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Mechanistic Analysis of Muraymycin Analogues: A Guide to the Design of MraY Inhibitors

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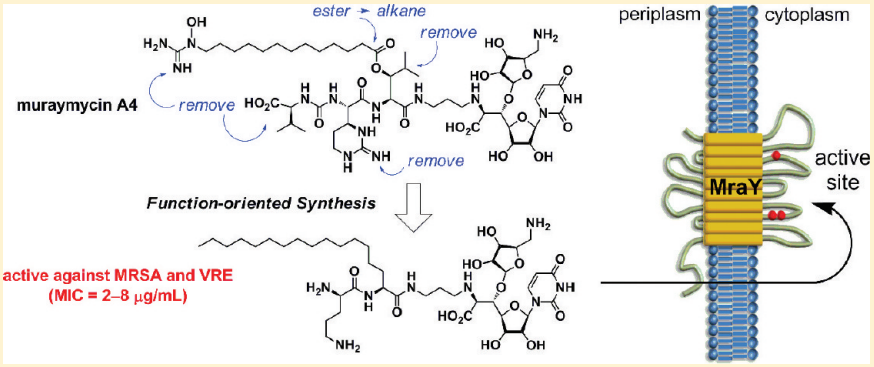
Supporting Information



ABSTRACT: The systematic structure−activity relationship (SAR) of the muraymycins (MRYs) using an Ugi four-component reaction (U4CR) was investigated. The impact of the lipophilic substituent on antibacterial activity was significant, and the analogues **8** and **9** having a lipophilic side chain exhibited good activity against a range of Gram-positive bacterial pathogens, including MRSA and VRE. Further investigation of compounds **8** and **9** revealed these analogues to be selective inhibitors of the MraY transferase and nontoxic to HepG2 cells. The SAR of the accessory urea−peptide moiety indicated that it could be simplified. Our SAR study of the MRYs suggests a probable mechanism for inhibition of the MraY, where the inner moiety of the urea−dipeptide motif interacts with the carbohydrate recognition domain in the cytoplasmic loop 5. The predicted binding model would provide 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 MRYs.

INTRODUCTION

Natural products are still a rich source for drug development;[1] however, some biologically relevant natural products possess rather large, complex, or labile chemical structures compared to synthetic drugs, which limits chemical modification in a process pursuing a structure−activity relationship (SAR). One of the solutions to these drawbacks is function-oriented synthesis (FOS),[2] namely, the design of less complex targets with comparable or superior function that could be made in a practical and even synthetically novel manner. Although challenging, simpler scaffold designs would provide a practical synthesis of a set of analogues and synthetic innovation in current medicinal chemistry.

The muraymycins (MRYs) (Figure 1, **1**), isolated from a culture broth of *Streptomyces* sp.,[3] are members of a class of naturally occurring 6′-*N*-alkyl-5′-*β*-*O*-aminoribosyl-*C*-glycyluridine antibiotics.[4] The MRYs, having 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 extensive use of antibiotics has raised a serious global public health problem. Since 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.[5] Although two new classes of antibiotics have been launched in the clinic since 2000 to treat these infections, clinical resistance to both has already emerged.[6,7] In choosing novel antibacterial agents to address this problem, the target must be essential for growth, the agent different from existing drugs, and the initial "hit" scaffold amenable to structural changes that allow for optimization of the potency and efficacy to generate "lead" compounds.[8−10] The MRYs inhibit the formation of lipid II and peptidoglycan

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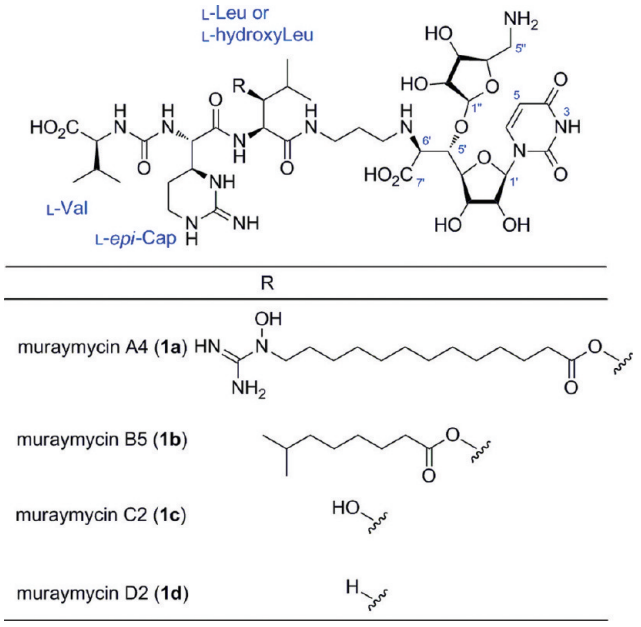

Figure 1. Structures of muraymycins (MRYs).

and are believed to be inhibitors of phospho-MurNAc-pentapeptide transferase (MraY), which is responsible for the formation of lipid I in the peptidoglycan biosynthesis pathway (Figure 2).[11−14] The enzymes involved in the biosynthesis of peptidoglycan are essential for bacterial life and represent important targets for antibacterial chemotherapy. The MraY transferase catalyzes the transfer of the phospho-MurNAc-pentapeptide motif from UDP-MurNAc-pentapeptide onto a lipid carrier, undecaprenyl phosphate (C_{55}-P), leading to the formation of undecaprenylpyrophosphoryl-MurNAc-pentapeptide (lipid **I**) with the subsequent release of UMP.[13,14] Since MraY is an essential enzyme in bacteria,[15] 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.[16] Recently, we have accomplished the first total synthesis of MRY D2 (Figure 3, **1d**)

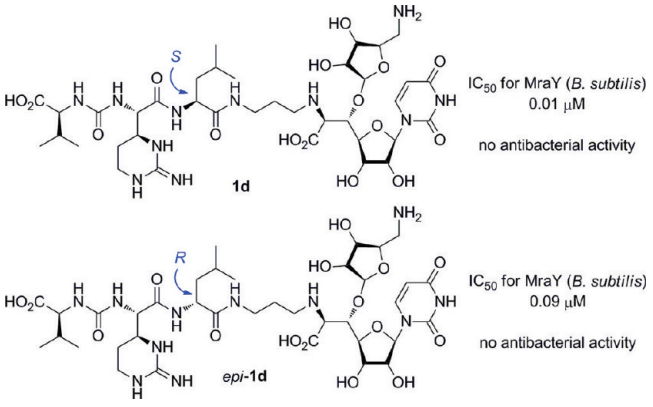

Figure 3. Structures and biological activity of muraymycin D2 and its epimer.

and its epimer (*epi*-**1d**).[17] However, when **1d** and *epi*-**1d** were subjected to a range of Gram-positive bacteria up to 64 μg/mL, they showed no antibacterial activity whereas against their target enzyme MraY (IC_{50} = 0.01 and 0.09 μM), respectively, exhibited potent inhibitory activity. Herein we describe the full details of our efforts to increase the antibacterial activity and to simplify the chemical structure based on the FOS of MRYs, as well as our discovery of simplified analogues that proved to be effective against a range of bacteria including drug-resistant

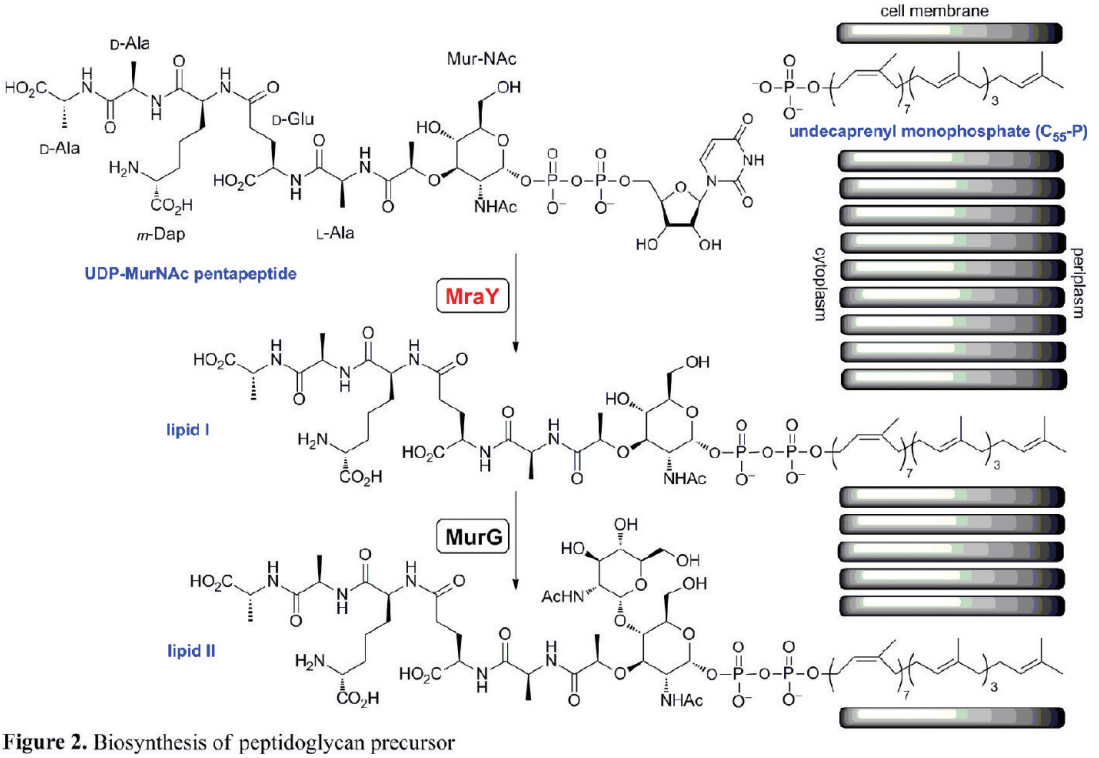

Figure 2. Biosynthesis of peptidoglycan precursor.

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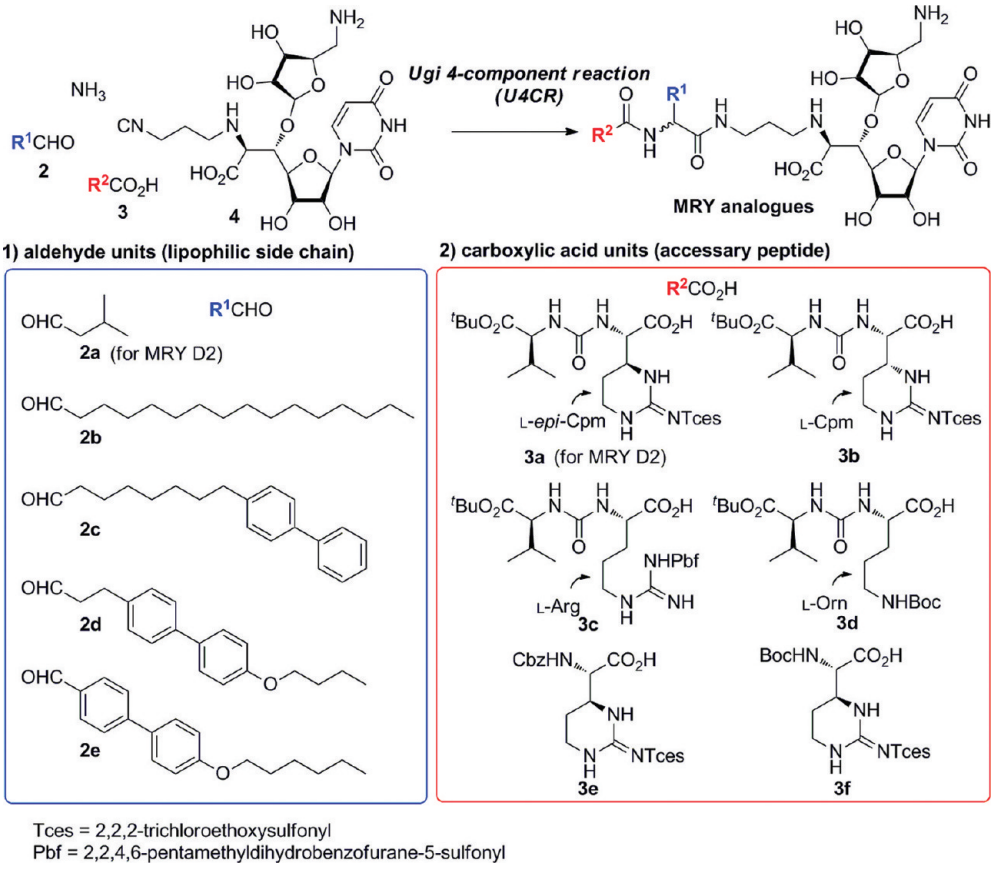

Figure 4. Two-directional optimization via U4CR.

strains. Our SAR study provides an insight into a binding model of the MRYs with the target MraY. The study of the lipophilic side chain has recently been reported,[18] and its details are also described.

RESULTS AND DISCUSSION

Two-Directional Optimization. The structure of the muraymycins can be characterized by three components that are responsible for their biological activity: the aminoribosyluridine, the lipophilic side chain, and the accessory urea–peptide moieties. Previous studies by us[19] and others[20] associated with antibacterial nucleoside natural products indicated that the aminoribosyluridine moiety is the essential structural feature that interacts with the active site of the MraY enzyme. That the lipophilic side chain is not essential for MraY inhibition but rather for antibacterial activity clearly indicates that the side chain contributes to the membrane permeability of the molecule because MraY is an integral membrane protein and its active site exists on the cytoplasmic side of the membrane. Since the lipid bilayer of the cytoplasmic membrane is thought to be a common barrier in bacteria, a lipophilic side chain is necessarily attached to the MRY D2 in order to acquire antibacterial activity. Incorporating a lipophilic side chain onto molecules that inhibit bacterial cell wall synthesis is a known concept.[21] However, little is known about the structure–activity relationship of the lipophilic moiety. The urea–peptide moiety, the third structural component, is regarded as an accessory motif linking the aminoribosyluridine moiety to the lipophilic moiety. Its role and structural requirement for the biological activity are totally unknown. Therefore, an investigation of the lipophilic side chain as well as the accessory urea–peptide moiety is necessary to pursue FOS. Ideally we hope to optimize multiple sites by a common synthetic strategy to chemically supply the analogues. We have accomplished the total synthesis of **1d** and *epi*-**1d**,[17] which features the convergent assemblage of isovaleraldehyde (**2a**), the urea–dipeptide carboxylic acid **3a**, 2,4-dimethoxybenzylamine, and the isonitrile derivative of the aminoribosyluridine **4** by an Ugi four-component reaction[22] (U4CR) as shown in Figure 4. Basically, the U4CR is nonstereoselective at the newly formed stereogenic center, resulting in a mixture of products. From a medicinal chemistry point of view, this strategy works well for examining the structure–activity relationship. Thus, the U4CR gives us two diastereomers, which are useful compounds in order to examine the structure–activity relationship. In conjunction with the nature of the multicomponent assemblage, this strategy allows us to diversify accessible analogues simply by altering each component. We planned a two-directional optimization on both the lipophilic side chain and the urea–peptide moieties (Figure 4).

Investigation of the Lipophilic Side Chain. The precursor aldehydes **2c–e**, which all contain a biphenyl moiety, were prepared by a conventional method as shown in Scheme S1 of Supporting Information. With the components for the U4CR in hand, we started the optimization from the lipophilic moiety (Figure 4, left). Because the β-acyloxyleucine moiety found in the parent MRY A and B classes could be susceptible to β-elimination or hydrolysis by enzymes such as esterases, we therefore designed and synthesized analogues such as **8** and **9**, 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 (Scheme 1). The molecular design

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Scheme 1

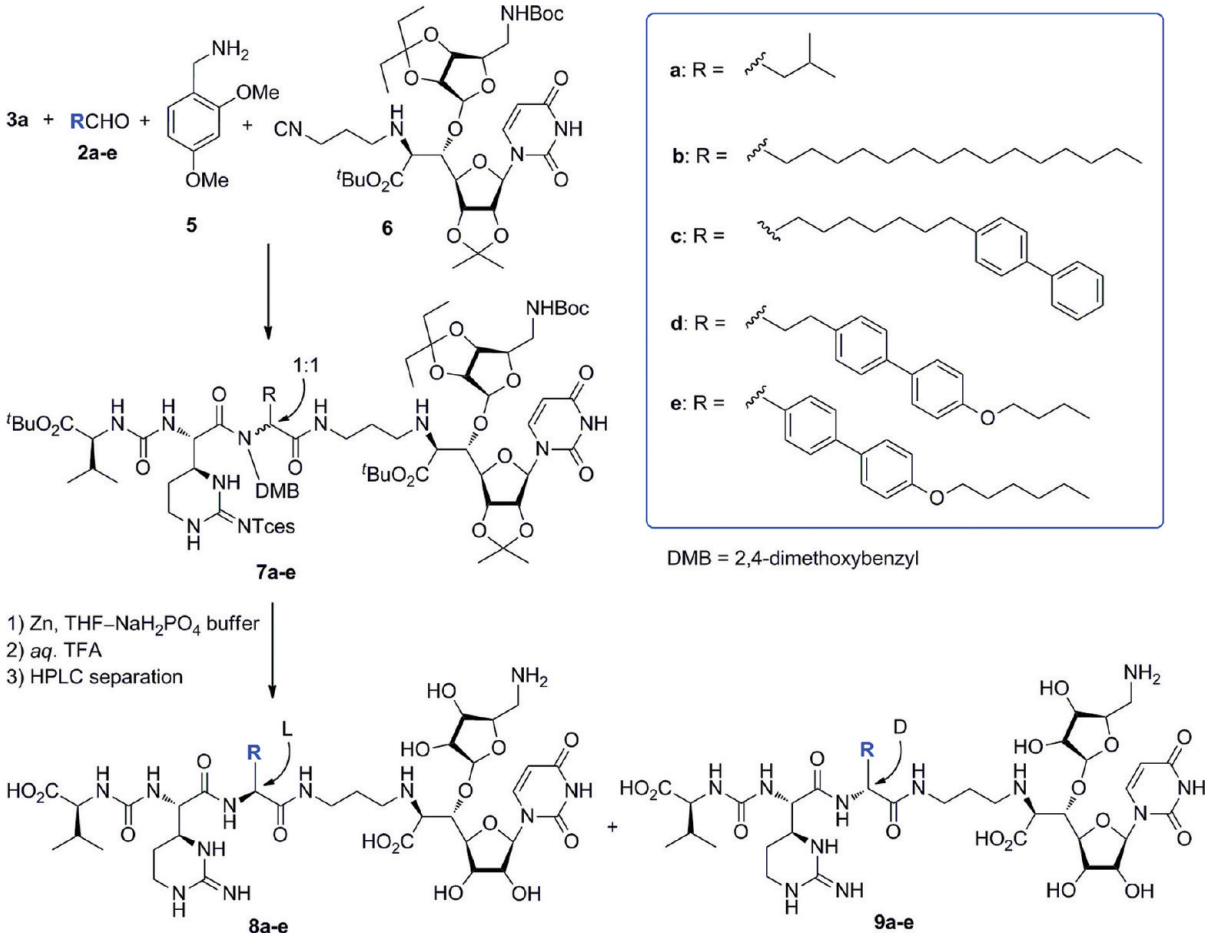

Table 1. Inhibitory Activities of the Synthesized Compounds against MraY[a]

	IC_{50} (μM)			mode of inhibition	
	MraY	WecA	K_i (nM)	UDP-MurNAc-pentapeptide	C_{55}-P
1d	0.01	1230	7.6	competitive	noncompetitive
epi-**1d**	0.09	1560	49	competitive	noncompetitive
8b	0.33	970	247	competitive	noncompetitive
9b	0.74	1340	698	competitive	noncompetitive

[a]The activities of the compounds were tested against purified MraY from *B. subtilis*. The assay was performed in a reaction mixture of 10 μL 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*-lauroylsarcosine. 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. Lineweaver–Burk plots were used to determination of the K_i values and the type of inhibition assuming a ping-pong Bi Bi kinetic mechanism as proposed previously by Heydanek et al.[26]

was easily transferred to the analogue synthesis by the use of the simple aldehydes shown in Scheme S1 in the U4CR. Thus, **2b**, **3a**,[17] **5**, and **6**[17] were admixed in EtOH to give **7b** containing a pentadecylglycine residue in its structure in 37% yield as a 1:1 mixture of diastereomers. Deprotection of **7b** was achieved by a two-step sequence (Zn, 1 M aqueous NaH_2PO_4, THF, then 80% aqueous TFA) to afford the hydrophobic analogue **8b** and its diastereomer **9b**, which were easily separated by reverse-phase HPLC. The newly formed stereogenic center at the pentadecylglycine residue of each diastereomer was determined by conventional amino acid analysis[23] using L- or D-pentadecylglycine as the reference compound (see Supporting Information). Analogues incorporating a biphenyl moiety at the end (**8c** and **9c**), in the middle (**8d** and **9d**), and at the junction (**8e** and **9e**) of the lipophilic moiety were also synthesized. In HPLC analysis, a similar behavior was observed between compounds with the *R*-configuration (**1d** and **8b**, having shorter retention times) and those with the *S*-configuration (*epi*-**1d** and **9b**, having longer retention times) at the newly formed stereogenic center in the U4CR. According to the relative retention time in the HPLC analysis, the absolute stereochemistries of **8c–e** and **9c–e** were tentatively assigned to be the *R*- and *S*-configurations, respectively.

First, the inhibitory activity of **8b** and **9b** on the purified MraY enzyme (*B. subtilis*) was examined by quantifying the

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Table 2. Antibacterial Activity of Lipophilic Side Chain Analogues

	MIC (μg/mL)[a]					
	S. aureus		*E. faecalis*		*E. faecium*	
compd	ATCC 29213 (MSSA)	SR3637 (MRSA)	ATCC 29212	SR7914 (VRE)	ATCC19434	SR7917 (VRE)
1d	>64	>64	>64	>64	>64	>64
epi-**1d**	>64	>64	>64	>64	>64	>64
8b	2	4	4	4	4	2
9b	2	4	2	4	0.5	0.25
8c	4	4	4	16	4	8
9c	8	16	8	16	4	4
8d	16	32	16	64	16	32
9d	64	64	32	64	4	8
8e	4	8	8	8	4	4
9e	16	16	16	16	4	8
vancomycin	1	1	1	>64	1	>64

[a]MICs were determined by a microdilution broth method as recommended by the NCCLS with cation-adjusted Mueller–Hinton broth (CA-MHB). 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.

incorporation of MurNAc-[^{14}C]pentapeptide by MraY from UDP-MurNAc-[^{14}C]pentapeptide into lipid **I**, the product of MraY (Table 1).[14,24] The lipophilic analogues **8b** and **9b** were found to be weaker inhibitors of MraY than **1d** but still potent (IC_{50} of 0.33 and 0.74 μM, respectively). Therefore, introducing the long lipophilic side chain was still acceptable for MraY inhibition.

Next, the type of inhibition of **8b** and **9b** as well as of **1d** toward the MraY transferase was investigated. In order to determine the K_i values and the type of inhibition, initial rates were measured at different concentrations of UDP-MurNAc-[^{14}C]pentapeptide (0.25–1.5 mM) with a fixed concentration of C_{55}-P (1 mM) in the presence of different concentrations of the inhibitors. Similarly, initial rates were also measured at different concentrations of C_{55}-P (0.1–0.6 mM) with a fixed concentration of UDP-MurNAc-[^{14}C]pentapeptide (0.25 mM) in the presence of different concentrations of the inhibitors. In each case, Lineweaver–Burk plots revealed that these compounds present a common behavior with respect to the substrates of MraY: they were all competitive and non-competitive inhibitors toward the nucleotide and the lipid substrates, respectively. This is consistent with the structures of these compounds that share certain structural moieties with the nucleotide substrate. However, it is known that liposidomycin B, a natural inhibitor of MraY, is noncompetitive toward the nucleotide substrate despite the presence of the uridine moiety.[25] Lineweaver–Burk plots were used to determine of the K_i values and the type of inhibition assuming a ping-pong Bi Bi kinetic mechanism as proposed previously by Heydanek et al.[26] Among the muraymycin analogues, the most active compound is **1d**, which has a K_i value 6 times lower than that of *epi*-**1d**. The replacement of D-leucine with L-leucine leads to a decrease in its affinity for the MraY target. Moreover, the substitution of the leucine side chain by a long carbon chain decreased the K_i by factors of 30 and 90 for compounds **8b** and **9b**, respectively. Therefore, in each case the compound with the amino acid residue having the L-configuration displayed a higher affinity for the enzyme target compared to that with the D-configuration. Furthermore, the extended length of the side chain limited the binding of the inhibitor in the catalytic site of the enzyme, whereas the type of inhibition remained unchanged.

The antibacterial activity of this series of compounds was then evaluated,[27] and the results are summarized in Table 2. The impact of the lipophilic substituent on antibacterial activity was very good, and both **8b** and **9b** exhibited good activity against a range of Gram-positive bacterial pathogens including *S. aureus* SR3637 (MRSA) and *E. faecium* SR7917 (VRE) with MIC values of 0.25–4 μg/mL. The activity was comparable to that reported for the MRY A and B classes.[3] Thus, membrane permeability plays an important role in terms of the antibacterial activity among this class of natural products. Considering the inhibitory properties of these compounds for MraY, the observed impact of the lipophilic substituent on the antibacterial activity of **8b** in vivo and **9b** could be attributed to its role in facilitating the transfer of the molecule through the lipophilic membrane (log *P* values from octanol/water partitioning calculated with Multiple minimization program in MacroModel program, version 9.2, are −5.20 for **1d** and 0.265 for **8b**). Of significance is the discovery of **9b** with the "unnatural" D-pentadecylglycine residue, which exhibited 8 times more potent antibacterial activity against *E. faecium* SR7917 than **8b** with the "natural" stereochemistry. However, analogues with the "natural" stereochemistry were similar to or slightly more potent than those with the "unnatural" stereochemistry against *Staphylococci*. Introducing the rigid biphenyl group into the lipophilic side chain had no advantage, and the analogues **8c–e** and **9c–e** did not improve antibacterial activity but rather reduced potency in the cases of **8d** and **9d**, which contain the biphenyl group in the middle of the side chain. Thus, the simple long alkyl group proved to be the best lipophilic side chain among the five groups tested in this study although further optimization will be necessary.

Selective toxicity is a key concern in the chemotherapy for infectious diseases. Consequently, determining the specificity of the synthesized compounds for inhibition of MraY activity is an important part of clinical drug development. Among the paralogues of MraY is WecA, which is an integral membrane protein that initiates the biosynthesis of the enterobacterial common antigen and the *O*-antigen lipopolysaccharide.[28,29] This enzyme catalyzes the transfer of the phospho-GlcNAc moiety from the nucleotide precursor UDP-GlcNAc onto C_{55}-P, leading to the formation of C_{55}-PP-GlcNAc (lipid intermediate **I**) with the subsequent release of UMP. The MraY and WecA enzymatic reactions share the same lipid

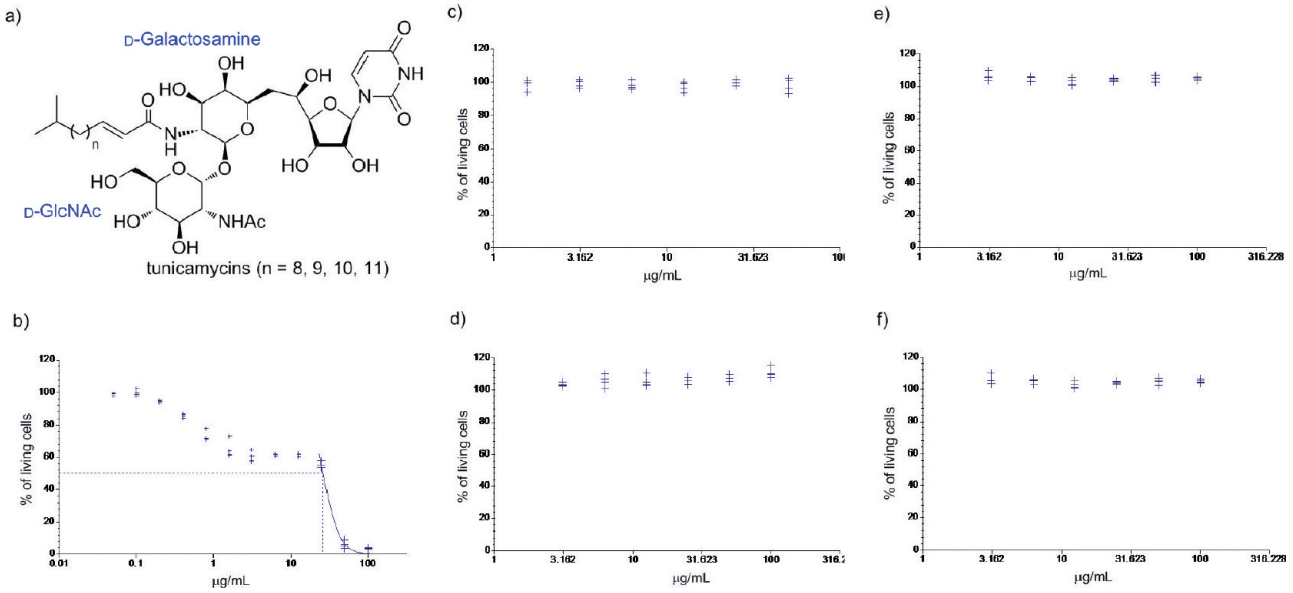
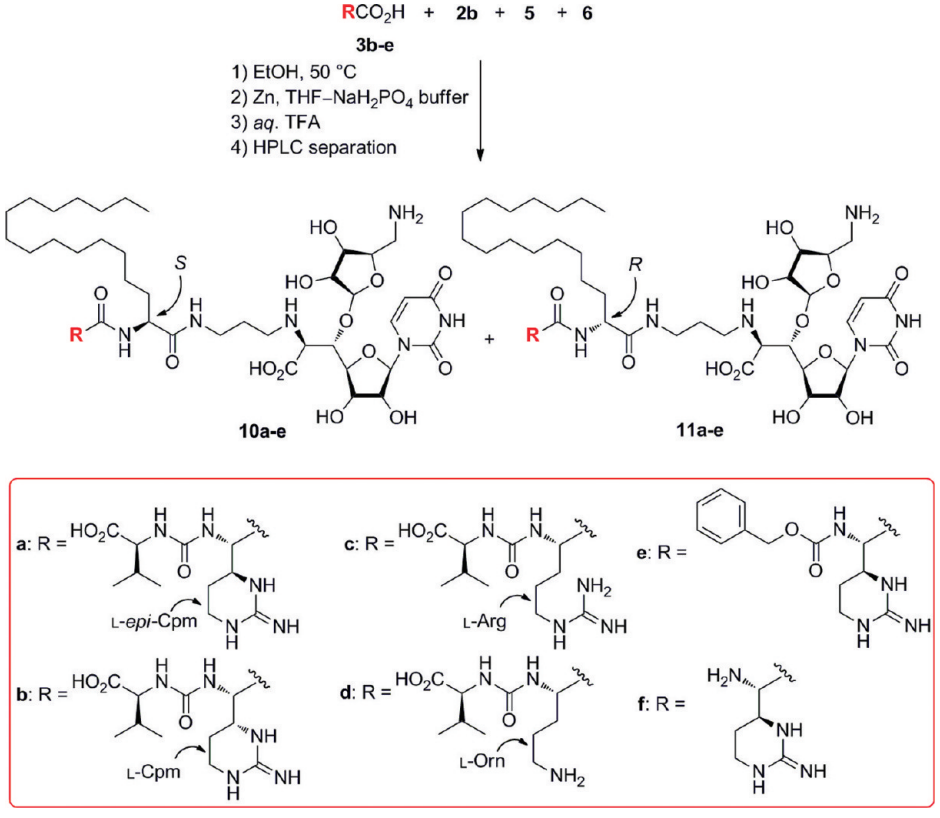


Figure 5. Cytotoxicity of selected lipophilic analogues against HepG2 cells. (a) Chemical structure of tunicamycins. HepG2 cells were seeded in 96-well tissue culture plates at a 1×10^4 cells/well. After 24 h, cells were treated with varying concentrations of (b) tunicamycin (a positive control), (c) **9b**, (d) **8c**, (e) **8d**, and (f) **8e** for 48 h. After the treatment, the cell viability was measured by WST-8 assay as the absorbance at 450 nm, and percentage inhibition in growth was calculated against that of cells treated without those compounds. Tunicamycin exhibited cytotoxicity with an IC_{50} of 26.8 μg/mL.

Scheme 2



substrate, C_{55}-P. However, they differ in the nature of the nucleotide substrate, which is UDP-MurNAc-pentapeptide and UDP-GlcNAc for MraY and WecA, respectively. Contrary to the MraY activity which is specific to bacteria, the reaction catalyzed by the WecA enzyme is also conserved in eukaryotic cells. Tunicamycins[30] (Figure 5) are known to be nonselective inhibitors of both MraY and WecA and show antibacterial activity.[31] Tunicamycins also inhibit the eukaryotic UDP-GlcNAc:dolichol-P GlcNAc-1-P transferase, which transfers *N*-acetylglucosamine 1-phosphate (GlcNAc-1-P) from UDP-GlcNAc to the polyisoprenoid acceptor dolichol phosphate to form GlcNAc-PP-dolichol,[32,33] and are not suitable candidates

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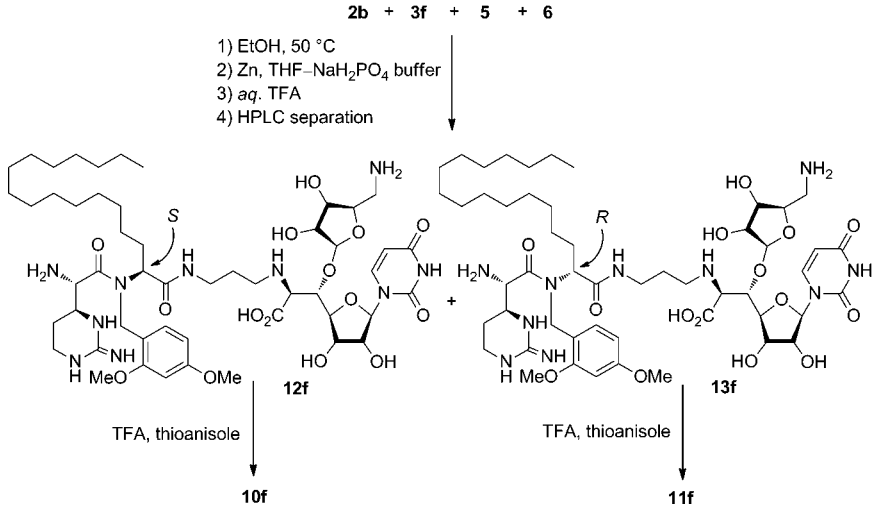
Scheme 3



as antibacterial drugs because of their cytotoxicity. Nonselective inhibition could be one of the factors for toxicity against host cells. In order to investigate the selectivity of **8b** and **9b** for the MraY enzyme, both compounds were tested on the purified WecA enzyme from *Thermatoga maritima*. The standard WecA assay was performed by quantifying the incorporation of [^{14}C]GlcNAc by WecA from UDP-[^{14}C]GlcNAc into C_{55}-PP-GlcNAc as previously described.[28,29] Residual activities and IC_{50} values were calculated with respect to a control assay without inhibitors. Our results showed that the observed activity of the compounds synthesized in this study was highly selective toward MraY, since these compounds revealed very weak inhibitions and IC_{50} values in the millimolar range (Table 1) when tested on the WecA paralogue. Thus, the inhibitory effects are 3−5 orders of magnitude lower than those observed in MraY. It is also suspected that the analogues **8** and **9** act as detergents, which, because of micelle formation, are sometimes cytotoxic. Accordingly, their cytotoxicity against human hepatocellular carcinoma (HepG2) cells was then evaluated (Figure 5). Tunicamycins were also tested as a positive control and showed cytotoxicity with an IC_{50} of 26.8 μg/mL. Under these conditions, all the compounds prepared in this study exhibited no cytotoxicity (IC_{50} > 100 μg/mL), the selected data of which are shown in Figure 5. In conjunction with the observed selectivity among the MraY paralogues, these results clearly indicate that **8** and **9** showed selective toxicity against bacterial strains. The observed high therapeutic index, together with the high selectivity against the MraY enzyme, is a desirable property for antibacterial agents, and analogues **8** and **9** are suitable candidates for further development.

Investigation of the Accessory Urea−Peptide Moiety. With these results in hands, we next investigated the impact of the accessory urea−peptide moiety on antibacterial activity with a pentadecyl group as the lipophilic side chain. Two sets of analogues were designed to address the structural requirement of the accessory peptide moiety of the MRYs. One of the structural features of the MRYs is the L-*epi*-capreomycidine (Cpm) contained in the accessory moiety. Thus, several mutated analogues, where the L-*epi*-Cpm was replaced by the L-Cpm, L-Arg, and L-ornithine (Orn), respectively, were first designed to investigate the role of the cyclic guanidine functionality (Figure 4, right). The carboxylic acid containing the L-Cpm **3b** was prepared as previously reported.[17] The carboxylic acids containing L-Arg **3c** or L-Orn **3d** were prepared by condensation of the isocyanate of L-Val-O^{t}Bu with either L-Arg or L-Orn (Scheme S2 of Supporting Information). As for the preparation of the *N*-protected L-*epi*-Cpm **3e** and **3f**, the synthetic route was improved as described previously.[34] Truncated analogues at the L-Val residue were also designed to observe the impact of the C-terminus of the MRYs on antibacterial activity. These analogues were also prepared by the U4CR by altering the carboxylic acid component (Scheme 2). With the carboxylic acid components **3b−f** in hand, we then conducted the U4CR assemblage in a manner similar to that for the preparation of **8** and **9**, and the desired mutated and truncated analogues **10** and **11** were obtained after deprotection (Zn, THF-NaH_2PO_4 buffer (pH 7), then 80% aqueous TFA) and HPLC separation of diastereomers (>95% purity). The newly formed stereogenic center was tentatively assigned as the same as that in **8** and **9**. Average overall yields were ∼30% over three steps. As for the deprotection of **10f** and **11f**, the 2,4-dimethoxybenzyl group at the newly formed carboxamide nitrogen atom was surprisingly tolerant, and only the *N*-2,4-dimethoxybenzyl derivatives **12f** and **13f** were obtained under typical deprotection conditions (80% aqueous TFA). After extensive efforts to remove the 2,4-dimethoxybenzyl group, it was found that using TFA and thioanisole, known as a push−pull system,[35] was effective, and the desired **10f** and **11f** were each obtained in 60% yield (Scheme 3).

The antibacterial activity of this series of compounds was evaluated, and the results are summarized in Table 3. Overall, the stereochemistry at the newly formed stereogenic center in the U4CR did not influence the antibacterial activity, and the diastereomers exhibited similar antibacterial potency within a factor of 2. All analogues were also active against drug-resistant bacteria such as MRSA or VRE as observed for **8** and **9**. Both of the L-Cpm and L-Arg mutated analogues **10b,c** and **11b,c** exhibited antibacterial activity to a range of Gram-positive bacteria with MICs of 1−4 μg/mL. These values are comparable to and, in some cases, twice as strong as those of the "natural"-type analogues **8b**. The results indicate that the stereochemistry or cyclic structure of the L-*epi*-Cpm residue found in MRYs is not important for antibacterial activity. Moreover, the activity of the L-Orn mutated analogues **10d** and **11d** was 2−8 μg/mL, namely, reduced by approximately 2-fold. Nonetheless, the analogues still showed potent antibacterial

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Table 3. Antibacterial Activity of Accessory Peptide Analogues

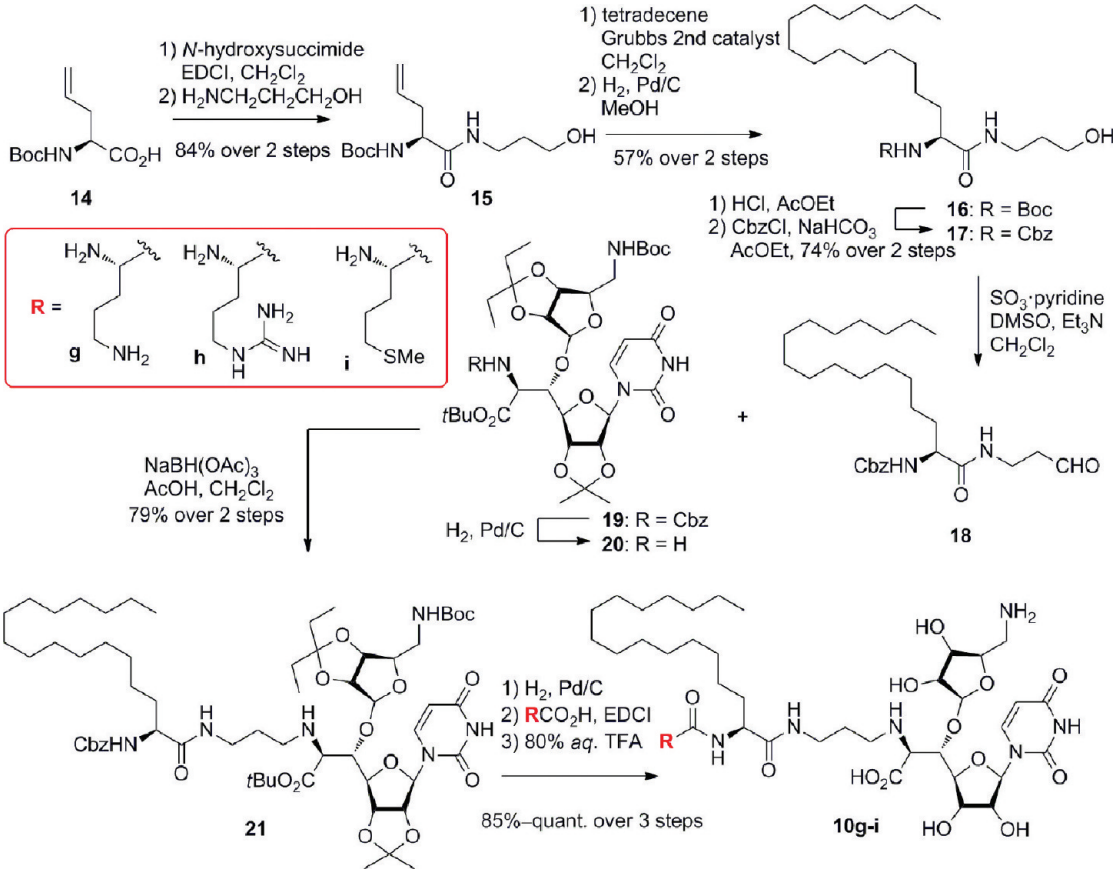
	MIC (μg/mL)[a]				
	S. aureus		*E. faecalis*		
compd	ATCC 29213 (MSSA)	SR3637 (MRSA)	ATCC 29212	SR7914 (VRE)	*E. facium* SR7917 (VRE)
10b	2	2	2	4	2
11b	4	2	2	4	4
10c	2	2	2	2	1
11c	4	4	4	4	2
10d	4	2	8	8	4
11d	4	8	8	8	8
10e	8	8	4	4	8
11e	8	8	4	4	4
10f	8	8	4	4	8
11f	8	8	4	8	8
10g	4	4	4	4	8
10h	4	4	4	4	4
10i	8	8	4	4	8
23	>64	>64	32	64	64
vancomycin	1	1	1	>64	>64

[a]MICs were determined by a microdilution broth method as recommended by the NCCLS with cation-adjusted Mueller−Hinton broth (CA-MHB). 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.

activity. Although the guanidine functionality is preferred, a less basic and simple amino functional group is tolerated enough to exhibit moderate antibacterial activity. From a medicinal chemical point of view, the presence of the L-*epi*-Cpm residue is unimportant. It is also noteworthy that the truncated analogues **10e**,**f** and **11e**,**f** exhibited potent antibacterial activity with MIC values of 4−8 μg/mL. The L-Val residue and/or the carboxylic acid would not contribute to the activity.

These results prompted us to examine the impact of the cyclic guanidine moiety of **10f** with the analogues replaced with L-Orn **10g**, L-Arg **10h**, or L-Met **10i** (Scheme 4). In this particular case, the stereochemistry of the pentadecylglycine moiety was set as the more active *S*-configuration, and the synthetic route was modified as shown in Scheme 4. *N*-Boc-allylglycine (**14**)[36] was condensed with aminoethanol to give the carboxamide **15** (*N*-hydroxysuccinimide, EDCI, CH_2Cl_2, then aminoethanol, 84% over two steps). The cross-metathesis of **15** with tetradecene catalyzed by Grubbs second generation catalyst[37] in refluxing CH_2Cl_2 followed by hydrogenation of the olefin provided the pentadecylglycin derivative **16** in 57% yield over two steps. After protecting group manipulation, the residual primary hydroxyl group of **17** was oxidized (IBX, MeCN) to give the aldehyde **18**. Reductive amination of **18** with **20** provided **21** ($NaBH(OAc)_3$, 79% over two steps from **19**). After removal of the Cbz group (H_2, Pd/C, MeOH), the resulting secondary amine was coupled with the suitably protected amino acids (EDCI, CH_2Cl_2) to afford **10g−i**, respectively, after global deprotection (80% aqueous TFA). The analogues **10g−i** exhibited similar or slightly improved antibacterial activity (Table 3). As indicated by the SAR study

Scheme 4



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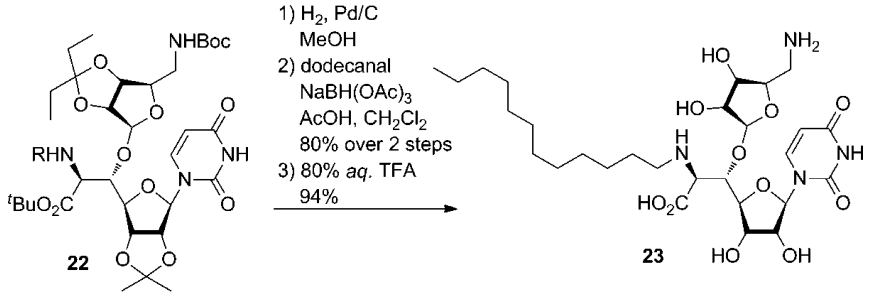
Scheme 5



of **10b–e** and **11b–e**, the cyclic guanidine of the L-*epi*-Cpm residue is not necessary for the antibacterial activity. In order to see the impact of the accessory motif, the analogue **23**, where the accessory motif was completely removed from **10** or **11**, was prepared from **22**[17] as shown in Scheme 5,[18] and its biological properties were compared. The truncated analogue **23** was found to be a much weaker MraY inhibitor with an IC_{50} of 5 μM, which was a 6- to 12-fold reduction of the inhibitory activity compared to **8b** and **9b** (Table 2). In addition to and apart from the binding pocket interacting with the 5′-*O*-aminoribosyl-5′-*C*-glycyluridine moiety, an additional binding site in MraY presumably exists, which recognizes the accessory urea–peptide motif. The antibacterial activity of **23** was greatly decreased with MICs ranging from 32 to 64 μg/mL, although **23** possessed a hydrophobic substituent (Table 3). These results clearly show that the partial structure of the urea–peptide accessory motif is a contributing factor in the interaction with the MraY enzyme to result in strong antibacterial activity. Two-directional optimization was effectively achieved by the SAR of several analogues resulting from the U4CR.

Proposed Binding Model of MRY with MraY. Two-directional optimization was effectively achieved by the SAR of several analogues, and these results indicate that (i) the lipophilic side chain is necessary to exhibit antibacterial activity and the synthetically accessible simple alkyl chain is preferred and (ii) the stereochemistry or cyclic structure of the L-*epi*-Cpm residue is not important and the C-terminal L-Val is not necessary. These initial SAR studies provide an insight into a binding model of the MRYs with the target MraY. The active conformation when the MRYs bind to the MraY is not yet known because the three-dimensional structure of this integral membrane protein has not been reported. However, the membrane topologies of the MraY transferases from both the Gram-negative *E. coli* and Gram-positive *S. aureus* have been established to generate a topological model.[38] The proposed model indicates that the MraY transferases contain 10 transmembrane domains, with the N- and C-termini exposed on the periplasmic face of the bacterial membrane. The topology and distribution are highly conserved in other MraY subfamily members with five cytoplasmic loops (CL) 1–5 and four periplasmic loops (PL) 1–4.[12,39,40] The five CLs form an active site. It was revealed that several Asp residues in the CL 2 that are invariant in the whole superfamily are important to the catalytic activity and the CL 5 is expected to be in proximity to the CL2 and responsible for the UDP-*N*-acetyl-D-hexosamine substrate specificity as a carbohydrate recognition (CR) domain.[39]

Our SAR study of the MRYs suggests a probable mechanism for the inhibition of the MraY. It is indicated that the ammonium group of the aminoribose moiety of this class of natural products is important to exhibit antibacterial activity.[19a,c,20] Previous active site mapping of *B. subtilis* MraY with 19 mutants strongly suggests that the transfer reaction would proceed via direct attack of the phosphate group of C_{55}-P substrate to the diphosphate moiety of UDP-MurNAc-peptapeptide and the Asp 98 in the CL2 should be involved in the deprotonation of the phosphate during the catalytic process.[14] Interaction of the Asp residues with the aminoribosyl moiety of MRYs might be probable, and this arrangement would block the binding of the UDP-MurNAc pentapeptide to the active site in a competitive fashion. On the other hand, the lipophilic side chain is not involved in the interaction with MraY because the analogues **8** and **9** were noncompetitive to the other substrate, C_{55}-P. Moreover, that the enzymatic inhibition by **23**, with a partial structure of the urea–peptide accessory motif, was largely decreased is an additional contributing factor in the interaction of the inhibitor with the MraY. This was supported by conformational calculations and comparison of UDP-MurNAc pentapeptide and the MRY as follows. A three-dimentional orientation of the MRY and UDP-MurNAc-pentapeptide was simulated because a solution structure of these molecules, which are long linear and rather flexible, was difficult to analyze by NMR. The selected low-energy conformers of **24** and **25** are shown in Figure 6a,b.[41] As indicated by previous studies, structural comparison of **24** and **25** revealed that the ammonium group of the aminoribose moiety of **25** was positioned near the diphosphate moiety of **24** (Figure 6c). This is indicative of the interaction of the aminoribose moiety of MRYs to the Asp residues in the CL2. The hydroxyl-Leu residue could be freely rotating making the position of the Val, *epi*-Cpm, and lipophilic side chain easily interconvertible. These moieties have a weaker contribution to enzymatic or antibacterial activity. On the other hand, the inner moiety of the urea–dipeptide motif (marked by a circle) of **25** is less flexible than the Val, *epi*-Cpm, and lipophilic side chain and superimposable on the MurNAc moiety of **24**. Since the MurNAc moiety is recognized by the CR domain in CL5, it is probable that the inner moiety of the urea–dipeptide motif interacts with the CR domain. The MraY CR domain contains a highly conserved motif of 13 amino acids (MAPIHHH-FELKGW for *E. coli*) for UDP-MurNAc-pentapeptide recognition. This motif is not found in any of the other subgroups within the transferase family. The WecA CR domain contains a different motif (RRxxxGxSPFSPDxxHIHH), which is selectively used for UDP-GlcNAc recognition.[37,38,40,42] This is supported by the high selectivity of **8** and **9** to the MraY observed in this study. However, the minimum structural determinant of the accessory motif found in the MRYs for recognition by the CR domain is not expected to be large

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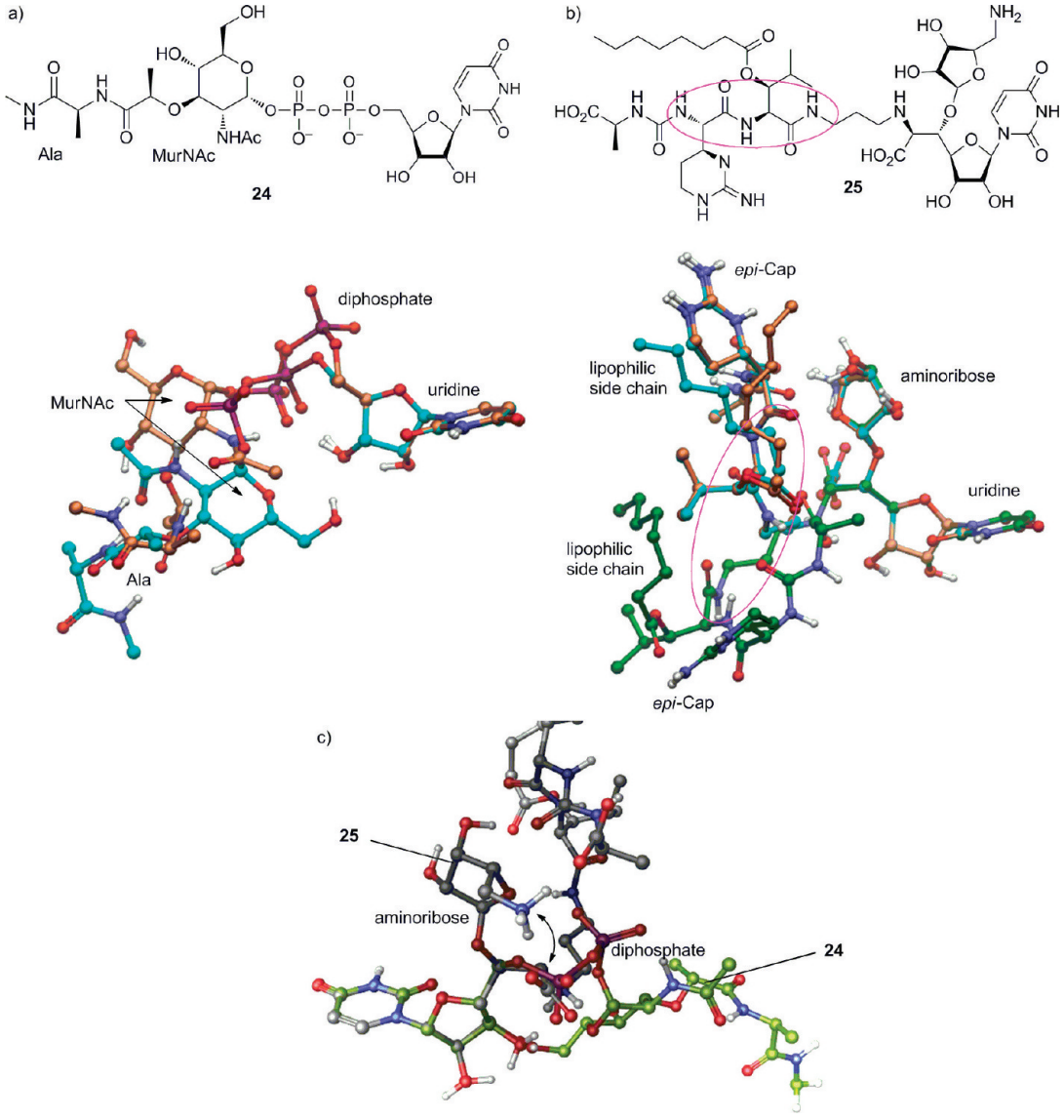

Figure 6. Predicted energy-minimum conformations of (a) UDP-MurNAc-Ala-amide (**24**) and (b) MRY analogue **25**. The lipophilic side chain and Val residue of the urea–peptide moiety were replaced with an octanoyl group and Ala residue, respectively, for the MRY, and the tetrapeptide was omitted for UDP-MurNAc pentapeptide to simplify the calculations. MacroModel program, version 9.0, was used for conformational search. Ionization status in H_2O under pH 7 ± 1 was predicted by the Epik program, and these structures were used for the following conformational analysis. Conformational searching was carried out using the Monte Carlo multiple minimum (MCMM) method (100 000 steps), followed by Polak–Ribiere conjugate gradient (PRCG) minimization with the OPLS 2005 force field. Water was chosen for a solvent with the GB/SA model. The other settings were used as default. The lowest 1000 conformers were clustered by multiple minimization. Representative conformers were superimposed by N-1, C-1′, and C-4′ atoms. (c) The representative conformer of **24** or **25** was merged.

considering the simplification at the urea–peptide moiety provided by the SAR of **10** and **11**. Of note is the presumed interaction of the urea–peptide motif with the CR domain. The CR domain contains several Lys and His residues and is highly basic with calculated isoelectric points (pI) ranging from 10–12.[31] The fact that the analogues **10e–g**, which lack the carboxylate functionality, retained biological activity indicates that the urea–peptide motif does not interact with the basic residues in the CR by a simple acid–base interaction. Rather, one of the possible binding modes of a partial structure of the urea–peptide is to interact with other residues such as the Phe, Ile, or Trp by a hydrophobic and/or a cation–π interaction or with the backbone of the CR domain by hydrogen bonding. The overall predicted binding mode of the MRYs to MraY is summarized in Figure 7. An interactive SAR study with both the MRY analogues and the MraY mutants at the CR domain would provide further insight into the precise mode of action of the MRYs and clarify the MraY "machinery". This proposed binding mode of the MRYs could be a guide to the rational design of selective inhibitors for MraY.

CONCLUSION

The systematic SAR study of the MRYs was investigated. The MRY analogues on both the lipophilic side chain and the urea–peptide moiety were synthesized via the U4CR assemblage. By virtue of the multicomponent assemblage at the late stage of the synthesis and despite the challenges this imposes, this approach was quite effective and has provided ready access to a range of analogues simply by altering the aldehyde or carboxylic acid component.

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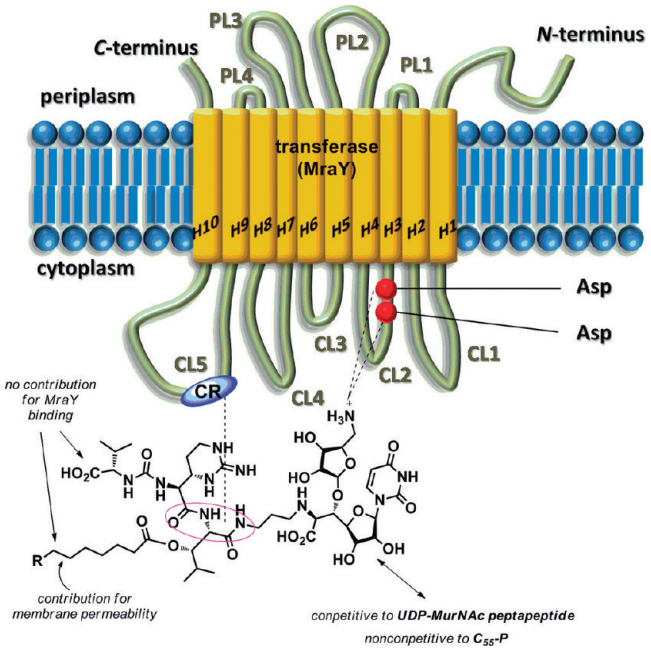

Figure 7. Schematic model for muraymycin inhibition of MraY transferase. The Asp residues in cytoplasmic loop (CL) 2 form ion or hydrogen bonding to muraymycins at aminoribose moiety, occupying the binding site for the UDP-MurNAc pentapeptide substrate. A partial urea−peptide motif is recognized by the carbohydrate recognition (CR) region.

The impact of the lipophilic substituent on antibacterial activity was very large, and analogues **8b−e** and **9b−e** exhibited good activity against a range of Gram-positive bacterial pathogens, including MRSA and VRE. Other biological properties of **8b−e** and **9b−e** were investigated, and it was found that these analogues are selective inhibitors of the MraY and are nontoxic to HepG2 cells. The SAR of the accessory urea−peptide moiety suggested that it could be simplified to a large extent. On the basis of these SAR studies, the binding mode of the MRYs was proposed. This could be a guide to the rational design of selective inhibitors for MraY, whose three-dimensional structure is not yet known. Since the analogues prepared in this study are effective against a range of bacterial pathogens, including MRSA and VRE, selective MraY inhibitors are promising candidates for antibacterial agents to treat drug-resistant bacteria.

Compared to synthetic drugs, many biologically relevant natural products possess large, complex, or labile chemical structures, which may restrict chemical modifications in a structure−activity relationship study. Therefore, it is important to pursue FOS, a strategy for the design of less structurally complex targets with comparable or superior activity that could be made in a practical manner. This study revealed that the molecular complexity of the MRYs could be significantly reduced (the molecular weight of **10f** is two-thirds that of **1a**) with comparable antibacterial activity. This is a good contrast to the tunicamycins, where the truncation of either the lipophilic side chain or the GluNAc moiety results in complete loss of biological activity.[30] We have also studied a rational and drastic simplification of the common molecular architecture of MRY congeners by decoding the role of the aminoribofuranose moieties, which is another important issue associated with the simplification of the MRYs.[19a] In summary, a drastic simplification of the chemical structure of the MRYs is feasible to establish a synthetic chemical approach based on rational drug design using FOS. The knowledge obtained from our SAR study of the MRYs would provide further direction toward the design of potent MraY inhibitors.

EXPERIMENTAL SECTION

General Experimental Methods. NMR spectra were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard unless otherwise noted. Coupling constants (J) were reported in herz (Hz). Abbreviations of multiplicity were as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Data were presented as follows: chemical shift (multiplicity, integration, coupling constant). Assignments were based on ^{1}H−^{1}H COSY, HMBC, and HMQC NMR spectra. Purity of all the compounds tested for biological evaluation was confirmed to be >95% by HPLC and ^{1}H NMR analyses.

Compounds 8b and 9b (General Procedure of the Preparation of 8 and 9 by the U4CR). Carboxylic acid **3a** (42.7 mg, 0.073 mmol), **2b** (89.8 mg, 0.37 mmol), and **5** (53.9 μL, 0.37 mmol) were dissolved in EtOH (1 mL), and the solution was concentrated in vacuo. The residue was coevaporated with EtOH three times. The residue and the isonitrile **6**[17] (56.9 mg, 0.073 mmol) in EtOH (1 mL) were concentrated in vacuo, and the resulting syrup was kept at room temperature for 48 h. The mixture was diluted with AcOEt, and the solution was washed with H_2O, saturated aqueous NaCl and dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (0.5 cm × 2 cm, 66% AcOEt−hexane) to afford the U4CR product (47.6 mg, 37%) as a white foam. The product (7.0 mg, 0.0040 mmol) in THF (1 mL) and 1 M aqueous NaH_2PO_4 (500 μL) was treated with Zn (4.3 mg, 0.080 mmol) for 40 h. After the resulting mixture was concentrated in vacuo, the residue was suspended in AcOEt. The insoluble portion was filtered off through a short silica gel pad, and the filtrate was concentrated in vacuo. The residue was treated with 80% aqueous TFA (2 mL) for 8 h, and the resulting mixture was concentrated in vacuo. The residue was purified by HPLC (YMC J'sphere ODS M80, 4.6 mm × 150 mm, 0.1% TFA, a linear gradient with MeOH from 73% to 80% - 20 min, 7.09 min - **9b**, 9.53 min for **8b**) to afford **8b** (1.5 mg, 35%) and **9b** (1.5 mg, 35%) as a white foam. Data for **8b**: $[\alpha]^{22}_D$ +294.3 (c 0.053, MeOH); ^{1}H NMR (D_2O containing CD_3OD, 500 MHz) δ 7.78 (br s, 1H, H-6), 5.91 (br s, 1H, H-5), 5.85 (br s, 1H, H-1′), 5.25 (br s, 1H, H-1″), 4.65 (br s, 1H, H-5′), 4.44 (br s, H, H-2′, H-2-*epi*-Cpm), 4.35 (m, 2H, H-3′ and 4′), 4.20 (m, 4H, H-2″, -3″, -4″, and C*H*CH$_2$(CH$_2$)$_{13}$CH$_3$), 4.09 (br s, 1H, H-2-Val), 3.01 (m, 2H, H-6′ and H-3-*epi*-Cpm), 3.37 (m, 4H, H-10′ and H-5-*epi*-Cpm), 3.23 (m, 4H, H-8′ and 5″), 2.17 (m, 1H, H-3-Val), 2.00 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.72 (m, 2H, −CHC*H*$_2$(CH$_2$)$_{13}$CH$_3$), 1.26 (m, 26H, −CHCH$_2$(C*H*$_2$)$_{13}$CH$_3$), 0.97 (m, 3H, H-4-Val), 0.93 (br s, 3H, H-4-Val), 0.86 (s, 3H, −CHCH$_2$(CH$_2$)$_{13}$C*H*$_3$); ^{13}C NMR (D_2O, 125 MHz) δ 177.2, 172.2, 166.6, 159.8, 154.9, 152.1, 143.2, 141.9, 109.8, 103.1, 92.6, 85.5, 80.4, 76.8, 75.8, 73.7, 72.8, 70.2, 64.9, 59.7, 56.7, 55.1, 47.6, 47.0, 43.4, 37.1, 32.8, 30.7, 30.6, 30.3, 26.6, 23.5, 20.0, 18.1, 14.7, 9.13; ESIMS-LR m/z 1071 [(M + H)$^+$]; ESIMS-HR calcd for $C_{48}H_{84}N_{11}O_{16}$ 1070.6098, found 1070.6069. Data for **9b**: $[\alpha]^{22}_D$ +297.8 (c 0.059, MeOH); ^{1}H NMR (D_2O, 600 MHz) δ 7.90 (br s, 1H, H-6), 6.04 (br s, 1H, H-5), 6.00 (br s, 1H, H-1′), 5.38 (br s, 1H, H-1″), 4.80 (br s, 1H, H-5′), 4.68 (br s, 1H, H-2′), 4.49 (m, 3H, H-3′, -4′, and H-2-*epi*-Cpm), 4.33 (m, 4H, H-2″, 3″, 4″, and C*H*CH$_2$(CH$_2$)$_{13}$CH$_3$), 4.16 (br s, 1H, H-2-Val), 4.00 (m, 2H, H-6′ and H-3-*epi*-Cpm), 3.52 (m, 4H, H-10′ and H-5-*epi*-Cpm), 3.37 (m, 4H, H-8′ and H-5″), 2.29 (m, 1H, H-3-Val), 2.10 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.80 (m, 2H, −CHC*H*$_2$(CH$_2$)$_{13}$CH$_3$), 1.37 (m, 26H, −CHCH$_2$(C*H*$_2$)$_{13}$CH$_3$), 1.08 (br s, 3H, H-4-Val), 1.03 (br s, 3H, H-4-Val), 0.98 (s, 3H, −CHCH$_2$(CH$_2$)$_{13}$C*H*$_3$); ^{13}C NMR (D_2O containing CD_3OD, 150 MHz) δ 177.8, 173.9, 167.2, 160.4, 155.5, 152.6, 143.8, 142.5, 110.4, 103.7, 93.2, 86.1, 80.6, 77.4, 76.4, 74.3, 73.4, 70.7, 65.5, 60.2, 57.3, 55.7, 48.2, 47.6, 43.9, 37.7, 33.4, 31.3, 31.2, 30.9, 27.2, 24.0, 20.6, 18.7, 15.3, 9.7; ESIMS-LR m/z 1070 [(M +

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H)$^+$]; ESIMS-HR calcd for $C_{48}H_{84}N_{11}O_{16}$ 1070.6098, found 1070.6073.

Compounds 8c and 9c. According to the general procedure, **8c** (5.1 mg, 27%) and **9c** (5.1 mg, 27%) were obtained as a white foam from **3** (17.4 mg, 0.03 mmol), **2c** (85.0 mg, 0.30 mmol), **5** (45.0 μL, 0.3 mmol), and **6** (23.1 mg, 0.03 mmol) after purification by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, 62% MeOH−H_2O, 8.6 min - **9c**, 14.0 min - **8c**). Data for **8c**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.56 (d, 2H, H-1′-biphenyl and H-6′-biphenyl, J = 8.0 Hz), 7.49 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.0 Hz), 7.39 (dd, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 7.5, 8.0 Hz), 7.28 (t, 2H, H-4′-biphenyl, J = 7.5 Hz), 7.22 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.0 Hz), 5.75 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 1H, H-1″), 4.56 (d, 1H, H-5′, $J_{5',4'}$ = 5.2 Hz), 4.31 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 7.4 Hz), 4.28 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.2 Hz), 4.23 (m, 2H, H-3′ and H-4′), 4.15 (m, 1H, H-2-8-(biphenyl-4-yl)heptylglycine), 4.12 (d, 1H, H-2-Val, $J_{2,3}$ = 4.6 Hz), 4.08−4.02 (m, 3H, H-2″, -3″, and -4″), 3.96 (s, 1H, H-6′), 3.87 (dd, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = 4.6, $J_{3,4}$ = 12.3 Hz), 3.40 (m, 1H, H-5a-*epi*-Cpm), 3.29 (m, 2H, H-10′a and H-5b-*epi*-Cpm), 3.18 (m, 4H, H-5″, H-8′a, and H-10′b), 3.04 (m, 1H, H-8′b), 2.62 (t, 2H, H-9-8-(biphenyl-4-yl)heptylglycine, J = 8.0 Hz), 2.18 (m, 1H, H-3-Val), 1.86 (m, 5H, H-9′, H-4-*epi*-Cpm and H-3a-8-(biphenyl-4-yl)heptylglycine), 1.64 (m, 3H, H-3b-8-(4-biphenylyl-4-yl)heptylglycine and H-4-8-(biphenyl-4-yl)heptylglycine), 1.34 (m, 8H, H-5, H-6, H-7, and H-8-8-(biphenyl-4-yl)heptylglycine), 0.98 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.94 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 176.4, 175.2, 172.5, 166.3, 166.0, 160.4, 155.6, 152.1, 143.3, 143.1, 142.4, 139.9, 130.1, 129.9, 129.8, 128.0, 127.8, 127.8, 110.2, 103.1, 93.9, 85.7, 80.4, 77.6, 76.4, 74.4, 73.9, 73.8, 71.1, 60.1, 59.2, 56.9, 55.7, 43.9, 37.5, 36.7, 36.4, 32.6, 32.4, 31.5, 30.7, 30.6, 30.4, 30.2, 27.2, 22.5, 19.8, 18.2; ESIMS-LR m/z 1110 [(M + H)$^+$]; ESIMS-HR calcd for $C_{52}H_{76}N_{11}O_{16}$ 1110.5466, found 1110.5465. Data for **9c**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.55 (d, 2H, H-1′ and 6′-biphenyl, J = 8.0 Hz), 7.49 (d, 2H, H-2 and 6-biphenyl, J = 8.0 Hz), 7.39 (dd, 2H, H-3′ and 5′-biphenyl, J = 7.5, 8.0 Hz), 7.28 (t, 2H, H-4′-biphenyl, J = 7.5 Hz), 7.22 (d, 2H, H-2 and 6-biphenyl, J = 8.0 Hz), 5.74 (d, 1H, H-1′, $J_{1',2'}$ = 2.8 Hz), 5.71 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.18 (s, 1H, H-1″), 4.58 (d, 1H, H-5′, $J_{5',4'}$ = 4.9 Hz), 4.43 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 5.5 Hz), 4.30 (dd, 1H, H-2′, $J_{2',1'}$ = 2.8, $J_{2',3'}$ = 5.5 Hz), 4.24 (m, 2H, H-3′ and H-4′), 4.16 (d, 2H, H-2-8-(biphenyl-4-yl)heptylglycine and H-2-Val, $J_{2,3}$ = 5.2 Hz), 4.08−4.00 (m, 3H, H-2″, -3″, and -4″), 3.93 (s, 1H, H-6′), 3.70 (dd, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = 5.5, $J_{3,4}$ = 13.6 Hz), 3.40 (m, 2H, H-5a-*epi*-Cpm), 3.29 (m, 2H, H-10′a), 3.18 (m, 4H, H-5″, 8′a, and H-10′b), 3.06 (m, 1H, H-8′b), 2.62 (t, 2H, H-9-8-(biphenyl-4-yl)heptylglycine, J = 8.1 Hz), 2.15 (m, 1H, H-3-Val), 1.93 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.69 (m, 3H, H-3-8-(biphenyl-4-yl)heptylglycine and H-4-8-(biphenyl-4-yl)heptylglycine), 1.34 (m, 8H, H-5, -6, -7, and H-8-8-(biphenyl-4-yl)heptylglycine), 0.95 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.91 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 189.2, 176.4, 173.1, 166.8, 161.0, 156.4, 152.9, 144.2, 143.9, 143.2, 140.6, 130.7, 130.6, 128.8, 128.6, 128.6, 111.0, 104.0, 98.5, 94.8, 93.6, 86.6, 81.2, 77.3, 75.1, 74.6, 72.0, 57.5, 56.7, 53.0, 44.7, 38.2, 37.8, 37.2, 33.5, 33.4, 32.6, 31.2, 31.0, 27.9, 23.1, 20.5, 18.8; ESIMS-LR m/z 1110 [(M + H)$^+$]; ESIMS-HR calcd for $C_{52}H_{76}N_{11}O_{16}$ 1110.5466, found 1110.5466.

Compounds 8d and 9d. According to the general procedure, **8d** (4.8 mg, 24%) and **9d** (4.8 mg, 24%) were obtained as a white foam from **3** (17.4 mg, 0.03 mmol), **2d** (84.6 mg, 0.30 mmol), **5** (45.0 μL, 0.3 mmol), and **6** (23.1 mg, 0.03 mmol) after purification by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, 60% MeOH−H_2O, 5.8 min - **9d**, 10.2 min - **8d**). Data for **8d**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.48 (m, 4H, H-3, 5, 2′ and H-6′-biphenyl), 7.24 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.6 Hz), 6.94 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 6.9 Hz), 5.76 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.16 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.27 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.2 Hz), 4.24 (m, 1H, H-2-*epi*-Cpm), 4.23 (dd, 1H, H-4′, $J_{4',5'}$ = 5.7, $J_{4',3'}$ = 9.8 Hz), 4.21 (m, 1H, H-4′), 4.19 (m, 1H, H-2-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 4.12 (d, 1H, H-2-Val, $J_{2,3}$ = 5.2 Hz), 4.06 (m, 1H, H-4″), 4.04 (m, 1H, H-3″), 4.02 (m, 1H, H-2″), 3.97 (t, 2H, $OCH_2CH_2CH_2CH_3$, J = 7.4 Hz), 3.92 (s, 1H, H-6′), 3.90 (m, 1H, H-3-*epi*-Cpm), 3.39 (m, 1H, H-10′a), 3.32 (m, 3H, H-5″a and H-5a-*epi*-Cpm), 3.22 (m, 1H, H-5″b), 3.16 (m, 2H, H-8′a and H-10′b), 3.04 (m, 1H, H-8′b), 2.75 (m, 1H, H-4a-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.64 (m, 1H, H-4b-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.18 (m, 2H, H-3-Val and H-3a-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.01 (m, 1H, H-3b-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 1.87 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.75 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.51 (m, 2H, $OCH_2CH_2CH_2CH_3$), 0.99 (d, 3H, H-4a-Val, $J_{4a,3}$ = 10.6 Hz), 0.96 (m, 3H, $OCH_2CH_2CH_2CH_3$), 0.95 (d, 3H, H-4b-Val, $J_{4b,3}$ = 8.8 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 175.8, 173.6, 161.3, 160.9, 156.3, 152.9, 144.2, 141.3, 140.8, 135.2, 130.8, 129.5, 128.4, 116.7, 111.1, 104.0, 94.6, 86.6, 81.3, 78.6, 77.3, 75.2, 74.5, 71.9, 69.6, 57.9, 56.0, 52.2, 48.7, 47.6, 44.7, 38.2, 37.6, 34.6, 33.7, 33.3, 32.3, 27.8, 23.4, 21.1, 20.6, 19.0, 15.0, 10.0; ESIMS-LR m/z 1111 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{74}N_{11}O_{17}$ 1111.5259, found 1111.5271. Data for **9d**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.62 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 7.46 (m, 4H, H-3, H-5, H-2′, and H-6′-bihenyl), 7.23 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.6 Hz), 6.94 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 9.2 Hz), 5.73 (d, 1H, H-1′, $J_{1',2'}$ = 2.8 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 5.17 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.45 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 8.6 Hz), 4.30 (dd, 1H, H-2′, $J_{2',1'}$ = 2.8, $J_{2',3'}$ = 5.2 Hz), 4.23 (m, 3H, H-3′, H-4′ and H-2-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 4.18 (d, 1H, H-2-Val, $J_{2,3}$ = 4.5 Hz), 4.04 (m, 3H, H-2″, H-3″ and H-4″), 3.99 (t, 2H, $OCH_2CH_2CH_2CH_3$, J = 6.9), 3.93 (s, 1H, H-6′), 3.75 (dd, 1H, H-3-*epi*-Cpm, $J_{3,4}$ = 5.7, $J_{3,2}$ = 8.6 Hz), 3.44 (m, 1H, H-5a-*epi*-Cpm), 3.36 (m, 1H, H-5b-*epi*-Cpm), 3.35 (m, 1H, H-10′a), 3.28 (m, 1H, H-10′b), 3.21−3.15 (m, 4H, H-8′a, 10′b, and H-5″), 3.06 (m, 1H, H-8′b), 2.78 (m, 1H, H-4a-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.66 (m, 1H, H-4b-4-(4′-butoxybiphenyl-4-yl)-ethylglycine), 2.16 (m, 1H, H-3-Val), 2.07 (m, 2H, H-3-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 1.96 (m, 2H, H-4-*epi*-Cpm), 1.88 (m, 2H, H-9′), 1.75 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.51 (m, 2H, $OCH_2CH_2CH_2CH_3$), 0.99 (m, 6H, H-4a-Val and $OCH_2CH_2CH_2CH_3$), 0.92 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.8 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 176.7, 176.1, 173.3, 166.8, 161.0, 160.9, 156.4, 152.9, 144.3, 141.2, 140.9, 135.3, 130.8, 129.6, 128.4, 116.7, 111.1, 104.0, 94.5, 86.6, 81.2, 78.6, 77.3, 75.0, 74.5, 72.0, 69.6, 60.5, 57.7, 56.1, 52.9, 48.0, 44.7, 38.2, 35.2, 33.5, 33.3, 32.6, 28.0, 23.2, 21.1, 20.5, 18.8, 15.0; ESIMS-LR m/z 1111 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{74}N_{11}O_{17}$ 1111.5259, found 1111.5272.

Compounds 10b and 11b. Compound **3b** (113 mg, 0.194 mmol), hexadecanal (46.6 mg, 0.194 mmol), and **5** (29.1 μL, 0.194 mmol) were dissolved in EtOH (1.0 mL), and the solution was concentrated in vacuo. The residue was coevaporated with EtOH (1.0 mL), and this was repeated 3 times. The residue and **6** (150 mg, 0.194 mmol) in EtOH (2.0 mL) were stirred at 50 °C for 12 h. Hexadecanal (46.6 mg, 0.194 mmol) and 2,4-dimethoxybenzylamine (29.1 μL, 0.194 mmol) were added to the solution, and the mixture was stirred further 18 h. The organic phase was diluted with AcOEt and washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, H_2O, saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2 cm × 10 cm, 40% AcOEt−hexane) to afford a mixture of U4CR products (114 mg, 37%) as a white foam. The products (114 mg, 0.065 mmol) in THF (1.6 mL) and 1 M aqueous NaH_2PO_4 (400 μL) were treated with Zn (204 mg, 3.25 mmol) for 12 h. After the resulting mixture was concentrated in vacuo, the residue was suspended in AcOEt. The insoluble was filtered off through a short silica gel pad, and the filtrate was concentrated in vacuo. The residue was treated with 80% aqueous TFA containing 5% Et_3SiH (2 mL) for 24 h. The solution was concentrated in vacuo, and the residue was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 70% to 75% MeOH−H_2O for 20 min, 10.9 min−**11b**, 15.0 min−**10b**) to afford lipophilic MRY derivative **10b** (33.0 mg, 46%) and **11b** (33.0 mg, 46%) as a white foam. Data for **10b**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.74 (d,

1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.73 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.18 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.44 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 5.7 Hz), 4.31 (dd, 1H, H-4′, $J_{4',3'}$ = 2.4, $J_{4',5'}$ = 5.7 Hz), 4.28 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.8 Hz), 4.23 (d, 1H, H-3′, $J_{3',2'}$ = 5.8 Hz), 4.18 (d, 1H, H-2-Val, $J_{2,3}$ = 4.6 Hz), 4.16 (m, 1H, $-CHCH_2(CH_2)_{13}CH_3$), 4.06 (2H, H-2″ and H-4″), 4.01 (s, 1H, H-3″), 3.99 (s, 1H, H-6′), 3.79 (dt, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = $J_{3,4a}$ = 5.7, $J_{3,4b}$ = 8.6 Hz), 3.43 (m, 1H, H-10′a), 3.32 (m, 3H, H-10′b and H-5-*epi*-Cpm), 3.25−3.18 (m, 3H, H-8′a and H-5″), 3.06 (m, 1H, H-8′b), 2.17 (dt, 1H, H-3-Va, $J_{3,2}$ = 4.6, $J_{3,4a}$ = $J_{3,4b}$ = 6.8 Hz), 2.05 (m, 1H, H-4a-*epi*-Cpm), 1.88 (m, 3H, H-9′ and H-4b-*epi*-Cpm), 1.73 (m, 2H, $-CHCH_2(CH_2)_{13}CH_3$), 1.26 (m, 26H, $-CHCH_2(CH_2)_{13}CH_3$), 0.97 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.92 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz), 0.87 (t, 3H, $-CHCH_2(CH_2)_{13}CH_3$, J = 7.2 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.7, 171.0, 170.2, 164.6, 161.3, 158.9, 154.7, 150.8, 142.1, 108.7, 101.8, 92.9, 84.3, 80.6, 78.9, 76.3, 75.1, 72.8, 72.6, 69.9, 63.5, 58.2, 55.6, 54.2, 50.9, 47.1, 42.7, 36.6, 35.3, 31.7, 31.6, 30.4, 29.4, 29.3, 29.1, 26.0, 25.8, 22.8, 22.4, 18.4, 16.5, 13.1; ESIMS-LR m/z 1068 [(M − H)$^-$]; ESIMS-HR calcd for $C_{48}H_{82}N_{11}O_{16}$ 1068.5947, found 1068.5956. Data for **11b**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.76 (d, 1H, H-1′, $J_{1',2'}$ = 2.9 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.18 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.2 Hz), 4.39 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 4.6 Hz), 4.29 (m, 2H, H-2′ and H-4′), 4.20 (m, 2H, H-3′ and $-CHCH_2(CH_2)_{13}CH_3$), 4.14 (d, 1H, H-2-Val, $J_{2,3}$ = 4.6 Hz), 4.07 (m, 2H, H-3″ and H-4″), 4.03 (s, 1H, H-2″), 3.94 (s, 1H, H-6′), 3.78 (dt, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = 4.6, $J_{3,4a}$ = $J_{3,4b}$ = 9.2 Hz), 3.42 (m, 2H, H-5-*epi*-Cpm), 3.29−3.18 (m, 5H, H-8′a, 10′, and H-5″), 3.04 (m, 1H, H-8′b), 2.17 (dt, 1H, H-3-Va, $J_{3,4a}$ = $J_{3,4b}$ = 6.9, $J_{3,2}$ = 12.0 Hz), 2.00 (m, 1H, H-4a-*epi*-Cpm), 1.91 (m, 1H, H-4b-*epi*-Cpm), 1.84 (m, 3H, H-9′a and $-CHCH_2(CH_2)_{13}CH_3$), 1.64 (m, 1H, H-9′b), 1.26 (m, 26H, $-CHCH_2(CH_2)_{13}CH_3$), 0.97 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.93 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz), 0.87 (t, 3H, $-CHCH_2(CH_2)_{13}CH_3$, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.8, 173.9, 170.9, 170.3, 164.7, 159.1, 154.6, 150.7, 142.0, 108.7, 101.8, 92.7, 84.1, 79.0, 76.3, 75.1, 74.5, 73.0 72.6, 69.9, 63.5, 58.4, 55.8, 54.0, 51.1, 45.3, 42.7, 36.7, 35.3, 36.7, 35.3, 31.7, 31.4, 30.3, 29.5, 29.3, 29.1, 29.0, 26.0, 25.8, 22.9, 22.4, 18.4, 16.7, 13.1; ESIMS-LR m/z 1068 [(M − H)$^-$]; ESIMS-HR calcd for $C_{48}H_{82}N_{11}O_{16}$ 1068.5947, found 1068.5957.

Compounds 10c and 11c. A solution of **3c** (32.2 mg, 0.05 mmol), hexadecanal (12.4 mg, 0.05 mmol), **5** (7.8 μL, 0.05 mmol), and ammonium chloride (4.0 mg, 0.075 mmol) in toluene (600 μL) were stirred at room temperature for 30 min. The isonitrile **6** (40 mg, 0.05 mmol) was added to the solution, and the suspension was stirred at 50 °C for 48 h. The organic phase was diluted with AcOEt and washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, H_2O, saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2 cm × 10 cm, 40% AcOEt−hexane) to afford a mixture of U4CR products (40 mg, 47%) as a white foam. The products (40 mg, 0.022 mmol) were treated with 80% aqueous TFA containing 5% Et_3SiH (2 mL) for 8 h. The solution was concentrated in vacuo, and the residue was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 70% to 75% MeOH−H_2O for 20 min, 10.1 min−**11c**, 14.0 min−**10c**) to afford lipophilic MRY derivative **10c** (7.5 mg, 32%) and **11c** (7.5 mg, 32%) as a white foam. Data for **10c**: $[\alpha]^{23}_D$ +1.59 (c 0.34, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ 7.60 (s, 1H, H-6), 5.73 (s, 1H, H-5), 5.67 (s, 1H, H-1′), 5.08 (s, 1H, H-1″), 4.49 (s, 1H, H-5′), 4.25−3.85 (m, 10H, H-2-Val, $-CHCH_2(CH_2)_{13}CH_3$, 2′, 3′, 4′, 6′, 2″, 3″, and H-4″), 1.99 (s, 1H, H-3-Va), 1.68−1.53 (m, 8H, 9′a, $-CHCH_2(CH_2)_{13}CH_3$, 3-Arg and H-4-Arg), 1.13 (m, 26H, $-CHCH_2(CH_2)_{13}CH_3$), 0.70 (m, 9H, H-4a-Val and $-CHCH_2(CH_2)_{13}CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 177.8, 177.0, 167.6, 162.2, 160.2, 153.7, 145.2, 111.9, 104.7, 95.7, 87.3, 82.0, 79.5, 78.0, 75.8, 75.3, 72.7, 57.1, 56.6, 48.6, 45.5, 43.5, 38.3, 34.6, 34.1, 33.4, 32.3, 32.3, 32.2, 32.1, 32.0, 32.0, 31.9, 28.6, 27.6, 25.2, 21.3, 19.6, 16.0; ESIMS-LR m/z 1072 [(M + H)$^+$]; ESIMS-HR calcd for $C_{48}H_{86}N_{11}O_{16}$ 1072.6249, found 1072.6264. Data for **11c**: $[\alpha]^{23}_D$ +4.34 (c 0.34, MeOH); ^{1}H NMR (D_2O, 600 MHz) δ 7.54 (s, 1H, H-6), 5.63 (s, 1H, H-5), 5.58 (s, 1H, H-1′), 5.03 (s, 1H, H-1″), 4.44 (s, 1H, H-5′), 4.11−3.83 (m, 10H, H-2-Val, H-2-Val, $-CHCH_2(CH_2)_{13}CH_3$, 2′, 3′, 4′, 6′, 2″, 3″, and H-4″), 1.98 (s, 1H, H-3-Va), 1.73−1.49 (m, 8H, H-9′a, $-CHCH_2(CH_2)_{13}CH_3$, 3-Arg, and H-4-Arg), 1.12 (m, 26H, $-CHCH_2(CH_2)_{13}CH_3$), 0.72 (m, 9H, H-4a-Val and $-CHCH_2(CH_2)_{13}CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 177.1, 167.7, 162.0, 160.3, 160.2, 153.7, 145.0, 112.0, 104.7, 95.5, 87.2, 82.0, 79.4, 78.0, 75.9, 75.2, 72.6, 61.4, 56.8, 49.4, 48.2, 45.4, 43.5, 38.2, 34.6, 34.0, 33.2, 32.3, 32.2, 32.2, 32.1, 32.0, 31.8, 28.8, 28.6, 27.8, 25.2, 21.3, 19.7, 16.0; ESIMS-LR m/z 1072 [(M + H)$^+$]; ESIMS-HR calcd for $C_{48}H_{86}N_{11}O_{16}$ 1072.6249, found 1072.6259.

Compounds 10d and 11d. A solution of **3d** (56.0 mg, 0.13 mmol), hexadecanal (31.2 mg, 0.13 mmol), **5** (19.5 μL, 0.13 mmol), and ammonium chloride (20 mg, 0.39 mmol) were suspended in toluene (2.0 mL), and the suspension was concentrated in vacuo. The residue was coevaporated with EtOH (1.0 mL), and this was repeated 3 times. The residue and **6** (100 mg, 0.13 mmol) in toluene (2.0 mL) were stirred at 50 °C for 12 h. Hexadecanal (31.2 mg, 0.13 mmol) and **5** (19.5 μL, 0.13 mmol) was added to the solution, and the mixture was stirred a further 36 h. The organic phase was diluted with AcOEt and washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, H_2O, saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2 cm × 10 cm, 40% AcOEt−hexane) to afford a mixture of U4CR products (119 mg, 56%) as a white foam. The products (119 mg, 0.074 mmol) were treated with 80% aqueousTFA containing 5% Et_3SiH (2 mL) for 8 h. The solution was concentrated in vacuo, and the residue was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 70% to 75% MeOH−H_2O for 20 min, 11.3 min−**11d**, 15.3 min−**10d**) to afford lipophilic MRY derivative **10d** (33.0 mg, 45%) and **11d** (33.0 mg, 45%) as a white foam. Data for **10d**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.8 Hz), 5.76 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.8 Hz), 5.17 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 4.0 Hz), 4.26 (m, 2H, H-2′ and H-4′), 4.22 (m, 1H, H-3′), 4.17 (m, 2H, H-2-Orn and $-CHCH_2(CH_2)_{13}CH_3$), 4.11 (d, 1H, H-2-Val, $J_{2,3}$ = 4.6 Hz), 4.07 (m, 2H, H-2″ and H-4″), 4.04 (m, 2H, H-6′ and H-3″), 3.33 (m, 1H, H-10′a), 3.24 (m, 4H, H-10′b, 8′a, and H-5″), 3.07 (m, 1H, H-8′b), 2.95 (t, 2H, H-5-Orn), 2.15 (dt, 1H, H-3-Va, $J_{3,2}$ = 4.6, $J_{3,4a}$ = $J_{3,4b}$ = 6.9 Hz), 1.86 (m, 5H, H-9′a, Orn, and H-4-Orn), 1.69 (m, 3H, H-9′b and $-CHCH_2(CH_2)_{13}CH_3$), 1.26 (m, 26H, $-CHCH_2(CH_2)_{13}CH_3$), 0.96 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.93 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz), 0.87 (t, 3H, $-CHCH_2(CH_2)_{13}CH_3$, J = 7.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.8, 174.3, 173.9, 170.0, 164.7, 159.1, 150.7, 142.1, 109.0, 101.7, 92.8, 84.1, 79.1, 76.3, 75.0, 73.0, 72.4, 69.8, 62.9, 58.4, 54.0, 53.3, 45.2, 42.7, 38.9, 35.2, 31.7, 31.3, 30.4, 29.5, 29.3, 29.2, 29.1, 29.0, 25.8, 23.7, 22.4, 18.4, 16.7, 13.1; ESIMS-LR m/z 1028 [(M − H)$^-$]; ESIMS-HR calcd for $C_{47}H_{82}N_9O_{16}$ 1028.5885, Ffound 1028.5903. Data for **11d**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 5.74 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.72 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 5.17 (s, 1H, H-1″), 4.58 (d, 1H, H-5′, $J_{5',4'}$ = 5.2 Hz), 4.31 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.8 Hz), 4.27 (d, 1H, H-4′, $J_{4',5'}$ = 5.2 Hz), 4.23 (d, 2H, H-3′, $J_{3',2'}$ = 5.8 Hz), 4.20 (m, 1H, H-2-Orn), 4.17 (d, 2H, H-2-Val and $-CHCH_2(CH_2)_{13}CH_3$), 4.06 (m, 2H, H-3″ and H-4″), 4.03 (m, 2H, H-6′ and H-2″), 3.29 (m, 5H, H-8′a, 10′, and H-5″), 3.06 (m, 1H, H-8′b), 2.96 (t, 2H, H-5-Orn), 2.16 (dt, 1H, H-3-Va, $J_{3,2}$ = 5.2, $J_{3,4a}$ = $J_{3,4b}$ = 6.9, Hz), 1.86 (m, 3H, H-9′ and H-4a-Orn), 1.74 (m, 5H, H-4b, 3-Orn, and $-CHCH_2(CH_2)_{13}CH_3$), 1.26 (m, 26H, $-CHCH_2(CH_2)_{13}CH_3$), 0.96 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.92 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz), 0.87 (t, 3H, $-CHCH_2(CH_2)_{13}CH_3$, J = 7.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.6, 174.2, 173.8, 170.0, 164.7, 159.1, 150.8, 142.3, 108.9, 101.8, 93.0, 84.1, 79.0, 76.4, 75.0, 72.8, 72.5, 69.9, 63.0, 58.2, 54.2, 53.3, 45.6, 42.7, 38.9, 35.4, 31.7, 31.4, 30.6, 29.5, 29.4, 29.3, 29.1, 29.1, 25.8, 23.5, 22.4, 18.4, 16.6, 13.1; ESIMS-LR m/z 1028 [(M − H)$^-$]; ESIMS-HR calcd for $C_{47}H_{82}N_9O_{16}$ 1028.5885, found 1028.5904.

Compounds 10e and 11e. A solution of **3e** (15.3 mg, 0.3 mmol), hexadecanal (71.3 mg, 3.0 mmol), and **5** (44.6 μL, 3.0 mmol) in toluene (2.0 mL) was stirred at room temperature for 30 min. The isonitrile **6** (22.8 mg, 0.03 mmol) was added to the solution and stirred

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at 50 °C for a week. The reaction mixture was partitioned between AcOEt and 1 M aqueous HCl, and the organic phase was washed with saturated aqueous $NaHCO_3$, H_2O, saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2 cm × 6 cm, 40% AcOEt−hexane) to afford a mixture of U4CR products (16 mg, 31%) as a white foam. The products (16 mg, 0.009 mmol) in THF (2.0 mL) and 1 M aqueous NaH_2PO_4 (500 μL) were treated with Zn (59.8 mg, 0.92 mmol) for 48 h. After the resulting mixture was concentrated in vacuo, the residue was suspended in AcOEt. The insoluble portion was filtered off through a short silica gel pad, and the filtrate was concentrated in vacuo. The residue was treated with 80% aqueous TFA for 18 h. The solution was concentrated in vacuo, and the residue was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 70% to 85% MeOH−H_2O for 20 min, 13.2 min−**11e**, 15.7 min−**10e**) to afford lipophilic MRY derivative **10e** (2.2 mg, 23%) and **11e** (2.2 mg, 23%) as a white foam. Data for **10e**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.60 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.33 (m, 5H, phenyl), 5.74 (s, 1H, H-1′), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 1H, H-1″), 5.10 (d, 2H, CH_2Ph, J = 12.8 Hz), 4.55 (d, 1H, H-5′, $J_{5',4'}$ = 4.0 Hz), 4.33 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 8.6 Hz), 4.25 (m, 3H, H-2′, -3′, and -4′), 4.15 (dd, 1H, −CHCH_2(CH_2)$_{13}$CH_3, J = 5.2, J = 10.6 Hz), 4.07 (m, 1H, H-4″), 4.02 (m, 2H, H-2′ and H-3″), 3.87 (s, 1H, H-6′), 3.75 (m, 1H, H-3-*epi*-Cpm), 3.40 (m, 2H, H-5-*epi*-Cpm), 3.29 (m, 3H, H-5″a and H-10′a), 3.19 (m, 2H, H-10′b and H-8′a), 3.07 (m, 1H, H-8′b), 1.93 (m, 4H, H-9′ and −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.71 (m, 2H, H-4-*epi*-Cpm), 1.35 (m, 26H, −CHCH$_2$(CH_2)$_{13}$CH$_3$), 0.86 (t, 3H, −CHCH$_2$(CH$_2$)$_{13}$CH_3, J = 6.9 Hz); ESIMS-LR m/z 1061 [(M + H)$^+$]; ESIMS-HR calcd for $C_{50}H_{81}N_{10}O_{15}$ 1061.5880, found 1061.5886. Data for **11e**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.57 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.34 (m, 5H, phenyl), 5.74 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.69 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 2H, H-1″ and CH_2Ph), 5.10 (d, 1H, CH_2Ph, J = 12.6 Hz), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.34 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 7.4 Hz), 4.24 (dd, 1H, H-2′, $J_{2'1'}$ = 2.3, $J_{2',3'}$ = 5.2 Hz), 4.22 (dd, 1H, H-4′, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 5.7 Hz), 4.18 (dd, 1H, H-3′, $J_{3',2'}$ = 5.1, $J_{3',4'}$ = 5.1 Hz), 4.13 (dd, 1H, −CHCH$_2$(CH$_2$)$_{13}$CH$_3$, J = 5.5, J = 8.3 Hz), 4.07 (dd, 1H, H-4″, $J_{4'',3''}$ = 2.8, $J_{4'',5''}$ = 6.3 Hz), 4.05 (d, 1H, H-2″, $J_{2'',3''}$ = 4.0 Hz), 4.01 (dd, 1H, H-3″, $J_{3'',4''}$ = 2.8, $J_{3'',2''}$ = 4.0 Hz), 3.87 (m, 2H, H-6′ and H-3-*epi*-Cpm), 3.48 (m, 1H, H-5a-*epi*-Cpm), 3.39 (m, 1H, H-5b-*epi*-Cpm), 3.30 (m, 2H, H-10′a and H-5″a), 3.23 (m, 1H, H-10′b), 3.14 (m, 2H, H-8′a and H-5″b), 3.04 (m, 1H, H-8′b), 1.91 (m, 3H, H-9′a and H-4-*epi*-Cpm), 1.80 (m, 2H, H-9′b and −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.26 (m, 26H, −CHCH$_2$(CH_2)$_{13}$CH$_3$), 0.88 (t, 3H, −CHCH$_2$(CH$_2$)$_{13}$CH_3, J = 6.8 Hz); ESIMS-LR m/z 1061 [(M + H)$^+$]; ESIMS-HR calcd for $C_{50}H_{81}N_{10}O_{15}$ 1061.5877, found 1061.5886.

Compounds 12f and 13f. A solution of **3f** (20.0 mg, 0.042 mmol), hexadecanal (99.6 mg, 0.42 mmol), and **5** (62.3 μL, 0.42 mmol) in EtOH (2.0 mL) was stirred at room temperature for 30 min. The isonitrile **6** (32.0 mg, 0.042 mmol) was added to the solution and stirred at 50 °C for 24 h. The reaction mixture was partitioned between AcOEt and 1 M aqueous HCl, and the organic phase was washed with saturated aqueousNaHCO$_3$, H_2O, saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2 cm × 6 cm, 40% AcOEt−hexane) to afford a mixture of U4CR products (18 mg, 26%) as a white foam. The products (18 mg, 0.011 mmol) in THF (2.0 mL) and 1 M aqueous NaH_2PO_4 (500 μL) was treated with Zn (74.8 mg, 1.15 mmol) for 48 h. After the resulting mixture was concentrated in vacuo, the residue was suspended in AcOEt. The insoluble was filtered off through a short silica gel pad, and the filtrate was concentrated in vacuo. The residue was treated with 80% aqueous TFA for 18 h. The solution was concentrated in vacuo, and the residue was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, 71% MeOH−H_2O for 20 min, 16.7 min−**13f**, 18.5 min−**12f**) to afford lipophilic MRY derivative **12f** (3.3 mg, 29%) and **13f** (3.3 mg, 29%) as a white foam. Data for **12f**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.16 (d, 1H, aromatic, J = 8.6 Hz), 6.60 (s, 1H, aromatic), 6.56 (d, 1H, aromatic, J = 8.6 Hz), 5.72 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.69 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 1H, H-1″), 4.80 (d, 1H, H-5′, $J_{5',4'}$ = 4.5 Hz), 4.70 (d, 1H, CH_2Ph, J = 16.0 Hz), 4.56 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 4.1 Hz), 4.50 (d, 1H, CH_2Ph, J = 16.0 Hz), 4.30 (m, 2H, H-2′ and −CHCH$_2$(CH$_2$)$_{13}$CH$_3$, $J_{2,3a}$ = 5.2, $J_{2,3b}$ = 10.6 Hz), 4.22 (m, 2H, H-3′ and 4′), 4.17 (m, 1H, H-3-*epi*-Cpm), 4.06 (m, 1H, H-3″), 4.04 (m, 1H, H-4″), 4.01 (t, 1H, H-2″, J = 2.3 Hz), 3.88 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.42 (m, 1H, H-10′a), 3.38 (m, 1H, H-5a-*epi*-Cpm), 3.29 (m, 3H, H-5″a and H-5b-*epi*-Cpm), 3.25 (m, 2H, H-5″b and H-10′b), 3.16 (m, 1H, H-8′a), 3.03 (m, 1H, H-8′b), 1.99 (m, 2H, H-4-*epi*-Cpm), 1.89 (m, 1H, H-9′a), 1.82 (m, 1H, H-9′b), 1.79 (m, 2H, −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.30 (m, 26H, −CHCH$_2$(CH_2)$_{13}$CH$_3$), 0.88 (m, 3H, −CHCH$_2$(CH$_2$)$_{13}$CH_3, J = 6.9 Hz); ESIMS-LR m/z 1077 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{85}N_{10}O_{15}$ 1077.6190, found 1077.6182. Data for **13f**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.66 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 7.21 (d, 1H, aromatic, J = 8.6 Hz), 6.58 (s, 1H, aromatic), 6.54 (d, 1H, aromatic, J = 8.6 Hz), 5.73 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.72 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.16 (s, 1H, H-1″), 4.95 (m, 1H, H-2-*epi*-Cpm), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 4.6 Hz), 4.28 (m, 1H, H-2′), 4.22 (m, 2H, H-3′ and H-4′), 4.17 (m, 1H, CH_2Ph), 4.12 (m, 1H, H-3-*epi*-Cpm), 4.07 (m, 1H, H-4″), 4.04 (m, 2H, H-3″ and CH_2Ph), 4.01 (m, 1H, H-2″), 3.92 (m, 2H, H-6′ and −CHCH$_2$(CH$_2$)$_{13}$CH$_3$), 3.87 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.45 (m, 3H, H-10′a and H-5a-*epi*-Cpm), 3.29 (m, 1H, H-10′b), 3.20 (m, 2H, H-5″), 3.07 (m, 2H, H-8′), 2.13 (m, 1H, H-4a-*epi*-Cpm), 2.02 (m, 1H, H-4b-*epi*-Cpm), 1.86 (m, 2H, H-9′), 1.78 (m, 2H, −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.27−0.99 (m, 26H, −CHCH$_2$(CH_2)$_{13}$CH$_3$), 0.88 (t, 3H, −CHCH$_2$(CH$_2$)$_{13}$CH_3, J = 6.8 Hz); ESIMS-LR m/z 1077 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{85}N_{10}O_{15}$ 1077.6190, found 1077.6184.

Compound 10f. A solution of **12f** (3.3 mg, 0.0031 mmol) and thioanisole (100 μL, 0.085 mmol) was treated with 80% aqueous TFA for 48 h. The solution was concentrated in vacuo. The residue was diluted with H_2O, and the aqueous phase was washed with AcOEt. The solution was concentrated in vacuo. The residued was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, 71% MeOH−H_2O for 20 min, 6.3 min) to afford lipophilic MRY derivative **10f** (1.8 mg, 60%) as a white foam. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.74 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.72 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 4.6 Hz), 4.29 (m, 1H, H-2′), 4.23 (m, 2H, H-3′ and 4′), 4.19 (dd, 1H, −CHCH$_2$(CH$_2$)$_{13}$CH$_3$, J = 5.8, 8.6 Hz), 4.07 (m, 1H, H-4″), 4.04 (m, 1H, H-3″), 4.01 (m, 2H, H-2″ and H-2-*epi*-Cpm), 3.89 (m, 2H, H-6′ and H-3-*epi*-Cpm), 3.48 (m, 1H, H-10′a), 3.42 (m, 2H, H-5-*epi*-Cpm), 3.19 (m, 2H, H-5″), 3.15 (m, 2H, H-8′), 3.11 (m, 1H, H-10′b), 2.12 (m, 1H, H-4a-*epi*-Cpm), 1.97 (m, 1H, H-4b-*epi*-Cpm), 1.90 (m, 2H, H-9′), 1.73 (m, 2H, −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.30 (m, 26H, −CHCH$_2$(CH_2)$_{13}$CH$_3$), 0.88 (t, 3H, −CHCH$_2$(CH$_2$)$_{13}$CH_3, J = 6.3 Hz); ESIMS-LR m/z 927 [(M + H)$^+$]; ESIMS-HR calcd for $C_{42}H_{75}N_{10}O_{13}$ 927.5510, found 927.5517.

Compound 11f. A solution of **13f** (3.3 mg, 0.0031 mmol) and thioanisole (100 μL, 0.085 mmol) was treated with 80% aqueous TFA for 48 h. The solution was concentrated in vacuo. The residue was diluted with H_2O, and the aqueous phase was washed with AcOEt. The solution was concentrated in vacuo. The residue was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, 71% MeOH−H_2O for 20 min, 6.1 min) to afford lipophilic MRY derivative **11f** (1.8 mg, 60%) as a white foam. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.74 (d, 1H, H-1′, $J_{1',2'}$ = 2.9 Hz), 5.71 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.19 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 4.6 Hz), 4.29 (m, 1H, H-2′), 4.23 (m, 2H, H-3′ and -4′), 4.18 (d, 1H, H-2-*epi*-Cpm, J = 4.6 Hz), 4.12 (dd, 1H, −CHCH$_2$(CH$_2$)$_{13}$CH$_3$, J = 6.8, 8.4 Hz), 4.07 (m, 1H, H-3″), 4.05 (m, 1H, H-2″), 4.00 (m, 2H, H-4″ and H-3-*epi*-Cpm), 3.85 (s, 1H, H-6′), 3.48 (m, 2H, H-10′), 3.32 (m, 2H, H-5-*epi*-Cpm), 3.28 (m, 2H, H-5″), 3.11 (m, 2H, H-8′), 2.01 (m, 2H, H-9′a and H-4a-*epi*-Cpm), 1.90 (m, 1H, H-4b-*epi*-Cpm), 1.81 (m, 1H, H-9′b), 1.74 (m, 1H, −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.69 (m, 1H, −CHCH_2(CH$_2$)$_{13}$CH$_3$), 1.29 (m, 26H, −CHCH$_2$(CH_2)$_{13}$CH$_3$), 0.88 (t, 3H, −CHCH$_2$(CH$_2$)$_{13}$CH_3, J = 6.8 Hz); ESIMS-LR m/z 927 [(M + H)$^+$]; ESIMS-HR calcd for $C_{42}H_{75}N_{10}O_{13}$ 927.5510, found 927.5519.

(2*S*)-2-*tert*-Butoxycarbonylamino-*N*′-3-hydroxylpropylpent-4-enamide (15). A mixture of **14**[41] (3.8 g, 17.5 mmol) and *N*-hydroxysuccinimide (3.0 g, 26.2 mmol) in CH_2Cl_2 (100 mL) and THF (50 mL) at 0 °C was treated with EDCI (5.0 g, 26.2 mmol), and the mixture was stirred at the same temperature for 12 h. The reaction was quenched with H_2O, and the mixture was partitioned between AcOEt and H_2O. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue in CH_2Cl_2 (100 mL) was treated with 3-aminopropanol (2.0 mL, 26.2 mmol) at 0 °C and stirred at room temperature for 8 h. The resulting mixture was partitioned between AcOEt and H_2O. The organic phase was washed with H_2O (twice), saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5 cm × 10 cm, 40% AcOEt−hexane) to afford **15** as a colorless syrup (4.0 g, 84% over two steps). $[\alpha]^{21}_D$ −5.94 (*c* 2.41, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.97 (br s, 1H, NH-1′), 5.69 (m, 1H, H-4), 5.24 (br d, 1H, NH-Boc, $J_{NH,2}$ = 7.5 Hz), 5.11 (dd, 1H, H-5a, $J_{5a,5b}$ = 3.8, $J_{5a,4}$ = 14.5 Hz), 5.09 (dd, 1H, H-5b, $J_{5b,5a}$ = 3.8, $J_{5b,4}$ = 5.2 Hz), 4.12 (m, 1H, H-2), 3.58 (m, 2H, H-3′), 3.36 (m, 2H, H-1′), 2.48 (dt, 1H, H-3a, *J* = 6.3, 14.3 Hz), 2.42 (dt, 1H, H-3b, *J* = 7.6, 14.3 Hz), 1.65 (t, 2H, H-2′, *J* = 5.3 Hz), 1.40 (s, 9H, ^{t}Bu); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.8, 155.8, 133.1,119.0, 80.3, 59.3, 54.1, 37.0, 36.3, 32.2, 28.4; ESIMS-LR *m*/*z* 295 [(M + H)$^+$]; ESIMS-HR calcd for $C_{13}H_{24}N_2O_4Na$ 295.1628, found 295.1629.

(2*S*)-2-*tert*-Butoxycarbonylamino-*N*′-3-hydroxylpropylhexadecanamide (16). A mixture of tetradecene (37 mL, 147 mmol) and **15** (4.3 g, 14.7 mmol) and Grubbs catalyst second generation (623 mg, 0.74 mmol) in CH_2Cl_2 (150 mL) were refluxed at 40 °C for 92 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (10 cm × 10 cm, 40% AcOEt−hexane) to afford cross-metathesis products as a brown syrup (5.0 g, 77%, 1.7:1 mixture of geometric isomers). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25 (br s, 1H, NH-1′), 5.43 (m, 3H, H-3 and H-4), 4.04 (m, 1H, H-2), 3.95 (m, 2H, NH-Boc and OH), 3.51 (m, 2H, H-3′), 3.28 (m, 2H, H-1′), 2.33 (m, 1H, H-6a), 2.26 (m, 2H, H-6b), 1.87 (m, 2H, H-7), 1.58 (m, 2H, H-2′), 1.56 (s, 9H, ^{t}Bu), 1.33 (m, 18H, $CHCH_2CHCHCH_2(CH_2)_{10}CH_3$), 0.77 (t, 3H, $CHCH_2CHCHCH_2(CH_2)_{10}CH_3$, *J* = 6.9 Hz); ESIMS-LR *m*/*z* 463 [(M + Na)$^+$]. The olefins and 10% Pd/C (500 mg) in MeOH (5 mL) were vigorously stirred under H_2 atmosphere at room temperature for 24 h. The insoluble was filtered off through Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (10 cm × 10 cm, 40% AcOEt−hexane) to afford **16** (3.7 g, 57% over two steps) as a colorless oil. $[\alpha]^{21}_D$ −7.05 (*c* 1.80, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.92 (br s, 1H, NH-1′), 5.27 (br d, 1H, NH-Boc, $J_{NH,2}$ = 7.4 Hz), 4.02 (d, 1H, H-2, $J_{2,3}$ = 6.3 Hz), 3.58 (m, 3H, H-3′ and OH), 3.38 (m, 2H, H-1′), 1.74 (m, 1H, H-3a), 1.66 (m, 2H, H-2′), 1.56 (m, 1H, H-3b), 1.40 (s, 9H, ^{t}Bu), 1.33 (m, 26H, $CHCH_2(CH_2)_{13}CH_3$), 0.85 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, *J* = 6.8 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 178.3, 173.8, 156.0, 80.1, 59.1, 54.9, 36.1, 32.7, 32.2, 32.0, 29.8, 29.7, 29.7, 29.6, 29.4, 28.4, 25.8, 22.8, 14.2; ESIMS-LR *m*/*z* 465 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{25}H_{50}N_2O_4Na$ 465.3663, found 465.3671.

(2*S*)-2-Benzyloxycarbonylamino-*N*′-3-hydroxylpropylhexadecanamide (17). Compound **16** (2.0 g, 4.52 mmol) was treated with 4.0 M HCl in AcOEt (40 mL) at room temperature for 3 h. The resulting mixture was concentrated in vacuo. The residue in AcOEt (100 mL) was treated with saturated aqueous $NaHCO_3$ (100 mL) and benzyl chloroformate (760 μL, 5.3 mmol) at 0 °C for 8 h. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5 cm × 10 cm, 67% AcOEt−hexane) to afford **17** (1.6 g, 74% over two steps) as a white wax. $[\alpha]^{21}_D$ +4.47 (*c* 0.37, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.82 (t, 1H, NH-1′, *J* = 5.5 Hz), 7.30 (m, 6H, phenyl and NH-Cbz), 4.98 (m, 2H, CH_2Ph), 4.39 (br s, 1H, OH), 3.88 (dd, 1H, H-2, $J_{2,3a}$ = 8.6, $J_{2,3b}$ = 13.8 Hz), 3.35 (m, 2H, H-3′), 3.04 (m, 2H, H-1′), 1.50 (m, 4H, H-3′ and H-3), 1.21 (m, 26H, $CHCH_2(CH_2)_{13}CH_3$), 0.80 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, *J* = 7.5 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 179.9, 172.4, 156.4, 137.6, 128.8, 128.3, 128.2, 65.8, 58.9, 55.2, 36.2, 32.9, 32.6, 31.8, 30.1, 29.6, 29.6, 29.5, 29.3, 29.2, 25.9,22.6, 14.5; ESIMS-LR *m*/*z* 499 [(M + Na)$^+$]; ESIMS-HR calcd for $C_{28}H_{48}N_2O_4Na$ 499.3506, found 499.3510.

***tert*-Butyl 5-*O*-[5-*tert*-Butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribo-pentofuranosyl]-6-*N*-[3-(2*S*-benzyloxycarbonylaminohexadecanoyl)aminopropyl]amino-6-deoxy-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-glycelo-L-*talo*-heptofuranuronate (21).** Aldehyde **18** was prepared as follows. A mixture of compound **17** (309 mg, 0.70 mmol) and Et_3N (389 μL, 2.8 mmol) in CH_2Cl_2 (4 mL) and DMSO (4 mL) was treated with pyridine−sulfur trioxide complex (35.0 mg, 1.4 mmol) at room temperature for 2 h. The mixture was partitioned between AcOEt and 0.1 M aqueous HCl. The organic phase was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo to afford **18** (308 mg, quantitative) as a white wax. The compound was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.77 (s, 1H, formyl), 7.34 (m, 5H, phenyl), 6.37 (br s, NH-1′), 5.20 (br s, NH-Cbz), 5.08 (s, 2H, CH_2Ph), 4.04 (m, 1H, H-2), 3.52 (m, 2H, H-1′), 2.74 (m, 2H, H-2′), 2.04 (m, 1H, H-3a), 1.56 (m, 1H, H-3a), 1.24 (m, 26H, $CHCH_2(CH_2)_{13}CH_3$), 0.86 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, *J* = 11.5 Hz); ESIMS-LR *m*/*z* 497 [(M + Na)$^+$]. A mixture of **19**[4] (692 mg, 0.70 mmol) and 10% Pd/C (70 mg) in MeOH (7 mL) was vigorously stirred under H_2 atmosphere at room temperature for 30 min. The catalyst was filtered off through Celite pad, and the filtrate was concentrated in vacuo to give a crude amine **20**. A solution of **20** (500 mg, 0.70 mmol) and **18** (308 mg, 0.70 mmol) and AcOH (384 μL, 7.0 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 30 min. The mixture was treated with $NaBH(OAc)_3$ (443 mg, 2.1 mmol) at room temperature for 1.5 h. The reaction was quenched by saturated aqueous $NaHCO_3$ (7 mL), and the whole mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5 cm × 10 cm, 50% AcOEt−hexane) to afford **21** (629 mg, 79%) as a white foam. $[\alpha]^{21}_D$ +15.7 (*c* 1.40, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.33 (m, 5H, phenyl), 5.65 (s, 1H, H-1′), 5.61 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (d, 1H, H-2′, $J_{2',3'}$ = 6.3 Hz), 5.09 (d, 1H, CH_2Ph, *J* = 12.0 Hz), 5.04 (d, 1H, CH_2Ph, *J* = 12.0 Hz), 4.99 (s, 1H, H-1″), 4.85 (m, 1H, H-6′), 4.81 (t, 1H, H-3′, $J_{3',2'} = J_{3',4'}$ = 6.3 Hz), 4.62 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.49 (d, 1H, H-2″, $J_{2'',3''}$ = 9.3 Hz), 4.45 (dd, 1H, H-4′, $J_{4',5'}$ = 5.7, $J_{4',3'}$ = 6.3 Hz), 4.18 (d, 1H, H-3″, $J_{3'',2''}$ = 9.3 Hz), 4.09 (t, 1H, H-4″, $J_{4'',5''a} = J_{4'',5''b}$ = 6.9 Hz,), 4.01 (m, 1H, $-CHCH_2(CH_2)_{13}CH_3$), 3.28 (m, 2H, H-10′), 3.17 (m, 1H, H-5″a), 2.99 (m, 1H, H-5″b), 2.75 (m, 1H, H-8′a), 2.37 (m, 1H, H-8′b), 1.60 (m, 2H, H-9′), 1.52 (m, 15H, $(CH_3CH_2)_2CH$, ^{t}Bu and $CHCH_2(CH_2)_{13}CH_3$), 1.42 (m, 12H, ^{t}Bu and acetonide), 1.26 (m, 29H, acetonide and $CHCH_2(CH_2)_{13}CH_3$), 0.88 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, *J* = 6.9 Hz), 1.26 (m, 29H, acetonide and $CHCH_2(CH_2)_{13}CH_3$), 0.88 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, *J* = 6.9 Hz), 0.76 (m, 6H, $(CH_3CH_2)_2CH$); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.5, 172.5, 165.1, 157.0, 156.9, 150.7, 145.0, 136.9, 128.2, 127.7, 127.6, 115.8, 114.2, 112.9, 101.4, 96.1, 88.0, 86.5, 86.1, 84.9, 82.4, 82.2, 82.0, 78.9, 66.3, 61.7, 55.3, 45.3, 43.1, 37.1, 32.2, 31.8, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.9, 28.5, 27.5, 27.4, 26.3, 25.6, 24.4, 22.5, 13.2, 7.6, 6.3; ESIMS-LR *m*/*z* 1171 [(M + H)$^+$]; ESIMS-HR calcd for $C_{61}H_{99}N_6O_{16}$ 1171.7112, found 1171.7094.

Compound 10g. Compound **21** (17.0 mg, 0.014 mmol) and 10% $Pd(OH)_2$/C (6 mg) in MeOH (1 mL) were vigorously stirred under H_2 atmosphere at room temperature for 5 h. The insoluble was filtered off through Celite pad, and the filtrate was concentrated in vacuo to afford the crude amine. Boc-Orn(Cbz)-OH (6.36 mg, 0.017 mmol) and HOSu (2.0 mg, 0.014 mmol) in CH_2Cl_2 (1 mL) were treated with EDCI (5.36 mg, 0.028 mmol) at 0 °C for 1 h. The amine in CH_2Cl_2 (1 mL) was added to the solution of the amino acid at 0 °C, and the mixture was stirred at room temperature for 8 h. The reaction mixture was partitioned between AcOEt and 1 M aqueous HCl. The organic phase was washed with H_2O, saturated aqueous $NaHCO_3$, and

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saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was treated with 80% aqueous TFA (2 mL) for 8 h, and the solution was concentrated in vacuo. The residue was triturated from cyclopentyl methyl ether to afford **10g** (15.4 mg, quantitative) as a white foam. Compound for biological assays was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 60% to 75% MeOH−H_2O for 20 min, 10.5 min). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.67 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 5.75 (d, 1H, H-1′), 5.71 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 5.15 (s, 1H, H-1″), 4.55 (m, 1H, H-5′), 4.26 (m, 3H, H-2′, H-3′ and H-4′), 4.04 (m, 5H, H-2″, H-3″, H-4″, H-2-Orn, and C*H*$CH_2(CH_2)_{13}CH_3$), 3.89 (s, 1H, H-6′), 3.45 (m, 1H, H-10′a), 3.23−2.97 (m, 7H, H-8′, H-10′b, H-5″, and H-5-Orn), 1.93 (m, 4H, H-9′ and $CHCH_2(CH_2)_{13}CH_3$), 1.68 (m, 2H, H-3-Orn), 1.25 (m, 26H, $CHCH_2(CH_2)_{13}CH_3$), 0.87 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, J = 7.0 Hz); ESIMS-LR m/z 887 [(M + H)$^+$]; ESIMS-HR calcd for $C_{41}H_{75}N_8O_{13}$ 887.5454, found 887.5456.

Compound 10h. Compound **21** (30 mg, 0.025 mmol) and 10% $Pd(OH)_2/C$ (10 mg) in MeOH (1 mL) were vigorously stirred under H_2 atmosphere at room temperature for 5 h. The insoluble was filtered off through Celite pad, and the filtrate was concentrated in vacuo. Boc-Arg-Pbf-OH (13.2 mg, 0.025 mmol) and HOBt (3.4 mg, 0.025 mmol) in DMF (1 mL) was treated with EDCI (7.8 mg, 0.050 mmol) at 0 °C and stirred at the same temperature for 1 h. The amine in DMF (1 mL) was added to the solution of the amino acid at 0 °C, and the mixture was stirred at room temperature for 8 h. The mixture was partitioned between AcOEt and H_2O. The organic phase was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was treated with 80% aqueous TFA (2 mL) for 8 h, and the solution was concentrated in vacuo. The residue was triturated from MeOH to afford **10h** (19.8 mg, 85%) as a white foam. Compound for biological assays was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 60% to 75% MeOH−H_2O for 20 min, 10.6 min). ^{1}H NMR (D_2O, 500 MHz) δ 7.59 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.75 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.66 (s, 1H, H-1′), 5.07 (s, 1H, H-1″), 4.47 (d, 1H, H-5′, $J_{5',4'}$ = 9.7 Hz), 4.28 (m, 1H, H-2′), 4.17 (m, 2H, H-3′ and H-4′), 4.01 (m, 4H, H-2″, H-3″, H-4″ and C*H*$CH_2(CH_2)_{13}CH_3$), 3.91 (m, 1H, H-2-Arg), 3.80 (s, 1H, H-6′), 3.20−2.95 (m, 8H, H-8′, H-10′, H-5″, and H-3-Arg), 1.57 (m, 4H, H-9′ and $CHCH_2(CH_2)_{13}CH_3$), 1.10 (m, 26H, $CHCH_2(CH_2)_{13}CH_3$), 0.70 (s, 3H, $CHCH_2(CH_2)_{13}CH_3$); ESIMS-LR m/z 929 [(M + H)$^+$]; ESIMS-HR calcd for $C_{42}H_{77}N_{10}O_{13}$ 929.5666, found 929.5664.

Compound 10i. Compound **21** (29.3 mg, 0.025 mmol) and 10% $Pd(OH)_2/C$ (10 mg) in MeOH (3 mL) were vigorously stirred under H_2 atmosphere at room temperature for 5 h. The insoluble was filtered off through Celite pad, and the filtrate was concentrated in vacuo. The residue was suspended in AcOEt, and the insoluble was filtered off through a short silica gel pad. The filtrate was concentrated in vacuo to afford the crude amine. Boc-Met-OH (6.86 mg, 0.025 mmol) and HOBt (3.38 mg, 0.025 mmol) in DMF (2 mL) were treated with EDCI (5.36 mg, 0.028 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1 h. The crude amine in DMF (1 mL) was added to the solution of the amino acid at 0 °C, and the mixture was stirred at room temperature for 8 h. The reaction mixture was partitioned between AcOEt and 1 M aqueous HCl. The organic phase was washed with H_2O, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was treated with 80% aqueous TFA (2 mL) for 8 h, and the solution was concentrated in vacuo. The residue was triturated from diethyl ether to afford **10i** (16.2 mg, 72%) as a white foam. Compound for biological assays was purified by HPLC (YMC J'sphere ODS M80, 10 mm × 150 mm, 0.1% TFA, a linear gradient from 60% to 80% MeOH−H_2O for 14 min). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.76 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.71 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 1H, H-1″), 4.56 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.27 (m, 3H, H-2′, H-4′ and C*H*$CH_2(CH_2)_{13}CH_3$), 4.16 (m, 1H, H-3′), 4.03 (m, 4H, H-2″, H-3″, H-4″, H-2-Met), 3.89 (s, 1H, H-6′), 3.33 (m, 1H, H-10′a), 3.24 (m, 4H, H-8′a, H-10′b, H-5″), 2.61 (m, 2H, H-4-Met), 2.11 (m, 5H, H-3-Met, SMe), 1.87 (m, 2H, H-9′), 1.71 (m, 2H, $CHCH_2(CH_2)_{13}CH_3$), 1.26 (m, 26H, $CHCH_2(CH_2)_{13}CH_3$), 0.86 (t, 3H, $CHCH_2(CH_2)_{13}CH_3$, J = 7.0 Hz); ESIMS-LR m/z 904 [(M + H)$^+$]; ESIMS-HR calcd for $C_{41}H_{73}N_7O_{13}S$ 904.5060, found 904.5081.

***tert*-Butyl 5-*O*-[5-*tert*-Butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-6-deoxy-6-dodecylamino-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-glycero-L-*talo*-heptofuranuronate.** A mixture of **22**[12] (85 mg, 0.1 mmol) and 10% Pd/C (10 mg) in MeOH (3 mL) was vigorously stirred for 1 h under H_2 atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated in vacuo to give the free amine. The amine and dodecanal (24 μL, 0.1 mmol) in CH_2Cl_2 (1 mL) were treated with AcOH (28 μL) and $NaBH(OAc)_3$ (84 mg, 0.40 mmol) at room temperature for 1 h. The reaction mixture was diluted with AcOEt (50 mL), which was washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (2 cm × 10 cm, 50% AcOEt/hexane) to give the title compound (71 mg, 80% over two steps) as a white foam. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.70 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.70 (d, 1H, H-1′, $J_{1'',2''}$ = 1.7 Hz), 5.66 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.21 (dd, 1H, H-2′, $J_{2',1'}$ = 1.7, $J_{2',3'}$ = 6.3 Hz), 5.04 (s, 1H, H-1″), 4.83 (dd, 1H, H-3′, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 4.0 Hz), 4.67 (d, 1H, H-2″, $J_{2'',3''}$ = 5.7 Hz), 4.55 (d, 1H, H-3″, $J_{3'',2''}$ = 5.7 Hz), 4.48 (dd, 1H, H-4′, $J_{4',3'}$ = 9.7, $J_{4',5'}$ = 4.6 Hz), 4.42 (d, 1H, H-5′, $J_{5',4'}$ = 4.6 Hz), 4.14 (t, 1H, H-4″, $J_{4'',5''}$ = 6.3 Hz), 3.25 (br s, 1H, H-6′), 3.21 (dd, 1H, H-5″a, $J_{5''a,4''}$ = 5.7, $J_{5''a,5''b}$ = 14.3 Hz), 3.05 (dd, 1H, H-5″b, $J_{5''b,4''}$ = 7.5, $J_{5''b,5''a}$ = 14.3 Hz), 2.68 (m, 1H, $CH_3(CH_2)_{10}CH_2NH$), 2.42 (m, 1H, $CH_3(CH_2)_{10}CH_2NH$), 1.58−1.32 (m, 39H, $CH_3(CH_2)_{10}CH_2NH$, *tert*-butyl, CH_2CH_3 × 2, and acetonide), 0.93 (t, 3H, $CH_3(CH_2)_{10}CH_2NH$, J = 6.9 Hz), 0.83, 0.81 (each t, each 3H, CH_2CH_3, J = 7.4 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.0, 166.4, 158.2, 152.2, 146.3, 117.1, 115.4, 114.2, 102.7, 97.3, 89.1, 87.7, 87.4, 86.2, 83.7, 83.6, 83.4, 83.3, 80.2, 62.9, 44.4, 33.1, 30.8, 30.7, 30.6, 30.5, 30.4, 29.7, 28.8, 28.6, 28.5, 28.3, 27.6, 25.7, 23.8, 14.5, 8.9, 7.6; ESIMS-HR m/z calcd for $C_{45}H_{77}N_4O_{13}$ 881.5409, found 881.5480.

5-*O*-(5-Amino-5-deoxy-β-D-ribofuranosyl)-6-deoxy-6-dodecylamino-1-(uracil-1-yl)-β-D-glycero-L-*talo*-heptofuranuronic Acid Trifluoroacetic Salt (23). *tert*-Butyl 5-*O*-[5-*tert*-butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-6-deoxy-6-dodecylamino-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-glycero-L-*talo*-heptofuranuronate (50 mg, 0.057 mmol) was treated with 80% aqueous TFA (1 mL) at room temperature for 6 h. The reaction mixture was concentrated in vacuo, and the residue was purified by C18 reverse phase column chromatography (1.5 cm × 10 cm, 80% aqueous MeOH containing 0.5% TFA) to afford **23** (33 mg, 94%) as a TFA salt. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.69 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.77 (s, 1H, H-1′), 5.76 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.23 (s, 1H, H-1″), 4.61 (br s, 1H, H-5′), 4.33 (br s, 1H, H-2′), 4.27 (m, 2H, H-6′ and H-3″), 4.21 (br s, 1H, H-4′), 4.14−4.06 (m, 3H, H-3′, H-2″, and H-4″), 3.24 (br s, 2H, H-5″a and H-5″b), 3.22−3.11 (m, 2H, $CH_3(CH_2)_9CH_2CH_2NH$), 1.75 (m, 2H, $CH_3(CH_2)_9CH_2CH_2NH$), 1.36−1.32 (m, 18H, $CH_3(CH_2)_9CH_2CH_2NH$), 0.93 (t, 3H, $CH_3(CH_2)_9CH_2CH_2NH$, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 170.9, 165.9, 152.0, 143.5, 110.1, 103.1, 94.5, 85.3, 80.3, 77.7, 76.3, 74.2, 73.7, 71.2, 63.8, 44.0, 33.1, 30.7, 30.6, 30.5, 30.2, 28.2, 27.6, 26.8, 23.7, 14.4; ESIMS-HR m/z calcd for $C_{28}H_{49}N_4O_{11}$ 617.3320, found 617.3381.

MraY Enzymatic Assay. The activities of the compounds were tested against purified MraY from *B. subtilis*.[23] The assay was performed in a reaction mixture (10 μL) 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-Mur*N*Ac-[^{14}C]pentapeptide (337 Bq), and 8.4 mM *N*-lauroylsarcosine. The reaction was initiated by the addition of MraY enzyme, and the mixture was incubated for 30 min at 37 °C under shaking with a thermomixer (Eppendorf). The reaction was stopped by heating at 100 °C for 1 min. The radiolabeled substrate UDP-Mur*N*Ac-pentapeptide and reaction product (lipid **I**, product of MraY) were separated by TLC on silica gel plates LK6D (Whatman) using 2-propanol/concentrated ammonium hydroxide/water (6:3:1; v/v/v) as a mobile phase. The radioactive spots were located and quantified with a radioactivity scanner (model Multi-

Tracemaster LB285; EG&G Wallac/Berthold). IC_{50} values were calculated with respect to a control assay without the inhibitor. Data represent the mean of independent triplicate determinations.

WecA Enzymatic Assay. A standard WecA assay[26,27] was performed in a reaction mixture (10 μL) containing, in final concentrations, 100 mM Tris-HCl buffer, pH 8, 10 mM $MgCl_2$, 1.1 mM C_{55}-P, 0.16 mM UDP-[^{14}C]GlcNAc (550 Bq), and 92.7 mM Triton X-100. The reaction was initiated by the addition of WecA enzyme, and the mixture was incubated for 30 min at 65 °C. The reaction was stopped by heating at 100 °C for 1 min, and the radiolabeled substrate and product, UDP-GlcNAc and C_{55}-PP-GlcNAc, were separated by thin-layer chromatography on silica gel plates LK6D (Whatman) using 2-propanol−ammonium hydroxide−water (6:3:1; v/v/v) as a mobile phase. The radioactive spots were located and quantified with a radioactivity scanner (model Multi-Tracemaster LB285; Berthold- France). For WecA activity, residual activities and IC_{50} values were calculated with respect to a control assay without the inhibitors. Data represent the mean of independent triplicate determinations, and the standard deviation was less than 20%.

Antibacterial Activity Evaluation. Vancomycin-resistant *Enterococcus faecalis* SR7914 (VanA) and *Entercoccus faecium* SR7917 (VanA) and methicillin-resistant *Staphylococcus aureus* SR3637 were clinical isolates collected from hospitals of Japan and kindly provided by Shionogi & Co., Ltd. (Osaka, Japan).[25] MICs were determined by a microdilution broth method as recommended by the NCCLS (National Committee for Clinical Laboratory Standards, 2000, National Committee for Clinical Laboratory Standards, Wayne, PA) with cation-adjusted Mueller−Hinton broth (CA-MHB) (Becton Dickinson, Sparks, MD). 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.

Assay of Cytotoxicity. HepG2 cells were suspended in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and then seeded in 96-well tissue culture plates at 1×10^4 cells/well. After 24 h, cells were treated with varying concentrations of **8b**−**e**, **9b**−**e**, or tunicamycin (as a positive control) for 48 h. After the treatment, WST-8 reagent (Kishida Chemical) was added to each well, and cells were incubated for 1.5 h at 37 °C. The cell viability was measured as the absorbance at 450 nm, and percentage inhibition in growth was calculated against that of cells treated without those compounds. Tunicamycin exhibited cytotoxicity with an IC_{50} of 26.8 μg/mL. IC_{50} values of all the compounds tested were >100 μg/mL.

Conformation Analysis. The number of carbon atom of the lipophilic side chain was reduced for the MRY, and the tetrapeptide was omitted for UDP-MurNAc to simplify the calculation. The energy-minimized conformations of **24** and **25** were calculated by a conformational search by a MacroModel program, version 9.2.[43] The ionization status in H_2O at pH 7 ± 1 was first predicted by Epik,[44] which is an empirically based pK_a predictor and ionization state generator based upon the Hammett and Taft methodologies. These structures were used for the following conformational analysis. Conformational searching was carried out using the Monte Carlo multiple minimum (MCMM) method[45] (100 000 steps), followed by Polak−Ribiere conjugate gradient (PRCG) minimization[46] with the OPLS 2005 force field. Water was chosen for a solvent with the GB/SA model.[47] The other settings were used as default. Structural analysis of energy-minimum conformers calculated for **24** and **25** indicates several conformers within 25 kJ/mol (6.2 kcal/mol). Among them, the calculated conformers where the uracil base interacts with the remaining residue in the molecule were not used for further analysis because the MraY should include the nucleotide-binding motif considering the reaction mechanism and the uracil base posed at the clef of the motif. Finally the calculated conformers were refined by density functional theory (DFT) quantum mechanical calculations at the BL3LYP/6-31G* level.[48]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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.

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■ ABBREVIATIONS USED

Cpm, L-*epi*-capreomycidine; CR, carbohydrate recognition; CL, cytoplasmic loop; FOS, function-oriented synthesis; HepG2, human hepatocellular liver carcinoma; MRY, muraymycin; PL, periplasmic loop; MraY, phospho-MurNAc-pentapeptide transferase; MRSA, *Staphylococcus aureus*; SAR, structure−activity relationship; U4CR, Ugi four-component reaction; C_{55}-P, undecaprenyl phosphate; VRSA, vancomycin-resistant *Staphylococcus aureus*

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