This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.

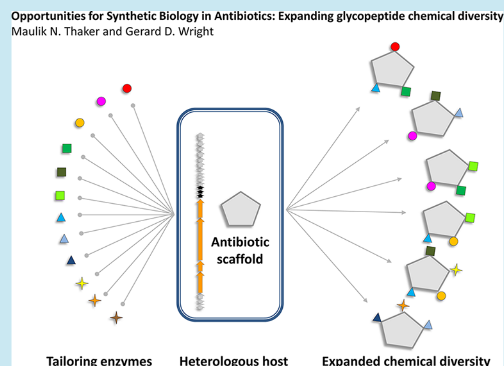
ACS Synthetic Biology
Review
pubs.acs.org/synthbio

Opportunities for Synthetic Biology in Antibiotics: Expanding Glycopeptide Chemical Diversity

Maulik N. Thaker and Gerard D. Wright*

M.G. DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, L8S 4K1 Canada

ABSTRACT: Synthetic biology offers a new path for the exploitation and improvement of natural products to address the growing crisis in antibiotic resistance. All antibiotics in clinical use are facing eventual obsolesce as a result of the evolution and dissemination of resistance mechanisms, yet there are few new drug leads forthcoming from the pharmaceutical sector. Natural products of microbial origin have proven over the past 70 years to be the wellspring of antimicrobial drugs. Harnessing synthetic biology thinking and strategies can provide new molecules and expand chemical diversity of known antibiotic scaffolds to provide much needed new drug leads. The glycopeptide antibiotics offer paradigmatic scaffolds suitable for such an approach. We review these strategies here using the glycopeptides as an example and demonstrate how synthetic biology can expand antibiotic chemical diversity to help address the growing resistance crisis.



KEYWORDS: *antibiotic scaffold, tailoring enzymes, heterologous host, synthetic biology, glycopeptides, resistance*

Antibiotics are among the most successful drugs discovered and developed over the past century. The first clinically useful antimicrobial compounds were synthetic molecules including dyes, organometallic complexes, and structurally simple aromatic compounds. The discovery and purification of antibiotics (a term first used to describe antimicrobial compounds derived from living organisms) such as penicillin, gramicidin, tyrocidine, and streptomycin in the late 1930s and early 1940s ushered in what has become known as the Golden Age of antibiotics: a decade and a half where the products of microbial secondary metabolism were systematically screened for antibiotic activity.[1] The vast majority of chemical scaffolds in current clinical use can be traced back to this relatively short period of time. The remarkable richness of microbial natural products as sources of antibiotics reflects the fact that they are products of evolution, selected over millennia for interaction with biological targets. When compared to synthetic molecules that are generally the result of experimental campaigns based on chemical diversity that is readily accessible in the lab, the privileged nature of microbially derived natural products as antibiotics becomes clear.

A hallmark of antibiotic natural products is their chemical diversity and complexity (Figure 1). This chemical diversity is based on the production of chemical scaffolds consisting of peptide, polyketide, carbohydrate, alkaloid, or terpene backbones that serve as the core structure. These scaffolds are then modified by a variety of chemical reactions catalyzed by a broad spectrum of tailoring enzymes that are co-produced with the scaffold assembly machinery. Scaffold-tailoring reactions include isomerization and racemization, reduction and oxidation (including hydroxylation), as well as group transfer such as acylation, methylation, glycosylation, sulfation, phosphorylation, and halogenation.[2] As a result the end products are often chemically complex, stereochemically intricate, and rich in hydrogen bond donors and acceptors. For example, the antibiotic erythromycin has 18 chiral centers compared to none for the synthetic antibiotic ciprofloxacin. Moreover the macrocyclic polyketide scaffold of erythromycin, 6-deoxyerythronolide, is further modified by glycosylation by two unusual carbohydrates that contribute to bioactivity and bioavailability to complete the antibiotic. Nature's ability to generate a large number of natural product scaffolds, including the combination of scaffolds to produce hybrids, and to tailor them in combinatorial fashion results in near limitless bioactive chemical diversity. Nevertheless, despite the proven track record of natural products in drug and antibiotic discovery, they have largely been abandoned by the pharmaceutical sector in favor of large libraries of synthetic molecules.

The rich bioactivity of natural products is the result of chemical diversity that is intrinsically compatible with interaction with proteins, nucleic acids, and membranes under physiological conditions and fine-tuned by natural selection. It is therefore not surprising then that natural products and their derivatives dominate the molecules that find use as drugs and drug leads including antibiotics.[3,4] Yet despite their success, the drug discovery sector has turned away from these molecules over the past two decades in favor of small synthetic molecules. As outlined by Li and Vederas,[4] the reasons for the abandonment of natural products by the pharmaceutical industry are multifactorial, yet there are two significant reasons

Received: September 21, 2012
Published: December 17, 2012

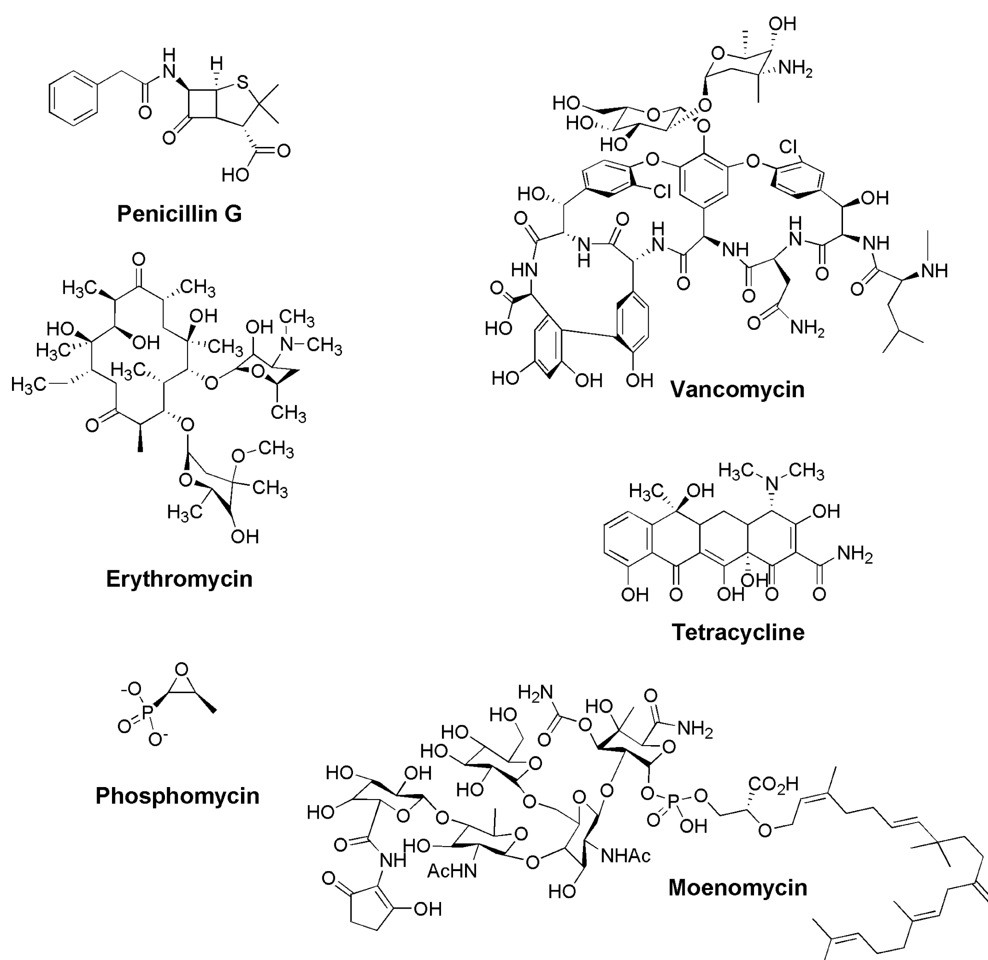


Figure 1. Chemical diversity and complexity of antibiotics in nature. Antibiotics derived from microbial sources are rich in chiral centers and hydrogen bond donors and acceptors and span an order of magnitude in molecular weight.

for the current emphasis on small synthetic molecules over natural products. First is the limited number of natural scaffolds discovered that have proven suitable for downstream antibiotic development. Second are the significant technical challenges in the identification, purification, synthesis, and scale up production of natural products for the preparation of diverse libraries suitable for screening and lead optimization. On the other hand, synthetic and combinatorial chemistry provide tractable molecules that, while often lacking in innate bioactivity, can be readily modified in the lab by medicinal chemists. Drug discovery firms therefore have largely chosen to forego the rich bioactivity of natural products, and their associated chemical challenges, in favor of the predictable outcomes of synthetic chemistry. The fruits of this emphasis on synthetic molecules are evident in the absence of new antibiotics brought to market over the past 20 years.

There is a growing clinical need for new antibiotics that is driven by the emergence and prevalence of multidrug resistance.[5] As noted by Fischbach and Walsh, our current arsenal of broad spectrum antibiotics is dominated by only four major scaffolds:[6] cephalosporins, penicillins, quinolones, and macrolides, all of which were discovered almost half a century ago. Other distinct chemical scaffolds are also important, among them glycopeptides, lipopeptides, aminoglycosides, and tetracyclines, but these generally have more narrow application than the "big four". The innovation gap in new antibiotic scaffold identification over the past 50 years has been obscured by the great success in tailoring of existing scaffolds either by the discovery of natural variants or by medicinal chemical modification in the lab. The challenges faced by antibiotic field are emblematic of a general crisis in the generation of new bioactive chemical matter in drug discovery.

Fortunately, new genomic technologies including rapid and low-cost DNA sequencing and a growing understanding of the mechanistic details of natural product biosynthesis, along with innovation in chemical synthesis and analytical methods, can be combined to expand bioactive chemical diversity in a fashion that is compatible with high-throughput technology and begin to overcome the innovation gap in antibiotic discovery. In order to fully exploit the privileged nature of natural products in antibiotic drug discovery, what is required is the ability to rapidly identify scaffolds, tailor them using a collection of modifying enzymes, and rapidly purify the resulting compounds in sufficient quantity to generate libraries of molecules suitable for high-throughput screening. The rise of synthetic biology offers a tremendous opportunity to address these issues. At the same time, medicinal chemical modification of natural product scaffolds and *de novo* compound synthesis of new natural product-inspired scaffolds will increase the value of synthetic libraries in drug discovery.

SYNTHETIC BIOLOGY AND ANTIBIOTICS

Natural products are genetically encoded small molecules. They represent a rich source of privileged chemical structures that

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206

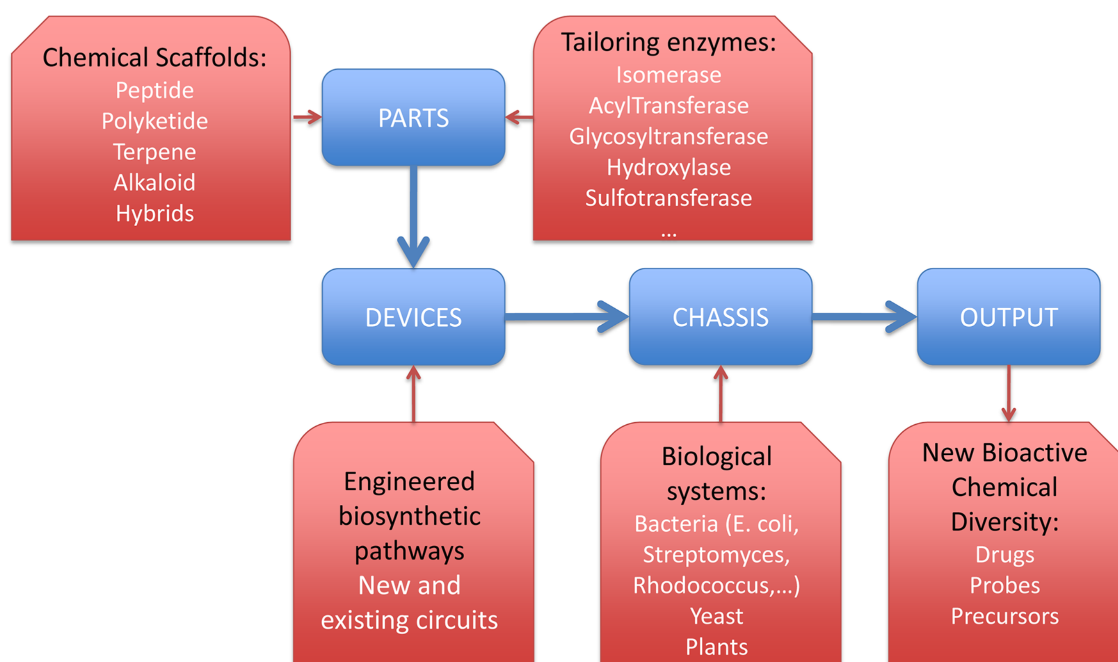


Figure 2. Representative model for the application of synthetic biology to expand antibiotic chemical diversity. Various tailoring enzymes and backbones (parts) can be assembled in biosynthetic circuits (devices) in a versatile host (chasis) to generate novel compounds. Synthetic chemistry can expand chemical diversity orthogonally to further increase diversity.

have evolved over millennia specifically to interact with biological macromolecules. Nevertheless, there remain some significant barriers for the widespread integration of natural products in modern high-throughput antibiotic discovery including issues of compound availability, purity, and chemical complexity. Fortuitously, the renewed interest in natural products as drug leads is concurrent with the development of new technologies in genomics, bioinformatics, analytical chemistry, and chemical synthesis that are enabling a new assessment of the compatibility of natural products with modern drug discovery. In particular, synthetic biology has the potential to play a leading role in this area and holds real promise to help overcome some of the challenges for increased integration of natural products in antibiotic drug discovery.

The genes encoding natural products are generally clustered together in the genomes of microbes that produce natural products. The low cost of DNA sequencing, continuing improvements in predicting natural product chemical structures from biosynthetic gene sequences,[7,8] and a growing understanding of the molecular details of biosynthetic chemistry[9] have dramatically increased the number of well-characterized natural product biosynthetic pathways in the past 15 years. This work is not limited to cases where a producing organism is known and cultivated, and indeed whole biosynthetic pathways have been successfully reconstructed from metagenomic libraries bypassing the frequent bottleneck of successfully culturing natural product-producing microbes.[10] Furthermore, there have been several examples of gene mutation, deletion, replacement, and introduction into natural product producing bacteria that have generated new compounds, an approach termed combinatorial biosynthesis.[11] This approach was pioneered by Hopwood and colleagues, who first genetically manipulated the antibiotic pigment actinorhodin in the producing organism *Streptomyces coelicolor*.[12] Researchers at Kosan later refined the combinatorial biosynthesis approach and demonstrated that systematic and rational mutation of an antibiotic biosynthetic pathway could generate libraries of novel and predicted compounds and that this biosynthetic machinery could be recapitulated in a heterologous host, in this case *Escherichia coli*.[13]

What has been lacking in these early efforts to make what we now would term "synthetic biological strategies" generally applicable and compatible with modern drug discovery is a series of predictable and unifying rules of assembly and production such that "unnatural" natural products can be designed and expressed in large quantities with minimal purification steps. Nevertheless, the field is now well suited for a coordinated synthetic biology effort to increase natural product antibiotic diversity and the preparation of large libraries suitable for drug discovery.

In the vernacular of synthetic biology,[14] the genes encoding scaffolds (peptides, polyketides, etc.) and various tailoring enzymes are **Parts** that can be assembled to generate **Devices**, genes assemblies that encode specific compounds. These are coexpressed in a **Chassis**, a suitable host (bacteria, yeast) for generation of engineered natural products. By systematically manipulating **Parts** and **Devices** within an appropriate **Chassis**, the resulting compounds can be assembled in a library for drug discovery (Figure 2).

The increasingly affordable cost of DNA synthesis and a growing understanding of natural product gene expression as well as biosynthetic biochemistry make this approach realistic. Improvements in heterologous gene expression and the availability of a growing number of candidates for "universal" natural product producers including yeast, *E. coli*, *Streptomyces* spp., *Rhodococcus* sp. and *Pseudomonas* sp. are serving to build the appropriate hosts.[15−18] The early success of this concept with the macrolide antibiotics[19] and the antimalarial compound artemisinin[20] demonstrate the promise of synthetic biology approaches in expanding bioactive chemical diversity and accessibility of privileged natural product structures.

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206

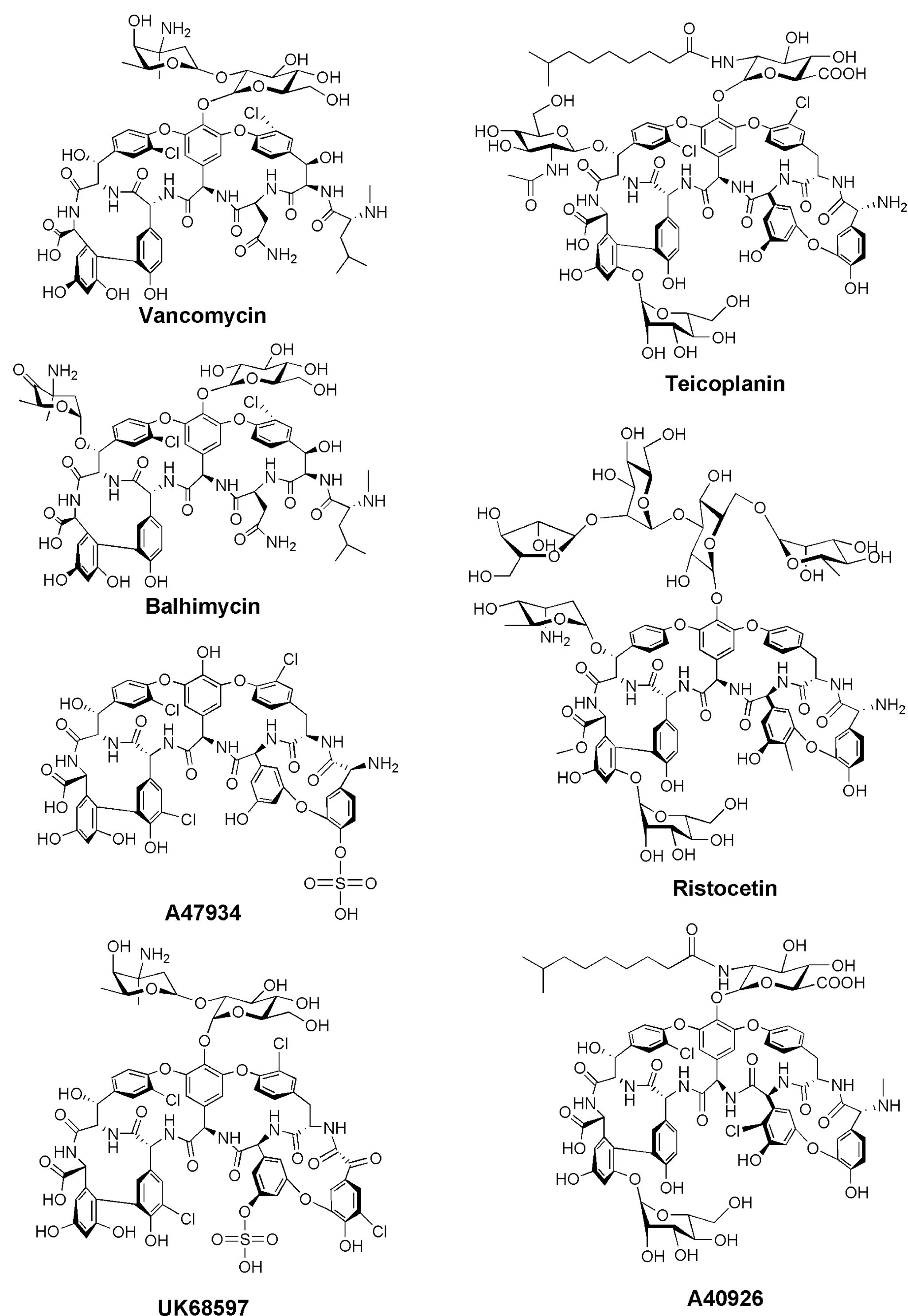


Figure 3. GPAs: a structurally diverse class of bacterially produced antibiotics.

To fully realize the promise of synthetic biology applications in natural product biosynthesis requires investment in the following areas:

1. A complete toolkit. This includes all of the tailoring enzymes and all of the scaffolds. There has been great progress in this area over the past decade. For example, glycosyltransferases,[21,22] sulfotransferases,[2,23] and acyltransferases[24] have all be used to generate "unnatural" natural products. Access to new biological diversity (microbial and plant) and understanding of substrate specificity of tailoring enzymes and scaffold biochemistry will expand these resources.

2. Dependable circuit diagrams. This will require improved understanding of the regulation of natural product genes to generate efficient biosynthetic circuits suitable for production of compounds.[25] Recent evidence suggests that gene expression is precisely staged during natural product biosynthesis with the small molecule products of early gene expression inducing the expression of late gene expression such as transporters and resistance elements.[26] Also essential is an understanding of how

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206

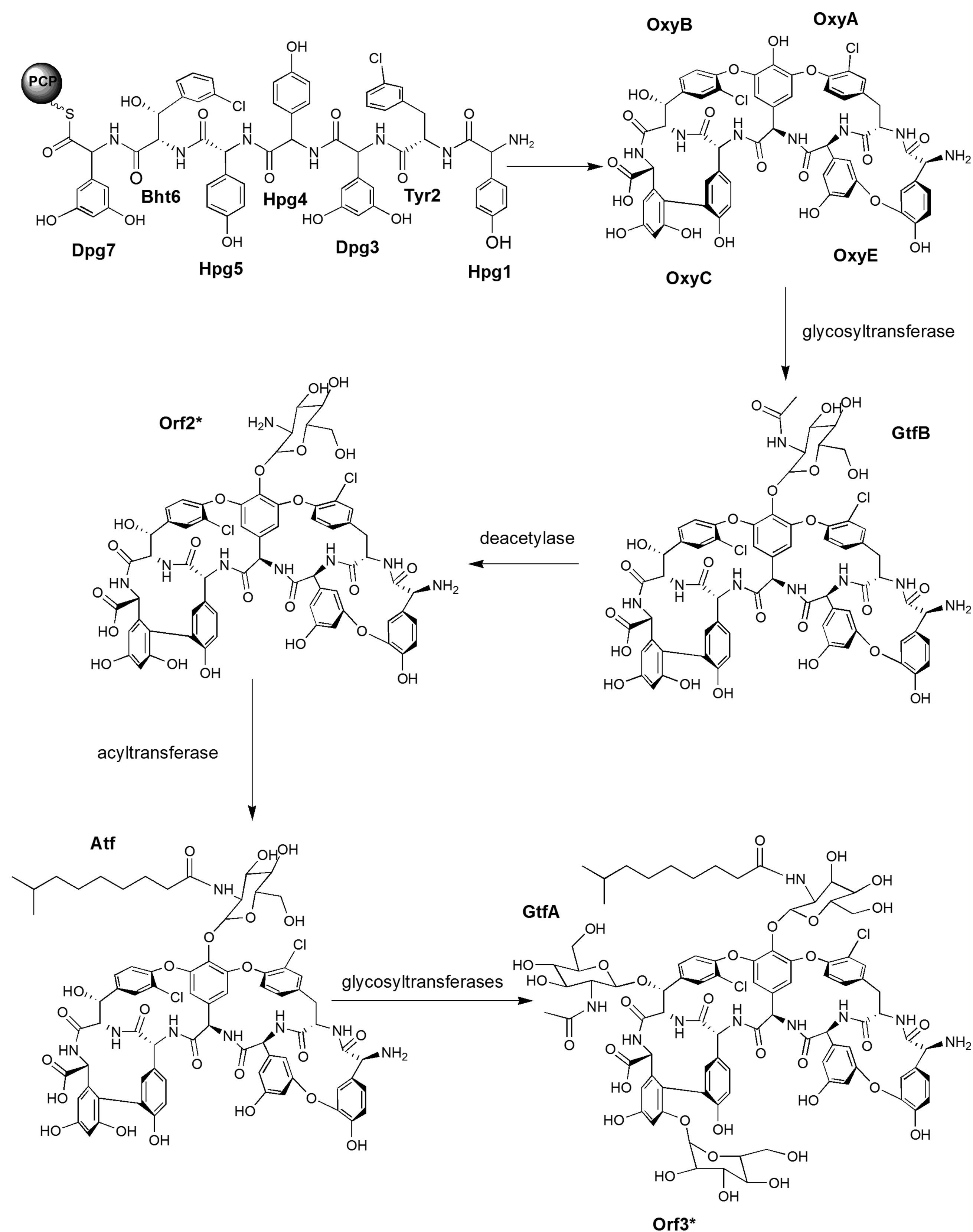


Figure 4. The maturation of GPAs includes a number of distinct enzyme activities and chemical modifications. The maturation of teicoplanin is shown as an example. In a series of enzymatic steps, the linear heptapeptide is cross-linked via the activity of monooxygenases followed by a tandem action of scaffold modifying enzymes to create the final teicoplanin antibiotic.

these pathways draw on host metabolism and what impact this has on cell growth.

3. Robust chassis. Hosts that have been engineered to produce a single compound (or family) that can be easily

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195–206

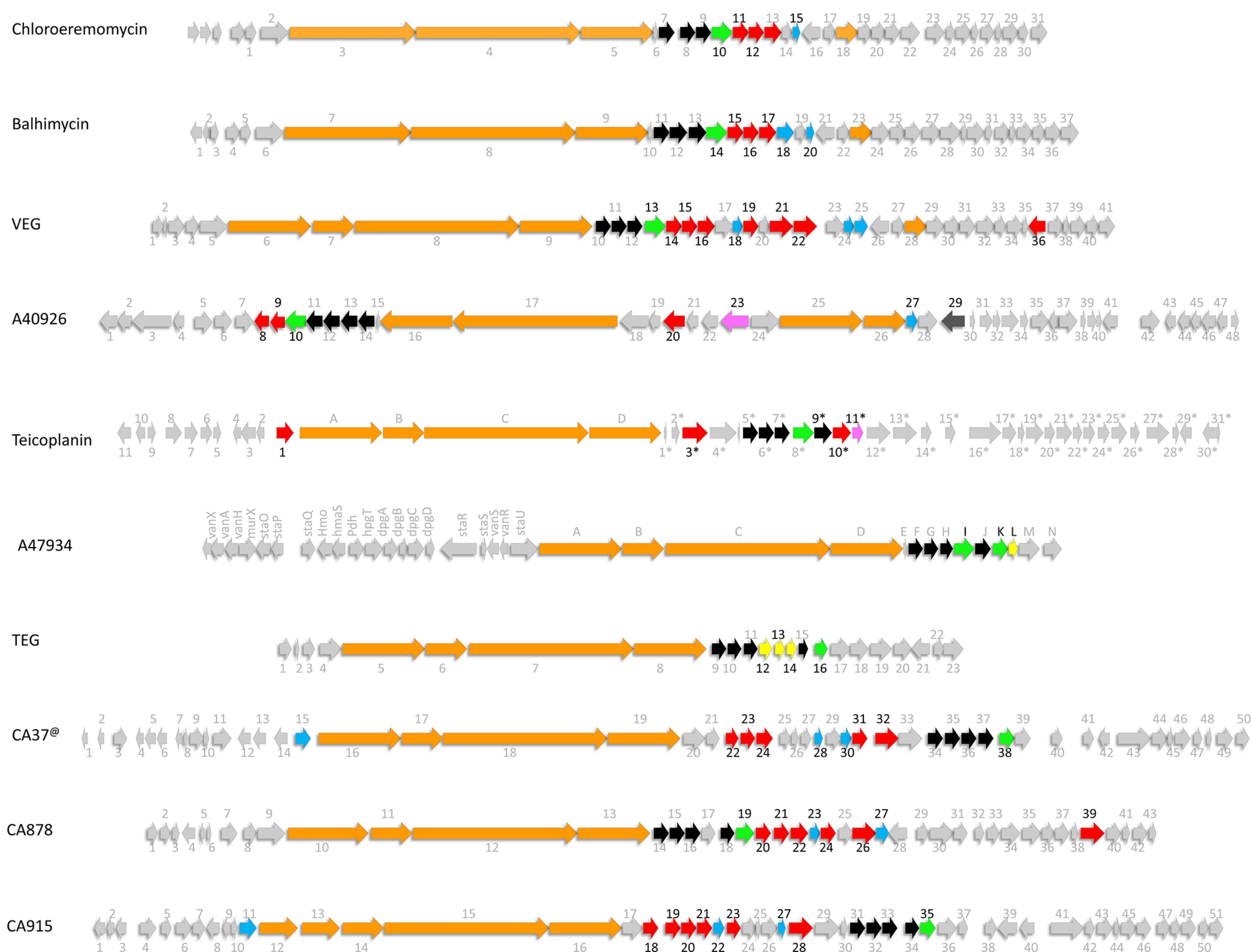


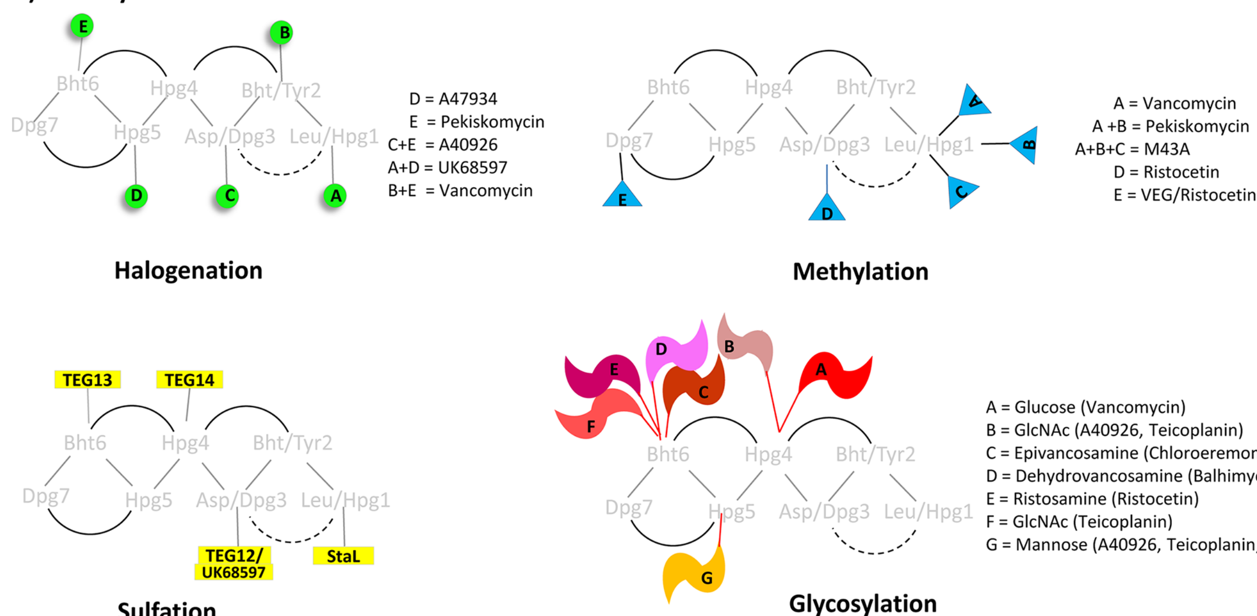
Figure 5. Examples of GPA biosynthetic gene clusters. Scaffold biosynthesis is encoded by nonribosomal peptide synthetases (orange) producing peptides that are cross-linked by P450 monooxygenases (black). Tailoring enzyme are encoded by genes for glycosylation (red), halogenation (green), sulfation (yellow), methylation (blue), and acylation (pink) are shown.

purified will greatly improve the recovery of the pure molecules that are important for reproducible high-throughput testing. Organisms that are robust producers of natural products (e.g., *Streptomyces*) offer advantages of intrinsic metabolism that supports secondary metabolite production, while nonproducers such as *E. coli* have been successfully manipulated for natural product production. A collection of optimized potential hosts will no doubt be required to maximize the production potential of various scaffolds.

Finally, new scaffolds need not be derived from natural products, and there remains an important role for synthetic chemistry to expand bioactive chemical space suitable for drug discovery. Growing efforts to generate libraries of complex synthetic molecules that emulate natural product chemical diversity such as diversity-oriented synthesis are yielding new chemical matter with novel activity (e.g., ref 27). Furthermore, new chemical catalysts with the ability to expand natural product diversity are proving robust and useful.[28,29] Indeed, chemical synthesis and synthetic biology strategies to expand bioactive chemical diversity are not mutually exclusive and can be complementary (Figure 2). In fact there is a long history of merging synthesis and natural product biosynthesis in the form of mutasynthesis, precursor-directed biosynthesis, and semi-synthetic medicinal chemistry.[30] All of these strategies can be brought together to help address the antibiotic crisis.

■ GLYCOPEPTIDES: A MODEL PLATFORM TO EXPLORE ANTIBIOTIC SYNTHETIC BIOLOGY

Glycopeptide antibiotics (GPAs) offer a useful example where we and others have initiated efforts to explore the applicability of synthetic biology approaches in an effort to expand the chemical diversity of a natural product class. GPAs (Figure 3) are critically important antibiotics that target Gram-positive bacteria by binding to the acyl-D-alanyl-D-alanine terminus of growing peptidoglycan. This interaction physically blocks cell wall biosynthetic enzymes, inhibiting cell growth and division. They remain essential drugs for the treatment of life-threatening infections caused by important human pathogens such as *Staphylococcus aureus* and *Enterococcus* sp. The antibiotics consist of a heptapeptide scaffold that is matured to the active antibiotic via a series of tailoring enzymes that oxidatively catalyze 3−4 intramolecular cyclizations and a variety of modifications including glycosylation, halogenation, acylation, etc. (Figure 4). Two distinct peptide scaffolds that include two unusual amino acids, 4-hydroxylphenylglycine (Hpg) and 3,5-dihydroxyphenylglycine (Dpg), are in current



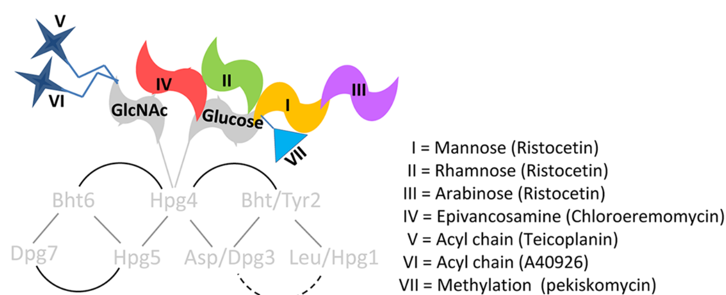


Figure 6. The diversity of GPA modifications offers tremendous opportunity for chemical diversification. The GPA heptapeptide backbone is numbered, and arcs represent regions of cross-linking. (A) Sites of primary modification of the backbone in the form of halogenation, glycosylation, methylation, and sulfation. Homologues of the modifying enzymes act on one or more different amino acid as indicated. (B) Secondary modifications are confined to the Hpg_4 sugar (glucose or GlcNAc) in the form of methylation, acylation, or glycosylation.

clinical use exemplified by vancomycin (Leu-Bht-Asn-Hpg-Hpg-Bht-Dpg) and teicoplanin (Hpg-Tyr-Dpg-Hpg-Hpg-Bht-Dpg). Since the discovery of vancomycin in 1953, a large number of GPAs have been isolated. These molecules generally retain the canonical heptapeptide cores and vary in their accessorization by various functional groups. Nicolaou and colleagues have catalogued an exhaustive list of all of the functional groups attached at various positions in different GPAs.[31] The first reported GPA biosynthetic gene clusters revealed a predicted set of nonribosomal peptide synthetase units required for assembling the peptide scaffold along with genes encoding amino acid and sugar biosynthesis, self-resistance, export, and tailoring enzymes.[32,33] This pattern is repeated in all other GPA clusters (Figure 5). Here we will focus on the molecules with known biosynthetic machinery, which can be utilized to assemble novel GPAs using a synthetic biology approach. These include the A47934, A40926, teicoplanin, chloroeremomycin, vancomycin, balhimycin, VEG, TEG, CA37, CA878, and CA915 GPA biosynthetic clusters along with some information from our unpublished work on UK68597, ristocetin, and the new GPA pekiskomycin.

Various tailoring enzymes including glycosyltransferases, methyltransferases, sulfotransferases, halogenases, and acyltransferases decorate the heptapeptide scaffolds of GPAs. The modifications resulting from the action of these enzymes have been shown or speculated to impart stability, increase solubility, affect dimerization constants, limit conformational flexibility, avoid degradation, and evade resistance.[34−37] GPA tailoring modifications can be grouped in two categories: (1) primary modifications, where the amino acid components of the GPA scaffold are directly modified (Figure 6A), and (2) secondary modifications, referring to tailoring of primary modifications (Figure 6B). Thus, the presence of a primary modification of the amino acid is a prerequisite for the action of this latter group of enzymes.

GPA Primary Modifications. *Halogenation.* Halogenation by means of chlorination is the most prevalent modification observed in GPA scaffolds. This functionalization is also common among the glycopeptide variants such as actinoidin, complestatin, and kistamycin. Chlorination has been implicated in imparting rotamer stability to the molecule during the assembly stage and also in the intermolecular dimerization of GPAs in solution.[31] The GPA biosynthetic clusters sequenced so far show the presence of either one or two halogenases in every cluster (Figure 5). They are implicated in the addition of chlorine(s) ranging from one Cl atom per GPA molecule in pekiskomycin to four in the case of UK68597. Chemical diversity is also achieved by the ability of halogenases

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206

to add chlorine to different amino acids of the heptapeptide. Tyr (or its β-hydroxy version, Bht) at positions 2 and 6 are the most commonly chlorinated amino acids as seen in chloroeremomycin, vancomycin, balhimycin, teicoplanin, UK68597, and A47934. Both chlorine molecules are added by the same halogenase as demonstrated by the deletion of *bhaA*, the sole halogenase in the balhimycin biosynthetic gene cluster, resulting in complete absence of Cl from the resulting antibiotic.[38] The halogenase in A40926 biosynthetic cluster of *Nonomuraea* sp. shows some variation in function where it chlorinates Dpg_3 instead of Tyr_2. Pekiskomycin is a monohalogenated GPA where the putative halogenase Pek27 adds a chlorine atom on Bht_6. An additional chlorination on Hpg_5 of A47934 is proposed to occur by another halogenase present in the cluster, StaK.[33] Similarly, a second halogenase is present in the UK68597 cluster, which we attribute to chlorination of Hpg_1 and Hpg_5. Thus, the use of these enzymes in selected combinations provides enough choices to create many backbone variations. Furthermore, degeneracy in reagent specificity of these enzymes allows additional leverage in modifications. In the balhimycin producer *Amycolatopsis balhimycina*, bromobalhimycin and bromochlorobalhimycin were obtained by simply regulating the concentration of bromine and chlorine salts in the growth media.[39]

Sulfation. Sulfation is a relatively uncommon yet very interesting modification on GPAs. By loading a sulfate molecule on the GPA backbone, the sulfotransferases impart a negative charge to the molecule. Until recently the role of this modification in the GPAs was not understood, but experiments in our lab show that GPA sulfation plays an important role in evading the induction of resistance.[40] Only two naturally occurring sulfated GPAs, namely, A47934 and UK68597, have been so far reported. In UK68597 the sulfation occurs on the free hydroxyl moiety of Dpg_3 that is not involved in the *oxyE* mediated aryl cross-linking with Hpg_1. The sulfotransferase StaL in *Streptomyces toyocaensis* has been shown to decorate Hpg_1 of A47934. This substitution is noteworthy since the modification on Hpg_1 is very rare. Additional sulfotransferases have been discovered recently from screening of soil metagenomic cosmid libraries. Out of four novel sulfotransferase identified, three belong to the TEG cluster.[10] Enzymes Teg12, Teg13, and Teg14 have been shown to modify Dpg_3, Bht_6, and Hpg_4, respectively, on the A47934 backbone. The fourth sulfotransferase, identified on a partial GPA cluster of cosmid AZ205, is a Bht_6 modifying analogue of Teg13.[41] By using these enzymes in different combinations, 15 modifications of teicoplanin-type GPA with mono-, di-, and trisulfated derivatives have been created.[41] Interestingly all of the sulfotransferases, including those identified from metagenomes, belong to the teicoplanin type of GPA.

StaL was the first GPA sulfotransferase to have its 3D structure determined by X-ray crystallography, which showed that the protein exists as a dimer and has a dimerization motif unique from the other known sulfotransferases.[42] The subsequent determination of the structures of two additional sulfotransferases, Teg12[43] and Teg14[44] with alternate regiospecificity, suggests that these enzymes must have a unique substrate binding pattern in their variable regions, to orient the active site toward different parts of the heptapeptide scaffold. Recent updates from co-crystallization of StaL bound to the substrate (desulfo-A47934) and product of sufate donation (3′-phosphoadenosine 5′-phosphate) have improved our understanding of the reaction.[45] It clearly shows that even though the protein exists as a dimer, only one of the active sites can accommodate the GPA backbone in proper orientation. Interestingly, StaL also demands a conformational flexibility from the canonical cup shape of the heptapeptide core as the enzyme can only recognize the more planar conformation observed in desulfo-A47934. The information provides a deeper understanding useful in predicting the behavior of these group transferases.

Methylation. GPAs can be modified by SAM-dependent *N*-methyltransferases that cap the amino terminus of Leu_1 of the vancomycin class antibiotics. MtfA from the chloroeremomycin cluster is a well-studied example representing the group. The crystal structure of MtfA reveals that the protein exists as a dimer with wing-like extensions and the GPA substrate is proposed to bind in the cleft located at the dimer interface.[46] Furthermore, MtfA has some substrate elasticity and can modify the *N*-terminal Hpg_1 of teicoplanin-class antibiotics. Other variants of this protein exist; for example, M43A is a vancomycin analogue from *Amycolatopsis orientalis*, which differs from the parent molecule in trimethylation of Leu_1.[47] We have recently identified a methyltransferase in the pekiskomycin cluster that adds two methyl groups to the amino-terminus of the heptapeptide. Ristocetin is decorated by two methyl groups at different positions: one is an unusual C-methylation at the *para* position of the phenol ring of Dpg_3, and the other is on the carboxyl group of Dpg_7 forming a methyl ester. An orthologuous methyltransferase is also found in the VEG cluster obtained from an environmental metagenome study.[10] The cosmid when expressed in *S. toyocaensis* conferred methylation of the carboxy terminus of A47934.[41] This chemical modification resulted in improvement of the MIC of the GPA against *S. aureus* and *Enterococcus faecalis*. This demonstrates that even modest alteration to the molecule indeed can increase antibiotic efficacy. It is noteworthy that dalbavancin (Zeven), a semisynthetic GPA currently in phase III clinical trials, also includes an aliphatic tail at the C-terminus of the scaffold heptapeptide.[48]

Glycosylation. Sugar derivatization is the most studied GPA tailoring mechanism. The spectrum of glycosylation ranges from the aglycone antibiotic A47934 to ristocetin, which bears a six-sugared scaffold. The addition of carbohydrate has been implicated in imparting solubility and structural rigidity, as well as impacting the antibiotic activity of GPAs.[34−36] It is noteworthy that even though the glycosyltransferases provide the broadest chemical diversity to the scaffold, they exhibit rather modest versatility in their choice for amino acid on the GPA. All of the sugar modifying enzymes identified so far act on one of amino acids 4, 6, or 7 of the heptapeptide. The phenolic OH of Hpg_4 serves as the “hot seat” for loading sugar moieties on GPAs; this reflects its relative availability for modification given its spatial separation from the acyl-D-alanyl-D-alanine binding region of the antibiotic as well as the role that sugars at this position play in back-to-back dimerization. The glucosyltransferases in the chloroeremomycin (GtfB), vancomycin (GtfE), and balhimycin (bGtfB) clusters transfer glucose from the nucleoside diphosphosugar (dTDP-D-glucose) to the phenolic hydroxyl of Hpg_4. The crystal structure for GtfB has been solved and provides insights into the sugar and aglycone substrate binding sites, creating possibilities for remodeling the protein for different sugar or substrate binding.[49] The substrate promiscuity of GtfE with variant heptapeptide aglycones has been demonstrated. The *in vitro* studies have identified the elasticity of GtfE in recognizing variant aglycons and utilizing

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206

over 30 natural as well as synthetic sugar residues including the aminosugars on vancomycin.[50−53] The aminosugars present an independent platform for further secondary modifications either naturally (e.g., acylation in teicoplanin) or by semi-synthetic derivatization (e.g., alkylation in oritavancin) for improved GPA activity. Thus, glycosylation serves as an excellent example of the potential of synthetic biology in harnessing these enzymes in creating diverse GPA libraries.

The four-ringed heptapeptide scaffolds of UK68597 and ristocetin are also glucosylated at Hpg_4, whereas teicoplanin (GtfB) and A40926 (Dbv9) have a GlcNAc moiety at Hpg_4. The biosynthetic clusters of the latter two, along with ristocetin, also include a mannosyltransferase that modifies Dpg_7 with a mannose. The most unusual sugars associated with GPAs modify the β-hydroxyl of Bht_6. In teicoplanin biosynthesis modification of the position is catalyzed by a glycosyltransferase (Orf1) that attaches a GlcNAc moiety. A customized sugar, 4-*epi*-L-vancosamine, adorns the Bht_6 in chloroeremomycin. A set of five dedicated enzymes are required to mature a putative substrate TDP-4-keto-6-deoxyglucose in a series of steps involving C2 deoxygenation (EvaA), C3 amination (EvaB), C3 methylation (EvaC), and C5 epimerization (EvaD) followed by C4 ketoreduction (EvaE) to form 4-*epi*-vancosamine.[54] An intermediate form of this sugar, L-dehydrovancosamine, decorates the sixth amino acid in balhimycin. This is due to a nonfunctional DvaE (EvaE) in the balhimycin cluster required for the final maturation of the sugar. Another variant of 4-*epi*-vancosamine, observed in the form of ristosamine, is present on the ristocetin Bht_6. The lack of a C3 methyl group on ristosamine suggests that the producer might not have a functional EvaC homologue. A40926 and vancomycin molecules are devoid of any sugar at Bht_6, a key feature differentiating them from similar GPAs chloroeremomycin and teicoplanin, respectively.

GPA Secondary Modifications. All of the secondary tailoring enzymes identified so far act on the sugar linked to Hpg_4 of the backbone. The modifications such as the methyltransferases (EvaC, DvaC) and the aminotransferases (EvaB, DvaB) involved in maturation of vancosamine and its analogues in vancomycin, chloroeremomycin, balhimycin, and ristocetin do not qualify as secondary modifications since these modifications occur independently toward maturation of the sugar, before it is attached to the GPA backbone. In teicoplanin and A40926 the primary sugar GlcNAc undergoes deacetylation mediated by Orf2* and Dbv21, respectively.[55] This modification paves the way for an acyltransferase to add an acyl chain on the glucosamine.[24] The acyltransferase is promiscuous in recognizing and loading different acyl chains, thereby generating many natural variants. Teicoplanin is clinically used as a mixture of five molecules with acyl tails of varying lengths (C10−C11), and four more forms have been identified as related substances produced in relatively minor amounts.[56] The lipophilic nature of the aliphatic tail has been suggested to impart superior pharmacokinetic profile to teicoplanin.

The crystal structures solved for two deacetylases (Orf2* and Dbv21) show a unique capping loop involved in substrate binding.[57] Recently the crystal structure of Orf2* bound to teicoplanin has also been solved, revealing a cavity lined by hydrophobic residues that can host the long aliphatic acyl chain.[37] Furthermore, the enzyme also shows a higher affinity and specificity for long chain carbons compared to C2. This explains the role of Orf2*as a deacylase shown previously in *in vitro* assays. In A40926 biosynthetic pathway sugar oxidation by a hexose oxidase (Dbv29) is an additional modification that oxidizes C6 of the *N*-acylglucosaminyl substituent to *N*-acylglucuronic acid. The minor change serves as a block and safeguards the molecule from any further deacylation attack by Dbv21 in A40926, which was observed in teicoplanin. Interestingly, during recent structure determination studies of the flavin-containing hexose oxidase (Dbv29), Li et al. serendipitously discovered a solvent-exposed reaction intermediate that they cleverly used in generating a series of teicoplanin analogues with amidated and aminated lipid chains.[58] Some of these derivatives showed excellent activity against vancomycin- and teicoplanin-resistant *E. faecalis* strains. This provides yet another example of small chemical modifications to the molecule yielding major impact in antibiotic improvement.

Another common secondary modification is glycosylation, again confined only to the primary sugar (invariably a glucose) on Hpg_4. A vancosamine sugar is added on the hexose of the pseudoaglycone vancomycin by GtfD, to form vancomycin. The same modification is also observed in UK68597, whereas chloroeremomycin glycosyltransferase (GtfC) transfers 4-*epi*-vancosamine on the glucose. Ristocetin has a crown of three different sugars attached to the primary glucose. Two putative glycosyltransferases attach a rhamnose and a mannose to the glucose moiety. A third glycosyltransferase attaches an arabinose to the mannose. It is not known if the two sugars are assembled before loading on the GPA or act in a stepwise fashion as in case of other secondary sugars. The crystal structure of the vancosamyl transferase GtfD has been solved.[59] Walsh et al. have shown that the enzyme can recognize alternate primary sugar substrates attached to Hpg_4 creating several variants of the disaccharides including a 4-*epi*-vancosaminyl-4-aminoglucosyl moiety on the vancomycin backbone. The availability of two nucleophilic amino handles (one on each sugar) creates new opportunity for testing addition of functional groups (e.g., acylation) to create semisynthetic GPAs with potential better pathogen susceptibility.

GPA Production Chassis. A fundamental requirement for successful application of synthetic biology to GPA assembly is the need for a robust platform for production. Most of the efforts to expand GPA chemical diversity have so far involved *in vitro* or synthetic approaches. These serve as important tools in developing good leads but are limited in the ability to be scaled up. Thus it is essential to develop a robust chassis on which the new GPA molecules can be constructed.

The concept of a versatile host system has evolved gradually over the decades. After the Golden Era of antibiotics, when new antibiotics were hard to find and sufficient genetic understanding and tools were available, the first foray in the development of "hybrid" antibiotics was reported by Hopwood and colleagues.[12] The use of combinatorial biosynthesis was rapidly adapted to create libraries of unnatural natural products. Methods for heterologous transfer of large clusters on multiple cosmids using recombination techniques were also evolved. Most of the early work was done using the well-characterized model system of *Streptomyces coelicolor* as the host. The constraint with this blue pigment (actinorhodin) producer was a very high pigment background that often masked the production of new compounds and siphoned off precursor elements necessary for expression of heterologous pathways. Alternative strains including *Streptomyces lividans* and *Streptomyces albus* that showed fewer background host secondary

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206

metabolites and receptiveness for foreign DNA were successfully employed. The CH999 strain of *S. coelicolor*, with most of the actinorhodin cluster removed, was among the first designer strains developed as a "clean" host dedicated for expression of foreign natural product clusters. Several tools and techniques have been evolved or adapted (such as *E. coli-Streptomyces* artificial chromosomes, Transformation Associated Recombination, ReDirect technology among others)[60−62] over the years that benefit the process. During this time, a phenomenal accomplishment was achieved when the Khosla group and colleagues at Kosan successfully expressed the genes necessary for the biosynthesis of the erythromycin polyketide scaffold (6-deoxyerythronolide) from *Saccharopolyspora erythrea* in *E. coli*. To achieve this, they reengineered the *E. coli* strain and added appropriate promoters to the biosynthetic genes, which could be readily identified by the host system. Genes for alternate substrates required for building the polyketide, absent in *E. coli*, were also added.[63] Various bacterial host systems besides *Streptomyces* and *E. coli* such as *Bacillus* sp., *Pseudomonas* sp., *Myxococcus xanthus*, and others have been developed over the years.[64] In the post genomics era the enormous natural product wealth of the Actinomycetes has been unleashed. It has opened the genome-wide view of strains and developed patterns that reveal a relatively conserved essential genomic core and dispensable arms with natural product pathways. On the basis of such information and advances in molecular engineering, improved host systems have been developed in recent years, including a genome minimized strain of *S. avermitilis* where 1.4 Mb of the dispensable genomic region has been removed to develop an efficient and versatile host.[15] The genome reduction channels metabolic resources toward the production of heterologous molecules as was shown with streptomycin and cephamycin C overproduction. Similarly the M1154 strain of *S. coelicolor* has been developed by Bibb's group at John Innes Centre, where the four major secondary metabolite pathways (*act*, *red*, *cdA*, *cpk*) have been systematically deleted generating a host with greater heterologous biosynthetic potential.[18] Further, mutations in *rpoB* and *rpsL* genes that stimulate secondary metabolite production discovered by the Ochi group[65] have also been incorporated in this strain, further improving heterologous expression. Another strain of *S. coelicolor* devoid of all of the 10 PKS and NRPS biosynthetic pathways in the strain, along with 900 kb subtelomeric DNA, has also been recently developed offering yet more potential biosynthetic capacity as an efficient chassis for synthetic biology.[66]

In the current scenario, the best available option for development of a GPA synthetic biology platform is to build a genomic library of a producer using one of the *E. coli-Streptomyces* artificial chromosomes (pPAC-S2, pSTREPTOBAC V, pSBAC).[67] The advantage of using such a system instead of a cosmid vector lies in its capacity to carry large "payload" (up to 300 kb) enough to accommodate virtually any bacterial natural product biosynthetic apparatus in a single clone. Heterologous expression of the entire cluster in one of the available Streptomyces hosts should offer the opportunity to produce many different scaffolds and variants. The resulting system serves as an optimal chassis on which a variety of modifications can be designed using the toolbox of the ancillary genes. These tailoring enzymes can be sourced by isolating new GPA producers or from the environmental metagenomes.

CONCLUSIONS AND CAVEATS

A synthetic biology approach is an ideal strategy for expansion of the chemical diversity of many natural products. GPA antibiotics in particular offer an optimal scaffold for this approach since the necessary assembly lines are known and various tailoring enzymes have been tested successfully in the manufacture of new derivatives. The pioneering work done by the Baltz group at Eli Lilly some 15 years ago anticipated this strategy and offers the first proof of principle.[21] By expressing the glycosyltransferases from the chloroeremomycin producer *Amycolatopsis orientalis* in *S. toyocaensis* they for the first time showed production of two hybrid GPAs in the form of glycosylated derivatives of A47934. Our subsequent work has helped to decipher the biosynthetic pathway of A47934 and has led to better understanding of the structure and functioning of the ancillary proteins, especially sulfotransferases. Furthermore, the creative exploration of environmental metagenomic cosmid megalibraries as a source of novel tailoring enzymes has yielded several novel GPA variants. Given the number of scaffolds, tailoring enzymes, and the impact that various chemical modifications have on antibiotic activity, a synthetic biology strategy can yield libraries of GPAs suitable for screens for bioactivity.

Nevertheless, there are some significant challenges to the routine application of synthetic biology strategies in expanding natural product diversity. Among them are difficulties in heterologous expression of biosynthetic gene clusters, competing metabolic pathways that siphon off precious precursors, unpredictable substrate specificity and/or efficiency of tailoring enzymes, unknown specificity of efflux systems to deliver new derivatives out of the cell, and the potential generation of toxic compounds unable to be averted by associated resistance mechanisms. Some of these difficulties may be insurmountable for some scaffolds; however, there is reason to be hopeful that for many complex compounds that are practically inaccessible by total synthesis methods synthetic biology approaches offer a fresh strategy to explore new chemistry.

While natural products may have fallen out of favor in modern drug and antibiotic discovery, the need for new medicines, the rich bioactivity of these molecules, the dramatic advancements in our understanding of natural product biosynthesis, and the resulting amenability of the field to synthetic biology solutions are poised to relaunch a new era in natural product-focused pharmaceuticals.

AUTHOR INFORMATION

Corresponding Author

*E-mail: wrightge@mcmaster.ca.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Our research on GPA biosynthesis is funded by grants from the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes of Health Research (CIHR) Grant MT-14981 and by a Canada Research Chair (to G.D.W.). We thank Prof. Greg Challis for helpful input into Figure 2.

REFERENCES

(1) Walsh, C. T. (2003) *Antibiotics: Actions, Origins, Resistance*, ASM press, Washington, DC.

(2) Lamb, S. S., and Wright, G. D. (2005) Accessorizing natural products: adding to nature's toolbox. *Proc. Natl. Acad. Sci. U.S.A. 102*, 519–520.

(3) Miller, S. J., and Clardy, J. (2009) Natural products: Beyond grind and find. *Nat. Chem. 1*, 261–263.

(4) Li, J. W., and Vederas, J. C. (2009) Drug discovery and natural products: end of an era or an endless frontier? *Science 325*, 161–165.

(5) Wright, G. D. (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat. Rev. Microbiol. 5*, 175–186.

(6) Fischbach, M. A., and Walsh, C. T. (2009) Antibiotics for emerging pathogens. *Science 325*, 1089–1093.

(7) McAlpine, J. B., Bachmann, B. O., Piraee, M., Tremblay, S., Alarco, A. M., Zazopoulos, E., and Farnet, C. M. (2005) Microbial genomics as a guide to drug discovery and structural elucidation: ECO-02301, a novel antifungal agent, as an example. *J. Nat. Prod. 68*, 493–496.

(8) Lautru, S., Deeth, R. J., Bailey, L. M., and Challis, G. L. (2005) Discovery of a new peptide natural product by Streptomyces coelicolor genome mining. *Nat. Chem. Biol. 1*, 265–269.

(9) Sattely, E. S., Fischbach, M. A., and Walsh, C. T. (2008) Total biosynthesis: *in vitro* reconstitution of polyketide and nonribosomal peptide pathways. *Nat. Prod. Rep. 25*, 757–793.

(10) Banik, J. J., and Brady, S. F. (2008) Cloning and characterization of new glycopeptide gene clusters found in an environmental DNA megalibrary. *Proc. Natl. Acad. Sci. U.S.A. 105*, 17273–17277.

(11) Floss, H. G. (2006) Combinatorial biosynthesis-potential and problems. *J. Biotechnol. 124*, 242–257.

(12) Hopwood, D. A., Malpartida, F., Kieser, H. M., Ikeda, H., Duncan, J., Fujii, I., Rudd, B. A., Floss, H. G., and Omura, S. (1985) Production of 'hybrid' antibiotics by genetic engineering. *Nature 314*, 642–644.

(13) McCarthy, A. A. (2002) Kosan biosciences. Better chemistry through genetics. *Chem. Biol 9*, 849–850.

(14) Koide, T., Pang, W. L., and Baliga, N. S. (2009) The role of predictive modelling in rationally re-engineering biological systems. *Nat. Rev. Microbiol. 7*, 297–305.

(15) Komatsu, M., Uchiyama, T., Omura, S., Cane, D. E., and Ikeda, H. (2010) Genome-minimized Streptomyces host for the heterologous expression of secondary metabolism. *Proc. Natl. Acad. Sci. U.S.A. 107*, 2646–2651.

(16) Martinez, A., Kolvek, S. J., Yip, C. L., Hopke, J., Brown, K. A., MacNeil, I. A., and Osburne, M. S. (2004) Genetically modified bacterial strains and novel bacterial artificial chromosome shuttle vectors for constructing environmental libraries and detecting heterologous natural products in multiple expression hosts. *Appl. Environ. Microbiol. 70*, 2452–2463.

(17) Pfeifer, B., Hu, Z., Licari, P., and Khosla, C. (2002) Process and metabolic strategies for improved production of *Escherichia coli*-derived 6-deoxyerythronolide B. *Appl. Environ. Microbiol. 68*, 3287–3292.

(18) Gomez-Escribano, J. P., and Bibb, M. J. (2011) Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. *Microb. Biotechnol. 4*, 207–215.

(19) Lee, H. Y., and Khosla, C. (2007) Bioassay-guided evolution of glycosylated macrolide antibiotics in *Escherichia coli*. *PLoS Biol. 5*, e45.

(20) Ro, D. K., Paradise, E. M., Ouellet, M., Fisher, K. J., Newman, K. L., Ndungu, J. M., Ho, K. A., Eachus, R. A., Ham, T. S., Kirby, J., Chang, M. C., Withers, S. T., Shiba, Y., Sarpong, R., and Keasling, J. D. (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature 440*, 940–943.

(21) Solenberg, P. J., Matsushima, P., Stack, D. R., Wilkie, S. C., Thompson, R. C., and Baltz, R. H. (1997) Production of hybrid glycopeptide antibiotics in vitro and in *Streptomyces toyocaensis*. *Chem. Biol. 4*, 195–202.

(22) Oberthur, M., Leimkuhler, C., Kruger, R. G., Lu, W., Walsh, C. T., and Kahne, D. (2005) A systematic investigation of the synthetic utility of glycopeptide glycosyltransferases. *J. Am. Chem. Soc. 127*, 10747–10752.

(23) Lamb, S. S., Patel, T., Koteva, K. P., and Wright, G. D. (2006) Biosynthesis of sulfated glycopeptide antibiotics by using the sulfotransferase StaL. *Chem. Biol 13*, 171–181.

(24) Kruger, R. G., Lu, W., Oberthur, M., Tao, J., Kahne, D., and Walsh, C. T. (2005) Tailoring of glycopeptide scaffolds by the acyltransferases from the teicoplanin and A-40,926 biosynthetic operons. *Chem. Biol 12*, 131–140.

(25) Michalodimitrakis, K., and Isalan, M. (2009) Engineering prokaryotic gene circuits. *FEMS Microbiol. Rev. 33*, 27–37.

(26) Tahlan, K., Ahn, S. K., Sing, A., Bodnaruk, T. D., Willems, A. R., Davidson, A. R., and Nodwell, J. R. (2007) Initiation of actinorhodin export in *Streptomyces coelicolor*. *Mol. Microbiol. 63*, 951–961.

(27) Morton, D., Leach, S., Cordier, C., Warriner, S., and Nelson, A. (2009) Synthesis of natural-product-like molecules with over eighty distinct scaffolds. *Angew. Chem. 48*, 104–109.

(28) Lewis, C. A., and Miller, S. J. (2006) Site-selective derivatization and remodeling of erythromycin A by using simple peptide-based chiral catalysts. *Angew. Chem. 45*, 5616–5619.

(29) Lewis, C. A., Merkel, J., and Miller, S. J. (2008) Catalytic site-selective synthesis and evaluation of a series of erythromycin analogs. *Bioorg. Med. Chem. Lett. 18*, 6007–6011.

(30) Kennedy, J. (2008) Mutasynthesis, chemobiosynthesis, and back to semi-synthesis: combining synthetic chemistry and biosynthetic engineering for diversifying natural products. *Nat. Prod. Rep. 25*, 25–34.

(31) Nicolaou, K. C., Boddy, C. N., Brase, S., and Winssinger, N. (1999) Chemistry, biology, and medicine of the glycopeptide antibiotics. *Angew. Chem. 38*, 2096–2152.

(32) van Wageningen, A. A., Kirkpatrick, P. N., Williams, D. H., Harris, B. R., Kershaw, J. K., Lennard, N. J., Jones, M., Jones, S. J. M., and Solenberg, P. J. (1998) Sequencing and analysis of genes involved in the biosynthesis of a vancomycin group antibiotic. *Chem. Biol. 5*, 155–162.

(33) Pootoolal, J., Thomas, M. G., Marshall, C. G., Neu, J. M., Hubbard, B. K., Walsh, C. T., and Wright, G. D. (2002) Assembling the glycopeptide antibiotic scaffold: The biosynthesis of A47934 from *Streptomyces toyocaensis* NRRL15009. *Proc. Natl. Acad. Sci. U.S.A. 99*, 8962–8967.

(34) Grdadolnik, S. G., Pristovsek, P., and Mierke, D. F. (1998) Vancomycin: conformational consequences of the sugar substituent. *J. Med. Chem. 41*, 2090–2099.

(35) Kannan, R., Harris, C. M., Harris, T. M., Waltho, J. P., Skelton, N. J., and Williams, D. H. (1988) Function of the amino sugar and N-terminal amino acid of the antibiotic vancomycin in its complexation with cell wall peptides. *J. Am. Chem. Soc. 110*, 2946–2953.

(36) Mackay, J. P., Gerhard, U., Beauregard, D. A., Maplestone, R. A., and Williams, D. H. (1994) Dissection of the contributions toward dimerization of glycopeptide antibiotics. *J. Am. Chem. Soc. 116*, 4573–4580.

(37) Chan, H. C., Huang, Y. T., Lyu, S. Y., Huang, C. J., Li, Y. S., Liu, Y. C., Chou, C. C., Tsai, M. D., and Li, T. L. (2011) Regioselective deacetylation based on teicoplanin-complexed Orf2* crystal structures. *Mol. Biosyst. 7*, 1224–1231.

(38) Puk, O., Huber, P., Bischoff, D., Recktenwald, J., Jung, G., Sussmuth, R. D., van Pee, K. H., Wohlleben, W., and Pelzer, S. (2002) Glycopeptide biosynthesis in *Amycolatopsis mediterranei* DSM5908: function of a halogenase and a haloperoxidase/perhydrolase. *Chem. Biol 9*, 225–235.

(39) Bister, B., Bischoff, D., Nicholson, G. J., Stockert, S., Wink, J., Brunati, C., Donadio, S., Pelzer, S., Wohlleben, W., and Sussmuth, R. D. (2003) Bromobalhimycin and chlorobromobalhimycins—illuminating the potential of halogenases in glycopeptide antibiotic biosyntheses. *ChemBioChem 4*, 658–662.

(40) Kalan, L., Perry, J., Koteva, K., Thaker, M. N., and Wright, G. (2012) Glycopeptide sulfation evades resistance. *J. Bacteriol. 195*, 167–171.

(41) Banik, J. J., Craig, J. W., Calle, P. Y., and Brady, S. F. (2010) Tailoring enzyme-rich environmental DNA clones: a source of

enzymes for generating libraries of unnatural natural products. *J. Am. Chem. Soc. 132*, 15661−15670.
(42) Shi, R., Lamb, S. S., Bhat, S., Sulea, T., Wright, G. D., Matte, A., and Cygler, M. (2007) Crystal structure of StaL, a glycopeptide antibiotic sulfotransferase from *Streptomyces toyocaensis*. *J. Biol. Chem. 282*, 13073−13086.
(43) Bick, M. J., Banik, J. J., Darst, S. A., and Brady, S. F. (2010) Crystal structures of the glycopeptide sulfotransferase Teg12 in a complex with the teicoplanin aglycone. *Biochemistry 49*, 4159−4168.
(44) Bick, M. J., Banik, J. J., Darst, S. A., and Brady, S. F. (2010) The 2.7 A resolution structure of the glycopeptide sulfotransferase Teg14. *Acta Crystallogr., Sect. D 66*, 1278−1286.
(45) Shi, R., Munger, C., Kalan, L., Sulea, T., Wright, G. D., and Cygler, M. (2012) Sulfonation of glycopeptide antibiotics by sulfotransferase StaL depends on conformational flexibility of aglycone scaffold. *Proc. Natl. Acad. Sci. U.S.A. 109*, 11824−11829.
(46) Shi, R., Lamb, S. S., Zakeri, B., Proteau, A., Cui, Q., Sulea, T., Matte, A., Wright, G. D., and Cygler, M. (2009) Structure and function of the glycopeptide N-methyltransferase MtfA, a tool for the biosynthesis of modified glycopeptide antibiotics. *Chem. Biol. 16*, 401−410.
(47) Nagarajan, R., Merkel, K. E., Michel, K. H., Higgins, H. M., Hoehn, M. M., Hunt, A. H., Jones, N. D., Occolowitz, J. L., Schabel, A. A., and Swartzendruber, J. K. (1988) M43 antibiotics: methylated vancomycins and unrearranged CDP-I analogs. *J. Am. Chem. Soc. 110*, 7896−7897.
(48) Malabarba, A., and Goldstein, B. P. (2005) Origin, structure, and activity in vitro and in vivo of dalbavancin. *J. Antimicrob. Chemother. 55*, ii15−ii20.
(49) Mulichak, A. M., Losey, H. C., Walsh, C. T., and Garavito, R. M. (2001) Structure of the UDP-glucosyltransferase GtfB that modifies the heptapeptide aglycone in the biosynthesis of vancomycin group antibiotics. *Structure 9*, 547−557.
(50) Fu, X., Albermann, C., Jiang, J., Liao, J., Zhang, C., and Thorson, J. S. (2003) Antibiotic optimization via in vitro glycorandomization. *Nat. Biotechnol. 21*, 1467−1469.
(51) Hubbard, B. K., and Walsh, C. T. (2003) Vancomycin assembly: Nature's way. *Angew. Chem., Int. Ed. 42*, 730−765.
(52) Losey, H. C., Jiang, J., Biggins, J. B., Oberthür, M., Ye, X.-Y., Dong, S. D., Kahne, D., Thorson, J. S., and Walsh, C. T. (2002) Incorporation of glucose analogs by GtfE and GtfD from the vancomycin biosynthetic pathway to generate variant glycopeptides. *Chem. Biol. 9*, 1305−1314.
(53) Losey, H. C., Peczuh, M. W., Chen, Z., Eggert, U. S., Dong, S. D., Pelczer, I., Kahne, D., and Walsh, C. T. (2001) Tandem action of glycosyltransferases in the maturation of vancomycin and teicoplanin aglycones: Novel glycopeptides. *Biochemistry 40*, 4745−4755.
(54) Chen, H., Thomas, M. G., Hubbard, B. K., Losey, H. C., Walsh, C. T., and Burkart, M. D. (2000) Deoxysugars in glycopeptide antibiotics: Enzymatic synthesis of TDP-l-epivancosamine in chloroeremomycin biosynthesis. *Proc. Natl. Acad. Sci. U.S.A. 97*, 11942−11947.
(55) Ho, J.-Y., Huang, Y.-T., Wu, C.-J., Li, Y.-S., Tsai, M.-D., and Li, T.-L. (2006) Glycopeptide biosynthesis: Dbv21/Orf2* from dbv/tcp gene clusters are N-Ac-Glm teicoplanin pseudoaglycone deacetylases and Orf15 from cep gene cluster is a Glc-1-P thymidyltransferase. *J. Am. Chem. Soc. 128*, 13694−13695.
(56) Li, T. L., Huang, F., Haydock, S. F., Mironenko, T., Leadlay, P. F., and Spencer, J. B. (2004) Biosynthetic gene cluster of the glycopeptide antibiotic teicoplanin: characterization of two glycosyltransferases and the key acyltransferase. *Chem. Biol. 11*, 107−119.
(57) Zou, Y., Brunzelle, J. S., and Nair, S. K. (2008) Crystal structures of lipoglycopeptide antibiotic deacetylases: Implications for the biosynthesis of A40926 and teicoplanin. *Chem. Biol. 15*, 533−545.
(58) Liu, Y.-C., Li, Y.-S., Lyu, S.-Y., Hsu, L.-J., Chen, Y.-H., Huang, Y.-T., Chan, H.-C., Huang, C.-J., Chen, G.-H., Chou, C.-C., Tsai, M.-D., and Li, T.-L. (2011) Interception of teicoplanin oxidation intermediates yields new antimicrobial scaffolds. *Nat. Chem. Biol. 7*, 304−309.
(59) Mulichak, A. M., Lu, W., Losey, H. C., Walsh, C. T., and Garavito, R. M. (2004) Crystal structure of vancosaminyltransferase GtfD from the vancomycin biosynthetic pathway: interactions with acceptor and nucleotide ligands. *Biochemistry 43*, 5170−5180.
(60) Sosio, M., Giusino, F., Cappellano, C., Bossi, E., Puglia, A. M., and Donadio, S. (2000) Artificial chromosomes for antibiotic-producing actinomycetes. *Nat. Biotechnol. 18*, 343−345.
(61) Gust, B., Chandra, G., Jakimowicz, D., Yuqing, T., Bruton, C. J., and Chater, K. F. (2004) λ Red-mediated genetic manipulation of antibiotic-producing Streptomyces, In *Advances in Applied Microbiology*, pp 107−128, Academic Press, New York.
(62) Kim, J. H., Feng, Z., Bauer, J. D., Kallifidas, D., Calle, P. Y., and Brady, S. F. (2010) Cloning large natural product gene clusters from the environment: piecing environmental DNA gene clusters back together with TAR. *Biopolymers 93*, 833−844.
(63) Pfeifer, B. A., Admiraal, S. J., Gramajo, H., Cane, D. E., and Khosla, C. (2001) Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli*. *Science 291*, 1790−1792.
(64) Zhang, H., Wang, Y., and Pfeifer, B. A. (2008) Bacterial hosts for natural product production. *Mol. Pharm. 5*, 212−225.
(65) Hosaka, T., Ohnishi-Kameyama, M., Muramatsu, H., Murakami, K., Tsurumi, Y., Kodani, S., Yoshida, M., Fujie, A., and Ochi, K. (2009) Antibacterial discovery in actinomycetes strains with mutations in RNA polymerase or ribosomal protein S12. *Nat. Biotechnol. 27*, 462−464.
(66) Zhou, M., Jing, X., Xie, P., Chen, W., Wang, T., Xia, H., and Qin, Z. (2012) Sequential deletion of all the polyketide synthase and nonribosomal peptide synthetase biosynthetic gene clusters and a 900-kb subtelomeric sequence of the linear chromosome of *Streptomyces coelicolor*. *FEMS Microbiol. Lett. 333*, 169−179.
(67) Alduina, R., and Gallo, G. (2012) Artificial chromosomes to explore and to exploit biosynthetic capabilities of actinomycetes. *J. Biomed. Biotechnol. 2012*, 462049.

DOI: 10.1021/sb300092n
ACS Synth. Biol. 2015, 4, 195−206