Semisynthetic Aminoglycoside Antibacterials. Part III.[1] Synthesis of Analogues of Gentamicin X_2 modified at the 3′-Position

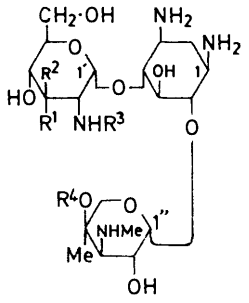
By **Max Kugelman, Alan K. Mallams,* and H. Frederick Vernay,** Research Division, Schering Corporation, Bloomfield, New Jersey 07003, U.S.A.

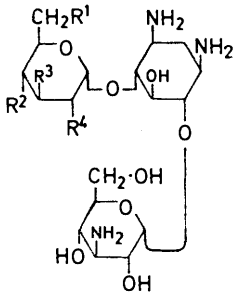
In order to prevent inactivation of gentamicin X_2 by phosphorylation, a series of 3′-modified derivatives was prepared. These included 3′-deoxygentamicin X_2, *O*-2-amino-2-deoxy-α-D-allopyranosyl-(1 ⟶ 4)-garamine, and 3′-*O*-methylgentamicin X_2, all of which were synthesized by application of the Lemieux-Nagabhushan reaction, to a suitably protected garamine intermediate. The formation of *O*-2-amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl-(1 ⟶ 4)-garamine during the synthesis of 3′-*O*-methylgentamicin X_2 by the Lemieux-Nagabhushan reaction is reported. The solution conformations of the novel 4-*O*-β-D-mannopyranosyl and 4-*O*-β-D-glucopyranosyl derivatives are discussed. The formation of nitroglucals during the preparation of the nitroso-chloro-adducts is described.

THE synthesis of a variety of novel analogues of gentamicin X_2 (1) by glycosylation of appropriately protected garamine derivatives [2] has been described.[1] In that study, modifications were effected in the 4-*O*-glycosyl unit, primarily at sites remote from the site of inactivation of the antibiotic. In the present work, modifications were effected at the 3′-position, which is one of the sites of inactivation in gentamicin X_2 (1). Resistant strains of bacteria that carry R-factors are known to phosphorylate the 3′-hydroxy-group of these antibiotics. Similar inactivation of aminoglycoside antibiotics such as kanamycins A and B, neomycin B, and paromomycin by resistant strains of bacteria carrying R-factors, has been demonstrated to involve phosphorylation of the 3′-hydroxy-groups in these substrates.[3-9] At the outset of this work it therefore seemed appropriate to modify the 3′-position in gentamicin X_2 (1) to determine what effects such modifications would have on the spectrum of biological activity of these antibiotics. During our work 3′-deoxykanamycin (8),[10,11] 3′,4′-dideoxykanamycin B (9),[12] 3′-*O*-methylkanamycin (10),[10,13] 3′-amino-3′-deoxykanamycin (11),[14]

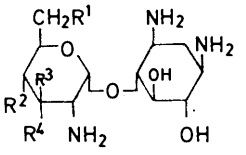
[1] Part II, M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, preceding paper.
[2] M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, and M. Tanabe, *J.C.S. Perkin I*, 1976, 1088.
[3] H. Umezawa, M. Okanishi, S. Kondo, K. Hamana, R. Utahara, K. Maeda, and S. Mitsuhashi, *Science*, 1967, **157**, 1559.
[4] S. Kondo, M. Okanishi, R. Utahara, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1968, **21**, 22.
[5] M. Okanishi, S. Kondo, R. Utahara, and H. Umezawa, *J. Antibiotics*, 1968, **21**, 13.
[6] M. Brzezinska and J. Davies, *Antimicrobial Agents Chemother.*, 1973, **3**, 266.
[7] J. E. Davies and R. Rownd, *Science*, 1972, **176**, 758.
[8] H. Naganawa, S. Kondo, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 823.
[9] M. Yagisawa, H. Yamamoto, H. Naganawa, S. Kondo, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 748.
[10] S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 274.
[11] S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2847.
[12] S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1972, **45**, 3624.
[13] H. Umezawa, T. Tsuchiya, R. Muto, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2842.
[14] S. Inouye, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 573.

4′-deoxykanamycin (12),[15,16] 3′,4′-dideoxyneamine (13),[17,18] 3′-*O*-methylneamine (14),[17,19] 4′-*O*-methylneamine (15),[17,19] and 3′-*epi*-paromamine (16) [20] have





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

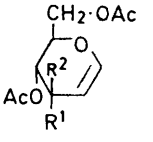


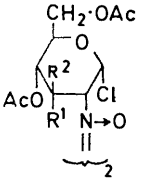
(13) $R^1=NH_2$, $R^2=R^3=R^4=H$
(14) $R^1=NH_2$, $R^2=OH$, $R^3=OMe$, $R^4=H$
(15) $R^1=NH_2$, $R^2=OMe$, $R^3=OH$, $R^4=H$
(16) $R^1=R^2=R^4=OH$, $R^3=H$

been synthesized by other groups with similar objectives in mind.

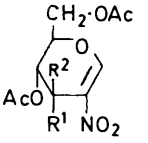
The application of the Lemieux–Nagabhushan reaction to the preparation of 3-deoxy-sugars has not been reported previously. However, in view of our success in utilizing this reaction to prepare gentamicin X_2 (1) and a variety of analogues,[1] we were encouraged to apply the reaction to the synthesis of 3′-deoxygentamicin X_2 (2). 4,6-Di-*O*-acetyl-1,2,3-trideoxy-D-*erythro*-hex-1-enopyranose (17) was prepared in high yield by reduction with lithium aluminium hydride of methyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside [21] followed by acetylation of the 3-deoxy-D-glucal thus obtained. When the glucal (17) was treated with an excess of nitrosyl chloride [22,23] in ethyl acetate at −5 °C for *ca.* 3 h a quantitative yield of dimeric 4,6-di-*O*-acetyl-2,3-dideoxy-2-nitroso-α-D-*ribo*-hexopyranosyl chloride (21) was obtained, as a colourless glass which could not be induced to crystallize. The ^1H n.m.r. spectrum of (21) revealed two doublets in the ratio 7 : 3 at δ 6.70 and 6.82, with $J_{1,2}$ 4 Hz in each instance, the peaks being ascribed to the anomeric H-1′. The i.r. spectrum showed no vinyl ether absorption, indicating complete conversion of the glucal (17) into the nitroso-chloro-adduct (21). There was no evidence for the formation of any nitroglucal (24) under the conditions used. The poor resolution observed in the ^1H n.m.r. spectrum of (21), reminiscent of a rotamer spectrum, was characteristic of all the 3′-deoxy-nitroso-chloro-adducts prepared, and must be related to the physical state in which these adducts exist. When the glucal (17) was treated with an excess of nitrosyl chloride in ethyl acetate for longer than 3 h, a clean conversion into 4,6-di-*O*-acetyl-1,2,3-trideoxy-2-nitro-D-*erythro*-hex-1-enopyranose (24) occurred. The vinyl ether and nitro-groups gave rise to bands at 1 600 and 1 510 cm^{-1} in the i.r. spectrum. The ^1H n.m.r. spectrum revealed a characteristic singlet at δ 8.12 due to H-1. The conversion of 3,4-di-*O*-acetyl-2-deoxy-2-nitroso-α-D-xylopyranosyl chloride (27) into 3,4-di-*O*-acetyl-1,2-dideoxy-2-nitro-D-*erythro*-hex-1-enopyranose (28) during purification has been reported.[24]

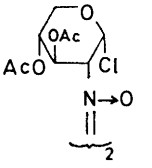
The nitroso-chloro-adduct (21) on treatment with propan-2-ol in dimethylformamide afforded isopropyl 4,6-di-*O*-acetyl-3-deoxy-2-hydroxyimino-α-D-*erythro*-hexopyranoside (29). The ^1H n.m.r. spectrum of (29)





(17) $R^1=R^2=H$
(18) $R^1=H$, $R^2=OAc$
(19) $R^1=OAc$, $R^2=H$
(20) $R^1=H$, $R^2=OMe$
(21) $R^1=R^2=H$
(22) $R^1=H$, $R^2=OAc$
(23) $R^1=H$, $R^2=OMe$





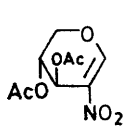
(24) $R^1=R^2=H$
(25) $R^1=H$, $R^2=OAc$
(26) $R^1=OAc$, $R^2=H$

(27)

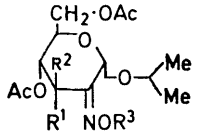
revealed two anomeric proton singlets, at δ 5.14 due to the *E*-isomer and at δ 5.99 due to the *Z*-isomer, in the ratio 5 : 1. The absence of the 3-equatorial substituent in (29) enables the oxime to exist predominantly in the

[15] S. Umezawa, Y. Nishimura, Y. Hata, T. Tsuchiya, M. Yagisawa, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 722.
[16] T. Naito, S. Nakagawa, Y. Abe, K. Fujisawa, and H. Kawaguchi, *J. Antibiotics*, 1974, **27**, 838.
[17] T. Tikihara, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 3507.
[18] S. Umezawa, T. Tsuchiya, T. Jikihara, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 711.
[19] S. Umezawa, T. Jikihara, T. Tsuchiya, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 322.
[20] S. Hanessian, R. F. Butterworth, and T. Nakagawa, *Carbohydrate Res.*, 1973, **26**, 261.
[21] B. Fraser-Reid and B. Radatus, *J. Amer. Chem. Soc.*, 1970, **92**, 6661.
[22] R. U. Lemieux, T. L. Nagabhushan, and I. K. O'Neill, *Tetrahedron Letters*, 1964, 1909.
[23] R. U. Lemieux and T. L. Nagabhushan, *Methods Carbohydrate Chem.*, 1972, **6**, 487.
[24] R. U. Lemieux, T. L. Nagabhushan, and I. K. O'Neill, *Canad. J. Chem.*, 1968, **46**, 413.

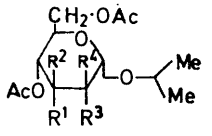
E-configuration, whereas previous work [25] has demonstrated that with a 3-equatorial acetyl group the oxime exists entirely in the *Z*-configuration. Acetylation of the oxime (29) gave the acetate (30), which on reduction



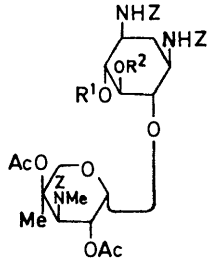
(28)



(29) $R^1=R^2=R^3=H$
(30) $R^1=R^2=H$, $R^3=Ac$
(31) $R^1=OAc$, $R^2=R^3=H$
(32) $R^1=OAc$, $R^2=H$, $R^3=Ac$
(33) $R^1=R^3=H$, $R^2=OMe$
(34) $R^1=H$, $R^2=OMe$, $R^3=Ac$



(35) $R^1=R^2=R^4=H$, $R^3=NHAc$
(36) $R^1=R^2=R^3=H$, $R^4=NHAc$
(37) $R^1=OAc$, $R^2=R^4=H$, $R^3=NHAc$
(38) $R^1=OAc$, $R^2=R^3=H$, $R^4=NHAc$
(39) $R^1=R^4=H$, $R^2=OMe$, $R^3=NHAc$



Z = $PhCH_2\cdot O\cdot CO$

(40) $R^1=H$, $R^2=Ac$
(41) $R^1=Ac$, $R^2=H$

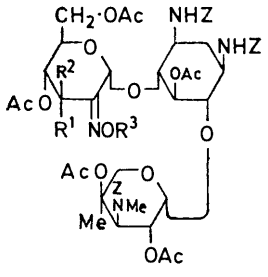
with borane in tetrahydrofuran followed by *N*-acetylation gave a 30% yield of isopropyl 2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*ribo*-hexopyranoside (35), together with a 7% yield of isopropyl 2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*arabino*-hexopyranoside (36). The ^1H n.m.r. spectrum of (35) contained a doublet due to the anomeric proton at δ 4.83 ($J_{1,2}$ 3.5 Hz) consistent with an α-D-*ribo*-configuration; the spectrum of (36) showed a doublet at δ 4.74 ($J_{1,2}$ 1.5 Hz) indicating an α-D-*arabino*-configuration.

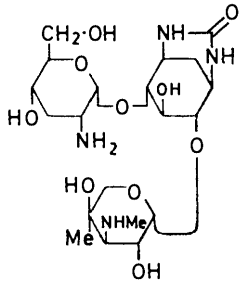
The synthesis of 3′-deoxygentamicin X_2 (2) was then undertaken as follows. The nitroso-chloro-adduct (21) was condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (40) [2] in dimethylformamide to give a 51% yield of *O*-4,6-di-*O*-acetyl-3-deoxy-2-hydroxyimino-α-D-*erythro*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (42).* The absence of an anomeric proton signal due to H-1′ at δ *ca.* 5.9—6.3 in the ^1H n.m.r. spectrum of (42) indicated that the oxime existed predominantly in the *E*-configuration, as in the case of the monosaccharide model (29). Acetylation of the oxime (42) afforded the acetate (43), which on reduction with 18 equiv. of borane in tetrahydrofuran followed sodium in liquid ammonia, and basic hydrolysis, gave 3′-deoxygentamicin X_2 (2) in 16% yield based on (43).

* Whenever the triacetate (40) was used in a Lemieux–Nagabhushan reaction some of it was recovered as well as the transacylation product (41).[1,2] For simplicity, details of the isolation of these products are not included in the Experimental section.

The ^1H n.m.r. spectrum of (2) exhibited a doublet at δ 5.10 ($J_{1',2'}$ 3 Hz) due to the H-1′ in agreement with the assigned α-D-*ribo*-configuration. The mass spectrum of (2) (Table 1) showed the expected fragment ions and contained the protonated formyl sequence of ions A_1—A_4 [26] indicating that the newly formed glycoside unit was linked to the deoxystreptamine ring. The c.d. spectrum of (2) in TACu and in Cupra A solution confirmed the 4- and 6-glycosidic linkages of the sugars about the deoxystreptamine ring. Over-reduction of the tertiary 4″-acetyl group occurred, as observed previously,[1] during the treatment with borane to give a 44% yield of 3′-deoxy-4″-*O*-ethylgentamicin X_2 (3) as the principal product. The ^1H n.m.r. spectrum of (3) revealed a triplet at δ 1.14 and a quartet at δ 3.46 (*J* 7 Hz) due to an *O*-ethyl group. The anomeric H-1′ signal occurred as a doublet at δ 5.09 ($J_{1',2'}$ 3.5 Hz), in agreement with an α-D-*ribo*-configuration for the 4-*O*-glycoside unit. The mass spectral fragmentation pattern (Table 1) agreed with the proposed structure. In contrast to 3′-deoxygentamicin X_2 (2), 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 4″-*O*-ethyl derivative (3) did not exhibit such fragment ions. Instead a prominent $M-74$ ion was observed at *m/e* 420 due to the fragment ion E_5,[1] which in turn underwent glycosyl cleavage accompanied by hydrogen transfer to give the ion E_6 at *m/e* 275. The c.d. data for (3) also agreed with the proposed structure.

Recently it has been reported [27] that when the Lemieux–Nagabhushan reaction was carried out in the





(48)

(42) $R^1=R^2=R^3=H$
(43) $R^1=R^2=H$, $R^3=Ac$
(44) $R^1=OAc$, $R^2=R^3=H$
(45) $R^1=OAc$, $R^2=H$, $R^3=Ac$
(46) $R^1=R^3=H$, $R^2=OMe$
(47) $R^1=H$, $R^2=OMe$, $R^3=Ac$

presence of a mild base such as *NN*,2,4,6-pentamethylaniline or *NN*,2,6-tetramethylaniline to neutralize the hydrogen chloride formed during the reaction, enhanced yields of the glycoside were obtained. Indeed when the

[25] R. U. Lemieux, R. A. Earl, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 19.
[26] P. J. L. Daniels, A. K. Mallams, J. Weinstein, J. J. Wright, and G. W. A. Milne, *J.C.S. Perkin I*, 1976, 1078.
[27] K. Miyai and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **21**, 45.

nitroso-chloro-adduct (21) was condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarboxylgaramine (40) in dimethylformamide in the presence of *NN*,2,6-tetramethylaniline, a greater yield (70%) of the oxime (42) was obtained. Acetylation of the oxime (42) gave the acetate (43), which on reduction with 10 equiv. of borane at 7 °C followed by basic hydrolysis with refluxing aqueous 5% sodium hydroxide gave some 3′-deoxygentamicin X_2 (2) together with 1,3-*N*-carbonyl-3′-deoxygentamicin X_2 (48) as a by-product. The cyclic urea (48) showed carbonyl i.r. absorption at 1 660 cm^{-1}. The ^1H n.m.r. spectrum showed deshielding of the axial 2-proton of the deoxystreptamine ring [28] which is characteristic of these cyclic ureas. The c.d. data and the mass spectral fragment ions (Table 1) further supported the proposed structure. The cyclic urea (48) was hydrolysed to give 3′-deoxygentamicin X_2 (2) by heating with 90% hydrazine hydrate at 130 °C for 89 h. The total yield of 3′-deoxygentamicin X_2 (2) obtained in the above reaction was 20% based on (43).

When the oxime acetate (43) was reduced with 10 equiv. of borane in tetrahydrofuran at 7 °C for 30 h and then deprotected by refluxing with aqueous 5% sodium hydroxide followed by treatment with 90% hydrazine hydrate at 130 °C for 130 h, a 22% yield of 3′-deoxygentamicin X_2 (2) was obtained. The above reaction also produced 3′-deoxy-2′-*N*-ethylgentamicin X_2 (4) as a by-product [1] in 7% yield. The ^1H n.m.r. spectrum of (4) contained a triplet at δ 1.07 and a quartet at δ 2.71 (*J* 7 Hz) due to the 2′-*N*-ethyl group. The anomeric H-1′ signal occurred as a doublet at δ 5.21 ($J_{1',2'}$ 3.5 Hz), consistent with an α-D-*ribo*-configuration. The c.d. data and the mass spectral fragment ions (Table 1) agreed with the proposed structure.

Having synthesized 3′-deoxygentamicin X_2, we then decided to prepare *O*-2-amino-2-deoxy-α-D-allopyranosyl-(1 ⟶ 4)-garamine (5), in which the stereochemistry of the 3′-hydroxy-group is inverted relative to gentamicin X_2 (1). In order to use the Lemieux–Nagabhushan reaction it was necessary first to prepare 3,4,6-tri-*O*-acetyl-D-allal (19). This was done by treating 1,2,3,4,6-penta-*O*-acetyl-β-D-allopyranose (49) [29] with titanium tetrabromide, which afforded a 2 : 1 mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranosyl bromide (50) and 2,3,4,6-tetra-*O*-acetyl-D-allopyranose (51). The latter was treated directly with zinc in acetic acid, affording 3,4,6-tri-*O*-acetyl-D-allal (19) in a 50% yield together with 2,3,4,6-tetra-*O*-acetyl-D-allopyranose (51) in 15% yield. The latter (51) on acetylation gave 1,2,3,4,6-penta-*O*-acetyl-β-D-allopyranose (49). Similar results were obtained when the penta-acetate (49) was treated with hydrogen bromide in acetic acid. When aluminium tribromide in bromoform [30] was used, tarry products were formed and the reaction was not further investigated. The reaction of the glycal (19) with 1 equiv. of nitrosyl chloride in ethyl acetate was slow; after several hours a considerable amount of unchanged

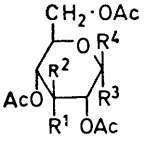
TABLE 1

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

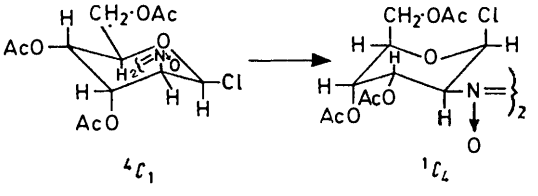
Compd.	$(M+1)^+$	M+·	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}	A_{11}	A_{12}
(2)	467 (1)	466 (1)	336 (15)	318 (2)	308 (7)	290 (7)	350 (17)	332 (5)	322 (26)	304 (24)	191 (80)	173 (16)	163 (49)	145 (80)
(3)	495 (2)	494 (3)	336 (30)	318 (6)	308 (16)	290 (15)	378 (22)	360 (4)	350 (25)	332 (23)	191 (70)	173 (47)	163 (37)	145 (65)
(4)	495 (2)	494 (4)	364 (26)	346 (2)	336 (7)	318 (4)	350 (26)	332 (4)	322 (8)	304 (36)	191 (24)	173 (31)	163 (15)	145 (25)
(5)	483 (0.3)		352 (4)	334 (1)	324 (2)	306 (3)	350 (6)	332 (2)	322 (4)	304 (6)	191 (100)	173 (22)	163 (80)	145 (80)
(6)	511 (3)	510 (3)	380 (38)	362 (2)	352 (11)	334 (16)	350 (36)	332 (8)	322 (12)	304 (64)	191 (70)	173 (25)	163 (19)	145 (70)
(7)	497 (2)	496 (0.4)	366 (36)	348 (4)	338 (18)	320 (15)	350 (41)	332 (22)	322 (27)	304 (52)	191 (100)	173 (70)	163 (70)	145 (65)
(58)	497 (1)	496 (0.1)	366 (21)	348 (2)	338 (10)	320 (10)	350 (17)	332 (12)	322 (22)	304 (18)	191 (63)	173 (22)	163 (40)	145 (70)

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
(2)	146 (80)	160 (100)	362 (2)	203 (4)	391 (3)	246 (12)	349 (5)	204 (20)			275 (12)	289 (8)
(3)	146 (100)	188 (85)	390 (5)	203 (8)			349 (8)	204 (18)	420 (16)	275 (25)	275 (25)	317 (14)
(4)	174 (100)	160 (37)	390 (4)		419 (5)	246 (4)	377 (4)	204 (3)			303 (32)	289 (21)
(5)	162 (39)	160 (70)	362 (70)	203 (4)	407 (0.5)	246 (30)	365 (1)	204 (45)			291 (4)	289 (2)
(6)	190 (100)	160 (80)	390 (13)	231 (5)	435 (6)	246 (11)	393 (7)				319 (46)	289 (19)
(7)	176 (75)	160 (80)	362 (4)	203 (14)	421 (7)	246 (24)	379 (9)	204 (25)			305 (51)	289 (15)
(58)	176 (90)	160 (100)	362 (1)	203 (2)	421 (1)	246 (9)	379 (2)	204 (7)			305 (16)	289 (7)

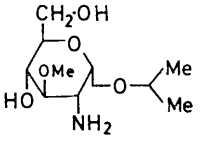
* The structures and designations of all fragment ions are identical with those described in ref. 26. The ions E_5 and E_6 are the same as those described in ref. 1.



(49) $R^1 = R^4 = OAc, R^2 = R^3 = H$
(50) $R^1 = OAc, R^2 = R^4 = H, R^3 = Br$
(51) $R^1 = OAc, R^2 = H, R^3, R^4 = H, OH$
(52) $R^1 = R^3 = H, R^2 = OMe, R^4 = OAc$
(53) $R^1 = R^4 = H, R^2 = OMe, R^3 = Br$



(54)



(55)

[28] J. K. Jenkins and H. Reimann, unpublished observations.
[29] J. D. Stevens, *Methods Carbohydrate Chem.*, 1972, **6**, 123.
[30] R. E. Harmon, T. Lin, and S. K. Gupta, 166th American Chemical Society Meeting, Chicago, Illinois, August 27—30, 1973, Carbohydrate Division, paper 23.

glycal (19) remained. In order to convert all the glycal (19) it was necessary to use 28 equiv. of nitrosyl chloride in ethyl acetate, the reaction being carried out under nitrogen at 0 °C for 1.9 h. Under these conditions most of the glycal (19) reacted to give a 47% yield of crystalline 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-altropyranosyl chloride (54) and a 10% yield of crystalline 3,4,6-tri-*O*-acetyl-1,2-dideoxy-2-nitro-D-*ribo*-hex-1-enopyranose (26) as well as some unchanged glycal (19). The ^{1}H n.m.r. spectrum of the nitroso-chloro-adduct (54) showed the anomeric proton signal as a doublet at δ 6.54 ($J_{1,2}$ 3.5 Hz). The 2-proton signal occurred as a doublet of doublets at δ 5.77 ($J_{2,3}$ 9.5 Hz), as did those of the 3- and 4-protons at δ 6.00 and 5.60, respectively, the latter having $J_{3,4} = J_{4,5} = 3$ Hz. The above data indicated that addition of the nitrosyl chloride to the glycal (19) had occurred from the 'top' face of the molecule, with a subsequent conformational change from the $^4C_1$ to the $^1C_4$ conformation. The i.r. spectrum of the nitroallal (26) exhibited a band at 1 650 cm^{-1} due to the vinylic ether double bond, and the nitro-group stretching band appeared at 1 510 cm^{-1}. The ^{1}H n.m.r. spectrum of (26) revealed a singlet at δ 8.31 due to the anomeric proton, and the H-3 signal occurred as a doublet at δ 6.43 ($J_{3,4}$ 4 Hz). The nitroglycal (26) is probably formed as described later; it is difficult to prevent its formation in the above reaction owing to the need for an excess of nitrosyl chloride to cause formation of the initial nitroso-chloro-adduct (54). Tri-*O*-acetyl-D-allal (19) appears to be less reactive towards nitrosyl chloride than tri-*O*-acetyl-D-glucal (18), probably because *cis*-addition to the double bond occurs, with the formation of the equatorial 1-chloride in the former case, and this then rapidly undergoes inversion to the $^1C_4$ conformation in which the 1-chloride is axial.

Treatment of the nitroso-chloro-adduct (54) with propan-2-ol in dimethylformamide afforded a 69% yield of isopropyl 3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*ribo*-hexopyranoside (31). The ^{1}H n.m.r. spectrum of the oxime (31) indicated that the product was a mixture of *Z*- and *E*-isomers in the ratio 2 : 1. The anomeric proton of the *Z*-isomer gave rise to a singlet at δ 6.03; that of the *E*-isomer resonated at higher field (δ 5.26). The presence of the axial 3-acetyl group in (31) clearly enabled the oxime to exist partially in the *E*-configuration. Acetylation of the oxime (31) gave the acetate (32), which on reduction with 10 equiv. of borane in tetrahydrofuran, followed by acetylation, gave a 45% yield of isopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-allopyranoside (37), together with a 13% yield of isopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-altropyranoside (38). The ^{1}H n.m.r. spectrum of (37) was in agreement with an α-D-glycoside having a $^4C_1$ conformation. The ^{1}H n.m.r. spectrum of the *altro*-product (38) also indicated that it was an α-D-glycoside, and that the molecule existed mainly in the $^4C_1$ conformation. The preparation of the above model α-glycosides from the *altro*-nitroso-chloro-adduct (54) demonstrated as anticipated that the Lemieux–Nagabhushan reaction could be successfully applied to prepare α-D-allopyranosides.

The synthesis of an *allo*-analogue of gentamicin X_2 was therefore undertaken. Condensation of the *altro*-nitroso-chloro-adduct (54) with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (40) in dimethylformamide gave a 17% yield of *O*-3,4,6-tri-*O*-acetyl-2-hydroxyimino-α-D-*ribo*-hexopyranosyl-(1 → 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (44), which existed predominantly as the *E*-isomer. The anomeric H-1′ singlet was not visible for the *E*-isomer; however the corresponding signal for the *Z*-isomer at δ 5.99 integrated for less than one proton, indicating that there was some *E*-isomer present. Acetylation of the oxime (44) gave the acetate (45), which was reduced with 15 equiv. of borane in tetrahydrofuran. The product was deprotected by refluxing with aqueous 5% sodium hydroxide to give *O*-2-amino-2-deoxy-α-D-allopyranosyl-(1 → 4)-garamine (5) in 5% yield from (44). The ^{1}H n.m.r. spectrum of (5) exhibited a doublet at δ 5.12 with ($J_{1′,2′}$ 4 Hz) due to H-1′. The 2′-proton gave rise to a doublet of doublets at δ 2.99 ($J_{1′,2′}$ 4, $J_{2′,3′}$ 3.5 Hz). Irradiation at the frequency of H-1′ caused the H-2′ signal to collapse to a doublet ($J_{2′,3′}$ 3.5 Hz). The above data are consistent with an α-D-allopyranosyl unit. The c.d. spectrum of (5) in TACu solution showed $[\theta]_{290}$ −4 493, the amplitude less than that of gentamicin X_2 (1) owing to a positive contribution from the complex formed at the 2′- and 3′-positions. The mass spectral fragment ions of (5) (Table 1) agreed with the proposed structure. *O*-2-Deoxy-2-ethylamino-α-D-allopyranosyl-(1 → 4)-garamine (6) (9%) was also formed. The presence of a triplet at δ 1.07 and a quartet at δ 2.75 (*J* 7 Hz) in the ^{1}H n.m.r. spectrum of (6) confirmed the presence of an *N*-ethyl group. Its location at the 2′-position was indicated by the mass spectral cracking pattern (Table 1). The anomeric H-1′ of (6) gave rise to a doublet at δ 5.19 ($J_{1′,2′}$ 4 Hz), confirming the α-glycosidic linkage at the 4-position. The c.d. spectrum (TACu solution) agreed with structure (6).

The final 3′-modified derivative described here is 3′-*O*-methylgentamicin X_2 (7), which was also prepared by a Lemieux–Nagabhushan reaction. The preparation of 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose (52) was effected by a slight modification of the published procedure.[31] Thus 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose was methylated at the 3-position with methyl iodide and sodium hydride and the product was subjected to acidic hydrolysis and peracetylation to give (52). The acetate (52) was treated with titanium tetrabromide to give 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl bromide (53) in quantitative yield. Reduction of the bromide (53) with zinc in aqueous acetic acid afforded a 51% yield of 4,6-di-*O*-acetyl-3-*O*-methyl-D-glucal (20).

Treatment of the glycal (20) with an excess of nitrosyl

[31] E. L. Hirst and E. Percival, *Methods Carbohydrate Chem.*, 1963, **2**, 145.

chloride in ethyl acetate at 0 °C afforded an 85% yield of crystalline dimeric 4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-2-nitroso-α-D-glucopyranosyl chloride (23). The ^{1}H n.m.r. spectrum of (23) exhibited a doublet at δ 6.60 ($J_{1,2}$ 3.5 Hz) as expected for the anomeric proton of an α-D-chloro-sugar. The 2-proton gave rise to a doublet of doublets at δ 5.35 ($J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz) in agreement with *cis*-addition of the nitrosyl chloride to the glycal to give the α-D-chloride, as anticipated.

reflux with aqueous 5% sodium hydroxide to give isopropyl 2-amino-2-deoxy-3-*O*-methyl-α-D-glucopyranoside (55), the rotation of which was recorded for use in subsequent molecular rotation calculations (Table 2).

The synthesis of 3′-*O*-methylgentamicin X_2 (7) was then undertaken. Condensation of the nitroso-chloro-adduct (23) with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (40) in dimethylformamide at 25 °C for 90 h gave two pseudotrisaccharide products.

TABLE 2

Molecular rotations {$[M]_D$ (in H_2O)}

	Obs.	Calc.
Garamine (59) [1,2]	+435°	
Isopropyl 2-amino-2-deoxy-3-*O*-methyl-α-D-glucopyranoside (55)	+349°	
3′-*O*-Methylgentamicin X_2 (7)	+804°	+784°
O-2-Amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl-(1 ⟶ 4)-garamine (58)	+435°	

TABLE 3

^{13}C Chemical shifts [a]

Carbon atom	(59) [3]	Δ[DOS ⟶ (59)]	(60) [33]	Δ[DOS ⟶ (60)]	(1) [25]	Δ[DOS ⟶ (1)]	(7)	Δ[(1) ⟶ (7)]	Δ[DOS ⟶ (7)]	(61) [b]
C-1	51.7	+0.1	51.1	−0.5	51.4	−0.2	51.5		−0.1	51.5
C-2	36.6		36.7		36.6		36.6			36.1
C-3	51.4	−0.2	50.3	−1.3	50.2	−1.4	50.3		−1.3	50.4
C-4	78.8		88.8		88.6		88.2			88.6
C-5	75.1	−1.5	76.8	+0.2	75.1	−1.5	75.2		−1.4	73.1
C-6	87.9		78.3		87.5		87.6			87.4
C-1′			102.0		101.6		101.5			103.8
C-2′			56.1		56.2		55.1	−1.1		57.7
C-3′			74.6		74.6		84.3	+9.7		76.6
C-4′			70.8		70.9		70.3	−0.6		70.6
C-5′			73.8		73.8		73.9			77.1
C-6′			61.6		61.5		61.4			61.6
3′-OCH_3							60.6			
C-1″	101.4				101.2		101.3			101.3
C-2″	70.0				70.1		70.2			70.0
C-3″	64.3				64.2		64.2			64.3
C-4″	73.2				73.2		73.2			73.1
C-5″	68.5				68.7		68.6			68.5
3″-NCH_3	38.0				37.9		37.8			37.5
4″-CH_3	22.9				22.7		22.5			22.3

Carbon atom	(61) pH 1	Δ[Base ⟶ pH 1]	Δ[(59) ⟶ (61)]	Δ[DOS ⟶ (61)]	(58)	(58) pH 1	Δ[Base ⟶ pH 1]	Δ[(59) ⟶ (58)]	Δ[DOS ⟶ (58)]
C-1	50.3	−1.2	−0.2	−0.1	51.6	51.8	+0.2	−0.1	0
C-2	28.4	−7.7			36.3	30.2	−6.1		
C-3	49.7	−0.7	−1.0	−1.2	50.3	49.8	−0.5	−1.1	−1.3
C-4	77.7	−10.9			89.3	83.2	−6.1		
C-5	72.7	−0.4	−2.0	−3.5	73.4	72.6	−0.8	−1.7	−3.2
C-6	84.5	−2.9			87.3	84.7	−2.6		
C-1′	96.4	−7.4			102.0	97.3	−4.7		
C-2′	55.6	−2.1			50.7	50.9	+0.2		
C-3′	71.7	−4.9			83.0	80.1	−2.9		
C-4′	70.1				66.1	66.1			
C-5′	77.1				77.3	77.3			
C-6′	61.0				61.6	61.2			
3′-OCH_3					57.0	58.6	+1.6		
C-1″	101.8	+0.5			101.3	101.7	+0.4		
C-2″	67.0	−3.0			70.3	67.4 [c]	−2.9		
C-3″	64.1	−0.2			64.2	64.5	+0.3		
C-4″	70.7	−2.4			73.3	70.8	−2.5		
C-5″	68.7				68.5	68.4			
3″-NCH_3	35.4	−2.1			37.7	35.5	−2.2		
4″-CH_3	21.7				22.3	21.8			

[a] δ$_C$ in p.p.m. downfield from external Me_4Si[δ(Me_4Si) = δ(dioxan) + 67.4] for the free base in D_2O. Deoxystreptamine (DOS) values were those reported by Daniels.[25] [b] Sample kindly provided by Dr. M. Tanabe.[1] [c] Signal obscured by dioxan reference peak.

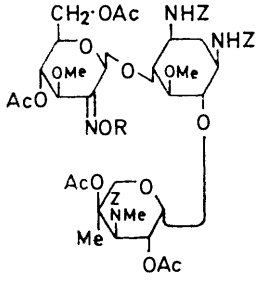
The nitroso-chloro-adduct (23) was treated with propan-2-ol in dimethylformamide to give isopropyl 4,6-di-*O*-acetyl-2-hydroxyimino-3-*O*-methyl-α-D-*arabino*-hexopyranoside (33) as the principal product in 51% yield. The ^{1}H n.m.r. spectrum of the oxime (33) showed a singlet at δ 6.13 for the anomeric proton indicating that the compound was the *Z*-isomer. Acetylation gave the acetate (34), which on reduction with *ca.* 9 equiv. of borane in tetrahydrofuran and acetylation afforded an 81% yield of isopropyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-α-D-glucopyranoside (39). The product (39) was shown to be the α-D-glucopyranoside from its ^{1}H n.m.r. spectrum, which contained a doublet at δ 4.96 ($J_{1,2}$ 4 Hz) due to the anomeric proton. The acetate (39) was deacetylated by heating under

The first was *O*-4,6-di-*O*-acetyl-2-hydroxyimino-3-*O*-methyl-β-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (56), isolated in a 37% yield. The axial anomeric H-1′ signal in the ^{1}H n.m.r. spectrum of (56) was not visible in a clear region of the spectrum, so that the configuration could not be ascertained. The second product was the expected *O*-4,6-di-*O*-acetyl-2-hydroxyimino-3-*O*-methyl-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (46), isolated in a 26% yield. The ^{1}H n.m.r. spectrum of (46) revealed a singlet at δ 6.08 due to the anomeric H-1′, indicating that the oxime was in the *Z*-configuration.

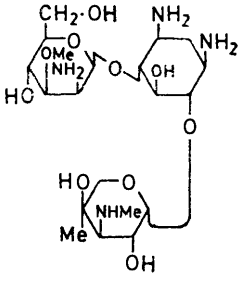
The β-oxime (56) was acetylated in the usual way to

give the acetate (57). Reduction of the latter with 10 equiv. of borane in tetrahydrofuran followed by alkaline hydrolysis and treatment with 90% hydrazine hydrate afforded *O*-2-amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl-(1 ⟶ 4)-garamine (58) in 25% yield from (56). The molecular rotation of (58) (Table 2) suggested that it was a β-glycoside. The ^{1}H n.m.r. spectrum exhibited a doublet at δ 4.77 ($J_{1',2'}$ 1 Hz) consistent with a β-D-*manno*-configuration for the 4-*O*-glycoside. Further confirmation of the β-D-*manno*-structure (58) was obtained from the ^{13}C n.m.r. spectrum (Table 3) which will be discussed presently. The mass spectral fragment ions (Table 1) were as expected. The c.d. spectrum (TACu solution) showed the expected amplitude for 4- and 6-glycosidic linkages about the deoxystreptamine ring, but, owing to the presence of the 3'-*O*-methyl group, gave no clue as to the stereochemistry at C-2'.

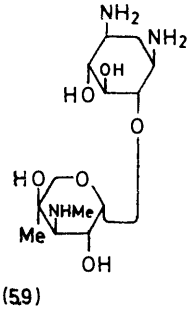
The α-oxime (46) was acetylated to give the acetate (47), which on reduction with *ca.* 9 equiv. of borane in tetrahydrofuran followed by deprotection as for the β-anomer, afforded 3'-*O*-methylgentamicin X_2 (7) in 32% yield based on (46). The molecular rotation of (7) (Table 2) indicated that the newly formed glycosidic linkage had the α-configuration. The ^{1}H n.m.r. spectrum further supported the α-glycoside structure as it contained a doublet at δ 5.22 ($J_{1',2'}$ 3.5 Hz) due to the



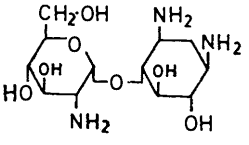
(56) R = H
(57) R = Ac



(58)

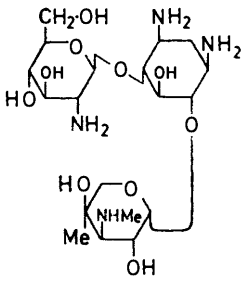


(59)

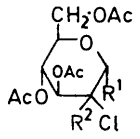


(60)

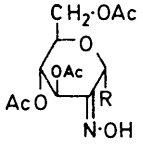
anomeric H-1'. The coupling constant was in agreement with a *gluco*-stereochemistry. The ^{13}C n.m.r. spectrum of (7) (Table 3) further supported the assigned stereochemistry. The mass spectral fragment ions (Table 1) and the c.d. spectrum (TACu solution) agreed with structure (7).



(61)



(62) R^1 = Cl, R^2 = N(OH)NO
(63) R^1 = Cl, R^2 = NO
(64) R^1 = OPr^i, R^2 = N(OH)NO
(65) R^1 = OPr^i, R^2 = NO



(66) R = Cl
(67) R = OPr^i

The ^{13}C n.m.r. parameters of the 3'-*O*-methyl derivatives (58) and (7), together with those of garamine (59),[2] paromamine (60),[32] gentamicin X_2 (1),[33] and *O*-2-amino-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-garamine (61) [1] for comparison purposes, are given in Table 3. The assignments for gentamicin X_2 (1) [33] agree well with those for the component pseudodisaccharide units, namely garamine (59) [2] and paromamine (60).[32] Methylation of the 3'-hydroxy-group in the analogue (7) produced upfield β-shifts of 1.1 and 0.6 p.p.m. for the C-2' and C-4' resonances, and the C-3' resonance experienced a downfield α-shift of 9.7 p.p.m. Negligible γ-shifts were observed for the C-1' and C-5' resonances. These shifts agree with those found upon methylation of the 3-hydroxy-group of α-D-glucopyranose.[34] The ^{13}C n.m.r. spectrum of *O*-2-amino-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-garamine (61) [1] showed the expected garosamine resonances.[35] The carbon resonances of the D-glucosamine ring in (61) showed downfield shifts of 2.2, 1.5, 2.0, and 3.3 p.p.m. for C-1', -2', -3', and -5', respectively, relative to the corresponding resonances in gentamicin X_2 (1). These shifts are in general agreement with those expected for an α- *versus* a β-D-glucopyranoside.[34]

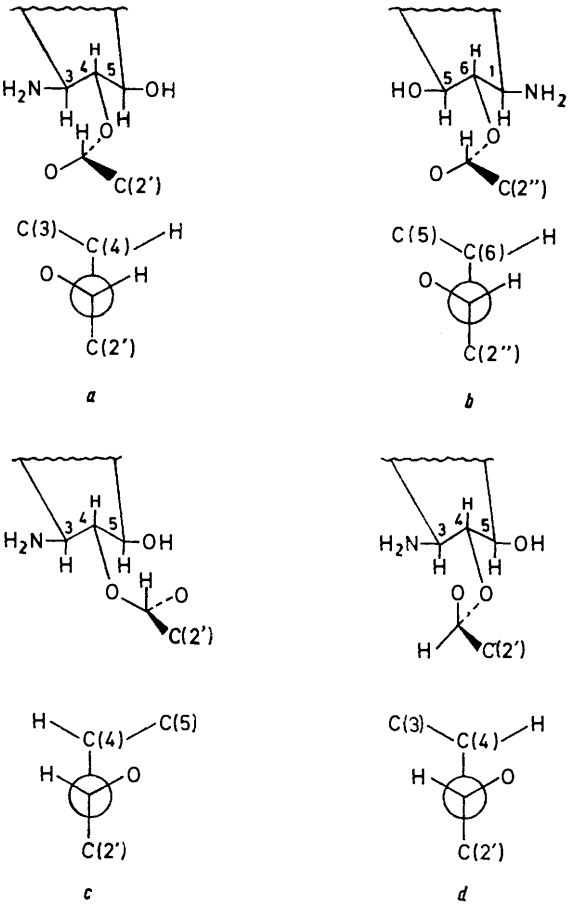
The solution conformations of aminoglycoside antibiotics are of interest in view of the biological activity of these compounds and their clinical importance. Previous ^{1}H n.m.r. studies on the kanamycins [36] and related synthetic analogues, as well as ^{13}C n.m.r. studies on the gentamicins [35] and on tobramycin,[37] have defined the

[32] T. L. Nagabhushan, W. N. Turner, P. J. L. Daniels, and J. B. Morton, *J. Org. Chem.*, 1975, **40**, 2830.
[33] P. J. L. Daniels, unpublished observations.
[34] D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1970, **92**, 1355.
[35] J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1973, **95**, 7464.
[36] R. U. Lemieux, T. L. Nagabhushan, K. J. Clemetson, and L. C. N. Tucker, *Canad. J. Chem.*, 1973, **51**, 53.
[37] K. F. Koch, J. A. Rhoades, E. W. Hagaman, and E. Wenkert, *J. Amer. Chem. Soc.*, 1974, **96**, 3300.

solution conformations of several 4-*O*-α-D-glycosyl, 6-*O*-α-D-glycosyl, and 6-*O*-β-L-glycosyl units with respect to their orientation about the C(4)–O and C(6)–O bonds of the deoxystreptamine ring. Similar conformations have been proposed for sisomicin,[35] antibiotics 66-40B and D,[38] and garamine (59).[2] In 3′-*O*-methylgentamicin X_2 (7) the shielding of C-3 by 1.3 p.p.m. due to the 4-*O*-glycoside unit and of C-5 by 1.4 p.p.m. due to the 6-*O*-glycoside unit, together with the absence of any shielding of C-1, suggested that the preferred rotamers about the C(4)–O and C(6)–O bonds were *a* and *b*, respectively. Both rotamers satisfy the requirements of the *exo*-anomeric effect,[36,39-42] and are the same as those observed for gentamicin X_2 (1).[33]

To the best of our knowledge no information has been published on the conformation of a 4-*O*-β-D-glycosyl derivative of deoxystreptamine. The preparation of *O*-2-amino-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-garamine (61) [1] and of *O*-2-amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl-(1 ⟶ 4)-garamine (58) afforded us the opportunity of studying the change in conformation due to the presence of a 4-*O*-β-D-glycoside in an aminoglycoside. The Δδ values for C-1, -3, and -5 for *O*-2-amino-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-garamine (61) [1] and *O*-2-amino-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl-(1 ⟶ 4)-garamine (58) are given in Table 3. The two 4-*O*-β-D-glycosides (61) and (58), which now have a 4-*O*-equatorial glycosidic bond, exhibited striking differences in the shielding of the β-carbons C-3 and -5, indicating a different solution conformation for these molecules relative to the 4-*O*-α-D-glycosides. In (61) C-3 was shielded by 1.2 p.p.m. whereas C-5 was shielded by a total of 3.5 p.p.m. of which 2.0 p.p.m. was attributable to the 4-*O*-β-D-glycoside unit, the remainder being due to the 6-*O*-garosaminyl unit. Similar results were observed with the 4-*O*-β-glycoside (58). In both (61) and (58) negligible shielding of C-1 was observed. By comparison the 4-*O*-α-D-glycoside unit in gentamicin X_2 (1) shields C-3 by 1.2 p.p.m. but does not shield C-5, while the 6-*O*-β-L-glycoside unit shields C-5 by 1.5 p.p.m. and has a negligible shielding effect on C-1. The most probable conformations of the rotamers about the C(4)–O bond for the 4-*O*-β-D-glycosides (61) and (58) would be represented by rotamer *c*. The latter would be expected to produce the observed shielding of C-5 of 1.7—2.0 p.p.m. in the β-glycosides (61) and (58), and and would also satisfy the requirements of the *exo*-anomeric effect.[36,39-42] The origin of the observed shielding of C-3 in these 4-*O*-β-glycosides is uncertain at present. The conformations about the C(6)–O glycosidic bond in (61) and (58) are best represented by rotamer *b*, which would produce the observed shielding of C-5 but not C-1, as observed with these and other aminoglycosides.[2,35-38] Rotamer *b* would also satisfy the requirements of the *exo*-anomeric effect.[36,39-42]

The characteristic Δδ values for C-3 and -5 supported the 4-*O*-β-D-glycosyl structure for (58), whereas the chemical shift of the anomeric C-1′ (102.0 p.p.m) could not be used to establish unambiguously the presence of the 4-*O*-β-D-glycosidic linkage, owing to the close similarity between the chemical shifts of the anomeric carbon atoms of α- and β-mannopyranosides.[34] The observed resonances for C-2′, -3′, -4′, and -5′ agreed closely with the predicted chemical shifts relative to (61), taking into consideration the inversion at C-2′ in



the *manno*-configuration and the methylation of the 3′-hydroxy-group.[34]

The carbon resonances β to the amino-groups in (61) and (58) showed the expected upfield shifts when the spectra were run at pH 1, due to protonation of the amino-groups.[43] The Δδ values observed upon protonation of the free bases (61) and (58) are given in Table 3. The Δδ values for the asymmetric carbons C-1′, -4, and -6 observed upon protonation of an aminoglycoside have recently been shown [44] to be useful in assigning the absolute configuration of the anomeric centre provided sufficient stereochemical information is known about the

[38] D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, *J.C.S. Perkin I*, 1975, 814.
[39] R. U. Lemieux, A. A. Pavia, J. C. Martin, and K. A. Watanabe, *Canad. J. Chem.*, 1969, **47**, 4427.
[40] R. U. Lemieux and J. C. Martin, *Carbohydrate Res.*, 1970, **13**, 139.
[41] R. U. Lemieux, *Ann. New York Acad. Sci.*, 1973, **222**, 915.
[42] R. U. Lemieux and S. Koto, *Tetrahedron*, 1974, **30**, 1933.
[43] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669.
[44] T. L. Nagabhushan and P. J. L. Daniels, *Tetrahedron Letters*, 1975, 747.

glycoside. In keeping with these observations the $\Delta\delta$ values for the β-glycosides (61) and (58) (Table 3) are clearly affected both by the change in the anomeric configuration and by the stereochemistry at C-2′ in the 4-*O*-glycosyl unit. The $\Delta\delta$ value for C-1′ in the α-D-*manno*-analogue of gentamicin X_2 reported earlier [1] was also affected by the stereochemistry at C-2′.

Although the Lemieux–Nagabhushan reaction invariably gives rise to the α-glycoside, isolated cases have been noted where β-glycosides were also produced. In one case the condensation of the nitroso-chloro-adduct (22) with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose gave rise to an 8.7% yield of the β-hydroxyiminoglycoside [45] together with an 80% yield of the α-anomer. The other reported instance where both α- and β-glycosides were formed in the Lemieux–Nagabhushan reaction was during the condensation of (22) with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside.[27] It has been suggested [45] that the β-hydroxyiminoglycoside may indeed be formed and then isomerise to the α-hydroxyiminoglycoside *via* an αβ-unsaturated nitrosoglycoside intermediate. In our experience with the Lemieux–Nagabhushan reaction we obtained exclusively α-glycosides, with the exception of the reaction of the 3-*O*-methyl nitroso-chloro-adduct (23), which gave the β-glycoside as the major product on condensation with the garamine derivative (40). The only other β-glycosides isolated in our work were obtained during condensations with the nitroso-chloro-adduct of D-arabinal.[46]

The ready formation of nitroglucals during the preparation of the nitroso-chloro-adducts was further investigated. The formation of nitro-derivatives during the addition of nitrosyl chloride to olefins has been reported previously.[47-49] Thus cholesteryl acetate has been demonstrated to react slowly with an excess of pure nitrosyl chloride to give the 5α-chloro-6β-nitro-derivative and it has been suggested that the reaction proceeds *via* addition of nitrosyl chloride to the double bond, followed by oxidation.[48] Impure nitrosyl chloride was found to react rapidly to give the nitro-derivative, and the direct free-radical addition of nitrogen dioxide was suggested as the mechanism of the latter reaction. Treatment of tri-*O*-acetyl-D-glucal (18) with an excess of commercial nitrosyl chloride under published conditions [22-24] with ethyl acetate as solvent gave reproducibly high yields of the nitroso-chloro-adduct (22). However, when the reaction was allowed to continue for 48 h, conversion of the nitroso-chloro-adduct (22) into 3,4,6-tri-*O*-acetyl-1,2-dideoxy-2-nitro-D-*arabino*-hex-1-enopyranose (25) occurred. The nitroglucal (25) was identical with the product prepared by treating tri-*O*-acetyl-D-glucal (18) with dinitrogen tetraoxide.[24] Similar results were obtained with purified, triply distilled nitrosyl chloride [50,51] in either methylene chloride or deuteriochloroform. When a solution of tri-*O*-acetyl-D-glucal (18) in purified nitrosyl chloride in methylene chloride * was left at 25 °C for 48 h under argon and samples were worked up at intervals of 1 h, i.r. spectra (in chloroform) showed initial rapid formation of the nitroso-chloro-adduct (22), as demonstrated by the disappearance of the vinylic ether absorption of the glucal at 1 650 cm^{-1}. The gradual formation of the nitroglucal (25) was indicated by the appearance of bands at 1 650 and 1 510 cm^{-1} due to the vinylic ether grouping and the nitro-stretching frequency, respectively. In order to gain a better insight into the reaction, tri-*O*-acetyl-D-glucal (18) was treated with pure nitrosyl chloride in deuteriochloroform † under argon, the reaction being monitored in an n.m.r. tube at 37 °C over 50 h. It was apparent that the initial reaction involved the rapid conversion of tri-*O*-acetyl-D-glucal (18) into the nitroso-chloro-adduct (22). The characteristic ^1H n.m.r. signals for the nitroglucal (25) gradually appeared and increased in intensity until they accounted for *ca.* 50% of the mixture after 50 h. The intensity of the signals due to the nitroso-chloro-adduct (22) gradually decreased, until no nitroso-chloro-adduct (22) remained after 50 h. In addition to the signals arising from the above compounds, it was also apparent that two other discrete products were being formed. The first of these to be formed is thought to be the 2-chloro-2-*N*-nitrosohydroxyamino-derivative (62), which eliminates nitrosyl hydride to give the *gem*-2-chloro-2-nitroso-derivative (63). The ^1H n.m.r. parameters for these products are given in Table 4. After 50 h the only products remaining in the mixture were the nitroglucal

TABLE 4

^1H N.m.r. parameters for nitrosyl chloride addition products [a]

Compd.	H-1	H-3	H-4
(62)	6.08 (s)	6.99 (d, $J_{3,4}$ 9.5 Hz)	5.37 (dd, $J_{3,4} = J_{4,5} = 9.5$ Hz)
(63)	6.23 (s)	5.82 (d, $J_{3,4}$ 9.5 Hz)	5.32 (dd, $J_{3,4} = J_{4,5} = 9.5$ Hz)
(64)	5.18 (s)	6.80 (d, $J_{3,4}$ 9 Hz)	5.31 (dd, $J_{3,4} = J_{4,5} = 9$ Hz)
(65)	5.10 (s)	5.67 (d, $J_{3,4}$ 9 Hz)	5.24 (dd, $J_{3,4} = J_{4,5} = 9$ Hz)

[a] Recorded at 100 MHz in $CDCl_3$; assignments were confirmed by off-resonance decoupling.

* A solution of tri-*O*-acetyl-D-glucal (18) (300 mg) in a 5.26N-solution (6 ml) of pure nitrosyl chloride in methylene chloride was used.

† A solution of tri-*O*-acetyl-D-glucal (18) (20 mg) in a 5.25N-solution (0.4 ml) of pure nitrosyl chloride in deuteriochloroform was used.

[45] R. U. Lemieux, Y. Ito, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 7.
[46] A. K. Mallams, S. S. Saluja, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J.C.S. Perkin I*, 1976, 1135.
[47] K. Tanabe and R. Hayashi, *Chem. and Pharm. Bull.* (*Japan*), 1962, **10**, 1177.
[48] W. A. Harrison, E. R. H. Jones, G. D. Meakins, and P. A. Wilkinson, *J. Chem. Soc.*, 1964, 3210.
[49] A. Hassner and C. Heathcock, *J. Org. Chem.*, 1964, **29**, 1350.
[50] G. H. Coleman, G. A. Lillis, and G. E. Goheen, *Inorg. Synth.*, 1939, **1**, 55.
[51] J. R. Morton and H. W. Wilcox, *Inorg. Synth.*, 1953, **4**, 48.

(25) and the *gem*-2-chloro-2-nitroso-derivative (63), in approximately equal amounts. After work-up followed by chromatography the only product that could be isolated was the nitroglucal (25), the *gem*-2-chloro-2-nitroso-derivative (63) having decomposed. It therefore seems that the initially formed nitroso-chloro-adduct (22) undergoes two reactions with an excess of nitrosyl chloride. The first involves oxidation and elimination of hydrogen chloride to give the nitroglucal (25), isolated in 47% yield. The second involves initial formation of the chloro-oxime (66),[52] which would be expected to undergo rapid reaction with the excess of nitrosyl chloride present to give (62), which in turn would react to give the *gem*-2-chloro-2-nitroso-derivative (63). At no time during the reaction were any ^{1}H n.m.r. signals attributable to the chloro-oxime (66) detected. The addition of nitrosyl chloride to oximes has been shown to lead to *gem*-chloro-nitroso-derivatives,[53,54] and intermediate *N*-nitrosohydroxylamines have been claimed as intermediates in the reaction.[54] *N*-Nitrosohydroxylamines have been isolated as unstable solids which decompose to nitroso-derivatives.[55] By analogy, the oxime (67) when treated with an excess of pure nitrosyl chloride in deuteriochloroform,* under argon in an n.m.r. tube at 37 °C, underwent rapid conversion into the 2-chloro-2-*N*-nitrosohydroxylamine derivative (64), which reacted further to give the *gem*-2-chloro-2-nitroso-derivative (65) as a pale blue gum, the conversion being complete after 24 h. The i.r. spectrum of the crude product after 24 h showed no nitro-stretching bands, indicating that no oxidation of the oxime (67) was occurring. The ^{1}H n.m.r. parameters for the 2-chloro-2-*N*-nitrosohydroxylamine (64) and for the *gem*-2-chloro-2-nitroso-derivative (65) (Table 4) agree well with the values for the 1-chloro-analogues (62) and (63). All attempts at preparative isolation of the *gem*-2-chloro-2-nitroso-derivative (65), either on a silica gel column or on silica gel thin layer plates with benzene–hexane–acetone (2 : 2 : 1) as eluant, resulted in extensive decomposition.

The novel aminoglycoside antibacterials prepared 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.[2]

4,6-Di-O-acetyl-2,3-dideoxy-2-nitroso-α-D-ribo-hexopyranosyl Chloride (21).—4,6-Di-*O*-acetyl-1,2,3-trideoxy-D-*erythro*-hex-1-enopyranose (17) (41.7 g) dissolved in dry ethyl acetate (1 250 ml) was flushed with dry nitrogen and cooled to 0 °C. A solution of nitrosyl chloride (25.5 g) in dry ethyl acetate (100 ml) was added and the mixture was kept at −5 °C for 2.75 h. The reaction was then complete (t.l.c.). The solution was evaporated to dryness and the gum was triturated with dry ether and then dried under vacuum to give the *chloride* (21) (54 g, 99%) as a glass (Found: C, 42.9; H, 5.10; Cl, 12.15; N, 4.8. $C_{20}H_{28}Cl_2N_2O_{12}$ requires C, 42.95; H, 5.05; Cl, 12.7; N, 5.0%) $[\alpha]_D$ +75.5° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 1 740, 1 220, and 1 040 cm^{-1}, δ($CDCl_3$) 2.10 (6 H, s, OAc) and 6.70 and 6.82 [1 H, d, $J_{1,2}$ 4 Hz, H-1 (3 : 2)].

4,6-Di-O-acetyl-1,2,3-trideoxy-2-nitro-D-erythro-hex-1-enopyranose (24).—A solution of 4,6-di-*O*-acetyl-3-deoxy-D-glucal (17) (200 mg) in dry ethyl acetate (10 ml) was saturated with nitrosyl chloride at 0 °C, then allowed to warm to 25 °C. After 23 h it was evaporated to dryness and the residue chromatographed on silica gel plates [hexane–benzene–acetone (2 : 2 : 1) as eluant] to give the *hexenopyranose* (24) (105 mg, 43%) as a glass (Found: C, 44.8; H, 5.1; N, 5.1. $C_{10}H_{13}NO_7$ requires C, 46.3; H, 5.1; N, 5.4%), $[\alpha]_D$ +22.0° (in $CHCl_3$), $\lambda_{max.}$ 289 nm (ε 5 807), $\nu_{max.}$ (film) 1 750, 1 660, 1 510, 1 220, and 1 040 cm^{-1}, δ($CDCl_3$) 2.08 (6 H, s, OAc) and 8.12 (1 H, s, H-1).

3,4,6-Tri-O-acetyl-1,2-dideoxy-2-nitro-D-arabino-hex-1-enopyranose (25).—A solution of 3,4,6-tri-*O*-acetyl-D-glucal (18) (192 mg) in dry ethyl acetate (20 ml) was cooled to 0 °C and purged with dry nitrogen for 0.5 h. A slow stream of nitrosyl chloride was bubbled through at 0 °C for 2 h. The mixture was then kept under dry nitrogen at 0—25 °C for a total of 22 h. The solution was evaporated to dryness and the residue was chromatographed on preparative silica gel plates [hexane–benzene–acetone (2 : 2 : 1) as eluant] to give the hexenopyranose (25) [24] as a glass (105 mg, 47%), $\nu_{max.}$($CHCl_3$) 1 750, 1 650, 1 510, 1 220, and 1 025 cm^{-1}, δ($CDCl_3$) 2.10 (3 H, s, OAc), 2.12 (6 H, s, OAc), 4.24 (1 H, dd, $J_{6a,6b}$ 12, $J_{5,6a}$ 5 Hz, H-6a), 4.50 (1 H, dd, $J_{6a,6b}$ 12, $J_{5,6b}$, 8 Hz, H-6b), 4.77 (1 H, m, H-5), 5.30 (1 H, dd, $J_{3,4}$ 3, $J_{4,5}$ 2 Hz, H-4), 6.01 (1 H, dd, $J_{3,4}$ 3, $J_{3,5}$ *ca.* 2 Hz, H-3), and 8.36 (1 H, s, H-1).

Isopropyl 4,6-Di-O-acetyl-3-deoxy-2-hydroxyimino-α-D-erythro-hexopyranoside (29).—A solution of 4,6-di-*O*-acetyl-2,3-dideoxy-2-nitroso-α-D-glucopyranosyl chloride (21) (610 mg) and propan-2-ol (0.84 ml) in dry dimethylformamide (24 ml) was kept at 25 °C for 41 h, then evaporated to dryness. The residue was chromatographed on preparative silica gel plates [hexane–benzene–acetone (2 : 2 : 1) as eluant] to give the *hexopyranoside* (29) (352 mg, 53%) as a gum (Found: C, 51.7; H, 6.9; N, 4.6. $C_{13}H_{21}NO_7$ requires C, 51.5; H, 7.0; N, 4.6%), *m/e* 286 ($M^{+\cdot}$ − OH), $[\alpha]_D$ +120.5° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 300, 1 740, 1 230, and 1 030 cm^{-1}, δ($CDCl_3$) 1.19 and 1.25 (6 H, d, *J* 2 Hz, $OCHMe_2$), 2.06 (6 H, s, OAc), 5.14 (0.8 H, s, H-1, *E*-isomer), 5.99 (0.2 H, s, H-1, *Z*-isomer), and 9.05br (1 H, s, :NOH, disappears on deuteriation) (*E* : *Z* ratio 5 : 1).

Isopropyl 2-Acetamido-4,6-di-O-acetyl-2,3-dideoxy-α-D-ribo-hexopyranoside (35) *and its* arabino-*Isomer* (36).—The oxime (29) (750 mg) and acetic anhydride (3.2 ml) were dissolved in dry pyridine (8.5 ml) and the solution was kept at 25 °C for 18 h. The mixture was poured into ice–water and the acetate (30) (731 mg) was filtered off, dried, and dissolved in dry tetrahydrofuran (25 ml). The solution was cooled to 0 °C and M-borane in tetrahydrofuran (21.2 ml) was added dropwise; the solution was then stored at 7 °C for 30 h. The excess of borane was destroyed by dropwise addition of water, the solution was evaporated to dryness, and the residue was azeotroped with benzene. The solid was dissolved in dry pyridine (20 ml), acetic

* A solution of isopropyl 3,4,6-tri-*O*-acetyl-2-hydroxyimino-D-*arabino*-hexopyranoside (67) (20 mg) in a 5.25N-solution (0.4 ml) of pure nitrosyl chloride in deuteriochloroform was used.

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

[53] H. Rheinboldt and M. Dewald, *Annalen*, 1972, **455**, 300.

[54] L. W. Kissinger and H. E. Ungnade, *J. Org. Chem.*, 1958, **23**, 1517.

[55] E. Muller and H. Metzger, *Chem. Ber.*, 1956, **89** 396.

anhydride (6 ml) was added, and the solution was kept at 25 °C for 18 h. Methanol was added and after 0.5 h the solution was evaporated to dryness and the residue azeotroped with toluene. Chromatography on a silica gel column (110 × 2.5 cm) (0.5% methanol–chloroform as eluant) gave the α-D-ribo-*hexopyranoside* (35) (249 mg, 30%) as a glass (Found: C, 55.0; H, 7.7; N, 4.3. $C_{15}H_{25}NO_7$ requires C, 54.4; H, 7.6; N, 4.2%), *m/e* 332 $(M + 1)^+$, $[\alpha]_D$ +132.3° (in MeOH), δ($CDCl_3$) 1.17 and 1.26 (6 H, d, *J* 6 Hz, CH*Me*$_2$), 1.96, 2.02, and 2.06 (9 H, s, OAc), 4.83 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1), and 5.71br (1 H, d, *J* 8.5 Hz, N*H*Ac), and the α-D-arabino-*hexopyranoside* (36) (61 mg, 7%) as a glass (Found: C, 54.2; H, 7.5; N, 4.1%), *m/e* 332 $(M + 1)^+$, $[\alpha]_D$ +66.0° (in MeOH), δ($CDCl_3$) 1.18 and 1.23 (6 H, d, *J* 6 Hz, CH*Me*$_2$), 2.00, 2.04, and 2.07 (9 H, s, OAc), 4.74 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1), and 6.42br (1 H, d, *J* 8 Hz, N*H*Ac).

3′-Deoxygentamicin X$_2$ (2).—(i) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (40) (7.28 g) and 4,6-di-*O*-acetyl-2,3-dideoxy-2-nitroso-α-D-glucopyranosyl chloride (21) (4.8 g) were dissolved in dry, redistilled dimethylformamide (200 ml) and the solution was kept at 25 °C for 46 h. The mixture was worked up as before and the product chromatographed on a silica gel column (160 × 2.5 cm) (1% methanol–chloroform as eluant) to give O-4,6-*di*-O-*acetyl*-3-*deoxy*-2-*hydroxyimino*-α-D-erythro-*hexopyranosyl* (1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonyl*-*garamine* (42) (4.8 g, 51%) as an amorphous solid, m.p. 123—133° (Found: C, 58.0; H, 5.7; N, 5.0. $C_{53}H_{64}N_4O_{21}$ requires C, 58.2; H, 5.9; N, 5.1%), $[\alpha]_D$ +117.0° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 400, 1 740, 1 710, 1 220, 1 030, and 695 cm^{-1}, δ($CDCl_3$) * 1.25br and 1.37br (3 H, 2 s, 4″-CH_3), 1.90br, 1.96br, and 2.01br (15 H, 3 s, OAc), 2.83br (3 H, s, 3″-NCH_3), 5.07br (6 H, s, C*H*$_2$Ph), and 7.28br (15 H, s, CH_2*Ph*).

The oxime (42) (3.3 g) was acetylated and the acetate (43) (3.2 g) reduced with 1M-borane in tetrahydrofuran (55.3 ml) as described before. The resulting solid was dissolved in liquid ammonia (250 ml) at −70 °C and sodium (5 g) was added in small portions. The deep blue solution was stirred at −70 °C for 2 h and the reaction was then quenched by careful addition of water. The ammonia was allowed to evaporate at room temperature and sufficient water was added to form a viscous paste, which was kept at 25 °C for 18 h. Water was then added and the solution was neutralized with Amberlite IRC 50 (H^+) resin; the slurry was poured onto a column, the resin was washed with water, and the products were then eluted with 1.5N-ammonium hydroxide. The eluate was evaporated 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 two principal fractions. The less polar was rechromatographed on a silica gel column (110 × 1 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1)] to give *3′-deoxy*-4″-O-*ethylgentamicin X*$_2$ (3) (617 mg, 44%) (Found: C, 50.8; H, 8.6; N, 11.1. $C_{21}H_{42}N_4O_9$ requires C, 51.0; H, 8.6; N, 11.3%), $[\alpha]_D$ +146.8° (in H_2O), $[\theta]_{290}$ −8 060 (TACu), $[\theta]_{290}$ −3 730 (Cupra A), $\nu_{max.}$(KCl) 3 330 and 1 045 cm^{-1}, δ(D_2O) 1.14 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.20 (3 H, s, 4″-CH_3), 2.52 (3 H, s, 3″-NCH_3), 3.46 (2 H, q, *J* 7 Hz, OCH_2CH_3), 5.05 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.09 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′).

The more polar fraction from the original column was rechromatographed on a silica gel column (110 × 1 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1)] to give *3′-deoxygentamicin X*$_2$ (2) (210 mg, 16%), m.p. 131—141° (Found: C, 48.7; H, 8.1; N, 11.9. $C_{19}H_{38}N_4O_9$ requires C, 48.9; H, 8.2; N, 12.0%), $[\alpha]_D$ +171.6° (in H_2O), $[\theta]_{290}$ −9 040 (TACu), $[\theta]_{290}$ −7 550 (Cupra A), $\nu_{max.}$(KCl) 3 340 and 1 030 cm^{-1}, δ(D_2O) 1.20 (3 H, s, 4″-CH_3), 2.51 (3 H, s, 3″-NCH_3), 5.07 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.10 (1 H, d, $J_{1',2'}$ 3 Hz, H-1′).

(ii) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (40) (44.5 g), 4,6-di-*O*-acetyl-2,3-dideoxy-2-nitroso-α-D-glucopyranosyl chloride (21) (29.3 g), and *NN*,2,6-tetramethylaniline (9 g) were dissolved in dry, redistilled dimethylformamide (1 200 ml) and the solution was kept at 25 °C for 59 h. The mixture was worked up as before and the product chromatographed on a silica gel column (160 × 7.5 cm) (1% methanol–chloroform as eluant) to give *O*-4,6-di-*O*-acetyl-3-deoxy-2-hydroxyimino-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (42) (40 g, 70%), identical with that prepared in (i).

The oxime (42) (40 g) was acetylated to give the acetate (43) (36 g, 87%). The acetate (43) (48.7 g) was reduced with M-borane in tetrahydrofuran (428 ml) as described before (30 h). The solid product was dissolved in a 5% solution of sodium hydroxide in aqueous dioxan (1 : 1) (2.4 l) and the solution was heated under reflux for 18 h. The mixture was cooled and neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water and then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give two fractions. The less polar was rechromatographed on a silica gel column (160 × 5 cm) [chloroform–methanol–7% ammonium hydroxide (15 : 27 : 15) as eluant] to give 1,3-N-*carbonyl*-*3′-deoxygentamicin X*$_2$ (48) (3.5 g, 17%) (Found: C, 47.6; H, 7.5; N, 11.1. $C_{20}H_{36}N_4O_{10}$,H_2O requires C, 47.05; H, 7.50; N, 11.0%), *m/e* 493 $(M + 1)^+$, $[\alpha]_D$ +171.6° (in H_2O), $[\theta]_{287}$ −7 490 (TACu), $[\theta]_{290}$ −6 020 (Cupra A), $\nu_{max.}$ (KCl) 3 330, 1 660, 1 060, and 1 030 cm^{-1}, δ(D_2O) 1.24 (3 H, s, 4″-CH_3), 2.52 (3 H, s, 3″-NCH_3), 4.93 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.03 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′).

The more polar fraction from the first column was rechromatographed on a silica gel column (160 × 5 cm) [chloroform–methanol–7% ammonium hydroxide solution (15 : 27 : 15)] to give 3′-deoxygentamicin X_2 (2) (2.5 g).

The cyclic urea (48) (3.5 g) was dissolved in 90% hydrazine hydrate (50 ml) and the solution was heated in a bomb at 130 °C for 89 h. The solution was evaporated *in vacuo* and the residue was repeatedly dissolved in water and methanol and recovered by evaporation. The 3′-deoxygentamicin X_2 (2) (1.56 g) was purified as described above (total yield 4.06 g, 20%).

(iii) *O*-2,4,6-Tri-*O*-acetyl-3-deoxy-2-hydroxyimino-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (43) (81.3 g) was dissolved in dry tetrahydrofuran (4.18 l) and cooled to 4 °C. M-Borane in tetrahydrofuran (714 ml) was added dropwise and the solution was stored at 7 °C for 30 h. The reaction was worked up as in (ii) and the residue was heated under reflux with a 5% solution of sodium hydroxide in aqueous dioxan (1 : 1; 2 l) for 18 h. The reaction was worked up as in (ii) and the resulting solid was dissolved in 90%

* Mixture of rotamers at ambient temperatures.

hydrazine hydrate (80 ml) and heated in a bomb at 130 °C for 130 h. The reaction was worked up as in (ii). Chromatography on silica gel columns (160 × 5 cm), with chloroform–methanol–7% ammonium hydroxide (15 : 27 : 15) as eluant, followed by rechromatography of the two fractions with the lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant in each case, afforded, as the less polar product, 3′-*deoxy*-2′-N-*ethylgentamicin* X_2 (4) (2.56 g, 7%) (Found: C, 50.9; H, 8.4; N, 11.3. $C_{21}H_{42}N_4O_9$ requires C, 51.0; H, 8.6; N, 11.3%), $[\alpha]_D$ +147.9° (in H_2O), $[\theta]_{290}$ −8 800 (TACu), $[\theta]_{290}$ −7 280 (Cupra A), $\nu_{max.}$(KCl) 3 300 and 1 050 cm^{-1}, δ(D_2O) 1.07 (3 H, t, *J* 7 Hz, $NHCH_2CH_3$), 1.24 (3 H, s, 4″-CH_3), 2.54 (3 H, s, 3″-NCH_3), 2.71 (2 H, q, *J* 7 Hz, $NHCH_2CH_3$), 5.12 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.21 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′). The more polar product was 3′-deoxygentamicin X_2 (2) (7.48 g, 22%), identical with that described above.

3,4,6-*Tri*-O-*acetyl*-D-*allal* (19).—1,2,3,4,6-Penta-*O*-acetyl-β-D-allopyranose (49) (2.71 g) was dissolved in chloroform (38.7 ml) (ethanol-free) and a solution of titanium tetrabromide (3.49 g) in chloroform (13.3 ml) (ethanol-free) was added. The mixture was heated under reflux for 2 h. The chloroform solution was cooled, washed with water, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and evaporated to give an amber gum (2.9 g) comprising 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranosyl bromide (50) and 2,3,4,6-tetra-*O*-acetyl-D-allopyranose (51) (2 : 1) (as assessed by t.l.c.). The crude gum (2.9 g) was dissolved in aqueous acetic acid (1 : 1) (32 ml) and zinc (5.13 g) was added in portions at 0 °C. The mixture was stirred for 1.5 h. Ethyl acetate (100 ml) was added, the slurry was filtered, and insoluble material was washed with ethyl acetate. The combined ethyl acetate filtrates were washed with water, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and evaporated. Chromatography on a silica gel column (110 × 2.5 cm) (20% acetone–hexane as eluant) gave 3,4,6-*tri*-O-*acetyl*-D-*allal* (19) (0.95 g, 50%), m.p. 83—85° (from ether–hexane) (Found: C, 53.2; H, 6.4. $C_{12}H_{16}O_7$ requires C, 52.9; H, 5.9%), *m/e* 272 ($M^{+\cdot}$), $[\alpha]_D$ +308.1° (in EtOH), $\nu_{max.}$($CHCl_3$) 1 740, 1 650, 1 220, and 1 070 cm^{-1}, δ($CDCl_3$) 2.03 (3 H, s, OAc), 2.08 (6 H, s, OAc), 4.95 (1 H, dd, $J_{1.2} = J_{2.3}$ = 6 Hz, H-2), 5.48 (1 H, dd, $J_{2.3}$ 6, $J_{3.4}$ 4 Hz, H-3), and 6.55 (1 H, d, $J_{1.2}$ 6 Hz, H-1), and 2,3,4,6-tetra-*O*-acetyl-D-allopyranose (51) (0.37 g, 15%). The latter on acetylation gave 1,2,3,4,6-penta-*O*-acetyl-β-D-allopyranose (49), m.p. 89—91°.

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*nitroso*-β-D-*altropyranosyl Chloride* (54).—3,4,6-Tri-*O*-acetyl-D-allal (19) (1.46 g) was dissolved in dry ethyl acetate (42 ml) and cooled to 0 °C under dry nitrogen. 2.99M-Nitrosyl chloride in ethyl acetate (50 ml) was added and the mixture was kept at 0 °C for 1.9 h. The solution was evaporated to dryness and the residue crystallized from ether to give the *altropyranosyl chloride* (54) (852 mg, 47%), m.p. 71—73° (decomp.) (from methylene chloride–ether) (Found: C, 42.45; H, 4.8; Cl, 10.6; N, 4.2. $C_{24}H_{32}Cl_2N_2O_{16}$ requires C, 42.7; H, 4.8; Cl, 10.5; N, 4.15%), $[\alpha]_D$ −67.8° ($CHCl_3$), $\nu_{max.}$($CHCl_3$) 1 750, 1 220, and 1 050 cm^{-1}, δ($CDCl_3$) 2.07, 2.12, and 2.15 (9 H, 3 s, OAc), 5.60 (1 H, dd, $J_{3.4} = J_{4.5}$ = 3 Hz, H-4), 5.77 (1 H, dd, $J_{1.2}$ 3.5, $J_{2.3}$ 9.5 Hz, H-2), 6.00 (1 H, dd, $J_{3.4}$ 3, $J_{2.3}$ 9.5 Hz, H-3), and 6.54 (1 H, d, $J_{1.2}$ 3.5 Hz, H-1). The mother liquors afforded material that was recrystallized from ether to give 3,4,6-*tri*-O-*acetyl*-1,2-*dideoxy*-2-*nitro*-D-*ribohex*-1-*enopyranose* (26) (168 mg, 10%), m.p. 150—151° (from ethyl acetate–ether) (Found: C, 45.2; H, 4.95. $C_{12}H_{15}NO_9$ requires C, 45.4; H, 4.8%), $[\alpha]_D$ +272.0° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 1 760, 1 650, 1 510, 1 220, and 1 050 cm^{-1}, δ($CDCl_3$) 2.04, 2.08, and 2.10 (9 H, 3 s, OAc), 6.43 (1 H, d, $J_{3.4}$ 4 Hz, H-3), and 8.31 (1 H, s, H-1). The residual mother liquors comprised a mixture of compounds (19), (54), and (26) in the ratio 1 : 1 : 1 (as assessed by t.l.c.).

Isopropyl 3,4,6-*Tri*-O-*acetyl*-2-*hydroxyimino*-α-D-*ribohexopyranoside* (31).—The β-D-altropyranosyl chloride (54) (852 mg) and dry propan-2-ol (1.17 ml) were dissolved in dry dimethylformamide (33 ml) and kept under argon at 25 °C for 20 h. The solution was evaporated to dryness and the residue was chromatographed on a silica gel column (47 × 2.5 cm) (20% acetone–hexane as eluant) to give the *pyranoside* (31) (628 mg, 69%) as a gum (Found: C, 49.8; H, 6.4; N, 3.7. $C_{15}H_{23}NO_9$ requires C, 49.9; H, 6.4; N, 3.9%), *m/e* 344 (*M* − 17), $[\alpha]_D$ +111.4° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 300, 1 740, 1 220, 1 040, and 1 020 cm^{-1}, δ(D_2O) 1.22 (*E*-isomer, 0.33 × 3 H, d, *J* 7 Hz, $OCHMe_2$), 1.24 (*Z*-isomer, 0.66 × 3 H, d, *J* 7 Hz, $OCHMe_2$), 1.28 (3 H, d, *J* 7 Hz, $OCHMe_2$), 2.04, 2.10, and 2.13 (9 H, s, OAc), 4.96 (*E*-isomer, 0.33 × 1 H, dd, $J_{3.4}$ 3.5, $J_{4.5}$ 10.5 Hz, H-4), 5.03 (*Z*-isomer, 0.66 × 1 H, dd, $J_{3.4}$ 3.5, $J_{4.5}$ 10 Hz, H-4), 5.26 (*E*-isomer, 0.33 × 1 H, s, H-1), 5.84 (*Z*-isomer, 0.66 × 1 H, d, $J_{3.4}$ 3.5 Hz, H-3), 6.03 (*Z*-isomer, 0.66 × 1 H, s, H-1), 6.54 (*E*-isomer, 0.33 × 1 H, d, $J_{3.4}$ 3.5 Hz, H-3), and 8.56br (1 H, s, NOH) (*Z* : *E* ratio 2 : 1).

Isopropyl 2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-α-D-*allopyranoside* (37) *and the Altropyranoside* (38).—The oxime (31) (600 mg) and acetic anhydride (2.6 ml) were dissolved in dry pyridine (6.8 ml) and kept at 25 °C for 17 h. Ethanol was added and after 0.5 h the mixture was evaporated to dryness and the residue azeotroped with toluene. The oxime acetate (32) was dissolved in dry tetrahydrofuran (21.6 ml) and cooled to 0 °C. M-Borane in tetrahydrofuran (18.4 ml) was added dropwise and the solution was stored at 7 °C for 30 h. The reaction was worked up as before. The resulting solid was taken up in pyridine (20 ml), acetic anhydride (6 ml) was added, and the solution was kept at 25 °C for 19 h. The product was worked up and chromatographed on a silica gel column (110 × 2.5 cm) (1% methanol–chloroform as eluant) to give the *allopyranoside* (37) (288 mg, 45%) [Found: $(M + 1)^+$, 390.1688. $C_{17}H_{28}NO_9$ requires $(M + 1)$, 390.1686], $[\alpha]_D$ +63.8° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 330, 1 740, 1 675, 1 210, and 1 040 cm^{-1}, δ($CDCl_3$) 1.16 (3 H, d, *J* 6 Hz, $OCHMe_2$), 1.27 (3 H, d, *J* 6 Hz, $OCHMe_2$), 2.00 (6 H, s, OAc), 2.10 (3 H, s, OAc), 2.15 (3 H, s, NHAc), 4.44 (1 H, ddd, $J_{1.2}$ 4, $J_{2.3}$ 3.5, $J_{2.NH}$ 9 Hz, H-2), 4.89 (1 H, d, $J_{1.2}$ 4 Hz, H-1), 4.97 (1 H, dd, $J_{3.4}$ 3.5, $J_{4.5}$ 9.5 Hz, H-4), 5.51 (1 H, dd, $J_{2.3} = J_{3.4}$ = 3.5 Hz, H-3), and 5.72br (1 H, d, $J_{2.NH}$ 9 Hz, NHAc), and the *altropyranoside* (38) (83 mg, 13%) (Found: C, 52.4; H, 7.1; N, 3.7%), *m/e* 390 $(M + 1)^+$, $[\alpha]_D$ +96.0° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 330, 1 730, 1 670, 1 210, and 1 040 cm^{-1}, δ($CDCl_3$) 1.18 (3 H, d, *J* 6 Hz, $OCHMe_2$), 1.30 (3 H, d, *J* 6 Hz, $OCHMe_2$), 2.04 (3 H, s, OAc), 2.10 (6 H, s, OAc), 2.13 (3 H, s, NHAc), 4.87 (1 H, dd, $J_{3.4}$ 2, $J_{4.5}$ 9.5 Hz, H-4), 5.07 (1 H, d, $J_{1.2}$ 3 Hz, H-1), and 6.21br (1 H, d, $J_{2.NH}$ 8 Hz, NHAc).

O-2-*Amino*-2-*deoxy*-α-D-*allopyranosyl*-(1 ⟶ 4)-*garamine* (5).— 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonyl-garamine (40) (8.96 g) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-β-D-altropyranosyl chloride (54) (6.6 g) were dissolved in dry, redistilled dimethylformamide (295 ml) and kept under argon at 25 °C for 137 h. The reaction was

worked up as before. The solid was chromatographed on a silica gel column (160 × 5 cm) [ethyl acetate–hexane (2 : 1) as eluant] to give O-3,4,6-*tri*-O-*acetyl*-2-*hydroxyimino*-α-D-ribo-*hexopyranosyl*-(1→4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (44) (1.68 g, 17%) (Found: C, 57.6; H, 5.7; N, 4.8. $C_{55}H_{66}N_4O_{23}$ requires C, 57.4; H, 5.8; N, 4.9%), $[\alpha]_D$ +113.7° (in MeOH), $\nu_{max.}$(CHCl$_3$) 3 330, 1 740, 1 690, 1 200, and 1 040 cm^{-1}, δ(CDCl$_3$) † 1.26br and 1.38br (3 H, 2 s, 4″-CH$_3$), 1.98br, 2.01br, 2.06br, and 2.12br (18 H, 4 s, OAc), 2.86br (3 H, s, 3″-NCH$_3$), 5.08br (6 H, s, C*H*$_2$Ph), 5.99br (≪1 H, s, H-1′, *Z*-isomer), and 7.33br (15 H, s, CH$_2$*Ph*).

The oxime (44) (1.2 g) was acetylated and the acetate (45) (1.18 g) reduced with borane in tetrahydrofuran (15.05 ml) as before (30 h). The residue was taken up in aqueous 5% sodium hydroxide (60 ml) and the mixture was heated under reflux for 14 h. The solution was cooled and neutralized with Amberlite IRC 50 (H$^+$) resin. The resin was washed with water and then 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) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give O-2-*amino*-2-*deoxy*-α-D-*allopyranosyl*-(1→4)-*garamine* (5) (25 mg, 5%) [Found: $(M + 1)^+$, 483.2510. $C_{19}H_{39}N_4O_{10}$ requires $(M + 1)$, 483.2522], $[\alpha]_D$ +131.0° (H_2O), $[\theta]_{290}$ −4,493 (TACu), δ(D_2O) 1.19 (3 H, s, 4″-CH$_3$), 2.50 (3 H, s, 3″-NCH$_3$), 2.99 (1 H, dd, $J_{1',2'}$ 4, $J_{2',3'}$ 3.5 Hz, H-2′), 5.08 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.12 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′). The less polar fraction was rechromatographed on a silica gel column (110 × 1 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant] to give O-2-*deoxy*-2-(*ethylamino*)-α-D-*allopyranosyl*-(1→4)-*garamine* (6) (46 mg, 9%) (Found: $M^{+\cdot}$, 510.2874. $C_{21}H_{42}N_4O_{10}$ requires M, 510.2901), $[\alpha]_D$ +139.1° (in H_2O), $[\theta]_{318}$ −2 588 (TACu), δ(D_2O) 1.07 (3 H, t, J 7 Hz, NCH$_2$C*H*$_3$), 1.19 (3 H, s, 4″-CH$_3$), 2.51 (3 H, s, 3″-NCH$_3$), 2.75 (2 H, q, J 7 Hz, NC*H*$_2$CH$_3$), 2.86 (1 H, dd, $J_{1',2'}$ 4, $J_{2',3'}$ 3.5 Hz, H-2′), 5.08 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.19 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′).

4,6-*Di*-O-*acetyl*-3-O-*methyl*-D-*glucal* (20).—1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose (52) (42 g) was dissolved in ethanol-free chloroform and a solution of titanium tetrabromide (54.1 g) in ethanol-free chloroform (250 ml) was added. The mixture was heated under reflux for 2.75 h, and then cooled and poured into water. The chloroform extract was washed with aqueous sodium hydrogen carbonate and water, dried (MgSO$_4$), and evaporated to give 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl bromide (53) (44 g, 99%) as an amber gum. The bromo-sugar (53) (44 g) was dissolved in 50% aqueous acetic acid (500 ml) and cooled to 0 °C. Powdered zinc (80 g) was added in portions with stirring and the mixture was stirred at 5 °C for 1.5 h. The zinc was filtered off and the solids were washed with water, ethyl acetate, and then water again. The water–ethyl acetate filtrates were combined and more ethyl acetate was added. The ethyl acetate layer was washed with aqueous sodium hydrogen carbonate and water and dried (MgSO$_4$). The solution was evaporated to dryness and the resulting gum was chromatographed on a silica gel column (160 × 5 cm) with acetone–hexane (1 : 8) as eluant to give 4,6-*di*-O-*acetyl*-3-O-*methyl*-D-*glucal* (20) (14.5 g, 51%) as a gum (Found: C, 53.8; H, 6.7. $C_{11}H_{16}O_6$ requires C, 54.1; H, 6.6%), *m/e* 213 $(M - 31)$, $[\alpha]_D$ +2.6° (in EtOH), $\nu_{max.}$ (film) 1 760, 1 650, 1 220, and 1 040 cm^{-1}, δ(CDCl$_3$) 2.05 (3 H, s, OAc), 2.06 (3 H, s, OAc), 3.32 (3 H, s, OCH$_3$), 3.80 (1 H, dddd, $J_{2.3} = J_{3.4} = 4$, $J_{1,3} = J_{1,5} = 1.5$ Hz, H-3), 4.90 (1 H, ddd, $J_{1,2}$ 6.5, $J_{2.3}$ 4, $J_{2.4}$ 1 Hz, H-2), 5.16 (1 H, ddd, $J_{3.4}$ 4, $J_{4.5}$ 5, $J_{2.4}$ 1 Hz, H-4), and 6.41 (1 H, dd, $J_{1.2}$ 6.5, $J_{1,3}$ 1.5 Hz, H-1).

4,6-*Di*-O-*acetyl*-2-*deoxy*-3-O-*methyl*-2-*nitroso*-α-D-*glucopyranosyl Chloride* (23).—4,6-Di-*O*-acetyl-3-*O*-methyl-D-glucal (20) (12.5 g) was dissolved in ethyl acetate (125 ml) and cooled to 0 °C. The solution was saturated with nitrosyl chloride (1.5 h) and then stirred at 0 °C for 1.5 h. The excess of nitrosyl chloride was removed by passing dry nitrogen for 2 h. On evaporation to a small volume the mixture crystallized and the crystals were washed with ether and collected, giving the *chloride* (23) (13.4 g, 85%) as needles, m.p. 117—118° (Found: C, 43.35; H, 5.3; Cl, 11.4; N, 4.3. $C_{22}H_{32}Cl_2N_2O_{14}$ requires C, 42.65; H, 5.2; Cl, 11.4; N, 4.5%), $[\alpha]_D$ +166.1° (in CHCl$_3$), $\nu_{max.}$(CHCl$_3$) 1 750, 1 220, and 1 040 cm^{-1}, δ(CDCl$_3$) 2.05 (3 H, s, OAc), 2.11 (3 H, s, OAc), 3.47 (3 H, s, OCH$_3$), 5.35 (1 H, dd, $J_{1,2}$ 3.5, $J_{2.3}$ 10 Hz, H-2), and 6.60 (1 H, d, $J_{1.2}$ 3.5 Hz, H-1).

Isopropyl 4,6-*Di*-O-*acetyl*-3-O-*methyl*-2-*hydroxyimino*-α-D-arabino-*hexopyranoside* (33).—The glucopyranosyl chloride (23) (850 mg) and propan-2-ol (1.17 ml) were dissolved in dry redistilled dimethylformamide (33 ml) and the solution was kept under argon at 25 °C for 42 h. The solution was evaporated to dryness and the residue was chromatographed on a silica gel column (110 × 2.5 cm) (20% acetone–hexane as eluant) to give the *oxime* (33) (465 mg, 51%) (Found: C, 50.6; H, 6.85; N, 4.3. $C_{14}H_{23}NO_8$ requires C, 50.45; H, 7.0; N, 4.2%), *m/e* 333 ($M^{+\cdot}$), $[\alpha]_D$ +46.3° (in CHCl$_3$), $\nu_{max.}$(CHCl$_3$) 3 200, 1 740, 1 220, and 1 030 cm^{-1}, δ(CDCl$_3$) 1.24 (3 H, d, J 6.5 Hz, OCH*Me*$_2$), 1.26 (3 H, d, J 6.5 Hz OCH*Me*$_2$), 2.10 (3 H, s, OAc), 2.12 (3 H, s, OAc), 3.53 (3 H, s, OCH$_3$), 4.20br (2 H, m, 6-CH$_2$), 4.24 (1 H, d, $J_{3.4}$ 9 Hz, H-3), 5.11 (1 H, dd, $J_{3.4} = J_{4.5} = 9$ Hz, H-4), 6.13 (1 H, s, H-1), and 9.15br (1 H, NOH).

Isopropyl 2-*Acetamido*-4,6-*di*-O-*acetyl*-2-*deoxy*-3-O-*methyl*-α-D-*glucopyranoside* (39).—The oxime (33) (380 mg) and acetic anhydride (2.2 ml) were dissolved in dry pyridine (6 ml) and the solution was kept at 25 °C for 17 h. The reaction was worked up as before. The oxime acetate (34) (345 mg) was dissolved in dry tetrahydrofuran (10 ml) and cooled to 0°. M-Borane in tetrahydrofuran (8.6 ml) was added and the solution was stored at 7 °C for 30 h. The reaction was worked up as before. The solid product was dissolved in dry pyridine (15 ml), acetic anhydride (5 ml) was added, and the reaction was allowed to proceed at 25 °C for 20 h. The product was worked up as before. Chromatography on a silica gel column (110 × 2.5 cm) (2% methanol–chloroform as eluant) gave the *amide* (39) (332 mg, 81%) as crystals (from methanol–chloroform), m.p. 108—110° (Found: C, 53.1; H, 7.6; N, 3.8. $C_{16}H_{27}NO_8$ requires C, 53.2; H, 7.5; N, 3.9%), *m/e* 362 $(M + 1)^+$, $[\alpha]_D$ +98.7° (in MeOH), $\nu_{max.}$(CHCl$_3$) 1 750, 1 680, 1 250, and 1 030 cm^{-1}, δ(CDCl$_3$) 1.16 (3 H, d, J 7 Hz, OCH*Me*$_2$), 1.24 (3 H, d, J 7 Hz, OCH*Me*$_2$), 2.03, 2.09, and 2.10 (9 H, s, OAc and NHAc), 3.38 (3 H, s, OCH$_3$), 4.96 (1 H, d, $J_{1.2}$ 4 Hz, H-1), and 5.70 (1 H, d, $J_{2.NH}$ 9 Hz, N*H*Ac).

Isopropyl 2-*Amino*-2-*deoxy*-3-O-*methyl*-α-D-*glucopyranoside* (55).—The amide (39) (41 mg) was dissolved in aqueous 5% sodium hydroxide (4 ml) and the solution was heated

† Same footnote as on page 1123.

under reflux for 17 h, cooled, and neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water and then eluted with 1.5N-ammonium hydroxide, and the basic eluate was evaporated to dryness. The solid was chromatographed on a silica gel column (53 × 0.7 cm) [lower phase of chloroform–methanol–15% ammonium hydroxide solution (2 : 1 : 1) as eluant] to give the *amine* (55) (14 mg, 52%) as a colourless amorphous solid after lyophilization [Found: $(M + 1)^+$, 236.1514. $C_{10}H_{22}NO_5$ requires $(M + 1)$, 236.1498], $[\alpha]_D$ +148.3° (in H_2O).

3′-O-*Methylgentamicin* X_2 (7).—2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (40) (18.2 g) and 4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-2-nitroso-α-D-glucopyranosyl chloride (23) (13.4 g) were dissolved in dry, redistilled dimethylformamide (600 ml) and the solution was kept at 25 °C for 90 h. The mixture was poured into ice–water and the solid was filtered off, washed with water, and dried. Chromatography on a silica gel column (160 × 7.5 cm), (1% methanol–chloroform as eluant) gave O-4,6-*di*-O-*acetyl*-3-O-*methyl*-2-*hydroxyimino*-β-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (56) (8.81 g, 37%) (Found: C, 57.55; H, 6.2; N, 4.8. $C_{54}H_{66}N_4O_{22}$ requires C, 57.75; H, 5.9; N, 5.0%), $[\alpha]_D$ +57.5° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 380, 1 750, 1 610, 1 220, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.27br and 1.38br (3 H, 2 s, 4″-CH_3), 1.92br, 2.01br, and 2.03br (15 H, 3 s, OAc), 2.85br (3 H, s, 3″-NCH_3), 3.09br (3 H, s, 3′-OCH_3), 5.04br (6 H, s, CH_2Ph), and 7.29br (15 H, s, $CH_2C_6H_5$), and O-4,6-*di*-O-*acetyl*-3-O-*methyl*-2-*hydroxyimino*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (46) (6.27 g, 26%) (Found: C, 57.4; H, 6.2; N, 4.9%), $[\alpha]_D$ +81.6° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 380, 1 740, 1 720, 1 220, and 1 030 cm^{-1}, δ($CDCl_3$) † 1.27br and 1.38br (3 H, 2 s, 4″-CH_3), 1.90br, 1.93br, and 2.02br (15 H, 3 s, OAc), 2.87br (3 H, s, 3″-NCH_3), 3.44br (3H, s, 3′-OCH_3), 5.07br (6 H, s, C$H_2C_6H_5$), 6.08br (1 H, s, H-1′), and 7.29br and 7.31br (15 H, 2 s, $CH_2C_6H_5$).

The β-oxime (56) (7.46 g) was acetylated and the acetate (57) (7.02 g) was reduced with M-borane in tetrahydrofuran (60.1 ml) as before (30 h). The resulting solid was dissolved in a 5% solution of sodium hydroxide in aqueous dioxan (1 : 1; 560 ml) and the solution was heated under reflux for 17 h, cooled, and neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water and then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness and the residue was taken up in 90% hydrazine hydrate (70 ml) and heated in a sealed vessel at 130 °C for 65 h. The solution was evaporated to dryness and the residue was taken up in first water and then methanol and recovered each time by evaporation. Chromatography on a silica gel column (160 × 5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] gave O-2-*amino*-2-*deoxy*-3-O-*methyl*-β-D-*mannopyranosyl*-(1 ⟶ 4)-*garamine* (58) (820 mg, 25%) (Found: C, 48.6; H, 8.2; N, 11.1. $C_{20}H_{40}N_4O_{10}$ requires C, 48.4; H, 8.1; N, 11.3%), $[\alpha]_D$ +87.8° (in H_2O), $[\theta]_{290}$ −8 390 (TACu), $[\theta]_{290}$ −6 980 (Cupra A), $\nu_{max.}$(KCl) 3 300 and 1 050 cm^{-1}, δ(D_2O) 1.18 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 3.41 (3 H, s, 3′-OCH_3), 4.77 (1 H, d, $J_{1',2}$ 1 Hz, H-1′), and 5.03 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″).

The α-oxime (46) (6.27 g) was acetylated and the acetate (47) (5.85 g) was reduced with M-borane in tetrahydrofuran (50.2 ml) as before (30 h). The product was deprotected as for the β-isomer. Chromatography on a silica gel column (160 × 5 cm) [chloroform–methanol–7% ammonium hydroxide solution (1 : 2 : 1) as eluant] gave 3′-O-*methylgentamixin* X_2 (7) (862 mg, 32%) (Found: C, 48.15; H, 8.2; N, 11.1. $C_{20}H_{40}N_4O_{10}$ requires C, 48.4; H, 8.1; N, 11.3%), $[\alpha]_D$ +162.0° (in H_2O), $[\theta]_{290}$ −8 380 (TACu), $[\theta]_{290}$ −6 920 (Cupra A), $\nu_{max.}$(KCl) 3 300 and 1 020 cm^{-1}, δ(D_2O) 1.18 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 3.58 (3 H, s, 3′-OCH_3), 5.04 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.22 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′).

We thank J. McGlotten and J. B. Morton and their staff for spectral and analytical services, and, particularly, R. Brambilla for kinetic data and off-resonance-decoupled n.m.r. spectra for the reaction products of nitrosyl chloride and the nitroso-chloro-adducts.

[5/1897 *Received*, 30*th September*, 1975]

† Same footnote as on page 1123.