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Studies on the synthesis of neamine-dinucleosides and neamine-PNA conjugates and their interaction with RNA

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ABSTRACT

Two types of neamine derivatives, neamine-dinucleotide conjugates **8a–g** and neamine-PNA conjugates **12a–c** and **14a–d**, were synthesized. Compound **8a–g** were synthesized by the condensation of azido-neamine with dinucleotide-5′-carboxylic acids, followed by reduction and deprotection. Compound **12a–c** and **14a–d** were synthesized by the similar strategy. The binding affinities of conjugates **8a–g, 12a–c**, and **14a–d** towards 16S RNA, 18S RNA, and TAR RNA were evaluated by SPR. It indicates that conjugates **12a–c** and **14a–d** interact with 16S, 18S RNA at the same level as that of neamine, **14a** and **14d** show about twofold binding affinities to TAR RNA compared to that of neamine. However, the neamine-dinucleotide conjugates **8a–g** exhibit very weak binding affinities to 16S, 18S, and TAR RNA, computer modelling results that negative–negative electrostatic repulsion of phosphate group in compound **8a–g** and RNA leads to a sharp decrease of the binding affinities compared with that of neamine, neamine-nucleoside and neamine-PNA conjugates.

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Aminoglycosides, such as neomycin or paramycin, are well-known natural products as clinical used important antibiotics. The mechanism of their anti-bacteria activities is the interaction of these molecules and ribosome RNA of bacteria, accordingly interfering with protein biosynthesis.[1] Unfortunately, aminoglycosides are far from ideal antibiotics due to their toxicities and adverse affects which are caused by the lack of selectivity towards different RNAs.[2] Recently, many efforts have been made to design specific RNA binders. With the determination of structure of RNA-aminoglycoside complex by X-ray or NMR,[3] there have been more specific RNA binders since then.[4]

Other study shows that PNAs (peptide nucleic acid), which are DNA analogues with a 2-aminoethyl-glycine linkage replacing the phosphodiester backbone, are capable of sequence specific recognition of DNA and RNA, obeying the Watson–Crick hydrogen bonding or/and Hoogsteen schemes.[5] Short PNA probes were shown to be able to disturb and finally to bind folded RNA structures.[6] Gregory Upert and coworkers synthesized a series of constrained cyclic PNA derivatives containing the octameric PNA fragment 5′-GTCCCAGA-3′, and UV-monitored thermal denaturation studies demonstrate that these cyclic PNAs are able to strongly interact with their trans-activation response (TAR) RNA target, very likely through the formation of a 6-bp stable complex.[7] Based on the NMR structure of TAR RNA, Raphael Terreux designed and synthesized two peptide nucleic (PNA) analogue based molecules which connected with arginine through a linker. CD spectra indicate that both designed molecules do interact with TAR and they were active against HIV-1 at micro-molar level.[8]

Based on the rich of adenine base in the binding pocket of A-site rRNA, we synthesized a series of neamine-nucleoside conjugates to improve their binding affinity and specificity to 16S and 18S RNA.[9] It was found that compound **1** (Fig. 1) could bind specifically to 16S RNA and computer simulation indicated that the thymine moiety of compound **1** could form hydrogen bonding with adenine residue of 16S RNA.

To understand more information on the interaction between the designed molecules and RNA, we synthesized neamine-dinucleosides (type I) as well as neamine-PNA heteroconjugates (type II), aiming to increase their binding affinity and selectivity towards RNA. Herein, the synthesis and their binding properties to 16S RNA, 18S RNA, and TAR RNA were reported.

*Synthesis of dinucleosides-5′-carboxylic acids **6a–d**.* Dinucleosides were synthesized through the phosphoramidite strategy (Scheme 1).[10] Phosphoramidite **4** was coupled with the free 5′-hydroxyl group of deoxynucleoside **3** followed by iodine oxidation and selective removal of the DMTr group using trifluoroacetic acid to afford dinucleosides **5** in 76–84% yield. The structures of **5a–d** were confirmed by ^{1}H, ^{13}C, ^{31}P NMR, and HRMS. The 5′-OH of dinucleosides **5a–d** were oxidized by 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) and [bis(acetoxy)-iodo]benzene (BAIB) in acetonitrile/water (1:1, less than 1 mL) at ~40 °C to give dinucleosides-5′-carboxylic acids **6a–d** in good yield (78–85%).[11]

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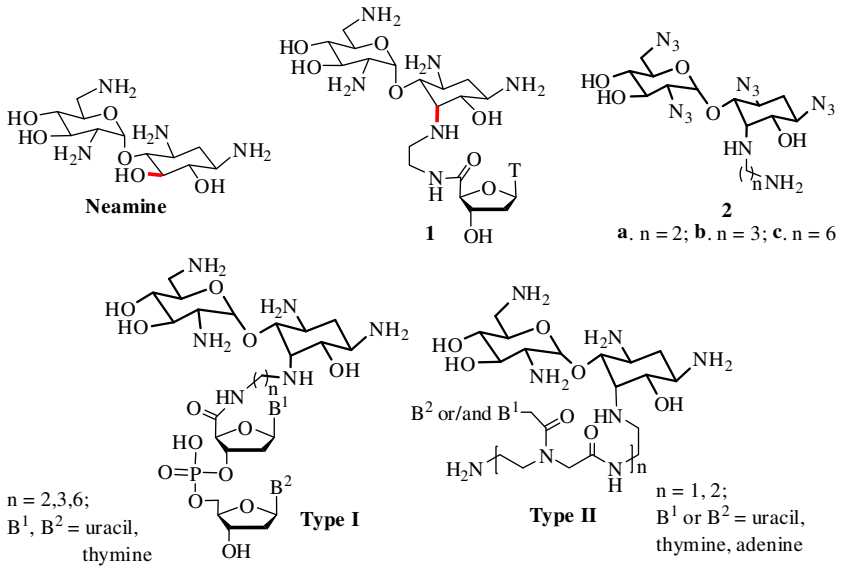
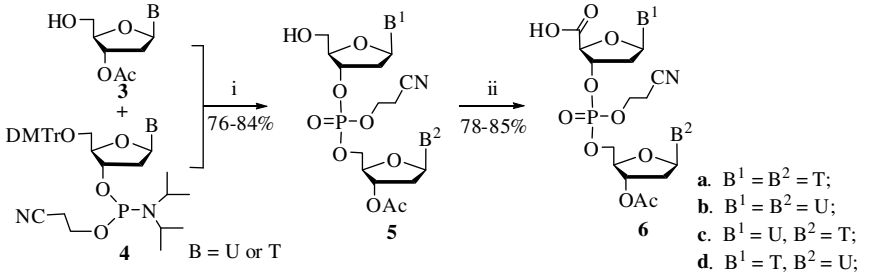


Figure 1. Structures of aminoglycosides, neamine, neamine-dinucleotide conjugates (type I) and neamine-PNA conjugates (type II), and aminated azido-neamines.

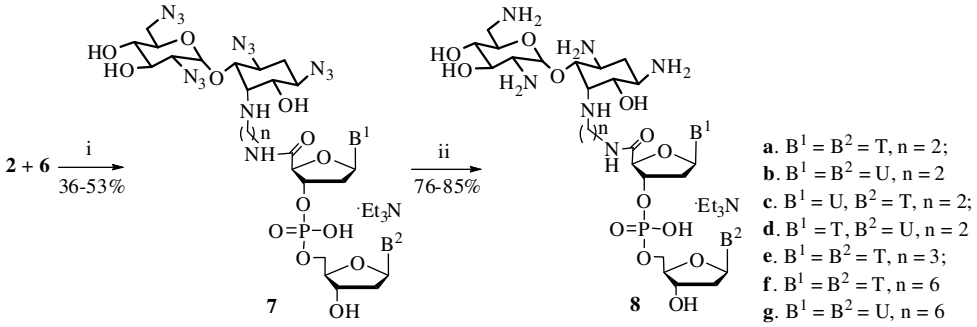


Scheme 1. Synthesis of 5′-carboxyl-di-nucleotide **6a–6d**. Reagents and conditions: (i) a—1H-Tetrazole/CH_2Cl_2; b—I_2/THF/H_2O/Py; c—3% CF_3COOH/CH_2Cl_2; (ii) TEMPO/BAIB, H_2O/CH_3CN, 35–40 °C.

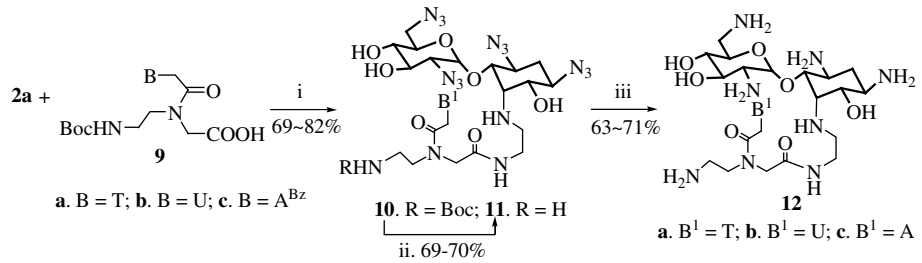
*Synthesis of neamine-dinucleotide **8a–g** and neamine-PNA conjugates **12a–c** and **14a–d**.* Condensation of azidodisaccharide **2** and compound **6** was run with DCC/HOBt strategy. After removal of cyanoethyl and acetyl group with concd aq ammonia, compound **7** was afforded in 36–53% yield. The azido group of intermediate **7** was reduced with H_2S, followed by HPLC purification to obtain neamine-dinucleosides conjugates **8a–g** in 76–85% yield (Scheme 2).

Neamine-PNA conjugates were synthesized by the following procedure. Compound **2a** was condensed with **9a–c**, respectively, to give **10a–c** in good yield.[9b,12] After deprotection of Boc and benzoyl of compound **10** with TFA followed with saturated ammonia in methanol, the intermediate **11** was reduced by H_2S to yield compound **12a–c** (Scheme 3). Neamine-di-PNA conjugates **13a–d** were obtained by condensation of **11a–b** with **9a–b**, respectively, in the presence of DCC/HOBT. After deprotection and reduction as described above, **14a–d** were afforded in good yield (Scheme 4).

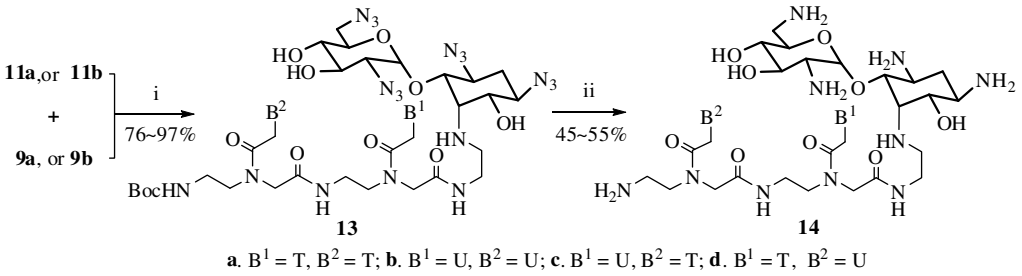
Study on the interaction of target compounds with 16S, 18S, and TAR RNA by SPR. The interaction between aminoglycoside and RNA has been successfully observed by Surface Plasmon Resonance (SPR).[13] The dissociation constants (K_d values in μM) were calculated from the slope of the Scatchard plot.[14] The dissociation con-



Scheme 2. Synthesisof amino disaccharide-dinucleotide conjugates. Reagents and conditions: (i) a—DCC, HOBt, DMF, 0 °C to rt; b—concd NH_4OH, rt, then HPLC; (ii) a—H_2S, Pyridine/Et_3N/H_2O, rt, then HPLC; b—1 N HCl.



Scheme 3. General synthesis of neamine-PNA conjugates **12a–c**. Reagents and conditions: (i) DCC, HOBt, DMF, rt; (ii) TFA, DCM, rt; (iii) H_2S, Pyridine/Et_3N/H_2O, rt.



Scheme 4. General synthesis of neamine-PNA conjugates **14a–d**. Reagents and conditions: (i) DCC, HOBt, HBTU, DMF, rt; (ii) a—TFA, DCM, rt; b—H_2S, Pyridine/Et_3N/H_2O, rt.

stants (K_d) of compounds **8a–g**, **12a–c**, and **14a–d** to 16S, 18S, and TAR RNA were listed in Table 1, respectively. Neamine-PNA conjugates **12a** and **14a** indicate the stronger binding activities to 16S and 18S RNA compared with the activity of neamine, the other conjugates interact with 16S or 18S RNA at the same level as that of neamine. However, **14a** and **d** show about twofold binding affinities to TAR RNA compared to that of neamine. It seems that the structure of PNA unit in compounds **12a–c** and **14a–14d** gives some contributions to the interaction with 16S, 18S and TAR RNA. Neamine-dinucleosides **8a–g** conjugates show as much as 10–30 higher dissociation constants than that of neamine, with less relationship with the kind of nucleobase or the length of linker. Compared with compound **1**, **8a–g** displayed a sharp decrease of binding affinity to 16S RNA. This implied that some modified function-groups in **8a–g** decrease significantly the binding affinity of aminoglycoside to the A-site of 16S RNA. It was reported that 16S RNA is highly discriminatory of the structure and conformation of aminoglycoside and their derivatives.[15] Obviously, the additional amino groups in compounds **12a–c** and **14a–d** may increase the electrostatic interactions with RNA, on the contrary, phosphate groups in compounds **8a–g** make the repulsion between such interactions.

Table 1
Interaction between neamine-dinucleotide, neamine-PNA conjugates and 16S, 18S, or TAR RNA

Structure	Entry	K_d (16S)	K_d (18S)	S.F.*	K_d (TAR)
Type I[a]	**8a**	304	548	1.80	634
	8b	180	404	2.24	417
	8c	352	1214	3.45	1000
	8d	227	479	2.11	476
	8e	316	555	1.76	533
	8f	208	266	1.28	115
	8g	N/A#	N/A#	N/A#	N/A#
Neamine[b]		11	26	2.36	11
Type II[a]	**12a**	8	11	1.38	10
	12b	14	17	1.21	24
	12c	16	12	0.75	12
	14a	9	10	1.11	5
	14b	17	35	2.06	11
	14c	12	25	2.08	8
	14d	11	20	1.81	6
Neamine[b]		11	26	2.36	11

* Specific factor (S.F. = $K_{d\ 18S}/K_{d\ 16S}$). K_d in μM.
\# Not available.
[a] See Figure 2.
[b] See Figure 1.

Study on computer modelling. For elucidating the binding mode of neamine-dinucleosides and neamine-PNA conjugates to 16S and 18S RNA, we carried out molecular docking study for compounds **12a**, **14a**, and **8e** to RNA using the program AutoDock 3.0. The target structure of 16S RNA was extracted from the protein databank (PDB code 1PBR). Because there is no crystal structure for human 18S ribosomal RNA, the target structure of it was produced from molecular dynamics simulation. Binding ability of compound to 16S and 18S RNA has been evaluated by the free energy. And the conformation with the lowest free energy was used as the final docked conformation for analysis. In this model, compounds **12a**, **14a**, and **8e** can fit into the major groove of 16S or 18S ribosomal RNA and the dinucleosides and PNA parts bind towards the bulge region (except for **12a** binding to 18S). As expected, some amino groups of aminosugar interact strongly with the negative charges of phosphate in RNA leading to strong electrostatic interaction. Moreover, hydrogen bonding was also observed when **12a**, **14a**, and **8e** binding to 16S or 18S RNA. One of carbonyl oxygen is strongly hydrogen bonded with the N^4 atom of C1407 on the major groove in **14a**. Both of electrostatic interaction and hydrogen bonding made all compounds binding to the pocket of RNA. However, the phosphate group in **8e** interacted with the phosphate negative charges of residue G 1491 (16S RNA) or G 1405 (18S RNA). The negative–negative electrostatic repulsive interaction kept the dinucleosides part of **8e** a tendency to far from the bulge region of RNA, which was responsible for the lower binding affinity of the neamine-dinucleosides conjugates to 16S or 18S RNA than the neamine-PNA ones (Fig. 2).

The free energy of the complexes reflected the binding affinity of the compounds to 16S and 18S RNA. The negative free energy suggested that the compounds **12a**, **14a**, and **8e** could all bind to 16S RNA and 18S RNA. Compared to compound **12a** and **14a**, the lower

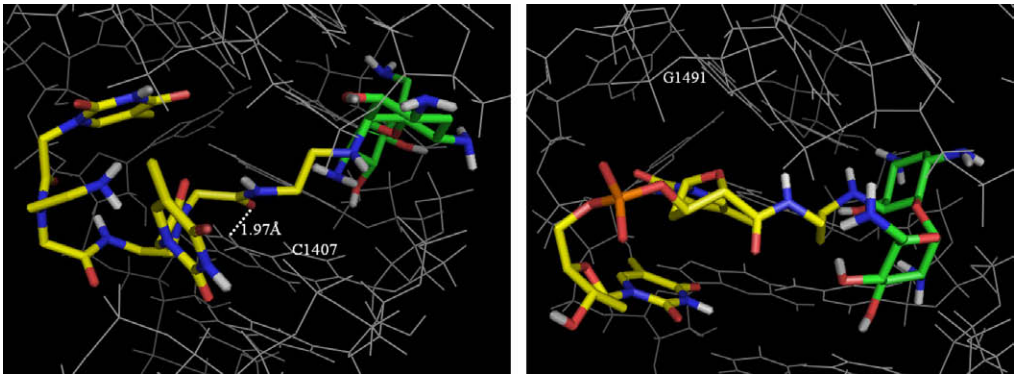


Figure 2. The binding mode of compound **14a** (left), or **8e** (right) with 16S RNA. Hydrogen bond between **14a** and 16S RNA is shown as dotted line.

free energy for compound **8e** shows its very weak binding interactions with 16S and 18S RNA. Compound **12a** and **14a** showed the similar binding affinity according to the free energy values. The calculated results were in good agreement with SPR analysis.

In conclusion, two types of aminodisaccharide-dinucleosides and aminodisaccharide-peptide nucleic acids conjugates were synthesized by the condensation of azidodisaccharide and dinucleosides/PNA monomer using aliphatic diamine as linker. Results from the SPR evaluation suggested that aminodisaccharide-PNA conjugate **12a–c** and **14a–d** were favourable for 16S, 18S, and TAR RNA binding, while neamine-dinucleotides conjugate **8a–g** displayed a sharp decrease of binding affinity to 16S, 18S, and TAR RNA. Molecular modelling indicated that the PNA unit in conjugates **12a–c** and **14a–d** gives some contributions for the interaction between the designed compounds and RNA, but nucleotide unit in compound **8a–g** results a negative–negative electrostatic repulsive interaction with RNA which keeps the designed compounds far from the bulge region of RNA.

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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.2008.09.062.

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