Semisynthetic Aminoglycoside Antibacterials. Part II.[1] Synthesis of Gentamicin X_2 and Related Compounds

By **Max Kugelman, Alan K. Mallams,*** and **H. Frederick Vernay,** Research Division, Schering Corporation, Bloomfield, New Jersey 07003, U.S.A.
David F. Crowe, George Detre, Masato Tanabe, and **Dennis M. Yasuda,** Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025, U.S.A.

Gentamicin X_2, a naturally occurring aminoglycoside antibiotic produced as a minor component together with the clinically important gentamicin C complex by *Micromonospora purpurea*, has been synthesized by glycosylation of suitably protected garamine derivatives. The synthesis of the α-glycoside was accomplished by means of the Lemieux–Nagabhushan reaction as well as by using a Koenigs–Knorr reaction. The latter reaction was also used to prepare the 4-*O*-β-analogue of gentamicin X_2. The syntheses of other analogues of gentamicin X_2, namely 4-*O*-[2-amino-2-deoxy-α-D-mannopyranosyl]. 4-*O*-[2-amino-2-deoxy-α-D-galactopyranosyl]. 4-*O*-[2-amino-2-deoxy-α- and -β-D-glucofuranosyl]. 4'-*O*-[2-amino-2-deoxy-α-D-glucopyranosyl]. 4-*O*-α-D-glucopyranosyl, 4-*O*-α-D-talopyranosyl, and 4-*O*-2-deoxy-α-D-galactopyranosyl derivatives of garamine, are also described.

DURING the past decade the growth of multiple drug resistant strains of bacteria carrying extrachromosomal elements commonly referred to as R factors [2] has given considerable impetus to the search for new semisynthetic aminoglycoside antibiotics active against such strains. The ready availability of suitably protected garamine derivatives [1] made it possible to contemplate the synthesis not only of the naturally occurring aminoglycoside gentamicin X_2 (1),[3,4] but also of a variety of analogues. This would broaden our understanding of the structure–activity requirements of the 4-*O*-glycosyl unit in aminoglycoside antibiotics containing the garamine unit. In view of the fact that gentamicin X_2 (1) has been shown to possess not only broad spectrum antibacterial activity, but also antiprotozoal and anthelmintic activity,[3] we decided initially to concentrate our attention on this series of compounds.

The synthesis of α-glycosides is a long-standing problem in carbohydrate chemistry; three synthetic reactions are commonly used. The Lemieux–Nagabhushan reaction [5-16] leads selectively to the α-glycoside in most instances and may be used to prepare both 2-amino- and 2-hydroxy-glycosides from a common intermediate. The Koenigs–Knorr reaction,[17-19] although less selective for α-glycoside formation, may be successfully used to prepare reasonable yields of the α-glycoside provided care is exercised in the choice of protecting groups for the glycosyl halide. This reaction may also be used to obtain β-glycosides. Acid-catalysed addition of a glycal to the hydroxy-group invariably leads to both α- and β-glycosides.[17,20,21]

The Lemieux–Nagabhushan reaction was applied to the synthesis of gentamicin X_2 (1) as follows. Tri-*O*-acetyl-D-glucal (13) on treatment with nitrosyl chloride afforded the dimer (15) of 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride [5] in high yield. Initially the nitroso-chloro-adduct (15) was condensed with 1,3,3'-tris-*N*-benzyloxycarbonylgaramine (17) [1] in dimethylformamide at 25 °C to give a 46% yield of *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (24), together with a less polar

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[19] R. J. Ferrier and D. Prasad, *J. Chem. Soc.*, 1965, 7429.
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TABLE 1

Aminoglycoside mass spectral ions [*m/e* (%)] ‡

Compd.	$(M+1)^+$	$M^{+\cdot}$	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}	A_{11}	A_{12}
(1)	483 (1)	482 (0.2)	352 (10)	334 (2)	324 (7)	306 (4)	350 (14)	332 (4)	322 (5)	304 (10)	191 (74)	173 (22)	163 (50)	145 (100)
(2)	511 (2)	510 (2)	380 (27)	362 (2)	352 (9)	334 (6)	350 (21)	332 (8)	322 (7)	304 (41)	191 (53)	173 (19)	163 (18)	145 (45)
(3)	511 (1)		352 (11)	334 (3)	324 (7)	306 (6)	378 (10)	360 (3)	350 (3)	332 (6)	191 (72)	173 (27)	163 (29)	145 (79)
(4)	483 (1)	482 (0.3)	352 (15)	334 (2)	324 (7)	306 (6)	350 (18)	332 (5)	322 (7)	304 (16)	191 (63)	173 (18)	163 (46)	145 (65)
(5)	511 (3)	510 (5)	380 (28)	362 (2)	352 (9)	334 (6)	350 (27)	332 (8)	322 (15)	304 (47)	191 (50)	173 (17)	163 (18)	145 (37)
(42)	483 (1)		352 (34)	334 (4)	324 (16)	306 (11)	350 (18)	332 (7)	322 (6)	304 (13)	191 (100)	173 (45)	163 (77)	145 (85)
(6)	483 (0.2)	482 (0.1)	352 (8)	334 (2)	324 (3)	306 (4)	350 (12)	332 (3)	322 (8)	304 (12)	191 (100)	173 (25)	163 (80)	145 (95)
(7)	511 (2)	510 (2)	380 (11)	362 (2)	352 (5)	334 (4)	350 (9)	332 (5)	322 (4)	304 (22)	191 (46)	173 (14)	163 (20)	145 (46)
(45)	483 (0.4)		352 (5)	334 (1)	324 (2)	360 (2)	350 (7)	332 (1)	322 (2)	304 (5)	191 (100)	173 (17)	163 (65)	145 (70)
(49)	483 (1)										191 (90)	173 (19)	163 (60)	145 (100)
(8)	484 (0.4)		353 (7)	335 (2)	325 (6)	307 (6)	350 (2)	332 (0.5)	322 (2)	304 (2)	191 (74)	173 (7)	163 (60)	145 (100)
(9)	468 (2)	467 (1)	353 (5)	335 (3)	325 (70)	307 (38)	334 (7)	316 (2)	306 (5)	288 (13)	191 (65)	173 (15)	163 (75)	145 (80)
(10)	484 (0.2)		353 (19)	335 (3)	325 (10)	307 (8)	350 (3)	332 (1)	322 (5)	304 (2)	191 (74)	173 (11)	163 (65)	145 (72)
(11)	484 (0.2)		353 (16)	335 (3)	325 (8)	307 (8)	350 (4)	332 (2)	322 (3)	304 (3)	191 (74)	173 (13)	163 (51)	145 (83)
(59)	484 (0.1)		353 (2)	335 (0.5)	325 (1)	307 (1)	350 (0.1)	332 (4)	322 (6)	304 (1)	191 (68)	173 (10)	163 (40)	145 (84)
(12)	468 (1)	467 (0.5)	337 (26)	319 (4)	309 (12)	291 (11)	350 (5)	332 (4)	322 (2)	304 (2)	191 (100)	173 (24)	163 (65)	145 (90)
(32)	350 (4)	349 (0.5)									191 (100)	173 (20)	163 (23)	145 (80)
(34)	306 (11)	305 (14)									191 (36)	173 (14)	163 (40)	145 (60)

Compd.	B_1	C_1	D_9	D_{10}	E_1	E_2	E_3	E_4	E_5	E_6	F_1	F_2
(1)	162 (39)	160 (80)	362 (2)	203 (3)	407 (4)	246 (10)	365 (4)	204 (13)			291 (10)	289 (7)
(2)	190 (100)	160 (80)	390 (7)	231 (4)	435 (5)	246 (8)	393 (5)				319 (26)	289 (12
(3)	162 (37)	188 (71)	390 (2)	231 (3)			365 (3)	204 (13)	436 (6)	275 (5)	291 (9)	316 (6)
(4)	162 (56)	160 (100)	362 (3)	203 (4)	407 (3)	246 (12)	365 (4)	204 (14)			291 (11)	289 (7)
(5)	190 (100)	160 (80)	390 (9)	231 (3)	435 (5)	246 (7)	393 (6)				319 (22)	289 (13)
(42)	162 (78)	160 (85)	362 (1)	203 (5)	407 (3)	246 (20)	365 (5)	204 (26)			291 (15)	289 (9)
(6)	162 (42)	160 (90)	362 (2)	203 (10)	407 (1)	246 (25)	365 (2)	204 (22)			291 (11)	289 (7)
(7)	190 (72)	160 (100)	390 (3)	231 (4)	435 (2)	246 (6)	393 (2)				319 (15)	289 (6)
(45)	162 (12)	160 (85)	362 (1)		407 (1)	246 (19)	365 (2)	204 (17)			291 (4)	289 (3)
(49)	162 (80)	160 (38)				246 (28)		204 (20)				
(8)	163 (60)	160 (84)		204 (12)	408 (0.5)	246 (7)	366 (2)	204 (12)			292 (7)	289 (2)
(9)	163 (75)	144 (100)		204 (39)			366 (30)	204 (39)			292 (65)	273 (7)
(10)	163 (65)	160 (100)		204 (11)	408 (2)	246 (8)	366 (3)	204 (11)			292 (15)	289 (6)
(11)	163 (51)	160 (78)	363 (1)	204 (12)	408 (2)	246 (7)	366 (3)	204 (12)			292 (15)	289 (7)
(59)	163 (40)	160 (54)		204 (5)	408 (0.1)	246 (8)		204 (5)			292 (0.5)	289 (1)
(12)	147 (3)	160 (85)			392 (3)	246 (20)	350 (5)	204 (40)			276 (25)	289 (7)
(32)		188 (30)						204 (55)		275 (18)		
(34)		144 (40)						204 (45)				

‡ The structures and designations of all fragment ions are identical with those described in our publication on the mass spectra of aminocyclitol antibiotics.[22] Only fragment ions not previously described are included in full detail in this text.

TABLE 2

Molecular rotations $[M]_D$ (°) (in H_2O)

Compound	Exptl.	Calc.
Garamine (33)	+435	
Gentamicin X_2 (1)	+746	+667
Methyl 2-amino-2-deoxy-α-D-glucopyranoside [23]	+232	
O-2-Amino-2-deoxy-α-D-mannopyranosyl-(1 ⟶ 4)-garamine (4)	+638 [a]	+559
Isopropyl 2-amino-2-deoxy-α-D-mannopyranoside	+124	
O-2-Amino-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-garamine (42)	+427	+381
Methyl 2-amino-2-deoxy-β-D-glucopyranoside [23]	−54	
O-2-Amino-2-deoxy-α-D-galactopyranosyl-(1 ⟶ 4)-garamine (6)	+751	+790
Isopropyl 2-amino-2-deoxy-α-D-galactopyranoside hydrochloride [13]	+355	
O-2-Amino-2-deoxy-α-D-glucofuranosyl-(1 ⟶ 4)-garamine (45)	+645	+494
Methyl 2-amino-2-deoxy-α-D-glucofuranoside [24]	+59 [b]	
O-2-Amino-2-deoxy-β-D-glucofuranosyl-(1 ⟶ 4)-garamine (47)	+300	
O-2-Amino-2-deoxy-α-D-glucopyranosyl-(1 ⟶ 4′)-garamine (49)	+694	+667
O-α-D-Glucopyranosyl-(1 ⟶ 4)-garamine (8)	+707	+742
Methyl α-D-glucopyranoside [24]	+307	
O-α-D-Talopyranosyl-(1 ⟶ 4)-garamine (10)	+839	+842
Methyl α-D-talopyranoside [25]	+407	
O-α-D-Mannopyranosyl-(1 ⟶ 4)-garamine (11)	+624	+589
Methyl α-D-mannopyranoside [26]	+154	
O-β-D-Mannopyranosyl-(1 ⟶ 4)-garamine (59)	+491	+340
Methyl β-D-mannopyranoside [27]	−95	

[a] In methanol. [b] In acetone.

tetrasaccharide tentatively assigned structure (31) on the basis of the known reactivities of the hydroxy-groups in garamine.[1] The formation of the tetrasaccharide (31) is reasonable in view of the presence of 2 equiv. of the nitroso-chloro-adduct (monomer) (15) per equiv. of the garamine derivative (17). When the amount of (15) was reduced to 1 equiv. some tetrasaccharide (31) was still obtained, indicating that there was competitive reaction between the 4- and 2′-hydroxy-groups in (17), although the former was more reactive. The ^1H n.m.r. spectrum of the oxime (24) exhibited a broad singlet at δ 6.35 due to the anomeric H-1′, indicating that the oxime was in the *Z*-configuration.[11] Had the oxime possessed the *E*-configuration the signal for H-1′ would have occurred at higher field.[11] The oxime (24) on acetylation with acetic anhydride in pyridine at 25 °C gave the acetate (26). Reduction of the latter with 4.5 equiv. of borane in tetrahydrofuran at 25 °C for 18 h, followed by catalytic hydrogenation over 30%

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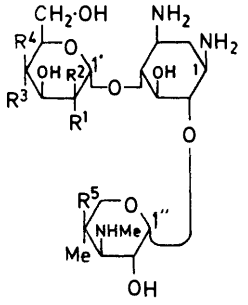
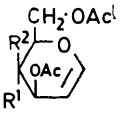
[24] E. Fischer, *Ber.*, 1893, **26**, 2406.
[25] P. A. J. Gorin, *Canad. J. Chem.*, 1960, **38**, 641.
[26] E. Fischer and L. Beench, *Ber.*, 1896, **29**, 2927.
[27] B. Helferich and G. Duve, *Chem. Ber.*, 1958, **91**, 1790.

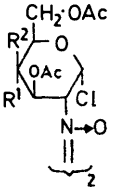
palladium–carbon in glacial acetic acid and then refluxing with 90% hydrazine hydrate at 120 °C for 24 h,



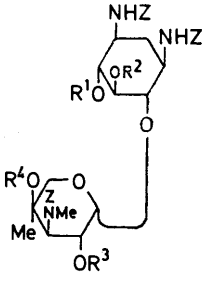
(1) $R^1 = NH_2, R^2 = R^4 = H, R^3 = R^5 = OH$
(2) $R^1 = NHEt, R^2 = R^4 = H, R^3 = R^5 = OH$
(3) $R^1 = NH_2, R^2 = R^4 = H, R^3 = OH, R^5 = OEt$
(4) $R^1 = R^4 = H, R^2 = NH_2, R^3 = R^5 = OH$
(5) $R^1 = R^4 = H, R^2 = NHEt, R^3 = R^5 = OH$
(6) $R^1 = NH_2, R^2 = R^3 = H, R^4 = R^5 = OH$
(7) $R^1 = NHEt, R^2 = R^3 = H, R^4 = R^5 = OH$
(8) $R^1 = R^3 = R^5 = OH, R^2 = R^4 = H$
(9) $R^1 = R^3 = OH, R^2 = R^4 = R^5 = H$
(10) $R^1 = R^3 = H, R^2 = R^4 = R^5 = OH$
(11) $R^1 = R^4 = H, R^2 = R^3 = R^5 = OH$
(12) $R^1 = R^2 = R^3 = H, R^4 = R^5 = OH$



(13) $R^1 = OAc, R^2 = H$
(14) $R^1 = H, R^2 = OAc$



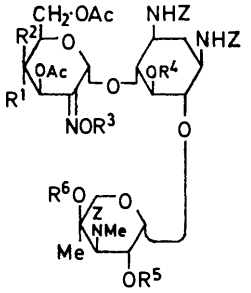
(15) $R^1 = OAc, R^2 = H$
(16) $R^1 = H, R^2 = OAc$



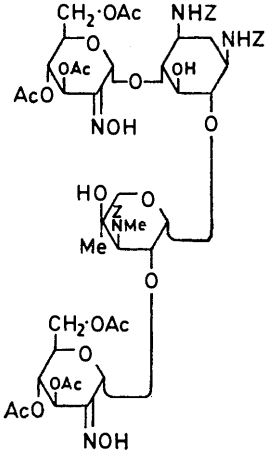
(17) $R^1 = R^2 = R^3 = R^4 = H$
(18) $R^1 = R^2 = R^4 = H, R^3 = Ac$
(19) $R^1 = H, R^2 = R^3 = R^4 = Ac$
(20) $R^1 = R^3 = R^4 = Ac, R^2 = H$
(21) $R^1 = R^2 = R^3 = H, R^4 = Ac$
(22) $R^1 = R^2 = R^3 = Ac, R^4 = H$
(23) $R^1 = R^2 = R^3 = R^4 = Ac$

$Z = PhCH_2 \cdot O \cdot CO$

afforded gentamicin X_2 (1) as the principal product in 10% yield from the oxime (24). The physical and biological data were identical with those of the authentic natural antibiotic.[3,4] The formation of the protonated formyl sequences of ions A_1—A_4 in the mass spectrum of (1) (Table 1), together with the c.d. spectrum which exhibited $[\theta]_{290}$ −12 500 in TACu (tetra-amminecopper sulphate) solution, clearly established the linkage of the glucosamine unit to the 4-hydroxy-group of garamine. The coupling constant of the anomeric H-1′ was 4 Hz, confirming the α-*gluco*-stereochemistry in the product. The molecular rotation is given in Table 2. The borane reduction was not, however, stereoselective; the other major product was *O*-2-amino-2-deoxy-α-D-mannopyranosyl-(1 ⟶ 4)-garamine (4), formed in 9% yield based on the oxime (24). The mass spectrum of (4) (Table 1) was consistent with the proposed structure, and the presence of the protonated formyl ions A_1—A_4, together with the c.d. spectrum ($[\theta]_{290}$ −3 860 in TACu solution), constituted evidence for the location of the mannosamine unit at the 4-position of garamine. The newly formed anomeric proton gave rise to a doublet at δ 5.17 with $J_{1',2'}$ 2 Hz, consistent with a *manno*-configuration. The molecular rotation of (4) (Table 2) was in agreement with a 4-*O*-α-D-mannosyl structure. The ^{13}C n.m.r. spectrum of (4) revealed resonances at δ_C 87.7 due to C-4 and C-6 which on protonation of the amino-groups shifted upfield[28] to δ_C 81.0 and 84.1, respectively, consistent with a 4,6-*O*-glycosidic structure (Table 3). The resonances due to the garosamine carbon atoms were in good agreement with those reported for the gentamicins.[29]



(24) $R^1 = OAc, R^2 = R^3 = R^4 = R^5 = R^6 = H$
(25) $R^1 = OAc, R^5 = Ac, R^2 = R^3 = R^4 = R^6 = H$
(26) $R^1 = OAc, R^3 = R^5 = Ac, R^2 = R^4 = R^6 = H$
(27) $R^1 = OAc, R^4 = R^5 = R^6 = Ac, R^2 = R^3 = H$
(28) $R^1 = OAc, R^3 = R^4 = R^5 = R^6 = Ac, R^2 = H$
(29) $R^1 = R^3 = H, R^2 = OAc, R^4 = R^5 = R^6 = Ac$
(30) $R^1 = H, R^2 = OAc, R^3 = R^4 = R^5 = R^6 = Ac$



(31)

The chemical shift differences between the α-D-mannosamine C-1′ to -6′ in (4) and those of the corresponding α-D-glucosamine carbon atoms in gentamicin X_2 (1)[4]

[28] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669.
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were in general agreement with results obtained by others [30,31] with methyl α-D-mannopyranoside and methyl α-D-glucopyranoside. The shielding of 1.5 p.p.m. for C-3 and of 1.4 p.p.m. for C-5 relative to

TABLE 3

^{13}C Chemical shifts [a]

Carbon atom	(4)	Δ[DOS → (4)] [b]	(4) (pH 1)	Δ(Base → pH 1)
C-1	51.5	−0.1	50.6	−0.9
C-2	36.3		28.4	−7.9
C-3	50.1	−1.5	49.4	−0.7
C-4	87.7		81.0	−6.7
C-5	75.2	−1.4	76.7	+1.5
C-6	87.7		84.1	−3.6
C-1′	103.4		97.7	−5.7
C-2′	54.7		53.7	−1.0
C-3′	71.2		68.1	−3.1
C-4′	67.4 [c]		67.4 [c]	
C-5′	74.5		74.3	−0.2
C-6′	61.7		60.9	−0.8
C-1″	101.5		102.0	+0.5
C-2″	69.8		67.4 [c]	*ca.* −2.4
C-3″	64.3		64.2	−0.1
C-4″	72.9		70.8	−2.1
C-5″	68.4		68.7	+0.3
3″-NCH_3	37.4		35.3	−2.1
4″-CH_3	22.3		21.7	−0.6

[a] δ_C in p.p.m. downfield from external Me_4Si; [$\delta_C(Me_4Si)$ = δ_C (dioxan) + 67.4] for the free base in D_2O. [b] DOS = deoxystreptamine. [c] Signal obscured by dioxan reference peak.

deoxystreptamine indicated that the α-D-mannosyl derivative (4) had a solution conformation similar to those observed for the gentamicins [29] and for tobramycin.[32] In addition to the above products, two by-products were also formed. One was 2′-*N*-ethylgentamicin X_2 (2), obtained in 3% yield based on the oxime (24). The mass spectrum of (2) exhibited a molecular ion at *m/e* 510, 28 mass units higher than in gentamicin X_2 (1). A high resolution measurement confirmed the presence of an extra C_2H_4 unit. The presence of a triplet in the ^{1}H n.m.r. spectrum at δ 1.10 with *J* 7 Hz as well as a quartet at δ 2.79 with *J* 7 Hz clearly demonstrated the presence of an *N*-ethyl unit. A careful analysis of the mass spectrum of (2) (Table 1) demonstrated that the *N*-ethyl group was located at the 2′-position. The coupling constant for the anomeric H-1′ (3.5 Hz) confirmed the *gluco*-stereochemistry. The presence of the protonated formyl ions A_1—A_4 in the mass spectrum as well as the c.d. spectrum ($[\theta]_{290}$ −11 400 in TACu solution) supported the location of the glycoside unit at the 4-position of garamine. The other by-product, formed in 2% yield from the oxime (24), was *O*-2-deoxy-2-ethylamino-α-D-mannopyranosyl-(1 → 4)-garamine (5). A high resolution mass measurement on the molecular ion indicated that the molecule contained an additional C_2H_4 unit relative to (4). This was again shown to be an *N*-ethyl group [δ_H 1.12 (t, *J* 7 Hz) and 2.72 (q, *J* 7 Hz)]. An analysis of the mass spectrum of (5) (Table 1) showed that the *N*-ethyl group was at the 2′-position. The coupling constant for the anomeric H-1′ proton was 2 Hz, consistent with a *manno*-configuration. The presence of the protonated formyl ions A_1—A_4 in the mass spectrum, together with the c.d. spectrum ($[\theta]_{290}$ −6 470 in TACu solution), indicated that the glycoside unit was at the 4-position of garamine.† All four products [(1), (2), (4), and (5)] were clearly α-glycosides from their ^{1}H n.m.r. spectra. The formation of the 2′-*N*-ethyl derivatives (2) and (5) is most easily explained by *O* → *N* migration of an acetyl group during the reduction, with further reduction in the medium.

Several attempts were made at reducing the oxime (24) by hydrogenation over a variety of catalysts,[7,13] but no isolable amounts of gentamicin X_2 (1) were obtained, the principal product after deblocking being garamine in each instance.

In order to prevent formation of the tetrasaccharide (31) during the reaction, 2 equiv. of the nitroso-chloro-adduct (15) (monomer) were condensed with 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) [1] in dimethylformamide at 25 °C, to give a 42% yield of *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (25). The presence of a signal due to the anomeric H-1′ at δ 6.39 indicated that the oxime existed in the *Z*-configuration.[11] Acetylation of the oxime (25) gave the acetate (26), which on reduction with borane and deprotection as before gave gentamicin X_2 (1) in 25% yield based on the oxime (25). The failure to isolate any tetrasaccharide along with the oxime (25) further supported the location of the fourth sugar unit at the 2″-position in (31).

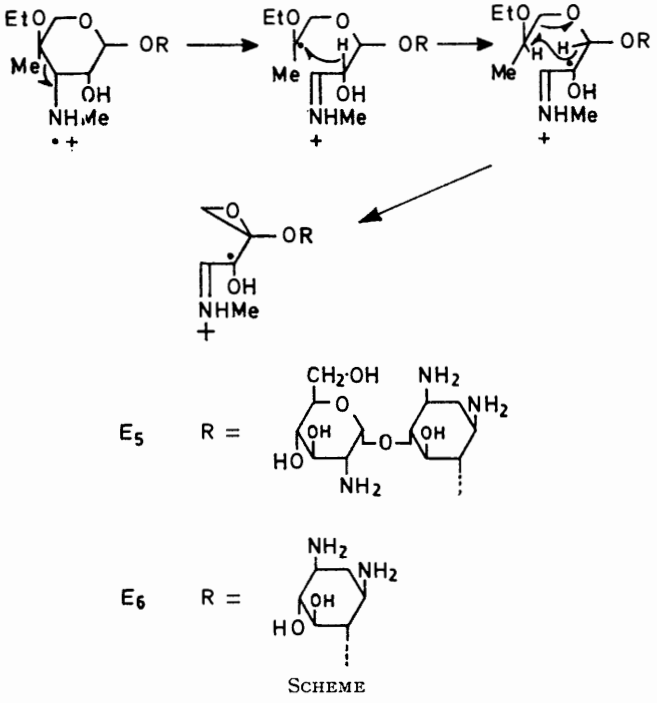
When 2 equiv. of the nitroso-chloro-adduct (15) (monomer) were condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) [1] in dimethylformamide, a 40% yield of *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27) was obtained. The anomeric H-1′ signal occurred as a singlet at δ 6.19, indicating that (27) existed in the *Z*-configuration.[11] Unchanged 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19), as well as the transacylation product 2′,4,4′-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (20) [1] were the only other compounds isolated.‡ Attempted condensation of the nitroso-chloro-adduct (15) with (20) in dimethylformamide failed to effect any glycosylation of

† Additional proof for the 4,6-glycosidic linkage of the sugars was obtained by per-*N*-acetylation followed by per-*ON*-methylation of the trisaccharide. The fully derivatized trisaccharide was hydrolysed with 6N-hydrochloric acid and the product after *N*-acetylation was purified by preparative t.l.c. to give 1,3-di-*N*-acetyl-2-deoxy-1,3-di-*N*-methyl-5-*O*-methyl-D-streptamine, identical (t.l.c. and mass spectrum) with an authentic sample kindly supplied by Dr. P. J. L. Daniels.

‡ Whenever the triacetate (19) was used in a Lemieux–Nagabhushan reaction some was recovered as well as the transacylation product (20). For simplicity, details of the isolation of these products are not included in the Experimental section.

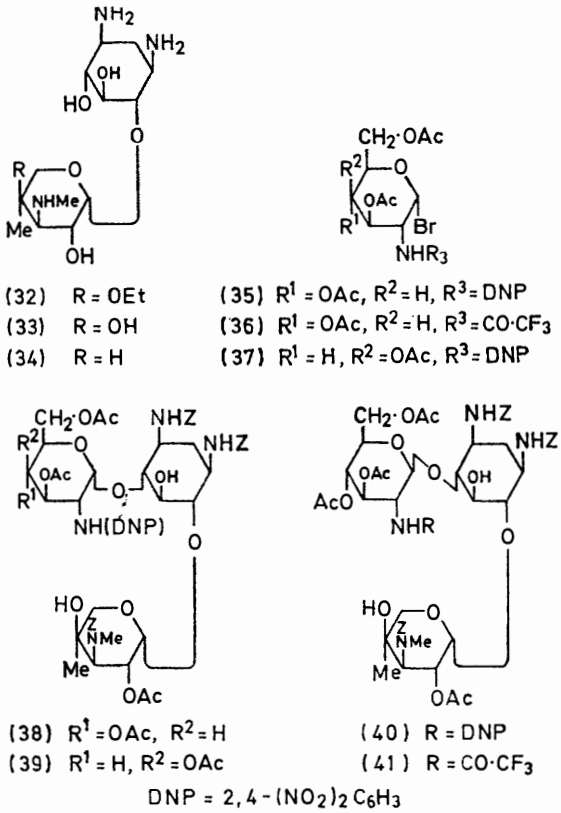
[30] D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1970, **92**, 1355.
[31] A. S. Perlin, B. Casu, and H. J. Koch, *Canad. J. Chem.*, 1970, **48**, 2596.
[32] K. F. Koch, J. A. Rhoades, E. W. Hagaman, and E. Wenkert, *J. Amer. Chem. Soc.*, 1974, **96**, 3300.

the 5-hydroxy-group under conditions similar to those described above. Acetylation of the oxime (27) gave the acetate (28), a portion of which was chromatographed to obtain analytical data. In general the oxime acetates were labile on chromatography and were used without purification. Reduction of the oxime acetate (28) with 20 equiv. of borane in tetrahydrofuran at 7 °C for 18 h followed by deprotection with sodium in liquid ammonia afforded gentamicin X_2 (1) in 21% yield based on the oxime acetate (28). A less polar by-product (8% yield) was 4''-*O*-ethylgentamicin X_2 (3). The mass spectrum of (3) exhibited an $(M + 1)^+$ ion at *m/e* 511 (Table 1) and the key fragment ions indicated that the ethyl group was located at the 4''-position. In contrast to gentamicin X_2 (1), which shows a prominent $M - 75$ peak due to fragment ion E_1 as well as a fragment ion E_2 formed by glycosyl cleavage of E_1, the



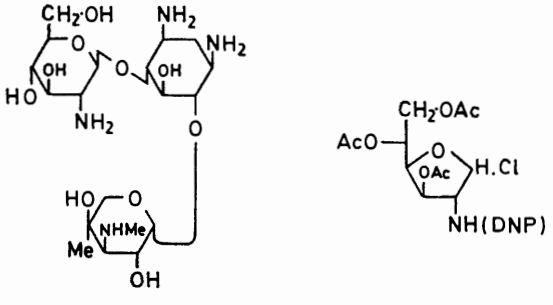
SCHEME

4''-*O*-ethyl derivative (3) did not exhibit such fragment ions. Instead a prominent $M - 74$ ion was observed at *m/e* 436 which may be due to the fragment ion E_5 (Scheme); this in turn underwent glycosyl cleavage with hydrogen transfer to give the ion E_6 at *m/e* 275. The formation of these new ions E_5 and E_6 indicated that the fragmentation pathway leading to ions E_1 and E_2 was no longer favoured in the 4''-*O*-ethyl derivative (3). The ^{1}H n.m.r. spectrum of (3) showed a triplet at δ 1.15 (*J* 7 Hz) and a quartet at δ 3.48 (*J* 7 Hz) consistent with an *O*-ethyl group. The 4''-*O*-ethylgentamicin X_2 (3) presumably arises by reduction of the tertiary 4''-*O*-acetyl group by borane. No *manno*-derivatives were isolated when 20 equiv. of borane were used in the reduction of the oxime. When 4'-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (21) [1] was reduced with 30 equiv. of borane in tetrahydrofuran at 7 °C for 18 h and the product was deprotected by heating



with aqueous 5% sodium hydroxide, 4'-*O*-ethylgaramine (32) was obtained in 20% yield. The mass and ^{1}H n.m.r. spectra were consistent with the proposed structure (32). When the reaction was repeated with 10 equiv. of borane, only traces of 4'-*O*-ethylgaramine (32) were formed, the major product being garamine (33).

Reduction of the oxime acetate (28) with 20 equiv. of borane in tetrahydrofuran at 7 °C for 18 h, followed by removal of the benzyloxycarbonyl groups in glacial acetic acid over 30% palladium–carbon and subsequent



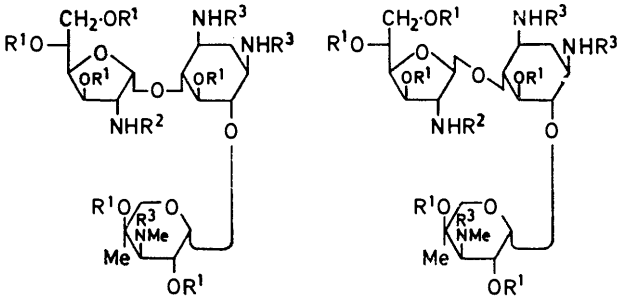
heating with 10% barium hydroxide, afforded gentamicin X_2 (1) in 13% yield and an almost equal amount (14% yield) of 4''-*O*-ethylgentamicin X_2 (3).

The above reactions demonstrated the utility of the Lemieux–Nagabhushan reaction as applied to a variety

of protected garamine derivatives. Some limitations due to the nature of the garamine substrate were evident, but in general the reaction afforded reasonable yields of the desired α-glycosides.

The Koenigs–Knorr reaction has been utilized to synthesize kanamycins A, B, and C,[33-36] and in order to determine the utility of the reaction as applied to garamine derivatives, the synthesis of gentamicin X_2 (1) was undertaken; the modified Helferich conditions [18] were employed to ensure reasonable yields of the desired α-glycoside. 2-*N*-(2,4-Dinitrophenyl)-D-glucosamine [37] was prepared and converted by known procedures [38] into 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (35). The latter was treated with 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) in the presence of Drierite and mercury(II) cyanide in toluene at 110 °C for 24 h to give a 35% yield of *O*-3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl-(1 → 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (38) and a 6% yield of the 4-*O*-β-anomer (40). The presence of a non-participating dinitrophenyl group at N-2 in (35) led to the expected α-glycoside (38) as the major product, although some of the β-glycoside (40) was also produced. The α-glycoside (38) was subjected to ammonolysis and treatment with Amberlite IRA 400 (OH^-) resin, and the product was then treated with sodium in liquid ammonia to give gentamicin X_2 (1) in 60% yield based on (38). The product was identical with both natural gentamicin X_2 [3,4] and the sample prepared by the Lemieux–Nagabhushan reaction.

In order to achieve a more efficient synthesis of the 4-*O*-β-analogue (42) of gentamicin X_2 (1), the trifluoroacetyl group was selected for protection of the 2-amino-group in the monosaccharide. Thus D-glucosamine was converted by literature procedures [39-41] into 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (36). Condensation of the latter with 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) in the presence of Drierite and mercury(II) cyanide in toluene–dioxan afforded a 13% yield of *O*-3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl-(1 → 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (41). Ammonolysis of the latter followed by treatment with sodium in liquid ammonia afforded an 86% yield of *O*-2-amino-2-deoxy-β-D-glucopyranosyl-(1 → 4)-garamine (42). The ^1H n.m.r. spectrum of (42) clearly supported the β-glycosidic structure and exhibited a doublet at δ 4.67 ($J_{1',2'}$ 8 Hz) due to the anomeric H-1′. The molecular rotation (Table 2) was also in agreement with the value expected for a β-glycoside. The mass spectrum of (42) exhibited the expected protonated formyl ions A_1—A_4 and the c.d. spectrum showed $[\theta]_{290}$ −9 070 in TACu solution, indicating that the glycoside was at the 4-position of garamine.



(44) R^1 = Ac, R^2 = DNP, R^3 = Z (46) R^1 = Ac, R^2 = DNP, R^3 = Z
(45) R^1 = R^2 = R^3 = H (47) R^1 = R^2 = R^3 = H

In order to investigate the effect of inversion of stereochemistry at the 4′-position on the biological activity, the *galacto*-analogue of gentamicin X_2 was synthesized, by two routes. Tri-*O*-acetyl-D-galactal (14) was converted into the nitroso-chloro-adduct (16) by standard procedures.[5] When the latter was condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) a 19% yield of *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*lyxo*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29) was obtained. The ^1H n.m.r. spectrum of the oxime (29) exhibited a broad singlet at δ 6.32 due to the anomeric H-1′, indicating a *Z*-configuration.[11] Acetylation of (29) gave the acetate (30), which on reduction with 13 equiv. of borane in tetrahydrofuran followed by ammonolysis and alkaline hydrolysis (aqueous 5% sodium hydroxide), gave *O*-2-amino-2-deoxy-α-D-galactopyranosyl-(1 → 4)-garamine (6) in 14% yield based on the oxime (29). The ^1H n.m.r. spectrum of (6) showed a doublet at δ 5.23 ($J_{1',2'}$ 4 Hz) due to the anomeric H-1′ proton, as expected for an α-glycoside. The stereochemistry at C-2′ was confirmed by INDOR experiments in which the H-1′ and H-2′ frequencies were irradiated. The mass spectrum showed the expected protonated formyl ions A_1—A_4 (Table 1) and the c.d. spectrum showed $[\theta]_{290}$ −9 630 in TACu solution, thus confirming the linkage of the glycoside to the 4-position of garamine. A 2% yield of *O*-2-deoxy-2-ethylamino-α-D-galactopyranosyl-(1 → 4)-garamine (7) was obtained as a by-product. The ^1H n.m.r. and mass spectra supported the presence of a 2′-*N*-ethyl group.

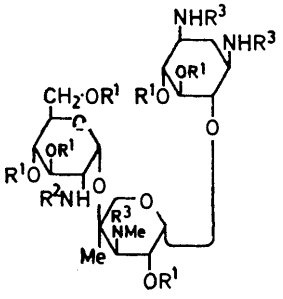
The *galacto*-analogue (6) was also prepared by a Koenigs–Knorr reaction. The preparation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-galactopyranosyl bromide (37) from D-glucosamine was accomplished by literature procedures.[37,38,42] Conden-

[33] S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, *J. Antibiotics*, 1968, **21**, 162.
[34] S. Umezawa, K. Tatsuta, and S. Koto, *J. Antibiotics*, 1968, **21**, 367.
[35] S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, *J. Antibiotics*, 1968, **21**, 424.
[36] M. Nakajima, A. Hasegawa, N. Kurihara, H. Shibata, T. Ueno, and D. Nishimura, *Tetrahedron Letters*, 1968, 623.
[37] P. F. Lloyd and M. Stacey, *Tetrahedron*, 1960, **9**, 116.
[38] D. Horton and M. L. Wolfrom, *J. Org. Chem.*, 1962, **27**, 1794.
[39] M. Bergmann and L. Zervas, *Ber.*, 1931, **64***B*, 975.
[40] D. Horton, *J. Org. Chem.*, 1964, **29**, 1776.
[41] M. L. Wolfrom and H. B. Bhat, *J. Org. Chem.*, 1967, **32**, 1821.
[42] E. F. Annison, A. T. James, and W. T. J. Morgan, *Biochem. J.*, 1951, **48**, 477.

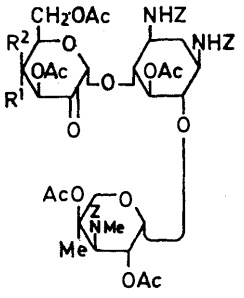
sation of the bromide (37) with 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) in the presence of Drierite and mercury(II) cyanide in toluene afforded *O*-3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-galactopyranosyl-(1 ⟶ 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (39) in 22% yield. The product (39) was subjected to ammonolysis, basic hydrolysis [Amberlite IRA 400 (OH^-) resin], and finally reduction with sodium in liquid ammonia to give *O*-2-amino-2-deoxy-α-D-galactopyranosyl-(1 ⟶ 4)-garamine (6) in 59% yield from (39). The product was identical with that isolated from the Lemieux–Nagabhushan reaction.

No aminoglycoside antibiotics isolated hitherto from micro-organisms have been found to contain a furanoside linked to the 4-position of the deoxystreptamine ring. It therefore seemed worthwhile to prepare the glucofuranosyl analogue of gentamicin X_2 by a Koenigs–Knorr reaction. 3,5,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α- and -β-D-glucofuranosyl chloride (43) were prepared by known procedures.[43,44] The chlorides (43) were condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) in the presence of calcium sulphate and mercury(II) cyanide in toluene to give both *O*-3,5,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucofuranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (44) in 8% yield and the β-anomer (46) in 10% yield. The α-anomer (44) was subjected to ammonolysis, basic hydrolysis [Amberlite IRA 400 (OH^-) resin], and reduction with sodium and liquid ammonia to give *O*-2-amino-2-deoxy-α-D-glucofuranosyl-(1 ⟶ 4)-garamine (45) in 76% yield based on (44). The ^1H n.m.r. spectrum of (45) contained a doublet at δ 5.52 ($J_{1',2'}$ 5 Hz) attributed to the anomeric H-1′, the coupling constant being consistent with that predicted for an α-glycoside. The molecular rotation (Table 2) was also in agreement with such an assignment. The mass spectral fragment ions (Table 1) and the c.d. spectrum ($[\theta]_{290}$ −8 090 in TACu solution) were consistent with the proposed structure. The β-anomer (46) was deprotected in a similar fashion to give a 69% yield of *O*-2-amino-2-deoxy-β-D-glucofuranosyl-(1 ⟶ 4)-garamine (47). The ^1H n.m.r. spectrum of (47) showed a singlet at δ 5.24 due to the anomeric H-1′, as expected for a β-glycoside. The c.d. spectrum and the molecular rotation value (Table 2) were in accord with the proposed β-glycoside structure.

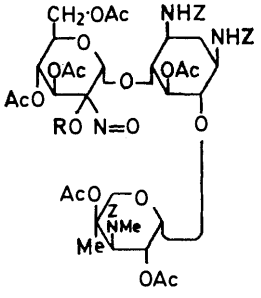
A novel aminoglycoside having D-glucosamine glycosidically attached to the 4′-position of garamine was prepared in the following way. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (35) [37,38] was condensed with 2′,4,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (22) [1] in the presence of Drierite and mercury(II) cyanide in toluene to give a 10% yield of *O*-3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl-(1 ⟶ 4′)-2′,4,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (48). Deprotection by ammonolysis, basic hydrolysis with Amberlite IRA 400 (OH^-) resin, and reduction with sodium in liquid ammonia gave *O*-2-amino-2-deoxy-α-D-glucopyranosyl-(1 ⟶ 4′)-garamine (49) in a 65% yield from (47). The ^1H n.m.r. spectrum of (49) showed a doublet at δ 5.28 ($J_{1'',2''}$ 4 Hz) due to the anomeric H-1″, in accord with a 4′-*O*-α-D-glycosidic linkage. As



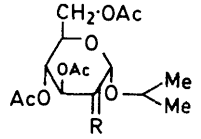
(48) R^1 = Ac, R^2 = DNP, R^3 = Z
(49) R^1 = R^2 = R^3 = H



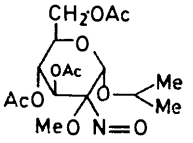
(50) R^1 = OAc, R^2 = H
(51) R^1 = H, R^2 = OAc



(52) R = Ac
(53) R = Me



(54) R = NOH
(55) R = O



(56)

anticipated, the mass spectrum of (49) (Table 1) revealed significant differences from the usual mass spectra of 4,6-linked aminoglycosides.[22] The usual sequences of protonated formyl ions A_1—A_4 and A_5—A_8 were absent; only those due to the deoxystreptamine series A_9—A_{12} were present. The monosaccharide glycosyl ions B_1 and C_1 were both present. A prominent ion at *m/e* 246 due to ion E_2 indicated that this fragmentation pathway had not been suppressed in the case of (49), in contrast with the behaviour of 4″-*O*-ethylgentamicin X_2 (3) and 4′-*O*-ethylgaramine (32). The c.d. spectrum of (49) showed $[\theta]_{290}$ — 13 300 in TACu solution.

Although several aminoglycosides containing a D-glucopyranosyl unit attached to the deoxystreptamine

[43] M. L. Wolfrom and M. W. Winkley, *J. Org. Chem.*, 1966, **31**, 1169.

[44] M. L. Wolfrom and M. W. Winkley, *J. Org. Chem.*, 1967, **32**, 1823.

ring have been synthesized,[16] no such products containing a 4-*O*-α-D-glucopyranosyl unit have been isolated from microbial sources. The synthesis of *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) was therefore undertaken in order to study the changes in biological activity of gentamicin X_2 (1) that result upon replacement of the 2′-amino-group by a hydroxy-group. As stated earlier, one of the inherent advantages of the Lemieux–Nagabhushan reaction is the ability to go from the intermediate oxime either to a 2-aminoglycoside or (by deoximation and subsequent reduction) to a 2-hydroxyglycoside. Several commonly used deoximating reagents have been used to prepare the 2-oxo-derivatives by the Lemieux group [11,16] and all were found to give satisfactory results on the substrates that were used.

Deoximation of *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27) with levulinic acid, followed by reduction of the intermediate 2′-ketone with sodium borohydride and deprotection by refluxing with aqueous 5% sodium hydroxide, gave the desired *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) in 32% yield. The ^{1}H n.m.r. spectrum of (8) revealed a doublet at δ 5.19 ($J_{1',2'}$ 3.5 Hz) due to the anomeric H-1′, indicating that reduction had occurred to give the α-D-*gluco*-configuration. The c.d. and mass spectra were in full accord with structure (8). When the above reaction was repeated and the deprotection was effected with sodium in liquid ammonia instead of aqueous base, *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) was formed in 19% yield, together with *O*-α-D-glucopyranosyl-(1 → 4)-4′-deoxygaramine (9) (see later). When titanium trichloride was used to effect the deoximation of (27) and the ketone was reduced with sodium borohydride and deprotected by treatment with sodium in liquid ammonia, the yield of *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) was only 7%. Similar yields of (8) were obtained when the deoximation was carried out with thallium(III) nitrate [45] (TTN).

In view of the disappointing yields of (8) obtained by using titanium trichloride or TTN it was decided to investigate alternative deoximation procedures. Recently Sugden [46] reported that oximes could easily be converted in high yields into the corresponding ketones by treatment with aluminium isopropoxide in propan-2-ol. As the reaction had never been applied to a sugar oxime we tested it on the oxime (27). When the latter was heated with aluminium isopropoxide in propan-2-ol for 21 h and then reacetylated with acetic anhydride–concentrated hydrochloric acid (10 : 1), a 41% yield of *O*-3,4,6-tri-*O*-acetyl-α-D-*arabino*-hexopyran-2-ulosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzoyloxycarbonylgaramine (50) was obtained. The product was isolated by preparative layer chromatography since it showed instability upon column chromatography. These results would suggest that this deoximation procedure works as well on the oxime (27) as the levulinic acid process. The ketone was reacetylated after the aluminium isopropoxide treatment as some loss of acetyl groups occurred owing to the basic pH of the reaction medium.

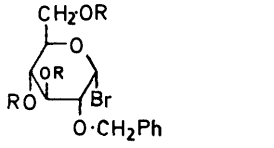
Deoximation by lead tetra-acetate [47,48] was also investigated. Treatment of the oxime (27) with lead tetra-acetate followed by acetylation with acetic anhydride–concentrated hydrochloric acid (10 : 1) afforded three products, which were separated by preparative layer chromatography. These were 2′,4,4′,5-tetra-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (23), *O*-2,3,4,6-tetra-*O*-acetyl-2-nitroso-α-D-*arabino*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (52), and *O*-3,4,6-tri-*O*-acetyl-α-D-*arabino*-2-hexopyranulosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (50). The samples of (23) and (50) were identical with authentic samples prepared by other routes. The pale blue tint of (52) suggested that the molecule was the intermediate nitroso-acetate [49,50] and the physical data confirmed this. The i.r. spectrum contained nitroso-absorption at 1 580 cm^{-1}. Similar results were obtained when the reaction mixture was hydrolysed with aqueous 70% perchloric acid at 25 °C. More vigorous hydrolytic conditions resulted in extensive decomposition of the product. The stability of the nitroso-acetate (52), particularly when treated at 25 °C with aqueous perchloric acid, was unexpected.

When the oxime (27) was deoximated with TTN and the reaction mixture was hydrolysed with aqueous 70% perchloric acid at 25 °C and peracetylated as above, three products were obtained: 2′,4,4′,5-tetra-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (23), *O*-3,4,6-tri-*O*-acetyl-1-methoxy-1-nitroso-α-D-*arabino*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (53) and *O*-3,4,6-tri-*O*-acetyl-α-D-*arabino*-2-hexopyranulosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (50). The ^{1}H n.m.r. spectrum of (53) revealed a broad singlet at δ 3.44 due to the 2′-OCH_3 group. The i.r. spectrum showed a band at 1 560 cm^{-1} due to the nitroso-group. The relative stability of the nitroso-methoxy-intermediate (53) was interesting in view of the fact that Taylor [45] had postulated the intermediacy of such a species in the reaction of TTN with simple oximes, but had not been able to isolate the intermediate owing to its instability. We therefore investigated the reaction of TTN with a simple monosaccharide oxime isopropyl 3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranoside (54).[8] Treatment with TTN in methanol and work-up with dilute hydrochloric acid gave isopropyl 3,4,6-tri-*O*-acetyl-1-methoxy-1-nitroso-α-D-*arabino*-hexopyranoside (56), which was rather unstable,

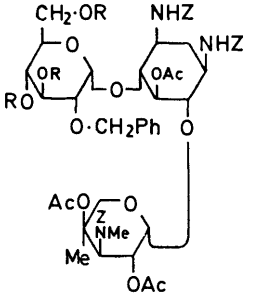
[45] A. McKillop, J. D. Hunt, R. D. Naylor, and E. C. Taylor, *J. Amer. Chem. Soc.*, 1971, **93**, 4918.
[46] J. K. Sugden, *Chem. and Ind.*, 1972, 680.
[47] D. C. Iffland and G. X. Criner, *Chem. and Ind.*, 1956, 176.
[48] H. Kropf and R. Lambeck, *Annalen*, 1966, **700**, 1.
[49] M. M. Frojmovic and G. Just, *Canad. J. Chem.*, 1968, **46**, 3719.
[50] Y. Yukawa, M. Sakai, and S. Suzuki, *Bull. Chem. Soc. Japan*, 1966. **39**, 2266.

but could be isolated by preparative layer chromatography on silica gel in *ca.* 70% purity. The ^{1}H n.m.r. spectrum of this sample in deuteriochloroform showed a singlet at δ 3.10 for the 2-*O*-methyl group. When aqueous 70% perchloric acid was used instead of dilute hydrochloric acid in the work-up, the ketone (55) [11] was the principal product. It was evident that the nitroso-methoxy-intermediate was much less stable in the monosaccharide case than in that of the trisaccharide. The formation of these relatively stable intermediates during the deoximation of the oxime (27) could well account for the low yields of the desired glycosyl compound (8).

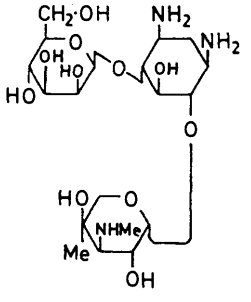
The synthesis of *O*-α-D-glucopyranosyl-(1 ⟶ 4)-garamine (8) by the Koenigs–Knorr route was also undertaken. Thus 2-*O*-benzyl-3,4,6-tris-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide (57) [51,52] was condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) in the presence of calcium



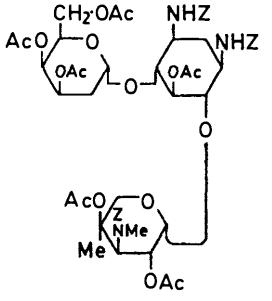
(57) R = CO·C$_6$H$_4$·NO$_2$—*p*



(58) R = CO·C$_6$H$_4$·NO$_2$—*p*



(59)



(60)

sulphate and mercury(II) cyanide in toluene to give a 26% yield of *O*-2-*O*-benzyl-3,4,6-tris-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (58). Deprotection of (58) by ammonolysis followed by reduction with sodium in liquid ammonia, afforded *O*-α-D-glucopyranosyl-(1 ⟶ 4)-garamine (8) in 34% yield, together with *O*-α-D-glucopyranosyl-(1 ⟶ 4)-4″-deoxygaramine (9) in 22% yield. The 4″-deoxy-derivative (9), although homogeneous on t.l.c., was found to be a 95 : 5 mixture of compounds with the 4″-methyl group equatorial and axial, respectively. The lack of the 4″-hydroxy-group was evident from the ^{1}H n.m.r. spectrum, as the equatorial 4″-methyl group gave rise to a doublet at δ 0.97 (*J* 6 Hz). The mass spectral fragmentation pattern is shown in Table 1. The fragment ions E_1 and E_2, formed by cleavage between C-3″ and C-4″ in garosamine-containing aminoglycosides, were absent in the mass spectrum of the 4″-deoxy-compound (9). When 4′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (21) was reduced with sodium in liquid ammonia the principal product was garamine (33), obtained together with a 22% yield of 4′-deoxygaramine (34).[53] The ^{1}H n.m.r. spectrum * of the latter indicated it to be a 7 : 3 mixture of isomers having the 4′-methyl group equatorial and axial, respectively, which could not be separated by chromatography. It showed doublets at δ 0.76 and 0.93 (*J* 6.5 Hz) due to the axial and equatorial 4′-methyl groups. The 3′-proton in the equatorial compound gave rise to a doublet of doublets at δ 2.64 ($J_{2',3'} = J_{3',4'} = 10$ Hz). The reaction occurs with compounds having a tertiary acyl group [53] and may proceed by way of a tertiary radical intermediate.

The synthesis of *O*-α-D-talopyranosyl-(1 ⟶ 4)-garamine (10) from *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*lyxo*-hexopyranosyl-(1′ ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29) was achieved by use of either levulinic acid or aluminium isopropoxide to effect the deoximation step. The intermediate ketone obtained by using levulinic acid was reduced with sodium borohydride and then deprotected by ammonolysis followed by hydrolysis with aqueous 5% sodium hydroxide to give (10) in 21% yield. The *galacto*-isomer could be detected in the reaction product, indicating a high degree of stereoselective reduction to give the 2′-axial alcohol. The c.d. spectrum (TACu solution) agreed with that expected for a 4-linked glycoside, and the mass spectral fragmentation pattern (Table 1) lent further support to structure (10). The molecular rotation (Table 2) confirmed the presence of an α-glycosidic linkage at the 4-position. The ^{1}H n.m.r. spectrum revealed a doublet at δ 5.24 ($J_{1',2'}$ 1.5 Hz) due to the anomeric H-1′, consistent with the proposed *talo*-structure (10). When the ketone intermediate obtained from the aluminium isopropoxide reaction was reduced with borane and then deprotected by hydrolysis with aqueous 5% sodium hydroxide, a 31% yield of (10) was obtained, the reduction at position 2′ again occurring to give the *talo*-isomer. The product of deoximation of the oxime (29) was isolated by using preparative silica gel plates and shown to be a ketone triacetate; it was very labile towards column chromatography on silica gel. Peracetylation of the ketone triacetate with acetic anhydride–concentrated hydrochloric acid (10 : 1) afforded the peracetyl ketone (51), identical

* Values reported earlier [53] were quoted with reference to HOD at δ 4.61; those given here were corrected for pH (HOD at δ 4.68).

[51] A. Klemer, *Chem. Ber.*, 1963, **96**, 634.
[52] T. Ishikawa, and H. G. Fletcher, *J. Org. Chem.*, 1969, **34**, 563.
[53] A. K. Mallams, H. F. Vernay, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J. Antibiotics*, 1973, **26**, 782.

with a sample prepared (16% yield) by deoximation of the oxime (29) with sodium nitrite in aqueous acetic acid.

The preparation of *O*-α-D-mannopyranosyl-(1 → 4)-garamine (11) and the β-anomer (59) was undertaken by a Koenigs–Knorr reaction. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl chloride [54] was condensed with 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) in the presence of mercury(II) bromide and mercury(II) cyanide, the reaction being carried out in nitromethane, to give a 16% yield of *O*-2,3,4,6-tetra-*O*-acetyl-α- and -β-D-mannopyranosyl-(1 → 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine. The latter was hydrogenated over 10% palladium–carbon in the presence of hydrazine hydrate at 39 °C to give *O*-α-D-mannopyranosyl-(1 → 4)-garamine (11) in 12% yield and the β-anomer (59) in 8% yield. Both anomers showed reasonable molecular rotations (Table 2) corresponding to the proposed structures (11) and (59). The ^{1}H n.m.r. spectrum of the α-anomer (11) showed a doublet at δ 5.25 with $J_{1',2'}$ 2 Hz due to the anomeric H-1′; the β-anomer (59) gave rise to a doublet at δ 5.17 having $J_{1',2'}$ 1 Hz. The mass spectra confirmed that the mannosyl residue was glycosidically attached to the deoxystreptamine ring and in the case of the α-glycoside (11) this was unambiguously established.*

Direct acid-catalysed addition of 3,4,6-tri-*O*-acetyl-D-galactal (14) to 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) afforded, under appropriate conditions, a 59% yield of *O*-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (60) as the major product. The latter was subjected to ammonolysis followed by alkaline hydrolysis with barium hydroxide to give *O*-2-deoxy-α-D-*lyxo*-hexopyranosyl-(1 → 4)-garamine (12) in 10% yield from (60). The ^{1}H n.m.r. spectrum of (12) showed an unresolved multiplet at δ 5.50 due to the anomeric H-1′.

The novel aminoglycoside antibacterials were subjected to a variety of antibacterial and anti-protozoal tests; the results will be discussed elsewhere.

EXPERIMENTAL

All physical data were recorded as described in Part I.[1]

Gentamicin X$_2$ (1).—(i) 1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (17) (30 g) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (15) [5] (23 g) were dissolved in dry, redistilled dimethylformamide (120 ml). The solution was kept at 25 °C for 60 h, then concentrated *in vacuo* and partitioned between chloroform and water. The chloroform extract was dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on a silica gel column (160 × 5 cm) (3% methanol–chloroform as eluant) to give O-3,4,6-*tri*-O-*acetyl*-2-*hydroxyimino*-α-D-arabino-*hexopyranosyl*-(1 → 4)-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (24) (19.7 g, 46%), m.p. 171—173° (Found: C, 57.0; H, 5.9; N, 5.65. $C_{49}H_{60}N_4O_{20}$ requires C, 57.4; H, 5.9; N, 5.5%), $[\alpha]_D$ +86.7° (in MeOH), $\nu_{max.}$(CHCl$_3$) 3 380, 1 750, 1 720, 1 230, 1 030, and 694 cm^{-1}, δ(CDCl$_3$) † 1.01br (3 H, s, 4″-CH$_3$), 1.95br (9 H, s, OAc), 3.02br (3 H, s, 3″-NCH$_3$), 5.02br (6 H, s, C*H*$_2$C$_6$H$_5$), 6.35br (1 H, s, H-1′), and 7.24br (15 H, s, CH$_2$C$_6$*H*$_5$). A less polar *tetrasaccharide*, tentatively assigned structure (31), was also obtained (8.9 g, 16%) (Found: C, 54.5; H, 5.7; N, 5.35. $C_{61}H_{75}N_5O_{28}$ requires C, 55.2; H, 5.7; N, 5.3%), $[\alpha]_D$ +87.1° (in MeOH), $\nu_{max.}$(CHCl$_3$) 3 400, 1 750, 1 730, 1 720, 1 230, and 1 030 cm^{-1}, δ(CDCl$_3$) † 1.13br (3 H, s, 4″-CH$_3$), 2.00br (18 H, s, OAc), 3.04br (3 H, s, 3″-NCH$_3$), 5.09br (6 H, s, C*H*$_2$C$_6$H$_5$), and 6.30br and 6.43br (2 H, s, H-1′ and -1‴), and 7.32br (15 H, s, CH$_2$C$_6$*H*$_5$).

The trisaccharide oxime (24) (12.22 g) and acetic anhydride (14 ml) were dissolved in dry pyridine (35 ml) and the solution was left at 25 °C for 16 h. The mixture was poured into ice-water and the oxime acetate (26) was filtered off, dried, dissolved in dry tetrahydrofuran (50 ml), and cooled to 0 °C. A 1M-solution of borane in tetrahydrofuran (55 ml) was added and the mixture was left at 25 °C for 16 h. The excess of reagent was destroyed by dropwise addition of water, and when no further effervescence occurred a saturated solution of ammonia in methanol (250 ml) was added. The solution was then left at 25 °C for 18 h, and evaporated *in vacuo*, and the residue was taken up in glacial acetic acid (200 ml) and hydrogenated over 30% palladium–carbon at 25 °C and 55 lb in^{-2} for 18 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was taken up in 90% hydrazine hydrate and the mixture was heated at 120 °C for 24 h, then evaporated to dryness. Repetitive chromatography on silica gel columns [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] afforded 2′-N-*ethylgentamicin X*$_2$ (2) (160 mg, 3%) (Found: $M^{+\cdot}$, 510.2920. $C_{21}H_{42}N_4O_{10}$ requires *M*, 510.2901), $[\theta]_{290}$ −11 400 (TACu), δ(D$_2$O) 1.10 (3 H, t, *J* 7 Hz, NHCH$_2$C*H*$_3$), 1.24 (3 H, s, 4″-CH$_3$), 2.54 (3 H, s, 3″-NCH$_3$), 2.79 (2 H, q, *J* 7 Hz, NHC*H*$_2$CH$_3$), 5.11 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.23 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′); O-2-*deoxy*-2-*ethylamino*-α-D-*mannopyranosyl*-(1 → 4)-*garamine* (5) (100 mg, 2%) (Found: $M^{+\cdot}$, 510.2926. $C_{21}H_{42}N_4O_{10}$ requires *M*, 510.2901), $[\theta]_{290}$ −6 470 (TACu), δ(D$_2$O) 1.12 (3 H, t, *J* 7 Hz, NHCH$_2$C*H*$_3$), 1.24 (3 H, s, 4″-CH$_3$), 2.54 (3 H, s, 3″-NCH$_3$), 2.72 (2 H, q, *J* 7 Hz, NHC*H*$_2$CH$_3$), 5.10 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.24 (1 H, d, $J_{1',2'}$ 2 Hz, H-1′); *gentamicin X*$_2$ (1) [4] (600 mg, 10%) (Found: C, 45.0; H, 7.8; N, 11.0. $C_{19}H_{38}N_4O_{10}$,H_2O requires C, 45.6; H, 8.1; N, 11.2%), $[\alpha]_D$ +154.8° (in H$_2$O), $[\theta]_{290}$ −12 500 (TACu), $\nu_{max.}$(KCl) 3 350 and 1 040 cm^{-1}, δ(D$_2$O) 1.19 (3 H, s, 4″-CH$_3$), 2.49 (3 H, s, 3″-NCH$_3$), 5.08 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.22 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′); and O-2-*amino*-2-*deoxy*-α-D-*mannopyranosyl*-(1 → 4)-*garamine* (4) (530 mg, 9%) (Found: C, 44.5; H, 7.6; N, 10.9. $C_{19}H_{38}N_4O_{10}$,H_2O requires C, 45.6; H, 8.1; N, 11.2%), $[\alpha]_D$ +132.3° (in MeOH), $[\theta]_{290}$ −3 860 (TACu), δ(D$_2$O) 1.25 (3 H, s, 4″-CH$_3$), 2.62 (3 H, s, 3″-NCH$_3$), 5.09 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.17 (1 H, d, $J_{1',2'}$ 2 Hz, H-1′).

(ii) 2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) (0.91 g) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-

* Additional proof for the 4- and 6-glycosidic linkages of the sugars was obtained by per-*N*-acetylation followed by per-*ON*-methylation of the trisaccharide. The fully derivatized trisaccharide was subjected to acidic hydrolysis (6N-hydrochloric acid) and the product after *N*-acetylation was purified by preparative t.l.c. to give 1,3-di-*N*-acetyl-2-deoxy-1,3-di-*N*-methyl-5-*O*-methyl-D-streptamine, identical (t.l.c. and mass spectrum) with an authentic sample kindly supplied by Dr. P. J. L. Daniels.

† Mixture of rotamers at ambient temperatures.

[54] E. Pascu, *Chem. Ber.*, 1928, **61**, 1508.

glucopyranosyl chloride (15) [5] (0.61 g) were dissolved in dry, redistilled dimethylformamide (4 ml) and the solution was maintained at 25 °C for 115 h. The reaction was worked up as before and the residue was chromatographed on a silica gel column (60 × 2.5 cm) (3% methanol–chloroform as eluant) to give O-3,4,6-*tri*-O-*acetyl*-2-*hydroxyimino*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (25) (0.53 g, 42%), m.p. 116—127° (Found: C, 56.7; H, 6.0; N, 5.3. $C_{51}H_{62}N_4O_{21}$ requires C, 57.4; H, 5.9; N, 5.25%), $[\alpha]_D$ +92.6° (in EtOH), $\nu_{max.}$(CHCl$_3$) 3 330, 1 740, 1 700, 1 210, 1 045, 1 030, and 696 cm^{-1}, δ(CDCl$_3$) † 1.03br (3 H, s, 4″-CH$_3$), 1.91—2.17br (12 H, s, OAc), 2.90br (3 H, s, 3″-NCH$_3$), 6.39br (1 H, s, H-1′), and 7.21—7.29br (15 H, s, CH$_2$C$_6$*H*$_5$). The oxime (25) was converted into the acetate (26), reduced with borane, deblocked, and chromatographed as in (i) above to give gentamicin X$_2$ (1) [4] (60 mg, 25%). The by-products were not isolated.

(iii) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) (9.17 g), 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (15) [5] (7.33 g), and *NN*,2,6-tetramethylaniline (1.5 g) were dissolved in dry, redistilled dimethylformamide (300 ml) and the solution was left at 25 °C for 95 h. The mixture was poured into ice–water (5 l) and the precipitate was filtered off, dried, and chromatographed on a silica gel column (160 × 5 cm) (1% methanol–chloroform as eluant) to give O-3,4,6-*tri*-O-*acetyl*-2-*hydroxyimino*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzoyloxycarbonylgaramine* (27) (5 g, 40%), m.p. 134—139° (Found: C, 57.6; H, 5.9; N, 4.8. $C_{55}H_{66}N_4O_{23}$ requires C, 57.4; H, 5.8; N, 4.9%), $[\alpha]_D$ +90.0° (in MeOH), $\nu_{max.}$(CHCl$_3$) 3 350, 1 740, 1 710, 1 220, 1 030, and 695 cm^{-1}, δ(CDCl$_3$) † 1.29br and 1.39br (3 H, 2 s, 4″-CH$_3$), 1.99br (18 H, s, OAc), 2.85br (3 H, s, 3″-NCH$_3$), 5.08br (6 H, s, C*H*$_2$C$_6$H$_5$), 6.19br (1 H, s, H-1′), and 3.28br (15 H, s, CH$_2$C$_6$*H*$_5$).

The oxime (27) (1 g) was acetylated as before to give the *acetate* (28) (828 mg, 80%). Chromatography on a silica gel column (110 × 2.5 cm) (1% methanol–chloroform as eluant) gave a sample of m.p. 115—123° (Found: C, 57.4; H, 6.0; N, 4.7. $C_{57}H_{68}N_4O_{24}$ requires C, 57.4; H, 5.7; N, 4.7%), $[\alpha]_D$ +88.0° (in MeOH), $\nu_{max.}$(CHCl$_3$) 3 338, 1 740, 1 710, 1 220, 1 030, and 695 cm^{-1}, δ(CDCl$_3$) † 1.27br and 1.37br (3 H, 2 s, 4″-CH$_3$), 1.92br, 2.01br, 2.09br, and 2.17br (21 H, 4 s, OAc), 2.85br (3 H, s, 3″-NCH$_3$), 5.07br (6 H, s, C*H*$_2$C$_6$H$_5$), and 3.31br (15 H, s, CH$_2$C$_6$*H*$_5$).

The acetate (28) (386 mg) in dry tetrahydrofuran (10 ml) was reduced with a 1M-solution of borane in tetrahydrofuran (6.67 ml) as before. The dry solid was added to a mixture of sodium (0.66 g) in liquid ammonia (40 ml) at −70° and the mixture was stirred for 2 h. The reaction was quenched by dropwise addition of water and the ammonia was allowed to evaporate at 25 °C overnight. The residue was dissolved in ice-cold water (70 ml) and neutralized with Amberlite IRC 50 (H$^+$) resin. The slurry was transferred to a column, washed thoroughly with water, and then eluted with 1.5N-ammonium hydroxide. The eluate was evaporated to dryness and the residue was chromatographed on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give 4″-O-*ethylgentamicin X*$_2$ (3) (13 mg, 8%), δ(D$_2$O) 1.15 (3 H, t, *J* 7 Hz, OCH$_2$·C*H*$_3$), 1.22 (3 H, s, 4″-CH$_3$), 2.51 (3 H, s, 3″-NCH$_3$), 2.60 (1 H, d, $J_{2'',3''}$ 10 Hz, H-3″), 3.48 (2 H, q, *J* 7 Hz, OC*H*$_2$CH$_3$), 5.06 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.27 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′) and gentamicin X$_2$ (1) [4] (34 mg, 21%).

(iv) The oxime acetate (28) (450 mg) was dissolved in dry tetrahydrofuran (12 ml) and reduced with a 1M-solution of borane in tetrahydrofuran (7.75 ml) as before. The resulting solid was taken up in glacial acetic acid (70 ml) and hydrogenated over 30% palladium–carbon at 25 °C and 55 lb in^{-2} for 17 h. The catalyst was filtered off and washed with water. The combined filtrates were evaporated and the residue was azeotroped with benzene. The resulting solid was taken up in aqueous 10% barium hydroxide (w/v) (50 ml) and the solution was heated under reflux at 130 °C for 16 h, cooled, and saturated with carbon dioxide. The barium carbonate was filtered off and washed with water. The filtrate was passed down Amberlite IRA 401S (OH$^-$) resin and the eluate was evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give 4″-*O*-ethylgentamicin X$_2$ (3) (27 mg, 14%) and gentamicin X$_2$ (1) [4] (24 mg, 13%), identical with the products isolated in (iii).

(v) 2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) (1.5 g) in dry toluene (100 ml) was treated with Drierite (freshly ground and baked out on a hotplate) (8.6 g), mercury(II) cyanide (0.83 g), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (35) [37,38] (1.25 g) and the mixture was heated at 110 °C for 24 h. The solution was cooled and filtered through a Celite pad, and the filter cake was washed with ethyl acetate. The combined filtrates were evaporated and the residue in ethyl acetate was washed with 20% potassium bromide and water, dried (MgSO$_4$), filtered, and evaporated. The residue was chromatographed on a silica gel column (100 g) [1% methanol in benzene–ether (1 : 1) as eluant] to give O-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-α-D-*glucopyranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (38) (838 mg, 35%), which crystallized from 95% ethanol–water; m.p. 128—130° (Found: C, 56.5; H, 5.2; N, 6.9. $C_{57}H_{66}N_6O_{24}$ requires C, 56.2; H, 5.5; N, 6.9%), $[\alpha]_D^{19}$ +98.0° (in CHCl$_3$), $\nu_{max.}$ (Nujol) 3 448, 3 333, 1 754—1 709, 1 618, 1 592, and 1 527 cm^{-1}, δ(CDCl$_3$–CD$_3$OD, 3 : 1) † 1.18br (3 H, s, 4″-CH$_3$), 1.85br, 2.02br, and 2.08br (12 H, 3 s, OAc), 2.95br (3 H, s, 3″-NCH$_3$), 7.33br and 7.38br (16 H, s, CH$_2$C$_6$*H*$_5$ and DNP), and 8.25br and 9.03br (2 H, 2 s, DNP). Further elution, with 2% methanol in benzene–ether (1 : 1), gave a mixed fraction which was further purified by preparative layer chromatography on silica gel plates [chloroform–ethyl acetate (1 : 2) as eluant] to give O-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-β-D-*glucopyranosyl*-(1 ⟶ 4)-2 -O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (40) (150 mg, 6%), which crystallized from 95% ethanol–water; m.p. 133—135° (Found: C, 57.0; H, 5.65; N, 6.8. $C_{57}H_{66}N_6O_{24}$ requires C, 56.2; H, 5.5; N, 6.9%), $[\alpha]_D^{19}$ +39.5° (in CHCl$_3$), $\nu_{max.}$ (Nujol) 3 448, 3 333, 1 754—1 709, 1 618, 1 592, and 1 527 cm^{-1}, δ(CDCl$_3$) † 1.10br (3 H, s, 4″-CH$_3$), 1.88br, 2.03br, and 2.10br (12 H, 3 s, OAc), 2.92br (3 H, s, 3″-NCH$_3$), 7.25br, 7.28br, 7.30br, and 7.33br (16 H, 4 s, CH$_2$C$_6$*H*$_5$ and DNP), and 8.20br and 9.00br (2 H, s, DNP).

Compound (38) (160 mg) was dissolved in methanol (32 ml) and the solution was saturated with ammonia at 0 °C. After 16 h at 25 °C the solution was evaporated to

† Same footnote as on page 1106.

afford 1,3,3″-tris-*N*-benzyloxycarbonyl-2′-*N*-(2,4-dinitrophenyl)gentamicin X_2, which was dissolved in a mixture of acetone (20 ml) and water (10 ml), treated with Amberlite IRA 400 (OH^-) resin (5 ml), and stirred at 25 °C for 16 h. The mixture was filtered, the resin was washed with acetone–water (2 : 1; 100 ml), and the combined filtrates were evaporated to afford 1,3,3″-tris-*N*-benzyloxycarbonylgentamicin X_2. This was dissolved in liquid ammonia (20 ml) at −70 °C and sodium (230 mg) was added, the mixture being stirred for 2 h at −70 °C. Water was added dropwise, and after the blue colour had disappeared the ammonia was allowed to evaporate. The residue in water (5 ml) was cooled to 0 °C and transferred to an Amberlite IRC 50 (H^+) resin column, which was left for 1.5 h. The neutral impurities were eluted with water (140 ml) and the gentamicin X_2 (1) [4] (36 mg, 60%) was then eluted with 1.5N-ammonium hydroxide. The n.m.r. and mass spectra and t.l.c. mobility [silica gel plate; lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] were identical with those of an authentic sample.

O-2-*Amino*-2-*deoxy*-β-D-*glucopyranosyl*-(1 ⟶ 4)-*garamine* (42).—2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) (940 mg) in a mixture of dry toluene (47 ml) and dioxan (15 ml) was treated with Drierite (5.4 g), mercury(II) cyanide (515 mg), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (36) [39-41] (684 mg), and the mixture was heated at 110 °C for 96 h. The reaction was worked up as in (v) above and the residue was chromatographed on a silica gel column (50 g). Benzene–ether (9 : 1 ⟶ 1 : 3) was used to elute the less polar impurities, then 1 ⟶ 3% methanol in benzene–ether (1 : 1) eluted the desired product. The latter was rechromatographed on silica gel plates [chloroform–ethyl acetate (1 : 4)] to give O-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-(*trifluoroacetamido*)-β-D-*glucopyranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (41) (180 mg, 13%), which crystallized from methylene chloride–ether; m.p. 203—205° (Found: C, 55.8; H, 5.6; N, 4.9. $C_{53}H_{60}F_3N_4O_{20}$ requires C, 56.3; H, 5.35; N, 5.0%), $[\alpha]_D^{19}$ +41.5° (in $CHCl_3$), $\nu_{max.}$ (Nujol) 3 448, 3 333, 1 748, 1 739—1 681, and 1 527 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.08br (3 H, s, 4″-CH_3), 1.95br, 2.03br, 2.07br, and 2.08br (12 H, 4 s, OAc), 2.93br (3 H, s, 3″-NCH_3), and 7.37br (15 H, s, $CH_2C_6H_5$).

The product (41) (210 mg) was dissolved in methanol (40 ml) and the solution was saturated with ammonia at 0 °C. After 48 h at 25 °C the solution was evaporated to give O-2-*amino*-2-*deoxy*-β-D-*glucopyranosyl*-(1 ⟶ 4)-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine*, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.00br (3 H, s, 4″-CH_3), 3.08br (3H, s, 3″-NCH_3), and 7.32br and 7.35br (15 H, 2 s, $CH_2C_6H_5$).

The latter in liquid ammonia (40 ml) at −70 °C was treated with sodium (400 mg) and the mixture was stirred at −70° for 2 h. The reaction was worked up as in (v) above to give O-2-*amino*-2-*deoxy*-β-D-*glucopyranosyl*-(1 ⟶ 4)-*garamine* (42) (77 mg, 86%), which was homogeneous on t.l.c. [silica gel; lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1)] [Found: $(M + 1)^+$, 483.2625. $C_{19}H_{39}N_4O_{10}$ requires $(M + 1)$, 483.2666], $[\alpha]_D$ +88.6° (in H_2O), $[\theta]_{290}$ −9 070 (TACu), $\nu_{max.}$(KCl) 3 300 and 1 050 cm^{-1}, δ(D_2O) 1.30 (3 H, s, 4″-CH_3), 2.60 (3 H, s, 3″-NCH_3), 4.67 (1 H, d, $J_{1',2'}$ 8 Hz, H-1′), and 5.19 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″).

4′-O-*Ethylgaramine* (32).—4′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (21) (500 mg) was dissolved in dry tetrahydrofuran (25 ml) and cooled to 0 °C. A 1M-solution of borane in tetrahydrofuran (13 ml) (30 equiv.) was added dropwise and the mixture was kept at 7 °C for 18 h. Water was added dropwise and the solution was evaporated to dryness. The residue was dissolved in 5% sodium hydroxide in aqueous dioxan (1 : 1) (40 ml) and heated under reflux for 16 h. The solution was neutralized with Amberlite IRC 50 (H^+) resin; work-up and chromatography as in (i) gave 4′-O-*ethylgaramine* (32) (45 mg, 20%) [Found: $(M + 1)^+$, 350.2312. $C_{15}H_{32}N_3O_6$ requires $(M + 1)$, 350.2291], $[\alpha]_D$ +123.1° (in H_2O), δ(D_2O) 1.14 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.20 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 2.58 (1 H, d, $J_{2',3'}$ 10 Hz, H-3′), 3.46 (2 H, q, *J* 7 Hz, OCH_2CH_3), 3.86 (1 H, d, *J* 13.5 Hz, H-5′e), 3.90 (1 H, dd, $J_{1',2'}$ 4, $J_{2',3'}$ 10 Hz, H-2′), and 5.14 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′), and garamine (33) (70 mg, 33%), identical with an authentic sample.

When the above reaction was repeated with 10 equiv. of borane only traces of 4′-*O*-ethylgaramine (32) were formed, the principal product being garamine (33).

O-2-*Amino*-2-*deoxy*-α-D-*galactopyranosyl*-(1 ⟶ 4)-*garamine* (6).—(i) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) (35 g) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-galactopyranosyl chloride (16) [5] (27.8 g) were dissolved in dry, redistilled dimethylformamide (1 l) and the solution was kept at 25 °C for 92 h. The product was precipitated as before and the solid was chromatographed on a silica gel column (152 × 7.5 cm) (40% acetone–hexane as eluant) to give O-3,4,6-*tri*-O-*acetyl*-2-*hydroxyimino*-α-D-lyxo-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (29) (9.2 g, 19%) (Found: C, 57.1; H, 5.8; N, 4.8. $C_{55}H_{66}N_4O_{23}$ requires C, 57.4; H, 5.8; N, 4.9%), $[\alpha]_D$ +101.5° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 300, 1 750, 1 720, 1 220, and 1 030 cm^{-1}, δ($CDCl_3$) † 1.24br and 1.37br (3 H, s, 4″-CH_3), 1.82br, 1.99br, 2.05br, and 2.16br (18 H, 4 s, OAc), 2.87br (3 H, s, 3″-NCH_3), 5.08br (6H, s, $CH_2C_6H_5$), 6.32br (1 H, s, H-1′), and 7.27br and 7.30br (15 H, 2 s, $CH_2C_6H_5$).

The oxime (29) (3 g) was acetylated as before, and the acetate (30) was dissolved in dry tetrahydrofuran (100 ml) and cooled to 0 °C. A 1M-solution of borane in tetrahydrofuran (30 ml) was added dropwise and the solution was kept at 7 °C for 30 h. The reaction was worked up as before. The residue was taken up in a mixture of methanol (20 ml) and concentrated ammonium hydroxide (40 ml) and heated in a sealed vessel at 100 °C for 18 h. The solution was evaporated to dryness and the product was taken up in aqueous 5% sodium hydroxide (30 ml) and heated under reflux for 16 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water and then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness and the solid was chromatographed on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give O-2-*deoxy*-2-*ethylamino*-α-D-*galactopyranosyl*-(1 ⟶ 4)-*garamine* (7) (30 mg, 2%) [Found: $(M + 1)^+$, 510.2901. $C_{21}H_{43}N_4O_{10}$ requires $(M + 1)$, 510.2901], $[\alpha]_D$ +144.2° (in H_2O), δ(D_2O) 1.06 (3 H, t, *J* 7.5 Hz, NCH_2CH_3), 1.20 (3 H, s, 4″-CH_3), 2.52 (3 H, s, 3″-NCH_3), 2.74 (2 H, q, *J* 7.5 Hz, NCH_2CH_3), 5.08 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.20 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′), and O-2-*amino*-2-*deoxy*-α-D-*galactopyranosyl*-(1 ⟶ 4)-*garamine* (6) (181 mg,

† Same footnote as on page 1106.

14%) (Found: C, 44.5; H, 7.9; N, 10.5. $C_{19}H_{38}N_4O_{10},H_2O,CO_2$ requires C, 44.1; H, 7.4; N, 10.3%), $[\alpha]_D^{28}$ +155.8° (in H_2O), $[\theta]_{285}$ −9 630 (TACu), $[\theta]_{290}$ −7 580 (Cupra A), $\nu_{max.}$(KCl) 3 330 and 1 060 cm^{-1}, δ(D_2O) 1.18 (3 H, s, 4″-CH_3), 2.49 (3 H, s, 3″-NCH_3), 3.01 (1 H, dd, $J_{1',2'}$ 4, $J_{2',3'}$ 10.5 Hz, H-2′), 5.04 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.23 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′).

(ii) 2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) (575 mg) in dry toluene (38 ml) was treated with Drierite (freshly ground and baked out on a hotplate) (3.8 g), mercury(II) cyanide (316 mg), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-galactopyranosyl bromide (37) [37,38,42] (800 mg), and the mixture was heated at reflux temperature under nitrogen for 64 h. The reaction was worked up as before. The residue was chromatographed on preparative silica gel plates (50% ethyl acetate–chloroform as eluant) to give O-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-α-D-*galactopyranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (39) (205 mg, 22%) as a yellow amorphous solid, m.p. 136—139° (Found: C, 56.0; H, 5.6; N, 7.1. $C_{57}H_{66}N_6O_{24}$ requires C, 56.2; H, 5.5; N, 6.9%), $[\alpha]_D$ +75.3° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 754, 1 724, 1 695, 1 613, 1 587, 1 538, 1 333, 746, and 694 cm^{-1}, δ($CDCl_3$–CD_3OD, 2 : 1) † 1.18br (3 H, s, 4″-CH_3), 1.85br, 1.88br, and 2.23br (12 H, 3 s, OAc), 2.92br (3 H, s, 3″-NCH_3), 7.30br and 7.35br (16 H, s, $CH_2C_6H_5$ and DNP), and 8.28br and 9.03br (2 H, s, DNP).

The product (39) (180 mg) was dissolved in methanol (36 ml) and the solution was saturated with ammonia at 0 °C. After 18 h at 25 °C the solution was evaporated to afford *O*-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-galactopyranosyl-(1 ⟶ 4)-1,3,3′-tris-*N*-benzyloxycarbonylgaramine, which was dissolved in a mixture of acetone (20 ml) and water (10 ml), treated with Amberlite IRA 400 (OH^-) resin, and stirred at 25 °C for 24 h. The mixture was filtered and the resin was washed with acetone–water (2 : 1) (30 ml), and the combined filtrates were evaporated to afford *O*-2-amino-2-deoxy-α-D-galactopyranosyl-(1 ⟶ 4)-1,3,3′-tris-*N*-benzyloxycarbonylgaramine. The latter was dissolved in liquid ammonia (30 ml) at −70 °C and sodium (400 mg) was added; the mixture was then stirred for 2 h at −80 °C. Water was added dropwise and the ammonia was allowed to evaporate. The residue was taken up in water (5 ml), cooled to 0 °C, and transferred to an Amberlite IRC 50 (H^+) resin column. The neutral impurities were eluted with water (250 ml) and the *O*-2-amino-2-deoxy-α-D-galactopyranosyl-(1 ⟶ 4)-garamine (6) (42 mg, 59%) was then eluted with 1.5N-ammonium hydroxide. The n.m.r., mass, and i.r. spectra and t.l.c. mobility were identical with those of the sample described in (*i*).

O-2-*Amino*-2-*deoxy*-α- *and* -β-D-*glucofuranosyl*-(1 ⟶ 4)-*garamine* [(45) *and* (47)].—2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) (1 g) in dry toluene (75 ml) was treated with calcium sulphate (freshly ground and baked out on a hotplate) (5 g), mercury(II) cyanide (505 mg), and 3,5,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α- and -β-D-glucofuranosyl chloride (43) [43,44] (695 mg), and the mixture was heated at 70 °C under nitrogen for 3 days. The reaction was worked up as before. The residue was chromatographed on silica gel plates [benzene–ether–methanol (49 : 49 : 2) as eluant] to give O-3,5,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-α-D-*glucofuranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (44) (121 mg, 8%) as a yellow amorphous solid, m.p. 123—125° (Found: C, 56.5; H, 5.5; N, 6.6. $C_{61}H_{70}N_6O_{26}$ requires C, 56.2; H, 5.4; N, 6.45%), $[\alpha]_D$ +77.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, 1 613, 1 515, 1 325, and 1 235 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.22br and 1.37br (3 H, 2 s, 4″-CH_3), 1.95br, 1.97br, 2.00br, and 2.02br (18 H, 4 s, OAc), 2.88br (3 H, s, 3″-NCH_3), 7.05br (1 H, s, DNP), 7.37br (15 H, s, $CH_2C_6H_5$), 8.37br (1 H, s, DNP), and 9.17br (1 H, s, DNP), and O-3,5,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-β-D-*glucofuranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (46) (157 mg, 10%) as a yellow amorphous solid, m.p. 111—115° (Found: C, 58.8; H, 5.25; N, 7.4. $C_{61}H_{70}N_6O_{26}$ requires C, 56.2; H, 5.4; N, 6.45%), $[\alpha]_D$ +40.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, 1 613, 1 515, 1 333, and 1 235 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.32br and 1.42br (3 H, 2 s, 4″-CH_3), 1.97br, 2.00br, 2.10br, and 2.20br (18 H, 4 s, OAc), 2.90br (3 H, s, 3″-NCH_3), 7.10br (1 H, s, DNP), 7.20br, 7.23br, and 7.37br (15 H, 3 s, $CH_2C_6H_5$), 8.25br (1 H, s, DNP), and 8.97br (1 H, s, DNP).

The α-anomer (44) (105 mg) was deprotected as described for compound (39). The residue was taken up in water and transferred to a BioRex 70 (H^+) resin column. The neutral impurities were eluted with water (30 ml). Elution with 1.5N-ammonium hydroxide afforded O-2-*amino*-2-*deoxy*-α-D-*glucofuranosyl*-(1 ⟶ 4)-*garamine* (45) (30 mg, 76%) (Found: C, 47.3; H, 8.1; N, 11.4. $C_{19}H_{38}N_4O_{10}$ requires C, 47.3; H, 7.9; N, 11.6%), $[\alpha]_D$ +133.8° (in H_2O), $[\theta]_{290}$ −8 090 (TACu), $\nu_{max.}$(KCl) 3 330, 1 050, and 1 020 cm^{-1}, δ(D_2O) 1.30 (3 H, s, 4″-CH_3), 2.64 (3 H, s, 3″-NCH_3), 5.14 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.52 (1 H, d, $J_{1',2'}$ 5 Hz, H-1′).

The β-anomer (46) (170 mg) was deprotected as above to give O-2-*amino*-2-*deoxy*-β-D-*glucofuranosyl*-(1 ⟶ 4)-*garamine* (47) (44 mg, 69%), $[\alpha]_D$ +62.3° (in H_2O), $[\theta]_{290}$ −8 280 (TACu), $\nu_{max.}$(KCl) 3 333, 1 099, and 1 053 cm^{-1}, δ (D_2O) 1.31 (3 H, s, 4″-CH_3), 2.69 (3 H, s, 3″-NCH_3), 5.21 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.24 (1 H, s, H-1′).

O-2-*Amino*-2-*deoxy*-α-D-*glucopyranosyl*-(1 ⟶ 4′)-*garamine* (49).—2′,4,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (22) (10 g) in dry toluene (590 ml) was treated with Drierite (freshly ground and baked out on a hotplate) (50 g), mercury(II) cyanide (4.85 g), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (35) [37,38] (7.5 g), and the mixture was heated at reflux temperature under nitrogen for 120 h. The reaction was worked up as before. The residue was chromatographed on a silica gel column (450 g) (10 ⟶ 50% ether–benzene as eluant) to give O-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitrophenylamino*)-α-D-*glucopyranosyl*-(1 ⟶ 4′)-2′,4,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (48) (1.56 g, 10%), which crystallized from ethanol–water; m.p. 120—123° (Found: C, 55.6; H, 5.5; N, 6.4. $C_{61}H_{70}N_6O_{26},H_2O$ requires C, 55.45; H, 5.5; N, 6.4%), $[\alpha]_D$ +115.8° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 754, 1 724, 1 709, 1 695, 1 613, 1 587, 1 538, 1 348, 746, and 694 cm^{-1}, δ($CDCl_3$–CD_3OD, 2 : 1) † 1.17br (3 H, s, 4″-CH_3), 1.77br, 1.90br, 1.93br, and 2.07br (18 H, s, OAc), 3.08br (3 H, s, 3″-NCH_3), 7.37br (16 H, s, $CH_2C_6H_5$ and DNP), and 8.33br and 9.12br (2 H, s, DNP).

The product (48) (1.5 g) was deprotected as described for compound (39). The residue was taken up in water (15 ml), cooled to 0 °C, and transferred to a BioRex 70 (H^+) resin

† Same footnote as on page 1106.

column. The neutral impurities were eluted with water (160 ml). Elution with 1.5N-ammonium hydroxide afforded O-2-*amino*-2-*deoxy*-α-D-*glucopyranosyl*-(1 → 4')-*garamine* (49) (365 mg, 65%) (Found: C, 47.4; H, 7.9; N, 11.4. $C_{19}H_{38}N_4O_{10}$ requires C, 47.3; H, 7.9; N, 11.6%), $[\alpha]_D$ +143.9° (in H_2O), $[\theta]_{290}$ −13 300 (TACu), $[\theta]_{290}$ −8 190 (Cupra A), $\nu_{max.}$(KCl) 3 300 and 1 020 cm^{-1}, δ(D_2O) 1.38 (3 H, s, 4'-CH_3), 2.60 (3 H, s, 3'-NCH_3), 5.18 (1 H, d, $J_{1',2'}$ 4 Hz, H-1'), and 5.28 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1'').

O-α-D-*Glucopyranosyl*-(1 → 4)-*garamine* (8).—(i) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2',4',5-tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (27) (1.85 g) in glacial acetic acid (28 ml) was treated with levulinic acid (2.8 ml) and 1N-hydrochloric acid (4 ml), and the mixture was stirred at 25 °C for 18 h. The mixture was poured into methylene chloride and the latter extract was washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and evaporated to dryness. The solid was dissolved in dioxan (20 ml) and water (2 ml) and cooled to 5 °C. Sodium borohydride (250 mg) in dioxan (4 ml) and water (8 ml) was added dropwise with stirring. The mixture was stirred at 5 °C for 0.5 h and at 25 °C for 1 h. Acetic acid was added and the solution was evaporated to dryness. The solid was dissolved in a 5% solution of sodium hydroxide in aqueous dioxan (1 : 1; 60 ml) and the mixture was heated under reflux for 17 h, cooled, and neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water and eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated and the residue was chromatographed on a silica gel column (160 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give O-α-D-*glucopyranosyl*-(1 → 4)-*garamine* (8) (250 mg, 32%) (Found: C, 46.9; H, 7.8; N, 8.9. $C_{19}H_{37}N_3O_{11}$ requires C, 47.20; H, 7.7; N, 8.7%), $[\alpha]_D$ +146.3° (in H_2O), $[\theta]_{290}$ −7 380 (TACu), $\nu_{max.}$(KCl) 3 300 and 1 050 cm^{-1}, δ(D_2O) 1.23 (3 H, s, 4''-CH_3), 2.54 (3 H, s, 3''-NCH_3), 5.08 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1''), and 5.19 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1').

(ii) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2',4',5-tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (27) (500 mg) in glacial acetic acid (7 ml) was treated with levulinic acid (1 g) and 1N-hydrochloric acid (1 ml) and the mixture was stirred at 25 °C for 18 h. The reaction was worked up as in (i). The solid was dissolved in dioxan (10 ml) and water (1 ml) and cooled to 5 °C. Sodium borohydride (100 mg) in dioxan (2 ml) and water (4 ml) was added dropwise with stirring. The mixture was stirred at 5 °C for 1.5 h and at 25 °C for 1 h. Acetic acid was added and the solution was evaporated to dryness and azeotroped with toluene. The solid was dissolved in liquid ammonia (80 ml) at −70 °C and sodium (1 g) was added. After stirring at −70 °C for 3 h the reaction was quenched by dropwise addition of water and the ammonia was allowed to evaporate overnight. The basic slurry was taken up in water and neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water and eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2.5 cm) with chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant to give *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) (39 mg, 19%), identical with that described in (i). *O*-α-D-Glucopyranosyl-(1 → 4)-4'-deoxygaramine (9) was also formed [mixed t.l.c. on silica gel; lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant], but it was not isolated.

(iii) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2',4',5-tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (27) (500 mg) was dissolved in dioxan (10 ml). Ammonium acetate (5 g) dissolved in 50% aqueous acetic acid (2 ml) was added and the mixture was stirred under nitrogen. Titanium trichloride (20% solution) (12 ml) was added gradually and the mixture was stirred at 25 °C for 1 h. Water was added and the mixture was extracted with chloroform; the extract was washed with water, dried ($MgSO_4$), and evaporated to dryness. The solid was dissolved in dioxan (10 ml) and water (1 ml) and cooled to 5 °C. Sodium borohydride (100 mg) in dioxan (2 ml) and water (4 ml) was added slowly with stirring. The mixture was stirred at 5 °C for 0.5 h and at 25 °C for 1 h. Acetic acid was added and the solution was evaporated to dryness. The residue was treated with sodium in liquid ammonia and chromatographed as in (ii) to give *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) (14 mg, 7%), identical with that described in (i).

(iv) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 → 4)-2',4',5-tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (27) (500 mg) was dissolved in bis-(2-methoxyethyl) ether (10 ml), and water (0.3 ml) and 70% perchloric acid (0.4 ml) were added. Thallium(III) nitrate (1.5 g) in bis-(2-methoxyethyl) ether (10 ml) was added and the mixture was stirred at 25 °C for 19 h, then diluted with chloroform. The organic solution was washed with water, dried ($MgSO_4$), and evaporated to dryness. The solid was reduced with sodium borohydride, deblocked with sodium in liquid ammonia, and chromatographed as in (iii) to give *O*-α-D-glucopyranosyl-(1 → 4)-garamine (8) (15 mg, 7%), identical with that described in (i).

(v) 2',4',5-Tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (19) (800 mg), 2-*O*-benzyl-3,4,6-tris-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide (57) [51,52] (871 mg), dry mercury(II) cyanide (406 mg), and anhydrous calcium sulphate (4.2 g) in dry toluene (75 ml) were stirred and heated under dry nitrogen at 70 °C for 48 h. The reaction was worked up as before and the residue was subjected to preparative layer chromatography on silica gel plates [benzene–ether–methanol (49.5 : 49.5 : 1) as eluant] to give O-2-O-*benzyl*-3,4,6-*tris*-O-p-*nitrobenzoyl*-α-D-*glucopyranosyl*-(1 → 4)-2',4',5-*tri*-O-*acetyl*-1,3,3'-*tris*-N-*benzyloxycarbonylgaramine* (58) (375 mg, 26%), m.p. 135—138° (Found: C, 60.1; H, 5.0; N, 5.7. $C_{77}H_{76}N_6O_{29}$ requires C, 59.7; H, 4.9; N, 5.4%), $[\alpha]_D$ +91.3° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, 1 613, 1 527, 1 342, and 1 235 cm^{-1}, δ($CDCl_3$) † 1.28br, 1.42br (3 H, 2 s, 4''-CH_3), 1.95br, 1.98br, and 2.05br (9 H, 3 s, OAc), 2.90br (3 H, s, 3''-NCH_3), 7.20br (5 H, s, $OCH_2C_6H_5$), 7.35br (15 H, s, $CO_2CH_2C_6H_5$), and 8.17br (12 H, s, $COC_6H_4NO_2$).

The product (58) (320 mg) was dissolved in a mixture of methanol (180 ml) and concentrated ammonium hydroxide (20 ml) and stirred at 25 °C for 18 h. The solution was evaporated to dryness and the dried solid was taken up in liquid ammonia (100 ml) and cooled to −70 °C. Sodium (400 mg) was added and the mixture was stirred for 2 h. Water (10 ml) was added dropwise and the solution was allowed to warm gradually to 25 °C. The solution was neutralized with BioRex 70 (H^+) resin and the resin was washed with water and then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness

† Same footnote as on page 1106.

and the residue was chromatographed on a silica gel column (5 g) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give O-α-D-*glucopyranosyl*-(1 ⟶ 4)-4′-*deoxygaramine* (9) (21 mg, 22%) (Found: $M^{+\cdot}$, 467.2471. $C_{19}H_{37}N_3O_{10}$ requires M, 467.2479), $[\theta]_{282}$ −13 900 (TACu), $\nu_{max.}$ (Nujol) 3 333, 1 099, and 1 053 cm^{-1}, δ(D_2O) 0.97 (3 H, d, J 6 Hz, 4″e-CH_3), 2.47 (3 H, s, 3″-NCH_3), 5.18, (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.38 (1 H, d, $J_{1,'2'}$ 4 Hz, H-1′) and O-α-D-glucopyranosyl-(1 ⟶ 4)-garamine (8) (34 mg, 34%), identical with that described in (i).

O-3,4,6-*Tri*-O-*acetyl*-α-D-arabino-*hexopyran*-2-*ulosyl*-(1⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine*(50).—(i) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27) (500 mg) and aluminium isopropoxide (1 g) in dry propan-2-ol (35 ml) were heated under reflux for 21 h. The solution was evaporated to small volume, diluted with water, and then acidified with 2N-hydrochloric acid. The mixture was extracted with chloroform and the extracts were washed with water, dried ($MgSO_4$), and evaporated. The solid was taken up in acetic anhydride–concentrated hydrochloric acid (10 : 1; 5 ml) and the mixture was left at 25 °C for 16 h. The solution was evaporated to dryness and azeotroped with toluene. Preparative layer chromatography on silica gel (5% methanol–chloroform as eluant) afforded the *ketose* (50) (204 mg, 41%) (Found: C, 58.6; H, 6.30; N, 3.9. $C_{55}H_{65}N_3O_{23}$ requires C, 58.15; H, 5.8; N, 3.70%), $[\alpha]_D$ +76.5° ($CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 380, 1 740, 1 720, 1 700, 1 210, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.28br and 1.40br (3 H, 2 s, 4″-CH_3), 1.95br, 2.00br, 2.01br, 2.02br, 2.12br, and 2.21br (18 H, 6 s, OAc), 2.88br (3 H, s, 3″-NCH_3), 5.11br (6 H, s, C$H_2C_6H_5$), 6.48br (1 H, s, H-1′), and 7.32br and 7.37br (15 H, 2 s, $CH_2C_6H_5$).

(ii) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27) (500 mg) was dissolved in glacial acetic acid (6 ml). Lead tetra-acetate (100 mg) was added and the mixture was stirred at 25 °C for 19 h. The blue solution was diluted with water and extracted with chloroform and the extract was dried and evaporated to dryness. The residue was dissolved in acetic anhydride–concentrated hydrochloric acid (10 : 1; 5.5 ml) and the mixture was left at 25 °C for 19 h, then evaporated to dryness, and azeotroped with toluene. The residue was chromatographed on preparative silica gel plates (50% acetone–hexane as eluant) to afford 2′,4,4′,5-tetra-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (23) [1] (63 mg, 13%), O-2,3,4,6-*tetra*-O-*acetyl*-2-*nitroso*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (52) (76 mg, 14%) as a blue-tinted amorphous solid (Found: C, 56.5; H, 6.0; N, 4.2. $C_{57}H_{66}N_4O_{25}$ requires C, 56.6; H, 5.7; N, 4.6%), $[\alpha]_D$ +81.4° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 1 740, 1 710, 1 580, 1 220, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.29br and 1.40br (3 H, 2 s, 4″-CH_3), 1.96—2.25br (21 H, s, OAc), 2.89br (3 H, s, 3″-NCH_3), 5.14br (6 H, s, C$H_2C_6H_5$), and 7.34br and 7.37br (15 H, s, $CH_2C_6H_5$), and compound (50) (88 mg, 18%). Compounds (23) and (50) were identical with authentic samples.

Similar results were obtained when the reaction mixture was treated with aqueous 70% perchloric acid prior to acetylation.

(iii) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27) (500 mg) was dissolved in methanol (10 ml). Thallium(III) nitrate (200 mg) in methanol (10 ml) was added and the mixture was stirred at 25 °C for 3.5 h. Perchloric acid (70%; 3 ml) was added and the solution was left at 25 °C for 0.75 h, then diluted with water, and extracted with chloroform. The extracts were dried ($MgSO_4$) and evaporated to dryness to give a pale blue solid (480 mg). This was dissolved in acetic anhydride–concentrated hydrochloric acid (10 : 1; 3 ml) and kept at 25 °C for 16 h. The solution was evaporated to dryness and azeotroped with toluene to give a solid. Preparative layer chromatography on silica gel (50% acetone–hexane as eluant) gave compound (23) (40 mg, 10%), O-3,4,6-*tri*-O-*acetyl*-1-*methoxy*-1-*nitroso*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (53) (140 mg, 28%), as a blue tinted amorphous solid (Found: C, 56.7; H, 6.1; N, 4.75. $C_{56}H_{68}N_4O_{24}$ requires C, 56.95; H, 5.8; N, 4.7%), $[\alpha]_D$ +94.8° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 1 730, 1 700, 1 560, 1 220, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.29br and 1.40br (3 H, s, 4″-CH_3), 1.97—2.18br (18 H, s, OAc), 2.88br (3 H, s, 3″-NCH_3), 3.44br (3 H, s, 2′-OCH_3), 5.00—5.12br (6 H, s, C$H_2C_6H_5$), and 7.29—7.36br (15 H, s, $CH_2C_6H_5$), and compound (50) (145 mg, 29%). Compounds (23) and (50) were identical with authentic samples.

4′-*Deoxygaramine* (34).—4′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (21) (500 mg) was dissolved in liquid ammonia (100 ml) at −70 °C. Sodium (0.95 g) was added and the mixture was stirred at −70 °C for 2 h. Water was added dropwise and the ammonia was allowed to evaporate at 25 °C overnight. The solution was neutralized with Amberlite IRC 50 (H^+) resin and the resin was washed with water and eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness and chromatographed on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give 4′-*deoxygaramine* (34) (44 mg, 22%) (Found: $M^{+\cdot}$, 305.1962. $C_{13}H_{27}N_3O_5$ requires M, 305.1951), δ(D_2O) 0.76 (0.3 × 3 H, d, J 6.5 Hz, 4′a-CH_3), 0.93 (0.7 × 3 H, d, J 6.5 Hz, 4′e-CH_3), 2.39 (0.7 × 3 H, s, 3′e-NCH_3, 2.70 (0.3 × 3 H, s, 3′a-NCH_3), 5.09 (0.7 × 1 H, d, $J_{1',2'}$ 3.5 Hz, 1′e-H), and 5.15 (0.3 × 1 H, d, $J_{1',2'}$ 3.5 Hz, 1′a-H), and garamine (33) (110 mg, 52%), identical with an authentic sample.

O-α-D-*Talopyranosyl*-(1 ⟶ 4)-*garamine* (10).—(i) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*lyxo*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29) (2 g) was dissolved in glacial acetic acid (60 ml), and levulinic acid (6 ml) and 1N-hydrochloric acid (8 ml) were added. The mixture was left at 25 °C for 20 h, then poured into chloroform (600 ml). The chloroform extract was washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and evaporated to dryness. The resulting solid was dissolved in a mixture of dioxan (60 ml) and water (6 ml) and cooled to 5 °C. Sodium borohydride (1.2 g) dissolved in dioxan (12 ml) and water (24 ml) was added dropwise with stirring. The mixture was stirred at 5 °C for 0.5 h and at 25 °C for 1 h. Acetic acid was added dropwise and the solution was evaporated to dryness. The solid was dissolved in methanol (20 ml) and concentrated ammonium hydroxide (40 ml), and heated in a bomb at 100 °C for 18 h. The solution was evaporated and the solid was then dissolved in aqueous

† Same footnote as on page 1106.

5% sodium hydroxide (100 ml) and heated under reflux for 17 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin and the resin was washed with water and then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness and chromatographed on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give O-α-*talopyranosyl*-(1 ⟶ 4)-*garamine* (10) (176 mg, 21%) (Found: C, 47.1; H, 7.6; N, 8.6. $C_{19}H_{37}N_3O_{11}$ requires C, 47.20; H, 7.7; N, 8.7%), $[\alpha]_D$ +173.8° (in H_2O), $[\theta]_{285}$ −8 190 (TACu), $[\theta]_{285}$ −7 940 (Cupra A), $\nu_{max.}$(KCl) 3 330 and 1 050 cm^{-1}, δ(D_2O) 1.18 (3 H, s, 4″-CH_3), 2.49 (3 H, s, 3″-NCH_3), 5.03 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.24 (1 H, d, $J_{1',2'}$ 1.5 Hz, H-1′).

(ii) *O*-3,4,6-Tri-*O*-acetyl-2-hydroxyimino-α-D-*lyxo*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29) (4 g) and aluminium isopropoxide (8 g) were dissolved in dry propan-2-ol (280 ml) and the mixture was heated under reflux for 23 h. The solution was evaporated to small volume, diluted with water, and then acidified with 2N-hydrochloric acid. The mixture was extracted with chloroform and the extracts were washed with water, dried ($MgSO_4$), and evaporated. The ketone (3.15 g) was dissolved in dry tetrahydrofuran (160 ml) and cooled to 0 °C. A 1M-solution of borane in tetrahydrofuran (40 ml) was added dropwise and the mixture was left at 7 °C for 27 h. Water was added dropwise and the solution was evaporated to dryness. The solid was dissolved in aqueous 5% sodium hydroxide (50 ml) and heated under reflux for 17 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water and eluted with 1.5N-ammonium hydroxide. Ths basic eluate was evaporated and the product was chromatographed on a silica gel column (160 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give *O*-α-D-talopyranosyl-(1 ⟶ 4)-garamine (10) (520 mg, 31%), identical with that described in (i).

Studies on the Deoximation of O-3,4,6-*Tri*-O-*acetyl*-2-*hydroxyimino*-α-D-lyxo-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (29).—(i) The oxime (29) (750 mg) was deoximated as in (ii) above to give, after preparative layer chromatography on silica gel [hexane–benzene–acetone (1 : 1 : 2)], the *ketone triacetate* (190 mg, 29%) (Found: C, 58.0; H, 5.8; N, 4.2. $C_{49}H_{59}N_3O_{20}$ requires C, 58.3; H, 5.9; N, 4.2%), $[\alpha]_D$ +93.2° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$ 3 400, 1 750, 1 740, 1 720, 1 230, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.24br and 1.37br (3 H, 2 s, 4″-CH_3), 1.91—2.15br (9 H, s, OAc), 2.84br (3 H, s, 3″-NCH_3), 5.07br (6 H, s, $CH_2C_6H_5$), and 7.30br (15 H, s, $CH_2C_6H_5$). Attempts at purification of the ketone on silica gel columns resulted in extensive decomposition.

The ketone triacetate (5 mg) was dissolved in concentrated hydrochloric acid–acetic anhydride (1 : 10 v/v; 1 ml) and left at 25 °C for 18 h. The mixture was evaporated to dryness and azeotroped with toluene to give a product identical with the peracetyl ketone (51) (see later) (t.l.c. on silica gel; 3% methanol–chloroform).

(ii) The oxime (29) (300 mg) was dissolved in glacial acetic acid (12 ml). Sodium nitrite (1.6 g) in water (4 ml) was added dropwise and the mixture was left at 25 °C for 20 h. The solution was poured into chloroform and the resulting solution was washed with aqueous 5% sodium hydroxide and water, dried ($MgSO_4$), and evaporated to dryness. Preparative layer chromatography on silica gel (45% acetone–hexane as eluant) gave O-3,4,6-*tri*-O-*acetyl*-α-D-lyxo-2-*hexopyranulosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (51) (47 mg, 16%) (Found: C, 57.8; H, 6.0; N, 3.7. $C_{55}H_{65}N_3O_{23}$ requires C, 58.15; H, 5.8; N, 3.70%), $[\alpha]_D$ +69.6° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 330, 1 750, 1 730, 1 720, 1 230, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.28br and 1.40br (3 H, 2 s, 4″-CH_3), 1,94—2.15br (18 H, s, OAc), 2.86br (3 H, s, 3″-NCH_3), 5.06br (6 H, s, $CH_2C_6H_5$), and 7.33br (15 H, s, $CH_2C_6H_5$).

O-α- *and* -β-D-*Mannopyranosyl*-(1 ⟶ 4)-*garamine* [(11) *and* (59)].—2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (18) (4.2 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride [54] (8.9 g) were dissolved in dry, redistilled nitromethane (500 ml). Dry mercury(II) cyanide (1.68 g) and dry mercury(II) bromide (1.68 g) were added and the mixture was heated at 100 °C for 7 days. The solution was evaporated to dryness and the residue taken up in methanol; hydrogen sulphide was bubbled through the solution until no further precipitation of mercury(II) salts occurred. The mixture was filtered and the filtrate passed through Amberlite IRA 45 (OH^-) resin. The eluate was evaporated and the residue was chromatographed on a silica gel column (160 × 5 cm) (3% methanol–chloroform as eluant) to give O-2,3,4,6-*tetra*-O-*acetyl*-α- and -β-D-*mannopyranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (0.96 g, 16%), m.p. 90—95° (Found: C, 58.0; H, 6.0; N, 3.7. $C_{53}H_{65}N_3O_{22}$ requires C, 58.1; H, 6.0; N, 3.8%), $[\alpha]_D$ +83.1° (in EtOH), $\nu_{max.}$ ($CHCl_3$) 3 400, 1 740, 1 700, 1 230, 1 040, and 695 cm^{-1}, δ($CDCl_3$) † 1.08br and 1.24br (3 H, 2 s, 4″-CH_3), 1.92br, 1.98br, 2.07br, and 2.08br (15 H, 4 s, OAc), 2.91br and 2.98br (3 H, 2 s, 3″-NCH_3), 5.08br (6 H, s, $CH_2C_6H_5$), and 7.29br (15 H, s, $CH_2C_6H_5$).

This product (900 mg) and hydrazine hydrate (1 ml) were dissolved in methanol (100 ml) and hydrogenated over 10% palladium–carbon (1 g) at 39 °C and 50 lb in^{-2} for 16 h. The catalyst was filtered off and washed, and the filtrates were evaporated to dryness. Chromatography on a silica gel column (110 × 2.5 cm) [chloroform–methanol–concentrated ammonium hydroxide (3 : 4 : 3) as eluant] gave two products. The less polar was rechromatographed on a silica gel column (110 × 1 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) to which was added methanol (0.5 parts) as eluant] to give O-α-D-*mannopyranosylgaramine* (11) (49 mg, 12%) [Found: $(M + 1)^+$, 484.2527. $C_{19}H_{38}N_3O_{11}$ requires $(M + 1)$, 484.2506], $[\alpha]_D$ +129.1° (in H_2O), $\nu_{max.}$ (Nujol) 3 300 and 1 050 cm^{-1}, δ(D_2O) 1.18 (3 H, s, 4″-CH_3), 2.59 (3 H, s, 3″-NCH_3), 5.03 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.25 (1 H, d, $J_{1',2'}$ 2 Hz, H-1′). The polar product was rechromatographed in a similar manner to give O-β-D-*mannopyranosyl*-(1 ⟶ 4)-*garamine* (59) (32 mg, 8%), $[\alpha]_D$ +101.6° (in H_2O), δ(D_2O) 1.31 (3 H, s, 4″-CH_3), 2.60 (3 H, s, 3″-NCH_3), 5.04 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.17 (1 H, d, $J_{1',2'}$ 1 Hz, H-1′).

O-2-*Deoxy*-α-D-lyxo-*hexopyranosyl*-(1 ⟶ 4)-*garamine* (12).—2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19) (2 g), 3,4,6-tri-*O*-acetyl-D-galactal (14) (7.5 g), and anhydrous toluene-*p*-sulphonic acid (20 mg) dissolved in dry benzene (108 ml) were stirred at 25 °C under nitrogen for 48 h. The mixture was diluted with ether (200 ml) and washed with saturated sodium hydrogen carbonate (2 × 200 ml) and water, dried (Na_2SO_4), and evaporated to

† Same footnote as on page 1106.

dryness. The resulting oil was triturated with hot hexane (5 × 200 ml) and the resulting solid was chromatographed on a dry silica gel column (450 g) [ethyl acetate–chloroform (4 : 6) as eluant] to give O-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-α-D-lyxo-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (60) (1.57 g, 59%), which crystallized from ether; m.p. 118—122° (Found: C, 58.6; H, 6.1; N, 3.9. $C_{55}H_{67}N_3O_{22}$ requires C, 58.9; H, 6.0; N, 3.7%), $\nu_{max.}$ (Nujol) 3 333, 1 739, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$–CH_3OD, 3 : 1) † 1.28br and 1.42br (3 H, 2 s, 4″-CH_3), 1.87br, 1.97br, 2.03br, and 2.08br (18 H, 4 s, OAc), 2.90br (3 H, s, 3″-NCH_3), and 7.33br (15 H, s, $CH_2C_6H_5$).

The trisaccharide (60) (550 mg) dissolved in dry methanol (200 ml) was cooled to 0 °C and saturated with ammonia. The mixture was stirred at 25 °C for 18 h and then evaporated to dryness. The residue was dissolved in propan-1-ol (25 ml) and a solution of barium hydroxide octahydrate (32 g) in water (75 ml) was added. The solution was heated under reflux for 18 h, then cooled and diluted with methanol (100 ml). Carbon dioxide was passed through until the solution was neutral. The solid were filtered off and washed with methanol (100 ml) and the combined filtrates were then neutralized with BioRex 70 (H^+) resin (30 ml). The resin was washed with water and eluted with 1.5N-ammonium hydroxide (60 ml), and the basic eluate was evaporated to dryness. The residue was chromatographed on a silica gel (10 g) column [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1) as eluant] to give O-2-*deoxy*-α-D-lyxo-*hexopyranosyl*-(1 ⟶ 4)-*garamine* (12) (24 mg, 10%) [Found: $(M + 1)^+$, 468.2535. $C_{19}H_{38}N_3O_{10}$ requires $(M + 1)$, 468.2557], δ(D_2O) 1.27 (3 H, s, 4″-CH_3), 2.60 (3 H, s, 3″-NCH_3), 5.14 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.50 (1 H, m, H-1′).

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† Same footnote as on page 1106.