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Synthesis of (+),(−)-Neamine and Their Positional Isomers as Potential Antibiotics

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Abstract—The syntheses of (+)-neamine **1**, (−)-neamine ***ent*-1** and their positional isomers **2**, **3**, ***ent*-2** and ***ent*-3** are reported as potential new scaffolds for novel aminoglycoside antibiotics. These isomers exhibit similar inhibitory activities, as shown using an in vitro translation assay. A simple model is proposed to explain this lack of stereospecific binding to the ribosomal RNA.

Aminoglycoside antibiotics are clinically useful drugs known to function by binding to the A-site decoding region on bacterial 16S rRNA.[1] This binding causes mistranslation and premature termination during protein synthesis in bacteria, leading to the bacteriocidal effects of this class of drug.[2] However, the high toxicity and rapid emergence of resistance has limited their use, and resulted in the need for novel aminoglycosides devoid of these limitations.[3] Since several aminoglycosides such as neomycin B, ribostamycin and kanamycin B share a common pseudodisaccharide, (+)-neamine **1**, it has been used as a scaffold for the synthesis of diverse small molecules for developing new aminoglycoside antibiotics.[3c,3d,4]

In order to design a more potent antibiotics, the question of stereospecificity of this class of drug needs to be addressed. Recently, we synthesized (+)-neamine **1** and (−)-neamine ***ent*-1** and binding measurements with the A-site rRNA constructs demonstrated weak stereospecific binding.[5] Functional studies, including in vitro translation assays, antibiotic disk assays and minimum inhibitory concentration studies, also reveal a low level of stereospecificity. Interestingly, (−)-neamine ***ent*-1**, inhibits the growth of aminoglycoside resistant *Escherichia coli*.[5,6]

These results led us to synthesize positional isomers **2**, **3** of (+)-neamine and their enantiomers ***ent*-2**, ***ent*-3** to investigate the origin of the weak stereospecific interactions, and to search for new scaffolds for novel antibiotics. We describe here the synthesis of (+)-neamine **1**, (−)-neamine ***ent*-1**, all positional isomers **2**, **3**, ***ent*-2**, ***ent*-3** (Fig. 1) and a working model to explain the source of weak stereospecificity.

The synthesis of pseudodisaccharide **1**–**3** and ***ent*-1**–***ent*-3** was accomplished by glycosidation of protected 2-deoxystreptamine acceptors **4** and **6** with thioglycoside donors **12** and ***ent*-12** (Scheme 1). Methoxymethyl protected 2-deoxystreptamine acceptor **6** was prepared from the known diacetylated 2-deoxystreptamine derivative **4**.[4a] The D-thioglycoside donor **12** was synthesized from the protected azidoglycoside **7**, derived from D-glucosamine.[7] Azidoglycoside **7** (α/β, 1:3.5) was converted to the phenylthioglycoside **8** (α/β, 1.6:1) by treatment with phenylthiotrimethylsilane and zinc iodide.[8] Acetate deprotection of **8** afforded triol **9**, which was monotosylated to give **10**. The tosylate **10** was displaced with sodium azide providing **11** in quantitative yield. Dibenzylation of **11** gave D-thioglycoside donor **12** (α/β, 1:1). The enantiomer of **12**, ***ent*-12** (α/β, 1:1) was obtained from ***ent*-7**,[9] a L-glucosamine derivative, using the same protocol just described.

Glycosylation of the 2-deoxystreptamine acceptor **4** with D-thioglycoside donor **12** (Scheme 2) and acetate deprotection provided the pseudodisaccharide **13** in 74% yield (α/β, 10:1). Coupling of donor **12** and acceptor **6** gave pseudodisaccharide **14** and **15** after deprotection of the MOM group and chromatographic

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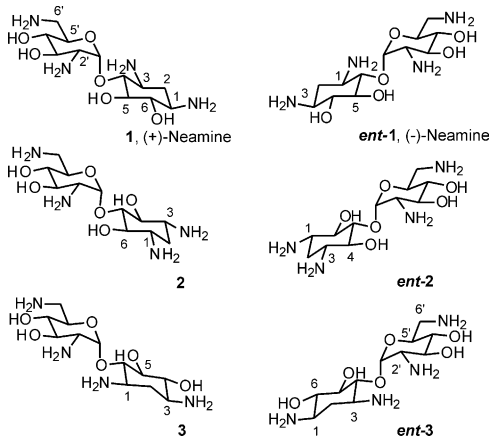
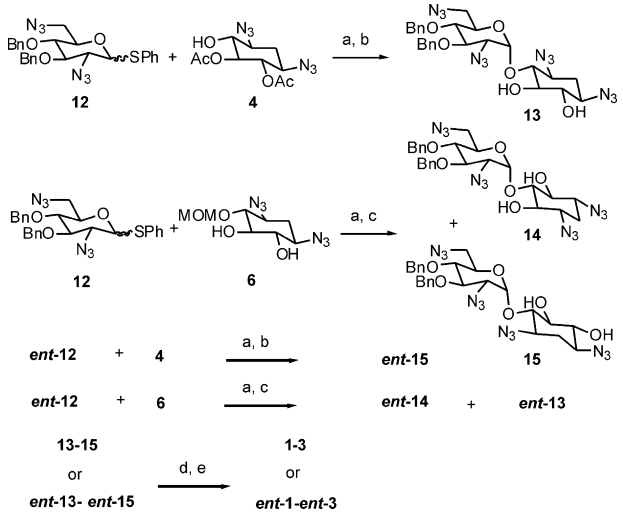


Figure 1. Structures of (+)-neamine **1**, (−)-neamine ***ent*-1** and their positional isomers (**2**, **3**, ***ent*-2** and ***ent*-3**).



Scheme 1. Synthesis of glycosyl acceptors **4**, **6** and donor **12** and ***ent*-12.** Reagents and conditions: (a) $CH_2(OCH_3)_2$, $CHCl_3$, P_2O_5; 96% (b) NaOMe, MeOH, 100%; (c) $PhSSi(CH_3)_3$, ZnI_2, $ClCH_2CH_2Cl$, 50 °C, 90%; (d) NaOMe, MeOH, 98%; (e) toluenesulfonyl chloride, pyridine, 94%; (f) NaN_3, DMF, 80 °C, 100%; (g) BnBr, NaH, DMF, 86%.

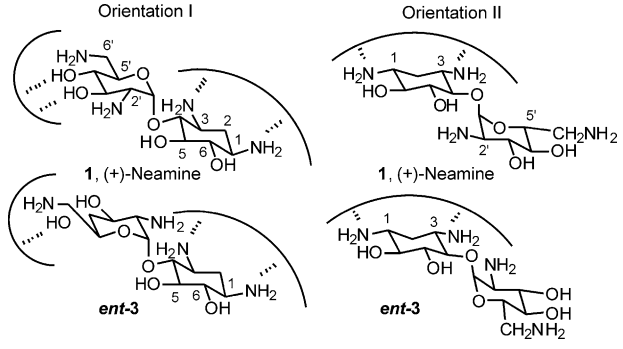


Figure 2. A simple model of (+)-neamine **1** and ***ent*-3**'s interaction with the A-site of rRNA.

separation. Pseudodisaccharides **14** and **15** were obtained in 30 and 37% yields, respectively, along with a 15% yield of a mixture of β isomers. Azidoglycosides **13–15** were individually subjected to Staudinger reaction[4a] conditions followed by benzyl deprotection to give (+)-neamine **1**, its 5-positional isomer **2** and its 6-positional isomer **3** in 90, 77 and 91% yields, respectively.

Using the same coupling conditions, ***ent*-12** and **4** gave ***ent*-15** in 73% yield with a better selectivity (α/β, 50:1). Similarly, **6** and ***ent*-12** coupled to provide ***ent*-14** and ***ent*-13** in 14 and 46% yield respectively, with 15% of the β isomeric mixture after deprotection and chromatographic separation. Azidoglycosides ***ent*-13–*ent*-15** were also individually deprotected to give the 4-positional isomer of (−)-neamine ***ent*-3**, its 5-positional isomer ***ent*-2** and (−)-neamine ***ent*-1** in 92, 78, and 90% yields, respectively.

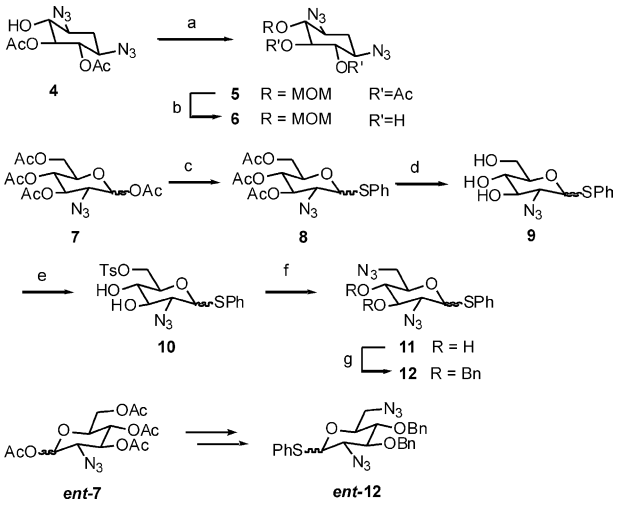
Binding assays of the aminoglycosides with either A-site decoding region rRNA constructs or ribosomes were found not to be quantitatively predictive of the bacteriocidal activity of these antibiotics, compared to in vitro translation assays.[5] In vitro translation studies of (+)-neamine **1**, (−)-neamine ***ent*-1**, and their positional isomers **2**, **3**, ***ent*-2**, ***ent*-3** were determined using Ambion PROTEINscript^{TM}-PRO kit (Table 1). The translation assay utilized highly active *E. coli* S30 extracts and a plasmid containing a gene expressing truncated lecithin retinol acyltransferase (tLRAT).[10] Various concentrations of the antibiotics were added to inhibit translation.

The translation assay revealed that (+)-neamine is approximately a 5-fold better inhibitor than (−)-neamine. The assay also revealed two trends. The inhibitory ability of the 4-positional isomers **1** and ***ent*-3** > 5-positional isomers **2** and ***ent*-2** > 6-positional isomers **3** and ***ent*-1**. The (+)-neamine and its isomers are more potent inhibitors than (−)-neamine and its isomers.

Footprinting studies of (+)-neamine **1** and (−)-neamine ***ent*-1** with the A-site rRNA construct, revealed that they bind to the same site.[5] NMR studies of the A-site of 16S rRNA and (+)-neamine by Puglisi[11] showed the presence of intermolecular NOEs between 3′OH, 4′OH, $1NH_2$ and $3NH_2$ of (+)-neamine **1** and the rRNA (Orientation I, Fig. 2). An alternative binding mode was also proposed (Orientation II, Fig. 2). In both binding modes, $1NH_2$ and $3NH_2$ are essential for binding. The 5-positional isomers, **2** and ***ent*-2**, has its deoxystreptamine arranged such that one of its two amino groups point away from the active site, resulting in much less effective binding. The least potent 6-positional isomers, **3** and (−)-neamine ***ent*-1**, have both amino groups pointing away. The most effective inhibitor in the enantiomeric series, ***ent*-3**, adopted conformations that mimics (+)-neamine and allows $1NH_2$ and $3NH_2$ to face the active site (Fig. 2). These alter-

Table 1. IC_{50} (50% inhibition of translation, μM) of the in vitro translation assay of (+)-neamine, (−)-neamine and their positional isomers

Aminoglycoside	IC_{50}, (μM)
1	38.6±4.6
2	132±9.0
3	197±10
ent-1	198±17
ent-2	152±12
ent-3	102±6
Neomycin B	14.9±0.1



Scheme 2. Synthesis of (+)-neamine **1**, (−)-neamine ***ent*-1** and their positional isomers (**2**, **3**, ***ent*-2** and ***ent*-3**). Reagents and conditions: (a) *N*-iodosuccinimide, silver trifluoromethanesulfonate, Et_2O/CH_2Cl_2 (3:1), −20 °C; (b) NaOMe, MeOH; (c) 1 N HCl, MeOH, 75 °C; (d) $P(CH_3)_3$, THF/0.1 N NaOH (9:1); (e) H_2, 20% $Pd(OH)_2/C$, AcOH/H_2O (1:1).

native conformations could explain the relative inhibitory power of these antibiotics and also the low level of stereospecificity observed.

In conclusion, the syntheses of (+)-neamine **1**, (−)-neamine ***ent*-1** and their positional isomers **2**, **3**, ***ent*-2** and ***ent*-3** are reported. These isomers exhibit similar inhibitory activities as shown using the translation assay. We also proposed that these low level of stereospecificity could be due to possible alternative conformations that ***ent*-1**, ***ent*-2** and ***ent*-3** may adopt. These results may serve as the basis for the preparation of novel unnatural aminoglycosides.

Supporting information available: Experimental procedure for the preparation of **1**, **2**, **3**, ***ent*-1**, ***ent*-2**, and ***ent*-3**, their spectroscopic data, and in vitro translation assay.

Acknowledgements

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