A SYNTHESIS ENABLED UNDERSTANDING OF AMPHOTERICIN B LEADING TO DERIVATIVES WITH IMPROVED THERAPEUTIC INDICES

BY

BRICE E. UNO

DISsertATION

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Urbana, Illinois

Doctoral Committee:

Professor Martin D. Burke, Chair
Professor Paul J. Hergenrother
Professor Wilfred A. van der Donk
Professor Peter E. Orlean
ABSTRACT

Systemic fungal infections represent a significant contributor to human morbidity and mortality. To lower the impact of invasive fungal pathogens on the global population, more progress must be made in the areas of diagnostic tests and safe and effective new therapies. Although Amphotericin B (AmB) has served as the last line of defense against invasive fungal infections without significant pathogen resistance, its clinical application is restricted by severe dose-limiting toxicity. Efforts to improve AmB’s therapeutic index (a comparison between the dose that causes the desired therapeutic effect to the dose that causes toxicity) have not been successful. This lack of progress is in large part attributable to an incorrect understanding of AmB’s primary mechanism of action.

For more than 5 decades AmB has been perceived to primarily exist and operate as a self assembled ion channel complex—exerting cytotoxicity through the efflux of intracellular ions leading to the disruption of critical electrochemical gradients. Although a beautiful and rare phenomenon for a small molecule, the ion channel model has masked AmB’s underlying mechanism of action.

In this body of work we will discuss how, through the systematic synthesis of single functional group deficient derivatives of AmB, coupled with state-of-the-art biophysical and biological experiments we have been able to fully elucidate AmB’s mechanism of action. We discuss how AmB predominantly exists as an extramembranous sterol sponge that primarily kills yeast and human cells by binding and extracting sterols in a mycosamine dependent fashion. Additionally, the C2’-OH and C3’-NH$_3^+$ are critical residues on the mycosamine appendage, which are potentially responsible for stabilizing a conformation that allows for the binding of both ergosterol and cholesterol. When either of these residues is deleted, AmB can still bind ergosterol but can no longer bind cholesterol. This shift in sterol binding directly correlates to a substantial loss of toxicity. These results suggest that the C2’-OH and C3’-NH$_3^+$ do not directly bind sterols but are potential sites of allosteric modification.

Empowered with an accurate macroscopic and atomistic understanding of AmB, we were able to rationally guide the development of a novel derivative with an increased therapeutic index.
To my Mom, Merrill Uno
I would like to start by thanking my advisor Professor Martin D. Burke for his thoughtful guidance and unwavering support of my endeavors as a young scientist, for which I am immensely grateful. His confidence, eagerness, brilliance, and passion for solving problems will continue to inspire me for the rest of my career. I would also like to thank my committee Professor Paul Hengerrother, Professor Wilfred A. van der Donk, and Professor Peter A. Orlean for their patience and belief in me. They have continuously held me to the highest levels of scholarship and excellence, and I am a much better scientist because of their efforts.

I must express my utmost appreciation for the departmental secretaries past and present Becky Duffield, Gayle Adkinsson, Lori Johnson, Susan Lighty, and Stacy Olson. Their hard work, support, and positivity are why this department is as successful as it is and I am eternally grateful for their help over the years.

I want to acknowledge the members of the Burke group who have served as mentors to me during my graduate career. It is because of their guidance and willingness to help me when they were just as busy that it was possible for me to complete this degree. I need to mention Dr. Dan Palacios for showing me the ropes of dark lab and how to successfully manipulate amphotericin. I also need to thank my lab mate Dr. Pulin Wang for always looking out for me and teaching me to never talk myself out of running an experiment. It’s because of his guidance and camaraderie that I was successful in lab. I am also grateful to Dr. Brandon Wilcock for being a friend and mentor on numerous projects. His willingness to share his wisdom even after he left the lab has been instrumental in my completion of this degree. I have been privileged to work with Dr. Justin Struble who has always patiently steered me in the right direction with his immense knowledge of the literature and prowess in lab. I also need to thank Dr. Tom Anderson, Dr. Arjun Palyam, and Dr. Souvik Rakshit for their friendship and wisdom.

Beyond a doubt, the friends that I have made here in the Burke group have shaped who I am as a scientist. I will always cherish the time spent with Dr. Steven Ballmer over the years. From being friends with him since day one of grad school to being best men at each other’s wedding makes me feel as if we’ve really grown up together. I also need to
acknowledge Matt Endo for his help and intellectual contribution, without it, none of these projects would be published in the high-impact journals that they are. I am also appreciative of Stephen Davis for a lot of things but namely teaching me how to interpret ampho NMR spectra, seriously very helpful.

I have made many friends while being here and their friendship has made my life full and worthwhile. Namely, I need to acknowledge Heath Timmons, Stacy Alikakos, an Ellen Dahlke for being true blue friends.

Most importantly, I need to thank all of my family. Every one of my family members has provided infinite love and support throughout all of my life, and I cannot even begin to express my gratitude. I am blessed to have wonderful and loving parents in Michael and Merrill Uno. They have always supported me in all of my endeavors and inspired me to work hard and love life. I dedicated this body of work to my mom because she is the source of my curiosity, tenacity and creativity. I need to thank my grandparents Gene and Hirobumi Uno for always believing in me. I am also extremely fortunate to have two amazing and brilliant brothers, Curran and Neal. I love you guys. I need to thank my aunts and uncles, who will find the smallest excuse to drop everything and show their love for me. I need to thank all of the Moyers and Cantors for whose love and warmth I am eternally grateful. I am truly blessed and lucky to be a part of your wonderful family. I cannot acknowledge Aimee and John Moyer enough for their endless love and support. I am also grateful to Patricia & Sandy Cantor along with Mary & Ed Moyer who have shown me overwhelming love and warmth. I must also thank Hannah, Zach, and Danielle Moyer who are truly wonderful siblings, a source of positivity, hilarity, and my biggest fans.

Finally, I must thank my loving wife and partner Rachel Moyer. I am unable to fully express my gratitude for the boundless love and support that you have always shown me, especially throughout this process. You are the reason I have achieved this degree. You inspire me and I love you dearly.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ac</td>
<td>acetate</td>
</tr>
<tr>
<td>AmB</td>
<td>amphotericin B</td>
</tr>
<tr>
<td>AmdeB</td>
<td>amphoteronolide B</td>
</tr>
<tr>
<td>AmE</td>
<td>amphotericin B methyl ester</td>
</tr>
<tr>
<td>C35deOAmB</td>
<td>C35-deoxy amphotericin B</td>
</tr>
<tr>
<td>C2’deOAmB</td>
<td>C2’-deoxy amphotericin B</td>
</tr>
<tr>
<td>C2’epiAmB</td>
<td>C2’-epi amphotericin B</td>
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</tbody>
</table>
C2'epiAmE  |  C2’epi amphotericin B methyl ester

Chol     |  cholesterol

CSA      |  (±)-10-camphorsulfonic acid

DCM      |  dichloromethane

DEIPS    |  diethylisopropylsilyl

DIAD     |  diisopropyl azodicarboxylate

DDQ      |  2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DMAP     |  4-(dimethylamino)-pyridine

DMF      |  dimethyl formamide

DMP      |  Dess-Martin periodinane

DMSO     |  dimethyl sulfoxide
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Erg</td>
<td>ergosterol</td>
</tr>
<tr>
<td>HMPA</td>
<td>hexamethylphosphoramide</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>ICC</td>
<td>iterative cross-coupling</td>
</tr>
<tr>
<td>ITC</td>
<td>isothermal titration calorimetry</td>
</tr>
<tr>
<td>LUV</td>
<td>large unilamellar vesicle</td>
</tr>
<tr>
<td>MeAmB</td>
<td>C41-methyl amphotericinB</td>
</tr>
<tr>
<td>MeAmdeB</td>
<td>C41-methyl amphoteronolide B</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MIDA</td>
<td>N-methyliminodiacetic acid</td>
</tr>
<tr>
<td>MIDA salt</td>
<td>bis-sodium N-methyliminodiacetate</td>
</tr>
<tr>
<td>NOE</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>Ph₃P</td>
<td>triphenylphosphine</td>
</tr>
<tr>
<td>PMP</td>
<td>p-methoxyphenyl</td>
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</table>
POPC 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine

pyr pyridine

S-Phos 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl

TBS t-butyldimethylsilyl

TES triethylsilyl

Tf trifluoromethane sulfonate

THF tetrahydrofuran

TLC thin layer chromatography

TMSE 2-(trimethylsilyl)ethyl

UV ultraviolet
CHAPTER 1: TREATMENT OF SYSTEMIC FUNGAL INFECTIONS

1-1: GLOBAL IMPACT OF SYSTEMIC FUNGAL INFECTIONS

As the population with immunodeficiency has expanded over the past four decades, systemic fungal infections have emerged as one of the leading causes of human morbidity and mortality worldwide. Between 1979 and 2000, the annual number of sepsis cases caused by fungal organisms has increased by 207%.

Patients who are immunocompromised (e.g. HIV/AIDS infection, chemotherapy, organ transplantation with long-term administration of corticosteroids, or the elderly) are substantially more susceptible to contracting invasive fungal infections. *Candida albicans* infections are the fourth most common hospital acquired blood-stream infections with an estimated 300,000 cases globally per year. *Cryptococcus neoformans* a pathogenic fungus responsible for Cryptococcal meningitis—a fungal infection of the meninges, the tissue covering the brain—can infect immuno-competent persons but is typically found in patients with AIDS.

It is estimated that there are 1,000,000 cases of *Cryptococcus* infections globally per year, and it is the second most common AIDS-defining illness in Africa. *Aspergillus fumigatus*, the most common species responsible for invasive Aspergillus infections, is estimated to cause 350,000 cases per year globally. The average mortality rate for all of these infections is 50% for patients receiving treatment. Worldwide deaths from fungal infections outnumber those of tuberculosis and malaria annually (Fig. 1.1 A). In the US alone the annual cost associated with these infections will top $1 billion. Furthermore, quickly emerging resistance to nearly all pharmaceutical treatments for invasive fungal infections poses an alarming threat of immediate global concern.

![Figure 1.1 A) Total deaths per year from invasive fungal infections (blue) compared to total deaths per year from tuberculosis and malaria respectively. B) Average mortality rates for invasive fungal infections by species.](image-url)
The polyene macrolide natural product Amphotericin B (AmB) is a powerful broad-spectrum antifungal drug that has served as the prototypical treatment for systemic fungal infections. Discovered in 1955 by Gold and coworkers and rapidly approved by the FDA in 1957, AmB has marked a paradigm shift in the treatment of systemic fungal infections. Prior to its discovery, the prognosis for such infections was exceptionally bleak, with a mortality rate close to 100%. Furthermore, pathogen resistance to AmB is exceptionally rare despite its clinical use for nearly 60 years. Unfortunately, however AmB is so highly toxic that its effective utilization as the last line of defense against life-threatening systemic fungal infections is often precluded. Intravenous AmB treatment can cause severe and irreversible damage to multiple organs, most notably nephrotoxicity (kidney) and hepatotoxicity (liver). AmB will also disrupt the soft muscle tissue of the heart, causing serious cardiac arrhythmias and, in some cases, sudden cardiac failure. Additionally, a course of AmB treatment will cause anemia and electrolyte imbalances such as hypoalkemia and hypomagnesemia as a result of systemic hemolysis of erythrocytes (red blood cells). The severity of these treatment effects have underlined how critically important it is to develop a way to improve the therapeutic index of Amphotericin B.

Despite more than five decades of extensive efforts worldwide, a clinically viable derivative of AmB with an improved therapeutic index has yet to emerge. In 1972, Mechlinski and Schaffner discovered that methylation of the C41 carboxylate of AmB to generate amphotericin B methyl ester (AME) somewhat improved the therapeutic index in vitro. These studies showed that AME had decreased hemolytic activity (lysis of red blood cells) while maintaining comparable broad-spectrum antifungal activity. In 1976, researchers at the Squibb Institute for Medical Research conducted a 1-month comparative toxicology study of AME and AmB in mouse, rat and dog models. Importantly, the material used in these studies was only 57% pure. It was concluded from these experiments that less toxic that AmB. In 1978, the same only 57% pure material was expedited into human clinical trials as an experimental new treatment for systemic fungal infections. However, the trial was prematurely halted due to numerous patient deaths resulting from unexpected leukoencephalopathy. The causes of this side effect remains unclear.

Aside from covalently modifying the structure of AmB to improve its therapeutic index, another strategy has been to incorporate AmB into liposomes. Due to their
higher cost and associated storage requirements, LFAmB treatments are much less accessible to impoverished populations in the global south where these pathogens have the highest impact. Additionally, LFAmBs are similarly toxic to the AmBD formulation if the required course of treatment is extended.

Two other classes of broad-spectrum antifungal drugs have also been contemporaneously developed for the treatment of systemic fungal infections: Azoles and echinocandins. Azole drugs like fluconazole and itraconazole were discovered to inhibit the ergosterol biosynthesis pathway by inhibiting the enzyme lanosterol 14 α-demethylase, a cytochrome P450 enzyme responsible for the conversion of lanosterol into ergosterol (a critical sterol in fungal cell membranes). Echinocandins like caspofungin inhibit fungal cell wall construction by targeting the 1,3-β-glucan synthase, a glycosyltransferase responsible for β-glucan synthesis. Although these drugs have been shown to be efficacious and less toxic compared to AmB, they suffer from a narrower spectrum of application and significant clinical resistance appearing shortly after their approval (Fig. 1.2).

Therefore, due to the rise of significant microbial resistance to alternative therapies for the treatment of systemic fungal infections, efforts to create a less toxic derivative of AmB are of utmost importance. However, a major contributor to the lack of progress towards this endeavor has been the poor understanding of the mechanisms by which AmB is cytotoxic to yeast and human cells. A thorough mechanistic understanding of this natural product stands to enable the rational design of antifungal drugs with improved therapeutic indices. Furthermore, a deeper understanding of AmB’s mechanism may also lead to the development of other resistance-refractory antibiotics, thereby combating the growing threat of resistance to the modern pharmaceutical arsenal.
11. Resistance to AmB is exceptionally rare despite its clinical use for nearly 60 years


15. Fisher, P.B.; Goldstein, N.I.; Bryson, V.; Schaffner, C.P. In Vitro 1976, 12,133-140.


CHAPTER 2: A SYNTHESIS ENABLED ELUCIDATION OF AMB’S PRIMARY MECHANISM OF ACTION

2-1: BACKGROUND

For more than 50 years, the prevailing explanation for AmB’s mechanism of action was permeabilization of lipid membranes via the formation of ion channels. Evidence for this model came from early observations that AmB and other mycosamine containing polyene macrolides caused efflux of intracellular ions from yeast cells and liposomes. These findings were supported by electrophysiology experiments using planar black lipid membranes where both AmB and nystatin form discrete single ion channels.\textsuperscript{1,2} Similar experiments with other membrane active polyenes such as filipin, which in contrast do not contain mycosamine, caused global disruption of cell membranes rather than discrete ion channel formation.\textsuperscript{3} Andreoli and coworkers demonstrated that the size of the pores formed by AmB, were between 7 and 10 Å by the examination of the relative permeability of the AmB channel to solutes of increasing size.\textsuperscript{4}

Collectively these experiments led to the proposal of the classic barrel-stave model for AmB ion channel formation (Fig.2.1).\textsuperscript{3,5,6} Further computational modeling predicted that a collection of eight AmB monomers self-assemble into an ion channel complex where the hydrophilic polyol regions form a pore that allows the passage of hydrated ions. The exterior of the channel complex is lined by the hydrophobic polyenes and engage in van der Waals interactions with the hydrophobic lipid tails of the lipid bilayer. It has been proposed that two specific functional groups are critical for the stabilization of the AmB ion
channel: the C41 carboxylate and the C19 mycosamine.\textsuperscript{7}

Efforts to probe the role of these functional groups in the formation of ion channels have relied on covalent modifications, i.e. methylation of the carboxylate or acylation of the C3'-amine on the sugar.\textsuperscript{1,7,8} However, the results garnered from these experiments are complicated by new steric interactions in the corresponding derivatives, which may disrupt intermolecular contacts. Additionally, the potential for these groups to maintain hydrogen bonding potential (i.e. the carbonyl of the C41 methyl ester interacting with the ammonium, or the acyl group on the amide with the carboxylate) further complicates the interpretation of these results.\textsuperscript{8} Palacios et al. employed a functional group deletion strategy—similar to alanine scanning in protein science—to determine the role of the C41 carboxylate and C19 mycosamine in AmB's mechanism of action.\textsuperscript{9} They synthesized three AmB derivatives: amphotronolide B (AmdeB, AmB aglycone—lacking the mycosamine), C41 methyl AmB (C41MeAmB, C41-COOH fully reduced to a methyl group) and the hybrid C41 methyl amphotronlide B (C41MeAmdeB, lacking both mycosamine and the C41-COOH). Deletion of the C41 carboxylate (C41MeAmB) did not attenuate the antifungal activity of AmB, however, both derivatives lacking mycosamine (AmdeB and C41MeAmdeB) were completely inactive.\textsuperscript{9} These results indicated that the C19 mycosamine was clearly required for biological activity and that the C41 carboxylate was not.\textsuperscript{9}

Palacios et al. further investigated the mycosamine's relationship to the underlying cause of AmB's biological activity. Early reports have underlined the fact that sterols are necessary for AmB's ability to kill yeast cells and form channels.\textsuperscript{7} However, it was unclear how the membrane embedded sterols were interacting with AmB. There are various reports supporting indirect sterol effects, the direct binding of AmB and sterols, or both as plausible roles for sterols.\textsuperscript{10} Palacios et al. conducted isothermal titration calorimetry (ITC) with the aforementioned AmB derivatives in liposomes embedded with and without ergosterol.\textsuperscript{11} A significant exotherm was observed with AmB and C41MeAmB in the erosterol-containing liposomes, indicative of a direct binding event. AmdeB and MeAmdeB did not produce significant exotherms, strongly suggesting that the mycosamine is critical to bind sterol and that this key small molecule-small molecule interaction is absolutely required for AmB's biological activity.\textsuperscript{11}
At this point two main mechanisms of action were possible: \( i \) permeabilization of lipid bilayers mediated by sterol binding causes cytotoxicity, or \( ii \) sterol binding alone is responsible for cytotoxicity (Fig. 2.2 B). In order to differentiate between these possible mechanisms we needed to synthesize a derivative of AmB that maintains the ability to bind ergosterol but lacks the ability to form channels.\(^{12}\)

The hydroxyl group at C35 on AmB has been predicted to be critical in the formation of ion channels. Hypothetically, the C35-OH stabilizes the ion channel complex through hydrogen bonding to the phospholipid head groups (single barrel model) or to another octomeric complex of AmB molecules (double barrel model) (Fig. 2.2 C). If this prediction were correct, an AmB derivative lacking the C35-OH group (C35deOAmB) would be unable to form channels.\(^{12}\)

2-2: SYNTHESIS OF C35-DEOXY AMB BUILDING BLOCKS, INSPIRING A NOVEL METHODOLOGY FOR VINYL MIDA BORONATE SYNTHESIS

We synthesized C35deOAmB through the iterative cross-coupling of three building blocks BB1, BB2, and BB3 (Fig. 2.3A). Our synthetic strategy hinged on the synthesis of N-
methyllimmino diaacetate (MIDA) protected boronic acid building blocks. MIDA boronates are inert to anhydrous Suzuki-Miyaura cross-coupling conditions, but can be deprotected to reveal the free boronic acid under mild aqueous basic conditions. These characteristics allow for the sequentially assembly of each building block (Fig. 2.3B). My main contributions to the synthesis of C35deOAmB were in the large-scale production of BB1 and BB2. BB1 was derived via chemical degradation of the natural product. BB2 was synthesized via iterative Stille couplings, which required large quantities of MIDA boronate 2.1 (Scheme 2.1 A). Large scale access to trans-(2-bromovinyl) MIDA boronate 2.1 proved to be difficult with our synthetic procedure at the time. The first generation synthesis of 2.1, initiated with the highly exothermic addition of BBr3 across acetylene gas. The resulting intermediate dibromoborane would then need to be purified away from polymeric byproducts via distillation under reduced pressure while ensuring the rigorous exclusion of air and water Once purified the dibromoborane intermediate was transferred to a DMSO suspension of MIDA in the presence of 2,6-lutidine. MIDA boronate 2.1 was afforded after aqueous work-up and chromatographic purification (Scheme 2.1 B).

Difficulty accessing 2.1 in large quantities inspired us to investigate alternative synthetic methods for the construction of 2.1 that could also be employed to generate other inaccessible small vinyl MIDA boronates. We therefore hypothesized that the interesting, yet synthetically challenging vinyl MIDA boronate 2.3, would be an excellent model system for this investigation.

Most MIDA boronates can be easily prepared by complexation of the corresponding boronic acids with MIDA under Dean–Stark-type conditions. Alternatively, vinyl MIDA boronates can be prepared through the hydroboration of alkynes, or vinyl Grignard additions to trialkoxyboranes, followed by the same dean-stark MIDA complexation
conditions. However, our initial efforts to prepare vinyl MIDA boronate 2.3 using the above approaches were thwarted by rapid decomposition of vinyl boronic acid/ester intermediate 19 during MIDA complexation. A ‘hot protocol’ was later developed by my colleague in the context of 2-pyridyl MIDA boronate synthesis and other unstable heteroaryl MIDA boronates.20 Synthesis of these MIDA boronates was previously inaccessible due to the extremely unstable nature of the intermediate 2-pyridyl-boronic acids/esters. Here, slow addition of the intermediate lithium 2-pyridyl-trisopropoxyboronate salt was added to a DMSO solution of MIDA at 120 °C, which rapidly facilitated the complexation to 2-pyridyl MIDA boronate.20 In principle, this operationally complex procedure could successfully generate 2.3. However, vinylmagnesium bromide is an unstable reagent and unreliable from commercial sources, further complicating the procedure.21

Based on our prior experience with the first generation synthesis of 2.1, we knew that vinyl-dibromoboranes were efficiently trapped by MIDA in the presence of exogenous base. Silicon to boron transmetalation of vinyl silanes has also been previously reported by Singleton and coworkers.16 We therefore attempted to prepare vinyl MIDA boronate 2.3 via $\text{BBr}_3$-promoted transmetalation16,22,23 of the readily available vinyltrimethylsilane 2.4 into the corresponding dibromoborane intermediate 2.5,16,23,24 followed by trapping of 2.5 with MIDA in the presence of 2,6-lutidine and DMSO (Table 2.1, entry 1). We were encouraged to observe the formation of significant quantities of 2.3 using this protocol, yet important limitations still remained. Specifically, in addition to providing only moderate yield, this procedure requires the utilization of 2.0 equivalents of 2,6-lutidine to scavenge the HBr generated upon MIDA complexation. We reasoned that alternatively trapping this same dibromoborane intermediate 2.5 with the pre-formed bis-sodium salt of MIDA ($\text{MIDA}^{2-}$-Na$_{2}$)25 would instead generate

![Scheme 2.2: MIDA salt is solubilized by trimethylsilation and demonstrated to be necessary in the synthesis of Br-B(MIDA) and H-B(MIDA)](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ligand</th>
<th>Base</th>
<th>Solvent</th>
<th>% Yield</th>
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<tr>
<td>1</td>
<td>MIDA</td>
<td>2,6-lutidine</td>
<td>DMSO</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>MIDA Salt</td>
<td>None</td>
<td>DMSO</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>MIDA Salt</td>
<td>None</td>
<td>CH$_3$CN</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 2.1: Discovery and optimization of vinyl MIDA boronate 2.3
access	 to sodium hydroxide followed by a simple filtration procedure. Transformed MIDA into the corresponding bis-sodium salt due to the fact the by $^1$H NMR, MIDA and—the less soluble MIDA$^2$-Na$^+$$_2$ salt—is completely insoluble in MeCN and that these conditions do not translate to MIDA complexation of other boronic acid or ester intermediates.

Through a series of $^1$H NMR experiments, I discovered that the stoichiometric byproduct in the Si$\rightarrow$B transmetalation step, bromotrimethylsilane was fully silylating the MIDA$^2$-Na$^+$$_2$ salt and responsible for solubilizing it to a small extent, thus facilitating complexation. To further demonstrate the necessity for TMS-X in MIDA$^2$-Na$^+$$_2$ salt complexation of haloboranes in MeCN, we targeted the synthesis of Br-B(MIDA).

Adapting the complexation procedure to prepare 2.3, without the addition of TMS-Cl, the complexation of BB$_3$ with MIDA$^2$-Na$^+$$_2$ salt in MeCN was not productive. However, with 1.0 equivalent of TMS-Cl under the same conditions, Br-B(MIDA) boronate was afforded in 64% yield (Scheme 2.2). This procedure was also amenable in the preparation H-B(MIDA) boronate from dibromoborane-DMS complex in 55% yield. However, these MIDA boronates proved to be as inert as they are novel. Because the boron center is pyramidalized, these compounds were unreactive at boron to any useful functionalization condition.

Given the simple nature of the procedure to generate 2.3, we were keenly interested to explore its scalability. We first developed a convenient, >100 g scale procedure for transforming MIDA into the corresponding bis-sodium salt via the treatment with aqueous sodium hydroxide followed by a simple filtration procedure. Having secured large-scale access to MIDA$^2$-Na$^+$$_2$, we translated the conditions described above for the synthesis of 2.3.

| Table 2.1, entry 2). Switching to MeCN resulted in a robust and high-yielding synthesis of 2.3 (Table 2.1, entry 3). By employing MeCN a solvent, we obviated all of the procedural burdens when DMSO is the solvent. However, we questioned how the MIDA-MeCN suspension was allowing complexation due to the fact the by $^1$H NMR, MIDA and—the less soluble MIDA$^2$-Na$^+$$_2$ salt—is completely insoluble in MeCN and that these conditions do not translate to MIDA complexation of other boronic acid or ester intermediates.

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Given the simple nature of the procedure to generate 2.3, we were keenly interested to explore its scalability. We first developed a convenient, >100 g scale procedure for transforming MIDA into the corresponding bis-sodium salt via the treatment with aqueous sodium hydroxide followed by a simple filtration procedure. Having secured large-scale access to MIDA$^2$-Na$^+$$_2$, we translated the conditions described above for the synthesis of 2.3.
to the 30 mmol scale (Scheme 2.3). The product was purified by filtration and recrystallization—No aqueous work-up or chromatography—affording 2.3 as a colorless, free-flowing, crystalline solid. Single crystal X-ray analysis confirmed the predicted structure of 2.3 having a pyramidalized boron center. We have stored novel vinyl borane 2.3 on the benchtop under air for more than 3 months without any noticeable decomposition.

I then applied the identical conditions used to synthesize 2.3 to synthesize 2.1. The only difference was the use of commercially available 2-bromovinyl silane 2.2 instead of vinyl trimethylsilane. Although 2-bromovinyl silane 2.2 is typically prepared (or purchased) as a mixture of E and Z isomers, it has been previously observed that transmetalation of Z-alkenyl silanes with BBr₃ yields predominantly the corresponding E-alkenyl boranes (E:Z ~ 9:1)¹⁶. Fortuitously, we found that transmetalation of 2.2 (E:Z 9:1) with BBr₃ at 0 °C followed by trapping with MIDA²⁻Na⁺ yields trans-(2-bromovinyl) MIDA boronate 2.1 as a single stereoisomer (Scheme 2.2). We were able to run this much more convenient procedure on 30 mmol scale to prepare 4.8 g (61% yield) of 2.1 in a single step without the need for an aqueous workup or chromatographic purification.

2.3: FUNCTIONALIZATION OF VINYL MIDA BORONATE

With a simple and readily scalable synthesis of 2.3 in hand, we have preliminarily explored its utility as a starting material for the preparation of a range of new MIDA boronate building blocks. For example, due to its sensitivity to protodeboronation, cyclopropyl boronic acid 2.6 can be difficult to synthesize and store.²⁶ Circumventing this challenge, the cyclopropanation²⁷ of 2.3

![Scheme 2.4: 2nd generation synthesis of trans-bromovinyl MIDA boronate](image)

![Scheme 2.5: Cyclopropanation and epoxidation of 2.3](image)
proceeds in excellent yields using Pd(OAc)$_2$ and diazomethane to yield cyclopropyl MIDA boronate 2.7 as an air- and chromatographically stable solid (Scheme 2.5). The success of this formal [2+1] cycloaddition prompted us to consider the related epoxidation reaction. In pioneering studies, Molander and co-workers have demonstrated the compatibility of substituted alkenyltrifluoroborate salts with epoxidation mediated by DMDO. mCPBA was also effective in these studies, but the incompatibility of trifluoroborate salts with chromatography precluded the separation of the epoxide products from the benzoic acid byproducts. In contrast, mCPBA promoted the epoxidation of 1 and, remarkably, the resulting novel oxiranyl MIDA boronate 2.8 is stable to silica gel chromatography and can be isolated in pure form. We are unaware of any prior synthesis of an unsubstituted oxiranylborane. Single crystal X-ray analysis confirmed unambiguously the structure of this very interesting and potentially highly versatile new building block.

We further explored the compatibility of vinyl MIDA boronate 2.3 with a series of transition metal-mediated transformations of the vinyl handle (Scheme 2.6). Whiting and co-workers have developed a series of selective Heck-type couplings of aryl and vinyl halides with sterically bulky vinyl boronic esters to generate styrenyl and polyenyl boranes, respectively. We have found that in the absence of water MIDA boronates are unreactive toward cross-coupling, which led us to question whether it might be possible to similarly achieve high selectivity for a Heck reaction between organohalides and 2.3 under anhydrous conditions. In fact, as shown in Scheme 2.6, p-bromoacetophenone readily coupled with 2.3 with to yield MIDA boronate 2.9 as a single regio and stereoisomer.

The oxidative Heck reaction provides a complementary opportunity to generate similar products from boronic acid rather than organohalide starting materials. For example, it has been demonstrated that tolylboronic acid and vinyl pinacol boronic ester can be effectively coupled using this reaction...
The White catalyst PdII-bissulfoxide (2.12) was recently found to be a powerful and highly selective promoter of oxidative Heck reactions with a wide range of aryl and alkenyl boranes. We found that 2.12 also promotes the oxidative coupling of 2.3 and phenylboronic acid to yield styrenyl MIDA boronate 2.12 as a single regio and stereoisomer. Importantly, products 2.9 and 2.10 retain the capacity for subsequent cross-coupling via the MIDA boronate-masked boronic acid. In this way, vinyl MIDA boronate 2.3 represents a new type of bifunctional MIDA boronate building block, rich with potential for a variety of iterative cross-coupling based applications.

The cross-metathesis of terminal olefins developed by Grubbs and co-workers with vinyl or propenyl pinacol boronic esters represents a powerful approach for the preparation of alkenyl boranes with many advantages over conventional methods, including the use of readily available and chemically robust terminal olefins as starting materials, excellent functional group compatibility, and generally good yields and stereoselectivities. However, there are some important limitations of this method, including instabilities of many alkenyl pinacol boronic esters to long-term storage and/or silica gel chromatography and suboptimal E:Z ratios for cross-metathesis with some important olefin classes, including unfunctionalized terminal olefins.

Given that alkenyl MIDA boronates are invariably stable to benchtop storage under air and chromatography, we were attracted to the use of 2.3 as an alternative vinyl borane for cross-metathesis applications. Moreover, we hypothesized that the sterically bulky nature of the MIDA boronate group would cause 2.3 to behave like a type III olefin (analogous to tert-butylethylene), thereby avoiding any homodimerization and providing high yields and stereoselectivities with alkenes of types I and II. Our initial experiments indicated that this approach was very promising. For example, as shown in Scheme 2.6,
cross-metathesis between 2.3 and unfunctionalized terminal olefin 1-octene, generated the corresponding octenyl MIDA boronate 2.11 as an air- and chromatographically stable crystalline solid in good yield and with a >20:1 E:Z ratio (the Z isomer was not observed by \(^1\)H NMR).

A similar cross-metathesis reaction using propenyl pinacol boronic ester has been reported to proceed with only a 9:1 E:Z ratio.\(^{34b}\) Moreover, the alkenyl pinacol boronic ester products are somewhat unstable to silica gel chromatography.\(^{34b}\) Encouraged by the exceptional yield and stereoselectivity observed for the preparation of 2.11, we decided to preliminarily explore the scope of the cross-metathesis with 2.3. As shown in Scheme 2.7, this reaction is in fact highly effective with a range of olefin substrates, invariably providing the corresponding alkenyl MIDA boronates in good to excellent yields and outstanding stereoselectivities (all >20:1, i.e., Z isomers were not observed by \(^1\)H NMR). Specifically, cross-metathesis with allyltriisopropylsilane and a pair of 2-butenediol derivatives were all highly effective, yielding a new collection of potentially bifunctional MIDA boronate building blocks 2.13, 2.14 and 2.15. As is common with many cross-metathesis reactions, increased substitution at the allylic position was also well tolerated and styrene derivatives proved to be excellent substrates. Specifically, all the regioisomers of bromostyrene were successfully employed to yield a series of halo MIDA boronates 2.19, 2.20 and 2.21, which represent excellent building blocks for iterative cross-coupling applications.\(^{18a}\) Moreover, given that alkenyl MIDA boronates can be readily converted into the corresponding boronic acids under mild conditions (NaOH/THF or NaHCO\(_3\)/MeOH)\(^{18}\) or used directly as cross-coupling partners under aqueous basic Suzuki–Miyaura conditions,\(^{36,18}\) cross-metathesis with 2.3 represents an excellent entry into these valuable
building blocks for synthesis. In Furstner and coworker’s synthesis of Leiodermatolide, they took advantage of vinyl MIDA boronate as a bifunctional building block. They installed $3$ with cross-metathesis and coupled the resulting alkenyl MIDA boronate building block to a late stage complex vinyl iodide (Scheme 2.8). $37$

![Scheme 2.8: Vinyl MIDA boronate 2.3 is a robust vinylation reagent capable of coupling a range of deactivated aryl chlorides under 'slow-release' cross-coupling conditions](image)

Furthermore, MIDA boronate 2.3 is successfully cross-coupled under ‘slow-release’ Suzuki-Miyaura conditions to a variety of aryl and vinyl chlorides and bromides (Scheme 2.9). $36$ Because is capable of efficiently cross coupling with a variety of aryl halides, vinyl MIDA boronate is arguably the most robust and best vinylating reagent. Additionally, 2.3 is a non-toxic crystalline solid that is easily synthesized or commercially available in bulk from at least 3 chemical companies including Sigma-Aldrich and Allychem.

2-4: C35DEOAMB REVEALS AMPHOTERICIN B PRIMARILY BINDS STEROLS AND EXISTS AS AN EXTRAMEMBRANEOUS STEROL SPONGE

With a robust and scalable route to the three building blocks for C35deOAmB, my colleagues assembled them via iterative Suzuki-Miyaura cross-coupling, macrolactonization and global deprotection, arriving at milligram quantities of C35deOAmB. $12$ The synthesis of this complex derivative proved to be quite challenging. Since removal of the C35-OH engendered more conformational flexibility in the polyene core, the final deprotection steps previously optimized and modeled on AmB, caused decomposition in the case of C35deOAmB. Therefore, the optimization of the protecting group ensemble proved to be a central theme in this synthesis and subsequent AmB derivatives.
Gratifyingly, biophysical studies revealed that C35deOAmB is incapable of forming channels yet readily binds ergosterol (Erg). Furthermore, C35deOAmB maintains potent fungicidal activity (MIC in *S. cerevisiae* 3.0 µM and 0.5 µM respectively). These findings demonstrate that AmB primarily kills yeast cells by simply binding Erg (Fig. 2.4 A) and that channel formation provides an additional enhancement of cytotoxicity. However, the structural and biophysical underpinnings of this small molecule-small molecule interaction (AmB-Erg) and its connection to cell killing remained unclear.

Sterols, including Erg in yeast, have many essential roles in eukaryotic cell physiology, including functional regulation of membrane proteins, micro-domain formation, endocytosis, vacuole fusion, cell division and cell signaling. We thus hypothesized that sequestering Erg and thereby concomitantly precluding its participation in multiple cellular functions may underlie the fungicidal action of AmB. Guided by this hypothesis, we considered three possible models for the primary structure and function of AmB in the presence of Erg-containing phospholipid membranes (Fig. 2.4 B–D): (i) in the classic channel model, AmB primarily exists in the form of small (~1 nm) ion channel aggregates inserted into the membrane, perpendicular to the membrane surface, with Erg molecules interdigitated between AmB molecules (Fig. 2.4 B) (ii) In an alternative surface adsorption model, AmB is primarily positioned in the intermediate–head group region, oriented parallel to the plane of the membrane, sequestering Erg to the membrane surface (Fig. 2.4 C) (iii) In a new sterol sponge model, AmB primarily exists as large extramembranous aggregates that extract Erg from lipid bilayers (Fig. 2.4 D). In the latter two models, we envisioned that membrane-permeabilizing ion channels represent relatively minor contributors to both the structure and cytoidal activity of AmB. Through an extensive series of SSNMR, TEM and...
cell-based experiments we have determined that AmB exists as an extramembranous sterol sponge model (Fig. 2.4 D).

Based on the classic ion channel model, many efforts over the past several decades to improve the therapeutic index of AmB have focused on selectively permeabлизing yeast versus human cells. The sterol sponge model on the other hand suggests that the much simpler extraction of cholesterol (Chol) by large extramembranous aggregates of AmB may be primarily responsible for toxicity to human cells (Fig. 2.4 D). This, in turn, suggests a much more actionable roadmap to an improved therapeutic index that involves simply maximizing the relative binding affinity of AmB aggregates for Erg versus Chol.

2-5: EXPERIMENTAL SECTION

Materials
Commercial reagents were purchased from Sigma–Aldrich, Fisher Scientific, Alfa Aesar, TCI America, or Frontier Scientific, and were used without further purification unless otherwise noted. Solvents were purified via passage through packed columns as described by Pangborn and co-workers (THF, Et₂O, CH₃CN, CH₂Cl₂: dry neutral alumina; hexane, benzene, and toluene, dry neutral alumina and Q5 reactant; DMSO, DMF: activated molecular sieves). All water was deionized prior to use. Triethylamine, diisopropylamine, diethylamine, pyridine, 2,6-lutidine, and ethanol were freshly distilled under an atmosphere of nitrogen from CaH₂. Grubbs II catalyst (Aldrich 569747) and Quadrasil (Aldrich 680427) silica supported Ru scavenger kits [TA (Aldrich 679496), MTU (Aldrich 679518), MP (Aldrich 679526), and AP (Aldrich 679534)] were generous gifts from Sigma–Aldrich (Milwaukee, WI).

General experimental procedures
Unless noted, all reactions were performed in flame-dried round-bottom or modified Schlenk flasks fitted with rubber septa under a positive pressure of argon. Organic solutions were concentrated via rotary evaporation under reduced pressure with a bath temperature of 40 °C. Reactions were monitored by analytical thin layer chromatography
(TLC) performed using the indicated solvent on E. Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by exposure to a UV lamp (λ=254 nm), a glass chamber containing iodine, and/or a solution of KMnO₄, an acidic solution of p-anisaldehyde, or a solution of ceric ammonium molybdate (CAM) followed by brief heating using a Varitemp heat gun. MIDA boronates are compatible with standard silica gel chromatography, including standard loading techniques. For this study, chromatography was performed on a Teledyne-Isco CombiFlash Rf purification system using Merck silica gel grade 9385 (60 Å, 230–400 mesh). For loading, compounds were adsorbed onto Celite in vacuo from an acetone solution. Specifically, for a 1 g mixture of crude material the sample is dissolved in reagent grade acetone (25–50 mL) and to the flask is added Celite 545 Filter Aid (5–15 g). The mixture is then concentrated in vacuo to afford a powder, which is then loaded on top of a silica gel column. The procedure is typically repeated with a small amount of acetone (5 mL) and Celite (2 g) to ensure quantitative transfer. Purification was generally performed using a gradient of Et₂O→Et₂O/CH₃CN 3:2.

**Structural analysis**

¹H NMR spectra were recorded at 23 °C on one of the following instruments: Varian Unity 400, Varian Unity 500, Varian Unity Inova 500NB. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to residual protium in the NMR solvent (CHCl₃, δ=7.26; CD₂HCN, δ=1.93, center line; CD₃C(O)CD₃δ=2.04, center line) or to added tetramethylsilane (δ= 0.00). Data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, sept=septet, m=multiplet, br=broad, app=apparent), coupling constant (J) in hertz (Hz), and integration. ¹³C NMR spectra were recorded at 23 °C on a Varian Unity 500. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced to carbon resonances in the NMR solvent (CDCl₃, δ=77.0, center line; CD₃CN, δ=1.30, center line, CD₃C(O)CD₃δ=29.80, center line) or to added tetramethylsilane (δ= 0.00). Carbons bearing boron substituents were not observed (quadrupolar relaxation). ¹¹B NMR were recorded using a General Electric GN300WB instrument and referenced to an external standard of (BF₃·Et₂O). High-resolution mass spectra (HRMS) were performed by Furong Sun and Dr. Steve Mullen at the University of Illinois School of Chemical Sciences Mass Spectrometry.
Laboratory. Infrared spectra were collected from a thin film on NaCl plates (a solution of each compound was placed on the salt plate and then evaporated to dryness) on a Perkin–Elmer Spectrum BX FT-IR spectrometer. Absorption maxima ($\nu_{\text{max}}$) are reported in wavenumbers (cm$^{-1}$). X-ray crystallographic analyses of 2.3 and 2.8 were carried out by Dr. Scott Wilson and Mr. Heath Timmons at the University of Illinois George L. Clark X-ray facility.

Synthesis of intermediate 2.1

To a 500 mL Schlenk flask equipped a stir bar was added BBr$_3$ (1.0 M in CH$_2$Cl$_2$, 30 mmol) and CH$_2$Cl$_2$ (270 mL) and the solution was cooled to 0 °C. To the solution was added dropwise 2-bromo-vinyltrimethylsilane 2.2 (5.0 mL, 33 mmol). The stirred solution was maintained at 0 °C for 3 h and then added dropwise via cannula to a mixture of sodium salt 6 (10.0 g, 52.3 mmol) in MeCN (300 mL) stirred at 0 °C. The rate of addition was controlled such that the internal temperature did not exceed 5 °C. Following the addition, the mixture was stirred at 0 °C for 30 min and was then filtered through a fine glass-fritted funnel. The filter cake was extracted with acetone. The filtrate was concentrated in vacuo and the resulting residue was purified via flash chromatography (SiO$_2$, Et$_2$O→Et$_2$O/MeCN 65:45) to yield 2.1 as a colorless, crystalline solid (4.79 g, 60%). For characterization of 2.1 see Ref. 18b. This building block is now commercially available from Sigma–Aldrich (703478).
Synthesis of intermediate 2.3

A 50 mL Schlenk flask equipped with a stir bar was charged with BBr₃ (1.0 M in CH₂Cl₂, 30 mmol) and the solution was cooled to 0 °C. To this solution was added dropwise vinyltrimethylsilane 2.4 (4.49 mL, 31.5 mmol). The solution was maintained at 0 °C for 20 min and then was allowed to warm to 23 °C with stirring for an additional 2 h. The resulting solution was added dropwise via cannula to a suspension of bis sodium MIDA salt (5.73 g, 30.0 mmol) in MeCN (50 mL) stirred at 0 °C. The rate of addition was controlled such that the internal temperature did not exceed 5 °C. Following the addition, the mixture was allowed to warm to 23 °C with stirring for 1 h. The resulting white suspension was filtered through a pad of Celite and the filter cake was extracted three times with acetone. To the orange filtrate was added Et₂O causing the crystallization of 2.3 as a colorless solid (4.74 g, 86%). This building block is now commercially available from Sigma–Aldrich (704415).

1H NMR (500 MHz, CD₃C(O)CD₃) δ 5.96 (dd, J=19.0, 13.5 Hz, 1H), 5.72–5.63 (m, 2H), 4.21 (d, J=17.0 Hz, 2H), 4.01 (d, J=17.0 Hz, 2H), 3.0 (s, 3H)

13C NMR (125 MHz, CD₃C(O)CD₃) δ 168.3, 128.7, 61.6, 46.7
IR (thin film, cm⁻¹) 3063, 2997, 2960, 1755, 1455, 1420, 1345, 1312, 1251, 1175, 1155, 1134, 1117, 1090, 1033, 987, 964, 951, 865

HRMS (EI⁺) calculated for C₇H₁₀BNO₄ (M⁺): 183.0703
Found: 183.0700

TLC (EtOAc) Rf = 0.26, visualized by KMnO₄.

Synthesis of intermediate 2.7

To a 100 mL Schlenk flask equipped with a stir bar was added vinyl MIDA boronate 2.3 (183 mg, 1.00 mmol), Pd(OAc)₂ (9 mg, 0.04 mmol), and THF (40 mL). The solution was cooled to 0 °C. To the solution was added dropwise diazomethane (0.3 M in Et₂O, 5 mmol, freshly prepared) and the resulting solution was stirred for 10 min. To the flask was added additional Pd(OAc)₂ (18 mg, 0.080 mmol) and diazomethane (0.3 M in Et₂O, 5 mmol). The solution was allowed to warm to 23 °C with stirring for 1 h. The solution was sparged with N₂ and was further quenched via the addition of glacial acetic acid (0.5 mL). The dark mixture was concentrated in vacuo and the resulting residue was purified via flash chromatography (SiO₂, Et₂O/CH₃CN) to yield 2.7 as a colorless crystalline solid (187 mg, 93%).
$^1$H NMR (500 MHz, CD$_3$CN) $\delta$ 3.92 (d, $J$=17.0 Hz, 2H), 3.80 (d, $J$=17.0 Hz, 2H), 2.98 (s, 3H), 0.46 (dq, $J$=9.5, 3.0 Hz, 2H), 0.12 (m, 2H), −0.33 (m, 1H)

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) $\delta$ 169.0, 62.7, 46.8, 1.2

$^{11}$B NMR (96 MHz, CD$_3$CN) $\delta$ 13.2

HRMS (FAB$^+$) calculated for C$_8$H$_{13}$BNO$_4$ (M+H)$^+$: 198.0938
Found: 198.0937

IR (thin film, cm$^{-1}$) 2998, 1744, 1457, 1358, 1337, 2197, 1246, 1129, 1048, 985, 956, 892, 880, 845, 865

Synthesis of intermediate 2.8

A 50 mL Schlenk flask equipped with a stir bar was charged with vinyl MIDA boronate 2.3 (183 mg, 1.00 mmol) and CH$_2$Cl$_2$ (20 mL) and the resulting suspension was cooled to 0 °C. To this suspension was added in one portion solid 3-chloroperbenzoic acid (77% mCPBA, 713 mg, 3.18 mmol). The mixture was allowed to warm to 23 °C with stirring for 18 h. The mixture was poured into a separatory funnel charged with satd aq NaHCO$_3$ (10 mL) and the mixture was diluted with EtOAc (40 mL). The mixture was shaken and the phases were separated. The aqueous phase was extracted with EtOAc (2×40 mL). The combined organic phases were washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The resulting residue was purified via flash chromatography (SiO$_2$, Et$_2$O→Et$_2$O/CH$_3$CN 3:2) to yield 2.8 as a colorless crystalline solid (147 mg, 74%).
1H NMR (500 MHz, acetone-d₆) δ 4.29 (d, J=17.0 Hz, 1H), 4.21 (d, J=17.0 Hz, 1H), 4.13 (d, J=17.0 Hz, 1H), 3.93 (d, J=17.0 Hz, 1H), 3.27 (s, 3H), 2.76 (dd, J=6.0, 5.0 Hz, 1H), 2.56 (dd, J=6.5, 3.5 Hz, 1H), 2.24 (t, J=4.0 Hz, 1H)

13C NMR (125 MHz, CD₃C(O)CD₃) δ 169.4, 168.1, 62.9, 62.7, 46.8, 44.9

HRMS (EI⁺) calculated for C₇H₁₀BNO₅ (M)⁺: 199.0652
Found: 199.0570

TLC (EtOAc) Rf= 0.25, visualized by KMnO₄

Synthesis of intermediate 2.9

In a glove box, to a 20 mL vial equipped with a stir bar was added vinyl MIDA boronate 2.3 (183 mg, 1.00 mmol), 4-bromoacetophenone (198 mg, 1.00 mmol), Pd(PPh₃)₄ (116 mg, 0.100 mmol), Ag₂PO₄ (838 mg, 2.00 mmol), and THF (5 mL). The vial was sealed with a PTFE-lined cap, removed from the glove box, and maintained in a 100 °C oil bath with stirring for 24 h. The mixture was concentrated in vacuo and the resulting residue was purified via flash chromatography (SiO₂, Et₂O/MeCN) to yield 2.9 as a colorless, crystalline solid (193 mg, 64%).
\**1H NMR** (500 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta 7.97\) (d, \(J=8.5\) Hz, 2H), 7.65 (d, \(J=8.5\) Hz, 2H), 7.03 (d, \(J=18.0\) Hz, 1H), 6.55 (d, \(J=18.0\) Hz, 1H), 4.29 (d, \(J=17.0\) Hz, 2H), 4.12 (d, \(J=17.0\) Hz, 2H), 3.09 (s, 3H), 2.57 (s, 3H)

\**13C NMR** (125 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta 197.3, 169.0, 143.4, 141.7, 137.3, 129.4, 127.5, 62.4, 47.4, 26.6\)

**HRMS (EI\(^+\))** calculated for C\(_7\)H\(_{10}\)BNO\(_4\)\((M)^{+}\): 301.1122

Found: 301.1126

**Synthesis of intermediate 2.10**

Under ambient atmosphere, to a 7 mL vial equipped with a stir bar was added vinyl MIDA boronate 2.3 (183 mg, 1.00 mmol), phenylboronic acid (305 mg, 2.50 mmol), 1,2-bis(phenylsulfanyl)ethane palladium(II) acetate 2.12 (White catalyst, 25 mg, 0.050 mmol), benzoquinone (216 mg, 2.00 mmol), glacial acetic acid (0.23 mL, 4.0 mmol), and dioxane (3.0 mL). The vial was sealed with a PTFE-lined cap and maintained in a 45 °C oil bath for 48 h. The solution was concentrated in vacuo and the resulting residue was purified via flash chromatography (SiO\(_2\), Et\(_2\)O/CH\(_3\)CN) to yield 2.10 as a colorless, crystalline solid (183 mg, 68%).

\**1H NMR** (500 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta 7.51\) (d, \(J=9.0\) Hz, 2H), 7.33 (m, 3H), 6.94 (d, \(J=18.0\) Hz, 1H), 6.35 (d, \(J=18.5\) Hz, 1H), 4.25 (d, \(J=17.0\) Hz, 2H), 4.07 (d, \(J=17.0\) Hz, 2H), 3.05 (s, 3H)

\**13C NMR** (125 MHz, CD\(_3\)CN) \(\delta 169.6, 143.3, 139.0, 129.5, 129.0, 127.6, 62.3, 47.6\)
HRMS (EI+) calculated for C$_{13}$H$_{14}$BNO$_4$ (M)$^+$: 259.1016

Found: 259.1017

General procedure for olefin cross-metathesis with 2.3

In a glove box, to a 25 mL Schlenk flask equipped with a stir bar was added vinyl MIDA boronate 2.3 (183 mg, 1.00 mmol), Grubbs II catalyst (85 mg, 0.10 mmol) and olefin (1.50–2.50 mmol). The flask was sealed with a septum and removed from the glove box. To the flask was added CH$_2$Cl$_2$ (10 mL). The flask was fitted with a water-cooled reflux condenser and the reaction was heated to reflux with stirring for 24 h. The mixture was cooled to 23 °C and to the mixture was added 400 mg of Quadrasil (TA, MTU, or AP) silica supported metal scavenger (Sigma–Aldrich), which caused significant decolorization. The mixture was stirred for 15 min and was then concentrated in vacuo. The resulting residue was purified via flash chromatography (SiO$_2$, Et$_2$O→Et$_2$O/CH$_3$CN 3:2). The products were thus obtained as colorless, crystalline solids. Some of the purified alkenyl MIDA boronate products contained small amounts of styrenyl MIDA boronate, presumably derived from reactions with the initial catalyst

Synthesis of intermediate 2.11
The general procedure was followed using 1-octene (280 mg, 2.50 mmol) to yield 2.11 (214 mg, 80%).

\[ ^1H\text{ NMR} (500\text{ MHz, } \text{CD}_3\text{C(O)CD}_3) \delta 6.07 \text{ (dt, } J=17.5, 6.5 \text{ Hz, 1H, } 5.46 \text{ (d, } J=17.5 \text{ Hz 1H, } 4.16 \text{ (d, } J=17.0 \text{ Hz, 2H, } 3.97 \text{ (d, } J=17.0 \text{ Hz, 2H, } 2.97 \text{ (s, 3H, } 2.10 \text{ (q, } J=7.0 \text{ Hz, 2H, } 1.40 \text{ (m, 2H, 1.29 (m, 6H, 0.86 (t, } J=5.0 \text{ Hz, 3H) }}\]

\[ ^{13}C\text{ NMR} (125\text{ MHz, } \text{CD}_3\text{C(O)CD}_3) \delta 169.0, 146.0, 62.1, 47.3, 36.1, 32.4, 32.2, 30.5, 23.2, 14.3 \]

HRMS (EI\(^+\)) calculated for C\(_{13}\)H\(_{22}\)O\(_4\)NB (M\(^+\)) 267.1642

Found: 267.1644

Synthesis of intermediate 2.13

The general procedure was followed using allyltrisopropylsilane (496 mg, 2.50 mmol) to yield 2.13 (299 mg, 85%).

\[ ^1H\text{ NMR} (500\text{ MHz, } \text{CD}_3\text{C(O)CD}_3) \delta 6.19 \text{ (dt, } J=17.5, 8 \text{ Hz, 1H, } 5.38 \text{ (d, } J=17.5 \text{ Hz, 1H, } 4.15 \text{ (d, } J=17.0 \text{ Hz, 2H, } 3.93 \text{ (d, } J=17.0 \text{ Hz, 2H, } 2.97 \text{ (s, 3H, } 1.78 \text{ (dd, } J=10.0, 1.0 \text{ Hz, 2H, } 1.07 \text{ (s, 18H, 1.07 \text{ (s, 3H) }}\]

\[ ^{13}C\text{ NMR} (125\text{ MHz, } \text{CD}_3\text{C(O)CD}_3) \delta 169.1, 143.2, 62.3, 47.2, 20.2, 19.0, 11.7 \]

HRMS (EI\(^+\)) calculated for C\(_{17}\)H\(_{32}\)O\(_4\)NSiB (M\(^+\)) 353.2194

Found: 353.2193
Synthesis of intermediate 2.14

The general procedure was followed using cis-1,4-diacetoxy-2-butene (431 mg, 2.50 mmol) to yield 2.14 (213 mg, 84%).

$^1$H NMR (500 MHz, CD$_3$C(O)CD$_3$) δ 6.12 (dt, J=17.5, 5.0 Hz, 1H), 5.76 (dt, J=18.0, 1.5 Hz, 1H), 4.58 (dd, J=5.0, 1.5 Hz, 2H), 4.21 (d, J=17.0 Hz, 2H), 4.02 (d, J=17.0 Hz, 2H), 2.99 (s, 3H), 2.01 (s, 3H)

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) δ 170.6, 168.9, 139.8, 66.3, 62.3, 47.4, 20.7

HRMS (EI⁺) calculated for C$_{10}$H$_{14}$O$_6$NB (M)⁺: 255.09142
Found: 255.09137

Synthesis of intermediate 2.15

The general procedure was followed using 1,4-dibenzoyloxy-2-butene (E:Z ~1:1, 444 mg, 1.50 mmol) to yield 2.15 (310 mg, 98%).

$^1$H NMR (500 MHz, CD$_3$C(O)CD$_3$) δ 8.05 (d, J=8.5 Hz, 2H), 7.63 (tt, J=7.0, 1.5 Hz, 1H), 7.51 (t, J=7.5 Hz, 2H), 6.28 (dt, J=18.0, 5.0 Hz, 1H), 5.90 (dt, J=16.0, 1.5 Hz, 1H), 4.87 (dd, J=4.5, 1.0 Hz, 2H), 4.23 (d, J=17.0 Hz, 2H), 4.04 (d, J=17.0 Hz, 2H), 3.01 (s, 3H)

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) δ 169.0, 166.4, 139.0, 133.9, 131.2, 130.2, 129.4, 66.9, 62.3, 47.4
**HRMS (EI^+) calculated for C_{15}H_{16}O_6NB (M)^+**: 317.10707

*Found:* 317.10738

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**Synthesis of intermediate 2.16**

![Image of intermediate 2.16](image)

The general procedure was followed using vinylcyclohexane (165 mg, 1.50 mmol) to yield 2.16 (253 mg, 96%).

**^1H NMR** (500 MHz, CD$_3$C(O)CD$_3$) $\delta$ 6.03 (dd, $J$=18.0, 6.5 Hz, 1H), 5.42 (dd, $J$=18.0, 1.5 Hz, 1H), 4.17 (d, $J$=17.0 Hz, 2H), 3.97 (d, $J$=17.0 Hz, 2H), 2.96 (s, 3H), 1.99 (m, 1H), 1.71 (m, 4H), 1.62 (m, 1H), 1.28 (m, 2H), 1.20–1.05 (m, 3H)

**^13C NMR** (125 MHz, CD$_3$C(O)CD$_3$) $\delta$ 169.1, 151.4, 62.1, 47.3, 43.7, 43.2, 33.2, 26.8

**HRMS (EI^+) calculated for C_{13}H_{20}O_{4}NB (M)^+**: 265.1485

*Found:* 265.1488

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**Synthesis of intermediate 2.17**

![Image of intermediate 2.17](image)

The general procedure was followed using 2-methylbut-3-en-2-ol (215 mg, 2.50 mmol) to yield 2.17 (227 mg, 94%).
\(^1\)H NMR (500 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta\) 6.22 (d, \(J=18.0\) Hz, 1H), 5.63 (d, \(J=18.0\) Hz, 1H), 4.18 (d, \(J=17.0\) Hz, 2H), 3.98 (d, \(J=17.0\) Hz, 2H), 3.46 (s, 1H), 2.97 (s, 3H), 1.23 (s, 6H)

\(^{13}\)C NMR (125 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta\) 169.2, 153.7, 71.1, 62.1, 47.3, 30.6

Synthesis of intermediate 2.18

The general procedure was followed using styrene (260 mg, 2.50 mmol) to yield 2.18 (240 mg, 93%). For characterization of 2.18, see above.

Synthesis of intermediate 2.19

The general procedure was followed using 2-bromostyrene (275 mg, 1.50 mmol) to yield 2.19 (274 mg, 81%).

\(^1\)H NMR (500 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta\) 7.73 (dd, \(J=7.5, 1.5\) Hz, 1H), 7.59 (dd, \(J=8.0, 1.0\) Hz, 1H), 7.36 (t, \(J=7.5\) Hz, 1H), 7.29 (d, \(J=18.0\) Hz, 1H), 7.20 (td, \(J=7.5, 1.5\) Hz, 1H), 6.36 (d, \(J=18.0\) Hz, 1H), 4.29 (d, \(J=17.0\) Hz, 2H), 4.11 (d, \(J=17.0\) Hz, 2H), 3.09 (s, 3H)

\(^{13}\)C NMR (125 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta\) 169.0, 140.9, 138.8, 133.7, 130.3, 128.6, 128.3, 124.1, 62.4, 47.5

HRMS (EI\(^+\)) calculated for C\(_{13}\)H\(_{13}\)O\(_4\)NBrB (M\(^+\)): 337.0121
Synthesis of intermediate 2.20

The general procedure was followed using 3-bromostyrene (275 mg, 1.50 mmol) to yield 2.20 (305 mg, 91%). For characterization of 16h see ref 4a.

Synthesis of intermediate 2.21

The general procedure was followed using 4-bromostyrene (275 mg, 1.50 mmol) to yield 2.21 (240 mg, 93%).

$^{1}$H NMR (500 MHz, CD$_3$C(O)CD$_3$) δ 7.52 (d, J=8.5 Hz, 2H), 7.47 (d, J=8.5 Hz, 2H), 6.91 (d, J=18.0 Hz, 1H), 6.39 (d, J=18.0 Hz, 1H), 4.26 (d, J=17.0 Hz, 2H), 4.08 (d, J=17.0 Hz, 2H), 3.06 (s, 3H)

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) δ 169.1, 141.4, 138.3, 132.3, 129.2, 121.9, 62.3, 47.4

HRMS (EI$^+$) calculated for C$_{13}$H$_{13}$O$_4$NBrB (M$^+$): 337.0121

Found: 337.0119
Synthesis of Br-B(MIDA).

To a 300 mL round bottom flask charged with bis sodium MIDA salt (2.293 g, 12.0 mmol, 1.2 equiv), MeCN (100 mL), and chlorotrimethylsilane (1.517 mL, 12.0 mmol, 1.2 equiv) stirred at 23 °C for 1 h then cooled to 0 °C. BBr₃ (1.0 M in DCM: 10 mL, 10.0 mmol, 1.0 equiv) was added dropwise into the rapidly stirring suspension of MIDA salt. Following the addition, the mixture was allowed to warm to 23 °C with stirring for 1 h. The resulting white suspension was filtered through a pad of Celite and the filter cake was extracted three times with acetone. To the orange filtrate was added Et₂O causing the crystallization of Br-B(MIDA) as a colorless solid (1.50 g, 64%).

¹H NMR (500 MHz, CD₃C(O)CD₃) δ 4.55 (d, J=17.5 Hz, 2H), 4.30 (d, J=17.0 Hz, 2H), 3.33 (s, 3H)

TLC (EtOAc) Rf = 0.21, visualized by KMnO₄.

Crystal structure:
Synthesis of H-B(MIDA).

To a 300 mL round bottom flask charged with bis sodium MIDA salt (210.0 mg, 1.1 mmol, 1.1 equiv), MeCN (20 mL), and chlorotrimethylsilane (139 mL, 1.1 mmol, 1.1 equiv) stirred at 23 °C for 1 h then cooled to 0 °C. HBBr₂-DMS complex (1.0 M in DCM: 120 mL, 10.0 mmol, 1.0 equiv) was added dropwise into the rapidly stirring suspension of MIDA salt. Following the addition, the mixture was allowed to warm to 23 °C with stirring for 1 h. The resulting white suspension was filtered through a pad of Celite and the filter cake was extracted three times with acetone. To the orange filtrate was added Et₂O causing the crystallization of H-B(MIDA) as a colorless solid (85.9 mg, 55%).

\[ \text{H-B(MIDA)} \]

\(^1\text{H NMR}\) (500 MHz, CD₃C(O)CD₃) δ 4.21 (d, \(J=17.5\) Hz, 2H), 4.02 (d, \(J=17.0\) Hz, 2H), 3.16 (s, 3H).

\(\text{TLC (EtOAc) } R_f = 0.21, \text{ visualized by KMnO}_4.\)

\(\text{HRMS (EI*) calculated for C}_5\text{H}_7\text{O}_4\text{NB (M*)}: 156.04682\)

\(\text{Found: 156.04656}\)

2-6 REFERENCES


21. Personal experience with sigma-aldrich vinyl magnesium bromide solutions


For half a century, AmB has widely been accepted to kill both yeast and human cells through ion channel-mediated membrane permeabilization.\textsuperscript{1-3} These results have been interpreted through the lens of the ion channel model. As a consequence, little progress has been made toward solving the challenging problem of selectively forming ion channels in yeast vs. human cells.\textsuperscript{2,3}

Through our synthesis of C35deOAmB we recently determined that AmB kills yeast primarily by simply binding sterols. Ergosterol (Erg), the main sterol in yeast has many essential roles in eukaryotic cell physiology, including functional regulation of membrane proteins, microdomain formation, endocytosis, vacuole fusion, cell division and cell signaling.\textsuperscript{4-7} Based on a series of SSNMR and TEM experiments we have reported that AmB primarily exists in the form of large extramembrane aggregates that kill yeast by extracting Erg from lipid bilayers (Fig. 3.1 A).\textsuperscript{8c}

These results indicate that channel formation is not primarily responsible for killing yeast\textsuperscript{8c}. This realization strongly suggests that binding cholesterol (Chol) may account primarily for the toxicity of AmB to human cells. Moreover, efforts to improve its therapeutic index should focus directly on the relatively simpler problem of maximizing the binding affinity for Erg over Chol.\textsuperscript{8}

Palacios et al. previously reported that the mycosamine is critical for sterol binding. Deletion of the mycosamine from AmB eliminates its capacity to bind both sterols.\textsuperscript{8b} However, the atomistic underpinnings of the mycosamine-sterol interaction were
undefined. There are four heteroatomic functional groups in the mycosamine appendage, C2’-OH, C3’-NH3+, C4’-OH, and the oxygen contained in the tetrahydropyran ring. One or more of these heteroatomic functional groups might participate in a polar interaction with the 3β-hydroxyl group of each sterol (Fig. 3.1 B). Computational models and NMR studies have indicated that AmB interacts with both Erg and Chol via a similar binding mode in which either the C2’-OH or C3’-NH3+ group of AmB form a critical H-bond to the 3β-hydroxyl group of the A-ring of the sterol (Fig. 3.1 B)9. To test these binding models, we initially aimed to remove the C2’-OH from AmB and determine its impact on binding Erg and Chol.10,11

Synthesis of the targeted C2’deoxy AmB (C2’deOAmB), however, represented a major challenge. This is because, in addition to all of the other problems associated with chemically manipulating this complex and sensitive natural product, 2-deoxy sugars are substantially more acid-sensitive than their oxygenated counterparts.12

3-2: SITE-SELECTIVE ACYLATION OF THE C2’-OH

To access C2’deOAmB we initially employed a site-selective deoxygenation of the decahydroxylated natural product. This led to the discovery that site-selective and site-divergent functionalizations can be achieved simply by modifying the electronic properties of achiral reagents.10 My colleague Dr. Brandon Wilcock discovered that under DMAP catalyzed conditions, the acylation selectivity for the C2’-OH over the other hydroxyl groups increased with more electronically rich acyl chloride donors. For example, the acylation of 3.1 with electron poor p-nitrobenzoyl chloride and DMAP resulted in 39% yield of the C2’ acylated product. In comparison, the yield increases when the electron rich p-N,N-dimethylaminobenzoyl chloride is employed, affording the C2’ product in 72% yield.10

We hypothesized that this phenomenon was linked to the Hammond postulate,13 which predicts that the transition state of the acylation reaction will become more product-like as the reaction becomes less exothermic. In a more product-like transition state, the site-discriminating interactions between the acylating complex and substrate will be
magnified leading to increased site-selectivity. Thus, Dr. Wilcock observed that electron-rich acyl donors created a milder catalyst-reagent complex, which resulted in a less exothermic reaction with greater site selectivity (Fig. 3.2).10

Harnessing this phenomenon into a preparatively useful procedure we achieved the site-selective acylation of the C2′-OH of Amb 3.2. Following silylation of the remaining 4 hydroxyl groups with DEIPS groups (3.3), the C2′-benzoyl group was hydrolyzed with KCN revealing the free C2′-OH 3.4. We then proceeded to functionalize the free C2′-OH 3.4 by conversion to the C2′-I 3.5 and further reduction to the C2′-methylene 3.6. I converted the free C2′-OH 3.4 into intermediates en route to C2′epiAmb 3.7 and a C2′Erg conjugate 3.8 (Scheme 3.1).

With C2′-methylene 3.6 in hand we proceeded with the final global deprotection steps. However, once the C2′-OH group is removed, the subsequent glycosidic intermediates are significantly less stable compared to the parent natural product. As a result the final deprotection steps resulted in a low yield of C2′de0Amb as an inseparable mixture of deglycosylated byproducts (Scheme 3.2).10
In an attempt to circumvent the issues with the current deprotection steps, we investigated other protecting groups, namely at the C3’-nitrogen. Since amides require forcing conditions to remove, i.e. strong base or strong nucleophiles/hydrides, carbamates were a logical alternative. Although they are easily removed under a variety of mild conditions (depending on the carbamate), their presence at the C3’-position completely prevented the AgOAc mediated NaBH₄ de-iodination of the neighboring C2’-I 3.5.

With this knowledge, we proceeded to investigate new amides that would potentially allow for both the de-iodination of the C2’-I 3.5 and their own mild removal in the final step (scheme 3.2). Several derivatives were synthesized (3.9 and 3.10 and 3.11). We had predicted that steric constraints would position the tethered nucleophile directly over the π* orbital of the amide carbonyl prior to activation. However, these acyl groups were incapable of providing efficient access to their corresponding de-iodinated products.

3-3: HYBRID SYNTHESIS OF C2’DEOAMB

Although the site-selective acylation route in its present form is unable to produce assayable quantities of C2’deOAmB, extensive knowledge was gained during these studies¹¹. Not only would it inform future plans to mycosamine modified derivatives by it also bolstered an alternative semisynthetic approach that was more productive and general (Fig. 3.3). The second-generation synthesis of C2’deOAmB was designed around the
glycosylation of the AmB aglycone with a mycosamine analog lacking the C2’-OH (acosamine). This route was beneficial because it 1) obviated the robust protecting groups required in the first-generation route, and 2) it was highly convergent. The C2-deoxy sugar donor 3.12 was synthesized from 2-acylfuran and the aglycone 3.13 was derived from AmB.

When C2’deOAmB was initially targeted in 2006, this hybrid route was disfavored out based on synthetic access to the C2-deoxy sugar donor 3.12. A paucity of methodological progress toward β-selective glycosylation in the absence of a C2’-substituent presented a further challenge14. Normally these types of β-glycosylations are carried out with participating groups located at the C2-position of the sugar donor. For example, Nicolaou15,16 and coworkers have synthesized β-glycosides with amphoteronolide B substrates using anchimeric assistance from the neighboring equatorial group at the C2’-position followed by inversion at the C2 position. In the case of a C2-deoxysugar this method is not an option to achieve β-selective glycosylation.

Although the glycosylation step was still a concern, we were encouraged by the report of a highly efficient and scalable epoxy-alcohol 3.14 synthesis by O’Doherty and coworkers17. As a result, the hybrid route to C2’deOAmB gained significant feasibility.
We first generated a C2’-deoxygenated mycosamine (L-acosamine) donor 3.12 from known intermediate 3.14 (Scheme 3.3)\textsuperscript{17}. I determined that the TBS-protected derivative of this 2,3-epoxy alcohol (3.15) can be regioselectively opened at C2’ using lithium triethylborohydride in THF at 60 °C affording 3.16 in 58% yield. The resulting β-C3’-alcohol of was mesylated to generate 2.17 in 68% yield. Displacement of the secondary mesylate group by sodium azide provided 3.18 (80% yield). Subsequent removal of the PMB group generated the deoxysugar donor 3.12 (65% yield). Importantly, deoxysugar donor 3.12 is protected in such a way that the functional groups at C3’ and C4’ are inert to all of the subsequently required transformations yet readily unmasked at the end of the synthesis using mild conditions\textsuperscript{15}. We also prepared a similarly protected macrolide acceptor 3.13, having suitably stable yet readily cleavable silyl groups, protecting all of the seven secondary hydroxyl groups and the C41 carboxylic acid. Glycosylation of persilylated-aglycone 3.13 with deoxysugar donor 3.12 proceeded to yield the protected C2’deOAmB derivative 3.19 as a 2:1 mixture of α and β anomers. This molecule proved to be much more amenable to the requisite deprotection conditions than in the previous generation allowing synthetic access to C2’deOAmB (Scheme 3.3).\textsuperscript{10}

3-4: THE C2’-OH IS NOT REQUIRED TO BIND ERGOSTEROL BUT IS CRITICAL FOR CHOLESTEROL BINDING

With C2’deOAmB in hand, we then went on to assay its ability to bind Erg and Chol. via an optimized isothermal titration calorimetry (ITC)-based assay with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) large unilamellar vesicles (LUVs). Surprisingly, C2’deOAmB had a significant exotherm in Erg containing LUVs demonstrating a retained capacity for this derivative to bind Erg (Fig. 3.4). Even more surprisingly, C2’deOAmB showed no evidence of binding Chol in the analogous experiment (Fig. 3.4 A). Therefore, in contrast to the computational modeling studies, the C2’-OH of AmB plays a major role in binding Chol but is not required to bind Erg.\textsuperscript{11}
When we tested the minimum inhibitory concentration (MICs) of C2′deOAmB against two Erg-containing strains of yeast, \textit{S. cerevisiae} and \textit{C. albicans} (the latter representing the most common cause of fungal infections in humans), we observed potent antifungal activity (Fig. 3.4 B). Finally we probed the activity against human cells. One of the most prevalent toxic side effects associated with AmB is anemia caused by damage to red blood cells. AmB causes 90% hemolysis of human red blood cells at 8.5µM (MHC) (Fig. 3.4 C). In stark contrast, we found the corresponding MHCs for C2′deOAmB, which does not bind Chol in our ITC assay, to be >500µM.\textsuperscript{11} Consistent with the lack of toxicity to human red blood cells, human renal epithelial cells were also found to be viable after being exposed to C2′deOAmB. With these exciting results in hand we have pursued a scaled up synthesis of C2′deOAmB in order to determine this derivative’s viability as a potential therapeutic replacement for AmB. In order to do this, I recently prepared more of this material to preliminarily assay its efficacy and toxicity in a \textit{C. albicans} infected mouse model.\textsuperscript{18}

These findings collectively demonstrate that the leading atomistic model for AmB-sterol binding (Fig. 3.1 B) is not correct.\textsuperscript{9} We favor an alternative model in which the C2′-OH is potentially involved in the stabilization of a conformer of AmB that readily binds both Erg and Chol. We hypothesize that deletion of the C2′-OH favors a shift to a different conformer or set of conformers which retain the capacity to bind Erg but not Chol due to the bulkier nature of the latter (Fig. 3.5). This model predicts deletion of the C2′-OH of AmB causes a small-molecule-based allosteric effect resulting in ligand-selective binding.\textsuperscript{19}
Although further studies are required to test this hypothesis, we note that in the X-ray crystal structure of N-iodoacetyl AmB\textsuperscript{20} there is a prominent water-bridged H-bond between the hydroxyl groups at C2′ and C13 that may serve to stabilize a particular conformation or conformations of the mycosamine relative to the macrolide core that is able to bind both sterols (Fig. 3.5 B).

3-5: EXPERIMENTAL SECTION

**General Methods Materials**
Amphotericin B was a gift from the Bristol-Myers Squibb Company. All other commercially available reagents were obtained from Sigma-Aldrich, TCI America, Fischer Scientific, Combi-Blocks Inc., and Oakwood Products. Chemicals were used without further purification unless otherwise specified. Camphorsulfonic acid was purified before use by recrystallization with ethyl acetate. Triethyl amine, diisopropylethyl amine, pyridine, and 2,6-lutidine were freshly distilled over calcium hydride under nitrogen atmosphere. All solvents were obtained from a solvent purification system utilizing packed columns as described by Pangborn and coworkers.\textsuperscript{21}

**Reactions**
All reactions were performed under argon atmosphere in low light conditions with flame-dried glassware unless otherwise indicated. All compounds were stored in the dark under argon atmosphere. Thin layer chromatography or reverse phase HPLC was used to monitor reaction progress. Thin layer chromatography was performed on silica gel 60 F254 plates from Merck with the indicated solvent. Visualization of the compounds was accomplished with a UV lamp (\textlambda 254) and ceric ammonium molybdate (CAM) stain. Analytical HPLC was done on an Agilent 1100 Series HPLC with a C\textsubscript{18} 5 μm, 4.6 x 150 mm, Symmetry® column from Waters Corp at a flow rate of 1 mL/min with the indicated solvent and gradient. The detection wavelength was set to 383 nm.
Purification and Analysis

Merck silica gel 60 230-400 mesh and SiliCycle reverse phase C$_{18}$ (17%) 40-63 μm 60 angstrom silica gel was used for flash chromatography with the indicated solvent. HPLC reverse phase purification was done on a waters C$_{18}$ 5 μm, 30 x 150 mm Sunfire column at a flow rate of 25 mL/min with the indicated solvent and gradient. The detection wavelength was set to 383 nm. $^1$H NMR spectra were taken at 23 °C on a Varian Unity Inova Narrow Bore spectrometer at 101a 1H frequency of 500 MHz with a Varian 5 mm $^1$H{$^{13}$C/$^{15}$N} pulsed-field gradient Z probe. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced internally to the residual protium in the NMR solvent (CHD$_2$OD, δ = 3.30, center line; CD$_3$C(O)CHD$_2$, δ = 2.04, center line; CD$_3$S(O)CHD$_2$, δ = 2.50, center line; CCl$_3$H, δ = 7.26, center line). Data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, td = triplet of doublets, m = multiplet, b = broad, app = apparent), coupling constant ($J$) in Hertz (Hz) and integration. $^{13}$C spectra were obtained at 23 °C with a Varian Unity Inova spectrometer at a $^{13}$C frequency of 125 MHz with a 5 mm Nalorac gradient {$^{13}$C/$^{15}$N}$^1$H quad probe. Chemical shifts (δ) are reported downfield of tetramethylsilane and are referenced to the carbon resonances in the NMR solvent (CD$_3$OD, δ = 49.0, center line; CD$_3$C(O)CD$_3$, δ = 29.8, center line; CD$_3$S(O)CD$_3$, δ = 39.5, center line; CDCI$_3$, δ = 77.0, center line). ESI high-resolution mass spectra (HRMS), ESI low resolution mass spectra (LRMS) and matrix-assisted laser desorption/ionization (MALDI) spectra were obtained at the University of Illinois mass spectrometry facility.
Synthesis of intermediate SI3.1

Phenyl acetic acid (662 mg, 1.62 mmol, 3.0 equiv) was dissolved in THF (30 mL). Trimethyl acetyl chloride (400 μL, 3.25 mmol, 2.0 equiv) was added followed by triethyl amine (900 μL, 6.46 mmol, 4 equiv). The reaction was allowed to stir for 8 hours at room temperature. The reaction was placed in an ice bath, and DMSO (30 mL) was added over 2 minutes as it cooled. Once the reaction reached 0 °C, AmB (1.5 g, 1.62 mmol, 1.0 equiv) was added. The yellow-tan suspension slowly became soluble over 90 minutes stirring at 0 °C. The reaction was poured slowly into rapidly stirring diethyl ether (1.8 L) at 0 °C. After 15 minutes of stirring, the resulting yellow precipitate was vacuum filtered with a Buchner funnel equipped with Whatman 50 filter paper and washed 3 times with diethyl ether (200 mL). The yellow powder was dried under vacuum for 8 hours.

The powder was then suspended in THF:MeOH 1:1 (60 mL) and cooled to 0 °C. Camphorsulfonic acid (94 mg, 405 μmol, 0.25 equiv) was added, and the yellow-tan suspension slowly became soluble over 45 minutes of stirring at 0 °C. The reaction was quenched by triethyl amine (57 μL, 405 μmol, 0.25 equiv) at 0 °C. The reaction solution was concentrated by approximately 2/5 by rotary evaporation and poured into diethyl ether:hexane 1:1 (1.2 L) while stirring rapidly. After stirring 15 minutes, the yellow precipitate was collected in a Buchner funnel equipped with Whatman 50 filter paper by vacuum filtration. The precipitate was washed 3 times with diethyl ether (200 mL). The powder was dried under vacuum for 8 hours.
The powder was suspended in THF (60 mL) and cooled to 0 °C. Freshly distilled diazomethane (8.10 mmol, 5 equiv) was added drop wise to the suspension over 20 minutes at 0 °C. The reaction was allowed to stir for 30 additional minutes at 0 °C. After quenching with acetic acid (8.10 mmol, 5 equiv) at 0 °C, the solution was then concentrated under reduced pressure and purified by flash chromatography (SiO₂; DCM:MeOH 9:1) to give SI3.1 as a yellow solid (971 mg, 907 μmol, 56 %).

TLC (DCM:MeOH 9:1) Rₓ = 0.2, stained by CAM

HPLC Rₓ = 18.1 min; flow rate = 1mL/min, gradient = 5 → 95 % MeCN in water over 30 min.

¹H NMR (500 MHz, pyridine d-5:CD₃OD 10:1) δ 9.01 (d, J = 8.5 Hz, 1H), 7.53 (m, 2H), 7.25 (m, 3H), 6.58-6.32 (m, 12H), 6.23 (m, 1H), 5.69 (m, 2H), 4.95 (m, 1H), 4.90 (s, 1H), 4.83 (m, 1H), 4.67 (m, 2H), 4.46 (m, 2H), 4.38 (app d, J = 3 Hz, 1H), 4.17 (m, 1H), 4.01 (m, 2H), 3.86 (m, 2H), 3.74 (m, 5H), 3.56 (m, 1H), 3.26 (s, 3H), 2.94 (m, 1H), 2.84 (t, J = 10.5 Hz, 1H), 2.69 (m, 2H), 2.54 (m, 1H), 2.31-1.81 (m, 13 H), 1.72 (m, 1H), 1.57 (d, J = 6 Hz, 3H), 1.44 (d, J = 6 Hz, 3H), 1.32 (d, J = 6.5 Hz, 3H), 1.24 (d, J = 7 Hz, 3H)

¹³C NMR (125 MHz, pyridine d-5:CD₃OD 10:1) δ 174.4, 174.2, 172.3, 171.9, 137.9, 137.5, 134.8, 134.7, 134.3, 134.2, 133.8, 133.7, 133.6, 133.5, 133.1, 132.9, 132.4, 130.5, 130.1, 129.1, 127.3, 102.3, 99.4, 78.2, 75.8, 75.7, 75.4, 75.0, 72.1, 71.4, 71.0, 68.5, 67.8, 67.7, 67.1, 57.8, 56.8, 52.2, 45.2, 44.1, 43.8, 43.6, 42.1, 36.6, 31.0, 19.2, 18.9, 18.0, 12.8.

HRMS (ESI) Calculated for C₅₇H₈₃NO₁₈ (M + Na)+: 1092.5508
Found: 1092.5515
Synthesis of 3.1

To a suspension of **SI3.1** (1.50 g, 1.40 mmol, 1.0 equiv) in MeOH:THF 2:1 (17 mL) was added anisaldehyde dimethyl acetal (2 mL) followed by camphorsulfonic acid (81 mg, 0.35 mmol, 0.25 equiv). The solution was stirred for 20 min. The reaction was quenched with triethylamine drop wise until the dark tan solution underwent a color change to light tan. The reaction was poured into saturated sodium bicarbonate and extracted 3 times with ethyl acetate. The organic layers were washed with water followed by a wash with saturated sodium chloride. The organic layers were combined and dried over sodium sulfate, filtered, and concentrated under reduced pressure. Flash chromatography (SiO₂; EtOAc:Hexane:MeOH 77:20:3) purification yielded **3.1** as a yellow-orange solid (1.10 g, 0.84 mmol, 60%).

**TLC** (EtOAc:Hexane:MeOH 77:20:3) $R_f = 0.25$, stained by CAM

**HPLC** $R_t = 15.4$ min; flow rate = 1mL/min, gradient = 5% MeCN in water for 2 min then 5 → 54% MeCN in water over 3 min then 54 → 95% MeCN in water over 13 min.
**1H NMR** (500 MHz, CD$_3$C(O)CD$_3$) δ 7.42 (m, 2H), 7.35 (m, 4H), 7.29 (m, 2H), 7.21 (m, 2H), 6.86 (m, 4H), 6.43-6.20 (m, 12H), 5.88 (m, 1H), 5.58 (m, 1H), 5.51 (s, 1H), 5.46 (s, 1H), 5.26 (m, 1H), 4.64 (m, 1H), 4.58 (app s, 1H), 4.20-4.10 (m, 2H), 4.02 (m, 1H), 3.95-3.86 (m, 3H), 3.78 (m, 6H), 3.75 (m, 2H), 3.66 (s, 3H), 3.45 (m, 1H), 3.36 (m, 1H), 3.30 (m, 2H), 3.05 (s, 3H), 2.57 (m, 1H), 2.40 (m, 1H), 2.31-2.24 (m, 3H), 1.96 (m, 1H), 1.89-1.45 (m, 9H), 1.37 (m, 2H), 1.22 (m, 4H), 1.19 (d, J = 6 Hz, 3H), 1.17 (m, 1H), 1.11 (d, J = 6.5 Hz, 3H), 1.01 (d, J = 7.5 Hz, 3H)

**13C NMR** (125 MHz, CD$_3$C(O)CD$_3$) δ 173.6, 172.7, 169.7, 160.6, 160.5, 137.5, 136.9, 136.2, 134.1, 134.0, 133.9, 133.7, 133.6, 133.5, 132.9, 132.6, 132.5, 132.2, 129.9, 129.8, 128.9, 128.3, 128.2, 127.2, 120.9, 117.6, 113.8, 101.0, 100.7, 100.6, 98.1, 81.1, 77.9, 76.2, 74.7, 74.4, 73.2, 73.1, 72.9, 72.8, 70.7, 70.5, 67.2, 66.9, 57.3, 56.4, 55.4, 51.8, 48.6, 43.4, 43.3, 42.6, 41.8, 41.5, 37.8, 36.8, 33.8, 33.2, 28.7, 18.7, 18.0, 17.4, 11.8.

**HRMS (ESI)** Calculated for C$_{73}$H$_{95}$NO$_{20}$ (M + Na)$^+$: 1328.6369

Found: 1328.6388

**Synthesis of 3.2**

THF (160 mL) was added to a flask containing 3.1 (6.16 g, 4.72 mmol, 1.0 equiv). DMAP (922 mg) was added to a separate flask and dissolved in THF (100 mL). 4-tertbutylbenzoyl chloride (1.29 mL, 6.60 mmol, 1.4 equiv) was added drop wise to the DMAP solution creating a white suspension. DIPEA (1.31 mL, 7.54 mmol, 1.6 equiv) was added to the
solution of 3.1. A portion of the white suspension was then transferred drop wise to the solution of 3.1 and DIPEA (over approximately 1 h) until the majority of 3.1 had been consumed as evidenced by TLC. The reaction was pored into EtOAc and washed with water followed by saturated sodium bicarbonate. Two more washes with water were performed followed by a wash with saturated sodium chloride. The organic layer was then dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO₂; EtOAc:Hexane:MeOH 60:37:3) purification yielded 3.2 as a yellow-orange solid (3.11 g, 2.12 mmol, 45 %) as well as unreacted 3.2.

TLC (EtOAc:Hexane:MeOH 60:37:3) \( R_f = 0.22 \), stained by CAM

HPLC \( R_t = 19.4 \text{ min} \); flow rate = 1mL/min, gradient = 5% MeCN in water for 2 min then 5 → 54% MeCN in water over 3 min then 54 → 95% MeCN in water over 13 min.

\( ^1\text{H NMR} \) (500 MHz, CD₃C(O)CD₃) \( \delta \): 7.99 (d, \( J = 8.5 \text{ Hz}, 2\text{H} \)), 7.59 (d, \( J = 8.5 \text{ Hz}, 2\text{H} \)), 7.39 (m, 3H), 7.34 (m, 2H), 7.23 (m, 2H), 7.17 (m, 2H), 7.12 (m, 1H), 6.85 (m, 4H), 6.39-6.13 (m, 10H), 6.07 (m, 1H), 5.92 (m, 1H), 5.76 (m, 1H), 5.68 (m, 1H), 5.56 (m, 1H), 5.48 (s, 1H), 5.43 (s, 1H), 5.14 (m, 1H), 4.88 (app s, 1H), 4.65 (m, 1H), 4.24 (m, 1H), 4.15 (m, 2H), 3.97 (m, 1H), 3.91-3.82 (m, 2H), 3.77 (s, 6H), 3.68 (m, 5H), 3.51 (m, 2H), 3.47 (m, 1H), 3.40 (m, 2H), 2.84 (s, 3H), 2.54 (m, 1H), 2.41 (m, 1H), 2.27 (m, 1H), 2.13 (m, 1H), 1.95 (m, 1H), 1.86 (m, 1H), 1.78-1.42 (m, 10H), 1.40-1.31 (m, 13H), 1.30-1.19 (m, 2H), 1.18 (d, \( J = 6.5 \text{ Hz}, 3\text{H} \)), 1.11 (d, \( J = 6.5 \text{ Hz}, 3\text{H} \)), 1.01 (d, \( J = 7.0 \text{ Hz}, 3\text{H} \))
$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) δ 172.7, 171.1, 169.2, 165.3, 160.1, 160.0, 156.7, 137.1, 136.4, 136.3, 133.6, 133.5, 133.4, 133.3, 133.2, 133.1, 132.5, 132.1, 132.0, 131.2, 131.1, 130.0, 129.4, 128.3, 127.7, 126.5, 125.6, 120.7, 113.3, 100.5, 96.1, 80.5, 77.2, 75.7, 74.1, 73.1, 72.6, 72.4, 71.7, 70.6, 66.4, 66.2, 57.2, 54.8, 54.1, 51.3, 47.9, 43.0, 42.7, 41.9, 40.9, 37.2, 36.3, 35.1, 33.4, 32.6, 30.8, 17.8, 17.4, 11.3.

**HRMS (ESI)** Calculated for C$_{94}$H$_{107}$NO$_{21}$ (M + Na)+: 1488.7233

Found: 1488.7212

**Synthesis of 3.3**

3.2 (2.30 g, 1.57 mmol, 1.0 equiv) was azeotropically dried with acetonitrile and left under vacuum overnight. DCM (40 mL) was added followed by hexane (40 mL) slowly while stirring to prevent 3.2 from crashing out of solution. 2,6-lutidine (2.4 mL, 20.4 mmol, 13 equiv) was added and the solution was cooled to 0 °C. Diethylisopropylsilyl triflate (DEIPSOTf) (2.5 mL, 12.5 mmol, 8.0 equiv) was added drop wise at 0 °C over 20 min. The reaction was stirred for an additional 30 min. The reaction was diluted with diethyl ether and quenched with saturated sodium bicarbonate at 0 °C. The reaction was extracted with diethyl ether and washed with 1.0 M copper sulfate until no white precipitate was observed. The organic layers were washed twice with water and then once with saturated sodium chloride. The organic layers were then dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO$_2$;
EtOAc:Hexane 1:9 → 1:4) purification yielded **3.3** as a yellow-orange solid (2.24 g, 1.13 mmol, 72%).

\[
\text{TLC (EtOAc:Hexane 1:3) } R_f = 0.25, \text{ stained by CAM}
\]

\[
\textbf{1H NMR} \ (500 \text{ MHz, CD}_3\text{C(O)CD}_3) \ \delta \ 8.01 \ (d, J = 8.5 \text{ Hz, 2H}), \ 7.63 \ (d, J = 8.5 \text{ Hz, 2H}), \ 7.34 \ (m, 4H), \ 7.22 \ (m, 3H), \ 7.17 \ (m, 3H), \ 6.84 \ (m, 4H), \ 6.35-6.13 \ (m, 9H), \ 6.04 \ (m, 1H), \ 5.91 \ (m, 1H), \ 5.74 \ (m, 2H), \ 5.49 \ (m, 1H), \ 5.41 \ (s, 1H), \ 5.39 \ (s, 1H), \ 4.92 \ (app s, 1H), \ 4.75 \ (m, 1H), \ 4.66 \ (m, 1H), \ 4.31 \ (m, 1H), \ 4.25 \ (m, 1H), \ 4.12 \ (m, 1H), \ 3.84 \ (m, 1H), \ 3.81-3.77 \ (m, 9H), \ 3.68 \ (m, 4H), \ 3.64 \ (s, 3H), \ 3.57-3.45 \ (m, 3H), \ 2.74 \ (s, 3H), \ 2.45 \ (m, 2H), \ 2.26 \ (m, 1H), \ 2.17 \ (m, 1H), \ 2.09 \ (m, 1H), \ 1.90 \ (m, 2H), \ 1.73-1.59 \ (m, 4H), \ 1.51-1.34 \ (m, 18H), \ 1.26-1.11 \ (m, 6H), \ 1.08-0.76 \ (m, 54H), \ 0.73-0.39 \ (m, 19H).
\]

\[
\textbf{13C NMR} \ (125 \text{ MHz, CD}_3\text{C(O)CD}_3) \ \delta \ 173.1, \ 171.1, \ 169.8, \ 165.9, \ 160.7, \ 157.4, \ 138.2, \ 136.5, \ 134.6, \ 134.4, \ 134.2, \ 133.9, \ 133.8, \ 133.4, \ 132.8, \ 132.7, \ 132.5, \ 132.1, \ 131.1, \ 130.7, \ 130.6, \ 130.2, \ 129.0, \ 128.9, \ 128.8, \ 128.6, \ 128.2, \ 127.1, \ 126.2, \ 121.3, \ 117.9, \ 115.1, \ 113.8, \ 113.7, \ 101.7, \ 100.9, \ 100.5, \ 96.6, \ 81.3, \ 75.6, \ 74.7, \ 73.7, \ 73.2, \ 72.9, \ 72.7, \ 72.6, \ 68.6, \ 66.6, \ 58.1, \ 55.4, \ 54.5, \ 51.8, \ 48.3, \ 43.4, \ 41.0, \ 37.9, \ 36.8, \ 35.7, \ 31.4, \ 19.0, \ 18.0, \ 17.9, \ 17.8, \ 17.7, \ 17.6, \ 17.4, \ 14.0, \ 13.8, \ 13.4, \ 7.7, \ 7.6, \ 7.5, \ 7.4, \ 7.1, \ 5.1, \ 4.8, \ 4.7, \ 4.6, \ 4.4, \ 4.1.
\]

\[
\textbf{HRMS} \ (ESI) \text{ Calculated for } \text{C}_{112}\text{H}_{171}\text{NO}_{21}\text{Si}_{4} \text{ (M + Na)+: 2001.1318}
\]

\[
\text{Found: 2001.1221}
\]
**Synthesis of 3.4**

3.3 (550 mg, 278 µmol, 1.0 equiv) was dissolved in THF:MeOH 1:2 (13.5 mL), and KCN (27.0 mg, 417 µmol, 1.5 equiv) was added. The reaction was heated to 40 °C for 2 days. The reaction was diluted with diethyl ether and washed with water three times followed by a wash of saturated sodium chloride. The organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO₂; EtOAc:Hexane 1:4 → 3:7) purification yielded 3.4 as a yellow-orange solid (329 mg, 181 µmol, 65%).

\[ 3.4 \]

**TLC** (EtOAc:Hexane 3:7) \( R_f = 0.22 \), stained by CAM

\[ \]

**\(^1\)H NMR** (500 MHz, CD₃C(O)CD₃) \( \delta \) 7.35 (m, 6H), 7.29 (m, 2H), 7.22 (m, 1H), 6.93 (d, \( J = 9.5 \) Hz, 1H), 6.85 (m, 4H), 6.40-6.17 (m, 11H), 6.09 (m, 1H), 5.82 (m, 1H), 5.77 (m, 1H), 5.43 (s, 1H), 5.42 (s, 1H), 4.80 (m, 1H), 4.62 (m, 1H), 4.60 (app s, 1H), 4.23 (m, 1H), 4.15 (m, 1H), 3.98 (m, 1H), 3.85 (m, 3H), 3.78 (m, 7H), 3.71 (m, 4H), 3.66 (m, 2H), 3.62 (s, 3H), 3.58-3.50 (m, 2H), 3.32 (m, 1H), 3.02 (s, 3H), 2.48 (m, 1H), 2.42 (m, 1H), 2.29-2.19 (m, 3H), 1.95-1.87 (m, 3H), 1.74 (m, 2H), 1.62-1.28 (m, 7H), 1.24-1.15 (m, 7H), 1.04-0.76 (m, 56H), 0.72-0.50 (m, 13H), 0.44-0.36 (m, 4H).
$^{13}$C NMR (125 MHz, CD$_3$(O)CD$_3$) $\delta$ 173.4, 170.8, 169.9, 160.8, 160.6, 137.2, 136.9, 134.6, 134.5, 134.1, 133.9, 133.8, 133.6, 133.2, 132.7, 132.6, 132.2, 130.9, 130.3, 129.5, 129.1, 128.7, 128.3, 127.3, 121.3, 117.9, 113.9, 113.8, 110.6, 101.8, 101.1, 100.8, 98.5, 81.4, 75.9, 75.0, 74.7, 74.6, 73.4, 73.0, 72.8, 71.5, 68.8, 67.1, 57.7, 55.8, 55.5, 52.0, 48.5, 43.8, 43.0, 41.2, 37.9, 36.7, 33.5, 32.7, 28.1, 18.9, 18.0, 17.9, 17.8, 17.4, 14.0, 13.9, 13.8, 13.5, 7.7, 7.6, 7.5, 7.2, 5.1, 4.9, 4.7, 4.6, 4.4, 4.1.

HRMS (ESI) Calculated for C$_{101}$H$_{159}$NO$_{20}$Si$_4$ (M + Na)+: 1841.0430
Found: 1841.0464

Synthesis of 3.5

3.4 (350 mg, 192 μmol, 1.0 equiv), triiodoimidazole (130 mg, 288 μmol, 1.5 equiv), triphenylphosphine (152 mg, 378 μmol, 3.0 equiv), and imidazole (60 mg, 866 μmol, 4.5 equiv) were placed in a flask and dissolved in toluene (9.6 mL). The reaction was heated to 70 °C for 3 h. The reaction was diluted with diethyl ether and washed with saturated sodium bicarbonate followed by water four times. A final wash of saturated sodium chloride was performed, and the organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO$_2$; EtOAc:Hexane 3:17) purification yielded 3.5 as a yellow-orange solid (296 mg, 154 μmol, 80%).
TLC (EtOAc:Hexane 3:7) $R_f = 0.55$, stained by CAM

$^1$H NMR (500 MHz, CD$_3$C(O)CD$_3$) $\delta$ 7.38-7.28 (m, 9H), 7.22 (m, 1H), 6.86 (m, 4H), 6.40-6.09 (m, 12H), 5.83 (m, 2H), 5.43 (s, 1H), 5.41 (s, 1H), 4.79 (m, 1H), 4.69 (app d, $J = 7.5$ Hz, 1H), 4.57 (m, 1H), 4.28 (m, 1H), 4.15 (m, 1H), 4.04 (m, 1H), 3.89 (m, 1H), 3.84 (m, 1H), 3.77 (m, 7H), 3.72 (m, 3H), 3.65 (s, 3H), 3.57 (m, 2H), 3.40 (m, 1H), 3.05 (s, 3H), 2.51-2.40 (m, 2H), 2.25 (m, 3H), 2.00 (m, 1H), 1.88 (m, 2H), 1.74 (m, 2H), 1.62-1.40 (m, 7H), 1.31-1.15 (m, 9H), 1.07-0.76 (m, 58H), 0.72-0.50 (m, 13H), 0.44-0.36 (m, 4H).

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) $\delta$ 172.5, 169.3, 160.6, 160.2, 160.0, 158.4, 137.5, 134.2, 134.1, 133.9, 133.6, 133.4, 133.2, 132.9, 132.5, 132.1, 132.0, 131.9, 131.3, 130.2, 130.0, 128.3, 128.1, 127.7, 126.8, 113.3, 113.2, 102.1, 101.3, 100.5, 100.1, 94.2, 80.8, 76.3, 75.4, 74.4, 74.0, 72.5, 72.2, 68.1, 66.7, 57.0, 54.8, 51.4, 47.9, 43.8, 43.4, 42.5, 40.6, 37.3, 36.8, 32.9, 27.5, 18.1, 17.4, 17.3, 17.2, 16.8, 13.4, 13.2, 12.9, 7.1, 7.0, 6.9, 6.6, 4.5, 4.3, 4.2, 4.1, 3.8, 3.6.

HRMS (ESI) Calculated for C$_{101}$H$_{158}$NO$_{19}$Si$_{4}$I (M + Na)+: 1950.9448

Found: 1950.9543
Synthesis of 3.6

\[
\begin{align*}
&3.5 \text{ (320 mg, 166 μmol, 1.0 equiv)} \text{ was placed in a vial and azeotropically dried with} \\
&\text{toluene and placed under vacuum overnight. The vial was backfilled with argon and DMPU} \\
&(6.6 \text{ mL}) \text{ was added. Sodium borohydride (50 mg, 1.33 mmol, 8.0 equiv)} \\
&\text{and silver(I) acetate (42 mg, 249 μmol, 1.5 equiv) was added in a glovebox. The reaction was heated} \\
&\text{in the range of 50-55 °C for 3 h. After 3 h, an aliquot was removed in the glovebox every 30} \\
&\text{min to monitor the reaction by TLC. The reaction was allowed to run to approximately 85} \\
&\text{% conversion until the rate of decomposition exceeded conversion of the starting material.} \\
&\text{The reaction was cooled to room temperature and then diluted with dry diethyl ether that} \\
&\text{had been cooled to 0 °C. The reaction was quenched with saturated sodium bicarbonate} \\
&\text{cooled to 0 °C. Room temperature diethyl ether was used to extract the aqueous layer. The} \\
&\text{organic layer was then washed with water twice. A final wash of saturated sodium chloride} \\
&\text{was performed, and the organic layers were dried over sodium sulfate and filtered. The} \\
&\text{solvent was removed under reduced pressure and column chromatography (SiO}_2; \\
&\text{EtOAc:Hexane 3:17) purification yielded 3.6 as a yellow-orange solid (89.8 mg, 49.8 μmol,} \\
&\text{30 %).}
\end{align*}
\]

This reaction is quite sensitive to water and air. DMPU was obtained from Aldrich absolute 
over molecular sieves H$_2$O ≤ 0.03%. The product is unstable to the reaction conditions and 
decomposes over time; the best yields are obtained by stopping the reaction before 
complete conversion is reached. The reaction was found to be dependent upon the identity 
of the protecting group on the C3’ amine. Extensive elimination or inactivity was observed 
for other protecting groups.
TLC (EtOAc:Hexane 1:3) $R_f = 0.47$, stained by CAM

$^1$H NMR (500 MHz, CD$_3$C(O)CD$_3$) $\delta$ 7.40-7.33 (m, 9H), 7.26 (m, 1H), 6.87 (m, 4H), 6.42-6.06 (m, 12H), 5.70 (m, 2H), 5.46 (s, 1H), 5.44 (s, 1H), 4.69 (app d, $J = 5$ Hz, 1H), 4.97 (m, 1H), 4.23 (m, 3H), 3.93 (m, 1H), 3.82-3.70 (m, 10H), 3.66 (m, 4H), 3.58 (m, 2H), 3.39 (m, 1H), 3.17 (m, 1H), 3.04 (s, 3H), 2.63 (m, 2H), 2.42 (m, 1H), 2.30 (m, 3H), 1.98 (m, 1H), 1.88 (m, 1H), 1.76-1.34 (m, 10H), 1.29-1.14 (m, 8H), 1.05-0.76 (m, 59H), 0.72-0.50 (m, 13H), 0.44-0.36 (m, 4H).

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) $\delta$ 173.6, 170.0, 160.8, 160.7, 136.9, 134.6, 134.3, 133.6, 136.4, 133.0, 132.6, 132.5, 130.0, 129.4, 128.7, 128.4, 127.6, 113.9, 113.8, 102.0, 101.2, 81.7, 80.9, 76.5, 75.2, 73.4, 73.1, 55.5, 51.7, 44.1, 43.8, 42.7, 41.5, 37.9, 33.6, 32.6, 28.5, 18.0, 17.9, 17.8, 17.6, 17.4, 13.9, 13.5, 7.7, 7.6, 7.5, 7.4, 7.2, 4.8, 4.7, 4.4, 4.2.

HRMS (ESI) Calculated for C$_{101}$H$_{159}$NO$_{19}$Si$_4$ (M + Na)$^+$: 1825.0481

Found: 1825.0496
Synthesis of 3.7

3.5 (18.2 mg, 10 μmol, 1.0 equiv), triphenylphosphine (4.0 mg, 15 μmol, 1.5 equiv), and p-nitrobenzoic acid (2.0 mg, 12 μmol, 1.15 equiv) were placed in a flask and azeotroped in toluene to dryness (3x 0.5 mL). The reaction was then dissolved in toluene (0.3 mL) and cooled to 0 °C for the drop wise addition of DIAD (3.0 μL, 15 μmol, 1.5 equiv). The reaction was stirred for 20 min at 0 °C then heated to 70 °C for 2 h. The reaction was diluted with diethyl ether (10 mL) and washed with saturated sodium bicarbonate (3.0 mL). The aqueous phase was extracted with diethyl ether (10 mL). A final wash of saturated sodium chloride was performed, and the organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO₂; EtOAc:Hexane 5:95 to 1:3) purification yielded the C2’ nitrobenzoate ester as a yellow-orange solid. Two reactions were run: (11.7 mg, 5.9 μmol, 59 %) (13.5 mg, 6.8 μmol, 68%)

The C2’ p-nitrobenzoate ester was combined (25.2 mg, 12.8 μmol, 1.0 equiv) and taken up in MeOH:THF 2:1 (435 μL). Potassium cyanide (2.5 mg, 38 μmol, 3.0 equiv) was then added and the reaction was stirred for 72 h at 30 °C. The reaction was then diluted with diethyl ether (10 mL) and washed with saturated sodium bicarbonate (3.0 mL). The aqueous phase was extracted with diethyl ether (2x 10 ml). A final wash of saturated sodium chloride was performed, and the organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO₂; EtOAc:Hexane 1:4 → 3:7) purification yielded 3.7 as a yellow-orange solid (15.8 mg, 8.7 μmol, 68%).
**TLC** (EtOAc:Hexane 3:7) $R_f = 0.2$, stained by CAM

**$^1H$ NMR** (500 MHz, CD$_3$C(O)CD$_3$) $\delta$ 7.36 (m, 6H), 7.28 (m, 2H), 7.21 (m, 1H), 7.15 (m, 1H), 6.86 (m, 4H), 6.41-6.18 (m, 11H), 6.10 (m, 1H), 5.86 (m, 1H), 5.77 (m, 1H), 5.44 (s, 1H), 5.43 (s, 1H), 4.81 (m, 1H), 4.56 (m, 1H), 4.36 (app d, $J = 7.5$, 1H), 4.26 (m, 1H), 4.17 (m, 1H), 3.97-3.85 (m, 4H), 3.79 (m, 7H), 3.73 (m, 4H), 3.66 (s, 3H), 3.61-3.48 (m, 3H), 3.43 (m, 1H), 3.33 (m, 1H), 3.04 (s, 3H), 2.49 (m, 1H), 2.42 (m, 1H), 2.30-2.21 (m, 3H), 1.95-1.87 (m, 3H), 1.80-1.67 (m, 3H), 1.64-1.27 (m, 6H), 1.24-1.16 (m, 7H), 1.04-0.76 (m, 56H), 0.73-0.51 (m, 13H), 0.46-0.37 (m, 4H).

**$^{13}C$ NMR** (125 MHz, CD$_3$C(O)CD$_3$) $\delta$ 173.9, 172.1, 170.1, 161.0, 137.9, 137.0, 134.8, 134.7, 134.3, 134.1, 133.9, 133.7, 133.3, 132.9, 132.4, 131.1, 130.5, 129.7, 129.1, 128.9, 128.5, 127.3, 114.1, 114.0, 103.4, 102.0, 101.3, 100.9, 81.6, 76.5, 76.1, 75.2, 74.7, 73.3, 73.0, 69.0, 67.6, 57.8, 55.6, 52.1, 48.6, 44.3, 44.2, 38.1, 33.7, 32.9, 19.1, 18.2, 18.1, 18.0, 17.6, 14.3, 14.2, 14.1, 13.7, 7.8, 7.7, 7.6, 7.3, 5.4, 5.1, 5.0, 4.9, 4.6, 4.4

**HRMS** (ESI) Calculated for C$_{101}$H$_{159}$NO$_{20}$Si$_4$ (M + Na)$+:$ 1841.0430

Found: 1841.0464
Synthesis of 3.8

Ergosterol (400 mg, 1.01 mmol, 1.0 equiv) and succinic anhydride (1.01 g, 10.1 mmol, 10.0 equiv) were azeotroped with toluene (3x 1.0 mL) in a 40 mL vial. Dry pyridine (20 mL 0.05 M) was then added followed by dimethylaminopyridine (DMAP) (154.2 mg, 1.26 mmol, 1.25 equiv). The reaction was sealed with a Teflon lined cap and heated to 140 °C for 16 h. The resulting black solution was extracted with HCl (10% v/v) and EtOAc. The organic phase was dried with sodium sulfate, filtered, and concentrated. Chromatography (SiO$_2$; EtOAc:Hexane 1:5 with 1% AcOH) purification yielded A as a white solid (282 mg, 0.57 mmol, 56%).

A (12.5 mg, 25 μmol, 2.5 equiv) was dissolved in toluene (0.3 mL) and oxalyl chloride (10.0 μL, 118 μmol, 4.75 equiv) was added and the reaction was heated to 50 °C and stirred for 15 min. The resulting yellow solution was azeotroped with toluene (3x 0.3 mL) to dryness. The resulting off-white solid B was then dissolved in THF (0.3 mL) whereupon DMAP (3.1 mg, 25 μmol, 2.5 equiv) was added to generate a cloudy white suspension. In a separate vial 3.4 (18.2 mg, 10 μmol, 1.0 equiv) was dissolved in THF (0.15 mL) and diethylisopropylamine (10 μL, 57 μmol, 5.7 equiv) was added. The resulting yellow/orange solution was added drop wise via cannula to the first suspension and stirred for 3 h at room temperature. The reaction was diluted with diethyl ether (10 mL) and washed with saturated sodium bicarbonate (3.0 mL). The aqueous phase was extracted with diethyl
ether (2x 10 mL). A final wash of saturated sodium chloride was performed, and the organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and column chromatography (SiO$_2$; EtOAc:Hexane 5:95 $\rightarrow$ 1:3) purification yielded the 3.8 as a yellow-orange solid (14.5 mg, 6.3 μmol, 63%)

![Chemical structure](image)

TLC (EtOAc:Hexane 1:3) $R_f$ = 0.76, stained by CAM

$^1$H NMR (500 MHz, CD$_3$C(O)CD$_3$) δ 7.36 (m, 4H), 7.28 (m, 4H), 7.23 (m, 1H), 6.93 (m, 1H), 6.86 (m, 4H), 6.39-6.12 (m, 12H), 5.84 (m, 2H), 5.61 (m, 1H), 5.41 (m, 3H), 5.26 (m, 3H), 5.83-4.61 (m, 4H), 4.29-4.13 (m, 3H), 3.90-3.81 (m, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.72 (m, 2H), 3.67 (s, 3H), 3.56 (m, 2H), 3.47 (m, 2H), 3.00 (s, 3H), 2.71-2.34 (m, 9H), 2.31-2.08 (m, 5H), 2.00-1.45 (m, 30H), 1.41-0.38 (m, 98H)

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) δ 173.4, 173.2, 172.3, 171.2, 170.1, 160.8, 142.3, 139.6, 137.8, 136.8, 136.7, 134.8, 134.5, 134.1, 133.7, 133.3, 132.9, 132.6, 132.1, 131.0, 130.5, 129.2, 128.9, 128.5, 127.5, 126.2, 121.4, 117.5, 114.1, 114.0, 102.0, 101.3, 100.9, 81.6, 76.2, 75.2, 75.1, 74.1, 73.9, 73.3, 73.1, 72.3, 68.9, 67.1, 58.1, 56.7, 55.7, 55.4, 54.6 52.1, 48.8, 47.1, 44.2, 43.9, 43.8, 43.7, 43.2, 41.4, 39.9, 38.8, 38.0, 37.6, 34.0, 33.8, 30.9, 29.1, 29.0, 28.4, 23.9, 21.7, 20.5, 20.2, 19.2, 18.3, 18.2, 18.1, 18.0, 17.9, 17.6, 16.6, 14.2, 14.1, 13.7, 12.6, 7.9, 7.8, 7.7, 7.6, 7.3, 5.4, 5.1, 5.0, 4.9, 4.7, 4.4, 1.5

MS (MALDI) Calculated for C$_{133}$H$_{205}$NO$_{23}$Si$_4$ (M + Na)$^+$: 2319

Found: 2319
Synthesis of 3.16

Epoxide intermediate 3.15 (8 g, 21 mmol, 1.0 equiv) was dissolved in THF (263 mL). The resulting solution was cooled to 0 °C, and LiHBEt$_3$ (1M in THF) (105 mL, 105 mmol, 5.0 equiv) was added slowly. The reaction heated to 60 °C for 2.5 h. The reaction was cooled to 0 °C and quenched with 1M ammonium chloride. The mixture was extracted with ether. The organic layer was washed with water and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO$_2$; Ether:Hexane 1:4 → 1:3) purification yielded 3.16 as an oil (5.47 g, 14.3 mmol, 68 %).

**TLC** (Ether:Hexane 3:7) $R_f = 0.38$, stained by CAM

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.27 (d, $J = 8.5$ Hz, 2H), 6.88 (d, $J = 9$ Hz, 2H), 4.87 (d, $J = 4$ Hz, 1H), 4.66 (d, $J = 12$ Hz, 1H), 4.44 (d, $J = 11.5$ Hz, 1H), 4.04 (m, 1H), 3.92 (m, 1H), 3.81 (s, 3H), 3.32 (dd, $J = 3$ Hz, $J = 9.5$ Hz, 1H), 3.19 (m, 1H), 2.14 (dd, $J = 3.5$ Hz, $J = 15$ Hz, 1H), 1.89 (td, $J = 3.5$ Hz, $J = 14.5$ Hz, 1H), 1.26 (d, $J = 6.5$ Hz, 3H), 0.93 (s, 9H), 0.12 (s, 6H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 159.5, 129.9, 114.0, 95.7, 75.1, 68.9, 68.0, 63.7, 55.5, 35.7, 26.1, 18.4, -4.0, -4.4

**HRMS** (ESI) Calculated for C$_{20}$H$_{34}$O$_5$Si (M + Na)$^+$: 405.2073

Found: 405.2078
Synthesis of 3.17

3.16 (4.83 g, 12.6 mmol, 1 equiv) was dissolved in THF (15 mL). Pyridine (10.2 mL, 126 mmol, 10 equiv) and MsCl (3.17 mL, 41 mmol, 3.25 equiv) were added. The reaction was stirred overnight. The reaction was then quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with 1M ammonium chloride, water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO$_2$; Ether:Hexane 2:3) purification yielded 3.17 as a solid (4.24 g, 9.2 mmol, 73 %).

TLC (Ether:Hexane 2:3) $R_f = 0.27$, stained by CAM

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.27 (d, $J = 9$ Hz, 2H), 6.88 (d, $J = 8.5$ Hz, 2H), 4.90 (dd, $J = 3$ Hz, $J = 8$ Hz, 2H), 4.66 (d, $J = 11.5$ Hz, 1H), 4.35 (d, $J = 11$ Hz, 1H), 4.13 (m, 1H), 3.80 (s, 3H), 3.43 (dd, $J = 3$ Hz, $J = 9$ Hz, 1H), 2.91 (s, 3H), 2.41 (dd, $J = 3$ Hz, $J = 15$ Hz, 1H), 1.96 (m, 1H), 1.23 (d, $J = 6.5$ Hz, 3H), 0.93 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 159.4, 130.2, 129.6, 113.9, 94.8, 77.5, 72.7, 69.1, 64.3, 55.5, 39.9, 34.5, 26.0, 18.3, -3.9, -4.6

HRMS (ESI) Calculated for C$_{21}$H$_{36}$O$_5$Si (M + Na)+: 483.1849

Found: 483.1848
Synthesis of 3.18

3.17 (1.6 g, 3.47 mmol, 1.0 equiv) was dissolved in DMF (15 mL). Sodium azide (1.6 g, 24.3 mmol, 7 equiv) was added. The reaction heated to 160 °C for 1.5 h. The reaction was cooled to room temperature. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO₂; Ether:Hexane 1:19) purification yielded 3.18 as a solid (1.13 g, 2.78 mmol, 80 %).

TLC (Ether:Hexane 1:19) Rf = 0.30, stained by CAM

^1H NMR (500 MHz, CDCl₃) δ 7.29 (d, J = 9 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 4.91 (d, J = 3 Hz, 2H), 4.61 (d, J = 11.5 Hz, 1H), 4.39 (d, J = 11 Hz, 1H), 3.82 (s, 3H), 3.71 (m, 2H), 3.10 (t, J = 9 Hz, 1H), 2.19 (dd, J = 5 Hz, J = 13.5 Hz, 1H), 1.73 (td, J = 4 Hz, J = 12.5 Hz, 1H), 1.27 (d, J = 6 Hz, 3H), 0.94 (s, 9H), 0.22 (s, 3H), 0.13 (s, 3H).

^13C NMR (125 MHz, CDCl₃) δ 159.6, 129.9, 129.8, 114.1, 95.4, 76.7, 68.9, 68.8, 61.8, 55.5, 35.9, 26.2, 18.7, 18.4, -3.9, -4.0.

HRMS (ESI) Calculated for C₂₀H₃₃N₃O₄Si (M + Na)+: 430.2138
Found: 430.2156
Synthesis of 3.12

3.18 (6.5 g, 15.9 mmol, 1.0 equiv) was dissolved in DCM:H₂O 9:1 (160 mL). The solution was cooled to 0 °C, and DDQ (4.3 g, 19.1 mmol, 1.2 equiv) was added. The reaction was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO₂; Ether:Hexane 1:19) purification followed by (C₁₈ SiO₂, water:MeCN 1:4) yielded 3.12 as a solid (3.47 g, 12.1 mmol, 76 %).

TLC (EtOAc:Hexane 1:4) Rᶠ = 0.31, stained by CAM (H₂O:MeCN 1:4) Rᶠ = 0.50, stained by CAM

HRMS (ESI) Calculated for C₁₂H₂₅N₃O₃Si (M + Na)+: 310.1563
Found: 310.1566
Synthesis of SI3.3

Intermediate SI3.2 (15.8 g, 7.20 mmol, 1.0 equiv) was azeotropically dried with toluene and placed under vacuum overnight. Hexane (240 mL) and 2,6-lutidine (2.9 mL, 25.2 mmol, 3.5eq) were added. The resulting solution was cooled to 0 °C and triisopropylsilyl triflate (2.9 mL, 10.8 mmol, 1.5eq) was added slowly over 15 min. The reaction was quenched after 1 h with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with copper sulfate, water, and finally saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO2; Ether:Hexane 5:95 → 1:4) purification yielded the SI3.3 as a yellow-orange solid (15.2 g, 6.5 mmol, 90%).

**TLC** (Ether:Hexane 0.1% Et3N 3:7) \( R_f \) = 0.72, stained by CAM

**\(^{1}\)H NMR** (500 MHz, CD3C(O)CD3)\( \delta \) 7.86 (d, \( J = 7.5 \) Hz, 2H), 7.69 (d, \( J = 7.5 \) Hz, 2H), 7.41 (t, \( J = 7.5 \) Hz, 2H), 7.33 (t, \( J = 7.5 \) Hz, 2H), 6.53-6.05 (m, 12H), 5.51 (m, 1H), 5.34 (m, 1H), 4.65 (m, 2H), 4.47 (m, 3H), 4.34 (m, 2H), 4.24 (m, 2H), 4.13 (m, 1H), 3.98 (m, 2H), 3.90 (m, 1H), 3.83 (m, 1H), 3.66 (m, 2H), 3.45 (m, 1H), 3.27 (m, 1H), 3.15 (s, 3H), 2.56 (m, 1H), 2.42 (m, 2H),
2.10-2.01 (m, 3H), 1.94-1.59 (m, 12H), 1.50 (m, 1H), 1.38-1.30 (m, 4H), 1.23 (m, 4H), 1.16 (m, 20H), 1.07-0.89 (m, 85H), 0.78-0.55 (m, 56H).

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) $\delta$ 172.3, 170.5, 156.2, 145.0, 142.1, 139.0, 135.7, 135.3, 135.2, 134.7, 133.8, 132.9, 132.8, 132.7, 132.5, 131.4, 130.7, 130.6, 128.4, 127.8, 125.8, 125.7, 120.7, 101.2, 99.5, 76.7, 74.6, 74.0, 73.9, 73.2, 71.1, 69.4, 68.0, 67.5, 67.3, 67.2, 59.0, 58.2, 48.2, 48.0, 47.8, 44.3, 43.4, 42.1, 41.2, 37.2, 35.6, 27.4, 19.9, 19.2, 19.0, 18.4, 18.2, 12.9, 11.3, 7.6, 7.5, 7.4, 7.3, 6.4, 6.2, 6.1, 6.0, 5.9, 5.8.

LRMS (ESI) Calculated for C$_{126}$H$_{231}$NO$_{19}$Si$_{10}$ (M + Na)+: 2365.5  
Found: 2365.1

Synthesis of 3.13

Intermediate SI3.3 (12.5 g, 5.35 mmol, 1.0 equiv) was azeotropically dried with toluene and placed under vacuum overnight. THF (100 mL) was added. The resulting solution was cooled to 0 °C, and DDQ (1.82 g, 8.03 mmol, 1.5eq) and CaCO$_3$ (5.3 g, 53.5 mmol, 10 equiv) were added. The reaction was warmed to room temperature and quenched after 30 min with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with water and then saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and flash column chromatography (SiO$_2$; Ether:Hexane 1:4) purification yielded the enone as a dark red solid. This intermediate is sensitive to silica gel and was immediately subjected to the next reaction conditions.
TLC (Ether:Hexane 3:17) \( R_f = 0.35 \), stained by CAM

LRMS (ESI) Calculated for C\(_{93}\)H\(_{180}\)NO\(_{14}\)Si\(_{8}\) (M + Na)+: 1768.1

Found: 1768.0

The enone intermediate was azeotropically dried with toluene. THF (10 mL) and MeOH (20 mL) was added. The resulting solution was cooled to 0 °C, and NaBH\(_4\) (1.08 g, 28.6 mmol, 5.3 equiv) was added. The reaction was quenched after 30 min with 1M aqueous ammonium chloride and extracted with ether. The organic layer was washed with water and then saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and flash column chromatography (SiO\(_2\); Ether:Hexane 1:9 → 1:4) purification yielded the 3.13 as a yellow-orange solid. This intermediate is not stable to long term storage and extended periods on silica gel (4.5 g, 2.57 mmol, 48 % 2 steps).

TLC (Ether:Hexane 1:4) \( R_f = 0.44 \), stained by CAM

\(^1\)H NMR (500 MHz, CD\(_3\)C(O)CD\(_3\)) \( \delta \) 6.49-6.10 (m, 13H), 5.53 (m, 1H), 4.68 (m, 1H), 4.50 (m, 2H), 4.22 (m, 1H), 4.15 (m, 1H), 4.06 (m, 1H), 4.00 (m, 1H), 3.91 (d, \( J = 4 \) Hz, 1H), 3.83 (m, 1H), 3.68 (m, 1H), 3.63 (m, 1H), 3.15 (s, 3H), 2.55 (m, 2H), 2.42 (m, 1H), 2.36 (m, 1H), 2.13
(m, 1H), 2.01 (m, 2H), 1.95-1.70 (m, 8H), 1.63 (m, 3H), 1.49 (m, 1H), 1.31 (m, 3H), 1.18-1.14 (m, 20H), 1.07-0.96 (m, 69H), 0.77-0.61 (m, 43H).

$^{13}$C NMR (125 MHz, CD$_3$C(O)CD$_3$) δ 172.2, 170.5, 139.6, 138.6, 134.8, 134.7, 134.0, 133.3, 133.1, 133.0, 132.8, 132.7, 131.7, 131.6, 130.8, 127.9, 101.1, 76.7, 74.0, 73.2, 71.0, 69.3, 69.1, 67.4, 67.3, 59.2, 48.0, 47.8, 44.4, 43.5, 41.3, 40.6, 35.5, 27.4, 19.8, 19.2, 18.4, 18.3, 12.8, 11.2, 7.7, 7.6, 7.5, 7.4, 7.3, 6.4, 6.2, 6.1, 5.9, 5.8.

LRMS (ESI) Calculated for C$_{93}$H$_{182}$NO$_{14}$Si$_{8}$ (M + Na)$^+$: 1770.2

Found: 1770.2

Synthesis of 3.19

Intermediate 3.13 (2.5 g, 1.29 mmol, 1.0 equiv) was azeotropically dried with toluene and placed under vacuum overnight. Hexane (80 mL) was added followed by activated 4-angstrom molecular sieves. The resulting solution was allowed to stir at room temperature while the sugar donor was prepared. The sugar donor 3.12 (739 mg, 2.57 mmol, 2.0 equiv) was dissolved in DCM (26 mL). Diphenyl sulfoxide (911 mg, 4.50 mmol, 3.5 equiv) and activated 4-angstrom molecular sieves were added. The reaction was stirred for 4 hours at room temperature. 2,6-lutidine (675 μL, 5.79 mmol, 4.5 equiv) was added, and the reaction was cooled to -60 °C. Triflic anhydride (1M in DCM) (2.57 mL, 2.57 mmol, 2.0 equiv) was added slowly. The reaction was warmed to -20 °C and stirred for 1.5 h. 2,6-lutidine (600 μL, 5.15 mmol, 4.0 equiv) was added to the solution of 3.12, and it was cooled to -30 °C. The sugar donor reaction was transferred via cannula to the solution of 3.12. The reaction was
warmed to 0 °C for 1hr. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ether. The organic layer was washed with copper sulfate, water, and saturated sodium chloride. The organic layer was dried with sodium sulfate and filtered. The solvent was removed under reduced pressure, and column chromatography (SiO₂; Ether:Hexane 3:47) purification yielded the glycosylated product 3.19 as a mixture of isomers ranging from 2:1 α:β (2.12 g, 1.06 mmol, 82 %). The isomers were inseparable at this stage and were taken directly on the next reaction.

\[ \text{TLC (Ether:Hexane 1:19)} \quad R_f = 0.25, \text{stained by CAM} \]

\[ \text{LRMS (ESI)} \quad \text{Calculated for } C_{105}H_{205}N_3O_{16}Si_9 (M + Na)^+: \quad 2039.9 \]

\[ \text{Found:} \quad 2039.3 \]

Synthesis of 3.20

The glycosidated product 3.19 (710 mg, 352 μmol, 1.0 equiv) was azeotropically dried with toluene in a Teflon vial. THF (3 mL) was added, and the solution was cooled to 0 °C. Pyridine (3 mL) in a Teflon vial was cooled to 0 °C, and MeOH (0.5 mL) was added. 70% HF-
pyridine was added slowly to the pyridine-MeOH solution at 0 °C. This solution was transferred slowly to the THF solution of glycosylated intermediate. The reaction was allowed to stir for 12 hours at room temperature. The reaction was quenched at 0 °C with excess MeOTMS and diluted with toluene. The solution was concentrated under reduced pressure and diluted again with toluene. This process was repeated 3 times to remove all of the pyridine. The product is base sensitive, especially if water is present, – care must be taken not to concentrate directly to solid with pyridine present. Reversed phase HPLC purification (C_{18} SiO_{2}; MeCN:5 mM NH_{4}OAc in H_{2}O 1:19 → 19:1 over 30 minutes) allowed the α and β isomers to be separated and yielded 260 mg, 275 μmol, 78 %. (86.7 mg, 91.7 mmol, 26% β isomer 3.20 and 173 mg, 183 mmol, 52% a isomer).

**HPLC** (C_{18} SiO_{2}; MeCN:5 mM NH_{4}OAc in H_{2}O 1:19 → 19:1 over 30 minutes)

R_{\alpha} = 17.1 min, α

R_{\beta} = 16.2 min, β

**1H NMR** (500 MHz, CD_{3}S(O)CD_{3}) δ 6.32-6.05 (m, 12H), 5.81 (m, 1H), 5.60 (m, 1H), 4.97 (m, 1H), 4.58 (m, 1H), 4.43 (m, 1H), 3.99 (m, 1H), 3.84 (m, 1H), 3.73 (m, 2H), 3.52 (m, 2H), 3.32 (m, 1H), 3.21 (m, 1H), 3.10 (m, 1H), 3.00 (s, 3H), 2.93 (m, 2H), 2.29 (m, 1H), 2.16 (m, 2H), 2.01 (m, 2H), 1.76 (m, 1H), 1.68 (m, 1H), 1.52-1.23 (m, 14H), 1.15 (d, J = 5.5 Hz, 3H), 1.11 (d, J = 5.5 Hz, 3H), 1.03 (d, J = 6 Hz, 3H), 0.89 (d, J = 7 Hz, 3H).

**HRMS** (ESI)

Calculated for C_{48}H_{73}N_{3}O_{16} (M + Na)^+: 970.4889

Found: 970.4897
Synthesis of 3.21

3.20 (19 mg, 20 μmol, 1.0 equiv) was dissolved in DMSO (657 μL). Added water (36 μL, 200 μmol, 100 equiv) and trimethyl phosphine (1M) (60 μL, 60 μmol, 3.0 equiv). The reaction was heated to 55 °C for 3 h. Reversed phase HPLC purification (C18 SiO2; MeCN:5 mM NH₄OAc in H₂O 1:19 → 19:1 over 30 minutes) yielded 3.21 (10.5 mg, 11.4 μmol, 57%).

HPLC (C18 SiO2; MeCN:5 mM NH₄OAc in H₂O 1:19 → 19:1 over 30 minutes)

$R_t = 14.3 \text{ min}$

$^1H$ NMR (500 MHz, CD₃S(O)CD₃) δ 6.34-6.06 (m, 12H), 5.90 (m, 1H), 5.62 (m, 1H), 4.94 (m, 1H), 4.63 (m, 1H), 4.52 (m, 1H), 3.97 (m, 1H), 3.90 (m, 1H), 3.73 (m, 2H), 3.56 (m, 1H), 3.38 (m, 1H), 3.30 (m, 1H), 3.25 (m, 1H), 3.15 (m, 1H), 2.95 (m, 5H), 2.25 (m, 4H), 2.03 (m, 1H), 1.77 (m, 3H), 1.53-1.24 (m, 13H), 1.17 (d, J = 5 Hz, 3H), 1.11 (d, J = 6 Hz, 3H), 1.03 (d, J = 6 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H).

HRMS (ESI) Calculated for C₄₈H₇₅NO₁₆ (M + H)+: 922.5164

Found: 922.5169
Synthesis of C2’deoOAmB

3.21 (5 mg, 5.42 μmol, 1.0 equiv) was placed in a vial. 180 μL of a 180 mM solution of CSA in 2:1 THF:H₂O was added. The reaction was stirred for 30 min. Reversed phase HPLC purification (C₁₈ SiO₂; MeCN:5 mM NH₄OAc in H₂O 1:19 → 19:1 over 30 minutes) yielded C2’-deoxyAmB (3.9 mg, 4.34 μmol, 80%).

HPLC (C₁₈ SiO₂; MeCN:5 mM NH₄OAc in H₂O 1:19 → 19:1 over 30 minutes)
Rₜ = 15.1 min

¹H NMR (500 MHz, CD₃S(O)CD₃) δ 6.47-5.94 (m, 11H), 5.73 (m, 1H), 5.42 (m, 2H), 5.23 (m, 1H), 4.77 (m, 1H), 4.61 (m, 1H), 4.38 (m, 1H), 4.26 (m, 1H), 4.15 (m, 1H), 4.06 (m, 1H), 3.99 (m, 1H), 3.70-3.20 (m, 4H), 3.09 (m, 1H), 2.92 (m, 1H), 2.36-2.16 (m, 5H), 1.99 (m, 1H), 1.83-1.72 (m, 4H), 1.56-1.51 (m, 4H), 1.39-1.23 (m, 7H), 1.15 (d, J = 5.5 Hz, 3H), 1.11 (d, J = 6 Hz, 3H), 1.03 (d, J = 6 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H).

HRMS (ESI) Calculated for C₄₇H₇₃NO₁₆ (M + H)+: 908.5008
Found: 908.5007
3-6: REFERENCES


CHAPTER 4: THE C3’-AMMONIUM OF AMB IS ANOTHER POTENTIAL SITE OF ALLOSTERIC MODIFICATION, LEADING TO LIGAND SELECTIVE-BINDING OF ERGOSTEROL OVER CHOLESTEROL

4-1: UNDERSTANDING THE ROLE OF THE C3’-NH₃⁺

A major contributor to the lack of progress toward a clinically viable therapeutic replacement for AmB has been poor understanding of the mechanism(s) by which AmB impacts yeast and human cells. It has for half a century been widely accepted that AmB kills both types of cells primarily via ion channel mediated membrane permeabilization. Guided by this model, extensive efforts have focused on the challenging problem of selectively forming ion channels in yeast vs. human cells.

In contrast to this classic model, we recently discovered that AmB primarily kills yeast cells by simply binding ergosterol (Erg); channel formation is not required. This suggests that binding cholesterol (Chol) may account primarily for the toxicity of AmB to human cells and that efforts to improve AmB can focus on the simpler problem of maximizing the relative binding affinity for Erg vs. Chol.

As we have discussed in the previous chapters when the mycosamine appendage is deleted, the resulting derivative can no longer bind either Erg or Chol. This inability to bind sterols leads to a derivative that is nontoxic to both yeast and human cells. With the mycosamine highlighted as being critical for AmB’s ability to bind sterols, we hypothesized that one or more of the heteroatoms contained on it was responsible for this phenomenon.

In the leading structural model, AmB interacts with both Erg and Chol via a similar binding mode in which the C2’-hydroxyl group of AmB forms a critical H-bond to the 3β-hydroxyl group on each sterol (fig 4.1). However, in contrast to this leading model, we recently discovered that the C2’-OH of AmB plays an important role in binding Chol but not Erg. Because of these findings, we favor an alternative model in which the C2’-OH stabilizes a conformer...
of AmB that readily binds both Erg and Chol. Deletion of this hydroxyl group, we propose, favors a shift to a different conformer or set of conformers which retain the capacity to bind Erg but not Chol. Alternatively stated, this model predicts that deletion of the C2'-OH of AmB causes a small-molecule-based allosteric effect that results in ligand-selective binding.

However, it is still possible that AmB interacts with these two sterols via distinct binding modes. It is possible that the C2'-OH uniquely participates in a direct binding interaction with Chol, and that the C3'-NH$_3^+$ uniquely participates in a direct binding interaction with Erg. This model could explain the observed difference in binding between the sterols for C2'deOAmB. Furthermore it has been previously proposed in the literature that the C3'-NH$_3^+$ is “indispensable for [AmB’s] biological activity and antibiotic-sterol interaction”. Additionally, other reports have indicated that the C3'-NH$_3^+$ is critical for channel formation.

In order to differentiate between the proposed mycosamine mediated binding models—a direct binding model or an allosteric binding model—we needed to synthesize a derivative lacking the C3'-NH$_3^+$. Furthermore, we would also be in a position to investigate the C3'-NH$_3^+$’s role in ion channel formation.

4-2: SYNTHESIS AND STUDY OF C3’-DEAMINO AMB

![Diagram ofScheme 4.1: Divergent route to C2’d eOAmB and C3’d eNHAmB sugar donors from common epoxide intermediate](image)

I realized that a similar hybrid synthetic strategy successfully employed in the synthesis of C2’d eOAmB would be amenable for the construction of C3’d eNHAmB. Additionally the common intermediate, epoxy-alcohol identical to 3.16—would readily lead to a C3’deamino sugar donor (Scheme 4.1). From epoxy-alcohol 4.1, lithium aluminum hydride would open the epoxide selectively at the C3’-position (turned over
from LiEt₃BH opening at the C2'-position) with yielding 2,4-diol 4.2 in 70% yield. With an equatorial hydroxyl group at the C2'-position we could now take advantage of well precedented anchimeric assistance methodology to selectively favor β over α glycoside formation.¹¹,¹²

We contemporaneously developed a pivolate-like directing group with a primary azide on the end of an ethyl tail. The azido-dimethyl butyrate (AzDMB) group is designed to maximize the formation of the β-glycoside, prevent orthoester formation and be readily removed under mild Staudinger deprotection conditions (Fig. 4.2).¹³ As the acid chloride, the AzDMB group was installed to afford 4.3 in 89% yield with 93:7 C2:C4 hydroxyl selectivity. Subsequent silylation at the C4-OH with TBSCI afforded 4.4 in 91% yield. DDQ mediated PMB hydrolysis (4.5) and 2,2,2-trichloroacetimidate activation at C1 provided a robust and scalable route to the C3’denH₂ sugar donor 4.6 (Scheme 4.2).

The persylilated macrolide acceptor 4.7, previously reported by Palacios et al.,⁵ is similar to the macrolide acceptor 3.13.⁷ We envisioned the more robust allyl ester would be required since an inversion of stereochemistry at C2’ via a tandem oxidation reduction sequence was needed to complete the synthesis of C3’denHAmB. Glycosylation of aglycone 4.7 with sugar donor 4.6 proceeded under 2-chloro-6-methyl pyridine and 2-chloro-6-methyl pyridine triflic acid salt buffered conditions¹² and resulted in a 65% yield of 4.8 with >20:1 α:β selectivity and with no detectable amount of orthoester. The AzDMB group
was readily removed with a two-step reduction, hydrolysis procedure. Staudinger-like conditions using PMe₃ with 100 equivalents of water at 55 °C completely reduced the azide (74% yield) and resulted in a minimal formation of 4.9 (25%) where the auxiliary was removed. The free amine was converted to 4.9 with a sub-stoichiometric amount of KOH in THF water at ambient temperature. This hydrolysis step was cycled an additional time to afford 4.9 in 57% yield (yield reported over two steps from azido-β-glycoside 4.8) (Scheme 4.3).

It is noteworthy that, in the context of the total synthesis, if the AzDMB-mycosamine donor was selectively glycosylated to the aglycone, the AzDMB group could not be removed from the C2’-OH if the C3’-N₃ was present. We hypothesized that the bulky AzDMB group cannot achieve the required conformation for lactam formation if a neighboring equatorial substituent is present. Therefore, other mycosamine modified AmB derivatives where the C3’-amine is present, should be synthesized through other means—either with a different anchimeric assistance auxiliary, or a degenerate strategy.

The inversion of the C2’-stereocenter of 4.9 was achieved only after some optimization. In Nicolaou’s total synthesis of AmB, inversion of the equatorial C2-OH to axial C2’-OH proceeded in good yields and >20:1 selectivity, employing a tandem Swern oxidation - sodium borohydride reduction. However, in the absence of a C3’-N₃, these conditions resulted in no selectivity for the desired axial C2’-OH. Furthermore, upon global deprotection, the two C2’-OH epimers were completely inseparable by HPLC. Fortuitously, stereoselective hydride reductions of conformationally rigid cyclic ketones have been previously addressed in the literature. In 1956, Noyce and coworkers described how bulky hydrides attack cyclic ketones from the more sterically accessible equatorial trajectory and override torsional strain, thus favoring the axial product. Based on this well established rational, the bulky L-selectride was employed to afford 4.10 in >20:1 axial:equatorial selectivity in 30% over two steps.

Since our strategy was informed by the successful synthesis of C2’dEOAmB, we employed labile TES groups that were removed under mild HF-pyridine conditions to afforded 4.11 in 27% yield. Subsequent hydrolysis of the C41-allylester under mild Pd(PPh₃)₄ with thiosalysilic acid in DMF for 1 hour, afforded C3’dENHAmB in 47% yield.
after HPLC purification (MeCN: H₂O with 0.1% formic acid). Fortuitously, the hydrolysis to the hemiketal—the actual final step—was carried out cleanly and quantitatively while concentrating aqueous acidic HPLC fractions containing the penultimate methyliketonal.

In our MIC assays, C3’denHAmB is active against two yeast strains *S. cerevisiae* and *C. albicans* at 3 uM and 4 uM respectively. These data reveal that, in contrast to prior predictions, the C3’-NH₃⁺ group is in fact not required for biological activity. Additionally, we have assayed C3’denHAmB against human red blood cells and have observed that this compound—like C2’denOAmB—shows no hemolytic activity up to the limits of solubility (MHC: >500 mM). Based on our previous work that establishes a strong correlation between sterol binding and biological activity, these preliminary biological data suggests that C3’denHAmB will still bind Erg but not bind Chol. My colleague is currently concluding the ITC experiments to test this prediction along with potassium efflux experiments to confirm that C3’denHAmB still capable of permeabilizing membranes.

Collectively these findings suggest that neither the C2’-OH nor the C3’-NH₃⁺ are directly responsible for AmB’s ability to bind sterols. Although ITC data is needed to determine C3’-NH₃⁺’s ability to bind sterols, we can infer from these preliminary biological data that these residues could be involved in stabilizing a conformation of AmB that binds both Erg and Chol. If either of these intramolecular polar interactions were removed, such an allosteric modification would change the shape of the binding site on the molecule.
magnifying Erg selectivity, and in effect, reducing human toxicity. This realization helps illuminate a roadmap to improve the therapeutic index of this important natural product.\textsuperscript{16b}

4-3: EXPERIMENTAL SECTION

Materials. Commercial reagents were purchased from Sigma-Aldrich, Strem, Alfa Aesar, and Fisher Scientific and used without further purification unless otherwise noted. Solvents were purified by passage through packed columns by the method of Pangborn and coworkers\textsuperscript{17} (THF, Et\textsubscript{2}O, CH\textsubscript{3}CN, CH\textsubscript{2}Cl\textsubscript{2}: dry neutral alumina; benzene, toluene: dry neutral alumina and Q5 reactant; DMSO, DMF, CH\textsubscript{3}OH: activated molecular sieves). Water was obtained from a Millipore (Billerica, MA) MilliQ water purification system. Triethylamine was freshly distilled under an atmosphere of nitrogen from CaH\textsubscript{2}. (±)-10-Camphorsulfonic acid was recrystallized from EtOAc.

General Experimental Procedures. All reactions were performed in flame- or oven (125 °C)-dried glassware under an atmosphere of dry nitrogen or argon unless otherwise stated. Reactions were monitored by analytical thin layer chromatography (TLC) on Merck silica gel 60 F254 plates (0.25 mm) using the indicated solvent system. Compounds were visualized by exposure to UV light (254 nm), or by an acidic solution of \textit{p}-anisaldehyde followed by heating with a Varitemp heat gun. Flash column chromatography was performed as described by Still and coworkers\textsuperscript{2} using Merck silica gel 60 (230-400 mesh).

Structural Analysis. \textit{\textsuperscript{1}H} NMR spectra were recorded at ambient temperature using one of the following instruments: Varian Unity 500 (500 MHz), Varian VXR 500 (500 MHz), or Varian Unity Inova 500NB (500 MHz). Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to residual protium in the NMR solvent (CD\textsubscript{3}Cl, $\delta = 7.26$; (CD\textsubscript{3})\textsubscript{2}CO, $\delta = 2.05$, center line). Spectral data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sext = sextet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of
doublets, m = multiplet, b = broad, app = apparent), coupling constant (J), and integration. 

$^{13}$C NMR spectra were recorded at ambient temperature using one of the following instruments: Varian VXR 500 (125 MHz), Varian Unity 500 (125 MHz), or Varian Unity 400 (101 MHz) instrument. Chemical shifts are reported in ppm downfield from tetramethylsilane and referenced to carbon resonances in the NMR solvent (CDCl$_3$, δ = 77.16, center line; (CD$_3$)$_2$CO, δ = 29.84, center line). High-resolution mass spectra (HRMS) were acquired by Mr. Pulin Wang, Mr. Furong Sun, or Dr. Haijun Yao at the University of Illinois School of Chemical Sciences Mass Spectrometry Laboratory. Data are reported in the form of m/z. Gas chromatography analysis was conducted on an Agilent Technologies 7890A instrument. The diastereoselectivity of lactonization was determined by GC analysis with an Agilent Technologies chiral β-cyclodextrin stationary phase (part number 112-2532). X-ray crystallographic analysis was carried out by Dr. Danielle gray at the University of Illinois George L. Clark X-Ray facility.

Synthesis of intermediate 4.2

Epoxy alcohol 4.1 (2.45 g, 9.21 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 5 mL) and placed under high vac overnight in a 250 mL round bottom flask. A rigorously dried 500 mL single-neck round bottom flask under an nitrogen atmosphere was charged with dry uninhibited THF (100 mL) and subsequently cooled to 0 °C. Lithiumaluminum hydride (700 mg, 18.42 mmol, 2.0 equiv) was carefully added as a solid powder to the 500 mL flask forming a dark grey slurry. 4.1 was then dissolved in dry THF (90 mL) and the subsequent solution was added drop wise via cannula to the cold stirring LiAlH$_4$ THF slurry. The addition was complete in approximately 10 min whereupon the ice bath was removed and the stirring solution was allowed to warm to 23 °C over 20 h. The reaction was then cooled to 0 °C for 10 min followed by slow sequential addition of dry Et$_2$O (60 mL), deionized water (1.0 mL), very slowly aqueous NaOH (15% w/w), and deionized water
(3.0 mL). The quenched reaction was allowed to warm up to 23 °C by removal of the ice bath and stirred for an additional 30 min followed by excess solid sodium sulfate powder. The slurry was filtered, filter cake was washed with Et₂O, and the resulting filtrate was concentrated under reduced pressure. Purification via flash chromatography (SiO₂, gradient elution: 1:1 Hex:EtOAc to 1:2 Hex:EtOAc to 100% EtOAc) afforded PMB-diol 4.2 (1.79 g, 6.67 mmol, 72%) as a clear colorless oil.

\[
\text{R}_f = 0.25 \text{ (1:1 Hex:EtOAc, CAM stain)}
\]

\[
\text{H NMR} \quad (500 \text{ MHz, CDCl}_3) \delta 7.27 \text{ (dd, } J = 9.4, 2.8 \text{ Hz, 2H)}, 6.92 - 6.86 \text{ (m, 2H)}, 4.79 \text{ (d, } J = 3.7 \text{ Hz, 1H)}, 4.70 \text{ (d, } J = 11.4 \text{ Hz, 1H)}, 4.47 \text{ (d, } J = 11.4 \text{ Hz, 1H)}, 3.81 \text{ (d, } J = 0.8 \text{ Hz, 3H)}, 3.73 - 3.65 \text{ (m, 1H)}, 3.57 \text{ (dq, } J = 9.0, 6.2 \text{ Hz, 1H)}, 3.28 \text{ (dddd, } J = 10.6, 9.2, 5.9, 4.5 \text{ Hz, 1H)}, 2.17 \text{ (dt, } J = 11.5, 4.6 \text{ Hz, 1H)}, 2.07 \text{ (dd, } J = 11.0, 1.1 \text{ Hz, 1H)}, 1.78 \text{ (dd, } J = 5.9, 1.8 \text{ Hz, 1H)}, 1.66 \text{ (q, } J = 11.4 \text{ Hz, 1H)}, 1.24 \text{ (dd, } J = 6.3, 0.8 \text{ Hz, 3H}).
\]

\[
\text{C NMR} \quad (126 \text{ MHz, CDCl}_3) \delta 159.53, 129.70, 114.03, 96.54, 70.90, 69.27, 69.11, 67.64, 55.43, 37.11, 17.47
\]

**HRMS (ESI)**

Calculated for C₁₄H₂₀O₅ (M + Na)+: 291.1208

Found: 291.1212
X-ray-crystal structure

Synthesis of intermediate 4.3

AzDMB acid (4.72 g, 30.04 mmol, 2.0 equiv) was azeotropically dried with benzene (3x 5 mL) in a 100 mL round bottom flask equipped with a reflux condenser. Dry benzene (12 mL) was then added under inert atmosphere followed by oxallylchloride (9.5 mL, 75.1 mmol, 5.0 equiv) and heated to 50 °C for 15 min (until gas formation ceases). The excess oxallylchloride was then removed under reduced pressure followed by azeotropic removal with benzene (3x 10 mL) to yield a yellow oil. Pyridine (60 mL, 0.5 M relative to AzDMB) was then added to the AzDMB acid chloride and stirred at 23 °C while the substrate was prepared. To a separate 100 mL round bottom flask, PMB-diol 4.2 (4.0 g, 15.02 mmol, 1.0 equiv) was added and azeotropically dried with benzene (3x 10 mL), placed under inert atmosphere, then charged with pyridine (40 mL, 0.375M) and cooled to 0 °C. The AzDMB acid chloride pyridine solution was then added to the solution containing the substrate drop wise via cannula at 0 °C over 15 min. The reaction was stirred for an additional 3 h at 0 °C then transferred to a seperatory funnel containing saturated aqueous NH₄Cl and Et₂O. Organics were washed 2 more times with NH₄Cl then aqueous phases were back extracted with Et₂O. The combined organics were washed with saturated aqueous bicarbonate 3x
then the resulting aqueous phases were back extracted with Et₂O. The combined organics were washed with saturated brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography (SiO₂ gradient elution: 3:1 Hex:EtOAc to 1:1 Hex:EtOAc to 100% EtOAc) yielded C2'-AzDMB-alcohol **4.3** (5.432 g, 13.37 mmol, 89%, 93:7 C2':C4' selectivity) as a clear colorless oil.

\[ R_f = 0.22 \text{ (3:1 Hex:EtOAc, CAM stain)} \]

**¹H NMR:** (500 MHz, CDCl₃) \( \delta \) 7.26 – 7.24 (m, 2H), 6.88 – 6.84 (m, 2H), 4.89 (d, \( J = 3.5 \) Hz, 1H), 4.75 (ddd, \( J = 12.3, 4.9, 3.5 \) Hz, 1H), 4.64 (d, \( J = 11.7 \) Hz, 1H), 4.44 (d, \( J = 11.7 \) Hz, 1H), 3.62 (dq, \( J = 9.2, 6.2 \) Hz, 1H), 3.37 (ddd, \( J = 11.2, 9.3, 4.6 \) Hz, 1H), 3.26 – 3.13 (m, 2H), 2.11 (dt, \( J = 11.3, 4.7 \) Hz, 1H), 1.98 – 1.89 (m, 1H), 1.79 (ddd, \( J = 8.2, 7.1, 1.2 \) Hz, 2H), 1.25 (d, \( J = 6.0 \) Hz, 4H), 1.16 (s, 6H).

**¹³C NMR:** (126 MHz, CDCl₃) \( \delta \) 176.37, 159.46, 129.73, 113.91, 93.94, 71.03, 69.27, 68.88, 68.87, 55.43, 47.95, 38.75, 32.85, 25.35, 17.50.

**Synthesis of intermediate **4.4**

To a stirring solution of alcohol **4.3** (5.4 g, 13.25 mmol, 1.0 equiv) in DMF (26.5 mL) in a 100 mL round bottom flask at 23 °C, was added sequentially imidazole (2.95 g, 46.4 mmol, 3.5 equiv) and TBSCl (6.0 g, 39.8 mmol, 3.0 equiv). The reaction was stirred at 23 °C for 18
h then transferred to a separatory funnel containing Et₂O and saturated aqueous bicarbonate and extracted with more Et₂O. The combined organics were then washed with deionized water, back extracted with Et₂O, and washed with brine. The combined organics were dried over sodium sulfate, filtered concentrated under reduced pressure. Purification via flash chromatography (SiO₂, gradient elution: 10:1 Hex:EtOAc to 5:1 EtOAc) afforded C2’AzDMB-TBS ether 4.4 (6.32 g, 12.06 mmol, 91%) as a clear colorless oil.

\[ R_f = 0.93 \text{ (3:1 Hex:EtOAc, CAM stain)} \]

\(^1\)H NMR: (500 MHz, CDCl₃) δ 7.29 – 7.24 (m, 2H), 6.90 – 6.82 (m, 2H), 4.88 (d, J = 3.6 Hz, 1H), 4.77 – 4.69 (m, 1H), 4.64 (d, J = 11.7 Hz, 1H), 4.41 (d, J = 11.7 Hz, 1H), 3.78 (s, 3H), 3.64 (dq, J = 9.0, 6.2 Hz, 1H), 3.33 (td, J = 8.9, 6.9 Hz, 1H), 3.29 – 3.14 (m, 2H), 2.33 (s, 1H), 2.15 (s, 2H), 1.95 (td, J = 8.8, 2.1 Hz, 2H), 1.82 – 1.76 (m, 2H), 1.18 (d, J = 2.0 Hz, 3H), 1.16 (d, J = 1.2 Hz, 6H), 0.86 (s, 9H).

\(^{13}\)CNMR: (126 MHz, CDCl₃) δ 176.35, 159.38, 129.67, 113.84, 93.85, 71.59, 69.26, 68.95, 68.69, 55.36, 47.93, 38.73, 33.40, 25.82, 25.32, 25.30, 17.99, 17.83, -4.03, -4.66.

HRMS (ESI)

Calculated for C₂₆H₄₃N₅O₆Si (M + Na)+: 544.2819

Found: 544.2820
Synthesis of intermediate 4.5

To a stirring biphasic solution of C2’AzDMB-TBS ether 4.4 (2.34 g, 4.5 mmol, 1.0 equiv) in DCM:H₂O (41 mL: 4.5 mL) in a 100 mL round bottom flask at 23°C was added DDQ (2.0 g, 9.0 mmol, 2.0 equiv). The reaction was sealed and heated to 40 °C for 4 h then transferred to a separatory funnel containing saturated aqueous bicarbonate and Et₂O. The aqueous phase was extracted with Et₂O until the resulting Et₂O layer was clear. The combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography (SiO₂ gradient elution: 9:1 Hex:EtOAc isocratic) afforded hemiketal 4.5 (1.61 g, 4.01 mmol, ~1:1 mixture of anomers, 89%) as a slightly yellow clear oil.

\[ \text{R}_f = 0.13 \text{ (9:1 Hex:EtOAc, CAM stain)} \]

**¹H NMR:** (500 MHz, CDCl₃) δ 5.25 (t, \( J = 3.5 \text{ Hz, 2H} \)), 4.80 – 4.72 (m, 2H), 4.69 – 4.58 (m, 5H), 3.81 (dq, \( J = 9.0, 6.2 \text{ Hz, 2H} \)), 3.44 – 3.23 (m, 21H), 2.72 (d, \( J = 3.4 \text{ Hz, 2H} \)), 2.20 (dt, \( J = 12.4, 4.1 \text{ Hz, 2H} \)), 2.02 – 1.78 (m, 16H), 1.58 (s, 7H), 1.36 – 1.14 (m, 65H), 0.94 – 0.85 (m, 60H), 0.07 (t, \( J = 2.3 \text{ Hz, 32H} \)).

**¹³C NMR:** (126 MHz, CDCl₃) δ 177.30, 176.38, 97.15, 89.27, 71.76, 71.39, 71.22, 69.61, 68.95, 48.13, 48.02, 41.18, 40.99, 38.99, 38.92, 37.43, 32.68, 31.74, 25.84, 25.83, 25.61, 25.53, 25.09, 22.81, 18.10, 17.94, 14.28, -3.97, -4.06, -4.61, -4.66.
**HRMS (ESI)**

Calculated for $\text{C}_{18}\text{H}_{35}\text{N}_{3}\text{O}_{5}\text{Si}$ (M + Na)$^+$: 424.2244

Found: 424.2242

Synthesis of intermediate 4.6

Hemiketal 4.5 (1.60 g, 3.99 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and left on the high vac over night in a 100 mL round bottom flask. DCM (40 mL) was then added to the flask containing 3.5 followed by $\text{Cs}_2\text{CO}_3$ (651 mg, 2.0 mmol, 0.5 equiv) and lastly trichloroacetonitrile (4.01 mL, 40.0 mmol, 10.0 equiv) at 23 °C and stirred for 1h. The reaction was then transferred to a separatory funnel containing saturated aqueous bicarbonate and Et$_2$O. The aqueous phase was extracted with Et$_2$O and the combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography ($\text{SiO}_2$, gradient elution: 95:5 Hex:EtOAc isocratic) afforded hemiketal 4.6 (1.91 g, 3.52 mmol, 88%) as a slightly yellow clear oil.

$$R_f = 0.95 \text{ (95:5 Hex:EtOAc, w/0.1% Et}_3\text{N CAM stain)}$$
$^1$H NMR: (500 MHz, CDCl$_3$) $\delta$ 8.64 (s, 1H), 4.95 (tdd, $J$ = 7.9, 6.0, 4.8 Hz, 1H), 3.49 (dddd, $J$ = 18.8, 9.7, 8.2, 5.8, 4.6 Hz, 2H), 3.26 (td, $J$ = 7.7, 1.2 Hz, 3H), 2.49 – 2.33 (m, 1H), 1.83 (pd, $J$ = 7.6, 6.9, 1.3 Hz, 2H), 1.66 – 1.52 (m, 3H), 1.34 – 1.25 (m, 4H), 1.25 – 1.11 (m, 9H), 0.97 – 0.78 (m, 14H), 0.19 – -0.03 (m, 9H).

$^{13}$CNMR: (126 MHz, CDCl$_3$) $\delta$ 175.65, 161.33, 128.45, 97.28, 77.47, 70.50, 69.11, 47.91, 41.09, 38.66, 37.35, 25.83 (d, $J$ = 4.2 Hz), 25.39, 25.15, 17.99, -4.07, -4.68

Synthesis of intermediate 4.8

Previously reported protected AmdeB 4.7 (1.45 g, 2.66 mmol, 2.0 equiv) was azeotropically dried with benzene (3x 5 mL) and placed under high vac overnight. The frozen Ar benzene matrix containing trichloroacetimidate 4.6 (2.17 g, 1.33 mmol, 1.0) was thawed at 23 °C then concentrated under reduced pressure and added to a 250 mL round bottom flask containing 4.7 and the two were azeotropically dried together with benzene (3x 5 mL). The flask was then placed under an inert atmosphere and charged with dry hexanes (67 mL) and 2-chloro-6-methylpyridine (145 mL, 1.33 mmol, 1.0 equiv) and cooled to 0 °C. Once the flask was at 0 °C, 2-chloro-6-methylpyridinium triflate (185 mL, 0.66 mmol, 0.5 equiv) was added as a solid in one portion. After 10 min the reaction turned greenish yellow from bright yellow. The reaction was stirred for 1 h total at 0 °C, then transferred to a separatory funnel containing saturated aqueous bicarbonate and hexane. The aqueous phase was extracted with hexanes and the combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography (SiO$_2$, gradient elution: 95:5 Hex:EtOAc isocratic) afforded AzDMB-glycoside 4.8 (1.72 g, 0.88 mmol, 66%) as a yellow/orange glassy solid.
\( R_f = 0.79 \) (5:1 Hex:EtOAc, w/0.1% Et\(_3\)N CAM stain)

**\(^1\text{H NMR}:** (500 MHz, CDCl\(_3\)) \( \delta \) 6.38 – 6.10 (m, 14H), 6.10 – 5.92 (m, 3H), 5.80 (dd, \( J = 14.2, 7.5 \) Hz, 1H), 5.45 – 5.36 (m, 2H), 5.28 (dq, \( J = 10.5, 1.2 \) Hz, 1H), 4.94 (s, 1H), 4.62 (ddtd, \( J = 10.9, 7.1, 5.8, 2.1 \) Hz, 4H), 4.52 – 4.45 (m, 1H), 4.42 (d, \( J = 7.9 \) Hz, 1H), 4.29 (dd, \( J = 10.4, 8.6, 4.5 \) Hz, 2H), 3.90 (t, \( J = 10.3 \) Hz, 1H), 3.86 – 3.78 (m, 1H), 3.74 (q, \( J = 6.0 \) Hz, 1H), 3.52 (t, \( J = 6.9 \) Hz, 2H), 3.46 (dd, \( J = 10.4, 4.3 \) Hz, 1H), 3.34 (ddt, \( J = 12.7, 7.0, 3.3 \) Hz, 1H), 3.26 (tdt, \( J = 15.0, 6.5, 2.8 \) Hz, 4H), 3.03 (s, 4H), 2.48 – 2.30 (m, 5H), 2.26 (dt, \( J = 12.0, 5.0 \) Hz, 1H), 2.12 (dd, \( J = 13.1, 4.7 \) Hz, 1H), 1.95 – 1.78 (m, 6H), 1.73 (ddd, \( J = 7.8, 5.5, 2.5 \) Hz, 4H), 1.70 – 1.56 (m, 5H), 1.55 – 1.34 (m, 6H), 1.33 – 1.14 (m, 22H), 1.07 – 0.78 (m, 113H), 0.74 – 0.47 (m, 60H).

**\(^{13}\text{C NMR}:** NMR (126 MHz, CDCl\(_3\)) \( \delta \) 175.83, 172.54, 170.93, 137.94, 135.53, 133.80, 133.70, 133.13, 132.93, 132.66, 132.57, 132.26, 132.17, 132.07, 131.97, 131.01, 130.40, 130.16, 119.07, 100.86, 100.21, 76.27, 75.87, 73.34, 71.06, 70.33, 70.12, 68.33, 67.25, 67.07, 66.46, 65.54, 55.92, 47.99, 47.79, 46.40, 43.53, 42.93, 42.53, 41.14, 40.31, 38.88, 37.77, 37.21, 35.95, 26.25, 25.85, 25.50, 25.20, 19.20, 18.27, 18.05, 18.00, 11.86, 7.36, 7.23, 7.18, 7.06, 6.96, 5.74, 5.58, 5.50, 5.43, 5.25, 5.16.

**HRMS (ESI)**

Calculated for C\(_{105}\)H\(_{199}\)N\(_3\)O\(_{18}\)Si\(_8\) (M + Na): 2037.2801

Found: 2037.2787
Synthesis of intermediate 4.9

To a stirred solution of AzDMB-glycoside 4.8 (1.77 g, 0.88 mmol, 1.0 equiv) in THF (30 mL) and H₂O (1.58 mL, 87.8 mmol, 100.0 equiv) at 50 °C in a 100 mL round bottom flask equipped with a septum was added PMe₃ (1.0 M in THF: 2.63 mL, 2.63 mmol, 3.0 equiv). The reaction was sealed and not submerged in the oil bath above the solution level. The reaction was stirred for 4 h total at 50 °C, then transferred to a separatory funnel containing saturated aqueous bicarbonate and hexane. The aqueous phase was extracted with hexanes and the combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography (SiO₂, gradient elution: 95:5 Hex:EtOAc isocratic) afforded C3’deNH-C2’bOH 4.9 (397 mg, 0.21 mmol, 24%) and aminoDMB-glycoside SI4.1 (1.286 g, 0.65 mmol, 74%) a yellow/orange glassy solid.

\[ R_f = 0.23 \text{ (9:1 Hex:EtOAc, w/0.1% Et₃N CAM stain)} \]

\(^1\text{H NMR: (500 MHz, CDCl}_3\) δ 6.37 – 5.90 (m, 13H), 5.81 (dd, \( J = 14.0, 7.7 \) Hz, 1H), 5.45 – 5.33 (m, 2H), 5.29 (dd, \( J = 10.3, 1.5 \) Hz, 1H), 4.95 (s, 1H), 4.69 – 4.55 (m, 3H), 4.46 (t, \( J = 8.4 \) Hz, 1H), 4.42 (d, \( J = 8.0 \) Hz, 1H), 4.28 (td, \( J = 10.2, 4.4 \) Hz, 2H), 3.88 (d, \( J = 10.2 \) Hz, 1H), 3.86 – 3.79 (m, 1H), 3.72 (d, \( J = 7.3 \) Hz, 1H), 3.51 (s, 2H), 3.46 (dd, \( J = 10.4, 4.3 \) Hz, 1H), 3.38 – 3.28 (m, 2H), 3.27 – 3.20 (m, 1H), 3.03 (d, \( J = 2.9 \) Hz, 3H), 2.73 (s, 1H), 2.49 – 2.30 (m, 4H), 2.26
(dt, J = 10.7, 5.0 Hz, 1H), 2.12 (dd, J = 13.1, 4.6 Hz, 1H), 1.96 – 1.70 (m, 8H), 1.70 – 1.35 (m, 11H), 1.27 (dd, J = 15.6, 6.9 Hz, 4H), 1.23 – 1.10 (m, 13H), 1.07 – 0.79 (m, 81H), 0.72 – 0.48 (m, 45H).

**LRMS (ESI)**

Calculated for C_{105}H_{202}N_{18}O_{18}Si_{8}(M + H)^+: 1989.3

Found: 1989.3

To a stirred solution of intermediate aminoDMB-glycoside **SI4.1** (1.281 g, 0.643 mmol, 1.0 equiv) in THF (20.8 mL) in a 40 mL iChem vial at 23 °C was added KOH (0.0167 M solution in DI water: 3.9 mL, 0.064 mmol, 0.1 equiv) and stirred for 2 h at 23 °C then transferred to a separatory funnel containing saturated aqueous bicarbonate and hexane. The aqueous phase was extracted with hexanes and the combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography (SiO₂, gradient elution: 95:5 Hex:EtOAc isocratic) afforded C3’denH-C2’bOH **4.9** (248 mg, 0.21 mmol, 20%) a yellow/orange glassy solid and recovered aminoDMB-glycoside **SI4.1** starting material (650 mg, 0.326 mmol, 51%) a yellow/orange glassy solid. The recovered **SI4.1** starting material was recycled under proportionally identical conditions to afford C3’denH-alcohol **4.9** (289 mg, 0.15 mmol, 47%) and recovered aminoDMB-glycoside **SI4.1** starting material (248 mg, 0.125 mmol, 38%). C3’denH-C2’bOH **4.9** (total of 933 mg, 0.497 mmol, 57% over 2 steps) was synthesized.

\[
R_f = 0.85 \text{ (9:1 Hex:EtOAc, w/0.1% Et}_3\text{N CAM stain)}
\]
\[ ^1H \text{NMR:} (500 \text{ MHz}, \text{CD}_3\text{C}(0)\text{CD}_3) \delta 6.60 - 5.97 \text{ (m, 15H)}, 5.51 \text{ (dd, } J = 14.9, 9.4 \text{ Hz, 1H)}, 5.41 \text{ (dt, } J = 17.2, 1.6 \text{ Hz, 1H)}, 5.33 - 5.23 \text{ (m, 1H)}, 4.74 - 4.58 \text{ (m, 3H)}, 4.57 - 4.49 \text{ (m, 1H)}, 4.42 \text{ (dt, } J = 10.5, 5.3 \text{ Hz, 1H)}, 4.21 \text{ (d, } J = 7.5 \text{ Hz, 2H)}, 4.14 \text{ (t, } J = 9.7 \text{ Hz, 1H)}, 4.04 \text{ (ddd, } J = 17.2, 11.3, 5.6 \text{ Hz, 2H)}, 3.86 \text{ (dd, } J = 8.9, 2.9 \text{ Hz, 1H)}, 3.70 \text{ (dt, } J = 9.0, 4.0 \text{ Hz, 1H)}, 3.62 \text{ (dt, } J = 10.6, 4.6 \text{ Hz, 1H)}, 3.44 - 3.28 \text{ (m, 2H)}, 3.23 \text{ (dq, } J = 8.7, 6.1 \text{ Hz, 1H)}, 3.12 \text{ (s, 3H)}, 2.59 \text{ (dd, } J = 6.5, 3.8 \text{ Hz, 2H)}, 2.43 \text{ (td, } J = 9.2, 6.5 \text{ Hz, 1H)}, 2.38 - 2.31 \text{ (m, 1H)}, 2.22 \text{ (dt, } J = 12.2, 4.8 \text{ Hz, 1H)}, 2.12 - 1.98 \text{ (m, 45H)}, 1.98 - 1.86 \text{ (m, 5H)}, 1.86 - 1.70 \text{ (m, 6H)}, 1.70 - 1.57 \text{ (m, 4H)}, 1.52 \text{ (d, } J = 12.4 \text{ Hz, 1H)}, 1.42 \text{ (d, } J = 11.1 \text{ Hz, 1H)}, 1.29 \text{ (s, 6H)}, 1.17 \text{ (dd, } J = 6.1, 3.3 \text{ Hz, 7H)}, 1.11 - 0.81 \text{ (m, 86H)}, 0.80 - 0.54 \text{ (m, 48H)}, 0.11 \text{ (d, } J = 5.0 \text{ Hz, 7H}).
\]

\[ ^{13} \text{C NMR:} (126 \text{ MHz, CDCl}_3) \delta 173.93, 170.18, 138.21, 134.53, 134.18, 133.65, 133.49, 132.05, 131.80, 131.54, 131.50, 130.75, 129.99, 119.21, 104.97, 100.76, 77.54, 76.33, 75.78, 73.25, 72.54, 71.50, 70.19, 68.79, 68.29, 66.66, 66.6, 66.5, 65.81, 56.44, 53.48, 47.82, 47.08, 43.39, 43.02, 41.53, 40.54, 39.45, 37.04, 35.16, 34.29, 30.39, 29.80, 26.59, 25.79, 19.50, 18.75, 18.07, 17.99, 11.27, 7.28, 7.25, 7.17, 7.13, 7.08, 7.01, 6.87, 5.73, 5.52, 5.35, 5.30, 5.28, 5.21, 5.03, 1.09, -4.19, -4.74.
\]

**HRMS (ESI)**

Calculated for C\(_{99}\)H\(_{19}\)O\(_{17}\)Si\(_{8}\) (M + Na\(^+\)):

1898.2055

Found: 1898.2061

Synthesis of intermediate 4.10

To a stirred solution of C\(^3\)deNH-C\(^2\)bOH 4.9 (70.5 mg, 37.6 mmol, 1.0 equiv) in CDCl\(_3\) (1.25 mL) at 0 °C in a 7 mL vial, was sequentially added the following solid reagents: sodium
bicarbonate (63 mg, 750 mmol, 20.0 equiv) Dess-Martin periodinane (32 mg, 75 mmol, 2.0 equiv). After 1 h at 0 °C the reaction was checked for conversion by monitoring the disappearance of the C1’ proton (5.1 ppm, d 1H) by 1H NMR. The reaction was transferred to a separatory funnel containing saturated aqueous bicarbonate and hexane. The aqueous phase was extracted with hexanes and the combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Due to the sensitive nature of ketone SI4.2, after azeotropic drying with benzene (3x 2 mL), it was taken on to the subsequent reaction without further purification.

$R_f = 0.55$ (9:1 Hex:EtOAc, CAM stain)

**HRMS (ESI)**

Calculated for C$_{97}$H$_{190}$O$_{17}$Si$_8$ (M + Na)$^+$: 1874.2055

Found: 1874.2053

To a stirred solution of ketone SI4.2 (assumed 37 mmol, 1.0 equiv) in THF (1.25 mL) cooled to -78 °C in a dry-ice/acetone bath was slowly added L-selectride (1.0 M in THF: 40 mL, 40 mmol, 1.05 equiv). After 30 min stirring at -78 °C, TLC showed full conversion to the reduced product and was transferred to a separatory funnel containing saturated aqueous bicarbonate and hexane. The aqueous phase was extracted with hexanes and the combined organic fractions were washed with saturated aqueous brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification via flash chromatography (SiO$_2$, gradient elution: 95:5 Hex:EtOAc isocratic) afforded C3’denH-C2’aOH 4.10 (18.5 mg, 9 mmol, 24%).
$R_f = 0.85$ (9:1 Hex:EtOAc, w/0.1% Et$_3$N CAM stain)

$^1$H NMR: (500 MHz, CD$_3$C(O)CD$_3$) $\delta$ 6.60 - 5.95 (m, 14H), 5.51 (dd, $J = 14.9$, 9.4 Hz, 1H), 5.41 (dq, $J = 17.2$, 1.6 Hz, 1H), 5.28 (dq, $J = 10.4$, 1.3 Hz, 1H), 4.75 - 4.57 (m, 4H), 4.50 (d, $J = 1.3$ Hz, 1H), 4.43 (td, $J = 10.4$, 4.8 Hz, 1H), 4.21 (qd, $J = 8.6$, 8.1, 3.2 Hz, 1H), 4.14 (td, $J = 8.2$, 4.1 Hz, 1H), 4.00 (ddddd, $J = 12.8$, 10.3, 8.7, 2.6 Hz, 2H), 3.87 (dd, $J = 9.0$, 2.9 Hz, 1H), 3.81 (p, $J = 2.7$, 2.3 Hz, 1H), 3.66 (ddddd, $J = 28.5$, 15.3, 7.7, 3.7 Hz, 3H), 3.27 (dq, $J = 8.4$, 6.1 Hz, 1H), 3.12 (s, 3H), 2.78 (d, $J = 15.9$ Hz, 1H), 2.65 - 2.52 (m, 2H), 2.44 (ddt, $J = 16.1$, 9.3, 7.0 Hz, 1H), 2.35 (t, $J = 10.4$ Hz, 1H), 2.18 - 2.02 (m, 4H), 2.02 - 1.81 (m, 6H), 1.81 - 1.68 (m, 5H), 1.68 - 1.59 (m, 3H), 1.52 (ddddd, $J = 19.0$, 14.5, 8.7, 2.9 Hz, 3H), 1.30 (dt, $J = 12.3$, 7.6 Hz, 5H), 1.18 (dd, $J = 8.0$, 6.0 Hz, 7H), 1.14 - 0.82 (m, 96H), 0.82 - 0.48 (m, 53H), 0.09 (t, $J = 2.6$ Hz, 6H).

$^{13}$CNMR: (126 MHz, CD$_3$C(O)CD$_3$) $\delta$ 173.40, 170.65, 139.43, 135.68, 135.59, 135.42, 135.07, 134.24, 133.65, 133.37, 132.95, 132.87, 132.72, 132.63, 131.59, 130.88, 130.70, 119.14, 101.60, 99.15, 77.01, 76.93, 74.67, 74.23, 73.76, 71.40, 69.77, 69.31, 69.01, 67.75, 67.51, 67.40, 66.16, 57.94, 48.36, 48.28, 44.44, 43.71, 42.14, 41.56, 36.36, 35.72, 35.45, 32.51, 27.81, 26.37, 23.50, 20.21, 19.56, 19.02, 18.69, 14.57, 11.35, 7.90, 7.88, 7.75, 7.56, 7.53, 7.37, 6.66, 6.41, 6.13, 6.08, 6.07, 6.02, 5.87, -3.75, -4.34.

HRMS (ESI)

  Calculated for C$_{99}$H$_{190}$O$_{17}$Si$_8$ (M + Na)$^+$: 1898.2055
  Found: 1898.2047
Synthesis of intermediate 4.11.

C3’deNH-C2’aOH 4.10 (130 mg, 69 mmol, 1.0 equiv) was azeotropically dried and placed on the high vac over night in a 7 mL vial. To a stirred solution of freshly distilled pyridine (690 mL, 0.1 M) in MeOH (300 mL) at 0 °C in a 40 mL Teflon vial was added HF-pyridine 70% complex (690 mL, 0.1 M). C3’deNH-C2’aOH 4.10 was then dissolved in THF (1.0 mL) and transferred via cannula to the HF-pyridine/pyridine solution at 0 °C. The vial containing 3.10 was then rinsed with THF (2x 500 mL) and then allowed to warm to 23 °C for 18 h. The reaction was quenched at 0 °C by the slow addition of excess TMSOMe (~25 mL) and allowed to warm to 23 °C by the removal of the ice bath and stirred for 1 h. The reaction was then transferred to a 100 mL round bottom flask and concentrated under reduced pressure. Pyridine was removed azeotropically with benzene (5x 2 mL). Preparative HPLC purification (C18SiO2, gradient elution: 5:95 MeCN:H2O to 95:5 MeCN:H2O over 25 min at 25 mL/min) afforded C3’deNH-methylketal-Allylester 4.11 (18.1 mg, 18.6 mmol, 27%) as a flaky yellow powder.

**HRMS (ESI)**

Calculated for C$_{51}$H$_{78}$O$_{17}$ (M + Na)$^+$: 985.5137

Found: 985.5146
Synthesis of C3’deNHAmB.

C3’deNH-methylketal-Allylester 4.11 (7 mg, 7.3 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 1 mL) in a 7 mL vial, taken into the glove box where, Pd(PPh₃)₄ (2.5 mg, 2.2 mmol, 30 mol%) was added followed by thiosalicylic acid (3.36 mg, 22 mmol, 3.0 equiv) then dissolved in DMF (240 mL, 0.03M) and stirred for 1 h at 23 °C. The reaction was then added drop-wise to rapidly stirring Et₂O (7 mL) and the precipitate was filtered. The resulting filtrate was concentrated under reduced pressure to yield a dark orange/red oil that was diluted with Et₂O and filtered again. The filtered precipitates were then dissolved in a minimal amount of DMSO and preparatively HPLC purified (C₁₈SiO₂, gradient elution: 5:95 to 95:5 MeCN:H₂O with 0.3% formic acid over 25 min at 25 mL/min). Concentration of the product containing aqueous acidic fractions afforded C3’deNHAmB (3.1 mg, 3.4 mmol, 47% over 2 steps) as a flaky yellow powder.

C3’deNHAmB

Rᵣ = 16.7 min; flow rate = 1mL/min, gradient = 0→5% MeCN in 0.1% aqueous formic acid at 2 min, 5 → 95% MeCN in 0.1% aqueous formic acid at 30 min.

¹H NMR: (500 MHz, CD₃S(O)CD₃) δ 6.52 – 6.03 (m, 6H), 5.86 (s, 0H), 5.40 (d, J = 7.5 Hz, 1H), 5.23 (d, J = 6.2 Hz, 1H), 4.80 (dd, J = 17.6, 4.5 Hz, 2H), 4.66 (s, 1H), 4.44 (s, 1H), 4.37 (d, J = 9.6 Hz, 1H), 4.24 (dt, J = 19.3, 9.7 Hz, 2H), 4.05 (d, J = 26.8 Hz, 2H), 3.10 (s, 2H), 2.26 – 2.14
(m, 1H), 1.93 – 1.81 (m, 2H), 1.71 (s, 1H), 1.56 (dd, J = 22.1, 11.8 Hz, 2H), 1.50 – 1.37 (m, 2H), 1.37 – 1.19 (m, 3H), 1.13 (dd, J = 13.8, 6.3 Hz, 4H), 1.04 (dd, J = 6.7, 3.9 Hz, 2H), 0.95 – 0.89 (m, 2H).

**HRMS (ESI)**

Calculated for C_{47}H_{72}O_{17} (M + Na)^+: 931.4667

Found: 931.4672

4-4: REFERENCES


13. Castelli, R.; Overkleeft, H. S.; van der Marel, G. A.; Codee, J. D. C. *Org. Lett.* **2013**, *2270*


16. (a) Uno, B. E.; Endo, M. M.; Struble, J. R.; Knapp, D. M.; Burke, M. D. manuscript in preparation. (b) Uno, B.E. Dissertation chapter 5

AmB self assembles into an extramembranous ‘sterol-sponge’\(^1\) (Fig. 5.1 A) that primarily kills cells by binding and extracting sterols\(^2\) in a mycosamine-dependent fashion.\(^3\) Evidence described in the previous chapters supports a model in which the C2’-OH and C3’-NH\(^{3+}\) on the mycosamine appendage\(^4,5\) are involved in stabilizing a ground state conformation of AmB that allows for the binding of both ergosterol (Erg) and cholesterol (Chol) (Fig. 5.1 B). When either the C2’-OH or C3’-NH\(^{3+}\) is deleted, AmB still binds Erg but can no longer bind Chol.\(^4,5\) Furthermore, this shift in sterol binding directly correlates to a substantial loss of observed toxicity to human cells.\(^4,5\) Alternatively stated, these results are consistent with ligand-selective allosteric effects in these prototypical small molecule-small molecule interactions.\(^\text{(Fig. 5.1 C)}\)

The relative exotherms observed in ITC assays and MIC vs. MHC concentrations suggest that AmB preferentially binds Erg over Chol. It is suggested that this predisposition is due to Chol being slightly bulkier than Erg. Furthermore, when allosteric modifications are made to AmB (i.e. deletion of either the C2’-OH or the C3’-NH\(^{3+}\)) this natural sterol selectivity is magnified to only favor Erg binding (Fig. 5.2 B). With these two potential sites of allostery identified, we proceeded to investigate how subtle modifications to the C2’-position could further magnify Erg binding selectivity.
We hypothesized that epimerization of the C2'-OH would lead to a similar magnification of Erg binding as observed when the C2'-OH is deleted. Specifically, based on our understanding of a possible ground-state conformation of AmB (Fig. 5.2 A)—informed by the N-iodoacylAmB crystal structure—\(^8\)—we proposed that a potential hydrogen bonding interaction between the C2'-OH, a molecule of water, and the C13-OH is implicated in AmB’s ability to bind both Erg and Chol. Similar to deletion of the C2' hydroxyl group, we predicted that epimerization at the C2'-position would potentially cause a disruption or alteration of this putative ‘water bridge,’ possibly leading to a change in shape of the sterol binding pocket that would result in a magnification of Erg binding (5.2 C).

The synthesis of doubly modified C2'epiAmB methyl ester (C2'epiAmE) was previously reported by Carreira and coworkers in 2011 (Fig. 5.3).\(^9\) Interestingly, they observed that C2'epiAmE was equipotent to AmB in their yeast MIC assays (Fig. 5.3 B). They also observed that it caused efflux of potassium ions from both Erg and Chol containing POPC liposomes at 1 \(\mu\)M. From these data, Carreira and coworkers concluded that “the configuration of C2'-position was inconsequential.”\(^9\) Carreira and coworkers did not assay C2'epiAmE for human toxicity or Chol binding. Guided by our new mechanistic insight: ligand-selective allosteric effects model, we aimed
to test the hypothesis that the singly modified AmB derivative having only one sterogenic center inverted, C2’epiAmB, would show little or no toxicity to human cells while retaining potent antifungal activity.

5-2: SYNTHESIS AND STUDY OF THE C2’-EPIMER OF AMPHOTERICIN B

In our first generation synthesis of C2’epiAmB, we adapted the previously reported synthesis of C2’epiAmE with one key change that allowed us to access the targeted deprotected material. Specifically, we employed a readily removable allyl ester at the C41-position. Employing our previously reported route to the fully protected aglycone 5.5—identical to 4.7\textsuperscript{3b,5} with the mycosamine donor and glycosylation conditions previously used in the construction of C3’denHAmB,\textsuperscript{5} I synthesized C2’epiAmB (Scheme 5.1).

Although the synthesis of C2’epiAmB was possible from a hybrid glycosylation route similar to C2’d0AmB\textsuperscript{4} and C3’denHAmB\textsuperscript{5}, we realized that our previously reported site-selective acylation methodology of AmB could provide a more efficient and practical synthesis of C2’epiAmB.\textsuperscript{11}

![Scheme 5.1: Hybrid synthesis of C2’epiAmB. 22 total steps](image)

In the second-generation synthesis of C2’epiAmB, a different protecting group strategy was employed based on all of the lessons learned from the previously synthesized AmB derivatives.\textsuperscript{1-5} Alloc was installed as the protecting group on the amine. The C41 carboxylate was protected with an allyl group. Both of these groups would be simultaneously removed in the penultimate step with Pd(PPh\textsubscript{3})\textsubscript{4} and thiosalicylic acid. The PMP ketals were critical for the selective acylation methodology and could be simultaneously removed with the C13 methylketal as the final step under mild acidic
conditions. Diethylisopropyl silyl (DEIPS) ether groups were also necessary as they are robust enough to survive the KCN mediated hydrolysis of both C2’benzoate intermediates, yet easily removed with HF-pyridine conditions.  

In the forward sense, the Alloc group, hemiketal, PMP ketals, and allyl groups were installed in three steps from AmB with one chromatographic separation affording 5.11 in 30% yield. At this point the C2’-OH of 5.11 was selectively acylated with p-tertbutylbenzoyl chloride under the previously reported conditions to generated 4.12 in a preparatively useful 45% yield. DEIPS groups were installed using the corresponding triflate, affording 5.13 in 72% yield. Subsequent KCN mediated hydrolysis of the C2’ p-tertbutylbenzoate provided free C2’-OH 5.14 in 65% yield. Inverting the C2’-OH of 5.14 proceeded under Mitsunobu conditions affording C2’ equatorial p-nitrobenzoate 5.15 in 84% yield. The resulting C2’-nitrobenzoate 5.15 was then cleaved with identical KCN conditions to generate intermediate 5.16. Global deprotection commenced with HF-pyridine mediated desilylation to yield intermediate 5.17 in 68% yield. The Alloc and allyl groups on the C3’-amine and C41-carboxylate respectively, were simultaneously removed with Pd(PPh₃)₄ and thiosalicylic acid to yield zwitterionic intermediate 5.18 in 87% yield as a single peak by HPLC. It is noteworthy that this reaction generates clean material without chromatography. The two PMP ketals along with the methyl ketal at C13 are hydrolyzed in a single step with CSA in 20:1 mixture of MeCN to H₂O at 0 °C. These conditions afford C2’epiAmB in 30% yield.
With scalable access to this key probe, my colleague Matt Endo tested its biophysical and biological properties. Gratifyingly, C2’epiAmB was able to bind Erg and not Chol within the limits of our assays. These sterol-binding results predicted the following biological activity. C2’epiAmB has potent antifungal activity against *S. cerevisiae* (MIC = 2 µM) and *C. albicans* (MIC = 2 µM) and within the limits of detection in our assays, complete lack of toxicity to human cells *in vitro* (MHC >500 µM, MTC >80 µM). These results show that C2’epiAmB is very similar to C2’déOAmB *in vitro* (Fig. 5.4) 10.

With these promising *in vitro* results in hand we are in the process of testing its efficacy and toxicity in a mouse study against AmB and C2’déOAmB. We predicted that C2’epiAmB will be equipotent to AmB yet substantially less-toxic to mice. Furthermore, since there is a hydroxyl group at the C2’-position, we predict that C2’epiAmB should behave otherwise similarly to AmB *in vivo*.

These results lend further evidence in support of our ligand selective allosteric binding model4,5. More importantly, we harnessed this understanding to guide the rational development of a new efficacious non-toxic derivative that has thus far shown substantial potential as a clinically viable therapeutic replacement for AmB. C2’epiAmB retains its zwitterionic character and is essentially identical to AmB with the only difference being the inversion of a single stereogenic center. Based on our proposed ligand selective allosteric binding model, C2’epiAmB adds to a growing list of rationally guided AmB derivatives with increased therapeutic indices.

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<th>AmB</th>
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Figure 5.4 A) ITC binding data, B) MIC data, C) MHC and MTC data against human red blood cells (RBC) and human renal epithelial cells (REC), respectively for AmB, C2’déOAmB, and C2’epiAmB. Concentrations given in µM.
5-3: EXPERIMENTAL SECTION

General methods

Materials. Commercial reagents were purchased from Sigma-Aldrich, Strem, Alfa Aesar, and Fisher Scientific and used without further purification unless otherwise noted. Solvents were purified by passage through packed columns by the method of Pangborn and coworkers\textsuperscript{12} (THF, Et\textsubscript{2}O, CH\textsubscript{3}CN, CH\textsubscript{2}Cl\textsubscript{2}: dry neutral alumina; benzene, toluene: dry neutral alumina and Q5 reactant; DMSO, DMF, CH\textsubscript{3}OH: activated molecular sieves). Water was obtained from a Millipore (Billerica, MA) MilliQ water purification system. Triethylamine was freshly distilled under an atmosphere of nitrogen from CaH\textsubscript{2}. (±)-10-Camphorsulfonic acid was recrystallized from EtOAc.

General Experimental Procedures. All reactions were performed in flame- or oven (125 °C)-dried glassware under an atmosphere of dry nitrogen or argon unless otherwise stated. Reactions were monitored by analytical thin layer chromatography (TLC) on Merck silica gel 60 F254 plates (0.25 mm) using the indicated solvent system. Compounds were visualized by exposure to UV light (254 nm), or by an acidic solution of p-anisaldehyde followed by heating with a Varitemp heat gun. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh).

Structural Analysis. \textsuperscript{1}H NMR spectra were recorded at ambient temperature using one of the following instruments: Varian Unity 500 (500 MHz), Varian VXR 500 (500 MHz), or Varian Unity Inova 500NB (500 MHz). Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to residual protium in the NMR solvent (CDCl\textsubscript{3}, \(\delta = 7.26\); (CD\textsubscript{3})\textsubscript{2}CO, \(\delta = 2.05\), center line). Spectral data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sext = sextet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, m = multiplet, b = broad, app = apparent), coupling constant \(J\), and integration. \textsuperscript{13}C NMR spectra were recorded at ambient temperature using one of the following instruments: Varian VXR 500 (125 MHz), Varian Unity 500 (125 MHz), or Varian Unity 400 (101 MHz) instrument. Chemical shifts are reported in ppm downfield from
tetramethylsilane and referenced to carbon resonances in the NMR solvent (CDCl₃, δ = 77.16, center line; CD₃C(O)CD₃, δ = 29.84, center line). High-resolution mass spectra (HRMS) were acquired by Mr. Pulin Wang, Mr. Furong Sun, or Dr. Haijun Yao at the University of Illinois School of Chemical Sciences Mass Spectrometry Laboratory. Data are reported in the form of m/z. Gas chromatography analysis was conducted on an Agilent Technologies 7890A instrument.

Synthesis of intermediate 5.2.

To a stirred solution of azido alcohol 5.1 (1.14 g, 2.69 mmol, 1.0 equiv) and pyridine (2.17 mL, 26.87 mmol, 10.0 equiv) in 27 mL DCM at 0 °C in a 100 mL round bottom flask were sequentially added acetic anhydride (1.27 mL, 13.4 mmol, 5.0 equiv) and DMAP (16.4 mg, 0.135 mmol, 0.05 equiv). After 15 min the solution was warmed to 23 °C, stirred for 10 min, poured into a separatory funnel containing Et₂O and saturated aqueous bicarbonate. The aqueous layer was extracted with Et₂O (3x 20 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The pyridine was removed azeotropically with benzene (3x15 mL). Purified by flash chromatography (gradient elution, 5% EtOAc:Hex to 10% EtoAc:Hex) afforded acetate 5.2 (1.09 g, 2.34 mmol, 87%) as a clear, colorless oil.

Rₛ = 0.65 (1:1 Et₂O/Hex, CAM stain)
\textbf{\textsuperscript{1}H NMR:} (500 MHz, CD\textsubscript{3}C(O)CD\textsubscript{3}) \(\delta\) 7.33 – 7.28 (m, 2H), 6.95 – 6.90 (m, 2H), 4.97 (d, \(J = 3.7\) Hz, 1H), 4.68 (d, \(J = 11.8\) Hz, 1H), 4.64 (dd, \(J = 10.6, 3.7\) Hz, 1H), 4.47 (d, \(J = 11.7\) Hz, 1H), 3.80 (s, 3H), 3.77 – 3.72 (m, 2H), 3.23 (t, \(J = 9.2\) Hz, 1H), 2.09 (s, 1H), 2.07 (s, 3H), 1.24 (d, \(J = 6.2\) Hz, 3H), 0.93 (s, 11H).

\textbf{HRMS (ESI)}

Calculated for C\textsubscript{22}H\textsubscript{35}N\textsubscript{3}O\textsubscript{6}Si (M + Na)+: 488.2193

Found: 488.2193

\textbf{Synthesis of intermediate 5.3}

To a stirred solution of acetate 5.2 (1.09 g, 2.34 mmol, 1.0 equiv) in a mixture of DCM:H\textsubscript{2}O (23.4 mL, 10:1) at 0 °C in a foil covered 40 mL iChem vial was added DDQ (623 mg, 2.81 mmol, 1.2 equiv). After 5 min, the reaction was warmed to 23 °C, stirred for 12 h, and poured into a separatory funnel containing Et\textsubscript{2}O and saturated aqueous bicarbonate. Organics were washed with saturated brine. The combined aqueous layers were extracted with Et\textsubscript{2}O (3x 20 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purified by flash chromatography (SiO\textsubscript{2}, gradient elution, 10% EtoAc:Hex to 15% EtoAc:Hex to 20% EtoAc:Hex) afforded hemiketal 5.3 (716 mg, 2.07 mmol, 89%) as a clear, colorless oil.

\textbf{R}_{f} = 0.45 (1:1 Et\textsubscript{2}O/Hex, CAM stain)
\textbf{\textsuperscript{1}H NMR:} \textsuperscript{1}H NMR (500 MHz, CD$_3$C(O)CD$_3$) δ 5.23 (d, $J = 3.7$ Hz, 1H), 4.61 (dd, $J = 10.5$, 3.6 Hz, 1H), 3.92 (dq, $J = 9.2$, 6.3 Hz, 1H), 3.77 (dd, $J = 10.5$, 9.2 Hz, 1H), 3.19 (t, $J = 9.2$ Hz, 1H), 2.09 (s, 3H), 2.08 (d, $J = 1.7$ Hz, 1H), 1.24 (d, $J = 6.2$ Hz, 0H), 1.19 (d, $J = 6.3$ Hz, 4H), 0.94 (s, 10H), 0.93 (s, 2H), 0.21 (d, $J = 3.3$ Hz, 4H), 0.15 (s, 4H).

\textbf{HRMS (ESI)}

Calculated for C$_{14}$H$_{27}$N$_3$O$_5$Si (M + Na)$^+$: 368.1618

Found: 368.1620

Synthesis of intermediate \textbf{5.4}

To a stirred solution of Hemiketal \textbf{5.3} (716 mg, 2.07 mmol, 1.0 equiv) in 10.35 mL DCM, at 23 °C in a 40 mL iChem vial were sequentially added trichloroacetonitrile (1.04 mL, 10.35 mmol, 5.0 equiv) and cesium carbonate (337.2 mg, 1.03 mmol, 0.5 equiv). After 30 min, the reaction was poured into a separatory funnel containing hexanes and water. The layers were separated, the aqueous phase was extracted with hexane (3x 30 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Exogenous water was azeotropically removed with benzene (3x 10 mL) and trichloroacetimidate \textbf{5.4} was used without further purification in the subsequent reaction. Since this product was not stable, it was either used immediately after formation or frozen in a benzene argon matrix.
$R_f = 0.95 \ (1:1 \text{ Et}_2\text{O}/\text{Hex} \text{ with } 0.1\% \text{ Et}_3\text{N CAM stain})$

Synthesis of intermediate 5.6:

AmB aglycone 5.5 (2.19 g, 1.34 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and left on high vac overnight in a 500 mL round bottom flask. Trichloroacetimidate 5.4 (944 mg, 1.93 mmol, 1.44 equiv) was added to the flask containing 5.5 as a solution in benzene and concentrated down. Hexanes (70 mL) was added and subsequently cooled to 0 °C after the system was placed under an N$_2$ atmosphere. 2-chloro-6-methylpyridine (147 mL, 1.34 mmol, 1.0 equiv) was added followed by 2-chloro-6-methylpyridinium triflate (186.0 mg, 0.67 mmol, 0.5 equiv) as a solid in one portion. After 8 min a color change was observed from orange to greenish yellow and slight precipitate formation. The reaction was quenched at 30 min after addition of triflate salt by pouring into a separatory funnel containing hexanes and saturated aqueous sodium bicarbonate. The aqueous phase was extracted with hexanes
(2x 50 mL) and the subsequent organic phases were washed with saturated brine then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (gradient elution 5:95 EtOAc:Hex to 1:9 EtOAc:Hex) afforded an inseparable 1:1 mixture of β-glycoside 5.6 and its orthoester (1.21 g 46% yield) as a yellowish-orange solid. This mixture was carried on to the subsequent reaction where cleavage of the acetate group provides an isolable product.

$$R_f = 0.73$$ (1:9 EtOAc:Hex, CAM stain)

**1H NMR:** (500 MHz, CD$_3$C(O)CD$_3$) δ 6.52 (ddd, J = 14.0, 10.5, 3.3 Hz, 5H), 6.46 – 5.97 (m, 31H), 5.53 (ddd, J = 14.3, 9.5, 3.6 Hz, 2H), 4.76 – 4.54 (m, 11H), 4.45 (td, J = 10.5, 4.7 Hz, 3H), 4.29 – 4.19 (m, 4H), 4.15 (s, 3H), 4.07 – 3.99 (m, 4H), 3.92 – 3.83 (m, 3H), 3.77 – 3.67 (m, 4H), 3.67 – 3.59 (m, 4H), 3.15 (s, 3H), 3.07 (s, 4H), 2.68 – 2.52 (m, 5H), 2.44 (q, J = 8.3 Hz, 2H), 2.24 (s, 3H), 2.13 – 1.98 (m, 150H), 1.98 – 1.59 (m, 39H), 1.52 (d, J = 12.6 Hz, 3H), 1.28 (d, J = 9.1 Hz, 3H), 1.25 (d, J = 6.2 Hz, 7H), 1.18 (d, J = 6.0 Hz, 8H), 1.13 – 0.91 (m, 191H), 0.91 – 0.82 (m, 5H), 0.81 – 0.56 (m, 112H), 0.22 (d, J = 1.2 Hz, 7H), 0.16 (d, J = 3.4 Hz, 7H).

**13C NMR:** (126 MHz, Acetone) δ 172.89, 139.33, 135.50, 134.89, 133.85, 133.69, 133.06, 132.81, 132.76, 131.64, 130.31, 129.83, 119.34, 119.04, 101.55, 98.52, 77.20, 76.93, 75.92, 74.28, 74.20, 72.97, 71.35, 70.50, 69.63, 69.27, 68.93, 67.71, 67.11, 66.27, 66.17, 58.17, 48.33, 48.26, 43.72, 41.49, 32.51, 30.51, 30.35, 30.20, 30.05, 29.89, 29.74, 29.66, 29.58, 27.74, 26.46, 26.38, 24.37, 21.12, 20.18, 19.53, 18.88, 18.81, 18.75, 18.73, 14.58, 11.33, 7.91, 7.88, 7.87, 7.75, 7.69, 7.56, 7.54, 7.48, 7.38, 7.37, 6.68, 6.65, 6.41, 6.13, 6.08, 6.04, 6.02, 5.96, 5.90, 5.86, 1.33, -3.83, -3.86, -4.01, -4.04.
HRMS (ESI)

Calculated for C\textsubscript{101}H\textsubscript{191}N\textsubscript{3}O\textsubscript{18}Si\textsubscript{8} (M + Na): 1981.2175

Found: 1981.2169

Synthesis of intermediate 5.7.

To a stirred solution of a mixture of 5.6 and the corresponding orthoester as a 1:1 mixture (1.01 g, 0.515 mmol, 1.0 equiv) in THF:MeOH (51 mL: 51 mL) at 0 °C in a 200 mL round bottom flask was added K\textsubscript{2}CO\textsubscript{3} (2.85 g, 20.6 mmol, 40.0 equiv). After stirring at 0 °C for 2.5 hours the reaction was allowed to warm to 23 °C and stir for an additional 1.5 h. The reaction was then worked up by transferring to a separatory funnel containing saturated brine and hexanes. The combined organic phases were washed with saturated aqueous bicarbonate, followed by DI water, saturated brine, and then they were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (gradient elution 5:95 EtOAc:Hex isocratic) afforded pure persilyl-C41allyl-C2’epiOH-methylketal-azidoAmB 5.7 (333 mg, 0.174 mmol 68% based on 0.2575 mmol) as an orange-yellow solid.

\[ R_f = 0.62 \ (1:9 \text{ EtOAc:Hex, CAM stain}) \]
\[^1H\] NMR: (500 MHz, CD\textsubscript{3}C(O)CD\textsubscript{3}) δ 6.57 – 5.97 (m, 14H), 4.74 – 4.59 (m, 3H), 4.43 (q, \(J = 6.2, 5.3 \text{ Hz}, 1\text{H}\)), 4.38 (d, \(J = 7.5 \text{ Hz}, 1\text{H}\)), 4.21 (s, 1H), 4.05 (qd, \(J = 8.1, 7.6, 4.8 \text{ Hz}, 3\text{H}\)), 3.71 (dt, \(J = 6.5, 4.5 \text{ Hz}, 1\text{H}\)), 3.64 (dd, \(J = 10.6, 4.7 \text{ Hz}, 1\text{H}\)), 3.35 (ddt, \(J = 10.3, 4.1, 2.6 \text{ Hz}, 2\text{H}\)), 3.28 (t, \(J = 9.4 \text{ Hz}, 1\text{H}\)), 3.15 (s, 3H), 3.08 (t, \(J = 9.0 \text{ Hz}, 1\text{H}\)), 2.63 – 2.52 (m, 2H), 2.09 (s, 1H), 1.99 – 1.69 (m, 14H), 1.69 – 1.57 (m, 5H), 1.57 – 1.46 (m, 3H), 1.40 – 1.23 (m, 33H), 1.23 – 1.09 (m, 10H), 1.09 – 0.91 (m, 90H), 0.91 – 0.82 (m, 29H), 0.80 – 0.55 (m, 48H), 0.21 (s, 3H), 0.14 (s, 3H).

\[^{13}C\] NMR: (126 MHz, CD\textsubscript{3}C(O)CD\textsubscript{3}) δ 172.81, 169.91, 134.76, 134.17, 133.32, 132.81, 132.48, 132.00, 131.80, 131.69, 130.73, 130.06, 129.88, 118.41, 101.82, 100.77, 76.58, 76.07, 75.14, 73.90, 73.40, 72.96, 70.94, 70.47, 68.41, 66.87, 66.71, 65.53, 59.87, 56.85, 47.52, 43.65, 42.83, 40.70, 36.83, 36.21, 34.60, 31.66, 29.65, 28.73, 26.87, 25.66, 25.52, 25.15, 22.64, 20.26, 20.17, 19.31, 18.37, 18.10, 18.07, 13.85, 13.71, 11.04, 10.56, 7.03, 7.01, 6.89, 6.77, 6.70, 6.67, 6.53, 5.77, 5.71, 5.55, 5.29, 5.27, 5.24, 5.21, 5.17, 5.02, -4.53, -4.73.

HRMS (ESI)

Calculated for C\(_{99}\)H\(_{189}\)N\(_3\)O\(_{17}\)Si\(_8\) (M + Na)\(^+\): 1939.2069

Found: 1939.2126

Synthesis of intermediate 5.8:

To a stirred solution of pyridine (5 mL, 62 mmol, 351 equiv) in MeOH 250 μL in a 50 mL Teflon vial at 0 °C was added drop-wise HF-pyridine 70% complex (1.04 mL, 328 equiv).
To this solution was added via cannula 5.7 (333 mg, 174 µmol, 1.0 equiv) as a solution in THF (1.5 mL). The vial containing 5.7 was washed with THF (3x 500 µL) to ensure quantitative transfer of material. The reaction was then allowed to warm to 23 °C and stirred for 18 h. The reaction was then cooled to 0 °C and quenched via slow addition of MeOSiMe₃ (gross excess) then allowed to warm to 23 °C and stirred for 1 h. The reaction was then concentrated under reduced pressure and pyridine was azeotropically removed with benzene (3x 15 mL). Purification by preparative reverse phase HPLC (C₁₈ SiO₂, 5:95 to 95:5 MeCN:H₂O 25 mL/min over 20 min) afforded C₄₁allyl-C²'epiOH-methylketal-azidoAmB 5.8 (48.6 mg, 0.047 mmol, 27% yield) as a flaky yellow solid. Material with extra silyl groups remaining was also recovered (111 mg). This material was re-subjected to similar reaction conditions (assuming fully silylated 5.7 as a molecular weight: pyridine 585 µL, 7.25 mmol, 125 equiv; HF-pyr 70%, 345 µL, 19 mmol, 328 equiv; 1.2 mL:0.2 mL THF:MeOH). A second cycle and HPLC purification yielded 5.8 (152.6 mg, 152 µmol, 88% combined yield) as a yellow flaky solid.

Rₛ = 15.68 min (C₁₈SiO₂ analytical HPLC, 5:95 to 95:5 MeCN:H₂O over 20 min, 1 mL/min)

¹H NMR: (500 MHz, CD₃C(O)CD₃) δ 6.55 – 6.15 (m, 23H), 6.06 – 5.89 (m, 3H), 5.54 – 5.46 (m, 2H), 5.41 (dq, J = 17.3, 1.7 Hz, 1H), 5.38 – 5.31 (m, 1H), 5.24 (dq, J = 10.5, 1.5 Hz, 2H), 4.76 – 4.61 (m, 8H), 4.36 – 4.32 (m, 1H), 4.32 – 4.15 (m, 4H), 4.15 – 4.05 (m, 5H), 3.97 (dt, J = 19.0, 4.2 Hz, 3H), 3.91 – 3.84 (m, 2H), 3.84 – 3.71 (m, 5H), 3.64 – 3.49 (m, 12H), 3.44 – 3.26 (m, 9H), 3.22 (d, J = 6.3 Hz, 7H), 3.01 (td, J = 9.0, 5.1 Hz, 2H), 2.49 – 2.20 (m, 8H), 2.17 – 2.08 (m, 3H), 2.04 – 1.72 (m, 13H), 1.71 – 1.53 (m, 16H), 1.53 – 1.39 (m, 12H), 1.21 (qd, J = 7.2, 6.4, 3.1 Hz, 12H), 1.12 (dd, J = 6.9, 3.7 Hz, 6H), 1.02 (t, J = 8.0 Hz, 6H).
\(^{13}\text{CNMR}\): (126 MHz, CD\(_3\)C(O)CD\(_3\)) δ 172.92, 172.30, 137.60, 136.91, 135.04, 134.98, 134.35, 133.89, 133.82, 133.76, 133.68, 133.36, 133.18, 132.79, 131.24, 118.46, 104.39, 102.54, 102.51, 78.81, 77.90, 76.00, 75.24, 75.17, 73.99, 73.54, 72.91, 71.14, 70.63, 69.47, 68.66, 68.30, 67.49, 67.18, 67.06, 65.94, 62.57, 62.36, 56.45, 48.68, 44.49, 43.60, 42.74, 42.43, 41.38, 38.46, 36.82, 33.03, 31.79, 30.66, 30.63, 30.40, 30.31, 30.25, 30.23, 30.19, 30.09, 30.07, 30.01, 27.21, 24.24, 18.97, 18.20, 17.52, 12.46.

**HRMS (ESI)**

Calculated for C\(_{51}\)H\(_{77}\)N\(_3\)O\(_{17}\) (M + Na): 1026.5151

Found: 1026.5115

Synthesis of intermediate 5.9

Intermediate 5.8 (104 mg, 0.135 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 2 mL) placed on high vac over night in a 20 mL iChem vial. In the glove box, Pd(PPh\(_3\))\(_4\) (35.9 mg, 0.03105 mmol, 30 mol%) and thiosalicylic acid (79.8 mg, 0.517 mmol, 5.0 equiv) was added followed by DMF (3.5 mL) and sealed under Ar atmosphere and stirred for 1 h at 23 °C. The reaction was transferred drop-wise into rapidly stirring Et\(_2\)O (100 mL). The precipitate was filtered through a 5” pipette containing a small piece of a kim-wipe\textsuperscript{TM} as a filter. The filter cake was then washed with additional Et\(_2\)O then eluted through the filter with DMSO. The filter was washed further with minimal DMSO. The combined DMSO fractions were lyophilized to yield 5.9 (68.9 mg, 0.714 mmol, 69%) as a yellow powder and taken on to the next reaction without additional purification. By analytical HPLC full conversion to a single peak was observed.
$R_f = 18.7 \text{ min (C}_{18}\text{SiO}_2 \text{ analytical HPLC, 5:95 to 95:5 MeCN:H}_2\text{O w/0.1\% formic acid over 20 min, 1 mL/min)}$

**HRMS (ESI)**

Calculated for $C_{48}H_{73}N_3O_{17} \text{ (M + Na)}^+: 986.4838$

Found: 986.4825

**Synthesis of intermediate 5.10**

To a stirred solution of 5.9 (68.9 mg, 0.0715 mmol, 1.0 equiv) in THF:H$_2$O (1.59 mL: 0.8 mL 2:1) in a 7 mL vial at 23 °C was added CSA (4.5 mg, 0.0178 mmol, 0.25 equiv) and stirred for 2 h. Aqueous saturated bicarbonate (0.5 mL) was added and then filtered through HPLC filters (need Fischer brand part No.) followed by preparative reverse phase HPLC purification (C$_{18}$SiO$_2$, 5:95 to 95:5 MeCN:H$_2$O with 0.1\% formic acid for 25 min at 25 mL/min) yielded 5.10 (30.8 mg, 32.2 µmol, 45%) as a yellow powder.
**R_f** = 19.3 min (C_{18}SiO_{2} analytical HPLC, 5:95 to 95:5 MeCN:H_{2}O over 20 min, 1 mL/min)

**HRMS (ESI)**

Calculated for C_{47}H_{71}N_{3}O_{17} (M + Na)^+: 972.4681  
Found: 972.4661

**Synthesis of C2’epiAmB:**

To a stirred solution of **5.10** (30.8 mg, 32.2 µmol, 1.0 equiv) in DMSO (1.1 mL) and H_{2}O (58 µL, 100 equiv) in a 7 mL vial at 23 °C under Ar atmosphere was added PMe_{3} as a 1.0 M solution in THF (97 µL, 97.0 µmol, 3.0 equiv) and then warmed to 55 °C for 6 h. The reaction was then concentrated under reduced pressure followed by preparative reverse phase HPLC purification (C_{18}SiO_{2}, 5:95 to 95:5 MeCN:NH_{4}OAc (15 mM) for 20 min at 25 mL/min) yielded **C2’epiAmB** (11.2 mg, 17.2 µmol, 54%) as a yellow powder.
$R_f = 11.17$ min ($C_{18}$SiO$_2$ analytical HPLC, 5:95 to 95:5 MeCN:NH$_4$OAc (5 mM) over 20 min, 1 mL/min)

Extinction coefficient: 92,000 cm$^2$/mol

$^1$H NMR: (500 MHz, CD$_3$S(O)CD$_3$) $\delta$ 6.55 – 6.03 (m, 10H), 5.97 (dd, $J = 15.5$, 8.7 Hz, 1H), 5.75 (d, $J = 10.9$ Hz, 1H), 5.44 (dd, $J = 15.0$, 10.1 Hz, 1H), 5.34 (s, 1H), 5.21 (d, $J = 7.9$ Hz, 1H), 4.89 – 4.71 (m, 3H), 4.62 (d, $J = 5.7$ Hz, 1H), 4.41 (d, $J = 6.3$ Hz, 1H), 4.39 – 4.30 (m, 2H), 4.25 (t, $J = 10.5$ Hz, 2H), 4.06 (s, 1H), 3.91 (d, $J = 10.4$ Hz, 1H), 3.49 (d, $J = 31.6$ Hz, 2H), 3.17 – 3.04 (m, 2H), 3.04 – 2.84 (m, 2H), 2.66 (d, $J = 11.9$ Hz, 1H), 2.40 (s, 1H), 2.28 (dd, $J = 14.6$, 7.5 Hz, 1H), 2.17 (t, $J = 8.5$ Hz, 2H), 2.05 – 1.68 (m, 5H), 1.65 – 1.47 (m, 5H), 1.47 – 1.29 (m, 7H), 1.24 (q, $J = 5.6$, 4.6 Hz, 6H), 1.20 – 1.08 (m, 6H), 1.04 (t, $J = 7.4$ Hz, 3H), 0.91 (d, $J = 7.1$ Hz, 3H), 0.86 (td, $J = 7.1$, 4.2 Hz, 1H).

HRMS (ESI)

Calculated for C$_{47}$H$_{73}$NO$_{17}$ (M + H)$^+$: 924.4957

Found: 924.4960
Synthesis of intermediate 5.11

To a stirred suspension of AmB (4.0 g, 4.3 mmol, 1.0 equiv) in DMF:MeOH (75 mL: 75 mL) in a 300 mL round bottom at 23 °C, was added sequentially, pyridine (5.0 mL, 50.0 mmol, 11.5 equiv) and alloc-succinimide (2.4 g, 12.05 mmol, 2.8 equiv). After stirring for 16 h at 23 °C, the dark orange, homogeneous solution was slowly poured into rapidly stirring Et₂O (3.5 L). The yellow suspension was filtered through Whatman™ 42 filter paper and washed with Et₂O (3x 100 mL) before the cake was allowed to fully dry. The fully dried alloc-AmB yellow powder (4.3 mmol, quantitative) was taken on to the subsequent reaction without further purification.

To a stirred suspension of alloc-AmB (4.3 mmol, 1.0 equiv) in MeOH (35 mL, 0.1 M) in a 300 mL round bottom flask at 23 °C was added anisaldehyde dimethylacetal (4.0 mL, 23.5 mmol, 5.5 equiv) and stirred for 10 min until a very fine, uniform suspension formed. CSA (250 mg, 1.08 mmol, 0.25 equiv) as a white crystalline solid was then added in one portion. After stirring at 23 °C for 30 min, Et₃N was added (~160 μL) followed by THF (81 mL to dilute down to 0.03M). The reaction was slowly poured into rapidly stirring hexane (3.5 L). The subsequent yellow suspension was filtered through Whatman 42 filter paper and washed with Et₂O (3x 100 mL) before the cake was allowed to fully dry. The fully dried alloc-bisPMP-methylketal (4.3 mmol, quantitative) was taken on to the subsequent reaction as a yellow powder without further purification.

To a stirred suspension of alloc-bisPMP-methylketal (4.0 g, 4.3 mmol, 1.0 equiv) in DMF:MeOH (10:1) in a 300 mL round bottom at 23 °C, was added sequentially, Hunig’s
base (3.75 mL, 21.5 mmol, 5.0 equiv) and allyl bromide (11.2 mL, 129.0 mmol, 30 equiv). After stirring for 8 h at 23 °C, the dark orange, homogeneous solution was transferred into a separatory funnel containing EtOAc and deionized H₂O (1:1). The organic phase was washed with water (3x 200 mL) followed by brine. The combined aqueous phases were extracted with EtOAc. The combined organic phases were washed with saturated brine and dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (SiO₂, gradient elution 50:49:1 EtOAc:Hex:MeOH to 75:24:1 EtOAc:Hex:MeOH) afforded 5.12 (1.42 g, 1.09 mmol, 30%) as an orange solid.

\[ R_f = 0.21 \ (50:49:1 \text{ EtOAc:Hex:MeOH}) \]

\[^{1}H \text{ NMR:} \ (500 \text{ MHz, CD}_3\text{C(O)CD}_3) \delta \ 7.43 \ (d, \ J = 8.5 \text{ Hz, 2H}), \ 7.38 - 7.33 \ (m, \ 2H), \ 6.90 - 6.82 \ (m, \ 4H), \ 6.48 - 6.18 \ (m, \ 11H), \ 6.05 - 5.84 \ (m, \ 3H), \ 5.59 \ (dd, \ J = 14.3, \ 9.3 \text{ Hz, 1H}), \ 5.52 \ (s, \ 1H), \ 5.46 \ (s, \ 1H), \ 5.45 - 5.38 \ (m, \ 1H), \ 5.28 - 5.22 \ (m, \ 1H), \ 4.71 - 4.62 \ (m, \ 3H), \ 4.60 \ (d, \ J = 7.0 \text{ Hz, 1H}), \ 4.53 \ (q, \ J = 7.2, \ 4.6 \text{ Hz, 2H}), \ 4.17 \ (tt, \ J = 10.4, \ 6.0 \text{ Hz, 2H}), \ 3.95 \ (dd, \ J = 9.9, \ 6.9 \text{ Hz, 3H}), \ 3.79 \ (d, \ J = 2.9 \text{ Hz, 7H}), \ 3.77 - 3.66 \ (m, \ 3H), \ 3.61 \ (td, \ J = 9.0, \ 3.2 \text{ Hz, 1H}), \ 3.45 \ (d, \ J = 8.0 \text{ Hz, 1H}), \ 3.39 \ (p, \ J = 6.8 \text{ Hz, 2H}), \ 3.33 \ (q, \ J = 8.6 \text{ Hz, 3H}), \ 3.08 \ (s, \ 2H), \ 2.36 - 2.25 \ (m, \ 3H), \ 1.96 - 1.88 \ (m, \ 2H), \ 1.88 - 1.78 \ (m, \ 3H), \ 1.73 \ (dt, \ J = 16.4, \ 8.1 \text{ Hz, 3H}), \ 1.69 - 1.42 \ (m, \ 8H), \ 1.41 - 1.21 \ (m, \ 28H), \ 1.19 \ (p, \ J = 5.2 \text{ Hz, 4H}), \ 1.13 - 1.08 \ (m, \ 5H), \ 1.02 \ (d, \ J = 7.1 \text{ Hz, 4H}), \ 0.95 \ (d, \ J = 6.6 \text{ Hz, 2H}), \ 0.87 \ (dt, \ J = 12.0, \ 7.0 \text{ Hz, 22H}). \]

HRMS (ESI)

Calculated for C_{71}H_{95}NO_{21} (M + Na)^+: 1320.6294

Found: 1320.6285

120
Synthesis of intermediate 5.12

Intermediate **5.11** (2.83 g, 2.18 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and placed on high vac overnight in a 300 mL round bottom flask. To intermediate **5.11** was added THF (74 mL) followed by DIPEA (0.61 mL, 3.49 mmol, 1.6 equiv). In a separate 200 mL round bottom flask was added sequentially, THF (46 mL), DMAP (426 mg, 3.49 mmol, 1.6 equiv), and drop-wise *p*-tertbutylbenzoylchloride (595 µL, 3.05 mmol, 1.4 equiv) forming a fine, white suspension. Most of this suspension was slowly added drop wise via cannula to the THF, DIPEA and **5.11** solution over 50 min until a majority of the starting material was converted as judged by TLC. The reaction was diluted with EtOAc and transferred to a separatory funnel containing aqueous saturated sodium bicarbonate and extracted with EtOAc. The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (SiO₂, gradient eluent 65:33:2 EtOAc:Hex:MeOH isocratic) afforded **5.11** (930 g, 0.654 mmol, 30% yield) as an orange solid.

\[ R_f = 0.24 \ (65:33:2 \ \text{EtOAc:Hex:MeOH}) \]
**1H NMR**: (500 MHz, CD$_3$C(O)CD$_3$) δ 8.07 – 7.89 (m, 2H), 7.64 – 7.48 (m, 2H), 7.38 (ddt, $J$ = 25.4, 8.0, 2.2 Hz, 4H), 6.86 (ddd, $J$ = 9.5, 4.6, 2.4 Hz, 4H), 6.46 – 6.11 (m, 10H), 6.10 – 5.96 (m, 3H), 5.96 – 5.82 (m, 3H), 5.82 – 5.65 (m, 1H), 5.58 (d, $J$ = 3.7 Hz, 1H), 5.52 – 5.38 (m, 2H), 5.33 – 5.18 (m, 1H), 5.11 (td, $J$ = 9.2, 7.5, 3.9 Hz, 1H), 4.88 (s, 0H), 4.73 – 4.56 (m, 2H), 4.49 (t, $J$ = 5.9 Hz, 1H), 4.24 – 4.10 (m, 1H), 4.01 – 3.82 (m, 2H), 3.82 – 3.75 (m, 4H), 3.75 – 3.63 (m, 1H), 3.59 (td, $J$ = 9.6, 6.1 Hz, 1H), 3.56 – 3.46 (m, 1H), 3.45 – 3.34 (m, 1H), 2.85 (s, 1H), 2.60 (s, 1H), 2.45 – 2.35 (m, 1H), 2.35 – 2.23 (m, 1H), 2.02 – 1.94 (m, 1H), 1.91 – 1.82 (m, 1H), 1.80 – 1.40 (m, 6H), 1.36 (d, $J$ = 3.6 Hz, 8H), 1.32 – 1.26 (m, 3H), 1.22 – 1.15 (m, 2H), 1.12 (d, $J$ = 6.7 Hz, 2H), 1.01 (d, $J$ = 7.1 Hz, 2H).

**HRMS (ESI)**

Calculated for C$_{82}$H$_{107}$NO$_{22}$ (M + Na)$^+$: 1480.7182

Found: 1480.7172

Synthesis of intermediate 5.13

Intermediate 5.12 (910 mg, 0.624 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and placed on high vac overnight in a 300 mL round bottom flask. To intermediate 5.13 was added DCM (10.5 mL) and hexanes (10.5 mL) followed by freshly distilled 2,6-lutidine (654 µL, 5.61 mmol, 9.1 equiv) and cooled to 0 ºC. DEIPSOTf (743 µL, 3.74 mmol, 6.0 equiv) was added dropwise over 10 min and stirred for another hour. The reaction transferred to a separatory funnel containing Et$_2$O and aqueous saturated
bicarbonate and extracted with Et₂O. The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (SiO₂, gradient eluent 1:9 EtOAc:Hex to 1:4 EtOAc:Hex) afforded 5.13 (980 mg, 0.5 mmol, 80% yield) as an orange solid.

\[ \text{R}_f = 0.21 \text{ (1:4 EtOAc:Hex)} \]

\[ ^{1}H \text{ NMR: (500 MHz, CD}_3\text{C(O)CD}_3 \text{) } \delta 8.07 - 7.95 \text{ (m, 2H), 7.65 - 7.54 \text{ (m, 2H), 7.37 - 7.31 \text{ (m, 4H), 6.94 - 6.81 \text{ (m, 6H), 6.41 - 6.32 \text{ (m, 5H), 6.32 - 6.24 \text{ (m, 3H), 6.20 - 6.13 \text{ (m, 3H), 6.10 - 5.84 \text{ (m, 4H), 5.72 \text{ (ddd, } J = 21.6, 15.2, 6.4 \text{ Hz, 2H), 5.52 \text{ (d, } J = 3.3 \text{ Hz, 1H), 5.45 \text{ (q, } J = 1.6 \text{ Hz, 0H), 5.41 \text{ (d, } J = 10.3 \text{ Hz, 3H), 5.34 \text{ (dt, } J = 10.3, 1.4 \text{ Hz, 1H), 5.27 \text{ (dq, } J = 17.3, 1.8 \text{ Hz, 1H), 5.13 \text{ (dq, } J = 10.4, 1.5 \text{ Hz, 1H), 4.91 \text{ (d, } J = 1.1 \text{ Hz, 1H), 4.75 \text{ (s, 1H), 4.71 - 4.62 \text{ (m, 2H), 4.62 - 4.55 \text{ (m, 2H), 4.52 \text{ (dt, } J = 5.6, 1.6 \text{ Hz, 2H), 4.33 - 4.25 \text{ (m, 1H), 4.19 - 4.08 \text{ (m, 1H), 4.07 - 3.94 \text{ (m, 1H), 3.93 - 3.81 \text{ (m, 3H), 3.81 - 3.73 \text{ (m, 10H), 3.72 - 3.60 \text{ (m, 4H), 3.51 \text{ (dq, } J = 8.8, 6.1 \text{ Hz, 1H), 2.75 \text{ (s, 3H), 2.53 - 2.39 \text{ (m, 2H), 2.27 \text{ (dd, } J = 17.7, 4.4 \text{ Hz, 1H), 2.23 - 2.11 \text{ (m, 2H), 2.09 \text{ (s, 7H), 1.99 - 1.94 \text{ (m, 1H), 1.89 \text{ (ddt, } J = 12.5, 8.0, 3.9 \text{ Hz, 1H), 1.78 - 1.56 \text{ (m, 5H), 1.56 - 1.41 \text{ (m, 4H), 1.37 \text{ (d, } J = 3.4 \text{ Hz, 14H), 1.32 - 1.21 \text{ (m, 6H), 1.21 - 1.11 \text{ (m, 7H), 1.09 \text{ (d, } J = 6.8 \text{ Hz, 3H), 1.07 - 0.76 \text{ (m, 79H), 0.76 - 0.65 \text{ (m, 12H), 0.61 - 0.49 \text{ (m, 7H), 0.43 \text{ (dqd, } J = 14.1, 7.9, 1.7 \text{ Hz, 5H).}}}}

\[ ^{13}C \text{ NMR: (126 MHz, CD}_3\text{C(O)CD}_3 \text{) } \delta 172.60, 170.01, 166.28, 160.93, 160.80, 157.48, 157.01, 138.66, 135.17, 134.93, 134.66, 134.40, 134.27, 134.01, 133.67, 133.05, 132.92, 132.79, 132.29, 131.26, 130.93, 130.90, 129.29, 129.12, 128.87, 128.47, 127.24, 126.28, 119.43, 117.28, 114.09, 114.08, 113.99, 102.02, 101.18, 100.78, 96.73, 81.57, 75.89, 75.03, 74.97, \]
74.17, 73.14, 73.02, 72.98, 68.92, 66.82, 65.95, 65.84, 58.56, 57.01, 55.68, 48.58, 43.99, 42.91, 38.08, 36.90, 35.90, 33.75, 32.97, 31.64, 30.77, 28.14, 19.27, 18.24, 18.19, 18.07, 18.01, 17.70, 17.68, 14.19, 14.17, 14.03, 13.76, 7.94, 7.90, 7.82, 7.77, 7.72, 7.71, 7.48, 7.36, 5.21, 5.10, 4.94, 4.89, 4.69, 4.44.

HRMS (ESI)

Calculated for C_{110}H_{171}NO_{22} (M + Na)^+:  1993.1268
Found: 1993.1189

Synthesis of intermediate 5.14

Intermediate 5.13 (980 mg, 0.497 mmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and placed on high vac overnight in a 40 mL iChem. To intermediate 5.13 was added THF (6.2 mL) and MeOH (12.3 mL) followed by KCN (48.5 mg, 0.745 mmol, 1.5 equiv) placed under Ar atmosphere and warmed to 40 °C and stirred for 72 h. The reaction transferred to a separatory funnel containing Et₂O and aqueous saturated bicarbonate. The organic phase was washed with water followed by brine. The combined aqueous phases were extracted with Et₂O. The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (SiO₂, gradient eluent 1:9 EtOAc:Hex to 1:4 EtOAc:Hex) afforded 5.14 (542 mg, 0.298 mmol, 60% yield) as an orange solid.
R_f = 0.22 (3:7 EtOAc:Hex)

\(^1\text{H NMR:}\) NMR (500 MHz, CD\(_3\)C(O)CD\(_3\)) \(\delta\) 7.43 – 7.30 (m, 5H), 6.92 – 6.79 (m, 5H), 6.48 – 6.14 (m, 12H), 6.11 (dd, \(J = 15.0, 10.0\) Hz, 1H), 6.03 – 5.89 (m, 3H), 5.88 – 5.73 (m, 2H), 5.43 (d, \(J = 3.6\) Hz, 3H), 5.37 (dq, \(J = 21.8, 1.6\) Hz, 1H), 5.33 – 5.26 (m, 2H), 5.17 (dq, \(J = 10.6, 1.5\) Hz, 1H), 4.79 (s, 1H), 4.71 – 4.48 (m, 7H), 4.27 (td, \(J = 10.6, 4.7\) Hz, 1H), 4.21 – 4.11 (m, 1H), 3.95 – 3.82 (m, 4H), 3.79 (s, 4H), 3.78 (s, 4H), 3.77 – 3.63 (m, 6H), 3.54 (t, \(J = 9.2\) Hz, 1H), 3.38 – 3.26 (m, 1H), 2.49 (dd, \(J = 17.6, 7.6\) Hz, 1H), 2.43 (q, \(J = 7.1\) Hz, 1H), 2.32 – 2.24 (m, 3H), 1.96 (s, 3H), 1.94 – 1.86 (m, 2H), 1.82 – 1.67 (m, 3H), 1.66 – 1.57 (m, 2H), 1.58 – 1.27 (m, 7H), 1.26 (d, \(J = 6.1\) Hz, 4H), 1.23 – 1.10 (m, 8H), 1.10 – 0.86 (m, 58H), 0.86 – 0.76 (m, 15H), 0.70 (tdt, \(J = 8.2, 4.4, 2.9\) Hz, 11H), 0.63 – 0.48 (m, 5H), 0.48 – 0.36 (m, 4H).

\(\text{HRMS (ESI)}\)

Calculated for C\(_{99}\)H\(_{159}\)NO\(_{21}\) (M + Na\(^+\)):\ 1833.0379

Found: 1833.0355
Synthesis of intermediate 5.15

Intermediate 5.14 (## mg, ## mmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and placed on high vac overnight in a 40 mL iChem. To intermediate 5.14 was added p-nitrobenzoic acid (# mg, 0.## mmol, 6.0 equiv), PPh₃ (# mg, ## mmol, 6.0 equiv) and toluene (# mL). The solution was cooled to 0 °C and DIAD (## µl, 0.## mmol, 6.0 equiv) was added drop-wise and stirred at 0 °C for 1 h. The reaction was then heated to 70 °C for 3 h. The reaction was transferred to a separatory funnel containing Et₂O and aqueous saturated sodium bicarbonate. The organic phase was washed with water followed by brine. The combined aqueous phases were extracted with Et₂O. The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (SiO₂, gradient eluent 1:9 EtOAc:Hex to 1:4 EtOAc:Hex) afforded C2’epi nitrobenzoate 5.15 (# mg, ## µmol, 84% yield) as an orange solid.

\[ R_f = 0.2 \ (1:4 \text{EtOAc:Hex}) \]

\[ ^1H \text{ NMR:} \ (500 \text{ MHz, CD}_3\text{C(O)CD}_3) \delta \ 8.37 \ (s, \ 4H), \ 7.37 - 7.30 \ (m, \ 4H), \ 6.89 - 6.81 \ (m, \ 5H), \ 6.50 \ (d, \ J = 9.8 \text{ Hz, 1H}), \ 6.45 - 6.09 \ (m, \ 15H), \ 6.07 - 5.95 \ (m, \ 1H), \ 5.86 \ (ddd, \ J = 19.1, 14.5, 10.0 \text{ Hz, 1H}) \]
5.8 Hz, 2H), 5.67 (ddt, J = 17.3, 10.6, 5.4 Hz, 1H), 5.47 – 5.39 (m, 2H), 5.35 (s, 1H), 5.30 (dq, J = 10.4, 1.3 Hz, 1H), 5.15 (dd, J = 10.4, 7.9 Hz, 1H), 5.08 (dq, J = 17.2, 1.7 Hz, 1H), 4.92 (dq, J = 10.5, 1.4 Hz, 1H), 4.82 (d, J = 7.8 Hz, 1H), 4.79 – 4.69 (m, 2H), 4.61 (qdt, J = 13.1, 6.0, 1.4 Hz, 3H), 4.33 (qdt, J = 13.6, 5.4, 1.5 Hz, 2H), 4.18 – 4.09 (m, 1H), 3.97 (td, J = 10.6, 4.6 Hz, 1H), 3.90 – 3.81 (m, 3H), 3.77 (d, J = 2.9 Hz, 8H), 3.75 – 3.63 (m, 7H), 3.52 (dq, J = 9.0, 6.1 Hz, 1H), 2.69 (s, 3H), 2.53 – 2.39 (m, 2H), 2.34 – 2.21 (m, 1H), 2.19 – 2.07 (m, 2H), 2.04 – 1.98 (m, 1H), 1.88 (dddd, J = 12.9, 10.2, 6.6, 3.8 Hz, 1H), 1.79 (d, J = 15.5 Hz, 1H), 1.76 – 1.64 (m, 2H), 1.61 (dt, J = 13.0, 2.5 Hz, 1H), 1.56 – 1.40 (m, 5H), 1.37 – 1.24 (m, 14H), 1.23 – 1.12 (m, 8H), 1.10 – 0.95 (m, 45H), 0.94 – 0.84 (m, 19H), 0.84 – 0.76 (m, 13H), 0.74 – 0.60 (m, 15H), 0.53 (dqd, J = 26.8, 7.8, 3.2 Hz, 5H), 0.42 – 0.28 (m, 5H).

$^{13}$CNMR: (126 MHz, CD$_3$C(O)CD$_3$) δ 173.00, 170.05, 164.87, 160.93, 160.79, 157.06, 151.67, 138.05, 136.54, 134.87, 134.73, 134.64, 134.56, 134.45, 134.16, 133.82, 133.65, 133.35, 132.91, 132.75, 132.48, 132.40, 131.84, 130.96, 128.86, 128.47, 127.65, 124.39, 119.57, 117.11, 114.07, 113.98, 101.97, 101.21, 100.71, 98.47, 81.53, 76.09, 76.00, 75.09, 74.92, 73.67, 73.04, 72.94, 68.84, 66.84, 66.12, 65.56, 59.60, 58.12, 55.66, 55.12, 48.39, 43.94, 42.99, 41.32, 38.08, 36.35, 33.68, 32.96, 28.21, 22.01, 18.87, 18.20, 18.14, 18.00, 17.98, 17.93, 17.62, 17.60, 14.15, 14.12, 14.02, 13.67, 7.90, 7.86, 7.76, 7.73, 7.69, 7.66, 7.36, 5.15, 5.06, 4.93, 4.91, 4.88, 4.63, 4.36.

HRMS (ESI)

Calculated for C$_{106}$H$_{162}$N$_2$O$_{24}$Si$_4$ (M + Na)$^+$: 1982.0492

Found: 1982.0464
Synthesis of intermediate 5.16

Intermediate 5.15 (80.4 g, 40.4 µmol, 1.0 equiv) was azeotropically dried with benzene (3x 10 mL) and placed on high vac overnight in a 7 mL iChem. To intermediate 5.15 was added THF (1.0 mL) and MeOH (0.5 mL) followed by KCN (4.08 mg, 61.4 µmol, 1.5 equiv) placed under Ar atmosphere and warmed to 40 °C and stirred for 72 h. The reaction transferred to a separatory funnel containing Et₂O and aqueous saturated bicarbonate. The organic phase was washed with water followed by brine. The combined aqueous phases were extracted with Et₂O. The combined organic phases were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by flash chromatography (SiO₂, gradient eluent 1:9 EtOAc:Hex to 1:4 EtOAc:Hex) afforded 5.16 (42.6 mg, 23.4 µmol, 57% yield) as an orange solid.

Rₐ = 0.2 (3:7 EtOAc:Hex)

¹H NMR: (500 MHz, CD₃C(O)CD₃) δ 7.43 - 7.32 (m, 4H), 6.87 (ddd, J = 13.9, 8.9, 2.1 Hz, 4H), 6.47 - 6.15 (m, 13H), 6.10 (dd, J = 15.1, 10.0 Hz, 1H), 6.06 - 5.82 (m, 3H), 5.78 (dd, J = 15.1, 8.6 Hz, 1H), 5.43 (d, J = 6.0 Hz, 3H), 5.36 (dt, J = 31.2, 1.6 Hz, 1H), 5.31 - 5.25 (m, 1H), 5.16
(dt, J = 10.7, 1.5 Hz, 1H), 4.81 (s, 1H), 4.66 – 4.55 (m, 3H), 4.51 (td, J = 4.9, 3.9, 1.5 Hz, 2H), 4.37 (d, J = 6.5 Hz, 1H), 4.33 – 4.23 (m, 1H), 4.22 – 4.12 (m, 1H), 4.01 – 3.82 (m, 3H), 3.79 (d, J = 1.8 Hz, 3H), 3.78 (d, J = 1.9 Hz, 3H), 3.76 – 3.66 (m, 4H), 3.43 (tt, J = 9.2, 3.9 Hz, 3H), 3.34 (h, J = 6.3 Hz, 1H), 3.05 (d, J = 1.9 Hz, 3H), 2.49 (dd, J = 17.6, 7.7 Hz, 1H), 2.46 – 2.38 (m, 1H), 2.27 (dt, J = 14.3, 4.6 Hz, 3H), 2.09 (d, J = 1.6 Hz, 4H), 2.01 – 1.93 (m, 1H), 1.93 – 1.85 (m, 2H), 1.85 – 1.77 (m, 1H), 1.73 (q, J = 10.2, 9.4 Hz, 1H), 1.68 – 1.38 (m, 7H), 1.31 (q, J = 10.9 Hz, 5H), 1.24 (t, J = 5.4 Hz, 4H), 1.22 – 1.16 (m, 6H), 1.10 – 0.86 (m, 52H), 0.86 – 0.75 (m, 14H), 0.69 (dddd, J = 13.6, 11.6, 8.0, 3.8 Hz, 10H), 0.63 – 0.49 (m, 4H), 0.49 – 0.34 (m, 4H).

\[^{13}C\text{NMR:}\ (126 \text{ MHz, } CD_3\text{C(O)CD}_3) \delta 173.37, 170.15, 160.95, 160.81, 157.34, 137.97, 134.87, 134.84, 134.77, 134.74, 134.35, 134.15, 133.96, 133.77, 133.56, 133.36, 132.90, 132.78, 132.42, 131.08, 129.69, 128.90, 128.50, 119.55, 117.30, 114.08, 114.01, 103.12, 102.07, 101.27, 100.90, 81.60, 76.29, 76.20, 75.23, 74.59, 73.32, 73.28, 72.97, 69.07, 67.63, 66.27, 65.64, 61.38, 57.67, 55.66, 48.58, 44.14, 43.33, 41.41, 38.08, 37.66, 33.73, 32.93, 30.76, 28.33, 19.26, 19.11, 18.21, 18.14, 18.05, 18.02, 18.00, 17.69, 17.67, 14.15, 14.04, 13.72, 7.90, 7.87, 7.80, 7.78, 7.75, 7.71, 7.47, 7.45, 5.18, 5.06, 5.02, 4.96, 4.90, 4.88, 4.66, 4.43.

HRMS (ESI)

Calculated for C\(^{99}\)H\(^{159}\)NO\(^{21}\)Si\(^4\) (M + Na)+: 1833.0379

Found: 1833.0309

Synthesis of intermediate 5.17

To a 30 mL Teflon vial charged with MeOH (14.5 mL) at 0 °C was added HF-pyridine 70% solution (2.05 mL). To a separate 100 mL Teflon vial containing 5.16 (0.65 mmol 1.05 g) as an azeotropically dried solid was charged with THF (7.2 mL) and cooled to 0 °C. The
contents of the 1st vial were transferred slowly via cannula to the second vial over 20 min. At this point the ice bath was removed and the reaction was allowed to stir at 23 °C for 6 h. Upon completion, the reaction was cooled to 0 °C and quenched by slow addition of saturated aqueous sodium bicarbonate (60 mL) and allowed to warm to 23 °C for 1 h. The biphasic suspension was then transferred to a separatory funnel containing saturated aqueous sodium bicarbonate and EtOAc. The combined organics were washed with H2O, saturated brine, dried with sodium sulfate, filtered and concentrated under vacuum. Purification by flash chromatography (SiO2, gradient eluent 49:1 DCM:MeOH to 97:3 DCM:MeOH to 19:1 DCM:MeOH) afforded 5.17 (473.3 mg, µmol, 68% yield) as an orange solid.

\[ R_f = 0.2 \text{ (19:1 DCM:MeOH)} \]

\textbf{1H NMR} (500 MHz, Acetone-$d_6$) \( \delta \begin{align*} 7.48 - 7.32 & (m, 5H), 6.91 - 6.81 (m, 5H), 6.35 \text{ (ddddd, } J = 53.1, 19.0, 13.1, 8.3 \text{ Hz, 18H}), 6.06 - 5.87 (m, 3H), 5.60 \text{ (p, } J = 8.4, 7.5 \text{ Hz, 1H}), 5.54 - 5.38 (m, 3H), 5.35 - 5.20 (m, 3H), 5.19 - 5.11 (m, 1H), 4.70 - 4.56 (m, 4H), 4.52 \text{ (d, } J = 5.4 \text{ Hz, 3H}), 4.38 \text{ (d, } J = 7.5 \text{ Hz, 2H}), 4.27 - 4.08 (m, 3H), 4.10 - 3.90 (m, 3H), 3.89 - 3.83 (m, 1H), 3.77 \text{ (d, } J = 3.6 \text{ Hz, 9H)}, 3.56 \text{ (d, } J = 5.6 \text{ Hz, 1H}), 3.52 - 3.33 (m, 6H), 3.22 - 3.13 (m, 1H), 3.08 (s, 3H), 2.59 \text{ (dt, } J = 14.5, 7.3 \text{ Hz, 1H}), 2.36 \text{ (tdd, } J = 27.1, 16.2, 7.3 \text{ Hz, 5H}), 2.09 \text{ (s, 1H)}, 2.07 - 1.99 (m, 2H), 1.97 (s, 1H), 1.96 - 1.82 (m, 3H), 1.83 - 1.64 (m, 3H), 1.62 - 1.56 (m, 1H), 1.38 \text{ (d, } J = 11.8 \text{ Hz, 1H}), 1.28 - 1.19 (m, 8H), 1.15 \text{ (d, } J = 6.4 \text{ Hz, 4H}), 1.04 \text{ (d, } J = 7.0 \text{ Hz, 4H}). \end{align*} \]

\textbf{13C NMR} (126 MHz, Acetone) \( \delta \begin{align*} 206.32, & 173.28, 170.86, 169.77, 160.51, 160.43, 158.26, 150.38, 137.62, 137.01, 134.35, 134.24, 134.18, 134.06, 133.88, 133.77, 133.42, 133.05, 132.81, 132.57, 132.51, 132.32, 130.13, 128.29, 128.24, 128.19, 124.55, 118.50, 117.18, 114.18, 113.87, 102.86, 101.05, 100.73, 100.55, 81.03, 77.97, 76.51, 76.34, 75.25, 73.92, 73.25, 73.19, 72.92, 70.58, 67.58, 67.10, 65.74, 65.67, 60.88, 60.46, 56.96, 55.45, 48.64, 43.54, 42.66, 41.80, 41.47, 37.89, 37.66, 33.85, 33.34, 30.58, 28.76, 23.21, 20.80, 18.99, 18.34, 17.60, 14.44, 14.32, 11.97. \end{align*} \)
Synthesis of intermediate 5.18

To a 40 mL iChem vial charged with 5.17 (## mg, ## mmol, 1.0 eq) was added in the glove box Pd(PPh₃)₄ (## mg, ## mmol, 0.3 eq), thiosalicylic acid (## mg, ## mmol, 1.0 eq) and sealed. Outside of the glove box was then added DMF (## mL) at 23 °C and stirred for 1 h. The reaction was then added dropwise to stirring Et₂O (# mL). The resulting yellow precipitate was then filtered through Whatman 50 filter paper and rinsed with excess Et₂O. The filtrate was then concentrated and slowly added to stirring Et₂O (# mL). This process was repeated until all of the precipitate was collected. The orange-yellow solid intermediate 5.18 was taken on to the next reaction without further purification.

Rₜ = 15.9 min (C₁₈SiO₂ 5:95 -> 95:5 MeCN:H₂O 5mM NH₄OAc over 20 min @ 1 mL/min)

¹H NMR (500 MHz, Methanol-d₄) δ 7.51 – 7.39 (m, 4H), 6.84 – 6.75 (m, 4H), 6.32 (dddd, J = 36.2, 31.5, 17.4, 9.9 Hz, 13H), 6.01 (dd, J = 14.5, 6.0 Hz, 1H), 5.57 (s, 1H), 5.52 (q, J = 4.5 Hz, 2H), 5.40 – 5.35 (m, 1H), 5.30 (s, 18H), 4.81 – 4.74 (m, 1H), 4.72 (d, J = 7.5 Hz, 1H), 4.47 (td, J = 10.7, 4.6 Hz, 1H), 4.36 – 4.27 (m, 2H), 4.08 – 4.00 (m, 1H), 3.86 – 3.72 (m, 2H), 3.69 – 3.54 (m, 9H), 3.47 – 3.35 (m, 3H), 3.33 (dq, J = 3.1, 1.4 Hz, 7H), 3.09 (d, J = 0.9 Hz, 3H), 2.64 (dd, J = 16.8, 6.6 Hz, 1H), 2.52 (dd, J = 13.1, 4.6 Hz, 1H), 2.49 – 2.37 (m, 2H), 2.34 (dd, J = 16.9, 6.1 Hz, 1H), 2.15 – 2.04 (m, 1H), 2.02 – 1.83 (m, 5H), 1.70 (q, J = 11.7 Hz, 1H), 1.61 – 1.52 (m, 4H), 1.44 – 1.36 (m, 1H), 1.33 (d, J = 6.0 Hz, 3H), 1.28 (dd, J = 12.9, 10.4 Hz, 1H), 1.22 (d, J = 6.3 Hz, 3H), 1.15 (d, J = 6.4 Hz, 4H), 1.12 – 1.08 (m, 1H), 1.06 (d, J = 7.0 Hz, 3H).
\(^{13}\text{C NMR}\) (126 MHz, CD\textsubscript{3}OD) \(\delta\) 179.76, 170.66, 160.94, 160.80, 135.39, 135.30, 134.42, 134.30, 134.16, 134.40, 133.98, 133.54, 133.45, 132.89, 132.67, 131.25, 128.72, 128.59, 114.22, 114.17, 102.51, 101.14, 81.52, 78.40, 77.94, 77.00, 74.38, 73.37, 73.66, 73.49, 72.28, 71.21, 68.59, 67.89, 60.19, 59.72, 55.62, 55.60, 41.82, 38.22, 33.80, 19.31, 18.29, 17.75, 12.37.

**HRMS (ESI)**

Calculated for C\textsubscript{64}H\textsubscript{88}NO\textsubscript{19}: 1174.5941

Found: 1174.5951

**Synthesis of C2’epiAmB**

To a 300 mL round bottom flask containing in azeotropically dried 5.18 (## mg, ## mmol, 1.0 eq) was added MeCN (## mL) and DI H\textsubscript{2}O (## mL). The suspension was then cooled to 0 °C whereupon CSA (## mg, ## mmol, 150 eq) was added in one portion. The yellow orange suspension became a yellow orange clear solution upon the addition of CSA. Overtime a fine precipitate forms. After stirring for 3 h at 0 °C, triethyl amine (## mL, ## mmol, 300 eq) was added. The reaction was then concentrated and purified by preparative HPLC (C\textsubscript{18}SiO\textsubscript{2} 5:95 -> 95:5 MeCN:H\textsubscript{2}O 5mM NH\textsubscript{4}OAc over 20 min @ 1 mL/min) to yield C2’epiAmB (## mg, ## mmol, ##%yield) as a yellow powder.

5-4: REFERENCES

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