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The synthesis and 16S A-site rRNA recognition of carbohydrate-free aminoglycosides

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Abstract—The first carbohydrate-free aminoglycoside analogs bearing the 2-deoxystreptamine moiety were synthesized from asymmetrically protected 2-deoxystrepamine and subsequently demonstrated to have significant binding to the 16S A-site rRNA target and moderate functional activity.

Although aminoglycosides were discovered over 60 years ago, they are still among the most potent antibiotics in the clinic.[1] However, aminoglycosides have poor oral bioavailability and pharmacokinetics, and their oto- and nephro-toxicity remain obstacles that limit their use to 'last resort' or to combination therapies.[2,3] In addition, bacterial resistance has been increasing, a serious problem that requires the development of better aminoglycosides with structural motifs that block the many aminoglycoside modifying enzymes.[1,4] Therefore, research to discover modified aminoglycosides has been an ongoing focus of several research groups.[5–10]

One approach[11–13] is to replace one or more carbohydrate rings with an open alkylamino chain. In one example, a crystal structure[14] of an analog bound to the 16S A-site rRNA was offered together with good antibacterial activity as evidence of the potential for success of this strategy.[13] Our own work, focusing on replacing the A-ring with a suitable aromatic moiety, has been previously demonstrated as another strategy for producing analogs lacking the easily modified carbohydrate rings.[15–17] Such A-ring replacements have been demonstrated to have good binding to the 16S A-site rRNA along with antibacterial effects. Moreover, computer modeling suggests that this strategy would not be mutually exclusive with the carbohydrate ring replacement strategy. Therefore, we sought to combine both of these strategies and prepare aminoglycoside analogs lacking one sugar ring. Herein, we report the synthesis of aminoglycoside analogs and their initial evaluation in an FTICR mass spectrometer binding assay.

Our initial synthetic strategy focused on the protected 2-deoxystreptamine (2-DOS) analog **1**. We found this previously reported compound easy to prepare, scalable, and suitably enantiopure.[11] First, we sought to avoid basic conditions to avoid any acetyl group migration. To this end, the Pummerer rearrangement[18,19] was adopted successfully to functionalize position 4 of the 2-DOS analog **1** with a methylthiomethylene group. Subsequent chlorination by sulfuryl chloride and reaction with thiolquinoline gave intermediate **3a** in an 85% yield. On the other hand, *p*-methoxybenzyl imidate[20,21] and compound **1** were treated with camphosulfonic acid under anhydrous conditions to yield compound **3b** in a good yield. Deacetylation of **3a**,**b** went smoothly to give the key diol intermediates **4a**,**b**, individually. Reduction of **4a**,**b** by transfer hydrogenation using hydrazine and Raney Ni gave the diamine **5a**,**b** in good to excellent yields (see Scheme 1).

Previous carbohydrate ring replacement strategies have relied on a successful alcohol allylation followed by a subsequent oxidative cleavage to introduce an aldehyde moiety.[11–13] However, the presence of a thiol group prone to oxidation prompted us to look for an alternative route. Alkylation of compounds **4a**,**b** with ^{t}butyl-α-bromoacetate proved to be feasible, while iodoacetonitrile and bromoacetaldehyde diethyl acetal failed to give the desired products. After alkylation, carboxylic acids **6a** and **7a** were obtained exclusively, probably due to hydrolysis facilitated by the neighboring hydroxy group. Although the yield for **6a** and **7a** was relatively low

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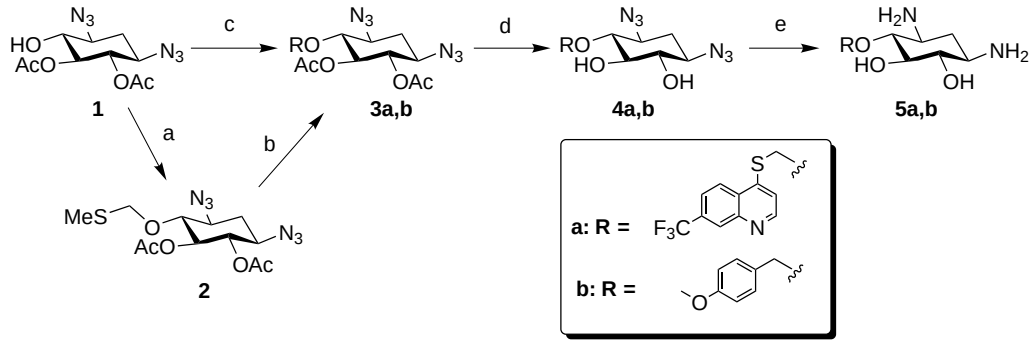
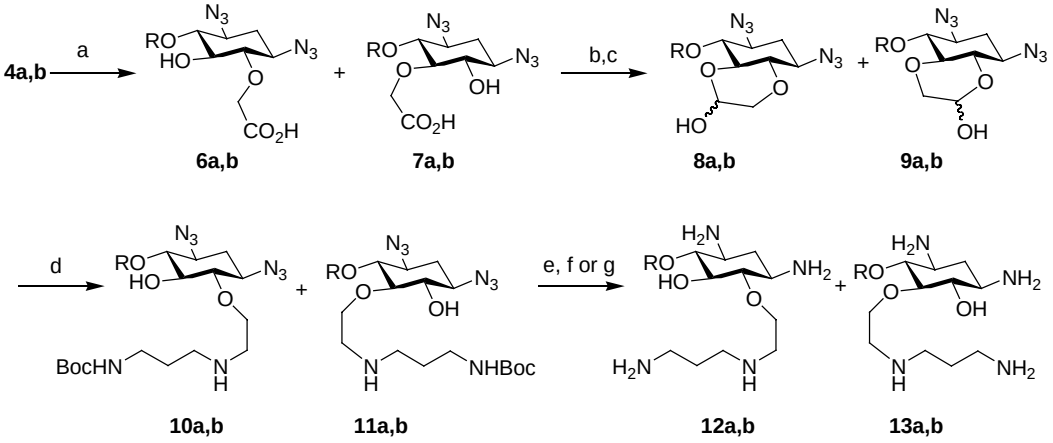
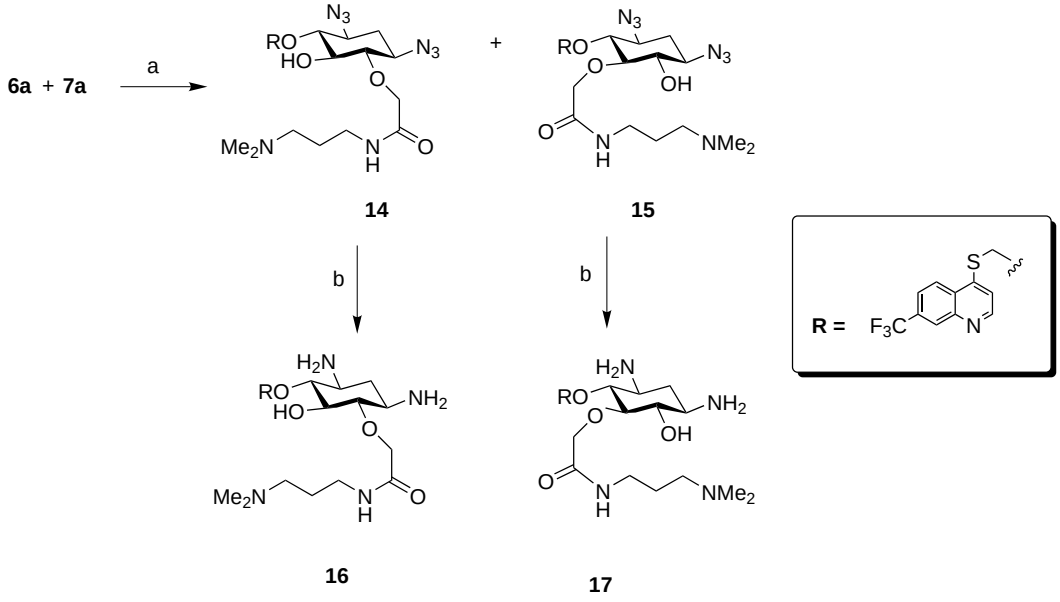


Scheme 1. Reagents and conditions: (a) DMSO, AcOH, Ac_2O, rt, 2.5 d (80%); (b) (i) SO_2Cl_2, DCM, rt, 30 min, (ii) NaH, 7-trifluoromethyl-4-quinolinethiol, CH_3CN, rt, 2.5 h (85%); (c) *p*MeOBnOC(NH)CCl_3, CSA, DCM, rt, 2 d; (d) LiOH, THF, *i*PrOH, H_2O, rt, 2 h (72% **1**–**4b**); (e) NH_4OH, MeOH, rt, 16 h (91% for **4a**); (f) NH_2NH_2, Raney Ni, 1 h (82% for **5a**, 92% for **5b**).



Scheme 2. R = a,b same as in Scheme 1. Reagents and conditions: (a) NaH, $BrCH_2CO_2tBu$, THF (45% for **6a** and **7a**, 85% for **6b** and **7b**); (b) EDAC·HCl, DMAP, THF, rt, 4 h; (c) DIBAL, toluene, −78 °C, 1.5 h (86% for **8a**, 78% for **9a**, 85% for **8b** and **9b**); (d) $BocNH_2(CH_2)_3NH_2$, AcOH, $NaCNBH_3$, MeOH, rt, 4 h (quantitative); (e) NH_2NH_2, Raney Ni, EtOH rt, 2 h; (f) TFA, $(iPr)_3SiH$, rt, 5 min (for **12a** and **13a**); (g) 2,6-lutidine, TMSOTf, Et_3N, DCM, MeOH, rt, 16 h (for **12b** and **13b**).



Scheme 3. Reagents and conditions: (a) HATU, DIPEA, $NH_2(CH_2)_3N(CH_3)_2$, DCM (72% for **14**, 81% for **15**); (b) NH_2NH_2, Raney Ni, EtOH, rt, 2 h (75% for **16**, 73% for **17**).

(45%) due to partial decomposition, incomplete conversion, and a small amount of di-alkylation, compounds **6a** and **7a** could easily be obtained, individually, after column chromatography. Intramolecular lactonization was achieved quantitatively using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC·HCl); however, attempts to isolate and purify the lactone were unsuccessful. Alternatively, the lactone could be reduced by DIBAL, without purification of the starting material, cleanly and smoothly to give intermediates **8a** and **9a**, individually. A subsequent reductive amination, azido reduction, and Boc removal gave the final compounds **12a** and **13a**, which were purified by preparative LC–MS. The *p*-methoxybenzyl (PMB) substituted analogs were made in a similar manner, except intermediates **6b** and **7b** were not separable by silica gel chromatography; however, the final mixture of compounds **12b** and **13b** was easily separated by preparative LC–MS. Regioselective synthesis of **8b** or **9b** from **4b** was attempted using Bu_2SnO and allyl bromide and then oxidative cleavage. Only one regioisomer **9b** was obtained in 18% yield. The regiochemistries of compounds **12a**,**b** and **13a**,**b** were ascertained by 1-D NMR (^1H, ^{13}C, and DEPT) and 2-D NMR (COSY, HMQC, and HMBC) (see Scheme 2).

The carboxylic acid regioisomers **6a** and **7a** were also directly coupled with primary amines to form amides **14** and **15**. A subsequent azido reduction using transfer hydrogenation yielded final compounds **16** and **17**. The regiochemistries of compounds **16** and **17** were confirmed by 2-D NMR (see Scheme 3).

The noncovalent binding interaction between the synthetic aminoglycosides and RNA was studied by high-resolution FTICR mass spectrometry.[22] As shown in Table 1, the addition of the alkyl amino chain (**12a**,**b** and **13a**,**b**) improved the binding activity over the unsubstituted **5a** and **5b** by a factor of 10. Quinoline-substituted analogs bind stronger than the PMB substituted (**12a** vs **12b** and **13a** vs **13b**) versions. The presence of the amide moiety dramatically decreases the binding affinity (**16** vs **12a** and **17** vs **13a**). A cell-free bacterial transcription/translation (T/T) assay was run to measure the functional activity of these compounds inhibiting at the translation level. To our delight, the two tightest binders **12a** and **13a** from the mass spectrometry assay did show moderate functional activity.

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

Compound	K_d (μM)	IC_{50} (μM)
Paromomycin	0.1	0.6
Neamine	5	>50
5a	35	>50
12a	1.5	15.0
13a	2	2.1
16	241	>50
17	88	>50
5b	315	>50
12b	27	>50
13b	10	>50

[a] Ligands (7.5, 2.5, 0.75, and 0.25 μM); target RNA (0.1 μM).

In conclusion, aminoglycoside analogs lacking a carbohydrate ring have been prepared, demonstrated to have significant binding to its 16S A-site rRNA target, and shown to have the ability to inhibit bacterial translations. This opens up the possibilities of generating libraries of synthetic compounds mimicking the conformation and function of aminoglycosides lacking the structural features most recognized by aminoglycoside modifying enzymes. These compounds offer a new strategy to combat prevalent and persistent bacterial resistance emerging in the clinic.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.027

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