Studies on Aminosugars. XXIX. The Synthesis of 3′-*O*-Methylkanamycin[1,2]

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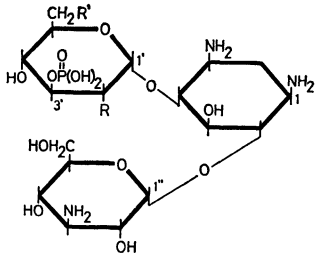
6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine, previously synthesized, was masked with ethoxycarbonyl, isopropylidene, and benzyl groups to give 6-*O*-(2-*O*-benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2-deoxystreptamine, which was condensed with 6-azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-glucopyranosyl chloride by a modified Koenigs-Knorr reaction to give a mixture of α,α- and α,β-diglycoside compounds. Hydrogenation of the azido group and removal of the masking groups gave a mixture of 3′-*O*-methylkanamycin and its diastereomer. The 3′-*O*-methylkanamycin was devoid of antibacterial activity.

Recently, Umezawa and co-workers[3] have clarified the mechanism of the resistance of resistant bacteria in patients to kanamycins and streptomycin, and it was found that these resistant bacteria produce enzymes which inactivate these antibiotics. In the case of kanamycins, 3′-hydroxyl groups are phosphorylated (Fig. 1) by the enzymic reaction and, in the case of streptomycin, the 3-hydroxyl group of *N*-methyl-L-glucosamine moiety is adenylylated[4] by ATP or phosphorylated.[5] It was also proved that resistant *Pseudomonas aeruginosa* to kanamycin produces a similar enzyme[6] which phosphorylates kanamycin from ATP to give kanamycin-3′-phosphate. Furthermore, studies[6] on the substrate specificity of the phosphorylating enzyme using a variety of aminoglycosides showed that



Kanamycin A 3′-phosphate: R=OH, R′=NH_2
Kanamycin B 3′-phosphate: R=NH_2, R′=NH_2
Kanamycin C 3′-phosphate: R=NH_2, R′=OH

Fig. 1.

the whole kanamycin structure is not required for the enzymic reaction and that only 4-*O*-glycosyl-2-deoxystreptamine is required for the enzymic reaction, whereas 3-amino-3-deoxy-D-glucose (3AG) moiety is not involved in binding of the antibiotics with the enzyme.

The above mentioned phosphorylation resistance mechanism suggested that variation of the surroundings of C-3 in the aminosugar moiety attached to C-4 of deoxystreptamine of kanamycins may lead to derivatives that are not susceptible to phosphorylation by the enzyme and active against the resistant bacteria. Our initial approach to such a derivative was the total synthesis of 3′-*O*-methylkanamycin and 3′-deoxykanamycin.

The synthesis has been accomplished by the condensation of 6-azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-glucopyranosyl chloride (**7**) with a masked derivative (**12**) of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-dexoystreptamine. In this synthesis, we tried to use an azido derivative of glucose for the condensation with an aglycone.

An anomeric mixture of methyl 3-*O*-methyl-D-glucopyranoside (**2**) was prepared by acidic methanolysis of 1,2: 5,6-di-*O*-isopropylidene-3-*O*-methyl-D-glucose[7] (**1**) and the anomers (**2a**, **2b**) were separated by resin column chromatography using Dowex 1×2. The procedure for the separation of the anomers was found to be more convenient than an alternative method[8] through a 4,6-*O*-benzylidene derivative. Preferential tosylation of **2a** and **2b** gave 6-*O*-tosylated product (**3a**, **3b**) in about 60% yields, respectively, and their structures were confirmed by NMR spectra. The tosyl group of **3a** and **3b** was displaced by an azido group with sodium azide in dimethyl formamide (DMF) to give 6-azido derivatives (**4a**, **4b**). Benzylation of **4a** with benzyl chloride and potassium hydroxide in DMF gave 2,4-di-*O*-benzylated derivative (**5a**) in a yield of 82%.

An anomeric mixture of **2** was led to an anomeric mixture of **5** in a yield of 50%, which was hydrolyzed

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; A short communication was reported: S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *J. Antibiotics*, **24**(4), 274 (1971).

2) The numbering of the kanamycin structure was followed by that described in the following paper: T. Kobayashi, T. Tsuchiya, K. Tatsuta, and S. Umezawa, *ibid.*, **23**(5), 225 (1970).

3) H. Umezawa, *Progress in Antimicrobial And Anticancer Chemotherapy*, **2**, 567 (1970), University of Tokyo Press.; Related references are cited therein; H. Umezawa, M. Okanishi, S. Kondo, K. Hamana, R. Utahara, K. Maeda, and S. Mitsuhashi, *Science*, **157**, Sept. 29 (1967), 1559.

4) H. Umezawa, S. Takazawa, R. Utahara, M. Okanishi, and K. Maeda, *J. Antibiotics*, **21** (1), 81 (1968); *ibid.*, **21**(8), 477 (1968).

5) H. Naganawa, S. Kondo, K. Maeda, and H. Umezawa, *ibid.*, **24** (12), 823 (1971).

6) O. Doi, S. Kondo, N. Tanaka, and H. Umezawa, *ibid.*, **22**(6), 273 (1969).

7) "Methods in Carbohydrate Chemistry," Vol. II, edited by R. L. Whistler and M. L. Wolfrom, Academic Press Inc., New York (1963), p. 147.

8) R. W. Jeanloz and M. Gut, *J. Amer. Chem. Soc.*, **76**, 5793 (1954).

with hydrochloric acid and acetic acid in aqueous methanol to give 6-azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-D-glucopyranose (**6**), mp 112—113°C, $[\alpha]_D^{20}$ +108° (*c* 0.9, acetone). Expectedly,[9] in this reaction, the β-anomer (**5b**) was found to be more readily hydrolyzed than the α-anomer (**5a**). Chlorination of **6** with thionyl chloride gave the desired 6-azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-glucopyranosyl chloride (**7**), mp 33—35°C, $[\alpha]_D^{15}$ +184° (*c* 1.1, acetone). The α-anomeric configuration of the chlorine atom was confirmed by the NMR spectrum and optical rotation of **7**.

On the other hand, a partially masked derivative of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine was prepared. Previously in the total syntheses of kanamycins,[10] we prepared a suitably masked derivative of this glycoside and this derivative was expected to be usable again for the present synthesis, however, in the present synthesis, we turned hopefully to use an *N*-ethoxycarbonyl derivative instead of the carbobenzoxy derivative.

Treatment of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine with ethyl chloroformate in aqueous acetone in the presence of sodium carbonate gave tri-*N*-ethoxycarbonyl derivative (**8**) in a 82% yield. Isopropylidenation of **8** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid at about 40°C afforded a di-*O*-isopropylidene derivative (**9**) in a 84% yield. Benzylation of **9** with benzyl chloride in the presence of barium oxide and barium hydroxide in DMF afforded the benzyl derivative (**10**), which, on deacetonation by treatment with 80% aqueous acetic acid, afforded 6-*O*-(2-*O*-benzyl-3-ethoxycarbonylamino-3-deoxy-α-D-glucopyranosyl)-*N*,*N*'-diethoxycarbonyl-2-deoxystreptamine (**11**), mp 149°C, $[\alpha]_D^{15}$ +77° (*c* 1, pyridine). Controlled isopropylidenation of **11** by treatment with 2,2-dimethoxypropane at 0°C for three hours successfully afforded the desired mono-*O*-isopropylidene derivative (**12**), mp 263°C, $[\alpha]_D^{20}$ +54° (*c* 1, pyridine), in a 60% yield. The orientation of the isopropylidene group in **12** was confirmed by the tritylation of **12** and **11**. When **11** which contains a primary hydroxyl group was tritylated with trityl chloride in pyridine, the product showed the characteristic color-reaction of a trityl derivative when sprayed with sulfuric acid on tlc, while **12** underwent no tritylation by the same procedure. In this connection, we would like to mention about the characteristic feature of the isopropylidene groups in the NMR spectra of the above mentioned compounds. When the NMR spectra of **10** and **12** were compared, it was found that methyl signals ascribable to the 4,5-*O*-isopropylidene group of deoxystreptamine moiety appeared at $\tau\sim8.6$, whereas those of 4,6-*O*-isopropylidene group of 3AG moiety appeared separately at $\tau\sim8.6$ and ~8.7. This clear-cut separation of signals was recognizable only in DMSO-d_6 and not in pyridine-d_5.

In the next place, the monoisopropylidene derivative (**12**) was condensed with the glycosyl chloride (**7**). In analogy to the previous studies on the total syntheses of kanamycins,[10] the condensation was effected by use of mercuric cyanide and Drierite in dioxane–benzene under an extremely anhydrous condition. The product was chromatographed on a silica gel column, affording the desired condensation product (**13**), mp 226°C $[\alpha]_D^{15}$ +92.6° (*c* 0.4, chloroform), in a 14% yield. Expectedly, **13** showed, in the NMR spectrum, the methyl signals of the isopropylidene group separated at τ 8.75 and 8.63, indicating that the group is attached at C-4,6 of the 3AG moiety in conformity with the above-mentioned observation.

Finally, **13** was treated with aqueous acetic acid to remove the isopropylidene group, hydrogenated over palladium black to remove the benzyl groups and treated with barium hydroxide to hydrolyze the *N*-ethoxycarbonylamino groups, affording an anomeric mixture of the 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-3-*O*-methyl-D-glucopyranosyl)-2-deoxystreptamine (**14**), mp 167—169°C, $[\alpha]_D^{15}$ +106° (*c* 0.5, water), in a 55% yield. Although paper chromatography of **14** with *n*-butanol–pyridine–water–acetic acid (6:4:3:1) showed a single spot by ninhydrin coloration, the NMR spectrum of **14** showed approximately 0.5 proton of the equatorial H-1 of 6-amino-6-deoxy-3-*O*-methyl-D-glucose moiety at τ 4.63 as doublet ($J\sim3$ Hz), indicating that **14** was an anomeric mixture of a ratio about 1:1.

The position of attachment of 6-amino-6-deoxy-3-*O*-methyl-D-glucosyl moiety to the 2-deoxystreptamine was demonstrated by the periodate oxidation of **14**. The survival of deoxystreptamine in the hydrolyzate of the oxidation mixture was shown by paper chromatography with ninhydrin indicating that the glycosidation did not occur at the C-5 of 2-deoxystreptamine, but at the C-4.

The 3'-*O*-methylkanamycin (**14**) showed no inhibition to *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Mycobacterium* 607 at 100 μg/ml in a nutrient agar, but inhibited *B. subtilis* PCI 219 at 25 μg/ml. It showed partial inhibition of *E. coli* carrying R factor at 100 μg/ml. As reported in the succeeding paper, 3'-deoxykanamycin exhibits a strong antibacterial activity in the similar strength as kanamycin. 3'-*O*-Methyl group was suggested to hinder the binding of 3'-*O*-methyl kanamycin with bacterial ribosomes, because it showed almost no inhibition of poly phenylalanine synthesis on poly U-ribosome system.

Experimental

Infrared spectra (IR) were recorded with a Perkin-Elmer Infrared Spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian A-60 spectrometer. Tetramethylsilane was used as the internal standard.

9) See, for examples, 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.

10) S. Umezawa, S. Koto, T. Tatsuta, and T. Tsumura, *J. Antibiotics*, **21**, 162 (1968); S. Umezawa, K. Tatsuta and S. Koto, *ibid.*, **21**, 367 (1968); S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, *ibid.*, **21**, 424 (1968); S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, This Bulletin, **42**, 529 (1969); S. Umezawa, K. Tatsuta, and S. Koto, *ibid.*, **42**, 533 (1969); S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, *ibid.*, **42**, 537 (1969).

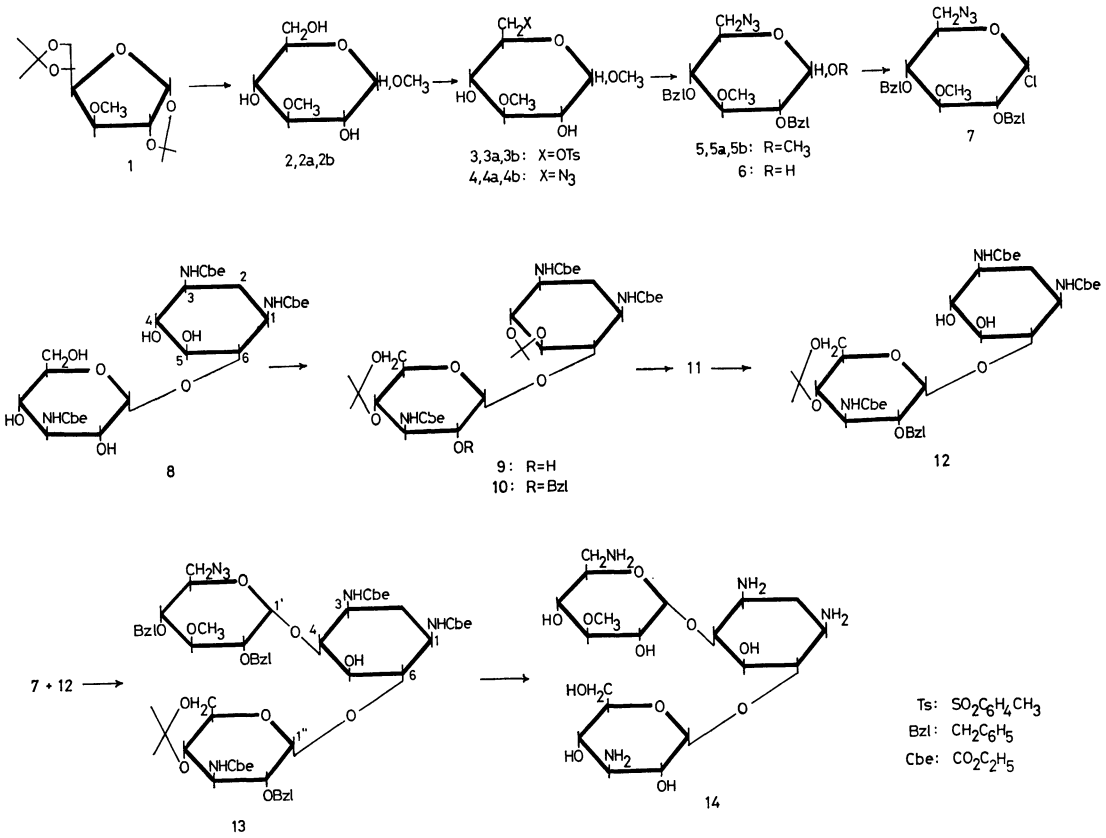


Chart 1.

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°.

Methyl 3-O-Methyl-α-D-glucopyranoside (2a) and its β-anomer (2b). A solution of **1** (5.1 g) in methanolic 1% hydrochloric acid (120 m*l*) was refluxed for 7 hr and coevaporated with benzene. The residue was dissolved in methanol and the solution was neutralized with Amberlite IRA 400 (OH form), treated with charcoal and evaporated to give a syrup (3.51 g, 91%). On tlc (acetone–ethyl acetate 3:2), the product showed a single spot (*Rf* 0.6), indicating no contamination with the starting material (R_f 1.0). The syrup (2.76 g) was then chromatographed on a column of Dowex 1×2 (OH form, 400 m*l*) with water. After the first fraction (400 m*l*) was discarded, the eluate was cut into 10 g each. α-Anomer (**2a**) was eluted between tube Nos. 10—17 and β-anomer (**2b**) between Nos. 20—35. Evaporation of the former gave a syrup (1.42 g, 47%) which, on storage, crystallized in needles, mp 80—82°C (lit,[8] 80—81°C), $[\alpha]_D^{25}$ +161° (*c* 1.4, acetone) (lit, +164°); IR (KBr): 840 cm^{-1} (type 2a).

Found: C, 46.30; H, 8.02%. Calcd for $C_8H_{16}O_6$: C, 46.15; H, 7.75%.

Evaporation of the latter fraction gave a syrup (0.98 g, 32%), $[\alpha]_D^{25}$ −33° (*c* 0.8, acetone) (lit [8] −26°); IR 885 cm^{-1} (type 2b).

Found: C, 46.34; H, 7.80%.

Mthyl 3-O-Methyl-6-O-tosyl-α-D-glucopyranoside (3a). To an ice-cold solution of **2a** (1.42 g) in dry pyridine (28 m*l*, dried over fused sodium hydroxide), tosyl chloride (2 g) was added and the solution was allowed to stand in a refrigerater for 5 hr. After a few drops of water was added, the solution was concentrated. The residue was dissolved in chloroform (200 m*l*) and the solution was washed successively with 5% potassium bisulfate solution, water, saturated sodium bicarbonate solution and water, dried over sodium sulfate and evaporated, giving a syrup. Tlc (ethyl acetate) showed that the syrup contained three components of R_f 0.93 (ditosylated product), 0.61 (**3a**, major) and 0.17 (**2a**). The syrup was then chromatographed on silica gel column (70 g) with ethyl acetate. The ditosylated product was eluted between 160—190 m*l* and **3a** between 240—370 m*l*. The latter fraction was evaporated and the residue was dissolved in benzene. Addition of petroleum ether gave a solid (1.48 g, 60%), mp 100—103°C, $[\alpha]_D^{15}$ +107° (*c* 1, acetone); IR (KBr): 1360, 1170 cm^{-1} (ν_{as} and ν_s SO_2, respectively).

Found: C, 49.98; H, 6.37; S, 8.64%. Calcd for $C_{15}H_{22}O_8S$: C, 49.71; H, 6.12: S, 8.85%.

NMR (in $CDCl_3$): τ 7.54 (3H s, the methyl of a tosyl group), 6.61 (3H s, C(1)-OCH_3), 6.35 (3H s, 3(C)-OCH_3), 6.1—6.8 (4H, m H-2,3,4,5), 5.70 (2H d, H-6,6′; This shows that 6-OH was tosylated. Corresponding signals are not recognized in **2a** and **4a**), 5.33 (1H d, H-1), 2.05—2.75 (4H, AB quartet-like pattern, J~8 Hz, tosyl); $J_{1,2}$ 3 Hz, $J_{5,6'}$ ~$J_{5,6'}$ ~3 Hz.

Methyl 3-O-Methyl-6-O-tosyl-β-D-glucopyranoside (3b). Compound **2b** (0.98 g) was treated likewise as in the case of **2a** and the product (**3b**) was recrystallized from benzene–petroleum ether to give crystals (1.0 g, 59%), mp 132—133°C, $[\alpha]_D^{15}$ −13.6° (*c* 1.1, acetone).

Found: C, 49.91; H, 6.34; S, 8.99%. Calcd for $C_{15}H_{22}O_8S$: C, 49.71; H, 6.12; S, 8.85%.

Methyl 6-Azido-6-deoxy-3-O-methyl-α-D-glucopyranoside (4a) and its β-Anomer (4b). The anomeric mixture of methyl 3-*O*-methyl-D-glucopyranoside (**2**) was tosylated likewise as described in the case of **3a**, and the product (5.0 g) was dissolved in dry DMF (dried over calcium hydride and supernatant layer was used without distillation). After addition of sodium azide (1.8 g), the mixture was refluxed

for 1 hr. The resulting brown solution was filtered and the filtrate was evaporated with several additions of toluene to remove the remaining DMF. The syrupy residue was dissolved in ethyl acetate and the solution was passed through a short column of silica gel (15 g) with the same solvent; the fraction containing the products (**4a** and **4b**) was collected and evaporated to give a pale yellow syrup (3.14 g, 98%), which had the same R_f-value with that of **3a** or **3b** (R_f 0.61 with ethyl acetate, 0.6 with ethyl acetate–benzene 5: 1 and 0.8 with methyl ethyl ketone), however, the infrared spectrum of the syrup showed no absorption of sulfonyl group but showed that of an azide group (2100 cm^{-1}), indicating that the reaction was completed. Anomers (2.05 g) were separated by chromatography on a column of Dowex 1×2 (OH form, 33×450 mm) with water. After the first fraction (400 m*l*) had been discarded, the eluate was cut into 10 g each. The α-anomer was eluted between tube Nos. 22—28 and the β-anomer between Nos. 34—48. Evaporation of the former afforded a solid (0.56 g), which was recrystallized from benzene to give colorless crystals (0.51 g), mp 88—89°C, $[\alpha]_D^{20}$ +129° (*c* 1, acetone); IR (KBr): 2100 (N_3), 845 cm^{-1} (type 2a).

Found: C, 41.39; H, 6.61; N, 18.30%. Calcd for $C_8H_{15}O_5N_3$: C, 41.20; H, 6.48; N, 18.02%.

Evaporation of the latter fraction followed by recrystallization from benzene gave colorless crystals (0.56 g), mp 110—111°C, $[\alpha]_D^{20}$ −87° (*c* 1, acetone); IR (KBr): 2100 (N_3), 885 cm^{-1} (type 2b).

Found: C, 41.51; H, 6.73; N, 17.89%.

These α- and β-anomers (**4a** and **4b**) were identical with those prepared from the anomerically pure tosylated compounds (**3a** and **3b**), respectively.

*Methyl 6-Azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-*D*-glucopyranoside (5a) and its β-anomer (5b).* To an ice-cold solution of **4a** (207 mg) in dry DMF (4 m*l*), powdered potassium hydroxide (590 mg) and benzyl chloride (0.6 m*l*) were added in turn and the mixture was stirred for 30 min. at room temperature. Tlc (benzene–methyl ethyl ketone 25: 1) of the reaction mixture showed that the starting material (**4a**, R_f 0) disappeared and **5a** (R_f 0.55) appeared as a sole product. Filtration followed by evaporation gave a syrup, which was coevaporated with toluene several times. The resultant syrup was dissolved in chloroform and the solution was washed with water, dried over sodium sulfate, and evaporated to give a solid (302 mg, 82%), which gradually crystallized on storage, mp 59—61°C, $[\alpha]_D^{15}$ +111° (*c* 1, acetone); IR (KBr): 2100 (N_3), 840 (type 2a; 735, 690 cm^{-1} (phenyl).

Found: C, 64.20; H, 6.58; N, 10.07%. Calcd for $C_{22}H_{27}O_5N_3$: C, 63.90; H, 6.58; N, 10.16%.

Likewise, from the β-anomer (**4b**, 282 mg) **5b** was obtained as needles (392 mg, 79%), mp 62—65°C, $[\alpha]_D^{15}$ +1.7° (*c* 0.9, acetone); IR (KBr): 2100 (N_3), 885 (type 2b); 745, 695 cm^{-1} (phenyl).

Found: C, 64.11; H, 6.94; N, 10.06%.

*6-Azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-*D*-glucopyranose (6)* To an anomeric mixture of **5** (8.12 g) in acetic acid (46 m*l*), 2N hydrochloric acid in 50% aqueous–methanol (15.5 m*l*) was added and the solution was refluxed for 3 hr. Tlc (benzene–methyl ethyl ketone 15: 1) of the reaction mixture showed that the solution contained four components of R_f 0.76 (**5b**), 0.71 (**5a**), 0.5 (**6**), and 0. Evaporation *in vacuo* followed by coevaporation with toluene gave a syrup (2.86 g), which was then chromatographed on a column of silica gel (100 g) with benzene–methyl ethyl ketone (15: 1). After the first fraction (160 m*l*) was discarded, the eluate was cut into 5 g each. A mixture of **5a** and **5b** (rich in **5a**) was eluted between tube Nos. 3—10 and **6** between Nos. 16—35. Evaporation of the latter fraction gave a syrup (1.19 g), which was recrystallized from benzene–petroleum ether to give **6** (1.15 g), mp 112—113°C, $[\alpha]_D^{20}$ +108° (*c* 0.9, acetone; final value); IR (KBr): 3380, 2100; 750, 740, 697 cm^{-1}.

Found: C, 63.36; H, 6.52; N, 10.52%. Calcd for $C_{21}H_{25}O_5N_3$: C, 63.14; H, 6.31; N, 10.52%.

The former fraction was evaporated to give a syrup (1.18 g), which could again be used for hydrolysis to **6**.

When the reaction period exceeded the limit of 3 hr, the product was observed to increase in the undesirable product of R_f 0, decreasing the yield of **6**. NMR of **6** (in $CDCl_3$): τ 6.32 (3H, s, OCH_3), 5.26 (2H s, one of methylenes of benzyls), 5.13 and 5.37 (2H AB quartet; another methylene of benzyls; *J* 11 Hz).

*6-Azido-2,4-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-*D*-glucopyranosyl Chloride (7).* A solution of **6** (5.0 g) in thionyl chloride (55 m*l*) was allowed to stand for 1 hr at room temperature. Tlc (benzene–methyl ethyl ketone 30: 1) of the reaction mixture showed that the starting material (**6**, R_f 0.15) disappeared and **7** (R_f 0.82) appeared as a main product. Evaporation of the solution followed by coevaporation with toluene gave a syrup, which was then chromatographed on a column of silica gel (90 g) with benzene. The fraction containing **7** (500—800 m*l* portion) was evaporated and the resultant syrup (3.2 g, 61%) crystallized to needles after 2 weeks storage. Mp 33—35°C, $[\alpha]_D^{15}$ +184° (*c* 1.1, acetone).

Found: C, 60.18; H, 6.05; N, 9.85; Cl, 8.78%. Calcd for $C_{21}H_{24}O_4N_3Cl$: C, 60.36; H, 5.79; N, 10.06; Cl, 8.48%.

NMR (in $CDCl_3$): τ 6.20—6.62 (5H, m, H-2,3,4,6,6′), 6.30 (3H s, OCH_3), 5.90 (1H doublet of triplets, H-5), 5.28 (2H s, $C\underline{H}_2C_6H_5$), 5.12 and 5.36 (2H AB quartet, *J* 11 Hz, $C\underline{H}_2C_6H_5$), 3.98 (1H d, H-1), 2.64 and 2.60 (5H singlets, phenyl of benzyls); $J_{1,2}$ 3.2 Hz, $J_{4,5}$ 9.0 Hz, $J_{5,6}=J_{5,6'}\sim3$ Hz.

N,N′-*Diethoxycarbonyl-6-*O*-(3-deoxy-3-ethoxycarbonylamino-α-*D*-glucopyranosyl)-2-deoxystreptamine (8).* To a solution of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (3AD) (5.52 g) and anhydrous sodium carbonate (5.30 g) in water (55 m*l*), acetone (55 m*l*) was added with vigorous stirring and to the suspension, ethyl chloroformate (5.8 g) was added all at once. An almost clear solution was soon formed with evolution of gas, after that precipitates appeared. Regardless of precipitation, agitation was continued for 18 hr at room temperature. Tlc (benzene–methyl ethyl ketone 4: 1) of the reaction mixture showed a single spot (R_f 0.4). After addition of 2N hydrochloric acid (22 m*l*), the reaction mixture was evaporated *in vacuo*. The residue was then suspended in DMF (200 m*l*), filtered from an insoluble matter and the filtrate was evaporated with several additions of toluene. The resultant residue was dissolved in DMF (40 m*l*) and worked up again as above. The desalted residue was boiled with ethanol (150 m*l*) for a while, cooled, and the insoluble product was taken by filtration; 7.50 g (82%), mp>250°C, $[\alpha]_D^{20}$ +64° (*c* 1, DMF); IR (KBr): 1695 (amide I), 1545 cm^{-1} (amide II).

Found: C, 47.01; H, 7.12; N, 7.83%. Calcd for $C_{12}H_{37}O_{13}N_3$: C, 46.75; H, 6.91; N, 7.79%.

N,N′-*Diethoxycarbonyl-6-*O*-(3-deoxy-3-ethoxycarbonylamino-4,6-*O*-isopropylidene-α-*D*-glucopyranosyl)-4,5-*O*-isopropylidene-2-deoxystreptamine (9).* To a solution of well dried **8** (973 mg) in dry DMF (dried over calcium hydride), dehydrated *p*-toluenesulfonic acid (30 mg, dried *in vacuo* at 100°C in the presence of P_2O_5) and 2,2-dimethoxypropane (1.4 m*l*) were added, and the mixture was allowed to stand for 5 hr at 40°C. Tlc (benzene–methanol 3: 1) of the reaction mixture showed that **8** (R_f 0.37) had completely been consumed and the main product (**9**, R_f 0.74) appeared, being accompanied

by a trace of mono-isopropylidene product (R_f 0.54). After treatment with Amberlite IRA-400 (OH form, 2 ml) resin, the mixture was filtered and the filtrate was evaporated with several additions of toluene to give a thick syrup (1.06 g). Purification was accomplished by dissolving the syrup in methanol–ethanol (1:1) with subsequent addition of petroleum ether; a colorless solid (940 mg, 84%), mp 237—238°C, $[\alpha]_D^{20}$ +41° (*c* 1, pyridine); IR (KBr): 1705, 1540, 1310 (isopropyl), 845 cm^{-1} (type 2a).

Found: C, 52.23; H, 7.55; N, 6.81%. Calcd for $C_{27}H_{45}O_{13}N_3$: C, 52.33: H, 7.32; N, 6.78%.

NMR (in DMSO-d_6): τ 8.85 (9H t, *J* 7 Hz, $CH_2C\underline{H}_3$), 8.70 (3H s, one of methyls of 4,6-*O*-isopropylidene group), 8.61—8.63 (9H singlet-like pattern, other methyls of isopropylidene groups).

*6-O-(2-O-Benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-O-isopropylidene-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-4,5-O-isopropylidene-2-deoxystreptamine (**10**).* To a mixture of **9** (102 mg), powdered barium oxide (62 mg) and powdered barium hydroxide octahydrate (123 mg) in DMF (2 ml), benzyl chloride (0.14 ml) was added and the suspension was agitated vigorously for 1 hr at room temperature. Tlc (benzene–methanol 5: 1) of the reaction mixture showed that the starting material (R_f 0.52) disappeared and instead, a new spot (**9**, R_f 0.70) appeared. The suspension was filtered with the aid of chloroform (20 ml) and the filtrate was evaporated with several additions of toluene. The chloroform solution of the resultant residue was washed with saturated sodium bicarbonate solution, then with water, dried over sodium sulfate and concentrated. Addition of petroleum ether afforded a colorless solid (112 mg, 96%), which was recrystallized from methanol, mp 286°C, $[\alpha]_D^{20}$ +47° (*c* 1, pyridine); IR (KBr): 1695, 1545, 1375, 845; 735, 700 cm^{-1}.

Found: C, 57.20; H, 7.44; N, 5.81%. Calcd for $C_{34}H_{51}O_{13}N_3$: C, 57.53; H, 7.24; N, 5.92%.

NMR (in DMSO-d_6): τ 8.98 (3H t, *J* 7 Hz, $CH_2C\underline{H}_3$), 8.85 (6H t, *J* 7 Hz,$CH_2C\underline{H}_3$), 8.74 (3H s, isopropylidene), 8.63 (9H s, isopropylidene), 2.77 (5H s, phenyl).

*6-O-(2-O-Benzyl-3-deoxy-3-ethoxycarbonylamino-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2-deoxystreptamine (**11**).* A suspension of **10** (3.36 g) in 80% aqueous acetic acid (100 ml) was heated for 10 min at 80°C. Tlc (benzene–methanol 5: 2) of the solution showed that the starting material (**10**, R_f 0.80) disappeared and instead, **11** (R_f 0.58) appeared as a sole product. The solution was evaporated with several additions of toluene-methanol. The resultant residue was dissolved in methanol, and the solution was treated with Amberlite IRA-400 (OH form), then with active charcoal. Concentration of the solution to about 100 ml followed by cooling gave a colorless crystals (2.58 g, 87%), mp 149°C, $[\alpha]_D^{15}$ +77° (*c* 1, pyridine); IR (KBr): 3340, 1690, 1545 cm^{-1}.

Found: C, 53.15; H, 6.93; N, 6.53%. Calcd for $C_{28}H_{43}O_{13}N_3$: C, 53.41; H, 6.88; N, 6.67%.

*6-O-(2-O-Benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-O-isopropylidene-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2-deoxystreptamine (**12**).* To an ice-cold solution of well dried **11** (1.76 g, 2.8 mmol) in dry DMF (35 ml, dried over calcium hydride), dehydrated *p*-toluenesulfonic acid (24 mg) and 2,2-dimethoxypropane (1.46 g, 14 mmol) were added and the mixture was allowed to stand for 3 hr at 0°C. Tlc (benzene–methanol 5: 1) of the reaction mixture showed that three components, namely, **10** (R_f 0.66), **11** (R_f 0.30) and **12** (R_f 0.54, major) were contained. After treatment with methanol-washed Amberlite IRA-400 (OH form), the mixture was filtered and the filtrate was evaporated with several additions of toluene to give a colorless powder (1.8 g). Since the powder had only very limited solubility against the solvent systems tested, several attempts to separate the components by column chromatography, however, were unsuccessful. The suspension of the powder (1.8 g) in benzene (60 ml) was refluxed for 15 min, cooled and filtered. Tlc showed that the benzene–insoluble material (1.50 g) contained **11** and **12**. The insoluble material was treated with warm chloroform and an insoluble part was removed. The chloroform-soluble portion (1.28 g) was then dissolved in dioxane (15 ml) and the solution was diluted with petroleum ether (100 ml) to give pure **12** (1.12 g, 60%), mp 263°C, $[\alpha]_D^{20}$ +54° (*c* 1, pyridine); IR (KBr): 3340, 1695, 1545, 1375; 750, 690 cm^{-1}.

Found: C, 55.12; H, 7.14; N, 6.15%. Calcd for $C_{31}H_{47}O_{13}N_3$: C, 55.60; H, 7.07; N, 6.27%.

NMR (in DMSO-d_6): τ 8.95 (3H, t, *J* 7 Hz, $CH_2C\underline{H}_3$), 8.84 (6H, t, *J* 7 Hz, $CH_2C\underline{H}_3$), 8.72 (3H s, isopropylidene), 8.61 (3H s, isopropylidene), 2.63 (5H, s, phenyl).

*Tritylation of **12** and **11**.* To a solution of **12** (one mol. equiv.) in pyridine, trityl chloride (2 mol. equiv.) was added and the solution was allowed to stand for 4 days at 27°C. Tlc (benzene–methanol 5: 1) of the reaction mixture showed that the starting material (**12**) was left unchanged throughout the reaction. On the other hand, by the same tritylation as mentioned above, the deacetonated compound **11** (R_f 0.30) was changed to a derivative which had R_f 0.56 and showed the characteristic yellow coloration on spraying sulfuric acid on tlc, indicating the presence of trityl group. These facts indicated that, in the compound **12**, the isopropylidene group is not attached to the deoxystreptamine moiety, but to C-4,6 positions of the 3-amino-3-deoxyglucose moiety.

*4-O-(6-Azido-2,4-di-O-benzyl-6-deoxy-3-O-methyl-D-glucopyranosyl)-6-O-(2-O-benzyl-3-deoxy-3-ethoxycarbonylamino-4,6-O-isopyropylidene-α-D-glucopranosyl)-N,N′-diethoxycarbonyl-2-deoxy streptamine (**13**).* Solvents used in this reaction were thoroughly dried as follows: purified dioxane was refluxed with sodium metal for 1 hr just before use and the supernatant layer was used; purified benzene was dried over $LiAlH_4$ overnight and the supernatant layer was used.

To a mixture of **12** (558 mg, 1 mol. equiv.), mercuric cyanide (836 mg) and freshly prepared Drierite (800 mg) in dry dioxane (6 ml), a solution of **7** (455 mg, 1.3 mol. equiv.) in dry benzene (6 ml) was added and the mixture was heated under vigorous stirring for 5 hr at 100—105°C in a pressure bottle. During the reaction period, absorption of the moisture from the air was avoided strictly, otherwise the yield of **13** become very poor with the formation of undesirable compound (R_f 0.58) mentioned below. The reaction mixture was poured into a saturated sodium bicarbonate solution with stirring and the product was extracted with chloroform. After dried over sodium sulfate, the chloroform solution was concentrated and diluted with petroleum ether to give a colorless powder (500 mg). Tlc (benzene–methanol 5: 1) of the product showed the presence of at least four components: The desired product (**13**, R_f 0.68, major), a deacetonated product (R_f 0.58) and the starring materials **12** (R_f 0.54) and **7** (R_f 1.0; **6** will also be included). The powder was chromatographed, without delay, on a column of silica gel (50 g) with benzene–methanol–ammonia saturated methanol[11] (5: 0.75: 0.25) and the fraction containing **13** (85—110 ml portion) was evaporated to dryness. The residue was treated with dioxane–petroleum ether to give a colorless solid (119 mg, 14%), mp 226°C, $[\alpha]_D^{15}$ +92.6° (*c* 0.4, $CHCl_3$); IR (KBr): 2100, 1700, 1535, 1370; 740, 695 cm^{-1}.

Found: C, 59.23; H, 6.78; N, 8.11%. Calcd for $C_{52}H_{70}$-

11) Since the silica gel is somewhat acidic and was found to cause the hydrolysis of the isopropylidene group, a small quantity of ammonia was added to the developer.

$O_{17}N_6$: C, 59.42; H, 6.71; N, 8.00%.

NMR (in DMSO-d_6): τ 8.94 (3H t, J 7 Hz, $CH_2C\underline{H}_3$), 8.86 (6H, t, J 7 Hz, $CH_2C\underline{H}_3$), 8.75 (3H s, isopropylidene), 8.63 (3H s, isopropylidene), 2.72, 2.73, 2.76 (5H, singlets, phenyl).

*6-O-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-O-(6-amino-6-deoxy-3-O-methyl-D-glucopyranosyl)-2-deoxystreptmine (**14**).* A suspension of **13** (212 mg) in 80% aqueous acetic acid (4 m*l*) was heated at 80°C for 5 min. The resultant solution was evaporated with several additions of toluene to give a solid (200 mg), which had a R_f-value of 0.58 (by tlc with benzene–methanol 5:1) and, in its NMR spectrum (in DMSO-d_6), showed no signals corresponding to an isopropylidene group. Other clear signals were: τ 8.95 (3H t, J 7 Hz, $CH_2C\underline{H}_3$), 8.83 (6H t, J 7 Hz, $CH_2C\underline{H}_3$), 2.61, 2.62, 2.65 (each 5-proton singlet, phenyl of benzyls). A methoxyl signal was not clear by being superimposed with other signals.

The solid was then suspended in a mixture of water (4.3 m*l*) and methanol (7 m*l*), and after the addition of a few drops of acetic acid, the mixture was hydrogenated with freshly prepared palladium black and hydrogen under pressure (30 lb/sq. inch) at 45°C for 2 hr. Water (5 m*l*) was added and the reaction was continued for additional 10 hr. The resultant solution was filtered and the filtrate was evaporated to give a solid (~100 mg), which had a R_f-value 0.26 on tlc with benzene–methanol (1:2). Its infrared spectrum showed no absorption of azide (2100 cm^{-1}) and benzyl groups (~700 cm^{-1}) but showed the absorptions of amide groups (1700, 1550 cm^{-1}). By NMR spectroscopy (in D_2O), it showed also no aromatic peaks (τ 2—3). Other clear signals were: τ 8.78 (3H t, J 7 Hz), 8.74 (6H t, J 7 Hz), 4.84 (1H d, J ~3 Hz, H-1 of 3AG moiety), 4.62 (approximately 0.5 proton doublet, J ~ 3 Hz, H_{eq}-1 of 6-amino-6-deoxy-D-glucose moiety).

The solid (100 mg) was then heated with 10% solution of barium hydroxide octahydrate (5 m*l*) at 100°C for 7 hr, and then neutralized with sulfuric acid. The resultant precipitate was removed by filtration and the filtrate was concentrated. The residue was chromatographed on a column Dowex 1×2 (OH form, 10×100 mm) with water. Ninhydrin-positive fractions were collected and evaporated to give a crystalline product (**14**); 55 mg (55%), mp 167—169°C, $[\alpha]_D^{15}$ +106° (*c* 0.5, water). By paper chromatography with *n*-butanol–pyridine–water–acetic acid (6:4:3:1), it showed single spot of R_{fKM}[12] 2.2.

Found: C, 46.20; H, 7.47; N, 11.00%. Calcd for $C_{14}H_{38}O_{11}N_4$: C, 45.78; H, 7.68; N, 11.24%.

NMR (in D_2O): τ 6.37 (3H s, OCH_3); this singal is absent in the NMR of kanamycin; 4.95 (1H d, J ~3 Hz), 4.63 (~0.5 H d, J ~3 Hz). The whole pattern was closely resemble to that of kanamycin free base.

Bioassay. The anomeric mixture (approximately 1:1) of 3′-*O*-methylkanamycin (**14**) only inhibited the growth of *Bacillus subtilis* PCI 219 completely at a dilution 25 mcg/m*l*, but showed no inhibition against several kinds of strains such as *M. pyogenes* var. *aureus* 209P, *E. coli* K-12, *K. pneumoniae*, *Ps. aeruginosa*, *Mycobacterum* 607 and *M. phlei*, respectively, at 100 mcg/m*l* in nutrient agar.

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12) R_f-value relative to kanamycin, which is taken as 1.