Published in final edited form as:
ACS Chem Biol. 2011 October 21; 6(10): 1000–1007. doi:10.1021/cb200284p.

Chemical Logic and Enzymatic Machinery for Biological Assembly of Peptidyl Nucleoside Antibiotics

Christopher T. Walsh[†,*] and **Wenjun Zhang**[‡,*]

[†]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

[‡]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720

Abstract

Peptidyl nucleoside antibiotics are a group of natural products targeting MraY, a bacterial translocase involved in the lipid-linked cycle in peptidoglycan biosynthesis. In this Perspective, we explore how Nature builds complex peptidyl nucleoside antibiotics scaffolds from simple nucleoside and amino acid building blocks. We discuss the current stage of research on biosynthetic pathways for peptidyl nucleoside antibiotics, primarily focusing on chemical logic and enzymatic machinery for uridine transformation and coupling to peptides. We further survey the nonribosomal biosynthetic paradigm for a subgroup of uridyl peptide antibiotics represented by pacidamycins, concluded by diversification opportunities for antibiotic optimization.

Keywords

Peptidyl nucleoside antibiotics; MraY; Nonribosomal peptide synthetase (NRPS); Nonproteinogenic amino acid

Microbes make a variety of natural products with antibiotic activity, including molecules with polyketide frameworks (erythromycins and tetracyclines), oligosaccharide frameworks (aminoglycosides), and those with peptide scaffolds. The peptide scaffolds can be of ribosomal origin with extensive posttranslational modification to rigidify the architecture, as in lantibiotics (1) and thiazolyl peptides (2), or they can be made on nonribosomal peptide synthetase (NRPS) assembly lines, most famously the tripeptide precursors to penicillins and cephalosporins (3). The nonribosomal peptide antibiotics are often elaborated during biosynthesis to lipopeptides such as daptomycin (4) or to glycopeptides such as vancomycin (5) to alter physical properties and provide targeting.

Microbes can also join nucleosides to peptide scaffolds to create peptidyl nucleosides that function as antibiotics. Examples include the cytosine derivative blasticidin S and the adenine derivative puromycin which target bacterial and fungal ribosomes (6-8), and uridine-based nikkomycins and polyoxins which target cellulose biosynthesis in fungi (9, 10). A larger group of uridyl peptide natural product antibiotics, including pacidamycins and mureidomycins, liposidomycins and caprazamycins, capuramycin and muraymycins (Figure 1), target the enzyme MraY involved in the first membrane-dependent step in bacterial peptidoglycan assembly (11).

[*]**Corresponding Author** christopher_walsh@hms.harvard.edu; wjzhang@berkeley.edu .

MraY and Bacterial Cell Wall Biosynthesis

MraY is an essential bacterial enzyme with ten predicted transmembrane helices that embed it within the cytoplasmic membrane of bacteria (12) where it can interact with one of its substrates, the C_{55} isoprenoid carrier lipid known as bactoprenol-phosphate (bactoprenol-P). The cosubstrate is the UDP-*N*-acetylmuramoyl (UDP-MurNAc) pentapeptide (-L-Ala_1-D-γ-Glu_2-Lys/DAP_3-D-Ala_4-D-Ala_5-COOH) that accumulates from action of the enzymes MurA-F in the bacterial cytoplasm. The MurNAc-pentapeptide will need to be subsequently glycosylated with an *N*-acetylglycosamine (GlcNAc) residue and flipped to the outer surface of the cytoplasmic membrane where the disaccharyl pentapeptide units can be used to elongate peptidoglycan chains necessary for cell division (Figure 2). The UDP moiety, the canonical biologic sugar activating group, has two negatively charged phosphate groups and cannot cross membranes. Thus transfer of the MurNAc-pentapeptide to the membrane soluble C_{55} bactoprenol-P by MraY is a necessary prequel to getting peptidoglycan building blocks to the outer surface of the membrane. For this reason, MraY is sometimes termed a translocase because it moves the sugar pentapeptide unit from the cytoplasm to the membrane. The *Bacillus subtilis* MraY has been solubilized, purified and characterized kinetically, but there are no structural data to guide the MraY inhibitor design (13). It is also unclear if the phosphor-MurNAc-pentapeptide group is directly translocated to bactoprenol-P or this transfer process involves a covalent enzyme intermediate. If direct, this would be a precedented displacement of UMP as a good leaving group by a nucleophilic phosphate oxygen, in this instance the C_{55} phosphate monoester (Figure 2).

Uridyl Peptide Antibiotics Targeting MraY

As often the case for antibiotics Nature has served up some scaffolds that give insights into mode of action and further directions in the various classes of uridyl peptides that inhibit MraY. These uridyl peptides can be divided into three subclasses based on the connectivity of the uridine moiety to the rest of the scaffold (Figure 1) (11). The subfamily that encompasses pacidamycins, mureidomycins, napsamycins and sansanmycins have an unusual 3′-deoxy-4′,5′-enamino-uridine nucleoside which is attached through the 5′-nitrogen in carboxamido linkage to a tetra- or pentapeptide scaffold. The liposidomycins, caprazamycins, and muraymycins have a 5′-alcohol group, but now it is a secondary rather than the starting primary alcohol (e.g. of the common metabolite uridine), with a peptide chain as one substituent and a 5-aminoribose in β-1″,5′-linkage. Fatty acid chains of various lengths can be esterified, giving rise to the name liposidomycins. Capuramycin forms a third subgroup in which C-5′ of the uridine moiety has a pendant one carbon carboxamide moiety that has been introduced during biosynthesis. As one might anticipate from these structures, the producing microbes have thus built molecules that mimic the MraY substrate UDP-MurNAc-pentapeptide, and act as competitive inhibitors with the uridine moiety as a key recognition element. None of the molecules is a potent broad spectrum antibiotic ready for human clinical use, but they have selectivity against bacterial MraY and are generally not toxic (14), suggesting they could be improved. This is in contrast to tunicamycins and related metabolites (Figure 1): these uridyl-sugar-lipids lack peptide moieties, and are prohibitively toxic by inhibiting a wide variety of membrane-associated glycosyl transferases including those involved in eukaryotic glycoprotein maturation.

Biosynthetic Gene Clusters to Provide Assembly Hypotheses

In the past two years genome sequencing, gene cloning, and bioinformatics-guided analyses have identified biosynthetic gene clusters for liposidomycins, caprazamycins, capuramycin, pacidamycins, and napsamycins (15-21). A combination of individual gene mutagenesis, heterologous expression of gene clusters in *Streptomycete* hosts, and mechanistic study of

purified pathway enzymes have given insight into the chemical logic for coupling of uridine moieties to the peptide backbones as well as appreciation for the nonribosomal peptide assembly lines used to build the noncanonical peptide scaffolds. These pathways have also turned up novel chemistry in biological systems.

Uridine-5′-aldehyde and Uridine-5′-amine to Provide Electrophilic and Nucleophilic Condensation Partners

Inspection of C-5′ of the ribose moiety in liposidomycins, caprazamycins, muraymycins (and also tunicamycins) has suggested that enzymatic oxidation of the 5′-alcohol to the aldehyde level would create an electrophilic center for C-C bond formation. This uridine metabolite, the 5′-aldehyde, had never been reported in the literature, and several efforts to find a uridine-specific alcohol dehydrogenase/oxidase activity have not succeeded. In 2011, Van Lanen and colleagues reported that an oxygenative rather than a dehydrogenative metabolic strategy is in play (22). The enzyme LipL encoded in the A90289 cluster (a caprazamycin type antibiotic) is a mononuclear iron-containing, α-ketoglutarate-dependent hydroxylase acting on C-5′ of UMP (not free uridine) to generate what is presumed to be the phospho form of the aldehyde hydrate, which decomposes to inorganic phosphate and uridine-5′-aldehyde (Figure 3**, panel a**). Characterization of the LipL reaction is a transformative event in uridine oxidative metabolism and places uridine-5′-aldehyde firmly on the metabolic board as a player. Whether other pathways also use this oxygenative strategy on UMP or act on uridine via a NADH-dependent dehydrogenation has yet to be resolved.

Inspection of C-5′ in the mureidomycin and pacidamycin subgroup suggests uridine-5′-amine could be on pathway, which is bolstered by the observation that the liposidomycins, caprazamycins and muraymycins all have 5-aminoribose groups in β-1″,5′-linkage to the uridine moieties. These glycosidic linkages could arise by displacement of uracil from a 5′-aminouridine by the 5′-OH of an intermediate via C-1′-N cleavage. Thus it is highly likely that the initial uridine-5′-aldehyde or its derivative undergoes enzymatic transamination by pyridoxal-phosphate (PLP)-dependent enzymes to yield uridine-5′-amine building blocks in both subfamilies of antibiotic producers (Figure 3**, panel b**).

The uridine-5′-aldhyde can now function as an *electrophilic* partner not just with amine nucleophiles, as in the postulated transamination, but also with carbon nucleophiles on amino acid frameworks. The classic route to stabilize α-amino acid carbanions in biology is to form aldimines in the active sites of PLP-dependent enzymes and subtract Hα to give the delocalized carbanion equivalent. As shown in Figure 3, panel b, reaction with such a glycine carbanion equivalent would build the key C-C bond and lead to uridyl-5′-glycine as a prototypic building block in the muraymycin framework. Analogously the value of uridine-5′-amine partner, such as 3′-deoxy-4′,5′-enamino-uridine, is that it can be used with reverse polarity, as a nucleophile, to build the urdine-carboxamide bond to the peptide backbone in all the pacidamycins, mureidomycins, napsamycins and sansanmycins. As we will note below, the electrophilic partner in those amide-forming condensations are tetrapeptidyl- or pentapeptidyl thioesters tethered to the phosphopantetheinyl arms of thiolation domain proteins in NRPS assembly lines.

Capuramycin and polyoxin mysteries

Still mysterious are the transformations that occur at C-5′ of uridines to build the connectivity in such uridyl peptides as capuramycin and polyoxins, e.g. polyoxin C (Figure 4**, panel a**). In a formal sense the one carbon carboxamide branching at C-5′ in capuramycin could arise by action of a carbamoyltransferase with the primary metabolite carbamoyl

phosphate as an electrophilic acyl donor. This requires C-5′ of the uridine moiety to be nucleophilic if it is a typical carbamoyl transfer reaction. It is not clear how carbanion character would be generated, nor is it clear when the hexuronic acid glycoside link is made to the C-5′ alcohol. Another route to the one carbon carboxamide might involve uridyl-5′-glycine, the same intermediate proposed in muraymycin assembly. Decarboxylation followed by oxygenation at 5′-glycine would lead to the C-5′ carboxamide branching. For polyoxins and related nikkomycins, C-5′ has both an amine and a carboxylate substituent. Surprisingly the carboxylate derives from C-3 of glycerate (23), and 3′-*O*-enolpyruvyl UMP appears to be an early biosynthetic intermediate (24, 25). It has been proposed, but not shown, that the pyruvyl moiety could migrate and rearrange, presumably with C-5′ at the aldehyde oxidation state. The four carbon γ-hydroxy-α-keto acid side chain would need to be oxidatively decarboxylated, trimmed, and reductively aminated to get to polyoxin C (Figure 4**, panel b**).

Pacidamycin Peptide Scaffold: Nonribosomal Provenance

In the first subgroup of uridyl peptide antibiotics, the pacidamycins have been most thoroughly examined and most probably represent the paradigm for the other members. Depending on fermentation conditions, both tetrapeptide and pentapeptide versions of pacidamycins can be isolated. The pentapeptide versions have Ala or Gly as residue 1 at the *N*-terminus. The tetrapeptide versions are all missing that first residue, and have a nonproteinogenic *meta*-Tyr_2 (*m*-Tyr_2), an Ala_2, or a tetrahydroisoquinoline moiety seen as the predominant residue in the napsamycin series at the *N*-terminus (26-28). Residue 3 is the nonproteinogenic *2S,3S*-diaminobutyrate (DABA); residue 4 is Ala; residue 5 is any one of aromatic amino acids: Phe, Trp, or *m*-Tyr. The *m*-Tyr building block is produced by a novel regiospecific iron(II)-dependent phenylalanine-3-hydroxylase that resembles its phenylalanine-4-hydroxylase counterpart, using molecular oxygen and tetrahydrobiopterin as cosubstrates (29). One might have expected that the tetrapeptides arose by aminopeptidase-mediated degradation of the pentapeptides during or after secretion, but that expectation was invalidated during careful study of the assembly line logic as described below.

The multiple forms and the utilization of nonproteinogenic amino acid building blocks raised the expectation that the peptide scaffolds would be put together by mRNA-independent NRPS assembly lines. Furthermore, residues 4 and 5 are connected not by a normal peptide bond, but by a highly unusual ureido linkage (hence the name mureidomycins). Closer inspection of the peptide scaffold running from *N*-terminus to *C*-terminus indicates the chain direction is actually reversed twice. First, the connectivity between Ala_2/m-Tyr_2 and $DABA_3$ is not to the α-amine of $DABA_3$ to form a normal peptide bond but instead to the β-amine (a β-peptide bond); then, the next residue Ala_4 is not connected to the $DABA_3$ carboxyl but instead to the α-amine of $DABA_3$ through the carboxyl group of Ala_4. Ala_4 is thus in a retro direction and offers its amino to the 5^{th} and last residue. The last peptidyl chain connectivity is through the ureido linkage which again reverses the polarity, so that *m*-Tyr_5 is back in the correct direction and the peptide chain ends with a free carboxyl moiety.

One last consequence of the use of $DABA_3$, not as a regular amino acid building block but effectively as a diamine spacer is that its carboxyl is not tied up in an amide linkage and becomes available for reaction with the uridine-5′-amine. The uridine amide linkage is built as a branch point at residue 3 using carboxyl of $DABA_3$, rather than using the *C*-terminal carboxylate of residue 5. A side by side comparison of a pacidamycin pentapeptide with the UDP-MurNAc-pentapeptide substrate for MraY (Figure 5**, panel a**) shows a branched

versus linear connectivity in the molecular scaffolds, and a replacement of the diphosphate linker in the substrate with the enamide linkage in the antibiotic.

A highly Fragmented NRPS Assembly Line

Examination of the Pac proteins revealed a highly dissociated assembly line (Figure 5**, panel b**), in contrast to typical NRPS organizational logic where multiple modules are typically encoded within a single protein, the prototype being the three-module ACV synthetase in penicillin biosynthesis. There are four proteins containing adenylation (A) domains, two of them free-standing. There are likewise four proteins containing predicted 8-10 kDa thiolation (T) domains with a conserved serine residue likely to undergo posttranslational phosphopantetheinylation, adding a thiol group for covalent tethering of amino acids and the growing peptide chain as a series of elongating thioesters. There are three proteins with condensation (C) domains that typically make amide bonds during NRP chain elongation on the pantetheinyl arms of T domains, even though five amino acid monomers need to be joined. Ultimately a combination of genetic deletions, the heterologous expression and purification of nine Pac proteins from *E. coli* and the following assays in various combinations and permutations have allowed complete *in vitro* reconstruction of the highly fragmented NRPS assembly line (20, 30, 31).

Unusual Building Blocks and Ureido Linkage

Notable biosynthetic features include the fact that the tetrapeptidyl chain (residues 2-5) is built on the free-standing T domain PacH (10 kDa), allowing high resolution mass spectrometry (HRMS) characterization of all the covalently tethered intermediates. The chain is built from the middle outwards in both directions, via $DABA_3$ tethered through its carboxyl as a thioester to PacH. The *m*-Tyr_2 is regiospecifically coupled to the β-amine of the $DABA_3$ residue, while the Ala_4-ureido-*m*-Tyr_5 dipeptidyl piece is coupled to the α-amine of the tethered $DABA_3$. The ureido linkage is most probably introduced during chain elongation, via reaction of CO_2 with the amine of *m*-Tyr_5 to yield the *N*-carboxyaminoacyl-thioester. This may cyclize to a Leuchs' anhydride and diffuse to the active site of the Ala_4-S-PacN (C-T didomain protein), where it can be regiospecifically opened to give only the indicated PacN-*S*-Ala_4-ureido-*m*-Tyr_5-COOH (Figure 5**, panel c**) (20, 32). Ureido linkages have been seen in some other peptide natural products including syringolins which are mechanism-based inactivators of the proteasome, and *N*-carboxyaminoacyl-thioesters are also intermediates for those ureido linkages (33-35). The resultant *m*-Tyr_2-$DABA_3$-Ala_4-ureido-*m*-Tyr_5-COOH is stable in thioester linkage to PacH, and the cluster does not encode a standard thioesterase for chain release. Instead the free-standing condensation protein PacI catalyzes the aminolysis reaction with a synthetic 5′-aminouridine to build a tetrapeptidyl nucleoside scaffold (Figure 5, panel b).

Alanyl-tRNA as Donor to Complete Pentapeptide Scaffold

A combination of genetic deletions and structure-based predictions suggested that PacB might be the missing enzyme for converting uridyl tetrapeptide to uridyl pentapeptide frameworks in the pacidmaycin and related subfamilies (36). This was validated with purified PacB from *E. coli* heterologous expression: pure PacB uses alanyl-tRNA as the aminoacyl donor to *m*-Tyr_2-$DABA_3$-*S*-PacH to create Ala_1-*m*-Tyr_2-$DABA_3$-*S*-PacH for subsequent coupling with the Ala_4-ureido-*m*-Tyr5-COOH moiety (Figure 5**, panel d**). Thus, these peptidyl nucleoside assembly lines show an intersection between an RNA-templated protein synthesis component: an aminoacyl-tRNA, and the mRNA-independent NRPS assembly line machinery. Furthermore, it is remarkable that these assembly lines do not start at residue 1 and proceed downstream to the C-terminal residue 5, but instead build in both directions from the trifunctional DABA in the middle.

Mechanisms for Diversification

The producing *Streptomycetes* already practice combinatorial biosynthesis at residue 2 (Ala, *m*-Tyr from two different adenylation enzymes) (31) and residue 5 (Phe, Trp, *m*-Tyr) in these uridyl peptide antibiotic families, and will incorporate exogenous building blocks such as halotryptophans during fermentations for mutasynthesis (37). The ten protein assembly line for pacidamycins suggests some of the A and C domains will be evolvable and/or interchangeable with proteins from other NRPS assembly lines. It will be worth exploring for promiscuity to use the *Streptomyces coeruleorubidus* $tRNA^{Ala}$ loaded with different aminoacyl moieties to see what variation can be achieved at residue 1. Other diamino acids may serve as central building blocks in place of $DABA_3$. Determining how a β-peptide coupling is achieved at Ala_2-$DABA_3$ may allow export of this machinery to other NRPS antibiotic assembly lines. Finally, the tetra/pentapeptidyl offloading PacI condensation enzyme will use uridines in place of aminouridines (31), suggesting further exploration of nucleoside specificity for antibiotic optimization. In summary, characterization of the ten protein assembly line for pacidamycin antibiotics has revealed new principles of chemical logic to couple nucleosides and peptides, and enzymatic machinery to build morphed peptide frameworks.

Acknowledgments

This work was supported by NIH Grant GM49338 (to C.T.W.).

REFERENCES

1. Xie L, van der Donk WA. Post-translational modifications during lantibiotic biosynthesis. Curr. Opin. Chem. Biol. 2004; 8:498–507. [PubMed: 15450492]
2. Walsh CT, Acker MG, Bowers AA. Thiazolyl peptide antibiotic biosynthesis: a cascade of post-translational modifications on ribosomal nascent proteins. J. Biol. Chem. 2010; 285:27525–27531. [PubMed: 20522549]
3. Martin JF. Alpha-aminoadipyl-cysteinyl-valine synthetases in beta-lactam producing organisms. From Abraham's discoveries to novel concepts of non-ribosomal peptide synthesis. J. Antibiot. (Tokyo). 2000; 53:1008–1021. [PubMed: 11132945]
4. Baltz RH. Biosynthesis and genetic engineering of lipopeptide antibiotics related to daptomycin. Curr. Top. Med. Chem. 2008; 8:618–638. [PubMed: 18473888]
5. Hubbard BK, Walsh CT. Vancomycin assembly: nature's way. Angew. Chem. Int. Ed. Engl. 2003; 42:730–765. [PubMed: 12596194]
6. Cone MC, Petrich AK, Gould SJ, Zabriskie TM. Cloning and heterologous expression of blasticidin S biosynthetic genes from Streptomyces griseochromogenes. J. Antibiot. (Tokyo). 1998; 51:570–578. [PubMed: 9711220]
7. Tercero JA, Espinosa JC, Lacalle RA, Jimenez A. The biosynthetic pathway of the aminonucleoside antibiotic puromycin, as deduced from the molecular analysis of the pur cluster of Streptomyces alboniger. J. Biol. Chem. 1996; 271:1579–1590. [PubMed: 8576156]
8. Hansen JL, Moore PB, Steitz TA. Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. J. Mol. Biol. 2003; 330:1061–1075. [PubMed: 12860128]
9. Endo A, Kakiki K, Misato T. Mechanism of action of the antifugal agent polyoxin D. J. Bacteriol. 1970; 104:189–196. [PubMed: 5473886]
10. Dahn U, Hagenmaier H, Hohne H, Konig WA, Wolf G, Zahner H. Metabolic products of microorganisms. 154 Nikkomycin, a new inhibitor of fungal chitin synthesis. Arch. Microbiol. 1976; 107:143–160. [PubMed: 1259515]
11. Winn M, Goss RJ, Kimura K, Bugg TD. Antimicrobial nucleoside antibiotics targeting cell wall assembly: recent advances in structure-function studies and nucleoside biosynthesis. Nat. Prod. Rep. 2010; 27:279–304. [PubMed: 20111805]

12. Bouhss A, Mengin-Lecreulx D, Le Beller D, Van Heijenoort J. Topological analysis of the MraY protein catalysing the first membrane step of peptidoglycan synthesis. Mol. Microbiol. 1999; 34:576–585. [PubMed: 10564498]
13. Bouhss A, Crouvoisier M, Blanot D, Mengin-Lecreulx D. Purification and characterization of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan biosynthesis. J. Biol. Chem. 2004; 279:29974–29980. [PubMed: 15131133]
14. Bryskier, A. Antimicrobial agents: antibacterials and antifungals. ASM Press; Washington, USA: 2005.
15. Funabashi M, Nonaka K, Yada C, Hosobuchi M, Masuda N, Shibata T, Van Lanen SG. Identification of the biosynthetic gene cluster of A-500359s in Streptomyces griseus SANK60196. J. Antibiot. (Tokyo). 2009; 62:325–332. [PubMed: 19478828]
16. Kaysser L, Lutsch L, Siebenberg S, Wemakor E, Kammerer B, Gust B. Identification and manipulation of the caprazamycin gene cluster lead to new simplified liponucleoside antibiotics and give insights into the biosynthetic pathway. J. Biol. Chem. 2009; 284:14987–14996. [PubMed: 19351877]
17. Funabashi M, Baba S, Nonaka K, Hosobuchi M, Fujita Y, Shibata T, Van Lanen SG. The biosynthesis of liposidomycin-like A-90289 antibiotics featuring a new type of sulfotransferase. Chembiochem. 2010; 11:184–190. [PubMed: 20043306]
18. Kaysser L, Siebenberg S, Kammerer B, Gust B. Analysis of the liposidomycin gene cluster leads to the identification of new caprazamycin derivatives. Chembiochem. 2010; 11:191–196. [PubMed: 20039253]
19. Rackham EJ, Gruschow S, Ragab AE, Dickens S, Goss RJ. Pacidamycin biosynthesis: identification and heterologous expression of the first uridyl peptide antibiotic gene cluster. Chembiochem. 2010; 11:1700–1709. [PubMed: 20665770]
20. Zhang W, Ostash B, Walsh CT. Identification of the biosynthetic gene cluster for the pacidamycin group of peptidyl nucleoside antibiotics. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:16828–16833. [PubMed: 20826445]
21. Kaysser L, Tang X, Wemakor E, Sedding K, Hennig S, Siebenberg S, Gust B. Identification of a napsamycin biosynthesis gene cluster by genome mining. Chembiochem. 2011; 12:477–487. [PubMed: 21290549]
22. Yang Z, Chi X, Funabashi M, Baba S, Nonaka K, Pahari P, Unrine J, Jacobsen JM, Elliott GI, Rohr J, Van Lanen SG. Characterization of LipL as a non-heme, Fe(II)-dependent alpha-ketoglutarate:UMP dioxygenase that generates uridine-5′-aldehyde during A-90289 biosynthesis. J. Biol. Chem. 2011; 286:7885–7892. [PubMed: 21216959]
23. Isono K, Sato T, Hirasawa K, Funayama S, Suzuki S. Biosynthesis of the nucleoside skeleton of polyoxins. J. Am. Chem. Soc. 1978; 100:3937–3939.
24. Ginj C, Ruegger H, Amrhein N, Macheroux P. 3′-Enolpyruvyl-UMP, a novel and unexpected metabolite in nikkomycin biosynthesis. Chembiochem. 2005; 6:1974–1976. [PubMed: 16206325]
25. Chen W, Huang T, He X, Meng Q, You D, Bai L, Li J, Wu M, Li R, Xie Z, Zhou H, Zhou X, Tan H, Deng Z. Characterization of the polyoxin biosynthetic gene cluster from Streptomyces cacaoi and engineered production of polyoxin H. J. Biol. Chem. 2009; 284:10627–10638. [PubMed: 19233844]
26. Chen RH, Buko AM, Whittern DN, McAlpine JB. Pacidamycins, a novel series of antibiotics with anti-Pseudomonas aeruginosa activity. II. Isolation and structural elucidation. J. Antibiot. (Tokyo). 1989; 42:512–520. [PubMed: 2498264]
27. Fronko RM, Lee JC, Galazzo JG, Chamberland S, Malouin F, Lee MD. New pacidamycins produced by Streptomyces coeruleorubidus, NRRL 18370. J. Antibiot. (Tokyo). 2000; 53:1405–1410. [PubMed: 11217807]
28. Chatterjee S, Nadkarni SR, Vijayakumar EK, Patel MV, Ganguli BN, Fehlhaber HW, Vertesy L. Napsamycins, new Pseudomonas active antibiotics of the mureidomycin family from Streptomyces sp. HIL Y-82,11372. J. Antibiot. (Tokyo). 1994; 47:595–598. [PubMed: 8040059]
29. Zhang W, Ames BD, Walsh CT. Identification of Phenylalanine 3-Hydroxylase for meta-Tyrosine Biosynthesis. Biochemistry. 2011; 50:5401–5403. [PubMed: 21615132]

30. Zhang W, Heemstra JR Jr. Walsh CT, Imker HJ. Activation of the pacidamycin PacL adenylation domain by MbtH-like proteins. Biochemistry. 2010; 49:9946–9947. [PubMed: 20964365]

31. Zhang W, Ntai I, Bolla ML, Malcolmson SJ, Kahne D, Kelleher NL, Walsh CT. Nine enzymes are required for assembly of the pacidamycin group of peptidyl nucleoside antibiotics. J. Am. Chem. Soc. 2011; 133:5240–5243. [PubMed: 21417270]

32. Hirschma R, Strachan RG, Schwam H, Schoenew Ef, Joshua H, Barkemey B, Veber DF, Paleveda WJ, Jacob TA, Beesley TE, Denkewal Rg. Controlled synthesis of peptides in aqueous medium. 3. Use of leuchs anhydrides in synthesis of dipeptides. Mechanism and control of side reactions. J. Org. Chem. 1967; 32:3415. [PubMed: 5622457]

33. Itou Y, Suzuki S, Ishida K, Murakami M. Anabaenopeptins G and H, potent carboxypeptidase A inhibitors from the cyanobacterium Oscillatoria agardhii (NIES-595). Bioorg. Med. Chem. Lett. 1999; 9:1243–1246. [PubMed: 10340607]

34. Imker HJ, Walsh CT, Wuest WM. SylC catalyzes ureido-bond formation during biosynthesis of the proteasome inhibitor syringolin A. J. Am. Chem. Soc. 2009; 131:18263–18265. [PubMed: 19968303]

35. Walther T, Renner S, Waldmann H, Arndt HD. Synthesis and structure-activity correlation of a brunsvicamide-inspired cyclopeptide collection. Chembiochem. 2009; 10:1153–1162. [PubMed: 19360807]

36. Zhang W, Ntai I, Kelleher NL, Walsh CT. tRNA-dependent peptide bond formation by the transferase PacB in biosynthesis of the pacidamycin group of pentapeptidyl nucleoside antibiotics. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:12249–12253. [PubMed: 21746899]

37. Gruschow S, Rackham EJ, Elkins B, Newill PL, Hill LM, Goss RJ. New pacidamycin antibiotics through precursor-directed biosynthesis. Chembiochem. 2009; 10:355–360. [PubMed: 19090518]

Peptidyl nucleoside antibiotics: A group of natural products with both nucleoside and peptide in molecular scaffolds that have antibiotic activities.

MraY: an essential bacterial cytoplasmic membrane enzyme catalyzing the formation of lipid intermediate I in peptidoglycan biosynthesis by transferring phosphor-MurNAc-pentapeptide from UDP-MurNAc-pentapeptide to bactoprenol-phosphate

Nonribosomal peptide synthetase (NRPS): A multimodular enzyme catalyzing biosynthesis of peptides without the aid of mRNA and ribosome.

Nonproteinogenic amino acid: An amino acid not typically involved in protein synthesis, and often present in nonribosomal peptides.

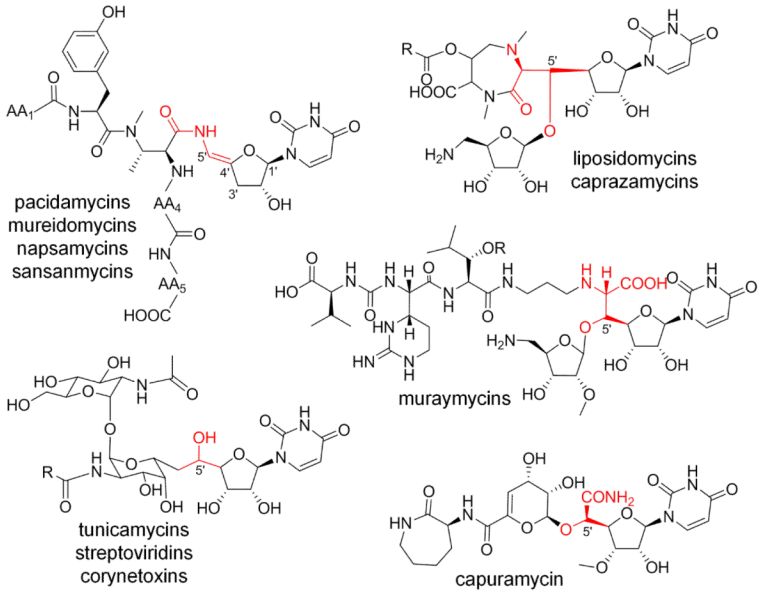

Figure 1.

Structures of selected nucleoside antibiotics targeting cell wall assembly. R = fatty acid moiety. The linkage of the nucleoside moiety to the rest of the scaffold is highlighted in red.

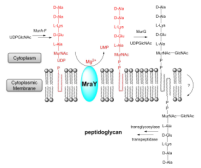

Figure 2.
Peptidoglycan biosynthetic pathway. The integral membrane protein MraY catalyzes transfer of the phospho-MurNAc-pentapeptide to the membrane soluble C_{55} bactoprenol-phosphate. The MraY-catalyzed reaction is highlighted in red.

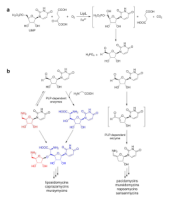

Figure 3.
Schematic of nucleoside modification for peptidyl nucleoside antibiotics biosynthesis. a) Characterization of LipL as a Fe(II)- and α-ketoglutarate-dependent hydroxylase catalyzing uridine-5′-aldehyde formation from UMP. b) Proposed roles of uridine-5′-aldehyde and uridine-5′-amine as electrophilic and nucleophilic condensation partners.

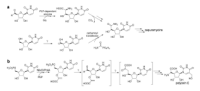

Figure 4.
Proposed nucleoside transformations leading to capuramycin (a) and polyoxin C (b).

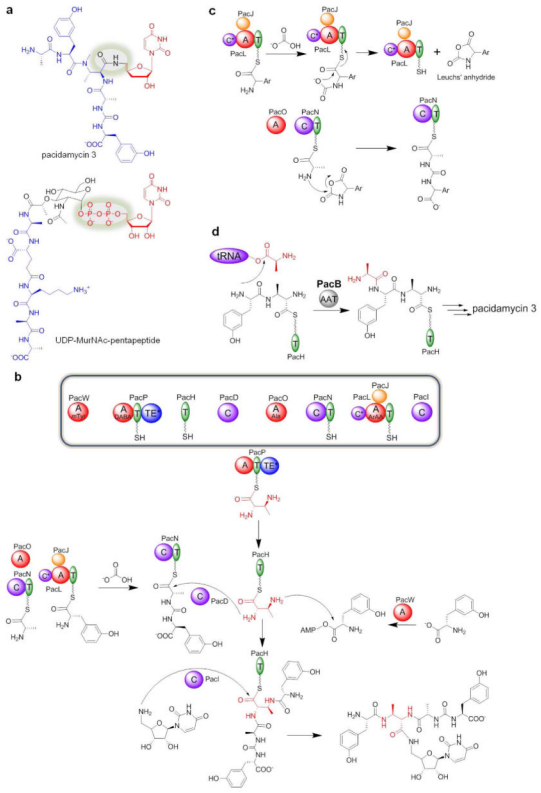

Figure 5.

Pacidamycin uridyl peptide scaffold assembly. a) Structure comparison of MraY inhibitor pacidamycin pentapeptide with MraY substrate UDP-MurNAc-pentapeptide. b) Schematic of pacidamycin highly fragmented NRPS assembly line. c) Proposed mechanism leading to ureido linkage found in pacidamycins. Ar = aromatic side chain of Phe, *m*-Tyr or Trp. d) Characterization of PacB as a tRNA-dependent aminoacyltransferase acting on NRPS assembly line PacH.