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A New Class of Branched Aminoglycosides: Pseudo-Pentasaccharide Derivatives of Neomycin B

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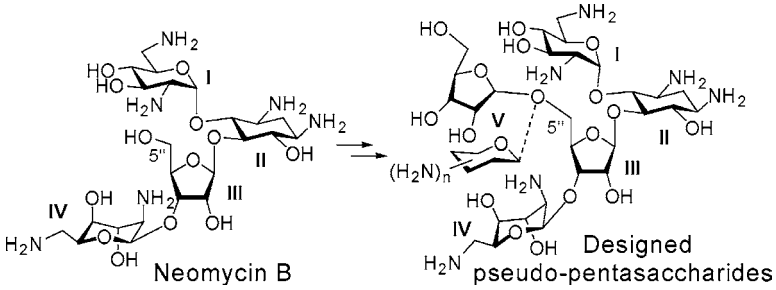
ABSTRACT



The clinically important antibiotic neomycin B was modified at position C5″ by adding one extra sugar ring in the β-configuration, and the observed pseudo-pentasaccharides were tested against various bacterial strains, including pathogenic and resistant strains. The designed antibiotics show antibacterial activity superior to that of neomycin B against pathogenic and resistant bacterial strains.

Aminoglycoside antibiotics are clinically important drugs that function through binding to specific sites in prokaryotic ribosomal RNA (rRNA) and affecting the fidelity of protein synthesis.[1] However, rapid emergence of resistance and high human toxicity has limited their use and resulted in the need for novel structures devoid of these limitations.[2] Hence, many structural analogues of natural aminoglycosides have been synthesized over the past decade.[3]

In the majority of these studies, a minimal structural motif, which is common for a series of structurally related aminoglycosides, has been identified and used as a scaffold for the construction of diverse analogues as potential new antibiotics.[4] Some of the designed structures showed considerable antibacterial activities. Unlike this strategy, we hypothesized that since aminoglycoside antibiotics exert their antibacterial activity by selectively recognizing and binding to rRNA, it is likely that keeping the whole antibiotic constitution intact while adding an additional recognition/binding elements will result in superior binding to rRNA and probably better anti-

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(1) Davis, B. D. *Microbiol. Rev.* **1987**, *51*, 341−350.

(2) Wright, G. D.; Berghuis, A. M.; Mobashery, S. *Adv. Exp. Med. Biol.* **1998**, *456*, 27−69.

(3) For a recent review, see: Ye, X.-S.; Zhang, L.-H. *Curr. Med. Chem.* **2002**, *9*, 929−939.

(4) For representative studies, see: (a) Kumar, V.; Jones, G. S., Jr.; Blacksberg, I.; Remers, W. A. *J. Med. Chem.* **1980**, *23*, 42−49. (b) Yoshikawa, M.; Ikeda, Y.; Takenaka, K. *Chem. Lett.* **1984**, *13*, 2097−2100. (c) Girodeau, J.-M.; Pineau, R.; Masson, M.; Le Goffic, F. *J. Antibiot.* **1984**, *37*, 150−158. (d) Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1965−1978. (e) 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*, 6527−6541. (f) Wang, J.; Li, J.; Tuttle, D.; Takemoto, J. Y.; Chang, C.-W. T. *Org. Lett.* **2002**, *4*, 3997−4000. (g) Hanessian, S.; Tremblay, M.; Swayze, E. *Tetrahedron* **2003**, *59*, 983−993 and references therein.

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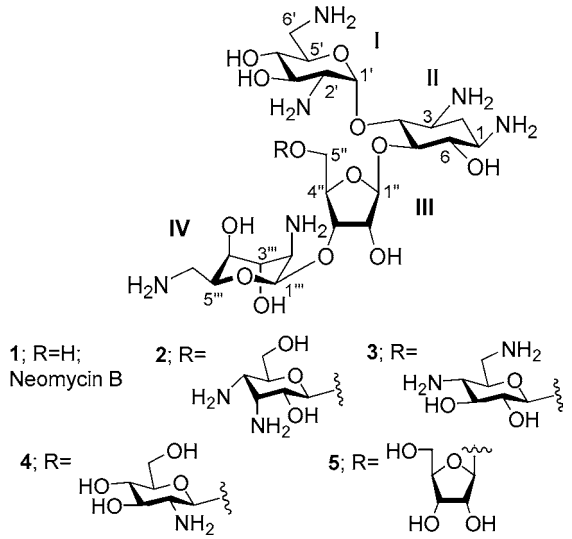


Figure 1.

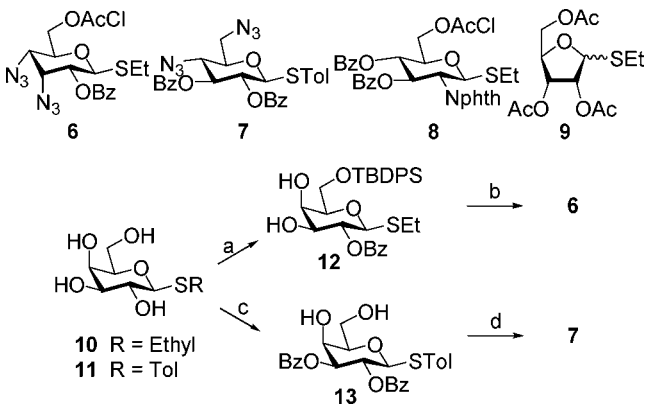
bacterial performance. Enhancement of RNA binding by using dimerized aminoglycosides,[5] bifunctional aminoglycosides,[6] and amino-aminoglycosides[7] supports this hypothesis.

To this end, we have modified neomycin B (**1**, Figure 1) by adding an additional sugar as a ring V at C5″-OH and prepared a series of pseudo-pentasaccharides **2**−**5**. These structures keep the whole antibiotic constitution intact as a recognition element to the rRNA, while the extended sugar ring (V) of each structure was designed in a manner that incorporates either *cis*-1,2-dimine (**2**), flexible 1,3-diamine (**3**), *cis*-1,3-hydroxyamine (**4**), or ribofuranose ring (**5**) as potential functionalities directed for the recognition of the phosphodiester bond of RNA.[8−10]

In selecting the modification site in neomycin B and the degree of modification, we have taken into consideration the following recently found structural information. Superposition of neomycin B bound to the aminoglycoside kinase APH(3′) ternary complex with ADP,[11] as well as paromomycin I (containing C6′-OH instead of C6′-NH_2 as in neomycin B) bound to A-site bacterial ribosome,[12] reveals that all the functional groups of aminoglycosides utilized for binding are identical in both, with the exception of two, which are not employed for binding in the antibiotic-resistance enzyme.[11] One of these groups is the C5″-OH of neomycin B, which is phased toward the second substrate, ATP, and may have a crucial role for the formation of the reactive ternary complex prior the phosphorylation step. Therefore, we anticipated that incorporation of gross changes such as addition of an extra rigid sugar ring in this region might have a dramatic effect on the formation of the precise ternary complex required for enzymatic catalysis. Taken together with the relative ease of derivatizing a primary alcohol, we selected position C5″ in neomycin B and prepared the new generation of pseude-pentasaccharides **2**−**5** with the expectation that they will function better than neomycin B against both the resistant and non-resistant organisms.

Our strategy for the construction of **2**−**5** (Schemes 1 and 2) was to convert commercial neomycin B to a common

Scheme 1[a]



[a] Reagents and conditions: (a) (i) TBDPSCl, pyridine, DMAP, 60 °C; (ii) 2,2-dimethoxypropane, acetone, CSA; (iii) BzCl, pyridine, DMAP; (iv) AcOH/H_2O 9:1, THF, 60 °C, 55% for four steps. (b) (i) Tf_2O, pyridine; (ii) NaN_3, DMF, HMPA, 63% for two steps; (iii) HF/pyridine; (iv) ClAcCl, pyridine, 91% for two steps. (c) (i) Anisaldehyde−dimethylacetal, CSA, THF; (ii) BzCl, pyridine; (iii) AcOH/H_2O 9:1, THF, 60 °C, 58% for three steps. (d) (i) Tf_2O, pyridine; (ii) NaN_3, DMF, HMPA, 86% for two steps.

acceptor (**14**) to which the donors **6**−**9**[13] can be connected. The protecting groups used in this study served admirably in terms of the ease of attachment and removal and survivability under the reaction conditions, whereas the thioglycoside−NIS glycosidation method[14] proved to be both rapid and efficient. The N-phth and ester protections at C-2 of the monosaccharide donors (**6**−**9**) were designed to allow, through neighboring group participation, selective β-glycoside bond formation between rings V and III.

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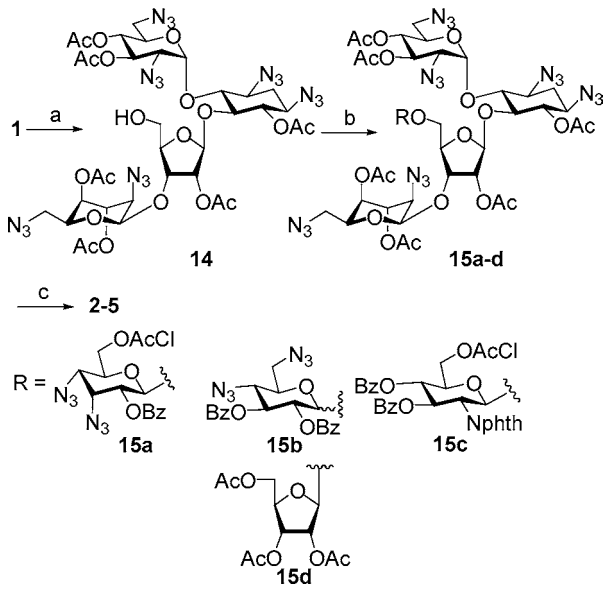
(13) Monosaccharides **8** and **9** were prepared by standard methods. All new compounds exhibited satisfactory spectral and analytical data. Yields refer to spectroscopically and chromatographically homogeneous materials.

(14) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331−1334.

Scheme 2[a]



[a] Reagents and conditions: (a) (i) TfN_3, Et_3N, $CuSO_4$, in CH_2Cl_2/MeOH/H_2O 3:10:3; (ii) TBDMSCl, pyridine, DMAP; (iii) Ac_2O, pyridine, DMAP; (iv) HF/pyridine, 57% for the four steps. (b) NIS, TfOH; **6** → **15a** (81%), **7** → **15b** (89%), **8** → **15c** (58%), **9** → **15d** (71%). (c) For **2**−**4**: (i) $MeNH_2$ (33% in EtOH); (ii) PMe_3, NaOH 0.1 M, THF/H_2O 3:1. For **5**: (i) NaOMe/MeOH; (ii) PMe_3, NaOH 0.1 M, THF/H_2O 3:1, afforded **2** (81%), **3** (96%), **4** (84%), and **5** (70%).

The diazido monosaccharides **6** and **7**, having D-allo and D-gluco configurations, respectively, were constructed from the common D-galactose derivatives (Scheme 1) by selectively inverting the configurations at C3 and C4 (in **6**) and at C4 (in **7**). The diol **12** was prepared from the known thioglycoside **10**[15] in four steps (selective silylation of the primary hydroxyl, acetonide formation at C3−OH and C4−OH, benzoylation, and removal of the acetonide) without isolation of intermediate products in an overall yield of 55%. Simultaneous triflation of both hydroxyls in **12** was followed by nucleophilic displacement with azide (without isolation of the intermediate ditriflate) to afford the corresponding diazide (63% isolated yield for two steps). Desilylation was then followed with a chloracetylation step to produce the allo-donor **6**. Alternatively, selective protection of C6 and C4 hydroxyls in the galactoside **11** by *p*-methoxybenzylidene, followed by benzoylation and hydrolysis of the benzylidene, gave the diol **13** in an overall 58% yield for three steps. This diol was then subjected to a similar triflation and azidation steps as for **12** to afford the 4,6-diazido donor **7** in an isolated yield of 86% for two steps.

The neomycin acceptor **14** was readily accessible in four chemical steps from the commercial neomycin B (Scheme 2) in an overall yield of 57%.[16] NIS-promoted coupling of **14** with thioglycosides **6**−**9** furnished the designed protected pseudo-pentasaccharides **15a**−**d** in 58−89% yields.[17] These protected compounds were subjected to a two-step deprotection, removal of all the ester and phthalimido groups by treatment with methylamine (33% solution in EtOH) and reduction of all the azido groups by Staudinger reaction, to furnish the final products **2**−**5** with excellent purity and isolated yields.[18]

The new analogues were tested for antibacterial activities against both Gram-negative and Gram-positive bacteria, including pathogenic and resistant strains,[19] and the minimal inhibitory concentrations (MIC)[20] were determined using a microdilution assay with neomycin B and kanamycin as controls (Table 1).

From the MIC values, it turns out that among the four analogues, only compound **5** having a ribose substituent at ring V is as potent as neomycin B against *E. coli* strains. The activity of this analogue against *E. coli* XL1(pET9d) having kanamycin resistance is even more impressive, exhibiting better activity than neomycin B. Analogue **5** is also effective against Gram-positive bacteria, *Staphylococcus epidermidis* and *Bacilus subtilis*. Furthermore, **5** demonstrates better activity than other analogues against pathogenic bacterium *Salmonella virchow* that is resistant to kanamycin and neomycin B. In this case **5** is about 5 times more effective than kanamycin and 2 times more effective than neomycin B. Finally, we also examined the susceptibility of enterobacterium *Pseudomonas aeuriginosa*, which is often very difficult to treat, sometimes requiring use of a combination of aminoglycosides with other antibiotics.[2,21] Interestingly, in this particular case, while **5** demonstrates activity close to that of neomycin B, the 2-glucosamino derivative **4** is even more effective than **5** and the diamino derivative **3** is superior to both.

Although we still did not examine the similar analogues with the plain pyranose ring and with the furanose ring bearing amino group(s),[22] the observed preliminary data of **2**−**5** indicate that merely increasing the number of amino

(15) Pozsgay, V.; Jennings, H. J. *Tetrahedron Lett.* **1987**, *28*, 1375−1376.

(16) These steps included perazidation of the commercial neomycin B (Sigma) with TfN_3 according to the procedure of Wong,[4a,b] selective silylation of the primary hydroxyl at C5″, acetylation of all the remaining hydroxyls, and desilylation as depicted in Scheme 2.

(17) Purity and exclusive β-stereochemistry of new glycosidic bonds in **15a**,**b** were confirmed by ^{1}H NMR spectroscopy (**15a**: H-1, δ 4.86 ppm, $J_{1,2}$ = 8.0 Hz. **15b**: H-1, δ 4.81 ppm, $J_{1,2}$ = 7.5 Hz. **15c**: H-1, δ 5.62 ppm, $J_{1,2}$ = 8.5 Hz. **15d**: H-1, δ 5.95 ppm, $J_{1,2}$ = 4.5 Hz).

(18) Complete NMR assignments for the monosaccharides **6**−**9**, along with the protected pseudo-pentasaccharides **15a**−**d**, and selected data for the unprotected **2**−**5** are given in Supporting Information.

(19) Resistant strains included *E. coli* XL1(pET9d), *Pseudomonas aeuriginosa* (ATCC 27853), and *Salmonella virchow* (SV49). *E. coli* XL1-(pET9d) is an antibiotic-sensitive laboratory strain of *E. coli* that harbors plasmid pET9d with the cloned *orf2* gene, which codes for aminoglycoside kinase APH(3′). *P. aeuriginosa* is a Gram-negative pathogen. The *aph(3′)-IIb* gene, which codes for APH(3′), is a chromosomal gene that was found in many clinical isolates of *P. aeruginosa*, including the ATCC 27853 strain, and likely accounts at least partly for the resistance of *Pseudomonas* to aminoglycosides (Hachler, H.; Santanam, P.; Kayser, F. H. *Antimicrob. Agen. Chemother.* **1996**, *40*, 1254−1256). *S. virchow* (SV49) is a clinical multidrug-resistant strain obtained from poultry and found to be resistant to streptomycin, tetracycline, ampicillin, sulfa, kanamycin, and neomycin. The mechanism(s) of resistance of this strain is still not known.

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Table 1. MICs of **1**−**5** against Various Bacterial Strains

bacterial strain	MIC (μg/mL)					
	KAN[a]	**1**	**2**	**3**	**4**	**5**
E. coli (R47-100)	ND[b]	4−5.5	85	40−50	35−40	4.5−6
E. coli (ATCC 25922)	ND	8−10	95	40−50	25−30	10−11
E. coli XL1 blue (pET9d)	260−270	50−60	>200	>200	>200	35−45
Staphylococcus epidermidis (ATCC 12228)	ND	0.3−0.4	5.5−7	1.5−1.8	1.4−1.8	0.2−0.4
Bacilus subtilis (ATCC 6633)	ND	0.8−0.9	8.5−10	3.5−4	1.4−1.8	0.6−0.8
Salmonella virchow (SV49)	500−570	200−250	>1250	>1250	>1250	75−125
Pseudomonas aeuriginosa (ATCC 27853)	450−500	55−60	110−130	30−35	40−50	60−65

[a] KAN = kanamycin. [b] ND = not determined.

groups on the natural drug may not lead to an increase in antibacterial activity, even though the binding affinity of these analogues to RNA is likely to increase in vitro.[5−7] However, the excellent activities observed for the amino derivatives **3** and **4** against *Pseudomonas* but significantly weak activities against other bacterial strains imply that the structural and functional requirements for this family of drugs are not similar in order to reach analogous high antibacterial performance against different organisms. While this is in agreement with earlier reported data on other aminoglycoside analogues,[2,21] clearly further structure−activity studies within more diverse structures at ring V, along with more structural data on the interaction between aminoglycosides and rRNA, are required to better understand this issue in detail.[22]

In summary, the neomycin B derivatives prepared in this study represent a new class of branched aminoglycoside antibiotics that show antibacterial activity superior to that of neomycin B against pathogenic and resistant bacterial strains. Here we demonstrate that the precise incorporation of additional rigidity into the natural drug while the parent structure remains intact leads to improved antibacterial performance. This research thus provides a new direction for the development of novel antibiotics that target at once both the bacterial RNA and resistance-causing enzymes.

(22) Studies on the complete structure−activity relationship of ring V of designer pseudo-pentasaccharides are currently under investigation. We also intend to examine these newly designed structures as substrates/inhibitors of aminoglycoside-modifying enzymes to validate the postulated reduced interaction.

Acknowledgment. We thank Dr. Smadar Shulami for antibacterial tests and insightful discussions, Dr. Yael Balazs for technical support in monitoring oligosaccharides by NMR, and Shay Yogev for help in preparation of donor **7**. This research was supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities (Grant 214/00) and in part by the Foundation for Promotion of Research at the Technion. V.B. acknowledges the financial Support by the Center of Absorption in Science, the Ministry of Immigration Absorption and the Ministry of Science and Arts, Israel (Kamea Program).

Supporting Information Available: Selected procedures and complete analytical data for compounds **6**−**9**, **15a**−**d**, and selected data for **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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