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Synthesis of a library of stereo- and regiochemically diverse aminoglycoside derivatives†

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A library of forty modified aminoglycosides was prepared in which the configuration and regiochemistry of two or three rings was widely varied. The library was based around three core ring systems: the 2-deoxystreptamine ring system found in the natural products, and both enantiomers of (1*R**,2*R**,4*R**,5*R**)-2,5-diamino-cyclohexane-1,4-diol and (1*R**,3*R**,4*R**,6*R**)-4,6-diaminocyclohexane-1,3-diol. In each case, the core was modified by glycosylation with one or two sugar rings. The absolute configuration of the sugar substituents (D or L), the configuration of the anomeric centres (α or β), and the regiochemical arrangement of the amine(s) were varied.

Introduction

Libraries of stereo-and regioisomeric ligands can probe large areas of conformational space, and can be used to identify unnatural ligands for macromolecular targets.[1] A key milestone in this area has been the synthesis of a 1,300-member library of acylated amino di- and tri-saccharides.[2] This approach enabled the identification of two compounds that exhibited a higher affinity for a bacterial lectin from *Bauhinia purpurea* than the known natural ligand. Furthermore, modifying the glycosylation patterns of natural products can alter their targeting, biological activity and pharmacology.[3] To this end, a chemoenzymatic 'glycorandomisation' strategy has been developed in which the glycosylation patterns of natural products may be varied combinatorially.[3]

The aminoglycoside antibiotics (such as **1**–**5**) interfere with protein synthesis by interacting with the 16S subunit of the bacterial ribosome, thereby inhibiting translation and causing miscoding.[4] The structural basis of the recognition of aminoglycosides by the prokaryotic A site has been determined.[5] In addition, the aminoglycosides are recognised by a range of other RNA sequences: aminoglycosides inhibit the splicing of group I introns,[6] and can disrupt key protein–RNA recognition events; for example, the formation of the RRE–Rev[7] and TAR–Tat[8] complexes required in the life cycle of the HIV virus.

A wide range of modified aminoglycosides, in which substitution[9] of one or more carbohydrates[10] was varied systematically, have been prepared previously. Furthermore, orthogonally-protected sugar diamino acids have been exploited as building blocks in the synthesis of linear and branched aminoglycoside derivatives.[11] In addition, macrocyclic ^{15}N-labelled oligoaminodeoxydisaccharides have been prepared to probe RNA binding events.[12] These compounds, which have been derived from or have been inspired by aminoglycoside structure and function, are valuable tools for investigating RNA-mediated biological processes.

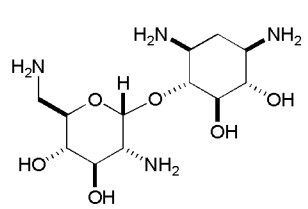
Here, we describe the synthesis of a library of aminoglycoside derivatives in which the configuration and regiochemistry of two or three rings has been more widely varied. These compounds retain the general structural features which enable the aminoglycosides to recognise specific RNA sequences. However, by exploring regions of conformational space that are not available to the natural products, it was expected that novel ligands for other important RNA sequences might be discovered. Such ligands could be exploited as chemical tools for investigating RNA-mediated biological processes.

The library is based around three core ring systems: the 2-deoxystreptamine ring system found in the natural products (see structures **6**‡ and **9** / **9′**,§ Fig. 1), and two alternative cores (see

† Electronic supplementary information (ESI) available: characterisation data. See http://dx.doi.org/10.1039/b505865a

‡ The 2-deoxystreptamine core is prochiral, so its symmetrically substituted derivatives may be designated as **6XY**; the order of the substituents denotes how the enantiotopic 4- and 6-hydoxy groups have been substituted (first letter and second letter respectively). Throughout the paper, the descriptors **X** and **Y** refer to generic glycosyl substituents.

§ The 4,5- and 5,6-disubstituted derivatives of 2-deoxystreptamine are designated **9XY** and **9′XY** respectively; the second letter denotes the 5-substituent of the core.



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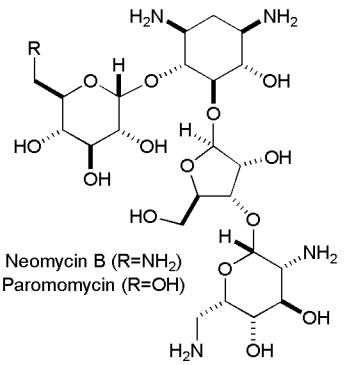
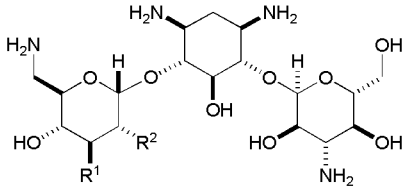


2; Neomycin B (R=NH_2)
3; Paromomycin (R=OH)



4; Kanamycin A (R^1=R^2=OH)
5; Tobramycin (R^1=H, R^2=NH_2)

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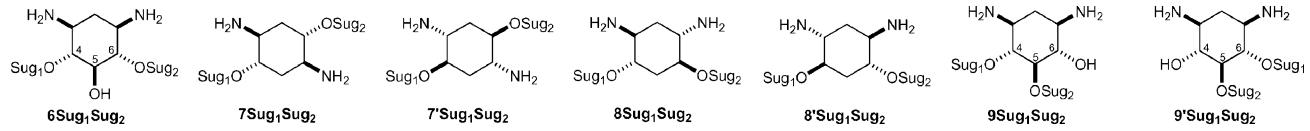


Fig. 1 Core ring systems in the aminoglycoside derivatives. Sug_1 and Sug_2 denote the generic substituents: either hydrogen or the sugars A–H/A′–H′.

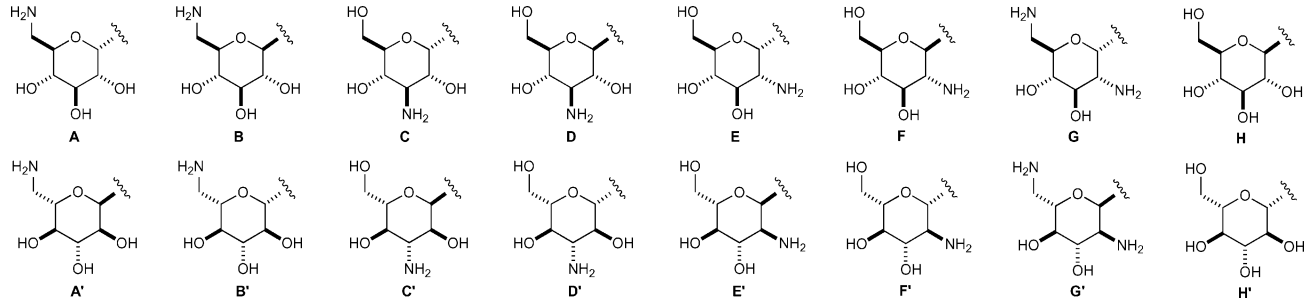


Fig. 2 Variations in the sugar substituents.

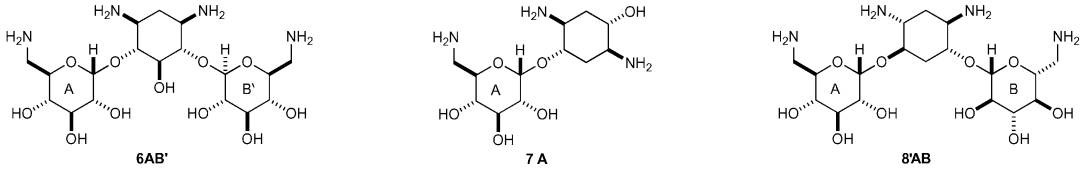
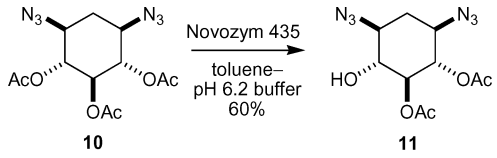


Fig. 3 Examples of possible aminoglycoside derivatives.

structures **7** / **7′** and **8** / **8′**,¶ Fig. 1). In each case, the core has been further modified by glycosylation with one or two of the substituents A–H/A′–H′ (see Fig. 2). The absolute configuration of the sugar substituents (D or L), the configuration of the anomeric centres (α or β), and the regiochemical arrangement of amine(s) have been varied widely.* Examples of possible aminoglycoside derivatives include those shown in Fig. 3.

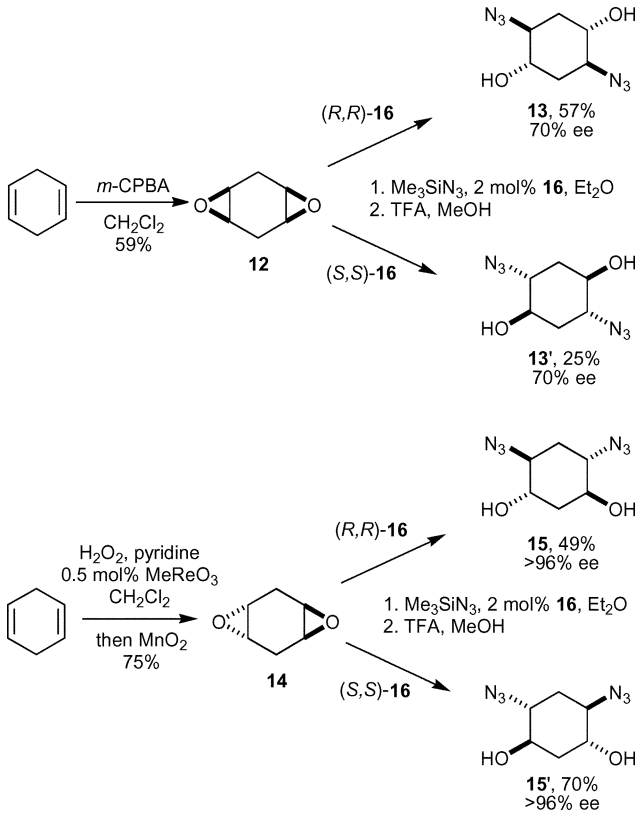
Synthesis of the cores

The 2-deoxystreptamine core, prepared by degradation of neomycin,[13] was desymmetrised by enantioselective ester hydrolysis. Using this approach, the *meso*-triacetate **10** was converted into the chiral derivative **11** with >95% ee (Scheme 1).[10b]



Scheme 1

Both enantiomers of the C_2-symmetrical diols **13** and **15** were prepared by desymmetrisation of the corresponding bisepoxides[14] **12** and **14** by double enantioselective epoxide opening with Me_3SiN_3 (Scheme 2).[15] The enantiomeric diols **13** and **13′** were prepared by desymmetrisation of the bisepoxide **12** using 2 mol% of the catalysts (*R*,*R*)- and (*S*,*S*)-**16**; the products **13** / **13′** were shown to have 70% ee by chiral HPLC. The desymmetrisation of the bisepoxide **14** using (*R*,*R*)- and (*S*,*S*)-**16**

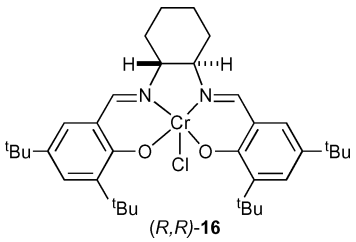


Scheme 2

gave the required diols, **15** and **15′**, in good yield, together with the centrosymmetric diol **17** (5% yield). The major products **15** and **15′** were shown by chiral HPLC to have >96% ee. The absolute configurations of **13** / **13′** and **15** / **15′** have been assigned by analogy with many previous examples of enantioselective epoxide openings.[15]

¶ The cores of **7** and **8** are C_2-symmetrical, and two enantiomeric cores are possible in each case (**7** / **7′** and **8** / **8′**). Here, the order of the substituents is irrelevant (**7′AB** and **7′BA** would be the same compound); however, by convention, we have listed the substituents in alphabetical order.

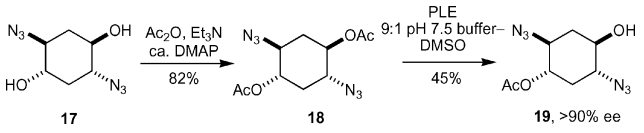
* All D sugars are designated A–H and L sugars A′-H′; the substituents A, C, E and G are α-configured and B, D, F and H are β-configured.



(*R,R*)-**16**

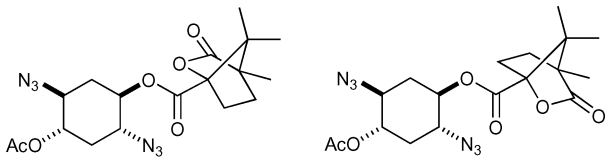
Desymmetrisation of a centrosymmetric diacetate

The centrosymmetric diol **17** was converted into the corresponding diacetate **18**, and desymmetrised[16] by enantioselective ester hydrolysis using pig liver esterase (Scheme 3). The corresponding hydroxy acetate **19** was obtained in 45% yield, and was shown to have >90% ee by conversion into the diastereomeric esters **20a** and **20b** derived from (+)- and (−)-camphanic acid. The absolute configuration of **17** has been assigned by analogy with the desymmetrisation of a similar centrosymmetric diacetate using the same enzyme.[16f] The hydroxy acetate **19** is a protected version of a diastereoisomer of **13**, and could be used as an additional core for the synthesis of aminoglycoside derivatives.



17 **18** **19**, >90% ee

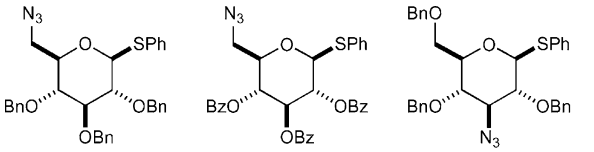
Scheme 3



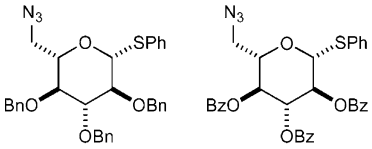
20a **20b**

Preparation of the glycosyl donors

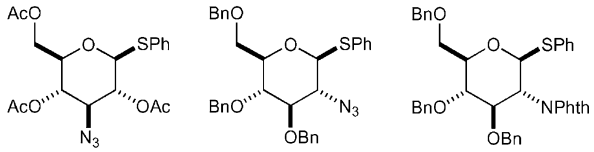
The glycosyl donors **21** were prepared using literature procedures,[10b,17] or using the synthetic approaches described in Scheme 4. The donor **21B** was prepared by benzoylation of **23**. Global deacetylation of **24**, diazo transfer[17] and then *p*-tosylation gave the *p*-tosylate **25**; displacement using sodium azide (→**26**) and benzylation gave the donor **21G**. The donors **21A′**, **21B′** and **21H′** were prepared from L-glucose pentaacetate using methods which were analogous to those used to prepare the enantiomeric donors.[10b,16,18]



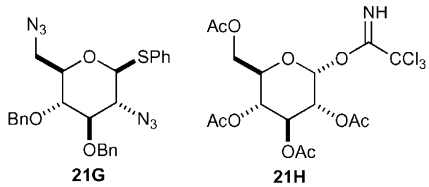
21A **21B** **21C**



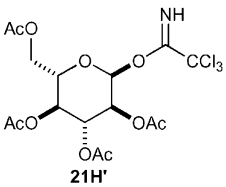
21A′ **21B′**



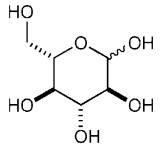
21D **21E** **21F**



21G **21H**



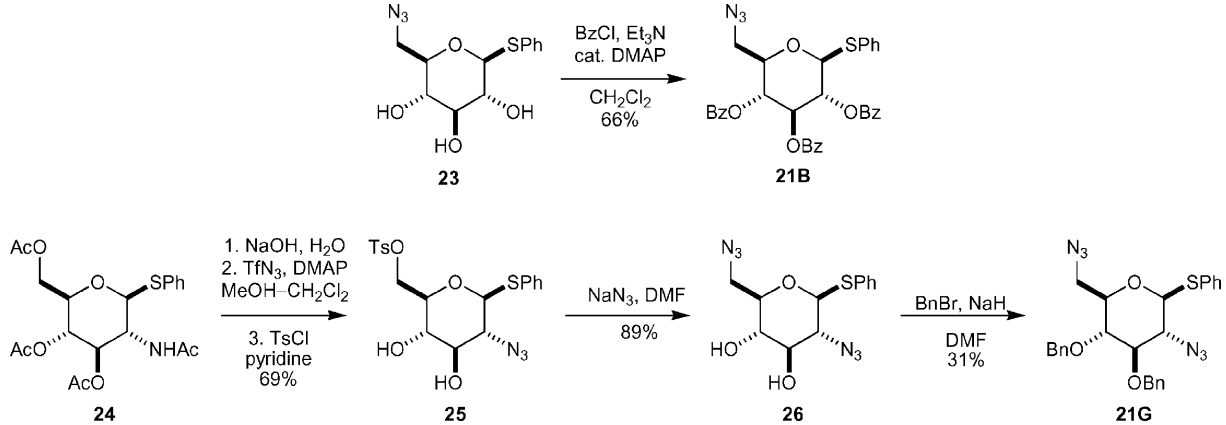
21H′



22A′

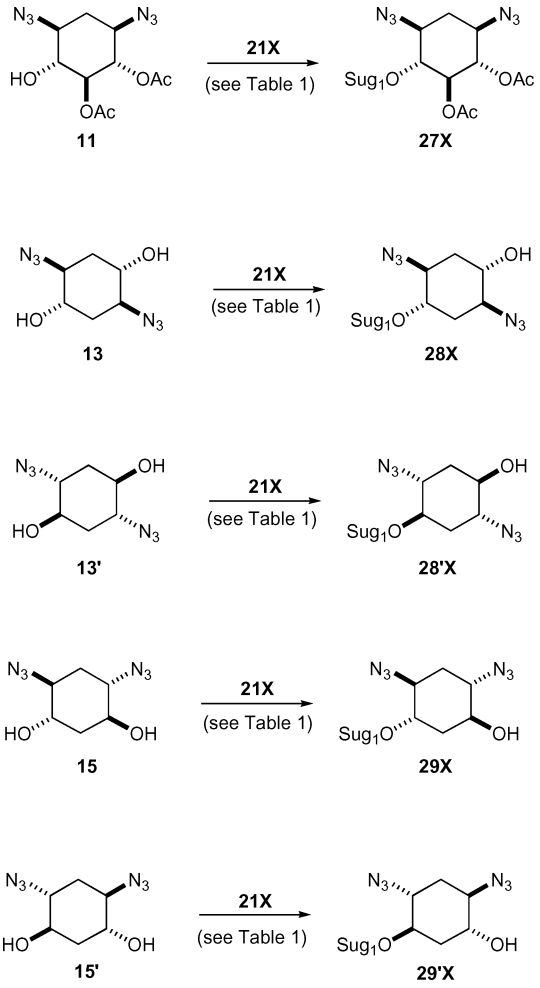
Glycosylation of the cores

The cores **11**, **13**, **13′**, **15** and **15′** were exploited in the preparation of the monoglycosylated aminoglycoside derivatives **27X**–**29X** (see Scheme 5 and Table 1). In order to minimise the number of individual intermediates to be prepared, it was decided to introduce greater structural diversity in the second glycosylation step (see below). Accordingly, the cores were glycosylated with around three of the appropriate donors **21X** under the reaction



23 **21B** **24** **25** **26** **21G**

Scheme 4



Scheme 5

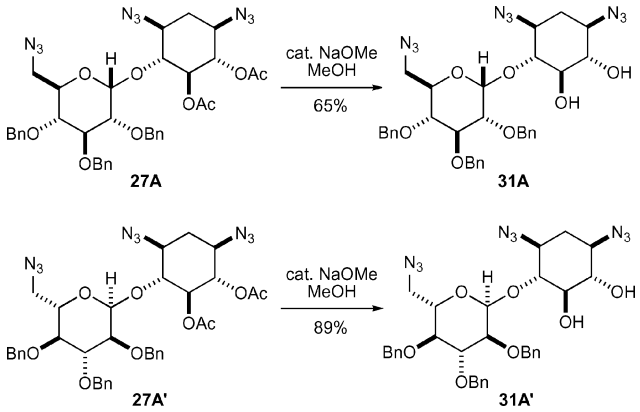
Table 1 Synthesis of the protected aminoglycosides **27**, **28** and **29**

Entry	Starting material	Donor	Conditions[a]	Product	Yield[b] (%)
1a	**11**	21A	A	**27A**	65
1b	**11**	21A′	A	**27A′**	55
1c	**11**	21B	A	**27B**	75
2	**13**[c]	21A	B	**28A**[d]	13
				28′A[d]	3
				28b[d]	4
				28AA[e]	15
				28′AA[e]	2
				28′Ab[e]	5
3a	**15**	21A	A	**29A**	29
3b	**15′**	21A	B	**29′A**	15
				29′AA	2
				29′Ab	1
3c	**15′**	21B	A	**29′B**	8
3d	**15′**	21F	A	**29′F**	45
3e	**15′**	21F[f]	A	**29′FF**	33
3f	**15**	21G	B	**29G**	14
3g	**15′**	21G	B	**29′G**	26

[a] A: NIS, 10 mol% AgOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C; B: NIS, 10 mol% AgOTf, 4 Å molecular sieves, Et_2O–CH_2Cl_2, 0 °C. [b] Yield of purified product. [c] The starting material had 70% ee. [d] 32% of a diastereomeric mixture of **28A**, **28′A** and **28b** was obtained; an aliquot of the product was purified by preparative HPLC to give the yields of purified products shown. [e] 32% of a diastereomeric mixture of **28AA**, **28′AA** and **28Ab** was obtained; an aliquot of the product was purified by preparative HPLC to give the yields of purified products shown. [f] 2.0 equivalents of the donor was used.

conditions which are summarized in Table 1; the products were purified, using preparative HPLC where necessary.

Under our preferred reaction conditions (NIS, 10 mol%, AgOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C), the stereoselectivity of the glycosylations with **21A** / **21A′** and **21B** were complementary and highly stereoselective (entries 1a–c, Table 1): glycosylation of the core **11** with **21A** / **21A′** and **21B** gave the required products **27A** / **27A′** and **27B** as the only detectable diastereoisomers (α and β, respectively). The protected aminoglycoside derivatives **27A** and **27A′** were deacetylated under standard conditions to give **31A** and **31A′** (Scheme 6).



Scheme 6

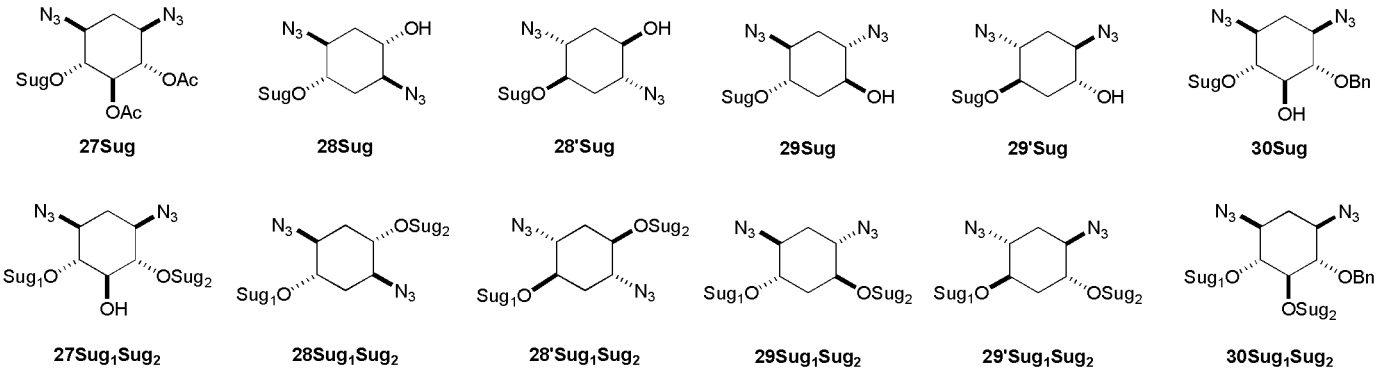
The C_2 symmetry of **13** / **13′** and **15** / **15′**, and the hence homotopicity of their hydroxyl groups, led us to glycosylate these cores without first protecting one of the alcohol groups. However, the functionalisations of **15** / **15′** were still complicated by the possibility of a second glycosylation and by their insolubility in dichloromethane (entries 3a–g, Table 1). With these cores, the glycosylation reactions were performed using a mixed solvent system, ether–CH_2Cl_2. Under these conditions, glycosylation was less stereoselective.[19] For example, **15′** was glycosylated with **21A** to give the expected products **29′A** and **29′AA**, as well as the side product†† **29′Ab** in which one of the sugar substituents is β-linked (compare entries 1a–b with entry 3b).

The ratio of mono-and di-glycosylated products could be varied by changing the equivalents of donor used. For example, with 1.2 equivalents of **21F** relative to the acceptor **15′**, the monoglycosylated product, **29′F**, was obtained in 45% yield. However, with 2.0 equivalents of the donor, an optimized 33% yield of the diglycosylated product, **29′FF**, was obtained.

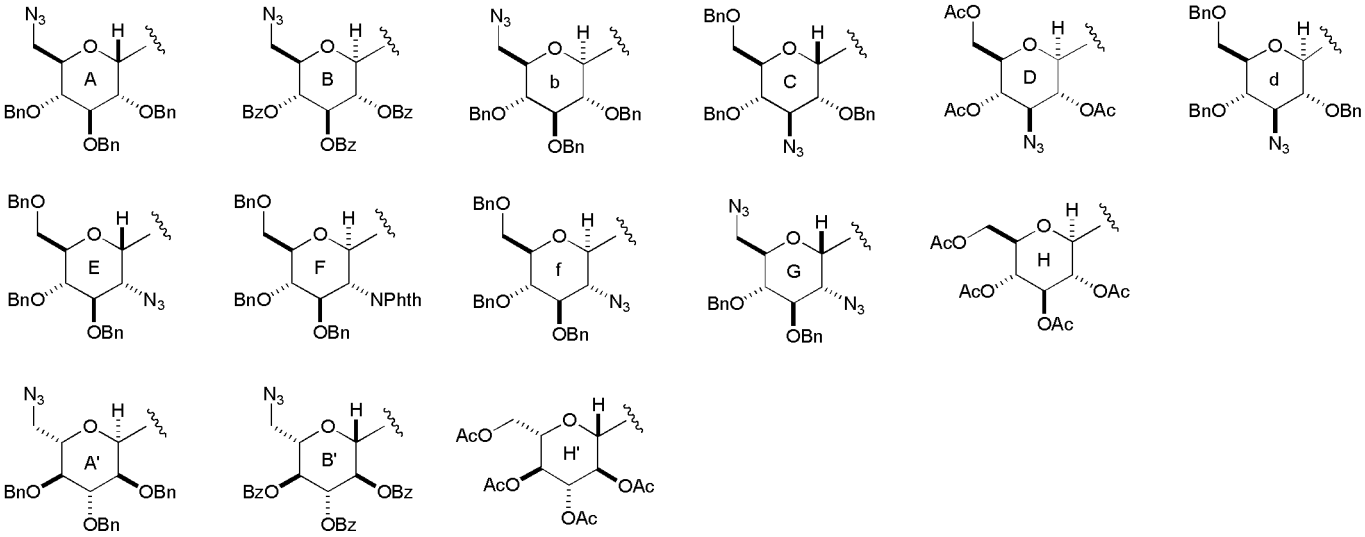
The reactions of the cores **13** and **13′** were further complicated by the poor enantiomeric excesses (70% ee) of these compounds. Glycosylation of the acceptor **13** with the donor **21A** (entry 2, Table 1) gave mixtures of mono-glycosylated products (32%) and di-glycosylated products (32%) (which were then separated by preparative HPLC for characterisation). A summary of the purified products of the reaction is provided in Scheme 7. Not only was the reaction poorly diastereoselective, leading to **28b** and **28Ab**, but significant yields of the products **28′A** and **28′AA**, stemming from the minor enantiomer of the acceptor, were also obtained.

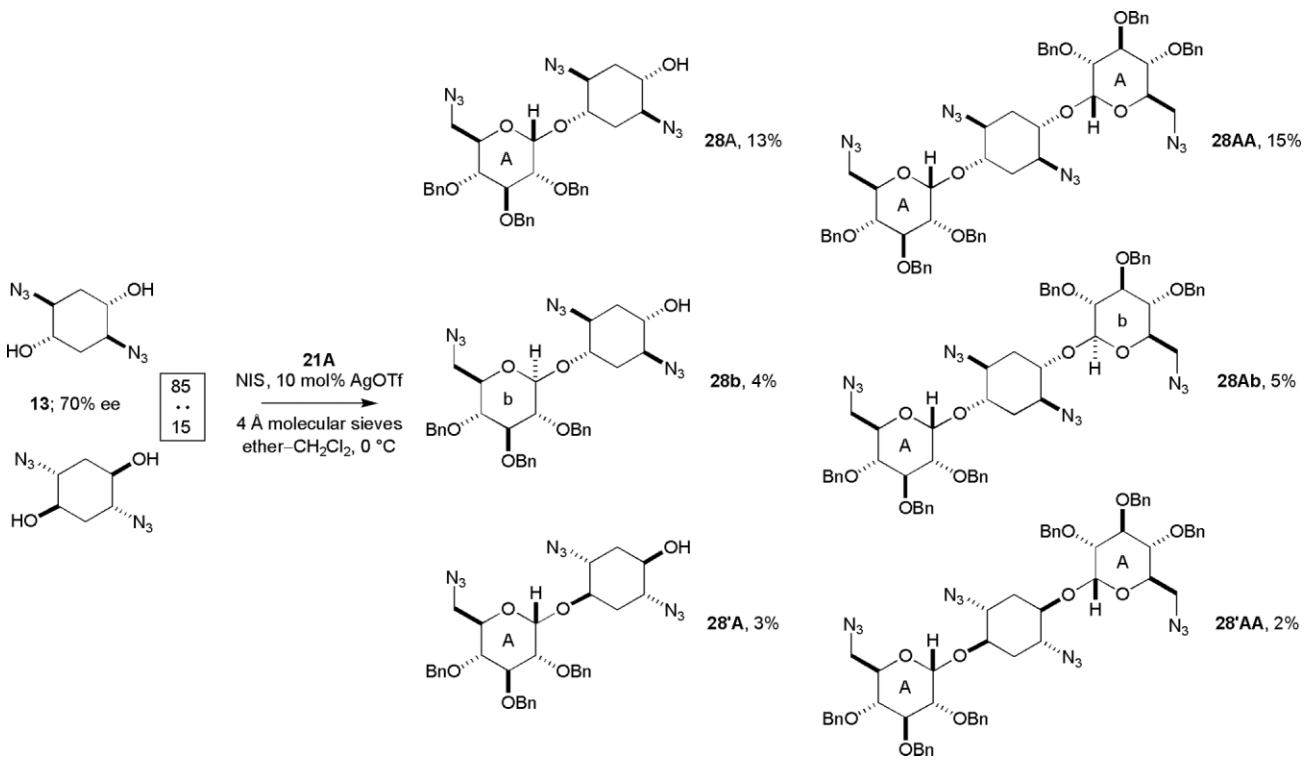
The monoglycosylated products, **28X**, **29X** and **31X**, together with **30G**, prepared by the controlled degradation of a derivative of neomycin,[20] were further modified by glycosylation (see Scheme 8 and Table 2). The glycosylations of **31A** and **31A′** were, on the whole, rather straightforward (entries 1–16). Some

†† Lower case letters are used to describe the side-products of unselective glycosylation reactions. Here, in **29′Ab**, the second sugar ring is β-linked. The use of the lower case descriptor signifies that the protecting group pattern is not the same as that found in the donor **21B**. Hence,**29′AB** and **29′Ab** bear different protecting groups, but deprotection would give the same product, **8′AB**.

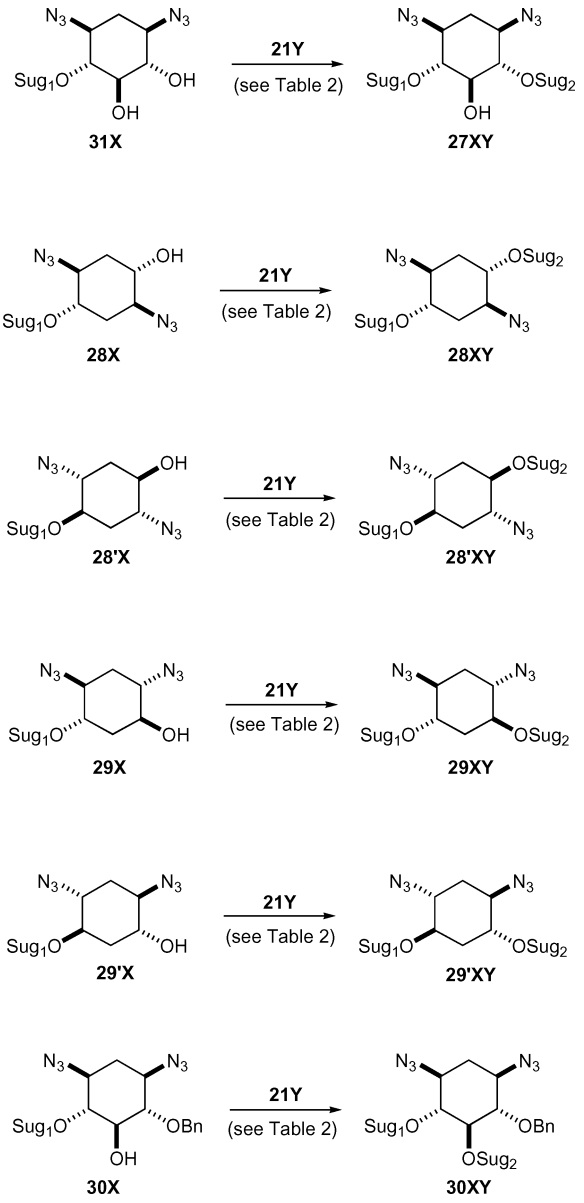


where Sug, Sug_1 and Sug_2 =





Scheme 7



Scheme 8

of the reactions were complicated by poor stereoselectivity: in particular, glycosylations involving the donors **21C** and **21E** gave *ca.* 3 : 1 mixtures of α and β anomers (see entries 9–10 and 13–14).

For the second glycosylation reaction, of the core **28**, we found it easiest to use directly the *ca.* 70 : 15 : 15 diastereomeric mixture of **28A**, **28′A** and **28b** which had been prepared by glycosylation of acceptor **13** (see Scheme 7). Accordingly, the mixture of acceptors was glycosylated with four different donors, and the products were purified by preparative HPLC (entries 17 and 21). Here, the glycosylations were performed in neat dichloromethane; under these conditions,[19] high levels of diastereoselectivity were observed with donor **21A′**, and the corresponding α-linked products **28AA′**, **28′AA′** and **28A′b** were obtained (compare entry 17, Table 2 with entry 2, Table 1). With donor **21C**, a lower level of diastereoselectivity was observed, resulting in a significant (11%) yield of the β-linked product **28Ad** (entry 20, Table 2). The glycosylations of the acceptors **29X** were straightforward, and the corresponding diglycosylated products were easily obtained in each case (see entries 22–28, Table 2).

The acceptor **30G**, prepared by controlled degradation of a neomycin derivative,[20] was rather more hindered than those previously used. A reasonable (25%) yield of **30GF** was obtained with donor **21F** under our standard glycosylation conditions. In order the install the H and H′ rings, however, it proved necessary to use the imidate[21,22] donors **21H** and **21H′** in conjunction with a Lewis acid (boron trifluoride etherate):[10c] under these conditions, reasonable yields of the required products **30GH** and **30GH′** were obtained.

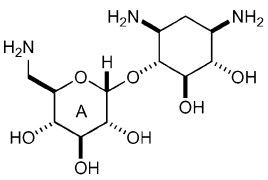
Table 2 Synthesis of the protected aminoglycosides **27**, **28**, **29** and **30**

Entry	Starting material	Donor	Conditions[a]	Product	Yield[b] (%)
1	**31A**	**21A**	A	**27AA**	62
2	**31A**	**21A′**	A	**27AA′**	50
3	**31A′**	**21A**	A	**27A′A**	21
4	**31A′**	**21A′**	A	**27A′A′**	26
5	**31A**	**21B**	A	**27AB**	51
6	**31A**	**21B′**	A	**27AB′**	28
7	**31A′**	**21B**	A	**27A′B**	32
8	**31A′**	**21B′**	A	**27A′B′**	26
9	**31A**	**21C**	A	**27AC**	30
				27Ad	11
10	**31A′**	**21C**	A	**27A′C**	40
				27A′d	12
11	**31A**	**21D**	A	**27AD**	54
12	**31A′**	**21D**	A	**27A′D**	37
13	**31A**	**21E**	A	**27AE**	24
				27Af	24
14	**31A′**	**21E**	A	**27A′E**	19
				27A′f	26
15	**31A**	**21F**	A	**27AF**	51
16	**31A′**	**21F**	A	**27A′F**	42
17	**28A**[c]	**21A′**	A	**28AA′**	21
				28′AA′	5
				28A′b	10
18	**28A**[c]	**21B**	A	**28AB**	45
				28′AB	3
				28Bb	5
19	**28A**[c]	**21B′**	A	**28AB′**	42
				28′AB′	3
				28bB′	9
20	**28A**[c]	**21C**	A	**28AC**	30
				28′AC	4
				28Ad	11
				28bC	8
21	**28A**[c]	**21D**	A	**28AD**	8
22	**29A**	**21A**	B	**29AA**	18
23	**29′A**	**21A′**	B	**29′AA′**	28
24	**29′A**	**21B**	A	**29′AB**	37
25	**29′A**	**21B′**	A	**29′AB′**	34
26	**29′A**	**21C**	B	**29′AC**	48
27	**29′F**	**21B**	A	**29′BF**	11
28	**29′F**	**21F**	A	**29′FF**	33
29	**30G**	**21F**	A	**30GF**	25
30	**30G**	**21H**	B	**30GH**	22
31	**30G**	**21H′**	B	**30GH′**	38

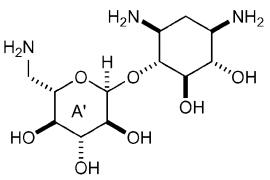
[a] A: NIS, 10 mol% AgOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C; B: $BF_3{\cdot}Et_2O$, CH_2Cl_2, −60 °C, 4 Å molecular sieves. [b] Yield of purified product. [c] The starting material was a *ca.* 70 : 15 : 15 diastereomeric mixture of **28A**, **28′A** and **28b**.

Deprotection of the aminoglycoside derivatives

The protected aminoglycosides **27**–**30** were deprotected under standard conditions to give the aminoglycoside derivatives **6**–**9** (Table 3) . First, acyl and/or phthalimido protecting groups were removed, if necessary. Two alternative procedures were used for the reduction of the azides: either Staudinger[10b] reaction with PMe_3, followed by purification through a short silica–celite column, or hydrogenolysis[10c] in 1 : 1 : 1 EtOAc–MeOH–H_2O. Finally, the remaining benzyl groups were removed by hydrogenation in 1 : 1 H_2O–AcOH to give the unprotected aminoglycoside derivatives as their peracetate salts. In practical terms, our preferred method for azide reduction and benzyl deprotection involved the two hydrogenolysis reactions: first in 1 : 1 : 1 EtOAc–MeOH–H_2O and then in 1 : 1 H_2O–AcOH.



6A



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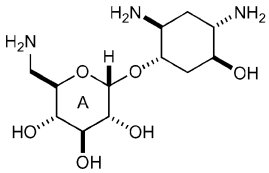
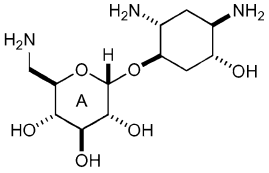


8A



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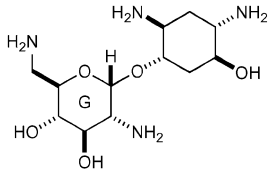
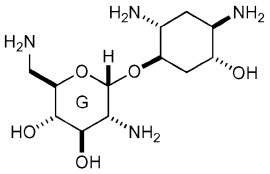
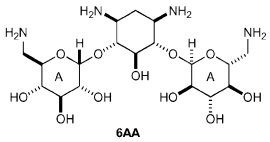


8G



8'G



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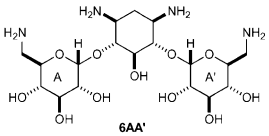

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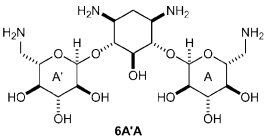
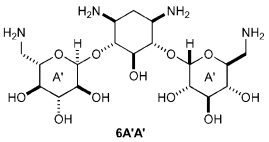


6A'A



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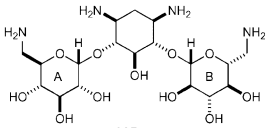

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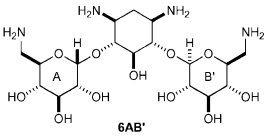

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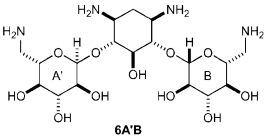

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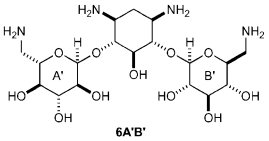

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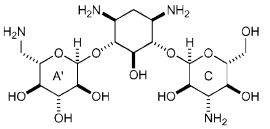

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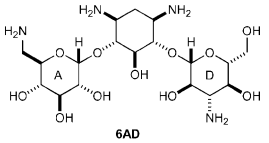

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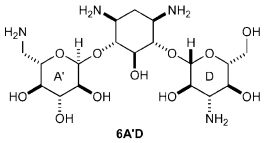
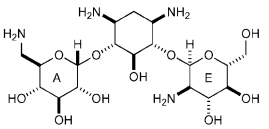


6A'D



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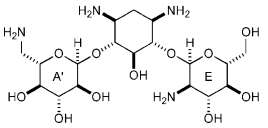

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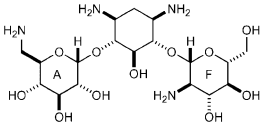

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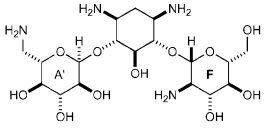

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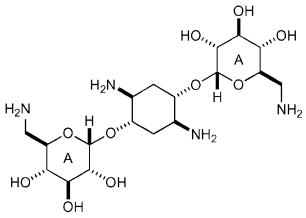

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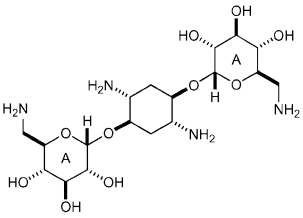

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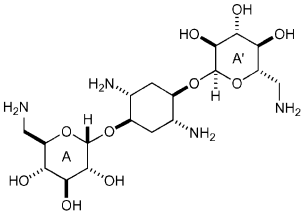

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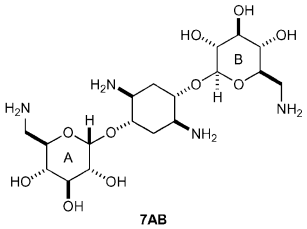

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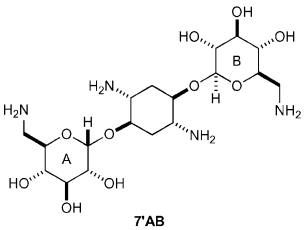

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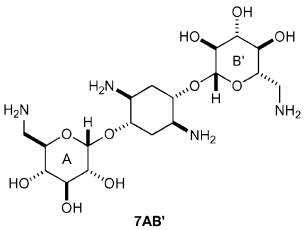

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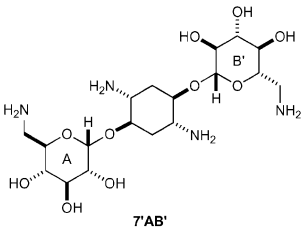

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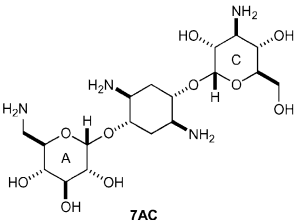

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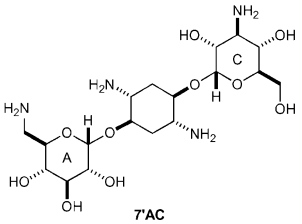

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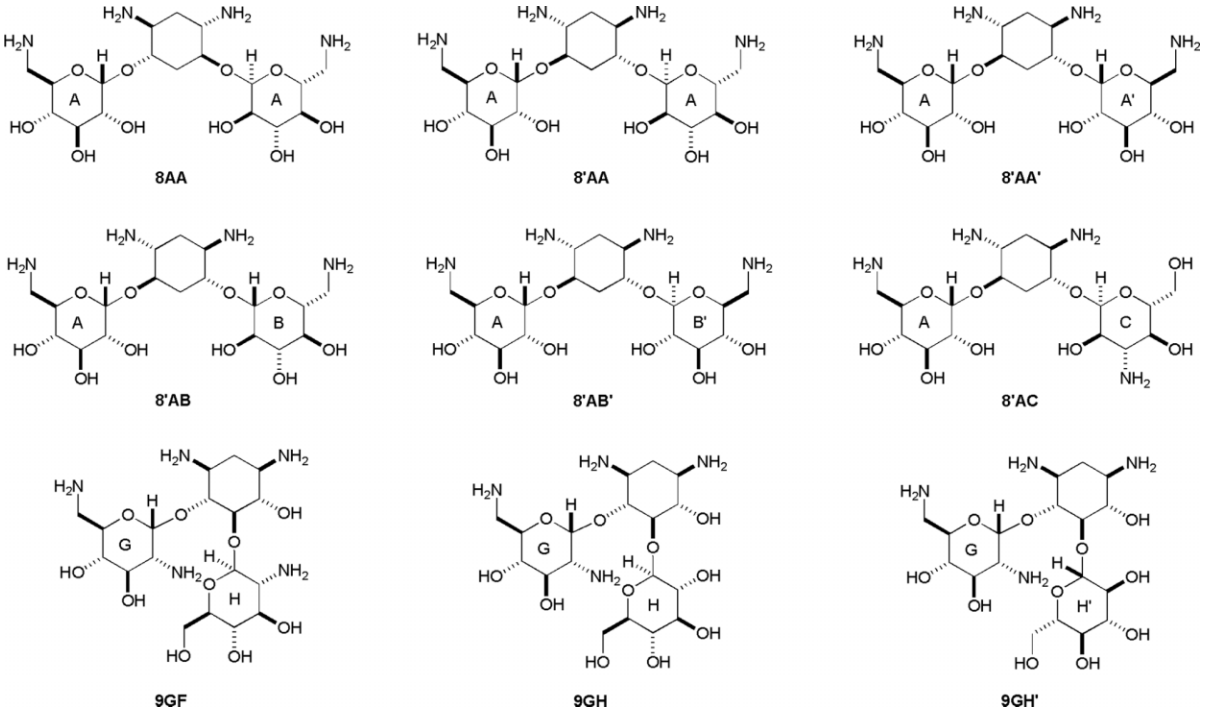

Table 3 Deprotection reactions to yield the aminoglycosides **6**, **7**, **8** and **9**

Entry	Starting material	Conditions[a]	Product	Yield[b] (%)
1	**31A**	a,b	**6A**	86
2	**31A′**	a,b	**6A′**	51
3	**29A**	e,b	**8A**	42
4	**29′A**	e,b	**8′A**	100
5	**29G**	e,b	**7G**	67
6	**29′G**	e,b	**7′G**	100
7	**27AA**	a,b	**6AA**	77
8	**27AA′**	a,b	**6AA′**	25
9	**27A′A**	a,b	**6A′A**	46
10	**27A′A′**	a,b	**6A′A′**	41
11	**27AB**	c,a,b	**6AB**	37
12	**27AB′**	c,a,b	**6AB′**	58
13	**27A′B**	a,b	**6A′B**	48
14	**27A′B′**	a,b	**6A′B′**	33
15	**27A′C**	a,b	**6A′C**	88
16	**27AD**	a,b	**6AD**	56
17	**27A′D**	a,b	**6A′D**	67
18	**27AE**	a,b	**6AE**	63
19	**27A′E**	a,b	**6A′E**	6
20	**27AF**	d,a,b	**6AF**	4
21	**27A′F**	d,a,b	**6A′F**	4
22	**28AA**	e,b	**7AA**	85
23	**28′AA**	e,b	**7′AA**	84
24	**28′AA′**	e,b	**7′AA′**	68
25	**28AB**	c,e,b	**7AB**	77
26	**28′AB**	c,e,b	**7′AB**	98
27	**28AB′**	c,e,b	**7AB′**	83
28	**28′AB′**	c,e,b	**7′AB′**	98
29	**28AC**	e,b	**7AC**	79
30	**28′AC**	e,b	**7′AC**	73
31	**29′AA**	e,b	**8′AA**	58
32	**29AA**	e,b	**8AA**	98
33	**29′AA′**	e,b	**8′AA′**	77
34	**29′AB**	c,e,b	**8′AB**	59
35	**29′Ab**	e,b	**8′AB**	82
36	**29′AB′**	c,e,b	**8′AB′**	97
37	**29′AC**	e,b	**8′AC**	59
38	**30GF**	d,a,b	**9GF**	42
39	**30GH**	c,a,b	**9GH**	48
40	**30GH′**	c,a,b	**9GH′**	61

[a] (a) PMe_3, THF–0.1 M NaOH solution; (b) H_2, $Pd(OH)_2/C$ (20% Degussa type), 1 : 1AcOH–H_2O; (c) NaOMe, MeOH; (d) N_2H_4, toluene–EtOH, 110 °C; (e) H_2, $Pd(OH)_2/C$ (20% Degussa type), 1 : 1 : 1 EtOAc–MeOH–H_2O. [b] Yield of purified products.

Summary

A total of 40 aminoglycoside derivatives were prepared that might be expected to probe large regions of conformational space. The most dramatic variation was the central core of the derivatives: although most of the derivatives (**6** and **9**) had the 2-deoxystreptamine core found in the aminoglycoside antibiotics, C_2-symmetrical cores were also incorporated in the derivatives **7** and **8**. The configuration of the aminoglycosides was varied widely: both D- and L-sugars were attached to the cores through both α- and β-glycosidic linkages. Furthermore, the number and regiochemistry of the amino substituents in the sugar rings were varied.

The library might be exploited as a refined tool for the analysis of many RNA-mediated biological processes. Since the library is based on structures which are largely isomeric, the specificity of interactions with RNA may be probed critically by *in vitro* study. Furthermore, the library may be a valuable resource in chemical genetic studies, with isomeric structures providing useful controls in cell-based assays. The details of these experiments will be reported in due course.

Experimental

Characterisation data for all of the compounds described in this paper is provided in the electronic supplementary information. General procedures for the synthesis of the aminoglycoside library are provided below.

General procedure for glycosylation (Method A)

The glycosyl donor (0.43 mmol) and the acceptor (0.34 mmol), both freshly dried azeotropically by removal of toluene, were dissolved in dry dichloromethane (2.6 mL) and transferred *via* syringe into a flame dried round bottom flask containing activated 4 Å molecular sieves. The reaction mixture was cooled to 0 °C, *N*-iodosuccinimide (104 mg, 0.46 mmol) and silver(I) trifluoromethanesulfonate (9 mg, 0.03 mmol) were added simultaneously, the reaction was stirred for 2 h and then quenched with Et_3N (1 mL). The reaction mixture was filtered through celite, eluting with dichloromethane (15 mL), then washed with 10% aqueous $Na_2S_2O_3$ solution (2 × 10 mL) and brine (2 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product.

General procedure for glycosylation (Method B)

The glycosyl donor (5.57 mmol) and the acceptor (7.06 mmol), both freshly dried azeotropically by removal of toluene, were dissolved in dry dichloromethane (13 mL) and diethyl ether (40 mL), respectively, and transferred *via* syringe into a flame dried round bottom flask containing activated 4 Å molecular sieves. The reaction mixture was cooled to 0 °C, *N*-iodosuccinimide (1.63 g, 7.23 mmol) and silver(I) trifluoromethanesulfonate (286 mg, 1.11 mmol) were added simultaneously, the reaction was stirred for 3 h and then quenched with Et_3N (5 mL). The reaction mixture was filtered through celite, eluting with dichloromethane (50 mL), then washed with 10% aqueous $Na_2S_2O_3$ solution (2 × 50 mL) and brine (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product.

General procedure for glycosylation with a trichloroacetimidate donor (Method C)

A solution of the trichloroacetimidate donor (1 equivalent), the acceptor (1.2 equivalents) and powdered 4 Å molecular sieves (80 mg) in dichloromethane (5 mL) were stirred at room temperature for 1 h. The reaction mixture was cooled to −60 °C and boron trifluoride diethyl etherate (0.1 equivalents) was added dropwise. The reaction was stirred at −60 °C for 2 h and then quenched with solid $NaHCO_3$ and stirred for 15 min. The mixture was filtered through celite, washing with dichloromethane and EtOAc, and the solvent was removed under reduced pressure to yield the crude product.

General procedure for the deprotection of benzylated aminoglycoside derivatives (Method D)

The perbenzylated azidoaminoglycoside (0.12 mmol) was dissolved in a solution of THF (3.6 mL), and 0.1 M aqueous sodium hydroxide solution (0.3 mL) and trimethylphosphine (0.82 mL, 1 M in THF, 6 eq., 0.82 mmol) were added. The reaction was stirred at 50 °C and monitored by TLC (elution: 2 : 1 *i*PrOH–NH_4OH). After 2 h the reaction mixture was cooled to room temperature, loaded onto a short column (4 cm silica and 1 cm of celite) and then eluted (gradient; 1 : 0 : 0 → 1 : 1 : 0 → 0 : 1 : 0 → 0 : 2 : 1 THF–MeOH–NH_4OH). The fractions containing the required product were collected, concentrated under reduced pressure and dissolved in a degassed solution of 1 : 1 AcOH–H_2O (4 mL). To this solution, $Pd(OH)_2/C$ (20% Degussa type) was added, and the reaction was stirred at room temperature under atmospheric pressure of hydrogen. After 2 d, the reaction mixture was filtered through a short pad of celite, eluted with water, and the filtrate was then concentrated under reduced pressure.

General procedure for the debenzoylation (Method E)

Sodium methoxide (0.5 eq.) was added to a solution of benzylated aminoglycoside (0.22 mmol) in dry MeOH (1.4 mL). The reaction mixture was stirred at room temperature for 18 h and then concentrated under reduced pressure.

General procedure for the removal of a phthalimide group (Methods F)

Hydrazine acetate (83.0 mg, 0.90 mmol) was added in one portion to a stirred solution of the protected aminoglycoside (55.6 mg, 0.45 mmol) in toluene (0.8 mL) and ethanol (1.2 mL). The reaction mixture was heated at reflux at 110 °C for 5 d. The reaction was allowed to cool to room temperature and the solvent was removed under reduced pressure. The resulting residue was redissolved in 1 : 1 dichloromethane–ethanol and washed with water (20 mL), and the aqueous layer was back-extracted with 1 : 1 dichloromethane–ethanol (10 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent removed under reduced pressure to give the crude product which was purified by column chromatography (elution: 7 : 3 petrol–EtOAc) to yield a solution of the crude product which was evaporated under reduced pressure.

Alternative procedure for the deprotection of benzylated aminoglycosides (Method G)

The perbenzylated azidoaminoglycoside (142.4 mg, 0.12 mmol) was dissolved in 1 : 1 : 1 EtOAc–MeOH–H_2O (6 mL), $Pd(OH)_2/C$ (150 mg) was added and the reaction was stirred under an atmospheric pressure of hydrogen. After 2 d, the reaction mixture was filtered through a short pad of celite, eluting sequentially with ethyl acetate, methanol and water. The filtrate was concentrated under reduced pressure, redissolved in a degassed solution of 1 : 1 AcOH–H_2O (4 mL), $Pd(OH)_2/C$ (20% Degussa type) added and the reaction mixture stirred under an atmospheric of hydrogen. After 2 d, the reaction mixture was filtered under a short pad of celite, eluting with water. The filtrate was concentrated under reduced pressure to give the crude product.

Acknowledgements

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