Semisynthetic Aminoglycoside Antibacterials. Part IV.[1] Synthesis of Antibiotic JI-20A, Gentamicin B, and Related Compounds

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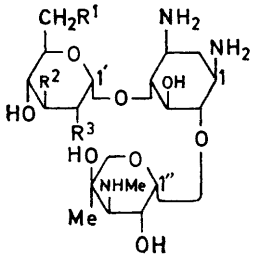
The application of the Lemieux-Nagabhushan reaction to the synthesis of antibiotic JI-20A and gentamicin B from a suitably protected garamine derivative is described. 3'-Deoxy-6'-*N*-methyl analogues of antibiotic JI-20A and gentamicin B have also been prepared from garamine intermediates. The transformation of 3'-deoxygentamicin X_2 into the 6'-deoxy-6'-methylamino-derivative is discussed.

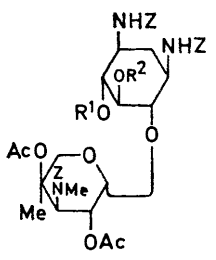
ANTIBIOTIC JI-20A (1) has been isolated as a minor component of the fermentation of a mutant strain of *Micromonospora purpurea*, identified as *M. purpurea* JI-20, and its structure has been elucidated.[2,3] Fermentation of *M. purpurea* has been shown to produce gentamicin B (2)[4] together with the gentamicin C complex, and the structure of compound (2) has been elucidated.[5] In view of the close structural similarities between antibiotic JI-20A (1) and gentamicin B (2), the application of the Lemieux–Nagabhushan reaction

[1] Part III, M. Kugelman, A. K. Mallams, and H. F. Vernay, preceding paper.
[2] H. Reimann, unpublished observations.
[3] Patent NL 7,308,046/1973 (Derwent 80078U).
[4] Patent BE 768,796/1971 (Derwent 81416S).
[5] P. J. L. Daniels, unpublished observations.

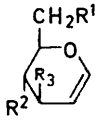
to their synthesis from 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) by using common glycal precursors seemed appropriate.



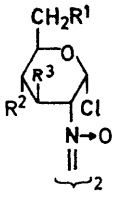
(1) $R^1=R^3=NH_2$, R^2-OH
(2) $R^1=NH_2$, $R^2=R^3$=OH
(3) $R^1=R^3=NH_2$, R^2=H
(4) $R^1=NH_2$, R^2=H, R^3=OH
(5) R^1=NHMe, R^2=H, R^3=OH
(6) R^1=OH, R^2=H, $R^3=NH_2$
(7) R^1=NHMe, R^2=H, $R^3=NH_2$



(8) R^1=H, R^2=Ac
(9) R^1=Ac, R^2=H
Z=$PhCH_2O\cdot CO$



(10) $R^1=R^2=R^3$=OAc
(11) $R^1=R^2=R^3$=OH
(12) R^1=OTs, $R^2=R^3$=OAc
(13) $R^1=N_3$, $R^2=R^3$=OAc
(14) $R^1=R^2$=OH, R^3=H
(15) R^1=OTs, R^2=OAc, R^3=H
(16) $R^1=R^2$=OTs, R^3=H.
(17) $R^1=N_3$, R^2=OAc, R^3=H
(18) $R^1=NH_2$, R^2=OH, R^3=H
(19) R^1=NHAc, R^2=OAc, R^3=H

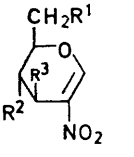


(20) $R^1=N_3$, $R^2=R^3$=OAc
(21) R^1=OTs, R^2=OAc, R^3=H
(22) $R^1=N_3$, R^2=OAc, R^3=H
(23) R^1=NHAc, R^2=OAc, R^3=H

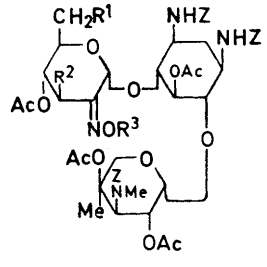
Ammonolysis of tri-*O*-acetyl-D-glucal (10) afforded D-glucal (11), which on treatment with 3 equiv. of tosyl chloride at 7 °C followed by acetylation, gave a 50% yield of 3,4-di-*O*-acetyl-6-*O*-tosyl-D-glucal (12).[6] Treatment of the latter with sodium azide gave 3,4-di-*O*-acetyl-6-azido-6-deoxy-D-glucal (13), which reacted with an excess of nitrosyl chloride to give crystalline dimeric 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-nitroso-α-D-glucopyranosyl chloride (20). When the reaction was carried out at −30 °C for 1.25 h, an 81% yield of the nitroso-chloro-adduct (20) was obtained. However, when the reaction was carried out at 0 °C over 4 h, the yield of the nitroso-chloro-adduct (20) was only 52%, and 3,4-di-*O*-acetyl-6-azido-1,2,6-trideoxy-2-nitro-D-*arabino*-hex-1-enopyranose (24) was also formed, in 46% yield. The nitroglucal (24) showed the expected u.v. maximum at 274 nm due to the αβ-unsaturated nitro-chromophore. The i.r. spectrum contained a vinylic ether band at 1 650 cm^{-1} and a nitro-stretching absorption at 1 520 cm^{-1}. The ^1H n.m.r. spectrum showed a characteristic H-1 singlet at δ 8.35; assignments were confirmed by decoupling experiments. The formation of the nitroglucal (24) probably occurs as described previously.[1] When the glucal (13) reacted with an excess of nitrosyl chloride for 24 h, the principal product isolated was the nitroglucal (24).

Condensation of the nitroso-chloro-adduct (20) with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8), under the usual conditions of the Lemieux–Nagabhushan reaction, afforded a 29% yield of *O*-3,4-di-*O*-acetyl-6-azido-6-deoxy-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27).* The ^1H n.m.r. spectrum of the oxime (27) showed a broad singlet at δ 6.12 indicating that it existed in the *Z*-configuration.[8] Acetylation gave the acetate (28), which on reduction with 23 equiv. of borane, followed by catalytic hydrogenation over 30% palladium–carbon and alkaline hydrolysis, gave antibiotic JI-20A (1) in 6% yield based on (27). The mass spectrum of the product (1) (Table) was in agreement[9,10] with the structure and the c.d. data supported the 4-*O*-glycosyl linkage. The ^1H n.m.r. spectrum revealed a doublet at δ 5.31 ($J_{1',2'}$ 3.5 Hz) in agreement with a 4-*O*-α-D-*gluco*-structure. The synthetic sample was identical with the natural antibiotic JI-20A (1).[2]

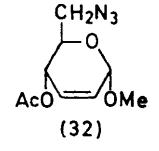
Gentamicin B (2) was synthesised from the oxime (27) as follows. Treatment with aluminium isopropoxide in propan-2-ol[11] gave the 2′-oxo-derivative, which was



(24) $R^1=N_3$, $R^2=R^3$=OAc
(25) $R^1=N_3$, R^2=OAc, R^3=H
(26) R^1=NHAc, R^2=OAc, R^3=H



(27) $R^1=N_3$, R^2=OAc, R^3=H
(28) $R^1=N_3$, R^2=OAc, R^3=Ac
(29) R^1=OTs, $R^2=R^3$=H
(30) $R^1=N_3$, $R^2=R^3$=H
(31) $R^1=N_3$, R^2=H, R^3=Ac



(32)

reduced with sodium borohydride. The product was hydrogenated over 10% palladium–carbon and deprotected by heating with aqueous 5% sodium hydroxide

* Whenever the triacetate (8) was used in a Lemieux–Nagabhushan reaction some unchanged (8) was isolated as well as the transacylation product (9).[7] Details of the isolation of these products are not included in the Experimental section.

[6] T. L. Nagabhushan, *Canad. J. Chem.*, 1970, **48**, 257.
[7] M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, and M. Tanate, *J.C.S. Perkin I*, 1976, 1088.
[8] R. U. Lemieux, R. A. Earl, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 19.
[9] P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Comm.*, 1971, 1629.
[10] P. J. L. Daniels, A. K. Mallams, J. Weinstein, J. J. Wright, and G. W. A. Milne, *J.C.S. Perkin I*, 1976, 1078.
[11] J. K. Sugden, *Chem. and Ind.*, 1972, 680.

to give gentamicin B (2) in a 6% yield. The physical constants were in agreement with those of an authentic sample.[5]

The synthesis of 3′-deoxy-analogues of antibiotic JI-20A and gentamicin B was undertaken in view of the fact that both these antibiotics are susceptible to inactivation by resistant strains of bacteria carrying R-factor enzymes, some of which phosphorylate the 3′-hydroxy-groups whereas others acetylate the 6′-amino-groups. In order to use the Lemieux–Nagabhushan reaction it was necessary first to obtain the glycal precursors. 3′-Deoxy-D-glucal (14) was prepared [1] by a literature procedure;[11] on treatment with 3 equiv. of tosyl chloride at 7 °C followed by acetylation, a 76% yield of 4-*O*-acetyl-3-deoxy-6-*O*-tosyl-D-glucal (15) was obtained. The by-product was 3-deoxy-4,6-di-*O*-tosyl-D-glucal (16), isolated in 14% yield. Treatment of (15) with 1.04 equiv. of nitrosyl chloride at 0 °C for 1.5 h afforded the nitroso-chloro-adduct (21) in 74% yield. The ^{1}H n.m.r. spectrum at 60 MHz showed the characteristic lack of definition previously observed with 3-deoxy-nitroso-chloro-adducts,[1] and the anomeric proton gave rise to a pair of doublets at δ 6.53 and 6.65 ($J_{1,2}$ 3.5 Hz), consistent with an α-chloro-substituent at C-1.

When 4-*O*-acetyl-3-deoxy-6-*O*-tosyl-D-glucal (15) was treated with sodium azide, a 60% yield of 4-*O*-acetyl-6-azido-3,6-dideoxy-D-glucal (17) was obtained. The latter exhibited a strong azide i.r. band at 2 100 cm^{-1}. Treatment of the glucal (17) with 1.5 equiv. of nitrosyl chloride at 0 °C for 0.5 h gave dimeric 4-*O*-acetyl-6-azido-2,3,6-trideoxy-2-nitroso-α-D-*ribo*-hexopyranosyl chloride (22) as a gum. The ^{1}H n.m.r. spectrum of (22) again showed a pair of doublets, at δ 6.71 and 6.84 ($J_{1,2}$ 3.5 Hz) due to the anomeric proton.[1] Crystallization of the gum from ether afforded low yields of a crystalline form of (22), the i.r. spectrum of which was identical with that of the gum. The ^{1}H n.m.r. spectrum of the crystalline sample showed a single anomeric proton signal as a doublet at δ 6.73 ($J_{1,2}$ 3.5 Hz). The crystalline sample showed a high positive specific rotation. When the nitroso-chloro-adduct (22) was dissolved in ethyl acetate and the solution was saturated with nitrosyl chloride at 0 °C, gradual conversion into 4-*O*-acetyl-6-azido-2,3,6-trideoxy-2-nitro-D-*erythro*-hex-1-enopyranose (25) occurred. The nitroglucal (25) showed a vinylic ether absorption at 1 650 cm^{-1} and a nitro stretching band at 1 500 cm^{-1} in the i.r. spectrum. The ^{1}H n.m.r. spectrum showed a characteristic singlet at δ 8.21 due to the anomeric proton.

The reaction of 3,4-di-*O*-acetyl-6-azido-6-deoxy-D-glucal (13) with methanol in the presence of boron trifluoride–ether in benzene afforded methyl 4-*O*-acetyl-6-azido-2,3,6-trideoxy-α-D-*erythro*-hex-2-enopyranoside (32), which on reduction with lithium aluminium hydride in refluxing dioxan [12] gave 6-amino-6-deoxy-D-glucal (18). Acetylation of the amine (18) gave the diacetate (19). The i.r. spectrum of (19) contained a single band at 1 670 cm^{-1} due to the vinylic ether and amide groups. The ^{1}H n.m.r. spectrum showed a singlet at δ 2.09 due to the *N*-acetyl group and was in full agreement with the 3-deoxyglucal structure. The glucal (19) reacted with 1.5 equiv. of nitrosyl chloride at 0 °C for 0.5 h to give the nitroso-chloro-adduct (23) in 90% yield. The i.r. spectrum of (23) showed an *N*-acetyl band at 1 660 cm^{-1}. The ^{1}H n.m.r. spectrum of (23) in [$^2H_7$]dimethylformamide showed a singlet at δ 2.08 (NAc) and a doublet at δ 6.75 ($J_{1,2}$ 3.5 Hz, H-1). Under the above conditions no *N*-nitroso-derivative was isolated. The nitroso-chloro-adduct (23) was very labile in solution: almost complete conversion into the nitroglucal (26) was achieved after 20 min in dimethyl sulphoxide or 68 h in dimethylformamide. The presence of the product (26) was indicated by the disappearance of the doublet due to the anomeric proton of (23) and the formation of a singlet at δ 8.28 in either solvent. Attempted condensations of the nitroso-chloro-adduct (23) with either propan-2-ol or 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) in dimethylformamide gave no α-glycosides, probably owing to the ready formation of the unreactive nitroglucal (26).

Condensation of 4-*O*-acetyl-2,3-dideoxy-2-nitroso-6-*O*-tosyl-α-D-*ribo*-hexopyranosyl chloride (21) with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) in dimethylformamide at 25 °C afforded a 50% yield of *O*-4-*O*-acetyl-3-deoxy-2-hydroxyimino-6-*O*-tosyl-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29). The absence of any ^{1}H n.m.r. singlet due to the anomeric H-1′ at δ *ca.* 6.0 suggested that the oxime existed in the *E*-configuration,[8] as demonstrated earlier for 3′-deoxy-oximes.[1] The oxime (29) reacted with sodium azide to

[12] B. Fraser-Reid and B. Radatus, *J. Amer. Chem. Soc.*, 1970, **92**, 6661.

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

Compd.	$(M+1)^+$	$M^{+\cdot}$	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}	A_{11}	A_{12}
(1)	482 (0.7)		351 (8)	333 (1)	323 (6)	305 (8)	350 (8)	332 (5)	322 (10)	304 (22)	191 (85)	173 (35)	163 (75)	145 (100)
(2)	483 (1)	482 (0.2)	352 (16)	334 (0.5)	324 (8)	306 (6)	350 (0.1)	332 (0.2)	322 (6)	304 (9)	191 (85)	173 (12)	163 (60)	145 (100)
(3)	466 (1)		335 (13)	317 (2)	307 (5)	289 (11)	350 (8)	332 (4)	322 (33)	304 (49)	191 (38)	173 (16)	163 (50)	145 (90)
(4)	467 (0.2)		336 (7)	318 (2)	308 (4)	290 (4)	350 (2)	332 (1)	322 (7)	304 (7)	191 (100)	173 (20)	163 (13)	145 (90)
(5)	481 (0.5)		350 (21)	332 (3)	322 (14)	304 (9)	350 (21)	332 (3)	322 (14)	304 (9)	191 (29)	173 (11)	163 (23)	145 (39)
(7)	480 (2)	479 (0.4)	349 (29)	331 (2)	321 (10)	303 (6)	350 (11)	332 (6)	322 (19)	304 (27)	191 (22)	173 (15)	163 (24)	145 (56)

Compd.	B_1	C_1	D_9	D_{10}	D_{11}	E_1	E_2	E_3	E_4	F_1	F_2
(1)	161 (70)	160 (80)	362 (2)	203 (3)		406 (2)	246 (21)	364 (3)	204 (36)	290 (10)	289 (5)
(2)	162 (67)	160 (77)			422 (1)	407 (2)	246 (10)	365 (3)	204 (10)	291 (12)	289 (5)
(3)	145 (90)	160 (100)	362 (2)	203 (4)	405 (1)	390 (2)	246 (12)	348 (5)	204 (7)	274 (17)	289 (11)
(4)	146 (69)	160 (74)			406 (1)	391 (1)	246 (15)	349 (2)	204 (19)	275 (8)	289 (6)
(5)	160 (100)	160 (100)			406 (1)	405 (1)	246 (4)	363 (4)	204 (4)	289 (33)	289 (33)
(7)	150 (100)	160 (95)	362 (10)	203 (8)	405 (1)	404 (2)	246 (8)	362 (10)	204 (4)	288 (42)	289 (17)

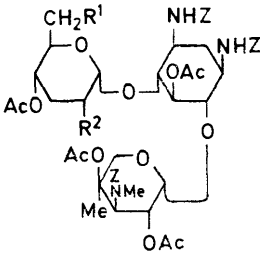
* The structures and designations of all fragment ions are identical with those described in ref. 10.

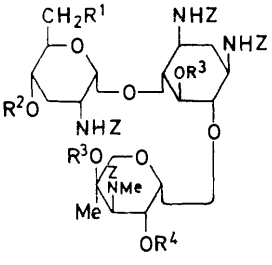
give the 6′-azide (30). Reduction of the oxime acetate (31), prepared in the usual manner, with 11 equiv. of borane, followed by catalytic hydrogenation over 10% palladium–carbon and deprotection by basic hydrolysis, afforded a 4% yield [based on (29)] of *O*-2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (3′-deoxy-antibiotic JI-20A) (3). The mass spectrum of (3) (Table) exhibited the expected fragment ions.[9,10] The ^{1}H n.m.r. spectrum contained a doublet at δ 5.14 ($J_{1',2'}$ 3.5 Hz) due to H-1′ indicating an α-D-*ribo*-configuration. The c.d. data were also in accord with structure (3).

When 4-*O*-acetyl-6-azido-2,3,6-trideoxy-2-nitroso-α-D-ribopyranosyl chloride (22) was condensed with 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) in dimethylformamide in the presence of *NN*,2,6-tetramethylaniline, a 70% yield of *O*-4-*O*-acetyl-6-azido-3,6-dideoxy-2-hydroxyimino-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (30) was obtained; this existed in the *E*-configuration. The azide (30) was reduced and deprotected as previously to give 3′-deoxy-antibiotic JI-20A (3).

The synthesis of 3′-deoxygentamicin B (4) was undertaken and two deoximation procedures were investigated. The oxime (30) was converted into the acetate (31), which on treatment with chromium(II) acetate[13,14] afforded the 2′-ketone; this was reduced directly with sodium borohydride and acetylated to give a 10% yield of *O*-6-acetamido-2,4-di-*O*-acetyl-3,6-dideoxy-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (33). In view of the low yield of (33), an alternative deoximation procedure was investigated. Thus *O*-4-*O*-acetyl-3-deoxy-2-hydroxyimino-6-*O*-tosyl-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29) on treatment with levulinic acid [8] followed by reduction of the 2′-ketone with sodium borohydride gave a 35% yield of the 2′-alcohol (34). Acetylation of (34) afforded the 2′-acetate (35). The displacement of the 6′-tosyl group in (34) was effected with ammonia in methanol in a bomb at 135 °C, and the product was deprotected by heating with 90% hydrazine hydrate followed by aqueous 5% sodium hydroxide to give 3′-deoxygentamicin B (4) in 20% yield based on (34). When the displacement of the tosyl group in (34) was effected with methylamine and the product was deprotected as described above, a 48% yield of 3′-deoxy-6′-*N*-methylgentamicin B (5) was obtained. The ^{1}H n.m.r. spectrum of (5) showed a singlet at δ 2.33 due to the 6′-*N*-methyl group.

The relative ease of preparation of 3′-deoxygentamicin X_2 (6) [1] by the Lemieux–Nagabhushan reaction made it an attractive starting material from which to synthesize *O*-2-amino-2,3,6-trideoxy-6-methylamino-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (7) by direct transformation of the pseudotrisaccharide. 3′-Deoxygentamicin X_2 (6) was converted in high yield by standard procedures into 1,2′,3,3″-tetrakis-*N*-benzyloxycarbonyl-3′-deoxygentamicin X_2 (36). The latter was converted into the 6′-*O*-trityl derivative, which on acetylation with acetic anhydride in pyridine at 25 °C afforded 2″,4′-di-*O*-acetyl-1,2′,3,3″-tetrakis-*N*-benzyloxycarbonyl-3′-deoxy-6′-*O*-tritylgentamicin X_2 (37). The latter on heating with glacial acetic acid was detritylated to give (38), which on





(33) R^1 = NHAc, R^2 = OAc
(34) R^1 = OTs, R^2 = OH
(35) R^1 = OTs, R^2 = OAc

(36) R^1 = OH, R^2 = R^3 = R^4 = H
(37) R^1 = OCPh$_3$, R^2 = R^4 = Ac, R^3 = H
(38) R^1 = OH, R^2 = R^4 = Ac, R^3 = H
(39) R^1 = OTs, R^2 = R^4 = Ac, R^3 = H
(40) R^1 = OTs, R^2 = R^3 = R^4 = H
(41) R^1 = OTs, R^2 = Ts, R^3 = R^4 = H
(42) R^1 = OTs, R^2 = R^3 = R^4 = Ac

treatment with tosyl chloride in pyridine afforded 2″,4′-di-*O*-acetyl-1,2′,3,3″-tetrakis-*N*-benzyloxycarbonyl-3′-deoxy-6′-*O*-tosylgentamicin X_2 (39). The latter on heating with methylamine in methanol in a bomb at 135 °C, followed by deprotection by heating first with aqueous 5% sodium hydroxide and then with 90% hydrazine hydrate, gave a 36% yield of *O*-2-amino-2,3,6-trideoxy-6-methylamino-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (7).

An alternative procedure for the preparation of (7), in higher yield, was as follows. Direct tosylation of 1,2′,3,3″-tetrakis-*N*-benzyloxycarbonyl-3′-deoxygentamicin X_2 (36) with 4 equiv. of tosyl chloride in pyridine at 25 °C gave a 56% yield of the 6′-*O*-tosyl derivative (40) together with a 38% yield of the di-*O*-tosyl derivative (41), which was tentatively assigned the 4′,6′-structure. When the reaction was carried out at 7 °C with 2.7 equiv. of tosyl chloride the yield of the 6′-*O*-tosyl derivative (40) was 66%. The 6′-*O*-tosyl derivative (40), on acetylation with acetic anhydride–concentrated hydrochloric acid (9 : 1) at 25 °C, was converted into 2″,4′,4″,5-tetra-*O*-acetyl-1,2′,3,3″-tetrakis-*N*-benzyloxycarbonyl-3′-deoxy-6′-*O*-tosylgentamicin X_2 (42) in 95% yield. The acetate (42) on heating with methylamine in methanol in a bomb at 135 °C, followed by deprotection by heating with 90% hydrazine hydrate, afforded *O*-2-amino-2,3,6-trideoxy-6-methylamino-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (7) in 40% yield. Similarly the alcohol (40) on treatment with methylamine in methanol in a bomb, followed by alkaline hydrolysis with aqueous 5% sodium hydroxide and then 90%

[13] M. R. Hatfield, *Inorg. Synth.*, 1950, **3**, 148.
[14] E. J. Corey and J. E. Richman, *J. Amer. Chem. Soc* 1970, **92**, 5276.

hydrazine hydrate, gave a 38% yield of (7). The ^1H n.m.r. spectrum showed a singlet at δ 2.26 due to the 6′-*N*-methyl group.

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

EXPERIMENTAL

All physical data were recorded as described in Part I.[7]

3,4-Di-O-acetyl-6-O-tosyl-D-glucal (12).—3,4,6-Tri-*O*-acetyl-D-glucal (10) (25 g) dissolved in a saturated solution of ammonia in methanol (800 ml) was kept at 25 °C for 20 h. The solution was concentrated and azeotroped with toluene. The D-glucal (11) was dissolved in pyridine (300 ml) and tosyl chloride (52 g) dissolved in pyridine (500 ml) was added to the solution at 0°; the mixture was kept at 7 °C for 25 h. Acetic anhydride (172 ml) was added and the mixture was kept at 7 °C for 24 h. The solution was evaporated to dryness and the resulting gum was azeotroped with toluene and then chromatographed on a silica gel column (157 × 7.5 cm) (1% methanol–chloroform as eluant) to give 3,4-di-*O*-acetyl-6-*O*-tosyl-D-glucal (12) [6] (17.7 g, 50%) as crystals, m.p. 104—106° (from ether) (Found: C, 53.25; H, 5.4; N, 8.4. Calc. for $C_{17}H_{20}O_8S$: C, 53.1; H, 5.2; N, 8.3%), *m/e* 384 ($M^{+\cdot}$), $[\alpha]_D$ +38.3° (in MeOH), $\nu_{max.}$($CHCl_3$) 1 750, 1 660, 1 600, 1 370, 1 220, and 1 180 cm^{-1}, δ($CDCl_3$) 2.02 (6 H, s, OAc), 2.45 (3 H, s, $C_6H_4CH_3$), 4.25 (2 H, m, 6-H_2), 6.38 (1 H, dd, $J_{1,2}$ 6.5 *J* 1.5 Hz, H-1), and 7.37 and 7.83 (each 2 H, d, *J* 6.5 Hz, $C_6H_4CH_3$).

3,4-Di-O-acetyl-6-azido-6-deoxy-D-glucal (13).—3,4-Di-*O*-acetyl-6-*O*-tosyl-D-glucal (12) (50 g) was dissolved in hexamethylphosphoramide (800 ml), sodium azide (34 g) was added, and the solution was stirred at 25 °C for 24 h. The hexamethylphosphoramide was distilled off under high vacuum and the resulting gum was taken up in chloroform. The solution was washed with water, dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on a silica gel column (150 × 7.5 cm) (7% acetone–hexane as eluant) to give 3,4-*di*-O-*acetyl*-6-*azido*-6-*deoxy*-D-*glucal* (13) (25 g, 75%) as a gum (Found: C, 47.2; H, 5.3; N, 16.4. $C_{10}H_{13}N_3O_5$ requires C, 47.1; H, 5.1; N, 16.5%), *m/e* 255 ($M^{+\cdot}$), $[\alpha]_D$ +25.1° (in MeOH), $\nu_{max.}$($CHCl_3$) 2 110, 1 755, 1 660, 1 220, and 1 040 cm^{-1}, δ($CDCl_3$) 2.06 (3 H, s, OAc), 2.09 (3 H, s, OAc), 4.91 (1 H, ddd, $J_{1,2}$ 6.5, $J_{2,3}$ 3, *J* 1.5 Hz, H-2), and 6.53 (1 H, dd, $J_{1,2}$ 6.5 Hz, *J* 1.5 Hz, H-1).

3,4-Di-O-acetyl-6-azido-2,6-dideoxy-2-nitroso-α-D-glucopyranosyl Chloride (20).—(i) 3,4-Di-*O*-acetyl-6-azido-6-deoxy-D-glucal (13) (4.5 g) was dissolved in dry ether (70 ml) and cooled to *ca.* −30 °C. Liquid nitrosyl chloride (12 ml) was added with stirring and the solution was kept at −30 °C for 1.25 h. It was then concentrated *in vacuo* to give 3,4-*di*-O-*acetyl*-6-*azido*-2,6-*dideoxy*-2-*nitroso*-α-D-*glucopyranosyl chloride* (20) (4.55 g, 81%) as crystals, m.p. 109—111° (from ether) (Found: C, 37.4; H, 3.9; Cl, 11.1; N, 17.4. $C_{20}H_{26}Cl_2N_8O_{12}$ requires C, 37.45; H, 4.1; Cl, 11.05; N, 17.5%), $[\alpha]_D$ +248.8° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 2 100, 1 760, 1 220, and 1 050 cm^{-1}, δ($CDCl_3$) 2.00 (3H s, OAc), 2.07 (3 H, s, OAc), and 6.70 (1 H, d, $J_{1,2}$ 3.7 Hz, H-1).

(ii) A solution of 3,4-di-*O*-acetyl-6-azido-6-deoxy-D-glucal (13) (7 g) in dry ethyl acetate (80 ml) was flushed with dry nitrogen. Nitrosyl chloride gas was bubbled slowly through with stirring at 0 °C for 1 h, and stirring was continued for a further 1.5 h. The solution was flushed with dry nitrogen for 1.5 h and then evaporated to dryness. The residue was crystallized from dry ether to give the adduct (20) (4.55 g, 52%) as crystals, identical with those described in (i). The mother liquors were concentrated to afford 3,4-di-*O*-acetyl-6-azido-1,2,6-trideoxy-2-nitro-D-*arabino*-hex-1-enopyranose (24) * (3.6 g, 46%) as a gum. A portion of the gum was subjected to preparative layer chromatography on silica gel plates (40% acetone–hexane) to give a sample, $[\alpha]_D$ +11.2° (in $CHCl_3$), $\lambda_{max.}$($CHCl_3$) 274 nm (ε 6 350), $\nu_{max.}$($CHCl_3$) 2 110, 1 760, 1 650, 1 520, 1 370, 1 350, 1 200, and 1 030 cm^{-1}, δ($CDCl_3$) 2.12 (6 H, s, OAc), 3.52 (1 H, dd, $J_{5,6a}$ 6, $J_{6a,6b}$ 14 Hz, H-6a), 3.80 (1 H, dd, $J_{5,6b}$ 8, $J_{6a,6b}$ 14 Hz, H-6b), 4.66 (1 H, dddd, $J_{4,5}$ 2.5, $J_{3,5}$ 2, $J_{5,6b}$ 8, $J_{5,6a}$ 6 Hz, H-5), 5.27 (1 H, dd, $J_{3,4}$ 2.5, $J_{4,5}$ 2.5 Hz, H-4), 6.02 (1 H, dd, $J_{3,4}$ 2.5, $J_{3,5}$ 2 Hz, H-3), and 8.35 (1 H, s, H-1).

Antibiotic JI-20A (1).—2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) (6 g) and 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-nitroso-α-D-glucopyranosyl chloride (20) (4.5 g) were dissolved in dry redistilled dimethylformamide (205 ml) and kept at 25 °C for 94 h. The product was worked up as before and chromatographed on a silica gel column (160 × 2.5 cm) (1% methanol–chloroform as eluant) to give O-3,4-*di*-O-*acetyl*-6-*azido*-6-*deoxy*-2-*hydroxyimino*-α-D-arabino-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (27) (2.4 g, 29%), m.p. 129—137° (Found: C, 56.05; H, 6.0; N, 8.5. $C_{53}H_{63}N_7O_{21}$ requires C, 56.15; H, 5.6; N, 8.65%), $[\alpha]_D$ +112.0° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 350, 2 110, 1 740, 1 710, 1 220, and 1 035 cm^{-1}, δ($CDCl_3$) † 1.23br and 1.36br (3 H, 2 s, 4″-CH_3), 1.92br (15 H, s, OAc), 2.82br (3 H, s, 3″-NCH_3), 5.02br (6 H, s, CH_2Ph), 6.12br (1 H, s, H-1′), and 7.23br (15 H, s, Ph).

The oxime (27) (1.3 g) was acetylated and the acetate (28) was reduced with M-borane in tetrahydrofuran (18 ml) as before. The residue was taken up in methanol and hydrogenated over 30% palladium–carbon at 60 lb in^{-2} at 25 °C for 18 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in aqueous dioxan (1 : 1; 60 ml) containing sodium hydroxide (3 g) and the solution was heated under reflux at 120 °C for 16 h, cooled, and neutralized with Amberlite IRC 50 (H^+) resin. After washing with water, the resin was eluted with 1.5N-ammonium hydroxide and the eluate was evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm) [chloroform–methanol–7% ammonium hydroxide (2 : 1 : 1) as eluant] to give *antibiotic JI-20A* (1) (34 mg, 6%) (Found: C, 45.4; H, 7.7; N, 13.1. $C_{19}H_{39}N_5O_9,CO_2$ requires C, 45.7; H, 7.5; N, 13.3%), $[\alpha]_D$ +149.8° (in H_2O), $[\theta]_{290}$ 11 000 (TACu), $[\theta]_{290}$ −8 840 (Cupra A), $\nu_{max.}$(KCl) 3 350 and 1 050 cm^{-1}, δ(D_2O) 1.21 (3 H, s, 4″-CH_3), 2.53 (3 H, s, 3″-NCH_3), 5.07 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.31 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′), identical with natural antibiotic JI-20A (1).[2]

Gentamicin B (2).—*O*-3,4-Di-*O*-acetyl-6-azido-6-deoxy-2-hydroxyimino-α-D-*arabino*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (27) (4.2 g) was dissolved in dry propan-2-ol (350 ml). Aluminium isopropoxide (13.6 g) was added and the mixture was heated under reflux for 23 h. The solution was concentrated to half its volume, shaken with 2N-hydrochloric acid, and then evaporated to a slurry. The slurry was diluted with

* Satisfactory C,H,N analyses were not to be obtained for this compound.

† Mixture of rotamers at ambient temperatures.

water (50 ml) and extracted with chloroform. The extract was dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was dissolved in dioxan (200 ml) and water (20 ml) and cooled to 0 °C. Sodium borohydride (4 g) dissolved in water (80 ml) and dioxan (40 ml) was added, and the solution was stirred at 0 °C for 0.5 h and at 25 °C for 1 h. The excess of hydride was destroyed with acetic acid and the mixture was concentrated to dryness. The residue was dissolved in methanol and hydrogenated over 10% palladium–carbon at 60 lb in^{-2} and 25 °C for 18 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in aqueous 5% sodium hydroxide (60 ml) and heated under reflux for 18 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin, the resin was eluted with water, and the eluate was discarded. The resin was then eluted with 1.5N-ammonium hydroxide and the eluate was evaporated to dryness. The residue was chromatographed on a silica gel column (160 × 2.5 cm) [the lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give *gentamicin B* (2). Rechromatography on a silica gel column (110 × 1 cm) with the same eluant afforded a sample (100 mg, 6%), $[\alpha]_D$ +108.1° (in H_2O), $[\theta]_{282}$ −8 320 (TACu), $[\theta]_{290}$ −6 262 (Cupra A), $\nu_{max.}$(KCl) 3 300 and 1 050 cm^{-1}, $\delta(D_2O)$ 1.20 (3 H, s, 4″-CH_3), 2.51 (3 H, s, 3″-NCH_3), 5.08 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.33 (1 H, d, $J_{1',2'}$ 3 Hz, H-1′), identical with the natural product (2).[5]

4-O-*Acetyl*-3-*deoxy*-6-O-*tosyl*-D-*glucal* (15).—3-Deoxy-D-glucal (14) (2 g) was dissolved in dry pyridine (50 ml) and cooled in an ice-bath. Tosyl chloride (8.8 g) in dry pyridine (100 ml) at 0 °C was added and the mixture was maintained at 7 °C for 22 h. Acetic anhydride (10 ml) was then added and the mixture was kept at 7 °C for 19 h, concentrated to small volume, poured into water, and extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), filtered, and evaporated. The residue was azeotroped with toluene and then chromatographed on a silica gel column (160 × 2.5 cm) (chloroform as eluant) to give 4-O-*acetyl*-3-*deoxy*-6-O-*tosyl*-D-*glucal* (15) (3.83 g, 76%) as plates (from chloroform), m.p. 76—79° (Found: C, 55.0; H, 5.8; N, 10.0. $C_{15}H_{18}O_6S$ requires C, 55.20; H, 5.6; N, 9.8%), *m/e* 327 $(M + 1)^+$, $[\alpha]_D$ +105.5° (in MeOH), $\nu_{max.}$ (film) 1 740, 1 660, 1 360, and 1 240 cm^{-1}, $\delta(CDCl_3)$ 2.01 (3 H, s, OAc), 2.43 (3 H, s, $SO_2C_6H_4CH_3$), 4.66 (1 H, ddd, $J_{1,2}$ 6, $J_{2,3}$ 4.5, $J_{2,3'}$ 3 Hz, H-2), and 6.25 (1 H, ddd, $J_{1,2}$ 6, J 2, J 2 Hz, H-1). The less polar fractions from the column afforded 3-*deoxy*-4,6-*di*-O-*tosyl*-D-*glucal* (16) (0.93 g, 14%) as a gum (Found: C, 53.1; H, 4.8; S, 13.9. $C_{20}H_{22}O_7S_2$ requires C, 54.8; H, 5.1; S, 14.6%), *m/e* 438 $(M^{+\cdot})$, $[\alpha]_D$ +72.6° (in MeOH), $\nu_{max.}$ (film) 1 670, 1 610, 1 490, 1 450, 1 370, 1 240, 1 190, and 1 180 cm^{-1}, $\delta(CDCl_3)$ 2.46 (6 H, s, $SO_2C_6H_4CH_3$), 6.20 (1 H, ddd, $J_{1,2}$ 6, J 2, J 2 Hz, H-1), 7.35 (4 H, d, J 8.5 Hz, $SO_2C_6H_4CH_3$), 7.78 (2 H, d, J 8.5 Hz, $SO_2C_6H_4CH_3$), and 7.80 (2 H, d, J 8.5 Hz, $SO_2C_6H_4CH_3$).

4-O-*Acetyl*-2,3-*dideoxy*-2-*nitroso*-6-O-*tosyl*-α-D-ribo-*hexopyranosyl Chloride* (21).—4-*O*-Acetyl-3-deoxy-6-*O*-tosyl-D-glucal (15) (0.79 g) was dissolved in dry ethyl acetate (50 ml) and cooled in an ice-bath. 2.5M-Nitrosyl chloride in dry ethyl acetate (9.7 ml) was added and the mixture was stirred at 0 °C for 1.5 h. The green solution was evaporated *in vacuo* and the residual gum was triturated with dry ether and dried to give the *adduct* (21) (0.7 g, 74%). (Found: C, 46.2; H, 4.8; Cl, 8.0; N, 3.2; S, 7.8, $C_{30}H_{36}Cl_2N_2O_{14}S_2$ requires C, 46.0; H, 4.6; Cl, 9.05; N, 3.6; S, 8.2%), $[\alpha]_D$ +85.3° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 1 740, 1 360, 1 220, and 1 040 cm^{-1}, $\delta(CDCl_3)$ 2.02 and 2.09 (3 H, s, OAc), 2.45 (3 H, s, $SO_2C_6H_4CH_3$), and 6.53 and 6.65 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1) (3 : 2).

4-O-*Acetyl*-6-*azido*-3,6-*dideoxy*-D-*glucal* (17).—4-*O*-Acetyl-3-deoxy-6-*O*-tosyl-D-glucal (15) (550 mg) and sodium azide (550 mg) were dissolved in hexamethylphosphoramide (50 ml); the mixture was stirred at 25 °C for 24 h, then poured into ether, and the solution was washed with water. The ethereal extract was dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on a silica gel column (58 × 2.5 cm) (7% acetone–hexane as eluant) to give 4-O-*acetyl*-6-*azido*-3,6-*dideoxy*-D-*glucal* (17) (200 mg, 60%) as a gum (Found: C, 48.8; H, 5.3; N, 21.5. $C_8H_{11}N_3O_3$ requires C, 48.7; H, 5.6; N, 21.3%), $[\alpha]_D$ +158.6° (in MeOH), $\nu_{max.}$ (film) 2 100, 1 720, 1 660, 1 220, and 1 050 cm^{-1}, $\delta(CDCl_3)$ 2.09 (3 H, s, OAc), 4.72 (1 H, ddd, $J_{1,2}$ 6, $J_{2,3}$ 4.5, $J_{2,3'}$ 3 Hz, H-2), and 6.39 (1 H, ddd, $J_{1,2}$ 6, J 2, J 2 Hz, H-1).

4-O-*Acetyl*-6-*azido*-2,3,6-*trideoxy*-2-*nitroso*-α-D-ribo-*hexopyranosyl Chloride* (22).—4-*O*-Acetyl-6-azido-3,6-dideoxy-D-glucal (17) (112 mg) was dissolved in dry ethyl acetate (15 ml) and cooled to 0 °C. A solution of nitrosyl chloride (55 mg) in dry ethyl acetate (0.53 ml) was added and the mixture was stirred at 0 °C for 0.5 h, then evaporated to dryness. The residue was triturated with dry ether and dried *in vacuo* to give the *adduct* (22) (146 mg, 98%) as a gum (Found: C, 36.7; H, 4.3; Cl, 12.5; N, 19.9. $C_{16}H_{22}Cl_2N_8O_8$ requires C, 36.6; H, 4.2; Cl, 13.5; N, 21.3%), $[\alpha]_D$ +98.8° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 2 110, 1 750, 1 220, and 1 040 cm^{-1}, $\delta(CDCl_3)$ 2.09 and 2.11 (3 H, s, OAc) and 6.71 and 6.84 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1) (3 : 2). Crystallization of the gum from ether afforded needles, m.p. 95—96° (decomp.), $[\alpha]_D$ +255.8° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 2 110, 1 750, 1 220, and 1 040 cm^{-1}, $\delta(CDCl_3)$ 2.12 (3H, s, OAc) and 6.73 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1).

4-O-*Acetyl*-6-*azido*-2,3,6-*trideoxy*-2-*nitro*-D-erythro-*hex*-1-*enopyranose* (25).—The adduct (22) (380 mg) was dissolved in dry ethyl acetate; the solution was cooled to 0 °C and flushed with dry nitrogen. The solution was then saturated with nitrosyl chloride at 0 °C for 2 h and allowed to warm to 25 °C. After 17 h the solution was evaporated to dryness and the resulting gum was subjected to preparative layer chromatography on silica gel [benzene–hexane–acetone (2 : 2 : 1) as eluant] to give the *nitroglucal* (25) (52 mg, 15%) as a gum (Found: $M^{+\cdot}$, 242.0623. $C_8H_{16}N_4O_5$ requires M, 242.0651), $[\alpha]_D$ +111.6° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 2 100, 1 740, 1 650, 1 500, 1 215, and 1 040 cm^{-1}, $\delta(CDCl_3)$ 2.10 (3 H, s, OAc) and 8.21 (1 H, s, H-1).

6-*Acetamido*-4-O-*acetyl*-3,6-*dideoxy*-D-*glucal* (19).—3,4-Di-*O*-acetyl-6-azido-6-deoxy-D-glucal (13) (23 g) and methanol (8 ml) were dissolved in dry benzene (80 ml). Boron trifluoride–ether (6 ml) in dry benzene (16 ml) was added dropwise to the stirred solution and stirring was continued for 3.5 h at 25 °C. Solid sodium carbonate was added, the mixture was filtered, and the filtrate was evaporated to dryness to give crude methyl 4-*O*-acetyl-6-azido-2,3,6-trideoxy-α-D-*erythro*-hex-2-enopyranoside (32) (16.1 g). The pseudoglucal (32) dissolved in dry dioxan (300 ml) was added slowly to a stirred mixture of lithium aluminium hydride (12 g) and dry dioxan (400 ml); the resulting mixture was heated under reflux for 1.5 h, then kept at 25 °C for 16 h. The excess of hydride was destroyed by careful addition of acetone–water. The slurry was then

concentrated and dissolved in water. The solution was neutralized with Amberlite IRC 50 (H^+) resin and the resin was then washed thoroughly with water. Elution with 1.5N-ammonium hydroxide followed by evaporation of the eluate afforded crude 6-amino-2,6-dideoxy-D-glucal (18) (9.65 g). The amine (18) (9.65 g) was dissolved in dry pyridine (350 ml) and acetic anhydride (120 ml) was added. The solution was kept at 25 °C for 4.5 h, then evaporated to dryness *in vacuo*, and the residue was azeotroped with toluene. The residue was chromatographed on a silica gel column (160 × 5 cm) (0.8% methanol–chloroform as eluant) to give 6-*acetamido*-4-O-*acetyl*-3,6-*dideoxy*-D-*glucal* (19) (7.7 g, 40%) which crystallized from ether; m.p 105—108° (Found: C, 56.7; H, 7.1; N, 6.8. $C_{10}H_{15}NO_4$ requires C, 56.3; H, 7.1; N, 6.6%), *m/e* 213 ($M^{+\cdot}$), $[\alpha]_D$ +112.0° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 430, 3 330, 1 740, 1 670, 1 220, and 1 040 cm^{-1}, $\delta(D_2O)$ 1.97 (3 H, s, OAc), 2.09 (3 H, s, NHAc), 2.30 (2 H, complex m, 3-H_2), 3.30 (1 H, dd, $J_{5,6a}$ 4, $J_{6a,6b}$ 14 Hz, H-6a), 3.51 (1 H, dd, $J_{5,6b}$ 6.5, $J_{6a,6b}$ 14 Hz, H-6b), 4.06 (1 H, ddd, $J_{5,6b}$ 6.5, $J_{5,6a}$ 4, $J_{4,5}$ 5.5 Hz, H-5), 4.93 (1 H, ddd, $J_{3a,4} = J_{3b,4} = J_{4,5} =$ 5.5 Hz, H-4), 4.81 (1 H, ddd, $J_{1,2}$ 6, $J_{2,3a} = J_{2,3b} = 3.5$ Hz, H-2), and 6.34 (1 H, ddd, $J_{1,2}$ 6, *J* 2, *J* 2 Hz, H-1).

6-*Acetamido*-4-O-*acetyl*-2,3,6-*trideoxy*-2-*nitroso*-α-D-ribo-*hexopyranosyl Chloride* (23).—6-Acetamido-4-*O*-acetyl-2,6-dideoxy-D-glucal (19) (300 mg) dissolved in dry ethyl acetate (10 ml) was cooled to 0 °C. A solution of nitrosyl chloride (45 mg) in ethyl acetate (0.85 ml) was added and the solution was stirred at 0 °C for 0.5 h. A solid separated upon addition of the nitrosyl chloride. The mixture was evaporated to dryness and triturated with dry ether to give the *adduct* (23) (352 mg, 90%), m.p. 105° (decomp.) (Found: C, 43.5; H, 5.7; Cl, 11.3; N, 9.75. $C_{20}H_{30}Cl_2N_4O_{10}$ requires C, 43.1; H, 5.4; Cl, 12.7; N, 10.05%), $[\alpha]_D$ −61.6° (in $Me_2N{\cdot}CHO$), $\nu_{max.}$ (Nujol) 3 220, 1 750, 1 660, 1 220, and 1 040 cm^{-1}, $\delta[(CD_3)_2N{\cdot}CDO]$ 1.91 (3 H, s, OAc), 2.08 (3 H, s, NHAc), and 6.75 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1).

O-2,6-*Diamino*-2,3,6-*trideoxy*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-*garamine* (3).—(i) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) (25.5 g) and 4-*O*-acetyl-2,3-dideoxy-2-nitroso-6-*O*-tosyl-α-D-ribopyranosyl chloride (21) (23.5 g) were dissolved in dry, redistilled dimethylformamide (650 ml) and the solution was kept at 25 °C for 91 h. The product was worked up as before and chromatographed on a silica gel column (160 × 7.5 cm) (0.5% methanol–chloroform as eluant) to give O-4-O-*acetyl*-3-*deoxy*-2-*hydroxyimino*-6-O-*tosyl*-α-D-erythro-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonyl*-*garamine* (29) (18 g, 50%), m.p. 123—132° (Found: C, 57.7; H, 5.8; N, 4.6; S, 2.5. $C_{58}H_{68}N_4O_{22}S$ requires C, 57.8; H, 5.7; N, 4.65; S, 2.7%), $[\alpha]_D$ +116.2° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 330, 1 740, 1 710, 1 360, 1 220, and 1 030 cm^{-1}, $\delta(CDCl_3)$ † 1.23br and 1.37br (3 H, 2 s, 4″-CH_3), 1.88br, 1.90br, and 1.99br (12 H, 3 s, OAc), 2.38br (3 H, s, $SO_2C_6H_4CH_3$), 2.84br (3 H, s, 3″-NCH_3), 5.02br and 5.14br (6 H, 2 m, CH_2Ph), 7.23 (2 H, d, *J* 8 Hz, $SO_2C_6H_4CH_3$), 7.26br (15 H, s, Ph), and 7.68 (2 H, d, *J* 8 Hz, $SO_2C_6H_4CH_3$).

The oxime (29) (5 g) and sodium azide (5.7 g) were dissolved in hexamethylphosphoramide (180 ml) and stirred at 25 °C for 65 h. The mixture was poured into water and extracted with ether and the extract was dried ($MgSO_4$) and evaporated to give *O*-4-*O*-acetyl-6-azido-3,6-dideoxy-2-hydroxyimino-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (30). The crude azide (30) was acetylated as before and the acetate (31) was reduced with M-borane in tetrahydrofuran (41.5 ml) as before (36 h). The residue was taken up in methanol and hydrogenated over 10% palladium–carbon at 60 lb in^{-2} and 25 °C for 19 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was taken up in methanol–concentrated ammonium hydroxide (1 : 2; 60 ml) and heated in a bomb at 100 °C for 16 h. The solution was evaporated and the resulting glass was heated under reflux for 16 h with aqueous 5% sodium hydroxide (60 ml). The mixture was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water and eluted with 1.5N-ammonium hydroxide. The eluate was evaporated to dryness. The residue was chromatographed first on a silica gel column (160 × 5 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1)] then on a silica gel column (100 × 1 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1)] to give O-2,6-*diamino*-2,3,6-*trideoxy*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-*garamine* (3) (69 mg, 4%) (Found: C, 47.85; H, 8.2; N, 13.5. $C_{19}H_{39}N_5O_8,CO_2$ requires C, 47.1; H, 7.7; N, 13.7%), $[\alpha]_D$ +158.4° (in H_2O), $[\theta]_{285}$ −7 970 (TACu), $[\theta]_{285}$ −6 480 (Cupra A), $\nu_{max.}$(KCl) 3 280, 1 050, and 1 020 cm^{-1}, $\delta(D_2O)$ 1.21 (3 H, s, 4″-CH_3), 2.52 (3 H, s, 3″-NCH_3), 5.08 (1 H, d, $J_{1″,2″}$ 4 Hz, H-1″), and 5.14 (1 H, d, $J_{1′,2′}$ 3.5 Hz, H-1′).

(ii) 2′,4′,5-Tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (8) (7.1 g) and 4-*O*-acetyl-6-azido-2,3,6-trideoxy-2-nitroso-α-D-ribopyranosyl chloride (22) (4.2 g) were dissolved in dry, redistilled dimethylformamide (160 ml) containing *NN*,2,6-tetramethylaniline (0.9 g) and the solution was kept at 25 °C for 94 h. The product was worked up as before and chromatographed on a silica gel column (150 × 5 cm) (1% methanol–chloroform as eluant) to give O-4-O-*acetyl*-6-*azido*-3,6-*dideoxy*-2-*hydroxyimino*-α-D-erythro-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (30) (6.3 g, 70%), m.p. 125—133° (Found: C, 57.1; H, 5.8; N, 8.65. $C_{51}H_{61}N_7O_{19}$ requires C, 56.9; H, 5.7; N, 9.1%), $[\alpha]_D$ +150.5° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 340, 2 110, 1 740, 1 700, 1 220, and 1 030 cm^{-1}, $\delta(CDCl_3)$ † 1.27br and 1.38br (3 H, 2 s, 4″-CH_3), 1.91br and 2.01br (12 H, 2 s, OAc), 2.86br (3 H, s, 3″-NCH_3), 5.04br, 5.11br, and 5.17br (6 H, 3 s, CH_2Ph), and 7.30 and 7.31 (15 H, 2 s, Ph). The azide (30) was reduced and deprotected as in (i) above to give *O*-2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (3).

O-6-*Acetamido*-2,4-*di*-O-*acetyl*-3,6-*dideoxy*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (33).—*O*-4-*O*-Acetyl-6-azido-2,3,6-trideoxy-2-hydroxyimino-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (30) (1 g) was dissolved in dry pyridine (10 ml) and acetic anhydride (1 ml) was added; the mixture was kept at 25 °C for 19 h. The solution was evaporated *in vacuo* and the residue was azeotroped with toluene. The acetate (31) was dissolved in tetrahydrofuran–water (9 : 1; 21 ml) and the solution was buffered to pH 5 (sodium acetate buffer). Chromium(II) acetate [13] (0.89 g) was added and the mixture was stirred under nitrogen at 25 °C for 18 h. The solution was concentrated, diluted with water, and extracted with chloroform. The extract was concentrated to dryness and the residue was dissolved in dioxan–water (10 : 1; 33 ml) and cooled to 5 °C. Sodium borohydride (600 mg) in dioxan–water (1 : 2; 22 ml) was added and the

† Mixture of rotamers at ambient temperatures.

mixture was stirred at 5 °C for 0.5 h and at 25 °C for 1 h. Acetic acid was added and the solution was concentrated to dryness. The residue was taken up in acetic anhydride–concentrated hydrochloric acid (10 : 1; 10 ml) and kept at 25° for 24 h. The mixture was azeotroped with toluene and the product chromatographed on a silica gel column (55 × 2.5 cm) (1% methanol–chloroform as eluant) to give the *amide* (33) (99 mg, 10%) (Found: C, 58.65; H, 6.35; N, 4.6. $C_{55}H_{68}N_4O_{21}$ requires C, 58.9; H, 6.1; N, 5.0%), $[\alpha]_D$ +75.0° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 380, 1 730, 1 690, 1 235, 1 050, and 1 035 cm^{-1}, δ($CDCl_3$) † 1.27br and 1.42br (3 H, 2 s, 4″-CH_3), 1.91br, 1.97br, 2.00br, and 2.03br (18 H, 4 s, OAc and NHAc), 2.88—2.95br (3 H, s, 3″-NCH_3), 5.05br and 5.17br (6 H, s, CH_2Ph), and 7.31br (15 H, s, Ph).

3′-*Deoxygentamicin B* (4).—*O*-4-*O*-Acetyl-3-deoxy-2-hydroxyimino-6-*O*-tosyl-α-D-*erythro*-hexopyranosyl-(1 ⟶ 4)-2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (29) (4 g) was dissolved in glacial acetic acid (100 ml), and levulinic acid (10 ml) and N-hydrochloric acid (13.3 ml) were added. The mixture was kept at 25 °C for 20 h, then poured into methylene chloride (1 l). The methylene chloride solution was then washed with aqueous 5% sodium hydrogen carbonate and water, dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in dioxan–water (10 : 1; 110 ml) and cooled to 5 °C. Sodium borohydride (2.5 g) dissolved in dioxan–water (1 : 2; 75 ml) was added and the solution was stirred at 5 °C for 0.5 h and at 25 °C for 1 h. Acetic acid was added and the mixture was evaporated to dryness. The residue was taken up in chloroform and the solution was washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on a silica gel column (103 × 3.5 cm) (30—50% acetone–hexane as eluant) to give O-4-O-*acetyl*-3-*deoxy*-6-O-*tosyl*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (34) (1.43 g, 35%) (Found: C, 58.25; H, 5.9; N, 3.65. $C_{58}H_{69}N_3O_{22}S$ requires C, 58.45; H, 5.8; N, 3.5%), $[\alpha]_D$ +85.3° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 250, 1 740, 1 700, 1 230, and 1 040 cm^{-1}, δ($CDCl_3$) † 1.25—1.42br (3 H, s, 4″-CH_3), 1.83br, 1.89br, 1.99br, and 2.05br (12 H, 4 s, OAc), 2.40br (3 H, s, $SO_2C_6H_4CH_3$), 2.88br and 3.00br (3 H, s, 3″-NCH_3), 5.04br (6 H, s, CH_2Ph), 7.29br (17 H, s, Ph and $SO_2C_6H_4CH_3$), and 7.70br (2 H, d, *J* 8.5 Hz, $SO_2C_6H_4CH_3$).

A portion of the alcohol (34) was acetylated (acetic anhydride–pyridine at 25 °C for 18 h) and the product purified as above to give O-2,4-*di*-O-*acetyl*-3-*deoxy*-6-O-*tosyl*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-2′,4′,5-*tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (35) (Found: C, 58.6; H, 6.0; N, 4.6; S, 2.5. $C_{60}H_{71}N_3O_{23}S$ requires C, 58.4; H, 5.8; N, 3.4; S, 2.6%), $[\alpha]_D$ +81.3° (in $CHCl_3$), $\nu_{max.}$($CHCl_3$) 3 330, 1 730, 1 700, 1 220, and 1 030 cm^{-1}, δ($CDCl_3$) † 1.23br and 1.32br (3 H, 2 s, 4″-CH_3), 1.82br, 1.87br, 1.97br, 2.04br, and 2.13br (15 H, 5 s, OAc), 2.39br (3 H, s, $SO_2C_6H_4CH_3$), 2.87br and 2.99br (3 H, 2 s, 3″-NCH_3), 5.04br (6 H, s, CH_2Ph), 7.26br (17 H, s, Ph and $SO_2C_6H_4CH_3$), and 7.69br (2 H, d, *J* 8.5 Hz, $SO_2C_6H_4CH_3$).

The alcohol (34) (670 mg) was dissolved in anhydrous methanol and saturated at 0 °C with dry ammonia gas. The resulting solution was heated in a bomb at *ca.* 135 °C for 18 h and then evaporated to dryness. The residue was dissolved in 90% hydrazine hydrate (3 ml) and heated in a bomb at 130 °C for 67 h. The hydrazine solution was diluted with water–methanol and evaporated to dryness. The residue was dissolved in aqueous 5% sodium hydroxide (3 ml) and the solution was heated under reflux for 16 h. The mixture was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water and eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness. The residue was chromatographed three times on silica gel columns (twice on 140 × 2.5 cm; once on 135 × 4.5 cm), with chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant in each case, to give 3′-*deoxygentamicin B* (4) (78 mg, 20%) [Found: $(M - H_2O)^{+\cdot}$, 448.2533. $C_{19}H_{38}N_4O_9 - H_2O$ requires 448.2524,] $\nu_{max.}$(KCl) 3 100 and 1 060 cm^{-1}, δ(D_2O–DCl) 1.30 (3 H, s, 4″-CH_3), 2.89 (3 H, s, 3″-NCH_3), 5.08 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.41 (1 H, d, $J_{1',2'}$ 4 Hz, H-1′).

3′-*Deoxy*-6′-N-*methylgentamicin B* (5).—The alcohol (34) (335 mg) was dissolved in anhydrous methanol and the solution was saturated with methylamine at 0 °C, then heated in a bomb at *ca.* 135 °C for 18 h, and evaporated to dryness. The residue was dissolved in aqueous 5% sodium hydroxide (3 ml) and heated under reflux for 16 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water, then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness. The residue was taken up in 90% hydrazine hydrate (3 ml) and heated in a bomb at *ca.* 130 °C for 17 h. The hydrazine solution was diluted with aqueous methanol and evaporated to dryness and the residue was chromatographed on a silica gel column (130 × 2.5 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant] to give 3′-*deoxy*-6′-N-*methylgentamicin B* (5) (65 mg, 48%) [Found: $(M + 1)^+$, 481.2834. $C_{20}H_{41}N_4O_9$ requires $M + 1$, 481.2873], $[\alpha]_D$ +122.4° (in H_2O), $[\theta]_{287}$ −7 066 (TACu), $[\theta]_{290}$ −5 222 (Cupra A), $\nu_{max.}$(KCl) 3 200 and 1 020 cm^{-1}, δ(D_2O) 1.21 (3 H, s, 4″-CH_3), 2.33 (3 H, s, 6′-NCH_3), 2.52 (3 H, s, 3″-NCH_3), 5.08 (1 H, d, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.16 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1′).

1,2′,3,3″-*Tetrakis*-N-*benzyloxycarbonyl*-3′-*deoxygentamicin* X_2 (36).—3′-Deoxygentamicin X_2 (6) (1 g) [1] and potassium carbonate (0.8 g) were dissolved in aqueous methanol (1 : 1; 20 ml) and stirred at 0 °C. Benzyloxycarbonyl chloride (1.52 ml) was added and the mixture was stirred at 0 °C for 1.5 h and then at 25 °C for 3.5 h. The solid was filtered off, washed with water, triturated with ether, and dried. The product was chromatographed on a silica gel column (110 × 2.5 cm) (9% methanol–chloroform as eluant) to give 1,2′,3,3″-*tetrakis*-N-*benzyloxycarbonyl*-3′-*deoxygentamicin* X_2 (36) (1.93 g, 91%) (Found: C, 60.9; H, 6.4; N, 5.7. $C_{51}H_{62}N_4O_{17}$ requires C, 61.1; H, 6.2; N, 5.6%), $[\alpha]_D$ +76.5° (in Me_2SO), $\nu_{max.}$($CHCl_3$) 3 300, 1 690, 1 540, and 1 035 cm^{-1}, δ[$(CD_3)_2SO$] † 0.90br (3 H, s, 4″-CH_3), 2.98br (3 H, s, 3″-NCH_3), 5.02br (8 H, s, CH_2Ph), and 7.32br (20 H, s, Ph).

2″,4′-*Di*-O-*acetyl*-1,2′,3,3″-*tetrakis*-N-*benzyloxycarbonyl*-3′-*deoxy*-6′-O-*tosylgentamicin* X_2 (39).—1,2′,3,3″-Tetrakis-*N*-benzyloxycarbonyl-3′-deoxygentamicin X_2 (36) (990 mg) and trityl chloride (293 mg) were dissolved in dry pyridine (10 ml) and kept at 25 °C for 24 h. More trityl chloride (114 mg) was then added, and after 46 h the mixture was poured into ice–water and the trityl derivative was filtered off, dried (1.22 g), and taken up in dry pyridine (25 ml). Acetic anhydride (4 ml) was added and the mixture was kept at 25 °C for 18 h. Chromatography on a silica gel column (110 × 2.5 cm) (2% methanol–chloroform as

† Same footnote as on page 1132.

eluant) gave 2'',4'-di-*O*-acetyl-1,2',3,3''-tetrakis-*N*-benzyloxycarbonyl-3'-deoxy-6'-*O*-tritylgentamicin X_2 (37) (0.9 g). This was dissolved in glacial acetic acid (35 ml) and heated on a steam-bath for 3 h. The solution was evaporated to dryness and the residue was taken up in chloroform; this solution was washed with water, dried ($MgSO_4$), and evaporated to give 2'',4'-di-*O*-acetyl-1,2',3,3''-tetrakis-*N*-benzyloxycarbonyl-3'-deoxygentamicin X_2 (38) (0.61 g). The product (38) was dissolved in pyridine (10 ml) and tosyl chloride (0.6 g) was added. The mixture was kept at 25 °C for 23 h then poured into ice–water and extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm) (3.5% methanol–chloroform as eluant) to give 2'',4'-*di*-O-*acetyl*-1,2',3,3''-*tetrakis*-N-*benzyloxycarbonyl*-3'-*deoxy*-6'-O-*tosylgentamicin* X_2 (39) (163 mg, 13%) (Found: C, 59.9; H, 5.9; N, 4.3; S 2.75. $C_{62}H_{72}N_4O_{21}S$ requires C, 60.0; H. 5.85; N, 4.5; S 2.6%), $\nu_{max.}$($CHCl_3$) 3 400, 3 300, 1 725, 1 500, 1 220, and 1 030 cm^{-1}, δ($CDCl_3$) † 0.99br (3 H, s, 4''-CH_3), 1.83br (6 H, s, OAc), 2.41br (3 H, s, $SO_2C_6H_4CH_3$), 2.88br (3 H, s, 3''-NCH_3), 5.02br (8 H, m, CH_2Ph), 7.27br and 7.31br (22 H, 2 s, Ph and $SO_2C_6H_4CH_3$), and 7.76br (2 H, d, *J* 8.5 Hz, $SO_2C_6H_4CH_3$).

1,2',3,3''-*Tetrakis*-N-*benzyloxycarbonyl*-3'-*deoxy*-6'-O-*tosylgentamicin* X_2 (40).—1,2',3,3''-Tetrakis-*N*-benzyloxycarbonyl-3'-deoxygentamicin X_2 (36) (500 mg) was dissolved in dry pyridine (9 ml), tosyl chloride (376 mg) was added, and the mixture was kept at 25 °C for 16.5 h. Ethanol (100 ml) was added and the solution was kept at 25 °C for 0.5 h. The solution was evaporated to dryness, the product was azeotroped with toluene, and the residue was subjected to chromatography on a silica gel column (110 × 2.5 cm) (6% methanol–chloroform as eluant) followed by preparative layer chromatography of some of the overlap fractions on silica gel plates (5% methanol–chloroform) to give 1,2',3,3''-*tetrakis*-N-*benzyloxycarbonyl*-3'-*deoxy*-6'-O-*tosylgentamicin* X_2 (40) (325 mg, 56%) (Found: C, 60.45; H, 6.05; N, 5.0; S, 3.0. $C_{58}H_{68}N_4O_{19}S$ requires C, 60.2; H, 5.9; N, 4.8; S 2.8%), $[\alpha]_D$ +74.1° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 330, 1 720, 1 340, 1 180, 1 050, and 694 cm^{-1}, δ($CDCl_3$) † 0.95—1.05br (3 H, s, 4''-CH_3), 2.36br (3 H, s, 6'-$SO_2C_6H_4CH_3$), 3.01br (3 H, s, 3''-NCH_3), 5.00br (8 H, m, CH_2Ph), 7.27br (22 H, s, Ph and $SO_2C_6H_4CH_3$), and 7.70br (2 H, d, *J* 8.5 Hz, $SO_2C_6H_4CH_3$); and 1,2',3,3''-*tetrakis*-N-*benzyloxycarbonyl*-3'-*deoxy*-4',6'-*di*-O-*tosylgentamicin* X_2 (41) (140 mg, 38%) (Found: C, 59.2; H, 5.85, N, 4.0; S, 4.1. $C_{65}H_{74}N_4O_{21}S_2$ requires C, 59.6; H, 5.7; N, 4.3; S, 4.9%), $[\alpha]_D$ +71.1° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 300, 1 720, 1 340, 1 180, 1 040, and 697 cm^{-1}, δ($CDCl_3$) † 1.0—1.1br (3 H, s, 4''-CH_3), 2.40br (6 H, s, $SO_2C_6H_4CH_3$), 3.02br (3 H, s, 3''-NCH_3), 5.02br (8 H, m, CH_2Ph), 7.27br and 7.30br (24 H, s, Ph and $SO_2C_6H_4CH_3$), 7.68br (2 H, d, *J* 8.5 Hz, $SO_2C_6H_4CH_3$), and 7.71br (2 H, d, *J* 8.5 Hz, $SO_2C_6H_4CH_3$).

When the reaction was repeated at 7 °C for 18 h with 2.7 mol. equiv. of tosyl chloride, a 66% yield of the 6'-*O*-tosyl derivative (40) was obtained.

O-2-*Amino*-2,3,6-*trideoxy*-6-*methylamino*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-*garamine* (7).—(i) 1,2',3,3''-Tetrakis-*N*-benzyloxycarbonyl-3'-deoxy-6-*O*-tosylgentamicin X_2 (40) (2.17 g) was dissolved in acetic anhydride–concentrated hydrochloric acid (9 : 1; 40 ml) at 0 °C and the mixture was allowed to warm to 25 °C over 17 h. The solution was evaporated to dryness and the residue was azeotroped with toluene to give 2'',4',4'',5-tetra-*O*-acetyl-1,2',3,3''-tetrakis-*N*-benzyloxycarbonyl-3'-deoxy-6'-*O*-tosylgentamicin X_2 (42) (2.37 g, 95%).

The acetate (42) (870 mg) was dissolved in anhydrous methanol (15 ml) and the solution was saturated with methylamine at 0 °, heated in a bomb at *ca.* 135 °C for 18 h, and then evaporated to dryness. The residue was dissolved in 90% hydrazine hydrate (3 ml) and heated in a bomb at 130 °C for 88 h. The solution was diluted with aqueous methanol and evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant] to give O-2-*amino*-2,3,6-*trideoxy*-6-*methylamino*-α-D-ribo-*hexopyranosyl*-(1 ⟶ 4)-*garamine* (7) (126 mg, 40%) (Found: C, 47.4; H, 8.1; N, 13.3. $C_{20}H_{41}N_5O_8$,-CO_2,0.5H_2O requires C, 47.4; H, 7.95; N, 13.15%), $[\alpha]_D$ +167.9° (in H_2O), $[\theta]_{290}$ −8 170 (TACu), $[\theta]_{290}$ −6 220 (Cupra A), δ(D_2O) 1.14 (3 H, s, 4''-CH_3), 2.26 (3 H, s, 6'-NCH_3), 2.44 (3 H, s, 3''-NCH_3), 5.02 (1 H, d, $J_{1'',2''}$ 4 Hz, H-1''), and 5.07 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1').

(ii) 1,2',3,3''-Tetrakis-*N*-benzyloxycarbonyl-3'-deoxy-6'-*O*-tosylgentamicin X_2 (40) (300 mg) was dissolved in anhydrous methanol (20 ml) and the solution was saturated with methylamine at 0 °C, heated in a bomb at *ca.* 135 °C for 18 h, then evaporated to dryness. The residue was dissolved in aqueous 5% sodium hydroxide (7 ml) containing methanol (1 ml) and heated under reflux for 17 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin and the resin was washed with water, then eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness. The residue was dissolved in 90% hydrazine hydrate (3 ml) and heated in a bomb at 120 °C for 17 h. The solution was diluted with aqueous methanol and evaporated to dryness and the residue was chromatographed on a silica gel column (110 × 2.5 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant] to give *O*-2-amino-2,3,6-trideoxy-6-methylamino-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (7) (47 mg, 38%), identical with that described in (i).

(iii) 2'',4'-Di-*O*-acetyl-1,2',3,3''-tetrakis-*N*-benzyloxycarbonyl-3'-deoxy-6'-*O*-tosylgentamicin X_2 (39) (144 mg) was dissolved in dry methanol (2 ml) and the solution was saturated with methylamine at 0 °C, heated in a bomb at 135 °C for 18 h, and then evaporated to dryness. The residue was dissolved in aqueous 5% sodium hydroxide (3 ml) and the mixture was heated under reflux for 18 h. The solution was cooled and neutralized with Amberlite IRC 50 (H^+) resin, and the resin was washed with water and eluted with 1.5N-ammonium hydroxide. The basic eluate was evaporated to dryness. The residue was dissolved in 90% hydrazine hydrate (1 ml) and heated in a bomb at 125 °C for 24 h. The mixture was diluted with aqueous methanol and evaporated to dryness and the residue was chromatographed on a silica gel column (110 × 1 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give *O*-2-amino-2,3,6-trideoxy-6-methylamino-α-D-*ribo*-hexopyranosyl-(1 ⟶ 4)-garamine (7) (20 mg, 36%), identical with that described in (i) and (ii).

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† Same footnote as on page 1132.