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SYNTHESIS OF 6-DEOXYDIHYDROSTREPTOMYCIN

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ABSTRACT

6-Deoxydihydrostreptomycin (**8**) has been prepared from dihydrostreptomycin by a sequence of reactions involving 2″-*N*-benzyloxycarbonylation, 3a′,6″-ditritylation, selective 6-*O*-tosylation, displacement of the tosyloxy group with chloride, and hydrogenolysis of the chloro, benzyloxycarbonyl, and trityl groups with sodium in liquid ammonia. Tritylation was effective in converting dihydrostreptomycin into derivatives readily soluble in common organic solvents, which permitted their purification by column chromatography. Confirmation of the structure of **8** was effected by hydrolysis, which gave methyl dihydrostreptobiosaminide and 6-deoxystreptidine (**9**). The structure of di-*N*-acetyl-6-deoxystreptamine derived from **9** was determined by a copper-complexing method.

INTRODUCTION

Streptomycin is inactivated by resistant bacteria and resistant *Pseudomonas*, which produce enzymes that adenylylate[1] or phosphorylate[2] the 3″-hydroxyl group, or adenylylate[3] or phosphorylate[4] the 6-hydroxyl group of streptomycin, to give the corresponding *O*-adenylyl or *O*-phosphono derivatives. In order to obtain a derivative active against resistant bacteria producing 3″-modifying enzymes, 3″-deoxydihydrostreptomycin** has been prepared[5,6]. This compound proved to have satisfactory activity against the resistant bacteria owing to the lack[7] of a 3″-hydroxyl group that could be esterified. This paper describes the synthesis of another deoxy derivative, 6-deoxydihydrostreptomycin, which was expected to show activity against resistant bacteria that produce 6-modifying enzymes.

RESULTS AND DISCUSSION

In order to prepare 6-deoxydihydrostreptomycin (**8**) from dihydrostreptomycin, we began with selective tosylation of the 6-hydroxyl group of 2″-*N*-benzyloxycarbo-

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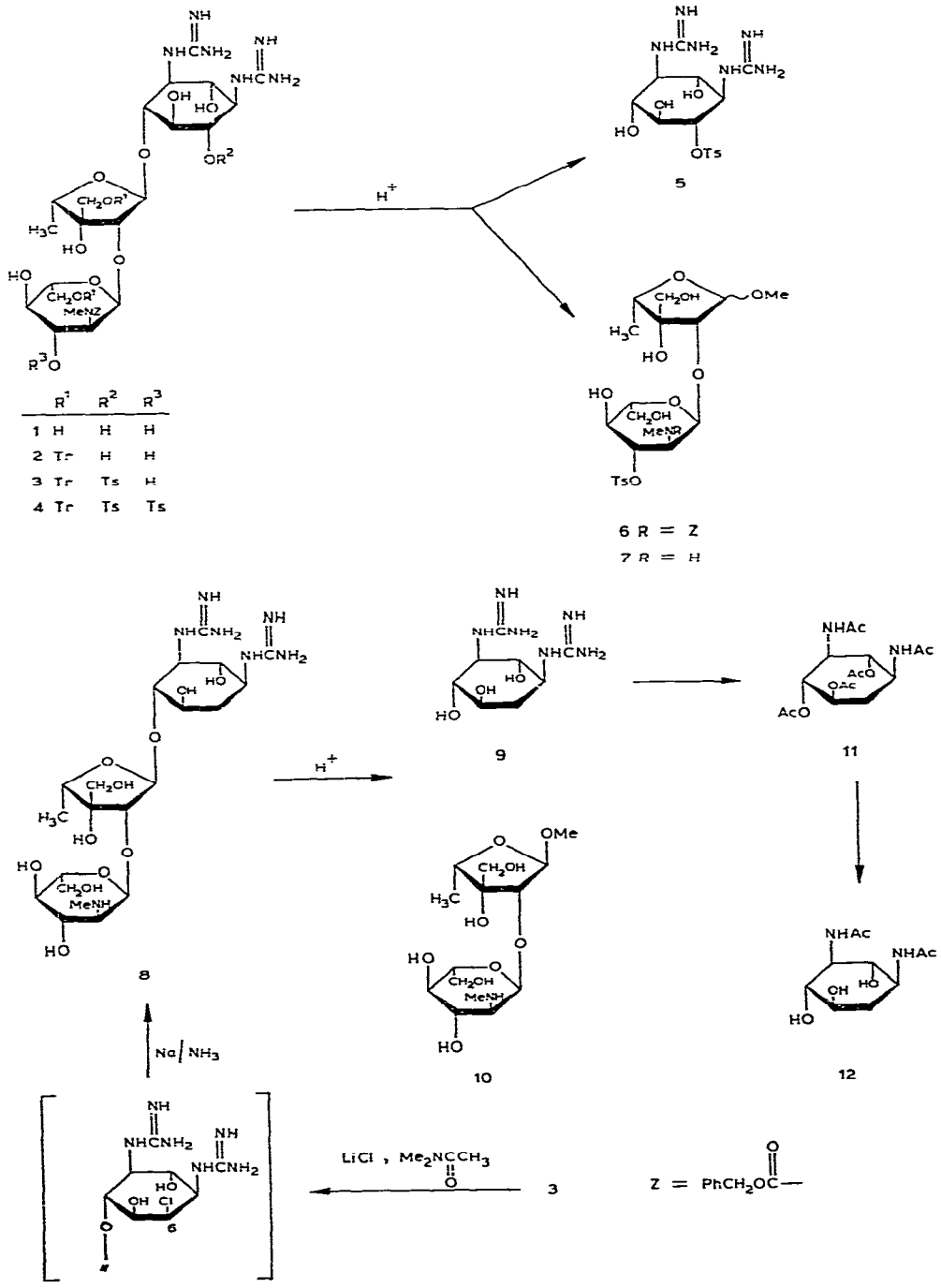
**As the name dihydrostreptomycin has been widely used, we have used this trivial name.



	R^1	R^2	R^3
1	H	H	H
2	Tr	H	H
3	Tr	Ts	H
4	Tr	Ts	Ts

nyldihydrostreptomycin[8] (**1**), expecting that the tosyl derivative could later be converted into the 6-deoxy derivative. Prior to tosylation, benzoylation of **1** was examined to protect selectively the reactive functional groups. However, several benzoyl derivatives were produced simultaneously without selectivity, and separation of the products was troublesome, mainly on account of low solubility of the resulting products in common organic solvents. To enhance both the solubility of products and selectivity in the reaction, compound **1** was tritylated. Tritylation was found to occur selectively at the 3a'- and 6″-hydroxyl groups to afford the di-trityl ether **2**. This compound, despite the presence of strongly hydrophilic guanidium groups, is highly soluble in common organic solvents, so that purification by column chromatography was feasible. It should be mentioned that 3',3a';4″,6″-di-*O*-benzylidene-2″-*N*-phenoxycarbonyl-dihydrostreptomycin[6], reported recently, is also soluble in common organic solvents, but not sufficiently to permit column chromatography. The positions at which tritylation occurred were suggested by the fact that **2** could not be benzylidenated by applying the procedure[6] which gave the 3',3a';4″,6″-di-*O*-benzylidene derivative[6] from the corresponding 2″-*N*-(phenoxycarbonyl)dihydrostreptomycin[6].

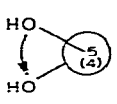
Tosylation of **2** in pyridine gave two main products, readily separable by column chromatography. The major one (46%) was the desired 6-*O*-tosyl derivative (**3**), and the minor one (19%), the 6,3″-di-*O*-tosyl derivative (**4**). It may be noted that the tosyl groups reacted mainly with the hydroxyl groups and not the guanidino groups, and that, among the six hydroxyl groups in **2**, that at C-6 was the most reactive for tosylation. Acidic methanolysis of **4** gave two products, which proved to be a streptidine derivative (**5**) and a dihydrostreptobiosaminide derivative (**6**), both of which retained a tosyl group. Catalytic hydrogenolysis of **6** gave methyl 3″-*O*-tosyldihydrostreptobiosaminide (**7**). The low $\Delta[M]_{TACu}$[9] value of **7** indicates the absence of copper complexing between the 2″-methylamino and 3″-hydroxyl groups, thus confirming that the tosyloxy group was present at C-3″ of **6** and **7**. Similar methanolysis followed by hydrogenolysis of **3** gave the mono-*O*-tosylstreptidine (**5**), identical to that from **4** and methyl dihydrostreptobiosaminide[10], indicating that the tosyl group of **3** is present in the streptidine portion.

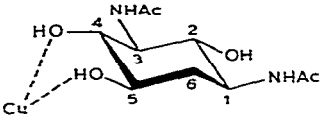
Displacement of the tosyloxy group of **3** with iodide was initially attempted by treatment with sodium iodide in *N*,*N*-dimethylformamide at several temperatures. By this procedure, however, a product having neither tosyl groups nor iodine resulted*, suggesting that the guanidino group at C-1 may have participated in the reaction. After several attempts, an iodo derivative was obtained (~30%) when **3** was treated (70 h, 50°) with sodium iodide in *N*,*N*-dimethylacetamide. It was further found that, when lithium chloride was used instead of sodium iodide in the same solvent (12 h, 75°), the corresponding chloro derivative was obtained in better yield. As the chloro derivative was rather labile and could not be purified, the crude product

*Results obtained by treatment of cyclohexyl 2-*O*-tosylguanidine with iodide ion will be reported elsewhere.

was used immediately in the next step. Treatment of the crude chloro derivative with sodium in liquid ammonia at −50° gave the desired 6-deoxydihydrostreptomycin (**8**, 48%), with simultaneous hydrogenolysis of the chloro, benzyloxycarbonyl, and trityl groups. In the ^{1}H-n.m.r. spectrum, H-6ax resonated as a quartet having large spacings (11–12 Hz), indicating that the substituents at C-1 and C-5 are both equatorial in the chairlike cyclohexane ring. Thus, it may be assumed that neighboring-group participation* by the guanidino group did not occur, at least in the main reaction-pathway (3 to the chloro derivative).

In order to confirm its structure, compound **8** was hydrolyzed, whereupon two products resulted. One of them was found to be methyl dihydrostreptobiosaminide[10] (**10**) and the other, 6-deoxystreptidine, that is, 1,3,4-trideoxy-1,3-diguanidino-*scyllo*-inositol (**9**). To confirm the structure of **9**, it was hydrolyzed under basic conditions and then acetylated to give 6-deoxystreptamine pentaacetate (**11**), identical with the compound reported by Suami *et al.*[11]. Treatment of **11** with ammonia in methanol gave di-*N*-acetyl-6-deoxystreptamine[11] (**12**). The $\Delta[M]_{CuAm}$[9] value (+1840°)** of **12** indicates that copper (Cu^+) complexing occurred between the equatorial hydroxyl groups at C-4 and -5, showing **12** to be a 6-deoxy derivative. The structure of **8** was thus completely established.





The antibacterial spectrum of **8** showed activity of 1/10–1/80 of that of dihydrostreptomycin. This suggests that, in dihydrostreptomycin, the hydroxyl group at C-6 or the equivalent is necessary for antibacterial activity.

EXPERIMENTAL

General. — Melting points were determined on a Kofler block and are uncorrected. Specific rotations were measured, in a 0.1-dm tube, with a Perkin–Elmer Model 241 polarimeter. ^{1}H-N.m.r. spectra were recorded at 90 MHz with a Varian EM-390 spectrometer. T.l.c. was performed on Wakogel B-5, unless otherwise stated, with sulfuric acid as the spray for detection. Silica gel (Wakogel C-200) and microcrystalline cellulose powder (Avicel SF, Funakoshi Co. Tokyo) were used, unless otherwise stated, for separation of the products by column chromatography.

2″-N-Benzyloxycarbonyldihydrostreptomycin[8] (**1**). — To an ice-cold mixture

*See footnote p. 71.

**A solution of 0.16M CuCl in 15M aqueous ammonia (CuAm) was used. If a copper(I) complex is formed between two adjacent equatorial hydroxyl groups in a chair cyclohexane ring, $\Delta[M]_{CuAm}^{436}$ = ($[\alpha]_{436}$ in CuAm − $[\alpha]_{436}$ in water) × mol. wt./100 is expected to be ~ ±2000° (the same value expected from that measured in Cupra B[12]), the sign being decided by the chirality of the dihedral angle (~ 60°) between the two hydroxyl groups.

of dihydrostreptomycin trihydrochloride (30 g) and anhydrous sodium carbonate (9 g) in 50% aqueous acetone (780 mL), benzyl chloroformate (20 mL) was added and the mixture was stirred for 15 h at room temperature. Filtration followed by neutralization of the filtrate with M aqueous hydrochloric acid and evaporation gave a residue. Extraction of the product from the residue with ethanol (3 × 100 mL) and evaporation of the solution gave a syrup, which was treated with ether to give a solid (34 g) of the dihydrochloride of **1**, contaminated slightly with salt. T.l.c. showed R_F 0.46 (Avicel, 6:4:3:1 butanol–pyridine–water–acetic acid).

2″-N-Benzyloxycarbonyl-3a′,6″-di-O-(triphenylmethyl)dihydrostreptomycin (**2**). — To a solution of the dihydrochloride of **1** (10.35 g) in dry pyridine (150 mL) was added chlorotriphenylmethane (26 g) and the mixture was heated for 15 h at 50°. After the addition of sodium hydrogencarbonate (9.2 g) and water (4 mL), the mixture was evaporated and the residue washed thoroughly with ether, and then extracted with chloroform. The chloroform-soluble products (20 g) were chromatographed on a column of silica gel with 6:3:2:0.8 benzene–pyridine–ethanol–25% acetic acid. The eluate containing the main fraction (R_F 0.37 in t.l.c. with the same solvent-mixture) was evaporated and the residue dissolved in chloroform. The solution was successively washed with saturated aqueous sodium hydrogencarbonate and saturated aqueous sodium chloride, dried over sodium sulfate, and evaporated. Addition of ether to the residue gave the dihydrochloride of **2** as the solid dihydrate; 11.63 g (68% based on dihydrostreptomycin · 3 HCl), $[\alpha]_D^{25}$ −71° (*c* 1, chloroform); ^{1}H-n.m.r. (CD_3OD): δ 1.0–1.2 (3 H, CCH_3), 2.95 and 3.07 (each s, 3 H in total, NCH_3), and 7.15–7.65 (35 H, phenyl).

Anal. Calc. for $C_{67}H_{75}N_7O_{14} \cdot 2\,HCl \cdot 2\,H_2O$: 61.37; H, 6.23; N, 7.48. Found: C, 61.28; H, 6.36; N, 7.47.

2″-N-Benzyloxycarbonyl-6-O-(p-tolylsulfonyl)-3a′,6″-di-O-(triphenylmethyl)dihydrostreptomycin (**3**) *and 2″-N-benzyloxycarbonyl-6,3″-di-O-p-(tolylsulfonyl)-3a′,6″-di-O-(triphenylmethyl)dihydrostreptomycin* (**4**). — To an ice-cold solution of the dihydrochloride of **2** (1.44 g, 1.13 mmol, dried *in vacuo* to constant weight) in dry pyridine (30 mL) was added *p*-toluenesulfonyl chloride (430 mg, 2.26 mmol) and the mixture was kept for 22 h at room temperature. *p*-Toluenesulfonyl chloride (430 mg) was added and the solution was kept for a further 30 h. After the addition of water (3 mL) followed by storage for 0.5 h, sodium hydrogencarbonate (1.5 g) and water (10 mL) were added and the mixture was evaporated *in vacuo*. The residue was extracted with chloroform and the organic solution (400 mL) washed with saturated aqueous ammonium chloride (30 mL × 2), saturated aqueous sodium chloride (30 mL × 3) and water, dried over sodium sulfate, and evaporated to a solid (1.8 g). T.l.c. with 4:5:3:1 pyridine–ethyl acetate–chloroform–25% acetic acid showed two major spots for the solid at R_F 0.26 (**3**) and 0.48 (**4**), accompanied by minor spots at 0.18 (**2**) 0.58 and 0.67. The mixture of products were chromatographed over silica gel (360 g) with the same solvent-mixture already described and the fractions containing **3** and **4** were individually evaporated. The residues were dissolved, separately, in chloroform (2 mL), and, after addition of sodium acetate saturated in 50%

aqueous methanol (1 mL), the mixture was stirred vigorously for a short period. Chloroform (50 mL) was added and the solution was washed with water, dried, and concentrated to low volume. Addition of ether gave a solid of the acetic acid salt.

The acetic acid salt of **3**: 786 mg (46%) had $[\alpha]_D^{25}$ −44° (*c* 1, methanol); ^{1}H-n.m.r. (CD_3OD): δ 0.9–1.15 (3 H, CCH_3), 1.93 (6 H s, $CH_3CO_2^-$), 2.40 (3 H s, TsMe), 3.00 and 3.07 (each s, 3 H, in total, NCH_3) and 7.0–8.0 (39 H aromatic).

Anal. Calc. for $C_{74}H_{81}N_7O_{16}S \cdot 2\ CH_3CO_2H \cdot 2\ H_2O$: C, 61.27; H, 6.43; N, 6.01; S, 2.45. Found: C, 61.20; H, 6.26; N, 6.41; S, 2.09.

The acetic acid salt of **4**; 358 mg (19%) had $[\alpha]_D^{25}$ −47° (*c* 1, methanol); ^{1}H-n.m.r. (CD_3OD): δ 1.20 (3 H br, CCH_3), 1.93 (6 H s, $CH_3CO_2^-$), 2.30 and 2.43 (each 3 H s, Ts), 2.83 and 2.97 (each s, 3 H, in total, NCH_3), and 7.0–8.0 (43 H, aromatic).

Anal. Calc. for $C_{81}H_{87}N_7O_{18}S_2 \cdot 2\ CH_3CO_2H \cdot 2\ H_2O$: C, 61.25; H, 5.99; N, 5.88; S, 3.85. Found: C, 61.46; H, 5.78; N, 5.69; S, 3.73.

6-Deoxydihydrostreptomycin (**8**). — A solution of the acetic acid salt **3** (1.04 g) in 50% aqueous methanol was passed through a column of Dowex 1 × 2 (Cl^- form, 20 mL) with the aid of the same solvent-mixture, and the biacetyl-positive fractions were evaporated to a solid, which was dried well. To a solution of the resulting hydrochloride of **3** (1.00 g) in dry *N*,*N*-dimethylacetamide (10 mL) was added anhydrous lithium chloride (3.0 g) and the mixture was stirred for 12 h at 75°. Chloroform (150 mL) was added and the organic solution washed with water (100 mL × 3), dried over sodium sulfate, and concentrated. To the concentrate (~10 mL), was added ether to give a precipitate of the crude 6-chloro derivative (885 mg), R_F 0.26 in t.l.c. with 4:5:3:1 pyridine–ethyl acetate–chloroform–25% aqueous acetic acid. The precipitate, dissolved in tetrahydrofuran (4 mL), was added to a solution of sodium metal (−500 mg) in liquid ammonia (−50°, ~40 mL) and the solution was gently stirred for 1 h at ~50°. Methanol was added until the blue color of the mixture disappeared. Evaporation of ammonia by raising the temperature gave a residue that was made neutral with M aqueous hydrochloric acid. After filtration, the filtrate solution was charged onto a column of Amberlite CG-50 (NH_4^+, 15 mL) and the column washed with water, and then with 2% aqueous ammonium carbonate (100 mL). Products were eluted with 2→10% aqueous ammonium carbonate with gradual increase in concentration. Biacetyl-positive fractions were combined and evaporated. T.l.c. (Avicel) with 6:4:3:1 1-butanol–pyridine–water–acetic acid, showed one major spot (R_F 0.14) accompanied by several minor spots. An aqueous solution of the residue was repeatedly evaporated with additions of water until ammonium carbonate-free solid was obtained. The solid was then chromatographed on a column of Avicel (~40 g) with the same solvent mixture described for t.l.c., and fractions containing **8** were evaporated. The residue was further subjected to column chromatography on Amberlite CG 50 with 2→10% ammonium carbonate as developer to give solid **8** as the carbonate; 232 mg (48%), $[\alpha]_D^{23}$ −86° (*c* 1, water); ^{1}H-n.m.r. [to a solution of the carbonate of **8** in D_2O was added DCl in D_2O until it became strongly acidic]: δ 1.29 (3 H d, *J* 7 Hz, CCH_3), 1.62 (1 H q, *J* 11–12 Hz, H-6ax), 2.34

(1 H dt, *J* 4 and 12 Hz, H-6eq), 2.96 (3 H s, NCH_3), 3.44 (1 H dd, *J* 3.5 and 10 Hz, H-2″), 5.40 (1 H d, *J* ~1 Hz, H-1′), and 5.63 (1 H d, *J* 3.5 Hz, H-1″).

Anal. Calc. for $C_{21}H_{41}N_7O_{11} \cdot 2\ H_2CO_3$: C, 39.94; H, 6.56; N, 14.18. Found: C, 39.70; H, 6.57; N, 14.08.

Acid hydrolysis of **8**. — A solution of **8** (84 mg as the carbonate) in 2M methanolic hydrochloric acid (2 mL) was boiled under reflux for 3 h. Concentration followed by repeated concentration with several additions of methanol–toluene gave a residue that was chromatographed on a column of Dowex 1 × 2 (OH^- form) eluted with water. Fractions containing a biacetyl-positive product (**9**), and methyl dihydrostreptobiosaminide[10] (**10**), were eluted in that order. The former fractions were concentrated and the concentrate was passed through a column of Dowex 1 × 2 (Cl^- form) with water to give a glassy solid of the dihydrochloride of **9**, 38 mg (98%), which was recrystallized from 90% aqueous ethanol, m.p. 272–274° (dec.), $[\alpha]_D^{17}$ −11° (*c* 1, water); ^{1}H-n.m.r. (D_2O): δ 1.58 (1 H q, *J* 11–12 Hz, H-6ax), and 2.27 (1 H dt, *J* 4 and 12 Hz, H-6eq).

Anal. Calc. for $C_8H_{18}N_6O_3 \cdot 2$ HCl: C, 30.10; H, 6.32; N, 26.33; Cl, 22.22. Found: C, 30.37; H, 6.25; N, 26.04; Cl, 22.65.

The later fractions were evaporated and the residue was charged onto a column of CM-Sephadex C-25 (after pretreatment with 0.1M aqueous ammonia followed by thorough washing with water). After washing the column with water, **10** (as the base) was eluted with 0→0.03M aqueous ammonia to give a glassy solid, 31.4 mg (72%), $[\alpha]_D^{18}$ −150° (*c* 1, water) [lit.[10] $[\alpha]_D^{25}$ −164° (*c* 1, water)]. The ^{1}H-n.m.r. spectrum (in D_2O) of **10** was identical with that[10] of methyl α-dihydrostreptobiosaminide[10] obtained by similar hydrolysis of dihydrostreptomycin · 3 HCl.

(*1S*)-*Penta*-N,O-*acetyl-1,3-diamino-1,3,4-trideoxy*-scyllo-*inositol* [*1,3-Di*-N-*acetyl-2,4,5-tri*-O-*acetyl-6-deoxystreptamine*] (**11**). — A mixture of the dihydrochloride of **9** (40 mg), $Ba(OH)_2 \cdot 8\ H_2O$ (0.6 g), and water (1.2 mL) was gently boiled under reflux for 43 h. Neutralization with M sulfuric acid followed by removal of the precipitate gave a solution which, after concentration, was charged on an Amberlite CG-50 (NH_4^+ form) column and the column was washed with 2% aqueous ammonium carbonate. Ninhydrin-positive fractions were evaporated and the residue was acetylated with acetic anhydride (1 mL) containing anhydrous sodium acetate (80 mg) for 20 h at 100°. Concentration of the mixture with several additions of xylene gave a residue, which was extracted with chloroform. The chloroform-soluble product was recrystallized from ethanol to give needles of **11**, 24 mg (52%), m.p. 315–317° (dec.) (lit.[11] 309–310°), $[\alpha]_D^{22}$ −5° (*c* 0.8, pyridine) (lit.[11] −13°, *c* 1, pyridine). In its ^{1}H-n.m.r. spectrum, the chemical shifts of the methyl protons of the acetyl groups were identical with those reported[11].

(*1S*)-*1,3-Di*(*acetamido*)-*1,3,4-trideoxy*-scyllo-*inositol* [*1,3-Di*-N-*acetyl-6-deoxystreptamine*] (**12**). — Compound **11** (21 mg) was treated with methanol saturated with ammonia. The product obtained was recrystallized from aqueous methanol (1:10)–acetone to give needles, 11 mg (78%), m.p. 291–292° (dec.) (lit.[11], 288–289°), $[\alpha]_D^{24}$ −4° (*c* 0.9, water) (lit.[11] − 5°, water); $\Delta[M]_{CuAm}{}^9$ +1840°.

Acid hydrolysis of **4**. — A solution of the acetic acid salt dihydrate of **4** (196 mg) in M methanolic hydrochloric acid (2 mL) was kept for 3 h at room temperature. Addition of anhydrous sodium carbonate (170 mg) followed by evaporation gave a solid, which was extracted with chloroform. The chloroform-soluble product was purified by chromatography on a column of silica gel with 9 : 1 : 1 chloroform–methanol–ethyl acetate to give a mixture of methyl 2″-*N*-benzyloxycarbonyl-3″-*O*-(*p*-tolylsulfonyl)-α- and -β-dihydrostreptobiosaminides (**6**), 64.8 mg (86%), $[\alpha]_D^{25}$ −75° (*c* 1, methanol); ^{1}H-n.m.r. (CD_3OD): δ 1.20 and 1.23 (~3:2, each d, 3 H total, *J* 6 Hz, CCH_3), 2.34 and 2.43 [~3:2 each s, 3 H total, Ts (CH_3)], 3.05 and 3.08 (each s, 3 H in total, NCH_3), 3.41 and 3.47 (each s, OCH_3; overlapped with solvent peaks), 5.09 (2 H s, CH_2Ph), and 5.0–5.2 (2 H, H-1′,1″).

Anal. Calc. for $C_{29}H_{39}NO_{13}S$: C, 54.28; H, 6.13; N, 2.18; S, 5.00. Found: C, 54.43; H, 6.07; N, 2.48; S, 5.00.

The chloroform-insoluble part from the foregoing experiment was extracted with *N*,*N*-dimethylformamide (0.5 mL × 10). After evaporation, the residue was chromatographed on a column of Avicel with 5 : 3 : 2 : 1 pyridine–water–ethyl acetate–acetic acid as eluant and the biacetyl-positive eluate was again chromatographed on a column of Dowex 1 × 2 (AcO^- form) with 0.5M aqueous acetic acid to give solid 6-*O*-(*p*-tolylsulfonyl)streptidine (**5**), which gave a single spot on the chromatogram. Its elemental analysis, however, was not satisfactory, possibly because of contamination by inorganic salts; ν_{max}^{KBr} 1190 and 1340 cm^{-1} (sulfonic ester); ^{1}H-n.m.r. (CD_3OD): δ 2.00 (6 H s, AcO^-), 2.49 (3 H s, TsMe), and 7.3–8.0 (four peaks, 4 H, Ts).

Methyl 3″-O-(p-*tolylsulfonyl*)*dihydrostreptobiosaminide* (**7**). — A solution of **6** (50 mg) in 50% aqueous 1,4-dioxane (1 mL) containing one drop of acetic acid was hydrogenated under 3.4 kg/cm^2 pressure in the presence of palladium black to give solid **7** as the acetic acid salt; yield 44 mg (100%), $[\alpha]_D$ −90° (*c* 0.5, water), $\Delta[M]_{TACu}^{436}$ −20°; ^{1}H-n.m.r. (CD_3OD): δ 1.24 (3 H d, *J* 6 Hz, CCH_3), 2.35–2.6 [four singlets, 6 H in total, NCH_3 and TsMe], and 2.93 (1 H dd, *J* 3 and 10 Hz, H-2″).

Anal. Calc. for $C_{21}H_{33}NO_{11}S \cdot CH_3CO_2H$: C, 48.67; H, 6.57; N, 2.47; S, 5.65. Found: C, 48.82; H, 6.67; N, 2.42; S, 5.76.

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