Spectinomycin Chemistry. 1. Characterization of a 5a,9a-*epi*-4(*R*)-Dihydrospectinomycin Derivative

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The identification of a diastereomeric derivative of 4(*R*)-dihydrospectinomycin, having the reversed absolute stereochemistry in the cyclitol ring, is reported. The chemical transformations providing the unequivocal proof of structure **11** for this compound (Schemes II and III) take advantage of the instability of the diastereomeric skeleton relative to that of spectinomycin.

Spectinomycin (**1a**)[1,2] has a structure unique among aminocyclitol antibiotics in that its single sugar component (actinospectose) is fused to the cyclitol portion (actinamine) by both a β-glycosidic bond as well as a hemiketal bond.

As depicted in Scheme I, opening of the hemiketal bond of **1a** would generate the hydrated diketone **2a,** which then could be expected to be in equilibrium with the three isomeric compounds **3a, 4a,** and **5a,** as well as with spectinomycin (**1a**). Such interconversions would also be conceivable for the dihydrospectinomycins **1b** and **1c**.[3] However, to date, only compounds possessing skeleton **1** have been reported.[2-5]

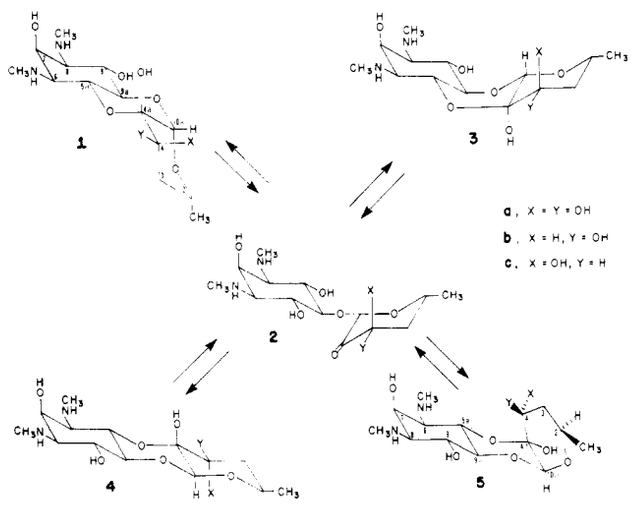
The instability of structure **3** relative to **1** is most likely due to the presence of the high energy boat conformation in its central 1,4-dioxin ring. The predominance of **1** over **4** and **5** may reflect, in addition to steric interactions, the configurational preferences arising from the "anomeric effect".[6]

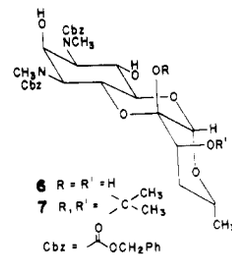
Despite the obvious preference for structure **1,** we continued to seek compounds derived from structures **4** and **5** and now report the isolation and structure determination of the dihydrospectinomycin diastereomer **11,** a derivative of structure **5.**

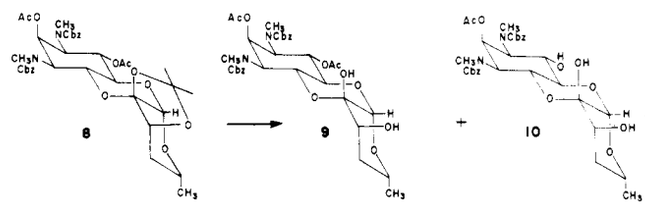
Acid-catalyzed reaction of *N,N'*-dicarbobenzoxy-4(*R*)-dihydrospectinomycin (**6**)[7] with 2,2-dimethoxypropane in dimethylformamide gave, in addition to the reported product **7**,[4b] a small amount of a second acetonide in crystalline form (subsequently shown to be **11**). Acid hydrolysis of both **7** and the unknown acetonide generated the same tetrol **6.**

The 7,9-di-*O*-acetyl-*N,N'*-dicarbobenzoxy-4(*R*)-dihydrospectinomycin acetonide (**8**), prepared by treatment of **7** with excess acetic anhydride in pyridine, on acid hydrolysis yielded the diol **9** in addition to 7-*O*-acetyl-*N,N'*-dicarbobenzoxy-4(*R*)-dihydrospectinomycin (**10**). Both were characterized as their acetonide derivatives. Under identical conditions, the diacetate derived from the unknown acetonide afforded a diol

Scheme I







different from **9** as well as the triol **10**. Further hydrolysis of either diol, **9** or that obtained from the unknown acetonide, gave only the known triol **10,** also characterized as its acetonide derivative.

These results suggest that the structure of the unknown acetonide is that of the dihydrospectinomycin diastereomer **11**. Thus, it is evident that hydrolysis of the diastereomeric acetonide **11** resulted in the rearrangement of its skeleton back to the more stable one of the dihydrospectinomycin **6**. Presumably this transformation involved the intermediacy of the tetrol **12** and the ketone **13,** of which the latter upon rotation about the glycoside bond underwent recyclization to the hemiketal **6** (Scheme II). The diacetate **14** afforded, upon acidic hydrolysis, a mixture of the novel diol **15** and the triol **10** (formed via **16**).

The chemistry outlined in Scheme III allowed the establishment of the regioisomeric nature of the cyclitol hydroxyl groups involved in the hemiketal linkages in the dihydrospectinomycin derivative **7** and the diastereomeric acetonide **11**. Thus, if the structure of **11** is correct, the isomeric cyclic carbamates **21a** and **22a** would be expected to afford the rearrangement products **17a** and **18a,** respectively.

The *N,N'*-dicarbobenzoxy-4(*R*)-dihydrospectinomycin acetonide (**7**) on treatment with potassium carbonate in dimethylformamide at 90 °C yielded a mixture of the 7,8- and 6,7-cyclic carbamates **17a** and **18a**. Spin decoupling experiments carried out on **17b** and **18b,** the hydrogenolysis products of **17a** and **18a,** allowed the unequivocal assignment of the 6,7-cyclic carbamate structure **18a** to the less polar component and confirmed the 7,8-cyclic carbamate structure **17a** of the more polar compound.[8] The pure cyclic carbamates **17a** and **18a** were separately hydrolyzed to yield the triols **19** and **20,** respectively. Reintroduction of the isopropylidene group regenerated only the corresponding starting cyclic carbamates **17a** and **18a,** without evidence of rearrangements or side reactions.

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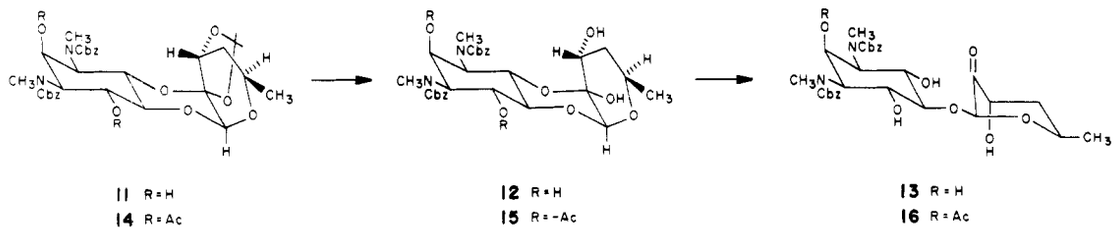
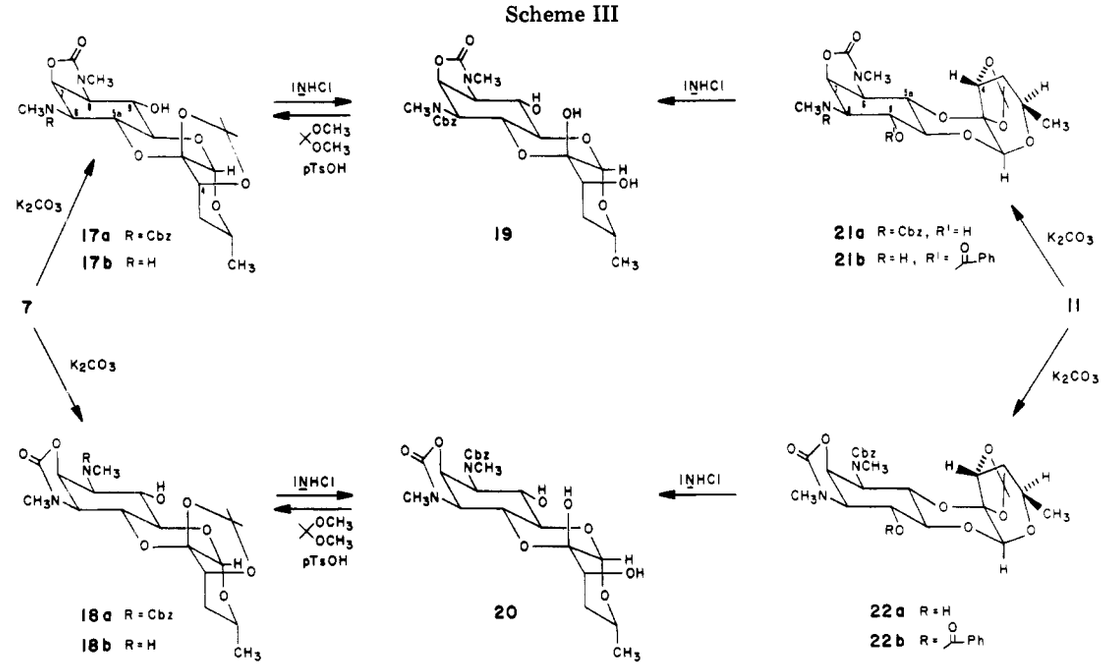
Scheme II



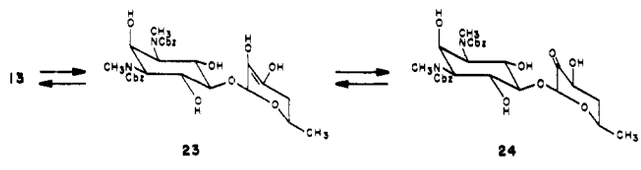
Scheme III



Analogous base treatment of the diastereomeric acetonide **11** resulted in the formation of a mixture of the two new cyclic carbamates **21a** and **22a**. Conversion of the more polar component into the 9-*O*-benzoyl derivative **22b** allowed definitive structure assignment by spin decoupling experiments.[8] Again the more polar compound was found to be a 7,8-cyclic carbamate, in this case **22a**. Confirmation of the 6,7-cyclic carbamate structure **21a** for the less polar component by spin decoupling required the preparation of **21b** by treatment with benzoyl chloride in pyridine followed by the hydrogenolysis of the remaining carbobenzoxy group.[8]

The pure 7,8-cyclic carbamate **22a** was hydrolyzed with 1 N hydrochloric acid in refluxing methanol to yield a triol. Without purification this crude triol was reacted with 2,2-dimethoxypropane in dimethylformamide to generate the expected 6,7-cyclic carbamate **18a**. As anticipated, similar treatment of the 6,7-cyclic carbamate **21a** resulted in formation of the 7,8-cyclic carbamate **17a**. These reactions, as outlined in Schemes II and III, together with the preservation of the 4*R* stereochemistry throughout these transformations firmly established the proposed structure **11**[9] for the novel dihydrospectinomycin diastereomer and ruled out an alternative structure derived from **4**.

The absence of products with 4*S* configuration makes it unlikely that the keto alcohol **13** is in equilibrium with the enediol **23**, which in turn would be expected to produce the keto alcohol **24**.[10] Failure to observe this interconversion or the rearrangement to the 2-hydroxy-3-ulose derivative is indeed surprising in view of the well-known isomerization of 3-hydroxy-2-uloses to the more stable 2-hydroxy-3-uloses via an intermediate enediol.[11,12]



These findings also cast doubt on a proposed biosynthetic scheme for spectinomycin involving the rearrangement of a 2-hydroxy-3-ulose to a 3-hydroxy-2-ulose via an enediol similar to **23**.[13] Additionally, the observation that a symmetrical cyclitol intermediate, such as **2**, will preferentially cyclize to form the spectinomycin skeleton greatly simplifies any synthetic approach to this molecule.

Experimental Section

General. Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Infrared (IR) spectra were recorded on a Digilab FTS 14 spectrometer. Proton NMR spectra were obtained with Varian XL-100 and HA-100 instruments and are reported in parts per million downfield from internal tetramethylsilane. Mass spectra (MS) were obtained on a CEC-110 mass spectrometer. Rotations were measured on a Perkin-Elmer 241 polarimeter.

Silica gel 60 (0.063–0.200 mm) and plates precoated with silica gel 60 F-254 (both from E. Merck) were used for column and thin-layer chromatography (TLC), respectively. Silica gel PF-254 was used for preparative TLC.

Pyridine and dimethylformamide (DMF) were dried by prolonged storage over Davidson 4A molecular sieves and filtered just prior to use.

p-Toluenesulfonic acid was dried at 80 °C at 10 mm for 4 h prior to use.

Hydrogenolyses were carried out in the Parr apparatus at the pressures noted.

(2*R*)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6,8-bis[*N*-(benzyloxycarbonyl)-*N*-methylamino]-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide (7) and (2*R*)-2α,4β,4aβ,5aα,6α,7α,8α,9β,9aβ,10aβ-Decahydro-2-methyl-6,8-bis[*N*-(benzyloxycarbonyl)-*N*-methylamino]-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide

(11). The pure 4(R)-dihydrospectinomycin derivative 6[4b] (14.8 g, 0.025 mol) was dissolved in dry DMF (65 mL) and 100 mL of 2,2-dimethoxypropane (84.8 g, 0.814 mol) and p-toluenesulfonic acid (0.2 g) were added. The resulting solution was stirred at room temperature for 21 h. The low boiling solvents were then removed in vacuo, and the residue was treated with AG 1-X8 (OH^-) (30 mL) in MeOH (100 mL). After stirring for 15 min, the resin was filtered off and the filtrate concentrated in vacuo to leave 18.5 g of a white foam. Chromatography on a column containing 900 g of silica gel and development with n-hexane–EtOAc (3:7) gave 15.0 g (95%) of the known N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin 4,4a-acetonide (7):[4b] R_f 0.68; $[\alpha]^{25}_D$ +31.17° (1.0167, $CHCl_3$).

Later fractions contained the diastereomeric acetonide **11**. Crystallization from MeOH gave 0.21 g (1.3%) as very fine needles: mp 232–233 °C; R_f 0.50; $[\alpha]^{25}_D$ +3.90° (0.9989, $CHCl_3$); IR (KBr) 3490, 1683 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.28 (d, 3 H, C-2 CH_3), 1.38 and 1.49 [2s, 6 H, $C(CH_3)_2$], 1.80–2.40 (m, 2 H, H-3), 3.04 and 3.10 (2 s, 6 H, N–CH_3), 3.30–4.60 (m, 8 H), 4.85 (s, 1 H, H-10a), 5.12 (s, 4 H, CH_2Ph), 7.33 (s, 10 H, arom); MS *m/e* 642 (M^+), 627 (M − CH_3).

Anal. Calcd for $C_{33}H_{42}N_2O_{11}$: C, 61.67; H, 6.59; N, 4.36. Found: C, 61.68; H, 6.51; N, 4.59.

Rearrangement of 11 to Give 7. A solution of 11.4 mg (0.018 mmol) of **11** in MeOH (1 mL) and 1 N HCl (1 mL) was heated to reflux on a steam bath for 1 h. Concentration of the reaction solution in vacuo gave 11 mg of a glass, identical to **6** by IR and TLC. This glass was dissolved in a mixture of dry DMF (1 mL), 2,2-dimethoxypropane (5 mL), and p-toluenesulfonic acid (1 mg), and this solution was stirred at room temperature for 24 h. The total reaction solution was concentrated in vacuo, and the residue was dissolved in 10 mL of MeOH and stirred with AG 1-X8 (OH^-) (1 mL) for 5 min. The resin was filtered off and the filtrate concentrated under vacuum to afford 11 mg of a glass, whose IR, TLC, and MS data were identical to those of **7**.

(2R)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6,8-bis[N-(benzyloxycarbonyl)-N-methylamino]-7,9-bis(acetyloxy)-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a-diol4,4a-Acetonide (8). A solution of the acetonide **7** (37.5 g, 0.058 mol) in dry pyridine (400 mL) and acetic anhydride (30 mL) was stirred at room temperature for 4 days. The total reaction solution was diluted with toluene (400 mL) and concentrated in vacuo. The remaining oil was redissolved in 500 mL of toluene and reconcentrated. The residue was dissolved in CH_2Cl_2 and washed twice with H_2O. Drying and concentration of the CH_2Cl_2 solution gave 36.7 g of crude product. Purification of 27 g by column chromatography on 865 g of silica gel using n-hexane–EtOAc (1:1) gave 17 g of 7,9-di-O-acetyl-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin 4,4a-acetonide (**8**) as a glass: IR (KBr) 1755 and 1710 cm^{-1}; NMR ($CDCl_3$) (two rotamers present) δ 1.24 (d, 3 H, C-2 CH_3), 1.46 [broad s, 6 H, $C(CH_3)_2$], 1.60–2.10 (m, 2 H, H-3), 1.97 and 2.08 (2s, 6 H, Ac), 2.73, 2.86, and 2.91 (3s, 6 H, N–CH_3), 3.60–4.50 (m, 6 H), 4.59 (s, 1 H, H-10a), 5.10 (broad s, 4 H, CH_2Ph), 5.46 (t, 1 H, *J* = 10 Hz, H-9), 5.74 (broad s, 1 H, H-7), 7.34 (s, 10 H, arom); MS *m/e* 726 (M^+), 711 (M − CH_3), 683 (M − Ac), 591 (M − $OCOCH_2Ph$).

Anal. Calcd for $C_{37}H_{46}N_2O_{13}$: C, 61.15; H, 6.38; N, 3.85. Found: C, 61.04; H, 6.45; N, 3.76.

(2R)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6,8-bis[N-(benzyloxycarbonyl)-N-methylamino]-7,9-bis(acetyloxy)-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a-diol (9) and (2R)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6,8-bis[N-(benzyloxycarbonyl)-N-methylamino]-7-acetyloxy-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a,9-triol (10). A mixture of 0.2 g (0.28 mmol) of the diacetate **8** in MeOH (4 mL) and 1 N HCl (3 mL) was heated to reflux on a steam bath for 0.5 h. Concentration of the total reaction solution in vacuo followed by purification by preparative TLC on silica gel using n-hexane–EtOAc (3:7) gave 0.15 g of 7,9-di-O-acetyl-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**9**) as a glass: IR (KBr) 3460, 1756, 1710, 1460, 1385, 1350, 1220, 1175, 1085, 1060, 950, 780, 750, 740, 705 cm^{-1}. 7-O-Acetyl-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**10**) was also obtained as a glass (0.04 g): IR (KBr) 3440, 1754, 1700, 1455, 1350, 1220, 1180, 1060, 950, 780, 750, 740, 705 cm^{-1}.

Reintroduction of the isopropylidene group by treatment of **9** with 2,2-dimethoxypropane in DMF containing a catalytic amount of p-toluenesulfonic acid (as described for **7** above) regenerated **8**.

Similar treatment of **10** (0.51 g, 0.79 mmol) gave 0.51 g (94%) of the 7-O-acetyl-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin 4,4a-acetonide as a glass: IR (KBr) 3590, 1752, 1698 cm^{-1}; NMR ($CDCl_3$–D_2O) (two rotamers present) δ 1.25 (d, 3 H, C-2 CH_3), 1.45 and 1.49 [2s, 6 H, $C(CH_3)_2$], 2.70–2.90 (m, 2 H, H-3), 2.04 (s, 3 H, Ac), 2.86, 2.90, and 2.92 (3s, 6 H, N–CH_3), 3.70–4.50 (m, 7 H), 4.67 (s, 1 H, H-10a), 5.16 (broad s, 4 H, CH_2Ph), 5.78 (broad s, 1 H, H-7), 7.26 (s, 10 H, arom); MS *m/e* 684 (M^+), 669 (M − CH_3), 549 (M − $OCOCH_2Ph$).

Anal. Calcd for $C_{35}H_{44}N_2O_{12}$: C, 61.39; H, 6.48; N, 4.09. Found: C, 61.63; H, 6.52; N, 3.92.

(2R)-2α,4β,4aβ,5aα,6α,7α,8α,9β,9aβ,10aβ-Decahydro-2-methyl-6,8-bis[N-(benzyloxycarbonyl)-N-methylamino]-7,9-bis(acetyloxy)-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a-diol 4,4a-Acetonide (14). A solution of the diastereomeric acetonide **11** (23 mg, 0.036 mmol) in pyridine (2 mL) containing distilled acetic anhydride (0.5 mL) was stirred at room temperature for 3 days. The total reaction solution was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ and washed once with H_2O. Drying and concentration of the $CHCl_3$ solution gave 35 mg of an oil. Pure **14**, 25 mg (96%), was obtained by preparative TLC on silica gel using n-hexane–EtOAc (3:7): IR (KBr) 1753, 1708 cm^{-1}; NMR ($CDCl_3$) δ 1.24 (d, 3 H, C-2 CH_3), 1.40 and 1.48 [2s, 6 H, $C(CH_3)_2$], 1.90–2.20 (m, 8 H, H-3 and 2Ac), 2.76 and 2.92 (2s, 6 H, N–CH_3), 3.50–4.70 (m, 6 H), 4.73 (s, 1 H, H-10a), 5.10–5.20 (m, 4 H, CH_2Ph), 5.50 (t, 1 H, *J* = 10 Hz, H-9), 5.74 (broad s, 1 H, H-7), 7.33 (s, 10 H, arom); MS *m/e* 726 (M^+), 711 (M − CH_3), 683 (M − Ac).

Anal. Calcd for $C_{37}H_{46}N_2O_{13}$: C, 61.15; H, 6.38; N, 3.85. Found: C, 60.88; H, 6.45; N, 3.54.

Hydrolysis of the Diacetate 14 to Give the Diol 15 and Triol 10. A solution of 37 mg (0.051 mmol) of **14** in 1.5 mL of MeOH containing 1 mL of 1 N HCl solution was heated to reflux for 20 min. The reaction solution was concentrated, and preparative TLC on silica gel using n-hexane–EtOAc (3:7) gave the diol **15** (23 mg) as a glass (IR (KBr) 3505, 3460, 1755, 1705, 1455, 1380, 1350, 1335, 1220, 1165, 1140, 1120, 1080, 1065, 945, 780, 755, 705 cm^{-1}) and the triol **10** (6 mg) (IR (KBr) 3440, 1754, 1700, 1455, 1350, 1220, 1180, 1060, 950, 780, 750, 740, 705 cm^{-1}).

Treatment of the diol **15** in dry DMF with 2,2-dimethoxypropane and a catalytic amount of p-toluenesulfonic acid (as described for **7** above) afforded a glass identical in all respects with the starting diacetate **14**.

Identical treatment of the triol **10** yielded the monoacetate acetonide, identical in all respects with that obtained from **8**.

(2R)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6-[N-(benzyloxycarbonyl)-N-methylamino]-8-methylamino-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide 7,8-Cyclic Carbamate (17a) and (2R)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-8-[N-(benzyloxycarbonyl)-N-methylamino]-6-methylamino-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide 6,7-Cyclic Carbamate (18a). A solution of 2.0 g (3.11 mmol) of the acetonide **7** in dry DMF (10 mL) containing K_2CO_3 (0.4 g) was heated in an oil bath at 90 °C for 24 h. After cooling to room temperature, the solids were filtered off and washed with toluene. Concentration of the combined filtrates in vacuo gave 1.85 g of a foam.

Chromatography on a silica gel column (80 g) developed with n-hexane–EtOAc (3:7) gave pure 6,7-cyclic carbamate **18a**, 0.5 g (30%), as a glass: R_f 0.55; IR (KBr) 3460, 1773, 1700 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.26 (d, 3 H, C-2 CH_3), 1.96 [s, 6 H, $C(CH_3)_2$], 1.60–2.10 (m, 2 H, H-3), 2.98 and 3.03 (2s, 6 H, N–CH_3), 3.54 (dd, 1 H, $J_{5a,6}$ = 8 Hz, $J_{6,7}$ = 6 Hz, H-6), 3.60–4.10 (m, 4 H), 4.14 (m, 1 H, H-4), 4.44 (dd, 1 H, $J_{7,8}$ = 5 Hz, $J_{8,9}$ = 11 Hz, H-8), 4.60 (s, 1 H, H-10a), 4.70 (dd, 1 H, $J_{6,7}$ = 6 Hz, $J_{7,8}$ = 5 Hz, H-7), 5.15 (s, 2 H, CH_2Ph), 7.34 (s, 5 H, arom); MS *m/e* 534 (M^+), 519 (M − CH_3), 427 (M − OCH_2Ph), 399 (M − $OCOCH_2Ph$).

Anal. Calcd for $C_{26}H_{34}N_2O_{10}$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.35; H, 6.62; N, 5.33.

Further elution gave the 7,8-cyclic carbamate **17a**, 1.0 g (60%), as a glass: R_f 0.37; IR (KBr) 3450, 1770, 1700 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.26 (d, 3 H, C-2 CH_3), 1.44 and 1.47 [2s, 6 H, $C(CH_3)_2$], 1.60–2.00 (m, 2 H, H-3), 3.03 (broad s, 6 H, N–CH_3), 3.53 (dd, 1 H, $J_{7,8}$ = 6.5 Hz, $J_{8,9}$ = 8 Hz, H-8), 3.60–4.30 (m, 5 H), 4.45 (dd, 1 H, $J_{5a,6}$ = 11 Hz, $J_{6,7}$ = 4 Hz, H-6), 4.61 (s, 1 H, H-10a), 4.70 (dd, 1 H, $J_{6,7}$ = 4 Hz, $J_{7,8}$ = 6.5 Hz, H-7), 5.17 (s, 2 H, CH_2Ph), 7.36 (s, 5 H, arom); MS *m/e* 534 (M^+), 519 (M − CH_3), 443 (M − CH_2Ph), 427 (M − OCH_2Ph).

Anal. Calcd for $C_{26}H_{34}N_2O_{10}$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.59; H, 6.39; N, 5.29.

(2R)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6,8-bis(methylamino)-2H-pyrano[2,3-b][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide 7,8-Cyclic Carbamate (17b). Hydrogenolysis of the 7,8-cyclic carbamate **17a** (67.5 mg, 0.0126 mmol) in 2-propanol (30 mL) using 5% Pd/C (58 mg) at room temperature and 50 psi of hydrogenation for 4 h gave, after filtration of the catalyst and concentration of the filtrate, 50 mg of **17b** as a glass: IR (KBr) 3325, 1763 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.27 (d, 3 H, C-2 CH_3), 1.47 [s, 6 H, $C(CH_3)_2$], 1.65–2.10 (m, 2 H, H-3), 2.54 (s, 3 H, C-6 N–CH_3), 2.82 (dd, 1 H, $J_{5a,6}$ = 10 Hz, $J_{6,7}$ = 4.5 Hz, H-6), 3.02 (s, 3 H, C-8 N–

CH_3), 3.47 (dd, 1 H, $J_{7,8}$ = 6 Hz, $J_{8,9}$ = 8 Hz, H-8), 3.66 (dd, 1 H, $J_{8,9}$ = 8 Hz, $J_{9,9a}$ = 8.5 Hz, H-9), 3.75 (t, 1 H, $J_{9,9a}$ = $J_{9a,5a}$ = 8.5 Hz, H-9a), 3.85 (m, 1 H, H-2), 3.93 (dd, 1 H, $J_{9a,5a}$ = 8.5 Hz, $J_{5a,6}$ = 10 Hz, H-5a), 4.18 (m, 1 H, H-4), 4.59 (s, 1 H, H-10a), 4.67 (dd, 1 H, $J_{6,7}$ = 4.5 Hz, $J_{7,8}$ = 6 Hz, H-7); MS *m/e* 385 (M − CH_3), 382 (M − H_2O).

Spin decoupling: irradiation at δ 2.82 (H-6) caused the d of d at δ 3.93 (H-5a) to collapse to a doublet (J = 8.5 Hz) and the d of d centered at δ 4.67 (H-7) to collapse to a doublet (J = 6 Hz); irradiation at δ 4.67 (H-7) caused the collapse of the d of d at δ 2.82 (H-6) and 3.47 (H-8) to doublets (J = 10 and 8 Hz, respectively).

(2*R*)-2α,4β,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ-Decahydro-2-methyl-6,8-bis(methylamino)-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide 6,7-Cyclic Carbamate (18b). The hydrogenolysis of the 6,7-cyclic carbamate **18a** (30 mg, 0.056 mmol) in 2-propanol (20 mL) containing 5% Pd/C (30 mg) was carried out as described for **17a** to afford 20 mg of **18b** as an oil: IR (KBr) 3330, 1765 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.26 (d, 3 H, C-2 CH_3), 1.46 [s, 6 H, $C(CH_3)_2$], 1.50–2.10 (m, 2 H, H-3), 2.51 (s, 3 H, C-8 N–CH_3), 2.59 (dd, 1 H, $J_{7,8}$ = 4.5 Hz, $J_{8,9}$ = 9.5 Hz, H-8), 3.00 (s, 3 H, C-6 N–CH_3), 3.50 (t, 1 H, $J_{8,9}$ = 9.5 Hz, $J_{9,9a}$ = 10 Hz, H-9), 3.52 (dd, 1 H, $J_{5a,6}$ = 8 Hz, $J_{6,7}$ = 6.5 Hz, H-6), 3.70 (t, 1 H, $J_{9a,5a}$ = $J_{9,9a}$ = 10 Hz, H-9a), 3.80 (m, 1 H, H-2), 4.02 (dd, 1 H, $J_{5a,6}$ = 8 Hz, $J_{5a,9a}$ = 10 Hz, H-5a), 4.12 (m, 1 H, H-4), 4.60 (s, 1 H, H-10a), 4.75 (dd, 1 H, $J_{6,7}$ = 6.5 Hz, $J_{7,8}$ = 4.5 Hz, H-7); MS *m/e* 400 (M^+), 385 (M − CH_3).

Spin decoupling: irradiation at δ 2.59 (H-8) collapsed the t at δ 3.50 (H-9) to a doublet (J = 10 Hz) and collapsed the d of d at δ 4.75 (H-7) to a doublet (J = 6.5 Hz); irradiation at δ 4.75 (H-7) caused the collapse of the d of d at δ 2.59 (H-8) and 3.52 (H-6) to give doublets (J = 9.5 and 8 Hz, respectively).

Hydrolysis and Regeneration of 17a. A methanol solution (1 mL) of the 7,8-cyclic carbamate **17a** (18.5 mg, 0.0346 mmol) containing 1 N HCl solution (1 mL) was heated to reflux on a steam bath for 25 min. Concentration of the total reaction solution followed by purification by preparative TLC using *n*-hexane–EtOAc (3:7) gave 17.1 mg (100%) of pure **19**: IR (KBr) 3400, 1784, 1695 cm^{-1}; MS *m/e* 366 (M − 128).[3]

A dry DMF solution (1 mL) of **19** (13.3 mg, 0.027 mmol) was allowed to react with 2,2-dimethoxypropane (1.5 mL) and *p*-toluenesulfonic acid (1 mg) for 24 h. Removal of the low boiling solvents in vacuo and treatment of the residue with methanol (10 mL) containing AG 1-X8 (OH^-) afforded, after filtration and concentration of the filtrate, 14.2 mg (99%) of **17a.**

Hydrolysis and Regeneration of 18a. Hydrolysis of the 6,7-cyclic carbamate **18a** (22 mg, 0.041 mmol) as described for **17a** above yielded 18 mg (90%) of pure **20**: IR (KBr) 3440, 1750, 1694 cm^{-1}; MS *m/e* 366 (M − 128).[3]

Reintroduction of the isopropylidene group as described for **19** above afforded 10 mg (100%) of **18a** from 93 mg of the triol **20.**

(2*R*)-2α,4β,4aβ,5aα,6α,7α,8α,9β,9aβ,10aβ-Decahydro-2-methyl-8-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-6-methylamino-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide 6,7-Cyclic Carbamate (21a) and (2*R*)-2α,4β,4aβ,5aα,6α,7α,8α,9β,9aβ,10aβ-Decahydro-2-methyl-6-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-8-methylamino-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7,9-tetrol 4,4a-Acetonide 7,8-Cyclic Carbamate (22a). A mixture of the diastereomeric acetonide **11** (130 mg, 0.202 mmol) in dry DMF (3 mL) containing K_2CO_3 (60 mg) was heated at 95 °C for 4 h. After cooling to room temperature, the solids were filtered off and washed with toluene and the combined filtrates were concentrated in vacuo to leave 110 mg of an oil.

Preparative TLC on silica gel developed with *n*-hexane–EtOAc (3:7) afforded 51 mg of the 6,7-cyclic carbamate **21a** as a glass: R_f 0.46; IR (KBr) 3450, 1768, 1699 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.28 (d, 3 H, C-2 CH_3), 1.51 [s, 6 H, $C(CH_3)_2$], 1.90–2.10 (m, 2 H, H-3), 3.02 and 3.04 (2s, 6 H, N–CH_3), 3.50–4.30 (m, 5 H), 4.41 (m, 1 H, H-4), 4.47 (dd, 1 H, $J_{7,8}$ = 4.5 Hz, $J_{8,9}$ = 8 Hz, H-8), 4.71 (dd, 1 H, $J_{6,7}$ = 6 Hz, $J_{7,8}$ = 4.5 Hz, H-7), 4.81 (s, 1 H, H-10a), 5.13 (s, 2 H, CH_2Ph), 7.32 (s, 5 H, arom); MS *m/e* 534 (M^+), 519 (M − CH_3), 427 (M − OCH_2Ph), 399 (M − $OCOCH_2Ph$).

Anal. Calcd for $C_{26}H_{34}N_2O_{10}$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.20; H, 6.51; N, 5.21.

The 7,8-cyclic carbamate **22a,** 54 mg, was obtained as a glass: R_f 0.25; IR (KBr) 3440, 1765, 1700 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.27 (d, 3 H, C-2 CH_3), 1.41 and 1.48 [2s, 6 H, $C(CH_3)_2$], 1.90–2.10 (m, 2 H, H-3), 3.04 (s, 6 H, N–CH_3), 3.34–4.20 (m, 5 H), 4.41 (m, 1 H, H-4), 4.56 (dd, 1 H, $J_{5a,6}$ = 10.5 Hz, $J_{6,7}$ = 4 Hz, H-6), 4.70 (dd, 1 H, $J_{6,7}$ = 4 Hz, $J_{7,8}$ = 6 Hz, H-7), 4.86 (s, 1 H, H-10a), 5.14 (s, 2 H, CH_2Ph), 7.32 (s, 5 H, arom); MS *m/e* 534 (M^+), 519 (M − CH_3), 427 (M − OCH_2Ph), 399 (M − $OCOCH_2Ph$).

Anal. Calcd for $C_{26}H_{34}N_2O_{10}$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.32; H, 6.21; N, 5.30.

(2*R*)-2α,4β,4aβ,5aα,6α,7α,8α,9β,9aβ,10aβ-Decahydro-2-methyl-6,8-bis(methylamino)-9-benzoyloxy-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7-triol 4,4a-Acetonide 6,7-Cyclic Carbamate (21b). A solution of the 6,7-cyclic carbamate **21a** (53 mg, 0.0991 mmol) in pyridine (2 mL) containing benzoyl chloride (0.042 mL, 52 mg, 0.3 mmol) was stirred at room temperature for 24 h. The reaction solution was concentrated, and the residue was dissolved in $CHCl_3$, washed once with H_2O, dried, and concentrated. The crude product was purified by preparative TLC on silica gel using *n*-hexane–EtOAc (3:7) to yield 56 mg (89%) of (2*R*)-2α,4β,4aβ,5aα,6α,7α,8α,-9β,9aβ,10aβ-decahydro-2-methyl-8-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-6-methylamino-9-benzoyloxy-2*H*-pyrano[2,3-*b*]-[1,4]benzodioxin-4,4a,7-triol 4,4a-acetonide 6,7-cyclic carbamate as a foam: IR (KBr) 1773, 1730, 1694 cm^{-1}; NMR ($CDCl_3$) (two rotamers) δ 1.18 (d, 3 H, C-2 CH_3), 1.49 [broad s, 6 H, $C(CH_3)_2$], 1.95–2.20 (m, 2 H, H-3), 2.84, 2.90, and 3.03 (3s, 6 H, N–CH_3), 3.50–3.80 (m, 3 H), 4.02 (m, 1 H, H-2), 4.46 (broad t, 1 H, J = 3 Hz, H-4), 4.66 (s, 1 H, H-10a), 4.70–5.00 (m, 2 H), 5.07 (s, 2 H, CH_2Ph), 5.66 (dd, 1 H, $J_{8,9}$ = 10 Hz, $J_{9,9a}$ = 8 Hz, H-9), 7.34 (s, 5 H, arom), 7.20–7.60 (m, 3 H, O_2CPh), 7.97 (m, 2 H, O_2CPh); MS *m/e* 638 (M^+), 623 (M − CH_3), 516 (M − $PhCO_2H$).

Hydrogenolysis of the above benzoate (54 mg, 0.0845 mmol) in 2-propanol (30 mL) containing 5% Pd/C (40 mg), as described for **17b,** gave 30 mg (70%) of **21b** as a glass: IR (KBr) 1768, 1727 cm^{-1}; NMR ($CDCl_3$–D_2O) δ 1.21 (d, 3 H, C-2 CH_3), 1.51 and 1.52 [2s, 6 H, $C(CH_3)_2$], 1.95–2.40 (m, 2 H, H-3), 2.53 (s, 3 H, C-8 N–CH_3), 3.04 (s, 3 H, C-6 N–CH_3), 3.06 (dd, 1 H, $J_{7,8}$ = 4 Hz, $J_{8,9}$ = 6 Hz, H-8), 3.65 (dd, 1 H, $J_{5a,9a}$ = 10.5 Hz, $J_{9,9a}$ = 7.5 Hz, H-9a), 3.74 (t, 1 H, $J_{5a,6}$ = 8 Hz, $J_{6,7}$ = 7.5 Hz, H-6), 4.03 (m, 1 H, H-2), 4.20 (dd, 1 H, $J_{5a,6}$ = 8 Hz, $J_{5a,9a}$ = 10.5 Hz, H-5a), 4.50 (broad t, 1 H, J = ~3 Hz, H-4), 4.72 (s, 1 H, H-10a), 4.85 (dd, 1 H, $J_{6,7}$ = 7.5 Hz, $J_{7,8}$ = 4 Hz, H-7), 5.34 (dd, 1 H, $J_{8,9}$ = 6 Hz, $J_{9,9a}$ = 7.5 Hz, H-9), 7.30–7.60 (m, 3 H, arom), 8.02 (m, 2 H, arom); MS *m/e* 489 (M − CH_3), 382 (M − $PhCO_2H$).

Spin decoupling: irradiation at δ 5.34 (H-9) caused the collapse of the d of d centered at δ 3.06 (H-8) to a doublet (J = 4 Hz) and the d of d at δ 3.65 (H-9a) to a doublet (J = 10.5 Hz).

(2*R*)-2α,4β,4aβ,5aα,6α,7α,8α,9β,9aβ,10aβ-Decahydro-2-methyl-6-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-8-methylamino-9-benzoyloxy-2*H*-pyrano[2,3-*b*][1,4]benzodioxin-4,4a,7-triol 4,4a-Acetonide 7,8-Cyclic Carbamate (22b). The 7,8-cyclic carbamate **22a** (54 mg, 0.101 mmol) in dry pyridine (1.5 mL) was treated with benzoyl chloride (0.042 mL, 52 mg, 0.3 mmol), and this solution was stirred at room temperature for 20 h. The reaction mixture was concentrated and the residue was dissolved in $CHCl_3$ and washed once with H_2O. Drying and concentration of the $CHCl_3$ solution left 63 mg (98%) of pure **22b** as a foam: IR (KBr) 1773, 1733, 1703 cm^{-1}; NMR ($CDCl_3$) δ 1.18 (d, 3 H, C-2 CH_3), 1.44 [broad s, 6 H, $C(CH_3)_2$], 1.80–2.40 (m, 2 H, H-3), 2.82 and 3.07 (2s, 6 H, N–CH_3), 3.70 (t, 1 H, $J_{9,9a}$ = $J_{9a,5a}$ = 10 Hz, H-9a), 3.87 (dd, 1 H, $J_{7,8}$ = 6 Hz, $J_{8,9}$ = 8 Hz, H-8), 4.00 (t, 1 H, $J_{5a,6}$ = 9 Hz, $J_{5a,9a}$ = 10 Hz, H-5a), 4.47 (broad t, 1 H, J = ~3 Hz, H-4), 4.65 (dd, 1 H, $J_{6,7}$ = 4 Hz, $J_{5a,6}$ = 9 Hz, H-6), 4.69 (s, 1 H, H-10a), 4.82 (dd, 1 H, $J_{6,7}$ = 4 Hz, $J_{7,8}$ = 6 Hz, H-7), 5.18 (s, 2 H, CH_2Ph), 5.48 (dd, 1 H, $J_{8,9}$ = 8 Hz, $J_{9,9a}$ = 10 Hz, H-9), 7.34 (s, 5 H, arom), 7.51 (m, 3 H, O_2CPh), 8.04 (m, 2 H, O_2CPh); MS *m/e* 638 (M^+), 623 (M − CH_3), 593 (M − $PhCO_2H$).

Spin decoupling: irradiation at δ 5.48 (H-9) caused the collapse of the t centered at δ 3.70 (H-9a) to a doublet (J = 10 Hz), and the d of d at δ 3.87 (H-8) collapsed to a doublet (J = 6 Hz).

Anal. Calcd for $C_{33}H_{38}N_2O_{11}$: C, 62.06; H, 6.00; N, 4.39. Found: C, 62.23; H, 6.22; N, 4.30.

Rearrangement of the 6,7-Cyclic Carbamate 21a into the 7,8-Cyclic Carbamate 17a. The 6,7-cyclic carbamate **21a** (5 mg, 0.009 mmol) was dissolved in 0.5 mL of MeOH, 1 N HCl solution (0.5 mL) was added, and this solution was heated to reflux on a steam bath for 20 min. The total reaction solution was concentrated, and the residue was redissolved in MeOH–toluene (1:1) and reconcentrated. The crude product was then dissolved in a mixture of dry DMF (0.5 mL), 2,2-dimethoxypropane (1 mL), and *p*-toluenesulfonic acid (0.1 mg) and stirred overnight at room temperature. After concentration of the reaction solution in vacuo, the residue was dissolved in MeOH (10 mL) and stirred for 10 min with AG 1-X8 (OH^-) (1 mL). The resin was filtered off, the filtrate concentrated, and the product purified by preparative TLC using *n*-hexane–EtOAc (3:7) to yield 5 mg of pure product identical with **17a** by TLC, IR, and MS.

Rearrangement of the 7,8-Cyclic Carbamate 22a into the 6,7-Cyclic Carbamate 18a. Treatment of the 7,8-cyclic carbamate **22a** (5 mg, 0.009 mmol) as described for **21a** above afforded 5 mg of a foam, whose TLC, IR, and MS data were identical with those of **18a.**

Acknowledgment. We wish to thank David Greeley and

Gino Sasso of the NMR Laboratory (under the direction of Dr. T. Williams) for their assistance in obtaining 100-MHz NMR spectra and carrying out numerous decoupling experiments. The skilled assistance of Mr. Jack Lin in preparing compound i[5] and isolating some of the initial quantities of **11** is gratefully acknowledged. We also thank Dr. J. F. Blount for the X-ray analysis confirming the structure of compound i.

Registry No.—**1a,** 1695-77-8; **6,** 56782-21-9; **7,** 58515-30-3; **8,** 67421-50-5; **9,** 67421-51-6; **10,** 67421-52-7; **11,** 67462-78-6; **11** 7-*O*-acetyl derivative, 67421-53-8; **14,** 67462-79-7; **15,** 67462-80-0; **17a,** 67421-54-9; **17b,** 67421-55-0; **18a,** 67421-56-1; **18b,** 67421-57-2; **19,** 67421-58-3; **20,** 67421-59-4; **21a,** 67462-81-1; **21b** 9-benzoate, 67421-62-9; **21b,** 67421-60-7; **22a,** 67462-82-2; **22b,** 67421-61-8; 2,2-dimethoxypropane, 77-76-9.

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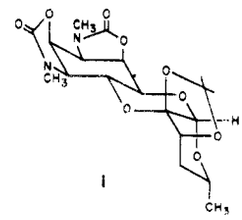
(5) An X-ray analysis carried out on the bis(carbamate) i, prepared in a series of steps from the acetonide **7,** confirmed its structure as well as that of **7** (J. Lin, unpublished results).



i

(6) For recent discussions of the "anomeric effect" see, Romers, C.; Altona, C.; Buys, H. R.; Havinga, E. *Top. Stereochem.* **1969,** *4,* 73–7. David, S.; Einstein, O.; Hehre, W. J.; Salem, L.; Hoffmann, R. *J. Am. Chem. Soc.,* **1973,** *95,* 3806–7. Bailey, W. F.; Eliel, E. L. *ibid.* **1974,** *96,* 1798–1806.
(7) The numbering system used in this paper is that of the 2*H*-pyrano[2,3-*b*][1,4]benzodioxin ring system. This numbering system also corresponds to the one used for spectinomycin.
(8) Details of the spin decoupling experiments may be found in the Experimental Section.
(9) The narrow line width of the triplet, $J = 2.5$–3 Hz, observed for H-4 indicates that the actinospectose ring exists in the boat conformation. Examination of Dreiding models reveals that the chair conformation would result in considerable steric interaction between the C-2 methyl group and the oxygen atom of the 1,4-benzodioxin ring.
(10) The unlikely possibility that the keto alcohol **13** represents the most stable form of this molecule may be discounted since the 4(*S*)-dihydrospectinomycin **1c** or its derivatives do not rearrange under conditions used for preparing the acetonides or on treatment with mild base (unpublished results).
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Malyngamides D and E, Two *trans*-7-Methoxy-9-methylhexadec-4-enamides from a Deep Water Variety of the Marine Cyanophyte *Lyngbya majuscula*

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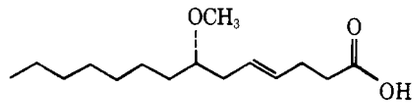
Malyngamides D and E are two *trans*-7-methoxy-9-methylhexadec-4-enamides that have been isolated from the lipid extract of a deep water variety of the marine blue-green alga *Lyngbya majuscula*. Detailed spectral analysis, mostly NMR, and chemical degradation show that malyngamides D and E have the gross structures **2** and **3**, respectively. Malyngamides D and E produce the same diacetate on acetylation. The ring stereochemistry of **2** and **3** has been defined from NMR and chemical reactivity data.

Malyngamides A, B, and C are chlorine-containing *trans*-7(*S*)-methoxytetradec-4-enamides that are present in shallow-water varieties of the marine blue-green alga *Lyngbya majuscula*.[1,2] Free *trans*-7(*S*)-methoxytetradec-4-enoic acid (**1**) is also a lipophilic constituent of the shallow-water strains.[1]



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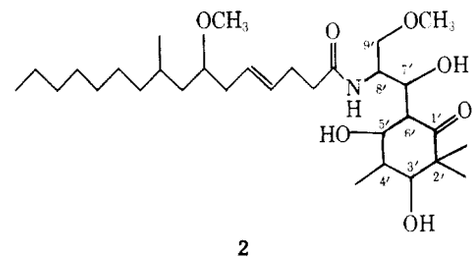
Neither **1** nor amides of **1** have been found in a toxic, deep-water variety of *L. majuscula* from Enewetak.[3,4] Instead two closely related *trans*-7-methoxy-9-methylhexadec-4-enamides, malyngamides D (**2**) and E (**3**),[5] are present in this alga. This paper describes the gross structure elucidations of malyngamides D and E.

Structure Determination

Mass spectral analysis showed that amides **2,** $[\alpha]_D$ −33.0° in $CHCl_3$, and **3,** $[\alpha]_D$ +24.2° in $CHCl_3$, differed in molecular composition by the elements of H_2O. Except for a small M^+ ion at *m/e* 555 for **2,** the mass spectra of **2** and **3** were essentially identical, with compound **3** showing a M^+ ion at *m/e* 537.40235 for $C_{31}H_{55}NO_6$ (calcd 537.40295). The



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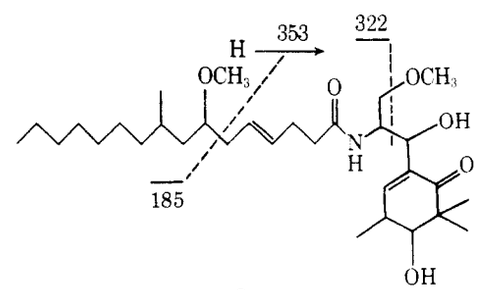

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molecular formula of **2** was therefore $C_{31}H_{57}NO_7$ and this agreed with the formula determined from ^{13}C NMR (5 CH_3 bonded to carbon, 2

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