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Synthesis of Linked Carbohydrates and Evaluation of Their Binding for 16S RNA by Mass Spectrometry

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Abstract—A library of linked molecules were synthesized from the common sugar moieties existing in the natural amino glycosides. These linked molecules were screened against bacterial 16S RNA for their binding affinity using a mass spectrometry-based technology. Some of these compounds exhibited low micromolar affinity and could serve as leads for further development as antibacterial agents.

The potential of RNA as a new drug target has recently come to the core, with the recognition that RNA molecules can adopt complex three-dimensional structures that, as with proteins, enable the design of specific ligands.[1–7] Another reason for the present interest comes from the fact that many pathogenic agents, such as retroviruses, encode their genetic information in RNA strands.[8–10] Amino glycoside antibiotics (Fig. 1) have long been used as very efficient drugs against Gram-positive and Gram-negative bacteria, and against mycobacterial infections.[11] These molecules, however, impair hearing and kidney functions at high doses and resistant strains are appearing at an increasing rate.[12–15] In the meanwhile, their complex chemical structures have impeded studies to discover aminoglycoside analogues with improved pharmaceutical properties. It is of great interest to find compounds with improved properties and simplified chemical structures.[16–23]

The aminoglycoside antibiotics are thought to function by binding to the decoding region of bacterial 16S ribosomal RNA, thus causing premature termination and mistranslation of proteins and consequently, bacterial death.[24–26] The interaction between aminoglycosides and the decoding region was recently characterized using a 27-nucleotide RNA molecule containing the target site for these antibiotics. The oligonucleotide mimics the binding affinity and specificity of aminoglycosides binding to the ribosome.[27,28] The structure of the RNA oligonucleotide complex with the aminoglycoside paromomycin has been determined by NMR and X-ray crystallography.[29] The 27 nucleotide construct has been used by several research groups in the RNA binding studies (Fig. 2).

In search for new and more specific compounds, we have developed mass-spectrometry based high-throughput screening technologies.[1,30–32] These technologies allow us to discover ligands that bind to the target RNA and to carry out SAR studies around the lead

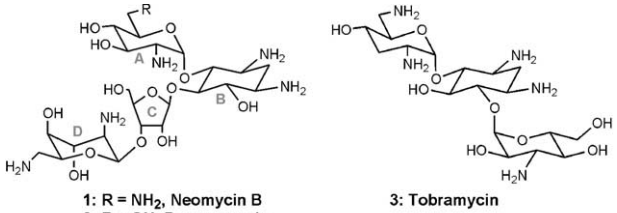

Figure 1. Structures of neomycin B, paromomycin and tobramycin.

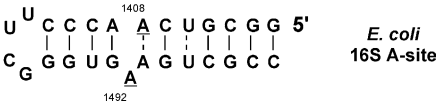

Figure 2. Sequence of the *Escherichia coli* 16S RNA A-site.

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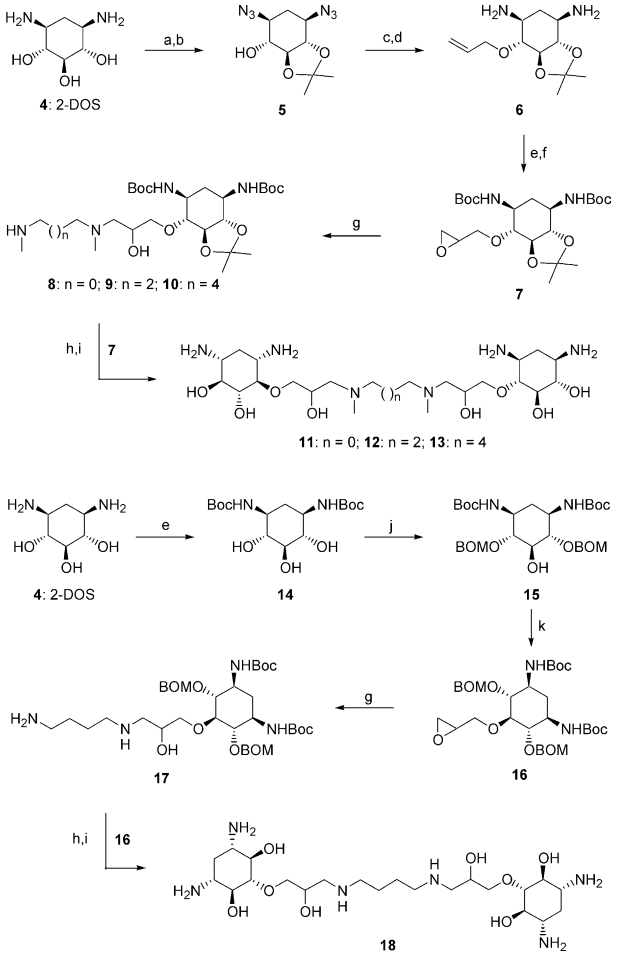
compounds. We have been using the 27-nucleotide construct for the discovery of RNA binding molecules with potential application in developing novel antibacterial agents.[22,23]

Several major classes of natural aminoglycosides have been discovered. These include the neomycin class, kanamycin class and gentamicin class. Many of these aminoglycosides have a common 2-deoxystreptamine (2-DOS, **4**) moiety, which suggest the important role of this moiety. Previous studies have established the direct involvement of the neomycin A ring and paromomycin A ring in causing the misreading in the bacterial translation. Both A rings were shown to contribute significantly to the binding affinity and specificity for the target 16S RNA.[25] We have found that 2-DOS and neomycin A ring binds to the target 16S RNA with micromolar affinity. Since these aminosugar moieties bind to the target with two or more binding sites, it was perceived that linking these aminosugar moieties with a proper linker could enhance the binding affinity and specificity. Recently, Wong and coworkers have synthesized a series of neamine dimers that showed significant binding to the target RNA and exhibited low micromolar MIC activity against bacteria.[33] Encouraged by their results, we synthesized a library of linked molecules with different linker length based on these motifs to study their binding properties for the 16S RNA.

The synthesis of the 4-O substituted 2-DOS dimers **11**–**13** and the related intermediates is shown in Scheme 1. 2-DOS (**4**) was first treated with TfN_3 followed by the protection of the adjacent hydroxy groups as an acetonide. The free hydroxy group was then allowed to react with allyl bromide. Expoxidation of the resulted allyl ether led to the formation of the corresponding epoxide as a mixture of two diastereomers in a 1:1 ratio. For the purpose of diversity synthesis and quick screening, all the linked compounds were synthesized as a mixture of diastereomers and used directly for the RNA binding assays in this study. Reaction of epoxide **7** with three different diamines gave the corresponding intermediates **8**–**10** with proper linkers that are ready for coupling with another sugar moiety. Although reaction of two equivalents of epoxide **7** with one equivalent of linker diamine gave the corresponding dimers **11**–**13**, it was more efficient to react **8**–**10** with **7**, which gave the desired products in excellent yields and purity after deprotection. These compounds were used directly for our MS-based screening assay.

The synthesis of the 5-O substituted 2-DOS dimer **18** started from **4** again (Scheme 1). The selective introduction of susbtituents at 5-hydroxy group was not a trivial task. No efficient process has been reported in the literature. We have developed a highly efficient and practical route towards this problem. The two amino groups in 2-DOS were first protected with Boc. Treatment of **14** with BOMCl in the presence of DIEA and catalytic amount of TBAI led to the formation of the desired intermediate **15** in 68% yield together with its regioisomer (15%). In a similar fashion to the synthesis of **8**–**10**, the desired intermediate **17** was prepared in three steps from **15** by allylation, epoxidation and amination. Again, the corresponding dimer **18** was obtained in excellent yield and purity by reacting **17** with one equivalent of **16** followed by the deprotection of the Boc and acetonide protecting groups.

The synthesis of the paromomycin A-ring dimers **26**–**28** started from readily available *N*-acetyl glucosamine (Scheme 2). Selective allylation of *N*-acetyl glucosamine with allyl alcohol and $BF_3{\cdot}Et_2O$ gave the α-anomer in 71%. The acetate was removed with calcium carbonate and the C4 and C6 hydroxy groups were protected as an acetonide. Protection of the amino group in **21** with Boc followed by epoxidation led to **22** in 78% overall yield, which was converted to the corresponding monomers **23**–**25** with different lengths of linkers. The corresponding dimers **26**–**28** were then obtained by reacting with the corresponding linker amines and removal of the protecting groups.

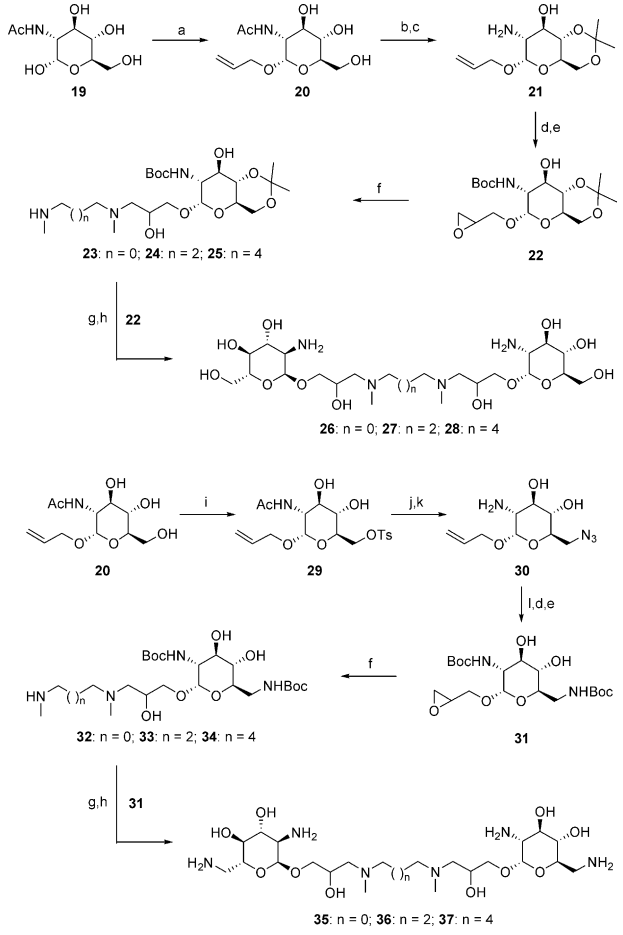


Scheme 1. Synthesis of 2-deoxystreptamine (2-DOS) dimers. Reagents and conditions: (a) TfN_3, $CuSO_4$, MeOH, Et_3N, 85%; (b) $Me_2C(OMe)_2$, PPTS, DMF, 100 °C, 98%; (c) NaH, allyl bromide, DMF, 0 °C, 93%; (d) Me_3P, NaOH, MeOH, 88%; (e) Boc_2O, NaOH, dioxane, 90–95%; (f) *m*CPBA, CH_2Cl_2, $NaHCO_3$, 82%; (g) 10 equiv $MeNH(CH_2)_4NHMe$, EtOH, reflux, 95%; (h) 1.0 equiv epoxide (**7** or **16**), EtOH, reflux, 90–95%; (i) 4.0 M HCl, dioxane, 90–95%; (j) BOMCl, EtN^iPr_2, TBAI, 80 °C, 68% plus 15% regioisomer; (k) NaH, DMF, glycidol triflate, 85%.

The synthesis of the neomycin A-ring dimers **35**–**37** started from **20**. Selective tosylation and displacement of the resulted tosylate converted the primary alcohol into the corresponding azide. The azide was then reduced with PMe_3, and the amino groups were protected with Boc. Again, the terminal olefin was converted into epoxide with *m*CPBA, which reacted with three linker amines to give the corresponding intermediates **32**–**34** and dimers **35**–**37** by following the same procedures as described above.

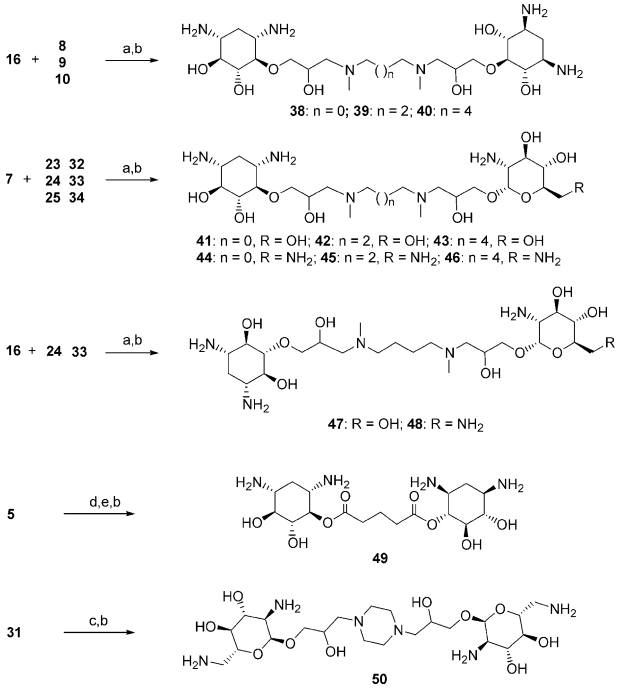
The crossly linked molecules **38**–**48** were easily prepared by following similar procedures as for the dimer synthesis described in Schemes 1 and 2 (Scheme 3). All these linked compounds were obtained in excellent yields and purity. To explore alternative linkers for the library synthesis, compounds **49** and **50** were also synthesized, which have a bisester linker and a piperazine linker respectively.

For comparison, the aminosugar intermediates in their free form were prepared by easily removing the acid labile protecting groups with hydrochloric acid (Scheme 4).

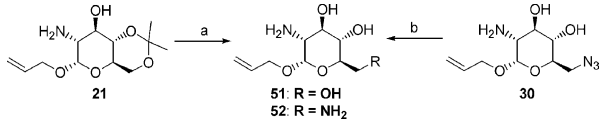


Scheme 2. Synthesis of neomycin A ring and glucosamine dimers. Reagents and conditions: (a) allyl alcohol, $BF_3{\cdot}Et_2O$, 71%; (b) $Me_2C(OMe)_2$, PPTS, 95%; (c) $Ca(OH)_2$, 89%; (d) Boc_2O, NaOH, dioxane, 90–90%; (e) *m*CPBA, $NaHCO_3$, 75–80%; (f) 10 equiv $MeNH(CH_2)_nNHMe$, EtOH, reflux, 90–95%; (g) EtOH, epoxide (**22** or **31**) reflux, 90–95%; (h) HCl, MeOH, 90–95%; (i) TsCl, EtN^iPr_2, 82%; (j) NaN_3, 91%; (k) $Ca(OH)_2$, 87%; (l) PMe_3, NaOH, MeOH, 91%.

The linked molecules were screened against 16S RNA for their binding affinity using a mass spectrometry-based assay and their estimated K_d are shown in Table 1.[32] Data suggest that all the linked molecules tested have improved binding affinity for 16S RNA compared to the corresponding monomers. While the 2-DOS dimmers (**11**–**13**, **18**, **49**) showed moderate improvement over 2-DOS (**4**), the glucosamine dimmers acquired significant improvement in potency over the corresponding monomers (**26**–**28** vs **51**; **35**–**37** and **50** vs **52**). In particular, **27** exhibited more than 35-fold improvement in potency over **51**. The cross-linked molecules (**38**–**48**) between 2-DOS and glucosoamine also exhibited improved binding affinity over the corresponding monomers (**4**, **51** and **52**). The data also suggest that the glucosamine dimmers have higher potential for binding affinity improvement as compared to the 2-DOS dimmers. In general, compounds with relatively longer linkers ($n = 2$ and 4) exhibited higher potency (**11** vs **12** and **13**, **26** vs **27** and **28**, **41** vs **42** and **43**, **44** vs **45** and **46**). Among these compounds, **12**, **36**, **37** and **45** showed highest potency with K_d in the low μM range. Studies to further improve the potency of these compounds for



Scheme 3. Synthesis of cross-linked molecules from 2-DOS, glucosamine and neomycin A ring, and dimers with piperazine and ester linkers. Reagents and conditions: (a) EtOH, reflux, >95%; (b) HCl, MeOH, >95%; (c) 0.5 equiv piperazine, ethanol, reflux, >95%; (d) $HO_2C(CH_2)_3CO_2H$, PS-carbodiimide, EtN^iPr_2, 75%; (e) PMe_3, NaOH, MeOH, 90%.



Scheme 4. (a) HCl, MeOH, 95%; (b) Me_3P, NaOH, MeOH, 91%.

Table 1. Binding affinity of the linked molecules for 16S RNA (μM)

Compd	K_d	Compd	K_d
4	130	**40**	36
11	57	**41**	335
12	16	**42**	54
13	21	**43**	39
18	96	**44**	47
26	146	**45**	13
27	45	**46**	32
28	52	**47**	29
35	20	**48**	25
36	16	**49**	105
37	11	**50**	32
38	44	**51**	1587
39	26	**52**	327

16S RNA and to evaluate the potential of these compounds application in antibacterial research shall be reported in due course.

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