Total Synthesis of Dihydrostreptomycin[1)]

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(Received May 28, 1974)

Dihydrostreptomycin has been synthesized from dihydrostreptobiosamine and streptidine. A glycosyl chloride derivative (**10**) of the former was coupled with a protected derivative (**26**) of the latter to give a mixture of four isomeric glycosides, which were separated by chromatography. Deblocking of the desired glycoside led to dihydrostreptomycin. Several other protected derivatives of dihydrostreptobiosamine have also been described.

Streptomycin is the first useful streptomyces antibiotic discovered by Waksman[2)] in 1943. Dihydrostreptomycin[3,4)] is produced by hydrogenation of streptomycin or by fermentation.[5)] Its structure was established by 1948 except for the glycosidic linkage between streptose and streptidine, which was again revised[6,7)] to be α in 1965. We wish here to describe the total synthesis of dihydrostreptomycin in detail as the first synthesis of an antibiotic of streptomycin series.

There appeared to be two possible routes for synthetic approach to dihydrostreptomycin; one of which involves the synthesis of dihydrostreptobiosamine and subsequent coupling of its derivative with protected streptidine, and the other involves the coupling of protected derivatives of dihydrostreptose and streptidine followed by formation of second glycosidic linkage with a protected derivative of 2-deoxy-2-methylamino-L-glucose or 2-amino-2-deoxyl-L-glucose derivative. We chose the former route because the α glycosidic linkage between dihydrostreptose and streptidine is less stable for hydrolytic treatment than that between 2-deoxy-2-methylamino-L-glucose and dihydrostreptose owing to the presence of the methylamino group at C-2 of the latter glycoside moiety. Thus, we have already completed the total synthesis of dihydrostreptobiosamine[1,8)] and the preparation of an appropriate derivative of streptidine.[1,9)]

The next aspect of the synthesis of dihydrostreptomycin was concerned with the preparation of a protected glycosyl halide of dihydrostreptobiosamine. As for a per-*N*,*O*-acylated glycosyl halide of benzyl dihydrostreptobiosaminide,[8)] it was considered to be unfavorable for the deblocking of the condensation product with a streptidine derivative because the guanidino groups are susceptible to alkaline hydrolysis to give urethane. Consequently, the dihydrostreptobiosamine was protected as follows: Treatment of the benzyl α-dihydrostreptobiosaminide (**1**) with 2,2-dimethoxypropane by the usual ketal-exchange procedure[10,11)] gave the diisopropylidene derivative (**2**) in an 81% yield. **2** was further blocked by treatment with *p*-nitrophenyl chloroformate (NPCF) and 1 M sodium hydroxide to give the carbamate (**4**) in a 75% yield, which was alternatively prepared through the intermediate (**3**). We have previously reported[12)] the protecting method of *trans*-vicinal hydroxyl and amino groups. We can confirm the presence of the carbamate ring by an IR absorption band near 1770 cm^{-1}.

In the next step, we faced a problem arising from the isopropylidenated dihydrostreptose moiety, which was labile in the reaction of the anomeric hydroxyl

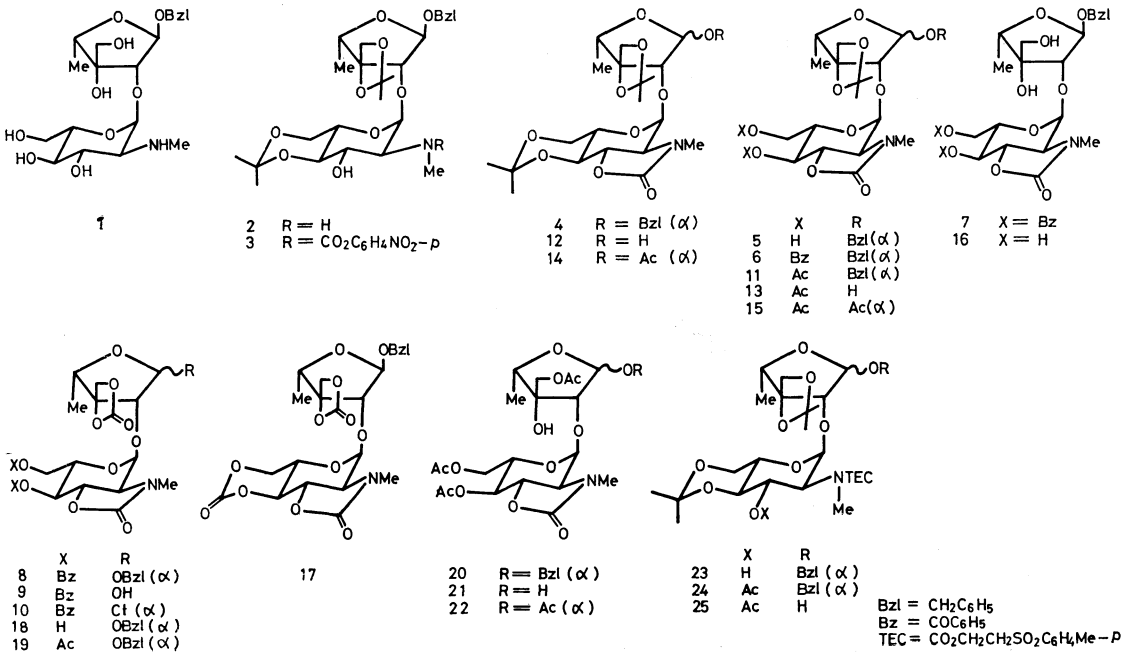

Chart 1.

group with halogenating agents. In a preliminary experiment with a dihydrostreptose derivative, we have observed that transketalization occurred in the chlorination of 2-*O*-acetyl-3,3'-*O*-isopropylidenedihydrostreptose with thionyl chloride. Therefore, we had to take a round-about way as follows: Treatment of **4** with 25% methanolic acetic acid under reflux overnight selectively removed the 4', 6'-*O*-isopropylidene group to give the monoisopropylidene derivative **5** in an 80% yield. This deisopropylidenation was found delicate; when **4** was treated with 50% acetic acid at 70 °C for 4 hr, both of the isopropylidene groups were hydrolyzed to give **16**. The free hydroxyl groups of **5** were then benzoylated to give the dibenzoyl derivative (**6**) in a 90% yield; **6** was found to have larger solubility in benzene. Treatment of **6** with aqueous 75% acetic acid at 70 °C for 6 hr removed the 3,3'-*O*-isopropylidene group to give the diol (**7**) in an 83% yield. When **7** was treated with NPCF in pyridine at room temperatures overnight, the carbonate (**8**) was obtained in a 91% yield. The infrared spectrum of **8** exhibited three distinct carbonyl absorptions for the cyclic carbonate, cyclic carbamate and benzoic esters. Direct treatment of benzyl α-dihydrostreptobiosaminide with NPCF or phosgene followed by benzoylation gave a poor yield of **8.** Hydrogenolysis of the glycosidic linkage of **8** on palladium black in dioxane gave the 1-OH-free disaccharide (**9**) quantitatively. Conversion of **9** into a glycosyl halide was achieved by the thionyl chloride procedure in a usual way. The fully protected glycosyl chloride (**10**) was obtained in a 68% yield. Glycosidation of **10** with benzyl alcohol gave **8** in yield of 59%.

Before proceeding along the main synthetic pathway, we prepared several other derivatives of dihydrostreptobiosamine which were found to be unsatisfactory for the glycoside synthesis.

Acetylation of **5** gave the di-*O*-acetyl derivative (**11**). Hydrogenolysis of **4** and **11** on palladium black gave the free disaccharides **12** and **13**, respectively, and they were respectively acetylated to give the glycosyl acetates, **14** and **15**. However, when the isopropylidene derivatives **14** and **15** were respectively treated with hydrogen bromide in acetic acid, and when **12** and **13** were respectively allowed to react with thionyl chloride, no glycosyl halide was isolated from these reaction products. It is noteworthy that an *N*-anisylidene derivative is useful for C-1 halogenation with hydrogen bromide–acetic acid as described in a preceding paper.[8]

Hydrolysis of **4** or **5** with 50% acetic acid gave the tetrol (**16**), which, on treatment with NPCF, gave the dicarbonate (**17**). When **17** was treated with silica gel in 80% aqueous acetone under reflux, the 4',6'-diol (**18**) was obtained, and this was converted into **8** by benzoylation. Thus, this constitutes an alternative route to **8** which is an intermediate in the main synthetic pathway. Acetylation of **18** gave **19**, which is the diacetyl analog of the above-mentioned useful dibenzoyl derivative (**8**). However, its solubility in organic solvents was still not satisfactory for the further coupling reaction.

Acetylation of **16** gave the triacetate (**20**), which led to the glycosyl acetate (**22**) by way of the free disaccharide (**21**). We have again here experienced that the glycosyl bromide of **21** could not be obtained from **22** by treatment with hydrogen bromide–acetic acid, and that the reaction of **21** with thionyl chloride did not give the glycosyl chloride.

Protection of the methylamino group with (β-*p*-tolylsulfonylethoxy)carbonyl group, which can easily be removed by alkaline hydrolysis,[13] was also studied. Acetylation of the derivative (**23**) gave **24**, which was led to the free disaccharide (**25**) by hydrogenolysis. After reaction with thionyl chloride in pyridine or without pyridine, the glycosyl chloride, which could not be isolated in pure form, was allowed to react with benzyl alcohol in order to test the glycoside formation. However, the benzyl glycoside (**24**) was obtained in a poor yield (13%) only in the presence of pyridine.

Returning to the final stage leading to dihydrostreptomycin, condensation of the glycosyl chloride (**10**) with the protected derivative (**26**)* of streptidine was carried out in anhydrous benzene in the presence of silver carbonate, silver perchlorate, and molecular sieves at 50 °C overnight. Examination of the products by tlc proved the formation of four products, *A*, *B*, *C*, and *D* in their order of mobility. Their separation was accomplished by repeated column chromatography on silica gel. Total yield of the condensation products was 81%. Their IR and NMR spectra respectively supported the structures of these products.

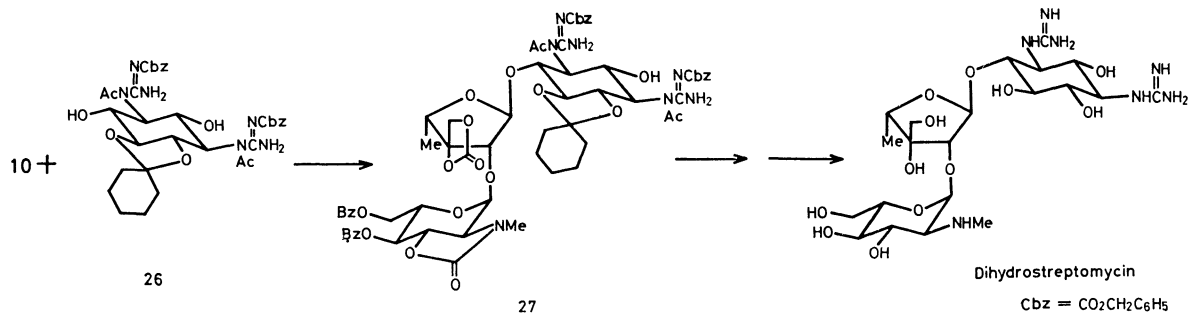

Chart 2.

* The positions of acetyl and benzyloxycarbonyl groups are arbitrarily assigned. See Ref. 9.

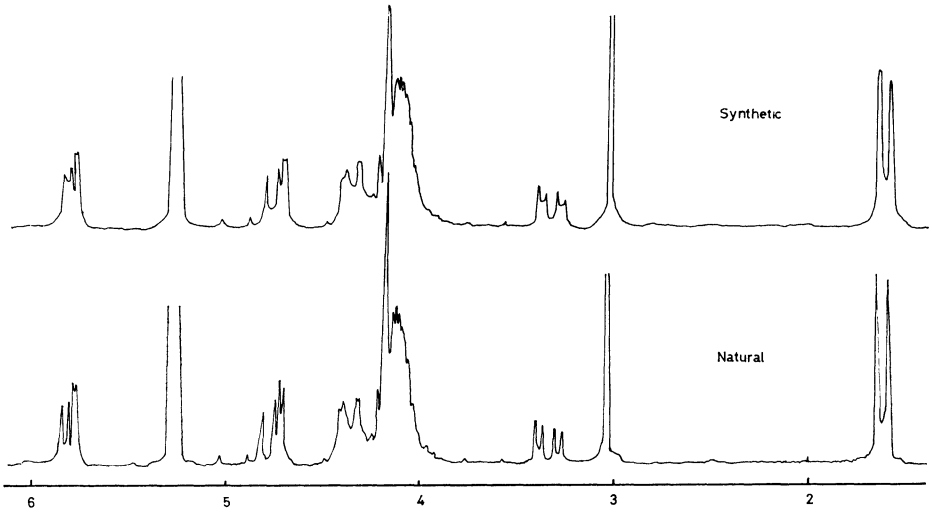

Fig. 1. NMR spectra of natural and synthetic dihydrostreptomycin sesquisulfate (in D_2O at 100 MHz).

Deblocking of the product A (**27**)* led to dihydrostreptomycin. By its treatment with a mixture of 0.025 M barium hydroxide and dioxane at 50 °C, the acetyl, benzoyl, carbonate, and carbamate groups were removed. The remaining cyclohexylidene group was removed by treatment with 50% acetic acid, and the benzyloxycarbonyl groups were removed by hydrogenolysis on palladium black in aqueous dioxane in a usual way to afford dihydrostreptomycin, which was converted into its sesquisulfate. The synthetic dihydrostreptomycin was identical in every respect with natural specimen. The identity was also established by optical rotational shift due to copper complexing ($\Delta[M]_{CuAm}$),[14] specific rotation, IR and NMR spectra (Fig. 1), thin layer and paper chromatographic behavior, paper electrophoresis mobility, and antibacterial spectra (Table 1).

Table 1. Antibacterial spectra of natural and synthetic dihydrostreptomycin

Test organisms[a]	MIC (mcg/ml)	
	Natural	Synthetic
Staphylococcus aureus FDA 209P	1.56	1.56
FDA 209P SM STf	>50	>50
Bacillus subtilis NRRL B-558	6.25	6.25
PCI 219	<0.2	0.2
Bacillus agri	>50	>50
Escherichia coli K-12	0.39	0.78
K-12 R-5	>50	>50
K-12 ML1629	>50	>50
K-12 ML1410	0.78	0.78
K-12 ML1410 R81	>50	>50
K-12 LA290 R55	1.56	1.56
K-12 W677	0.78	0.78
K-12 JR66/W677	>50	>50
NIHJ	3.12	3.12
NIHJ SMf	>50	>50
Pseudomonas aeruginosa A3	25	25
No. 12	50	50
Mycobacterium smegmatis ATCC 607[b]	0.39	0.39

a) Agar dilution streak method, nutrient agar 37 °C, 18 hr. b) 47 hr.

Similar procedures were carried out for deblocking of the other products, *B*, *C*, and *D*, respectively, giving three isomers of dihydrostreptomycin (**29**, **30**, and **31**) which showed no antibacterial activity.

Experimental

General procedure was the same as described in the foregoing paper.

Benzyl 2-O-(2-Deoxy-4,6-O-isopropylidene-2-methylamino-α-L-glucopyranosyl)-3,3'-O-isopropylidenedihydrostreptoside (2). To a solution of the hydrochloride (5.00 g) of **1** in dry DMF, 2,2-dimethoxypropane (8 ml), anhydrous *p*-toluenesulfonic acid (500 mg) and freshly dried Molecular Sieves 5A (30 g, Wako Pure Chem. Ind., Ltd., Osaka) were added, and the mixture was stirred at 60 °C overnight. To the mixture triethylamine (5 ml) was added and the mixture was evaporated. The residue was chromatographed on a short column of silica gel with chloroform–acetone (1 : 1) to give a syrup, 4.90 g (89%), $[\alpha]_D^{18}$ −121° (*c* 0.5, acetone).

Found: C, 61.03; H, 7.60; N, 2.55%. Calcd for $C_{26}H_{39}NO_9$: C, 61.28; H, 7.71; N, 2.75%.

NMR (in $CDCl_3$): δ 1.28 (3H d, $CHC\underline{H}_3$), 1.42 and 1.50 (9H and 3H s, respectively, isopropylidene), 2.50 (3H s, NCH_3), 5.05 (1H d, J ~1 Hz, H-1), 5.21 (1H d, J 3.5 Hz, H-1').

Benzyl 2-O-[2-Deoxy-4,6-O-isopropylidene-2-(N-methyl-p-nitrophenoxylcarboxamido)-α-L-glucopyranosyl]-3,3'-O-isopropylidenedihydrostreptoside (3). To a solution of **2** (260 mg) in aqueous acetone (1 : 2, 3 ml), triethylamine (60 mg) was added and the solution was cooled to −10 °C. A solution of NPCF (125 mg) in acetone (2 ml) was added and the solution was stirred for 15 min under cooling and at room temperature for additional 2 hr. After addition of triethylamine (70 mg), the solution was evaporated. A solution of the residue in chloroform was washed with saturated sodium hydrogen carbonate solution and with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to give a syrup, 355 mg (98%), $[\alpha]_D^{21}$ −122° (*c* 1, $CHCl_3$); IR (KBr): $\nu_{C=O}$ 1775, 1732 cm^{-1}, ν_{NO_2} 1530, 1350 cm^{-1}.

Found: C, 58.83; H, 6.23; N, 4.11%. Calcd for $C_{33}H_{42}N_2O_{13}$: C, 58.75; H, 6.27; N, 4.15%.

Benzyl 2-O-(2,3-N,O-Carbonyl-2-deoxy-4,6-O-isopropylidene-2-methylamino-α-L-glucopyranosyl)-3,3'-O-isopropylidenedihydrostreptoside (4). To a solution of **2** (1.61 g) in acetone (16 ml) maintained at −10 °C, a cold solution of NPCF (770 mg) in acetone (8 ml) and 1 M aqueous sodium hydroxide (4 ml) were simultaneously added dropwise with stirring. tlc of the reaction mixture with benzene–ether (1 : 1) showed the single spot of **3** at R_f 0.75. An aliquot (4 ml) of the sodium hydroxide solution was added and after standing at −10 °C for 15 min, another aliquot (2 ml) was added and the solution was allowed to stand for further 15 min. On tlc with the above-mentioned solvent system, the spot of R_f 0.75 disappeared and the spot of **4** (R_f 0.55) appeared. After neutralization with 10% acetic acid, the solution was extracted with ether. The ethereal solution was washed with cold 1 M sodium hydroxide until the yellow color of sodium *p*-nitrophenoxide disappeared. The solution was then washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated. The solid was recrystallized from ether–petroleum ether to give needles, 1.27 g (75%), mp 169—170 °C, $[\alpha]_D^{23}$ −152° (*c* 1, MeOH); IR (KBr): 1770 cm^{-1}.

Found: C, 60.42; H, 6.90; N, 2.60%. Calcd for $C_{27}H_{37}NO_{10}$: C, 60.55; H, 6.96; N, 2.62%.

NMR (in $CDCl_3$): δ 1.28 (3H d), 1.43 and 1.53 (9H and 3H s, respectively, isopropylidene), 2.87 (3H s, NCH_3), 3.37 (1H q, *J* 3 and 11.5 Hz, H-2'), 5.11 (1H d, *J* ~1 Hz, H-1), 5.42 (1H d, *J* 3 Hz, H-1'), 7.32 (5H s, Ph).

Benzyl 2-O-(2,3-N,O-Carbonyl-2-deoxy-2-methylamino-α-L-glycopyranosyl)-3,3'-O-isopropylidenedihydrostreptoside (5). A solution of the diisopropylidene derivative **4** (2.24 g) in 25% acetic acid and methanol (45 ml) was refluxed overnight. The solution was evaporated with toluene to give a solid, which was recrystallized from methanol, 1.75 g (80%), mp 190—190.5 °C, $[\alpha]_D^{23}$ −177° (*c* 1, MeOH); IR (KBr): 1775 cm^{-1}.

Found: C, 58.10; H, 6.78; N, 2.88%. Calcd for $C_{24}H_{33}NO_{10}$: C, 58.17; H, 6.71; N, 2.83%.

NMR (in pyridine-d_5): δ 1.40 (6H s, isopropylidene).

Benzyl 2-O-(4,6-Di-O-benzoyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3'-O-isopropylidenedihydrostreptoside (6). A sample of **5** (1.17 g) was treated with benzoyl chloride and pyridine to give a syrup, which was chromatographed on silica gel (50 g) with chloroform to yield a solid, 1.30 mg, (90%), mp 78—81 °C, $[\alpha]_D^{18}$ −145° (*c* 1, $CHCl_3$); IR (KBr): 1775, 1725 cm^{-1}.

Found: C, 64.59; H, 5.83; N, 1.90%. Calcd for $C_{38}H_{41}NO_{12}$: C, 64.87; H, 5.87; N, 1.99%.

Benzyl 2-O-(4,6-Di-O-benzoyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptoside (7). A sample of **6** (1.20 g) was treated with 75% acetic acid at 70 °C for 6 hr, and the reaction mixture was evaporated. To the resulting syrup was added toluene and the mixture was again evaporated to remove the remaining acetic acid. The residue was chromatographed on a silica gel with benzene–ethyl acetate (1 : 1) to yield a solid, 937 mg (83%), $[\alpha]_D^{18}$ −177° (*c* 1, $CHCl_3$).

Found: C, 63.63; H, 5.67; N, 1.95%. Calcd for $C_{35}H_{37}NO_{12}$: C, 63.14; H, 5.61; N, 2.11%.

Benzyl 2-O-(4,6-Di-O-benzoyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl-3,3'-O-carbonyldihydrostreptoside (8).

a) From 7. To a solution of **7** (830 mg) in pyridine (20 ml), NPCF (800 mg) was added and the solution was stirred at room temperature overnight. The solution was filtered and, to the filtrate, methanol (1 ml) was added. After standing for 30 min, the solution was evaporated. To the residue was added toluene and the mixture was again evaporated. The residue was chromatographed on a short column of silica gel (40 g) with benzene–ethyl acetate (1 : 2) to give a solid, 798 mg (91%), $[\alpha]_D^{18}$ −145° (*c* 1, $CHCl_3$); IR (KBr): $\nu_{C=O}$ 1815 (carbonate), 1715 (cyclic carbamate), 1725 cm^{-1} (ester).

Found: C, 62.88; H, 5.30; N, 1.91%. Calcd for $C_{36}H_{35}NC_{13}$: C, 62.69; H, 5.12; N, 2.03%.

NMR (in $CDCl_3$): δ 1.40 (3H d, $CHCH_3$), 2.91 (3H s, NCH_3), 3.57 (1H q, *J* 3 and 11.5 Hz, H-2'), 5.32 (1H d, *J* ~2 Hz, H-1), 5.33 (1H d, *J* 3 Hz, H-1'), 5.70 (1H t, *J* 10 Hz, H-4').

b) From 18. Treatment of **18** with benzoyl chloride and pyridine in a usual manner gave **8** in yield of 95%.

2-O-(4,6-Di-O-benzoyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3'-O-carbonyldihydrostreptose (9). A solution of **8** (798 mg) in dioxane (20 ml) was hydrogenated (50 psi) with palladium black (prepared from 2 g of palladium chloride), and the product was chromatographed on a 30 g of silica gel with chloroform–acetone (4 : 1) to give a solid, 681 mg, (98%), mp 150 °C, $[\alpha]_D^{18}$ −95° (*c* 1, $CHCl_3$); IR (KBr): 1820, 1775, 1730 cm^{-1}.

Found: C, 58.38; H, 5.07; H, 2.19%. Calcd for $C_{29}H_{29}NO_{13}$: C, 58.10; H, 4.88; N, 23.3%.

2-O-(4,6-Di-O-benzoyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3'-O-carbonyldihydrostreptosyl Chloride (10). A solution of **9** (200 mg) in thionyl chloride (2.0 ml) was allowed to stand at room temperature for 3 hr, and evaporated under reduced pressure (<1 Torr). The residue was dissolved in a mixture of benzene and ethyl acetate (1 : 1, dried over Molecular Sieves 4A). The solution was evaporated to give a syrup, which was chromatographed on 10 g of silica gel (activated at 260 °C *in vacuo* for 5 hr) by elution with dry benzene–ethyl acetate (1 : 1). The product was reprecipitated from dry benzene–*n*-hexane, 141 mg (68%), mp 114—116 °C (decomp), $[\alpha]_D^{20}$ −170° (*c* 1, $CHCl_3$); IR (KBr): 1820, 1770, 1725 cm^{-1}.

Found: C, 55.96; H, 4.59; N, 1.95; Cl, 5.89%. Calcd for $C_{29}H_{28}NO_{12}Cl$: C, 56.36; H, 4.57; N, 2.27; Cl, 5.74%.

NMR (in $CDCl_3$): δ 1.43 (3H d), 2.94 (3H s), 5.32 (1H d, *J* 3 Hz, H-1'), 5.70 (1H t, H-4'), 6.28 (1H d, *J* ~1 Hz, H-1).

Formation of 8 from 10. A mixture of **10** (48 mg), benzyl alcohol (14 mg), and Molecular Sieves 3A (200 mg) in dry benzene (1 ml) was stirred at room temperature for 3 hr. When checked by tlc, hardly any progress of reaction was observed. Freshly prepared and well dried (at 120 °C) silver carbonate (180 mg) and silver perchlorate (20 mg) were added and the mixture was vigorously stirred at room temperature for 2 hr. The reaction mixture was filtered with the aid of benzene and the filtrate was evaporated. The residue was chromatographed on a short column of silica gel with benzene–ethyl acetate (2 : 1) to give a solid, 31 mg (59 %). Its optical rotation and IR and NMR spectra were identical with those of authentic **8**.

Benzyl 2-O-(4,6-Di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3'-O-isopropylidenedihydrostreptoside (11). A sample of **5** was treated with acetic anhydride and pyridine at room temperature overnight and worked up to give a solid in 98% yield, mp 53—56 °C, $[\alpha]_D^{20}$ −176° (*c* 1, MeOH); IR (KBr): 1750, 1780 cm^{-1}.

Found: C, 57.83; H, 6.53; N, 2.39%. Calcd for $C_{28}H_{37}NO_{12}$: C, 58.02; H, 6.43; N, 2.42%.

NMR (in $CDCl_3$): δ 2.05 and 2.10 (each 3H s, Ac).

2-O-(2,3-N,O-Carbonyl-2-deoxy-4,6-O-isopropylidene-2-methylamino-α-L-glucopyranosyl)-3,3'-O-isopropylidenedihydrostreptose (12). A solution of **4** (1.48 g) in dioxan–methanol (1 : 5, 12 ml) was treated with palladium black and hydrogen (50 psi) at

room temperature overnight to yield a syrup, which was reprecipitated from chloroform–*n*-hexane (1 : 3). The solid was chromatographed on silica gel with chloroform–acetone (1 : 1) to give needles, 887 mg (85%), which was recrystallized from chloroform–*n*-hexane, mp 188—190 °C, $[\alpha]_D^{20}$ −113° (*c* 1, $CHCl_3$); IR (KBr): 1765 cm^{-1}.

Found: C, 54.24; H, 6.92; N, 3.22%. Calcd for $C_{20}H_{31}NO_{10}$: C, 53.93; H, 7.02; N, 3.14%.

2-O-(4,6-Di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3′-O-isopropylidenedihydrostreptose (**13**). Compound **11** was hydrogenated by a method similar to that described for the preparation of **12** from **4**. Mp 185—186 °C, $[\alpha]_D^{20}$ −108° (*c* 1, EtOAc); IR (KBr): 1750, 1780 cm^{-1}.

Found: C, 51.66; H, 6.12; N, 2.85%. Calcd for $C_{21}H_{31}NO_{12}$: C, 51.54; H, 6.38; N, 2.87%.

1-O-Acetyl-2-O-(2,3-N,O-carbonyl-2-deoxy-4,6-O-isopropylidene-2-methylamino-α-L-glucopyranosyl)-3,3′-O-isopropylidenedihydrostreptose (**14**). A sample of **12** (887 mg) was treated with acetic anhydride and pyridine and the product was recrystallized from chloroform–*n*-hexane to give crystals, 783 mg (81%), mp 247—250 °C (decomp), $[\alpha]_D^{21}$ −130° (*c* 1, $CHCl_3$).

Found: C, 54.01; H, 6.88; N, 2.80%. Calcd for $C_{22}H_{33}NO_{11}$: C, 54.20; H, 6.82; N, 2.87%.

NMR (in $CDCl_3$): δ 1.30 (3H d, *J* 6.5 Hz, $CHC\underline{H}_3$), 1.43 and 1.52 (9H and 3H s, respectively, isopropylidene), 2.07 (3H s, Ac), 2.84 (3H s, NCH_3), 5.36 (1H d, *J* 3 Hz, H-1′), 6.13 (1H d, *J* 1.5 Hz, H-1).

1-O-Acetyl-2-O-(4,6-di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3′-O-isopropylidenedihydrostreptose (**15**). A sample of **13** was acetylated by a method to that similar described for the preparation of **14** from **12**. Mp 137—138 °C, $[\alpha]_D^{21}$ −142° (*c* 1, $CHCl_3$).

Found: C, 51.73; H, 6.21; N, 2.37%. Calcd for $C_{23}H_{33}NO_{13}$: C, 51.97; H, 6.26; N, 2.64%.

NMR (in $CDCl_3$): δ 2.10 and 2.13 (6H and 3H s, respectively, Ac), 6.14 (1H d, *J* ~1 Hz, H-1).

Benzyl 2-O-(2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptoside (**16**). A solution of **4** (1.00 g) in 50% aqueous acetic acid (20 ml) was heated at 70 °C for 4 hr. Toluene was added and the solution was evaporated. The residue was recrystallized from methanol–ether, 632 mg (74%), mp 199—202 °C, $[\alpha]_D^{20}$ −186° (*c* 1, MeOH); IR (KBr): 1760 cm^{-1}.

Found: C, 55.30; H, 6.34; N, 3.12%. Calcd for $C_{21}H_{29}NO_{10}$: C, 55.38; H, 6.42; N, 3.07%.

Benzyl 3,3′-O-Carbonyl-2-O-(2,3-N,O-carbonyl-4,6-O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptoside (**17**). A sample of **16** was treated with NPCF by a method similar to that described for the preparation of **8** from **7**. On monitoring with tlc with benzene–acetone (2 : 1), the spot of **16** (R_f 0.1) gradually disappeared and two spots of R_f 0.17 and 0.46 (dicabonyl derivatives) appeared. Finally there appeared only one spot of **17** (R_f 0.55). The crude product was recrystallized from acetone–benzene, yielding **17** in 40% yield, mp 269—272 °C (decomp), $[\alpha]_D^{17}$ −120° (*c* 1, acetone); IR (KBr): 1800, 1765 cm^{-1}.

Found: C, 54.39; H, 4.93; N, 2.82%. Calcd for $C_{23}H_{25}NO_{12}$: C, 54.44; H, 4.97; N, 2.76%.

Compound **17** is soluble in acetone, scarcely soluble in methanol, ethyl acetate, and chloroform and insoluble in benzene and ether.

Benzyl 3,3′-O-Carbonyl-2-O-(2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptoside (**18**). To a solution of **17** (100 mg) in 80% aqueous acetone (2 ml), silica gel (1.0 g) was added and the mixture was refluxed for 2 hr. The mixture was filtered and the filtrate was evaporated to give a solid, which was recrystallized from acetone–*n*-hexane, 86 mg (90%), mp 196—197 °C, $[\alpha]_D^{25}$ −160° (*c* 1, MeOH), IR; 1805, 1775 cm^{-1}.

Found: C, 54.61; H, 5.62; N, 2.73%. Calcd for $C_{22}H_{27}NO_{11}$: C, 54.88; H, 5.65; N, 2.90%.

Conversion of **18** *into* **8**. A sample of **18** was treated with benzoyl chloride, pyridine, and triethylamine, and worked up in a usual way. The product was reprecipitated from benzene–*n*-hexane to give **8**, quantitatively, which was identical with the specimen prepared from **7**.

Benzyl 2-O-(4,6-Di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)-3,3′-O-carbonyldihydrostreptoside (**19**). A sample of **18** was treated with acetic anhydride and pyridine in a usual manner to yield **19** (80%), $[\alpha]_D^{21}$ −150° (*c* 1, $CHCl_3$); IR (KBr): 1815, 1765, 1740 cm^{-1}.

Found: C, 55.35; H, 5.62; N, 2.42%. Calcd for $C_{26}H_{31}NO_{13}$: C, 55.21; H, 5.53; N, 2.48%.

NMR (in $CDCl_3$): δ 2.05 and 2.11 (each 3H s, Ac).

Benzyl 3′-O-Acetyl-2-O-(4,6-di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptoside (**20**). A sample of **16** was treated with acetic anhydride and pyridine and worked up to give a solid of **20** in 98% yield, which was reprecipitated from ethyl acetate–*n*-hexane, mp 35—36 °C, $[\alpha]_D^{18}$ −149° (*c* 1, MeOH); IR (KBr): 1750, 1775 cm^{-1}.

Found: C, 55.49; H, 6.02; N, 2.40%. Calcd for $C_{27}H_{35}NO_{13}$: C, 55.76; H, 6.07; N, 2.41%.

NMR (in $CDCl_3$): δ 2.03, 2.08, and 2.11 (each 3H s, Ac).

3′-O-Acetyl-2-O-(4,6-di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptose (**21**). A sample of **20** was dissolved in methanol and hydrogenated with palladium black in a usual manner to give **21**, quantitatively, mp 170—172 °C, $[\alpha]_D^{20}$ −103° (*c* 1, MeOH); IR (KBr): 1780, 1745 cm^{-1}.

Found: C, 49.07; H, 5.98; N, 2.75%. Calcd for $C_{20}H_{29}NO_{13}$: C, 48.88; H, 5.95; N, 2.85%.

1,3′-Di-O-acetyl-2-O-(4,6-di-O-acetyl-2,3-N,O-carbonyl-2-deoxy-2-methylamino-α-L-glucopyranosyl)dihydrostreptose (**22**). A sample of **21** was treated with acetic anhydride and pyridine to give **22**, quantitatively, mp 152—153 °C, $[\alpha]_D^{20}$ −128° (*c* 1, $CHCl_3$); IR (KBr): 3600 (sharp), 1780, 1740 cm^{-1}.

Found: C, 49.58; H, 5.77; N, 2.66%. Calcd for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.62%.

Benzyl 2-O-[2-Deoxy-4,6-O-isopropylidene-2-(N-methyl-β-(p-toluenesulfonyl)ethoxycarboxamido)-α-L-glucopyranosyl]-3,3′-O-isopropylidenedihydrostreptoside (**23**). To an ice-cold solution of **2** (2.00 g) in tetrahydrofuran (15 ml), ice-cold sodium hydrogen carbonate solution (600 mg in 15 ml water) and β-(*p*-toluenesulfonyl)ethyl chloroformate[13] (1.4 g) in acetone (15 ml) were added and the mixture was stirred at room temperature for 2 hr. Evaporation of the mixture gave a syrup, which was dissolved in chloroform. The solution was washed with water, dried with anhydrous sodium sulfate and evaporated to give a syrup. The syrup was chromatographed on a short column of silica gel with chloroform–ether (1 : 1) to give a solid, 2.39 g (83%); $[\alpha]_D^{13}$ −91° (*c* 0.4, $CHCl_3$); IR (KBr): 1700 cm^{-1}.

Found: C, 58.73; H, 6.54; N, 1.90%. Calcd for $C_{36}H_{49}NO_{13}S$: C, 58.76; H, 6.71; N, 1.98%.

Benzyl 2-O-[3-O-Acetyl-2-deoxy-4,6-O-isopropylidene-2-(N-methyl-β-(p-toluenesulfonyl)ethoxycarboxamido)-α-L-glucopyranosyl]-3,3′-O-isopropylidenedihydrostreptoside (**24**). A sample of **23** (2.28 g) was treated with acetic anhydride in pyridine at room temperature overnight. The reaction mixture was poured into cold water (5 ml) and then the mixture was evaporated. The residue was stirred with a mixture of water (200 ml) and chloroform (100 ml). The aqueous layer was separated and extracted with chloroform. The chloroform

layers were combined, washed with saturated aqueous solution of potassium hydrogen sulfate, dried over magnesium sulfate and evaporated to give a solid in 90% yield, $[\alpha]_D^{18}$ −74° (*c* 1, $CHCl_3$); IR (KBr): 1753, 1705 cm^{-1}.

Found: C, 58.82; H, 6.51; N, 2.11; S, 4.05%. Calcd for $C_{38}H_{51}NO_{14}S$: C, 58.67; H, 6.61; N, 1.80; S, 4.12%.

NMR (in $CDCl_3$): δ 1.26 (3H d, *J* 6.5 Hz, $CHC\underline{H}_3$), 1.31, 1.35, 1.36, and 1.45 (each 3H s, isopropylidene), 2.03 (3H s, Ac), 2.44 (3H s, $C\underline{H}_3Ph$), 2.75 (~1.8 H s) and 2.94 (~1.2H s) (both NCH_3, rotamers), 5.02 (1H d, *J* ~1 Hz, H-1), 5.09 (1H d, *J* 3.5 Hz, H-1').

*2-O-[3-O-Acetyl-2-deoxy-4,6-O-isopropylidene-2-(N-methyl-β-(p-toluenesulfonyl)ethoxycarboxamido)-α-L-glucopyranosyl]-3,3'-O-isopropylidenedihydrostreptose (**25**).* A sample of **24** (1.70 g), dissolved in dioxane (10 ml), was treated with palladium black and hydrogen in a usual manner. The product was purified by chromatography on a short column of silica gel by elution with chloroform–ether (1 : 1) to yield **25**, 827 mg (55%), $[\alpha]_D^{21}$ −57° (*c* 1, $CHCl_3$); IR (KBr): 1750, 1705 cm^{-1}.

Found: C, 54.03; H, 6.45; N, 1.72; S, 4.89%. Calcd for $C_{31}H_{45}NO_{14}S$: C, 54.13; H, 6.60; N, 2.04; S, 4.66%.

*Formation of **24** from **25**.* A sample of **25** (100 mg) was dissolved in a mixture (0.5 ml) of thionyl chloride, dry benzene, and dry pyridine (0.25 : 10 : 0.31 by volume), Molecular Sieves 3A was added and the mixture was allowed to stand at room temperature for 1 hr. A solution (0.5 ml) of benzyl alcohol in benzene (3 : 100) was added and the mixture was allowed to stand at room temperature overnight. Saturated sodium hydrogen carbonate solution and chloroform were added with stirring and the organic layer was washed with water, dried over sodium sulfate and evaporated to give a syrup, which was chromatographed on a column of silica gel by elution with benzene–MEK (10 : 1) to give **24** (15 mg, 13%). The IR and NMR spectra of the product was quite superimposable on those of authentic **24** prepared from **23**.

*Condensation of **10** with **26**.* To a solution of **26** (1.20 g) in dry benzene (30 ml; dried over $LiAlH_4$ and distilled before use) was added freshly prepared and well dried silver carbonate (3.6 g), well dried silver perchlorate (0.4 g) and Molecular Sieves 3A (dried at 250 °C) and the mixture was stirred at room temperature overnight in the dark. The glycosyl chloride **26** (1.60 g) was added and the mixture was stirred at 50 °C overnight in the dark. The mixture was filtered with the aid of chloroform and the filtrate was evaporated. On tlc with benzene–chloroform–ethanol–concentrated ammonium hydroxide (50 : 50 : 4 : 0.5) (Solvent A), the residue gave four spots other than those of starting materials **10** and **26** and **9** derived from **10**. The condensation products were designated **A**, **B**, **C**, and **D** in their order of mobility. The chromatography of the residue on a column of silica gel (100 g) with Solvent A afforded fractions containing **A**+**B** (434 mg), **C** (669 mg), and **D** (730 mg); total yield 81%. The first fraction was further chromatographed three times on a column of silica gel (20 g) with benzene–90% ethanol (25 : 1) to give **A** and **B**. The second fraction **C** was further purified by chromatography on silica gel (35 g) with Solvent A. The third fraction **D** was also further chromatographed on silica gel (40 g) with chloroform–90% ethanol (10 : 1). Each product, which is chromatographically homogeneous was reprecipitated from benzene–petroleum ether to give **A** (196 mg, 11% based on **26**), **B** (85 mg), **C** (371 mg), and **D** (416 mg; still containing a trace of impurity).

A: $[\alpha]_D^{23}$ −70° (*c* 2, $CHCl_3$); IR (KBr): 1810, 1775, 1725, 1640, 1570 cm^{-1}; NMR (in $CDCl_3$): δ 1.29 (3H d, $CHC\underline{H}_3$), 1.6 (10H broad, cyclohexylidene), 2.07 and 2.20 (each 3H s, Ac), 2.83 (3H s, NCH_3).

Found: C, 59.29; H, 5.58; N, 7.85%. Calcd for $C_{61}H_{69}N_7O_{22}$: C, 59.29; H, 5.45; N, 7.68%.

B: $[\alpha]_D^{23}$ −70° (*c* 2, $CHCl_3$); IR: Almost identical with that of **A**; NMR (in $CDCl_3$): δ 1.24 (3H d), 1.45 (10H), 2.15 (6H s), 2.87 (3H s).

Found: C, 59.13; H, 5.51; N, 7.45%.

C: $[\alpha]_D^{22}$ −8° (*c* 2, $CHCl_3$); IR: Almost identical with that of **A**; NMR (in $CDCl_3$): δ 1.27 (3H d), 1.6 (10H), 2.10 and 2.21 (each 3H s), 2.85 (3H s).

Found: C, 59.21; H, 5.45; N, 7.42%.

D: $[\alpha]_D^{23}$ −10° (*c* 2, $CHCl_3$); IR: Almost identical with that of **A**; NMR (in $CDCl_3$): δ 1.30 (3H d), 1.58 (10H), 2.15 and 2.22 (each 3H s), 2.90 (3H s).

Found: C, 58.88; H, 5.49%.

*Deblocking of the Condensation Products. a) Deblocking of **A**.* To a solution of compound **A** (120 mg) in dioxane (20 ml), 0.025 M barium hydroxide (8 ml×4) was added at 1 hr intervals and the mixture was heated at 50 °C for 3 hr. Carbon dioxide was introduced and the precipitate was filtered off. The filtrate was evaporated and the residue was chromatographed on a column of silica gel with chloroform–ethanol–17% ammonia (20 : 10 : 1) to give a solid (**28**, 38 mg, 44%). In the NMR spectrum of **28**, peaks attributable to the carbobenzoxy and cyclohexylidene groups were observed, and peaks corresponding to acetyl and benzoyl groups disappeared. Additionally, in the IR spectrum of **28**, no trace of the carbonyl absorptions of acetyl, benzoyl, carbonate, and carbamate groups was observed.

Next, a solution of **28** (38 mg) in 50% acetic acid (2 ml) was heated at 60 °C overnight and evaporated. The residue was dissolved in a mixture of water and dioxane (2 : 1) and the solution was hydrogenated (50 psi) with palladium black (about 0.3 ml) at room temperature overnight. The mixture was filtered, and the filtrate was evaporated. The residue was chromatographed on 20 ml of Amberlite CG–50 (NH_4 form) with 5% ammonium carbonate to give a solid (18 mg). An aqueous solution of the solid was passed through a column of Dowex 1×2 (OH form) with water to give the free base of dihydrostreptomycin, which was transformed into its sesquisulfate by neutralizing with sulfuric acid, 14 mg (48%), $[\alpha]_D^{17}$ −85° (*c* 0.1, H_2O); natural specimen: $[\alpha]_D^{17}$ −85° (*c* 0.1, H_2O) [lit,[15] $[\alpha]_D^{25}$ −88.5° (*c* 1, H_2O); $\Delta[M]_{436(CuAm)}^{10}$ −1200°; natural specimen: $\Delta[M]_{436(GuAm)}^{17}$ −1170° [lit,[16] dihydrostreptomycin: $\Delta[M]_{436(CupraB)}$ −1180°].

Found: C, 33.50; H, 6.52; N, 12.37%. Calcd for $C_{21}H_{41}N_7O_{12}\cdot 3/2H_2SO_4\cdot 3/2H_2O$: C, 33.29; H, 6.25; N, 12.94%.

*b) Deblocking of **B**, **C**, and **D**.* Compounds **B**, **C**, and **D** were respectively treated in the same way as described above for **A** to give sesquisulfates **29**, **30**, and **31** respectively.

29: $[\alpha]_D^{17}$ −88° (*c* 0.1, H_2O); $\Delta[M]_{436(CuAm)}^{17}$ +320°.
30: $[\alpha]_D^{17}$ −35° (*c* 0.1, H_2O); $\Delta[M]_{436(CuAm)}^{17}$ +580°.
31: $[\alpha]_D^{17}$ −35° (*c* 0.1, H_2O); $\Delta[M]_{436(CuAm)}^{17}$ −3200°.

The authors are grateful to Professor Hamao Umezawa of The University of Tokyo and of the Institute of Microbial Chemistry for his important ideas and encouragement.

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