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DESIGN AND SYNTHESIS OF NOVEL RING-EXPANDED ARBEKACIN ANALOGUES

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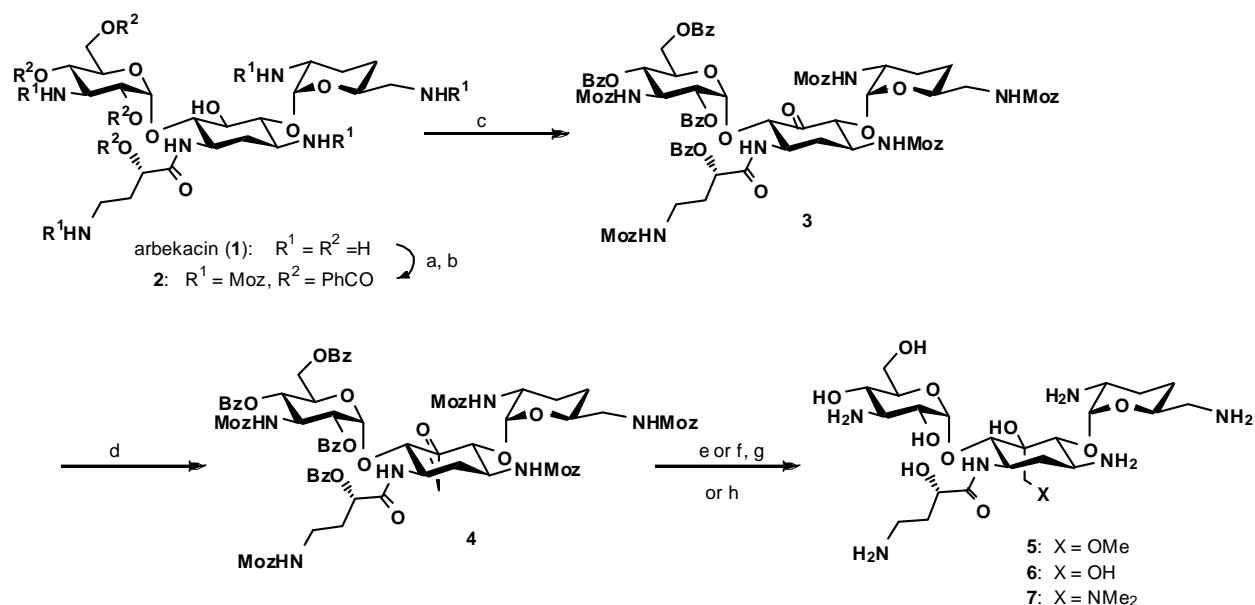
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Abstract – Novel homologated aminoglycosides having a seven-membered ring were designed and synthesized by treatment of 5-keto arbekacin with diazomethane in CH₂Cl₂-Et₂O. The ring-expanded arbekacin analogue showed good antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* including aminoglycoside-resistant bacterial strain.

Aminoglycoside antibiotics are a clinically important class of natural products with antibacterial activity against Gram-positive and Gram-negative bacteria.¹ The aminoglycosides bind specifically to the A-site of the decoding region of the 16S bacterial ribosome RNA (rRNA) and interfere with protein biosynthesis, leading to bacterial cell death.² The recent studies of X-ray crystal structures of several aminoglycosides complexed to the rRNA suggest that the central 2-deoxystreptamine ring of aminoglycoside structures play a crucial role in the interactions with 16S rRNA.³

Recently ring-expanded analogues of natural pyranose sugars have been reported as new efficient tools for the investigation of protein-carbohydrate interactions.⁴ Further, the ring-expanded carbohydrate analogues are useful for the development of glycosidase inhibitors and the investigation of glycosidase mechanisms.^{4c} Based on these findings, we have been interested in the ring-expansion of the 2-deoxystreptamine ring of aminoglycosides in our continuous search for novel aminoglycosides with efficient antibacterial activity.⁵ However, to the best of our knowledge, ring-expanded aminoglycosides have not been reported so far. Herein, we disclose the first example of the synthesis of 2-deoxystreptamine ring-expanded aminoglycosides and its antibacterial activity.

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Scheme 1. Reagents and conditions: a) Moz-S, Et₃N, H₂O, IPA, THF, 60 °C, 79%; b) BzCl, Py, rt, 97%; c) DMSO, Ac₂O, rt, 99%; d) excess CH₂N₂, 1:1 MeOH/Et₂O, 0 °C, 86%; e) (1) 1M NaOMe, MeOH, rt; (2) 90%TFA, **5** 41%; f) 1N NaOH, MeOH, CHCl₃, rt, 73%; g) (1) CsOAc, DMF, 80 °C; (2) 1N NaOH, H₂O, rt; (3) 90% TFA, rt, **6** 76%; (h) (1) excess Me₂NH, H₂O, EtOH, 80 °C; (2) 1M NaOMe, MeOH, rt; (3) 90% TFA, **7** 79%.

We focused on arbekacin⁶ (ABK, **1**) as a starting material and attempted expansion of the 2-deoxystreptamine ring of 5-keto ABK **3** by treatment with diazomethane.⁷ Compound **3**, having a 5-keto group, was prepared from **1** in three steps (Scheme 1). *p*-Methoxybenzyloxycarbonyl⁸ (Moz) protection of the amino groups of **1** with *S-p*-methoxybenzyloxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine (Moz-S)⁹ followed by treatment of the resulting penta-*N*-Moz arbekacin with benzoyl chloride (BzCl) afforded **2**, which was treated with DMSO-Ac₂O¹⁰ to provide the 5-keto **3**. The first attempt to expand the deoxystreptamine ring of **3** by treatment with diazomethane⁷ in MeOH-Et₂O¹¹ provided only the undesired epoxide **4** in good yield as a single stereoisomer; no ring-expanded product was obtained (Scheme 1). We assume that the selective formation of epoxide in this reaction is attributable to the pyranosyl substituents at positions C4 and C6 of

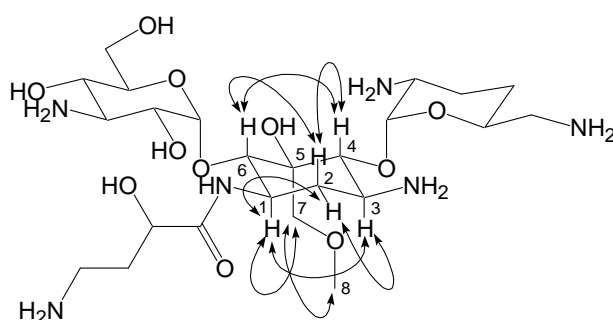
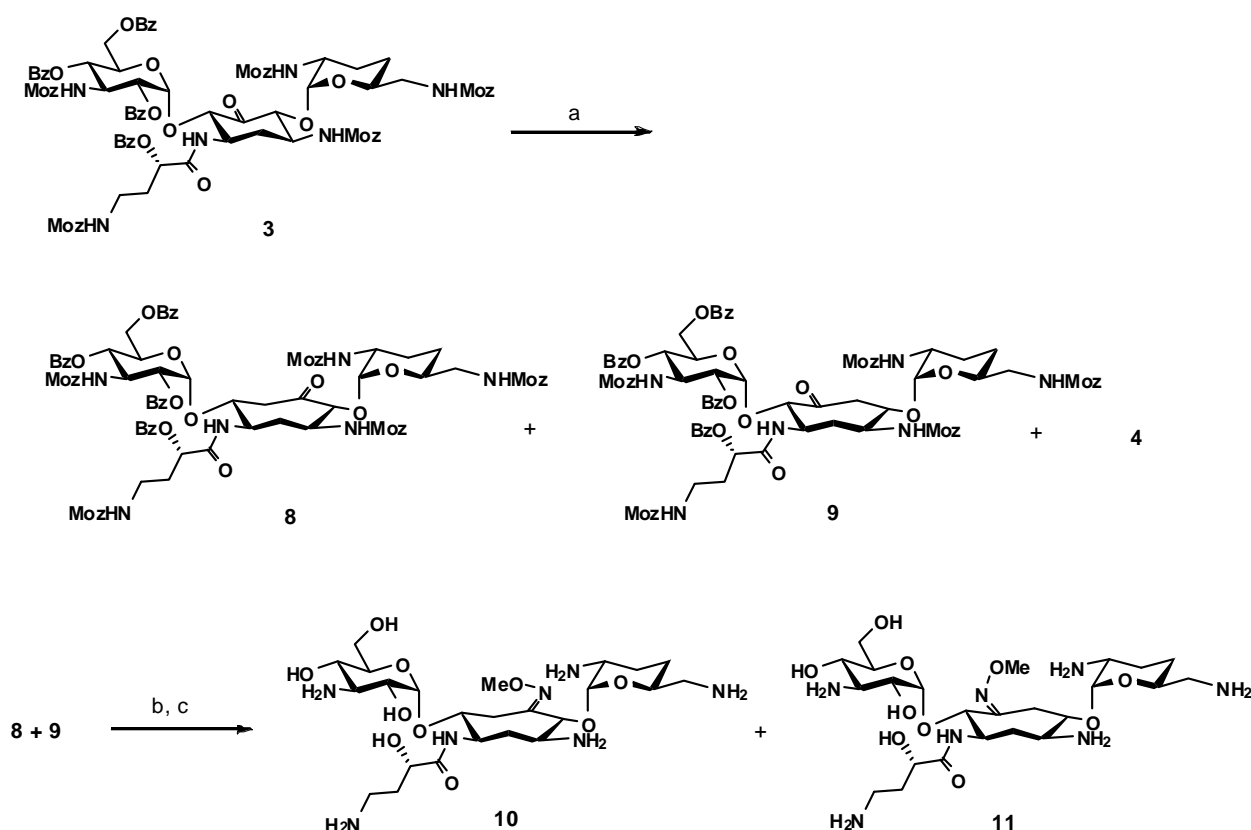


Figure 1. Selective ROESY correlations of **5**.

ketone **3**.¹² To confirm the configuration of **4** at position C5, the epoxide was converted into the ring-opened derivative. Thus, treatment of **4** with 1M NaOMe followed by deprotection of the Moz groups with trifluoroacetic acid (TFA) afforded 5-methoxymethyl ABK **5**.¹³ The results of ROE experiments on **5** indicated that the 5-OH of **5** was equatorial in orientation and the 5-methoxymethyl group was axial in configuration (Figure 1). Therefore, the configuration of **4** at position C5 was determined to be as illustrated in Scheme 1. Presumably, nucleophilic attack of diazomethane on the ketone at position C5 would take place stereoselectively from the less hindered α face to afford **4**. Further, we attempted several ring opening reactions to investigate the reactivity of the epoxide **4**. Deprotection of the Bz groups of **4** with 1N NaOH followed by treatment with CsOAc at 80 °C in DMF gave penta-*N*-Moz-5-epiacetoxymethyl ABK. Then, stepwise deprotections of the Ac and Moz groups gave the 5-hydroxymethyl ABK **6**.¹³ While, cleavage of epoxy group of **4** with excess dimethylamine at 80 °C followed by stepwise deprotections of Bz and Moz groups provided the 5-dimethylaminomethyl ABK **7**.¹³ These results suggested that the epoxide **4** would be a useful intermediate for preparation of various 5 α -substituted aminoglycoside derivatives.



Scheme 2. Reagents and conditions: a) excess CH_2N_2 , 1:1 $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$, rt, **8** + **9** 36%, **4** 40%; b) $\text{MeONH}_2 \cdot \text{HCl}$, Py, 60 °C, a mixture of oximes 74%; c) (1) 1M NaOCH₃, MeOH, CHCl_3 , rt; (2) 90% TFA, rt, **10** 65%, **11** 16%.

After considerable experimentation, it was found that the product ratio of the ring-expanded compounds to the undesired epoxide was significantly influenced by the choice of solvent. Thus, ring expansion was achieved by treatment of **3** with diazomethane in the aprotic solvent mixture, $\text{CH}_2\text{Cl}_2\text{-Et}_2\text{O}$, to give the desired **8** and **9** as an inseparable regioisomeric mixture in 36% yield, and the epoxide **4** in 40% yield (Scheme 2). It is assumed that use of CH_2Cl_2 in place of MeOH resulted in a decrease in the relative rate of the reaction to form the thermodynamically stable ring-expanded product.^{11,12} After the keto group of the mixture of **8** and **9** was converted into the apparently stable oxime in 74% yield, by treatment with hydroxylamine, deprotection of the Moz and Bz groups afforded the ring-expanded **10**¹³ and **11**¹³ in 65% and 16% yield respectively.¹⁴ These results suggested that the ring expansion of **3** with diazomethane in $\text{CH}_2\text{Cl}_2\text{-Et}_2\text{O}$ proceeds stereoselectively to afford **8** as the major isomer. Presumably, the selective formation of **8** is explained by considering the relative stabilities of the conformations of the intermediates A and B (Figure 2).¹⁵

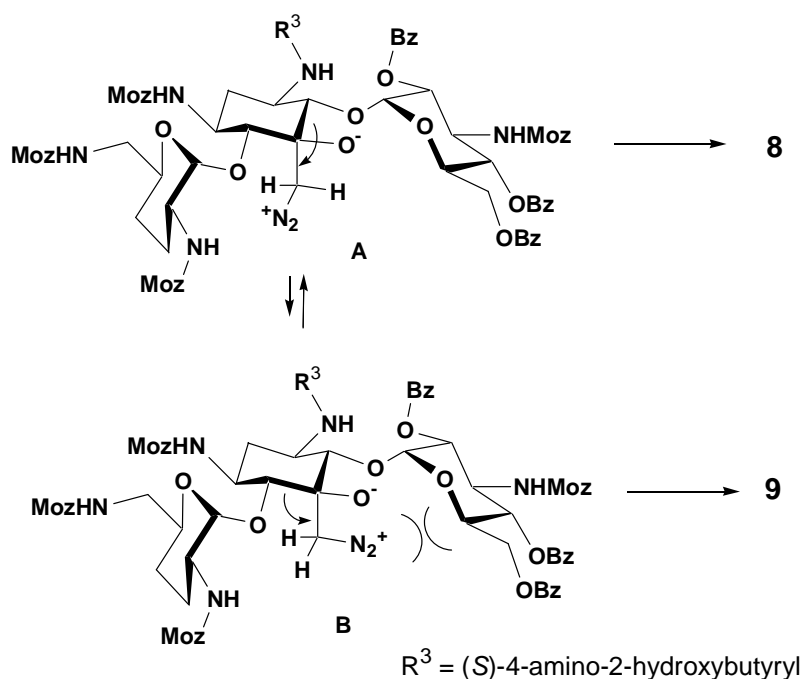


Figure 2. Pathways to ring-expanded products via intermediates A and B.

The antibacterial activity of **10** was tested against several strains. Interestingly, the 2-deoxystreptamine ring-expanded arbekacin **10** showed good antibacterial activity against *Staphylococcus aureus* 209P JC-1, *S. aureus* RN4220 and *Escherichia coli* JM109 (minimum inhibitory concentrations (MICs) 2, 8, and 4 $\mu\text{g/mL}$, respectively).¹⁶ Further, **10** retained activity against *S. aureus* RN4220/pCR1948, which is a aminoglycoside-resistant strain, expressing aminoglycoside-modifying enzyme AAC(6')-APH(2'')^{1,6,17} (MIC 16 $\mu\text{g/mL}$). These results seem to indicate that 2-deoxystreptamine ring-expanded arbekacin would be an attractive lead compound for development of novel aminoglycosides.

In summary, the novel ring-expanded aminoglycoside synthesized in this study represents a new class of aminoglycoside antibiotic, which is active against *E. coli* and *S. aureus* including aminoglycoside-resistant bacterial strain. The design would be useful for future development of novel aminoglycosides with good antibacterial activity, while, the undesired epoxide **4** would be a useful intermediate for preparation of various 5 α -substituted aminoglycoside derivatives. The structure-activity relationship studies of the 2-deoxystreptamine ring-expanded aminoglycosides are currently in progress.

ACKNOWLEDGEMENTS

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13. Compound **5**: ^1H NMR (400MHz, $\text{D}_2\text{O} + \text{DCl}$): δ 1.69-1.75 (m, 1H, H-4'), 1.90 (q, $J = 12.7$ Hz, 1H, H-2a), 1.99-2.06 (m, 2H, H-4', H-3''a), 2.08-2.16 (m, 2H, H-3'), 2.24 (ddt, $J = 14.4, 7.3, 3.9$ Hz, 1H, H-3''b), 2.39 (dt, $J = 12.9, 4.6$ Hz, 1H, H-2b), 3.20-3.24 (m, 1H, H-6'a), 3.24 (t, $J = 7.4$ Hz, 2H, H-4'') 3.35 (dd, $J = 13.6, 3.4$ Hz, 1H, H-6'b), 3.44 (t, $J = 10.5$ Hz, 1H, H-3''), 3.50 (s, 3H, H-8), 3.63-3.68 (m, 1H, H-2'), 3.77 (t, $J = 10.0$ Hz, 1H, H-4''), 3.82 (d, $J = 10.7$ Hz, 1H, H-7a), 3.86 (dd, $J = 10.0, 3.7$ Hz, 1H, H-2''), 3.87 (d, $J = 2.9$ Hz, 2H, H-6''), 3.87-3.92 (m, 1H, H-3), 3.94 (d, $J = 10.8$ Hz, 1H, H-7b), 4.02 (d, $J = 10.7$ Hz, 1H, H-6), 4.06 (dt, $J = 10.0, 2.9$ Hz, 1H, H-5''), 4.12 (d, $J = 11.0$ Hz, 1H, H-4), 4.23-4.29 (m, 1H, H-5''), 4.33 (dd, $J = 9.0, 3.6$ Hz, 1H, H-2'''), 4.35-4.40 (m, 1H, H-1), 5.19 (d, $J = 3.6$ Hz, 1H, H-1''), 5.76 (d, $J = 3.2$ Hz, 1H, H-1'); ^{13}C NMR (100MHz, $\text{D}_2\text{O} + \text{DCl}$): δ 21.5 (C-3'), 26.0 (C-4'), 31.2 (C-2), 31.7 (C-11), 37.9 (C-12), 43.3 (C-6'), 48.4 (C-1), 48.9 (C-3), 49.9 (C-2'), 56.3 (C-3''), 60.0 (C-8), 60.6 (C-6''), 66.4 (C-4''), 67.2 (C-5'), 69.0 (C-2''), 70.5 (C-10), 71.1 (C-7), 73.5 (C-5''), 77.7 (C-5), 81.8 (C-4), 84.0 (C-6), 96.6 (C-1'), 100.4 (C-1''), 176.4 (C-9). Compound **6**: ^1H NMR (400MHz, $\text{D}_2\text{O} + \text{ND}_3$): δ 1.32-1.50 (m, 2H, H-2a, H-4'a), 1.65-1.80 (m, 4H, H-3', H-4'b, H-3''a), 1.85-1.95 (m, 1H, H-3''b), 1.97-2.05 (m, 1H, H-2b), 2.60-2.70 (m, 2H, H-6'), 2.70-2.80 (m, 2H, H-4''), 2.85-2.92 (m, 1H, H-2'), 2.98 (t, $J = 10.0$ Hz, 1H, H-3''), 3.15-3.25 (m, 1H, H-3), 3.32 (t, $J = 10.0$ Hz, 1H, H-4''), 3.39 (dd, $J = 10.5, 3.9$ Hz, 1H, H-2''), 3.45 (d, $J = 10.3$ Hz, 1H, H-4), 3.76-3.78 (m, 2H, H-6''), 3.81 (d, $J = 10.7$ Hz, 1H, H-6), 3.85-3.94 (m, 1H, H-5'), 3.90 (d, $J = 11.7$ Hz, 1H, H-7a), 3.99 (d, $J = 11.7$ Hz, 1H, H-7b), 3.99-4.05 (m, 1H, H-5''), 4.18 (dd, $J = 9.2, 3.6$ Hz, 1H, H-2'''), 4.32-4.40 (m, 1H, H-1), 5.05 (d, $J = 3.9$ Hz, 1H, H-1''), 5.11 (d, $J = 3.2$ Hz, 1H, H-1'). Compound **7**: ^1H NMR (400MHz, $\text{D}_2\text{O} + \text{ND}_3$): δ 1.36 (q, $J = 12.5$ Hz, 1H, H-2a), 1.42-1.52 (m, 1H, H-4'a), 1.66-1.81 (m, 4H, H-3', H-4'b, H-3''a), 1.87-1.96 (m, 1H, H-3''b), 2.02 (dt, $J = 13.2, 4.6$ Hz, 1H, H-2b), 2.60 (s, 6H, H-8, H-9), 2.63-2.70 (m, 2H, H-6'), 2.73-2.82 (m, 2H, H-4''), 2.97 (t, $J = 9.8$ Hz, 1H, H-3''), 3.02 (d, $J = 14.4$ Hz, 1H, H-7a), 3.03-3.10 (m, 2H, H-3, H-2'), 3.15 (d, $J = 14.2$ Hz, 1H, H-7b), 3.33 (t, $J = 9.7$ Hz, 1H, H-4''), 3.40 (d, $J = 10.5$ Hz, 1H, H-2''), 3.41 (d, $J = 10.5$ Hz, 1H, H-4), 3.75-3.82 (m, 3H, H-6, H-6''), 3.90-3.98 (m, 2H, H-1, H-5'), 4.00-4.04 (m, 1H, H-5''), 4.18 (dd, $J = 9.5, 3.6$ Hz, 1H, H-2'''), 5.05 (s, 1H, H-1' or H-1''), 5.06 (s, 1H, H-1' or H-1''). Compound **10**: ^1H NMR (400MHz, $\text{D}_2\text{O} + \text{ND}_3$): δ 1.40-1.54 (m, 1H, H-4'a), 1.60-1.88 (m, 6H, H-2, H-3', H-4'b, H-3''a), 1.96-2.05 (m, 1H, H-3''b), 2.46 (t, $J = 11.9$ Hz, 1H, H-6a), 2.71 (dd, $J = 13.4, 7.8$ Hz, 1H, H-6'a), 2.79 (dd, $J = 13.4, 3.9$ Hz, 1H, H-6'b), 2.82-2.92 (m, 3H, H-2', H-4''), 3.04 (t, $J = 9.8$ Hz, 1H, H-3''), 3.34-3.38 (m, 1H, H-6b), 3.38 (t, $J = 9.8$ Hz, 1H, H-4''), 3.42-3.45 (m, 1H, H-3), 3.46 (dd, $J = 10.2, 3.9$ Hz, 1H, H-2''), 3.81-3.94 (m, 4H,

H-5', H-5'', H-6''), 3.94-3.96 (m, 1H, H-7), 3.99 (d, $J = 8.1$ Hz, 1H, H-4), 4.07 (s, 3H, OCH₃), 4.08-4.14 (m, 1H, H-1), 4.27 (dd, $J = 9.2, 3.9$ Hz, 1H, H-2'''), 4.86 (d, $J = 3.4$ Hz, 1H, H-1'), 5.10 (d, $J = 3.9$ Hz, 1H, H-1''); ¹³C NMR (100MHz, D₂O + ND₃): δ 29.0 (C-3'), 30.5 (C-4'), 31.7 (C-6), 38.7 (C-2), 38.9 (C-3'''), 40.2 (C-4'''), 48.0 (C-6'), 52.1 (C-2'), 54.6 (C-3), 57.1 (C-3''), 58.4 (C-1), 63.5 (C-6''), 64.7 (OCH₃), 72.5 (C-4''), 72.8 (C-2'''), 73.5 (C-5'), 74.6 (C-2''), 75.4 (C-5''), 81.0 (C-7), 83.2 (C-4), 98.1 (C-1'), 102.4 (C-1''), 157.2 (C-5), 179.1 (C-1'''). NMR assignments were made by interpretation of COSY experiments.

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