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Studies on Aminosugars. XXX. The Synthesis of 3′-Deoxykanamycin[1,2]

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3′-Deoxykanamycin has been totally synthesized from 6-*O*-(2-*O*-benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2-deoxystreptamine and 6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranosyl chloride. It has been found that the 3′-deoxykanamycin has strong antibacterial activity not only against common bacteria but also against kanamycin-resistant bacteria.

In a preceding paper[3] we described the synthesis of 3′-*O*-methylkanamycin as an initial approach to an active kanamycin derivative against resistant bacteria. However, the derivative was found to be inactive. The lack of activity is thought to be due to the presence of the *O*-methyl group at C-3′ hindering its binding to bacterial ribosome. Consequently, we undertook to replace the 3′-hydroxyl group by hydrogen, namely the synthesis of 3′-deoxykanamycin.

The synthesis has been accomplished by the condensation of 6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-α-D-

1) A part of this paper was read by S. Umezawa at Symposium of New Natural Product Syntheses, the 23rd International Congress of Pure and Applied Chemistry at Boston, U.S.A., July 28, 1971.

2) Short communication: S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *J. Antibiotics*, **24**, 274 (1971).

3) H. Umezawa, T. Tsuchiya, R. Muto, and S. Umezawa, This Bulletin, **45**, 2842 (1972).

ribo-hexopyranosyl chloride (**10**) with masked derivative[3] (**11**) of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (3AD).

The starting material, methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside (**1**) was prepared from methyl 4,6-*O*-benzylidene-2,3-di-*O*-tosyl-α-D-glucopyranoside and lithium aluminium hydride according to a modified procedure[4] of the method of Vis and Karrer.[5] Debenzylidenation of **1** in aqueous acetic acid gave methyl 3-deoxy-α-D-*ribo*-hexopyranoside (**2**). Preferential tosylation of **2** followed by acetylation gave a 6-*O*-tosyl-2,4-di-*O*-acetyl derivative (**3**) in a yield of 39%. The tosyl group of **3** was then displaced by an azide group with sodium azide in DMF giving a 6-azide derivative (**4**) in a yield of 76%. Deacetylation of **4** followed by benzylation with benzyl bromide and barium oxide-barium hydroxide octahydrate in DMF gave a 6-azido-2,4-di-*O*-benzyl derivative (**6**) in a yield of 81%. The NMR spectrum of **6** showed the following coupling constants: $J_{2,3ax}=J_{3ax,4}=J_{3eq,3ax}$ ~11 Hz and these values indicated that **6** had a ribopyranoside structure. The retension of configuration in **6** showed that the displacement of the tosyl group of **3** by azide ion occured at C-6, indicating preferential tosylation occured at C-6 of 2. Hydrolysis of **6** by refluxing with hydrochloric acid in water–methanol–acetic acid gave 6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-D-*ribo*-hexopyranose (**7**) in a yield of 75%. The high yield of the hydrolysis product (**7**) is noteworthy, since hydrolysis of glycosidic bond of a 2-*O*-benzylated glycoside is generally fairly slow[6] and the prolonged reaction causes the decomposition lowering the yield. The high yield of **7** may be ascribed to the absence of any substituents at C-3, causing an easy approach of hydrogen ion to the anomeric center. Chlorination of **7** with cold thionyl chloride gave a syrup of the desired 6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hexopyranosyl chloride (**10**) in a yield of 61%. The α-anomeric configuration of the chloride atom was confirmed by the NMR spectrum, in which H-1_{eq} resonated at τ 3.83 (J 3.5 Hz) and no other signals ascribable to H-1 were observed.

Since, as described above, the yield of the step of the preferential tosylation of **2** was low, an alternative method was searched. Treatment of **1** with *N*-bromosuccinimide according to the method of Hanessian[7] gave methyl 4-*O*-benzoyl-6-bromo-3,6-dideoxy-α-D-*ribo*-hexopyranoside (**8**) in a yield of 85%. Displacement of the bromine atom with an azide ion in DMF proceeded smoothly and the corresponding 6-azido derivative (**9**) was obtained in a yield of 81%. Removal of the benzoyl group gave a derivative identical with **5** (methyl 6-azido-3,6-dideoxy-α-D-*ribo*-hexopyranoside) prepared by the former route. Thus the new route was proved to be superior to the former.

The condensing partner, 6-*O*-(2-*O*-benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2-deoxystreptamine (**11**) has been prepared by the method described in a preceding paper[3] starting from 3AD. Condensation of **10** with **11** was carried out in a strictly anhydrous condition as described in a preceding paper.[3] The solution of **10** and **11** in dry benzene–dioxane (3:1) was refluxed for 15 hr in the presence of mercuric cyanide and Drierite and the main condensation product, 4-*O*-(6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-α-D-

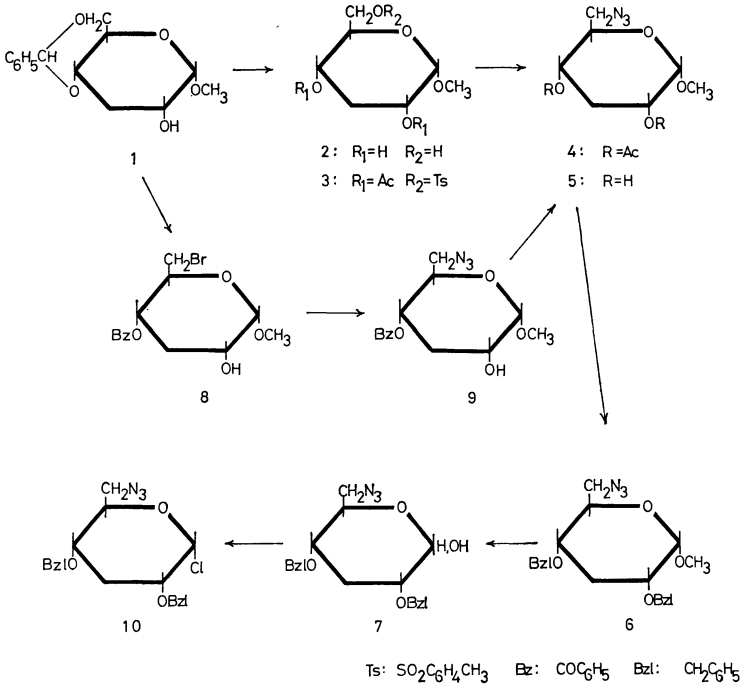

Chart 1.

4) S. Umezawa, T. Tsuchiya, and H. Hineno, This Bulletin, **43**, 1212 (1970).

5) E. Vis and P. Karrer, *Helv. Chim. Acta*, **37**, 378 (1954).

6) See, for example, P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, **1965**, 1419; R. Gigg and C. D. Warren, *ibid.*, **1965**, 2205.

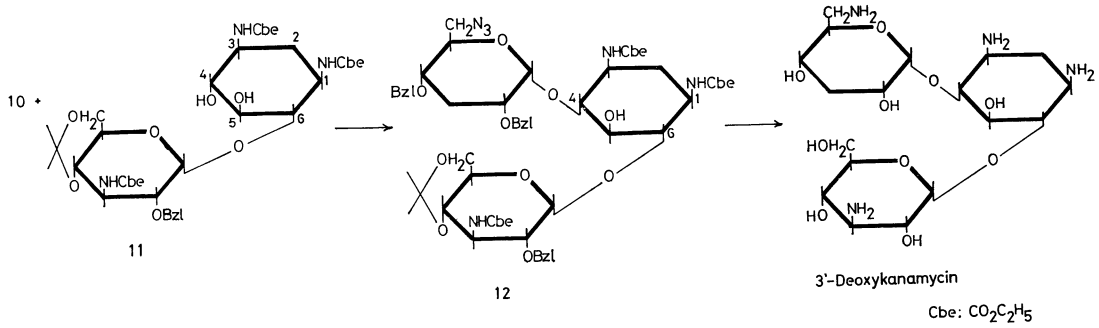

Chart 2.

ribo-hexopyranosyl)-6-*O*-(2-*O*-benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2-deoxystreptamine (**12**) was isolated by column chromatography in a yield of 25%. The structural proof of the product was obtained by its elemental analysis, infrared and NMR spectra, confirming the presence of an azide, three *O*-ethyls, three benzyls, and an isopropylidene group. The sharp melting point, the chromatographycal homogeneity and the high positive rotation ($[\alpha]_D^{20}=+97°$, *c* 0.9, pyridine) indicated that the product was in a highly pure state and had 4-*O*-α-D-glycoside configuration. In the NMR spectrum, the signals assignable to the anomeric proton (H-1′) were not clear, overlapping with other signals.

The condensation product (**12**) was then deacetonated with 80% acetic acid and the azido group was reduced with Raney nickel and hydrogen to an amino group. The product was *N*-acylated with ethoxycarbonyl chloride and debenzylated with palladium black and hydrogen to give the tetra-*N*-ethoxycarbonyl derivative. Since the reduction of an azido group with consecutive removal of benzyl groups with palladium black sometimes[8] causes the damage[9] of the amino group liberated, the amino group was acylated in advance of the debenzylation. Finally, de-*N*-acylation followed by column chromatography with Amberlite CG50 and 0—0.3N ammonia yielded 3′-deoxykanamycin, manely, 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-2-deoxystreptamine, $[\alpha]_D^{20}$ +146° (*c* 0.2, water).

3′-Deoxykanamycin thus synthesized showed[2] antibacterial activity in the similar strength as kanamycin and moreover, it showed inhibition against resistant *E. coli* carrying R factor, resistant *Staphylococci*, and *Pseudomonas aeruginosa*. This result is in accord with expectation based on the resistant mechanism.

Experimental

Infrared spectra (IR) were recorded with a Perkin-Elmer Infrared Spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian A-60D spectrometer. Tetramethylsilane was used as the internal standard. Thin layer chromatography (tlc) was performed on silica gel and the spots were visualized with sulfuric acid. Paper chromatography was performed on Toyo-Roshi paper No. 50 and the spots were detected with 0.5% ninhydrin in pyridine and heating to 110°C.

Methyl 3-Deoxy-α-D-ribo-hexopyranoside (2). A solution of **1** (1.59 g) in aqueous acetic acid (1:2, 50 m*l*) was heated at 100°C for 10 min. The solution was evaporated and the residue was treated with water and ether. After the ether layer was discarded, the aqueous solution was filtered, evaporated and coevaporated with toluene to give a syrup, 1.00 g, $[\alpha]_D^{20}$ +124° (*c* 1, water) (lit,[10] +125.1° (*c* 2.3, water)).

Methyl 2,4-Di-O-acetyl-3-deoxy-6-O-tosyl-α-D-ribo-hexopyranoside (3). To a cold (−10°C) solution of **2** (1.61 g) in dry pyridine (75 m*l*), *p*-toluenesulfonyl chloride (1.81 g) was added and the mixture was stirred at the temperature for 3 hr and allowed to stand at 0°C overnight. Tlc (ethyl acetate) of the reaction mixture showed three spots corresponding to **2** (R_f 0.21), the monotosyl derivative (R_f 0.55, major) and the ditosyl derivative (R_f 0.93). Acetic anhydride (5.6 m*l*) was added and the mixture was allowed to stand at room temperature overnight. The solution was evaporated and the residue was extracted with chloroform. The solution was washed with water, dried over sodium sulfate and evaporated to give a syrup (3.47 g). Tlc (benzene–ethyl acetate 6:1) of the syrup showed three spots of R_f 0.34, 0.45 (major) and 0.57. The syrup was chromatographed on a column of silica gel (100 g) with benzene–ethyl acetate (5:1) and the fraction containing **3** (360—510 m*l*, R_f 0.45) was evaporated to give a syrup, 1.47 g (39%), $[\alpha]_D^{18}$ +108° (*c* 1, $CHCl_3$).

Found: C, 52.07; H, 5.92; S, 7.58%. Calcd for $C_{18}H_{24}O_9S$: C, 51.91; H, 5.81; S, 7.70%.

NMR (in $CDCl_3$): τ 8.00 and 7.94 (both 3H singlets, OAc), 8.3—7.7 (2H m, H-3,3′), 7.56 (3H s, CH_3Ph), 6.68 (3H s, OCH_3), 6.35—6.0 (1H m, H-5?), 6.0—5.7 (2H m, H-6,6′?), 5.6—5.15 (3H m; the signal pattern in this area was quite resemble with that of **4** at τ 5.5—5.0. Therefore these signals could be assigned to H-1,2 and 4.

Methyl 2,4-Di-O-acetyl-6-azido-3,6-dideoxy-α-D-ribo-hexopyranoside (4). A mixture of **3** (0.98 g) and sodium azide (1.6 g) in dry DMF (40 m*l*, dried over calcium hydride) was stirred at 145°C for 1.5 hr. The resulting yellow solution was filtered, evaporated, and coevaporated with toluene to give a syrup. Tlc (benzene–ethyl acetate 6:1) of the syrup showed two spots of R_f 0.55 (major, **4**) and 0.45 (**3**), the former being orange when sprayed with sulfuric acid. Chromatography with silica gel (100 g) and the same solvent system gave a pale yellow syrup, 0.51 g (76%), $[\alpha]_D^{18}$ +121°C (*c* 1, $CHCl_3$); IR: 2100, 1745, 1445, 1370 cm^{-1}.

Found: C, 46.07; H, 6.03; N, 14.65%. Calcd for $C_{11}H_{17}N_3O_6$: C, 45.99; H, 5.97; N, 14.62%.

8) Y. Nishimura, T. Tsuchiya, and S. Umezawa, This Bulletin **44**, 2521 (1971).

9) Use of fresh palladium black prepared just before use also is of importance, as described in the preceding paper.[3]

10) E. J. Hedgley, W. G. Overend, and R. A. C. Rennie, *J. Chem. Soc.*, **1963**, 4701.

NMR (in $CDCl_3$): τ 8.00 and 7.97 (both 3H singlets, OAc), 8.4—7.5 (2H m, H-3.3′), 6.74 (2H d, J 5 Hz, H-6,6′), 6.58 (3H s, OCH_3), 6.16 (1H quintet, J 5,5 and ~10 Hz, H-5), 5.5—5.0 (3H m, H-1,2,4); $J_{4,5}$~10 Hz, $J_{5,6}=J_{5,6'}$ 5Hz. Comparison of the signal pattern of **3** and **4** showed that the H-6,6′ protons which appeared in **4**, as a doublet at τ 6.74, resonated at τ 5.85 in **3**, indicating the presence of a 6-*O*-tosyl group in **3**.

*Methyl 6-Azido-3,6-dideoxy-α-*D*-ribo-hexopyranoside (5).*

a) From 4: To a solution of **4** (0.51 g) in methanol (10 m*l*), a piece of sodium metal was added and subsequent procedures were carried out in a usual manner; a pale yellow syrup, 0.30 g (84%), $[\alpha]_D^{18}$ +142° (*c* 1, $CHCl_3$); IR: 3420 (s), 2960, 2100 (sharp), 1450, 1280, 1150, 1050, 1015, 970, 910, 840, 755 cm^{-1}.

Found: C, 41.45; H, 6.53; N, 20.49%. Calcd for $C_7H_{13}N_3O_4$: 41.37; H, 6.45; N, 20.68%.

NMR (in $CDCl_3$): τ 6.47 (3H s, OCH_3), 5.30 (1H d, J 3.5 Hz. H-1),

b) From 9: A solution of **9** in methanol was treated likewise as described above. Yield 97%.

*Methyl 6-Azido-2,4-di-*O*-benzyl-3,6-dideoxy-α-*D*-ribo-hexopyranoside (6).* To a solution of **5** (0.57 g) in DMF (12 m*l*), powdered barium oxide (2.2 g) and powdered barium hydroxide octahydrate (4.6 g) were added and to the suspension, benzyl bromide (5 m*l*) was stirred in dropwise under vigorous stirring at room temperature and the mixture was agitated overnight. Chloroform (80 m*l*) was added and the mixture was filtered through a layer of celite. The filtrate was evaporated and coevaporated with toluene to give a syrup, which showed four spots of R_f 0.80 (**6**, major), 0.20 (slight), 0.05 (slight) and 0 (slight) on tlc with benzene–ethyl acetate (10: 1). The syrup was chromatographed on a short column of silica gel (30 g) with the same solvent system and the fraction containing **6** was collected and evaporated to give a pale yellow syrup, 0.87 g (81%), $[\alpha]_D^{19}$ +90° (*c* 1, $CHCl_3$); IR: 2100, 735, 700 cm^{-1}.

Found: C, 65.84; H, 6.49; N, 10.99%. Calcd for $C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96%.

NMR (in $CDCl_3$): τ 8.10 (1H q, J ~11 Hz, H_{ax}–3), 7.69 (1H sextet, J 4.5, 4.5, and 11 Hz, H_{eq}–3), 6.56 (3H s, OCH_3), 6.9—6.0 (5H m, H-2,4,5,6,6′), 5.58 and 5.38 (each 1H doublet (J 11.5 Hz) forming an AB quartet centered at 5.48, C(2?) $OC\underline{H}_2C_6H_5$), 5.40 (2H s, C(4?)$OC\underline{H}_2C_6H_5$), 5.28 (1H d, J 3.5 Hz, H-1), 2.65 and 2.62 (both 5H singlets, $OCH_2C_6\underline{H}_5$).

*6-Azido-2,4-di-*O*-benzyl-3,6-dideoxy-*D*-ribo-hexopyranose (7).* To a solution of **6** (0.51 g) in acetic acid (18 m*l*), 2N hydrochloric acid in 50% aqueous methanol (6 m*l*) was added and the solution was refluxed for 1.5 hr. After neutralization with sodium hydrogen carbonate, the solution was filtered, and the filtrate was evaporated to give a residue. The chloroform solution of the residue was washed with water, dried over sodium sulfate and evaporated to give a syrup, which, on tlc with benzene–ethyl acetate (10:1), showed three spots of R_f 0.80 (**6**, minor), 0.40 (**7**, major) and 0.0. The syrup was chromatographed on a short column of silica gel with the same solvent system and the fraction containing **7** was collected and evaporated to give a pale yellow syrup, 0.37 g (75%), $[\alpha]_D^{18}$ +75° (*c* 1, $CHCl_3$; the final value); IR: 2100, 740, 700 cm^{-1}.

NMR (in $CDCl_3$): The whole pattern of **7** was fairly resemble to that of **6**. However an anomeric proton appeared at τ 4.75 (J 3.5 Hz) in the strength of approximately 0.55 proton. Hence, **7** will be an anomeric mixture of approximately α:β-anomer 55: 45 at least in chloroform solution.

Found: C, 64.90; H, 6.61; N, 11.12%. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.02; H, 6.28; N, 11.38%.

*Methyl 4-*O*-Benzoyl-6-bromo-3,6-dideoxy-α-*D*-ribo-hexopyranoside (8).* To a solution of **1** (19.6 g) in dry carbon tetrachloride (500 m*l*), *N*-bromosuccinimide (14.4 g) and barium carbonate (30 g) were added and the mixture was refluxed for 1.5 hr. The resulting yellow solution was filtered and the filtrate was washed with 2% sodium hydrogen carbonate solution and with water, dried over sodium sulfate, and evaporated to give a syrup, 21.7 g (85%), $[\alpha]_D^{18}$ +169° (*c* 1, $CHCl_3$); R_f 0.54 on tlc with benzene–ethyl acetate (1: 1) (**1**, R_f 0.45); IR (KBr): 3430, 1725, 710 cm^{-1}.

Found: C, 48.98; H, 4.92; Br, 23.47%. Calcd for $C_{14}H_{17}O_5Br$: C, 48.71; H, 4.97; Br, 23.15%.

NMR (in $CDCl_3$); τ 8.13 (1H, q, J 11.5 Hz, H_{ax}–3), 7.56 (1H sextet, J 5,5 and 11.5 Hz, H_{eq}–3), 6.45 (3H s, OCH_3), 6.8—5.8 (4H m), 5.22 (1H d, J 3.5 Hz, H-1), 5.03 (1H octet, J 5,10 and 11.5 Hz, H-4), 2.8—1.8 (5H m, typical for benzoyl protons).

*Methyl 6-Azido-4-*O*-benzoyl-3,6-dideoxy-α-*D*-ribo-hexopyranoside (9).* To a solution of **8** (9.5 g) in dry DMF (180 m*l*), sodium azide (9.0 g) was added and the mixture was stirred at 140°C for 1.5 hr. Tlc (benzene–ethyl acetate 1: 1) showed that **8** and **9** had an identical R_f value, but they were distinguished each other by the difference in coloration. After filtration, the solution was evaporated and the residue was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate and evaporated to give a syrup. To remove color impurities, the solution of the syrup in benzene–ethyl acetate (1: 1) was passed through a short column of silica gel with the aid of the same solvent system and the fraction containing **9** was evaporated to give a pale yellow syrup, 6.90 g (81%), $[\alpha]_D^{18}$ +189° (*c* 1, $CHCl_3$); IR: 3440, 2100, 1725, 710 cm^{-1}.

Found: C, 54.95; H, 5.75; N, 14.01%. Calcd for $C_{14}H_{17}N_3O_5$: C, 54.71; H, 5.58; N, 13.68%.

*6-Azido-2,4-di-*O*-benzyl-3,6-dideoxy-α-*D*-ribo-hexopyranosyl Chloride (10).* To ice-cold thionyl chloride (5 m*l*), **7** (197 mg) was added gradually and the solution was allowed to stand in the cold for 1 hr and then at room temperature overnight. The solution was coevaporated with dry toluene *in vacuo* and the residue was dissolved in dry benzene. The solution was passed through a short column of silica gel (3 g), which was preactivated at 120°C *in vacuo* for 5 hr before use. The eluate containing **10** was collected and evaporated to give a pale yellow syrup, 125 mg (61%), $[\alpha]_D^{20}$ +170° (*c* 1, $CHCl_3$); R_f 0.5 on tlc with benzene (**7**, R_f 0); IR: 2930, 2870, 2100 (sharp, N_3), 1500, 1460, 1370, 1350, 1290, 1110 (s), 1000, 895, 740 (s), 700 cm^{-1}.

Found: C, 62.14; H, 5.98; N, 11.07; Cl, 9.61%. Calcd for $C_{20}H_{22}N_3O_3Cl$: C, 61.93; H, 5.72; N, 10.83; Cl, 9.14%.

NMR (in $CDCl_3$): τ 8.12 (1H q, J ~11.5 Hz, H_{ax}–3), 7.58 (1H sextet, J ~5, ~5, and ~12 Hz, H_{eq}–3), 6.8—5.8 (5H m, H-2,4,5,6,6′), 5.53 and 5.40 (both 1H doublets (J 11.5 Hz) forming an AB quartet centered at 5.46, C(2?) $OC\underline{H}_2C_6H_5$), 5.43 (2H s, C(4?) $OC\underline{H}_2C_6H_5$), 3.83 (1H d, J 3.5 Hz, H-1), 2.68 and 2.65 (both 5H singlets, $OCH_2C_6\underline{H}_5$).

*4-O-(6-Azido-2,4-O-di-O-benzyl-3,6-dideoxy-α-*D*-ribo-hexopyranosyl)-6-O-(2-O-benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-O-isopropylidene-α-*D*-glucopyranosyl)-N,N′-diethoxycarbonyl-2-deoxystreptamine (12).* A mixture of **10** (400 mg, 1.6 mol and freshly prepared Drierite (1.5 g) in strictly dried[3] benzene–dioxane (3: 1, 16 m*l*) was refluxed for 30 min and to the mixture, **11**[3] (450 mg, 1 mol) and dried mercuric cyanide (0.57 g) were added. The mixture was refluxed for 6 hr. Mercuric cyanide (0.57 g) was added and the mixture was again refluxed for 6 hr. The procedure was repeated once again. The resulting reaction mixture was poured into a saturated sodium hydrogen carbonate

solution with stirring and the product was extracted with chloroform. After dried over sodium sulfate, the chloroform solution was evaporated to give a syrup (0.9 g). Tlc (chloroform–acetone 5: 1) of the syrup showed six spots of R_f 0.02 (**11**), 0.58, 0.49 (major, **12**), 0.47. 0.31, and 0.9 (**10** and **7**). The syrup was chromatographed on a column of alumina (E. Merck, neutral, 60 g) with chloroform–acetone (5: 1) initially, and after **10** and **7** (both, R_f 0.9) had been eluted (42—88 m*l*), with ethanol. The ethanol eluate was evaporated to give a solid (0.49 g), which contained all components cited above other than **10** and **7**. The solid was then chromatographed on a column of silica gel (Wako gel, 50 g) with chloroform–acetone (5: 1). Compound **12** (R_f 0.49, 250 mg, 36%), a mixture of **12** and the substance having R_f 0.47 (0.10 g), a mixture of the substance having R_f 0.47 and 0.3 (0.06 g) and the substance having R_f 0.31 (0.08 g) were eluted in this order in the fractions of 187—287, 287—400, 400—500, and 500—700 m*l*, respectively. Compound **12** was recrystallized from a mixture of ethanol–acetone (1:1) containing a small amount of ammonia: 170 mg (25%), mp 263—264°C, $[\alpha]_D^{20}$ +97° (*c* 0.9, pyridine); IR (KBr): 3360, 2940, 2110 (N_3), 1705 (amide I), 1545 (amide II); 1075, 1045, 860, 780, 745, 700 cm^{-1}.

Found: C, 59.79; H, 6.76; N, 7.91%. Calcd for $C_{51}H_{68}N_6O_{16}$: C, 59.98; H, 6.71; N, 8.28%.

NMR (in pyridine-d_5): τ 8.90, 8.87, and 8.85 (each 3H s, $CH_2C\underline{H}_3$), 8.57 and 8.54 (each 3H s, isopropylidene), 2.4—2.7 (15H m, phenyl).

*6-O-(3-Amino-3-deoxy-α-*D*-glucopyranosyl)-4-O-(6-amino-3,6-dideoxy-α-*D*-ribo-hexopyranosyl)-2-deoxystreptamine (3′-Deoxykanamycin).* A suspension of **12** (82 mg) in aqueous acetic acid (1: 4, 2 m*l*) was heated at 90°C for 5 min. Tlc (chloroform–acetone 5: 1) of the resulting solution showed that **12** (R_f 0.85) had disappeared and a deacetonation product (R_f 0.07) appeared. The solution was poured into water and the precipitates were filtered to give a solid (79 mg). The solid was dissolved in hot ethanol–dioxane (5: 1, 12 m*l*) and the solution was hydrogenated with Raney nickel (T-4) and hydrogen under pressure (50 *l*b/sq. inch) at 42°C for 3 hr. The reaction mixture was filtered and the filtrate was evaporated to give a solid (60 mg), which had an R_f value 0.22 on tlc with benzene–ethanol (5: 1) (the starting material, R_f 0.75). Its infrared spectrum showed no absorption corresponding to an azide (~2100 cm^{-1}).

To a suspension of the solid in aqueous acetone (1: 1, 3 m*l*), anhydrous sodium carbonate (70 mg) and ethoxycarbonyl chloride (36 mg) were added and the mixture was stirred at room temperature for 30 min, whereupon, on tlc with benzene–ethanol (5: 1), the starting material (R_f 0.22) disappeared and a product (R_f 0.71) appeared. The resulting solution was evaporated *in vacuo* and the residue was extracted with chloroform. The chloroform solution was washed with water, dried over sodium sulfate and evaporated to give a solid (53 mg).

The solid was dissolved in hot aqueous ethanol (1: 8, 9 m*l*) and, after addition of a few drops of acetic acid, the solution was hydrogenated with palladium black and hydrogen under pressure (50 *l*b/sq. inch) at 30°C for 12 hr. The reaction mixture was filtered and evaporated to give a solid (39 mg), which gave R_f 0 on tlc with benzene–ethanol (5:1). Its NMR spectrum showed no absorption peaks corresponding to benzyl protons.

The solid was then heated with 1N barium hydroxide solution at 90°C for 9 hr and the solution was neutralized with carbon dioxide. After filtration, the solution was evaporated *in vacuo*. The residue was dissolved in water to give a slightly turbid solution, which was filtered and evaporated. To remove barium carbonate thoroughly, the procedure was repeated untill a clear solution was obtained. Colorless solid, 24 mg. Paper chromatography (Toyo Roshi No. 50) of the solid with *n*-butanol–pyridine–water–acetic acid (6:4:3:1) showed that the solid was composed mainly of 3′-deoxykanamycin ($R_{f\,\text{kanamycin}}$ 2.2) being accompanied with a slight amount of 2-deoxystreptamine and by-products (R_f 0). The solid was then chromatographed with a column of Amberlite CG50 (NH_4^+ form, 5.5×55 mm); water (30 m*l*), 0.05N (70 m*l*) and 0.1N ammonia (100 m*l*) eluted impurities. Subsequent development with 0.3 N ammonia eluted 3′-deoxykanamycin in 99—135 m*l* fraction, colorless solid, 10 mg (27%). It was recrystallized from water–acetone, $[\alpha]_D^{20}$ +146° (*c* 0.2, H_2O).

Found: C, 46.10; H, 7.70%. Calcd for $C_{18}H_{16}H_4O_{10}$: C, 46.14; H, 7.75%.

Proofs for the Structure of 3′-Deoxykanamycin. *a) By Periodate Oxidation*: 3′-Deoxykanamycin (1.0 mg) was dissolved in 10% sodium periodate solution (0.09 m*l*) and the solution was allowed to stand at room temperature for 12 hr. After addition of ethylene glycol (0.09 m*l*), an equal volume of concentrated hydrochloric acid with that of the solution was added and the solution was heated at 90°C for 1 hr. Paper chromatography with *n*-butanol–pyridien–water–acetic acid (6:4:3:1) showed the presence of 2-deoxystreptamine. Parallel to this experiment, kanamycin and neomycin were treated likewise and it was shown that kanamycin gave 2-deoxystreptamine, whereas neomycin did not give it.

b) By Hydrolysis: A solution of 3′-deoxykanamycin in 6N hydrochloric acid was heated at 90°C for 40 min. Paper chromatography with the above-mentioned solvent-system of the resulting solution showed the presence of 2-deoxystreptamine and 3-amino-3-deoxy-D-glucose. Other products could not be clearly discerned because of diffusion of the spots.

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