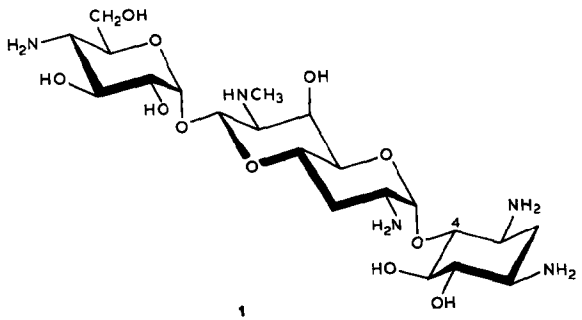
Carbohydrate Research, 165 (1987) 129–133
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Apramycin analogues

ANTONIO CANAS-RODRIGUEZ AND LUIS ANTONIO CORONEL-BORGES
Chelsea Department of Pharmacy, King's College, Manresa Road, London SW3 6XL (Great Britain)
(Received January 6th, 1987; accepted for publication, February 11th, 1987)

Two factors have motivated our synthetic studies[1] of aminoglycoside antibiotic analogues, especially those related to apramycin (**1**), namely, the high stability[2] of this antibiotic to enzymic deactivation by aminoglycoside-resistant bacterial strains and the efficiency of the glycosidation procedure (both in terms of yield and stereoselectivity) leading to α-linked glycosides[1,3].



1

In exploring the structure–activity relationships in this family of antibiotics, we have synthesised an α-linked pseudotrisaccharide analogue of apramycin.

BF_3-Catalysed glycosidation of 1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-1,3-di-*N*-methoxycarbonylstreptamine[3] (**3**) with hexa-*O*-acetyl-D-maltal[4] (**2**) in 1,2-dichloromethane and catalytic hydrogenation of the product, without purification, gave the pseudotrisaccharide α-glycoside **4** together with some polymeric products which were separated by semi-preparative reverse-phase h.p.l.c. The ^{13}C-n.m.r. spectrum of **4** confirmed the anomeric purity since there was only one signal for each of the anomeric carbons (C-1′ and C-1″), occurring at δ 93.4 and 94.8 p.p.m. Because of overlapping signals, the stereochemistry of the newly formed glycosidic bond could not be deduced from the ^{1}H-n.m.r. spectrum, but the high, positive $[\alpha]_D^{22}$ value (+103°) of **4** indicated an α-glycoside. Conventional deacetylation of **4** led to **5** which, after reverse-phase h.p.l.c., was

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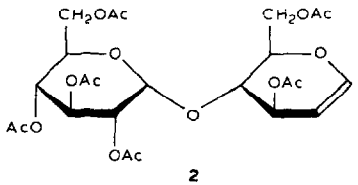
obtained as a glassy product. Its ^{1}H-n.m.r. spectrum contained two signals for anomeric protons, δ 5.06 (bs, H-1′) and 5.32 (d, H-1″).

Selective formation of phosphonium salts at the primary hydroxyl groups of **5**, using tris(dimethylamino)phosphine[5] in *N*,*N*-dimethylformamide, followed by their nucleophilic displacement with azide ion, gave 60% of the diazide **6**, the ^{1}H-n.m.r. spectrum of which exhibited the two signals for anomeric protons at δ 5.00 (bs, H-1′) and 5.23 (d, H-1″).



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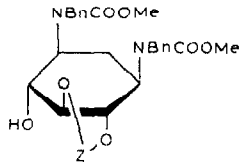
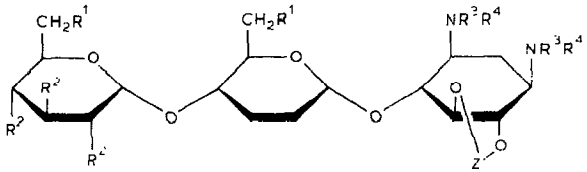


3

Z = cyclohexylidene



4 $R^1 = R^2 = OAc, R^3 = COOMe, R^4 = Bn$

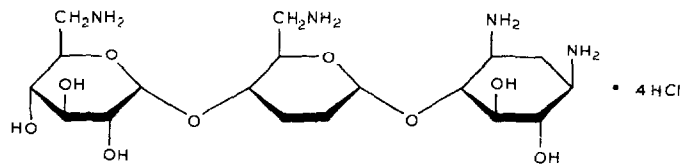
5 $R^1 = R^2 = OH, R^3 = COOMe, R^4 = Bn$

6 $R^1 = N_3, R^2 = OH, R^3 = COOMe, R^4 = Bn$

7 $R^1 = NH_2, R^2 = OH, R^3 = R^4 = H$

Simultaneous reduction of the azido groups and saponification of the carbamate groups of **6** in boiling 1-propanol, with hydrazine hydrate in the presence of 10% Pd/C, gave a product which was not purified but hydrogenolysed over 20% $Pd(OH)_2$ to produce the tetra-amine **7**. The ^{1}H-n.m.r. spectrum of **7** had signals for the two anomeric protons at δ 5.06 (dd, H-1′) and 5.42 (d, H-1″). The equivalent weight of **7** accorded with the presence of four amino groups.

Hydrolysis of the cyclohexylidene acetal group from **7** with aqueous HCl, followed by t.l.c., afforded the pseudotrisaccharide **8** as a homogeneous glassy solid with a high, positive $[\alpha]_D^{22}$ value (+140°) and the expected equivalent weight. C.i.



8

(isobutane)-m.s. of **8** gave, *inter alia*, a peak for $(M + 1)^+$ at *m/z* 453. The ^{13}C-n.m.r. spectrum of **8** included signals for the anomeric carbons at δ 93.4 (C-1′) and 94.8 (C-1″), which, together with the signals for C-5′ and C-5″ (δ 69.7 and 71.6, respectively), supported the α,α-structure.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Organic solutions were dried with anhydrous Na_2SO_4 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), 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 Chromatospac prep. 100 instrument. Identifications were based on m.p.s, i.r. and ^1H-n.m.r. spectra, and R_F and $[\alpha]_D^{22}$ values (measured with a Bellingham and Stanley polarimeter). I.r. spectra were recorded with a Perkin–Elmer 157 G spectrometer on films. ^1H-N.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.

4-O-[6-O-Acetyl-2,3-dideoxy-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-α-D-erythro-hexopyranosyl]-1,3-di-N-benzyl-5,6-O-cyclohexylidene-2-deoxy-1,3-di-N-methoxycarbonylstreptamine (**4**). — A stirred solution of **3** (ref. 3) (4.3 g, 8 mmol) in dry 1,2-dichloroethane (100 mL) at −30° was treated in sequence with BF_3-etherate (0.2 mL) and a solution of hexa-*O*-acetyl-D-maltal[4] (**2**; 8.96 g, 16 mmol) in the same solvent (20 mL). The mixture was stirred at −5° for 2 h, then neutralised with triethylamine (1 mL), stirred for a further 0.5 h, washed with water (3 × 30 mL), dried, and concentrated. T.l.c. (benzene–EtOAc, 2:1) of the residue showed the absence of **2** and **3**. A solution of the residue in MeOH (150 mL) was hydrogenated over 10% Pd/C catalyst (0.1 g) at 3.5 atm. for 48 h, then filtered, and concentrated. Semi-preparative h.p.l.c. [RP-18, MeOH–H_2O (183:17) at 6 mL/min] of the residue (14 g) gave, first, material, *T* 0–9 min (negative test with anisaldehyde), which contained self-condensation products of **2**. Eluted second was **4** (7.07 g, 85%), positive anisaldehyde test, *T* 13 min, $[\alpha]_D^{22}$ +102° (*c* 0.8, chloroform), R_F 0.59 (EtOAc–benzene, 2:1), *T* 4 min [analytical h.p.l.c., RP-18, MeOH–water (187:13) at 1 mL/min]; ν_{max} 3030, 2970, 2880 (C–H), 1750 (OAc), 1697 cm^{-1} (NCOOMe). N.m.r. data: ^1H (90 MHz, 80°): δ 1.58 (m, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 2.00 (5 s, each 3 H, 5 OAc), 3.65, 3.68 (2 s, each 3 H, 2 COOMe), 5.17 (m, 6 H, H-1′,1″ and 4 CH_2Ph), 7.22 (m, 10 H, 2 Ph); appropriate resonances for the other 15 skeletal protons were observed;

^{13}C, δ 14.1, 20.7, 23.8, 25.0, 28.9, 29.3, 30.9, 31.7, 36.3 (5 *Me*CO, C-2,2',3' and 5 CH_2 cyclohexylidene), 47.7 (2 MeO), 52.8 (2 CH_2O), 54.7, 54.8 (NCH_2Ph), 61.7, 63.0, 66.0, 68.3, 69.0, 70.1, 71.0, 75.7 (13 CH–O), 94.3 (C-1'), 94.8 (C-1''), 112.1 (C-acetal), 127.4, 128.7, 131.1, 139.1 (aromatic), 157.3 (N*C*OOMe), 169.9, 170.5, 170.9, 170.95, 171.0 (O*C*O–Me).

Anal. Calc. for $C_{52}H_{68}N_2O_{20}$: C, 59.99; H, 6.58; N, 2.69. Found: C, 60.33; H, 6.70; N, 2.51.

Deacetylation of **4**. — A solution of **4** (0.187 g, 0.179 mmol) in dry MeOH (3 mL) was treated with MeONa (20 mg) for 48 h. After the usual work-up, the residue (0.16 g) was extracted with $CHCl_3$ (4 × 1 mL). Concentration of the combined extracts gave a solid (0.145 g, 99%) which was purified by semi-preparative reverse-phase h.p.l.c. [*T* 2 min, RP-18, MeOH–water (187:13) at 1 mL/min] to give 1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-4-*O*-[2,3-dideoxy-4-*O*-α-D-glucopyranosyl-α-D-*erythro*-hexopyranosyl]-1,3-di-*N*-methoxycarbonylstreptamine (**5**), isolated as a glass which had m.p. 89°, $[\alpha]_D^{22}$ +108° (*c* 1, methanol); R_F 0.65 (MeOH–$CHCl_3$, 1:2); ν_{max} 3400 (OH), 2960, 2880 (C–H), 1695 cm^{-1} (NCOOMe). ^1H-N.m.r. data (90 MHz, D_2O, 80°): δ 1.58 (m, 16 H, H-2,2,2',2',3',3' and 10 cyclohexylidene protons), 3.34, 3.37 (2 s, each 3 H, 2 NCOOMe), 5.06 (bs, 1 H, H-1'), 5.32 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''), 7.21 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{42}H_{58}N_2O_{15}$: C, 60.71; H, 7.03; N, 3.37. Found: C, 60.84; H, 7.10; N, 3.50.

*4-O-[6-Azido-4-O-(6-azido-6-deoxy-α-*D-glucopyranosyl*)-2,3,6-trideoxy-α-*D-erythro-*hexopyranosyl*]-1,3-di-*N*-benzyl-5,6-*O*-cyclohexylidene-2-deoxy-1,3-di-*N*-methoxycarbonylstreptamine* (**6**). — A mixture of **5** (42 mg, 0.05 mmol), CCl_4 (124 mg, 0.08 mmol), and dry *N,N*-dimethylformamide (4 mL) at −55° under N_2 was treated with tris(dimethylamino)phosphine (49 mg, 0.3 mmol). The temperature was allowed to rise to −40° and stirring was continued for 2 h. Sodium azide (0.13 g, 2 mmol) was then added, and the mixture was stirred at 60° under N_2 for 48 h and then concentrated. The residue was triturated with water (4 mL) and extracted with $CHCl_3$ (3 × 2 mL), and the combined extracts were washed with water, dried, and concentrated. Preparative t.l.c. (ether–methanol), 9:1) of the residue gave **6** (24 mg, 60%), $[\alpha]_D^{22}$ +123° (*c* 1, chloroform); R_F 0.5 (EtOAc–MeOH, 9:1), 0.3 (Et_2O–MeOH, 9:1); ν_{max} 3480 (OH), 2940 (C–H), 2100 (N_3), 1700 (NCOOMe), 1600, 770, 700 cm^{-1} (Ph). ^1H-N.m.r. data (400 MHz): δ 1.20–1.90 (m, 16 H, H-2,2,2',2',3',3' and 10 cyclohexylidene protons), 3.64 (2 s, each 3 H, 2 NCOOMe), 4.50 (m, 4 H, 2 NCH_2Ph), 5.00 (bs, 1 H, H-1'), 5.23 (d, 2 H, $J_{1'',2''}$ 4 Hz, H-1''), 7.20 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{42}H_{56}N_8O_{13}$: C, 57.26; H, 6.40; N, 12.72. Found: C, 57.11; H, 6.50; N, 12.46.

*4-O-[6-Amino-4-O-(6-amino-6-deoxy-α-*D-glucopyranosyl*)-2,3,6-trideoxy-α-*D-erythro-*hexopyranosyl*]-5,6-*O*-cyclohexylidene-2-deoxystreptamine* (**7**). — A mixture of **6** (0.197 g, 0.22 mmol), 1-propanol (2 mL), hydrazine hydrate (90%, 2 mL), and 10% Pd/C catalyst (30 mg) was heated to reflux under N_2 for 48 h, then

filtered, and concentrated, and the residue was azeotroped with water to remove traces of hydrazine. The glassy product (110 mg, no i.r. bands for N_3 and NCOOMe) gave a positive colour test with anisaldehyde spray (presence of cyclohexylidene group). Hydrogenation of this material over 20% $Pd(OH)_2/C$ at 3 atm. for 24 h gave a glassy product (0.1 g), column chromatography (MeOH–conc. ammonia, 100:1) of which gave **7** (95 mg, 81%), $[\alpha]_D^{22}$ +131° (*c* 1, methanol), R_F 0.5 (above solvent); ν_{max} 3400 (NH, OH), 1620 cm^{-1} (NH). ^{1}H-N.m.r. data (D_2O, 400 MHz): δ 1.20–2.00 (m, 16 H, H-2,2,2′,2′,3′,3′ and 10 cyclohexylidene protons), 5.05 (dd, 1 H, $J_{1',2'e}$ 3.5 Hz, $J_{1',2'a}$ 1 Hz, H-1′), 5.42 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″).

Anal. Calc. for $C_{24}H_{44}N_4O_9$: equiv. wt. (in glacial acetic acid), 133.16; C, 54.12; H, 8.32; N, 10.51. Found: equiv. wt., 132; C, 54.34; H, 8.42; N, 10.17.

*4-O-[6-Amino-4-O-(6-amino-6-deoxy-α-*D-glucopyranosyl*)-2,3,6-trideoxy-α-*D-erythro-*hexopyranosyl*]-2-deoxystreptamine tetrahydrochloride or 2-deoxy-4-O-(6,6′-diamino-2,3,6,6′-tetradeoxy-α-*D-*maltosyl)streptamine tetrahydrochloride* (**8**). — A solution of **7** (0.12 g, 0.225 mmol) in hydrochloric acid (1.4M, 2 mL) was heated at 80° for 3 h, the reaction being monitored by t.l.c. (solvent as below). The mixture was concentrated to dryness, water (1 mL) was added, and the solution was heated at 80° for 1 h and then concentrated to a syrup. T.l.c. (MeOH–conc. ammonia, 100:1) of the product (0.136 g), R_F 0.2, followed by neutralisation with HCl, gave **8** as a glassy solid (0.12 g, 90%), which had $[\alpha]_D^{22}$ +140° (*c* 2, water); ν_{max} 3400 (OH,NH), 1620 cm^{-1} (NH). N.m.r. data: ^{1}H (D_2O, 400 MHz), δ 1.50–2.25 (m, 6 H, H-2,2,2′,2′,3′,3′), 3.48–4.20 (m, 15 H, other skeletal protons), 5.20 (t, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.30 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″); ^{13}C, δ 26.5, 28.8 (C-2′,3′), 34.0 (C-2), 41.7, 42.3 (C-6′,6″), 48.6 (C-1), 53.1 (C-3), 69.7 (C-5′), 69.8 (C-5), 70.5, 70.6 (C-6,4″), 71.6, 72.3, 72.5, 73.5, 74.0 (C-4,4′,2″,3″,5″, order undefined), 93.4 (C-1′), 94.8 (C-1″). C.i. mass spectrum (isobutane): *m/z* 453 $(M + 1)^+$, 291 $(C_{12}H_{23}N_2O_6)^+$, 290 $(C_{12}H_{24}N_3O_5)^+$, 161 $(C_6H_{13}N_2O_3)^+$.

Anal. Calc. for $C_{18}H_{36}N_4O_9 \cdot 4$ HCl: equiv. wt. (in glacial AcOH), 149.67; C, 36.11; H, 6.73; N, 9.35. Found: equiv. wt., 151; C, 35.95; H, 6.86; N, 9.08.

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