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Probing the functional requirements of the L-haba side-chain of amikacin—synthesis, 16S A-site rRNA binding, and antibacterial activity

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Abstract—The l-amino group in amikacin was acylated with a variety of 2-hydroxy aminocarboxylic acids to probe the effect of acylation on ribosomal binding and antibacterial activity. The *N*-hydroxy urea analogue of amikacin (**8a**) in which the 2-*S*-hydroxyl-bearing carbon was replaced by an *N*–OH group was equally active against *S. aureus* and *E. coli* in vitro. The analogous tobramycin variant **9** was more active than amikacin.

The aminoglycosides have been known to be potent antibacterial drugs for nearly half a century since the discovery of streptomycin.[1] Their widespread use in clinical practice has been significantly compromised by their oto- and nephrotoxicity[2] unless drug levels are carefully monitored. The emergence of aminoglycoside-modifying enzymes[3] in resistant strains of certain pathogenic bacteria has further curtailed their use as primary treatments for life threatening infections. This has instigated intensive efforts in the chemical modification of naturally occurring aminoglycosides such as kanamycin,[4] so as to alter its recognition sites by *O*-phosphorylating, adenylylating, and *N*-acetylating enzymes.[3,5] Indeed, dibekacin, a 3″,4″-dideoxykanamycin which cannot be *O*-phosphorylated, and amikacin, an 1-*N*-(2*S*-hydroxy-4-aminobutyryl) (L-haba side-chain) analog of kanamycin, are better antibiotics against resistant bacteria.[4] Although related aminoglycosides such as gentamicin C_{1a} and tobramycin are used in clinical practice, the prospects of resistance are omnipresent which limits their use to specific pathogeneses.[2,4]

Aminoglycosides exert their antibacterial action by inhibiting protein biosynthesis at the prokaryotic ribosomal RNA level.[6] Codon misreading in the decoding region of the 16S ribosomal RNA(rRNA)[7] affects translocation which results in aberrant translation. Recent interest in RNA function[8] and the quest for recognition by small-molecules has rekindled interest in aminoglycosides and their analogs as probes.[9] In fact they can specifically recognize components of rRNA as evidenced from biochemical,[10] spectroscopic,[11] biophysical, and mass spectroscopic[12] methods. The three-dimensional solution structures of paromomycin, neomycin and related aminoglycosides bound to the prokarotic 16S rRNA has been determined by elegant NMR studies.[13] A crystal structure of paromomycin complexed with the 30 S subunit of rRNA from *Thermus thermophilus* has been described.[14] Another crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site was recently reported.[15] Modelling studies of aminoglycoside–RNA interactions have been reported with interesting predictive insights.[16] The binding of tobramycin to an RNA aptamer with high affinity has been reported[17] and studied by solution NMR.[18]

Aminoglycosides have been generally classified based on the glycosylation sites of the symmetrical 2-deoxystreptamine unit. A 4,6-bis-glycosidic type is exemplified by kanamycin, amikacin, tobramycin and the gentamicin family (Fig. 1). A 5,6-bis-glycosidic type can be found in paromomycin, neomycin, ribostamycin, and butirosin (Fig. 1). The scholarly spectroscopic and biochemical studies already reported[10–18] with some of these aminoglycosides, and the availability of smaller subunits derived by degradation[19] have provided a molecular blueprint for the study of their interactions with rRNA. Thus, the 5,6-substituted pseudotetrasaccharides such as paromomycin adopt an L-shaped structure in their bound conformation with RNA, while the 4,6-pseudotrisaccharide types such as gentamicin C_{1a} and kanamycin are 'linear',[16a] The high resolution structure of paromomycin with the 27-nucleotide RNA containing the A-site of *E. coli* 16S rRNA has provided great insights in the nature of specific contacts.[13] These consist of intermolecular H-bonds of hydroxyl and

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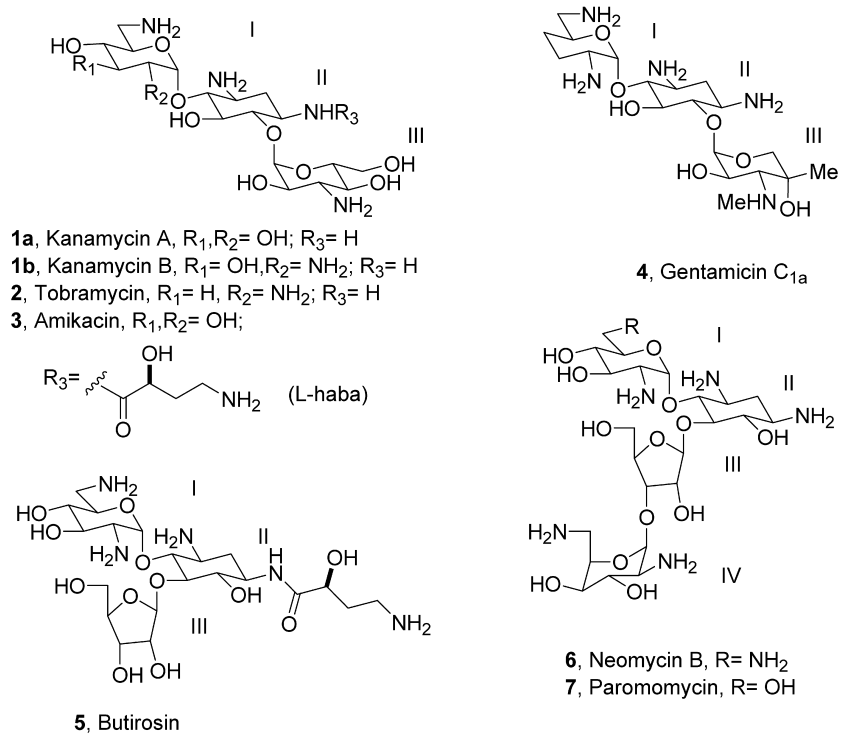


Figure 1. Structures or representative aminoglycosides. Roman numerals refer to individual rings.

amino groups to base edges and intramolecular H-bonds to sugar ring oxygens. The aminoglycoside is anchored within the major groove of the rRNA by contact of amino groups with backbone phosphates. Paromomycin is one of the best binders among the aminoglycosides, and results in the displacement of the conserved residues A^{1492} and A^{1493} towards the minor groove. Rings I and II in both classes appear to make the same contacts and are important for recognition, binding, and stabilization (Fig. 1).[13,16a] Rings III and IV in the paromomycin-type contribute to the binding affinity, in part by direct electrostatic contact of charged amino groups with phosphates in the lower stem, and by correctly orienting rings I and II for effective binding.[13] Ring III in gentamicin C_{1a} appears to make sequence specific contacts in the upper stem of the RNA.[20]

Butirosin was the original aminoglycoside found to contain the L-haba side-chain at $N-1$ of its 2-deoxystreptamine subunit.[21] Extensive chemical modifications of the haba unit showed that there were critical spacial and stereochemical requirements for antibacterial activity. Incorporation of this unique amino acid unit in kanamycin to produce amikacin[22] was a logical extension with gratifying results.

It has been reported that the kanamycin class of aminoglycosides has lower binding specificity toward 16S rRNA,[12d,16a] compared to the paromomycin–neomycin class. This has been attributed to more than one binding orientation within the rRNA,[16a] one of which could change the local conformation within the major groove, displacing A^{1492} and A^{1493} toward the minor groove.[13] In fact, Puglisi[20] has shown by high resolution NMR structure elucidation of a gentamicin C_{1a}–RNA complex that A^{1492} and A^{1493} are displaced. Improved specificity of gentamicin C_{1a}, a member of the kanamycin-class of antibiotics may explain its prevalence as a historically better drug compared to paromomycin or neomycin.[20] Subtle functional differences within one and the same class of aminoglycosides may also account for the observed variance in binding specificities.

Although complexes of amikacin with RNAs have not been studied to the best of our knowledge, it is most likely that the L-haba side-chain makes a productive contact within the major groove, and further contributes to stabilize the intrinsic binding of the kanamycin portion. The importance of 1,2- and 1,3-aminoalcohol units as binding sites for phosphate esters of RNAs has been pointed out for simple molecules[23] as well as for aminoglycosides.[24] Specific contacts made by rings I, II, and III of gentamicin C_{1a} for example, within the major groove of a rRNA have been identified.[20] Inter- and intramolecular H-bonds, as well as ammonium-phosphate contacts delineate a bioactive conformation that is consistent with previous data on paromomycin.[13] Best fit superpositions of the gentamicin C_{1a}–RNA complex and the corresponding paromomycin complex reveals a convergence of rings I and II in both cases, but also distinctly different spaces occupied by the remaining saccharide units.[15b,20] We therefore adopted a working hypothesis in which amikacin may, in one of its binding conformations, acquire an orientation that resembles that of gentamicin C_{1a}.

The purpose of this study was to probe the nature of functional and stereochemical requirements of the L-haba side-chain in amikacin in hitherto unexplored ways. Thus, our primary target compounds were the *N*-hydroxyurea isosteric analogs[25] **8a–e** and **9** of amikacin and tobramycin, respectively (Fig. 2). We also wished to introduce terminal aminomethyl and hydroxymethyl appendages (**10–15**) on

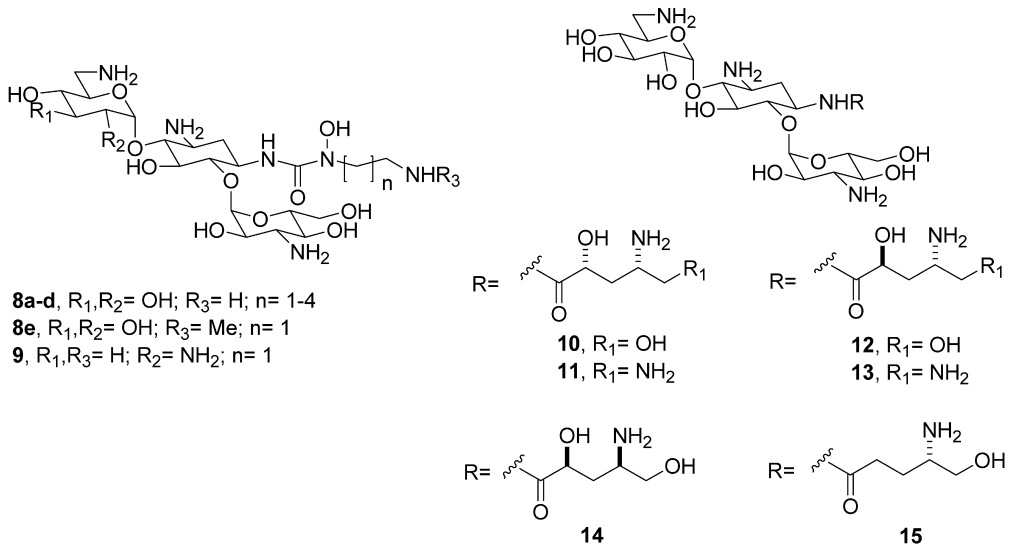


Figure 2. Structures of amikacin side-chain analogues.

the original haba unit to study the effect of a potential new binding site, as well as consequences of stereochemical variations. In the case of **8a**–**e** and **9**, the stereogenic carbon which is known to be important for in vitro activity[21] would be replaced by an isosteric *N*-hydroxy group. We reasoned that this replacement would introduce a new binding site while offering possibilities for inter- or intramolecular H-bonding as suggested for amikacin (Fig. 3(A) and (B)). Such preorganized motifs involving flexible 1,2- and 1,3-aminodiol or diamino alcohol groups by intra- and intermolecular H-bonding are well known in the solid state[26] and from NMR studies[27] (Fig. 3(C) and (D)). Bidentate binding[24] may also occur depending on the extent of electrostatic contact with phosphate diesters of specific nucleotide sequences (Fig. 3(D) and (E)). Unfortunately, it is difficult to ascertain the contribution, if any, of such H-bonded and charged motifs in aqueous solution. It has been shown that dimethylphosphate and neamine form complexes in DMSO, but that such interactions are not detectable in aqueous solution by NMR.[24]

As previously mentioned, the goal of our study was to probe the electronic, functional, and stereochemical requirements of the L-haba side-chain in amikacin, and to correlate them to antibacterial activity as well as to ribosomal binding. Very recently, Mobashery and co-workers[28] have reported L-haba derivatives of 6-aminoalkyl neamines with impressive antibacterial activity and ribosomal binding.

1. Chemistry

Scheme 1 shows the synthetic route to *N*-hydroxyurea analogs of amikacin with different chain lengths. Treatment of kanamycin with benzyloxycarbonyloxy succinimide (Cbz-NOS) in the presence of zinc acetate, followed by addition of ethyl trifluoroacetate in DMSO gave the selectively protected kanamycin derivative **16** (Scheme 1).[29] Preferential protection of aminoglycosides by in situ complexation with metal salts has been known from many years.[30]

The active ester reagents, **17a**–**e** were easily available from the corresponding mono-*N*-Cbz diamines by treatment with benzoyl peroxide followed by disuccinimidyl carbonate. Condensation with **16** in aqueous THF afforded the *N*−1

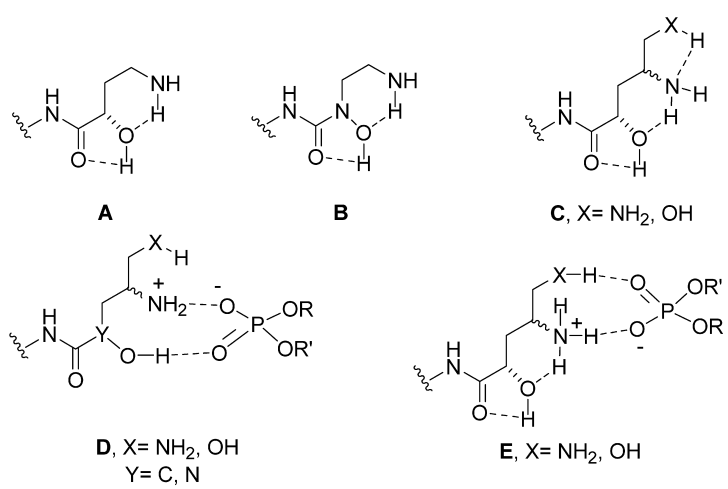
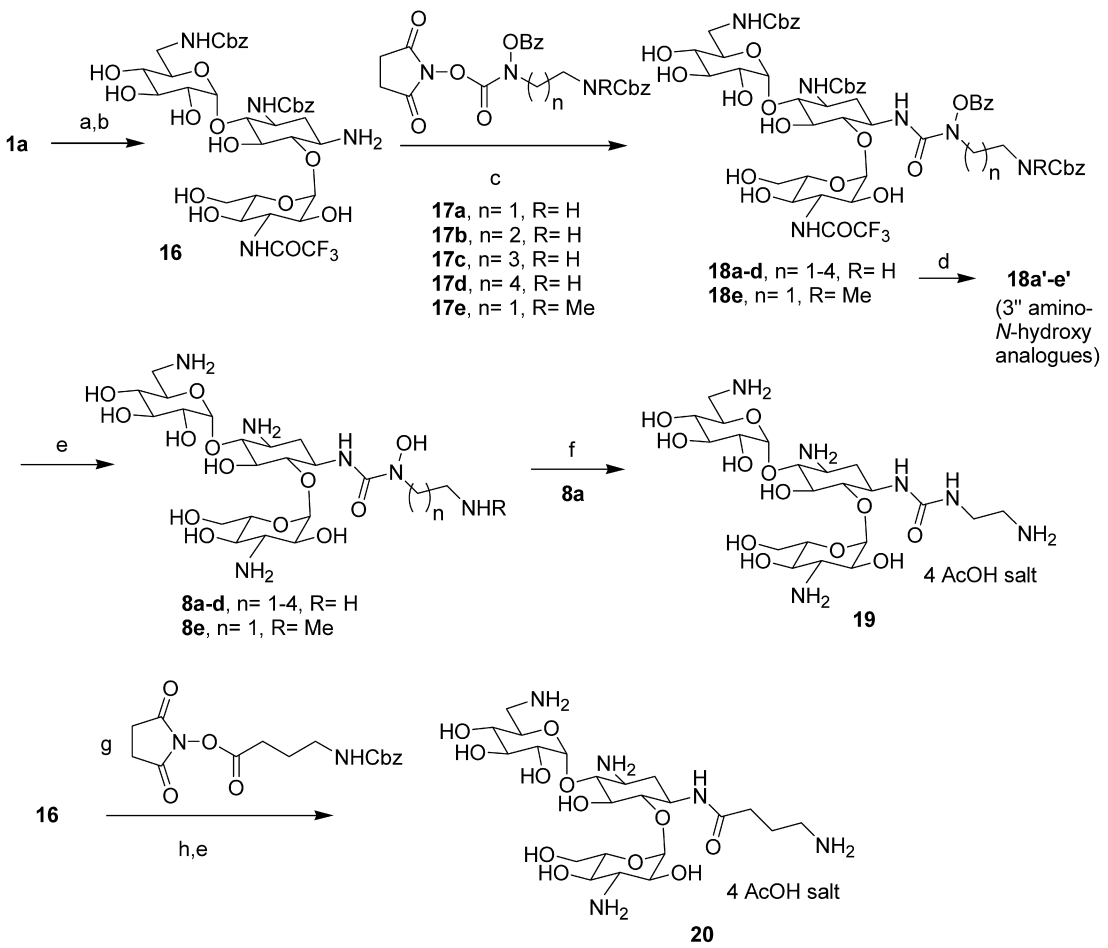
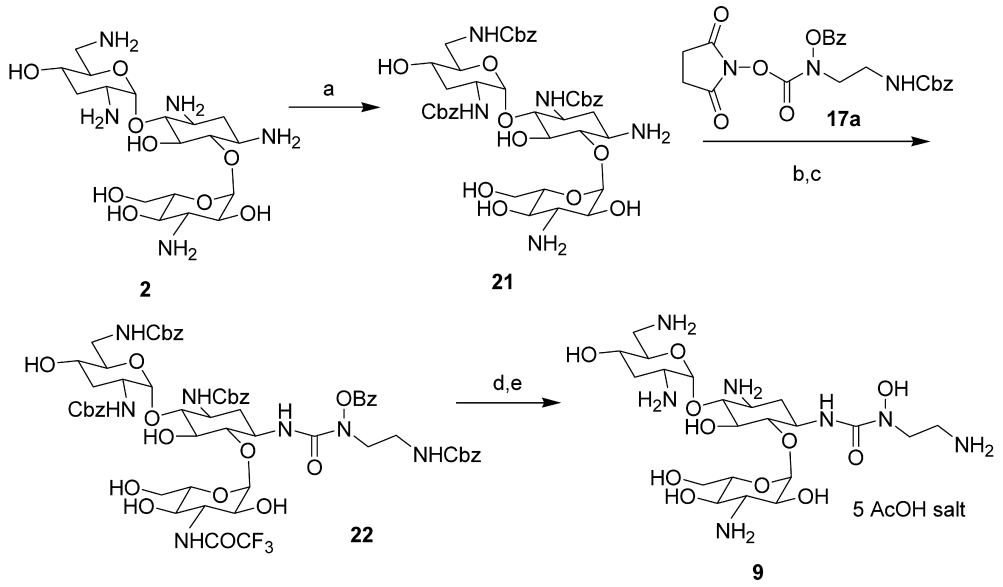


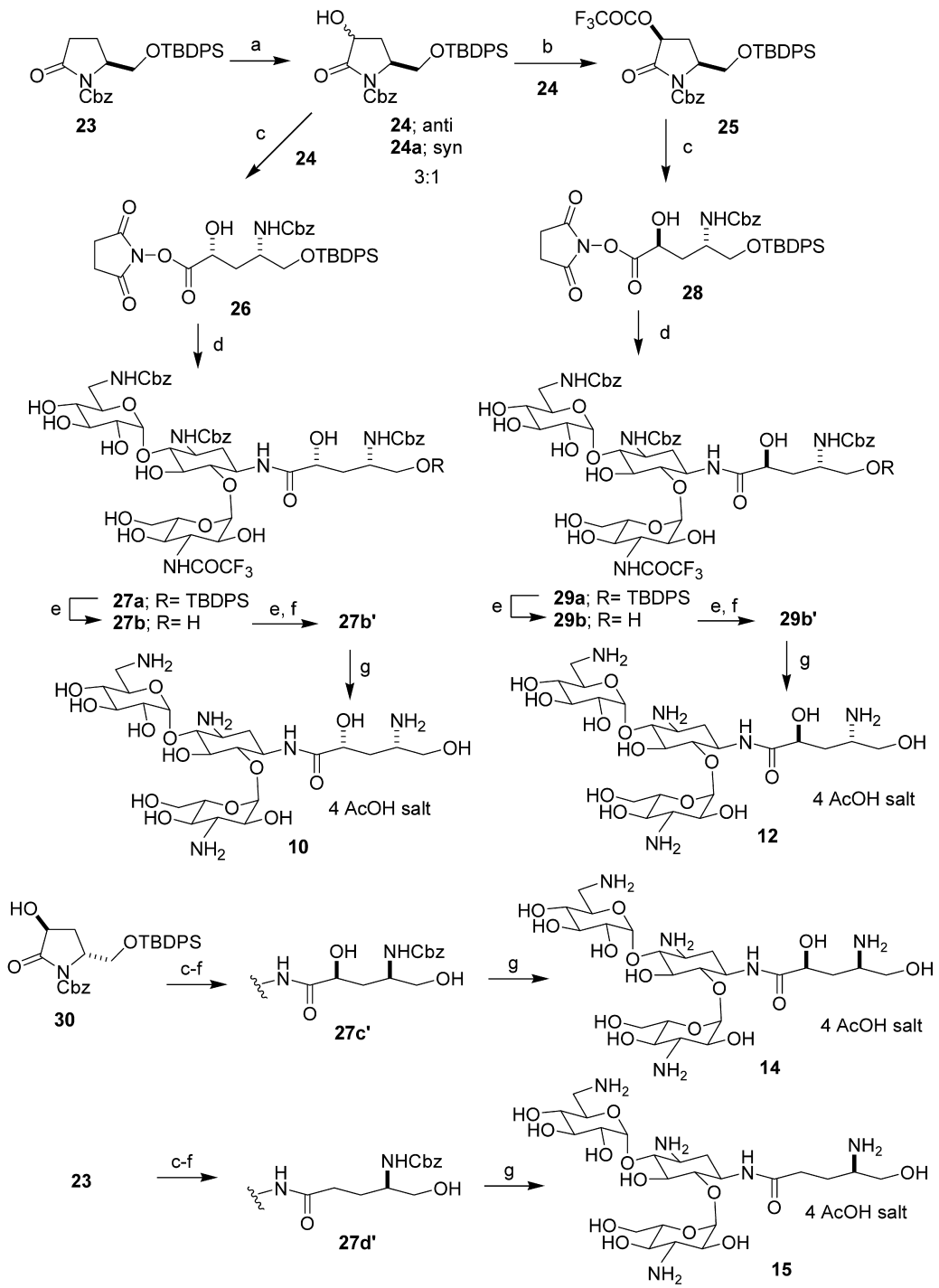
Figure 3. Intramolecular H-bond and backbone phosphodiester contacts with neutral and charged L-haba analogues.



Scheme 1. *Reagents and conditions*: (a) *N*-benzyloxycarbonyloxy succinimide, $Zn(OAc)_2$, DMSO; (b) ethyl trifluoroacetate, DMSO, 35–45% for two steps; (c) reagent prepared from mono *N*-Cbz diamine, benzoyl peroxide, pH 10.4, then *N*,*N*′-disuccinimidyl carbonate in MeCN, followed by condensation in THF–H_2O (2:1), rt, 2 days, 37–57%; (d) aq. MeOH, 1% NaOH, then Amberlite IR-120 (H^+); (e) Pd/C, H_2, aq. dioxane, AcOH, 96–98% for two steps; (f) Ra-Ni, H_2, H_2O, 70%; (g) reagent prepared from *N*-Cbz 4-aminobutyric acid as in (c); (h) aq. NH_4OH, THF, then (e), 65% for three steps.



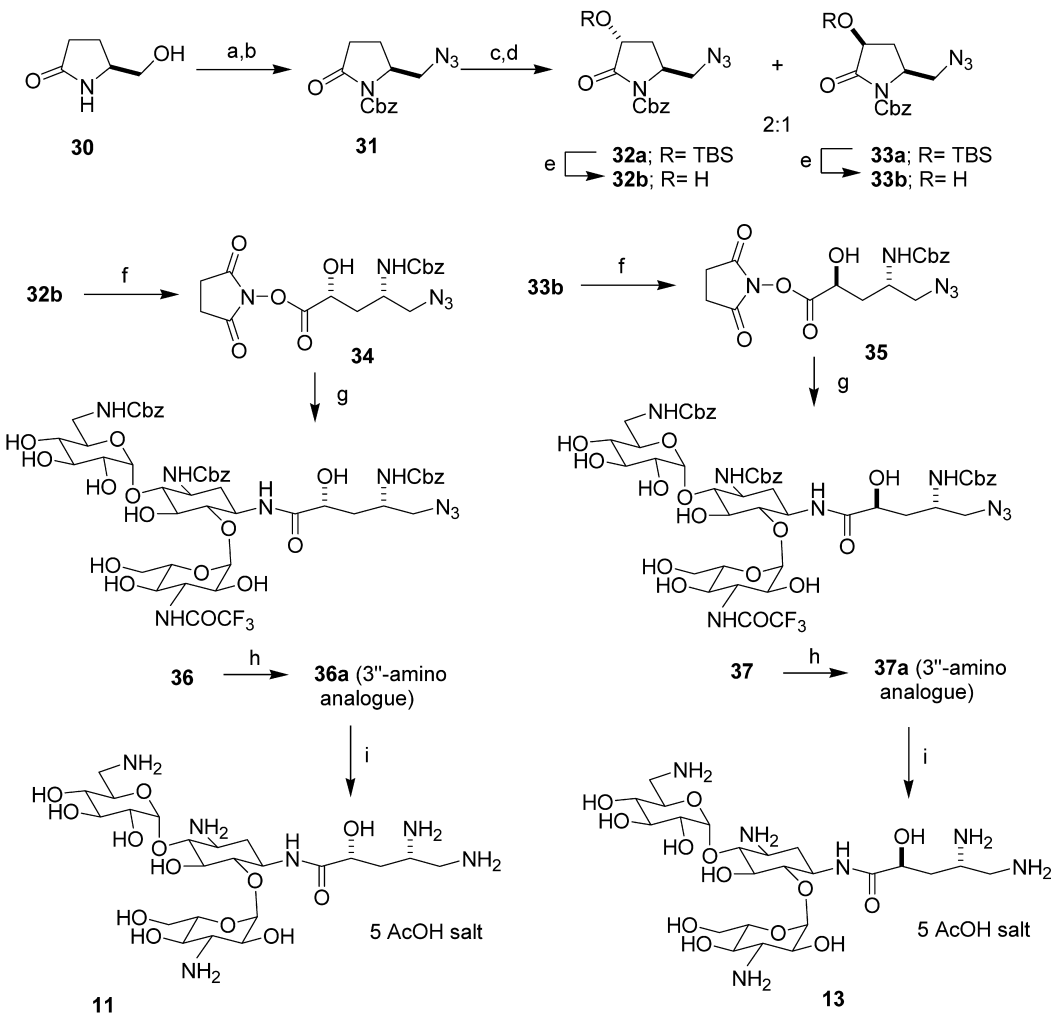
Scheme 2. *Reagents and conditions*: (a) *N*-benzyloxycarbonyloxy succinimide, $Zn(OAc)_2$, DMSO, 18–25%; (b) ethyl trifluoroacetate, DMSO, 60%; (c) aq. THF, rt, 4 days, 35%; (d) aq. MeOH, 1% NaOH, then Amberlite IR-120 (H^+); (e) Pd/C, H_2, aq. dioxane, AcOH, 95%.



Scheme 3. *Reagents and conditions*: (a) LiHMDS, Davis oxaziridine, THF, −78°C, 65% (3:1 *anti*/*syn*); (b) PPh_3, DEAD, CF_3CO_2H, CH_2Cl_2, 90%; (c) aq. LiOH, then 2N HCl, pH 4–5, workup then treat with *N*-hydroxy succinimide, DCC; (d) add **16**, aq. THF, rt, 2 days, 73% (2 steps); (e) TBAF, THF, 3 h; (f) aq. NH_4OH, THF; (g) Pd/C, H_2, aq. dioxane, AcOH, 97–99% (3 steps).

acylated ureas **18a**–**e**. Cleavage of the benzoate and *N*-trifluoroacetyl groups followed by catalytic hydrogenation afforded the *N*-hydroxy-ω-aminoalkyl urea derivatives of kanamycin **8a**–**e** as the peracetate salts. Treatment of **8a** with Raney-nickel under a hydrogen atmosphere gave the urea analogue **19**. *N*-Acylation of **16** with the active NOS ester of 4-*N*-Cbz butyric acid afforded the corresponding amide **20** after hydrogenolysis. Preferential *N*-protection of tobramycin **2** with Cbz-NOS in the presence of zinc acetate afforded the selectively protected tobramycin derivative **21** (Scheme 2). Treatment with ethyl trifluoroacetate in DMSO followed by condensation with **17a** in aqueous THF gave the *N*-benzoyloxy urea derivative **22**, which was treated with aqueous base, then hydrogenated to give **9** as the pentaacetate salt.

Access to the diastereomeric 2-hydroxy-4-amino-5-hydroxypentanoic acids was realized by utilizing 4*R*- and 4*S*-hydroxymethyl *N*-Cbz pyrrolidinones, readily available from the corresponding D- and L-pyroglutamic acids



Scheme 4. *Reagents and conditions*: (a) PPh_3, DEAD, $(PhO)_2PON_3$, CH_2Cl_2; (b) LiHMDS, Cbz-Cl, THF, 70% (2 steps); (c) LiHMDS, Davis oxaziridine, THF, −78°C; (d) TBSCl, imidazole, DMF, **32a** in 32% from **31** (2 steps), **33a** in 20% from **31** (2 steps); (e) TBAF, THF; (f) aq. LiOH, then 2N HCl, then *N*-hydroxy succinimide, DCC; (g) aq. THF, 55–60% (2 steps); (h) aq. NH_4OH, THF; (i) Pd/C, H_2, aq. dioxane, AcOH, 97% (2 steps).

(Scheme 3).[31,32] Treatment of **23** with LiHMDS followed by the Davis oxaziridine reagent[33] led to a 3:1 *anti*/*syn* mixture of 2-hydroxyl lactams **24** and **24a** which could be separated by column chromatography. The major isomer **24** was converted to the trifluoroacetate **25** via a Mitsunobu reaction.[34] Hydrolysis of **24** with aqueous LiOH, followed by treatment with *N*-hydroxysuccinimide and DCC gave the corresponding active ester **26** which was then condensed with **16** to give the corresponding amide **27**. Deprotection and hydrogenolysis afforded the 2*R*,4*S*-5-hydroxypentanoyl analogue of amikacin **10**. Similar treatment of the active ester **28** prepared from **24a** or **25** afforded the 2*S*,4*S*-amide **12** as described for **10**. Starting from D-pyroglutamic acid and proceeding through the same sequence via intermediate **30** shown in Scheme 3 afforded the 2*S*,4*R*-amide **14**. Finally, the 4*R*-amide **15** was also prepared as a control from L-pyroglutamic acid via the 5-hydroxy analogue **23**.

Amikacin analogues containing a terminal vicinal diamino moiety were also prepared (Scheme 4). Thus Mitsunobu azidation[35] of the primary hydroxyl group of L-pyroglutamic acid afforded the azido lactam **31**. Treatment of the corresponding enolate with the Davis oxaziridine reagent,[33] gave a mixture of *anti*- and *syn*-4-hydroxy lactams in a 2:1 ratio, respectively, which were conveniently separated as their TBS ethers **32a** and **33a**. Formation of the NOS-active esters **34** and **35** from **32b** and **33b**, and condensation with **16** afforded the corresponding *N*-acylated analogues **36** and **37**, respectively. Finally, removal of the *N*-trifluoroacetyl group, followed by hydrogenolysis led to the 2*R*,4*S*, and 2*S*,4*S*-diamino-5-hydroxypentanoyl amides **11** and **13**, respectively.

2. Results

The chemically modified analogues described in this paper were subjected to RNA binding studies and to antibacterial testing. Table 1 lists the data expressed as K_D for the 16S A-Site rRNA subdomain,[36] IC_{50} for bacterial transcription/translation (T/T), and MIC values for *E. coli* (ATCC 25922) and *S. aureus* (ATCC 13709).[37] Control samples were paromomycin, bekanomycin, kanamycin, amikacin, and tobramycin. The control aminoglycosides all bound to the A-site model and inhibited bacterial growth at the expected concentrations, which are consistent with values previously reported.[38–40] Interestingly, all control aminoglycosides except the 4,5-substituted paromomycin inhibited bacterial

Table 1. Activities of modified aminoglycosides

Entry	Compound	K_D (μM)	Translation T/T IC_{50} (μM)	*E. coli*[a] MIC (μM)	*S. aureus*[b] nMIC (μM)
1	Kanamycin A, **1a**	4.82	0.57	2.5–5	1.2–2.5
2	Kanamycin B, **1b**	1.48	0.36	1.2–2.5	0.3–0.6
3	Tobramycin, **2**	2.39	0.45	0.6–1.2	0.3–0.6
4	Amikacin, **3**	2.45	0.53	1.2–2.5	1.2–2.5
5	Paromomycin, **7**	0.15	0.56	2.5–5	1.2–2.5
6	**8a**	8.24	0.67	2.5–5	2.5–5
7	**8b**	22.20	1.54	2.5–5	>10
8	**8c**	39.82	>10	>10	2.5–5
9	**8d**	19.29	>10	>10	>10
10	**8e**	25.29	4.43	>10	2.5–5
11	**9**	1.86	0.31	0.6–1.2	0.6–1.2
12	**10**	19.49	2.66	>10	>10
13	**11**	6.64	0.67	5–10	1.2–2.5
14	**12**	4.11	0.93	2.5–5	1.2–2.5
15	**13**	4.55	0.67	2.5–5	>10
16	**14**	20.36	2.02	5–10	>10
17	**15**	33.53	>10	>10	>10

[a] ATCC 25922.
[b] ATCC 13709.

transcription/translation at concentrations lower than their binding K_Ds, a result consistent with previous studies of aminoglycoside analogs.[41] While it is possible that this is indicative of a second binding site for the 4,6-substituted aminoglycoside class which is responsible for much of their antibacterial effect, there is biochemical and structural evidence[6c,20] which suggests that both the paromomycin and kanamycin classes of aminoglycosides function by binding to the A-site. More likely, these discrepancies are a result of subtle structural differences between the model 27mer RNA subdomain used in the K_D measurements and the native rRNA target. They may also reflect the ability of the aminoglycosides to cause miscoding, as a single binding event of an aminoglycoside to the A-site region during protein synthesis could cause production of the incorrect reporter.

The *N*-hydroxyurea isostere of amikacin (**8a**) shows a 3–4 decrease in affinity for the RNA, but only a small decrease in T/T and MIC activities. This discrepancy in activity between binding and function is 10-fold for **8a**, whereas the difference is 5-fold for amikacin (Table 1, entries 4 and 6). Lengthening the side chain by a single methylene results in roughly a 3-fold drop in binding and T/T activity, while adding either 2 or 3 methylene groups provides compounds with similarly reduced binding (K_Ds 20–40 μM) and an even greater loss of functional activity (Table 1, entries 7–10). These data suggest that substitution of an *N*-hydroxyurea for the L-haba chain results in a slight to drastic reduction of antibacterial activity, depending on the chain length, likely as a result of reduction in affinity for the target RNA. In contrast, the *N*-hydroxyurea analogue **9** has activity nearly identical to amikacin and tobramycin. The 5-fold better binding observed for **9** relative to **8a** suggests that removal of the hydroxyl group on ring I compensates for the reduction in binding imparted by the substitution of an *N*-hydroxyurea for the L-haba chain of amikacin **8a** (Table 1, entries 1, 3 and 9). This improvement in affinity is not observed in the natural aminoglycoside series upon removal of the ring I 3-hydroxy group (compare **1a** and **2**), nor is a loss in affinity observed upon introduction of the L-haba chain (compare **1a** and **3**, Table 1, entries 1 and 3). However, substitution of an L-haba chain onto $N-1$ of tobramycin has been previously found to increase antibacterial activity by approximately 5-fold,[42] which is largely consistent with our data. These differences may be a result of conformational changes induces by the $N-1$ substitution in concert with the ring I changes from kanamycin A to tobramycin. Taken in total, these observations suggest that the kanamycin A class is not optimally suited to accept side chains at $N-1$, and that the tobramycin skeleton may be the optimal parent for future SAR studies at this position.

Substitution off the 4-position of the L-haba chain in amikacin resulted in lower affinity, T/T potency, and antibacterial activity in all cases. Introduction of an aminomethyl or hydroxymethyl group to provide the 2*S*,4*S* configuration (**12** and **13**) results in a 2–3 fold decrease in affinity and T/T activity relative to amikacin (Table 1, entries 14 and 15). The decrease in activities is greater for the (2*S*,4*R*) hydroxymethyl derivative **14**, which shows a 10-fold reduction, while inversion of the configuration of 2-hydroxyl on the L-haba chain as in **10**, was, as expected, also detrimental (Table 1, entries 12 and 16). This effect is somewhat overcome by substitution of an aminomethyl for the hydroxymethyl (**11** vs. **10**) (Table 1, entries 12 and 13). Removal of the 2-hydroxyl group in **12** to give **15** dramatically decreased binding, and resulted in a loss of both T/T and antibacterial activity at the doses tested as observed in the original series (Table 1, entry 17).[21] Unfortunately, the binding affinity, T/T IC_{50}, and antibacterial activities were not well correlated in this series of 4-substituted L-haba analogues of amikacin.

3. Conclusions

We have described the preparation of *N*-hydroxyurea isosteres of the L-haba side-chain in the aminoglycoside antibiotic amikacin, as well as chain-extended analogues. These seemingly subtle modifications have dramatic effects upon their binding to RNA and on the antibacterial activity. While extended L-haba chains of amikacin result in

reduction or loss in activity, the *N*-hydroxyurea analogue maintains the antibacterial activity against *S. aureus* and *E. coli* compared to amikacin. The corresponding *N*-hydroxyurea analogue of L-haba tobramycin exhibits equal activity to tobramycin with a slight superiority over amikacin. The *N*-hydroxyurea analogs **8a** and **9** were active against *P. aeruginosa* ATCC 27853 at IC_{50} 6.25 μg/mL. However, they were inactive (IC_{50}>50 μg/mL) against resistant strains of the same organism (CF clincal isolate) as well as against *S. aureus* ATCC 33591 (MRSA).[43] In this study we attempted to correlate the ribosomal binding affinities, and T/T potency with the antibacterial activities of several known aminoglycosides. Although a consistently linear relationship could not be found, a K_D range between 0.15 μM (paromomycin) and 4.82 μM (kanamycin) resulted in good activities.

4. Experimental

4.1. 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 (^1H,^{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 bombardment (FAB), electrospray and MALDI-FTMS techniques. 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 constants for the 16S A-site model RNA, IC_{50}s for inhibition of bacterial transcription/translation, and MIC values were determined as described previously.[37]

4.1.1. 3,6′-Bis-*N*-benzyloxycarbonyl-3″-*N*-trifluoroacetyl kanamycin, 16. To a stirred suspension of kanamycin (200 mg, 0.43 mmol) in DMSO (3 mL) was added $Zn(OAc)_2{\cdot}2H_2O$ (0.37 g, 1.7 mmol, washed with THF prior to use). After stirring for 12 h at rt, *N*-benzyloxycarbonyloxy succinimide (0.36 g, 1.46 mmol) was added in one portion. The reaction mixture was stirred for 2 h, then diluted with dioxane (3 mL). Amberlite–120(H^+) (2 g) was added, the mixture was stirred for 1 h, then poured onto a porous glass filter containing 1 g of the same resin and washed with a mixture of dioxane–H_2O (1:1) until *N*-hydroxysuccinimide was no longer detectable by TLC. The product was eluted from the resin with 1 M solution of NH_3 in dioxane–H_2O (1:1, and monitored by TLC ($CHCl_3$–MeOH–5N NH_4OH). After all the fractions containing pure 3,6′-bis-*N*-Cbz derivative (R_f 0.2) were collected and pooled the solution was saturated with carbon dioxide (by bubbling gas through the solution for 30 min), and lyophilized to yield pure product as white fluffy solid. ^1H NMR (DMSO) δ 8.42 (br s, 1H), 7.42–6.38 (m, 15H), 5.15–4.82 (m, 8H), 3.62–3.29 (m, 10H), 3.22 (m, 2H), 3.05 (br t, 1H), 2.92 (br t, 1H), 2.73 (br t, 1H), 1.88 (m, 2H), 1.60 (br dd, 1H), 1.38 (br dd, 1H); FAB MS for $C_{42}H_{55}N_5O_{15}$ calcd ($M+H^+$) 870.4 found 870.4. To a stirred solution of the above product (100 mg, 0.22 mmol) in DMSO (1 mL) was added CF_3-COOEt (13.5 μL, 0.22 mmol). The reaction mixture was stirred for 2 h at rt, after which time DMSO was removed by lyophilization to give **16** as a white solid which was used in the next step.

4.2. Preparation of *N*-benzyloxycarbonyloxy succinimides 17a–e

The required mono-Cbz-protected diamine (2.6 mmol) was stirred with benzoyl peroxide (1.2 g, 5.2 mmol) in a mixture of CH_2Cl_2–H_2O (60 mL) buffered at pH 10.4 with $NaHCO_3$–NaOH for 1 h. The mixture was extracted with CH_2Cl_2 (2×30 mL), the organic extracts combined, dried (Na_2SO_4) and evaporated. The residue was dissolved in CH_3CN (40 mL) and added dropwise over a period of 6 h to a solution of *N*,*N*′-disuccinimidyl carbonate (1.28 g, 5 mmol) in CH_3CN (40 mL). The mixture was stirred for 2 days and evaporated, $CHCl_3$ was added and the excess reagent was filtered off. Purification by column chromatography (CH_2Cl_2–ethyl acetate, 4:1) afforded carbamates **17a–e** contaminated with ureas as minor side-products. Due to their instability compounds **17a–e** were characterized by ^1H NMR and immediately used in the next step.

4.3. Preparation of compounds 18a–e

Compound **16** (34 mg, 0.04 mmol) was dissolved in THF–H_2O (3 mL, 2:1) and a quantity of **17a** (15 mg, 0.08 mmol) was added. The reaction mixture was stirred for 2 days after which time the solvent was evaporated and the residue chromatographed (CH_2Cl_2–MeOH, 9:1 then 4:1) to give pure **18a** (11 mg, 35% over two steps). This compound was not characterized by NMR due to severe peak broadening caused by the presence of multiple rotational isomers. TLC R_f 0.62 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{54}H_{63}F_3N_6O_{21}$ ($M+H^+$) 1189.4; found 1189.2.

4.3.1. Compound 18b. *n*=2: (52%), TLC R_f 0.57 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{55}H_{65}F_3N_6O_{21}$ ($M+H^+$) 1203.4; found 1203.4.

4.3.2. Compound 18c. *n*=3: (55%), TLC R_f 0.68 (CH_2Cl_2–MeOH, 4:1; FAB MS calcd for $C_{56}H_{67}F_3N_6O_{21}$ ($M+H^+$) 1217.4; found 1217.4.

4.3.3. Compound 18d. *n*=4: (48%), TLC R_f 0.70 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{57}H_{69}F_3N_6O_{21}$ ($M+H^+$) 1231.4; found 1231.4.

4.3.4. Compound 18e. *n*=1: R=Me: (36%), TLC R_f 0.65 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{55}H_{65}F_3N_6O_{21}$ ($M+H^+$) 1203.4; found 1203.4.

4.4. Preparation of compounds 8a–e

The *N*-trifluoroacetyl and OBz groups in the protected aminoglycosides (**18a–e**) (20 mg) were removed by treatment with 1% methanolic NaOH (2 mL). The solutions were stirred for 20 h, after which time Amberlite IR 120

(H^+) was added in small portions until the solutions became neutral. The resin was filtered and the filtrate was evaporated to dryness to yield the corresponding 3″-amino-*N*-hydroxy analogues: **18a′** (FAB MS calcd for $C_{45}H_{60}N_6O_{19}$ ($M+Na^+$) 1011.4; found 1011.4), **18b′** (FAB MS calcd for $C_{46}H_{62}N_6O_{19}$ ($M+Na^+$) 1025.4; found 1025.4), **18c′** (FAB MS calcd for $C_{47}H_{64}N_6O_{19}$ ($M+Na^+$) 1039.4; found 1039.6), **18d′** (FAB MS calcd for $C_{48}H_{66}N_6O_{19}$ ($M+Na^+$) 1051.4; found 1051.3), **18e′** (FAB MS calcd for $C_{46}H_{62}N_6O_{19}$ ($M+Na^+$) 1025.4; found 1025.4) as white solids. These were hydrogenolyzed over H_2 (balloon) in the presence of 10% Pd/C (7 mg) in dioxane–H_2O–acetic acid (20:20:1) for 40 min. Filtration followed by lyophilization of the solvent gave pure *N*-hydroxyureas **8a**–**e** as white solids.

4.4.1. Compound 8a (95%). ^1H NMR (D_2O) δ 5.50 (d, J=4.2 Hz, 1H), 5.18 (d, J=4.4 Hz, 1H), 4.09–3.49 (m, 20H), 3.42–3.29 (m, 4H), 2.21 (m, 1H), 1.96 (s, 12H), 1.74 (dd, J=14.2, 1.2 Hz, 1H); ^{13}C NMR (D_2O) δ 180.5, 165.0, 98.5, 96.3, 81.5, 80.0, 73.1, 73.0, 72.6, 71.5, 69.4, 68.8, 66.2, 60.3, 56.0, 50.3, 48.7, 40.9, 38.2, 31.6, 25.9, 23.0; MALDI-FTMS calcd for $C_{21}H_{42}N_6O_{13}Na$ ($M+Na^+$) 609.2702; found 609.2714.

4.4.2. Compound 8b (98%). ^1H NMR (D_2O) δ 5.49 (d, J=4.4 Hz, 1H), 5.14 (d, J=4.3 Hz, 1H), 4.08–3.24 (m, 24H), 3.14 (dd, J=10.2, 6.8 Hz, 1H), 2.9 (t, J=5.2 Hz, 2H), 2.17 (m, 1H), 1.94 (s, 12H), 1.92 (m, 2H), 1.72 (dd, J=14.1, 7.4 Hz, 1H); ^{13}C NMR (D_2O) δ 181.2, 177.8, 162.0, 98.6, 96.3, 81.4, 79.9, 73.1, 72.9, 72.5, 71.5, 69.4, 68.8, 66.2, 63.2, 60.2, 55.9, 50.3, 48.5, 47.9, 40.9, 37.8, 25.8, 25.3, 23.4; ES-MS calcd for $C_{22}H_{45}N_6O_{13}$ ($M+H^+$) 601.3; found 601.3.

4.4.3. Compound 8c (96%). ^1H NMR (D_2O) δ 5.49 (d, J=4.3 Hz, 1H), 5.16 (d, J=4.4 Hz, 1H), 4.09–3.19 (m, 24H), 3.13 (dd, J=10.4, 6.6 Hz, 1H), 3.0 (m, 2H), 2.18 (m, 1H), 1.92 (s, 12H), 1.71 (dd, J=14.0, 7.4 Hz, 1H), 1.62 (m, 4H); ^{13}C NMR (D_2O) δ 180.3, 161.9, 98.5, 96.2, 81.0, 79.9, 73.0, 72.9, 72.6, 71.5, 69.4, 68.8, 66.1, 63.2, 60.3, 55.9, 50.3, 49.9, 48.5, 40.9, 39.8, 24.7, 24.7, 24.0, 22.9; ES-MS calcd for $C_{23}H_{47}N_6O_{13}$ ($M+H^+$) 615.3; found 615.3.

4.4.4. Compound 8d (97%). ^1H NMR (D_2O) δ 5.48 (d, J=4.3 Hz, 1H), 5.14 (d, J=4.2 Hz, 1H), 4.06–3.25 (m, 24H), 3.11 (dd, J=10.5, 6.6 Hz, 1H), 2.93 (t, J=5.1 Hz, 2H), 2.16 (m, 1H), 1.95 (s, 12H), 1.58 (m, 4H), 1.35 (m, 2H); ^{13}C NMR (D_2O) δ 179.8, 161.9, 98.5, 96.2, 81.0, 79.9, 73.0, 72.5, 71.5, 69.4, 68.7, 66.1, 63.2, 60.3, 55.9, 50.3, 50.2, 48.5, 40.9, 40.0, 31.7, 27.1, 26.3, 25.9, 23.4, 22.6; ES-MS calcd for $C_{24}H_{49}N_6O_{13}$ ($M+H^+$) 629.3; found 629.3.

4.4.5. Compound 8e (97%). ^1H NMR (D_2O) δ 5.48 (d, J=3.9 Hz, 1H), 5.15 (d, J=3.9 Hz, 1H), 4.10–3.32 (m, 24H), 3.28 (t, J=3.8 Hz, 2H), 3.12 (dd, J=10.5, 6.7 Hz, 1H), 2.71 (s, 3H), 2.19 (m, 1H), 1.92 (s, 12H), 1.72 (dd, J=14.0, 7.5 Hz, 1H); ^{13}C NMR (D_2O) δ 180.7, 161.9, 98.8, 96.2, 81.4, 80.0, 73.0, 72.6, 71.5, 69.4, 68.8, 66.2, 60.3, 55.9, 50.4, 48.5, 47.5, 40.9, 33.6, 31.6, 23.1; ES-MS calcd for $C_{22}H_{45}N_6O_{13}$ ($M+H^+$) 601.3; found 601.2.

4.4.6. Compound 19. A solution of **8a** (20 mg) in H_2O (3 mL) was treated with Ra-Ni (5 mg of 50% dispersion in H_2O/AcOH). The mixture was stirred over H_2 (balloon) for 5 h at rt, then filtered and lyophilized to give pure **19** (14 mg, 70%). ^1H NMR (D_2O) δ 5.50 (d, J=3.9 Hz, 1H), 5.18 (d, J=3.9 Hz, 1H), 4.10–3.99 (m, 2H), 3.88–3.52 (m, 16H), 3.45–3.30 (m, 7H), 3.18 (dd, J=10.3, 6.7 Hz, 1H); ^{13}C NMR (D_2O) δ 160.5, 98.8, 97.1, 82.2, 81.5, 73.7, 73.0, 72.5, 71.7, 71.6, 69.4, 69.2, 66.5, 60.3, 55.9, 50.4, 48.7, 41.0, 40.6, 38.1, 33.0; FAB MS calcd for $C_{53}H_{66}N_6O_{20}$ ($M+Na^+$) 1129.4; found 1129.4.

4.4.7. Compound 20. To the mixture of *N*-Cbz-4-amino-butyric acid (1 g, 4.22 mmol) and *N*-hydroxysuccinimide (0.49 g, 4.22 mmol) in dioxane (15 mL) was added DCC (0.87 g, 4.22 mmol) at 0°C. The mixture was stirred overnight, filtered, the solvent was removed in vacuo, the residue was dissolved in benzene (40 mL) and ether (40 mL), washed with $NaHCO_3$, dried ($MgSO_4$) and evaporated to dryness. The NOS ester was sufficiently pure for coupling with the aminoglycoside. A solution containing 65 mg (0.2 mmol of ester) was added to **16** (85 mg, 0.1 mmol) in THF–H_2O (2:1, 3 mL). The resulting solution was stirred for 24 h, then evaporated and chromatographed (CH_2Cl_2–MeOH, 4:1) to give 22 mg the amide (FAB MS calcd for $C_{48}H_{60}F_3N_5O_{19}$ ($M+H^+$) 1068.38; found 1068.3).

A portion of the above amide (15 mg, 0.015 mmol) was dissolved in the solution of 1 M NH_3 in THF–H_2O (3:1, 1.5 mL). The mixture was stirred for 44 h, after which time it was evaporated to dryness. The resulting *N*-Cbz intermediate (FAB MS calcd for $C_{46}H_{61}N_5O_{18}$ ($M+H^+$) 972.4; found 972.4) was subjected to hydrogenolysis with Pd/C 10% (5 mg) over H_2 (balloon) in H_2O–dioxane–AcOH (20:20:1, 1.5 mL) to give 11 mg of pure **20** (96%). ^1H NMR (D_2O) δ 5.52 (d, J=5.4 Hz, 1H), 5.13 (d, J=5.6 Hz, 1H), 4.12–3.99 (m, 3H), 3.91–3.62 (m, 11H), 3.48–3.32 (m, 3H), 3.18 (dd, J=8.0, 13.2 Hz, 1H) 3.05 (dd, J=7.4, 7.2 Hz, 2H), 2.39 (t, J=7.0 Hz), 1.93 (s, 12H); ^{13}C NMR (D_2O) δ 182.1, 175.5, 99.0, 96.6, 81.8, 80.5, 73.3, 73.0, 72.7, 71.6, 71.5, 69.4, 68.8, 66.3, 60.3, 55.9, 49.4, 48.6, 41.0, 39.6, 33.3, 31.4, 25.7, 23.9, 23.4

4.4.8. Compound 21. To a stirred suspension of tobramycin (200 mg, 0.43 mmol) in DMSO (3 mL) was added $Zn(OAc)_2{\cdot}2H_2O$ (0.37 g, 1.7 mmol, washed with THF prior to use). The mixture was stirred for 12 h, after which time *N*-benzyloxycarbonyloxy succinimide (0.36 g, 1.46 mmol) was added in one portion. The reaction mixture was stirred for 2 h, diluted with dioxane (3 mL), Amberlite IR-120 (H+) (2 g) was added and the mixture was stirred for another hour. The resin was poured onto a porous glass filter containing 1 g of the same resin, which was washed with a mixture of dioxane–H_2O (1:1) until *N*-hydroxysuccinimide was no longer detectable by TLC. The product was then eluted with 1 M NH_3 in dioxane–H_2O (1:1); (TLC: $CHCl_3$–MeOH–5N NH_4OH), and the fractions containing 3,2′,6′-tris-*N*-benzyloxycarbonyl tobramycin (R_f 0.2) were collected and pooled, the solution was saturated with carbon dioxide (by bubbling gas through the solution for 30 min) and lyophilized to give **21** as a white fluffy solid, (62–70 mg, 18–25%). ^1H NMR (DMSO) δ 8.42 (br s, 1H), 7.42–6.38 (m, 15H), 5.15–4.82 (m, 8H), 3.62–3.29 (m,

10H), 3.22 (m, 2H), 3.05 (br t, 1H), 2.92 (br t, 1H), 2.73 (br t, 1H), 1.88 (m, 2H), 1.60 (br dd, 1H), 1.38 (br dd, 1H); FAB MS calcd for $C_{42}H_{55}N_5O_{15}$ (M+H$^+$) 870.4; found 870.4.

4.4.9. Compound 22. To a stirred solution of the above product (20 mg, 0.024 mmol) in DMSO (0.2 mL) was added CF_3COOEt (2.7 μL, 0.024 mmol). The reaction mixture was stirred for 2 h, then lyophilized. The residue was dissolved in THF–H_2O (3 mL, 2:1) and treated with **17a** (15 mg, 0.032 mmol). The reaction mixture was stirred for 4 days, after which time the solvent was evaporated and the residue chromatographed (CH_2Cl_2–MeOH, 95:5) to give pure **22** (11 mg, 35% over two steps). TLC R_f 0.55 (CH_2Cl_2–MeOH, 9:1); FAB MS calcd for $C_{62}H_{70}F_3N_7O_{21}$ (M+H$^+$) 1306.4; found 1306.1.

4.4.10. Compound 9. The aminoglycoside **22** (11 mg, 0.008 mmol) was treated with 1% methanolic NaOH (2 mL) and the solution was stirred for 20 h. Amberlite IR-120 (H$^+$) was added in small portions until the solution became neutral then the mixture was filtered and evaporated to dryness to yield 3″-amino *N*-hydroxy intermediate as a white solid (FAB MS calcd for $C_{53}H_{67}N_7O_{19}$ (M+H$^+$) 1106.4; found 1106.4). This was hydrogenolyzed over H_2 (balloon) in the presence of 10% Pd/C (3 mg) in dioxane–H_2O–acetic acid (2 mL, 20:20:1) for 40 min. Filtration followed by lyophilization gave pure **9** (4.5 mg, 95%) as a white solid. ^{1}H NMR (D_2O) δ 5.72 (d, J=4.5 Hz, 1H), 5.17 (d, J=4.4 Hz, 1H), 4.03–3.62 (m, 21H), 3.48–3.32 (m, 4H), 3.27 (m, 3H), 2.28 (m, 2H), 2.05 (m, 1H), 1.96 (s, 15H), 1.78 (m, 1H); ES-MS calcd for $C_{21}H_{44}N_7O_{11}$ (M+H$^+$) 570.3; found 570.2.

4.4.11. (3*R*,4*S*)-4-(*t*-Butyldiphenylsilyl)oxymethyl-3-hydroxy-*N*-benzyloxycarbonyl-pyrrolidine-2-one (24). To a solution of **23** (120 mg, 0.24 mmol) in THF (6 mL) was added LiHMDS (0.30 mmol, 0.30 mL of 1 M solution in THF) dropwise at −78°C. The mixture was stirred for 30 min, after which time a solution of the Davis oxaziridine[33] (98 mg, 0.36 mmol) in THF (2 mL) was added dropwise to the above cold mixture. The reaction mixture was stirred at −78°C for 1 h, then at −40°C for 2 h. To the cold mixture was added aqueous NH_4Cl (2 mL of 1 M solution), reaction mixture was allowed to warm up to rt, extracted with ether (3×3 mL), dried ($MgSO_4$) and concentrated in vacuo. Purification by column chromatography (hexane–ethyl acetate, 3:2) afforded 36 mg of **24** (R_f 0.25) and 12 mg of the 3*S*-epimer (R_f 0.2) (40%). ^{1}H NMR ($CDCl_3$) δ 7.95 (m, 1H), 7.63–7.28 (m, 14H), 5.20 (d, J=11.2 Hz, 1H), 5.16 (d, J=11.2 Hz, 1H), 4.81 (dd, J=8.2, 8.0 Hz, 1H), 4.27 (m, 1H), 3.91 (dd, J=12.3, 2.8 Hz, 1H), 3.68 (dd, J=12.3, 3.2 Hz, 1H), 2.56 (dd, J=8.2, 10.0 Hz, 1H), 2.14 (ddd, J=8.0, 10.0, 5.6 Hz, 1H), 0.98 (s, 9H); FAB MS calcd for $C_{29}H_{33}NO_5Si$ (M+Na$^+$) 526.2; found 526.1.

4.4.12. (3*R*,4*S*)-4-(*t*-Butyldiphenylsilyl)oxymethyl-3-trifluoroacetoxy-*N*-benzyloxycarbonyl- pyrrolidine-2-one (25). To a stirred mixture of **24** (334 mg, 0.65 mmol), PPh_3 (184 mg, 0.7 mmol) and diethylazodicarboxylate (111 mg, 1.4 mmol) in THF (10 mL) was added trifluoroacetic acid (228 mg, 2 mmol). After stirring overnight the solution was evaporated to dryness and chromatographed ($CHCl_3$–MeOH, 10:1) to give **25** (351 mg, 90%); ^{1}H NMR ($CDCl_3$) δ 8.12 (dd, J=2.5, 8.1 Hz, 1H), 7.95 (m, 1H, 7.67–7.28 (m, 14H), 5.18 (d, J=11.3 Hz, 1H), 5.10 (d, J=11.3 Hz, 1H), 4.29 (m, 1H), 4.07 (dd, J=10.7, 2.6 Hz, 1H), 3.68 (dd, J=10.7, 2.9 Hz, 1H), 2.52 (m, 1H), 2.12 (m, 1H), 1.01 (s, 9H); FAB MS calcd for $C_{31}H_{32}F_3NO_6Si$ (M+H$^+$) 599.2; found 599.1.

4.5. General procedure for the preparation of 27a and 29a

To a stirred solution of the appropriate lactams **24** or **25** (0.3 mmol) in THF (7 mL) was added 1 M aqueous LiOH (7 mL) at 0°C. The mixture was stirred for 10 min at the same temperature and then acidified with 2 M HCl till pH 4–5. The reaction mixture was extracted with ether (2×10 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was coevaporated with toluene (2×5 mL) and treated with *N*-hydroxysuccinimide (35 mg, 0.3 mmol) and DCC (60 mg, 0.3 mmol) in ethyl acetate at 0°C overnight. Filtration of the reaction mixture, followed by evaporation of the solvent gave *N*-hydroxysuccinimide esters **26** and **28**, respectively, which were added to **16** (170 mg, 0.2 mmol) in THF–H_2O (6 mL, 2:1) and the mixture was stirred at rt for 48 h. Evaporation of the solvent followed by chromatographic purification (CH_2Cl_2–MeOH, 7:1) provided pure products as white solids.

4.5.1. Compound 27a (72%). R_f 0.78 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{65}H_{80}F_3N_5O_{21}Si$ (M+H$^+$) 1352.5; found 1352.3.

4.5.2. Compound 29a (53%). R_f 0.78 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{65}H_{80}F_3N_5O_{21}Si$ (M+H$^+$) 1352.5; found 1352.7.

4.6. General procedure for the preparation of 27b and 29b

To a stirred solution of **27a** or **29a** (0.06 mmol) in DMF (0.5 mL) was added 1 M solution of TBAF in THF (0.4 mL, 0.4 mmol). The reaction mixture was stirred for 3 h at rt after which time MeOH (2 mL) was added followed by silica. The solvent was evaporated and the residue purified by flash column chromatography (CH_2Cl_2–MeOH, 4:1).

4.6.1. Compound 27b (41%). R_f 0.25 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{49}H_{62}F_3N_5O_{21}$ (M+H$^+$) 1114.4; found 1114.2.

4.6.2. Compound 29b (52%). R_f 0.25 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{49}H_{62}F_3N_5O_{21}$ (M+H$^+$) 1114.4; found 1114.3.

4.7. General procedure for the preparation of 10, 12, 14, 15

The appropriate aminoglycoside **27b**, **29b** and the corresponding analogues prepared from **23** and **30** (0.02 mmol) was treated with 1 M NH_3 solution in THF–H_2O (3:1) at rt for 10 h, after which time it was evaporated to dryness to give the corresponding pure de-*N*-trifluoroacetylated intermediates **27b′** (FAB MS calcd for $C_{47}H_{63}N_5O_{20}$ (M+H$^+$)

1018.4; found 1018.4), **29b′** (FAB MS calcd for $C_{47}H_{63}N_5O_{20}$ ($M+H^+$) 1018.4; found 1018.4), **27c′** (FAB MS calcd for $C_{47}H_{63}N_5O_{20}$ ($M+H^+$) 1018.4; found 1018.4), **27d′** (FAB MS calcd for $C_{47}H_{63}N_5O_{19}$ ($M+H^+$) 1002.4; found 1002.4). These were hydrogenolyzed over an H_2 balloon with 10% Pd/C (5 mg) in dioxane–H_2O–AcOH (20:20:1, 2 mL) for 2 h. Filtration through a layer of Celite followed by lyophilization gave pure products as white fluffy solids.

4.7.1. Compound 10 (97%). ^{1}H NMR (D_2O) δ 5.51 (d, J=3.9 Hz, 1H), 5.09 (d, J=4.0 Hz, 1H), 4.32–3.33 (m, 19H), 3.18 (m, 3H), 2.12 (m, 2H), 1.91 (s, 12H); ^{13}C NMR (D_2O) δ 194.2, 179.3, 73.7, 72.9, 71.6, 69.9, 69.4, 68.8, 66.3, 61.2, 58.8, 55.9, 41.1, 31.5, 25.7, 23.9, 19.9, 13.5; MALDI-FTMS calcd for $C_{23}H_{45}N_5O_{14}Na$ ($M+Na^+$) 638.2855; found 638.2862.

4.7.2. Compound 12 (99%). ^{1}H NMR (D_2O) δ 5.49 (d, J=4.0 Hz, 1H), 5.14 (d, J=4.2 Hz, 1H), 4.31 (m, 1H), 4.14–3.29 (m, 20H), 3.12 (m, 1H), 2.22–2.06 (m, 2H), 1.95 (s, 12H); ^{13}C NMR (D_2O) δ 181.2, 176.2, 98.8, 96.3, 80.9, 79.9, 73.2, 73.0, 72.7, 71.5, 69.4, 69.3, 68.8, 66.2, 62.1, 60.3, 56.0, 51.3, 49.6, 48.5, 41.0, 33.4, 30.8, 25.8, 23.4; MALDI-FTMS calcd for $C_{23}H_{45}N_5O_{14}Na$ ($M+Na^+$) 638.2855; found 638.2855.

4.7.3. Compound 14 (96%). ^{1}H NMR (D_2O) δ 5.51 (d, J=3.7 Hz, 0.4H), 5.15 (d, J=3.9 Hz, 0.6H), 4.91 (d, J=3.5 Hz, 0.6H), 4.28–3.12 (m, 24H), 2.59–2.12 (m, 4H), 1.92 (s, 12H); ^{13}C NMR (D_2O) δ 180.8, 177.6, 176.2, 99.0, 98.7, 96.3, 95.7, 90.8, 80.8, 79.9, 73.2, 73.1, 73.0, 72.7, 71.5, 70.0, 69.9, 69.4, 69.3, 68.7, 67.5, 66.3, 66.2, 63.3, 63.0, 61.2, 60.8, 60.3, 56.0, 55.6, 52.2, 52.1, 49.5, 48.5, 48.3, 41.0, 39.4, 33.1, 30.8, 25.9, 23.2; MALDI-FTMS calcd for $C_{23}H_{45}N_5O_{14}Na$ ($M+Na^+$) 638.2855; found 638.2841.

4.7.4. Compound 15 (97%). ^{1}H NMR (D_2O) δ 5.44 (d, J=3.9 Hz, 1H), 5.03 (d, J=4.1 Hz, 1H), 4.10–3.22 (m, 28H), 2.41–2.09 (m, 2H), 1.82 (s, 12H); ^{13}C NMR (D_2O) δ 178.9, 175.2, 99.0, 96.6, 81.9, 80.4, 73.4, 73.0, 72.6, 71.5, 69.4, 68.7, 66.2, 61.0, 60.3, 55.9, 53.1, 49.4, 48.6, 41.0, 32.2, 25.7, 25.0, 23.9, 19.9, 13.5; MALDI-FTMS calcd for $C_{23}H_{45}N_5O_{13}Na$ ($M+Na^+$) 622.2906; found 622.2905.

4.7.5. Lactams 32a and 33a. To a stirred mixture of **30** (150 mg, 130 mmol), PPh_3 (368 mg, 1.4 mmol) and diethylazodicarboxylate (222 μL, 1.4 mmol) in THF (10 mL) was added diphenylphosphoryl azide (302 μL, 1.4 mmol) at rt. The reaction mixture was stirred overnight and concentrated in vacuo. Column chromatography ($CHCl_3$ followed by $CHCl_3$–MeOH, 95:5→9:1) gave 240 mg of the crude azide and 70 mg of recovered starting lactam. To a solution of the above crude azide (150 mg) in THF (6 mL) was added a 1 M solution of LiHMDS in THF (1.4 mL, 1.4 mmol) at −78°C. The reaction mixture was stirred for 30 min, after which time CbzCl (181 μL, 1.28 mmol) was added dropwise. The reaction mixture was warmed-up to rt, stirred overnight, then treated with 1 M aqueous NH_4Cl (5 mL), followed by extraction with ethyl acetate (2×10 mL). The organic extracts were dried ($MgSO_4$) and concentrated. Column chromatography (CH_2Cl_2–EtOAc, 100:1 followed by 9:1) gave 60 mg of **31** (70% based on the recovered **30**). ^{1}H NMR ($CDCl_3$) δ 7.5–7.3 (m, 5H), 5.26 (d, J=11.2 Hz, 1H), 5.33 (d, J=11.2 Hz, 1H), 4.32 (m, 1H), 3.69 (dd, J=5.9, 10.2 Hz, 1H), 3.52 (dd, J=3.3, 10.2 Hz, 1H), 2.73 (m, 1H) 2.48 (m, 1H) 2.18 (m, 1H) 1.92 (m, 1H).

To a stirred solution of lactam **31** (0.54 g, 2 mmol) in THF (50 mL) was added 1 M solution of LiHMDS in THF (2.2 mL, 2.2 mmol) at −78°C. After 40 min of stirring at the same temperature a solution of Davis oxaziridine (0.78 g, 3 mmol) in THF (10 mL) was added dropwise. The resulting mixture was stirred for 3 h, after which time it was quenched with 1 M NH_4Cl (20 mL), the mixture was extracted with EtOAc (2×50 mL) and the organic extracts combined and dried ($MgSO_4$). After evaporation of the solvent the ^{1}H NMR analysis showed that **32b** and **33b** were present in 1.7:1 diastereomeric ratio. The crude material was treated with TBSCl (1 g, 6.75 mmol) and imidazole (0.92 g, 13.5 mmol) in DMF (5 mL) overnight. Aqueous work-up followed by column chromatography (hexane–EtOAc, 9:1) gave 270 mg of **32a** (32% from **31**) and 160 mg (19% from **31**) of **33a**.

4.7.6. Compound 32a. ^{1}H NMR ($CDCl_3$) δ 7.49–7.32 (m, 5H), 5.35 (d, J=12.4 Hz, 1H), 5.29 (d, J=12.4 Hz, 1H), 4.62 (t, J=9.2 Hz, 1H), 4.28 (m, 1H), 3.74 (dd, J=4.5, 12.6 Hz, 1H), 3.52 (dd, J=2.6, 12.6 Hz, 1H), 2.28 (m, 1H), 2.13 (m, 1H), 0.92 (s, 9H), 0.19 (s, 3H), 0.14 (s, 3H).

4.7.7. Compound 33a. ^{1}H NMR ($CDCl_3$) δ 7.58–7.32 (m, 5H), 5.35 (d, J=12.3 Hz, 1H), 5.29 (d, J=12.3 Hz, 1H), 4.27 (dd, J=4.7, 7.2 Hz, 1H), 4.15 (m, 1H), 3.67 (m, 2H), 2.30 (m, 1H), 1.98 (m, 1H), 0.93 (s, 9H), 0.21 (s, 3H), 0.15 (s, 3H).

4.8. General procedure for the preparation of 36 and 37

To a stirred solution of lactam **32a** or **33a** (0.5 mmol) and AcOH (173 μL) in THF (12 mL) was added 1 M solution of TBAF in THF (1.5 mL, 1.5 mmol) at 0°C. The mixture was stirred overnight, and the product (**32b** and **33b** respectively) was treated with 1 M aqueous LiOH in THF–H_2O (12 mL, 2:1) at 0°C for 5 min. Acidification with 2N HCl (pH 4–5) and extraction with EtOAc gave the crude acids, which were mixed with DCC (0.1 g, 0.5 mmol) and *N*-hydroxysuccinimide (57 mg, 0.5 mmol) in EtOAc (5 mL) at 0°C. The mixture was placed in the refrigerator for 4 days, after which time it was filtered and the filtrate evaporated to provide crude esters **34** and **35**, respectively. These were mixed with **16** (150 mg, 0.2 mmol) in THF–H_2O (15 mL, 2:1) and stirred for 3 days. Evaporation of the solvent followed by the purification by column chromatography (CH_2Cl_2–MeOH, 9:1) provided **36** and **37** as white solids.

4.8.1. Compound 36 (45%). R_f 0.79 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{49}H_{61}F_3N_8O_{20}$ ($M+H^+$) 1139.4; found 1139.5.

4.8.2. Compound 37 (40%). R_f 0.81 (CH_2Cl_2–MeOH, 4:1); FAB MS calcd for $C_{49}H_{61}F_3N_8O_{20}$ ($M+H^+$) 1139.4; found 1139.7.

4.9. General procedure for the preparation of 11, 13

The appropriate aminoglycoside **36** and **37** (0.02 mmol) was treated with 1 M NH_3 solution in THF–H_2O (3:1) at rt for 10 h., after which time it was evaporated to dryness to give pure de-*N*-trifluoroacetylated intermediates **36a** (FAB MS calcd for $C_{47}H_{62}N_8O_{19}$ ($M+H^+$) 1043.4; found 1043.5) and **37a** (FAB MS calcd for $C_{47}H_{62}N_8O_{19}$ ($M+H^+$) 1043.4; found 1043.4). These were hydrogenolyzed over H_2 (balloon) with 10% Pd/C (5 mg) in dioxane–H_2O–AcOH (20:20:1, 2 mL) for 2 h. Filtration through a layer of Celite followed by lyophilization of solvent gave pure products as white fluffy solids.

4.9.1. Compound 11 (97%). ^{1}H NMR (D_2O) δ 5.51 (d, J=4.6 Hz, 1H), 5.13 (d, J=4.5 Hz, 1H), 4.15–3.13 (m, 24H), 2.49 (m, 1H), 2.22 (m, 1H), 1.92 (s, 12H); MALDI-FTMS calcd for $C_{23}H_{46}N_6O_{13}Na$ ($M+Na^+$) 637.3015; found 637.3019.

4.9.2. Compound 13 (98%). ^{1}H NMR (D_2O) δ 5.50 (d, J=4.5 Hz, 1H), 5.10 (d, J=4.2 Hz, 1H), 4.12–3.15 (m, 24H), 2.45 (m, 1H), 2.23 (m, 1H), 1.98 (s, 12H); MALDI-FTMS calcd for $C_{23}H_{46}N_6O_{13}Na$ ($M+Na^+$) 637.3015; found 637.3022.

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