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Fixing the Unfixable: The Art of Optimizing Natural Products for Human Medicine

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Abstract

Molecules isolated from natural sources including bacteria, fungi, and plants are a long-standing source of therapeutics that continue to add to our medicinal arsenal today. Despite their potency and prominence in the clinic, complex natural products often exhibit a number of liabilities that hinder their development as therapeutics, which may be partially responsible for the current trend away from natural product discovery, research, and development. However, advances in synthetic biology and organic synthesis have inspired a new generation of natural product chemists to tackle powerful undeveloped scaffolds. In this Perspective, we will present case studies demonstrating the historical and current focus on making targeted, but significant, changes to natural product scaffolds via biosynthetic gene cluster manipulation, total synthesis, semisynthesis, or a combination of these methods, with a focus on increasing activity, decreasing toxicity, or improving chemical and pharmacological properties.

Graphical Abstract

INTRODUCTION

The complex molecular architecture and high functional density of natural products are responsible for their selective and potent interactions with a wide variety of clinically relevant biomolecular targets. These properties contribute to the large proportion of natural-product-based pharmaceuticals in the clinic.¹ However, as isolated from their source organisms, natural products sometimes possess suboptimal pharmacological properties including high toxicity, poor pharmacokinetics, rapid metabolism, and low solubility or chemical stability. Natural products have acquired a reputation for being intransigent to the

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contemporary optimization workflows favored by medicinal chemists due to the perception of technical challenges involved in generating targeted changes in complex natural scaffolds. Additionally, questions of supply may raise concerns for the development of natural products and their analogs for clinical applications. In this Perspective, we endeavor to counter this prevailing wisdom by providing support for our opinion that technological advances in synthetic biology and in the chemical synthesis of natural products render these concerns obsolete. Decades of research focused on natural product biosynthesis and synthetic biology, coupled with the great strides taken in the field of organic synthesis, provide powerful tools that demonstrate the ability to salvage natural product scaffolds of high complexity. We will provide case studies demonstrating how several natural product scaffolds have been rescued via biosynthetic manipulation, total synthesis, semisynthesis, or a combination of these methods. We will discuss the development of pactamycin derivatives harnessing the power of the biosynthetic machinery to access analogs with differential activity. The polyene macrolides will be presented as an example of semisynthetic and biosynthetic manipulations applied to decrease the mammalian toxicity of a powerful natural product scaffold. The long history of semisynthetic manipulations of the tetracycline core will also be reviewed, with an eye toward highlighting completely synthetic analogs currently in development. Geldanamycin will be considered as an example of an anticancer therapeutic developed via semisynthetic manipulation and biosynthetic engineering to bypass toxicity and low solubility. Finally, the thiopeptides will act as an example of the advanced biosynthetic and selective semisynthetic manipulations that can be employed to access potent antimicrobials with increased solubility. These examples of a rapidly growing number of success stories highlight the potential for a renaissance in adapting the largely untapped pharmacopeia of natural products currently not optimized for application in human medicine. While the liabilities of many natural products may have appeared insurmountable in the past, our current capacity to precisely manipulate genomes encoding biosynthetic pathways and efficiently synthesize core scaffolds should motivate medicinal chemists to re-embrace natural products. Our ability to adapt natural products' molecular complexity to improve selectivity, potency, and pharmacological properties is now only limited by our ability to see past initial structural liabilities and embrace the now conjoined arts of natural product synthesis and synthetic biology.

CASE STUDIES IN IMPROVING NATURAL PRODUCTS

Pactamycin.

The aminocyclopentitol pactamycin is a clear example of the intricate and complex molecular diversity available from the natural product catalog. Pactamycin's highly substituted five-membered aminocyclitol core is elaborated with 6-methylsalicylic acid (6-MSA), 3-aminoacetophenone, and a 1,1-dimethylurea.² This complex structure directly traces to pactamycin's potent activity across all three domains as a universal protein synthesis inhibitor.^{3,4} Crystal structures of pactamycin bound to the 16S rRNA region of the ribosome illustrate that the two aromatic rings lie in the E-site of the 30S ribosome, acting as mRNA mimics to prevent initiation of protein biosynthesis.^{5,6} The significant structural similarity between kingdoms at the pactamycin ribosomal binding site likely accounts for its broad and potent activity across Gram-positive and negative bacteria, as well as its antitumor

activity. Unfortunately, pactamycin's broad activity is a double-edged sword, as it is also highly cytotoxic to healthy mammalian cells.⁷ Therefore, development of pactamycin as a therapeutic hinges on the ability to access less toxic and/or more selective analogs. The complex architecture, dense functionalization, and chemical instability of pactamycin make derivatization via semisynthesis particularly challenging. However, the ability to genetically manipulate the biosynthetic machinery of the producing organism, *Streptomyces pactum*, has provided a rich source of analogs with differentiated activity profiles.

Early studies utilizing isotope labeled substrates identified the key pactamycin biosynthetic precursors for the aminocyclopentitol core, the 3-aminoacetophenone ring, and the 6-MSA ring.⁸ In particular, 3-aminobenzoic acid (3-ABA) was identified as a key intermediate in 3-aminoacetophenone biosynthesis from the shikimate pathway.⁹ This discovery led to chemical bypass studies of non-natural fluorinated 3-ABA derivatives to yield fluorinated pactamycin analogs, which did not demonstrate decreased cytotoxicity or differentiated activity.¹⁰ However, these early experiments revealed the possibility of utilizing the biosynthetic machinery to access pactamycin analogs. Advances in genome editing methodology allowed the Mahmud group to develop a robust system for gene inactivation in the pactamycin producer *S. pactum*. Using this system, Mahmud and co-workers were able to sequentially delete key biosynthetic genes to elucidate the pactamycin biosynthetic pathway and generate a number of analogs with differentiated activity (Figure 1).¹¹ The deletion of the gene encoding the iterative polyketide synthase (iPKS) PtmQ, encoding 6-MSA biosynthesis, revealed that this moiety may not be necessary for pactamycin bioactivity. Through further gene deletions, the Mahmud group discovered that replacement of the hydroxyethyl group at the C-1 position of the aminocyclitol core with a methyl group resulted in pactamycin analogs with significantly different activity across domains. For example, pactamycin analogs TM-025/026 completely lacked antibacterial activity but retained eukaryotic activity against the parasite *Plasmodium falciparum*.¹² These analogs also showed divergent activity across kingdoms with significantly decreased cytotoxicity against mammalian cells. In order to further derivatize the already enhanced analogs TM-025/026, the Mahmud group deleted the gene encoding for the pyridoxal phosphate-dependent enzyme PtmT, which is responsible for the conversion of 3-dehydroshikimate to 3-ABA.¹³ Thus, fluorinated 3-ABA derivatives were able to chemically bypass the 3-ABA deletion strains. The fluorinated analogs showed similar antiplasmodial activity as their nonfluorinated equivalents.¹⁴ Additional deletion of genes encoding late-stage tailoring enzymes in the pactamycin biosynthetic gene cluster elucidated the key biosynthetic steps, as well provided access to additional pactamycin analogs. Double deletion strains lacking the SAM-dependent *N*-methyltransferase encoded by *ptmD* with previously investigated genes produced key analogs TM-101/102. Even though the des-methyl analogs were less active against *P. falciparum*, they were also less cytotoxic, demonstrating a widening therapeutic window for these typically highly toxic compounds.¹⁵ Current progress in the development of pactamycin derivatives with decreased cytotoxicity has focused on taking advantage of the extremely flexible β -ketoacyl-ACP synthase III-like protein PtmR that installs the 6-MSA moiety at the C-9 position. As previous work has demonstrated that the 6-MSA moiety is not required for bioactivity, it is possible to envision pactamycin analogs functionalized with diverse acyl moieties to modulate activity.¹⁶ Interestingly, jogyamycin is

a natural product analog of pactamycin that lacks the 6-MSA and the hydroxylethyl group at C-1.¹⁷ This compound shows decreased antiprotozoal activity with increased cytotoxicity, indicating that while the 6-MSA may not be necessary for bioactivity, it may affect selectivity, thus providing a handle for designing pactamycin analogs. In addition to the analogs accessed via biosynthesis, the total synthesis of pactamycin was accomplished by Hanessian and co-workers in 2011.¹⁸ More recently, the Hanessian and Johnson groups have synthesized analogs of this complex class of molecules to gain a clearer understanding of structure–activity relationships (SAR).^{19,20} While many of the synthetic analogs have not demonstrated increased or differentiated activity to this point, the ability to efficiently access such analogs synthetically greatly increases the pharmaceutical potential for this potent scaffold.

Polyene Macrolides.

The need for less toxic natural product analogs is especially evident in the field of antifungals. The physiological similarity between mammalian and fungal cells makes developing selective antifungals a difficult needle to thread.²¹ Since their discovery in the early 1950s, the polyene macrolides, including nystatin and the amphotericins, have been used as antifungals both topically and systemically. In particular, amphotericin B (AmpB) has broad activity against a number of pathogenic fungi such as *Candida albicans*, *Candida neoformans*, and *Blastomyces dermatitidis*.²² Unfortunately, AmpB has significant nephrotoxicity coupled with poor solubility, relegating it to a drug of last-resort for systemic fungal infections.^{23,24} Originally, AmpB was believed to kill fungi via assembly with sterols in the lipid membrane to create channels leading to ion and solute leakage and ultimately to cell death.²⁵ More recent work from the Burke laboratory suggests another potential mechanism where AmpB acts as a sterol sponge to extract ergosterol from fungal lipid membranes.²⁶ Due to its mammalian toxicity and chemical lability to light, oxygen, and acid, generating AmpB or other polyene macrolide analogs with improved properties has been a long-standing goal in the natural product field.

Due to their molecular complexity, the total synthesis of polyene macrolides has been difficult, with several groups developing methods to access these potent compounds synthetically.^{27,28} However, the ability to access analogs from bacterial sources via genetic manipulation coupled with elegant semisynthetic derivatization has provided polyene macrolide analogs with increased activity and decreased toxicity. Polyene macrolide derivatization has focused on three main components of the core structure: the exocyclic carbonyl at C-16, the polyol region, and glycosyl variants at the C-19 position (Figure 2). Removal of the exocyclic carboxylic acid at the C-16 position via genetic deletion of the gene encoding a P450 monooxygenase in both the AmpB and nystatin biosynthetic clusters provided the C-16 methylated analogs.^{29,30} The Burke group accessed this same AmpB methylated analog (MeAmB) via targeted semisynthetic methods.³¹ In all cases, the loss of the carboxylic acid did not decrease antifungal activity and instead resulted in decreased mammalian hemolysis (Figure 2A). Leveraging chemical synthesis, the Burke group generated a number of analogs at the C-16 position via a Curtius rearrangement to yield an oxazolidinone intermediate, which could then be opened by a variety of primary amines. In particular, the urea analogs showed increased water solubility and in vivo activity.³² Zotchev

and co-workers were able to investigate similar C-16 nystatin analogs by coupling biosynthetic manipulations with semisynthesis. By deleting the gene encoding the enoylreductase domain of the NysC polyketide synthase (PKS) in nystatin producer *Streptomyces noursei*, the Zotchev group was able to access a heptaenic version of nystatin, termed S44HP, with improved antifungal activity.³³ The S44HP metabolite was further derivatized to access a large number of C-16 nystatin analogs (S44HPSZ6,7,14) that showed similar antifungal activity to AmpB but with reduced toxicity.³⁴ The polyol region of the polyene macrolides has also proven to be a convenient area for derivatization via biosynthetic manipulations. The oxidation pattern between C-7 and C-10 is one of the primary differences between AmpB and nystatin. Editing portions of the genes encoding individual ketoreductase or dehydratase domains within the PKS modules, as well as post-PKS tailoring enzymes, has afforded access to analogs with diverse oxidation patterns in this region. A number of these analogs, such as 7-oxo-AmpB, retained antifungal activity with decreased hemolytic activity (Figure 2B).^{30,35,36} The production of these derivatives through genetic manipulation, in light of the evident challenges that their synthetic production would entail, demonstrates the power of this method. A final portion of the polyene macrolide structure under study is the mycosamine sugar at the C-19 position. Deletion of this moiety, either synthetically or biosynthetically, results in a complete loss of antifungal activity.^{31,37} However, there is significant room for mycosamine derivatization despite its importance in polyene macrolide activity (Figure 2C). Burke and co-workers were able to achieve site-selective acylation of the C-2' position of mycosamine via electronic tuning of acylation reagents.³⁸ This site-selective functionalization allowed for a clearer understanding of how the C-2' hydroxyl group modulates sterol binding and mammalian toxicity.³⁹ More complex semisynthetic transformations, such as double reductive alkylation of the mycosamine moiety, were also found to be well tolerated.^{40,41} Synthetic biology approaches have also been successful in providing access to mycosamine derivatives geared toward increasing selectivity. The Thorson group has investigated the AmpB and nystatin glycosyltransferases (GTs) for their potential to introduce glycosyl variants chemoenzymatically.⁴² The in vivo bioengineering of polyene GTs also provided AmpB derivatives with perosamine and 6-deoxyhexose in place of mycosamine via a hybrid GT.⁴³ Interestingly, nature has revealed its own ability to produce analogs at this position, as the identification of nystatin-like *Pseudonocardia* polyene (NPP) illustrates. The Kim group identified NPP using targeted genome screening for polyene cytochrome P450 hydroxylases to identify novel polyene natural products.⁴⁴ Produced by the rare actinomycete *Pseudonocardia autotrophica*, NPP bears the disaccharide mycosamine (α 1-4)-*N*-acetyl-glucosamine at C-19, which results in greatly increased water solubility and decreased hemolytic activity.⁴⁵ The identification of new analogs within known natural product classes via genome mining also has the potential to revitalize underdeveloped scaffolds as these previously unknown analogs may exhibit improved activity or pharmacological properties. The polyene macrolides remain a therapeutic of last resort for serious fungal infections despite their toxicity and poor solubility. Biosynthetic and semisynthetic techniques, however, have generated polyene macrolides with improved activity profiles. The development of AmpB and nystatin derivatives with decreased toxicity and retained antifungal properties is quite promising for the continued development of these natural products.

Tetracyclines.

The tetracycline polyketides are a class of potent natural products that have provided broad-spectrum antibacterial therapeutics for decades. Along with the eponymous tetracycline, there are three related natural products that differ only on the north side of the scaffold; these analogs initially provided a rich source of antibiotics (Figure 3A).^{46,47} However, all natural tetracycline analogs are oxidized to introduce a hydroxyl at C-6, which is known to undergo acid-catalyzed dehydration to yield anhydrotetracycline analogs with reported nephrotoxicity.⁴⁸ Due to this structural liability, semisynthetic analogs of the tetracycline core have been of significant interest in the field. Early work led to the discovery that the tetracyclines undergo acidic hydrogenolysis to yield 6-deoxy analogs that are resistant to dehydration while maintaining antibacterial activity.⁴⁹ This conservative change resulted in the development of the terramycin derivative, doxycycline, which remains one of the most prescribed generic antibiotics in use (Figure 3B). This transformation also increases the reactivity of the C-7 and C-9 positions of the 6-deoxytetracycline core. Nitration of the C-7 position allowed for reductive methylation to yield the alkylated aminotetracycline analog, minocycline, with increased activity against tetracycline sensitive and resistant bacterial strains.⁵⁰

While these second generation semisynthetic analogs expanded the range of potential applications for the tetracyclines in the clinic, their widespread prescription has also contributed to growing tetracycline resistance. The tetracyclines inhibit protein translation by binding to the 30S ribosomal subunit to block aminoacylated tRNA from binding the ribosomal A-site.⁵ Resistance to the tetracyclines is acquired via *tet* genes that encode for efflux pumps, ribosomal protection proteins, or tetracycline destructases.^{51,52} Increasing acquisition of *tet* genes prompted new investigations into tetracycline derivatization, particularly at the C-9 position. The American Cyanamid Company led the way with expanded investigations into the potent glycylcyclines by developing an efficient route of nitration, reduction, and acylation of minocycline at the C-9 position to provide analogs with increased antibacterial activity against tetracycline resistant strains.^{53,54} A 9-*tert*-butylglycylamidominocycline derivative bypassed major tetracycline resistance mechanisms in vivo and in vitro, resulting in its approval for use in the clinic as tigecycline (Figure 3C).⁵⁵ Due to an FDA black-box warning, tigecycline is now an antibiotic of last resort used to combat only the most serious of bacterial infections.⁵⁶ Recently, omadacycline, a novel minocycline derivative, was approved by the FDA for treatment of acute skin infections and bacterial pneumonia.⁵⁷ Developed at Paratek Pharmaceuticals, omadacycline is a C9-aminomethylcycline with potent activity against tetracycline resistant strains and improved oral bioavailability compared to tigecycline.⁵⁸ Another novel aminomethylcycline, sarecycline, was also recently approved as a targeted spectrum tetracycline for treatment of acne vulgaris. Sarecycline shows decreased activity against Gram-negative bacilli, which are associated with healthy intestinal flora.⁵⁹ The development of novel semisynthetic tetracycline analogs demonstrates the continued importance of this natural product scaffold in the clinic.

Despite the decades of semisynthetic derivatization, a scalable and efficient fully synthetic route to the tetracyclines has lagged due to their molecular complexity. From Woodward's

first racemic synthesis of sancycline in 1968 to the first enantioselective synthesis of (–)-tetracycline from the Takahashi group in 2000, total synthesis of the tetracyclines has been stymied by lengthy synthetic sequences and low overall yields.^{46,60,61} However, this trend was disrupted in 2005 with work from the Myers laboratory. Their approach focused on a tricyclic AB enone precursor that allowed access to enantiopure tetracycline in a total of 17 steps with 1.1% overall yield.⁶² Importantly, this route also allowed for the facile synthesis of a number of tetracycline derivatives through a late-stage addition of the D-ring (Figure 3D). A number of these novel tetracycline derivatives showed increased antimicrobial activity against a broad bacterial panel.⁶³ Along with the development of a more efficient route to the AB enone, the Myers group was able to access approximately 50 tetracycline analogs, including pentacyclines, with excellent antibacterial properties.⁶⁴ This work eventually led to the founding of Tetraphase, which continues to develop tetracycline antibiotics. In particular, Tetraphase has focused on the glycylicycline-related fluorocycline analogs, which demonstrate potent antibacterial activity.⁶⁵ One such analog, eravacycline, was recently approved for treatment of complicated intra-abdominal infection and urinary tract infections.^{66,67} The successful derivatization of the tetracyclines should serve as a powerful reminder of the potential for semisynthesis and total synthesis to complement one another in the development of natural products for pharmaceutical applications. The past and continued successes of semisynthetic techniques to develop improved tetracycline analogs informed the total synthesis endeavors by identifying vital points of derivatization. Neither of these techniques must stand alone as we continue to search for new therapeutics from the natural product arsenal.

Geldanamycin.

Natural products with anticancer activity often stall in development due to toxicity to noncancerous cells. A prime example of such a natural product is the macrocyclic lactam geldanamycin. Since its discovery from *Streptomyces hygroscopicus* extracts in 1970, its potency as an extremely active anticancer agent has made geldanamycin a compound of intense study.⁶⁸ Despite this, hepatotoxicity and low stability and bioavailability have made natural geldanamycin a poor choice as a therapeutic.⁶⁹ Geldanamycin's antitumor activity, however, spurred countless investigations into its mechanism of action, eventually leading to identification of its target. Geldanamycin prevents binding of ATP to the N-terminus of heat shock protein 90 (Hsp90), which is a prevalent molecular chaperone.^{70,71} Cancer cells are especially sensitive to geldanamycin due to increased binding to the Hsp90 heterodimers that are overexpressed in tumors.⁷² Therefore, the search for improved geldanamycin analogs lacking hepatotoxicity but retaining activity became an area of intense focus.

Multiple groups have investigated total synthetic routes not only to geldanamycin but also to related ansamycin scaffolds.⁷³ The first total synthesis of an ansamycin natural product, herbimycin A, was completed by the Tatsuta group.⁷⁴ Subsequently, Andrus and co-workers completed the first total synthesis of geldanamycin with their work demonstrating the difficulty in accessing the final C-17 methoxy benzoquinone moiety.^{75,76} A number of different protecting groups were investigated by Andrus, as well as the Panek group, to reliably achieve the final deprotection to yield the desired *p*-benzoquinone.^{77,78} Panek and co-workers were able to extend their synthetic route to access other ansamycin natural

product, with a particular focus on non-benzoquinone analogs such as reblastatin and autolytimycin.⁷⁹ Despite these robust efforts, a facile method to access geldanamycin analogs has thus far eluded the field, with only a handful of synthetic analogs reported.⁸⁰

Semisynthetic methods, however, have demonstrated significant utility for the derivatization of geldanamycin. The α,β -unsaturated quinone was immediately recognized as a redox liability of the core structure but also as a potentially powerful site of reactivity. A large number of analogs were readily accessed at the C-17 position by displacing the methoxy group to form 17-amino-17-demethoxygeldanamycin analogs. Derivatization at this position provided analogs with increased in vitro activity and decreased overall toxicity, especially the 17-allylamino derivative (17-AAG).⁸¹ Subsequent work focused on increasing solubility and bioavailability, with the 17-(2-dimethylaminoethyl)amino derivative (17-DMAG) showing potent activity and increased solubility (Figure 4B).⁸² Overall, the C-17 position appears to be particularly open to derivatization with a large panel of moieties being well-tolerated.⁸³ Other areas of the geldanamycin scaffold proved less amenable to modification, such as the C-7 carbamate of the ansa-ring, which was determined to be crucial for Hsp-90 binding following screening of various substituents.⁸⁴ The geldanamycin derivatives 17-AAG and 17-DMAG were eventually brought to clinical trials as tanespimycin and alvespimycin, respectively, but neither was advanced for use in the clinic.⁸⁵

Contemporaneously, Kosan Biosciences developed genomic tools to study and manipulate the biosynthetic pathway of geldanamycin in the producer *S. hygroscopicus*. Analogues were accessed by substituting heterologous acyltransferase (AT) domains within the geldanamycin PKS via gene editing. This work yielded a number of geldanamycin analogs with derivatized ansa chains (C-1 to C-16) and contributed to the understanding of the vital interactions between AT domains within PKS proteins (Figure 4C). Most importantly perhaps, these compounds illustrated that the hydroquinone form of geldanamycin was as active as the quinone form, thus providing a new avenue for derivatization.^{86,87} The Kirschning group focused on accessing related geldanamycin analogs via feeding precursors to a 3-amino-5-hydroxybenzoic acid (AHBA) blocked mutant of the producer strain (Figure 4D). Removal of AHBA production via mutagenesis allowed for supplementation of diverse aminobenzoic acid starting units for geldanamycin incorporation, as AHBA serves as the starter unit for the type I PKS amide synthase in geldanamycin biosynthesis.⁸⁸ This work facilitated access to a range of geldanamycin analogs showing differential activity across a number of cancer cell lines and providing a more nuanced understanding of the hydroquinone's SAR in Hsp90 binding.^{89,90} Previous semisynthetic manipulations of the geldanamycin scaffold eventually resulted in the advancement of a number of clinical candidates. Despite not advancing to the clinic, the SAR data gathered from previous efforts continue to inform the current work toward biosynthetically generated analogs focused on leveraging the Hsp90 target in anticancer treatment.

Thiopeptides.

The thiopeptides are a class of complex ribosomally synthesized and post-translationally modified peptides (RiPPs).^{91,92} These compounds all contain a polyazole cluster with a central nitrogen heterocycle, the oxidation state of which provides the structural

classification system.⁹³ Thiopeptides are sulfur-rich and contain heavily modified side chains off the peptide core. Despite their ubiquity and high potency against Gram-positive pathogenic strains, no thiopeptide is currently approved for use in the clinic. This is likely due to their extremely poor solubility and bioavailability. However, the thiopeptides are known to inhibit bacterial protein synthesis through novel binding sites at the ribosome. Certain thiopeptide classes bind the 23S rRNA at the L11 binding domain of the 50S ribosomal subunit to abrogate tRNA translocation.^{94,95} Conversely, other classes of thiopeptides have been shown to bind the bacterial elongation factor Tu, preventing the initiation of peptide synthesis.⁹⁶ There have been extensive investigations into the derivatization of thiopeptides with the goal of increasing activity and solubility in order to capitalize on their unique mechanism of action and potent activity.⁹⁷

Thiostrepton (Tsr), a series b thiopeptide, is the parent of this class of macrocycles. It was first isolated in 1954 from *Streptomyces azureus* and has subsequently been found to be produced by *Streptomyces hawaiiensis* and *Streptomyces laurentii*.⁹⁸ Similar to all thiopeptides, Tsr suffers from poor solubility, preventing its use in the clinic. Unfortunately, access to analogs via total synthesis is difficult with only one total synthesis published from Nicolaou and co-workers.⁹⁹ However, valuable Tsr analogs have been accessed via biosynthetic manipulations and targeted semisynthesis. The Kelly group developed a fosmid Tsr expression strain in the homologous producer *S. laurentii* to allow for engineering of the biosynthetic gene cluster.¹⁰⁰ Using this system, Kelly and co-workers replaced amino acids throughout the macrocyclic core in order to investigate the flexibility of the biosynthetic machinery as well as access novel Tsr analogs. Initial replacement of the threonine at the 7-position of the Tsr propeptide stunted the biosynthesis of the quinaldic acid (QA) macrocycle to provide analogs with significantly decreased antibacterial activity.¹⁰¹ However, subsequent amino acid substitutions of the alanines at the 4- and 2-position of the propeptide provided Tsr analogs with increased aqueous solubility as compared to the parent compound.^{102,103} Importantly, many of these analogs retain the potent antibacterial activity observed with Tsr. More thorough investigations into the QA macrocycle have also yielded valuable Tsr analogs. To this end, the Liu group performed genetic functional analysis to identify the five enzymes involved in converting L-tryptophan to QA in *S. laurentii*.¹⁰⁴ It was confirmed that production of Tsr could be restored to these mutant strains via chemical complementation with the quinaldic acid ketone precursor. Encouraged by these results, the Liu group investigated the incorporation of non-natural QA precursors through chemical complementation in mutated strains to access Tsr analogs. Their work focused on modulating the interactions between the QA portion of Tsr and the 23S rRNA of the 50S ribosome to generate pharmaceutically relevant Tsr analogs with increased antibacterial activity and solubility (Figure 5).¹⁰⁵ Further work combined these improvements with modifications to the C-terminus of Tsr via a double mutant strain lacking TsrB, a carboxylesterase responsible for C-terminal tailoring.¹⁰⁶ Therefore, Liu and co-workers were able to access Tsr analogs with differentiated QA moieties and methyl ester C-termini that demonstrated potency against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE).¹⁰⁷ Biosynthetic manipulations have provided access to a variety of Tsr analogs with increased activity and drug-like properties, which would have been difficult to achieve synthetically.

Due to the complexity and dense functionalization of the thiopeptides, targeted semisynthesis of the scaffold is difficult to achieve. Recently, however, the Miller group successfully utilized catalyst-controlled site- and stereoselective functionalization to access Tsr derivatives. A rhodium catalyzed conjugate arylation was optimized to selectively functionalize the subterminal dehydroalanine residue at the C-terminus in a fully deprotected Tsr substrate (Figure 5). Further, diastereoselectivity was also achieved by varying the proton source and base to provide Tsr derivatives with retained antibiotic activity.¹⁰⁸ Catalyst-controlled semisynthetic routes have also been successful for the derivatization of the *e* series thiopeptide nocathiacin. Nocathiacin is produced by *Nocardia* sp. and *Amycolatopsis* sp. MJ347–81F4 and is highly active against a number of drug-resistant Gram-positive bacteria.^{109,110} Bristol-Meyers Squibb dedicated considerable semisynthetic effort to increasing the solubility of nocathiacin in an attempt to capitalize on its activity. While nocathiacin is less complex than thiostrepton, it contains multiple functional groups that make selective late-stage functionalization challenging. Initial conjugations to the C-terminus dehydroalanine side-chain introduced a variety of amines and thiol Michael adducts, resulting in derivatives with increased water solubility.^{111,112} However, all of these nocathiacin analogs were produced as diastereomeric mixtures, thus increasing production costs. Therefore, a method for asymmetric hydrogenation of the dehydroalanine olefin was developed using a rhodium catalyst to achieve high diastereomeric excess.¹¹³ Subsequent derivatization of the C-terminus end provided compounds with greatly increased solubility and high antibacterial activity (Figure 5, gray portion).

Similar strategies have also been employed for the development of series *d* thiopeptides. Initial investigations into the thiocillin thiopeptides revealed the overall biosynthetic logic of these natural products.^{114,115} These discoveries aided in the biosynthetic manipulation of the producing strain, *Bacillus cereus* ATCC 14579, to allow for SAR studies to be performed on the thiocillins. The Walsh laboratory substituted alanine for all residues in the thiocillin prepeptide and substituted serine for all cysteine residues to evaluate scaffold incorporation and, when successful, the antibiotic activity of analogs.^{116,117} The inherent flexibility in these pathways has allowed for more recent work from Bower and co-workers focusing on the promiscuous enzymes involved in thiopeptide biosynthesis to access novel analogs.^{118,119} Conversely, a semisynthetic strategy was employed by Novartis for the development of related thiopeptide GE2270A. Modifications at the C-terminus allowed for the introduction of an N-alkylated urethane moiety, greatly increasing aqueous solubility with a retention of antimicrobial activity.^{120,121} One of these derivatives, LFF571, was advanced to clinical trials as an oral treatment for *Clostridium difficile*, where it showed non-inferiority to vancomycin.^{122,123} These examples show the power of the selective functionalization of complex natural products to access derivatives with improved pharmacological properties.

ADDITIONAL CASE STUDIES

The five case studies illustrated in this Perspective represent only a small portion of the many examples of therapeutics derived from natural products via synthesis, semisynthesis, and/or biosynthetic manipulation. An important example of continued development of natural products is the macrolides, which have provided a rich source of therapeutics since their discovery. Multiple generations of macrolide derivatives have now been accessed via

semisynthesis from the parent compound erythromycin.^{124,125} Additionally, investigations into the biosynthetic machinery deoxyerythronolide B synthase (DEBS) yielded the discovery of multimodular PKS systems responsible for the biosynthesis of many natural products and allowed for manipulation of these pathways to access macrolide analogs.^{126,127} Despite multiple generations of macrolides currently in the clinic, there is a continued need for analogs that can overcome widespread resistance. Novel screening techniques that use the bacterial ribosome to template in situ click chemistry may provide improved macrolide scaffolds with increased potency against resistant strains.^{128,129} In addition, semisynthetic techniques now stand to be complemented by total synthetic methods from the Myers laboratory using a scalable route to easily access macrolides of varying ring size and substitution patterns.^{130,131} Thus, the ability to access novel macrolides able to bypass growing resistance is likely on the horizon.

The glycopeptide antibiotics are another well-known example of successful semisynthetic derivatization of a natural product scaffold. Vancomycin and teicoplanin antibiotics are used in the clinic in their natural form. However, both of these scaffolds have been improved upon to introduce additional generations of glycopeptides into the clinic with increased potency and in vivo stability.¹³² Telavancin, a vancomycin derivative, shows enhanced potency and improved bioavailability (Figure 6).¹³³ Other second generation glycopeptides, such as dalbavancin and oritavancin, benefit from greatly extended half-lives to allow for less frequent dosing. Oritavancin is a derivative of chloroeremomycin with an alkyl chain on the vancosamine amino group and an aminosugar on the phenylserine hydroxyl. These changes resulted in extended drug half-life and retention of activity against vancomycin-resistant *Staphylococcus aureus* (VRSA) and enterococci (VRE).¹³⁴ Total synthesis of the glycopeptides has been advanced by a number of groups allowing access to more complex analogs and the development of powerful synthetic tools.¹³⁵ Boger and co-workers have used total synthesis to generate vancomycin derivatives specifically designed to act via multiple mechanisms of action. These analogs show increased potency against vancomycin-resistant strains and decreased susceptibility to resistance due to their multiple bacterial targets.¹³⁶ Additionally, site-selective alterations of multiple glycopeptide scaffolds via nonenzymatic catalysis have allowed for targeted changes in the pursuit of active analogs.^{137–139} Recently, further elucidation of key late-stage biosynthetic steps from the Seyedsayamdost group has allowed for in vitro biosynthesis of the aglycone core from the 7-mer precursor peptide. This chemoenzymatic approach may allow access to additional vancomycin analogs in the future.^{140,141}

The lipopeptides are a powerful class of natural products that have undergone a recent renaissance for clinical use. The cyclic lipopeptide daptomycin showed potent antibacterial activity in vitro and in vivo but was discontinued due to adverse side effects. However, later development by Cubist Pharmaceuticals was able to overcome these adverse outcomes by altering the dosing schedule to allow daptomycin to make it to the clinic.¹⁴² Additional members of the lipopeptide class, the arylomycins, were first isolated from *Streptomyces* sp. Tü 6075 in 2002 and demonstrated activity against Gram-positive organisms.¹⁴³ It was eventually discovered that the arylomycins act via inhibition of the essential bacterial type I signal peptidase (SPase). Subsequently, Romesberg and co-workers investigated which portions of the arylomycin scaffold are necessary for activity via the total synthesis of

arylomycin analogs.^{144–146} This work led to a scalable synthetic route focusing on C–H functionalization of the aarylomycin core to access analogs with increased potency that bypass resistance incurred by SPase mutations.¹⁴⁷ Genentech has also investigated the aarylomycins, specifically aiming to broaden their activity for Gram-negative organisms. Targeted modifications of the N-terminal lipopeptide tail, the C-terminal carboxylic acid, and the two phenols located on the macrocycle resulted in the compound G0775, which is significantly more potent than the parent compound, aarylomycin A-C₁₆, against Gram-negative ESKAPE pathogens.¹⁴⁸ Specifically, G0775 showed a 500-fold increase in potency against *E. coli* and a 32-fold increase against *A. baumannii* (Figure 6, gray portion).

The ability to access analogs of complex scaffolds via semisynthesis has also been applied to the mutilins, fungal diterpene natural products. The mutilins inhibit protein synthesis by binding to a unique site on the 50S ribosome, which overlaps both the A- and P-sites of the peptidyl transfer center (PTC).¹⁴⁹ The mutilins were not initially developed for human clinical use due to issues of instability, toxicity, and poor bioavailability.¹⁵⁰

Semisynthetically derived analogs of the C-14 side chain allowed for the diversification of this class and led to the development of tiamulin for veterinary use.¹⁵¹ Subsequently, a topical formulation of the semisynthetic retapamulin was developed at GlaxoSmithKline, also using the C-14 side chain as the primary area of derivatization.^{152,153} More recent work from Nabriva Therapeutics introduced a thioether at the C-14 side chain and led to the development of lefamulin (BC-3781), an orally available mutilin derivative currently in clinical trials for community-acquired bacterial pneumonia (Figure 6).^{154,155} While these semisynthetic derivatives have brought the mutilins into the clinic, the investigation of more diverse mutilin analogs requires a scalable synthetic route. Modular synthetic routes to the mutilins allow for increased scale with the potential to allow development of more complex derivatives.^{156,157} Additionally, the Herzon group has developed a route utilizing C–H activation to access analogs at the C-17 and C-20 positions while also allowing for further investigations into the activity of the Gram-negative 12-*epi*-pleuromutilins.¹⁵⁸ These efforts will likely allow access to more diverse mutilin analogs with potential for clinical use.

Natural products continue to be a rich source of antimicrobials in the clinic. However, natural product development is not restricted to antibiotics, as shown by the development of the antiplatelet therapeutic vorapaxar. Developed at Schering-Plough, vorapaxar (SCH 530348) is an analog of the tetracyclic piperidine alkaloid himbacine (Figure 6).¹⁵⁹ Initially investigated for its potent antagonist effects for muscarine receptors, research was stymied by a lack of reliable availability of the compound. Therefore, access to himbacine, and subsequent analogs, was reliant on total synthesis.^{160,161} Chackalamannil and co-workers used their scalable synthetic route to develop substituted pyridine derivatives of himbacine that showed increased binding to the protease-activated receptor-1 (PAR-1).^{162,163} PAR-1 is a thrombin activated G-protein-coupled receptor involved in platelet activation, making it a valuable antiplatelet target for the treatment of cardiovascular disease. Subsequent derivatization of himbacine added an ethyl carbamate at the C-7 position of the tricyclic core, which further improved potency and oral availability.¹⁶⁴ Vorapaxar is a first-in-class PAR-1 antagonist and is a vital tool in the treatment of coronary heart disease.¹⁶⁵

In the area of cancer therapeutics, the alkaloid indolocarbazoles were identified as promising inhibitors of protein kinases.^{166,167} Staurosporine, the first indolocarbazole described, is a potent, but promiscuous, inhibitor of protein kinases. A biosynthetic precursor of staurosporine (K252a), however, was found to specifically inhibit protein kinase C (PKC), which is commonly dysregulated in cancers.¹⁶⁸ Therefore, development of the indolocarbazoles focused on increasing selectivity to inhibit specific PKC isozymes.¹⁶⁹ Semisynthetic modifications of staurosporine via N-alkylation/acylation allowed access to a number of analogs with increased selectivity for PKC.¹⁷⁰ Subsequently, midostaurin, an *N*-benzoyl-staurosporine derivative, was found to have in vivo antitumor activity and to inhibit angiogenesis, eventually leading to its development as a therapeutic to treat acute myeloid leukemia (Figure 6).^{171,172} While other indolocarbazole derivatives were developed and showed increased protein kinase selectivity, they did not advance to the clinic due to lack of efficacy.^{173,174} However, a number of indolocarbazoles are of continued interest due to their antibacterial activity and DNA topoisomerase inhibition, potentially leaving space for additional therapeutic analogs to be advanced.¹⁷⁵

DISCUSSION

Recent innovations in synthetic biology and chemical synthesis have lowered many perceived barriers to natural-product-based drug development. Across all classes of secondary metabolites it is now possible to produce scale quantities of natural products and targeted structural analogs using these technologies. Eliciting changes in potency, toxicity, cell-type selectivity, and pharmacokinetic properties is now well precedented using these methods. Innovations in synthetic methodology now permit rapid and efficient access to potent natural product cores, allowing for the production of analogs that can overcome the structural liabilities of their parent compounds.^{176,177} No compound exemplifies these innovations more clearly than the most structurally complex drug accessed via total synthesis on the market, eribulin. A simplified derivative of the natural product halichondrin B, eribulin is an antimicrotubule agent for the treatment of metastatic breast cancer.¹⁷⁸ Its advancement to the clinic was directly due to the ability to access analogs via total synthesis since it was only available in limited amounts from the natural source, a marine sponge. In conjunction with advances in synthetic chemistry, the ongoing innovations in the biosynthetic arts include inexpensive genome sequencing, gene synthesis, and increasingly facile genetic manipulation of the biosynthetic machinery via advances in genomic editing, such as CRISPR/Cas9 technology.^{179–181} Additionally, the increased competence to selectively alter natural products via targeted semisynthesis also has the potential to revitalize the field.¹⁸² By combining these efforts through collaboration and cross-disciplinary training, we can continue to develop natural product analogs with novel activity and improved profiles.^{183,184}

There are pressing clinical needs that require the adaption and improvement of natural products for human medicine. Antibiotic resistance is a critical threat, and historically, natural products have provided the most successful entrée. There is also a need for target and cell-type selectivity in noninfectious diseases such as cancer and neurodegenerative diseases.¹⁸⁵ As we argue in this Perspective, it is now feasible to greatly expand and improve existing scaffolds to develop new generations of therapeutics and to potentially adapt previously

discarded scaffolds using the current state of the art. Within the complexity and functional richness of natural products' structures are the necessary properties to engender target specificity. In particular, the large size and conformation of many natural products facilitate addressing “undruggable” targets, such as protein–protein interactions, that are not easily affected by small molecules. By taking advantage of advances in chemical synthesis and synthetic biology, we can continue to make optimal use of natural products to improve human health.

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ABBREVIATIONS USED

6-MSA	6-methylsalicylic acid
3-ABA	3-aminobenzoic acid
iPKS	iterative polyketide synthase
PKS	polyketide synthase
SAM	<i>S</i> -adenosyl methionine
GT	glycosyltransferase
AmpB	amphotericin B
NPP	nystatin-like <i>Pseudonocardia</i> polyene
17-AAG	17-allylamino geldanamycin
17-DMAG	17-(2-dimethylaminoethyl)amino geldanamycin
AT	acyltransferase

AHBA	3-amino-5-hydroxybenzoic acid
RiPP	ribosomally synthesized and post-translationally modified peptide
Tsr	thiostrepton
QA	quinaldic acid
DEBS	deoxyerythronolide B synthase
VRSA	vancomycin-resistant <i>Staphylococcus aureus</i>
PTC	peptidyl transfer center
SPase	signal peptidase
PKC	protein kinase C

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Figure 2.

Overview of polyene macrolide analogs. (A) Semisynthetic analogs at the C-16 exocyclic carbonyl (blue). Urea functionalized compounds show similar antifungal properties with decreased hemolytic properties as compared to AmpB (shown). (B) Analogs at the polyol region (red) accessed via functional deletion of genes encoding ketoreductase (KR) and/or dehydratase (DH) domains within nystatin (*nysJ*) and AmpB (*amphJ*) PKS modules. (C) Polyene macrolides with diverse sugar moieties (green) from natural sources and via derivatization.

Figure 3.

Profile of tetracycline analogs. (A) First generation of tetracycline and related natural products. (B) Second generation semisynthetic 6-deoxy analogs. (C) Third generation semisynthetic glycylicyclines showing increased activity toward resistant bacteria (shown). Minocycline semisynthetic derivatives, omadacycline and sarecycline, are also highlighted (blue box). (D) Fourth generation fluorocycline derivatives accessed via total synthesis with increased activity toward tetracycline-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) (shown).

Figure 4.

Overview of geldanamycin analogs. (A) Geldanamycin biosynthesis. (B) Geldanamycin analogs accessed via semisynthesis. Alkylamine analogs show similar activity against human breast cancer cell line (SKBr-3) and increased aqueous solubility as compared to the wild-type geldanamycin compound (shown). (C) Geldanamycin derivatives with ansa chain modifications from acyltransferase (AT) domain switching in the polyketide synthase (PKS) modules of Gdm A1, A2, and A3. (D) Reblastatin analogs accessed via mutasynthetic manipulations and precursor supplementation.

Figure 5. Overview of thiopeptide analogs accessed via biosynthetic manipulation and semisynthesis. Thiostrepton QA-analogs show increased aqueous solubility and antimicrobial activity against *B. subtilis* as compared to the parent compound (shown). Derivatives of nocathiacin I, accessed via stereoselective catalysis, have greatly increased solubility with retention of activity against *E. faecalis*.

Figure 6.

Natural product derivatives accessed via biosynthetic manipulation, semisynthesis, or total synthesis (changes to scaffold highlighted in red). Telavancin is a first-generation glycopeptide derivative accessed via semisynthesis from vancomycin. The macrolide solithromycin is accessed in nine steps from clarithromycin. Midostaurin is an *N*-benzoyl-staurosporine analog approved for treatment of acute myeloid leukemia. Total synthesis allows access to vorapaxar, a himbacine analog, employed in the treatment of coronary heart disease. Generation of arylomycin A-C₁₆ analogs yielded compound G0775, which shows increased activity against Gram-negative pathogen *P. aeruginosa* (gray portion).