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Novel FR-900493 Analogues That Inhibit the Outgrowth of *Clostridium difficile* Spores

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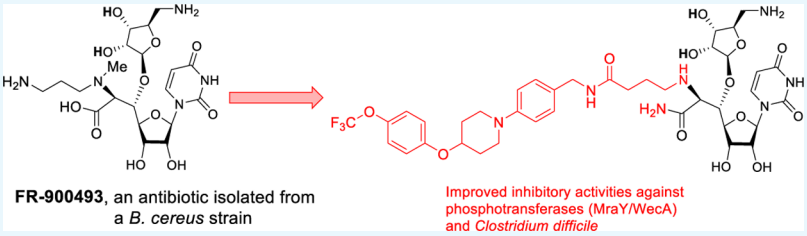
Supporting Information



ABSTRACT: The spectrum of antibacterial activity for the nucleoside antibiotic FR-900493 (**1**) can be extended by chemical modifications. We have generated a small focused library based on the structure of **1** and identified UT-17415 (**9**), UT-17455 (**10**), UT-17460 (**11**), and UT-17465 (**12**), which exhibit anti-*Clostridium difficile* growth inhibitory activity. These analogues also inhibit the outgrowth of *C. difficile* spores at 2× minimum inhibitory concentration. One of these analogues, **11**, relative to **1** exhibits over 180-fold and 15-fold greater activity against the enzymes, phospho-MurNAc-pentapeptide translocase (MraY) and polyprenyl phosphate-GlcNAc-1-phosphate transferase (WecA), respectively. The phosphotransferase inhibitor **11** displays antimicrobial activity against several tested bacteria including *Bacillus subtilis*, *Clostridium spp.*, and *Mycobacterium smegmatis*, but no growth inhibitory activity is observed against the other Gram-positive and Gram-negative bacteria. The selectivity index (Vero cell cytotoxicity/*C. difficile*antimicrobial activity) of **11** is approximately 17, and **11** does not induce hemolysis even at a 100 μM concentration.

INTRODUCTION

Clostridium difficile, a Gram-positive bacterium, is transmitted by the fecal-oral route. *C. difficile* infection (CDI) can cause illness ranging from diarrhea, colitis, and toxic inflammatory bowel disease to death. *C. difficile* has become one of the most common causes of health care-associated infections in U.S. hospitals.[1] Approximately 250 000 people are hospitalized in the U.S. every year from CDI.[2] The infective form of *C. difficile* is the spore, and its germination is the first committed step in CDI onset. *C. difficile* is found in abundance in the environment, and colonizes in the gut where it produces toxins that cause *C. difficile*-associated diarrhea (CDAD).[3−5] Frequently, treatment with broad-spectrum antibiotic(s) has the adverse effect of increasing the incidence of recurrent CDIs because of the disruption of the normal balance of gut flora.[6] A recently described highly toxic strain, BI/NAP1/027, exhibits resistance to fluoroquinolones and produces >20 times more toxins than historical strains.[7] Antibiotic treatment of CDI is difficult because of the antibiotic resistance and bacterial physiology (e.g., spore formation and protective effects of pseudomembranous colitis).[8,9] Currently, there are only a limited number of drugs available for the treatment of CDAD.[10] Metronidazole and vancomycin are the primary therapy options for CDI. Vancomycin is recommended for severe infections that do not respond to metronidazole. Vancomycin, rifaximin, and fidaxomicin are used in recurrent or persistent cases.[11] For severe recurrent CDIs, fecal microbiota transplantation, which involves instillation of stool from a healthy donor into the gastrointestinal (GI) tract of the patient, has been highlighted to restore the gut microbiome to a healthy state.[12] At therapeutic concentrations, currently available drugs for the treatment of CDI are not effective in inhibiting the germination or outgrowth of *C. difficile* spores. Only fidaxomicin has been reported to inhibit spore production in *C. difficile*.[13] To date, bile acid derivatives and a few organic molecules have been studied for their efficacy in the reduction of spore viability or inhibition of spore germination.[14−19]

In our continued efforts to identify strong inhibitors of bacterial phosphotransferases [phospho-MurNAc-pentapeptide translocase (translocase I or MraY) and polyprenyl phosphate-GlcNAc-1-phosphate transferase (WecA)] (Figure 1),[20−25] we

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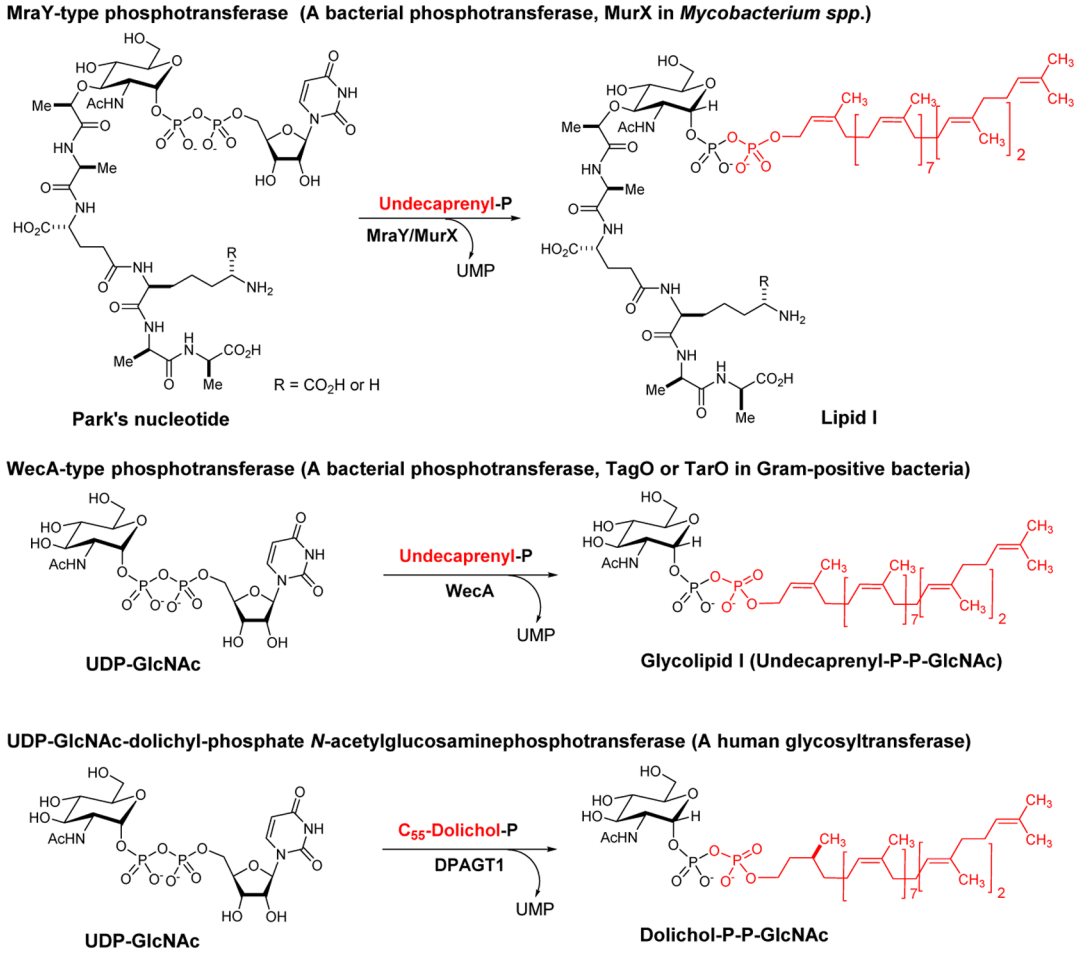

Figure 1. Bacterial phosphotransferases and a human glycosyltransferase. MraY (MurX) is an established drug target for Gram-positive and Gram-negative bacterial infections. WecA is essential in the growth of *Mycobacterium* spp. and some Gram-positive bacteria (TagO or TarO). DPAGT1 is a human glycosyltransferase. Strong inhibition of DPAGT1 may cause cytotoxicity in mammalian cells.

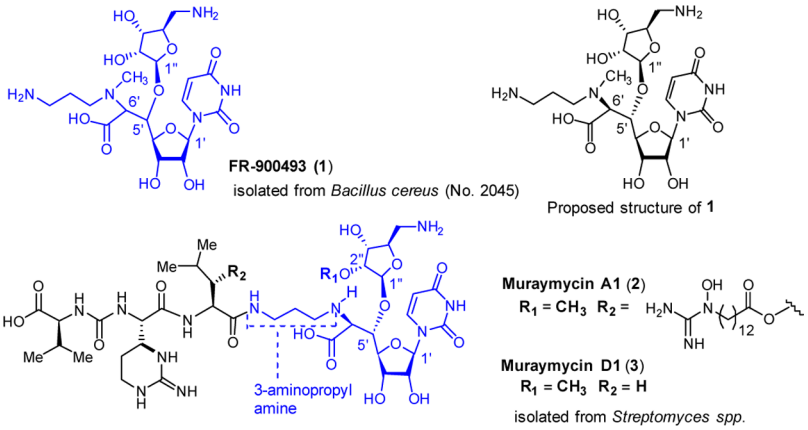

Figure 2. Structures of FR-900493 (**1**) and representative muraymycins, muraymycin A1 (**2**) and D1 (**3**). FR-900493 has only the right half of the muraymycins (the highlighted portion in blue). The unknown stereochemistries of the 5′- and 6′-positions are determined unequivocally in this study.

have generated a small focused library based on the structure of the known MraY inhibitor FR-900493 (**1**, Figure 2)[26] and assayed against the vegetative and spore forms of a *C. difficile* strain. MraY is specific for UDP-*N*-acetylmuramyl-pentapeptide (Park's nucleotide) forming undecaprenyl diphosphoryl-*N*-acetylmuramate-pentapeptide (lipid I). WecA and its homologues (e.g., TagO or TarO) in Gram-negative bacteria are responsible for the first step of wall teichoic acid synthesis that anchors the phospho-GlcNAc moiety of UDP-GlcNAc to undecaprenyl phosphate (C_{55}−P). MraY enzymatic activity is essential for the growth of both Gram-positive and Gram-negative bacteria, and thus is considered as a target of interest for the discovery of novel antibacterial agents. The major source of MraY inhibitors resides in the nucleoside-based antibiotic group. This group has been subdivided into four classes: tunicamycins, ribosaminouridines, uridyl peptides, and capuramycins. Analysis

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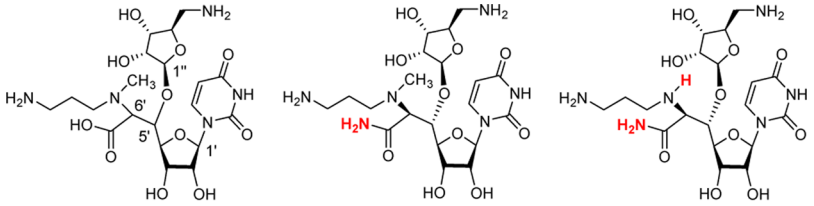
Table 1. Inhibitory Activity of Bacterial Phosphotransferases (MraY and WecA) and *C. difficile* Growth by the C5′- and 6′-Diastereomers of FR-900493[a]



compound	WecA inhibition IC_{50} (μM)[b]	MraY inhibition IC_{50} (μM)[c]	*C. difficile* ATCC 43596 MIC (μg/mL)[d]
FR-900493 (**1**)	5.0 ± 5.44	25.0 ± 8.67	>25.0
5′*S*,6′*R*-diastereomer (**4**)	>100	>100	>25.0
5′*R*,6′*S*-diastereomer (**5**)	>100	>100	>25.0
5′*R*,6′*R*-diastereomer (**6**)	>100	>100	>25.0
FR-900493-amide (**7**)	5.0 ± 6.34	25.0 ± 9.67	>25.0
8	>100	>100	>25.0
tunicamycin	0.15 ± 7.80	3.38 ± 7.32	>25.0

[a]WecA and MraY assays (see the Supporting Information). [b]*E. coli* WecA-containing membrane was used. [c]*Hydrogenivirga* spp. MraY was used. [d]A microdilution broth method was used.

of the pharmacological behaviors observed with several compounds in these classes shows a broad spectrum of antibacterial activity, including relevant drug-resistant strains, and in vivo efficacy without apparent toxicity. Ribosaminouridine antibiotics, which include muraymycin, liposidomycins, caprazamycin, and FR-900493 (**1**), exhibit the most promising biological profiles.[27] On the other hand, essentiality of the WecA/TagO transferase subfamily is not completely understood in the growth of many bacteria.[28,29] Only a few molecules have been reported to exhibit WecA inhibitory activity.[28,29] Herein, we report a structure−activity relationship (SAR) obtained from a small focused library of FR-900493 (**1**) in a range of enzymes (MraY, WecA, and AglH) and bacterial growth inhibitory assays. Efficacy of these new MraY inhibitors against *C. difficile* spores along with in vitro toxicity assessments of the selected anti-*C. difficile* molecules is discussed.

RESULTS AND DISCUSSION

Chemistry and SAR of FR-900493. Aminoribosyl-uridyl peptide antibiotics such as FR-900493 (**1**) and muraymycins are an important class of natural products for the development of novel antibacterial agents.[30,31] Chemical syntheses of **1** and muraymycin analogues are essential to perform exhaustive SAR studies.[32] Muraymycin A1 (**2**) is one of the most active members of this family against both Gram-positive and Gram-negative bacteria. The fatty acid side chain (R_2) of **2** is critical for antimicrobial activity as muraymycin D1 (**3**) and the other related molecules lacking the R_2 group are poorly active (Figure 2).[25,33] Interestingly, we have demonstrated that muraymycin D1 shows strong bacteriostatic activity against *Mycobacterium tuberculosis* by targeting the bacterial phosphotransferases (MurX and WecA).[25] Although FR-900493 (**1**) possesses only one half of the structure of the muraymycins, it displays antistaphylococcal activity [minimum inhibitory concentration (MIC) 3.13 μg/mL] in vitro and in vivo. The LD_{50} value of **1** is over 500 mg/kg, which was determined via intravenous administrations in mice, indicating that **1** is an ideal scaffold to develop into new antibacterial agents.[26] It is interesting to note that **1** was isolated from the culture broth of *Bacillus cereus* (no. 2045), whereas other aminoribosyl-uridyl peptide antibiotics including muraymycins were isolated from *Streptomyces* spp.[31,33] Structurally, the C6′-amino group of FR-900493 is methylated, whereas *O*-methylation at the C2″-position is observed in muraymycin A1 and D1. Absolute stereochemistries at the C5′- and C6′-positions are speculated to be 5′*S* and 6′*S*, respectively, based on the correlations with stereochemistries of muraymycins and structurally related molecules, caprazamycins. On the basis of the proposed structure, FR-900493 was first synthesized by Hirano and co-workers who only reported physical chemistry data (e.g., NMR and optical rotation) for the synthetic molecule.[34] We have synthesized the four diastereomers of FR-900493 with respect to the C5′- and C6′-stereocenters according to the synthetic scheme developed for muraymycin D1 (**3**)[25] with minor modifications and compared their physical and biological data with those of the natural product. Chemical shifts and coupling constants of the 5′*S*,6′*S*-diastereomer **1** showed good agreement with those of the natural FR-900493 (see the Supporting Information).[26] The four diastereomers of FR-900493 were evaluated against the bacterial phosphotransferases MraY and WecA (Table 1).[29,35]

Surprisingly, **1** exhibited a weak MraY inhibitory activity (IC_{50} 25.0 μM) but a moderate WecA inhibitory activity (IC_{50} 5.0 μM). All unnatural diastereomers (**4**, **5**, and **6**) did not display either MraY or WecA inhibitory activity, even at a 100 μM concentration. The results of these enzymatic assays unequivocally determined the absolute stereochemistry of FR-900493 to be 5′*S* and 6′*S* configurations. We have observed that amidation of the C6′-carboxylic group in muraymycin D1 (**3**) does not decrease the MraY/WecA activity.[25] Similarly, FR-900493-amide (**7**) exhibited an MraY/WecA inhibitory activity equal to that of **1**. The *N*-methyl group of **1** is essential to inhibit the MraY and WecA enzymes; the de-*N*-methyl analogue **8** completely lost the MraY/WecA inhibitory activities. FR-900493 and its analogues shown in Table 1 were not effective in killing *C. difficile* (ATCC 43596) at 25.0 μg/mL or lower concentrations. As exemplified in the antibacterial activity of the muraymycin family molecules,[30] the hydrophobic residues appended on the 3-aminopropylamine portion play a key role in selectivity and susceptibility against

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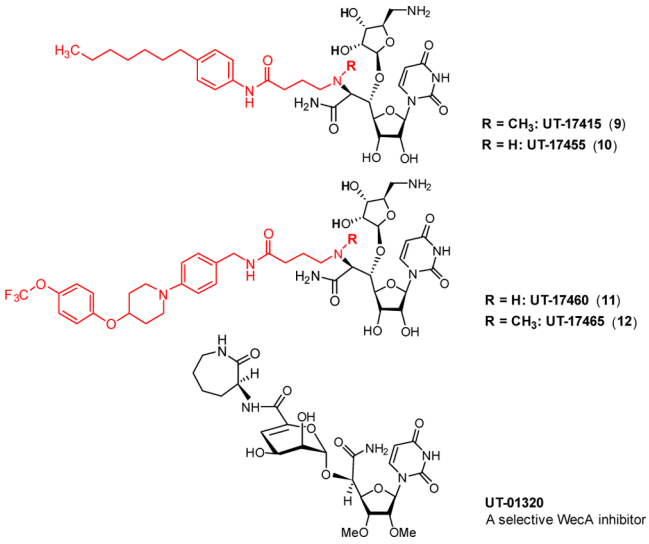
bacteria (Figure 2). We have generated a small forced library based on the core structures of **7** and **8**, and the generated molecules were assayed against *C. difficile* (ATCC 43596) at a single concentration of 50.0 μg/mL. Four molecules (**9**, **10**, **11**, and **12**) displayed anti-*C. difficile* activity (Table 2), and sufficient amounts of these molecules were resynthesized for thorough in vitro profiling.

Table 2. Inhibitory Activity of Bacterial Phosphotransferases (MraY and WecA) and *C. difficile* Growth by 9−12[a]



compound	WecA inhibition IC_{50} (μM)[b]	MraY inhibition IC_{50} (μM)[c]	*C. difficile* ATCC 43596 MIC (μg/mL)[d]
FR-900493 (**1**)	5.0 ± 5.44	25 ± 8.67	>50.0
UT-17415 (**9**)	0.85 ± 7.50	0.69 ± 7.32	25.0 ± 7.64
UT-17455 (**10**)	12.5 ± 6.01	0.25 ± 5.80	12.5 ± 5.93
UT-17460 (**11**)	**0.32 ± 4.09**	**0.08 ± 4.33**	**3.25 ± 4.40**
UT-17465 (**12**)	12.3 ± 5.75	7.70 ± 5.47	50.0 ± 5.81
UT-01320	0.035 ± 9.14	>100	>50.0
tunicamycin	0.15 ± 7.80	3.38 ± 7.32	>50.0
vancomycin	>50.0	>50.0	2.5 ± 7.25

[a]WecA and MraY assays (see the Supporting Information). [b]*E. coli* WecA-containing membrane was used. [c]*Hydrogenivirga* spp. MraY was used. [d]A microdilution broth method was used.

Syntheses of FR-900493 Analogues, 9, 10, 11, and 12. In our synthesis of muraymycin D1 (**3**), β-ribosylation of the C2-ether-protected ribose donor (R_1 = Me in **15**) and Strecker reaction with mono-protected 1,3-diaminopropane to form *secondary* amine were performed stereoselectively.[25] Our revised synthetic routes for FR-900493 analogues (**9**−**12**) are illustrated in Schemes 1 and 2. The monomethoxytetrachlorodiphenylmethoxymethyl (MTPM)-protected uridine **13** was prepared according to the previously reported procedure.[36] The *primary* alcohol of **13** was oxidized by a modified Swern condition to provide the corresponding aldehyde in a quantitative yield, which was then subjected to Carreira's asymmetric alkynylation reaction using (−)-*N*-methylephedrine,[37] furnishing the (*S*)-propargyl alcohol **14** in 80% yield with a selectivity of >98:2. The stereochemistry of the *secondary* alcohol of **14** generated via Carreira's alkynylation was determined by the advanced Mosher's method (see the Supporting Information).[38] The newly devised glycosyl donor **15** was designed to provide β-riboside whose protecting groups can be deprotected under mild acidic conditions. *N*-Iodosuccinimide (NIS)−$AgBF_4$-promoted ribosylation of (*S*)-propargyl alcohol **14** with **15** furnished the β-riboside **16** exclusively in 95% yield.[23,25,39] The azido group of **16** was reduced with Zn metal in the presence of aq NH_4Cl, and the generated free-amine was protected with $(Boc)_2O$ to furnish **17** in 90−92% overall yield. The alkyne moiety of **17** was subjected to a three-step procedure (partial reduction with a Lindlar catalyst, osmylation, and oxidative cleavage with $Pb(OAc)_4$), providing the crude aldehyde **18**. In a systematic screening of catalysts for Strecker reaction of **18** with the 4-aminobutanamide derivatives **19** (Scheme 1) or **20** (Scheme 2), it was realized that $(BnO)_2P(O)CH_2P(O)(OBn)OH$ provided a ∼4:1 mixture of the 6′*S*- and 6′*R*-diastereomers (**21*S*** and **21*R***) in greater than 80% yield. The desired diastereomer **21*S*** was subjected to hydration reaction with $HgCl_2$-acetaldoxime to furnish the amide **22** in 95% overall yield. *N*-methylation of **22** was performed via reductive amination with paraformaldehyde and $NaB(CN)H_3$ to afford **23** in 95% yield. Global deprotection of **22** and **23** to form the desired products **9** and **10**, respectively, was performed in a one-pot two-step reaction using 30% trifluoroacetic acid (TFA) followed by 80% TFA at 40 °C; the crude products were purified by C_{18} reverse-phase high-performance liquid chromatography (HPLC) ($MeOH/H_2O$ = 75:25) to yield **9** and **10** in 85−90% yield. The stereochemistry of the C6′-stereocenter for **21*S*** generated via Strecker reaction was unequivocally determined via its conformational analyses (see the Supporting Information). Similarly, the analogues **11** and **12** were synthesized with the *primary* amine **20** via the synthetic scheme developed for **9** and **10** (Scheme 2).

Enzyme and Bacterial Growth Inhibitory Activities of 9, 10, 11, and 12. Despite the extensive efforts to obtain membrane fractions from *C. difficile* (ATCC 43596), we could not obtain sufficient amounts of active *C. difficile* membrane containing MraY and WecA homolog. In this study, for assays, we used purified MraY from the thermophilic bacterium *Hydrogenivirga* (*Hy*MraY) and the active membrane fraction (P-60) from *Escherichia coli*. Protein sequence alignment via BLAST[40] of *Hy*MraY and *Ec*WecA against *C. difficile* (strain F501) revealed that MraY between *Hydrogenivirga* spp. and *C. difficile* showed 57% similarity/38% identity and WecA/putative WecA homolog between *E. coli* and *C. difficile* showed 47% similarity/28% identity. We previously confirmed that the activities of inhibitor molecules against MraY and WecA are similar across various species: *Mycobacterium smegmatis*, *M. tuberculosis*, *E. coli*, and *Hydrogenivirga* spp.[21,29,35] The synthetic molecules (**9**, **10**, **11**, and **12**) were evaluated against the phosphotransferases (MraY and WecA) and *C. difficile* strain (ATCC 43596) (Table 2). The *N*-methyl analogues, **9** and **12**, exhibited weak anti-*C. difficile* growth inhibitory activity, although their MraY inhibitory activities were over 30- and 3-fold, respectively, more potent than that of FR-900493 (**1**). In sharp contrast, the de-*N*-methyl analogues, **10** and **11**, were over 100 and 300-times more potent in the MraY inhibitory activity than **1**. The WecA inhibitory activity of **11** was ∼15-fold more than that of **1**. Therefore, the anti-*C. difficile* activity of **11** is likely attributed to the inhibition of the MraY enzyme. This hypothesis was further supported by the fact that a selective WecA inhibitor, UT-01320,[20] did not inhibit growth of *C. difficile* at >50.0 μg/mL concentrations. The analogue **11** was identified to be a strong MraY/WecA inhibitor, whose activity was significantly better than that of a known MraY/WecA inhibitor, tunicamycin.[28,29,35] The anti-*C. difficile* activity is correlated with the enzyme inhibitory activity of MraY; **10** and **11** displayed MIC values of 12.5 and 3.25 μg/mL,

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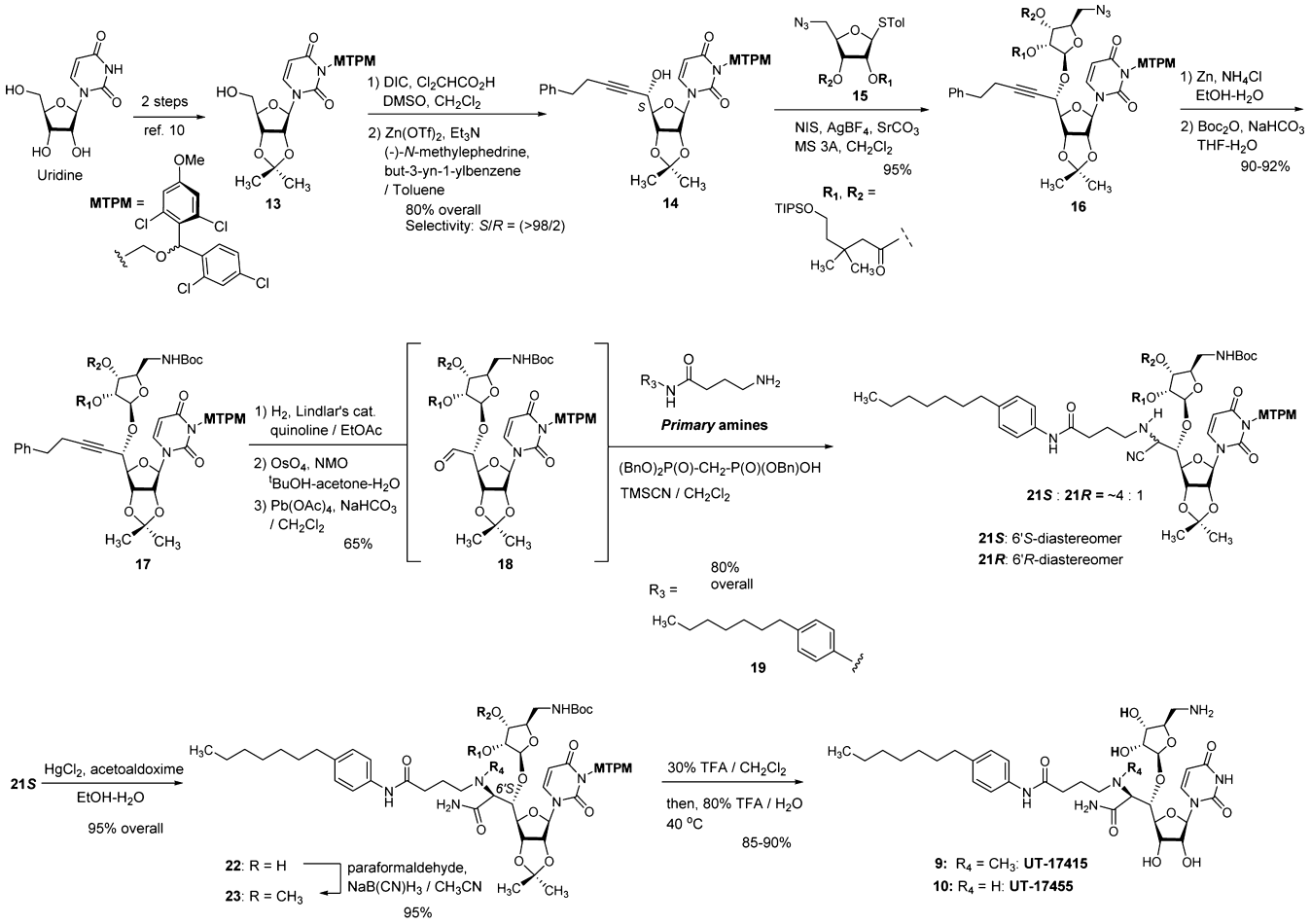
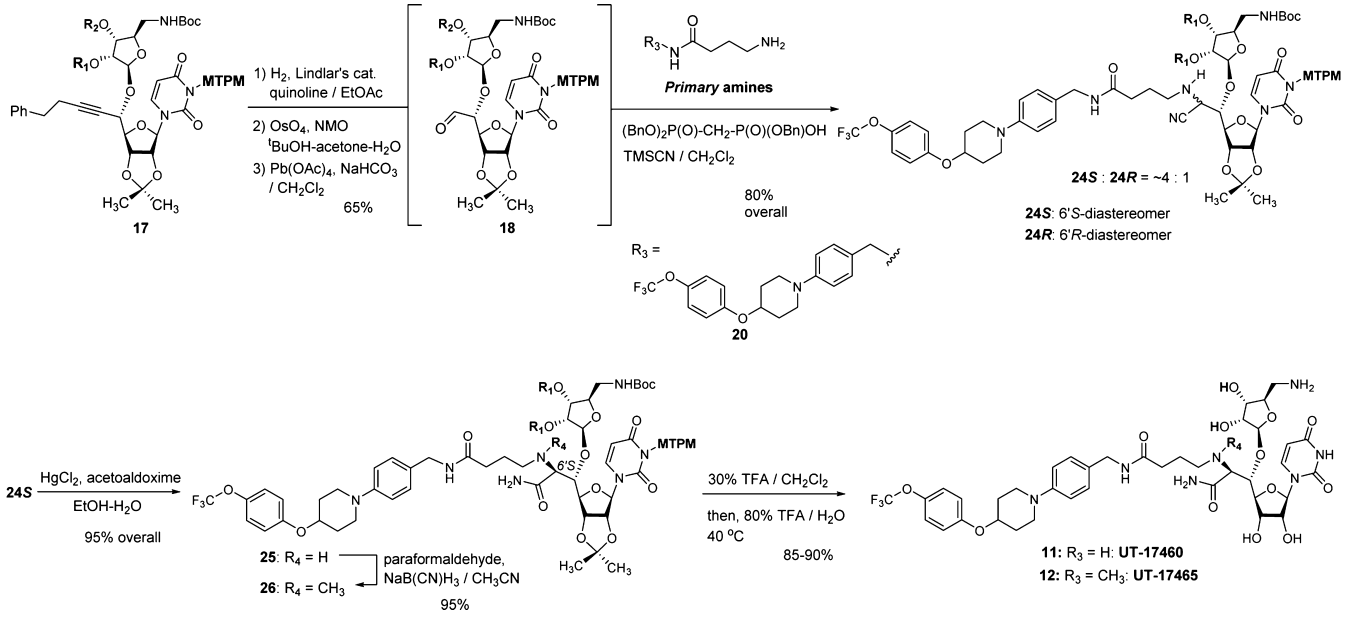
Scheme 1. Syntheses of Anti-*C. difficile* FR-900493 Analogues 9 and 10



Scheme 2. Syntheses of Anti-*C. difficile* FR-900493 Analogues 11 and 12



respectively, against *C. difficile* (e.g., an MIC of 2.50 μg/mL for vancomycin).

Effect of 9, 10, and 11 on *C. difficile* Spores. The effect of **9**, **10**, and **11** on *C. difficile* spores was determined by counting colony-forming units (CFUs) of the spore germination on taurocholate-containing agar plates after the treatment of the *C. difficile* spores with these analogues (2× MIC) for 24 h. *C. difficile* spores show resistance to most known anti-*C. difficile* agents.[19]

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Indeed, in these studies, vancomycin, metronidazole, and linezolid did not inhibit the spore outgrowth, even at 5× MIC. On the contrary, the new MraY inhibitors **9**, **10**, and **11** prevented the outgrowth of the *C. difficile* spores into colonies at 2× MIC (Figure 3).

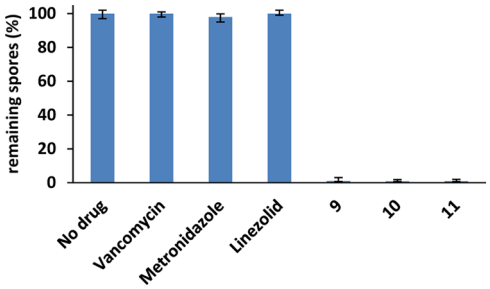

Figure 3. Viability of *C. difficile* (ATCC 43596) spores treated with the MraY inhibitors, **9**, **10**, and **11**, and representative anti-*C. difficile* drugs. The spores treated with molecules (2× or 5× MIC) in a BHI medium or saline (24 h) were plated on a BHI agar plate containing sodium taurocholate and incubated anaerobically for 48 h. Vancomycin, metronidazole, and linezolid were treated with 5× MIC, and **9**, **10**, and **11** were treated with 2× MIC. Germinated spores were counted (CFUs) ($p \leq 0.05$).

Physicochemical Properties and in Vitro Toxicity of 11. UT-17460 (**11**) exhibited pharmacological characteristics superior to those of UT-17455 (**10**); (1) water solubility of **11** (22.0 mg/mL) is 200 times greater than that of **10** (0.11 mg/mL), (2) **11** is approximately 8 times less cytotoxic than **10** against Vero cells (African green monkey kidney epithelial cells, IC_{50} 65.0 μM for **10**), and (3) **11** showed relatively low induction of hemolysis (IC_{50} 205.6 μM), whereas **10** lysed blood cells at a much lower concentration (IC_{50} 70.0 μM) than **11** (Table 3). Thus, we selected **11** for further in vitro pharmacological evaluation. WecA enzyme inhibitors have the potential to interfere with a human homologue, dolichyl-phosphate GlcNAc-1-phosphotransferase 1 (DPAGT1), which catalyzes the first step of the protein *N*-glycosylation process in humans (Figure 1). Thus, strong inhibition of DPAGT1 may cause cytotoxicity in vitro and in vivo.[41] We have established a counterselection assay using a thermophilic dolichyl-phosphate GlcNAc-1-phosphotransferase (AglH from *Methanocaldococcus jannaschii*) to assess the toxicity level of antibacterial phosphotransferase inhibitors (Table 3). Interestingly, **11** exhibited a relatively stronger AglH inhibitory activity (IC_{50} 3.61 μM) than **10** and tunicamycin; however, the IC_{50} level of **11** against Vero cells was much higher than those of **10** and tunicamycin. The FR-900493 analogue **11** exhibited low permeability across Caco-2 epithelial monolayers (P_{app} rate coefficient < 1×10^{-6} cm/s) with moderate efflux (an efflux ratio of 5.5), predicting that **11** is poorly absorbed from the GI tract.

Spectrum of Antibacterial Activity of 11. As summarized in Figure 4, UT-17460 (**11**) exhibited a very narrow spectrum of

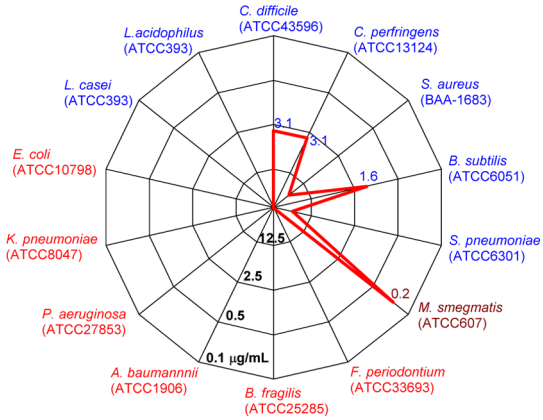

Figure 4. Antibacterial activity of UT-17460 (**11**, line in red).

antibacterial activity; **11** also killed the other *Clostridium* spp., *Clostridium perfringens* (MIC 3.1 μg/mL), *Bacillus subtilis* (MIC 1.6 μg/mL), and *M. smegmatis* (MIC 0.2 μg/mL) but did not inhibit the growth of *Lactobacillus* spp., *Staphylococcus aureus*, *Staphylococcus pneumoniae*, and all Gram-negative bacteria tested at 25.0 μg/mL or higher concentrations.

CONCLUSIONS

FR-900493 (**1**) has long been known as an MraY inhibitor for which we have firmly established the stereochemistry. In our thorough enzyme inhibitory assays of the phosphotransferases, we concluded that the antibacterial activity of **1** is attributed to a combination of WecA and MraY inhibitory activities, but **1** is a weak MraY inhibitor (IC_{50} 25.0 μM). Because WecA is not essential in the growth of many bacteria except for a few pathogens (e.g., *Mycobacterium* spp.),[28,42] improving MraY inhibitory activity of **1** should be a beneficial direction to identify effective antibacterial agents. Stereochemistry of the C5′- and C6′-positions of **1** is required to be the natural configuration (5′*S* and 6′*S*) to exhibit bacterial growth and MraY/WecA enzyme inhibitory activities. The C6′-CO_2H group of **1** can be masked as its primary amide without decreasing MraY/WecA activity; however, the *N*-methyl group is essential for **1** to display phosphotransferase inhibitory activities. Muraymycins lack the *N*-methyl group but exhibit a strong MraY enzyme inhibitory activity. Importantly, only the muraymycin analogues having a hydrophobic side chain [e.g., muraymycin A1 (**2**)] exhibit a strong antimicrobial activity. We have generated a small focused library based on the structures of **7** and **8** and screened against the

Table 3. Counterselection Assays Based on Prenyl phosphate-GlcNAc-1-phosphotransferases (WecA vs AglH) and in Vitro Cytotoxicities

	WecA inhibition IC_{50} (μM)[a]				
compound	*E. coli* WecA	*M. smegmatis* WecA	AglH inhibition IC_{50} (μM)[a] *M. jannaschii* AglH	Vero cells IC_{50} (μM)[a]	hemolysis IC_{50} (μM) sheep blood[a]
UT-17455 (**10**)	12.5 ± 6.01	12.5 ± 5.42	12.5 ± 5.18	7.08 ± 5.67	70 ± 5.71
UT-17460 (**11**)	**0.32±4.09**	**0.25±4.84**	**3.61±4.43**	**65±4.65**	**205.8±4.33**
tunicamycin	0.15 ± 7.80	0.15 ± 7.22	13.27 ± 8.10	0.12 ± 7.78	15 ± 7.83

[a]All assay procedures are described in the Supporting Information.

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vegetative form of *C. difficile* strain (ATCC 43596) under anaerobic conditions. We identified four anti-*C. difficile* FR-900493 analogues, **9**, **10**, **11**, and **12**. These analogues were resynthesized via the synthetic procedures reported previously with modifications of selective ribosylation (**14** → **16**) and Strecker reactions (**18** → **21** and **24**) (Schemes 1 and 2). The resynthesized analogues were characterized by *C. difficile* growth and the bacterial phosphotransferase inhibitory assays. The analogue **11** exhibited strong MraY/WecA inhibitory activity (IC_{50} 0.08 and 0.30 μM, respectively) and killed the vegetative state of *C. difficile* with an MIC value of 3.25 μg/mL. In vitro cytotoxicity level of **11** was much lower than those of tunicamycin and **10**; although **11** inhibited AglH at low concentrations. Toxicity of tunicamycin and **10** in mammalian cells may be largely due to the membrane disruption as demonstrated by the hemolysis assay. On the other hand, the hemolytic activity of **11** is attenuated by the pharmacologically benign hydrophobic group.[43] Interestingly, the MraY inhibitors **9**, **10**, and **11** inhibited the outgrowth of the *C. difficile* spores (endospores) at 2× MIC concentrations (Figure 3). The inhibitory mechanism of outgrowth of the spores by the MraY inhibitors is far from completely understood. Recently, a strong lipid II-binding antibacterial peptide, nisin, was reported to inhibit the viability of the *C. difficile* spores at high concentrations.[44] The effect of fidaxomicin was also observed in the inhibition of the *C. difficile* spore outgrowth at high concentrations (4.0−14 μg/mL, MIC_{90} 0.5 μg/mL against vegetative *C. difficile*).[45] *C. difficile* spores are metabolically dormant and are known to exhibit impermeability to a wide range of organic molecules.[46] Thus, organic molecules with a high molecular mass (e.g., M_w = 881.3 for **11**) are not likely to permeate the spore walls and directly inhibit the viability of the spores. Certain peptides or nucleoside derivatives are recognized by the *Bacillus anthracis* germination machinery, increasing their susceptibility to geminated spores or outgrowing spores.[47,48] On the other hand, germination machineries of *C. difficile* spores remain less well-understood because of the significantly lower levels of homologues of the spore coat proteins than those of other well-studied bacteria (e.g., *B. subtilis* and *B. anthracis*).[48] Recent studies demonstrated that *C. difficile* uses subtilisin-like serine proteases (Csp) that regulate spore germination.[49] In *C. difficile*, CspC is likely to transmit the bile acid signal to CspB, which activates the spore cortex lytic enzyme (i.e., SleC) by cleavage of pro-SlecC. SlecC alters the structure of the spore cortex and induces Ca-dipicolinate release, which triggers the outgrowth of *C. difficile* spores.[50] Our MraY inhibitors may be involved in the induction from the spore forms to the germinating state. The germinated or outgrowing spores require MraY, which will be inhibited by the new inhibitors **9**−**12**. We are currently evaluating the effectiveness of structurally distinct MraY inhibitors against the *C. difficile* spores. Correlation of nucleoside-based MraY inhibitor and ability of the *C. difficile* spore germination are being investigated. In preliminary bacterial growth inhibitory assays against a series of bacteria, **11** displayed a very narrow-spectrum antibiotic activity (Figure 4), which is advantageous in the development of selective anti-*C. difficile* agents that do not disrupt the gut microbiota in humans. In addition, in vitro pharmacokinetic data obtained via the Caco-2 permeability assay may indicate that **11** is poorly absorbed from the GI tract, which may reduce the toxicity of **11** when given orally. Pharmacokinetics and oral bioavailability, susceptibility to clinically isolated *C. difficile* and in vivo efficacy of **11** (using preclinical animal models), and selectivity of antibacterial activity of **11** at higher concentrations (1000 μg/mL) are the object of future studies.

EXPERIMENTAL SECTION

Chemistry: General Information. All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. Tetrahydrofuran (THF), CH_2Cl_2, and *N*,*N*-dimethylformamide were purified via an Innovative Technology's Pure-Solve System. All reactions were performed under an argon atmosphere. All stirring procedures were performed with an internal magnetic stirrer. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm coated commercial silica gel plates (EMD, silica gel $60F_{254}$). TLC spots were visualized by UV light at 254 nm or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using Shimadzu LCMS-2020 with the following solvents: A: 0.1% formic acid in water and B: acetonitrile. Flash chromatography was performed with SiliCycle silica gel (Purasil 60 Å, 230−400 mesh). Proton magnetic resonance (^{1}H NMR) spectral data were recorded on 400 and 500 MHz instruments. Carbon magnetic resonance (^{13}C NMR) spectral data were recorded on 100 and 125 MHz instruments. For all NMR spectra, chemical shifts (δH, δC) were quoted in parts per million (ppm) and *J* values were quoted in Hz. ^{1}H and ^{13}C NMR spectra were calibrated with a residual undeuterated solvent ($CDCl_3$: δH = 7.26 ppm, δC = 77.16 ppm; CD_3CN: δH = 1.94 ppm, δC = 1.32 ppm; CD_3OD: δH = 3.31 ppm, δC = 49.00 ppm; DMSO-d_6: δH = 2.50 ppm, δC = 39.52 ppm; D_2O: δH = 4.79 ppm) as an internal reference. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, quin = quintet, hept = heptet, m = multiplet, and br = broad. Infrared (IR) spectra were recorded on a PerkinElmer FT1600 spectrometer. HPLC analyses were performed with a Shimadzu LC-20AD HPLC system. All compounds were purified by reverse HPLC to be ≥95% purity. High-resolution mass spectrometry (HRMS) data were obtained from a Waters SYNAPT G2-Si (ion mobility mass spectrometer with nano-electrospray ionization).

FR-900493 (1). ^{1}H NMR (400 MHz, D_2O) δ: 7.79 (d, *J* = 7.9 Hz, 1H), 5.83 (d, *J* = 7.8 Hz, 1H), 5.75 (d, *J* = 2.3 Hz, 1H), 5.20 (s, 1H), 4.33 (dd, *J* = 2.3, 5.1 Hz, 1H), 4.28 (dd, *J* = 2.3, 8.4 Hz, 1H), 4.23−4.19 (m, 2H), 4.16 (dd, *J* = 2.8, 6.1 Hz, 1H), 4.13−4.07 (m, 2H), 3.49 (d, *J* = 8.3 Hz, 1H), 3.18 (dd, *J* = 3.6, 13.7 Hz, 1H), 3.05−3.00 (m, 3H), 2.88−2.83 (m, 1H), 2.64−2.61 (m, 1H), 2.41 (s, 3H), 1.94−1.88 (m, 1H), 1.82−1.78 (m, 1H); ^{13}C NMR (101 MHz, D_2O) δ: 175.72, 171.35, 155.21, 141.90, 110.08, 102.46, 91.42, 83.74, 80.76, 78.53, 75.41, 74.20, 71.41, 71.34, 69.98, 52.35, 41.81, 39.02, 38.65, 24.99; HRMS (ESI^+) *m*/*z*: calcd for $C_{20}H_{34}N_5O_{11}$ [M + H], 520.2255; found, 520.2262.

FR-900493-5′S, 6′R-Diastereomer (4). ^{1}H NMR (400 MHz, D_2O) δ: 7.83 (d, *J* = 8.0 Hz, 1H), 5.90 (d, *J* = 8.3 Hz, 1H), 5.73 (d, *J* = 2.1 Hz, 1H), 5.25 (s, 1H), 4.36 (d, *J* = 7.7 Hz, 1H), 4.28−4.19 (m, 2H), 4.18 (d, *J* = 10.4 Hz, 1H), 4.12−4.06 (m, 2H), 3.45 (d, *J* = 10.4 Hz, 1H), 3.32 (dd, *J* = 14.1, 3.7 Hz, 1H), 3.15 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.10−3.03 (m, 3H), 2.97−2.87 (m, 1H), 2.59−2.51 (m, 1H), 2.43 (s, 3H), 1.99−1.93 (m, 1H), 1.87−1.77 (m, 1H); ^{13}C NMR (101 MHz, D_2O) δ: 175.72, 171.35, 155.21, 141.90, 110.08, 102.46, 91.42, 83.74, 80.76, 78.53, 75.41, 74.20, 71.41, 71.34, 69.98, 52.35, 41.81, 39.02, 38.65, 24.99; HRMS (ESI^+) *m*/*z*: calcd for $C_{20}H_{34}N_5O_{11}$ [M + H], 520.2255; found, 520.2272.

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*FR-900493-5′R,6′S-Diastereomer (**5**).* ^{1}H NMR (400 MHz, D_2O) δ: 7.68 (d, J = 8.2 Hz, 1H), 5.93 (d, J = 8.1 Hz, 1H), 5.77 (d, J = 4.3 Hz, 1H), 5.33 (s, 1H), 4.50−4.44 (m, 3H), 4.20−4.11 (m, 3H), 4.06 (dd, J = 5.2, 4.7 Hz, 1H), 3.61 (d, J = 6.9 Hz, 1H), 3.37 (d, J = 13.2 Hz, 1H), 3.13 (dd, J = 13.2, 8.7 Hz, 1H), 3.06 (t, J = 7.3 Hz, 2H), 2.84 (dt, J = 13.5, 6.6 Hz, 1H), 2.74 (dt, J = 12.8, 6.7 Hz, 1H), 2.44 (s, 3H), 1.89 (dq, J = 13.8, 6.6 Hz, 2H); ^{13}C NMR (101 MHz, D_2O) δ: 175.72, 171.35, 155.21, 141.90, 110.08, 102.46, 91.42, 83.74, 80.76, 78.53, 75.41, 74.20, 71.41, 71.34, 69.98, 52.35, 41.81, 39.02, 38.65, 24.99; HRMS (ESI$^+$) m/z: calcd for $C_{20}H_{34}N_5O_{11}$ [M + H], 520.2255; found, 520.2251.

*FR-900493-5′R, 6′R-Diastereomer (**6**).* ^{1}H NMR (400 MHz, D_2O) δ: 7.67 (d, J = 8.0 Hz, 1H), 5.91 (d, J = 8.0 Hz, 1H), 5.78 (d, J = 5.2 Hz, 1H), 5.31 (s, 1H), 4.49−4.44 (m, 1H), 4.43 (t, J = 5.3 Hz, 2H), 4.16−4.07 (m, 3H), 4.05 (t, J = 5.3 Hz, 1H), 3.54 (d, J = 7.3 Hz, 1H), 3.23 (dd, J = 13.3, 3.0 Hz, 1H), 3.10−2.98 (m, 3H), 2.80 (dt, J = 13.3, 6.7 Hz, 1H), 2.67 (dt, J = 13.3, 6.9 Hz, 1H), 2.38 (s, 3H), 1.86 (dq, J = 13.9, 7.1 Hz, 2H); HRMS (ESI$^+$) m/z: calcd for $C_{20}H_{34}N_5O_{11}$ [M + H], 520.2255; found, 520.2247.

*FR-900493-Amide (**7**).* ^{1}H NMR (400 MHz, D_2O) δ: 7.83 (d, J = 8.1 Hz, 1H), 5.91 (d, J = 8.0 Hz, 1H), 5.72 (s, 1H), 5.27 (s, 1H), 4.44−4.37 (m, 1H), 4.32−4.25 (m, 2H), 4.23 (t, J = 6.5 Hz, 1H), 4.20−4.14 (m, 1H), 4.11 (d, J = 8.2 Hz, 1H), 3.68 (dd, J = 16.3, 8.1 Hz, 1H), 3.43 (d, J = 14.0 Hz, 1H), 3.26 (dd, J = 13.6, 5.2 Hz, 2H), 3.07 (t, J = 7.3 Hz, 2H), 3.03−2.95 (m, 1H), 2.64−2.56 (m, 1H), 2.50 (s, 3H), 2.04−1.94 (m, 1H), 1.88−1.79 (m, 1H); ^{13}C NMR (101 MHz, D_2O) δ: 175.72, 171.35, 155.21, 141.90, 110.08, 102.46, 91.42, 83.74, 80.76, 78.53, 75.41, 74.20, 71.41, 71.34, 69.98, 52.35, 41.81, 39.02, 38.65, 24.99; HRMS (ESI$^+$) m/z: calcd for $C_{20}H_{35}N_6O_{10}$ [M + H], 519.2415; found, 519.2432.

*De N-Methyl FR-900493 (**8**).* ^{1}H NMR (400 MHz, D_2O) δ: 7.78 (d, J = 8.1 Hz, 1H), 5.89 (d, J = 7.8 Hz, 1H), 5.78 (d, J = 3.1 Hz, 1H), 5.22 (s, 1H), 4.40 (dd, J = 5.5, 3.2 Hz, 1H), 4.29 (t, J = 6.3 Hz, 1H), 4.22 (dd, J = 6.8, 5.1 Hz, 1H), 4.18 (t, J = 4.5 Hz, 1H), 4.16−4.08 (m, 3H), 3.59 (d, J = 3.8 Hz, 1H), 3.28 (d, J = 12.6 Hz, 1H), 3.11−3.02 (m, 3H), 2.77 (dt, J = 12.8, 6.8 Hz, 1H), 2.64 (dt, J = 12.7, 7.0 Hz, 1H), 1.83 (quin, J = 7.1 Hz, 2H); ^{13}C NMR (101 MHz, D_2O) δ: 175.72, 171.35, 155.21, 141.90, 110.08, 102.46, 91.42, 83.74, 80.76, 78.53, 75.41, 74.20, 71.41, 71.34, 69.98, 52.35, 39.02, 38.65, 24.99; HRMS (ESI$^+$) m/z: calcd for $C_{19}H_{33}N_6O_{10}$ [M + H], 505.2258; found, 505.2277.

*Synthesis of UT-17415 (**9**).* To a stirred solution of **22** (2.9 mg, 1.6 μmol) in CH_2Cl_2 (0.70 mL) was added TFA (0.30 mL). The reaction mixture was stirred for 2 h at room temperature (rt), and all volatiles were evaporated in vacuo. To a stirred solution of the crude mixture in H_2O (0.2 mL) was added TFA (0.8 mL). The reaction mixture was stirred for 4 h at 40 °C, and all volatiles were evaporated in vacuo. The crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH 80:20 to $CHCl_3$/MeOH/H_2O/50% aq ammonia 56:42:7:3) to afford UT-17415 (**9**) (1.2 mg, 1.6 μmol, 100%): TLC (*n*-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.55; HPLC condition, column: Phenomenex Kinetex 1.7 μ XB-C18 100 Å 150 × 2.10 mm column, solvents: 80:20 MeOH/0.05 M NH_4HCO_3 in water, UV: 254 nm; $[\alpha]_D^{20}$ +0.038 (c = 0.12, methanol); IR (thin film) ν_{max}: 3333 (br), 2955, 2926, 2855, 1676, 1541, 1515, 1467, 1413, 1273, 1204, 1135, 1063, 1010, 840, 801, 722 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.82 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.1 Hz, 2H), 5.76 (d, J = 8.0 Hz, 1H), 5.70 (s, 1H), 5.18 (s, 1H), 4.58 (s, 1H), 4.28 (d, J = 9.3 Hz, 1H), 4.21−4.16 (m, 3H), 4.14−4.07 (m, 3H), 3.61 (d, J = 9.4 Hz, 1H), 3.21 (dd, J = 13.6, 3.4 Hz, 1H), 2.83 (td, J = 12.1, 11.7, 5.0 Hz, 1H), 2.57 (t, J = 7.6 Hz, 2H), 2.46 (s, 3H), 2.46−2.40 (m, 2H), 2.00−1.89 (m, 1H), 1.79 (d, J = 12.4 Hz, 1H), 1.63−1.55 (m, 2H), 1.37−1.23 (m, 10H), 0.91−0.87 (m, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ: 173.86, 172.29, 142.40, 140.18, 137.30, 129.69 (2C), 121.48 (2C), 112.07, 102.41, 92.48, 84.18, 80.50, 78.40, 76.43, 75.35, 71.95, 70.75, 68.52, 54.47, 39.45, 36.33, 35.02, 33.01, 32.78, 30.29, 30.26, 24.30, 23.70, 14.42; HRMS (ESI$^+$) m/z: calcd for $C_{34}H_{53}N_6O_{11}$ [M + H], 721.3772; found, 721.3761.

*Synthesis of UT-17455 (**10**).* To a stirred solution of **21** (7.9 mg, 4.5 μmol) in CH_2Cl_2 (0.70 mL) was added TFA (0.30 mL). The reaction mixture was stirred for 1 h at rt, and all volatiles were evaporated in vacuo. To a stirred solution of the crude mixture in H_2O (0.2 mL) was added TFA (0.8 mL). The reaction mixture was stirred for 2 h at 40 °C, and all volatiles were evaporated in vacuo. The crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH 80:20 to $CHCl_3$/MeOH/H_2O/50% aq ammonia 56:42:7:3) to afford UT-17455 (**10**) (2.4 mg, 3.4 μmol, 76%): TLC (*n*-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.50; HPLC condition: column: Phenomenex Kinetex 1.7 μ XB-C18 100 Å 150 × 2.10 mm column, solvents: 80:20 MeOH/0.05 M NH_4HCO_3 in water, UV: 254 nm; $[\alpha]_D^{21}$ +0.538 (c = 0.24, methanol); IR (thin film) ν_{max}: 3302 (br), 2955, 2926, 2855, 1672, 1605, 1542, 1515, 1466, 1412, 1271, 1203, 1185, 1131, 1111, 1062, 1010, 819, 721 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.77 (d, J = 8.1 Hz, 1H), 7.43 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 5.74 (s, 1H), 5.73 (d, J = 12.6 Hz, 1H), 5.14 (s, 1H), 4.21 (dd, J = 4.7, 4.2 Hz, 1H), 4.19−4.13 (m, 2H), 4.11 (t, J = 4.7 Hz, 1H), 4.08 (s, 2H), 4.02−3.99 (m, 1H), 3.50 (d, J = 8.9 Hz, 1H), 3.24 (d, J = 13.0 Hz, 1H), 3.16−3.09 (m, 1H), 2.73−2.60 (m, 2H), 2.57 (t, J = 7.7 Hz, 2H), 2.43 (dd, J = 7.4, 4.0 Hz, 2H), 1.86 (quin, J = 7.2 Hz, 2H), 1.59 (quin, J = 6.4, 5.7 Hz, 2H), 1.35−1.26 (m, 8H), 0.92−0.87 (m, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ: 177.20, 174.18, 166.14, 152.10, 142.63, 140.13, 137.35, 129.68 (2C), 121.45 (2C), 110.58, 102.67, 92.49, 85.22, 80.48, 76.41, 75.02, 72.97, 71.23, 64.28, 43.15, 36.34, 35.45, 33.01, 32.78, 30.29, 26.60, 23.70, 14.42; HRMS (ESI$^+$) m/z: calcd for $C_{33}H_{51}N_6O_{11}$ [M + H], 707.3616; found, 707.3624.

*Synthesis of UT-17460 (**11**).* To a stirred solution of **23** (5.3 mg, 2.7 μmol) in CH_2Cl_2 (0.70 mL) was added TFA (0.30 mL). The reaction mixture was stirred for 1 h at rt, and all volatiles were evaporated in vacuo. To a stirred solution of the crude mixture in H_2O (0.2 mL) was added TFA (0.8 mL). The reaction mixture was stirred for 2 h at 40 °C, and all volatiles were evaporated in vacuo. The crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH 80:20 to $CHCl_3$/MeOH/H_2O/50% aq ammonia 56:42:7:3) to afford UT-17460 (**11**) (2.2 mg, 2.5 μmol, 91%): TLC (*n*-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.50; HPLC condition: column, Phenomenex Kinetex 1.7 μ XB-C18 100 Å 150 × 2.10 mm column, solvents: 75:25 MeOH/0.05 M NH_4HCO_3 in water, UV: 254 nm; $[\alpha]_D^{21}$ +0.375 (c = 0.30, methanol); IR (thin film) ν_{max}: 3352 (br), 2932, 1677, 1505, 1270, 1243, 1201, 1136, 1060, 840, 801, 722 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.78 (d, J = 8.1 Hz, 1H), 7.18 (dd, J = 9.0, 3.5 Hz, 4H), 7.00 (dd, J = 16.0, 8.6 Hz, 4H), 5.77 (d, J = 2.9 Hz, 1H), 5.73 (d, J = 8.1 Hz, 1H), 5.14 (s, 1H), 4.57−4.50 (m, 1H), 4.28 (s, 2H), 4.22−4.13 (m, 3H), 4.10 (dd, J = 8.6, 4.4 Hz, 1H), 4.07−3.98 (m, 2H), 3.52−3.46 (m, 3H), 3.44 (d, J = 8.8 Hz, 1H), 3.17 (d, J = 13.0 Hz, 1H), 3.14−3.02 (m, 3H), 2.60 (ddq, J = 18.4, 11.8, 6.9 Hz, 2H), 2.29 (td, J = 7.3, 2.8 Hz, 2H), 2.12 (dd, J = 14.5, 5.6 Hz, 2H), 1.93−1.73 (m, 4H), 1.39−1.25 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ: 175.58, 166.16, 157.62, 152.01, 142.55, 131.22,

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129.62 (2C), 123.56 (2C), 118.11 (2C), 118.07 (2C), 110.51, 102.67, 92.31, 85.27, 81.41, 80.43, 76.47, 75.08, 74.07 (2C), 73.01, 71.26, 64.44, 43.72, 43.62, 34.66, 31.48, 26.89; HRMS (ESI$^+$) m/z: calcd for $C_{39}H_{51}F_3N_7O_{13}$ [M + H], 882.3497; found, 882.3512.

*UT-17465 (**12**).* To a stirred solution of **24** (4.7 mg, 2.4 μmol) in CH_2Cl_2 (0.70 mL) was added TFA (0.30 mL). The reaction mixture was stirred for 2 h at rt, and all volatiles were evaporated in vacuo. To a stirred solution of the crude mixture in H_2O (0.2 mL) was added TFA (0.8 mL). The reaction mixture was stirred for 4 h at 40 °C, and all volatiles were evaporated in vacuo. The crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH 80:20 to $CHCl_3$/MeOH/H_2O/50% aq ammonia 56:42:7:3) to afford UT-17465 (**12**) (2.0 mg, 2.2 μmol, 92%): TLC (*n*-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.55; HPLC condition: column: Phenomenex Kinetex 1.7 μ XB-C18 100 Å 150 × 2.10 mm column, solvents: 70:30 MeOH/0.05 M NH_4HCO_3 in water, UV: 254 nm; $[\alpha]_D^{20}$ +0.246 (c = 0.24, methanol); IR (thin film) ν_{max}: 3276 (br), 2933, 1675, 1505, 1465, 1424, 1271, 1243, 1199, 1111 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.84 (d, J = 7.7 Hz, 1H), 7.19 (dd, J = 8.5, 3.3 Hz, 4H), 7.01 (dd, J = 13.1, 8.6 Hz, 4H), 5.86 (d, J = 7.8 Hz, 1H), 5.73 (d, J = 2.4 Hz, 1H), 5.16 (s, 1H), 4.54 (tt, J = 7.3, 3.1 Hz, 1H), 4.30−4.25 (m, 3H), 4.26 (d, J = 9.2 Hz, 1H), 4.22−4.05 (m, 6H), 3.70 (d, J = 9.2 Hz, 1H), 3.52−3.44 (m, 2H), 3.09 (ddt, J = 12.3, 8.6, 4.3 Hz, 2H), 2.91−2.82 (m, 1H), 2.58−2.53 (m, 1H), 2.50 (s, 3H), 2.30 (q, J = 6.9 Hz, 2H), 2.15−2.08 (m, 2H), 1.96−1.82 (m, 3H), 1.81−1.71 (m, 1H), 1.39−1.25 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ: 175.34, 172.18, 157.63, 151.97, 142.38, 131.33, 129.66 (2C), 123.55 (2C), 118.12 (2C), 118.08 (2C), 111.86, 92.05, 84.35, 80.28, 78.36, 76.42, 75.37, 74.08 (2C), 71.54, 70.80, 68.28, 43.73, 39.72, 34.50, 31.48 (2C), 24.52; HRMS (ESI$^+$) m/z: calcd for $C_{40}H_{53}F_3N_7O_{13}$ [M + H], 896.3653; found, 896.3640.

*MTPM-Protected Uridine **13**.* The MTPM-protected uridine **13** was synthesized according to the reported procedure.[24,34] TLC (hexane/EtOAc 33:67) R_f = 0.50; $[\alpha]_D^{22}$ −2.315 (c = 8.98, $CHCl_3$); IR (thin film) ν_{max}: 3478 (br), 3057, 2986, 2940, 1715, 1663, 1598, 1556, 1455, 1374, 1265, 1212, 1066, 1039, 853, 808, 787, 732, 702 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (ddd, J = 17.3, 8.5, 0.7 Hz, 1H), 7.33−7.27 (m, 2H), 7.18 (ddd, J = 8.8, 6.9, 2.1 Hz, 1H), 6.85 (d, J = 5.1 Hz, 2H), 6.51 (d, J = 3.7 Hz, 1H), 5.72 (dd, J = 8.1, 4.0 Hz, 1H), 5.60−5.51 (m, 2H), 5.48 (dd, J = 12.6, 2.1 Hz, 1H), 4.95−4.93 (m, 1H), 4.92 (t, J = 2.4 Hz, 1H), 4.30−4.25 (m, 1H), 3.89 (ddd, J = 11.7, 9.1, 2.6 Hz, 1H), 3.82−3.73 (m, 4H), 1.57 (s, 3H), 1.36 (d, J = 2.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.10, 162.05, 159.51, 150.97, 150.91, 141.55, 141.31, 136.88, 135.28, 135.22, 134.02, 134.00, 133.84, 133.71, 131.17, 129.38, 126.22, 126.18, 125.44, 125.40, 115.33, 114.29, 114.26, 102.05, 102.01, 97.16, 96.82, 87.15, 87.06, 83.76, 83.65, 80.29, 69.50, 62.76, 62.72, 55.69, 27.25, 25.25; HRMS (ESI$^+$) m/z: calcd for $C_{27}H_{26}N_2O_8NaCl_4$ [M + Na], 669.0341; found, 669.0324.

*Propargyl Alcohol **14**.* To a stirred solution of **13** (1.54 g, 2.37 mmol) and dichloroacetic acid (0.29 mL, 3.56 mmol) in CH_2Cl_2 (11.9 mL) and DMSO (2.37 mL) was added *N*,*N*′-diisopropylcarbodiimide (0.56 mL, 3.56 mmol) at 0 °C, and the reaction mixture was warmed to rt. After 8 h, the reaction was quenched with saturated aq $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The precipitates were filtered, and the crude mixture was used for the next reaction without purification. To a suspension of $Zn(OTf)_2$ (3.45 g, 9.48 mmol) and (+)-*N*-methylephedrine (1.70 g, 9.48 mmol) in toluene (7.1 mL) was added Et_3N (1.32 mL, 9.48 mmol) at rt. After 3 h, 4-phenyl-1-butyne (1.33 mL, 9.48 mmol) was added. After 4 h, a solution of the crude aldehyde in toluene (5 mL) was added. The reaction mixture was stirred for 15 h, quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexane/EtOAc 60:40 to 50:50) to afford **14** (1.48 g, 1.90 mmol, 80% for 2 steps): TLC (hexane/EtOAc 50:50) R_f = 0.30; $[\alpha]_D^{22}$ −0.116 (c = 2.17, $CHCl_3$); IR (thin film) ν_{max}: 3387 (br), 3087, 2981, 2937, 1716, 1664, 1597, 1556, 1454, 1374, 1276, 1211, 1156, 1065, 1039, 916, 856, 807, 786, 733, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (ddd, J = 20.4, 8.5, 0.7 Hz, 1H), 7.35−7.27 (m, 4H), 7.24−7.15 (m, 4H), 6.85 (d, J = 5.1 Hz, 2H), 6.51 (d, J = 5.4 Hz, 1H), 5.68 (dd, J = 8.1, 4.1 Hz, 1H), 5.60−5.50 (m, 3H), 4.89−4.78 (m, 2H), 4.57 (ddt, J = 12.0, 4.3, 2.0 Hz, 1H), 4.24 (dd, J = 4.4, 3.1 Hz, 1H), 3.78 (d, J = 3.3 Hz, 3H), 2.83 (t, J = 7.5 Hz, 2H), 2.53 (td, J = 7.4, 2.0 Hz, 2H), 1.57 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.11, 162.08, 159.50, 150.87, 150.85, 141.07, 140.84, 140.30, 140.27, 136.90, 135.36, 135.29, 133.99, 133.95, 133.79, 133.64, 131.21, 129.37, 129.34, 128.41, 128.39, 126.40, 126.21, 126.18, 125.49, 125.44, 115.34, 115.32, 114.28, 114.24, 101.79, 101.74, 96.69, 96.37, 89.23, 89.19, 86.83, 86.73, 84.09, 83.93, 80.91, 69.46, 63.02, 62.99, 55.68, 34.72, 34.70, 27.16, 25.29, 20.87, 20.85; HRMS (ESI$^+$) m/z: calcd for $C_{37}H_{34}N_2O_8NaCl_4$ [M + Na], 797.0967; found, 797.0994.

*Ribosylation of **14**.* To a stirred suspension of **14** (227 mg, 0.292 mmol), **15** (497 mg, 0.584 mmol), MS3Å (900 mg), and $SrCO_3$ (431 mg, 2.920 mmol) in CH_2Cl_2 (12.0 mL) were added $AgBF_4$ (28.5 mg, 0.146 mmol) and NIS (131 mg, 0.584 mmol) at 0 °C. After 24 h, the reaction mixture was added to Et_3N (2 mL) and passed through a silica gel pad (hexane/EtOAc 1:1). The combined organic phase was concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexane/EtOAc 90:10 to 80:20 to 70:30) to afford **16** (416 mg, 0.277 mmol, 95%): TLC (hexane/EtOAc 67:33) R_f = 0.70; $[\alpha]_D^{21}$ +0.100 (c = 2.09, $CHCl_3$); IR (thin film) ν_{max}: 2942, 2892, 2866, 2102, 1743, 1724, 1675, 1600, 1556, 1456, 1382, 1371, 1278, 1218, 1099, 1070, 1049, 1013, 999, 882, 807, 772, 745, 681 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (dd, J = 23.1, 8.5 Hz, 1H), 7.32−7.27 (m, 4H), 7.24−7.16 (m, 4H), 6.84 (d, J = 7.3 Hz, 2H), 6.51 (d, J = 3.7 Hz, 1H), 5.71−5.64 (m, 2H), 5.60−5.49 (m, 2H), 5.20−5.16 (m, 3H), 4.79 (ddd, J = 7.5, 6.5, 3.1 Hz, 1H), 4.64 (td, J = 5.9, 2.6 Hz, 1H), 4.57 (ddt, J = 11.4, 6.3, 1.9 Hz, 1H), 4.28 (dt, J = 6.2, 2.8 Hz, 1H), 4.19 (tt, J = 6.1, 3.0 Hz, 1H), 3.79−3.72 (m, 7H), 3.50 (ddd, J = 13.0, 7.6, 3.3 Hz, 1H), 3.35 (dd, J = 13.0, 3.4 Hz, 1H), 2.83 (t, J = 7.4 Hz, 2H), 2.55 (td, J = 7.4, 1.8 Hz, 2H), 2.29 (t, J = 1.6 Hz, 2H), 2.24 (dd, J = 5.1, 2.1 Hz, 2H), 1.62−1.55 (m, 7H), 1.36 (d, J = 2.0 Hz, 3H), 1.08−1.00 (m, 54H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 175.61, 170.98, 170.88, 170.71, 170.70, 170.64, 162.17, 162.14, 159.45, 150.75, 150.72, 140.39, 140.19, 140.15, 140.13, 136.92, 136.91, 135.43, 135.29, 133.94, 133.80, 133.65, 131.24, 129.36, 129.31, 128.46 (2C), 128.40 (2C), 126.46, 126.44, 126.19, 126.10, 125.56, 125.45, 115.29, 115.25, 114.23, 114.22, 104.61, 104.55, 101.83, 101.82, 88.84, 88.20, 84.44, 84.35, 83.93, 81.43, 81.33, 80.57, 79.88, 76.49, 75.86, 75.83, 74.12, 71.78, 71.73, 71.40, 70.73, 69.60, 69.47, 68.85, 68.78, 59.97, 59.96, 55.67, 46.15, 45.97, 44.72, 44.58, 34.65, 34.51, 34.49, 32.67, 32.61, 32.57, 28.00, 27.38, 27.35, 27.30, 27.07, 25.34, 25.27, 20.89, 18.05 (12C), 11.91 (6C);

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HRMS (ESI$^+$) m/z: calcd for $C_{74}H_{106}Cl_4N_5O_{15}Si_2$ [M + H], 1500.5978; found, 1500.5992.

Synthesis of ***17***. A suspension of **16** (286 mg, 0.19 mmol), NH_4Cl (305 mg, 5.70 mmol), and Zn (373 mg, 5.70 mmol) in EtOH/H_2O (9:1, 9.5 mL) was stirred at 80 °C for 12 h and cooled to rt. The precipitates were filtered, and the combined organic solution was concentrated in vacuo. The crude mixture was used for the next reaction without purification. To a stirred solution of the crude amide in THF (9.5 mL) were added saturated aq $NaHCO_3$ (9.5 mL) and Boc_2O (124 mg, 0.57 mmol). The reaction mixture was stirred for 6 h at rt, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexane/EtOAc 85:15 to 80:20 to 67:33) to afford **17** (258 mg, 0.16 mmol, 86% for 2 steps): TLC (hexane/EtOAc 70:30) R_f = 0.30; $[\alpha]_D^{21}$ +0.012 (c = 0.90, $CHCl_3$); IR (thin film) ν_{max}: 3387 (br), 3090, 2941, 2866, 1742, 1720, 1676, 1600, 1556, 1505, 1456, 1366, 1278, 1219, 1161, 1100, 1070, 1049, 1013, 998, 882, 806, 772, 745, 681 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (dd, J = 19.9, 8.5 Hz, 1H), 7.33−7.27 (m, 4H), 7.24−7.16 (m, 4H), 6.85 (d, J = 7.3 Hz, 2H), 6.51 (d, J = 4.8 Hz, 1H), 5.72−5.64 (m, 2H), 5.60−5.48 (m, 2H), 5.26 (d, J = 6.0 Hz, 1H), 5.17 (d, J = 8.6 Hz, 2H), 5.13−5.08 (m, 1H), 4.82−4.76 (m, 1H), 4.65 (t, J = 7.0 Hz, 1H), 4.51 (dd, J = 13.8, 6.0 Hz, 1H), 4.31−4.26 (m, 1H), 4.23−4.17 (m, 1H), 3.78 (d, J = 2.7 Hz, 3H), 3.74 (d, J = 6.9 Hz, 4H), 3.48−3.40 (m, 1H), 3.36−3.26 (m, 1H), 2.83 (t, J = 7.4 Hz, 2H), 2.56 (t, J = 7.5 Hz, 2H), 2.27 (t, J = 2.6 Hz, 2H), 2.23 (t, J = 3.0 Hz, 2H), 1.62−1.55 (m, 7H), 1.42 (s, 9H), 1.37 (d, J = 2.6 Hz, 3H), 1.11−0.99 (m, 54H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.46, 150.79, 136.91, 131.26, 129.33, 128.48 (2C), 128.37 (2C), 126.48, 126.11, 125.44, 115.30, 115.26, 79.95, 59.98, 55.68, 46.42, 46.16, 45.95, 44.83, 44.78, 42.49, 34.51, 34.49, 32.60, 32.55, 31.91, 29.69, 28.71, 28.40, 28.34, 27.37, 27.33, 27.29, 27.26, 27.09, 27.05, 25.36, 22.68, 22.63, 20.87, 18.06 (6C), 17.90 (6C), 14.12, 11.92 (3C), 11.78 (3C); HRMS (ESI$^+$) m/z: calcd for $C_{79}H_{116}Cl_4N_3O_{17}Si_2$ [M + H], 1574.6597; found, 1574.6609.

Synthesis of ***21S***. To a stirred solution of **17** (258 mg, 0.16 mmol) and quinoline (38.7 μL, 0.33 mmol) in EtOAc (50 mL) and MeOH (50 mL) was added Lindlar catalyst (300 mg). H_2 gas was introduced, and the reaction mixture was stirred under a H_2 atmosphere (600 psi) at rt. The reaction mixture was stirred for 11 h under a H_2 atmosphere (600 psi) at rt. The reaction mixture was filtered through Celite, and the filtrate was washed with 1 N HCl. The combined organic solution was dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was used for the next reaction without purification. To a stirred solution of the crude mixture in t-BuOH/acetone (1:1, 2.1 mL) were added NMO (192 mg, 1.64 mmol) and OsO_4 (4% in water, 1.04 mL, 0.16 mmol) at rt. After 2 h, the reaction solution was diluted with EtOAc and quenched with saturated aq $NaHCO_3$/saturated aq Na_2SO_3 (2:1). The heterogeneous mixture was stirred for 30 min and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was passed through a silica gel pad (hexane/EtOAc 33:67) to afford the diols as a diastereomeric mixture. This mixture was used for the next reaction without further purification. To a stirred solution of the diols (22.1 mg, 0.014 mmol) and $NaHCO_3$ (11.5 mg, 0.14 mmol) in CH_2Cl_2 (0.7 mL) was added $Pb(OAc)_4$ (12.1 mg, 0.027 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude aldehyde **18** was used for the next reaction without purification. To a stirred solution of $(BnO)_2P(O)-CH_2-P(O)(OBn)OH$ (30.6 mg, 0.069 mmol) in CH_2Cl_2 (0.4 mL) was added a CH_2Cl_2 (0.3 mL) solution of **18** and **19**. To the reaction mixture was added TMSCN (17.1 μL, 0.14 mmol) and stirred for 9 h at rt. After completion, the reaction mixture was quenched with saturated aq $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane/EtOAc 80:20 to 60:40) to afford the Strecker products. To a stirred solution of the desired Strecker product (8.8 mg, 5.0 μmol) in EtOH/H_2O (9:1, 0.5 mL) were added $HgCl_2$ (2.7 mg, 0.010 mmol) and acetaldoxime (3.0 μL, 0.050 mmol) at rt. After being stirred for 6 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated aq $NaHCO_3$ and extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane/EtOAc 80:20 to 60:40) to afford ***21S*** (16.7 mg, 9.49 μmol, 69% for two steps) and ***21R*** (4.1 mg, 2.34 μmol, 17% for two steps): ***21S***: TLC (hexane/EtOAc 60:40) R_f = 0.40; $[\alpha]_D^{21}$ +0.075 (c = 0.73, $CHCl_3$); IR (thin film) ν_{max}: 3317 (br), 2930, 2865, 1719, 1675, 1600, 1462, 1102, 1071, 882, 772, 683 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (s, 1H), 7.49 (dd, J = 11.4, 8.8 Hz, 1H), 7.39 (d, J = 7.9 Hz, 2H), 7.32 (s, 1H), 7.19 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 9.3 Hz, 2H), 6.50 (d, J = 15.4 Hz, 1H), 5.73 (dd, J = 23.0, 8.0 Hz, 1H), 5.59 (d, J = 5.9 Hz, 1H), 5.54 (d, J = 9.4 Hz, 2H), 5.42 (t, J = 10.1 Hz, 1H), 5.25 (s, 1H), 5.08−5.00 (m, 2H), 4.96−4.82 (m, 2H), 4.50−4.45 (m, 1H), 4.25−4.19 (m, 1H), 4.15−4.06 (m, 1H), 3.94−3.83 (m, 1H), 3.80−3.63 (m, 10H), 3.49−3.41 (m, 1H), 3.39−3.31 (m, 1H), 3.03 (dt, J = 12.0, 6.1 Hz, 1H), 2.71−2.61 (m, 1H), 2.54 (t, J = 7.3 Hz, 2H), 2.51−2.45 (m, 1H), 2.29−2.17 (m, 4H), 1.67−1.51 (m, 10H), 1.41 (s, 9H), 1.28 (dd, J = 15.7, 8.1 Hz, 10H), 1.05 (s, 42H), 1.01 (s, 6H), 0.95 (s, 6H), 0.87 (t, J = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 170.9, 159.5, 136.9, 136.8, 131.3, 131.2, 129.42, 129.36, 128.84 (2C), 128.82 (2C), 128.80 (2C), 126.4, 126.2, 125.1, 120.09, 120.05, 115.4, 115.31, 115.30, 114.84, 114.81, 84.90, 84.87, 80.84, 80.78, 80.2, 79.8, 79.4, 78.2, 76.1, 74.3, 60.0, 59.9, 55.8, 55.7, 52.0, 46.2, 46.0, 44.83, 44.77, 35.4, 32.56, 32.55, 31.8, 31.5, 29.7, 29.19, 29.16, 28.4, 28.3, 27.3, 27.2, 22.7, 18.1 (12C), 14.1, 11.9 (6C); HRMS (ESI$^+$) m/z: calcd for $C_{88}H_{135}Cl_4N_6O_{18}Si_2$ [M + H], 1759.8126; found, 1759.8135.

21R. TLC (hexane/EtOAc 60:40) R_f = 0.30; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (dd, J = 16.8, 11.4 Hz, 1H), 7.51 (dd, J = 11.7, 8.5 Hz, 1H), 7.47−7.29 (m, 3H), 7.23−7.15 (m, 2H), 7.10 (t, J = 9.5 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.49 (d, J = 4.6 Hz, 1H), 5.76 (dd, J = 21.1, 8.1 Hz, 1H), 5.65 (d, J = 23.4 Hz, 1H), 5.56 (d, J = 9.2 Hz, 1H), 5.52 (d, J = 3.9 Hz, 1H), 5.46 (t, J = 10.8 Hz, 1H), 5.40−5.23 (m, 2H), 5.23−5.16 (m, 1H), 5.10−5.05 (m, 1H), 5.02 (s, 1H), 4.90−4.78 (m, 1H), 4.26 (t, J = 6.3 Hz, 1H), 4.21 (d, J = 8.9 Hz, 1H), 3.96−3.89 (m, 1H), 3.80−3.71 (m, 10H), 3.68−3.63 (m, 1H), 3.48−3.39 (m, 1H), 3.04−2.95 (m, 1H), 2.75−2.66 (m, 1H), 2.53 (t, J = 7.7 Hz, 2H), 2.44 (d, J = 12.1 Hz, 1H), 2.37−2.27 (m, 2H), 2.23 (d, J = 14.7 Hz, 2H), 1.65−1.51 (m, 10H), 1.42 (s, 9H), 1.36−1.23 (m, 10H), 1.08−1.01 (m, 42H), 0.97 (s, 6H), 0.90−0.85 (m, 9H); HRMS (ESI$^+$) m/z: calcd for $C_{88}H_{135}Cl_4N_6O_{18}Si_2$ [M + H], 1759.8126; found, 1759.8113.

Synthesis of ***22***. To a stirred solution of **21S** (8.8 mg, 5.0 μmol) in EtOH/H_2O (9:1, 0.5 mL) were added $HgCl_2$ (2.7 mg, 0.010 mmol) and acetaldoxime (3.0 μL, 0.050 mmol) at rt. After

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being stirred for 6 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated aq $NaHCO_3$ and extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH 99.5:0.5 to 99.2:0.8 to 98.8:1.2) to afford **22** (7.9 mg, 4.5 μmol, 89%): TLC ($CHCl_3$/MeOH 95:5) R_f = 0.40; IR (thin film) ν_{max}: 3335 (br), 2927, 2865, 1668, 1601, 1460, 1099, 1071, 882, 681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.59 (dd, J = 19.5, 8.5 Hz, 1H), 7.40 (d, J = 8.0 Hz, 2H), 7.30 (t, J = 2.6 Hz, 1H), 7.24−7.18 (m, 1H), 7.11 (d, J = 8.0 Hz, 2H), 6.84 (d, J = 7.2 Hz, 2H), 6.50 (s, 1H), 5.84 (br s, 1H), 5.59−5.47 (m, 3H), 5.26−5.14 (m, 2H), 5.06−4.97 (m, 1H), 4.96−4.87 (m, 1H), 4.84−4.73 (m, 1H), 4.55 (t, J = 5.0 Hz, 1H), 4.28−4.14 (m, 2H), 3.80−3.70 (m, 7H), 3.59−3.46 (m, 1H), 3.41 (brs, 2H), 2.83 (brs, 2H), 2.54 (t, J = 7.7 Hz, 3H), 2.50−2.43 (m, 1H), 2.29−2.21 (m, 4H), 1.99 (brs, 2H), 1.65−1.53 (m, 10H), 1.43 (s, 9H), 1.35 (d, J = 5.2 Hz, 3H), 1.32−1.24 (m, 10H), 1.05 (d, J = 3.2 Hz, 48H), 1.00−0.97 (m, 6H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.6, 159.5, 136.87, 136.85, 136.4, 135.2, 134.0, 133.64, 133.59, 131.3, 129.42, 129.40, 128.9 (2C), 126.2, 125.3, 120.2, 120.1, 115.4 (2C), 74.5, 60.0, 59.9, 55.73, 55.72, 46.2, 46.1, 46.0, 44.8, 35.4, 32.7, 32.6, 31.8, 31.5, 29.69, 29.67, 29.6, 29.5, 29.4, 29.3, 29.24, 29.16, 29.09, 28.51, 28.49, 28.48, 28.45, 28.43, 28.42, 28.36, 28.33, 28.31, 28.28, 27.33, 27.30, 27.25, 27.2, 25.3, 22.7, 18.1 (12C), 14.1, 11.9 (6C); HRMS (ESI^+) m/z: calcd for $C_{88}H_{137}Cl_4N_6O_{19}Si_2$ [M + H], 1777.8231; found, 1777.8219.

Synthesis of ***23***. To a stirred solution of **22** (5.8 mg, 3.3 μmol) and paraformaldehyde (2.9 mg, 0.098 mmol) in CH_3CN (0.5 mL) was added $NaB(CN)H_3$ (6.2 mg, 0.098 mmol). After being stirred for 4 h at rt, the reaction mixture was quenched with saturated aq $NaHCO_3$ and extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane/EtOAc 40:60) to afford **23** (5.5 mg, 3.1 μmol, 95%): TLC (hexane/EtOAc 33:67) R_f = 0.60; $[\alpha]_D^{21}$ +0.022 (c = 0.28, $CHCl_3$); IR (thin film) ν_{max}: 2932, 2866, 1718, 1672, 1601, 1463, 1101, 1071, 884 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, J = 17.0 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H), 7.43 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 1H), 7.30 (s, 2H), 7.20 (dt, J = 8.5, 2.0 Hz, 1H), 7.10 (d, J = 8.1 Hz, 2H), 6.85 (s, 2H), 6.51 (d, J = 7.9 Hz, 1H), 6.28 (brs, 1H), 5.95 (d, J = 21.6 Hz, 1H), 5.84−5.78 (m, 1H), 5.74 (d, J = 23.3 Hz, 1H), 5.54 (s, 2H), 5.49 (d, J = 9.6 Hz, 1H), 5.18 (brs, 1H), 5.11 (s, 2H), 5.02 (brs, 1H), 4.88−4.83 (m, 1H), 4.80−4.74 (m, 1H), 4.39−4.31 (m, 2H), 4.24−4.18 (m, 1H), 3.92 (t, J = 5.8 Hz, 1H), 3.78 (s, 3H), 3.74 (q, J = 6.6 Hz, 4H), 3.68−3.63 (m, 1H), 3.50−3.40 (m, 2H), 3.37−3.30 (m, 1H), 2.83−2.74 (m, 1H), 2.68−2.59 (m, 1H), 2.54 (t, J = 7.8 Hz, 2H), 2.49 (s, 3H), 2.37 (q, J = 8.0, 7.6 Hz, 2H), 2.29−2.20 (m, 4H), 1.98−1.88 (m, 2H), 1.62−1.52 (m, 6H), 1.40 (s, 9H), 1.36 (brs, 3H), 1.33−1.23 (m, 6H), 1.09−1.01 (m, 48H), 0.98 (s, 6H), 0.87 (t, J = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.0, 162.0, 159.5, 157.1, 136.9, 131.3, 129.4, 128.7 (2C), 119.9 (2C), 115.3, 115.1, 114.2, 70.6, 70.1, 69.9, 67.1, 60.4, 60.1, 59.96, 59.95, 58.9, 55.8, 55.7, 54.4, 54.1, 46.22, 46.16, 46.1, 45.3, 44.9, 44.8, 44.7, 42.3, 41.2, 39.93, 39.86, 39.6, 39.04, 38.97, 35.4, 32.7, 32.64, 32.63, 32.62, 32.58, 31.9, 31.8, 31.7, 31.6, 31.53, 31.48, 29.69, 29.67, 29.6, 29.4, 29.22, 29.17, 28.50, 28.49, 28.4, 27.29, 27.28, 27.21, 27.17, 25.23, 25.20, 22.68, 22.66, 18.1 (12C), 14.1, 11.9 (6C); HRMS (ESI^+) m/z: calcd for $C_{89}H_{139}Cl_4N_6O_{19}Si_2$ [M + H], 1791.8388; found, 1791.8404.

Synthesis of ***24S***. To a stirred solution of the diols (32.5 mg, 0.020 mmol) synthesized for **21*S*** and $NaHCO_3$ (16.9 mg, 0.20 mmol) in CH_2Cl_2 (1.0 mL) was added $Pb(OAc)_4$ (17.9 mg, 0.040 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude aldehyde **18** was used for the next reaction without purification. To a stirred solution of $(BnO)_2P(O)-CH_2-P(O)(OBn)OH$ (45.0 mg, 0.10 mmol) in CH_2Cl_2 (0.5 mL) was added a CH_2Cl_2 (0.5 mL) solution of **18** and **20**. After 6 h, to the reaction mixture was added TMSCN (25.2 μL, 0.20 mmol) and stirred for 12 h at rt. After completion, the reaction mixture was quenched with saturated aq $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane/EtOAc 80:20 to 60:40) to afford **24*S*** (23.9 mg, 12.0 μmol, 61% for two steps) and **24*R*** (5.1 mg, 2.6 μmol, 13% for two steps). **24*S***: TLC (hexane/EtOAc 50:50) R_f = 0.40; $[\alpha]_D^{21}$ +0.102 (c = 0.75, $CHCl_3$); IR (thin film) ν_{max}: 3342 (br), 2941, 2866, 1718, 1675, 1505, 1464, 1243, 1164, 1101, 1071, 883, 772, 688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.49 (dd, J = 8.5, 4.3 Hz, 1H), 7.32 (d, J = 2.0 Hz, 1H), 7.22−7.11 (m, 7H), 6.94−6.88 (m, 5H), 6.86 (d, J = 6.5 Hz, 2H), 6.50 (d, J = 8.6 Hz, 1H), 6.25−6.16 (m, 1H), 5.73 (dd, J = 22.2, 8.0 Hz, 1H), 5.60 (t, J = 8.8 Hz, 1H), 5.56−5.41 (m, 3H), 5.21 (d, J = 4.4 Hz, 1H), 5.05−4.98 (m, 2H), 4.94−4.77 (m, 2H), 4.53−4.37 (m, 3H), 4.25−4.16 (m, 2H), 4.05−3.98 (m, 1H), 3.80−3.69 (m, 6H), 3.68−3.63 (m, 1H), 3.56 (dd, J = 17.3, 3.4 Hz, 1H), 3.48 (ddt, J = 11.6, 7.2, 4.0 Hz, 2H), 3.44−3.29 (m, 1H), 3.08 (dq, J = 9.5, 5.3, 4.8 Hz, 2H), 2.95 (dt, J = 11.4, 5.5 Hz, 1H), 2.47 (td, J = 12.0, 11.4, 5.7 Hz, 1H), 2.36−2.14 (m, 5H), 2.13−2.05 (m, 2H), 1.97−1.85 (m, 3H), 1.84−1.75 (m, 1H), 1.58 (t, J = 6.9 Hz, 2H), 1.55−1.50 (m, 4H), 1.40 (s, 9H), 1.33 (d, J = 4.8 Hz, 3H), 1.28−1.23 (m, 3H), 1.08−1.02 (m, 42H), 1.01 (s, 6H), 0.94 (d, J = 2.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.4, 171.0, 170.9, 159.5, 155.8, 150.9, 150.7, 142.8, 136.9, 136.8, 135.3, 135.1, 134.13, 134.05, 133.86, 133.85, 133.78, 131.2, 131.1, 129.42, 129.37, 129.0, 126.4, 126.2, 125.5, 125.2, 122.5 (2C), 121.8, 119.3, 118.4, 116.8 (2C), 116.6 (2C), 115.4, 115.3, 114.71, 114.66, 106.4, 102.3, 102.2, 84.8, 80.7, 80.6, 79.9, 79.8, 79.3, 76.2, 74.32, 74.30, 72.9, 60.38, 60.35, 60.0, 59.9, 55.72, 55.71, 52.0, 46.6, 46.2, 45.9, 44.84, 44.77, 42.99, 42.96, 42.4, 41.2, 33.53, 33.49, 32.6, 32.5, 30.3, 28.4, 27.3 (2C), 27.17, 27.16, 27.1, 25.4, 18.1 (12C), 14.2, 14.1, 11.91 (3C), 11.90 (3C); HRMS (ESI^+) m/z: calcd for $C_{94}H_{135}Cl_4F_3N_7O_{20}Si_2$ [M + H], 1934.8007; found, 1934.8021. **24*R***: TLC (hexane/EtOAc 60:40) R_f = 0.30; ^1H NMR (400 MHz, chloroform-d) δ: 7.53 (d, J = 8.7 Hz, 1H), 7.33−7.29 (m, 1H), 7.21−7.07 (m, 7H), 6.94−6.88 (m, 5H), 6.85 (d, J = 6.8 Hz, 2H), 6.54 (s, 1H), 5.71 (d, J = 7.9 Hz, 1H), 5.58−5.49 (m, 3H), 5.46 (t, J = 8.9 Hz, 1H), 5.24−5.20 (m, 1H), 5.15−5.08 (m, 1H), 5.08−5.00 (m, 1H), 4.92 (dd, J = 11.5, 5.6 Hz, 1H), 4.85−4.78 (m, 1H), 4.49−4.39 (m, 2H), 4.39−4.18 (m, 3H), 3.98 (dd, J = 11.1, 5.4 Hz, 1H), 3.81−3.70 (m, 6H), 3.69−3.61 (m, 2H), 3.52−3.43 (m, 2H), 3.37−3.32 (m, 1H), 3.08 (ddd, J = 12.4, 8.5, 3.8 Hz, 2H), 2.99−2.91 (m, 1H), 2.65 (dd, J = 12.8, 6.5 Hz, 1H), 2.31−2.18 (m, 5H), 2.13−2.04 (m, 2H), 1.96−1.85 (m, 4H), 1.67−1.52 (m, 6H), 1.40 (s, 9H), 1.38−1.29 (m, 3H), 1.25 (s, 6H), 1.10−0.98 (m, 42H), 0.97 (s, 6H), 0.88 (t, J = 6.7 Hz, 3H); HRMS (ESI^+) m/z: calcd for $C_{94}H_{135}Cl_4F_3N_7O_{20}Si_2$ [M + H], 1934.8007; found, 1934.8000.

Synthesis of ***25***. To a stirred solution of **24*S*** (15.4 mg, 8.0 μmol) in EtOH/H_2O (9:1, 0.5 mL) were added $HgCl_2$ (4.3 mg,

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0.016 mmol) and acetaldoxime (4.9 μL, 0.080 mmol) at rt. After being stirred for 6 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated aq $NaHCO_3$ and extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH 99.5:0.5 to 99.2:0.8 to 98.8:1.2) to afford **25** (15.3 mg, 7.8 μmol, 98%): TLC ($CHCl_3$/MeOH 95:5) R_f = 0.30; $[\alpha]_D^{21}$ +0.144 (c = 0.53, $CHCl_3$); IR (thin film) ν_{max}: 3335 (br), 2940, 2866, 1719, 1676, 1505, 1464, 1367, 1242, 1162, 1101, 1070, 882, 681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.53 (dd, J = 8.6, 5.1 Hz, 1H), 7.30 (s, 1H), 7.28−7.22 (m, 2H), 7.21−7.12 (m, 6H), 6.91 (d, J = 8.5 Hz, 4H), 6.86 (d, J = 2.6 Hz, 2H), 6.51 (d, J = 8.7 Hz, 1H), 5.94 (brs, 1H), 5.79−5.67 (m, 3H), 5.56−5.47 (m, 2H), 5.17 (brs, 1H), 5.06 (s, 1H), 4.96 (brs, 1H), 4.82−4.73 (m, 2H), 4.43 (tt, J = 7.8, 3.8 Hz, 1H), 4.39−4.28 (m, 3H), 4.21 (brs, 1H), 4.13 (brs, 1H), 3.78 (s, 3H), 3.73 (q, J = 7.4 Hz, 5H), 3.67 (brs, 1H), 3.48 (ddd, J = 11.7, 7.2, 3.7 Hz, 2H), 3.41−3.28 (m, 1H), 3.17 (s, 1H), 3.09 (ddd, J = 12.2, 8.2, 3.3 Hz, 2H), 2.80−2.60 (m, 2H), 2.38−2.15 (m, 7H), 2.13−2.05 (m, 2H), 1.93 (ddd, J = 12.8, 8.0, 3.7 Hz, 2H), 1.85−1.79 (m, 2H), 1.54 (s, 3H), 1.42 (s, 9H), 1.34 (s, 3H), 1.04 (d, J = 2.8 Hz, 42H), 1.01 (s, 6H), 0.96 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.1, 162.0, 159.6, 159.5, 156.2, 155.8, 150.9, 150.4, 142.80, 142.78, 136.88, 136.86, 135.23, 135.21, 133.9, 133.6, 131.33, 131.30, 131.29, 129.40, 129.37, 129.2, 129.1, 129.02, 128.98, 126.24, 126.22, 126.21, 125.40, 125.36, 124.5, 124.4, 123.20, 123.19, 122.5 (2C), 121.8, 120.1, 119.3, 116.8 (2C), 115.4, 80.4, 80.02, 79.99, 79.96, 79.95, 79.92, 79.87, 79.85, 79.83, 74.51, 74.50, 72.7, 70.4, 70.3, 69.5, 60.0, 59.9, 55.73, 55.72, 46.7, 46.19, 46.15, 46.13, 46.11, 46.10, 46.07, 46.0, 44.8, 34.7, 34.5, 32.61, 32.58, 30.2, 29.7, 29.64, 29.60, 28.50, 28.45, 28.42, 28.38, 28.34, 27.25 (2C), 27.19, 27.16, 25.31, 25.29, 25.27, 18.1 (12C), 14.1, 12.2, 11.9 (6C); HRMS (ESI^+) m/z: calcd for $C_{94}H_{137}Cl_4F_3N_7O_{21}Si_2$ [M + H], 1952.8112; found, 1952.8098.

Synthesis of ***26***. To a stirred solution of **25** (7.8 mg, 4.0 μmol) and paraformaldehyde (3.6 mg, 0.12 mmol) in CH_3CN (0.5 mL) was added $NaB(CN)H_3$ (7.5 mg, 0.12 mmol). After being stirred for 17 h at rt, the reaction mixture was quenched with saturated aq $NaHCO_3$ and extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane/EtOAc 33:67) to afford **26** (4.7 mg, 2.4 μmol, 59%): TLC (hexane/EtOAc 20:80) R_f = 0.50; ^1H NMR (400 MHz, chloroform-*d*) δ: 7.57 (d, J = 8.8 Hz, 1H), 7.38 (dd, J = 19.7, 7.9 Hz, 1H), 7.29 (s, 1H), 7.22−7.10 (m, 5H), 6.90 (d, J = 9.1 Hz, 4H), 6.85 (d, J = 3.6 Hz, 2H), 6.51 (d, J = 5.1 Hz, 1H), 6.25 (d, J = 27.7 Hz, 1H), 5.84 (dd, J = 13.4, 8.0 Hz, 1H), 5.55 (s, 1H), 5.48 (brs, 1H), 5.13 (brs, 1H), 5.09 (s, 1H), 4.99 (brs, 1H), 4.86 (d, J = 6.3 Hz, 1H), 4.74 (d, J = 7.0 Hz, 1H), 4.43 (tt, J = 7.5, 3.6 Hz, 1H), 4.36−4.28 (m, 4H), 4.20 (dd, J = 8.6, 3.5 Hz, 1H), 3.77 (s, 3H), 3.74 (t, J = 6.5 Hz, 4H), 3.69−3.63 (m, 2H), 3.51−3.42 (m, 4H), 3.29 (d, J = 14.5 Hz, 1H), 3.08 (ddd, J = 12.2, 8.4, 3.4 Hz, 2H), 2.76−2.68 (m, 1H), 2.61−2.51 (m, 1H), 2.45 (s, 3H), 2.29−2.14 (m, 5H), 2.12−2.05 (m, 2H), 1.96−1.83 (m, 4H), 1.55 (s, 3H), 1.39 (s, 9H), 1.37 (s, 3H), 1.26 (s, 3H), 1.04 (d, J = 4.6 Hz, 42H), 1.01 (s, 6H), 0.99 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.0, 172.3, 171.22, 171.15, 162.0, 159.5, 157.5, 155.8, 150.6, 142.83, 142.81, 136.9, 135.4, 131.3, 129.36, 129.35, 129.31, 129.30, 129.03, 128.99, 128.95, 128.93, 126.1, 122.5 (2C), 116.8 (2C), 116.6, 115.33, 115.29, 107.3, 106.9, 84.1, 79.30, 79.28, 79.26, 79.24, 79.23, 74.88, 74.87, 73.6, 72.83, 72.80, 70.61, 70.56, 69.8, 67.3, 60.39, 60.36, 60.0, 59.9, 55.70 (2C), 54.2, 46.6 (2C), 46.1, 46.0, 45.0, 44.9, 44.7, 43.1, 41.2, 32.61, 32.59, 30.33 (2C), 30.27, 30.25, 29.69, 29.67, 29.65, 29.60, 28.52, 28.45, 27.31, 27.28, 27.24, 27.23, 27.22, 27.15, 25.14, 25.11, 22.7, 18.1 (12C), 14.2, 14.1, 11.9 (6C); HRMS (ESI^+) m/z: calcd for $C_{95}H_{139}Cl_4F_3N_7O_{21}Si_2$ [M + H], 1966.8269; found, 1966.8288.

MIC Assays. *M. smegmatis* (ATCC 607), *Klebsiella pneumoniae* (ATCC 8047), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 19606), *S. aureus* (BAA-1683), *C. difficile* (ATCC 43596), *Enterococcus faecium* (ATCC 349), *Fusobacterium periodontium* ATCC 33693), *Bacteroides fragilis* (ATCC 25285), *Streptococcus pneumoniae* (ATCC 6301), *Bacillus subtilis* (ATCC 6051), *C. perfringens* (ATCC 13124), *Lactobacillus casei* (ATCC 393), *Lactobacillus acidophilus* (ATCC 4356), and *E. coli* (ATCC 10798) were obtained from American Type Culture Collection (ATCC). A single colony of *M. smegmatis* was obtained on Difco Middlebrook 7H10 nutrient agar enriched with albumin, dextrose, and catalase. Single colonies of *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecium*, and *E. coli* were grown on tryptic soy agar for 24 h at 37 °C in a static incubator and cultured in tryptic soy broth until log phase to be an optical density (OD) of 0.2−0.5. The OD was monitored at 600 nm using a 96-well microplate reader. A single colony of *C. difficile* was obtained on a brain−heart infusion (BHI) agar plate and incubated at 37 °C under anaerobic conditions for 48 h. Seed cultures and larger cultures were obtained using a BHI broth. The flasks were incubated anaerobically for 48 h at 37 °C and cultured to mid-log phase (OD_{600} 0.4). The other bacteria were cultured in the recommended conditions by ATCC.[48] The inhibitors were dissolved in polyethylene glycol 300−H_2O (1/1, a final concentration of 1 mg per 100 μL). This concentration was used as the stock solution for all studies. Bacterial cultures were treated with serial dilutions of inhibitors and incubated at 37 °C for 48 h. The MIC was determined by a 96-well plate reader (BioTek Synergy XT, Winooski, VT, USA) at 570 and 600 nm. If necessary, viable bacteria in each well (96-well plate) were measured via CFUs on a BHI agar plate. The absorbance measurements were also performed using a BioTek Synergy XT (Winooski, VT, USA) 96-well plate reader at 570 and 600 nm.

Cytotoxicity Assays. Cytotoxicity assays were performed using Vero monkey kidney (ATCC CCL-81) and HepG2 human hepatoblastoma cell (ATCC HB-8065) lines. Vero or HepG2 cells were cultured in 75 cm^2 flasks and transferred to 96-well cell culture plates using ATCC-formulated Eagle's minimum essential medium containing 10% fetal bovine serum and penicillin−streptomycin. Serially diluted aliquots of each test compound at concentrations ranging from 0.78 to 200 μg/mL were added to the cells. Control compounds with known toxicity, such as tunicamycin, colistin, or tobramycin, were included on each plate. The plates were incubated, and cytotoxic effects were determined via the MTT assay.

Spore Preparation. *C. difficile* (ATCC 43596) was inoculated on a BHI agar plate and incubated at 37 °C under anaerobic conditions for 14 days. The spores were collected from the agar using sterile distilled water and purified according to the procedures described in the literature.[17] The vegetative forms of *C. difficile* were killed upon heating at 50 °C for 30 min. The prepared spores were suspended in sterile distilled water at 4 °C.

Spore Viability Testing. A solution of test compound was added to a suspension containing *C. difficile* spores (2×10^5 mL^{-1}), and the mixture was incubated at 37 °C for 24 h. The spore suspension treated with the test compound was

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centrifuged (4700*g*), and the pellet was washed with sterile distilled water, plated on a BHI agar containing 0.1% sodium taurocholate (a germination agent), incubated at 37 °C for 48 h under anaerobic conations. The resulting colonies were counted.

MraY (MurX), WecA, and AglH Assays. Preparations of the membrane fractions from *E. coli*, *M. smegmatis*, and *Hydrogenivirga* spp. were performed according to the procedures previously described.[27,33] Procedures for the purification of MraY and AglH and inhibitory assays using these phosphotransferases are described in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01740.

Some assay data, copies of NMR spectra, HPLC chromatogram of new compounds, and assay procedures (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

THF, tetrahydrofuran; TFA, trifluoroacetic acid; CH_2Cl_2, methylene chloride; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; EtOAc, ethyl acetate; $CHCl_3$, chloroform; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; Bu, *n*-butyl; Ts, *p*-toluenesulfonyl; DMAP, *N*,*N*-dimethyl-4-aminopyridine; Boc, *tert*-butoxycarbonyl; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DIC, *N*,*N*′-diisopropylcarbodiimide; NIS, *N*-iodosuccinimide; NMO, *N*-methylmorpholine; TIPS, triisopropylsilyl; Ph, phenyl; ATCC, American Type Culture Collection; MIC, minimum inhibitory concentration; FIC, fractional inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OD, optical density; FBS, fetal bovine serum; NADPH, nicotinamide adenine dinucleotide phosphate; SAR, structure−activity relationship; *C. difficile*, *Clostridium difficile*; CDI, *Clostridium difficile* infection; CDAD, *C. difficile*-associated diarrhea; MraY, phospho-MurNAc-pentapeptide translocase (translocase I); WecA, polyprenyl phosphate-GlcNAc-1-phosphate transferase; AglH, dolichyl-phosphate GlcNAc-1-phosphotransferase; DPAGT1, dolichyl-phosphate GlcNAc-1-phosphotransferase 1; CFU, colony-forming unit; BHI, brain−heart infusion medium; Vero cells, African green monkey kidney cells; Caco-2 cells, heterogeneous human epithelial colorectal adenocarcinoma cells; *C. perfringens*, *Clostridium perfringens*; *S. aureus*, *Staphylococcus aureus*; *B. subtilis*, *Bacillus subtilis*; *S. pneumoniae*, *Streptococcus pneumoniae*; *M. smegmatis*, *Mycobacterium smegmatis*; *L. acidophilus*, *Lactobacillus acidophilus*; *L. casei*, *Lactobacillus casei*; *F. periodontium*, *Fusobacterium periodontium*; *B. fragilis*, *Bacteroides fragilis*; *A. baumannii*, *Acinetobacter baumannii*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. pneumoniae*, *Klebsiella pneumoniae*; *E. coli*, *Escherichia coli*

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