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The Structure of Sisomicin, a Novel Unsaturated Aminocyclitol Antibiotic from *Micromonospora inyoensis*

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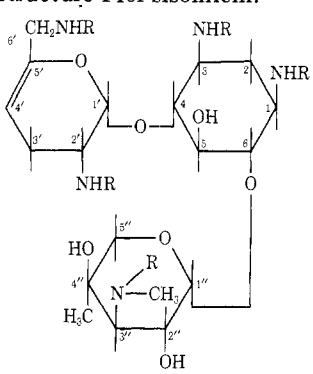
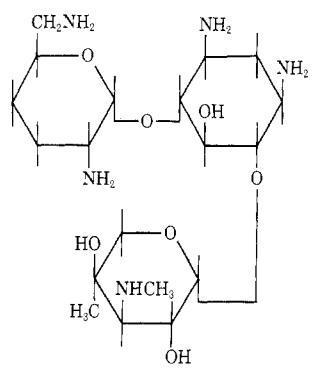
Sisomicin, the principal antibiotic produced in the fermentation of *Micromonospora inyoensis*, has been shown to be *O*-2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl(1→4)-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl(1→6)]-2-deoxy-D-streptamine (**1**). Sisomicin contains a novel unsaturated sugar unit, not previously encountered in any aminocyclitol antibiotic.

Submerged fermentations of *Micromonospora inyoensis* (NRRL 3292) produce sisomicin,[1] a novel unsaturated aminocyclitol antibiotic[2,3] having broad spectrum antibacterial activity.[4] Sisomicin is the major component of the crude antibiotic complex, which was isolated from the fermentation broth by ion-exchange chromatography.[3] Column chromatography of the crude antibiotic on silica gel afforded pure sisomicin, which was crystallized from ethanol. Chemical and physical studies have established structure **1** for sisomicin.[5]



1, R = H
5, R = Ac
21, R = Cbz



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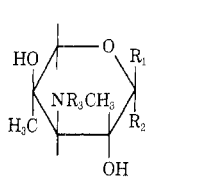
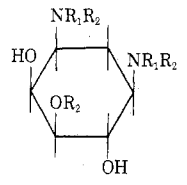
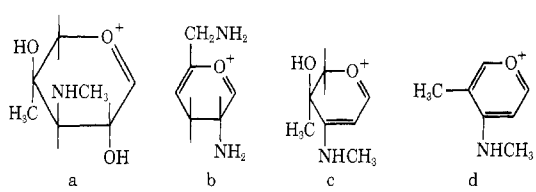


3, R_1, R_2 = H, OH; R_3 = H
11, R_1, R_2 = H, OH; R_3 = Ac
15, R_1 = OCH_3; R_2 = R_3 = H
16, R_1 = R_3 = H; R_2 = OCH_3

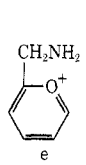


4, R_1 = R_2 = H
6, R_1 = Ac; R_2 = H
26, R_1 = Ac; R_2 = OCH_3

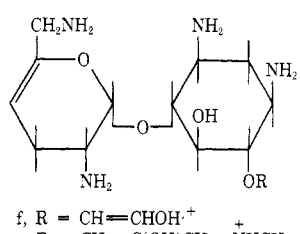
The molecular composition of sisomicin (**1**) was shown to be $C_{19}H_{37}N_5O_7$ by high-resolution mass spectrometry, and microanalyses were in agreement with a monohydrate of the above composition. The molecular weight and composition of sisomicin suggested that it was a dehydro derivative of gentamicin C_{1a} (**2**),[6-10] or an isomer thereof. The ir spectrum of sisomicin showed an absorption at 1690 cm^{-1} consistent with the presence of a vinylic ether group in the molecule. Further evidence for the presence of unsaturation in the molecule and for the location of the site of the unsaturation was obtained from a detailed study of the high-resolution mass spectrum. The latter exhibited prominent peaks at *m/e* 160 and 127 due to the ions[11] a and b formed by glycosidic cleavage of the sugar moieties in the molecule. Subsequent losses of water from the ion a gave rise to ions c and d at *m/e* 142 and 124, respectively. Loss of ammonia from the ion b gave rise to ion *e* at *m/e* 110. The formation of the ions f and g at *m/e*



a b c d



e



f, R = $CH{=}CHOH^{\cdot+}$
g, R = $CH{=}C(OH)CH{=}\overset{+}{N}HCH_3$

330 and 372 due to cleavages adjacent to the 3″-amino group was consistent with structure **1** for sisomicin. Conclusive evidence for the location of the double bond at the 4′,5′ position in sisomicin was obtained by the presence of a prominent peak at *m/e* 362 for the ion h formed by retro-Diels–Alder cleavage of the enopyranoside moiety. The characteristic protonated formyl ions formed in the

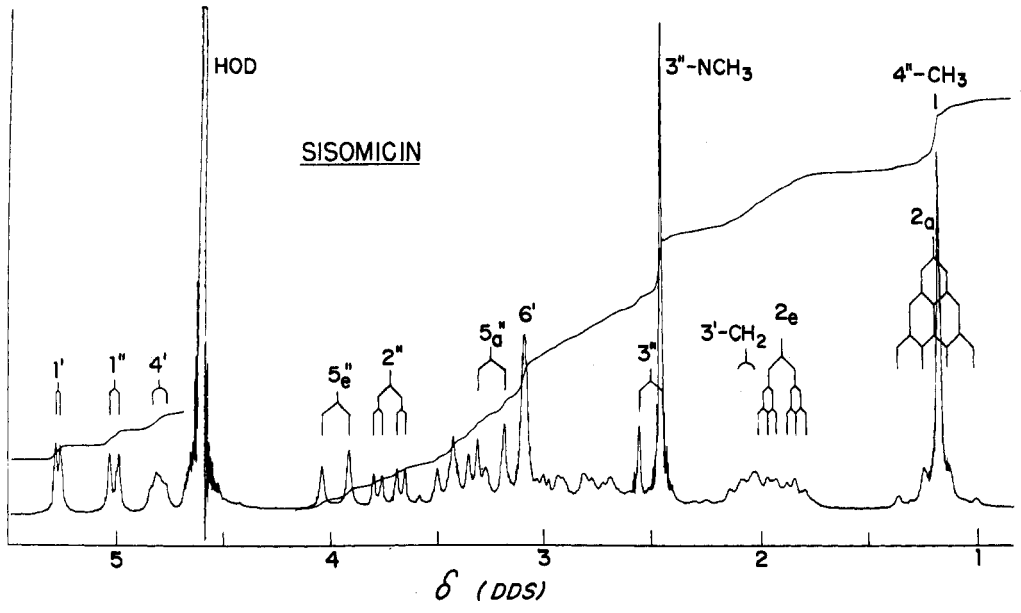
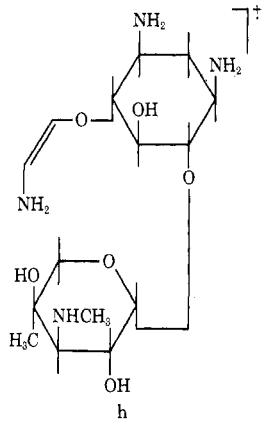


Figure 1.

mass spectra of aminoglycoside antibiotics[12] by initial cleavage of the C_1-C_2 bonds in the sugars followed by

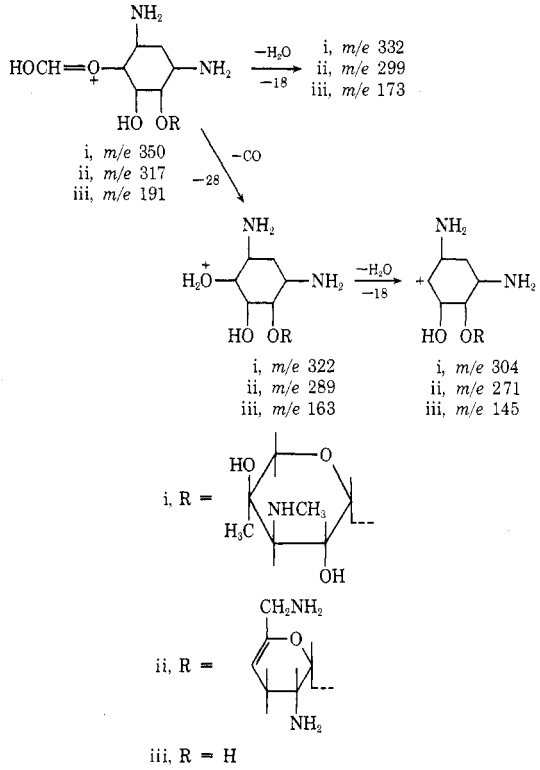


h

losses of carbon monoxide and water were also observed and are shown in Scheme I.

The 100-MHz nmr spectrum (Figure 1) of sisomicin (1) was consistent with the proposed structure and revealed the presence of one *C*-methyl group at δ 1.18 and one *N*-methyl group at δ 2.48. The anomeric proton of the saturated sugar moiety occurred as a doublet at δ 5.04 with $J_{1'',2''}$ = 4 Hz consistent with an axial glycosidic linkage for that sugar. Proton $H_{2''}$ gave rise to a doublet of doublets at δ 3.76 with $J_{2'',3''}$ = 10.0 Hz, while $H_{3''}$ occurred as a doublet at δ 2.53. These assignments confirmed the relative stereochemistry of C_1-C_3 of this unit. The $H_{5''a}$ and $H_{5''e}$ signals occurred as doublets at δ 3.28 and 4.00, respectively, with $J_{5''a,5''e}$ = 12.2 Hz. Irradiation of the $H_{5''e}$ signal caused the doublet at δ 3.28 to collapse to a singlet. The above assignments were consistent with the presence of garosamine (3)[7] as one of the sugar components of sisomicin. The anomeric proton of the enopyranoside moiety gave rise to a doublet at δ 5.30 having $J_{1',2'}$ = 2 Hz, consistent, with an axial glycosidic linkage. A multiplet at δ 3.13 due to $H_{2'}$ was in agreement with the presence of a 2'-equatorial amino group in the enopyranoside. Irradiation of this signal caused the signal due to $H_{1'}$ to collapse to a singlet. The 3'-methylene protons occurred as a complex multiplet at *ca.* δ 1.6. Irradiation of this signal caused the multiplet due to $H_{2'}$ to simplify and at the same time collapsed the multiplet at δ 4.83, due to

Scheme I



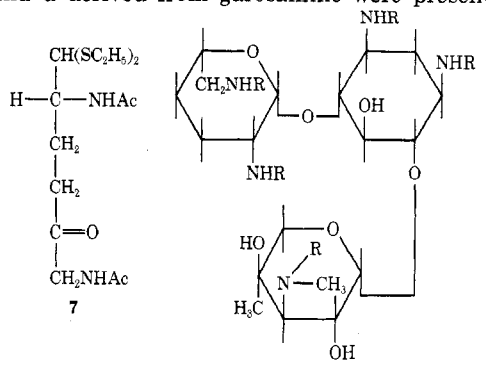
the vinylic 4' proton, to a singlet. The 6'-methylene group appeared as a broad singlet at δ 3.12. Long-range coupling between the 6'-methylene group and the vinylic 4' proton was demonstrated by irradiating the signal at δ 3.12, which sharpened the multiplet at δ 4.83. The above nmr observations established the essential structural features and relative stereochemistry of the novel enopyranoside unit of sisomicin. The presence of deoxystreptamine (4) was supported in the nmr spectrum by a doublet of a doublet of doublets at δ 1.19 having $J_{1a,2a} = J_{2a,3a} = J_{2a,2e}$ = 13 Hz due to H_{2a} and at δ 1.92 having $J_{2a,2e}$ = 13 and $J_{1a,2e} = J_{2e,3a}$ = 4 Hz due to H_{2e}.

The CD spectra of sisomicin (1) recorded in cuprammonium A and in TACu gave values for $[\theta]_{290}$ of −5890 and −8500, respectively. These were consistent with a 4,6 linkage of the sugar units, the 3-aminopentopyranoside

being in the β-L-arabino configuration, thus confirming that it is garosamine.

Acetylation of sisomicin (1) using acetic anhydride in methanol gave a penta-*N*-acetate (5). Mercaptolysis of the *N*-acetate 5 using ethanethiol and concentrated hydrochloric acid gave 1,3-di-*N*-acetyl-2-deoxystreptamine (6), which was identical with an authentic sample,[8] and (2*R*)-2,6-diacetamido-5-ketohexanal diethyl dithioacetal (7). The ir spectrum of the latter revealed the presence of keto and amide groups in the molecule. The nmr spectrum of 7 showed the presence of two ethyl groups and two *N*-acetyl groups. The chemical shifts of the 6- and 4-methylene groups were consistent with the location of the keto group at C_5 and of one of the amino groups at C_6. The occurrence of H_1 as a doublet and the chemical shift of the 2-methine proton at *ca.* δ 4.30 confirmed that the second amino group was situated at C_2. The mass spectrum of 7 showed the expected molecular ion at *m/e* 334 and a prominent ion at *m/e* 275 due to elimination of acetamide (M − 59) clearly supported the location of an amino group at C_2.[13] Subsequent cleavage of the C_3–C_4 bond to give an ion at *m/e* 161 confirmed the presence of a methylene group at C_3. These and other prominent ions are shown in Scheme II.

Hydrogenation of sisomicin (1) was expected to give gentamicin C_{1a} (2), thus affording a complete proof of structure, including stereochemistry and linkages to the 2-deoxystreptamine ring. However, when the catalytic reduction was carried out, an isomeric compound, dihydrosisomicin (8), was obtained and no gentamicin C_{1a} (2) could be demonstrated in the reduction product on careful chromatographic examination. The absence of a vinylic ether band in the ir spectrum of 8 and the presence of an $M^+ + 1$ peak at *m/e* 450 in the mass spectrum indicated that the double bond had been reduced. The expected ions a, c, and d derived from garosamine were present in the



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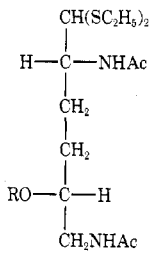
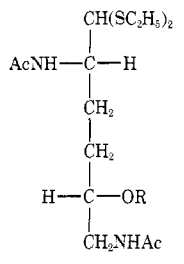
8, R = H
9, R = Ac

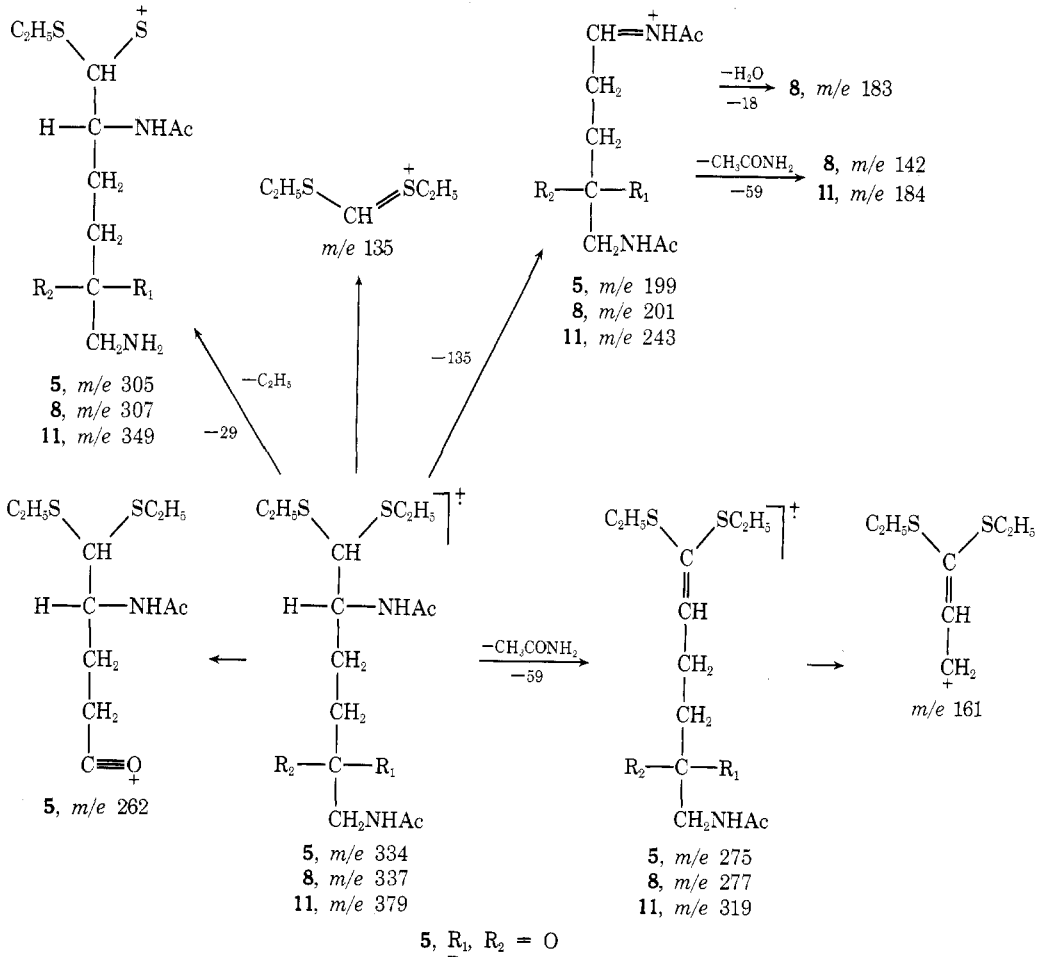


10, R = H
13, R = Ac



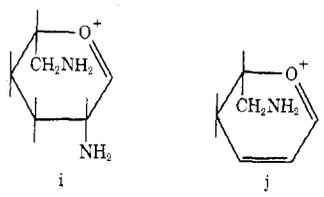
12, R = H
14, R = Ac

Scheme II



5, R_1, R_2 = O
8, R_1 = H; R_2 = OH
11, R_1 = H; R_2 = OAc

spectrum, while the new sugar moiety gave rise to the ions i and j at m/e 129 and 112, respectively. The prominent

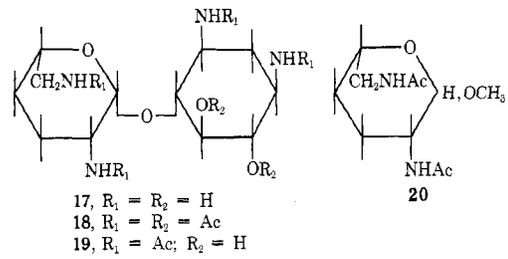


i j

ion h formed by retro-Diels–Alder cleavage of the enopyranoside in sisomicin was absent in the spectrum of **8**. Ions corresponding to saturated derivatives of f and g were observed in the spectrum of **8**, at m/e 332 and 374, respectively. The characteristic ions in Scheme I for series i and iii were identical with those of sisomicin (**1**), while the series ii was shifted to higher mass by two mass units, corresponding to the saturated ions at m/e 319, 301, 291, and 273. The nmr spectrum of **8** showed the anomeric proton of garosamine as a doublet with J = 4 Hz at δ 5.10. The anomeric proton of the remaining sugar occurred as a doublet at δ 4.78 with J = 1.7 Hz. The chemical shift of the latter clearly indicated that $H_{1'}$ was not in an equatorial orientation, which would have resulted if reduction had occurred from the lower face of the enopyranoside to give gentamicin C_{1a}. Instead, reduction had taken place exclusively from the top face of the enopyranoside to give an L sugar having an axial CH_2NH_2 substituent at $C_{5'}$ and an axial glycosidic linkage at $C_{1'}$. The chemical shift of the anomeric proton may now be explained by a conformational inversion from the $^4C_1$ to the $^1C_4$ conformation, in order to relieve excessive 1,3-diaxial interaction. This resulted in an axial orientation for $H_{1'}$ causing an upfield shift for $H_{1'}$ relative to the corresponding proton in the isomeric gentamicin C_{1a} (**2**).[6] The signal due to $H_{1'}$ in the latter occurred as a doublet at *ca.* δ 5.10 with J = 4 Hz, consistent with an $H_{1'e}$, $H_{2'a}$ orientation in gentamicin C_{1a} (**2**).[6] The coupling constant of 1.7 Hz observed for $H_{1'a}$,$H_{2'e}$ in the spectrum of **8** was consistent with the above observations. Dihydrosisomicin (**8**) and gentamicin C_{1a} (**2**) could be distinguished by tlc on silica gel plates using chloroform–methanol–7% ammonium hydroxide (1:2:1) as the eluent. The compounds could also be distinguished by the color produced on spraying with ninhydrin.[3] Dihydrosisomicin (**8**) is inactive as an antibacterial agent.

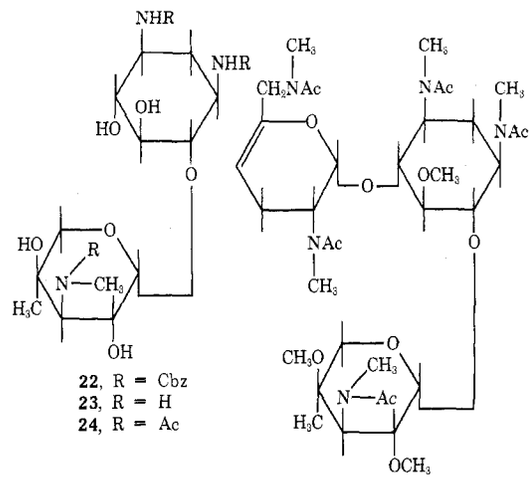
Acetylation of dihydrosisomicin (**8**) in methanol afforded the penta-*N*-acetyl derivative **9**. Mercaptolysis of the latter with ethanethiol and concentrated hydrochloric acid gave, after re-N-acetylation, 2,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose diethyl dithioacetal (**10**), 1,3-di-*N*-acetyl-2-deoxystreptamine (**6**), and 3-*N*-acetylgarosamine (anomeric mixture) (**11**). The nmr spectrum of the dithioacetal **10** supported the assigned structure and was identical with that of the D-threo enantiomer (**12**) which had been prepared by unambiguous synthesis.[14] The dithioacetals **10** and **12** were identical on tlc. The L-threo enantiomer (**10**) showed a rotation of +32° in methanol while the D-threo enantiomer (**12**) had a rotation of −30°.[14] Acetylation of **10** gave the *N,N,O*-triacetyl derivative (**13**) as a syrup having a rotation of +21.6° in methanol. The corresponding *N,N,O*-triacetyl-D-threo enantiomer (**14**)[14] had a rotation of −19° in the same solvent. The mass spectrometric fragmentation patterns of **10** and **13** supported the assigned structures and are given in Scheme II.

Methanolysis of dihydrosisomicin (**8**) gave methyl garosaminide (**15** and **16**)[7] and 5′-epigentamine C_{1a} (**17**), which was characterized as the hydrochloride salt. The spectroscopic and analytical data were in accord with the



17, $R_1 = R_2 = H$
18, $R_1 = R_2 = Ac$
19, $R_1 = Ac$; $R_2 = H$

20



22, R = Cbz
23, R = H
24, R = Ac

25

assigned structure. The free base **17**, obtained from the hydrochloride salt by passage over Amberlite IR 45 resin, was acetylated to give the *N,O*-peracetyl derivative (**18**), which on saponification with sodium methoxide in methanol gave the tetra-*N*-acetyl derivative (**19**). The CD spectrum of **19** run in cuprammonium A showed a negative extremum at 290 nm and a positive extremum at 575 nm consistent with a negative dihedral angle for the glycol system.[15,16] The above observation demonstrated that the sugar moiety is located at the 4 position of the deoxystreptamine ring in 5′-epigentamine C_{1a} (**17**). It follows that the enopyranoside moiety in sisomicin (**1**) is glycosidically linked to the 4-hydroxy group of deoxystreptamine.

Methanolysis of penta-*N*-acetyldihydrosisomicin (**9**) followed by re-N-acetylation gave 1,3-di-*N*-acetyl-2-deoxystreptamine (**6**), methyl 3-*N*-acetylgarosaminide, and methyl 2,6-di-*N*-acetyl-5-epipurpurosaminide C as an anomeric mixture (**20**). The two anomeric protons in **20** gave rise to doublets in the nmr spectrum at δ 4.50 with $J_{1e,2e}$ = 1.5 Hz due to the α-L anomer and at δ 4.40 with $J_{1a,2e}$ = 2 Hz due to the β-L anomer. Both signals were consistent with a $^1C_4$ configuration for this sugar, and with the assigned stereochemistry at C_2.

Sisomicin (**1**) was converted into the penta-*N*-carbobenzoxy derivative (**21**), which was found to be extremely labile to mildly acidic conditions. Thus hydrolysis of **21** with sulfuric acid in tetrahydrofuran, or with Amberlite IR 120 (H^+) resin in tetrahydrofuran at ambient temperature, gave 1,3,3′-tri-*N*-carbobenzoxygaramine (**22**), a novel pseudodisaccharide derivative, in high yield. Hydrogenolysis of tri-*N*-carbobenzoxygaramine (**22**) over 10% palladium on carbon gave the underivatized pseudo-disaccharide, garamine (**23**). The nmr and mass spectra were in accord with the assigned structure. Methanolysis of garamine (**23**) gave 2-deoxystreptamine (**4**) and methyl α- and β-garosaminides (**15** and **16**) which were identical with authentic samples obtained from the gentamicins.[7]

Garamine (**23**) was converted into the tri-*N*-acetyl derivative (**24**) in the usual manner. The CD spectrum of a cuprammonium A complex of **24** showed a positive extremum at 290 nm and a negative extremum at 560 nm con-

sistent with a positive dihedral angle for the 4,5-glycol.[15,16] The garosamine moiety is therefore located at the 6 position of deoxystreptamine in sisomicin (**1**).

Further confirmation of the location of the enopyranoside at the 4 position and of garosamine at the 6 position in sisomicin was obtained chemically as follows. Permethylation of penta-*N*-acetylsisomicin (**5**) gave the permethylated derivative **25**, which on vigorous acidic hydrolysis gave the symmetrical 1,3-di-*N*-acetyl-1,3-di-*N*-methyl-5-*O*-methyl-2-deoxystreptamine (**26**), which was identical with an authentic sample.[8] The latter showed a prominent ion at *m/e* 270 due to loss of water from the molecular ion, which is characteristic[17] of a deoxystreptamine derivative containing hydroxyl groups at the 4 and/or 6 positions. If the *O*-methyl group had been at the 4 or 6 position a loss of methanol would also have been evident from the molecular ion, which was not the case. The CD spectrum of **26** in cuprammonium A showed only base-line absorption, indicating that the molecule contained no vicinal glycol system.

The total structure and absolute stereochemistry of sisomicin may therefore be represented by the structure **1**. The ^{13}C magnetic resonance spectrum of sisomicin obtained subsequently[18] was in full accord with this structure.

Experimental Section

Optical rotations were measured at *c* 0.3. Ir spectra were recorded either on a Perkin-Elmer Model 221 or on an Infracord 137 spectrometer. Nmr spectra were obtained at 60 or 100 MHz on a Varian A-60A or on an XL-100-15 spectrometer, respectively. Chemical shifts in D_2O solution are reported in parts per million downfield from internal or external DSS. All other chemical shifts are reported in parts per million downfield from internal TMS. CD spectra were run on a Cary 61 spectrometer. Mass spectra were recorded on a Perkin-Elmer RMU-6D instrument, a Varian MAT CH5 instrument, or on an AEI MS 902B spectrometer.

Isolation and Characterization of Sisomicin (1). Chromatography of the crude antibiotic complex produced by submerged fermentation of *Micromonospora inyoensis*[2] on silica gel using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide (1:1:1) system as the eluent gave sisomicin (**1**), which after passage over Amberlite IRA 401S (OH^-) resin, crystallized as needles from ethanol: mp 198–201°; $[\alpha]^{26}D$ +188.9° (H_2O); $[\theta]_{290}$ −8500 (TACu), −5800 (Cupra A); ν_{max} (KCl) 3370 (NH, OH), 1690 (CH=COC), 1065 cm^{-1} (COC); nmr (D_2O) δ 1.18 (s, 3, 4″-CH_3), 2.48 (s, 3, 3″-NCH_3), 2.53 (d, $J_{2'',3''}$ = 10.0 Hz, 1, $H_{3''}$), 3.12 (broad s, 2, 6′-CH_2), 3.28 (d, $J_{5''a,5''e}$ = 12.2 Hz, 1, $H_{5''a}$), 3.76 (dd, $J_{1'',2''}$ = 4, $J_{2'',3''}$ = 10 Hz, 1, $H_{2''}$), 4.00 (d, $J_{5''a,5''e}$ = 12.2 Hz, 1, $H_{5''e}$), 4.83 (broad t, 1, $H_{4'}$), 5.04 (d, $J_{1'',2''}$ = 4 Hz, 1, $H_{1''}$) and 5.30 (d, $J_{1',2'}$ = 2 Hz, 1, $H_{1'}$); *m/e* 447.262 ($M^{\cdot+}$) (calcd for $C_{19}H_{37}N_5O_7$, *m/e* 447.269).

Anal. Calcd for $C_{19}H_{37}N_5O_7 \cdot H_2O$: C, 49.01; H, 8.46; N, 15.04. Found: C, 49.56; H, 8.26; N, 14.89.

1,3,2′,6′,3″-Penta-*N*-acetylsisomicin (5). Sisomicin (**1**, 500 mg) was dissolved in a mixture of methanol (25 ml) and acetone (25 ml) containing acetic anhydride (8 ml) and the solution was allowed to remain at 25° for 30 min. The solution was evaporated *in vacuo* and the residue, after azeotroping with toluene, was chromatographed on a silica gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide (1:1:1) system as the eluent to give the acetate **5**: 660 mg (90%); mp 188–198° dec; $[\alpha]^{26}D$ +194.6° (CH_3OH), +200° (H_2O); ν_{max} (Nujol) 3240 (OH, NH), 1650, 1550 ($NCOCH_3$), 1025 cm^{-1} (COC); nmr (CD_3OD)[19] δ 1.01, 1.10 (s, 3, 4″-CH_3), 1.90, 1.96, 1.99, 2.15 (s, 15, $NCOCH_3$), 3.01, 3.13 (s, 3, 3″-NCH_3), 5.19 (d, $J_{1'',2''}$ = 4 Hz, 1 $H_{1''}$), 5.54 (d, $J_{1',2'}$ = 2.5 Hz, 1, $H_{1'}$), δ (DMSO, at 140°) 0.97 (s, 3, 4″-CH_3), 1.76, 1.79, 1.82, 1.86, 1.99 (s, 15, $NCOCH_3$), 2.97 (s, 3, 3′-NCH_3), 5.09 (broad d, $J_{1'',2''}$ = 4 Hz, 1, $H_{1''}$), 5.32 (d, $J_{1',2'}$ = 2.5 Hz, 1, $H_{1'}$).

Anal. Calcd for $C_{29}H_{47}N_5O_{12}$: C, 52.95; H, 7.20; N, 10.65. Found: C, 52.45; H, 7.26; N, 10.44.

Mercaptolysis of 1,3,2′,6′,3″-Penta-*N*-acetylsisomicin (5). 1,3,2′,6′,3″-Penta-*N*-acetylsisomicin (**5**, 3 g) was added to a solution of ethanethiol (6 ml) and concentrated hydrochloric acid (6 ml) and the mixture was stirred at 7° for 24 hr. The solution was diluted with water (500 ml), neutralized with an excess of basic lead carbonate, filtered, and lyophilized. The solid was dissolved in methanol (100 ml), acetic anhydride (3 ml) was added, and the solution was allowed to remain at 25° for 1 hr. The solution was evaporated, the residue was triturated with chloroform (50 ml), and the insoluble 1,3-di-*N*-acetyl-2-deoxystreptamine (**6**), 1.07 g (96%), was filtered off. Trituration of the latter with chloroform gave colorless crystals which were identical (melting point and mixture melting point) with an authentic sample.[8] The filtrate was evaporated to dryness and the residue (1.75 g) was recrystallized repeatedly from benzene–methanol and then from ethanol to give (2*R*)-2,6-diacetamido-5-ketohexanal diethyl dithioacetal (**7**): 930 mg (61%); mp 153–154°; $[\alpha]^{26}D$ +33.7° ($CHCl_3$); ν_{max} ($CHCl_3$) 3380 (NH), 1740 (C=O), 1670, 1500 cm^{-1} ($NHCOCH_3$); nmr ($CDCl_3$) δ 1.28 (t, J = 7 Hz, 3, CH_3CH_2S), 1.30 (t, J = 7 Hz, 3, CH_3CH_2S), 1.98 (s, 3, NHAc), 2.02 (s, 3, NHAc), *ca.* 2.50 (m, 2, 4-CH_2), 2.69 (q, J = 7 Hz, 2, CH_3CH_2S), 2.70 (q, J = 7 Hz, 2, CH_3CH_2S), 3.92 (d, J = 4 Hz, 1, H_1), 4.11 (d, J = 5 Hz, 2, 6-CH_2), 6.00 (broad d, J = 9 Hz, 1, 2-NHAc), and 6.38 (broad m, $W_{1/2} \cong$ 9 Hz, 1, 6-NHAc; *m/e* 334 ($M^{\cdot+}$).

Dihydrosisomicin (8). Sisomicin (**1**, 1.79 g) in methanol (40 ml) was hydrogenated over 10% palladium on carbon (400 mg) at 25° (1 atm) for 23 hr. The catalyst was filtered off and washed with methanol and the combined filtrates were evaporated. The solid was chromatographed on a silica gel column (160 × 1 cm) using the lower phase of a chloroform–2-propanol–concentrated ammonium hydroxide (2:1:1) system as the eluent to give, after passage over Amberlite IRA 401S (OH^-) followed by lyophilization, the dihydrosisomicin (**8**), 1.05 g (58%), as a colorless solid: $[\alpha]^{26}D$ +145.0° (H_2O); ν_{max} ($CHCl_3$) 3550, 3200 cm^{-1} (NH, OH); nmr (D_2O) δ 1.21 (s, 3, 4″-CH_3), 2.52 (s, 3, 3″-NCH_3), 4.78 (d, $J_{1',2'}$ = 1.7 Hz, 1, $H_{1'}$) and 5.10 (d, $J_{1'',2''}$ = 4 Hz, 1, $H_{1''}$); *m/e* 450 [(M + 1)$^+$].

Anal. Calcd for $C_{19}H_{39}N_5O_7 \cdot \frac{1}{2}CO_2$:[20] C, 49.67; H, 8.33; N, 14.85. Found: C, 50.12; H, 8.47; N, 14.32.

1,3,2′,6′,3″-Penta-*N*-acetyldihydrosisomicin (9). Dihydrosisomicin (**8**, 400 mg) was dissolved in methanol (10 ml), and acetic anhydride (1 ml) was added. After standing at 25° for 5 hr the mixture was poured into ether and the precipitate was filtered off and washed with ether to give the acetate **9** as a colorless, amorphous solid: 440 mg (75%); mp 192–200°; $[\alpha]^{26}D$ +98.0° (C_2H_5OH); ν_{max} (Nujol) 3250 (NH, OH), 1640, 1540 cm^{-1} ($NCOCH_3$).

Anal. Calcd for $C_{29}H_{49}N_5O_{12} \cdot H_2O$: C, 51.39; H, 7.59; N, 10.33. Found: C, 51.49; H, 7.43; N, 10.20.

Mercaptolysis of 1,3,2′,6′,3″-Penta-*N*-acetyldihydrosisomicin (9). 1,3,2′,6′,3″-Penta-*N*-acetyldihydrosisomicin (**9**, 600 mg) was dissolved in a mixture of 6 *N* hydrochloric acid (3 ml) and ethanethiol (3 ml) and the reaction mixture was stirred at 25° for 48 hr. The reaction mixture was worked up as before to give 1,3-di-*N*-acetyl-2-deoxystreptamine (**6**), 210 mg (94%), which was insoluble in chloroform. The chloroform-soluble material was chromatographed on a silica gel column (110 × 2.5 cm) using 15% methanol in benzene as the eluent to give 2,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose diethyl dithioacetal (**10**) as a low-melting crystalline solid: 235 mg (77%); mp 81–84°; $[\alpha]^{26}D$ +32.0° (CH_3OH); ν_{max} ($CHCl_3$) 3400, 3300 (OH, NH), 1650 cm^{-1} ($NCOCH_3$); nmr ([$^2H_5$]pyridine) δ 1.19 (t, J = 7.5 Hz, 3, CH_3CH_2S), 1.26 (t, J = 7.5 Hz, 3, CH_3CH_2S), 2.05 (s, 3, NAc), 2.12 (s, 3, NAc), 2.74 (q, J = 7.5 Hz, 2, CH_3CH_2S), 2.78 (q, J = 7.5 Hz, 2, CH_3CH_2S), 4.09 (m, 1, H_5), 4.40 (d, $J_{1,2}$ = 4 Hz, 1, H_1), and 4.70 (dt, $J_{1,2}$ = 4, $J_{2,3}$ = 9 Hz, 1, H_2); *m/e* 337 [(M + 1)$^+$].

Anal. Calcd for $C_{14}H_{28}N_2S_2O_3$: C, 49.97; H, 8.39; N, 8.33; S, 19.06. Found: C, 49.85; H, 8.44; N, 8.39; S, 18.22.

The more polar fractions from the column afforded 3-*N*-acetylgarosamine (**11**), which crystallized from methanol–benzene, 17 mg (9%), mp 183–185°, *m/e* 220 [(M + 1)$^+$] and was identical with an authentic sample.

5-*O*-Acetyl-2,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose Diethyl Dithioacetal (13). 2,6-Diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexose diethyl dithioacetal (**10**, 48 mg) was dissolved in dry pyridine (0.5 ml), and acetic anhydride (0.05 ml) was added. The mixture was allowed to remain at 25° for 16 hr. The solution was evaporated and the residue was chromatographed on a silica gel column (110 × 1 cm) using 15% methanol in chloroform as the eluent to give the acetate **13** as a colorless syrup: 32 mg (59%); $[\alpha]^{26}D$ +22.0° (CH_3OH); ν_{max} ($CHCl_3$) 3450 (NH), 1740, 1240 (CH_3COO), 1660 cm^{-1} ($NCOCH_3$); nmr ([$^2H_5$]pyridine) δ 1.20 (t, J = 7.5 Hz, 3, CH_3CH_2S), 1.25 (t, J = 7.5 Hz, 3, CH_3CH_2S), 1.88 (s, 3, OAc), 2.02 (s, 3, NAc), 2.11 (s, 3, NAc), 2.72 (q, J = 7.5 Hz, 2, CH_3CH_2S), 2.77 (q, J = 7.5 Hz, 2, CH_3CH_2S), 3.52 (dd, $J_{6,6'}$

= 14, $J_{5,6}$ = 6 Hz, 1, H_6), 3.73 (dd, $J_{6,6'}$ = 14 Hz, $J_{5,6'}$ = 5 Hz, 1, $H_{6'}$), 4.37 (d, $J_{1,2}$ = 4 Hz, 1, H_1), 4.70 (m, 1, H_2), and 5.30 (m, 1, H_5); *m/e* 379 [(M + 1)$^+$].

Methanolysis of Dihydrosisomicin (8). Dihydrosisomicin (**8**, 1.25 g) was heated under reflux with 6 *N* hydrogen chloride in methanol (125 ml) for 8 hr. The reaction mixture was cooled to 7° and the crystalline 5′-epigentamine C_{1a} (**17**) hydrochloride precipitate was filtered off, washed with cold methanol, and dried: 591 mg (49%); mp 270–275°; $[\alpha]^{26}D$ +24.5° (H_2O); ν_{max} (Nujol) 3350–3320 (OH, NH), 1975 cm^{-1} (NH^+Cl^-).

Anal. Calcd for $C_{12}H_{26}N_4O_4 \cdot 4HCl \cdot H_2O$: C, 31.72; H, 7.10; N, 12.33; Cl, 31.22. Found: C, 31.41; H, 7.11; N, 12.30; Cl, 30.82.

The hydrochloride (350 mg) was converted to the free base by passage over Amberlite IR 45 resin followed by lyophilization to give **17**: 255 mg; $[\alpha]^{26}D$ +31.0° (H_2O); nmr (D_2O) δ 4.87 (d, J = 1.5 Hz, 1, $H_{1'}$); *m/e* 291 [(M + 1)$^+$], 191, 173, 163, 145, 129.

The mother liquors after crystallization of 5′-epigentamine C_{1a} (**17**) hydrochloride on evaporation followed by chromatography on silica gel using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide (1:1:1) system as the eluent, gave additional 5′-epigentamine C_{1a} (**17**), 415 mg (51%), and methyl garosaminide (**15** and **16**) as a syrup, 400 mg (76%), which was identical with an authentic sample (tlc, ir, nmr, and mass spectrum).

1,3,2′,6′-Tetra-*N*-acetyl-5,6-di-*O*-acetyl-5′-epigentamine C_{1a} (18). 5′-Epigentamine C_{1a} (**17**, 90 mg) in pyridine (2 ml) was treated with acetic anhydride (0.5 ml) and the mixture was stirred at 25° for 24 hr. The solution was evaporated and the residue was dissolved in acetone and filtered. The hexaacetate **18** crystallized as a monohydrate, monoacetone solvate: 76 mg (45%); mp 161–164°; $[\alpha]^{26}D$ +8.1° (H_2O); ν_{max} (Nujol) 3440, 3290, 3100 (NH), 1755, 1720, 1690, 1240, 1225 (OAc), 1650 cm^{-1} (NAc); nmr (D_2O) δ 1.91 (s, 3, OAc), 1.97–2.00 (s, 12, NAc), 2.09 (s, 3, OAc) and 2.18 (s, 6, acetone); *m/e* 542 (M·$^+$), 483 (M − 59), 359, 313, 212.

Anal. Calcd for $C_{24}H_{38}N_4O_{10} \cdot H_2O \cdot C_3H_6O$: C, 52.41; H, 7.50; N, 9.06. Found: C, 52.68; H, 7.42; N, 9.41.

1,3,2′,6′-Tetra-*N*-acetyl-5′-epigentamine C_{1a} (19). 1,3,2′,6′-Tetra-*N*-acetyl-5,6-di-*O*-acetyl-5′-epigentamine C_{1a} (**18**, 40 mg) was dissolved in methanol (1.5 ml) and a catalytic amount of sodium metal was added to the stirred solution. After stirring at 25° for 1 hr, Dry Ice was added, the solution was concentrated, and the residue was triturated with ethanol. The sodium carbonate was filtered off and the filtrate was concentrated to give the crystalline tetra-*N*-acetate **19**: 21 mg (62%); mp 250–260° dec; ν_{max} (Nujol) 3500, 3450, 3300, 3100 (OH, NH), 1640, 1600 cm^{-1} (NAc); nmr (D_2O) δ 1.98–2.01 (s, 12, NAc) and 4.80 (d, J = 1.5 Hz, 1, $H_{1'}$); *m/e* 441 (M − 17), 399 (M − 59), 381 (M − 17 − 59), 275 (191 + 2CH_2CO), 229 (145 + 2CH_2CO), 257 (275 − 18), 239 (257 − 18), 211 (229 − 18), 213 (129 + 2CH_2CO).

Methanolysis of 1,3,2′,6′,3″-Penta-*N*-acetyldihydrosisomicin (9). 1,3,2′,6′3″-Penta-*N*-acetyldihydrosisomicin (**9**, 2.5 g) was heated under reflux with 6 *N* hydrogen chloride in methanol (125 ml) for 7 hr and then allowed to remain at 25° for 16 hr. The reaction mixture was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2.5 cm) using the lower phase of a chloroform–2-propanol–concentrated ammonium hydroxide (2:1:1) system as the eluent. The least polar component, methyl 3-*N*-acetylgarosaminide, was isolated as a crystalline solid, 190 mg (22%), mp 198–201°, $[\alpha]^{26}D$ +206.0° (H_2O), *m/e* 233 (M·$^+$), which was identical with an authentic sample.

The remaining fractions from the column were re-*N*-acetylated and then rechromatographed on a silica gel column (110 × 2.5 cm) using the same eluent as before to give additional methyl 3-*N*-acetylgarosaminide, 386 mg (44%), and methyl 2,6-di-*N*-acetyl-5′-epipurpurosaminide C (**20**) as a syrup: 628 mg (68%); $[\alpha]^{26}D$ −34.6° (H_2O); ν_{max} ($CHCl_3$) 3450 (NH), 1670 cm^{-1} (NAc); nmr ($CDCl_3$) δ 2.00 (s, 6, NAc), 3.35, 3.48 (s, 3, α- and β-1-OCH_3), 4.40 (d, $J_{1a,2e}$ = 2 Hz, 0.3, H_{1a}) and 4.50 (d, $J_{1e,2e}$ = 1.5 Hz, 0.7, H_{1e}); *m/e* 245 [(M + 1)$^+$], 213 (M − 31), 185 (M − 59, 184 (M − 60), 154 (M − 59 − 60), 153 (M − 60 − 31).

1,3,2′,6′,3″-Penta-*N*-carbobenzoxysisomicin (21). Sisomicin (**1**, 25 g) and sodium carbonate (13 g) were dissolved in distilled water (625 ml). Carbobenzoxy chloride (100 ml) was added to the stirred solution at 25° and the mixture was stirred for 16 hr. The solid was filtered off, washed thoroughly with water, dried *in vacuo*, and then washed with hexane to give **25**, 62 g (99%), as a colorless, amorphous solid. Preparative tlc on silica gel plates using 40% acetone in benzene as the eluent gave an analytically pure sample of **21**: mp 165–173° dec; $[\alpha]^{26}D$ +96.2° (CH_3OH); ν_{max} ($CHCl_3$) 3400 (OH, NH), 1720, 1515, 1215 (NHCOO), 1050 (COC), 695 cm^{-1} (C_6H_5); nmr ($CDCl_3$)[19] δ 1.03 (broad s, 3, 4″-CH_3), 3.02 (broad s, 3, 3″-NCH_3), 5.02 (broad s, 10, -$CH_2C_6H_5$), 3.28, 3.30 (broad s, 25, -$CH_2C_6H_5$).

Anal. Calcd for $C_{59}H_{67}N_5O_{17}$: C, 63.41; H, 5.99; N, 6.27. Found: C, 63.53; H, 6.23; N, 6.28.

1,3,3′-Tri-*N*-carbobenzoxygaramine (22). 1,3,2′,6′,3″-Penta-*N*-carbobenzoxysisomicin (**21**, 436 g) was dissolved in tetrahydrofuran (3 l.), and Amberlite IR 120 (H^+) resin (1 kg) was added. The mixture was allowed to stand at 25° for 3 days and was then filtered and the resin was washed with tetrahydrofuran. The combined filtrates were evaporated *in vacuo* in the presence of a few milliliters of water to give the crude product as a gum. Chromatography on a silica gel column (200 × 10 cm) using 10% methanol in chloroform as the eluent gave **22**, 200 g (71%), as a colorless, amorphous solid: mp 104–112°: $[\alpha]^{26}D$ + 69.6° (C_2H_5OH); λ_{max} (CH_3OH) 206 nm (ϵ 28,000) and 258 (538); ν_{max} ($CHCl_3$) 3350 (OH, NH), 1700, 1525 (NHCOO), 694 cm^{-1} (C_6H_5); nmr ($CDCl_3$)[19] δ 0.99 (broad s, 3, 4′-CH_3), 3.00 (broad s, 3, 3′-NCH_3), 5.00 (broad s, 6, -$CH_2C_6H_5$), 7.20 (m, 15, -$CH_2C_6H_5$), δ (DMSO, at 120°) 0.96 (s, 3, 4′-CH_3), 2.99 (s, 3, 3′-NCH_3), 5.02 (s, 2, -$CH_2C_6H_5$), 5.05 (s, 2, -$CH_2C_6H_5$), 5.11 (s, 2, -$CH_2C_6H_5$), 3.31 (s, 15, -$CH_2C_6H_5$).

Anal. Calcd for $C_{37}H_{45}N_3O_{12} \cdot H_2O$: C, 59.92; H, 6.34; N, 5.67. Found: C, 60.12; H, 5.83; N, 5.63.

Garamine (23). 1,3,3′-Tri-*N*-carbobenzoxygaramine (**22**, 2.02 g) was dissolved in methanol (100 ml) and hydrogenated over 10% palladium on carbon (1.0 g) at 25° (50 psi) for 16 hr. The catalyst was filtered off and rinsed with methanol and the combined filtrates were evaporated and chromatographed on a silica gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide (2:1:1) system as the eluent to give **23**. The latter, after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization, was obtained as a colorless amorphous solid: 750 mg (84%); mp 89–99°; $[\alpha]^{26}D$ +135.4° (H_2O); pK_a 8.5; $[\theta]_{290}$ −15,600 (TACu); ν_{max} (Nujol) 3300 (OH, NH), 1060 cm^{-1} (COC); nmr (D_2O) δ 1.19 (s, 3, 4′-CH_3), 2.51 (s, 3, 3′-NCH_3), 2.57 (d, $J_{2',3'}$ = 10.5 Hz, 1, $H_{3'}$), 3.30 (d, $J_{5'a,5'e}$ = 12.5 Hz, 1, $H_{5'a}$), 3.79 (dd, $J_{2',3'}$ = 10.5, $J_{1',2'}$ = 4 Hz, 1, $H_{2'}$), 4.03 (d, $J_{5'a,5'e}$ = 12.5 Hz, 1, $H_{5'e}$), 5.06 (d, $J_{1',2'}$ = 4 Hz, 1, $H_{1'}$); *m/e* 322 [(M + 1)$^+$], 246, 191, 173, 163, 145, 160.

Anal. Calcd for $C_{13}H_{27}N_3O_6$: C, 48.60; H, 8.41; N, 13.08. Found: C, 48.31; H, 8.54; N, 12.87.

Methanolysis of Garamine (23). Garamine (**23**, 484 mg) was dissolved in 6 *N* hydrogen chloride in methanol (30 ml) and the solution was heated on a steam bath for 6 hr. The insoluble 2-deoxystreptamine dihydrochloride, 223 mg (63%), mp 281–286° dec, was filtered off. The latter was dissolved in water and passed over Amberlite IR 45 resin and the eluate on evaporation gave 2-deoxystreptamine (**4**) which crystallized from ethanol, mp 220° dec, and which was identical (tlc, ir, nmr, melting point, analysis) with an authentic sample.

The filtrate after removal of 4 was passed over Amberlite IR 45 resin and the eluate was evaporated to give an oil. The latter was chromatographed on a silica gel column (110 × 1 cm) using the lower phase of a chloroform–methanol–17% ammonium hydroxide (2:1:1) system as the eluent to give a mixture of methyl α- and β-garosaminide (**15** and **16**), 225 mg (78%), as a colorless gum, which was identical (tlc, ir, nmr) with authentic samples.[7]

1,3,3′-Tri-*N*-acetylgaramine (24). Garamine (**23**, 500 mg) was dissolved in methanol (17 ml) containing acetic anhydride (2.5 ml) and the solution was allowed to remain at 25° for 20 min. The solution was evaporated to dryness and the resulting solid was chromatographed on preparative tlc plates using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide (1:1:1) system as the eluent. The acetate **24** was extracted from the silica gel with 25% methanol in chloroform, which on evaporation gave a colorless, amorphous solid, 450 mg (65%), which crystallized from ethyl acetate–methanol–acetone: mp 190–195°; $[\alpha]^{26}D$ +106.6° (C_2H_5OH), +108.2° (H_2O); $[\theta]_{290}$ +1970 (Cupra A); ν_{max} (Nujol) 3240 (OH, NH), 1650, 1550 (NHCOCH$_3$), 1050 cm^{-1} (COC); nmr (CD_3OD)[19] δ 1.02, 1.12 (s, 3, 4′-CH_3), 1.93, 1.98, 2.18 (s, 9, $NCOCH_3$), 3.05, 3.17 (s, 3, 3′-NCH_3), 5.22 (d, $J_{1',2'}$ = 4 Hz, 1, $H_{1'}$).

Anal. Calcd for $C_{19}H_{33}N_3O_9 \cdot H_2O$: C, 49.02; H, 7.58; N, 9.03. Found: C, 49.48; H, 7.51; N, 9.47.

Methylation and Acid Hydrolysis of 1,3,2′,6′,3″-Penta-*N*-acetylsisomicin (5). 1,3,2′,6′,3″-Penta-*N*-acetylsisomicin (**5**, 102 mg) was dissolved in dry DMF (5 ml) and sodium hydride (hexane washed, 214 mg) was added. The mixture was warmed to 50° and stirred for 1 hr. The mixture was cooled, methyl iodide (1.4 ml) was added, and the mixture was stirred at 25° for 24 hr. The

solids were filtered off and washed with tetrahydrofuran and the combined filtrates were evaporated to dryness. The residue was taken up in chloroform and washed with water. The chloroform extract was dried ($MgSO_4$) and evaporated to give 1,3,2′,6′,3″-penta-*N*-acetyl-1,3,2′,6′-tetra-*N*-methyl-5,2″,4″-tri-*O*-methylsisomicin (**25**) as a clear gum, *m/e* 755 ($M^{\cdot+}$), 614, 509, 500, 271, 239, 230.

The pseudotrisaccharide **25** was heated under reflux on a steam bath with 6 *N* hydrochloric acid (30 ml) for 2 hr. The solution was cooled and passed over Amberlite IR45 resin and the eluate was evaporated to dryness. The latter was taken up in methanol (5 ml), and acetic anhydride (1 ml) was added. After 25 min at 25° the mixture was evaporated to dryness and the residue was azeotroped with toluene and then chromatographed on a silica gel column (50 × 1 cm) using the lower phase of a chloroform–methanol–7% ammonium hydroxide (2:1:1) system as the eluent to give 1,3-di-*N*-acetyl-1,3-di-*N*-methyl-5-*O*-methyl-2-deoxystreptamine (**26**) as a colorless, amorphous solid, 11 mg (25%), *m/e* 288 ($M^{\cdot+}$), 270 ($M - H_2O$), which was identical (melting point, tlc, mass spectrum, ir) with an authentic sample.[9] The deoxystreptamine derivative (**26**) showed no CD in Cupra A solution.

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Preparative Routes to 4-Amino-4-deoxy-D-galactose[1]

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The elaboration of two synthetic routes to methyl 4-acetamido-4-deoxy-α-D-galactopyranoside (**15**) is described, involving the displacement of the sulfonyloxy group by azide in methyl 4-*O*-methanesulfonyl-α-D-glucosides, in which the hydroxyl functions are blocked by benzoyl groups (**4**, route A) or by benzyl and trityl moieties (**6**, route B). With respect to yields and crystallinity of products, route A (**4** → **5** → **8** → **15**) proved to be the more efficient. Free 4-amino-4-deoxy-D-galactose, characterized in the form of its hydrochloride (**18**), its highly crystalline *N*-acetate (**20**), and its α- and β-pentaacetyl derivatives (**19**), was readily obtained by acetolysis of methyl tri-*O*-acetyl-4-azido-4-deoxy-α-D-galactoside **9** and subsequent hydrogenation. Nmr data and rotations unambiguously confirm the assigned structures and configurations, and are in excellent agreement with those of the respective 2-amino-2-deoxy and 3-amino-3-deoxy derivatives of D-galactose.

As a prelude to the synthesis of 4-aminogalactosyl nucleosides,[2] required for further assessing structure–activity relationships in the aminoacyl aminohexosyl cytosine group of antibiotics,[3,4] the elaboration of an adequate preparative sequence was considered essential, that not only would make 4-amino-4-deoxy-D-galactose accessible in a form suitable for subsequent nucleosidation, but also would be applicable to simple hexopyranosyl nucleosides without major modifications. We have, by consequence, initiated work on synthetic routes meeting these requirements,[1a] and herein report the details of these investigations. The preliminary published portions thereof[1c] already had sufficed to disprove an earlier structure of the nucleoside antibiotic gougerotin, the sugar part of which had erroneously been assigned the 4-aminogalacto configuration.[5]

Of the conceivable synthetic approaches to 4-aminogalactose or its derivatives, the oxidation of readily available methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside[6] to its 4-hexuloside followed by oximation, reduction of the oxime, and removal of the protecting groups appeared to be the most propitious. Although this procedure has proved effective with hex-4-uloses carrying alkylidene protecting groups,[7-10] including a synthesis of 4-amino-4-deoxy-D-galactose from open-chain sugar derivatives,[10] its success appeared doubtful with an acylated glycopyranosid-4-ulose owing to extreme sensitivity toward β elimination under acidic and basic conditions.[11,12] Hence, another approach was deemed more promising, involving azide displacement of a 4-sulfonyl ester group in a suitable protected glucopyranoside. This route, which, at the outset of this work,[1] had been utilized in preparing 4-amino-4,6-dideoxy-[13] 4,6-diamino-4,6-dideoxy-,[14] and 2,3,4,6-tetramino-D-galactose derivatives,[15] was also used with two methyl 4-*O*-methylsulfonylglucopyranosides, in which the hydroxyl functions at C-2, C-3, and C-6 were