

**Manuscript version: Author's Accepted Manuscript**

The version presented in WRAP is the author's accepted manuscript and may differ from the published version or Version of Record.

**Persistent WRAP URL:**

<http://wrap.warwick.ac.uk/124569>

**How to cite:**

Please refer to published version for the most recent bibliographic citation information. If a published version is known of, the repository item page linked to above, will contain details on accessing it.

**Copyright and reuse:**

The Warwick Research Archive Portal (WRAP) makes this work by researchers of the University of Warwick available open access under the following conditions.

Copyright © and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable the material made available in WRAP has been checked for eligibility before being made available.

Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

**Publisher's statement:**

Please refer to the repository item page, publisher's statement section, for further information.

For more information, please contact the WRAP Team at: [wrap@warwick.ac.uk](mailto:wrap@warwick.ac.uk).

# 1 Mechanism of Action of Nucleoside Antibacterial Natural Product Antibiotics

2  
3 Timothy D. H. Bugg and Rachel V. Kerr

4 Department of Chemistry, University of Warwick, Coventry CV4 7AL, U.K.

5 Email T.D.Bugg@warwick.ac.uk

6

## 7 Contents

8 1. Antibacterial nucleoside antibiotics targetting bacterial peptidoglycan biosynthesis

9 1.1 The tunicamycin group of GlcNAc-tunicamine nucleoside antibiotics (tunicamycins,  
10 streptovirudins, corynetoxins)

11 1.2 The mureidomycin group of ureidyl-peptide nucleoside antibiotics (mureidomycins,  
12 pacidamycins, napsamycins, sansanmycins)

13 1.3 The liposidomycin group of liponucleoside antibiotics (liposidomycins, caprazamycins)

14 1.4 The muraymycin group of lipo-ureidylpeptide nucleoside antibiotics

15 1.5 The capuramycin group of caprolactam nucleoside antibiotics (capuramycin, A-  
16 500359A)

17 1.6 Comparison of antibacterial spectrum of nucleoside antibiotics

18 2. Mechanism of inhibition of translocase *MraY* by nucleoside antibacterial antibiotics

19 2.1 Kinetic mechanism of inhibition of translocase *MraY*

20 2.2 Structure of *Aquifex aeolicus* *MraY* and its complexes with nucleoside antibiotics

21 2.3 Interaction with protein-protein interaction site for bacteriophage lysis protein E

22 3. Inhibition of other bacterial phospho-nucleotide transferase enzymes by nucleoside natural  
23 product analogues.

24

## 25 Abstract

26 This article reviews the structures and biological activities of several classes of uridine-containing  
27 nucleoside antibiotics (tunicamycins, mureidomycins/pacidamycins/sansanmycins,  
28 liposidomycins/caprazamycins, muraymycins, capuramycins) that target translocase *MraY* on the  
29 peptidoglycan biosynthetic pathway. In particular, recent advances in structure-function studies, and  
30 recent X-ray crystal structures of translocase *MraY* complexed with muraymycin D2 and tunicamycin  
31 are described. The inhibition of other phospho-nucleotide transferase enzymes related to *MraY* by  
32 nucleoside antibiotics and analogues is also reviewed.

33

34 **Dedication.** This article is part of a Special Issue commemorating Dr Kiyoshi Isono and his important  
35 contributions to the study of nucleoside antibiotics. Dr. Isono led the discovery of the liposidomycin

1 natural products in 1985, one of the first studies in this field, which established that nucleoside  
2 antibiotics could be selective antibacterial agents.

3

4 The discovery of the liposidomycin nucleoside antibiotics by Dr. Kiyoshi Isono and co-workers  
5 in 1985 [1], and the nucleoside antibiotic tunicamycin by Tamura and co-workers [2], has led to the  
6 identification and study of a related collection of uridine-containing nucleoside antibiotics with potent  
7 antibacterial activity, targeting the enzyme phospho-MurNAc-pentapeptide translocase (MraY) on the  
8 peptidoglycan cell wall biosynthetic pathway. The structures of each family have been reviewed in  
9 detail in reviews in 2003 [3] and 2010 [4]. This review will discuss recent structure-activity studies on  
10 each group of nucleoside antibiotics, and the mechanism of inhibition of translocase MraY, in  
11 particular, the recent crystal structures of nucleoside antibiotics bound to MraY.

12

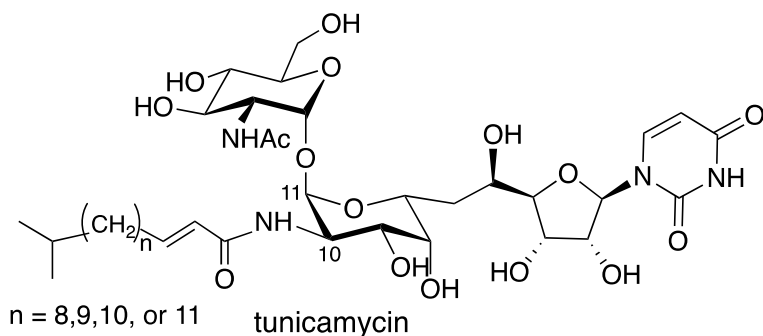
13

## 14 **1. Antibacterial nucleoside antibiotics targeting bacterial peptidoglycan** 15 **biosynthesis**

16

### 17 **1.1 The tunicamycin group of GlcNAc-tunicamine nucleoside antibiotics (tunicamycins,** 18 **streptovirudins, corynetoxins)**

19 The tunicamycin group of nucleoside antibiotics were isolated in 1971 from *Streptomyces*  
20 *lysosuperficus* by Tamara and co-workers [2]. They contain a uracil base attached to a C<sub>11</sub>  
21 tunicamine sugar, glycosylated at C<sub>11</sub> by a GlcNAc sugar and N-acylated at C<sub>10</sub> by a C<sub>12</sub>-C<sub>15</sub> fatty  
22 acid (see Figure 1). They showed antibacterial activity against a range of Gram-positive bacteria,  
23 especially those in the *Bacillus* genus (MIC 0.1-20 µg/ml) [2], but also showed toxicity towards  
24 eukaryotic cells, due to inhibition of eukaryotic N-linked glycoprotein biosynthesis [5]. The  
25 streptovirudins and corynetoxins contain the same uracil-tunicamine skeleton, but are acylated by  
26 different fatty acids [3]. The biosynthetic gene cluster for the tunicamycin antibiotics has been  
27 identified in *Streptomyces chartreusis* [6], and the biosynthetic pathway has been shown to involve  
28 an unusual radical SAM enzyme TunM in the assembly of the tunicamine sugar [7].



1 Figure 1. Structures of tunicamycins

2

3 A total synthesis of tunicamycin V was reported in 2017 by Ichikawa and co-workers [8],  
4 which has enabled the synthesis of tunicamycin analogues for structure-activity study [9]. A lipid-  
5 truncated analogue and an analogue lacking the GlcNAc sugar both lost 1000-fold in *MraY*  
6 inhibition activity but retained some enzyme inhibition, while an analogue lacking the nucleoside  
7 base was completely inactive [9]. The presence of the uracil base has been shown to be required in  
8 other nucleoside antibiotic families [3,4], which can be rationalised by the *MraY* structural studies  
9 described in Section 2.2.

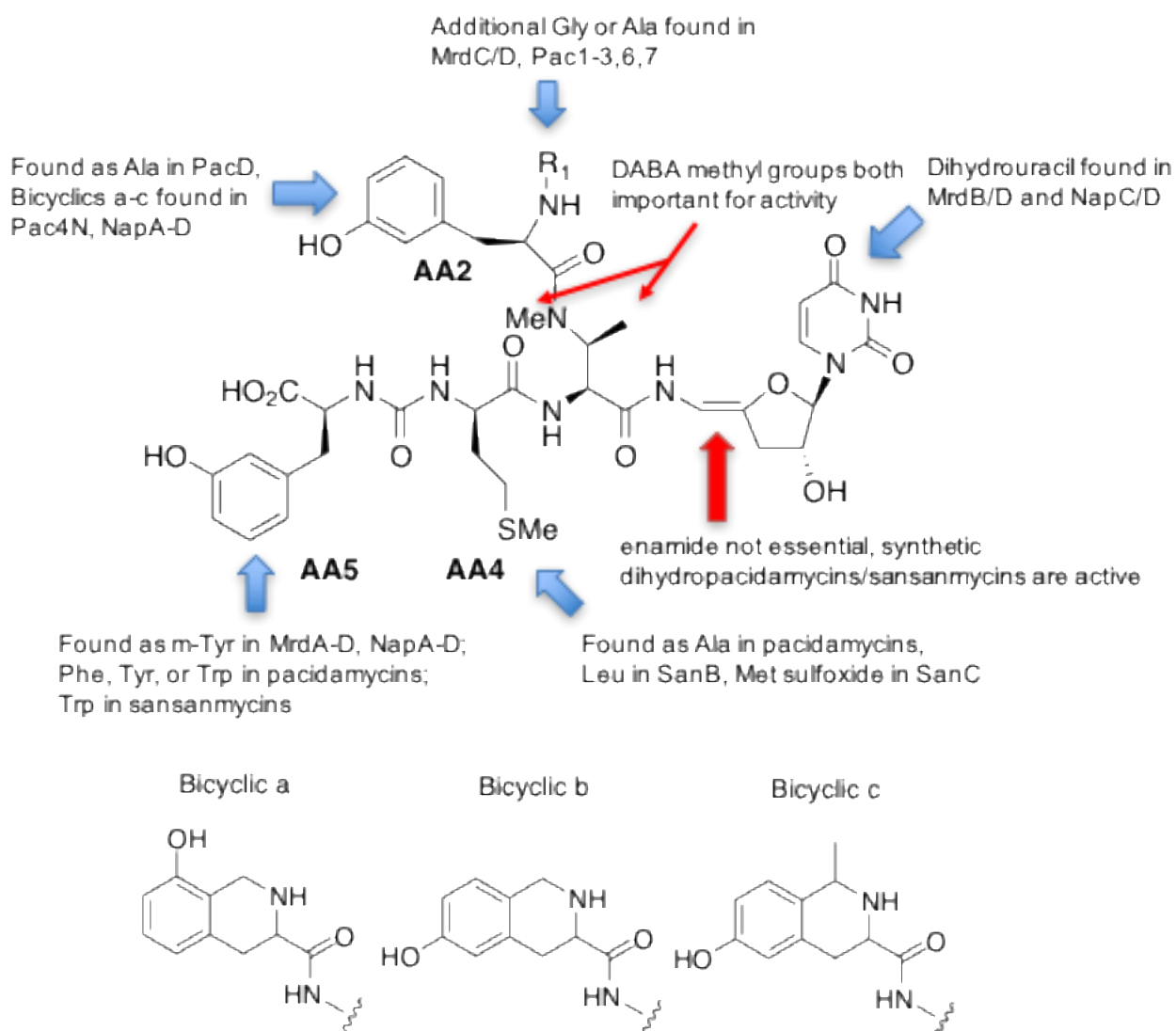
10

11 **1.2 The mureidomycin group of ureidyl-peptide nucleoside antibiotics (mureidomycins,**  
12 **pacidamycins, napsamycins, sansanmycins)**

13 Mureidomycins A-D were isolated from *Streptomyces flavidoviridens* SANK 60486, and  
14 first reported in 1989 [10]. They showed potent antimicrobial activity against a range of  
15 *Pseudomonas* strains (MIC 0.1 – 3 µg/mL), and protected mice against infection by *Pseudomonas*  
16 *aeruginosa* (ED<sub>50</sub> 50 mg/kg for MrdC) [11,12]. Phospho-MurNAc-pentapeptide translocase (*MraY*)  
17 on the bacterial peptidoglycan biosynthetic pathway was identified as the molecular target of these  
18 compounds [11]. A closely related series of pacidamycins 1–7, isolated from *Streptomyces*  
19 *coeruleorubidus* strain AB 1183F-64, were also reported in 1989 [13-15]. The pacidamycins also  
20 showed antimicrobial activity against *Pseudomonas* strains (MIC's 8 – 64 µg / mL), but they were  
21 found not to protect mice against infection by *Pseudomonas aeruginosa* [15].

22 The structures of both families contain a 3'-deoxyuridine sugar attached via an 4',5'-  
23 enamide linkage to the carboxyl group of an N-methyl 2,3-diaminobutyric acid (DABA) residue, to  
24 which amino acids are attached on both nitrogen substituents (see Figure 2). To the α-amino group  
25 of DABA is attached either Met (mureidomycins) or Ala (pacidamycins), which is in turn attached  
26 via a urea linkage to a C-terminal aromatic amino acid, either *meta*-tyrosine (mureidomycins), or  
27 Trp or Phe (pacidamycins). To the β-amino group of the DABA residue is attached in most cases a  
28 *meta*-tyrosine residue, except in pacidamycin D, which contains Ala. Two further mureidomycins E  
29 and F were later reported, containing a bicyclic derivative of *meta*-tyrosine at the amino-terminal  
30 position [16], also found in the closely related napsamycins, which were reported in 1994 [17]. The  
31 sansanmycins were reported in 2007, and contain the same structural skeleton as the  
32 mureidomycins, but contain Trp at the C-terminal position, and contain either Met, Leu, or  
33 methionine sulfoxide at position 4 [18,19]. The sansanmycins showed antipseudomonal activity,  
34 but also showed activity against *Mycobacterium tuberculosis* (MIC 8-20 µg/ml) [19]. The structures  
35 of these classes of uridyl peptide antibiotics are shown in Figure 2.

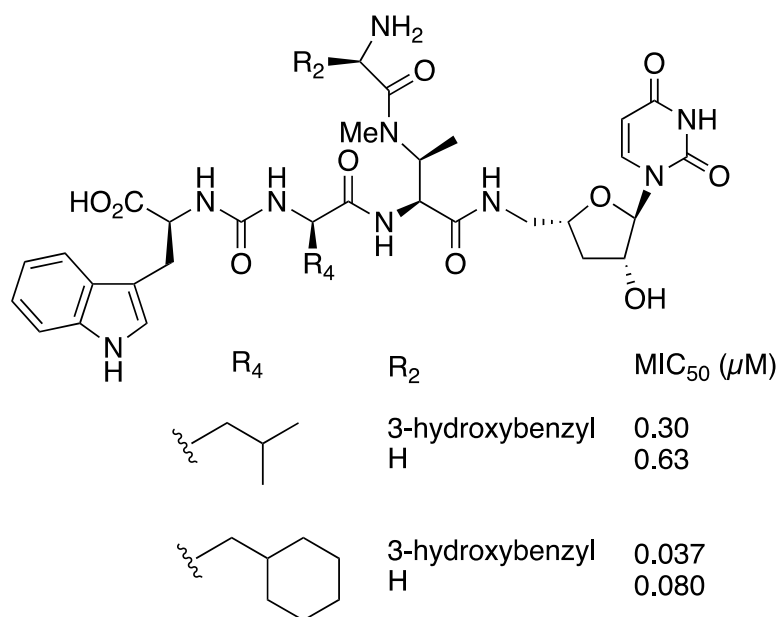
1 A series of synthetic dihydropacidamycin analogues in which the 4'-5' enamide was absent  
 2 were prepared by Microcide Inc. The parent 4*R*-dihydropacidamycin retained antipseudomonal  
 3 activity, but with somewhat reduced MIC (64 µg/ml) compared to pacidamycin D [20]. Synthetic  
 4 analogues containing Phe or Leu at position 4, and Trp or Tyr at position 5, showed best  
 5 antipseudomonal activity (MIC 4-16 µg/ml) [20]. An analogue containing 4-fluorophenylalanine in  
 6 place of Met at position 4 showed antimicrobial activity against clinical *E. coli* strains (MIC 4-8  
 7 µg/ml), as well as *Mycobacterium tuberculosis* (MIC 4-10 µg/ml) [21]. In 2011, Okamoto *et al*  
 8 published a total synthesis of pacidamycin D [22], which they have used to synthesise further  
 9 analogues varying the N-terminal dipeptide chain [23]. They have reported that meta-tyrosine in the  
 10 amino-terminal position is considerably more active than L-Tyr, and that the stereochemistry of the  
 11 2,3-diaminobutyric acid is important for both MraY inhibition and antimicrobial activity [23].



12  
 13 Figure 2. Structural features of mureidomycin, pacidamycin, napsamycin, and sansanmycin families  
 14 of ureidyl peptide antibiotics. Structural variation show by blue arrows, structure-activity  
 15 observations shown by red arrows.

16

1 The anti-TB activity of the sansanmycin series has been developed significantly by Payne  
 2 and co-workers, via chemical synthesis of a set of dihydrosansanmycin analogues [24]. They found  
 3 that dihydrosansanmycin B had significantly improved anti-TB activity (MIC<sub>50</sub> 0.3 μM) compared  
 4 with sansanmycin B (MIC<sub>50</sub> 9.5 μM). Structure-activity studies revealed that analogues containing  
 5 glycine at the N-terminal amino acid showed comparable activity (MIC<sub>50</sub> 0.63 μM), and that a  
 6 further modification of a cyclohexyl group at position 4 led to an analogue with MIC<sub>50</sub> 80 nM that  
 7 was a potent *MraY* inhibitor (IC<sub>50</sub> 30 nM), as shown in Figure 3 [24].



8  
 9 Figure 3. Antimicrobial activity of synthetic dihydrosansanmycin analogues modified at positions 2  
 10 and 4 against *Mycobacterium tuberculosis* H37Rv.

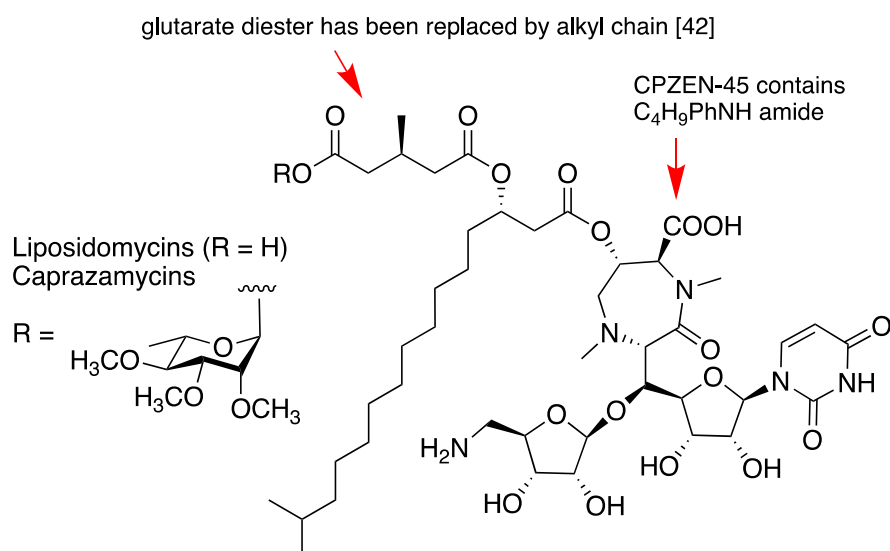
11  
 12 The biosynthetic gene cluster for the pacidamycin antibiotics, containing a number of non-  
 13 ribosomal peptide synthetase genes, was identified in *Streptomyces coeruleorubidus* in 2010 by the  
 14 groups of Goss [25] and Walsh [26]. The unusual ureidopeptide moiety at the carboxyl terminus of  
 15 the peptide chain is assembled via carboxyl activation of Ala by PacN, followed by carboxylation,  
 16 and then peptide bond formation catalysed by ligase PacL [27]. The diamino acid DABA is  
 17 biosynthesised from L-threonine by a pyridoxal 5'-phosphate-dependent β-replacement reaction,  
 18 also observed for mureidomycin biosynthesis in *Streptomyces flavidovirens* [28], using L-aspartate  
 19 as a nucleophile, followed by a β-elimination reaction [26]. The modified uridine nucleoside is  
 20 formed via oxidation of uridine to the 5'-aldehyde, followed by transamination to 5'-amino-uridine,  
 21 followed dehydration of the 4'-hydroxyl group [29]. The crystal structure of the novel dehydratase  
 22 enzyme Pac13 has been determined, implicating His-42 in the catalytic mechanism [30]. The  
 23 additional N-terminal Ala found in some pacidamycins and mureidomycins is added by ligase  
 24 PacB, that uses Ala-tRNA as an amino acid donor [31]. The unusual amino acid meta-tyrosine is

1 biosynthesised from L-Phe by a novel non-heme iron- and tetrahydrobiopterin-dependent  
2 hydroxylase [32]. Mutasyntesis has been used to generate novel chlorinated pacidamycin  
3 derivatives [33], and modified sansanmycins [34]. The modified sansanmycins were reported to  
4 retain antimicrobial activity, in some cases with reduced activity, but MX-6 containing 4-  
5 fluorophenylalanine at the C-terminus showed enhanced antimicrobial activity against *B. subtilis*  
6 and *M. tuberculosis* [34].

7

### 8 1.3 The liposidomycin group of liponucleoside antibiotics (liposidomycins, caprazamycins)

9 The liposidomycins are liponucleoside natural products containing an aminoglycoside  
10 sugar, which were reported by K. Isono and coworkers in 1985 [1], and their molecular structures  
11 reported in 1988 [35]. They show antimicrobial activity against *Mycobacterium* strains (MIC 1.6  
12  $\mu\text{g/ml}$ ) [1]. The caprazamycins were reported in 2003: they share the same structural skeleton as the  
13 liposidomycins, as shown in Figure 4, but the 3-methylglutaryl substituent is glycosylated by an  
14 additional L-rhamnose sugar [36,37].



15

16 Figure 4. Structures of the liposidomycins and caprazamycins, and synthetic analogues

17

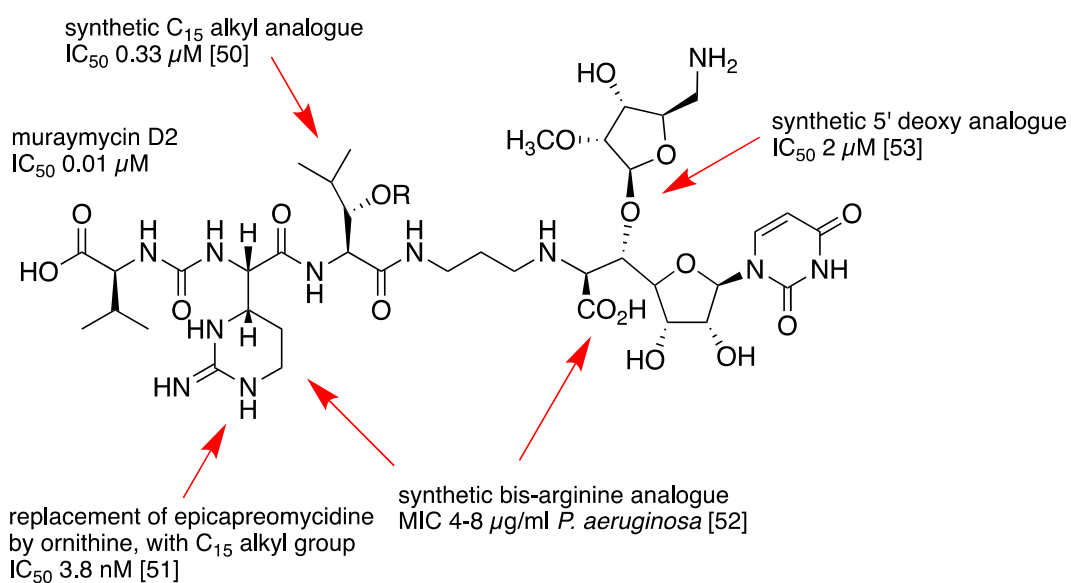
18 Synthetic uridine-based analogues of the liposidomycins containing the  
19 aminoribofuranoside sugar retain *MraY* inhibition activity ( $\text{IC}_{50}$  0.14-50  $\mu\text{M}$ ), but show weaker  
20 antimicrobial activity, demonstrating the importance of the lipophilic substituent, probably needed  
21 for cellular uptake [38-40]. Fer et al have published a further series of uridine-based analogues  
22 containing a lipophilic group linked via a triazole heterocycle, which show antimicrobial activity  
23 against *Staphylococcus aureus*, and inhibit *MraY* with  $\text{IC}_{50}$  values in the range 100-1000  $\mu\text{M}$  [41].  
24 Matsuda and coworkers have synthesised analogues of caprazamycin containing an alkyl chain in  
25 place of the glutarate diester sidechain, which retain antimicrobial activity but show enhanced  
26 stability [42]. A semisynthetic caprazamycin derivative CPZEN-45 is active in animal models for

1 protection against tuberculosis infection, and Ichizaki et al published in 2013 that CPZEN-45  
2 inhibits transferase WecA in *M. tuberculosis*, involved in lipopolysaccharide biosynthesis, rather  
3 than MraY [43].

4 The biosynthetic gene cluster for production of the caprazamycins in *Streptomyces* sp.  
5 MK730-62F2 was identified in 2009 by Kaysser et al [44]. The biosynthetic pathway involves the  
6 formation of uridine 5'-aldehyde, followed by a pyridoxal-5'-phosphate dependent reaction with  
7 glycine to form a uridine-amino acid adduct, followed by an *S*-adenosylmethionine-dependent  
8 reaction transferring a 3-amino-3-carboxypropyl group [44]. A gene deletion strain, in which *cpz21*  
9 encoding an acyltransferase enzyme acting late in the biosynthetic pathway had been deleted, was  
10 found to accumulate the caprazamycin aglycone [44]. The genes responsible for addition of the L-  
11 rhamnose sugar found in caprazamycins have been identified, allowing the heterologous gene  
12 expression of intact caprazamycins [45]. The biosynthetic pathway for the aminoribosyl sugar  
13 moiety found in the caprazamycin, muraymycin and other nucleoside natural products has also been  
14 shown to proceed via uridine 5'-aldehyde, and was reported by van Lanen and co-workers [46-48].  
15

#### 16 1.4 The muraymycin group of lipo-ureidylpeptide nucleoside antibiotics

17 The muraymycins were reported in 2002 by McDonald et al, isolated from a *Streptomyces*  
18 *sp.* strain [49]. Their structure contains an aminoribofuranoside monosaccharide attached to the 5'-  
19 position of a uridine-amino acid, similar to that found in the liposidomycins & caprazamycins, as  
20 shown in Figure 5, and a ureidopeptide structure linked via a 3-aminopropyl moiety [49]. The  
21 muraymycins also target translocase MraY (IC<sub>50</sub> 0.027 µg/ml), show antimicrobial activity against  
22 strains of *Staphylococcus aureus* (MIC 2-16 µg/ml) and *Enterococcus* (MIC 16-64 µg/ml), and  
23 were reported to protect mice against *S. aureus* infection (ED<sub>50</sub> 1.1 mg/kg) [49].



24  
25 Figure 5. Structures of muraymycin antibiotics and synthetic analogues



1  
2 Several bioactive muraymycin analogues have been generated via total chemical synthesis.  
3 Tanino *et al* have synthesised analogues in which the hydroxyleucine residue is replaced by an  
4 alkyl sidechain, which show MraY inhibition activity (IC<sub>50</sub> 0.33 μM), and retain antimicrobial  
5 activity [50]. The same group have reported that the epicapreomycidine amino acid (a cyclic  
6 analogue of arginine) can be replaced by arginine, lysine or ornithine residues, and that these  
7 synthetic analogues retain antimicrobial activity [51]. Takeoka *et al.* have prepared further  
8 analogues with L-arginine in place of epicapreomycidine, in which the C-terminal amino acid is  
9 removed, which retain full MraY inhibition activity, and show enhanced antimicrobial activity  
10 against *Pseudomonas* strains [52]. Spork *et al* have synthesised an analogue of muraymycin lacking  
11 the aminoribose sugar which retains activity for MraY inhibition (IC<sub>50</sub> 2 μM) [53]. The ω-  
12 guanylated fatty acid which is found in the most active muraymycins has been shown to assist  
13 localisation of the antibiotic into the cell membrane [54]. The total synthesis of muraymycin D1  
14 was reported in 2016 by Mitachi *et al* [55], enabling the synthesis of further analogues. The  
15 biosynthetic gene cluster for the biosynthesis of muraymycin in *Streptomyces* sp. NRRL 30471 was  
16 reported in 2011 [56].

17

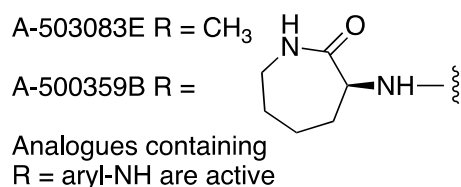
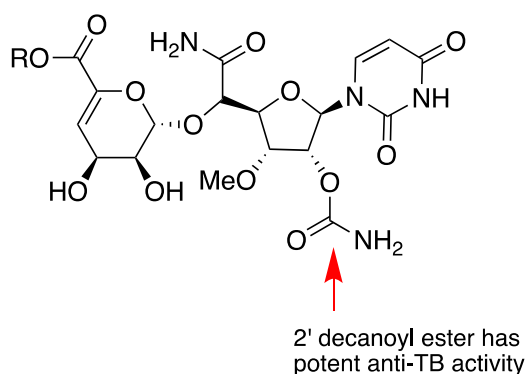
### 18 **1.5 The capuramycin group of caprolactam nucleoside antibiotics (capuramycin, A-500359A)**

19 Capuramycin, a nucleoside antibiotic produced by *Streptomyces griseus*, containing a uronic  
20 acid monosaccharide attached to the 5' position of a modified uridine nucleoside, to which is  
21 attached a 7-membered caprolactam ring, as shown in Figure 6, was first reported in 1986 [57,58].  
22 Capuramycin and a methylated derivative A-500359A which shows antimicrobial activity against  
23 *Mycobacterium smegmatis* (MIC 2-16 μg/ml) and potent MraY inhibition (IC<sub>50</sub> 0.017 μg/ml) was  
24 then reported in 2003 [59,60]. A-500359E, which lacks the aminocaprolactam ring, shows potent  
25 inhibition of MraY (IC<sub>50</sub> 0.027 μM), but lacks antimicrobial activity [61]. Semi-synthetic  
26 derivatives of A-500359E have been reported, in which the aminocaprolactam is replaced by  
27 synthetic arylamines, which show potent MraY inhibition (IC<sub>50</sub> 10-40 ng/ml), and antimicrobial  
28 activity against *Mycobacterium* strains (MIC 0.5-2 μg/ml) [62]. Acylation of capuramycin on the 2'  
29 hydroxyl group gave a further series of bioactive derivatives, including a decanoyl derivative which  
30 shows very potent activity against *M. tuberculosis* (MIC 0.06 μg/ml) [63].

31 A biosynthetic gene cluster for a closely related capuramycin antibiotic A-503083B in  
32 *Streptomyces* sp. SANK 62799 was reported in 2010 [64]. The biosynthetic steps for attachment of  
33 a caprolactam moiety were elucidated, via carboxy methyltransferase CapS and transferase CapW  
34 [64]. The biosynthetic gene cluster for capuramycin A-102395 has also been reported, involving the  
35 incorporation of L-threonine into uridine 5'-carboxamide [65]. Transferase CapW has been used to

1 prepare of a set of 43 semi-synthetic bioactive capuramycin derivatives [66]. Several of these  
 2 analogues retained similar antimicrobial activity to the parent compound, with three analogues  
 3 showing enhanced activity against *M. smegmatis* and *M. tuberculosis* [66].

4



5

6 Figure 6. Capuramycin natural products and synthetic analogues

7

## 8 1.6 Comparison of antimicrobial activities of nucleoside natural products.

9 The nucleoside natural product antibiotics show very interesting and varied antimicrobial  
 10 activities. The mureidomycins show particularly potent antimicrobial activity against *Pseudomonas*  
 11 *aeruginosa* (MIC 0.1 – 3 µg/mL), a bacterium responsible for antibiotic-resistant infections around  
 12 the world, and can protect mice against infection by *Pseudomonas aeruginosa* [11,12]. The  
 13 pacidamycins and napsamycins also show anti-pseudomonal activity, but synthetic  
 14 dihydropacidamycins containing modifications at position 4 (see Figure 2) showed new  
 15 antimicrobial spectrum against *Escherichia coli* (MIC 4-8 µg/mL) and *Citrobacter freundii* (1.0  
 16 µg/mL) [20,21]. Given that the *MraY* sequences from these organisms are quite closely related, it  
 17 seems likely that these changes in antibacterial spectrum are caused by changes in uptake.

18 The liposidomycin and caprazamycin liponucleosides show activity against strains of  
 19 *Mycobacterium* (MIC 1.6 µg/ml) [2]. The synthetic caprazamycin derivative CPZEN-45 has shown  
 20 efficacy against both drug-sensitive and extremely drug-resistant (XDR) *Mtb* in a mouse model of  
 21 acute tuberculosis, and is in clinical trials against TB infection [43]. Capuramycins also show  
 22 potent activity against *Mycobacterium smegmatis* (MIC 2-16 µg/ml) [59,60], and semi-synthetic  
 23 derivatives show enhanced anti-*Mtb* activity [62,63]. The activity of the sansanmycins against  
 24 *Mycobacterium tuberculosis* has been greatly enhanced in synthetic dihydrosansanmycins  
 25 containing modifications at position 4 (MIC<sub>50</sub> 0.04-0.6 µM) [24].

1 The muraymycin antibiotics show antimicrobial activity against *Staphylococcus aureus* (MIC 2-  
2 16 µg/ml) and *Enterococcus* (MIC 16-64 µg/ml), and can protect mice against *S. aureus* infection  
3 [49]. Synthetic analogues containing two L-arginine residues show modified antimicrobial  
4 spectrum, notably against *Pseudomonas* strains (MIC 4-8 µg/mL) [52].

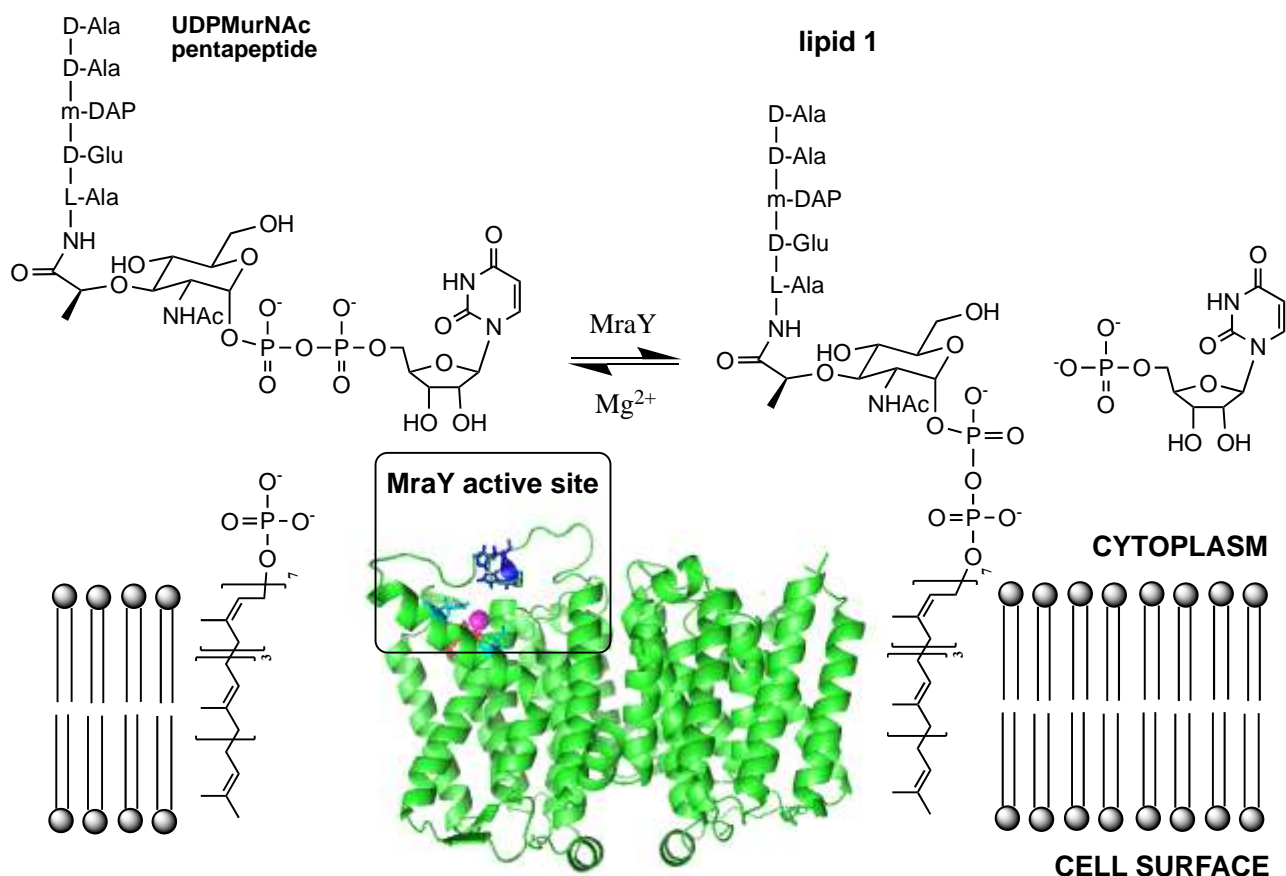
5

## 6 **2. Mechanism of inhibition of translocase *MraY* by nucleoside antibiotics**

### 7 **2.1 Kinetic mechanism of inhibition of translocase *MraY***

8 Translocase *MraY* catalyses the first step of the lipid cycle of bacterial peptidoglycan  
9 biosynthesis, namely the reaction of UDPMurNAc-L-Ala-γ-D-Glu-m-DAP-D-Ala-D-Ala  
10 (UDPMurNAc-pentapeptide) with lipid carrier undecaprenyl phosphate, to form lipid intermediate  
11 1 (undecaprenyl-diphospho-MurNAc-pentapeptide), releasing uridine 5'-monophosphate (UMP)  
12 [67]. Translocase *MraY* is an integral membrane protein, shown to contain ten transmembrane  
13 helices [68]. The *MraY*-catalysed reaction is a phosphotransfer reaction, shown in Figure 7, whose  
14 catalytic mechanism could either proceed via a single step phosphotransfer, or a two-step  
15 mechanism involving an active site nucleophile [67]. Three aspartic acid residues in *E. coli* *MraY*  
16 (Asp-115, Asp-116, Asp-267), found on cytoplasmic loops, were shown to be essential for activity,  
17 and it has been proposed that two Asp residues bind the active site Mg<sup>2+</sup> cofactor, while the third  
18 may be a catalytic nucleophile [69].

19



1

2 Figure 7. Reaction catalysed by translocase MraY, showing MraY dimer structure and location of  
 3 MraY active site.

4

5 Mureidomycin A has been found to act as a slow-binding inhibitor ( $K_i$  35 nM,  $K_i^*$  2 nM) for  
 6 solubilised *E. coli* MraY, using a continuous fluorescence enhancement assay, showing competitive  
 7 enzyme inhibition towards both UDPMurNAc-pentapeptide and polyprenyl phosphate substrates  
 8 [70]. Liposidomycin B also acts as a slow-binding inhibitor of *E. coli* MraY ( $K_i^*$  90 nM), showing  
 9 non-competitive enzyme inhibition towards UDPMurNAc-pentapeptide, but competitive inhibition  
 10 towards dodecaprenyl phosphate [71]. By contrast, tunicamycin is a reversible inhibitor of *E. coli*  
 11 MraY ( $K_i$  0.6  $\mu$ M) showing competitive enzyme inhibition towards UDPMurNAc-pentapeptide, but  
 12 non-competitive inhibition towards dodecaprenyl phosphate [71]. Muraymycin D2 and synthetic  
 13 analogues thereof were found by Tanino et al to show competitive inhibition towards  
 14 UDPMurNAc-pentapeptide ( $K_i$  7.6 nM), but non-competitive inhibition versus undecaprenyl  
 15 phosphate, against *B. subtilis* MraY using a radiochemical assay [51]. Hence there are some  
 16 differences in the kinetic mechanism of MraY inhibition shown by the different classes of  
 17 nucleoside antibiotics.

18 The possibility that the mureidomycins might be mechanism-based inhibitors, reacting via  
 19 the enamide functional group, which might be expected to be chemically reactive, has been found  
 20 not to be the case, from studies on model enamide-containing analogues [72], and since synthetic

1 dihydropacidamycin and dihydrosansanmycin analogues retain *MraY* inhibition [20,21,24]. The  
2 observed slow-binding inhibition is therefore probably due to a conformational change in the  
3 protein structure, discussed in Section 2.2. Studies on analogues of mureidomycins have found that  
4 the amino terminus of the peptide chain and the N-methyl amide group of DABA are both  
5 important for *MraY* inhibition [73,74], leading to a proposal that the amino terminus might bind in  
6 place of the  $Mg^{2+}$  cofactor, positioned via a *cis*-amide rotamer in the peptide chain [74]. The amino  
7 group of the aminoribofuranose monosaccharide of liposidomycins and caprazamycins is also  
8 known to be important for activity [39].

9

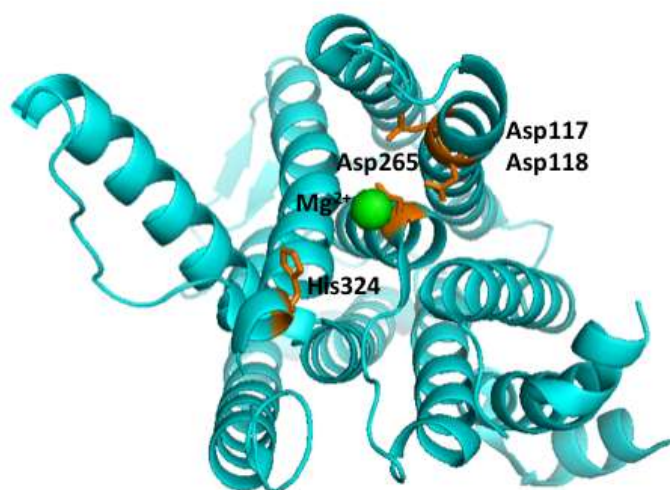
## 10 **2.2 Structure of *Aquifex aeolicus* *MraY* and its complexes with nucleoside antibiotics**

11 In 2013 the crystal structure of the *Aquifex aeolicus* *MraY* was determined, confirming the  
12 arrangement of ten transmembrane  $\alpha$ -helices [75]. The protein was found to crystallise as a dimer,  
13 with transmembrane helix 9 strongly bent and protruding into the membrane. The active site  
14 contained the three catalytic Asp residues, close to the  $Mg^{2+}$  cofactor, with Asp-265 positioned  
15 closest to the  $Mg^{2+}$  ion [75]. There is a triad of three histidine residues (His-324, His-325, His-326;  
16 HHH motif) conserved in bacterial sequences of the polyprenyl-phosphate N-acetylhexosamine 1-  
17 phosphate transferase (PNPT) superfamily, which are positioned on loop E on the opposite side of  
18 the active site, 10-13 Å from the three catalytic Asp residues, shown in Figure 8.

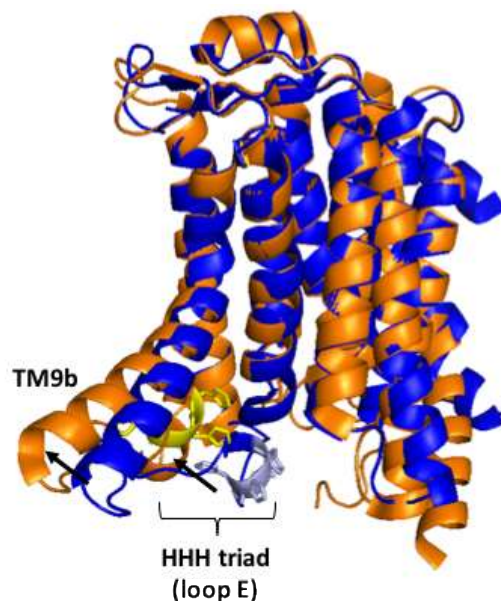
19 The structure of a complex of *A. aeolicus* *MraY* with muraymycin D2 was published in  
20 2016 [76]. Upon binding of muraymycin D2, transmembrane helix 9b (TM9b) moves away from  
21 the active site and the HHH motif (conserved across the PNPT family) in loop E extends, which  
22 widens and reshapes the active site, hence there is a significant conformational change upon ligand  
23 binding [76], which may be important for the catalytic cycle of *MraY*, and potentially could explain  
24 the slow-binding inhibition of *MraY* observed for some nucleoside natural product inhibitors  
25 [70,71]. Binding of muraymycin D2 to the *MraY* active site does not involve the catalytic Asp  
26 residues and does not require  $Mg^{2+}$ , but several other binding interactions were elucidated [76]. The  
27 uracil base is bound via  $\pi$ - $\pi$  stacking interactions to Phe-262 (see Figure 9A), as well as hydrogen-  
28 bonding interactions to the uracil carbonyl and NH groups. The amino group of the aminoribose  
29 moiety is bound by Asp-193, whose mutation greatly reduces affinity for muraymycin D2, and  
30 Asn-190. The strong binding of the amino group of the aminoribose helps to rationalise why this  
31 group is important for activity in several nucleoside antibiotics [39,73]. The carboxyl terminus of  
32 the peptide chain is bound by Gln-305, a residue that is conserved in bacterial *MraY* homologues,  
33 and the *epi*-capreomycidine amino acid is bound by His-324 and His-325 [76].

34

A. AaMraY



B. AaMraY overlaid with AaMraY-MD2



1

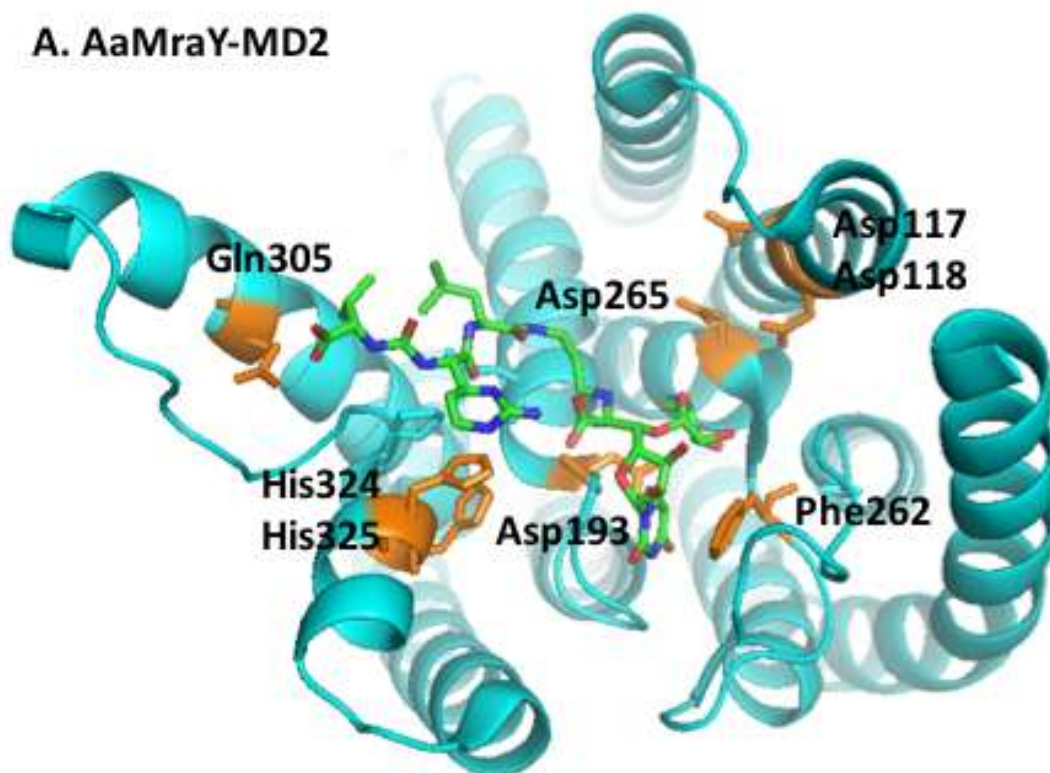
2 Figure 8. Structures of *A. aeolicus* MraY (A) containing no ligand, (B) complexed with  
 3 muraymycin D2, showing active site residues (A) and conformational change upon muraymycin  
 4 binding (B).

5

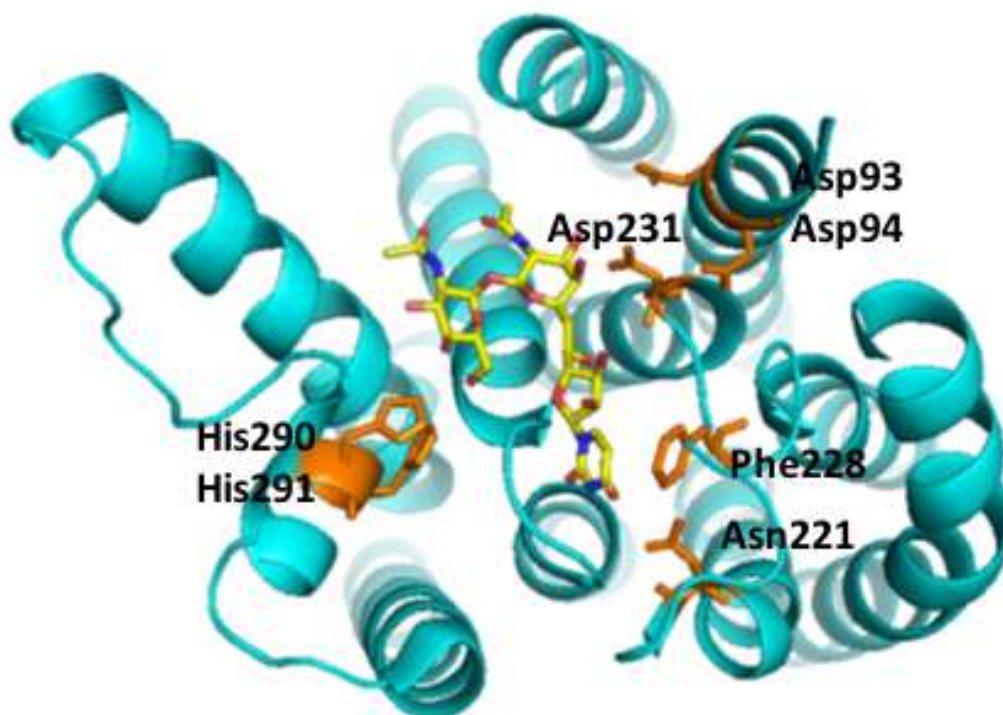
6 In 2017 the structure of a complex of *Clostridium boltae* MraY with tunicamycin was  
 7 published [77]. The overall conformation of the protein was similar to the MraY-muraymycin  
 8 complex, but some similarities and differences in the enzyme-ligand binding interactions were  
 9 observed [77,78]. As in the MraY-muraymycin complex, the uracil base was bound in a small  
 10 cavity, interacting via  $\pi$ - $\pi$  stacking interactions to Phe-228 (see Figure 9B), and the 4-carbonyl  
 11 group binding to Asn-221. The HHH motif (His-290, His-291) are also involved in ligand binding,  
 12 in this case to the GlcNAc 4'- and 6'-hydroxyl groups. However, unlike the MraY-muraymycin  
 13 complex, tunicamycin was found to interact with the catalytic Asp residues, with the tunicamine 9'-  
 14 hydroxyl group interacting with Asp-231, but in the absence of  $Mg^{2+}$  [77]. The structure of  
 15 tunicamycin bound to its eukaryotic target enzyme GlcNAc-1-phosphate transferase involved in N-  
 16 linked glycoprotein biosynthesis was also reported in 2018, showing some differences in active site  
 17 binding, compared to MraY [79].

18 Hence both nucleosides bind to an MraY structure that has undergone a conformational  
 19 change, both show specific binding for the uracil base and some involvement of the HHH motif in  
 20 ligand binding, but muraymycin and tunicamycin show different polar contacts in the MraY active  
 21 site [78].

**A. AaMraY-MD2**



**B. CbMraY-tunicamycin**

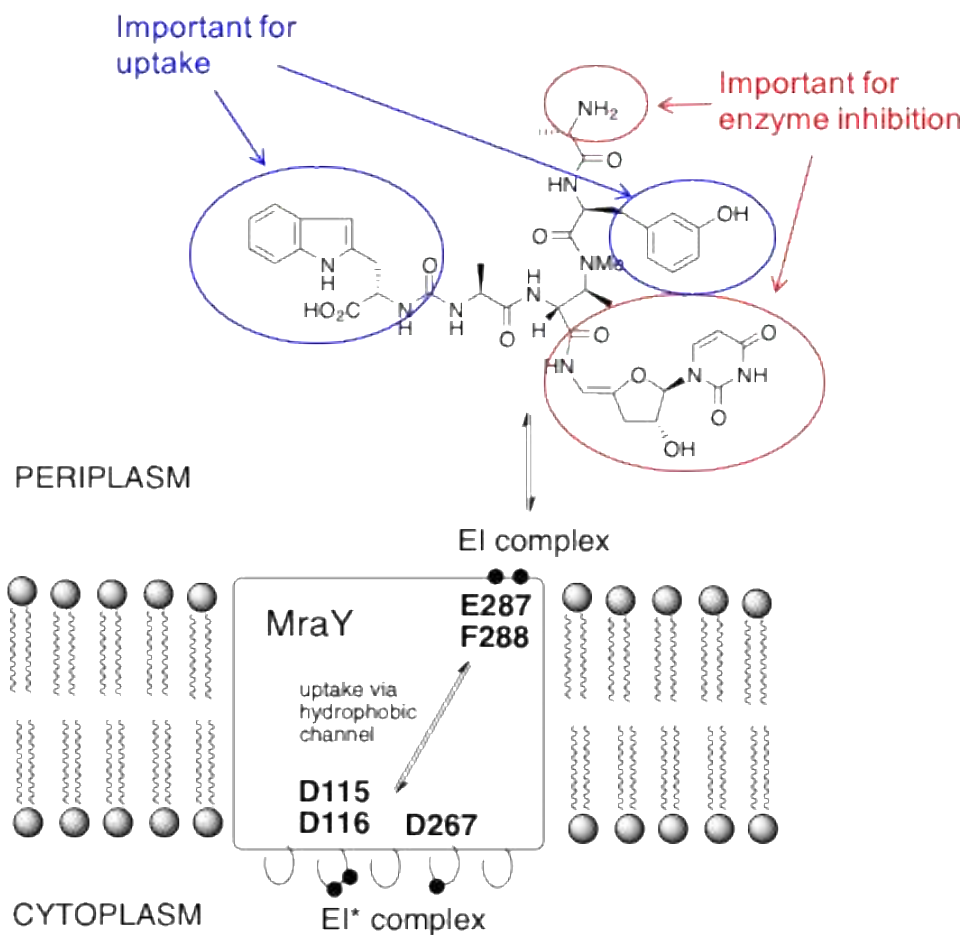


1  
2 Figure 9. Structures of MraY-ligand complexes, showing the location of selected binding  
3 interactions. A. *A. aeolicus* MraY complexed with muraymycin D2. B. *C. boltae* MraY complexed  
4 with tunicamycin.

5

1 **2.3 Interaction with protein-protein interaction site for bacteriophage lysis protein E**

2 *E. coli* MraY is also targetted by an antibacterial lysis protein E from bacteriophage  $\phi$ X174,  
3 which interacts with Phe-288 and Glu-287 of MraY, on the exterior face of transmembrane 9 of  
4 MraY, via an Arg-Trp-x-x-Trp sequence motif near the N-terminus of the E protein [80]. The  
5 presence of a guanidine-containing amino acid *epi*-capreomycin in muraymycin, and two  
6 aromatic residues (Trp or m-Tyr and a second m-Tyr) in the mureidomycin/pacidamycin structures,  
7 is reminiscent of this Arg-Trp-x-x-Trp motif. Rodolis et al have shown that pacidamycin 1 and a  
8 synthetic muraymycin analogue showed significantly reduced activity against site-directed F288L  
9 and E287A MraY mutant enzymes [81], suggesting that parts of the antibiotic structure somehow  
10 aid the targetting of MraY *in vivo*, perhaps aiding uptake into the cell via a hydrophobic channel  
11 present in the structure of MraY [75], as shown in Figure 10. This hypothesis might explain how  
12 these agents of molecular weight 600-1200 Da are able to access the MraY active site on the inner  
13 face of the cytoplasmic membrane, and also why it has proved difficult to design small analogues of  
14 these nucleoside antibiotics that retain both MraY inhibition and antimicrobial activity.



15 Figure 10. Interaction with the E protein binding site in MraY (Phe-288 and Glu-287) by nucleoside  
16 antibiotics, and hypothesis for uptake to the MraY active site.  
17  
18



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35

### 3. Inhibition of other bacterial phospho-nucleotide transferase enzymes by nucleoside natural product analogues.

There are homologues of MraY involved in lipid-linked cycles responsible for the biosynthesis of enterobacterial common antigen and O-antigen lipopolysaccharide (LPS) in Gram-negative bacteria (WecA) [82], and teichoic acid in Gram-positive bacteria (TagO), both of which are integral membrane proteins that utilise UDP-GlcNAc and undecaprenyl phosphate as substrates [83]. CPZEN-45, a semisynthetic caprazamycin derivative undergoing clinical trials for treatment of tuberculosis, was found to inhibit transferase WecA in *M. tuberculosis* >20 fold more strongly than MraY, and CPZEN-45 also inhibits *B. subtilis* TagO 8-fold more tightly than *B. subtilis* MraY [43]. Selective synthetic inhibitors for TagO have also been published, based on the existing drug ticlopidine [84]. In contrast, the muraymycin analogues prepared by Tanino *et al* were highly selective for MraY inhibition over *E. coli* WecA [51], as were the dihydrosansanmycin analogues of Tan et al [24].

A homologue of WecA (GacO) has been found to catalyse the formation of undecaprenyl-diphospho-GlcNAc in the biosynthesis of the Lancefield group A carbohydrate in *Streptococcus pyogenes* [85]. A further group of small 20-25 kDa phospho-sugar transferase enzymes utilising a UDP-di-*N*-acetyl-bacillosamine substrate are involved in *N*-glycoconjugate biosynthesis in *Campylobacter jejuni* [86]. Synthetic peptidyl-uridine inhibitors have been synthesised as inhibitors of *C. jejuni* PglC, with IC<sub>50</sub> values in the range 40-250 µM [87].

In conclusion, Nature produces several classes of uridine-based nucleoside antibiotics that target translocase MraY in the bacterial peptidoglycan biosynthetic pathway. Total synthesis of modified nucleoside analogues, and exploitation of the biosynthetic machinery for these natural products, offer considerable promise for the development of highly active antimicrobial agents to treat antibiotic-resistant infections. Understanding of the MraY structure will aid the development of selective MraY inhibitors, and the discovery of MraY homologues such as WecA and TagO has identified further interesting targets for antibacterial drug discovery by nucleoside analogues.

**Acknowledgements.** Research in the author's laboratory was supported by an EPSRC CASE PhD studentship (to RVK) with LifeArc Ltd.

### References

1. Isono K, Uramoto M, Kusakabe H, Kimura K, Izaki K, Nelson CC, McCloskey JA. Liposidomycins: novel nucleoside antibiotics which inhibit bacterial peptidoglycan

- 1 biosynthesis. *J. Antibiotics* 1985; 38:1617-1621.
- 2 2. Takatsuki A, Arima K, Tamura G. Tunicamycin, a new antibiotic. I. Isolation and  
3 characterization of tunicamycin. *J. Antibiotics* 1971; 24:215-223.
- 4 3. Kimura KI, Bugg TDH. Recent advances in antimicrobial nucleoside antibiotics targeting cell  
5 wall biosynthesis. *Nat. Prod. Reports* 2003; 20:252-273.
- 6 4. Winn M, Goss RJM, Kimura KI, Bugg TDH. Antimicrobial nucleoside antibiotics targeting cell  
7 wall assembly: recent advances in structure-function studies and nucleoside biosynthesis. *Nat.*  
8 *Prod. Reports* 2010; 27:279-304.
- 9 5. Heifetz A, Keenan RW, Elbein AD. Mechanism of action of tunicamycin on the UDP-  
10 GlcNAc:dolichyl-phosphate GlcNAc-1-phosphate transferase. *Biochemistry* 1979; 18:2186-  
11 2192.
- 12 6. Wyszynski FJ, Hesketh AR, Bibb MJ, Davis BG. Dissecting tunicamycin biosynthesis by  
13 genome mining: cloning and heterologous expression of a minimal gene cluster. *Chem. Sci.*  
14 2010; 1:581-589.
- 15 7. Wyszynski FJ, Lee SS, Yabe T, Wang H, Gomez-Escribano JP, Bibb MJ, Lee SJ, Davies GJ,  
16 Davis BG. Biosynthesis of the tunicamycin antibiotics proceeds via a unique exo-glycal  
17 intermediate. *Nature Chem.* 2012; 4:539-546.
- 18 8. Yamamoto K, Yakushiji F, Matsumaru T, Ichikawa S. Total synthesis of tunicamycin V. *Org.*  
19 *Lett.* 2018; 20:256-259.
- 20 9. Yamamoto K, Katsuyama A, Ichikawa S. Structural requirement of tunicamycin V for MraY  
21 inhibition. *Bio-Org. Med. Chem.* 2019; 27:1714-1719.
- 22 10. Isono F, Inukai M, Takahashi S, Haneishi T, Kinoshita T, Kuwano H. Mureidomycins A-D,  
23 novel peptidylnucleoside antibiotics with spheroplast forming activity. II. Structural elucidation.  
24 *J. Antibiotics* 1989; 42:667-673.
- 25 11. Inukai M, Isono F, Takahashi R. Selective inhibition of the bacterial translocase reaction in  
26 peptidoglycan synthesis by mureidomycins. *Antimicrob. Agents Chemother.* 1993; 37:980-983.
- 27 12. Isono, F.; Katayama, T.; Inukai M.; Haneishi, T. Mureidomycins A-D, novel peptidylnucleoside  
28 antibiotics with spheroplast forming activity. III. Biological properties. *J. Antibiotics* 1989;  
29 42:674-679.
- 30 13. Karwowski JP, Jackson M, Theriault RJ, Chen RH, Barlow GJ, Maus ML. Pacidamycins, a  
31 novel series of antibiotics with anti-*Pseudomonas aeruginosa* activity. I. Taxonomy of the  
32 producing organism and fermentation. *J. Antibiotics* 1989; 42:506-511.
- 33 14. Chen RH, Buko AM, Whittern DN, McAlpine JB. Pacidamycins, a novel series of antibiotics  
34 with anti-*Pseudomonas aeruginosa* activity. II. Isolation and structural elucidation. *J. Antibiotics*  
35 1989; 42:512-520.

- 1 15. Fernandes PB, Swanson RN, Hardy DJ, Hanson CW, Coen L, Rasmussen RR, Chen RH.  
2 Pacidamycins, a novel series of antibiotics with anti-*Pseudomonas aeruginosa* activity. III.  
3 Microbiological profile. *J. Antibiotics* 1989; 42:521-526.
- 4 16. Isono F, Sakaida Y, Takahashi S, Kinoshita T, Nakamura T, Inukai M. Mureidomycins E and F,  
5 minor components of mureidomycins. *J. Antibiotics* 1993; 46:1203-1207.
- 6 17. Chatterjee S, Nadkarni SR, Vijayakumar EKS, Patel MV, Ganguli BN. Napsamycins, new  
7 *Pseudomonas* active antibiotics of the mureidomycin family from *Streptomyces* sp. HIL Y-82,  
8 11372. *J. Antibiotics* 1994; 47:595-598.
- 9 18. Xie XY, Chen RX, Si SY, Sun CH, Xu HZ. A new nucleosidyl-peptide antibiotic, sansanmycin.  
10 *J. Antibiotics* 2007; 60:158-162.
- 11 19. Xie XY, Xu HZ, Si SY, Sun CH, Chen RX. Sansanycins B and C, new components of  
12 sansanmycins. *J. Antibiotics* 2008; 61:237-240.
- 13 20. Booramra CG, Lemoine RC, Lee JC, Léger R, Stein KA, Vernier NG, Magon A, Lomovskaya  
14 O, Martin PK, Chamberland S, Lee MD, Hecker SJ, Lee VJ. Stereochemical elucidation and  
15 total synthesis of dihydropacidamycin D, a semisynthetic pacidamycin. *J. Am. Chem. Soc.*  
16 2001; 123:870-874.
- 17 21. Booramra CG, Lemoine RC, Blais J, Vernier NG, Stein KA, Magon A, Chamberland S, Hecker  
18 SJ, Lee VJ. Synthetic dihydropacidamycin antibiotics: a modified spectrum of activity for the  
19 pacidamycin class. *Bio-Org. Med. Chem. Lett.* 2003; 13:3305-3309.
- 20 22. Okamoto K, Sakagami M, Feng F, Togame H, Takemoto H, Ichikawa S, Matsuda A. Total  
21 synthesis of pacidamycin D by Cu(I)-catalyzed oxy enamide formation. *Org. Lett.* 2011;  
22 13:5240-5243.
- 23 23. Okamoto K, Sakagami M, Feng F, Takahashi F, Uotani K, Togame H, Takemoto H, Ichikawa  
24 S, Matsuda A. Synthesis of pacidamycin analogues via an Ugi-multicomponent reaction.  
25 *Bioorg. Med. Chem. Lett.* 2012; 22:4810-4815.
- 26 24. Tran AT, Watson EE, Pujari V, Conroy T, Dowman LJ, Giltrap AM, Pang A, Wong WR,  
27 Linington RG, Saunders J, Charman SA, West NP, Bugg TDH, Tod J, Dowson CG, Roper DI,  
28 Crick DC, Britton WJ, Payne RJ. Synthesis of sansanmycin natural product analogues as potent  
29 and selective anti-mycobacterials that inhibit lipid I biosynthesis. *Nature Comm.* 2017, 14414.
- 30 25. Rackham EJ, Gruschow S, Ragab AE, Dickens S, Goss RJM. Pacidamycin biosynthesis:  
31 identification and heterologous expression of the first uridyl peptide antibiotic gene cluster.  
32 *ChemBioChem* 2010; 11:1700-1709.
- 33 26. Zhang W, Ostash B, Walsh CT. Identification of the biosynthetic gene cluster for the  
34 pacidamycin group of peptidyl nucleoside antibiotics. *Proc. Natl. Acad. Sci. USA* 2010;  
35 107:16828-16833.

- 1 27. Zhang W, Ntai I, Bolla ML, Malcolmson SJ, Kahne D, Kelleher NL, Walsh CT. Nine enzymes  
2 are required for assembly of the pacidamycin group of pentapeptidyl nucleoside antibiotics. *J.*  
3 *Am. Chem. Soc.* 2011; 133:5240-5243.
- 4 28. Lam WH, Rychli K, Bugg TDH. Identification of a novel  $\beta$ -replacement reaction in the  
5 biosynthesis of 2,3-diaminobutyric acid in peptidylnucleoside mureidomycin A. *Org. Biomol.*  
6 *Chem.* 2008; 6:1912-1917.
- 7 29. Ragab A, Grüşchow S, Tromans DR, Goss RJM. Biogenesis of the unique 4',5'-dehydro-  
8 nucleoside of the uridylpeptide antibiotic pacidamycin. *J. Am. Chem. Soc.* 2011; 133:15288-  
9 15291.
- 10 30. Zhang W, Ntai I, Kelleher NL, Walsh CT. tRNA-dependent peptide bond formation by the  
11 transferase PacB in the biosynthesis of the pacidamycin group of pentapeptidyl nucleoside  
12 antibiotics. *Proc. Natl. Acad. Sci. USA* 2011; 108:12249-12253.
- 13 31. Michailidou F, Chung C, Brown MJB, Bent AF, Naismith JH, Leavens WJ, Lynn SN, Sharma  
14 SV, Goss RJM. Pac13 is a small, monomeric dehydratase that mediates the formation of the  
15 3'-deoxy nucleoside of pacidamycins. *Angew. Chem. Intl. Ed.* 2017; 56:12492-12497.
- 16 32. Zhang W, Ames BD, Walsh CT. Identification of phenylalanine 3-hydroxylase for meta-  
17 tyrosine biosynthesis. *Biochemistry* 2011, 50:5401-5403.
- 18 33. Deb Roy A, Grüşchow S, Cairns N, Goss RJM. Gene expression enabling synthetic  
19 diversification of natural products: chemogenetic generation of pacidamycin analogues. *J. Am.*  
20 *Chem. Soc.* 2010; 132:12243-12245.
- 21 34. Shi YY, Jiang Z, Lei X, Zhang NN, Cai Q, Li Q, Wang LF, Si S, Xie Y, Hong B. Improving the  
22 N-terminal diversity of sansanmycin through mutasynthesis. *Microb. Cell Factories* 2016;  
23 15:77.
- 24 35. Ubukata M, Isono K, Kimura K, Nelson CC, McCloskey JA. The structure of liposidomycin B,  
25 an inhibitor of bacterial peptidoglycan biosynthesis. *J. Am. Chem. Soc.* 1988; 110:4416-4417.
- 26 36. Igarashi M, Nakagawa N, Doi N, Hattori S, Naganawa H, Hamada M. Caprazamycin B, a novel  
27 anti-tuberculosis antibiotic from *Streptomyces* sp. *J. Antibiotics* 2003; 56:580-583.
- 28 37. Igarashi M, Takahashi Y, Shitara T, Nakamura H, Nakagawa H, Miyake T, Akamatsu Y.  
29 Caprazamycins, novel lipo-nucleoside antibiotics from *Streptomyces* sp. II. Structure  
30 elucidation of caprazamycins. *J. Antibiotics* 2005; 58:327-337.
- 31 38. Dini C, Collette P, Drochon N, Guillot JC, Lemoine G, Mauvais P, Aszodi J. Synthesis of the  
32 nucleoside moiety of liposidomycins: elucidation of the pharmacophore of this family of MraY  
33 inhibitors. *Bio-Org. Med. Chem. Lett.* 2000; 10:1839-1843.
- 34 39. Dini, C.; Drochon, N.; Feteanu, S.; Guillot, J.C.; Peixoto, C.; Aszodi, J. Synthesis of analogues  
35 of the *O*- $\beta$ -D-ribofuranosyl nucleoside moiety of liposidomycins. Part 1: contribution of the

- 1 amino group and the uracil moiety upon the inhibition of *MraY*. *Bio-Org. Med. Chem. Lett.*  
2 2001; 11:529-531.
- 3 40. Dini C, Didier-Laurent S, Drochon N, Feteanu S, Guillot JC, Monti F, Uridat E, Zhang J,  
4 Aszodi J. Synthesis of sub-micromolar inhibitors of *MraY* by exploring the region originally  
5 occupied by the diazepamone ring in the liposidomycin structure. *Bio-Org. Med. Chem. Lett.*  
6 2002; 12:1209-1213.
- 7 41. Fer MJ, Bouhss A, Patrao M, Le Corre L, Pietrancosta N, Amoroso A, Joris B, Mengin-  
8 Lecreulx D, Calvet-Vitale S, Gravier-Pelletier C. 5'-Methylene-traizole-substituted-  
9 aminoribosyl uridines as *MraY* inhibitors: synthesis, biological evaluation and molecular  
10 modeling. *Org. Biomol. Chem.* 2015; 13:7193-7222.
- 11 42. Ichikawa S, Yamaguchi M, Hsuan LS, Kato Y, Matsuda A. Carbaprazamycins: chemically  
12 stable analogues of the caprazamycin nucleoside antibiotics. *ACS Infect. Dis.* 2015; 1:151-156.
- 13 43. Ishizaki Y, Hayashi C, Inoue K, Igarashi M, Takahashi Y, Pujari V, Crick DC, Brennan PJ,  
14 Nomoto A. Inhibition of the first step in synthesis of the mycobacterial cell wall core, catalyzed  
15 by the GlcNAc-1-phosphate transferase *WecA*, by the novel caprazamycin derivative CPZEN-  
16 45. *J. Biol. Chem.* 2013; 288:30309-30319.
- 17 44. Kaysser L, Lutsch L, Siebenberg S, Wemakor E, Kammerer B, Gust B. Identification and  
18 manipulation of the caprazamycin gene cluster lead to new simplified liponucleoside  
19 antibiotics and give insights into the biosynthetic pathway. *J. Biol. Chem.* 2009; 284:14987-  
20 14996.
- 21 45. Kaysser L, Wemakor E, Siebenberg S, Salas JA, Sohng JK, Kammerer B, Gust B. Formation  
22 and attachment of the deoxysugar moiety and assembly of the gene cluster for caprazamycin  
23 biosynthesis. *Appl. Environ. Microbiol.* 2010; 76:4008-4018.
- 24 46. Yang Z, Chi X, Funabashi M, Baba S, Nonaka K, Pahari P, Unrine J, Jacobsen JM, Elliott GI,  
25 Rohr J, Van Lanen SG. Characterization of *LipL* as a non-heme, Fe(II)-dependent  $\alpha$ -  
26 ketoglutarate:UMP dioxygenase that generates uridine-5'-aldehyde during A-90289  
27 biosynthesis. *J. Biol. Chem.* 2011; 286:7885-7892.
- 28 47. Barnard-Britson S, Chi X, Nonaka K, Spork AP, Tibrewal N, Goswami A, Pahari P, Ducho C,  
29 Rohr J, Van Lanen SG. Amalgamation of nucleosides and amino acids in antibiotic  
30 biosynthesis: discovery of an L-threonine:uridine-5'-aldehyde transaldolase. *J. Am. Chem. Soc.*  
31 2012; 134:18514-18517.
- 32 48. Chi X, Pahari P, Nonaka K, Van Lanen SG. Biosynthetic origin and mechanism of formation of  
33 the aminoribosyl moiety of peptidyl nucleoside antibiotics. *J. Am. Chem. Soc.* 2011;  
34 133:14452-14459.
- 35 49. McDonald LA, Barbieri LR, Carter GT, Lenoy E, Lotvin J, Petersen PJ, Siegel MM, Singh G,

- 1 Williamson RT. Structures of the muraymycins, novel peptidoglycan biosynthesis inhibitors. J.  
2 Am. Chem. Soc. 2002; 124:10260-10261.
- 3 50. Tanino T, Ichikawa S, Al-Dabbagh B, Bouhss A, Oyama H, Matsuda A. Synthesis and  
4 biological evaluation of muraymycin analogues active against anti-drug-resistant bacteria. ACS  
5 Med. Chem. Lett. 2010; 1:258-262.
- 6 51. Tanino T, Al-Dabbagh B, Mengin-Lecreulx D, Bouhss A, Oyama H, Ichikawa S, Matsuda A.  
7 Mechanistic analysis of muraymycin analogues: a guide to the design of MraY inhibitors. J.  
8 Med. Chem. 2011; 54:8421-8439.
- 9 52. Takeoka Y, Tanino T, Sekiguchi M, Yonezawa S, Sakagami M, Takahashi F, Togame H,  
10 Tanaka Y, Takemoto H, Ichikawa S, Matsuda A. Expansion of antibacterial spectrum of  
11 muraymycins towards *Pseudomonas aeruginosa*. ACS Med. Chem. Lett. 2014; 5:556-560.
- 12 53. Spork AP, Büschleb M, Ries O, Wiegmann D, Boettcher S, Mihalyi A, Bugg TDH, Ducho C.  
13 Lead structures for new antibacterials: stereocontrolled synthesis of a bioactive muraymycin  
14 analogue. Chem. Eur. J. 2014; 20:15292-15297.
- 15 54. Ries O, Carnarius C, Steinem C, Ducho C. Membrane-interacting properties of the  
16 functionalised fatty acid moiety of muraymycin antibiotics. Med. Chem. Comm. 2015; 6:879-  
17 886.
- 18 55. Mitachi K, Aleiwi BA, Schneider CM, Siricilla S, Kurosu M. Stereocontrolled total synthesis of  
19 muraymycin D1 having a dual mode of action against *Mycobacterium tuberculosis*. J. Am.  
20 Chem. Soc. 2016; 138:12975–12980.
- 21 56. Cheng L, Chen W, Zhai L, Xu D, Huang T, Lin S, Zhou X, Deng Z. Identification of the gene  
22 cluster involved in muraymycin biosynthesis from *Streptomyces* sp. NRRL 30471. Mol.  
23 Biosyst. 2011; 7:920-927.
- 24 57. Yamaguchi H, Sato S, Yoshida S, Takada K, Itoh M, Seto H. Capuramycin, a new nucleoside  
25 antibiotic. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 1986;  
26 39:1047-1053.
- 27 58. Seto H, Otake N, Sato S, Yamaguchi H, Takada K, Itoh M, Lu HSM, Clardy J. The structure of  
28 a new nucleoside antibiotic, capuramycin. Tetrahedron Lett. 1988; 29:2343-2346.
- 29 59. Muramatsu Y, Muramatsu A, Ohnuki T, Ishii MM, Kizuka M, Enokita R, Tsutsumi S, Arai M,  
30 Ogawa Y, Suzuki T, Takatsu T, Inukai M. Studies on novel bacterial translocase I inhibitors, A-  
31 500359s. I. Taxonomy, fermentation, isolation, physico-chemical properties and structure  
32 elucidation of A-500359 A, C, D, and G. J. Antibiotics 2003; 56:243-252.
- 33 60. Muramatsu Y, Ishii MM, Inukai M. Studies on novel bacterial translocase I inhibitors, A-  
34 500359s. II. Biological activities of of A-500359 A, C, D, and G. J. Antibiotics 2003; 56:253-  
35 258.

- 1 61. Muramatsu Y, Miyakoshi S, Ogawa Y, Ohnuki T, Ishii MM, Arai M, Takatsu T, Inukai M.  
2 Studies on novel bacterial translocase I inhibitors, A-500359s. III. Deaminocaprolactam  
3 derivatives of capuramycin: of A-500359 E, F, H, M-1 and M-2. J. Antibiotics 2003; 56:259-  
4 267.
- 5 62. Hotoda H, Furukawa M, Daigo M, Murayama K, Kaneko M, Muramatsu Y, Ishii MM,  
6 Miyakoshi SI, Takatsu T, Inukai M, Kakuta M, Abe T, Harasaki T, Fukuoka T, Utsui Y, Ohya  
7 S. Synthesis and antimycobacterial activity of capuramycin analogues. Part 1: substitution of  
8 the azepan-2-one moiety of capuramycin. Bio-Org. Med. Chem. Lett. 2003; 13:2829-2832.
- 9 63. Hotoda H, Daigo M, Furukawa M, Murayama K, Hasegawa CA, Kaneko M, Muramatsu Y,  
10 Ishii MM, Miyakoshi SI, Takatsu T, Inukai M, Kakuta M, Abe T, Fukuoka T, Utsui Y, Ohya S.  
11 Synthesis and antimycobacterial activity of capuramycin analogues. Part 2: acylated derivatives  
12 of capuramycin-related compounds. Bio-Org. Med. Chem. Lett. 2003; 13:2833-2836.
- 13 64. Funabashi M, Yang Z, Nonaka K, Hosobuchi M, Fujita Y, Shibata T, Chi X, Van Lanen SG. An  
14 ATP-independent strategy for amide bond formation in antibiotic biosynthesis. Nat. Chem.  
15 Biol. 2010; 6:581-586.
- 16 65. Cai W, Goswami A, Yang Z, Liu X, Green KD, Barnard-Britson S, Baba S, Funabashi M,  
17 Nonaka K, Sunkara M, Morris AJ, Spork AP, Ducho C, Garneau-Tsodikova S, Thorson JS, Van  
18 Lanen SG. The biosynthesis of capuramycin-type antibiotics: identification of the A-102395  
19 biosynthetic gene cluster, mechanism of self-resistance, and formation of uridine-5'-  
20 carboxamide. J. Biol. Chem. 2015; 290:13710-13724.
- 21 66. Liu X, Jin Y, Cai W, Green K, Goswami A, Garneau-Tsodikova S, Nonaka K, Baba S,  
22 Funabashi M, Yang Z, Van Lanen SG. A biocatalytic approach to capuramycin analogues by  
23 exploiting a substrate permissive N-transacylase CapW. Org. Biomol. Chem. 2016; 14:3956-  
24 3962.
- 25 67. Bouhss A, Trunkfield AE, Bugg TDH, Mengin-Lecreulx D. The biosynthesis of peptidoglycan  
26 lipid-linked intermediates. FEMS Microbiol. Rev. 2008; 32:208-233.
- 27 68. Bouhss A, Mengin-Lecreulx D, Le Beller D, van Heijenoort J. Topological analysis of the  
28 MraY protein catalysing the first membrane step of peptidoglycan synthesis. J. Mol. Microbiol.  
29 1999; 34:576-585.
- 30 69. Lloyd AJ, Brandish PE, Gilbey AM, Bugg TDH. Phospho-MurNAc-pentapeptide translocase  
31 (MraY) from *Escherichia coli*: catalytic role of conserved aspartic acid residues J. Bacteriol.  
32 2004; 186:1747-1757.
- 33 70. Brandish PE, Burnham MK, Lonsdale JT, Southgate R, Inukai M, Bugg TDH. Slow-binding  
34 inhibition of phospho-MurNAc-pentapeptide translocase (*Escherichia coli*) by mureidomycin  
35 A. J. Biol. Chem. 1996; 271:7609-7614.

- 1 71. Brandish PE, Kimura K, Inukai M, Southgate R, Lonsdale JT, Bugg TDH. Modes of action of  
2 tunicamycin, liposidomycin B and mureidomycin A: inhibition of phospho-MurNAc-  
3 pentapeptide translocase from *Escherichia coli*. *Antimicrob. Agents Chemother.* 1996; 40:1640-  
4 1644.
- 5 72. Gentle CA, Bugg TDH. Role of the enamide linkage of nucleoside antibiotic mureidomycin A:  
6 synthesis and reactivity of enamide-containing analogues. *J. Chem. Soc. Perkin Trans. 1* 1999;  
7 1279-1286.
- 8 73. Gentle CA, Harrison SA, Inukai M, Bugg TDH. Structure-function studies on nucleoside  
9 antibiotic mureidomycin A: synthesis of 5'-functionalised uridine analogues *J. Chem. Soc.*  
10 *Perkin Trans. 1* 1999:1287-1294.
- 11 74. Howard NI, Bugg TDH. Synthesis and activity of 5'-uridinyl dipeptide analogues mimicking  
12 the amino-terminal peptide chain of nucleoside antibiotic mureidomycin A *Bio-Org. Med.*  
13 *Chem.* 2003; 11:3083-3099.
- 14 75. Chung BC, Zhao J, Gillespie RA, Kwon D, Guan Z, Hong J, Zhou P, Lee S. Crystal structure of  
15 MraY, an essential membrane enzyme for bacterial cell wall synthesis. *Science* 2013;  
16 341:1012–1016.
- 17 76. Chung BC, Mashalidis EH, Tanino T, Kim M, Matsuda A, Hong J, Ichikawa S, Lee S.  
18 Structural insights into inhibition of lipid I production in bacterial cell wall synthesis. *Nature*  
19 2016; 533:557-560.
- 20 77. Hakulinen JK, Hering J, Brändén G, Chen H, Snijder A, Ek M, Johansson P. MraY-antibiotic  
21 complex reveals details of tunicamycin mode of action. *Nature Chem. Biol.* 2017; 13:265-267.
- 22 78. Hering J, Dunevall E, Ek M, Brändén G. Structural basis for selective inhibition of antibacterial  
23 target MraY, a membrane-bound enzyme involved in peptidoglycan synthesis. *Drug Discovery*  
24 *Today* 2018; 23:1426-1435.
- 25 79. Yoo J, Mashalides EH, Kuk ACY, Yamamoto K, Kaeser B, Ichikawa S, Lee SY. GlcNAc-1-P  
26 transferase-tunicamycin complex structure reveals basis for inhibition of N-glycosylation. *Nat.*  
27 *Str. Biol.* 2018; 25:217-224.
- 28 80. Rodolis MT, Mihalyi A, O'Reilly A, Slikas J, Roper DI, Hancock REM, Bugg TDH.  
29 Identification of a novel inhibition site in translocase MraY based upon the site of interaction  
30 with lysis protein E from bacteriophage  $\phi$ X174. *ChemBioChem* 2014; 15:1300-1308.
- 31 81. Rodolis MT, Mihalyi A, Ducho C, Eitel K, Gust B, Goss RJM, Bugg TDH. Mechanism of  
32 action of the uridyl peptide antibiotics: an unexpected link to a protein-protein interaction site in  
33 translocase MraY. *Chem Commun.* 2014; 50:13023-13025.
- 34 82. Lehrer J, Vigeant KA, Tatar LD, Valvano MA. Functional characterization and membrane  
35 topology of *Escherichia coli* WecA, a sugar-phosphate transferase initiating the biosynthesis of



- 1 enterobacterial common antigen and O-antigen lipopolysaccharide. J. Bacteriol. 2007;  
2 189:2618-2628.
- 3 83. Soldo B, Lazarevic V, Karamata D. *tagO* is involved in the synthesis of all anionic cell-wall  
4 polymers in *Bacillus subtilis* 168. Microbiology 2002; 148:2079-2087.
- 5 84. Farha MA, Koteva K, Gale RT, Sewell EW, Wright GD, Brown ED. Designing analogues of  
6 ticlopidine, a wall teichoic acid inhibitor, to avoid formation of its oxidative metabolites.  
7 Bioorg. Med. Chem. Lett. 2014; 24:905-910.
- 8 85. Rush JS, Edgar RJ, Deng P, Chen J, Zhu H, van Sorge NM, Morris AJ, Korotkov KV,  
9 Korotkova N. The molecular mechanism of N-acetylglucosamine side-chain attachment to the  
10 Lancefield group A carbohydrate in *Streptococcus pyogenes*. J. Biol. Chem. 2017; 292:19441-  
11 19457.
- 12 86. Glover KJ, Weerapana E, Chen MM, Imperiali, B. Direct biochemical evidence for the  
13 utilization of UDP-bacillosamine by PglC, an essential glycosyl-1-phosphate transferase in the  
14 *Campylobacter jejuni* N-linked glycosylation pathway. Biochemistry 2006; 45:5343-5350.
- 15 87. Walvoort MTC, Lukose V, Imperiali B. A modular approach to phosphoglycosyltransferase  
16 inhibitors inspired by nucleoside antibiotics. Chem. Eur. J. 2015; 22:3856-3864

