A Synthesis of 3′,4′-Dideoxykanamycin B

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3′,4′-Dideoxykanamycin B was prepared from kanamycin B *via* 4″,6″-*O*-cyclohexylidenation, *N*-benzyloxycarbonylation, 3′,4′,2″-tri-*O*-benzylsulfonylation, double bond formation at C-3′,4′, removal of the cyclohexylidene group, simultaneous removal of the *N*,*O*-protecting groups with sodium metal in liquid ammonia and hydrogenation of the resulting 3′,4′-dideoxy-3′-eno-kanamycin B.

3′,4′-Dideoxykanamycin B[1] (Dibekacin) (**10**) has strong antibacterial activities against usual and resistant bacteria including *Pseudomonas aeruginosa*. The first[1] and an improved synthesis[2] have been reported. This paper describes another synthesis of **10**.

In this synthesis, various protecting groups used in transformation of aminoglycoside antibiotics were first studied to increase the yield of **10**.

In order to prepare kanamycin derivatives, protection of the amino groups is generally the requisite step which comes first, but we tried, in this paper, to protect the 4″- and 6″-hydroxyl groups in advance to the protection of the amino groups. In this attempt, kanamycin B (KMB) was converted to its penta-*p*-toluenesulfonate (**1**) in order to raise the solubility of kanamycin B in organic solvents. The pentasulfonate was treated with 1,1-dimethoxycyclohexane in *N*,*N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid in a manner as described in a previous paper[2] to give 4″,6″-*O*-cyclohexylidene-kanamycin B (**2**) in 85% yield. Similar treatment of the pentasulfonate (**1**) with 2,2-dimethoxypropane gave the corresponding 4″,6″-*O*-isopropylidene derivative (**3**). The low yield (48%) of the latter compound will be due to[3] the low boiling point of the ketal reagent. It was surprising that **2** and **3** (especially **3**) retained antibacterial activity as shown in Table 1. As already reported, even minor modifications of 6-amino-6-deoxyglucose and 2,6-diamino-2,6-dideoxyglucose moieties of kanamycin (3′-*O*-methylkanamycin[4]) and neamine (3′- and 4′-*O*-methylneamines[5]) gave derivatives almost devoid of antibacterial activity. However, it seems that the antibacterial activity is not strongly reduced by minor modifications of the 3-amino-3-deoxyglucose moiety of kanamycins or other related antibiotics.

The amino groups of **2** were protected with benzyl chloroformate in a usual manner to give **4** in 94% yield. To carry out 3′,4′-di-*O*-sulfonylation, which is the requisite step for making 3′,4′-unsaturation bond, benzylsulfonylation was adopted from the following reason. In *N*-tosyl derivatives[2] of kanamycin B, 3′,4′-dibenzylsulfonylation can smoothly be carried out and the 2″-*O*-benzylsulfonyl group, which is simultaneously formed, can readily be removed in addition to the *N*-tosyl groups in a later step without formation of 2″,3″-epimine. On the other hand, 3′,4′-di-*O*-tosylation was shown to be difficult to attain on account of steric hindrance caused by the first tosyl group introduced at C-3′ or C-4′ of the *N*-ethoxycarbonyl derivative[6] of kanamycin B. Instead of tosylation, 3′,4′-di-*O*-

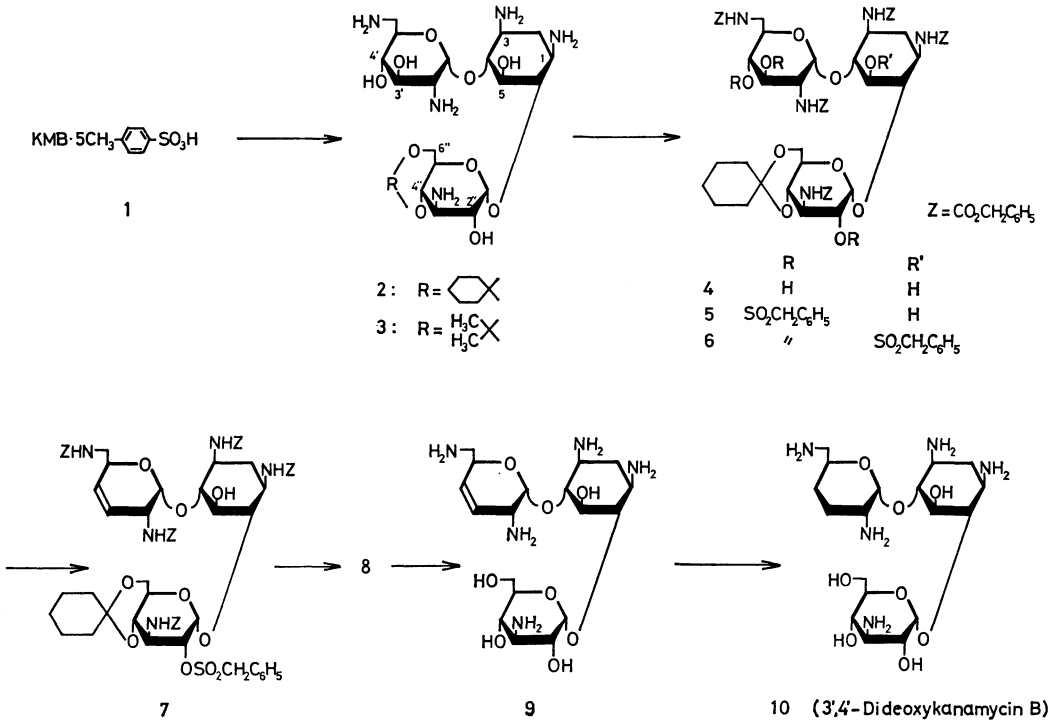

Table 1. Antibacterial spectra of **2**, **3**, **9**, **10**, authentic sample of **10**, and kanamycin B (KMB)

Test organisms[a]	Minimal inhibitory concentration (mcg/ml)					
	KMB	**2**	**3**	**9**	**10**	**10** (authentic)
Staphylococcus aureus FDA 209P	0.39	1.56	0.78	1.56	<0.2	<0.2
Sarcina lutea PCI 1001	12.5	50	12.5	>100	12.5	12.5
Bacillus subtilis NRRL B-558	<0.2	0.78	0.4	0.78	<0.2	<0.2
Klebsiella pneumoniae PCI 602	0.39	6.25	1.56	12.5	0.78	0.78
Salmonella typhi T-63	<0.2	0.39	0.78	12.5	0.78	0.39
Escherichia coli K-12	0.78	1.56	0.78	12.5	0.39	0.39
Escherichia coli K-12 ML 1629[b]	>100	>100	>100	25	0.78	0.78
Pseudomonas aeruginosa A3	25	>100	>100	12.5	0.39	0.39
Mycobacterium smegmatis ATCC 607[c]	1.56	25	12.5	3.12	0.39	0.39

a) Agar dilution streak method (nutrient agar, 37 °C, 18 h). b) A strain of clinical origin having the ability of phosphorylating the 3′-hydroxyl group of kanamycin B. c) 48 h.

mesylation is also possible, however, 2″-*O*-mesyl group, which is simultaneously formed, resists[7] sometimes to elimination in treatment with sodium metal in liquid ammonia in comparison to the 2″-*O*-benzylsulfonyl group. In view of these results benzylsulfonylation was selected. When **4** was treated with benzylsulfonyl chloride in pyridine–collidine (1: 1), 3′,4′,2″-tri-*O*- (**5**) and 5,3′,4′,2″-tetra-*O*-benzylsulfonyl (**6**) derivatives were formed. The formation of the 5-*O*-sulfonate was unexpected, because 5-*O*-acylation of kanamycins in pyridine occurs scarcely, in general. Since **6** gave, in the next unsaturation step, an unidentified product, **5** was selected for the precursor for unsaturation and an effort was made to increase the yield of **5**. When **4** was treated with four equivalents of benzylsulfonyl chloride for **4** at −20 °C for 4.5 h, **5** was obtained in 98% yield.

3′,4′-Unsaturation of **5** was tried firstly with sodium iodide in DMF in a similar fashion as described[2] for 3′,4′-di-*O*-benzylsulfonyl-penta-*N*-tosylkanamycin B derivative, however, no unsaturated compound was obtained as previously[2] experienced. Raising the reaction temperature or prolongation of the reaction period also failed to give the desired product. Addition of zinc dust, however, readily gave the 3′,4′-unsaturated derivative (**7**) in 86% yield. The presence of the 3′,4′-unsaturation bond was supported by the optical rotation[1,2,8] and also by the PMR spectrum, in which a 2-proton AB quartet ($J \approx 11$ Hz) assignable to protons of *cis* double bond[8] was observed. In this reaction, no 2″,3″-aziridine as experienced in the above-described *N*-tosyl derivative[2] of kanamycin B was formed. These features of reactions may be due to the nature of the *N*-protecting groups, *i.e.* the benzyloxycarbonyl and tosyl groups.

Acidic hydrolysis of **7** gave the decyclohexylidenated product (**8**). Treatment of **8**, which has been carefully purified to remove inorganic impurities,[9] with sodium metal in liquid ammonia at −50 °C for 1 h gave the desired 3′,4′-unsaturated derivative (**9**), both the *N*-benzyloxycarbonyl[10] and *O*-benzylsulfonyl groups being readily removed. Catalytic hydrogenation of **9** gave 3′,4′-dideoxykanamycin B (**10**) in 95% yield. The physical constants, IR and PMR spectra, and antibacterial activity were identical with those of specimen previously prepared.[1,2] Overall yield of **10** from kanamycin B was over than 50% of the theoretical.

Antibacterial activities of the synthesized products and related antibiotics are ahown in Table 1. The antibacterial activity of **9** was found to be fairly weaker than that of 3′,4′-dideoxykanamycin B (**10**), although **9** inhibited growth of resistant bacteria and *pseudomonas*

Experimental

PMR spectra were recorded at 60, 90, and 100 MHz with Hitachi R-24A, Varian EM-390, and Varian XL-100 spectrometers, respectively. Thin-layer chromatography (TLC) was performed on Wakogel B-5 with sulfuric acid spray for detection. Paper chromatography (PPC) was carried out on Toyo-Roshi No.50 descending with 1-butanol–pyridine–water–acetic acid=6: 4: 3: 1 and detected by 0.5% ninhydrin in pyridine. For column chromatography, silica gel (Wakogel C-200) was used. For experiments at lower than 0 °C, cooling assembly of Haake constant temperature circulator KS60W was used.

*Kanamycin B Penta-*p*-toluenesulfonate (1).* To an aqueous solution (5 ml) of kanamycin B monocarbonate (4.1 g), *p*-toluenesulfonic acid monohydrate (8.5 g) was added and to the acidic solution (pH≈1), acetone (300 ml) was added. Precipitates were collected, washed thoroughly with acetone and dried *in vacuo* in a desiccator to give kanamycin B penta-*p*-toluenesulfonate as tetrahydrate, 10.17 g (96%), $[\alpha]_D^{25}$ +55° (*c* 1, water).

Found: C, 45.15; H, 5.65; N, 4.83; S, 11.29%. Calcd for $C_{18}H_{37}N_5O_{10}\cdot 5C_7H_8O_3S\cdot 4H_2O$: C, 44.94; H, 6.05; N, 4.94; S, 11.32%.

4″,6″-O-Cyclohexylidenekanamycin B (2). Compound **1** tetrahydrate (5.16 g) was dried at 60 °C *in vacuo* in the presence of CaH_2 for 2.5 h and the dried **1** was dissolved in dry DMF (26 ml). To the solution, anhydrous *p*-toluenesulfonic acid (1.85 g) and 1,1-dimethoxycyclohexane (4.2 ml) were added and the solution was kept at room temperature overnight, further at 50 °C for 30 min and at 65 °C for 15 min *in vacuo* (both at 20 Torr). The solution contained two components, **2** (major, TLC, R_f 0.38 with 1-BuOH–EtOH–$CHCl_3$–17% NH_4OH=4: 4: 2: 3, doubly developed; *cf.* **1**, R_f 0.05) and dicyclohexylidene derivative (R_f 0.52). Water (0.015 ml) was added and the solution was kept at room temperature overnight. Dicyclohexylidene derivative almost disappeared. The solution was poured into 8 M ammonia solution (20 ml) with vigorous stirring and concentrated *in vacuo*. The residue

was dissolved in water and the solution was passed through a column of Amberlite IRA 900 (OH form, 50 ml) with water. The ninhydrin-positive fractions were concentrated to give a solid (2.8 g), which was further chromatographed over CM-Sephadex C-25 (NH_4 form, 300 ml) with 0.03→0.15 M ammonia (gradually changed). After minor product was eluted (≈0.2 g), **2** was eluted, 1.93 g (85% as monocarbonate), $[\alpha]_D^{25}$ +114° (*c* 1, water); PMR (D_2O) δ: 1.0—2.2 (12H, H-2,2 and cyclohexylidene), 4.98 and 5.32 (each 1H d, $J\approx$ 3 Hz, anomeric).

Found: C, 48.31; H, 7.77; N, 11.31%. Calcd for $C_{24}H_{45}N_5O_{10}\cdot H_2CO_3$: C, 47.97; H, 7.57; N, 11.20%.

4″,6″-O-Isopropylidenekanamycin B (3). Compound **1** tetrahydrate (100 mg) was treated with 2,2-dimethoxypropane (0.1 ml) similarly as described for **2** to give **3** as a solid of monocarbonate, 20.0 mg (48%). Recovered **2** was 13 mg.

Compound **3**: $[\alpha]_D^{25}$ +110° (*c* 0.5, water); PMR (D_2O) δ: 1.94 and 2.07 (each 3H s, $(CH_3)_2C$).

Found: C, 45.10; H, 7.47; N, 11.74%. Calcd for $C_{21}H_{41}N_5O_{10}\cdot H_2CO_3$: C, 45.12; H, 7.40; N, 11.96%.

Penta-N-benzyloxycarbonyl-4″,6″-O-cyclohexylidenekanamycin B (4). To a stirred mixture of **2** (1.60 g) and anhydrous sodium carbonate (1.57 g) in acetone (16 ml)–water (16 ml)–methanol (1.6 ml), benzyl chloroformate (2.05 ml) was added and the mixture was stirred at room temperature for 2 h. After concentration, the residue was washed successively with ether and water and dried, 2.97 g (94%), $[\alpha]_D^{25}$ +47° (*c* 1, pyridine); IR (KBr): 1700, 1520 cm^{-1}.

Found: C, 62.06; H, 6.11; N, 5.61%. Calcd for $C_{64}H_{75}N_5O_{20}$: C, 62.28; H, 6.12; N, 5.67%.

Penta-N-benzyloxycarbonyl-3′,4′,2″-tri-O- and 5,3′,4′,2″-tetra-O-benzylsulfonyl-4″,6″-O-cyclohexylidenekanamycin B (6 and 5).

Reaction 1. To a cold solution (−20 °C) of **4** (1.00 g) in dry pyridine–collidine (1: 1, 20 ml), benzylsulfonyl chloride (925 mg, 6 equivalents for **4**) was added and the solution was kept at −20 °C for 20 h. The solution was gradually warmed to 3 °C in 20 min and further kept at the temperature for 2 h. Water (0.1 ml) was added to stop the reaction and the solution was allowed to stand at 3 °C for 1 h. The solution was poured into aqueous 0.05% sodium carbonate solution. Slightly yellow precipitates were collected, washed with water, and dissolved in chloroform. The solution was washed with water, dried (Na_2SO_4), and concentrated to give a solid (1.67 g). The solid was chromatographed over silica gel with chloroform–acetone=12: 1 to give **5** (847 mg, 62%, R_f 0.5 with benzene–ethyl acetate=2: 1) and **6** (327 mg, 22%, R_f 0.77).

5: mp 198—205 °C (dec), $[\alpha]_D^{25}$ +59° (*c* 0.8, dioxane).

Found: C, 59.90; H, 5.45; N, 4.03; S, 5.67%. Calcd for $C_{85}H_{93}N_5O_{26}S_3$: C, 60.17; H, 5.52; N, 4.13; S, 5.67%.

6: mp 110—114 °C, $[\alpha]_D^{25}$ +52° (*c* 0.8, dioxane).

Found: C, 59.68; H, 5.40; N, 3.69; S, 6.79%. Calcd for $C_{92}H_{99}N_5O_{28}S_4$: C, 59.70; H, 5.39; N, 3.78; S, 6.93%.

Reaction 2. To a cold solution (−20 °C) of **4** (200 mg) in dry pyridine (4 ml), benzylsulfonyl chloride (123 mg, 4 equivalents for **4**) was added and the solution was kept at −20 °C for 4.5 h. After addition of water (0.02 ml), the solution was further kept at the temperature for 1 h. The solution contained, on checked by TLC, **5** accompanied by only a slight amont of **6**. The solution was poured into aqueous 0.05% sodium carbonate solution. Colorless solid precipitated was then treated similarly as described in Reaction 1 (but without column chromatography) to give a solid, which was purified by reprecipitation with chloroform–hexane to give **5**, 270 mg (98%).

Penta-N-benzyloxycarbonyl-2″-O-benzylsulfonyl-4″,6″-O-cyclohexylidene-3′,4′-dideoxy-3′-eno-kanamycin B (7). A mixture of **5** (169 mg), sodium iodide (1.7 g), and zinc dust (840 mg) in dry DMF (3.4 ml) was stirred at 95 °C for 35 min. The solution, which soon solidified on cooling, was extracted with chloroform (7 ml×6). The solution was washed with water, dried (Na_2SO_4), and concentrated. The resulting syrup was dissolved in a mixture of hot chloroform (16 ml) and hexane (6 ml) and gradually cooled to give precipitates. After filtration, hexane was added to the filtrate to give additional precipitates. The process was further repeated three times. Precipitates combined were chromatographically homogeneous colorless solid, 115 mg (86%), $[\alpha]_D^{25}$ +12.5° (*c* 0.8, dioxane); PMR ($CDCl_3$): δ 1.1—1.9 (12H, H-2,2 and cyclohexylidene), $\delta\approx$5.5 (12H, CH_2Ph), a deformed AB quartet (2H, $J\approx$11 Hz) centered at δ 5.6 (H-3′,4′).

Found: C, 62.73; H, 5.96; N, 5.04; S, 2.32%. Calcd for $C_{71}H_{79}N_5O_{20}S$: C, 62.96; H, 5.88; N, 5.17; S, 2.32%.

Penta-N-benzyloxycarbonyl-2″-O-benzylsulfonyl-3′,4′-dideoxy-3′-eno-kanamycin B (8). A suspension of **7** (115 mg) in a mixture of dioxane (0.25 ml)–water (0.15 ml)–acetic acid (1.6 ml) was heated at 80 °C for 2.5 h. The solution showed, on TLC with chloroform–acetone=2: 1, a spot at R_f 0.15 (**8**), and the spot of R_f 0.85 (**7**) disappeared. The solution was concentrated with several additions of toluene to give a residue, which was dissolved in dioxane. Addition of water gave precipitates, which were washed with hexane and with water to give a solid, 104 mg (96%), $[\alpha]_D^{25}$ +10° (*c* 0.2, dioxane).

Found: C, 61.43; H, 5.71; N, 5.70; S, 2.78%. Calcd for $C_{65}H_{71}N_5O_{20}S$: C, 61.26; H, 5.62; N, 5.50; S, 2.52%.

3′,4′-Dideoxy-3′-eno-kanamycin B (9). A hot solution of **8** (61 mg) in dioxane (4 ml) was filtered with aid of absorbent cotton in close distance of a bar magnet, and to the filtrate, Dowex 50W X2 resin (H form, 1 ml) pretreated with dioxane was added. After agitation for a while, the mixture was filtered and the filtrate was concentrated. The residual solid was dried at 65 °C *in vacuo* for 2 h. An air-proof vessel containing the above solid was cooled to −60 °C and to it, ammonia was introduced. To the resulting solution (≈18 ml), a piece of sodium metal (≈120 mg) was added and the solution was warmed to −50 °C with stirring within 15 min. The deep-blue solution was kept at the temperature for 1 h. After addition of methanol, ammonia was evaporated with gradual warming to room temperature. To an aqueous solution of the residue, Dowex 50W X2 resin (H form, 4 ml) was added and after agitation for a while, the mixture was poured into a column containing the same resin (3.5 ml). The column was washed thoroughly with water and the product was eluted with 1 M aqueous ammonia. The ninhydrin-positive fractions were collected and concentrated to give a solid, which was thoroughly dried *in vacuo* to give **9** as monocarbonate, 23.8 mg (97%), $[\alpha]_D^{25}$ +44° (*c* 0.4, water); **9** has slightly slower mobility than that of **10** on PPC, and **9** gives bluish brown coloration (*cf.* **10**, blue) with ninhydrin in pyridine. PMR (D_2O+slight DCl) δ: 1.3 (1H q, $J\approx$12 Hz, H-2_{ax}), 2.0 (1H double t, J=3—4, 3—4, and 12 Hz, H-2_{eq}), 5.0 (1H m, H-1′), 5.4 (1H, d, J=3 Hz, H-1″), 5.82 (2H, slightly broadened s, H-3′,4′).

Found: C, 44.76; H, 7.51; N, 13.75%. Calcd for $C_{18}H_{35}N_5O_8\cdot H_2CO_3$: C, 44.61; H, 7.29; N, 13.69%.

3′,4′-Dideoxykanamycin B (10). To an aqueous solution (0.3 ml) of **9** monocarbonate (12.1 mg), platinum oxide (≈3 mg) was added and the mixture was hydrogenated with hydrogen under pressure (3.5 kg/cm^2) at room temperature for 1.5 h. Filtration followed by concentration gave a solid, which was dried thoroughly to give **10** as monocarbonate, 11.5 mg (95%), $[\alpha]_D^{25}$ +109° (*c* 1, water) (lit,[1] +132° as free base).

Found: C, 44.91; H, 7.96; N, 13.63%. Calcd for $C_{18}H_{37}N_5O_8 \cdot H_2CO_3$: C, 44.44; H, 7.65; N, 13.64%.

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