Syntheses of 4′-Deoxykanamycin[1] and 4′-Deoxykanamycin B

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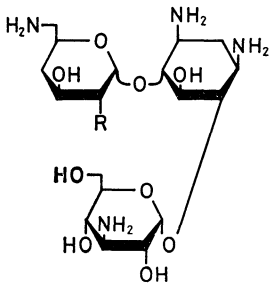
4′-Deoxykanamycin (**9**) was prepared from kanamycin *via* *N*-benzyloxycarbonylation, 4″,6″-*O*-cyclohexylidenation, selective benzoylation at 2′,3′, and 2″-hydroxyl groups, 4′-*O*-mesylation, 4′-iodination, 4′-hydrogenation, and removal of the protective groups. 4′-Deoxykanamycin B (**19**) was analogously prepared from kanamycin B. 4′-*O*-Tosyl derivative of kanamycin B was also led to **19**. Selective formylation of the 3′-hydroxyl group of a protected kanamycin B derivative with *N*,*N*-dimethylformamide-tosyl chloride followed by mesylation of the unstable 3′-*O*-formyl derivative also led to 4′-deoxykanamycin B. Throughout the syntheses, the 4′-hydroxyl group of the protected kanamycins was found to be least reactive for benzoylation among the 2′,3′,4′, and 2″-hydroxyl groups. The ^{13}C NMR spectrum of **9** was also measured. It was concluded that 4′-deoxygenation of antibiotics structurally related to kanamycin caused the resonances of ^{13}C-6′ downfield shift.

As reported[2] previously, 4′-deoxykanamycin (**9**) synthesized by a condensation method has been found to have antibacterial activity in the similar strength as that of parent antibiotic, kanamycin and moreover to have activity against some strains of *Pseudomonas aeruginosa*. Naito *et al.*[3] have also prepared **9** starting from kanamycin *via* the 4′,6′-carbamate formation[4] followed by selective liberation of the 4′-hydroxyl group or *via* the 4′-*O*-acetyl migration to the 6′-amino group. This paper describes another synthesis of 4′-deoxykanamycin (**9**) starting from kanamycin and three processes for the synthesis of 4′-deoxykanamycin B (**19**) strating from kanamycin B.

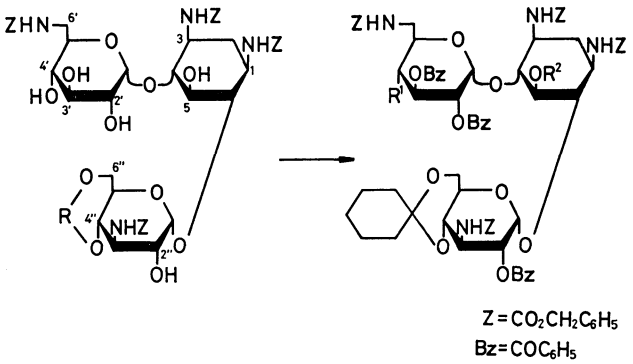


9 R=OH
19 R=NH_2

In preliminary experiments we have found that the 4′-hydroxyl groups of kanamycin, kanamycin B and their derivatives were least reactive for benzoylation among the 2′,3′,4′, and 2″-hydroxyl groups. We, therefore, utilized this finding for the syntheses of **9** and **19**.

Synthesis of 4′-Deoxykanamycin (9). Tetrakis(*N*-benzyloxycarbonyl)kanamycin (**1**) was treated with 1,1-dimethoxycyclohexane in *N*,*N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid. The 4″,6″-*O*-cyclohexylidenation was achieved almost quantitatively without formation of other mono-*O*-cyclohexylidene or di-*O*-cyclohexylidene derivatives. This exclusive 4″,6″-*O*-cyclohexylidenation was rather unexpected, because in a similar treatment of a *N*-protected kanamycin B derivative, the 3′,4′-*O*-cyclohexylidenation occurred[5] more or less in addition to the 4″,6″-*O*-cyclohexylidenation.

Treatment of the 4″,6″-*O*-cyclohexylidene derivative (**2**) with 6 mol equivalents of benzoyl chloride in pyridine gave a mixture of tri- and tetra-*O*-benzoyl (**4**) derivatives. If the amount of benzoyl chloride used



Z=$CO_2CH_2C_6H_5$
Bz=COC_6H_5

1 R=H, H
2 R=$C(CH_2)_5$

	R^1	R^2
3	OH	H
4	$OCOC_6H_5$	H
5	OSO_2CH_3	H
6	OSO_2CH_3	SO_2CH_3
7	I*	H
8	H	H

* A mixture of epimers

was reduced, the production of tri-*O*-benzoyl derivatives was increased with the decrease of **4**. However, the apparent increase of tri-*O*-benzoyl derivatives did not necessarily mean the yield increase of the desired 2′,3′,2″-tri-*O*-benzoyl derivative (**3**), since **3** was found to be contaminated with other minor tri-*O*-benzoyl isomer(s) and this made the purification of **3** difficult even by column chromatography (**3** and the isomer(s) had the same mobility). The contamination was shown by the PMR spectrum of the mesylated mixture of the tri-*O*-benzoylated products; that is, in the spectrum, at least two peaks assignable to the methyl protons of mesyls were observed, indicating that the tri-*O*-benzoyl products were a mixture of the position isomers. By increasing the amount of benzoyl chloride up to 6 mol equivalents for **2**, a mixture of **3** and **4** was formed exclusively. The tetra-*O*-benzoyl isomer (**4**), which was formed concomitantly in high proportion, could be converted again to **2** in 90% yield by treatment with sodium methoxide. The doubtless structure of **3** was deduced from the fact that **3** was led to 4′-deoxykanamycin after a sequence of reactions described below.

Mesylation of **3** gave 4′-*O*-mesyl (**5**, 65%) and 5,4′-di-*O*-mesyl (**6**, 9.5%) derivatives. It should be noted that mesylation of an *N*-ethoxycarbonyl-kanamycin B

derivative[6] (compound **5** in that literature) gave 3′,4′-di-*O*-mesyl derivative in 96% yield without formation of the 5-*O*-mesyl derivative. This means that 4′-*O*-mesylation of **3** is somewhat hindered by the neighboring groups (possibly by 3′-*O*-benzoyl and 6′-*N*-benzyloxycarbonyl groups) and consequently the mesylation can occur at the 4′- and 5-hydroxyl groups in a similar level. Tosylation of **3**, in contrast to mesylation, occurred scarcely. Treatment of **5** with 50% (w/v) sodium iodide in DMF at 100 °C gave an epimeric mixture of 4′-iodo derivatives (**7**). This displacement reaction required 40 h heating. Since the similar iodination of **22** and **26** described later required a shorter reaction period for completion, the displacement reaction of **5** was considered to be somewhat hindered by the groups in the vicinity of the 4′-mesyloxy group. Hydrogenolysis of **7** with Raney nickel gave the 4′-deoxy derivative (**8**), which, after deblocking, gave 4′-deoxykanamycin (**9**).

The structure of **9** was confirmed by acidic solvolysis of the tetrakis(*N*-benzyloxycarbonyl) derivative of **9**. One of the alcoholyzed products which showed the same mobility was identified to the anomeric mixture of benzyl 6-benzyloxycarbonylamino-4,6-dideoxy-D-*xylo*-hexopyranosides (**10**) which consumed periodate, indicating that **10** has a pair of vicinal diols. Acidic hydrolysis of **9** followed by paper chromatography also supported the conclusion (Chart 1).

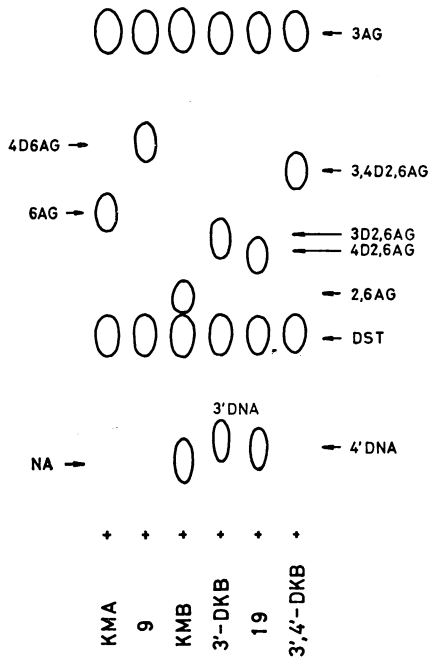


Chart 1. The paper chromatogram of acidic hydrolyzates (6M HCl 100 °C 0.5 h for KMA and **9**, and 6.5 h for other compounds) of kanamycin (KMA), 4′-deoxykanamycin (**9**), kanamycin B (KMB), 3′-deoxykanamycin B (3′-DKB), 4′-deoxykanamycin B (**19**), and 3′,4′-dideoxykanamycin B, descending for 3 days. Abbreviations are: NA (neamine), DST (2-deoxystreptamine), 3AG (3-amino-3-deoxy-D-glucose), 6AG (6-amino-6-deoxy-D-glucose), 2,6AG (2,6-diamino-2,6-dideoxy-D-glucose); the denotions of 3D, 3′D, 4D, 4′D, and 3,4D mean that the numbered positions of the parent compounds (cited after the letter D) are deoxygenated.

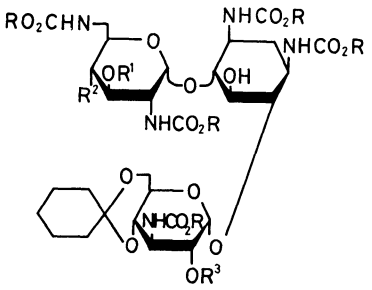
Table 1. The ^{13}C chemical shifts[a] of kanamycin (KMA),[b] 4′-deoxykanamycin (**9**), 3′-deoxykanamycin B (3′-DKB),[c] and 3′,4′-dideoxykanamycin B (3′,4′-DKB) (all as free bases)

Carbon	KMA	**9**	3′-DKB	3′,4′-DKB
1′	100.8	101.2	100.8	101.1
2′	72.7	74.5	50.3	50.6
3′	73.7	70.5[d]	35.7	26.2[e]
4′	71.9	36.3	67.1	28.1[e]
5′	73.7	68.0[d]	74.1	70.2
6′	42.4	45.3	42.4	45.4
1	51.2	51.3	51.2	51.1
2	36.3	36.3	36.4	36.3
3	49.8	49.9	50.0	50.3
4	88.2	88.1	87.1	86.9
5	74.9	75.1	75.3	75.4
6	88.7	88.6	89.0	89.0
1″	100.4	100.8	100.3	100.7
2″	72.7	72.6	72.6	72.6
3″	55.1	55.1	55.1	55.1
4″	70.2	70.2	70.2	70.2
5″	73.0	73.0	73.0	73.1
6″	61.2	61.2	61.3	61.3

a) In ppm downfield from TMS calculated as $\delta^{TMS} = \delta^{dioxane} + 67.4$ ppm. b) Shift assignments were based on the shifts of kanamycin (pH 9.6) reported.[8] c) The shifts were substantially the same with those reported by Koch *et al.*[9] d) The values of C-3′ and C-5′ may well be reversed. e) Shift assignments of C-3′ and C-4′ were made based on the corresponding shifts[10] of gentamicin C_{1a} and gentamin C_{1a}.

^{13}C NMR spectral studies were further made. The data of **9**, kanamycin, 3′-deoxykanamycin B (tobramycin), and 3′,4′-dideoxykanamycin B[7] were shown in Table 1. By the spectrum of **9**, the presence of two deoxy groups (δ 36.3 at C-2 and 4′) was clearly shown. Comparison of the C-6′ resonances of kanamycin and 3′-deoxykanamycin B with those of **9** and 3′,4′-dideoxykanamycin B showed downfield shifts $\approx$3 ppm of the latter. By taking the shift difference ($\Delta\delta$) between C-6′ and C-2 of structurally related antibiotics as a measure of downfield shift (the shift of C-2 was selected as a standard because the shift was thought to remain relatively constant throughout kanamycin series), following results were obtained: kanamycin ($\Delta\delta$ 6.1), 3′-deoxykanamycin B (6.0),[9] 3′,4′-dideoxykanamycin B (9.1), gentamicin C_{1a} (9.4),[10] ribostamycin (6.0),[11] 4′-deoxyneamine (9.1),[12] and seldomycin factor 5 (9.2).[13] These results show that the lack of 4′-hydroxyl group causes downfield shift of the resonance of C-6′ by $\approx$3 ppm. This fact is useful to discern the presence of 3′- or 4′-deoxy group. Similar downfield shifts were reported[14] on mannose and galactose, in which the C-4 resonances shifted downfield ($\Delta\delta$ 2.5—5.3) on 6-deoxygenation.

Synthesis of 4′-Deoxykanamycin B. Pentakis(*N*-benzyloxycarbonyl)kanamycin B (**11**), which was prepared from kanamycin B, was treated with 1,1-dimethoxycyclohexane similarly as described for **2** to give the 4″,6″-*O*-cyclohexylidene derivative (**12**).[15] In this reac-



	R	R^1	R^2	R^3
12	$CH_2C_6H_5$	H	OH	H
13	$CH_2C_6H_5$	COC_6H_5	OH	COC_6H_5
14	$CH_2C_6H_5$	COC_6H_5	$OCOC_6H_5$	COC_6H_5
15	$CH_2C_6H_5$	COC_6H_5	OSO_2CH_3	COC_6H_5
17	$CH_2C_6H_5$	COC_6H_5	I*	COC_6H_5
18	$CH_2C_6H_5$	COC_6H_5	H	COC_6H_5
20	C_2H_5	H	OH	COC_6H_5
21	C_2H_5	$SO_2C_6H_4CH_3$-(*p*)	OH	COC_6H_5
22	C_2H_5	H	$SO_2C_6H_4CH_3$-(*p*)	COC_6H_5
24	C_2H_5	CHO	OH	COC_6H_5
25	C_2H_5	CHO	OSO_2CH_3	COC_6H_5
26	C_2H_5	H	OSO_2CH_3	COC_6H_5
27	C_2H_5	H	I*	COC_6H_5
28	C_2H_5	H	H	COC_6H_5

* A mixture of epimers

tion, undesirable 3′,4′-*O*-cyclohexylidenation was inevitable, but this product was mostly hydrolyzed to **12** by addition of water.[5] Benzoylation of **12** with benzoyl chloride gave a mixture of 3′,2″-di-*O*-(**13**) and 3′,4′,2″-tri-*O*-benzoyl (**14**) derivatives, from which **13** was readily separated by chromatography. Mesylation of **13** gave 4′-*O*-mesyl derivative (**15**) and subsequent iodination gave 4′-iodo derivative (**17**). Hydrogenation of **17** with tributyltin hydride[16] or with hydrogen-Raney nickel gave the 4′-deoxy derivative (**18**). Finally, removal of the protecting groups gave 4′-deoxykanamycin B (**19**).

The structure of **19** was confirmed by another synthesis, that is, the 4′-*O*-tosyl derivative[17] (**22**) of 2″-*O*-benzoyl-4″,6″-*O*-cyclohexylidene-pentakis(*N*-ethoxycarbonyl)kanamycin B (**20**), was led to **19**. Iodination of **22** gave the 4′-iodo derivative (**27**) and it was hydrogenated with Raney nickel to give the 4′-deoxy derivative (**28**), which after deblocking gave 4′-deoxykanamycin B (**19**) identical with that obtained by the first synthesis. Paper chromatogram of the acidic hydrolyzates of **19** was shown in Chart 1.

To further improve the synthesis of **19**, we tried to protect the 3′-hydroxyl group of **20** by formylation with *N*,*N*-dimethylformamide in the presence[18] of tosyl chloride. Treatment of **20** in DMF with tosyl chloride in the presence of pyridine gave a product (**24**) which was considered to be a mono-*O*-formyl compound. Since the compound could not be purified by column chromatography owing to its instability, **24** was mesylated without purification and the mesyl derivative (**25**) was treated with aqueous ammonia to remove the formyl group. The 4′-*O*-mesyl derivative (**26**) was thus obtained in a yield of 62% from **20**. This formylation reaction did not proceed smoothly, if pyridine was omitted, and higher temperature and longer reaction period were required for completion of the reaction and the yield of **26** was much lower. The structure of **26** was confirmed by leading it to **27** by treatment with sodium iodide. Above results indicate that the formylation occured at 3′-hydroxyl group fairly selectively, although the mode of acylation was different from that of benzoylation for the preparation of **13**. In the above 4′-*O*-sulfonylation step, if tosyl chloride was used instead of mesyl chloride, the 4′-*O*-tosylation hardly occurred. This suggests that the 3′-*O*-formyl group of **24** fairly prevents the 4′-*O*-tosylation as in the case of 3′-*O*-benzoyl group in **3** described before.

The structure of **19** was further confirmed by determination of $\Delta[M]_{TACu}$[19] values (Table 2). TACu can form complex only with a pair of vicinal amino and hydroxyl groups having relative spacial orientations of ≈60° dihedral angle and the $\Delta[M]_{TACu}$ shows a value

Table 2. The $\Delta[M]_{TACu}$[19] values measured at 20 °C

Kanamycin	+870°
4′-Deoxykanamycin (**9**)	+890°
Kanamycin B	−500°
3′-Deoxykanamycin B	+780°
4′-Deoxykanamycin B (**19**)	−570°
3′,4′-Dideoxykanamycin B	+820°

Table 3. Antibacterial spectra of **9**, **19**, kanamycin (KMA), and kanamycin B (KMB)

Test organisms[a]	Minimal inhibitory concentration (mcg/ml)			
	9	KMA	**19**	KMB
Staphylococcus aureus 209P	0.78	0.78	0.39	0.39
Sarcina lutea PCI 1001	12.5	12.5	1.56	12.5
Klebsiella pneumoniae PCI 602	1.56	0.78	0.39	0.39
Escherichia coli K-12	3.12	1.56	0.78	0.78
Escherichia coli K-12 ML 1629[b]	>100	>100	>100	>100
Pseudomonas aeruginosa A3	1.56	50	6.25	25
Pseudomonas aernginosa A3 No. 12	6.25	12.5	12.5	12.5
Pseudomonas aeruginosa TI-13	6.25	>100	50	100
Mycobacterium smegmatis ATCC 607[c]	0.78	0.78	0.78	0.78

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 groups of kanamycins. c) 48 h.

of $\approx\pm900°$. The accordance of the difference of the $\Delta[M]_{TACu}$ values between kanamycin and kanamycin B with that between 3′-deoxy- and 4′-deoxykanamycin B (**19**) shows that a copper complex was formed between 2′-amino and 3′-hydroxyl groups in **19**.

Antibacterial activities of 4′-deoxykanamycin[2] (**9**) and 4′-deoxykanamycin B (**19**) are shown in Table 3.

Experimental

PMR spectra were recorded at 60, 90, and 100 MHz with Hitachi R-24A, Varian EM-390, and Varian XL-100 spectrometers, respectively. ^{13}C NMR spectra were recorded on a Varian XL-100 spectrometer with Varian 620-L data processing system (25.2 MHz) in $\approx$0.5 M deuterium oxide solution containing dioxane as internal reference. Thin-layer chromatography (TLC) was performed on Wakogel B-5 unless otherwise stated or on E. Merck silica gel 60 F_{254}. Paper chromatography (PPC) was carried out on Toyo-Roshi paper No. 50 with 1-butanol–pyridine–water–acetic acid=6 : 4 : 3 : 1, descending for 7—8 days unless otherwise stated and spots were visualized 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 circulator KS60W was used. Reprecipitation was carried out by adding the last-cited solvent to a solution of the first-cited solvent (or the mixture of the first and the second-cited solvents).

*Tetrakis(*N*-benzyloxycarbonyl)kanamycin (**1**).* To an ice-cold suspension of kanamycin sulfate (54.2 g as kanamycin·H_2SO_4·H_2O) and anhydrous sodium carbonate (52 g) in aqueous acetone (1 : 1, 1 l), benzyl chloroformate (50 ml) was gradually added under vigorous stirring and the mixture was stirred for 1.5 h in the cold. Precipitates occurred were filtered, washed with water and then with ether to give a solid of **1**, 78.0 g (90%), $[\alpha]_D^{25}$ +68° (*c* 2, DMF); IR (KBr): 1690, 1530 cm^{-1}; PMR (DMSO-d_6) δ: 5.05 (8H s, C_6H_5-CH_2).

Found: C, 58.89; H, 6.18; N, 5.41%. Calcd for $C_{50}H_{60}N_4O_{19}$: C, 58.81; H, 5.92; N, 5.49%.

*Tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidenekanamycin (**2**).* To a solution of **1** (297 mg) in dry DMF (3 ml), anhydrous *p*-toluenesulfonic acid (11 mg) and 1,1-dimethoxycyclohexane (0.07 ml, $\approx$1.5 mol equivalents for **1**) were added and the solution was heated at 50 °C under reduced pressure (25—30 Torr) for 1 h. The resulting solution showed, on TLC with $CHCl_3$–EtOH (6 : 1), a single spot at R_f 0.5 (*cf.* **1**, R_f 0.12). The solution was poured into aqueous sodium hydrogencarbonate solution with stirring and the precipitates occurred were filtered, washed with water, and dried. The solid was reprecipitated from dioxane–water, 314 mg (98%), $[\alpha]_D^{20}$ +60° (*c* 1, DMF).

Found: C, 60.78; H, 6.14; N, 4.82%. Calcd for $C_{56}H_{68}N_4O_{19}$: C, 61.08; H, 6.22; N, 5.09%.

*2′,3′,2″-Tri-*O*-(**3**) and 2′,3′,4′,2″-Tetra-*O*-benzoyl-tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidenekanamycin (**4**).* To an ice-cold solution of **2** (39.4 g) in dry pyridine (800 ml), benzoyl chloride (8.35 ml, 2 mol equivalents for **2**) was added and the solution was kept in the cold for 1 h. Another benzoyl chloride (8.35 ml) was added and the solution was kept in the cold for further 1 h. Benzoyl chloride (8.35 ml) was again added and the solution was kept at room temperature overnight. The resulting solution showed, on TLC with $CHCl_3$–2-propanol (IPA)(40 : 1), spots of **3** (R_f=0.15) and **4** (R_f=0.2) in almost equal color strength. After addition of water (20 ml) followed by heating at 40 °C for a while, the solution was concentrated. The chloroform solution (1.2 l) of the residual syrup was washed with 5% aqueous potassium hydrogensulfate, 2% aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated. The pale-brown solid was chromatographed over silica gel with the same solvent system to give a colorless solid of **3**, 24.3 g (48%) and **4**. Since **4** thus obtained was contaminated with color impurities, it was purified by column chromatography again as described above and the pale-yellow solid obtained was reprecipitated from $CHCl_3$–ether–hexane to give a colorless solid of **4**, 341 mg (50%).

3: $[\alpha]_D^{25}$ +106° (*c* 1, $CHCl_3$).

Found: C, 65.61; H, 5.79; N, 3.77%. Calcd for $C_{77}H_{80}N_4O_{22}$: C, 65.43; H, 5.70; N, 3.96%.

4: $[\alpha]_D^{25}$ +109° (*c* 1, $CHCl_3$); PMR ($CDCl_3$): the areal ratio of the signals at δ 6.8—7.7 ($CH_2C_6H_5$ and *m* and *p* of COC_6H_5) and those at δ 7.7—8.2 (*o* of COC_6H_5) were $\approx$4 : 1.

Found: C, 66.16; H, 5.69; N, 3.74%. Calcd for $C_{84}H_{84}N_4O_{23}$: C, 66.48; H, 5.58; N, 3.69%.

*Conversion of **4** to **2**.* To a solution of **4** (68.5 mg) in dioxane (1.4 ml), 1 M sodium methoxide in methanol (0.14 ml) was added and the solution was kept at room temperature for 1.5 h. After addition of Dowex 50Wx8 (H form, pretreated with methanol) the mixture was filtered and the filtrate was concentrated to give a syrup. Washing the syrup thoroughly with water gave a solid (45.0 mg, 90%) identical with **2**.

*2′,3′,2″-Tri-*O*-benzoyl-tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidene-4′-*O*-mesylkanamycin (**5**).* To a solution of **3** (20.0 g) in dry pyridine (300 ml), mesyl chloride (3.32 ml, $\approx$3 mol equivalents for **3**) were added and the solution was kept at 50 °C overnight. The solution showed, on TLC with $CHCl_3$–IPA (25 : 1), three spots of R_f 0.22 (**3**), 0.3 (major, **5**) and 0.45 (**6**). After addition of water (3.8 ml), the solution was concentrated *in vacuo* and the chloroform solution (600 ml) of the residual syrup was washed with 5% aqueous potassium hydrogensulfate, 2% aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated. The brown solid (21.3 g) was vigorously stirred with benzene (100 ml) and the mixture was centrifuged. The solid was treated likewise twice more to give a colorless solid of **5**, 13.8 g (65%). The benzene-soluble part (6.4 g), which was a mixture of **3**, **5**, and **6**, was repeatedly chromatographed over silica gel with $CHCl_3$–IPA to give a colorless solid of **6**, 2.1 g (9.5%).

5: $[\alpha]_D^{23}$ +120° (*c* 1, $CHCl_3$); PMR ($CDCl_3$): δ 2.85 (3H s, SO_2CH_3).

Found: C, 62.81; H, 5.62; N, 3.52; S, 2.07%. Calcd for $C_{78}H_{82}N_4O_{24}S$: C, 62.81; H, 5.54; N, 3.75; S, 2.15%.

6: $[\alpha]_D^{25}$ +82° (*c* 1, $CHCl_3$); PMR ($CDCl_3$): δ 2.87 and 2.96 (each 3H s, SO_2CH_3).

Found: C, 60.26; H, 5.26; N, 3.37; S, 3.83%. Calcd for $C_{79}H_{84}N_4O_{26}S_2$: C, 60.45; H, 5.39; N, 3.57; S, 4.08%.

*2′,3′,2″-Tri-*O*-benzoyl-tetrakis(*N*-benzyloxycarbonyl)-4″,6″-*O*-cyclohexylidene-4′-deoxy-4′-iodokanamycin (**7**).* A mixture of **5** (5.00 g) and sodium iodide (50 g) in DMF (100 ml) was heated at 100 °C for 40 h in an atmosphere of nitrogen. The solution, which solidified on cooling, was shaken with chloroform and the organic layer was concentrated *in vacuo* with several additions of toluene (to remove DMF) to give a residue. The solution of the residue in chloroform was washed with aqueous sodium thiosulfate and water, dried ($MgSO_4$), and concentrated. The brown solid was chromatographed over silica gel with $CHCl_3$–IPA (45 : 1) to give a pale-yellow solid, 3.34 g (65%). The solid contained two components, on checked by TLC, of R_f 0.3 (major) and 0.4 (the 3′-epimer); $[\alpha]_D^{23}$ +102° (*c* 1, $CHCl_3$).

Found: C, 60.70; H, 5.41; N, 3.69; I, 8.17%. Calcd for $C_{77}H_{79}N_4O_{21}I$: C, 60.71; H, 5.23; N, 3.68; I, 8.33%.

2',3',2''-Tri-O-benzoyl-tetrakis(N-benzyloxycarbonyl)-4'',6''-O-cyclohexylidene-4'-deoxykanamycin (8). A solution of **7** (2.38 g) in dioxane–methanol (1 : 1, 14 ml) containing a few drops of triethylamine was hydrogenated with hydrogen under pressure (50 lb/in^2) with Raney nickel at room temperature. After 1 h, the catalyst was replaced with fresh one and the mixture was treated likewise for further 1 h, then the procedure was repeated again. Filtration followed by evaporation gave a solid, which was chromatographed over silica gel with $CHCl_3$–IPA (45 : 1) to give a solid of **8**, 1.93 g (89%). The solid was reprecipitated from dioxane–water, $[\alpha]_D^{20}$ +160° (*c* 0.5, $CHCl_3$).

Found: C, 66.33; H, 5.87; N, 3.85%. Calcd for $C_{77}H_{80}N_4O_{21}$: C, 66.18; H, 5.77; N, 4.01%.

4'-Deoxykanamycin (9). To a solution of **8** (502 mg) in dry dioxane–methanol (1 : 1, 10 ml), 1 M sodium methylate in methanol (0.5 ml) was added and the solution was kept at room temperature for 4.5 h. Dowex 50Wx8 (H form) resin pretreated with methanol was added and the neutral solution was filtered and the filtrate was concentrated. The solid was washed with vigorous stirring with ether and then with hot acetone to give a solid, 304 mg (78% as debenzoyl product). A suspension of the solid in 80% aqueous acetic acid (11 ml) was heated at 80 °C for 1 h. The resulting clear solution was concentrated *in vacuo* to give a solid (295 mg). The decyclohexylidenated product was suspended in a mixture of dioxane–acetic acid–water (2 : 2 : 1, 20 ml) and the mixture was hydrogenated with atmospheric hydrogen in the presence of palladium black at room temperature for 20 min. Filtration followed by evaporation *in vacuo* gave a pale-brown syrup, which was chromatographed over CM-Sephadex C-25 (NH_4 form) with aqueous ammonia (0.02→0.15 M, gradually increased). The ninhydrin-positive fractions were concentrated to give a solid, 121 mg (62% as monocarbonate hemihydrate), $[\alpha]_D^{20}$ +137° (*c* 1, H_2O) (lit[2] +129° as 2/3 hydrate; +134°[3] as free base); PPC: $R_{f\ kanamycin}$ 1.2, TLC: $R_{f\ kanamycin}$ 1.7 (Avicel (microcrystalline cellulose powder, Funakoshi Co.), BuOH–EtOH–$CHCl_3$–17% NH_3=4 : 4 : 2 : 3, doubly developed).

Found: C, 42.45; H, 7.30; N, 10.08%. Calcd for $C_{18}H_{36}N_4O_{10}\cdot H_2CO_3\cdot 1/2H_2O$: C, 42.29; H, 7.29; N, 10.38%.

Benzyl 6-Benzyloxycarbonylamino-4,6-dideoxy-α- and β-D-xylo-hexopyranosides (10). A suspension of **9** (51.1 mg as $H_2CO_3\cdot 1/2H_2O$ salt) and anhydrous sodium carbonate (57 mg) in aqueous methanol (1 : 1, 1 ml) was treated with benzyl chloroformate (0.07 ml) in a similar manner as described to give tetrakis-(*N*-benzyloxycarbonyl)-4'-deoxykanamycin (85 mg, 89%). This was suspended in 2 M hydrogen chloride in benzyl alcohol (1.5 ml) and the mixture was heated at 100 °C for 15 min. The resulting clear solution showed, on TLC with $CHCl_3$–EtOH (10 : 1), three spots of R_f 0.05 (2-deoxystreptamine), 0.30 (3-amino-3-deoxy-D-glucose), and 0.38 (**10**). Basic lead carbonate (0.8 g) was added and the mixture was vigorously stirred for hours. Centrifugation followed by concentration of the upper layer gave a syrup, which was chromatographed over silica gel with $CHCl_3$–EtOH (40 : 1) to give a colorless solid of **10**, 32 mg (98%). PMR ($CDCl_3$–D_2O): δ 1.36 (≈0.7H q, J≈12 Hz, H-4_{ax} (α-anomer)), 1.39 (≈0.3H q, H-4_{ax} (β-anomer)), 1.75—2.05 (1H, m H-4_{eq}), 4.24 (≈0.3H d, J=7.5 Hz, H-1 (β)), 4.53 (≈1.4H AB q, J_{AB}=11.5 Hz, $C_6H_5C\underline{H}_2OC(1)(\alpha)$), 4.69 (≈0.6H AB q, J_{AB}=11.5 Hz, $C_6H_5C\underline{H}_2OC(1)(\beta)$), 4.93 (≈0.7H d, J=4.0 Hz, H-1(α)), 5.85 (2H s, $C_6H_5C\underline{H}_2OCO$).

Periodate Oxidation of 10. A sample of **10** (≈1 mg) was dissolved in a drop of dioxane and a drop of 0.01 M aqueous metaperiodate solution was added. The solution was checked by TLC with $CHCl_3$–EtOH (10 : 1). After keeping at room temperature for 10 min, the solution showed a spot of R_f 0.33 (**10**) and that of 0.43 in almost equal color strength, and after 1 h, the former spot disappeared. The latter spot was active for triphenyltetrazolium chloride, a reagent for reducing substances.

Pentakis(N-benzyloxycarbonyl)kanamycin B (11). Kanamycin B sulfate was treated similarly as described for **1** to give **11** in a yield of 85%, $[\alpha]_D^{25}$ +72° (*c* 1, DMF).

Found: C, 60.13; H, 5.81; N, 6.05%. Calcd for $C_{58}H_{67}N_5O_{20}$: C, 60.36; H, 5.85; N, 6.07%.

Pentakis(N-benzyloxycarbonyl)-4'',6''-O-cyclohexylidenekanamycin B (12). A mixture of **11** (6.65 g), 1,1-dimethoxycyclohexane (1.7 ml, 2 mol equivalents for **11**), and anhydrous *p*-toluenesulfonic acid (220 mg) was treated in a similar manner as described for **2**. The resulting sloution showed, on TLC with $CHCl_3$–EtOH (10 : 1), two spots of R_f 0.3 (**12**) and 0.7 (dicyclohexylidene isomer, major) (*cf.* **11**, R_f 0.12). After addition of water (0.05 ml, 0.5 mol equivalent for **11**), the solution was kept at room temperature overnight. The formation of **12** became major. Aqueous sodium hydrogencarbonate (480 mg) was added with vigorous stirring and the mixture was concentrated *in vacuo* with several additions of toluene. The residual colorless solid (5.9 g) was stirred with hot benzene (30 ml×3), centrifuged, and dried to give pure **12**, 5.67 g (80%). The benzene-soluble portion (217 mg) contained the dicyclohexylidene derivative as a major component.

12: $[\alpha]_D^{25}$ +62° (*c* 1, DMF) (lit,[15] +47° in pyridine).

Found: C, 62.43; H, 6.18; N. 5.68%. Calcd for $C_{64}H_{75}N_5O_{20}$: C, 62.28; H, 6.12; N, 5.67%.

3',2''-Di-O- (13) and 3',4',2''-Tri-O-benzoyl-tetrakis(N-benzyloxycarbonyl)-4'',6''-O-cyclohexylidenekanamycin B (14). To a solution of **12** (495 mg) in dry pyridine (10 ml), benzoyl chloride (0.12 ml, 2.5 mol equivalents for **12**) was added and the solution was kept at room temperature overnight. This reaction conditions were established by mesylating the product followed by examining its PMR spectrum several times, as in the preparation of **3**. The solution showed, on TLC (E. Merck) with $CHCl_3$–methyl ethyl ketone (MEK) (3 : 1), two spots of R_f 0.15 (**13**) and 0.22 (**14**) in almost equal strength. Work up as described for **3** gave a mixture of **13** and **14** (611 mg). The solid (195 mg) was chromatographed over silica gel (E. Merck silica gel 60, prepacked column 2.5×25 cm) with $CHCl_3$–MEK (3 : 1) to give **13** (81.6 mg, 44%), **14** (56.9 mg, 29%) and a mixture of **13** and **14** (25.7 mg).

13: mp 237—238 °C, $[\alpha]_D^{25}$ +112° (*c* 0.8, $CHCl_3$).

Found: C, 64.70; H, 5.75; N, 4.82%. Calcd for $C_{78}H_{83}N_5O_{22}$: C, 64.94; H, 5.80; N, 4.86%.

14: mp 139—140 °C, $[\alpha]_D^{25}$ +96° (*c* 1, $CHCl_3$).

Found: C, 65.74; H, 5.74; N, 4.45%. Calcd for $C_{85}H_{87}N_5O_{23}$: C, 66.01; H, 5.67; N, 4.53%.

3',2''-Di-O-benzoyl-pentakis(N-benzyloxycarbonyl)-4'',6''-O-cyclohexylidene-4'-O-mesylkanamycin B (15). A solution of **13** (197 mg) and mesyl chloride (0.05 ml, 5 mol equivalents for **13**) in dry pyridine (4 ml) was kept at room temperature for 2 h. The solution showed, on TLC (E. Merck) with $CHCl_3$–MEK (3 : 1), two spots of R_f 0.23 (**15**) and 0.44 (minor, di-*O*-mesyl derivative). Work up as described for **5** gave a solid (195 mg), which was chromatographed over silica gel with $CHCl_3$–MEK (3 : 1) to give a solid of **15**, 155 mg (75%) and a solid of 5,4'-di-*O*-mesyl derivative (**16**), 18 mg (8.3%).

15: mp 211—212 °C, $[\alpha]_D^{25}$ +97° (*c* 1, $CHCl_3$); PMR ($CDCl_3$): δ 2.78 (3H s, CH_3SO_2).

Found: C, 62.66; H, 5.73; N, 4.58; S, 1.88%. Calcd for $C_{79}H_{85}N_5O_{24}S$: C, 62.40; H, 5.63; N, 4.61; S, 2.11%.

16: mp 162—163 °C, $[\alpha]_D^{25}$ +70° (*c* 0.5, $CHCl_3$); PMR ($CDCl_3$): δ 2.78 and 3.07 (each 3H s, CH_3SO_2).

Found: C, 59.86; H, 5.43; N, 4.46; S, 4.01%. Calcd for $C_{80}H_{87}N_5O_{26}S_2$: C, 60.10; H, 5.49; N, 4.38; S, 4.01%.

*3′,2″-Di-O-benzoyl-pentakis(N-benzyloxycarbonyl)-4″,6″-O-cyclohexylidene-4′-deoxy-4′-iodokanamycin B (**17**).* Compound **15** (147 mg) was treated with sodium iodide (1.5 g) in DMF (3 ml) at 100 °C for 40 h. Work up as described for **7** gave a mixture of 4′-iodo epimers (R_f 0.2 and 0.38 (minor) with $CHCl_3$–MEK=3 : 1), 68 mg (45%).

*3′,2″-Di-O-benzoyl-pentakis(N-benzyloxycarbonyl)-4″,6″-O-cyclohexylidene-4′-deoxykanamycin B (**18**).* To a solution of **17** (35.6 mg) in dry dioxane (0.7 ml), tributyltin hydride (0.07 ml) and α,α′-azobisisobutyronitrile (≈3 mg) were added and the solution was heated at 80 °C for 2 h under the atmosphere of nitrogen. The solution showed, on TLC with $CHCl_3$–MEK (3 : 1), a single spot at R_f 0.2. Concentration gave a residue, which was chromatographed over silica gel with $CHCl_3$–MEK (3 : 1) to give a solid, which was reprecipitated from chloroform–ether, 26.5 mg (81%), mp 247—248 °C, $[\alpha]_D^{25}$ +120° (*c* 0.8, $CHCl_3$).

Found: C, 65.62; H, 5.84; N, 4.85%. Calcd for $C_{78}H_{83}N_5O_{21}$: C, 65.67; H, 5.86; N, 4.91%.

*4′-Deoxykanamycin B (**19**).* *A. From **18***: Compound **18** (26.5 mg) was treated likewise as described in the preparation of 4′-deoxykanamycin (**9**) to yield **19**, 7.0 mg (64% as dicarbonate), $[\alpha]_D^{20}$ +117° (*c* 0.5, H_2O); PPC: $R_{f\ kanamycin\ B}$ 1.2 (*cf.* tobramycin, 1.3); PMR (D_2O): δ 1.0—1.5 (2H two overlapped q, $J\approx12$ Hz, H-2_{ax}, H-$4'_{ax}$), 1.75—2.1 (2H m, H-2_{eq}, H-$4'_{eq}$), 4.99 (1H d, J=3.5 Hz, H-1′ or 1″), 5.35 (1H d, J=3.5 Hz, H-1′ or 1″).

Found: C, 40.75; H, 6.96; N, 11.90%. Calcd for $C_{18}H_{37}N_5O_9\cdot2H_2CO_3$: C, 40.61; H, 6.99; N, 11.84%.

*B. From **28***: Compound **28** (97.4 mg) was treated similarly as described[17] (preparation of **10** from **7** in that litrature) to give **19**, 20.0 mg (35% as dicarbonate) and a ureylene derivative,[20] 15.6 mg.

*2″-O-Benzoyl-4″,6″-O-cyclohexylidene-pentakis(N-ethoxycarbonyl)-3′-O-(**21**), 4′-O- (**22**), and di-O-tosylkanamycin B (**23**).* A solution of **20**[17] (4.17 g) and *p*-toluenesulfonyl chloride (3.90 g, 5 mol equivalents for **20**) in dry pyridine (50 ml) was kept at room temperature overnight. The solution showed, on TLC with $CHCl_3$–IPA (15 : 1), three spots of R_f 0.30 (**21**, major), 0.36 (**22**), and 0.38 (**23**). After addition of water, the reaction mixture was treated likewise as descibed[17] (preparation of **5** in that literature) to give **21** (2.76 g, 58%; mp 149—150 °C (lit, [17]149—150 °C), $[\alpha]_D^{20}$ +88° (*c* 1, MeOH) (lit,[17] +88°)), **22** (0.60 g, 12.5%), and **23** (0.36 g, 6%).

22: mp 152—153 °C (dec), $[\alpha]_D^{20}$ +118° (*c* 1, DMF); PMR ($(CD_3)_2SO$): δ 2.42 (3H s, CH_3(Ts)).

Found: C, 53.78; H, 6.15; N, 5.88; S, 2.85%. Calcd for $C_{53}H_{75}N_5O_{23}S$: C, 53.84; H, 6.40; N, 5.93; S, 2.71%.

23: $[\alpha]_D^{25}$ +79° (*c* 1, MeOH); PMR ($CDCl_3$): δ 2.47 (6H s, CH_3(Ts)).

Found: C, 54.08; H, 6.12; N, 5.36; S, 4.71%. Calcd for $C_{60}H_{81}N_5O_{25}S_2$: C, 53.92; H, 6.11; N, 5.24; S, 4.80%.

*2″-O-Benzoyl-4″,6″-O-cyclohexylidene-pentakis(N-ethoxycarbonyl)-4′-O-mesylkanamycin B (**26**).* To a cold (−20 °C) solution of **20** (101 mg) in dry DMF (1 ml) containing dry pyridine (0.048 ml, 6 mol equivalents for **20**), tosyl chloride (38.2 mg) was added and the solution was kept for 2 h in the cold. The solution showed, on TLC with $CHCl_3$–IPA (10 : 1), a single spot at R_f 0.2 (mainly **24**) (*cf.* **19**, R_f 0.1). Concentration of the solution below 30 °C gave a syrup, which was dissolved in chloroform. The solution was washed with 5% aqueous sodium hydrogencarbonate and water, dried ($MgSO_4$), and concentrated *in vacuo* with several additions of toluene to give a colorless solid (114 mg). The solid was vigorously stirred with ether and filtered. The solid (108 mg) contained a trace amount of di-*O*-formyl(?) product (R_f 0.21). To a solution of the solid in pyridine (2 ml), mesyl chloride (0.024 ml, 3 mol equivalents for **20**) was added and the solution was kept at room temperature for 4 h. The solution showed, on TLC (E. Merck), a single spot at R_f 0.49 (mainly **25**) *cf.* mono-*O*-formyl product, R_f 0.42). Work up as described for **5** gave a solid (112 mg). A solution of the solid in dioxane (1 ml) and 28% aqueous ammonia (0.1 ml) was kept at room temperature for 15 min. The solution showed, on TLC, a single spot at R_f 0.40. Concentration followed by washing of the residue with water gave a solid (100 mg). The solid was washed with ethyl acetate (1 ml×2) with vigorous stirring to give a colorless solid of **26**, 68 mg (62% based on **20**). The ethyl acetate-soluble part (30 mg) is mainly composed of **20**. Compound **26** has fairly low solubility in usual organic solvents.

26: $[\alpha]_D^{25}$+105° (*c* 1, DMF); PMR (Py-d_5): δ 3.43 (3H s, CH_3SO_2).

Found: C, 51.14; H, 6.44; N, 6.16; S, 2.88%. Calcd for $C_{47}H_{71}N_5O_{23}S$: C, 51.03; H, 6.47; N, 6.33; S, 2.90%.

*2″-O-Benzoyl-4″,6″-O-cyclohexylidene-4′-deoxy-pentakis(N-ethoxycarbonyl)-4′-iodokanamycin B (**27**).* *A. From **22***: A mixture of **22** (517 mg) and sodium iodide (5.0 g) in DMF (10 ml) was heated at 100 °C for 3 h. Work up as described for **7** gave a solid of **27**, 359 mg (72%), $[\alpha]_D^{20}$ +78° (*c* 1, MeOH).

Found: C, 48.69; H, 5.91; N, 6.01; I, 10.80%. Calcd for $C_{46}H_{68}N_5O_{20}I$: C, 48.55; H, 6.02; N, 6.15; I, 11.15%.

*B. From **26***: A mixture of **26** (49.3 mg) and sodium iodide (500 mg) in DMF (1 ml) was heated at 100 °C for 10 h. Work up as described in A gave **27**, 46.8 mg (92%). Compounds **27** obtained by A and B were identical in every respect.

*2″-O-Benzoyl-4″,6″-O-cyclohexylidene-4′-deoxy-pentakis(N-ethoxycarbonyl)kanamycin B (**28**).* Compound **27** (49.6 mg) was treated as described for **8** to give a solid of **28**, 37.3 mg (85%), mp 256—257 °C, $[\alpha]_D^{20}$ +93° (*c* 1, MeOH),

Found: C, 54.47; H, 6.73; N, 6.65%. Calcd for $C_{46}H_{69}N_5O_{20}$: C, 54.59; H, 6.87; N, 6.92%.

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