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Transformation of Dihydrostreptomycin into 3″-Deoxydihydrostreptomycin

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3″-Deoxydihydrostreptomycin active against resistant bacteria was prepared from dihydrostreptomycin (DHSM). The key intermediate in this synthesis is a protected derivative of DHSM in which all hydroxyl groups except the 3″-hydroxyl group are protected, namely, 2″-*N*-(benzyloxycarbonyl)-2,5,6-tris(*O*-tetrahydropyran-2-yl)-3′,3$_a$′:4″,6″-bis(*O*-*p*-methylbenzylidene)-1,3-di-N^G-tosyldihydrostreptomycin. 3″-Deoxygenation was successfully performed by 3″-*O*-imidazolylthiocarbonylation followed by treatment with tributylstannane. The $\Delta[M]_{TACu}$ value of the synthetic 3″-deoxydihydrostreptomycin supports the structure.

3″-Deoxydihydrostreptomycin (3″-DDHSM) (**14**) prepared by a total synthesis[1] exhibits remarkable activity against resistant as well as sensitive bacteria except *Pseudomonas aeruginosa*. The present paper describes another synthesis of the 3″-DDHSM by regiospecific deoxygenation of dihydrostreptomycin (DHSM).

Results and Discussion

The 2″-methylamino group of DHSM was protected by the phenoxycarbonyl group in order to obtain a 2″,3″-cyclic carbamate in a later step, since the phenoxycarbonyl derivative gives a better yield of the cyclic carbamate (**3**) than benzyloxycarbonyl derivative.[2] The *N*-phenoxycarbonyl DHSM (**1**) was isolated as its *p*-toluenesulfonic acid salt. The *p*-toluenesulfonic acid salts of **1** and other guanidino derivatives (**2**—**4**) showed much higher solubility in usual organic solvents than the corresponding hydrochlorides, acetates or carbonates.

Selective acetalation of **1** with *p*-tolualdehyde dimethyl acetal was achieved by a controlled procedure to give 3′,3$_a$′:4″,6″-bis(*O*-*p*-methylbenzylidene) derivative (**2**) in high yield. The purity of the *p*-methylbenzylidene derivatives (**2**—**10**) was easily estimated by the signals of the *p*-methylbenzylidene methyls in their NMR spectra.

The positions of the two cyclic acetals in **2** were presumed from the fact that the 3′,3$_a$′-positions of the dihydrostreptose portion of DHSM were acetalated in preference to the 4″,6″-positions[3] which are generally acetalated more readily than *trans*-diequatorial 5,6-positions. Attempts were also made to prepare 1-*N*, 2-*O*-(or 2-*O*, 3-*N*)-(*p*-methylbenzylidene)-5,6:3′,3$_a$′:4″,6″-tris(*O*-*p*-methylbenzylidene) derivative,[4] in which only the 3″-hydroxyl group is not protected. However, the compound could not be separated from another product suggested to be 5,6:3′,3$_a$′:4″,6″-tris(*O*-*p*-methylbenzylidene) derivative judging from its R_f value on TLC and NMR spectrum.

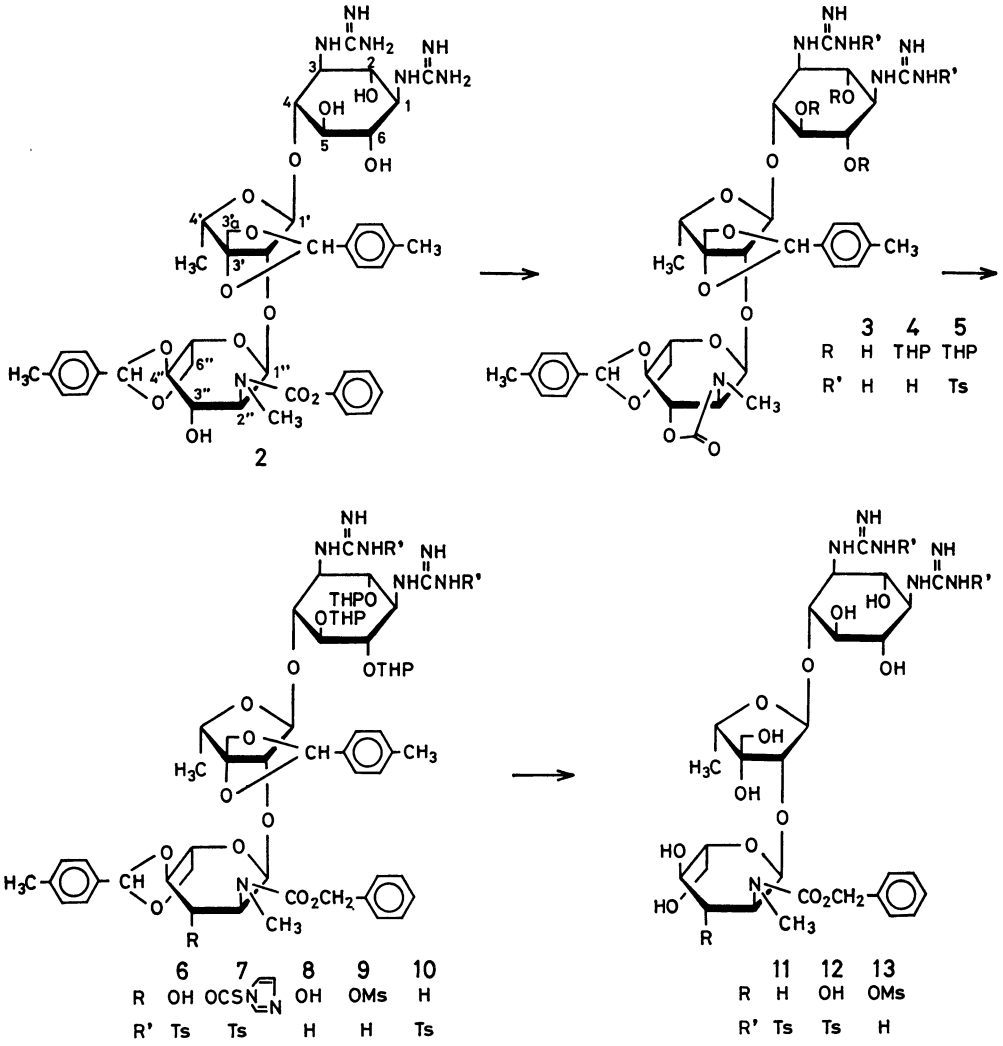
Treatment of the bis(*O*-*p*-methylbenzylidene) derivative (**2**) with potassium *t*-butoxide in *N*,*N*-dimethylformamide gave the cyclic carbamate (**3**). This reagent was found to give better yield than sodium hydride usually used.[2] In the NMR spectra of **2** and **3**, each peak attributable to 5′-methyl, 2″-*N*-methyl and one of the methyl groups of the *p*-methylbenzylidene groups appeared as two peaks in the ratio of approximately 6:4, indicating the mixtures of two diastereomers originating from the new-born chirality at the methine carbon of the 3′,3$_a$′-*O*-(*p*-methylbenzylidene) portion.

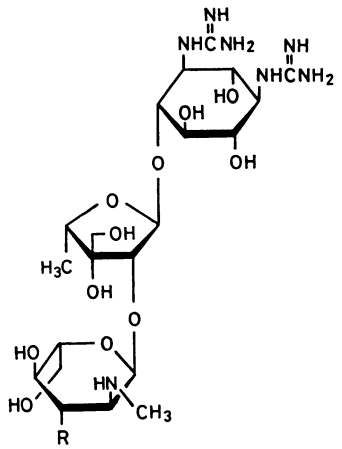
Tetrahydropyranyl protection of **3** by the conventional procedure gave a mixture of pertetrahydropyranyl derivatives. The mixture was negative for diacetyl-coloration test for guanidine and found to have more than five tetrahydropyranyl groups (mainly seven, judging from the NMR spectrum), indicating that the tetrahydropyranyl groups are introduced into the guanidine nitrogens as well as the hydroxyl groups. However, treatment of the mixture with ethanolic ammonia gave tris(tetrahydropyranyl) derivative (**4**) which was diacetyl-positive. This shows that the tetrahydropyranyl groups intoduced into guanidine are unusually sensitive to base.

Benzyloxycarbonylation of the guanidino groups of **4** was unsuccessful, yielding no definite product. However, tosylation was successful to give the di-N^G-tosyl derivative (**5**) in pure state and good yield. Use of excess sodium hydride and tosyl chloride resulted in ditosylation at each guanidino group, the yield of the tetra-N^G-tosyl derivative[5] being 50% at most. From a comparison of the IR spectra of **1**—**4** with those of **5**—**7**, we could determine the absorption peaks assignable to the *N*-tosylguanidine of DHSM derivatives to be 1540 and 1260 cm^{-1}.

In the derivative (**5**), the cyclic carbamate group is the only function sensitive to alkaline treatment. Cleavage of the cyclic carbamate (**5**) by alkaline hydrolysis followed by 2″-*N*-benzyloxycarbonylation gave the desired product (**6**) having a free function only at C-3″. The structure of **6** was confirmed by absence of the absorption peak at 1770 cm^{-1} (cyclic carbamate).[6]

Since deoxygenation at C-3″ of **6** through S_N2 process was presumed to be difficult[7] on account of the 1,3-diaxial interaction between the axial substituent at C-1″ and the approaching nucleophile at C-3″, several radical-type deoxygenation reactions were attempted. Among the preliminary experiments tested, 3″-*O*-dimethylsulfamoylation[7] followed by treatment of the product with sodium in liquid ammonia, and another method reported by Barton and McCombie[8] involving thiocarbonylation followed by reduction with tributylstannane gave 3″-DDHSM (**14**). The latter was found to be superior to the former in the ease of purification and yield. Treatment of **6** with 1,1′-thiocarbonyldiimidazole in boiling 1,2-dichloroethane gave the 3″-*O*-imidazolylthiocarbonyl derivative (**7**) in good yield. This procedure was found to be superior to other methods of thiocarbonylation without use of sodium





hydride.[8] Most of the other methods require sodium hydride which causes thiocarbonylation at the guanidino groups as well as at the 3″-hydroxyl.

Treatment of **7** with tributylstannane in boiling toluene gave a mixture of 3″-deoxy (**10**) and 3″-hydroxy (**6**) derivatives in a ≈5:1 ratio which was determined by the yield ratio of deprotected products, 3″-DDHSM (**14**) and DHSM. Since isomers **10** and **6** have the same mobility in column chromatography, they could not be separated. Treatment of a mixture of **10** and **6** with aqueous acetic acid to remove the acid-labile protecting groups gave a mixture of 2″-*N*-(benzyloxycarbonyl)-3″-deoxy-di-N^G-tosyl-DHSM (**11**), its 3″-hydroxy derivative (**12**) and a slight amount of 1,3-di-*N*-tosylstreptidine, which were separated by column chromatography. Formation of the last product showed that the glycoside linkage between streptidine and dihydrostreptose is notably unstable to acid.

Finally, **11** was treated with sodium in liquid ammonia to give 3″-DDHSM (**14**), the absence of the 3″-hydroxyl group being shown by its NMR spectrum. The 3″-deoxy structure was confirmed by its small value (−50°) of $\Delta[M]_{TACu}$; DHSM has a large value +1020.[9] The 3″-DDHSM (**14**) showed antibacterial activity against resistant bacteria producing 3″-adenylyltransferase and 3″-phosphotransferase, the antibacterial spectrum being the same as that of 3″-DDHSM prepared by a total synthesis.[1]

It may be worthwhile to describe another attempted route from **4** involving intermediates having unprotected guanidino groups. The cyclic carbamate (**4**) was treated with benzyl alcohol in the presence of sodium hydride to give 2″-*N*-benzyloxycarbonyl-3″-hydroxy derivative (**8**) which was mesylated to give

9. Removal of the *p*-methylbenzylidene and tetrahydropyranyl groups from **9** gave the 3″-*O*-mesyl derivative (**13**) having free guanidino groups, the yield not being high. The absence of the mesyl group on the guanidine portion of **13** was shown by the result of the acidic hydrolysis (2 M HCl in 80% methanol 55 °C, 2 h) of **13**; streptidine was produced quantitatively, indicating that the mesyl group was introduced to C-3″. However, when the 3″-hydroxy compound (**8**) was treated with dimethylsulfamoyl chloride in the presence of sodium hydride by the procedure as described above[7] or with 1,1′-thiocarbonyldiimidazole[8,10] in boiling tetrahydrofuran, no definite product was obtained.

Experimental

^{1}H-NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer except for **14**, the spectra of which being recorded at 100 MHz with a Varian XL-100A Spectrometer. Thin-layer chromatography (TLC) was performed on Kieselgel H (Type 60) using a sulfuric acid spray for detection. Silica gel (Kieselgel 60, 230—400 mesh, E Merck Darmstadt, W. Germany) was used for separation of products by column chromatography.

2″-N-(Phenoxycarbonyl)dihydrostreptomycin (1). Anhydrous sodium carbonate (225 mg) and phenyl chloroformate (0.26 ml) were added to an ice-cold solution of DHSM trihydrochloride hydrate (990 mg) in aqueous acetone (2:1, 30 ml), and the mixture was vigorously stirred for 5 min. Ether (20 ml) was added and, after stirring, the aqueous solution was separated and washed with ether twice more. The aqueous solution was neutralized (pH 7) with hydrochloric acid and concentrated to 1 ml. Addition of ethanol (10 ml) gave a precipitate, which was removed by filtration. Concentration of the organic solution gave a solid (Solid A, 1.18 g). The solid, after being well dried, was dissolved in dry DMF (10 ml), filtered, and the solution was concentrated to give a solid. An aqueous ethanol (1:1) solution of the solid was passed through a Dowex 1×2 column (TsO^- form, 200 ml, 200—400 mesh, pretreated with aqueous ethanol (1:1)) with the same solvent mixture. Concentration of the diacetyl-positive fractions gave a solid[11] of bis(*p*-toluenesulfonate) of **1**, 1.31 g (88%), $[\alpha]_D^{25}$ −48° (*c* 1, methanol); IR(KBr): 1670 cm^{-1}.

^{1}H-NMR (D_2O at 25 °C): δ 1.22 (3H d, J=6.5 Hz, CCH_3), 2.37 (6H s, CH_3 of TsOH), 3.10 and 3.21 (each ≈1.5 H s, NCH_3); at 80 °C: δ 1.32 (3H d), 2.47 (6H s), 3.25 (3H s, NCH_3).

Found: C, 47.36; H, 5.98; N, 8.64; S, 6.47%. Calcd for $C_{28}H_{45}N_7O_{14}\cdot 2C_7H_8O_3S\cdot H_2O$: C, 47.32; H, 5.96; N, 9.20; S, 6.01%.

2″-N-(Phenoxycarbonyl)-3′,3′$_a$:4″,6″-bis(O-p-methylbenzylidene)dihydrostreptomycin (2). Anhydrous *p*-toluenesulfonic acid (95 mg) and *p*-tolualdehyde dimethyl acetal (1.85 ml, ≈10 mol eq for **1**) were added to a solution of well dried crude **1** (Solid A, 874 mg) in dry DMF (9 ml, dried over molecular sieves 4A), and the solution was kept at room temperature for 1 h. On TLC with benzene–pyridine–ethanol–water–acetic acid (6:3:3:1:0.5) the solution showed a strong spot at R_f 0.47 with a slight spot at R_f 0.2 (mono(*p*-methylbenzylidene) products) (*cf* **1**, R_f 0.1). Triethylamine (0.08 ml) was added and the solution was concentrated *in vacuo* to give a syrup. The chloroform solution of the syrup was washed with saturated aqueous sodium hydrogencarbonate (10 ml) and saturated aqueous sodium chloride and dried over sodium sulfate. Concentration gave a residue, which was dissolved in acetone (10 ml). After filtration, the acetone solution was treated with ether and the precipitate was thoroughly washed with ether. The solid (980 mg) was passed through a Dowex 1×2 column (TsO^- form, 50 ml) with aqueous ethanol (1:3) as a developer. The fractions containing **2** were collected and concentrated to give a solid, which was extracted with chloroform (*cf.* hydrochloride of **2** did not dissolve in chloroform). The chloroform solution was washed with water which dissolved the mono(*p*-methylbenzylidene) products. After being dried over sodium sulfate, the chloroform solution was concentrated to give a solid. The solid, after being dried well *in vacuo*, was suspended in hot water (0.5 ml) and stirred vigorously. After cooling, the suspension was filtered and the solid was again treated similarly and dried to give **2** as the bis(*p*-toluenesulfonate). Slight occlusion of chloroform was removed by this procedure. Yield, 980 mg (75%, based on DHSM·3HCl·H_2O), $[\alpha]_D^{25}$ −52° (*c* 1, chloroform); IR(KBr): 1670 cm^{-1}.

^{1}H-NMR (CD_3OD): δ 1.28 and 1.40 (≈6:4 in strength, 3H in total, each d, J=6.5 Hz, CCH_3), 2.24 and 2.31 (each s, 4:6 in strength, 3H in total, *p-methyl*benzylidene), 2.39 (9H s, CH_3 of Ts and *p-methyl*benzylidene). The peak of NCH_3 could not be discerned by overlapping with other signals.

Found: C, 54.85; H, 6.22; N, 7.76; S, 5.15%. Calcd for $C_{44}H_{57}N_7O_{14}\cdot 2C_7H_8O_3S\cdot H_2O$: C, 54.84; H, 5.95; N, 7.72; S, 5.05%.

In pyridine-d_5–D_2O (5:1 at 70 °C): δ 1.43 and 1.54 (≈6:4 in strength, CCH_3); 2.20 (≈7.2H s, CH_3 of Ts and a part of *p-methyl*benzylidene), 2.32 (≈4.8H s, *p-methyl*benzylidene); 3.43 and 3.58 (each s, in the ratio of 4:6, 3H in total, NCH_3).

An aqueous ethanol (1:3) solution of the above toluenesulfonic acid salt of **2** (190 mg) was passed through a Dowex 1×2 column (Cl^- form, 5 ml) with the same aqueous ethanol to give dihydrochloride of **2**, 143 mg (96%), R_f 0.37 on TLC with benzene–ethanol–pyridine–water–acetic acid 6:3:3:1:0.5 (*cf.* bis(*p*-toluenesulfonate) of **2**: 0.47) $[\alpha]_D^{25}$ −55° (*c* 1, methanol).

^{1}H-NMR (pyridine-d_5–D_2O 5:1 at 70 °C): δ 1.41 and 1.50 (≈6:4, CCH_3); 2.18 (≈1.2H s) and 2.28 (≈4.8H s) (*p-methyl*benzylidene); 3.43 and 3.60 (each s, 4:6 in strength, 3H in total, NCH_3).

Found: C, 52.43; H, 5.96; N, 9.33 Cl, 7.77%. Calcd for $C_{44}H_{57}N_7O_{14}\cdot 2HCl\cdot H_2O$: C, 52.90; H, 6.15; N, 9.82; Cl, 7.10%.

2″-N:3″-O-Carbonyl-3′,3′$_a$:4″,6″-bis(O-p-methylbenzylidene)-dihydrostreptomycin (3). Fresh potassium *t*-butoxide (110 mg, ≈3.3 mol eq for **2**) was added under nitrogen atmosphere to an ice-cold solution of bis(*p*-toluenesulfonate) of **2**, (380 mg) in dry DMF (3.8 ml), and the solution was kept in the cold for 20 min. *p*-Toluenesulfonic acid monohydrate (190 mg) in ethanol (1 ml) was added and the solution was concentrated *in vacuo*. The chloroform (20 ml) solution of the residue was washed successively with saturated aqueous sodium hydrogencarbonate, water, 40% aqueous sodium *p*-toluenesulfonate and water, dried over sodium sulfate and concentrated. Ether was added to the chloroform (3 ml) solution of the residue, and the resulting precipitate was washed thoroughly with ether to give a solid of bis(*p*-toluenesulfonate), which was further washed with water thoroughly as described for **2** and dried. Yield, 311 mg (88%), $[\alpha]_D^{25}$ −63° (*c* 1, chloroform); IR(KBr): 1770 (cyclic carbamate), 1680 cm^{-1}.

^{1}H-NMR (pyridine-d_5–D_2O 5:1 at 70 °C): δ 1.3—1.6 (two kinds of d, 3H in total, J=6.5 Hz, CCH_3); 2.22 (6H

s, CH_3 of Ts), 2.27 and 2.30 (6H in total, *p-methyl*benzylidene); 2.78 and 3.11 (each s, 4:6 in strength, 3H in total, NCH_3).

Found: C, 52.83; H, 5.88; N, 8.02; S, 5.34%. Calcd for $C_{38}H_{51}N_7O_{13}\cdot 2C_7H_8O_3S\cdot H_2O$: C, 53.10; H, 5.91; N, 8.34; S, 5.45%.

Dihydrochloride of **3** was prepared by passing the aqueous methanol (1:9) solution of bis(*p*-toluenesulfonate) (181 mg) of **3** with a column of Dowex 1×2 (Cl^- form, 5 ml, 200—400 mesh). Yield, 136 mg (97%).

^{1}H-NMR (pyridine-d_5–D_2O 5:1): δ 1.47 (3H, CCH_3); 2.28 and 2.30 (6H, each s, *p-methyl*benzylidene); 2.90 and 3.20 (each s, 4:6 in strength, 3H in total, NCH_3).

Found: C, 50.41; S, 5.96; N, 10.58; Cl, 7.95%. Calcd for $C_{38}H_{51}N_7O_{13}\cdot 2HCl\cdot H_2O$: C, 50.44; H, 6.13; N, 10.84; Cl, 7.84%.

*2″-*N*:3″-*O*-Carbonyl-2,5,6-tris(*O*-tetrahydropyran-2-yl)-3′,3′*$_a$*:4″,6″-bis(*O*-p-methylbenzylidene)dihydrostreptomycin (**4**).* Fuse-dried anhydrons *p*-toluenesulfonic acid (12 mg) and freshly distilled 3,4-dihydro-2*H*-pyrane (0.8 ml) were added to a solution of bis(*p*-toluenesulfonate) of **3** (235 mg, dried at 60° *in vacuo*) in 1,2-dichloroethane (4 ml, after distillation the solvent was dried over molecular sieves 4A activated at 350 °C under a stream of nitrogen). The solution was kept at room temperature for 10 min. Triethylamine (0.02 ml) was added and the solution was poured into saturated aqueous sodium hydrogencarbonate (3 ml) with vigorous stirring. The lower layer separated was washed with 40% aqueous sodium *p*-toluenesulfonate and water, dried over sodium sulfate and concentrated. The syrup was triturated with cyclohexane to give a solid, which was thoroughly washed with cyclohexane. On TLC with chloroform–methanol–pyridine (5:1:0.2), the solid showed one spot (R_f 0.68) which was negative for diacetyl-coloration. The solid was dissolved in 1.5 M ethanolic ammonia (8 ml), heated at 70 °C for 1.5 h and concentrated. On TLC with the same solvent mixture as described above, the residue showed a diacetyl-positive spot at R_f 0.27. The chloroform solution of the residue was washed with 40% aqueous sodium *p*-toluenesulfonate, water, dried over sodium sulfate and concentrated. The residue was successively treated with chloroform, ether and water as described for **3** to give a solid of bis(*p*-toluenesulfonate), 240 mg (84%), $[\alpha]_D^{25}$ −42° (*c* 1, chloroform); IR(KBr): 1770, 1670 cm^{-1}.

^{1}H-NMR (CD_3OD): δ 1.1—2.0 (21H, CCH_3 and H-3,4,5 of THP).

Found: C, 56.38; H, 6.38; N, 6.88; S, 4.29%. Calcd for $C_{53}H_{75}N_7O_{16}\cdot 2C_7H_8O_3S\cdot H_2O$: C, 56.33; H, 6.56; N, 6.86; S, 4.49%.

*2″-*N*:3″-*O*-Carbonyl-2,5,6-tris(*O*-tetrahydropyran-2-yl)-3′,3′*$_a$*:4″,6″-bis(*O*-p-methylbenzylidene)-1,3-di-*N^G*-tosyldihydrostreptomycin (**5**).* Dry toluene (1 ml) was added to a solution of bis(*p*-toluenesulfonate) (144 mg) of **4** in dry DMF (4.5 ml), and the mixture was concentrated *in vacuo* to a volume of ≈4 ml. After ice-cooling, 50% oily sodium hydride (net 12 mg) was added and the mixture was stirred under an atmosphere of nitrogen for 1 h in the cold. *p*-Toluenesulfonyl chloride (42 mg) was added, stirring being continued for 1 h. The mixture was poured into phosphate buffer (pH 7, 10 ml) and the whole mixture was extracted with chloroform. The organic solution was washed with saturated aqueous sodium hydrogencarbonate and water, dried over sodium sulfate and concentrated to give a solid. The solid was chromatographed on silica gel with chloroform–ethanol–pyridine (15:1:0.3). The fractions containing **5** were concentrated to give a solid, which was thoroughly washed with water to give diacetyl-negative product, 99 mg (71%), $[\alpha]_D^{25}$ −46° (*c* 0.5, chloroform); IR(KBr) (the figures in parenthesis are for **4**): 1770(1770), (1670), 1620(1630), 1540(1440), 1380(1380), 1260, (1180), 1130(1120), 1070 (1070), 1030(1030), 810(810) cm^{-1}.

Found: C, 57.50; H, 6.39; N, 6.88; S, 4.49%. Calcd for $C_{67}H_{87}N_7O_{20}S_2\cdot H_2O$: C, 57.78; H, 6.44; N, 7.04; S, 4.60%.

*2″-*N*-(Benzyloxycarbonyl)-2,5,6-tris(*O*-tetrahydropyran-2-yl)-3′,3′*$_a$*:4″,6″-bis(*O*-p-methylbenzylidene)-1,3-di-*N^G*-tosyldihydrostreptomycin (**6**).* A solution of **5** (278 mg) in a mixture of dioxane (18 ml) and 0.2 M aqueous sodium hydroxide (10 ml) was heated at 80 °C for 5 h. After being cooled to room temperature, benzyloxycarbonyl chloride (0.17 ml) was added and the mixture was stirred vigorously for 5 min. After 1 M aqueous ammonium hydroxide (2 ml) had been added (to decompose the reagent chloride) with subsequent stirring for 3 h, the mixture was concentrated and the residue was extracted with chloroform. The chloroform-soluble portion was chromatographed on silica gel (8.5 g) at first with chloroform–pyridine (50:1, 40 ml), then with chloroform–ethanol–pyridine (15:1:0.3) to give a solid, which was thoroughly washed with water and dried, 270 mg(90 %), $[\alpha]_D^{25}$ −50° (*c* 1, chloroform); IR(KBr): 1690, 1625, 1540, 1440, 1380, 1270, 1130, 1075, 1020, 810 cm^{-1}.

Found: C, 59.25; H, 6.47; N, 6.29; S, 4.12%. Calcd for $C_{74}H_{95}N_7O_{21}S_2\cdot H_2O$: C, 59.22; H, 6.51; N, 6.53; S, 4.27%.

*2″-*N*-(Benzyloxycarbonyl)-3″-*O*-(imidazolylthiocarbonyl)-2,5,6-tris(*O*-tetrahydropyran-2-yl)-3′,3′*$_a$*:4″,6″-bis(*O*-p-methylbenzylidene)-1,3-di-*N^G*-tosyldihydrostreptomycin (**7**).* To a solution of **6** (300 mg) in 1,2-dichloroethane (10 ml) were added 1,1′-thiocarbonyldiimidazole (270 mg) and imidazole (30 mg) and the solution was gently refluxed for 6 h under nitrogen atmosphere. Concentration by addition of dioxane gave a residue, which was suspended in water, agitated for 3 h (yellow color disappeared), and then filtered. The solid collected was dried and chromatographed on silica gel (6 g) with chloroform–ethanol–pyridine (15:1:0.3) to give a solid, which was dissolved in benzene. The solution was washed with water, dried over sodium sulfate, and after concentration to a small volume, cyclohexane was added to cause precipitation, 273 mg (86%), $[\alpha]_D^{25}$ −43° (*c* 1, chloroform); on TLC with chloroform–ethanol (15:1) it gave the same R_f value (0.5) with that of **6**; IR(KBr): 1700, 1625, 1540, 1440, 1390, 1320, 1280, 1225, 1130, 1080, 1030, 990, 810 cm^{-1}.

^{1}H-NMR (acetone-d_6–D_2O 8:1): δ 8.4—8.5 (1H, H-2 of imidazolyl group).

Found: C, 58.53; H, 6.06; N, 7.74; S, 5.80%. Calcd for $C_{78}H_{97}N_9O_{21}S_3$: C, 58.81; H, 6.14; N, 7.91; S, 6.04%.

*2″-*N*-(Benzyloxycarbonyl)-2,5,6-tris(*O*-tetrahydropyran-2-yl)-3′,3′*$_a$*:4″,6″-bis(*O*-p-methylbenzylidene)dihydrostreptomycin (**8**).* Benzyl alcohol (0.1 ml) and 50% oily sodium hydride (net 55 mg) were added to a solution of bis(*p*-toluenesulfonate) (285 mg) of **4** in dry dioxane (3 ml), and the mixture was stirred under nitrogen atmosphere at room temperature for 30 min. The solution was poured into cold water (4 ml) containing *p*-toluenesulfonic acid monohydrate (450 mg) and the mixture was extracted with chloroform. The chloroform solution was washed with aqueous 40% sodium *p*-toluenesulfonate solution (5 ml) and then with water, dried over sodium sulfate and concentrated. The residue was chromatographed on a silica gel (6 g) column firstly with chloroform (15 ml) and then with chloroform–ethanol (3:1). The diacetyl-positive fractions were concentrated and the chloroform solution of the residue was washed with aqueous 40% sodium *p*-toluenesulfonate, then with water, dried over sodium sulfate and concentrated. Addition of cyclohexane

to the concentrate gave a solid, which was thoroughly washed with water and dried well to give bis(*p*-toluenesulfonate) of **8**, 251 mg (82%), $[\alpha]_D^{25}$ −31° (*c* 1, chloroform).

Found: C, 57.84; H, 6.51; N, 6.19; S, 3.91%. Calcd for $C_{60}H_{83}N_7O_{17}\cdot 2C_7H_8O_3S\cdot H_2O$: C, 57.83; H, 6.62; N, 6.38; S, 4.17%.

*2″-N-(Benzyloxycarbonyl)-3″-O-mesyldihydrostreptomycin (**13**).* Methanesulfonyl chloride (0.072 ml, 8 mol equiv for **8**) was added to an ice-cold solution of bis(*p*-toluenesulfonate) (180 mg) of **8** in dry pyridine (1.8 ml), and the solution was kept at the temperature for 1 h, and then at room temperature for 1 h. After addition of water (0.03 ml) followed by standing for 1 h at room temperature, the solution was concentrated. Addition of water to the concentrate gave a thick syrup which was collected by centrifugation. Chloroform solution of the syrup was washed successively with water, saturated aqueous sodium hydrogencarbonate, 10% aqueous potassium hydrogensulfate, water, 40% aqueous sodium *p*-toluenesulfonate and water, centrifuged to remove accompanying water, and concentrated to give bis(*p*-toluenesulfonate) of 3″-*O*-mesylate (**9**), 197 mg. The solid (195 mg) was dissolved in 90% aqueous acetic acid (6 ml) heated at 55 °C for 3 h and the solution was concentrated with occasional addition of toluene to give a syrup. Addition of ether gave a crude solid of **13**, 135 mg, which showed, on TLC (benzene–pyridine–ethanol–water–acetic acid 6:3:3:1:0.5), spots at 0.4, 0.37, 0.18 (slight), 0.1 and 0.05 (very slight, 2″-*N*-(benzyloxycarbonyl)dihydrostreptomycin[3]). Since the appearance of multiple spots on TLC was suspected to be caused by the formation of salts of **13** with anions (possibly by *p*-toluenesulfonate and acetate anions), the crude **13** was transformed into acetic acid salt. The crude solid (134 mg) was passed through a column of Dowex 1×2 (AcO^- form, 4 ml, 200—400 mesh) with aqueous methanol (3:1). Concentration of the eluate gave a solid (104 mg) which on being subjected to TLC, showed spots at R_f 0.22 (slight), 0.18(minor), 0.1(major) and 0.05(very slight), the spots at R_f 0.4 and 0.37 (possibly caused by the *p*-toluenesulfonic acid salts) disappearing. The solid (103 mg) was then chromatographed (6 g silica gel) with chloroform–methanol–pyridine–25% aqueous acetic acid (6:3:4:1) and the fractions (38—53 ml) containing the major component (R_f 0.1) were collected and concentrated. The resulting residue was thoroughly washed with ether to give a solid, 48 mg, which was chromatographically pure and contained no *p*-toluenesulfonic acid as proved by the NMR spectrum. The solid (47 mg) was passed through a column of Dowex 1×2 (Cl^- form, 9 ml) with water. Concentration of the eluate (4—5.5 ml) gave the solid of dihydrochloride of **13**, 39 mg (38% based on **8**), $[\alpha]_D^{25}$ −69° (*c* 0.6, water). The solid showed the same R_f value (0.1) with that of acetic acid salt described above.

^{1}H-NMR (D_2O): δ 1.24 (3H d, J=6.5 Hz, CCH_3) [2″-*N*-(benzyloxycarbonyl)dihydrostreptomycin dihydrochloride (D_2O): 1.22], 3.10 (3H s, NCH_3) [3.06], 3.20 (3H s, SO_2CH_3), 5.38 (1H d, J=3 Hz, H-1″) [5.26]; on heating to 60 °C, all the above peaks of **13** became sharpened.

Found: C, 40.73; H, 5.91; N, 10.81%. Calcd for $C_{30}H_{49}N_7O_{16}S\cdot 2HCl\cdot H_2O$: C, 40.63; H, 6.02; N, 11.06%.

*2″-N-(Benzyloxycarbonyl)-3″-deoxy-1,3-di-N^G-tosyldihydrostreptomycin (**11**).* Tributylstannane (0.4 ml) was added to a solution of **7** (280 mg) in dry toluene (7 ml), and the solution was gently refluxed under nitrogen atmosphere for 2 h. Concentration gave a syrup which was thoroughly washed with cyclohexane. The resulting solid was dissolved in benzene, washed with water, dried over sodium sulfate and concentrated to give a solid, which gave, on TLC with chloroform–ethanol (15:1), the same R_f value (=0.5) as that of **6** or **7**. In the NMR spectrum no peak near δ 8.4—8.5 (see the description of **7**) was observed. The solid was chromatographed on silica gel (5.5 g) first with chloroform–pyridine (50:1, 20 ml) (to remove an impurity) and then with chloroform–ethanol–pyridine (15:1:0.3) to give crude **10**, 210 mg.

A solution of the crude **10** (180 mg) in aqueous acetic acid (1:9, 18 ml) was heated at 55 °C for 4 h, and then concentrated with addition of toluene to give a residue. The residue, on TLC with chloroform–methanol (3:1), gave spots at R_f 0.54(minor), 0.46(slight), 0.34(major, **11**), 0.16 (minor **12**) and 0.11 (minor, 1,3-di-*N*-tosylstreptidine). The mixture was chromatographed on silica gel (15 g) with chloroform–methanol (3:1) to give **11**, **12**, and 1,3-di-*N*-tosylstreptidine in amounts of 65, 13, and 10 mg, respectively. Ether was added to **11** dissolved in methanol to cause precipitation, 62 mg (40% based on **7**), $[\alpha]_D^{25}$ −35° (*c* 0.6, methanol); IR(KBr): 1680, 1625, 1580, 1540, 1440, 1400, 1260, 1130, 1075, 1030, 1010, 830, 810 cm^{-1}.

^{1}H-NMR (CD_3OD at 50 °C): δ 1.23 (3H d, J=6.5 Hz, CCH_3), 2.40 (6H s, CH_3 of Ts), 3.01 (3H s, NCH_3), 4.90 (1H d, J=3.5 Hz, H-1″), 5.17 (2H s, CO_2CH_2Ph), 5.23 (1H d, J=2 Hz, H-1′).

Found: C, 50.43; H, 5.88; N, 9.22; S, 6.31%. Calcd for $C_{43}H_{59}N_7O_{17}S_2\cdot H_2O$: C, 50.23; H, 5.98; N, 9.53; S, 6.24%.

*3″-Deoxydihydrostreptomycin (**14**).* A solution of **11** (28 mg) in methanol was passed through a column (0.5 ml) of Sephadex LH-20 with the aid of methanol and the eluate was concentrated to give a solid, which was thoroughly dried *in vacuo* at 50 °C. Ammonia (≈1.5 ml as the liquid) was introduced to a solution of the solid in THF (0.5 ml, freshly distilled over LAH) cooled at −50 °C, sodium metal (≈30 mg) being added to the solution. The resulting deep-blue solution was kept at the same temperature for 1 h. Methanol was added until the color disappeared and the solution was gradually warmed with gradual decrease in pressure. The methanol solution of the residue was filtered and the filtrate was concentrated. The strongly alkaline aqueous solution of the resulting syrup was charged on a column of Amberlite CG50 (NH_4^+ form 2.5 ml) and the column was washed with water (25 ml). The column was then washed with 0.5% aqueous ammonium carbonate until the eluate gave no flame reaction for sodium. The eluting solution was then replaced by 6% aqueous ammonium carbonate, when a diacetyl-positive product was eluted. The eluate was concentrated *in vacuo* with addition of water and dried to give a constant weight (≈11 mg). An aqueous solution of the solid almost free from ammonium carbonate was passed through a Dowex 1×2 column (OH^- form, 1 ml) with water, and the strongly alkaline, diacetyl-positive fractions were concentrated, after bubbling carbon dioxide for a while, to give a solid, 10 mg (53%), $[\alpha]_D^{25}$ −67° (*c* 1, water) (lit,[1] −85° as carbonate); $\Delta[M]_{TACu}$ −50° (*cf.* DHSM +970°, lit,[9] +1020°).

^{1}H-NMR (20% ND_3 in D_2O): δ 1.20 (3H d, J=6.5 Hz, CCH_3), 1.55 (1H q, J ≈12 Hz, H_{ax}-3″), 2.15 (1H double t, J ≈4.5, ≈4.5, 12 Hz, H_{eq}-3″), 2.31 (3H s, NCH_3), 2.71 (1H incomplete double t, J ≈4, ≈4, 12 Hz, H-2″), 5.05 (1H d, J=3.5 Hz, H-1″), 5.31 (1H d, J=1.5 Hz, H-1′).

Irradiation of H-1″ caused the signals of H-2″ to become double doublets (J ≈4.5 and 12 Hz). Irradiation of H_{ax}-3″ caused the signals of H-2″ and H_{eq}-3″ to become a narrow triplet, respectively.

Found: C, 40.15; H, 6.66; N, 13.87%. Calcd for $C_{21}H_{41}N_7O_{11}\cdot 2H_2CO_3$: C, 39.94; H, 6.56; N, 14.17%.

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11) This product contained a small amount of sodium *p*-toluenesulfonate which was difficult to remove completely. This was confirmed by the flame reaction.