Semisynthetic Aminoglycoside Antibacterials. Part 7.[1,2] Synthesis of Novel Hexopyranosyl and Hexofuranosyl Derivatives of Gentamine C_1 and C_{1a}

By **Peter J. L. Daniels,* Charles E. Luce, Alan K. Mallams,* James B. Morton, Surinderjit S. Saluja, Hsingan Tsai, Jay Weinstein,** and **John J. Wright,** Research Division, Schering–Plough Corporation, Bloomfield, New Jersey 07003, U.S.A.

George Detre, Masato Tanabe, and **Dennis M. Yasuda,** Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025, U.S.A.

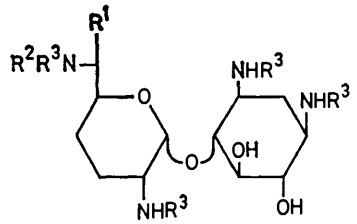
Acidic hydrolysis of gentamicin C_1 and C_{1a} provides ready access to the pseudodisaccharides gentamine C_1 and C_{1a} respectively. Glycosylation of tetrakis-*N*-benzyloxycarbonylgentamine C_1 and C_{1a} using the Koenigs–Knorr or Lemieux–Nagabhushan reactions has led to the preparation of a series of novel 6-*O*-α- and -β-3-amino-3-deoxy- and 2-amino-2-deoxy-D-hexopyranosyl and hexofuranosyl analogues of the gentamicins. The ^{13}C n.m.r. properties of representative compounds are described and the rotamer populations about the C-4–*O* and C-6–*O* glycosidic bonds are discussed.

THE occurrence and increasing incidence of multiple drug resistant strains of bacteria carrying extrachromosomal elements commonly referred to as R-factors [3] has given considerable impetus to the search for new semisynthetic aminoglycoside antibacterials. These efforts have yielded amikacin,[4] which is in clinical use and netilmicin,[5] which is currently undergoing clinical trials. In order to broaden our understanding of the structure–activity relationships that govern antibacterial activity, we embarked on an extensive semisynthetic aminoglycoside programme. Thus, the availability of garamine [6-8] made it possible to prepare a wide range of novel semisynthetic 4-*O*-glycosyl derivatives [9-13] related to the gentamicins, while the gentamines [14] provided useful substrates for the preparation of a variety of novel 6-*O*-glycosyl derivatives, some of which have previously been described.[15,16] We describe here and in the following paper [17] the syntheses of a number of novel 5-*O*- and 6-*O*-glycosyl derivatives of gentamine C_1 (1) and C_{1a} (2), which have further contributed to our understanding of the structure–activity relationships of these important antibiotics. After completion of this study there were reports of the glycosylation of derivatives of gentamine C_{1a} (2), which have led to the preparation of 3′,4′-dideoxykanamycin B (5),[18] *O*-3,4,6-trideoxy-3-methylamino-α-D-xylohexopyranosyl-(1 → 6)-gentamine C_{1a} (6) [19] and *O*-3-deoxy-3-methylamino-α-D-xylopyranosyl-(1 → 6)-gentamine C_{1a} (7).[20]

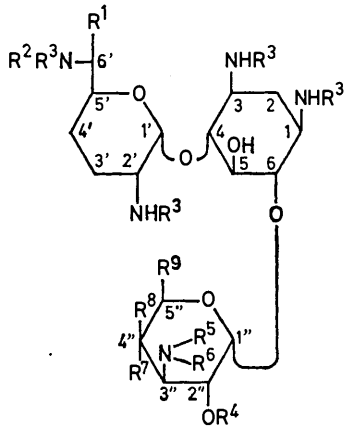
Acidic hydrolysis of gentamicin C_1 (8) and C_{1a} (9) provides ready access to the pseudodisaccharides gentamine C_1 (1) and C_{1a} (2) [14] respectively, which on treatment with benzyl chloroformate afforded 1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) and 1,3,-2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_{1a} (4) respectively. These derivatives were used directly for the glycosylation reactions without further protection of the hydroxy-groups. Thus (3) on treatment with 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (17),[21] under the conditions of the Koenigs–Knorr reaction as modified by Helferich,[22] afforded a mixture of the protected α- (10) and β-glycosides (29) which could not be separated. Reduction with sodium in liquid ammonia followed by basic hydrolysis afforded *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1 → 6)-gentamine C_1 (11) as the principal product of the reaction. The rotation was in agreement with an α-glycoside and the occurrence of a signal at δ_H 4.96 ($J_{1''eq,2''ax}$ 3.5 Hz) clearly supported the above assignment. The c.d. spectrum run in TACu and Cupra A supported the 1 → 6 linkage to the deoxystreptamine ring. Further support for the structure was obtained from the mass spectrum (Table 1) [23] and from the ^{13}C n.m.r. spectrum (Table 2).[24,25] The minor product of the reaction was *O*-3-amino-3-deoxy-β-D-glucopyranosyl-(1 → 6)-gentamine C_1 (30). The low positive rotation and the appearance of a doublet at δ_H 4.57 with $J_{1ax'',2ax''}$ 8.5 Hz, due to the newly formed anomeric proton, were consistent with values expected for a β-glycoside. The ^{13}C n.m.r. spectrum (Table 2) also revealed characteristic deshielding of C-1″, C-3″, and C-5″ relative to the α-glycoside (11) and also confirmed the 1 → 6 linkage. The c.d. data in TACu were also in agreement with such a linkage. The mass spectrum is detailed in Table 1.

The α-anomer (11) was also synthesized by the Lemieux–Nagabhushan reaction.[26-38] Thus 3-acetamido-4,6-di-*O*-acetyl-1,2,3-trideoxy-D-*arabino*-hex-1-enopyranose (31) [34] upon treatment with nitrosyl chloride gave the nitroso-chloro-adduct (32). The latter on condensation with 1,3,2′,6′-tetra-*N*-benzyloxycarbonylgentamine C_1 (3) in dimethylformamide afforded *O*-3-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 6)-1,3,2′,6′-tetra-*N*-benzyloxycarbonylgentamine C_1 (34). Deoximation gave the 2″-ketone which was reduced with sodium borohydride and then deprotected by treatment with sodium in liquid ammonia followed by basic hydrolysis to give the α-anomer (11), which was identical with that prepared by the Koenigs–Knorr reaction.

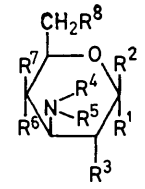
Although many aminoglycoside antibiotics are known that contain a 5-*O*-pentofuranosyl moiety,[35] none have yet been found to contain a 6-*O*-pento-, or -hexofuranosyl unit. It was therefore of interest to prepare



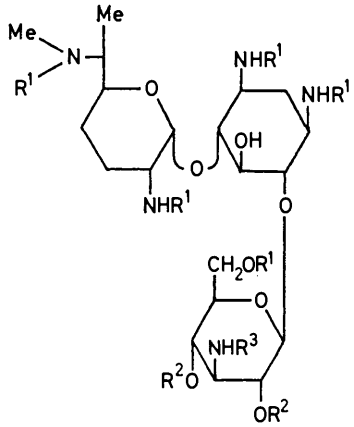
(1) $R^1 = R^2 = Me$, $R^3 = H$
(2) $R^1 = R^2 = R^3 = H$
(3) $R^1 = R^2 = Me$, $R^3 = Z$
(4) $R^1 = R^2 = H$, $R^3 = Z$



(5) $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = R^8 = H$, $R^7 = OH$, $R^9 = CH_2OH$
(6) $R^1 = R^2 = R^3 = R^4 = R^5 = R^7 = R^8 = H$, $R^6 = R^9 = Me$
(7) $R^1 = R^2 = R^3 = R^4 = R^5 = R^8 = R^9 = H$, $R^6 = Me$, $R^7 = OH$
(8) $R^1 = R^2 = R^6 = R^7 = Me$, $R^3 = R^4 = R^5 = R^9 = H$, $R^8 = OH$
(9) $R^1 = R^2 = R^3 = R^4 = R^5 = R^9 = H$, $R^6 = R^7 = Me$, $R^8 = OH$
(10) $R^1 = R^2 = Me$, $R^3 = Z$, $R^4 = CH_2Ph$, $R^5 = Ac$, $R^6 = R^8 = H$, $R^7 = OCH_2Ph$, $R^9 = CH_2OCH_2Ph$
(11) $R^1 = R^2 = Me$, $R^3 = R^4 = R^5 = R^6 = R^8 = H$, $R^7 = OH$, $R^9 = CH_2OH$
(12) $R^1 = R^2 = R^6 = Me$, $R^3 = Z$, $R^4 = CH_2Ph$, $R^5 = Ac$, $R^7 = OCH_2Ph$, $R^8 = H$, $R^9 = CH_2OCH_2Ph$
(13) $R^1 = R^2 = R^6 = Me$, $R^3 = R^4 = R^5 = R^8 = H$, $R^7 = OH$, $R^9 = CH_2OH$
(14) $R^1 = R^2 = R^6 = R^8 = H$, $R^3 = Z$, $R^4 = CH_2Ph$, $R^5 = Ac$, $R^7 = OCH_2Ph$, $R^9 = CH_2OCH_2Ph$
(15) $R^1 = R^2 = Me$, $R^3 = Z$, $R^4 = CH_2Ph$, $R^5 = Ac$, $R^6 = R^7 = H$, $R^8 = OCH_2Ph$, $R^9 = CH_2OCH_2Ph$
(16) $R^1 = R^2 = Me$, $R^3 = R^4 = R^5 = R^6 = R^7 = H$, $R^8 = OH$, $R^9 = CH_2OH$



(17) $R^1 = Cl$, $R^2 = R^4 = R^7 = H$, $R^3 = R^6 = R^8 = OCH_2Ph$, $R^5 = Ac$
(18) $R^1 = OMe$, $R^2 = R^7 = H$, $R^3 = OH$, $R^4 = Me$, $R^5 = Ac$, $R^6 = R^8 = OCH_2Ph$
(19) $R^1 = R^7 = H$, $R^2 = OMe$, $R^3 = OH$, $R^4 = Me$, $R^5 = Ac$, $R^6 = R^8 = OCH_2Ph$
(20) $R^1 = OMe$, $R^2 = R^7 = H$, $R^3 = R^6 = R^8 = OCH_2Ph$, $R^4 = Me$, $R^5 = Ac$
(21) $R^1 = R^7 = H$, $R^2 = OMe$, $R^3 = R^6 = R^8 = OCH_2Ph$, $R^4 = Me$, $R^5 = Ac$
(22) $R^1 = OAc$, $R^2 = R^7 = H$, $R^3 = R^6 = R^8 = OCH_2Ph$, $R^4 = Me$, $R^5 = Ac$
(23) $R^1 = R^7 = H$, $R^2 = OAc$, $R^3 = R^6 = R^8 = OCH_2Ph$, $R^4 = Me$, $R^5 = Ac$
(24) $R^1 = Cl$, $R^2 = R^7 = H$, $R^3 = R^6 = R^8 = OCH_2Ph$, $R^4 = Me$, $R^5 = Ac$
(25) $R^1 = R^4 = R^6 = H$, $R^2 = OMe$, $R^3 = R^7 = R^8 = OH$, $R^5 = Ac$
(26) $R^1 = R^4 = R^6 = H$, $R^2 = OMe$, $R^3 = R^7 = R^8 = OCH_2Ph$, $R^5 = Ac$
(27) $R^1 = R^4 = R^6 = H$, $R^2 = OAc$, $R^3 = R^7 = R^8 = OCH_2Ph$, $R^5 = Ac$
(28) $R^1 = Cl$, $R^2 = R^4 = R^6 = H$, $R^3 = R^7 = R^8 = OCH_2Ph$, $R^5 = Ac$



(29) $R^1 = Z$, $R^2 = CH_2Ph$, $R^3 = Ac$
(30) $R^1 = R^2 = R^3 = H$

$Z = PhCH_2O \cdot CO$

a furanosyl 3-aminoglycoside from gentamine. This was accomplished as follows. 1,2-*O*-Isopropylidene-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranose (37) [17] was converted into the 5,6-di-*O*-benzyl derivative (38), which was subjected to aqueous acidic hyrolysis followed by methanolysis to give methyl 5,6-di-*O*-benzyl-3-deoxy-3-methylamino-α-D-glucofuranoside (39) and the corresponding β-anomer (40). The rotations and ^1H n.m.r. spectra were in accord with the assigned anomeric configurations. Thus (39) gave a doublet at δ_H 4.88 with $J_{1,2}$ 4.2 Hz due to 1-H, while (40) gave a singlet at δ_H 4.74. The mass spectra of (39) and (40) also revealed characteristic fragments at *m/e* 356 (*a*), 296 (*d*), and 146 (*f*) consistent with the methyl furanoside structures (Figure 1). The above products were characterized as the de-acetyl derivatives because of the complex rotamer spectra obtained on the *N*-acetyl derivatives. When the reaction was repeated on a preparative scale, the products were *N*-acetylated prior to chromatography. Only small amounts of methyl 5,6-di-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranoside (41) and its β-anomer (42) were obtained pure. The assignment of the structures was confirmed by *N*-acetylation of (39) to give a sample identical with (41), and of (40) to give a sample identical with (42). The bulk of the material was obtained as a mixture consisting mainly of

TABLE 1

Mass-spectral fragment ions [*m/e* (%)]

Compound	$M^+ + 1$	M^+	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}
(11)	480(1)	479(1)	347(7)		319(3)	301(4)	352(2)	334(7)	324(7)	306(24)	191(18)	173(12)
(30)	480(0.3)	479(0.5)	347(4)		319(3)	301(3)	352(2)	334(2)	324(3)	306(8)	191(16)	173(19)
(13)	494(1)	493(1)	347(6)		319(11)	301(4)	366(5)	348(3)	338(8)	320(11)	191(12)	173(15)
(51)	494(1)	493(1)	347(2)		319(5)	301(3)	366(4)	348(3)	338(7)	320(7)	191(9)	173(17)
(5)	542(0.2)		319(2)		291(2)	273(4)	352(2)	334(2)	324(4)	306(4)	191(33)	173(35)
(16)	480(2)	479(1)	347(8)		319(1)	301(2)	352(2)	334(3)	324(9)	306(21)	191(18)	173(22)
(52)	480(1)	479(1)	347(4)	329(1)	319(1)	301(2)	352(4)	334(1)	324(4)	306(11)	191(12)	173(10)
(57)	480(1)	479(1)	347(6)	329(2)	319(2)	301(5)	352(2)	334(1)	324(4)	306(10)	191(23)	173(22)
(59)	480(0.7)	479(0.2)	347(4)	329(2)	319(8)	301(10)	352(3)	334(1)	324(3)	306(5)	191(56)	173(46)

Compound	A_{11}	A_{12}	B_1	C_1	D_1	D_2	D_7	D_8	E_1	E_3	F_1	F_2
(11)	163(26)	145(34)	157(100)	162(44)	462(0.5)	301(4)	422(6)	261(4)		360(1)	286(19)	291(8)
(30)	163(34)	145(53)	157(100)	162(45)	462(0.5)	301(3)	422(2)	261(13)		360(2)	286(12)	291(4)
(13)	163(20)	145(40)	157(100)	176(47)	476(1)	301(4)	436(7)	262(12)	402(1)	360(6)	286(12)	305(14)
(51)	163(20)	145(43)	157(100)	176(48)	476(1)	301(3)	436(5)	261(10)	402(1)	360(2)	286(10)	305(7)
(5)	163(62)	145(100)	129(61)	162(9)	434(1)					332(18)	258(4)	291(2)
(16)	163(26)	145(40)	157(100)	162(45)	462(1)	301(2)	422(6)	262(2)		360(2)	286(26)	291(8)
(52)	163(12)	145(20)	157(42)	162(16)	462(1)	301(2)	422(5)	261(5)			286(4)	291(3)
(57)	163(15)	145(56)	157(100)	162(13)	462(1)	301(5)	422(4)	261(11)			286(6)	291(3)
(59)	163(70)	145(99)	157(98)	162(21)	462(0.3)	301(10)	422(2)	263(36)			286(6)	291(6)

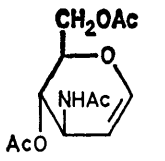
TABLE 2

^{13}C N.m.r. chemical shifts (δ_C, p.p.m. downfield from tetramethylsilane in D_2O)

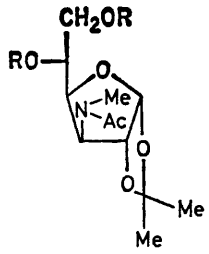
Carbon	(11)	(30)	(30)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(13)	(13)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(51)	(51)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(5)	(5)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(16)	(52)	(52)H$^+$	$\Delta\delta_C$(Base→H)
C-1	51.5	49.6	49.6 *a*		51.2	50.4	−0.8	50.7 *a*	50.0	−0.7	51.1	50.5	−0.6	52.0	50.7 *a*	49.6	−1.1
C-2	36.6	36.3	28.5	−7.8	36.5	28.5	−8.0	36.8	28.6	−8.2	36.3	28.5	−7.8	36.4	36.3	29.0	−7.3
C-3	50.8	50.7 *a*	49.2 *a*	−1.5	50.9 *a*	49.4 *a*.	−1.5	50.8 *a*	49.4 *a*	−1.4	50.6 *a*	49.5	−1.1	50.9 *a*	50.5 *a*	49.0	−1.5
C-4	89.0	87.9	76.9	−11.0	88.8	77.4	−11.4	88.1	77.5	−10.6	89.0	77.4	−11.6	89.2	88.0	78.2	−9.8
C-5	75.4	76.5	75.6	−0.9	75.3	75.2	−0.1	75.4	75.1	−0.3	75.4	75.2	−0.2	75.5	75.3	74.6	−0.7
C-6	87.9	87.9	80.4	−7.5	88.3	84.6	−3.7	87.9	82.3	−5.6	87.3	84.5	−2.8	88.1	86.2	82.2	−4.0
C-1′	102.5	102.7	95.7	−7.0	102.7	96.0	−6.7	102.7	96.1	−6.6	101.6	95.7	−5.9	102.7	100.8	95.7	−5.1
C-2′	50.8	50.3 *a*	49.5 *a*	−0.8	50.5 *a*	49.6 *a*	−0.9	50.4 *a*	49.6 *a*	−0.8	50.3 *a*	49.5	−0.8	50.8 *a*	50.2 *a*	49.6	−0.6
C-3′	26.8	26.9	21.3	−5.6	26.9	21.3	−5.6	26.8	21.3	−5.5	26.3	21.2	−5.1	26.7	25.9	21.4	−4.5
C-4′	25.5	25.8	23.4	−2.4	25.8	23.3	−2.5	25.7	23.3	−2.4	28.1	26.2	−1.9	25.7	25.2	23.2	−2.0
C-5′	72.7	72.8	70.0	−2.8	73.0	70.1	−2.9	73.0	70.1	−2.9	70.7	66.8	−3.9	72.8	70.3 *b*	70.2 *a*	−0.1
C-6′	58.1	58.0	58.5	+0.5	58.0	58.6	+0.6	58.0	58.5	+0.5	45.5	43.5	−2.0	58.0	58.6	58.5	−0.1
C-7′	14.2	14.7	10.8	−3.9	14.8	10.9	−3.9	14.7	10.8	−3.9				14.6	12.2	11.0	−1.2
6′-NCH$_3$	33.1	33.3	32.0	−1.3	33.4	32.1	−1.3	33.3	32.0	−1.3				33.3	32.3	32.1	−0.2
C-1″	100.8	104.6	103.6	−1.0	100.9	101.5	+0.6	104.6	103.1	−1.5	100.8	101.4	+0.6	101.0	100.6	96.5	−4.1
C-2″	72.5	74.6	70.3	−4.3	70.8	67.0	−3.8	75.9	74.3	−1.6	72.7	68.9	−3.8	70.6	56.3	54.7	−1.6
C-3″	55.0	58.7	58.3	−0.4	62.4	60.8	−1.6	66.4	64.4	−2.0	55.0	55.8	+0.8	50.5 *a*	74.5	70.4 *a*	−4.1
C-4″	70.3	70.4	66.5	−3.9	68.5	64.3	−4.2	78.1	76.5	−1.6	70.2	66.3	−3.9	69.7	70.7 *b*	69.8	−0.9
C-5″	73.0	78.2	77.6	−0.6	73.3	73.9	+0.6	70.7	70.9	+0.2	73.0	73.7	+0.7	72.8	73.4	74.5	+1.1
C-6″	61.3	61.6	60.9	−0.7	61.3	61.2	−0.1	64.1	63.4	−0.7	61.2	60.8	−0.4	61.8	61.3	61.3	
3″-NCH$_3$					33.9	30.1	−3.8	34.4	33.5	−0.9							

a,*b* May be interchanged in any vertical column.

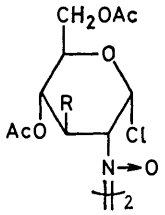
(41) and (42), with about 24% of the corresponding pyranosides (18) and (19), as determined by the presence of an additional 3-*N*-acetyl group at δ_H 2.07 due to the pyranosyl form of the sugar. The pyranosides appear to be forming by migration of the 5-*O*-benzyl group to the 4-position under the acidic hydrolysis conditions although we know of no precedent for such a reaction. Benzylation of the above mixture of methyl glycosides afforded methyl 2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranoside (43) and the β-anomer (44), together with the corresponding pyranosides (20) and (21). The mixture of methyl glycosides was subjected to acidic hydrolysis using a mixture of 1M-hydrochloric acid and glacial acetic acid and the products were acetylated to give a mixture of 1-*O*-acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranose (45) and the β-anomer (46) which could not be separated. The mass spectra showed the characteristic fragment ions *c*, *k*, and *l* (Figure 1). A mixture of 1-*O*-acetyl-2,4,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucopyranose (22) and its β-anomer (23) was also isolated. The more polar fractions from the column afforded 1,4-di-*O*-acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-D-*threo*-hex-1-enose (49), the spectral data of which were in accord with the assigned structure. On treatment with triethylamine in methanol, (49) was converted into the acetyl furanoses (45) and (46). The above mixture of 1-*O*-acetyl furanoses and pyranoses was converted into a mixture of α- (47) and β-furanosyl (48), and α-pyranosyl (24) chlorides by treatment with acetyl chloride and hydrogen chloride. The mixture of chloro-sugars was condensed with 1,3,2′,6′-tetra-*N*-benzyloxycarbonylgentamine C_1 (3) in the presence of silver toluene-*p*-sulphonate in dichloromethane to give a mixture of *O*-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (50) and *O*-2,4,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucopyranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (12) which could not be separated under the chromatographic conditions used. Deprotection of the mixture with sodium in liquid ammonia followed by basic hydrolysis gave after chromatography, *O*-3-deoxy-3-methylamino-α-D-gluco-



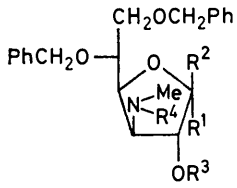
(31)



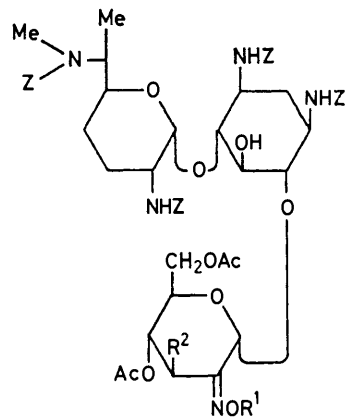
(37) R = H
(38) R = CH_2Ph



(32) R = NHAc
(33) R = OAc



(39) R^1 = OMe, R^2 = R^3 = R^4 = H
(40) R^1 = R^3 = R^4 = H, R^2 = OMe
(41) R^1 = OMe, R^2 = R^3 = H, R^4 = Ac
(42) R^1 = R^3 = H, R^2 = OMe, R^4 = Ac
(43) R^1 = OMe, R^2 = H, R^3 = CH_2Ph, R^4 = Ac
(44) R^1 = H, R^2 = OMe, R^3 = CH_2Ph, R^4 = Ac
(45) R^1 = OAc, R^2 = H, R^3 = CH_2Ph, R^4 = Ac
(46) R^1 = H, R^2 = Ac, R^3 = CH_2Ph, R^4 = Ac
(47) R^1 = Cl, R^2 = H, R^3 = CH_2Ph, R^4 = Ac
(48) R^1 = H, R^2 = Cl, R^3 = CH_2Ph, R^4 = Ac



(34) R^1 = H, R^2 = NHAc
(35) R^1 = H, R^2 = OAc
(36) R^1 = Ac, R^2 = OAc

furanosyl-(1 → 6)-gentamine C_1 (51) and *O*-3-deoxy-3-methylamino-α-D-glucopyranosyl-(1 → 6)-gentamine C_1 (13), which separated readily. Both compounds exhibited typical aminoglycoside mass spectra (Table 1). The furanoside (51) exhibited a doublet at δ_H 5.28 with $J_{1'',2''}$ 4.5 Hz, while the pyranoside (13) exhibited a doublet at δ_H 4.91 with $J_{1eq'',2ax''}$ 4 Hz, consistent with α-glycosidic linkages for the newly introduced sugars. The ^{13}C n.m.r. data (Table 2) were in excellent agreement with the assigned structures.

3′,4′-Dideoxykanamycin B (5) is a clinically useful semisynthetic aminoglycoside which has been prepared from kanamycin B [36–38] as well as from 3′,4′-dideoxyneamine.[18] We wish also to report the synthesis of (5) by condensation of 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (17) [21] with 1,3,2′,3′-tetra-*N*-benzyloxycarbonylgentamine C_{1a} (4) in the presence of toluene-*p*-sulphonate to give *O*-3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_{1a} (14) which on deprotection with sodium in liquid ammonia followed by basic hydrolysis afforded (5). The physical data (Tables 1 and 2) were in accord with the structure and the product was identical with an authentic sample (t.l.c.).

The synthesis of a galactosyl analogue of (11) was undertaken next. Methyl 3-acetamido-3-deoxy-β-D-galactopyranoside (25) [39] was converted into the benzyl derivative (26) which on acetolysis gave 3-acetamido-1-*O*-acetyl-2,4,6-tri-*O*-benzyl-3-deoxy-β-D-galactopyranose (27). The latter on treatment with acetyl chloride in the presence of hydrogen chloride gave 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-galactopyranosyl chloride (28) which was condensed with 1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) in the presence of silver toluene-*p*-sulphonate to give *O*-3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-galactopyranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (15).

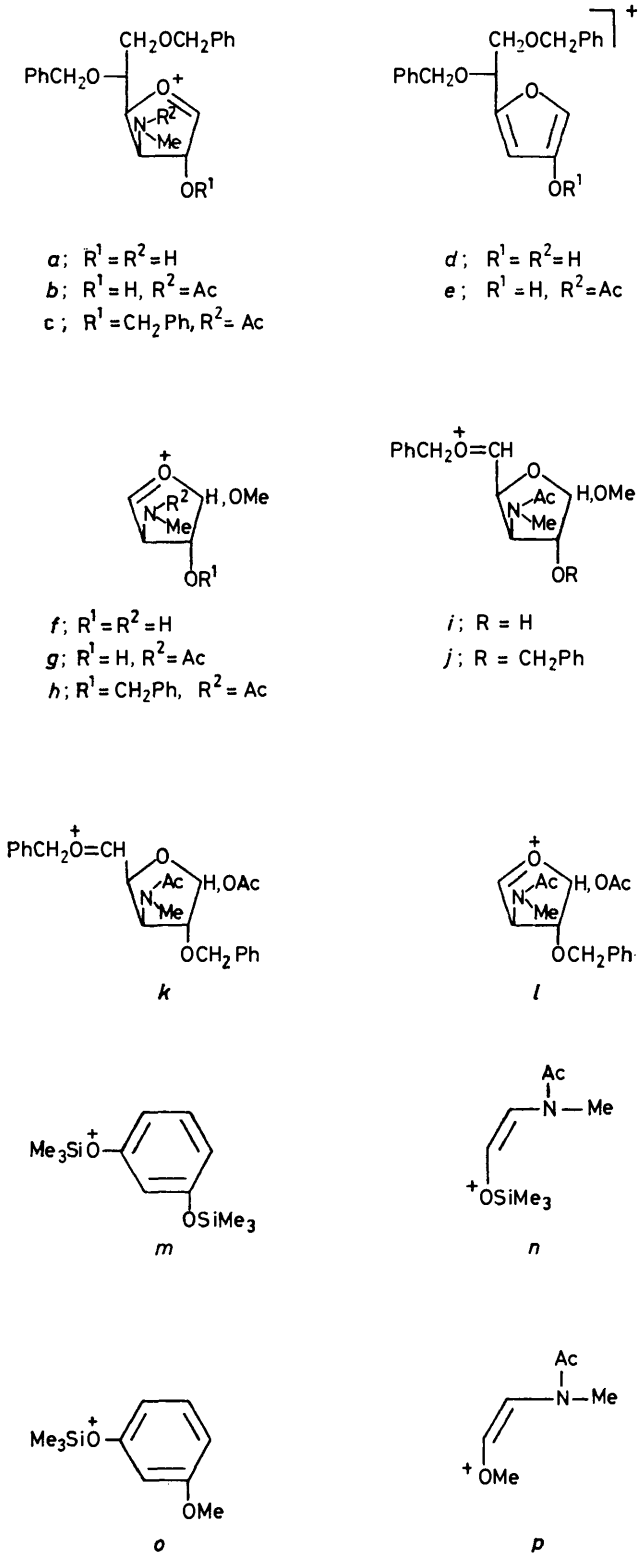


FIGURE 1 Mass-spectral fragment ions

Deprotection of the latter as described above gave *O*-3-amino-3-deoxy-α-D-galactopyranosyl-(1 ⟶ 6)-gentamine C_1 (16). The rotation, as well as the presence of a doublet with $J_{1eq'',2ax''}$ 4 Hz at δ_H 5.04 in the ^1H n.m.r. spectrum, supported the presence of the α-glycosidic linkage. The ^{13}C n.m.r. spectrum (Table 2) confirmed the anomeric linkage and showed that the product was a 6-*O*-glycosyl derivative. The synthesis of a galactopyranosyl analogue of tobramycin has previously been reported.[40]

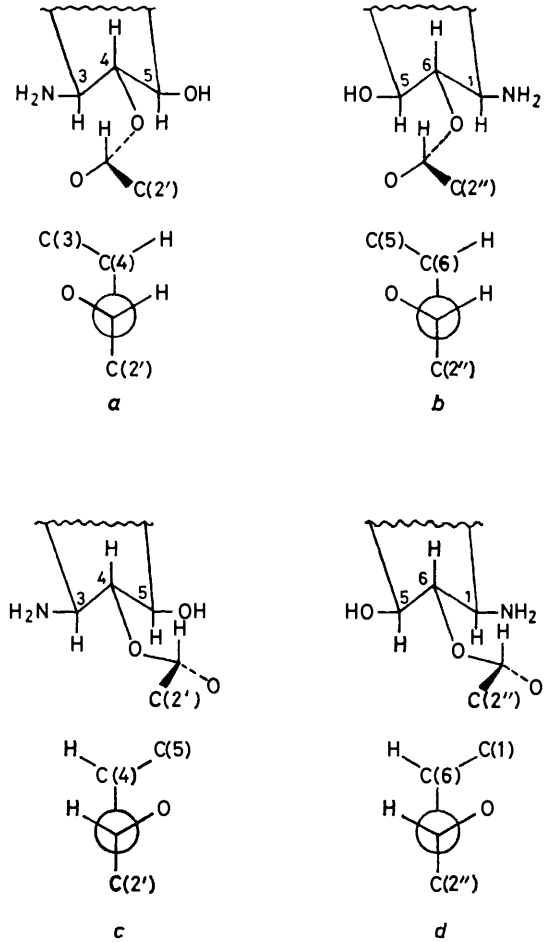
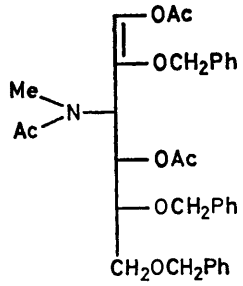
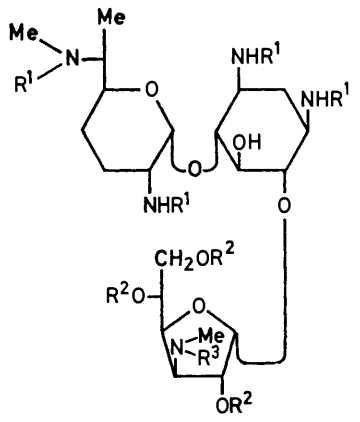


FIGURE 2 Preferred rotamer conformations

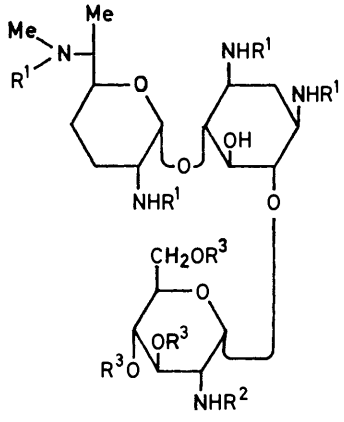
We next turned our attention to the synthesis of novel 6-*O*-glucosaminyl derivatives from gentamine C_1. At the time there were no known naturally occurring aminoglycoside antibiotics having a 2-amino-2-deoxy-sugar linked at the 6-position. Subsequently the seldomycins were discovered. Seldomycin factor 5 contains a 6-*O*-(2,3-diamino-2,3-dideoxy-3-*O*-methyl-α-D-xylopyranosyl) unit,[41] while seldomycin factors 1 and 3 contain a 6-*O*-(2-amino-2-deoxy-α-D-xylopyranosyl) unit in the molecule.[42] When 1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) was treated with 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (33) [43] under the conditions of the Lemieux–Nagabhushan reaction, the oxime (35) was obtained. Acetylation of the latter gave the oxime acetate (36) which was reduced with diborane and deprotected by treatment with sodium in liquid ammonia followed by basic hydrolysis to give *O*-2-amino-2-deoxy-α-D-glucopyranosyl-(1 ⟶



(49)



(50) R^1 = Z, R^2 = CH_2Ph, R^3 = Ac
(51) R^1 = R^2 = R^3 = H

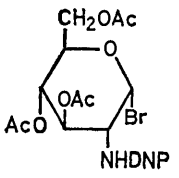


(52) R^1 = R^2 = R^3 = H
(53) R^1 = Z, R^2 = DNP, R^3 = Ac
DNP = 2,4-$(NO_2)_2C_6H_3$

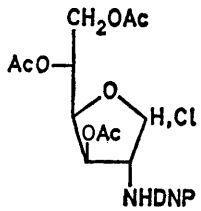
6)-gentamine C_1 (52). The rotation, as well as the presence of a doublet with $J_{1eq'',2ax''}$ 4 Hz at δ_H 5.35 in the ^1H n.m.r. spectrum, supported the presence of an α-glycosidic linkage for the glucosaminyl unit. The ^{13}C n.m.r. spectrum confirmed this assignment (Table 2) and also indicated that glycosylation had again occurred at the 6-position. The aminoglycoside (52) was also prepared by the Koenigs–Knorr route as follows. 1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) was condensed with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (54) [44,45] in the presence of mercury(II) cyanide and Drierite to give the α-glycoside (53) as the major product of the reaction. Deprotection of the latter by basic hydrolysis followed by reduction with sodium in liquid ammonia afforded the α-glycoside (52) which was identical with that prepared above.

When 1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) was treated with 3,5,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α- and -β-D-glucofuranosyl chloride (55) [46,47] in the presence of mercury(II) cyanide and calcium sulphate, *O*-3,5,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucofuranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (56) and the β-anomer (58) were obtained. Both were deprotected by basic hydrolysis followed by reduction with sodium in liquid ammonia. The α-anomer (56) afforded *O*-2-amino-2-deoxy-α-D-glucofuranosyl-(1 → 6)-gentamine C_1 (57) which exhibited a doublet at δ_H 5.37 having $J_{1'',2''}$ 5 Hz due to the anomeric proton of the α-furanosyl moiety. The β-anomer (58) on similar treatment gave *O*-2-amino-2-deoxy-β-D-glucofuranosyl-(1 → 6)-gentamine C_1 (59) which exhibited at singlet at δ_H 5.17 due to the β-furanosyl anomeric proton. The mass spectra were in agreement with the proposed structures (Table 1). The proof that (57) and (59) contained 1 → 6-linked glycosides was obtained by the application of the following g.l.c.–mass spectral technique. The trisaccharides (57) and (59) were each *N*-acetylated and then per-*NO*-methylated. The resulting products were subjected to acidic hydrolysis to remove both sugar moieties and the remaining deoxystreptamine derivative was then re-*N*-acetylated and subjected to g.l.c.–mass spectral analysis as the trimethylsilyl derivative. The presence of prominent ions at *m/e* 254 (*m*) and 187 (*n*) in the mass spectra (Figure 1) due to the formation of (60) clearly supported the 1 → 6 linkages in both (57) and (59). Had the linkages been 1 → 5 (61) would have been produced, and prominent ions would have been visible at *m/e* 196 (*o*) and 129 (*p*) (Table 3).

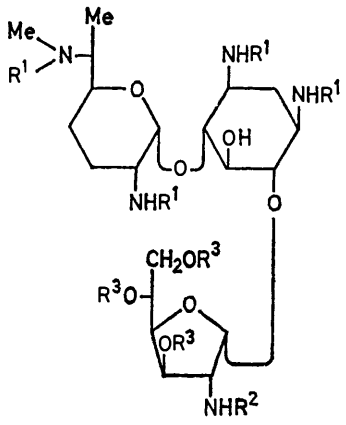
In all of the above, as well as subsequent condensations [17] with gentamine and neamine,[40] we have observed that when the 5- and 6-hydroxy-groups are



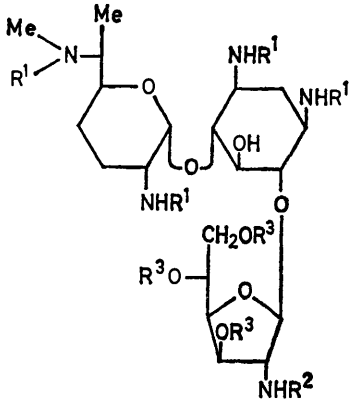
(54)



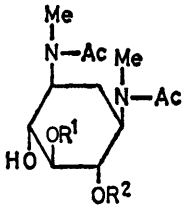
(55)



(56) R^1 = Z, R^2 = DNP, R^3 = Ac
(57) $R^1 = R^2 = R^3 = H$



(58) R^1 = Z, R^2 = DNP, R^3 = Ac
(59) $R^1 = R^2 = R^3 = H$



(60) R^1 = Me, R^2 = H
(61) R^1 = H, R^2 = Me
DNP = $2,4\text{-}(NO_2)_2C_6H_3$

available for glycosylation, reaction occurs predominantly at the 6-hydroxy-group. Similar preference for 6-*O*-glycosylation was also reported recently for paromamine.[48] These results are in contrast to those observed during the synthesis of ribostamycin, where predominant 5-*O*-glycosylation was reported.[49]

Pioneering studies by Lemieux [50] have defined the solution conformations of simple glycosides and in recent years these concepts have been extended to enable the solution conformations of aminoglycoside antibiotics to be defined. Thus ^1H n.m.r. studies on the kanamycins and related synthetic analogues,[33] as well as ^{13}C n.m.r. studies on the gentamicins,[24] tobramycin,[25] 66–40 B and D,[51] and a number of semisynthetic aminoglycosides derived from garamine,[10,12,13] have defined the solution conformations of 4-*O*-α-D-glycosyl, 4-*O*-β-D-glycosyl, 4-*O*-α-L-glycosyl, 4-*O*-β-L-glycosyl, 6-*O*-α-D-glycosyl, and 6-*O*-β-L-glycosyl units with respect to their orientation about the C-4–O and C-6–O bonds respectively, of the dexoystreptamine ring. No information has yet been published on the solution conformation of a 6-*O*-β-D-glycosyl derivative of deoxystreptamine and we now report our results.

The ^{13}C n.m.r. chemical shifts for seven of the compounds described earlier are given in Table 2. The $\Delta\delta_O$

TABLE 3

Linkage determination by g.l.c.–mass spectroscopy

	Fragments (*m/e*)			
Derivative	*m*	*n*	*o*	*p*
Reference (60)	254	187		
Reference (61)			196	129
(57) ⟶ (60)	254	187		
(59) ⟶ (60)	254	187		

TABLE 4

$\Delta\delta_O$ Values for DOS [24] ⟶ trisaccharide

Carbon	(11)	(30)	(13)	(51)	(5)	(16)	(52)
C-1	−0.1	−2.0	−0.4	−0.9	−0.5	+0.4	−0.9
C-3	−0.8	−0.9	−0.7	−0.8	−1.0	−0.7	−1.1
C-5	−1.2	−0.1	−1.3	−1.2	−1.2	−1.1	−1.3

values in going from deoxystreptamine to the appropriate trisaccharide are given in Table 4. It is evident from the $\Delta\delta_C$ values that the aminoglycosides which contain 6-*O*-α-D-glycosyl units, namely (11), (13), (51), (5), (16), and (52), adopt the usual rotamer *a* about the C-4–O glycosidic bond resulting in shielding of C-3, and the usual rotamer *b* about the C-6–O glycosidic bond resulting in shielding of C-5. Somewhat greater shielding than usual (−0.1 to −0.5) was observed at C-1 (−0.9) in the 6-*O*-α-D-furanosyl derivative (51) and in the 6-*O*-α-D-glucosaminyl derivative (52), which is reminiscent of what was observed earlier [10,12] with certain 4-*O*-β-D-glycosyl derivatives that adopt rotamer *c* about the C-4–O glycosidic bond. On the other hand the 6-*O*-β-D-glycosyl derivative (30) exhibited $\Delta\delta_C$ values (Table 4) that clearly indicated the presence of the usual rotamer *a* about the C-4–O glycosidic bond as evidenced by the observed shielding of C-3. No shielding was observed at C-5. However, strong shielding (−2.0) was observed at C-1 indicating that rotamer *d* is the preferred rotamer about the C-6–O glycosidic bond in the 6-*O*-β-D-glucosyl derivatives. Rotamer *d* also satisfies the requirements of the *exo*-anomeric effect.[33,50,52-54] These results are in full agreement with those which we would have predicted on the basis of our earlier work [10,12] on 4-*O*-β-D-glycosyl derivatives of garamine and upon subsequent studies on 4-*O*-α-L-glycosyl derivatives of garamine.[13] Further examples of the occurrence of rotamer *d* in other semisynthetic aminoglycosides will be described in the following paper.[17] Other novel rotamers will also be described there [17] and in the subsequent paper.[55]

The antibacterial activity of these novel aminoglycoside antibacterials will be described elsewhere.

EXPERIMENTAL

Optical rotations were recorded at 26 °C at *c* 0.3. I.r. spectra were recorded on a Perkin-Elmer model 221 spectrometer. ^{1}H N.m.r. spectra were obtained at 60 MHz on a Varian A-60A, or at 100 MHz on a Varian XL-100-15 spectrometer. Chemical shifts are reported in p.p.m. downfield from external sodium 4,4-dimethyl-4-silapentane sulphonate or internal tetramethylsilane as standards. ^{13}C N.m.r. spectra were obtained on a Varian XL-100-15 spectrometer in the Fourier transform mode using a Varian 620L-100 16K computer equipped with a 2.5 Megabyte disc system. An internal dioxan, or tetramethylsilane standard was used and chemical shifts are reported in p.p.m. downfield from tetramethylsilane. Mass spectra were recorded on a Varian MAT CH 5 spectrometer.

1,3,2′,6′-*Tetrakis*-N-*benzyloxycarbonylgentamine* C_1 (3).—Gentamine C_1 (1)[14] (113 g) was dissolved in methanol (290 ml) and a saturated solution of aqueous sodium hydrogencarbonate (73 ml) was added. Benzyl chloroformate (278 g) was added dropwise to the stirred mixture at 0 °C with periodic addition of sodium hydrogencarbonate to maintain the pH at *ca.* 9 (*ca.* 650 ml). The mixture was stirred at 25 °C for 16 h. The precipitate was filtered off and washed with water and then ether. The precipitate was dried to give 1,3,2′,6′-*tetrakis*-N-*benzyloxycarbonylgentamine* C_1 (3) (231.8 g, 76%) as an amorphous solid which crystallized from benzene, m.p. 188—190 °C (Found: C, 64.6; H, 6.4; N, 6.7. $C_{46}H_{54}N_4O_{12}$ requires C, 64.6; H, 6.4; N, 6.55%), $[\alpha]_D$ +72.0° ($CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 400, 3 300, 1 690, 1 500, and 1 025 cm^{-1}, δ_H($CDCl_3$) 1.11 (3 H d, $J_{6',7'}$ 6.5 Hz, 7′-CH_3), 2.60 (3 H, s, 6′-NCH_3), 5.03 and 5.06 (8 H, s, $CH_2C_6H_5$), and 7.27 (20 H, s, $CH_2C_6H_5$).

1,3,2′,6′-*Tetrakis*-N-*benzyloxycarbonylgentamine* C_{1a} (4).—Gentamine C_{1a} (2)[14] (29.1 g) was dissolved in methanol (93 ml) and a saturated solution of aqueous sodium hydrogencarbonate (23 ml) was added. Benzyl chloroformate (77.1 g) was added and the reaction was carried out as described above. Crystallization from methanol and then benzene afforded 1,3,2′,6′-*tetrakis*-N-*benzyloxycarbonylgentamine* C_{1a} (4) (72 g, 87%) as crystals, m.p. 228—230 °C (Found: C, 63.0; H, 6.1; N, 6.8. $C_{44}H_{50}N_4O_{12}$ requires C, 63.9; H, 6.10; N, 6.8%), $[\alpha]_D$ +51.0° ($CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 675, 3 565, 3 420, 3 320, 1 700, 1 500, 1 240, 1 220, and 1 020 cm^{-1}, δ_H ($CDCl_3$) 5.02 (8 H, s, $CH_2C_6H_5$) and 7.25 (20 H, s, $CH_2C_6H_5$).

3-*Acetamido*-4,6-*di*-O-*acetyl*-2,3-*dideoxy*-2-*nitroso*-α-D-*glucopyranosyl Chloride* (32).—3-Acetamido-4,6-di-*O*-acetyl-1,2,3-trideoxy-D-*arabino*-hex-1-enopyranose (31) [34] (325 mg) was dissolved in dry ethyl acetate (10 ml) and the mixture was cooled to 0 °C and purged with nitrogen. Nitrosyl chloride gas was passed through the solution for 30 min. Dry ether was added and the mixture was purged with dry nitrogen. The solid *nitroso-chloro-adduct* (32) (330 mg, 82%) was filtered off and dried (Found: C, 42.8; H, 5.15; N, 7.5. $C_{24}H_{34}Cl_2N_4O_{14}$ requires C, 42.5; H, 5.17; N, 7.6%), $\nu_{max.}$ (Nujol) 3 450, 1 745, and 1 705 cm^{-1}.

O-3-*Amino*-3-*deoxy*-α-D-*glucopyranosyl*-(1 → 6)-*gentamine* C_1 (11) *and* O-3-*Amino*-3-*deoxy*-β-D-*glucopyranosyl*-(1 → 6)-*gentamine* C_1 (30).—(i) 1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) (4.3 g) and anhydrous silver toluene-*p*-sulphonate (2.8 g) were stirred in dry benzene (250 ml) and the volume was reduced to 150 ml by distillation under reduced pressure. 3-Acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (17) [21] (3.9 g) was dissolved in dry benzene (100 ml) and concentrated to 50 ml. The solutions were combined and collidine (0.6 g) was added and the mixture was stirred under anhydrous conditions at 45—50 °C for 18 h. After filtration and evaporation of the solvent, the residue was chromatographed on silica gel using 0.5% methanol in chloroform as the eluant to give a mixture of *O*-3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (10) and O-3-*acetamido*-2,4,6-*tri*-O-*benzyl*-3-*deoxy*-β-D-*glucopyranosyl*-(1 → 6)-1,3,2′,6′-*tetrakis*-N-*benzyloxycarbonylgentamine* C_1 (29), m.p. 185—187 °C (Found: C, 67.5; H, 6.55; N, 5.2. $C_{75}H_{85}N_5O_{17}$ requires C, 67.8; H, 6.4; N, 5.3%), $[\alpha]_D$ +55.4° (EtOH). Ammonia (250 ml) was distilled from sodium into the mixture of (10) and (29), and sodium (2 g) was added in portions. After 3 h at −80 °C the excess of sodium was destroyed by addition of water. The ammonia was allowed to evaporate under a stream of nitrogen over 48 h. Water was added and the solution was neutralized with Amberlite IRC 50 (H^+) resin and washed with water. Elution with 1.5M-ammonium hydroxide followed by lyophilization of the concentrated solution gave a solid residue (1.5 g) which was chromatographed on silica gel using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1) as the eluant to give O-3-*amino*-3-*deoxy*-α-D-*glucopyranosyl*-(1 → 6)-*gentamine* C_1 (11) (0.5 g, 21%) as an amorphous solid

after passage over Amberlite IRA 401S (OH^-) resin followed by lyophization (Found: $M^{+\cdot}$, 479.3006. $C_{20}H_{41}N_5O_8$ requires M, 479.2954), $[\alpha]_D$ +107.2° (H_2O), $[\theta]_{290}$ +2 140 (TACu), $[\theta]_{290}$ +1 790 (Cupra A), δ_H (D_2O) 0.95 (3 H, d, $J_{6',7'}$ 6.5 Hz, 7'-CH_3), 2.23 (3 H, s, 6'-NCH_3), 4.96 (1 H, d, $J_{1eq'',2ax''}$ 3.5 Hz, $H_{1eq''}$) and 5.05 (1 H, d, $J_{1eq',2ax'}$ 3.5 Hz, $H_{1eq'}$), and *O*-3-amino-3-deoxy-β-D-glucopyranosyl-(1 → 6)-gentamine C_1 (30) (0.25 g, 10%) as a colourless amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization, $[\alpha]_D$ +31.5° (H_2O), $[\theta]_{290}$ −890 (TACu), δ_H (D_2O) 1.15 (3 H, d, $J_{6',7'}$ 6.5 Hz, 7'-CH_3), 2.57 (3 H, s, 6'-NCH_3), 4.57 (1 H, d, $J_{1ax'',2ax''}$ 8.5 Hz, $H_{1ax''}$), and 5.25 (1 H, d, $J_{1eq',3ax'}$ 4 Hz, $H_{1eq'}$).

(ii) 1,3,2',6'-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) (1.0 g) and 3-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-2-nitroso-α-D-glycopyranosyl chloride (32) (0.73 g) were stirred in dry dimethylformamide (9 ml) at 25 °C for 24 h. The reaction mixture was poured into water and the precipitate was filtered off, dried, and chromatographed on silica gel using 1% v/v ethanol–chloroform as the eluant to give *O*-3-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 6)-1,3,2',6'-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (34) (0.45 g) as an amorphous solid. The oxime (34) (0.3 g), acetaldehyde (4 ml), acetonitrile (3 ml), tetrahydrofuran (1 ml), and 1M hydrochloric acid (1 ml) were combined and stirred at 25 °C for 18 h. The acetaldehyde was removed by evaporation and the residue was partitioned between water and ethyl acetate. The organic phase was dried and evaporated. The residue was dissolved in dioxan–water (3 : 1 v/v) (3 ml) and cooled to 0 °C. Sodium borohydride (50 mg) in dioxan–water (3 : 1 v/v) (2 ml) was added at 0 °C. After 1.5 h glacial acetic acid was added to destroy the excess of sodium borohydride and the solvent was removed under reduced pressure. The residue in tetrahydrofuran (3 ml) was treated with liquid ammonia (30 ml) and sodium (0.3 g) was added in portions. After 2 h at −80 °C, water was added to destroy the excess of sodium. The ammonia was allowed to evaporate over 18 h. Water (1 ml) was added and the solution was heated at 100 °C for 4 h, cooled, and neutralized with Amberlite IRC 50 (H^+) resin. The slurry was poured onto a column and washed with water. Elution with 1.5M-ammonium hydroxide followed by concentration of the eluant and passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization afforded *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1 → 6)-gentamine C_1 (11) (30 mg, 5%) as an amorphous solid, identical with that prepared in (i) above.

5,6-*Di*-O-*benzyl*-3-*deoxy*-1,2-O-*isopropylidene*-3-N-*methylacetamido*-α-D-*glucofuranose* (38).—1,2-*O*-Isopropylidene-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranose (37) [36] (30 g) was dissolved in dry dimethylformamide (700 ml) and the solution was cooled to 0 °C. Barium oxide (137.2 g), barium hydroxide octahydrate (70.6 g), and benzyl bromide (280 ml) were added and the mixture was stirred at 0 °C for 0.5 h and then at 25 °C for 16 h. The mixture was diluted with chloroform, filtered, and the combined filtrate and chloroform wash were evaporated to dryness. The resulting syrup was chromatographed (×2) on silica gel (1.7 kg) using chloroform–hexane (1 : 1 v/v) as the eluant to give the 5,6-*di*-O-*benzyl derivative* (38) (27.9 g, 56%) as a syrup (Found: $M^{+\cdot}$, 455.2296. $C_{26}H_{33}NO_6$ requires M, 455.2308), $[\alpha]_D$ +20.7° ($CHCl_3$), δ_H ($CDCl_3$) * 1.30 and 1.50 [6 H, 2 s, $OC(CH_3)_2O$], 1.98 (3 H, s, 3-NAc), 2.86 [3 H, s, 3-NCH_3(Ac)], 4.56 (2 H, s, 6-$OCH_2C_6H_5$), 5.94 (1 H, d, $J_{1,2}$ 4 Hz, 1-H) and 7.29 and 7.30 (10 H, 2 s, $OCH_2C_6H_5$).

Methyl 5,6-*Di*-O-*benzyl*-3-*deoxy*-3-*methylamino*-α- *and* -β-D-*glucofuranoside* (39) *and* (40).—5,6-Di-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methylacetamido-α-D-glucofuranose (38) (1 g) was dissolved in dioxan (25 ml) and 0.1M-hydrochloric acid (25 ml) and the solution was heated at 95 °C for 4 h. The solution was evaporated to dryness and then co-evaporated several times with methanol. The resulting syrup was dissolved in 1% methanolic hydrogen chloride (70 ml) and the solution was heated at 85 °C for 2 h. The solution was concentrated, passed over Amberlite IRA 401S (OH^-) resin and the eluate was evaporated to dryness. The resulting syrup was chromatographed on silica gel (50 g) using chloroform as the eluant to give *methyl* 5,6-*di*-O-*benzyl*-3-*deoxy*-3-*methylamino*-α-D-*glucofuranoside* (39) (103 mg, 12%) as a colourless syrup (Found: $M^{+\cdot}$, 387.2038. $C_{22}H_{29}NO_5$ requires M, 387.2046), *m/e* 388 ($M^+ + 1$), 387 ($M^{+\cdot}$), 356 (*a*), 296 (*d*), and 146 (*f*), $[\alpha]_D$ +49.9° ($CHCl_3$), δ_H ($CDCl_3$) 2.35 (3 H, s, 3-NCH_3), 2.65 (1 H, s, exchanges with D_2O, 2-OH), 3.10 (1 H, dd, $J_{2,3}$ 4.2, $J_{3,4}$ 6 Hz, 3-H), 3.40 (3 H, s, 1-OCH_3), 3.77 (2 H, m, 6-CH_2), 4.05 (1 H, dd, $J_{1,2} = J_{2,3} = 4.2$ Hz, 2-H), 4.29 (1 H, dd, $J_{3,4} = J_{4,5} = 6$ Hz, 4-H), 4.53 (2 H, s, 6-$OCH_2C_6H_5$), 4.67 and 4.71 (2 H, s, 5-$OCH_2C_6H_5$), 4.88 (1 H, d, $J_{1,2}$ 4.2 Hz, 1-H) and 7.30 (10 H, s, $OCH_2C_6H_5$), and the β-*anomer* (40) (129 mg, 15%) as a syrup (Found: $M^{+\cdot}$, 387.2018. $C_{22}H_{29}NO_5$ requires M, 387.2046), *m/e* 388 [$(M + 1)^+$], 387 ($M^{+\cdot}$), 356 (*a*), 296 (*d*), and 146 (*f*), $[\alpha]_D$ −14.6° ($CHCl_3$), δ_H ($CDCl_3$) 2.36 (3 H, s, 3-NCH_3), 2.61br (1 H, s, exchanges with D_2O), 3.27 (3 H, s, 1-OCH_3), 4.58 (2 H, s, 6-$OCH_2C_6H_5$), 4.68 and 4.73 (2 H, s, 5-$OCH_2C_6H_5$), 4.74 (1 H, s, H_1), and 7.31 (10 H, s, $OCH_2C_6H_5$).

Methyl 5,6-*Di*-O-*benzyl*-3-*deoxy*-3-N-*methylacetamido*-α- *and* -β-D-*glucofuranoside* (41) *and* (42).—(*i*) 5,6-Di-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methylacetamido-α-D-glucofuranose (38) (25 g) was dissolved in dioxan (300 ml) and 0.1M-hydrochloric acid (400 ml), and the solution was heated at 95 °C for 6 h. The solution was evaporated to dryness and then co-evaporated several times with methanol. The resulting syrup was dissolved in 1% methanolic hydrogen chloride (500 ml) and the solution was heated under reflux for 2 h. The solution was concentrated, passed over Amberlite IRA 401S (OH^-) resin and the eluate was evaporated to dryness. The resulting syrup was dissolved in dry methanol (300 ml) and acetic anhydride (100 ml) was added. The mixture was allowed to remain at 25 °C for 1 h. The solution was evaporated to dryness, azeotroped with toluene and chromatographed on silica gel (1 kg) using 1% v/v methanol–chloroform as the eluant to give the *methyl* α-D-*glucofuranoside* (41) as a syrup (Found: $M^{+\cdot}$, 429.2141. $C_{24}H_{31}NO_6$ requires M, 429.2151), *m/e* 429 ($M^{+\cdot}$), 398 (*b*), 338 (*e*), 308 (*i*), and 188 (*g*), $[\alpha]_D$ +118.3° ($CHCl_3$), δ_H ($CDCl_3$) * 1.96 and 2.01 (3 H, 2 s, 3-NAc), 2.94 [3 H, s, 3-NCH_3(Ac)], 3.47 and 3.48 (3 H, 2 s, 1-OCH_3), 5.05 (1 H, d, $J_{1,2}$ 4 Hz, 1-H), and 7.32 and 7.34 (10 H, 2 s, $OCH_2C_6H_5$), and the β-*anomer* (42) as a syrup (Found: $M^{+\cdot}$, 429.2131. $C_{24}H_{31}NO_6$ requires M, 429.2151), *m/e* 429 ($M^{+\cdot}$), 398 (*b*), 338 (*e*), 308 (*i*), and 188 (*g*), $[\alpha]_D^{26}$ +1.3° ($CHCl_3$), δ_H ($CDCl_3$) * 1.88 and 1.98 (3 H, 2 s, 3-NAc), 2.93 and 2.97 [3 H, 2 s, 3-NCH_3(Ac)], 3.44 and 3.46 (3 H, 2 s, 1-OCH_3), and 7.30, 7.32, and 7.34 (10 H, s, $OCH_2C_6H_5$). All fractions containing the above products were combined to afford (41) and (42) as a syrup (12 g, 51%) containing a total of *ca.* 24% of

* Mixture of rotamers at ambient temperatures.

the pyranosides (18) and (19) as determined from the ^{1}H n.m.r. spectrum.*

(*ii*) Methyl 5,6-di-*O*-benzyl-3-deoxy-3-methylamino-α-D-glucofuranoside (39) (55 mg) was dissolved in dry methanol (2 ml). Acetic anhydride (1 ml) was added and the mixture was allowed to remain at 25 °C for 1 h. The solution was evaporated to dryness and azeotroped with toluene to give the α-glycoside (41) (60 mg, 98%) as a syrup. The product was identical (^{1}H n.m.r. and mass spectra, $[\alpha]_D$, t.l.c.) with that prepared in (*i*) above.

Methyl 5,6-di-*O*-benzyl-3-deoxy-3-methylamino-α-D-glucofuranoside (40) (40 mg) was acetylated as described above to give the β-anomer (42) (43 mg, 97%) as a syrup. The product was identical (^{1}H n.m.r. and mass spectra, $[\alpha]_D$, t.l.c.) with that prepared in (*i*) above.

*Methyl 2,5,6-Tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-*D*-glucofuranoside* (43) *and* (44).—Methyl 5,6-di-*O*-benzyl-3-deoxy-3-methylacetamido-α- and -β-D-glucofuranoside (41) and (42) (11 g) containing *ca.* 24% of the corresponding pyranosides (18) and (19) were dissolved in dry dimethylformamide (200 ml). Barium oxide (74 g), barium hydroxide octahydrate (34 g), and benzyl bromide (100 ml) were added to the stirred solution at 0 °C and the mixture was then stirred at 25 °C for 17 h. The mixture was diluted with chloroform and filtered and the combined chloroform filtrate and washings were evaporated to dryness. The syrup was chromatographed on silica gel (600 g) using initially hexane, followed by chloroform–hexane (1 : 1 v/v) as the eluant to give the *methyl* α-D-*glucofuranoside* (43) as a syrup (Found: $M^{+\cdot}$, 519.2595. $C_{31}H_{37}NO_6$ requires M, 519.2621), *m/e* 519 ($M^{+\cdot}$), 488 (*c*), 398 (*j*), and 278 (*h*), $[\alpha]_D$ −2.2° ($CHCl_3$), δ_H ($CDCl_3$) † 1.94 and 1.95 (3 H, 2 s, 3-NAc), 2.87 and 2.99 [3 H, 2 s, 3-NCH_3(Ac)], 3.46 and 3.47 (3 H, 2 s, 1-OCH_3), 4.89 and 4.92 (1 H, 2 d, $J_{1,2}$ 3.5 Hz, 1-H), and 7.34 (15 H, s, $OCH_2C_6H_5$), and the β-*anomer* (44) as a syrup (Found: $M^{+\cdot}$, 519.2590. $C_{31}H_{37}NO_6$ requires M, 519.2621), *m/e* 519 ($M^{+\cdot}$), 488 (*c*), 398 (*j*), and 278 (*h*), $[\alpha]_D$ +77.6° ($CHCl_3$), δ_H ($CDCl_3$) † 1.91 and 1.97 (3 H, 2 s, 3-NAc), 2.75 and 2.85 [3 H, 2 s, 3-NCH_3(Ac)], 3.40 (3 H, s, 1-OCH_3), 4.84 and 4.90 (1 H, 2 d, $J_{1,2}$ 4.5 Hz, 1-H), and 7.34 (15 H, s, $OCH_2C_6H_5$). All fractions containing the above products were combined to afford (43) and (44) as a syrup (10.2 g, 77%) containing a total of *ca.* 24% of the corresponding pyranosides (20) and (21) (^{1}H n.m.r. spectrum).

1-O-*Acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-*D*-glucofuranose* (45) *and* (46) *and* 1-O-*Acetyl-2,4,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-*D*-glucopyranose* (22) *and* (23).—Methyl 2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-D-glucofuranoside (43) and (44) (8.6 g) containing *ca.* 24% of the corresponding pyranosides (20) and (21) were dissolved in glacial acetic acid (150 ml) and 1M-hydrochloric acid (200 ml), and the solution was heated at 85 °C for 3 h. The solution was cooled and extracted with chloroform, and the chloroform extracts were washed with water, dried (Na_2SO_4), and evaporated to dryness. The resulting syrup was dissolved in acetic anhydride (100 ml) and anhydrous sodium acetate (8 g) was added and the mixture was heated at 85 °C for 1 h. The mixture was cooled, poured into ice–water and extracted with chloroform. The chloroform extracts were washed with water, dried (Na_2SO_4), and evaporated to dryness. The syrup was azeotroped with toluene and chromatographed on silica gel (300 g) using 5% acetone in hexane as the eluant to give a mixture of the *acetyl* α- and -β-D-*glucofuranoses* (45) and (46) as a syrup [Found: $(M + 1)^+$, 548.2818. $C_{32}H_{37}NO_7$ requires $M + 1$, 548.2848], *m/e* 547 ($M^{+\cdot}$), 488 (*c*), 426 (*k*), 306 (*l*), δ_H ($CDCl_3$) † 1.75, 1.86, 1.89, 2.00, and 2.04 (6 H, 5 s, 3-NAc and 1-OAc), 2.80, 2.93 [3 H, 2 s, 3-NCH_3(Ac)], 6.09 [<1 H, d, $J_{1,2}$ 2 Hz, 1-H (α-anomer)], 6.11 [<1 H s, 1-H (β-anomer)], and 7.30 and 7.33 (15 H, 2 s, $OCH_2C_6H_5$), and the *acetyl* α- and -β-D-*glucopyranoses* (22) and (23) as a syrup (Found: $(M + 1)^+$, 548.2619. $C_{32}H_{37}NO_7$ requires $M + 1$, 548.2848), *m/e* 547 ($M^{+\cdot}$), δ_H ($CDCl_3$) † 2.10, 2.12, 2.16, and 2.18 (6 H, 4 s, 3-NAc and 1-OAc), 2.60 and 2.63 [3 H, 2 s, 3-NCH_3-(Ac)], 5.64 [<1 H, d, $J_{1,2}$ 7.5 Hz, 1-H (β-anomer)], 6.48 [<1 H, d, $J_{1,2}$ 3.5 Hz, 1-H (α-anomer)], and 7.28 and 7.36 (15 H, 2 s, $OCH_2C_6H_5$). All fractions containing (45), (46), (22), and (23) were combined to give a syrup (3.0 g, 33%). The more polar fractions from the column afforded 1,4-*di-*O*-acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-*D-threo-*hex-*1-*enose* (49) (0.84 g, 9%) as a syrup (Found: *m/e*, 588.2558. $C_{34}H_{38}NO_8$ requires $M - 1$, 588.2597), *m/e* 590 [$(M + 1)^+$], 589 ($M^{+\cdot}$), 530 (M − OAc), and 498 (M − 91), $[\alpha]_D$ +4.0° ($CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 740, 1 640, 1 220, 1 080, 1 030, and 693 cm^{-1}, δ_H ($CDCl_3$) † 1.90—2.15 (9 H, s, 1-OAc, 4-OAc, and 3-NAc), 2.85 and 2.91 [3 H, 2 s, 3-NCH_3(Ac)], 4.53 and 4.57 (4 H, 2 s, $OCH_2C_6H_5$), 5.05 and 5.11 (2 H, 2 s, 5-$OCH_2C_6H_5$), 5.70 (1 H, d, J 1 Hz, 1-H), and 7.30 and 7.34 (15 H, 2 s, $OCH_2C_6H_5$).

1-O-*Acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-*D*-glucofuranose* (45) *and* (46).—1,4-Di-*O*-acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-D-*threo*-hex-1-enose (49) (42 mg) was dissolved in 50% aqueous methanol (5 ml) and triethylamine (10 drops) was added. The solution was allowed to remain at 25 °C for 16 h. The solution was evaporated to dryness and the syrup was dissolved in dry pyridine (5 ml) and acetic anhydride (1 ml) was added. The mixture was allowed to remain at 25 °C for 2.5 h. The solution was evaporated to dryness and azeotroped with toluene to give the acetyl α- and -β-D-glucofuranoses (45) and (46) as a syrup, identical (^{1}H n.m.r. and mass spectra, t.l.c.) with the samples prepared earlier.

*2,5,6-Tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-*D*-glucofuranosyl Chloride* (47) *and* (48) *and* 2,4,6-*Tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-*D*-glucopyranosyl Chloride* (24).—1-*O*-Acetyl-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-D-glucofuranose (45) and (46) and 1-*O*-acetyl-2,4,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-D-glucopyranose (22) and (23) (1.85 g) were dissolved in a saturated solution of hydrogen chloride in dry dioxan (150 ml). Acetyl chloride (75 ml) was added and the solution was allowed to remain at 25 °C for 25 h. The solution was evaporated to dryness and azeotroped with toluene to give a mixture of the α- and -β-furanosyl chlorides (47) and (48) and the α-pyranosyl chloride (24) (1.8 g, 100%) as a labile syrup, δ_H ($CDCl_3$) † 1.70—2.20 (3 H, s, 3-NAc), 2.50—3.00 [3 H, s, 3-NCH_3(Ac)], 5.52 [<1 H, d, $J_{1,2}$ 4 Hz, 1-H (α-furanoside)], 5.68 [<1 H, s, 1-H (β-furanoside)], 6.16 and 6.24 [<1 H, d, $C_{1,2}$ 4 Hz, (α-pyranoside)], and 7.20 and 7.30 (15 H, 2 s, $OCH_2C_6H_5$). The chloro-sugars were used without further purification.

O-3-*Deoxy-3-methylamino-α-D-glucofuranosyl-*(1 ⟶ 6)-*gentamine* C_1 (51) *and* O-3-*Deoxy-3-methylamino-α-D-glucopyranosyl-*(1 ⟶ 6)-*gentamine* C_1 (13).—1,3,2′,6′-Tetra-*N*-benzyloxycarbonylgentamine C_1 (3) (3.4 g) was dissolved

* As determined by the presence of an additional 3-NAc group at δ_H 2.07 arising from the pyranosyl form of the sugar.

† Mixture of rotamers at ambient temperatures.

in dry dichloromethane (200 ml) and dry silver toluene-*p*-sulphonate (1.05 g) was added. A mixture of 2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α- and -β-D-glucofuranosyl chloride (47) and (48) and 2,4,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucopyranosyl chloride (24) (1.8 g) dissolved in dry dichloromethane (150 ml) were added and the mixture was stirred at 25 °C for 159 h. The solids were filtered off and washed with dichloromethane and the combined filtrates were evaporated to dryness. The resulting solid was chromatographed on silica gel (300 g) using 1% v/v methanol–chloroform as the eluant to give a mixture of *O*-2,5,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucofuranosyl-(1 ⟶ 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (50) and *O*-2,4,6-tri-*O*-benzyl-3-deoxy-3-*N*-methylacetamido-α-D-glucopyranosyl-(1 ⟶ 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (12) (2.5 g, 47%) as an amorphous solid (Found: C, 65.9; H, 6.2; N, 4.9. $C_{76}H_{87}N_5O_{17}\cdot 3H_2O$ requires C, 65.6; H, 6.7; N, 5.0%), δ_H ($CDCl_3$) * 1.11br and 1.22br (3 H, 2 s, 6′-CH_3), 1.80—2.20br (3 H, s, 3-NAc), 2.60—2.86br [6 H, s, 3-NCH_3(Ac) and 6′-NCH_3], and 7.36br (35 H, s, $OCH_2C_6H_5$).

The protected pseudotrisaccharides (50) and (12) (2.4 g) were dissolved in dry tetrahydrofuran (25 ml) and the solution was added to liquid ammonia (400 ml) at −79 °C. Sodium metal was added until the blue colour persisted and the mixture was stirred at −79 °C for 0.5 h. The excess of sodium was destroyed by dropwise addition of methanol and the ammonia was allowed to distil off at 25 °C for 17 h. 1M-Sodium hydroxide (100 ml) was added and the mixture was heated under reflux for 7 h. The solution was cooled, neutralized with Amberlite IRC 50 (H^+) resin and the resin was eluted as described previously. The residue was chromatographed on silica gel (300 g) using chloroform–methanol–3.5% increasing to 7% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant and the products were each rechromatographed on silica gel (30 g and 40 g) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant in each case to give the *furanoside* (51) (16 mg, 2%) as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 493.3092. $C_{21}H_{43}N_5O_8$ requires *M*, 493.3111), $[\alpha]_D$ +54.4° (H_2O), δ_H (D_2O), 1.04 (3 H, d, *J* 6.5 Hz, 7′-CH_3), 2.31 (3 H, s, 6′-NCH_3), 2.52 (3 H, s, 3″-NCH_3), 5.14 (1 H, d, $J_{1eq',2ax'}$ 3.5 Hz, 1*eq*′-H), and 5.28 (1 H, d, $J_{1'',2''}$ 4.5 Hz, 1″-H), and the *pyranoside* (13) (38 mg, 4%) as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization (Found: $M^{+\cdot}$, 493.3105. $C_{21}H_{43}N_5O_8$ requires *M*, 493.3111), $[\alpha]_D$ +84.4° (H_2O), δ_H (D_2O–DCl) 1.04 (3 H, d, *J* 6.5 Hz, 7′-CH_3), 2.50 (3 H, S, 6′-NCH_3), 2.56 (3 H, s, 3″-NCH_3), 4.91 (1 H, d, $J_{1eq'',2ax''}$ 4 Hz, 1*eq*″-H), and 5.62 (1 H, d, $J_{1eq',2ax'}$ 3.5 Hz, 1*eq*-H).

O-3-*Amino*-3-*deoxy*-α-D-*glucopyranosyl*-(1 ⟶ 6)-*gentamine* C_{1a} (3′,4′-*Dideoxykanamycin B*) (5).—1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_{1a} (4) (5.2 g) and silver toluene-*p*-sulphonate (3.4 g) were stirred in dry benzene (500 ml) and the volume was reduced to 250 ml under reduced pressure. 3-Acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (17) [21] (5.6 g) in dry dichloromethane (250 ml) was added and the mixture was stirred at 40 °C for 24 h and then at 25 °C for 72 h. The mixture was filtered and the solids were washed with dichloromethane. The filtrate was evaporated to dryness and the residue was chromatographed on silica gel (900 g) using 0.75% v/v ethanol–chloroform as the eluant to give *O*-3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl-(1 ⟶ 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_{1a} (14) (3.7 g) as an amorphous solid. The latter was dissolved in tetrahydrofuran (38.4 ml) and ammonia (670 ml) distilled over sodium (7.2 g) was added. Sodium (3.59 g) was added in portions and the mixture was stirred at −80 °C for 45 min. The ammonia was allowed to evaporate over 18 h. Ethanol (5 ml) and water (100 ml) were added and the mixture was heated at 80 °C for 2 h. The solution was neutralized with Amberlite IRC 50 (H^+) resin and the slurry was poured onto a column and washed with water (11). Elution with 2M-ammonium hydroxide (1.5 l) followed by evaporation gave a residue which was chromatographed on silica gel (250 g) using the lower phase of a chloroform–methanol–10% ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant. The appropriate fractions were combined and evaporated to dryness and the residue was heated under reflux with hydrazine hydrate (3 ml) for 24 h. The mixture was evaporated to dryness and the residue was chromatographed on silica gel (7.5 g) using a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 2.5 : 1 v/v) as the eluant to give *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1 ⟶ 6)-gentamine C_{1a} (5) (30 mg, 1%) as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization, $[\alpha]_D$ +112.0° (H_2O), δ_H (D_2O) 4.98 (1 H, d, $J_{1eq'',2ax''}$ 4 Hz, 1*eq*″-H), and 5.16 (1 H, d, $J_{1eq',2ax'}$ 3.5 Hz, 1*eq*-H). The sample was identical with an authentic sample of 3′,4′-dideoxykanamycin B (5).[36-38]

Methyl 3-*Acetamido*-2,4,6-*tri*-O-*benzyl*-3-*deoxy*-β-D-*galactopyranoside* (26).—Methyl 3-acetamido-3-deoxy-β-D-galactopyranoside (25) [39] (15 g) was dissolved in dimethylformamide (300 ml). Barium oxide (111 g) and barium hydroxide (51 g) were added and the mixture was cooled to 0 °C. Benzyl bromide (150 ml) was added dropwise to the stirred solution and the mixture was stirred for 4 h at 0 °C and for 16 h at 25 °C. The mixture was diluted with chloroform, filtered, and the filtrate was evaporated to dryness. The resulting syrup was extracted with ethyl acetate (500 ml) and the mixture was filtered. The filtrate was evaporated to dryness and dissolved in chloroform (200 ml). The solution was washed with water (3 × 100 ml), dried ($MgSO_4$), filtered, and diluted with hexane to give the crystalline *benzyl derivative* (26) (29.5 g, 92%), m.p. 147—151 °C (Found: C, 71.2; H, 6.85; N, 2.7. $C_{30}H_{35}NO_6$ requires C, 71.3; H, 6.9; N, 2.8%), *m/e* 505 ($M^{+\cdot}$) and 474 (*M* − 31), $[\alpha]_D$ +52.5° ($CHCl_3$), $\nu_{max.}$ (Nujol) 3 300, 1 640, 1 540, 1 100, and 1 060 cm^{-1}, δ_H ($CDCl_3$) 1.47 (3 H, s, 3-NHAc), 3.54 (3 H, s, 1-OCH_3), 3.67br (2 H, s, 6-CH_2), 4.68 (1 H, d, $J_{1ax,2ax}$ 7.5 Hz, 1*ax*-H), and 7.29 (15 H, s, $CH_2C_6H_5$).

3-*Acetamido*-1-O-*acetyl*-2,4,6-*tri*-O-*benzyl*-3-*deoxy*-β-D-*galactopyranose* (27).—Methyl 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-β-D-galactopyranoside (26) (5 g) was dissolved in a mixture of glacial acetic acid (120 ml) and 1M-sulphuric acid (30 ml) and the solution was heated at 100 °C for 6 h. The mixture was cooled and poured into water (4 l). The precipitate was filtered off, dissolved in methanol (200 ml) and the solution was added slowly to hexane. The precipitate was filtered off and dried (4 g), m.p. 165—167 °C. The latter (3 g) was dissolved in acetic anhydride (20 ml) and pyridine (20 ml) and the mixture was allowed to remain at 25 °C for 16 h. The solution was evaporated

* Mixture of rotamers at ambient temperatures.

to dryness and the residue was crystallized from chloroform–isopropyl alcohol to give the crystalline β-D-*galactopyranose* (27) (2.53 g, 64%), m.p. 147—148 °C (Found: C, 69.7; H, 6.8; N, 2.3. $C_{31}H_{35}NO_7$ requires C, 69.8; H, 6.6; N, 2.6%), *m/e* 533 ($M^{+\cdot}$) and 474 (M − 59), $[\alpha]_D$ +90.9° ($CHCl_3$), δ_H ($CDCl_3$) 1.44 (3 H, s, 3-NHAc), 2.08 (3 H, s, 1-OAc), 3.68br (2 H, s, 6-CH_2), 5.63 (1 H, d, $J_{1ax,2ax}$ 8 Hz, 1*ax*-H), and 7.33 (15 H, s, $CH_2C_6H_5$).

3-*Acetamido*-2,4,6-*tri*-O-*benzyl*-3-*deoxy*-α-D-*galactopyranosyl Chloride* (28).—3-Acetamido-1-*O*-acetyl-2,4,6-tri-*O*-benzyl-3-deoxy-β-D-galactopyranose (27) (1.5 g) was dissolved in dioxan (75 ml) containing 4% by weight of hydrogen chloride and acetyl chloride (35 ml). The mixture was allowed to remain under dry nitrogen at 40 °C for 16 h. The solution was evaporated to dryness and repeatedly co-distilled with toluene to remove the hydrogen chloride. Crystallization from hexane gave the crystalline *chloride* (28) (1.1 g, 77%), m.p. 158—159 °C (Found: C, 67.8; H, 6.5; Cl, 7.2; N, 2.6. $C_{29}H_{32}ClNO_5$ requires C, 68.30; H, 6.3; Cl, 7.0; N, 2.75%), $[\alpha]_D$ +169.9° ($CHCl_3$), δ_H ($CDCl_3$) 1.58br (3 H, s, NHAc), 4.52br (6 H, s, 6-CH_2), 6.32 (1 H, d, $J_{1eq,2ax}$ 3.3 Hz, 1-*eq*-H), and 7.33 (15 H, s, $CH_2C_6H_5$).

O-3-*Amino*-3-*deoxy*-α-D-*galactopyranosyl*-(1 ⟶ 6)-*gentamine* C_1 (16).—1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) (2.2 g) and silver toluene-*p*-sulphonate (1.4 g) were added to dry benzene (500 ml) and the volume was reduced to 250 ml by distillation. 3-Acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-galactopyranosyl chloride (28) (2 g) was added and the mixture was stirred at 40 °C for 72 h. The mixture was filtered and evaporated to dryness. The residue was dissolved in chloroform and added to 25% v/v ether–hexane and the precipitate was filtered off. The solid was chromatographed on silica gel (300 g) using 2% v/v ethanol–chloroform as the eluant to give the product. The latter was rechromatographed on silica gel (200 g) using 1.5% v/v ethanol–chloroform as the eluant to give O-3-*acetamido*-2,4,6-*tri*-O-*benzyl*-3-*deoxy*-α-D-*galactopyranosyl*-(1 ⟶ 6)-1,3,2′,6′-*tetrakis*-N-*benzyloxycarbonylgentamine* C_1 (15) (770 mg) as an amorphous solid (Found: C, 67.7; H, 6.4; N, 5.4. $C_{75}H_{85}N_5O_{17}$ requires C, 67.8; H, 6.4; N, 5.3%), $[\alpha]_D$ +82.7° ($CHCl_3$), δ_H ($CDCl_3$) 1.15 (3 H, d, $J_{6',7'}$ 6.5 Hz, 7′-CH_3), 1.45 (3 H, s, 3″-NHAc), 2.67 (3 H, s, 6′-NCH_3), 5.04br (14 H, s, $CH_2C_6H_5$), and 7.27, 7.30 and 7.32 (35 H, s, $CH_2C_6H_5$). The latter (15) (20 mg) was dissolved in tetrahydrofuran (7.5 ml) and ammonia (100 ml; distilled over sodium) was added. Sodium (0.7 g) was added in portions and the mixture was stirred at −80 °C for 45 min. The ammonia was allowed to distil off at room temperature. Water (50 ml) containing potassium hydroxide (0.4 g) was added to the residue and the mixture was heated at 100 °C for 72 h. The mixture was neutralized with Amberlite IRC 50 (H^+) resin and the resin was eluted as described previously. The eluate was evaporated to dryness and the residue was chromatographed on silica gel (30 g) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give *O*-3-*amino*-3-*deoxy*-α-D-*galactopyranosyl*-(1 ⟶ 6)-*gentamine* C_1 (16) (175 mg, 14%) as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization (Found: C, 47.8; H, 8.70; N, 13.6. $C_{22}H_{43}N_5O_9 \cdot 2.5H_2O$ requires C, 47.4; H, 8.70; N, 13.8%), $[\alpha]_D$ +119.6° (H_2O), δ_H (D_2O) 0.96 (3 H, d, $J_{6',7'}$ 6.5 Hz, 7′-CH_3), 2.25 (3 H, s, 6′-NCH_3), 5.04 (1 H, d, $J_{1eq'',2ax''}$ 4 Hz, 1*eq*″-H) and 5.08 (1 H, d, $J_{1eq',2ax'}$ 3.5 Hz, 1*eq*′-H).

O-2-*Amino*-2-*deoxy*-α-D-*glucopyranosyl*-(1 ⟶ 6)-*gentamine* C_1 (52).—(*i*) 1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) (10 g) (dried) was dissolved in dry, redistilled dimethylformamide (70 ml) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (33) [43] (4.36 g) was added. The mixture was allowed to remain under argon at 25 °C for 72 h. The reaction mixture was concentrated and water (200 ml) was added. The mixture was extracted with chloroform and the latter was dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed on silica gel (90 × 5.5 cm) using chloroform, then chloroform–acetone (12 : 1 v/v), and finally chloroform–acetone (8 : 1 v/v) as the eluant to give *O*-3,4,6-tri-*O*-acetyl-2-deoxy-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 6)-1,3,2′,6′-tetra-*N*-benzyloxycarbonylgentamine C_1 (35) (1.35 g) as an amorphous solid. The oxime (35) (1.3 g) was dissolved in dry pyridine (4.2 ml) and acetic anhydride (1.5 ml) was added. The mixture was allowed to remain at 25 °C for 16 h. The mixture was poured into water and the precipitate was filtered off and dried affording *O*-2,3,4,6-*tetra*-*O*-*acetyl*-2-*deoxy*-2-*hydroxyimino*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 6)-1,3,2′,6′-*tetrakis*-*N*-*benzyloxycarbonylgentamine* C_1 (36) (1.31 g) as an amorphous solid (Found: C, 59.2; H, 5.95; N, 5.55. $C_{59}H_{69}N_5O_{21}$ requires C, 59.8; H, 5.9; N, 5.9%), $[\alpha]_D$ +75.3° ($CHCl_3$). The oxime acetate (36) (1.23 g) was dissolved in dry tetrahydrofuran (4 ml) at 0 °C. A 1M-solution of borane in tetrahydrofuran (13 ml) was added dropwise to the stirred solution at 0 °C and the mixture was then allowed to remain at 7 °C for 19 h. The excess of borane was destroyed by dropwise addition of water and the mixture was then evaporated to dryness. The residue (1.2 g) was dissolved in tetrahydrofuran (10 ml) and ammonia (60 ml; distilled over sodium) was added to the mixture at −80 °C. Sufficient sodium was added to maintain a blue colour and the mixture was stirred for 30 min. Methanol (4 ml) was added and the ammonia was allowed to distil off overnight. The reaction was acidified to pH 10.5 with 1M-sulphuric acid and then neutralized with Amberlite IRC 50 (H^+) resin. The resin was eluted as described previously and evaporation gave a product, which was chromatographed on silica gel (60 × 1.4 cm) using a chloroform–methanol–concentrated ammonium hydroxide solution (3 : 4 : 2 v/v) as the eluant to give O-2-*amino*-2-*deoxy*-α-D-*glucopyranosyl*-(1 ⟶ 6)-*gentamine* C_1 (52) (46 mg, 1%) as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization (Found: C, 46.2; H, 8.4; N, 13.0. $C_{20}H_{41}N_5O_8 \cdot H_2CO_3$ requires C, 46.6; H, 8.00; N, 12.9%), $[\alpha]_D$ +112.1° (H_2O), δ_H (D_2O, pD 5.85) 1.26 (3 H, d, $J_{6',7'}$ 7 Hz, 7′-CH_3), 2.71 (3 H, s, 6′-NCH_3), 5.35 (1 H, d, $J_{1eq'',2ax''}$ 4 Hz, 1-*eq*-H), and 5.73 (1 H, d, $J_{1eq',2ax'}$ 3.5 Hz, 1*eq*′-H).

(*ii*) 1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) (10.9 g) was dissolved in dry toluene (600 ml), and Drierite (freshly ground and baked out on a hot-plate) (50 g), mercury(II) cyanide (4.75 g), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (54) [44,45] (6.35 g) were added. The mixture was heated at 100 °C for 18 h and at 110 °C for 6 h. The solution was cooled and filtered through a Celite pad, and the latter was washed with 10% v/v methanol–chloroform. The combined filtrates were evaporated to dryness and the residue (20 g) was chromatographed on silica gel dry columns (5 × 600 g) using 40% v/v ethyl acetate–chloroform as the eluant to give *O*-3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-gluco-

pyranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (53) * (2.63 g, 20%) as a pale yellow amorphous solid, δ_H [$CDCl_3$–CD_3OD (3 : 1 v/v)] 1.17 (3 H, d, $J_{6',7'}$ 6 Hz, 7′-CH_3), 1.87, 2.00, 2.03, and 2.10 (9 H, s, OAc), 2.67 (3 H, s, 6′-NCH_3), 7.17, 7.33, 7.37, and 7.38 (21 H, s, $CH_2C_6H_5$ and DNP †), and 8.17, 8.75, and 8.98 (2 H, m, DNP). The compound (53) was dissolved in methanol (500 ml) and the solution was saturated with ammonia at 0 °C. After 44 h at 25 °C the solution was evaporated to dryness and the residue was dissolved in acetone (300 ml) and water (150 ml). Bio Rad AG 1 × 2 (OH^-) resin (50 ml) was added and the mixture was stirred at 25 °C for 72 h. The resin was filtered off and washed with acetone–water (2 : 1 v/v) (100 ml) and the combined filtrates were evaporated to dryness. The residue was dissolved in liquid ammonia (100 ml) at −70 °C and sodium (1.5 g) was added and the mixture was stirred at −70 °C for 2 h. The excess of sodium was destroyed by dropwise addition of water and the ammonia was allowed to evaporate off. The residue was cooled to 0 °C and transferred to a Bio Rex 70 (H^+) column and the column was washed with water and then eluted with 1.5M-ammonium hydroxide. The eluate was evaporated to dryness and the residue was chromatographed on silica gel using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give *O*-2-amino-2-deoxy-α-D-glucopyranoside (52) (217 mg, 24%) as an amorphous solid, identical with that prepared in (i) above.

O-2-*Amino*-2-*deoxy*-α-D-*glucofuranosyl*-(1 → 6)-*gentamine* C_1 (57) *and* O-2-*Amino*-2-*deoxy*-β-D-*glucofuranosyl*-(1 → 6)-*gentamine* C_1 (59).—1,3,2′,6′-Tetrakis-*N*-benzyloxycarbonylgentamine C_1 (3) (5.5 g) was dissolved in a mixture of dry toluene (200 ml) and dry dioxan (70 ml) and calcium sulphate (dried and baked out on a hot-plate) (29 g), mercury(II) cyanide (8.3 g) and 3,5,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α- and -β-D-glucofuranosyl chloride (55) [46,47] (6.5 g) were added. The mixture was heated under nitrogen at 100 °C for 7 days. The mixture was cooled and filtered, and the residue was washed with ethyl acetate. The combined filtrates were washed with 20% aqueous potassium bromide and water, dried (Na_2SO_4), filtered, and evaporated to dryness. The residue (11 g) was chromatographed on silica gel dry columns (3 × 1 kg) using 50% v/v ethyl acetate–chloroform as the eluant. The chromatography was repeated to give pure samples of *O*-3,5,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-α-D-*glucofuranosyl*-(1 → 6)-1,3,2′,6′-*tetrakis*-N-*benzyloxycarbonylgentamine* C_1 (56) (1.62 g, 19%) as a pale yellow solid which crystallized from acetone–ether (Found: C, 58.5; H, 5.9; N, 7.75. $C_{64}H_{73}N_7O_{23}$ requires C, 58.75; H, 5.6; N, 7.50%), δ_H [$CDCl_3$–CD_3OD (3 : 1 v/v)] 1.15 (3 H, d, $J_{6',7'}$ 7 Hz, 7′-CH_3), 1.98, 2.02, and 2.15 (9 H, br, s, OAc), 2.69br (3 H, s, 6′-NCH_3), 6.90 (1 H, d, *J* 9 Hz, DNP), 7.47 (20 H, s, $CH_2C_6H_6$), 8.25 (1 H, d, *J* 9 Hz, DNP), and 9.02 (1 H, d, *J* 2 Hz, DNP), and *O*-3,5,6-tri-*O*-acetyl-2-(2,4-dinitrophenylamino)-β-D-glucofuranosyl-(1 → 6)-1,3,2′,6′-tetrakis-*N*-benzyloxycarbonylgentamine C_1 (58) (1.22 g, 15%) as a pale yellow amorphous solid, δ_H [$CDCl_3$–CD_3OD (3 : 1 v/v)] 1.16 (3 H, d, $J_{6',7'}$ 7 Hz, 7′-CH_3), 2.03br and 2.09br (9 H, s, OAc), 2.67br (3 H, s, 6′-NCH_3), 7.16 (1 H, d, *J* 9 Hz, DNP), 7.42 (20 H, s, $CH_2C_6H_5$), 8.41 (1 H, d, *J* 9 Hz, DNP), and 9.12 (1 H, d, *J* 2 Hz, DNP).

* A minor isomeric component (2%) was also isolated, but no further work was done on it.

† DNP = 2,4-dinitrophenyl.

The α-anomer (56) (464 mg) was dissolved in methanol (90 ml) containing ammonium hydroxide (10 ml) and after 3 days at 25 °C the solution was evaporated to dryness. The residue was dissolved in warm acetone (198 ml), and water (102 ml) and Amberlite IRA 400 (OH^-) resin were added and the mixture was stirred at 25 °C for 24 h. The resin was filtered off and washed with acetone–water (2 : 1 v/v) (100 ml) and the combined filtrates were evaporated to dryness. The residue was dissolved in liquid ammonia (100 ml) at −78 °C and sodium (400 mg) was added. The mixture was stirred at −78 °C for 2 h. The reaction was worked up as described for (52). The eluate was evaporated to dryness and the residue was chromatographed on silica gel (5 g) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give *O*-2-*amino*-2-*deoxy*-α-D-*glucofuranosyl*-(1 → 6)-*gentamine* C_1 (57) (77 mg, 46%) as an amorphous solid [Found: $(M + 1)^+$ 480.3056. $C_{20}H_{42}N_5O_8$ requires *M* + 1, 480.3032], δ_H (D_2O) 1.16 (3 H, d, $J_{6',7'}$ 7 Hz, 7′-CH_3), 2.49 (3 H, s, 6′-NCH_3), 5.23 (1 H, d, $J_{1eq',2ax'}$ 4 Hz, 1*eq*′-H), and 5.37 (1 H, d, $J_{1'',2''}$ 5 Hz, 1″-H).

The β-anomer (58) (1.2 g) was deprotected and purified as above to give *O*-2-amino-2-deoxy-β-D-glucofuranosyl-(1 → 6)-gentamine C_1 (59) (41 mg, 9%) as an amorphous solid, δ_H (D_2O) 1.14 (3 H, d, $J_{6',7'}$ 7 Hz, 7′-CH_3), 2.43 (3 H, s, 6′-NCH_3), 5.17 (1 H, s, 1″-H), and 5.18 (1 H, d, $J_{1eq',2ax'}$ 4 Hz, 1*eq*′-H).

G.l.c.–Mass Spectral Determination of Linkages to the Deoxystreptamine.—General procedure. A solution of the aminoglycoside (10 mg) in methanol (3 ml) was treated with acetic anhydride (1 ml) and the mixture was allowed to stand at 25 °C for 18 h. The mixture was evaporated to dryness and the residue was dissolved in dry dimethylformamide (5 ml) and added to a mixture of sodium hydride (6.5 mg) and dry dimethylformamide (2 ml). The reaction was stirred at 25 °C for 0.5 h and methyl iodide (0.2 ml) was added. The mixture was stirred at 25 °C for 4 days, whereupon methanol (10 ml) was added. The solution was filtered and evaporated to dryness. The residue was dissolved in a mixture of chloroform (20 ml) and water (10 ml) and the layers were separated. The aqueous layer was extracted with chloroform (3 × 20 ml) and the combined chloroform extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The resulting per-*N*-acetyl-per-*NO*-methylaminoglycoside was treated with 6M-hydrochloric acid (5 ml) and the mixture was heated on a steam-bath for 2 h and then evaporated to dryness. The residue was neutralized with Amberlite IRA 401S (OH^-) resin and the latter was then washed with water (3 × 5 ml) and the combined aqueous filtrates were evaporated to dryness. The residue was dissolved in methanol (3 ml) and acetic anhydride (1 ml) and allowed to stand at 25 °C for 1 h. The solution was evaporated to dryness to afford the 1,3-di-*N*-acetylmono-*O*-methyl-1,3-di-*N*-methyl-2-deoxystreptamine (*ca.* 1 mg). The g.l.c.–mass spectrum of the trimethylsilyl derivative was then obtained. 4,6-Di-*O*-glycosyl derivatives gave 1,3-di-*N*-acetyl-5-*O*-methyl-1,3-di-*N*-methyl-2-deoxystreptamine (60) which exhibited characteristic peaks at *m/e* 254 (*m*) and 187 (*n*) (Table 3) (Figure 1). 4,5-Di-*O*-glycosyl derivatives, on the other hand, gave 1,3-di-*N*-acetyl-6-*O*-methyl-1,3-di-*N*-methyl-2-deoxystreptamine (61) which exhibited characteristic peaks at *m/e* 196 (*o*) and 129 (*p*) (Table 3) (Figure 1).

We thank Mr. J. McGlotten and his staff for providing

spectral and analytical data. Thanks are also extended to Mr. R. W. Tkach and Dr. H. Buchoff for providing the gentamine intermediates.

[0/1618 *Received*, 21*st October*, 1980]

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