LESS TOXIC YET STILL RESISTANCE EVASIVE AMPHOTERICINS AND
ATOMISTIC PROBING OF THE AMPHOTERICIN B ION CHANNEL

BY

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DISSERTATION

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ABSTRACT

Amphotericin B (AmB) is a polyene macrolide natural product with two major functions: antifungal activity and ion channel formation. For over 50 years AmB has remained the last line of defense against systemic fungal infections. Remarkably, AmB has evaded the development of drug resistance during this extensive clinical lifetime. Unfortunately, AmB’s utility is impaired by severe toxicities. A less toxic AmB derivative thus stands to have a major impact on global human health. Similar to most small molecule medicines, AmB exerts its antifungal activity by binding and disabling a specific molecular target. In contrast, some diseases are caused by the malfunctioning of proteins, and thus lie outside this traditional paradigm. For example, treating the underlying cause of cystic fibrosis requires a small molecule capable of replicating the function of the CFTR chloride ion channel. AmB is the prototypical small molecule capable of ion channel formation. Although it has been known for over 40 years that AmB forms ion channels, the structure of these channels, as well as the functional groups responsible for ion conductance and selectivity remain unknown. Understanding these basic tenants of AmB mediated ion channel activity is the first step towards harnessing AmB to replace a missing or malfunctioning protein ion channel in a living system. There is thus a rich opportunity to harness synthesis to understand and optimize both the ion channel and antifungal functions of AmB.

The polyol region of AmB has been predicted line channel interior, creating a hyrophillic environment for ions and water. Furthermore, the channel is proposed to be funnel shaped, with the narrowest region near the C3 hydroxyl. Based on this model, we hypothesized that removal of the C3 hydroxyl would impact ion channel conductance. The C3 alcohol was promptly excised, synthesizing C3deoxyAmB in only 9 steps from AmB. NMR characterization revealed that there were no significant alterations in the overall conformation of the of the AmB macrocycle upon removal of the C3 alcohol. Single ion channels of C3deoxyAmB in planar lipid bilayers revealed that C3deoxyAmB is capable of ion channel formation, however its conductance is significantly reduced relative to AmB. This is consistent with models that place the C-3 hydroxyl group at a critical point for ion conductance.

During efforts to understand the molecular basis of ion selectivity, an efficient 3-step synthesis of a series of AmB urea derivatives was discovered. Although not useful for ion channel study, they were important probes to test an emerging allosteric modification model for
non-toxic AmB derivatization. These derivatives selectively bound ergosterol (the primary sterol in yeast), but not cholesterol (the primary sterol in human cells), and maintained antifungal activity but were significantly less toxic to human cells. Additionally, these derivatives were more efficacious than AmB in a mouse model of disseminated candidiasis, and drastically less toxic in acute toxicity studies in mice. More selective pharmacological action is generally associated with decreased toxicity, but also with increased vulnerability to resistance. This creates an important question. Would a less toxic AmB derivative still evade drug resistance? The AmB urea derivatives were ideal candidates to evaluate this question. AmB urea resistant yeast were generated using mutagenesis and evaluated with a suite a fitness and genomic tests. Despite increased sterol selectivity and decreased toxicity, the development resistance to the AmB ureas was accompanied by significant fitness trade-offs, resulting in completely avirulent yeast. Therefore, it is possible to have a less-toxic yet resistance evasive antifungals. Based on their more selective, non-toxic, and resistance evasive profile, two of these compounds, AmBMU and AmBAU, are exceptionally exciting prospects as a clinical replacement for AmB.

Degradative synthesis was vital in accessing both C3deoxyAmB and the AmB urea derivatives leveraged to probe AmB’s ion channel and antifungal functions. However, not all desired derivatives are accessible from this platform. For example, installing $^{13}$C labels into the backbone of the AmB framework would enable solid-state NMR studies capable of mapping the interaction between AmB and sterols at the atomistic level. An efficient and flexible total synthesis of AmB was designed grounded in the iterative cross coupling (ICC) strategy for small molecule synthesis. The synthesis was divided into three main phases, building block construction, ICC, and protecting group removal. Advances were made in all three phases. A scalable synthesis of building block one, and its cross coupling with building block two was developed. The planned final deprotection was additionally optimized. These advances contributed to the completion of the total synthesis of a fully protected doubly $^{13}$C-labeled AmB.
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List of Abbreviations

Ac  acetate

ACME  amplitude constrained multiplet evaluation

AmB  amphotericin B

AmBAU  amphotericin B aminoethylurea

AmBCU  amphotericin B carboxylethylurea

AmdeB  amphoteronolide B

AmBME  amphotericin B methyl ester

AmBMU  amphotericin B methyl urea
BLM  black lipid membrane

C16amAmB  C16 amino amphotericin B

C2’deOAmB  C2’ deoxy amphotericin B

C3deOAmB  C3 deoxy amphotericin B

C35deOAmB  C35-deoxy amphotericin B

C41amAmB  C41 amino amphotericin B
CBS  Corey-Bakshi-Shibata

CSA  (±)-10-camphorsulfonic acid

DBU  1,8-diazabicyclo[5.4.0]undec-7-ene

DCM  dichloromethane

DIAD  diisopropyl azodicarboxylate

DMAP  4-(dimethylamino)-pyridine

DMEU  1,3-dimethyl-2-imidazolidinone

DMF  dimethyl formamide

DMP  Dess-Martin periodinane

DMSO  dimethyl sulfoxide

DPhPC  1,2-diphtanoyl-sn-glycero-3-phosphocholine
DPPA Diphenyl phosphoryl azide
HMPA hexamethylphosphoramide
HPLC high performance liquid chromatography
ICC iterative cross-coupling
ITC isothermal titration calorimetry
LUV large unilamellar vesicle
MeAmB C41-methyl amphotericin B
MeAmdeB C41-methyl amphoteronolide B
MHC minimum hemolytic concentration
MIC minimum inhibitory concentration
MIDA $N$-methyliminodiacetic acid
MTC  minimum toxic concentration
NaHMDS  sodium hexamethyldisilazide
NMP  N-methyl-2-pyrrolidone
NOE  nuclear Overhauser effect
Ph₃P  triphenylphosphine
PMP  p-methoxyphenyl
POPC  1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
PPTS  pyridinium para-toluenesulfonate
pyr  pyridine
S-Phos  2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
TBAF  tetra-n-butylammonium fluoride
TBS  t-butyldimethylsilyl
TEM  transmission electron microscopy
TEMPO  2,2,6,6-tetramethylpiperidine 1-oxyl

TES  triethylsilyl

Tf  trifluoromethane sulfonate

THF  tetrahydrofuran

TLC  thin layer chromatography

TMS  trimethylsilyl

TMSE  2-(trimethylsilyl)ethyl

UV  ultraviolet

X-Phos  2-dicyclohexylphosphino-2’,4’,6’-triisopropylbiphenyl
Chapter 1
Current Understanding of Amphotericin B’s Antifungal and Ion Channel Functions

1-1 DUAL FUNCTIONS OF THE SMALL MOLECULE AMPHOTERICIN B

The natural product Amphotericin B (Fig. 1.1, AmB, 1.1) AmB has been used clinically for over 50 years as the last line of defense against systemic fungal infections.\(^1\) Despite AmB’s extensive tenure as a clinically vital antimycotic, it has remarkably evaded drug resistance.\(^1\) Unfortunately, AmB’s utility is impaired by severe dose-limiting toxicities. In part due to these toxicities, an estimated 1.5 million people die worldwide annually from invasive mycoses, more than either malaria or tuberculosis.\(^2\) Therefore, a less toxic AmB derivative stands to have a significant impact on global human health.

AmB is also the archetypical small molecule capable of ion channel formation in living systems. Protein ion channel activity underlies a variety of critical cellular functions including electrical signaling, cell volume regulation, pH balance, water transport, and electrochemical gradient maintenance.\(^3\) Malfunctioning or missing protein ion channels cause a number of diseases, known as the channelopathies. These diseases, such as cystic fibrosis,\(^4\) lie outside of the traditional paradigm of small molecule medicines in which drugs inhibit the function of their cellular target. If AmB’s ion channel activity could be harnessed, it could potentially replace a malfunctioning protein ion channel and thereby serve as a prosthetic on the molecular level. Rational optimization of AmB’s ion channel properties have been limited by a lack of understanding of the functional groups that govern AmB’s ion channel formation, conductance, and selectivity.

![Chemical structure of amphotericin B](image)

**Figure 1.1:** Chemical structure of amphotericin B
1-2 AMPHOTERICIN B’S HISTORICAL CONTEXT, FOUNDATIONAL STUDIES, AND MECHANISM OF ACTION

Prior to the 1950’s invasive fungal infections had a mortality rate near 100%, with very few effective treatments available.\textsuperscript{5} The isolation\textsuperscript{6} of the antifungal nystatin from the bacterium \textit{Streptomyces noursei} and its subsequent marketing as the first broad-spectrum antifungal agent led to an explosion of research into antifungal agents produced by actinomycetes.\textsuperscript{7-10} One such soil bacterium, \textit{Streptomyces nodosus}, was found to produce a compound with broad spectrum and highly potent activity, amphotericin B (AmB).\textsuperscript{11-14} Soon after its discovery, AmB was introduced into the clinic, marking a paradigm shift in the treatment of invasive mycoses.\textsuperscript{15,16} The scientific community then collectively sought to understand AmB’s mechanism of action. Early efforts by Kinsky demonstrated that AmB’s activity was sterol dependent. Both ergosterol (1.2) rich \textit{Neurospora crassa} protoplasts\textsuperscript{17} and cholesterol (1.3) rich rat erythrocytes\textsuperscript{18} were susceptible to AmB, while sterol deficient \textit{Bacillus megaterium} were unaffected by AmB.\textsuperscript{19} Consistent with this finding, Feingold discovered that only \textit{Acholeplasma laidlwalli} grown in cholesterol rich media were susceptible to AmB.\textsuperscript{20}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Chemical structures of ergosterol and cholesterol the primary sterols in yeast and human cells respectively.}
\end{figure}

Early studies showed that AmB induced intracellular potassium excretion from both cholesterol laden \textit{A. laidlwalli}\textsuperscript{20} and the pathogenic yeast \textit{C. albicans}.\textsuperscript{21} It was suggested that sterol-binding-mediated membrane permeabilization was responsible for the cell killing activity of AmB. Membrane permeabilization by AmB was proposed to be caused by several different mechanisms including gross membrane disruption, ion transport activity, or discrete single ion channel formation.

Voltage-clamp electrophysiological studies aimed to distinguish between these possibilities.\textsuperscript{3} Andreoli and coworkers first demonstrated that AmB induces a decrease in electrical resistance in planar lipid bilayers in a sterol dependent manner.\textsuperscript{22} Importantly, the integrity of the membrane was uncompromised, strongly suggesting that global membrane
disruption was inoperative. To distinguish between ion channel and ion transport activities, Finkelstein compared AmB’s electrical activity to the known K\textsuperscript{+} ion transporter valinomycin.\textsuperscript{23} Increasing concentrations of valinomycin, as well as increasing temperature both resulted in a linear increase in conductance. In contrast, increasing AmB’s concentration resulted in a power increase in conductance while increasing temperatures resulted in decreases in conductance. AmB’s electrical properties are therefore inconsistent with the known ionophore valinomycin, and more consistent with a multimeric self-assembling aggregate that forms discrete ion channels. Andreoli and coworkers further found a strong correlation between the hydrodynamic radius organic solutes and membrane permeability.\textsuperscript{24} For example AmB mediated the passage of small solutes such as urea and glycerol, however, larger solutes such as glucose remained impermeable. Andreoli thus concluded that AmB forms large water filled channels with a diameter between 7 and 10.5 Å.\textsuperscript{25}

In a landmark study, Ermishkin and coworkers conclusively demonstrated that AmB forms sterol dependent discrete single ion channels in planar lipid bilayers (Figure 1.3).\textsuperscript{26} Furthermore, AmB produced ion channel phenomena such as gating and ion selectivity like that demonstrated by protein ion channels.\textsuperscript{27} Follow-up studies by Ermishkin demonstrated that AmB forms single ion channels capable of transporting a variety of different monovalent cations and anions.\textsuperscript{28} Similar to protein ion channels,\textsuperscript{29} tetraalkylammonium ions block the AmB ion channel,\textsuperscript{30} with tetraethylammonium being most effective. Ermishkin also investigated the basis of ion selectivity and demonstrated that monovalent cations are only permeable in the presence of a permeable anion.\textsuperscript{31}

![Figure 1.3: The first example of discrete AmB single ion channel activity in voltage-clamp studies. Single ion channels are formed in a 20:1 lipid:cholesterol ratio with either a 2M KCl or b 2M KNO\textsubscript{3} in the bathing solutions. From Ermishkin et al. Nature 1976, 262, 698-699. Copyright 1976. Nature Publishing Group.](image-url)
The establishment of single ion channel formation by AmB stimulated efforts to understand the structure of the AmB ion channel, and its role in antifungal activity. Multiple groups simultaneously proposed the now classic barrel-stave model of the AmB ion channel (Figure 1.4 A).\textsuperscript{32-35} In this model, eight different AmB monomers self assemble in lipid bilayers forming an octomeric aggregate stabilized by interdigitating sterol molecules. The mycosamine and polyene motifs are predicted to stabilize the ion channel by interacting with sterols and lipids, while the polyol region is predicted to line the interior of the ion channel, creating a hydrophilic environment for ions and water. Furthermore, AmB is approximately 20 Å in length,\textsuperscript{36} roughly half the length of a lipid bilayer. This model proposed that to span the length of the bilayer, either the membrane could thin to the width of a single barrel channel, or the AmB barrel stave could dimerize to form a double barrel structure (Figure 1.4 A).

Modeling studies predicted that several of AmB’s polar functional groups play critical roles in ion channel self-assembly (Figure 1.4 B,C). The C41 acid and mycosamine appendage were proposed to form stabilizing interactions with either neighboring AmB molecules,\textsuperscript{37,38} phospholipids,\textsuperscript{38-41} or membrane embedded sterols.\textsuperscript{38,42-47} The polyol region has been implicated in a stabilizing intramolecular hydrogen bond network between the C8 and C9 alcohols of neighboring AmB molecules.\textsuperscript{39} Additionally, the C35 alcohol was predicted to stabilize the single barrel channel via membrane anchoring, or the double barrel channel via AmB dimerization.\textsuperscript{48}
Figure 1.4: Classic “barrel-stave” structure of AmB ion channel. A.) Schematic representation of AmB ion channel as either a single barrel (left) or double barrel (right). B.) Birds-eye view of proposed AmB ion channel.

Definitive experiments to evaluate these structure function relationships, and thereby understand both the structure of the AmB ion channel and its role in antifungal activity, have been difficult to design. The proposed multimeric, self-aggregating, and membrane associated nature of the AmB ion channel challenge the limitations of computational\textsuperscript{38,39,42} as well as spectroscopic\textsuperscript{40,49,50} techniques. Many studies have alternatively utilized covalently modified AmB derivatives to investigate the roles of each of the proposed critical functional groups on ion channel formation and assembly. A small selection of these molecules is depicted in Figure 1.5.\textsuperscript{37,44,51,52} For example, N-acyl AmB (NACAmB) has significantly reduced antifungal and membrane permeabilizing activity.\textsuperscript{51,53} This has been used to support the necessity of an
intramolecular salt bridge ring to stabilize the AmB ion channel. However, it has remained unclear whether this reduction in activity results from removal of a critical polar interaction, or alternatively introduction of steric clashing. Covalent modification is inherently limited as in contrast to larger peptides, small molecules self assembly can be exquisitely sensitive to the introduction of even a small degree of steric bulk.\textsuperscript{54,55} To overcome this limitation the Burke group has adopted a functional group deletion strategy to systematically explore the role each functional group plays in AmB mediated antifungal and ion channel activities.

![Chemical structures](image1.png)

**Figure 1.5:** Covalently modified AmB derivatives used to probe AmB ion channel properties.

To probe the roles of the acid and mycosamine functionalities, Palacios et al. synthesized a series of AmB derivatives in which either the C41 carboxylate (C41MeAmB), the mycosamine appendage (AmdeB), or both (C41MeAmdeB)(Figure 1.6 A) were synthetically deleted.\textsuperscript{56,57} The derivatives were then evaluated for the ability to kill yeast, form ion channels in planar lipid bilayers, and bind sterols via isothermal titration calorimetry (Figure 1.6A). Both derivatives lacking the mycosamine appendage (AmdeB and C41MeAmdeB) did not kill yeast or form ion channels in planar lipid bilayers. These derivatives were also incapable of binding sterol as determined from isothermal titration calorimetry (ITC), and showed zero antifungal activity. C41MeAmB on the other hand surprisingly maintained antifungal activity and formed ion channels very similar to AmB. Importantly, only the derivatives containing the mycosamine, AmB and C41MeAmB, were capable of binding ergosterol (Figure 1.6B). Thus, a direct binding
interaction between AmB and ergosterol is required for antifungal and ion channel activities, and this binding interaction is mediated by the mycosamine appendage (Figure 1.6C).

Figure 1.6: Functional group deletion strategy has illuminated several structure activity relationships governing AmB ion channel assembly. A.) Structures of AmB, MeAmB, AmdeB, and MeAmdeB. Displayed beneath each structure are their antifungal capacities and single ion channel forming capabilities in planar lipid bilayers. B.) Ergosterol binding capabilities of all four derivatives as determined by ITC. Displayed are the differences in net exotherms solutions of each compound titrated with sterol-free followed by 10% ergosterol containing LUVs. C. Structure of C35deOAmB, and its sterol binding and antifungal activities. D.) C35deOAmB is incapable of K+ efflux from either liposomes or yeast cells. Adapted from refs 57.

Two possible mechanisms of action can explain AmB’s antifungal activity. Either ergosterol-binding-dependant channel formation is required to kill yeast, or ergosterol binding alone is sufficient to exert antifungal activity. To distinguish between these two models further structure activity relationships were required. The C35 hydroxyl was predicted to be critical to ion channel activity in both the single and double barrel models (Figure 1.4A). Gray and coworkers synthesized a derivative lacking just this hydroxyl, C35deOAmB (Figure 1.6 C), and evaluated its antifungal, membrane permeabilization, and sterol binding capacities.58-60 The C35 was critical for ion channel formation, as C35deOAmB did not cause K+ efflux from either
liposomes or yeast (Figure 1.6 D). Surprisingly, despite this lack of ion channel activity, C35deOAmB maintained antifungal activity and was capable of binding ergosterol as determined by ITC. Furthermore, Anderson and coworkers recently demonstrated that AmB primarily exists as a large extramembranous aggregate via transmission electron microscopy (TEM) visualization and solid-state NMR experiments. They additionally demonstrated that the aggregate kills yeast by extracting ergosterol from the lipid bilayer. Combined, these two studies strongly suggest that in contrast to the widely accepted ion channel model, AmB primarily kills yeast by simply binding ergosterol. The implications of this finding on designing less toxic AmB analogs will be explored in greater detail in later sections.

![Figure 1.7: Distinguishing between the two possible mechanisms of action of AmB, ergosterol binding and membrane permeabilization. A.) Schematic representation of ergosterol binding and membrane permeabilization. B.) C35deOAmB binds sterol and is toxic to to yeast cells. C.) C35deOAmB does not induce K⁺ efflux from yeast cells. Adapted from ref. 58.](image)

The functional group deletion strategy has illuminated three key structure activity relationships governing AmB ion channel formation. The mycosamine appendage and C35-hydroxyl are required for ion channel formation, while oxidation at C41 is not. The functional groups responsible for ion conductance and selectivity still largely remain unknown. Modeling
studies place the polyol region lining the channel interior, playing a pivotal role in ion conductance. Furthermore, the channel is predicted to be funnel shaped, with its narrowest region near the C3 alcohol (Figure 1.8).\cite{39,42} This structural model leads to the prediction that removal of the C3 hydroxyl group would alter AmB ion channel conductance. This functional motif has largely remained refractory to synthetic manipulation, therefore new synthetic strategies were required to access such a derivative. Additionally, the basis of AmB’s ion selectivity is still unknown.\cite{26,28,31,62-66} The ion selectivity is pH dependant,\cite{67} but the specific functional groups involved in ion selectivity are unknown. Developing these structure activity relationships would illuminate the molecular underpinnings of AmB ion conductance and selectivity, establishing guidelines for the rational optimization of AmB’s ion channel properties. The development of such a molecular understanding would additionally shed light on the structure of the AmB ion channel, which has remained elusive for over 40 years.

![Figure 1.8: A.) Structure of the AmB ion channel with polyol region highlighted. B.) The AmB ion channel is predicted to be funnel shaped, narrowing to the C3-hydroxyl (highlighted in blue). Protic functional groups (highlighted in red) could play a critical role in ion selectivity.](image)

1-3 AMPHOTERICIN B – THE ARCHETYPICAL RESISTANCE REFRACTORY ANTIMICROBIAL

Drug resistance to virtually all antimicrobials including antifungals,\cite{68,69} antibiotics,\cite{70} antiparasitics,\cite{71} HIV therapeutics,\cite{72} and cancer\cite{73} chemotherapies is a global health threat and is presumed to be inevitable. In contrast, AmB has evaded drug resistance despite extensive use for over 50 years as the last line of defense against systemic fungal infections.\cite{1} Thus, AmB is the archetypical small molecule that is inherently refractory to antimicrobial resistance.

Several factors combine which likely play critical roles in AmB’s ability to evade drug resistance. AmB is not affected by most of the common drug-resistance mechanisms including
drug-efflux pumps and the polyene macrolide framework is not a substrate for enzymatic degradation. In contrast to most small molecule therapeutics, AmB does not target a mutable protein, but instead exerts its cytotoxic effect by binding the polyfunctional lipid ergosterol. Ergosterol is a central processes in yeast physiology, playing multiple roles in healthy yeast function including vacuole fusion, endocytosis, pheromone signaling, membrane compartmentalization, and the proper functioning of membrane proteins. AmB is a promiscuous sterol binder, thus to avoid binding by AmB, yeast must modify their sterol structure to a significant degree. Mutations in ergosterol biosynthesis mutations can engender AmB resistance, however the resulting fitness trade-offs cripple fungal virulence.

Unfortunately AmB’s therapeutic potential is severely limited by infusion related toxicities including fever and tremors, and devastating dose dependent and irreversible renal toxicity. This toxicity is likely also caused by AmB’s non-selective sterol binding, as AmB can also bind cholesterol, the primary human sterol. In part because AmB’s utility is limited by it’s toxicity, an estimated 1.5 million people worldwide still die from systemic fungal infections annually. This number is anticipated to rise in parallel with a expanding immunocompromised population. Thus, there is desperate need for new antifungal therapies. Liposomal formulations have emerged as less-toxic AmB alternatives, however these are often prohibitively expensive, and significant toxicities still remain. AmB has been replaced as the frontline therapy against fungal infections with less toxic alternatives such as fluconazole and the echinocandins. However, shortly after their clinical introduction resistance began to emerge. Fluconazole resistant C. albicans was recently recognized by the CDC as a serious health risk which deserves significant attention. A less toxic AmB is an extremely attractive strategy to overcome these challenges. However, less selective pharmacological action is generally associated with decreased vulnerability to resistance, but also with increased toxicity. Therefore, would a more selective and less toxic amphotericin become vulnerable to the onset of resistance?

Much effort has been exerted in the search for a less-toxic AmB replacement therapy. For example, AmB methyl ester (Figure 1.5, AmBME) demonstrated a modestly improved therapeutic index and was thus advanced to clinical trials. Unfortunately, in these trials devastating neurotoxic side effects were observed. However, analysis of this trial is complicated by the fact that some of the AmBME used in this study was only ~50% pure. The search for less toxic amphotericin derivatives has largely been guided by a model in which AmB exerts its
cytotoxic activity through ion channel formation. In this model, a less toxic derivative would have to selectively form ion channels in yeast, but not human cells. This challenging problem has been difficult to approach rationally. As mentioned previously, two recent pieces of evidence enabled a re-evaluation of this model. C35deOAmB binds ergosterol and is toxic to yeast, but is incapable of ion channel formation (Figure 1.7), and Anderson and coworkers recently reported that AmB primarily exists as a large extramembranous aggregate which kills yeast by extracting sterol from the cell membrane. Combined, this suggests that ergosterol binding alone is sufficient to kill yeast cells. It is also likely that cholesterol binding causes AmB’s human cell toxicity.

This new mechanistic understanding enabled a focus on sterol binding, rather than channel formation, en route to a less-toxic derivative. Specifically, this model predicts that an AmB derivative that selectively binds ergosterol, the primary sterol in yeast, but not cholesterol, the primary sterol in human cells, would be selectively toxic to yeast but not human cells.

In this vein, Wilcock and coworkers selectively removed the C2’ alcohol from the mycosamine, creating C2’deOAmB (Figure 1.9A). C2’deOAmB was capable of binding ergosterol but surprisingly not cholesterol. Consistent with the selective ergosterol binding hypothesis, C2’deOAmB was toxic to yeast but not human cells (Figure 1.9 B). C2’deOAmB is therefore an exciting potential therapeutic compound, however its synthesis is not amenable to large-scale production. Could an AmB derivative that selectively binds ergosterol and is therefore selectively toxic to yeast be synthetically accessed on large scale?

![Figure 1.9](image-url)

**Figure 1.9:** C2’deOAmB selectively binds ergosterol but not cholesterol, and is selectively toxic to yeast but not human cells. A.) Chemical structure of C2’deOAmB. B.) Sterol binding affinity (determined via ITC) and cell toxicity of AmB and C2’deOAmB. Adapted from ref 80.

I-4 SYNTHESIS OF AMPHOTERICIN B DERIVATIVES
Several factors combine to make the synthesis of AmB derivatives very difficult. The AmB structure is complex, being both amphipathic and amphoteric, and AmB is sensitive to light, oxygen, protic acids, and is insoluble in most common organic solvents.\textsuperscript{56} Despite these challenges, a number of different strategies have emerged which grant access to AmB derivatives, including degradative synthesis, semisynthesis, biosynthesis, and total synthesis.\textsuperscript{104} These strategies complement each other, with each strategy having inherent benefits and limitations.

Enabled by a fermentation of the natural product, degradative synthesis has been the most productive, yielding the largest number of AmB derivatives. However, AmB’s complex architecture and functional group variety make site selective derivatization challenging. Therefore, degradative synthesis has been limited to modifications of the C16 carboxylate, C13 ketal, and mycosamine appendage. A survey of these derivatives is outlined in Figure 1.10. The first reported AmB derivatives modified the carboxylate via simple reactions such as esterification (1.9)\textsuperscript{94,95} and amide (1.10)\textsuperscript{105-110} bond formation. Later the oxidation state of the C41 carbon was explored, including reduction to a ketone (1.11),\textsuperscript{111} hydroxy methyl (1.12)\textsuperscript{112} or even simple methyl group (1.13).\textsuperscript{56,57} A group working at Smith-Kline-Beechum synthesized a number of derivatives where the C41 was modified to a number of more complex functionalities including oximes and vinyl groups (1.14).\textsuperscript{112,113} The C-13 position is also readily accessible and different ketals (1.15),\textsuperscript{114,115} thio ketals (1.16),\textsuperscript{115} and oximes (1.17)\textsuperscript{113,116} have all been prepared. It was also found that the C-13 hemiketal can undergo an elimination, forming a dihydropyran\textsuperscript{117} which could undergo subsequent reaction to a variety of functionalized pyran derivatives (1.18).\textsuperscript{113,114}

The mycosamine sugar has been predicted to play critical roles in AmB activity, and thus was the target of study. The entire mycosamine appendage can be cleaved oxidatively, generating amphoteronolide 1.19.\textsuperscript{56} Single atom selectivity can also be achieved, most notably via the C3’ amine. Initial studies involved simple acylation (1.20) and alkylation of the amine (1.21).\textsuperscript{51,53,118} Carriera and coworkers discovered that a bis-reductive alkylation of the mycosamine to a polyamine tail moderately improved the therapeutic index (1.22).\textsuperscript{119,120} Further studies looked more closely at the C2’ alcohol both through inversion of the stereocenter accompanied with other structural changes (1.23),\textsuperscript{121} and selectively removing just the C2’ oxygen (1.24).\textsuperscript{80}
Degradative synthesis thus provides efficient access to an assortment of AmB derivatives, but is limited to modifications to a small subset of functional groups. Furthermore the carbon backbone cannot be manipulated via this strategy. Semi-synthesis allows access to a much larger portion of the AmB framework, but is accompanied by a trade-off of a more involved and typically less efficient synthetic effort. Nicolaou demonstrated that ozonolytic cleavage of protected intermediate 1.25 excises the entire polyene, generating bis-aldehyde 1.26. Methanolysis then removed the western half 1.27 leaving 1.28 containing the entire polyol region, pyran, and mycosamine (Figure 1.11 A). Using this hybrid semisynthesis strategy the polyene and western half can be rebuilt and modified, allowing for synthetic manipulation of the entire fragment from C20 to C37 (Fig 1.6B). Rychnovsky and coworkers leveraged this strategy to synthesize the rigid non-polyene amphotericin 1.29. Similarly, Murata and coworkers installed a fluorine label in the polyene core (1.30, appended to C28) for use in solid state NMR.
Gray and coworkers employed this strategy to complete the synthesis of C35deoxyAmB.\textsuperscript{58}

Both degradative and semisynthesis use chemical means to alter AmB’s structure. Alternatively, the polyketide synthase machinery responsible for the biosynthesis of AmB can be modified to produce AmB derivatives.\textsuperscript{124} For example the polyketide synthetase responsible for the biosynthesis of AmB initially produces macrolide 1.32. Then a series of posttranslational modifications oxidize the C-8 and C41 carbons, and glycosylation installs the mycosamine sugar (Figure 1.12). By deleting these genes Caffrey’s group was able to produce C8deoxyAmB (1.33)\textsuperscript{125,126} and C41MeAmB (1.34), although the latter was not able to be purified.\textsuperscript{127} Caffrey has also shown that the glycosyltransferase which installs the mycosamine can be swapped with the glycosyltransferase from perimycin to replace mycosamine with perosamine (1.35).\textsuperscript{128} Further modification of the AmB gene cluster enabled access to derivatives such as C7-oxo-AmB (1.36), and C15-oxo-AmB (1.37).\textsuperscript{129} Using similar biosynthetic techniques the related
polyene macrolide nystatin has been converted from a tetraene to a heptaene, yielding polyol modified amphotericins (1.38) (Figure 1.12). Similar to degradative synthesis, biosynthetic manipulation is limited as it is only capable of modifying a select number of functional groups. Additionally, the isolation of polyenes from the fermentation broth is often low yielding, limiting the quantity of isolable polyenes. 

Figure 1.12: Biosynthesis of AmB (top) and AmB derivatives (bottom) accessible via biosynthetic modification.

Degradative synthesis, semi-synthesis, and biosynthesis strategies enable access to a variety of AmB derivatives, however, these strategies are not general and are incapable of constructing any desired derivative. For example, nearly half of the AmB structure has remained refractory to modification. An efficient and flexible total synthesis of AmB would overcome this limitation. The structural complexity of AmB has captivated synthetic chemists for decades, and despite numerous efforts, only K. C. Nicolaou and coworkers in the late 1980s were able to complete this total synthesis. Their retrosynthesis began with removal of the
mycosamine, arriving at aglycone 1.39 (Figure 1.13). The macrolactone was then split in two, with Horner-Wadsworth-Emmons (HWE) and lactonization transforms disconnecting the molecule back to polyene 1.40 and polyol 1.41. Additional applications of HWE transforms then disconnected these two fragments into the five building blocks 1.42, 1.43, 1.44, 1.45, and 1.46. Sharpless asymmetric epoxidation was utilized to install the stereocenters on building blocks 1.42 and 1.43, while the chiral pool starting material (+) diethyl tartrate was used as the source of chirality for building blocks 1.44 and 1.46.

![Figure 1.13: Nicolaou and coworkers retrosynthesis of AmB.](image)

**1-5 ITERATIVE CROSS-COUPLING STRATEGY FOR AMPHOTERICIN B SYNTHESIS**

We pursued an alternate strategy for the total synthesis of AmB with the goal of achieving more efficient and flexible access to a number of different AmB derivatives. In 2007 Gillis et al. described a simple and modular strategy for the construction of small molecules through iterative cross-coupling (ICC) (Figure 1.14 A).\(^\text{143}\) Conceptually, this strategy is similar to iterative amide bond formation used to construct peptides (Figure 1.14 B).\(^\text{144}\) The ICC strategy utilizes bifunctional haloboronic acid building blocks protected as their corresponding N-methyliminodiacetic acid (MIDA) boronate esters to assemble small molecules through iterative application of the Suzuki-Miyaura cross coupling reaction. The MIDA boronate protecting group provides several advantageous properties. Complexation of a boronic acid with the MIDA ligand cage renders the typically reactive boronic acid inert towards a number of typical organic transformations, allowing boronic acids to be carried through multiple reactions. This tolerance allows all functionality, desired stereochemistry, and proper oxidation states to be pre-installed in
the building blocks prior to cross coupling. Purification and storage of MIDA boronates is simple, as they are typically free-flowing crystalline solids which are stable on the benchtop under ambient conditions, and are amenable to flash chromatography. The MIDA boronate can easily be readied for cross-coupling in the ICC cycle by deprotection under aqueous basic conditions to the free boronic acid, or directly converted to a boronic ester.

The ICC strategy has proven to be quite useful for the construction of a number of natural products including ratanhine, retinal, parinaric acid, crocacin C, peridinin, synechoxanthin, the polyene cores of AmB and vacidin A, as well as the polyene cores of >75% of all polyenes ever isolated. We envisioned that the many benefits of the ICC strategy could greatly simplify the construction of AmB in a total synthesis effort.

![Figure 1.14: Iterative Cross-Coupling Strategy for the synthesis of small molecules. A.) Peptides are assembled via iterative amide bond formation using bifunctional amino acids. B.) Small molecules can be synthesized with iterative Suzuki Miyaura cross couplings using bifunctional halo-boron acids protected as their corresponding MIDA boronates.](image)

1-6 SUMMARY

AmB has been used clinically for over 50 years as the last line of defense against systemic fungal infections, which still cause 1.5 million deaths worldwide each year. AmB’s utility is limited by severe toxicities. Although less toxic AmB derivatives have been synthesized, none have yet made a clinical impact, and leading candidates for a clinical AmB replacement, such as C2’deOAmB, are currently inaccessible on large scale. Could a less-toxic AmB derivative be synthesized on large scale? Less toxic drugs are also generally more vulnerable to the onset of drug resistance. Would a less toxic amphotericin become more vulnerable to the onset of drug resistance?

AmB is also the prototypical small molecule capable of ion channel formation. If this unique ability of AmB could be harnessed, potentially it could replace dysfunctional protein ion
channels, and thereby treat the underlying cause of diseases such as cystic fibrosis. Despite decades of research on the AmB ion channel, there is still a fundamental lack of knowledge of many aspects of this channel. What is the structure of the AmB ion channel? What functional groups are responsible for simple ion channel properties such as conductance and ion selectivity? The following chapters describe our efforts to synthesize AmB derivatives capable of investigating and answering these questions critical to advancing the understanding of both AmB’s antifungal and ion channel functions.

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Chapter 2

Atomistic Probing of the Amphotericin B Ion Channel

Amphotericin B (AmB) is the textbook example of a small molecule that forms ion channels in living systems. Despite extensive investigation into this molecule for over 50 years, the functional groups responsible for ion channel conductance and selectivity largely remain unknown. Leading modeling studies predict that AmB aggregates in lipid membranes with the polyol region lining the channel interior, funneling to its narrowest region near the C3 hydroxyl. These models suggest that the C3 alcohol lies at a critical point in the ion conductance pathway. To evaluate this model, the C3 alcohol was selectively removed via an efficient 9-step degradative synthesis, forming C3deOAmB. NMR analysis of both C3deOAmB and AmB revealed that removal of the C3 alcohol does not significantly alter the overall conformation of the macrocycle. In voltage clamp single ion channel studies C3deOAmB was still capable of ion channel formation, however the channels were significantly lower in conductance than channels formed by AmB. Thus, consistent with the leading structural models the C3 alcohol is located at a critical position in the AmB ion conductance pathway.

In an attempt to determine the molecular basis of AmB’s ion selectivity, efforts were made to replace AmB’s carboxylate with an amine. These compounds ultimately proved too unstable for isolation, however, during the course of these investigations a Curtius rearrangement was discovered that allowed for efficient access to C16 urea amphotericins. These compounds will be utilized in Chapter 3 to explore an evolving allosteric modification model for the development of non-toxic amphotericins.

Bowie Hu synthesized compounds 2.12-2.14 and C3deOAmB. NMR analysis was performed jointly with Bowie Hu. Portions of this chapter were adapted from Stephen A. Davis, Bowie Hu, Lisa Della Ripa, Chad Reinstra, and Martin D. Burke, manuscript in preparation.
2-1 CURRENT UNDERSTANDING OF THE AMPHOTERICIN B ION CHANNEL

The traditional role of small molecules in medicine has been to bind a specific protein target, and disable that target’s function. In contrast, some diseases are caused not by an excess of protein function, but by a lack of function. For example the malfunctioning of specific protein ion channels causes the entire channelopathy family of diseases.\(^1\) Cystic fibrosis, the most well known channelopathy, is caused by the malfunctioning of the CFTR chloride ion channel.\(^2\) If a small molecule were to treat the underlying cause of this disease, it would need to replicate the function of a protein ion channel. Such a small molecule could be viewed as a prosthetic on the molecular level, replacing an inactive or functionally deficient protein ion channel. To achieve this goal a small molecule is required which is capable of transporting ions in living systems.

Several small peptides, including gramicidin and alamethicin, are known to form ion channels.\(^3,4\) However, the immune system will recognize and degrade these peptides. In addition to peptides, several classes of fully synthetic small molecule ion channels have recently been created.\(^5-7\) Although these synthetic channels have displayed a variety of intriguing channel properties, challenges remain to translate these initial findings into \textit{in vivo} activity and overcome potential issues such as toxicity. It has remained unclear whether such small molecule mimics of missing proteins can restore physiology. Natural products have often been a source of inspiration in drug development, and several naturally occurring small molecules have been shown to possess ion channel activity. One such natural product, amphotericin B (AmB), is the prototypical small molecule capable of forming ion channels in living systems.\(^8-17\)

It has been known since the mid-1970’s that AmB can form discrete ion channels in lipid bilayers.\(^13\) This ion channel has several advantageous properties. Most importantly, AmB has a 50 year history as a clinically vital antimycotic,\(^18\) and consequently is already known to be tolerated \textit{in vivo}. The polyene macrolide structure is undetected by the immune system and is not a substrate for enzymatic degradation.\(^19\) The ion channel activity of AmB has been leveraged in perforated patch clamp electrophysiology to enable understand the electrical systems of intact living cells.\(^20\) For all of these reasons, AmB is an excellent starting point for the development of a small molecule capable of replicating the function of a protein ion channel.

The AmB channel is capable of transporting both monovalent anions and cations,\(^14\) and has been reported in some studies to be slightly cation selective.\(^13,17,21-23\) Similar to some potassium selective protein ion channels,\(^24\) the AmB channel can be blocked by
tetraalkylammonium ions. In contrast to most protein ion channels, it's been suggested from permeability studies that the AmB channel is rather wide, at nearly 8Å in diameter. In order to expand AmB’s therapeutic potential as a protein ion channel surrogate, several challenges need to be overcome. Primarily, a detailed understanding of the molecular features that govern AmB’s ion channel formation, conductance, and selectivity is required. Leading modeling studies predict that AmB self-aggregates into multimeric “barrel-stave” structures. In these models, AmB’s polyene and mycosamine motifs interact with lipids and sterols while the polyol region lines the water-filled channel interior.

Many features of protein ion channels have been elucidated through developing structure activity relationships. Although protein ion channels are much larger, and arguably more complex than AmB, the synthesis of protein analogs using techniques such as site directed mutagenesis is now routine. In contrast, chemoselectively altering AmB’s structure in order to probe specific structure activity relationships is quite challenging. Because of these difficulties, there have only been a limited number of studies investigating the roles of specific functional groups on AmB ion channel formation.

Leading modeling studies predicted an intermolecular salt bridge between the C41 carboxylate and the C3’ ammonium of a neighboring AmB was critical for AmB channel stability. Removal of the entire mycosamine yielded a derivative incapable of either binding ergosterol or forming channels. Intriguingly, reduction of the C41 carboxylic acid to a methyl group did not preclude channel formation (Figure 1.6A,B). Moreover, the C35 hydroxyl group was predicted to stabilize both the single-barrel as well as double barrel ion channel models (Fig. 1.6C). Consistent with this model, a derivative lacking the C35 alcohol, C35deOAmB, did not permeabilize cell membranes or induce K⁺ efflux from either liposomes or S. cerevisiae, even at 10× the concentration at which AmB readily permabilizes. Thus, both the mycosamine appendage and C35 hydroxyl are required for ion channel formation, while oxidation at C41 is not. Despite these advances, the functional groups responsible for ion channel conductance and ion selectivity still remain unknown.

As mentioned above, the polyol region of AmB is predicted to line the AmB channel interior, creating a hydrophilic environment for ions and water. Furthermore, the channel is predicted to be funnel shaped with a wide entrance near the C15 alcohol, tapering to its narrowest region near the C3 alcohol (Figure 2.1). It would stand to reason then that the C3
alcohol plays a critical role in ion conductance. We aimed to investigate this prediction by synthetically deleting just the C3 hydroxyl and evaluating the impact of the loss of this protic functional group on ion channel conductance.

The site-selective removal of a single one of the ten alcohols from the AmB is a challenging synthetic task. A handful of polyol modified derivatives have been reported, including synthetic modification of the C13 hemiketal\(^{38,39}\) as well as biosynthetic manipulation of both AmB\(^{40-42}\) and nystatin.\(^{43-45}\) The impacts of these modifications on ion channel formation or conductance have not been investigated. To gain a better understanding of AmB’s ion channel conductance, we set out to synthesize C3deOAmB.

**Figure 2.1:** A.) Chemical structure of AmB and C3deOAmB. B.) Proposed AmB ion channel model placing C3 alcohol at the narrowest region of the channel.

### 2-2 SYNTHESIS OF C3DEOXYAMB AND UNDERSTANDING AMPHOTERICIN B’S ION CHANNEL CONDUCTANCE

Initial efforts to synthesize C3deOAmB centered on developing a total synthesis capable of accessing any desired polyol modified derivative (see chapter 4). Concurrently two of my colleagues, Dan Palacios and Kaitlyn Gray, were completing the semi-synthesis of a different AmB derivative, C35deoxyAmB (Scheme 2.1).\(^{32}\) In this synthesis they attempted a TBAF mediated desilylation of TMSE ester 2.1. Intriguingly, they isolated an unexpected enone (2.2) byproduct from this reaction. Presumably during this reaction TBAF acted as a base, selectively eliminating the C3-PMP via an E1cB type mechanism. Although this was an undesired byproduct for the C35deOAmB synthesis, it was an excellent opportunity to potentially access C3deoxyAmB via a degradative synthesis strategy.
Several challenges had to be overcome to transform the observation of an enone byproduct into a viable synthesis of C3deOAmB. First, the elimination required optimization. Two important issues were identification of a base optimally suited to achieve the elimination, and the step during the synthesis to best perform the elimination. Second, upon enone generation, how could this enone be selectively reduced in the presence of a sensitive polyene framework? Lastly, protecting group strategy is critical in any synthesis, and protecting group removal from deoxygenated AmB’s is especially challenging. Therefore, protecting groups are required which could survive the synthesis, but also undergo facile removal during the end game strategy.

First, the elimination was optimized (Scheme 2.2). Minimally protected intermediate 2.3 was quickly generated, mimicking the protecting group strategy used in the C35deOAmB synthesis. Compound 2.3 was exposed to a variety of bases (TBAF-3H2O, NaH, NaHMDS, DBU) to promote elimination to 2.4. The hexamethyldisilazide base did show some product formation, but was accompanied with a significant amount of decomposition. We hypothesized that this decomposition was due to the presence of free alcohols. To minimize this undesired side reactivity, fully silylated intermediate 2.5 was generated (Scheme 2.3). This strategy was successful, as KHMDS in THF competently performed this elimination, cleanly yielding enone 2.6 on small scale. With enone 2.6 in hand, we turned our attention to the selective reduction of an enone in the presence of the sensitive heptaene framework. Fortunately, the first reagent attempted, Stryker’s reagent, efficiently reduced the α, β-unsaturated enone providing lactone 2.7.

Scheme 2.1: Enone byproduct isolated by Dan Palacios and Kaitlyn Gray during synthesis of C35deoxyAmB inspiring the degradative synthesis of C3deoxyAmB.

Scheme 2.2: Initial attempts of elimination on minimally protected amphotericin 2.3.
With a robust elimination and reduction protocol in hand, the protecting group strategy was re-evaluated. Two protecting groups, those on the C3’ amine and C41 carboxylate, deserved special attention. During the synthesis of C35deOAmB the phenylacyl group on the mycosamine amine was very challenging to remove. To avoid this difficulty, the phenylacyl was replaced with an alloc carbamate which should be stable to elimination, but also be easily removed.

With respect to the C41 protecting group, Carreira’s group was unsuccessful in cleaving a simple methyl ester from a deoxygenated AmB derivative. We elected to protect the C41 acid as its corresponding TMSE ester, a strategy that proved fruitful during the synthesis of C35deOAmB.

To evaluate this protecting group strategy, fully protected intermediate 2.8 was targeted.

The first generation synthesis of C3deOAmB is depicted in Scheme 2.4. The synthesis of 2.8 began with protection of the mycosamine amine as an alloc carbamate, followed by methyl ketal formation, and installation of p-methoxyphenyl acetics of both the C3/C5 and C9/C11 alcohols. The remaining five alcohols were protected as triethylsilyl ethers, and lastly Mitsunobu esterification with 2-(trimethylsilyl)-ethanol formed TMSE ester 2.8. Under optimized conditions, 2.8 was exposed to NaHMDS at -78°C and slowly warmed to 0°C, eliminating the C3 p-methoxyacetal and yielding enone 2.10. HF promoted desilylation of 2.11 proceeded smoothly. Next, the TMSE ester was then targeted for removal, however, this reaction presented several difficulties. Removal of excess tetrabutylammonium leftover upon reaction completion proved to be difficult. A workup developed by Kishi using DOWEX 50WX8 resin combined with CaCO₃ aided in this problem, but the combination of a challenging workup and
irreproducibility in the desilylation led us to re-evaluate the protecting group choice for the C41 acid.

**Scheme 2.4:** 1st generation synthesis of C3deoxyAmB.

The Alloc carbamate proved stable during the 1st generation synthesis, and mild conditions for the removal of alloc carbamates,50 as well as allyl esters36 from AmB were both known. Based on this precedence we elected to exchange the TMSE ester for a simple allyl ester. We envisioned carrying both protecting groups through the elimination, reduction and deprotection sequence, setting the stage for a final double deallylation to remove both the Alloc and allyl groups simultaneously.

With this new protecting group strategy in hand, a 2nd generation synthesis of C3deOAmB was explored. First the synthesis of fully protected intermediate 2.12 was targeted (Scheme 2.5). Synthesis of 2.12 was achieved with a sequence similar to that used to access 2.8 with a couple of slight modifications. In this sequence, the methyl ketal formation and bis PMP ketalizations were accomplished simultaneously. Additionally, the Mitsunobu esterification was replaced with a simple alkylation of the C41 acid with allyl bromide, yielding 2.12 in only 4 steps from AmB. Gratifyingly, both the elimination of fully protected 2.12 with NaHMDS, as well as Stryker reduction of enone 2.13 worked efficiently, providing lactone 2.14. The triethylsilyl ethers were removed with HF-pyridine, followed by CSA-promoted ketal hydrolysis. Satisfyingly, exposure of this penultimate doubly allylated intermediate to palladium tetrakis and
thiosalicylic acid cleanly removed the two remaining protecting groups. This completed the efficient degradative synthesis of C3deOAmB in only 9 steps from AmB.

Scheme 2.5: Degradative synthesis of C3deOAmB in only 9 steps from AmB.

One potential consequence of removing the C3 alcohol is a conformational change in the macrolide. The ground state conformation of a series of AmB derivatives, albeit with modifications exocyclic of the macrocycle, revealed that the macrolide conformation is rigid. Because the C3 hydroxyl is endocyclic, it potentially plays a greater role in conformational rigidity. In addition, the crystal structure of N-Iodoacyl AmB reveals a trans-decalin like hydrogen bonding framework between the C1 carbonyl, C3 alcohol, and C5 alcohol. Deletion of the C3 hydroxyl would abolish this hydrogen-bonding network, potentially conferring greater flexibility to the macrocycle. To test this hypothesis, extensive NOESY characterization of both AmB and C3deOAmB was performed. Figure 2.2 shows the NOESY correlations observed during this experiment. As can be seen, the NOE pattern for both AmB and C3deOAmB are very similar. Importantly, both molecules show a series of transannular interactions between the polynene and polyl frameworks. Given the similarities between these two spectra, it is unlikely that removal of the C3 alcohol causes a significant conformational change in AmB’s macrocyclic structure. Consequently, any observed change in AmB’s ion channel properties is due to removal of the C3 alcohol, not conformational differences in the AmB macrolactone. Simulated annealing calculations constrained by this NOESY data as well as dihedral angles determined by ACME analysis of the macrolide backbone are targeted to more accurately determine the ground state conformations of these two molecules.
Figure 2.2: NOE correlation of both AmB and C3deOAmB showing only peaks under 3.5Å. Both compounds show strong transannular NOEs between the polyol and polyene subunits. Some functional groups have been removed for clarity.

To test the key hypothesis that the C3 hydroxyl plays a critical role in AmB’s ion channel conductance, we utilized voltage clamp electrophysiology experiments in planar lipid bilayers. First, we asked whether both AmB and C3deOAmB were capable of ion channel formation. To interrogate this hypothesis, AmB and C3deOAmB were compared head to head in single ion channel studies. Planar lipid bilayers were formed from a 70:30 ratio of diphytanoyl phosphatidylcholine (DPhPC) lipids to ergosterol in 1M KCl at pH=7.0. Then AmB or C3deOAmB was added to both sides of the membrane as a solution in DMSO, and channel activity was investigated at +150 mV. Shown in Figure 2.3A are representative traces from these experiments. As can be seen, both AmB and C3deOAmB form single ion channels. Therefore, the C3 alcohol is not critical for ion channel formation. We then determined the conductance of these two molecules by plotting the current of each channel at various applied potentials (Fig. 2.3B). Both molecules display a linear response to applied voltage ranging from +90 to +210 mV, with AmB having a single ion channel conductance of 17.2 ± 0.5 pS. Under identical conditions, C3deOAmB’s single ion channel conductance decreased significantly to only 5.2 ± 0.9 pS.

The reduction in current upon removal of the C3 hydroxyl is consistent with the leading models which place the C3 alcohol at a critical position in the ion channel conductance pathway. However, this is not conclusive proof of the barrel-stave structure, as the reduced conductance is consistent with any channel model that places the C3 alcohol at a critical juncture. This study for the first time demonstrates that manipulation of the polyol motif affects ion channel activity. This
structure activity is step towards determining the elusive structure of the AmB ion channel. Further polyol manipulations, especially at the C3 position, are an exciting prospect for the optimization or tuning of other AmB ion channel properties, such as ion selectivity.

**Figure 2.3:** The C3 alcohol plays an important role in ion channel conductance. A) Representative single ion channel recordings comparing AmB (top) to C3deOAmB (bottom) in planar lipid bilayers. Recordings were performed in a 70:30 DPhPC:Erg lipid environment with 1M KCl bathing solutions on cis and trans side at +150 mV. B) Current vs Voltage plot reveals that C3deOAmB has a lower conductance than AmB. Recordings were measured in a 70:30 DPhPC:Erg lipid environment with 2M KCl bathing solutions on cis and trans side at +90, 120, 150, 180, and 210 mV.

### 2-3 UNDERSTANDING AMPHOTERICIN B’S ION CHANNEL SELECTIVITY

Protein ion channels demonstrate a wide range of ion selectivities. For example, the cystic fibrosis transmembrane regulatory protein (CFTR) whose malfunction causes cystic fibrosis, is a chloride selective channel. Our ultimate goal is to be able to replace malfunctioning protein ion channels which possess a variety of ion selectivities with AmB, or one of its derivatives. To enable the rational design of AmB derivatives with altered ion selectivities the molecular basis for ion selectivity must be understood. Similar to the molecular basis of AmB’s ion channel conductance, the underpinnings of ion selectivity are also unknown. The AmB ion channel has been reported as slightly $K^+$ selective depending upon experimental setup. It has been demonstrated that AmB’s ion selectivity is pH dependant, suggesting that protonizable functional groups may play a role in ion selectivity.

In protein ion channels, charged residues have been shown to play critical roles in ion selectivity. In the ligand-gated superfamily of ion channels, both the nicotinic acetylcholine receptor (nAChR) and glycine receptor (GlyR) are very structurally similar. The cation selective nAChR channels display a glutamate at the extracellular entrance of the transmembrane conductance pathway. Conversely, in anion selective GlyR channels, this glutamate is exchanged for a positively charged arginine. Furthermore, the cation selective peptide ion channel
alamethicin, which like AmB has been predicted to form barrel-stave like channels,\textsuperscript{57} was similarly inverted to anion selective by exchanging a single glutamine residue for a lysine.\textsuperscript{58} Based on these protein and peptide channel models, the cation selectivity of AmB could be explained by the model outlined in Figure 2.4. Computational studies places the C41 carboxylic acid, one of AmB’s protonizable groups, at the entrance to the ion channel.\textsuperscript{37} At physiological pH this would create a ring of negatively charged carboxylates at the channel entrance. This electrostatically negative area could concentrate cations and repel anions, thereby explaining the observed potassium selectivity (Figure 2.4A). Following this logic, replacing the negatively charged carboxylate with a methyl group would abolish the ring of negative charges, thereby reducing the observed ion selectivity (Figure 2.4B). Alternatively, replacing the carboxylate with an amine would create a positively charged ring at physiological pH, potentially inverting the ion selectivity to chloride selective (Figure 2.4C). To test this hypothesis, both C41 methyl amphotericin B (C41MeAmB) and C41 aminomethyl amphotericin B (C41amAmB) were required.

![Diagram of AmB, C41MeAmB, and C41amAmB](image)

**Figure 2.4:** We hypothesize that AmB’s carboxylate plays a critical role in ion channel selectivity. Modification from A.) a carboxylate to B.) a methyl group to C.) an amine could cause a change in AmB’s ion selectivity.

Dan Palacios and Tom Anderson previously completed the synthesis of one of these derivatives, C41MeAmB (Figure 2.5).\textsuperscript{36} We thought we might be able to intercept an
intermediate on this pathway, methyl iodide 2.15, and divert this synthesis to access C41amAmB. Displacement of the primary iodide with a nitrogen nucleophile could install a nitrogen functionality at the desired position. A series of deprotections then could complete the synthesis of C41amAmB.

![Diagram](image)

**Figure 2.5:** Convergent synthetic strategy to access both C41MeAmB and C41amAmB from common intermediate 2.14.

**Scheme 2.6:** First generation synthesis of C41aminomethyl AmB

Following this synthetic strategy, iodide 2.15 was quickly generated (Scheme 2.6). The iodide then readily underwent displacement with sodium azide in DMF, generating azide 2.16. Perdesilylation of 2.16 was achieved upon exposure to HF-pyridine, followed by ketal hydrolysis under aqueous acidic conditions with CSA to generate azide 2.18. The final two remaining steps were removal of the two amine protecting groups. First, the azide was reduced under Staudinger conditions with tributylphosphine in a THF/MeOH/H2O mixture. Upon initial phosphine attack and azide reduction, the neighboring C-15 alcohol trapped the intermediate phosphorous ylide to yield bicycle 2.19. A number of trialkylphosphines were screened (trimethylphosphine, triethylphosphine, tributylphosphine, triphenylphosphine, trimethylphosphite) in search of conditions capable of avoiding this undesired byproduct. Ultimately this strategy proved
ineffective as a second major byproduct, C41 hydroxymethyl 2.20, resulting from displacement of the ylide with water formed.

Scheme 2.7: Alternative azide reduction strategy.

To overcome this problematic reaction, alternative azide reduction conditions were explored (Scheme 2.7). Hydrolysis of methyl ketal 2.17, followed by quench with excess piperidine neutralized the CSA while concomitantly removing the Fmoc group, generating azide 2.21. Propane dithiol mediated reduction of 2.21 was possible, but unfortunately upon amine formation, the product rapidly rearranged to a derivative such as 2.22. This destructive pathway is non-productive, as to test the ion selectivity hypothesis it is necessary for the nitrogen to remain exocyclic. Intriguingly, a group at Smith-Kline-Beechum similarly attempted to install nitrogen appended to the C41 position, but only acetylated amines and azides were reported as final compounds.

Figure 2.6: Retrosynthesis of C16aminoAmB from AmB highlighted by a Curtius rearrangement.

To circumvent this undesired rearrangement we targeted C16 amino AmB (Figure 2.11, C16amAmB) in which the nitrogen is moved in one atom. In this molecule, if a rearrangement were to occur, a five membered ring pyrrole would be formed instead of the six-membered pyridine. Because the AmB macrolactone is rigid, rearrangement to a pyrrole would potentially be higher in energy and consequently less likely to form. We aimed to achieve this synthesis via a degradative strategy in which AmB would be suitably protected such that the carboxylic acid was the only accessible functional group (2.25, Figure 2.11). A Curtius rearrangement would then generate an isocyanate intermediate such as 2.24, which could be converted to a protected amine (2.23). Subsequent deprotection would then generate C-16amAmB.
Scheme 2.8: Synthesis of AmB-isocyanate and carbamates

Exploration of this strategy is depicted in Scheme 2.8. Persilylated intermediate 2.26 was quickly generated following known conditions. Multiple pathways were then investigated to convert the free carboxylic acid into an acyl azide ready for Curtius rearrangement. Activating the carboxylic acid as either an acyl chloride or mixed anhydride proved inefficient. Alternatively, simply mixing 2.26 with Shioiri’s reagent, diphenyl phosphoryl azide (DPPA), at elevated temperatures in benzene cleanly converted 2.26 into desired isocyanate 2.27. We next attempted to convert isocyanate 2.27 into an easily deprotected form, such as Fmoc carbamate 2.28. Simply trapping 2.27 with Fmoc alcohol alone, or in the presence of Lewis acids, such as CuCl or Ti(OtBu)4, yielded very little product. Intriguingly, mixing 2.27 with titanium isopropoxide very cleanly transferred the isopropoxide ligand to the isocyanate, forming isopropyl carbamate 2.29. We hypothesized that steric bulk posed by the neighboring C-15 TES silyl ether was hindering formation of the desired product. To test this hypothesis we explored Curtius rearrangements on AmB derivatives lacking protecting groups at the C15 position.
To keep the C15 alcohol unprotected, a two-step procedure involving Fmoc protection of
the mycosamine amine followed by methyl ketal formation generated intermediate 2.30 (Scheme
2.9A). Exposure of 2.30 to DPPA at 50°C effected a Curtius rearrangement, presumably
generating intermediate isocyanate 2.31 which was trapped by the neighboring C-15 alcohol to
yield oxazolidinone 2.32. Katz and coworkers reported that 2-oxazolidinones can be mildly
deprotected up exposure to ethylene diamine, which undergoes cyclization to reveal the desired
free amine (Scheme 2.9B). 64 Upon exposure of 2.32 to ethylene diamine, the oxazolidinone
readily opened to aminoethyl urea 2.33, with concomitant Fmoc deprotection. Upon heating to
100°C for extended periods of time no cyclization to reveal amine 2.34 was observed. To take
advantage of the Thorpe-Ingold effect, 2.32 was exposed to 2,2-dimethyl-1,3-propanediamine
and converted into urea 2.35. Again, heating to 100°C did not result in any formation of free
amine 2.34. Alternatively, hydrolysis of oxazolidinone with hydroxide bases can directly reveal
free amines. 64 Hydrolysis of 2.32 with various hydroxide sources did provide the free amine, but
unfortunately competitively opened the macrolactone generating 2.36 (Scheme 2.9C).
In efforts to determine the minimum protecting groups required for Curtius rearrangement on AmB, I attempted a Curtius rearrangement on unprotected AmB (Scheme 2.10). Exposure of AmB to DPPA and Et₃N in DMF did effect a Curtius rearrangement, but unfortunately oxazolidinone 2.37 was highly unstable, and rapidly rearranged under the reaction conditions. Analysis of the resulting products revealed pyrrole formation was indeed occurring, accompanied with translactonization generating a mixture of C37 (2.38) and C35 (2.39) lactone pyrroles. This is now a second compound possessing a nitrogen appended to the C16 carbon that subsequently rearranged in the presence of a C13 hemiketal. An alternative strategy will likely be needed to evaluate the molecular basis of AmB’s ion selectivity.

2-4 SUMMARY

Expanding on the functional group deletion strategy, the C3 oxygen was selectively removed from the AmB framework via an efficient 9-step degradative synthesis. Extensive NMR analysis revealed that both AmB and C3deOAmB have very similar NOE patterns, suggesting that the overall macrolide conformation does not change upon removal of the C3 alcohol. C3deOAmB was still capable of ion channel formation, however its conductance was significantly reduced relative to the natural product, AmB. This is the first study to conclusively demonstrate that the polyol region impacts ion conductance, and supports the leading models that place the C3 alcohol at a critical point in the ion conductance pathway. This structure activity
relationship is the first step towards understanding the structure of the AmB ion channel. Furthermore, the demonstration that AmB’s ion channel conductance can be manipulated by modification of a polyol alcohol suggests that further polyol modifications could tune other channel properties, such as ion selectivity. This has important implications in the development of small molecule surrogates for missing or deficient protein ion channels.

Simultaneously as I was exploring the chemistry of the C16 acid in search of AmB derivatives capable of probing the molecular basis of AmB’s ion selectivity, our group made several important advances in understanding the molecular underpinnings of AmB’s antifungal function.\textsuperscript{31,32,65,66} It was discovered that an AmB derivative, C2’deOAmB, which selectively binds ergosterol (the primary yeast sterol), but not cholesterol (the primary human sterol) was selectively toxic to yeast but not human cells.\textsuperscript{65} Efforts to understand this selective sterol binding and improved therapeutic index led to the development of a new model for the rational synthesis of non-toxic AmB derivatives. The installation of a urea appended to the C-16 carbon was an intriguing new motif to probe this emerging model. The following chapter will describe efforts to test this model with AmB ureas in the search of a scalably accessible non-toxic yet resistance-evasive AmB derivative.

2-5 METHODS

Materials

Commercially available materials were purchased from Aldrich Chemical Co. (Milwaukee, WI), AKSci (Union City, CA), Fisher Scientific (Hampton, NH), Alfa Aesar (Ward Hill, MA), TCI America (Portland, OR), Oakwood Chemical (Wes Columbia, SC), and Silicycle (Quebec, Canada) and used without further purification unless noted otherwise. Solvents were purified via passage through packed columns as described by Pangborn and coworkers (THF, Et\textsubscript{2}O, CH\textsubscript{3}CN, CH\textsubscript{2}Cl\textsubscript{2}: dry neutral alumina; hexane, benzene, and toluene, dry neutral alumina and Q5 reactant; DMSO, DMF: activated molecular sieves).\textsuperscript{67} Triethylamine, pyridine, 2,6-lutidine, diisopropylamine, and piperidine were freshly distilled under nitrogen from CaH\textsubscript{2}. Camphorsulfonic acid was recrystallized from ethanol. Water was doubly distilled or obtained from a Millipore (Billerica, MA) MilliQ water purification system.
General Experimental Procedures

Due to the light and air sensitivity of amphotericin B (AmB), all manipulations were carried out under low light conditions and compounds were stored under an anaerobic atmosphere. All reactions were performed in oven- or flame-dried glassware under an atmosphere of argon unless otherwise indicated. Reactions were monitored by either analytical thin layer chromatography (TLC) or RP-HPLC. TLC was performed using the indicated solvent on E. Merck silica gel 60 F254 plates (0.25mm). Compounds were visualized by exposure to a UV lamp ($\lambda = 254$ nm), a glass chamber containing KMnO4, or an acidic solution of p-anisaldehyde, followed by brief heating using a Varitemp heat gun. Monitoring via RP-HPLC was performed using an Agilent 1200 Series HPLC system equipped with an Agilent Zorbax Eclipse C$_{18}$ 3.5 µm, 4.6 x 75 mm column or Waters Sunfire Eclipse C$_{18}$ 5 µm, 6x150 mm column with UV detection at 383 nm at 1.2 mL/min or an Agilent 6230 ESI TOF LC/MS system equipped with an Agilent Zorbax Eclipse C$_{18}$ 1.8-µm, 2.1 x 50 mm column with UV detection at 383 nm at 0.4 mL/min. Organic solutions were concentrated via rotary evaporation under reduced pressure with a bath temperature of 40° C.

Compound Purification

Column chromatography was performed using standard methods. Purification of late stage and highly polar AmB derivatives was carried out by preparative HPLC purification using either an agilent 1100 or 1200 series instrument equipped with a Waters SunFire Prep C$_{18}$ OBD 5-µm, 30 x 150 mm column at 25 mL/min.

Structural analysis.

$^1$H NMR spectra were recorded at room temperature on a Varian Unity 500, Varian Unity Inova Narrow Bore spectrometer operating at a $^1$H frequency of 500 MHz with a Varian 5 mm $^1$H$^{13}$C$/^{15}$N} pulsed-field gradient Z probe or an Agilent VNMRS 750 MHz spectrometer with a Varian 5mm indirect-detection probe $^1$H$^{13}$C$/^{15}$N} probe equipped with X,Y,Z-field gradient capability. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethysilane and referenced to residual protium in the NMR solvent (CHCl$_3$, δ = 7.26; CD$_2$HCN, δ = 1.94, center line; acetone- d$_6$, δ = 2.05, center line) or to added tetramethysilane (δ = 0.00). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, b = broad, app = apparent), coupling constant ($J$) in Hertz (Hz) and integration. $^{13}$C spectra were recorded at room temperature with a Varian
Unity Inova spectrometer operating at a $^{13}$C frequency of 125 MHz with a 5 mm Nalorac gradient $^{13}$C/$^{15}$N$^1$H quad probe or a Varian Unity Inova spectrometer operating at a $^{13}$C frequency of 150 MHz and equipped with a Varian 5 mm 600 DB Auto X X probe. Chemical shifts (δ) are reported downfield of added tetramethylsilane (δ = 0.00) or are referenced to the carbon resonances in the NMR solvent ((CD$_3$)$_2$SO, δ = 39.52, center line). MS analysis was performed with an Applied Biosystems Micromass Ultima system with ESI ionization. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All synthesized compounds gave HRMS within 5 ppm of the calculated values.

**Compound synthesis**

**Bis-paramethoxy acetal 2.3**

Mixed Anhydride Formation: All manipulations to prepare 2.3a were performed in triplicate. To a 200 mL Schlenk flask was added phenylacetic acid (670 mg, 4.92 mmol, 1.51 eq), THF (35 mL), trimethyl acetyl chloride (400 µL, 3.25 mmol, 1 eq), and lastly triethylamine (900 µL, 6.46 mmol, 2 eq) at room temperature. A fine white solid immediately began precipitating. The reaction was allowed to stir for 12 hours at room temperature and carried forward into the following reaction without purification.

The crude mixed anhydride solution was cooled to 0°C and then DMSO (30 mL) was added. After stirring for 10 minutes AmB (1.70 g, 1.84 mmol) was added, and the reaction stirred at 0°C for 2 hours. The reaction was then poured into 1.8 L diethyl ether and allowed to stir for 30 minutes. Then the yellow suspension was filtered through Whatman #50 filter paper (making sure filter cake is not allowed to dry until filtration completion). The filter cake was washed with ether, and dried on the Buchner funnel for 5 minutes. The semi crude product was then transferred to a flame dried 200 mL round bottom flask dried in vacuo for 1 hour yielding an orange solid (2.66 g, 138% crude yield).

To the crude dried solid was added THF (30 mL) and MeOH (35 mL). The red solution was cooled to 0°C, and then CSA (125 mg, 0.538 mmol, 0.29 eq) was added in a single portion. The reaction was then allowed to stir at 0°C for 1.5 hours. Triethylamine (0.100 mL) was then
added to quench the CSA. The reaction was then poured into a stirring solution of 1.8 L 2:1 hexanes:diethyl ether. After stirring for ten minutes the yellow suspension was passed over Whatman #50 filter paper. The filter cake was washed twice with diethyl ether, and then dried for 5 minutes before transfer to a flame dried 200 mL round bottom flask and dried in vacuo 1 hour yielding a crude red/brown solid (2.09 g, 108% crude yield).

The crude solid was added MeOH (40 mL), THF (14 mL), and CSA (200 mg, 0.860 mmol). Then para-anisaldehyde dimethylacetal (6 mL, 35.2 mmol) was added and the reaction stirred at room temperature for 40 minutes. Triethylamine (0.20 mL) was then added to quench the CSA. The volatiles were then removed via rotovap and the crude oil was purified by SiO₂ chromatography (95:5 → 92.5:7.5 → 90:10 DCM:MeOH with 0.1% acetic acid) yielding 2.3a (5.96 g, 4.61 mmol, 84% yield over 3 steps).

\[
\begin{align*}
\[\text{2.3a}\]
\end{align*}
\]

\(^{1}\)H NMR (500 MHz, Acetone-\(d_6\))

\(\delta\) 7.43 (d, \(J = 8.5 \text{ Hz}, 2\text{H})\), 7.40 – 7.34 (m, 4H), 7.33 – 7.25 (m, 2H), 7.26 – 7.18 (m, 1H), 6.87 (app dd, \(J = 8.7, 3.2 \text{ Hz}, 4\text{H})\), 6.49 – 6.16 (m, 12H), 5.88 (dd, \(J = 14.6, 5.3 \text{ Hz}, 1\text{H})\), 5.57 (dd, \(J = 14.3, 9.9 \text{ Hz}, 1\text{H})\), 5.52 (s, 1H), 5.46 (s, 1H), 5.32 – 5.23 (m, 1H), 4.68 (app t, \(J = 5.8 \text{ Hz}, 1\text{H})\), 4.61 (s, 1H), 4.23 – 4.08 (m, 4H), 3.99 – 3.89 (m, 4H), 3.79 (app d, \(J = 3.7 \text{ Hz}, 6\text{H})\), 3.76 – 3.69 (m, 1H), 3.65 (app d, \(J = 1.9 \text{ Hz}, 2\text{H})\), 3.48 – 3.40 (m, 2H), 3.35 (app d, \(J = 10.2 \text{ Hz}, 1\text{H})\), 3.31 (s, 3H), 2.64 (d, \(J = 14.7 \text{ Hz}, 1\text{H})\), 2.58 (dd, \(J = 16.4, 6.0 \text{ Hz}, 1\text{H})\), 2.45 – 2.35 (m, 3H), 2.34 – 2.28 (m, 2H), 2.03 – 1.98 (m, 1H), 1.89 (app d, \(J = 15.1 \text{ Hz}, 1\text{H})\), 1.86 – 1.84 (m, 1H), 1.84 – 1.79 (m, 1H), 1.79 – 1.68 (m, 3H), 1.56 (app d, \(J = 12.8 \text{ Hz}, 1\text{H})\), 1.48 (qd, \(J = 10.7, 9.2, 6.1 \text{ Hz}, 3\text{H})\), 1.21 (d, \(J = 5.8 \text{ Hz}, 3\text{H})\), 1.19 (d, \(J = 6.4 \text{ Hz}, 3\text{H})\), 1.11 (d, \(J = 6.5 \text{ Hz}, 3\text{H})\), 1.02 (d, \(J = 7.2 \text{ Hz}, 3\text{H})\).
Carboxylic Acid 2.3b

2.3a (5.96 g, 4.61 mmol, 1 eq) was added to a 200 mL round bottom flask and azeotropically dried with PhMe. Then DCM (60 mL) was added and the solution cooled to 0°C. 2,6 lutidine (8.05 mL, 69.2 mmol, 15 eq) was added followed by TESOTf (12.5 mL, 55.3 mmol, 12 eq) slowly over 5 minutes directly into the center of the stirring solution. The reaction was then allowed to stir at 0°C for 1.5 hours before NaHCO$_3$ (sat aq., 150 mL) was added and the reaction mixture transferred to a separatory funnel with 400 mL diethyl ether. The layers were separated and the aqueous layer was extracted with ether (1x400mL). The combined organic phase was washed with CuSO$_4$ (3x333mL), water (1x333mL), brine (333mL), dried over Na$_2$SO$_4$, filtered, and concentrated. The crude yellow oil was purified by SiO$_2$ chromatography (9:1 → 4:1 → 7:3 hexanes:EtOAc with 1% AcOH) isolating 2.3b as a yellow solid (2.15 g, 2.25 mmol, 25% yield)

TLC (hexanes : EtOAc 1:1)

$R_f = 0.80$, visualized by UV and stained by p-anisaldehyde

$^1$H NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.44 – 7.38 (m, 2H), 7.38 – 7.33 (m, 4H), 7.23 (t, $J = 7.6$ Hz, 2H), 7.17 (d, $J = 7.5$ Hz, 1H), 6.92 – 6.83 (m, 4H), 6.50 – 6.18 (m, 11H), 6.11 (dd, $J = 15.0$, 9.8 Hz, 1H), 5.82 (dd, $J = 15.0$, 6.0 Hz, 1H), 5.64 (dd, $J = 14.9$, 9.4 Hz, 1H), 5.45 (s, 2H), 5.00 – 4.89 (m, 1H), 4.67 (app t, $J = 6.5$ Hz, 1H), 4.53 (s, 1H), 4.27 (app td, $J = 10.7$, 4.7 Hz, 1H), 4.24 – 4.15 (m, 1H), 3.97 – 3.87 (m, 4H), 3.82 (app d, $J = 2.8$ Hz, 1H), 3.79 (app d, $J = 5.6$ Hz, 7H), 3.74 – 3.69 (m, 2H), 3.62 (app d, $J = 7.6$ Hz, 1H), 3.50 (app t, $J = 9.0$ Hz, 1H), 3.36 – 3.29 (m, 1H), 3.06 (s, 3H), 2.53 (dd, $J = 17.3$, 7.3 Hz, 1H), 2.46 – 2.36 (m, 2H), 2.30 – 2.21 (m, 3H), 2.20 – 2.16 (m, 1H)
2.08 (m, 2H), 1.97 – 1.82 (m, 4H), 1.66 (dd, \( J = 15.9, 10.0 \) Hz, 1H), 1.61 (d, \( J = 14.0 \) Hz, 1H), 1.48 – 1.39 (m, 2H), 1.30 – 1.27 m, 1H), 1.23 (d, \( J = 6.2 \) Hz, 3H), 1.19 (d, \( J = 6.1 \) Hz, 3H), 1.05 – 0.79 (m, 51H), 0.70 – 0.39 (m, 30H).

HRMS (ESI)
Calculated for C_{102}H_{164}NO_{20}Si_{5} (M + Na\(^{+}\)): 1885.0512
Found: 1885.0509

**TMSE Ester 2.5**

A flame dried 200mL round bottom flask was charged with 2.3b (2.1586 g, 1.16 mmol, 1 eq) triphenylphosphine (768.9 mg, 2.93 mmol, 2.53 eq), and 2-trimethylsilyl ethanol (0.496 mL, 3.48 mmol, 3 eq). THF (53.4 mL, 0.217 M) was then added and the solution was cooled to 0°C. Diisopropyl azodicarboxylate (0.504 mL, 2.56 mmol, 2.2 eq) was then dropwise added to the stirring solution. The reaction was then placed in a preheated oil bath at 45°C and allowed to stir for 2 hours. The reaction was then cooled to room temperature and the volatiles were removed via rotovap. Hexanes (50mL) then was added, and the resulting suspension was filtered through a Buchner funnel. The filtrate was collected, concentrated via rotovap, and purified by SiO\(_2\) chromatography (19:1 → 9:1 → 4:1 hexanes:EtOAc) isolating 2.5 as a yellow solid (1.3829 g, 0.704 mmol, 61% yield)

TLC (hexanes : EtOAc 4:1)

\( R_f = 0.56 \), visualized by UV and stained by \( \rho \)-anisaldehyde

\(^{1}\)H NMR (500 MHz, acetone-\( d_6 \))
δ 7.45 – 7.23 (m, 9H), 6.92 – 6.84 (m, 4H), 6.48 – 6.18 (m, 11H), 6.11 (dd, J = 14.8, 10.2 Hz, 1H), 5.83 (dd, J = 15.4, 6.1 Hz, 1H), 5.65 (dd, J = 15.0, 9.2 Hz, 1H), 5.45 (app d, J = 3.3 Hz, 2H), 4.99 – 4.90 (m, 1H), 4.63 (app t, J = 5.9 Hz, 1H), 4.52 (s, 1H), 4.21 (app dt, J = 13.1, 7.5 Hz, 5H), 4.04 – 3.96 (m, 1H), 3.96 – 3.88 (m, 1H), 3.89 – 3.82 (m, 1H), 3.79 (app d, J = 5.3 Hz, 6H), 3.73 – 3.69 (m, 2H), 3.60 (app d, J = 6.1 Hz, 2H), 3.49 (app t, J = 8.9 Hz, 1H), 3.39 – 3.27 (m, 1H), 3.07 (s, 3H), 2.53 (dd, J = 17.4, 7.1 Hz, 1H), 2.46 – 2.36 (m, 1H), 2.30 (dd, J = 16.8, 5.3 Hz, 2H), 2.27 – 2.23 (m, 1H), 1.96 – 1.42 (m, 14H), 1.23 (d, J = 6.1 Hz, 3H), 1.18 (d, J = 6.2 Hz, 3H), 1.08 – 0.78 (m, 53H), 0.70 – 0.38 (m, 30H), 0.08 (s, 9H).

HRMS (ESI)
Calculated for C₁₀₇H₁₇₅NO₂₀Si₆ (M + Na⁻): 1985.1221
Found: 1985.1229

Enone 2.6

In the glovebox, two flame dried 1.5 mL vials were charged with KHMDS (1.5 mg, 7.63 µmol, 3 eq) followed by THF (0.125 mL). The vial was sealed with a septa cap, removed from the glovebox and cooled to 0°C.

Prior to reaction 2.5 (10.6 mg) was azeotropically dried by co-evaporation with toluene (2x100 µL) in a 7mL vial. THF (260 µL) was then added and the solution was cooled to 0°C. Next, 125 µL of the 2.5 THF stock solution was added to each of the KHMDS solutions, immediately changing colors to dark red. Then one vial stirred at 0°C for 2 hours, while the other vial was warmed to room temperature and stirred for 2 hours. The reactions were then combined and passed through a SiO₂ plug with EtOAc as the eluent. The crude solid was then purified by SiO₂ chromatography (19:1 → 9:1 → 17:3 → 4:1 hexanes:EtOAc) isolating 2.6 as a yellow solid (5.7 mg, 3.12 µmol, 61% yield).
TLC (hexanes : EtOAc 4:1)

R<sub>f</sub> = 0.30, visualized by UV and stained by ρ-anisaldehyde

<sup>1</sup>H NMR (500 MHz, acetone-<em>d<sub>6</sub></em>)

δ 7.42 – 7.37 (m, 2H), 7.37 – 7.33 (m, 4H), 7.30 – 7.25 (m, 1H), 7.15 – 7.08 (m, 2H), 6.98 (app dt, J = 14.9, 7.2 Hz, 1H), 6.42 – 6.26 (m, 10H), 6.24 – 6.09 (m, 2H), 5.87 (dd, J = 14.4, 7.2 Hz, 1H), 5.80 (d, J = 15.6 Hz, 1H), 5.60 (dd, J = 14.1, 9.2 Hz, 1H), 5.47 (s, 1H), 5.15 – 5.06 (m, 1H), 4.61 (app t, J = 8.2 Hz, 1H), 4.53 (s, 1H), 4.29 – 4.16 (m, 4H), 4.00 (app td, J = 9.4, 2.8 Hz, 1H), 3.92 (s, 3H), 3.91 – 3.85 (m, 2H), 3.85 – 3.83 (m, 1H), 3.72 – 3.66 (m, 1H), 3.66 – 3.63 (m, 2H), 3.61 (app d, J = 6.5 Hz, 2H), 3.49 (app t, J = 8.9 Hz, 1H), 3.34 – 3.28 (m, 1H), 3.12 (s, 3H), 2.47 – 2.40 (m, 2H), 2.31 – 2.21 (m, 3H), 2.02 – 1.91 (m, 2H), 1.87 – 1.80 (m, 1H), 1.67 (app dd, J = 15.5, 9.4 Hz, 1H), 1.62 – 1.42 (m, 4H), 1.38 – 1.25 (m, 5H), 1.23 (d, J = 6.0 Hz, 3H), 1.20 (d, J = 6.1 Hz, 3H), 1.06 (d, J = 6.5 Hz, 3H), 1.02 – 0.78 (m, 50H), 0.71 – 0.38 (m, 30H), 0.08 (s, 9H).

HRMS (ESI)

Calculated for C<sub>99</sub>H<sub>167</sub>N<sub>18</sub>O<sub>18</sub>Si<sub>6</sub> (M + Na<sup>+</sup>): 1849.0696

Found: 1849.0699

**Macrolactone 2.7**

A flame dried 1.5 mL vial was brought into the glovebox and charged with Stryker’s reagent (1.8 mg, 0.917 µmol, 0.34 eq). The vial was then sealed with a septa cap and removed from the glovebox. In a separate 1.5 mL vial, 2.6 (5.0 mg, 2.73 µmol, 1 eq) was dissolved in toluene (225 µL) and then added to vial containing Stryker’s reagent followed by a single drop of water. The
reaction was allowed to stir at room temperature for 2 hours. The vial cap was removed and hexanes (0.5 mL) was added. The reaction then stirred open to air for 30 minutes. The reaction was then passed through a SiO₂ plug of celite using EtOAc as the eluent. The filtrate was concentrated and purified by SiO₂ chromatography (19:1 → 9:1 → 17:3 → 4:1 hexanes:EtOAc) isolating 2.7 as a yellow solid (3.5 mg, 1.91 µmol, 70% yield).

TLC (hexanes : EtOAc 4:1)

\[ R_f = 0.38, \text{ visualized by UV and stained by } \rho\text{-anisaldehyde} \]

\(^1\)H NMR (500 MHz, acetone-\(d_6\))

\[
\delta 7.49 – 7.31 (m, 6H), 7.28 (d, J = 6.1 Hz, 1H), 6.90 (d, J = 8.5 Hz, 2H), 6.45 – 6.07 (m, 12H), 5.87 (dd, J = 14.1, 7.5 Hz, 1H), 5.61 (dd, J = 14.6, 9.3 Hz, 1H), 5.47 (s, 1H), 5.01 – 4.93 (m, 1H), 4.62 (app t, J = 6.5 Hz, 1H), 4.52 (s, 1H), 4.29 – 4.14 (m, 3H), 4.00 (app td, J = 9.1, 2.5 Hz, 1H), 3.95 – 3.87 (m, 2H), 3.87 – 3.83 (m, 1H), 3.79 (s, 3H), 3.79 – 3.67 (m, 3H), 3.60 (d, J = 6.3 Hz, 2H), 3.49 (app t, J = 8.9 Hz, 1H), 3.45 – 3.39 (m, 1H), 3.33 – 3.28 (m, 1H), 3.25 (app d, J = 5.6 Hz, 1H), 3.12 (s, 3H), 2.42 (s, 1H), 2.31 – 2.22 (m, 2H), 2.00 – 1.57 (m, 11H), 1.54 – 1.33 (m, 6H), 1.23 (d, J = 6.1 Hz, 3H), 1.16 (d, J = 6.2 Hz, 3H), 1.08 – 0.78 (m, 53H), 0.71 – 0.38 (m, 30H), 0.08 (s, 9H).

HRMS (ESI)

Calculated for \(C_{99}H_{169}NO_{18}Si_6\) (M + Na\(^+\)): 1851.0853

Found: 1851.0835
Alloc carbamate 2.8a

A 250 mL round bottom flask was charged with Amphotericin B (1 eq, 1.56 g, 1.69 mmol, ~60% pure), DMF (60 mL), MeOH (30 mL) and alloc-succinimide (0.505 g, 2.54 mmol, 1.5 eq). Pyridine (0.82 mL, 10.1 mmol, 6 eq) was then added and the reaction was allowed to stir at room temperature for 14 hours, gradually turning clear red. The reaction was then poured into 1.8 L diethyl ether and allowed to stir for 30 minutes. Then the yellow suspension was filtered through Whatman #50 filter paper (making sure filter cake was not allowed to dry until filtration completion). The filter cake was washed with ether, and dried on the Buchner funnel for 5 minutes. The semi crude product was then transferred to a flame dried round bottom flask and left under vacuum for 1 hour isolating 2.8a as an red/brown solid (1.90 g, 1.88 mmol, 111% crude yield).

HRMS (ESI)

Calculated for C_{51}H_{77}NO_{19} (M + Na^+): 1030.4987

Found: 1030.4993

Methyl ketal 2.8b

A 250mL round bottom flask was charged with 2.8a (1.90 g crude, 1.69 mmol, 1eq), THF (30 mL) and MeOH (30 mL). The solution was cooled to 0°C and CSA (250 mg, 1.08 mmol, 0.64 eq) was added in a single portion and the reaction was allowed to stir at 0°C for 1 hour. Triethylamine (250 µL) was added then the reaction concentrated by ~1/2 on the rotovap. The concentrated solution was then poured into 1.8 L of a stirring solution of hexanes:ether 2:1. After stirring for ten minutes the yellow suspension was filtered through Whatman #50 filter paper. The filter cake was washed twice with ether, and then dried for 5 minutes before transfer.
to round bottom flask to dry under vacuum for 1 hour isolating 2.8b as a red/brown solid (1.54 g, 1.50 mmol, 89% 2-step crude yield).

HRMS (ESI)
Calculated for C$_{52}$H$_{79}$NO$_{19}$ (M + Na$^+$): 1044.5144
Found: 1044.5146

Bis-PMP ketal 2.8c
An oven dried 250mL round bottom flask was charged with 2.8b (4.63 g crude, 4.53 mmol, 1 eq). The solid substrate was then crushed by rapidly stirring with a mechanical stir bar before MeOH (40 mL) and THF (10 mL) were added. Then, anisaldehyde dimethyl acetal (2.30 mL, 13.59 mmol, 3 eq) was added followed by CSA (527 mg, 2.27 mmol, 0.50 eq). The reaction stirred at room temperature for 20 minutes before triethyl amine (650 µL) was added. The reaction was then concentrated on rotovap and purified by SiO$_2$ chromatography (19:1 → 18.5:1.5 → 9:1 DCM:MeOH with 0.1%AcOH) isolating 2.8c as a yellow solid (1.75 g, 1.40 mmol, 30.7 % over 3 steps)

TLC (DCM : MeOH 9:1, with .1%AcOH)
$R_f$ = 0.29, visualized by UV and stained by ρ-anisaldehyde
\[ ^1\text{H NMR} (500 \text{ MHz, Acetone-}d_6) \]

\[ \delta 7.46 – 7.31 (m, 4H), 6.90 – 6.85 (m, 4H), 6.49 – 6.17 (m, 12H), 6.03 (\text{app d, } J = 8.8 \text{ Hz, 1H}), 5.99 – 5.86 (m, 3H), 5.61 – 5.54 (m, 1H), 5.53 (s, 1H), 5.46 (s, 1H), 5.34 – 5.28 (m, 1H), 5.28 – 5.25 (m, 1H), 5.18 – 5.13 (m, 1H), 4.70 (\text{app t, } J = 5.8 \text{ Hz, 1H}), 4.64 (s, 1H), 4.57 – 4.45 (m, 3H), 4.23 – 4.14 (m, 1H), 3.99 – 3.90 (m, 3H), 3.79 (\text{app d, } J = 3.2 \text{ Hz, 6H}), 3.76 – 3.68 (m, 1H), 3.63 – 3.56 (m, 1H), 3.47 – 3.42 (m, 1H), 3.38 – 3.29 (m, 2H), 3.07 (s, 3H), 2.58 (dd, \text{ } J = 16.2, 5.7 \text{ Hz, 1H}), 2.46 – 2.35 (m, 1H), 2.36 – 2.28 (m, 2H), 2.28 – 2.18 (m, 1H), 2.19 – 2.07 (m, 1H), 1.94 – 1.80 (m, 5H), 1.80 – 1.70 (m, 2H), 1.70 – 1.60 (m, 2H), 1.57 (\text{app dt, } J = 12.9, 2.3 \text{ Hz, 1H}), 1.55 – 1.41 (m, 2H), 1.39 – 1.33 (m, 1H), 1.24 (\text{d, } J = 5.2 \text{ Hz, 3H}), 1.19 (\text{d, } J = 5.3 \text{ Hz, 3H}), 1.11 (\text{d, } J = 6.5 \text{ Hz, 3H}), 1.02 (\text{d, } J = 6.9 \text{ Hz, 3H}). \]

\[ ^{13}\text{C NMR} (125 \text{ MHz, acetone-}d_6) \]

\[ \delta 174.46, 169.87, 160.78, 160.69, 157.41, 137.70, 136.45, 134.65, 134.55, 134.28 (2), 134.19 (2), 134.08, 133.90, 133.80, 133.73, 133.13, 132.85, 132.78, 133.45, 130.05, 128.48, 128.43, 117.25, 114.03, 101.27, 100.98, 100.80, 98.18, 94.99, 81.34, 76.99, 76.34, 74.74, 74.35, 73.40, 73.10, 71.14, 70.69, 69.38, 67.41, 67.13, 65.80, 65.67, 57.97, 57.20, 55.04, 48.75, 43.66, 43.60, 41.96, 41.72, 38.08, 37.03, 34.09, 33.37, 18.88, 18.51, 18.40, 17.63, 12.03 \]

HRMS (ESI)

Calculated for $C_{68}H_{91}NO_{21} (M + Na^+)$: 1280.5981

Found: 1280.5983

**Pentasilyl Ether 2.8d**

A 250 mL round bottom flask was charged with $2.8c$ (1.0383 g, 0.825 mmol, 1 eq), imidazole (845 mg, 12.375 mmol, 15 eq), and DCM (41 mL). The reaction for 5 minutes and then TESCl (1.05 mL, 6.189 mmol, 7.5 eq), was added and the reaction stirred for 1 hour. Then additional imidazole (280 mg) and TESCl (350 µL) were added and the reaction stirred an additional 1
hour. The reaction was then transferred to a 250 mL separatory funnel with 75 mL ether and 50 mL sat. aq. NH₄Cl. The layers were separated and the aqueous layer was extracted with ether (50mL). The combined organic phase was washed with sat. aq. NaHCO₃ (50 mL), water (50mL), brine (50mL), dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude yellow oil was the purified by SiO₂ chromatography (9:1 → 4:1 → 7:3 hexanes:EtOAc with 0.1% AcOH) yielding **2.8d** as a yellow solid (1.0696 g, 0.585 mmol, 71% yield).

TLC (hexanes : EtOAc 7:3, with .1% AcOH)  
Rₓ = .70, visualized by UV and stained by ρ-anisaldehyde

**¹H NMR (500 MHz, Acetone-d₆)**

δ 7.43 – 7.33 (m, 4H), 6.92 – 6.83 (m, 4H), 6.48 – 6.17 (m, 11H), 6.11 (dd, J = 15.0, 10.0 Hz, 1H), 5.95 (app ddd, J = 22.8, 10.7, 5.5 Hz, 1H), 5.85 (dd, J = 14.9, 5.9 Hz, 1H), 5.65 (dd, J = 15.0, 9.3 Hz, 1H), 5.62 (s, 1H), 5.45 (s, 1H), 5.38 (bd, J = 9.8 Hz, 1H), 5.32 (app dq, J = 17.2, 1.7 Hz, 1H), 5.19 (app dq, J = 10.4, 1.5 Hz, 1H), 4.99 – 4.91 (m, 1H), 4.70 (app t, J = 6.6 Hz, 1H), 4.61 – 4.50 (m, 3H), 4.26 (app td, J = 10.6, 4.6 Hz, 1H), 4.22 - 4.16 (m, 1H), 3.97 – 3.87 (m, 4H), 3.79 (d, J = 5.8 Hz, 8H), 3.75 – 3.68 (m, 2H), 3.59 (app td, J = 9.7, 3.0 Hz, 1H), 3.47 (app t, J = 9.0 Hz, 1H), 3.35 – 3.29 (m, 1H), 3.08 (s, 3H), 2.53 (dd, J = 17.4, 7.2 Hz, 1H), 2.46 – 2.37 (m, 1H), 2.33 – 2.24 (m, 2H), 2.14 – 2.07 (m, 1H), 1.97 – 1.5 (m, 2H), 1.78 – 1.53 (m, 5H), 1.52 – 1.40 (m, 3H), 1.37 – 1.27 (m, 2H), 1.24 (d, J = 6.3 Hz, 3H), 1.19 (d, J = 6.1 Hz, 3H), 1.04 (d, J = 6.8 Hz, 3H), 1.01 – 0.78 (m, 48H), 0.75 – 0.88 (m, 30H).

**¹³C NMR (126 MHz, acetone-d6)**

δ 174.07, 169.99, 160.68, 156.32, 136.44, 134.68, 134.61, 134.41, 134.37, 134.22, 133.65, 133.57, 133.40, 133.14, 132.90, 132.75, 132.66, 131.43, 131.18, 128.68, 128.36, 117.36, 113.95, 113.86, 101.74, 101.13, 100.89, 98.19, 81.64, 76.20, 75.20, 74.54, 73.94, 73.31, 73.16,
HRMS (ESI)

Calculated for C\textsubscript{98}H\textsubscript{161}NO\textsubscript{21}Si\textsubscript{5} (M + Na\textsuperscript{+}): 1851.0305

Found: 1851.0310

**TMSE Ester 2.8**

A flame dried 100mL round bottom flask was charged with 2.8\textsubscript{d} (2.3925 g, 1.307 mmol, 1 eq) and triphenylphosphine (858 mg, 3.269 mmol, 2.5 eq). THF (65.5 mL) was then added and the solution was cooled to 0°C. After 5 minutes trimethylsilyl ethanol (560 µL, 3.923 mmol, 3 eq) was added followed by dropwise addition of DIAD (565 µL, 2.877 mmol, 2.2 eq). The reaction was then transferred to a preheated oil bath at 45°C and allowed to stir for 2 hours. The reaction was then cooled to room temperature and the volatiles were removed via rotovap. Hexanes (100mL) was added and the resulting suspension was filtered through a Buchner funnel. The filtrate was collected, concentrated via rotovap, and purified by SiO\textsubscript{2} chromatography (1:0 → 19:1 → 9:1 → 4:1 hexanes:EtOAc) isolating 2.8 as a yellow solid (2.1916 g, 1.14 mmol, 87% yield)

TLC (hexanes : EtOAc 4:1)

\[ R_f = 0.67, \text{ visualized by UV and stained by } \rho-\text{anisaldehyde} \]

\(^1\text{H NMR (500 MHz, acetone-}d_6\text{)}\)
δ 7.44 – 7.32 (m, 4H), 6.92 – 6.83 (m, 4H), 6.48 – 6.17 (m, 11H), 6.12 (dd, J = 15.0, 10.0 Hz, 1H), 6.01 – 5.89 (m, 1H), 5.86 (dd, J = 14.5, 6.0 Hz, 1H), 5.66 (dd, J = 14.9, 9.2 Hz, 1H), 5.45 (app d, J = 2.0 Hz, 2H), 5.36 (bd, J = 9.9 Hz, 1H), 5.35 – 5.29 (m, 1H), 5.18 (app dq, J = 10.5, 1.4 Hz, 1H), 4.99 – 4.90 (m, 1H), 4.65 (app t, J = 7.1 Hz, 1H), 4.58 – 4.51 (m, 3H), 4.29 – 4.16 (m, 5H), 3.95 – 3.85 (m, 2H), 3.81 – 3.71 (m, 2H), 3.79 (s, 3H), 3.78 (s, 3H), 3.72 (app d, J = 6.4 Hz, 2H), 3.68 – 3.61 (m, 1H), 3.48 (app t, J = 9.0 Hz, 1H), 3.34 – 3.28 (m, 1H), 3.08 (s, 3H), 2.59 – 2.49 (m, 2H), 2.45 – 2.37 (m, 1H), 2.33 – 2.23 (m, 3H), 2.19 – 2.16 (m, 1H), 1.96 – 1.90 (m, 1H), 1.90 – 1.82 (m, 2H), 1.79 – 1.69 (m, 2H), 1.70 – 1.52 (m, 3H), 1.53 – 1.40 (m, 3H), 1.37 – 1.27 (m, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H), 1.07 – 0.78 (m, 51H), 0.75 – 0.38 (m, 30H), 0.09 (s, 9H).

$^{13}$C NMR (126 MHz, acetone-$d_6$)

δ 173.18, 169.88, 160.72, 160.59, 156.10, 138.65, 136.65, 134.64, 134.59, 134.36, 134.32, 134.23, 133.66, 133.57, 133.54, 133.32, 133.08, 132.73, 132.66, 132.56, 131.42, 130.84, 128.62, 128.29, 117.32, 113.88, 113.80, 101.74, 101.10, 101.06, 100.80, 98.54, 81.58, 76.08, 75.54, 75.07, 74.67, 73.75, 73.10, 72.85, 71.75, 68.77, 67.68, 65.72, 63.23, 58.32, 57.27, 55.46, 48.39, 44.00, 43.22, 41.31, 37.92, 36.53, 33.52, 32.91, 28.18, 19.04, 18.56, 18.37, 18.32, 11.31, 7.46, 7.38, 7.35, 7.28, 7.20, 7.08, 5.92, 5.89, 5.83, 5.77, 5.53, -1.38.

HRMS (ESI)

Calculated for $C_{103}H_{173}NO_{21}Si_6$ (M + Na$^+$): 1951.1014

Found: 1951.1022

Enone 2.9
Reaction was performed in 4x. In the glovebox, a flame dried 20mL vial was charged with NaHMDS (56.8 mg, 309.9 mmol, 3 eq). The vial was sealed with a septa cap, removed from the glovebox. Then THF (5.17 mL) was was added and the solution was cooled to -78°C.

To a separate 40 mL ichem vial was added 2.8 (199.3 mg, 103.3 mmol, 1 eq) was azeotropically dried by coevaporation with toluene (3x200µL) in a 7mL vial. THF (5.17 mL) was then added and the solution was cooled to -78°C. Next, the NaHMDS stock solution was cannula transferred to the stirring substrate solution over 10 minutes. The reaction was then warmed to 0°C and allowed to stir for 2 hours. 5 mL sat. aq. NH₄Cl was added and the reactions were combined in a separatory funnel with NH₄Cl (300 mL) and ether (300 mL). The layers were separated and the organic layer was washed with water (300 mL) and brine (300 mL). The aqueous layer was then back extracted with ether (3x100mL). The combined organic phase was then dried over Na₂SO₄, filtered, concentrated, and purified by SiO₂ chromatograph (19:1 → 9:1 → 17:3 → 4:1 hexanes:EtOAc) isolating 2.9 as a yellow solid (327.9 mg, 0.183 µmol, 44% yield).

TLC (hexanes : EtOAc 4:1)

R_f = 0.31, visualized by UV and stained by ρ-anisaldehyde

¹H NMR (500 MHz, acetone-d₆)

δ 7.42 – 7.37 (m, 2H), 6.97 (app dt, J = 15.7, 7.2 Hz, 1H), 6.91 – 6.87 (m, 2H), 6.44 – 6.26 (m, 9H), 6.24 – 6.09 (m, 3H), 6.00 – 5.93 (m, 1H), 5.90 (dd, J = 14.4, 6.6 Hz, 1H), 5.79 (app dd, J = 15.6, 1.6 Hz, 1H), 5.65 – 5.58 (m, 1H), 5.47 (s, 1H), 5.36 (d, J = 9.8 Hz, 1H), 5.34 – 5.29 (m, 1H), 5.18 (app dq, J = 10.4, 1.5 Hz, 1H), 5.10 (app p, J = 6.2 Hz, 1H), 4.67 – 4.61 (m, 1H), 4.60 – 4.49 (m, 2H), 4.30 – 4.15 (m, 2H), 3.96 – 3.87 (m, 2H), 3.79 (s, 3H), 3.78 – 3.75 (m, 1H), 3.72 – 3.67 (m, 2H), 3.67 – 3.60 (m, 3H), 3.48 (app t, J = 9.0 Hz, 1H), 3.35 – 3.24 (m, 1H), 3.12 (s, 3H), 2.47 – 2.39 (m, 1H), 2.39 – 2.34 (m, 1H), 2.32 – 2.22 (m, 2H), 2.03 – 1.99 (m, 1H),
1.97 (s, 1H), 1.95 – 1.90 (m, 1H), 1.84 (app ddd, \( J = 14.9, 6.9, 2.4 \) Hz, 1H), 1.67 (app dd, \( J = 15.5, 9.4 \) Hz, 1H), 1.63 – 1.53 (m, 2H), 1.53 (s, 2H), 1.40 – 1.34 (m, 1H), 1.25 (d, \( J = 6.1 \) Hz, 2H), 1.22 (d, \( J = 4.1 \) Hz, 3H), 1.19 (d, \( J = 6.2 \) Hz, 3H), 1.06 (d, \( J = 6.7 \) Hz, 3H), 1.03 – 0.79 (m, 50H), 0.76 – 0.38 (m, 30H), 0.09 (s, 9H).

\(^{13}\)C NMR (126 MHz, acetone-\( d_6 \))

\[ \delta 173.19, 165.92, 160.80, 156.20, 147.55, 138.01, 136.92, 134.45, 134.22, 134.07, 133.77, 133.71, 133.56, 133.43, 132.76, 132.64, 131.75, 130.54, 128.67, 128.67, 123.63, 120.65, 117.33, 113.87, 101.77, 101.02, 98.66, 81.47, 75.93, 75.29, 74.71, 73.78, 73.41, 73.24, 70.96, 70.52, 68.78, 67.85, 65.77, 63.31, 58.39, 57.21, 55.50, 54.97, 48.50, 44.11, 43.12, 41.99, 37.26, 34.71, 33.30, 19.04, 18.54, 18.31, 18.18, 7.40, 7.36, 7.27, 7.09, 5.89, 5.83, 5.80, 5.55, -1.39. \]

HRMS (ESI)

Calculated for \( C_{95}H_{165}NO_{19}Si_{6} \) (M + Na\(^+\)): 1815.0489

Found: 1815.0490

Macrolactone 2.10

A 20 mL vial containing 2.9 (270.7 mg, 150.9 µmol, 1 eq) was brought into the glovebox and Stryker’s reagent (295.9 mg, 150.9 µmol, 1 eq) was added. The vial was then sealed with a septa cap and removed from the glovebox. Toluene (7.5 mL) and water (50 µL) were added and the reaction was allowed to stir at room temperature for 12 hours. Then 50 mL ether was added followed by Na\(_2\)SO\(_4\) and celite. The reaction mixture was then filtered, concentrated and purified directly by SiO\(_2\) chromatography (1:0 \(\rightarrow\) 4:1 \(\rightarrow\) 7:3 \(\rightarrow\) 13:7 \(\rightarrow\) 3:2 hexanes:EtOAc) isolating 2.10 as a yellow solid (241.3 mg, 134.4 µmol, 89% yield).
TLC (hexanes : EtOAc 3:1)
\[ R_f = 0.23 \], visualized by UV and stained by \( \rho \)-anisaldehyde

\[
\begin{align*}
^1H \text{ NMR} & \quad (500 \text{ MHz, Acetone-}\text{d}_6) \\
\delta & \quad 7.43 - 7.37 (m, 2H), 6.92 - 6.87 (m, 2H), 6.45 - 6.28 (m, 9H), 6.28 - 6.08 (m, 3H), \\
& \quad 6.02 - 5.92 (m, 1H), 5.93 - 5.86 (m, 1H), 5.66 - 5.58 (m, 1H), 5.47 (s, 1H), 5.38 (app bd, \( J = 9.8 \) Hz, 1H), 5.32 (app dq, \( J = 17.3, 1.7 \text{ Hz}, 1H), 5.18 (app dq, \( J = 10.5, 1.5 \text{ Hz}, 1H), 4.97 (app p, \( J = 6.4 \text{ Hz}, 1H), 4.68 - 4.61 (m, 1H), 4.61 - 4.50 (m, 3H), 4.31 - 4.14 (m, 3H), 3.96 - 3.88 (m, 3H), 3.79 (s, 3H), 3.78 - 3.67 (m, 3H), 3.64 (app td, \( J = 9.6, 2.9 \text{ Hz}, 1H), 3.48 (app t, \( J = 9.0 \text{ Hz}, 1H), 3.46 - 3.38 (m, 1H), 3.35 - 3.26 (m, 1H), 3.25 (app d, \( J = 5.6 \text{ Hz}, 1H), 3.12 (s, 3H), 2.46 - 2.38 (m, 1H), 2.31 - 2.19 (m, 3H), 2.19 - 2.10 (m, 1H), 2.04 - 1.97 (m, 1H), 1.98 - 1.92 (m, 1H), 1.80 - 1.81 (m, 2H), 1.78 - 1.70 (m, 1H), 1.66 (app dd, \( J = 15.6, 9.5 \text{ Hz}, 1H), 1.57 - 1.48 (m, 3H), 1.47 - 1.39 (m, 2H), 1.38 - 1.31 (m, 2H), 1.30 - 1.28 (m, 2H), 1.25 (d, \( J = 6.1 \text{ Hz}, 3H), 1.24 - 1.19 (m, 2H), 1.17 (d, \( J = 6.2 \text{ Hz}, 3H), 1.09 (app d, \( J = 6.2 \text{ Hz}, 1H), 1.05 (d, \( J = 6.7 \text{ Hz}, 3H), 1.03 - 0.78 (m, 50H), 0.77 - 0.38 (m, 30H), 0.09 (s, 9H).
\end{align*}
\]

\[
\begin{align*}
^13C \text{ NMR} & \quad (126 \text{ MHz, acetone-}\text{d}_6) \\
\delta & \quad 173.22, 172.72, 160.81, 156.20, 138.38, 136.77, 134.45, 134.17, 134.07, 133.96, \\
& \quad 133.75, 133.70, 133.62, 133.49, 132.83, 132.73, 132.65, 131.49, 130.47, 128.69, 117.34, 113.88, \\
& \quad 101.82, 100.98, 98.59, 81.79, 75.70, 75.29, 74.74, 73.80, 73.40, 73.19, 71.19, 71.11, 70.98, \\
& \quad 68.80, 67.79, 65.78, 63.30, 58.39, 57.37, 55.51, 48.53, 44.15, 43.20, 39.24, 37.07, 35.15, 34.57, \\
& \quad 33.34, 21.71, 19.05, 18.57, 18.34, 7.42, 7.36, 7.28, 7.09, 5.90, 5.81, 5.57, -1.38.
\end{align*}
\]

\[
\text{HRMS (ESI)} \\
\text{Calculated for C}_{95}H_{167}NO_{19}Si_6 (M + Na^+): \quad 1817.0646 \\
\text{Found:} \quad 1817.0637
\]
Ketal 2.11a

An oven dried 40 mL Teflon vial was charged with 2.10 (186.1 mg, 103.6 µmol, 1 eq) and THF (3.45 mL) and cooled to 0°C. A separate oven dried Teflon vial was sealed with a septum and charged with THF (9.2 mL), pyridine (5.44 mL), and HF-pyridine (820 µL, 36.3 mmol, 350 eq). After cooling to 0°C the HF solution was cannula transferred slowly to the substrate solution. The reaction stirred for 5 minutes before warming to room temperature and stirring for 4 hours. Then NaHCO₃ (sat. aq.) was added dropwise until bubbling ceased. The reaction was then transferred to a separatory funnel with 80mL NaHCO₃, 20mL H₂O and 80mL DCM. The layers were separated and the aqueous layer was extracted with DCM (3x20mL). The combined organic phase was washed with brine (40 mL), dried (Na₂SO₄), filtered, concentrated, and purified by SiO₂ chromatography (1:0 → 99:1 → 97:3 → 19:1 DCM:MeOH) isolating 2.11a as a yellow solid (111.7 mg, 91.2 µmol, 88% yield).

TLC (DCM : MeOH 9:1)

Rᵣ = 0.55, visualized by UV and stained by ρ-anisaldehyde

¹H NMR (500 MHz, acetone-d₆)

δ 7.46 – 7.41 (m, 2H), 6.92 – 6.86 (m, 2H), 6.42 – 6.16 (m, 12H), 6.03 (d, J = 8.7 Hz, 1H), 5.99 – 5.89 (m, 2H), 5.58 (dd, J = 14.5, 9.2 Hz, 1H), 5.54 (s, 1H), 5.31 (app dq, J = 17.2, 1.8 Hz, 1H), 5.23 – 5.17 (m, 1H), 5.16 (app dq, J = 10.5, 1.5 Hz, 1H), 4.69 – 4.64 (m, 1H), 4.62 (s, 1H), 4.52 (bd, J = 5.4 Hz, 2H), 4.29 – 4.13 (m, 3H), 4.09 (app d, J = 5.0 Hz, 1H), 4.01 (app d,
$J = \text{6.2 Hz, 1H}$, 3.99 – 3.81 (m, 3H), 3.87 – 3.84 (m, 2H), 3.79 (s, 3H), 3.78 – 3.68 (m, 2H), 3.62 (app td, $J = \text{9.2, 3.2 Hz, 1H}$), 3.47 – 3.39 (m, 2H), 3.39 – 3.28 (m, 2H), 3.11 (s, 3H), 2.46 – 2.37 (m, 1H), 2.33 (dd, $J = \text{13.2, 4.6 Hz, 1H}$), 2.27 – 2.18 (m, 2H), 2.19 – 2.11 (m, 1H), 2.01 (dd, $J = \text{6.7, 1.8 Hz, 1H}$), 1.93 – 1.79 (m, 2H), 1.79 – 1.70 (m, 2H), 1.64 – 1.55 (m, 2H), 1.54 – 1.47 (m, 5H), 1.43 – 1.29 (m, 3H), 1.25 (d, $J = \text{5.8 Hz, 3H}$), 1.18 (d, $J = \text{6.4 Hz, 3H}$), 1.11 (d, $J = \text{6.5 Hz, 3H}$), 1.09 – 1.03 (m, 1H), 1.01 (d, $J = \text{7.1 Hz, 3H}$), 0.08 (s, 9H).

$^{13}$C NMR (126 MHz, acetone-$d_6$)

$\delta$ 206.15, 205.98, 173.33, 172.74, 160.65, 157.27, 137.76, 136.87, 134.51, 134.07, 133.98, 133.94, 133.85, 133.65, 132.84, 132.77, 132.54, 132.16, 129.48, 128.38, 117.17, 113.96, 100.95, 100.78, 98.37, 81.15, 78.29, 74.95, 74.40, 74.20, 73.16, 72.20, 71.89, 71.16, 70.23, 67.38, 67.12, 65.70, 63.19, 58.04, 57.65, 55.53, 48.82, 43.65, 43.60, 42.61, 41.65, 39.15, 37.45, 35.75, 35.35, 33.91, 22.11, 18.82, 18.36, 18.11, 17.57, 12.24, -1.33.

HRMS (ESI)

Calculated for C$_{65}$H$_{97}$NO$_{19}$Si (M + Na$^+$): 1246.6322

Found: 1246.6332

Carboxylic Acid 2.11b

To a 7 mL vial was added TMSE ester 2.11a (10 mg, 8.17 µmol, 1 eq) and THF (410 µL). To a separate vial was added TBAF (24.5 µL of a 1M THF solution, 24.5 µmol, 3 eq) and additional THF (1.23 mL). Both vials were cooled to 0°C and the TBAF solution was transferred to the vial containing 2.11a. After stirring for 3 hours, the reaction was transferred to a 7 mL vial containing DOWEX 50WX8 200-400 resin (210 mg), CaCO3 (70 mg) and MeOH (1 mL). This stirred at 23°C for 30 minutes before filtering through a pad of celite. The filtrate was concentrated and carried forward crude into the next reaction.
TLC (DCM : MeOH 9:1, with 0.1%AcOH)

$R_f = 0.53$, stained by CAM

HRMS (ESI)

Calculated for C$_{60}$H$_{85}$NO$_{19}$ (M + Na$^+$): 1146.5613

Found: 1146.5610

Alloc carbamate 2.11

To a 40 mL vial was added acetonitrile (20 mL), H$_2$O (1 mL), and acetyl chloride (200 µL). This stirred at room temperature for 20 minutes before cooling to 0°C. 5 mL of this solution was then added to a vial containing ketal 2.11b (20 mg, 17.8 µmol, 1 eq) at 0°C. The reaction stirred for 1 hour before 50 µL triethylamine was added. The volatiles were removed and the reaction was purified by preparative RP-HPLC (Waters SunFire Prep C$_{18}$ OBD 5 micron 30 x 150 mm; 35mL/min flow rate, 25mM NH$_4$OAc in H$_2$O : MeCN 19:1 → 2:3 over 23 minutes) yielding 2.11 as a yellow solid (2.8 mg, 2.82 µmol, 15.7%).

$^1$H NMR (499 MHz, pyridine $d$-5:CD$_3$OD 1:1)

δ 6.75 – 6.26 (m, 13H), 5.31 – 5.22 (m, 1H), 5.93 (s, 1H), 5.66 (d, $J = 6.9$ Hz, 1H), 5.31 (bd, $J = 17.3$ Hz, 2H), 5.15 (bd, $J = 10.5$ Hz, 1H), 4.94 (s, 1H), 4.92 – 4.85 (m, 1H), 4.76 – 4.65
(s, 3H), 4.59 (bs, 1H), 4.31 (s, 1H), 4.11 – 4.06 (m, 1H), 3.92 – 3.85 (m, 1H), 3.77 – 3.69 (m, 1H), 3.67 – 3.57 (m, 2H), 3.42 – 3.38 (m, 1H), 3.34 (app d, J = 9.6 Hz, 1H), 2.77 – 2.66 (m, 1H), 2.63 – 2.55 (m, 1H), 2.53 – 2.46 (m, 1H), 2.46 – 2.35 (m, 1H), 2.37 – 2.27 (m, 2H), 2.27 – 2.16 (m, 2H), 2.02 (app t, J = 9.9 Hz, 3H), 1.98 – 1.91 (m, 2H), 1.90 – 1.78 (m, 4H), 1.70 – 1.52 (m, 5H), 1.46 (d, J = 5.9 Hz, 3H), 1.37 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 6.3 Hz, 3H), 1.19 (d, J = 7.1 Hz, 3H).

HRMS (ESI)

Calculated for C_{51}H_{77}NO_{18} (M + Na^+): 1014.5038

Found: 1014.5038

HPLC (analytical, C_{18} SiO_{2}; flow rate= 1.2 mL/min; 25mM NH_{4}OAc in H_{2}O : MeCN 19:1 → 2:3 over 23 minutes)

Retention time: 18.08 min

Allyl ester 2.12
To a stirred solution of 2.8d (1.62 g, 0.885 mmol, 1eq) in dimethylformamide (29.5 mL) was added allyl bromide (3.1 mL, 35.4 mmol, 40 eq) and N,N-diisopropylethylamine (0.62 mL, 3.54 mmol, 4 eq). The solution was stirred for 4 hours at room temperature and then diluted with diethyl ether (300 mL). The resulting mixture was washed with water: saturated aqueous ammonium chloride 1:1 (3 x75 mL) and water: brine 1:1 (1 x75 mL). The organic layer was dried over sodium sulfate and then concentrated in vacuo. Purification by SiO_{2} chromatography (19:1→ 9:1 hexanes:EtOAc) afforded 2.12 as an orange solid (1.42 g, 0.763 mmol, 86%).
TLC (hexanes : EtOAc 3:7)

$R_f=0.78$, visualized by UV

$^1$H NMR (500MHz, acetone $d$-$6$)

$\delta$ 7.40 (m, 2H), 7.36 (m, 2H), 6.88 (m, 4H), 6.52-6.17 (m, 11H), 6.12 (dd, $J = 15.0, 9.8$ Hz 1H), 6.05-5.90 (m, 2H), 5.86 (m, 1H), 5.66 (dd, $J = 14.9, 9.2$ 14.9 Hz, 1H), 5.45 (m, 2H), 5.42-5.26 (m, 4H), 5.19 (app dq, $J = 10.5, 1.5$ Hz, 1H), 4.96 (m, 1H), 4.76-4.51 (m, 5H), 4.50 (s, 1H), 4.28 (app dt, $J = 10.6, 4.6$ Hz, 1H), 4.20 (m, 1H), 3.97-3.87 (m, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.77-3.67 (m, 4H), 3.65 (app dt, $J = 9.6, 3.2$ Hz, 1H), 3.48 (app t, $J = 8.9$ Hz, 1H), 3.33 (m, 1H), 3.08 (s, 3H), 2.54 (dd, $J = 17.5, 7.3$ Hz, 1H), 2.42 (m, 1H), 2.36-2.25 (m, 3H), 2.02 (m, 1H), 1.93 (app d, $J = 15.6$Hz, 1H), 1.87 (m, 2H), 1.80-1.53 (m, 6H), 1.53-1.40 (m, 2H), 1.33 (m, 1H), 1.24 (d, $J = 6.2$ Hz, 3H), 1.19 (d, $J = 6.2$ Hz, 3H), 1.17 (m, 1H), 1.08-0.86 (m, 51H), 0.76-0.37 (m, 30H)

$^{13}$C NMR (125MHz, acetone $d$-$6$)

$\delta$ 172.7, 169.7, 160.6, 160.5, 156.1, 134.6, 134.5, 134.3, 134.2, 134.1, 133.6, 133.5 (2C), 133.3, 133.1, 132.7, 132.5, 132.4, 131.4, 131.0, 128.5, 128.2, 118.9, 117.3, 114.1, 113.8, 113.7, 101.7, 101.0, 100.7, 98.7, 81.5, 75.9, 75.7, 74.9, 74.5, 73.7, 73.0, 72.7, 68.7, 67.5, 65.7 (2C), 58.1, 57.7, 55.4, 48.4, 43.8, 43.1, 41.3, 37.8, 36.6, 33.4, 32.7, 28.0, 19.0, 18.4, 11.3, 7.4 (2C), 7.3 (2C), 7.1, 5.9 (2C), 5.8, 5.7, 5.5

HRMS (ESI)

Calculated for C$_{101}$H$_{165}$NO$_{21}$Si$_{5}$ (M+Na)$^+$: 1891.0618

Found: 1891.0547
Enone 2.13

Prior to the reaction, 2.12 (1.63 g, 0.872 mmol, 1 eq) was azeotropically dried via co-evaporation with toluene. The resulting orange solid was dissolved in THF (14.5 mL) and cooled to -78°C. Sodium bis(trimethylsilyl)amide (800 mg, 4.36 mmol, 5 eq) in THF (14.5 mL) was cooled to -78°C and added dropwise into the solution of 2.12 over 5 minutes via syringe. The mixture was stirred for 1 hour at -78°C and quenched with saturated aqueous ammonium chloride (29 mL) at -78°C, which was then extracted with diethyl ether (300 mL). The organic layer was washed with water: saturated aqueous ammonium chloride 1:1 (3x 75 mL) and water: brine 1:1 (1 x 75 mL), dried over sodium sulfate, and concentrated in vacuo. Purification of the crude orange oil by SiO₂ chromatography (19:1 → 17:3 hexanes:EtOAc) afforded 2.13 as a yellow solid (1.14 g, 0.658 mmol, 75%).

TLC (hexanes : EtOAc 3:7)

R_f=0.74, visualized by UV

¹H NMR (500MHz, acetone d-6)

δ 7.40 (app d, J = 6.7 Hz, 2H), 6.97 (app dt, J = 15.6, 7.0 Hz, 1H), 6.90 (app d, J = 6.7 Hz, 2H), 6.47-6.07 (m, 12H), 6.04-5.85 (m, 3H), 5.80 (d, J = 15.6 Hz, 1H), 5.62 (dd, J = 8.9 Hz, J=14.0 Hz, 1H), 5.47 (s, 1H), 5.46-5.25 (m, 4H), 5.19 (app dq, J = 10.5, 4.6 Hz, 1H), 5.09 (m, 1H), 4.69 (app dd, J = 13.6, 6.0 Hz, 1H), 4.66-4.53 (m, 4H), 4.52 (s, 1H), 4.28 (app dt, J = 10.6, 4.7 Hz, 1H), 3.99-3.89 (m, 3H), 3.80 (s, 3H), 3.77 (m, 1H), 3.71 (m, 2H), 3.68-3.60 (m, 2H), 3.48 (app t, J = 8.9 Hz, 1H), 3.31 (m, 1H), 3.13 (s,3H), 2.49-2.25 (m, 6H), 2.03-1.89 (m, 4H),
1.88-1.80 (m, 1H), 1.73-1.43 (m, 5H), 1.37 (m, 1H), 1.22 (d, J = 6.2 Hz, 3H), 1.20 (d, J = 6.1 Hz, 3H), 1.07 (d, J = 6.8 Hz, 3H), 1.05-0.77 (m, 48H), 0.75-0.38 (m, 30H)

$^{13}$C NMR (125MHz, acetone $d_6$)

\[ \delta 172.6, 165.7, 160.6, 156.0, 147.3, 137.7, 136.5, 134.2, 134.1, 134.0, 133.9, 133.7 \text{(2C)}, 133.5, 133.3, 133.2, 132.8, 132.6, 132.4, 131.8, 130.4, 128.5, 123.5, 118.9, 117.2, 113.7, 101.6, 100.8, 98.7, 81.4, 78.9, 75.9, 75.1, 74.4, 73.7, 73.2, 73.0, 70.7, 70.3, 68.6, 67.5, 65.7, 58.1, 57.1, 55.4, 48.5, 43.8, 42.9, 41.9, 37.2, 34.3, 33.2, 28.9, 18.9, 18.6, 18.1, 12.2, 7.4 \text{(2C)}, 7.3, 7.2, 7.0, 5.8, 5.7 \text{(2C)}, 5.4 \]

HRMS (ESI)

Calculated for $C_{93}H_{157}NO_{19}Si_{5}$ (M+Na)$^+$: 1755.0094

Found: 1755.0094

Macrolactone 2.14

A round bottom flask was purged with argon and charged with 2.13 (500 mg, 0.288 mmol, 1 eq), Stryker’s reagent (566 mg, 0.288 mmol, 1 eq). Toluene (14.4 mL) and water (45 mL) was added and the mixture was stirred for 14 hours at 23°C. The mixture was then stirred under air for 30 minutes before it was filtered through celite plug with ethyl acetate (600 mL). The filtrate was washed with water: saturated aqueous ammonium chloride 1:1 (2x150 mL), water: saturated aqueous sodium bicarbonate 1:1 (2x150 mL) and water:brine 1:1 (1x150 mL). The organic layer was dried over sodium sulfate and concentrated in vacuo. Purification by SiO$_2$ chromatography (19:1 → 17:3 hexanes:EtOAc) afforded 2.14 as a yellow solid (424 mg, 0.244 mmol, 85%).
TLC (hexanes : EtOAc 3:7)  
R_f=0.68, visualized by UV

^1^H NMR (500MHz, acetone d-6)  
δ 7.40 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 6.46-6.08 (m, 12H), 6.05-5.92 (m, 2H), 5.89 (m, 1H), 5.61 (dd, J = 14.5, 9.2 Hz, 1H), 5.47 (s, 1H), 5.46-5.26 (m, 4H), 5.19 (app dd, J = 10.5, 1.5 Hz, 1H), 4.98 (m, 1H), 4.69 (dd, J = 13.4, 5.8 Hz, 1H), 4.66-4.52 (m, 4H), 4.50 (s, 1H), 4.28 (app dt, J = 10.7, 4.6 Hz, 1H), 3.98-3.88 (m, 3H), 3.79 (s, 3H), 3.77-3.67 (m, 4H), 3.64 (m, 1H), 3.48 (app t, J = 8.9 Hz, 1H), 3.31 (m, 1H), 3.11 (s, 3H), 2.42 (m, 1H), 2.34-2.18 (m, 4H), 2.15 (m, 1H), 2.12 (m, 1H), 2.01 (m, 1H), 1.95 (m, 1H), 1.90-1.82 (m, 4H), 1.80-1.58 (m, 5H), 1.55-1.48 (m, 3H), 1.24 (d, J = 6.0 Hz, 3H), 1.18 (d, J = 6.2 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H), 1.04-0.78 (m, 48H), 0.76-0.36 (m, 30H)

^1^3^C NMR (125MHz, acetone d-6)  
δ 172.9, 172.7, 160.9, 156.3, 138.5, 136.7, 134.5, 134.2, 134.1, 134.0, 133.8, 133.7, 133.6, 133.5 (2C), 132.9, 132.8, 132.7, 132.6, 131.5, 130.5, 129.5, 129.4, 128.7, 126.1, 119.0, 117.3, 113.9, 110.6, 101.9, 101.0, 98.9, 81.8, 78.4, 76.0, 75.3, 74.7, 73.9, 73.4, 73.3, 71.2, 71.1, 68.8, 67.8, 65.9, 65.8, 58.4, 57.4, 55.6, 48.6, 44.1, 43.2, 41.3, 39.2, 37.4, 35.2, 34.5, 33.4, 29.1, 21.7, 19.0, 18.6, 18.4, 11.8, 7.4 (2C), 7.3, 7.1, 6.0 (2C), 5.9(2C), 5.6

HRMS (ESI)  
Calculated for C\textsubscript{93}H\textsubscript{159}NO\textsubscript{19}Si\textsubscript{5} (M+Na): 1757.0250  
Found: 1757.0303

Polyol 2.14a
A HDPE bottle was charged with 2.14 (1 g, 0.576 mmol, 1 eq). THF (20 mL) was added and the solution was cooled to 0°C. To a separate HDPE bottle was added pyridine (36.8 mL) and THF (60 mL) and cooled to 0°C. 70% HF-pyridine was added to the pyridine-THF solution at 0°C. This solution was transferred slowly to the THF solution of 2.14 via a cannula. The reaction was allowed to stir for 12 hours at 23°C. The reaction was quenched at 0°C with saturated aqueous sodium bicarbonate and diluted with methylene chloride (1.2 L). The organic layer was washed with water: saturated aqueous sodium bicarbonate 1:1 (2x600 mL) and water:brine 6:1 (600 mL). The organic layer was dried with sodium sulfate and concentrated in vacuo. Purification by SiO\textsubscript{2} chromatography (99:1→95:5 DCM:MeOH) afforded 2.14a as a yellow solid (587.2 mg, 0.504 mmol, 88%).

\[
\text{TLC (DCM : MeOH 9:1)}
\]
\[
\text{R}_f = 0.46, \text{ visualized by UV}
\]

\[
^1\text{H NMR (500MHz, acetone} \text{d-6)}
\]
\[
\delta 7.45 \text{ (d, } J = 8.7 \text{ Hz, 2H)}, \ 6.89 \text{ (d, } J = 8.6 \text{ Hz, 2H)}, \ 6.47-6.29 \text{ (m, 9H)}, \ 6.27-6.17 \text{ (m, 3H)}, \ 6.08 \text{ (app d, } J = 8.8 \text{ Hz,1H),} \ 6.03-5.90 \text{ (m, 3H)}, \ 5.59 \text{ (dd, } J = 14.3, 9.1 \text{ Hz, 1H)}, \ 5.54 \text{ (s, 1H)}, \ 5.43 \text{ (app dd, } J = 17.2, 1.6 \text{ Hz, 1H)}, \ 5.32 \text{ (app dd, } J = 17.2, 1.4 \text{ Hz, 1H)}, \ 5.26 \text{ (app dd, } J = 10.6, 1.4 \text{ Hz, 1H)}, \ 5.19 \text{ (m, 1H)}, \ 5.16 \text{ (app dq, } J = 10.6, 1.5 \text{ Hz, 1H)}, \ 4.66 \text{ (m, 3H)}, \ 4.59 \text{ (s, 1H)}, \ 4.53 \text{ (m, 2H)}, \ 4.19 \text{ (app dt, } J = 10.1, 4.8 \text{ Hz, 1H)}, \ 3.97 \text{ (m, 2H)}, \ 3.84 \text{ (app d, } J = 2.0\text{Hz, 1H)}, \ 3.79 \text{ (s, 3H)}, \ 3.75 \text{ (m, 1H)}, \ 3.63 \text{ (m, 1H)}, \ 3.48-3.28 \text{ (m, 5H)}, \ 3.11 \text{ (s, 3H)}, \ 2.43 \text{ (m, 1H)}, \ 2.37 \text{ (m, 1H)}, \ 2.31 \text{ (app t, } J = 10.1 \text{ Hz, 1H)}, \ 2.26-2.18 \text{ (m, 2H)}, \ 2.18-2.11 \text{ (m, 2H)}, \ 2.02 \text{ (m, 1H)}, \ 1.88 \text{ (m, 3H)}, \ 1.76 \text{ (m, 2H)}, \ 1.62 \text{ (m, 1H)}, \ 1.52 \text{ (m, 4H)}, \ 1.43-1.31 \text{ (m, 3H)}, \ 1.26 \text{ (d, } J = 5.2 \text{ Hz, 3H)}, \ 1.20 \text{ (d, } J = 6.0 \text{ Hz, 3H)}, \ 1.13 \text{ (d, } J = 6.4 \text{ Hz, 3H)}, \ 1.02 \text{ (d, } J = 7.2 \text{ Hz, 3H)}
\]

\[
^{13}\text{C NMR (125MHz, acetone} \text{d-6)}
\]
δ 172.9, 172.7, 160.6, 157.3, 136.8, 134.6, 134.5 (2C), 134.0 (2C), 133.9 (2C),
133.8 (2C), 133.6 (2C), 132.8, 132.7, 132.5, 132.2, 129.5, 128.4, 118.5, 117.1, 113.9, 101.0,
100.8, 98.3, 95.0, 81.2, 78.3, 74.9, 74.3, 74.2, 73.1, 72.1, 71.9, 71.2 (2C), 70.2, 69.3, 67.3, 67.2,
65.7, 65.6 (2C), 58.0, 57.5, 55.5, 48.9, 43.6, 43.5, 42.6, 41.6, 39.1, 37.4, 35.7, 35.3, 33.9, 30.6,
27.2, 22.1, 18.8, 18.3, 17.6, 12.2

HRMS (ESI)

Calculated for C_{63}H_{89}NO_{19} (M+Na)^+: 1186.5926
Found: 1186.5951

**Allyl Ester 2.14b**

A round bottle flask was charged with 2.14a (200 mg, 0.194 mmol, 1 eq). THF: H_{2}O 2:1 (6.5 mL) was added followed by camphorsulfonic acid (345 mg, 1.49 mmol, 7.7 eq). The reaction was allowed to stir at 23°C for 3h before quenched with triethyl amine (414 mL, 2.98 mmol, 15.4 eq). The reaction was dried over sodium sulfate and then concentrated in vacuo. Purification of the resulting residue by SiO_{2} chromatography (99:1 → 90:10 DCM:MeOH) afforded 2.14b as a yellow solid (80.9 mg, 0.078 mmol, 40%).

TLC (DCM : MeOH 9:1)

R_{f}=0.24, visualized by UV

{\textsuperscript{1}}H NMR (750MHz, pyridine d-5: CD_{3}OD 1:1)

δ 6.70-6.27 (m, 13H), 6.06 (m, 1H), 5.93 (m, 1H), 5.65 (app q, J = 6.6 Hz, 1H), 5.52 (app d, J = 17.3 Hz, 1H), 5.46 (dd, J = 15.2, 10.4 Hz, 1H), 5.32 (dd, J = 17.4, 1.1 Hz, 1H), 5.28(app d,
$J = 10.3 \text{ Hz}, 1\text{H}$), 5.14 (app d, $J = 10.4 \text{ Hz}, 1\text{H}$), 4.88 (m, 1H), 4.77 (s, 1H), 4.73 (m, 5H), 4.66 (app t, $J = 11.0 \text{ Hz}, 1\text{H}$), 4.58 (m, 1H), 4.22 (app d, $J = 3.1 \text{ Hz}, 1\text{H}$), 4.05 (app dd, $J = 10.2, 3.0 \text{ Hz}, 1\text{H}$), 3.88 (app d, $J = 11.1 \text{ Hz}, 1\text{H}$), 3.73 (app t, $J = 9.6 \text{ Hz}, 1\text{H}$), 3.63 (m, 1H), 3.58 (m, 1H), 3.46 (m, 1H), 3.34 (app d, $J = 9.3 \text{ Hz}, 1\text{H}$), 2.59 (m, 2H), 2.33 (m, 2H), 2.30-2.16 (m, 3H), 2.06-1.98 (m, 2H), 1.89 (app dd, $J = 15.2, 11.0 \text{ Hz}, 1\text{H}$), 1.85 (app dd, $J = 14.3, 11.3 \text{ Hz}, 1\text{H}$), 1.81 (m, 2H), 1.69 (app dd, $J = 13.8, 2.0 \text{ Hz}, 1\text{H}$), 1.64-1.52 (m, 4H), 1.48 (d, $J = 6.1 \text{ Hz}, 3\text{H}$), 1.45 (m, 1H), 1.41 (m, 2H), 1.37 (d, $J = 6.6 \text{ Hz}, 3\text{H}$), 1.24 (d, $J = 6.4 \text{ Hz}, 3\text{H}$), 1.19 (d, $J = 7.1 \text{ Hz}, 3\text{H}$)

$^{13}$C NMR (125MHz, acetone $d$-6)

$\delta$ 173.2, 172.8, 150.5, 150.3, 150.0, 137.4, 137.0, 134.9, 134.8, 134.6, 134.5, 134.0, 133.8 (3C), 133.7, 133.5 (2C), 133.3, 133.0, 118.2, 117.1, 98.5, 98.4, 98.0, 79.3, 79.1, 77.1, 76.9, 74.4, 70.2, 69.4, 67.0, 66.1, 65.7, 65.6, 58.6, 58.0, 47.3, 45.5, 43.9, 40.9, 40.8, 39.6, 38.8, 36.5, 35.2, 32.8, 22.8, 18.9, 18.4, 17.1, 12.5

HRMS (ESI)

Calculated for C$_{54}$H$_{81}$NO$_{18}$ (M+Na)$^+$: 1054.5351

Found: 1054.5387

C$_{3}$deoxyAmB

A round bottom flask was charged with **2.14b** (35 mg, 0.034 mmol, 1 eq), thiosalicylic acid (26.1 mg, 0.170 mmol, 5 eq) and palladium tetrakis(triphenylphosphine) (11.6 mg, 0.0100 mmol, 0.3 eq). DMF (1.75 mL) was added and the reaction was allowed to stir at 23°C for 15 minutes before poured into diethyl ether (35 mL). The reaction mixture was then transferred to a 50 mL centrifuge tube and centrifuged at 3700g for 5 minutes. The red pellet was dissolved in DMSO (4 mL) and purified by preparative RP-HPLC (Waters SunFire Prep C$_{18}$ OBD 5 micron 30 x 150 mm; 25mL/min flow rate, MeCN: 15mM NH$_4$OAc in H$_2$O 1:19 → 19:1 over 9 minutes, 19:1 for
1 minute, 19:1 → 1:19 over 2 minutes) to afford **C3deOAmB** (16.2 mg, 0.018 mmol, 53%) as a yellow solid.

HPLC (analytical, C\textsubscript{18} SiO\textsubscript{2}; flow rate= 1.2 mL/min; MeCN: 25mM NH\textsubscript{4}OAc in H\textsubscript{2}O 1:19 → 19:1 over 8 minutes)

Retention time: 5.1 min

\[^{1}\text{H} \text{NMR} (750MHz, \text{pyridine} \, d-5: \text{CD}_2\text{OD} 1:1)\]

\[\delta \, 6.68 \, (dd, \, J = 14.9, \, 11.3 \, Hz, \, 1H), \, 6.64 \, (dd, \, J = 14.9, \, 10.9 \, Hz, \, 1H), \, 6.56 \, (dd, \, J = 14.6, \, 11.3 \, Hz, \, 1H), \, 6.51 \, (dd, \, J = 13.4, \, 11.4 \, Hz, \, 1H), \, 6.47-6.35 \, (m, \, 7H), \, 6.33-6.25 \, (m, \, 2H), \, 5.66 \, (m, \, 1H), \, 5.45 \, (m, \, 1H), \, 5.03 \, (s, \, 1H), \, 4.87 \, (app \, t, \, J = 10.7 \, Hz, \, 1H), \, 4.79-4.73 \, (m, \, 2H), \, 4.69 \, (app \, t, \, J = 10.7 \, Hz, \, 1H), \, 4.57 \, (app \, d, \, J = 3.2 \, Hz, \, 1H), \, 3.87 \, (app \, d, \, J = 11.0 \, Hz, \, 1H), \, 3.85 \, (app \, t, \, J = 9.8 \, Hz, \, 1H), \, 3.70-3.68 \, (m, \, 2H), \, 3.62 \, (m, \, 1H), \, 3.45 \, (m, \, 1H), \, 3.34 \, (m, \, 1H), \, 2.82 \, (app \, dd, \, J = 4.5 \, Hz, \, J = 15.2 \, Hz, \, 1H), \, 2.53 \, (m, \, 1H), \, 2.46 \, (app \, t, \, J = 10.4 \, Hz, \, 1H), \, 2.34-2.16 \, (m, \, 4H), \, 2.05-1.97 \, (m, \, 3H), \, 1.90 \, (m, \, J = 14.0, \, 9.1 \, Hz, \, 1H), \, 1.86-1.78 \, (m, \, 2H), \, 1.66 \, (app \, dd, \, J = 13.9, \, 2.3 \, Hz, \, 1H), \, 1.64-1.51 \, (m, \, 4H), \, 1.46 \, (d, \, J = 6.2 \, Hz, \, 3H), \, 1.45-1.38 \, (m, \, 3H), \, 1.37 \, (d, \, J = 6.5 \, Hz, \, 3H), \, 1.14 \, (d, \, J = 6.4 \, Hz, \, 3H), \, 1.19 \, (d, \, J = 7.1 \, Hz, \, 3H)\]

\[^{13}\text{C} \text{NMR} (187.5MHz, \text{pyridine} \, d-5:\text{CD}_2\text{OD} 1:1)\]
δ 173.7, 134.9 (2C), 134.6, 134.3, 134.1, 134.0, 133.9, 133.7, 133.1, 130.5, 98.7, 98.4, 79.4, 78.5, 76.9, 75.4, 74.5, 72.5, 70.9, 70.2, 70.0, 69.5, 67.8, 67.1, 57.6, 48.0, 45.6, 44.3, 41.2, 41.0, 39.7, 39.0, 36.7, .5.5, 32.3, 23.1, 19.3, 18.4, 17.4, 12.9

HRMS (ESI)
Calculated for C_{47}H_{73}NO_{16} (M+H)^{+}: 908.5008
Found: 908.5007

Azide 2.16
Iodide 2.15^{36} (783.5 mg, 0.3433 mmol, 1 eq) was added to a 40 mL vial with DMF (17.2 mL). Sodium azide (178 mg, 2.75 mmol, 8 eq) was added and the solution was placed in a preheated heating block at 40°C. After stirring for 2 hours the reaction had turned cloudy white. The reaction was then transferred to a 250 mL separatory funnel with 60 mL ether and 60 mL water. The layers were separated and the organic layers was washed with water (2x60 mL), brine (50 mL), dried over Na$_2$SO$_4$, filtered, concentrated. The crude oil was then purified by SiO$_2$ chromatography (1:0 → 19:1 → 9:1 → 17:3 hexanes:Et$_2$O) yielding 2.16 as a yellow solid (458.2 mg, 208.2 µmol, 61% yield).

TLC (hexanes : Et$_2$O 4:1)
R$_f$ = 0.40, visualized by UV and stained by CAM

$^1$H NMR (500 MHz, acetone-$d_6$)
δ 7.87 (d, J = 7.6 Hz, 2H), 7.69 (d, J = 7.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 6.57 – 6.10 (m, 12H), 6.10 – 6.00 (m, 1H), 5.51 (dd, J = 14.9, 9.5 Hz, 1H), 5.33 (bd,
$J = 9.9$ Hz, 1H), 4.73 (s, 1H), 4.73 – 4.64 (m, 2H), 4.47 (dd, $J = 10.5$, 6.4 Hz, 1H), 4.34 (dd, $J = 10.5$, 6.5 Hz, 1H), 4.30 – 4.21 (m, 2H), 4.16 – 4.09 (m, 2H), 4.04 – 3.96 (m, 1H), 3.96 (app d, $J = 2.9$ Hz, 1H), 3.88 – 3.80 (m, 2H), 3.77 (app bs, 1H), 3.74 – 3.66 (m, 2H), 3.69 – 3.60 (m, 2H), 3.48 (app t, $J = 9.1$ Hz, 1H), 3.40 – 3.31 (m, 1H), 3.14 (s, 3H), 2.61 – 2.52 (m, 2H), 2.53 – 2.47 (m, 1H), 2.49 – 2.39 (m, 1H), 2.31 – 2.21 (m, 1H), 2.19 – 2.11 (m, 1H), 1.99 – 1.85 (m, 3H), 1.85 – 1.72 (m, 3H), 1.70 – 1.59 (m, 3H), 1.55 – 1.44 (m, 2H), 1.29 – 1.22 (m, 2H), 1.18 (d, $J = 5.9$ Hz, 3H), 1.12 – 0.88 (m, 90H), 0.82 – 0.56 (m, 54H).

$^{13}$C NMR (126 MHz, acetone-$d_6$)

$\delta$ 170.63, 156.28, 145.07, 142.20, 139.15, 135.80, 135.32, 135.26, 134.75, 133.93, 133.11, 132.89, 132.82, 132.56, 132.52, 131.45, 131.04, 130.80, 128.48, 127.85, 127.83, 125.85, 125.74, 120.79, 101.05, 98.75, 76.76, 76.17, 74.56, 74.12, 74.04, 73.40, 73.22, 71.09, 67.82, 67.53, 67.45, 67.25, 67.09, 58.20, 49.35, 48.07, 48.05, 47.77, 44.56, 43.26, 41.40, 41.04, 35.83, 35.24, 30.73, 29.84, 27.44, 19.89, 19.20, 19.05, 11.42, 7.70, 7.68, 7.56, 7.40, 7.36, 7.33, 7.32, 6.46, 6.23, 5.97, 5.89, 5.83.

HRMS (ESI)

Calculated for C$_{117}$H$_{212}$N$_4$O$_{17}$Si$_9$ (M + Na$^+$): 2220.3669

Found: 2220.3606

Polyol 2.17

A HDPE bottle was charged with 2.16 (223.8 mg, 101.7 µmol, 1 eq) and THF (6.8 mL) and cooled to 0°C. A separate HDPE bottle was charged with THF (11.2 mL), pyridine (6.7 mL), and HF-pyridine (980 µL, 35.6 mmol, 350 eq). After cooling to 0°C the HF solution was cannula transferred slowly to the substrate solution. The reaction stirred for 5 minutes before warming to room temperature and stirring for 5 hours. The reaction was then cooled to 0°C and NaHCO$_3$ (sat. aq.) was added dropwise until bubbling ceased. The reaction was then transferred to a separatory funnel, the layers separated, and the organic phase washed with NaHCO$_3$ (40 mL).
The combined aqueous phase was then back extracted with DCM (3×60 mL). The organic phase was then washed with brine (2×50 mL), dried (Na$_2$SO$_4$), filtered, concentrated, and then azeotroped with toluene (30 mL). The crude yellow/orange solid was then purified by SiO$_2$ chromatography (97:3 → 19:1 → 92.5:7.5 → 9:1 DCM:MeOH) isolating 2.17 as a yellow solid (102.8 mg, 87.7 µmol, 86% yield).

TLC (DCM:MeOH 9:1)

$R_f = 0.47$, stained by CAM

$^1$H NMR (500 MHz, acetone-$d_6$)

$\delta$ 7.86 (d, $J = 7.6$ Hz, 2H), 7.74 (d, $J = 7.5$ Hz, 2H), 7.41 (t, $J = 7.5$ Hz, 2H), 7.36 – 7.30 (m, 2H), 6.52 – 6.13 (m, 12H), 5.91 (dd, $J = 15.1$, 7.6 Hz, 1H), 5.51 (dd, $J = 14.4$, 9.6 Hz, 1H), 5.37 – 5.31 (m, 1H), 4.77 (d, $J = 8.3$ Hz, 2H), 4.68 (s, 1H), 4.64 (app t, $J = 8.1$ Hz, 1H), 4.34 – 4.30 (m, 2H), 4.24 (app t, $J = 7.3$ Hz, 1H), 4.19 – 4.13 (m, 1H), 4.11 (app d, $J = 2.2$ Hz, 1H), 4.06 – 4.03 (m, 1H), 3.98 (app d, $J = 5.4$ Hz, 1H), 3.96 – 3.88 (m, 4H), 3.85 (app dd, $J = 12.8$, 3.5 Hz, 1H), 3.77 – 3.68 (m, 3H), 3.69 – 3.61 (m, 1H), 3.58 (app d, $J = 10.1$ Hz, 1H), 3.42 – 3.27 (m, 2H), 3.19 (s, 3H), 2.48 – 2.37 (m, 1H), 2.34 (dd, $J = 16.7$, 8.9 Hz, 1H), 2.30 – 2.21 (m, 2H), 2.02 – 1.90 (m, 2H), 1.89 – 1.83 (m, 1H), 1.71 – 1.57 (m, 1H), 1.57 – 1.38 (m, 6H), 1.26 (d, $J = 5.6$ Hz, 3H), 1.20 (d, $J = 6.4$ Hz, 3H), 1.11 (d, $J = 6.5$ Hz, 3H), 1.01 (d, $J = 7.2$ Hz, 3H).

$^{13}$C NMR (126 MHz, pyridine $d_5$:CD$_3$OD 10:1)

$\delta$ 172.20, 158.35, 145.45, 145.31, 142.31, 138.14, 138.03, 138.05, 136.05, 135.14, 135.00, 134.79, 134.49, 134.04, 133.98, 133.84, 133.71, 133.34, 133.07, 132.75, 130.82, 128.70, 128.14, 126.46, 126.39, 121.04, 102.27, 99.86, 78.58, 76.47, 76.07, 75.52, 75.35, 72.40, 72.23, 71.94, 71.20, 68.82, 68.52, 68.31, 67.45, 64.90, 59.16, 48.45, 45.48, 44.50, 43.99, 43.42, 42.13, 41.97, 37.08, 37.00, 31.39, 30.66, 30.58, 19.48, 19.32, 18.21, 13.11.

LRMS (ESI)
Calculated for C_{63}H_{86}N_{4}O_{17} (M + H'):

Found: 1193.5886 1193.5879

Hemiketal 2.18

Methyl ketal 2.17 (19.4 mg, 16.6 µmol, 1 eq) was dissolved in THF (1.46 mL) and H_{2}O (0.73 mL) in a 7 mL vial. A single crystal of CSA was added and the reaction was allowed to stir at room temperature for 3 hours. Then solid NaHCO_{3} was added to quench the CSA. The reaction filtered and the filtrate was collected, the volatiles removed in vacuo. The resulting oil was dissolved in DMSO and purified by preparative RP-HPLC yielding 2.18 as a yellow solid (7.8 mg, 6.74 µmol, 41%).

HPLC (analytical, 5 µm, 6x150 mm, C_{18}SiO_{2}; flow rate= 1.2 mL/min; 5mM NH_{4}OAc in H_{2}O : MeCN 19:1 → 1:19 over 26 minutes)

Retention time: 22.05 min

^1H NMR (500 MHz, DMSO-d_{6})

δ 7.89 (d, J = 7.6 Hz, 2H), 7.78 – 7.73 (m, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (td, J = 7.5, 3.6 Hz, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.51 – 6.22 (m, 8H), 6.19 – 6.03 (m, 4H), 5.94 (dd, J = 15.1, 8.8 Hz, 1H), 5.44 (dd, J = 14.9, 9.9 Hz, 1H), 5.34 – 5.28 (m, 1H), 5.23 – 5.17 (m, 1H), 4.84 – 4.73 (m, 1H), 4.65 – 4.59 (m, 1H), 4.53 (s, 1H), 4.43 (bd, J = 5.9 Hz, 1H), 4.39 – 4.33 (m, 1H),
4.28 – 4.17 (m, 2H), 4.10 – 4.01 (m, 2H), 3.74 – 3.59 (m, 1H), 3.69 – 3.66 (m, 2H), 3.62 (bd, J = 3.8 Hz, 2H), 3.55 – 3.48 (m, 1H), 3.49 – 3.40 (m, 1H), 3.33 (s, 1H), 3.30 (s, 1H), 3.23 – 3.13 (m, 1H), 3.12 – 3.07 (m, 1H), 2.32 – 2.24 (m, 1H), 2.16 (app d, J = 6.2 Hz, 3H), 2.04 – 1.96 (m, 1H), 1.94 – 1.87 (m, 1H), 1.76 – 1.69 (m, 1H), 1.63 – 1.49 (m, 6H), 1.48 – 1.35 (m, 3H), 1.35 – 1.28
(m, 2H), 1.17 (d, J = 5.8 Hz, 3H), 1.11 (d, J = 6.3 Hz, 3H), 1.04 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 7.1 Hz, 3H).

HRMS (ESI)

Calculated for C_{62}H_{83}N_{4}O_{17} (M + Na^+): 1179.5729

Found: 1179.5723

Ylide 2.19 and hydroxy methyl 2.20

To a 1.5 mL vial was added 2.18 (1 mg as a solution in 50 µL THF, 0.864 µmol, 1 eq) and PBu \text{3} (0.64 µL as a solution in 50 µL THF, 2.59 µmol, 1 eq) followed by a single drop of water. The reaction was then allowed to stir at room temperature for 3 hours. The reaction was monitored by LC/MS (1.8 µm, 2.1 x 50 mm, C_{18}; flow rate= 0.4 mL/min; H\textsubscript{2}O : MeCN (both containing 0.1 % HCO\textsubscript{2}H) 19:1 → 1:19 over 8 minutes). Representative LC/MS traces are shown below. In the bottom trace PBu \text{3} was exchanged with PMe \text{3}.

2.19 LRMS (ESI from LC/MS)

Calculated for C_{74}H_{111}N_{2}O_{17} (M + H^+): 1331.77

Found: 1331.76
2.20 LRMS (ESI from LC/MS)

Calculated for C\textsubscript{62}H\textsubscript{85}NO\textsubscript{18} (M + Na\textsuperscript{+}): 1154.57

Found: 1154.67

Azide 2.21

Methyl ketal 2.17 (50.6 mg, 42.3 µmol, 1 eq) was dissolved in THF (3.79 mL) and H\textsubscript{2}O (1.89 mL) in a 7 mL vial. A single crystal of CSA was added and the reaction was allowed to stir at room temperature for 1 hour and 15 minutes. Then piperidine (85 µL, 860 µmol, 20 eq) was added and the reaction stirred an additional 2 hour at room temperature. THF was removed \textit{in vacuo} and then toluene was added remove the H\textsubscript{2}O by co-evaporation. The resulting oil was dissolved in DMSO and purified by preparative RP-HPLC (5 µm, 30x250 mm, C\textsubscript{18} SiO\textsubscript{2}; flow rate= 25 mL/min; 0.1% Et\textsubscript{3}N in H\textsubscript{2}O : MeCN 19:1 → 1:19 over 18 minutes) yielding 2.21 as a yellow solid (25.7 mg, 27.4 µmol, 64%).
HPLC (analytical, 3.5 µm, 4.6x75 mm, C18 SiO2; flow rate= 1.2 mL/min; 0.1% Et3N in H2O : MeCN 19:1 → 1:19 over 8 minutes)
Retention time: 5.7 min

1H NMR (500 MHz, pyridine d-5:CD3OD 10:1)

δ 6.84 - 6.73 (m, 2H), 6.66 - 6.35 (m, 11H), 5.86 - 5.80 (m, 1H), 5.55 (dd, J = 15.2, 10.1 Hz, 1H), 5.06 (s, 1H), 4.92 (app t, J = 7.0 Hz, 1H), 4.89 - 4.75 (m, 2H), 4.68 (app t, J = 9.4 Hz, 1H), 4.55 - 4.46 (m, 2H), 4.15 (app t, J = 10.0 Hz, 1H), 4.06 (app d, J = 11.0 Hz, 1H), 4.00 (dd, J = 12.7, 3.9 Hz, 1H), 3.92 - 3.83 (m, 2H), 3.74 - 3.67 (m, 1H), 3.67 - 3.61 (m, 1H), 3.44 (app d, J = 9.5 Hz, 1H), 3.28 (app d, J = 9.5 Hz, 1H), 2.75 - 2.60 (m, 3H), 2.57 - 2.48 (m, 3H), 2.48 - 2.38 (m, 2H), 2.25 - 2.18 (m, 1H), 2.13 - 2.09 (m, 1H), 2.06 - 2.00 (m, 2H), 1.96 (dd, J = 14.2, 10.2 Hz, 2H), 1.91 - 1.69 (m, 6H), 1.64 (app d, J = 14.3 Hz, 1H), 1.57 (d, J = 6.2 Hz, 3H), 1.48 (d, J = 6.4 Hz, 3H), 1.32 (d, J = 6.3 Hz, 3H), 1.26 (d, J = 7.0 Hz, 3H).

13C NMR (126 MHz, pyridine d-5:CD3OD 10:1)

δ 174.69, 172.24, 138.13, 137.69, 135.03, 134.91, 134.53, 134.25, 133.83, 133.67, 133.56, 133.53, 133.26, 132.99, 130.29, 126.71, 123.57, 99.42, 98.29, 78.79, 77.22, 76.53, 75.34, 74.89, 73.95, 72.23, 71.52, 70.42, 69.86, 68.61, 66.99, 64.74, 58.30, 50.07, 47.84, 46.93, 45.41, 44.03, 43.24, 41.21, 41.07, 37.27, 36.79, 32.01, 30.38, 22.21, 19.31, 18.88, 17.64, 13.03.

HRMS (ESI)

Calculated for C47H74N4O15 (M + H+): 935.5229
Found: 935.5215

C41amAmB
To a 1.5 mL vial was added 2.21 (4 mg, 4.28 µmol, 1 eq) followed by propane dithiol (6.4 µL as a solution in 400 µL DMSO, 64.2 µmol, 15 eq) and triethylamine (8.95 µL as a solution in 400 µL DMSO, 64.2 µmol, 15 eq). The reaction was then allowed to stir at room temperature for 21 hours. The crude reaction mixture was then diluted with DMSO (~3 mL) and directly purified by preparatory RP-HPLC (5 µm, 30x150 mm, C18; flow rate= 25 mL/min; 0.1% Et3N in H2O : MeCN 19:1 → 1:19 over 18 minutes). C41amAmB could presumably be isolated, but then easily decomposed to a number of byproducts.

Isocyanate 2.27

To a 40 mL vial was added 2.26 (602.6 mg, 275.3 µmol, 1 eq),36 and benzene (13.7 mL). Next, triethylamine (115 µL, 0.822 mmol, 3 eq) was added followed by DPPA (71 µL, 33.0 mmol, 1.2 eq). The reaction was then placed in a preheated heating block at 80°C and allowed to stir for 3.5 hours. The reaction was then transferred to a 125 mL separatory funnel with water (25 mL) and diethyl ether (50 mL). The layers were separated and the organic layer was washed with brine (25 mL), dried over Na2SO4, filtered and concentrated in vacuo. The resulting red/orange oil was then purified by SiO2 chromatography (100:0 to 0:100 Hexane:Et2O) yielding 2.27 as an orange solid (168.7 mg, 0.077 mmol, 28% yield).

TLC (hexanes : Et2O 7:3)

Rf = 0.64, visualized by CAM
$^1$H NMR (500 MHz, Acetone-$d_6$)

$\delta$ 7.87 (d, $J = 7.5$ Hz, 2H), 7.70 (d, $J = 7.5$ Hz, 2H), 7.42 (t, $J = 7.4$ Hz, 2H), 7.37 – 7.29 (m, 2H), 6.59 – 6.11 (m, 12H), 6.06 (dd, $J = 15.6$, 5.9 Hz, 1H), 5.51 (dd, $J = 14.8$, 9.4 Hz, 1H), 5.36 (d, $J = 9.7$ Hz, 1H), 4.70 (s, 1H), 4.69 – 4.64 (m, 2H), 4.48 (dd, $J = 10.5$, 6.5 Hz, 1H), 4.35 (dd, $J = 10.5$, 6.6 Hz, 1H), 4.24 (app t, $J = 6.6$ Hz, 1H), 4.18 (s, 1H), 4.16 – 4.05 (m, 2H), 4.04 – 3.98 (m, 1H), 3.97 (app d, $J = 3.1$ Hz, 1H), 3.86 (app dd, $J = 9.0$, 2.9 Hz, 1H), 3.80 (app t, $J = 9.4$ Hz, 1H), 3.73 – 3.60 (m, 2H), 3.48 (app t, $J = 9.0$ Hz, 1H), 3.38 – 3.31 (m, 1H), 3.29 (app t, $J = 9.7$ Hz, 1H), 3.16 (s, 3H), 2.48 – 2.41 (m, 1H), 2.38 (dd, $J = 14.8$, 6.2 Hz, 1H), 2.20 – 2.14 (m, 1H), 1.96 – 1.87 (m, 2H), 1.87 – 1.81 (m, 4H), 1.69 – 1.60 (m, 3H), 1.57 – 1.47 (m, 1H), 1.29 (s, 4H), 1.25 (d, $J = 6.2$ Hz, 3H), 1.18 (d, $J = 6.0$ Hz, 3H), 1.10 – 0.88 (m, 89H), 0.80 – 0.50 (m, 54H).

IR (thin film, cm$^{-1}$)

2956.35, 2912.00, 2877.29, 2250.53, 1731.77, 1590.99, 1488.78, 1457.93, 1415.50, 1378.86, 1303.65, 1272.79, 1238.08, 1205.29, 1184.08, 1160.94, 1108.87, 1076.09, 1008.59, 966.16, 738.60.

HRMS (ESI)

Calculated for C$_{117}$H$_{210}$N$_2$O$_{18}$Si$_9$ (M$+\text{Na}^+$): 2206.3400

Found: 2206.3413

Isopropyl Carbamate 2.28

To a 1.5 mL vial was added 2.27 (as a stock solution (100 µL of 150 mg in 1.5 mL benzene) 10 mg, 4.57 µmol, 1 eq), and Titanium isopropoxide (as a stock solution (50 µL of 25 µL in 4.6 mL benzene) 0.27 µL, 0.914 µmol, 0.2 eq) and THF (80 µL). The reaction was then allowed to stir at room temperature for 1 hour. The reaction was then diluted with water (1.5 mL)
and diethyl ether (1.5 mL). The layers were separated and the organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The resulting red/orange oil was then purified by SiO₂ chromatography (100:0 to 80:20 hexane:Et₂O) yielding 2.28 as an orange solid.

**TLC (hexanes : Et₂O 7:3)**

$R_f = 0.51$, stained by CAM

$^1$H NMR (500 MHz, Acetone-$d_6$)

\[ \delta \text{ ppm} \]

- $7.88$ (d, $J = 7.5$ Hz, 2H), $7.70$ (d, $J = 7.5$ Hz, 2H), $7.43$ (t, $J = 7.3$ Hz, 2H), $7.36 - 7.32$ (m, 2H), $6.59 - 6.08$ (m, 12H), $6.03$ (dd, $J = 15.5, 6.1$ Hz, 1H), $5.51$ (dd, $J = 14.9, 9.5$ Hz, 1H), $5.35$ (d, $J = 9.9$ Hz, 1H), $4.87$ (p, $J = 6.3$ Hz, 1H), $4.77 - 4.73$ (m, 1H), $4.71 - 4.67$ (m, 1H), $4.65$ (s, 1H), $4.48$ (dd, $J = 10.5, 6.5$ Hz, 1H), $4.37$ (dd, $J = 10.4, 6.5$ Hz, 1H), $4.25$ (app t, $J = 6.3$ Hz, 2H), $4.18 - 4.09$ (m, 1H), $4.07 - 3.97$ (m, 2H), $3.87 - 3.84$ (m, 1H), $3.76$ (app dd, $J = 11.8, 6.9$ Hz, 1H), $3.70$ (d, $J = 8.9$ Hz, 1H), $3.74 - 3.66$ (m, 2H), $3.47 - 3.34$ (m, 2H), $3.35 - 3.28$ (m, 1H), $3.15$ (s, 3H), $2.58$ (d, $J = 6.6$ Hz, 1H), $2.47 - 2.40$ (m, 2H), $2.26$ (app dd, $J = 15.6, 7.4$ Hz, 2H), $2.20 - 2.15$ (m, 1H), $1.94 - 1.85$ (m, 4H), $1.84 - 1.80$ (d, $J = 13.1$ Hz, 3H), $1.79 - 1.68$ (m, 4H), $1.68 - 1.61$ (d, $J = 9.3$ Hz, 2H), $1.54 - 1.56$ (s, 1H), $1.26$ (app dd, $J = 6.2, 3.2$ Hz, 6H), $1.22$ (d, $J = 6.2$ Hz, 3H), $1.18$ (d, $J = 6.0$ Hz, 3H), $1.14 - 0.83$ (m, 87H), $0.81 - 0.53$ (m, 54H).

**LRMS (ESI)**

Calculated for $C_{120}H_{218}N_2O_{19}Si_9$ (M+ Na$^+$): 2266.4

Found: 2266.6.
Pyrroles 2.38 and 2.39

First, stock solutions of DPPA (3.49 μL in 540 μL DMF, 16.2 μmol, 1.5 eq) and triethylamine (4.52 μL in 540 μL DMF, 32.5 μmol, 3 eq) were made. Then, 540 μL of each solution was added to a 1.5 mL vial containing AmB (10 mg, 10.8 μmol, 1 eq), and the vial was heated to 50°C. After stirring for 3 hours the reaction was poured into 40 mL 1:1 hexanes : diethyl ether. The suspension was spun down in a centrifuge at 4000 G for 5 minutes. The supernatant was decanted and the resulting yellow pellet was dissolved in DMSO and purified by preparatory RP-HPLC (Waters SunFire Prep C\textsubscript{18} OBD 5 micron 30 x 150 mm; 25mL/min flow rate, 0.1 % Et\textsubscript{3}N in H\textsubscript{2}O : MeCN 19:1 \rightarrow 29:71 over 12.5 minutes).

HRMS (ESI)

Calculated for C\textsubscript{46}H\textsubscript{71}N\textsubscript{2}O\textsubscript{3} (M+ H\textsuperscript{+}): 859.4956

Found: 859.4972

HPLC (analytical, C\textsubscript{18} SiO\textsubscript{2}; flow rate= 1.2 mL/min; 0.1% Et3N in H\textsubscript{2}O : MeCN 19:1 \rightarrow 1:19 over 8 minutes)

Retention time: 5.21 min

\textsuperscript{1}H NMR (500MHz, pyridine d-5: CD\textsubscript{3}OD 1:1)

NMR analysis was performed on an approximate 1:1 mixture of 2.38 and 2.39. Doublets at 6.33 and 6.18, both with a coupling constant of 3.1 Hz, are consistent with pyrrole formation. COSY analysis of this mixture revealed that the C17 \textsuperscript{1}H is pulled downfield to 5.07 and 5.23 ppm. Furthermore the mixture is of two different constitutional isomers, C35 and C37.
macrolactones. Cross peaks corresponding to 2.38 are designated as 33, 34, etc…, while cross peaks corresponding to 2.39 are designated 33a, 34a, etc… The $^1$H attached to the carbon bearing the macrolactone oxygen is shifted substantially downfield. For example, in 2.38 the C37 $^1$H can be found at 5.73 ppm, but is at 4.26 ppm in 2.39.

Figure 2.7: COSY analysis of a mixture of pyrroles 2.38 and 2.39.

Synthesis and characterization of oxazolidinone 2.32 and urea 2.33 will be detailed in Chapter 3.

Planar Lipid Bilayer Experiments

General Information.

All data were acquired using a Warner Instruments (Hamden, CT) BC-535 amplifier and the data were filtered using a built in 4 pole Bessel filter with a cutoff frequency of 5 kHz. The headstage and delrin cell were housed within a Warner Instruments model FC-1 Faraday cage. The solutions were stirred using a Warner Instruments SUNstir-3 stirplate. The signal was passed through a Warner Instruments low pass 8 pole Bessel filter with a frequency cutoff of 1 kHz. The filtered data were sampled at a rate of 10 kHz using a Molecular Devices (Sunnyvale, CA)
Digidata 1440 data acquisition system and the data were analyzed using Molecular Devices pClamp 10 software. Salt bridges were prepared monthly and were fabricated from 1.5 mm OD, 0.86 mm ID borosilicate capillary tubing and were filled with 1 M aqueous KCl with 2.5% agar. Prior to a day’s experiments, silver electrodes were plated by submerging in commercial bleach for 15 to 30 minutes. The electrodes were plated periodically throughout the day.

**Preparation of Lipid Solution**

Lipids were obtained from Avanti Polar Lipids as 10 mg/ml solutions in CHCl₃. The solutions were stored at -20 °C under dry argon and used within 3 months. A 4 mg/mL solution of ergosterol in CHCl₃ was prepared monthly and stored at -20 °C under dry argon. Lipid films were prepared by charging a 1.5 mL vial with 60 µL DPhPC, and 30 µL ergosterol. The solvent was removed with a gentle stream of nitrogen. The lipid film was then dissolved in 30 µL n-decane to give the 20 mg/ml solution of lipids used for the electrophysiology experiments. The decane solutions were used within 3 hours of preparation.

**Formation of planar lipid bilayers.**

Custom manufactured polysulfone cups were machined with a 150 µm aperture (modeled after Warner Instruments model 64-0416, Hamden, CT). The area around the hole was then primed with 1 µL of the decane lipid solution. The primed cup was left to stand for approximately 10 minutes such that most of the decane evaporated. Then 1 mL of 1 M KCl, 5 mM HEPES pH 7.0 buffer was added to each chamber. The membrane was formed by sequential vertical swabs across the hole using a flame polished glass applicator that had been previously dipped into the lipid solution. The formation of a membrane was detected by a reduction in the current to 0 pA. The integrity of this membrane was confirmed by applying a potential of 150 mV for approximately one minute. If the current increased by >1 pA upon voltage introduction, the membrane was rejected. Membranes were between 45 and 105 pF in size.

**Interrogating Channel Formation.**

If the membrane was acceptable, 0.25 - 1 µL of a compound in DMSO was added to both chambers and the solutions were stirred with zero applied potential for 4 minutes. After 4 minutes the stirring was stopped, and 150 mV of potential was applied across the membrane. The
formation of single AmB channels under similar conditions has been well documented.\textsuperscript{13-17,68-70} The concentration of AmB and C3deOAmB required to observe channel activity varied from membrane to membrane and appeared to depend on the membrane capacitance. For both AmB and C3deOAmB, single channel formation was observed at concentrations between 0.25 and 5 nM.

To measure conductance of AmB and C3deOAmB the same basic experimental setup was used with the following slight modifications. A 2M KCl, 5 mM HEPES pH=7.0 bathing solution was used instead of the 1 M KCl, 5 mM HEPES pH=7.0 solution. Upon bilayer formation and addition of compound to both sides of the membrane channel activity was investigated at 90, 120, 150, 180, and 210 mV applied potential.

**Single Channel Characterization**
All data processing was performed within the pClamp v10 software suite. Following acquisition, the data was digitally filtered to 20 Hz. Then using the single channel search feature (ignore short level changes of 25 ms or less, update baseline automatically) the amplitude of the open and closed states was determined. The delta between the open and closed levels represents the single channel current at the given applied voltage.
Figure 2.8: Three representative single ion traces formed by AmB in 1M KCl, 5 mM HEPES pH=7.0, +150 mV.
Figure 2.9: Three representative single ion traces formed C3deOA’mB in 1M KCl, 5 mM HEPES pH=7.0, +150 mV.
Figure 2.10: Single ion channel recordings of both AmB (left) and C3deOAmB (right) at 90, 120, 150, 180, and 210 mV in 2M KCl, 5 mM HEPES pH=7.0.

2-6 REFERENCES

50 M. J. Driver; W. S. Maclachlan; A. R. Greenlees, *1990 Patent*
CHAPTER 3: NON-TOXIC ANTIMICROBIALS THAT EVADE DRUG-RESISTANCE

The evolution of resistance to antimicrobials is a global health crisis and is widely presumed to be inevitable. Yet, the antifungal drug amphotericin B (AmB) has eluded resistance despite 50 years of worldwide, single-agent use. AmB is unusual amongst antimicrobials in that it kills fungi by binding a membrane sterol (ergosterol), rather than a mutable protein. Mutations that alter sterol biosynthesis in a manner that confers resistance abrogate fungal virulence, explaining the failure of fungi to evolve AmB resistance in the clinic. Unfortunately, AmB also binds cholesterol, the primary sterol in humans, likely resulting in its dose-limiting and irreversible renal toxicity. Thus, more specific and less toxic derivatives of AmB would have tremendous clinical impact. However, it is generally assumed that more selective compounds are more vulnerable to resistance. Guided by a model in which ergosterol selective binding could be achieved via allostERIC-modification, families of AmB urea derivatives were scalably synthesized in just three synthetic steps from the fermented natural product (chemistry originally developed in Chapter 2). The new derivatives were far less toxic to human cells and to mice than AmB and were more effective in a mammalian model of systemic candidiasis. Surprisingly, the high specificity of these compounds did not compromise their ability to evade resistance. Exhaustive selections for resistance yielded only strains that concomitantly lost virulence. Therefore, highly selective antimicrobial action and evasion of resistance are not mutually exclusive, and this new class of amphotericins has exceptional potential to yield a less toxic treatment for life-threatening invasive fungal infections.

ITC experiments, MIC in S. cerevisiae, red blood cell hemolysis (MHC), and kidney cell toxicity (MTC) experiments were performed by Matt Endo. Invasive Candida MIC panel, and mouse efficacy and toxicity studies were performed by David Andes’ lab at the University of Wisconsin. All resistance and fitness studies were performed by Ben Vincent in Susan Lindquist’s lab at Massachusetts Institute of Technology. Secondary ureas were synthesized by Sarah Tucker. Portions of this chapter were adapted from Stephen A. Davis, Benjamin M. Vincent, Matthew M. Endo, Luke Whitesell, Karen Marchillo, David R. Andes, Susan Lindquist, and Martin D. Burke, Non-Toxic Antimicrobials that Evade Drug Resistance, submitted.
3-1 Background

Less selective pharmacological action is generally associated with decreased vulnerability to resistance, but also with increased toxicity.\textsuperscript{1,2} The classic example is amphotericin B (AmB), an exceptionally resistance-evasive but also highly toxic antifungal agent that has remained the last line of defense in treating invasive fungal infections for over half a century.\textsuperscript{3} An excess of 1.5 million people die from such infections each year,\textsuperscript{4} in part because the extreme toxicity of AmB is dose-limiting.\textsuperscript{3} Liposomal formulations of AmB have shown modestly decreased toxicity, but these are often prohibitively expensive and substantial toxicities still remains.\textsuperscript{5} Extensive efforts to develop a clinically viable, less toxic amphotericin have been made, but without success.\textsuperscript{6} Moreover, it has remained unclear whether such a decrease in toxicity would come at the cost of an increase in vulnerability to pathogen resistance.

Resistance to antimicrobials is driving an escalating global health crisis, and is presumed to be inevitable. Most antimicrobials bind microbe-specific but mutable proteins and thus may have a high therapeutic index, yet remain vulnerable to the rapid evolution of target-related resistance. For example, AmB was replaced as a frontline therapy by the less toxic triazoles, which target ergosterol biosynthesis proteins.\textsuperscript{7} Resistance to these less toxic therapies began arising shortly after their clinical introduction.\textsuperscript{8} Additionally, fluconazole-resistant candidiasis has been recognized by the CDC as a serious health threat.\textsuperscript{9} In contrast, AmB has no known protein target. Instead, AmB kills by binding the lipid ergosterol,\textsuperscript{10} which is critical for many aspects of fungal cell physiology.\textsuperscript{11-15} Likely due to promiscuous sterol binding,\textsuperscript{16} substantial changes in sterol structure are required for yeast to become resistant to AmB in vitro. The associated fitness costs render such strains non-pathogenic.\textsuperscript{17} AmB also binds cholesterol, the primary human sterol, likely resulting in its severe toxicity to human cells.\textsuperscript{16} This analysis leads to the unwelcome prediction that a more ergosterol-selective could lose the capacity to evade drug-resistance.

For decades the pursuit of less toxic amphotericins was guided by the widely accepted model that AmB kills cells via membrane permeabilization.\textsuperscript{6,18} The search for less toxic AmB derivatives focused on the challenging task of selectively forming ion channels in yeast but not human cells. Recent mechanistic advances have shown that AmB primarily exists as a large extramembranous aggregate which kills yeast by binding\textsuperscript{10} and extracting\textsuperscript{19} ergosterol from the lipid membrane. These advances in mechanistic understanding suggest a simplified roadmap to
improve the therapeutic index of AmB. A non-toxic AmB derivative would simply bind ergosterol, but not cholesterol.

Knowing that the mycosamine appendage is critical for binding sterols, Wilcock et al. synthesized an AmB derivative in which only the C2’ oxygen was removed. This derivative, C2’deoxyAmB (C2’deOAmB) (Figure 3.1A), binds ergosterol but surprisingly did not bind cholesterol. Consistent with the selective sterol binding hypothesis, C2’deOAmB was toxic to yeast but not human cells, making C2’deOAmB an exciting potential clinical replacement of AmB. Unfortunately, limited synthetic access to this derivative has so far hindered further preclinical development as well as testing the impact of increased sterol selectivity and decreased toxicity on its ability to evade resistance.

3-2 An allosteric modification model for sterol selective binding

To rationalize the greater ergosterol-selective binding observed with C2’deOAmB, we took into consideration several new structural insights regarding these prototypical small molecule-small molecule interactions. First, the mycosamine appendage is critical for binding both ergosterol and cholesterol (Figure 3.1A). Recent solid-state NMR evidence also confirms direct contact between the ergosterol tetracyclic core and the AmB polyene motif in the sterol sponge complex. Furthermore, a recent crystal structure of an AmB derivative (Figure 3.1C) reveals a water-bridged hydrogen bond between the C2’ and the C13 hydroxyl groups. We propose that such intramolecular hydrogen bonding fixes the relative positions of the mycosamine appendage and the polyene motif, enabling AmB to bind both ergosterol and cholesterol (Figure 3.2B). Deletion of the C2’ hydroxyl group could favor a shift to an alternate conformer that selectively binds ergosterol (Figure 3.1A, 3.2C), but not cholesterol due to it’s larger steric profile resulting from an extra degree of saturation in the B ring. Alternatively stated, this model predicts that a protein-like ligand-selective allosteric effect underlies these small molecule-small molecule interactions. Based on this model, disruption of any rigidifying interaction between the macrolide and mycosamine could cause a similar conformational shift and therefore sterol selective binding (Figure 3.2D). A putative salt bridge between the C41 carboxylate and C3’ ammonium (Fig 3.1A) thus becomes an intriguing target for modification (Figure 3.1A).
Figure 3.1: Allosteric modification model of AmB-Sterol binding. A.) Chemical structures of AmB and C2’deOAmB. B.) Structures of both ergosterol (top) and cholesterol (bottom) depicted as both chemdraw and X-ray structures C.) X-ray crystal structure of N-iodoacylAmB showing an intramolecular water bridged hydrogen bond between the C2’ and C-13 hydroxyl groups.

Figure 3.2: Schematic representation of allosteric modification binding model. A.) AmB, ergosterol, and cholesterol structures. B.) AmB is capable of binding both ergosterol and cholesterol. C.) Removal of C2’ alcohol abolishes intramolecular hydrogen bond between mycosamine and macrolide resulting in a conformational change. D.) Modifying the C16 carboxylate could disrupt the rigidifying intramolecular salt bridge between C16 and C3’ causing a similar conformational.

Many derivatives of the C41 carboxylate have previously been synthesized, resulting in modest but promising improvements in therapeutic index. Intriguingly, all previously reported derivatives maintained a C16–C41 carbon–carbon bond. As detailed in Chapter 2, while investigating the molecular basis of ion selectivity in the AmB ion channel, a Curtius rearrangement was discovered which cleaved this carbon-carbon bond and appended a nitrogen atom to the C-16 carbon. This new AmB-urea (Figure 3.3, AmBAU) represented an intriguing opportunity to test this allosteric modification model. The yeast and human cell toxicity of
AmBAU was compared head to head against AmB and AmBME (Figure 3.3). AmB is a potent antifungal with an MIC against *S. cerevisiae* of 0.5 µM, and is highly toxic against human cells with an MHC of only 8.5 µM. AmBME, the only AmB derivative to undergo clinical trial as a potential replacement therapy, had a modestly improved therapeutic index maintaining antifungal activity (MIC 0.25 µM) while being slightly less toxic to human cells (MHC 31 µM). AmBAU also maintained potent antifungal activity (MIC 0.25 µM), but was strikingly less toxic to red blood cells with an MHC >100 µM.

This dramatic increase in therapeutic index of AmBAU, accompanied with an efficient 4-step synthesis from AmB was immediately exciting. We wanted to understand at the molecular level if the same sterol selective binding observed with C2’deOAmB was underlying this improvement in therapeutic index of AmBAU. Furthermore, we asked if improving the therapeutic index by replacing the carboxylate with a urea was a general phenomenon, thereby allowing for drug optimization by modification of the urea. Most importantly, the efficient synthesis of AmBAU would allow quantities sufficient to explore the relationship between AmB’s target selectivity, cell toxicity, and ability to evade resistance.

![Molecule structure](image)

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<th>MHC (µM) red blood cells</th>
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<td></td>
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<td>&gt;100</td>
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*Figure 3.3:* Initial therapeutic index assay comparing AmB, AmBME, and AmBAU showing both minimum inhibitory concentration (MIC) against *S. cerevisiae* and minimum hemolytic concentration (MHC) causing 90% hemolysis against human red blood cells.
3-3 The synthesis of a family of amphotericin B ureas

We first set out to synthesize a family of AmB urea compounds (Scheme 3.1). To achieve this goal, a minimally protected variant of AmB (2.29) was treated with diphenyl phosphoryl azide (DPPA) promoting the stereospecific Curtius rearrangement that cleaves the C16–C41 bond. The resulting isocyanate (2.30) is then intramolecularly trapped by the neighboring C15 alcohol\(^{30}\) to form the versatile oxazolidinone 2.31. This particular oxazolidinone was surprisingly reactive to ring-opening with primary amines under mild conditions. Exposure of 2.31 to simple methylvamine in THF at room temperature cleanly opened the oxazolidinone to a urea while cleaving the Fmoc group. Acidic aqueous workup then hydrolyzed the methyl ketal, generating AmBMU. Alternatively employing ethylene diamine, as previously mentioned, similarly grants access to AmBAU. Protected amino acids also can be utilized in this process, as β-alanine, protected as its allyl ester, is capable of forming the carboxylethyl urea (AmBCU) upon allyl deprotection.

Scheme 3.1: Synthesis of a family of AmB ureas.

We further found that 2.29 can be directly converted into AmBMU or AmBAU in a scalable one-pot operation involving serial addition of DPPA, an amine, and aqueous acid (Scheme 3.2). Starting with 1 g of fermented AmB and using methylvamine as the nucleophile, this overall three-step sequence yielded 264 mg of AmB methyl urea (AmBMU). Employing
ethylene diamine produced 236 mg of AmB amino urea (AmBAU). In a four step-variant, reaction with β-alanine allylester followed by deallylation yielded 124 mg AmB carboxylatoethyl urea (AmBCU), thus providing rapid access to these new derivatives. We further anticipate this route should be very scalable. The starting material, AmB, is already fermented on the metric ton scale. All of the reagents used in this synthesis are already employed on the process scale to prepare other pharmaceuticals, including diphenyl phosphoryl azide, which has important but manageable safety considerations. Additionally this three-step synthesis requires only a single HPLC purification, mirroring the industrial process which provides global supply of the clinically vital antimycotic Caspofungin.34

Scheme 3.2: Large-scale access of AmB ureas enabled by efficient 3-step synthesis from fermented AmB.

3-4 AmB ureas selectively bind ergosterol, maintain antifungal activity, and are significantly less toxic in vitro

With this new series of AmB derivatives in hand, we determined their sterol binding properties via isothermal titration calorimetry (ITC) (Figure 3.4).20 Titrating a suspension of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) large unilamellar vesicles (LUVs) or POPC LUVs containing 10% ergosterol into an aqueous solution of AmB revealed an increase in net exotherm for the ergosterol-containing LUVs. When cholesterol containing LUVs were used, a change in net exotherm was also observed. Therefore, AmB binds both ergosterol and cholesterol.16,20 The aglycone, amphoteronolide B (AmdeB), as previously reported binds neither sterol.16 Like C2’dEOAmB,20 all of the new C16 urea-containing derivatives retained the
capacity to bind ergosterol but, within the limits of detection of this experiment, showed no binding to cholesterol.

Figure 3.4: Total net isotherms from ITC showing AmB or derivatives binding to ergosterol (top) or cholesterol (bottom). Values represent the mean of at least three experiments ± SD. *, p<0.05; **, p<0.001; NS, not significant.

We next determined if the improved sterol selectivity would translate into a major improvement in therapeutic index *in vitro* for all ureas (Figure 3.5). Specifically, we determined the minimum inhibitory concentration (MIC) against *Saccharomyces cerevisiae* and the minimum hemolytic concentration (MHC) against human red blood cells for AmB, a series of previously reported C41 and/or C3'-modified derivatives, and the new AmB ureas. AmB is a potent antifungal (MIC 0.5 µM), but is also highly toxic to human red blood cells (MHC 8.6 µM) (entry 1). AmdeB is non-toxic to both (entry 2). Previously reported modifications including methyl esterification (AmBME), carboxylic acid reduction (C41MeAmB), methyl amidation (AmBMA), and double modification to form a triazoloethyl amide bis-aminopropyl derivative (AmBTABA) produced modest improvements in therapeutic index (entries 3-6), with the best results obtained with AmBTABA (MIC 0.25 µM, MHC 49 µM) (entry 6). In contrast, all of the AmB urea derivatives retained antifungal activity but showed dramatically reduced toxicity to human red blood cells. The MHC of AmBMU and AmBAU exceeded the limits of solubility in this assay (500 µM) (entries 7,8). Remarkably, the only structural difference between AmBMA (entry 5) and AmBMU (entry 7) is the insertion of a protonated nitrogen atom between the C16 and C41 carbons (Figure 3.1A,C).
The activities of the AmB urea derivatives were further tested against a series of clinically relevant yeast and human cell lines (Figure 3.6). Against a panel of pathogenic Candida strains, both AmBMU and AmBAU demonstrated potent antifungal activity against all strains tested (Fig. 2c). AmBCU retained activity, but was in general somewhat less potent. The compounds were also tested for toxicity against human renal proximal tubule epithelial cells (RPTEC), the critical site of toxicity for AmB in patients. They all showed little or no toxicity to hTERT1 RPTEC, and substantially reduced toxicity to the more sensitive primary RPTEC.
Figure 3.6: MIC against clinically relevant yeast pathogens (C. albicans, C. glabrata, C. tropicalis, C. parapsilosis) and minimum toxic concentration (MTC) causing 90% toxicity against human hTERT1 renal proximal tubule epithelial cells (RPTEC) and human primary RPTECs.

3-5 AmB ureas are more efficacious and less toxic than AmB in mice

Due to AmBMU and AmBAU’s enhanced antifungal activity relative to AmBCU, accompanied with drastically reduced human cell toxicity, these compounds were judged to be especially promising and further evaluated for efficacy and toxicity in vivo. Efficacy was evaluated in a mouse model of disseminated candidiasis (Figure 3.7). In this model, neutropenic mice were inoculated with C. albicans, then two hours post infection a single IP injection of compound was administered. Efficacy was then evaluated by determining the kidney fungal burden at various time points (2, 6, 12, and 24 hours) post inoculation. Both AmBMU and AmBAU were substantially more effective than AmB at reducing fungal burden in the kidneys at all three tested doses (1, 4 and 16 mg/kg). The differences in efficacy were most pronounced at the 16 mg/kg dose at 24 hours post inoculation. Relative to AmB, AmBMU reduced the fungal burden by 1.2 log units (p ≤ 0.0001), and AmBAU reduced the fungal burden by nearly 3 log units (p ≤ 0.0001). We speculate that an improved pharmacological profile, potentially due to a >20 fold increase in water solubility relative to AmB, may contribute to this unexpected dramatic improvement in antifungal activity for the new compounds in vivo.

We next determined if the decreased in vitro human cell toxicity would translate into decreased toxicity in a mouse model. Acute toxicity was determined by single intravenous administration of AmB or its derivatives to healthy, uninfected mice and monitoring for lethality up to 24 hours (Figure 3.8). All mice in the 4 mg/kg AmB dosage group died within seconds. AmBAU was drastically less toxic, with >50% lethality only observed at the 64 mg/kg dosage group. Strikingly, all mice dosed with even 64 mg/kg AmBMU survived with no observable toxicity.
Figure 3.7: Quantification of the fungal burden in the kidneys of *C. albicans* infected neutropenic mice 2, 6, 12, and 24 hours after a single IP injection of AmB, AmBMU, or AmBAU at dosages of a. 1 b. 4 or c. 16 mg/kg. P-values are relative to AmB at each indicated time point, *, p≤0.05, **, p≤0.001, ***, p≤0.0001. All compounds were HPLC purified.

Figure 3.8: Dose response toxicity assessed via determination of lethality upon single IV injection of AmB, AmBMU, and AmBAU to healthy mice at doses of ranging from 0.5 to 64 mg/kg (5 mice per dosage). Mice were monitored for survival up to one day.

These results support the novel ligand-selective allosteric-effect model for guiding the rational development of non-toxic amphotericins. This model predicts that disruption of intramolecular polar interactions between functional groups on the macrolide core and the mycosamine appendage cause a conformational shift in the molecule, from one that binds both ergosterol and cholesterol to one that selectively binds ergosterol. Intriguingly, the portion of AmB that contains all of these functional groups, i.e., the module comprised of C12-C23, is 100% conserved in every member of the mycosamine-bearing polyene macrolide family of natural products (Figure 3.9).\textsuperscript{44} This includes a collection of “aromatic polyenes” that are reported to be orders of magnitude more potent than AmB\textsuperscript{45} and effective against *Aspergillus*
infections which are often very challenging to treat. Synthesizing the analogous urea derivatives of other polyene macrolide natural products could lead to ultrapotent and/or broader spectrum, yet still minimally toxic new antifungal agents.

Because all of the amphotericin B (AmB) urea derivatives we have prepared so far showed no detectable binding to cholesterol and dramatic decreases in toxicity (Figures 3.6, 3.7, 3.10), it is reasonable to expect that many additional AmB urea derivatives will share these critical features. Moreover, oxazolidinone 2.31 represents an easily accessible and highly versatile intermediate, and its surprising reactivity with nucleophiles suggests the potential to access many such derivatives using a wide range of commercially available amines. This strategy represents a substantial opportunity for extensive optimization of the pharmacological properties of this new family of less toxic amphotericins.

In this vein, it was discovered that a suite of secondary amines are capable of opening oxazolidinone 2.31. Using this strategy, a number of secondary ureas were synthesized including: AmB dimethylurea (3.2), AmB diethylurea (3.3), AmB diisopropylurea (3.4), AmB piperidinourea (3.5), AmB morpholinourea (3.6), and AmB N-methylpiperazinourea (3.7) (Scheme 3.3 A). Many of these urea derivatives maintain potent antifungal activity. Intriguingly, increasing steric bulk with acyclic amines appears to decrease antifungal activity as 3.3 has reduced antifungal activity (5 µM) and 3.4 was inactive up to 10 µM. Consistent with the previously synthesized ureas, five out of the six compounds are drastically less toxic than AmB, not causing red blood cell hemolysis even at 500 µM (Scheme 3.3 B). Surprisingly, 3.4 was toxic at the low concentration of 60 µM. Further investigation will be required to understand the basis of this increase in toxicity. This initial therapeutic index screen reveals one important structure activity relationship. The urea hydrogen indicated in blue in Scheme 3.3 C is not required for
antifungal activity. Additional studies are targeted to determine if the carbonyl oxygen, or other urea hydrogen are required.

Scheme 3.3: A.) Synthesis of a variety of secondary AmB ureas. B.) Initial biological activity screen of AmB ureas against both \textit{S. cerevisiae} and red blood cells. C.) One of the urea hydrogens (indicated in blue) is unnecessary for antifungal activity.

3-6 Less toxic AmB ureas maintain ability to evade drug-resistance

AmBAU and AmBMU both show increased ergosterol selectivity, potent antifungal activity, significant decreases in human cell toxicity in cell culture, and drastically reduced acute toxicity in mice. We asked if the improved sterol selectivity and decreased toxicity of AmBMU and AmBAU would render them more vulnerable to the evolution of resistance. Due to its unique mode of action,\textsuperscript{10} AmB is not susceptible to most of the common mechanisms of
resistance to other antimicrobials. AmB is unaffected by efflux pumps, and its polyene macrolide structure is not a substrate for secretion via drug-detoxifying enzymes. Its cellular target, ergosterol, is not as readily mutable as proteins or RNA. Moreover, ergosterol plays a critical role in multiple aspects of yeast physiology. Mutations in genes involved in ergosterol biosynthesis can change sterol structures in ways that confer AmB resistance in vitro, however, these mutations have enormous fitness costs in vivo, crippling fungal virulence. Consequently, resistance rarely, if ever appears in the clinic.

As mutations in the ergosterol biosynthesis pathway most commonly confer AmB resistance, we first determined the MIC of AmB, AmBMU and AmBAU against a panel of lab-generated strains carrying mutations in all seven of the non-essential C. albicans late-stage ergosterol biosynthesis genes (Figure 3.10A). As for AmB, only erg2, erg6 or erg3/erg11 mutants showed substantial resistance, and all of these mutants are known to be avirulent. Surprisingly, AmBMU and AmBAU displayed an in vitro resistance profile that was very similar to AmB (Figure 3.10A). Consequently, known ergosterol biosynthesis mutations do not appear to be a threat to the efficacy of AmBAU and AmBMU.

Next, to ask if any other mutations could confer resistance to AmBMU or AmBAU, we used exhaustive unbiased selections for survival of C. albicans in the presence of the compounds. To generate highly resistant mutants we utilized a gradual resistance-selection protocol in liquid culture, with serial two-fold increases in drug concentration. Most selections ended in extinction of the lineage. In spite of these challenges, 5-8 mutants for each drug were recovered that exhibited a ≥4-fold increase in MIC. Importantly, all of these highly resistant mutants were cross-resistant to all three compounds, suggesting no new mechanisms of resistance unique to AmBMU or AmBAU.

To identify the mutations responsible for resistance in these selections, we used whole genome sequencing of the WT strain as well as all 11 of the in vitro-evolved resistant mutants. Included in this genome mapping were both strongly and weakly-resistant isolates. Most mutants with strong resistance to AmB or the derivatives contained mutations in the ERG2 or ERG6 locus (Figure 3.10B). Unexpectedly, three independent mutants were identified with low-level (~2-fold) AmB resistance mediated through a recurrent substitution in ORF19.7285 (D216Y), an uncharacterized WD40 repeat protein conserved across fungi. These mutants were no more resistant to AmBMU and AmBAU than to AmB.
We then asked if any of the mutants with substantial resistance to AmBMU or AmBAU (>4-fold MIC increase) could elude the dramatic fitness defects previously demonstrated for AmB-resistance. As previously reported, AmB-resistant yeast become hypersensitive to host-like defenses such as oxidative stress. Additionally, they also become hyperdependant upon the molecular chaperone Hsp90, which supports a variety of fungal stress responses. All of the mutants resistant to AmBMU or AmBAU were likewise severely sensitized to the oxidative stressor tert-butyl hydrogen peroxide and the Hsp90 inhibitor geldanamycin (Figure 3.10C,D). Furthermore, WT yeast readily filament in vitro, a key driver of virulence in Candida, and mutations which engender AmB-resistance disable filamentation. In response to stimulation with fetal bovine serum at 37°C, all mutants with strong resistance to AmBMU or AmBAU were unable to form the filaments observed in the wild-type (Figure 3.10E).

We next asked if resistance to AmBMU or AmBAU reduced competitive fitness in vivo. To do so, mice were infected with a pool of strains consisting of the wild-type parent (AmBMU and AmBAU-sensitive) and 15 AmBMU or AmBAU-resistant mutants (with each strain comprising 1/16th of the total population). After allowing the infection to progress for four days (in the absence of drug treatment), fungal colonies were isolated from the kidneys and tested for drug sensitivity. We then determined the AmBMU and AmBAU-resistant fraction of the final population. Even over this short period of infection, the percentage of the surviving population resistant to AmBMU or AmBAU dropped dramatically, and the drug-sensitive parent rapidly overtook the population (Figure 3.10F).

As a final test we asked if any of our AmBMU or AmBAU-resistant mutants retained the capacity to cause lethal infection. To do so, we inoculated mice with pools of resistant mutants and compared their survival to mice infected with wild-type strains. At a low inoculum the wild-type strain killed all infected mice (Figure 3.10G). Wild-type strains subjected to the same mutagenesis and in vitro passaging used to generate the resistant strains also killed all of the mice. In stark contrast, all mice infected with pools of mutants selected for resistance to AmB, AmBMU or AmBAU survived the infection (Figure 3.10G). Thus, AmBMU and AmBAU are no more vulnerable to resistance than AmB, which has evaded resistance despite widespread clinical utilization for over half a century.
Figure 3.10: Characterization of mechanisms and costs of resistance to AmB ureas. A) Activity of AmB, AmBMU and AmBAU against *C. albicans* ergosterol biosynthesis mutants. Growth (as judged by OD<sub>600</sub> at 24 h) is shown relative to WT with
no compound added. Scale bar indicates relative growth ranging from bright green (equal to WT growth, 1.00) to black (zero growth, 0.00). B.) Complete genome sequencing of parental wild-type (SC5314) and mutants selected for resistance to AmB, AmBMU or AmBAU. ERG2 ORF is shown; color coded mutations represent blue=C, red=T, brown=G, green=A in the mutant strain. C,D.) MIC of tert-butyl peroxide and geldanamycin compared to AmBMU for all resistant isolates selected; each point indicates one or more isolates. e. Filamentation in response to serum at 37°C. Representative images of wild-type and mutants selected in AmB, AmBMU or AmBAU, (all cross-resistant), stained with Calcofluor White. Scale bar = 10 mm F.) Competitive infection of mice with 16 different strains (1 WT and 15 that are resistant to AmBMU and AmBAU). 3x10^4 cells of each strain injected per mouse (4.8x10^5 total inoculum). Fraction of pool sensitive or resistant to AmBMU and AmBAU determined before tail-vein injection and 4d later after isolation from kidneys. G.) Mouse overall survival after tail-vein injection. Mice were injected with a pool of 5 passaged and mutagenized WT strains each at 1.6x10^5 (8x10^5 total inoculum), 1.6x10^5 cells of parental WT (WT-Low), or pools of 5 resistant mutants with 1.6x10^5 cells of each mutant.

3-7 Summary

AmB has been used for over 50 years as the last line of defense against systemic fungal infections. Unfortunately, AmB’s clinical utility is limited by severe toxicities. AmB exerts its antifungal activity by binding ergosterol, but also can bind cholesterol, likely explaining its human toxicity. Substantial changes in sterol structure are required to engender resistance, however the accompanying fitness trade offs render the yeast avirulent. An AmB derivative that selectively binds ergosterol would be non-toxic, but may lose the ability to evade drug resistance. To explore this relationship between sterol binding, toxicity, and drug resistance a series of AmB urea derivatives were synthesized in only 3-steps from the parent AmB natural product. These derivatives selectively bind ergosterol but not cholesterol, maintain antifungal activity, yet were drastically less toxic than AmB to both red blood cells and kidney cells in vitro. In a mouse model of disseminated candidiasis, two of these derivatives, AmBMU and AmBAU, were more effective than AmB. Acute mice toxicity experiments revealed that AmBMU and AmBAU are drastically less toxic than AmB. Using mutagenesis C. albicans mutants resistant to AmBMU and AmBAU were generated. Surprisingly, the onset of resistance to AmBMU and AmBAU was accompanied by the same drastic fitness costs demonstrated by AmB resistant yeast.

These findings collectively reveal that selective antimicrobial action and evasion of resistance are not mutually exclusive. Here, compounds that bind a pathogen-specific lipid with high selectivity evade the emergence of virulent resistant strains, suggesting major costs in fitness are caused by even small changes in the structure of this lipid. This is likely because ergosterol is a central molecular node in yeast physiology, being critical for the function of membrane proteins, endocytosis, vacuole fusion, membrane compartmentalization, and pheromone signaling.

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More broadly, our results show that targeting pathogen specific and polyfunctional lipids represents a promising blueprint for achieving the highly sought combination of low toxicity and evasion of resistance. Intriguingly, nisin, which binds a bacterial polyfunctional lipid, lipid II, has similarly evaded resistance despite half a century of use as a food preservative.\textsuperscript{52} It was recently discovered that binding of the same lipid underlies the action of defensin peptides,\textsuperscript{53} critical components of innate immunity that have retained antibiotic activity over more than two billion years of evolution. Other recent studies increasingly show that specific lipid-transmembrane protein interactions are critical for diverse cellular functions.\textsuperscript{54} Consequently, as new microbe-specific and polyfunctional lipids are discovered,\textsuperscript{55} they present outstanding targets for the rational development of other non-toxic and resistance-evasive antimicrobials.

AmBMU and AmBAU are exceptionally promising candidates for replacing AmB as a less toxic treatment for invasive fungal infections. These new derivatives bind ergosterol but not cholesterol, are substantially more effective and less toxic than AmB in vivo, yet still evade resistance. Moreover, these compounds are accessed in just three steps from AmB, which is already fermented and commercially available on the metric ton scale.

3-8 METHODS

Materials

Commercially available materials were purchased from Aldrich Chemical Co. (Milwaukee, WI), AKSci (Union City, CA), Fisher Scientific (Hampton, NH), Lipoid (Luwigshafen, Germany), and Silicycle (Quebec, Canada) and used without further purification unless noted otherwise. All solvents were dispensed from a solvent purification as described by Pangborn and coworkers\textsuperscript{56} (THF, Et\textsubscript{2}O: dry neutral alumina; DMSO, DMF, CH\textsubscript{3}OH : activated molecular sieves). Triethylamine and pyridine were freshly distilled under nitrogen from CaH\textsubscript{2}. Camphorsulfonic acid was recrystallized from ethanol. Water was doubly distilled or obtained from a Millipore (Billerica, MA) MilliQ water purification system.

Reactions

Due to the light and air sensitivity of amphotericin B (AmB), all manipulations were carried out under low light conditions and compounds were stored under an anaerobic atmosphere. All reactions were performed in oven- or flame-dried glassware under an
atmosphere of argon unless otherwise indicated. Reactions were monitored by RP-HPLC using an Agilent 1200 Series HPLC system equipped with an Agilent Zorbax Eclipse C_{18} 3.5-µm, 4.6 x 75 mm column with UV detection at 383 nm at 1.2 mL/min, or an Agilent 6230 ESI TOF LC/MS system equipped with an Agilent Zorbax Eclipse C_{18} 1.8-µm, 2.1 x 50 mm column with UV detection at 383 nm at 0.4 mL/min.

**Purification and Analysis**

$^1$H NMR spectra were recorded at room temperature on a Varian Unity Inova Narrow Bore spectrometer operating at a $^1$H frequency of 500 MHz with a Varian 5 mm $^1$H{$^{13}$C/$^{15}$N} pulsed-field gradient Z probe or an Agilent VNMRS 750 MHz spectrometer with a Varian 5 mm indirect-detection probe $^1$H{$^{13}$C/$^{15}$N} probe equipped with X,Y,Z-field gradient capability. Chemical shifts ($\delta$) are reported in parts per million (ppm) downfield added tetramethylsilane ($\delta = 0.0$). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, b = broad, app = apparent), coupling constant ($J$) in Hertz (Hz) and integration. $^{13}$C spectra were recorded at room temperature with a Varian Unity Inova spectrometer operating at a $^{13}$C frequency of 125 MHz with a 5 mm Nalorac gradient $^{13}$C/$^{15}$N$^1$H quad probe or a Varian Unity Inova spectrometer operating at a $^{13}$C frequency of 150 MHz and equipped with a Varian 5 mm 600 DB Auto X probe. Chemical shifts ($\delta$) are reported downfield of added tetramethylsilane ($\delta = 0.0$) or are referenced to the carbon resonances in the NMR solvent ((CD$_3$)$_2$SO, $\delta = 39.52$, center line). MS analysis was performed with an Applied Biosystems Micromass Ultima system with ESI ionization. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All synthesized compounds gave HRMS within 5 ppm of the calculated values. Large scale prep purification of amphotericin B and its derivatives was carried out by preparative HPLC purification using either an agilent 1100 or 1200 series instrument equipped with either a Waters SunFire Prep C$_{18}$ OBD 5-µm, 30 x 150 mm at 25 mL/min, or a Waters Sunfire Prep C$_{18}$ OBD 5-µm, 50 x 250 mm column at 75 mL/min with detection at 383 nm and an eluent of acetonitrile and 0.3% formic acid. The purity of amphotericin B and its derivatives was determined by HPLC analysis using an Agilent Zorbax Eclipse C$_{18}$ 1.8-µm, 2.1 x 50 mm column with detection at 383 nm and an eluent of acetonitrile and 0.1% formic acid in water unless otherwise indicated.
Synthesis of AmB Derivatives

Oxazolidinone 2.31

A round bottom flask was charged with amphotericin B (4 g, ca. 4.32 mmol, 1eq) and Fmoc-succinimide (2.19 g, 6.48 mmol, 1.5 eq) which were dissolved in a mixture of DMF:MeOH 2:1 (135 mL) at 23 °C. Pyridine (2.0 mL, 24.8 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (4.0 L). After stirring for 30 minutes the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour. The resulting powder was dissolved in THF:MeOH 1:1 (140 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (526 mg, 2.24 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0 °C. The reaction was then quenched at 0 °C with triethylamine (0.52 mL, 0.438 mmol, 0.55 eq). The reaction was concentrated in vacuo removing approximately half of the solvent. The resulting saturated solution was poured into hexanes:diethyl ether 1:1 4.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter paper and washed with diethyl ether (200 mL) to yield 2.29 as a yellow solid (5.1 g).

A portion of the resulting solid (0.204 g, ca. 0.1724 mmol, 1 eq) was added to a 20mL vial followed by THF (8.6mL, 0.2M), triethylamine (0.072 mL, 0.517 mmol, 3 eq), and lastly diphenyl phosphoryl azide (0.055 mL, 0.258 mmol, 1.5 eq). The reaction was then heated to 50°C and stirred for 3 hours. The reaction was cooled to room temperature and then all volatiles were removed via rotovap and the crude orange solid was purified by flash chromatography (SiO₂; DCM:MeOH 97:3 → 95:5 → 92.5:7.5 → 90:10) followed by reverse phase mPLC.
purification (H₂O:MeCN 95:5 → 5:95) yielding 2.31 as a yellow/orange solid (0.0775 g, 0.067 mmol, 38% yield over 3 steps).

TLC (DCM : MeOH 9:1)

Rᵣ = 0.55, stained by CAM

¹H NMR (500 MHz, THF-d₈)

δ 7.78 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H), 7.35 (app t, J = 7.5 Hz, 2H), 7.27 (app t, J = 7.4 Hz, 2H), 6.75 (s, 1H), 6.60 (d, J = 8.9 Hz, 1H), 6.45 – 6.17 (m, 11H), 6.18-6.07 (m, 1H), 6.02 (dd, J = 15.2, 6.8 Hz, 1H), 5.43 (dd, J = 15.1, 9.6 Hz, 1H), 5.31-5.26 (m, 1H), 4.83 (s, 1H), 4.75 (d, J = 2.6 Hz, 1H), 4.58 – 4.53 (m, 1H), 4.37-4.26 (m, 4H), 4.22 (app t, J = 7.1 Hz, 1H), 4.16 – 4.09 (m, 1H), 4.01-3.94 (m, 5H), 3.88 (app d, J = 2.9 Hz, 1H), 3.82 (app d, J = 4.3 Hz, 1H), 3.77 (app t, J = 3.6 Hz, 1H), 3.74-3.70 (m, 1H), 3.51 – 3.42 (m, 1H), 3.24 – 3.21 (m, 1H), 3.22-3.12 (m, 3H), 3.07 (app t, J = 10.3 Hz, 1H), 2.43 – 2.34 (m, 1H), 2.29 (dd, J = 16.8, 8.6 Hz, 1H), 2.20 (dd, J=16.9, 3.6) 2.18 – 2.13 (d, J = 6.1 Hz, 1H), 1.86 – 1.80 (m, 4H), 1.61-35 (m, 10H), 1.24 (d, J = 5.0 Hz, 3H), 1.19 (d, J = 6.4 Hz, 3H), 1.09 (d, J = 6.4 Hz, 3H), 0.99 (d, J = 7.1 Hz, 3H).

¹³C NMR (500 MHz, THF-d₈)

δ 172.02, 160.72, 157.90, 145.50, 142.39, 137.41, 136.89, 135.03, 134.97, 134.49, 134.38, 133.75, 133.67, 133.59, 133.54, 133.40, 133.02, 132.96, 130.21, 128.46, 127.93, 126.27, 120.74, 103.45, 99.70, 78.89, 76.42, 74.94, 72.93, 72.22, 71.56, 71.02, 70.79, 69.45, 69.38, 58.53, 49.19, 48.47, 44.67, 44.13, 43.44, 43.21, 42.08, 39.51, 36.94, 33.27, 31.64, 30.99, 30.57, 19.02, 18.60, 17.61, 12.46.
HRMS (ESI)

Calculated for C_{63}H_{84}N_{2}O_{18} (M + Na)^{+}: 1179.5617
Found: 1179.5627

AmBMU

A 7 mL vial was charged with 2.31 (51.4 mg, 0.044 mmol, 1 eq), THF (2.16 mL, 0.02M), and methylamine as a 2.0 M solution in THF (0.108 mL, 0.216 mmol, 4.9 eq). The reaction was allowed to stir for 6 hours, slowly evolving a yellow precipitate. The volatiles were then removed in vacuo and the resulting orange solid was purified by prep-HPLC (C_{18}, 10-µm, 30 x 150 mm, 25 mL/min, 95:5 to 50:50 0.3% HCO_2H (aq):MeCN over 13 minutes). After HPLC purification the solvent was removed in vacuo at 40°C. Upon complete solvent removal, residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding AmBMU as a yellow solid (15.4 mg, 0.016 mmol, 36% yield).

Alternate 3-step synthesis from AmB

A round bottom flask was charged with amphotericin B (1 g, ca. 1.082 mmol, 1 eq) and Fmoc-succinimide (0.55 g, 1.62 mmol, 1.5 eq) which were dissolved in a 2:1 mixture of DMF:MeOH (33.8 mL) at room temperature. Pyridine (0.5 mL, 6.21 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (1.0 L). After stirring for 30 minutes, the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour.

The resulting powder was dissolved in 1:1 THF:MeOH (35 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (138 mg, 0.59 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0°C. The reaction was then quenched at 0°C with triethylamine (0.14 mL, 0.59 mmol, 0.55 eq). The reaction was concentrated in vacuo removing approximately half of the solvent. The resulting saturated solution was poured into 1:1 hexanes:diethyl ether (1.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter.
paper and washed with diethyl ether (200 mL) to yield **2.29** as a yellow solid.

The resulting solid was dissolved in THF (54 mL, 0.02M). To this solution was added triethylamine (0.15 mL, 1.08 mmol, 1 eq) and then diphenyl phosphoryl azide (0.70 mL, 3.25 mmol, 3 eq). The reaction was heated to 50°C and stirred for 12 hours. After 12 hours the reaction was cooled to room temperature and methylamine (2.0M in THF, 4.33 mL, 8.8 mmol, 8 eq) was added. The reaction then stirred at room temperature for 8 hours, slowly evolving a yellow precipitate. The reaction mixture was then poured into diethyl ether (1.0 L), and the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The solid was dissolved in DMSO (~100 mg/mL) and purified by a single prep-HPLC purification (C<sub>18</sub>, 5-µm, 50 x 250 mm, 75 mL/min, 80:20 to 59:41 0.3% HCO<sub>2</sub>H (aq):MeCN over 9 minutes). Similarly, global supply of the antifungal caspofungin is supplied requiring only a single HPLC purification<sup>34</sup>. Following HPLC purification, the solvent was removed *in vacuo* at 40°C. Upon complete solvent removal, residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding AmBMU as a yellow solid (264.3 mg, 0.278 mmol, 64% average yield per step).

![AmBMU structure](image)

<sup>1</sup>H NMR (750 MHz, 1:1 Pyridine <i>d</i>-5: Methanol <i>d</i>-4)

δ 6.64 (dd, <i>J</i> = 14.7, 11.2 Hz, 1H), 6.60 (dd, <i>J</i> = 14.8, 10.0 Hz, 1H), 6.52 (t, <i>J</i> = 12.1 Hz, 1H), 6.48 – 6.37 (m, 6H), 6.36 – 6.25 (m, 4H), 5.69 – 5.63 (m, 1H), 5.53 – 5.47 (dd, <i>J</i> = 14.3, 9.8 Hz, 1H), 4.97 (s, 1H), 4.82-4.76 (m, 1H), 4.65 (app t, <i>J</i> = 10.3 Hz, 1H), 4.53 (bs, 1H), 4.49 (app tt, <i>J</i> = 9.8, 2.9 Hz, 1H), 4.42 (app t, <i>J</i> = 9.1 Hz, 1H), 4.29 – 4.23 (m, 1H), 3.98 (app t, <i>J</i> = 10.0 Hz, 1H), 3.90 – 3.84 (m, 2H), 3.80 – 3.72 (m, 1H), 3.62 – 3.56 (m, 1H), 3.56 – 3.51 (m, 1H), 3.47 – 3.44 (m, 1H), 3.38 (app d, <i>J</i> = 9.5 Hz, 1H), 2.79 (s, 3H), 2.71 – 2.65 (m, 1H), 2.61 – 2.55 (m, 1H), 2.51 (dd, <i>J</i> = 16.7, 9.8 Hz, 1H), 2.39 – 2.34 (m, 2H), 2.21 – 2.14 (m, 1H), 2.06 – 1.99 (m, 2H), 1.96 (dd, <i>J</i> = 14.8, 7.3 Hz, 1H), 1.85 (dd, <i>J</i> = 13.9, 10.9 Hz, 1H), 1.84-1.79 (m, 1H), 1.73 – 1.65 (m, 3H), 1.66 – 1.61 (m, 1H), 1.61 – 1.56 (m, 1H), 1.53 (app dt, <i>J</i> = 14.0, 3.0 Hz,
1H), 1.47-1.45 (m, 1H), 1.46 (d, J = 6.2 Hz, 3H), 1.37 (d, J = 6.5 Hz, 3H), 1.25 (d, J = 6.4 Hz, 3H), 1.18 (d, J = 7.1 Hz, 3H).

$^{13}$C NMR (125 MHz, 1:1 Pyridine $d$-5: Methanol $d$-4)

\[ \delta 172.31, 161.63, 137.57, 137.33, 134.82, 134.20, 134.17, 133.99, 133.91, 133.67, 133.59, 133.28, 132.98, 131.04, 129.73, 128.99, 98.04, 98.26, 79.10, 77.44, 76.32, 75.24, 74.64, 72.32, 70.92, 70.45, 69.98, 69.26, 68.97, 68.69, 68.46, 58.35, 57.35, 47.22, 45.74, 44.90, 44.06, 42.89, 41.28, 40.80, 36.72, 36.38, 31.53, 27.02, 19.11, 18.10, 17.35, 12.64. \]

HRMS (ESI)

Calculated for $C_{48}H_{77}N_{16}O_{16} + H^+$: 952.5382

Found: 952.5385

Analytical HPLC (Zorbax Eclipse C$_{18}$, 1.8-μm, 2.1 x 50mm, 0.4 mL/min, 95:5 to 5:95 H$_2$O:MeCN (both containing 0.1% HCO$_2$H) over 8 minutes)

Retention Time = 5.7 min.
AmBAU
A 7 mL vial was charged with 2.31 (53.0 mg, 0.046 mmol, 1 eq), THF (2.16 mL, 0.02M), and ethylene diamine (0.0057 mL, 0.086 mmol, 1.86 eq). The reaction was then placed in a preheated heating block at 40°C and allowed to stir for 5 hours, slowly evolving a yellow precipitate. The volatiles were then removed in vacuo and the resulting orange solid was purified by prep-HPLC (C₁₈, 10-µm, 30 x 150 mm, 25 mL/min, 95:5 to 50:50 0.3% HCO₂H (aq):MeCN over 13 minutes). After HPLC purification the solvent was removed in vacuo at 40°C. Upon complete solvent removal, residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding AmBAU as a yellow solid (18.8 mg, 0.019 mmol, 42% yield).

Alternate 3-step synthesis from AmB
A round bottom flask was charged with amphotericin B (1 g, ca. 1.082 mmol, 1 eq) and Fmoc-succinimide (0.55 g, 1.62 mmol, 1.5 eq) which were dissolved in a 2:1 mixture of DMF:MeOH (33.8 mL) at room temperature. Pyridine (0.5 mL, 6.21 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (1.0 L). After stirring for 30 minutes, the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour.

The resulting powder was dissolved in 1:1 THF:MeOH (35 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (138 mg, 0.59 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0°C. The reaction was then quenched at 0°C with triethylamine (0.14 mL, 0.59 mmol, 0.55 eq). The reaction was concentrated in vacuo removing approximately half of the solvent. The resulting saturated solution was poured into 1:1 hexanes:diethyl ether (1.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter paper and washed with diethyl ether (200 mL) to yield 2.31 as a yellow solid.

The resulting solid was dissolved in THF (54 mL, 0.02M). To this solution was added triethylamine (0.15 mL, 1.08 mmol, 1 eq) and then diphenyl phosphoryl azide (0.70 mL, 3.25 mmol, 3 eq). The reaction was heated to 50°C and stirred for 12 hours. After 12 hours, ethylene diamine (0.29 mL, 4.33 mmol, 4 eq) was added, and the reaction continued stirring at 50°C for 3 hours, slowly evolving a yellow precipitate. The reaction mixture was then poured into diethyl ether (1.0 L), and the resulting yellow precipitate was isolated via Büchner filtration using
Whatman #50 filter paper to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C\textsubscript{18}, 5-µm, 50 x 250 mm, 75 mL/min, 80:20 to 50:50 0.3% HCO\textsubscript{2}H (aq):MeCN over 9 minutes). After HPLC purification the solvent was removed \textit{in vacuo} at 40°C. Upon complete solvent removal, residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding AmBAU as a yellow solid (236.2 mg, 0.241 mmol, 61% average yield per step).

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

\textsuperscript{1}H NMR (750 MHz, 1:1 Pyridine \textit{d}-5: Methanol \textit{d}-4)

\[\delta 6.64 \text{ (dd, } J = 14.7, 11.2 \text{ Hz, 1H}), 6.60 \text{ (dd, } J = 15.2, 8.8 \text{ Hz, 1H}), 6.55 - 6.47 \text{ (m, 1H), 6.47 - 6.35 \text{ (m, 7H), 6.35 - 6.25 \text{ (m, 3H), 5.68 - 5.61 \text{ (m, 1H), 5.49 \text{ (dd, } J = 15.0, 10.2 \text{ Hz, 1H), 4.91 \text{ (s, 1H), 4.79 - 4.73 \text{ (m, 1H), 4.64 \text{ (app t, } J = 10.7 \text{ Hz, 1H), 4.49 - 4.42 \text{ (m, 3H), 4.28 - 4.23 \text{ (m, 1H), 3.96 \text{ (app t, } J = 10.4 \text{ Hz, 1H), 3.88 - 3.82 \text{ (m, 1H), 3.82 - 3.73 \text{ (m, 3H), 3.55 - 3.50 \text{ (m, 1H), 3.51 - 3.46 \text{ (m, 1H), 3.47 - 3.43 \text{ (m, 1H), 3.39 \text{ (app d, } J = 8.8 \text{ Hz, 1H), 3.36 \text{ (app d, } J = 9.2 \text{ Hz, 1H), 3.32 - 3.26 \text{ (m, 1H), 3.23 - 3.16 \text{ (m, 1H), 2.66 \text{ (dd, } J = 15.4, 4.9 \text{ Hz, 1H), 2.59 - 2.53 \text{ (m, 1H), 2.49 \text{ (dd, } J = 16.8, 9.8 \text{ Hz, 1H), 2.38 - 2.33 \text{ (m, 2H), 2.18 - 2.12 \text{ (m, 1H), 2.04 - 1.97 \text{ (m, 2H), 1.90 \text{ (dd, } J = 14.9, 7.8 \text{ Hz, 1H), 1.84 \text{ (dd, } J = 14.0, 11.0 \text{ Hz, 1H), 1.82 - 1.75 \text{ (m, 1H), 1.71 - 1.65 \text{ (m, 3H), 1.65 - 1.60 \text{ (m, 1H), 1.60 - 1.55 \text{ (m, 1H), 1.52 \text{ (app dt, } J = 13.8, 2.9 \text{ Hz, 1H), 1.48 - 1.44 \text{ (m, 1H), 1.44 \text{ (d, } J = 6.2 \text{ Hz, 3H), 1.36 \text{ (d, } J = 6.5 \text{ Hz, 3H), 1.24 \text{ (d, } J = 6.4 \text{ Hz, 3H), 1.17 \text{ (d, } J = 7.1 \text{ Hz, 3H).}}}

\textsuperscript{13}C NMR (150 HMz, 1:1 Pyridine \textit{d}-5: Methanol \textit{d}-4)

\[\delta 172.34, 161.35, 137.58, 137.08, 134.78, 134.16, 134.11, 134.02, 133.93, 133.71, 133.62, 133.26, 133.00, 130.85, 130.62, 98.26, 98.07, 79.12, 77.60, 76.37, 75.24, 74.63, 72.28,
71.43, 70.47, 70.00, 69.23, 69.23, 68.69, 68.62, 58.16, 57.32, 47.18, 45.77, 44.93, 44.05, 42.86, 41.67, 40.80, 40.49, 39.31, 36.73, 36.35, 31.56, 19.08, 18.08, 17.32, 12.63.

HRMS (ESI)

Calculated for C_{49}H_{80}N_{4}O_{16} + H^+: 981.5648

Found: 981.5641

Analytical HPLC (Zorbax Eclipse C_{18}, 1.8-µm, 2.1 x 50 mm, 0.4 mL/min, 95:5 to 5:95 H_{2}O:MeCN (both containing 0.1% HCO_{2}H) over 8 minutes)

Retention Time = 5.2 min.

AmBCU-allylester

To a 20 mL vial was added β-alanine allylester hydrochloride (1.125 g, 6.79 mmol, 39 eq), sodium carbonate (2.19 g, 20.66 mmol, 120 eq), and DMF (8.6 mL). The resulting suspension stirred then at room temperature for 15 minutes. The suspension was then filtered through celite followed by filtration through a syringe tip 0.2-µm filter. The resulting β-alanine allylester free base was then added to a 20 mL vial containing 2.31 (200 mg, 0.174 mmol, 1 eq). The reaction was then placed in a preheated heating block at 40°C reaction mixture and allowed to stir for 5 hours. The reaction was then directly purified directly by prep HPLC (C_{18}, 5-µm, 30 x 150 mm,
25 mL/min, 95:5 to 40:60 0.3% HCO₂H (aq):MeCN over 10 minutes). Upon removal of the acetonitrile and aqueous formic acid solution in vacuo at 35°C, the C-13 methyl ketal is converted to a hemiketal yielding AmBCU-allylester as a yellow solid (59.4 mg, 0.056 mmol, 32.5% yield).

Alternate 3-step synthesis from AmB

A round bottom flask was charged with amphotericin B (1 g, ca. 1.08 mmol, 1 eq) and Fmoc-succinimide (0.55 g, 1.62 mmol, 1.5 eq) which were dissolved in a mixture of 2:1 DMF:MeOH (33.8 mL) at room temperature. Pyridine (0.5 mL, 6.21 mmol, 5.74 eq) was subsequently added and the reaction was stirred for 12 hours at room temperature. The reaction mixture was then poured into diethyl ether (1.0 L). After stirring for 30 minutes, the resulting yellow precipitate was isolated via Büchner filtration using Whatman #50 filter paper to afford a yellow solid. The filter cake was dried on the filter for 10 minutes and then stored under vacuum for one hour.

The resulting powder was dissolved in 1:1 THF:MeOH (35 mL) and cooled to 0°C. To this solution was added camphorsulfonic acid (138 mg, 0.60 mmol, 0.55 eq) and the resulting mixture was stirred for 1 hour at 0°C. The reaction was then quenched at 0°C with triethylamine (0.14 mL). The reaction was concentrated in vacuo removing approximately half of the solvent. The resulting saturated solution was poured into 1:1 hexanes:diethyl ether (1.0 L) and the yellow precipitate was collected via Büchner filtration using Whatman #50 filter paper and washed with diethyl ether (20 mL) to yield 2.31 as a yellow solid.

The resulting solid (1.06 g, ca. 1.08 mmol, 1 eq) was added to a 40 mL vial followed by THF (54 mL, 0.02M), triethylamine (0.16 mL, 1.14 mmol, 1.05 eq), and lastly diphenyl phosphoryl azide (0.70 mL, 3.25 mmol, 3 eq). The reaction was then heated to 50°C and stirred for 15 hours.

To a separate 40 mL vial was added β-alanine allylester hydrochloride (7.16 g, 43.3 mmol, 40 eq), sodium carbonate (13.75 g, 129.8 mmol, 120 eq), and THF (14 mL). The resulting suspension stirred then at room temperature for 20 minutes. The suspension was then filtered through celite followed by filtration through a syringe tip 0.2-μm filter. The resulting β-alanine allylester free base was then added to the 50°C reaction mixture and allowed to stir for 8 hours. After 8 hours, the volatiles were removed in vacuo yielding a red oil. This was dissolved in DMSO and purified directly by prep HPLC (C₁₈, 5-μm, 50 x 250 mm, 80 mL/min, 80:20 to 40:60 0.3% HCO₂H (aq):MeCN over 9 minutes). Upon removal of the acetonitrile and aqueous
formic acid solution in vacuo at 35°C, the C-13 methyl ketal is converted to a hemiketal yielding AmBCU-allylester as a yellow solid (370 mg, 0.352 mmol, 68% average yield per step).

$^1$H NMR (500 MHz, 10:1 Pyridine d-5: Methanol d-4)

$\delta$ 6.71 – 6.25 (m, 13H), 6.01 – 5.89 (m, 1H), 5.70 – 5.64 (m, 1H), 5.54 – 5.48 (m, 1H), 5.33 (m, 1H), 5.20 (m, 1H), 4.97 (s, 1H), 4.79 (bs, 1H), 4.70 – 4.59 (m, 4H), 4.50 (app t, $J = 10.0$ Hz, 1H), 4.43 (app t, $J = 8.8$ Hz, 1H), 4.26 – 4.20 (m, 1H), 3.99 (app t, $J = 10.0$ Hz, 1H), 3.88 (app d, $J = 10.8$ Hz, 1H), 3.65-3.60 (m, 3H), 3.47 (m, 1H), 3.42 – 3.35 (m, 1H), 3.35 – 3.31 (m, 1H), 2.71 – 2.62 (m, 3H), 2.58 (m, 1H), 2.52 (dd, $J = 16.8, 9.7$ Hz, 1H), 2.41 – 2.33 (m, 2H), 2.23-2.13 (m, 1H), 2.07 – 1.91 (m, 3H), 1.91 – 1.77 (m, 2H), 1.75 – 1.57 (m, 5H), 1.57 – 1.51 (m, 1H), 1.48 – 1.44 (m, 4H), 1.37 (d, $J = 6.3$ Hz, 3H), 1.26 (d, $J = 6.3$ Hz, 3H), 1.18 (d, $J = 7.1$ Hz, 3H).

$^{13}$C NMR (125 MHz, 10:1 Pyridine-$d_5$ : MeOH-$d_4$)

$\delta$ 172.31, 171.92, 160.41, 136.83, 136.75, 134.46, 133.84, 133.57, 133.47, 133.45, 133.20, 133.03, 132.91, 132.61, 130.57, 129.55, 117.73, 98.06, 97.90, 77.08, 75.96, 74.89, 74.31, 71.92, 71.61, 70.08, 69.59, 68.65, 68.29, 68.19, 65.31, 57.96, 57.09, 46.84, 45.53, 44.62, 42.59, 40.89, 40.48, 36.41, 36.07, 35.42, 31.22, 18.74, 17.89, 17.02, 12.32.

HRMS (ESI)

Calculated for $C_{53}H_{83}N_3O_{18} + H^+$: 1050.5750

Found: 1050.5756

Analytical HPLC (Zorbax Eclipse C$_{18}$, 1.8-µm, 2.1 x 50 mm, 0.4 mL/min, 95:5 to 5:95 H$_2$O:MeCN (both containing 0.1% HCO$_2$H) over 8 minutes)

Retention Time = 6.4 min
AmBCU
To a 40 mL vial was added AmBCU-allylester (370 mg, 352.3 µmol, 1 eq), and thiosalicylic acid (203.4 mg, 1.76 mmol, 5 eq). The vial was then brought into a glove box and Pd(PPh₃)₄ was added (205 mg, 0.18 mmol, 0.5 eq). The vial was sealed with a septa cap, removed from the glovebox, and DMF was added (17.6 mL, 0.2 M) via syringe. The reaction then stirred at room temperature for 1 hour. The reaction was then poured into Et₂O (370 mL) in multiple 50 mL centrifuge tubes. The resulting suspension was then centrifuged at 3700 G for 5 minutes. The pale red supernatant was decanted and the resulting yellow/orange solid was dissolved in DMSO and purified by prep HPLC (C₁₈, 5-µm, 50 x 250 mm, 80 mL/min, 80:20 to 40:60 0.3% HCO₂H (aq):MeCN over 9 minutes) yielding AmBCU as a yellow solid (124.4 mg, 0.123 mmol, 35% yield, 58% average yield per step from 1 g AmB).

¹H NMR (500 MHz, 1:1 Pyridine d-5: Methanol d-4)
δ 6.69 – 6.23 (m, 13H), 5.70 – 5.64 (m, 1H), 5.54-5.50 (m, 1H), 5.00 (s, 1H), 4.76 (bs, 1H), 4.65 (app t, J = 10.4 Hz, 1H), 4.57 (app bs, 1H), 4.49 (app t, J = 9.0 Hz, 1H), 4.45 – 4.39 (m, 1H), 4.30-4.23 (m, 1H), 4.01-3.95 (m, 1H), 3.92-3.84 (m, 2H), 3.82-3.77 (m, 1H), 3.73-3.67 (m, 2H), 3.67-3.63 (m, 2H), 3.57-3.51 (m, 2H), 3.49-3.45 (m, 1H), 3.38 (app d, J = 9.8 Hz, 1H), 2.74-2.63 (m, 2H), 2.51 (dd, J = 17.0, 9.5 Hz 1H), 2.43 – 2.34 (m, 2H), 2.23 - 2.14 (m, 1H), 2.05-1.98 (m, 2H), 2.94-1.89 (m, 1H), 1.88-1.80 (m, 2H), 1.73-1.59 (m, 5H), 1.57 – 1.51 (m, 1H), 1.46 (d, J = 6.1 Hz, 4H), 1.44 – 1.40 (m, 1H), 1.38 (d, J = 6.3 Hz, 3H), 1.26 (d, J = 6.3 Hz, 3H), 1.19 (d, J = 7.1 Hz, 3H).

¹³C NMR (150 MHz, DMSO-d₆)
δ 175.38, 170.58, 158.41, 136.81, 136.33, 133.92, 133.76, 133.64, 133.25, 133.16, 132.57, 132.40, 132.25, 132.17, 131.87, 131.23, 130.28, 129.00, 97.04, 96.87, 77.20, 76.06, 73.86, 73.48, 73.05, 69.50, 69.16, 68.80, 67.98, 67.79, 67.44, 66.84, 66.20, 59.75, 56.23, 55.25, 45.84, 44.88, 44.80, 42.52, 42.01, 40.43, 39.52, 35.11, 30.96, 29.05, 18.52, 17.80, 16.96, 12.10.
HRMS (ESI)

Calculated for C_{50}H_{79}N_{18}O + H^+: 1010.5437
Found: 1010.5449.

Analytical HPLC (Zorbax Eclipse C_{18}, 1.8-µm, 2.1 x 50 mm, 0.4 mL/min, 95:5 to 5:95 H_{2}O:MeCN (both containing 0.1% HCO_{2}H) over 8 minutes)
Retention Time = 6.07 min

**Dimethyl urea 3.2**

Oxazolidinone 2.31 (50.0 mg, 43.2 µmol) was dissolved in THF (2.16 mL, 0.02M). To this solution was added dimethylamine (0.108 mL, 0.216 mmol, 5 eq). The reaction was heated to 40°C and stirred for 12 hours, yielding a yellow precipitate. The reaction mixture was then poured into diethyl ether (30 mL), and the resulting yellow precipitate was isolated via pipetting off the diethyl ether to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C_{18}, 5-µm, 50 x 250 mm, 25 mL/min, 95:5 to 5:95 0.3% HCO_{2}H (aq):MeCN over 15 minutes). After HPLC purification the desired fractions were collected in a round bottom flask. Residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (150 mL). This process was repeated three times yielding 3.2 as a yellow solid (12.7 mg, 13.2 µmol, 25% yield).
$^1$H NMR (600 MHz, 1:1 Pyridine $d$-5: Methanol $d$-4)

$\delta$ 6.70 – 6.24 (m, 13H), 5.67 – 5.61 (m, 1H), 5.49 (dd, $J = 15.1, 10.3$ Hz, 1H), 4.85 (s, 1H), 4.78 – 4.71 (m, 1H), 4.68 – 4.61 (m, 1H), 4.55 – 4.50 (m, 1H), 4.50 – 4.44 (m, 1H), 4.39 – 4.31 (m, 2H), 4.30 – 4.22 (s, 2H), 4.00 – 3.93 (m, 1H), 3.91 – 3.84 (m, 2H), 3.65 – 3.58 (m, 1H), 3.37 (d, $J = 10.0$ Hz, 1H), 2.94 (s, 6H), 2.58 (appd $J = 11.6$ Hz, 2H), 2.49 (dd, $J = 16.9, 9.8$ Hz, 1H), 2.40 – 2.32 (m, 2H), 2.21 – 2.06 (m, 2H), 1.99 (d, $J = 9.2$ Hz, 1H), 1.90 – 1.72 (m, 6H), 1.73 – 1.62 (m, 4H), 1.52 (appd $J = 13.9$ Hz, 1H), 1.43 (d, $J = 6.3$ Hz, 3H), 1.36 (d, $J = 6.3$ Hz, 3H), 1.25 (d, $J = 6.5$ Hz, 3H), 1.17 (d, $J = 7.1$ Hz, 3H).

$^{13}$C NMR (150 MHz, 1:1 Pyridine $d$-5: Methanol $d$-4)

$\delta$ 172.28, 160.65, 137.65, 134.87, 134.82, 134.26, 134.23, 133.91, 133.83, 133.79, 133.52, 133.48, 133.30, 132.96, 98.86, 98.26, 79.05, 77.60, 76.27, 75.28, 74.68, 72.31, 70.44, 69.94, 68.89, 68.66, 68.28, 58.94, 57.57, 47.29, 45.96, 44.92, 43.99, 42.94, 40.82, 36.55, 36.41, 19.08, 18.24, 17.36, 12.64.

Analytical HPLC (Zorbax Eclipse C$_{18}$, 3.5-µm, 4.6 x 75 mm, 1.2 mL/min, 95:5 to 5:95 H$_2$O(0.1% HCO$_2$H):MeCN over 8 minutes)

Retention Time = 4.54 min
Diethyl urea 3.3

Oxazolidinone 2.31 (20.0 mg, 17.3 µmol) was dissolved in THF (.864 mL, 0.02M). To this solution was added diethylamine (8.9 µL, 86.4 µmol, 5 eq). The reaction was heated to 40°C and stirred for 12 hours, yielding a yellow precipitate. The reaction mixture was then poured into diethyl ether (30 mL), and the resulting yellow precipitate was isolated via pipetting off the diethyl ether to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C\textsubscript{18}, 5-µm, 50 x 250 mm, 25 mL/min, 95:5 to 5:95 0.3% HCO\textsubscript{2}H (aq):MeCN over 15 minutes). After HPLC purification the desired fractions were collected in a round bottom flask. Residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding 3.3 as a yellow solid (8.6 mg, 8.66 µmol, 43% yield).

HRMS (ESI)

- Calculated for C\textsubscript{51}H\textsubscript{83}N\textsubscript{3}O\textsubscript{16} (M + H\textsuperscript{+}): 994.5852
- Found: 994.5852

Analytical HPLC (Zorbax Eclipse C\textsubscript{18}, 3.5-µm, 4.6 x 75 mm, 1.2 mL/min, 95:5 to 5:95 H\textsubscript{2}O(0.1% HCO\textsubscript{2}H):MeCN over 8 minutes)

Retention Time = 5.00 min
Diisopropyl urea 3.4

Oxazolidinone 2.31 (20.0 mg, 17.3 µmol, 1eq) was dissolved in THF (.864 mL, 0.02M). To this solution was added diisopropylamine (310 µL, .691 mmol, 160 eq). The reaction was heated to 60°C and stirred for 12 hours, yielding a yellow precipitate. The reaction mixture was then poured into diethyl ether (30 mL), and the resulting yellow precipitate was isolated via pipetting off the diethyl ether to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C\textsubscript{18}, 5-µm, 50 x 250 mm, 25 mL/min, 95:5 to 5:95 0.3% HCO\textsubscript{2}H\textsubscript{(aq):MeCN over 15 minutes). After HPLC purification the desired fractions were collected in a round bottom flask. Residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding 3.4 as a yellow solid (5.6 mg, 5.48 µmol, 28% yield).

HRMS (ESI)

Calculated for C\textsubscript{53}H\textsubscript{87}N\textsubscript{3}O\textsubscript{16} (M + H\textsuperscript{+}): 1022.6165
Analytical HPLC (Zorbax Eclipse C$_{18}$, 3.5-µm, 4.6 x 75 mm, 1.2 mL/min, 95:5 to 5:95 H$_2$O(0.1% HCO$_2$H):MeCN over 8 minutes)
Retention Time = 5.00 min

Piperdino urea 3.5
Oxazolidinone 2.31 (50.0 mg, 43.2 µmol, 1 eq) was dissolved in THF (2.16 mL, 0.02M). To this solution was added piperidine (21.3 µL, .216 mmol, 5 eq). The reaction was heated to 40°C and stirred for 8 hours, yielding a yellow precipitate. The reaction mixture was then poured into diethyl ether (30 mL), and the resulting yellow precipitate was isolated via pipetting off the diethyl ether to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C$_{18}$, 5-µm, 50 x 250 mm, 25 mL/min, 95:5 to 5:95 0.3% HCO$_2$H (aq):MeCN over 15 minutes). After HPLC purification the desired fractions were collected in a round bottom flask. Residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (150 mL). This process was repeated three times yielding 3.5 as a yellow solid (18.0 mg, 16.3 µmol, 36% yield).
$^1$H NMR (600 MHz, 1:1 Pyridine $d$-5: Methanol $d$-4)

$\delta$ 6.68 – 6.26 (m, 13H), 5.67 – 5.62 (m, 1H), 5.53 – 5.47 (m, 1H), 4.87 (s, 1H), 4.79 – 4.74 (m, 1H), 4.65 (app t, $J = 10.5$ Hz, 1H), 4.56 – 4.49 (m, 1H), 4.51 – 4.45 (m, 1H), 4.35 (app dt, $J = 14.9$, 5.0 Hz, 1H), 4.31 – 4.26 (m, 1H), 3.97 (app t, $J = 10.4$ Hz, 1H), 3.92 (app t, $J = 10.0$ Hz, 1H), 3.87 (app d, $J = 10.9$ Hz, 1H), 3.64 (app t, $J = 9.4$ Hz, 1H), 3.49 (app d, $J = 16.0$ Hz, 6H), 3.38 (app d, $J = 13.4$ Hz, 1H), 2.63 – 2.53 (m, 3H), 2.50 (dd, $J = 16.8$, 9.8 Hz, 1H), 2.42 – 2.32 (m, 2H), 2.22 – 2.13 (m, 1H), 2.06 – 1.93 (m, 4H), 1.89 – 1.76 (m, 6H), 1.74 – 1.62 (m, 7H), 1.62 – 1.52 (m, 2H), 1.44 (d, $J = 6.1$ Hz, 3H), 1.36 (d, $J = 6.3$ Hz, 3H), 1.25 (d, $J = 6.5$ Hz, 3H), 1.18 (d, $J = 7.1$ Hz, 3H).

$^{13}$C NMR (151 MHz, 1:1 Pyridine $d$-5: Methanol $d$-4)

$\delta$ 172.26, 160.06, 137.81, 134.89, 134.83, 134.32, 134.22, 133.88, 133.78, 133.72, 133.50, 133.39, 133.29, 132.96, 130.92, 98.89, 98.26, 79.04, 77.43, 76.27, 75.27, 74.68, 72.30, 70.43, 69.92, 68.90, 68.65, 68.33, 58.81, 57.62, 47.30, 46.10, 45.79, 44.92, 44.00, 42.94, 41.25, 40.82, 36.97, 36.43, 26.44, 25.14, 19.08, 18.25, 17.36, 12.64.

Analytical HPLC (Zorbax Eclipse C$_{18}$, 3.5-µm, 4.6 x 75 mm, 1.2 mL/min, 95:5 to 5:95 H$_2$O(0.1% HCO$_2$H):MeCN over 8 minutes)

Retention Time = 4.73 min
Morpholino urea 3.6

Oxazolidinone 2.31 (20.0 mg, 17.3 µmol, 1eq) was dissolved in THF (.864 mL, 0.02M). To this solution was added morpholine (30.2 µL, .346 mmol, 20 eq). The reaction was heated to 40°C and stirred for 12 hours, yielding a yellow precipitate. The reaction mixture was then poured into diethyl ether (30 mL), and the resulting yellow precipitate was isolated via pipetting off the diethyl ether to afford a yellow solid which was dissolved in DMSO (~66 mg/mL) and purified by prep-HPLC (C\textsubscript{18}, 5-µm, 50 x 250 mm, 25 mL/min, 95:5 to 5:95 0.3% HCO\textsubscript{2}H (aq):MeCN over 15 minutes). After HPLC purification the desired fractions were collected in a round bottom flask. Residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding 3.6 as a yellow solid (9.5 mg, 9.43 µmol, 48% yield).

\textsuperscript{1}H NMR (600 MHz, 1:1 Pyridine d-5: Methanol d-4)

\[ \delta 6.66 - 6.24 (m, 13H), 5.66 - 5.60 (m, 1H), 5.49 (dd, J = 15.3, 10.4 Hz, 1H), 4.89 (s, 1H), 4.78 - 4.73 (m, 1H), 4.64 (app t, J = 10.5 Hz, 1H), 4.52 (app t, J = 9.0 Hz, 1H), 4.47 (app t, J = 9.9 Hz, 1H), 4.39 - 4.33 (m, 2H), 4.00 - 3.93 (m, 1H), 3.92 (app t, J = 10.7 Hz, 2H), 3.86 (app d, J = 11.1 Hz, 1H), 3.76 - 3.69 (m, 1H), 3.69 - 3.62 (s, 4H), 3.54 - 3.49 (m, 4H), 3.37 (app d, J = 9.6 Hz, 1H), 3.27 - 3.19 (m, 1H), 2.61 - 2.53 (m, 2H), 2.49 (dd, J = 16.9, 9.8 Hz, 1H), 2.40 - 2.32 (m, 2H), 2.19 - 2.10 (m, 1H), 2.06 - 1.94 (m, 3H), 1.89 - 1.83 (m, 1H), 1.83 - 1.73 (m, 1H), 1.73 - 1.63 (m, 4H), 1.64 - 1.55 (m, 3H), 1.55 - 1.49 (m, 1H), 1.43 (d, J = 5.7 Hz, 3H), 1.36 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 7.1 Hz, 3H).
$^{13}$C NMR (151 MHz, 1:1 Pyridine d-5: Methanol d-4)

$\delta$ 172.28, 160.30, 137.62, 136.60, 134.88, 134.83, 134.27, 134.25, 133.92, 133.85, 133.81, 133.52, 133.51, 133.31, 132.97, 131.20, 98.65, 98.29, 79.08, 77.65, 76.25, 75.30, 74.67, 72.33, 70.47, 69.93, 68.85, 68.67, 67.16, 58.84, 57.51, 47.27, 46.00, 45.21, 44.90, 44.02, 42.96, 41.31, 40.83, 40.48, 37.04, 36.42, 31.48, 19.08, 18.15, 17.37, 12.63.

HRMS (ESI)

Calculated for $C_{51}H_{81}N_{3}O_{17}$ (M + $H^+$): 1008.5644

Found: 1008.5668

Analytical HPLC (Zorbax Eclipse C$_{18}$, 3.5-$\mu$m, 4.6 x 75 mm, 1.2 mL/min, 95:5 to 5:95 H$_2$O(0.1% HCO$_2$H):MeCN over 8 minutes)

Retention Time = 4.51 min

Piperazino urea 3.7

Oxazolidinone 2.31 (20.0 mg, 17.3 $\mu$mol, 1 eq) was dissolved in THF (0.864 mL, 0.02M). To this solution was added N-methylpiperazine (9.58 $\mu$L, 86.4 $\mu$mol, 5 eq). The reaction was heated to 40°C and stirred for 12 hours, yielding a yellow precipitate. The reaction mixture was then poured into diethyl ether (30 mL), and the resulting yellow precipitate was isolated via pipetting off the diethyl ether to afford a yellow solid which was dissolved in DMSO ($\sim$66 mg/mL) and
purified by prep-HPLC ($C_{18}$, 5-µm, 50 x 250 mm, 25 mL/min, 95:5 to 5:95 0.3% HCO$_2$H (aq):MeCN over 15 minutes). After HPLC purification the desired fractions were collected in a round bottom flask. Residual formic acid was removed via azeotroping with milliQ water (10 mL) and toluene (50 mL). This process was repeated three times yielding 3.7 as a yellow solid (4.4 mg, 4.31 µmol, 22% yield).

$^1$H NMR (600 MHz, Pyridine $d$-5: Methanol $d$-4)

$\delta$ 6.65 – 6.21 (m, 13H), 5.67 – 5.58 (m, 1H), 5.49 (dd, $J = 14.8, 10.5$ Hz, 1H), 4.93 (s, 1H), 4.79 – 4.73 (m, 1H), 4.67 – 4.60 (m, 1H), 4.54 – 4.44 (m, 2H), 4.44 – 4.36 (m, 1H), 4.40 – 4.32 (m, 1H), 3.99 – 3.93 (m, 2H), 3.91 (app t, $J = 10.2$ Hz, 1H), 3.88 – 3.79 (m, 2H), 3.61 – 3.50 (m, 1H), 3.37 (app d, $J = 8.4$ Hz, 1H), 2.60 – 2.53 (m, 2H), 2.49 (dd, $J = 16.9, 9.7$ Hz, 1H), 2.40 – 2.28 (m, 4H), 2.03 – 1.96 (m, 4H), 1.93 – 1.72 (m, 4H), 1.71 – 1.64 (m, 2H), 1.62 – 1.55 (m, 2H), 1.55 – 1.48 (m, 1H), 1.43 (d, $J = 5.9$ Hz, 3H), 1.35 (d, $J = 6.6$ Hz, 3H), 1.24 (d, $J = 6.5$ Hz, 3H), 1.17 (d, $J = 7.2$ Hz, 3H).

$^{13}$C NMR (150 MHz, Pyridine $d$-5: Methanol $d$-4)

$\delta$ 172.26, 160.03, 134.87, 134.83, 134.27, 133.91, 133.84, 133.51, 133.34, 132.97, 131.33, 98.49, 98.30, 77.69, 76.20, 75.33, 74.61, 72.35, 69.24, 68.67, 68.05, 46.05, 45.96, 44.89, 44.42, 43.01, 40.84, 40.48, 36.43, 19.08, 18.18, 18.07, 12.63.

HRMS (ESI)

Calculated for $C_{52}H_{84}N_4O_{16}$ (M + H$^+$): 1021.5691

Found: 1021.5942

Analytical HPLC (Zorbax Eclipse $C_{18}$, 3.5-µm, 4.6 x 75 mm, 1.2 mL/min, 95:5 to 5:95 H$_2$O(0.1% HCO$_2$H):MeCN over 8 minutes)

Retention Time = 4.03 min
Synthesis of AmdeB,\textsuperscript{35} AmBME,\textsuperscript{36} C41MeAmB,\textsuperscript{35} AmBMA,\textsuperscript{38} and AmBTABA\textsuperscript{39} was accomplished using previously reported procedures.

**Extinction Coefficient Determination**

Extinction coefficients (L mol\textsuperscript{-1} cm\textsuperscript{-1}) were determined as previously reported\textsuperscript{9} and were as follows: AmB (\(\varepsilon_{406} = 164,000\)), AmdeB (\(\varepsilon_{406} = 102,000\)), AmBME (\(\varepsilon_{406} = 117,000\)), C41MeAmB (\(\varepsilon_{406} = 102,000\)), AmBMA (\(\varepsilon_{406} = 114,000\)), AmBTABA (\(\varepsilon_{406} = 121,000\)), AmBMU (\(\varepsilon_{406} = 87,000\)), AmBAU (\(\varepsilon_{406} = 87,000\)), AmBCU (\(\varepsilon_{406} = 58,000\)).

**Isothermal Titration Calorimetry**

ITC was performed as previously reported.\textsuperscript{20}

**Growth Conditions for *S. cerevisiae*.**

*S. cerevisiae* was maintained with yeast peptone dextrose (YPD) growth media consisting of 10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, and 20 g/L agar for solid media. The media was sterilized by autoclaving at 250°F for 30 min. Dextrose was subsequently added as a sterile 40% w/v solution in water (dextrose solutions were filter sterilized). Solid media was prepared by pouring sterile media containing agar (20 g/L) onto Corning (Corning, NY) 100 x 20 mm polystyrene plates. Liquid cultures were incubated at 30°C on a rotary shaker and solid cultures were maintained at 30°C in an incubator.

The organisms were maintained, grown, subcultured, and quantified on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI). Twenty-four hours prior to the study, the organisms were subcultured at 35°C.

Minimum Inhibitory Concentration (MIC) Determination

MIC determinations were performed in duplicate on at least two occasions using the Clinical and Laboratory Standards Institute M27-A3 microbroth methodology.57

Hemolysis Assays

Hemolysis experiments were performed following known procedures.20 Whole human blood (sodium heparin) was purchased from BioreclamationIVT (Westbury, NY) and stored at 4°C and used within two days of receipt.

WST-8 Cell Proliferation Assays

Primary Renal Proximal Tubule Epithelial Cells Preparation.

Primary human renal proximal tubule epithelial cells (RPTECs) were purchased from ATCC (PCS-400-010, Manassas, VA) and immediately cultured upon receipt. Complete growth media was prepared using renal epithelial cell basal medium (ATCC, PCS-400-030), renal epithelial cell growth kit (ATCC, PCS-400-040), and penicillin-streptomycin (10 units/mL and 10 μg/mL). Complete media was stored at 4°C in the dark and used within 28 days. Primary RPTECs were grown in CO2 incubator at 37 °C with an atmosphere of 95% air/5% CO2.

TERT1 Renal Proximal Tubule Epithelial Cells Preparation.

TERT1 human renal proximal tubule epithelial cells (RPTECs) were purchased from ATCC (CRL-4031, Manassas, VA) and immediately cultured upon receipt. Complete growth media was prepared using DMEM:F12 media (ATCC, 30-2006), triido-L-thyronine (Sigma, T6397), recombinant human EGF (Life Technologies, PHG0311), ascorbic acid (Sigma, A4403), human transferrin (Sigma, T8158), insulin (Sigma I9278), prostaglandin E1 (Sigma, P7527), hydrocortisone (Sigma, H0888), sodium selenite (Sigma, S5261), and G418 (Sigma, A1720).
Complete media was stored at 4°C in the dark and used within 28 days. TERT1 RPTECs were grown in CO\(_2\) incubator at 37°C with an atmosphere of 95% air/5% CO\(_2\).

**WST-8 Reagent Preparation.**

WST-8 cell proliferation assay kit (10010199) was purchased from Cayman Chemical Company (Ann Arbor, MI) and stored at -20°C and used within 6 months of receipt. WST-8 reagent and electron mediator solution were thawed and mixed to prepare the WST-8 reagent solution. The solution was stored at -20°C and used within one week.

**WST-8 Assay.**

A suspension of primary or TERT1 RPTECs in complete growth media was brought to a concentration of 1 x 10\(^5\) cells/mL. A 96-well plate was seeded with 99 µL of the cell suspension and incubated at 37°C with an atmosphere of 95% air/5% CO\(_2\) for 3 hours. Positive and negative controls were prepared by seeding with 100 µL of the cell suspension or 100 µL of the complete media. Compounds were prepared as 5 mM (AmB) and 8 mM (AmBAU, AmBMU, AmBCU, and AmdeB) stock solutions in DMSO and serially diluted to the following concentrations with DMSO: 8000, 6000, 4000, 3000, 2000, 1500, 1000, 800, 600, 400, 300, 200, 100, 50, 25, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 µM. 1 µL aliquots of each solution were added to the 96-well plate in triplicate, with each column representing a different concentration of the test compound. The 96-well plate was incubated at 37°C with an atmosphere of 95% air/5% CO\(_2\) for 24 hours. After incubation, the media was aspirated and 100 µL of serum-free media was added and 10 µL of the WST-8 reagent solution was added to each well. The 96-well plate was mixed in a shaking incubator at 200 rpm for 1 minute and incubated at 37°C with an atmosphere of 95% air/5% CO\(_2\) for 2 hours. Following incubation, the 96-well plate was mixed in a shaking incubator at 200 rpm for 1 minute and absorbances were read at 450 nm using a Biotek H1 Synergy Hybrid Reader (Wanooski, VT). Experiments were performed in triplicate and the reported cytotoxicity represents an average of three experiments.

**Data Analysis.**

Percent hemolysis was determined according to the following equation:
Concentration vs. percent hemolysis was plotted and fitted to 4-parameter logistic (4PL)\textsuperscript{58} dose response fit using OriginPro 8.6. The MTC was defined as the concentration to cause 90% loss of cell viability.

**Ethics Statement**

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin according to the guidelines of the Animal Welfare Act, The Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals, and Public Health Service Policy.

**In Vivo Murine Efficacy Study**

All studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital (Madison, WI). Efficacy was assessed by CFU count in the kidneys of neutropenic mice with a disseminated fungal infection as described previously by Andes et al\textsuperscript{32,50,51}. A clinical isolate of *Candida albicans* (K-1) was grown and quantified on SDA. For 24 hours prior to infection, the organism was subcultured at 35°C on SDA slants. A $10^6$ CFU/mL inoculum (CFU, colony forming units) was prepared by placing six fungal colonies into 5 mL of sterile, depyrogenated normal (0.9%) saline warmed to 35°C. Six-week-old ICR/Swiss specific-pathogen-free female mice were obtained from Harlan Sprague Dawley (Madison, WI). The mice were weighed (23–27 g) and given intraperitoneal injections of cyclophosphamide to render neutropenia (defined as <100 polymorphonuclear leukocytes/mm$^3$). Each mouse was dosed with 150 mg/kg of cyclophosphamide 4 days prior to infection and 100 mg/kg 1 day before infection. Disseminated candidiasis was induced via tail vein injection of 100 µL of inoculum. AmB, AmBAU, or AmBMU were reconstituted with 1.0 mL of 5% dextrose. Each animal in the treatment group was given a single 200 µL intraperitoneal (ip) injection of reconstituted AmB, AmBAU, or AmBMU 2 hours post-infection. Doses were calculated in terms of mg of compound/kg of body weight. At each time point (6, 12, and 24 hours post-infection), three animals per experimental condition were sacrificed by CO$_2$ asphyxiation. The kidneys from each animal were removed and homogenized. The homogenate was diluted serially 10-fold with 9% saline and plated on SDA. The plates were then incubated.
for 24 hours at 35°C and inspected for CFU viable counts. The lower limit of detection for this technique is 100 CFU/mL. All results are expressed as the mean log_{10} CFU per kidney for three animals.

**In Vivo Murine Toxicity Study**

All studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital (Madison, WI). Uninfected Swiss ICR mice were used for assessment of infusion toxicity. Groups of five mice were treated with single intravenous doses of AmB, AmBAU, AmBMU (reconstituted with 1.0 mL of 5% dextrose), or sterile pyrogen-free 0.85% NaCl administered via the lateral tail vein over 30 seconds. Dose levels studies included 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/kg. Following administration mice were observed continuously for one hour and then every 6 hours up to 24 hours for signs of distress or death.

**Resistance Studies**

**Minimum Inhibitory Concentration and Growth Assays**

Susceptibility of wild-type and resistant strains to AmB, AmBAU, AmBMU, tert-butyl peroxide (Sigma-Aldrich), geldanamycin and radicicol (A.G. Scientific) was determined in flat bottom, 96-well microtiter plates (Costar) using a broth microdilution protocol adapted from CLSI M27-A3. Overnight cultures (14-20hr) were grown at 30°C in YPD, and approximately 5x10^3 cells were seeded per well. For AmB, AmBAU, and AmBMU, MIC assays were performed at 37°C in RPMI buffered with MOPS (0.165M) with 10% fetal bovine serum (Sigma-Aldrich) added; for tert-butyl peroxide, geldanamycin, and radicicol, MIC’s were determined in YPD at 30°C. MIC’s were determined after 24h incubation as the concentration of compound resulting in no visible growth in wells. For quantitative display of growth at drug dilutions, OD_{600} was measured in a spectrophotometer (Tecan) and displayed as heat maps using Java TreeView 1.1.3 (http://jtreeview.sourceforge.net).

**Media and Growth Conditions**

*C. albicans* was generally grown and maintained as described previously\(^1\) Stocks were stored in 15% glycerol at -80°C; strains were generally grown in YPD media at 30°C. Drugs were added directly to media from DMSO stocks.
**In Vitro Gradual Selection of AmB, AmBAU, or AmBMU Resistance**

Selection of resistance to AmB, AmBAU, and AmBMU was performed as follows. 1 mL overnight (14-20hr) cultures of SC5314 (WT) were washed in PBS, then treated with 3% EMS (Ethyl Methanesulfonate, Sigma-Aldrich) for 45 min. Cells were then washed 4x in YPD and resuspended in YPD and allowed to recover for 3h. Cells were then inoculated to an OD600 of approximately 0.025-0.05 in 100mL YPD containing 0.25µM AmB or AmB-AU, or 0.375µM AmB-MU. After 24-72 hours, a 1mL aliquot was removed from any culture that had grown to saturation and subjected to another round of mutagenesis in the same manner as described above. After recovery, cells were then inoculated into a new YPD flask containing 2x higher concentration of the same drug. Cultures that grew were subjected to one more round of EMS mutagenesis before inoculating into a 2-fold higher drug concentration (total of 3 rounds of EMS mutagenesis) and then passaged at 2-fold higher increments of drug concentration until reaching 2µM AmB or AmB-AU, or 3µM AmB-MU. Cultures were passaged once more at 2µM AmB or AmBAU or 3µM AmBMU, then plated onto YPD media and frozen in glycerol stocks before further evaluation.

**Filamentation Assay**

Hyphal induction was performed by growing *C. albicans* overnight at 30°C in YPD, washing in PBS, and diluting 1:100 into RPMI+10% fetal bovine serum (Sigma-Aldrich) at 37°C in a culture tube on a rotating wheel. After 3h, cultures were washed in PBS and resuspended in 250µg/mL Calcofluor white in a microcentrifuge tube, and shaken at 30°C for 10 min. Cells were then washed twice in PBS, concentrated 10-fold, briefly sonicated in a water bath, and mounted on slides for visualization under a DAPI filter set at 60X magnification.

**Murine Model of Systemic Infection**

All animal protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animals were maintained according to the guidelines of the MIT Committee on Animal Care (CAC). These studies were approved by the MIT CAC (protocol #0312-024-15). We used 7-12-week-old female Balb/c mice ordered from Taconic farms for all mouse virulence studies. All strains were prepared for inoculation by
diluting overnight cultures (14-20h) 1:100 into YPD and growing into log phase for 4-5 hours, then washing 3x in PBS before. Strains were injected into the lateral tail vein in a volume of 100 µl. For mouse survival experiments, strains were grouped as follows: The wild-type Mutagenized pool consisted of 5 SC5314 colonies subjected in parallel to mutagenesis and passaging (as described above) without drug exposure, injected as of 1.6x10^5 cfu per strain (8x10^5 cfu total inoculum per mouse); the Wild-type low inoculum was the SC5314 parental strain injected at 1.6x10^5 cfu. AmB-Resistant, AmBAU-Resistant, and AmBMU-resistant pools were comprised of strains isolated from each selection in the presence of the indicated drug, using strains that exhibited >4-fold MIC increase for the drug used. Individual resistant strains were present in the pools at 1.6x10^5 cfu per mouse (8x10^5 total inoculum per mouse when pooled). Each strain or pool of strains was tested in at least two independent experiments, and data were pooled. Mice were weighed daily and monitored for signs of morbidity and sacrificed when body weight decreased by 20%, or when signs of extreme distress were apparent. For the competitive infection with quantification of kidney burden, a pool comprised of 16 strains at equal fraction of the population, one SC5314 wild-type and 5 strains each from selections for resistance to AmB, AmBAU, and AmBMU was used, with 3x10^4 cells of each strain inoculated per mouse (4.8x10^5 total inoculum). Three mice were used per experiment, in a total of two experiments. 4d after infection, mice were sacrificed and kidneys were removed aseptically, homogenized, and plated onto YPD plates. Pools of the inoculum immediately before injection were also plated. 184 colonies were randomly selected from the pre-infection and 184 from the post-infection plates and tested for growth in 96-well plates in the presence of 1 µM AmBAU or 1.5 µM AmBMU, and the fraction of wells from the pre and post-infection pools exhibiting growth in either drug was determined.

**Whole Genome Sequencing, Alignment, Mapping, and Variant Calling**

Whole genome sequencing and analysis was performed as previously described.\(^\text{17}\)

**3-9 References**

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CHAPTER 4: A BUILDING BLOCK BASED APPROACH TO AMPHOTERICIN B SYNTHESIS

As demonstrated in both chapters 2 and 3, degradative synthesis enables access to numerous AmB derivatives. However, over half of the AmB backbone remains refractory to synthetic manipulation. An efficient and flexible total synthesis of AmB would permit access to derivatives not accessible by current synthetic strategies. For example, the binding of sterols underlies both AmB’s antifungal and ion channel functions. An atomistic understanding of this unique small-molecule small-molecule interaction is therefore required to optimize both functions. Site selectively placing $^{13}$C labels on the AmB backbone would enable solid-state NMR studies to map this interaction with atomic resolution. A $^{13}$C$_2$-AmB was therefore targeted. Furthermore, several polyol alcohols are predicted to play critical roles in AmB ion channel formation and conductance, notably the C9 alcohol. To test this hypothesis C9deoxyAmB is required. Neither C9deoxyAmB nor $^{13}$C$_2$-AmB are accessible using existing degradative or semisynthetic methodologies.

Taking advantage of the iterative cross coupling (ICC) platform for small molecule synthesis, AmB was retrosynthetically disconnected into four building blocks. The total synthesis then proceeded in three phases: 1.) building block construction, 2.) ICC and macrolactonization, 3.) protecting group removal. This chapter describes synthetic efforts in all three phases. Scalable access to one half of BB1 was achieved, significantly improving upon the existing methodology. With access to BB1 established, cross coupling conditions were developed in a model system for the BB1-BB2 cross coupling, the most difficult cross coupling in the ICC sequence. Gratifyingly, these conditions translated to the fully elaborated BB1-BB2 cross coupling. Lastly, optimized conditions for the final deprotection step, the reduction of the mycosamine azide to a free amine, were developed. The ICC strategy is especially conducive to derivatization as a new AmB analog could be accessed by simply exchanging a single building block. Demonstrating this flexibility, the fully oxidized backbone of a deoxygenated BB1, C9deoxyBB1, was synthesized.

Pulin Wang provided 4.18 and BB1 4.2 for cross coupling studies. Ian Dailey provided BB2 MOM ether 4.24. Dr. Justin Struble performed the ICC sequence and macrolactonization. Matt Endo prepared AmB-azide 4.30.
4-1 Background

The interface between AmB and sterol is the foundational interaction necessary for both AmB’s ion channel and antifungal functions. To understand and optimize both of these functions, it will be critical to understand this interaction at the atomistic level. Modeling studies have previously attempted to understand this interaction,\textsuperscript{1-4} however they lacked the structural constraints required to develop a reliable high-resolution structure. The mycosamine appendage is critical for sterol binding,\textsuperscript{5} and recent solid-state NMR experiments confirm direct contact between the AmB polyene and ergosterol tetracyclic core.\textsuperscript{6} If intermolecular distances between AmB and ergosterol could be quantified, then parameters obtained from such experiments could constrain modeling studies and inform AmB–sterol structure calculations. AmB’s complex architecture complicates this analysis as many of the $^{13}$C signals in a solid state NMR spectrum overlap. Consequently, site selectively $^{13}$C labeling the AmB framework is required (Figure 4.1A). To provide intermolecular interactions between AmB and sterol a position near the putative sterol binding pocket is needed, such as C19. AmB is also known to primarily exists as a large extramembranous aggregate (Figure 4.1B).\textsuperscript{6} Observing intermolecular AmB-AmB interactions would illuminate the structure of this fungicidal aggregate. Labeling the C13 position could give such structural information, as the crystal structure of N-iodoacetyl AmB reveals a water bridged hydrogen bond between the C13 hydroxyl and a neighboring AmB monomer.\textsuperscript{7} The C13 and C19 positions are ideally suited for this experiment as they are well resolved in the $^{13}$C spectrum. Current synthetic strategies are incapable of accessing such a doubly $^{13}$C labeled AmB derivative.

Figure 4.1: The AmB – sterol interaction is critical for both AmB’s ion channel and cell killing functions. A.) Crystal structures of N-Iodoacetyl AmB and ergosterol with ergosterol positioned in putative binding pocket between mycosamine appendage and polyene frameworks. Proposed $^{13}$C labels highlighted in red. B.) Sterol sponge model from which AmB exerts its fungicidal activity.
Much of the carbon backbone of the AmB framework, stretching from C1-C20, is still inaccessible using degradative or semisynthesis platforms (Figure 4.2). Several important critical functionalities are contained within this segment including both the 6-membered hemiketal ring and mycosamine appendage. Together these moities are critical for sterol binding and antifungal activity. Intriguingly, this functional motif is conserved across all members of the polyene macrolide family of natural products. Consequently, $^{13}$C labeling AmB in this highly conserved region may give insight not only into the interaction between AmB and sterols, but all polyene macrolides and sterols.

![Figure 4.2: Section of AmB inaccessible by current degradative or semisynthetic strategies. The universally conserved structural motif of all polyene macrolides is highlighted in blue.](image)

Also contained within fragment 4.1 is the polyol region. The selective removal of a single alcohol in chapter 2, the C3 alcohol, revealed the critical role this hydroxyl plays in AmB’s ion channel conductance. This structure activity relationship is the first step towards elucidating the structure of the AmB ion channel, which has remained elusive for 40 years. The remaining polyol alcohols have also been predicted to play critical roles in ion channel formation and conductance. Modeling studies have predicted that the C8 and C9 alcohols help stabilize the AmB ion channel by forming an intermolecular hydrogen-bonding network (Figure 1.4B,C). It would stand to reason then removing either the C8 or C9 alcohol would abolish this intermolecular interaction and thereby alter AmB’s ion channel properties. The C8 alcohol has been removed biosynthetically, but C8deoxyAmB’s ion channel properties have not been investigated. Current synthetic strategies are incapable of selectively modifying the C9 alcohol.

For these reasons, a total synthesis is required to access to both C9deOAmB and $^{13}$C$_2$-AmB. If the total synthesis strategy were efficient and flexible then virtually any desired AmB derivative could be accessed on scales commensurate with biological testing. Because of the complex and intriguing structure of AmB, a number of groups have attempted to access this molecule via total synthesis. Only the Nicolaou group was successful in this endeavor. We
pursued an alternate strategy for the total synthesis of AmB aiming to achieve efficient and flexible access to a number of different AmB derivatives. The iterative cross coupling (ICC) platform developed by the Burke group has found great success accessing a number of different small molecules efficiently and flexibly.\textsuperscript{14-20} Similar to peptide coupling, ICC relies on a single reaction, the Suzuki-Miyaura cross coupling, to join bifunctional building blocks under mild conditions.\textsuperscript{14} In this instance, the building blocks are halo-boronic acids protected as their corresponding MIDA boronate esters with all necessary functionality and stereochemical information preinstalled. The building blocks can easily be exchanged, making the construction of derivatives facile. Based on these reasons, we aimed to leverage the ICC strategy as a platform for the total synthesis of AmB.

4-2 Synthetic strategy and the synthesis of BB1

As depicted in Figure 4.3, based on the ICC platform AmB was retrosynthetically disconnected into four building blocks. Contained within BB1 is the entire polyol subunit. The first bifunctional building block, BB2, contains both the pyran and mycosamine appendages, two functionalities that are highly conserved across all polyene macrolides. Consequently, BB2 is a universal building block for the construction of all polyene macrolide natural products.\textsuperscript{8} The second bifunctional building block, iodo triene BB3, has already been successfully employed in the synthesis of multiple polyene natural products.\textsuperscript{17,20,21} Lastly, BB4 contains the western half of AmB. Using Suzuki cross coupling, we aimed to forge carbon-carbon bonds between BB1 and BB2, BB2 and BB3, and BB3 and BB4. A final macrolactonization would then complete the full macrolide core of AmB. This strategy greatly simplifies the synthesis of any desired AmB derivative. For example, the synthesis of C9deoxyAmB could be achieved by exchanging BB1 for a suitably deoxygenated BB1. All other components in the ICC and macrolactonization sequence remain unchanged, thereby reducing the synthesis of a new derivative to the creation of a single new building block.
Using this strategy the total synthesis of AmB can be divided into three phases: 1.) building block synthesis 2.) ICC and macrolactonization 3.) protecting group removal. Scalable access to both BB3\textsuperscript{17,20,21} and BB4,\textsuperscript{22} as well as the construction of the polyene core of AmB via ICC, has already been established.\textsuperscript{16} The synthesis and coupling of BB1 has several notable challenges. First, scalable access to BB1 is especially crucial as it is carried throughout the entire ICC sequence. Moreover, the coupling of BB1 to BB2 is predicted to be especially challenging as it is the only sp2-sp3 cross coupling. To address these concerns a scalable synthesis of BB1 was sought. As depicted in Figure 4.4, BB1, 4.2, could be accessed by hydroboration and reduction of enone 4.3. Application of the Horner-Wadsworth-Emmons transform to enone 4.3 divides this building block into aldehyde 4.4 and β-keto phosphonate 4.5. Efforts were first focused on developing an efficient and scalable synthesis of aldehyde 4.4.

The 1\textsuperscript{st} generation synthesis of aldehyde 4.4 is depicted in Scheme 4.1.\textsuperscript{23} Silyl enolate formation of dioxenone 4.6 enabled a Mukaiyama aldol addition between 4.7 and acrolein, forming allylic alcohol 4.8. A Sharpless kinetic resolution then set the stereocenter, forming enantioenriched allylic alcohol 4.9. Methanolysis of the dioxenone ring then generated β-keto ester 4.10, which upon reduction revealed syn 1,3 diol 4.11. Subsequent ketalization and ozonolysis of terminal olefin 4.12 completed the synthesis of aldehyde 4.4. This synthesis proceeded in 7 steps with a 3% overall yield. To enable large-scale access to this critical...
fragment, several aspects of this 1st generation synthesis needed improvement. First, the allylic stereocenter on 4.8 was set via kinetic resolution, by definition only having a maximum 50% yield. An alternative methodology could greatly improve efficiency and yield. Using TiCl₄ and the toxic reagent acrolein could also prove problematic on large scale.

To address these concerns, a 2nd generation synthesis of aldehyde 4.4 was designed (Scheme 4.2A). Enantioselective vinylogous aldol methodologies are typically used to access motifs such as 4.8. A report by Soriente et al. demonstrated that combining Chan’s diene 4.13 with cinnamaldehyde 4.14 in the presence of Titanium isopropoxide and (S)-BINOL effected an enantioselective extended aldol reaction to form β-keto ester 4.15. This intermediate then intercepted chemistry similar to the 1st generation synthesis. 1,3-Syn reduction, ketalization, and subsequent ozonolysis of styrene 4.17 completed the 2nd generation synthesis of aldehyde 4.4. This route substantially improved access to 4.4, improving the yield to 40% overall. Furthermore, efficiency was greatly increased as this sequence proceeds in four steps overall. Additionally, the toxic reagent acrolein has been replaced with the much more benign cinnamaldehyde. Styrene 4.17 proved to be crystalline (Scheme 4.2B), making it a shelf stable storage point. Large quantities of 4.17 could be synthesized and stored for extended periods of time. When β-keto ester 4.5 was available for Horner-Wadsworth-Emmons coupling, substantial quantities of aldehyde 4.4 could be prepared on a days notice.
4-3 BB1-BB2 cross coupling, ICC sequence, and final deprotection

The efficient synthesis of 4.4 enabled gram scale access to BB1. With access to this key building block in hand we focused on the BB1-BB2 cross coupling. We anticipated this reaction would be especially challenging as it is the only sp2-sp3 coupling in the ICC sequence. To develop initial conditions we first attempted cross-coupling on a simplified model system. Dioxene 4.18 was used as a BB1 surrogate while and the ketene acetal phosphate derived from valerelactone (4.20) was employed as a simplified model substrate for BB2. Upon hydroboration of 4.18 to borane 4.19, the catalyst species and base identity were explored to effect coupling with 4.20. Optimal production of 4.21 was observed when Pd(OAc)$_2$ was combined with Buchwald’s biaryl phosphine ligands and K$_3$PO$_4$ as base. Unfortunately, numerous attempts to translate these conditions to the fully functionalized BB1 did not initially yield the desired dihydropyran 4.22. Water was rigorously excluded to avoid ketene acetal phosphate hydrolysis, consequently inhibiting productive cross-coupling. The addition of three equivalents of exogenous water to the reaction mixture (Scheme 4.4) promoted formation of 4.22. This additional water causes a potential problem, as MIDA boronates are typically deprotected using aqueous basic conditions. Consequently, we needed to determine how the rate of MIDA boronate hydrolysis is impacted by the equivalence of water.

Scheme 4.3: Initial exploration of sp2-sp3 cross coupling on model system.
To investigate this water dependence, a simple hydrolysis study was performed in which vinyl MIDA boronate 4.23 was exposed to K₃PO₄ in THF with varying equivalents of water (1 eq, 3 eq, and excess water) and monitored for hydrolysis over 24 hours (Scheme 4.5). Solvent quantities of water rapidly hydrolyzed the MIDA boronate. In contrast, after 24 hours with 3 equivalents of water, 75% of the MIDA boronate remained intact. This suggested there was a window in which productive cross-coupling could occur without MIDA boronate hydrolysis. When these conditions were translated to the coupling between alkyl borane 4.23 and BB2 mom ether 4.24, the desired product 4.26 was observed (Scheme 4.6).
Gratifyingly, the coupling conditions optimized on the BB2 model system translated to the fully functionalized BB1-BB2 cross coupling (Scheme 4.7). 9BBN-borane 4.27 was combined with $^{13}$C$_2$-BB2 4.28 in the presence of the 2$^{nd}$ generation SPhos Palladacycle, K$_3$PO$_4$, and 3 equivalents of water to form the desired BB1-BB2 adduct 4.29. Cross coupling of 4.29 with iodotriene BB3, followed by coupling with dienyl iodide BB4 and finally macrolactonization completed the ICC sequence. This sequence completed the synthesis of the fully protected $^{13}$C$_2$-AmB (Scheme 4.6).

Scheme 4.7: Completion of the ICC sequence and macrolactonization to fully protected doubly $^{13}$C labeled AmB.

Only four deprotection steps remain to complete the total synthesis of a doubly $^{13}$C labeled AmB. Several steps including TBS desilylation, TMSE removal, and ketal hydrolysis were previously optimized on a model system, and during the synthesis of C35deOAmB. The final step, reduction of the mycosamine azide 4.32 to a free amine, still required optimization (Scheme 4.8). Typical azide reduction methodologies, such as hydrogenation, are incompatible with the polyene motif, and initial efforts using propane dithiol and triethylamine to perform this reduction were irreproducible. We thus turned to the Staudinger reduction. Multiple parameters in this reaction including phosphine identity, solvent, temperature, and water equivalents were investigated. Triphenylphosphine showed no reactivity, while varying reaction rates and selectivities were observed with trialkyl phosphines (PBu$_3$, PEt$_3$, and PMe$_3$). Of these, trimethyl phosphine performed the best. Solvent effects played a major role as some caused significant decomposition (DMEU, NMP, HMPA) while DMSO showed clean conversion. Temperature only appeared to effect reaction rate, while the equivalence of water affected both
reaction rate and the extent of decomposition. Ultimately it was discovered that 3 equivalents of PMe₃, 100 equivalents of H₂O, in DMSO at 50°C cleanly and efficiently reduced azide 4.31 to AmB (Scheme 4.7). Although we have not yet had the opportunity to evaluate these conditions on ¹³C₂-AmB, they gratifyingly translated to an even more sensitive substrate, C2’deOAmB. Using conditions almost identical to those above, Brandon Wilcock was able to reduce azide 4.32 to the desired penultimate free amine 4.33 in 57% yield (Scheme 4.9).³¹

![Scheme 4.8: Optimization of the final reaction, reduction of the C3’ azide to the free amine, AmB. Depicted below the reaction scheme is the HPLC conversion of 4.30 (top) into AmB.](image)

![Scheme 4.9: Conditions for AmB azide deprotection translated to the more sensitive C2’deoxyAmB derivative. Adapted from ref. 29.](image)

**4-4 Synthesis of deoxygenated building blocks**

The completion of the total synthesis of fully protected ¹³C₂-AmB validates the ICC platform as a strategy for the construction of AmB derivatives. The flexible nature of the ICC strategy is especially conducive to derivatization, as synthesis of a deoxygenated analog only requires exchange of a single building block. The C8 and C9 alcohols are especially intriguing for two reasons. As demonstrated in chapter 2, AmB’s polyol alcohols play a critical role in ion conductance through the ion channel. Additionally, the C8 and C9 alcohols are predicted to form
an intermolecular hydrogen bond between neighboring AmB molecules, stabilizing the AmB ion channel. Based on this model one would predict that removal of either the C-8 or C-9 alcohol would alter AmB’s ion channel properties. The C8 alcohol has been removed biosynthetically, but removal of the C9 alcohol is only achievable using total synthesis. The retrosynthesis of C9deOAmB is illustrated in Figure 4.5. Based on the ICC platform, only the C9 deoxygenated variant of BB1 needs to be created to achieve this synthesis.

The synthesis of C9deoxyBB1 presents a set of challenges. The installation of boron into this motif is critical, as it is required for the Suzuki cross coupling. In the synthesis of BB1, boron is installed during the last step of the synthesis via a 9BBN hydroboration. As shown in Figure 4.6A, the C9 and C-11 alcohols are ketalized, forming a six membered ring (4.34). Hydroboration can occur from either the exo (top) or endo (bottom) face of the exocyclic olefin. The axial product (4.35) resulting from hydroboration of the exo face is not observed as this would proceed through a high energy twist-boat conformation. Conversely, endo face attack proceeds through a low energy chair-like transition state, stereoselectively yielding equatorial adduct 4.36. Alternatively, if the C-9 alcohol were absent the six-membered ring would be abolished, as in enol 4.37. The resulting hydroboration would be rendered non-selective. Two strategies could overcome this challenge. A diastereoselective hydroboration of a protected enol, such as 4.37, would access the desired deoxy BB1 ready for coupling (Figure 4.6B). Although enantioselective transition metal catalyzed hydroborations have been developed, this methodology is yet to be extended to simple linear enolates. Alternatively, the MIDA boronate is stable to a number of common synthetic transformations. An achiral MIDA boronate, such as 4.39 (Figure 4.6C), could be elaborated using well precededent asymmetric methodologies into
fully functionalized C9deoxyBB1 4.40. Then, just prior to Suzuki coupling the free boronic acid could be revealed. We elected to use the latter strategy to access C9deoxyBB1.

![Diagram](image_url)

**Figure 4.6:** Installation of boron functionality to BB1. A.) Hydroboration of dioxenone olefin proceeds stereoselectively due to attack from the endo face leading to a chair like transition state. Therefore, only the equatorial hydroboration product is observed. B.) Without the C9 alcohol such a hydroboration would be non-selective. A stereoselective hydroboration would be required to access a boron adduct ready for coupling. C.) C9deoxyBB1 4.40 could be synthesized by elaboration of a simple MIDA boronate building block such as 4.39.

As depicted in Scheme 4.10, ozonolysis the terminal olefin of allyl MIDA boronate 4.39 generated alpha boryl aldehyde 4.41. Similar alpha boryl aldehydes have found great utility in the construction of chiral MIDA boronate building blocks.33 A Brown asymmetric allylation then formed homoallylic alcohol 4.42. This procedure required additional optimization as oxidative removal of the Brown reagent post reaction under typical conditions, H2O2 and NaOH, caused MIDA boronate degradation. After screening a number of different oxidation conditions,34-36 simple household bleach, sodium hypochlorite, was discovered to mildly oxidize the boryl adduct without MIDA deprotection. With this oxidation protocol established, 4.42 was protected as its corresponding TBS silyl ether, 4.43. A 9BBN mediated hydroboration of the terminal olefin, followed by the same oxidative workup procedure from the Brown allylation, formed alcohol 4.44. Dess-Martin periodinane mediated oxidation of 4.44 converted the alcohol into
aldehyde 4.45. Nucleophilic attack of aldehyde 4.45 with lithiated dimethyl methyl phosphonate 4.46, followed by Dess-Martin oxidation completed the synthesis of β-keto phosphonate 4.47, the deoxygenated half C9deoxyBB1.

The convergent nature of this synthesis is advantageous as aldehyde 4.4, used in the synthesis of BB1, can also be utilized to construct C9deoxyBB1. The Roush-Masamune variant of the Horner-Wadsworth Emmons reaction mildly couples 4.4 and 4.47 together forming enone 4.48. The C-8 stereocenter was then set via CBS reduction, generating allylic alcohol 4.49. Hydrogenation of the internal olefin completed the synthesis of C9deoxyBB1 4.50 with all carbons in their proper oxidation states (Scheme 4.11). Only a single protection remains to complete the synthesis of C9deoxy BB1 ready for cross coupling. The synthesis of 4.50 highlights the robustness of the MIDA boronate protecting group. Starting with allyl MIDA boronate 4.39, boron was carried ten-steps through variety of reaction conditions including ozonolysis, oxidation, reduction, hydrogenation, and attack by hard nucleophiles.

Scheme 4.11: End game synthesis of C9-Deoxy BB1.
4-5 Thesis summary

This dissertation describes the development of structure activity relationships that advance the understanding of AmB’s ion channel and antifungal functions. Towards this aim synthetic strategies were developed to gain access to site specifically modified AmB derivatives. Both degradative and semi-synthesis strategies have yielded many AmB derivatives, however many derivatives are inaccessible from these platforms. Thus, an efficient and flexible total synthesis based on the ICC platform was sought. Key advances were made in both the construction and cross coupling of BB1 as well as the final deprotection. Access to a key BB1 precursor was improved by replacing a multi-step sequence with a single enantioselective vinylogous aldol reaction. With access to BB1 established productive cross coupling between BB1 and BB2 was required. On a model system it was discovered that the addition of three equivalents of water was critical to encourage productive coupling. Gratifyingly this finding transferred to the fully functionalized BB1-BB2 coupling. The final deprotection, Staudinger reduction of the mycosamine azide, was systematically optimized. These findings collectively enabled the construction of a fully protected $^{13}$C$_2$-AmB.

Degradative synthesis played a crucial role in accessing derivatives to probe AmB’s ion channel and antifungal properties. Inspired by a fortuitous byproduct, the 9-step degradative synthesis of C3deOAmB from the parent AmB was achieved. In voltage clamp single ion channel studies C3deOAmB had a significantly decreased conductance relative to AmB. This result begins to shed light on the structure of the AmB ion channel, which has remained elusive for over 40 years. A change in single ion channel conductance is consistent with the leading barrel stave model, or any other ion channel model, that places the C3 alcohol at a critical point in the ion channel conductance pathway.

In further efforts to understand the ion selectivity of the AmB ion channel, AmB derivatives were sought in which the carboxylate was replaced with an amine. During these efforts, an efficient 3-step synthesis to a family of AmB urea derivatives was discovered. This new structural motif provided an intriguing way to test the emerging allosteric modification model as a rational platform for the non-toxic derivatization of AmB. Like C2’deOAmB, these derivatives selectively bound ergosterol but not cholesterol, maintained antifungal efficacy, but were drastically less toxic to human cells in culture. Furthermore two ureas, AmBMU and AmBAU, were more efficacious than AmB in a mouse model of disseminated candidiasis and
were significantly less toxic in acute mice toxicity studies. Despite their increased ergosterol specificity and decreased toxicity, AmBMU and AmBAU were no more susceptible to the onset of drug-resistance than AmB. The scalable 3-step synthesis, potent antifungal activity, drastically reduced toxicity, and resistance evasive profile make AmBMU and AmBAU exciting potential clinical replacements for AmB.

4-6 Methods

Materials

Commercially available materials were purchased from Aldrich Chemical Co. (Milwaukee, WI), Fisher Scientific (Hampton, NH), Alfa Aesar (Ward Hill, MA), TCI America (Portland, OR), Oakwood Chemical (Wes Columbia, SC), and Silicycle (Quebec, Canada) and used without further purification unless noted otherwise. Solvents were purified via passage through packed columns as described by Pangborn and coworkers (THF, Et₂O, CH₃CN, CH₂Cl₂: dry neutral alumina; hexane, benzene, and toluene, dry neutral alumina and Q5 reactant; DMSO, DMF: activated molecular sieves).³⁸ Triethylamine, pyridine, 2,6-lutidine, diisopropylamine, and piperidine were freshly distilled under nitrogen from CaH₂. Water was doubly distilled or obtained from a Millipore (Billerica, MA) MilliQ water purification system.

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 (0.25mm). Compounds were visualized by exposure to a UV lamp (λ = 254 nm), a glass chamber containing KMnO₄, or an acidic solution of p-anisaldehyde, followed by brief heating using a Varitemp heat gun. MIDA boronates are compatible with standard silica gel chromatography, including standard loading techniques. Reaction monitoring via RP-HPLC was performed using an Agilent 1200 Series HPLC system equipped with an Agilent Zorbax Eclipse C₁₈ 3.5 µm, 4.6 x 75 mm column or with UV detection at 383 nm at 1.2 mL/min. Column chromatography was performed using standard methods or on a Teledyne-Isco CombiFlash Rf purification system using Merck silica gel grade
9385 60Å (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 to 50 mL) and to the flask is added Celite 454 Filter Aid (5 to 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.

**Structural analysis.**

$^1$H 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 (CHCl$_3$, δ = 7.26; CD$_2$HCN, δ = 1.94, center line; acetone- d$_6$, δ = 2.05, 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, sext = sextet, sept = septet, m = multiplet, b = broad, app = apparent), coupling constant (J) in Hertz (Hz), and integration. $^{13}$C NMR spectra were recorded at 23 °C on a Varian Unity 400 or Varian Unity 500. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane and referenced to carbon resonances in the NMR solvent (CDCl$_3$, δ = 77.0, center line; CD$_3$CN, δ = 1.30, center line; acetone-d$_6$, δ = 29.80, center line) or to added tetramethylsilane (δ = 0.00). Carbons bearing boron substituents were not observed (quadrupolar relaxation). $^{11}$B NMR were recorded at room temperature on a General Electric GN300WB or a Varian Unity Inova 400 instrument and referenced to an external standard of BF$_3$•Et$_2$O. High resolution mass spectra (HRMS) were obtained at the University of Illinois mass spectrometry facility. All synthesized compounds gave HRMS within 5 ppm of the calculated values. X-ray crystallographic analyses were carried out by Dr. Danielle Gray at the University of Illinois George L. Clark X-Ray facility.
Experimental Procedures

1, 3 diol 4.16

β-keto ester 4.15 was synthesized following literature precedent. A flame dried 500 mL 3-neck round bottom flask was charged with 4.15 (12.12 g, 43.5 mmol, 1 eq), THF (170 mL), and MeOH (43 mL) and was cooled to -78°C. Then to this stirring yellow solution Diethylmethoxyborane (6 mL, 45.7 mmol, 1.05 eq) was added and the reaction was allowed to stir for 30 minutes. Then sodium borohydride (1.7197 g, 45.7 mmol, 1.05 eq) was added in a single portion. Gas evolved while the reaction was stirring at -78°C over 2 hours. Acetic acid (10 mL) was added and the reaction was warmed to room temperature and stirred until bubbling ceased, then sat. NaHCO₃ (150 mL) was added dropwise. The reaction was then transferred to a separatory funnel and the layers were separated. The aq phase was extracted with ether (3x100 mL) and then washed with EtOAc (3x50 mL). The combined organic phase was then washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The crude oil was then dissolved in MeOH (175 mL), and distilled (head temp 65°C) until 95% dry. The remaining MeOH was removed in vacuo revealing 4.16 as a yellow oil. 4.16 was carried forward into the following reaction without further purification.

TLC (1:1 hexanes:EtOAc,)

\[ R_f = 0.20, \text{stained with } p\text{-anisaldehyde} \]

\(^1\)H NMR (500 MHz, CDCl₃)

\[ \delta 7.21-7.39 (m, 5H), \delta 6.49 (d, J = 16 Hz, 1H), \delta 6.21 (dd, J = 16Hz, 6.5Hz), \delta 4.601 (M, 1H), \delta 4.35 (m, 1H), \delta 3.72 (s, 1H), \delta 2.53 (m, 2H), \delta 1.71-1.84 (m, 2H). \]
$^{13}$C NMR (125 MHz, CDCl$_3$)

$\delta$ 173.1, 136.8, 131.7, 130.5, 128.8, 127.9, 126.7, 72.9, 68.6, 52.1, 43.0, 41.7

HRMS (ESI)

Calculated for C$_{14}$H$_{18}$O$_4$ (M + Na$^+$): 273.1103

Found: 273.1100

Cyclopentylidene ketal 4.17

A flame dried 500 mL 3-neck round bottom flask was charged with 4.16 (43.5 mmol, 1 eq), DCM (175 mL), PPTS (544.1 mg, 2.175 mmol, 0.05 eq), and 1-methoxycyclopent-1-ene (4.75 mL, 45.7 mmol, 1.05 eq). The reaction was allowed to stir at room temperature for 2 hours before an additional 2 mL 1-methoxycyclopent-1-ene was added. After stirring an additional hour, 150 mL sat. NaHCO$_3$ was added dropwise until bubbling ceased. The reaction was transferred to a separatory funnel, the layers were separated, and the aqueous layer was extracted with DCM (3x100 mL). The combined organic phase was washed with brine, dried over MgSO$_4$, filtered, and concentrated. The crude oil was then absorbed on silica gel from acetone and purified by SiO$_2$ chromatography (9:1 hexanes:EtOAc then 100% Et$_2$O) revealing 4.17 as a white crystalline solid (11.4 g, 36.28 mmol, 83% yield).

TLC (hexanes : EtOAc 1:1)

$R_f = 0.86$, stained with $p$-anisaldehyde
\(^1\)H NMR (500 MHz, CDCl\(_3\))
\[\delta 7.37 (d, J = 7.5Hz, 2H), \delta 7.30 (t, J = 7 Hz, 2H), \delta 7.23 (t, J = 7Hz, 1H), \delta 6.60 (d, J = 16Hz, 1H), \delta 6.18 (dd, J = 16, 6 Hz, 1H), \delta 4.44-4.50 (m, 1H), \delta 4.27-4.34 (m, 1H), \delta 3.70 (s, 3H), \delta 2.62 (dd, J = 16Hz, 7.5Hz, 1H), \delta 2.44 (dd, J = 15.5, 5.5Hz, 1H), \delta 1.87-2.06 (m, 4H), \delta 1.60-1.76 (m, 5H), \delta 1.49 (q, J = 12Hz, 1H)\]

\(^13\)C NMR (125 MHz, CDCl\(_3\))
\[\delta 171.6, 136.9, 131.0, 129.7, 128.7, 127.9, 126.8, 111.1, 71.8, 67.4, 51.9, 41.3, 40.7, 37.0, 31.7, 24.8, 22.8\]

HRMS (ESI)
Calculated for C\(_{19}\)H\(_{24}\)O\(_4\) (M + Na\(^+\)): 339.1572
Found: 339.1575

\[\text{MeO} \quad \text{O} \quad \text{O} \quad \text{MeO} \quad \text{O} \quad \text{H} \]

\[\text{O}_3, \text{EtOAc, -78°C; PPh}_3, 23°C, 60% \]

**Aldehyde 4.4**
A 250mL Schlenk flask was charged with 4.4 (1.6658g, 24.22 mmol, 1 eq) dissolved in EtOAc (250 mL), cooled to -78°C, and stoppered with a gas sparger. Ozone was bubbled through the solution until it turned from clear colorless to blue. The reaction was then sparged with argon clear color returned. Triphenyl phosphine (21 g, 80.1mmol, 3.3 eq) was added and the reaction stirred for 12 hours at room temperature. The volatiles were then removed in vacuo, and the resulting crude oil was taken up in Hexanes with a white precipitate crashing out. The precipitate was filtered via Buchner funnel filtration and the filtrate was concentrated in vacuo. The resulting crude oil was then purified by SiO\(_2\) chromatography (9:1 → 7:3 → 1:1 → 3:7 hexanes:Et\(_2\)O) providing 4.4 as a colorless oil (5.0153g, 20.69 mmol, 85% yield).
TLC (hexanes : EtOAc 1:1)

\[ R_f = 0.45 \], stained with \( p \)-anisaldehyde

\(^1\text{H NMR} (500 \text{ MHz, CDCl}_3)\)

\[ \delta 9.59 (s, 1\text{H}), \delta 4.26-4.28 (m, 1\text{H}), \delta 4.24 (dd, J = 12.5, 3.5\text{Hz}, 1\text{H}), \delta 3.69 (s, 3\text{H}), \delta 2.59 (dd, J =15.5, 7.5 \text{ Hz}, 1\text{H}), \delta 2.44 (dd, J =15.5, 5.5\text{Hz}, 1\text{H}), \delta 1.83-1.97 (m, 4\text{H}), \delta 1.58-1.83 (m, 5\text{H}), \delta 1.41 (q, J =12\text{Hz}, 1\text{H}) \]

\(^{13}\text{C NMR} (125 \text{ MHz, CDCl}_3)\)

\[ \delta 201.2, 171.2, 111.4, 75.4, 67.2, 52.0, 41.0, 40.3, 31.3, 31.0, 24.6, 22.7 \]

HRMS (ESI)

Calculated for \( \text{C}_{19}\text{H}_{24}\text{O}_4 \) (M + \( \text{Na}^+ \)): 243.1232

Found: 243.1236

**Dihydropyran 4.21**

To a 7 mL vial was added dioxene 4.18 (15.9 mg, 75 \( \mu \text{mol} \)) followed by 9BBN borane (150 \( \mu \text{L} \) of a 0.5 M solution in THF). The reaction was then placed in a preheated heating block at room temperature and allowed to stir for 2 hours. A separate 1.5 mL vial was charged with palladium acetate (0.11 mg, 0.49 \( \mu \text{mol} \), 0.05 eq), SPhos (0.41 mg, 1.02 \( \mu \text{mol} \), 0.10 eq), \( \text{K}_3\text{PO}_4 \) (6.4 mg, 30 \( \mu \text{mol} \), 3 eq) and THF (387 \( \mu \text{L} \)). 12.5 \( \mu \text{L} \) of the borane solution was then added to the stirring
palladium mixture and the reaction was heated to 50°C and stirred for 4 hours. The reaction was then passed through a pipette containing celite and fluorisil and eluted with EtOAc. The eluent was concentrated and observed by $^1$H NMR.

$^1$H NMR (500 MHz, Chloroform-$d$)

$\delta$ 4.54 (app t, $J = 3.7$ Hz, 1H), 4.40 (app dd, $J = 12.1, 2.7$ Hz, 1H), 4.38 – 4.32 (m, 1H), 3.97 – 3.91 (m, 2H), 3.76 (s, 3H), 2.44 – 2.38 (m, 1H), 2.35 (dd, $J = 13.8, 5.6$ Hz, 1H), 2.07 – 1.03 (m, 14H).

Dihydropyran 4.22

To a 1.5 mL vial was added 4.22a (6.76 mg, 12.5 µmol, 1 eq) and 9BBN borane (27 µL of a 0.5 M solution in THF, 13.8 µmol, 1.1 eq). This hydroboration stirred for 2 hours at room temperature. In a separate 1.5 mL vial was added Pd(OAc)$_2$ (0.11 mg, 0.5 µmol, 0.05 eq), SPhos (0.41 mg, 1.0 µmol, 0.10 eq), K$_3$PO$_4$ (6.4 mg, 30.1 µmol, 2.4 eq) and THF (150 µL). The hydroborated 4.22a solution was then added to the vial containing the palladium mixture. Lastly water (0.54 µL in 72.5 µL THF, 37.5 µmol, 3 eq) was added to the reaction and it was heated to 50°C and allowed to stir for 6 hours. The reaction was passed through a plug of celite and fluorisil and eluted with EtOAc. The eluent was concentrated and observed by $^1$H NMR.

$^1$H NMR (500 MHz, Chloroform-$d$)

$\delta$ 4.53 (app t, $J = 3.6$ Hz, 1H), 4.37 (app bs, 1H), 4.22 – 4.15 (m, 1H), 4.13 (app s, 1H), 3.94 (app td, $J = 5.0, 2.6$ Hz, 2H), 3.94 – 3.84 (m, 1H), 3.68 (s, 3H), 3.62 – 3.54 (m, 1H), 2.56 (dd, $J = 15.6, 7.3$ Hz, 1H), 2.44 – 2.36 (m, 2H), 2.32 (dd, $J = 14.0, 6.2$ Hz, 1H), 2.04 – 1.36 (m, 28H), 0.87 (s, 9H), 0.04 (d, $J = 3.1$ Hz, 6H).

LRMS (ESI)

Calculated for C$_{34}$H$_{58}$O$_8$Si (M + Na$^+$): 645.4
Hydrolysis study

To a 20 mL vial was added vinyl MIDA boronate 4.23 (200 mg, 1.02 mmol, 1 eq), p-chloroanisole (125 µL, 1.02 mmol, 1 eq), and THF (7 mL). A stock solution of 100 µL H₂O in 5.46 mL THF was then made. Nine different 7 mL vials were each charged with K₃PO₄ (64.95 mg, 0.306 mmol, 3 eq). Then, to each vial was added 700 µL of the MIDA boronate solution. To three of these vials was added 100 µL of the H₂O solution and 200 µL THF. To three of these vials was added 300 µL of the H₂O solution and to the last three vials was added 167 µL pure H₂O and 133 µL THF. Then one vial from each group was left at room temperature, one heated to 35°C, and one heated to 50°C. All vials were then allowed to stir for 24 hours. An aliquot was removed after 6 hours, and after 24 hours, for analysis by ¹H NMR.

Mom ether 4.26

To a 1.5 mL vial was added 4.22a (1.34 mg, 2.5 µmol, 1.25 eq), toluene (10 µL), and 9BBN borane (11 µL, 2.75 µmol, 1.1 eq). This hydroboration stirred at room temperature for 2 hours. In a separate 1.5 mL vial was added Pd(OAc)₂ (0.02 mg, 0.1 µmol, 0.05 eq), SPhos (0.08 mg, 0.2 µmol, 0.10 eq), K₃PO₄ (1.27 mg, 6 µmol, 3 eq) and PhMe (100 µL). Then ketene acetal phosphate 4.25 (1.94 mg, 2.0 µmol, 1 eq) then added to the Pd(OAc)₂ solution followed by the hydroborated 4.22a solution. Lastly H₂O was added (10 µL of a 50 µL solution in 4.6 mL toluene) and the reaction stirred at 50°C for 6 hours. The reaction was then passed through a florisoril and celite pipette column and eluted with EtOAc. The eluent was the concentrated and then observed for conversion by mass spectrometry.
LRMS (ESI)

Calculated for C_{59}H_{102}BNO_{17}Si_{3} (M + Na\(^{+}\)): 1214.6
Found: 1214.2

**AmB azide reduction**

AmB azide **4.30** (4.9 mg, 5.16 µmol, 1 eq) was added to a 1.5 mL vial. Then PMe3 (15.79 µL of a 1.0 M solution in THF in 263 µL DMSO, 15.79 µmol, 3 eq) and H2O (9.48 µL in 263 µL DMSO, 526 µmol, 100 eq) was added to **4.30**. The reaction was then placed in a preheated heating block at 50°C and allowed to stir for 4 hours. The reaction was poured into a centrifuge tube containing 45 mL diethyl ether, and then spun down at 3700 G for 5 minutes. The resulting yellow pellet was dissolved in DMSO and purified by preparatory HPLC yielding AmB as a yellow solid (3.6 mg, 3.90 µmol, 75%). All characterization matched AmB.

**Allyl MIDA boronate 4.39**

A flame dried 500 mL RBF equipped with mechanical stirring and a pressure equalizing dropwise addition funnel under and Argon atmosphere was charged with ether (154 mL) and trimethyl borate (5.65 mL, 50.7 mmol, 1 eq). The mixture was cooled to -78°C and kept at this temperature for the reaction duration. Then allyl Grignard (24.5 mL of a 2.07M THF solution, 50.7 mmol, 1 eq) was added dropwise over 20 minutes followed by vigorous stirring for 3 hours. During the course of stirring the reaction a thick white precipitate evolves. The reaction was then warmed to 0°C, HCl (76 mL of a 2N solution, 152 mmol, 3 eq) was added and the reaction was allowed to stir for 50 minutes before warming to room temperature for 10 minutes. The reaction
was then transferred to a separatory funnel. The layers were separated the aqueous layer was extracted with ether (3x50 mL). The combined organic layer was then dried over Na$_2$SO$_4$, filtered, and concentrated partially in vacuo. Toluene (10 mL) was then added and the solution was partially evaporated in vacuo.

The resulting boronic acid solution in toluene was transferred to a 250mL round bottom flask and diluted with toluene (50 mL), DMSO (9 mL). N-methyl iminodiacetic acid (8.95 g, 60.8 mmol, 1.2 eq) was then added to the flask. A dean stark apparatus with attached reflux condenser open to air was attached to the flask and the solution was heated to 130°C and maintained at this temperature for 12 hours. The reaction was then cooled to room temperature and transferred to a 500 mL separatory funnel with 100 mL ethyl acetate and 300 mL water. The layers were separated and the aqueous layer was extracted with ethyl acetate (3x100 mL) followed by washing with ethyl acetate: acetone 1:1 (3x100 mL). The organic phase was washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The resulting white solid was recrystallized from acetone/ether yielding 4.39 as a crystalline white solid (7.49 g, 38.0 mmol 75% yield)

![Diagram](image)

4.39

TLC (hexanes : acetone 1:1)

R$_f$ = 0.15, stained with p-anisaldehyde

$^1$H NMR (500 MHz, acetone-$d_6$)

$\delta$ 5.87 (ddt, $J = 17.6, 10.1, 7.6$ Hz, 1H), 4.98 (dd, $J = 17.2, 1.9$ Hz, 1H), 4.92 – 4.85 (m, 1H), 4.20 (d, $J = 16.9$ Hz, 2H), 3.98 (d, $J = 16.8$ Hz, 2H), 3.13 (s, 3H), 1.64 (bd, $J = 7.6$ Hz, 2H).

$^{13}$C NMR (125 MHz, acetone-$d_6$)

$\delta$ 168.7, 137.0, 115.3, 62.9, 46.3
**α-boryl aldehyde 4.41**

A 500 mL schlenk flask equipped with mechanical stirring was charged with **4.39** (2.2655 g, 12.68 mmol, 1 eq) and DCM (250 mL). The reaction was cooled to -78°C and ozone was bubbled through until the solution turned blue. Nitrogen was bubbled through the reaction to purge excess ozone until the blue color disappeared. Triphenyl phosphine (13.412 g, 50.72 mmol, 4 eq) was then added in one portion and the reaction was warmed to room temperature and allowed to stir for 16 hours. The volatiles were removed *in vacuo* and the mixture was absorbed onto celite from acetone. The crude product was purified on SiO\(_2\) using an mPLC yielding a white solid. The product was then recrystallized from acetone/ether yielding **4.41** as a white crystalline solid (1.67 g, 8.38 mmol, 73%).

\[
\text{TLC (hexanes : acetone 1:1)}
\]
\[
\text{R}_f = 0.13, \text{stained with KMnO}_4
\]

\(^1\)H NMR (500 MHz, Acetone-\(d_6\))
\[
\delta 9.76 (t, J = 3.5 \text{ Hz}, 1\text{H}), 4.29 (d, J = 17.0 \text{ Hz}, 2\text{H}), 4.10 (d, J = 16.9 \text{ Hz}, 2\text{H}), 3.18 (s, 3\text{H}), 2.23 (s, 2\text{H}).
\]

\(^{13}\)C NMR (125 MHz, acetone-\(d_6\))
\[
\delta 202.9, 167.7, 62.3, 46.4
\]

\(^{11}\)B NMR (400 MHz, acetone-\(d_6\))
\[
\delta 11.36
\]

HRMS (ESI)
\[
\text{Calculated for C}_{7}H_{10}BNO}_{5} (M + Na^+): \quad 222.0550
\]
Figure 4.7: X-ray crystal structure of α-boryl aldehyde 4.41

Homoallylic alcohol 4.42

A flame dried 25 mL 2-neck pear vial equipped with mechanical stirring was brought into the glovebox. Then (-)DIP-Cl (2.4431 g, 7.54 mmol, 1.5 eq) was added, the vial was sealed with septa and removed from the glovebox. The vial was then charged with THF (18 mL) and the solution cooled to -78°C. Allyl Grignard (3.2 mL of a 2.07 M solution, 6.28 mmol, 1.25 eq) was then added dropwise over 5 minutes followed by warming to 0°C and stirring for 2 hours.

A separate flame dried 200 mL round bottom flask equipped with mechanical stirring was charged with 4.41 (0.9684 g, 4.86 mmol, 1 eq, ground with mortar and pestle) and THF (200 mL) and cooled to -78°C. After 2 hours of stirring the Ipc₂Ballyl solution was cannula transferred dropwise to the stirring MIDA boronate suspension. The reaction was then allowed to stir at -78°C for 1 hour before warming to 0°C and stirring for 5 hours. During the course of reaction the solution changes from a white suspension to a clear colorless solution. Then NaOCl (Clorox, 7.7 mL of a 0.688 M solution, 5.271 mmol, 1.05 eq) was added and the reaction allowed to stir at 0°C for 45 minutes. The reaction was then transferred to a 250 mL separatory funnel
and the aqueous layer was extracted with EtOAc:acetone 5:1 (3x50 mL). The combined organic layer was dried over MgSO₄, filtered, and concentrated in vacuo. The crude oil was then concentrated on celite from acetone and purified by mPLC SiO₂ chromatography (gradient 100% ether → 3:2 Et₂O:MeCN) yielding 4.42 as a foamy white solid (0.809 g, 3.36 mmol, 69% yield).

TLC (hexanes : acetone 1:1)  
R_f = 0.24, stained by KMnO₄

¹H NMR (500 MHz, acetone-d₆)  
δ 5.87 (ddt, J = 17.3, 10.2, 7.1 Hz, 1H), 5.09 – 4.96 (m, 2H), 4.20 – 3.90 (m, 4H), 3.85 – 3.75 (m, 1H), 3.66 – 3.61 (m, 1H), 3.16 (s, 3H), 2.31 – 2.19 (m, 2H), 0.97 – 0.86 (m, 2H).

TBS silyl ether 4.43  
Prior to the reaction homoallylic alcohol 4.42 was azeotropically dried by co-evaporation with MeCN (2x10 mL). A flame dried 40 mL vial equipped with mechanical stirring under an Argon atmosphere was charged with 4.42 (567 mg, 2.35 mmol, 1 eq), TBSCl (532 mg, 3.53 mmol, 1.5 eq), and imidazole (483.6 mg, 7.06 mmol, 3 eq). DMF (24 mL) was then added and the reaction was warmed to 45°C and allowed to stir for 1 hour. The reaction was then cooled to room temperature and transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted with EtOAc (3x30 mL). The combined organic layer was then rinsed with brine, dried with MgSO₄, filtered, and concentrated in vacuo. The crude oil was transferred to a separatory funnel with EtOAc and 20 mL H₂O. The layers were separated and the organic layer was washed with 20 mL water. The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo yielding 4.43 as a white solid (802 mg, 2.26 mmol, 96%).
TLC (Et₂O : MeCN 9: 1)

Rₜ = 0.55, stained by KMnO₄

¹H NMR (500 MHz, acetone-d₆)

δ 5.91 (ddt, J = 17.3, 10.3, 7.1 Hz, 1H), 5.05 – 5.00 (m, 1H), 4.98 (dd, J = 10.2, 2.5 Hz, 1H), 4.18 (d, J = 16.8 Hz, 2H), 4.04 (d, J = 12.8 Hz, 5H), 4.0 (d, J=15 Hz, 1H), 3.09 (s, 3H), 2.46 – 2.40 (m, 1H), 2.25 – 2.17 (m, 1H), 1.03 – 0.92 (m, 2H), 0.89 (s, 9H), 0.07 (d, J = 2.5 Hz, 6H).

Alcohol 4.44

Prior to reaction 4.43 was azeotropically dried by co-evaporation with MeCN (3x5 mL). A flame-dried vial equipped with mechanical stirring under an Argon atmosphere was charged with 4.43 (151 mg, 0.427 mmol, 1 eq) and THF (3.0 mL). 9BN (1.11 mL of a 0.5 M solution in THF, 1.11 mL, 1.2 eq) was added dropwise and the reaction was allowed to stir for 2 hours. The reaction was cooled to 0°C and then NaOCl (2.48 mL) was added dropwise. After warming to room temperature and stirring for 1.5 hours the reaction was transferred to a separatory funnel with EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (3x20 mL). The combined organic layer was dried over MgSO₄, filtered, and concentrated in vacuo. The crude oil was absorbed onto celite from acetone and purified by mPLC SiO₂ chromatography (9:1 → 4:1 → 3:2 Et₂O:MeCN) providing 4.44 as a white solid (131 mg, 0.35 mmol, 82%).
TLC (Et₂O: MeCN 4: 1)

\[ R_f = 0.46 \text{ stained by KMnO}_4 \]

\(^1\)H NMR (500 MHz, acetone-\(d_6\))

\[ \delta 4.17 \text{ (d, } J = 16.8 \text{ Hz, } 2\text{H}), 4.07 - 3.98 \text{ (m, } 3\text{H}), 3.53 \text{ (t, } J = 6.1 \text{ Hz, } 2\text{H}), 3.09 \text{ (s, } 3\text{H}), 2.02 \text{ (s, } 4\text{H}), 1.73 - 1.62 \text{ (m, } 2\text{H}), 1.60 - 1.52 \text{ (m, } 1\text{H}), 1.51 - 1.43 \text{ (m, } 1\text{H}), 0.97 \text{ (d, } J = 6.8 \text{ Hz, } 2\text{H}), 0.89 \text{ (s, } 9\text{H}), 0.07 \text{ (d, } J = 2.0 \text{ Hz, } 6\text{H}). \]

\textbf{Aldehyde 4.45}

A 20 mL vial was charged with 4.44 (107.8 mg, 0.289 mmol, 1 eq) and Dess-Martin periodinane (147 mg, 0.347 mmol, 1.2 eq). DCM (3 mL) was added the reaction was allowed to stir for 1 hour at room temperature. The reaction was then transferred to a separatory funnel with ethyl acetate and water. The layers were separated and the aqueous layer was extracted with ethyl acetate (3x10 mL). The combined organic phase was then dried over MgSO₄, filtered, and concentrated \textit{in vacuo}. The crude oil was then passed through a plug of silica gel using ether as the eluent. Then the plug was rinsed with acetone into a separate collection flask. The acetone rinse was collected and concentrated revealing 4.45 as a white solid (91.3 mg, 0.246 mmol, 85%).

TLC (Et₂O : MeCN 9:1)

\[ R_f = 0.44 \text{, stained by KMnO}_4 \]

\(^1\)H NMR (500 MHz, acetone-\(d_6\))
δ 9.75 (t, J = 1.9 Hz, 1H), 4.19 (app dd, J = 16.8, 1.4 Hz, 2H), 4.11 – 4.00 (m, 3H), 3.10 (s, 3H), 2.50 (app tt, J = 7.6, 1.9 Hz, 2H), 1.80 – 1.71 (m, 2H), 1.05 – 0.93 (m, 2H), 0.89 (s, 9H), 0.07 (d, J = 3.8 Hz, 6H).

**Alcohol 4.47a**

Prior to reaction 4.45 was azeotropically dried by co-evaporation with MeCN. A flame-dried vial equipped with mechanical stirring under an Argon atmosphere was charged with methyl dimethyl phosphate (37.3 µL, 0.344 mmol, 6 eq) and THF (3.4 mL). The solution was cooled to -78°C and nBuLi (145 µL of a 2.43 M solution, 0.344 mmol, 6 eq) was added dropwise and allowed to stir for 30 minutes. A separate vial was charged with 4.45 (21.3 mg, 57.4 µmol, 1 eq), placed under an Argon atmosphere, THF (574 µL) added and cooled to -78°C. This solution was then cannula transferred into the stirring lithiated phosphate solution. The reaction was allowed to stir at -78°C for 2.5 hours before 6:1 acetic acid: THF (500 µL) was added and the reaction allowed to warm to room temperature. The reaction was transferred to a separatory funnel with 1:1 sat. ammonium chloride:water and ethyl acetate. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layer was then dried over MgSO₄, filtered, and concentrated. The crude oil was then purified by silica gel column chromatography (acetone:hexanes 1:4, followed by acetone:hexanes 1:1) yielding 4.47a as a pale yellow oil (15.7 mg, 31.7 µmol, 55%).

**TLC (hexanes : acetone 1:1)**

Rₜ = 0.05 stained by p-Anisaldehyde

**1H NMR (500 MHz, acetone-d₆)**
δ 4.18 (app dd, J = 16.8, 1.0 Hz, 3H), 4.08 – 3.98 (m, 3H), 3.96 (bs, 1H), 3.72 – 3.70 (m, 3H), 3.69 – 3.67 (m, 3H), 3.09 (s, 3H), 2.04 – 1.94 (m, 2H), 1.94 – 1.77 (m, 3H), 1.75 – 1.64 (m, 1H), 1.04 – 0.95 (m, 2H), 0.94 – 0.84 (m, 9H), 0.13 – 0.03 (m, 6H).

β-keto phosphonate 4.47

4.47a (15.2 mg, 30.7 µmol, 1eq.) and Dess-Martin Periodinane (18.0 mg, 36.8 µmol, 1.2 eq) were combined in a vial with DCM (0.3 mL). The reaction was allowed to stir at room temperature for 30 minutes before 500 µL sat. Sodium thiosulfate was added. This mixture was allowed to stir for 15 minutes before transfer to a separatory funnel with EtOAc and water. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layer was washed with brine, dried over MgSO₄, filtered and concentrated. The crude oil was then absorbed onto celite from acetone and placed on a SiO₂ plug. The plug was washed with ether, followed by acetone. The acetone rinse was collected and concentrated yielding 4.47 as a white solid (14.2 mg, 28.8 µmol, 94%).

TLC (hexanes : acetone 1:1)

Rf = 0.21, stained by p-Anisaldehyde

¹H NMR (500 MHz, acetone-d₆)

δ 4.09 – 4.05 (m, 1H), 4.02 (app dd, J = 16.9, 4.9 Hz, 3H), 3.89 (app dd, J = 17.0, 4.6 Hz, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.23 (dd, J = 22.2, 3.9 Hz, 2H), 2.93 (s, 3H), 2.85 (ddd, J = 17.9, 10.0, 5.3 Hz, 1H), 2.77 – 2.67 (m, 1H), 2.02 – 1.92 (m, 1H), 1.74 – 1.65 (m, 1H), 1.00 (s, 9H), 0.98 (app d, J = 6.9 Hz, 2H), 0.18 (s, 6H).
Enone 4.48

A flame-dried vial equipped with mechanical stirring under an Argon atmosphere was charged with 4.47 (~0.05 M solution in MeCN, 2.04 mg, 4.13 µmol, 1 eq) and LiCl (~2 mg, 12.4 µmol, 3 eq). The solution was allowed to stir for 15 minutes before DIPEA (3 drops) was added. The mixture then stirred for 15 minutes before cooling to 0°C. Next 4.4 (~0.05 M solution in MeCN, 1 mg, 4.13 µmol, 1 eq) was added and the reaction was allowed to stir for 2 hours at 0°C. The reaction was then warmed to room temperature and allowed to stir overnight. The reaction was then transferred to a separatory funnel with half saturated ammonium chloride and ethyl acetate. The layers were separated and the aqueous layer was extracted with ethyl acetate (3x7.5 mL). The combined organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The crude oil was then purified by silica gel chromatography EtOAc: Hexanes 4:1. A second column (EtOAc:hexanes 7:3) was necessary to reveal 4.48 as a pale yellow residue (1.1 mg, 1.80 µmol, 44%).

TLC (hexanes : acetone 1:1)

R<sub>f</sub> = 0.75, stained by KMnO<sub>4</sub>

<sup>1</sup>H NMR (500 MHz, acetone-<em>d</em><sub>6</sub>)

δ 6.78 (dd, <em>J</em> = 16.0, 4.2 Hz, 1H), 6.28 (dd, <em>J</em> = 16.0, 1.8 Hz, 1H), 4.64 – 4.56 (m, 1H), 4.37 – 4.26 (m, 1H), 4.19 (app dd, <em>J</em> = 16.8, 7.8 Hz, 2H), 4.10 – 3.99 (m, 3H), 3.63 (s, 3H), 3.11 (s, 3H), 2.79 – 2.71 (m, 1H), 2.69 – 2.60 (m, 1H), 2.48 – 2.45 (m, 2H), 1.88 – 1.77 (m, 4H), 1.70 – 1.55 (m, 4H), 1.00 (d, <em>J</em> = 8.3 Hz, 2H), 0.90 (d, <em>J</em> = 2.5 Hz, 9H), 0.08 (d, <em>J</em> = 1.5 Hz, 6H).
Enol 4.49

A flame dried 7 mL vial equipped with mechanical stirring under an Argon atmosphere was charged with (R)-CBS catalyst (2 µL, 1.8 µmol, 1 eq) and THF (3 mL). The vial was cooled to -10°C and maintained at this temperature for the reaction duration. Next, borane-methyl sulfide complex (2 drops, 2.2 µmol, 1.2 eq) was added and allowed to stir for 5 minutes. Then 4.48 (1.1 mg 1 eq, 1.8 µmol, 1.2 eq) was added as a solution in THF (1 mL) and allowed to stir for 1 hour. The reaction was quenched with saturated ammonium chloride before transferring to a separatory funnel with EtOAc and water. The layers were separated and the aqueous layer was extracted with EtOAc (3x7.5 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. The crude oil was then purified by silica gel chromatography (acetone: hexanes 3:7) yielding 4.49 as a pale yellow residue (0.8 mg, 1.31 mmol, 73%).

TLC (hexanes : acetone 3:2)

\[ R_f = 0.32, \text{ stained by KMnO}_4 \]

\[^1\text{H} \text{ NMR (500 MHz, acetone-}d_6)\]

\[ \delta 5.68 (dd, J = 15.5, 7.0 \text{ Hz}, 1H), 5.58 (dd, J = 15.5, 6.0 \text{ Hz}, 1H), 4.38 – 4.32 (m, 1H), 4.31 – 4.13 (m, 4H), 4.08 – 3.95 (m, 3H), 3.62 (s, 3H), 3.09 (s, 3H), 2.47 – 2.40 (m, 1H), 1.79 – 1.72 (m, 1H), 1.72 – 1.62 (m, 4H), 1.61 – 1.47 (m, 4H), 1.47 – 1.39 (m, 2H), 0.99 – 0.91 (m, 2H), 0.89 (s, 9H), 0.07 (d, J = 4.0 \text{ Hz}, 6H). \]

HRMS (ESI+)

Calculated for C_{20}H_{50}BNO_{10}Si (M + Na^+): 634.3195

Found: 634.3209
Alcohol 4.50

Prior to reaction allylic alcohol 4.49 was azeotropically dried by co-evaporation with MeCN (2x5 mL). A flame dried 7 mL vial equipped with mechanical stirring under an Argon atmosphere was charged with 4.49 (0.8 mg, 1.31 µmol, 1 eq) and Pd on carbon (10% Pd by wt. 1.4 mg, 0.195 µmol, 0.15 eq). EtOAc (1 mL) was added and the atmosphere was purged with hydrogen. Then a balloon of hydrogen was attached and the reaction was allowed to stir at room temperature for 14 hours. The balloon was removed and the reaction was the passed over a plug of celite using ethyl acetate to rinse the plug. The filtrate was collected and the volatiles removed in vacuo revealing 4.50 as a pale yellow residue (0.8 mg, 1.30 µmol, 99%).

$^1$H NMR (500 MHz, acetone-$d_6$)

$\delta$ 4.24 – 4.17 (m, 3H), 4.07 – 3.98 (m, 3H), 3.84 – 3.77 (m, 1H), 3.62 (s, 3H), 3.57 – 3.49 (m, 1H), 3.09 (d, $J = 3.5$ Hz, 3H), 2.45 – 2.39 (m, 2H), 1.96 – 1.89 (m, 4H), 1.76 – 1.69 (m, 3H), 1.59 – 1.53 (m, 5H), 1.51 (s, 3H), 1.44 – 1.36 (m, 4H), 0.99 – 0.91 (m, 2H), 0.90 (s, 9H), 0.08 (d, $J = 1.2$ Hz, 6H).

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