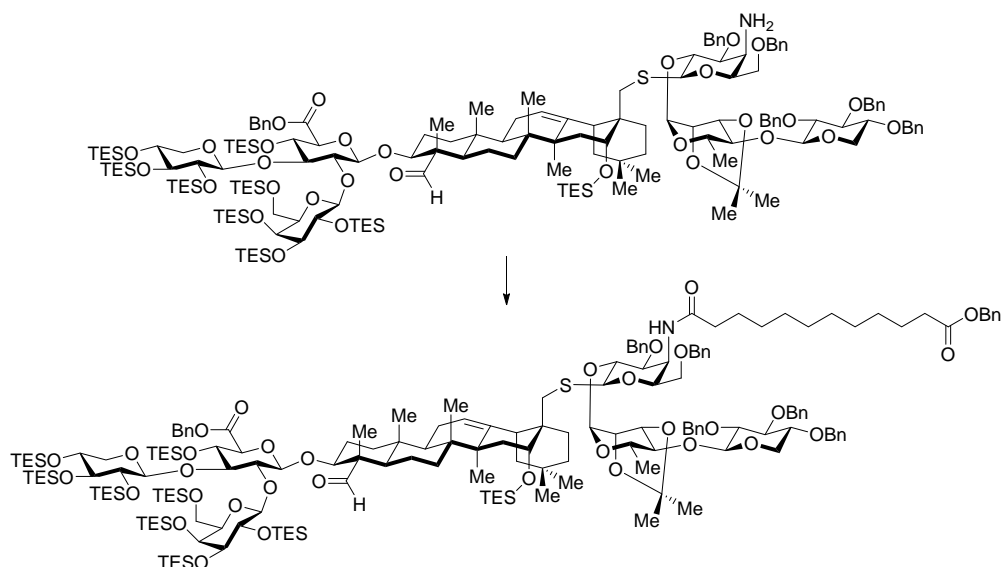
(96): Isobutyl chloroformate (6.4 µL, 0.049 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **106** (23.5 mg, 0.073 mmol, 6 equiv) and triethylamine (17 µL, 0.122 mmol, 10 equiv) in tetrahydrofuran (3 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine **89** (37 mg, 0.012 mmol, 1 equiv) in tetrahydrofuran (1 mL). After 16 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give glycosyl thioether **96** 27 mg, 67 % yield) as a colorless film

TLC R_f (hexanes:ethyl acetate, 2:1+0.5% triethylamine);**FTIR** (NaCl film) 2952, 2876, 1752, 1741, 1732, 1886, 1681, 1497, 1455, 1380, 1240, 1100, 1006, 826, 734, 697cm^{-1};
^{1}H-NMR (600 MHz, CDCl$_3$) δ 9.69 (s, 1H), 7.38 – 7.26 (m, 35H), 5.54 (s, 1H), 5.49 (d, J = 10.2 Hz, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.18 (s, 1H), 5.13 – 5.06 (m, 3H), 4.94 – 4.80 (m, 5H), 4.76 (d, J = 11.0 Hz, 1H), 4.71 (d, J = 11.7 Hz, 1H), 4.63 (dd, J = 13.9, 11.2 Hz,

2H), 4.56 (d, *J* = 7.4 Hz, 1H), 4.52 – 4.47 (m, 2H), 4.44 – 4.39 (m, 2H), 4.23 (d, *J* = 8.6 Hz, 1H), 4.20 – 4.16 (m, 2H), 4.11 (d, *J* = 5.7 Hz, 1H), 4.05 (dd, *J* = 10.1, 6.1 Hz, 1H), 3.96 – 3.90 (m, 4H), 3.88 – 3.72 (m, 5H), 3.67 – 3.53 (m, 9H), 3.52 – 3.45 (m, 3H), 3.40 (dd, *J* = 9.4, 2.5 Hz, 1H), 3.31 (dd, *J* = 9.0, 7.5 Hz, 3H), 3.25 (dd, *J* = 8.7, 7.4 Hz, 1H), 3.20 (dd, *J* = 12.1, 10.2 Hz, 1H), 3.13 (t, *J* = 10.9 Hz, 1H), 2.56 (d, *J* = 12.2 Hz, 1H), 2.39 (d, *J* = 12.2 Hz, 1H), 2.35 (t, *J* = 7.6 Hz, 2H), 2.15 (dtt, *J* = 18.0, 14.0, 8.5 Hz, 4H), 1.82 – 1.75 (m, 3H), 1.74 – 1.52 (m, 13H), 1.36 (s, 3H), 1.34 – 1.17 (m, 25H), 1.05 – 0.83 (m, 99H), 0.81 – 0.53 (m, 65H); ^{13}**C-NMR** (151 MHz, CDCl$_3$) δ 212.41, 173.72, 173.38, 168.38, 143.46, 138.79, 138.58, 138.56, 138.26, 137.76, 137.19, 137.17, 136.14, 135.26, 128.74, 128.55, 128.53, 128.48, 128.46, 128.45, 128.43, 128.35, 128.32, 128.31, 128.29, 128.25, 128.18, 128.16, 128.15, 128.11, 127.96, 127.94, 127.83, 127.81, 127.79, 127.77, 127.74, 127.73, 127.70, 127.68, 127.63, 127.56, 127.53, 122.34, 108.90, 103.53, 102.44, 101.39, 100.86, 98.66, 86.14, 85.30, 83.91, 82.26, 81.10, 78.81, 78.73, 78.25, 78.18, 78.01, 77.46, 76.45, 76.30, 75.98, 75.84, 75.82, 75.63, 75.09, 74.91, 74.76, 73.73, 73.71, 73.22, 72.62, 72.54, 71.41, 71.09, 70.71, 69.10, 66.86, 66.84, 66.07, 65.46, 65.35, 63.85, 60.29, 53.86, 49.25, 47.40, 46.47, 46.03, 45.22, 42.86, 42.37, 41.30, 41.22, 39.81, 39.58, 39.10, 37.96, 36.92, 36.40, 36.37, 36.09, 35.95, 34.68, 34.55, 34.36, 33.14, 32.78, 31.96, 31.83, 31.62, 30.78, 29.73, 29.51, 29.46, 29.42, 29.40, 29.37, 29.29, 29.24, 29.21, 29.16, 29.08, 27.83, 26.76, 26.64, 26.49, 26.45, 25.90, 25.34, 25.30, 24.99, 24.39, 23.37, 22.69, 20.73, 20.20, 18.79, 17.51, 17.48, 16.86, 16.12, 15.91, 14.17, 13.15, 12.20, 11.48, 7.59, 7.51, 7.49, 7.30, 7.28, 7.22, 7.20, 7.19, 7.16, 7.03, 7.01, 6.88, 6.82, 6.81, 5.94, 5.66, 5.46, 5.42, 5.39, 5.36, 5.34, 5.31, 5.30, 5.28, 5.25, 4.96, 4.44, 4.42; **HRMS** (ESI) *m/z*: Calcd for C$_{182}$H$_{295}$NO$_{33}$NaSi$_9$S 3329.8978 [M+Na]$^+$, found 3329.9033.
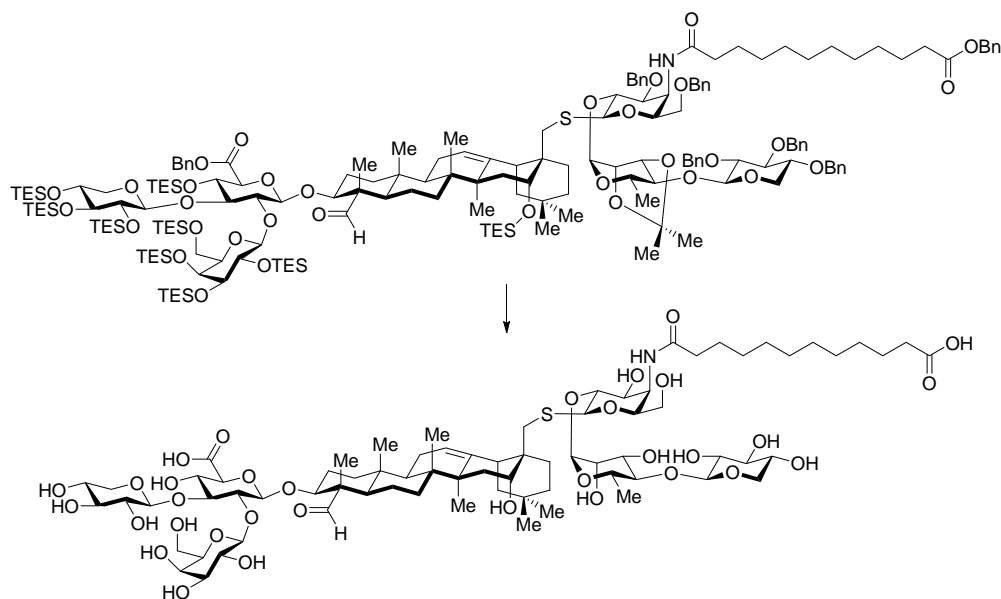
(103): A solution of fully protected thioether analogue (**96**) (26 mg, 0.008 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (33 mg, 0.031 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (4.0 mL, TFA/H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (4:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 20 · 75% acetonitrile (0.05% TFA) in water (0.05% TFA) over 19 min at a flow rate of 5 mL/min. The fraction

containing the major peak (tR = 12.60 min) was collected and lyophilized to dryness to afford SQS-0-14-5-5 (**103**) (5.8 mg, 46 % yield) as a white solid.

¹H-NMR (600 MHz, D$_2$O/CD$_3$CN, 1:1) δ 9.99 (s, 1H), 7.64 (d, J = 9.6 Hz, 1H), 5.89 (t, J = 3.7 Hz, 1H), 5.60 (d, J = 1.8 Hz, 1H), 5.28 (d, J = 7.8 Hz, 1H), 5.15 (d, J = 7.8 Hz, 1H), 5.08 (d, J = 7.8 Hz, 1H), 5.02 (d, J = 7.8 Hz, 1H), 4.80 (d, J = 9.7 Hz, 1H), 4.72 (dq, J = 9.7, 6.2 Hz, 1H), 4.58 – 4.54 (m, 1H), 4.52 – 4.50 (m, 1H), 4.50 – 4.45 (m, 1H), 4.42 – 4.29 (m, 7H), 4.25 – 4.21 (m, 2H), 4.16 – 4.05 (m, 8H), 4.05 – 4.00 (m, 1H), 3.98 – 3.88 (m, 4H), 3.85 – 3.79 (m, 3H), 3.79 – 3.74 (m, 1H), 3.20 (d, J = 11.8 Hz, 1H), 3.13 (d, J = 11.8 Hz, 1H), 2.88 (t, J = 7.5 Hz, 2H), 2.86 – 2.80 (m, 2H), 2.80 – 2.75 (m, 2H), 2.71 – 2.68 (m, 0H), 2.52 – 2.41 (m, 5H), 2.35 – 2.23 (m, 5H), 2.18 – 2.06 (m, 7H), 1.94 (s, 3H), 1.84 (d, J = 6.1 Hz, 3H), 1.77 – 1.73 (m, 1H), 1.72 (s, 3H), 1.57 (s, 3H), 1.50 (s, 3H), 1.48 (s, 3H), 1.45 (s, 3H); **HRMS** (ESI) m/z: Calcd for C$_{76}$H$_{123}$NO$_{33}$NaS 1632.7596 [M+Na]$^+$, found 1632.7648.
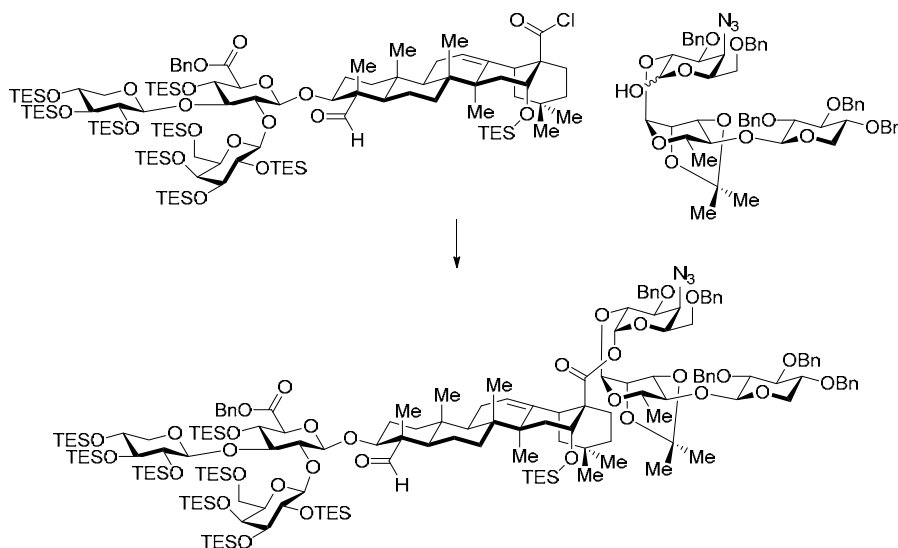
(83): Sodium hydride was added to a solution of hemiacetal **73** (28 mg, 0.029 mmol, 1.5 equiv) in tetrahydrofuran/dimethylformamide (2.0 mL, 1:1) at -20 C. After 5 min, a solution acyl chloride **65** (40 mg, 0.019 mmol, 1.0 equiv) in tetrahydrofuran (1.5 mL) was added over 1 min. After 10 min, concentrated aqueous ammonium chloride (0.5 mL) was added. Suspension was diluted with water and extracted with benzene (3 × 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes/ethyl acetate, 20:1 to 4:1) furnishing separable esters (α-35 mg (**83α**), β-6 mg **83β**, 70% total yield).

TLC R_f 0.55 (benzene:ethyl acetate, 20:1); **FTIR** (NaCl film) 2953, 2876, 2106, 1752, 1736, 1455, 1380, 1240, 1098, 1005, 825, 732, 696 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$-*d*) δ 9.74 (s, 1H), 7.47 – 7.26 (m, 30H), 6.12 (d, *J* = 3.7 Hz, 1H), 5.35 – 5.28 (m, 2H), 5.27 (t, *J* = 3.8 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 4.93 (d, *J* = 11.1 Hz, 1H), 4.89 (d, *J* = 7.4 Hz, 1H), 4.89 – 4.80 (m, 2H), 4.77 (d, *J* = 11.7 Hz, 1H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.70 (d, *J* = 11.1 Hz, 1H), 4.64 (dd, *J* = 11.7, 4.1 Hz, 2H), 4.59 (d, *J* = 7.5 Hz, 1H), 4.57 – 4.49

(m, 3H), 4.46 (d, $J = 7.2$ Hz, 1H), 4.23 (dd, $J = 9.9, 3.8$ Hz, 1H), 4.20 (d, $J = 7.4$ Hz, 1H),

4.17 (dd, $J = 3.5, 1.6$ Hz, 1H), 4.13 (dd, $J = 7.1, 5.6$ Hz, 1H), 4.05 (d, $J = 5.6$ Hz, 1H),

3.99 – 3.79 (m, 10H), 3.78 (t, $J = 9.2$ Hz, 1H), 3.68 – 3.54 (m, 9H), 3.55 – 3.45 (m, 2H),

3.42 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.41 – 3.33 (m, 2H), 3.35 – 3.29 (m, 1H), 3.28 (t, $J = 8.0$

Hz, 1H), 3.26 – 3.19 (m, 1H), 3.16 (t, $J = 11.0$ Hz, 1H), 2.94 (dd, $J = 14.4, 4.4$ Hz, 1H),

2.15 (t, $J = 13.6$ Hz, 1H), 1.86 – 1.75 (m, 4H), 1.77 – 1.55 (m, 7H), 1.52 – 1.47 (m, 2H),

1.38 (s, 4H), 1.33 (s, 6H), 1.24 (d, $J = 5.6$ Hz, 3H), 1.14 – 0.86 (m, 101H), 0.87 – 0.56

(m, 78H); ^{13}**C-NMR** (151 MHz, CDCl$_3$) δ 212.86, 174.19, 168.33, 142.70, 138.80,

138.41, 138.25, 137.45, 137.28, 135.26, 128.56, 128.52, 128.50, 128.48, 128.44, 128.40,

128.31, 128.28, 128.26, 128.23, 128.16, 128.13, 128.00, 127.96, 127.94, 127.90, 127.81,

127.78, 127.58, 127.50, 122.32, 109.19, 103.63, 102.83, 101.41, 100.85, 99.33, 91.33,

86.48, 83.78, 81.31, 78.83, 78.73, 78.32, 78.21, 77.98, 77.68, 76.65, 76.46, 76.09, 75.93,

75.82, 75.56, 75.08, 74.95, 74.51, 73.62, 73.15, 72.92, 72.62, 72.54, 72.41, 71.40, 71.09,

70.05, 68.30, 66.87, 65.35, 65.08, 63.70, 60.24, 59.95, 53.85, 49.60, 49.37, 46.37, 46.05,

41.50, 40.22, 39.53, 37.86, 36.09, 35.08, 34.69, 34.59, 34.55, 32.48, 32.40, 31.41, 30.36,

29.09, 27.83, 26.37, 26.32, 25.34, 25.30, 24.22, 23.21, 20.73, 20.27, 17.37, 17.13, 15.78,

12.27, 11.48, 7.59, 7.49, 7.27, 7.24, 7.22, 7.16, 7.01, 6.88, 6.81, 5.94, 5.66, 5.46, 5.39,

5.36, 5.33, 5.28, 5.25, 5.21, 5.04, 4.43; **HRMS** (ESI) *m/z*: Calcd for C$_{163}$H$_{265}$N$_3$O$_{32}$NaSi$_9$

3051.7023 [M+Na], found 3051.7041.
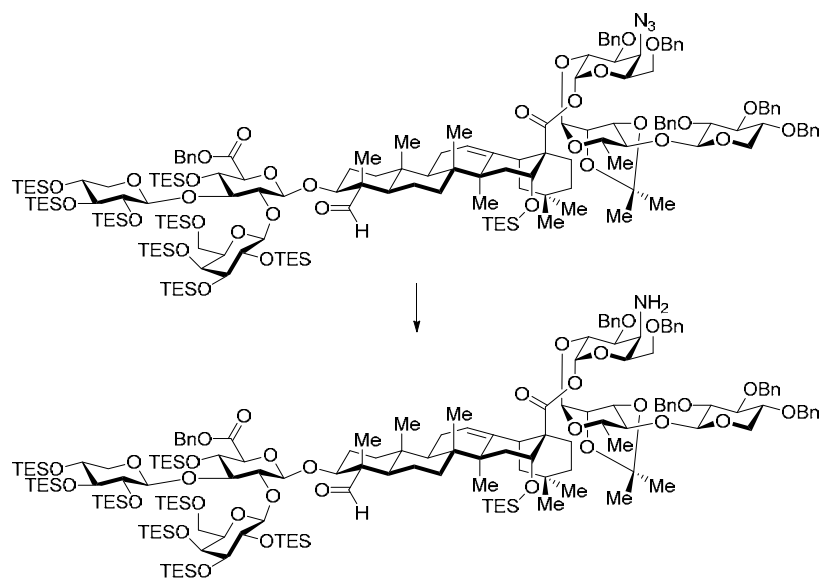
(90): Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **83** (44 mg, 0.015 mmol, 1.0 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to ambient temperature and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **90** (36 mg, 83 % yield) as a colorless oil.

TLC R_f 0.33 (hexanes:ethyl acetate, 2:1+0.5% triethylamine; **FTIR** (NaCl film) 2951, 2876, 1753, 1726 cm-1; **¹H-NMR** (600 MHz, CDCl$_3$-*d*) δ 9.70 (s, 1H), 7.42 − 7.25 (m, 33H), 6.15 (d, *J* = 3.8 Hz, 1H), 5.30 − 5.26 (m, 2H), 5.24 (t, *J* = 3.8 Hz, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 4.91 (d, *J* = 11.1 Hz, 1H), 4.87 (d, *J* = 7.4 Hz, 1H), 4.85 − 4.77 (m, 2H), 4.72 − 4.65 (m, 3H), 4.61 (d, *J* = 11.7 Hz, 1H), 4.58 − 4.52 (m, 4H), 4.47 (d, *J* = 12.1 Hz, 1H), 4.42 (d, *J* = 7.3 Hz, 1H), 4.19 − 4.13 (m, 2H), 4.11 (dd, *J* = 7.6, 5.5 Hz, 1H), 4.06 (d, *J* = 5.5 Hz, 1H), 3.98 − 3.89 (m, 4H), 3.88 − 3.71 (m, 6H), 3.67 − 3.51 (m, 10H), 3.51 −

3.45 (m, 2H), 3.41 – 3.28 (m, 5H), 3.25 (t, J = 8.0 Hz, 1H), 3.20 (ddd, J = 11.4, 7.6, 3.5 Hz, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.92 (dd, J = 14.4, 4.4 Hz, 1H), 2.12 (t, J = 13.6 Hz, 1H), 1.87 – 1.73 (m, 4H), 1.73 – 1.53 (m, 6H), 1.52 (s, 3H), 1.50 – 1.43 (m, 4H), 1.36 (s, 3H), 1.35 – 1.24 (m, 10H), 1.21 (d, J = 6.1 Hz, 3H), 1.08 – 0.89 (m, 92H), 0.88 (s, 3H), 0.80 (s, 3H), 0.78 (d, J = 7.8 Hz, 2H), 0.76 (s, 3H), 0.75 – 0.56 (m, 60H); [13]**C-NMR** (151 MHz, CDCl$_3$) δ 212.88, 174.27, 168.33, 142.87, 138.82, 138.40, 138.26, 137.75, 137.70, 135.26, 128.48, 128.47, 128.43, 128.39, 128.38, 128.36, 128.31, 128.27, 128.25, 128.18, 128.15, 127.95, 127.93, 127.81, 127.78, 127.77, 127.72, 127.68, 127.64, 127.59, 127.56, 127.49, 122.20, 109.15, 103.63, 102.88, 101.40, 100.85, 99.05, 91.41, 86.48, 83.76, 81.20, 78.83, 78.73, 78.33, 78.29, 78.13, 77.99, 76.46, 76.19, 75.94, 75.82, 75.54, 75.08, 74.99, 74.47, 73.41, 73.14, 72.62, 72.53, 71.97, 71.58, 71.46, 71.39, 71.09, 68.86, 66.86, 65.34, 64.96, 63.68, 60.23, 53.85, 49.59, 49.58, 49.43, 46.43, 46.07, 42.86, 41.48, 40.17, 39.99, 39.54, 37.86, 36.09, 35.12, 34.56, 32.41, 31.46, 30.37, 29.73, 27.87, 26.40, 26.37, 26.34, 25.35, 24.25, 23.21, 21.48, 20.28, 17.78, 17.77, 17.39, 17.06, 15.77, 14.20, 13.13, 12.28, 12.17, 7.59, 7.49, 7.27, 7.25, 7.16, 7.14, 7.01, 6.88, 6.81, 5.94, 5.66, 5.46, 5.39, 5.32, 5.28, 5.25, 5.04, 4.43; **HRMS** (ESI) m/z: Calcd for C$_{182}$H$_{293}$NO$_{35}$Si$_9$Na [M+Na]$^+$ 3327.8999, found 3327.9016.
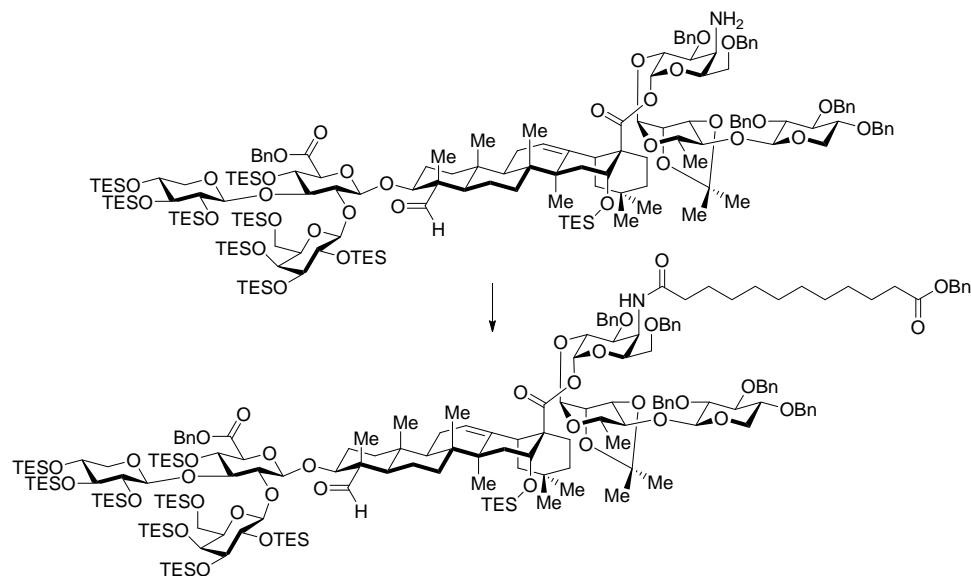
(97): Isobutyl chloroformate (6.3 µL, 0.048 mmol, 4 equiv) was added to an ice-cooled

solution of carboxylic acid **106** (23 mg, 0.072 mmol, 6 equiv) and triethylamine (17 µL,

0.122 mmol, 10 equiv) in tetrahydrofuran (3 mL) and stirred for 3 hours, then transferred

via cannula to an ice-cooled solution of amine **90** (36 mg, 0.012 mmol, 1 equiv) in

tetrahydrofuran (1 mL).   After 16 hr, suspension was diluted with saturated sodium

bicarbonate and then extracted with ethyl acetate (3×25 ml).   Combined organics were

washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel

chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give fully

protected α-ester analogue **97** (30 mg, 76 % yield) as a colorless film.

TLC R_f 0.60 (hexanes:ethyl acetate, 2:1+0.5% triethylamine; **^{1}H-NMR** (600 MHz,

CDCl$_3$-*d*) δ 9.69 (s, 1H), 7.40 − 7.21 (m, 35H), 6.15 (d, *J* = 3.7 Hz, 1H), 5.50 (d, *J* = 10.0

Hz, 1H), 5.30 − 5.26 (m, 2H), 5.19 (t, *J* = 3.8 Hz, 1H), 5.11 (s, 2H), 5.09 (d, *J* = 12.4 Hz,

1H), 4.91 (d, *J* = 11.2 Hz, 1H), 4.89 − 4.85 (m, 2H), 4.84 (d, *J* = 11.0 Hz, 1H), 4.79 (d, *J*

= 8.0 Hz, 1H), 4.77 (d, *J* = 8.7 Hz, 1H), 4.70 (d, *J* = 9.6 Hz, 1H), 4.68 (d, *J* = 9.2 Hz, 1H),

4.61 (d, 1H), 4.55 (d, J = 7.5 Hz, 1H), 4.53 (d, J = 3.2 Hz, 1H), 4.49 (d, J = 12.2 Hz, 1H), 4.44 – 4.39 (m, 3H), 4.16 (d, J = 7.4 Hz, 1H), 4.10 (dd, J = 7.6, 5.5 Hz, 1H), 4.08 – 4.04 (m, 2H), 3.94 – 3.89 (m, 3H), 3.87 – 3.76 (m, 6H), 3.74 (t, J = 9.2 Hz, 1H), 3.67 – 3.62 (m, 1H), 3.62 – 3.51 (m, 6H), 3.50 – 3.43 (m, 2H), 3.43 – 3.28 (m, 5H), 3.25 (t, J = 8.1 Hz, 1H), 3.22 – 3.17 (m, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.89 (dd, J = 14.4, 4.4 Hz, 1H), 2.35 (t, J = 7.5 Hz, 2H), 2.18 (t, J = 7.6 Hz, 2H), 2.11 (t, J = 13.6 Hz, 1H), 1.81 – 1.71 (m, 4H), 1.71 – 1.53 (m, 10H), 1.51 (s, 4H), 1.49 – 1.41 (m, 2H), 1.38 (s, 3H), 1.34 – 1.16 (m, 25H), 1.03 – 0.88 (m, 86H), 0.87 (s, 3H), 0.79 – 0.78 (m, 3H), 0.75 (s, 3H), 0.74 – 0.55 (m, 55H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.77, 174.17, 173.72, 173.57, 168.34, 142.80, 138.80, 138.34, 138.26, 137.68, 137.58, 136.13, 135.25, 128.55, 128.50, 128.48, 128.43, 128.38, 128.35, 128.30, 128.27, 128.25, 128.25, 128.21, 128.18, 128.17, 128.15, 128.02, 127.93, 127.77, 127.72, 127.70, 127.57, 127.50, 122.23, 109.16, 103.62, 102.88, 101.39, 100.84, 99.01, 90.84, 86.44, 83.75, 81.03, 78.81, 78.72, 78.23, 78.18, 77.97, 76.45, 76.04, 75.93, 75.85, 75.82, 75.55, 75.07, 74.92, 74.40, 73.35, 73.15, 72.62, 72.53, 72.12, 71.39, 71.27, 71.08, 70.92, 68.93, 66.86, 66.08, 65.34, 65.03, 63.69, 60.23, 53.83, 49.62, 49.40, 47.03, 46.38, 46.04, 41.47, 40.18, 39.52, 37.85, 36.99, 36.08, 35.09, 34.54, 34.35, 32.37, 32.32, 31.45, 30.33, 29.42, 29.38, 29.31, 29.27, 29.21, 29.18, 29.14, 27.85, 26.38, 26.33, 25.90, 25.33, 24.97, 24.21, 23.17, 20.24, 17.44, 17.00, 15.77, 12.27, 7.58, 7.48, 7.27, 7.22, 7.15, 7.14, 7.01, 6.87, 6.81, 5.94, 5.66, 5.46, 5.39, 5.32, 5.28, 5.24, 5.01, 4.43; **HRMS** (ESI) *m/z*: Calcd for C$_{182}$H$_{293}$NO$_{35}$Si$_9$Na [M+Na]$^+$ 3327.8999, found 3327.9016.
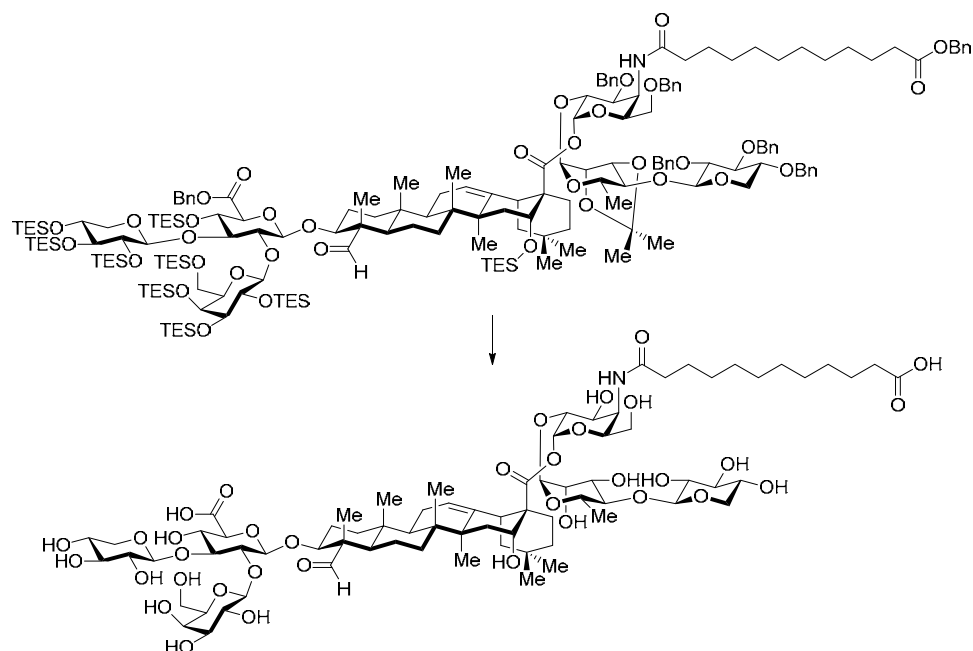
(104): A solution of fully protected α-ester analogue (**97**) (9 mg, 0.003 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (13 mg, 0.011 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (2.0 mL, TFA/H$_2$O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 20 · 75% acetonitrile (0.05% TFA) in over 19 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 10.10 min) was collected and lyophilized to dryness to afford SQS-0-0-8-5 (**104**) (3.3 mg, 77 % yield) as a fluffy white solid

¹H NMR (600 MHz, D₂O, CD₃CN, 1:1) δ 9.29 (s, 1H), 5.97 (d, J = 4.0 Hz, 1H), 5.26 (t, J = 3.5 Hz, 1H), 4.72 (d, J = 1.7 Hz, 1H), 4.60 (d, J = 7.7 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.39 (t, J = 4.2 Hz, 1H), 4.36 (d, J = 7.8 Hz, 1H), 4.34 (d, J = 4.6 Hz, 1H), 4.32 (d, J = 8.0 Hz, 1H), 3.97 (dd, J = 10.5, 4.6 Hz, 1H), 3.88 − 3.78 (m, 5H), 3.78 − 3.59 (m, 8H), 3.59 − 3.51 (m, 2H), 3.50 − 3.33 (m, 9H), 3.33 − 3.21 (m, 6H), 3.20 − 3.04 (m, 5H), 2.77 (dd, J = 14.1, 4.5 Hz, 1H), 2.20 (t, J = 7.5 Hz, 3H), 2.18 − 2.00 (m, 4H), 1.88 − 1.76 (m, 4H), 1.71 − 1.57 (m, 5H), 1.55 − 1.41 (m, 8H), 1.41 − 1.30 (m, 4H), 1.23 (s, 3H), 1.12 (d, J = 5.6 Hz, 3H), 1.01 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H), 0.78 − 0.73 (m, 1H), 0.62 (s, 3H); **HRMS** (ESI) m/z: Calcd for $C_{76}H_{121}NO_{35}Na$ [M+Na]⁺ 1630.7617, found 1630.7596.
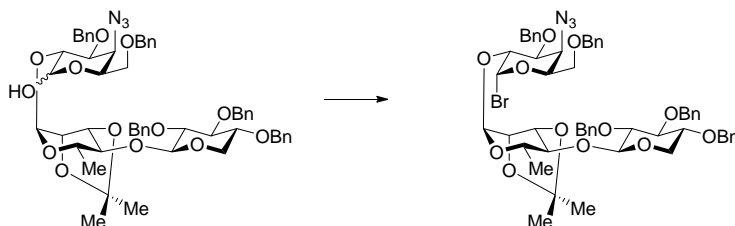
(76): Oxalyl bromide was added to an ice-cooled solution of hemiacetal **73** (125 mg, 0.128 mmol, 1.0 equiv), 2,4,6-tri-tertbutylpyridine (127 mg, 0.513 mmol, 4.0 equiv), and diemethylformamide (150 µL, 1.925 mmol, 15 equiv) in dichloromethane (2 mL) with immediate evolution of CO and CO_2. After five min, ice-bath was removed and warmed to ambient temperature. After three hours, solvent was removed with a stream of nitrogen and crude mixture was purified directly via silica gel chromatography (hexanes:ethyl acetate, 10:1 to 4:1) to give glycosyl bromide as a colorless, thin, and flaky film **76** (98 mg, 74% yield).

TLC R_f 0.43 (hexanes:ethyl acetate, 4:1); **FTIR** (NaCl film); **¹H-NMR** (600 MHz, C₆D₆-d_6) δ 7.49 − 7.06 (m, 25H), 6.62 (d, J = 3.7 Hz, 1H), 5.34 (s, 1H), 5.16 (d, J = 7.5 Hz, 1H), 5.02 (d, J = 11.3 Hz, 1H), 4.93 (d, J = 11.5 Hz, 1H), 4.86 (d, J = 11.5 Hz, 1H), 4.77 (d, J = 11.3 Hz, 1H), 4.48 (d, J = 12.0 Hz, 1H), 4.40 − 4.32 (m, 3H), 4.33 − 4.25 (m, 3H), 4.24 (d, J = 5.8 Hz, 1H), 4.20 (d, J = 11.7 Hz, 1H), 4.12 (dq, J = 9.9, 6.2 Hz, 1H), 4.07 (dd, J = 9.6, 3.8 Hz, 1H), 3.97 (dd, J = 9.9, 7.4 Hz, 1H), 3.92 (dd, J = 9.7, 3.6 Hz, 1H), 3.86 (dd, J = 3.6, 1.6 Hz, 1H), 3.82 (dd, J = 11.5, 5.3 Hz, 1H), 3.65 − 3.46 (m, 5H), 3.18 (dd, J = 11.6, 9.8 Hz, 1H), 1.45 − 1.40 (m, 6H), 1.20 (s, 3H); **¹³C-NMR** (151 MHz, C₆D₆-d_6) δ 139.63, 139.44, 139.01, 138.08, 137.99, 128.63, 128.44, 128.39, 128.34, 128.32, 128.23, 127.68, 127.67, 127.65, 127.55, 127.43, 109.18, 102.71, 100.72, 93.84, 84.15, 82.33, 78.60, 78.32, 78.10, 77.92, 76.85, 76.45, 75.43, 74.81, 73.55, 72.84, 72.76, 72.36, 67.98, 67.71, 66.32, 63.97, 60.24, 27.74, 26.18, 25.69, 17.73; **HRMS** (ESI) *m/z*: Calcd for
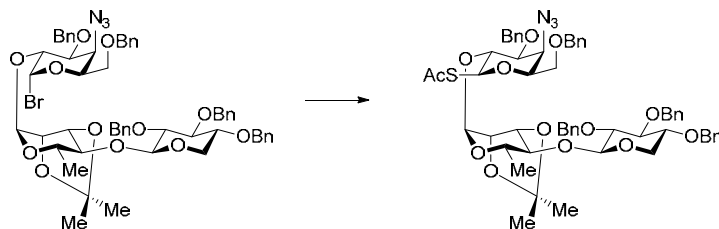
(S81): Cesium carbonate (77 mg, 0.237 mmol, 5 equiv) was added to an ice-cooled solution of thiolacetic acid (67 mL, 0.946 mmol, 20 equiv) and bromide **76** (49 mg, 0.047 mmol, 1 equiv) and in tetrahydrofuran/dimethylformamide (2 mL, 1:1). After one hour, reaction was diluted with ethyl acetate, washed with a saturated sodium bicarbonate and brine, dried over sodium sulfate, concentrated and purified with silica gel

chromatography (hexanes/ethyl acetate, 10:1 to 2:1) to give thioacetate as a colorless oil **S81** (42 mg, 87% yield).

TLC R_f 0.55 (hexanes:ethyl acetate, 2:1); **FTIR** (NaCl film) 3088, 3063, 3030, 2983, 2904, 2872, 2162, 2109, 1706, 1704, 1700, 1496, 1453, 1419, 1381, 1363, 1310, 1274, 1241, 1221, 1091, 1021, 989, 952, 912, 864, 862, 814, 790, 736, 697, 668, 625 cm-1; **^{1}H-NMR** (600 MHz, CDCl$_3$-d) δ 7.36 – 7.20 (m, 25H), 5.44 (s, 1H), 4.98 (d, J = 10.1 Hz, 1H), 4.85 (d, J = 7.6 Hz, 1H), 4.84 – 4.75 (m, 3H), 4.70 (d, J = 11.2 Hz, 1H), 4.66 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 11.0 Hz, 1H), 4.57 (d, J = 11.7 Hz, 1H), 4.55 – 4.45 (m, 3H), 4.11 (d, J = 2.8 Hz, 1H), 4.07 (dd, J = 7.4, 5.7 Hz, 1H), 4.02 (d, J = 5.7 Hz, 1H), 3.98 (t, J = 9.5 Hz, 1H), 3.89 (dd, J = 11.8, 4.1 Hz, 1H), 3.76 – 3.66 (m, 3H), 3.60 – 3.48 (m, 5H), 3.26 (t, J = 8.1 Hz, 1H), 3.19 – 3.11 (m, 1H), 2.21 (s, 3H), 1.44 (s, 3H), 1.25 (s, 3H), 1.23 (d, J = 6.2 Hz, 3H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 192.87, 138.78, 138.59, 138.23, 137.47, 136.58, 128.62, 128.56, 128.53, 128.45, 128.39, 128.36, 128.31, 128.29, 128.26, 128.11, 128.08, 128.04, 127.96, 127.94, 127.81, 127.78, 127.62, 127.56, 109.04, 101.99, 98.83, 83.86, 83.16, 81.94, 81.47, 78.09, 77.94, 77.72, 76.17, 75.70, 75.58, 74.70, 73.70, 73.21, 73.05, 71.80, 67.89, 65.32, 63.81, 58.58, 30.77, 27.75, 26.42, 17.17; **HRMS** (ESI) m/z: Calcd for C$_{57}$H$_{65}$N$_3$O$_{13}$SNa [M+Na]$^+$ 1054.4136, found 1054.4182.
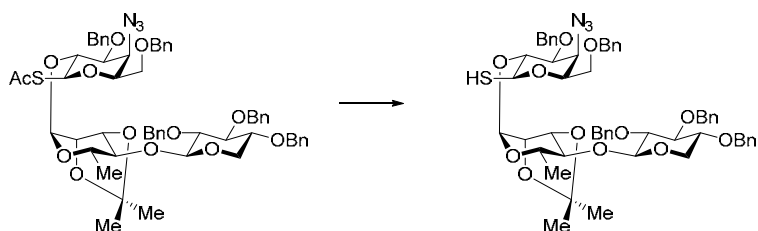
(81): Hydrazine (6.1 µL, 0.194 mmol, 5.0 equiv) was added to a solution of thioacetate **S81** (40 mg, 0.039 mmol, 1 equiv) and dithiothreitol (18 mg, 0.116 mmol, 3 equiv) in tetrahydrofuran/methanol (2 mL, 1:1). After 1 hr, reaction contents was diluted with ethyl acetate, washed with water and brine, dried over sodium sulfated, concentrated, and purified with silica gel chromatography to give thiohemiacetal as a clear oil **81** (36 mg, 94%).

TLC R_f 0.51 (hexanes:ethyl acetate, 2:1; **FTIR** (NaCl film) 3583, 3063, 3031, 2983, 2870, 2106, 1496, 1453, 1369, 1274, 1241, 1220, 1091, 1021, 990, 912, 862, 793, 736, 697, 665 cm^{-1}; **^{1}H-NMR** (500 MHz, CDCl$_3$) δ 7.40 – 7.26 (m, 25H), 5.52 (s, 1H), 4.93 – 4.80 (m, 4H), 4.71 (q, J = 11.2, 10.7 Hz, 3H), 4.62 (d, J = 11.7 Hz, 1H), 4.59 – 4.51 (m, 3H), 4.35 (t, J = 8.9 Hz, 1H), 4.17 (dd, J = 7.5, 5.7 Hz, 1H), 4.11 (d, J = 3.4 Hz, 1H), 4.08 (d, J = 5.7 Hz, 1H), 4.04 (dd, J = 9.9, 6.6 Hz, 1H), 3.94 (dd, J = 11.9, 4.1 Hz, 1H), 3.86 (t, J = 9.2 Hz, 1H), 3.66 – 3.57 (m, 7H), 3.31 (dd, J = 9.5, 8.7 Hz, 1H), 3.24 – 3.14 (m, 1H), 2.31 (d, J = 8.4 Hz, 1H), 1.49 (s, 3H), 1.31 (s, 3H), 1.23 (d, J = 6.2 Hz, 3H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 138.75, 138.71, 138.20, 137.39, 136.55, 128.57, 128.51, 128.40, 128.32, 128.27, 128.25, 128.22, 128.06, 128.05, 128.04, 127.91, 127.76, 127.75, 127.50, 127.48, 108.97, 101.96, 98.80, 83.86, 82.88, 81.90, 79.70, 78.14, 77.92, 77.69, 77.60, 76.27, 75.71, 75.53, 74.71, 73.73, 73.17, 71.66, 68.25, 65.38, 63.76, 58.53, 27.74,

26.42, 17.24; **HRMS** (ESI) *m/z*: Calcd for $C_{55}H_{63}N_3O_{12}SNa$ [M+Na]$^+$ 1012.4030, found 1012.4025.
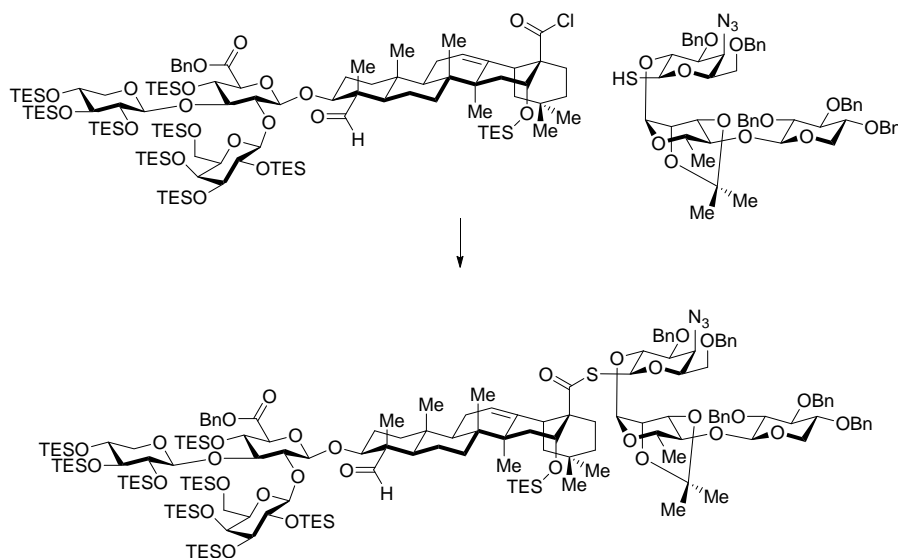
(84): Sodium hydride (60% dispersion in mineral oil, 4.7 mg, 0.115 mmol, 3 equiv) was added to an ice-cooled solution of thiohemiacetal **81** (38 mg, 0.038 mmol, 1.0 equiv) and acyl chloride **66** (88 mg, 0.042 mmol, 1.1 equiv) in tetrahydrofuran (5 mL). After two hours, saturated ammonium chloride (1 mL) was added and the mixture diluted with dichloromethane and washed with water and brine, then dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes/ethyl acetate, 20:1 to 4:1) to give glycosyl thioester **84** (102 mg, 87% yield) as a flaky white film. Characteristic chemical shift of newly formed thioester anomeric proton at 4.84 ppm, *J* = 10.0 Hz and carbon at 81.6 ppm.

TLC *R$_f$* 0.80 (hexanes:ethyl acetate, 2:1) **FTIR** (NaCl film) 2953, 2876, 2109, 1751, 1685, 1456, 1380, 1240, 1096, 1006, 900, 808, 733, 696, 665; **^{1}H-NMR** (500 MHz,

CDCl$_3$-d) δ 9.68 (s, 1H), 7.39 – 7.27 (m, 30H), 5.55 (s, 1H), 5.32 (t, J = 3.8 Hz, 1H), 5.29 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.4 Hz, 1H), 4.91 (d, J = 7.6 Hz, 1H), 4.86 – 4.80 (m, 3H), 4.76 – 4.70 (m, 2H), 4.63 (d, J = 9.3 Hz, 1H), 4.61 (d, J = 8.3 Hz, 1H), 4.58 – 4.51 (m, 4H), 4.49 (s, 1H), 4.42 (d, J = 7.2 Hz, 1H), 4.19 (d, J = 7.3 Hz, 1H), 4.15 (dd, J = 3.4, 1.4 Hz, 1H), 4.13 – 4.04 (m, 3H), 3.99 – 3.66 (m, 13H), 3.65 – 3.53 (m, 7H), 3.51 – 3.32 (m, 7H), 3.30 – 3.23 (m, 2H), 3.19 (dd, J = 11.5, 9.2 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.84 (dd, J = 13.3, 3.3 Hz, 1H), 2.22 (t, J = 13.3 Hz, 1H), 1.95 – 1.75 (m, 4H), 1.74 – 1.57 (m, 5H), 1.53 – 1.46 (m, 5H), 1.45 – 1.23 (m, 17H), 1.19 – 1.05 (m, 2H), 1.03 – 0.85 (m, 114H), 0.84 – 0.52 (m, 77H); ^{13}**C-NMR** (151 MHz, CDCl$_3$) δ 212.51, 204.16, 168.32, 142.00, 138.68, 138.48, 138.21, 137.60, 136.58, 135.23, 128.55, 128.46, 128.43, 128.42, 128.34, 128.32, 128.29, 128.24, 128.21, 128.14, 128.12, 128.11, 128.03, 128.01, 128.00, 127.88, 127.85, 127.79, 127.75, 127.60, 127.59, 123.87, 108.91, 103.51, 102.28, 101.37, 100.82, 98.50, 86.26, 83.92, 83.61, 82.46, 81.62, 78.78, 78.70, 78.23, 78.01, 77.80, 76.43, 76.16, 75.94, 75.80, 75.76, 75.57, 75.53, 75.05, 75.03, 73.60, 73.20, 72.59, 72.50, 71.64, 71.60, 71.38, 71.05, 67.93, 66.84, 65.38, 65.32, 63.84, 60.25, 58.80, 56.19, 53.84, 53.43, 49.30, 46.67, 46.09, 41.81, 41.21, 39.84, 37.85, 36.04, 35.01, 33.94, 32.56, 32.20, 32.04, 30.32, 27.74, 26.46, 26.39, 25.31, 24.64, 23.40, 20.21, 17.57, 17.10, 15.75, 12.22, 7.55, 7.46, 7.25, 7.16, 7.13, 7.09, 6.98, 6.85, 6.78, 5.91, 5.63, 5.43, 5.36, 5.33, 5.31, 5.25, 5.22, 4.88, 4.40; **HRMS** (ESI) m/z: Calcd for C$_{163}$H$_{265}$N$_3$O$_{31}$NaSi$_9$S [M+Na]$^+$ 3067.6794, found 3067.6711.
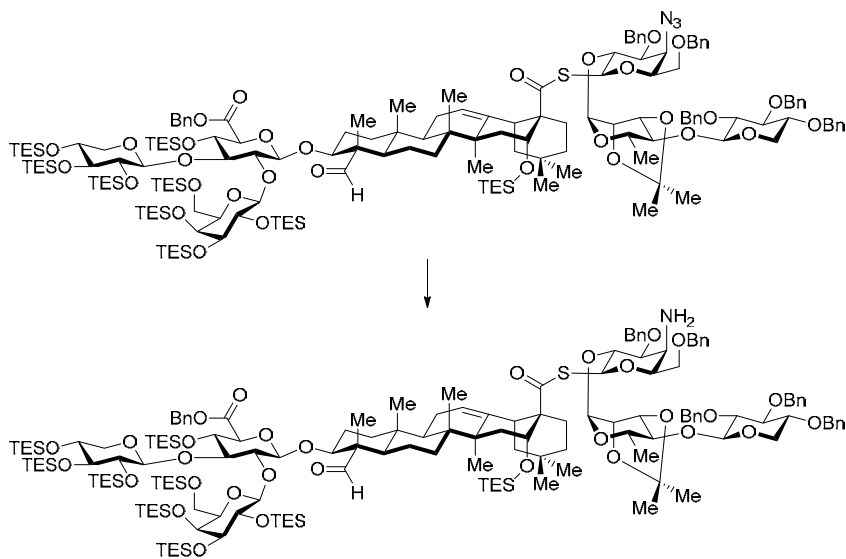
(85): An excess of hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **84** (80 mg, 0.026 mmol, 1.0 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to ambient temperature and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **85** (71 mg, 90 % yield).

TLC R_f 0.50 (hexanes:ethyl acetate, 2:1 +0.5% triethylamine); **FTIR** (NaCl film) 3583, 2951, 2876, 1751, 1724, 1685, 1496, 1457, 1380, 1240, 1097, 1006, 900, 807, 731 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$-d) δ 9.69 (s, 1H), 7.37 – 7.26 (m, 30H), 5.57 (s, 1H), 5.32 (t, J = 3.8 Hz, 1H), 5.29 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.4 Hz, 1H), 4.92 (d, J = 7.6 Hz, 1H), 4.88 – 4.81 (m, 4H), 4.74 – 4.68 (m, 2H), 4.64 (d, J = 8.3 Hz, 1H), 4.62 (d, J = 7.4 Hz, 1H), 4.58 (d, J = 11.8 Hz, 1H), 4.56 (d, J = 7.5 Hz, 1H), 4.53 – 4.49 (m, 3H), 4.42 (d, J = 7.3 Hz, 1H), 4.18 (d, J = 7.4 Hz, 1H), 4.13 (dd, J = 7.4, 5.6 Hz, 1H), 4.10 (d, J = 5.7

Hz, 1H), 4.00 (dd, J = 10.3, 8.5 Hz, 1H), 3.97 – 3.90 (m, 4H), 3.87 (d, J = 9.2 Hz, 1H),

3.85 – 3.72 (m, 6H), 3.68 – 3.53 (m, 10H), 3.48 (ddd, J = 10.5, 8.4, 5.1 Hz, 1H), 3.39 (dd,

J = 9.4, 2.5 Hz, 1H), 3.37 – 3.32 (m, 2H), 3.28 (dd, J = 8.7, 7.5 Hz, 1H), 3.25 (t, J = 8.0

Hz, 1H), 3.20 (dd, J = 11.7, 9.3 Hz, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.84 (dd, J = 13.4, 4.6

Hz, 1H), 2.22 (t, J = 13.2 Hz, 1H), 1.95 – 1.74 (m, 5H), 1.73 – 1.53 (m, 7H), 1.51 (s, 4H),

1.45 – 1.38 (m, 2H), 1.34 (s, 4H), 1.33 – 1.26 (m, 11H), 1.16 – 0.89 (m, 97H), 0.88 (s,

3H), 0.82 (s, 3H), 0.80 – 0.51 (m, 61H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.59, 204.03,

168.36, 142.34, 138.70, 138.50, 138.24, 137.93, 136.88, 135.26, 128.55, 128.52, 128.49,

128.46, 128.44, 128.38, 128.35, 128.33, 128.31, 128.24, 128.18, 128.16, 128.15, 128.07,

128.05, 127.82, 127.78, 127.72, 127.65, 127.63, 123.70, 108.96, 103.55, 102.32, 101.39,

100.85, 98.36, 86.28, 83.95, 82.49, 81.76, 78.81, 78.73, 78.22, 78.03, 77.85, 76.45, 76.24,

75.97, 75.83, 75.79, 75.68, 75.09, 75.07, 73.59, 73.23, 72.62, 72.53, 71.41, 71.08, 70.80,

69.05, 66.86, 65.35, 63.87, 60.28, 56.29, 53.86, 49.36, 49.04, 46.84, 46.10, 41.68, 41.28,

39.86, 37.88, 36.08, 35.06, 33.95, 32.58, 32.19, 32.10, 30.35, 29.73, 27.81, 26.46, 26.44,

25.34, 24.66, 23.46, 20.24, 17.62, 17.21, 15.80, 12.24, 7.58, 7.49, 7.27, 7.19, 7.16, 7.12,

7.01, 6.88, 6.81, 5.94, 5.66, 5.46, 5.39, 5.36, 5.28, 5.25, 4.92, 4.43; **HRMS** (ESI) *m/z*:

Calcd for C$_{163}$H$_{268}$NO$_{31}$Si$_9$S [M+H]$^+$ 3019.7070, found 3019.7112.
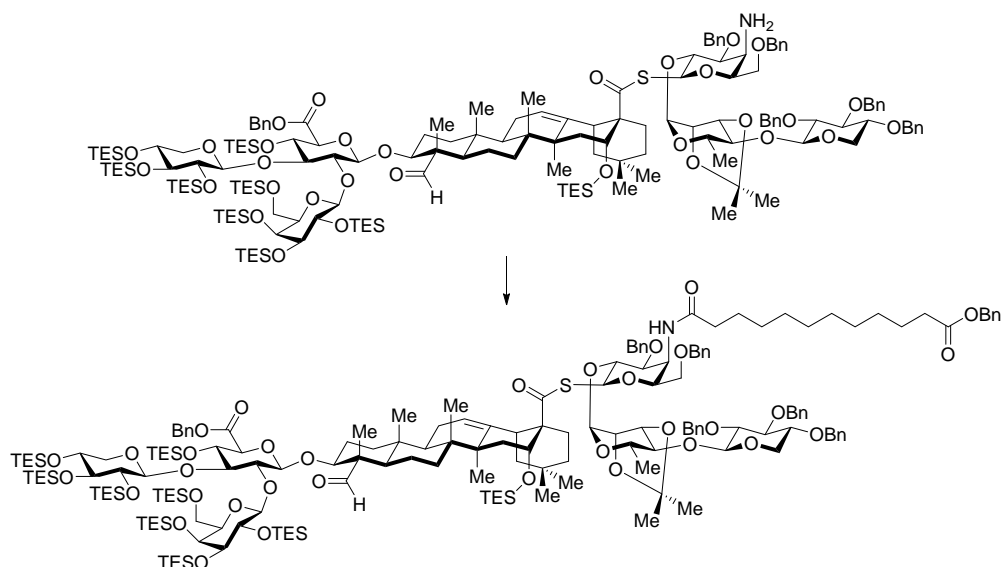
(92): Isobutyl chloroformate (3.5 µL, 0.0264 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **106** (11 mg, 0.033 mmol, 5 equiv) and triethylamine (9 µL, 0.066 mmol, 10 equiv) in tetrahydrofuran (3 mL) and stirred for 2 hours, then transferred via cannula to an ice-cooled solution of amine **85** (20 mg, 0.007 mmol, 1 equiv) in tetrahydrofuran (1 mL). After 2 hr, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give fully protected thioester analogue **92** (19 mg, 87 % yield) as a colorless film.

TLC R_f 0.57 (hexanes:dichloromethane:ethyl acetate, 4:2:1) **FTIR** (NaCl film) 3583, 3381, 2954, 2876, 1751, 1738, 1682, 1497, 1455, 1414, 1380, 1240, 1099, 1005, 901, 863, 806, 732, 696, 665 cm^{-1}; **^{1}H-NMR** (600 MHz, CDCl$_3$-d) δ 9.70 (s, 1H), 7.39 − 7.25 (m, 35H), 5.89 (s, 1H), 5.52 (s, 1H), 5.31 (d, J = 3.7 Hz, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.11 (s, 2H), 5.09 (d, J = 12.4 Hz, 1H), 4.93 − 4.87 (m, 2H), 4.89 − 4.74 (m, 6H), 4.72 (d,

J = 11.7 Hz, 1H), 4.64 (d, *J* = 3.4 Hz, 1H), 4.62 (d, *J* = 4.4 Hz, 1H), 4.55 (d, *J* = 7.4 Hz,

1H), 4.52 (d, *J* = 11.8 Hz, 2H), 4.47 – 4.40 (m, 3H), 4.17 (d, *J* = 7.4 Hz, 1H), 4.12 (dd, *J*

= 7.4, 5.6 Hz, 1H), 4.09 (d, *J* = 5.6 Hz, 1H), 3.97 – 3.72 (m, 12H), 3.65 – 3.51 (m, 8H),

3.48 (ddd, *J* = 10.4, 8.4, 5.1 Hz, 1H), 3.44 – 3.31 (m, 6H), 3.28 (dd, *J* = 8.7, 7.4 Hz, 1H),

3.25 (t, *J* = 8.0 Hz, 1H), 3.19 (dd, *J* = 11.6, 9.1 Hz, 1H), 3.13 (t, *J* = 11.0 Hz, 1H), 2.83

(dd, *J* = 13.6, 4.5 Hz, 1H), 2.34 (t, *J* = 7.6 Hz, 2H), 2.25 – 2.14 (m, 3H), 1.92 – 1.81 (m,

2H), 1.83 – 1.74 (m, 1H), 1.41 (ddd, *J* = 13.5, 9.3, 3.8 Hz, 1H), 1.71 – 1.53 (m, 12H) 1.50

(s, 3H) 1.35 (s, 3H), 1.33 – 1.03 (m, 31H), 1.03 – 0.87 (m, 99H), 0.87 (s, 3H), 0.83 (s,

4H), 0.81 – 0.50 (m, 70H); **^{13}C-NMR** (151 MHz, CDCl$_3$) δ 212.56, 203.39, 173.67,

173.31, 168.34, 142.85, 138.68, 138.49, 138.22, 137.85, 137.19, 136.12, 135.24, 128.72,

128.52, 128.44, 128.43, 128.39, 128.32, 128.31, 128.26, 128.25, 128.21, 128.15, 128.13,

128.08, 128.05, 128.02, 127.90, 127.79, 127.74, 127.63, 127.61, 127.59, 127.53, 122.89,

108.98, 103.57, 102.26, 101.37, 100.82, 98.39, 86.32, 83.91, 82.38, 81.95, 81.74, 78.79,

78.70, 78.06, 77.98, 77.92, 76.43, 76.12, 75.92, 75.84, 75.80, 75.74, 75.06, 74.99, 73.53,

73.20, 72.59, 72.50, 71.38, 71.07, 70.75, 68.76, 66.84, 66.03, 65.32, 65.26, 63.84, 60.25,

56.54, 53.80, 49.29, 46.86, 46.19, 45.99, 41.33, 41.28, 39.81, 37.83, 36.93, 36.03, 35.10,

34.33, 33.90, 32.53, 32.11, 32.08, 30.30, 29.70, 29.45, 29.40, 29.38, 29.24, 29.22, 29.14,

27.79, 26.42, 26.41, 25.87, 25.32, 24.97, 24.51, 23.40, 20.18, 17.67, 17.07, 15.79, 12.24,

7.55, 7.46, 7.24, 7.15, 7.13, 7.09, 6.98, 6.85, 6.78, 5.91, 5.63, 5.44, 5.36, 5.33, 5.25, 5.22,

4.90, 4.40; **HRMS** (ESI) *m/z*: Calcd for C$_{182}$H$_{293}$NO$_{34}$NaSi$_9$S [M+Na]+ 3343.8771, found

3343.8735.
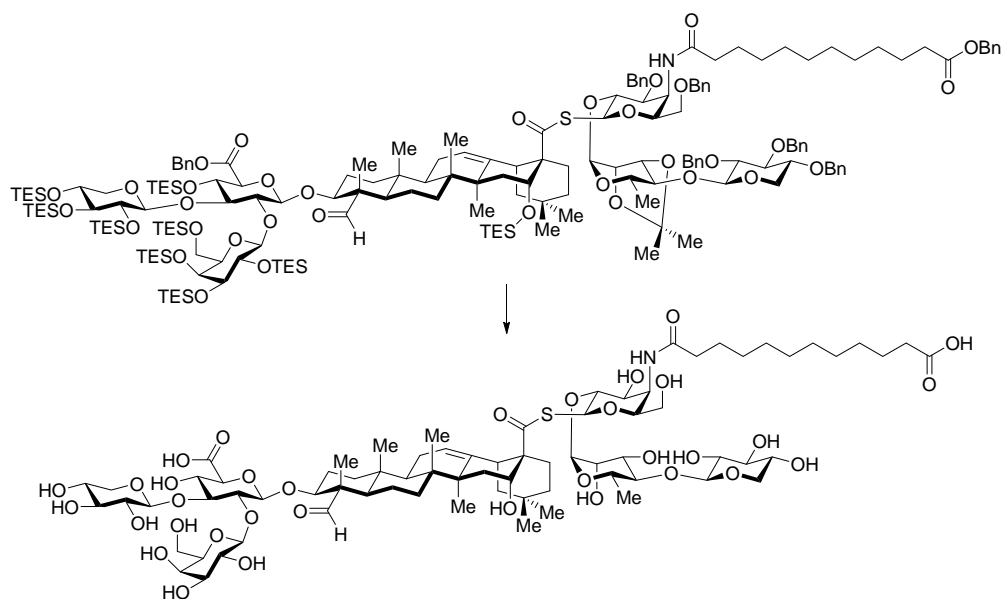
(99): A solution of fully protected β-thioester analogue (**92**) (18 mg, mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (13 mg, 0.011 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 °C) solution of trifluoroacetic acid (2.0 mL, TFA/H$_2$O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (4:1 water:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 20 · 66% acetonitrile (0.05% TFA) in over 16 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 13.2 min) was collected and lyophilized to dryness to afford SQS-0-13-5-5 (**99**) (3.1 mg, 33 % yield) as a fluffy white solid.

¹H NMR (600 MHz, D₂O/CD₃CN, 1:1) δ 9.91 (s, 1H), 5.87 (t, J = 4.1 Hz, 1H), 5.55 (d, J = 2.0 Hz, 1H), 5.38 (d, J = 10.0 Hz, 1H), 5.22 (d, J = 7.8 Hz, 1H), 5.10 (d, J = 7.8 Hz, 1H), 5.00 – 4.95 (m, 2H), 4.92 (d, J = 7.8 Hz, 1H), 4.46 – 4.36 (m, 5H), 4.35 – 4.25 (m, 4H), 4.24 – 4.12 (m, 6H), 4.09 – 3.95 (m, 6H), 3.93 – 3.84 (m, 4H), 3.82 (dd, J = 11.5, 6.3 Hz, 1H), 3.78 – 3.70 (m, 3H), 3.67 (dd, J = 9.3, 7.7 Hz, 1H), 3.45 (dd, J = 13.6, 2.9 Hz, 1H), 2.81 (t, J = 7.6 Hz, 3H), 2.79 – 2.67 (m, 3H), 2.47 – 2.32 (m, 5H), 2.32 – 2.14 (m, 6H), 2.07 (q, J = 6.9 Hz, 6H), 1.97 (t, J = 9.7 Hz, 2H), 1.89 (d, J = 15.4 Hz, 2H), 1.85 (s, 3H), 1.76 – 1.69 (m, 2H), 1.64 (s, 3H), 1.63 – 1.58 (m, 2H), 1.48 (s, 3H), 1.44 (s, 4H), 1.40 (s, 3H), 1.38 (d, J = 6.7 Hz, 2H), 1.19 (s, 3H); **HRMS** (ESI) *m/z*: Calcd for $C_{76}H_{121}NO_{34}NaS$ [M+Na]⁺ 1646.7388, found 1646.7373.
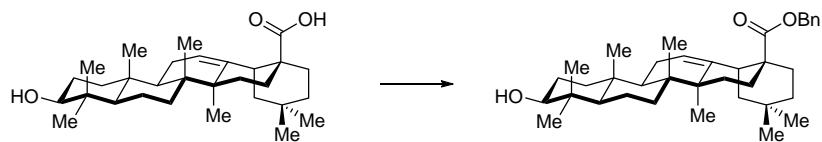
APPENDIX B

EXPERIMENTAL PROCEDURES FOR CHAPTER 3

General Procedures. Reactions were performed in flame-dried sealed-tubes or modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unlessotherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate and sulfoxide reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then flame-dried under vacuum. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed using glass plates pre-coated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm).

Materials. Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed columns of neutral alumina under an argon atmosphere. Methanol was distilled from magnesium at 760 Torr. Trifluoromethanesulfonic anhydride was distilled from phosphorus pentoxide at 760 Torr. Boron trifluoride diethyl etherate was distilled from calcium hydride at 760 Torr. All other chemicals were obtained from commercial vendors and were used without further purification unless noted otherwise.

Instrumentation. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer or a Bruker Tensor 27. Data are presented as the frequency of absorption (cm⁻¹). Proton and carbon-13 nuclear magnetic resonance (^{1}H NMR and ^{13}CNMR) spectra were recorded on a Bruker Avance III instrument; chemical

shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent (d-chloroform: δ 7.26 for ^{1}H NMR, δ 77.16 for ^{13}C NMR; d6-benzene: δ 7.16 for ^{1}H NMR, δ 128.06 for ^{13}C NMR; d4-methanol: δ 3.31 for ^{1}H NMR, δ 49.00 for ^{13}C NMR; d3-acetonitrile: δ 1.94 for ^{1}H NMR, δ 1.32 for ^{13}C NMR; deuterium oxide: δ 4.79 for ^{1}H NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment. RP-HPLC purification and analyses were carried out on a Waters 2545 binary gradient HPLC system equipped with a Waters 2996 photodiode array detector, and absorbances were monitored at wavelengths of 210–600 nm.

(124): To a suspension of oleanolic acid monohydrate (12 g, 25.3 mmol, 1 equiv) in dimethylformamide (50 mL) was added benzyl bromide (4.5 mL, 38.5 mmol, 1.5 equiv) followed by cesium carbonate (11 g, 25.3 mmol, 1 equiv). After 4 hrs, the solution was diluted with water (200 mL) and extracted with ethyl acetate (3 × 100 mL). Combined organics were washed with brine, then dried over magnesium sulfate, filtered, concentrated, and purified with silica gel chromatography to give benzyl ester **124** (11.1 g, 80% yield) as a white foam.

TLC R_f 0.24 (10:10:1 benzene:hexanes:ethyl acetate); **FTIR** (NaCl film) 3432, 2960, 2921, 1723, 1498, 1462, 1452, 1384, 1362, 1259, 1199, 1180, 1039, 1015 cm^{-1}; **^{1}H NMR**

(600 MHz, CDCl$_3$) δ 7.37 – 7.28 (m, 5H), 5.29 (t, *J* = 3.7 Hz, 1H), 5.12 – 5.02 (m, 2H), 3.20 (dd, *J* = 11.3, 4.3 Hz, 1H), 2.90 (dd, *J* = 13.8, 4.6 Hz, 1H), 1.98 (td, *J* = 13.6, 4.1 Hz, 1H), 1.85 (dd, *J* = 9.0, 3.7 Hz, 2H), 1.75 – 1.48 (m, 10H), 1.42 (td, *J* = 12.6, 4.0 Hz, 1H), 1.38 – 1.29 (m, 3H), 1.27 – 1.15 (m, 3H), 1.12 (s, 3H), 1.06 – 1.01 (m, 1H), 0.98 (s, 3H), 0.95 (d, *J* = 4.2 Hz, 1H), 0.92 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.71 (dd, *J* = 11.8, 2.0 Hz, 1H), 0.60 (s, 3H); ^{13}**C NMR** (151 MHz, CDCl$_3$) δ 177.42, 143.65, 136.39, 128.38, 127.94, 127.87, 122.46, 78.96, 65.90, 55.16, 47.57, 46.71, 45.83, 41.65, 41.33, 39.24, 38.72, 38.40, 36.97, 33.83, 33.10, 32.67, 32.34, 30.68, 28.08, 27.59, 27.16, 25.86, 23.63, 23.37, 23.02, 18.29, 16.85, 15.57, 15.28; **HRMS** *m/z* (ESI): Calcd for C$_{37}$H$_{55}$O$_3$ [M+] 547.4151, found 545.4124.
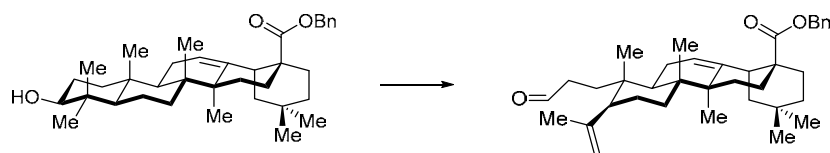

(123): To a suspension of benzyl oleanolate **124** (5.0 g, 9.1 mmol, 1 equiv), 2,4,6-tri-*tert*-butylpyridine (5.6 g, 22.9 mmol, 2.5 equiv), and PhI(OAc)$_2$ (3.5 g, 11 mmol, 1.2 equiv) in benzene (200 mL) in a 500 mL round bottomed flask was added crystalline iodine (1.15 g, 4.57 mmol, 0.5 equiv) and placed in a photo-box equipped with 10 visible full-spectrum visible light bulbs (wattage). After 50 min and 85 min, an additional portion of iodine was added (0.46 g, 1.8 mmol, 0.2 equiv, and 0.23, 0.9 mmol, 0.1 equiv, respectively). When TLC showed consumption of starting material (105 min total), reaction was diluted with saturated sodium thiosulfate, and extracted with ethyl acetate (2 × 100 mL). Combined organics were washed with brine, dried over sodium sulfate, decanted, concentrated, and purified with silica gel chromatography

((benzene/hexanes):ethyl acetate, 1:1:0 to 2:2:1) to give **123** (3.5 g, 70% yield) as a white foam.

TLC R_f 0.51 (10:10:1 benzene:hexanes:ethyl acetate); **FTIR** (NaCl film) 2949, 1726, 1639, 1499, 1456, 1387, 1303, 1263, 1161, 1123, 1082, 1034, 894, 738, 699 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.73 (t, J = 1.9 Hz, 1H), 7.41 – 7.28 (m, 5H), 5.29 (t, J = 3.5 Hz, 1H), 5.14 – 5.01 (m, 2H), 4.84 (bs, 1H), 4.62 (bs, 1H), 2.91 (ddd, J = 14.3, 4.9, 1.8 Hz, 1H), 2.47 (dddd, J = 16.3, 11.5, 5.0, 1.7 Hz, 1H), 2.30 (dddd, J = 16.7, 10.9, 6.3, 2.2 Hz, 1H), 2.03 – 1.88 (m, 3H), 1.77 – 1.72 (m, 2H), 1.72 (s, 3H), 1.70 – 1.56 (m, 4H), 1.55 – 1.48 (m, 2H), 1.45 (td, J = 13.0, 4.1 Hz, 1H), 1.40 – 1.30 (m, 2H), 1.24 – 1.16 (m, 3H), 1.15 (s, 3H), 1.08 (dt, J = 13.9, 3.4 Hz, 1H), 0.92 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.66 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 202.70, 177.40, 147.40, 143.71, 136.36, 128.40, 128.32, 127.99, 127.92, 122.16, 113.53, 65.95, 50.65, 46.73, 45.77, 42.19, 41.44, 39.02, 38.99, 38.30, 38.07, 33.84, 33.09, 32.31, 31.42, 30.75, 30.70, 27.58, 25.73, 24.32, 23.61, 23.55, 23.15, 23.01, 19.48, 16.92; **HRMS** m/z (ESI): Calcd for C$_{37}$H$_{53}$O$_3$ [M+] 545.3995, found 545.3988.
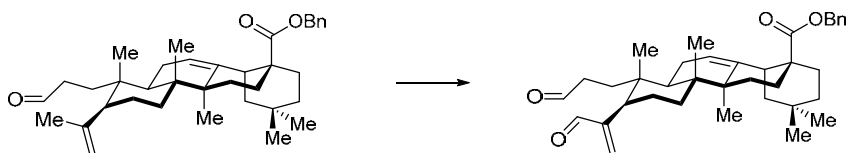
(122): Selenium dioxide (102 mg, 0.918 mmol, 0.5 equiv) was added to a flame dried 25 mL schlenk flask followed by addition of dichloromethane (4 mL) and *tert*-butanol hydroperioxide (5.5 M solution in decane, 1.0 mL, 5.50 mmol, 3 equiv). Enal **123** was

added as a solution in dichloromethane (5 mL). After 24 hr, reaction was diluted with sodium bicarbonate, then extracted with ethyl aceate (3×50 mL). Combined organics were washed with brine, then dried over sodium sulfate, concentrated, and purified with silica gel chromatography ([hexanes/benzene, 1:1]:ethyl acetate, 10:1 to 5:1) to give dialdehyde **122** (740 mg, 72% yield) as a white foam.

TLC R_f 0.20 (10:10:1 benzene:hexanes:ethyl acetate); **FTIR** (NaCl film) 2945, 1723, 1691, 1497, 1455, 1386, 1364, 1301, 1259, 1232, 1160, 1120, 1079, 1032, 968, 735, 696 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.67 (d, J = 1.8 Hz, 1H), 9.43 (s, 1H), 7.38 – 7.28 (m, 5H), 6.30 (s, 1H), 6.13 (s, 1H), 5.28 (t, J = 3.6 Hz, 1H), 5.12 – 5.02 (m, 2H), 2.96 – 2.83 (m, 2H), 2.68 (dd, J = 13.0, 2.7 Hz, 1H), 2.22 (dddd, J = 17.3, 12.0, 5.5, 2.1 Hz, 1H), 1.99 (td, J = 13.7, 4.1 Hz, 1H), 1.93 – 1.65 (m, 8H), 1.62 – 1.54 (m, 2H), 1.51 (td, J = 12.9, 3.6 Hz, 1H), 1.42 – 1.31 (m, 3H), 1.30 – 1.24 (m, 2H), 1.24 (s, 2H), 1.18 (s, 3H), 1.09 (dt, J = 13.7, 3.3 Hz, 1H), 0.92 (s, 3H), 0.91 (s, 3H), 0.84 (s, 3H), 0.68 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 202.87, 194.99, 177.36, 152.54, 143.69, 137.71, 136.34, 128.29, 127.97, 127.90, 122.04, 65.91, 46.71, 45.74, 42.25, 41.43, 38.99, 38.87, 38.47, 38.11, 37.79, 33.82, 33.07, 32.28, 31.44, 31.12, 30.67, 27.55, 25.75, 24.88, 23.80, 23.58, 22.99, 18.28, 16.91; **HRMS** m/z (ESI): Calcd for C$_{37}$H$_{51}$O$_4$ [M+H] 559.3787, found 559.3773.
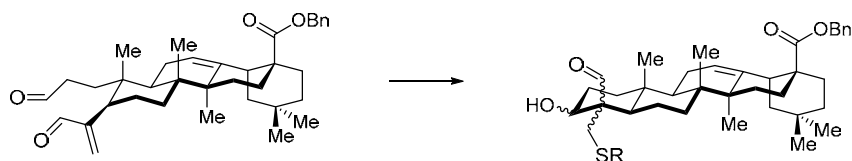
Representative procedure for tandem Michael–aldol reaction.

To an ice-cooled solution of enal **122** (148 mg, 0.265 mmol, 1.0 equiv) and *t*-BuSH (90 µL, 0.795 mmol, 3.0 equiv) in tetrahydrofuran (5 mL) was added *n*-BuLi (132 µL, 1.6 M solution in hexanes, 0.8 equiv). After 20 min, saturated ammonium chloride (1 mL) was added, then diluted with water. Mixture was extracted with ethyl acetate (3 × 50 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography ([2:1 hexanes:dichloromethane]:ethyl acetate, 1:0 to 5:1) to give thioether **129a** (121 mg, 70%) as a white foam.
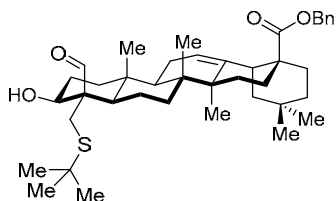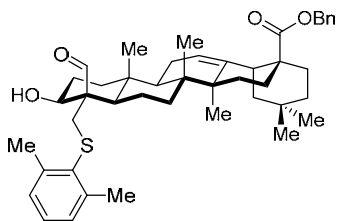

(129a): TLC R_f 0.45 (4:1 hexanes:ethyl acetate); **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.83 (d, *J* = 2.1 Hz, 1H), 7.37 − 7.28 (m, 5H), 5.28 (t, *J* = 3.7 Hz, 1H), 5.11 − 5.02 (m, 2H), 3.71 (ddd, *J* = 11.6, 5.6, 2.1 Hz, 1H), 2.94 − 2.83 (m, 3H), 2.01 − 1.93 (m, 1H), 1.92 − 1.75 (m, 4H), 1.76 − 1.39 (m, 13H), 1.35 (s, 9H), 1.32 − 1.14 (m, 8H), 1.13 (s, 3H), 1.08 − 0.98 (m, 3H), 0.97 (d, *J* = 6.7 Hz, 0H), 0.92 (s, 3H), 0.90 (s, 3H), 0.79 (s, 3H), 0.58 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 206.86, 177.38, 143.80, 136.35, 128.40, 128.38, 127.98, 127.90, 122.17, 71.44, 65.91, 55.31, 50.96, 46.67, 46.39, 45.76, 42.69, 41.74, 41.34, 39.04, 37.78, 36.93, 34.65, 34.51, 33.80, 33.08, 32.36, 32.31, 31.58, 30.80, 30.69,

27.86, 27.54, 27.51, 26.89, 25.84, 25.83, 25.26, 23.69, 23.62, 22.98, 22.65, 20.70, 18.52, 16.86, 14.92, 14.14; **HRMS** *m/z* (ESI): Calcd for $C_{41}H_{60}O_4NaS$ [M+Na] 671.4110, found 671.4121.
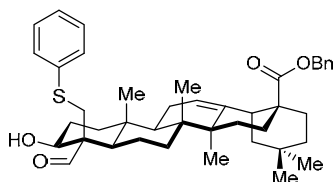
(121): Major Isomer, Shown Above TLC R_f 0.22 (4:1 hexanes:ethyl acetate); **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.80 (s, 1H), 7.39 – 7.27 (m, 9H), 7.24 – 7.18 (m, 1H), 5.28 (t, *J* = 3.7 Hz, 1H), 5.14 – 5.00 (m, 3H), 4.31 (q, *J* = 2.9 Hz, 1H), 3.34 (s, 2H), 3.08 (d, *J* = 2.7 Hz, 1H), 2.90 (dd, *J* = 13.7, 4.1 Hz, 1H), 2.03 – 1.93 (m, 2H), 1.92 – 1.80 (m, 3H), 1.78 – 1.62 (m, 7H), 1.62 – 1.60 (m, 1H), 1.60 – 1.41 (m, 8H), 1.37 – 1.23 (m, 7H), 1.14 (s, 4H), 0.99 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 1H), 0.91 (s, 4H), 0.90 (s, 4H), 0.60 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 208.57, 177.41, 143.70, 137.04, 136.37, 130.04, 129.15, 128.39, 127.96, 127.90, 126.75, 122.00, 68.86, 65.90, 55.80, 47.50, 46.68, 46.67, 45.80, 45.76, 45.18, 41.72, 41.32, 39.40, 36.90, 36.88, 35.63, 34.65, 34.51, 33.83, 33.81, 33.08, 32.30, 32.18, 32.10, 31.58, 30.69, 27.49, 26.90, 25.95, 25.92, 25.27, 24.83, 23.64, 23.62, 23.61, 23.20, 22.96, 22.65, 20.70, 19.72, 16.81, 16.24, 14.14; **HRMS** *m/z* (ESI): Calcd for $C_{43}H_{56}O_4NaS$ [M+Na] 691.3797, found 691.3804.

(131a): Major Isomer, Shown Above TLC R_f 0.29 (4:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3409, 2947, 1719, 1460, 1385, 1262, 1160, 1028, 773, 736, 697, 666 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.80 (d, J = 2.1 Hz, 1H), 7.39 – 7.31 (m, 5H), 7.17 – 7.09 (m, 3H), 5.32 (t, J = 3.8 Hz, 1H), 5.13 – 5.06 (m, 2H), 3.92 (ddd, J = 12.2, 5.2, 2.2 Hz, 1H), 3.12 (d, J = 12.3 Hz, 1H), 3.00 (d, J = 12.2 Hz, 1H), 2.94 (dd, J = 13.6, 4.2 Hz, 1H), 2.61 (s, 6H), 2.01 (td, J = 13.6, 4.1 Hz, 1H), 1.97 – 1.80 (m, 5H), 1.78 – 1.49 (m, 12H), 1.40 (dd, J = 12.8, 2.0 Hz, 3H), 1.26 – 1.18 (m, 3H), 1.16 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.82 (s, 3H), 0.60 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 206.05, 177.41, 143.80, 142.98, 136.37, 133.00, 128.43, 128.42, 128.36, 128.32, 128.04, 127.99, 127.95, 122.20, 72.19, 65.97, 65.96, 60.43, 56.05, 50.73, 46.71, 46.70, 46.56, 45.83, 41.79, 41.41, 39.06, 37.92, 37.06, 34.87, 33.85, 33.12, 32.34, 32.07, 30.73, 30.71, 27.59, 27.56, 25.83, 25.82, 23.75, 23.64, 23.02, 22.05, 22.03, 21.10, 18.74, 16.94, 15.01, 14.23.; **HRMS** m/z (ESI): Calcd for C$_{45}$H$_{60}$O$_4$NaS [M+Na] 719.4110, found 719.4132.
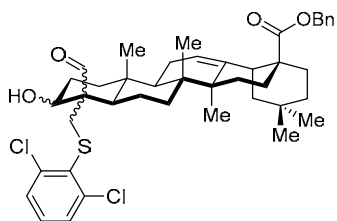

(130b): Major Isomer, Shown Above: **TLC** R_f 0.29 (4:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3508, 2945, 1722, 1553, 1454, 1262, 1159, 1083, 1032, 777, 736, 697,

666 cm^{-1}; ^{1}**H NMR** (600 MHz, CDCl$_3$) δ 9.89 (s, 1H), 7.39 (d, J = 8.0 Hz, 2H), 7.36 –

7.27 (m, 6H), 7.20 (t, J = 8.0 Hz, 1H), 5.27 (t, J = 3.6 Hz, 1H), 5.11 – 5.00 (m, 2H), 4.39

– 4.32 (m, 1H), 3.33 (d, J = 13.1 Hz, 1H), 3.26 (d, J = 13.1 Hz, 1H), 3.11 (d, J = 2.7 Hz,

1H), 2.89 (dd, J = 14.1, 4.1 Hz, 1H), 2.04 – 2.00 (m, 1H), 1.97 (td, J = 13.7, 4.1 Hz, 1H),

1.91 – 1.79 (m, 2H), 1.77 – 1.41 (m, 14H), 1.36 – 1.28 (m, 4H), 1.22 – 1.15 (m, 3H), 1.13

(s, 3H), 1.04 – 0.99 (m, 1H), 0.94 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.57 (s, 3H); ^{13}**C**

NMR (151 MHz, CDCl$_3$) δ 208.78, 177.40, 143.69, 140.74, 136.36, 134.09, 130.10,

128.78, 128.40, 128.38, 127.96, 127.90, 121.98, 68.61, 65.89, 60.40, 55.52, 47.51, 46.66,

45.79, 45.33, 41.71, 41.31, 39.39, 36.80, 36.51, 34.65, 33.81, 33.07, 32.30, 32.18, 32.06,

31.58, 30.68, 27.48, 26.90, 25.95, 25.27, 24.85, 23.62, 23.19, 22.96, 22.65, 21.07, 20.70,

19.71, 16.77, 16.23, 14.20, 14.14; **HRMS** m/z (ESI): Calcd for C$_{43}$H$_{54}$O$_4$SCl$_2$Na

[M+Na] 759.3018, found 759.2994.
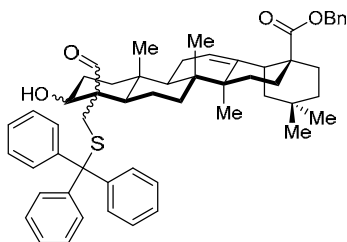
(**132**): **TLC** R_f 0.30 (4:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3532, 3057, 3030,

2945, 2864, 1719, 1594, 1489, 1445, 1385, 1364, 1319, 1302, 1209, 1159, 1121, 1080,

1030, 1006, 972, 907, 822, 741, 699, 666 cm^{-1}; ^{1}**H NMR** (600 MHz, CDCl$_3$) δ 9.55 (d, J

= 1.5 Hz, 1H), 7.52 – 7.45 (m, 6H), 7.36 – 7.27 (m, 10H), 7.24 – 7.20 (m, 3H), 5.26 (t, J

= 3.7 Hz, 1H), 5.10 – 5.02 (m, 3H), 3.71 – 3.62 (m, 1H), 2.92 – 2.87 (m, 1H), 2.51 (d, J =

11.9 Hz, 1H), 2.42 (d, J = 11.9 Hz, 1H), 2.11 (d, J = 8.4 Hz, 1H), 1.98 (td, J = 13.6, 4.0

Hz, 1H), 1.89 − 1.47 (m, 13H), 1.46 − 1.28 (m, 5H), 1.24 − 1.12 (m, 5H), 1.11 (s, 3H),

1.07 − 1.04 (m, 1H), 0.91 (s, 3H), 0.90 (s, 3H), 0.69 (s, 3H); **^{13}C NMR** (151 MHz,

CDCl$_3$) δ 205.78, 177.38, 171.14, 144.43, 143.82, 136.35, 129.70, 129.65, 129.52,

128.40, 128.39, 128.38, 128.01, 127.99, 127.98, 127.94, 127.91, 127.89, 127.87, 126.81,

126.77, 122.11, 72.03, 66.70, 65.92, 60.39, 55.72, 51.24, 46.66, 46.36, 45.78, 41.71,

41.30, 39.01, 37.67, 36.96, 33.80, 33.08, 32.39, 32.32, 31.72, 31.58, 30.69, 30.66, 27.54,

27.12, 25.94, 23.70, 23.63, 23.61, 22.97, 22.65, 21.07, 18.34, 16.77, 15.31, 14.20, 14.14;

**HRMS** *m/z* (ESI): Calcd for C$_{56}$H$_{66}$O$_4$NaS [M+Na] 857.4580, found 857.4573.
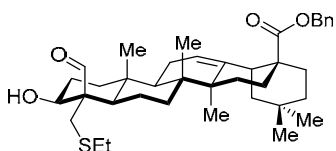
(133): TLC R_f 0.24 (4:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3519, 2947, 2867,

1713.1497, 1455, 1385, 1260, 1209, 1159, 1122, 1080, 1028, 1007, 970, 910, 821, 733,

696 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.82 (d, *J* = 2.1 Hz, 1H), 7.38 − 7.28 (m, 5H),

5.28 (t, *J* = 3.6 Hz, 1H), 5.12 − 5.02 (m, 2H), 3.67 (tdd, *J* = 11.7, 5.4, 2.1 Hz, 1H), 3.03

(d, *J* = 9.4 Hz, 1H), 2.98 (d, *J* = 12.9 Hz, 1H), 2.93 − 2.86 (m, 2H), 2.60 (qd, *J* = 7.4, 4.5

Hz, 2H), 1.98 (td, *J* = 13.7, 4.1 Hz, 1H), 1.92 − 1.77 (m, 4H), 1.74 − 1.53 (m, 10H), 1.51

(dd, *J* = 12.7, 3.1 Hz, 1H), 1.48 − 1.41 (m, 2H), 1.37 − 1.30 (m, 3H), 1.28 (t, *J* = 7.4 Hz,

4H), 1.22 − 1.14 (m, 2H), 1.13 (s, 3H), 1.09 − 0.99 (m, 3H), 0.92 (s, 3H), 0.90 (s, 3H),

0.80 (s, 3H), 0.58 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 206.92, 177.38, 143.78,

136.35, 128.39, 127.99, 127.91, 122.19, 72.07, 65.92, 55.76, 50.98, 46.67, 46.45, 45.77,

41.74, 41.36, 39.03, 37.82, 36.91, 34.65, 34.51, 33.81, 33.08, 32.38, 32.36, 32.31, 31.58,

30.69, 29.05, 27.95, 27.53, 27.49, 26.90, 25.81, 25.27, 23.71, 23.62, 22.98, 22.65, 20.70,

18.45, 16.88, 15.01, 14.97, 14.14, 11.45; **HRMS** *m/z* (ESI): Calcd for $C_{39}H_{56}O_4NaS$ [M+Na] 643.3797, found 643.3796.
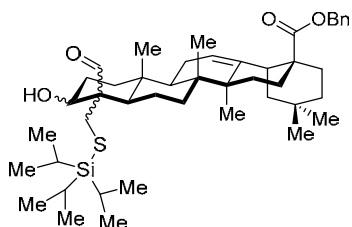
(134): TLC R_f 0.57 (4:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3517, 2944, 2865, 1723, 1461, 1384, 1260, 1122, 1081, 1012, 882, 823, 736, 696, 647 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 9.93 (s, 1H), 7.38 − 7.29 (m, 5H), 5.28 (t, *J* = 3.6 Hz, 1H), 5.12 − 5.00 (m, 2H), 4.52 (dt, *J* = 4.1, 2.2 Hz, 1H), 3.64 − 3.58 (m, 1H), 3.01 − 2.92 (m, 2H), 2.92 − 2.86 (m, 1H), 2.02 − 1.94 (m, 2H), 1.94 − 1.86 (m, 1H), 1.85 − 1.78 (m, 1H), 1.77 − 1.39 (m, 15H), 1.37 − 1.29 (m, 3H), 1.29 − 1.25 (m, 3H), 1.22 − 1.15 (m, 3H), 1.13 (s, 3H), 1.12 (d, *J* = 7.3 Hz, 17H), 1.05 (d, *J* = 1.2 Hz, 5H), 0.92 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.63 (s, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 209.91, 177.45, 143.66, 136.38, 128.40, 127.87, 127.84, 122.05, 67.52, 65.89, 54.76, 47.47, 46.69, 46.07, 45.77, 41.71, 41.31, 39.29, 37.06, 34.65, 33.81, 33.07, 32.31, 32.14, 32.11, 31.59, 30.69, 27.49, 26.90, 25.95, 25.44, 25.27, 24.39, 23.62, 23.20, 22.96, 22.66, 19.30, 18.62, 18.60, 18.57, 18.54, 17.70, 16.86, 16.27, 14.14, 12.60, 12.26; **HRMS** *m/z* (ESI): Calcd for $C_{46}H_{72}O_4NaSSi$ [M+Na] 771.4818, found 771.4833.
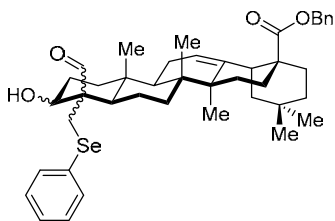
(135): Isomer A

TLC R_f 0.38 (4:1 hexanes:ethyl acetate); **¹H NMR** (600 MHz, CDCl₃) δ 9.74 (s, 1H), 7.55 − 7.50 (m, 2H), 7.37 − 7.26 (m, 7H), 5.28 (t, *J* = 3.7 Hz, 1H), 5.12 − 5.00 (m, 2H), 4.33 (q, *J* = 3.0 Hz, 1H), 3.32 (d, *J* = 12.7 Hz, 1H), 3.18 (d, *J* = 12.7 Hz, 1H), 2.93 − 2.84 (m, 2H), 2.03 − 1.99 (m, 1H), 1.99 − 1.93 (m, 1H), 1.91 − 1.78 (m, 2H), 1.77 − 1.69 (m, 2H), 1.69 − 1.58 (m, 4H), 1.56 − 1.41 (m, 6H), 1.36 − 1.23 (m, 5H), 1.21 − 1.15 (m, 3H), 1.14 (s, 3H), 1.04 − 1.00 (m, 1H), 0.95 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.58 (s, 3H); **¹³C NMR** (151 MHz, CDCl₃) δ 208.77, 177.40, 143.71, 136.37, 133.14, 131.58, 129.28, 128.41, 128.39, 127.97, 127.90, 127.41, 122.00, 69.91, 65.90, 55.89, 47.51, 46.66, 45.81, 45.41, 41.73, 41.32, 39.43, 36.82, 34.65, 33.81, 33.08, 32.30, 32.15, 32.13, 31.59, 30.69, 29.61, 27.49, 25.95, 25.27, 24.99, 23.62, 23.18, 22.96, 22.66, 19.75, 16.79, 16.33, 14.14;

Isomer B:

TLC R_f 0.32 (4:1 hexanes:ethyl acetate); **¹H NMR** (600 MHz, CDCl₃) δ 9.79 (s, 1H), 7.58 − 7.53 (m, 2H), 7.38 − 7.26 (m, 8H), 5.27 (t, *J* = 3.7 Hz, 1H), 5.12 − 5.00 (m, 2H), 4.11 (dt, *J* = 11.7, 4.7 Hz, 1H), 3.59 (d, *J* = 12.5 Hz, 1H), 3.19 (d, *J* = 12.5 Hz, 1H), 2.89 (dd, *J* = 13.8, 4.0 Hz, 1H), 2.23 (d, *J* = 4.9 Hz, 1H), 1.96 (td, *J* = 13.7, 4.1 Hz, 1H), 1.86 − 1.81 (m, 2H), 1.78 − 1.60 (m, 7H), 1.59 − 1.50 (m, 5H), 1.45 − 1.24 (m, 6H), 1.22 − 1.11 (m, 4H), 1.10 (s, 3H), 1.07 − 0.97 (m, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.57 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 206.42, 177.34, 143.67, 136.34, 133.42, 132.55, 129.21, 128.40, 128.00, 127.98, 127.92, 127.17, 121.91, 73.58, 65.92, 57.74, 52.04,

52.02, 47.54, 46.67, 46.66, 45.81, 41.66, 41.34, 39.28, 38.03, 36.90, 34.65, 34.52, 33.80, 33.09, 33.07, 32.31, 32.27, 32.16, 31.59, 30.70, 30.68, 29.05, 27.52, 26.90, 25.85, 25.81, 25.79, 25.49, 25.27, 23.61, 23.33, 22.95, 22.66, 20.70, 20.43, 16.72, 16.10, 14.14, 11.45; HRMS *m/z* (ESI): Calcd for $C_{43}H_{57}O_4Se$ [M+Na] 717.3422, found 717.3398.
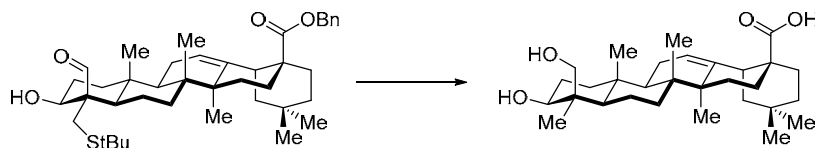
General Procedure for desulfurization:

(136): A slurry of Raney nickel (1 mL, washed with 3×5 mL ethanol) is added to a solution of thioether (10 mg, 0.015 mmol, 1 equiv) in ethanol (2 mL) were heated at 50 °C for 12 hr. After all TLC spots converge to one, reaction was filtered through celite and acidified to pH 3 with aqueous HCl (1.0 N), diluted with dichloromethane then washed with a saturated solution of EDTA. Organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated. Crude mixture was taken onto the next step without further purification.
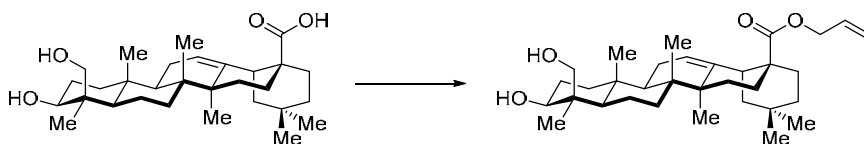
(S136): To the crude diol carboxylate **136** in dimethylformamide (1 mL) was added allyl bromide (6 µL, 0.053 mmol, 5 equiv) and cesium carbonate (3.5 mg, 0.011 mmol, 1 equiv). After 24 hr, reaction was diluted with water and washed with ethyl acetate (3×10 mL). Combined organics were washed with brine, dried with sodium sulfate,

concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 4:1 to 1:1) to give diol **S136**.

^{1}H NMR (500 MHz, CDCl$_3$) δ 5.89 – 5.78 (m, 0H), 5.28 – 5.21 (m, 2H), 5.14 (dd, J = 10.2, 0.9 Hz, 1H), 4.46 (s, 2H), 2.85 – 2.77 (m, 1H), 1.94 – 1.86 (m, 1H), 1.86 – 1.74 (m, 3H), 1.68 – 1.54 (m, 5H), 1.44 – 1.25 (m, 5H), 1.21 (s, 3H), 1.07 (s, 3H), 0.86 (s, 3H), 0.83 (s, 3H), 0.76 (s, 3H), 0.68 (s, 3H).
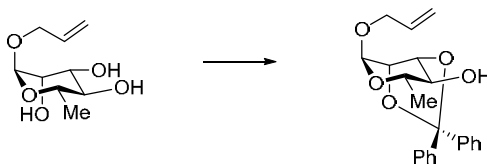
APPENDIX C

EXPERIMENTAL PROCEDURES FOR CHAPTER 4

General Procedures. Reactions were performed in flame-dried sealed-tubes or modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate and sulfoxide reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then flame-dried under vacuum. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed using glass plates pre-coated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm).

Materials. Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed columns of neutral alumina under an argon atmosphere. Methanol was distilled from magnesium at 760 Torr. Trifluoromethanesulfonic anhydride was distilled from phosphorus pentoxide at 760 Torr. Boron trifluoride diethyl etherate was distilled from calcium hydride at 760 Torr. All other chemicals were obtained from commercial vendors and were used without further purification unless noted otherwise.

Instrumentation. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer or a Bruker Tensor 27. Data are presented as the frequency of absorption (cm^{-1}). Proton and carbon-13 nuclear magnetic resonance (^{1}H NMR and ^{13}CNMR) spectra were recorded on a Bruker Avance III instrument; chemical

shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent (d-chloroform: δ 7.26 for ^{1}H NMR, δ 77.16 for ^{13}C NMR; d6-benzene: δ 7.16 for ^{1}H NMR, δ 128.06 for ^{13}C NMR; d4-methanol: δ 3.31 for ^{1}H NMR, δ 49.00 for ^{13}C NMR; d3-acetonitrile: δ 1.94 for ^{1}H NMR, δ 1.32 for ^{13}C NMR; deuterium oxide: δ 4.79 for ^{1}H NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment. RP-HPLC purification and analyses were carried out on a Waters 2545 binary gradient HPLC system equipped with a Waters 2996 photodiode array detector, and absorbances were monitored at wavelengths of 210–600 nm.

(144): Allyl glycoside **143** (7.0 g, 34.2 mmol, 1 equiv), benzophenone dimethyl acetal (19.6 g, 85.7 mmol, 2.5 equiv), camphorsulfonic acid (1.6 g, 6.8 mmol, 0.2 equiv), and dimethylformamide (80 mL) were combined in a 500 ml round-bottomed flask and placed on a rotary evaporator. Bath temperature was increased to 50 °C while decreasing pressure to 50 mTorr. After 15 hr, volatiles were removed on rotary evaporator. The remaining residue was taken up in ethyl acetate (200 mL) and wash with saturated sodium bicarbonate (100 mL) and brine, then dried with sodium sulfate, concentrated and the purified by silica gel chromatography (hexanes:ethyl acetate10:1 to 2:1) to give ketal **144** (8.4 g, 66.5% yield) as a clear oil.

TLC R_f 0.31 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3463, 3062, 2975, 2914, 1492, 1449, 1386, 1315, 1242, 1212, 1138, 1076, 996, 947, 919, 845, 819, 788, 754, 700, 642 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.54 – 7.48 (m, 4H), 7.34 – 7.22 (m, 6H), 5.87 (dddd, J = 16.9, 10.4, 6.2, 5.3 Hz, 1H), 5.27 (dd, J = 17.2, 1.6 Hz, 1H), 5.22 – 5.14 (m, 2H), 4.29 (dd, J = 7.0, 6.0 Hz, 1H), 4.21 – 4.14 (m, 1H), 4.08 (d, J = 6.0 Hz, 1H), 4.02 – 3.95 (m, 1H), 3.71 (dq, J = 9.2, 6.3 Hz, 1H), 3.38 (dd, J = 9.2, 7.0 Hz, 1H), 2.54 (bs, 1H), 1.24 (d, J = 6.3 Hz, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 142.82, 142.40, 133.48, 128.09, 128.07, 128.03, 126.04, 125.94, 117.84, 109.48, 96.11, 78.89, 75.93, 73.85, 68.03, 66.05, 17.47; **HRMS** m/z (ESI): Calcd for C$_{22}$H$_{25}$O$_5$ [M]$^+$ 369.1702, found 369.1711.
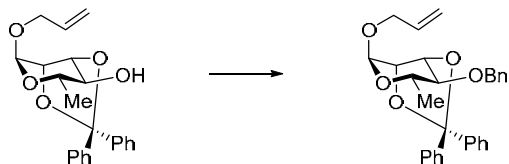
(S4.1): To an ice-cooled solution of **144** (4.6 g, 12.5 mmol, 1.0 equiv) and benzyl bromide (3 mL, 25 mmol, 2 equiv) in dimethylformamide (75 mL) was added sodium hydride (0.75 g, 18.7 mmol, 1.5 equiv). After 3 hr, reaction was diluted with saturated ammonium chloride (10 mL) and water (200 mL) and extracted with ethyl acetate (3x50 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 20:1 to 10:1) to give **S4.1** (4.9 g, 86%) as a clear oil.

TLC R_f 0.50 (10:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3376, 3062, 2917, 1492, 1450, 1389, 1211, 1131, 1072, 1027, 996, 947, 752, 698, 666, 641 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.59 – 7.51 (m, 1H), 7.35 – 7.17 (m, 3H), 5.86 (dddd, J = 16.9, 10.5, 6.2,

5.2 Hz, 0H), 5.26 (dt, J = 17.1, 1.6 Hz, 0H), 5.20 – 5.14 (m, 1H), 4.84 (d, J = 11.5 Hz, 0H), 4.50 (t, J = 6.5 Hz, 0H), 4.46 (d, J = 11.6 Hz, 0H), 4.15 (ddt, J = 12.8, 5.3, 1.5 Hz, 0H), 4.10 (d, J = 6.0 Hz, 0H), 3.96 (ddt, J = 12.9, 6.3, 1.4 Hz, 0H), 3.71 (dq, J = 10.1, 6.3 Hz, 0H), 3.20 – 3.14 (m, 0H), 1.17 (d, J = 6.2 Hz, 1H); ^{13}**C NMR** (151 MHz, CDCl$_3$) δ 143.13, 142.77, 138.23, 133.59, 128.31, 128.22, 128.11, 128.08, 128.08, 128.05, 128.00, 127.96, 127.55, 126.06, 126.02, 117.69, 109.21, 96.07, 80.62, 79.35, 76.29, 73.03, 67.87, 64.52, 17.71; **HRMS** *m/z* (ESI): Calcd for C$_{29}$H$_{30}$O$_5$Na [M+Na]$^+$ 481.1991, found 481.1980.
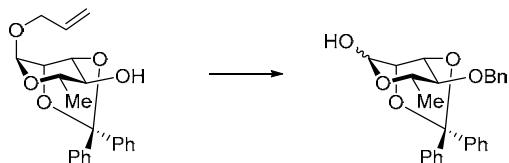
(145): To a solution of **S4.1** (394 mg, 0.860 mmol, 1.0 equiv) and pyrrolidine (0.564 mL, 6.87 mmol, 8.0 equiv) in dichloromethane/methanol (10 mL, 4:1) in a 25 mL schlenk flask was added palladium tetrakis (250 mg, 0.215 mmol, 0.25 equiv), then warmed to 35 °C with an oil bath. After 9 hr the reaction was concentrated then purified by silica gel chromatography (hexanes:ethyl acetate, 10:1 to 2:1) to give **145** (345 mg, 96%) as a pale yellow foam.

TLC R_f 0.41 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3407, 3062, 3030, 2933, 1492, 1450, 1380, 1315, 1243, 1211, 1131, 1071, 1028, 996, 947, 908, 844, 809, 751, 698, 642 cm^{-1}; ^{1}**H NMR** (500 MHz, CDCl$_3$) δ 7.54 (m, 4H), 7.34 – 7.17 (m, 11H), 5.49 (d, J = 3.9 Hz, 1H), 4.82 (d, J = 11.6 Hz, 1H), 4.53 – 4.45 (m, 2H), 4.10 (dd, J = 6.1, 0.9

Hz, 1H), 3.94 (dq, *J* = 9.2, 6.3 Hz, 1H), 3.23 (dd, *J* = 9.2, 6.7 Hz, 1H), 2.90 (d, *J* = 4.0

Hz, 1H), 1.17 (d, *J* = 6.3 Hz, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 171.23, 142.95,

142.58, 138.05, 128.25, 128.24, 128.22, 128.17, 128.11, 128.09, 128.07, 128.05, 128.03,

128.01, 127.95, 127.63, 127.58, 126.25, 126.15, 126.07, 126.06, 125.90, 125.87, 109.26,

91.92, 80.14, 78.65, 76.35, 73.06, 65.01, 60.43, 49.67, 48.81, 24.32, 23.93, 21.05, 18.00,

14.18; **HRMS** *m/z* (ESI): Calcd for C$_{26}$H$_{26}$O$_5$Na [M+Na] 441.1678, found 441.1680.
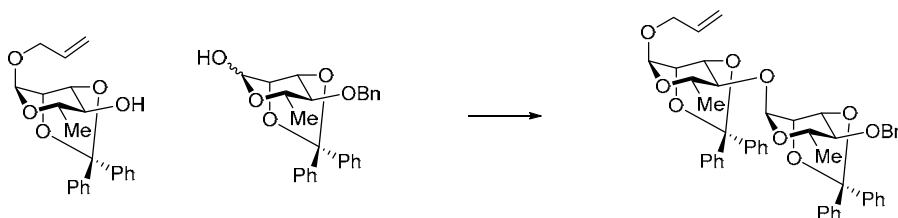
(146): Triflic an hydride (150 mL, 0.90 mmol, 1.5 equiv) was added to a solution of

hemiacetal **145** (250 mg, 0.60 mmol, 1.0 equiv), diphenyl sulfoxide (362 mg, 1.79 mmol,

3 equiv), and 2,4,6-tri-*tert*-butylpyridine (443 mg, 1.79 mmol, 3 equiv) in

dichloromethane (12 mL) cooled to −78 °C. After 15 min, reaction was warmed to −45

°C. After 85 min, acceptor **144** was added in dichloromethane (5 mL, then 2 mL wash)

via syringe. Cooling bath was removed after 1 hr and warmed to ambient temp for 15

min.   Reaction was concentrated then purified with silica gel chromatography

(hexanes:ethyl acetate 20:1 to 4:1) to give **146** (325 mg, 71% yield) and corresponding

β-anomer (75 mg, 16% yield) as white foams.

TLC *R$_f$* 0.66 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3061, 3030, 2973, 2932,

1491, 1449, 1387, 1313, 1212, 1138, 1070, 1027, 994, 947, 921, 811, 789, 752, 698, 666,

641 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.63 – 7.54 (m, 6H), 7.52 – 7.46 (m, 2H), 7.39 –

7.26 (m, 14H), 7.25 – 7.17 (m, 3H), 5.85 (dddd, J = 16.9, 10.4, 6.2, 5.3 Hz, 1H), 5.64 (s,

1H), 5.25 (dt, J = 17.2, 1.6 Hz, 1H), 5.17 (dq, J = 10.4, 1.4 Hz, 1H), 5.14 (s, 1H), 4.83 (d,

J = 11.5 Hz, 1H), 4.48 (d, J = 11.5 Hz, 1H), 4.44 (dd, J = 7.1, 6.0 Hz, 1H), 4.36 (t, J = 6.5

Hz, 1H), 4.16 – 4.10 (m, 2H), 4.09 (d, J = 6.2 Hz, 1H), 3.95 (ddt, J = 12.9, 6.2, 1.4 Hz,

1H), 3.61 (ddq, J = 16.3, 9.9, 6.2 Hz, 2H), 3.49 (dd, J = 9.9, 6.8 Hz, 1H), 3.19 (dd, J =

9.9, 7.1 Hz, 1H), 1.17 (d, J = 6.2 Hz, 3H), 1.05 (d, J = 6.2 Hz, 3H); ^{13}C NMR (151 MHz,

CDCl$_3$) δ 143.28, 143.12, 142.75, 142.21, 138.20, 133.52, 128.25, 128.18, 128.14,

128.11, 128.08, 128.02, 127.99, 127.98, 127.95, 127.89, 127.61, 126.14, 126.10, 126.06,

125.83, 117.69, 109.68, 109.19, 96.03, 95.47, 80.51, 79.29, 78.67, 76.48, 73.19, 67.92,

65.00, 63.88, 17.93, 17.43; **HRMS** m/z (ESI): Calcd for C$_{48}$H$_{48}$O$_9$Na [M+Na] 791.3196,

found 791.3197.
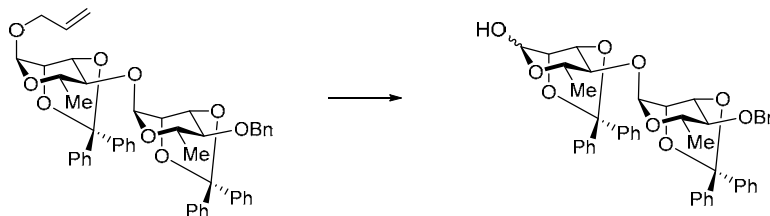
(147): To a solution of **146** (240 mg, 0.312 mmol, 1.0 equiv) and pyrrolidine (0.133 mL,

1.87 mmol, 6.0 equiv) in dichloromethane/methanol (5 mL, 4:1) in a 25 mL schlenk flask

was added tetraki(triphenylphosphine)palladium (54 mg, 0.047 mmol, 0.15 equiv), then

subjected to 3 cycles of freeze-pump-thaw, then warmed to 35 °C with an oil bath. After

5 hr the reaction was diluted with acetonitrile (precipitating palladium tetrakis), filtered

through celite, then concentrated and purified by silica gel chromatography

(hexanes:ethyl acetate, 10:1 to 2:1) to give **147** (226 mg, 99%) as a pale yellow foam.

TLC R_f 0.17 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3432, 3061, 3030, 2973, 2933, 1491, 1449, 1384, 1314, 1212, 1129, 1069, 1027, 995, 947, 908, 810, 751, 698, 641 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.63 – 7.47 (m, 8H), 7.35 – 7.16 (m, 17H), 5.63 (d, J = 2.0 Hz, 1H), 5.47 (d, J = 3.3 Hz, 1H), 4.83 (dd, J = 11.4, 1.7 Hz, 1H), 4.50 – 4.42 (m, 2H), 4.37 (t, J = 6.5 Hz, 1H), 4.16 – 4.12 (m, 1H), 4.09 (d, J = 6.2 Hz, 1H), 3.87 – 3.80 (m, 2H), 3.61 (qd, J = 6.3, 3.5 Hz, 1H), 3.54 – 3.49 (m, 1H), 3.19 (ddt, J = 9.3, 7.2, 2.3 Hz, 1H), 2.99 (d, J = 3.6 Hz, 1H), 2.96 – 2.91 (m, 0H), 1.16 (d, J = 6.2 Hz, 3H), 1.06 (d, J = 6.3 Hz, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 143.26, 142.98, 142.74, 142.10, 138.14, 128.30, 128.29, 128.26, 128.24, 128.20, 128.18, 128.17, 128.15, 128.13, 128.10, 128.08, 128.06, 128.04, 128.01, 128.00, 127.99, 127.97, 127.91, 127.62, 126.63, 126.13, 126.10, 126.08, 126.03, 125.99, 125.82, 109.68, 109.20, 95.63, 95.60, 91.82, 91.81, 80.46, 79.25, 76.75, 76.53, 73.18, 65.04, 64.28, 64.28, 53.67, 31.71, 29.20, 18.07, 17.43; **HRMS** m/z (ESI): Calcd for C$_{45}$H$_{44}$O$_9$Na [M+Na] 751.2883, found 751.2908.
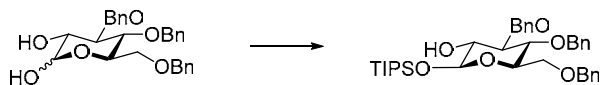
(149): Triisopropyl chloride (2.5 mL, 12 mmmol, 2 equiv) was added to a solution of diol **148** (2.7 g, 6.0 mmol, 1.0 equiv) and imidazole (1.6 g, 24 mmol, 4 equiv) in dimethylformamide (10 mL). After 75 min reaction was diluted with water (100 mL), then extracted with ethyl acetate (3 × 50 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 10:1 to 2:1) to give **149** (2.5 g, 69% yield) as a clear oil.

TLC R_f 0.61 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3474, 3030, 2942, 2865, 1496, 1453, 1358, 1114, 1065, 883, 808, 734, 695, 666 cm^{-1}; **^{1}H NMR** (500 MHz, CDCl$_3$) δ 7.41 – 7.18 (m, 17H), 4.95 (d, J = 11.2 Hz, 1H), 4.84 (t, J = 11.5 Hz, 2H), 4.62 – 4.55 (m, 3H), 4.51 (d, J = 12.2 Hz, 1H), 3.75 – 3.63 (m, 3H), 3.59 (t, J = 9.0 Hz, 1H), 3.53 – 3.43 (m, 2H), 2.27 (bs, 1H), 1.21 – 1.10 (m, 3H), 1.07 (d, J = 6.9 Hz, 18H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 138.77, 138.27, 138.26, 128.36, 128.35, 128.26, 127.88, 127.82, 127.67, 127.56, 127.54, 127.45, 97.45, 84.37, 77.56, 75.11, 75.03, 74.91, 73.39, 68.94, 17.86, 17.80, 12.19; **HRMS** m/z (ESI): Calcd for C$_{36}$H$_{50}$O$_6$NaSi [M+Na] 629.3274, found 629.3259.
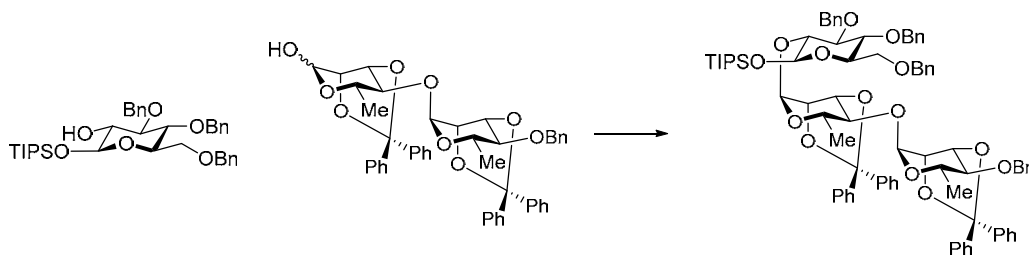
(150): Trifluoromethanesulfonic anhydride (38 μL, 0.226, 1.5 equiv) was added to a solution of hemiacetal **147** (110 mg, 0.151 mmol, 1.00 equiv), diphenyl sulfoxide (92 mg, 0.452 mmol, 3.0 equiv) and 2,4,6-tri-tertbutylpyridine (112 mg, 0.452 mmol, 3.0 equiv) in dichloromethane (4 mL) at −78 °C. The reaction stirred in a cold bath at −78 °C for 15 min and then was transferred to a bath at –40 °C for 90 min. A solution of acceptor **149** (110 mg, 0.181 mmol, 1.2 equiv) was added in dichloromethane (1.0 ml) via syringe. After 60 min, flask was transferred to an ice-bath and stirred for 15 min. Triethylamine was added, concentrated and purified via silica gel chromatography (hexanes:ethyl

acetate, 10:1 to 2:1) furnishing an analytical sample of β-glycoside **150** and mixture of **150** and acceptor **149**, which was taken on to the next step.

TLC R_f 0.54 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3062, 3030, 2941, 2866, 1726, 1599, 1495, 1451, 1364, 1313, 1253, 1210, 1063, 1027, 996, 948, 918, 863, 807, 750, 697, 641 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.63 – 7.58 (m, 4H), 7.55 – 7.51 (m, 2H), 7.41 – 7.17 (m, 35H), 5.82 (s, 1H), 5.61 (s, 1H), 4.88 (d, J = 11.0 Hz, 1H), 4.83 (d, J = 11.6 Hz, 1H), 4.78 (d, J = 10.9 Hz, 1H), 4.68 (d, J = 11.0 Hz, 1H), 4.59 (d, J = 11.0 Hz, 1H), 4.56 (dd, J = 9.7, 2.4 Hz, 2H), 4.49 (dd, J = 11.9, 5.8 Hz, 2H), 4.40 (t, J = 6.6 Hz, 1H), 4.24 (t, J = 6.4 Hz, 1H), 4.07 (dd, J = 9.2, 6.0 Hz, 2H), 3.99 (dt, J = 12.2, 6.0 Hz, 1H), 3.72 – 3.54 (m, 6H), 3.42 – 3.36 (m, 2H), 3.18 (dd, J = 9.9, 7.2 Hz, 1H), 1.12 (d, J = 6.1 Hz, 3H), 1.09 – 1.04 (m, 3H), 1.03 (d, J = 6.2 Hz, 3H), 0.98 (dd, J = 7.2, 2.7 Hz, 18H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 143.64, 143.27, 142.74, 142.57, 138.23, 138.08, 138.02, 137.84, 128.42, 128.39, 128.28, 128.23, 128.21, 128.16, 128.14, 128.11, 128.06, 127.96, 127.91, 127.79, 127.77, 127.73, 127.72, 127.69, 127.67, 127.62, 127.56, 127.51, 126.07, 126.06, 125.96, 125.61, 109.49, 109.19, 96.74, 95.96, 95.27, 85.78, 80.54, 79.35, 78.39, 78.21, 77.37, 76.88, 76.25, 75.56, 75.11, 74.85, 74.70, 73.43, 73.19, 68.67, 64.93, 63.69, 34.64, 34.50, 31.57, 29.04, 25.26, 22.65, 20.70, 18.34, 17.94, 17.86, 17.79, 17.76, 17.39, 14.13, 12.27, 12.18, 11.44; **HRMS** m/z (ESI): Calcd for C$_{81}$H$_{92}$O$_{14}$NaSi [M+Na] 1339.6154, found 1339.6169.
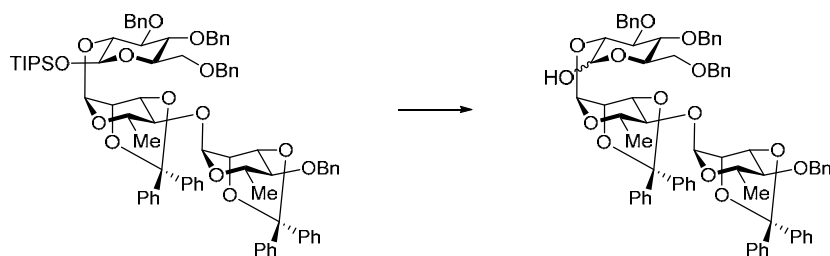
(151): A solution of tetrabutylammonium fluoride (180 mmol, 1.2 equiv) and acetic acid (10 μL, 180 mmol, 1.2 equiv) in tetrahydrofuran (5 mL) was added to a solution of trisaachride **150** monosaccharide **149** (*vide supra*) in tetrahydrofuran (25 mL). After 5 min, 2 drops of acetic acid was added, reaction concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 4:1 to 2:1) to give hemiacetal **151** (124 mg, 71% yield, two steps) as a white foam.

TLC R_f 0.40 (2:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3423, 3062, 3030, 2931, 1494, 1450, 1363, 1211, 1142, 1067, 1027, 995, 947, 750, 697, 666, 641 cm^{-1}; **^{1}H NMR** (500 MHz, CDCl$_3$) δ 7.64 – 7.49 (m, 6H), 7.42 – 7.11 (m, 34H), 5.64 (s, 1H), 5.40 (s, 1H), 5.26 (d, J = 3.6 Hz, 1H), 4.82 (t, J = 11.7 Hz, 2H), 4.75 – 4.68 (m, 2H), 4.61 – 4.56 (m, 1H), 4.54 – 4.47 (m, 3H), 4.44 (t, J = 6.5 Hz, 1H), 4.35 (t, J = 6.6 Hz, 1H), 4.18 – 4.12 (m, 2H), 4.00 (ddd, J = 10.0, 4.3, 2.2 Hz, 1H), 3.90 (t, J = 9.4 Hz, 1H), 3.79 – 3.71 (m, 2H), 3.70 – 3.54 (m, 5H), 3.51 (dd, J = 9.8, 6.9 Hz, 1H), 3.19 (dd, J = 9.9, 7.0 Hz, 1H), 2.81 (s, 1H), 1.14 (d, J = 6.2 Hz, 3H), 1.06 (d, J = 6.3 Hz, 3H); **HRMS** *m/z* (ESI): Calcd for C$_{72}$H$_{72}$O$_{14}$Na [M+Na] 1183.4820, found 1183.4800.
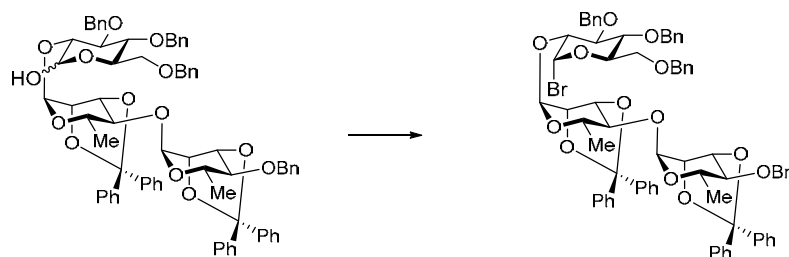
(152): Oxalyl bromide (13 mL, 0.142 mmol, 3 equiv) was added to an ice-cooled solution of hemiacetal **151** (55 mg, 0.047 mmol, 1.0 equiv), 2,4,6-tri-tertbutylpyridine (47 mg, 0.190 mmol, 4 equiv), and dimethylformamide (37 μL, 0..470 mmol, 10 equiv) in dichloromethane (2 mL). After 5 min the ice-bath was removed and warmed to ambient temperature. After 4 hr, reaction was diluted with benzene, filtered through celite, concentrated, and purified with silica gel chromatography ([hexanes:dichloromethane]:ethyl acetate, 1:0 to 6:1) to give bromide **152** (48 mg, 83 % yield) as a flaky white translucent film.

TLC R_f 0.68 (4:2:1 hexanes:dichloromethane:ethyl acetate); **FTIR** (NaCl film) 3030, 2931, 1597, 1494, 1450, 1363, 1211, 1068, 1027, 994, 908, 751, 697, 666 cm^{-1}; **^{1}H NMR** (600 MHz, Benzene-d_6) δ 7.80 – 7.75 (m, 6H), 7.48 (dd, J = 7.0, 1.8 Hz, 2H), 7.29 – 7.17 (m, 12H), 7.15 – 6.96 (m, 20H), 6.64 (d, J = 3.6 Hz, 1H), 6.06 (s, 1H), 5.42 (s, 1H), 4.97 (d, J = 11.7 Hz, 1H), 4.85 (d, J = 11.2 Hz, 1H), 4.68 (d, J = 11.1 Hz, 1H), 4.62 (d, J = 10.6 Hz, 2H), 4.60 – 4.55 (m, 3H), 4.35 – 4.30 (m, 2H), 4.26 – 4.18 (m, 3H), 4.11 (t, J = 9.2 Hz, 1H), 4.00 (dq, J = 9.9, 6.2 Hz, 1H), 3.93 (t, J = 6.7 Hz, 1H), 3.86 (t, J = 9.6 Hz, 1H), 3.83 – 3.75 (m, 2H), 3.64 (dd, J = 11.3, 3.2 Hz, 1H), 3.51 – 3.45 (m, 3H), 1.17 (d, J = 6.2 Hz, 3H), 1.16 (d, J = 6.2 Hz, 3H); **^{13}C NMR** (151 MHz, C$_6$D6) δ 143.96, 143.46, 143.36, 142.52, 138.84, 138.78, 138.35, 128.53, 128.52, 128.46, 128.43, 128.39, 128.36,

128.33, 128.25, 128.21, 128.19, 127.96, 127.84, 127.82, 127.59, 126.59, 126.54, 126.47, 126.21, 109.99, 109.74, 100.72, 95.48, 93.65, 81.80, 80.96, 80.80, 79.90, 78.68, 77.13, 76.52, 76.50, 75.98, 75.53, 75.08, 73.46, 73.12, 67.92, 65.62, 65.47, 30.75, 30.49, 17.76, 17.64; **HRMS** *m/z* (ESI): Calcd for $C_{72}H_{71}O_{13}BrNa$ [M+Na] 1245.3976, found 1245.3944.
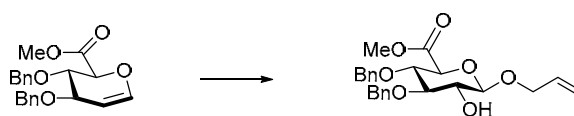
(154): A solution of dimethyldioxirane (DMDO) in acetone (40 mL, 3.5 mmol, 0.086 M, 2.5 equiv) was added to an ice-cooled solution of glycal **153** (510 mg, 1.44 mmol, 1.0 equiv) in dichloromethane (25 mL). After 2 hr, another aliquot of DMDO (20 mL, 1.75 mmol, 0.086 mmol, 1.25 equiv) was added. After 10 min, the solvent was removed and azeotroped with toluene (2 mL). A solution of allyl alcohol (0.98 mL, 14.4 mmol, 10 equiv) in dichloromethane was added, cool with an ice-bath, and treated with solid zinc (II) chloride (0.392 g, 2.88 mmol, 2 equiv). After 1 hr, reaction was warmed to ambient temperature for 15 min and diluted with dichloromethane (50 mL) and washed with water. Aqueous fraction extracted with dichloromethane (2 × 50 mL). Combined organics where washed with brine, dried over sodium sulfate, concentrated and purified with silica gel chromatography (hexanes:ethyl acetate, 10:1 to 2:1) to give **154** (365 mg, 59%) as a white amorphous solid.

TLC *R_f* 0.32 (4:2:1 hexanes:dichloromethane:ethyl acetate); **FTIR** (NaCl film) 3490, 3064, 3031, 2918, 1750, 1497, 1454, 1439, 1358, 1286, 1256, 1216, 1087, 1031, 913,

738, 698 cm^{-1}; ^{1}**H NMR** (600 MHz, CDCl$_3$) δ 7.39 – 7.20 (m, 10H), 5.90 (dddd, J = 17.1, 10.4, 6.5, 5.2 Hz, 1H), 5.31 (dq, J = 17.2, 1.5 Hz, 1H), 5.21 (dq, J = 10.4, 1.3 Hz, 1H), 4.91 (d, J = 11.3 Hz, 1H), 4.84 (d, J = 11.3 Hz, 1H), 4.80 (d, J = 10.9 Hz, 1H), 4.60 (d, J = 10.8 Hz, 1H), 4.41 – 4.31 (m, 2H), 4.09 (ddt, J = 12.8, 6.6, 1.3 Hz, 1H), 3.91 (d, J = 9.7 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.72 (s, 3H), 3.65 – 3.56 (m, 2H); ^{13}**C NMR** (151 MHz, CDCl$_3$) δ 168.89, 138.31, 137.70, 133.36, 128.45, 128.38, 127.97, 127.90, 127.85, 127.77, 118.27, 101.89, 83.51, 78.94, 75.20, 75.04, 74.56, 74.12, 70.41, 52.51; **HRMS** m/z (ESI): Calcd for C$_{24}$H$_{28}$O$_7$Na [M+Na] 451.1733, found 451.1725.
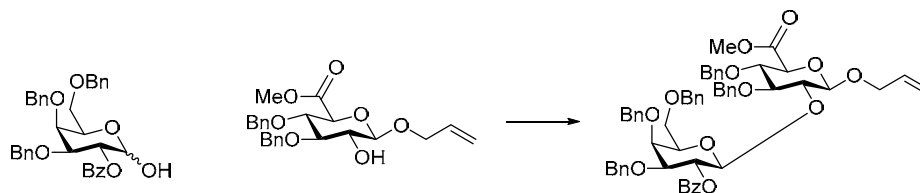


(156): Trifluoromethanesulfonic anhydride (365 μL, 0.2.16, 3.0 equiv) was added to a solution of hemiacetal **155** (800 mg, 1.44 mmol, 2.00 equiv), diphenyl sulfoxide (875 mg, 4.33 mmol, 6.0 equiv) and 2,4,6-tri-tertbutylpyridine (1.07 g, 4.33 mmol, 6.0 equiv) in dichloromethane (30 mL) at −78 °C. The reaction stirred in a cold bath at −78 °C for 10 min and then was transferred to a bath at −40 °C for 55 min. A solution of acceptor **154** (310 mg, 0.721 mmol, 1.0 equiv) was added in dichloromethane (10 mL, then 5 mL wash) via syringe. After 40min, flask was transferred to an ice-bath and stirred for 20 min. Reaction contents were diluted with water and extracted with dichloromethane (3 × 50 mL), washed with brine, dried over sodium sulfate, concentrated and purified via silica gel chromatography (benzene:ethyl acetate, 1:0 to 5:1) furnishing a separable

mixture of α/β-glycosides **156** (500 mg, 72%) and α-glycoside (85 mg, 12%) as colorless viscous oils.

TLC R_f 0.37 (4:2:1 hexanes:dichloromethane:ethyl acetate); **FTIR** (NaCl film) 3087, 3063, 3030, 3006, 2923, 2869, 1748, 1724, 1602, 1584, 1496, 1450, 1361, 1308, 1276, 1217, 1155, 1107, 1070, 1028, 1000, 936, 912, 844, 805, 732, 713, 699 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.86 – 7.81 (m, 2H), 7.49 (dd, J = 8.2, 6.5 Hz, 1H), 7.37 – 7.01 (m, 29H), 5.83 (ddt, J = 17.1, 10.6, 5.3 Hz, 1H), 5.69 (dd, J = 10.0, 8.0 Hz, 1H), 5.25 (dq, J = 17.3, 1.7 Hz, 1H), 5.08 (dt, J = 10.6, 1.6 Hz, 1H), 5.00 (d, J = 11.7 Hz, 1H), 4.94 (d, J = 8.1 Hz, 1H), 4.73 (d, J = 11.6 Hz, 1H), 4.63 (d, J = 11.7 Hz, 1H), 4.62 – 4.57 (m, 2H), 4.55 (d, J = 10.7 Hz, 1H), 4.50 (d, J = 11.6 Hz, 1H), 4.46 – 4.38 (m, 4H), 4.31 (ddt, J = 12.7, 4.8, 1.6 Hz, 1H), 4.04 – 3.97 (m, 2H), 3.85 (d, J = 9.4 Hz, 1H), 3.78 – 3.70 (m, 2H), 3.69 – 3.66 (m, 1H), 3.65 (s, 3H), 3.62 – 3.54 (m, 4H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 169.36, 165.17, 138.44, 137.71, 137.66, 137.48, 133.77, 132.70, 129.92, 129.77, 128.42, 128.29, 128.25, 128.23, 128.19, 128.16, 128.00, 127.97, 127.88, 127.86, 127.64, 127.59, 127.58, 127.51, 127.25, 127.01, 116.84, 102.10, 100.98, 83.26, 81.77, 80.08, 78.49, 75.21, 74.79, 74.40, 74.10, 73.56, 73.41, 72.38, 72.07, 71.47, 70.43, 68.32, 52.33; **HRMS** m/z (ESI): Calcd for C$_{58}$H$_{60}$O$_{13}$Na [M+Na] 987.3932, found 987.3936.
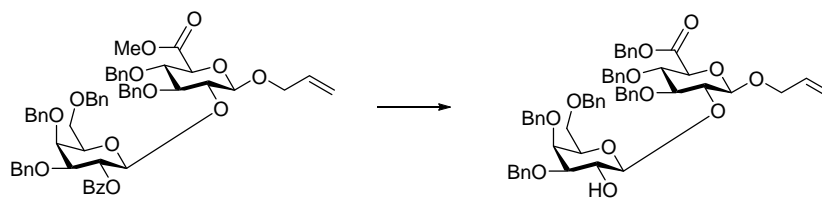
(157): Aqueous sodium hydroxide (2.15 mL, 2.15 mmol, 5 equiv) was added to a solution of **156** (520 mg, 0.539 mmol, 1.0 equiv) in 1,4-dioxane (10 mL) and heated to 50 °C. After methyl ester saponifcation complete by TLC (4 hr), cesium carbonate (0.874 g, 2.69 mmol, 5 equiv) and methanol were added. After 12 hr reaction was diluted with dichloromethane (50 mL) and filtered through celite, concentrated, taken up in dimethylformamide (12 mL) and added potassium bicarbonate (0.81 g, 8.1 mmol, 15 equiv) and benzyl bromide (0.64 mL, 5.38 mmol, 10 equiv). After 6 hr reaction was diluted with water and extracted with dichloromethane (3 × 50 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate 10:1 to 2:1) to give **157** (430 mg, 85%) as a white foam.

TLC R_f 0.49 (2:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3474, 3031, 2917, 1745, 1496, 1453, 1361, 1268, 1212, 1074, 1027, 735, 697 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.35 − 7.21 (m, 28H), 7.13 − 7.07 (m, 2H), 5.83 (ddt, J = 17.1, 10.6, 5.4 Hz, 1H), 5.22 (dq, J = 17.3, 1.7 Hz, 1H), 5.18 (d, J = 12.2 Hz, 1H), 5.13 (d, J = 12.2 Hz, 1H), 5.05 (dq, J = 10.5, 1.5 Hz, 1H), 4.91 (d, J = 11.5 Hz, 1H), 4.89 − 4.82 (m, 2H), 4.70 − 4.63 (m, 3H), 4.58 (d, J = 11.6 Hz, 1H), 4.54 − 4.50 (m, 1H), 4.49 (d, J = 7.7 Hz, 1H), 4.46 (d, J = 10.7 Hz, 1H), 4.39 (s, 2H), 4.33 − 4.28 (m, 1H), 4.07 − 4.02 (m, 1H), 3.94 − 3.84 (m, 4H), 3.77 − 3.69 (m, 2H), 3.63 (dd, J = 8.0, 6.9 Hz, 1H), 3.57 − 3.50 (m, 2H), 3.40 (dd, J = 9.8,

2.9 Hz, 1H), 2.91 (d, J = 2.2 Hz, 1H); ^{13}C **NMR** (151 MHz, CDCl$_3$) δ 168.38, 138.72, 138.44, 137.73, 137.53, 137.48, 134.90, 133.71, 128.52, 128.51, 128.46, 128.45, 128.34, 128.32, 128.30, 128.11, 128.03, 127.94, 127.91, 127.84, 127.75, 127.73, 127.51, 127.46, 127.33, 116.93, 104.64, 102.11, 82.86, 81.48, 81.26, 79.46, 75.87, 74.80, 74.48, 74.34, 73.71, 73.39, 73.32, 72.96, 72.45, 70.70, 68.31, 67.30; **HRMS** *m/z* (ESI): Calcd for C$_{57}$H$_{60}$O$_{12}$Na [M+Na] 959.3982, found 959.4026.
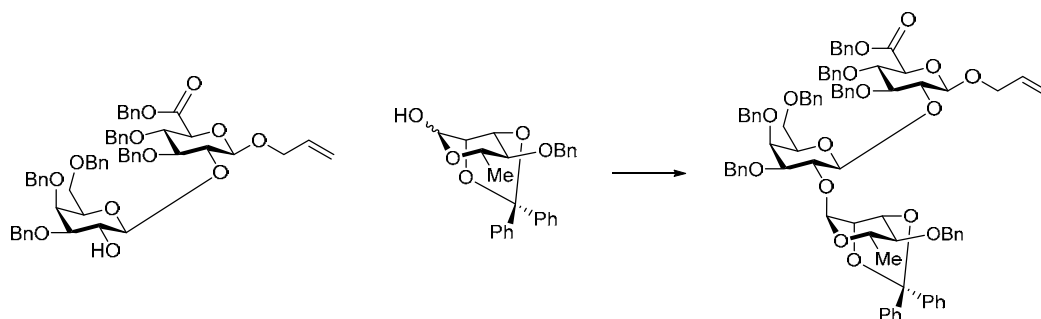

(158): Trifluoromethanesulfonic anhydride (260 μL, 1.54, 3.0 equiv) was added to a solution of hemiacetal **145** (433 mg, 1.03 mmol, 2.00 equiv), diphenyl sulfoxide (621 mg, 3.07 mmol, 6.0 equiv) and 2,4,6-tri-tertbutylpyridine (0.760 g, 3.07 mmol, 6.0 equiv) in dichloromethane (30 mL) at −78 °C. The reaction stirred in a cold bath at −78 °C for 15 min and then was transferred to a bath at −45 °C for 90 min. A solution of acceptor **157** (480 mg, 0.512 mmol, 1.0 equiv) was added in dichloromethane (6 mL, then 4 mL wash) via syringe. After 60 min, flask was transferred to an ice-bath and stirred for 30 min. Reaction contents were diluted with water and extracted with dichloromethane (3× 50 mL), washed with brine, dried over sodium sulfate, concentrated and purified via silica gel chromatography (hexanes:ethyl acetate, 20:1 to 4:1) furnishing α-glycoside **158** (653 mg, 95%) as a sticky colorless foam.

TLC R_f 0.57 (2:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3062, 3030, 2870, 1745, 1585, 1496, 1452, 1363, 1308, 1210, 1155, 1070, 1026, 996, 733, 697, 666 cm^{-1};

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.96 – 7.90 (m, 1H), 7.53 – 7.40 (m, 5H), 7.35 – 7.07 (m, 39H), 5.78 (ddt, J = 17.2, 10.4, 5.1 Hz, 1H), 5.74 (s, 1H), 5.30 (dd, J = 17.3, 1.8 Hz, 1H), 5.16 – 5.10 (m, 2H), 5.02 (dt, J = 10.6, 1.6 Hz, 1H), 4.88 (d, J = 11.6 Hz, 1H), 4.83 – 4.78 (m, 2H), 4.76 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 10.8 Hz, 1H), 4.65 (d, J = 7.8 Hz, 1H), 4.62 (d, J = 11.8 Hz, 1H), 4.57 (d, J = 11.6 Hz, 1H), 4.54 – 4.50 (m, 2H), 4.46 – 4.40 (m, 3H), 4.38 (d, J = 11.8 Hz, 1H), 4.26 – 4.21 (m, 2H), 4.14 (dq, J = 10.1, 6.2 Hz, 1H), 4.08 (dd, J = 9.8, 7.7 Hz, 1H), 4.05 – 3.98 (m, 3H), 3.96 – 3.90 (m, 2H), 3.88 (dd, J = 8.3, 5.7 Hz, 1H), 3.67 (dd, J = 9.5, 8.3 Hz, 1H), 3.63 (dd, J = 9.2, 7.7 Hz, 1H), 3.54 (dd, J = 9.2, 5.4 Hz, 1H), 3.45 – 3.40 (m, 1H), 3.35 (dd, J = 9.9, 2.8 Hz, 1H), 3.11 (dd, J = 10.1, 6.8 Hz, 1H), 1.11 (d, J = 6.2 Hz, 3H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 169.04, 167.11, 159.40, 143.34, 142.87, 141.52, 138.62, 138.47, 138.12, 137.84, 137.80, 137.43, 135.05, 133.66, 133.11, 129.21, 128.53, 128.50, 128.48, 128.42, 128.39, 128.37, 128.34, 128.29, 128.27, 128.25, 128.19, 128.17, 128.13, 128.10, 128.09, 128.07, 128.06, 128.03, 128.00, 127.97, 127.92, 127.88, 127.86, 127.83, 127.82, 127.79, 127.77, 127.75, 127.74, 127.72, 127.69, 127.67, 127.64, 127.59, 127.58, 127.57, 127.52, 127.40, 127.17, 126.02, 125.97, 116.73, 112.04, 108.97, 101.59, 101.14, 97.54, 84.26, 82.43, 80.15, 79.52, 78.76, 78.14, 76.06, 75.14, 74.70, 74.52, 74.32, 73.56, 73.55, 73.22, 72.37, 72.26, 71.33, 69.77, 68.25, 67.15, 64.76, 37.64, 34.87, 30.84, 30.26, 17.52; **HRMS** *m/z* (ESI): Calcd for C$_{83}$H$_{84}$O$_{16}$Na [M+Na] 1359.5657, found 1359.5670.
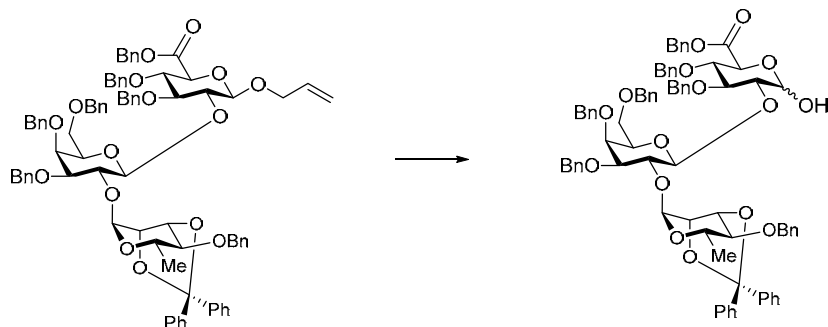
(159): To a solution of **158** (216 mg, 0.161 mmol, 1.0 equiv) and pyrrolidine (0.135 mL, 1.60 mmol, 10.0 equiv) in dichloromethane/methanol (5 mL, 4:1) in a 25 mL schlenk flask was added tetrakis(triphenylphosphine)palladium (9.2 mg, 0.008 mmol, 0.05 equiv), then subjected to 3 cycles of freeze-pump-thaw, then warmed to 35 °C with an oil bath. After 5 hr the reaction was diluted with acetonitrile (precipitating palladium tetrakis), filtered through celite, then passed through a short pad of silica gel to give crude **159**, which was taken to the next step without further purification.
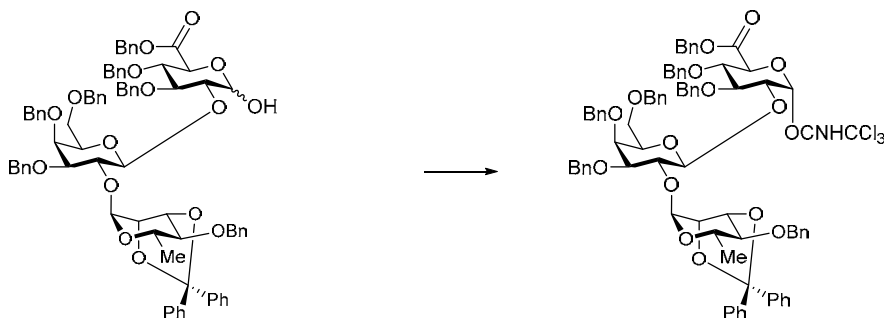
(160): To an ice-cooled solution of crude hemiacetal **159** (44 mg, 0.034 mmol, 1.0 equiv) in dichloromethane (3 mL) was added trichloroacetonitrile (68 µL, 0.678 mmol, 20 equiv) and DBU (5.6 µL, 0.037 mmol, 1.1 equiv). After 6 hr, rxn was concentrated, then purified with silica gel chromatography, (hexanes:[ethyl acetate+0.5% triethylamine], 20:1 to 4:1) to give imidate **160** (34 mg, 56 % yield, 2 steps).

TLC R_f 0.20 (4:1 hexanes:ethyl acetate); **FTIR** (NaCl, film) 3386, 3062, 3030, 2920, 1746, 1672, 1604, 1549, 1496, 1452, 1364, 1281, 1209, 1176, 1071, 1027, 910, 795, 734, 696, 665 cm^{-1}; **^{1}H NMR** (500 MHz, C$_6$D$_6$) δ 8.55 (s, 1H), 7.65 (d, J = 7.5 Hz, 2H), 7.54 (d, J = 7.2 Hz, 2H), 7.45 (d, J = 7.3 Hz, 2H), 7.35 (d, J = 7.3 Hz, 2H), 7.28 – 7.20 (m, 7H), 7.13 – 6.94 (m, 30H), 6.12 (s, 1H), 5.30 (d, J = 11.3 Hz, 1H), 5.05 – 5.00 (m, 2H), 4.88 (d, J = 10.1 Hz, 1H), 4.82 – 4.71 (m, 5H), 4.62 (d, J = 10.9 Hz, 1H), 4.56 (d, J = 7.5 Hz, 1H), 4.46 – 4.26 (m, 8H), 4.25 (d, J = 6.3 Hz, 1H), 4.24 – 4.15 (m, 3H), 4.09 – 4.04 (m, 2H), 4.00 (dd, J = 9.6, 3.9 Hz, 1H), 3.72 (s, 1H), 3.67 – 3.62 (m, 1H), 3.53 (dd, J = 8.9, 5.7 Hz, 1H), 3.34 (dd, J = 10.1, 6.8 Hz, 1H), 3.29 (t, J = 6.3 Hz, 1H), 3.24 (dd, J = 9.8, 2.6 Hz, 1H), 1.40 (d, J = 6.1 Hz, 3H).
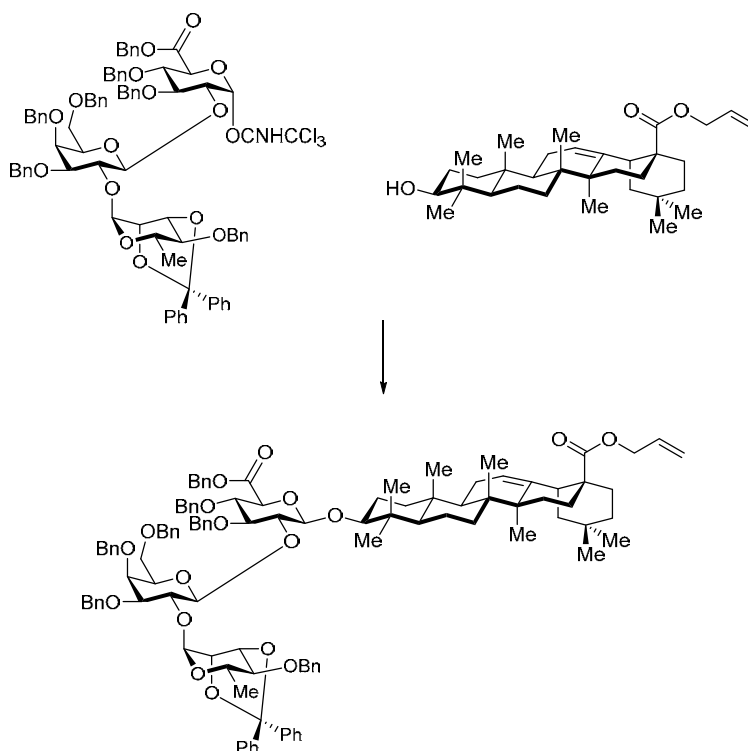
(165): A solution of tris-pentafluorophenylborane (0.45 mg, 0.0086 mmol, 0.05 equiv) in dichloromethane (50 µL) was added to a solution of imidate **160** (25 mg, 0.173 mmol, 1.0 equiv) and allyl oleanolate **164** (17 mg, 0.35 mmol, 2.0 equiv) in dichloromethane (1 mL). After 50 min, reaction and concentrated and purified with silica gel chromatography (2% acetone in 1:1 hexanes:benzene) to give **165** (22 mg, 72% yield). Analysis of crude NMR showed 14:1 β:α-glycosides.

TLC R_f 0.60 (20:1 benzene:ethyl acetate); **FTIR** (NaCl film) 3062, 3030, 2943, 2874, 1748, 1726, 1496, 1452, 1362, 1260, 1208, 1174, 1156, 1071, 1027, 912, 750, 733, 697 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$-d) δ 7.55 − 7.47 (m, 2H), 7.42 (dd, J = 6.7, 3.0 Hz, 2H), 7.33 − 7.04 (m, 41H), 5.92 (ddt, J = 17.3, 10.8, 5.5 Hz, 1H), 5.74 (s, 1H), 5.37 − 5.30 (m, 1H), 5.29 (t, J = 3.7 Hz, 1H), 5.22 (dq, J = 10.6, 1.4 Hz, 1H), 5.19 (d, J = 12.1 Hz, 1H), 5.10 (d, J = 12.3 Hz, 1H), 4.90 (d, J = 10.9 Hz, 1H), 4.82 (d, J = 10.0 Hz, 1H),

4.79 – 4.73 (m, 2H), 4.72 – 4.66 (m, 3H), 4.54 (td, J = 5.6, 1.6 Hz, 2H), 4.54 – 4.45 (m, 3H), 4.45 – 4.40 (m, 3H), 4.31 (d, J = 7.7 Hz, 1H), 4.24 (t, J = 6.4 Hz, 1H), 4.16 (dq, J = 10.0, 6.2 Hz, 1H), 4.04 (dd, J = 9.8, 7.7 Hz, 1H), 4.01 (d, J = 6.0 Hz, 1H), 3.94 (d, J = 2.7 Hz, 1H), 3.87 (d, J = 9.8 Hz, 1H), 3.84 (dd, J = 9.6, 7.9 Hz, 1H), 3.73 (t, J = 9.5 Hz, 1H), 3.67 (t, J = 8.7 Hz, 1H), 3.59 (t, J = 9.4 Hz, 1H), 3.51 (dd, J = 9.2, 5.2 Hz, 1H), 3.41 (dd, J = 8.2, 5.4 Hz, 1H), 3.36 (dd, J = 9.8, 2.7 Hz, 1H), 3.09 (dd, J = 10.1, 6.8 Hz, 1H), 2.94 (dd, J = 11.8, 4.6 Hz, 1H), 2.89 (dd, J = 13.9, 4.6 Hz, 1H), 1.99 (td, J = 14.3, 13.7, 4.1 Hz, 1H), 1.92 – 1.77 (m, 2H), 1.78 – 1.59 (m, 6H), 1.57 – 1.43 (m, 3H), 1.43 – 1.29 (m, 3H), 1.27 – 1.13 (m, 1H), 1.11 (s, 3H), 1.07 (d, J = 6.1 Hz, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.84 (s, 3H), 0.72 (s, 3H), 0.66 (s, 3H), 0.59 (dd, J = 11.8, 1.9 Hz, 1H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 177.38, 168.27, 143.73, 143.43, 143.04, 138.85, 138.43, 137.94, 137.92, 137.78, 137.50, 135.13, 132.57, 128.50, 128.42, 128.39, 128.37, 128.35, 128.33, 128.31, 128.24, 128.23, 128.08, 128.05, 128.01, 128.00, 127.97, 127.94, 127.86, 127.81, 127.74, 127.67, 127.64, 127.21, 127.17, 126.01, 125.98, 122.47, 117.64, 108.98, 104.96, 100.74, 97.52, 91.63, 85.32, 82.32, 79.98, 79.65, 79.53, 76.06, 75.99, 75.77, 74.91, 74.42, 74.28, 73.93, 73.68, 72.89, 72.35, 72.23, 71.01, 68.13, 67.06, 64.78, 64.72, 55.63, 47.46, 46.74, 45.87, 41.64, 41.30, 39.33, 39.19, 38.44, 36.61, 33.87, 33.11, 32.64, 32.44, 30.69, 29.25, 27.76, 27.65, 25.90, 25.87, 23.64, 23.41, 23.06, 18.17, 17.69, 16.96, 16.02, 15.24; **HRMS** *m/z* (ESI): Calcd for C$_{113}$H$_{130}$O$_{18}$Na [M+Na] 1797.9155, found 1797.9110.
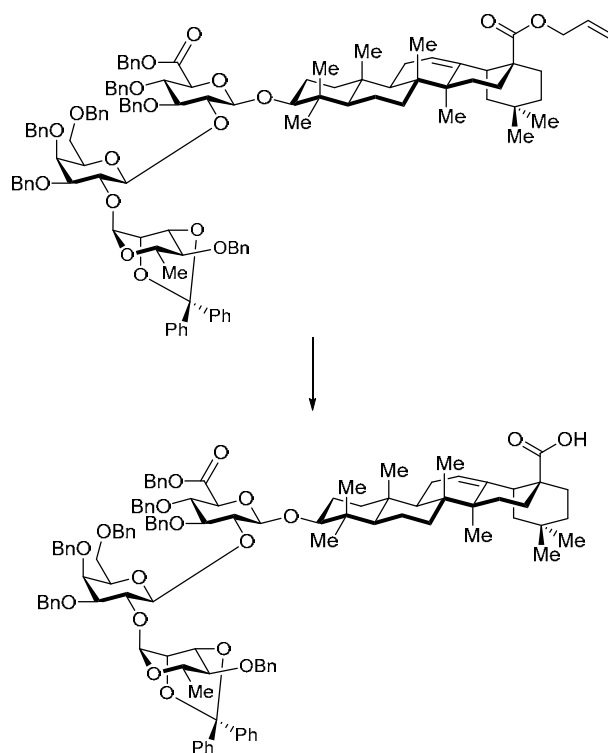
(166): Tetrakis(triphenylphosphine)palladium (1 mg, 0.0009 mmol, 0.05 equiv) was added to a solution of **165** (32 mg, 0.018 mmol, 1.0 equiv) and pyrrolidine (7.5 µL, 0.09 mmol, 5.0 equiv) in dichloromethane (1 mL). After 5 min, contents of reaction was purified directly with silica gel chromatography (hexanes:ethyl acetate, 4:1 to 2:1) to give **166** (31 mg, 99% yield) as a pale yellow foam.

TLC R_f 0.32 (2:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3030, 2941, 1749, 1697, 1653, 1558, 1540, 1966, 1454, 1362, 1208, 1071, 1027, 732, 696 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.53 – 7.49 (m, 2H), 7.44 – 7.40 (m, 2H), 7.33 – 7.03 (m, 47H), 5.74 (s, 1H), 5.29 (t, *J* = 3.6 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.82 (d, *J* = 10.0 Hz, 1H), 4.77 (d, *J* = 6.0 Hz, 1H), 4.76 (d, *J* = 8.2 Hz, 1H), 4.70 (d, *J* = 6.4 Hz, 1H), 4.68 (dd, *J* = 7.5, 1.8 Hz, 2H), 4.51 (d, *J* = 10.8 Hz, 1H), 4.49 – 4.44 (m, 2H), 4.44 – 4.39 (m, 3H), 4.31 (d, *J* = 7.6 Hz, 1H), 4.24 (t, *J* = 6.4

Hz, 1H), 4.16 (dq, J = 12.3, 6.2 Hz, 1H), 4.04 (dd, J = 9.8, 7.7 Hz, 1H), 4.01 (d, J = 6.0

Hz, 1H), 3.94 (d, J = 2.7 Hz, 1H), 3.87 (d, J = 9.6 Hz, 1H), 3.84 (dd, J = 9.4, 7.6 Hz, 1H),

3.72 (t, J = 9.4 Hz, 1H), 3.67 (t, J = 8.7 Hz, 1H), 3.59 (t, J = 9.4 Hz, 1H), 3.51 (dd, J =

9.2, 5.2 Hz, 1H), 3.40 (dd, J = 8.2, 5.4 Hz, 1H), 3.35 (dd, J = 9.7, 2.7 Hz, 1H), 3.09 (dd, J

= 10.1, 6.8 Hz, 1H), 2.95 (dd, J = 11.7, 4.7 Hz, 1H), 2.83 (dd, J = 13.8, 4.7 Hz, 1H), 1.98

(dt, J = 13.7, 6.9 Hz, 1H), 1.92 – 1.70 (m, 6H), 1.68 – 1.56 (m, 5H), 1.53 – 1.45 (m, 2H),

1.41 – 1.30 (m, 4H), 1.29 – 1.14 (m, 5H), 1.12 (s, 3H), 1.07 (d, J = 6.2 Hz, 3H), 0.94 (s,

3H), 0.91 (d, J = 4.3 Hz, 6H), 0.85 (s, 4H), 0.76 (s, 3H), 0.66 (s, 3H), 0.59 (dd, J = 11.8,

1.9 Hz, 1H); ^{13}**C NMR** (151 MHz, CDCl$_3$) δ 183.49, 168.27, 143.52, 143.43, 143.04,

138.84, 138.43, 137.94, 137.84, 137.79, 137.50, 135.13, 135.01, 129.01, 128.50, 128.45,

128.41, 128.38, 128.37, 128.34, 128.33, 128.30, 128.27, 128.22, 128.20, 128.08, 128.05,

128.01, 128.00, 127.97, 127.94, 127.86, 127.81, 127.74, 127.73, 127.67, 127.64, 127.20,

127.17, 126.01, 125.98, 125.27, 122.67, 108.98, 104.94, 100.73, 97.51, 91.64, 85.32,

82.29, 79.98, 79.65, 79.51, 76.06, 75.98, 75.76, 74.90, 74.41, 74.28, 73.92, 73.67, 72.91,

72.34, 72.21, 70.98, 68.15, 67.06, 64.71, 55.65, 50.77, 47.47, 46.55, 45.83, 41.62, 41.03,

39.26, 39.17, 38.43, 36.62, 33.79, 33.07, 32.58, 32.44, 30.67, 27.76, 27.60, 25.93, 25.91,

25.87, 23.70, 23.58, 23.40, 22.93, 21.46, 18.17, 17.70, 16.78, 16.02, 15.23; **HRMS** *m/z*

(ESI): Calcd for C$_{110}$H$_{126}$O$_{18}$Na [M+Na] 1757.8842, found 1757.8853.
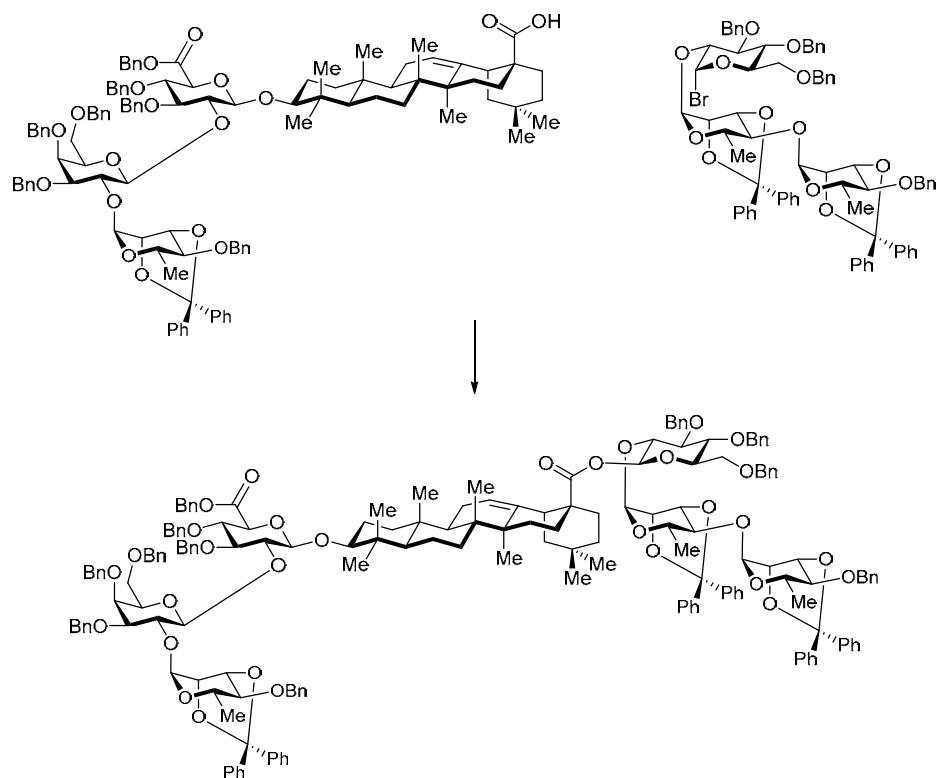
(167): Carboxylic acid **166** (21 mg, 0.12 mmol, 1 equiv) bromide **152** (45 mg, 0.036 mmol, 3 equiv), potassium carbonate (8.4 mg, 0.061 mmol, 5 equiv), and tetrabutylammonium bromide (12 mg, 0.036 mmol, 3 equiv) were combined in a biphasic mixture of ethyl acetate (1.5 mL) and water (1.5 mL) and heated to 45 °C. After 4 hr, another aliquot of bromide **152** (15 mg, 0.012 mmol, 1 equiv) in ethyl acetate (0.5 mL) was added. After 1 hr, rxn was diluted with ethyl acetate (25 mL) and sonicated for 5 min. Saturated aqueous sodium bicarbonate (25 mL) was added to the mixture, then extracted with ethyl acetate (2 × 50 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated and purified with silica gel chromatography (benzene:ethyl acetate, 1:0 to 10:1) to give **167** (24 mg, 69%) as a sticky white foam.

TLC R_f 0.53 (20:1 benzene:ethyl acetate); **FTIR** (NaCl film) 3062, 3030, 2934, 1750, 1586, 1548, 1495, 1451, 1363, 1314, 1259, 1210, 1145, 1070, 1027, 996, 948, 909, 791, 750, 735, 697, 666 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.63 – 7.04 (m, 85H), 5.75 (s, 1H), 5.63 (s, 1H), 5.56 (s, 1H), 5.54 (d, J = 7.0 Hz, 1H), 5.27 (t, J = 3.8 Hz, 1H), 5.20 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 4.90 (d, J = 11.0 Hz, 1H), 4.85 – 4.80 (m, 3H), 4.79 – 4.73 (m, 3H), 4.72 – 4.67 (m, 4H), 4.62 – 4.57 (m, 2H), 4.53 – 4.41 (m, 9H), 4.40 (d, J = 12.1 Hz, 1H), 4.30 (d, J = 7.7 Hz, 1H), 4.24 (t, J = 6.4 Hz, 1H), 4.21 (t, J = 6.6 Hz, 1H), 4.14 (dd, J = 10.6, 6.1 Hz, 1H), 4.10 (d, J = 6.0 Hz, 1H), 4.09 – 4.05 (m, 2H), 4.01 (d, J = 6.0 Hz, 1H), 3.95 (d, J = 2.7 Hz, 1H), 3.90 – 3.82 (m, 4H), 3.76 – 3.65 (m, 7H), 3.61 – 3.50 (m, 5H), 3.46 – 3.39 (m, 2H), 3.37 (dd, J = 9.8, 2.7 Hz, 1H), 3.17 (dd, J = 9.9, 7.0 Hz, 1H), 3.05 (dd, J = 10.1, 6.8 Hz, 1H), 2.93 (dd, J = 11.7, 4.7 Hz, 1H), 2.79 (dd, J = 13.6, 3.7 Hz, 1H), 1.95 – 1.58 (m, 8H), 1.55 – 1.44 (m, 4H), 1.43 – 1.38 (m, 1H), 1.37 – 1.22 (m, 5H), 1.19 (d, J = 6.2 Hz, 3H), 1.17 – 1.10 (m, 5H), 0.95 (s, 3H), 0.92 (s, 3H), 0.88 (s, 7H), 0.81 (s, 3H), 0.69 (s, 6H), 0.59 (dd, J = 11.8, 1.9 Hz, 1H); **^{13}C NMR** (151 MHz, CDCl$_3$) δ 176.10, 168.31, 143.45, 143.32, 143.27, 143.04, 142.85, 142.26, 138.70, 138.48, 138.29, 138.08, 137.96, 137.90, 137.81, 137.63, 137.54, 135.15, 130.52, 128.53, 128.47, 128.44, 128.42, 128.40, 128.36, 128.33, 128.27, 128.23, 128.20, 128.18, 128.11, 128.07, 128.06, 128.03, 127.99, 127.98, 127.95, 127.91, 127.89, 127.80, 127.76, 127.73, 127.71, 127.62, 127.57, 127.55, 127.39, 127.20, 126.12, 126.07, 126.02, 125.98, 125.96, 125.85, 122.45, 109.58, 109.27, 108.97, 104.94, 100.78, 97.49, 96.15, 95.83, 93.17, 91.62, 85.33, 84.72, 82.48, 80.49, 80.09, 79.65, 79.53, 79.36, 78.39, 77.38, 76.30, 76.13, 76.05, 75.75, 75.08, 74.92, 74.72, 74.49, 74.39, 74.28, 73.82, 73.69, 73.34, 73.08, 72.95, 72.36, 72.10, 71.09, 68.22, 68.18, 67.09, 64.99, 64.67, 64.62, 55.53, 47.45, 46.70,

45.98, 41.68, 41.27, 39.30, 39.22, 38.53, 36.63, 33.84, 33.02, 32.68, 31.86, 31.71, 30.61, 29.69, 27.95, 27.80, 25.92, 25.51, 23.62, 23.40, 22.79, 18.15, 17.93, 17.64, 17.43, 17.01, 16.08, 15.36; **HRMS** *m/z* (ESI): Calcd for $C_{182}H_{196}O_{31}Na$ [M+Na] 2900.3658, found 2900.3733.
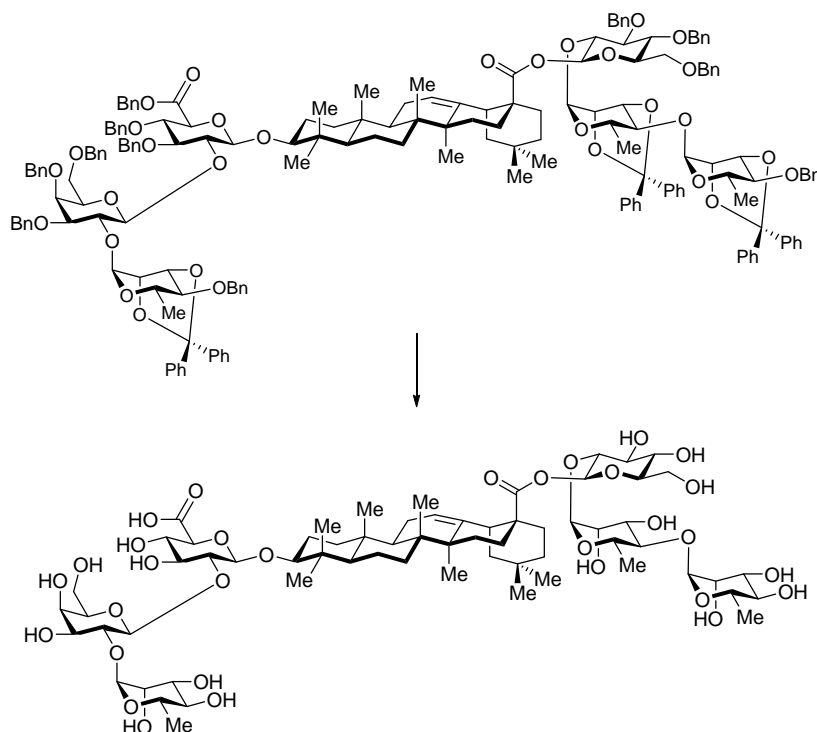
(168): A solution of fully protected lablaboside F **167** (15 mg, 0.005 mmol, 1.0 equiv) in tetrahydrofuran (3 mL) and methanol (3 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (56 mg, 0.026 mmol, 5 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) for 24 hr, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 H$_2$O:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 20 · 75%

acetonitrile (0.05% TFA) in over 19 min at a flow rate of 5 mL/min. The fraction containing the major peak (tR = 10.10 min) was collected and lyophilized to dryness to afford lablaboside F (**168**) (3.3 mg, 77 % yield) as a fluffy white solid.

$[\alpha]_D^{19}$: –44° (Lit –46°) (*c* 0.38, MeOH); **^{1}H NMR** (500 MHz, Methanol-*d*$_4$) δ 5.47 (d, *J* = 1.7 Hz, 1H), 5.26 (d, *J* = 8.1 Hz, 1H), 5.15 (d, *J* = 3.7 Hz, 1H), 5.10 (d, *J* = 1.7 Hz, 1H), 5.07 (d, *J* = 1.8 Hz, 1H), 4.70 (d, *J* = 7.7 Hz, 1H), 4.38 (d, *J* = 7.5 Hz, 1H), 4.03 (dq, *J* = 9.7, 6.2 Hz, 1H), 3.89 – 3.84 (m, 1H), 3.82 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.78 (dd, *J* = 9.5, 6.2 Hz, 1H), 3.75 (dd, *J* = 3.5, 1.7 Hz, 1H), 3.73 – 3.52 (m, 13H), 3.54 – 3.44 (m, 6H), 3.46 – 3.37 (m, 2H), 3.37 – 3.25 (m, 4H), 3.10 – 3.03 (m, 1H), 2.70 (dd, *J* = 14.1, 3.7 Hz, 1H), 1.83 – 1.74 (m, 3H), 1.70 – 1.60 (m, 3H), 1.57 – 1.40 (m, 7H), 1.38 – 1.23 (m, 2H), 1.21 (d, *J* = 6.2 Hz, 4H), 1.16 (d, *J* = 3.2 Hz, 4H), 1.15 (d, *J* = 3.2 Hz, 10H), 1.10 (s, 3H), 1.07 – 1.03 (m, 1H), 1.01 (s, 4H), 0.93 – 0.86 (m, 0H), 0.86 (s, 4H), 0.82 (s, 3H), 0.81 (s, 3H), 0.78 (s, 3H), 0.70 (s, 4H), 0.70 – 0.66 (m, 1H); **HRMS** *m/z* (ESI): Calcd for $C_{66}H_{106}O_{31}Na$ [M+Na] 1417.6616, found 1417.6649.
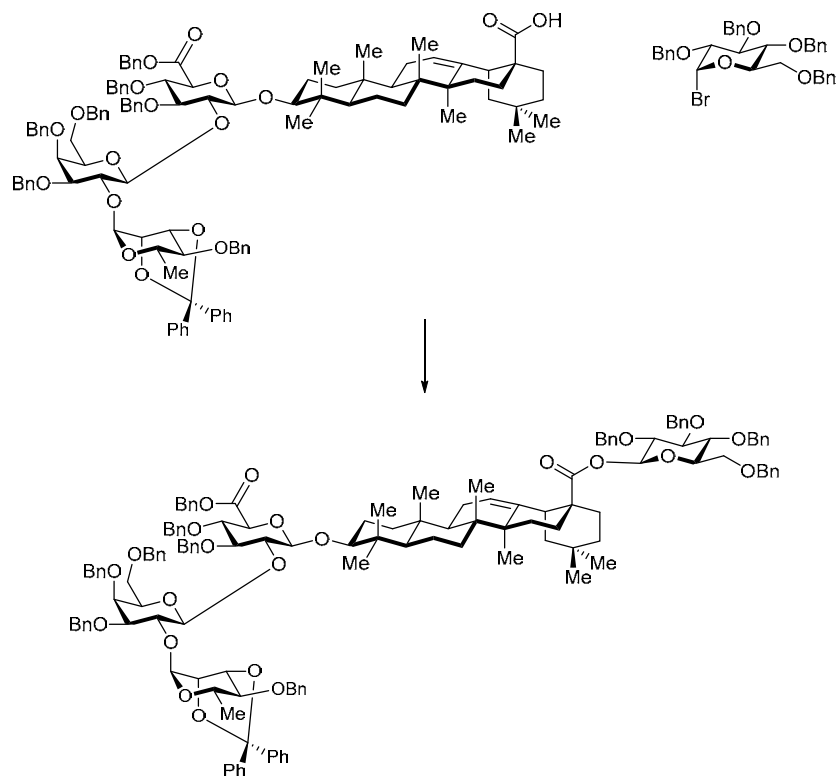
(170): Carboxylic acid **167** (15 mg, 0.0086 mmol, 1 equiv) bromide **169** (26 mg, 0.043 mmol, 5 equiv), potassium carbonate (3.6 mg, 0.026 mmol, 3 equiv), and tetrabutylammonium bromide (8 mg, 0.026 mmol, 3 equiv) were combined in a biphasic mixture of ethyl acetate (1 mL) and water (1 mL) and heated to 45 °C. After 4 hr, rxn was diluted with ethyl acetate (25 mL) and sonicated for 5 min. Saturated aqueous sodium bicarbonate (25 mL) was added to the mixture, then extracted with ethyl acetate (2× 50 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated and purified with silica gel chromatography (hexanes:ethyl acetate, 20:1 to 4:1) to give **170** (16 mg, 83%) as a sticky white foam.

TLC R_f 0.43 (20:1 benzene:ethyl acetate); **FTIR** (NaCl film) 2924, 1749, 1453, 1362, 1071, 1027, 733, 697, 666 cm^{-1}; **^{1}H NMR** (600 MHz, CDCl$_3$) δ 7.53 − 7.48 (m, 2H), 7.44

– 7.40 (m, 2H), 7.35 – 7.26 (m, 39H), 7.25 – 7.05 (m, 20H), 5.74 (s, 1H), 5.54 (d, *J* = 8.1 Hz, 1H), 5.30 (t, *J* = 3.7 Hz, 1H), 5.19 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 4.93 (d, *J* = 11.2 Hz, 1H), 4.88 (d, *J* = 10.9 Hz, 1H), 4.86 – 4.79 (m, 5H), 4.77 (d, *J* = 7.3 Hz, 1H), 4.76 (d, *J* = 9.3 Hz, 1H), 4.72 – 4.67 (m, 3H), 4.62 (d, *J* = 12.2 Hz, 1H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.54 – 4.49 (m, 3H), 4.48 (d, *J* = 11.6 Hz, 1H), 4.45 – 4.40 (m, 3H), 4.31 (d, *J* = 7.7 Hz, 1H), 4.24 (t, *J* = 6.4 Hz, 1H), 4.16 (dq, *J* = 10.0, 6.2 Hz, 1H), 4.05 (dd, *J* = 9.8, 7.7 Hz, 1H), 4.01 (d, *J* = 6.0 Hz, 1H), 3.97 – 3.93 (m, 1H), 3.89 – 3.82 (m, 2H), 3.81 – 3.66 (m, 6H), 3.64 – 3.50 (m, 4H), 3.42 (dd, *J* = 8.2, 5.4 Hz, 1H), 3.37 (dd, *J* = 9.9, 2.8 Hz, 1H), 3.09 (dd, *J* = 10.1, 6.8 Hz, 1H), 2.94 (dd, *J* = 11.7, 4.6 Hz, 1H), 2.86 (dd, *J* = 14.2, 4.3 Hz, 1H), 2.07 – 1.96 (m, 1H), 1.90 – 1.58 (m, 9H), 1.55 – 1.45 (m, 2H), 1.41 (dd, *J* = 11.3, 6.7 Hz, 1H), 1.35 (td, *J* = 13.4, 5.1 Hz, 1H), 1.32 – 1.09 (m, 7H), 1.07 (d, *J* = 6.2 Hz, 4H), 1.06 (s, 3H), 0.94 (s, 4H), 0.91 (s, 3H), 0.90 (s, 3H), 0.89 – 0.82 (m, 3H), 0.81 (s, 3H), 0.69 (s, 3H), 0.68 (s, 3H), 0.58 – 0.52 (m, 1H); ^{13}C NMR (151 MHz, CDCl$_3$) δ 176.04, 168.26, 143.43, 143.24, 143.05, 138.99, 138.44, 138.39, 138.19, 138.09, 138.07, 137.94, 137.92, 137.78, 137.50, 135.13, 128.51, 128.42, 128.40, 128.37, 128.36, 128.35, 128.34, 128.30, 128.28, 128.23, 128.05, 128.02, 128.00, 127.98, 127.96, 127.92, 127.87, 127.84, 127.82, 127.80, 127.76, 127.75, 127.74, 127.73, 127.68, 127.62, 127.54, 127.52, 127.42, 127.21, 127.11, 126.01, 125.99, 122.59, 108.98, 104.95, 100.79, 97.55, 94.13, 91.61, 85.34, 85.00, 82.33, 80.71, 79.98, 79.65, 79.54, 77.44, 77.24, 76.08, 76.00, 75.78, 75.68, 75.54, 74.96, 74.92, 74.71, 74.38, 74.28, 73.99, 73.69, 73.31, 72.93, 72.39, 72.35, 71.04, 68.13, 68.03, 67.07, 64.73, 55.64, 47.48, 46.71, 45.94, 41.71, 41.20, 39.20, 38.50, 36.60, 33.90, 33.04, 32.33, 31.85, 30.63, 27.89, 27.80, 25.89, 25.57, 23.61,

23.42, 23.21, 18.12, 17.70, 16.86, 16.06, 15.30, 14.14; **HRMS** *m/z* (ESI): Calcd for C$_{144}$H$_{160}$O$_{23}$Na [M+Na] 2280.1248, found 2280.1345.
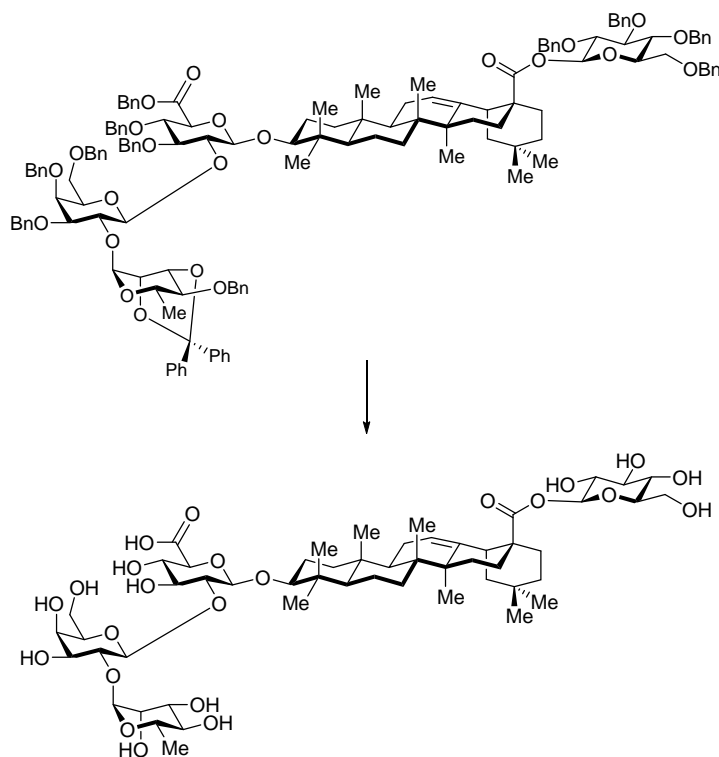
(171): A solution of fully protected lablaboside A **170** (19 mg, 0.007 mmol, 1.0 equiv) in tetrahydrofuran (3 mL) and methanol (3 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (60 mg, 0.028 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) for 24 hr, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 H$_2$O:acetonitrile) and purified by RP–HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 20 · 75% acetonitrile (0.05% TFA) in over 19 min at a flow rate of 5 mL/min. The fraction

containing the major peak (tR = 12.07 min) was collected and lyophilized to dryness to afford lablaboside A (**171**) (6.7 mg, 86 % yield) as a fluffy white solid.

$[\alpha]_D^{19}$: −10.1 ° (Lit −9.3 °) (*c* 0.6, MeOH); **^{1}H NMR** (600 MHz, Methanol-*d*$_4$) δ 5.40 (d, *J* = 8.1 Hz, 1H), 5.27 (t, *J* = 3.7 Hz, 1H), 5.20 (d, *J* = 1.7 Hz, 1H), 4.82 (d, *J* = 7.6 Hz, 1H), 4.50 (d, *J* = 7.6 Hz, 1H), 4.16 (dq, *J* = 9.7, 6.3 Hz, 1H), 3.94 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.86 − 3.66 (m, 8H), 3.65 − 3.58 (m, 3H), 3.53 (t, *J* = 9.4 Hz, 1H), 3.46 − 3.35 (m, 6H), 3.19 (dd, *J* = 11.8, 4.5 Hz, 1H), 2.87 (dd, *J* = 12.9, 3.2 Hz, 1H), 2.07 (td, *J* = 13.5, 3.9 Hz, 1H), 1.97 − 1.86 (m, 3H), 1.85 − 1.79 (m, 1H), 1.79 − 1.70 (m, 4H), 1.67 − 1.47 (m, 5H), 1.46 − 1.36 (m, 2H), 1.36 − 1.31 (m, 1H), 1.28 (d, *J* = 6.2 Hz, 3H), 1.26 − 1.21 (m, 1H), 1.18 (s, 3H), 1.17 − 1.15 (m, 1H), 1.13 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.82 (s, 3H), 0.80 − 0.77 (m, 1H); ^{13}C NMR (151 MHz, MeOD) δ 178.06, 172.59, 149.48, 149.30, 149.12, 144.84, 138.63, 138.46, 138.30, 125.40, 125.23, 125.06, 123.83, 105.84, 102.79, 102.08, 95.72, 92.44, 78.72, 78.48, 78.32, 78.27, 77.20, 76.92, 76.56, 76.10, 74.25, 73.93, 73.42, 72.28, 72.18, 71.10, 70.94, 69.50, 62.95, 62.40, 57.06, 49.58, 48.04, 47.23, 42.95, 42.63, 40.73, 40.48, 39.80, 37.91, 34.91, 33.96, 33.51, 33.15, 31.56, 28.92, 28.73, 26.97, 26.30, 24.58, 24.01, 23.97, 19.36, 18.26, 17.75, 16.85, 15.98; **HRMS** *m/z* (ESI): Calcd for $C_{54}H_{86}O_{23}Na$ [M+Na] 1125.5458, found 1125.5460.