Modification of Aminocyclitol Antibiotics. 6. Preparation of 5-Deoxykanamycin B

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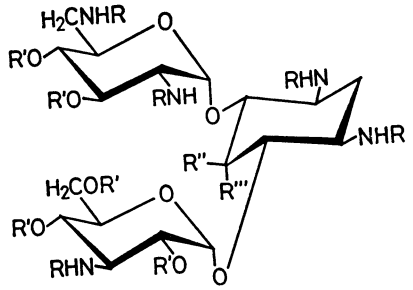
Aminocyclitol antibiotic kanamycin B has been modified as regards the aminocyclitol moiety by removing a hydroxyl group on C-5. 5-Deoxykanamycin B thus obtained was tested against several microorganisms. The structure of 5-deoxykanamycin B was established by mass and ^{13}C NMR spectrometry. 5,6,3′,4′-Tetradeoxyneamine was prepared as a reference compound for ^{13}C NMR spectrometry.

In connection with the preceding paper,[1] antibiotic kanamycin B[2-5] has been modified as regards the aminocyclitol moiety. It has been disclosed that removal of the hydroxyl group on C-5 of neamine enhances the antimicrobial activity relative to the parent neamine against kanamycin resistant strains.[6]

Recently, 5-deoxygentamicin complex[7] has been prepared by means of bioconversion[8] using 2,5-dideoxystreptamine[9] as a precursor in the culture medium. The antibiotic exhibits improved activity against gentamicin-acetylating strains of resistant bacteria. 5-Deoxysisomicin: Mutamicin 2[10] and 5-deoxykanamycin A[11] have also been prepared by means of bioconversion.

Since removal of the hydroxyl group on C-5 of kanamycin B might enhance its antimicrobial activity, we have attempted to prepare 5-deoxykanamycin B by using analogous reactions employed in the preparation of 5-deoxyneamine.[6]

When 1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B[12] (**1**) was treated with 22 molar equivalents of benzoyl chloride in pyridine, penta-*O*-benzoyl derivative (**2**) was obtained in 63% yield. As the hydroxyl group on C-5 of **1** is sterically highly hindered, compound **2** was deduced to be 3′,4′,2″,4″,6″-penta-*O*-benzoyl derivative. The proposed structure was rationalized by the fact that successive reactions gave 5-deoxykanamycin B. Chlorination of **2** with sulfuryl chloride in pyridine afforded chloro-deoxy compound (**3**) in which the chlorination occurred on C-5 with an inversion of configuration.[13] Dehalogenation of **3** was performed with tributylstannane[14] in toluene solution, giving deoxykanamycin B derivative (**4**) in 86% yield. Compound **4** was converted into deoxykanamycin B (**5**) by removing the protecting groups. *N*-Acetylation of **5** gave penta-*N*-acetyl-deoxykanamycin B (**6**).



1 R=Cbe, R′=R‴=H, R″=OH
2 R=Cbe, R′=Bz, R″=OH, R‴=H
3 R=Cbe, R′=Bz, R″=H, R‴=Cl
4 R=Cbe, R′=Bz, R″=R‴=H
5 R=R′=R″=R‴=H
6 R=Ac, R′=R″=R‴=H
Cbe=COOEt

Scheme 1.

The structure of **5** was determined by mass and ^{13}C NMR spectra as follows. The mass spectrum reveals the molecular ion and $[M+1]^+$ peak at *m/e* 467 and 468, respectively, equivalent to M−O and M+1−O ions of kanamycin B.[15] The peaks at *m/e* 335 and 336 are in line with protonated formyl ions. The amino sugar fragments reveal the peaks at *m/e* 161 and 162, observed in the spectrum of kanamycin B.[15] The aminocyclitol fragment ions appear at *m/e* 175, 157, 147, and 129. The results indicate that the aminocyclitol moiety of **5** has been deoxygenated, supporting the proposed structure. The structure of **5** was confirmed by ^{13}C NMR spectroscopy. The spectrum was determined at pD above 11, showing eighteen carbon signals. The signals are assigned in accordance with the data for kanamycin B,[16] neamine, 5-, 6-, 5,6-di- and 5,6,3′,4′-tetradeoxyneamine (**11**). Compound **11** was prepared by a five step reaction from neamine.

When the spectrum of **5** is compared with that of kanamycin B, it is obvious that deoxygenation causes no appreciable shifts of the signals of carbons in the amino sugar moieties, except for anomeric carbon C-1′. In contrast, the signals of the carbons in the aminocyclitol moiety are more or less shifted by deoxygenation.

In fact, the signal of C-1′ shifts toward a higher field by 4.3 ppm. An analogous upfield shift of the signal of C-1′ has been observed in the spectra of 5-, 5,6-dideoxyneamine, and **11**. However, no such shift of the signal of C-1′ was observed in the spectrum of 6-deoxyneamine.

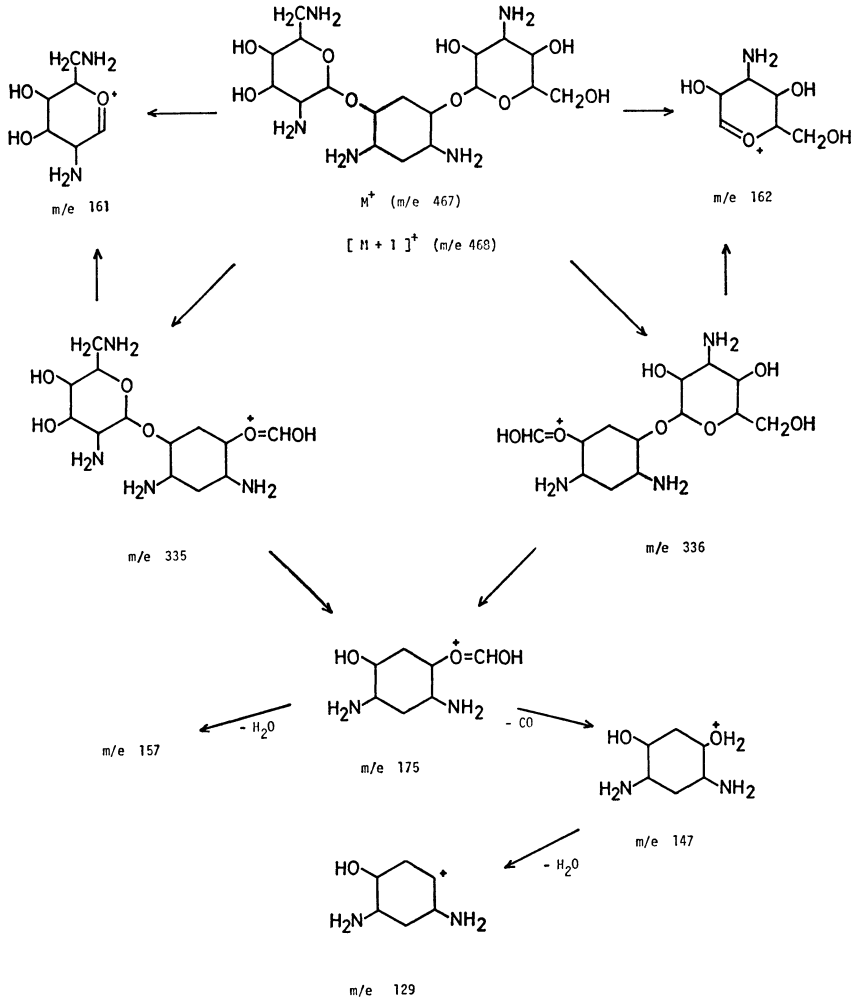
The signals of C-4 and 6 shift upfield by 9.3 and 4.5 ppm, respectively. This is in line with the fact that the signals of C-2 and 4 of methyl 3-deoxy-α-D-glucopyranoside appear in a higher field, as compared to those of methyl α-D-glucopyranoside.[17] The signals of C-1 and 3 shift to a lower field by 2.9 and 2.3 ppm, respectively. This is elucidated by the antiperiplanar arrangement of Eliel *et al.*[18]

The spectra were then determined at pD 1 in order to observe the N-protonation effect, which will supply valuable evidences for structure analysis.[16,19] At pD 1, the signal of C-2 of **5** shifts upfield a great deal (7.8 ppm), that of C-4 and 6 moderately (6.4 and 5.5 ppm) and that of C-5 toward a higher field (2.0 ppm) as compared

Table 1. The ^{13}C NMR chemical shifts[a] of 5-deoxykanamycin B, kanamycin B, and neamine derivatives

	5-Deoxykanamycin B (**5**)		Kanamycin B		5-Deoxyneamine[6]		6-Deoxyneamine[6]		5,6-Dideoxyneamine[6]		5,6,3′,4′-Tetradeoxyneamine (**11**)		Neamine	
	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1	pD 11	pD 1
C-1	53.4	52.2	50.5	50.5	54.9	53.5	45.6	45.3	48.6	48.0	48.7	48.2	51.8	50.7
C-2	36.9	29.1	36.5	28.6	37.0	29.3	42.1	32.6	41.9	32.9	41.9	32.9	36.9	29.0
C-3	52.6	51.7	50.3	49.3	53.0	52.0	50.7	50.0	53.1	52.4	53.3	52.7	50.3	49.5
C-4	78.1	71.7[c]	87.4	77.5	78.7	71.8[g]	91.1	80.4	80.4	73.8	80.5	73.1	88.9	77.7
C-5	34.9	32.9	75.2[d]	75.1	37.6	34.6	72.1	70.9	27.6	25.7	28.0	25.8	77.4	75.9
C-6	84.1	78.6	88.6	84.5	73.7	68.1	41.6	37.2	33.6	27.8	33.7	27.9	78.6	73.3
C-1′	96.2	91.4	100.5	96.2	96.4	91.6	102.3	96.6	96.2	91.5	96.6	90.5	102.2	96.1
C-2′	55.9	54.0	56.3	54.4	56.2	54.0	56.6	54.5	56.3	54.0	50.2	49.1	56.8	54.5
C-3′	75.4	69.6	74.9[d]	69.0	75.9[f]	69.5[h]	75.3[i]	69.2	76.0	69.5[j]	27.2	21.8	75.6	69.0
C-4′	73.0[b]	71.3[c]	73.3[e]	71.8	73.3	71.6[g]	73.2	71.7	73.4	71.6	28.4	26.6	73.4	71.7
C-5′	74.4	69.8	74.7	70.1	75.1[f]	69.7[h]	75.0[i]	69.9	75.2	69.6[j]	71.0	66.3	75.6	69.9
C-6′	42.7	41.1	42.8	41.2	42.9	41.1	42.9	41.3	43.0	41.0	46.0	43.7	43.0	41.3
C-1″	101.8	100.2	101.4	101.4										
C-2″	72.8[b]	68.8	73.0[e]	69.0										
C-3″	55.9	55.7	56.5	55.8										
C-4″	71.5	67.0	71.3	66.3										
C-5″	73.8	73.3	74.0	73.7										
C-6″	62.3	61.4	62.0	60.8										

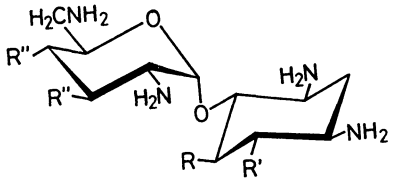
a) In parts per million downfield from tetramethylsilane. b—j) The signals may be reversed.



Scheme 2.

TABLE 2. ANTIMICROBIAL ACTIVITY OF 5-DEOXYKANAMYCIN B (**5**)

Test organisms	MIC (mcg/ml)
Staphylococcus aureus ATCC 6538P	0.78
Staphylococcus epidermidis ATCC 12228	0.39
Diplococcus pneumoniae Type 3	0.2
Bacillus subtilis ATCC 6633	0.2
Escherichia coli NIHJC-2	3.12
Klebsiella pneumoniae 602	3.12
Pseudomonas aeruginosa IAM 1007	100
Proteus vulgaris OX-19	0.39
Salmonella paratypin A 1015	1.56
Salmonella paratyphi B	12.5
Shigella flexneri 2a·SH-74-1	6.25



5-Deoxyneamine[6] R=H, R′=R″=OH
6-Deoxyneamine[6] R=R″=OH, R′=H
5,6-Dideoxyneamine[6] R=R′=H, R″=OH
5,6,3′,4′-Tetradeoxyneamine (**11**) R=R′=R″=H
Neamine R=R′=R″=OH

Scheme 3.

to those determined at pD 11. This is in line with the fact that the effect of N-protonation appears strongly on a β-carbon and weakly on a γ-carbon.[19] Thus, the proposed structure of **5** has been confirmed.

Antimicrobial activities of **5** were determined against several microorganisms. The MIC (minimum inhibition concentration) values, given in Table 2, are similar to those of kanamycin B against most of the microorganisms tested so far.

Experimental

General Methods. Melting points were determined in capillary tubes and are uncorrected. Solutions were concentrated under reduced pressure below 40 °C. Optical rotations were measured on a Japan Spectroscopic DIP-SL polarimeter. ^{1}H NMR spectra were recorded on a Varian A-60D spectrometer at 60 MHz. Deuteriochloroform was used as a solvent with an internal standard of tetramethylsilane. The peak positions are given in δ values. ^{13}C NMR spectra were determined on a Varian FT-80 spectrometer at 20 MHz. Deuterium oxide was used as a solvent with an internal standard of dioxane. The resonance signals are expressed in ppm downfield from the signal of tetramethylsilane (δ_C for dioxane= −67.4). Mass spectrum was recorded with a Hitachi RMU-6M single focusing mass spectrometer. TLC was performed on Wakogel B-10 plates (Wako Pure Chemical Co., Ltd.), and silica gel (Wakogel C-300) was employed for column chromatography.

*3′,4′,2″,4″,6″-Penta-*O*-benzoyl-1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B (2).* Benzoyl chloride (1.5 ml) was added gradually to a solution of 1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B[12] (**1**, 500 mg) in pyridine (15 ml) with stirring under ice cooling. After 18 h at ambient temperature, the solution was poured into 2.5% $NaHCO_3$ solution. The aqueous mixture was extracted with chloroform repeatedly, and the combined chloroform solution was washed with water. After being dried over Na_2SO_4, the solution was concentrated. The residue was purified on a silica-gel column using 7: 1 (v/v) benzene–methanol. Fractions homogeneous on TLC (R_f 0.32) in the same solvent were combined and concentrated to dryness. The residue was washed with ether and the product **2** (512 mg, 63%) was precipitated from a benzene solution by addition of hexane. Mp 137—143 °C, $[\alpha]_D^{25}$ +82.7° (*c* 0.4, chloroform).

Found: C, 59.61; H, 5.67; N, 5.08%. Calcd for $C_{68}H_{77}N_5O_{25}$: C, 59.86; H, 5.69; N, 5.13%.

*1D-4-O-(3,4-Di-*O*-benzoyl-2,6-dideoxy-2,6-bisethoxycarbonylamino-α-D-glucopyranosyl)-6-*O*-(2,4,6-tri-*O*-benzoyl-3-deoxy-3-ethoxycarbonylamino-α-D-glucopyranosyl)-5-chloro-1,2,3,5-tetradeoxy-1,3-bisethoxycarbonylamino-*neo*-inositol (3).* Sulfuryl chloride (0.8 ml) was added to a solution of **2** (868 mg) in pyridine (15 ml) with agitation under ice cooling. After being stirred at 0 °C overnight, the solution was poured into ice-cold water (100 ml). The aqueous solution was extracted with chloroform and the chloroform solution was washed with $NaHSO_4$ solution, $NaHCO_3$ solution and water successively. After being dried over Na_2SO_4, the solution was concentrated. The residue was purified on a silica gel column using 10: 1 (v/v) benzene–methanol. Fractions homogeneous on TLC (R_f 0.29) in the same solvent were combined and concentrated to give 735 mg (84%) of **3**, mp 111—116 °C, $[\alpha]_D^{21}$ +80.8° (*c* 0.4, chloroform).

Found: C, 58.61; H, 5.61; N, 5.00; Cl, 2.90%. Calcd for $C_{68}H_{76}N_5O_{24}Cl$: C, 59.06; H, 5.54; N, 5.06; Cl, 2.56%.

*3′,4′,2″,4″,6″-Penta-*O*-benzoyl-5-deoxy-1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B (4).* Tributylstannane[3] (1 ml) and bis(α-cyanoisopropyl)diazene (26 mg) were added to a solution of **3** (643 mg) in toluene (30 ml). The reaction mixture was heated at 78 °C for 1.5 h under nitrogen stream. The mixture was concentrated and the residue was washed with hexane. The crude product was purified on a silica-gel column using 7: 1 (v/v) benzene–methanol as an eluant. Fractions showing a single spot at R_f 0.32 in the same solvent were combined and concentrated to give 538 mg (86%) of **4**, mp 134—137 °C, $[\alpha]_D^{20}$ +81.5° (*c* 1.1, chloroform).

Found: C, 60.28; H, 5.65; N, 5.17%. Calcd for $C_{68}H_{77}N_5O_{24}$: C, 60.57; H, 5.76; N, 5.19%.

5-Deoxykanamycin B (5). A solution of **4** (529 mg) in methanol (6 ml) was added to a solution of barium hydroxide octahydrate (4 g) in water (14 ml). The mixture was heated under reflux for 8 h. Carbon dioxide was bubbled into the mixture and the precipitates were filtered off. The filtrate was concentrated and the residue was purified on a column of Amberlite CG-50(NH_4^+) resin. After being washed with water and 0.05 M aqueous ammonia, the column was eluted with 0.3 M aqueous ammonia to give 47 mg (26%) of **5**, mp 179—185 °C (dec), $[\alpha]_D^{22}$ +124° (*c* 0.7, water). The product showed a single spot on TLC (R_f 0.27) in 5: 8: 10: 7 (v/v) 28% ammonia–1-butanol–ethanol–water.

*1,3,2′,6′3,″-Penta-*N*-acetyl-5-deoxykanamycin B (6).* Compound **5** (15 mg) was acetylated with acetic anhydride (0.1 ml) in methanol (2 ml) to give 18 mg (81%) of **6**, mp above 260 °C, $[\alpha]_D^{21}$ +108° (*c* 0.4, water). ^{1}H NMR (D_2O): δ 1.96 (s, 3, NAc), 1.99 (s, 3, NAc), 2.03 (s, 9, 3×NAc), 4.96 (d, 1, J=3 Hz, anomeric H), 5.01 (d, 1, J=3.5 Hz, anomeric H).

Found: C, 48.54; H, 6.85; N, 10.01%. Calcd for $C_{28}H_{47}N_5O_{14}\cdot H_2O$: C, 48.33; H, 7.10; N, 10.07%.

*1,3,2′,6′-Tetrakis-*N*-(ethoxycarbonyl)neamine (7).* The compound was prepared by a similar reaction to that employed in the preparation of 1,3,2′,6′-tetrakis-*N*-(methoxycarbonyl)-neamine.[20] The product, mp 224—227 °C, $[\alpha]_D^{18}$ +45.2° (*c*

0.5, DMF), was obtained in 61% yield.

Found: C, 47.12; H, 6.77; N, 8.81%. Calcd for $C_{24}H_{42}N_4O_{14}$: C, 47.21; H, 6.93; N, 9.18%.

*1,3,2',6'-Tetrakis-*N*-(ethoxycarbonyl)-5,6,3',4'-tetra-*O*-mesylneamine (8).* Compound **7** (2.0 g) was treated with methanesulfonyl chloride (2.0 ml) in pyridine (20 ml). The product was recrystallized from 3:1 (v/v) acetone–ether to give 1.96 g (65%) of **8**, mp 192—193 °C, $[\alpha]_D^{21}$ +22.5° (*c* 1.06, chloroform). ^{1}H NMR: δ 3.06 (s, 3, SO_2CH_3), 3.11 (s, 3, SO_2CH_3) 3.24 (s, 3, SO_2CH_3), 3.27 (s, 3, SO_2CH_3), 5.21 (d, 1, J=3.5 Hz, H-1').

Found: C, 36.27; H, 5.47; N, 5.90; S, 13.62%. Calcd for $C_{28}H_{50}N_4S_4O_{22}$: C, 36.27; H, 5.47; N, 5.90; S, 13.62%.

*5,6,3',4'-Tetradeoxy-1,3,2',6'-tetrakis-*N*-(ethoxycarbonyl)neamine-5,3'-diene (9).* Compound **8** (1.44 g) was heated with zinc powder (11 g) and sodium iodide (20 g) in DMF (35 ml) at 95°C for 2.5 h. The mixture was diluted with chloroform, the insoluble matter being filtered off. The filtrate was washed with sodium chloride solution, sodium thiosulfate solution and water successively. After being dried over Na_2SO_4, the solution was concentrated. The residue was recrystallized from 1:4 (v/v) ether–methanol to give 0.38 g (45%) of **9**, mp 223—224 °C. From the mother liquor, a second crop of **9** (84 mg, 10%) was obtained. A part of the product was recrystallized for elemental analysis, mp 225—226 °C, $[\alpha]_D^{20}$ +73.6° (*c* 0.84, DMF). ^{1}H NMR (DMSO-d_6): δ 1.22 (*t*, 12, J=7.0 Hz, 4×$CO_2CH_2C\underline{H}_3$), 5.32 (d, 1, J=3.5 Hz, H-1'), 5.84 (broad s, 4, H-5, 6, 3' and 4').

Found: C, 53.20; H, 7.02; N, 10.20%. Calcd for $C_{24}H_{38}N_4O_{10}$: C, 53.15; H, 7.06; N, 10.33%.

*1,3,2',6'-Tetrakis-*N*-(ethoxycarbonyl)-5,6,3',4'-tetradeoxyneamine (10).* Compound **9** (306 mg) was hydrogenated in the presence of palladium black in aqueous dioxane (15 ml) in hydrogen atmosphere (3.4 kg/cm^2) for 26 h. The product was purified on a silica-gel column using 14:1 (v/v) benzene–isopropyl alcohol. Fractions homogeneous on TLC (R_f 0.49) in 15:1 (v/v) chloroform–ethanol were combined and concentrated to give 277 mg (90%) of **10**, mp 196—198 °C, $[\alpha]_D^{20}$ +97.9° (*c* 0.88, chloroform).

Found: C, 52.68; H, 7.62; N, 10.19%. Calcd for $C_{24}H_{42}N_4O_{10}$: C, 52.74; H, 7.74; N, 10.25%.

5,6,3',4'-Tetradeoxyneamine (11). Compound **10** (430 mg) was hydrolyzed in aqueous barium hydroxide solution as in the preparation of **5** to give 94 mg (46%) of **11**, mp 204 °C (dec), $[\alpha]_D^{19}$ +123.8° (*c* 0.63, water). The product was homogeneous on a paper chromatogram (R_f neamine 2.4) in 1:6:4:3 (v/v) acetic acid–1-butanol–pyridine–water. Lit,[21] the sulfate, mp 253—265 °C, $[\alpha]_D^{27}$ +80° (*c* 1, water).

N-Acetylation of **11** (18 mg) with acetic anhydride in methanol afforded 27 mg (91%) of tetra-*N*-acetyl derivative (**12**), mp above 264 °C (dec), $[\alpha]_D^{21}$ +138.3° (*c* 0.94, methanol). ^{1}H NMR (D_2O): δ 1.95 (s, 3, NAc), 1.96 (s, 6, 2×NAc), 2.01 (s, 3, NAc), 4.93 (d, 1, J=4 Hz, H-1').

Found: C, 56.02; H, 7.91; N, 12.89%. Calcd for $C_{20}H_{34}N_4O_6$: C, 56.32; H, 8.03; N, 13.14%.

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