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SYNTHESIS OF 3″-EPIDIHYDROSTREPTOMYCIN*†

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ABSTRACT

3″-Epidihydrostreptomycin has been prepared from dihydrostreptomycin (DHSM) by a sequence of reactions involving selective 3′,3′a;4″,6″-di-*O*-isopropylidenation of 2″-*N*-(benzyloxycarbonyl)-DHSM, N^G,*O*-peracetylation, *N*-de(benzyloxycarbonyl)ation, selective removal of the 3″-*O*-acetyl group, 2″-*N*-(benzyloxycarbonyl)ation, and epimerization of the 3″-hydroxyl group *via* the intermediary 2″-*N*-(benzyloxycarbonyl)-3″-*O*-(trifluoromethylsulfonyl) derivative, with the 2″,3″-*N*,*O*-carbonylallopyranoside as the intermediate. For use as a reference compound in the structural determination of 3″-epidihydrostreptomycin, methyl 2-deoxy-2-(methylamino)-α-D-allopyranoside was also prepared.

INTRODUCTION

Streptomycin is inactivated by various resistant bacteria that produce enzymes adenylylating[2,3] or phosphorylating[4,5] the 3″- or the 6-hydroxyl group. Synthetic 3″-deoxydihydrostreptomycin[6,7] had been proved to be active against resistant bacteria, producing 3″-modifying enzymes by the lack of the 3″-hydroxyl group. Synthetic 6-deoxydihydrostreptomycin[8], however, exhibited only weak activity, even against bacteria sensitive to dihydrostreptomycin (DHSM)**, suggesting that the 6-hydroxyl group is an important group in DHSM. We now describe the synthesis of another 3″-modified derivative, namely 3″-epidihydrostreptomycin, in order to clarify the epimerization effect of the 3″-hydroxyl group of DHSM against DHSM-sensitive and -resistant bacteria.

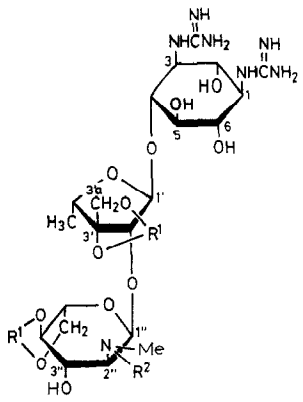
RESULTS AND DISCUSSION

The synthesis of 3″-epidihydrostreptomycin started from 2″-*N*-(benzyloxycarbonyl)dihydrostreptomycin[9] (**1**). In order to protect most of the hydroxyl groups by acetonation, **1** was treated with an excess of 2,2-dimethoxypropane in *N*,*N*-

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.
†For a preliminary report on a different route, see ref. 1.
**Since the trivial name dihydrostreptomycin has been widely used, we too have used it.

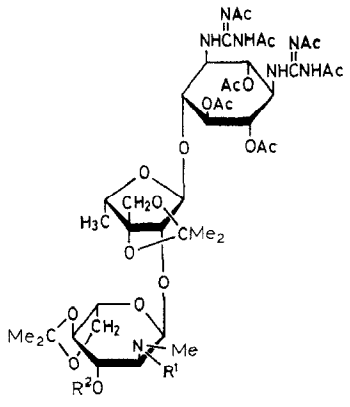
dimethylformamide (DMF) in the presence of an acid catalyst; however, a variety of products, including acetals participating[10] at the guanidino groups, were simultaneously produced; therefore, **1** was treated with a limited amount of the reagent under the conditions described in the Experimental part, to give the 3′,3′a;4″,6″-di-*O*-isopropylidene derivative (**2**) in a yield of ~40%, with some of a tri-*O*-isopropylidene derivative (**3**). Acetylation of **2** with acetic anhydride in the presence of sodium acetate gave the tetra-N^G-acetyltetra-*O*-acetyl derivative (**4**).

The positions at which the isopropylidene and the acetyl groups were introduced were determined from the ^{1}H-n.m.r. spectrum of **4** (see Tables I and II, and

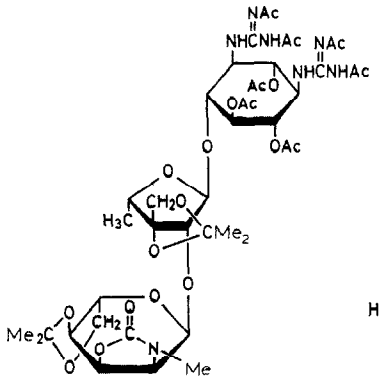


	R^1	R^2
DHSM	H,H	H
1	H,H	Z
2	CMe_2	Z

Z = $OCOCH_2Ph$



	R^1	R^2
4	Z	Ac
5	H	Ac
6	H	H
7	Z	H



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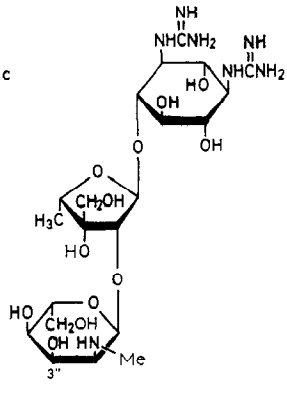
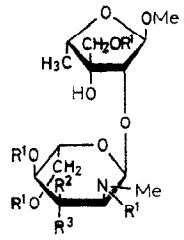


9 (3″-epiDHSM)



	R^1	R^2	R^3
10	H	OH	H
11	Ac	OAc	H
12	H	H	OH

TABLE I

^{1}H-N.M.R. CHEMICAL SHIFTS (δ) AND MULTIPLICITIES FOR COMPOUNDS **4–8** (IN $CDCl_3$)

Proton	*Temperature (degrees)*				
	53	24	24	55	24
	Compound				
	4	**5**	**6**	**7**	**8**
H-1(q)	4.60	4.65br	4.7br	4.61	4.7br
H-2(t)	5.50	~5.5br	5.51br	5.53	5.47br
H-3	~4.3br q	4.4	~4.45	4.27	~4.45
H-4	~3.85			~3.93	
H-5	5.17	~5.2	5.22t	5.17t	5.15
H-6	5.25t	5.24	5.27t	5.23t	5.3
H-1′(s)	4.95	4.92	4.92	~4.9	4.94
H-2′(s)	3.43	3.46	3.42	3.43	3.46
H-3′a(d)	3.82	3.90	3.94	3.81	3.93
H-3′a′(d)	4.15	4.14	4.15	4.14	4.16
H-4′(q)[a]	3.90	3.91	3.92	3.90	3.92
H-5′(d)[a]	1.18	1.21	1.18	1.17	1.19
H-1″(d)	4.88	4.94	4.95	~4.9	4.95
H-2″(dd)	4.48 (0.7H)[b]	2.55	2.35	~4.22	3.72t
H-3″(dd)	5.34	4.96	~3.5	3.97	4.54
H-4″	~3.7	3.56t		~3.6	3.76dd
NCH_3(s)	2.92	2.40	2.45	3.04	2.97
N*H*(=NAc)NHAc(d)	9.00, 9.18br	9.06, 9.23	9.06, 9.29	8.99, 9.17br	9.09, 9.22
NH(=NAc)N*H*Ac(s)	12.94, 12.96	12.98, 13.02	12.99(2)[c]	12.93, 12.94br	12.97, 13.00
CMe_2 (s, 3 H)	1.20, 1.28, 1.32 (weak), 1.37, 1.44	1.36(2), 1.37, 1.45	1.38(2), 1.42, 1.51	1.20br, 1.30br 1.43, 1.50	1.35(2), 1.44, 1.51
Ac (s, 3 H)	1.94br, 1.95 1.97, 2.08 2.12, 2.14 2.16, 2.17br	1.97, 1.99 2.08, 2.09 2.15, 2.16(2) 2.18	1.99, 2.00 2.08, 2.15 2.16, 2.18(2)	1.94, 1.96, 2.06, 2.12, 2.14, 2.16(2)	1.98, 2.00, 2.08, 2.16(3)br 2.19
NCO_2CH_2Ph(ABq)[d]	5.13			5.12	

[a] $J_{4',5'}$ 6.5 Hz. [b] Another H-2″ (0.3 H) signal appeared at δ ~4.3. [c] (2) and (3) means coalescence of two and three peaks. [d] $J_{A,B}$ 12–13 Hz.

Fig. 1). As compound **4**, and other *N*-(benzyloxycarbonyl) derivatives, including **15** (*quod vide*), described herein, gave, at room temperature, a set of signals for respective resonances owing to the presence of two rotamers (and this made assignment of the signals difficult), the spectrum was recorded at an elevated temperature, if necessary, so that assignments could be made. The spectrum of **4** was recorded at 53°, giving a fairly good spectrum, although conformational uniformity was not yet completely attained (see Fig. 1). Irradiation at δ 9 [one of the -N*H*-C(=NAc)NHAc protons] collapsed the quartet at δ 4.6 (J 10 Hz) to a triplet; the quartet, therefore, is assigned to H-1 or -3. Irradiation of the latter (δ 4.6), on the other hand, transformed the

TABLE II

^{1}H–^{1}H COUPLING CONSTANTS (Hz)[a] FOR COMPOUNDS **4–8** (IN $CDCl_3$), **9**, **10**, AND **18** (IN D_2O), AND **11** (IN C_6D_6)

Coupling constant	*Temperature (degrees)*								
	53	24	24	55	24	24	24	24	24
	Compound								
	4	**5**	**6**	**7**	**8**	**9**	**10**	**11**[b]	**18**
$J_{1,2}$	~10	~10	~10	~10	~10				
$J_{2,3}$	~10	~10	~10	~10	~10				
$J_{3,4}$									
$J_{4,5}$	~9.5		~9	~9					
$J_{5,6}$	~9.5		~9	~9.5					
$J_{6,1}$	~10	~10	~10	~10	~10				
$J_{1',2'}$	~0	~0	~0	~0	~0	2	3.5	2.5	
$J_{3',3'a}$	10	9.5	9.5	9.5	9.5		12	11.5	
$J_{1'',2''}$	3.5	3.5	3.5		~5.5	4	4	3.5	~3.5
$J_{2'',3''}$	11	10	10	10.5	~5.5	~3.5	3.5	3	~3.5
$J_{3'',4''}$	8.5	9		8	3.5	~3	3	3	3
$J_{4'',5''}$		~10			~9		10	10.5	10
J_{1,N^GH}	9	9	9	9	9				
J_{3,N^GH}	9	9	9	9	9				

[a]Accurate to within ±0.25 Hz, and ±0.5 Hz (for ~ figures). [b]$J_{4,5}$ 6.5, $J_{5',6'a}$ 5.5, $J_{5',6',b}$ 2, and $J_{6'a,6'b}$ 12 Hz.

downfield triplets at δ 5.5 (H-2) and 5.24 (H-6) into doublets, indicating that the proton (δ 4.6) has two vicinal protons in an acetoxyl group, and, therefore, it is assigned to H-1 (not H-3). By similar decoupling, the guanidino proton at δ 9.18 could be correlated to H-3 (δ 4.32), which has only one vicinal proton in an acetoxyl group, resonating downfield (δ 5.5, H-2). The presence of acetoxyl groups at C-5 and -3″ was also verified (see Experimental section). These results indicate that no isopropylidene group is present on the streptidine portion, and that the four acetoxyl groups are present at C-2, 5, 6, and 3″. The presence of two acetyl groups on each guanidino group was proved by the appearance of far-downfield resonances, at δ 9–9.2 (1 H) and ~13 (1 H).

The next process would be inversion of the configuration of C-3″ and the step preceding that would be selective *O*-deacetylation at O-3″ of **4**. Fodor *et al.*[11] reported that, in ethyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucoside, O→N acetyl migration and *O*-deacetylation occur, depending on the solvents used (methanol, ethanol, and acetone) and the reaction conditions (solvent dryness, reaction temperature). Yamasaki *et al.*[12] succeeded in selectively removing the 3″-*O*-acetyl group from tetra-N^G-acetyl-2,5,6,3′a,3″,4″,6″-hepta-*O*-acetyldihydrostreptomycin by allowing a solution of the compound in ethanol to stand for 24 h at room temperature. We applied

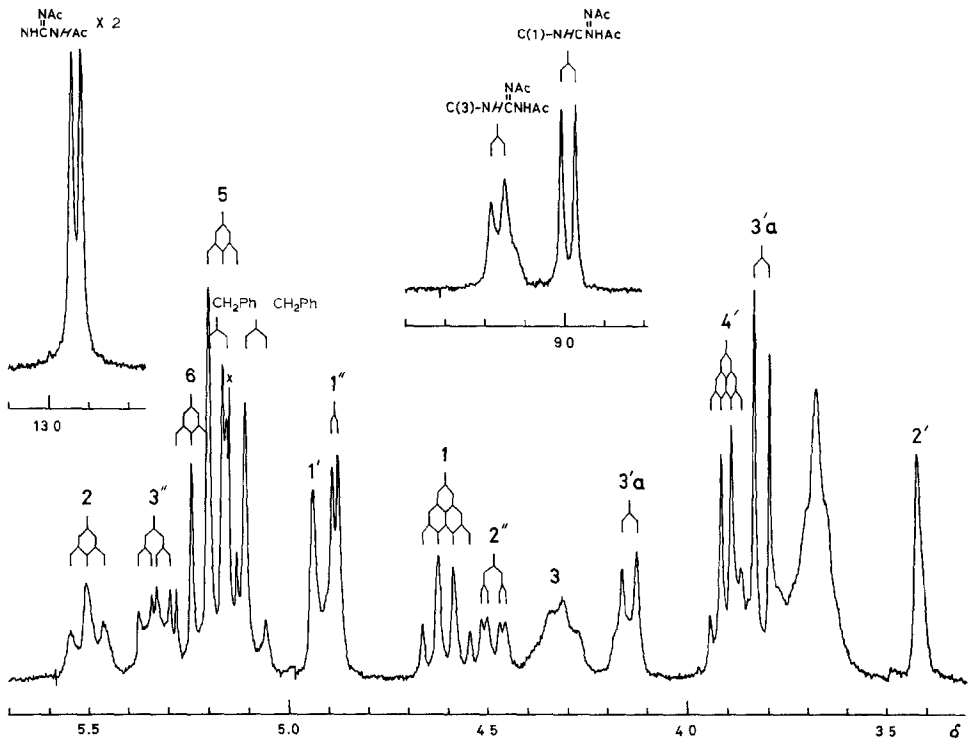


Fig. 1. 250-MHz, ^{1}H-n.m.r. spectrum of **4** in $CDCl_3$ at 53°.

that procedure in the present case, and, after the *N*-(benzyloxycarbonyl) group of **4** had been cleaved by hydrogenolysis, the resulting, free methylamino compound (**5**) was dissolved in ethanol, and the solution was allowed to stand for 3 days at 28°. 3″-*O*-Deacetylated compound (**6**) was obtained in 35% yield (the starting material recovered, 34%). The structure of **6** was confirmed from its ^{1}H-n.m.r. spectrum. The fairly decipherable spectrum measured at room temperature suggested that the *N*-(benzyloxycarbonyl) group had been removed; this was proved from the upfield shifts of the methylamino protons (δ 2.45) and H-2″ (δ 2.35), in comparison to those of **4** (δ 2.92 and 4.48). The absence of the 3″-*O*-acetyl group was verified from the upfield shift ($\delta \sim 3.5$; *cf.*, **4**: 5.34) of the C-3″ methine proton.

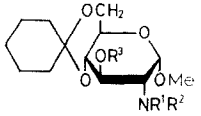
Sano *et al.*[13] prepared benzyl 2-*O*-(2,3-*N*,*O*-carbonyl-2-deoxy-4,6-*O*-isopropylidene-2-methylamino-α-L-allopyranosyl)-3,3a-*O*-isopropylidene-α-dihydrostreptoside from the corresponding 2-*N*-(benzyloxycarbonyl)-3-*O*-(methylsulfonyl)-α-L-glucosyl derivative by boiling the latter in 2-methoxyethanol, in the presence of sodium acetate, under reflux. We applied the procedure to the 3″-*O*-(methylsulfonyl) compound of the 2″-*N*-benzyloxycarbonyl derivative (**7**) of **6**, and the desired 3″-epimeric α-L-*allo*-2″,3″-cyclic carbamate (**8**) could be obtained in low yield. The reaction was found to proceed more readily when the 3″-hydroxyl group was (trifluoromethanesulfonyl)ated, instead of methanesulfonylated, and the unstable 3″-*O*-(trifluoro-

methylsulfonyl) derivative of **7** was, without purification, heated in pyridine, to give the *allo*-carbamate (**8**). The *allo* structure was confirmed by n.m.r. spectroscopy, as described later.

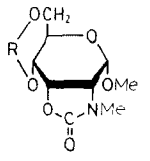
The final process remaining in this synthesis of 3″-epidihydrostreptomycin was that of deprotection. However, unexpected difficulty was experienced in attempted removal of the 2″,3″-cyclic carbamate group. The *cis*-carbamate, in comparison to the *trans*-carbamate[14], was found to be fairly stable in basic media; in an early stage of the treatment with base, the N^G- and *O*-acetyl groups of **8** were all cleaved, but cleavage of the carbamate required such a long time that part of the guanidino groups were simultaneously transformed into ureido groups. Another difficulty was encountered in the subsequent, acid hydrolysis; in the reaction, *O*-deisopropylidenation (especially at *O*-3′,3′a?) and cleavage of the bonding linking the streptidine and the dihydrostreptobiosamine portions competed, resulting in lowering of the yield of the product. For these two reasons, 3″-epidihydrostreptomycin (**9**) was obtained in only 17% yield from **8**.

The structure of **9**, having the allopyranoside structure, was clear from the synthetic pathway and from the ^{1}H-n.m.r. spectrum. In the spectrum, H-3″ resonated downfield (δ 4.25), with small splittings, indicating that H-3″ is equatorially attached. To confirm the structure of **9** further, compound **9** was methanolyzed, and the methyl 3′-epi-α-dihydrostreptobiosaminide (**10**) obtained was compared with methyl α-dihydrostreptobiosaminide[15] (**12**) prepared from DHSM. As reference compounds, methyl 2-deoxy-2-(methylamino)-α-D-allopyranoside (**20**) and the acetyl derivative (**11**) of **10** were prepared.

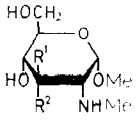
The synthesis of compound **20** is briefly described. Treatment of methyl 4,6-*O*-cyclohexylidene-2-deoxy-2-(methoxycarbonylamino)-α-D-glucopyranoside[16] (**13**) with lithium aluminum hydride in oxolane gave the *N*-methyl derivative (**14**). Protection of the methylamino group with benzyl chloroformate gave the *N*-(benzyloxycarbonyl)



	R^1	R^2	R^3
13	H	CO_2Me	H
14	H	Me	H
15	Z	Me	H
16	Z	Me	SO_2Me
17	Z	Me	SO_2CF_3



	R
18	$C(CH_3)_2$
19	H,H



	R^1	R^2
20	H	OH
21	OH	H

TABLE III

^{13}C-N.M.R. SPECTRA[a] OF COMPOUNDS **21**, **12**, **20**, AND **10** IN D_2O AT pD ~9 AND 34°

Atom	*Compound*			
	21	**12**	**20**	**10**
C-1		107.45		107.30
C-2		85.38[b]		86.58
C-3		81.02		80.97
C-3a		62.73		62.63
C-4		77.75[b]		77.59
C-5		13.09		12.99
C(1)-OCH_3		56.40		56.45
C-1′	98.76	98.86	99.52	99.47
C-2′	63.38	63.64	58.74	58.80
C-3′	73.38	73.30	68.38	67.75
C-4′	71.03	70.49	67.56	66.78
C-5′	72.40	72.64	67.96	68.44
C-6′	61.58	60.99	61.80	61.17
NCH_3	33.93	33.82	32.02	31.89
C(1′)-OCH_3	55.85		56.27	

[a]The chemical shifts were measured from 1,4-dioxane (67.4 p.p.m.) taken as the internal standard, and were confirmed by off-resonance, selective proton-, and long-range, selective proton-decoupling experiments, except for the shifts of C-3′ and -5′ of **12**. [b]The chemical shifts of C-2 and C-4 are the reverse of the results reported by Bock *et al.*[17] for those in dihydrostreptomycin sulfate.

derivative (**15**); it was a mixture of rotamers (on the n.m.r. time-scale), as experienced in the case of **4** and other *N*-(benzyloxycarbonyl) compounds already described. Treatment of **15** with trifluoromethanesulfonic anhydride in pyridine, followed by heating the unstable 3-*O*-sulfonyl intermediate (**17**) in pyridine gave the 2,3-*N*,*O*-carbonyl-α-D-allopyranoside (**18**) in 95% yield. The 3-*O*-(methylsulfonyl) derivative (**16**) of **15** was also prepared. Acid hydrolysis of **18** removed the cyclohexylidene group (to give **19**), and treatment of **19** with base gave the desired *N*-methyl-α-D-allopyranoside (**20**). As a reference compound, methyl 2-deoxy-2-(methylamino)-α-D-glucopyranoside (**21**) was also prepared. The structure of each compound described was confirmed by ^{1}H-n.m.r. spectroscopy. Comparison of the coupling constants, as between **9**, **10**, **11**, and **20** (see Table II), indicated that these compounds have the *allo* structure in their amino sugar portion.

The ^{13}C-n.m.r. spectra of **21**, **20**, methyl α-dihydrostreptobiosaminide[17] (**12**), and the 3′-epi isomer (**10**) at a basic pD-value were compared (see Table III). The chemical shifts of **21** and of the aminoglycoside portion of **12** coincided (within 0.7 p.p.m.) for the corresponding carbon atoms, and the situation was the same for **20** and **10**. The upfield shifts[18,19] of the signals of C-2–C-5 of **20** and **10**, relative to those of the corresponding carbon atoms of **21** and **12** ($\Delta\delta$ 3.5–5.5 p.p.m.) indicated that both **20** and **10** have allopyranoside structures.

Synthetic 3″-epidihydrostreptomycin (**9**) showed[1] strong antibacterial activities against bacteria both sensitive and resistant to dihydrostreptomycin.

EXPERIMENTAL

General. — Melting points were determined on a Kofler block, and are uncorrected. Specific rotations were measured with a Perkin–Elmer Model 241 polarimeter. Infrared spectra were recorded, for potassium bromide pellets, with a JASCO A-202 grating spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 250 MHz in the F.t. mode with a Bruker WM 250 spectrometer. ^{13}C-N.m.r. spectra were recorded in the F.t. mode with a Bruker WM 250 spectrometer operating at 62.9 MHz. Thin-layer chromatography (t.l.c.) was performed on precoated Kieselgel 60 F_{254}, Merck (for **1**–**10**), Wakogel B-5 (for other compounds), and microcrystalline, cellulose powder (Avicel SF, Funakoshi Co., Tokyo). For column-chromatographic separation of the products, silica gel (Wakogel C-200) was used.

*2″-*N*-(Benzyloxycarbonyl)-3′,3′a:4″,6″-di-*O*-isopropylidenedihydrostreptomycin* (**2**). — To a solution of the monosulfate of **1** (4.98 g) in dry DMF (100 mL) were added 2,2-dimethoxypropane (4.5 mL) and anhydrous *p*-toluenesulfonic acid (320 mg), and the mixture was heated at 40°. In order to keep the solution acidic (the pH of the solution gradually increased, to approach neutrality), *p*-toluenesulfonic acid (3 × 220 mg) was added at 5, 8, and 15 h from the start (total time of heating 18 h). On checking by t.l.c. with 12:6:6:2:1 benzene–ethanol–pyridine–water–acetic acid, the solution showed six spots after 2 h, and then, gradually, fewer spots, to give, finally, three spots of R_F 0.38 (tri-*O*-isopropylidene derivative), 0.2 (major, **2**), and 0.1. Addition of triethylamine (0.4 mL), followed by evaporation, gave a syrup that was thoroughly washed with ether, to give a solid. An aqueous solution of the solid was passed through a column of Dowex-1 X-2 (Cl^-) resin (70 mL) with water, and the biacetyl-positive fractions (biacetyl is a reagent for detection of a guanidino group) were evaporated to a solid (5.5 g of the hydrochlorides). The solid was chromatographed on a column of silica gel with 12:6:6:2:1 benzene–ethanol–pyridine–water–acetic acid, and the fractions containing only **2** were isolated and evaporated. The residue was passed through a column of Dowex-1 X-2 (OH^-) resin with water, and, into the fractions containing **2**, carbon dioxide was introduced. Evaporation of the solution gave solid **2** carbonate, 2.08 g (40%); $[\alpha]_D^{23}$ −67° (*c* 1, water); ^{1}H-n.m.r. (D_2O) at 24° (a singlet of ~3/5 and ~2/5 of 3 H in strength is expressed as *a* and *b*, respectively): δ 1.18 (*b*) and 1.27 (*a*), 1.36 (*a*) and 1.39 (*b*), 1.47 (s, 3 H), 1.58 (*b*), and 1.60 (*a*) were assigned for two $C(CH_3)_2$; 1.25 (d, 3 H, *J* 7.0 Hz, CCH_3); 3.01 (*b*) and 3.06 (*a*) (NCH_3); 7.44 and 7.45 (s each, 5 H total, Ph); at 70°: δ 1.24 (d, 3 H, *J* 6.5 Hz, CCH_3), 1.25 (sl. br), 1.35 (sl. br), 1.46, and 1.58 [s, 3 H each, two $C(CH_3)_2$], 3.04 (s, 3 H, NCH_3), 5.2 (m, 4 H, H-1′,-1″, CO_2CH_2Ph), and 7.44 (s, 5 H, Ph).

Anal. Calc. for $C_{35}H_{55}N_7O_{14} \cdot H_2CO_3$: C, 50.28; H, 6.68; N, 11.40. Found: C, 50.21; H, 6.74; N, 11.71.

From the earlier fractions of the chromatography, a solid (**3**) was obtained, 1.33 g; ^{1}H-n.m.r. (D_2O) at 24°: 11 sharp peaks at δ 1–1.6 (21 H), and 2 sharp peaks at δ 3–3.1 (3 H); at 70°: δ 1.24 (d, 3 H, *J* 6.5 Hz, CCH_3); 1.25 and 1.36 [br. s, 3 H

each, $C(CH_3)_2$]; 1.46, 1.49, 1.51 and 1.58 [sharp s, 3 H each, two $C(CH_3)_2$]; and 3.04 (br s, 3 H, NCH_3).

Tetra-N^G-*acetyl-2,5,6,3″-tetra*-O-*acetyl-2″*-N-(*benzyloxycarbonyl*)-*3′,3′a:4″,6″-di*-O-*isopropylidenedihydrostreptomycin* (**4**). — A suspension of the carbonate of **2** (3.75 g) and anhydrous sodium acetate (3.54 g) in acetic anhydride (38 mL) was vigorously stirred for 18 h at 75°. Evaporation gave a syrup that was washed with cyclohexane. The solid residue was extracted with chloroform, and the extract was successively washed with saturated, aqueous sodium hydrogencarbonate and water, dried (sodium sulfate), and evaporated to a solid, 4.75 g (96%). T.l.c. with 4:1 benzene–acetone (doubly developed) showed that the solid contained a slight impurity (R_F 0.35; *cf.*, **4**: R_F 0.4), and so the solid was chromatographed twice on a column of silica gel with 4:1 toluene–acetone, to give a solid, 3.08 g (62%); $[\alpha]_D^{23}$ −39° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710, and 1620 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$ at 53°): irradiation of H-6 collapsed the H-1 quartet to a triplet; irradiation at δ 3.85 (H-4) transformed the H-5 triplet into a doublet; irradiation at δ 4.88 (H-1″) collapsed the H-2″ double doublet to a doublet, and by irradiation of the latter, the H-3″ double doublet to a doublet. Irradiation at δ 3.7 (H-4″) collapsed the H-3″ double doublet to a doublet.

Anal. Calc. for $C_{51}H_{71}N_7O_{22} \cdot H_2O$: C, 53.16; H, 6.39; N, 8.51. Found: C, 53.15; H, 6.21; N, 8.40.

Tetra-N^G-*acetyl-2,5,6,3″-tetra*-O-*acetyl-3′,3′a:4″,6″-di*-O-*isopropylidenedihydrostreptomycin* (**5**). — A solution of **4** (221 mg) in ethanol (4 mL) was hydrogenated with hydrogen at atmospheric pressure in the presence of palladium black for 2 h at room temperature. The usual processing gave solid **5** carbonate, 190 mg (95%); $[\alpha]_D^{23}$ −48° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710 (vw), and 1620 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): irradiation of H-1 collapsed the doublet for C(1)N^G*H* (δ 9.06) to a singlet, and the broadened triplet for H-2 to a broadened doublet, and the multiplet at δ 5.16–5.3 (containing the H-6 signal) changed into a pattern containing three strong peaks; irradiation of H-3 collapsed the C(3)N^G*H* doublet to a singlet, and the signals for H-2 to a broadened doublet; irradiation at δ 4.14 (one of the H-3′a) collapsed the doublet at δ 3.90 (another H-3′a) to a singlet.

Anal. Calc. for $C_{43}H_{65}N_7O_{20} \cdot 0.5\ H_2CO_3 \cdot 0.5\ H_2O$: C, 50.23; H, 6.49; N, 9.43. Found: C, 50.35; H, 6.31; N, 9.38.

Tetra-N^G-*acetyl-2,5,6-tri*-O-*acetyl-3′,3′a:4″,6″-di*-O-*isopropylidenedihydrostreptomycin* (**6**). — A solution of **5** (298 mg) in ethanol (6 mL) was kept for 3 days at 28°. The solution became acidic (pH ~5) at the end of the first day, and was neutral after 3 days; the solution showed (t.l.c. with 10:1 chloroform–ethanol) three spots, R_F 0.5 (**5**), 0.3 (**6**), and 0 (minor), and the last spot increased on further standing. Evaporation of the solution gave a residue which was chromatographed on a column of silica gel with 15:1 chloroform–ethanol to give solid **6**, 100 mg (35%) and recovered **5**, 97 mg (34%). Compound **6**: $[\alpha]_D^{23}$ −48° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710 (vw), and 1620 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): irradiation at δ 3.5 transformed the H-2″ double doublet into a doublet having slight splittings.

Anal. Calc. for $C_{41}H_{63}N_7O_{19} \cdot 0.5\ H_2CO_3$: C, 50.40; H, 6.52; N, 9.91. Found: C, 49.95; H, 6.13; N, 10.17.

Tetra-N^G*-acetyl-2,5,6-tri-*O*-acetyl-2″-*N*-(benzyloxycarbonyl)-3′,3′a:4″,6″-di-*O*-isopropylidenedihydrostreptomycin* (**7**). — To a stirred mixture of **6** (232 mg) in chloroform (42 mL) and 0.7% aqueous sodium hydrogencarbonate (28 mL) was added benzyl chloroformate (0.13 mL), and stirring was continued for 1 h at room temperature. The crude product obtained was purified by chromatography on a column of silica gel with 7:3 benzene–acetone, to give solid **7**, 258 mg (98%); $[\alpha]_D^{23}$ −52° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710, and 1620 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$ at 24°): 11 peaks at δ 1–1.6; NCH_3, at δ 3.03 and 3.06; C(1)N^GH, a doublet at δ 9.06; C(3)N^GH, two doublets, at δ 9.22 and 9.27; N^GHAc, three peaks (2 H) at $\delta \sim 13$; at 55°: see Table I.

Anal. Calc. for $C_{49}H_{69}N_7O_{21}$: C, 53.89; H, 6.37; N, 8.98. Found: C, 53.47; H, 6.41; N, 9.30.

Tetra-N^G*-acetyl-2,5,6-tri-*O*-acetyl-2″-N,3″-*O*-carbonyl-3″-epi-3′,3′a:4″,6″-di-*O*-isopropylidenedihydrostreptomycin* (**8**). — To a cold (~ −50°) solution of **7** (592 mg) in dichloromethane (12 mL) containing pyridine (1.3 mL) was added trifluoromethanesulfonic anhydride (0.18 mL) under stirring, and the temperature was gradually raised to 5°. After 3.5 h, the deep-blue–brown solution was recooled to −50°, trifluoromethanesulfonic anhydride (0.05 mL) and pyridine (0.3 mL) were added, and the mixture was kept for 4.5 h at 5°. T.l.c. (20:1 chloroform–ethanol) then showed that most of the **7** (R_F 0.4) had disappeared, and that material of R_F 0.6 [3″-*O*-(trifluoromethylsulfonyl) compound] had become the major product. After addition of a few drops of water, the deep-colored solution was diluted with chloroform (30 mL), successively washed with 10% aqueous potassium hydrogensulfate, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and evaporated. The black residue was dissolved in pyridine (12 mL), and the solution was kept for 1 h at 65°. T.l.c. with 3:2 benzene–acetone then showed that the sulfonated compound (R_F 0.7) had disappeared, and **8** (R_F 0.3) had become preponderant. Evaporation gave a black residue, which was chromatographed on a column of silica gel with 9:5 benzene–acetone, to give pale-yellow, solid **8**, 244 mg (45%) and recovered **7**, 77 mg (13%); compound **8**: $[\alpha]_D^{23}$ −60° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710 (vw), and 1620 cm^{-1}.

Anal. Calc. for $C_{42}H_{61}N_7O_{20} \cdot H_2O$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.38; H, 5.86; N, 9.53.

3″-Epidihydrostreptomycin (**9**). — To a solution of **8** (193 mg) in oxolane (6 mL) was added 5.5% aqueous barium hydroxide (10 mL), and the mixture was stirred for 43 h at 40°. T.l.c. on Avicel with 6:4:3:1 butanol–pyridine–water–acetic acid then showed two spots, at R_F 0.45 [major, positive to both the biacetyl and the Ehrlich reagent (for detection of ureido group)] and 0.6 (positive to both the biacetyl and the Ehrlich reagent). Carbon dioxide was introduced, the suspension centrifuged, and the upper layer separated, and evaporated to give a yellow solid (270 mg). A solution of the solid in 75% aqueous acetic acid (6 mL) was heated for 70 h at 55°.

T.l.c. on Avicel with 5:3:2:1 pyridine–water–ethyl acetate–acetic acid then showed five spots [the major one (**9**) had R_F 0.35]. Evaporation, and coevaporation of a trace of solvent with toluene, gave a residue that was chromatographed on a column of Amberlite CG-50 (NH_4^+) resin (~40 mL) with 1→9% aqueous ammonium carbonate (linearly changed) as eluant, to give an Ehrlich-negative, colorless solid, 22.1 mg (17%); $[\alpha]_D^{23}$ −79° (*c* 0.9, water); ν_{max}^{KBr} ~1650 (v br) and 1630 cm^{-1}; ^{1}H-n.m.r. (D_2O, pD ~9): δ 1.24 (d, 3 H, CCH_3), 2.39 (s, 3 H, NCH_3), 2.81 (incomplete t, 1 H, H-2″), 4.12 (d, 1 H, H-2′), 4.25 (incomplete t, 1 H, H-3″), 4.29 (q, 1 H, H-4′), 5.08 (d, 1 H, H-1″), and 5.38 (d, 1 H, H-1′).

Anal. Calc. for $C_{21}H_{41}N_7O_{12} \cdot 1.5\ H_2CO_3$: C, 39.94; H, 6.55; N, 14.49. Found: C, 39.63; H, 6.47; N, 14.33.

Methyl 3′-epi-α-dihydrostreptobiosaminide (**10**). — A solution of the carbonate of **9** (26.7 mg) in 2M methanolic hydrogen chloride (0.6 mL) was kept for 3 h at room temperature. T.l.c. with 2:3:1 chloroform–methanol–17% aqueous ammonia then showed two spots, R_F 0 (streptidine; detected by biacetyl reagent) and 0.35 (**10**; *cf.*, methyl α-dihydrostreptobiosaminide: 0.4). Evaporation, with several additions of methanol–toluene, gave a syrup. Chromatography of the syrup on a column of Dowex-1 X-2 (OH^-) resin gave streptidine (9.0 mg, as the base (?); obtained from earlier fractions) and **10**. Carbon dioxide was passed into the aqueous fractions containing **10**, and the solution was evaporated, to give solid hemicarbonate, 9.9 mg (65%); $[\alpha]_D^{23}$ −124° (*c* 0.9, water); ^{1}H-n.m.r. (D_2O) of a sample before neutralization with carbon dioxide (pD ~9): δ 1.24 (d, 3 H, CCH_3), 2.39 (s, 3 H, NCH_3), 2.82 (dd, 1 H, H-2′), 3.44 (s, 3 H, OCH_3), ABq center at 3.59 (2 H, *J* 12 Hz, H-3a,a′), 3.68 (dd, 1 H, H-4′), 3.82–3.9 (2 H, H-5′,6′), 4.06 (d, 1 H, H-2), 4.23 (q, 1 H, H-4), 4.26 (t, 1 H, H-3′), 5.05 (d, 1 H, H-1′), and 5.08 (d, 1 H, H-1).

Anal. Calc. for $C_{14}H_{27}NO_9 \cdot 0.5\ H_2CO_3$: C, 45.31; H, 7.34; N, 3.64. Found: C, 45.43; H, 7.22; N, 3.43.

Methyl 2′-N-*acetyl-3a,3′,4′,6′-tetra*-O-*acetyl-3′-epi-α-dihydrostreptobiosaminide* (**11**). — A solution of the hemicarbonate of **10** (4.9 mg) and acetic anhydride (0.03 mL) in pyridine (0.1 mL) was heated for 35 h at 37°. The usual processing gave a thick syrup, 5.82 mg (81%); $[\alpha]_D^{23}$ −102° (*c* 0.5, chloroform); ^{1}H-n.m.r. (C_6D_6): δ 1.36 (d, CCH_3), 1.66, 1.68 (br), 1.69, 1.73, 1.74 (s, 3 H, Ac), 2.64 (s, 3 H, NCH_3), 3.28 (s, 3 H, OCH_3), 3.89 and 4.04 (ABq, H-3a), 3.99 (q, 1 H, H-4), 4.06 (d, 1 H, H-2), 4.17 (dd, 1 H, H-6′a), 4.33 (dd, 1 H, H-6′b), 4.35 (ddd, 1 H, H-5′), 5.05 (dd, 1 H, H-4′), 5.12 (br t, 1 H, H-2′), 5.21 (d, 1 H, H-1), and 5.27 (br d, 1 H, H-1′).

Anal. Calc. for $C_{24}H_{37}NO_{14}$: C, 51.15; H, 6.62; N, 2.49. Found: C, 50.97; H, 7.06; N, 2.60.

*Methyl 4,6-*O-*cyclohexylidene-2-deoxy-2-(methylamino)-α-*D-*glucopyranoside* (**14**). — A mixture of **13** (1.02 g) and lithium aluminum hydride (1.11 g) in dry oxolane (50 mL) was boiled under reflux for 2 h, and cooled; acetone was added, the mixture was filtered, and the filtrate was evaporated, to give a syrup that was extracted with chloroform–water. A by-product (t.l.c.: R_F 0.4 in 10:1 chloroform–methanol) was extracted into water, and **14** (R_F 0.3), into chloroform; aluminum hydroxide floated

between the two layers. The organic layer was separated, washed with water, dried (sodium sulfate), and evaporated to a syrup, the base of **14**, 866 mg (98%). Carbon dioxide was bubbled into an aqueous solution of the syrup, which was then passed through a column of Dowex-1 X-2 (Cl^-) resin with water, to give the solid hydrochloride of **14**, 917 mg (92%); $[\alpha]_D^{23}$ +91° (*c* 0.6, water); ^{1}H-n.m.r. (D_2O): δ 2.74 (s, 3 H, NCH_3), 3.27 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.5 Hz, H-2), 3.48 (s, 3 H, OCH_3), ~3.93 (H-3), and 5.16 (d, 1 H, H-1).

Anal. Calc. for $C_{14}H_{25}NO_5 \cdot HCl$: C, 51.93; H, 8.09; Cl, 10.95; N, 4.33. Found: C, 51.90; H, 7.78; Cl, 11.06; N, 4.19.

*Methyl 2-*N*-(benzyloxycarbonyl)-4,6-*O*-cyclohexylidene-2-deoxy-2-(methylamino)-α-*D*-glucopyranoside* (**15**). — To a solution of **14** (437 mg) in 1:1 water–acetone (9 mL) were added anhydrous sodium carbonate (370 mg) and benzyl chloroformate (0.32 mL), and the mixture was stirred for 1.5 h. The usual processing gave a solid, 508 mg (79%); $[\alpha]_D^{23}$ +89° (*c* 1, chloroform); ^{1}H-n.m.r. (5:1 pyridine-d_5–D_2O): δ (the ratio in strength *a*/*b* is ~1.8) 1.2–2.0 (10 H), 3.18 (*a*), 3.27 (*b*), 3.28 (*a*), and 3.30 (*b*) (s each, total 6 H, NCH_3 and OCH_3), 3.75–4.0 (4 H, H-4,5,6,6′), 4.34 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 8 Hz, H-3; unresolved m in the absence of D_2O), 4.54 (dd, $J_{1,2}$ 3.5 Hz, H-2*b*) and 4.77 (dd, H-2*a*), 4.94 (d, H-1*b*) and 5.01 (d, H-1a), and ABq centered at 5.25 (*a*) and 5.31 (*b*) (total 2 H, CO_2CH_2Ph).

Anal. Calc. for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.78; H, 7.31; N, 3.22.

*Methyl 2-*N*-(benzyloxycarbonyl)-4,6-*O*-cyclohexylidene-2-deoxy-2-(methylamino)-3-*O*-(methylsulfonyl)-α-*D*-glucopyranoside* (**16**). — This was a solid, $[\alpha]_D^{23}$ +73° (*c* 1, chloroform).

Anal. Calc. for $C_{23}H_{33}NO_9S$: C, 55.30; H, 6.66; N, 2.80; S, 6.42. Found: C, 55.53; H, 6.62; N, 2.75; S, 6.36.

*Methyl 2-*N*,3-*O*-carbonyl-4,6-*O*-cyclohexylidene-2-deoxy-2-(methylamino)-α-*D*-allopyranoside* (**18**). — To a cold (−20°) solution of **15** (852 mg) in pyridine (17 mL) was added trifluoromethanesulfonic anhydride (0.7 mL), and the solution was allowed to warm gradually to 20°, and kept for 2 h at that temperature. T.l.c. with 8:1 benzene–acetone then showed that **15** R_F (0.5) had disappeared, and that **17** (R_F 0.7) had appeared as the sole spot. Water (0.4 mL) was added, the solution was evaporated, and the residue was extracted with chloroform. The extract was successively washed with aqueous sodium hydrogencarbonate and water, dried (sodium sulfate), and evaporated to a syrup. A solution of the syrup in pyridine (15 mL) was heated for 1.5 h at 65°, cooled, and evaporated to a syrup (t.l.c.: R_F 0.2 with the same solvent-system as before); this was dissolved in chloroform, and the solution was washed as before, and evaporated. The syrup obtained was purified by chromatography on a column of silica gel with 8:1 benzene–acetone, to give a syrup that crystallized on standing, 602 mg (95%); m.p. 170–171.5°, $[\alpha]_D^{23}$ +149° (*c* 1, chloroform); ν_{max}^{KBr} 1750 cm^{-1} (cyclic carbamate); ^{1}H-n.m.r. ($CDCl_3$): δ 2.85 (s, 3 H, NCH_3), 3.39 (s, 3 H, OCH_3), 3.75 (t, 1 H, H-2), 3.84 (dd, 1 H, H-4), 4.58 (dd, 1 H, H-3), and 4.80 (d, 1 H, H-1); $J_{1,2}$ 5, $J_{2,3}$ 5.5, $J_{3,4}$ 3.5, and $J_{4,5}$ 9.5 Hz.

Anal. Calc. for $C_{15}H_{23}NO_6$: C, 57.50; H, 7.40; N, 4.47. Found: C, 57.74; H, 7.39; N, 4.58.

*Methyl 2-*N,*3-*O*-carbonyl-2-deoxy-2-(methylamino)-*α*-*D*-allopyranoside* (**19**). — A solution of **18** (573 mg) in 1 : 1 : 2 1,4-dioxane–water–acetic acid (17 mL) was heated for 3 h at 80°, cooled, and evaporated to a residue that was purified by chromatography on a column of silica gel with 8 : 1 chloroform–methanol, to give a syrup that crystallized on standing, 342 mg (80%); m.p. 140–140.5°, $[\alpha]_D^{23}$ +194° (*c* 1, water); ^{1}H-n.m.r. (D_2O): δ 2.86 (s, 3 H, NCH_3), 3.43 (s, 3 H, OCH_3), 4.08 (dd, 1 H, H-2), 4.84 (dd, 1 H, H-3), and 5.01 (d, 1 H, H-1); $J_{1,2}$ 5, $J_{2,3}$ 6.5, and $J_{3,4}$ 4 Hz.

Anal. Calc. for $C_9H_{15}NO_6$: C, 46.35; H, 6.48; N, 6.01. Found: C, 46.11; H, 6.26; N, 6.00.

*Methyl 2-deoxy-2-(methylamino)-*α*-*D*-allopyranoside* (**20**). — A solution of **19** (279 mg) in 5.5% aqueous barium hydroxide (14 mL) was heated for 6.5 h at 80°. Introduction of carbon dioxide followed by centrifugation, and evaporation of the supernatant liquor, gave a residue. Purification of the crude product by chromatography on a column of silica gel with 20 : 15 : 4 chloroform–ethanol–17% aqueous ammonia, and then by chromatography on a column of Dowex-1 X-2 (OH^-) resin with water gave syrupy **20** as the base, which crystallized on standing, 223 mg (90%); m.p. 122–123°, $[\alpha]_D^{23}$ +131° (*c* 1, water); ^{1}H-n.m.r. (D_2O): δ 2.36 (s, 3 H, NCH_3), 2.74 (t, 1 H, H-2), 3.40 (s, 3 H, OCH_3), 3.59 (dd, 1 H, H-4), 3.75–3.95 (3 H, H-5,6,6′), 4.20 (t, 1 H, H-3), and 4.81 (d, 1 H, H-1); $J_{1,2}$ ~3.5, $J_{2,3}$ ~3.5, $J_{3,4}$ 3, and $J_{4,5}$ 10 Hz.

Anal. Calc. for $C_8H_{17}NO_{15}$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.17; H, 8.06; N, 6.62.

*Methyl 2-deoxy-2-(methylamino)-*α*-*D*-glucopyranoside* (**21**). — A solution of **14** (304 mg) in 50% aqueous acetic acid (9 mL) was heated for 1.5 h at 80°, cooled, and evaporated to a syrup that was purified by chromatography on a column of Dowex-1 X-2 (OH^-) resin with water, to give a syrup, 202 mg (92%); $[\alpha]_D^{23}$ +165° (*c* 1, water); ^{1}H-n.m.r. (D_2O): δ 2.38 (s, 3 H, NCH_3), 2.57 (dd, 1 H, H-2), 3.39 (dd, 1 H, H-4), 3.41 (s, 3 H, OCH_3), 3.60 (dd, 1 H, H-3), 3.63 (ddd, 1 H, H-5), 3.76 (dd, 1 H, H-6), 3.87 (dd, 1 H, H-6′), and 4.92 (d, 1 H, H-1); $J_{1,2}$ 3.5, $J_{2,3}$ 10, $J_{3,4}$ 9, $J_{4,5}$ 10, $J_{5,6}$ 5, $J_{5,6'}$ 2, and $J_{6,6'}$ 12 Hz.

Anal. Calc. for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.12; H, 8.12; N, 6.58.

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