Journal of
Medicinal
Chemistry

Volume 46, Number 16 July 31, 2003

Award Address

Antibiotic Glycosyltransferases: Antibiotic Maturation and Prospects for Reprogramming†

Christopher Walsh,* Caren L. Freel Meyers, and Heather C. Losey

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115

Received May 29, 2003

Glycosylation Patterns in Natural Products

Many varieties of natural products elaborated by microorganisms are regio- and stereospecifically glycosylated. These include polyketides, both in the aromatic tetracyclic family as exemplified by daunomycin **1** (Figure 1) and in the macrocyclic lactones such as the 14-membered macrocycle erythromycin **2** and the 16-membered macrocycle tylosin **3.** The polyketide macrolides and the nonribosomal peptide NRPS natural products are biosynthesized by analogous assembly line enzymatic logic,[1,2] and glycosylation is a common postassembly line tailoring step. The antibiotics vancomycin (**4**) and ramoplanin (**5**) and the antitumor drug bleomycin (**6**) all have disaccharide chains added late in the biosynthetic pathways. While ramoplanin has a dimannosyl chain, the remaining polyketide and nonribosomal peptide natural products are more typically decorated with specialized deoxyhexose and aminodeoxyhexose units generated via pathways dedicated to antibiotic modifications.[3] The aminocoumarin antibiotics novobiocin (**7**, Figure 1), clorobiocin (**8**), and coumermycin A1 (**9**) target the B subunit of the type II topoisomerase DNA gyrase[4] and have an L-noviosyl sugar that presents 4′-*O*-carbamoyl or 4′-*O*-methylpyrrolyl substituents as competitive ligands for the ATP binding site in GyrB. Many other natural product scaffolds including enediyne antitumor agents and polyene antifungal agents[5] contain specialized hexosyl moieties.

Role of the Sugar Moieties in the Glycosylated Antibiotics

In some instances, the sugar moieties may serve mainly to solubilize the natural product by enabling the hydrophobic aglycone portions to partition into aqueous phases, thereby achieving useful intracellular and extracellular concentration. This may be the case for the housekeeping-type GlcNAc sugars added to the cross-linked heptapeptide core of teicoplanin (**12**, Figure 2) or of the dimannosyl chain in ramoplanin (**5**). Sequence analysis of the antibiotic biosynthetic cluster for A40926 reveals a dedicated glycosyltransferase gene as well as a protein mannosyltransferase ORF that may utilize a mannosyl-PP-C_{55} lipid rather than GDP-mannose as a sugar donor.[6] This observation is in concurrence with the prospect that mannosylation occurs during secretion of this antibiotic as well as for ramoplanin[7] and is in contrast to the mechanism of glycosyl transfer that occurs in compounds **1**−**4** and **6**−**9**.[8−13] Such glycosyl transfers may serve a protective function in the producer cell. This is the strategy used by the oleandomycin producer. Glycosylation with consequent temporary inactivation of the nascent antibiotic occurs intracellularly in the producing microorganism.[14] Once the latent antibiotic is secreted, hydrolytic glycosidases reactivate the antibiotic warhead via hexose removal.

In the remaining examples noted here, the hexoses attached to the antibiotic aglycone cores are specialized deoxy-, aminodeoxy-, and methylated deoxyhexoses that offer a mix of hydrophobic and hydrophilic surfaces as well as hydrogen-bonding capacity that most likely enable specific recognition by the biological targets. The functional importance of these specialized deoxyhexoses

† Adapted from the Alfred Bader Award Lecture in Bioinorganic or Bioorganic Chemistry. Presented at the 225th National Meeting of the American Chemical Society, New Orleans, LA, March 23−27, 2003.

* To whom correspondence should be addressed. E-mail: christopher_walsh@hms.harvard.edu. Phone: 617 432 0930. Fax: 617 432 0438.

10.1021/jm030257i CCC: $25.00
Published on Web 07/04/2003

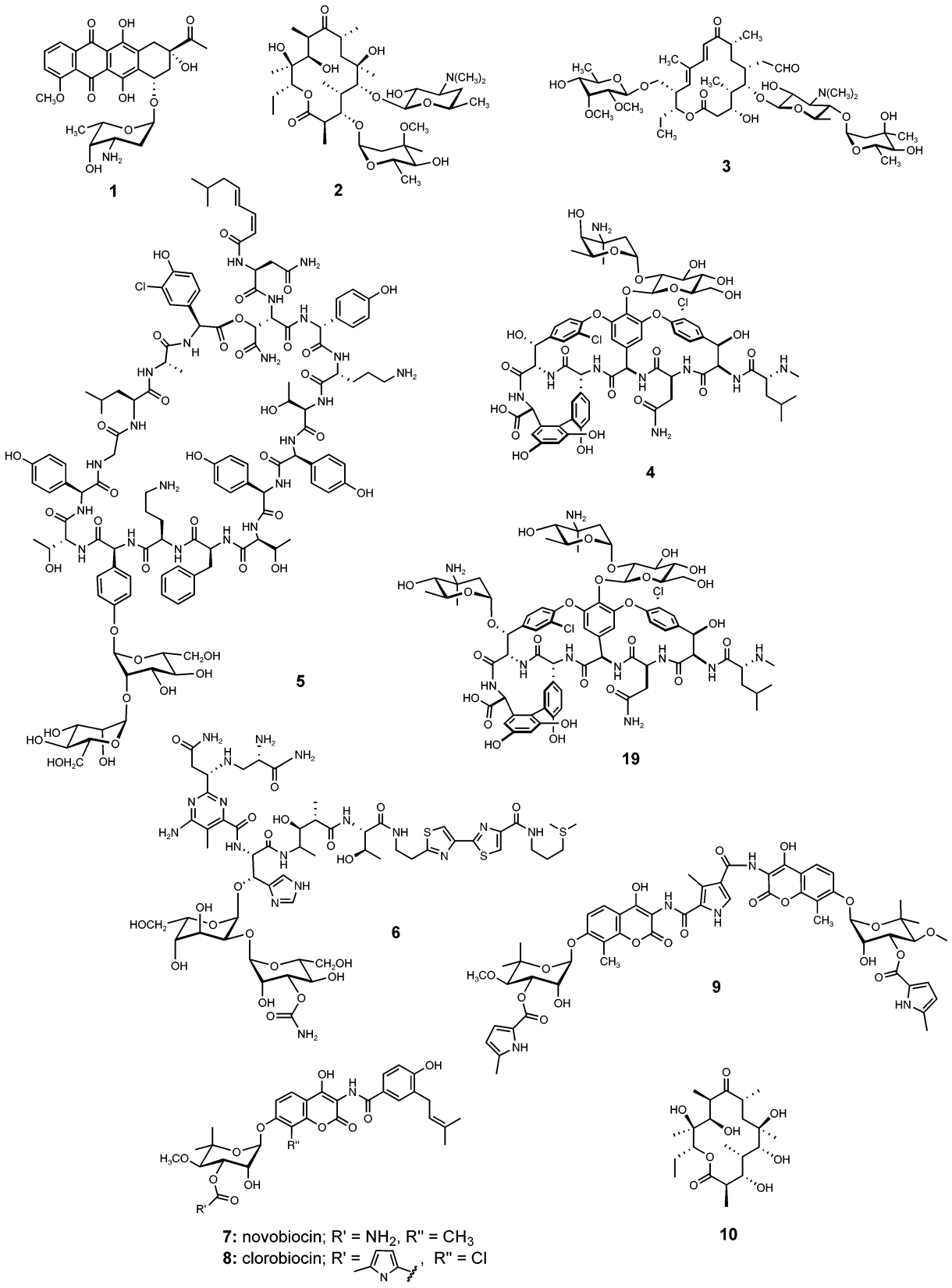
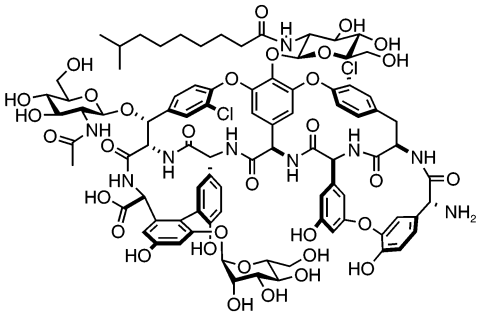
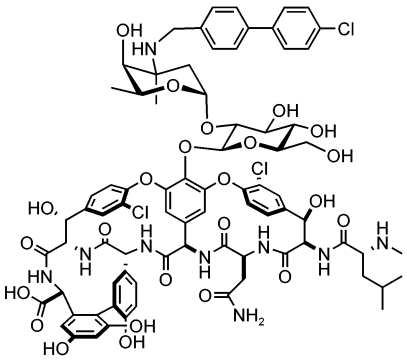


Figure 1. Glycosylated natural products.

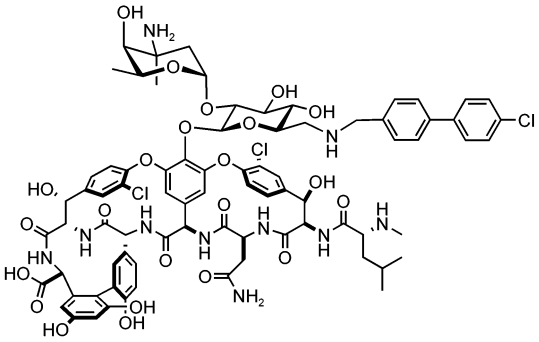
is particularly clear for the macrolide antibiotic family. The macrolactone aglycone 6-deoxyerythronolide **10** (Figure 1) exhibits no antibiotic activity. The sugars in erythromycin **2**, cladinose, and most particularly desosamine are crucial for antibiotic efficacy (Figure 2). The recent structural determinations of the 50S subunit of bacterial ribosomes complexed with macrolide antibiotics[15,16] have revealed the molecular basis for desosamine moiety recognition in blockade of bacterial protein synthesis. The 14- or 16-membered macrolactone is bound by 23S rRNA between the peptidyltransferase center (PTC) and the exit tunnel for the nascent polypeptide. The structure of carbomycin (**11**) in the 50S subunit confirms the positioning of the acyl disaccharide



12: Teicoplanin



13: Oritavancin

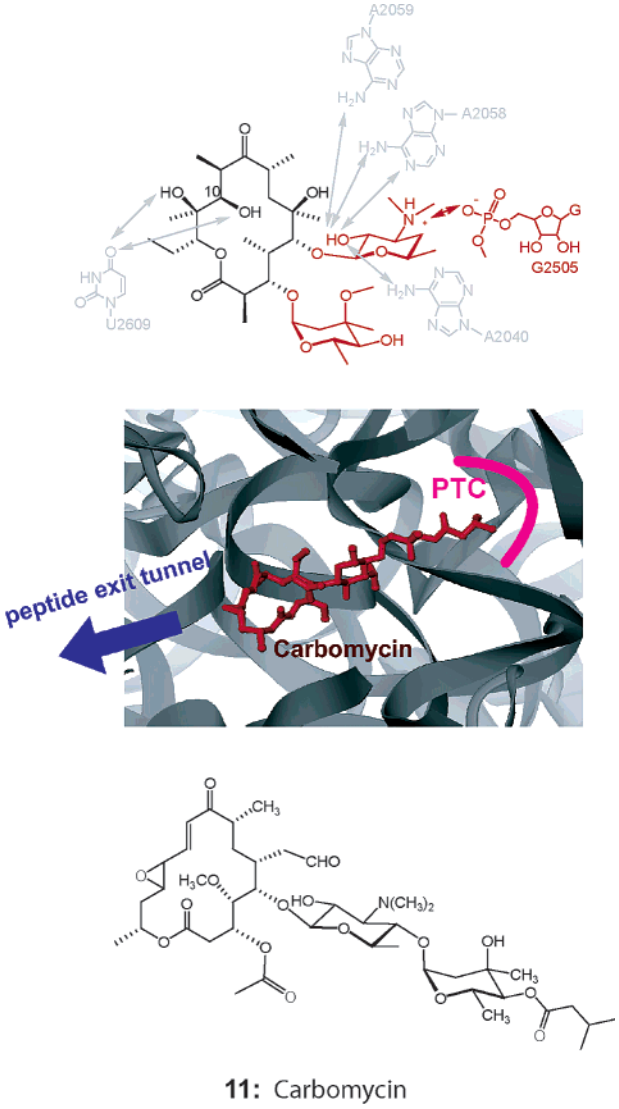


36: Chlorobiphenyl 6-aminoglucosyl Vancomycin

Figure 2. Glycopeptide antibiotics.

chain at the 5-position of the macrolactone reaching back to the PTC, thereby blocking peptide bond formation (Figure 3). It is likely there will be much effort to optimize the acyl and sugar substituents at the 5-hydroxyl of macrolides to occupy this region near the PTC. It is anticipated that these efforts will result in the generation of novel antibiotics.

In the glycopeptide antibiotic family, the emergence of clinically significant vancomycin-resistant enterococci (VRE) has prompted close examination of structural variants in an effort to overcome resistance.[17−19] The lipid chain in teicoplanin (**12**, Figure 2) enables resistance to the VRE of clinical phenotype B. Conversely, the synthetic analogue oritavancin (**13**, Figure 2) bearing a chlorobiphenyl moiety introduced via reductive alkylation at the amino group of the epivancosamine sugar is effective against both VanA and VanB phenotypes of VRE. These lipid modifications of the sugar may well serve to partition the glycopeptide portion of the molecule to the interface of the peptidoglycan and outer cell membrane, thereby altering the fraction of the molecule targeting the transpeptidase and transglycosylase steps of cell wall rigidification.[18]



11: Carbomycin

Figure 3. Carbomycin blocks peptide bond formation.

The L-noviosyl sugar in the aminocoumarins **7**−**9** serves as a platform for display of the 3′-*O*-acyl groups to the active site of the GyrB (Figure 4) and thereby blocks the ATP binding required for enzymatic topological isomerization of DNA during DNA replication.[4] In a real sense, the remainder of the molecule is a scaffold for precise presentation of the decorated L-deoxy sugar to the target enzyme.

Biosynthetic Logic: NDP-Hexose Formation and Glycosyltransferase-Mediated Transfers

Most often, the glycosylation steps occur late in the biosynthetic maturation of antibiotics following PKS and NRP assembly of the aglycones, as in erythromycin or vancomycin biosynthesis, and following release of the tetracyclic aglycone from the PKS machinery in the daunomycin-producing streptomycetes. Analogously, the L-noviosyl unit is transferred after the aminocoumarin and prenylbenzoyl moieties have been enzymatically

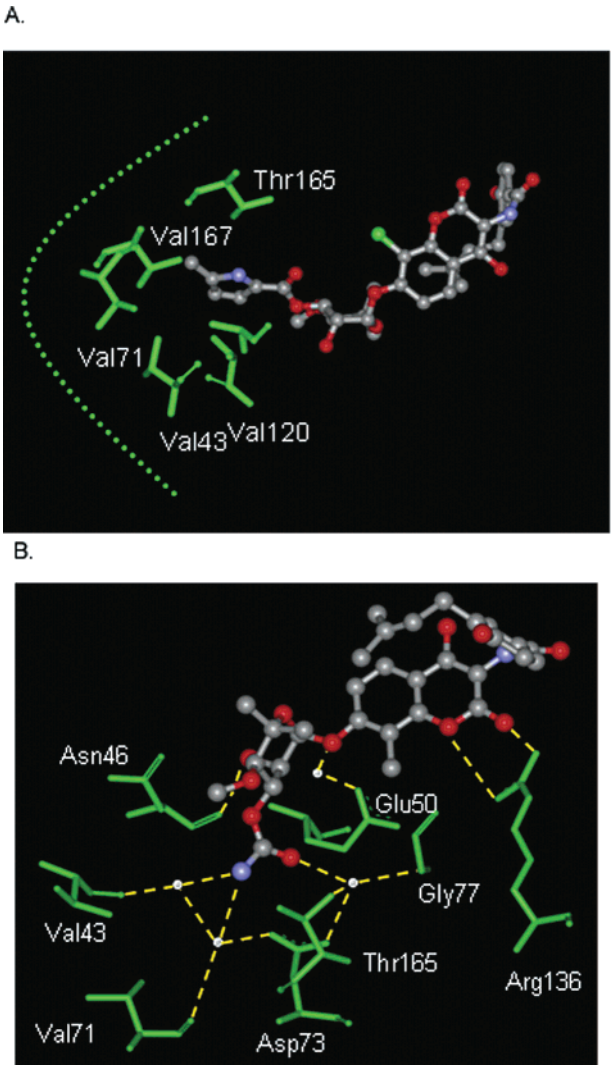


Figure 4. (A) Novobiocin bound to GyrB. (B) Clorobiocin bound to GyrB.

ligated.[12,20] Thus, the general stoichiometry for glycosyltransferase (Gtf) enzymes is as shown in Scheme 1 where a TDP-hexose is the donor substrate undergoing nucleophilic attack by a hydroxyl moiety on the aglycone. The universal biological reagents for hexosyl transfer, the NDP sugars, display the C_1 position of the sugar activated by the nucleoside diphospho moiety. The nucleoside diphospho moiety functions as a low-energy leaving group in Gtf-catalyzed reactions. Essentially all biological glycosyl transfers therefore involve capture of the glycosyl substrate at the electrophilic C_1 locus.

Two general characteristics of antibiotic glycosyltransferases are noteworthy. First, the late timing of Gtf action in the antibiotic biosynthetic processes makes these transformations optimally located for reprogramming and/or combinatorial biosynthetic approaches. The introduction of structural diversity late in the biosynthetic pathway can potentially lead to novel functions in new antibiotics. Second, the vast majority of glycosyltransferases catalyze activated hexosyl transfer to hydroxyl substituents on cosubstrate aglycones; however, amine and carbon nucleophiles exist such as those found in rebeccamycin (**14**, Figure 5) biosynthesis[21] and in urdamycin biosynthesis (**15**). In urdamycin biosynthesis, the carbon nucleophile is ortho to a phenolic hydroxyl leading to C-glycoside formation in the biogenesis of anguicycline classes of polyketide antibiotics (Figure 4).[3,22] As yet, these N- and C-glycosyltransferases have not been thoroughly studied for specificity or promiscuity.

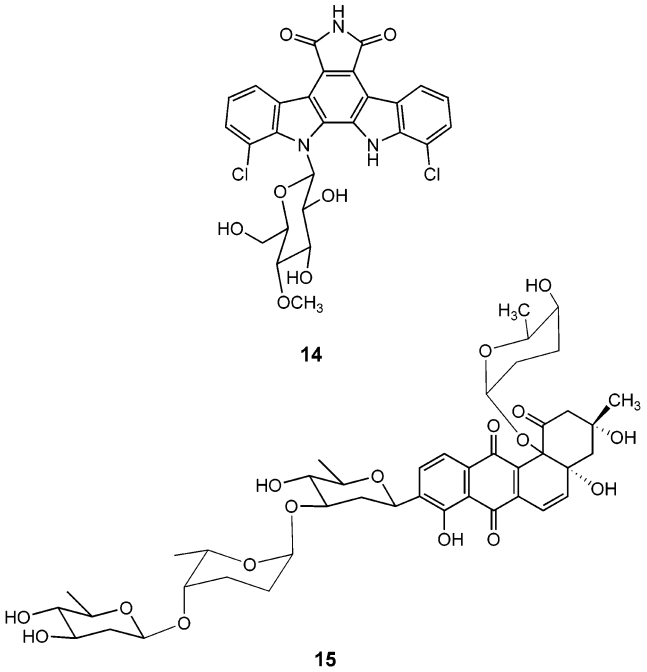
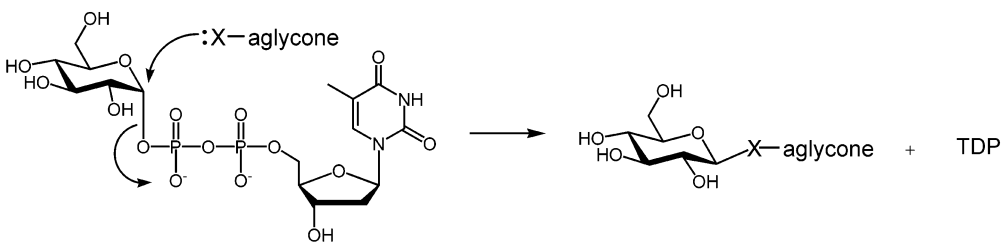


Figure 5. N- and C-glycosylated natural products.

TDP-D-glucose (**16**, Figure 6) and GDP-D-mannose (**17**) arise by the action of TTP and GTP pyrophosphorylases on sugar-1-P substrates, respectively,[23] and are common starting points for NDP sugar modification reactions. Some antibiotic Gtfs utilize D-glucose (e.g., in rebeccamycin biosynthesis and in the first step of vancomycin aglycone decoration), but the majority of antibiotic biosynthetic gene clusters contain genes that encode enzymes that catalyze the modification of TDP-D-glucose to afford TDP-deoxy and TDP-aminodeoxy sugars. Furthermore, TDP-hexose modification pathways often contain enzymes that act on TDP-4-keto sugar intermediates for epimerization at C_5, a transformation resulting in the generation of TDP L-sugars, as noted below.

Scheme 1. Glycosyltransferase Mechanism of Catalysis



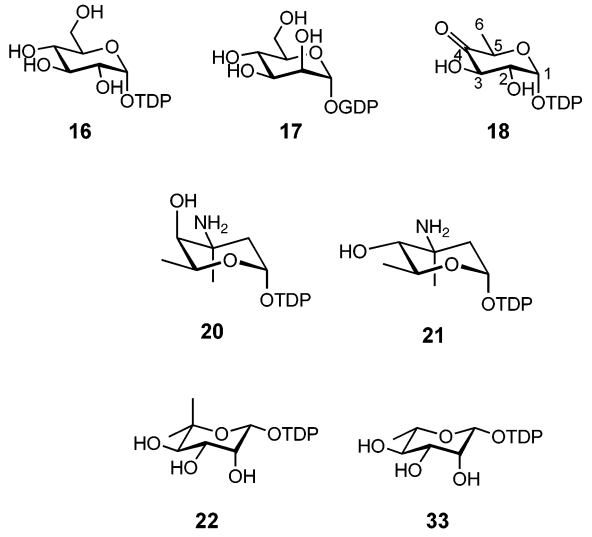


Figure 6. NDP-hexose structures.

TDP-Hexose Tailoring Enzymes

Over the past 2 decades, the enzymatic logic of TDP-deoxy- and aminodeoxyhexose formation has been deciphered, with sustained significant contributions by the Liu group.[24,25] These studies have shown that TDP-4-keto-6-deoxyglucose (**18**, Figure 6) is a common early intermediate and that the 4-keto functionality enables stabilization of carbanion formation at C_3 and C_5. In TDP-D-desosamine biosynthesis (Scheme 2), carbanion formation at C_3 leads to epimerization at C_3 or elimination of the C_2-hydroxyl in 2,6-dideoxyhexose and 3,4,-diketo intermediate biosynthesis. The 3-keto group can be reductively aminated to produce 3-amino-(2,6-di)-deoxyhexoses. The TDP-3-amino-4-keto-6-deoxyhexose then undergoes deoxygenation at C_4 and N,N-dimethylation to afford the TDP-D-desosamine as the key sugar donor in macrolide antibiotic decoration.[26] In TDP-vancosamine (**20**) biogenesis in the vancomycin pathway (Figure 6), the TDP-C_3-amino-2,6-dideoxy-4-keto intermediate instead undergoes C-methylation at the 3′-position. Conversion of TDP-D-deoxyaminohexoses to TDP-L-aminodeoxyhexoses requires C_5 epimerization accomplished via C_5 carbanion formation enabled by the adjacent C_4 ketone. Finally, the 4-keto undergoes enzymatic reduction in the last step. In the biosynthesis of TDP-L-vancosamine (vancomycin **4**, Figure 1), the resulting 4-hydroxyl is axial, while in the biosynthesis of TDP-L-epivancosamine (chloroeremomycin **19**), the corresponding 4-hydroxyl is equatorial. This difference in stereochemistry reflects the stereospecificity of the TDP-4-keto-L-hexose reductases in these pathways. The pervasive changes in the hexose skeleton during the six-step conversion of TDP-D-glucose to TDP-L-epivancosamine (**21**) are summarized in Table 1. Analogously, the biological generation of TDP-L-noviose **22** (Figure 6) proceeds through TDP-4-keto-6-deoxyglucose; however, the *C*-methyltransferase in this pathway is regioselective for C_5 methylation, and there is a putative C_3 epimerase that catalyzes the inversion of stereochemistry at C_3 prior to reduction at the C_4 ketone.[12]

Analysis of Antibiotic Gtf Specificity in Vivo and in Vitro

In Vivo. The burgeoning of microbial genome databases in the past half decade has revealed numerous antibiotic biosynthetic gene clusters containing putative genes for TDP-deoxyhexose production as well as the associated dedicated glycosyltransferases. Assignment of glycosyltransferase function has been validated in several pathways by insertional inactivation of specific genes.[7,10,27−32] These studies have, in turn, created opportunity for plasmid-mediated replacement and substitution of both TDP-deoxyhexose and glycosyltransferase biosynthetic genes such that the resulting mutant producer organisms may be used as cell factories[3] for the production of modified antibiotics. These studies include engineering the daunomycin pathway to make both 4′-epimers of glycosylated anthracyclines[33] and variation of sugar moieties in the pikromycin-producing *Streptomyces venezuleae*[34,35] and the urdamycin-producing *Streptomyces fradiae*.[36] In these cases, accumulating TDP-hexose pathway intermediates upstream of the gene disruption are transferred by glycosyltransferases. Alternatively the heterologous expression of Gtfs from one antibiotic producer in cells of a different antibiotic producer has led to fermentations that produce sugar-modified erythronolide and tylactone scaffolds. Examples include the generation of 3-*O*-rhamnosyl-6-DEB (**23**, Figure 7) via expression of the oleandomycin oleGII gene[37] and 5-*O*-desosaminyl tylactone (**24**, Figure 7) generation by the tylM2 gene in *S. erythrea*.[38] In aromatic polyketide glycosyl tailoring, a diolivosyltetracenomycin C (**25**, Figure 7) is produced in *S. argillaceus* via induced expression of the elmGT gene.[39] There is little doubt that the number of strategies of in vivo modification of the glycosylation patterns of polyketide, nonribosomal peptide, and hybrid PK/NRP antibiotics will continue to expand as more gtf genes are sequenced. Thus, the possibility increases for the establishment of microbial factories designed to produce natural products tailored with novel glycosyl moieties that can be screened for new activities.[3] A potential limitation in this process may be the availability of the TDP-D- and TDP-L-

Scheme 2. TDP-D-desosamine Biosynthetic Pathway

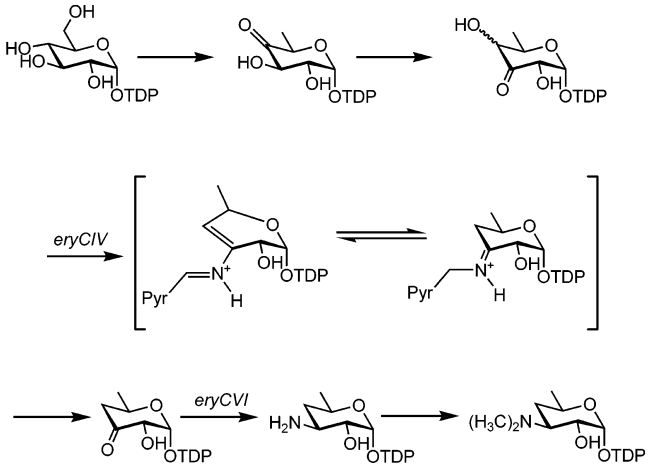


Table 1. Hexose Skeleton Manipulation in TDP-L-epivancosamine Biosynthetic Pathway

position	transformation	enzyme
C4 and C6	oxidation/reduction	RfbB
C2	deoxygenation (OH → H)	EvaA
C3	amination (OH → NH_2)	EvaB
C3	methylation (OH → CH_3)	EvaC
C5	epimerization (*R* → *S* and overall D → L)	EvaD
C4	keto reduction (overall *R* → *S*)	EvaE

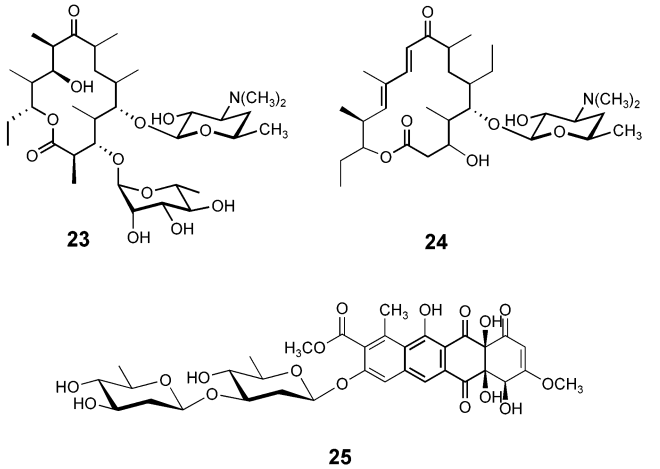


Figure 7. Sugar-modified natural products.

deoxyhexoses in such antibiotic producing cells, even when the corresponding gene pathways are engineered.

In Vitro. Evaluation of the synthetic potential of antibiotic Gtfs in vitro requires the availability of purified Gtfs in soluble, active form as well as the aglycone scaffolds and partially glycosylated intermediates. In addition, and most notably, access to the TDP-D-deoxyhexose and TDP-L-deoxyhexose substrates is required. Of these three limiting factors, the aglycones have been the most readily available reagents and are generally available via controlled, partial degradation of the parent antibiotics. Thus, the aglycones **26** (AGV) and **27** (AGT) are readily obtained from vancomycin and teicoplanin, respectively (Figure 8), and novobiocic acid **28** can be obtained by acid-catalyzed hydrolysis of novobiocin. Variant aglycone structures are also available via total synthesis efforts or from semisynthetic modification of the authentic aglycones.[40−43]

The Gtf compounds can, in principle, be obtained by large-scale growth of the antibiotic producer organisms followed by classical purification techniques. Alternatively, glycosyltransferases can be obtained by heterologous expression in more user-friendly organisms such as *E. coli* or *Streptomyces lividans*. Heterologous expression can yield preparatively useful amounts of enzyme, although expression in a soluble, active form is often challenging. We shall note two successful examples from our efforts in the following section.

The most challenging aspect of systematic in vitro exploration of antibiotic glycosyltransferase specificity has been the variable accessibility of NDP-sugar substrates. It has been possible to generate TDP-D-glucose analogues both synthetically and enzymatically[41] for the substrate specificity study of glycosyltransferases that utilize TDP-D-glucose as substrate, as is the case for GtfB and GtfE in the chloroeremomycin and vancomycin producers, respectively. Few other TDP-D-deoxyhexoses, such as TDP-D-desosamine used in the first step of 6-deoxyerythronolide decoration, have been prepared chemically. TDP-L-deoxyhexose sugars are, in general, less available. We have previously reported the reconstitution of the TDP-L-epivancosamine (**21**) pathway in a one-pot incubation of TDP-D-glucose in the presence of six purified enzymes.[44] This preparation resulted in the formation of TDP-L-epivancosamine in amounts sufficient for the in vitro characterization of GtfA and GtfC as catalysts for the transfer of this sugar to the heptapeptide aglycone scaffolds in the chloroeremomycin and vancomycin pathways.[45] However, it is a heroic endeavor to utilize six enzymes for the preparation of one NDP sugar to use for a single glycosyltransferase characterization and substrate specificity study. Therefore, chemical syntheses of UDP-L-β-epivancosamine and TDP-L-β-noviose **22** (Figure 6) have been crucial to the efforts noted below.[20,45]

Case Study 1: Gtfs in the Glycopeptide Antibiotic Pathways

Vancomycin (**4**) and chloroeremomycin (**19**, Figure 1) share the identical cross-linked heptapeptide aglycone

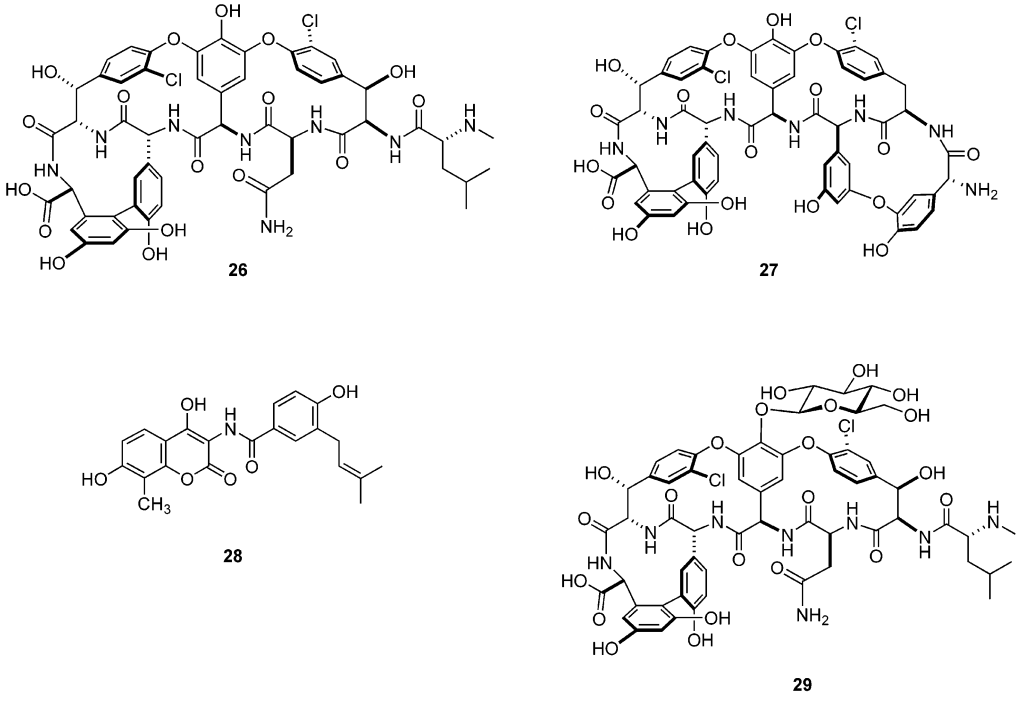
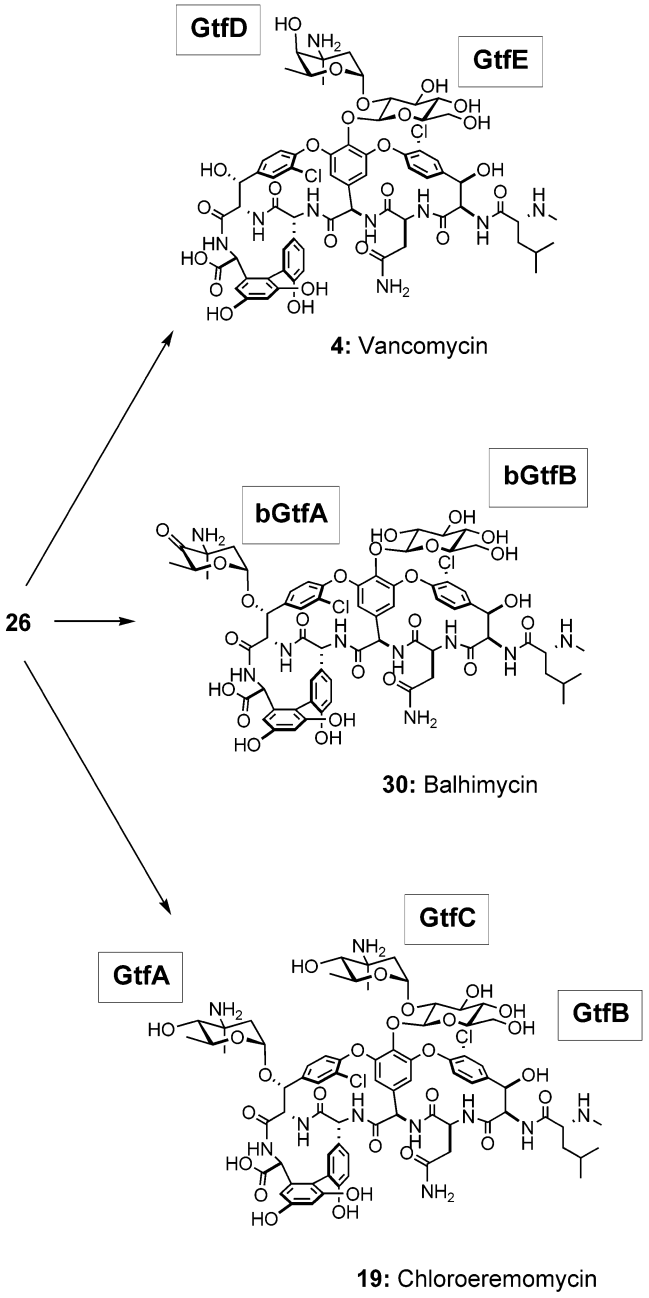
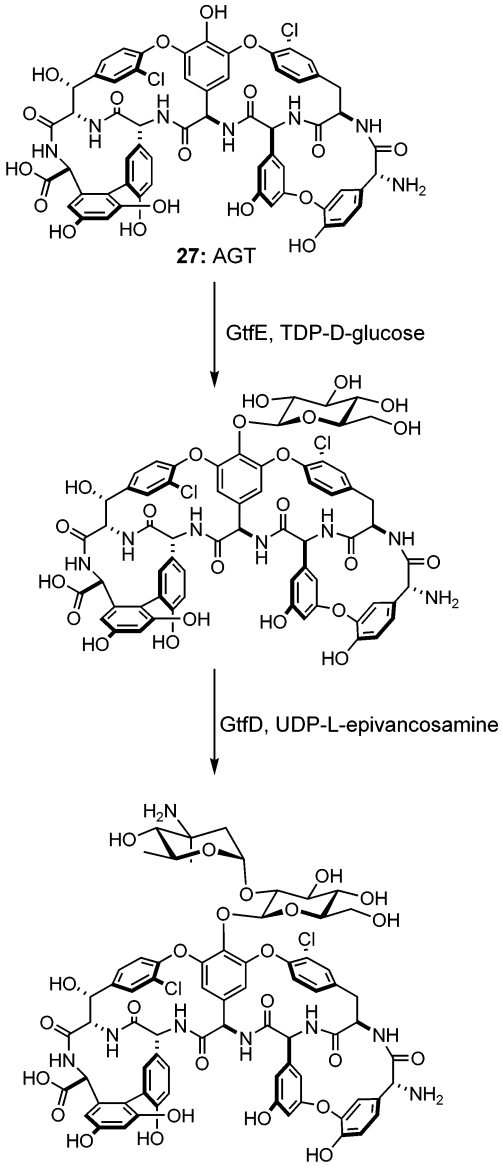


Figure 8. Aglycone structures.

Scheme 3. Gtf Compounds in Glycopeptide Antibiotic Pathways



Scheme 4. Aglycone Substrate Specificity of Glycopeptide Antibiotic Gtf Compounds



26 (Figure 8) but display differences in their glycosylation pattern. These differences reflect both the number and specificity of the tailoring Gtfs as well as differences in the TDP-L-aminodeoxyhexose biosynthetic pathways at the last, 4-keto reductive step. In the vancomycin and chloroeremomycin pathways, the initial glycosylation is the same (Scheme 3), by the action of GtfE or GtfB, respectively,[41,45] for the transfer of a glucosyl moiety from TDP-D-glucose to the phenolic hydroxyl of $Phegly_4$ of **26** to form the monoglucosylated product known as desvancosaminylvancomycin (**29**, DVV). In vancomycin maturation, GtfD catalyzes the regioselective transfer of L-vancosamine from TDP-L-vancosamine to the 2′-hydroxyl moiety of the glucosyl moiety in DVV, generating the vancosaminyl-1,2-glucosyl disaccharide chain and completing the last step in vancomycin production. In chloroeremomycin biosynthesis (Scheme 3), GtfC is the congener of GtfD, creating a similar linkage but utilizing the 4′-epimer of vancosamine as TDP-L-epivancosamine substrate. A third glycosyltransferase, GtfA (Scheme 3), exhibits distinct regioselectivity and is present in chloroeremomycin and balhimycin gene clusters but not in the vancomycin gene cluster. The nucleophilic substrate is the benzylic β-hydroxyl of the β-OH-Tyr_6 residue in DVV **29**. In chloroeremomycin biosynthesis, GtfA uses the same NDP-sugar substrate as GtfC (TDP-L-epivancosamine). However, the final 4-keto reductase in the TDP-epivancosamine biosynthetic pathway does not function in the balhimycin producer. This leads to accumulation of the TDP-4-keto intermediate (4-oxo-vancosamine), which is subsequently transferred by the action of GtfA to yield balhimycin **30**.

The D-glucosyltransferases GtfB and GtfE and the vancosaminyl- and epivancosaminyltransferases GtfC and GtfD have undergone preliminary examination for promiscuity with regard to both aglycone and NDP-sugar substrates. Studies carried out on aglycone variation have involved replacement of the natural substrate vancomycin aglycone (AGV) **28** (Figure 8) with the teicoplanin aglycone **27** (AGT) scaffold. AGT differs from

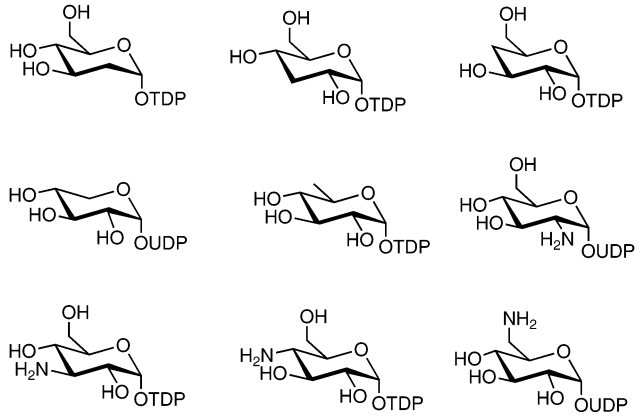


Figure 9. Deoxy- and amino-TDP-D-glucose analogues as alternative NDP-sugar substrates.

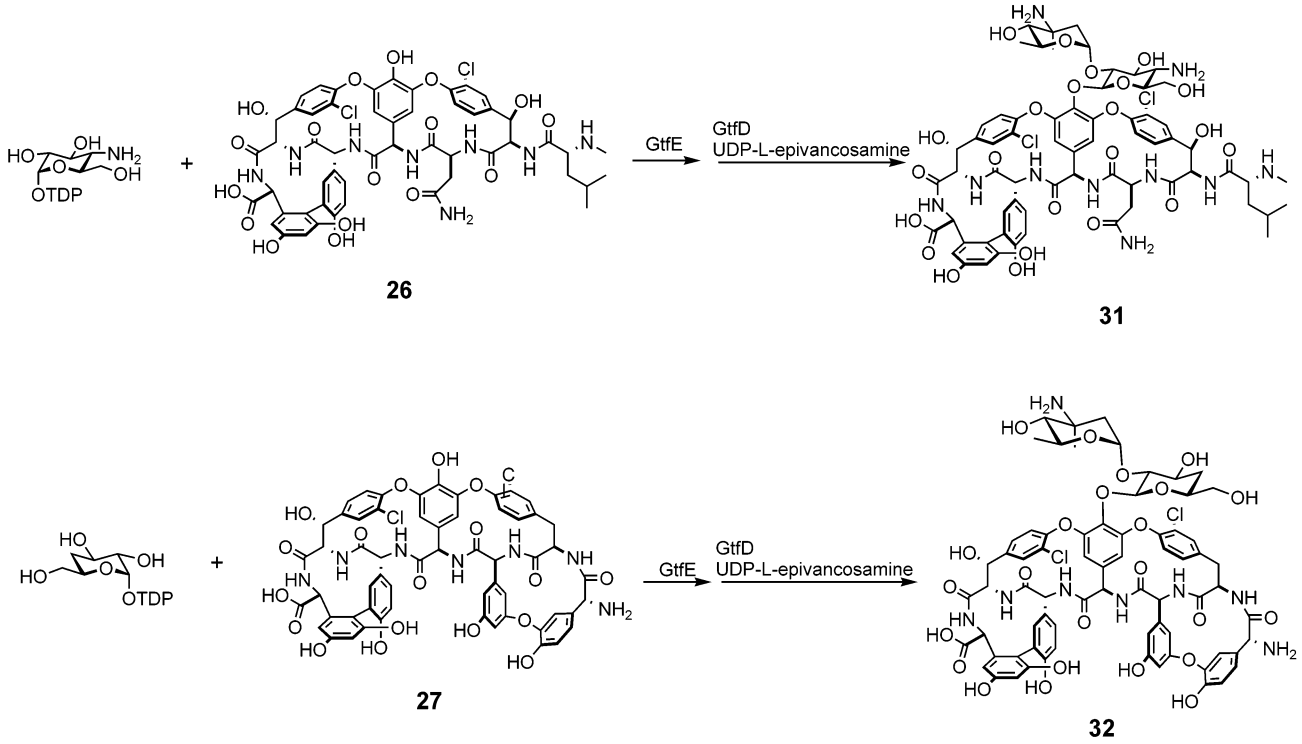
AGV at residues 1 and 3, where the side chains are cross-linked to produce a fourth cross-link in the AGT aglycone (1−3, 2−4−6, 5−7). AGT was processed by the GtfB/C combination and also the GtfE/D combination using TDP-D-glucose and UDP-L-epivancosamine as substrates to produce the epivancosaminyl-1,2-glucosyl disaccharide on the $Phegly_4$ of AGT (Scheme 4).

The assembly of a small library of TDP-D-glucose analogues both by synthesis and by enzymatic transfer of sugar-1-Ps to TTP[41] to make all four variants (2′-, 3′-, 4′-, 6′-) of TDP-D-deoxyglucose and the corresponding four regioisomers of TDP-D-aminoglucose (Figure 9) enabled a study of GtfD and GtfE in which variation of the NDP-hexose substrate was undertaken. Each TDP-D-glucose variant was transferred to AGV and AGT by GtfE. Selected analogues of the glucosylated scaffolds generated in this process were analyzed as substrates for GtfD with UDP-L-epivancosamine as cosubstrate. Thus, it was possible to produce **31** bearing an L-epivancosaminyl-1,2-D-4-aminoglucosyl disaccharide chain on AGV (Scheme 5) or **32** bearing an L-epivancosaminyl-1,2-D-4-deoxyglucosyl chain on the AGT scaffold.[41] The lack of a library of TDP-L-aminodeoxy sugars to date has precluded a study of the promiscuity of GtfC and GtfA toward TDP L-sugar donors. The 3-, 4-, and 6-aminoglucosyl analogues are of interest because it is known that reductive alkylation of the amino group in the 6-amino vancomycin analogue with chlorobiphenylaldehyde (**36**) leads to a regain in activity against VRE.[46] This analogue mimics the activity of the lipoglycopeptide oritavancin **13** where the chlorobiphenyl is on the epivancosamine ring (Figure 2) and suggests that additional lipoglycopeptide variants on either residue of the disaccharide moiety may have therapeutic utility against vancomycin-resistant pathogens.

Case Study 2: Gtf and Subsequent Tailoring Enzymes in Amincoumarin Antibiotic Maturation

The aminocoumarin antibiotics **7**−**9** (Figure 1) have not achieved wide clinical use because of suboptimal pharmacokinetic and toxicity properties and poor penetration into Gram-negative bacteria.[47−49] However, they target DNA gyrase, the same essential bacterial enzyme target of the quinolones ciprofloxacin and levofloxacin.[50−54] The quinolones target primarily the GyrA subunit, while the aminocoumarins block the ATPase activity of the GyrB subunit and may therefore be more useful as the number of quinolone-resistant bacterial pathogens increases. Novobiocin and clorobiocin comprise three components: the aminocoumarin, prenylbenzoate, and L-noviosyl units.[12] Each component is biosynthesized separately and ligated sequentially, with TDP-L-noviose **22** (Figure 6) and novobiocic acid **28** acting as substrates for the glycosyltransferase NovM. NovM was subcloned from *S. spheroides*, heterologously expressed in *E. coli*, and purified in soluble

Scheme 5. NDP-hexose Substrate Specificity of Glycopeptide Antibiotic Gtf Compounds



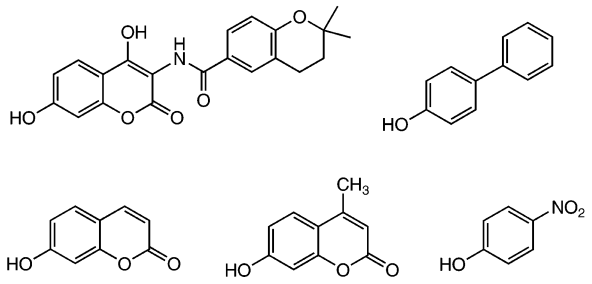


Figure 10. Alternative aglycone substrates for glycosyltransferase NovM.

form in good quantity.[20] The aglycone **28** was available via acid degradation of **7**, while TDP-L-noviose required an 11-step synthesis. NovM was found to be robustly active (>300 catalytic turnovers per minute). Notably, NovM exhibits some promiscuity toward some simplified coumarin scaffolds as aglycone substrates,[20] suggesting that a library approach to aminocoumarin analogue synthesis would be worthwhile (Figure 10). Surprisingly, NovM is inactive in the presence of the structurally related NDP-sugar analogue TDP-L-rhamnose **33** (Figure 6), indicating that the 5,5-dimethyl substituent may be critical for NovM recognition. Further evaluation of NovM specificity toward NDP sugars will require a library of TDP-L-deoxyhexoses.

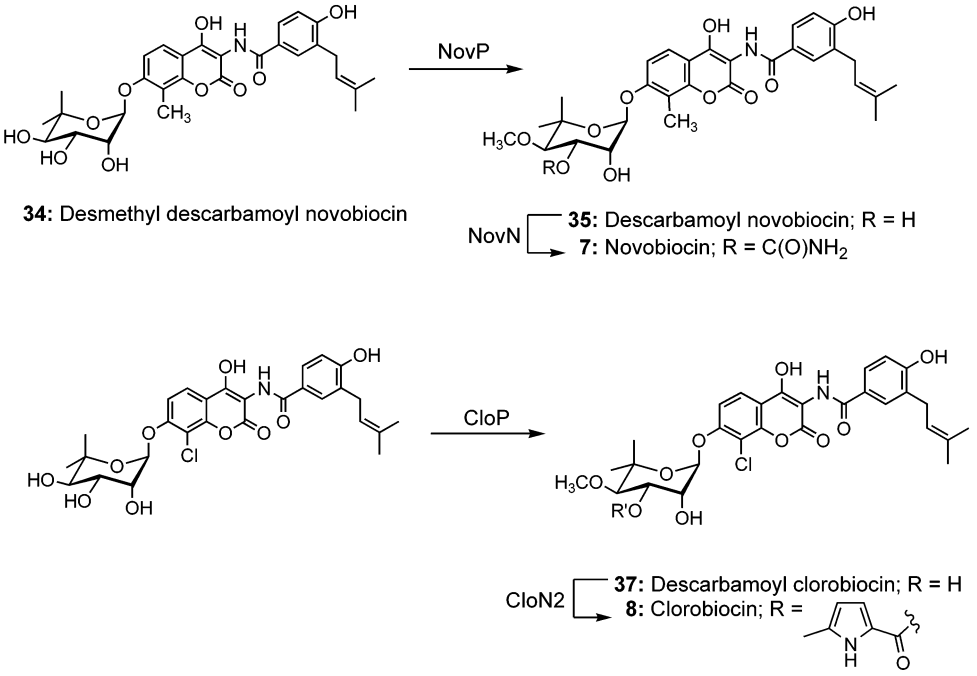
The product from NovM action is desmethyldescarbamoylnovobiocin **34** (Scheme 6) and is two enzymatic steps from the final antibiotic novobiocin. These enzymes are encoded by the novP and novN genes adjacent to the novM gene in the novobiocin gene cluster.[12] NovP can be expressed in *E. coli* and catalyzes O-methylation at the 4′-hydroxyl of **34**, yielding the descarbamoyl intermediate **35** (Scheme 6).[55] It is anticipated that NovN will carry out the 3′-O-carbamoylation and complete the biosynthetic pathway to novobiocin. Carbamoylation by the action of NovN is critical for antibiotic activity;[56] the functional importance of this substituent has been explained by cocrystallization of novobiocin with an N-terminal fragment of the GyrB subunit.[4] The crystallographic data suggest that the 3′-*O*-carbamoyl group on the noviosyl ring interacts with two structured water molecules in the ATP binding site (Figure 4). Clorobiocin **8** (Figure 1) binds roughly an order of magnitude more tightly than novobiocin to the same GyrB site.[4] Crystallographic studies reveal that the larger 3′-O-acyl moiety, in this case 5-methylpyrrolyl, displaces enzyme-bound water molecules and makes hydrophobic contacts to the enzyme. The final step of clorobiocin biosynthesis involves CloN2[57] in place of NovN, with altered specificity for acyl group transfer to **37** (Scheme 6). It is likely that a combination of chemoenzymatic approaches (NovM, -P, then chemical or enzymatic acylations by NovN or CouN2) will allow exploration and optimization of acyl group presentation by the L-noviosyl sugar platform to the GyrB subunit.

Prospects for Combinatorial Biosynthesis via Antibiotic Glycoysltransferases

Modulation of the identity, placement, and decoration of deoxy sugars on several classes of antibiotics holds promise for altered functional characteristics. Initial studies both in vivo and in vitro have provided evidence that permissivity may be anticipated for the glycosyltransferases that act in the late stages of antibiotic assembly. To date, the in vivo studies have been initiated with transfection of glycosyltransferases from one host to a separate producing cell system.

What are the prospects for the evolution of catalytic activity of antibiotic Gtfs to alter specificity for either the aglycone and/or the deoxysugar being transferred? In vitro studies with purified Gtfs in the glycopeptide systems have allowed crystallization and structure determination, revealing a common two-domain architecture for this Gtf family,[58−60] exemplified by the GtfB and GtfA structures in the chloroeremomycin maturation pathway (Figure 11) as well as for the MurG enzyme in lipid II formation in bacterial peptidoglycan

Scheme 6. Glycosylation and Tailoring of Novobiocic Acid and Clorobiocic Acid



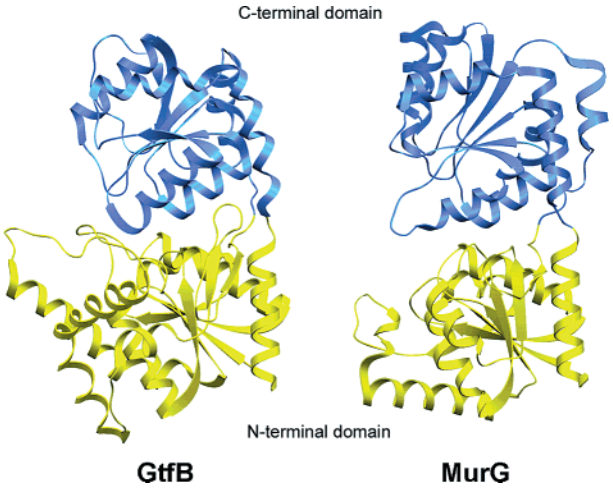


Figure 11. Glycosyltransferase superfamily exhibits a two-domain architecture.

assembly. The NDP sugar binds to the C-terminal domain,[58] while the aglycones (AGV in GtfB and DVV in GtfA) bind to the N-terminal domain. This bilobal architecture joined by only two peptide linkers suggests that it should be possible to mix and match autonomous domains. Thus, DNA shuffling or related enzyme evolution approaches could create chimeric Gtfs with separate altered specificities for the hexose moiety in the NDP-hexose and aglycone substrates. Such hybrid Gtfs could be tested both in vitro and in vivo for altered specificity.

In summary, three requirements need to be met for systematic diversification of antibiotic glycosylation patterns during biosynthesis. First, the glycosyltransferases encoded by gtf genes will be a source for late-stage antibiotic maturation and diversification of the sugars appended to the aglycone cores of bioactive natural products. So far, these genes are found in dozens of antibiotic biosynthetic clusters and are likely to number in the hundreds as more clusters are sequenced. The possibility exists for "mixing and matching" of N- and C-terminal Gtf domains to create hybrid catalysts for reprogramming aglycone and deoxyhexose recognition and should produce novel composition and attachment sites for the specialized deoxyhexose moieties. A short-term challenge will be to express both wild-type and mutated Gtfs in heterologous hosts in soluble active forms and in useful quantities.

The second requirement is the necessity for aglycones libraries. The particular aglycone structural set will vary for each natural product family to be enzymatically glycosylated. These can range from simple aminocoumarin scaffolds to libraries of nonribosomal peptide aglycones and aromatic polyketide aglycones, many of which can be generated via combinatorial biosynthesis from type II polyketide synthases.[38] For example, the tolerance exhibited by GtfB and GtfE for changes in the cross-linked heptapeptide platform of the vancomycin and teicoplanin families has not yet been widely explored. Aglycones that are hybrids of polyketide and nonribosomal peptide scaffolds, e.g., in bleomycins or in synthetic PK/NRP hybrids,[61−63] also remain to be studied for Gtf permissivity. Aglycones providing nucleophilic nitrogen and carbon sites for N- and C-glycosylation for cognate Gtfs also merit further examination.

Third is the need for libraries of NDP-D-deoxy- and aminodeoxyhexoses and NDP-L-deoxy- and aminodeoxyhexoses. These will be most useful initially for in vitro evaluation of Gtf activity profiles. Combinations of 6-deoxy, 2,6-dideoxy, 6-deoxy-3-amino, and 2,6-dideoxy-3-amino variants with C-methylation at the 3- and 5-positions, N-methylation, *O*-methyl regioisomers, *O*-carbamoyl, and other *O*-acyl moieties in both the D- and L-hexose series, and present as UDP and TDP sugars, are obvious places to start in NDP-sugar library generation because these represent natural deoxyhexoses appended to aglycones. In the event that a particular sugar confers desired novel properties to an aglycone scaffold, the enzymes to make that TDP-hexose will need to be assembled and moved into a producer microorganism to begin in vivo fermentations. Acylation of such deoxy sugars are found in polyketides (carbomycin), nonribosomal peptides (teicoplanin), and aminocoumarins (e.g., clorobiocin), so acylated regioisomers of all of the TDP-D- and TDP-L-deoxyhexose variants in such libraries would be worth preparation.

Because Gtf compounds act as late or even in the last steps in antibiotic maturation, often conferring the target recognition ability on the natural product, they are efficient catalytic vehicles to introduce diversity with a high likelihood of producing bioactivity. Gtf-mediated transformations should be ideal for combination with strategies that reprogram earlier steps in antibiotic assembly enzymatic pathways.

Acknowledgment. We gratefully acknowledge Dan Kahne and co-workers for collaborations involving NDP-hexose synthesis, and Michael Garavito and Anne Mulichak for collaborations involving the Gtf X-ray structures. We acknowledge funding from the NIH (Grants GM20011-32, GM 49338, and AI42738 for Christopher T. Walsh, Caren L. Freel Meyers, and Heather Losey).

Biographies

Christopher Walsh is the Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. His experience in academic administration includes Chairmanship of the Massachusetts Institute of Technology Chemistry Department (1982−1987) and the Harvard Medical School Biological Chemistry and Molecular Pharmacology Department (1987−1995). He has also served as President and CEO of the Dana Farber Cancer Institute (1992−1995). His research has focused on enzymes and enzyme inhibitors, with recent specialization on antibiotics. Professor Walsh and his group have published over 500 research papers, and he has authored a text entitled Enzymatic Reaction Mechanisms (1979) and a book entitled Antibiotics, Origins, Actions, Resistance (2003).

Caren L. Freel Meyers received her Ph.D. in Chemistry from the University of Rochester under the direction of Richard Borch. Her graduate research focused on synthesis of nucleotide phosphoramidate prodrugs and elucidation of their activation mechanisms. She continued in a teaching/research postdoctoral program in Medicinal Chemistry and Molecular Pharmacology at Purdue University where she instructed Organic Chemistry under the guidance of G. Marc Loudon and conducted research under the guidance of Richard Borch. Her research interests included development of novel methods for the preparation of nucleotide diphosphates and strategies for nucleotide immobilization. She is currently a postdoctoral fellow in the laboratory of Christopher Walsh in Biological Chemistry and Molecular Pharmacology at Harvard Medical

School where she is studying glycosyltransferase and sugar-tailoring enzymes in novobiocin biosynthesis.

Heather C. Losey received her Ph.D. in Biological Chemistry and Molecular Pharmacology from Harvard University in December 2002 under the guidance of Professor Christopher T. Walsh. Her research focused on glycosyltransferases involved in the biosynthesis of the glycopeptide antibiotics vancomycin and chloroeremomycin. She is currently a postdoctoral fellow with Professor Gregory L. Verdine in the Department of Chemistry and Chemical Biology at Harvard University, where she is studying the structure and function of DNA-modifying proteins.

References

(1) Khosla, C. Harnessing the biosynthetic potential of modular polyketide synthases. *Chem. Rev.* **1997**, *97*, 2577−2590.

(2) Mootz, H. D.; Marahiel, M. A. Biosynthetic systems for nonribosomal peptide antibiotic assembly. *Curr. Opin. Chem. Biol.* **1997**, *1*, 543−551.

(3) Mendez, C.; Salas, J. A. Altering the glycosylation pattern of bioactive compounds. *Trends Biotechnol.* **2001**, *19*, 449−456.

(4) Gellert, M.; O'Dea, M. H.; Itoh, T.; Tomizawa, J. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4474−4478.

(5) Thorson, J. S.; Hoster, T. J.; Jiang, J.; Biggins, J. B.; Ahlert, J. Nature's carbohydrate chemists: the enzymatic glycosylation of bioactive bacterial metabolites. *Curr. Org. Chem.* **2001**, *5*, 139−167.

(6) Sosio, M.; Stinchi, S.; Beltrametti, F.; Lazzarini, A.; Donadio, S. The gene cluster for the biosynthesis of the glycopeptide antibiotic A40926 by *Nonomuraea* sp. *Chem. Biol.*, in press.

(7) Bate, N.; Butler, A. R.; Smith, I. P.; Cundliffe, E. The mycarose-biosynthetic genes of Streptomyces fradiae, producer of tylosin. *Microbiology* **2000**, *146* (1), 139−146.

(8) Solenberg, P. J.; Matsushima, P.; Stack, D. R.; Wilkie, S. C.; Thompson, R. C.; et al. Production of hybrid glycopeptide antibiotics in vitro and in *Streptomyces toyocaensis*. *Chem. Biol.* **1997**, *4*, 195−202.

(9) Galm, U.; Schimana, J.; Fiedler, H. P.; Schmidt, J.; Li, S. M.; et al. Cloning and analysis of the simocyclinone biosynthetic gene cluster of *Streptomyces antibioticus* Tu 6040. *Arch. Microbiol.* **2002**, *178*, 102−114.

(10) Summers, R. G.; Donadio, S.; Staver, M. J.; Wendt-Pienkowski, E.; Hutchinson, C. R.; et al. Sequencing and mutagenesis of genes from the erythromycin biosynthetic gene cluster of Saccharopolyspora erythraea that are involved in L-mycarose and D-desosamine production. *Microbiology* **1997**, *143* (10), 3251−3262.

(11) van Wageningen, A. M.; Kirkpatrick, P. N.; Williams, D. H.; Harris, B. R.; Kershaw, J. K.; et al. Sequencing and analysis of genes involved in the biosynthesis of a vancomycin group antibiotic. *Chem. Biol.* **1998**, *5*, 155−162.

(12) Steffensky, M.; Muhlenweg, A.; Wang, Z. X.; Li, S. M.; Heide, L. Identification of the novobiocin biosynthetic gene cluster of *Streptomyces spheroides* NCIB 11891. *Antimicrob. Agents Chemother.* **2000**, *44*, 1214−1222.

(13) Otten, S. L.; Liu, X.; Ferguson, J.; Hutchinson, C. R. Cloning and characterization of the *Streptomyces peucetius dnrQS* genes encoding a daunosamine biosynthesis enzyme and a glycosyltransferase involved in daunorubicin biosynthesis. *J. Bacteriol.* **1995**, *177*, 6688−6692.

(14) Vilches, C.; Hernandez, C.; Mendez, C.; Salas, J. A. Role of glycosylation and deglycosylation in biosynthesis of and resistance to oleandomycin in the producer organism, *Streptomyces antibioticus*. *J. Bacteriol.* **1992**, *174*, 161−165.

(15) Hansen, J. L.; Ippolito, J. A.; Ban, N.; Nissen, P.; Moore, P. B.; et al. The structures of four macrolide antibiotics bound to the large ribosomal subunit. *Mol. Cell* **2002**, *10*, 117−128.

(16) Schlunzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; et al. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* **2001**, *413*, 814−821.

(17) Cooper, M. A.; Williams, D. H. Binding of glycopeptide antibiotics to a model of a vancomycin-resistant bacterium. *Chem. Biol.* **1999**, *6*, 891−899.

(18) Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; et al. Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. *Science* **1999**, *284*, 507−511.

(19) Rodriguez, M. J.; Snyder, N. J.; Zweifel, M. J.; Wilkie, S. C.; Stack, D. R.; et al. Novel glycopeptide antibiotics: N-alkylated derivatives active against vancomycin-resistant enterococci. *J. Antibiot.* **1998**, *51*, 560−569.

(20) Freel Meyers, C. L.; Anderson, J. W.; Kahne, D.; Walsh, C. T. Initial characterization of novobiocic acid noviosyl transferase activity of NovM in biosynthesis of the antibiotic novobiocin. *Biochemistry* **2002**, *42*, 4179−4189.

(21) Sanchez, C.; Butovich, I. A.; Brana, A. F.; Rohr, J.; Mendez, C.; et al. The biosynthetic gene cluster for the antitumor rebeccamycin: characterization and generation of indolocarbazole derivatives. *Chem. Biol.* **2002**, *9*, 519−531.

(22) Hoffmeister, D.; Ichinose, K.; Domann, S.; Faust, B.; Trefzer, A.; et al. The NDP-sugar co-substrate concentration and the enzyme expression level influence the substrate specificity of glycosyltransferases: cloning and characterization of deoxysugar biosynthetic genes of the urdamycin biosynthetic gene cluster. *Chem. Biol.* **2000**, *7*, 821−831.

(23) Johnson, D. A.; Liu, H.-w. *Comprehensive Natural Products Chemistry: Carbohydrates and Their Derivatives Including Tannins, Cellulose, and Related Lignins*; Elsevier: New York, 1999; pp 312−322.

(24) He, X. M.; Liu, H. W. Formation of unusual sugars: mechanistic studies and biosynthetic applications. *Annu. Rev. Biochem.* **2002**, *71*, 701−754.

(25) He, X.; Liu, H. W. Mechanisms of enzymatic CbondO bond cleavages in deoxyhexose biosynthesis. *Curr. Opin. Chem. Biol.* **2002**, *6*, 590−597.

(26) Zhao, L.; Borisova, S.; Yeung, S. M.; Liu, H. Study of C-4 deoxygenation in the biosynthesis of desosamine: evidence implicating a novel mechanism. *J. Am. Chem. Soc.* **2001**, *123*, 7909−7910.

(27) Jenkins, G.; Cundliffe, E. Cloning and characterization of two genes from *Streptomyces lividans* that confer inducible resistance to lincomycin and macrolide antibiotics. *Gene* **1991**, *108*, 55−62.

(28) Xue, Y.; Zhao, L.; Liu, H. W.; Sherman, D. H. A gene cluster for macrolide antibiotic biosynthesis in *Streptomyces venezuelae*: architecture of metabolic diversity. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12111−12116.

(29) Wilson, V. T.; Cundliffe, E. Characterization and targeted disruption of a glycosyltransferase gene in the tylosin producer, *Streptomyces fradiae*. *Gene* **1998**, *214*, 95−100.

(30) Gandecha, A. R.; Large, S. L.; Cundliffe, E. Analysis of four tylosin biosynthetic genes from the *tylLM* region of the *Streptomyces fradiae* genome. *Gene* **1997**, *184*, 197−203.

(31) Olano, C.; Rodriguez, A. M.; Michel, J. M.; Mendez, C.; Raynal, M. C.; et al. Analysis of a *Streptomyces antibioticus* chromosomal region involved in oleandomycin biosynthesis, which encodes two glycosyltransferases responsible for glycosylation of the macrolactone ring. *Mol. Gen. Genet.* **1998**, *259*, 299−308.

(32) Salah-Bey, K.; Doumith, M.; Michel, J. M.; Haydock, S.; Cortes, J.; et al. Targeted gene inactivation for the elucidation of deoxysugar biosynthesis in the erythromycin producer *Saccharopolyspora erythraea*. *Mol. Gen. Genet.* **1998**, *257*, 542−553.

(33) Madduri, K.; Kennedy, J.; Rivola, G.; Inventi-Solari, A.; Filippini, S.; et al. Production of the antitumor drug epirubicin (4′-epidoxorubicin) and its precursor by a genetically engineered strain of *Streptomyces peucetius*. *Nat. Biotechnol.* **1998**, *16*, 69−74.

(34) Zhao, Y.; Ahlert, J.; Xue, Y.; Thorson, J. S.; Sherman, D. H.; et al. Engineering a methymycin/pikromycin-calicheamicin hybrid: construction of two new macrolides carrying a designed sugar moiety. *J. Am. Chem. Soc.* **1999**, *121*, 9881−9882.

(35) Borisova, S. A.; Zhao, L.; Sherman, D. H.; Liu, H. W. Biosynthesis of desosamine: construction of a new macrolide carrying a genetically designed sugar moiety. *Org. Lett.* **1999**, *1*, 133−136.

(36) Hoffmeister, D.; Ichinose, K.; Domann, S.; Faust, B.; Trefzer, A. et al. The NDP-sugar co-substrate concentration and the enzyme expression level influence the substrate specificity of glycosyltransferases: cloning and characterization of deoxysugar biosynthetic genes of the urdamycin biosynthetic gene cluster. *Chem. Biol.* **2000**, *7*, 821−831.

(37) Doumith, M.; Legrand, R.; Lang, C.; Salas, J. A.; Raynal, M. C. Interspecies complementation in *Saccharopolyspora erythraea*: elucidation of the function of oleP1, oleG1 and oleG2 from the oleandomycin biosynthetic gene cluster of *Streptomyces antibioticus* and generation of new erythromycin derivatives. *Mol. Microbiol.* **1999**, *34*, 1039−1048.

(38) Gaisser, S.; Reather, J.; Wirtz, G.; Kellenberger, L.; Staunton, J.; et al. A defined system for hybrid macrolide biosynthesis in *Saccharopolyspora erythraea*. *Mol. Microbiol.* **2000**, *36*, 391−401.

(39) Blanco, G.; Patallo, E. P.; Brana, A. F.; Trefzer, A.; Bechthold, A.; et al. Identification of a sugar flexible glycosyltransferase from *Streptomyces olivaceus*, the producer of the antitumor polyketide elloramycin. *Chem. Biol.* **2001**, *8*, 253−263.

(40) Povarov, L. S.; Isaeva, N. N. [Synthesis and properties of the 14-amino derivatives of daunorubicin and carminomycin]. *Antibiotiki* **1979**, *24*, 92−96.

(41) Losey, H. C.; Jiang, J.; Biggins, J. B.; Oberthur, M.; Ye, X.-Y.; et al. Incorporation of glucose analogs by glycosyltransferases GtfE and GtfD from the vancomycin biosynthetic pathway to generate variant glycopeptides. *Chem. Biol.*, in press.

(42) Dufat-Trinh Van, H.; Seguin, E.; Tillequin, F.; Monneret, C.; Koch, M. Total synthesis of oxa-9-anthracyclines. *Chem. Pharm. Bull. (Tokyo)* **1989**, *37*, 3294−3300.
(43) Zou, Y.; Fahmi, N. E.; Vialas, C.; Miller, G. M.; Hecht, S. M. Total synthesis of deamido bleomycin a(2), the major catabolite of the antitumor agent bleomycin. *J. Am. Chem. Soc.* **2002**, *124*, 9476−9488.
(44) Chen, H.; Thomas, M. G.; Hubbard, B. K.; Losey, H. C.; Walsh, C. T.; et al. Deoxysugars in glycopeptide antibiotics: enzymatic synthesis of TDP-L-epivancosamine in chloroeremomycin biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11942−11947.
(45) Losey, H. C.; Peczuh, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; et al. Tandem action of glycosyltransferases in the maturation of vancomycin and teicoplanin aglycones: novel glycopeptides. *Biochemistry* **2001**, *40*, 4745−4755.
(46) Kerns, R.; Dong, S. D.; Fukuzawa, S.; Carbeck, J.; Kohler, J.; et al. The role of the hydrophobic constituents in the biological activity of glycopeptide antibiotics. *J. Am. Chem. Soc.* **2000**, *122*, 12608−12609.
(47) Downes, C. S.; Ord, M. J.; Mullinger, A. M.; Collins, A. R.; Johnson, R. T. Novobiocin inhibition of DNA excision repair may occur through effects on mitochondrial structure and ATP metabolism, not on repair topoisomerases. *Carcinogenesis* **1985**, *6*, 1343−1352.
(48) Castora, F. J.; Vissering, F. F.; Simpson, M. V. The effect of bacterial DNA gyrase inhibitors on DNA synthesis in mammalian mitochondria. *Biochim. Biophys. Acta* **1983**, *740*, 417−427.
(49) Sung, S. C. Effect of novobiocin on DNA-dependent DNA polymerases from developing rat brain. *Biochim. Biophys. Acta* **1974**, *361*, 115−117.
(50) Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; et al. The nature of inhibition of DNA gyrase by the coumarins and the cyclothialidines revealed by X-ray crystallography. *EMBO J.* **1996**, *15*, 1412−1420.
(51) Tsai, F. T.; Singh, O. M.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; et al. The high-resolution crystal structure of a 24-kDa gyrase B fragment from *E. coli* complexed with one of the most potent coumarin inhibitors, clorobiocin. *Proteins* **1997**, *28*, 41−52.
(52) Kampranis, S. C.; Gormley, N. A.; Tranter, R.; Orphanides, G.; Maxwell, A. Probing the binding of coumarins and cyclothialidines to DNA gyrase. *Biochemistry* **1999**, *38*, 1967−1976.
(53) Gormley, N. A.; Orphanides, G.; Meyer, A.; Cullis, P. M.; Maxwell, A. The interaction of coumarin antibiotics with fragments of DNA gyrase B protein. *Biochemistry* **1996**, *35*, 5083−5092.
(54) Chatterji, M.; Unniraman, S.; Maxwell, A.; Nagaraja, V. The additional 165 amino acids in the B protein of *Escherichia coli* DNA gyrase have an important role in DNA binding. *J. Biol. Chem.* **2000**, *275*, 22888−22894.
(55) Freel Meyers, C. L.; et al. Manuscript in preparation.
(56) Hooper, D. C.; Wolfson, J. S.; McHugh, G. L.; Winters, M. B.; Swartz, M. N. Effects of novobiocin, coumermycin A1, clorobiocin, and their analogs on *Escherichia coli* DNA gyrase and bacterial growth. *Antimicrob. Agents Chemother.* **1982**, *22*, 662−671.
(57) Pojer, F.; Li, S. M.; Heide, L. Molecular cloning and sequence analysis of the clorobiocin biosynthetic gene cluster: new insights into the biosynthesis of aminocoumarin antibiotics. *Microbiology* **2002**, *148*, 3901−3911.
(58) Mulichak, A. M.; Losey, H. C.; Walsh, C. T.; Garavito, R. M. Structure of the UDP-glucosyltransferase GtfB that modifies the heptapeptide aglycone in the biosynthesis of vancomycin group antibiotics. *Structure* **2001**, *9*, 547−557.
(59) Morera, S.; Imberty, A.; Aschke-Sonnenborn, U.; Ruger, W.; Freemont, P. S. T4 phage β-glucosyltransferase: substrate binding and proposed catalytic mechanism. *J. Mol. Biol.* **1999**, *292*, 717−730.
(60) Ha, S.; Walker, D.; Shi, Y.; Walker, S. The 1.9 Å crystal structure of *Escherichia coli* MurG, a membrane-associated glycosyltransferase involved in peptidoglycan biosynthesis. *Protein Sci.* **2000**, *9*, 1045−1052.
(61) Du, L.; Sanchez, C.; Chen, M.; Edwards, D. J.; Shen, B. The biosynthetic gene cluster for the antitumor drug bleomycin from Streptomyces verticillus ATCC15003 supporting functional interactions between nonribosomal peptide synthetases and a polyketide synthase. *Chem. Biol.* **2000**, *7*, 623−642.
(62) Du, L.; Sanchez, C.; Shen, B. Hybrid peptide−polyketide natural products: biosynthesis and prospects toward engineering novel molecules. *Metab. Eng.* **2001**, *3*, 78−95.
(63) Kohli, R. M.; Burke, M. D.; Tao, J.; Walsh, C. T. Chemoenzymatic route to macrocyclic hybrid peptide/polyketide-like molecules. *J. Am. Chem. Soc.* **2003**, *125* (24), 7160−7161.

JM030257I