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Probing the ribosomal RNA A-site with functionally diverse analogues of paromomycin—synthesis of ring I mimetics

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Abstract—Methods were developed to selectively cleave the 2-amino-2-deoxy-α-D-glucopyranosyl ring in paromomycin. The preferentially N- and O-protected products were alkylated on the liberated C4 hydroxyl group of the deoxystreptamine subunit. Further manipulation furnished a series of aromatic, heteroaromatic, and aliphatic appendages as spatial mimics of ring I. Modest inhibitory activity was found against *Staphylococcus aureus* with two analogues (**27** and **63**), although cell-free functional transcription/translation assays were similar to paromomycin for analogue **27**.

1. Introduction

The aminoglycoside (aminocyclitol) group of antibiotics are among the oldest known antibacterial agents, with streptomycin being discovered over half a century ago.[1] Although several members of this class are currently used in clinical practice, their administration must be carefully monitored because of potential dose-related nephrotoxicity and ototoxicity.[2] The emergence of resistant strains of Gram-positive and Gram-negative bacteria, capable of inactivating the aminoglycosides via enzymatic modification, has further compromised their use as primary treatments for life-threatening infections. Two of the more prevalent modes of inactivation involve O-phosphorylation at the C3′-hydroxyl in ring I and N1-acetylation in ring II (deoxystreptamine)[3] using paromomycin as a representative example (Fig. 1). This has instigated extensive research efforts on several fronts in search of natural or chemically modified aminoglycosides that are not affected by enzymatic deactivation.[4–6] Indeed, tobramycin, a 3′-deoxykanamycin is not a substrate for O-phosphorylation, and is used in an inhaler formulation for the prevention of infection in cystic fibrosis patients.[7] Other well-known clinically effective aminoglycosides are used for specific indications, but the prospects of developing resistance through a variety of mechanisms such as membrane impermeability and efflux are omnipresent.

The mode of antibacterial action of aminoglycosides is well understood.[8] They exert their bactericidal action by inhibiting protein biosynthesis at the prokaryotic rRNA level. A region of highly conserved nucleotides in the decoding 16S rRNA (A-site) region on the 30S subunit is the site of binding. Codon misreading affects translocation and misreading of the mRNA sequence, leading to aberrant translation and premature termination of protein synthesis.[9] This unique energy-dependent process is highly relevant in the design of new analogues, since mammalian cells do not have a 30S subunit in their ribosome. Exciting developments in the molecular structure of the ribosome[10] as well as its components have instigated extensive efforts in uncovering the nature of interactions of aminoglycosides in the 16S region in particular utilizing biochemical,[11] spectroscopic,[12] and mass spectrometric[13] methods. Elegant NMR[14] and X-ray crystallographic studies[15–17] have elucidated the binding interactions of several aminoglycosides. Molecular modeling has also offered predictive insights.[18] Paromomycin **1** exhibits strong binding to the A-site of the 16S region, by making effective contacts via amino and hydroxyl groups, and ring oxygen to specific bases. In the process, the conserved A^{1492} and A^{1493} residues are displaced toward the minor groove, which is a hallmark of effective binding.[14,16] Rings I and II in paromomycin are important for recognition, binding, and stabilization (Fig. 1). Rings III and IV contribute to the binding affinity, charged interactions with phosphates in the lower stem, and correctly orient rings I and II.[16] Thus, paromomycin can be depicted in its bioactive conformation, where the 2′-amino group of ring I is H-bonded to

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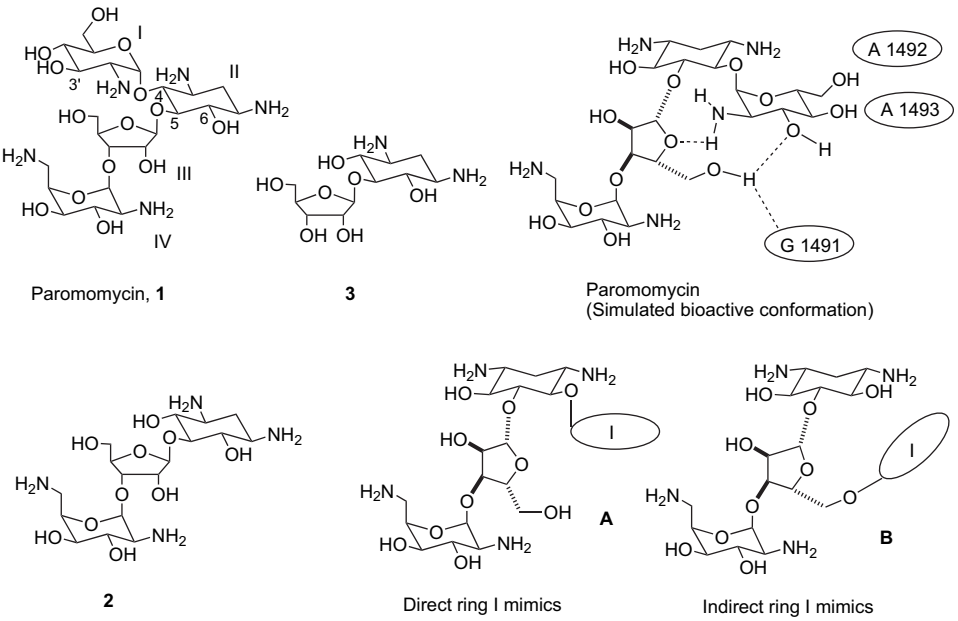


Figure 1.

the ring oxygen of the β-D-ribopyranosyl unit and the C3′ hydroxyl is H-bonded with the primary hydroxymethyl group of the same ring III subunit, giving rise to an L-shaped motif. NMR[14] and X-ray co-crystal structures[16] have also shown the disposition of strategically located bases at the site of binding. For example, ring I is deployed 'above' G[1491] possibly benefiting from a hydrophobic interaction.

Cognizant of these enormously informative structural insights pertaining to the three-dimensional interactions of paromomycin with the A-site of rRNA we have rekindled our interest in this area[19] with new objectives.[20] Herein, we describe methods for the selective removal of ring I of paromomycin, and its replacement with non-carbohydrate entities. Additionally, we report methods for the preparation of ring I analogues, which involve the construction of the ring II/ring III glycosidic bond.

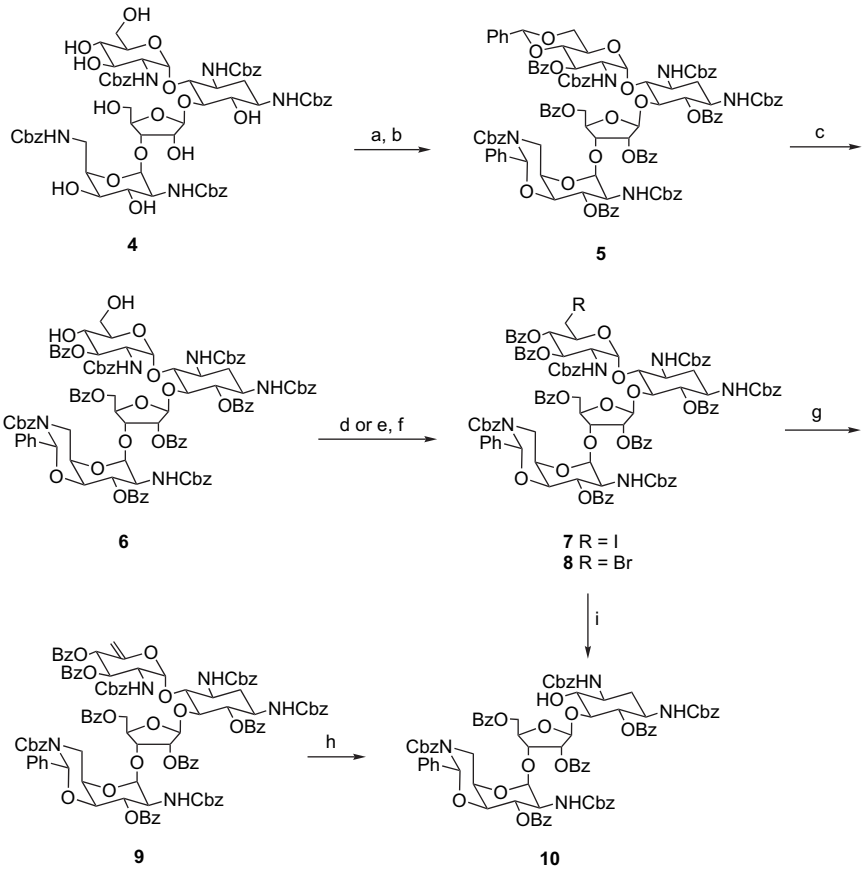
2. Results and discussion

Some years ago, we had described an oxidative method for the synthesis of truncated pseudosaccharides (nor- and bis nor-paromomycins) **2** and **3**, for assessing the possible role of each subunit in antibacterial activity.[19] However interesting, these subunits of paromomycin could not be put to good use as functional probes to study binding to ribosome at that time due to lack of structural and binding information.

Our first objective was to develop methods for the synthesis of a preferentially substituted pseudotrisaccharide corresponding to **2**, in which the 1,3-diol unit in the 2-deoxystreptamine (ring II) was differentiated. Another objective was to utilize such a versatile intermediate to install a diverse set of direct ring I mimetics as well as spatially indirect mimetics (Fig. 1, structure **A** and **B**, respectively), and to assess their ribosomal binding properties and potential antibacterial activities. The readily available penta-*N*-Cbz-paromomycin[19a] **4** was converted to the 4′,6′,4‴,6‴-bis-benzylidene acetal pentabenzoate **5**[19b] in moderate yield by treatment with $ZnCl_2$ and benzaldehyde, followed by benzoylation (Scheme 1). The 6-OH in the precursor benzylidene intermediate was found to be particularly difficult to benzoylate. Selective cleavage of the 4′,6′-*O*-acetal afforded the diol **6**, which was converted to the iodide **7** and bromide **8** under standard conditions using Ph_3P, imidazole, I_2,[21] and Ph_3P, NBS,[22] respectively followed by benzoylation. Treatment of **7** with AgF in pyridine,[23] resulted in smooth elimination to afford the exocyclic enol ether **9**. Cleavage with $HgCl_2$ in aqueous acetone[24] led to the selectively protected pseudotrisaccharide **10** in excellent yield.

An alternative route to **10** was also studied exploring two methods for a Grob-type fragmentation of 6-halo glycopyranosides (Scheme 1).[25] Treatment of either iodide **7** or bromide **8** with Zn dust and $CeCl_3$ heptahydrate as described by Ganem and co-workers[26] afforded a modest 34% yield of **10**. A more recent variation reported by Jäger and co-workers[27] utilizing Zn dust, and catalytic vitamin B-12 in aqueous ammonium chloride also gave 35% yield of **10** accompanied by 55% of the 6′-deoxy product. It is possible that the densely functionalized substrate is incompatible with the presence of Zn to allow a more effective conversion to the expected product. Recently, Vassella and co-workers[28] reported a Zn-mediated reductive fragmentation of a *N*-Boc perester analogue of **7** and **8** in 56% yield (73% conversion).

The most practical and chemically diverse modification at C4 in **10** was based on alkylations to a series of aryl methyl ethers (Scheme 2). Although devoid of heteroatoms or H-bonding functionality as found in ring I, such ethers would nevertheless provide a control with regard to space, and to the importance of hydrophobic interactions with G[1491]. The most effective alkylation conditions were found to be KHMDS in THF to afford representative benzyl ethers **11** and **12**. Treatment with methoxide followed by hydrogenolysis led to the ring I surrogates **13** and **14**. In another effort to diversify the pseudotrisaccharide scaffold, we

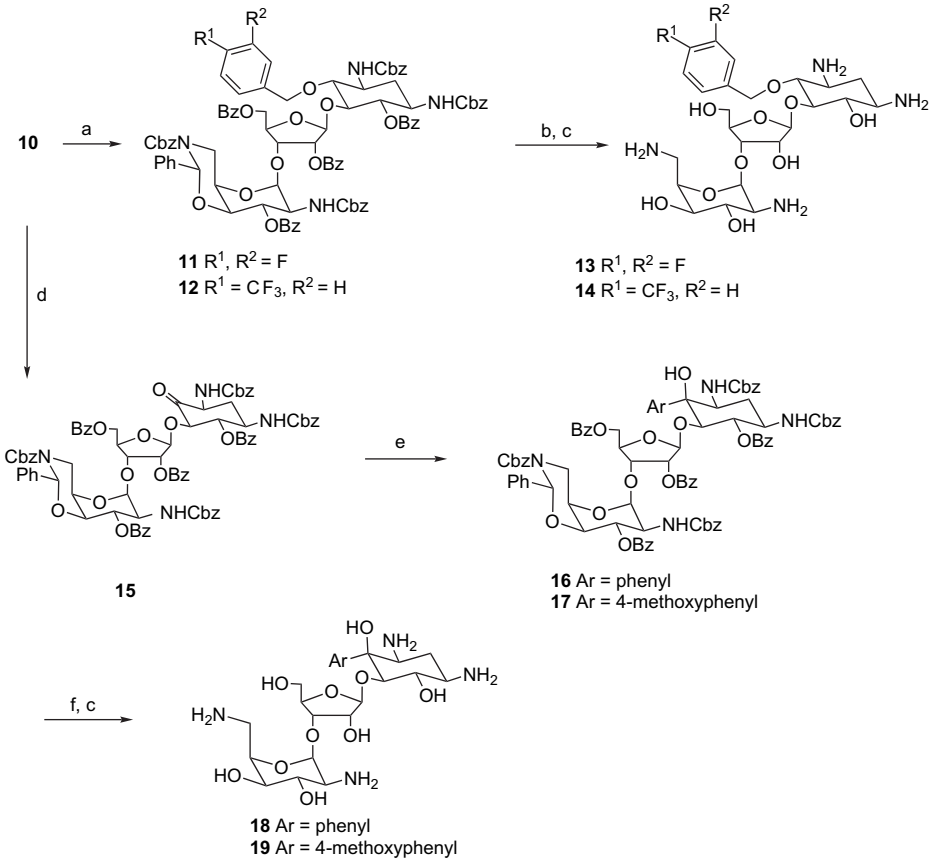


Scheme 1. Reagents and conditions: (a) PhCHO, $ZnCl_2$, 86%; (b) BzCl, pyridine, DMAP, 70 °C, 40%; (c) AcOH/H_2O (4:1), 55 °C, 70%; (d) Ph_3P, imidazole, I_2, 50 °C, 94%; (e) Ph_3P, NBS, DMF, 50 °C, 70%; (f) BzCl, pyridine, DMAP, 90% for **7** and **8**; (g) AgF, pyridine, 92%; (h) $HgCl_2$, aqueous acetone, reflux, 81%; (i) Zn dust, $CeCl_3$, MeOH, refflux or Zn dust, NH_4Cl, Vitamin B_{12} (cat.), MeOH, 35%.

succeeded in oxidizing the C4 hydroxyl group with the Dess–Martin periodinane reagent[29] in excellent yield to give the ketone **15** (Scheme 2). Addition of phenyl- and *p*-methoxyphenyl magnesium bromides proceeded smoothly to afford the tertiary alcohols **16** and **17**, respectively. Debenzoylation and hydrogenolysis gave the corresponding C4-aryl analogues **18** and **19**. The stereochemistry of the newly created center at C4 remains unknown, although an equatorial attack may be anticipated on steric grounds. Unfortunately, the C4 ether and aryl analogues **13**, **14**, **18**, and **19** had no binding affinity to the 16S rRNA fragment, and no antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was found (see Table 1).[30]

We then proceeded to expand the level of diversity at C4 by incorporating motifs that encompassed polar functionality, capable of H-bonding interactions. A reductive amination protocol[20a,c] on an aldehydo 2-carbon ether tether seemed to be a practical approach (Scheme 3). Thus, allylation of **10** with allyl iodide in the presence of KHMDS in THF gave the corresponding allyl ether, which was oxidatively cleaved with ozone[20a] to give the aldehyde intermediate **20**. A series of readily available amines were used in the reductive amination under standard conditions using $NaCNBH_3$ in methanol containing AcOH.[20a] Yields of the products **21**–**25** were moderate to high depending on the amine used. In the case of 2-aminopyrimidine, the reaction was accompanied by considerable formation of the alcohol **25**, which was also prepared as a control by direct reduction of the aldehyde **20**. We then proceeded to remove the benzoate esters and the *N*-Cbz groups by hydrogenolysis with Pearlman's catalyst[31] in aqueous AcOH/MeOH. Although the products **26**–**30** were obtained in high yields, we encountered unexpected reactivity with some of the heterocycles. Upon reduction of the 3-amino-6-methoxypyridyl (**22**) and 2-aminopyrimidinyl (**23**) products, the saturated analogues **27** and **28** were obtained (Scheme 3). A model reduction performed under the same conditions with phenylpropionaldehyde also gave the same results. This unexpected overeduction due to the electronic nature of the heterocycles was not unwelcome, since the products **27** and **28** now had functionality that could potentially engage in donor–acceptor H-bonding interactions, in addition to their intrinsically more Lewis basic character.

Next we turned to an alternative ring I–ring II construction strategy. We believed that the coupling of compounds **31**[30c] and **32** would give us the O5 and O6 coupled products directly (Scheme 4). Glycosylation of **31** with the thiol acceptor **32** promoted by AgOTf gave only one major compound, which after deacetylation proved to be the orthoester **33**. The regiochemistry of compound **33** was ascertained by 1D and 2D NMR spectra. In the HMBC spectrum, there was a strong correlation between H5 and C8. The absolute configuration of the new stereogenic center of the orthoester was unclear and only tentatively assigned. Other conditions using TfOH as the activator and corresponding trichloroacetimidate as the donor were not successful.



Scheme 2. Reagents and conditions: (a) $ArCH_2Br$, KHMDS, THF, 84% (**11**), 85% (**12**); (b) MeONa/MeOH, 90% (from **11**), 96% (from **12**); (c) $Pd(OH)_2/C$, H_2, MeOH, H_2O, AcOH, quant.; (d) Dess–Martin periodinane, CH_2Cl_2, 90%; (e) ArMgX, −78 °C, 85% (**16**), 80% (**17**); (f) MeONa/MeOH, 89% (from **16**), 98% (from **17**).

Table 1. Calculated dissociation constant (K_d) with 16S A-site rRNA,[a] cell-free functional transcription/translation (IC_{50}) and antimicrobial activity

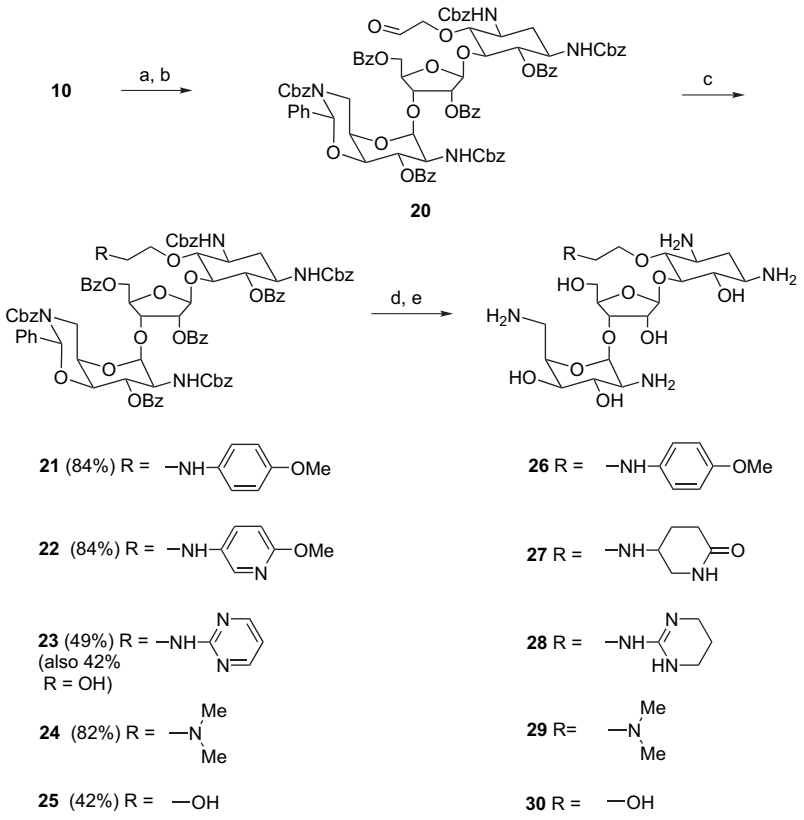
Compound	K_d (μM)	IC_{50} (μM)	MIC (μM)	
			Escherichia coli	*Staphylococcus aureus*
Paromomycin	0.1	0.6	3–6	1–2
13	>100	n.d.	>100	>100
14	>100	n.d.	>100	>100
18	>100	2.7	>100	>100
19	>100	1.5	>100	>100
26	>100	0.2	>100	>100
27	4.1	0.3	25–50	6–12
28	3.2	0.3	>100	>100
29	10	>50	>100	>100
30	6.1	2.2	>100	>100
39	11	>50	>100	>100
47a	4	4	25–50	>100
47b	14	>50	>100	>100
47c	0.3	>50	>100	>100
47d	2	>50	>100	>100
40	14	>50	>100	>100
48a	16	16	>100	25–50
48b	39	>50	>100	>100
48c	0.4	>50	>100	>100
48d	3	>50	>100	>100
54	n.d.	n.d.	>100	>100
55	n.d.	n.d.	>100	>100
62	n.d.	n.d.	6–12	25–50
63	n.d.	n.d.	6–12	3–6

n.d.: not determined.
[a] Ligands (7.5, 2.5, 0.75, 0.25 μM); target RNA (0.1 μM).

Therefore we turned to a somewhat more complex strategy, involving construction of the ring II/ring III bond.[33] Toward this end, glycosylation between *p*-methoxybenzyl (PMB) substituted analog **34** and thiolglycosyl donor **32**[32] under scrupulously anhydrous conditions gave the desired regioisomers **35a** and **35b** in almost equal ratio (Scheme 5). The mixture of the two isomers was then deacetylated and reduced with hydrazine and Raney Ni to give the final PMB-substituted analogues **39** and **40**. Compounds **39** and **40** were easily separated by preparative LC-MS using Luna C18 column, and individually characterized using 1D, 2D NMR spectra to determine the regiochemistry precisely.

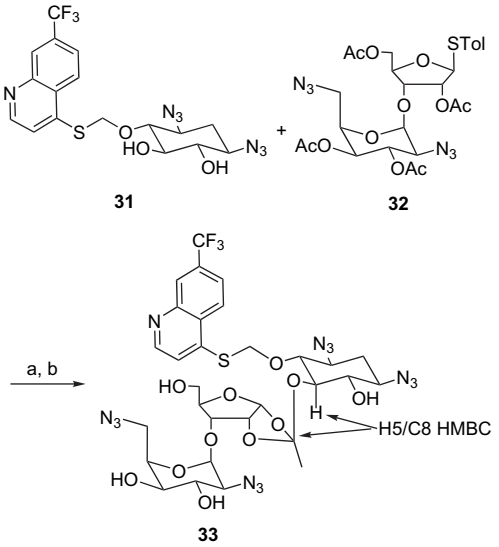
Next, the mixture of **35a** and **35b** was acetylated and treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give a 1:1 mixture of the desired protected II,III,IV-ring analogues **37** and **38**, which could be easily separated using silica gel flash chromatography (Scheme 5).

A Pummerer rearrangement (using acidic conditions that avoid a previously observed acetyl group migration) was adopted successfully to functionalize the free hydroxy group of compounds **37** or **38** to give intermediates **41** or **42** individually (Scheme 6). Subsequent chlorination by sulfuryl chloride and reaction with the sodium salt of thiol heterocycles **a–d** gave intermediates **45a–d** or **46a–d**. Deprotection to remove the acetyl groups using LiOH, hydrazine, and Raney Ni gave the desired final compounds **47a–d** or **48a–d**.



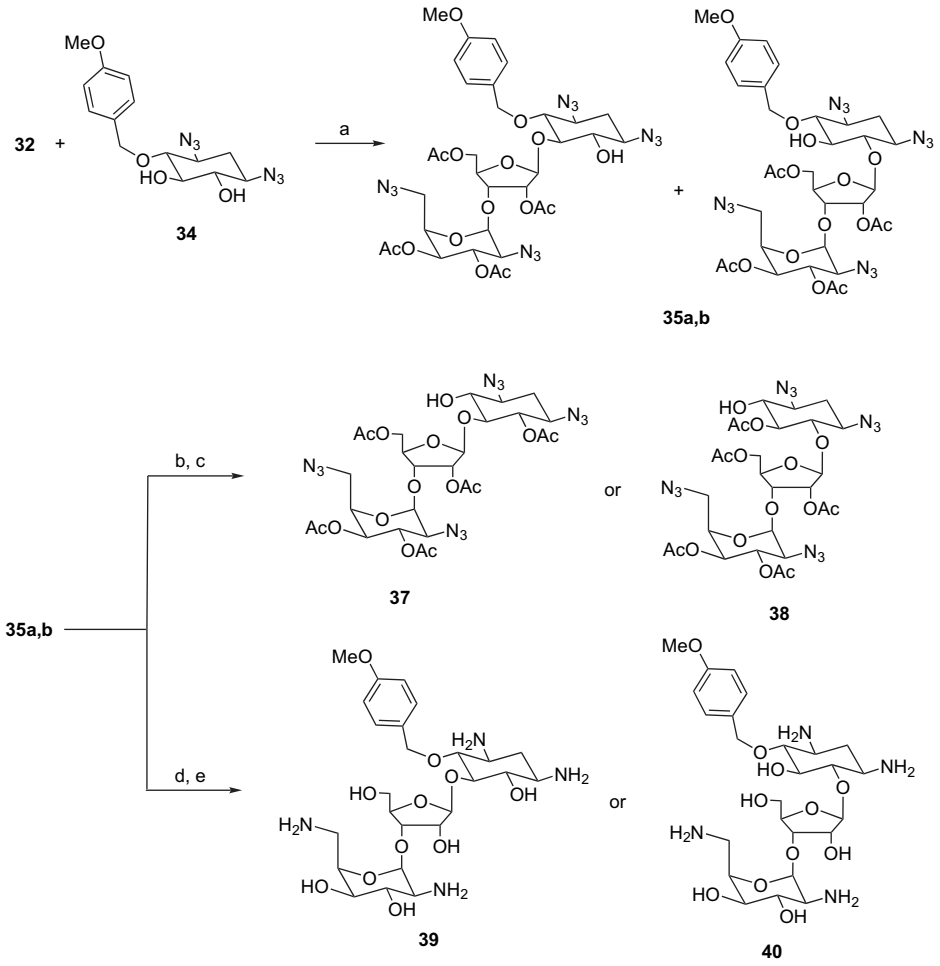
Scheme 3. Reagents and conditions: (a) $CH_2{=}CHCH_2I$, KHMDS, THF, 74%; (b) (i) O_3, CH_2Cl_2, −78 °C, (ii) Ph_3P, 84%; (c) amine, $NaBH_3CN$, AcOH, MeOH; (d) MeONa/MeOH; (e) $Pd(OH)_2/C$, H_2, AcOH, MeOH, H_2O.

High-resolution FTICR mass spectrometry was used to study the non-covalent binding interaction between the synthetic aminoglycosides and RNA (see Table 1).[34,35] Many of the analogues were shown to bind to 16S A-site; however, none were as good as paromomycin. To determine whether our compounds were binding in a functional manner, we tested them for intrinsic activity in a cell-free transcription/translation (T/T) assay. In general, we can classify our compounds into three categories: (1) those with 16S binding, but lacking T/T activity (e.g., **29**, **39**, **47b–d**, **48b–d**), this is most likely due to a non-functional binding event, which further suggests the difficulty in achieving hydrogen bond donor pairs in the precise orientation, (2) those lacking 16S binding but having T/T activity (e.g., **18**, **19**, **26**), which suggests an alternative mechanism of action from that typical of aminoglycosides, and (3) those with both 16S binding and T/T activity (**27**, **28**, **47a**, **48a**). Furthermore, compounds **26–30**, **39–40**, **47a–d**, and **48a–d** were tested against sensitive strains of *E. coli* and *S. aureus* (see Table 1), and a good correlation was seen with compounds with both good binding and T/T activity. Moderately weak activity was found in the case of compound **27** and all other compounds were inactive at 100 μM. In view of the biological results of **39** and **40**, we did not attempt to separate the mixture of glycosides **35a**,**b**.



Scheme 4. Reagents and conditions: (a) CH_2Cl_2, 3 Å molecular sieves, NIS, AgOTf, toluene, −10 °C, 59%; (b) NH_3/MeOH, 88%.

In the hope of finding new binding interactions, we explored branching at the 5″-hydroxyl site of ring III. We surmised that the branch motif would extend toward the vacated ring I space, and possibly engage in productive interactions (Fig. 1, B). In the process, the inherent non-symmetrical 4,5-disubstitution pattern in the deoxystreptamine ring of paromomycin would be lost. Nevertheless, we pursued the synthesis of the prototypical target structure starting with the nor-paromomycin intermediate **10**. Thus, debenzoylation followed by selective protection of the 5″-hydroxyl group and rebenzoylation gave **49** (Scheme 7). Removal of the silyl ether and alkylation in the presence of allyl iodide and KHMDS gave the corresponding 5″-*O*-allyl ether **50**. Ozonolytic cleavage led to the aldehyde **51**, which was reduced



Scheme 5. Reagents and conditions: (a) CH_2Cl_2, 3 Å molecular sieves, NIS, AgOTf, toluene, 89%; (b) pyridine, Ac_2O, DMAP, 95%; (c) DDQ, CH_2Cl_2, H_2O, 95%; (d) THF–^{i}PrOH, LiOH/H_2O, 69%; (e) EtOH, NH_2NH_2, Raney Ni, 97%.

to the alcohol **52**, and also reductively aminated to the branched bis-aminoethyl ether **53**. Deprotection as described above afforded the 5″-*O*-branched pseudotrisaccharides **54** and **55**, respectively, which have no antibacterial activity (see Table 1).

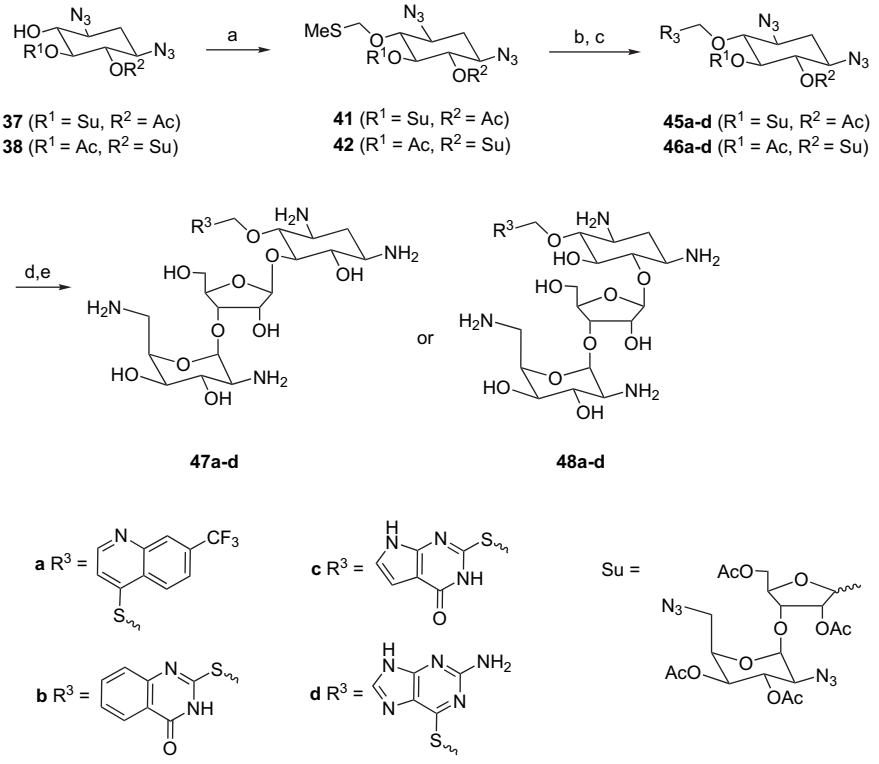
Application of analogous transformations to the versatile intermediate **56** afforded the 5″-dimethylaminoethyl and the 5″-phenylpropylaminoethyl ether analogues of paromomycin **62** and **63**, respectively (Scheme 8). The 5″-hydroxyl group in ring III of paromomycin has been previously derivatized principally by Tor[35] and co-workers in connection with dimeric analogues. Heteroconjugates derived from neomycin have also been studied by Yu.[36] Extensive studies by Wong[37] and Mobashery[4] on aminoglycosides bearing hydrophilic substituents have also been reported. Unfortunately, the 5″-*O*-branched analogue **62** did not show antibacterial activity (see Table 1). Interestingly, paromomycin analogue **63** showed low micromolar MIC against *S. aureus* (see Table 1). Studies are in progress to optimize binding interactions through the design of more effective mimics of ring I in paromomycin and to uncover new modes of binding to the A-site.[38]

A series of ether analogues at C6 and C5″ hydroxyl groups individually were prepared from paromomycin by selective functionalizations and degradations. A diverse set of aromatic, heteroaromatic, and aliphatic appendages were introduced in the ether chains to simulate the spatial disposition of ring I in the bioactive conformation of paromomycin. Starting with a deoxystreptamine derivative, we prepared a series of β-ribosyl glycosides containing heterocyclic appendages at C6 hydroxyl group. A total of 23 new analogues of paromomycin and its ring I truncated variant were prepared and tested for their inhibitory activities on sensitive strains of *S. aureus* and *E. coli*. Many of the analogues were also evaluated in a translation/transcription assay. Only two analogues, **27** and **63**, exhibited modest MIC values, which were two to three times weaker than the parent paromomycin. In spite of this, it is clear that the replacement of ring I with suitable mimics has the potential for the discovery of novel bioactive analogues as evidenced by the inhibitory activity of compound **27**. Appending a hydrophobic ether chain at C5″ of paromomycin may also open the way to better analogues compared to modestly active **63**.[39]

3. Experimental section

3.1. General procedures

^{1}H and ^{13}C spectra were recorded on a 300 and 75 MHz Bruker spectrometer, respectively (rotamers may exist for some intermediates). 2D NMR spectra were run on

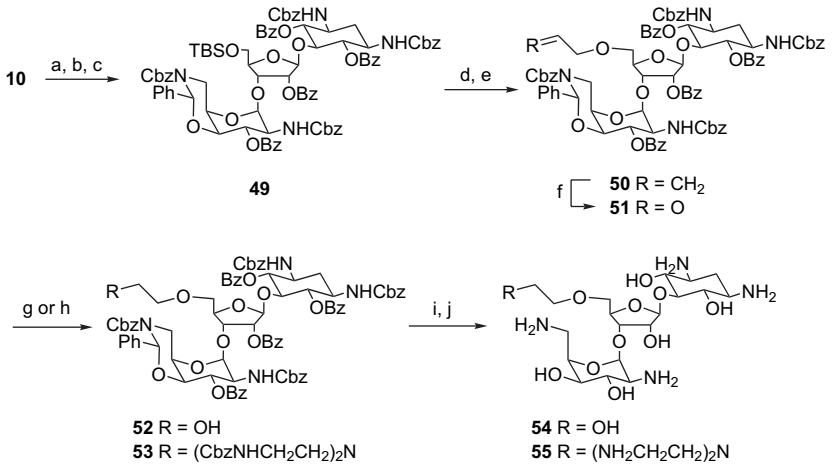


Scheme 6. Reagents and conditions: (a) DMSO, Ac_2O, AcOH, 98% for **41**, 100% for **42**; (b) CH_2Cl_2, SO_2Cl_2, CH_2Cl_2; (c) thiol in CH_3CN or DMF, NaH, 70–91%; (d) THF–^{i}PrOH, LiOH/H_2O, 69%; (e) EtOH, NH_2NH_2, Raney Ni, 41–83%.

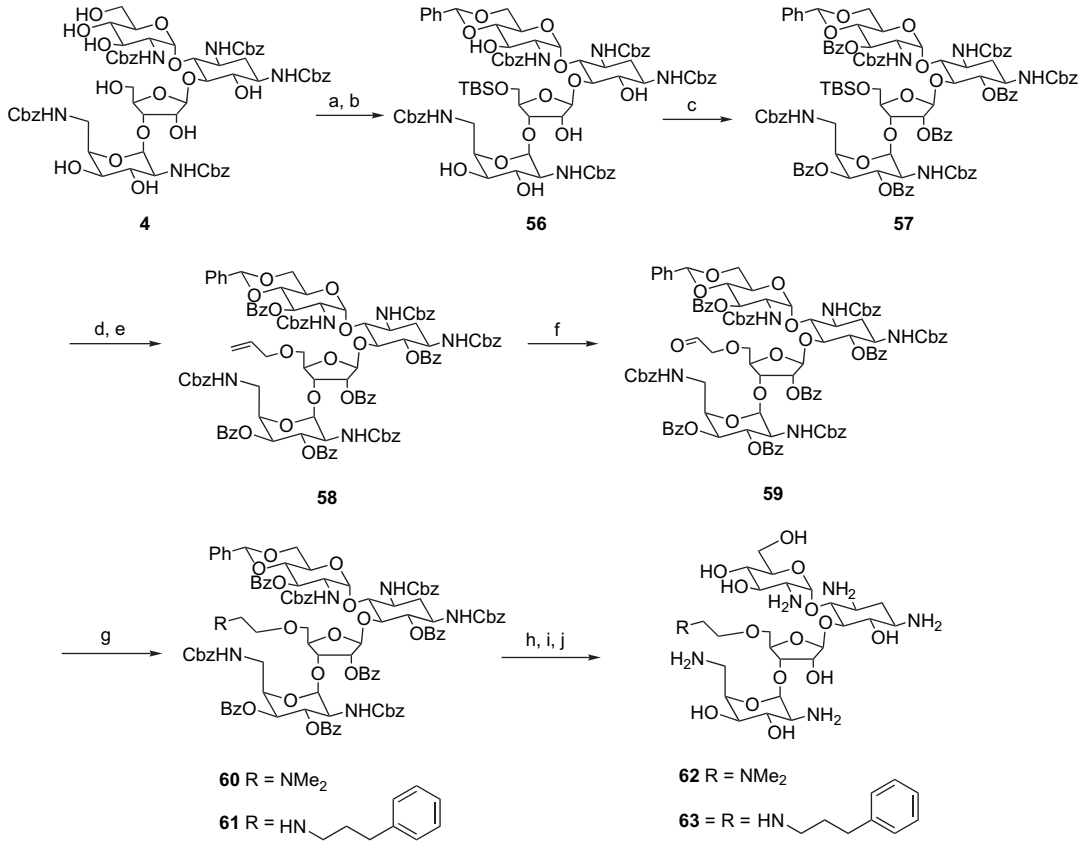
compounds (**33**, **39**, **40**, **42**, **45a**, **47a**, **48a**) to confirm the regiochemistry. Silica gel 60 from EM Science was used for purification. The column for preparative LC-MS (Agilent) was a Luna C18 column (10 μ, 250×21.20 mm) from Phenomenex. An isocratic gradient (1% AcOH in CH_3CN) was used as the mobile phase. All mass spectrometry data (API-ES) were obtained as a result of running the compounds through analytical LC-MS, which simultaneously provided ELSD (evaporative light scattering detectors) and UV (ultraviolet at 254 nm) data.

3.2. Minimum inhibitory concentrations (MIC bacterial assay)

The assays are carried out in 150 μL volume in duplicate in 96-well clear flat-bottom plates. The bacterial suspension from an overnight culture growth in appropriate medium is added to a solution of test compound in 2.5% DMSO in water. Final bacterial inoculum is approximately 10^2–10^3 CFU/well. The percentage growth of the bacteria in test wells relative to that observed for a control wells containing



Scheme 7. Reagents and conditions: (a) MeONa/MeOH, quant.; (b) TBS-OTf, 2,4,6-collidine, CH_2Cl_2, 75%; (c) BzCl, pyridine, 90%; (d) Bu_4NF, THF, 90%; (e) CH_2=$CHCH_2I$, NaH, THF, 75%; (f) (i) O_3, CH_2Cl_2, −78 °C, (ii) Ph_3P, 60%; (g) $NaBH_3CN$, AcOH, MeOH, 85%; (h) amine, $NaBH_3CN$, AcOH, MeOH, 78%; (i) MeONa/MeOH, 88% for **52** and **53**; (j) $Pd(OH)_2/C$, H_2, AcOH, MeOH, H_2O, quant.



Scheme 8. Reagents and conditions: (a) PhCHO, HCOOH, 4 Å molecular sieves, 73%; (b) TBS-OTf, 2,4,6-collidine, CH_2Cl_2, 75%; (c) BzCl, pyridine, DMAP, 70 °C, 70%; (d) Bu_4NF, THF, 51%; (e) $CH_2{=}CHCH_2I$, KHMDS, THF, 58%; (f) (i) O_3, CH_2Cl_2, −78 °C, (ii) Ph_3P, 60%; (g) amine, $NaBH_3CN$, MeOH, AcOH (85% for **60**, 80% for **61**); (h) MeONa/MeOH, 60% (R=NMe_2), 72% (R=3-propylamine); (i) AcOH/H_2O (4:1), 55 °C; (j) $Pd(OH)_2/C$, H_2, AcOH, MeOH, H_2O, quant.

no compound is determined by measuring absorbance at 595 nm (A_{595}) after 20–24 h at 37 °C. The MIC is determined as a range of concentration where complete inhibition of growth is observed at the higher concentration and bacterial cells are viable at the lower concentration. Both ampicillin and tetracycline are used as antibiotic positive controls in each screening assay for *E. coli* (ATCC25922) and *S. aureus* (ATCC13709).

3.3. Coupled bacterial transcription/translation assay (T/T assay)

The DNA template, pBest Luc™ (Promega), is a plasmid containing a reporter gene for firefly luciferase fused to a strong *tac* promoter and ribosome binding site. Messenger RNA from 1 μg pBestLuc was transcribed and translated in *E. coli* S30 bacterial extract in the presence or absence of test compound. Compounds were tested in a black 96-well microtiter plate with an assay volume of 35 μL. Each test well contained 5 μL test compound, 13 μL S30 premix (Promega), 4 μL 10X complete amino acid mix (1 mM each), 5 μL *E. coli* S30 extract, and 8 μL of 0.125 μg/μL pBest Luc™. The transcription/translation reaction was incubated for 35 min at 37 °C followed by detection of functional luciferase with the addition of 30 μL LucLite™ (Packard). Light output was quantitated on a Packard TopCount.

3.3.1. 4′,6′-*O*-Benzylidene-4‴,6‴-*N*,*O*-benzylidene penta-*O*-benzoyl penta-*N*-benzyloxycarbonyl paromomycin (5). Freshly distilled benzaldehyde (250 mL) was added to $ZnCl_2$ (9.53 g, 0.069 mol) fused under flame using a vacuum pump at 10 mmHg. To the stirred mixture was added 4 Å molecular sieves (25 g), followed by penta-*N*-benzyloxycarbonyl paromomycin **4** (6.0 g, 0.046 mol). After stirring for 12 h at room temperature, the mixture was added dropwise to a stirred ice-cold solution of satd aqueous Na_2CO_3, extracted with ethyl acetate (three times), and the organic layer was washed with water, brine, and dried over Na_2SO_4. The solvent was evaporated to dryness and excess benzaldehyde was removed under vacuum to afford a crude solid, which was purified by flash column chromatography over silica gel (1:19 MeOH/CH_2Cl_2) to obtain the *N*-Cbz-4′,6′,4‴,6‴-bis acetal as a white solid (5.86 g, 86%) with analytical data identical to those we published some years ago.[19a]

A solution containing the above compound (2.0 g, 1.36 mmol) and *N*,*N*-dimethylamino pyridine (0.834 g, 6.83 mmol) in dry pyridine (30 mL) was treated with benzoyl chloride (1.6 mL, 13.67 mmol) at 0 °C. The reaction mixture was stirred at room temperature for two days followed by 70 °C for 12 h when TLC examination indicated the formation of two products in 2:3 ratio. Water (1 mL) was added and after standing for 10 min, the solvent was removed under vacuum. The residue was dissolved in EtOAc/H_2O, the aqueous layer

was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to yield **5** (1.1 g, 40%); R_f 0.68 (7:3 EtOAc/hexane); $[\alpha]_D$ +51.2 (*c* 1.92, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.09–7.15 (m, 60H), 5.62–3.00 (m, 39H), 2.20 (m, 1H), 1.44 (m, 1H); HRMS calcd for $C_{112}H_{103}N_5O_{29}$ $[M+H]^+$ 1982.68170, found 1983.68231.

3.3.2. 4-*O*-[{3′-*O*-Benzoyl-2′-benzyloxycarbonylamino-2′-deoxy-α-D-glucopyranosyl}-5-*O*-{3-*O*-(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″,5″-di-*O*-benzoyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (6). The above ester **5** (640 mg, 0.032 mmol) was dissolved in acetic acid (80% in H_2O, 15 mL) and the reaction mixture was stirred at room temperature for 12 h, followed by 55 °C for 6 h for completion of the reaction. The solvent was removed under vacuum and the crude product was dissolved in EtOAc, washed with water, brine, and dried over Na_2SO_4. The solvent was removed under vacuum and the crude solid was purified by silica gel flash chromatography (1:1 EtOAc/hexane) to give diol **6** (450 mg, 70%) as a white solid; R_f 0.52 (7:3 EtOAc/hexane); $[\alpha]_D$ +67.1 (*c* 1.24, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.11–7.09 (m, 55H), 5.60–3.05 (m, 36H), 2.23 (m, 1H), 1.42 (m, 1H); HRMS calcd for $C_{105}H_{99}N_5O_{29}$ $[M+H]^+$ 1894.65040; found 1894.64579.

3.3.3. 4-*O*-[{3′,4′-Di-*O*-benzoyl-2′-benzyloxycarbonylamino-2′,6′-dideoxy-6′-iodo-α-D-glucopyranosyl}-5-*O*-{3-*O*-(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″,5″-di-*O*-benzoyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (7). To a stirred solution of **6** (1.10 g, 0.580 mmol) in dry toluene (30 mL) at 50 °C were added iodine (0.221 g, 0.871 mmol), triphenylphosphine (0.456 g, 1.742 mmol), and imidazole (236.96 mg, 3.484 mmol) successively and the solution was kept at the same temperature for 3 h. The solvent was removed by evaporation under vacuum and the resulting crude product was purified by silica gel flash chromatography using (2:3 EtOAc/hexane) to yield pure iodo derivative (1.0 g, 94%); R_f 0.44 (1:1 EtOAc/hexane); HRMS calcd for $C_{105}H_{98}N_5O_{28}I$ $[M+H]^+$ 2004.55213; found 2004.54789.

A solution containing the above iodo compound (1.0 g, 0.499 mmol) and catalytic *N*,*N*-dimethylamino pyridine (20 mg) in dry pyridine (20 mL) was treated with benzoyl chloride (0.12 mL, 0.998 mmol) at 0 °C. The reaction mixture was stirred for 12 h at room temperature and quenched with water (0.5 mL). The solvent was removed under vacuum and the residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (1:2 EtOAc/hexane) to yield **7** (1.0 g, 95%); R_f 0.30 (2:3 EtOAc/hexane); $[\alpha]_D$ +50.7 (*c* 1.65, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.14–7.19 (m, 60H), 5.69–2.99 (m, 36H), 2.22 (m, 1H), 1.47 (m, 1H); HRMS calcd for $C_{112}H_{102}N_5O_{29}I$ $[M+Na]^+$ 2130.56029; found 2130.55617.

3.3.4. 4-*O*-[{3′,4′-Di-*O*-benzoyl-2′-benzyloxycarbonylamino-2′,6′-dideoxy-6′-bromo-α-D-glucopyranosyl}-5-*O*-{3-*O*-(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″,5″-di-*O*-benzoyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (8). To a stirred solution of **6** (350 mg, 0.184 mmol) in dry DMF (20 mL) was added PPh_3 (155 mg, 0.59 mmol) and the solution was cooled to 0 °C. *N*-Bromosuccinimide (99.3 mg, 0.556 mmol) was added in portions, over a period of 5 min and the resulting pale yellow solution was stirred at 50 °C for three days, excess reagent was destroyed by the addition of methanol (3 mL), and the solvent was removed under vacuum. The dark yellow syrup was dissolved in EtOAc, washed with water, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel flash chromatography (1:2 EtOAc/hexane) to afford pure bromo derivative (253 mg, 70%); R_f 0.42 (1:1 EtOAc/hexane); MS calcd for $C_{105}H_{98}N_5O_{28}Br$ $[M+H]^+$ 1958.6; found 1958.8.

A solution containing the preceding bromo derivative (190 mg, 0.096 mmol) and *N*,*N*-dimethylamino pyridine (11.84 mg, 0.096 mmol) in dry pyridine (4 mL) was treated with benzoyl chloride (0.02 mL, 0.192 mmol) at 0 °C. The reaction mixture was stirred for 12 h at room temperature and quenched with water (0.2 mL). The solvent was removed under vacuum and the residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel flash chromatography (1:2 EtOAc/hexane) to yield **8** (180 mg, 90%); R_f 0.29 (2:3 EtOAc/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 8.12–7.11 (m, 60H), 5.66–3.02 (m, 36H), 2.24 (m, 1H), 1.45 (m, 1H); HRMS calcd for $C_{112}H_{102}N_5O_{29}Br$ $[M+H]^+$ 2060.59221; found 2060.58964.

3.3.5. 4-*O*-[{3′,4′-Di-*O*-benzoyl-2′-benzyloxycarbonylamino-2′-deoxy-5′,6′-didehydro-α-D-xylohexopyranosyl}-5-*O*-{3-*O*-(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″,5″-di-*O*-benzoyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (9). A mixture of **7** (1.0 g, 0.474 mmol) and silver fluoride (421.48 mg, 3.322 mmol) in dry pyridine (20 mL) was stirred in the dark overnight. The mixture was filtered over Celite and washed with EtOAc. Combined filtrates were removed under reduced pressure and the residue was dissolved in EtOAc, washed with water, brine, dried over Na_2SO_4, and concentrated to afford crude product, which was purified by silica gel flash chromatography (1:2 EtOAc/hexane) to afford enol ether **9** (865 mg, 92%); R_f 0.5 (1:1 EtOAc/hexane); $[\alpha]_D$ +38.6 (*c* 2.43, $CHCl_3$); MS calcd for $C_{112}H_{101}N_5O_{29}Na$ $[M+Na]^+$ 2002.6; found 2002.6.

3.3.6. 5-*O*-[3-*O*-(4‴,6‴-*N*,*O*-Benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″,5″-di-*O*-benzoyl-β-D-ribofuranosyl]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (10). *A. From enol ether **9***: The above-obtained enol ether derivative **9** (430 mg, 0.217 mmol) was dissolved in acetone/H_2O (2:1, 10 mL), $HgCl_2$ (58.93 mg, 0.217 mmol) was added and the mixture was refluxed for 6 h. The solvent was removed under vacuum and the crude residue was dissolved

in EtOAc, processed as usual, which upon purification by silica gel flash chromatography (1:2 EtOAc/hexane) afforded pure **10** (265 mg, 81%) as a white solid; $[\alpha]_D$ +42.9 (*c* 1.75, $CHCl_3$); R_f 0.48 (1:1 EtOAc/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 8.12–7.08 (m, 45H), 5.87 (m, 1H) 5.62–3.13 (m, 25H), 2.17 (m, 1H), 1.43 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.3, 167.1, 166.8, 165.4, 161.8, 161.7, 161.1, 160.3, 132.4–126.1 (54C), 111.8, 100.4, 88.5, 81.6, 80.9, 79.3, 78.5, 78.1, 76.6, 71.2, 70.4, 69.7, 68.6, 68.4, 68.1, 67.0, 66.8, 54.5, 52.3, 51.6, 45.2, 35.1; HRMS calcd for $C_{84}H_{78}N_4O_{22}$ $[M+H]^+$ 1495.51860; found 1495.51412.

B. From bromide ***8***: Zinc dust (prewashed with 5% HCl in water, water, Et_2O successively, dried under vacuum, 216 mg, 3.30 mmol), cerium chloride heptahydrate (123 mg, 0.33 mmol), and bromo derivative **8** (170 mg, 0.082 mmol) were suspended in dry MeOH (8 mL). After refluxing for 4 h and cooling for 30 min, the mixture was filtered to remove excess zinc. The residue was rinsed with MeOH and the combined filtrates were evaporated to dryness. The crude product was dissolved in EtOAc and the organic layer was washed with water, dried over Na_2SO_4, and concentrated under vacuum to afford a crude product, which was further purified by silica gel flash chromatography (1:2 EtOAc/hexane) to yield pure **10** (42 mg, 34%) with identical physical properties as described above.

C. From iodide ***7***: To a stirred suspension of zinc (30.84 mg, 0.474 mmol) and ammonium chloride (27.76 mg, 0.474 mmol) in dry MeOH (2 mL), a catalytic amount of vitamin B_{12} (2 mg) was added at room temperature. After 10 min, a solution of iodo compound **8** (56 mg, 0.026 mmol) in a mixture of dry THF (0.2 mL) and dry MeOH (2 mL) was added, and the resulting suspension was stirred for 24 h at room temperature. Undissolved material was filtered off, washed with MeOH, and the combined filtrate was evaporated to dryness and dissolved in EtOAc. The organic layer was washed with cold 1.5 M HCl, water, dried over Na_2SO_4, and concentrated, which was purified by silica gel flash chromatography to obtain **10** (25 mg, 35%) with identical physical properties as described above.

3.4. General procedure for alkylation (11,12)

To a solution of **10** (0.0234 mmol) in dry THF (2 mL) was added a solution of 0.5 M KHMDS in toluene (0.0351 mmol) at 0 °C. After stirring at room temperature for 15 min, a solution of aryl methyl bromide (0.0468 mmol) in dry THF (2 mL) was added and the resulting mixture was stirred for further 2 h, quenched with satd aqueous NH_4Cl solution. The solvent was removed under vacuum and the crude product was dissolved in EtOAc, processed as usual way. Upon purification by silica gel flash chromatography (1:2 EtOAc/hexane), the corresponding aryl ethers **11**,**12** as benzoate esters were obtained.

Compound ***11***: 84%; R_f 0.68 (1:1 EtOAc/hexane); $[\alpha]_D$ +29 (*c* 0.73, $CHCl_3$); MS calcd for $C_{91}H_{82}F_2N_4O_{22}$ $[M]^+$ 1620.5; found 1620.6.

Compound ***12***: 85%; R_f 0.60 (1:1 EtOAc/hexane); $[\alpha]_D$ +36.7 (*c* 0.84, $CHCl_3$); MS calcd for $C_{92}H_{83}F_3N_4O_{22}$ $[M]^+$ 1652.5; found 1652.6.

The individual pseudotrisaccharide derivative **11** or **12** was treated with a catalytic amount of NaOMe in MeOH (1:1, 2 mL, pH 8–9) and stirred at room temperature until the disappearance of starting material. Dry ice was added, solvent was removed under vacuum, and the residue was purified by silica gel flash chromatography (1:19 MeOH/CH_2Cl_2) to afford the corresponding polyol derivatives as white solids.

Polyol from ***11***: 90%; R_f 0.58 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −2.9 (*c* 0.99, MeOH); MS calcd for $C_{63}H_{66}F_2N_4O_{18}$ $[M+Na]^+$ 1227.4; found 1227.7.

Polyol from ***12***: 96%; R_f 0.63 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +0.4 (*c* 0.53, MeOH); MS calcd for $C_{64}H_{67}F_3N_4O_{18}$ $[M+Na]^+$ 1260.4; found 1260.8.

3.5. General procedure for hydrogenolysis (13,14)

To the solution of pseudotrisaccharide in MeOH/H_2O (1:1, 2 mL) was added 20% palladium hydroxide-on-carbon and the suspension was stirred at room temperature under an atmosphere of hydrogen (balloon) until disappearance of starting material as indicated by LC-MS. The mixture was filtered through a layer of Celite, concentrated under vacuum, and the residue was dissolved in AcOH/H_2O (2:1, 0.5 mL) and lyophilized to afford **13** and **14** as fluffy white solids.

Compound ***13***: quant.; $[\alpha]_D$ +13.3 (*c* 1.15, H_2O); ^1H NMR (400 MHz, D_2O) δ 7.30–7.20 (m 1H), 7.19–7.08 (m, 2H), 5.23 (s, 1H), 5.04 (s, 1H), 4.81 (d, *J*=11 Hz, 1H), 4.56 (d, *J*=11 Hz, 1H), 4.33–4.31 (m, 1H), 4.24–4.19 (m, 1H), 4.10–3.90 (m, 3H), 3.65–3.58 (m, 2H), 3.50–3.40 (m, 3H), 3.38–3.30 (m, 2H), 3.22–3.15 (m, 2H), 3.09–2.95 (m, 2H), 2.20–2.16 (m, 1H), 1.74 (s, 12H), 1.50–1.35 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 153.0, 149.3, 134.7, 125.4, 117.8, 117.5, 108.6, 96.4, 82.1, 81.9, 80.9, 76.9, 73.7, 73.5, 72.9, 70.5, 68.3, 67.7, 61.4, 51.3, 50.5, 49.2, 40.7, 30.6, 23.5; HRMS calcd for $C_{24}H_{38}F_2N_4O_{10}$ $[M+H]^+$ 581.26342; found 581.26230.

Compound ***14***: quant.; $[\alpha]_D$ +12.6 (*c* 0.7, H_2O); ^1H NMR (400 MHz, D_2O) δ 7.61 (d, *J*=7.7 Hz, 2H), 7.47 (d, *J*=7.7 Hz, 2H), 5.24 (s, 1H), 5.02 (s, 1H), 4.93 (d, *J*=11.9 Hz, 1H), 4.38 (d, *J*=11.9 Hz, 1H), 4.27–4.20 (m, 1H), 4.19–4.08 (m, 2H), 4.04–3.94 (m, 1H), 3.69–3.60 (m, 2H), 3.50–3.06 (m, 10H), 2.21–2.17 (m, 1H), 1.74 (s, 12H), 1.51–1.40 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 182.2, 142.2, 130.3, 130.0, 128.8 (2C), 126.2 (2C), 108.8, 96.9, 82.4, 82.2, 81.3, 77.7, 74.1, 74.0, 73.7, 70.8, 68.7, 68.2, 61.9, 51.7, 50.9, 49.6, 41.1, 30.8, 23.9; HRMS calcd for $C_{25}H_{39}F_3N_4O_{10}$ $[M+H]^+$ 613.2695; found 613.26995.

3.5.1. 4-Keto pseudotrisaccharide (15). A solution of **10** (130 mg, 0.086 mmol) in dry CH_2Cl_2 (4 mL) was added to a stirring solution of Dess–Martin periodinane (184.47 mg, 0.434 mmol) in dry CH_2Cl_2 (6 mL) at room temperature and the mixture was stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and quenched by the addition of a mixture of $Na_2S_2O_3$/$NaHCO_3$ (0.5 mL satd aqueous solution of $Na_2S_2O_3$ and 2 mL satd aqueous solution of $NaHCO_3$) and allowed to stir further for 15 min. The

reaction mixture was partitioned between CH_2Cl_2/H_2O, the organic layer was washed with water, brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel flash chromatography (1:2 EtOAc/hexane) to afford ketone **15** (117 mg, 90%) as a white solid; $[\alpha]_D$ +29.2 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.14–7.11 (m, 45H), 5.94 (m, 1H), 5.82–3.07 (m, 24H), 2.39 (m, 1H), 1.62 (m, 1H); HRMS calcd for $C_{84}H_{76}N_4O_{22}$ $[M+Na]^+$ 1515.48489; found 1515.48547.

3.6. General procedure for compounds (16,17)

To a solution of **15** (0.020 mmol) in dry THF (2 mL) was added a solution of 2 M PhMgCl (0.2 mmol, 0.2 mL) or 0.5 M *p*-MeOPhMgCl (0.2 mmol, 0.4 mL) in dry THF (2 mL) at −78 °C. The resulting mixture from each reaction was stirred for 1 h at −78 °C, quenched with satd aqueous NH_4Cl solution. The solvent was removed under vacuum and the crude product was dissolved in EtOAc, processed in a usual way and purification by silica gel flash chromatography (1:2 EtOAc/hexane) provided compounds **16** and **17**.

Compound ***16***: 85%; R_f 0.51 (1:1 EtOAc/hexane); $[\alpha]_D$ +28.2 (*c* 1.2, $CHCl_3$); MS calcd for $C_{90}H_{82}N_4O_{22}$ $[M+H]^+$ 1571.5; found 1571.9.

Compound ***17***: 80%; R_f 0.48 (1:1 EtOAc/hexane); $[\alpha]_D$ +30.1 (*c* 1.12, $CHCl_3$); MS calcd for $C_{91}H_{84}N_4O_{23}$ $[M+H]^+$ 1601.6; found 1601.9.

Compounds **16** and **17** in MeOH were debenzoylated in NaOMe and MeOH as previously described to give white solids.

Polyol from ***16***: 89%; R_f 0.47 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −12.4 (*c* 0.78, MeOH); MS calcd for $C_{62}H_{66}N_4O_{18}$ $[M+Na]^+$ 1177.4; found 1177.7.

Polyol from ***17***: 98%; R_f 0.45 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −10.4 (*c* 0.73, MeOH); MS calcd for $C_{63}H_{68}N_4O_{19}$ $[M+Na]^+$ 1185.4; found 1185.7.

Above polyol compounds were hydrogenolyzed as previously described to afford **18** and **19** as fluffy white solids.

Compound ***18***: quant.; $[\alpha]_D$ −8.3 (*c* 0.46, H_2O); ^1H NMR (400 MHz, D_2O) δ 7.46 (m, 1H), 7.43 (d, *J*=7.1 Hz, 2H), 7.38 (d, *J*=7.1 Hz, 2H), 5.08 (s, 1H), 5.05 (s, 1H), 4.18–4.15 (m, 2H), 4.08–3.94 (m, 3H), 3.76–3.62 (m, 4H), 3.39–3.29 (m, 2H), 3.27–3.24 (m, 2H), 2.90–2.87 (m, 1H), 2.58–2.55 (m, 1H), 2.21–2.18 (m, 1H), 2.08–2.05 (m, 1H), 1.81 (s, 15H); ^{13}C NMR (100 MHz, D_2O) δ 182.2, 140.2, 130.1, 129.7, 129.5, 128.5, 128, 126, 96.3, 82.7, 81.8, 78.1, 77.8, 74, 72.6, 70.7, 68.5, 68.2, 62, 54.5, 51.6, 50.8, 41.1, 29.3, 23.9; HRMS calcd for $C_{23}H_{38}N_4O_{10}$ $[M+H]^+$ 531.26662; found 531.26633.

Compound ***19***: quant.; $[\alpha]_D$ −6.7 (*c* 0.45, H_2O); ^1H NMR (400 MHz, D_2O) δ 7.40–7.20 (m, 2H), 7.0–6.95 (br s, 2H), 5.0 (s, 2H), 4.18–4.0 (m, 4H), 3.95–3.80 (m, 2H), 3.70 (s, 3H), 3.69–3.55 (m, 3H), 3.36–3.15 (m, 4H), 2.95–2.81 (m, 1H), 2.62–2.56 (m, 1H), 2.28–2.16 (m, 1H), 2.09–1.97 (m, 1H), 1.76 (s, 15H); ^{13}C NMR (100 MHz, D_2O) δ 181.7, 159.4, 132.0, 130.2, 129.6, 128.4, 126.8, 108.3, 95.9, 82.4, 81.5, 78.4, 77.7, 73.8, 73.2, 70.2, 68.8, 67.7, 61.5, 55.7, 54.2, 51.8, 50.3, 40.9, 28.8, 24.1; HRMS calcd for $C_{24}H_{40}N_4O_{11}$ $[M+H]^+$ 561.27718; found 561.27860.

3.6.1. 4-*O*-[(2-Oxo-ethyl)-5-*O*-{3-*O*-(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2‴,4‴-di-*O*-benzoyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (20). Compound **10** (210 mg, 0.140 mmol) was co-distilled with dry toluene (twice) and dissolved in dry THF (10 mL). The flask was cooled at 0 °C and allyl iodide (129 μL, 1.40 mmol) was added. A solution of 0.5 M KHMDS solution in toluene (337 μL, 0.168 mmol) was added dropwise, and the mixture was stirred for 3 h at room temperature by careful monitoring on TLC. The reaction mixture was quenched with an aqueous solution of NH_4Cl (satd, 0.2 mL) and the solvent was evaporated to dryness. The crude product was dissolved in EtOAc, processed as usual, and purified by silica gel flash chromatography (1:2 EtOAc/hexane) to give the corresponding allyl ether (160 mg, 74%); R_f 0.55 (1:1 EtOAc/hexane); $[\alpha]_D$ +22.6 (*c* 1.63, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.06–7.13 (m, 45H), 6.24–3.13 (m, 31H), 2.14 (m, 1H), 1.43 (m, 1H); HRMS calcd for $C_{87}H_{82}N_4O_{22}$ $[M+H]^+$ 1534.54207; found 1534.54374.

The allyl ether derivative (153 mg, 0.099 mmol) in CH_2Cl_2 (6 mL) was cooled at −78 °C and ozone was bubbled for 2 h after which argon was bubbled through. The mixture was treated with PPh_3 (78.4 mg, 0.30 mmol), warmed to the room temperature, solvent was removed under vacuum, and the crude aldehyde was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to give the aldehyde **20** (129 mg, 84%); R_f 0.39 (1:1 EtOAc/hexane); $[\alpha]_D$ +23.9 (*c* 1.61, $CHCl_3$).

3.7. General procedure for reductive amination (synthesis of 26–30)

To a mixture of **20** (0.024 mmol) and the appropriate amine (0.096 mmol) in dry 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 the disappearance of **20**. The reaction mixture was 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 solvents, the residue was purified by flash chromatography (1:2 EtOAc/hexane for *p*-anisidine, 5-aminoquinoline, 2-methoxy-5-aminopyridine, 3-aminopyrimidine, and hydroxyethyl derivative, respectively, and 1:19 MeOH/CH_2Cl_2 for the dimethylaminoethyl derivative) to give **21**–**25** as white solids.

Compound ***21***: 84%; R_f 0.5 (1:1 EtOAc/hexane); $[\alpha]_D$ +39.3 (*c* 0.96, $CHCl_3$); MS calcd for $C_{93}H_{89}N_5O_{23}$ $[M+H]^+$ 1644.6; found 1644.4.

Compound ***22***: 84%; R_f 0.34 (1:1 EtOAc/hexane); $[\alpha]_D$ +37.3 (*c* 1.25, $CHCl_3$); MS calcd for $C_{92}H_{88}N_6O_{23}$ $[M+H]^+$ 1645.7; found 1645.7.

Compound ***23***: 49%; R_f 0.20 (1:1 EtOAc/hexane); $[\alpha]_D$ +24.6 (*c* 0.7, $CHCl_3$); MS calcd for $C_{90}H_{85}N_7O_{22}$ $[M+H]^+$ 1616.6; found 1616.6.

Compound ***24***: 82%; R_f 0.34 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +26.2 (*c* 1.57, $CHCl_3$); MS calcd for $C_{88}H_{87}N_5O_{22}$ $[M+H]^+$ 1566.6; found 1566.4.

Compound ***25***: 42%; R_f 0.31 (1:1 EtOAc/hexane); $[\alpha]_D$ +32.3 (*c* 0.65, $CHCl_3$); MS calcd for $C_{86}H_{82}N_4O_{23}$ $[M+H]^+$ 1539.5; found 1539.5.

The individual pseudotrisaccharide derivatives (0.012 mmol) **21**–**25** were treated with a catalytic amount of NaOMe in MeOH (1:1, 3 mL, pH 8–9) and the mixture was stirred at room temperature until the disappearance of starting material. Dry ice was added, solvent was removed under vacuum, and purified by silica gel flash chromatography (1:19 MeOH/CH_2Cl_2 for *p*-anisidine, 5-aminoquinoline 2-methoxy-5-aminopyridine, 3-aminopyrimidine, and hydroxyethyl derivative, respectively, and 1:9 MeOH/CH_2Cl_2 for dimethylaminoethyl derivative) to afford the corresponding white solids.

From ***21***: 88%; R_f 0.44 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +2.2 (*c* 1.2, MeOH); MS calcd for $C_{65}H_{73}N_5O_{19}$ $[M+H]^+$ 1228.5; found 1228.3.

From ***22***: 86%; R_f 0.41 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +0.7 (*c* 0.7, MeOH); MS calcd for $C_{64}H_{72}N_6O_{19}$ $[M+H]^+$ 1229.3; found 1229.5.

From ***23***: 96%; R_f 0.39 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +0.6 (*c* 0.5, MeOH); MS calcd for $C_{62}H_{69}N_7O_{18}$ $[M+H]^+$ 1200.3; found 1200.5.

From ***24***: 80%; R_f 0.08 (6:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +2.6 (*c* 0.7, MeOH); MS calcd for $C_{60}H_{71}N_5O_{18}$ $[M+H]^+$ 1151.5; found 1151.3.

From ***25***: 95%; R_f 0.52 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +0.7 (*c* 0.5, MeOH); MS calcd for $C_{58}H_{66}N_4O_{19}$ $[M+Na]^+$ 1145.4; found 1145.4.

The above compounds were hydrogenolyzed according to the procedure previously described to afford **26**–**30** as fluffy white solids in quantitative yields.

Compound ***26***: quant.; $[\alpha]_D$ +15.2 (*c* 0.29, H_2O); ^1H NMR (400 MHz, D_2O) δ 6.79 (d, J=8.78 Hz, 2H), 6.72 (d, J=8.78 Hz, 2H), 5.20 (s, 1H), 5.09 (s, 1H), 4.35–4.00 (m, 6H), 3.71–3.64 (m, 2H), 3.62 (s, 3H), 3.59–3.51 (m, 2H), 3.45–3.14 (m, 10H), 2.20–2.12 (m, 1H), 1.70 (s, 15H), 1.52–1.4 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 151, 148.5, 117.1 (2C), 115.5 (2C), 109.1, 96.1, 85, 82.4, 81.8, 76.5, 73.5, 71.0, 70.5, 68.2, 67.6, 61.1, 56.1, 51.2, 50.3, 49.5, 45.4, 44.3, 40.7, 30, 23.5; HRMS calcd for $C_{26}H_{45}N_5O_{11}$ $[M+H]^+$ 604.31938; found 604.32007.

Compound ***27***: quant.; $[\alpha]_D$ +9.6 (*c* 0.5, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.18 (s, 1H), 5.08 (s, 1H), 4.40–4.36 (m, 1H), 4.31–4.25 (m, 1H), 4.15–4.06 (m, 1H), 4.05–3.81 (m, 3H), 3.78–3.58 (m, 5H), 3.52–3.43 (m, 2H), 3.39–3.20 (m, 6H), 3.19–3.10 (m, 2H), 3.0–2.90 (m, 4H), 2.32–2.30 (m, 2H), 2.20–2.10 (m, 1H), 1.78 (s, 15H), 1.45–1.39 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 175.0, 114.4, 96.7, 84.4, 83.5, 82.3, 81.8, 76.4, 74.0, 73.4, 70.7, 69.9 (2C), 68.7, 68.1, 61.6, 51.5, 50.5, 49.6, 46.0, 44.6, 40.8, 31.7, 27.8, 23.5; HRMS calcd for $C_{24}H_{46}N_6O_{11}$ $[M+H]^+$ 595.33028; found 595.33156.

Compound ***28***: quant.; $[\alpha]_D$ +7.4 (*c* 0.42, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.13 (s, 1H), 5.10 (s, 1H), 4.45–4.40 (m, 1H), 4.35–4.20 (m, 1H), 4.19–4.15 (m, 1H), 4.13–3.89 (m, 5H), 3.75–3.58 (m, 7H), 3.46–3.39 (m, 9H), 2.35–2.23 (m, 1H), 2.19–2.15 (m, 2H), 1.84 (s, 18H), 1.65–1.58 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 179.5, 153.5, 109.8, 95.7, 82.6, 81.9, 79.9, 76.3, 73.0, 72.0, 71.0, 70.5, 67.9, 67.5, 61.5, 51.1, 50.2, 49.1 (2C), 40.7, 38.6, 38.3, 28.3, 22.2, 19.5; HRMS calcd for $C_{23}H_{45}N_7O_{10}$ $[M+H]^+$ 579.32279; found 580.33046.

Compound ***29***: quant.; $[\alpha]_D$ +13.1 (*c* 0.62, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.17 (s, 1H), 5.14 (s, 1H), 4.40 (s, 2H), 4.36 (s, 1H), 4.24 (s, 1H), 4.16–4.04 (m, 2H), 3.78–3.43 (m, 8H), 3.27–3.14 (m, 6H), 2.78 (s, 6H), 2.27–2.24 (m, 1H), 1.76 (s, 15H), 1.66–1.60 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.9, 110.2, 96.3, 84.4, 82.3, 80.9, 76.9, 74.0, 73.0, 70.9, 68.4, 68.0, 66.8, 62.0, 58.0, 51.6, 50.6, 49.6, 43.8 (2C), 41.1, 29.4, 23.7; HRMS calcd for $C_{21}H_{43}N_5O_{10}$ $[M+H]^+$ 526.30882; found 526.30800.

Compound ***30***: quant.; $[\alpha]_D$ +4.4 (*c* 0.55, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.23 (s, 1H), 5.16 (s, 1H), 4.39–4.35 (m, 1H), 4.23–4.20 (m, 1H), 4.19–4.16 (m, 1H), 4.15–3.95 (m, 3H), 3.68–3.46 (m, 8H), 3.42–3.38 (m, 2H), 3.31–3.12 (m, 4H), 2.35–2.21 (m, 1H), 1.78 (s, 12H), 1.70–1.60 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.5, 108.8, 95.9, 82.0, 81.9, 79.9, 76.7, 73.6, 73.5, 72.6, 70.5, 68.0, 67.5, 61.3, 61.1, 51.1, 50.1, 49.4, 40.7, 28.4, 23.3; HRMS calcd for $C_{19}H_{38}N_4O_{11}$ $[M+H]^+$ 499.26153; found 499.26177.

3.7.1. 2-Deoxy-1,3,2‴,6‴-tetraazido-4-*O*-methylthiolmethyleneparomomycin orthoester (33). Substituted derivative **31**[30c] (137 mg, 0.3 mmol) and thiolglycosyl donor **32**[32] (191 mg, 0.30 mmol) were dissolved in anhydrous CH_2Cl_2 (15 mL) under nitrogen in the presence of 3 Å molecular sieves (~100 mg). The suspension was stirred at −40 °C for 10 min, before *N*-iodosuccinamide (202 mg, 0.9 mmol) was added in one portion. In another flask, AgOTf (78 mg, 0.3 mmol) was dissolved in anhydrous toluene (1.5 mL) in the presence of small amount of 3 Å molecular sieves. The suspension of AgOTf was then added to the suspension of compounds **31** and **32** dropwise at −40 °C. After complete addition, the reaction temperature was raised between −15 and 0 °C for 30 min. To the suspension was added Et_3N (8 mL) and stirred for 30 min before filtration and evaporation. The crude material was purified by silica gel chromatography using CH_2Cl_2/EtOAc (85:15) as the eluant to give 170 mg (59% yield) of the acetylated **33**. The acetylated intermediate was then dissolved in 7 N NH_3 in MeOH in a pressure tube and kept at room temperature for 16 h. Removal of the solvent gave the pure **33** (134 mg, 88%): ^1H NMR ($CDCl_3$) δ 1.10–1.40 (m, 1H), 1.83 (s, 3H), 2.07–2.21 (m, 1H), 3.21–3.47 (m, 4H), 3.47–3.67 (m, 2H), 3.70–3.88 (m, 4H), 3.93–4.19 (m, 4H), 4.43 (dd, J=5.2, 8.3 Hz, 1H), 4.99 (t, J=4.5 Hz, 1H), 5.21 (s, 1H), 5.63 (dd, J=11.8, 18.8 Hz, 2H), 6.06 (d, J=4.0 Hz, 1H), 7.73 (dd, J=1.6, 8.8 Hz, 1H), 7.83 (d, J=4.9 Hz, 1H), 8.24 (d, J=8.7 Hz, 1H), 8.37 (s, 1H), 8.80 (d, J=4.8 Hz,

1H); ^{13}C NMR ($CDCl_3$) δ 24.8, 29.6, 32.5, 51.4, 58.7, 59.2, 60.6, 60.9, 64.5, 69.0, 69.2, 73.1, 74.7, 74.8, 78.5, 79.2, 81.3, 98.0, 104.2, 119.3, 122.0 (q, J=11.7 Hz), 125.2, 125.3, 125.4 (q, J=25.8 Hz), 127.4 (q, J=4.2 Hz), 128.2, 131.6 (q, J=27.0 Hz), 146.3, 147.3, 150.6; MS calcd for $C_{30}H_{34}F_3N_{13}O_{11}S$ $[M+H]^+$ 841.2; found 842.0.

3.8. Procedure for glycosylation and subsequent deacetylation (35a,b)

p-Methoxybenzyl (PMB) protected derivative **34** (137 mg, 0.41 mmol) and thiolglycosyl donor **32** (260 mg, 0.41 mmol) were dissolved in anhydrous CH_2Cl_2 (12 mL) under nitrogen in the presence of 3 Å molecular sieves (~100 mg). The suspension was stirred at −40 °C for 10 min, before *N*-iodosuccinamide (138 mg, 0.61 mmol) was added in one portion. In another flask, AgOTf (51 mg, 0.2 mmol) was dissolved in anhydrous toluene (1 mL) in the presence of small amount of 3 Å molecular sieves. The suspension of AgOTf was then added to the suspension of compounds **34** and **32** dropwise at −40 °C. After complete addition, the reaction temperature was raised to −25 °C for 30 min. To the suspension was added Et_3N (8 mL) and stirred for 30 min before filtration and evaporation. The crude material was purified by silica gel chromatography using CH_2Cl_2/EtOAc (9:1) as the eluant to give 308 mg (89% yield) of the desired compounds **35** as a 1:1 mixture of regioisomers, which were characterized as per acetylated protected product (see below).

3.9. Preparation of the ring II–IV fragment (37 and 38)

The regioisomers of compounds **35** and catalytic amount of 4-*N*,*N*-dimethylamino pyridine (DMAP) were then dissolved in dry pyridine (4 mL) under N_2. At 0 °C, Ac_2O (0.4 mL) was added dropwise. The solution was stirred at room temperature for 16 h. After removal of the solvent, the crude material was purified by silica gel chromatography using CH_2Cl_2/EtOAc (9:1) as the eluant to give 308 mg (95% yield) of the desired per acetylated compounds as a yellow oil. MS calcd for $C_{35}H_{44}N_{12}O_{16}$ $[M+Na]^+$ 911.3; found 911.0.

A solution of the above per acetylated compounds (1.24 g, 1.4 mmol) in CH_2Cl_2 (25 mL) and H_2O (1.3 mL) was added with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (477 mg, 2.1 mmol, DDQ) at room temperature. The resulting emulsion was stirred for 1 h before another addition of DDQ (477 mg, 2.1 mmol) together with 6 mL CH_2Cl_2 and 0.3 mL H_2O. At 2.5 h, more DDQ (239 mg, 1.0 mmol), 6 mL CH_2Cl_2, and 0.3 mL H_2O were added. At 3.5 h, more DDQ (239 mg, 1.0 mmol) was added. At 4 h, satd $Na_2S_2O_3$ (200 mL) was added to the mixture, followed by 200 mL CH_2Cl_2. The suspension was stirred for 15 min before the filtration and separation. The aqueous layer was further extracted by CH_2Cl_2 (200 mL×2). Combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The crude material was purified by silica gel chromatography using a gradient of CH_2Cl_2/EtOAc (9:1 to 3:1) as an eluant to give 500 mg of **37** as a colorless oil, and 540 mg of **38** as a colorless oil.

Compound ***37***: ^{1}H NMR ($CDCl_3$) δ 1.42 (dd, J=12.3, 25.3 Hz, 1H), 2.12 (s, 3H), 2.14 (s, 3H), 2.16 (s, 3H), 2.18 (s, 6H), 2.18–2.23 (m, 1H), 3.22–3.49 (m, 6H), 3.52–3.62 (m, 1H), 4.09–4.16 (m, 1H), 4.33–4.36 (m, 2H), 4.42–4.48 (m, 1H), 4.51–4.62 (m, 1H), 4.72 (t, J=1.9 Hz, 1H), 4.89–4.95 (m, 2H), 4.98–5.12 (m, 3H); DEPT135 NMR ($CDCl_3$) δ 20.6 (2C), 20.7 (2C), 20.8, 31.9, 50.6, 56.5, 58.3, 59.3, 62.3, 65.7, 68.6, 73.3, 74.3, 74.5, 75.0, 75.1, 79.3, 84.7, 98.7, 107.0; MS calcd for $C_{27}H_{36}N_{12}O_{15}$ $[M+Na]^+$ 791.2; found 791.0.

Compound ***38***: ^{1}H NMR ($CDCl_3$) δ 1.42 (dd, J=12.6, 25.4 Hz, 1H), 2.10 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.16 (s, 3H), 2.17 (s, 3H), 2.27 (dt, J=3.7, 13.2 Hz, 1H), 3.18–3.50 (m, 5H), 3.50–3.74 (m, 2H), 4.06–4.11 (m, 1H), 4.28–4.37 (m, 2H), 4.44–4.50 (m, 2H), 4.71 (br s, 1H), 4.85–4.97 (m, 2H), 5.01–5.04 (m, 2H), 5.30 (s, 1H); DEPT135 NMR ($CDCl_3$) δ 20.6, 20.7, 20.8 (2C), 20.9, 31.9, 50.5, 56.5, 59.2, 60.6, 64.8, 65.7, 68.7, 73.4, 74.4, 75.0, 75.8, 76.1, 79.0, 79.2, 98.9, 105.6; MS calcd for $C_{27}H_{36}N_{12}O_{15}$ $[M+Na]^+$ 791.2; found 791.1.

3.10. Typical procedure for deprotection (39 and 40)

To a solution of compounds **35** (192 mg, 0.23 mmol) in THF (2 mL) and *i*-PrOH (2 mL) was added a solution of LiOH (44 mg, 1.84 mmol) in H_2O (1 mL) at 0 °C. The suspension was then stirred at 0 °C for 1 h and room temperature for 3 h. To the mixture, satd NH_4Cl (30 mL) and CH_2Cl_2 (30 mL) were added. After separation, the aqueous layer was extracted further by CH_2Cl_2 (30 mL×2). Combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The crude oil was purified by silica gel chromatography (CH_2Cl_2/MeOH, 95:5) to give 107 mg (69%) of the deacetylated intermediate, which was then dissolved in EtOH (20 mL). Hydrazine (117 μL, 1.92 mmol) was then added, followed by a tip of spatula of Raney Ni. The suspension was then stirred at room temperature under N_2 for 30 min. Filtration and evaporation gave the mixture of compounds **39** and **40** (88.3 mg, 97%). Preparative LC-MS was applied to separate compounds **39** and **40** as the individual acetate salts.

Compound **39** (white solid, 22 mg); ^{1}H NMR (MeOD-d_3) δ 1.43 (dd, J=12.4, 24.8 Hz, 1H), 2.05–2.22 (m, 1H), 2.84–3.02 (m, 2H), 3.02–3.16 (m, 1H), 3.22–3.45 (m, 4H), 3.46–3.67 (m, 4H), 3.69 (s, 3H), 3.92–4.20 (m, 3H), 4.25 (d, J=4.0 Hz, 1H), 4.38–4.67 (m, 2H), 4.77–4.90 (m, 1H), 5.07 (s, 1H), 5.27 (s, 1H), 6.81 (d, J=8.6 Hz, 2H), 7.29 (d, J=8.6 Hz, 2H); ^{13}C NMR (MeOD-d_3) δ 30.2, 38.4, 39.7, 48.7, 50.1, 51.1, 53.6, 60.3, 67.3, 67.6, 70.3, 72.4, 72.7, 72.9, 76.0, 80.6, 81.0, 95.8, 107.6, 112.7, 129.0, 129.5, 158.9; MS calcd for $C_{25}H_{42}N_4O_{11}$ $[M+H]^+$ 575.3; found 575.3.

Compound **40** (white solid, 17.8 mg); ^{1}H NMR (MeOD-d_3) δ 1.44 (dd, J=12.4, 24.7 Hz, 1H), 2.03–2.20 (m, 1H), 2.75–3.00 (m, 2H), 3.04–3.20 (m, 4H), 3.35 (dd, J=9.2, 18.8 Hz, 1H), 3.40 (dd, J=6.9, 15.8 Hz, 1H), 3.55 (s, 1H), 3.69 (s, 3H), 3.76 (s, 2H), 3.95–4.15 (m, 3H), 4.21 (d, J=4.3 Hz, 1H), 4.51–4.63 (m, 2H), 4.89 (d, J=10.7 Hz, 1H), 5.05 (s, 1H), 5.16 (s, 1H), 6.80 (d, J=8.6 Hz, 2H), 7.25 (d, J=8.6 Hz, 2H); ^{13}C NMR (MeOD-d_3) δ 32.6, 41.9, 50.5, 50.9, 53.4, 55.7, 59.8, 69.8, 69.9, 72.4, 74.7, 75.8, 76.5, 77.7, 82.5, 83.4, 83.9, 97.9, 110.3, 114.8,

131.0, 131.8, 161.0; MS calcd for $C_{25}H_{42}N_4O_{11}$ $[M+H]^+$ 575.3; found 575.3.

3.11. General procedure for Pummerer reaction

Compounds **37** and **38** (0.13 mmol) were individually dissolved in anhydrous DMSO (0.35 mL) under nitrogen. At 0 °C, AcOH (1.0 mL) and Ac_2O (0.33 mL) were added in sequence. The mixture was maintained at room temperature for two days. To the mixture were added satd $NaHCO_3$ (15 mL) and additional $NaHCO_3$ till there was no gas released and the mixture was extracted by CH_2Cl_2 (15 mL×3). Combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The crude material was purified by silica gel chromatography using CH_2Cl_2/EtOAc (9:1).

*Compound **41***: from **37**, 108 mg (98% yield) brown oil; ^{1}H NMR ($CDCl_3$) δ 1.48 (dd, J=12.6, 25.7 Hz, 1H), 2.11 (s, 3H), 2.12 (s, 3H), 2.16 (s, 6H), 2.17 (s, 3H), 2.26 (s, 3H), 2.21–2.35 (m, 1H), 3.22–3.47 (m, 4H), 3.47–3.67 (m, 2H), 3.74 (t, J=9.4 Hz, 1H), 4.00–4.10 (m, 1H), 4.27–4.34 (m, 2H), 4.36–4.42 (m, 2H), 4.70 (t, J=1.9 Hz, 1H), 4.87–4.98 (m, 5H), 5.03 (t, J=2.8 Hz, 1H), 5.23 (d, J=2.8 Hz, 1H); DEPT135 NMR ($CDCl_3$) δ 14.8, 20.5, 20.6, 20.7, 20.8 (2C), 32.0, 50.6, 56.6, 58.3, 59.6, 63.9, 65.6, 68.6, 73.3, 74.8, 75.2, 76.2, 77.4, 79.4, 79.6, 80.8, 99.3, 106.4; MS calcd for $C_{29}H_{40}N_{12}O_{15}S$ $[M+Na]^+$ calcd 851.2; found 851.0.

*Compound **42***: from **38**, 122 mg (quant.) colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.44 (dd, J=12.6, 25.3 Hz, 1H), 2.10 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.16 (s, 3H), 2.17 (s, 3H), 2.19 (s, 3H), 2.29 (dt, J=4.5, 14.0 Hz, 1H), 3.19–3.52 (m, 5H), 3.52–3.71 (m, 2H), 4.02–4.20 (m, 1H), 4.23–4.39 (m, 2H), 4.39–4.54 (m, 2H), 4.70 (s, 1H), 4.81 (dd, J=11.7, 22.4 Hz, 2H), 4.90 (d, J=1.7 Hz, 1H), 4.95–5.10 (m, 3H), 5.20 (d, J=1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.7, 20.5, 20.7, 20.8 (2C), 21.1, 32.3, 50.5, 56.5, 59.0, 60.1, 64.7, 65.7, 68.7, 73.4, 74.4, 74.5, 76.1, 77.2, 79.2, 79.2, 79.6, 79.9, 98.9, 105.7, 168.6, 169.6, 169.8, 170.0, 170.7; MS calcd for $C_{29}H_{40}N_{12}O_{15}S$ $[M+Na]^+$ calcd 851.2; found 851.0.

3.12. Typical procedure for coupling to heterocycle (45a–d and 46a–d)

To the solution of intermediate **41** or **42** in anhydrous CH_2Cl_2 (0.08 M) under nitrogen was added sulfuryl chloride (SO_2Cl_2, 1.04 equiv to **41** or **42**) in anhydrous CH_2Cl_2 (0.16 M) slowly. The solution was stirred at room temperature for 30 min before evaporation to a foamy solid (**43** or **44**), which was then dissolved in anhydrous acetonitrile (0.4 M). In another flask, 7-trifluoromethyl-4-quinolinethiol or other heterocycles in Scheme 6 (1.02 equiv to **41** or **42**) and NaH (1.02 equiv to **41** or **42**) were suspended in anhydrous acetonitrile (0.04 M) under nitrogen. This mixture was stirred at room temperature for 15 min before the solution of chloromethyl substituted intermediate **43** or **44** in acetonitrile was added in one portion. The mixture was stirred at room temperature for 2.5 h. To the mixture was added satd NH_4Cl and extracted with CH_2Cl_2 three times. The organic layers were dried (Na_2SO_4), filtered, and evaporated. The crude material was purified by silica gel on column using a mixture of CH_2Cl_2/EtOAc (4:1).

*Compound **45a***: yellow oil, 91%; ^{1}H NMR ($CDCl_3$) δ 1.48 (dd, J=12.2, 25.2 Hz, 1H), 2.06 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 2.18 (s, 6H), 2.27 (dt, J=4.1, 13.6 Hz, 1H), 3.23–3.49 (m, 4H), 3.52–3.63 (m, 2H), 3.77 (t, J=9.4 Hz, 1H), 4.01–4.18 (m, 1H), 4.22–4.45 (m, 4H), 4.71 (s, 1H), 4.90–5.05 (m, 4H), 5.42 (d, J=12.2 Hz, 1H), 5.70 (d, J=12.1 Hz, 1H), 7.65–7.77 (m, 2H), 8.24 (d, J=8.8 Hz, 1H), 8.38 (s, 1H), 8.84 (d, J=4.8 Hz, 1H); DEPT135 NMR ($CDCl_3$) δ 20.5, 20.7, 20.8 (3C), 32.0, 50.7, 56.6, 58.1, 59.5, 63.8, 65.7, 68.7, 73.6, 73.8, 74.7, 75.0, 76.0, 79.7, 80.5, 81.8, 99.3, 107.0, 119.1, 122.1, 125.1, 127.8, 150.8.

*Compound **46a***: yellow oil, 82%; ^{1}H NMR ($CDCl_3$) δ 1.46–1.58 (m, 1H), 2.03 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 2.16 (s, 3H), 2.27–2.48 (m, 1H), 3.08–3.31 (m, 2H), 3.34–3.42 (m, 1H), 3.51–3.65 (m, 4H), 4.04–4.11 (m, 1H), 4.28–4.33 (m, 2H), 4.41–4.47 (m, 2H), 4.69 (s, 1H), 4.85 (d, J=1.6 Hz, 1H), 4.91 (d, J=3.3 Hz, 1H), 4.97–5.03 (m, 2H), 5.11 (s, 1H), 5.42 (d, J=12.6 Hz, 1H), 5.57 (d, J=12.6 Hz, 1H), 7.59 (d, J=4.8 Hz, 1H), 7.75 (d, J=9.1 Hz, 1H), 8.18 (d, J=8.6 Hz, 1H), 8.39 (s, 1H), 8.84 (d, J=4.8 Hz, 1H).

*Compound **45b***: white solid, 71%; ^{1}H NMR ($CDCl_3$) δ 1.45 (dd, J=12.7, 25.7 Hz, 1H), 2.10 (s, 3H), 2.12 (s, 3H), 2.16 (s, 6H), 2.17 (s, 3H), 2.26–2.34 (m, 2H), 3.38–3.50 (m, 2H), 3.59 (dd, J=8.3, 13.0 Hz, 1H), 3.66–3.86 (m, 2H), 4.06–4.10 (m, 1H), 4.29–4.47 (m, 4H), 4.70 (t, J=1.9 Hz, 1H), 4.83–4.97 (m, 3H), 5.03 (t, J=2.8 Hz, 1H), 5.24 (d, J=2.4 Hz 1H), 5.64 (d, J=11.6 Hz, 1H), 5.81 (d, J=11.6 Hz, 1H), 7.43 (t, J=8.2 Hz, 1H), 7.62 (d, J=7.6 Hz, 1H), 7.74 (t, J=6.9 Hz, 1H), 8.22 (d, J=7.9 Hz, 1H); DEPT135 NMR ($CDCl_3$) δ 20.5, 20.7, 20.8, 20.9, 21.0, 31.8, 50.7, 56.6, 58.2, 59.2, 63.8, 65.7, 68.7, 73.3, 73.7, 74.6, 75.1, 75.9, 79.5, 80.3, 81.3, 99.2, 106.6, 126.4, 126.5, 126.7, 134.9.

*Compound **46b***: white solid, 70%; ^{1}H NMR ($CDCl_3$) δ 1.27 (dd, J=12.5, 25.6 Hz, 1H), 2.05 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 2.25–2.39 (m, 1H), 3.20–3.43 (m, 3H), 3.49–3.65 (m, 3H), 3.82 (t, J=9.7 Hz, 1H), 4.02–4.10 (m, 1H), 4.26–4.34 (m, 2H), 4.42–4.48 (m, 2H), 4.70 (s, 1H), 4.88 (d, J=1.8 Hz, 1H), 4.95–5.07 (m, 3H), 5.15 (d, J=1.4 Hz, 1H), 5.69 (dd, J=12.1, 19.7 Hz, 1H), 7.46 (t, J=8.0 Hz, 1H), 7.62 (d, J=8.2 Hz, 1H), 7.77 (t, J=6.9 Hz, 1H), 8.24 (d, J=7.9 Hz, 1H); DEPT135 NMR ($CDCl_3$) δ 20.5, 20.7, 20.8 (2C), 21.0, 32.2, 50.5, 56.5, 59.0, 59.8, 64.7, 65.7, 68.7, 73.2, 73.4, 74.0, 74.4, 76.1, 79.2, 79.5, 80.9, 98.9, 105.7, 126.6, 126.8 (2C), 135.3.

Compounds **45c**, **46c**, **45d**, and **46d** were directly carried to the next step without characterization.

3.13. Typical procedure for deprotection (47a–d and 48a–d)

Compounds **45a–d** and **46a–d** were individually dissolved in a 7 N NH_3 solution in MeOH (0.02 M). The solution was stirred in a pressure tube at room temperature for 16 h. After evaporation, the crude material was purified by silica gel using CH_2Cl_2/MeOH (95:5). The intermediate was then dissolved in EtOH (0.01 M), followed by the addition of hydrazine (12 equiv) and catalytic amount of Raney Ni. The suspension was stirred at room temperature under N_2

for 2.5 h. Filtration and evaporation gave the desired product, which was then purified by preparative LC-MS to yield the pure compound as an acetate salt.

Compound ***47a***: yellow solid, 78%; ^{1}H NMR (MeOD-d_3) δ 1.45 (dd, J=12.4, 24.9 Hz, 1H), 2.06–2.12 (m, 1H), 2.79–2.89 (m, 1H), 2.94–3.03 (m, 1H), 3.07–3.13 (m, 1H), 3.20–3.30 (m, 2H), 3.42–3.48 (m, 1H), 3.52–3.61 (m, 3H), 3.68 (d, J=3.9 Hz, 2H), 4.04–4.08 (m, 2H), 4.11–4.21 (m, 1H), 4.25–4.28 (m, 1H), 4.45 (t, J=5.2 Hz, 1H), 5.15 (d, J=1.4 Hz, 1H), 5.29 (d, J=2.3 Hz, 1H), 5.57 (d, J=12.4 Hz, 1H), 5.86 (d, J=12.4 Hz, 1H), 7.76 (dd, J=1.7, 8.9 Hz, 1H), 7.87 (d, J=5.0 Hz, 1H), 8.22 (s, 1H), 8.28 (d, J=8.8 Hz, 1H), 8.74 (d, J=4.9 Hz, 1H); ^{13}C NMR (MeOD-d_3) δ 32.3, 41.7, 50.6, 52.1, 53.1, 62.3, 69.3, 69.4, 72.3, 74.6, 74.7, 75.1, 78.1, 83.2 (2C), 84.4, 97.7, 110.6, 120.4, 123.3, 125.2 (q, J=27.0 Hz), 126.9, 127.6 (q, J=4.5 Hz), 129.6, 132.9 (q, J=32 Hz), 147.4, 149.6, 152.1; MS calcd for $C_{28}H_{40}F_3N_5O_{10}S$ $[M+H]^+$ 696.2; found 696.1.

Compound ***48a***: yellow solid, 67%; ^{1}H NMR (MeOD-d_3) δ 1.43 (dd, J=12.6, 25.1 Hz, 1H), 2.05–2.11 (m, 1H), 2.77–2.80 (m, 1H), 2.90–2.99 (m, 1H), 3.08–3.30 (m, 3H), 3.35–3.53 (m, 3H), 3.56 (s, 1H), 3.76 (s, 2H), 4.03–4.08 (m, 2H), 4.14 (s, 1H), 4.23 (d, J=4.3 Hz, 1H), 4.59 (dd, J=4.4, 7.9 Hz, 1H), 5.08 (s, 1H), 5.16 (s, 1H), 5.59 (d, J=12.1 Hz, 1H), 5.76 (d, J=12.0 Hz, 1H), 7.76 (dd, J=1.7, 8.9 Hz, 1H), 7.88 (d, J=5.0 Hz, 1H), 8.22 (s, 1H), 8.28 (d, J=8.8 Hz, 1H), 8.73 (d, J=4.9 Hz, 1H); ^{13}C NMR (MeOD-d_3) δ 33.0, 41.8, 49.3, 50.5 (2C), 53.4, 59.6, 69.6, 72.4, 74.3, 74.6, 76.6, 77.2, 82.5, 83.2, 84.3, 97.7, 110.4, 120.3, 123.3, 126.8, 125.2 (q, J=28.0 Hz), 127.0, 129.5, 132.9 (q, J=32 Hz), 147.3, 149.7, 152.1; MS calcd for $C_{28}H_{40}F_3N_5O_{10}S$ $[M+H]^+$ 696.2; found 696.1.

Compound ***47b***: white powder, 51%; ^{1}H NMR (D_2O) δ 1.49–1.61 (m, 1H), 2.15–2.31 (m, 1H), 3.15–3.30 (m, 5H), 3.40–3.54 (m, 2H), 3.58–3.73 (m, 4H), 4.02–4.08 (m, 2H), 4.10–4.17 (m, 1H), 4.19–4.28 (m, 1H), 4.32–4.42 (m, 1H), 5.13 (s, 1H), 5.26 (s, 1H), 5.42–5.58 (m, 1H), 7.39 (t, J=7.6 Hz, 1H), 7.45 (d, J=8.5 Hz, 1H), 7.71 (t, J=8.4 Hz, 1H), 7.98 (d, J=7.9 Hz, 1H); DEPT135 NMR (D_2O) δ 29.2, 40.3, 48.2, 48.9, 50.0, 50.9, 61.1, 67.3, 67.8, 70.2, 73.1, 73.3, 76.6, 78.7, 81.3, 81.7, 95.7, 108.5, 123.8, 126.1, 126.6, 135.2; MS calcd for $C_{26}H_{40}N_6O_{11}S$ $[M+H]^+$ 645.3; found 645.2.

Compound ***48b***: white powder, 53%; ^{1}H NMR (D_2O) δ 1.50 (dd, J=12.1, 25.6 Hz, 1H), 2.10–2.25 (m, 1H), 2.97–3.06 (m, 2H), 3.15–3.30 (m, 2H), 3.36 (s, 1H), 3.40–3.55 (m, 3H), 3.58–3.76 (m, 4H), 4.04–4.13 (m, 3H), 4.27 (d, J=4.3 Hz, 1H), 4.50–4.55 (m, 1H), 5.09 (d, J=1.4 Hz, 1H), 5.11 (s, 1H), 5.47 (dd, J=11.4, 22.6 Hz, 2H), 7.36 (t, J=7.2 Hz, 1H), 7.44 (d, J=8.2 Hz, 1H), 7.68 (t, J=7.0 Hz, 1H), 7.96 (d, J=8.0 Hz, 1H); DEPT135 NMR (D_2O) δ 29.2, 40.3, 48.2, 48.9, 50.0, 50.9, 61.1, 67.3, 67.8, 70.2, 73.1, 73.3, 76.6, 78.7, 81.3, 81.7, 95.7, 108.5, 123.8, 126.1, 126.6, 135.2; MS calcd for $C_{26}H_{40}N_6O_{11}S$ $[M+H]^+$ 645.3; found 645.1.

Compound ***47c***: white powder, 41%; ^{1}H NMR (D_2O) δ 1.55–1.66 (m, 1H), 2.17–2.31 (m, 1H), 3.05–3.30 (m, 4H), 3.43 (s, 1H), 3.49–3.66 (m, 5H), 3.79 (t, J=9.3 Hz, 1H), 3.94–4.20 (m, 4H), 4.33 (t, J=5.1 Hz, 1H), 5.13 (s, 1H), 5.23 (s, 1H), 5.43 (d, J=11.5 Hz, 1H), 5.58 (d, J=11.5 Hz, 1H), 6.48 (d, J=3.1 Hz, 1H), 6.98 (d, J=3.1 Hz, 1H); ^{13}C NMR (D_2O) δ 38.8, 40.5, 49.9, 50.9, 61.1, 67.3, 67.7, 70.3 (2C), 72.6, 73.4, 74.3, 76.8, 77.8, 81.8, 81.9, 95.8, 102.0, 108.7, 122.1, 148.6, 152.1, 161.8, 181.0; MS calcd for $C_{24}H_{39}N_7O_{11}S$ $[M+H]^+$ 634.3; found 634.1.

Compound ***48c***: white powder, 83%; ^{1}H NMR (D_2O) δ 1.75 (dd, J=12.6, 25.0 Hz, 1H), 2.36–2.40 (m, 1H), 3.22–3.41 (m, 4H), 3.52 (s, 1H), 3.57–3.69 (m, 2H), 3.72–3.90 (m, 4H), 4.11–4.22 (m, 2H), 4.26 (t, J=4.6 Hz, 1H), 4.36 (d, J=4.5 Hz, 1H), 4.62 (dd, J=4.6, 7.5 Hz, 1H), 5.19 (s, 1H), 5.23 (s, 1H), 5.53 (d, J=11.6 Hz, 1H), 5.62 (d, J=11.6 Hz, 1H), 6.52 (d, J=3.4 Hz, 1H), 7.03 (d, J=3.4 Hz, 1H); ^{13}C NMR (D_2O) δ 39.2, 40.8, 48.9, 49.1, 51.4, 59.2, 67.7, 68.1, 70.7, 73.2, 74.3, 75.2, 79.5, 81.3, 81.6, 95.6, 102.4, 105.4, 109.3, 122.3, 149.0, 152.5, 162.0, 181.7; MS calcd for $C_{24}H_{39}N_7O_{11}S$ $[M+H]^+$ 634.3; found 634.1.

Compound ***47d***: white solid, 45%; ^{1}H NMR (D_2O) δ 1.69 (dd, J=13.1, 25.3 Hz, 1H), 2.23–2.47 (m, 1H), 3.17–3.40 (m, 4H), 3.53 (s, 1H), 3.63 (t, J=9.8 Hz, 1H), 3.70–3.83 (m, 4H), 3.91 (t, J=10.0 Hz, 1H), 4.08–4.18 (m, 2H), 4.20–4.38 (m, 2H), 4.47 (t, J=5.6 Hz, 1H), 5.24 (s, 1H), 5.37 (d, J=1.8 Hz, 1H), 5.65 (d, J=11.6 Hz, 1H), 5.74 (d, J=11.1 Hz, 1H), 8.02 (s, 1H); DEPT135 NMR (D_2O) δ 38.7, 40.4, 48.9, 50.0, 50.9, 61.1, 67.2, 67.7, 70.3, 71.7, 72.8, 73.4, 76.7, 77.8, 81.4, 81.8, 95.7, 108.4, 141.8; MS calcd for $C_{23}H_{39}N_9O_{10}S$ $[M+H]^+$ 634.3; found 634.2.

Compound ***48d***: white solid, 48%; ^{1}H NMR (D_2O) δ 1.40 (dd, J=10.8, 22.8 Hz, 1H), 1.99–2.18 (m, 1H), 2.85–2.99 (m, 2H), 3.14–3.29 (m, 3H), 3.37–3.54 (m, 3H), 3.64–3.75 (m, 3H), 4.03–4.12 (m, 3H), 4.25 (d, J=4.3 Hz, 1H), 4.51 (dd, J=4.9, 7.9 Hz, 1H), 5.06 (s, 1H), 5.11 (s, 1H), 5.50 (d, J=11.4 Hz, 1H), 5.65 (d, J=11.3 Hz, 1H), 7.90 (s, 1H); MS calcd for $C_{23}H_{39}N_9O_{10}S$ $[M+H]^+$ 634.3; found 634.1.

3.13.1. 4′,6′-*O*-Benzylidene-penta-*N*-benzyloxycarbonyl-5″-*O*-*tert*-butyldimethylsilyl paromomycin (49). The perester **10** was treated with MeONa in MeOH and after 18 h dry ice was added, solvent evaporated and the resulting solid was taken in AcOEt and washed with water, dried with Na_2SO_4 and AcOEt was evaporated to get a known alcohol[19] in quantitative yield. This alcohol (900 mg, 0.834 mmol) was co-distilled (twice) with toluene and dissolved into CH_2Cl_2 (40 mL). The reaction was cooled at 0 °C and 2,4,6-collidine (165 μL, 1.252 mmol), followed by TBDMSOTf (191 μL, 0.834 mmol) was added into it and stirred for 12 h. At this stage, another 1.5 equiv of 2,4,6-collidine (165 μL, 1.252 mmol) and 1 equiv of TBDMSOTf (165 μL, 0.834 mmol) were added and after stirring for an additional 6 h, organic layer was washed with HCl (0.5 M twice), H_2O successively and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel flash chromatography (2% MeOH/CH_2Cl_2) to give pure compound as white solids (748 mg, 75%); R_f 0.45 (CH_2Cl_2/MeOH, 19:1); $[\alpha]_D$ −4.08 (*c* 1.69, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60–7.10 (m, 30H), 5.60–3.00 (m, 41H), 2.20 (m, 1H), 1.30 (m, 1H), 0.83 (s, 9H), 0.01 (s,

6H); HRMS calcd for $C_{62}H_{76}N_4O_{18}Si$ $[M+H]^+$ 1193.50021; found 1193.49783.

A solution containing preceding compound (560 mg, 0.469 mmol) and *N*,*N*-dimethylamino pyridine (229 mg, 1.87 mmol) in dry pyridine (15 mL) was treated with benzoyl chloride (0.44 mL, 3.75 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 24 h when TLC examination indicated the complete conversion of reaction. Water (1 mL) was added and after standing for 10 min, the solvent was removed under vacuum. The residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to yield fully compound **49** (680 mg, 90%); R_f 0.52 (2:3 EtOAc/hexane); $[\alpha]_D$ +26.28 (*c* 1.64, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.07–7.12 (m, 45H), 5.67–3.00 (m, 26H), 2.20 (m, 1H), 1.43 (m, 1H), 0.83 (s, 9H), 0.01 (s, 6H); HRMS calcd for $C_{90}H_{92}N_4O_{22}Si$ $[M+H]^+$ 1609.60507; found 1609.60828.

3.13.2. 5-*O*-[{3″-*O*-(4‴,6‴-*N*,*O*-Benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″-*O*-benzoyl-5″-*O*-allyl-β-D-ribofuranosyl}]-4,6-di-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (50). A solution containing compound **49** (540 mg, 0.335 mmol) in dry THF was treated with AcOH (0.38 mL, 6.712 mmol) and TBAF (3.4 mL, 3.356 mmol) successively at 0 °C. The reaction mixture was allowed to come to room temperature and further stirred for 24 h for completion of the reaction. The solvent was removed in Rotavapor and the residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (1:1 EtOAc/hexane) to yield pure compound (452 mg, 90%); R_f 0.31 (1:1 EtOAc/hexane); $[\alpha]_D$ +49.87 (*c* 2.39, $CHCl_3$); MS calcd for $C_{84}H_{78}N_4O_{22}$ $[M+H]^+$ 1495.5; found 1495.6.

The preceding compound (200 mg, 0.134 mmol) was co-distilled with toluene twice, dissolved in dry THF (15 mL), then transferred through a cannula to an ice-cooled solution of sodium hydride (6.4 mg, 0.267 mmol, 95% dispersion in oil) in dry THF (5 mL). Allyl iodide (19 μL, 0.20 mmol) was added at 0 °C to the reaction mixture and was allowed to come slowly at room temperature and further stirred for 1.5 h by careful monitoring on TLC. The reaction mixture was quenched with an aqueous solution of NH_4Cl (satd, 0.1 mL) and the solvent was evaporated to dryness in vacuo. The crude product was dissolved in EtOAc, processed as usual, which upon purification by silica gel flash chromatography (2:3 EtOAc/hexane) provided the corresponding allyl ether **50** (154 mg, 75%); R_f 0.62 (1:1 EtOAc/hexane); $[\alpha]_D$ +37.07 (*c* 1.47, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.07–7.12 (m, 45H), 6.24–3.05 (m, 31H), 2.20 (m, 1H), 1.37 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 167.5, 167.1, 165.7, 163.0, 162.6, 161.4, 160.9, 140.6, 132.7–125.8 (54C), 118.3, 111.9, 101.1, 88.7, 82.1, 81.3, 79.7, 78.6, 78.4, 76.7, 72.6, 71.5, 70.9, 70.1, 68.8, 68.5, 68.3, 67.4, 66.7, 54.7, 53.8, 52.7, 46.5, 37.3; HRMS calcd for $C_{87}H_{82}N_4O_{22}$ $[M+H]^+$ 1535.54990; found 1535.55109.

3.13.3. 5-*O*-[{3″-*O*-(4‴,6‴-*N*,*O*-Benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″-*O*-benzoyl-5″-*O*-oxo-ethyl-β-D-ribofuranosyl}]-4,6-di-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (51). The allyl ether derivative **50** (170 mg, 0.111 mmol) in CH_2Cl_2 (10 mL) was cooled at −78 °C and ozone was bubbled for 2 h after which argon was bubbled through. The mixture was treated with PPh_3 (87.1 mg, 0.332 mmol), warmed to room temperature, solvent was removed under vacuum and the crude aldehyde was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to give the aldehyde **51** (100 mg, 60%); R_f 0.52 (1:1 EtOAc/hexane), which was used as such.

3.13.4. 5″-*O*-Hydroxyethyl nor-paromomycin (54). The aldehyde **51** (24 mg, 0.0156 mmol) in dry MeOH (3 mL) was treated with $NaBH_3CN$ (1.0 M in THF, 156 μL, 0.156 mmol). The mixture was stirred at room temperature overnight, the solvent was removed, the reaction mixture was diluted with EtOAc (10 mL), washed with a solution of $NaHCO_3$ (satd, 2 mL), and dried over Na_2SO_4. After evaporation of the solvents, the residue was purified by flash chromatography (2:3 EtOAc/hexane) to give derivative **52** as a white solid (20.4 mg, 85%); R_f 0.27 (1:1 EtOAc/hexane); $[\alpha]_D$ +11.43 (*c* 0.7, $CHCl_3$); MS calcd for $C_{86}H_{82}N_4O_{23}$ $[M+H]^+$ 1539.5; found 1539.9.

A solution of above compound **52** (16 mg, 0.0082 mmol) in dry MeOH (2 mL) was treated with a catalytic amount of NaOMe in dry MeOH (1 mL, pH 8–9) and stirred at room temperature for 3 h for completion of the reaction. The reaction mixture was neutralized by the addition of dry ice, and the solvent was evaporated to dryness under vacuum to afford a crude product, which was purified by silica gel flash chromatography (1:19 MeOH/CH_2Cl_2) to obtain pure tetrol (9 mg, 88%); R_f 0.52 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D$ −5.5 (*c* 0.4, MeOH); MS calcd for $C_{58}H_{66}N_4O_{19}$ $[M+Na]^+$ 1145.4; found 1145.6.

To a solution of above compound (10 mg, 0.007 mmol) in MeOH/H_2O (1:1, 5 mL) was added 20% palladium hydroxide-on-carbon and the suspension was stirred at room temperature under an atmosphere of hydrogen (hydrogen balloon) until the disappearance of starting material as judged from the LC-MS. The mixture was filtered through a layer of Celite, concentrated under vacuum, the residue was dissolved in AcOH/H_2O (2:1, 0.5 mL) and lyophilized to afford **54** as a fluffy white solid (5.3 mg) in quantitative yield. $[\alpha]_D$ +4.8 (*c* 0.5, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.21 (s, 1H), 5.14 (s, 1H), 4.40–4.38 (m, 1H), 4.22–4.16 (m, 2H), 4.14–3.36 (m, 3H), 3.7–3.5 (m, 8H), 3.42–3.38 (m, 2H), 3.30–3.14 (m, 4H), 2.32–2.24 (m, 1H), 1.76 (s, 12H), 1.63–1.61 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 108.8, 95.9, 82.0, 81.9, 79.9, 76.6, 73.6, 73.4, 72.7, 70.5, 68.0, 67.5, 61.3, 61.0, 51.1, 50.1, 49.4, 40.7, 30.5, 23.4; HRMS calcd for $C_{19}H_{38}N_4O_{11}$ $[M+H]^+$ 499.26153; found 499.26123.

3.13.5. 5″-*O*-(Bis-2-aminoethyl)aminoethyl nor-paromomycin (55). To aldehyde **51** (30 mg, 0.0195 mmol) and bis(2-aminoethyl)amine (72.46 mg, 0.195 mmol) in dry MeOH (5 mL) was added AcOH (3–4 drops) followed by $NaBH_3CN$ (1.0 M in THF, 0.02 mL, 0.195 mmol). The

mixture was stirred at room temperature overnight until the disappearance of **51** and the solvent was evaporated to dryness. The crude reaction mixture was diluted with EtOAc (10 mL), washed with a solution of $NaHCO_3$ (satd, 2 mL), and dried over Na_2SO_4. After evaporation of the solvents, the residue was purified by flash chromatography (3:2 EtOAc/hexane) to give bis-amine derivative **53** as a white solid (29 mg, 78%); R_f 0.4 (19:1 MeOH/CH_2Cl_2); $[\alpha]_D$ +22.66 (*c* 0.9, $CHCl_3$); MS calcd for $C_{106}H_{106}N_7O_{26}$ $[M+H]^+$ 1892.7; found 1893.0.

A solution of **53** (22 mg, 0.0116 mmol) in dry MeOH (4 mL) was treated with a catalytic amount of NaOMe in dry MeOH (1 mL, pH 8–9) and stirred for 3 h for completion of the reaction. The reaction mixture was neutralized by the addition of dry ice, and the solvent was evaporated to dryness under vacuum to afford a crude product, which was purified by silica gel flash chromatography (1:9 MeOH/CH_2Cl_2) to obtain pure tetrol (15 mg, 88%); R_f 0.47 (1:12 MeOH/CH_2Cl_2); $[\alpha]_D$ −2.2 (*c* 0.5, MeOH); MS calcd for $C_{78}H_{90}N_7O_{22}$ $[M+H]^+$ 1476.6; found 1476.8.

To a solution of above compound (10 mg, 0.007 mmol) in MeOH/H_2O (1:1, 5 mL) was added 20% palladium hydroxide-on-carbon and the suspension was stirred at room temperature under an atmosphere of hydrogen (hydrogen balloon) until the disappearance of starting material (LC-MS). The mixture was filtered through a layer of Celite, concentrated under vacuum, and the residue was dissolved in AcOH/H_2O (2:1, 0.5 mL) and lyophilized to afford **55** as a fluffy white solid (6.4 mg) in quantitative yield; $[\alpha]_D$ +10.4 (*c* 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.13 (s, 1H), 5.09 (s, 1H), 4.39–4.35 (m, 1H), 4.26–4.22 (m, 1H), 4.18–4.15 (m, 1H), 4.14–3.38 (m, 3H), 3.80–3.50 (m, 5H), 3.42–3.35 (m, 3H), 3.31–3.16 (m, 3H), 3.08–2.85 (m, 5H), 2.80–2.40 (m, 6H), 2.20–2.12 (m, 1H), 1.76 (s, 21H), 1.42–1.35 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 109.3, 97.2, 88.5, 83.0, 82.5, 81.8, 80.5, 77.0, 76.5, 74.0, 73.5, 70.7, 68.7, 67.9, 62.0, 51.5, 51.1, 50.5, 49.8, 49.3, 47.0, 40.8, 37.0, 23.5; HRMS calcd for $C_{23}H_{49}N_7O_{10}$ $[M+H]^+$ 584.36192; found 584.36230.

3.13.6. 4′,6′-*O*-Benzylidene-penta-*N*-benzyloxycarbonyl-5″-*O*-*tert*-butyldimethylsilyl paromomycin (56). Freshly distilled benzaldehyde (400 mL) was added to a 1 L round-bottom flask containing pure penta-*N*-benzyloxycarbonyl paromomycin (20 g) and stirred vigorously to bring it into solution. To the stirred mixture were added 4 Å molecular sieves (15 g) and formic acid (20.00 mL, 0.530 mol). After 12 h at room temperature, the reaction mixture was added dropwise to a stirred ice-cold solution of satd aqueous Na_2CO_3, extracted with ethyl acetate, and the organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was evaporated to dryness and excess benzaldehyde was removed under vacuum to afford a crude solid, which was purified by flash column chromatography over silica gel (3% MeOH/CH_2Cl_2) to obtain pure 4′-6′-*O*-benzylidene acetal (16 g, 73%) with analytical data identical to those we published previously.[38]

The preceding compound (6.00 g, 4.367 mmol) was co-distilled (twice) with toluene, the residue was dissolved in CH_2Cl_2 (400 mL), the solution cooled to 0 °C and treated with 2,4,6-collidine (1.15 mL, 8.735 mmol), followed by TBDMSOTf (0.50 mL, 2.184 mmol). After stirring for 12 h, another 0.6 equiv of TBDMSOTf was added. After stirring for an additional 6 h, CH_2Cl_2 was evaporated to half of its initial volume and the solution washed successively with HCl (0.5 M twice), H_2O and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel flash chromatography (2% MeOH/CH_2Cl_2) to give **56** as a white solid (4.861 g, 75%); R_f 0.6 ($CHCl_3$/EtOAc/MeOH (20:5:3); $[\alpha]_D$ +41.8 (*c* 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60–7.10 (m, 30H), 5.62–3.00 (m, 41H), 2.20 (m, 1H), 1.30 (m, 1H), 0.83 (s, 9H), 0.01 (s, 6H); HRMS calcd $C_{76}H_{93}N_5O_{24}Si$ $[M+H]^+$ 1488.60580; found 1488.60258.

3.13.7. 4′,6′-*O*-Benzylidene-penta-*O*-benzoyl penta-*N*-benzyloxycarbonyl-5″-*O*-*tert*-butyldimethylsilyl paromomycin (57). A solution containing the above compound (540 mg, 0.362 mmol) and *N*,*N*-dimethylamino pyridine (176 mg, 1.44 mmol) in dry pyridine (20 mL) was treated with benzoyl chloride (0.85 mL, 7.25 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h followed by 70 °C for 24 h when TLC examination indicated the formation of two products with a 3:1 ratio. Water (1 mL) was added and after standing for 10 min, the solvent was removed under vacuum. The residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to yield **57** (510 mg, 70%); R_f 0.43 (1:1 EtOAc/hexane); $[\alpha]_D$ +37.9 (*c* 1.15, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11–7.08 (m, 55H), 6.28–3.05 (m, 36H), 2.21 (m, 1H), 1.42 (m, 1H), 0.81 (s, 9H), 0.02 (s, 6H); HRMS calcd for $C_{111}H_{113}N_5O_{29}Si$ $[M+H]^+$ 2008.73687; found 2008.73984.

3.13.8. 4-*O*-[{4′,6′-*O*-Benzylidene-3′-*O*-benzoyl-2′-benzyloxycarbonylamino-α-D-glucopyranosyl}-3″-*O*-{(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″-*O*-benzoyl-5″-*O*-allyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (58). A solution containing compound **57** (420 mg, 0.209 mmol) in dry THF was treated with AcOH (119.6 μL, 2.09 mmol) and TBAF successively at 0 °C. The reaction mixture was allowed to come to room temperature and further stirred for 24 h. The solvent was evaporated, the residue was dissolved in EtOAc/H_2O, the aqueous layer was extracted with EtOAc, and the combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to yield pure 5″-hydroxy derivative (202 mg, 51%); R_f 0.47 (3:2 EtOAc/hexane); $[\alpha]_D$ +25.16 (*c* 0.93, $CHCl_3$); MS calcd for $C_{105}H_{99}N_5O_{29}$ $[M+H]^+$ 1894.9; found 1895.0; Additional product containing two free hydroxyl groups was isolated from the column (125 mg, 33%); R_f 0.27 (3:2 EtOAc/hexane).

The 5″-hydroxy derivative (120 mg, 0.063 mmol) was co-distilled with toluene twice and dissolved in dry THF (3 mL) in a flask covered with aluminum foil. Allyl iodide (58.2 μL, 0.63 mmol) was added at 0 °C followed by the

dropwise addition of 0.5 M KHMDS solution in toluene (152 μL, 0.076 mmol). The mixture was stirred for 3 h at room temperature by careful monitoring on TLC. The reaction mixture was quenched with an aqueous solution of NH_4Cl (satd, 0.1 mL), the solvent was evaporated to dryness, the crude product was dissolved in EtOAc, processed as usual, then purified by silica gel flash chromatography (1:2 EtOAc/hexane) to give the corresponding allyl ether **58** (71 mg, 58%); R_f 0.62 (1:1 EtOAc/hexane); $[\alpha]_D$ +39.6 (*c* 0.84, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.16–7.11 (m, 55H), 6.30–3.00 (m, 41H), 2.17 (m, 1H), 1.41 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 168.3, 167.8, 167.7, 167.1, 162.3, 162.0, 161.8, 161.5, 161.2, 139.6, 117.5, 133.4–126.3 (66C), 110.7, 101.3, 100.8, 86.4, 84.2, 82.5, 81.8, 81.3, 79.7, 78.2, 76.8, 75.9, 75.6, 74.7, 74.1, 71.3, 70.9, 70.1, 69.6, 69.3, 68.7, 68.0, 63.6, 54.2, 51.7, 51.4, 49.3, 43.6, 36.2, 35.1; HRMS calcd for $C_{108}H_{103}N_5O_{29}$ $[M+H]^+$ 1934.68190; found 1934.68326.

3.13.9. 4-*O*-[{4′,6′-*O*-Benzylidene-3′-*O*-benzoyl-2′-benzyloxycarbonylamino-α-D-glucopyranosyl}-3″-*O*-{(4‴,6‴-*N*,*O*-benzylidene-3‴-*O*-benzoyl-2‴,6‴-dibenzyloxycarbonylamino-2‴,6‴-dideoxy-α-L-idopyranosyl)-2″-*O*-benzoyl-5″-*O*-oxo-ethyl-β-D-ribofuranosyl}]-6-*O*-benzoyl-1,3-di-*N*-benzyloxycarbonyl-2-deoxystreptamine (59). The preceding compound **58** (100 mg, 0.0517 mmol) in CH_2Cl_2 (4 mL) was cooled at −78 °C and ozone was bubbled for 2 h after which argon was bubbled through. The mixture was treated with PPh_3 (40.64 mg, 0.299 mmol), warmed to room temperature, the solvent was removed under vacuum, the crude product was purified by silica gel flash chromatography (2:3 EtOAc/hexane) to give the aldehyde **59** (60 mg, 60%); R_f 0.38 (1:1 EtOAc/hexane), which was used as such.

3.13.10. 5″-*O*-(2-*N*,*N*-Dimethylaminoethyl)paromomycin (62). To a mixture of **59** (30 mg, 0.0155 mmol) and *N*,*N*-dimethylamine (2.0 M in THF, 80 μL, 0.155 mmol) in dry MeOH (3 mL) was added AcOH (3–4 drops) followed by $NaBH_3CN$ (1.0 M in THF, 0.15 mL, 0.155 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (10 mL), washed with a solution of $NaHCO_3$ (satd, 2 mL), and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography (48:1 CH_2Cl_2/MeOH) to give the *N*,*N*-dimethylamino ethyl derivative **60** as a white solid (26 mg, 85%); R_f 0.67 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D$ +22 (*c* 1.57, $CHCl_3$); MS calcd for $C_{109}H_{108}N_6O_{29}$ $[M+H]^+$ 1965.7; found 1966.4.

A solution of **60** (20 mg, 0.0102 mmol) in dry MeOH (2 mL) was treated with a catalytic amount of NaOMe in dry MeOH (1 mL, pH 8–9) and stirred at room temperature for 3 h. The reaction mixture was neutralized by the addition of dry ice, and the solvent was evaporated to dryness under vacuum to afford a crude product, which was purified by silica gel flash chromatography (1:19 MeOH/CH_2Cl_2) to obtain pure pentol (8.8 mg, 60%); R_f 0.32 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D$ +18.5 (*c* 0.44, MeOH); MS calcd for $C_{74}H_{88}N_6O_{24}$ $[M+H]^+$ 1445.6; found 1445.9.

The methanolysis product (6 mg, 0.0041 mmol) was dissolved in AcOH/H_2O (4:1, 2 mL) and heated at 60 °C for 2 h. The solvent was removed and the crude product was dissolved in MeOH/H_2O (1:1, 2 mL) followed by the addition of 20% palladium hydroxide-on-carbon. The suspension was stirred at room temperature overnight under an atmosphere of hydrogen (hydrogen balloon), the mixture was filtered through a layer of Celite, concentrated under vacuum, and the residue was dissolved in AcOH/H_2O (2:1, 0.5 mL) and lyophilized to afford **62** (4.1 mg, quant.) as a white solid; $[\alpha]_D$ +33.1 (*c* 0.26, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.34 (s, 1H), 5.23 (s, 1H), 5.0 (s, 1H), 4.33–4.12 (m, 4H), 4.11–4.0 (m, 1H), 3.94–3.83 (m, 1H), 3.75–3.63 (m, 5H), 3.61–3.58 (m, 4H), 3.50–3.21 (m, 8H), 3.13–2.93 (m, 3H), 2.78 (s, 6H), 2.17–2.08 (m, 1H), 1.82 (s, 15H), 1.44–1.37 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 182.1, 108.9, 96.9, 95.5, 85.6, 81.7, 81.3, 78.5, 74.6, 74.4, 73.9, 71.5, 70.1, 69.9, 68.7, 68.3, 65.4, 61.3, 60.2, 57.7, 54.9, 51.7, 50.9, 49.8, 43.8 (2C), 41.3, 30.5, 23.9; HRMS calcd for $C_{27}H_{54}N_6O_{14}$ $[M+H]^+$ 687.37763; found 687.6, 687.37907.

3.13.11. 5″-*O*-(3-Phenylpropyl-1-aminoethyl)paromomycin (63). To a mixture of **59** (30 mg, 0.0155 mmol) and propylamine (2.0 M in THF, 80 μL, 0.155 mmol) in dry MeOH (3 mL) was added AcOH (3–4 drops) followed by $NaBH_3CN$ (1.0 M in THF, 0.15 mL, 0.155 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (10 mL), washed with a solution of $NaHCO_3$ (satd, 2 mL), and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography (3:2 AcOEt/hexane) to give **61** as a white solid (23 mg, 80%); R_f 0.15 (1:1 AcOEt/hexane); $[\alpha]_D$ +14 (*c* 1.0, $CHCl_3$); One benzoate group was removed during the reaction as indicated by MS analysis; MS calcd for $C_{109}H_{110}N_6O_{28}$ $[M+H]^+$ 1951.7; found 1952.2.

A solution of **61** (20 mg, 0.0102 mmol) in dry MeOH (2 mL) was treated with a catalytic amount of NaOMe in dry MeOH (1 mL, pH 8–9) and stirred at room temperature for 3 h. The reaction mixture was neutralized by the addition of dry ice, and the solvent was evaporated to dryness under vacuum to afford a crude product, which was purified by silica gel flash chromatography (5% MeOH/CH_2Cl_2) to obtain pure pentol (9 mg, 60%); R_f 0.3 (5% MeOH/CH_2Cl_2); $[\alpha]_D$ +12.3 (*c* 0.5, MeOH); MS calcd for $C_{81}H_{94}N_6O_{24}$ $[M+H]^+$ 1535.6; found 1536.0.

The methanolysis product was dissolved in AcOH/H_2O (4:1, 2 mL) and heated at 60 °C for 2 h. The solvent was removed and the crude product was dissolved in MeOH/H_2O (1:1, 2 mL) followed by the addition of 20% palladium hydroxide-on-carbon. The suspension was stirred at room temperature overnight under an atmosphere of hydrogen (hydrogen balloon), the mixture was filtered through a layer of Celite, concentrated under vacuum, and the residue was dissolved in AcOH/H_2O (2:1, 0.5 mL) and lyophilized to afford **63** (4 mg, quant.) as a white solid; $[\alpha]_D$ +13.6 (*c* 0.5, H_2O); ^1H NMR (400 MHz, D_2O) δ 7.24–7.15 (m, 5H), 5.32 (s, 1H), 5.22 (s, 1H), 5.03 (s, 1H), 4.34–4.09 (m, 4H), 4.10–4.02 (m, 1H), 3.94–3.83 (m, 1H), 3.75–3.63 (m, 5H), 3.61–3.58 (m, 4H), 3.50–3.21 (m, 8H), 3.13–2.93 (m, 3H), 2.18–2.05 (m, 1H), 1.81 (s, 15H), 1.49–1.41 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 182.5, 132.9, 129.7 (2C), 129.6 (2C), 127.0, 110.7, 97.5, 97.1, 85.8, 82.2, 81.9, 76.1, 74.7, 74.4, 74.1, 71.5, 71.3, 70.5, 69.3, 68.7, 61.9, 61.1,

55.1, 52.4, 51.1, 50.3, 41.3, 39.7, 33.3, 32.5, 30.8, 29.7, 28.9, 24.0; HRMS calcd for $C_{34}H_{60}N_6O_{14}$ $[M+H]^+$ 777.42758; found 777.42090.

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