AMINOGLYCOSIDE ANTIBIOTICS. VIII

SYNTHESIS AND ACTIVITY OF 4′-DEOXYKANAMYCIN A

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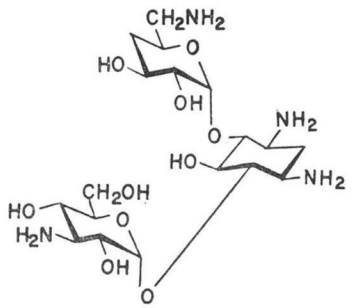
4′-Deoxykanamycin A (**12**) has been prepared by two different routes (Routes A and B) starting from 6′-N-benzyloxycarbonyl-kanamycin A (**1**) which was prepared by the selective benzyloxycarbonylation of kanamycin A. The key compounds for the 4′-deoxygenation, N,O-poly-blocked derivatives having a free hydroxy group on C-4′, were prepared either by the cleavage of the 4′,6′-cyclic carbamate (**7**) to the 6′-N-carbethoxy derivative (**8**) in Route A or by the O→N migration of an acetyl group from the 4′-hydroxy group to the 6′-amino group (**13**→**14**) in Route B. 4′-Deoxykanamycin A is significantly more active than kanamycin against *Pseudomonas* strains and also inhibits the resistant organisms which produce neomycin-kanamycin phosphotransferase II.

3′-O-Phosphorylation is one of the mechanisms by which the neomycin-kanamycin group of antibiotics may be inactivated. Two different enzymes, neomycin-kanamycin phosphotransferase I[1~3] and II[4,5] (NPT_1 and NPT_2), are known, which are differentiated from each other by the specific activity toward butirosin: NPT_2 phosphorylates the 3′-OH of butirosin while NPT_1 does not. Recently 4′-deoxybutirosins (Bu-1975 C_1 and C_2)[6,7] have been isolated in our laboratories from the fermentation broth of *Bacillus circulans*. The 4′-deoxybutirosins showed a broader antibacterial spectrum than butirosin in that the NPT_2-producing organisms were inhibited by the former but not by the latter[6]. However some of the bioactive degradation products of 4′-deoxybutirosins, *i.e.* 4′-deoxyneamine, 4′-deoxyribostamycin and its xylosyl isomer, all of which are 4′-deoxy aminoglycosides but with no acyl substitution at the C-1 position, were not active against the NPT_2-producing organisms[7,23]. Thus it seemed to be worthwhile to make 4′-deoxykanamycin and examine the 4′-deoxygenation effect in the kanamycin-group of antibiotics.

This paper reports the synthesis of 4′-deoxykanamycin A* by two different routes and its *in vitro* antimicrobial activity.



4′- Deoxykanamycin A

Chemistry

The 4′-hydroxy group of kanamycin A was removed by two methods (Routes A and B) starting from 6′-N-benzyloxycarbonyl-

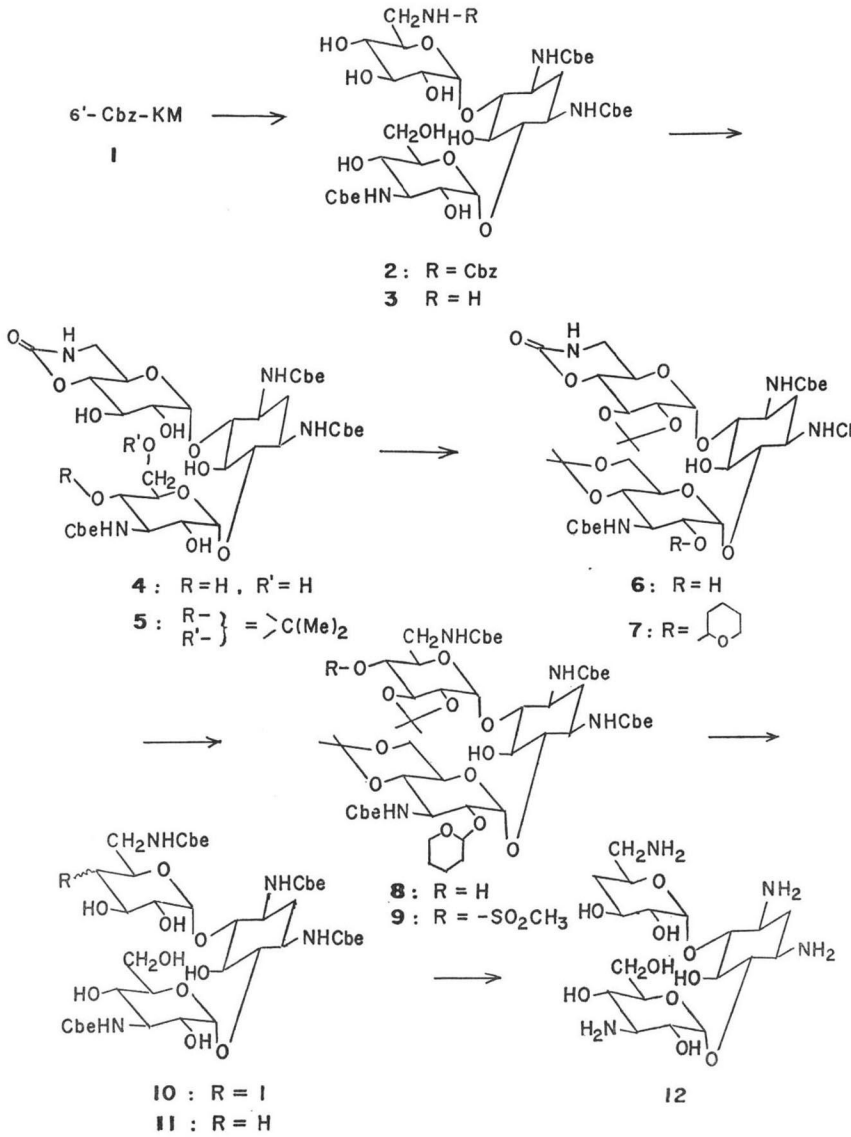
* A part of this report was briefly presented at the 192nd Meeting of Japan Antibiotics Research Association, March 22, 1974[8]. S. UMEZAWA *et al.* also reported the same compound at the same meeting.[9]

kanamycin A (1) which was prepared by the selective benzyloxycarbonylation of kanamycin A[10]. Key compounds for the 4′-deoxygenation, N,O-poly-blocked derivatives with a free hydroxy group on C-4′, were prepared either by the cleavage of the 4′,6′-cyclic carbamate (7) to the 6′-N-carbethoxy derivative (8) in Route A or by the O→N migration of an acetyl group on the 4′-hydroxy group to the 6′-amino group (13→14) in Route B.

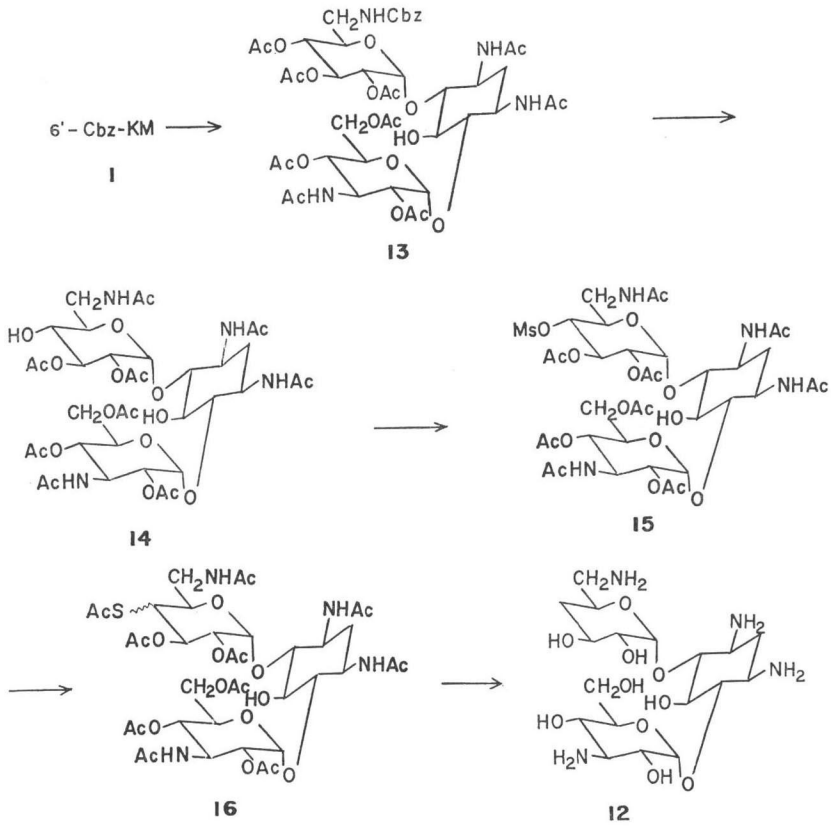
Route A (Scheme 1)

The starting material 1 was treated with ethyl chloroformate in aqueous acetone in the presence of sodium carbonate at room temperature to give the tri-N-ethoxycarbonyl derivative (2) in 93% yield. The benzyloxycarbonyl (Cbz) group of 2 was removed by hydrogenation with 10% palladium on charcoal to afford the 6′-amino derivative (3), which was reacted with

Scheme 1. Preparation of 4′-deoxykanamycin A by route A



Scheme 2. Preparation of 4′-deoxykanamycin A by route B



phenyl chloroformate[11] to form the 4′,6′-cyclic carbamate (4) in 93% yield. The five hydroxyl groups on C-2′, C-3′, C-2″, C-4″ and C-6″ were blocked by successive treatment with 2,2-dimethoxypropane in dimethylformamide (DMF) followed by dihydropyran in the same solvent to afford **7**. The cyclic carbamate group of **7** was selectively cleaved by the action of sodium ethoxide to give the key intermediate **8** in 57% yield. Mesylation of **8** with mesyl chloride in pyridine gave the 4′-monomesyl derivative **9** in 90% yield. In the NMR spectrum a three-proton singlet due to SO_2CH_3 appeared at δ 3.30 ppm. It was considered reasonable that the mesylation occurred predominantly at the C-4′ hydroxy group, because the hydroxy group at C-5 is sterically hindered and hence less reactive to the acylation as in the case observed by UMEZAWA *et al.* in the synthesis of 3′,4′-dideoxykanamycin B (DKB)[12,13] and tobramycin[14]. Displacement of the mesyl group of **9** by an iodo group was carried out by heating with sodium iodide in acetone at 110~115°C for 9 hours in a sealed tube. The iodination was accompanied by simultaneous removal of two isopropylidene groups and the tetrahydropyranyl group to afford BEILSTEIN-positive 4′-iodo-1, 3, 3″, 6′-tetra-N-ethoxycarbonyl kanamycin (**10**) in 72% yield. Its NMR spectrum (in DMSO-d_6) showed absence of the isopropylidene methyl-protons and tetrahydropyranyl methylenes. The iodo derivative (**10**) was hydrogenated in the presence of 10% palladium on charcoal to 4′-deoxy-tetra-N-ethoxycarbonyl-kanamycin A (**11**), which was heated with barium hydroxide in aqueous dioxane to remove the ethoxycarbonyl groups. The final

product was purified by Amberlite CG-50 column chromatography and then crystallized from water-methanol to give 4′-deoxykanamycin A (**12**) as colorless crystals, m.p. 271~273°C (in a sealed tube), $[\alpha]_D^{27}+134°$(*c* 0.5, water).

Route B (Scheme 2)

6′-Benzyloxycarbonylkanamycin was acetylated with acetic anhydride in dry pyridine to give the N, O-nona-acetate (**13**), in which only the C-5 hydroxy group in the deoxystreptamine moiety was presumed to be unprotected. Removal of the Cbz group on the 6′-amino group by catalytic hydrogenation resulted in an O→N shift of the O-acetyl group on the C-4′ position to the 6′-amino group to afford the ninhydrin negative-product **14** with a free hydroxy group on the 4′ position. The 4′-hydroxy group of **14** was mesylated with mesyl chloride in pyridine to give the monomesyl derivative (**15**) in 64% yield, indicated by a three-proton singlet at δ 3.15 ppm in the NMR spectrum. As in the case of Route A, no 4′, 5-dimesyl derivative was obtained, presumably because of the weak reactivity of the 5-hydroxy group. Removal of the mesyloxy group was carried out by a series of reaction different from those

Table 1. Thin-layer chromatography on acid hydrolysates of 4′-deoxykanamycin A and kanamycin A

Rf value of the hydrolysate		Identification
4′-Deoxykanamycin A	Kanamycin A	
0.05	0.05	Deoxystreptamine
	0.27	6-Amino-6-deoxyglucose
0.37	0.37	3-Amino-3-deoxyglucose
0.55		unknown
	0.58	A degradation product of 6-amino-6-deoxyglucose

Hydrolysis: refluxed in 4 N HCl for 45 minutes
TLC plate: silica gel plate F_{254}
Solvent: *n*-Propanol - pyridine - acetic acid - water (51 : 20 : 6 : 24)
Detection: ninhydrin

Fig. 1. NMR spectrum of 4′-deoxykanamycin A in D_2O (60 MHz)

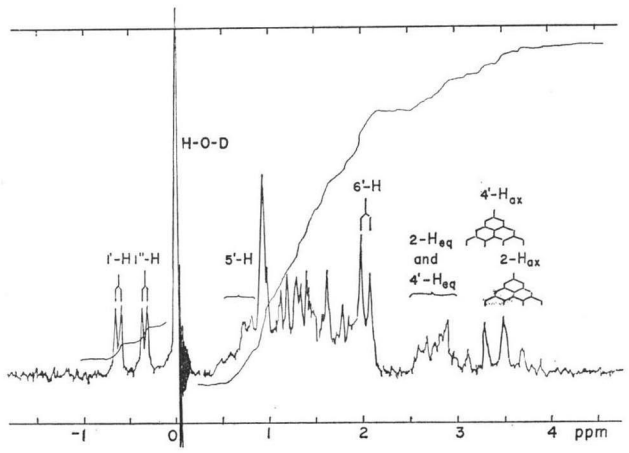


Table 2. Antibacterial spectra of 4′-deoxykanamycin A (**12**) and kanamycin A

Organism	Inactivating enzyme(s)*	MIC (mcg/ml)	
		4′-Deoxy-kanamycin A (**12**)	Kanamycin A
Escherichia coli NIHJ		3.1	1.6
〃 〃 JR35/C600	NPT_1[15]	>100	>100
〃 〃 A20107	NPT_2[16]**	6.3	>100
〃 〃 JR66/W677	NPT_2+GAS[4,5,17,18]	>100	>100
〃 〃 K12, R-5	KAT[19]	50	25
〃 〃 JR88	GAT_1[13]	3.1	1.6
〃 〃 A20732	GAS[16]**	100	50
Klebsiella pneumoniae D11		0.8	0.4
〃 〃 type 22 #3038	NPT_2+GAS[17]	>100	>100
Enterobacter cloacae A20364	NPT_1[16]**	>100	>100
〃 〃 A21006	NPT_2[16]**	6.3	>100
Proteus vulgaris A9436		0.8	0.2
Proteus morganii A20031		6.3	1.6
Proteus mirabilis A9554	GAT_2[20,25]	1.6	0.8
Providencia stuartii #164 A20894		1.6	0.8
Pseudomonas aeruginosa A9930		1.6	25
〃 〃 A20653	NPT_1+NPT_2[16]**	100	>100
〃 〃 #209	GAT_1+NPT[16,24]	25	>100
〃 〃 #130	GAT_1+NPT[16,24]	6.3	100
〃 〃 A20325		12.5	100
〃 〃 A20601	GAT_1[16]**	12.5	100
〃 〃 GN-315	KAT[21,22]	>100	>100
Serratia marcesens A20019		6.3	1.6
〃 〃 A21247	NPT_1+GAS[16]**	>100	>100
Staphylococcus aureus Smith		0.8	0.4
〃 〃 A20239	NPT_1+NPT_2[16]**	6.3	>100

* NPT_1: Neomycin-kanamycin 3′-O-phosphotransferase I
NPT_2: Neomycin-kanamycin-butirosin 3′-O-phosphotransferase II
KAT: Kanamycin 6′-N-acetyltransferase
GAT_1: Gentamicin 3-N-acetyltransferase I
GAT_2: Gentamicin 2′-N-acetyltransferase II
GAS: Gentamicin-DKB 2″-O-adenylate synthetase

** Enzymes identified on basis of substrate profile. Inactivated product was not actually isolated.

used in Route A. The mesylate **15** was treated with potassium thiolacetate in DMF at 110°C for 2 hours to afford the 4′-acetylthio derivative (**16**) in 23% yield. Desulfurization of **16** with RANEY nickel in ethanol followed by removal of acetyl groups with 80% hydrazine hydrate in a sealed tube gave the desired 4′-deoxykanamycin A (**12**), which was purified by Amberlite CG-50 (NH_4^+ form) column chromatography and then crystallized to give pure **12**, identical in IR, NMR and TLC with **12** prepared by Route A.

Characterization

The above-obtained 4′-deoxykanamycin (**12**) and kanamycin A were hydrolyzed in 4N

hydrochloric acid and the hydrolysates were examined by TLC. As shown in Table 1, the hydrolysate of **12** gave three ninhydrin-positive spots in TLC at Rf 0.05 (2-deoxystreptamine, DOS), 0.37 (3-amino-3-deoxy-D-glucose, 3-AG) and 0.55 (unknown), but did not give any spot around Rf 0.27 due to 6-amino-6-deoxy-D-glucose (6-AG) which was detected in the hydrolysate of kanamycin A.

In the NMR spectrum of **12** (Fig. 1), signals appeared in three separate regions. The lowest field contained two one-proton doublets due to anomeric protons at −0.61 ppm* (J= 3.8 Hz) and −0.32 ppm (J=3.8 Hz) similar to those of kanamycin A. The highest field shows two additional one-proton signals when compared to kanamycin A. One is a quartet centered at 3.4 ppm (J=12 Hz) overlapped with the C-2 axial proton of the DOS moiety and the other is a multiplet at 2.5~3.05 ppm overlapped with the C-2 equatorial proton. This indicates that an additional metylene group is present in **12** together with the C-2 metylene of the DOS moiety. The remaining protons attached to the carbon bearing $-NH_2$, -OH or -O- come together in the middle region (0.45 ppm~2.2 ppm). Irradiation on a multiplet at 0.68 ppm resulted in collapse of the quartet at 3.4 ppm to a triplet and also the doublet at 2.0 ppm (J= 6 Hz) assigned to the C-6′ methylene to a singlet, indicating that the signal of the C-5′ proton is in the area of 0.68 ppm and the quartet at 3.4 ppm is assigned to the C-4′ axial proton.

Antimicrobial Activity

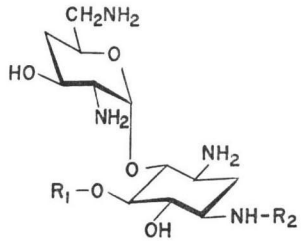
The minimal inhibitory concentrations of **12** were determined against a variety of Gram-positive and Gram-negative bacteria by the two-fold agar-dilution method on MUELLER-HINTON agar plates using the Steers' multi-inoculating apparatus. The results are shown in Table 2.

The antibacterial activity of **12** presents two interesting features, *i.e.* increased activity against *Pseudomonas* strains and resistance to the enzymatic action of NPT_2. Most of the *Pseudomonas* strains tested in the present studies were moderately sensitive to **12** (MIC: 6.3~ 25 mcg/ml), although they were highly resistant to kanamycin A (MIC: ≧100 mcg/ml). In addition, some of the aminoglycoside-resistant organisms which are known to produce NPT_2 enzyme showed significantly increased susceptibility to **12** as was seen with *Escherichia coli* A20107, *Enterobacter cloacae* A21006 and *Staphylococcus aureus* A20239 (MIC: 6.3~12.5 mcg/ml) which were highly resistant to kanamycin (MIC: >100 mcg/ml). However, the 4′-dehydroxylation appears to have no protective effect against the enzymatic action of the 3′-phosphorylative enzyme, NPT_1, which is produced by *E. coli* JR35/C600 and *E. cloacae* A20364. Some of the NPT_2-producing strains such as *E. coli* JR66/W677, *K. pneumoniae* type 22 #3038 and *P. aeruginosa* A20653 were still resistant to **12** probably due to the other inactivating enzymes (GAS or NPT_1) which are co-produced.

It is interesting to note that the 4′-deoxygenation effect in kanamycin is significantly different than in the ribostamycin-butirosin group of antibiotics. Table 3 shows the MICs of 4′-deoxyneamine (**17**), 1-[L(−)-γ-amino-α-hydroxybutyryl]-4′-deoxyneamine (**18**), 4′-deoxy-5-xylosylneamine (**19**) and 4′-deoxybutirosin A (**20**) against a selected group of aminoglycoside-resistant organisms. The structures of these 4′-deoxy aminoglycosides[7] are shown below: The

* In the NMR spectrum of **12** in D_2O, the H-O-D signal was used as an internal reference. Negative value means a proton appeared at lower magnetic field than the H-O-D signal.

comparative MIC data shown in Table 3 indicate that the 4′-deoxygenation improves the spectrum of the ribostamycin-butirosin class of antibiotics and makes them resistant to the enzymatic action of NPT_2 only when combined with the amino acid substitution. In kanamycin, 4′-deoxygenation was in itself sufficient to make the antibiotic a poor substrate of the NPT_2 enzyme.



Compoud	R_1	R_2
17	H	H
18	H	AHB*
19	D-Xylosyl	H
20	D-Xylosyl	AHB*

*AHB : L(−) $-CO-CH(OH)-CH_2-CH_2-NH_2$

Table 3. Antibacterial spectra of 4′-deoxy-aminoglycoside derivatives

Organism	Inactivating enzyme	MIC (mcg/ml)					
		17	18	19	20	Butirosin	KM
Staphylococcus aureus Smith	—	1.6	1.6	1.6	0.8	0.8	0.4
Escherichia coli NIHJ	—	25	6.3	1.6	1.6	1.6	1.6
〃 JR35/C600	NPT_1	>100	6.3	>100	0.8	0.8	>100
〃 A20207	NPT_2	>100	12.5	>100	3.1	50	>100
〃 A20732	GAS	6.3	6.3	1.6	1.6	0.8	50
〃 A20895	GAT_1	12.5	12.5	1.6	3.1	1.6	3.1
Providencia stuartii A20894	GAT_2	100	>100	>100	>100	>100	1.6
Escherichia coli R5	KAT	>100	100	50	25	25	50
Pseudomonas aeruginosa GN315	KAT	>100	>100	>100	>100	>100	>100

Experimental

Preparation of 4′-Deoxykanamycin A (**12**) by Route A (Scheme 1)

6′-N-Benzyloxycarbonyl-1, 3, 3″-tri-N-ethoxycarbonylkanamycin A (**2**)

To a stirred solution of 18.55 g (0.03 mole) of **1** and 8.33 g (0.08 mole) of Na_2CO_3 in 300 ml of aqueous acetone (1 : 1) was added dropwise 10.8 g (0.1 mole) of ethyl chloroformate at room temperature over 1.5 hours. The reaction mixture was allowed to stand overnight and then neutralized with 1 N HCl. The precipitated product was filtered, washed with 50 ml of water and dried to give 21.53 g (93%) of **2** which showed no melting point and darkened over 290°C. IR(nujol): 3310, 1700(sh), 1680, 1540, 1035 cm^{-1}. NMR(DMSO-d_6): δ 1.17 (9H, m), 3.35 (6H, m), 4.8～5.15 (2H, m), 5.05 (2H, s), 7.37 (5H, s). TLC*: Rf 0.57 (S-114**, anthrone).

Anal. Calcd. for $C_{35}H_{54}N_4O_{19}\cdot H_2O$: C, 49.29; H, 6.62; N, 6.57
Found: C, 49.52; H, 6.73; N, 6.59

1, 3, 3″-Tri-N-ethoxycarbonylkanamycin A (**3**)

A solution of 4.9 g (6.37 m moles) of **2** in 170 ml of THF-water (1 : 1) was hydrogenated overnight in the presence of 2 g of 10% palladium on charcoal at room temperature. The catalyst was removed by filtration. The filtrate was evaporated *in vacuo* to a small volume to give a precipitate, which was filtered and washed with a small amount of cold water to give 4.14 g (100%) of **3**, m.p. >300°C. IR(nujol): 3320, 1685, 1035 cm^{-1}. NMR(DMSO-d_6): δ 1.0～

* TLC plate: Merck, silica gel 60 F_{254} (0.25 mm)

** S-114 solvent system: MeOAc - *n*-PrOH - 28% NH_4OH (45 : 105 : 60)

1.3 (9H). TLC: Rf 0.42 (S-114, anthrone).

1, 3, 3''-Tri-N-ethoxycarbonylkanamycin A-4'-O, 6'-N-cyclic carbamate (**4**)

To a stirred solution of 19.59 g (0.028 mole) of **3** and 1.83 g (0.0173 mole) of Na_2CO_3 in 450 ml of THF-water (1 : 1) was added dropwise 4.87 g (0.031 mole) of phenyl chloroformate at room temperature. The mixture was stirred overnight to give a precipitate, which was filtered and washed with 50 ml of cold water to afford 16.96 g of **4**. The filtrate and wash were evaporated *in vacuo* to dryness. The residue was triturated with 5 ml of cold water to give an additional 2.02 g of **4**. Total 18.98 g (93%). The analytical sample was prepared by crystallization from water - MeOH (8 : 2), m.p. >320°C. IR(nujol): 3300, 1715(sh), 1700(sh), 1675, 1035 cm^{-1}. NMR(DMSO-d_6): δ 1.18 (9H, t, J=6.75 Hz, $-OCH_2\underline{CH_3}$), 4.0 (6H, q, J=6.75 Hz, $-O\underline{CH_2}CH_3$), 4.9~5.15 (2H, m, 1' and 1''-H). TLC: Rf 0.47 (S-114, anthrone).

Anal. Calcd. for $C_{25}H_{46}N_4O_{18}$: C, 46.28; H, 6.38; N, 7.71
Found: C, 45.95; H, 6.34; N, 7.20

1, 3, 3''-Tri-N-ethoxycarbonyl-4'', 6''-O-isopropylidenekanamycin A-4'-O, 6'-N-cyclic carbamate (**5**)

To a solution of 14.06 g (0.0194 mole) of **4** and 390 mg of *p*-toluenesulfonic acid in 270 ml of dry DMF was added 5.8 g of 2, 2-dimethoxypropane. The mixture was allowed to stand overnight at room temperature, then treated with 1 ml of triethylamine and evaporated to dryness *in vacuo*. The residue was triturated with 20 ml of water to give 13.05 g (88%) of **5**, m.p. >300°C. IR(nujol): 3350, 1695, 1530, 1375, 1050 cm^{-1}. NMR(DMSO-d_6): δ 1.16 (9H, t, J=6.75 Hz, $-CH_2\underline{CH_3}$), 1.27 (3H, s, $-\underline{CH_3}$), 1.39 (3H, s, $-\underline{CH_3}$), 3.96 (6H, q, J=6.75 Hz, $-O\underline{CH_2}CH_3$), 5.01 (2H, br). TLC : Rf 0.68 (S-114, anthrone), Rf 0.38 [EtOH–$CHCl_3$(1 : 3), anthrone].

Anal. Calcd. for $C_{31}H_{50}N_4O_{18}$: C, 48.51; H, 6.75; N, 7.31
Found: C, 48.39; H, 6.81; N, 6.88

1, 3, 3''-Tri-N-ethoxycarbonyl-2', 3'; 4'', 6''-di-O-isopropylidenekanamycin A-4'-O, 6'-N-cyclic carbamate (**6**)

A solution of 4.37 g (5.71 m moles) of **5**, 100 mg of *p*-toluenesulfonic acid and 7.5 g of 2, 2-dimethoxypropane in 100 ml of dry DMF was heated at 60°C for 30 minutes and then evaporated *in vacuo* until 20 ml of the distillate was obtained. The concentrate was again heated at 60°C for 30 minutes with an additional 7.5 g of 2, 2-dimethoxypropane and 40 mg of *p*-toluenesulfonic acid. The reaction mixture was treated with 200 mg of triethylamine and then evaporated to near dryness *in vacuo*. The residue was triturated with 30 ml of benzene and then 50 ml of water to afford 4.0 g (87%) of **6**, m.p. >300°C. IR(KBr): 3340, 1690, 1540, 1280, 1050 cm^{-1}. NMR(DMSO-d_6): δ 1.16 (9H, t, J=6.9 Hz, $-OCH_2\underline{CH_3}$), 1.28 (6H, s, isopropylidene), 1.39 (6H, s, isopropylidene), 3.98 (6H, q, J=6.9 Hz, $-O\underline{CH_2}CH_3$), 5.03 (2H, br). TLC : Rf 0.23 [EtOH–$CHCl_3$ (1 : 8), anthrone].

Anal. Calcd. for $C_{34}H_{54}N_4O_5 \cdot H_2O$: C, 49.51; H, 6.84; N, 6.79
Found: C, 49.22; H, 6.99; N, 7.08

1, 3, 3''-Tri-N-ethoxycarbonyl-2', 3'; 4'', 6''-di-O-isopropylidene-2''-O-tetrahydropyranylkanamycin A-4'-O, 6'-N-cyclic carbamate (**7**)

To a stirred solution of 403 mg (0.5 m mole) of **6** in 3 ml of dry DMF were added 3 ml of 2, 3-dihydropyran and 12 mg of *p*-toluenesulfonic acid. The mixture was stirred for an hour then treated with 0.05 ml of triethylamine and evaporated to dryness *in vacuo*. The residue was triturated with water to give 480 mg (100 %) of **7**, m.p. >300°C. IR(KBr): 3340, 1700, 1540, 1270, 1030 cm^{-1}. NMR(DMSO-d_6): δ 0.95~2.0 (27H, m, $-\underline{CH_2}\underline{CH_2}-$ and $-\underline{CH_3}$). TLC: Rf 0.53 (main), 0.58 [EtOH–$CHCl_3$ (1 : 8), anthrone].

1, 3, 3'', 6'-Tetra-N-ethoxycarbonyl-2', 3'; 4'', 6''-di-O-isopropylidene-2''-O-tetrahydropyranylkanamycin A (**8**)

A solution of 290 mg (0.32 m mole) of **7** and 0.058 N sodium ethoxide in ethanol (15 ml) in 40 ml of dry DMF was refluxed for 15 minutes, cooled to room temperature, neutralized with glacial acetic acid and concentrated *in vacuo* to 10 ml. The insoluble material was filtered off. The filtrate was evaporated *in vacuo* to 0.5 ml and the concentrate was dissolved in 30 ml of chloroform. The solution was chromatographed on a silica gel column (17 g) with ethanol-chloroform (1 : 20) as eluant to give 174 mg (57 %) of **8**, m.p. 157~160°C. The analytical sample was prepared by reprecipitation from methanol-water, m.p. 160~163°C. IR(KBr): 3440, 3340, 1700, 1535, 1265, 1030 cm^{-1}. NMR(DMSO-d_6): δ 0.95~1.9 (30H, m, $-\underline{CH_2CH_2}-$ & $-\underline{CH_3}$). TLC : Rf 0.49 [EtOH–$CHCl_3$ (1 : 15), anthrone].

Anal. Calcd. for $C_{41}H_{85}N_3O_{20}$: C, 52.55; H, 7.32; N, 5.92
Found: C, 52.91; H, 7.57; N, 5.36

1, 3, 3″, 6′-Tetra-N-ethoxycarbonyl-2′, 3′; 4″, 6″-di-O-isopropylidene-4′-O-mesyl-2″-O-tetrahydropyranylkanamycin A (**9**)

To a stirred solution of 1.09 g (1.15 m moles) of **8** in 11 ml of dry pyridine was added portionwise 393 mg (3.43 m moles) of methanesulfonyl chloride at 5°C. The mixture was allowed to stand for 2.5 hours and concentrated *in vacuo* to 4 ml. The concentrate was poured into 50 ml of water. The resulting precipitate was filtered and washed with water to give 1.05 g (90 %) of **9**. The analytical sample was prepared by crystallization from water-methanol, m.p. 158~161°C. IR(KBr): 3460~3340, 1710, 1535, 1260, 1170(νSO_2), 1040, 970 cm^{-1}. NMR (DMSO-d_6): δ 1.0~1.9 (30H, $-\underline{CH_2CH_2}-$ & $-\underline{CH_3}$), 3.30 (3H, s, $SO_2\underline{CH_3}$). TLC : Rf 0.56 [EtOH–$CHCl_3$ (1 : 15), anthrone].

Anal. Calcd. for $C_{42}H_{70}N_3O_{22}S$: C, 49.70; H, 6.95; N, 5.52; S, 3.11
Found: C, 49.74; H, 6.79; N, 4.82; S, 3.70

1, 3, 3″, 6′-Tetra-N-ethoxycarbonyl-4′-iodokanamycin A (**10**)

A solution of 875 mg (0.849 m mole) of **9** and 1.95 g of NaI in 50 ml dry acetone was heated at 110~115°C for 9 hours in a sealed tube, cooled to room temperature and evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml of water and the solution was filtered to remove the insoluble material. The filtrate was passed through a column of carbon (20 ml), which was developed with 140 ml of water and then ethanol-water (2 : 1). The anthrone positive fractions were combined and evaporated *in vacuo* to yield 539 mg (72 %) of **10**. IR(KBr): 3420~3320, 1695, 1310, 1270, 1080, 1040 cm^{-1}. NMR(DMSO-d_6): δ 1.11 (12H, t, J=6.75 Hz, $-OCH_2\underline{CH_3}$), 3.98 (8H, q, J=6.75 Hz, $-O\underline{CH_2}CH_3$). TLC : Rf 0.38 [EtOH–$CHCl_3$ (1 : 4), anthrone].

4′-Deoxy-1, 3, 3″, 6′-tetra-N-ethoxycarbonylkanamycin A (**11**)

A solution of 539 mg (0.612 m mole) of **10** and 190 mg of triethylamine in water-THF was hydrogenated overnight with 370 mg of 10 % palladium on charcoal at room temperature. The catalyst was filtered off. The filtrate was evaporated *in vacuo* to a small volume to precipitate **11**, which was filtered and washed with ice-cold water to give 220 mg of **11**. The filtrate was adsorbed on a column of carbon (10 ml), which was eluted with water and then water-ethanol (1 : 2). Evaporation of the anthrone-positive fraction gave 243 mg of additional **11**. Total yield 463 mg (100 %). TLC : Rf 0.46 [EtOH–$CHCl_3$ (1 : 4), anthrone].

4′-Deoxykanamycin A (**12**)

A stirred mixture of 1.957 g (2.59 m moles) of the N-Cbe-4′-deoxykanamycin (**11**) and 16.5 g of $Ba(OH)_2 \cdot 8H_2O$ in 120 ml of water-dioxane (4 : 5) was heated at reflux for 4.5 hours. The mixture was neutralized with dil. H_2SO_4 and filtered to remove the inorganic salt. The filtrate was evaporated *in vacuo*. The residue in 30 ml of water was passed through a column of CG-50 (NH_4^+, 55 ml), which was washed with 360 ml of water and then eluted stepwise with 860 ml of 0.1 N NH_4OH and 740 ml 0.2 N NH_4OH. The eluate was collected in 20-ml fractions. Fractions 64~76 showing a major spot at Rf 0.60 accompanied by two minor spots

by TLC on a silica gel plate (S-110*) were combined, evaporated *in vacuo* and lyophilized to give 281 mg of crude 4′-deoxykanamycin **12**, which was further purified on a column of CG-50 (cupra-ammonium form**) and then on CG-50 (NH_4^+) to give 259 mg (21 %) of the purified sample, m.p. 190~191°C.

Crystallization—A solution of 258 mg of the amorphous **12** in 5 ml of water was adsorbed on a column of CG-50 (NH_4^+, 10 ml), which was washed with 100 ml of water and eluted with 0.5 N NH_4OH. The eluate was collected in 10-ml fractions. Fractions 13~16 which showed ninhydrin-positive test were combined and evaporated *in vacuo* to give an oily syrup, to which 3 ml of methanol was added slowly. After standing overnight colorless crystals were filtered, washed with methanol - water (10 : 1) and dried overnight *in vacuo* over P_2O_5 to afford 189 mg (16 %) of 4′-deoxykanamycin free base, m.p. 271~273°C (dec. in a sealed tube) $[\alpha]_D^{27}$+ 134° (*c* 0.5, H_2O). IR(KBr): 3280, 1680, 1610, 1140, 1090, 1040. NMR(D_2O): δ (ppm from HOD) 3.6 (1H, q, J=12 Hz, 2-Hax), 3.4 (1H, q, J=12 Hz, 4′-Hax), 2.5~3.05 (2H, m, 2-Heq, & 4′-Heq), 2.04 (2H; d, J=6 Hz, 6′-H), 0.97 (2H, d, J=3 Hz, 6″-H), 0.45~2.2 (16H), −0.32 (1H, d, J=3.8 Hz, 1″-H), −0.61 (1H, d, J=3.8 Hz, 1′-H). TLC : Rf 0.60 (S-110, ninhydrin); cf kanamycin A, Rf 0.47.

Anal. Calcd. for $C_{18}H_{38}N_4O_{10}$: C, 46.15; H, 7.75; N, 11.96
Found: C, 45.89; H, 7.77; N, 11.55

Preparation of 4′-Deoxykanamycin A (**12**) by Route B (Scheme 2)

1, 2′, 2″, 3, 3′, 3″, 4′, 4″, 6″-Nona-N, O-acetyl-6′-N-benzyloxycarbonylkanamycin A (**13**)

To a stirred suspension of 5.0 g (0.008 mole) of 6′-N-Cbz-kanamycin (**1**) in 50 ml of dry pyridine was added 15 ml (0.159 mole) of acetic anhydride at ambient temperature. The temperature rose to 40°C. When the exothermic reaction subsided, the reaction mixture was stirred for 3 hours at 50°C, held overnight and evaporated *in vacuo*. The residue was co-evaporated with ethyl acetate several times to give a powder, which was refluxed with 150 ml of tetrahydrofuran (THF) for 20 minutes. The insoluble material (3.6 g) was filtered and the filtrate was concentrated *in vacuo* to one third volume to give a precipitate (3.35 g), which was identical with the insoluble material by IR spectroscopy and TLC : Rf 0.64 [EtOAc - EtOH (2 : 1)]. The insoluble material and the precipitate were combined and crystallized from EtOH to give 5.18 g (66 %) of crystalline **13**, m.p. 168°C (dec.). IR(KBr): 1730, 1650 cm^{-1}. $[\alpha]_D^{19.5}$+ 106.2° (*c* 2.5, MeOH).

Anal. Calcd. for $C_{44}H_{60}N_4O_{22}\cdot H_2O$: C, 52.07; H, 6.16; N, 5.52
Found: C, 52.28; H, 6.08; N, 5.47

1, 2′, 2″, 3, 3′, 3″, 4″, 6′, 6″-Nona-N, O-acethylkanamycin A (**14**)

A solution of 12.5 g (12.7 m moles) of **13** in 150 ml of methanol and 12 ml of acetic acid was hydrogenated overnight with 1.5 g of 10 % palladium carbon at atmospheric pressure and room temperature. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was co-evaporated three times with ethanol and ethyl acetate to give a solid, which was triturated with ethyl acetate, filtered off and washed with ethyl acetate. The crude powder (9.7 g) was crystallized from 80 ml of 95 % ethanol and 100 ml of ethyl acetate to give 9.30 g (85 %) of **14**, which showed a ninhydrin-negative spot at Rf 0.33 on TLC [EtOAc - EtOH (2 : 1), anthrone]. M.p. 210~213°C. IR(KBr): 3275, 1720, 1620, 1550, 1430, 1370, 1230, 1030 cm^{-1}.

Anal. Calcd. for $C_{36}H_{54}N_4O_{20}\cdot 2H_2O$: C, 48.10; H, 6.50; N, 6.23
Found: C, 48.22; H, 6.33; N, 6.15

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* S-110 solvent system: $CHCl_3$ - MeOH - 28% NH_4OH - H_2O (1 : 4 : 2 : 1)

** The cupra-ammonium form of CG-50 was prepared in the following way: to a stirred suspension of CG-50 (NH_4^+) in water was added 10% $CuSO_4$ solution to give the copper salt of CG-50 which was filtered. The resin was washed several times with water, then treated with 1 N NH_4OH under stirring, filtered and washed several times with water to give a deep blue, cupra-ammonium form of CG-50.

1, 2′, 2″, 3, 3′, 3″, 4″, 6′, 6″-Nona-N, O-acetyl-4′-O-mesylkanamycin A (**15**)

A solution of 1.0 g (1.16 m moles) of **14** in 10 ml of pyridine was evaporated *in vacuo* to remove the solvated water. The residue was dissolved in 10 ml of pyridine. The solution was chilled to 0°C and treated with 200 mg (1.74 m moles) of methanesulfonyl chloride with shaking. The mixture was allowed to stand at 0°C for an hour and then at room temperature for 2 hours, treated with a drop of water and evaporated *in vacuo*. The residue was treated with 5 ml of water and shaken with four 5-ml portions of water-saturated butanol. The butanol extracts were combined, washed with two 2-ml portions of water and evaporated *in vacuo* to give a powder, which was washed with ethyl acetate and dried to give 699 mg (64 %) of **15**, m.p. 217~220°C.

Anal. Calcd. for $C_{37}H_{56}N_4O_{22}S \cdot 3/2H_2O$: C, 45.91; H, 6.14; N, 5.79; S, 3.31
Found: C, 45.82; H, 6.08; N, 5.54; S, 3.85

1, 2′, 2″, 3, 3′, 3″, 4″, 6′, 6″-Nona-N, O-acetyl-4′-thioacetylkanamycin A (**16**)

A mixture of 100 mg (0.106 m mole) of **15** and 60 mg (0.5 m mole) of potassium thiolacetate in 1 ml of dry DMF was heated at 110°C for 2 hours. The reaction mixture was dried up *in vacuo*. The residue was dissolved in 2 ml of water. The solution was shaken with four 2-ml portions of water-saturated butanol. The combined butanol extracts were washed with two 1-ml portions of water and evaporated *in vacuo*. The residue was dissolved in 1 ml of ethanol and adsorbed on a silica gel column (7 g), which was developed with 200 ml of EtOAc and then 500 ml of EtOAc - EtOH (5 : 1). The eluate was collected in 10-ml fractions. Fractions 32~45 which showed Rf 0.64 (red color with anthrone - H_2SO_4 spray reagent) by TLC on a silica gel plate with EtOAc - EtOH (2 : 1) were combined and evaporated *in vacuo* to give 22 mg (23 %) of **16**, m.p. 198~203°C. IR(KBr): 1740, 1650, 1540, 1370, 1235, 1120, 1030 cm^{-1}.

Anal. Calcd. for $C_{38}H_{56}N_4O_{20}S \cdot 3/2H_2O$: C, 48.15; H, 6.27; N, 5.91; S, 3.38
Found: C, 48.19; H, 5.94; N, 5.13; S, 3.25

4′-Deoxykanamycin A (**12**) by Route B

A mixture of 380 mg (0.41 m mole) of **16**, 50 ml ethanol and *ca.* 2 g of RANEY Ni was refluxed for 4 hours and then filtered to remove the catalyst. The filter cake was washed with 30 ml of ethanol and 50 ml of water. The filtrate and wash were evaporated *in vacuo* to give a solid which was heated with 6 ml of 80 % hydrazine in a sealed tube at 140°C for 50 hours. The reaction mixture was co-evaporated with water several times to remove the hydrazine. The residue was dissolved in 3 ml of water. The solution was adjusted to pH 6 with 1 N HCl and passed through a column of CG-50 (NH_4^+, 27 ml). After washing with 100 ml of water, the column was eluted with 0.1 N NH_4OH. The eluate was collected in 10-ml fractions. Fractions 15~30 which showed a positive ninhydrin test were evaporated *in vacuo* to give 139 mg of crude product. Rechromatography of the crude product on a column of CG-50 (NH_4^+, 17 ml) was carried out using 0.5 N NH_4OH as eluent and 10-ml fractions were collected. Fractions 11~22 showing a spot at Rf 0.60 (identical with that of **12** by Route A) on TLC plate (S-110, ninhydrin) were evaporated *in vacuo* to a syrup, to which 2 ml of MeOH and 5 ml of EtOH were added. Crystals separated from the solution were filtered, washed with a small amount of MeOH and dried *in vacuo* over P_2O_5 at 80°C overnight to give 66 mg (34 %) of **12**, m.p. 269~271°C (dec. in a sealed tube). The mixed melting point with **12** by Route A showed no depression. Evaporation of the filtrate gave 14 mg (7 %) of amorphous **12**. The IR and NMR spectra of the crystalline **12** were superimposable with those of crystals obtained by route A.

Hydrolysis of 4′-deoxykanamycin A (**12**)

A solution of **12** in 4 N HCl was heated at reflux for 45 minutes. Under the same condition a sample of kanamycin A was hydrolyzed as reference. The resulting hydrolysates were subjected to TLC on a silica gel plate with *n*-PrOH - pyridine - AcOH - H_2O (51 : 20 : 6 : 24). The Rf values are shown in the Table 1.

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