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Synthesis and antibacterial activity of novel neamine derivatives

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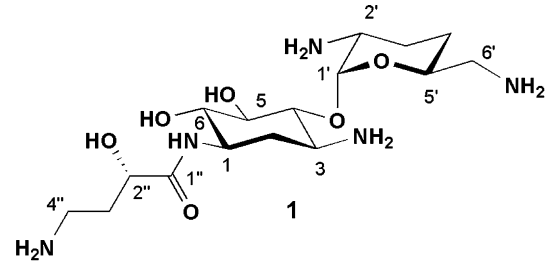
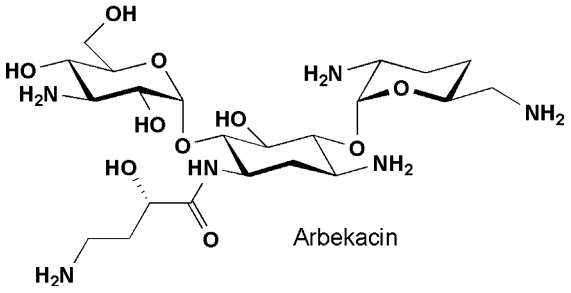
Abstract—Synthesis and activity of derivatives at the O5 or O6 positions of 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-3′,4′-dideoxyneamine, which is the neamine moiety of arbekacin, were reported. Among these results, the 5-*O*-aminoethylaminocarbonyl derivative showed effective activity against *Staphylococcus aureus* expressing a bifunctional aminoglycoside-modifying enzyme AAC(6′)-APH(2″).

Aminoglycoside antibiotics are clinically important drugs used to treat various infections caused by Gram-positive and Gram-negative bacteria.[1] It is well recognized that these antibiotics bind specifically in the bacterial ribosome to the A-site of the decoding region of 16S rRNA and interfere with protein biosynthesis, leading to bacterial cell death.[2] Recently, growing resistance to aminoglycosides has been observed among bacterial species such as methicillin-resistant *Staphylococcus aureus* (MRSA). The major mechanism of resistance to aminoglycosides is their inactivation by aminoglycoside-modifying enzymes (AMEs) such as aminoglycoside acetyltransferase (AAC), aminoglycoside phosphotransferase (APH), and aminoglycoside adenylyltransferase (AAD)[1] in the bacterial periplasm. Modification of the antibiotic by AME reduces its affinity for bacterial 16S rRNA or decreases the membrane permeability of the drug. To overcome this problem, the development of a novel class of AME-resistant aminoglycosides is essential. In the course of our work on such novel aminoglycosides, we became interested in 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-3′,4′-dideoxyneamine **1**, the neamine moiety of arbekacin (ABK),[3] as a lead substrate, since the neamine core in aminoglycosides plays a critical role in aminoglycoside binding to A-site RNA.[1,2] Further, neamine derivative **1** would be stable against AMEs such as APH(3′), AAD(4′), and the bifunctional enzyme AAC(6′)–APH(2″)[4] produced by MRSA. The antibacterial activities of various neamine derivatives have been studied to date;[5] however, to our knowledge, little has been known about the antibacterial activities of derivatives of **1**. In this paper, we describe the synthesis of 1-*N*-((*S*)-4-amino-2-hydroxybutyryl)-3′,4′-dideoxyneamine derivatives at the O5 or O6 positions and the antibacterial activity of these derivatives.





Keywords: Aminoglycoside; Neamine; Methicillin-resistant *Staphylococcus aureus*.
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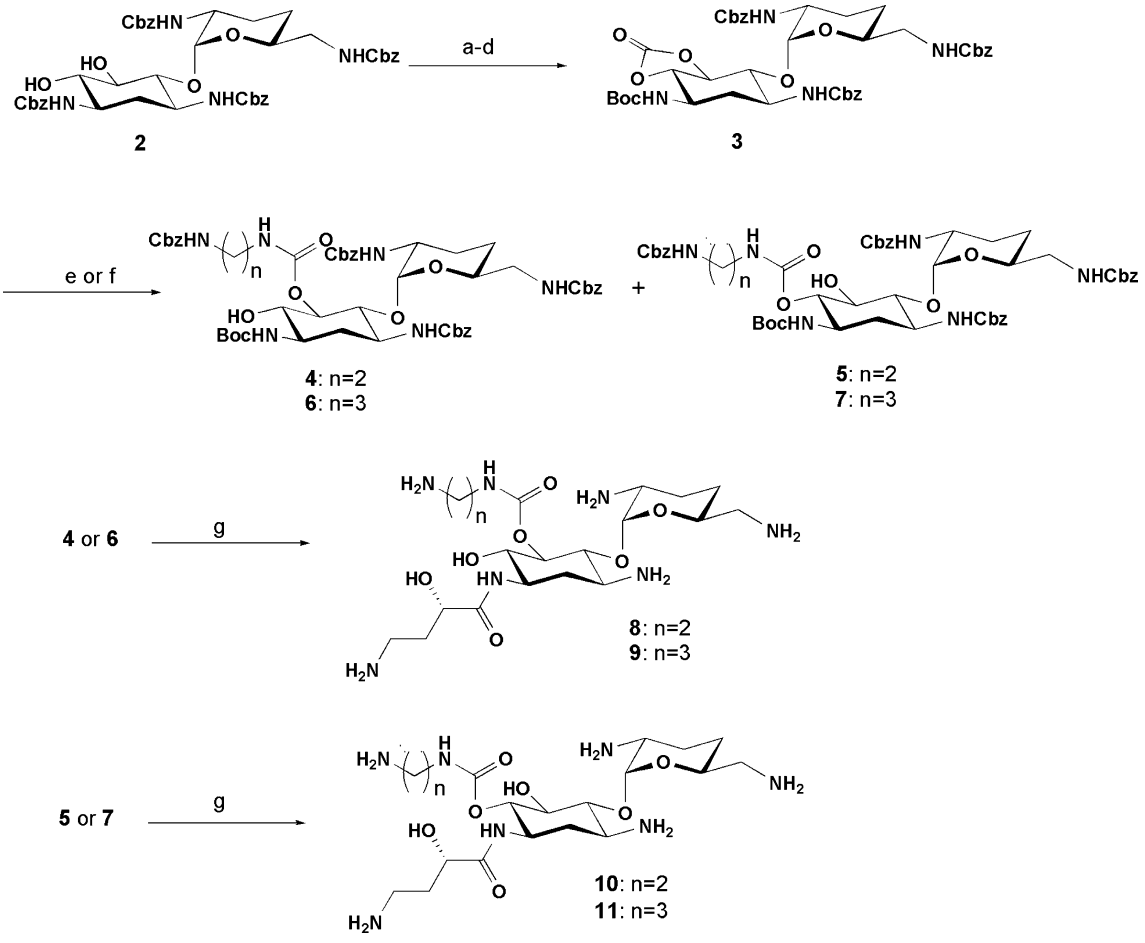
Derivatives **8**–**11** possessing an amino alkyl side chain at the O5 or O6 positions were prepared from **2** (Scheme 1). Selective deprotection[4b,4c] of **2** prepared from neamine gave 3,2′,6′-tri-*N*-benzyloxycarbonyl-3′, 4′-dideoxyneamine. Then, *N*-*tert*-butoxycarbonyl (Boc) protection of the amino group followed by treatment of the resulting diol with 1,1′-carbonyl diimidazole (CDI) afforded cyclic carbonate **3**. Reaction of **3** with *N*-(benzyloxycarbonyl)ethylenediamine (*N*-Cbz-ethylenediamine) gave carbamates **4** and **5** which could be separated by column chromatography on silica gel ($CHCl_3$/MeOH, 80:1), while the reaction of **3** with *N*-(benzyloxycarbonyl)propanediamine gave carbamates **6** and **7** which could be separated by flash chromatography on silica gel (CH_2Cl_2/MeOH, 50:1). Deprotection of the *N*-Boc group of **4** with formic acid followed by condensation with the *N*-hydroxysuccinimide ester of (*S*)-4-*p*-methoxybenzyloxycarbonylamino-2-hydroxybutanoic acid (PMZ-AHB) gave the corresponding amide, which was deprotected by Pd–C catalyzed hydrogenation to afford the desired **8**[6,7]. In a similar procedure, **5**, **6**, and **7** were converted to **10**,[7] **9**, and **11**, respectively.[6]

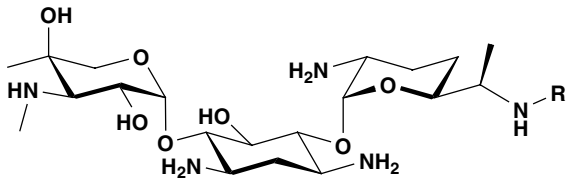
Antibacterial activities of **8**–**11** are shown in Table 1.[8] *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, including resistant strains, were tested. Lead compound **1**, the neamine moiety of ABK, showed a good antibacterial spectrum compared to 3′,4′-dideoxyneamine (DN). Introduction of the aminoethylaminocarbonyl group at the O5 position of **1** resulted in increased activity against both sensitive and resistant *S. aureus* (compound **8**). In particular, compound **8** showed effective activity against gentamicin (GM) and ABK-resistant strains such as *S. aureus* RN4220/pMF490 and MF490 expressing AAC(6′)-APH(2″) and AAD(4′). It is noteworthy that compound **8** is more active than **1** and GM against *P. aeruginosa* GN4925 expressing AAC(6′)-Ib. On the other hand, compounds **9**, **10**, and **11** showed significantly reduced activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. These results seem to indicate that the introduction of the 5-*O*-aminoethylaminocarbonyl group led to enhanced stability against modification by AMEs such as AAD(4′)-I, AAC(6′)-Ib, and AAC(6′)-APH(2″).

Further, to obtain information on how the O5 or O6 side chains of the derivatives bind to the A-site RNA, docking studies were performed for these derivatives. Structural models of compounds **8**–**11** bound to the A-site RNA were generated based on the crystal structure of the A-site RNA, in complex with paromomycin[2c] and

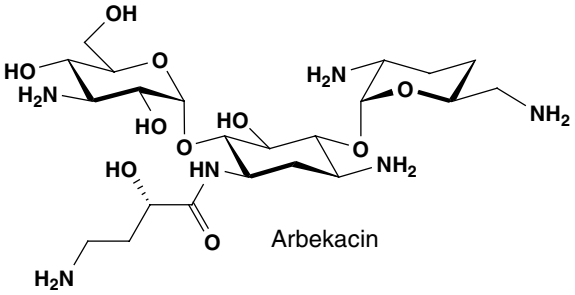


Scheme 1. Reagents and conditions: (a) NaH, DMF, 0 °C; (b) $Ba(OH)_2$, dioxane, H_2O, 80 °C; (c) $(Boc)_2O$, Et_3N, THF, H_2O, rt, 51% from **2**; (d) CDI, THF, rt, 91%; (e) *N*-Cbz-ethylenediamine, CH_2Cl_2, rt, **4**: 49%, **5**: 30%; (f) *N*-Cbz-propanediamine, CH_2Cl_2, rt, **6**: 24%, **7**: 20%; (g) 1—HCOOH, rt; 2—PMZ-AHB, *N*-hydroxysuccinimide, DCC, THF, rt; 3—10% Pd–C, EtOH, H_2O, rt, **8**: 52%, **9**: 42%, **10**: 15%, **11**: 32%.

Table 1. Minimum inhibitory concentrations (MICs) of compounds **8**–**11**



Gentamicin C_1: R=CH_3
Gentamicin C_2: R=H



Arbekacin

Test organism	AME	MIC (μg/ml)							
		8	**10**	**9**	**11**	DN	**1**	GM	ABK
Staphylococcus aureus RN4220		2	8	16	16	16	4	0.25	0.5
S.aureus RN4220/pMS520	AAD(4′)-I	2	8	16	16	16	4	0.25	0.5
S.aureus RN4220/pCR1948	AAC(6′)-APH(2″)	2	8	16	16	>128	2	64	1
S.aureus RN4220/pMF490	AAC(6′)-APH(2″)	2	16	32	16	>128	4	>128	8
S.aureus MF490 (MRSA)	AAD(4′), AAC(6′)-APH(2″)	8	32	128	64	>128	8	>128	64
Escherichia coli NIH JC-2		8	32	32	64	32	8	0.5	1
Pseudomonas aeruginosa PAO1		8	>32	32	32	32	8	4	4
P.aeruginosa GN4925	AAC(6′)-Ib	8	>32	128	128	>128	64	128	4
P.aeruginosa GN3054	AAC(3)-I	16	>32	64	128	64	16	128	8

DN, 3′,4′-dideoxyneamine; GM, gentamicin (gentamicin C_1, gentamicin C_2); ABK, arbekacin.

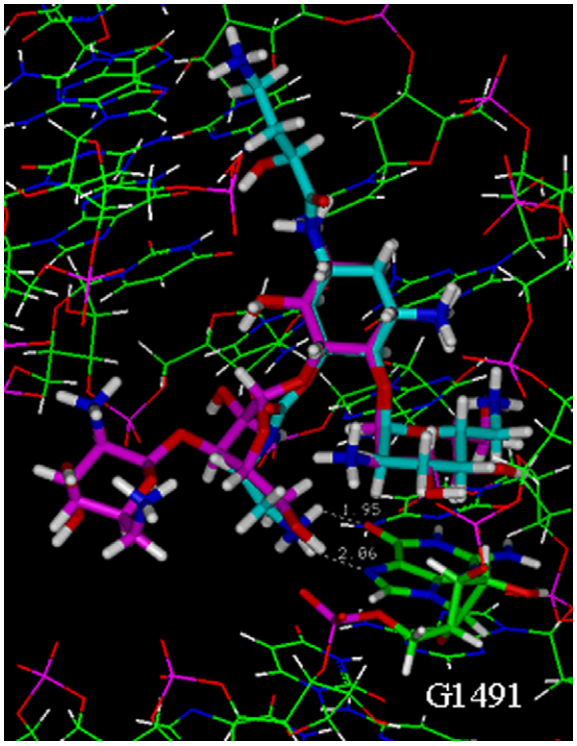


Figure 1. Modeling of compound **8** (blue) and paromomycin (red) bound to the A-site of 16S rRNA (green).

neamine derivative.[5d] As expected, the molecular modeling studies suggest that the O5 side chain of **8**, which shows effective antibacterial activity, interacts significantly with 16S rRNA, compared to the side chains of compounds **9**–**11**. Thus, the terminal amino group of the O5 side chain of **8** locates itself in the space near the hydroxyl group O5″ of paromomycin and forms two hydrogen bonds with O6 and N7 of G1491[2a,2c] (Fig. 1).

In summary, we chose the neamine derivative **1** as a lead compound and synthesized several derivatives with side chains at the O5 and O6 positions. Among these derivatives, **8** showed effective activity against *S. aureus* expressing AAC(6′)-APH(2″) and *P. aeruginosa* expressing AAC(6′)-Ib. This series of derivatives offers a new perspective for the development of novel aminoglycosides that will prove effective against resistant bacteria.

Acknowledgments

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6. Compounds **5**–**8** were purified by column chromatography on CM Sephadex (NH_4^+ form, gradient elution with 0.05–1.1 N NH_4OH).
7. Selected spectral data. Compound **8**: ^1H NMR (D_2O/ND_3, 400 MHz) δ 1.37–1.46 (m, 2H, H-2a, H-4′a), 1.53–1.59 (m, 1H, H-3′a), 1.66–1.75 (m, 2H, H-3′b, H-4′b), 1.80 (quin, J = 7.2 Hz, 1H, H-3″a), 1.88–1.96 (m, 1H, H-3″b), 1.99–2.03 (m, 1H, H-2b), 2.62–2.77 (m, 7H, H-2′, H-6′, H-4″, H-3‴), 2.96–3.02 (m, 1H, H-3), 3.17–3.22 (m, 2H, H-2‴), 3.51 (dd, J = 10.1, 8.9 Hz, 1H, H-4), 3.62 (t, J = 10.1 Hz, 1H, H-6), 3.87–3.94 (m, 2H, H-1, H-5′), 4.23 (dd, J = 7.8, 4.2 Hz, 1H, H-2″), 4.75–4.79 (1H, H-5, J value could not be correctly measured by overlapping with the solvent peaks), 4.92 (d, J = 2.9 Hz, 1H, H-1′); FAB MS: m/z 478 $(M+H)^+$. Compound **10**: ^1H NMR (D_2O/ND_3, 400 MHz) δ 1.37–1.46 (m, 1H, H-4′a), 1.55 (q, J = 12.5 Hz, 1H, H-2a), 1.63–1.78 (m, 4H, H-3′a, H-3′b, H-4′b, H-3″a), 1.81–1.89 (m, 1H, H-3″b), 2.01 (dt, J = 12.9, 2.9 Hz, 1H, H-2b), 2.62–2.74 (m, 6H, H-6′, H-4″, H-3‴), 2.88 (dt, J = 12.0, 3.9 Hz, 1H, H-2′), 2.94–2.97 (m, 1H, H-3), 3.08–3.14 (m, 1H, H-2‴a), 3.17–3.26 (m, 1H, H-2‴b), 3.40 (t, J = 9.3 Hz, 1H, H-4), 3.76 (t, J = 9.3 Hz, 1H, H-5), 3.87–3.89 (m, 1H, H-5′), 4.01–4.06 (m, 1H, H-1), 4.18 (dd, J = 8.6, 3.5 Hz, 1H, H-2″), 4.60–4.66 (1H, H-6, J value could not be correctly measured by overlapping with the solvent peaks), 5.15 (d, J = 2.7 Hz, 1H, H-1′); FAB MS: m/z 478 $(M+H)^+$. NMR assignments were made by interpretation of COSY experiments.
8. MICs were detemined by the two-fold agar dilution method according to NCCLS.