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Structure–activity relationship of truncated analogs of caprazamycins as potential anti-tuberculosis agents

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Abstract—Systematic structure–activity relationship studies of caprazamycin (CPZ) analogs, including the aminoribose-truncated **5** and the uridine-truncated **6**, have been carried out. Both **5** and **6** were synthesized efficiently via diazepanone ring construction by intramolecular reductive alkylation of aminoaldehyde derivatives. The antibacterial activity of a range of analogs, including **5** and **6**, against *Mycobacteriumosis* was evaluated, and it was found that the uridine, the aminoribose, and the fatty acyl side chains are crucial for antibacterial activity. This study would be a guide for designing novel anti-tuberculosis agents based on the 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-glycyluridine class of antibiotics including the CPZs.
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1. Introduction

Tuberculosis (TB) is a disease primarily of the respiratory system from which two million people die each year.[1] With resistant strains continuing to emerge,[2] the need for better anti-TB agents possessing new mechanisms of action remains critical.[3] Caprazamycins (CPZs) (Fig. 1, **1**), isolated from a culture broth of the Actinomycete strain *Streptomyces* sp. MK730-62F2 in 2003,[4] represent the newest members of a class of naturally occurring 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-glycyluridine antibiotics that have shown excellent anti-mycobacterial activity in vitro against drug-susceptible (MIC = 3.13 μg/mL) and multi drug-resistant *Mycobacterium tuberculosis* strains (MIC = 3.13 μg/mL) and that exhibit no significant toxicity in mice. With such excellent biological properties, CPZs are expected to become promising leads for the development of anti-tuberculosis agents with a novel mode of action. A biological target of the 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-glycyluridine class of antibiotics is believed to be the phospho-MurNAc-pentapeptide translocase (MraY, translocase I).[5] MraY catalyzes the first step of the lipid-linked cycle of the reactions, where UDP-MurNAc-pentapeptide is attacked by the undecaprenol monophosphate in the bacterial cell membrane providing the lipid I (Fig. 2). Since MraY is an essential enzyme among bacteria, it is a potential target for the development of anti-TB agents as well as general antibacterial agents.[6] Recently, we completed a total synthesis of (+)-caprazol (Fig. 3, **2**), a core structure of the CPZs.[7] These studies allowed us to access several analogs.[8,9] Among them, it was found that the palmitoyl caprazol **3**, where a fatty acyl side chain at the diazepanone moiety of the CPZs was replaced with a simple palmitoyl group, possesses antibacterial

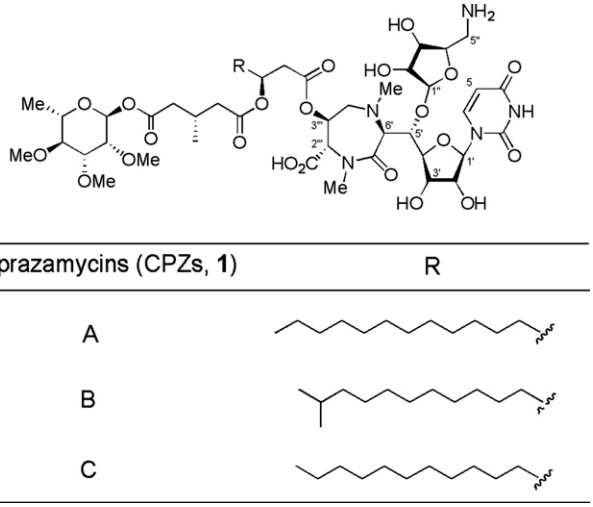

Figure 1. Structure of caprazamycins.

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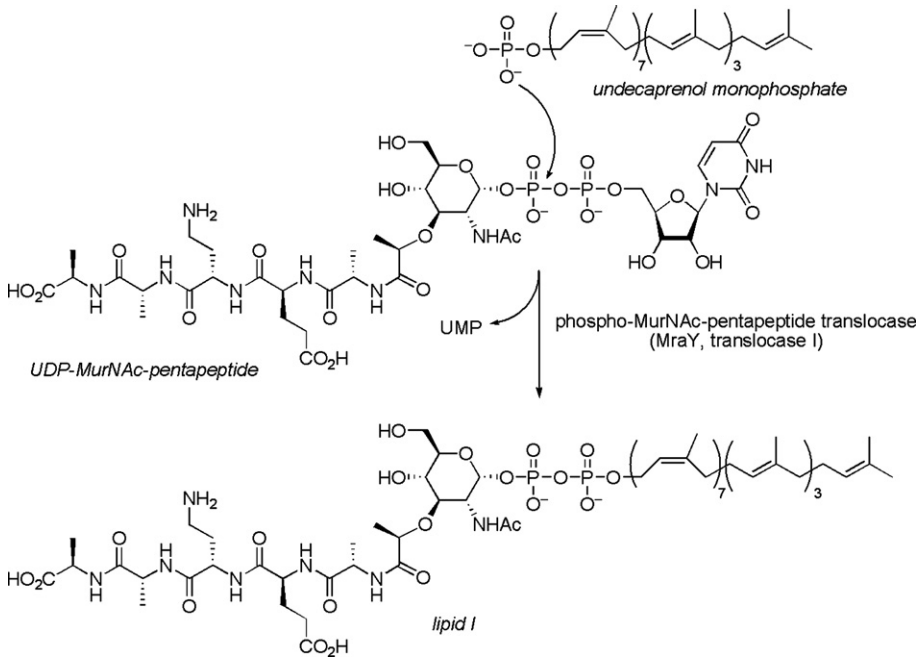


Figure 2. Formation of lipid I catalyzed by MraY (translocase I).

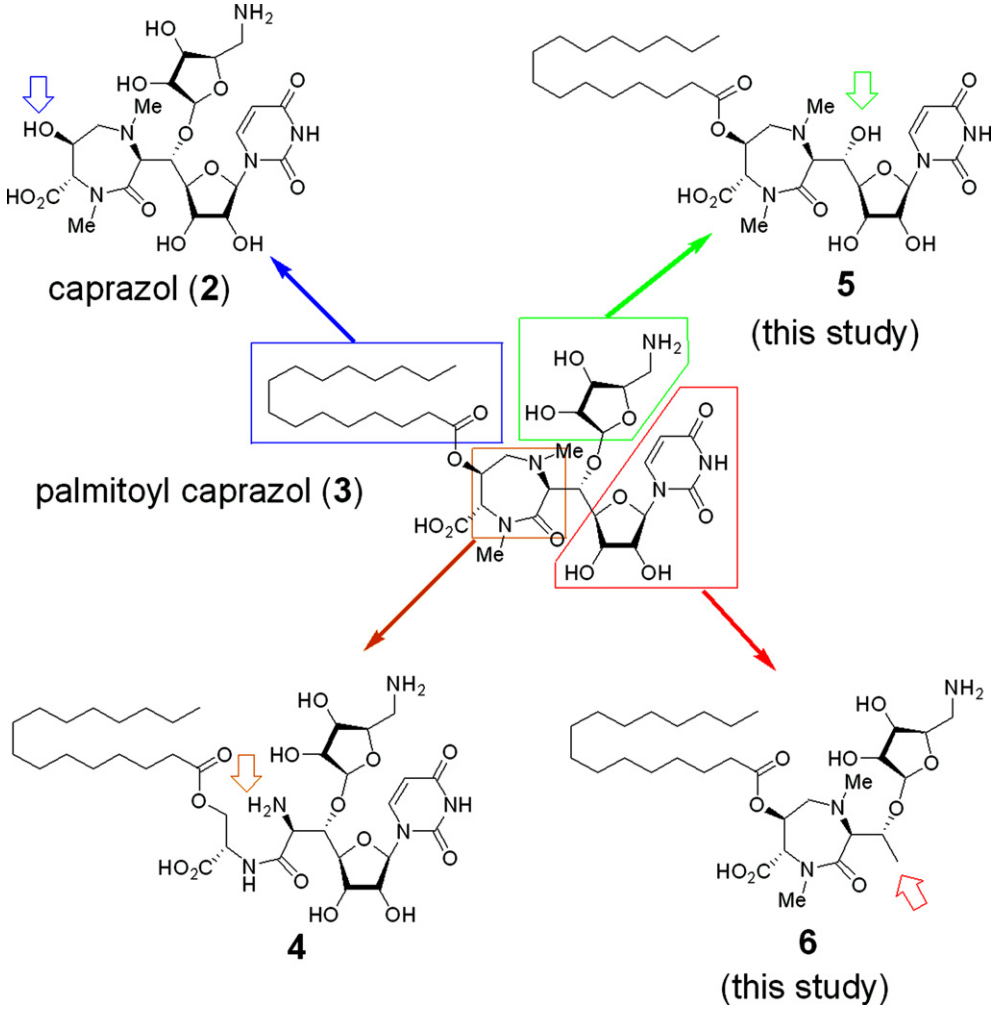


Figure 3. Structure of truncated analogs of CPZs.

activity against *Mycobacterium smegmatis* similar to that of CPZ B (MIC = 1.56 μg/mL for CPZ B, 6.25 μg/mL for **3**).[8] Thus, the complex fatty acyl side chain contained in the CPZs could be replaced by a simple acyl group. Since caprazol **2** exhibits no antibacterial activity against a range of bacterial strains, the hydrophobic fatty acyl side chain at the diazepanone moiety therefore plays an important role in antibacterial activity. Presumably, the lipophilic moiety is necessary in order to penetrate inside the bacterial cell membrane to exhibit antibacterial activity. Another aspect of the structure–activity relationship involves the characteristic diazepanone ring system. The antibacterial activity of the acyclic analog **4**, where the diazepanone ring is broken, was decreased but **4** still retains moderate antibacterial activity against several bacterial strains (12.5–50 μg/mL).[9] It has been suggested that the diazepanone ring might play an important role as a scaffold on which to hang the aminoribosyluridine and the fatty acyl moieties thus allowing them to be placed in the right orientation to interact with the target MraY. As part of a continuing structure–activity relationship study on the CPZs class of antibiotics, herein we describe the synthesis of the aminoribose-truncated analog **5** and the uridine-truncated analog **6** of the CPZs (Fig. 3). We also present an evaluation of their antibacterial activity against *M. tuberculosis* in order to understand the impact of the aminoribose and uridine moieties on the antibacterial activity and the determination of the minimum structural features of the CPZs required for anti-TB agent design.

2. Results and discussions

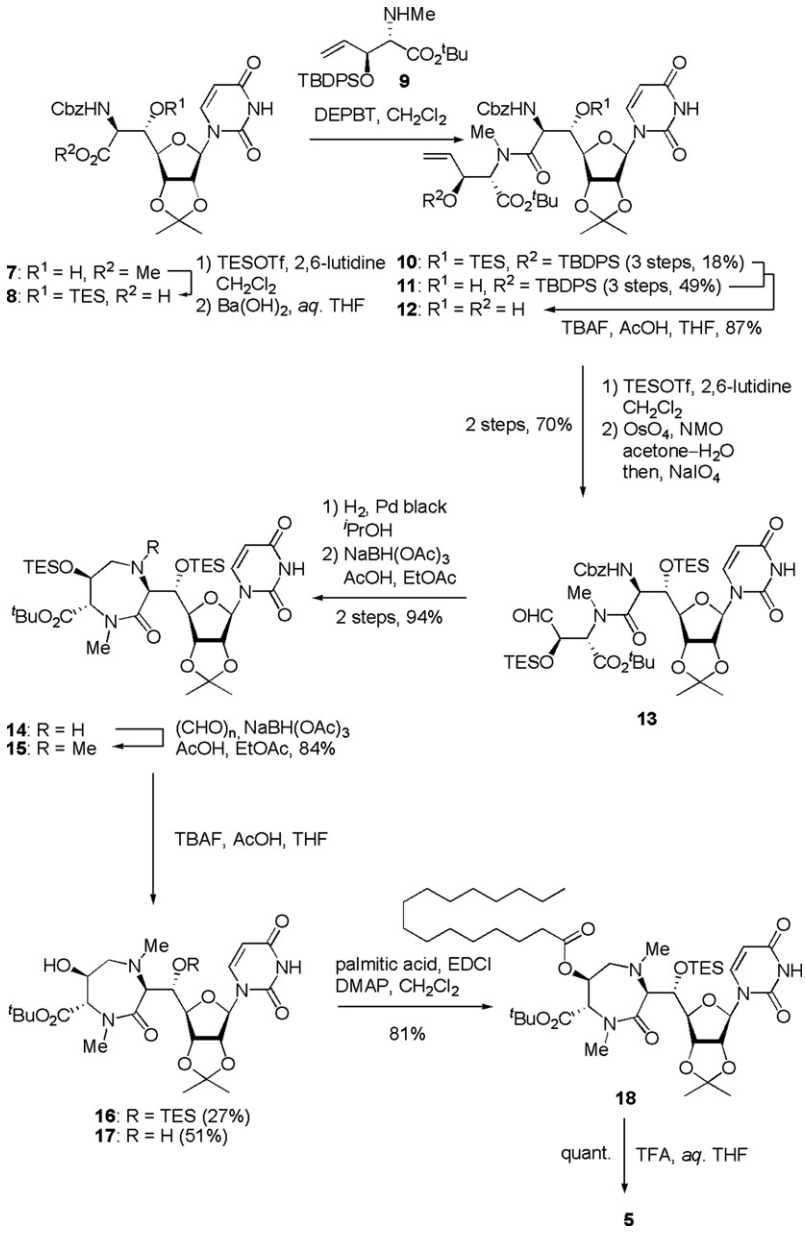
2.1. Chemistry

We have already developed efficient synthetic methods for the preparation of (+)-caprazol (**2**) and palmitoyl caprazol **3**,[7,8] which we employed in the synthesis of the aminoribose-truncated analog **5**, as shown in Scheme 1. The 5′-*C*-glycyluridine derivative **7** was prepared in four steps starting from uridine as previously reported.[8] The 5′-hydroxyl group of **7** was temporarily protected with a TES group (5 equiv of TESOTf, 5 equiv of 2,6-lutidine, CH_2Cl_2, 0 °C), and the methyl ester was saponified to give the carboxylic acid **8** (1 equiv of $Ba(OH)_2$, aqueous THF). Without the temporary TES-protection, it was difficult to recover the corresponding carboxylic acid from the aqueous phase in the work-up of the hydrolysis step. The resulting carboxylic acid **8** was coupled with the *N*-methylamine **9**[8] using 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one[10] (DEPBT) (4 equiv, 1.5 equiv of **9**, 4 equiv of $NaHCO_3$, THF, 0 °C) and gave the secondary amide **10** (18% overall) and its TES-deprotected derivative **11** (49% overall). These secondary amides were observed as a mixture of rotamers in ^1H NMR spectrum. Compounds **10** and **11** were combined and the silyl protecting groups were removed to afford **12** (excess TBAF, AcOH, THF, 78%). After re-protection of the two hydroxyl groups of **12** with TES groups (10 equiv of TESOTf, 5 equiv of 2,6-lutidine, CH_2Cl_2, 0 °C), conversion of the terminal olefin to the aldehyde via the two-step sequence provided **13** (0.5 mol % of OsO_4, 2.5 equiv of NMO, acetone–H_2O; 2.7 equiv of $NaIO_4$, acetone–phosphate buffer (pH 7)), the precursor for the cyclization reaction in 70% overall yield. Compounds **12** and **13** were also observed as a mixture of rotamers in ^1H NMR spectrum. The key diazepanone structure was constructed as follows. Hydrogenolysis of the Cbz group of **13** (H_2, Pd black, ^{i}PrOH) gave the free aminoaldehyde. The resulting aminoaldehyde derivative then was treated with $NaBH(OAc)_3$, which promoted both intramolecular imine formation and its reduction, to afford the desired diazepanone **14** in excellent yield (4 equiv of $NaBH(OAc)_3$, AcOH, AcOEt, 94% overall). Methylation of the secondary amine in **14** gave **15** (9 equiv of $(CH_2O)_n$, 9 equiv of $NaBH(OAc)_3$, AcOH, AcOEt, 84%).[11] Treatment of **15** with TBAF in the presence of AcOH afforded **16**, in which the TES group at the 3‴-hydroxyl group of the diazepanone moiety was removed; however, the yield was not high enough since the di-TES deprotected material **17** was obtained as the major product. Diol **17** can be recycled by re-protection with TES groups to provide **15**. Acylation of the resulting secondary hydroxyl group of **16** with palmitic acid gave **18** (6 equiv, 6 equiv of EDCI, 0.5 equiv of DMAP, CH_2Cl_2, 81%). Finally, a global deprotection of **18** (80% aqueous TFA, quant.) provided the aminoribose-truncated analog **5**.

The synthesis of **6** is illustrated in Scheme 2. β-Selective ribosylation[7] of the *N*-Cbz-L-threonine trichloroethyl (TCE) ester **19**[12] with the 3-pentylidene protected ribosyl donor **20**[7] gave the desired **21** with excellent β-selectivity (1.2 equiv of $BF_3{\cdot}OEt_2$, CH_2Cl_2, −30 °C, 84%, β/α = 97/3). The azide group in **21** was reduced to the corresponding amine (3 equiv of PPh_3, 5 equiv of H_2O, benzene–THF, 45 °C), which was protected with a Boc group to give **22** (2 equiv of Boc_2O, 2 equiv of $NaHCO_3$, 71% overall). Deprotection of the TCE group with zinc gave the carboxylic acid **23**, which was coupled with the secondary amine **9** using DEPBT (4 equiv, 1.5 equiv of **9**, 4 equiv of $NaHCO_3$, THF, 0 °C, 71% overall) to give amide **24**. Deprotection of the TBDPS group of **24** furnished **25** (5 equiv of TBAF, AcOH, THF, 96%). Acylation of the resulting secondary alcohol of **25** with palmitic acid (3 equiv, 3 equiv of EDCI, 0.3 equiv of DMAP, CH_2Cl_2) and conversion of a terminal olefin of the resulting **26** to the aldehyde via the two-step sequence provided **27** (O_3, CH_2Cl_2, −78 °C), the precursor for the cyclization reaction. The intramolecular reductive amination of **27**, afforded diazepanone **28** (H_2, Pd black, ^{i}PrOH, then 4 equiv of $NaBH(OAc)_3$, AcOH, AcOEt, 66% overall). Methylation of **28** gave **29** (9 equiv of $(CH_2O)_n$, 9 equiv of $NaBH(OAc)_3$, AcOH, AcOEt, 77%). Finally, a global deprotection of **29** (80% aqueous TFA, quant.) provided the desired uridine-truncated analog **6**.

2.2. Antibacterial activity

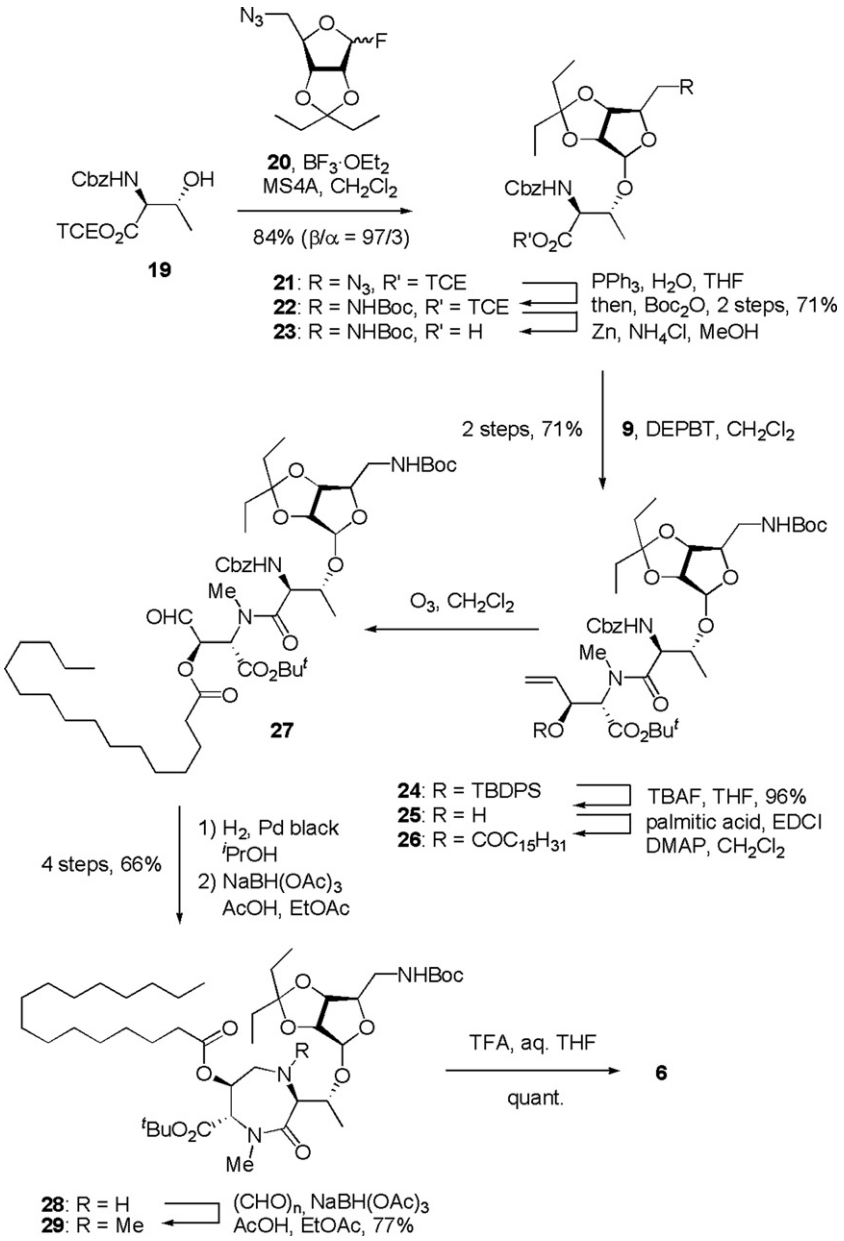
The antibacterial activity of the newly synthesized analogs against *M. tuberculosis* H37Rv was evaluated using the Alamar blue assay, and the 50% minimum inhibitory



Scheme 1. Synthesis of aminoribose-truncated analog **5**.

concentrations (MIC_{50}, μg/mL) are summarized in Table 1.[9] Antibacterial activity of palmitoyl caprazol **3** against *M. tuberculosis* has not been evaluated in the previous study. However, in this study, palmitoyl caprazol **3** was tested and exhibited antibacterial activity against *M. tuberculosis* H37Rv (MIC_{50} = 2.50 μg/mL). Consistent with these observations and with previous studies,[4d,8] simplification of the fatty acyl side chain in the CPZs to the palmitoyl group, which lacks substituents and stereocenters, was tolerated for antibacterial activity. Analog **4**, the acyclic analog in which the diazepanone ring is broken, exhibited some activity (MIC_{50} = 6.25 μg/mL) although it was less active than **3**. On the other hand, the aminoribose-truncated analog **5** exhibited a complete loss of activity, demonstrating therefore that the aminoribose moiety is crucial for antibacterial activity. Dini et al. found that the 3′-hydroxyl group, the uracil moiety, and the amino group of the ribose attached on the 5′-hydroxyl group of uridine are indeed necessary for MraY inhibition in a structure–activity relationship of the liposidomycins[13] (LPMs), the structures of which closely resemble those of the CPZs, with quite simple analogs.[14] Our results are consistent with those studies.

MraY is one of the key enzymes for peptidoglycan biosynthesis and appears to be conserved in both Gram-negative and Gram-positive bacteria. This enzyme catalyzes the first step of the lipid-linked cycle of reactions, where UDP-MurNAc-pentapeptide is attacked by the undecaprenol monophosphate in the bacterial cell membrane providing lipid I (Fig. 2). The 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-glycyluridine class of antibiotics is a strong inhibitor of MraY. Brandish et al. have studied the mode of action of the LPMs.[15] LPM B exhibited slow-binding inhibition which was competitive with respect to the lipid



Scheme 2. Synthesis of uridine-truncated analog **6**.

Table 1. Antibacterial activity of CPZ analogs against *Mycobacterium tuberculosis* H37Rv

	MIC_{50} (μg/mL)				
	2	**3**	**4**	**5**	**6**
Mycobacterium tuberculosis H37Rv	>100	2.50	6.25	>100	>100

acceptor substrate (the undecaprenol monophosphate) and noncompetitive with respect to the fluorescent substrate analog of UDP-MurNAc-pentapeptide (dansyl-UDP-MurNAc-pentapeptide) although LPM B contains a uridine moiety common to UDP-MurNAc-pentapeptide. These results indicate that, in MraY inhibition, the uridine moiety of LPM B may not be necessary. Indeed, the role of the uridine moiety in the structure–activity relationship is still largely unknown. However, the antibacterial activity of the uridine-truncated analog **6** is of particular interest since it offers a mechanistic insight into the mode of action of the CPZs and LPMs as lipid acceptor substrate analogs. The fact that uridine-truncated analog **6** lost antibacterial activity, therefore confirms that the uridine moiety is also indispensable for antibacterial activity. The slow-binding inhibition by LPM B is characterized by the rapid and reversible formation of the EI complex from the enzyme (E) plus inhibitor (I), followed by isomerization of the EI complex to a more tightly associated EI* complex.[15] Analog **6** may not be suitable for transition to the EI* complexation although a further mechanistic study of the MraY inhibition would be necessary to determine this.

Due to their relatively large molecular weights and their complex and chemically labile structure, the CPZs

themselves may not be appropriate as drug-like compounds. This is especially true for the basic conditions since the CPZs possess β-acyloxy- and ribofuranosyloxycarbonyl structures.[7,13c] Therefore, minimum structural requirements for the CPZs for anti-TB agent design would have to be established first. Our systematic structure–activity relationship studies of the CPZ analogs revealed that the uridine, the aminoribose, and the fatty acyl side chains are crucial for antibacterial activity. However, since the fatty acyl side chain can be simplified, this moiety would play a role in penetrating the bacterial cell membrane. The characteristic diazepanone moiety is necessary presumably to maintain the spatial position of the aminoribosyluridine and the fatty acyl moiety; this being the case, it could be replaced by an appropriate scaffold. Thus, the 5′-β-*O*-aminoribosyl-glycyluridine structure connected to a lipophilic group is predicted to be the pharmacophore of this class of natural products. Since simplification of the fatty acyl side chain of the CPZs is tolerated, we plan to simplify the hydrophilic pharmacophore in order to reduce the size of the molecules and to stabilize the chemically labile structure in order to discover novel antibacterial drugs. These studies are underway.

3. Conclusion

Systematic structure–activity relationship studies of CPZ analogs, including **5** and **6**, which have been designed and synthesized as truncated analogs of the CPZs, revealed that the uridine, the aminoribose, and the fatty acyl side chain are crucial for antibacterial activity. The characteristic diazepanone moieties are necessary presumably to maintain the spatial position of the aminoribosyluridine and the fatty acyl moieties. Thus, the 5′-β-*O*-aminoribosyl-glycyluridine structure connected to a lipophilic group is predicted to be the pharmacophore of this class of natural products. This study would be a guide for designing novel anti-TB agents based on the 6′-*N*-alkyl-5′-β-*O*-aminoribosyl-glycyluridine class of antibiotics including the CPZs.

4. Experimental

4.1. General experimental methods

NMR spectra were obtained on a JEOL EX270, JEOL GX270, JEOL AL400, or Bruker ARX-500, and were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (J) was reported in Hertz (Hz). Abbreviations of multiplicity were as follow: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Data were presented as follows: chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H COSY, HMBC, and HMQC NMR spectra. Optical rotations were recorded on JASCO DIP-370 digital polarimeter or JASCO P-1030 polarimeter. FAB-MS was obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60 N (neutral). Flash column chromatography was performed on Merck silica gel 60.

4.1.1. *N*-[(1*S*,2*S*)-2-*tert*-Butyldiphenylsiloxy-1-*tert*-butoxycarbonyl-3-butenyl]-*N*-methyl-6-benzyloxycarbonylamino-5-*O*-triethylsilyl-6-deoxy-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-*glycelo*-L-*talo*-heptofuranuronamide (10) and *N*-[(1*S*,2*S*)-2-*tert*-butyldiphenylsiloxy-1-*tert*-butoxycarbonyl-3-butenyl]-*N*-methyl-6-benzyloxycarbonylamino-6-deoxy-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-*glycelo*-L-*talo*-heptofuranuronamide (11). A solution of **7** (177 mg, 0.35 mmol) in CH_2Cl_2 (4 mL) was treated with 2,6-lutidine (203 μL, 1.75 mmol) and TESOTf (395 μL, 1.75 mmol) at 0 °C. After stirring for 10 min, MeOH (100 μL) was added. The mixture was partitioned between AcOEt and 0.5 N aqueous HCl. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue in aqueous THF (4:1, 4 mL) was treated with $Ba(OH)_2{\cdot}8H_2O$ (110 mg, 0.035 mmol) at room temperature for 4 h. The mixture was partitioned between AcOEt and 0.5 N aqueous HCl. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo to afford crude acid **8** as a white foam. A mixture of **8** and **9** (118 mg, 0.27 mmol) in THF (3 mL) was treated with $NaHCO_3$ (90 mg, 1.08 mmol) and DEPBT (322 mg, 1.08 mmol) at room temperature for 18 h. The mixture was partitioned between AcOEt and 0.5 N aqueous HCl, and the organic phase was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (3 × 22 cm, 16% AcOEt–hexane) to give **10** (50 mg, 18% in 3 steps) and **11** (120 mg, 49% in 3 steps): data for **10**; ^{1}H NMR ($CDCl_3$, 500 MHz, 10:1 mixture of the rotamers) δ 8.45 (br s, 1H, NH-3), 8.00 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 7.69–7.64 (m, 4H, phenyl), 7.41–7.26 (m, 11H, phenyl), 6.09 (d, 1H, H-1′, J = 3.9 Hz), 5.73 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 5.65 (m, 1H, H-11′), 5.32 (d, 1H, NHBoc, $J_{NH,6}$ = 10.2 Hz), 5.10 (d, 1H, benzyl, J = 12.1 Hz), 5.08 (d, 1H, H-2′, $J_{2',3'}$ = 7.4 Hz), 5.00 (d, 1H, benzyl, J = 12.1 Hz), 4.90 (dd, 1H, H-6′, $J_{6',NH} = 10.2, J_{6',5'} = 8.2$ Hz), 4.66 (m, 2H, H-12′a,b), 4.59 (d, 1H, H-9′, $J_{9',10'}$ = 6.2 Hz), 4.56 (d, 1H, H-3′, $J_{3',2'}$ = 7.8 Hz), 4.53 (dd, 1H, H-10′, $J_{10',11'} = 4.6, J_{10',9'} = 6.2$ Hz), 4.27 (m, 1H, H-4′), 4.12 (dd, 1H, H-5′, $J_{5',6'} = 8.2, J_{5',4'} = 1.7$ Hz), 2.98 (s, 3H, NMe), 1.55 (s, 3H, acetonide), 1.46 (s, 9H, *tert*-butyl), 1.31 (s, 3H, acetonide), 1.00 (s, 9H, *tert*-butyl), 0.95 (m, 9H, 3× $SiCH_2CH_3$), 0.61 (m, 6H, 3× $SiCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.2, 167.5, 163.1, 155.9, 150.2, 140.0, 136.5, 136.0, 136.0, 135.9, 133.7, 132.9, 129.7, 129.5, 128.5, 128.3, 128.2, 127.4, 127.2, 117.8, 114.1, 102.8, 91.0, 85.0, 84.3, 81.9, 81.4, 77.2, 74.2, 74.0, 67.1, 61.3, 52.3, 33.0, 28.0, 27.9, 27.1, 26.9, 25.2, 19.2, 6.8, 5.1, 4.8; FABMS-LR *m*/*z* 1027 (MH^+); FABMS-HR (NBA) calcd for $C_{54}H_{75}N_4O_{12}Si_2$ 1027.4920; found: 1027.4927. Data for **11**; ^{1}H NMR ($CDCl_3$, 500 MHz, 9:1 mixture of the rotamers) δ 9.18 (br s, 1H, NH-3), 7.68 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.41–7.26 (m, 11H, phenyl), 5.89 (d, 1H, H-1′, $J_{1',2'}$ = 3.6 Hz), 5.75–5.70 (m, 3H, H-5, H-11′, BocNH), 5.07–5.03 (m, 3H, benzyl, H-

2′), 4.86 (m, 2H, H-12′a,b), 4.76 (d, 1H, H-6′, $J_{6',NH} = 10.4$ Hz), 4.73 (dd, 1H, H-3′, $J_{3',4'} = 3.9, J_{3',2'} = 6.1$ Hz), 4.69 (m, 1H, H-9′), 4.62 (m, 1H, H-10′), 4.26 (m, 1H, H-4′), 4.05 (m, 2H, OH, H-5′), 3.04 (s, 3H, COMe), 1.54 (s, 3H, acetonide), 1.44 (s, 9H, *tert*-butyl), 1.31 (s, 3H, acetonide), 1.01 (s, 9H, *tert*-butyl); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.2, 167.5, 163.2, 156.4, 150.2, 141.3, 137.2, 136.8, 136.0, 135.2, 133.6, 133.1, 129.6, 128.5, 128.2, 128.0, 127.4, 114.4, 103.3, 102.1, 93.4, 92.4, 83.2, 82.1, 81.6, 73.9, 72.6, 67.1, 62.0, 52.8, 28.1, 28.0, 27.2, 27.0, 26.9, 25.3, 25.2, 19.2; FABMS-LR *m*/*z* 913 (MH^+); FABMS-HR (NBA) calcd for $C_{48}H_{61}N_4O_{12}Si$ 913.3977; found: 913.3980.

4.1.2. *N*-[(1*S*,2*S*)-1-*tert*-Butoxycarbonyl-2-hydroxy-3-butenyl]-*N*-methyl-6-benzyloxycarbonylamino-6-deoxy-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-*glycelo*-L-*talo*-heptofuranuronamide (12). A solution of **10** (50 mg, 0.048 mmol) and **11** (120 mg, 0.132 mmol) in THF (4 mL) was treated with AcOH (75 μL) and TBAF solution (1 M solution in THF, 1.3 mL) at room temperature for 1 week. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a neutral flash silica gel column (3 × 10 cm, 75% AcOEt–hexane) to give **12** (106 mg, 87%) as a colorless foam: ^{1}H NMR ($CDCl_3$, 500 MHz, 6.7:1 mixture of the rotamers) δ 7.75 (d, 1H, H-6, $J_{6,5} = 8.0$ Hz), 7.29–7.24 (m, 5H, phenyl), 6.01 (d, 1H, CbzNH, $J_{NH,6'} = 8.5$ Hz), 5.87 (s, 1H, H-1′), 5.79 (m, 1H, H-11′), 5.71 (d, 1H, H-5, $J_{5,6} = 8.0$ Hz), 5.30 (d, 1H, H-12′a, $J_{12'a,11'} = 17.1$ Hz), 5.10 (d, 1H, H-12′b, $J_{12'b,11'} = 10.5$ Hz), 5.06 (s, 2H, benzyl), 4.86 (m, 2H, H-6′, H-10′), 4.74 (m, 1H, H-9′), 4.61 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 4.24 (m, 1H, H-4′), 4.06 (d, 1H, H-5′, $J_{5',6'} = 6.5$ Hz), 2.84 (s, 3H, CONMe), 1.52 (s, 3H, acetonide), 1.45 (s, 9H, *tert*-butyl), 1.30 (s, 3H, acetonide); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 169.4, 163.6, 156.5, 150.3, 141.7, 136.0, 128.5, 128.3, 128.2, 128.0, 117.8, 114.4, 114.3, 102.7, 93.2, 85.0, 83.5, 83.2, 81.6, 77.3, 71.9, 71.4, 67.1, 63.5, 60.4, 52.7, 35.5, 27.9, 27.8, 27.2, 25.2, 14.1, 11.4; FABMS-LR *m*/*z* 675 (MH^+); FABMS-HR (NBA) calcd for $C_{32}H_{43}N_4O_{12}$ 675.2864; found: 675.2878.

4.1.3. *N*-[(1*S*,2*S*)-1-*tert*-Butoxycarbonyl-3-oxo-2-triethylsiloxy-propyl]-*N*-methyl-6-benzyloxycarbonylamino-6-deoxy-2,3-*O*-isopropylidene-5-*O*-triethylsilyl-1-(uracil-1-yl)-β-D-*glycelo*-L-*talo*-heptofuranuronamide (13). A solution of **12** (10 mg, 0.015 mmol) in CH_2Cl_2 (1 mL) was treated with 2,6-lutidine (17 μL, 0.075 mmol) and TESOTf (17 μL, 0.15 mmol) at 0 °C. After stirring for 5 h, MeOH (50 μL) was added. The mixture was partitioned between AcOEt and 0.5 N aqueous HCl. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue in 80% aqueous dioxane (1 mL) was treated with OsO_4 in *t*-BuOH (5 mg/mL, 235 μL) and NMO (13 mg, 0.061 mmol) at room temperature for 48 h. After $NaIO_4$ (19 mg, 0.089 mmol) was added, the mixture was stirred for an additional 5 h. The mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$, and the organic phase was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (1 × 3 cm, 33% AcOEt–hexane) to give **13** (9.5 mg, 70% in 3 steps) as a colorless foam: ^{1}H NMR ($CDCl_3$, 500 MHz, 9:1 mixture of the rotamers) δ 9.64 (s, 1H, H-11′), 8.20 (br s, 1H, NH-3), 7.69 (d, 1H, H-6, $J_{6,5} = 8.0$ Hz), 7.36–7.31 (m, 5H, phenyl), 6.06 (d, 1H, H-1′, $J_{1',2'} = 3.8$ Hz), 5.75 (d, 1H, H-5, $J_{5,6} = 8.0$ Hz), 5.43 (m, 2H, CbzNH, H-10′), 5.16 (d, 1H, benzyl, $J = 12.2$ Hz), 5.06 (m, 1H, H-6′), 5.05 (d, 1H, benzyl, $J = 12.2$ Hz), 4.71 (dd, 1H, H-3′, $J_{3',4'} = 2.5, J_{3',2'} = 6.1$ Hz), 4.55 (dd, 1H, H-2′, $J_{2',3'} = 6.1$, $J_{2',1'} = 3.8$ Hz), 4.33 (d, 1H, H-9′, $J_{9',10'} = 3.7$ Hz), 4.22 (m, 1H, H-4′), 4.14 (d, 1H, H-5′, $J_{5',6'} = 6.8$ Hz), 3.20 (s, 3H, CONMe), 1.55 (s, 3H, acetonide), 1.45 (s, 9H, *tert*-butyl), 1.30 (s, 3H, acetonide), 0.93 (m, 18H, 6× $SiCH_2CH_3$), 0.61 (m, 12H, 6× $SiCH_2CH_3$); FABMS-LR *m*/*z* 905 (MH^+); FABMS-HR (NBA) calcd for $C_{43}H_{69}N_4O_{13}Si_2$ 905.4400; found: 905.3995.

4.1.4. Diazepanone (14). A mixture of **13** (43.0 mg, 0.048 mmol) and Pd black (43 mg) in ^{i}-PrOH (4 mL) was vigorously stirred under H_2 atmosphere at room temperature for 4 h. The insoluble was filtered off through a Celite pad, and the filtrate was concentrated in vacuo. The residue in CH_2Cl_2 (2 mL) was treated with AcOH (20 μL) and $NaBH(OAc)_3$ (40 mg, 0.19 mmol) at room temperature for 24 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a neutral silica gel column (1 × 5 cm, 40% AcOEt–hexane) to give **14** (34.3 mg, 94%) as a white foam: $[\alpha]_D^{21}$ 29.8 (*c* 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.48 (br s, 1H, NH-3), 7.86 (d, 1H, H-6, $J_{6,5} = 8.1$ Hz), 6.03 (d, 1H, H-1′, $J_{1',2'} = 3.6$ Hz), 5.64 (dd, 1H, H-5, $J_{5,6} = 8.1$, $J_{5,NH} = 1.1$ Hz), 4.68 (m, 1H, H-4′), 4.67 (m, 1H, H-3′), 4.58 (m, 1H, H-2′), 4.27 (m, 2H, H-3″, H-5′), 3.93 (d, 1H, H-2″, $J_{2'',3''} = 5.3$ Hz), 3.09–3.07 (m, 2H, H-6′, H-4″a), 3.06 (s, 3H, CONMe), 2.85 (d, 1H, H-4″b, $J_{4''b,a} = 14.7$ Hz), 1.56 (s, 3H, acetonide), 1.40 (s, 9H, *tert*-butyl), 1.32 (s, 3H, acetonide), 0.95 (m, 18H, 6× $SiCH_2CH_3$), 0.52 (m, 12H, 6× $SiCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 167.6, 162.7, 149.7, 140.8, 114.2, 102.2, 90.8, 86.3, 84.6, 83.2, 82.0, 74.2, 68.6, 67.4, 61.8, 53.3, 39.7, 29.7, 28.0, 27.8, 27.4, 25.5, 7.1, 7.0, 6.9, 5.6, 5.4, 4.9, 1.1; FABMS-LR *m*/*z* 755 (MH^+); FABMS-HR (NBA) calcd for $C_{35}H_{63}N_4O_{10}Si_2$ 755.4077; found: 755.4077.

4.1.5. Diazepanone (15). A solution of **14** (33 mg, 0.043 mmol) in AcOEt (3 mL) was treated with paraformaldehyde (13 mg, 0.4 mmol), AcOH (75 μL), and $NaBH(OAc)_3$ (91 mg, 0.4 mmol) at room temperature for 72 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (1 × 5 cm, 33% AcOEt–hexane) to give **15** (28.2 mg, 84%) as a colorless foam: $[\alpha]_D^{23}$ 12.3 (*c* 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (br s, 1H, NH-3), 8.02 (d, 1H, H-6,

$J_{6,5}$ = 8.1 Hz), 6.13 (d, 1H, H-1′, $J_{1',2'}$ = 3.8 Hz), 5.62 (dd, 1H, H-5, $J_{5,6}$ = 8.1, $J_{5,NH}$ = 2.4 Hz), 4.93 (m, 1H, H-4′), 4.72 (dd, 1H, H-3′, $J_{3',2'} = 6.2, J_{3',4'} = 2.4$ Hz), 4.47 (dd, 1H, H-2′, $J_{2',1'} = 3.8, J_{2',3'} = 6.2$ Hz), 4.31 (m, 1H, H-3″), 4.20 (d, 1H, H-5′, $J_{5',6'} = 8.8$ Hz), 3.93 (d, 1H, H-2″, $J_{2'',3''} = 4.3$ Hz), 3.32 (d, 1H, H-6′, $J_{6',5'} = 8.8$ Hz), 3.12 (s, 3H, CONMe), 3.09 (d, 1H, H-4″a, $J_{4''a,b} = 14.0$ Hz), 2.99 (d, 1H, H-4″b, $J_{4''b,a} = 14.0$ Hz), 2.46 (s, 3H, NMe), 1.74 (s, 3H, acetonide), 1.42 (s, 9H, *tert*-butyl), 1.32 (s, 3H, acetonide), 0.98 (m, 18H, 6× $SiCH_2CH_3$), 0.62 (m, 12H, 6× $SiCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 167.5, 162.7, 149.7, 140.6, 114.0, 102.1, 90.5, 86.0, 84.8, 83.0, 82.2, 77.2, 71.7, 70.0, 69.2, 65.4, 60.6, 38.9, 37.4, 27.8, 27.8, 27.7, 27.6, 25.5, 25.4, 7.1, 7.1, 6.9, 6.8, 5.6, 5.3, 5.2, 5.1, 5.0, 4.8, 4.8, 4.5; FABMS-LR *m*/*z* 769 (MH^+); FABMS-HR (NBA) calcd for $C_{36}H_{65}N_4O_{10}Si_2$ 769.4239; found: 769.4233.

4.1.6. Diazepanones (16) and (17). A solution of **15** (22.3 mg, 0.029 mmol) and AcOH (4 μL, 0.069 mmol) in THF (1 mL) was treated with TBAF (1 M solution in THF, 64 μL, 0.064 mmol) at room temperature for 4 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a neutral flash silica gel column (1 × 3 cm, 33–90% AcOEt–hexane) to give **16** (5.2 mg, 27%) and **17** (13.4 mg, 51%) each as a colorless foam: data for **16**; ^{1}H NMR (CD_3CN, 400 MHz) δ 8.70 (br s, 1H, NH-3), 7.58 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 5.75 (d, 1H, H-1′, $J_{1',2'}$ = 3.4 Hz), 5.31 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 4.53 (dd, 1H, H-3′, $J_{3',2'} = 6.2, J_{3',4'} = 3.2$ Hz), 4.44 (m, 1H, H-3″), 4.33 (dd, 1H, H-2′, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 3.4 Hz), 4.01 (m, 1H, H-4′), 3.97 (d, 1H, H-2″, $J_{2'',3''}$ = 4.5 Hz), 3.91 (d, 1H, H-5′, $J_{5',6'}$ = 8.3 Hz), 3.00 (d, 1H, H-6′, $J_{6',5'}$ = 8.3 Hz), 2.83 (m, 2H, H-4″a,b), 2.80 (s, 3H, CONMe), 2.20 (s, 3H, NMe), 1.29 (s, 3H, acetonide), 1.10 (s, 9H, *tert*-butyl), 1.07 (s, 3H, acetonide), 0.69 (m, 9H, 3× $SiCH_2CH_3$), 0.43 (m, 6H, 3× $SiCH_2CH_3$); FABMS-LR *m*/*z* 655 (MH^+); FABMS-HR (NBA) calcd for $C_{30}H_{51}N_4O_{10}Si$ 655.3375; found: 655.3370. Data for **17**; $[\alpha]_D^{22}$ 37.5 (*c* 0.90, $CHCl_3$); ^{1}H NMR (CD_3CN, 400 MHz) δ 8.75 (br s, 1H, NH-3), 7.69 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 5.83 (d, 1H, H-1′, $J_{1',2'}$ = 4.2 Hz), 5.35 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 4.74 (dd, 1H, H-3′, $J_{3',2'} = 6.0, J_{3',4'} = 2.0$ Hz), 4.43 (dd, 1H, H-2′, $J_{2',3'} = 6.0, J_{2',1'} = 4.2$ Hz), 4.10 (m, 1H, H-4′), 4.07 (m, 1H, H-3″), 4.01 (d, 1H, H-2″, $J_{2'',3''}$ = 4.9 Hz), 3.83 (ddd, 1H, H-5′, $J_{5',6'} = 9.6, J_{5',4'} = 3.4, J_{5',OH} = 2.1$ Hz), 3.11 (d, 1H, OH, *J* = 4.2 Hz), 2.96 (m, 1H, OH), 2.93 (d, 1H, H-6′, $J_{6',5'}$ = 9.6 Hz), 2.83 (m, 2H, H-4″a,b), 2.80 (s, 3H, CONMe), 2.12 (s, 3H, NMe), 1.74 (s, 3H, acetonide), 1.30 (s, 3H, acetonide), 1.10 (s, 9H, *tert*-butyl); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3, 168.7, 163.5, 151.2, 141.6, 114.3, 103.1, 90.8, 84.8, 84.5, 83.5, 82.8, 70.0, 68.7, 67.7, 64.3, 60.6, 38.6, 36.9, 27.7, 27.6, 25.5; FABMS-LR *m*/*z* 541 (MH^+); FABMS-HR (NBA) calcd for $C_{24}H_{37}N_4O_{10}$ 541.2510; found: 541.2500.

4.1.7. Diazepanone (18). A solution of **17** (5.0 mg, 7.8 μmol) in CH_2Cl_2 (1 mL) was treated with palmitic acid (12 mg, 47 μmol), DMAP (1 mg, 3.9 μmol), and EDCI (8 mg, 47 μmol) at room temperature for 10 h. The reaction mixture was partitioned between AcOEt and 0.3 N aqueous HCl, and the organic phase was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (1 × 5 cm, 33% AcOEt–hexane) to give **18** (5.4 mg, 81%) as a colorless foam: $[\alpha]_D^{21}$ 8.2 (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (br s, 1H, NH-3), 7.98 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 6.15 (d, 1H, H-1′, $J_{1',2'}$ = 3.8 Hz), 5.64 (dd, 1H, H-5, $J_{5,6}$ = 8.1, $J_{5,NH}$ = 2.1 Hz), 5.31 (m, 1H, H-3″), 4.89 (br s, 1H, H-4′), 4.73 (dd, 1H, H-3′, $J_{3'',2''} = 6.1, J_{3',4'} = 2.4$ Hz), 4.48 (dd, 1H, H-2′, $J_{2',3'} = 6.1, J_{2',1'} = 3.8$ Hz), 4.22 (d, 1H, H-5′, $J_{5',6'}$ = 9.0 Hz), 4.22 (m, 1H, H-2″), 3.35 (d, 1H, H-6′, $J_{6',5'}$ = 9.0 Hz), 3.30 (dd, 1H, H-4″a, $J_{4''a,b} = 15.9, J_{4''a,3''} = 2.4$ Hz), 3.18 (d, 1H, H-4″b, $J_{4''b,3''}$ = 15.9 Hz), 3.0 (s, 3H, CONMe), 2.41 (s, 3H, NMe), 2.32 (m, 2H, $COCH_2$), 1.59 (m, 5H, acetonide, $COCH_2CH_2$), 1.37 (s, 9H, *tert*-butyl), 1.34 (s, 3H, acetonide), 1.25 (m, 24H, palmitoyl), 0.97 (m, 9H, 3× $SiCH_2CH_3$), 0.88 (m, 3H, palmitoyl terminal-Me), 0.63 (m, 6H, 3× $SiCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 171.4, 166.5, 162.4, 149.6, 140.4, 114.1, 102.2, 90.3, 85.7, 84.8, 83.7, 82.0, 77.3, 71.5, 71.0, 65.2, 65.0, 57.7, 38.4, 36.8, 34.6, 33.5, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.7, 27.4, 25.5, 24.9, 24.8, 22.8, 14.2, 7.1, 7.1, 5.3; FABMS-LR *m*/*z* 893 (MH^+); FABMS-HR (NBA) calcd for $C_{46}H_{81}N_4O_{11}Si$ 893.5671; found: 1893.5673.

4.1.8. Aminoribose-truncated analog (5). Compound **18** (5.0 mg, 5.6 μmol) was treated with 80% aqueous TFA (1 mL) at room temperature for 1 h. The mixture was concentrated in vacuo, and then the residue was dissolved in H_2O (1 mL) and freeze–dried to afford **5** (4.5 mg, quant.) as a white solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.99 (d, 1H, H-6, $J_{6,5}$ = 8.3 Hz), 5.92 (d, 1H, H-1′, $J_{1',2'}$ = 4.9 Hz), 5.68 (d, 1H, H-5, $J_{5,6}$ = 8.3 Hz), 5.65 (d, 1H, H-3″, $J_{3'',2''}$ = 5.1 Hz), 4.80 (d, 1H, H-2″, $J_{2'',3''}$ = 5.1 Hz), 4.36 (d, 1H, H-5′, $J_{5',6'}$ = 9.6 Hz), 4.24 (m, 2H, H-2′, H-3′), 4.11 (m, 1H, H-4′), 3.85 (d, 1H, H-6′, $J_{6',5'}$ = 9.6 Hz), 3.65 (m, 1H, H-4″a), 3.55 (m, 1H, H-4″b), 3.14 (s, 3H, CONMe), 2.72 (s, 3H, NMe), 2.42 (t, 2H, $COCH_2$, *J* = 7.2 Hz), 1.61 (m, 2H, $COCH_2CH_2$), 1.28 (m, 24H, palmitoyl), 0.89 (m, 3H, palmitoyl terminal-Me); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.3, 166.2, 152.6, 142.6, 103.1, 90.6, 85.3, 75.7, 73.7, 72.9, 67.3, 65.1, 64.8, 62.5, 58.7, 39.0, 30.8, 35.2, 33.3, 31.0, 30.9, 30.8, 30.7, 30.6, 30.5, 30.4, 26.3, 26.1, 24.0, 14.7; FABMS-LR (negative) *m*/*z* 681 (M−H); FABMS-HR (NBA) calcd for $C_{33}H_{53}N_4O_{11}$ 681.3711; found: 681.3705.

4.1.9. 2,2,2-Trichloroethyl (2*S*,3*S*)-3-*O*-[5-2-azido-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-2-benzyloxycarbonylamino-3-hydroxy butanoate (21). A mixture of **19** (924 mg, 2.52 mmol), **20** (1.23 g, 5.04 mmol), and MS4A (3.0 g) in CH_2Cl_2 (30 mL) was treated with $BF_3{\cdot}Et_2O$ (255 μL, 2.02 mmol) at 0 °C for 30 min. After addition of saturated aqueous $NaHCO_3$, the insoluble was filtered off through a Celite pad. The filtrate was

partitioned between AcOEt and H_2O, and the organic layer was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (3 × 15 cm, 14% AcOEt–hexane) to give **21** (1.29 g, 84%) as a colorless oil: $[\alpha]_D^{23}$ −3.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.32 (m, 5H, phenyl), 5.60 (d, 1H, NH, $J_{NH,2}$ = 9.5 Hz), 5.19 (s, 1H, H-1′), 5.15 (s, 2H, CH_2CCl_3), 4.85 (d, 1H, benzyl, J = 11.6 Hz), 4.67 (d, H, benzyl, J = 11.6 Hz), 4.56 (m, 2H, H-2′, H-3′), 4.51 (dd, 1H, H-2, $J_{2,NH}$ = 9.5, $J_{2,3}$ = 1.9 Hz), 4.45 (m, 1H, H-3), 4.28 (dd, 1H, H-4′, $J_{4',5'a} = 6.8, J_{4',5'b} = 6.8$ Hz), 3.33 (dd, 1H, H-5′a, $J_{5'a,5'b} = 12.6, J_{5'a,4'} = 6.8$ Hz), 3.23 (dd, 1H, H-5′b, $J_{5'b,5'a} = 12.6, J_{5'b,4'} = 6.8$ Hz), 1.68 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.55 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.32 (d, 3H, Me-4, J = 6.2 Hz), 0.90 (t, 3H, CH$_2$CH_3, J = 7.4 Hz), 0.86 (t, 3H, CH$_2$CH_3, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.1, 156.5, 136.0, 128.5, 128.3, 128.2, 117.3, 106.8, 94.2, 86.0, 85.3, 82.1, 75.0, 72.7, 67.3, 58.5, 53.0, 29.3, 28.8, 16.5, 8.4, 7.4; FABMS-HR (NBA) calcd for $C_{24}H_{32}Cl_3N_4O_8$ 609.1286; found: 609.1287.

4.1.10. 2,2,2-Trichloroethyl (2*S*,3*S*)-3-*O*-[5-*tert*-butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-2-benzyloxycarbonylamino-3-hydroxy butanoate (22). A mixture of **21** (1.20 g, 1.98 mmol), Ph_3P (1.56 g, 5.94 mmol), and H_2O (1.8 mL) in THF–benzene (1:1 solution, 15 mL) was stirred at 45 °C for 12 h. After the reaction mixture was cooled to room temperature, $(Boc)_2O$ (918 μL, 3.96 mmol) was added to the mixture, which was stirred for an additional 5 h. The reaction mixture was partitioned between AcOEt and H_2O, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (3 × 15 cm, 29% AcOEt–hexane) to give **22** (955 mg, 71% in 2 steps) as a colorless oil: $[\alpha]_D^{23}$ −16.5 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.32 (m, 5H, phenyl), 5.49 (d, 1H, CbzNH, $J_{NH,2}$ = 9.5 Hz), 5.16 (s, 1H, H-1′), 5.14 (s, 2H, CH_2CCl_3), 5.04 (m, 1H, BocNH), 4.86 (d, 1H, benzyl, J = 11.8 Hz), 4.74 (d, 1H, benzyl, J = 11.8 Hz), 4.54 (m, 3H, H-2′, H-3′, H-2), 4.44 (m, 1H, H-3), 4.24 (dd, 1H, H-4′, $J_{4',5'a} = 6.3, J_{4',5'b} = 6.2$ Hz), 3.25 (m, 1H, H-5′a), 3.00 (m, 1H, H-5′b), 1.67 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.55 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.43 (s, 9H, *tert*-butyl), 1.32 (d, 3H, Me-4, J = 6.5 Hz), 0.89 (t, 3H, CH$_2$CH_3, J = 7.4 Hz), 0.85 (t, 3H, CH$_2$CH_3, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.3, 156.4, 155.8, 135.8, 128.4, 128.2, 128.1, 116.7, 107.0, 94.2, 86.7, 86.1, 82.1, 79.5, 74.8, 72.7, 67.2, 58.6, 43.3, 29.2, 28.7, 28.3, 16.2, 8.3, 7.3; FABMS-HR (NBA) calcd for $C_{29}H_{42}Cl_3N_2O_{10}$ 683.1906; found: 683.1906.

4.1.11. *N*-[(1*S*,2*S*)-2-*tert*-Butyldiphenylsiloxy-1-*tert*-butoxycarbonyl-3-butenyl]-*N*-methyl-2-benzyloxycarbonylamino-3-*O*-[5-*tert*-butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-3-hydroxybutanamide (24). A solution of **22** (320 mg, 0.47 mmol) in MeOH (10 mL) was treated with NH_4Cl (354 mg) and Zn powder (85% purity, 255 mg) at room temperature for 3 h. The mixture was diluted with AcOEt, and the insoluble was filtered off through a Celite pad. The filtrate was concentrated in vacuo to afford the crude acid **23**. A mixture of **23** and **9** (205 mg, 0.47 mmol) in THF (5 mL) was treated with $NaHCO_3$ (559 mg, 1.87 mmol) and DEPBT (559 mg, 1.87 mmol) at room temperature for 48 h. The reaction mixture was partitioned between AcOEt and 0.3 N aqueous HCl, and the organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a silica gel column (3 × 10 cm, 33% AcOEt–hexane) to give **24** (325 mg, 71% in 3 steps) as a white foam: ^{1}H NMR ($CDCl_3$, 500 MHz, 5:1 mixture of the rotamers) δ 7.72–7.65 (m, 4H, phenyl), 7.43–7.30 (m, 11H, phenyl), 6.04 (m, 1H, BocNH), 5.85 (d, 1H, CbzNH, $J_{NH,7}$ = 8.0 Hz), 5.72 (ddd, 1H, H-2, $J_{2,1a}$ = 17.1, $J_{2,1b}$ = 10.3, $J_{2,3}$ = 1.7 Hz), 5.23 (d, 1H, H-4, $J_{4,3}$ = 7.4 Hz), 5.13 (d, 1H, benzyl, J = 15.5 Hz), 5.08 (s, 1H, H-1′), 5.06 (d, 1H, benzyl, J = 15.5 Hz), 4.77 (d, 1H, H-1b, $J_{1b,2}$ = 10.3 Hz), 4.67 (d, 1H, H-1a, $J_{1a,2}$ = 17.1 Hz), 4.58 (m, 2H, H-3, H-7), 4.54 (d, 1H, H-2′, $J_{2',3'}$ = 6.3 Hz), 4.46 (d, 1H, H-3′, $J_{3',2'}$ = 6.3 Hz), 4.33 (m, 1H, H-4″), 4.06 (m, 1H, H-8), 3.26 (m, 1H, H-5′a), 3.13 (m, 1H, H-5′b), 2.97 (s, 3H, NMe), 1.67 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.53 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.44 (s, 9H, *tert*-butyl), 1.42 (s, 9H, *tert*-butyl), 1.18 (d, 3H, Me-9, J = 6.3 Hz), 0.99 (s, 9H, *tert*-butyl), 0.89 (t, 3H, CH$_2$CH_3, J = 7.4 Hz), 0.84 (t, 3H, CH$_2$CH_3, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 167.8, 155.8, 136.6, 136.2, 136.1, 136.1, 137.0, 135.9, 133.7, 133.1, 129.7, 129.5, 128.5, 128.2, 128.2, 127.5, 127.4, 127.3, 127.3, 118.3, 116.6, 106.5, 99.9, 86.9, 86.2, 82.8, 82.3, 81.9, 78.9, 74.3, 73.1, 67.0, 61.6, 54.9, 43.8, 33.0, 29.3, 28.8, 28.5, 28.4, 28.1, 28.0, 27.8, 27.3, 27.0, 26.9, 19.3, 14.9, 8.4, 8.3, 7.4; FABMS-HR (NBA) calcd for $C_{53}H_{76}N_3O_{12}Si$ 974.5198; found: 974.5196.

4.1.12. *N*-[(1*S*,2*S*)-1-*tert*-Butoxycarbonyl-2-hydroxy-3-butenyl]-*N*-methyl-2-benzyloxycarbonylamino-3-*O*-[5-*tert*-butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-3-hydroxybutanamide (25). A solution of **24** (110 mg, 0.11 mmol) in THF (2 mL) was treated with AcOH (71 μL, 1.2 mmol) and TBAF solution (1 M solution in THF, 1.13 mL) at room temperature for 1 week. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a neutral flash silica gel column (3 × 10 cm, 33% AcOEt–hexane) to give **25** (79.8 mg, 96%) as a colorless foam: ^{1}H NMR ($CDCl_3$, 400 MHz, 5:1 mixture of the rotamers) δ 7.35–7.32 (m, 6H, phenyl), 6.03–5.79 (m, 2.9H), 5.50–5.29 (m, 2H), 5.19–5.07 (m, 6H), 4.79 (m, 0.2H), 4.65–4.44 (m, 7.2H), 4.31 (m, 1.8H), 4.06 (m, 1.6H), 3.65 (br s, 1H, OH), 3.26 (m, 1H, H-5′a), 3.18 (m, 1H, H-5′b), 3.12 (s, 3H, COMe), 2.82 (s, 0.6H, COMe), 2.45 (br s, 0.2H, OH), 1.67 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.53 (dd, 2H, CH_2CH$_3$, J = 7.4, 14.8 Hz), 1.43 (m, 24H, 2× *tert*-butyl), 1.19 (d, 3H, Me-9, J = 6.0 Hz), 0.93–0.83 (m, 7H, 2× CH$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 169.5, 155.9, 136.1, 135.5, 128.4, 128.4, 128.1, 118.6, 117.7, 116.7,

106.7, 99.7, 86.9, 86.3, 83.1, 82.3, 82.2, 73.2, 73.1, 71.3, 70.8, 67.1, 69.9, 64.1, 63.7, 54.9, 43.9, 35.8, 29.5, 29.4, 29.0, 28.5, 28.0, 27.8, 20.8, 15.5, 15.0, 14.1, 8.4, 7.5; FABMS-HR (NBA) calcd for $C_{37}H_{58}N_3O_{12}$ 736.4020; found: 736.4020.

4.1.13. Diazepanone (28). A solution of **25** (36 mg, 0.048 mmol) in CH_2Cl_2 (1 mL) was treated with DMAP (1.7 mg, 0.01 mmol), palmitic acid (37 mg, 0.14 mmol), and EDCI (28 mg, 0.14 mmol) at room temperature for 24 h. After the reaction was quenched by addition of MeOH, the mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with 0.3 N aqueous HCl and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue in CH_2Cl_2 (2 mL) was treated with O_3 gas at −78 °C for 10 min, and then Me_2S (2 drops) was added. The resulting mixture was slowly warmed to room temperature and concentrated in vacuo to give crude **27**. A mixture of **27** and Pd black (30 mg) in ^{i}PrOH (3 mL) was vigorously stirred under H_2 atmosphere at room temperature for 4 h. The insoluble was filtered off through a Celite pad, and the filtrate was concentrated in vacuo. The residue in CH_2Cl_2 (3 mL) was treated with AcOH (75 μL) and $NaBH(OAc)_3$ (51 mg, 0.24 mmol) at room temperature for 24 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a neutral silica gel column (1 × 5 cm, 29% AcOEt–hexane) to give **28** (26 mg, 66% in 4 steps) as a white foam: $[\alpha]_D^{23}$ −35.6 (*c* 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.97 (m, 1H, BocNH), 5.24 (m, 1H, H-3), 5.22 (s, 1H, H-1′), 4.64 (d, 1H, H-2′, $J_{2',3'} = 6.0$ Hz), 4.52 (d, 1H, H-3′, $J_{3',2'} = 6.0$ Hz), 4.39 (m, 2H, H-4′, H-2), 4.22 (m, 1H, H-8), 3.38 (m, 1H, H-5′a), 3.28 (d, 1H, H-4a, $J_{4a,b} = 14.3$ Hz), 3.14–3.08 (m, 2 H, H-4b, H-5′a), 2.30 (s, 3H, CONMe), 2.90 (d, 1H, H-6, $J_{6,NH} = 7.2$ Hz), 2.32 (m, 2H, $COCH_2$), 1.68 (dd, 2H, CH_2CH_3, $J = 7.4$, 14.8 Hz), 1.62 (m, 2H, $COCH_2CH_2$), 1.53 (dd, 2H, CH_2CH_3, $J = 7.4$, 14.8 Hz), 1.50 (s, 9H, *tert*-butyl), 1.43 (s, 9H, *tert*-butyl), 1.28–1.23 (m, 29H, Me-9, palmitoyl), 0.92–0.83 (m, 9H, 2× CH_2CH_3, palmitoyl); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6, 172.9, 167.7, 156.3, 116.2, 106.7, 86.8, 83.7, 82.5, 78.6, 72.1, 69.2, 65.4, 64.4, 50.5, 43.4, 38.8, 34.3, 33.7, 32.0, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.2, 29.1, 28.6, 28.4, 28.0, 24.9, 24.8, 22.7, 17.4, 14.2, 8.5, 7.5; FABMS-HR (NBA) calcd for $C_{44}H_{80}N_3O_{11}$ 826.5793; found: 826.5780.

4.1.14. Diazepanone (29). A solution of **28** (26 mg, 32 μmol) in AcOEt (2 mL) was treated with paraformaldehyde (10 mg, 0.03 mmol), AcOH (50 μL), and $NaBH(OAc)_3$ (68 mg, 0.03 mmol) at room temperature for 72 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by a short silica gel column (1 × 5 cm, 17% AcOEt–hexane) to give **29** (22.1 mg, 82%) as a colorless oil: $[\alpha]_D^{23}$ −8.90 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (m, 1H, BocNH), 5.33 (m, 1H, H-3), 5.19 (s, 1H, H-1′), 4.68 (d, 1H, H-2′, $J_{2',3'} = 6.0$ Hz), 4.48 (d, 1H, H-3′, $J_{3',2'} = 6.0$ Hz), 4.42 (m, 1H, H-4′), 4.32 (d, 1H, H-2, $J_{2,3} = 4.7$ Hz), 4.15 (m, 1H, H-8), 3.68 (ddd, 1H, H-5′a, $J_{5'a,b} = 14.2$, $J_{5'a,NH} = 8.5$, $J_{5'a,4'} = 2.8$ Hz), 3.41 (dd, 1H, H-4a, $J_{4a,b} = 15.8$, $J_{4a,3} = 1.5$ Hz), 3.15 (d, 1H, H-4 b, $J_{4b,a} = 15.8$ Hz), 3.10 (d, 1H, H-6, $J_{6,8} = 9.8$ Hz), 3.07 (s, 3H, CONMe), 2.94 (d, 1H, H-5′b, $J_{5'b,a} = 14.2$ Hz), 2.36 (s, 3H, NMe), 2.32 (m, 2H, $COCH_2$), 1.68 (dd, 2H, CH_2CH_3, $J = 7.4$, 14.8 Hz), 1.61 (m, 2H, $COCH_2CH_2$), 1.53 (dd, 2H, CH_2CH_3, $J = 7.4$, 14.8 Hz), 1.50 (s, 9H, *tert*-butyl), 1.44 (s, 9H, *tert*-butyl), 1.24 (m, 29H, Me-9, palmitoyl), 0.93–0.83 (m, 9H, 2× CH_2CH_3, palmitoyl terminal-Me); FABMS-LR *m*/*z* 840 (MH^+); FABMS-HR (NBA) calcd for $C_{45}H_{82}N_3O_{11}$ 840.5949; found: 840.5940.

4.1.15. Uridine-truncated analog (6). Compound **29** (22.0 mg, 0.026 mmol) was treated with 80% aqueous TFA (2 mL) for 24 h, and the reaction mixture was concentrated in vacuo. The residue was dissolved in H_2O (1 mL) and freeze–dried to afford **6** (a TFA salt, 19 mg, quant.) as a white powder: $[\alpha]_D^{23}$ −5.40 (*c* 1.00, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.43 (m, 1H, H-3), 5.02 (s, 1H, H-1′), 4.64 (d, 1H, H-2, $J_{2,3} = 4.6$ Hz), 4.10 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.90 (d, 1H, H-6, $J_{6,9} = 1.6$ Hz), 3.32–3.15 (m, 4H, H-4a, H-4b, H-5′a, H-5′b), 3.09 (s, 3H, CONMe), 2.42 (s, 3H, NMe), 3.37 (ddd, 2H, $COCH_2$, $J = 3.4$, 7.9, 10.6 Hz), 1.62 (m, 2H, $COCH_2C\underline{H}_2$), 1.28 (m, 24H, palmitoyl), 1.19 (d, 3H, Me-10, $J = 6.3$ Hz), 0.89 (m, 3H, palmitoyl terminal-Me); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.7, 173.9, 172.9, 144.2, 107.9, 80.0, 76.7, 73.6, 73.1, 72.5, 72.3, 69.6, 65.7, 57.8, 42.4, 38.5, 35.2, 35.0, 33.1, 30.8, 30.8, 30.8, 30.7, 30.6, 30.6, 30.5, 30.4, 30.4, 30.3, 30.2, 26.1, 25.9, 23.7, 19.0, 14.4; FABMS-HR (NBA) calcd for $C_{31}H_{56}N_3O_9$ 614.4017; found: 614.4011.

4.2. Antibacterial activity against *M. tuberculosis*

The initial screen is conducted against *M. tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium using the microplate Alamar blue assay (MABA). Compounds are tested in 10 twofold dilutions, usually from 100 to 0.19 μg/mL. Compounds exhibiting autofluorescence are sent for testing in an alternative assay at the University of Illinois at Chicago.

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