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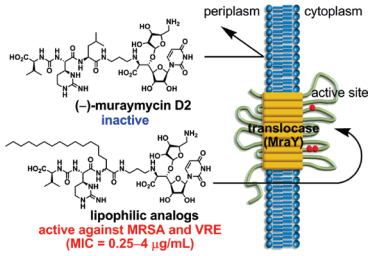
Synthesis and Biological Evaluation of Muraymycin Analogues Active against Anti-Drug-Resistant Bacteria

Tetsuya Tanino,[†] Satoshi Ichikawa,*[,†] Bayan Al-Dabbagh,[‡,∥] Ahmed Bouhss,[‡] Hiroshi Oyama,[§] and Akira Matsuda*[,†]

[†]Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan, [‡]Laboratoire des Enveloppes Bactériennes et Antibiotiques, Institut de Biochimie et Biophysique Moléculaire et Cellulaire, UMR 8619 CNRS, Université Paris-Sud, Bât. 430, Orsay F-91405, France, and [§]Shionogi Innovation Center for Drug Discovery, Shionogi & Co., LTD., Kita-21, Nishi-11, Kita-ku, Sapporo 001-0021, Japan

ABSTRACT Muraymycin analogues with a lipophilic substituent were synthesized using an Ugi four-component assemblage. This approach provides ready access to a range of analogues simply by altering the aldehyde component. The impact of the lipophilic substituent on the antibacterial activity was very large, and analogues **7b**−**e** and **8b**−**e** exhibited good activity against a range of Gram-positive bacterial pathogens including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. This study also showed that the accessory urea-dipeptide motif contributes to MraY inhibitory and antibacterial activity. The knowledge obtained from our structure−activity relationship study of muraymycins provides further direction toward the design of potent MraY inhibitors. This study has set the stage for the generation of novel antibacterial "lead" compounds based on muraymycins.

KEYWORDS Antibiotics, drug resistance, MraY, muraymycin, peptidoglycan, Ugi four-component reaction



The extensive use of antibiotics has raised a serious global public health problem. Because bacterial pathogens inevitably develop resistance to every new drug launched in the clinic, the need for new antibiotics to counteract drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) is critical.[1] In choosing novel antibacterial agents to address this problem, several criteria need to be considered as follows: The target must be essential for growth, the agent must be different from existing drugs, and the initial "hit" scaffold must be amenable to structural changes that allow for optimization of the potency and efficacy to generate "lead" compounds.[2−6]

The muraymycins (MRYs) (Figure 1, **1**), isolated from a culture broth of *Streptomyces* species,[7,8] are members of a class of naturally occurring 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-*C*-glycyluridine antibiotics.[9,10] The MRYs that have a lipophilic side chain have been shown to exhibit excellent antimicrobial activity against Gram-positive bacteria. In particular, the efficacy of the MRYs in *S. aureus*-infected mice represents a promising lead for the development of new antibacterial agents. The MRYs inhibit the formation of lipid II and peptidoglycan and are believed to be inhibitors of the phospho-MurNAc-pentapeptide transferase (MraY), which is responsible for the formation of lipid I in the peptidoglycan biosynthesis pathway.[11−15] Because MraY is an essential enzyme in bacteria,[16] it is a potential target for the development of general antibacterial agents. Because of these promising biological properties, the MRYs have become intriguing, challenging synthetic targets.[17−19] Recently, we have accomplished the first total synthesis of MRY D2 (**7a**) and its epimer (**8a**),[20] featuring the convergent assemblage of the urea-dipeptide carboxylic acid **2**, isovaleraldehyde (**3a**), 2,4-dimethoxybenzylamine (**4**), and the isonitrile derivative of the aminoribosyluridine **5** by an Ugi four-component reaction[21] (U4CR) as shown in Scheme 1. Herein, we describe the synthesis and biological evaluation of MRY analogues associated with a lipophilic side chain as an initial structure−activity relationship (SAR) study of MRYs, and the MRY analogues that we discovered were effective against drug-resistant bacterial pathogens.

First, the inhibitory activity of **7a** on the purified MraY enzyme (*Bacillus subtilis*) was examined by quantifying the incorporation of MurNAc-[^{14}C]pentapeptide by MraY from UDP-MurNAc-[^{14}C]pentapeptide into lipid I, the product of MraY (Table 1).[22] It turned out that it was a strong MraY inhibitor with an IC_{50} value of 0.01 μM. Compound **8a**, which is the epimer of **7a** at the α-position of the Leu residue, exhibited good MraY inhibitory activity (IC_{50} = 0.09 μM) albeit reduced by a factor of 9 as compared to that of **7a**. The antibacterial activity of **7a** and **8a** was then evaluated.[23] However, no antibacterial activity was exhibited by these compounds when subjected to a range of Gram-positive bacteria up to 64 μg/mL (Table 2), despite the fact that they did exhibit potent inhibitory activity against their target

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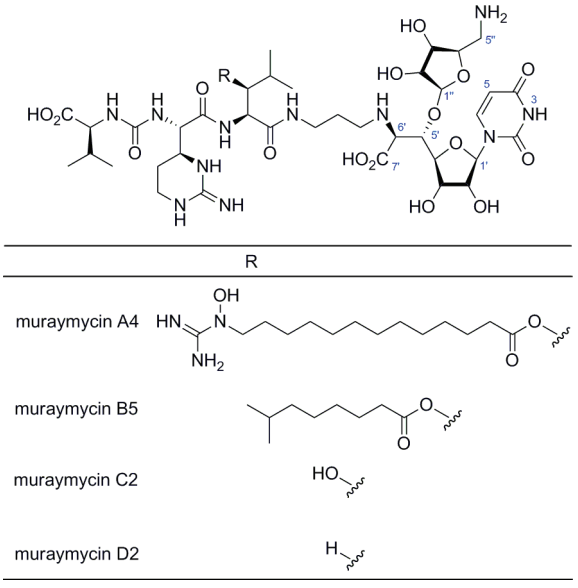

Figure 1. Structures of MRYs (**1**).

enzyme MraY. Because the lipid bilayer of the cytoplasmic membrane is thought to be a common barrier in bacteria, this observed membrane permeability; these compounds lack the hydrophobic side chain found in MRY A and B classes (Figure 1), which have good antibacterial activity. Although not essential to MraY inhibition, the fatty acyl side chain attached to

Table 1. Inhibitory Activities of the Synthesized Compounds against the MraY Enzyme[a]

	7a	8a	7b	8b	17
IC_{50} (μM)	0.01	0.09	0.33	0.74	5

[a] The activities of the compounds were tested against purified MraY from *B. subtitis*.[22] The assay was performed in a reaction mixture of 10 mL containing, in final concentrations, 100 mM Tris-HCl, pH 7.5, 40 mM $MgCl_2$, 1.1 mM C 55-P, 250 mM NaCl, 0.25 mM UDP-MurNAc-[^{14}C]pentapeptide (337 Bq), and 8.4 mM *N*-lauroyl sarcosine. The mixture was incubated for 30 min at 37 °C. The radiolabeled substrate UDP-MurNAc-pentapeptide and reaction product (lipid I, product of MraY) were separated by TLC on silica gel plates. The radioactive spots were located and quantified with a radioactivity scanner. IC_{50} values were calculated with respect to a control assay without the inhibitor. Data represent the mean of independent triplicate determinations.

Scheme 1

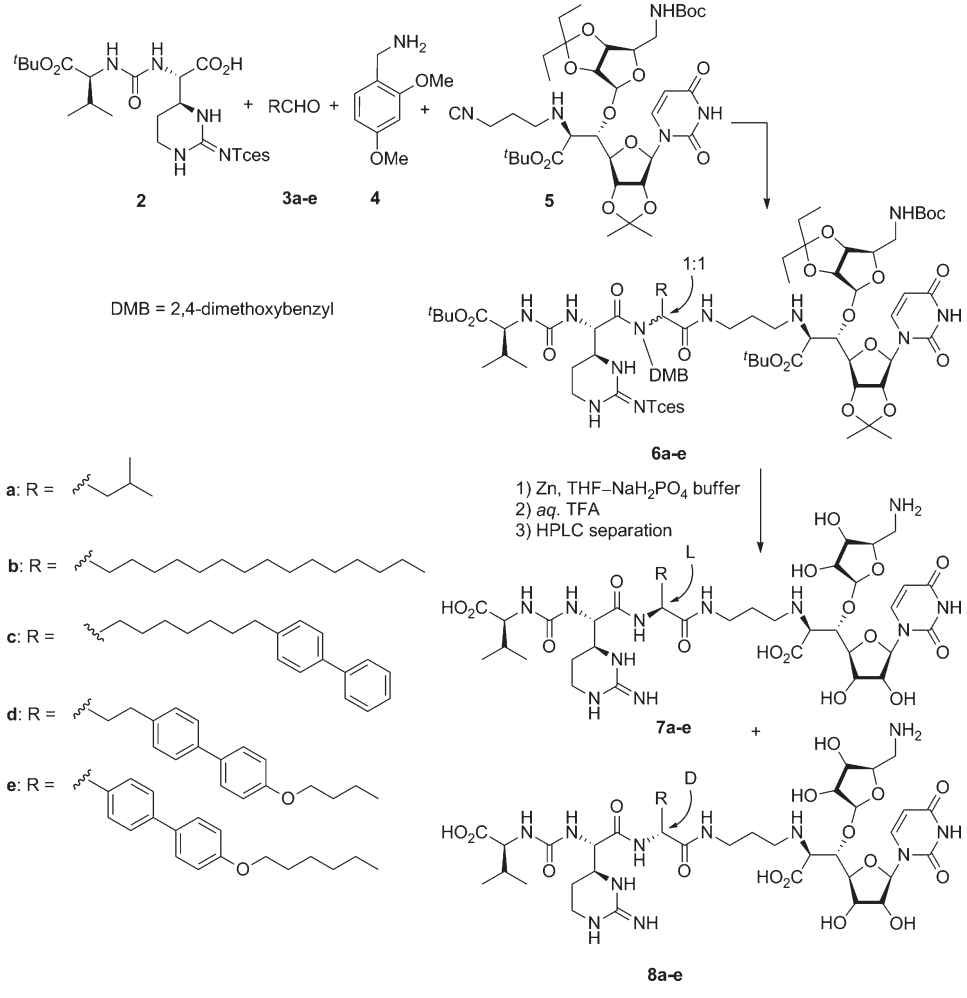

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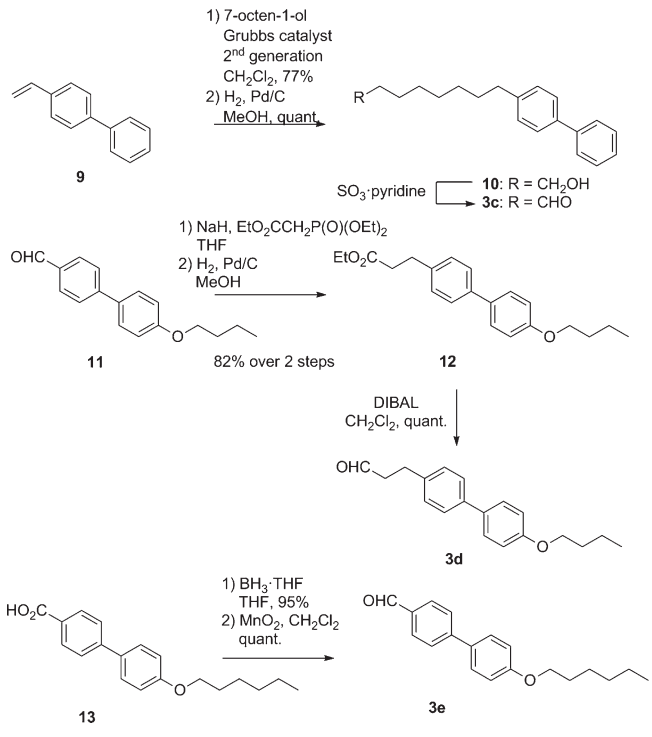
Table 2. Antibacterial Activity

	MIC (ug/mL)[a]					
compound	*S. aureus* ATCC 29213 (MSSA)	*S. aureus* SR3637 (MRSA)	*E. faecalis* ATCC 29212	*E. faecalis* SR7914 (VRE)	*E. faecium* ATCC 19434	*E. faecium* SR7917 (VRE)
7a	> 64	> 64	> 64	> 64	> 64	> 64
8a	> 64	> 64	> 64	> 64	> 64	> 64
7b	2	4	4	4	4	2
8b	2	4	2	4	0.5	0.25
7c	4	4	4	16	4	8
8c	8	16	8	16	4	4
7d	16	32	16	64	16	32
8e	64	64	32	64	4	8
7e	4	8	8	8	4	4
8e	16	16	16	16	4	8
17	> 64	> 64	32	64	64	64
vancomycin	1	1	1	> 64	0.5	> 64

[a] MICs were determined by a microdilution broth method as recommended by the NCCLS with cation-adjusted Mueller−Hinton broth (CA-MHB).[23] Serial 2-fold dilutions of each compound were made in appropriate broth, and the plates were inoculated with 5×10^4 CFU of each strain in a volume of 0.1 mL. Plates were incubated at 35 °C for 20 h, and then, MICs were scored.

the peptide moiety of MRYs is necessary for antibacterial activity. Because the β-acyloxyleucine moiety found in the MRY A and B classes could be susceptible to β-elimination or hydrolysis by enzymes such as esterases, these shortcomings would have to be overcome. Therefore, we designed and synthesized analogues, which were linked to a hydrophobic substituent on the MRY core structure via a C−C bond, as chemically and biologically stable isosteres of the MRYs. Our synthetic route to **7a** and **8a** provides ready access to a range of analogues containing an unnatural amino acid simply by altering the aldehyde component. Thus, **2**,[20] **4**, **5**,[20] and hexadecanal (**3b**) gave, via the U4CR in EtOH, **6b** containing a pentadecylglycine residue in its structure in 37% yield as a 1:1 mixture of diastereomers. Deprotection of **6b** was achieved by a two-step sequence [Zn, 1 M aqueous NaH_2PO_4, tetrahydrofuran (THF), then 80% aqueous TFA] to afford the hydrophobic analogue **7b** and its diastereomer **8b**, which were easily separated by reverse-phase high-performance liquid chromatography (HPLC). The newly formed stereogenic center at the pentadecylglycine residue of each diastereomer was determined by conventional amino acid analysis[24] by using L- or D-pentadecylglycine as the reference compound (see the Supporting Information, Scheme S1). The inhibitory effect of **7b** and **8b** on purified MraY activity was then evaluated (Table 1). The lipophilic analogues **7b** and **8b** were found to be weaker inhibitors of MraY than **7a** but still potent (IC_{50} = 0.33 and 0.74 μM, respectively). Introducing the long lipophilic side chain was still acceptable for MraY inhibition. The nature of the lipophilic side chain could influence the antibacterial activity by modulating the membrane permeability or the affinity to the target enzyme MraY. Therefore, analogues incorporating a biphenyl moiety at the end (**7c** and **8c**), in the middle (**7d** and **8d**), and at the junction (**7e** and **8e**) of the lipophilic moiety were also designed and synthesized. The corresponding aldehydes, precursors for the U4CR assemblage, were prepared as shown in Scheme 2. Cross-metathesis of 4-vinylbiphenyl (**9**) and 7-octen-1-ol in the presence of Grubbs

Scheme 2



second generation catalyst[25] (77% yield) was followed by catalytic hydrogenation to give the saturated biphenylalcohol **10** (quant.). The primary alcohol was oxidized to give the aldehyde **3c**. On the other hand, the aldehyde **11**[26] was bis-homologated by the Horner−Wadsworth−Emmons reaction to give the corresponding α,β-unsaturated ester, which was reduced by catalytic hydrogenation to afford the saturated ester **12** in 82% yield over two steps. The ethyl ester was reduced with DIBAL to give the aldehyde **3d** in quantitative yield. The

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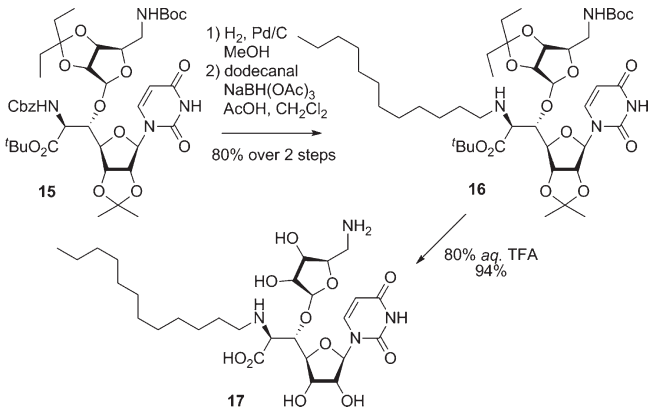
Scheme 3



last aldehyde **3e**[27] was prepared from the carboxylic acid **13** by reduction of the acid with BH_3·THF (95% yield) followed by oxidation with MnO_2 (quant.). With these aldehyde units in hand, we have synthesized the analogues **7c**−**e** and **8c**−**e** via the U4CR followed by global deprotection in a manner similar to the preparation of **7b** and **8b** as shown in Scheme 1.[28]

The antibacterial activity of this series of compounds was evaluated, and the results are summarized in Table 2. The impact of the lipophilic substituent on the antibacterial activity was very good, and both **7b** and **8b** exhibited good activity against a range of Gram-positive bacterial pathogens including *S. aureus* SR3637 (MRSA) and *Enterococcus faecium* SR7917 (VRE) with minimum inhibitory concentration (MIC) values of 0.25−4 μg/mL. The activity was comparable to that reported for MRY A and B classes.[7,8] Thus, the membrane permeability plays an important role in terms of the antibacterial activity among this class of natural products. Of significance is the discovery of **8b** with the "unnatural" D-pentadecylglycine residue, which exhibited eight times more potent antibacterial activity against *E. faecium* SR7917 than that of **7b** with the "natural" stereochemistry. Overall, analogues with the "natural" stereochemistry were slightly more potent than those with "unnatural" stereochemistry against Staphylococci. Introducing the rigid biphenyl group into the lipophilic side chain did not improve antibacterial activity but rather reduced potency in the case of **7d** and **8d**, which contain the biphenyl group in the middle of the side chain. Further optimization of the lipophilic side chain therefore will be necessary. All of the compounds prepared in this study exhibited no cytotoxicity against human hepatoceller liver carcinoma (HepG2) cells (IC_{50} > 100 μg/mL).

MRYs share the accessory urea-dipeptide motif in addition to the 5′-*O*-aminoribosyl-5′-*C*-glycyluridine moiety. To rapidly see the impact of the accessory motif, a truncated analogue **17**, where the accessory motif was completely removed from **7b** or **8b**, was prepared from **15**[29] as shown in Scheme 3, and the biological properties were compared. The truncated analogue **17** was found to be a much weaker MraY inhibitor with an IC_{50} value of 5 μM, which was a 6−12-fold reduction of the inhibitory activity as compared to **7b** and **8b** (Table 1). The antibacterial activity of **17** was greatly decreased with MICs ranging from 32−64 μg/mL, although **17** possessed a hydrophobic substituent (Table 2). These results clearly show that the urea-dipeptide accessory motif is also a contributing factor in the interaction with MraY to result in strong antibacterial inhibitory activity. In addition to and apart from the binding pocket interacting with the 5′-*O*-aminoribosyl-5′-*C*-glycyluridine moiety, it is noteworthy that there would be an additional binding site in MraY, which recognizes the accessory urea-dipeptide motif.

In summary, MRY analogues with a lipophilic substituent were synthesized by U4CR, which enabled us easily to prepare MRY analogues containing unnatural amino acids. The impact of the lipophilic substituent on the antibacterial activity was very large, and analogues **7b**−**e** and **8b**−**e** exhibited good activity against a range of Gram-positive bacterial pathogens, including MRSA and VRE. This study also indicated that the accessory urea-dipeptide motif contributes to MraY inhibitory and antibacterial activity. The knowledge obtained from our SAR study of MRYs would provide further direction toward the design of potent MraY inhibitors. Because MRYs possess relatively high molecular weight and polarity, these structural features are in good agreement with the property space characteristics of antibacterial agents.[30−33] This initial study has set the stage for the generation of novel antibacterial "lead" compounds based on MRYs and is currently being expanded.

SUPPORTING INFORMATION AVAILABLE

Full experimental procedures, compound purities by HPLC, and NMR data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *To whom correspondence should be addressed. (S.I.) Tel: (+81)11-706-3229. Fax: (+81)11-706-4980. E-mail: ichikawa@pharm.hokudai.ac.jp. (A.M.) Tel: (+81)11-706-3228. Fax: (+81)11-706-4980. E-mail: matuda@pharm.hokudai.ac.jp.

Present Addresses: ‖ Department of Internal Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates.

Author Contributions: T.T. contributed to the synthesis of muraymycin analogues. B.A.-D. contributed to the MraY inhibitory assay. S.I. was the PI of T.T., made significant writing and editing contributions, and provided significant intellectual input. A.B. was the PI of B.A.-D. and a collaborator of the Matsuda lab and provided significant intellectual input. H.O. contributed to the cytotoxicity assay. A.M. was the main PI of the entire project, was the overseeing PI of T.T., S.I., and A.B., made significant writing and editing contributions, and provided significant intellectual input.

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