Semisynthetic Aminoglycoside Antibacterials. Part V.[1] Synthesis of Pentosyl and Related Derivatives of Garamine

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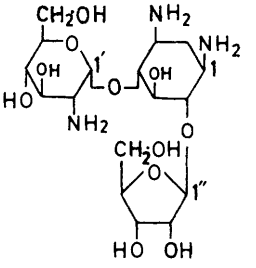
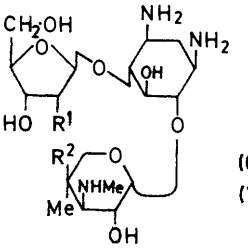
The application of the Koenigs–Knorr and Lemieux–Nagabhushan reactions to the synthesis of a variety of novel 4-*O*-pentofuranosyl and 4-*O*-pentopyranosyl derivatives of garamine is described. The solution conformations of these novel pentosyl aminocyclitols have been assigned on the basis of ^{13}C n.m.r. data.

THE occurrence in nature of a number of 5-*O*-β-D-ribofuranosyl aminocyclitol antibiotics such as the neomycins,[2] the paramomycins,[3] the lividomycins,[4] ribostamycin,[5] and the butirosins [6,7] encouraged us to prepare a series of novel 4-*O*-pentosyl derivatives of garamine in order to determine whether such compounds possess antibacterial activity. No 4-*O*-pentosyl-containing aminoglycosides have been isolated to date from microorganisms although *O*-β-D-ribofuranosyl-(1 → 6)-paromamine (1) has recently been synthesized from paromamine.[8]

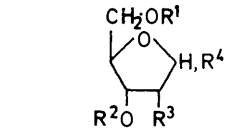
The synthesis of *O*-β-D-ribofuranosyl-(1 → 4)-garamine (2) by a Koenigs–Knorr reaction was undertaken. Thus when 2,3,5-tri-*O*-benzoyl-α- and -β-D-ribofuranosyl bromides (6) [9] were condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) [10] in the presence of mercury(II) cyanide in toluene at 55 °C, a 46% yield of *O*-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (11) was obtained. The latter on ammonolysis followed by treatment with sodium in liquid ammonia, afforded *O*-β-D-ribofuranosyl-(1 → 4)-garamine (2) in a 64% yield based on (11). The mass spectrum of (2) showed the expected glycosyl cleavages and protonated formyl fragment ions (Table 1), consistent with those observed for other aminocyclitol antibiotics.[11,12] In addition a prominent fragment ion was observed at *m/e* 422 due to an ion of the type D_{12}, formed by loss of 31 mass units from the molecular ion (*i.e.* loss of the hydroxymethylene group of the ribosyl unit). The c.d. maxima in TACu and Cupra A solutions were in accord with a 4-*O*-β-D-ribofuranosyl structure. Further support for the β-glycosidic structure was obtained from the ^1H n.m.r. spectrum, which showed a singlet at δ 5.36 due to the anomeric H-1′. *O*-β-D-Ribofuranosyl-(1 → 4)-



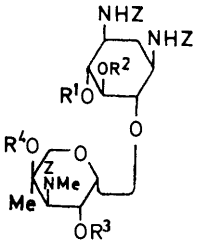
(1)



(2) $R^1=R^2=OH$
(3) $R^1=OH, R^2=H$
(4) $R^1=H, R^2=OH$
(5) $R^1=R^2=H$.



(6) $R^1=R^2=Bz, R^3=OBz, R^4=Br$
(7) $R^1=R^2=CO\cdot C_6H_4\ Me-p, R^3=H, R^4=Cl$



Z = $PhCH_2\cdot O\cdot CO$

(8) $R^1=H, R^2=R^3=R^4=Ac$
(9) $R^1=R^2=R^4=H, R^3=Ac$
(10) $R^1=R^3=R^4=Ac, R^2=H$

4′-deoxygaramine (3) was isolated in 20% yield as a by-product from the sodium–liquid ammonia reaction.[13,14]

[1] Part IV, M. Kugelman, A. K. Mallams, and H. F. Vernay, preceding paper.
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[3] T. H. Haskell, J. C. French, and Q. R. Bartz, *J. Amer. Chem. Soc.*, 1959, **81**, 3482.
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[7] M. Konishi, K. Numata, K. Shimoda, H. Tsukuira, and H. Kawaguchi, *J. Antibiotics*, 1974, **27**, 471.
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[10] M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, and M. Tanabe, *J.C.S. Perkin I*, 1976, 1088.
[11] P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Comm.*, 1971, 1629.
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[13] A. K. Mallams, H. F. Vernay, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J. Antibiotics*, 1973, **26**, 782.
[14] M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J.C.S. Perkin I*, 1976, 1097.

The principal isomer (3) showed a doublet at δ 0.98 (J 6.5 Hz) due to the equatorial 4''-methyl group. The mass spectral cracking pattern further confirmed the structure (3) (Table 1).

In order to prepare the 2'-deoxy-D-*erythro*-pentofuranosyl analogues, 2-deoxy-3,5-di-*O*-*p*-toluoyl-α- and -β-D-*erythro*-pentofuranosyl chlorides (7) were synthesized by standard procedures.[15] The latter on condensation with 2',4',5-tri-*O*-acetyl-1,3,3'-tri-*N*-benzyloxycarbonylgaramine (8) in the presence of mercury(II) cyanide in toluene at 25 °C afforded a 36% yield of *O*-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-2',4',5-tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (14), and a 23% yield of the β-anomer (12). The anomeric configurations were assigned on the basis of molecular rotations ($[M]_D$ + 1 312 and +867°, respectively). The α-anomer (14) was subjected to ammonolysis and reduction with sodium in liquid ammonia to give a 30% yield of *O*-2-deoxy-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (16). The ^{1}H n.m.r. spectrum revealed a doublet of doublets at δ 5.70 ($J_{1',2'}$ 1.5 and 5 Hz) consistent with an α-D-*erythro*-pentofuranosyl structure. The mass spectrum showed the expected fragment ions (Table 1), with the exception of the protonated formyl sequence of ions A_5—A_8 which arise following initial cleavage of the 1',2'-bond.[11,12] In the case of the 2'-deoxy-derivative (16) such a cleavage would be expected to be energetically less favoured than in the 2'-hydroxy-compounds, and the A_5—A_8 sequence of ions would be expected to be of low abundance, or even absent. Biemann [16] has offered an alternative explanation for the presence of protonated formyl ions in lower abundance in the spectra of 2'-deoxy-nucleosides than in those of their 2'-hydroxy-counterparts, *viz.* that it is the hydrogen atom from the 2'-hydroxy-group that is being transferred to give the observed ion. A 10% yield of *O*-2-deoxy-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-4'-deoxygaramine (17) was isolated as a by-product in the reaction.[13,14] Deprotection of the β-anomer (12) as described above gave a 52% yield of *O*-2-deoxy-β-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (4). The ^{1}H n.m.r. spectrum of (4) exhibited a doublet of doublets at δ 5.60 ($J_{1',2'}$ 4 Hz in each case) due to the anomeric H-1', in accordance with the presence of a β-anomeric linkage. A 13% yield of *O*-2-deoxy-β-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-4'-deoxygaramine (5) was obtained as a by-product of the sodium–liquid ammonia reaction.[13,14] The c.d. data for compounds (16), (17), (4), and (5) in TACu and Cupra A solutions supported the presence of the pentosyl unit at the 4-position of the deoxystreptamine ring.

Condensation of the chloro-sugar (7) with 2'-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (9) in the presence of mercury(II) cyanide in toluene at 25 °C afforded a 12% yield of *O*-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-2'-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (15) and an 11% yield of the β-anomer (13). The anomeric configurations were again

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}
(2)	454 (2)		323 (75)	305 (10)	295 (35)	277 (36)	350 (4)	332 (2)	322 (4)	304 (7)	191 (100)	173 (25)	163 (80)	145 (80)
(3)	438 (2)	437 (3)	323 (4)	305 (3)	295 (75)	277 (41)	334 (3)	316 (3)	306 (4)	288 (10)	191 (32)	173 (13)	163 (70)	145 (39)
(16)	438 (0.3)		307 (7)	289 (4)	279 (4)	261 (5)					191 (53)	173 (10)	163 (32)	145 (43)
(17)	422 (1)	421 (1)	307 (3)	289 (3)	279 (55)	261 (23)				288 (4)	191 (17)	173 (7)	165 (50)	145 (60)
(4)	438 (2)		307 (32)	289 (9)	279 (16)	261 (14)					191 (100)	173 (18)	163 (70)	145 (80)
(5)	422 (2)	421 (2)	307 (3)	289 (3)	279 (51)	261 (15)				288 (3)	191 (23)	173 (10)	163 (80)	145 (75)
(18)	466 (0.4)		335 (10)	317 (3)	307 (4)	289 (14)				316 (1)	219 (26)	201 (5)	191 (14)	173 (16)
(24)	454 (0.5)		323 (42)	305 (7)	295 (23)	277 (21)	350 (4)	332 (1)	322 (4)	304 (8)	191 (100)	173 (17)	163 (60)	145 (80)
(28)	454 (0.3)	453 (0.3)	323 (43)	305 (7)	295 (22)	277 (20)	350 (4)	332 (1)	322 (4)	304 (7)	191 (90)	173 (14)	163 (55)	145 (66)
(25)	406 (0.2)		275 (25)	257 (2)	247 (18)	229 (10)				304 (3)	191 (33)	173 (8)	163 (28)	145 (45)
(29)	406 (0.2)		275 (27)	257 (3)	247 (23)	229 (14)				304 (3)	191 (31)	173 (7)	163 (32)	145 (46)
(26)	453 (0.3)		322 (6)	304 (6)	294 (1)	276 (1)	350 (4)	332 (1)	322 (6)	304 (6)	191 (52)	173 (9)	163 (32)	145 (78)
(30)/(31)	453 (0.8)		322 (18)	304 (15)	294 (3)	276 (3)	350 (10)	332 (4)	322 (18)	304 (15)	191 (100)	173 (24)	163 (65)	145 (80)
g	481 (2)	480 (2)	350 (39)	332 (8)	322 (21)	304 (60)	350 (39)	332 (8)	322 (21)	304 (60)	191 (41)	173 (20)	163 (22)	145 (80)
(27)	453 (0.4)		322 (12)	304 (7)	294 (5)	276 (2)	350 (4)	332 (3)	322 (12)	304 (7)	191 (100)	173 (16)	163 (70)	145 (90)

Compd.	B_1	C_1	D_8	D_{10}	D_{12}	E_1	E_2	E_3	E_4	F_1	F_2
(2)	133 (37)	160 (95)			422 (2)	378 (6)	246 (15)	336 (10)	204 (40)	262 (20)	289 (14)
(3)	133 (30)	144 (80)			406 (1)			336 (27)	204 (70)	262 (31)	273 (12)
(16)	117 (44)	160 (39)			406 (0.5)	362 (0.5)	246 (13)	320 (2)	204 (16)	246 (13)	289 (4)
(17)	117 (60)	144 (70)			390 (2)			320 (14)	204 (55)	246 (39)	273 (12)
(4)	117 (28)	160 (80)			406 (2)	362 (4)	246 (12)	320 (4)	204 (36)	246 (12)	289 (9)
(5)	117 (70)	144 (90)			390 (2)			320 (13)	204 (69)	246 (19)	273 (3)
(18)	117 (55)	160 (61)			434 (0.8)	390 (1)	274 (13)	348 (2)	232 (4)	246 (14) *a,b*	317 (3) *a,e*
										274 (13) *d,e*	289 (14) *d,f*
(24)	133 (9)	160 (90)				378 (5)	246 (8)	336 (10)	204 (17)	262 (18)	289 (8)
(28)	133 (8)	160 (100)				378 (6)	246 (6)	336 (10)	204 (15)	262 (16)	289 (13)
(25)	85 (100)	160 (41)				330 (3)	246 (3)	288 (8)	204 (26)	214 (12)	289 (5)
(29)	85 (100)	160 (31)				330 (3)	246 (5)	288 (12)	204 (27)	214 (11)	289 (7)
(26)	132 (16)	160 (61)				377 (0.7)	246 (7)	335 (0.4)	204 (10)	261 (4)	289 (4)
(30)/(31)	132 (18)	160 (70)				377 (2)	246 (40)	335 (2)	204 (25)	261 (10)	289 (7)
g	160 (100)	160 (100)	390 (11)	231 (5		405 (5)	246 (6)	363 (4)		289 (54)	289 (54)
(27)	132 (19)	160 (80)				377 (1)	246 (23)	335 (1)	204 (16)	261 (4)	289 (2)

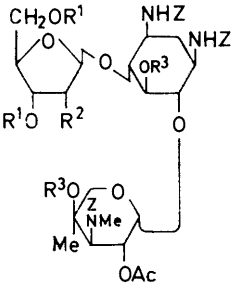
‡ The structures and designations of all fragment ions are identical with those described in ref. 12.

a 1-*N*-Ethyl derivative. *b* Found: 246.1337 ($C_{11}H_{20}NO_5$ requires 246.1341). *c* Found: 317.2086 ($C_{15}H_{29}N_2O_5$ requires 317.2076). *d* 3-*N*-Ethyl derivative, *e* Found: 274.1677 ($C_{13}H_{24}NO_5$ requires 274.1654). *f* Found: 289.177) ($C_{13}H_{25}N_2O_5$ requires 289.1763). *g* Mixture of 2'-*N*-ethyl pseudotrisaccharides from preparation of (26), (30), and (31).

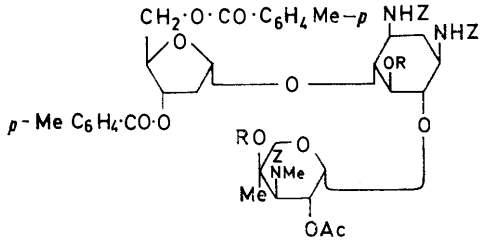
[15] 'Synthetic Procedures in Nucleic Acid Chemistry,' eds. W. W. Zorbach and R. S. Tipson, Wiley, New York, 1968, vol. 1, p. 521.

[16] K. Biemann and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1962, **84**, 2005.

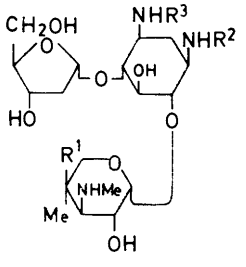
assigned on the basis of the molecular rotations {$[M]_D$ + 976° for (15) and +498° for (13), respectively}. Catalytic hydrogenation of the α-glycoside (15) over palladium black followed by ammonolysis gave a 79% yield of *O*-2-deoxy-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (16), identical with the product prepared from the fully protected garamine derivative (8). Similar treatment of



(11) R^1 = Bz, R^2 = OBz, R^3 = Ac
(12) R^1 = CO·C_6H_4Me-*p*(xz), R^2 = H, R^3 = Ac
(13) R^1 = CO·C_6H_4Me-*p*(xz), R^2 = R^3 = H



(14) R = Ac
(15) R = H



(16) R^1 = OH, R^2 = R^3 = H
(17) R^1 = R^2 = R^3 = H
(18) R^1 = OH, R^2 = Et, R^3 = H and R^2 = H, R^3 = Et

the β-glycoside (13) gave a 73% yield of *O*-2-deoxy-β-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (4). There was no evidence in the above reaction with the reactive pentofuranosyl chloride for any glucosylation at the 5-hydroxy-group of garamine. In view of the known solution conformation of garamine [10] it is reasonable to invoke steric hindrance by the 6-*O*-glycosyl unit as the factor preventing 5-*O*-glycosylation. Although from the known solution conformations of the 4-*O*-glycosyl pseudodisaccharides paromamine, neamine, and the gentamines, less steric hindrance would be predicted at the 5-hydroxy-group than in garamine, the absence of 5-*O*-glycosides has been noted during glycosylations of these compounds.[8,17–19] These results make Ito's synthesis of ribostamycin [20] surprising in view of his having obtained mainly 5-*O*-glycosylation of neamine in that reaction. When the α-glycoside (15) was hydrogenated under forcing conditions with palladium black, *O*- to *N*-transacylation occurred, with subsequent reduction of the *N*-acetyl derivatives to give, after ammonolysis, *O*-2-deoxy-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-1- and -3-*N*-ethylgaramine (18), in 25% yield. The ^{1}H n.m.r. spectrum of (18) revealed a triplet at δ 1.20 and a quartet at δ 2.78 (*J* 7 Hz) due to an *N*-ethyl group. The high resolution mass spectrum (Table 1) further confirmed the presence of an *N*-ethyl group and indicated that (18) was in fact a mixture of the 1-*N*-ethyl and 3-*N*-ethyl isomers. The fragmentation pattern indicated that ethyl groups were located only on the amino-groups of the deoxystreptamine ring.

It was of interest to determine the solution conformations of the 4-*O*-β-D-ribofuranosyl derivatives (2) and (4) and to compare these with the conformation of the 4-*O*-α-D-ribofuranosyl derivative (16), as these compounds represent novel aminocyclitol systems whose conformations have never been determined. The ^{13}C n.m.r. spectrum of *O*-β-D-ribofuranosyl-(1 ⟶ 4)-garamine (2) (Table 2) showed the expected resonances for the garosaminyl unit.[21] The ribosyl resonances were assigned by comparison with the most recent revised values reported for several ribosyl-containing nucleotides.[22] The assignments for the ribosyl carbon atoms were in agreement with those assigned to the corresponding carbon atoms in ribostamycin.[23] Although the ^{13}C n.m.r. spectra of the butirosins have been published,[24] the signals for the ribosyl carbon atoms were not unambiguously assigned. The chemical shift of the anomeric C-1′ (δ_C 109.3) was in good agreement with that reported for the β-D-ribofuranosyl unit of ribostamycin.[23] The deoxystreptamine resonances were in excellent agreement with the values reported [25] for *O*-2-amino-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-garamine. The similarity between the C-1, -3, and -5 resonances for these two β-glycosides indicates that they have similar rotamer conformations about the C(4)–O bond and suggests that

[17] M. Tanabe, unpublished observations.
[18] P. J. L. Daniels, A. K. Mallams, and J. J. Wright, *J.C.S. Chem. Comm.*, 1973, 675.
[19] P. J. L. Daniels, A. K. Mallams, M. Tanabe, and J. J. Wright, unpublished observations.
[20] T. Ito, E. Akita, T. Tsuruoka, and T. Niida, *Agric. and Biol. Chem.* (*Japan*), 1970, **34**, 980.
[21] J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1973, **95**, 7464.
[22] H. H. Mantsch and I. C. P. Smith, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 808.
[23] S. Omoto, S. Inouye, M. Kojima, and T. Niida, *J. Antibiotics*, 1973, **26**, 717.
[24] P. W. K. Woo and R. D. Westland, *Carbohydrate Res.*, 1973, **31**, 27.
[25] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J.C.S. Perkin I*, 1976, 1113.

the preferred rotamer at this bond in (2) is *a*.[25] In the β-D-ribofuranosyl derivative (2), C-3 is shielded by 0.9 p.p.m. relative to deoxystreptamine, and C-5 is shielded by 3.2 p.p.m., of which 1.7 p.p.m. is due to the presence of the β-D-ribofuranosyl unit. As discussed previously,[25] the origin of the shielding of C-3 remains unclear. The remainder of the shielding observed at C-5 (1.5 p.p.m.) is due to the garosaminyl unit at C-6 which adopts the usual rotamer conformation *b* [10,21,26,27] for a 6-*O*-axially linked glycoside. Negligible shielding of C-1 was observed. Both rotamers *a* and *b* satisfy the requirements of the *exo*-anomeric effect.[28,29] Protonation of the amino-groups resulted in the expected shielding of the carbon atoms β to the amino-groups, and the observed shielded a total of 3.9 p.p.m. Of this, 1.5 p.p.m. is due to the garosaminyl unit,[10,21] the remainder (2.4 p.p.m.) being due to the 4-*O*-β-D-ribofuranosyl unit. Negligible shielding of C-1 was observed. The above results may be interpreted as indicating a preference for rotamer *a* for the 4-*O*-glycoside [25] and for rotamer *b* for the 6-*O*-glycoside.[21,26] Both rotamers satisfy the requirements of the *exo*-anomeric effect.[28,29]

The ^{13}C n.m.r. spectrum of *O*-2-deoxy-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (16) showed the expected resonances for the garosaminyl unit.[21] The observed γ effect of −2.5 p.p.m. for C-4′ was in keeping with the anticipated effect in going from the 4-*O*-β-glycoside (4) to the 4-*O*-α-glycoside (16). The signal for

TABLE 2

^{13}C Chemical shifts

Carbon atom	(2) [a]	(2),[a] pH 1	Δ(Base ⟶ pH 1)	Δ[DOS ⟶ (2)]	(16) [b]	Δ[DOS ⟶ (16)]
C-1	51.5	50.7	−0.8	−0.1	51.3	−0.3
C-2	36.4	28.5	−7.9		36.7	
C-3	50.7	49.5	−1.2	−0.9	50.0	−1.6
C-4	88.5	81.5	−7.0		86.7	
C-5	73.4	72.4	−1.0	−3.2	75.5	1.1
C-6	87.6	84.0	−3.6		88.1	
C-1′	109.3	107.5	−1.8		105.0	
C-2′	75.7	75.7			41.2	
C-3′	70.4	70.3			72.1	
C-4′	83.5	83.7			84.9	
C-5′	61.7	61.5			62.9	
C-1″	101.3	101.9	+0.6		101.0	
C-2″	70.2	67.4 [c]	−2.8		70.4	
C-3″	64.2	64.2			64.7	
C-4″	73.2	70.8	−2.4		73.2	
C-5″	68.6	68.5			68.9	
3″-NCH_3	37.7	35.4	−2.3		37.6	
4″-CH_3	22.4	21.8			22.6	

Carbon atom	(4) [a]	Δ[DOS ⟶ (4)]	(24) [b]	Δ[DOS ⟶ (24)]	(28)[a]	Δ[DOS ⟶ (28)]
C-1	51.5	−0.1	51.2	−0.4	51.8	+0.2
C-2	36.0		36.7		36.6	
C-3	50.7	−0.9	49.8	−1.8	51.8	+0.2
C-4	87.0		87.5		88.4	
C-5	72.7	−3.9	75.0	−1.6	73.6	−3.0
C-6	87.7		87.9		88.1	
C-1′	106.6		104.7		101.9	
C-2′	41.4		72.5		69.6	
C-3′	71.1		73.5		69.8	
C-4′	87.4		69.3		69.8	
C-5′	62.3		67.3		64.2	
C-1″	101.1		101.0		101.5	
C-2″	69.6		70.2		70.3	
C-3″	64.4		64.5		64.2	
C-4″	73.4		73.2		73.3	
C-5″	68.5		68.7		68.6	
3″-NCH_3	37.3		37.8		37.9	
4″-CH_3	22.3		22.9		22.6	

[a] δ_C in p.p.m. downfield from external Me_4Si [$\delta(Me_4Si) = \delta$(dioxan) + 67.4] in D_2O solution. [b] External $^{13}CH_3I$ used as reference. [c] Signal obscured by dioxan. DOS = deoxystreptamine.

shielding of C-4 and C-6 clearly established the 4,6-*O*-glycosidic structure of the compound.

The ^{13}C n.m.r. spectrum of *O*-2-deoxy-β-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (4) revealed the expected garosamine resonances.[21] The carbon resonances for the furanosyl unit were assigned by analogy with those of the corresponding sugar in 2′-deoxynucleosides.[30] The chemical shift differences for the deoxystreptamine carbon atoms β to the glycosidic bonds were in agreement with those reported for 4-*O*-β-D-glucopyranosyl derivatives,[25] as well as for the 4-*O*-β-D-ribofuranosyl derivative (2). Thus C-3 was shielded by 0.9 p.p.m. relative to deoxystreptamine, and C-5 was the anomeric C-1′ in (16) occurred at δ_C 105.0 in keeping with an α-D-glycoside. The deoxystreptamine resonances were assigned by comparison with other garamine-containing aminocyclitols.[10,21,25,27] The chemical shift differences for the deoxystreptamine carbon atoms β to the glycosidic bonds in (16) were in agreement with those reported for other 4-*O*-α-D-glucopyranosyl aminocyclitols.[21,26] This C-3 was shielded by 1.6 p.p.m. relative to deoxystreptamine, and C-5 was shielded by 1.1 p.p.m.; C-1 experienced negligible shielding. The shieldings indicate that the preferred rotamers about the C(4)–O and C(6)–O bonds are *c* and *b* respectively.[21,26] Both

[26] K. F. Koch, J. A. Rhoades, E. W. Hagaman, and E. Wenkert, *J. Amer. Chem. Soc.*, 1974, **96**, 3300.

[27] D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, *J.C.S. Perkin I*, 1975, 814.

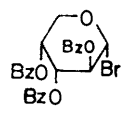
[28] R. U. Lemieux, T. L. Nagabhushan, K. J. Clemetson, and L. C. N. Tucker, *Canad. J. Chem.*, 1973, **51**, 53.

[29] R. U. Lemieux and S. Koto, *Tetrahedron*, 1974, **30**, 1933.

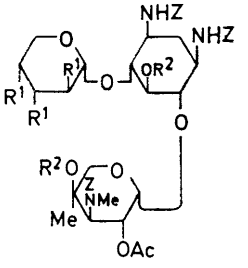
[30] A. J. Jones, D.M. Grant, M. W. Winkley, and R. K. Robins, *J. Amer. Chem. Soc.*, 1970, **92**, 4079.

rotamers would satisfy the requirements of the *exo*-anomeric effect.[28,29]

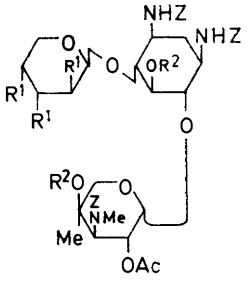
In order to determine the effect of the absence of either a 6′-hydroxymethylene or a 6′-aminomethylene group upon the biological spectrum of these compounds, a series of such derivatives was synthesized. 2,3,4-Tri-*O*-benzoyl-α-D-arabinopyranosyl bromide (**19**)[31] was condensed with 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (**9**) in the presence of mercury(II) cyanide in



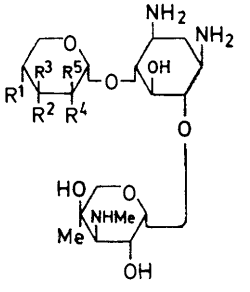
(19)



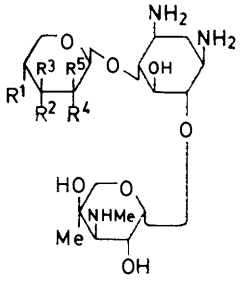
(20) R^1=OBz, R^2=H
(21) R^1=H, R^2=Ac

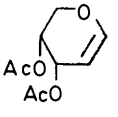


(22) R^1=OBz, R^2=H
(23) R^1=H, R^2=Ac



(24) R^1=R^2=R^5=OH, R^3=R^4=H
(25) R^1=R^2=R^3=R^4=R^5=H
(26) R^1=R^2=OH, R^3=R^5=H, R^4=NH_2
(27) R^1=R^3=OH, R^2=R^5=H, R^4=NH_2

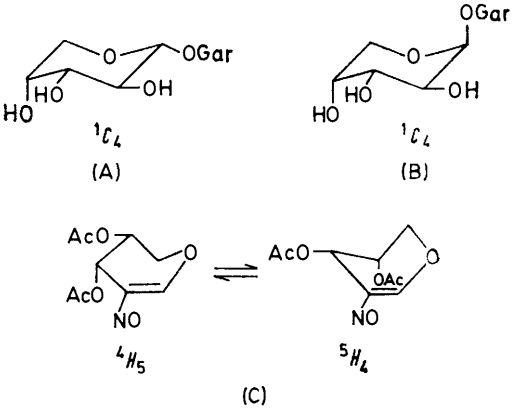




(32)

(28) R^1=R^2=R^5=OH, R^3=R^4=H
(29) R^1=R^2=R^3=R^4=R^5=H
(30) R^1=R^2=OH, R^3=R^5=H, R^4=NH_2
(31) R^1=R^2=OH, R^3=R^4=H, R^5=NH_2

refluxing benzene–dioxan to give a 5% yield of *O*-2,3,4-tri-*O*-benzoyl-β-D-arabinopyranosyl-(1 ⟶ 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (**22**) and a 33% yield of the α-anomer (**20**). The molecular rotations of (**22**) and (**20**) ($[M]_D$ — 942 and 0°, respectively) supported the assigned configurations for the 4-*O*-glycoside



(A) (B) (C)

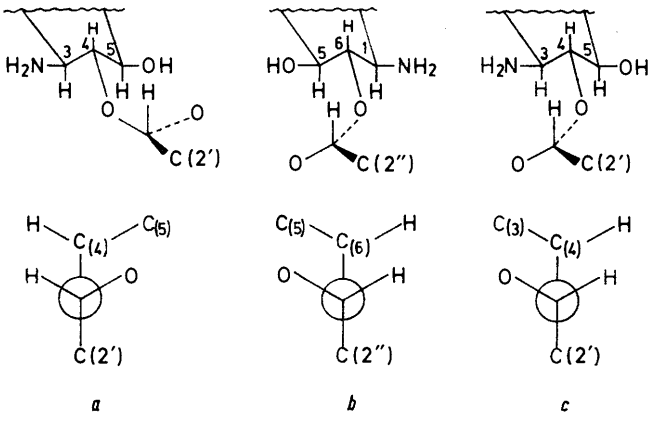
in these molecules. The α-glycoside (**20**) was subjected to ammonolysis followed by treatment with sodium in liquid ammonia to give a 95% yield of *O*-α-D-arabinopyranosyl-(1 ⟶ 4)-garamine (**24**) [based on (**20**)]. The mass spectrum of (**24**) showed the expected fragment ions (Table 1). The c.d. data were in accord with the proposed 4-*O*-glycosyl structure. The ^{1}H n.m.r. spectrum contained a doublet at δ 4.67 ($J_{1'a,2'a}$ 7 Hz) due to the axial H-1′, indicating that the α-D-arabinopyranosyl unit existed in the $^1C_4$ conformation (A). When the β-glycoside (**22**) was deprotected as described above a 91% yield of *O*-β-D-arabinopyranosyl-(1 ⟶ 4)-garamine (**28**) [based on (**22**)] was obtained. The mass spectrum, c.d. data, and rotation were in agreement with structure (**28**). The ^{1}H n.m.r. spectrum revealed a doublet at δ 5.20 ($J_{1'e,2'a}$ *ca.* 4 Hz) due to the equatorial H-1′, indicating that the 4-*O*-arabinopyranosyl unit existed primarily in the $^1C_4$ conformation (B).

It was of interest to determine the solution conformations of the two novel $^1C_4$ arabinopyranosyl glycosides (**24**) and (**28**), as such structures have not been encountered previously in known aminocyclitols. The ^{13}C n.m.r. parameters are given in Table 2. The α-glycoside (**24**) showed resonances for the carbon atoms of the garamine unit that were in good agreement with those observed for other garamine-containing aminoglycosides.[10,21] The anomeric C-1′ resonance occurred at δ_C 104.7, in agreement with an equatorial glycosidic linkage such as occurs in the $^1C_4$ conformation proposed for (**24**). The remaining arabinosyl resonances were similar to those assigned for methyl α-D-arabinoside.[32] The solution conformation of the α-glycoside (**24**) was determined by studying the shifts of the deoxystreptamine carbon atoms β to the glycosyl linkages (Table 2).[21,26] The absence of any significant shielding of C-1, together with the observed shielding of 1.8 p.p.m. for C-3 and of 1.6 p.p.m. for C-5, relative to deoxystreptamine, indicated that the preferred rotamer about the

[31] H. G. Fletcher and C. S. Hudson, *J. Amer. Chem. Soc.*, 1947, **69**, 1145.

[32] P. A. J. Gorin and M. Mazurek, *Canad. J. Chem.*, 1975, **53**, 1212.

C(4)–O bond was *c* and that the preferred rotamer about the C(6)–O bond was *b*. The results indicate that in (24) the equatorially linked 4-*O*-α-D-pentopyranoside, which is in the $^1C_4$ conformation, adopts essentially the same

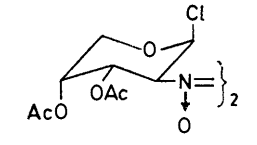


a *b* *c*

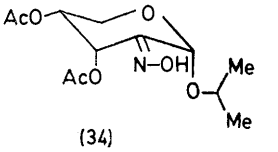
rotamer configuration about the C(4)–O bond as do the axially linked 4-*O*-α-D-hexopyranosides, which are in the $^4C_1$ conformation in most aminocyclitol antibiotics.[21,26-28] The garosaminyl unit adopts the same conformation as observed for other aminoglycosides which contain the garosamine unit.[10,21,25] Both rotamers *c* and *b* satisfy the requirements of the *exo*-anomeric effect.[28,29] The β-glycoside (28) showed resonances for the garosamine unit in good agreement with those reported for other garosamine-containing aminoglycosides.[21] The anomeric C-1′ resonance occurred at δ_C 101.9, in agreement with an axial glycosidic linkage such as occurs in the $^1C_4$ conformation in which (28) exists. The remaining arabinosyl resonances were assigned by analogy with those of methyl β-D-arabinoside.[32] The solution conformation of the β-glycoside (28) was determined by studying the shifts of the deoxystreptamine carbon atoms β to the glycosyl linkages (Table 2).[21,26] The absence of any significant shieldings for C-1 and C-3, together with the observed large shielding of 3.0 p.p.m. for C-5 relative to deoxystreptamine, indicated that the preferred rotamer about the C(4)–O bond was *a*, and that the preferred rotamer about the C(6)–O bond was again *b*. These results indicate that in (28) the axially linked 4-*O*-β-D-pentopyranoside, which is in the $^1C_4$ conformation, adopts a rotamer configuration about the C(4)–O bond depicted by *a*, in which C-5 of deoxystreptamine is shielded by 1.5 p.p.m. due to the presence of the 4-*O*-glycoside. The remaining 1.5 p.p.m. shielding of C-5 is due to the garosamine unit.[10,21,25,26] The conformation of (28) as determined above is such that both rotamers *a* and *b* satisfy the requirements of the *exo*-anomeric effect.[28,29]

The substitution of a tetrahydropyranyl ring for the 4-*O*-glycoside unit was also studied. Treatment of 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) with dihydropyran in the presence of toluene-*p*-sulphonic acid gave a 38% yield of *O*-α-tetrahydropyranyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (21) and a 9% yield of the β-anomer (23).* The configurational assignments at C-1′ were supported by the molecular rotations ($[M]_D$ + 820 and +704°, respectively). Deprotection of the α-anomer (21) by ammonolysis followed by treatment with sodium in liquid ammonia afforded a 43% yield of *O*-α-tetrahydropyranyl-(1 ⟶ 4)-garamine (25) [based on (21)]. The molecular rotation of (25) was +612° and the mass spectral fragmentation pattern (Table 1) and c.d. data agreed with the proposed structure. The equatorial H-1′ signal in the ^{1}H n.m.r. spectrum was obscured by the HOD peak. The β-anomer (23) was deprotected in a similar manner to give *O*-β-tetrahydropyranyl-(1 ⟶ 4)-garamine (29). The 4″-deoxy-by-products from the sodium–liquid ammonia reaction were not isolated.

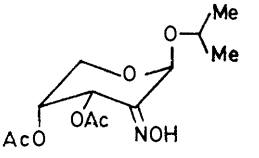
The preparation of 4-*O*-2-amino-2-deoxypentopyranosyl derivatives of garamine was undertaken by the Lemieux–Nagabhushan reaction. 3,4-Di-*O*-acetyl-D-arabinal (32) was converted into the nitroso-chloro-adduct (33), identical in physical characteristics with that described by Serfontein.[33] The ^{1}H n.m.r. spectrum of (33) clearly indicated that addition of the nitrosyl



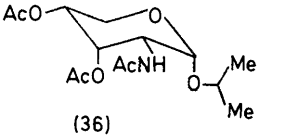
(33)



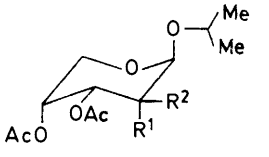
(34)



(35)



(36)



(37) R^1=NHAc, R^2=H
(38) R^1=H, R^2=NHAc

chloride had occurred in a *cis*-manner from the upper (β) face of the glucal, with subsequent ring inversion to the more favoured $^1C_4$ conformation (33) having the 1-chloro-group axial. In order to gain an insight into the nature of the products formed by reaction of the nitroso-chloro-adduct (33) with alcohols, it was first condensed

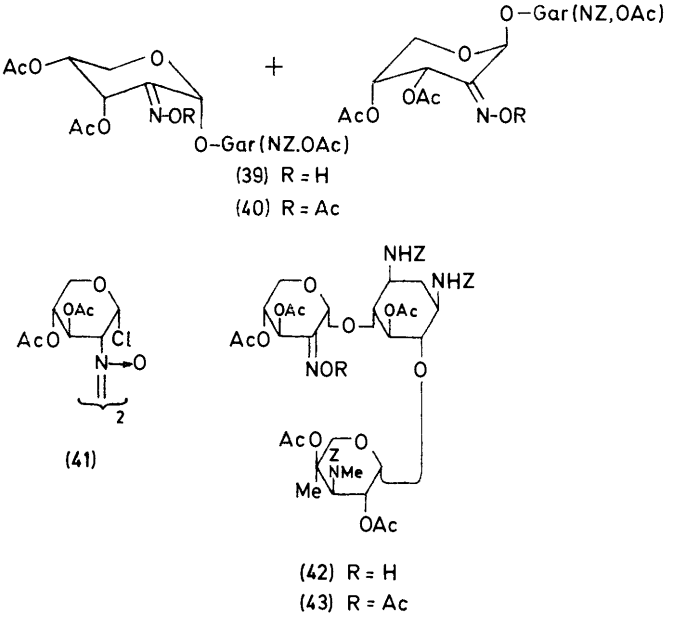
* For the purposes of this text the α-anomer is defined as having the (1′*R*)-stereochemistry and the β-anomer as having the (1′*S*)-stereochemistry for the tetrahydropyranyl derivatives.

[33] W. J. Serfontein, J. H. Jordaan, and J. White, *Tetrahedron Letters*, 1964, 1069.

with propan-2-ol in dimethylformamide at 25 °C. This gave a 27% yield of isopropyl 3,4-di-*O*-acetyl-2-hydroxyimino-α-D-*erythro*-pentopyranoside (34) together with a 52% yield of the β-anomer (35). The ^{1}H n.m.r. spectrum of the α-D-anomer (34) indicated that the molecule existed in the $^4C_1$ conformation. The equatorial anomeric proton gave rise to a singlet at δ 5.95, indicating that the oxime was in the *Z*-configuration.[25,34] On the basis of previous studies in the *allo*-series [25] such a configuration would be predicted in molecules having a 3′-axial acetyl group. The coupling constants, $J_{3,4}$ 3.5, $J_{4,5a}$ 10.5, and $J_{4,5e}$ 5 Hz, clearly were in agreement with the assigned $^4C_1$ conformation. The ^{1}H n.m.r. spectrum of the β-anomer (35) indicated that the molecule existed in the $^1C_4$ conformation. The equatorial anomeric proton gave rise to a singlet at δ 6.13 indicating that the oxime was in the *Z*-configuration,[34] as expected owing to the presence of the 3-equatorial acetyl group. The coupling constants, $J_{3,4}$ 3.5, $J_{4,5a}$ 2.5, and $J_{4,5e}$ 1 Hz, agreed with the assigned $^1C_4$ conformation. The ^{1}H n.m.r. assignments for (34) and (35) were confirmed by appropriate off-resonance decoupling experiments.

Acetylation of the oxime (34) gave isopropyl 2,3,4-tri-*O*-acetyl-2-hydroxyimino-α-D-*erythro*-pentopyranoside, which on reduction with 4 equiv. of borane in tetrahydrofuran and acetylation, gave a 29% yield of isopropyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-ribopyranoside (36). The ^{1}H n.m.r. spectrum of (36) showed a doublet at δ 4.74 ($J_{1,2}$ 3.5 Hz) due to the equatorial anomeric proton. The coupling constants, $J_{1,2}$ 3.5, $J_{2,3} = J_{3,4} = J_{4,5e} = 4$, and $J_{4,5a}$ 9 Hz, were in agreement with the structure (36) having a $^4C_1$ conformation. When the oxime (35) was similarly treated, a 38% yield of a syrup was obtained which was homogeneous in several t.l.c. systems. The ^{1}H n.m.r. spectrum revealed the syrup to be a 1 : 2 mixture of isopropyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-ribopyranoside (37) and -β-D-arabinopyranoside (38). The spectrum of the *ribo*-isomer (37) contained a doublet at δ 4.88 ($J_{1,2}$ 1.5 Hz) due to the equatorial anomeric proton. The coupling constants, $J_{1,2}$ 1.5 and $J_{2,3}$ 4 Hz, agreed with a β-D-ribopyranoside in the $^1C_4$ conformation. The spectrum of the *arabino*-isomer (38) contained a doublet at δ 4.97 ($J_{1,2}$ 3.5 Hz) due to the equatorial anomeric proton. The coupling constants, $J_{1,2}$ 3.5 and $J_{2,3}$ 10 Hz, agreed with a β-D-arabinopyranoside in the $^1C_4$ conformation.

The above results indicated that considerable complexity could be anticipated on condensing (33) with garamine derivatives; indeed this was the case. Condensation of the nitroso-chloro-adduct (33) with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) in dimethylformamide at 25 °C afforded a 58% yield of *O*-3,4-di-*O*-acetyl-2-hydroxyimino-D-*erythro*-pentopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (39).* The latter was a mixture of the α-D-*erythro*-oxime ($^4C_1$ conformation) and the β-D-*erythro*-oxime ($^1C_4$ conformation). The ^{1}H n.m.r. spectrum of (39) revealed two broad singlets at δ 6.00 due to the anomeric H-1′ of the α-D-*erythro*-oxime (*Z*-configuration) and at δ 6.46 due to H-1′ of the β-D-*erythro*-oxime (*Z*-configuration). These conclusions agreed with the results for the monosaccharides (34) and (35). Acetylation of the mixture of oximes (39) afforded the acetates (40), which on reduction with 13 equiv. of borane in tetrahydrofuran at 5 °C followed by alkaline hydrolysis gave, after chromatography, two homogeneous fractions (as assessed by t.l.c. in several systems).



The less polar fraction was shown by its ^{1}H n.m.r. spectrum to be a 2 : 1 mixture of *O*-2-amino-2-deoxy-β-D-ribopyranosyl-(1 ⟶ 4)-garamine (30) and the *arabino*-isomer (31), and was obtained in a 16% yield based on (40). The *ribo*-isomer (30) exhibited a ^{1}H n.m.r. doublet at δ 4.73 ($J_{1',2'}$ 1.5 Hz), whereas the *arabino*-isomer (31) exhibited a doublet at δ 4.93 having ($J_{1',2'}$ 3.5 Hz). As predicted from the monosaccharide model compounds (37) and (38), both the trisaccharides (30) and (31) existed in the $^1C_4$ conformation. The coupling constant $J_{2',3'}$ could not be observed for (30). However, the ^{1}H n.m.r. spectrum of (31) revealed a doublet of doublets at δ 3.13 ($J_{1',2'}$ 3.5, and $J_{2',3'}$ 6 Hz) for H-2′. An INDOR experiment confirmed the assignment, and the observed value for $J_{2',3'}$ in (31) was consistent with the proposed conformation.

A more polar product was also obtained, in 8% yield based on (40), and was found to be *O*-2-amino-2-deoxy-α-D-ribopyranosyl-(1 ⟶ 4)-garamine (26). The c.d. spectrum run in TACu was in agreement with the pro-

* Whenever the triacetate (8) was used in a Lemieux–Nagabhushan reaction some unchanged (8) was recovered as well as the transacylation product (10).[10] Details of the isolation of these products are not included in the Experimental section.

[34] R. U. Lemieux, R. A. Earl, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 19.

posed structure and the ^{1}H n.m.r. spectrum showed a doublet at δ 4.85 ($J_{1',2'}$ 3.5 Hz) due to H-1′. The H-2′ signal occurred as a doublet of doublets at δ 2.92 ($J_{1',2'} = J_{2',3'} = 3.5$ Hz), consistent with an α-D-*ribo*-glycoside having a $^4C_1$ conformation. An INDOR experiment confirmed the above coupling constants. A mixture of 2′-*N*-ethyl derivatives was also isolated, but was not further analysed owing to its complexity.

In view of what is known about the mechanism of the Lemieux–Nagabhushan reaction,[35-37] we conclude that the reaction between 3,4-di-*O*-acetyl-2-deoxy-2-nitroso-β-D-arabinopyranosyl chloride (33) with alcohols proceeds *via* the reactive unsaturated nitroso-intermediate (C). The conformational flexibility of the intermediate would enable it to exist in both the $^4H_5$ and $^5H_4$ conformations as shown. In the latter conformation the nitroso-group would be predicted to be *cis* to the double bond, whereas in the former conformation it could adopt either the *cis*- or the *trans*-configuration. On the basis of the mechanism proposed by Lemieux and Nagabhushan,[35-37] the $^4H_5$ conformation would be expected to lead to the α-glycoside, whereas the $^5H_4$ conformation would be expected to produce a β-glycoside. The formation of both α- and β-glycosides during the condensation of (33) with alcohols may be rationalized in the above manner.

The reaction of 3,4-di-*O*-acetyl-2-deoxy-2-nitroso-α-D-xylopyranosyl chloride (41) [38] with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) in dimethylformamide gave a 51% yield of a product consisting predominantly of *O*-3,4-di-*O*-acetyl-2-hydroxyimino-α-D-*threo*-pentopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (42). The ^{1}H n.m.r. spectrum of (42) revealed a broad singlet at δ 6.04 due to the anomeric H-1′, indicating that the oxime was in the *Z*-configuration.[34] The oxime (42) was acetylated to give the acetate (43), which on reduction with 11 equivalents of borane in tetrahydrofuran followed by basic hydrolysis gave a 9% yield of a homogeneous product (t.l.c.). This consisted mainly of *O*-2-amino-2-deoxy-α-D-xylopyranosyl-(1 ⟶ 4)-garamine (27). Its ^{1}H n.m.r. spectrum revealed a doublet at δ 5.11 ($J_{1',2'}$ 4 Hz) due to H-1′, confirming the α-D-*xylo*-configuration. The product could not be obtained pure and in view of the low yield no further studies were carried out on its preparation. Evidently the Lemieux–Nagabhushan reaction gives complex mixtures when applied to the synthesis of pentosyl glycosides.

The novel aminoglycosides described above were subjected to a variety of antibacterial and antiprotozoal tests; the results will be described elsewhere.

EXPERIMENTAL

Experimental data were recorded as described in Part I.[10]

O-β-D-*Ribofuranosyl*-(1 ⟶ 4)-*garamine* (2).—2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) (2.2 g) and 2,3,5-tri-*O*-benzoyl-α- and -β-D-ribofuranosyl bromides (6) (1.85 g) were dissolved in dry toluene (100 ml); mercury(II) cyanide (1.11 g) and calcium sulphate (11 g) (baked out on a hot-plate) were added and the mixture was stirred under nitrogen at 55 °C for 20 h. The product was worked up as before. The residue was chromatographed on a silica gel (180 g) column (25% ether–benzene as eluant) to give O-2,3,5-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (11) (1.55 g, 46%). A portion crystallized from ether had m.p. 108—110° (Found: C, 63.9; H, 5.5; N, 3.5. $C_{69}H_{71}N_3O_{22}$ requires C, 64.0; H, 5.5; N, 3.25%), $[\alpha]_D$ +70.5° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.28br and 1.42br (3 H, 2 s, 4″-CH_3), 1.97br and 2.05br (9 H, 2 s, OAc), 2.88br (3 H, s, 3″-NCH_3), and 7.35br and 8.00br (30 H, 2 s, aromatic).

The product (11) (1.5 g) was dissolved in methanol (150 ml) and concentrated ammonium hydroxide (15 ml) was added. The mixture was kept at 25 °C under nitrogen for 18 h. The solution was evaporated to dryness and the residue was dissolved in liquid ammonia (200 ml) at −78 °C. Sodium (2 g) was added and the mixture was stirred at −78 °C for 2 h. Water (10 ml) was added dropwise and the ammonia was allowed to evaporate at 25 °C. The residue was dissolved in water and neutralized with BioRex 70 (H^+) resin (125 ml). The resin was washed with water and eluted with 1.5N-ammonium hydroxide (250 ml); the basic eluate was evaporated to dryness. The residue was chromatographed on a silica gel (25 g) column [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1) as eluant] to give O-β-D-*ribofuranosyl*-(1 ⟶ 4)-4′-*deoxygaramine* (3) (104 mg, 20%) (Found: $M^{+\cdot}$, 437.2366. $C_{18}H_{35}N_3O_9$ requires *M*, 437.2373), $[\alpha]_D$ +52.2° (in H_2O), $[\theta]_{290}$ −17 612 (TACu), $[\theta]_{290}$ −16 500 (Cupra A), $\nu_{max.}$ (Nujol) 3 333, 1 099, and 1 053 cm^{-1}, δ(D_2O) 0.98 (3 H, d, *J* 6.5 Hz, 4″e-CH_3), 2.45 (3 H, s, 3″-NCH_3), 5.20 (1 H, d, *J* 4 Hz, H-1″), and 5.30 (1 H, s, H-1′). The more polar fractions afforded O-β-D-*ribofuranosyl*-(1 ⟶ 4)-*garamine* (2) (340 mg, 64%) (Found: C, 47.5; H, 7.85; N, 9.2. $C_{18}H_{35}N_3O_{10}$ requires C, 47.7; H, 7.8; N, 9.3%), $[\alpha]_D$ +74.5° (in H_2O), $[\theta]_{290}$ −14 870 (TACu), $[\theta]_{290}$ −11 300 (Cupra A), $\nu_{max.}$ (KCl) 3 330 and 1 055 cm^{-1}, δ(D_2O), 1.37 (3 H, s, 4″-CH_3), 2.68 (3 H, s, 3″-NCH_3), 5.26 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.36 (1 H, s, H-1′).

O-2-*Deoxy*-α- *and* -β-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-*garamine* [(16) *and* (4)].—(i) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) (4 g) and 2-deoxy-3,5-di-*O*-*p*-toluoyl-α- and -β-D-*erythro*-pentofuranosyl chlorides (7) (2.2 g) were dissolved in dry toluene (300 ml); mercury(II) cyanide (2.03 g) and calcium sulphate (21 g) (baked out on a hot-plate) were added and the mixture was stirred under nitrogen at 25 °C for 18 h. The product was worked up as before. The residue was chromatographed on preparative silica gel plates (52; 20 × 40 cm) [ether–benzene–methanol (50 : 50 : 0.5) as eluant; two developments] to give O-2-*deoxy*-3,5-*di*-O-p-*toluoyl*-α-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (14) (2.04 g, 36%), which crystallized from ether; m.p. 91—93° (Found: C, 64.0; H, 5.8; N, 3.7. $C_{64}H_{71}N_3O_{20}$

† Mixture of rotamers at ambient temperatures.

[35] R. U. Lemieux, T. L. Nagabhushan, and S. W. Gunner, *Canad. J. Chem.*, 1968, **46**, 405.

[36] R. U. Lemieux, T. L. Nagabhushan, and K. James, *Canad. J. Chem.*, 1973, **51**, 1.

[37] R. U. Lemieux, Y. Ito, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 7.

[38] R. U. Lemieux, T. L. Nagabhushan, and I. K. O'Neill, *Tetrahedron Letters*, 1964, 1909.

requires C, 63.9; H, 5.95; N, 3.5%), $[\alpha]_D$ +109.3° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 739, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.30br and 1.43br (3 H, 2 s, 4″-CH_3), 1.97br, 2.05br, and 2.07br (9 H, 3 s, OAc), 2.43br (6 H, s, $COC_6H_4CH_3$), 2.90br (3 H, s, 3″-NCH_3), 7.18 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), 7.32 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), 7.23br and 7.35br (15 H, 2 s, Ph), 7.93 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), and 7.95 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$). The more polar product was the β-*isomer* (12) (1.29 g, 23%), m.p. 103—108° (Found: C, 64.2; H, 5.9; N, 3.8%), $[\alpha]_D$ +72.7° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 739, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.28br and 1.42br (3 H, 2 s, 4″-CH_3), 1.97br, 2.05br, 2.07br (9 H, s, OAc), 2.43br (3 H, s, $COC_6H_4CH_3$), 2.90br (3 H, s, 3″-NCH_3), 7.20 (4 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), 7.31br and 7.33br (15 H, s, Ph), 7.92 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), and 8.00 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$).

The α-glycoside (14) (2 g) was deprotected as before. The residue was chromatographed on a silica gel (16.5 g) column [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1) as eluant] to give O-2-*deoxy*-α-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-4′-*deoxygaramine* (17) (68 mg, 10%) (Found: $M^{+\cdot}$, 421.2429. $C_{18}H_{35}N_3O_8$ requires *M*, 421.2440), $[\alpha]_D$ +138.2° (in H_2O), $[\theta]_{282}$ −9 390 (TACu), $\nu_{max.}$ (Nujol) 3 333, 1 099, and 1 053 cm^{-1}, δ(D_2O) 0.97 (3 H, d, *J* 7 Hz, 4″e-CH_3), 2.45 (3 H, s, 3″-NCH_3), 5.17 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.68 (1 H, dd, $J_{1',2'} = J_{1',2'} = 5$ Hz, H-1′). The more polar fractions afforded O-2-*deoxy*-α-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-*garamine* (16) (222 mg, 30%) (Found: C, 49.2; H, 8.2; N, 9.7. $C_{18}H_{35}N_3O_9$ requires C, 49.4; H, 8.1; N, 9.6%), $[\alpha]_D$ +173.6° (in H_2O), $[\theta]_{290}$ −9 300 (TACu), $[\theta]_{290}$ −5 520 (Cupra A), $\nu_{max.}$ (KCl) 3 333 and 1 050 cm^{-1}, δ(D_2O) 1.28 (3 H, s, 4″-CH_3), 2.58 (3 H, s, 3″-NCH_3), 5.15 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.70 (1 H, dd, $J_{1',2'}$ 1.5 and 5 Hz, H-1′).

The β-glycoside (12) (1.29 g) was deprotected as for the α-glycoside and the resulting mixture was chromatographed on a silica gel column (20 g) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1) as eluant] to give O-2-*deoxy*-β-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-4′-*deoxygaramine* (5) (60 mg, 13%) (Found: C, 51.4; H, 8.2; N, 9.8. $C_{18}H_{35}N_3O_8$ requires C, 51.3; H, 8.4; N, 10.0%), $[\alpha]_D$ +49.0° (in H_2O), $[\theta]_{287}$ — 12 600 (TACu), $[\theta]_{287}$ — 11 000 (Cupra A), $\nu_{max.}$ (KCl) 3 300 and 1 040 cm^{-1}, δ(D_2O) 0.99 (3 H, d, *J* 7 Hz, 4″e-CH_3), 2.48 (3 H, s, 3″-NCH_3), 5.18 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.59 (1 H, dd, $J_{1',2'} = J_{1',2'} = 4.5$ Hz, H-1′). The more polar fractions afforded O-2-*deoxy*-β-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-*garamine* (4) (240 mg, 52%) (Found: C, 48.0; H, 7.7; N, 9.4. $C_{18}H_{35}N_3O_9,H_2O$ requires C, 47.5; H, 8.2; N, 9.2%), $[\alpha]_D$ +80.8° (in H_2O), $[\theta]_{287}$ — 8 860 (TACu), $[\theta]_{287}$ −7 190 (Cupra A), $\nu_{max.}$ (KCl) 3 300 and 1 040 cm^{-1}, δ(D_2O) 1.30 (3 H, s, 4″-CH_3), 2.63 (3 H, s, 3″-NCH_3), 5.18 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.60 (1 H, dd, $J_{1',2'} = J_{1',2'} = 4$ Hz, H-1′).

(ii) 2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) (2 g) was dissolved in a mixture of dry dioxan (33 ml) and dry benzene (100 ml). Mercury(II) cyanide (1.1 g) and dry calcium sulphate (11.15 g) were added and the mixture was stirred under nitrogen. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α- and -β-D-*erythro*-pentofuranosyl chlorides (7) (1.09 g) in dry benzene (100 ml) were added and the mixture was stirred at 25° C for 24 h. The product was worked up as before. The residue was chromatographed on preparative silica gel plates (18; 20 × 20 cm) [benzene–ether–methanol (12 : 12 : 1) as eluant, developed twice] to give O-2-*deoxy*-3,5-*di*-O-p-*toluoyl*-α-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (15) (305 mg, 12%), m.p. 96—99° (Found: C, 64.55; H, 6.1; N, 3.6. $C_{60}H_{67}N_3O_{18}$ requires C, 64.45; H, 6.0; N, 3.8%), $[\alpha]_D^{21}$ +102.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$) † 0.97br and 1.05br (3 H, s, 4″-CH_3), 1.88br (3 H, s, OAc), 2.33br (6 H, s, $COC_6H_4CH_3$), 2.90br (3 H, s, 3″-NCH_3), 7.11 (4 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), 7.13, 7.22, and 7.28 (15 H, 3 s, Ph), 7.81 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), and 7.86 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$); and O-2-*deoxy*-3,5-*di*-O-*p*-*toluoyl*-β-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-2′-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (13) (287 mg, 11%), m.p. 96—99° (Found: C, 64.5; H, 6.15; N, 3.6. $C_{60}H_{67}N_3O_{18}$ requires C, 64.45; H, 6.0; N, 3.8%), $[\alpha]_D^{21}$ +57.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$) † 0.91br and 0.95br (3 H, 2 s, 4″-CH_3), 1.88br (3 H, s, OAc), 2.33br (6 H, s, $COC_6H_4CH_3$), 2.90br (3 H, s, 3″-NCH_3), 7.10 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), 7.17 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), 7.13, 7.22, and 7.28 (15 H, 3 s, Ph), 7.80 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$), and 7.89 (2 H, d, *J* 8 Hz, $OCOC_6H_4CH_3$).

The α-glycoside (15) (180 mg) was dissolved in 95% ethanol (25 ml) and hydrogenated over palladium black (450 mg) at 45 lb in^{-2} and 25 °C for 24 h. The catalyst was filtered off and washed with 95% ethanol and the combined filtrates were evaporated to dryness. The residue was dissolved in methanol (25 ml), the solution was saturated with ammonia at 5 °C, and the mixture was kept at 25 °C for 16 h. The solution was evaporated to dryness to give O-2-deoxy-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (16) (65 mg, 79%), which was homogeneous on t.l.c. on silica gel [lower phase of chloroform–methanol–concentrated ammonium hydroxide (3 : 4 : 3) as eluant], and identical (n.m.r., i.r., and t.l.c.) with the sample described in (i).

The β-glycoside (13) (180 mg) was deprotected as for the α-glycoside (15) to give O-2-deoxy-β-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-garamine (4) (60 mg, 73%), homogeneous on t.l.c. on silica gel [lower phase of chloroform–methanol–concentrated ammonium hydroxide (3 : 4 : 3) as eluant], and identical (n.m.r., i.r., and t.l.c.) with the sample described in (i).

O-2-*Deoxy*-α-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-1- and -3-*N*-*ethylgaramine* (18).—*O*-2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl-(1 ⟶ 4)-2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (15) (1.7 g) was dissolved in 95% ethanol (200 ml) and hydrogenated over palladium black (1.5 g) at 45 lb in^{-2} and 25 °C for 18 h. The catalyst was filtered off and the hydrogenation was repeated twice under the same conditions. The catalyst was filtered off and washed with ethanol, and the combined filtrates were evaporated to dryness. The residue (1.38 g) was dissolved in methanol (250 ml) and the solution was saturated with ammonia at 0 °C. The mixture was kept at 25 °C for 70 h, then evaporated to dryness. The residue was chromatographed on a silica gel (45 g) column [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give O-2-*deoxy*-α-D-erythro-*pentofuranosyl*-(1 ⟶ 4)-1- and -3-*N*-*ethylgaramine* (18) (210 mg, 25%) (Found: $M^{+\cdot}$, 465.2649. $C_{20}H_{39}N_3O_9$ requires *M*, 465.2686), $[\alpha]_D$ +158.7° (in H_2O), $[\theta]_{287}$ −5 034 (TACu), $[\theta]_{287}$ −3 780 (Cupra A), $\nu_{max.}$ (Nujol) 3 333, 1 099, and 1 053 cm^{-1}, δ(D_2O) 1.20 (3 H, t, *J* 7 Hz, $NHCH_2CH_3$), 1.30 (3 H, s, 4″-CH_3), 2.62 (3 H, s, 3″-NCH_3), 2.78 (2 H, q, *J* 7 Hz, $NHCH_2CH_3$), 5.17

† Same footnote as on page 1142.

(1 H, d, $J_{1'',2''}$ 4 Hz, H-1''), and 5.73 (1 H, dd, $J_{1',2'}$ 1.5 and 4 Hz, H-1').

O-α- *and* -β-D-*Arabinopyranosyl*-(1 ⟶ 4)-*garamine* [(24) *and* (28)].—2'-*O*-Acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (9) (6.6 g) and 2,3,4-tri-*O*-benzoyl-α-D-arabinopyranosyl bromide (19) (6.9 g) were dissolved in dry dioxan (100 ml) and dry benzene (330 ml). Mercury(II) cyanide (3.63 g) and Drierite (36.45 g) (freshly baked out on a hot-plate) were added and the mixture was heated under reflux under nitrogen for 18 h. More arabinosyl bromide (19) (2.3 g) was added and the mixture was heated under reflux for a further 5 h. The product was worked up as before. The residue was subjected to chromatography on a silica gel column [0—50% ether in benzene and then ether–benzene–methanol (100 : 100 : 1) as eluant] followed by preparative layer chromatography of the overlap fractions on silica gel plates (36; 20 × 40 cm) [ethyl acetate–chloroform (1 : 1)]. This gave O-2,3,4-*tri*-O-*benzoyl*-β-D-*arabinopyranosyl*-(1 ⟶ 4)-2'-O-*acetyl*-1,3,3'-*tris*-N-*benzyloxycarbonylgaramine* (22) (504 mg, 5%), which crystallized from petroleum (b.p. 30—60°)–ether; m.p. 132—134° (Found: C, 64.1; H, 5.5; N, 3.4. $C_{65}H_{67}N_3O_{20}$ requires C, 64.5; H, 5.6; N, 3.5%), $[\alpha]_D^{21}$ −77.9° (in MeOH), $\nu_{max.}$ (Nujol) 3 448, 3 333, 1 724, 1 695, 1 603, 1 587, 746, 709, and 694 cm^{-1}, δ($CDCl_3$) † 1.08br (3 H, s, 4''-CH_3), 1.88br (3 H, s, OAc), 2.93br (3 H, s, 3''-NCH_3), 7.20br (15 H, s, $PhCH_2$), and 7.43br, 7.83br, and 7.97br (15 H, 3 s, OBz); and the α-*isomer* (20) (3.52 g, 33%), which crystallized from petroleum (b.p. 30—60°)–ether; m.p. 135—137° (Found: C, 64.20; H, 5.6; N, 3.6%), $[\alpha]_D^{21}$ 0.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 448, 3 333, 1 724, 1 695, 1 603, 1 587, 746, 709, and 694 cm^{-1}, δ($CDCl_3$) † 0.97br and 1.08br (3 H, 2 s, 4''-CH_3), 1.80br (3 H, s, OAc), 2.88br (3 H, s, 3''-NCH_3), 7.20br (15 H, s, Ph), and 7.40br and 8.00br (15 H, 2 s, OBz).

The α-glycoside (20) (2.61 g) was deprotected as before with (11) to give O-α-D-*arabinopyranosyl*-(1 ⟶ 4)-*garamine* (24) (933 mg, 95%) (Found: C, 47.65; H, 8.0; N, 9.2. $C_{18}H_{35}N_3O_{10}$ requires C, 47.7; H, 7.8; N, 9.3%), $[\alpha]_D$ +98.4° (in H_2O), $[\theta]_{292}$ −5 436 (TACu), $\nu_{max.}$ (KCl) 3 330 and 1 060 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, $J_{1',2'}$ 7 Hz, H-1'), and 5.18 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1'').

The β-glycoside (22) (504 mg) was deprotected similarly to give the β-*isomer* (28) (170 mg, 91%) (Found: C, 47.5; H, 7.6; N, 9.1%), $[\alpha]_D^{21}$ +16.0° (in MeOH), $[\theta]_{290}$ −6 790 (TACu), $[\theta]_{290}$ −4 750 (Cupra A), $\nu_{max.}$ (Nujol) 3 448, 3 333, 1 087, 1 053, and 1 010 cm^{-1}, δ(D_2O) 1.28 (3 H, s, 4''-CH_3), 2.62 (3 H, s, 3''-NCH_3), 5.20 (1 H, d, $J_{1',2'}$ *ca.* 4 Hz, H-1'), and 5.20 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1'').

O-α- *and* -β-*Tetrahydropyranyl*-(1 ⟶ 4)-*garamine* [(25 and (29)].—2',4',5-Tri-*O*-acetyl-1,3,3'-tris-*N*-benzyloxycarbonylgaramine (8) (1 g), dihydropyran (5 ml), and anhydrous toluene-*p*-sulphonic acid (10 mg) were dissolved in dry benzene (50 ml) and stirred under nitrogen at 25 °C for 3 h. The mixture was diluted with ether (200 ml), washed with saturated aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on preparative silica gel plates (18; 20 × 20 cm) (50% ethyl acetate–chloroform as eluant) to give O-α-*tetrahydropyranyl*-(1 ⟶ 4)-2',4',5-*tri*-O-*acetyl*-1,3,3'-*tris*-N-*benzyloxycarbonylgaramine* (21) (402 mg, 38%), m.p. 101—104° (Found: C, 61.5; H, 6.45; N, 4.7. $C_{48}H_{59}N_3O_{16}$ requires C, 61.7; H, 6.4; N, 4.5%), $[\alpha]_D^{21}$ +92.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, and 1 235 cm^{-1}, $CDCl_3$–CD_3OD, 3 : 1) † 1.28br and 1.42br (3 H, 2 s, 4''-CH_3), 1.95br and 2.05br (9 H, 2 s, OAc), 2.88br (3 H, s, 3''-NCH_3), and 7.35br (15 H, s, Ph); the β-*isomer* (23) (91 mg, 9%), m.p. 106—111° (Found: C, 61.1; H, 6.4; N, 4.7%), $[\alpha]_D^{21}$ +79.0° (in MeOH), $\nu_{max.}$ (Nujol) 3 333, 1 724, 1 695, and 1 235 cm^{-1}, δ($CDCl_3$–CD_3OD, 3 : 1) † 1.28br and 1.42br (3 H, s, 4''-CH_3), 1.95br and 2.07br (9 H, s, OAc), 2.90br (3 H, s, 3''-NCH_3), and 7.37br (15 H, s, Ph).

The α-glycoside (21) (193 mg) was deprotected as before with (11). The residue was chromatographed on a silica gel column (8 g) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1) as eluant] to give O-α-*tetrahydropyranyl*-(1 ⟶ 4)-*garamine* (25) (38 mg, 43%) [Found: $(M + 1)^+$, 406.2531. $C_{18}H_{36}N_3O_7$ requires M + 1, 406.2553], $[\alpha]_D$ +151.0° (in H_2O), $[\theta]_{290}$ −11 490 (TACu), $\nu_{max.}$ (Nujol) 3 333, 1 099, and 1 053 cm^{-1}, δ(D_2O) 1.28 (3 H, s, 4''-CH_3), 2.62 (3 H, s, 3''-NCH_3), and 5.16 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1'').

The β-glycoside (23) (67 mg) was deprotected similarly to give the β-*isomer* (29) (28 mg, 92%) [Found: $(M + 1)^+$, 406.2535], $[\theta]_{290}$ −9 280 (TACu), $\nu_{max.}$ (Nujol) 3 333, 1 099, and 1 053 cm^{-1}, δ(D_2O) 1.30 (3 H, s, 4''-CH_3), 2.64 (3 H, s, 3''-NCH_3), and 5.17 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1'').

3,4-*Di*-O-*acetyl*-2-*deoxy*-2-*nitroso*-β-D-*arabinopyranosyl Chloride* (33).—3,4-Di-*O*-acetyl-D-arabinal (32) (4.07 g) dissolved in dry ethyl acetate (100 ml) was cooled to 0 °C. A 1.69M-solution of nitrosyl chloride in ethyl acetate (12 ml) was added and the mixture was stirred at 0 °C for 3.5 h. The solution was evaporated to dryness and the residue was taken up in dry ether (30 ml) to give the adduct (33) (5.24 g, 97%) as crystals, m.p. 126—128° (lit.,[33] 116—121°), $[\alpha]_D$ −211.6° (in $CHCl_3$) {lit.,[33] −203.2° (in $CHCl_3$)}, δ($CDCl_3$) 2.07 (3 H, s, OAc), 2.22 (3 H, s, OAc), 3.80—4.48 (2 H, m, $J_{4,5e}$, 1, $J_{4,5a}$ 2.5, $J_{5a,5e}$ 13 Hz, H-5a and -5e), 5.52 (1 H, m, H-4), 5.63 (1 H, dd, $J_{1,2}$ 2, $J_{2,3}$ 11 Hz, H-2), 5.95 (1 H, dd, $J_{2,3}$ 11, $J_{3,4}$ 2 Hz, H-3), and 6.74 (1 H, d, $J_{1,2}$ 2 Hz, H-1).

Isopropyl 3,4-*Di*-O-*acetyl*-2-*hydroxyimino*-α- *and* -β-D-erythro-*pentopyranoside* [(34) *and* (35)].—The adduct (33) (3.98) and dry propan-2-ol (3.5 ml) were dissolved in dry dimethylformamide (60 ml) and kept under argon at 25 °C for 90 h. The solution was concentrated *in vacuo* to a syrup which was dissolved in chloroform (100 ml); the solution was washed with water (3 × 50 ml), dried (Na_2SO_4), and evaporated to dryness. The resulting syrup was chromatographed on a silica gel column (600 g) (10% acetone–hexane as eluant) to give the α-*oxime* (34) (1.17 g, 27%) as a syrup (Found: C, 49.8; H, 6.6; N, 4.9. $C_{12}H_{19}NO_7$ requires C, 49.8; H, 6.6; N, 4.8%), $[\alpha]_D$ +182.8° (in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 560, 1 740, 1 240, and 1 030 cm^{-1}, δ($CDCl_3$) 1.21 and 1.26 (each 3 H, d, J 6 Hz, Me_2CH), 2.05 (3 H, s, OAc), 2.11 (3 H, s, OAc), 3.60 (1 H, m, $J_{4,5e}$ 5, $J_{5a,5e}$ 10.5 Hz, H-5e), 3.99 (1 H, m, Me_2CH), 4.18 (1 H, m, $J_{4,5a}$ 10.5 Hz, H-5a), 5.03 (1 H, m, H-4), 5.78 (1 H, dd, $J_{3,4}$ 3.5, $J_{3,5e}$ 1.5 Hz, H-3), and 5.95 (1 H, s, H-1); and the β-*oxime* (35) (2.27 g, 52%) as a syrup (Found: C, 49.4; H, 7.0; N, 4.6%), $[\alpha]_D$ −135.0° (in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 570, 1 740, 1 230, and 1 030 cm^{-1}, δ($CDCl_3$) 1.17 and 1.22 (each 3 H, d, J 6 Hz, Me_2CH), 2.05 (3 H, s, OAc), 2.11 (3 H, s, OAc), 3.74 (1 H, dd, $J_{4,5a}$ 2.5, $J_{5e,5a}$ 13 Hz, H-5a), 3.97 (1 H, m, Me_2CH), 4.20 (1 H, dd, $J_{4,5e}$ 1, $J_{5a,5e}$ 13 Hz, H-5e), 5.35 (1 H, m, H-4), 5.85 (1 H, d, $J_{3,4}$ 3.5 Hz, H-3), and 6.13 (1 H, s, H-1).

Isopropyl 2-*Acetamido*-3,4-*di*-O-*acetyl*-2-*deoxy*-α-D-*ribopyranoside* (36).—The α-oxime (34) (1 g) and acetic anhydride (7 ml) were dissolved in dry pyridine (15 ml) and kept at

† Same foonote as on page 1142.

25° C for 18 h. The solution was concentrated *in vacuo* and the syrup was dissolved in methylene chloride (50 ml); the solution was washed with water, dried (Na_2SO_4), and evaporated to dryness. The last traces of pyridine were removed by co-distillation with dry ethanol to give isopropyl 2,3,4-tri-*O*-acetyl-2-hydroxyimino-α-D-*erythro*-pentopyranoside as a syrup. The acetate (1 g) was dissolved in dry tetrahydrofuran (50 ml) and cooled to 0 °C. A 1M-solution of borane in tetrahydrofuran (12 ml) was added dropwise and the solution was kept at 5 °C for 28 h. The excess of reagent was destroyed by dropwise addition of water and the solution was evaporated to dryness. The residue was taken up in methanol (50 ml) and neutralized with Amberlite IRA 401S (OH^-) resin. The resin was eluted with methanol and the eluate was evaporated to dryness. The residue was dissolved in dry pyridine (10 ml), acetic anhydride (5 ml) was added, and the mixture was kept at 25 °C for 18 h. The solution was concentrated *in vacuo* to a syrup, which was dissolved in methylene chloride (30 ml); this solution was washed with aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated to dryness. The syrup was azeotroped with ethanol and then chromatographed on a silica gel column (70 g) (20% acetone–hexane as eluant) to give *isopropyl* 2-*acetamido*-3,4-*di*-O-*acetyl*-2-*deoxy*-α-D-*ribopyranoside* (36) (336 mg, 29%) as a syrup [Found: $(M + 1)^+$, 318.1540. $C_{14}H_{24}NO_7$ requires $M + 1$, 318.1553], $[\alpha]_D$ +56.5° (in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 440, 1 740, 1 670, 1 500, 1 240, and 1 030 cm^{-1}, δ($CDCl_3$) 1.15 and 1.26 (each 3 H, d, $CHMe_2$), 2.00 (3 H, s, OAc), 2.04 (3 H, s, OAc), 2.12 (3 H, s, NHAc), 3.53 (1 H, dd, $J_{4,5e}$ 4, $J_{5a,5e}$ 11 Hz, H-5e), 3.92 (1 H, m, $CHMe_2$), 3.99 (1 H, dd, $J_{4,5a}$ 9, $J_{5a,5e}$ 11 Hz, H-5a), 4.41 (1 H, m, $J_{1,2}$ 3.5, $J_{2,3}$ 4, $J_{2,NH}$ 9 Hz, H-2), 4.74 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1), 5.03 (1 H, m, $J_{3,4}$ 4, $J_{4,5a}$ 9, $J_{4,5e}$ 4 Hz, H-4), 5.37 (1 H, dd, $J_{2,3} = J_{3,4} = 4$ Hz, H-3), and 5.84 (1 H, d, $J_{2,NH}$ 9 Hz, NH).

Isopropyl 2-*Acetamido*-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*ribo-* *and* -β-D-*arabino*-*pyranoside* [(37) *and* (38)].—The β-oxime (35) (2 g) and acetic anhydride (10 ml) were dissolved in dry pyridine (20 ml). The solution was kept at 25 °C for 18 h, then poured into ice–water, and the solid was filtered off, washed with water, and dried to give isopropyl 2,3,4-tri-*O*-acetyl-2-hydroxyimino-β-D-*erythro*-pentopyranoside (1.6 g, 70%). The acetate (1 g) was reduced with borane in tetrahydrofuran and acetylated as for the α-anomer. The resulting syrup was chromatographed on a silica gel column (70 g) (20% acetone–hexane as eluant) to give a syrup (363 mg, 38%), homogeneous on t.l.c. The ^1H n.m.r. spectrum revealed it to be a 1 : 2 mixture of *isopropyl* 2-*acetamido*-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*ribo-* and -β-D-*arabino*-*pyranoside* [(37) and (38)] [Found: $(M + 1)^+$, 318.1558. $C_{14}H_{24}NO_7$ requires M + 1, 318.1553]. The *ribo*-isomer (37) showed δ($CDCl_3$) 1.19 and 1.25 (each 3 H, d, J 6.5 Hz, $CHMe_2$), 2.04 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.20 (3 H, s, NHAc), 4.41 (1 H, dd, $J_{1,2}$ 1.5, $J_{2,3}$ 4 Hz, H-2), 4.88 (1 H, d, $J_{1,2}$ 1.5 Hz. H-1), and 6.44 (1 H, d, $J_{2,NH}$ 9 Hz, NH). The *arabino*-isomer (38) showed δ($CDCl_3$) 1.15 and 1.25 (each 3 H, d, J 6.5 Hz, $CHMe_2$), 1.99 (3 H, s, OAc), 2.04 (3 H, s, OAc), 2.18 (3 H, s, NHAc), 4.62 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10, H-2), 4.97 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1), and 5.85 (1 H, d, $J_{2,NH}$ 9 Hz, NH).

Reaction of 3,4-*Di*-O-*acetyl*-2-*deoxy*-2-*nitroso*-β-D-*arabino*-*pyranosyl Chloride* (33) *with* 2′,4′,5-*Tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (8).—Compounds (8) (8.49 g) and (33) (5.31 g) were dissolved in dry, redistilled dimethylformamide (125 ml) and kept under argon at 25 °C for 138 h. The product was worked up as before and chromatographed on a silica gel column (1 kg) (40% acetone–hexane as eluant) to give O-3,4-*di*-O-*acetyl*-2-*hydroxyimino*-D-erythro-*pentopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (39) (6.21 g, 58%) (Found: C, 58.2; H, 5.6; N, 5.15. $C_{52}H_{62}N_4O_{21}$ requires C, 57.9; H, 5.8; N, 5.2%), δ($CDCl_3$)† 1.25br and 1.30br (3 H, s, 4″-CH_3), 1.70—2.30br (15 H, s, OAc), 2.86br and 2.88br (3 H, s, 3″-NCH_3), 6.00br and 6.46br (1 H, s, H-1′), and 7.30br (15 H, s, Ph).

The oximes (39) (3 g) were acetylated to give the acetates (40) (2.94 g, 94%). The acetates (40) (2.8 g) were reduced with M-borane in tetrahydrofuran (30 ml) as before (28 h). The residue was dissolved in a mixture of methanol (80 ml) and concentrated ammonium hydroxide (80 ml), and heated in a bomb at 100 °C for 20 h. The solution was cooled and evaporated to dryness, and the residue was taken up in aqueous 5% sodium hydroxide (100 ml) and heated at 90 °C for 20 h. The cooled solution was neutralized with Amberlite IRC 50 (H^+) resin and, after washing with water, the resin was eluted with 1.5N-ammonium hydroxide. The eluate was concentrated to dryness. The residue was chromatographed on a silica gel column (150 g) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give a product (181 mg, 16%) which, although homogeneous on t.l.c. was found by n.m.r. to be a 2 : 1 mixture of O-2-*amino*-2-*deoxy*-β-D-*ribo-* *and* -β-D-*arabino*-*pyranosyl*-(1 ⟶ 4)-*garamine* [(30) and (31)]. The *ribo*-isomer (30) showed δ(D_2O) 1.22 (3 H, s, 4″-CH_3), 2.54 (3 H, s, 3″-NCH_3), 4.73 (1 H, d, $J_{1,2'}$ 1.5 Hz, H-1′), and 5.06 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″). The *arabino*-isomer (31) showed δ(D_2O) 1.22 (3 H, s, 4″-CH_3), 2.54 (3 H, s, 3″-NCH_3), 3.13 (1 H, dd, $J_{1',2'}$ 3.5, $J_{2',3'}$ 6 Hz, H-2′), 4.93 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′), and 5.06 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″). Subsequent fractions from the column afforded O-2-*amino*-2-*deoxy*-α-D-*ribopyranosyl*-(1 ⟶ 4)-*garamine* (26) (95 mg, 8%) [Found: $(M - 75)^+$, 377.2036. $C_{18}H_{36}N_4O_9 - C_3H_7O_2$ requires 377.2035], $[\alpha]_D$ +139.1° (in H_2O), $[\theta]_{290}$ — 8 820 (TACu), δ(D_2O) 1.21 (3 H, s, 4″-CH_3), 2.51 (3 H, s, 3″-NCH_3) 2.92 (1 H, dd, $J_{1',2'} = J_{2',3'} = 3.5$ Hz, H-2′), 4.85 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′), and 4.97 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″). The least polar fractions afforded a t.l.c.-homogeneous mixture of 2′-*N*-ethylpseudotrisaccharides (119 mg, 10%), δ(D_2O) 1.10 (3 H, t, J 7.5 Hz, $NHCH_2CH_3$), 1.22 (3 H, s, 4″-CH_3), 2.53 (3 H, s, 3″-NCH_3), 4.75 (<1 H, m, H-1′), 4.97 (<1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′), and 5.09 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″).

Reaction of 3,4-*Di*-O-*acetyl*-2-*deoxy*-2-*nitroso*-α-D-*xylopyranosyl Chloride* (41) *with* 2′,4′,5-*Tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (8).—Compounds (8) (4.25 g) and (41) [38] (2.65 g) were dissolved in dry, redistilled dimethylformamide (60 ml) and kept at 25 °C for 90 h. The product was worked up as before and chromatographed on a silica gel column (600 g) (30% acetone–hexane as eluant) to give O-3,4-*di*-O-*acetyl*-2-*hydroxyimino*-α-D-threo-*pentopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (42) (2.73 g, 51%) (Found: C, 56.60; H, 6.2; N, 4.9. $C_{52}H_{62}N_4O_{21},H_2O$ requires C, 56.9; H, 5.9; N, 5.1%), δ($CDCl_3$) † 1.20br (3 H, s, 4″-CH_3), 1.7—2.2br (15 H, s, OAC), 2.86 br (3 H, s, 3″-NCH_3), 6.04br (1 H, s, H-1), and 7.30br (15 H, s, Ph).

The oxime (42) (2.53 g) was acetylated to give the acetate (43) (2.53 g, 96%). The acetate (43) (1.5 g) was reduced with borane in tetrahydrofuran (17 ml) as described

† Same footnote as on page 1142.

before (28 h). The residue was dissolved in a mixture of methanol (40 ml) and concentrated ammonium hydroxide (40 ml) and heated in a bomb at 100 °C for 50 h. The solution was cooled and evaporated to dryness and the residue was taken up in aqueous 5% sodium hydroxide (40 ml) and heated at 100 °C for 4.5 h. The cooled solution was neutralized with Amberlite IRC 50 (H^+) resin and, after washing with water, the resin was eluted with 1.5N-ammonium hydroxide. The eluate was concentrated to dryness and the residue was chromatographed on a silica gel column (70 g) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give a product (52 mg, 9%). Although homogeneous on t.l.c. the product contained only *ca.* 60% of *O*-2-amino-2-deoxy-α-D-xylopyranosyl-(1 ⟶ 4)-garamine (27), δ(D_2O) 1.24 (3 H, s, 4″-CH_3), 2.64 (3 H, s, 3″-NCH_3), 5.02 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.11 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′). The stereochemistry of the contaminating by-products could not be unambiguously assigned. The 2′-*N*-ethyl derivative was isolated as a by-product, but was not fully characterized.

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