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Neamine dimers targeting the HIV-1 TAR RNA

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Abstract—Natural aminoglycoside antibiotics, such as neomycin, target bacterial ribosomal RNA. Neomycin also binds strongly to HIV TAR and RRE RNA through the predominant interactions of its neamine core. In the search for antiviral agents targeting multiple binding sites for aminoglycosides in RNA, we report here the synthesis of new neamine dimers and a trimer in which the neamine cores are connected by different linking chains attached at the 4′- and/or 5-positions. Inhibition of TAR-Tat complexation by these oligomers was studied via fluorimetric binding assays performed under two ionic strengths. All dimers strongly inhibit TAR-Tat association, with IC_{50} values 17–85 times better than the value obtained with neomycin. These results demonstrate that modifying neamine at the 4′- or the 5-position is a promising strategy in the search for antiviral agents.

Ribosomal RNA (rRNA) is the target of clinically important antibiotics. Among them, aminoglycosides (aminosides) constitute a large family of natural antibiotics effective against a broad range of microorganisms.[1] These pseudo-oligosaccharides are multiply charged cationic molecules of high flexibility that bind specifically in the bacterial ribosome to the A-site of the decoding region of 16S rRNA and induce the synthesis of modified proteins.[2] The aminoglycoside antibiotic neomycin B (**1**) (Fig. 1) also binds to other RNA targets,[3,4] such as the HIV RNA recognition elements RRE (rev responsive element)[5–8] and TAR (trans-acting responsive sequence),[9] and blocks the HIV-Rev and HIV-Tat protein binding necessary for viral RNA transactivation.

Unfortunately, neomycin B is toxic and high level antibiotic resistance involving enzymatic modifications has been reported.[10–12] Detailed comparative NMR studies and biochemical experiments have shown that rings I and II of neomycin-class aminoglycosides (Fig. 1) are sufficient to confer the specificity of binding to a model

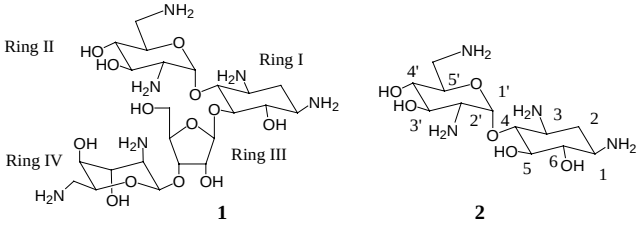

Figure 1. Structures of neomycin B **1** and neamine **2**.

A-site RNA and serve as a minimal structural motif for binding to the 16S subunit of rRNA[13] and to the RRE and TAR HIV RNA.[14,15] Therefore, in the search for new antibiotic or antiviral drugs, less toxic than neomycin, neamine **2** (Fig. 1) appears to be an attractive starting molecule.

In the last few years, neamine derivatives have been prepared by modifying the amino groups, or the 3′-, the 5- or the 6-hydroxyl function to increase the affinity for the RNA targets and/or to induce resistance to aminoglycoside-modifying enzymes.[16–26]

In an attempt to explore the multiple binding sites for aminoglycosides in RNA and target them,

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aminoglycoside antibiotics kanamycin A, tobramycin and neomycin B have been homo- and heterodimerized with linking chains of various lengths through disulfide bond formation.[27] These dimers inhibit the catalytic function of *Tetrahymena* ribozyme 20- to 12,000-fold more efficiently than the corresponding monomers.[28] Their dissociation constants to a dimerized A-site 16S RNA construct were about 20-fold higher than the monomeric aminoglycoside molecules.[29] Incorporation of a naphthalene diimide threading intercalator in the linking chain led to a 35-fold enhancement in binding affinity compared to the monomeric aminoglycosides.[30] Neomycin dimers also showed dissociation constants to RRE RNA to be approximately 17-fold higher than neomycin.[7,31] Combinatorial chemistry has been used to generate dimers of neamine, that target rRNA, in which the two subunits are linked by an amino chain attached to the 5-positions. Some of these dimers exhibit very interesting antibiotic effects and resistance to aminoglycoside-modifying enzymes.[24,26,32,33] Aminoglycoside dimers have also been shown to stabilize B-form duplex DNA.[34]

The deoxystreptamine core, corresponding to ring I in neamine, was recently dimerized and the resulting dimers were shown to bind strongly to RNA hairpin loops.[35]

Both mass spectrometry and gel mobility shift assays have revealed the existence of multiple neomycin binding sites in TAR RNA.[36] We also observed a biphasic profile in the melting temperature curve of mixtures TAR RNA-neomycin B, suggesting the presence of at least two binding sites. In the search for aminoglycosides having multiple binding sites in the TAR RNA, we report here the synthesis of four symmetrical 4′,4′-, and 5,5-neamine dimers **3**, **5**, **7**, **9**, and a 5,5,5-neamine trimer **11** (Fig. 2) using protecting groups optimized previously[37,38] and a comparative study of their affinity for TAR RNA through inhibition of the TAR-Tat association via fluorimetric binding assays.

We have recently reported a route for preparing, for the first time, 4′-neamine derivatives using trityl and *p*-methoxybenzyl protective groups for the amino and hydroxyl functions, respectively. Interestingly, a comparative study of the binding to TAR RNA of 4′- and 5-neamine-histidine, -phenanthroline, -flavin, and -adenine conjugates has shown that 4′-neamine derivatives have similar affinities and selectivities for TAR RNA than their corresponding 5-derivatives.[37] A 4′-neamine–histidine conjugate has also revealed a remarkable affinity and selectivity for TAR RNA better than those of the corresponding 5-derivative.[37]

The isomeric 5,5- and 4′,4′-dimers **3** and **7** possessing an uncharged linking chain at pH 7 in water were synthesized from the neamine derivatives **13** and **15**,[37,38] carrying an amino function at the end of a hexyl chain introduced at the 5 and 4′-positions, respectively (Schemes 1 and 2). Compounds **13** and **15** were converted to the corresponding carboxylic acids **14** and **16**, respectively, through the reaction with succinic anhydride (90% and 89% yields, respectively). The reaction

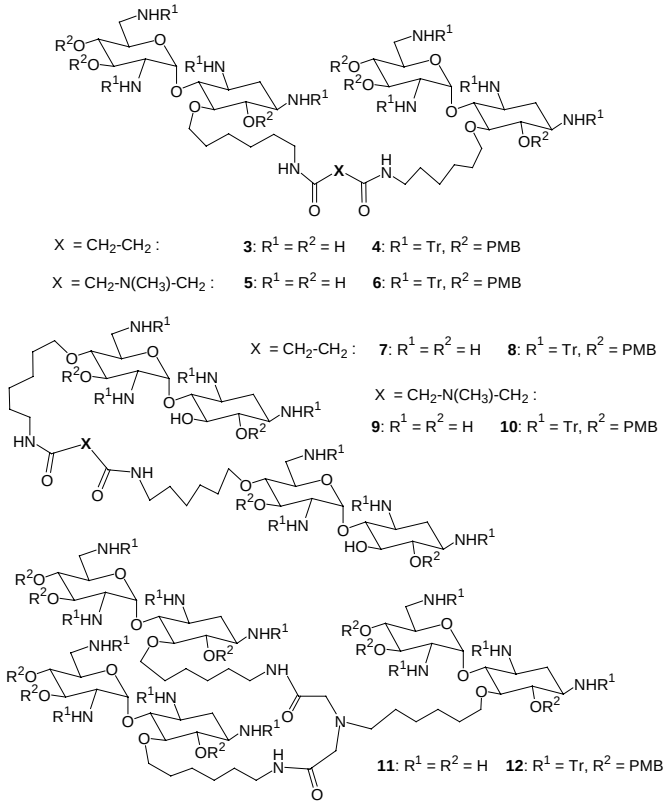
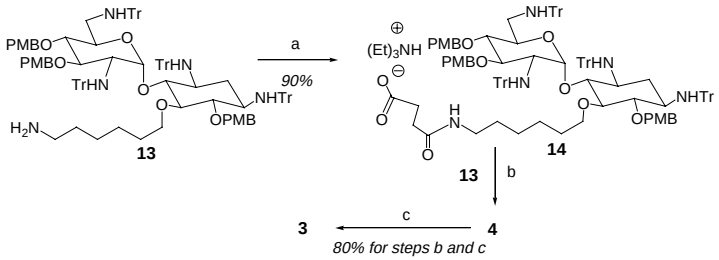
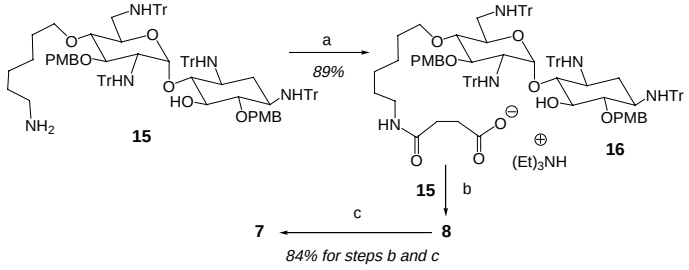


Figure 2. Structures of the neamine dimers **3**, **5**, **7**, **9**, trimer **11** and their protected derivatives **4**, **6**, **8**, **10**, and **12**, respectively.



Scheme 1. Synthesis of the 5,5-symmetrical neamine dimer **3**. Reagents: (a) succinic anhydride, Et_3N, CH_2Cl_2; (b) EDC, HOBt, CH_2Cl_2; (c) TFA, anisole.



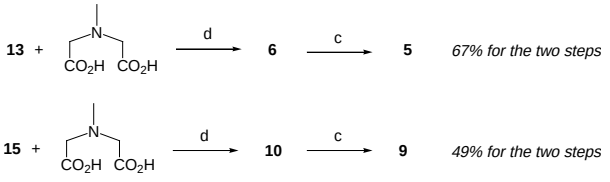
Scheme 2. Synthesis of the 4′,4′-symmetrical neamine dimer **7**. Reagents: (a) succinic anhydride, Et_3N, CH_2Cl_2; (b) EDC, HOBt, CH_2Cl_2; (c) TFA, anisole.

of these carboxylic acids with the corresponding amino derivatives **13** and **15** in the presence of EDC/HOBt and then deprotection with TFA/anisole led to the corresponding dimers **3** and **7** (80% and 84% yields, respectively, for the coupling and deprotection reactions).

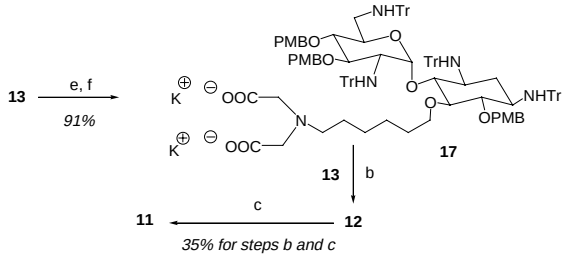
The 5,5- and 4′,4′-dimers **5** and **9** possessing a methylamino function in the center of the linking chain were obtained in one step through the reaction of the amino derivatives **13** and **15**, respectively, with methyliminodiacetic acid in the presence of EDC/HOBt and then deprotection (Scheme 3; 67% and 49% yields, respectively, for the coupling and deprotection reactions).

The 5,5,5-trimer **11** was prepared from the 5-hexylamino derivative **13** (Scheme 4). The reaction of this derivative with ethyl bromoacetate in THF/EtOH in the presence of triethylamine and, then treatment with KOH in THF led to the bis carboxylate salt intermediate **17** (91% yield for the two steps).

Condensation of this derivative with the 5-hexylamino derivative **13** led to the protected trimer **12**, which was deprotected in TFA/anisole to afford the trimer **11** (35% yield for the two steps). The four dimers and the



Scheme 3. Synthesis of the 4′,4′- and 5,5-symmetrical neamine dimers **5** and **9**, respectively, possessing an amino function in the linking chain. Reagents: (d) EDC, HOBt, Et_3N, CH_2Cl_2; (c) TFA, anisole.



Scheme 4. Synthesis of the 5,5,5-neamine trimer **11**. Reagents: (e) ethyl bromoacetate, Et_3N, THF/EtOH; (f) KOH, THF ; (b) EDC, HOBt, CH_2Cl_2; (c) TFA, anisole.

trimer prepared were characterized by ^1H and ^{13}C NMR and HRMS (see Supplementary data).

In the presence of trimer **11** (1 equiv), precipitation of 27mer TAR RNA models[37] at 2.5 μM was observed and, thus, this neamine derivative was not studied further.

To define the affinity of the dimers for TAR, the fluorimetric competition assay of Hamasaki and co-workers was applied to the 31mer oligonucleotide ($^{5'}$GGCCAGAUC UGAGCCUGGGAGCUCUCUGGCC) including the lateral UCU bulge found in the wild-type TAR RNA (Table 1).[39,40]

In this assay, a TAR-bound Tat_{49-57}–sequence fluorescein–AAARKKRRQRRRAAAC–rhodamine is titrated with a competitor RNA ligand. Upon displacement from the TAR complex, the peptide conformation changes from extended to random coil. This change is accompanied by significant quenching of the fluorescence emission. At pH 7.4 and 70 mM salts, IC_{50} values of all dimers were found in the 0.15–0.4 μM range (see Table 1 and Fig. 3 for dimers **5** and **9**) and appeared to be lower than the values obtained for neomycin

Table 1. Aminoglycoside concentrations required to decrease TAR-Tat binding by 50% (IC_{50}), as determined in a fluorimetric competition assay[40]

	Aminoglycoside					
	Neomycin **1**	Neamine **2**	5,5-Dimer **3**	4′,4′-Dimer **7**	5,5-Dimer **5**	4′,4′-Dimer **9**
IC_{50} μM pH 7.4, 50 mM Tris–HCl, 20 mM KCl	7.0 ± 0.2	10.0 ± 0.5	0.40 ± 0.01	0.15 ± 0.01	0.15 ± 0.02	0.33 ± 0.01
IC_{50} μM pH 7.5, 10 mM Tris–HCl, 150 mM KCl	35 ± 3	40 ± 4	0.9 ± 0.03	0.48 ± 0.02	1.0 ± 0.1	0.44 ± 0.05

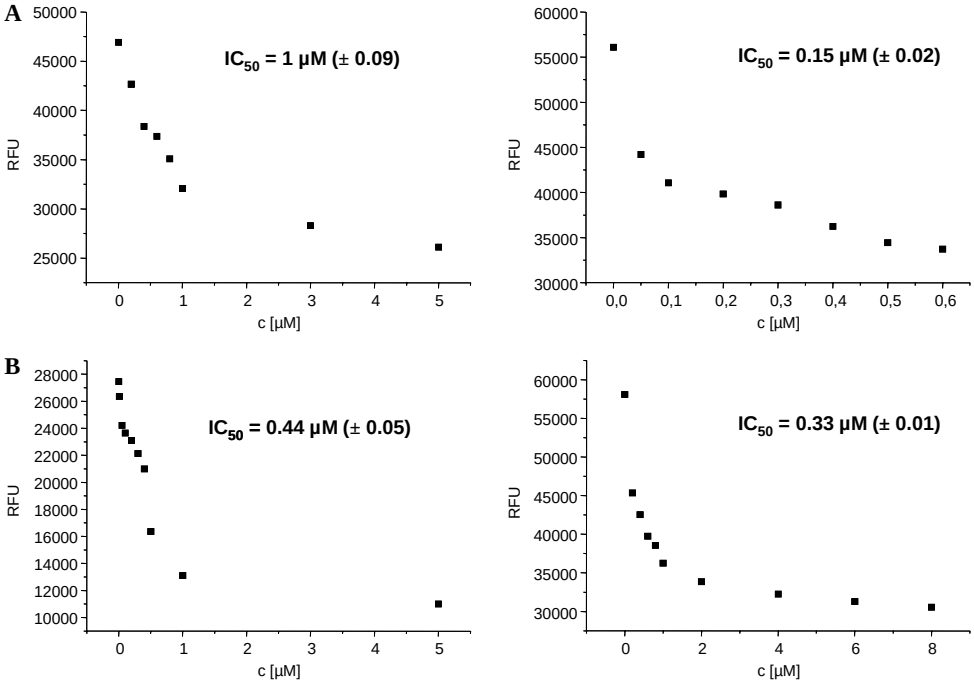


Figure 3. Determination of IC_{50} values. Typical titration curves are shown: (A) left: dimer **5** at pH 7.5, 10 mM Tris–HCl, 150 mM KCl; right: **5** at pH 7.0, 50 mM Tris–HCl, 20 mM KCl; (B) left: dimer **9** at pH 7.5, 10 mM Tris–HCl, 150 mM KCl, right: **9** at pH 7.0, 50 mM Tris–HCl, 20 mM KCl. The given IC_{50} numbers are mean values from three independent titrations.

(Sigma N1876, 7 μM) and neamine (10 μM). At a higher ionic strength (pH 7.5, 160 mM salts), IC_{50} values of the dimers were found in the μM range increasing from 1.3 (dimer **9**, Fig. 3B) to more than six times (dimer **5**, Fig. 3A). These values remain much lower than those observed under the same conditions for neamine (40 μM) and neomycin (35 μM). Biphasic titration curves were observed for dimers **3** and **7** (data not shown). This particular behavior indicates the presence of at least two independent binding sites in TAR occupied by these dimers with rather different affinities.

In conclusion, all synthesized dimers are able to inhibit the TAR-Tat binding at interesting submicromolar concentrations. These results point out that modifying neamine at the 4′- or the 5-position is a highly promising strategy to compete with the TAR-Tat binding in the search for antiviral agents. The dimers have now to be modified to allow their cellular uptake.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.07.082.

References and notes

1. Edson, R. S.; Terell, C. L. *Mayo. Clin. Proc.* **1999**, *74*, 519.
2. Wong, C.-H.; Hendrix, M.; Priestley, E. S.; Greenberg, W. A. *Chem. Biol.* **1998**, *5*, 397.
3. For a review, see: Maurel, M.-C.; Biard, D.; Moulinier, C.; Braz, D.; Nugier, J.; Chaumas, I.; Reboud-Ravaux, M.; Décout, J.-L. *J. Pharm. Pharmacol.* **2002**, *54*, 1019.
4. Neomycin also specifically binds to the kissing-loop complex formed by the dimerisation initiation site of the HIV genomic RNA: Ennifar, E.; Paillart, J.-C.; Marquet, R.; Ehresmann, B.; Ehresmann, C.; Dumas, P.; Walter, P. *J. Biol. Chem.* **2003**, *278*, 2723.
5. Werstuck, G.; Zapp, M. L.; Green, M. R. *Chem. Biol.* **1996**, *3*, 129.
6. Zapp, M. L.; Stern, S.; Green, M. R. *Cell* **1993**, *74*, 969.
7. Tok, J. B.-H.; Dunn, L. J.; Des Jean, R. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1127.
8. Wang, Y.; Hamasaki, K.; Rando, R. R. *Biochemistry* **1997**, *36*, 768.
9. Mei, H.-Y.; Galan, A. A.; Halim, N. S.; Mack, D. P.; Moreland, D. W.; Sanders, K. B.; Truong, H. N.; Czarnik, A. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2755.
10. Wright, G. D.; Berghuis, A. M.; Mobashery, S. *Adv. Exp. Med. Biol.* **1998**, *156*, 27.
11. Kondo, S.; Hotta, K. *J. Inf. Chemother.* **1999**, *5*, 1.
12. Mingeot-Leclerc, M.-P.; Glupczynski, Y.; Tulkens, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 727.

13. Fourmy, D.; Recht, M. I.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 347.
14. Leclerc, F.; Cedergren, R. *J. Med. Chem.* **1998**, *41*, 175.
15. Hermann, T.; Westhof, E. *J. Med. Chem.* **1999**, *42*, 1250.
16. Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother.* **2000**, *44*, 3249.
17. Kotra, L. P.; Mobashery, S. *Curr. Org. Chem.* **2001**, *5*, 193.
18. Roestamadji, J.; Grapsas, I.; Mobashery, S. *J. Am. Chem. Soc.* **1995**, *117*, 80.
19. Roestamadji, J.; Mobashery, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3483.
20. Liu, M.; Haddad, J.; Azucena, E.; Kotra, L. P.; Kirzhner, M.; Mobashery, S. *J. Org. Chem.* **2000**, *65*, 7422.
21. Haddad, J.; Kotra, L. P.; Llano-Sotelo, B.; Kim, C.; Azucena, E. F.; Liu, M.; Vakulenko, S. B.; Chow, C. S.; Mobashery, S. *J. Am. Chem. Soc.* **2002**, *124*, 3229.
22. Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150.
23. Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 6527.
24. Sucheck, S. J.; Wong, A. L.; Koeller, K. M.; Boehr, D. D.; Draker, K.-A.; Sears, P. S.; Wright, G. D.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 5230.
25. Sucheck, S. J.; Greenberg, W. A.; Tolbert, T. J.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2000**, *39*, 1080.
26. Agnelli, F.; Sucheck, S. J.; Marby, K. A.; Rabuka, D.; Yao, S.-L.; Sears, P. S.; Liang, F.-S.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1562.
27. Wang, H.; Tor, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1951.
28. Michael, K.; Wang, H.; Tor, Y. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 1361.
29. Tok, J. B.-H.; Huffman, G. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1593.
30. Tok, J. B.-H.; Fenker, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2987.
31. Luedtke, N. W.; Liu, Q.; Tor, Y. *Biochemistry* **2003**, *42*, 11391.
32. Miura, Y.; Ikeda, T.; Wada, N.; Kobayashi, K. *Macromol. Biosci.* **2003**, *3*, 662.
33. Liang, C.-H.; Romero, A.; Rabuka, D.; Sgarbi, P. W. M.; Marby, K. A.; Duffield, J.; Yao, S.; Cheng, M. L.; Ichikawa, Y.; Sears, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2123.
34. Arya, D. P.; Lane Coffee, R.; Xue, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4643.
35. Liu, X.; Thomas, J. R.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2004**, *126*, 9196.
36. Sannes-Lowery, K. A.; Mei, H.-Y.; Loo, J. A. *Int. J. Mass Spectrom.* **1999**, *193*, 115.
37. Riguet, E.; Désiré, J.; Bailly, C.; Décout, J.-L. *Tetrahedron* **2004**, *60*, 8053.
38. Riguet, E.; Tripathi, S.; Chaubey, B.; Désiré, J.; Pandey, V. N.; Décout, J.-L. *J. Med. Chem.* **2004**, *47*, 4806.
39. Matsumoto, C.; Hamasaki, K.; Mihara, H.; Ueno, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1857.
40. Renner, S.; Ludwig, V.; Boden, O.; Scheffer, U.; Göbel, M.; Schneider, G. *ChemBioChem* **2005**, *6*, 1.