Carbohydrate Research, 68 (1979) 43–53

THE SYNTHESIS OF 2,5-DIDEOXY-4,6-DI-*O*-(2,3-DIDEOXY-α-D-*erythro*-HEXOPYRANOSYL)STREPTAMINE AND 4,6-DI-*O*-(6-AMINO-2,3,6-TRIDEOXY-α-D-*erythro*-HEXOPYRANOSYL)-2,5-DIDEOXYSTREPTAMINE

A. CANAS-RODRIGUEZ AND A. MARTINEZ-TOBED
Pharmacy Department, Chelsea College (University of London), London SW3 6LX (Great Britain)
(Received December 6th, 1977; accepted for publication, January 9th, 1978)

ABSTRACT

The title pseudotrisaccharides, derived from 2,5-dideoxystreptamine, have been synthesized, in order to ascertain structure–activity relationships in aminoglycoside antibiotics. High yields of α-D-glycosides, virtually free of β anomers, were achieved by the BF_3-catalyzed addition of alcohols to glycals.

INTRODUCTION

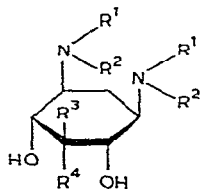
Although syntheses of such derivatives of aminoglycoside antibiotics as 3′,4′-dideoxykanamycin B[1] and 3′-deoxykanamycin B[2], together with their antibiotic activities against common and resistant bacteria, have been reported, showing that the 3′- and 4′-hydroxyl groups are not essential for antibacterial activity, the role of the various hydroxyl groups in the biological activity of these antibiotics has not yet been fully elucidated. Recently, Testa *et al.*[3] reported the preparation of mutamycin 2 by the addition of 2,5-dideoxystreptamine to the fermentation broth of a mutant of *Micromonospora inyoensis* (the sisomycin-producing organism) that exhibits broad-spectrum activity, including activity against gentamycin–sisomycin acetylating strains. Subsequently, Suami *et al.*[4] published syntheses of 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)- and 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine, and determined their biological activities against several organisms.

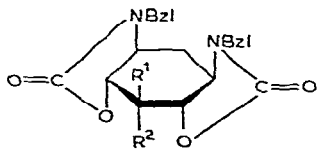
We now report the synthesis of two pseudotrisaccharides of 2,5-dideoxystreptamine, in which five hydroxyl groups have been replaced by hydrogen.

RESULTS AND DISCUSSION

1,3-Di-*N*-benzyl-2-deoxystreptamine dihydrochloride (**1**) was prepared from kanamycin A base *via* the reduction of the tetra-*N*-benzylidene derivative with sodium borohydride and hydrolysis of the resulting tetra-*N*-benzylkanamycin A in 6M hydrochloric acid. Treatment of **1** with phenyl chloroformate gave **2**, which was readily converted into the dicarbamate **3** with sodium hydride in *N*,*N*-dimethyl-

formamide. Treatment of **3** with bromine in triphenyl phosphite[5] gave **4** in high yield. Catalytic hydrogenolysis of **4** in the presence of palladium-on-charcoal and triethylamine[6] afforded **5**. Alkaline hydrolysis of **5**, followed by reaction of the crude product with methyl chloroformate, gave the important intermediate **6** which was used in the glycosidation reactions. 2,5-Dideoxystreptamine dihydrochloride (**7**) was obtained in 75–77% yield from the bromo derivative **4** (36% overall yield from kanamycin) if the hydrogenolysis of **4**, the alkaline hydrolysis of the carbamate groups, and the hydrogenolysis of the benzyl groups of **5** were carried out without isolating the intermediate products.





1 $R^1 = R^4 = H, R^2 = Bzl, R^3 = OH : 2\,HCl$
2 $R^1 = CO_2Ph, R^2 = Bzl, R^3 = OH, R^4 = H$
6 $R^1 = CO_2Me, R^2 = Bzl, R^3 = R^4 = H$
7 $R^1 = R^2 = R^3 = R^4 = H : 2\,HCl$

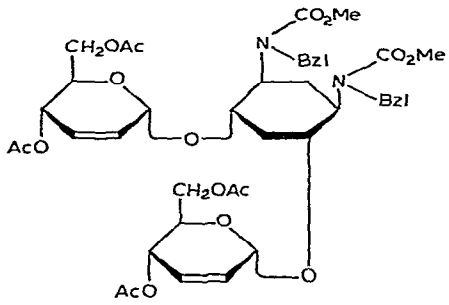
3 $R^1 = OH, R^2 = H$
4 $R^1 = H, R^2 = Br$
5 $R^1 = R^2 = H$

When **6** was treated with tri-*O*-acetyl-D-glucal in the presence of catalytic amounts of boron trifluoride[7] in benzene, the unsaturated pseudotrisaccharide **8** was obtained in 73.5% yield from the mixture containing small proportions of the corresponding pseudodisaccharides. If the condensation of **6** with tri-*O*-acetyl-D-glucal was followed by the hydrogenation of the double bonds over palladium catalyst, the separation of the pseudotrisaccharide **9** from the pseudodisaccharides **10** and **11** was accomplished more readily than for **8**, and crystalline **9** could be easily obtained. Catalytic deacetylation (MeO^-) of **9**, **10**, and **11** gave the pseudotrisaccharide **12** and the pseudodisaccharides **13** and **14**, respectively. Alkaline hydrolysis of the carbamate group of **12** with barium hydroxide or 90% hydrazine hydrate was then followed by hydrogenolysis of the *N*-benzyl groups in **15**, to produce **16** (57% overall yield).

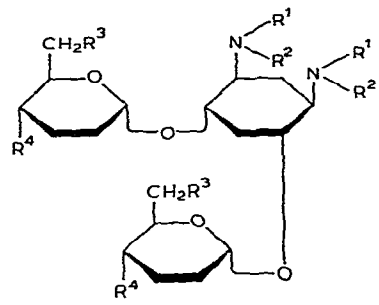
The 4-*O*-α-pseudodisaccharide **11** had higher $[\alpha]_D$, R_F, and δ (H-1′) values than the corresponding 6-*O*-α derivative **10**; these data are in agreement with those published[4] for 4-*O*- and 6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine. Similarly, the p.m.r. spectra of the pseudotrisaccharide **9** showed different chemical shifts for the two anomeric protons, that at the 4-*O* position having the higher value (δ 4.91, H-1′α; δ 4.60, H-1″α).

Two routes were used in the preparation of the diazido compound **19**. One involved the selective tosylation of HO-6′,6″ of **16**, which gave the ditosyl derivative **17**. Treatment of **17** with sodium azide in *N*,*N*-dimethylformamide yielded **19**.

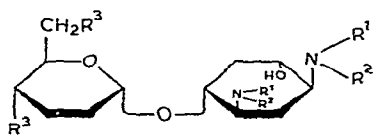
The other route made use of the selective reaction of primary hydroxyl groups with hexamethylphosphorous triamide–tris(dimethylamino)phosphine–carbon tetra-



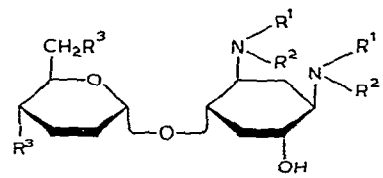
8



9 R^1 = Bzl, R^2 = CO_2Me, R^3 = R^4 = OAc
12 R^1 = Bzl, R^2 = CO_2Me, R^3 = R^4 = OH
15 R^1 = Bzl, R^2 = H, R^3 = R^4 = OH
16 R^1 = R^2 = H, R^3 = R^4 = OH
17 R^1 = Bzl, R^2 = CO_2Me, R^3 = OTs, R^4 = OH
18 R^1 = Bzl, R^2 = CO_2Me, R^3 = OTs, R^4 = OAc
19 R^1 = Bzl, R^2 = CO_2Me, R^3 = N_3, R^4 = OH
20 R^1 = Bzl, R^2 = CO_2Me, R^3 = N_3, R^4 = OAc
21 R^1 = Bzl, R^2 = H, R^3 = NH_2, R^4 = OH
22 R^1 = R^2 = H, R^3 = NH_2, R^4 = OH



10 R^1 = Bzl, R^2 = CO_2Me, R^3 = OAc
13 R^1 = Bzl, R^2 = CO_2Me, R^3 = OH



11 R^1 = Bzl, R^2 = CO_2Me, R^3 = OAc
14 R^1 = Bzl, R^2 = CO_2Me, R^3 = OH

chloride[8] in *N,N*-dimethylformamide to give the corresponding 6′,6″-di-*O*-tris(dimethylamino)phosphonium chloride derivative, which was directly transformed with sodium azide into **19**. Reduction of the azido functions in **19**, followed by removal of the *N*-methoxycarbonyl groups and hydrogenolysis of the *N*-benzyl protecting-groups without the isolation of the intermediates, afforded **22** in 43% overall yield.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Organic solutions were dried with anhydrous sodium sulphate and concentrated under diminished pressure at 40°. Light petroleum refers to the fraction having b.p. 40–60°. Preparative chromatography was performed with silica gel (70–270 mesh ASTM; Macherey Nagel), and paper chromatography on Whatman No. 1 paper. T.l.c. was performed on silica gel GF_{254}, and detection was effected by the following sequence: exposure to u.v. light (254 and 265 nm) and to I_2 vapours, use of spray reagents (ninhydrin, anisaldehyde), and charring with sulphuric acid. Optical rotations were measured with a Bellingham and Stanley polarimeter, i.r. spectra were recorded with a Perkin–

Elmer 157 G spectrophotometer, and p.m.r. spectra (90 MHz) were recorded for solutions in chloroform-*d* (internal Me_4Si), unless otherwise stated, with a Perkin–Elmer R 32 spectrometer. Mass spectra (direct inlet) were obtained with an ionization potential of 70 eV in a VG Micromass 12 F spectrometer.

*1,3-Di-*N*-benzyl-2-deoxystreptamine dihydrochloride* (**1**). — Benzaldehyde (15 ml) was added to a solution of kanamycin A sulphate (20 g) in water (100 ml), and the mixture was basified with 20% aqueous NaOH to pH 12 and then shaken for 3 h. The precipitated solid was filtered off, washed with water, dried, and then dissolved in methanol–chloroform (1 : 1, 200 ml). After the addition of benzaldehyde (15 ml), the mixture was stirred at 22° overnight, concentrated to a small volume, and poured into ether (100 ml). The resulting solid was washed with ether and water, and dried to give tetra-*N*-benzylidenekanamycin A (26 g), m p. 205–206°. A solution of this solid (26 g) in ethanol (700 ml) was treated cautiously with $NaBH_4$ (5 g) in water (25 ml), and the mixture was stirred overnight, filtered, and concentrated. Several distillations of methanolic HCl from the residue gave a borate-free, glassy residue (36 g) of tetra-*N*-benzylkanamycin tetrahydrochloride contaminated with NaCl. A solution of this product in 6M hydrochloric acid (1 litre) was heated on a water bath for 1 h, filtered, and concentrated. Addition of ethanol to the syrupy residue gave an inorganic precipitate that was discarded. After concentration of the filtrate, the residue was recrystallized from absolute ethanol–ether (20 : 1). The total yield (2 crops) of 1,3-di-*N*-benzyl-2-deoxystreptamine dihydrochloride (**1**) was 10 g (81.9%); m.p. 258–260°; R_F 0.45 (10 : 6 : 1 chloroform–methanol–conc. ammonia). P.m.r. data (D_2O): δ 2.05 (t, 1 H, H-2ax), 2.60 (m, 1 H, H-2eq), 3.35 (m, 2 H, H-1,3), 3.75 (m, 3 H, H-4,5,6), 4.43 (m, 4 H, 2 CH_2-Ph), and 7.57 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{20}H_{26}N_2O_3 \cdot 2HCl \cdot 3H_2O$: C, 51.17; H, 7.30; N, 5.97. Found: C, 51.19; H, 6.82; N, 5.68.

*1,3-Di-*N*-benzyl-2-deoxy-1,3-di-*N*-(phenoxycarbonyl)streptamine* (**2**). — A solution of **1** (6 g, 14.4 mmol) and sodium carbonate (10 g) in water–acetone (2 : 1, 300 ml) was treated dropwise with phenyl chloroformate (4.2 ml, 33 mmol) in acetone (50 ml) at −10° with stirring. After 3 h, the mixture was concentrated, water (200 ml) was added, and the precipitated product was filtered off, washed with water, and recrystallized from chloroform–ether–light petroleum to give **2** (7.3 g, 87%), m.p. 96–100°, R_F 0.30 (ethyl acetate). P.m.r. data: δ 1.50–1.90 (m, 2 H, H-2ax,2eq), 3.50–4.00 (m, 5 H, H-1,3,4,5,6), 4.42 (m, 4 H, 2 CH_2-Ph), and 6.90–7.30 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{34}H_{34}N_2O_7$: C, 70.09; H, 5.88; N, 4.81. Found: C, 70.20; H, 5.94; N, 4.74.

*1,3-Di-*N*-benzyl-2-deoxystreptamine 1,6 : 3,4-dicarbamate* (**3**). — A mixture of **2** (11.6 g, 19.2 mmol) in *N,N*-dimethylformamide (50 ml) and sodium hydride (60% oil-dispersed; 2.3 g, 58 mmol) was stirred at 0° for 4 h. Neutralization of the mixture with solid CO_2 and concentration (1 Torr) gave a syrup that was triturated with water. The resulting solid was filtered off and twice recrystallized from hot methanol, to give **3** (5.3 g, 70%), m.p. 249–252°, R_F 0.5 (ethyl acetate); $\nu_{max}^{CHCl_3}$ 3470 (OH), 1775

and 1765 (N-COO, cyclic), and 1610 cm^{-1} (Ph). P.m.r. data (methyl sulphoxide-d_6): δ 1.44 (m, 1 H, H-2ax), 2.13 (m, 1 H, H-2eq), 3.20 (m, 2 H, H-1,3), 3.95 (m, 2 H, H-4,6), 4.30 (m, 4 H, 2 CH_2-Ph), and 7.30 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{22}H_{22}N_2O_5$: C, 66.99, H, 5.62; N, 7.10. Found: C, 67.28; H, 5.77; N, 6.84.

*1,3-Di-*N*-benzyl-5-bromo-2,5-dideoxystreptamine 1,6:3,4-dicarbamate* (**4**) — To a warm (60°) solution of **3** (5 g, 12.7 mmol) in triphenyl phosphite (20 ml) was added bromine (1 ml, 19 mmol). After being shaken for 5 min, the mixture was stored at 22° for 14 h, diluted with chloroform (200 ml), and washed with aqueous 10% Na_2CO_3 (4 × 50 ml) and then water (3 × 50 ml). The organic phase was dried and concentrated, the oily residue was dissolved in benzene, and light petroleum was added to slight turbidity. Crystallization took place on storing the solution at 0° overnight. The solid was recrystallized from hot benzene to give **4** (5.6 g, 96%), m p. 176–178°, R_F 0.50 (1:1 chloroform–ethyl acetate). P.m r. data: δ 1.17 (q, 1 H, H-2ax), 1.80 (dt, 1 H, H-2eq), 3 52 (dt, 2 H, $J_{1,2ax} = J_{3,2ax} = 11$ Hz, $J_{1,6} = J_{3,4} = 11$ Hz, $J_{1,2eq} = J_{3,2eq} = 3.5$ Hz, H-1,3), 4.07 (dd, 2 H, $J_{4,3} = J_{6,5} = 11$ Hz, $J_{4,5} = J_{6,5} = 3$ Hz, H-4,6), 4.33 (m, 4 H, 2 CH_2-Ph), 4.91 (t, 1 H, $J_{5,4} = J_{5,6} = 3$ Hz, H-5), and 7.25 (m, 10 H, 2 Ph). Mass spectrum: *m/e* 457 (M^+, 0.5%), 337 (M—Br, 3.8), and 91 ($PhCH_2^+$, 100).

Anal. Calc. for $C_{22}H_{21}BrN_2O_4$: C, 57.77; H, 4 63; Br, 17.47; N, 6.13. Found: C, 57.65; H, 4.72; Br, 17.21; N, 6 00.

*1,3-Di-*N*-benzyl-2,5-dideoxystreptamine 1,6:3,4-dicarbamate* (**5**). — A solution of **4** (3 g, 6 5 mmol) in absolute ethanol (300 ml) containing triethylamine (5.5 ml) and 10% palladium-on-charcoal (2 g) was hydrogenated at 2 atmos. for 2 h. After removal of the catalyst, the solution was concentrated to small volume and kept at 0° overnight. The resulting product was recrystallized from ethanol to give **5** (1.98 g, 80%), m.p 193–194°; R_F 0.65 (ethyl acetate) and 0 47 (1 .1 chloroform–ethyl acetate). P.m.r. data: δ 1.17 (q, 1 H, $J_{2ax,1} = J_{1ax,3} = 11$ Hz, H-2ax), 1.90 (m, 2 H, H-2eq,5ax), 2.73 (dt, 1 H, H-5eq), 2.95 (dt, 2 H, $J_{1,2ax} = J_{3,2ax} = 11$ Hz, $J_{1,6} = J_{3,4} = 11$ Hz, $J_{1,2eq} = J_{3,2eq} = 3$ 5 Hz, H-1,3), 3 87 (dt, 2 H $J_{4,3} = J_{6,1} = 11$ Hz, $J_{4,5eq} = J_{6,5eq} = 3.7$ Hz, $J_{4,5ax} = J_{6,5ax} = 11$ Hz, H-4,6), 4.33 (m, 4 H, 2 CH_2-Ph), and 7.25 (m, 10 H, 2 Ph). Mass spectrum: *m/e* 379 (M + 1, 7.8%), 378 ($M^{+\cdot}$, 28.5), and 91 ($PhCH_2^+$, 100)

Anal. Calc. for $C_{22}H_{22}N_2O_4$: C, 69.82; H, 5.86; N, 7.40. Found: C, 69.68; H, 6.04; N, 7.00.

*1,3-Di-*N*-benzyl-2,5-dideoxy-1,3-di-*N*-(methoxycarbonyl)streptamine* (**6**). — A solution of **5** (5.45 g, 14.4 mmol) in M methanolic NaOH (25 ml) was heated at 100° for 10 h, made neutral with CO_2, and concentrated. The resulting residue was dissolved in acetone–water (1:1, 50 ml), Na_2CO_3 (5 g) was added, and the mixture was treated dropwise with methyl chloroformate (3 ml, 38.8 mmol) at 0° with stirring. After 4 h, the mixture was concentrated, and extracted with chloroform. The extracts were washed with water, dried, and concentrated to give a glassy material that was purified by chromatography on silica gel with ethyl acetate. Pure, glassy **6** (5.32 g,

84%) had R_F 0.55 (4:1 ethyl acetate–ethanol) and 0.15 (ethyl acetate). P.m.r. data: δ 1.25 (m, 1 H, H-2ax), 1.55 (m, 2 H, H-2eq,5ax), 2.15–2.45 (m, 3 H, 2 OH and H-5eq), 3.60 (m, 4 H, H-1,3,4,6), 3.70 (m, 6 H, 2 CO_2Me), 4.38 (m, 4 H, 2 CH_2-Ph), and 7.23 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 64.94; H, 6.89; N, 6.15.

The diacetate of **6** had m.p. 160–163° (from ethanol), R_F 0.45 (1:1 benzene–ethyl acetate) and 0.57 (1:1 chloroform–ethyl acetate). P.m.r. data: δ 1.20–1.80 (m, 3 H, H-2ax,2eq,5ax), 1.87 (s, 6H, 2 OAc), 2.37 (dt, 1 H, $J_{5ax,5eq}$ = 12 Hz, $J_{5eq,4} = J_{5eq,6}$ = 5 Hz, H-5eq), 3.69 (s, 6 H, 2 CO_2Me), 4.30 (m, 4 H, 2 CH_2-Ph), 5.00 (m, 2 H, H-4,6), and 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{28}H_{34}N_2O_8$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.70; H, 6.67; N, 5.15.

2,5-Dideoxystreptamine dihydrochloride (**7**). — A solution of **5** (1.77 g, 4.7 mmol) in M methanolic sodium methoxide (5 ml) was heated at 80° for 1 h. Water (5 ml) was then added, and the mixture was heated at 100° for 6 h. The solution was acidified to pH 2 with 2M HCl and concentrated to dryness. The residue was treated with methanol (25 ml), and the filtered solution was hydrogenated over palladium-on-charcoal (50 mg) at 2 atmos. for 12 h. Concentration of the filtered solution and recrystallization of the residue from aqueous ethanol gave **7** (1.0 g, 96%), m.p. 280° (dec.) (lit.[9] m.p. 290–295°); R_F 0.22 (8:1 methanol–conc. ammonia) and 0.40 (4:1 methanol–conc. ammonia); R_{GlcN} 0.6 (paper; 6·4·3:1 1-butanol–pyridine–water–acetic acid). P.m.r. data (D_2O): δ 1.72 (m, 2 H, H-2ax,5ax), 2.48 (m, H-2eq,5eq), 3.28 (m, 2 H, $J_{1,6} = J_{3,4}$ = 10 Hz, H-1,3), and 3.78 (dt, 2 H, $J_{4,5eq} = J_{6,5eq}$ = 5 Hz, H-4,6). Mass spectrum: *m/e* 147 (M + 1, 0.2%), 129 (M — 17, 14), and 59 (100).

Anal. Calc. for $C_6H_{14}N_2O_2 \cdot 2HCl$: C, 32.89; H, 7.36; N, 12.79. Found: C, 33.06; H, 7.21; N, 12.54.

The *N,O*-tetraacetyl derivative of **7** had m.p. 291–292° (from ethanol–ether) (lit.[9] m.p. 292–293°), R_F 0.15 (20:6:1 chloroform–methanol–conc. ammonia). P.m.r. data: δ 1.20–1.80 (m, 2 H, H-2ax,5ax), 1.95 (s, 6 H, 2 NAc), 2.07 (s, 6 H, 2 OAc), 2.20–2.55 (m, 2 H, H-2eq,5eq), 4.15 (m, 2 H, H-1,3), 4.87 (dt, 2 H, $J_{4,3} = J_{6,1}$ = 10.5 Hz, $J_{4,5eq} = J_{6,5eq}$ = 5 Hz, H-4,6), and 6.35 (d, 2 H, J = 9 Hz, 2 NH).

1,3-Di-N-*benzyl-2,5-dideoxy-4,6-di*-O-(*4,6-di*-O-*acetyl-2,3-dideoxy*-α-D-*erythro-hex-2-enopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**8**). — A stirred solution of **6** (0.51 g, 1.15 mmol) and tri-*O*-acetyl-D-glucal (0.78 g, 2.8 mmol) in dry benzene (20 ml) was treated with boron trifluoride etherate (0.03 ml). After 15 min, triethylamine (1 ml) was added, and the mixture was washed with water, dried, and concentrated. The syrupy residue showed three components in t.l.c.: R_F (ethyl acetate) 0.57 (major), 0.40 (minor), and 0.30 (minor). Elution of the mixture from silica gel with benzene–chloroform (1:1) gave the main component as a glassy product (0.75 g, 75.3%), R_F 0.30 (3:1 chloroform–ethyl acetate); $\nu_{max}^{CHCl_3}$ 1735 (OAc), 1690 (NCO_2Me), 1600 (Ph), and 1490 cm^{-1} (Ph). P.m.r. data: δ 1.40 (m, 3 H, H-2ax,2eq,5ax), 2.07 (q, 12 H, 4 OAc), 3.67 (m, 6 H, 2 COOMe), 4.25 (bm, 14 H,

2 CH_2Ph and 10 skeleton protons), 5.20 (m, 2 H, H-1′α,1″α), 5.36 (m, 2 H, H-4′,4″), 5.77 (m, 4 H, H-2′,2″,3′,3″), and 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{44}H_{54}N_2O_{16}$: C, 60.96; H, 6.21; N, 3.23. Found: C, 61.10; H, 6.41; N, 3.10.

1,3-Di-N-*benzyl-2,5-dideoxy-4,6-di*-O-(*4,6-di*-O-*acetyl-2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**9**). — A solution of **6** (442 mg, 1 mmol) and tri-*O*-acetyl-D-glucal (820 mg, 3 mmol) in benzene (25 ml) was treated with boron trifluoride etherate (0.1 ml) at 20° with stirring. After 0.5 h, triethylamine (0.5 ml) was added and the mixture was washed with water, dried, and concentrated. The residual syrup was dissolved in methanol (20 ml) and hydrogenated over 10% palladium-on-charcoal (0.2 g) at atmospheric pressure for 2 h. The filtered solution was concentrated, and the residue was chromatographed on silica gel with benzene–chloroform (1 : 2), to give three components having R_F (t.l.c.; 1 : 1 benzene–ethyl acetate) 0.30 (major, pseudotrisaccharide), 0.09 (minor, pseudodisaccharide), and 0 03 (minor, pseudodisaccharide). The major component was recrystallized from ether–light petroleum to give **9** (560 mg, 65%), m.p. 63–65°, $[\alpha]_D^{24}$ +71.5° (*c* 0.7, chloroform); R_F 0.65 (ethyl acetate) and 0.2 (3 : 1, chloroform–ethyl acetate). P.m.r. data (70°): δ 1.10–1.80 (bm, 12 H, methylene protons), 1.95 (3 s, 12 H, 4 OAc), 3.62 (2 s, 6 H, 2 NCO_2Me), 3.70–4.45 (bm, 16 H, 2 CH_2-Ph and 12 skeleton protons), 4.60 (m, 1 H, H-1″α), 4.91 (m, 1 H, H-1′α), and 7.13 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{44}H_{58}N_2O_{16}$: C, 60.68; H, 6.71; N, 3.21. Found: C, 60.81; H, 6.52; N, 3.05.

1,3-Di-N-*benzyl-2,5-dideoxy-6*-O-(*4,6-di*-O-*acetyl-2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**10**). — The minor component having R_F 0.09 (benzene–ethyl acetate, 1 : 1), chromatographically separated from **9** as a glass, was identified as **10**, $[\alpha]_D^{25}$ +32° (*c* 0.7, chloroform), R_F 0.45 (ethyl acetate); $\nu_{max}^{CHCl_3}$ 3440 (OH), 1725 (OAc), 1680 (NCO_2Me), 1487 (Ph), 1235 (C-O), 1037 (glycoside), and 705 cm^{-1} (Ph). P.m.r. data: δ 1.25 (m, 1 H, H-2ax), 1.65 (m, 4 H, H-2′,2′,3′,3′), 2.02 (s, 6 H, 2 OAc), 2.40 (m, 1 H, H-5eq), 3.68 (2 s, 6 H, 2 NCO_2Me), 4.10 (m, 4 H, 2 CH_2Ph), 4.65 (m, 1 H, H-1′α), and 7.24 (m, 10 H, 2 Ph)

Anal. Calc. for $C_{34}H_{44}N_2O_{11}$: C, 62.18; H, 6.75; N, 4.26. Found: C, 62.05; H, 6.60; N, 4.33.

1,3-Di-N-*benzyl-2,5-dideoxy-4*-O-(*4,6-di*-O-*acetyl-2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**11**). — The minor component having R_F 0.05 (benzene–ethyl acetate, 1 : 1), chromatographically separated from **9** as a glass, was identified as **11**, $[\alpha]_D^{25}$ +75.5° (*c* 0.6, chloroform), R_F 0.35 (ethyl acetate); $\nu_{max}^{CHCl_3}$ 3440 (OH), 1725 (OAc), 1680 (NCO_2Me), 1487 (Ph), 1240 (C-O), 1027 (glycoside), and 705 cm^{-1} (Ph). P.m.r. data: δ 1.25 (m, 1 H, H-2ax), 1.70–1.90 (m, 6 H, H-2eq,5ax,2′,2′,3′,3′), 2.02 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 3.68 (s, 6 H, 2 CO_2Me), 3.40–3.90 (bm, 4 H, H-1,3,4,6), 4.00–4.80 (m, 10 H, 2 CH_2-Ph and 6 skeleton protons), 4.94 (m, 1 H, H-1′α), and 7.23 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{34}H_{44}N_2O_{11}$: C, 62 18; H, 6.75; N, 4.26. Found: C, 62.28; H, 6.87; N, 4.20.

1,3-Di-N-*benzyl-2,5-dideoxy-4,6-di*-O-(*2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**12**). — A methanolic solution of **9** (0.36 g, 0.41 mmol) was treated with sodium methoxide (20 mg) at 20° for 12 h, made neutral with CO_2, and concentrated. The residue was extracted with chloroform, the extracts were evaporated, and the solid residue was recrystallized from chloroform–ether to yield pure **12** (0.28 g, 96%), m.p. 72°, $[\alpha]_D^{25}$ +66° (*c* 1.1, chloroform); R_F 0.12 (8:1 ethyl acetate–ethanol) and 0.50 (2:1 ethyl acetate–ethanol). P.m.r. data: δ 1.20–1.90 (m, 12 H, methylene protons), 2.40–2.80 (b, 4 H, 4 OH), 3.67 (m, 6 H, 2 CO_2Me), 4.93 (m, 1 H, H-1′α), and 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{50}N_2O_{12}$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.64; H, 7.02; N, 4.02.

1,3-Di-N-*benzyl-2,5-dideoxy-6*-O-(*2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**13**). — Catalytic deacetylation of **10** (65 mg, 0.1 mmol) in methanol (2 ml), as for **10**, gave **13** (54 mg, 95%), m.p. 87° (from chloroform–ether), $[\alpha]_D^{21}$ +29° (*c* 0.9, chloroform); R_F 0.20 (8:1 ethyl acetate–ethanol) and 0.20 (4:1 benzene–methanol). P.m.r. data (95°): δ 1.20–1.90 (m, 7 H, H-2ax,2eq,5ax,2′,2′,3′,3′), 2.25–2.60 (bm, 4 H, H-5eq and 3 OH), 3.70 (m, 6 H, 2 CO_2Me), 4.40 (m, 4 H, 2 CH_2Ph), 4.63 (m, 1 H, H-1′α), and 7.23 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{30}H_{40}N_2O_9$: C, 62.92; H, 7.04; N, 4.89. Found: C, 63.02; H, 7.16; N, 5.04.

1,3-Di-N-*benzyl-2,5-dideoxy-4*-O-(*2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**14**). — Catalytic deacetylation of **11** (50 mg) in methanol (2 ml), as for **12**, gave **14** (39 mg, 90%), m.p. 95° (from chloroform–ether), $[\alpha]_D^{21}$ +58° (*c* 0.5, chloroform); R_F 0.20 (4:1 benzene–methanol) and 0.20 (8:1 ethyl acetate–ethanol). P.m.r. data (100°): δ 1.20–1.65 (m, 3 H, H-2ax,2eq,5ax), 1.75 (m, 4 H, H-2′,2′,3′,3′), 2.10 (bm, 3 H, 3 OH), 2.40 (m, 1 H, H-5eq), 3.70 (2s, 6 H, 2 CO_2Me), 4.37 (m, 4 H, 2 CH_2Ph), 4.89 (m, 1 H, H-1′α), and 7.23 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{30}H_{40}N_2O_9$: C, 62.92; H, 7.04; N, 4.89. Found: C, 62.84; H, 7.08; N, 4.78.

1,3-Di-N-*benzyl-2,5-dideoxy-4,6-di*-O-(*2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-*streptamine* (**15**). — A mixture of either **12** or **9** (0.2 g, 0.28 mmol) and 90% hydrazine hydrate (2 ml) in ethanol (1 ml) was heated under reflux for 24 h at 130°. The solution was concentrated, and the residue was chromatographed on silica gel with chloroform–methanol mixtures. Pure **15** (0.147 g, 90%) was a glassy product, $[\alpha]_D^{25}$ +94° (*c* 1, methanol), R_F 0.50 (20:6:1 chloroform–methanol–conc. ammonia). P.m.r. data (D_2O): δ 1.95 (series of multiplets, 12 H, methylene protons), 3.70 (bm, 16 H, 2 CH_2Ph and skeleton protons), 4.98 (m, 2 H, H-1′α,1″α), and 7.22 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{32}H_{46}N_2O_8$: C, 65.50; H, 7.90; N, 4.77. Found: C, 65.65; H, 8.10; N, 4.85.

2,5-Dideoxy-4,6-di-O-(*2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)*streptamine* (**16**). — A solution of **15** (117 mg, 0.2 mmol) in methanol (20 ml) was hydrogenated over 10% palladium-on-charcoal (100 mg) at 2 atmos. for 12 h. The filtered solution and

washings of the catalyst were combined and concentrated, to give **16** (75 mg, 92%) as a clear glass, $[\alpha]_D^{25}$ +110° (*c* 0.6, methanol); R_F 0.06 (20:6:1 chloroform–methanol–conc. ammonia), 0.32 (8:1 methanol–conc. ammonia), and 0.55 (paper; 15:10:3:12 1-propanol–pyridine–acetic acid–water). Mass spectrum: *m/e* 407 (M + 1, 2.8%), 389 (1.2), 375 (1.4), 334 (0.9), 303 (0.8), 302 (0.8), 287 (1.3), 277 (1.5), 275 (1.7), 273 (1.1), 259 (18), 244 (11), 232 (5), 131 (44), 129 (16), 113 (21), 110 (43), 103 (50), 95 (21), 85 (29), 71 (100), 67 (23), 59 (26), 56 (25), and 45 (53).

Anal. Calc. for $C_{18}H_{34}N_2O_8$: C, 53.18; H, 8.43; N, 6.89. Found: C, 53.02; H, 8.35; N, 7.01.

By performing the steps illustrated for **15** and **16** on the acetyl derivative **9** (866 mg, 1 mmol), without the isolation of intermediates, the yield of **16** was 370 mg (91%).

1,3-Di-N-*benzyl-2,5-dideoxy-4,6-di*-O-(*2,3-dideoxy-6*-O-*toluene*-p-*sulphonyl*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**17**). — A mixture of **12** (0.5 g, 0.71 mmol) and toluene-*p*-sulphonyl chloride (0.298 g, 1.56 mmol) in dry pyridine (5 ml) was stirred at −20° for 1 h and at 20° for 12 h, poured into ice–water, and extracted with chloroform. The extracts were washed with water, dried, and concentrated, to give a glassy product which was purified by preparative t.l.c. (ethyl acetate). Pure **17** (0.503 g, 70%) had m.p. 105°, $[\alpha]_D^{24}$ +44° (*c* 0.5, chloroform), R_F 0.57 (ethyl acetate). P.m r. data: δ 1.25 (m, 1 H, H-2ax), 1.40–1.95 (m, 10 H, H-2eq,5ax and 8 methylene protons), 2.42 (2s, 6 H, 2 *Me*-Ph), 3.63 (s, 6 H, 2 CO_2Me), 4.18 (m, 4 H, 2 CH_2Ph), 4.93 (m, 1 H, H-1′α), 7.18 (m, 10 H, 2 Ph), 7.32 and 7.78 (m, 8 H, 2 Ts).

Anal. Calc. for $C_{50}H_{62}N_2O_{16}S_2$: C, 59.40; H, 6.18; N, 2.77, S, 6.33. Found: C, 59.60; H, 6.20; N, 2.95; S, 6.43.

Conventional acetylation of **17** (30 mg) with pyridine (0.5 ml) and acetic anhydride (0.05 ml) gave the diacetate **18** (23 mg, 71%), m.p. 85–88° (from ethanol), $[\alpha]_D^{22}$ +62° (*c* 0 5, chloroform); R_F 0.68 (1:2 benzene–ethyl acetate) and 0.50 (chloroform).

Anal. Calc. for $C_{54}H_{66}N_2O_{18}S_2$: C, 59.32; H, 6.06; N, 2.55; S, 5.83. Found: C, 59.20; H, 6.15; N, 2.70; S, 5.95.

4,6-Di-O-(*6-azido-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-*benzyl-2,5-dideoxy-1,3-di*-N-(*methoxycarbonyl*)*streptamine* (**19**). — (*a*) A mixture of **17** (48 mg, 0.047 mmol) and sodium azide (30 mg, 0.46 mmol) in dry *N,N*-dimethylformamide (1 ml) was stirred at 80° for 24 h, poured into ice–water, and extracted with ether. The ethereal extracts were washed with water, dried, and concentrated. The residue was recrystallized from ethanol–water to give **19** (31 mg, 87%), m.p. 80° (sinters), $[\alpha]_D^{24}$ +45° (*c* 0.8, chloroform); R_F 0.50 (ethyl acetate) and 0.11 (1:1 benzene–ethyl acetate); $\nu_{max}^{CHCl_3}$ 3420 (OH), 2100 (N_3), 1690 (NCO_2Me), 1500 (Ph), 1245 (C-O), 1040 (glycoside), and 710 cm^{-1} (Ph).

(*b*) Carbon tetrachloride (0.2 ml) and hexamethylphosphorous triamide (0.2 ml) were added to a solution of **12** (351 mg, 0.5 mmol) in dry *N,N*-dimethylformamide (2 ml). The mixture was stirred at −50° under N_2 for 1 h. Sodium azide was added

to the red solution, and the mixture was stirred at 80° for 8 h, poured into ice–water, and extracted with ether. The extracts were washed with water, dried, and concentrated, and the residue was chromatographed on silica gel with chloroform. The major component (R_F 0.50, ethyl acetate) was recrystallized from ethanol–water to give **19** (0.26 g, 70%), m.p. 80° (sinters), $[\alpha]_D^{27}$ +45° (*c* 1, chloroform). P.m.r. data (80°): δ 1.20–1.80 (m, 11 H, H-2ax,2eq,5ax and 8 methylene ring-protons), 1.98 (m, 2 H, 2 OH), 2.65 (m, 1 H, H-5eq), 3.46 (m, 4 H, H-6′,6′,6″,6″), 3.66 (2s, 6 H, 2 CO_2Me), 4.30 (m, 4 H, 2 CH_2Ph), 4.59 (m, 1 H, H-1″α), 4.96 (m, 1 H, H-1′α), and 7.17 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{48}N_8O_{10}$: C, 57.43; H, 6.43; N, 14.89. Found: C, 57.30; H, 6.50; N, 14.74.

Conventional acetylation of **19** (40 mg) in pyridine (0.5 ml) with acetic anhydride (0.1 ml) gave the diacetate **20** (39 mg), m.p. 85° (from ethanol–water), $[\alpha]_D^{24}$ +69° (*c* 0.55, chloroform), R_F 0.58 (1:1 benzene–ethyl acetate).

Anal. Calc. for $C_{40}H_{52}N_8O_{12}$: C, 57.40; H, 6.26; N, 13.39. Found: C, 57.12; H, 6.41; N, 13.50.

4,6-Di-O-(*6-amino-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl*)-*1,3-di*-N-*benzyl-2,5-dideoxystreptamine* (**21**). — A solution of **19** (0.2 g, 0.265 mmol) in methanol (10 ml) was hydrogenated over 10% palladium-on-charcoal (50 mg) at 1 atmos. for 20 h. The catalyst was filtered off and washed with methanolic ammonia. The combined organic liquids were concentrated to give a glass (0.192 g), R_F 0.25 (20:6:1 chloroform–methanol–conc. ammonia), which was heated under reflux for 48 h with ethanol–90% hydrazine hydrate (1:3, 2 ml). The mixture was then concentrated to dryness, and the chloroformic extracts of the residue were concentrated to give **21** as a glass (0.148 g, 95%), $[\alpha]_D^{24}$ +54° (*c* 0.17, methanol); R_F 0.10 (20:6:1 chloroform–ethanol–conc. ammonia) and 0.55 (8:1 methanol–conc. ammonia).

Anal. Calc. for $C_{32}H_{48}N_4O_6$: C, 65.73; H, 8.27; N, 9.58. Found: C, 65.91; H, 8.40; N, 9.63.

4,6-Di-O-(*6-amino-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl*)-*2,5-dideoxystreptamine* (**22**). — (*a*) A solution of **21** (0.23 g, 0.39 mmol) in methanol (25 ml) was hydrogenated over 10% palladium-on-charcoal (65 mg) at 1 atmos. for 16 h. The filtered solution was concentrated to give **22** as a glass (0.16 g, 100%), $[\alpha]_D^{24}$ +139° (*c* 0.37, methanol), R_F 0.10 (8:1 methanol–conc. ammonia).

(*b*) A mixture of **19** (0.7 g, 1 mmol), 90% hydrazine hydrate (3 ml), 10% palladium-on-charcoal (50 mg), and ethanol (2 ml) was heated under reflux for 6 h. After concentration, ethanol (10 ml) and more palladium catalyst (50 mg) were added, and the suspension was hydrogenated at 25° and 2 atmos. for 6 h. The filtered mixture was concentrated to give **22** as a glass (0.4 g, 99%), $[\alpha]_D^{24}$ +139.3° (*c* 0.5, methanol); R_F 0.10 (8:1 methanol–conc. ammonia), 0.47 (*cf.* kanamycin A, 0.25) (4:1 methanol–conc. ammonia); R_{GlcN} 0.26 (paper; 6:4:3:1 1-butanol–pyridine–water–acetic acid). P.m.r. data (D_2O): δ 1.10–2.20 (bm, 11 H, H-2ax,2eq,5ax and 8 methylene ring-protons), 3.30–3.70 (bm, skeleton protons), 5.04 (m, 1 H, H-1″α), and 5.06 (m, 1 H, H-1′α).

Anal. Calc. for $C_{18}H_{36}N_4O_6$: C, 53.44; H, 8.97; N, 13.85. Found: C, 53.56; H, 9.09; N, 13.76.

REFERENCES

1 S UMEZAWA, H. UMEZAWA, T. TSUCHIYA, AND Y. OKAZAKI, *Bull. Chem. Soc. Jpn*, 45 (1972) 3624–3628.
2 Y. TAKAGI, T. MIYAKE, T. TSUCHIYA, S. UMEZAWA, AND H UMEZAWA, *J Antibiot*, 26 (1973) 403–406.
3 R. T. TESTA, G. H. WAGMAN, P. J. L. DANIELS, AND M. J. WEINSTEIN, *J. Antibiot*, 27 (1974) 917–921
4 T. SUAMI, S. OGAWA, Y. FUNAKI, AND K. IWATA, *Bull Chem Soc Jpn.*, 49 (1976) 1975–1979
5 S R. LANDAUER AND H. N. RYDON, *J Chem Soc*, (1953) 2224–2234; D. G. COE, S. R. LANDAUER, AND H N. RYDON, *ibid*, (1954) 2281–2288.
6 M. G REINECKE, *J Org. Chem*, 29 (1964) 299–304, C. G OVERBERGER AND H KAYE, *J Am Chem. Soc*, 89 (1967) 5640–5645.
7 R. J. FERRIER AND N PRASAD, *J Chem. Soc., C*, (1969) 570–575.
8 B. CASTRO, Y. CHAPLEUR, AND B GROSS, *Bull. Soc. Chim Fr.*, (1973) 3034–3039
9 T. SUAMI, S. OGAWA, H. UCHINO, AND Y. FUNAKI, *J. Org. Chem*, 40 (1975) 456–461.