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SYNTHESIS OF APRAMYCIN ANALOGUES

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ABSTRACT

The synthesis is described of a pseudotrisaccharide analogue of apramycin from a suitably protected 2-deoxystreptamine derivative by the stepwise application of the BF_3-catalysed addition of alcohols to glycals.

INTRODUCTION

Apramycin[1] {*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)-(1→8)-*O*-[(8*R*)-2-amino-2,3,7-trideoxy-7-methylamino-D-*glycero*-α-D-*allo*-octodialdo-1,5:8,4-dipyranosyl]-(1→4)-2-deoxystreptamine}, an aminoglycoside having high antibacterial activity and low toxicity, is a pseudotrisaccharide in which the aglycon 2-deoxystreptamine is substituted at C-4 by a diamino-octodiose sugar possessing a rigid dipyranose, *trans*-decalin shaped, ring system. This in turn is also glycosidated through its second anomeric centre (C-8) by 4-amino-4-deoxy-D-glucose. The stability of this antibiotic to all but one (*N*-3 acetyltransferase AAC[3]IV) of the deactivating enzymes[2] produced by resistant strains of bacteria constitutes a basis for the establishment of structure–activity relationships and the preparation of more effective antibiotics.

As the octodiose is not readily accessible, 4-*O*-pseudotrisaccharides of 2-deoxystreptamine with glucose derivatives were prepared. The glycosidation procedure used, which involves the BF_3-catalysed addition of alcohols to glycals, was tested previously on this type of complex trisaccharide[3] and afforded high yields of α-glycosides.

RESULTS AND DISCUSSION

The optically active and suitably protected derivative (**4**) of 2-deoxystreptamine used for the synthesis of the pseudotrisaccharide **20** was obtained by a procedure similar to that[4] starting with 5,6-*O*-cyclohexylidene-tetra-*N*-methoxycarbonylneamine. Reaction of 3′,4′:5,6-di-*O*-cyclohexylidene-tetra-*N*-methoxycarbonylneamine[5] and benzyl bromide gave **1**. Selective removal of one of the cyclohexylidene groups then gave **2** which was oxidised with periodic acid to give **3**. A base-catalysed β-elimination[6] on **3** yielded a 3:1 mixture of the 2-deoxy-

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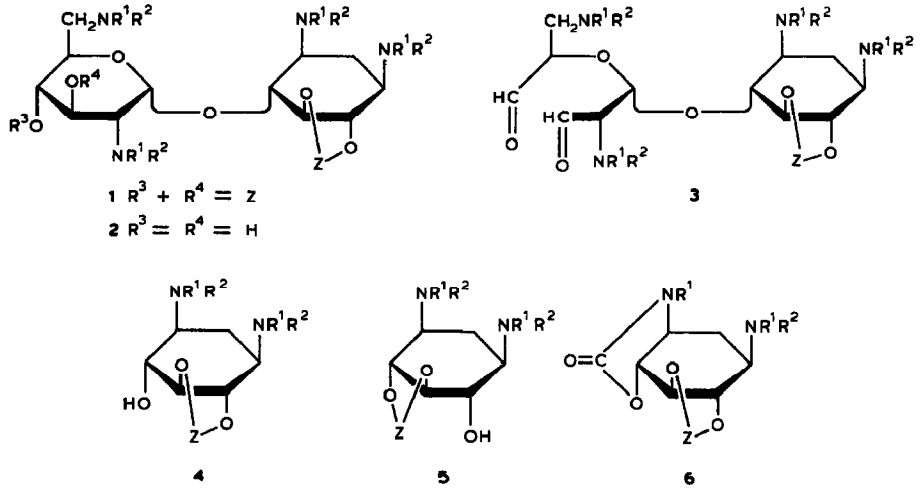
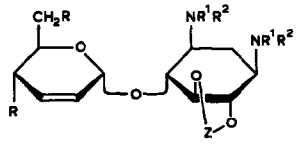


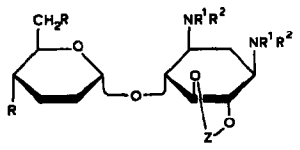
streptamine derivatives **4** and **6**. Compound **4** had HO-4 unprotected, $[\alpha]_D$ −9.5° (chloroform), and gave a positive carmine colour with anisaldehyde which indicated the presence of the cyclohexylidene group. The i.r. bands at 3520 (OH) and 1695 cm^{-1} (NCOOMe) confirmed the structure assigned to **4**.

The choice of the protecting groups (*N*-benzyl and *N*-methoxycarbonyl) for the 2-deoxystreptamine moiety met the requirements of (*a*) the solubility of the reagents in inert organic solvents (derivatives of 2-deoxystreptamine, unless fully protected, form insoluble complexes with BF_3), and (*b*) the identification of reaction products which is difficult by p.m.r. spectroscopy when dealing with a large number of protons, unless there are signals which can be used as markers. Compound **6**, the by-product of the β-elimination process produced by anchimeric attack on the methyl carbamate function (3-NCOOMe) in **4** by HO-4, was easily converted back into **4** with MeOH–MeONa. BF_3-Catalysed glycosidation of **4** with 3,4,6-tri-*O*-acetyl-D-glucal gave 75% of the pure α-glycoside **7**, the p.m.r. spectrum (90 and 400 MHz) of which showed a signal for H-1′ at δ 5.48 (bs). The high value of this chemical shift is indicative of an α-linkage, the $J_{1',2'}$ value of ~0 Hz is consistent with an H-1′/2′ dihedral angle of 90°, and the half-boat conformation of the unsaturated ring forces the glycosidic link to be quasi-equatorial. The ^{13}C-n.m.r. spectrum of **7** revealed the anomeric purity, there being only one signal for C-1′ (94.5 p.p.m.). This, together with the signal for C-5′ (69.4 p.p.m.), accorded with an α-linkage. Although 10–15% of the corresponding β-glycoside was present in the crude mixture, it could not be isolated pure by column chromatography. Conventional deacetylation of **7** afforded **8** and catalytic reduction of **7** or **8** readily gave **9** or **10**, respectively.

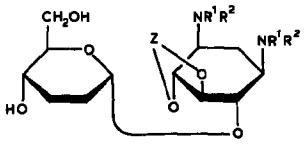
The racemic mixture[7] of **4** and **5**, easily prepared from kanamycin A, was used in the glycosidation step for large-scale preparations of **10**. This reaction and



7 R = OAc
8 R = OH



9 R = OAc
10 R = OH



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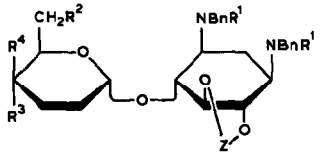
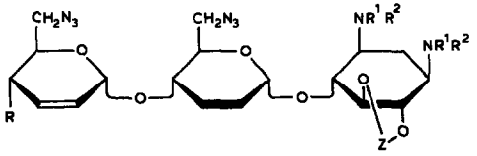
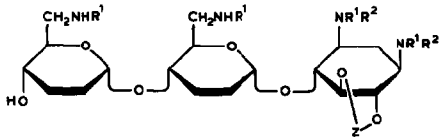
R^1 = Bn, R^2 = COOMe, Z = cyclohexylidene



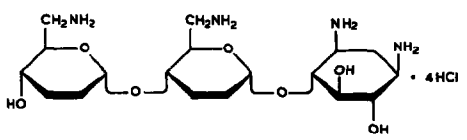
12 R^1 = COOMe, R^2 = N_3, R^3 = OH, R^4 = H
13 R^1 = COOMe, R^2 = N_3, R^3 = OAc, R^4 = H
14 R^1 = COOMe, R^2 = N_3, R^3 = H, R^4 = N_3
15 R^1 = H, R^2 = NH_2, R^3 = OH, R^4 = H
Z = cyclohexylidene



16 R = OAc
17 R = OH
R^1 = Bn, R^2 = COOMe, Z = cyclohexylidene



18 R^1 = Cbz
19 R^1 = R^2 = H
Z = cyclohexylidene



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the subsequent hydrogenation and deacetylation steps were telescoped without isolation of the intermediate products. The 4- and 6-linked isomeric disaccharides, thus generated, were separated by preparative h.p.l.c. and isolated in the ratio 2.4:1, in contrast to the 1:1 mixture of **4** and **5** used in the glycosidation. This result could be explained if the catalyst (BF_3-etherate) affected the ratio of the two isomeric cyclohexylidene derivatives *via* a migration during the reaction. Chemical shifts of the signals of the anomeric protons in these two isomers (5.25 and 5.10 p.p.m., respectively) as well as the larger $[\alpha]_D$ value exhibited by the 4-linked isomer accord with data reported[8] for similar types of pseudodisaccharide.

Selective formation of a phosphonium salt[9] at HO-6′ of **10** followed by reaction with azide ion in a one-pot reaction yielded 67% of the azido derivative **12** together with 20% of the 4′,6′-diazide **14**. Acetylation of **12** gave **13**. Hydrazinolysis of **12** in boiling 2-propanol in the presence of Pd–C for 38 h gave the amino derivative **15**. Glycosidation of **12** with 3,6-di-*O*-acetyl-6-azido-6-deoxy-D-glucal in the presence of BF_3-etherate in 1,2-dichloroethane gave the pseudotrisaccharide **16**.

Conventional deacetylation of **16** gave **17** which was then simultaneously saponified and reduced with hydrazine hydrate and Pd–C, and the product was isolated as the benzyloxycarbonylamino derivative **18**. Hydrogenolysis of **18** and removal of the *N*-benzyl substituent with palladium hydroxide afforded, after chromatography, pure **19** which gave positive tests with ninhydrin and anisaldehyde. Acid hydrolysis of the *O*-cyclohexylidene group of **19** yielded the pseudotrisaccharide **20**, isolated as the tetrahydrochloride, which had two ^{13}C-n.m.r. signals (92.7 and 93.6 p.p.m.) for anomeric carbons, thereby demonstrating the anomeric purity. One feature of these α-pseudo di- and tri-saccharides is that the chemical shift of the signal of the anomeric proton next to the aminocyclitol nucleus occurs at 5.00–5.25 p.p.m., whereas that next to the amino sugar in the trisaccharides occurs at 5.25–5.45 p.p.m.

The interpretation of the 90-MHz p.m.r. spectra was facilitated by measuring the spectra first at 20° and then at 80–100° when some multiplets coalesced. The spectra were then measured again at 20° in order to check for the absence of decomposition.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Organic solutions were dried with anhydrous sodium sulphate and concentrated at 10 Torr and 40°. Light petroleum refers to the fraction having b.p. 40–60°. Preparative chromatography was carried out on silica gel (Merck, 70–230 mesh) and t.l.c. on Silica Gel G (type 60) or GF_{254} (type 60) (Merck). Detection was effected by u.v. light (254 and 265 nm), I_2 vapour, ninhydrin, anisaldehyde, and charring with sulphuric acid. Analytical h.p.l.c. was performed on a column (250 × 4.6 mm) packed with ODS-Hypersyl (Magnus Scientific), semi-preparative h.p.l.c. with a column (250 × 16 mm, Knauer) packed with Lichrosorb RP-18 (7 μm), and preparative h.p.l.c. on a column (250 × 40 mm) packed with Lichroprep. RP-18 (25–40 μm, Merck) fitted in a Jobin Yvon Chromastopac instrument. Identifications were based on m.p.s., i.r. and p.m.r. spectra, R_F data, and $[\alpha]_D$ values (measured with a Bellingham and Stanley polarimeter). I.r. spectra were recorded with a Perkin–Elmer 157 G spectrometer on films. P.m.r. spectra were obtained with a Perkin–Elmer R32 (90 MHz) or Bruker (400 MHz) spectrometer for solutions in $CDCl_3$ (internal Me_4Si), unless otherwise stated. ^{13}C-N.m.r. spectra were measured at 62.9 MHz.

*Tetra-*N*-benzyl-3′,4′:5,6-di-*O*-cyclohexylidene-tetra-*N*-methoxycarbonylneamine* (**1**). — Sodium hydride (0.246 g, 10 mmol) was added gradually to a solution of 3′,4′:5,6-di-*O*-cyclohexylidene-tetra-*N*-methoxycarbonylneamine[5] (0.350 g, 0.5 mmol) in *N,N*-dimethylformamide (20 mL). The suspension was stirred for 1 h at 25° and then cooled to 0° whilst benzyl bromide (1 mL) was added. The mixture became solid after ~1 h and was then stored for 24 h at 25°. Pyridine (2 mL) was added with stirring during 1 h, and the mixture was then neutralised ($NaHCO_3$) and concentrated to dryness. The residue was extracted with light

petroleum (b.p. 80–100°, 3 × 10 mL), the combined extracts were concentrated, and the residue was partitioned between $CHCl_3$ (20 mL) and water (20 mL). The organic layer was washed with water (2 × 10 mL), dried, and concentrated. Preparative t.l.c. (EtOAc) of the solid residue (0.450 g, 83%) gave **1**, m.p. 97–100° (from ether–light petroleum), $[\alpha]_D^{22}$ +57° (*c* 1, chloroform); R_F 0.76 (ethyl acetate), 0.55 (chloroform), 0.40 (benzene–EtOAc, 9:2); ν_{max} 2930, 1690 (NCOOMe), 1600 cm^{-1} (Ph). P.m.r. data (80°): δ 1.20–1.60 (bm, 22 H, H-2*a*,2*e* and 20 cyclohexylidene protons), 3.58, 3.62, 3.65 (3 s, 3, 3, and 6 H, 4 COOMe), 5.45 (d, 1 H, H-1′), 7.00–7.30 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{60}H_{74}N_4O_{14}$: C, 67.02; H, 6.93; N, 5.21. Found: C, 66.90; H, 7.06; N, 4.96.

Tetra-N-*benzyl-5,6*-O-*cyclohexylidene-tetra*-N-*methoxycarbonylneamine* (**2**). — A solution of **1** (0.537 g, 0.5 mmol) in acetic acid (1:1, 15 mL) was heated at 56° for 7 h. T.l.c. (benzene–ethyl acetate, 1:1) then showed a major (R_F 0.30) and two minor components, R_F 0.75 (**1**) and 0.05. The mixture was neutralised ($NaHCO_3$) and concentrated, and the syrupy residue was partitioned between $CHCl_3$ (50 mL) and water (20 mL). The organic phase was washed with water (2 × 15 mL), dried, and concentrated. Trituration of the syrupy residue with hot light petroleum (b.p. 60–80°) gave a solid that was recrystallised from hot cyclohexane to give **2** (0.40 g, 81%), m.p. 113–116°, $[\alpha]_D^{22}$ +22° (*c* 1, chloroform); R_F 0.30 (benzene–EtOAc, 1:1), R_F 0.5 (EtOAc); ν_{max} 3400 (OH), 2935, 1690 (NCOOMe), 1600 cm^{-1} (Ph). N.m.r. data: ^1H (65°), δ 1.20–1.70 (bm, 12 H, H-2*a*,2*e* and 10 cyclohexylidene protons), 3.64, 3.69 (2 s, each 6 H, 4 COOMe), 5.35 (d, 1 H, H-1′), 7.10–7.40 (m, 20 H, 4 Ph); ^{13}C, δ 97 (C-1′), 112 (=C=, cyclohexylidene), 127 (CH_2–Ph), 156 (NCOO).

Anal. Calc. for $C_{54}H_{66}N_4O_{14}$: C, 65.19; H, 6.63; N, 5.53. Found: C, 65.70; H, 6.79; N, 5.80.

1,3-Di-N-*benzyl-4*-O-{*(1*R,2R*)-2-(*N-*benzylmethoxycarbonylamino)-1-*[*(1*R*)-2-(*N-*benzylmethoxycarbonylamino)-1-formylethoxy*]*-2-formylethyl*}*-5,6*-O-*cyclohexylidene-2-deoxy-1,3-di*-N-*methoxycarbonylstreptamine* (**3**). — A solution of **2** (1 g, 1 mmol) in tetrahydrofuran (10 mL) was treated with periodic acid (0.12 g) in the dark for 1 h, then neutralised ($NaHCO_3$), filtered, and concentrated. The residue was extracted with chloroform, and the dried extract was concentrated to give a glass which was purified by preparative t.l.c. to give **3**, m.p. 122° (from $CHCl_3$–light petroleum), $[\alpha]_D^{22}$ +27° (*c* 1, chloroform); R_F 0.9 (EtOAc), R_F 0.6 (benzene–EtOAc, 1:1), R_F 0.35 (benzene–EtOAc, 9:1); ν_{max} 2996 (CH), 1710 (CHO), 1695 (NCOOMe), 1600 (Ph), 1530 (C–N), 1460, 1250 cm^{-1}. P.m.r. data: δ 3.52 (m, 12 H, 4 NCOOMe), 5.62 (m, 1 H, H-1′), 7.52 (m, 20 H, 5 Ph), 9.70 and 9.80 (2 s, 2 H, 2 CHO).

Anal. Calc. for $C_{54}H_{66}N_4O_{15}$: C, 64.14; H, 6.58; N, 5.54. Found: C, 64.30; H, 6.70; N, 5.63.

1,3-Di-N-*benzyl-5,6*-O-*cyclohexylidene-2-deoxy-1,3-di*-N-*methoxycarbonylstreptamine* (**4**). — A solution of **3** (1.2 g, 1.1 mmol) in dry methanol (20 mL) was

heated with triethylamine (1 mL) at 25° for 24 h and then concentrated to dryness. The residue was washed with water and extracted into chloroform (3 × 30 mL), and the combined extracts were dried and concentrated to give a syrup, column chromatography (EtOAc) of which gave **4** (0.4 g) and 1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-1-*N*-methoxycarbonylstreptamine 3,4-carbamate (**6**, 0.15 g); compound **4** had m.p. 130–133° (from ether), $[\alpha]_D^{22}$ −9.5° (*c* 1, chloroform); R_F 0.4 (benzene–EtOAc, 1:1), R_F 0.51 (EtOAc), R_F 0.2 (benzene–EtOAc, 9:1); ν_{max} 3520 (OH), 2940, 1695 (NCOOMe), 1605 cm^{-1} (Ph). P.m.r. data: δ 1.55–1.80 (bm, 12 H, H-2*a*,2*e* and 10 cyclohexylidene protons), 2.54 (s, 1 H, OH), 3.66 and 3.69 (2 s, 6 H, 2 COOMe), 4.42 (m, 4 H, 2CH_2Ph), 7.23 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 66.80; H, 7.02; N, 5.11.

Compound **6** had m.p. 100–103° (from ether–EtOAc), $[\alpha]_D^{22}$ −35° (*c* 1, chloroform); R_F 0.3 (benzene–EtOAc, 9:1); ν_{max} 2960, 1760 (C=O, cyclic), 1690 (NCOOMe), 1605 cm^{-1} (Ph). P.m.r. data: δ 1.58 (bm, 12 H, H-2*a*,2*e* and 10 cyclohexylidene protons), 3.72 (s, 3 H, COOMe), 7.25 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{29}H_{34}N_2O_6$: C, 68.75; H, 6.76; N, 5.52. Found: C, 68.03; H, 6.75; N, 5.63.

A solution of **6** (0.15 g, 0.3 mmol) in dry methanol (15 mL) was treated at 70° for 2 h with sodium methoxide (20 mg), then neutralised (CO_2), and concentrated. The residue was extracted with chloroform and the extract was concentrated to give a solid residue (0.13 g, 81%) which was recrystallised from ether to give **4**, m.p. 130–132.5°.

*1,3-Di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-4-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-*D*-erythro-hex-2-enopyranosyl)-1,3-di-*N*-methoxycarbonylstreptamine* (**7**). — A mixture of **4** (0.306 g, 0.57 mmol), 3,4,6-tri-*O*-acetyl-D-glucal (0.24 g, 0.9 mmol), and chloroform (10 mL) was treated at −30° with boron trifluoride etherate (0.03 mL) and stirred at −5° for 2 h. Triethylamine (1 mL) was added, stirring was continued for 15 min, and the mixture was then washed with water (2 × 10 mL), dried, and concentrated. Column chromatography (benzene–chloroform) of the glassy product gave **7** (0.32 g, 75%), m.p. 104–108° (sintered at 74°) (from $CHCl_3$–light petroleum), $[\alpha]_D^{22}$ +49° (*c* 0.6, methanol); R_F 0.35 (benzene–EtOAc, 3:1); ν_{max} 1740 (OAc), 1695 (NCOOMe), 1605 (Ph), 915, 850 cm^{-1} (α-glycoside). N.m.r. data: ^1H (80°), δ 1.57 (m, 12 H, H-2*a*,2*e* and 10 cyclohexylidene protons), 2.04 (d, 6 H, 2 OAc), 3.68 (s, 6 H, 2 COOMe), 5.48 (bs, 1 H, H-1′), 5.84 (m, 2 H, H-2′,3′), 7.20 (m, 10 H, 2 Ph); ^{13}C, δ 20.7, 21.2 (2 Me–CO), 29.8 (C-2), 47.8, 47.9 (2 CH_2Ph), 58.0 (C-1), 58.2 (C-3), 62.3 (C-6′), 69.4 (C-5′), 73.1 (C-4′), 94.5 (C-1′), 109.4 [=C(O-)$_2$], 116.1 (C-2′), 115.5 (C-3′), 128.5 (Ph), 155.1 (NCOO), 168.5, 169.6 (2 Me–*C*O).

Anal. Calc. for $C_{40}H_{50}N_2O_{12}$: C, 63.99; H, 6.71; N, 3.73. Found: C, 64.15; H, 6.84; N, 3.69.

*1,3-Di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-4-*O*-(2,3-dideoxy-α-*D*-erythro-hex-2-enopyranosyl)-1,3-di-*N*-methoxycarbonylstreptamine* (**8**). — Conven-

tional deacetylation of **7** (0.1 g) with NaOMe–MeOH gave a glassy product (95%), column chromatography (C_6H_6–$CHCl_3$) of which gave **8**, m.p. 114–118° (sinters at 98°) (from EtOAc–light petroleum), $[\alpha]_D^{22}$ +36° (*c* 0.9, methanol); R_F 0.3 ($CHCl_3$–EtOAc, 1:1), 0.55 (EtOAc); ν_{max} 3440 (OH), 1695 (NCOOMe), 1605 and 1590 (Ph), 915 and 850 cm^{-1} (α-glycoside). ^{13}C-N.m.r. data: δ 25.1, 25.3, 26.1, 26.7, 36.4, 36.6 (C-2, and 5 cyclohexylidene carbons), 47.8, 48.0 (2 CH_2Ph), 51.0, 51.3 (OMe), 58.0, 58.2 (C-1,3), 62.1 (C-6'), 65.6, 65.9, 67.1, 67.3, 67.5 (C-4,4',5,5',6), 94.7 (C-1'), 109.5 (=C= cyclohexylidene), 125.4 (C-3'), 128.3, 128.4 (2 Ph), 129.2 (C-2'), 155.1, 155.4 (2 NCOO).

Anal. Calc. for $C_{36}H_{46}N_2O_{10}$: C, 64.84; H, 6.95; N, 4.20. Found: C, 64.71; H, 7.12; N, 4.13.

1,3-Di-N-*benzyl-5,6*-O-*cyclohexylidene-2-deoxy-4*-O-*(4,6-di*-O-*acetyl-2,3-dideoxy-α*-D-erythro-*hexopyranosyl)-1,3-di*-N-*methoxycarbonylstreptamine* (**9**). — A solution of **7** (0.1 g) in MeOH (15 mL) was hydrogenated over 10% Pd–C (10 mg) at 2 atm. for 3 h and then worked-up in the usual manner. Column chromatography (C_6H_6–$CHCl_3$) of the glassy product gave **9** (92 mg), m.p. 72–75° (from benzene–light petroleum), $[\alpha]_D^{22}$ +51° (*c* 1, chloroform); R_F 0.55 ($CHCl_3$–EtOAc, 5:1); ν_{max} 1730 (OAc), 1690 (NCOOMe), 1605 (Ph), 915 and 850 cm^{-1} (α-glycoside). P.m.r. data (95°): δ 1.26–1.89 (bm, 16 H, H-2,2',2',3',3' and 10 cyclohexylidene protons), 1.96, 1.99 (2 s, 6 H, 2 OAc), 3.63, 3.65 (2 s, 6 H, 2 COOMe), 5.27 (bs, 1 H, H-1'), 7.15 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{40}H_{52}N_2O_{12}$: C, 63.82; H, 6.96; N, 3.72. Found: C, 63.51; H, 7.16; N, 3.48.

1,3-Di-N-*benzyl-5,6*-O-*cyclohexylidene-2-deoxy-4*-O-*(2,3-dideoxy-α*-D-erythro-*hexopyranosyl)-1,3-di*-N-*methoxycarbonylstreptamine* (**10**). — (*a*) Deacetylation of **9** (50 mg) under the usual conditions (MeOH–MeONa) gave **10** (35 mg), m.p. 154–156° (from $CHCl_3$–light petroleum), $[\alpha]_D^{22}$ +49.5° (*c* 1, chloroform); R_F 0.5 (EtOAc), 0.5 ($CHCl_3$–EtOAc, 1:1); ν_{max} 3450 (OH), 1690 (NCOOMe), 1605 (Ph), 915 and 850 cm^{-1} (α-glycoside). P.m.r. data (80°): δ 1.15–2.00 (bm, 16 H, H-2,2,2',2',3',3' and 10 cyclohexylidene protons), 2.21 (bb, 2 H, 2 OH), 3.65, 3.66 (2 s, 3 H each, 2 COOMe), 5.24 (bs, 1 H, H-1'), 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{48}N_2O_{10}$: C, 64.65; H, 7.23; N, 4.18. Found: C, 64.82; H, 7.39; N, 4.25.

(*b*) A chloroform solution (10 mL) of the racemic mixture (0.5 g, 0.92 mmol) of **4** and **5** and 3,4,6-tri-*O*-acetyl-D-glucal (0.304 g, 1.11 mmol) was treated with BF_3-etherate (0.05 mL) at −30° and stirred at −5° for 2 h. Triethylamine (1 mL) was added, stirring was continued for 15 min, and then the mixture was washed with water, dried, and concentrated. The syrupy residue was hydrogenated in MeOH (50 mL) at 3 atm. for 24 h over 10% Pd–C (50 mg). The solution was filtered and concentrated, and the syrupy residue was taken up in dry MeOH (60 mL) and deacetylated with MeONa (10 mg) for 24 h. The mixture was neutralised (CO_2) and concentrated, and the residue was filtered through silica gel using EtOAc. Concentration of the eluate gave a glassy product (0.482 g, 80%). T.l.c.

(ethyl acetate) showed a major (R_F 0.45) and a minor component (R_F 0.6). Analytical r.p.h.p.l.c. (RP-18, MeOH–H_2O, 85:15) also showed two components, R_T 540 and 429 s, in the ratio 2:1. Preparative r.p.h.p.l.c. gave **10** (0.289 g, 60%, R_T 540 s) identical with the product in (*a*).

1,3-Di-N-*benzyl-4,5*-O-*cyclohexylidene-2-deoxy-6*-O-*(2,3-dideoxy-α*-D-erythro-*hexopyranosyl)-1,3-di*-N-*methoxycarbonylstreptamine* (**11**). — The component (0.12 g, 25%) with R_T 429 s, isolated in (*b*) above, was identified as **11**, m.p. 121–125° (from $CHCl_3$–light petroleum), $[\alpha]_D^{22}$ +29° (*c* 1, chloroform); ν_{max} 3470 (OH), 1695 (NCOOMe), 1605 cm^{-1} (Ph). P.m.r. data (80°): δ 1.55 (bm, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 2.25 (bb, 2 H, 2 OH), 3.66 (2 s, 3 H each, 2 COOMe), 5.10 (bs, 1 H, H-1′), 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{48}N_2O_{10}$: C, 64.65; H, 7.23; N, 4.19. Found: C, 64.59; H, 7.00; N, 4.08.

4-O-*(6-Azido-2,3,6-trideoxy-α*-D-erythro-*hexopyranosyl)-1,3-di*-N-*benzyl-5,6*-O-*cyclohexylidene-2-deoxy-1,3-di*-N-*methoxycarbonylstreptamine* (**12**). — To a cooled solution (−55°) of **10** (0.671 g, 1 mmol) and CCl_4 (0.614 g, 4 mmol) in *N,N*-dimethylformamide (5 mL) under N_2 was added tris(dimethylamino)phosphine (0.435 g, 2.66 mmol) dropwise with stirring. The temperature was then allowed to rise to −45°, stirring was continued for 1.5 h, and sodium azide (0.65 g, 10 mmol) was added. The mixture was stirred at 60° under N_2 for 48 h and then concentrated, and the residue was triturated with ice–water and extracted with ether (3 × 20 mL). The usual work-up of the combined extracts gave a glassy residue (0.747 g), column chromatography (ether) of which gave glassy **12** (0.466 g, 67%), m.p. 92°, $[\alpha]_D^{22}$ +32° (*c* 1, chloroform); R_F 0.7 (EtOAc), 0.6 (EtOAc–MeOH, 20:1), 0.35 ($CHCl_3$–EtOAc, 3:1); ν_{max} 3480 (OH), 2100 (N_3), 1690 (NCOOMe), 1605 (Ph), 915 and 855 cm^{-1} (α-glycoside). P.m.r. data (80°): δ 1.29–2.00 (bm, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 3.67 (2 s, 3 H each, 2 COOMe), 5.29 (m, 1 H, H-1′), 7.18 (bm, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{47}N_5O_9$: C, 62.32; H, 6.82; N, 10.09. Found: C, 62.48; H, 7.02; N, 9.73.

Conventional treatment of **12** with Ac_2O–pyridine gave **13** (90%), m.p. 73–75° (from benzene–light petroleum), $[\alpha]_D^{22}$ +56° (*c* 1, chloroform); R_F 0.6 ($CHCl_3$–EtOAc, 5:1), 0.5 (benzene–EtOAc, 5:1); ν_{max} 2105 (N_3), 1735 (OAc), 1690 (NCOOMe), 1610 (Ph), 915 and 850 cm^{-1} (α-glycoside). P.m.r. data (80°): δ 1.28–1.97 (bm, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 2.00 (s, 3 H, OAc), 3.28 (m, 2 H, CH_2N_3), 3.66, 3.68 (2 s, 3 H each, 2 COOMe), 4.42 (m, 4 H, 2 CH_2Ph), 4.73 (m, 1 H, H-4′), 5.33 (bs, 1 H, H-1′), 7.18 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{38}H_{49}N_5O_{10}$: C, 62.03; H, 6.71; N, 9.52. Found: C, 62.25; H, 6.73; N, 7.38.

1,3-Di-N-*benzyl-5,6*-O-*cyclohexylidene-2-deoxy-4*-O-*(4,6-diazido-2,3,4,6-tetradeoxy-α*-D-threo-*hexopyranosyl)-1,3-di*-N-*methoxycarbonylstreptamine* (**14**). — A second component was isolated as a glass from the above chromatography of **12**. Recrystallisation from cyclohexane–light petroleum gave **14** (0.15 g), m.p. 118–

121°, $[\alpha]_D^{22}$ +37° (*c* 0.75, chloroform); R_F 0.65 ($CHCl_3$–EtOAc, 5:1); ν_{max} 2105 (N_3), 1690 (NCOOMe), 1600 (Ph), 910 and 850 cm^{-1} (α-glycoside). P.m.r. data (100°): δ 1.57 (bm, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 3.66 (s, 6 H, 2 COOMe), 4.43 (m, 4 H, 2 CH_2Ph), 5.34 (bs, 1 H, H-1′), 7.19 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{36}H_{46}N_8O_8$: C, 60.15; H, 6.45; N, 15.59. Found: C, 60.35; H, 6.49; N, 15.37.

*4-*O*-(6-Amino-2,3,6-trideoxy-α-*D*-*erythro*-hexopyranosyl)-1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxystreptamine* (**15**). — A mixture of **12** (0.12 g, 0.18 mmol), hydrazine hydrate (90%, 5 mL), 2-propanol (4 mL), and 10% Pd–C (30 mg) was boiled under reflux under N_2 for 38 h and then filtered. The catalyst was washed with ethanol, the combined filtrate and washings were concentrated, and water (4 × 15 mL) was evaporated from the residue to remove hydrazine. The resulting syrup was extracted with $CHCl_3$ (2 × 10 mL), and the combined extracts were dried and concentrated. Preparative t.l.c. (MeOH–acetone, 6:1; R_F 0.25) of the residue gave **15**, m.p. 182–186° (from EtOH–ether), $[\alpha]_D^{22}$ +60° (*c* 1, methanol); ν_{max} 3300 (OH, NH, NH_2), 1600 (Ph), 915 cm^{-1} (α-glycoside). P.m.r. data: δ 1.58 (bm, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 3.85 (bm, 4 H, 2 CH_2Ph), 4.80 (b, 5 H, OH, 2 NH, NH_2), 5.37 (bs, 1 H, H-1′), 7.26 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{32}H_{47}N_3O_5$: C, 69.41; H, 8.55; N, 7.50. Found: C, 69.60; H, 8.42; N, 7.62.

*4-*O*-[4-O-(4-O-Acetyl-6-azido-2,3,6-trideoxy-α-*D*-*erythro*-hex-2-enopyranosyl)-6-azido-2,3,6-trideoxy-α-*D*-*erythro*-hexopyranosyl]-1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-1,3-di-*N*-methoxycarbonylstreptamine* (**16**). — A cooled (−15°) solution of **12** (0.35 g, 0.55 mmol) in dry 1,2-dichloromethane (5 mL) was stirred with BF_3-etherate (0.2 mL) and a solution of 3,4-di-*O*-acetyl-6-azido-6-deoxy-D-glucal [0.167 g, 0.64 mmol; prepared from the corresponding known[10] 6-toluene-*p*-sulphonate and sodium azide in *N,N*-dimethylformamide; b.p. 120–130°/1 Torr, $[\alpha]_D^{22}$ +25° (methanol)] in 1,2-dichloroethane (1 mL). The mixture was stirred at −5° for 1.5 h under N_2, triethylamine (1 mL) was then added, and stirring was continued for 0.5 h. The mixture was then washed with water (3 × 3 mL), dried, and concentrated. Preparative t.l.c. [light petroleum–ether (1:1) and then benzene–ethyl acetate] gave **16** as a glassy product (0.46 g, 88%), m.p. 129–133°, $[\alpha]_D^{22}$ +42.5° (*c* 0.5, chloroform); R_F 0.3 (benzene–ethyl acetate, 3:1); ν_{max} 2110 (N_3), 1740 (OAc), 1700 (NCOOMe), 1605 (Ph), 920 and 860 cm^{-1} (α-glycoside). P.m.r. data (80°): δ 1.58 (m, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 2.08 (s, 3 H, OAc), 3.67 (s, 6 H, 2 COOMe), 5.23 (bs, 1 H, H-1′), 5.34 (bs, 1 H, H-1″), 5.84 (m, 2 H, H-2″,3″), 7.21 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{44}H_{56}N_8O_{12}$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.31; H, 6.52; N, 12.59.

*4-*O*-[6-azido-4-O-(6-azido-2,3,6-trideoxy-α-*D*-*erythro*-hex-3-enopyranosyl)-2,3,6-trideoxy-α-*D*-*erythro*-hexopyranosyl]-1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-*

2-deoxy-1,3-di-N-*methoxycarbonylstreptamine* (**17**). — Compound **16** (0.25 g, 0.28 mmol) was deacetylated (MeOH–MeONa) to give **17** (0.23 g, 96%), m.p. 126–132° (from ether–light petroleum), $[\alpha]_D^{22}$ +34° (*c* 0.68, chloroform); R_F 0.5 (benzene–EtOAc), 0.25 ($CHCl_3$); ν_{max} 3420 (OH), 2100 (N_3), 1690 (NCOOMe), 1600 (Ph), 910 and 850 cm^{-1} (α-glycoside). P.m.r. data: δ 1.55 (bm, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 2.40 (b, 1 H, OH), 3.65 (s, 6 H, 2 COOMe), 5.15 (bs, 1 H, H-1′), 5.31 (bs, 1 H, H-1″), 5.85 (m, 2 H, H-2″,3″), 7.25 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{42}H_{54}N_8O_{11}$: C, 59.56; H, 6.43; N, 13.23. Found: C, 59.65; H, 6.63; N, 13.29.

1,3-Di-N-*Benzyl-1,3-di*-N-*benzyloxycarbonyl-4*-O-[*6-benzyloxycarbonylamino-4*-O-*(6-benzyloxycarbonylamino-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl)-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl*]-*5,6*-O-*cyclohexylidene-2-deoxystreptamine* (**18**). — A solution of **17** (0.192 g, 0.227 mmol) in MeOH (6 mL) was hydrogenated over 10% Pd–C (50 mg) at 3.5 atm. for 24 h, then filtered, and concentrated to give a glassy product (0.169 g, 89%), R_F 0.4 (t.l.c.; 1-propanol–pyridine–acetic acid–water, 15:10:3:12). This was heated under reflux and N_2 with aqueous 90% hydrazine hydrate (2 mL) and 1-propanol (3 mL) for 48 h. Concentration of the solution and azeotropic removal of traces of hydrazine with water left a glassy residue (0.13 g), R_F 0.05 (EtOAc–MeOH–conc. ammonia, 10:1:1), a solution of which in aqueous acetone (2:1, 4 mL) was treated with Na_2CO_3 (0.2 g) followed by the dropwise addition of benzyl chloroformate (0.3 mL) at 0° for 0.5 h and then storage at 20° for 8 h. The solid residue obtained on concentration of the mixture was extracted into $CHCl_3$ (4 × 2 mL). The usual work-up of the extracts gave a glassy product, preparative h.p.l.c. (R_T 762 s, RP:18; MeOH–H_2O, 12:1; 1 mL/min) of which gave **18** (0.231 g, 84%), $[\alpha]_D^{22}$ +136° (*c* 1, chloroform); R_F 0.6 (EtOAc); ν_{max} 3400 (OH, NH), 1700 (NCOOBn), 1600 cm^{-1} (Ph). P.m.r. data (400 MHz): δ 1.24–1.95 (m, 20 H, 10 CH_2), 3.50 (m, 10 H, 5 NCH_2), 3.80–4.70 (b, 12 H, OH, NH, CH-O), 4.80–5.45 (m, 10 H, 4 CH_2Ph, H-1′,1″), 7.25 (m, 30 H, 6 Ph).

Anal. Calc. for $C_{70}H_{80}N_4O_{15}$: C, 69.06; H, 6.62; N, 4.60. Found: C, 69.30; H, 6.51; N, 4.70.

4-O-[*6-Amino-4*-O-*(6-amino-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl)-2,3,6-trideoxy*-α-D-erythro-*hexopyranosyl*]-*5,6*-O-*cyclohexylidene-2-deoxystreptamine* (**19**). — A mixture of **18** (0.234 g, 0.19 mmol), 1-propanol (2 mL), aqueous 90% hydrazine hydrate (2 mL), and Pd–C (30 mg) was heated under reflux and N_2 for 24 h. The catalyst was collected and washed with ethanol (82 × 5 mL), and the combined filtrate and washings were hydrogenated over 20% palladium hydroxide (30 mg) at 3.5 atm. for 18 h. The mixture was filtered and concentrated, and column chromatography (methanol–conc. ammonia, 100:1; then methanol) of the residue gave **19** as a glass (98 mg, 95%), which gave positive colour tests with ninhydrin and anisaldehyde spray reagents and had $[\alpha]_D^{22}$ +124° (*c* 1, methanol); ν_{max} 3400 (NH, OH), 2980 cm^{-1} (C–H). P.m.r. data: δ 1.10–1.90 (m, 20 H, skeletal and cyclohexylidene protons), 5.00–5.50 (m, 2 H, H-1′,1″).

Anal. Calc. for $C_{24}H_{44}N_4O_7$: C, 57.58; H, 8.85; N, 11.24. Found: C, 57.38; H, 8.98; N, 11.02.

*4-O-[6-Amino-4-O-(6-amino-2,3,6-trideoxy-α-*D*-erythro-hexopyranosyl)-2,3,6-trideoxy-α-*D*-erythro-hexopyranosyl]*-2-deoxystreptamine (**20**). — A solution of **19** (82 mg, 0.164 mmol) in hydrochloric acid (15%, 2 mL) was heated at 80° for 3 h and then concentrated. A solution of the residue in water (1 mL) was heated at 80° for 1 h and then concentrated to give **20** as the glassy tetrahydrochloride (93 mg, 100%), which gave a positive ninhydrin colour test and had m.p. 250° (gradual decomposition), $[\alpha]_D^{22}$ +108° (*c* 1, water); R_F 0.25 (MeOH–conc. ammonia, 100:1), ν_{max} 3400 (OH, NH), 1620 cm^{-1} (NH). N.m.r. data: ^1H (D_2O, 400 MHz), δ 5.00 (m, 1 H, H-1′), 5.30 (m, 1 H, H-1″); ^{13}C (D_2O–DCl), δ 26.2, 26.4 (C-2′,2″), 28.3, 28.6 (C-3′,3″), 33.0 (C-2), 41.3, 41.7 (C-6′,6″), 48.2 (C-1), 52.8 (C-3), 69.0, 69.3, 69.8, 70.2 (C-4″,5,5′,6), 71.5 (C-5″), 73.3, 73.4 (C-4,4′), 92.7 (C-1′), 93.6 (C-1″).

Anal. Calc. for $C_{18}H_{36}N_4O_7 \cdot 4$ HCl: Equiv. wt. (in glacial acetic acid), 141.67; C, 38.16; H, 6.40; N, 9.80. Found: Equiv. wt., 139.86; C, 38.00; H, 6.20; N, 9.50.

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