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Tetrahedron 59 (2003) 983–993

TETRAHEDRON

Tobramycin analogues with C-5 aminoalkyl ether chains intended to mimic rings III and IV of paromomycin

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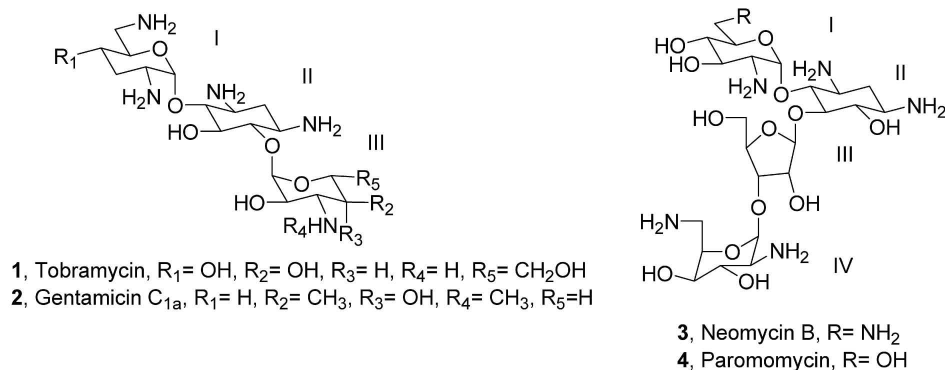
Received 23 October 2002; revised 16 December 2002; accepted 16 December 2002

Abstract—Based on available X-ray structural and modeling data, a series of tobramycin derivatives with C-5 ether chains bearing basic groups were synthesized. These were intended to be hybrid molecules that combine features of tobramycin and paromomycin. Their binding to ribosomes and their antibacterial activity were determined. The 5-*O*-(2-guanidylethyl) ether of tobramycin (**9g**) was the most active analogue in the series. © 2003 Elsevier Science Ltd. All rights reserved.

The binding of small organic molecules to RNAs of different origins has been the subject of extensive research on different fronts in recent years.[1] The diversity of structures, including biologically relevant compounds has focused more attention to RNAs as targets for therapeutic agents.[2] The aminoglycoside family, long known for their potent antibacterial action, are clinically used in a hospital environment where they can be monitored.[3] They are particularly attractive ligands to ribosomal and other types of RNAs, as exemplified by pioneering studies carried out in several laboratories world-wide.[4]

Paromomycin **4**, a member of the 4,5-linked aminoglycosides has been a superb substrate for binding studies to



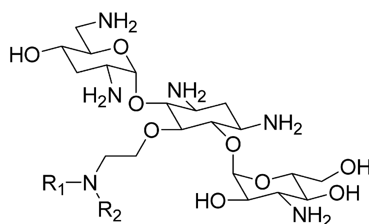


Figure 1. Structures of representative aminoglycosides. Roman numerals refer to individual rings.

Keywords: aminoglycoside; amidine; guanidine; ribosome; antibacterial activity.

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PII: S0040-4020(02)01624-1

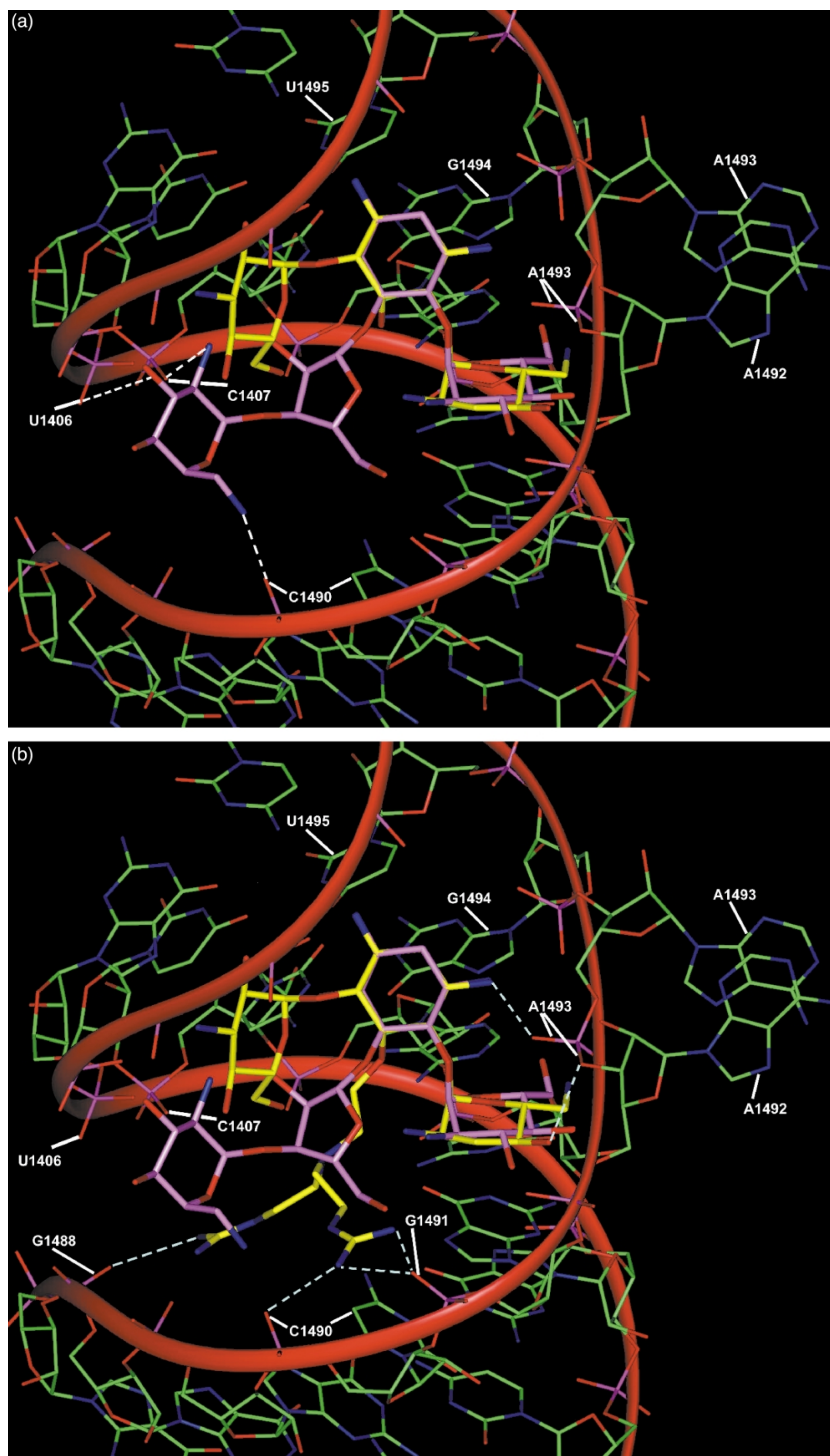


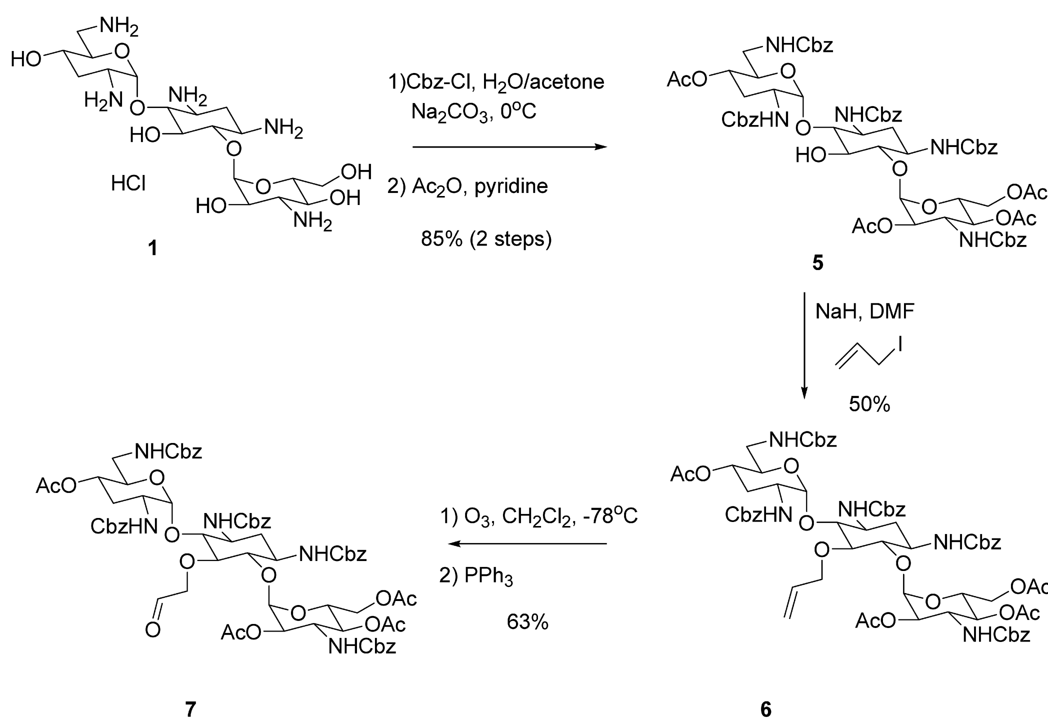
Figure. 2. Top panel. Superposition of paromomycin (pink) and tobramycin (yellow) in 16S RNA segment based on Puglisi[6] and Westhof.[8] Bottom panel. Tobramycin analogue **9g** with 5-*O*-(bis-guanidino)-alkyl chain (yellow), superposed on paromomycin (pink).

ribosomal RNA[5] (Fig. 1). Elegant NMR[6] and X-ray[7] crystallographic studies of paromomycin–RNA complexes have delineated the origins of the interactions of each of the four rings and their appended hydroxy and amino groups. Puglisi's NMR studies[6] originally identified several important contacts with specific nucleotide residues when bound in the major groove of the A-site of the 16S subunit ribosomal RNA. Rings I and II (paromamine subunit) make specific contacts by H-bonded, charge, and stacking interactions. Characteristically, ring I occupies the area left by bulging out of the A^{1492} and A^{1493} base pair which are critical for viable RNA function. Other contacts are made by ring III inter- and intramolecularly, while ring IV contributes in a less ordered manner by making H-bonds to the phosphate backbone of U^{1406}, C^{1407} and C^{1490}. It is reported that rings III and IV provide the requisite L-shape of paromomycin within the RNA complex to properly orient rings I and II for effective contacts.[6b] X-Ray crystallographic studies of paromomycin complexed with a sequence of oligonucleotides corresponding to the ribosomal A-site of *E. coli* at 2.5 Å resolution by Vicens and Westhof[8] have corroborated the NMR results and further characterized the nature of the contacts. For example, direct contacts of N-2‴ and O-4‴ of ring IV have been observed.[8] The importance of water molecules in H-bonding through hydration shells of paromomycin and the RNA has been demonstrated. X-Ray crystal structures of paromomycin complexed with mRNA and cognate tRNA in the A-site at 3.1–3.3 Å resolution have also been reported.[9]

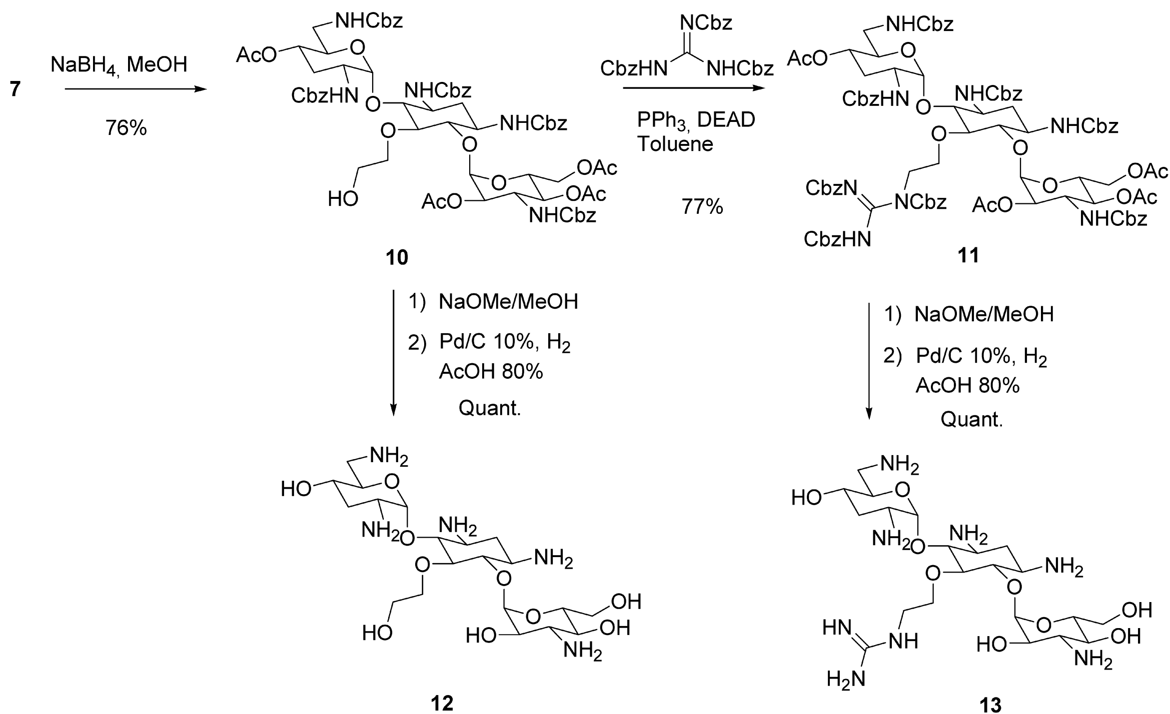
The interactions of 5,6-substituted aminoglycosides such as tobramycin **1**[10] and gentamicin C_{1a} **2**[11] with RNA sequences have also been studied by NMR and X-ray crystallography.[8b,12] The constant ring I and ring II pseudodisaccharide subunits occupy the same space as for paromomycin. However, whereas rings III and IV of paromomycin interact with the lower stem of the A-site RNA,[6] ring III of tobramycin and gentamicin C_{1a} makes contacts with the upper stem. Thus, we can qualitatively assume that rings III in the 4,6-disubstituted, and rings III, IV in the 4,5-disubstituted aminoglycosides, occupy different spaces while being anchored to their respective pseudodisaccharides within the major groove. (Fig. 2, top panel).

We reasoned that introduction of ether-linked basic groups at C-5 in tobramycin, and of varying chain length could produce hybrid molecules such that rings III and IV of paromomycin could be mimicked. Molecular modelling studies indicated that the amino or guanidino-containing side-chains originating at C-5 of tobramycin might reach far enough in the lower stem of the A-site of the 16S rRNA to interact with backbone phosphate linkages at U^{1406}, C^{1407}, and C^{1490}, or to alternative new contacts with C^{1498}, C^{1490}, and G^{1491} (Fig. 2, bottom panel). We report herein the results of our studies toward this goal.[13]

Acetylation of penta *N*-Cbz tobramycin[14] with excess acetic anhydride in pyridine afforded a partially acetylated derivative **5** in which the C-5 hydroxyl group of the deoxystreptamine unit was free (Scheme 1). Allylation under standard conditions afforded the C-5 allyl ether **6**, which was oxidatively cleaved to afford the aldehyde **7**. Detailed NMR studies on **6** indicated that acetyl migration had not taken place during the *O*-allylation step of **5**. Scheme 2 shows the synthesis of the C-5 hydroxyethyl ether **10** as well as its utility in a Mitsunobu-type guanidylation[15] reaction to afford the branched guanidino precursor **11**. Deprotection afforded the branched hybrid aminoglycoside analogues **12** and **13**. A series of reductive amination reactions performed on **7** led to a set of acylic and cyclic *N*-protected appendages in good to excellent yields

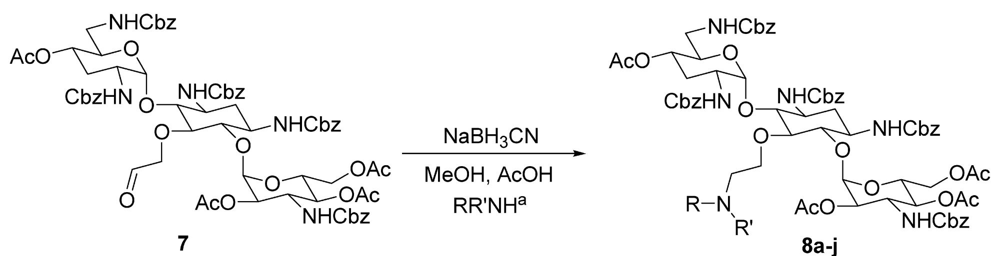


Scheme 1.



Scheme 2.

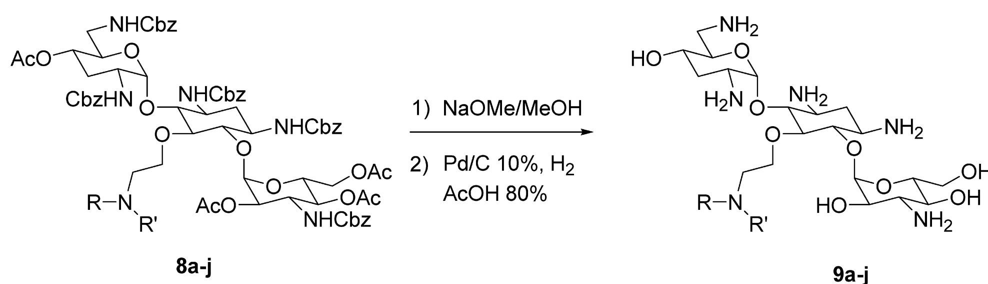
Table 1.



Amine used[a]	Product	R, (R′=H)	Yield (%)	Amine used[a]	Product	R,R′	Yield (%)
21	**8a**	CbzHN	54	**17**	**8g**	NBoc BocHN N H	46
23	**8b**	CbzHN	61				
–	**8c**	H_3C	92	**14b**	**8h**	CbzHN	54
26	**8d**	NBoc BocHN N H	90	**18**	**8i**	N Cbz	85
27	**8e**	NBoc BocHN N H	26				
20	**8f**	CbzHN NHCbz	81	**19**	**8j**	N BocN NHBoc	43

[a] See Scheme 3.

Table 2.



Starting material	Product	R, (R′=H)	Yield	Starting material	Product	R,R′	Yield
8a	**9a**	H_2N	Quant.	**8g**	**9g**	NH, H_2N, N, H	Quant.
8b	**9b**	H_2N	Quant.				Quant.
8c	**9c**	H	Quant.[a]	**8h**	**9h**	H_2N	Quant.
8d	**9d**	NH, H_2N, N, H	Quant.[a]	**8i**	**9i**	N, H	Quant.
8e	**9e**	NH, H_2N, N, H	Quant.[a]				
8f	**9f**	NH_2 NH_2	Quant.	**8j**	**9j**	N, HN, NH_2	Quant.[a]

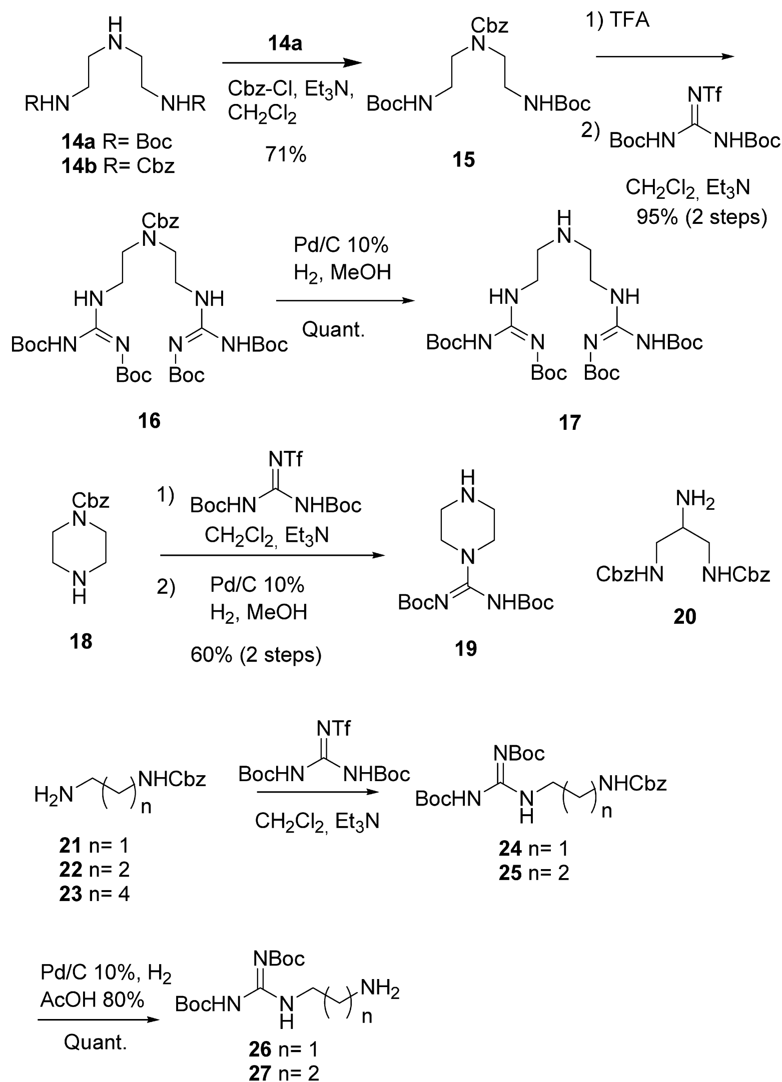
[a] Deprotection conditions: (1) TFA/H_2O (10:1) 1 h, (2) H_2, Pd/C in H_2O/dioxane/AcOH.

(Table 1). The low yield in the case of **8e** was due to a competing intramolecular cyclization to a cyclic guanidine. Depending on the *N*-protecting group, the hybrid analogues were first treated with NaOMe/MeOH to remove the acetates, then subjected to hydrogenolysis to remove all *N*-Cbz groups, or to TFA hydrolysis of *N*-Boc groups, followed by hydrogenolysis as shown in Table 2. The corresponding hybrid aminoglycosides were isolated as amorphous white solids as acetate salts. The synthesis of selected acyclic and cyclic amines used in the reductive amination reactions is illustrated in Scheme 3.

1. Results

As mentioned above, the basic premise for synthesizing hybrid aminoglycoside analogues using tobramycin as a prototype was to incorporate an appendage at C-5 to simulate the space and interactions made by rings III and IV of paromomycin. The choice of basic appendages relied on the diversity of pK_a, chain-length, branching and flexible topology. In Table 3 are listed the results of antibacterial activities against strains of *S. aureus*, and *E. coli*, the IC_{50} values of transcription/ translation, and the dissociation constants (K_D) for the binding of the analogues to a prototypical segment of 16S rRNA.[5,17]

Some of the analogues were as active as tobramycin exhibiting MIC values of 0.6–2.5 μM against *E. coli*. (Table 3, compounds **9f–i**). The guanidino analogues **9d** and **9g** also showed potentially interesting activity against *P. aeruginosa* (ATCC 27853) at MIC 12.5 μg/mL.[18] In general transcription/translation data and K_D values compared well with those obtained for tobramycin and paromomycin. A clear SAR correlating the nature of the appendage with MIC values could not be seen, although it appeared that analogues with more basic groups such as **9g** favored ribosomal binding and antibacterial activity. Some aminoglycosides such as paromomycin exhibit a good relationship between their binding affinity toward the 16S ribosome and transcription/translation activity.[16] The 4,6-disubstituted series such as kanamycin and tobramycin on the other hand show excellent MIC values, but do not bind as tightly as paromomycin. Good IC_{50} values for transcription/translation may reflect the efficiency of mis-coding and the formation of non-functional proteins. However low μM values for K_D may not necessarily result in potent compounds due to non-functional binding, efflux, or mechanisms not related to A-site ribosomal binding. Low



Scheme 3.

Table 3. Activities of aminoglycoside analogues

Entry	Compound	K_D (μM)	Translation, T/T IC_{50} (μM)	*E. coli*[a] MIC (μM)	*S. aureus*[b] MIC (μM)
1	Kanamycin A	4.82	0.57	2.5–5	1.2–2.5
2	Kanamycin B	1.48	0.36	1.2–2.5	0.3–0.6
3	Tobramycin	2.39	0.45	0.6–1.2	0.3–0.6
4	Amikacin	2.45	0.53	1.2–2.5	1.2–2.5
5	Paromomycin	0.15	0.56	2.5–5	1.2–2.5
6	Neamine	4.56	1.89	>10	>10
7	**9a**	3.05	1.01	2.5–5	>10
8	**9c**	1.37	2.17	5–10	>10
9	**9d**	2.86	2.33	2.5–5	>10
10	**9f**	3.97	0.86	1–2	5–10
11	**9g**	0.64	0.78	0.6–1.2	2.5–5
12	**9h**	2.29	0.72	1–2	5–10
13	**9i**	2.96	0.56	1–2	5–10
14	**9j**	9.58	1.09	2.5–5	>10
15	**12**	6.41	0.38	1–2	2.5–5
16	**28**	13.57	>10	>10	>10
17	**29**	1.15	3.62	>10	>10
18	**30**	[c]	>10	>10	>10

[a] ATCC 25922.
[b] ATCC 13709.
[c] Not determined.

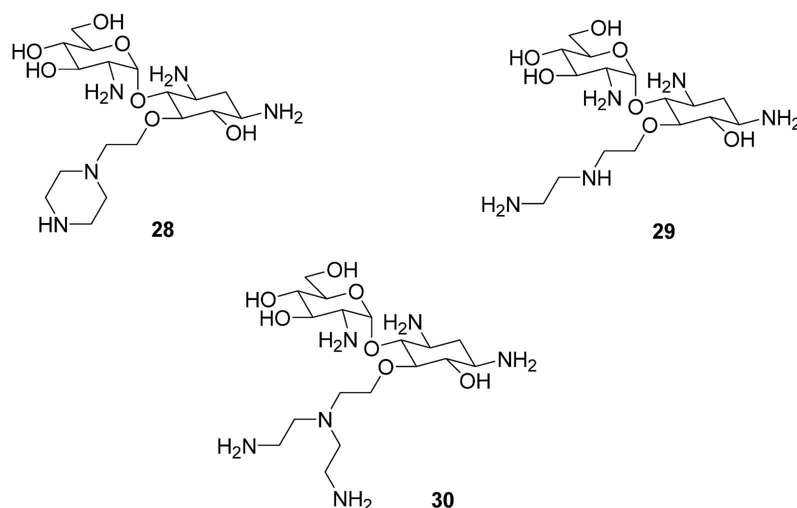


Figure 3. Structure of paromamine analogues.

binding affinity for 3″-OH substituted analogs of neomycin, in spite of their good antibacterial activity has been reported previously.[19] The promising antimicrobial activity against *E. coli* shown by some of the hybrid analogues, warrants further exploitation of the 'ring III/IV' space by more optimized appendages at C-5 of tobramycin.

We have recently reported the synthesis of a series of substituted analogues of paromamine **28**–**31**[20] (Fig. 3), which were found to have no inhibitory activity against a panel of sensitive strains (*P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis*).[17] These analogues resemble the 5-*O*-substituted tobramycin analogues **9a** and **9i**, except for the absence of the kanosamine ring, and the nature of ring I which delineates the well known need for a pseudotrisaccharide as a minimum structure if effective inhibition is expected.[13a] Nevertheless, the branched paromamine analogue **29** exhibited good ribosomal binding and/or transcription/translation activity (Table 3). Clearly, there is a need to better understand the structural and functional requirements that correlate ribosomal binding and antibacterial activity. Studies toward this goal are in progress in our laboratories.

2. General information

Solvents were distilled under positive pressure of dry nitrogen before use and dried by standard methods; THF and ether, from Na/benzophenone; and CH_2Cl_2, from $CaCl_2$. All commercially available reagents were used without further purification. All reactions were performed under nitrogen atmosphere. NMR (^{1}H,^{13}C) spectra were recorded on AMX-300, ARX-400 and DMX-600 spectrometers. Low- and high-resolution mass spectra were recorded on VG Micromass, AEI-MS 902 or Kratos MS-50 spectrometers using fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on Merck 60F_{254} pre-coated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate or ninhydrine. Flash column chromatography was performed using (40–60 μm) silica gel at increased pressure. Binding constant for the 16S A-site model RNA, IC_{50}s for inhibition of bacterial transcription/translation, and MIC values were determined as described previously.[16,17]

2.1.1. Compound 6

To a solution of **5**[14] (1.0 g, 0.76 mmol) in DMF (7 mL) was added allyl iodide (0.21 mL, 2.3 mmol). After stirring at 0°C for 15 min, a suspension of NaH (46 mg, 1.9 mmol) in DMF (6 mL) was added dropwise and the resulting mixture was stirred for a further 30 min, quenched with few drops of 1N NH_4Cl and the solvent was removed by evaporation. The residue was dissolved in EtOAc and washed with H_2O, brine and dried over Na_2SO_4. The solvent was evaporated under vacuum and the crude solid was purified by flash chromatography (CH_2Cl_2/acetone, 85:15) to give **6** (0.51 g, 50%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.20 (m, 25H), 5.82–5.60 (m, 1H), 5.50–3.00 (m, 22H), 2.08 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.3, 171.1, 170.5, 170.4, 157.2, 156.5, 156.3, 155.8, 136.9, 136.7, 136.6, 133.7, 129.0, 128.9, 128.9, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 117.8, 95.2, 83.6, 75.4, 72.0, 71.5, 70.9, 69.2, 68.6, 68.2, 67.5, 67.3, 67.2, 67.0, 66.9, 62.2, 52.2, 51.8, 50.4, 49.3, 32.1, 31.4, 31.2, 21.2, 21.1, 20.8, 19.7, 14.4; $[\alpha]_D$=+37 (*c* 1.1, $CHCl_3$); FAB *m/z* calcd (M+H^+) 1346.5; found 1346.0.

2.1.2. Compound 7

A solution of **6** (345 mg, 0.260 mmol) in CH_2Cl_2 (14 mL) was cooled at −78°C, ozone was bubbled until the solution turned light blue, after which argon was bubbled through. The solution was treated with PPh_3 (134 mg, 0.510 mmol) and warmed to room temperature. The solvent was removed under vacuum and the crude solid purified by flash chromatography (EtOAc/hexanes, 75:25) to give **7** (216 mg, 63%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.40–7.10 (m, 25H), 5.60–3.00 (m, 22H), 2.09 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 197.1, 171.2, 170.3, 157.3,

156.6, 155.9, 155.8, 136.9, 136.7, 132.6, 132.4, 128.9, 128.8, 128.6, 128.5, 128.3, 128.1, 128.0, 96.6, 94.8, 84.8, 79.2, 70.7, 68.6, 67.4, 67.0, 62.4, 51.9, 50.1, 49.3, 42.5, 35.6, 30.8, 21.1, 20.6, 19.5; $[\alpha]_D$=+65 (*c* 1.4, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1348.5; found 1348.1.

2.2. General procedure for reductive amination (8a–j)

To a mixture of **7** (0.034 mmol) and the appropriate amine (Scheme 3, **14b**,[21] **18**,[22] **20**,[23] **21–23**,[24] **26**[25] and **27**,[26]) (0.10 mmol) in MeOH (3 mL) was added AcOH (0.1 mL) followed by $NaBH_3CN$ (1.0 M in THF, 0.1 mL). The mixture was stirred at room temperature overnight until disappearance of **7**, diluted with EtOAc (15 mL), washed with a solution of $NaHCO_3$ (satd, 10 mL) and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give (**8a–j**) as white solids.

2.2.1. Compound 8a. (54%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.10 (m, 30H), 5.50–4.50 (m, 17H), 4.40–2.80 (m, 12H), 2.05 (s, 3H), 2.00 (s, 3H), 1.84 (s, 6H); $[\alpha]_D$=+59 (*c* 1.7, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1526.6; found 1525.9.

2.2.2. Compound 8b. (61%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.10 (m, 30H), 6.00–5.90 (large s, 1H), 5.50–4.50 (m, 17H), 4.40–3.00 (m, 12H), 2.70–2.45 (large, 2H), 2.07 (s, 3H), 2.03 (s, 3H), 1.84 (s, 6H), 1.70–1.40 (m, 4H), 1.35–1.20 (m, 2H); $[\alpha]_D$=+56 (*c* 1.1, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1568.7; found 1568.9.

2.2.3. Compound 8c. (92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.00 (m, 29H), 5.85–5.70 (large s, 1H), 5.40–4.45 (m, 17H), 4.30–3.20 (m, 10H), 3.00 (m, 1H), 2.78 (m, 1H), 2.45 (m, 1H), 2.33 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.82 (s, 6H), 1.49 (s, 18H); $[\alpha]_D$=+49 (*c* 1.2, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1634.7; found 1634.8.

2.2.4. Compound 8d. (90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 25H), 5.50–4.52 (m, 13H), 4.40–3.20 (m, 10H), 3.10–2.85 (m, 4H), 2.07 (s, 3H), 2.01 (s, 3H), 1.84 (s, 6H), 1.49 (s, 18H); $[\alpha]_D$=+46 (*c* 1.0, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1634.7; found 1634.8.

2.2.5. Compound 8e. (26%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.21 (m, 25H), 5.80–4.60 (m, 13H), 4.30–2.60 (m, 16H), 2.04 (s, 6H), 1.80 (s, 6H), 1.53 (s, 18H); $[\alpha]_D$=+47 (*c* 1.0, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1648.7; found 1649.0.

2.2.6. Compound 8f. (81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.15 (m, 35H), 5.70–4.42 (m, 20H), 4.38–2.80 (m, 16H), 2.70–2.60 (m, 2H), 2.05 (s, 3H), 1.99 (s, 3H), 1.84 (s, 3H), 1.82 (s, 3H); $[\alpha]_D$=+49 (*c* 1.2, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1689.7; found 1689.7.

2.2.7. Compound 8g. (46%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.20 (m, 25H), 5.80–3.20 (m, 36H), 2.80–2.45 (m, 4H), 2.06 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.82 (s, 3H), 1.47 (s, 36H); $[\alpha]_D$=+48 (*c* 1.1, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1919.9; found 1919.8.

2.2.8. Compound 8h. (34%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.10 (m, 35H), 5.50–4.50 (m, 17H), 4.40–2.80 (m, 15H), 2.70–2.12 (m, 6H), 2.05 (s, 3H), 1.97 (s, 3H), 1.86 (s, 6H); $[\alpha]_D$=+50 (*c* 1.2, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1703.7; found 1703.7.

2.2.9. Compound 8i. (85%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.10 (m, 30H), 5.60–4.55 (m, 22H), 4.40–3.20 (m, 18H), 3.02 (m, 2H), 2.50–2.10 (m, 4H), 2.04 (s, 3H), 1.98 (s, 3H), 1.88 (s, 3H), 1.80 (s, 3H); $[\alpha]_D$=+49 (*c* 1.4, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1552.6; found 1553.0.

2.2.10. Compound 8j. (43%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 25H), 5.70–4.50 (m, 20H), 4.40–3.20 (m, 16H), 3.10–2.85 (m, 2H), 2.60–2.30 (m, 4H), 2.05 (s, 3H), 1.98 (s, 3H), 1.89 (s, 3H), 1.80 (s, 3H), 1.49 (s, 18H); $[\alpha]_D$=+46 (*c* 1.0, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1634.7; found 1634.8.

2.3. General procedure for deprotection (9a–c, 9f, 9h and 9i)

The appropriate pseudo-disaccharide was treated with a catalytic amount of NaOMe in MeOH (2 mL). The reaction mixture was stirred at room temperature until completion (approximately 2 h) then neutralized by addition of Amberlite IR-120(H$^+$), the resin was filtered off and the filtrate was evaporated to dryness To the resulting residue in AcOH (80% in H_2O, 2 mL), was added 10% Pd/C (approximately 10 mg) and the mixture was stirred under 1 atm of hydrogen overnight. The mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford (**9a–c**, **9f**, **9h** and **9i**) as fluffy white solids.

2.3.1. Compound 9a. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.25 (s, 1H), 5.07 (s, 1H), 4.20–3.00 (m, 22H), 2.30 (m, 1H), 2.10–1.90 (m, 3H), 1.78 (s, 21H); ^{13}C NMR (75 MHz, D_2O) δ 181.5, 101.2, 93.4, 82.8, 82.4, 77.7, 73.9, 73.6, 68.9, 68.3, 66.1, 64.3, 60.8, 54.9, 50.1, 48.7, 48.2, 47.8, 45.5, 39.4, 36.8, 29.4, 23.5; $[\alpha]_D$=+25 (*c* 0.9, H_2O); MALDI-FTMS calcd for $C_{22}H_{47}N_7O_9Na$ (M+Na$^+$) 576.3327; found 576.3330.

2.3.2. Compound 9b. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.39 (s, 1H), 5.23 (s, 1H), 4.20–3.45 (m, 18H), 3.40–3.18 (m, 6H), 3.10–2.92 (m, 4H), 2.35 (m, 1H), 2.10–1.95 (m, 3H), 1.92 (s, 21H), 1.80–1.60 (m, 4H), 1.50–1.40 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 181.5, 101.2, 93.9, 83.0, 82.9, 78.1, 73.9, 73.2, 70.3, 68.9, 66.7, 64.5, 61.4, 54.7, 50.1, 48.9, 48.2, 47.9, 39.4, 30.6, 26.6, 25.5, 23.2,; $[\alpha]_D$=+16 (*c* 0.5, H_2O); MALDI-FTMS calcd for $C_{25}H_{54}N_7O_9$ (M+H$^+$) 596.3977; found 596.3980.

2.3.3. Compound 9c. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.35 (s, 1H), 5.19 (s, 1H), 4.20–3.36 (m, 17H), 3.35–3.15 (m, 6H), 2.25 (m, 1H), 2.20 (m, 1H), 2.00 (m, 1H), 1.88 (s, 18H), 1.70 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 181.7, 100.9, 93.4, 82.9, 82.6, 77.6, 73.9, 73.3, 68.8, 67.0, 66.3, 64.3, 61.1, 54.7, 50.0, 48.8, 47.9, 39.8, 39.4, 30.3, 29.8, 23.5; $[\alpha]_D$=+26 (*c* 1.0, H_2O); MALDI-FTMS calcd for $C_{20}H_{42}N_6O_9Na$ (M+Na$^+$) 533.2905; found 533.2909.

2.3.4. Compound 9f. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.36 (s, 1H), 5.08 (s, 1H), 4.00–2.60 (m, 22H), 2.25 (m,

1H), 2.27 (m, 1H), 1.92 (m, 1H), 1.92 (s, 1H), 1.78 (s, 24H) 1.62 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 182.1, 101.6, 93.5, 83.6, 83.1, 82.9, 82.7, 77.6, 74.1, 73.6, 71.8, 69.4, 66.1, 65.9, 64.8, 60.2, 55.5, 53.9, 50.5, 49.3, 48.4, 46.6, 45.7, 40.6, 40.5, 40.1, 30.2, 30.0, 29.9, 25.3, 24.0; $[\alpha]_D$=+25 (*c* 1.0, H_2O); MALDI-FTMS calcd for $C_{23}H_{50}N_8O_9Na$ (M+Na$^+$) 605.3593; found 605.3601.

2.3.5. Compound 9h. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.36 (s, 1H), 5.21 (s, 1H), 4.10–3.17 (m, 22H), 3.10–3.05 (m, 4H), 2.95–2.80 (m, 6H), 2.40–2.20 (m, 2H), 2.10–1.95 (m, 1H), 1.91 (s, 24H), 1.80–1.60 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 182.1, 101.1, 94.3, 83.5, 82.3, 78.2, 74.0, 72.4, 69.5, 65.9, 65.4, 60.2, 55.7, 52.5, 51.3, 50.8, 49.4, 48.7, 40.5, 37.3, 31.5, 31.3, 31.0, 24.0; $[\alpha]_D$=+21 (*c* 0.90, H_2O); MALDI-FTMS calcd for $C_{24}H_{53}N_8O_9$ (M+H$^+$) 597.3930; found 597.3938.

2.3.6. Compound 9i. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.30 (s, 1H), 5.05 (s, 1H), 3.92–3.00 (m, 24H), 2.75–2.52 (m, 8H), 2.17 (m, 2H), 1.88 (m, 1H), 1.76 (s, 21H) 1.55 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.7, 92.9, 82.7, 81.9, 77.0, 73.7, 71.9, 69.0, 66.5, 65.7, 64.8, 60.2, 57.0, 55.1, 50.4, 50.1, 48.7, 48.1, 43.1, 40.0, 30.7, 30.3, 23.5; $[\alpha]_D$=+35 (*c* 1.0, H_2O); MALDI-FTMS calcd for $C_{24}H_{49}N_7O_9Na$ (M+Na$^+$) 602.3484; found 602.3471.

2.4. General procedure for deprotection (9d,e, 9g and 9j)

The appropriate pseudo-disaccharide was treated with a catalytic amount of NaOMe in MeOH (2 mL). The reaction mixture was stirred at room temperature until completion (approximately 2 h) then reaction mixture was neutralized by addition of Amberlite IR-120(H$^+$), the resin was filtered off and the filtrate was evaporated to dryness The resulting residue was dissolved in TFA (2 mL), stirred for 2 h and concentrated under vacuum. To the resulting residue in AcOH (80% in H_2O, 2 mL), was added 10% Pd/C (approximately 10 mg) and the mixture was stirred under 1 atm of hydrogen overnight. The mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford (**9d**,**e**, **9g** and **9j**) as fluffy white solids.

2.4.1. Compound 9d. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.33 (s, 1H), 5.20 (s, 1H), 4.30 (m, 1H), 4.20–3.15 (m, 22H), 2.50 (m, 1H), 2.20 (m, 1H), 1.85 (s, 1H), 1.25 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 164.0, 163.5, 163.1, 162.6, 157.6, 122.5, 118.6, 114.7, 110.9, 101.5, 93.3, 82.6, 78.3, 77.3, 74.1, 68.7, 66.8, 63.1, 61.7, 54.5, 49.7, 48.5, 47.4, 46.9, 38.4, 38.1, 28.0, 27.8; $[\alpha]_D$=+27 (*c* 1.0, H_2O); MALDI-FTMS calcd for $C_{23}H_{49}N_9O_9Na$ (M+Na$^+$) 618.3545; found 618.3548.

2.4.2. Compound 9e. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.35 (s, 1H), 5.15 (s, 1H), 4.15–2.85 (m, 30H), 2.42 (m, 1H), 1.90 (m, 3H); ^{13}C NMR (75 MHz, D_2O) δ 165.8, 163.6, 115.3, 101.9, 94.1, 83.1, 82.3, 77.7, 74.4, 69.2, 67.1, 63.6, 55.1, 49.0, 47.9, 46.3, 38.9, 37.7, 32.2, 25.8; $[\alpha]_D$=+29 (*c* 1.0, H_2O).

2.4.3. Compound 9g. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.27 (s, 1H), 5.05 (s, 1H), 4.50–3.05 (m, 30H), 2.40 (m, 1H), 2.10 (m, 2H), 1.90 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 163.5, 163.0, 157.4, 118.6, 114.7, 101.1, 93.2, 83.6, 82.0, 78.4, 77.4, 74.3, 68.8, 65.8, 63.1, 54.9, 52.2, 49.6, 48.5, 47.4, 38.5, 36.9, 27.9; $[\alpha]_D$=+33 (*c* 1.1, H_2O); MALDI-FTMS calcd for $C_{26}H_{57}N_{12}O_9$ (M+H$^+$) 681.4366; found 681.4361.

2.4.4. Compound 9j. (quant.); ^{1}H NMR (400 MHz, D_2O) δ 5.26 (s, 1H), 5.08 (s, 1H), 4.50–3.10 (m, 30H), 2.40 (m, 1H), 2.10 (m, 2H), 1.89 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 163.9, 163.5, 163.1, 157.0, 118.2, 115.3, 101.4, 93.3, 83.1, 82.2, 78.5, 77.4, 74.4, 68.7, 68.5, 66.3, 63.1, 61.3, 57.5, 54.6, 52.6, 49.7, 48.5, 47.4, 43.1, 38.4, 27.9, 23.1; $[\alpha]_D$=+33 (*c* 1.1, H_2O); MALDI-FTMS calcd for $C_{25}H_{52}N_7O_9$ (M+H$^+$) 622.3882; found 622.3882.

2.4.5. Compound 10. A solution of **7** (200 mg, 0.148 mmol) in MeOH (2 mL) was cooled at 0°C and treated with $NaBH_4$ (22 mg, 0.590 mmol). The mixture was stirred for 15–20 min, few drops of AcOH were added to quench the reaction and the mixture was warmed to rt. The solvents were removed under vacuum, the residue was dissolved in EtOAc, washed with water, brine and dried over Na_2SO_4. The solvent was removed under vacuum and the crude solid purified by flash chromatography (CH_2Cl_2/acetone, 85:15) to give **10** (152 mg, 76%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 25H), 5.60–5.40 (m, 4H), 5.30–4.70 (m, 16H), 4.60 (m, 1H), 4.40–4.20 (m, 2H), 4.00–3.25 (m, 13H), 3.05 (m, 1H), 2.85 (m, 1H), 2.08 (s, 3H), 2.00 (s, 3H), 1.84 (s, 6H), 1.70 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 170.6, 157.3, 156.7, 156.4, 155.9, 136.9, 136.8, 136.7, 129.1, 129.0, 128.8, 128.6, 128.3, 128.2, 128.0, 95.8, 94.9, 84.6, 70.9, 68.9, 68.7, 67.5, 67.2, 67.0, 62.5, 61.7, 52.1, 51.7, 50.2, 49.3, 42.7, 35.6, 30.9, 21.2, 20.8; $[\alpha]_D$=+67 (*c* 1.4, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1350.5; found 1350.4.

2.4.6. Compound 11. To a suspension of **10** (50 mg, 0.037 mmol), tris(N,N',N''-benzyloxycarbamate)guanidine[15] (40 mg, 0.11 mmol) and PPh_3 (15 mg, 0.056 mmol) in toluene (1.5 mL) was added slowly diethylazodicarboxylate (8.7 μL, 0.056 mmol). The mixture was heated at 70°C during 1 h, cooled down at room temperature and concentrated under vacuum. The resulting residue was purified by flash chromatography (EtOAc/hexanes, 45:55) to give **11** (51 mg, 77%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.20 (m, 40H), 5.80 (m, 1H), 5.50 (m, 2H), 5.35–5.76 (m, 20H), 4.45 (m, 2H), 4.25–3.30 (m, 12H), 3.00 (m, 2H), 2.20 (m, 2H), 2.05 (m, 3H), 1.99 (m, 3H), 1.89 (s, 3H), 1.74 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.9, 169.7, 156.7, 155.7, 155.6, 155.5, 154.4, 136.6, 136.3, 134.4, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.6, 95.7, 94.9, 70.1, 69.2, 68.5, 68.2, 67.8, 66.7, 66.5, 66.2, 61.8, 51.9, 49.3, 49.0, 41.6, 29.9, 29.5, 20.7, 20.5, 20.4, 20.1; $[\alpha]_D$=+62 (*c* 1.0, $CHCl_3$); FAB *m/z* calcd (M+H$^+$) 1793.7; found 1793.8.

2.4.7. Compound 12. A solution of **10** (50.0 mg, 0.037 mmol) in MeOH (2 mL) was treated with a catalytic amount of NaOMe. The reaction mixture was stirred at room temperature until completion (approximately 2 h), neutralized by addition of Amberlite IR-120(H$^+$), the resin was filtered off and the filtrate was evaporated to dryness.

To the resulting residue in AcOH (80% in H_2O, 2 mL), was added 10% Pd/C (approximately 10 mg) and the mixture was stirred under 1 atm of hydrogen overnight. The mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford **12** (30 mg, quant.) as a fluffy white solid; ^1H NMR (400 MHz, D_2O) δ 5.45 (s, 1H), 5.06 (s, 1H), 4.03–3.53 (m, 16H), 3.50–3.30 (m, 4H), 3.20 (m, 2H), 2.37 (m, 1H), 2.10 (m, 1H), 2.01 (m, 1H), 1.84 (s, 15H), 1.78 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 182.1, 101.8, 94.1, 82.8, 82.5, 78.7, 73.8, 72.4, 72.3, 69.3, 65.8, 65.1, 61.4, 60.1, 55.5, 50.9, 49.3, 48.5, 40.2, 31.1, 30.0, 23.9; $[\alpha]_D=+42$ (*c* 1.1, H_2O); MALDI-FTMS calcd for $C_{20}H_{41}N_5O_{10}Na$ (M+Na^+) 534.2745; found 534.2745.

2.4.8. Compound 13. A solution of **11** (50.0 mg, 0.028 mmol) in MeOH (2 mL) was treated with a catalytic amount of NaOMe. The reaction mixture was stirred at room temperature until completion (approximately 2 h), neutralized by addition of Amberlite IR-120(H^+), the resin was filtered off and the filtrate was evaporated to dryness. To the resulting residue in AcOH (80% in H_2O, 2 mL), was added 10% Pd/C (approximately 10 mg) and the mixture was stirred under 1 atm of hydrogen overnight. The mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford **13** (25 mg, quant.) as a fluffy white solid; ^1H NMR (400 MHz, D_2O) δ 5.30 (s, 1H), 5.04 (s, 1H), 4.00–3.27 (m, 23H), 3.22–3.00 (m, 3H), 2.20 (m, 1H), 2.10 (m, 1H), 1.95 (m, 1H), 1.76 (s, 15H), 1.60 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.6, 164.1, 156.5, 101.2, 92.8, 83.2, 82.5, 77.2, 73.6, 72.9, 69.1, 65.6, 64.8, 64.4, 61.6, 60.1, 55.2, 54.1, 53.3, 50.2, 48.9, 48.1, 41.3, 30.7, 29.8, 23.5; $[\alpha]_D=+52$ (*c* 1.0, H_2O).

2.4.9. Compound 15. To a solution of **14a**[21] (300 mg, 0.989 mmol) in CH_2Cl_2 (6 mL) was added successively Et_3N (0.15 mL, 1.1 mmol), CbzCl (0.16 mL, 1.1 mmol) at 0°C and the solution was stirred overnight at room temperature. The solvent was removed under vacuum, the residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, the combined organic extracts were washed with water, brine and dried over Na_2SO_4. The solvent was removed under vacuum and the crude oil was purified by flash chromatography (EtOAc/hexanes, 30:70) to give **15** (307 mg, 71%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5H), 5.11 (s, 2H), 3.40–3.08 (m, 8H), 1.41 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 156.6, 156.4, 136.8, 129.0, 128.5, 128.3, 79.8, 67.9, 48.2, 39.8, 28.8; HRMS calcd for $C_{22}H_{36}N_3O_6$ (M+H^+) 438.26041; found 438.26025.

2.4.10. Compound 16. To a solution of **15** (244 mg, 0.558 mmol) in CH_2Cl_2 (12 mL) was added TFA (0.86 mL, 11 mmol) and stirred at room temperature for 2 h. The solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (7 mL), then Et_3N (0.39 mL, 2.8 mmol) followed by *N*,*N*′-di-Boc-*N*″-trifluoromethanesulfonylguanidine[26] (424 mg, 1.12 mmol) were added. The reaction mixture was stirred at room temperature for 1 h, and concentrated under vacuum. The resulting residue was purified by flash chromatography (EtOAc/hexanes, 25:75) to give **16** (384 mg, 95%) as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 8.50 (broad s, 2H), 7.40–7.23 (m, 5H), 5.16 (s, 2H), 3.63–3.42 (m, 8H), 1.49 (s, 36H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 156.8, 153.4, 136.6, 128.9, 128.5, 128.4, 83.8, 80.5, 80.0, 68.1, 47.1, 46.7, 28.4; FAB *m*/*z* calcd (M+H^+) 722.4; found 722.4.

2.4.11. Compound 17. To a solution of **16** (383 mg, 0.530 mmol) in MeOH (8 mL) was added 10% Pd/C (approximately 10 mg) and the mixture was stirred under 1 atm of hydrogen for 5 h, then few drops of AcOH were added. The mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford **17** (340 mg, quant.) as a fluffy white solid; ^1H NMR (400 MHz, CD_2OD) δ 3.70 (m, 4H), 3.30 (m, 4H), 1.99 (s, 3H), 1.54 (s, 18H), 1.49 (s, 18H); ^{13}C NMR (100 MHz, CD_2OD) δ 162.5, 157.7, 152.6, 83.9, 80.1, 38.1, 27.6, 27.2, 20.0; HRMS calcd for $C_{26}H_{50}N_7O_8$ (M+H^+) 588.37208; found 588.37269.

2.4.12. Compound 19. To a solution of **18** (203 mg, 0.921 mmol) in $CHCl_3$ (10 mL) was added successively Et_3N (0.26 mL, 1.8 mmol) followed by *N*,*N*′-di-Boc-*N*″-trifluoromethanesulfonylguanidine[15] (360 mg, 1.01 mmol). The reaction mixture was stirred at room temperature for 1.5 h, diluted with CH_2Cl_2, washed with HCl 1N, $NaHCO_3$ (sat.), brine, and dried over Na_2SO_4. The solvent was removed under vacuum and the crude oil was purified by flash chromatography (EtOAc/hexanes, 20:80). To a solution of the resulting oil in MeOH (6 mL) was added 10% Pd/C (approximately 10 mg), the mixture was stirred under 1 atm of hydrogen for 24 h, filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford **19** (180 mg, 60%) as a fluffy white solid; ^1H NMR (400 MHz, CD_2OD) δ 3.77 (m, 4H), 3.31 (m, 4H), 1.49 (s, 18H); ^{13}C NMR (100 MHz, CD_2OD) δ 152.8, 81.2, 43.4, 43.1, 40.2, 27.5; HRMS calcd for $C_{15}H_{28}N_4O_4$ (M+H^+) 328.21105; found 328.21155.

Acknowledgements

We thank NSERC for financial assistance through the Medicinal Chemistry Chair Program. M. T. acknowledges scholarships from NSERC and FCAR. We thank Dr William Baker (Pathogenesis) for his support at the inception of this project. We acknowledge the services of Lynn Barker (Pathogenesis, Seattle, WA, now Chiron Corporation, Emeryville, CA) for MIC determinations and Dr Kristin A. Sannes-Lowery for K_D determinations, and Ms L. M. Risen for T/T and MIC evaluations.

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18. We thank Ms Lynn Barker (Pathogenesis, Seattle, WA, now Chiron Corporation Emeryville, CA) for these tests.
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