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Strategies to Develop Antibiotics *via* Diversification from Natural Products

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Strategies to Develop Antibiotics *via* Diversification from Natural Products

Sean E. Rossiter

B.S., Moravian College, 2015

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An abstract of a thesis submitted to the Faculty of the

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in chemistry.

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Abstract

Strategies to Develop Antibiotics *via* Diversification from Natural Products

By Sean E. Rossiter

Given the recent proliferation of antibiotic-resistant pathogens, there is a profound need for scientific innovation to develop new therapeutics to combat these diseases which were previously thought to have been all but eradicated. I postulate that there are two key problems which must be addressed: in the short-term, chemists must develop antibiotics to which pathogenic bacteria are susceptible. In the long-term, however, there must be a radical shift in infection management and prevention to break the cycle of resistance. A brief survey of diversity-inspired strategies is conducted, and recent efforts in analog development towards establishing a thorough structure-activity relationship around the natural product promysalin are summarized herein.

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Chapter 1

Introduction: Diversity-Oriented Approaches towards Antibiotic Development

The long-foretold threat of antibiotic-resistant pathogens is no longer a distant fear.[1] Pathogens which resist colistin, a last-resort antibiotic, have been observed in the clinic.[2] We can identify two key problems moving forward: in the short term, we have an alarming lack of antibiotics able to overcome highly resistant bacteria. In the long term, we need to develop a fundamentally new approach to treating, managing, and preventing bacterial infections, especially given the inherent propensity for bacteria to evolve resistance to antibacterial drugs, as well as recent insights into the long-term effects of antibiotics disrupting the human gut microbiome. While the latter predicament requires extensive effort by clinicians and microbiologists to revolutionize the medical approach to treating infections, the first is very much a question answerable by synthetic chemists.[3]

Clinically used antibiotics target one of four key pathways in bacteria.[4] Two key families, including the β-lactams and glycopeptides, target various steps within peptidoglycan formation in cell wall synthesis, preventing the bacteria from surviving cell division. A second class, including macrolides, tetracyclines, and aminoglycosides, inhibits protein synthesis within the ribosome, stagnating bacterial growth. A third class includes the topoisomerase-inhibiting fluoroquinolones, which block DNA and RNA synthesis and replication. Finally, the dated sulfonamide antibiotics (sulfa drugs) act as *p*-aminobenzoate mimics and inhibit folate biosynthesis within bacteria.

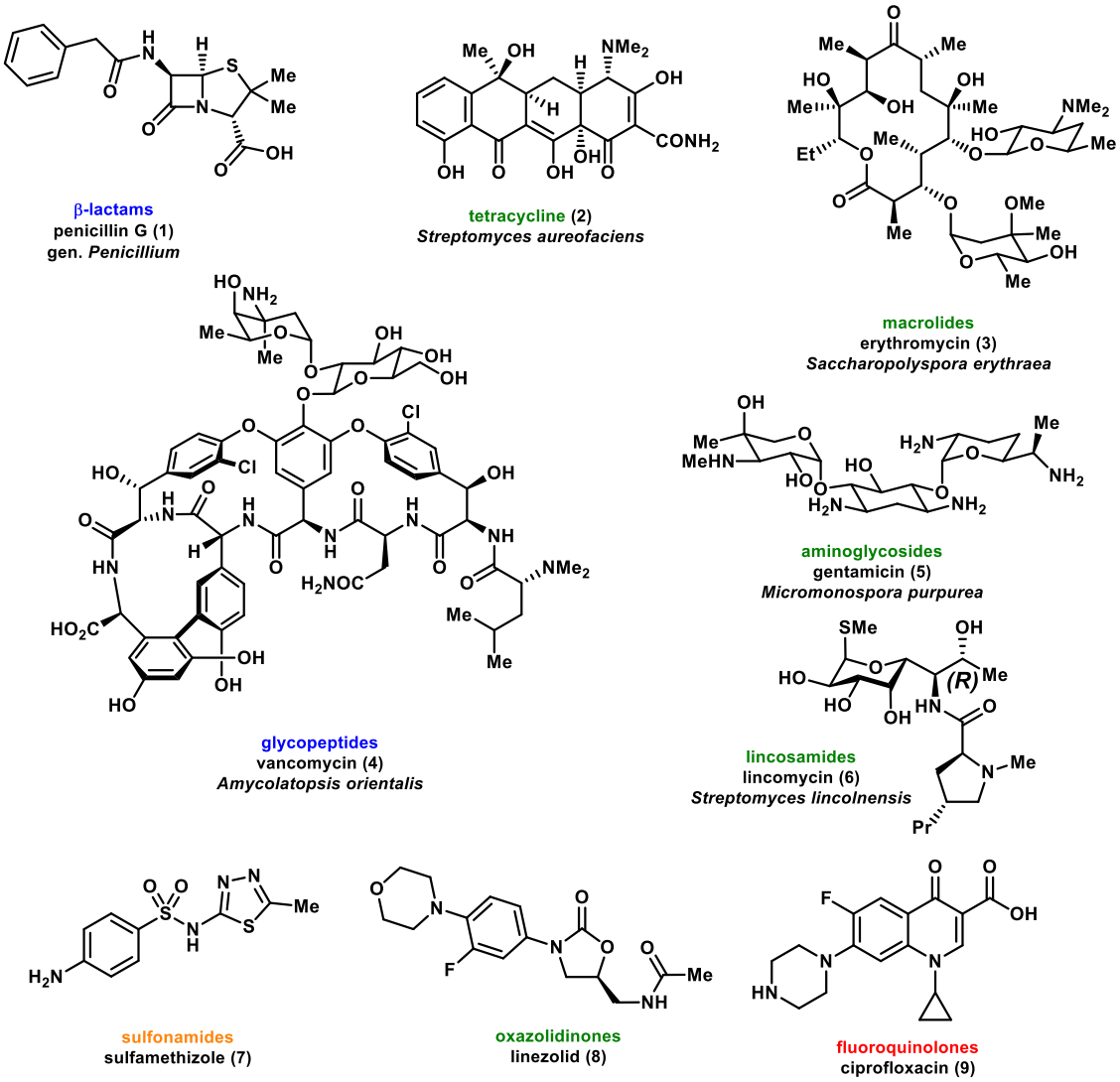


Figure 1. Representative classes of antibiotics with color coding by mechanism of action according to Figure 2. Natural products are denoted with their producing organisms.

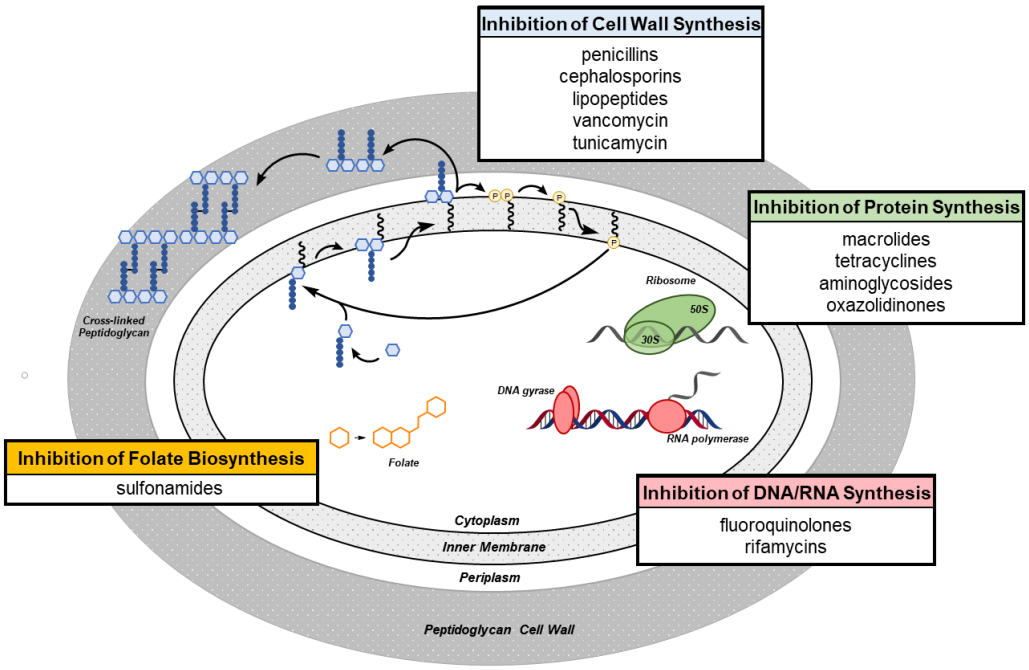


Figure 2. Schematic representation of mechanisms of action of clinically used antibiotics.

Given that the pathways discussed are critical for bacterial growth, it should be no surprise that resistance evolves so readily due to this selection pressure – bacteria have evolved three general strategies to allow these processes to continue relatively undisturbed by these antibiotics.[5] Efflux pumps, which are perhaps best associated with the tetracyclines given the use of the TetR/TetA system in inducible gene expression as a microbiology technique, actively remove drugs from the bacterial cytoplasm, preventing inhibition of a pathway. Bacteria also have evolved methods of modifying the target to prevent antibiotic binding at the molecular level. These methods can include mutations of a key residue of a protein target or an enzymatic modification of a substrate, such as the VanA-mediated replacement of D-Ala-D-Ala with D-Ala-D-Lac in peptidoglycan precursors, rendering the bacteria immune to vancomycin.[6] Finally, bacteria can deactivate

antibiotics, which is best known to occur by the ring-opening action of beta-lactamases on beta-lactams.[7]

The continuous evolution of resistance against antibiotics has necessitated that chemists modify drugs to restore activity, though the success is inherently temporary. Though the golden age of antibiotics resulted in the discovery of revolutionary drugs, both increasing resistance and pharmacological issues with the natural products necessitated molecular tinkering. Erythromycin, in particular, demonstrated excellent activity against a variety of Gram-positive pathogens, though unpleasant side effects were noted in many patients. Careful study identified that erythromycin undergoes acid-promoted addition of the C6 and C12 alcohols into the C9 carbonyl, resulting in a spiroketal that leads to gastrointestinal side effects.[3] To remedy this, two efforts led to clinically approved drugs,

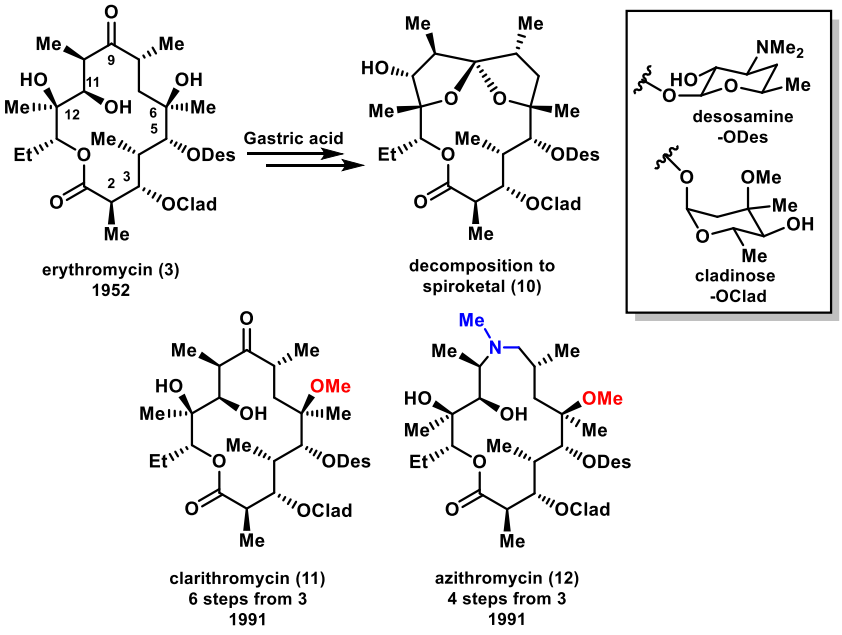


Figure 3. Successful application of semisynthesis to improve the pharmacological characteristics of erythromycin. FDA approval years are noted.

clarithromycin[8] and azithromycin,[9] both of which have the C6 alcohol capped with a methyl group. Azithromycin was also rendered particularly acid-stable and soluble by the replacement of the C9 carbonyl with an endocyclic amine, and azithromycin has been a particularly widely used antibiotic for a variety of minor infections.

For decades, semisynthesis has led to fruitful drugs which temporarily stave off resistance. However, one can argue that this relatively simplistic process is nearly exhausted. Three semisynthetic drugs face clear difficulties – tigecycline, a tetracycline derivative, carries an FDA black box warning for "all-cause mortality increase." Telithromycin, a ketolide brought to market in 2004, has been phased out of usage because of its hepatotoxicity.[3] Solithromycin, a promising clinical candidate intended to avoid telithromycin's toxicity issues, has recently been found to exhibit similar problems. It should also be noted that omadacycline, another semisynthetic tetracycline derivative, has been completing phase III trials without issue, and is likely to be approved in the near future. The state of antibiotics no longer permits the addition of a methyl group to an antibiotic to render it active – more extensive molecular remodeling is clearly necessary, and semisynthesis is inherently limited to the chemical reactivity of the natural product imposed by Nature.

To identify new antibiotic candidates, researchers often turn to high-throughput screening (HTS) approaches, based often on combinatorial chemistry approaches made possible by the advent of cross-coupling technologies. However, Wright and coworkers note in a recent review that "despite new genomic tools, the ability to identify high-priority targets using, for example, essential gene screens, and innovation in high-throughput screening technologies that enables millions of compounds to be probed in a short period

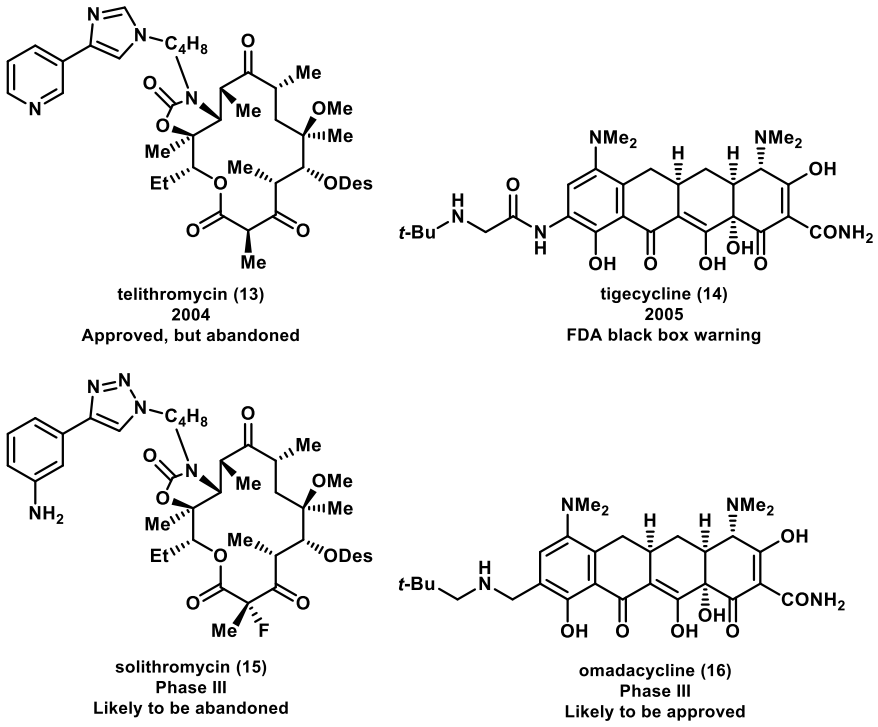


Figure 4. Selected semisynthetic drugs with regulatory approval and present status.

of time, no new antibiotic drugs have emerged."[10] Additionally, Lipinski's rules, which are so often invoked in medicinal chemistry, simply do not apply to antibiotics.[11] For these reasons, it is argued that natural products provide the necessary complexity and activity around which antibiotics must be based, though semisynthesis is a dated method for developing drug leads from a natural product hit.

During the 1990s, Danishefsky and coworkers reported a new method, termed diverted total synthesis (DTS), which allows the efficient design and preparation of analogs of natural products bearing structural modifications inaccessible through semisynthetic methods.[12] The Danishefsky group successfully applied this strategy to reach analogs of a potential cancer drug, epothilone, which possessed enhanced potency and stability and a widened therapeutic window. DTS, characterized by Danishefsky as "molecular editing through chemical synthesis," facilitated the replacement of an allylic methyl group with a

more stable trifluoromethyl group and unsaturation of the macrocycle, neither of which are feasible via isolation of natural epothilone and subsequent chemical modification.[13] In a similar vein, other scientists seek to explore chemical space through elaborately planned diversification to generate complex molecules with similarities to natural products, though themselves unknown to nature. Diversity-oriented synthesis (DOS) was described by Schreiber and coworkers as an innovative method to explore regions of chemical space not believed to be occupied by natural products.[14] In contrast to conventional target-oriented synthesis, the DOS strategy seeks to rapidly build complexity and diversity efficiently with the goal of constructing unnatural scaffolds with natural product-like characteristics, which then may be screened for biological activity. We can contrast DTS and DOS both in the application of each method as well as the overall philosophy of each strategy. DTS is best described as the adaptation of a total synthesis to arrive at an analog of the original target which yields some improved biological characteristic. DTS is fundamentally hypothesis-driven, seeking to link a pharmaceutical characteristic, whether toxicity, activity, or another pharmacokinetic attribute, to some molecular feature. DTS is therefore suited to modify a promising lead compound into a more desirable clinical candidate. DOS, inversely, is inherently exploratory in nature and is better suited for developing a library of unprecedented compounds from which a lead compound may be found. This demarcation is only apparent in considering the original reports of these strategies; many modern synthetic campaigns adopt principles from both DOS and DTS in developing analogs.[15,16,17]

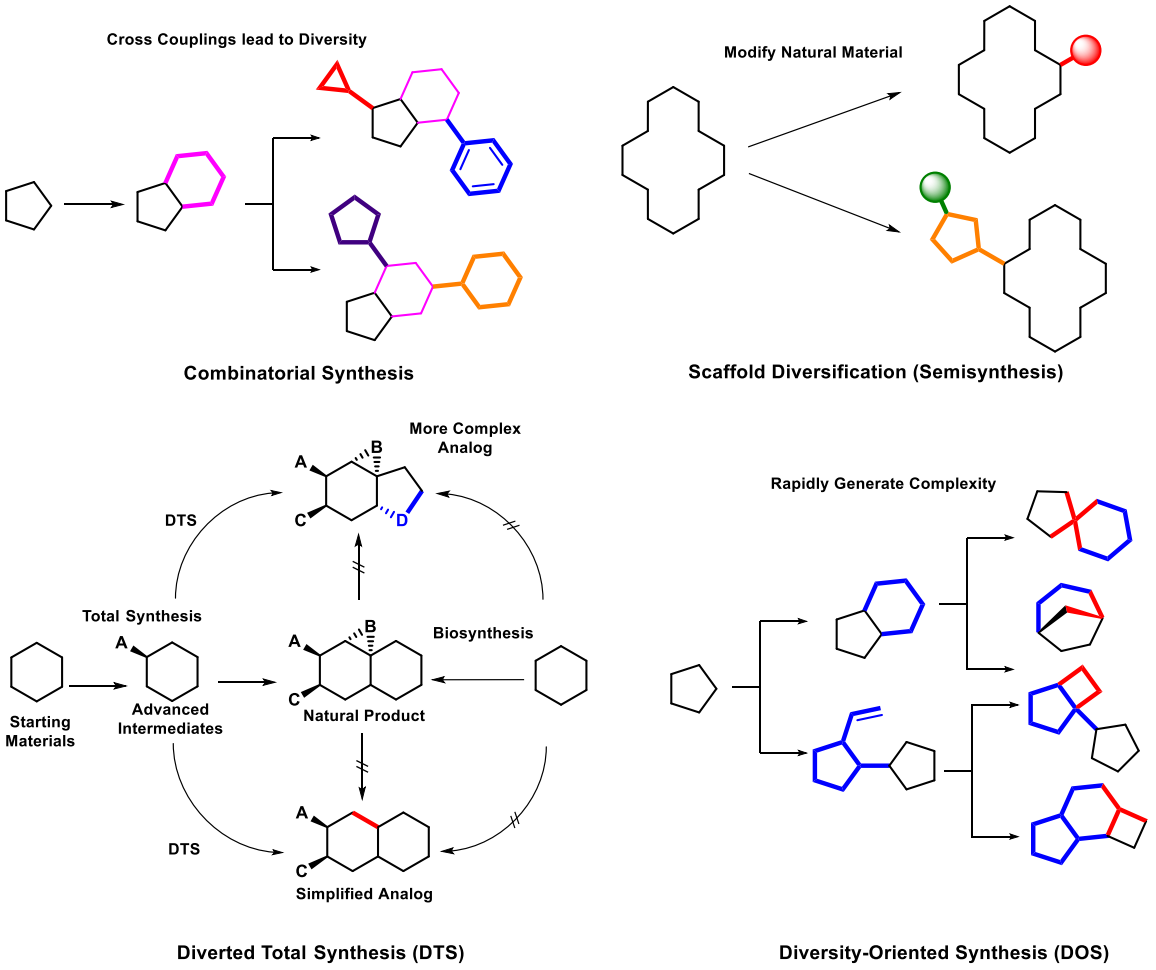
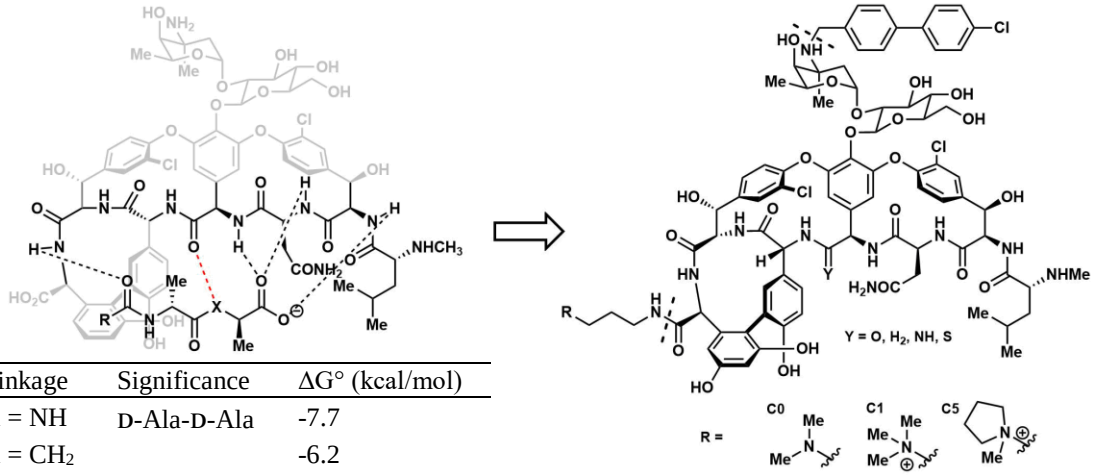


Figure 5. Comparison of traditional compound diversification strategies with modern, innovative approaches.

During the 1990s, vancomycin found itself at the center of a synthetic race undertaken by the Evans, Nicolaou, and Boger groups.[18] While the Evans group employed elegant methodology to construct the aglycon of this molecule[19], the Nicolaou and Boger groups continued on to determine if analogs of vancomycin could exhibit restored activity against vancomycin-resistant pathogens.



Linkage	Significance	ΔG° (kcal/mol)
X = NH	D-Ala-D-Ala	-7.7
X = CH_2		-6.2
X = O	D-Ala-D-Lac	-3.6

Figure 6. General schematic of Boger's approach to reengineer vancomycin to overcome the VanA mechanism of resistance. Dashed lines on right indicate key additions to the scaffold.

While Nicolaou and coworkers designed glycopeptides with modified terminal amino acids and tethered dimeric analogs,[20] the Boger group set out with a hypothesis-driven goal of engineering a vancomycin analog capable of specifically overcoming the VanA resistance phenotype.[21] In this resistance mechanism, the terminal D-alanine residue in the peptidoglycan precursor is replaced with lactate, effectively carrying out a single atom mutation from a peptide linkage to an ester. The NH to O replacement removes a hydrogen bond donor, which reduces the binding of vancomycin (to a model peptide) by a factor of 1400.[22] Boger and coworkers designed a series of vancomycin analogs which would avoid this electronic clash with a corresponding replacement of the peptide carbonyl, and these analogs were accessible by DTS from key intermediates in their vancomycin synthesis.[23] Three new analogs were developed to test this atom-mutation hypothesis: a methylene analog, which would remove any electronic clashes; a thioamide analog, which was expected to be less active, and an amidine analog, which could, in principle, make

hydrogen bonds between both the native D-Ala-D-Ala substrate and the modified D-Ala-D-Lac substrate.

The Boger laboratory confirmed this hypothesis with the successful synthesis of vancomycin aglycon analogs bearing these pocket modifications. Proof of concept was established as both the amidine and methylene analogs bound model substrates with little preference, though the amidine was more potent all-around given its restored hydrogen bond. These compelling results spurred the preparation of pocket-modified analogs of vancomycin proper[24] and lipoglycopeptides,[25] inspired by oritavancin, and further improvements to potency were observed. By tethering a quaternary amine to the periphery of the molecule, Boger and coworkers successfully rendered an analog with a nanogram per milliliter minimum inhibitory concentration (MIC), and this vancomycin derivative will likely find its way into clinical trials.[26]

The Boger group's success in applying DTS around vancomycin, an antibiotic which has been losing efficacy for decades, will surely inspire chemists to apply innovative strategies founded on the robustness of total synthesis to approach biological problems with newfound ferocity.[27] Not all structurally-driven attempts to rejuvenate antibiotics have been fruitful; Andrade and coworkers designed desmethyl telithromycin analogs hypothesized to overcome the resistance-causing A2058G mutation within the ribosome. Though these molecules were generally inactive, the Andrade group postulated that these methyl groups were critical to maintaining the native topography of the molecule, and their removal was correspondingly detrimental to bioactivity.[28] These findings illustrate a philosophical issue in science, as these negative results, which might not otherwise be

reported, are clearly valuable in indicating what molecular features within the macrolides are critical for activity.

Chapter 2

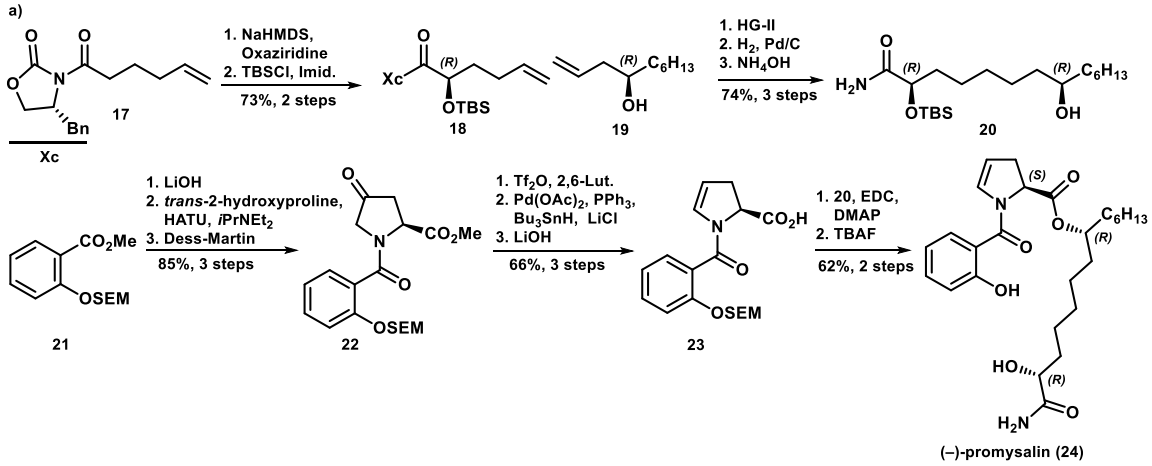
Promysalin and Analogs Thereof

In 2011, De Mot and coworkers reported a small molecule, promysalin, which exhibited intriguing biological activity within the genus *Pseudomonas*.[29] Isolated from *P. putida* colonizing soil near the roots of a Sri Lankan rice plant, promysalin demonstrated narrow-spectrum inhibition of the well-known pathogen *P. aeruginosa*, best known as an opportunistic pathogen in cystic fibrosis patients. No stereochemical information was reported in the structural determination. After applying the Stachelhaus code to predict L-dehydroproline, Wuest and coworkers embarked on a total synthesis of the remaining diastereomers to establish the absolute configuration of promysalin and further study this compound's intriguing biological attributes.[30] The synthetic strategy was highly modular and convergent, allowing chiral intermediates towards the right-hand 2-hydroxymyristate side chain to be joined in a "mix-and-match" approach via olefin cross-metathesis to reach the four necessary diastereomers (Scheme 1). Each of these side chains were united with the left-hand dehydroproline-salicylate fragment, which possessed defined chirality thanks to analysis of the biosynthetic gene cluster.

The left-hand portion was constructed through the amide coupling of trans-hydroxyproline and SEM-protected salicylic acid, which then underwent oxidation and selective formation of the enol triflate. Stille reduction and subsequent hydrolysis completed the left-hand precursor. The myristate fragment was envisioned to be formed through a cryptic hydrogenation/cross-metathesis, leveraging well-precedented chemistry to construct two building blocks in asymmetric fashion. The 6-carbon portion was furnished through the asymmetric Davis oxidation of the Evans-hexenoate conjugate and

following protection, and the 10-carbon chain was prepared through an enantioselective Keck allylation. After union using a Hoveyda-Grubbs metathesis catalyst, hydrogenation and removal of the Evans auxiliary completed the right-hand myristate portion. Finally, EDC-mediated esterification and painstakingly selected deprotection with TBAF in THF/DMPU completed this efficient synthesis of promysalin.

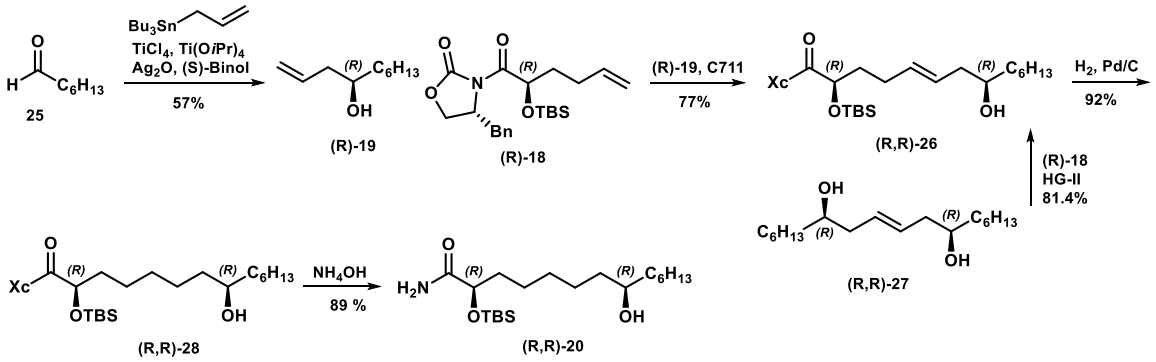
Careful comparison of the NMR spectra of the natural product and the four diastereomers suggested that the *R,R* configuration of the left-hand portion was correct, and this was corroborated by biological study, as this diastereomer shared the reported potency. Furthermore, Steele, et al reported quantitative biological data, with synthetic promysalin exhibiting IC_{50} (concentration inhibiting growth by 50%) values of 4.1 and 0.067 μM against *P. aeruginosa* strains PAO1 and PA14 (a highly virulent clinical isolate), respectively.[29] Furthermore, intriguing results concerning the inhibition of an unknown fluorescent species were obtained upon treatment of KT2440, a strain of the producing organism *P. putida*, with promysalin.



Scheme 1. Total synthesis of promysalin (**24**) by Wuest and coworkers.

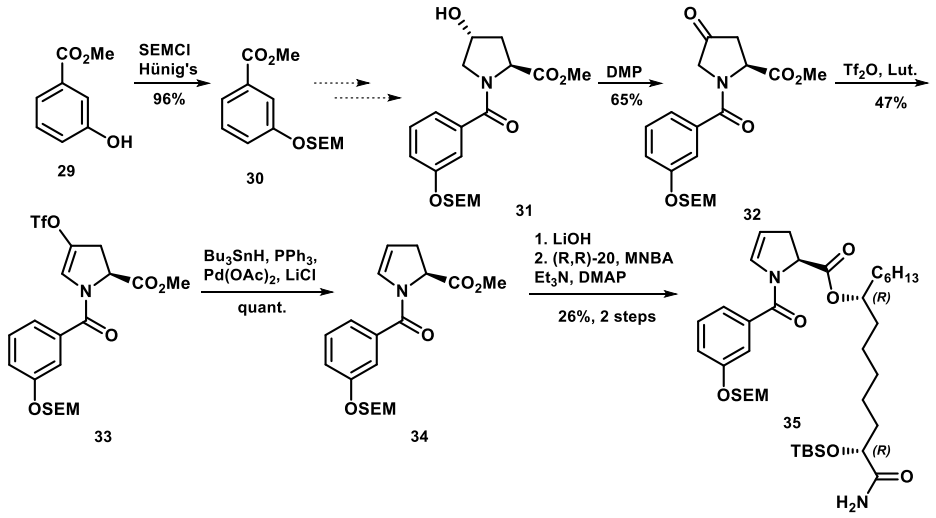
Given the importance of stereochemistry of the oxygens within the side chain, we hypothesized that promysalin's several hydrogen bond donors and acceptors could lead to

pseudo-macrocycle formation, which may have had biological relevance for binding in some active site. To test this hypothesis and establish a structure-activity relationship, a series of rationally designed analogs were prepared through a DTS approach, with changes to one of the three fragments well-tolerated by the modular synthesis.[31] Three questions stood out and could be tested in a straightforward manner: 1) is the 2-hydroxyl group on the myristate chain critical for activity? 2) are modifications to the salicylate portion tolerated? and 3) is unsaturation in the dehydroproline moiety necessary?

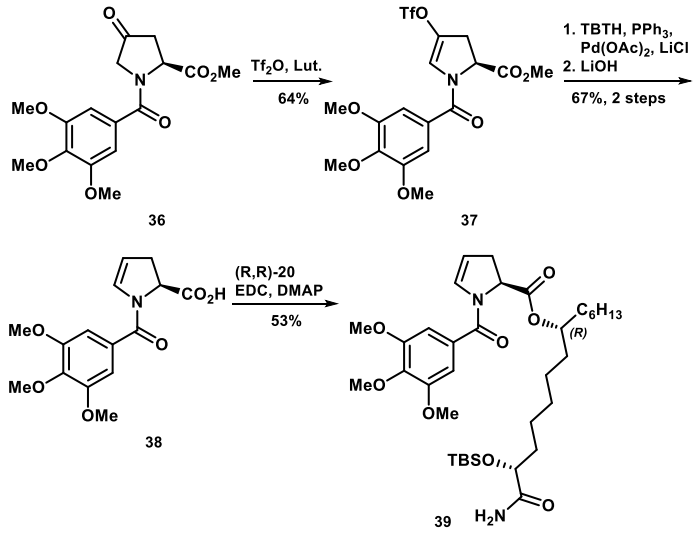


Scheme 2. Synthesis of promysalin side chain **20**.

To construct this library of compounds, I completed the synthesis of the (*R*,*R*)-side chain **20** with the best yields given in in Scheme 2. I should note that the final step was completed at gram-scale, enabling the preparation of many right-side analogues. I also note the efficiency of recycling the byproduct dimer (intermediate not characterized) of the homoallyl alcohol, improving the overall economy of this effort. I also contributed to the synthesis of the *meta*-salicylic promysalin analogue (Scheme 3). Additionally, a trimethylgallate analogue was conceived, and I successfully carried out many of the key late-stage steps at the direction of my senior colleagues (Scheme 4).

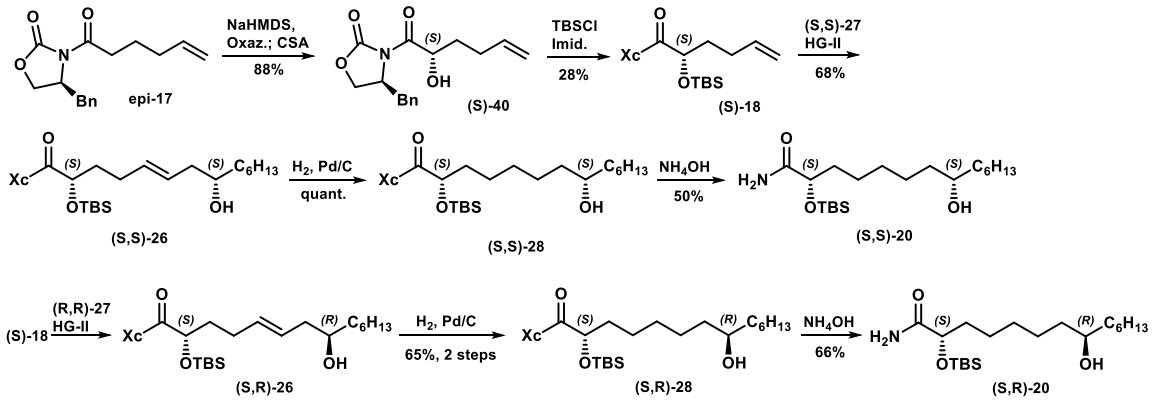


Scheme 3. Work towards *meta*-promysalin.

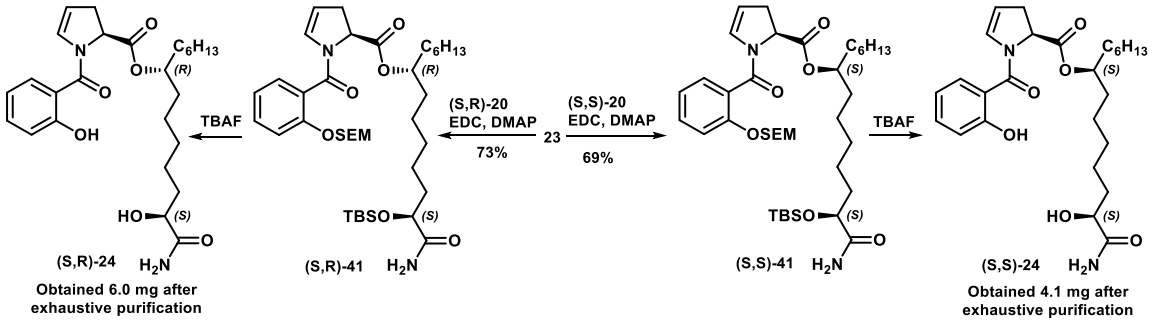


Scheme 4. Work towards gallate-derived promysalin analogue.

Another key contribution was the preparation of the (*S*,*S*)- and (*S*,*R*)-diasteromers of **24**, which were needed as negative controls for microbiological experiments (Schemes 5, 6). These syntheses worked as reported previously,[30] yielding milligram quantities of HPLC-purified material, suitable for biological assays.



Scheme 5. Syntheses of (*S*,*S*)- and (*S*,*R*)-side chains



Scheme 6. Synthesis of (*S*,*S*)- and (*S*,*R*)-diastereomers of promysalin

Screening of this library borne from an efficient diversity-oriented synthetic route led to several key insights (Figure 7). First, despite the importance of the stereochemistry of the C2 alcohol, deletion of this feature led to an analog (**46**) with a potency approximately the same as the natural product. Capping this alcohol with a methyl group (**47**) reduced activity, however. The orientation of the salicylate portion is indeed critical; no analog lacking the ortho-phenol was active. Finally, saturation of the dehydroproline ring (**42**) led to a modest decrease in potency; installation of a vinyl fluoride (**43**) was favorable. Finally, Steele, et al designed an analog bearing a propargyl group on the myristate amide as a surrogate for a promysalin photoprobe adduct.[32] This compound exhibited acceptable activity, and the diazirine photoprobe was synthesized for proteomic studies. That the C2 alcohol was entirely unnecessary for activity casted doubt on the

intramolecular hydrogen bonding hypothesis; and proteomic studies were conducted to unambiguously determine the protein target by C. Keohane in collaboration with Prof. Stefan Sieber (Technische Universität München).[32]

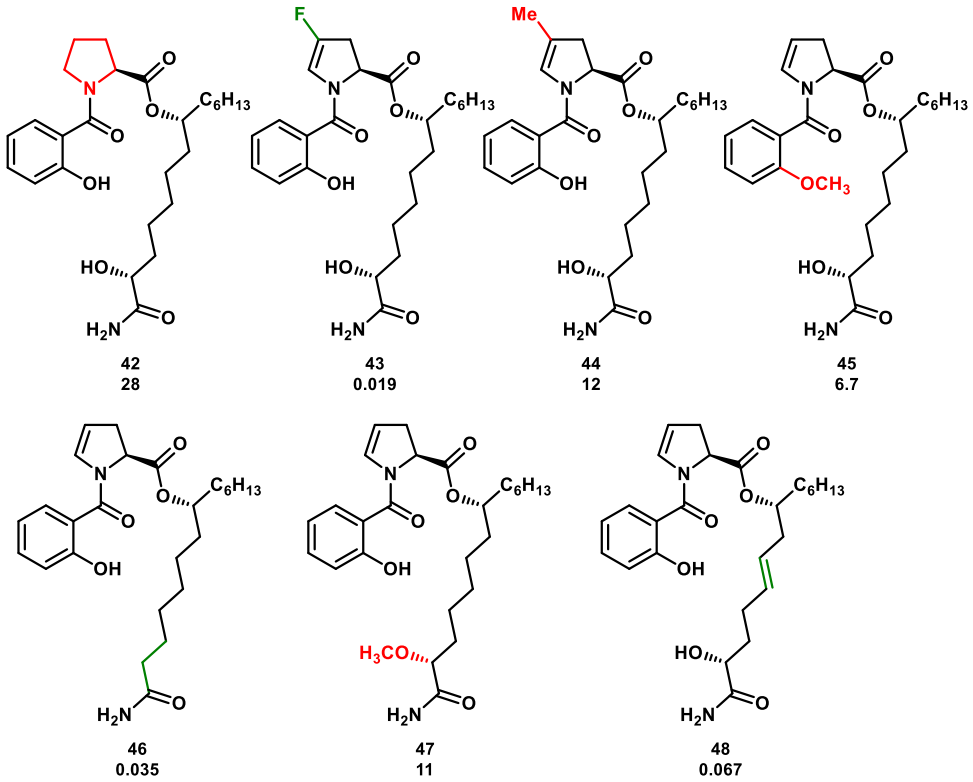


Figure 7. Selected promysalin analogs with IC_{50} values (μM) against *P. aeruginosa* PA14. Tolerated features in green; not tolerated in red.

Chapter 3

Promysalin as a Platform for Future Discovery: Proposed Future Directions

The aforementioned proteomic studies identified promysalin as a complex II inhibitor, targeting the membrane-bound succinate dehydrogenase C subunit, which carries out the oxidation of succinate to fumarate using FADH and ubiqunone (coenzyme Q) cofactors.[33] Structurally, the A subunit carries out the oxidation of succinate, and the resulting electrons pass through the iron-sulfur clusters in the B subunit. Coenzyme Q (ubiquinone), sitting at the interface of the B, C, and D subunits, then accepts these electrons and is reduced to ubiquinol.

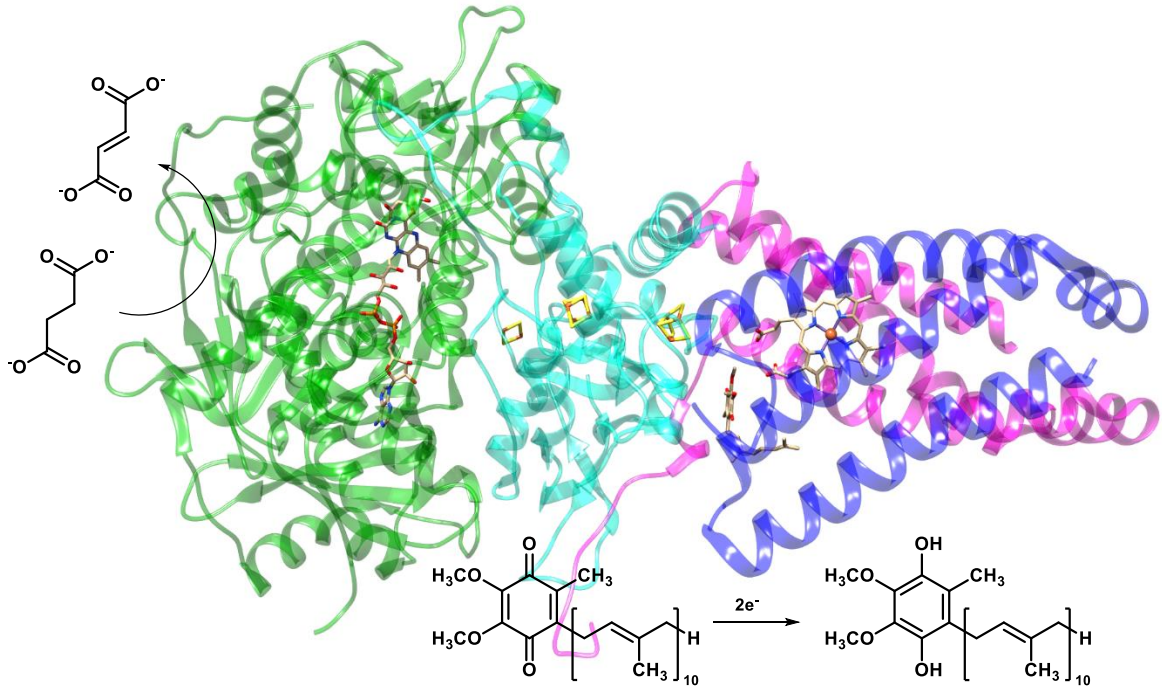


Figure 8. Structure of succinate dehydrogenase (PDB 1NEK) rendered in UCSF Chimera. A subunit in green, B in cyan, C in purple, D in blue.

Despite the conundrum that promysalin, a *narrow-spectrum* antibiotic, inhibits a target very well conserved across all domains of life, this result was particularly exciting given that complex II inhibitors are commercially useful fungicides, including the well-known carboxin. Based on structural similarities, it is reasonable to hypothesize that promysalin is a CoQ mimic.

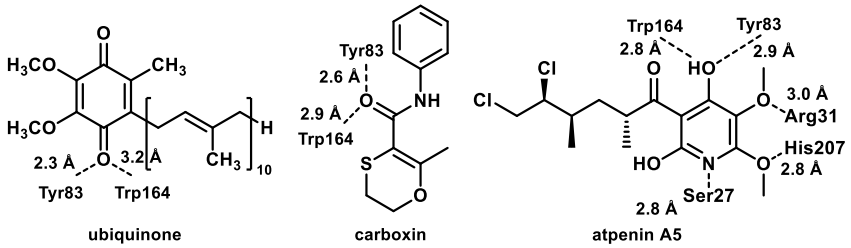
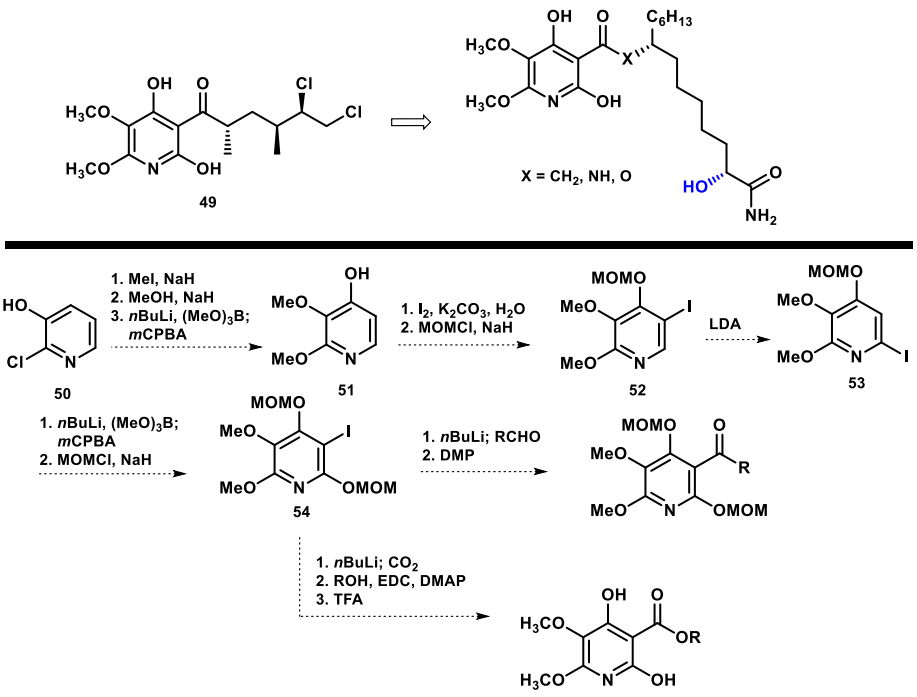


Figure 9. Key interactions between complex II and substrate and inhibitors. PDB 1NEK, 2WDQ, 2ACZ.

Natural inhibitors of complex II are also known. In 1988, Omura and coworkers discovered an antifungal agent, atpenin A5 (**49**), from *Penicillium.*[34] This compound exhibited excellent activity (MICs < 0.01 μg/ml) against some fungi, though it did not inhibit bacteria (>100 μg/ml). A later effort determined that atpenin A5 selectively targeted complex II of various eukaryotes *in vitro*.[35] The structural similarity to ubiquinone is obvious, and the mode of action was further confirmed by crystallization in *E. coli* SDH, despite its lack of inhibitory activity. Of note, the pyridine moiety makes an additional hydrogen bond to three amino acids, possibly explaining its outstanding potency. Carreira and coworkers recently reported an asymmetric synthesis of atpenin A5 and side chain analogs, two of which showed a small improvement in activity.[36] Furthermore, a pesticide research group at DuPont identified an atpenin analog with a highly simplified and branched side chain, which also showed activity.[37] With the highly oxidized pyridine

demonstrating such potent binding affinity for complex II, we hypothesized whether this structure could inspire an antibiotic.

We hypothesized that replacement of the atpenin side chain with the promysalin side chain could confer antibiotic activity. Molecular modeling analyses performed by Karanicolas and coworkers indicate subtle differences in the hydrophobic interactions, especially in *P. aeruginosa*, which could be significant enough to permit antibiotic activity.[32] Because the reported syntheses neatly divide the left-hand heterocycle and right-hand side chain (which supports the clear analogy with promysalin), we propose a diverted total synthesis approach to access unprecedented chimeric scaffolds.



Scheme 7. Planned synthesis of atpenin-promysalin chimeric compounds.

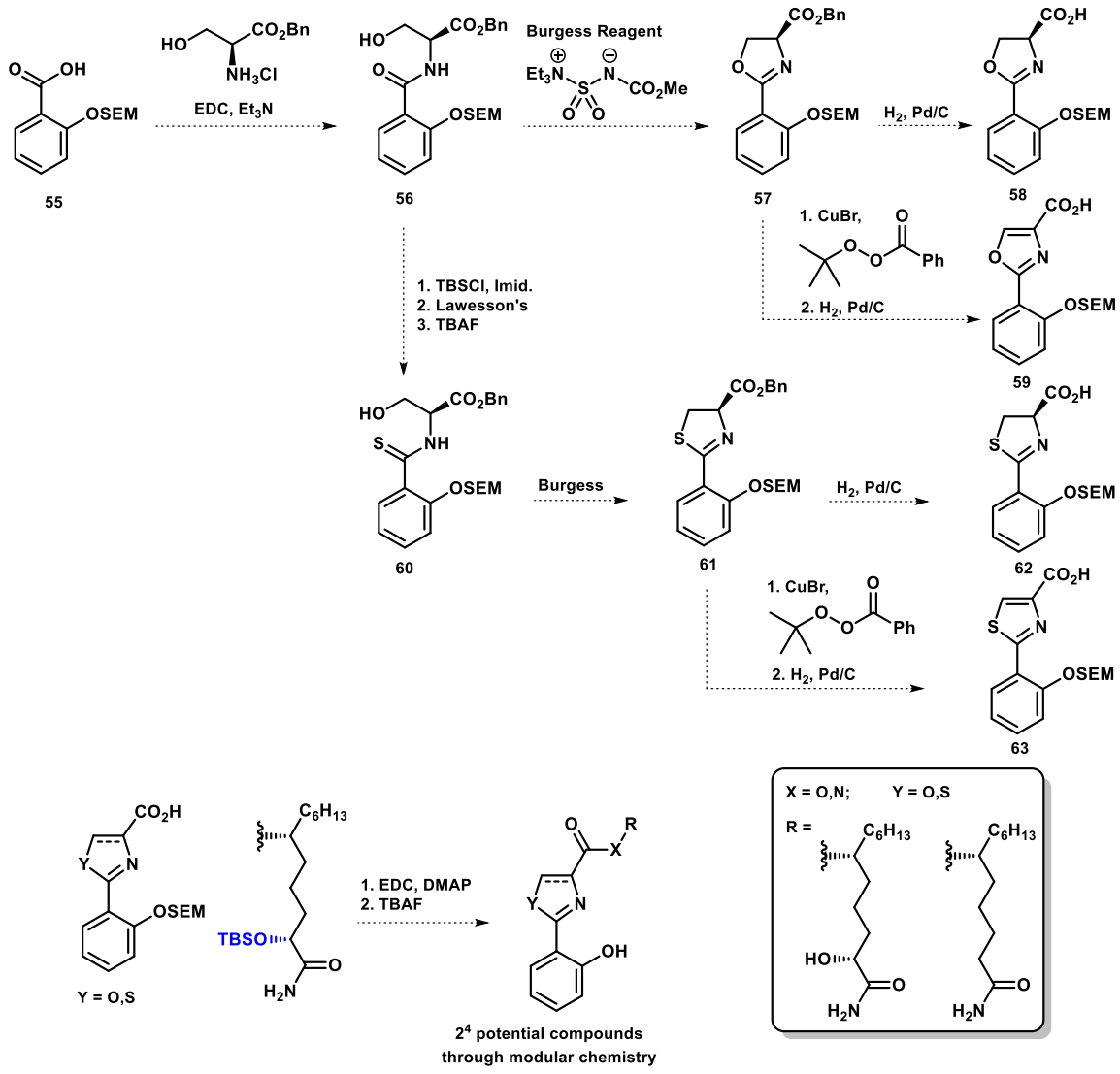
The synthetic approach adapts the generation of the challenging highly oxidized pyridine from Omura and coworkers' work, relying on a critical "halogen dance" mediated by LDA to permit the correct orientation of the subsequent oxidation. The key step towards the natural product generates an aryllithium species which adds to the appropriate

aldehyde, and oxidation yields the desired ketone. We propose the addition of this aryllithium reagent to carbon dioxide to yield the carboxylate, which then permits ester or amide coupling, allowing the incorporation of the promysalin side chain. Another possible avenue of study includes a ketone linkage between the pyridine and side chain from promysalin, though an asymmetric method of introducing the homologous aldehyde equivalent is needed. Additionally, modifying the promysalin ester into an amide was not tolerated in the analog screen, though there is no guarantee if this would remain the case.

Given the intense interest within the chemical biology community surrounding siderophores as a potential avenue of identifying antibiotics, we hypothesized preparing chimeric analogs of the promysalin side chain with a variety of heterocycles based around aeruginoic acid, and many of these molecular fragments occur in siderophores including pyochelin and mycobactin. As these structures share more than a superficial similarity to the left half of promysalin, we hypothesized that these may demonstrate antibiotic activity. We propose a highly divergent synthesis allowing for a "mix-and-match" approach between the left-half heterocycle and one of several side chains.

We draw inspiration from Miller and coworkers' synthesis of mycobactin S in beginning with the amide coupling between a protected salicylate derivative and L-serine, benzyl ester.[38] Similarly, we propose the use of Burgess' reagent[39] to close the oxazoline ring. To divert to aromatic heterocycles, Meyers reports the mild oxidation to the oxazole using *tert*-butyl peroxybenzoate with copper (I) bromide.[40] To install sulfur, the thioamide can be selectively formed by protecting the alcohol, treatment with Lawesson's reagent, and subsequent deprotection, allowing Burgess' reagent to form the thiazoline.[41] The same oxidation also permits access of the aromatic thiazole. After the hydrogenolysis of the

benzyl ester, each left-hand precursor can be united with the side chain through esterification, or additionally, amidation, with deprotection following. Given 4 binary variables (sulfur or oxygen heterocycle, aromatic or saturated, ester or amide, and native promysalin side chain or the deoxy-variant), up to 16 analogs are anticipated with well-precedented chemistry proposed.



Scheme 8. Planned modular synthesis of heterocyclic promysalin analogues.

Conclusion

The scientific ingenuity of chemists is critical to overcoming the short-term lack of new antibiotic agents, and diversity-oriented strategies will prove critical in expanding on the proven antibiotic capabilities of natural products while mitigating the key side effects that have sunk so many promising endeavors.

References

(1) Rossiter, S. E.; Fletcher, M. H.; Wuest, W. M. Natural Products as Platforms to Overcome Antibiotic Resistance. *Chem. Rev.* **2017**, *117*, 12415–12474.

(2) McGann, P.; Snesrud, E.; Maybank, R.; Corey, B.; Ong, A. C.; Clifford, R.; Hinkle, M.; Whitman, T.; Lesho, E.; Schaecher, K. E. Escherichia Coli Harboring Mcr-1 and Bla CTX-M on a Novel IncF Plasmid: First Report of Mcr-1 in the United States. *Antimicrob. Agents Chemother.* **2016**, *60*, 4420–4421.

(3) Wright, P. M.; Seiple, I. B.; Myers, A. G. The Evolving Role of Chemical Synthesis in Antibacterial Drug Discovery. *Angew. Chemie - Int. Ed.* **2014**, *53*, 8840–8869.

(4) Lewis, K. Platforms for Antibiotic Discovery. *Nat. Rev. Drug Discov.* **2013**, *12*, 371–387.

(5) Walsh, C. Molecular Mechanisms That Confer Antibacterial Drug Resistance. *Nature* **2000**, *406*, 775–781.

(6) Walsh, C. T.; Fisher, S. L.; Park, I.-S.; Prahalad, M.; Wu, Z. Bacterial Resistance to Vancomycin: Five Genes and One Missing Hydrogen Bond Tell the Story. *Chem. Biol.* **1996**, *3*, 21–28.

(7) Long, D. D.; Aggen, J. B.; Christensen, B. G.; Judice, J. K.; Hegde, S. S.; Kaniga, K.; Krause, K. M.; Linsell, M. S.; Moran, E. J.; Pace, J. L. A Multivalent Approach to Drug Discovery for Novel Antibiotics. *J. Antibiot. (Tokyo).* **2008**, *61*, 595–602.

(8) Morimoto, S.; Misawa, Y.; Adachi, T.; Nagate, T.; Watanabe, Y.; Omura, S. Chemical Modification of Erythromycins. II. Synthesis and Antibacterial Activity of O-Alkyl Derivatives of Erythromycin A. *J. Antibiot. (Tokyo).* **1990**, *43*, 286–294.

(9) Djokic, S.; Kobrehel, G.; Lazarevski, G.; Lopotar, N.; Tamburasev, Z.; Kamenar, B.; Nagl, A.; Vickovic, I. Erythromycin Series. Part 11. Ring Exansion of Erythromycin A Oxime by the Beckmann Rearrangement. *J. Chem. Soc. Perkin Trans. I* **1986**, 1881–1890.

(10) Pawlowski, A. C.; Johnson, J. W.; Wright, G. D. Evolving Medicinal Chemistry Strategies in Antibiotic Discovery. *Curr. Opin. Biotechnol.* **2016**, *42*, 108–117.

(11) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3–26.

(12) Danishefsky, S. On the potential of natural products in the discovery of pharma leads: A case for reassessment. *Nat. Prod. Rep.* **2010**, *27*, 1114−1116.

(13) Rivkin, A.; Chou, T.-C.; Danishefsky, S. J. On the Remarkable Antitumor Properties of Fludelone: How We Got There. *Angew. Chem., Int. Ed.* **2005**, *44*, 2838−2850.

(14) Schreiber, S. L. Target-Oriented and Diversity-Oriented Organic Synthesis in Drug

Discovery. *Science* **2000**, *287*, 1964−1969.

(15) Cordier, C.; Morton, D.; Murrison, S.; Nelson, A.;O'Leary-Steele, C. Natural Products as an Inspiration in the Diversity-Oriented Synthesis of Bioactive Compound Libraries. *Nat. Prod. Rep.* **2008**, *25*, 719−737.

(16) Thomas, G. L.; Spandl, R. J.; Glansdorp, F. G.; Welch, M.; Bender, A.; Cockfield, J.; Lindsay, J. A.; Bryant, C.; Brown, D. F. J.; Loiseleur, O.; et al. Anti-MRSA Agent Discovery Using Diversity-Oriented Synthesis. *Angew. Chem., Int. Ed.* **2008**, *47*, 2808−2812.

(17) Socha, A. M.; Tan, N. Y.; Laplante, K. L.; Sello, J. K. Diversity-Oriented Synthesis of Cyclic Acyldepsipeptides Leads to the Discovery of a Potent Antibacterial Agent. *Bioorg. Med. Chem.* **2010**, *18*, 7193− 7202.

(18) Nicolaou, K. C.; Boddy, C. N. C.; Bräse, S.; Winssinger, N. Chemistry, Biology, and Medicine of the Glycopeptide Antibiotics. *Angew. Chem. Int. Ed.* **1999**, *38*, 2096–2152.

(19) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. Total Syntheses of Vancomycin and Eremoycin Aglycons. *Angew. Chem. Int. Ed.* **1998**, *37*, 2700−2704.

(20) Nicolaou, K. C.; Hughes, R.; Cho, S. Y.; Winssinger, N.; Smethurst, C.; Labischinski, H.; Endermann, R. Target-Accelerated Combinatorial Synthesis and Discovery of Highly Potent Antibiotics Effective Against Vancomycin-Resistant Bacteria. *Angew. Chem., Int. Ed.* **2000**, *39*, 3823−3828.

(21) Walsh, C. T.; Fisher, S. L.; Park, I.-S.; Prahalad, M.; Wu, Z. Bacterial Resistance to Vancomycin: Five Genes and One Missing Hydrogen Bond Tell the Story.

Chem. Biol. **1996**, *3* (1), 21–28.

(22) McComas, C. C.; Crowley, B. M.; Boger, D. L. Partitioning the Loss in Vancomycin Binding Affinity for D-Ala-D-Lac into Lost H-Bond and Repulsiv Lone Pair Contributions. *J. Am. Chem. Soc.* **2003**, *5*, 9314–9315.

(23) Xie, J.; Pierce, J. G.; James, R. C.; Okano, A.; Boger, D. L. A Redesigned Vancomycin Engineered for Dual D-Ala-D-Ala and D-Ala-D-Lac Exhibits Potent Antimicrobial Activity Against Vancomycin-Resistant Bacteria. *J. Am. Chem. Soc.* **2011**, *133*, 13946–13949.

(24) Nakayama, A.; Okano, A.; Feng, Y.; Collins, J. C.; Collins, K. C.; Walsh, C. T.; Boger, D. L. Enzymatic Glycosylation of Vancomycin Aglycon: Completion of a Total Synthesis of Vancomycin and N- and C-Terminus Substituent Effects of the Aglycon Substrate. *Org. Lett.* **2014**, *16*, 3572–3575.

(25) Okano, A.; Nakayama, A.; Schammel, A. W.; Boger, D. L. Total Synthesis of [Ψ[C(=NH)NH]Tpg^4]Vancomycin and its 4-Chlorobiphenyl)methyl Derivative: Impact of Peripheral Modifications on Vancomycin Analogues Redesigned for Dual D-Ala-D-Ala and D-Ala-D-Lac Binding. *J. Am. Chem. Soc.* **2014**, *136*, 13522–13525.

(26) Okano, A.; Isley, N. A.; Boger, D. L. Peripheral Modifications of [Ψ[CH_2NH]Tpg^4]vancomycin with Added Synergistic Mechanisms of Action Provide Durable and Potent Antibiotics. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, E5053–E5061.

(27) Okano, A.; Isley, N. A.; Boger, D. L. Total Syntheses of Vancomycin-Related Glycopeptide Antibiotics and Key Analogues. *Chem. Rev.* **2017**, *117*, 11952–

11993.

(28) Andrade, R. B. Total Synthesis of Desmethyl Macrolide Antibiotics. *Synlett* **2015**, *26*, 2199–2215.

(29) Li, W.; Estrada-de los Santos, P.; Matthijs, S.; Xie, G.-L.; Busson, R.; Cornelis, P.; Rozenski, J.; De Mot, R. Promysalin, a Salicylate-Containing *Pseudomonas putida* Antibiotic, Promotes Surface Colonization and Selectively Targets Other *Pseudomonas*. *Chem. Biol.* **2011**, *18*, 1320–1330.

(30) Steele, A. D.; Knouse, K. W.; Keohane, C. E.; Wuest, W. M. Total Synthesis and Biological Investigation of (–)-Promysalin. *J. Am. Chem. Soc.* **2015**, *137*, 7314–7317.

(31) Steele, A. D.; Keohane, C. E.; Knouse, K. W.; Rossiter, S. E.; Williams, S. J.; Wuest, W. M. Diverted Total Synthesis of Promysalin Analogs Demonstrates That an Iron-Binding Motif Is Responsible for Its Narrow-Spectrum Antibacterial Activity. *J. Am. Chem. Soc.* **2016**, *138*, 5833–5836.

(32) Keohane, C. E.; Steele, A. D.; Fetzer, C. .; Khowsathit, J.; Van Tyne, D.; Moynié, L.; Gilmore, M. S.; Karanicolas, J.; Sieber, S. A.; Wuest, W. M. Promysalin Elicits Species-Selective Inhibition of *Pseudomonas aeruginosa* by Targeting Succinate Dehydrogenase. *J. Am. Chem. Soc.* **2018**, *140*, 1774–1782.

(33) Yankovskaya, V. Architecture of Succinate Dehydrogenase and Reactive Oxygen Species Generation. *Science.* **2003**, *299*, 700–704.

(34) Omura, S.; Tomoda, H.; Kimura, K.; Zhen, D.-Z.; Kumagai, H.; Igarashi, K.; Imamura, N.; Takahashi, Y.; Tanaka, Y.; Iwai, Y. Atpenins, New Antifungal Antibiotics Produced by *Penicillium* Sp. *J. Antibiot.* **1988**, *41*, 1769–1773.

(35) Miyadera, H.; Shiomi, K.; Ui, H.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Miyoshi, H.; Osanai, A.; Kita, K.; Omura, S. Atpenins, Potent and Specific Inhibitors of Mitochondrial Complex II (Succinate-Ubiquinone Oxidoreductase). *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 473–477.

(36) Krautwald, S.; Nilewski, C.; Mori, M.; Shiomi, K.; Omura, S.; Carreira, E. M. Bioisosteric Exchange of C Sp 3 -Chloro and Methyl Substituents: Synthesis and Initial Biological Studies of Atpenin A5 Analogues. *Angew. Chemie - Int. Ed.* **2016**, *55*, 4049–4053.

(37) Selby, T. P.; Hughes, K. A.; Rauh, J. J.; Hanna, W. S. Synthetic Atpenin Analogs: Potent Mitochondrial Inhibitors of Mammalian and Fungal Succinate-Ubiquinone Oxidoreductase. *Bioorganic Med. Chem. Lett.* **2010**, *20*, 1665–1668.

(38) Hu, J.; Miller, M. J. Total Synthesis of a Mycobactin S, a Siderophore and Growth Promoter of Mycobacterium Smegmatis, and Determination of Its Growth Inhibitory Activity against Mycobacterium Tuberculosis. *J. Am. Chem. Soc.* **1997**, *119*, 3462–3468.

(39) Taibi, P.; Mobashery, S. (Methoxycarbonylsulfamoyl)triethylammonium Hydroxide. *e-Eros* Encyclopedia of Reagents for Organic Synthesis.

(40) Meyers, A. I.; Tavares, F. The Oxidation of 2-Oxazolines to 1,3-Oxazoles. *Tetrahedron Lett.* **1994**, *35*, 2481–2484.

(41) Pattenden, G.; Thompson, T. Design and Synthesis of Novel Tubular and Cage Structures Based on Thiazole-Containing Macrolactams Related to Marine Cyclopeptides. *Chem. Commun.* **2001**, *3*, 717–718.

Experimental Information

General Procedures for Synthetic Steps:

General procedure A: SEM protection of methyl hydroxybenzoates.

To a methyl hydroxybenzoate (1 equiv) dissolved in CH_2Cl_2 (2 M solution) was added 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl, 2 equiv) and then cooled to 0 °C. Diisopropylethylamine (Hünig's base, 4 equiv) was added slowly and the solution was stirred and warmed to room temperature overnight. The following day, the mixture was poured into water and extracted three times with diethyl ether. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, concentrated, and purified by column chromatography.

General procedure B: Hydrolysis of methyl esters.

Methyl ester (1 equiv) was dissolved in a mixture of 3:1:1 tetrahydrofuran (THF)/methanol/water (1 M) and lithium hydroxide hydrate (5 equiv) was added as a solution in a small volume of water. The reaction was monitored by TLC and upon completion was carefully acidified by addition of either 1 M hydrochloric acid solution or 5% acetic acid until the pH reached 5-6. The solution was extracted three times with CH_2Cl_2, washed with brine, dried over magnesium sulfate, filtered, and concentrated.

General procedure C: HATU-promoted amide coupling of SEM-benzoic acids and hydroxyproline methyl ester.

Acid (1.0 equiv) was dissolved in *N*,*N*-dimethylformamide (DMF, 0.2 M) with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU, 1.2 equiv)

to which a solution of amine hydrochloride (1.2 equiv) and diisopropylethylamine (1.5 equiv) in an equal volume of DMF was added. Another portion of diisopropylethylamine (3 equiv) was added and the reaction was allowed to stir overnight, then was poured into water and extracted three times with ethyl acetate. The combined organic layers were washed with sat. NH_4Cl, sat. $NaHCO_3$, and twice each with water and brine, then dried over magnesium sulfate, filtered, concentrated, and purified by column chromatography (0 → 50% ethyl acetate/CH_2Cl_2).

General procedure D: DMP oxidation.

An acylated trans-L-hydroxyproline derivative (1 equiv) was dissolved in CH_2Cl_2 (0.05 M), and to this solution was added $NaHCO_3$ (20 equiv) and Dess-Martin periodinane (DMP, 2 equiv), and the reaction was allowed to stir overnight. The next day, the reaction was quenched with 2:1:1 water/sat. $NaHCO_3$/sat. $Na_2S_2O_3$ and allowed to stir for an hour. The mixture was then extracted three times with CH_2Cl_2 and the combined organic layers were washed with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, water, and brine, then dried over magnesium sulfate, filtered, concentrated, and purified by column chromatography.

General procedure E: Synthesis of enol triflates from ketones.

The ketone (1 equiv) was dissolved in CH_2Cl_2 (0.1 M) and cooled to -50 °C. 2,6-Lutidine (4 equiv) was added, and trifluoromethanesulfonic anhydride (2 equiv) was added dropwise. The reaction was allowed to warm to -35 °C. After 30 minutes the reaction was quenched with sat. $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layers were washed with sat. $NaHCO_3$, brine, dried over magnesium sulfate, concentrated, and purified by column chromatography (0 → 5% ethyl acetate/hexanes held at 5% until 2,6-lutidine finished eluting, then 5 → 20% ethyl acetate/hexanes).

General procedure F: Reductive cleavage of enol triflates.

To a solution of enol triflate (1 equiv) dissolved in tetrahydrofuran (0.1 M) was added triphenylphosphine (0.3 equiv), palladium (II) acetate (0.1 equiv), and flame-dried lithium chloride (1.5 equiv). Tributyltin hydride (1 equiv) was then added dropwise. The reactions turned orange or brown upon completion and were then quenched with a 1 M solution of potassium fluoride and extracted three times with ether. The combined organic layers were washed with 1 M KF, water, and brine, dried over sodium sulfate, filtered, concentrated, and purified by column chromatography (0 → 30% ethyl acetate/hexanes, loaded in CH_2Cl_2).

General procedure G: EDC esterification.

An acid (1.4 equiv) was dissolved in CH_2Cl_2 (0.2 M) was cooled to 0°C and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 2 equiv) was added. A solution of alcohol (1 equiv) and 4-(dimethylamino)pyridine (DMAP, 0.5 equiv) were dissolved in an equal volume of dry CH_2Cl_2, added to the first solution, and allowed to stir overnight. The next day, the reaction was poured into water and extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over magnesium sulfate, concentrated, and purified by column chromatography (0 → 30% ether/CH_2Cl_2).

General procedure H: Shiina esterification.

To a solution of the carboxylic acid (1.4 equiv) dissolved in CH_2Cl_2 (0.2 M) were added 2-methyl-6-nitrobenzoic anhydride (MNBA, 2.6 equiv) and triethylamine (3.3 equiv), and this solution was stirred for 10 minutes. Then the alcohol (1 equiv) and DMAP (0.1 equiv) dissolved in an equal volume of CH_2Cl_2 were added, and the reaction was stirred overnight. The reaction was poured into saturated ammonium chloride solution, extracted three times

with CH_2Cl_2, washed with brine, dried over magnesium sulfate, filtered, concentrated, and purified by column chromatography (0 → 30% ether/CH_2Cl_2).

Compounds not reported prior to Reference 31

Methyl 3-((2-(trimethylsilyl)ethoxy)methoxy)benzoate 30. Following general procedure A, methyl 3-hydroxybenzoate (300 mg, 2.00 mmol) yielded **30** as a clear oil (483 mg, 86% yield). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.71 – 7.66 (m, 2H), 7.34 (dd, J = 11.9, 4.2 Hz, 1H), 7.23 (ddd, J = 8.2, 2.6, 1.1 Hz, 1H), 5.26 (s, 2H), 3.91 (s, J = 2.9 Hz, 3H), 3.80 – 3.73 (m, 2H), 0.98 – 0.93 (m, 2H), -0.01 (s, J = 3.3 Hz, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 166.6, 157.3, 131.4, 129.3, 122.8, 120.9, 116.9, 92.7, 77.2, 66.2, 51.9, 17.9, -1.51; **IR** (film) 2952, 2897, 1723 (C=O), 1586, 1488, 1447, 1380, 1274, 1248, 1211, 1153, 1106, 1083, 1009, 994, 918, 857, 833, 783, 755, 683; **HRMS** Accurate mass (ES+): Found 305.1195 (+3.3 ppm), $C_{14}H_{22}O_4SiNa$ ($M+Na^+$) requires 305.1185.

Methyl (S)-4-oxo-1-(3-((2-(trimethylsilyl)ethoxy)methoxy)benzoyl)pyrrolidine-2-carboxylate 32. Using general procedure D, **31** yielded **32** as a yellow oil (244 mg, 65%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.37 – 7.32 (m, 1H), 7.19 – 7.11 (m, 3H), 5.37 – 5.27 (m, 1H), 5.24 (s, 2H), 3.85 – 3.70 (m, 5H), 2.97 (dd, J = 18.8, 10.6 Hz, 1H), 2.70 (d, J = 20.3 Hz, 1H), 0.98 – 0.91 (m, 2H), -0.00 (s, J = 3.4 Hz, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 207.2, 171.6, 170.2, 157.5, 136.2, 129.9, 120.2, 118.6, 114.9, 92.8, 66.4, 55.4, 52.9, 40.0, 18.0, -1.4; **[α]25D** +25.3 (c = 0.91 in $CHCl_3$); **IR** (film) 2950, 2395, 2342, 1757 (C=O), 1635 (C=O), 1575 (C=O), 1445, 1393, 1296, 1264, 1250, 1228, 1186, 1151, 1122, 1078, 1030, 1008, 990, 950, 862, 833, 817, 774, 753, 694, 600, 562; **HRMS** Accurate mass (ES+): Found 394.1700 (+3.6 ppm), $C_{19}H_{28}NO_6Si$ ($M+H^+$) requires 394.1686.

Methyl(S)-4-(((trifluoromethyl)sulfonyl)oxy)-1-(3-((2-(trimethylsilyl)ethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate 33. Using general procedure E, ketone **32** (111 mg, 0.280 mmol) yielded the triflate **33** as an orange oil (72 mg, 47% yield). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.37 (t, J = 7.9 Hz, 1H), 7.18 (dt, J = 24.8, 8.2 Hz, 3H), 6.81 (s, 1H), 5.24 (s, 2H), 5.08 (d, J = 6.5 Hz, 1H), 3.83 (s, 3H), 3.78 – 3.72 (m, 2H), 3.46 – 3.36 (m, 1H), 2.97 (ddd, J = 16.4, 4.8, 1.5 Hz, 1H), 0.99 – 0.92 (m, 2H), 0.00 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 169.7, 167.1, 159.7, 157.6, 144.3, 137.5, 134.6, 134.4, 130.0, 124.1, 123.3, 123.1, 120.9, 119.8, 119.4, 117.2, 115.6, 92.8, 66.6, 58.3, 57.6, 53.0, 33.2, 24.4, 18.1, -1.4; **[α]25D** –56.4 (c = 0.45 in $CHCl_3$); **IR** (film) 2954, 2359, 2341, 1749 (C=O), 1652 (C=O), 1581 (C=O), 1488, 1427, 1398, 1207, 1137, 1086, 1005, 990, 917, 857, 832, 744, 693, 667, 605; **HRMS** Accurate mass (ES+): Found 548.1028 (+5.5 ppm), $C_{20}H_{26}NO_8SSiNa$ ($M+Na^+$) requires 548.0998.

Methyl (S)-1-(3-((2-(trimethylsilyl)ethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate 34. Using general procedure F, triflate **33** (29 mg, 0.054 mmol) yielded olefin **34** as a yellow oil (22 mg, quantitative yield). **^{1}H NMR** (500 MHz, CDCl3) δ 7.33 (t, J = 7.9 Hz, 1H), 7.23 (s, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 6.58 – 6.52 (m, 1H), 5.23 (s, 2H), 5.11 (d, J = 5.1 Hz, 1H), 5.01 (dd, J = 11.6, 5.0 Hz, 1H), 3.80 (s, 3H), 3.77 – 3.72 (m, 2H), 3.15 – 3.06 (m, 1H), 2.76 – 2.67 (m, 1H), 0.98 – 0.93 (m, 2H), 0.00 (s, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.6, 166.8, 157.5, 136.2, 131.0, 129.7, 121.2, 118.6, 115.9, 109.0, 93.0, 66.5, 58.5, 52.6, 33.9, 18.1, -1.3; **[α]25D** –44.0 (c = 0.31 in $CHCl_3$); **IR** (film) 2953, 2359, 2341, 1749 (C=O), 1646, 1617, 1488, 1446, 1398, 1362, 1317, 1086, 1005, 989, 858, 834, 694, 668; **HRMS** Accurate mass (ES+): Found 378.1706 (-8.2 ppm), $C_{19}H_{28}NO_5Si$ ($M+H^+$) requires 378.1737.

(7R,13R)-14-amino-13-((tert-butyldimethylsilyl)oxy)-14-oxotetradecan-7-yl (S)-1-(3-((2- (trimethylsilyl)ethoxy)methoxy)benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate 35. Using general procedure B, methyl ester **34** (17 mg, 0.045 mmol) yielded the acid intermediate as a yellow oil. This compound was not of sufficient purity for characterization. Following general procedure H, (1.2 equiv. acid and MNBA), acid intermediate (~18 mg, 0.05 mmol) yielded the title compound as a yellow oil (8.0 mg, 26% yield, 2 steps). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 (q, J = 7.8 Hz, 1H), 7.20 (s, J = 11.1 Hz, 1H), 7.15 (dd, J = 15.5, 7.9 Hz, 2H), 6.52 (s, 2H), 5.54 – 5.44 (m, 1H), 5.23 (s, 2H), 5.08 (d, J = 1.9 Hz, 1H), 5.01 – 4.90 (m, 2H), 4.17 – 4.08 (m, 1H), 3.80 – 3.67 (m, 2H), 3.15 – 3.06 (m, 1H), 2.67 (d, J = 16.9 Hz, 1H), 1.81 – 1.70 (m, 1H), 1.69 – 1.47 (m, 7H), 1.26 (dd, J = 14.1, 6.9 Hz, 17H), 0.97 – 0.89 (m, 12H), 0.85 (t, J = 6.9 Hz, 3H), 0.10 – 0.06 (m, 6H), -0.01 (s, J = 3.2 Hz, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 176.9, 170.9, 166.6, 157.5, 136.5, 131.1, 129.7, 121.1, 118.5, 115.8, 108.9, 93.0, 75.6, 73.6, 66.5, 58.8, 35.1, 34.0, 31.8, 29.8, 29.5, 29.3, 25.9, 25.3, 25.1, 24.1, 22.7, 18.2, 14.2, -1.3, -4.7, -5.1; **[α]25D** –16.1 (c = 1.18 in $CHCl_3$); **IR (**film) 3480, 2927, 2867, 1739 (C=O), 1689 (C=O), 1651 (C=O), 1618, 1579, 1488, 1446, 1397, 1248, 1192, 1088, 1029, 1005, 991, 938, 857, 834, 778, 745, 694, 668; **HRMS** Accurate mass (ES+): Found 719.4445 (-5.8 ppm), $C_{38}H_{67}N_2O_7Si_2$ ($M+H_+$) requires 719.4487.

Methyl (2S)-4-(trifluoromethanesulfonyloxy)-1-(3,4,5-trimethoxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate 37. Using general procedure E, ketone **36** (145 mg, 0.431 mmol) yielded triflate **37** as an orange oil (129 mg, 64% yield). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.90 (s, 1H), 6.75 (s, 2H), 5.10 – 5.00 (m, 1H), 3.88 – 3.78 (m, 12H), 3.40 (dd, J = 15.2, 13.1 Hz, 1H), 3.00 – 2.90 (m, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 169.7, 167.2,

153.50, 140.84 134.5, 128.4, 123.3, 105.4, 61.0, 56.4, 53.1; **[α]25D** +7.3 (c = 0.26 in $CHCl_3$); **IR** (film) 2953, 2359, 1745 (C=O), 1636 (C=O), 1582, 1413, 1326, 1234, 1120, 999, 924, 819, 760, 725, 637, 605; **HRMS** Accurate mass (ES+): Found 470.0756 (+4.9 ppm), $C_{17}H_{19}F_3NO_9S$ ($M+H_+$) requires 470.0733.

(2S)-1-(3,4,5-trimethoxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylic acid 38. Following general procedure F, triflate **37** (110 mg, 0.234 mmol) yielded the desired olefin ester as a yellow oil, which was carried directly into the next step. Using general procedure B, the ester yielded acid **38** as a yellow oil (50 mg, 67% yield, 2 steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.83 – 6.76 (m, 2H), 6.55 (s, 1H), 5.28 (d, J = 11.1 Hz, 1H), 5.06 (d, J = 6.1 Hz, 1H), 3.92 – 3.83 (m, 12H), 3.14 – 3.00 (m, 2H); **^{13}C NMR (**100 MHz, $CDCl_3$) δ 173.5, 167.9, 153.3, 140.4, 132.3, 132.2, 130.3, 129.4, 128.8, 128.6, 111.0, 105.5, 68.0, 61.0, 59.2, 56.4; **[α]25D** –104.3 (c = 0.29 in $CHCl_3$); **IR** (film) 3269, 2954, 2899, 1747 (C=O), 1631 (C=O), 1605, 1467, 1425, 1363, 1311, 1208, 1136, 1028, 912, 833, 755, 693, 665, 605; **HRMS** Accurate mass (ES+): Found 308.1148 (+4.5 ppm), $C_{15}H_{18}NO_6$ (M+H+) requires 308.1134.

(1R,7R)-1-[(tert-butyldimethylsilyl)oxy]-1-carbamoyltridecan-7-yl (2S)-1-(3,4,5-trimethoxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate 39. Following general procedure G, acid **38** (26 mg, 0.085 mmol) yielded **39** as a yellow oil (21 mg, 53% yield). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.78 (s, 2H), 6.58 (s, 1H), 6.53 (d, J = 4.1 Hz, 1H), 5.47 (d, J = 4.1 Hz, 1H), 5.12 (s, 1H), 5.01 – 4.91 (m, 2H), 4.13 (t, J = 5.1 Hz, 1H), 3.87 (s, 9H), 3.16 – 3.06 (m, 1H), 2.68 (d, J = 16.5 Hz, 1H), 1.80 – 1.71 (m, 1H), 1.68 – 1.46 (m, 10H), 1.39 – 1.16 (m, 20H), 0.91 (s, J = 6.5 Hz, 9H), 0.86 (t, J = 7.0 Hz, 3H), 0.08 (d, J = 5.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.0, 170.8, 166.7, 153.3, 140.0, 131.1, 130.5, 129.1,

109.0, 105.2, 75.7, 73.5, 61.0, 58.8, 56.4, 35.1, 34.0, 31.8, 29.5, 29.3, 25.8, 25.3, 25.0, 24.0, 22.7, 18.1, 14.2, -4.7, -5.2; **[α]25D** –34.7 (c = 0.86 in $CHCl_3$); **IR** (film) 3480, 2927, 2856, 1738 (C=O), 1687 (C=O), 1645 (C=O), 1616, 1582, 1506, 1456, 1414, 1358, 1236, 1192, 1126, 1004, 951, 836, 810, 778, 720, 671; **HRMS** Accurate mass (ES+): Found 663.4066 (+3.8 ppm), $C_{35}H_{59}N_2O_8Si$ (M+H+) requires 663.4041.

Compounds prepared as described previously by Steele, et al.[30]

(4R)-dec-1-en-4-ol (R-19). Prepared as previously described (see Hanawa, et al., *J. Am. Chem. Soc.* **2003**, *125*, 1708) $TiCl_4$ (1 molar solution in CH_2Cl_2, 0.65 mL, 0.65 mmol), $Ti(OiPr)_4$ (0.36 mL, 1.17 mmol), Ag_2O (300 mg, 1.30 mmol), (S)-(–)-1,1'-bi(2-naphthol) (S-BINOL, 744 mg, 2.60 mmol), heptanal (1.83 mL, 13.0 mmol), and allyltributylstannane (5.23 mL, 16.9 mmol) yielded **R-19** as a yellow oil (1.165 g, 57%).

(4R)-4-benzyl-3-[(2R,8R)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradec-5-enoyl]-1,3-oxazolidin-2-one (R,R-26). A solution of **R-18** (484 mg, 1.20 mmol), and **R-19** (845 mg, 5.4 mmol) in CH_2Cl_2 (5 mL) was degassed with argon for at least 30 minutes. To the flask was added catalyst **C711** (Materia, CAS [635679-24- 2]) (85 mg, 0.12 mmol), degassed for another 5 minutes, then stirred under argon for 48 hours. The solution was immediately loaded onto a column of silica gel and subjected to chromatography, yielding **R,R-26** as a brown oil (576 mg, 77%).

(4R)-4-benzyl-3-[(2R,8R)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradecanoyl]-1,3-oxazolidin-2-one (R,R-28). To a solution of compound **R,R-26** (491 mg, 0.922 mmol) in ethyl acetate (10 mL) was added 5% Pd/C (100 mg), and stirred under a hydrogen atmosphere for 16 hours. The reaction was filtered through celite and concentrated, yielding **R,R-28** as a clear oil (502 mg, quant.).

(2R,8R)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradecanamide (R,R-20). To a sealed flask containing compound **R,R-28** (1.18 g, 2.21 mmol) dissolved in THF (30 mL) was added concentrated ammonium hydroxide solution (21 mL). The biphasic mixture was vigorously stirred for 2 days, then carefully vented, concentrated, and co-evaporated with methanol 3 times to remove residual water. After addition of hexanes, the solution was cooled in a freezer and then filtered to remove precipitated oxazolidinone. This process was repeated until no white solids precipitated. Concentration of the filtrate and column chromatography of the resultant residue (0→30% ether/ CH_2Cl_2→5% methanol/30% ether/65% CH_2Cl_2) yielded **R,R-20** as a faintly yellow oil (735 mg, 89% yield).

(4S)-4-benzyl-3-[(2S)-2-hydroxyhex-5-enoyl]-1,3-oxazolidin-2-one (S-40). Sodium bis(trimethylsilyl)amide **(**NaHMDS 2.14 mL, 1 M soln. in THF, 2.14 mmol) was diluted with THF (15 mL), and cooled to -78°C. **Epi-17** (487 mg, 1.78 mmol) was dissolved in THF (4 mL) and added dropwise at 0.2 mL/ min via syringe pump to the NaHMDS solution. The resulting solution was stirred for an hour at -78°C. Davis oxaziridine (650 mg, 2.49 mmol) was likewise dissolved in THF (4 mL) and added via syringe pump to the reaction at 0.2 mL/min. The reaction was stirred for another hour at -78°C. A solution of (±)-camphorsulfonic acid (2.0 g, 8.9 mmol) in THF (15 mL) was added, and the reaction was warmed to room temperature. Water was added, and the solution was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by column chromatography (0→10% ethyl acetate/hexanes) to yield **S-40** as a yellow oil (456 mg, 88%).

(4S)-4-benzyl-3-[(2S)-2-[(tert-butyldimethylsilyl)oxy]hex-5-enoyl]-1,3-oxazolidin-2-one (S-18). To a solution of **S-40** (456 mg, 1.58 mmol) in DMF (15 mL) cooled to 0°C

was added tert-butyldimethylsilyl chloride (360 mg, 2.36 mmol) and imidazole (146 mg, 2.05 mmol). The solution was stirred and warmed to room temperature overnight. The following day, the reaction was poured into water (15 mL) and extracted four times with a mixture of 1:1 ethyl acetate/hexanes (15 mL each). The combined organic layers were washed with water and brine, dried over Na_2SO4, filtered, concentrated, and purified by column chromatography to yield **S-18** as a clear oil (179 mg, 28%).

(4S)-4-benzyl-3-[(2S,8S)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradec-5-enoyl]-1,3-oxazolidin-2-one (S,S-26). Following a similar procedure as **R,R-26**; compound **S-18** (109 mg, 0.252 mmol), **S,S-27** (uncharacterized dimer, 152 mg, .76 mmol) in CH_2Cl_2 (10 mL) with catalyst Hoveyda-Grubbs II (8 mg, .012 mmol), yielded **S,S-26** (91 mg, 77%) as a brown oil.

(4S)-4-benzyl-3-[(2S,8S)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradecanoyl]-1,3-oxazolidin-2-one (S,S-28). Following the same procedure as **R,R-28: S,S-26** (92 mg, 0.172 mmol) in ethyl acetate (10 mL) treated with 5% Pd/C (100 mg) under an atmosphere of hydrogen yielded **S,R-28** as a clear oil (92 mg, quant.).

(2S,8S)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradecanamide (S,S-20). Following the same procedure as **R,R-20**; compound **S,S-28** (92 mg, 0.172 mmol), THF (3 mL), and NH_4OH (2 mL) yielded **S,S-20** as a clear oil (34 mg , 50% yield).

(4S)-4-benzyl-3-[(2S,8S)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradec-5-enoyl]-1,3-oxazolidin-2-one (S,R-26). Following the same procedure as **R,R-26**; compound **S,R-18 (**99 mg, 0.244 mmol), **S,R-27** (Uncharacterized dimer, 166 mg, .590 mmol) in CH_2Cl_2 (10 mL) with Hoveyda-Grubbs II (8 mg, .012 mmol), yielded **S,R-26,** which was carried directly into the next step.

(4S)-4-benzyl-3-[(2S,8R)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradecanoyl]-1,3-oxazolidin-2-one (S,R-28). Following the same procedure as **R,R-28: S,R-26** in ethyl acetate (10 mL) treated with 5% Pd/C (100 mg) under an atmosphere of hydrogen yielded **S,R-28** as a clear oil (85 mg, 65% over 2 steps).

(2S,8R)-2-[(tert-butyldimethylsilyl)oxy]-8-hydroxytetradecanamide (S,R-20). Following the same procedure as **R,R-20**: compound **S,R-28** (85 mg, 0.158 mmol), THF (3 mL), and NH_4OH (3 mL) yielded **S,R-20** as a clear oil (39 mg, 66% yield).

(1S,7S)-1-[(tert-butyldimethylsilyl)oxy]-1-carbamoyltridecan-7-yl(2S)-1-(2-{[2-(trimethylsilyl)ethoxy]methoxy}benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate (S,S-41). To a solution of **23** (53.0 mg, 0.145 mmol) in CH_2Cl_2 (4 mL) cooled to 0°C was added EDC (35 mg, 0.18 mmol), followed by DMAP (5.0 mg, 0.045 mmol); a solution of **S,S-28** (34 mg, 0.090 mmol) dissolved in CH_2Cl_2 (2 mL) was then added dropwise. The solution was stirred and warmed to room temperature overnight. The reaction was then poured into water and extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography, yielding **S,S-41** as a yellow oil (45 mg, 69%).

(1S,7S)-1-carbamoyl-1-hydroxytridecan-7-yl(2S)-1-(2-hydroxybenzoyl)-2,3-dihydro-1H-pyrrole-2- carboxylate (S,S-24). To a solution of compound **S,S-41** (45 mg, 0.062 mmol) dissolved in 1,3-dimethyl-1,3-diazinan-2-one (DMPU, 1.2 mL) was added tetrabutylammonium fluoride (TBAF, 1.2 mL 1M solution in THF, 1.2 mmol, 20 equiv.) dropwise. Both DMPU and TBAF were dried for at least 24 hours over freshly activated 3Å molecular sieves. The reaction was stirred at room temperature until LC-MS analysis indicated completion, typically after 30 to 60 minutes. At that time, the reaction was diluted

with saturated NH_4Cl solution (2 mL) and water (2 mL) and extracted with ether (10 mL). The organic layer was then washed 5 times with 1 M NH_4Cl solution (5 mL total), water, and brine. The organic layer was then dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (0→3% methanol/CH_2Cl_2) and high-performance liquid chromatography (40% to 95% acetonitrile/water) to yield **S,S-41** as a clear oil (4.1 mg, 14% yield after extensive purification).

(1S,7R)-1-[(tert-butyldimethylsilyl)oxy]-1-carbamoyltridecan-7-yl(2S)-1-(2-{[2-(trimethylsilyl)ethoxy]methoxy}benzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate (S,R-41). Following the same procedure as **S,S-41**; **23** (60 mg, 0.166 mmol) in CH_2Cl_2 (5 mL), EDC (40 mg, 0.208 mmol), DMAP (6 mg, 0.05 mmol), and **S,R-28** (38.8 mg, 0.104 mmol) yielded **S,R-41** as a yellow oil (75.5 mg, 73%).

(1S,7R)-1-carbamoyl-1-hydroxytridecan-7-yl(2S)-1-(2-hydroxybenzoyl)-2,3-dihydro-1H-pyrrole-2-carboxylate (S,R-24). Following the same procedure as **S,S-24: S,R-41** (28 mg, 0.040 mmol), DMPU (0.8 mL), and TBAF (0.8 mL, 1M solution in THF, 0.80 mmol) yielded **S,R-24** as a white translucent oil (6 mg, 32% yield after extensive purification).