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New antibiotics from bacterial natural products

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For the past five decades, the need for new antibiotics has been met largely by semisynthetic tailoring of natural product scaffolds discovered in the middle of the 20th century. More recently, however, advances in technology have sparked a resurgence in the discovery of natural product antibiotics from bacterial sources. In particular, efforts have refocused on finding new antibiotics from old sources (for example, streptomycetes) and new sources (for example, other actinomycetes, cyanobacteria and uncultured bacteria). This has resulted in several newly discovered antibiotics with unique scaffolds and/or novel mechanisms of action, with the potential to form a basis for new antibiotic classes addressing bacterial targets that are currently underexploited.

Because the use of an antibiotic inevitably selects for resistant microbes, there is a continuing and cyclical need for new antibiotics. The clock begins to tick down on an antibiotic's useful lifetime before clinically significant resistance emerges[1], impelling the need for new drugs to combat the current generation of resistant pathogens. In the 20th century, two parallel lines of discovery yielded success in the antibiotic arena: isolation of natural products with antibiotic activity and preparation of synthetic antibiotics. The golden age of antibiotic natural product discovery spanned only the 1940s to 1950s. In accord with Sir James Black's famous observation that "the most fruitful basis for the discovery of a new drug is to start with an old drug[2]," new antibiotics from the subsequent decades have come from iterative, semisynthetic tailoring of the natural scaffolds to create successive generations of antibiotics (for example, erythromycin → clarithromycin → telithromycin)[3].

This review examines the prospects for renewed discovery of natural antibiotics—especially those with new molecular templates and/or mechanisms of action—from two perspectives. First, we consider estimates of how many new molecules are still to be found and discuss strategies for finding them, with an emphasis on antibiotics from the uncultured microbial majority. Second, we use examples of recently discovered (or rediscovered) molecular classes and newly tailored scaffolds to discuss novel strategies for engaging old and new bacterial targets. Our emphasis on naturally occurring antibiotics has two motivations: first, the well-established ability to discover useful antibiotics from natural sources suggests that continued efforts are likely to be fruitful; and second, as antibiotics often reach their targets by transport rather than diffusion, antibiotic candidates benefit from having structural features rarely found in the synthetic libraries of 'drug-like' molecules used in most high-throughput screening facilities.

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How many natural antibiotics remain to be discovered?

Over the past two decades, pharmaceutical companies deemphasized microbial screening programs for many reasons, although they frequently cited declining productivity and diminishing discoveries of novel molecules[3]. Work by two research groups[4–6] allow at least a semiquantitative analysis of the chances for continued discovery from microbial sources.

In 2001, Watve *et al.*[4] estimated that from the first report of streptothricin in 1942 and streptomycin a year later, the order Actinomycetales had yielded ~3,000 known antibiotics (90% of those from *Streptomyces*, an Actinomycetales genus). On the basis of past experience, these authors proposed that if streptomycetes (exclusively) were screened as widely as they had been in 1995, 15–20 antibiotics would be discovered each year for the next 50 years. Over the subsequent five decades, these ~1,000 new molecules would yield 20–40 new antibiotics for human clinical use, assuming that the historical trend of one marketed antibiotic for 25–50 novel molecules remains the same. Apparently, this projected discovery rate was too low to be economically viable because several large pharmaceutical companies closed their antibiotic discovery programs shortly after its publication.

More recent (and complementary) analyses by Baltz[5,6] have estimated the frequency of antibiotic production by actinomycetes. In his 2005 paper, Baltz[5] noted a 1958 report from H.B. Woodruff and L.E. McDaniel at Merck (Whitehouse Station, NJ, USA) indicating that 10^4 strains would include 2,500 antibiotic producers. Of these 2,500, 2,250 would make streptothricin, 125, streptomycin and 40, tetracycline, with frequencies of 2×10^{-1}, 1×10^{-2} and 4×10^{-3}, respectively. In 1976, Arai[7] extended the Woodruff and McDaniel analysis to include vancomycin and erythromycin, and found frequencies of 1.5×10^{-5} and 5×10^{-6}, respectively[4]. Baltz[5] also points out that daptomycin (**Table 1** and **Fig. 1**) was found once in 10^7 actinomycete cultures. Thus, the frequency of discovery for antibiotics from the most productive bacteria, the actinomycetes, ranges 10^6-fold in the ~10^7 strains screened historically. In addition, Baltz[5] estimates that less than one part in 10^{12} of the earth's soil surface has been screened for actinomycetes.

Table 1 Selected natural product-derived antibiotics currently in clinical development.

Antibiotic (trade name)	Company	Description	Clinical phase
Daptomycin (LY 146032, Cubicin, Cidecin, Dapcin) C_{72} H_{101} N_{17} O_{26}	Cubist Pharmaceuticals (Lexington, MA, USA)	Cyclic lipopetide cell wall synthesis inhibitor, already approved for skin and soft tissue infections with activity against MRSA and other Gram-positive bacteria, now also registered against bacteremia and bacterial endocarditis.	Registered
Dalbavancin (Zeven, BI397, V-glycopeptide, VER001) C_{88} H_{100} Cl_2 N_{10} O_{28}	Pfizer (New York; formerly Vicuron Pharmaceuticals)	Glyopeptide cell wall synthesis inhibitor indicated against skin and soft tissue infections due to MRSA and other Gram-positive bacteria.	Preregistration
Telavancin (TD6424, Arbelic) C_{80} H_{106} Cl_2 N_{11} O_{27} P	Theravance (S. San Francisco, CA, USA) and Astellas Pharma (Colorado Springs, CO, USA)	Glycopeptide cell wall synthesis inhibitor indicated against complicated skin and soft tissue infections and nosocomial pneumonia due to MRSA and other Gram-positive bacteria.	Phase 3
Oritavancin (LY 333328) C_{86} H_{97} C_{13} N_{10} O_{26}	Targanta Therapeutics (St. Laurent, QC, Canada; in-licensed from Intermune)	N-alkyl-substituted glycopeptide cell wall synthesis inhibitor indicated against skin and soft tissue infections due to MRSA and other Gram-positive bacteria.	Phase 3
Ramoplanin (A 16686, A 16686A, MDL 62198) C_{98} H_{122} Cl N_{21} O_{29}	Pfizer (formerly Vicuron Pharmaceuticals)	Glycolipodepsipeptides derived from *Actinoplanes* spp. in trials against nosocomial infections and vancomycin-resistant enterococcal infections.	Phase 3
	Oscient Pharmaceuticals (Waltham, MA; USA; licensed from Pfizer)	In trials for treatment of *Clostridium difficile*-associated diarrhea.	Phase 2
Efiprestin (RPR106972)	Sanofi Aventis (Paris)	Oral streptogramin consisting of two synergistic components (pristinamycin IB and pristinamycin IIB) for the treatment of community-acquired Gram-positive bacterial infections.	Phase 2
Lyostaphin (intranasal creams BSYX L210, BSYX-L210; topical creams BSYX L310, BSYX-L310)	Biosynexus (Rockville, MD, USA)	Lanthocin class 27-kDa peptidase produced by *Staphylococcus simulans* indicated for staphylococcal infections.	Phase 1/2
WAP 8294A_2 (WAP 8294A_2, WAP 8294A2; topical JA 002) C_{73} H_{111} N_{17} O_{21}	aRigen (Tokyo)	Water-soluble depsipeptide membrane integrity inhibitor isolated from the fermentation broth of *Lysobacter* sp. in testing against methicillin-resistant *S. aureus* infections.	Phase 1

Although only 1–3% of all streptomycete antibiotics have been discovered, to find the remaining 97–99% will require a combination of high-throughput screening by modern technologies (10^8–10^9 strains per year), selection against the most common antibiotics, methods to enrich rare and slow-growing actinomycetes, a prodigious microbial collecting and culturing effort, and combinatorial biosynthesis in streptomycetes. As one example, Baltz[6] notes that screening strains of *Escherichia coli* K12—engineered to harbor 15 antibiotic-resistance genes to exclude the most common antibiotics produced by actinomycetes—can enhance the signal-to-noise ratio for new molecules with novel modes of action.

Accessing greater bacterial diversity and optimizing natural products

The impressive number of bioactive molecules isolated from actinomycetes reflects their historical importance in antibiotic discovery; however, as members of a single order in the high-GC Gram-positive bacteria, actinomycetes comprise a small minority of the microbial antibiotic producers. Given the importance of small-molecule antibiotics to the bacterial lifestyle, it is overwhelmingly likely that other bacteria could also contribute to the discovery of new antibiotics.

Efforts to expand the range of bacteria that can be tapped for antibiotic research are being facilitated by several strategies: expanded conventional culturing approaches, novel culture methods, heterologous DNA-based methods and metagenomics. Comparative genomics, combinatorial biosynthesis and more traditional structural studies (for example, X-ray crystallography and nuclear magnetic resonance (NMR)) of compounds bound to bacterial targets are also aiding the discovery of new, and improvement of existing, natural products. These approaches are summarized below.

Conventional culturing methods. Even among actinomycetes, most of the strains that have been screened for new antibiotics come from one genus, *Streptomyces*. This is spurring efforts to develop methods using selective growth media that can expand the number of actinomycetes cultured *in vitro*.

To pick two recent examples, two new genera of actinomycetes have been discovered in marine sediment samples from Papua New Guinea[8], and researchers at Vicuron[9] (Fremont, CA, USA) have isolated another two, *Catenulispor* and *Actinospica*, from forest soil in Gerenzano, Italy. These slow-growing genera likely define a widely distributed suborder. More importantly, they have ~8 Mb genomes, and one sequenced strain has genes encoding type I and II polyketide synthase (PKS) genes as well as nonribosomal peptide (NRP) synthetases (NRPSs).

Novel culturing environments. Only a small and unrepresentative minority of bacteria can be cultured by conventional methods. Estimates based on culture-free methods, such as ribosomal RNA (rRNA) sequencing, put the cultured minority at 1% or less of all bacteria[10,11], and approximately one-third of the bacterial divisions have no cultured representatives and are known only through rRNA sequences[12,13]. Actinomycetes have the largest percentage of cultured members[12], which probably reflects both their relative ease of culturing and their importance in antibiotic discovery efforts. Taken at face value, these estimates suggest that the number of antibiotics from uncultured sources is two to three orders of magnitude greater than those from

cultured sources. Not surprisingly, several laboratories have developed approaches to access this diversity, and although only a handful of molecules has been reported, the magnitude of the resource will lead to increased attention.

An ingenious approach to culturing the uncultured was reported by Epstein and colleagues[14]. Their strategy is based on the assumption that bacteria cannot be cultured by standard laboratory techniques because an essential agent—likely a molecule produced by other bacteria—in their natural environment is missing from standard culture media. To overcome this problem, the authors devised a new culturing method that allows the establishment of the molecular milieu of a multispecies community while a single strain is cultured in a small diffusion chamber located within this permissive environment. In this way, representative 'uncultured' marine bacteria can be grown in pure culture, and organisms that are unable to form colonies on artificial media form colonies in the molecular environment of other microorganisms. Although this approach has not yet led to new molecules, it seems likely it will in the future, and it supports the speculation that the uncultured majority reflect the necessity of community membership. Other promising approaches to culturing previously uncultured organisms have subsequently been reported[15].

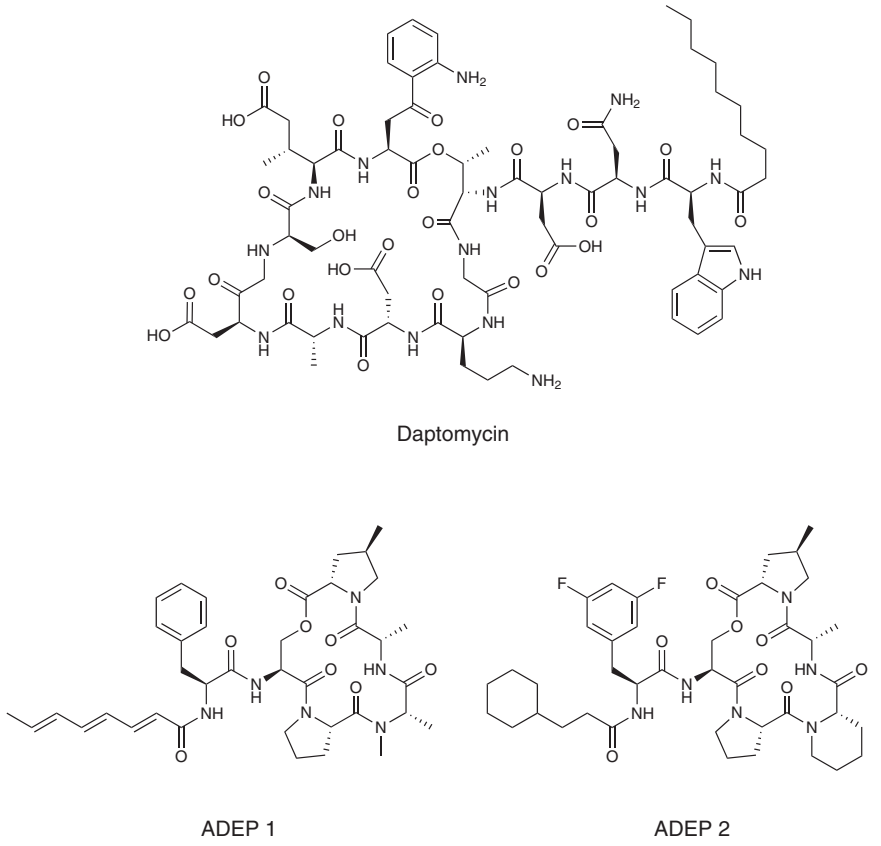

Figure 1 Structures of acyldepsipeptidolactones.

Heterologous DNA-based approaches. Heterologous expression of foreign biosynthetic genes in a host that can be cultured more easily and is amenable to genetic manipulation is a powerful approach for exploring the small-molecule repertoire of the bacterial world. In principle, a successful application requires that the biosynthetic, resistance and regulatory genes reside on a contiguous stretch of DNA and that the host has an appropriate metabolic and genetic background. Although complete pathways on contiguous stretches of DNA are common for bacterial natural products, deficiencies in background metabolism and differences in codon usage can be challenging barriers that render genes and pathways from the most prolific antibiotic producers incompatible with the most commonly used hosts. Nevertheless, there are now several successful attempts with engineered hosts (for example, *E. coli* and *Pseudomonas putida*) expressing drug candidates and other small molecules, and further progress on more metabolically versatile hosts is likely[16–19]. This work could set the stage for combinatorial biosynthetic efforts to engineer the production of natural product variants[20,21].

The pantocins, a group of antibiotics produced by *Pantoea agglomerans* and discovered in the early 1980s[22], are an early example of the power of this approach to identify new antibiotics. Although the existence of these antibiotics was well known, identifying individual molecules had frustrated several laboratories. Wright, Beer and their collaborators[23] made a clone library in *E. coli* from the genomic DNA of a *P. agglomerans* strain producing multiple antibiotics, identified antibiotic-producing clones through zones of inhibition in an *Erwinia amylovora* overlay assay, and used these clones to identify pantocins A and B (**Fig. 2**). This procedure worked because of the close phylogenetic relationship between *P. agglomerans* and *E. coli*. An important characteristic of these DNA-based studies is their reliance on a phenotypic (antibiotic) assay, in contrast to the target-based assays that will be discussed below.

Pantocin B, the antibiotic initially identified[24], is a linear molecule composed of L-alanine, a diamine probably derived from glycine, and a methylsulfone-containing dicarboxylic acid. Pantocin A, the other antibiotic identified in the initial clone library, has an unusual (and rather labile) bicyclic structure[25,26]. Despite the obvious differences between the two compounds, they share several features. Both must have free amino and carboxyl termini, which allow them to enter target cells via a peptide transporter. Both inhibit pyridoxal phosphate-dependent transaminases required for amino acid biosynthesis; pantocin A inhibits histidine formation, whereas pantocin B inhibits arginine production. Finally, their assembly shares an element of biosynthetic logic in which monomers—amino acids or related molecules—are initially linked through amide bonds.

Heterologous expression facilitates the discovery of biosynthetic gene clusters through subcloning and mutagenesis. The pantocin B gene cluster is large (~20 kb) and not completely annotated. In contrast, the pantocin A gene cluster is small (3.9 kb) and contains only three genes: two biosynthetic genes and one gene encoding a resistance-conferring pump[26]. The biosynthetic logic of the pantocins differs from that of PKSs and NRPSs, which tether nascent substrates as protein-bound thioesters[27]. In contrast, the pantocin pathways involve diffusible intermediates rather than protein-bound intermediates, and might therefore be more amenable to manipulation. Because their cellular targets are part of amino acid biosynthetic pathways, the pantocins are only effective in nutrient-poor environments and not likely to be useful in humans. But

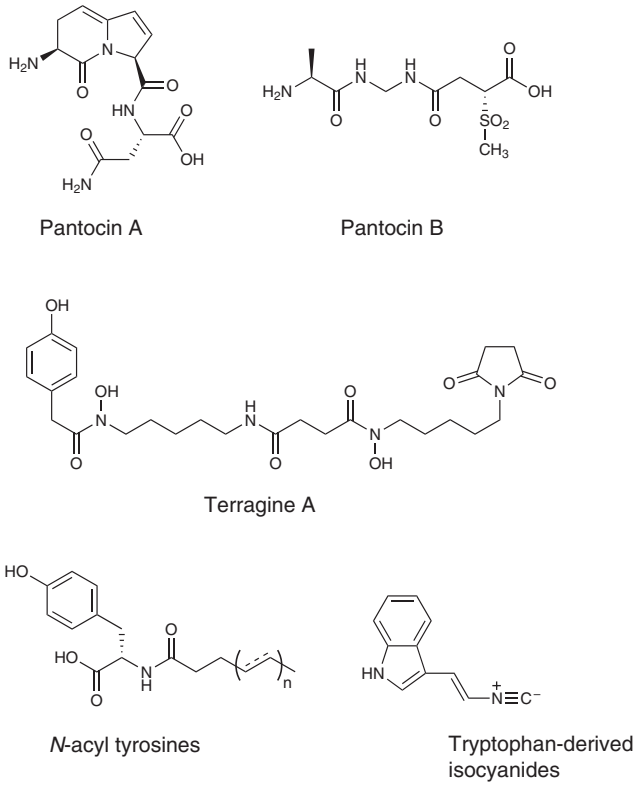

Figure 2 Natural products discovered by heterologous DNA-based approaches.

the general approach that led to their discovery could be a promising avenue for future exploration. The biosynthetic gene cluster for andrimid (a potent acetyl-CoA carboxylase inhibitor that is discussed below) was discovered in another *P. agglomerans*-derived library[28].

Metagenomics. Attempts to capture DNA from the environment and use it in heterologous expression systems have taken a variety of forms[29,30], and in this article we focus solely on work related to small-molecule antibiotics. The earliest report was the discovery of terragine A (**Fig. 2**) and related compounds in a 1,020-member library of soil DNA fragments expressed in *Streptomyces lividans*[31]. The relatively small size of the library allowed the small molecules made by each member to be screened by rapid high-performance liquid chromatography–mass spectrometry. Although terragine A is related to the metal-chelating hydroxamic acids, which are widely produced by bacteria and often show antibiotic activity, unfortunately neither terragine A nor any other terragines exhibit antibacterial properties.

In 2000, Brady and Clardy[32] reported another approach, featuring much larger libraries of ~7 × 10^5 metagenomic DNA clones from soil collected in Ithaca, New York, and an *E. coli* expression system. Because of the library size, the clones were screened on the selection plate with an antibiotic overlay assay using *Bacillus subtilis*. The resulting ~65 positive clones were cultured on a small scale, their organic extracts examined for antibiotic activity and the most active ones subsequently characterized. The antibiotic activity resided in a series of *N*-acyl tyrosines (**Fig. 2**), a family that differed in the length and degree of unsaturation of the fatty acid acyl groups. This family of antibiotics is the most frequently encountered in *E. coli* libraries, and the *N*-acyl tyrosines are produced by a single *N*-acyl synthase. These compounds—which had not been previously reported as natural products—are widespread, although the *N*-acyl synthase genes typically have little sequence similarity[33]. Some of them have low levels (<20%) of homology to the *N*-acyl-homoserine lactone synthases that produce the well-known quorum-sensing molecules of Gram-negative bacteria[34]. A related antibacterial molecule has been isolated from a Costa Rican sample of environmental DNA, in which putrescine, rather than an amino acid, had been *N*-acylated[35]. This same approach has also revealed unusual isocyanide derivatives of tryptophan (**Fig. 2**) that possess antibiotic activity[36,37].

Other notable attempts are the discovery of indirubin and related small molecules from metagenomic bacterial artificial chromosome (BAC) libraries in *E. coli* by an Aventis (now Sanofi-Aventis; Paris) group[38]. The same group used a shuttle vector to transfer an environmental DNA cosmid library from *E. coli* to *S. lividans* and reported several transformants that produced antibacterial activity, although no structures of active molecules were reported[39].

Finally, Gillespie *et al.*[40] reported that a screen of a soil DNA BAC library (24,545 members) identified two colored triarylcations with antibiotic activity[40]. This study linked small-molecule production to the introduction of a single gene, an apparent member of the 4-hydroxyphenylpyruvate dioxygenase family, which upregulates the production of triarylcations normally produced by *E. coli* to levels where they can be easily detected and isolated[40].

Genomics and combinatorial synthesis. As Bode and Muller[41] have recently reviewed the impact of bacterial genomics on the discovery and manipulation of natural product antibiotics, we do not discuss this (or combinatorial biosynthesis) in detail here. Suffice to say combinatorial biosynthesis promises to provide entirely novel structures; for example, a research group at Kosan (Hayward, CA, USA), Menzella *et al.*[42,43] has reduced to practice the synthesis of 5-kb stretches of DNA that encode PKS modules, reporting an initial exploration of combinations to yield novel condensation and chain-elongation patterns. Elsewhere, genome scanning of the bicyclomycin-producer *Streptomyces aizunensis* NRRL B-11277 has revealed a 35 open-reading-frame (ORF) gene cluster whose putative structure could be predicted bioinformatically[44,45]. Fermentation of this strain under 50 different culture conditions led to isolation and characterization of ECO-02301 by researchers at Ecopia Biosciences (Saint Laurent, QC, Canada), which has a molecular mass of 1,298 ($C_{70}H_{109}N_2O_{20}$) and shows activity against a broad spectrum of pathogenic mycoses, including drug-resistant strains of *Candida albicans* (**Fig. 3**).

Structural studies. The X-ray structure determination of small-molecule ligands bound to target proteins has become a widely used tool for drug discovery, both for initial screening and in lead-optimization stages. This includes work on natural product antibiotics bound to their targets (see discussion below of platensimycin bound to FabF C163→Q mutant in section 'Inhibitors of fatty acid biosynthesis').

Structure-based drug design and optimization is also being conducted against protein and RNA components of the bacterial ribosome. The elongation factor EF-Tu is a conditional GTPase that chaperones aminoacyl-tRNAs to the ribosome where hydrolysis of bound GTP to GDP sets up the release cycle for EF-Tu-GDP to bring the next aminoacyl-tRNA to the ribosome. Release of EF-Tu in its GDP-bound state is known to be blocked by natural products such as kirromycin, pulvomycin, GE2270 and enacyloxin IIa[46]. The determination of the structure of enacyloxin IIa bound to *Thermus thermophilus* EF-Tu-GDP[47,48] provides a view of how enacycloxin blocks EF-Tu release and suggests that hybrids of enacycloxin and kirromycin could have improved antibiotic activity.

The large (50S) ribosomal subunit contains the peptidyl transferase center where peptide bond formation occurs. Crystallization and

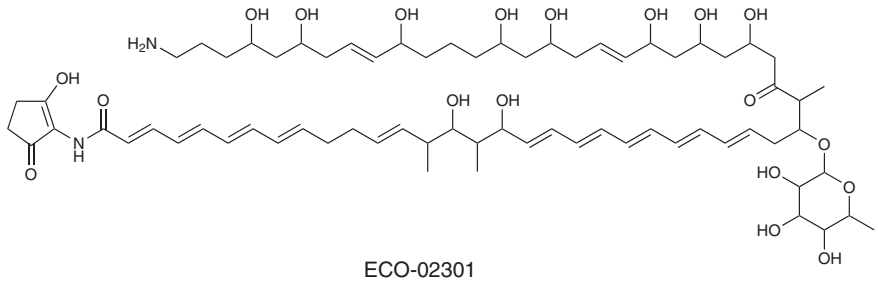

Figure 3 Structure of ECO-02301.

structure determination of the 50S subunit structure has elucidated the binding sites of all the antibiotics known to bind near the peptidyl transferase center[49–51]. The clustering of diverse antibiotic structures—both natural and semisynthetic molecules—within 10 Å of the peptidyl transferase center will inform the combinatorial design of hybrid molecules, including routes to overcome resistance to macrolides, lincosamides and streptogramins[52] in mutated ribosomes.

Novel and rediscovered natural product antibiotics

Many new natural products that show promise as antibiotics have been discovered by the approaches discussed above. We discuss these in more detail below.

Acyldepsipeptidolactones. Hundreds of NRP natural products are known. As the full-length peptidyl chain—usually tethered in thioester linkage to a series of carrier protein domains—reaches the final module of the NRPS, it is disconnected from the protein assembly line[27]. Chain release can be effected in three modes: hydrolysis, reduction or macrocyclization by thioesterase domains[53,54]. Macrocyclizing thioesterases are of particular interest because they impart conformational restraints on the released NRP scaffold when a nucleophilic side chain of the peptide attacks the C-terminal thioester[27,54]. When the side chain is an amino group (such as the C_5-NH_2 of ornithine or the C_3-NH_2 of diaminopropionate), a macrolactam is formed; and when the side chain is a hydroxyl group (such as the C_3-OH of serine or the phenolic-OH of tyrosine), a macrolactone or depsipeptide scaffold is generated. Many depsipeptide NRPs are *N*-acylated on their N-terminal amino group during the chain initiation step of NRP assembly, and the resulting NRPs are known as acyldepsipeptidolactones.

The most celebrated member of this class is daptomycin (Cubicin) (**Fig. 1**), which has been approved for human use to treat bacterial infections. The biosynthetic gene clusters for daptomycin[55,56] and related acyldepsipeptidolactones have been sequenced, and combinatorial biosynthesis has been performed by coexpressing alternative NRPS modules[57], providing a potential route to second-generation daptomycins. The acyl chain composition is controlled by feeding decanoic acid during fermentation[58].

A group at Bayer (Milford, CT, USA), Brotz-Oesterhelt *et al.*[59] has examined a complex of eight acyldepsipeptidolactones from *Streptomyces hawaiiensis* NRRL 15010 that were reported 20 years earlier in a 1985 patent[60]. The main component ADEP 1 (acyldepsipeptide 1; **Fig. 1**) is an *N*-acylhexapeptidolactone. The same group synthesized a synthetic congener with >tenfold increased bacteriocidal potency, ADEP 2 (**Fig. 1**), which features an alternative *N*-acyl chain, difluorophenylalanine instead of phenylalanine, and a pipecolyl residue instead of *N*-methyl-alanine to impart an amide conformational restraint. This semisynthetic acylpeptidolactone cures lethal *Staphylococcus aureus* sepsis in mice, lethal systemic murine infections by *Enterococcus fecalis* and *Streptococcus pneumoniae*–induced bacteremia in rats.

The mechanism of action of the ADEPs is novel and intriguing. Molecular characterization[59] of ADEP-resistant mutants in an efflux pump–deficient *E. coli* strain revealed that the target is ClpP (caseinolytic protease), the core catalytic subunit of a proteasome-like chambered bacterial protease. Typically, these widely distributed chambered proteases use an ATPase subunit to drive conformational changes that open the channel for proteins to be threaded into the chamber for proteolysis[61]. ADEPs act as allosteric activators in the absence of the ATPase subunit, stimulating ClpP-catalyzed hexapeptide cleavage 300-fold. Thus, ADEPs disregulate the highly regulated intracellular protease activity of the ClpPs, causing accelerated degradation of essential cellular proteins and leading to cell death. This novel mechanism of action makes cross-resistance to existing classes of antibiotics unlikely. Even so, a relatively high ADEP-resistance mutation frequency of 10^{-6} suggests ADEPs would be most useful in combination therapy[59].

Disruption or dysregulation of other bacterial ATP-using enzymes may be a more general strategy for testing natural product and synthetic chemical libraries for antibiotics. The synthetic diarylquinolones that have been recently reported by Johnson & Johnson's (Beerse, Belgium) Andries *et al.*[62] as promising antitubercular compounds target the *Mycobacterium tuberculosis* ATP synthase, and the coumarin antibiotics (novobiocin, clorobiocin, coumermycin) block the ATPase site of the DNA gyrase subunit GyrB[63,64].

Inhibitors of fatty acid biosynthesis. For some time, bacterial fatty acid biosynthesis has been of interest as an essential pathway (for membrane phospholipid production) that might be targeted by antibiotics. Whereas vertebrates have a type I fatty acid synthase with catalytic and carrier protein domains fused in a large multimodular polypeptide, most bacteria have type II fatty acid synthases with each domain a free-standing enzyme or carrier protein[65]. The natural products cerulenin and thiolactomycin are inhibitors of the FabB/F ketosynthases, and the synthetic molecules triclosan and isoniazid target the enoyl-ACP reductase FabI, suggesting that multiple steps in the fatty acid biosynthetic pathway would be effective antibacterial targets. We discuss below two bacterial natural products that inhibit fatty acid biosynthesis: platensimycin and andrimid.

Recently, Wang *et al.*[66] from Merck have reported that the natural product platensimycin (**Fig. 4**) possesses potent antibacterial activity. This molecule was detected in the culture extract of *Streptomyces platensis*, a South African soil isolate. The Merck program involved screening 83,000 bacterial culture extracts against a *Staphylococcus aureus* strain in which antisense RNA against *fabF* was used to lower its gene expression, making FabF activity rate-limiting for bacterial

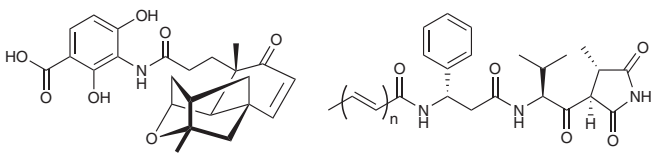

Figure 4 Structures of inhibitors of fatty acid biosynthesis.

growth. Platensimycin showed minimum inhibitory concentrations of 0.5–1 μg/ml against *S. aureus* and *S. pneumoniae* and caused a 10^4–10^5-fold reduction in bacterial count in a mouse model of disseminated *S. aureus* infection.

The structure of platensimycin is remarkable, with a dihydroxyaminobenzoate moiety linked by an amide bond to a pentacyclic scaffold. Biochemical assays of the antibiotic on FabF and FabH β-ketoacyl-*S*-acyl carrier protein (ACP) C-C bond formation showed mid-nanomolar potency on FabF but ~10^3-fold lower activity on FabH[66]. Careful detective work revealed that platensimycin binds specifically to the transient FabF acyl-*S*-enzyme intermediate. The C163→Q mutant of *E. coli* FabF mimicked the acyl-enzyme conformation well enough to yield good crystals in complex with platensimycin, revealing structural features of platensimycin's benzoate moiety and the novel architecture of its pentacyclic ketolide[66]. Although the complex structure of platensimycin offers challenges for biosynthesis and total synthesis, its potency and mode of action suggest further investigation is warranted.

Another group of fatty acid biosynthesis inhibitors that shows promise is the nonribosomal peptide pyrrolidine diones. The first committed step in fatty acid biosynthesis is C-C bond formation by the biotin-dependent acetyl-CoA carboxylase, generating malonyl-CoA as the donor of the C_2 elongation units for the decarboxylative Claisen condensations carried out by FabF/H[64]. Two related hybrid NRP-polyketide natural products that terminate in a pyrrolidine dione, moiramide B and andrimid (**Fig. 4**), have been found to be potent competitive inhibitors of malonyl-CoA in the carboxyltransferase reaction[67,68]. Moiramide B differs from andrimid only in the length of its *N*-acyl chain and is about tenfold more potent (K_i = 5 nM)[69]. These are the first potent inhibitors of the initial step in fatty acid assembly. The molecular basis of the recognition determinants—including the pyrrolidine dione, which is required for activity—are not yet deciphered. Recently, the biosynthetic gene cluster for andrimid has been isolated and sequenced[22], revealing an unusual NRP-polyketide synthase organization[54] and setting the stage for combinatorial biosynthetic manipulations to explore structure-activity relationships in the andrimid/moiramide series.

Lantibiotics/other modified peptides targeting lipid II. The peptidoglycan layer in Gram-positive and Gram-negative bacteria is a well-known target for antibiotics such as the beta-lactams and vancomycin, which exploit the fact that bacterial cell division requires synthesis of new peptidoglycan. The *N*-acetylmuramyl pentapeptide building block for peptidoglycan formation is assembled on the cytosolic side of the inner membrane as a UDP derivative and then transferred to membrane-associated bactoprenol-phosphate to form lipid I. Addition of *N*-acetylglucosamine by MurG completes the cytosolic phase and yields disaccharyl-pentapeptide-pyrophosphate-lipid II, which is subsequently translocated across the membrane to present the disaccharyl pentapeptide on its extracellular face. The disaccharyl pentapeptide is then crosslinked by transpeptidase and transglycosylase enzymes, ejecting the lipid II pyrophosphate anchor and generating mechanically-strong

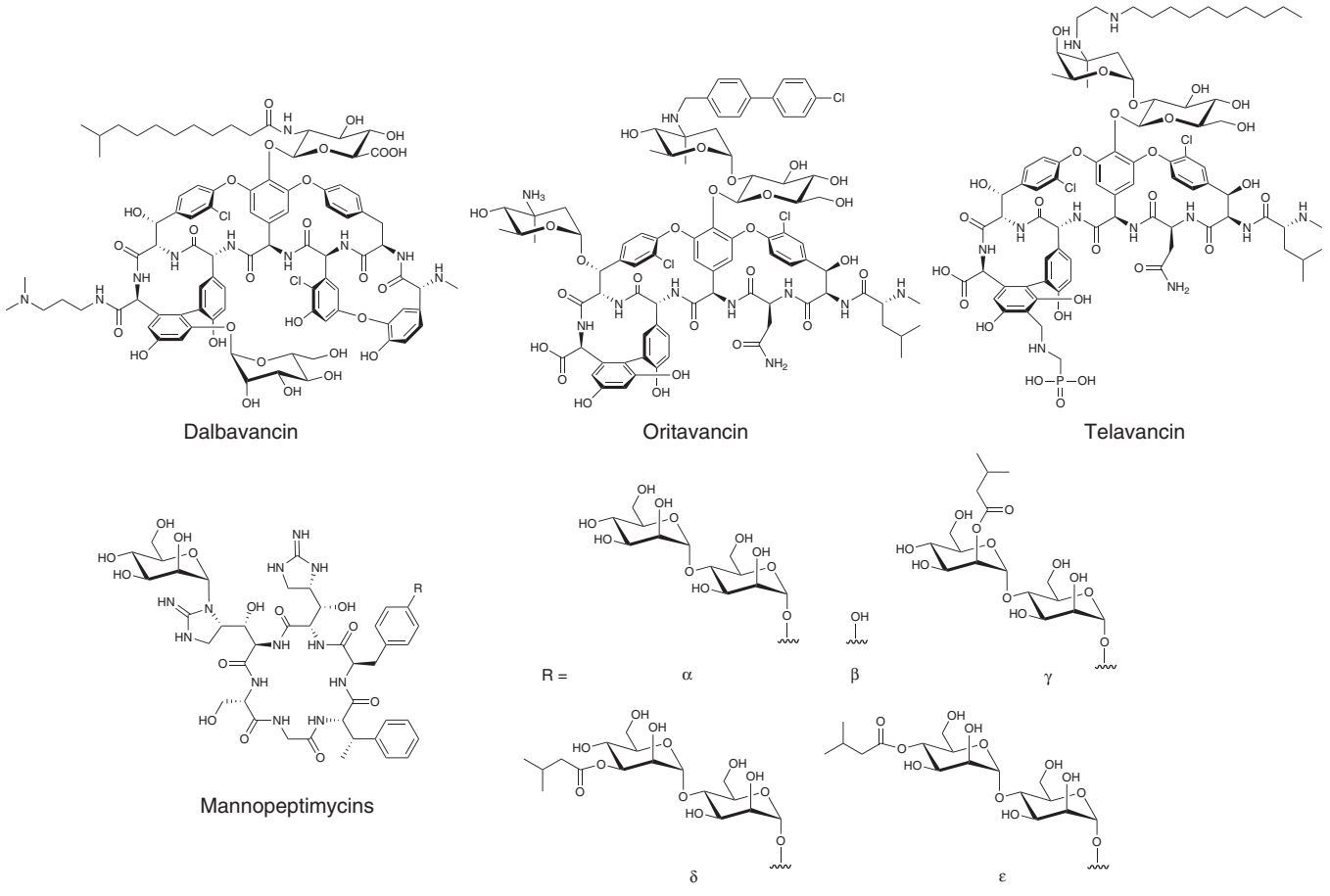

Figure 5 Structures of antibiotics that target lipid II.

peptidoglycan layers[1]. Finally, lipid II pyrophosphate is dephosphorylated and flipped back to the inner face of the membrane to begin another cycle. Availability of lipid II is thought to be a bottleneck for cell wall formation—as few as 2,000 lipid II molecules per cell are estimated to engage in the cycle[70]—so lipid II is a promising target for antibiotics.

Breukink and de Kruijff[70] note that five structurally distinct classes of natural products—vancomycin/teicoplanin-family glycopeptides, mannopeptimycins, ramoplanins, katanosins/plusbacins, and lantibiotics—act as antibiotics by binding lipid II on the extracellular face of the membrane, interdicting one or more steps of the lipid II cycle or blocking the flux of peptidoglycan precursors to the crosslinking transglycosylases and transpeptidases. All five of the classes are peptides with nonproteinogenic amino acids: four of them are formed by NRPS machinery[27], whereas the fifth class, lantibiotics, is made from a ribosomal peptide precursor that undergoes activity-conferring post-translational modifications[71]. The best known lipid II-targeting antibiotics are the vancomycin and teicoplanin class of glycopeptides[72] that bind the *N*-acyl-D-Ala-D-Ala termini of outward-facing lipid II molecules before transpeptidase-mediated crosslinking. Three second-generation glycopeptides—dalbavancin, telavancin and oritavancin (**Table 1**; **Fig. 5**)—have progressed to or through late-stage human clinical trials[70,72]. All are modified at one or more sites on the scaffold with synthetic hydrophobic substituents, which undoubtedly alter their partitioning and target recognition behavior.

A second class of glycopeptide antibiotics, the mannopeptimycins[73] (**Fig. 5**), block lipid II-dependent peptidoglycan maturations steps[74]. As with the ADEPs, the mannopeptimycins were discovered decades ago during the heyday of antibiotic natural product screening, but were put aside because they targeted only Gram-positive bacteria during an era in which broad-spectrum antibiotic activity was the goal. In recent years, multidrug-resistant Gram-positive bacterial pathogens have prompted a reexamination of the mannopeptimycin α-ε complex from *Streptomyces hygroscopicus* LL-AC98. The mannopeptimycins are cyclic hexapeptides with three nonproteinogenic amino acid residues: one β-methyl-phenylalanine and two β-OH-enduracidines[75]. Two mannoses, with variant placement of an isobutyryl moiety, are attached to the phenolic-OH of tyrosine-4 and another mannose is appended to enduracidine-6. Semisynthetic mannopeptimycins have improved activities and target a different portion of lipid II than vancomycin.

The other two classes of NRPs that target lipid II are the ramoplanins and the katanosins/plusbacins . None of these are new molecules; all are cyclic peptidolactones and ramoplanin and plusbacin A_3 are acyl-depsipeptidolactones. The data are quite convincing that ramoplanin, a 17-residue *N*-acyl macrolactone, targets lipid II[76] and like vancomycin exerts its activity by binding a small-molecule substrate rather than a protein. Much less is known about katanosin B and plusbacin A_3[70]. They are conformationally restricted cyclic peptidolactones that mimic ramoplanin with stretches of alternating D- and L-amino acid residues, and they have a high content of unusual hydroxylated amino acid side chains. Katanosin B has a Leu-Leu leader peptide, whereas plusbacin has an N-terminal β-OH fatty acyl moiety in which the β-OH group participates in macrolactone formation.

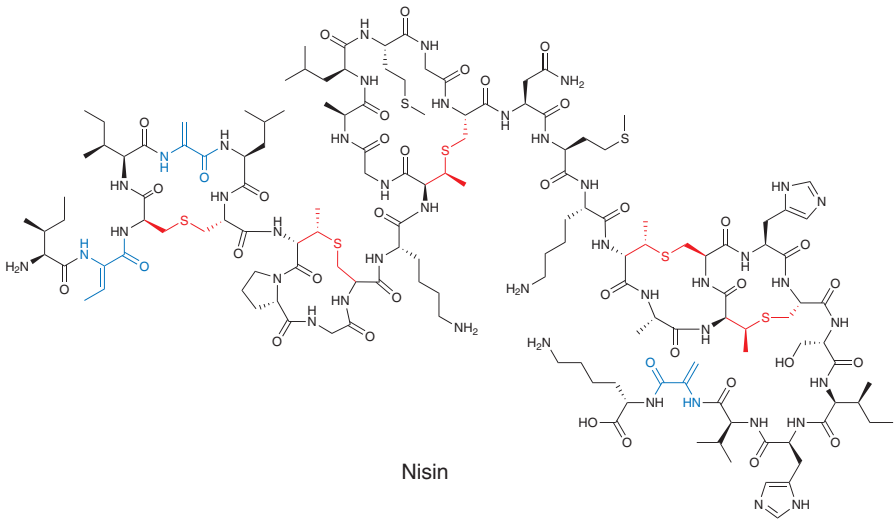

Figure 6 The lantibiotic nisin. Lanthionine and methyl-lanthionine thioether bridges are shown in red, and dehydroalanine and dehydrobutyrine residues are shown in blue.

The lantibiotics, a class of >50 peptide antibiotics synthesized by the ribosomes, have been known for decades. The prototype is nisin (**Fig. 6**), a 34-residue, five-ring molecule that is widely used as a food preservative[70,77]. Lantibiotics have been undergoing a renaissance, due in part to convincing recent evidence that nisin and homologs are mid-nanomolar binders to lipid II[78], and partly because the intricate post-translational enzymatic construction of the lanthionine-bridging rings has now been characterized biochemically[79,80]. NMR analysis indicates that the A and B rings of nisin form a pyrophosphate-binding cage with lipid II, thus interacting with a portion of this key peptidoglycan-forming intermediate which is different from that bound by vancomycin or ramoplanin[81].

Lipopeptide inhibitors of signal peptidase. Bacteria remove signal sequences from exported proteins by action of signal peptidase-1 (SP-1), an essential transmembrane enzyme with its active site on the periplasmic surface of the cytoplasmic membrane. Highly conserved in bacteria, SP-1—which uses a catalytic dyad of Ser and Lys—is not inhibited by most generic serine protease inhibitors. Two groups, Holtzel, Schmid, Fiedler and collaborators[82,83] and Kulanthaivel *et al.*[84], independently turned up families of aryl-bridged, *N*-acylated hexapeptides from *Streptomyces* natural product extracts that differ largely in the glycosylation and hydroxylation state of the *N*-methyl-hydroxyphenylglycine. The aryl-aryl coupling between hydroxyphenylglycine-4 and tyrosine-6—reminiscent of the oxidative coupling between residues 5 and 7 in vancomycin and teicoplanin—sets conformational constraints and is essential for subnanomolar inhibition of SP-1.

Fig. 7a shows the cocrystal structure of a soluble catalytic fragment of SP-1 with the acyl hexapeptide arylomycin, revealing that it forms a beta-sheet structure, which is presumed to mimic the binding of a signal peptide substrate[85]. The arylomycin C terminus makes hydrogen bonds to the side chains of two catalytic residues, serine-90 and lysine-145, accounting for its active-site placement. Arylomycin (**Fig. 7b**) and its glycosylated congeners—yet another subclass of lipoglycopeptides with a distinct antibacterial target—inhibit an accessible periplasmic enzyme and are unlikely to suffer from cross-resistance with other antibiotic classes.

Abyssomicin. The recently discovered natural product abyssomicin (**Fig. 7b**) is reminiscent of platensimycin: it comprises a novel polycyclic

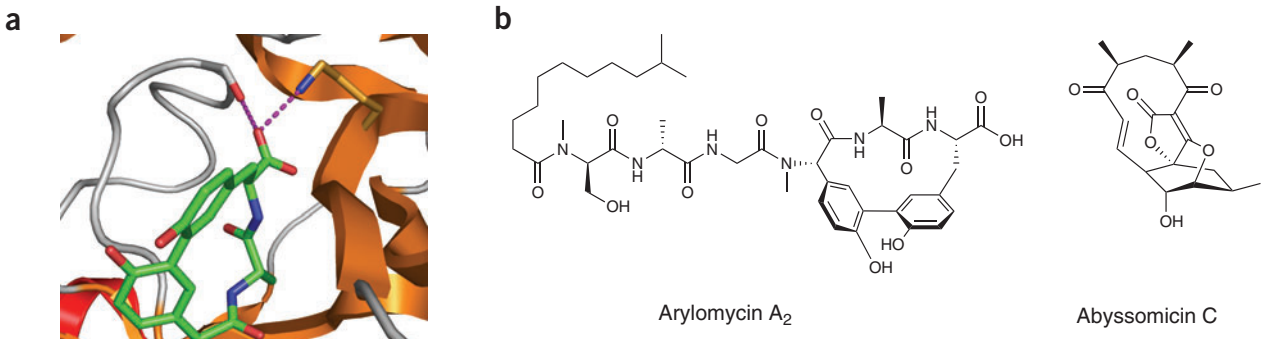

Figure 7 Structures of aryl-bridged, N-acylated hexapeptides from Streptomyces natural product extracts. (**a**) Arylomycin–signal peptidase cocrystal structure. (**b**) Structures of arylomycin and abyssomicin.

architecture in a polyketide-derived scaffold. In abyssomicin, an oxabicyclo-[2.2.2]-octane moiety is the key platform to which another eight-membered ring is attached. A suite of closely related abyssomicins was isolated from a marine sediment collected at a depth of 289 m under the sea of Japan and subsequently shown by Bister *et al.*[86] to be produced by a *Verrucosispora* species.

Abyssomicin C has recently been synthesized, relying on an intramolecular Diels-Alder cyclization that may mimic its natural mode of biosynthesis[87], opening the door for structure-activity relationship studies. Bister *et al.*[86] report that abyssomicin blocks *p*-aminobenzoate formation in the bacterial folate biosynthetic pathway. Although the exact target has not been determined yet, it has been hypothesized that abyssomicin inhibits *p*-aminobenzoic acid (PABA) synthase; this enzyme converts chorismate to PABA in a net amination, aromatization reaction. Abyssomicin could be a rigid transition-state analog that mimics a reactive conformer of chorismate during the addition-elimination steps.

Although more information on abyssomicin's mode of action is clearly required, its discovery reinforces two messages: first, sampling underexplored biological niches leads to detection of new natural product frameworks[88]; and second, the enzymes that catalyze the early steps in the folate pathway may serve as equally robust antibiotic targets as the better-known pteroate synthase and dihydrofolate reductase[1] enzymes that act later in the pathway.

Conclusions

Most clinically used antibiotics are either microbial natural products or semisynthetic derivatives of these molecules. On the basis of Baltz's prescriptions[5,6] for high-throughput screening of actinomycetes, the new raft of approaches for natural product discovery and the smattering of new compounds with interesting properties and mechanisms of action, it seems likely that continued discovery of new natural product antibiotics is a matter of expeditious deployment of resources.

Converging trends from microbial genomics and protein structural biology continue to drive new insights into the biosynthetic capacity of bacteria and fungi and the molecular recognition of small molecules with specific macromolecular targets. For example, the clustering of diverse antibiotic structures—both natural and semisynthetic molecules—in very close proximity to one another in the peptidyl transferase center of the bacterial ribosome illustrates how structural analysis can inform the engineering of hybrid molecules, in particular molecules capable of overcoming bacterial resistance mechanisms.

At the same time, successes with the heterologous expression of DNA provide a tantalizing glimpse of the potential benefits—and difficulties—of accessing uncultured microbes for antibiotics. To date, all reported small molecules from captured biosynthetic pathways have relied on heterologous expression. As a result, mismatches—particularly background metabolism, codon usage and promoter structure—between the host and the captured pathway have limited the range of small molecules that can be discovered, and new heterologous hosts will make wider screening efforts possible. Despite such problems, it seems likely that activity in this area will grow and that sequence-based screening, which has been successful in identifying both biocatalysts from environmental DNA libraries[89] and biologically active small molecules from cultured microbes[44], will be applied to small-molecule discovery from environmental DNA in the future. Furthermore, innovative new methods of culturing microorganisms and bioprospecting in environments that have been, as yet, only poorly sampled is likely to significantly expand the number of natural products that can be screened—platensimycin is just one example.

The examples of new natural products that are cited in this review also show the rich vein of leads that have resulted from natural product research. For example, work with acyldepsipeptidolactones suggests that other bacterial ATP-utilizing enzymes may be a promising targets for antibiotic discovery, and the remarkable array of conformationally constrained natural peptidic molecules and lantibiotics targeting lipid II indicate that this molecule will also be a useful focus point for antibacterial strategies.

Natural product research is also revealing new points for intervention in bacterial pathways (often pathways targeted at different points by existing antibiotics). For example, the enzymes that catalyze the early steps of fatty acid biosynthesis in the folate pathway may serve as equally robust antibiotic targets as the better-known pteroate synthase and dihydrofolate reductase[1] that act later in the pathway.

On this evidence, efforts to discover (or rediscover) natural product families seem likely to complement the decades-long trend of iterative semisynthetic modification of old antibiotic scaffolds in the drive for discovering new antibiotics. At the same time, many of these new antibiotics will provide valuable clues about currently unexploited targets against which natural product and synthetic chemical libraries should be screened. The ingenuity and success of the discovery efforts discussed above bode well for the future prospects of finding new antibiotics from natural sources.

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COMPETING INTERESTS STATEMENT

Christopher T. Walsh is on the board of directors of Kosan Biosciences, and Jon Clardy is on the scientific advisory boards on Novobiotics and Makoto Biosciences.

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