# Semisynthetic Aminoglycoside Antibacterials. Part I. Preparation of Selectively Protected Garamine Derivatives

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*N*-Alkoxycarbonyl and *N*-acyl derivatives of sisomicin, an unsaturated aminoglycoside antibiotic, have been demonstrated to undergo ready acid-catalysed hydrolysis to the novel pseudodisaccharide garamine [*O*-(3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl)-(1→6)-2-deoxy-D-streptamine]. Alternative procedures and routes for the selective protection of various hydroxy-groups in the garamine molecule are discussed. The selectively protected garamine derivatives constitute useful intermediates for the preparation of a wide variety of novel semisynthetic aminoglycoside antibacterials related to the clinically important gentamicins.

SUBMERGED fermentation of *Micromonospora inyoensis* (NRRL 3292) produces sisomicin (1),[1,2] a novel unsaturated aminoglycoside antibiotic the structure of which has been elucidated.[3-6] The occurrence of the novel vinylic ether system in the 2,6-diaminoglycosyl unit of sisomicin suggested a possible route for the conversion of the latter into the then unknown pseudodisaccharide *O*-β-L-garosaminyl-(1 → 6)-2-deoxy-D-streptamine, subsequently named garamine (8).[5-8] Although the garamine unit occurs in the clinically important gentamicins $C_1$ (25), $C_2$ (26), and $C_{1a}$ (27),[9-13] it cannot be prepared from these pseudotrisaccharides because of the greater stability of the purpurosamine glycosidic linkages towards acidic hydrolysis, due to the presence of 2-amino-groups.[14] The only pseudodisaccharides that can be isolated from the gentamicins by acidic hydrolysis are gentamines $C_1$ (33), $C_2$ (34), and $C_{1a}$ (35), respectively.[12] The occurrence of the garamine unit has also been demonstrated in several closely related antibiotics, namely gentamicins B (28),[15] $B_1$ (29),[15] $X_2$ (30),[15] $C_{2a}$ (36),[15] and $C_{2b}$ (31),[16] verdamicin (2),[17] and antibiotics G-52 (3),[18] G-418 (32),[19] JI-20A

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[15] P. J. L. Daniels, 'Drug Action and Drug Resistance in Bacteria,' ed. S. Mitsuhashi, University Park Press, Tokyo, 1975, p. 77.
[16] P. J. L. Daniels, C. Luce, T. L. Nagabhushan, R. S. Jaret, D. Schumacher, H. Reimann, and J. Ilavsky, *J. Antibiotics*, 1975, **28**, 35.
[17] P. J. L. Daniels and A. S. Yehaskel, Abstracts, 13th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., U.S.A., 19—21 September, 1973, paper 135.
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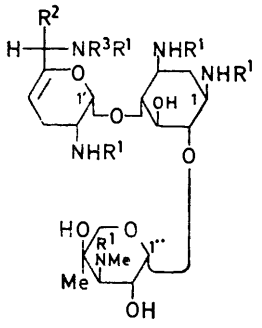
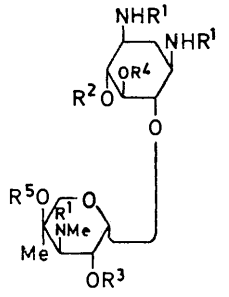
[19] P. J. L. Daniels, A. S. Yehasekl, and J. B. Morton, Abstracts, 13th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., U.S.A., 19—21 September, 1973, paper 137.

(37),[20] and JI-20B (38),[20] all of which are produced by various *Micromonospora* species. The structural differences between the above antibiotics reside in the nature



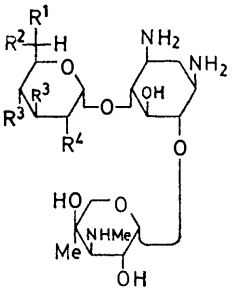
(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = R^3 = H, R^2 = Me$
(3) $R^1 = R^2 = H, R^3 = Me$
(4) $R^1 = Z, \quad R^2 = R^3 = H$
(5) $R^1 = EtO_2C, R^2 = R^3 = H$
(6) $R^1 = Ac, R^2 = R^3 = H$
(7) $R^1 = DNP, R^2 = R^3 = H$

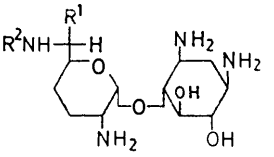


Z = $PhCH_2 \cdot O \cdot CO$
DNP = $2,4 \cdot (NO_2)_2C_6H_3$

(8) $R^1 = R^2 = R^3 = R^4 = R^5 = H$
(9) $R^1 = Z, R^2 = R^3 = R^4 = R^5 = H$
(10) $R^1 = EtO_2C, R^2 = R^3 = R^4 = R^5 = H$
(11) $R^1 = Z, R^2 = Ac, R^3 = R^4 = R^5 = H$
(12) $R^1 = Z, R^2 = R^3 = Ac, R^4 = R^5 = H$
(13) $R^1 = Z, R^2 = R^3 = R^4 = Ac, R^5 = H$
(14) $R^1 = Z, R^2 = R^3 = R^4 = R^5 = Ac,$
(15) $R^1 = Z, R^2 = R^3 = R^4 = H, R^5 = Ac$
(16) $R^1 = Z, R^2 = R^4 = R^5 = H, R^3 = Ac$
(17) $R^1 = Z, R^2 = Cl_3C \cdot CH_2 \cdot O \cdot CO, R^3 = R^4 = R^5 = H$
(18) $R^1 = Z, R^2 = Cl_3C \cdot CH_2 \cdot O \cdot CO, R^3 = R^4 = R^5 = Ac$
(19) $R^1 = Z, R^2 = H, R^3 = R^4 = R^5 = Ac$
(20) $R^1 = Z, R^4 = H, R^2 = R^3 = R^5 = Ac$
(21) $R^1 = Z, R^2 = Cl_3C \cdot CH_2 \cdot O \cdot CO, R^3 = R^4 = Ac, R^5 = H$
(22) $R^1 = Z, R^2 = R^5 = H, R^3 = R^4 = Ac$
(23) $R^1 = Ac, R^2 = R^3 = R^4 = R^5 = H$
(24) $R^1 = DNP, R^2 = R^3 = R^4 = R^5 = H$

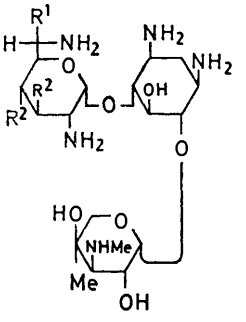
of the glycosyl units attached to the 4-position of garamine. Thus an efficient method for the preparation of garamine would provide a useful intermediate for the synthesis of a number of novel aminoglycoside antibacterials related to the gentamicins. By appropriate choice of sugar units the structure–activity requirements for the 4-glycoside unit could be extended beyond those currently known for the naturally occurring antibiotics. New antibacterials, hopefully with improved potency and improved antibacterial spectra, particularly against resistant organisms, could also be

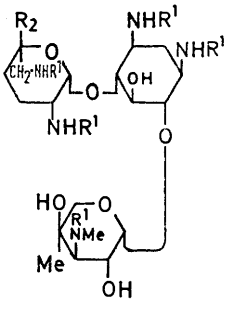




(33) $R^1 = R^2 = Me$
(34) $R^1 = Me, R^2 = H$
(35) $R^1 = R^2 = H$

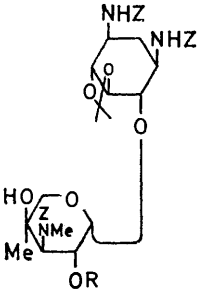
(25) $R^1 = Me, R^2 = MeNH, R^3 = H, R^4 = NH_2$
(26) $R^1 = Me, R^2 = R^4 = NH_2, R^3 = H$
(27) $R^1 = R^3 = H, R^2 = R^4 = NH_2$
(28) $R^1 = H, R^2 = NH_2, R^3 = R^4 = OH$
(29) $R^1 = Me, R^2 = NH_2, R^3 = R^4 = OH$
(30) $R^1 = H, R^2 = R^3 = OH, R^4 = NH_2$
(31) $R^1 = R^3 = H, R^2 = MeNH, R^4 = NH_2$
(32) $R^1 = Me, R^2 = R^3 = OH, R^4 = NH_2$



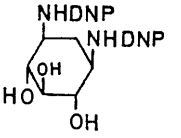


(36) $R^1 = Me, R^2 = H$
(37) $R^1 = H, R^2 = OH$
(38) $R^1 = Me, R^2 = OH$

(39) $R^1 = Z, R^2 = OMe$
(40) $R^1 = H, R^2 = OMe$
(41) $R^1 = R^2 = H$



(42) R = H
(43) R = Ac



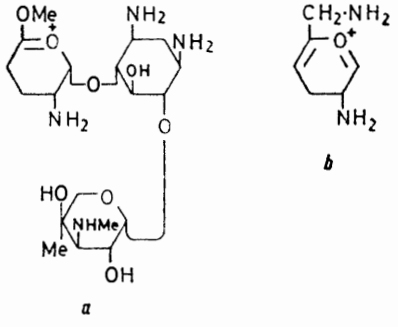
(44)

prepared. With these objectives in mind, the preparation and selective protection of garamine was undertaken.

[20] H. Reimann, unpublished observations.

Sisomicin (1) afforded a pentakis-*N*-benzyloxycarbonyl derivative (4) [5,6] that was extremely labile towards acidic reagents. When solutions of (4) in a suitable inert solvent such as tetrahydrofuran were treated with either Amberlite IR 120 ($H^+$) resin, or concentrated sulphuric acid until the pH reached 1, selective hydrolysis of the enopyranoside sugar occurred, affording high yields of 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9). The pentakis-*N*-ethoxycarbonyl derivative (5) was also acid-labile, affording 1,3,3′-tris-*N*-ethoxycarbonylgaramine (10) in high yield. In neither case did any further hydrolysis of the garamine occur. Attempted hydrolysis of sisomicin (1) under a variety of acidic conditions gave rise to no detectable amounts of garamine (8). The stability of the enopyranoside to glycosidic hydrolysis must be due to protonation of the 2′- and 6′-amino-groups in sisomicin (1). However, in the alkoxycarbonyl derivatives, where protonation of the 6′-group cannot occur, the vinylic ether grouping is rendered susceptible to acidic hydrolysis, leading to the formation of garamine.

When the hydrolysis of pentakis-*N*-benzyloxycarbonylsisomicin (4) was carried out in methanol the desired tris-*N*-benzyloxycarbonylgaramine (9) was obtained together with the methanol adduct (39). The latter exhibited a $^1$H n.m.r. signal at δ 3.21 due to the methoxy-group. Hydrogenation of (39) gave the deprotected adduct (40), which exhibited an $(M + 1)^+$ ion at *m/e* 480 in the mass spectrum, which lost water to give an ion at *m/e* 461. Loss of the 5′-aminomethyl side chain gave rise to the ion *a* at *m/e* 449. The ion $C_1$ produced by glycosidic cleavage of the garosamine unit [21] was present in the spectrum, but no ion corresponding to glycosidic cleavage of the 4-*O*-glycoside unit was detected although the ion *b* at *m/e* 127 produced by loss




*a* *b*

of methanol was evident. The characteristic protonated formyl deoxystreptamine ions $A_9$—$A_{12}$ formed in the mass spectra of aminocyclitol antibiotics [21,22] were observed. The corresponding pseudodisaccharide ions $A_5$—$A_8$ for the garamine portion of the molecule were present, but no ions $A_1$—$A_4$ due to the other protonated formyl pseudodisaccharide unit.[21,22] The ion $E_3$ was also present. The characteristic $M - 75$ ion, $E_1$, found in garosamine-containing aminocyclitol antibiotics, was not observed, but the related ion $E_2$ was present.[21] The mass spectral evidence supported the proposed gross structure of the methanol adduct (40) having the methoxy-group at the 5′-position. The i.r. spectrum of (40) showed no vinylic ether band. Its $^1$H n.m.r. spectrum exhibited the expected garosamine C·$CH_3$, N·$CH_3$, and H-1″ resonances, a methoxy-resonance δ 3.28, and no vinylic proton signal. The signal for the anomeric proton of the 4-glycoside unit occurred as a doublet at δ 4.93 ($J$ 2 Hz), consistent with that observed for dihydrosisomicin (41),[4,6] and indicating that addition of methanol to the vinylic ether system had occurred from the top face of the molecule. The resulting 1,3-diaxial interaction between the axial $CH_2$·$NH_2$ and the anomeric glycosidic bond was relieved by conformational inversion from the $^4C_1$ to the $^1C_4$ conformation, causing the signal for the anomeric proton to shift upfield. The $^{13}$C n.m.r. spectrum lent further support to structure (40) for the methanol adduct [the chemical shifts are recorded in the Table along with those of sisomicin (1) [23] and dihydrosisomicin (41) [15]] showing the absence of vinylic carbon atoms. The C-4′ signal was clearly that of a methylene carbon atom, and C-5′ gave a signal at $\delta_C$ 99.4, consistent with that expected for a hemiacetal carbon atom. The chemical shift differences between dihydrosisomicin (41) [15] and the methanol adduct (40) are also given in the Table. Thus replacement of the axial 5′-proton of (41) with a methoxy-group [in (40)] resulted in downfield α- and β-shifts of +20.6 and +1.8 p.p.m. for C-5′ and C-4′, respectively. Upfield γ-shifts of −4.6 and −1.2 p.p.m. for C-3′ and C-1′, respectively, and an upfield δ-shift of −0.7 p.p.m. for C-2′, were also observed. The methylene C-6′ exhibited an upfield shift of −0.9 p.p.m. These shift differences are compatible with structure (40).

Alternative procedures for the conversion of pentakis-*N*-benzyloxycarbonylsisomicin (4) into tris-*N*-benzyloxycarbonylgaramine (9) were developed, with *m*-chloroperbenzoic acid in the presence of aqueous sodium hydrogen carbonate, or diborane as reagent. The ready conversion of (4) into (9) by diborane probably occurs *via* reduction [24] of the reactive vinylic acetal group.

With the first objective achieved, namely the development of an efficient synthesis of 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) from sisomicin (1), the relative reactivities of the various hydroxy-groups towards acylation could be investigated. Acetylation of 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) with 1 mol. equiv. of acetic anhydride in pyridine at 100 °C gave the 4-*O*-acetyl derivative (11). The most reactive hydroxy-groups would be expected to be those at the 4- and 2′-positions, and subsequent preparation of the 2′-*O*-acetyl derivative (16) by an unambiguous route established

[21] P. J. L. Daniels, A. K. Mallams, J. Weinstein, J. J. Wright, and G. W. A Milne, preceding paper.
[22] P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Comm.*, 1971, 1969.
[23] J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1973, **95**, 7464.
[24] B. Fleming and H. I. Bolker, *Canad. J. Chem.*, 1974, **52**, 888.

compound (11) as the 4-*O*-acetyl derivative. No molecular ions were visible in the mass spectra of the *N*-benzyloxycarbonylgaramine derivatives, and meaningful mass spectra could not be obtained. Rotamers produced by the presence of the 3′-tertiary alkoxycarbonylamino-group resulted in complex $^1$H n.m.r. spectra at ambient temperatures. In general rotamer-free spectra were obtained by running the spectra in dimethyl sulphoxide at 100—140 °C. Although unambiguous assignments of the acetyl signals could not be made from the high-temperature spectra, some assignments are noted in the Experimental section.

$^{13}$C Chemical shifts [a]

| Carbon atom | (1) [23] | (41) [15] | (40) | Δ[(41) → (40)] | (8) | Δ[DOS → (8)] [b] | Δ[DOS → (41)] | Δ[DOS → (40)] |
|---|---|---|---|---|---|---|---|---|
| C-1 | 51.8 | 51.8 | 51.6 | | 51.7 | +0.1 | +0.2 | 0 |
| C-2 | 36.4 | 36.6 | 36.1 | | 36.6 | | | |
| C-3 | 50.4 | 50.1 | 49.9 | | 51.4 | −0.2 | −1.5 | −1.7 |
| C-4 | 85.3 | 86.7 | 87.4 | | 78.8 | | | |
| C-5 | 75.4 | 75.1 | 75.0 | | 75.1 | −1.5 | −1.5 | −1.6 |
| C-6 | 87.8 | 87.9 | 87.7 | | 87.9 | | | |
| C-1′ | 100.6 | 104.0 | 102.8 | −1.2 | | | | |
| C-2′ | 47.6 | 48.0 | 47.3 | −0.7 | | | | |
| C-3′ | 25.6 | 28.9 | 24.3 | −4.6 | | | | |
| C-4′ | 96.5 | 22.5 | 24.3 | +1.8 | | | | |
| C-5′ | 150.4 | 78.8 | 99.4 | +20.6 | | | | |
| C-6′ | 43.5 | 46.0 | 45.1 | −0.9 | | | | |
| 5′OCH$_3$ | | | 48.9 | | | | | |
| C-1″ | 101.5 | 101.5 | 101.5 | | 101.4 | | | |
| C-2″ | 70.0 | 70.1 | 70.1 | | 70.0 | | | |
| C-3″ | 64.3 | 64.4 | 64.2 | | 64.3 | | | |
| C-4″ | 73.0 | 73.1 | 73.1 | | 73.2 | | | |
| C-5″ | 68.5 | 68.5 | 68.5 | | 68.5 | | | |
| 3″-NCH$_3$ | 37.9 | 37.9 | 37.6 | | 38.0 | | | |
| 4″-CH$_3$ | 22.9 | 22.9 | 22.3 | | 22.9 | | | |

[a] $\delta_C$ in p.p.m. downfield from external $Me_4Si$ for the free base in $D_2O$. [b] DOS = deoxystreptamine.

Acetylation of 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) with an excess of acetic anhydride in pyridine at 25 °C gave the 2′,4-di-*O*-acetyl derivative (12). Similar results were obtained with acetic anhydride and anhydrous orthophosphoric acid in the presence of 4-dimethylaminopyridine (DMAP). The diacetate (12) was identical with that prepared by acetylation of 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (16) by using 1 mol. equiv. of acetic anhydride in pyridine at 25 °C. When 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) was acetylated under more vigorous conditions (an excess of refluxing acetic anhydride in pyridine in the presence of DMAP), the 2′,4,5-tri-*O*-acetyl derivative (13) was obtained as the principal product, together with the 2′,4,4′,5-tetra-*O*-acetyl derivative (14) as a by-product. The use of DMAP as a catalyst for acetylation of tertiary hydroxy-groups has been reported;[25] however under the above conditions no appreciable acylation of the tertiary 4′-hydroxy-group occurred. Higher yields of the triacetate (13) were obtained by refluxing (9) with acetic anhydride and orthophosphoric acid in the presence of DMAP. Only traces of the tetra-acetate (14) were observed with the above procedure. Acetylation of 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) with acetic anhydride containing concentrated hydrochloric acid (10 : 1) at ambient temperature afforded a high yield of the tetra-acetate (14). The triacetate (13) exhibited a $^1$H n.m.r. signal at δ 0.97 due to the 4′-$CH_3$ group on a carbon atom bearing a free hydroxy-group. In the tetra-acetate (14) the corresponding signal was shifted downfield to δ 1.30 due to the presence of the 4′-*O*-acetyl group. Ammonolysis of the tetra-acetate (14) effected smooth conversion into the 4′-*O*-acetyl derivative (15), in which only the tertiary hydroxy-group was protected. The latter exhibited the 4′-$CH_3$ signal at δ 1.30. The relative order of reactivity of the four hydroxy-groups in 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) was thus established as 4 > 2′ > 5 > 4′, and garamine derivatives could then be prepared having selected hydroxy-groups protected to prevent unwanted side reactions during subsequent glycosylation reactions to produce pseudotrisaccharides.

1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) was converted into the 4,5-*O*-isopropylidene derivative (42) in the usual way and the latter, on acetylation with 1 mol. equiv. of acetic anhydride in pyridine at 115 °C, gave the 2′-*O*-acetyl-4,5-*O*-isopropylidene derivative (43). Treatment of this with aqueous 80% acetic acid gave 2′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (16). The latter, having the second most reactive hydroxy-group protected, would be expected to undergo glycosylation at the 4-hydroxy-group.

A second protected garamine derivative, having only the 4-hydroxy-group available for glycosylation, was prepared as follows. Treatment of 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) with 1 mol. equiv. of 2,2,2-trichloroethyl chloroformate gave the 4-*O*-(2,2,2-trichloroethoxycarbonyl) derivative (17). The latter was extremely insoluble in chloroform and could readily be obtained pure in large scale experiments by simply triturating with chloroform followed by filtration. The carbonate (17) was then per-acetylated with acetic anhydride containing concentrated hydrochloric acid

[25] W. Steglich and G. Hofle, *Angew. Chem.*, 1969, **81**, 1001.

(10 : 1) at 25 °C to give 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)-garamine (18) in quantitative yield. Alternative acetylation procedures with acetic acid, trifluoroacetic anhydride, and toluene-*p*-sulphonic acid, or acetic anhydride, acetic acid, and concentrated hydrochloric acid gave similar results. Reductive cleavage of the trichloroethoxycarbonyl group with zinc in 90% acetic acid afforded 2′,4′,5-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (19), having only the 4-hydroxy-group available for subsequent glycosylation.

In order to demonstrate that no transacylation of the 5-*O*-acetyl group to the 4-hydroxy-group had occurred during the reaction with zinc in acetic acid, 2′,4,4′-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (20) was prepared in the following manner. Acetylation of 4′-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (15) with an excess of acetic anhydride and orthophosphoric acid in pyridine at 25 °C afforded 2′,4,4′-tri-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (20) as the principal product, along with the tetra-acetate (14). The two triacetyl derivatives (19) and (20) were readily distinguished by their physical characteristics and t.l.c. mobilities, the latter being less polar on silica gel.

Acetylation of 1,3,3′-tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)garamine (17) with an excess of acetic anhydride in pyridine afforded the 2′,5-di-*O*-acetyl derivative (21). The latter on reductive cleavage with zinc in 90% acetic acid gave 2′,5-di-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (22), another useful protected garamine intermediate.

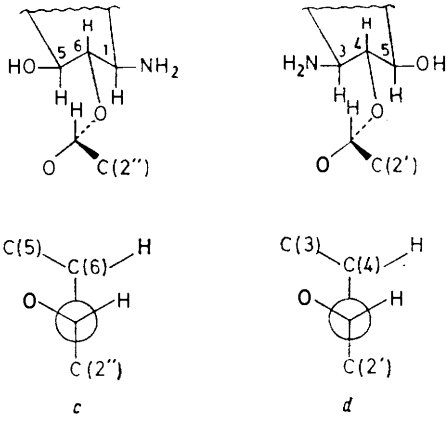
The reactivity of the *N*-alkoxycarbonyl derivatives of sisomicin towards acidic reagents was greater than that of the corresponding penta-*N*-acetyl derivative (6). When penta-*N*-acetylsisomicin (6) was treated with Amberlite IR 120 ($H^+$) resin in dimethylformamide solution the hydrolysis to tri-*N*-acetylgaramine (23) proceeded very slowly at 25 °C; after 300 h only a 50% conversion was effected. However, when penta-*N*-acetylsisomicin (6) in dimethylformamide solution was acidified with sulphuric acid to pH 1.2 and left at 25 °C, a smooth conversion into tri-*N*-acetylgaramine (23) was effected within 48 h.

When pentakis-*N*-(2,4-dinitrophenyl)sisomicin (7) was subjected to acidic hydrolysis at pH 1, the reaction proceeded slowly and in a non-selective manner to produce, after 90 h at 25 °C, 2-deoxy-1,3-bis-*N*-(2,4-dinitrophenyl)streptamine (44) as the principal product, together with some 1,3,3′-tris-*N*-(2,4-dinitrophenyl)-garamine (24). Both products were identical with authentic samples prepared from 2-deoxystreptamine and garamine (8), respectively. Treatment of pentakis-*N*-(2,4-dinitrophenyl)sisomicin (7) with *m*-chloroperbenzoic acid in the presence of aqueous sodium carbonate afforded acceptable yields of the garamine derivative (24).

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

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

The solution conformations of garamine (8), dihydrosisomicin (41),[6] and the 5′-methoxy-derivative (40) could readily be deduced from the $^{13}C$ n.m.r. parameters (Table). The $\Delta\delta$ values for the deoxystreptamine carbon atoms β to the glycosidic bonds have been shown to be of diagnostic value in determining the solution conformations of aminoglycoside antibiotics.[23,26] The presence of the 6-*O*-garosaminyl unit in garamine (8) resulted in the expected upfield shift of 1.5 p.p.m. for C-5 relative to deoxystreptamine, while the C-1 signal remained unchanged.[23] These results indicated that in garamine (8) the preferred rotamer about the C(6)–O




bond was *c*,[23,26] which satisfied the requirements of the *exo*-anomeric effect.[27,28] The solution conformations of the trisaccharides (41) and (40) were of interest in view of the novel $^1C_4$ conformation of the 4-*O*-glycosyl unit in these molecules. Both (41) and (40) showed negligible shielding of C-1 whereas C-5 was shielded by 1.5 p.p.m. in (41) and by 1.6 p.p.m. in (40), relative to deoxystreptamine. These results indicated that the preferred rotamer about the C(6)–O bond in (41) and (40) was *c*, as observed previously for all garosamine-containing aminoglycoside antibiotics.[23] The presence of the 4-*O*-glycosyl unit in the $^1C_4$ conformation having an equatorial glycosidic linkage in (41) resulted in an upfield shift of 1.5 p.p.m. for C-3, whereas in (40) an upfield shift of 1.7 p.p.m. for C-3 was observed. The 4-*O*-glycosyl unit produced no additional shielding of C-5 in these compounds. It was evident from the above findings that the preferred rotamer about the C(4)–O bond in both (41) and (40) was *d*. This rotamer satisfied the requirements of the *exo*-anomeric effect.[27,28] These novel 4-*O*-glycosides adopt similar rotamer conformations in aqueous solution to the kanamycins,[27,29] the gentamicins,[23] and tobramycin,[26] which have the monosaccharide units in the $^4C_1$ conformations and 4-*O*-axial glycosidic linkages.

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

[29] T. L. Nagabhushan, A. B. Cooper, J. B. Morton, R. Brambilla, and P. J. L. Daniels, Abstracts, 58th Chemical Conference and Exhibition, Toronto, Ontario, Canada, 25—28 May, 1975, paper 215.

EXPERIMENTAL

Unless otherwise stated optical rotations were recorded at 26 °C for 0.3% solutions. I.r. spectra were recorded with either a Perkin-Elmer 221 or an Infracord 137 spectrometer. $^1$H N.m.r. spectra were obtained at 60 or 100 MHz with either a Varian A60A or an XL 100-15 spectrometer. Chemical shifts for solutions in $D_2O$ are reported in p.p.m. downfield from internal or external sodium 4,4-dimethyl-4-silapentane-1-sulphonate. All other chemical shifts are reported in p.p.m. downfield from internal $Me_4Si$. $^{13}$C N.m.r. spectra were recorded with a Varian XL 100-15 spectrometer by Fourier transform with a Varian 620L-16K computer; chemical shifts are reported in p.p.m. downfield from $Me_4Si$ and internal dioxan was used as reference [$\delta_O(Me_4Si) = \delta_O(\text{dioxan}) + 67.4$]. Mass spectra were recorded with a Varian MAT CH5 spectrometer. U.v. spectra were obtained with a Cary 14 spectrometer. C.d. spectra were recorded with a Cary 61 spectrometer. All free aminoglycosides, after chromatographic purification, were passed over Amberlite IRA 40IS ($OH^-$) resin to remove carbon dioxide, and the eluant in each case was lyophilized to give a colourless amorphous solid.

1,3,3′-*Tris*-N-*benzyloxycarbonylgaramine* (9).[5,6]—In addition to the method published previously,[5,6] 1,2′,3,3″,6′-pentakis-*N*-benzyloxycarbonylsisomicin (4) [5,6] may be converted into 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (4) as follows.

(i) The pentakis-*N*-benzyloxycarbonylsisomicin (4) (100 mg) was dissolved in tetrahydrofuran (20 ml) and acidified to pH 1 with concentrated sulphuric acid, and the solution was kept at 25 °C for 16 h. The mixture was passed over Amberlite IR 45 ion-exchange resin and eluted with methanol. The product was chromatographed on preparative silica gel plates (10% methanol–chloroform as eluant) to give 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) (40 mg, 62%) with the physical properties described earlier.[5,6]

(ii) The pentakis-*N*-benzyloxycarbonylsisomicin (4) (3 g), sodium hydrogen carbonate (0.9 g), and *m*-chloroperbenzoic acid (0.9 g) were dissolved in tetrahydrofuran (75 ml) containing water (3.8 ml) and the mixture was stirred at 25 °C for 16 h. Evaporation gave a residue which was partitioned between water and chloroform. The chloroform layer was dried ($MgSO_4$) and evaporated to dryness, and the residue chromatographed on a silica gel column (110 × 2.5 cm) (7% methanol–chloroform as eluant) to give 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) (0.52 g, 27%).

(iii) The pentakis-*N*-benzyloxycarbonylsisomicin (4) (0.5 g) was dissolved in dry tetrahydrofuran (10 ml) and the solution was cooled to 0 °C in an ice-bath. A 1M-solution of borane in tetrahydrofuran (9 ml) (20 equiv.) was added and the solution was kept at 0 °C for 1.5 h. The excess of diborane was destroyed by careful addition of water, and after 1 h the solution was evaporated to small volume and extracted with chloroform. The extract was dried ($MgSO_4$) and evaporated to dryness, and the residue was chromatographed on a silica gel column (110 × 2.5 cm) (7% methanol–chloroform as eluant) to give 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) (0.3 g, 94%).

(iv) The pentakis-*N*-benzyloxycarbonylsisomicin (4) (234 g) was dissolved in methanol (5 l), the pH of the solution was adjusted to 1 by careful addition of concentrated sulphuric acid (1.2 ml), and the solution was kept at 25 °C for 26 h, neutralized with Amberlite IR 45 resin, filtered, and evaporated. The gum was chromatographed on a silica gel column to give 1,3,3′-tris-*N*-benzyloxycarbonylgaramine (9) (74 g, 49%). The less polar fractions were rechromatographed on a silica gel column (160 × 5 cm) (5% methanol–chloroform as eluant) to give the *pentakis-N-benzyloxycarbonyl derivative* of O-3-*deoxy*-4-C-*methyl*-3-*methylamino*-β-L-*arabinopyranosyl*-(1 ⟶ 6)-O-[2,6-*diamino*-2,3,4,6-*tetradeoxy*-5-C-*methoxy*-β-L-threo-*hexopyranosyl*-(1 ⟶ 4)]-2-*deoxy*-D-*streptamine* (39) (7.8 g, 3%) (Found: C, 62.5; H, 6.2; N, 6.0. $C_{60}H_{71}N_5O_{18}$ requires C, 62.65; H, 6.2; N, 6.1%), $[\alpha]_D$ +77.2° (in EtOH), $\lambda_{max.}$(MeOH) 208 nm (ε 27 800), $\nu_{max.}$($CHCl_3$) 3 420, 3 330, 1 710, 1 530, and 697 $cm^{-1}$, δ($CDCl_3$) † 1.07br (3 H, s, 4″-$CH_3$), 3.04br (3 H, s, 3″-$NCH_3$), 3.21br (3 H, s, 5′-$OCH_3$), and 7.20—7.28br (15 H, m, $NCO_2CH_2C_6H_5$).

O-3-*Deoxy*-4-C-*methyl*-3-*methylamino*-β-L-*arabinopyranosyl*-(1 ⟶ 6)-O-[2,6-*diamino*-2,3,4,6-*tetradeoxy*-5-C-*methoxy*-β-L-threo-*hexopyranosyl*-(1 ⟶ 4)]-2-*deoxy*-D-*streptamine* (40).—The pentakis-*N*-benzyloxycarbonyl derivative (39) was dissolved in methanol (100 ml) and hydrogenated over 30% palladium–carbon at 25 °C and 50 lb $in^{-2}$ for 16 h. The catalyst was filtered off and washed and the combined filtrates were evaporated. The residue was chromatographed on a silica gel column (110 × 1 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1) as eluant] to give the *methanol adduct* (40), m.p. 88—97° (Found: C, 46.9; H, 8.8; N, 13.2. $C_{20}H_{41}N_5O_8,H_2O,CH_3OH$ requires C, 47.6; H, 8.95; N, 13.2%), *m/e* 480 $(M + 1)^+$, $[\alpha]_D$ +153.0° (in $H_2O$), $\nu_{max.}$(KCl) 3 330 and 1 055 $cm^{-1}$, δ($D_2O$) 1.19 (3 H, s, 4″-$CH_3$), 2.50 (3 H, s, 3″-$NCH_3$), 2.59 (1 H, d, $J_{2'',3''}$ 10.5 Hz, H-3″), 3.28 (3 H, s, 5′-$OCH_3$), 3.30 (1 H, d, *J* 12.5 Hz, H-5″a), 3.30 (3 H, s, MeOH), 3.80 (1 H, dd, $J_{2'',3''}$ 10.5, $J_{1'',2''}$ 4 Hz, H-2″), 4.03 (1 H, d, *J* 12.5 Hz, H-5″e), 4.93 (1 H, d, $J_{1',2'}$ 2 Hz, H-1′), and 5.05 (1 H, d, $J_{1'',2''}$ 4 Hz H-1″).

1,3,3′-*Tris*-N-*ethoxycarbonylgaramine* (10).—Sisomicin (1) (2 g) was dissolved in water containing sodium hydrogen carbonate (4 g). Ethyl chloroformate (5 ml) was added and the mixture was stirred at 25 °C for 16 h. The mixture was extracted with chloroform and the extract was washed with water, dried ($MgSO_4$), and evaporated. The resulting gum was taken up in tetrahydrofuran (100 ml) and treated with Amberlite IR120 ($H^+$) resin (30 g), and the mixture was stirred at 25 °C for 72 h. The product was eluted from the resin with methanol; the solution was evaporated and the product chromatographed on a silica gel column (110 × 2.5 cm) (10% methanol–chloroform as eluant), to give 1,3,3′-*tris*-N-*ethoxycarbonylgaramine* (10) (1 g, 83%) m.p. 128—140° (Found: C, 48.6; H, 7.25; N, 7.6. $C_{22}H_{39}N_3O_{12}$ requires C, 49.2; H, 7.3; N, 7.8%), *m/e* 537 $(M^{+\cdot})$, $[\alpha]_D$ +99.2° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 330, 1 700, 1 530, 1 050, and 1 040 $cm^{-1}$, δ($CDCl_3$) † 1.20br (12 H, m, 4′-$CH_3$ and $NCO_2CH_2{\cdot}CH_3$), 3.01br (3 H, s, 3′-$NCH_3$), and 6.10br (6 H, m, $NCO_2CH_2{\cdot}CH_3$).

4-O-*Acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (11).—1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (5 g) was dissolved in dry pyridine (25 ml) and, after addition of acetic anhydride (0.7 g), the mixture was heated under reflux at 100 °C for 20 h. The solution was poured into water and extracted with chloroform, and the extract after drying ($MgSO_4$) was evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm)

† Mixture of rotamers at ambient temperatures.

(3% methanol–chloroform as eluant) to give 4-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (11) (3 g, 57%), m.p. 154—157° (Found: C, 61.4; H, 6.0; N, 5.5. $C_{39}H_{47}N_3O_{13}$ requires C, 61.2; H, 6.1; N, 5.5%), $[\alpha]_D$ +79.9° (in MeOH), $\lambda_{max.}$(MeOH) 205 nm (ε 26 250), $\nu_{max.}$ (Nujol) 3 430, 3 300, 1 740, 1 680, 1 540, 1 060, and 695 cm$^{-1}$, δ($CDCl_3$) † 1.00br and 1.07br (3 H, 2 s, 4′-$CH_3$), 1.92 (3 H, s, 4-OAc), 3.10br (3 H, s, 3′-$NCH_3$), and 7.32br (15 H, s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 100 °C] 0.97 (3 H, s, 4′-$CH_3$), 1.91 (3 H, s, 4-OAc), 3.00 (3 H, s, 3′-$NCH_3$), and 7.30 (15 H, s, $NCO_2CH_2C_6H_5$).

2′,4-*Di*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (12).—(i) 1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (10 g) was dissolved in dry pyridine (100 ml) and acetic anhydride (24.6 ml) was added. The solution was stirred at 25 °C for 24 h and then poured into ice–water. The precipitate was filtered off and taken up in ethyl acetate and the solution was washed with water, dried ($Na_2SO_4$), and evaporated to give an amorphous solid (10.8 g) comprising compounds (12), (11), and (13) in the approximate ratio 4 : 1 : 1. Chromatography on a silica gel (500 g) column [1—3% methanol in benzene–ether (1 : 1) as eluant] afforded the pure diacetate (12) (5.5 g, 49%), identical with that prepared in (ii) below. The remainder of the material was contained in overlap fractions and was not further purified.

(ii) 1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (720 mg) and 4-dimethylaminopyridine (6 mg) were dissolved in dry pyridine (10 ml). Acetic anhydride (2 ml) containing anhydrous phosphoric acid (0.5 ml) was added and the solution was kept at 25 °C for 16 h, then evaporated *in vacuo*. The residue in chloroform was washed with water, dried ($MgSO_4$), and evaporated. The residue was subjected to preparative layer chromatography on silica gel plates (5% methanol–chloroform as eluant) to give the *diacetate* (12) (460 mg, 57%), m.p. 108—114° (Found: C, 60.9; H, 6.1; N, 5.1. $C_{41}H_{49}N_3O_{14}$ requires C, 61.0; H, 6.1; N, 5.2%), $[\alpha]_D$ +75.6° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 520), $\nu_{max.}$($CHCl_3$) 3 400, 1 725, 1 520, 1 230, 1 040, and 694 cm$^{-1}$, δ($CDCl_3$) † 1.08br (3 H, s, 4′-$CH_3$), 1.89br and 1.97br (6 H, 2 s, 2 OAc), 2.92br (3 H, s, 3′-$NCH_3$), and 7.27br (15 H, s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 100 °C] 1.01 (3 H, s, 4′-$CH_3$), 1.89 (3 H, s, OAc), 1.93 (3 H, s, OAc), 2.88 (3 H, s, 3′-$NCH_3$), and 7.31 (15 H, s $NCO_2CH_2C_6H_5$). The less polar fraction afforded the triacetate (13) (51 mg, 6%), identical with that described below.

(iii) 2′-*O*-Acetyl-1,3,3′-tris-*N*-benzyloxycarbonylgaramine (16) (10 g) was dissolved in dry pyridine (85 ml) and after addition of acetic anhydride (1.26 ml) the mixture was kept at 25 °C for 48 h. The solution was evaporated and the residue in ethyl acetate was washed with water, dried ($Na_2SO_4$), and evaporated. The residue was chromatographed on a silica gel (500 g) column [1—3% methanol in benzene–ether (1 : 1) as eluant] to give the diacetate (12) (6.6 g, 63%), identical with that prepared in (i) and (ii).

2′,4,5-*Tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (13).—(i) 1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (7.2 g) and 4-dimethylaminopyridine (60 mg) were dissolved in dry pyridine (100 ml). Acetic anhydride (10 ml) was added and the solution was heated under reflux for 16 h, then evaporated *in vacuo*. The residue in chloroform was washed with water, dried ($MgSO_4$), and evaporated. Chromatography on a silica gel column (160 × 2.5 cm) (1% methanol–chloroform as eluant) gave the *triacetate* (13) (4.2 g, 49%), m.p. 108—114° (Found: C, 60.8; H, 6.0; N, 5.0. $C_{43}H_{51}N_3O_{15}$ requires C, 60.8; H, 6.0; N, 4.95%), $[\alpha]_D$ +77.8° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 350), $\nu_{max.}$($CHCl_3$) 3 400, 1 740, 1 520, 1 230, 1 040 and 695 cm$^{-1}$, δ($CDCl_3$) † 1.00br, 1.09br, and 1.12br (3 H, 3 s, 4′-$CH_3$), 1.90br and 2.00br (9 H, 2 s, 3 OAc), 2.89br (3 H, s, 3′-$NCH_3$), and 7.25br (15 H, s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 100 °C] 0.97 (3 H, s, 4′-$CH_3$), 1.86 (3 H, s, OAc), 1.90 (3 H, s, OAc), 1.95 (3 H, s, OAc), 2.85 (3 H, s, 3′-$NCH_3$), and 7.29 (15 H, s, $NCO_2CH_2C_6H_5$). The less polar fractions from the column afforded the tetra-acetate (14) 640 mg, 7%), identical with that described below.

(ii) 1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (720 mg) and 4-dimethylaminopyridine (6 mg) were dissolved in dry pyridine (50 ml). Acetic anhydride (2 ml) containing anhydrous phosphoric acid (0.5 ml) was added and the mixture was heated under reflux for 16 h. The solution was evaporated *in vacuo* and the residue in chloroform was washed with water, dried ($MgSO_4$), and evaporated. Chromatography on a silica gel column (100 × 2.5 cm) (1% methanol–chloroform as eluant) gave the triacetate (13) (840 mg, 99%), identical with that described in (i).

2′,4,4′,5-*Tetra*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (14).—1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (720 mg) was dissolved in acetic anhydride (10 ml) containing concentrated hydrochloric acid (1 ml) at 25 °C and the mixture was set aside for 17.5 h. The solution was concentrated *in vacuo* and the residue in chloroform was washed with water, dried ($MgSO_4$), and evaporated. The resulting residue was evaporated several times with methanol to give the *tetra-acetate* (14) (700 mg, 78%), m.p. 137—145° (from acetone–hexane) (Found: C, 60.4; H, 5.8; N, 4.6. $C_{45}H_{53}N_3O_{16}$ requires C, 60.6; H, 6.0; N, 4.7%), $[\alpha]_D$ +93.6° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 360), $\nu_{max.}$($CHCl_3$) 3 400, 1 750, 1 520, 1 220, 1 040, and 694 cm$^{-1}$, δ($CDCl_3$) † 1.30br and 1.40br (3 H, 2 s, 4′-$CH_3$), 1.90br, 1.93br, 1.98br, and 2.10br (12 H, 4 s, 4 OAc), 2.89 br (3 H, s, 3′-$NCH_3$), and 7.30br (15 H, s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 100 °C] 1.30 (3 H, s, 4′-$CH_3$), 1.86 (3 H, s, OAc), 1.92 (3 H, s, OAc), 1.96 (3 H, s, OAc), 2.03 (3 H, s, OAc), 2.84 (3 H, s, 3′-$NCH_3$), and 7.31 and 7.33 (15 H, 2 s, $NCO_2CH_2C_6H_5$).

4′-O-*Acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (15).—The tetra-acetate (14) (4.6 g) was dissolved in a 10% (v/v) solution of concentrated ammonium hydroxide in methanol (100 ml), and the solution was kept at 25 °C for 70 h, then evaporated to dryness. The residue in chloroform was washed with water, dried ($MgSO_4$), and evaporated to give the 4′-*acetate* (15) (3.9 g, 99%), m.p. 108—113° (Found: C, 61.2; H, 6.3; N, 5.5. $C_{39}H_{47}N_3O_{13}$ requires C, 61.2; H, 6.1; N, 5.5%), $[\alpha]_D$ +91.1° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 150), $\nu_{max.}$($CHCl_3$) 3 400, 3 300, 1 720, 1 700, 1 500, 1 210, and 693 cm$^{-1}$, δ($CDCl_3$) † 1.30br and 1.32br (3 H, 2 s, 4′-$CH_3$), 1.93br (3 H, s, OAc), 3.01br (3 H, s, 3′-$NCH_3$), and 7.23br and 7.28br (15 H, 2 s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 100 °C] 1.30 (3 H, s, 4′-$CH_3$), 2.00 (3 H, s, 4′-OAc), 3.01 (3 H, s, 3′-$NCH_3$), and 7.33 (15 H, s, $NCO_2CH_2{\cdot}C_6H_5$).

1,3,3′-*Tris*-N-*benzyloxycarbonyl*-4,5-O-*isopropylidenegaramine* (42).—1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (5 g), 2,2-dimethoxypropane (6.2 ml), and toluene-*p*-sulphonic acid (0.06 g) were dissolved in dry dimethylformamide (30 ml) and heated under reflux at 110 °C for 4 h. The mixture was cooled and passed through Dowex 1 × 2 ($OH^-$) resin, and the methanol eluate was concentrated and diluted with water to give the product. The

† Same footnote as on page 1093.

latter was chromatographed on a silica gel column (60 × 2.5 cm) (2% methanol–chloroform as eluant) to give the *acetonide* (42) (3.72 g, 70%), m.p. 126—129° (Found: C, 61.1; H, 6.3; N, 5.0. $C_{40}H_{49}N_3O_{12},H_2O$ requires C, 61.5; H, 6.5; N, 5.4%), $[\alpha]_D$ +87.3° (in EtOH), $\lambda_{max.}$(MeOH) 208 nm (ε 24 800), $\nu_{max.}$($CHCl_3$) 3 400, 3 280, 1 690, 1 540, 1 055, and 694 $cm^{-1}$, δ($CDCl_3$) † 1.03br (3 H, s, 4′-$CH_3$), 1.40br (6 H, s, $Me_2C$), 3.02br (3 H, s, 3′-$NCH_3$), and 7.08br and 7.30br (15 H, 2 s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 140 °C] 0.98 (3 H, s, 4′-$CH_3$), 1.40 (6 H, s, $Me_2C$), 3.02 (3 H, s, 3′-$NCH_3$), and 7.33 (15 H, s, $NCO_2CH_2C_6H_5$).

2′-O-*Acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonyl*-4,5-O-*isopropylidenegaramine* (43).—The acetonide (42) (1.6 g) in dry pyridine (10 ml) was treated with acetic anhydride (2 ml) and the mixture was heated at 100 °C for 20 h, then poured into ice–water. The solid that separated was filtered off and chromatographed on a silica gel column (110 × 2.5 cm) (7% methanol–chloroform as eluant) to give the 2′-*acetate* (43) (1.22 g, 72%), m.p. 105—108° (Found: C, 62.55; H, 6.5; N, 5.35. $C_{42}H_{51}N_3O_{13}$ requires C, 62.6; H, 6.3; N, 5.2%), $[\alpha]_D$ +77.5° (in EtOH), $\lambda_{max.}$(MeOH) 208 nm (ε 25 300), $\nu_{max.}$($CHCl_3$) 3 400, 3 300, 1 730, 1 700, 1 520, 1 220, 1 050, and 697 $cm^{-1}$, δ($CDCl_3$) † 1.08br (3 H, s, 4′-$CH_3$), 1.39br (6 H, s, $Me_2C$), 1.91br (3 H, s, 2′-OAc), 2.91br (3 H, s, 3′-$NCH_3$), and 7.23br and 7.31br (15 H, 2 s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 140 °C] 1.01 (3 H, s, 4′-$CH_3$), 1.37 (6 H, s, $Me_2C$), 1.90 (3 H, s, 2′-OAc), 2.90 (3 H, s, 3′-$NCH_3$), 5.33 (1 H, d, $J_{1',2'}$ 3 Hz, H-1′), and 7.32 (15 H, s, $NCO_2CH_2C_6H_5$).

2′-O-*Acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (16).—The acetonide 2′-acetate (43) (150 mg) was dissolved in 80% aqueous acetic acid (5 ml) and kept at 25 °C for 16 h. The mixture was evaporated to dryness and the residue was chromatographed on silica gel plates (10% methanol–chloroform as eluant) to give the 2′-*acetate* (16) (118 mg, 83%), m.p. 103—107° (Found: C, 60.8; H, 6.3; N, 5.5. $C_{39}H_{47}N_3O_{13}$ requires C, 61.2; H, 6.1; N, 5.5%), $[\alpha]_D$ +66.6° (in EtOH), $\lambda_{max.}$(MeOH) 208 nm (ε 24 800), $\nu_{max.}$($CHCl_3$) 3 410, 1 730, 1 700, 1 515, 1 220, 1 040, and 696 $cm^{-1}$, δ($CDCl_3$) † 1.04br (3 H, s, 4′-$CH_3$), 1.88br (3 H, s, 2′-OAc), 2.89br (3 H, s, 3′-$NCH_3$), and 7.23br (15 H, s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 140 °C] 1.04 (3 H, s, 4′-$CH_3$), 1.90 (3 H, s, 2′-OAc), 2.90 (3 H, s, 3′-$NCH_3$), 5.33 (1 H, d, $J_{1',2'}$ 3 Hz, H-1′), and 7.33 (15 H, s, $NCO_2CH_2C_6H_5$).

1,3,3′-*Tris*-N-*benzyloxycarbonyl*-4-O-(2,2,2-*trichloroethoxycarbonyl*)*garamine* (17).—1,3,3′-Tris-*N*-benzyloxycarbonylgaramine (9) (1 g) dissolved in dry pyridine (18 ml) was cooled to 0 °C. 2,2,2-Trichloroethyl chloroformate (dried over molecular sieves) (323 mg) was added dropwise with stirring. The funnel was rinsed with dry pyridine (4 ml) which was added to the mixture. Stirring was continued for 30 min (until the initially formed precipitate had dissolved) and the mixture was then stored at 7 °C for 45 h. The solution was poured into water and the precipitate was taken up in ethyl acetate. This solution was washed with water, 2N-hydrochloric acid, and water, dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The residue was azeotropically distilled with toluene; the residue was concentrated to small volume and triturated with chloroform. The insoluble *trichloroethoxycarbonyl derivative* (17) (1.1 g, 87%) was obtained as needles, m.p. 220—222° (Found: C, 53.5; H, 5.4; Cl, 12.3; N, 4.8. $C_{46}H_{46}Cl_3N_3O_{14}$ requires C, 53.4; H, 5.2; Cl, 11.8; N, 4.7%), $[\alpha]_D$ +65.9° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 200), $\nu_{max.}$ (Nujol) 3 450, 3 280, 1 770, 1 700, 1 680, 1 050, and 697 $cm^{-1}$, δ($CDCl_3$) † 1.26br (3 H, s, 4′-$CH_3$), 3.05br (3 H, s, 3′-$NCH_3$), 4.73 (2 H, s, $CO_2CH_2CCl_3$), and 7.33br (15 H, s, $NCO_2CH_2C_6H_5$).

2′,4′,5-*Tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonyl*-4-O-(2,2,2-*trichloroethoxycarbonyl*)*garamine* (18).—(i) 1,3,3′-Tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)-garamine (17) (16.5 g) was dissolved in glacial acetic acid (500 ml) and freshly redistilled trifluoroacetic anhydride (133 ml) and toluene-*p*-sulphonic acid (750 mg) were added with stirring. The mixture was stirred at 25 °C for 20 h, and was then poured into ice–water; the precipitate was extracted into ethyl acetate. The extract was washed with water, 5% sodium hydrogen carbonate, and water, dried ($MgSO_4$), filtered, and evaporated to give the *triacetate* (18) (18.8 g, 99%), m.p. 92—100° (Found: C, 54.2; H, 5.5; Cl, 9.55; N, 4.25. $C_{46}H_{52}Cl_3N_3O_{17}$ requires C, 54.15; H, 5.1; Cl, 10.4; N, 4.1%), $[\alpha]_D$ +69.0° (in $CHCl_3$), $\lambda_{max.}$(MeOH) 208 nm (ε 24 950), $\nu_{max.}$($CHCl_3$) 1 770, 1 740, 1 220, 1 055, and 695 $cm^{-1}$, δ($CDCl_3$) † 1.35br (3 H, s, 4′-$CH_3$), 1.93br and 2.04br (9 H, 2 s, OAc), 2.90br (3 H, s, 3′-$NCH_3$), 4.70br (2 H, s, $CO_2CH_2CCl_3$), and 7.33br (15 H, s, $NCO_2CH_2C_6H_5$).

(ii) 1,3,3′-Tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)garamine (17) (1 g) was dissolved in glacial acetic acid (50 ml) and a mixture of acetic anhydride (4.5 ml) and concentrated hydrochloric acid (0.5 ml) was added. The solution was heated on a steam-bath for 3 h and the reaction was worked up as in (i) to give the triacetate (18) (1.12 g, 99%), identical with that described in (i).

(iii) 1,3,3′-Tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)garamine (17) (1 g) and a mixture of acetic anhydride (9 ml) and concentrated hydrochloric acid (1 ml) were stirred at 25 °C. The initially insoluble material gradually went into solution and the solution was stirred for 18 h. The mixture was poured into water and extracted with ethyl acetate, and the extract was washed with aqueous 5% sodium hydrogen carbonate and water, dried ($MgSO_4$), filtered, and evaporated to give the triacetate (18) (1.1 g, 98%), identical with that described in (i).

2′,4′,5-*Tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (19).—The triacetate (18) (16.6 g) was dissolved in 90% acetic acid (650 ml), and powdered zinc (100 g) was added with stirring. Stirring was continued at 25 °C until no starting material remained (t.l.c.). The zinc was filtered off and the aqueous acetic acid was removed *in vacuo*; the residue in ethyl acetate was washed with water, dried ($MgSO_4$), filtered, and evaporated to give the *triacetate* (19), which was chromatographed on a silica gel column (100 × 5 cm) (1% methanol–chloroform as eluant) to give a solid (12.2 g, 89%), m.p. 101—105° (Found: C, 60.9; H, 6.4; N, 5.0. $C_{43}H_{51}N_3O_{15}$ requires C, 60.8; H, 6.05; N, 4.9%), $[\alpha]_D$ +83.1° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 500), $\nu_{max.}$($CHCl_3$) 3 480, 1 740, 1 710, 1 220, 1 035, and 694 $cm^{-1}$, δ($CDCl_3$) † 1.30br and 1.40br (3 H, s, 4′-$CH_3$), 1.95br, 2.05br, and 2.08br (9 H, 3 s, OAc), 2.88br (3 H, s, 3′-$NCH_3$), and 7.34br (15 H, s, $NCO_2CH_2C_6H_5$).

2′,4,4′-*Tri*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (20).—The 4′-acetate (15) (19.1 g) was dissolved in dry pyridine (125 ml) and a mixture of acetic anhydride (40 ml) and anhydrous phosphoric acid (10 ml) was added. The solution was kept at 25 °C for 16 h, then concentrated to small volume *in vacuo*. The concentrate, in chloroform, was washed with water, dried ($MgSO_4$), and evaporated.

† Same footnote as on page 1093.

The residue was chromatographed twice on silica gel columns (150 × 5 cm) (20—40% acetone in hexane as eluant in each case) to afford the tetra-acetate (14) (3.1 g, 14%), identical with that described above, and the *triacetate* (20) (12.9 g, 61%), m.p. 109—118° (Found: C, 60.5; H, 6.2; N, 5.0. $C_{43}H_{51}N_3O_{15}$ requires C, 60.8; H, 6.0; N, 4.95%), $[\alpha]_D$ +95.3° (in MeOH), $\lambda_{max.}$(MeOH) 207 nm (ε 26 120), $\nu_{max.}$($CHCl_3$) 3 400, 1 740, 1 710, 1 220, 1 030, and 694 $cm^{-1}$, δ($CDCl_3$) † 1.25br and 1.33br (3 H, 2 s, 4′-$CH_3$), 1.82br, 1.88br, and 1.92br (9 H, 3 s, OAc), 2.79br (3 H, s, 3′-$NCH_3$), and 7.19br and 7.23br (15 H, 2 s, $NCO_2CH_2C_6H_5$), δ[$(CD_3)_2SO$; 100 °C] 1.34 (3 H, s, 4′-$CH_3$), 1.91 (6 H, s, 4- and 2′-OAc), 2.02 (3 H, s, 4′-OAc), 2.87 (3 H, s, 3′-$NCH_3$), and 7.32 and 7.35 (15 H, s, $NCO_2CH_2C_6H_5$).

2′,5-*Di*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonyl*-4-O-(2,2,2-*trichloroethoxycarbonyl*)*garamine* (21).—(i) 1,3,3′-Tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)-garamine (17) (4.2 g) was dissolved in dry pyridine (100 ml) and treated with acetic anhydride (42 ml), and the mixture was kept at 25 °C for 6 days. The solution was poured into water and extracted with chloroform. The extract was dried ($MgSO_4$), filtered, and evaporated and the residue was chromatographed on a silica gel column (160 × 2.5 cm) (2% methanol–chloroform as eluant) to give the *diacetate* (21) (4.3 g, 96%), m.p. 115—120° (Found: C, 53.8; H, 5.1; Cl, 10.7; N, 3.9. $C_{44}H_{50}Cl_3N_3O_{16}$ requires C, 53.8; H, 5.1; Cl, 10.8; N, 4.3%), $[\alpha]_D$ +64.2° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 390, 1 760, 1 720, 1 230, and 1 050 $cm^{-1}$, δ($CDCl_3$) † 1.00br and 1.10br (3 H, 2 s, 4′-$CH_3$), 1.87br and 2.03br (6 H, 2 s, OAc), 2.89br (3 H, s, 3′-$NCH_3$), and 7.25br (15 H, m, $NCO_2CH_2C_6H_5$).

(ii) 1,3,3′-Tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)garamine (17) (500 mg) was dissolved in dry pyridine (25 ml) and treated with acetic anhydride (5 ml) and the mixture was heated under reflux on a steam-bath for 16 h. The solution was evaporated to small volume, poured into water, and extracted with chloroform. The extract was dried ($MgSO_4$), filtered, and evaporated and the residue was chromatographed on a silica gel column (110 × 2.5 cm) (1% methanol–chloroform as eluant) to give the diacetate (21) (480 mg, 90%), identical with that described in (i).

2′,5-*Di*-O-*acetyl*-1,3,3′-*tris*-N-*benzyloxycarbonylgaramine* (22).— 2′,5-Di-*O*-acetyl-1,3,3′-tris-*N*-benzyloxycarbonyl-4-*O*-(2,2,2-trichloroethoxycarbonyl)garamine (21) (1 g) was dissolved in 90% acetic acid (100 ml) and powdered zinc (7 g) was added with stirring. Stirring was continued at 25 °C until no starting material remained (t.l.c.; 2 h). The zinc was filtered off and the aqueous acetic acid was removed *in vacuo*; the residue in ethyl acetate was washed with water, dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on a silica gel column (160 × 2.5 cm) (4% methanol–chloroform as eluant) to give the *diacetate* (22) (0.8 g, 98%), m.p. 115—122° (Found: C, 60.2; H, 6.1; N, 5.2. $C_{41}H_{49}N_3O_{14}$ requires C, 61.0; H, 6.1; N, 5.2%), $[\alpha]_D$ +62.8° (in MeOH), $\nu_{max.}$($CHCl_3$) 3 350, 1 720, 1 220, and 1 040 $cm^{-1}$, δ($CDCl_3$) † 0.98br and 1.0br (3 H, 2 s, 4′-$CH_3$), 1.85br and 2.08br (6 H, 2 s, OAc), 2.87br (3 H, s, 3′-$NCH_3$), and 7.27br (15 H, s, $NCO_2CH_2C_6H_5$).

1,3,3′-*Tri*-N-*acetylgaramine* (23).—1,2′,3,3″,6′-Penta-*N*-acetylsisomicin (6) (735 mg) was dissolved in dimethylformamide (30 ml) and concentrated sulphuric acid was added dropwise until the pH reached 1.2. The mixture was kept at 25 °C for 170 h, then neutralized with Amberlite IRA 45 resin and filtered. The filtrate and methanol wash from the resin were combined and evaporated to dryness. The resulting gum was chromatographed on a silica gel colum (160 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) as eluant] to give 1,3,3′-tri-*N*-acetylgaramine (23) (460 mg, 92%), identical with an authentic sample.[5,6]

1,2′,3,3″,6′-*Pentakis*-N-(2,4-*dinitrophenyl*)*sisomicin* (7).—Sisomicin (1) (2 g), sodium hydrogen carbonate (3 g), and 1-fluoro-2,4-dinitrobenzene (8 g) were dissolved in acetone–water (3 : 1) (50 ml) and the mixture was stirred at 25 °C for 16 h, then evaporated to dryness *in vacuo*. The residue was extracted repeatedly with tetrahydrofuran and acetone and the insoluble inorganic salts were filtered off. Evaporation of the combined filtrates, followed by chromatography on a silica gel column (110 × 2.5 cm) (30% acetone–chloroform as eluant) gave a yellow solid which was washed with methanol affording 1,2′,3,3″,6′-*pentakis*-N-(2,4-*dinitrophenyl*)*sisomicin* (7) (5 g, 88%) as a yellow amorphous solid, m.p. 195—203° (Found: C, 45.8; H, 3.7; N, 16.6. $C_{49}H_{47}N_{15}O_{27}$ requires C, 46.2; H, 3.7; N, 16.5%), $[\alpha]_D$ +90.0° (in $Me_2CO$), $\lambda_{max.}$($Me_2CO$) 351 nm (ε 75 600), $\nu_{max.}$ (Nujol) 3 330, 1 630, 1 590, and 1 050 $cm^{-1}$.

1,3,3′-*Tris*-N-(2,4-*dinitrophenyl*)*garamine* (24).—(i) 1,2′,3,3″,6′-Pentakis-*N*-(2,4-dinitrophenyl)sisomicin (7) (1 g) was dissolved in tetrahydrofuran (50 ml) and concentrated sulphuric acid was added until the pH reached 1. The solution was kept at 25 °C for 90 h, then neutralized with lead carbonate, filtered, and evaporated. The residue was chromatographed on silica gel plates (first 4% methanol–chloroform, then 8% methanol–chloroform as eluant) to give 1,3,3′-tris-*N*-(2,4-dinitrophenyl)garamine (24) (133 mg, 21%) as a yellow amorphous solid, identical with an authentic sample prepared from garamine [see (ii)], and 2-deoxy-1,3-bis-*N*-(2,4-dinitrophenyl)-2-deoxystreptamine (44) (181 mg, 47%) as a yellow amorphous solid, identical with an authentic sample prepared from 2-deoxystreptamine.

(ii) Garamine (8) (100 mg), sodium hydrogen carbonate (250 mg), and 1-fluoro-2,4-dinitrobenzene (500 mg) were dissolved in acetone–water (3 : 1; 10 ml), and the mixture was stirred at 25 °C for 16 h, then evaporated to dryness. The residue was extracted with ethyl acetate and the extract was filtered to remove inorganic salts and evaporated. The residue was chromatographed on silica gel plates (25% methanol–chloroform as eluant) to give 1,3,3′-*tris*-N-(2,4-*dinitrophenyl*)*garamine* (24) (256 mg, 100%) as a yellow amorphous solid, m.p. 192—205° (Found: C, 45.2; H, 4.3; N, 14.2. $C_{31}H_{33}N_9O_{18}$ requires C, 45.4; H, 4.0; N, 15.4%), $[\alpha]_D$ +5.6° (in $Me_2CO$), $\lambda_{max.}$($Me_2CO$), 356 nm (ε 42 300), $\nu_{max.}$ (Nujol) 3 400, 1 630, 1 590, and 1 050 $cm^{-1}$, δ[$(CD_3)_2CO$] † 1.18 and 1.31 (3 H, 2 s, 4′-$CH_3$), 2.82 and 2.89 (3 H, 2 s, 3′-$NCH_3$), 5.50 (1 H, d, $J_{1',2'}$ 3 Hz, H-1′), and 7.35—9.00 (9 H, complex, aromatic).

(iii) 1,2′,3,3″,6′-Pentakis-*N*-(2,4-dinitrophenyl)sisomicin (7) (500 mg), sodium carbonate (1 g), and *m*-chloroperbenzoic acid (4.5 ml) were dissolved in tetrahydrofuran (22 ml) containing water (0.5 ml). The mixture was stirred at 25 °C for 7 days, then evaporated to small volume, taken up in chloroform, and shaken with aqueous 10% sodium sulphite, then with aqueous sodium hydrogen carbonate, and finally with water. The chloroform layer was dried ($MgSO_4$) and evaporated and the residue chromatographed on silica gel plates (20% methanol–chloroform

† Same footnote as on page 1093.

as eluant) to give 1,3,3′-tris-*N*-(2,4-dinitrophenyl)garamine (**24**) (194 mg, 60%) as a yellow amorphous solid, identical with that described in (i) and (ii).

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