Chemical Modification of Neamine. 5. Preparation of Amino-deoxyneamines

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An aminocyclitol antibiotic neamine has been modified in its 2-deoxystreptamine moiety by converting the hydroxyl group into an amino group to give three antibiotic amino-deoxyneamines. Their antimicrobial activities were determined against several microorganisms.

In connection with preceding papers[1-3] directed toward a synthesis of a more active antibiotic than the parent neamine, an amino group was introduced to the 2-deoxystreptamine moiety of neamine.

In the present article, we wish to report a preparation of three antibiotic amino-deoxyneamines (**3**, **7**, and **11**).

5-Amino-5-deoxyneamine (3). *O*-Deacetylation of 6,3′,4′-tri-*O*-acetyl-5-chloro-5-deoxy-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-5-epineamine[2] (**1**) in methanolic ammonia, followed by azidolysis and acetylation gave 6,3′,4′-tri-*O*-acetyl-5-azido-5-deoxy-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**2**) as crystals in 54% yield.

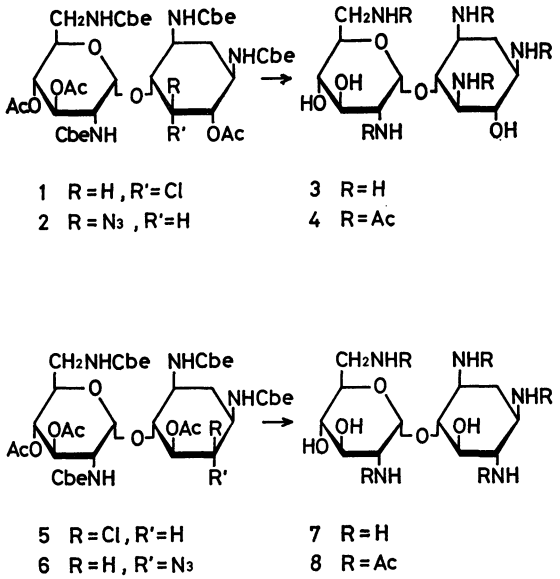
Catalytic hydrogenation of **2** in the presence of platinum oxide, followed by hydrolysis in aqueous barium hydroxide afforded 5-amino-5-deoxyneamine (**3**) as amorphous powder in 58% yield. *N*-Acetylation of **3** with acetic anhydride in methanol gave 1,3,5,2′,6′-penta-*N*-acetyl-5-amino-5-deoxyneamine (**4**).

The structure of **3** was established by a degradation of **3** in 8.8 M hydrobromic acid. From the degradation mixture, the known 1,3,5-triacetamido-4,6-di-*O*-acetyl-1,2,3,5-tetradeoxy-*scyllo*-inositol[4] was obtained.

6-Amino-6-deoxyneamine (7). When 5,3′,4′-tri-*O*-acetyl-6-chloro-6-deoxy-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-6-epineamine[2] (**5**) was *O*-deacetylated and subsequently treated with sodium azide in *N*,*N*-dimethylformamide, 5,3′,4′-tri-*O*-acetyl-6-azido-6-deoxy-1,3,-2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**6**) was obtained in 30% yield. Catalytic hydrogenation of **6** in the presence of platinum oxide, followed by hydrolysis in aqueous barium hydroxide gave 6-amino-6-deoxyneamine (**7**) as chromatographically homogeneous powder in 71% yield. *N*-Acetylation of **7** with acetic anhydride in methanol gave 1,3,6,2′,6′-penta-*N*-acetyl-6-amino-6-deoxyneamine (**8**).

The structure of **7** was established by the analogous degradation of **3** as described above, where L-1,3,4-triacetamido-5,6-di-*O*-acetyl-1,2,3,4-tetradeoxy-*scyllo*-inositol was obtained as crystals. The compound showed identical IR and ^{1}H NMR spectra with its racemate.[5]

5-Amino-5-deoxy-5,6-diepineamine (11). When 3′,4′-di-*O*-acetyl-5,6-anhydro-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-6-epineamine[6] (**9**) was treated with freshly distilled boron trifluoride etherate in acetonitrile, two products (**10a** and **10b**) were obtained in 35 and 28% yield respectively. Considering from the reaction mechanism proposed by Fox *et al.*[7] on the reaction of



Scheme 1.

nucleoside 2′,3′-epoxide with boron trifluoride etherate in acetonitrile, an introduction of an acetamido group into the 2-deoxystreptamine moiety of neamine might occur on C-5 or C-6. Therefore, compound **10a** and **10b** might be positional isomers, since both compounds gave a correct elemental analysis for the predicted structures.

However, degradation of **10a** or **10b** in 8.8 M hydrobromic acid gave the same compound: L-1,3,5-triacetamido-4,6-di-*O*-acetyl-1,2,3,5-tetradeoxy-*allo*-inositol. Its structure was established by the ^{1}H NMR spectra in comparison with its *O*-trideuterioacetyl derivative. This fact indicated that an introduction of an acetamido group occurred only at the C-5 position in a manner of trans diaxial opening. Therefore, the structures of **10a** and **10b** were comprehensible in terms of a possible migration of an ethoxycarbonyl group between an amino group on C-1 and a hydroxyl group on C-6.

An intact mixture of **10a** and **10b** was hydrolyzed in aqueous barium hydroxide and the hydrolyzate was purified by an ion-exchange resin chromatography to give 5-amino-5-deoxy-5,6-diepineamine (**11**) as amorphous powder.

Also, compound **11** was prepared by an alternative reaction process *via* an azido derivative in the following manner. When compound **9**[6] was heated with sodium

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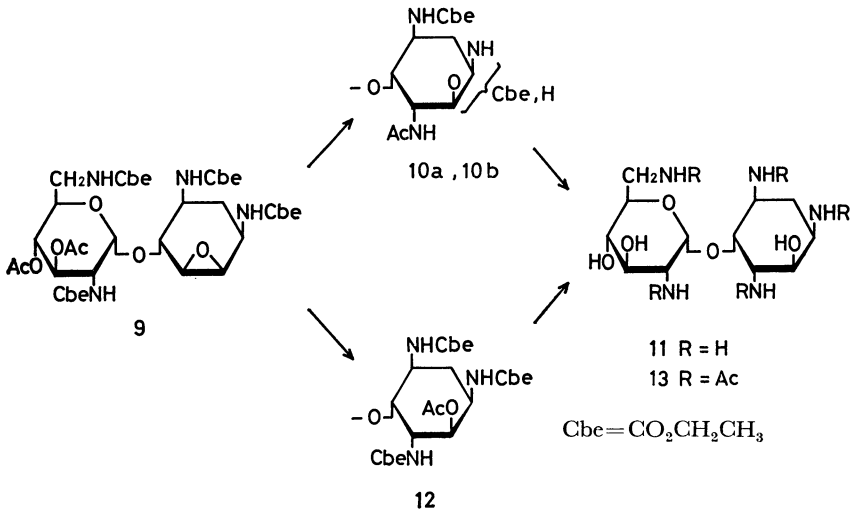

Scheme 2.

TABLE 1. ANTIMICROBIAL ACTIVITY OF 5-AMINO-5-DEOXY- (**3**), 6-AMINO-6-DEOXY- (**7**), 5-AMINO-5-DEOXY-5,6-DIEPI-NEAMINE (**11**), AND NEAMINE

Compound	Diameter of inhibition zone in mm, determined by the paper disk method					
Concentration 1 mg/ml	*Staphylococcus aureus* 6538P	*Bacillus subtilis* ATCC 6633	*Escherichia coli* *D*-12	*Mycobacterium smegmatis* ATCC 607	*Klebsiella*[a] *pneumoniae* 7	*Escherichia*[a] *coli* ML-1629
3	14.5	27.8	26.9	20.8[b]	0	13.8
7	12.8	23.0	21.0	0[b]	0	0
11	0	15.1	16.0	0[b]	0	0
Neamine	18.0	30.5	30.1	27.2[b]	0	0

a) Kanamycin resistant strains. b) Concentration 0.5 mg/ml.

azide and ammonium chloride in 2-methoxyethanol, an azido derivative was obtained. The product was hydrogenated in the presence of Raney nickel and the reduction product was treated with ethyl chloroformate in sodium carbonate solution. After acetylation, the product was purified by column chromatography to give 6,3',4'-tri-*O*-acetyl-5-amino-5-deoxy-1,3,5,2',6'-penta-*N*-(ethoxycarbonyl)-5,6-diepineamine (**12**) in 17% yield. Compound **12** was heated in aqueous barium hydroxide to give **11** as chromatographically homogeneous solid. *N*-Acetylation of **11** gave 1,3,5,2',6'-penta-*N*-acetyl-5-amino-5-deoxy-5,6-diepineamine (**13**).

Compound **3** was slightly less active than neamine, but active against *E. coli* ML-1629 resistant strain. Compounds **7** and **11** showed a considerably lower activity against the microorganisms tested in comparison with neamine (Table 1).

Experimental

General Methods. Melting points were determined in capillary tubes and are uncorrected. Solutions were evaporated under diminished pressure. Optical rotations were measured with a Japan Spectroscopic DIP-SL polarimeter. ^{1}H NMR spectra were recorded at 60 MHz with a Varian A-60D spectrometer in chloroform-*d*, unless otherwise noted, with tetramethylsilane as the internal standard and the peak positions are given in δ values. IR spectra were recorded on potassium bromide disks with a Hitachi-Perkin-Elmer 225 spectrophotometer. Acetylation was performed conventionally with acetic anhydride in pyridine. TLC was performed on Wakogel B-10 (Wako Pure Chemical Co., Ltd.) plates. Silica gel (Wakogel C-300) was employed for column chromatography. Elemental analyses were performed by Mr. Saburo Nakada.

6,3',4'-Tri-O-acetyl-5-azido-5-deoxy-1,3,2',6'-tetra-N-(ethoxycarbonyl)neamine (2). A 402 mg portion of 6,3',4'-tri-*O*-acethyl-5-chloro-5-deoxy-1,3,2',6'-tetra-*N*-(ethoxycarbonyl)-5-epineamine[2] (**1**) was dissolved in methanolic ammonia (7 ml) and the solution was settled in a refrigerator overnight to give 5-chloro-5-deoxy-1,3,2',6'-tetra-*N*-(ethoxycarbonyl)-5-epineamine (310 mg) as precipitates. The deacetylation product was heated with sodium azide (310 mg) in *N,N*-dimethylformamide (5 ml) at 100 °C for 19 h under agitation. The mixture was filtered and the filtrate was evaporated. The residue was acetylated and the product was recrystallized from ethanol–ether to give 193 mg of **2**, mp 228—229 °C; $[\alpha]_D^{23}$ +64.9° (*c* 0.97, chloroform). The second crop (24 mg) of the product was obtained from the mother liquor by column chromatography. Total yield was 54%. IR: 2175 cm^{-1} (N_3). ^{1}H NMR: δ 1.10—1.37 (m, 12, 4×$CO_2CH_2C\underline{H}_3$), 2.01 (s, 3, OAc), 2.03 (s, 3, OAc), 2.14 (s, 3, OAc).

Found: C, 47.05; H, 6.16; N, 12.59%. Calcd for $C_{30}H_{47}N_7O_{16}$: C, 47.30; H, 6.22; N, 12.87%.

5-Amino-5-deoxyneamine (3). Compound **2** (611 mg) was hydrogenated in the presence of platinum oxide (50 mg) in methanol (35 ml) under hydrogen atmosphere (3.4 kg/cm^2) for 45 h in a Parr apparatus. The mixture was filtered and the filtrate was evaporated. The residue was heated in water

(10 ml) containing barium hydroxide (7.6 g) for 10 h under reflux. Carbon dioxide was bubbled into the mixture, and the precipitate was filtered off. The filtrate was evaporated and the residue was purified on a column of Amberlite CG-50 (NH_4^+). The column was washed with water, 0.1M, and 0.2M aqueous ammonia and then eluted with 0.3M aqueous ammonia to give 150 mg (58%) of **3**, mp 135 °C (dec); $[\alpha]_D^{21}$ +92.0° (*c* 0.81, water). The product showed a single spot at R_f 0.23 on TLC in 5: 8: 10: 7 (v/v) 28% ammonia–1-butanol–ethanol–water. IR: 1570 cm^{-1} (NH_2). ^1H NMR (D_2O at pD 1): δ 5.86 (d, 1, J=3 Hz, H-1′).

1,3,5,2′,6′-Penta-N-*acetyl-5-amino-5-deoxyneamine (4).* Compound **3** (17 mg) was acetylated with acetic anhydride (0.5 ml) in methanol (1 ml) to give 24 mg (83%) of **4**, mp 255—260 °C (dec); $[\alpha]_D^{20}$ +71.2° (*c* 0.69, water). IR: 1630 (amide I), 1540 cm^{-1} (amide II). ^1H NMR (D_2O): δ 1.94 (s, 3, NAc), 1.99 (s, 6, 2×NAc), 2.03 (s, 3, NAc), 2.04 (s, 3, NAc), 5.18 (d, 1, J=3.5 Hz, H-1′).

Found: C, 48.25; H, 6.87; N, 12.51%. Calcd for $C_{22}H_{37}N_5O_{10}\cdot H_2O$: C, 48.08; H, 7.15; N, 12.74%.

Degradation of 3 in 8.8M Hydrobromic Acid. Compound **3** (72 mg) was heated in 8.8M hydrobromic acid (4 ml) for 43 h under reflux. The solution was diluted with water and neutralized with Dowex 1×2 (OH^-) resin. The solution was evaporated and the residue was purified on a column of Amberlite CG-50 (NH_4^+). After washed with water and 0.1M aqueous ammonia, the column was eluted with 0.3M aqueous ammonia and a ninhydrin positive fraction was collected and evaporated. The residue was acetylated and the product was recrystallized from ethanol–ethyl acetate to give 36 mg (43%) of 1,3,5-triacetamido-4,6-di-*O*-acetyl-1,2,3,5-tetradeoxy-*scyllo*-inositol which was identical with an authentic sample,[4] mp 308—309 °C (dec). (Found: C, 51.92; H, 6.77; N, 11.38%).

5,3′,4′-Tri-O-*acetyl-6-azido-6-deoxy-1,3,2′,6′-tetra*-N-*(ethoxycarbonyl)neamine (6).* 5,3′,4′-Tri-*O*-acetyl-6-chloro-6-deoxy-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-6-epineamine[2] (**5**, 1.48 g) was dissolved in methanolic ammnia (50 ml) and the solution was settled overnight at ambient temperature. The solution was evaporated and the residue was washed with ether, and then heated with sodium azide (1.28 g) in *N,N*-dimethylformamide (15 ml) at 95 °C for 16 h with mechanical agitation. The solution was evaporated and the residue was acetylated. The acetylation product was purified by a column chromatography using 30: 1 (v/v) chloroform–ethanol as eluant. Fractions showing a single spot at R_f 0.30 on TLC in 20: 1 (v/v) chloroform–ethanol were combined and evaporated to give 449 mg (30%) of **6** as amorphous powder, mp 110—117 °C; $[\alpha]_D^{20}$ +54.4° (*c* 1.3, chloroform). IR: 2140 cm^{-1} (N_3). ^1H NMR: δ 1.25 (t, 12, J=7 Hz, 4×$CO_2CH_2C\underline{H}_3$), 1.98 (s, 3, OAc), 2.02 (s, 3, OAc), 2.07 (s, 3, OAc).

Found: C, 47.25; H, 6.05; N, 12.48%. Calcd for $C_{30}H_{47}N_7O_{16}$: C, 47.30; H, 6.22; N, 12.87%.

6-Amino-6-deoxyneamine (7). Compound **6** (434 mg) was hydrogenated, followed by hydrolysis in barium hydroxide solution as described in the preparation of **3** to give 129 mg (71%) of **7** as amorphous powder, mp 118—121 °C (dec); $[\alpha]_D^{20}$ +81.9° (*c* 1.4, water). The product showed a single spot at R_f 0.32 on TLC in the same solvent as described for **3**. ^1H NMR (D_2O at pD 1): δ 6.09 (d, 1, J=3.5 Hz, H-1′).

1,3,6,2′,6′-Penta-N-*acetyl-6-amino-6-deoxyneamine (8).* Compound **7** (28 mg) was acetylated with acetic anhydride (0.1 ml) in methanol (1.5 ml) to give 35 mg (73%) of **8** as amorphous powder, mp above 290 °C; $[\alpha]_D^{17}$ +89.1° (*c* 0.9, water). ^1H NMR (D_2O): δ 1.95 (s, 3, NAc), 2.00 (s, 3, NAc), 2.02 (s, 3, NAc), 2.05 (s, 3, NAc), 2.07 (s, 3, NAc), 5.42 (d, 1, J=3 Hz, H-1′).

Found: C, 48.34; H, 7.05; N, 12.53%. Calcd for $C_{22}H_{37}N_5O_{10}\cdot H_2O$: C, 48.08; H, 7.15; N, 12.74%.

Degradation of 7 in 8.8M Hydrobromic Acid. Compound **7** (47 mg) was heated in 8.8M hydrobromic acid (2 ml) for 50 h under reflux. The solution was treated analogously as described in the degradation of **3** to give 20 mg (37%) of 1L-1,3,4-triacetamido-5,6-di-*O*-acetyl-1,2,3,4-tetradeoxy-*scyllo*-inositol, mp 298—302 °C (dec); $[\alpha]_D^{21}$ +4.4° (*c* 0.4, methanol). ^1H NMR (DMSO-d_6): δ 1.76 (s, 3, NAc), 1.78 (s, 6, 2×NAc), 1.91 (s, 3, OAc), 1.92 (s, 3, OAc).

Found: C, 51.78; H, 6.77; N, 11.20%. Calcd for $C_{16}H_{25}N_3O_7$: C, 51.74; H, 6.79; N, 11.31%.

Reaction of Epoxide 9 with Boron Trifluoride Etherate in Acetonitrile. 3′,4′-Di-*O*-acetyl-5,6-anhydro-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-6-epineamine[6] (**9**, 1.19 g) was dissolved in freshly distilled dry acetonitrile (15 ml), and freshly distilled boron trifluoride etherate (2.5 ml) was added gradually to the solution. The mixture was settled at ambient temperature for 20 h. The solution was poured into saturated sodium hydrogencarbonate solution (50 ml) and extracted with chloroform. After dried over anhydrous sodium sulfate, the chloroform solution was evaporated. The residue was purified by a column chromatography using 30: 1 (v/v) chloroform–ethanol.

Fractions showing a single spot at R_f 0.40 on TLC in 20: 1 (v/v) chloroform–ethanol were combined and evaporated. The residue was dissolved in benzene and hexane was added to the solution to give 450 mg (35%) of **10a**, mp 110—121 °C (dec); $[\alpha]_D^{16}$ +100.7° (*c* 1.1, chloroform). ^1H NMR: δ 1.1—1.4 (m, 12, 4×$CO_2CH_2C\underline{H}_3$), 2.00 (s, 3, OAc), 2.03 (s, 3, OAc), 2.12 (s, 3, NAc).

Found: C, 48.76; H, 6.57; N, 9.27%. Calcd for $C_{30}H_{49}N_5O_{16}$: C, 48.97; H, 6.71; N, 9.52%.

Fractions showing a single spot at R_f 0.15 on TLC in the same solvent were combined and evaporated to give 360 mg (28%) of **10b**, mp 149—157 °C (dec); $[\alpha]_D^{20}$ +72.5° (*c* 1.6, chloroform). ^1H NMR: δ 1.1—1.5 (m, 12, 4×$CO_2CH_2C\underline{H}_3$), 1.99 (s, 3, OAc), 2.01 (s, 3, OAc), 2.04 (s, 3, NAc).

Found: C, 48.83; H, 6.51; N, 9.32%. Calcd for $C_{30}H_{49}N_5O_{16}$: C, 48.97; H, 6.71; N, 9.52%.

Degradation of 10a and 10b in 8.8M Hydrobromic Acid.

a) Compound **10a** (184 mg) was heated in 8.8M hydrobromic acid (5 ml) under reflux for 48 h. The reaction mixture was treated analogously as described in the degradation of **3** to give 47 mg (51%) of 1L-1,3,5-triacetamido-4,6-di-*O*-acetyl-1,2,3,5-tetradeoxy-*allo*-inositol, mp 178—182 °C; $[\alpha]_D^{20}$ +14° (*c* 0.43, methanol). ^1H NMR (DMSO-d_6): δ 1.79 (s, 6, 2×NAc), 1.88 (s, 3, OAc), 1.91 (s, 3, NAc), 2.12 (s, 3, OAc).

Found: C, 51.95; H, 6.73; N, 11.36%. Calcd for $C_{16}H_{25}N_3O_7$: C, 51.74; H, 6.79; N, 11.31%.

b) Compound **10b** (159 mg) was treated with the above procedure to afford the same inosatriamine derivative (24 mg, 30%). (Found: C, 51.87; H, 6.61; N, 11.39%).

5L-1,3,5-triacetamido-1,2,3,5-tetradeoxy-4,6-di-O-trideuterioacetyl-allo-*inositol.* 1L-1,3,5-triacetamido-4,6-di-*O*-acetyl-1,2,3,5-tetradeoxy-*allo*-inositol (47 mg) was dissolved in 0.02M methanolic hydrogen chloride (2 ml) and the solution was settled overnight. The solution was evaporated and the residue was acylated with acetic anhydride-d_6 (0.3 ml) in pyridine (0.5 ml) to give 32 mg (67%) of crystalline product, mp 189—198 °C (dec); $[\alpha]_D^{20}$ +14° (*c* 0.5, methanol). ^1H NMR (DMSO-d_6): δ 1.79 (s, 6, 2×NAc), 1.91 (s, 3, NAc).

5-Amino-5-deoxy-5,6-diepineamine (11). a) Compound **9**[6] (800 mg) was treated analogously as described above to give a mixture of **10a** and **10b** (820 mg). The product was hydrolyzed in a mixture of barium hydroxide (9 g) and water (30 ml) at 100 °C for 24 h. The hydrolyzate was purified analogously as described in the preparation of **3** to give 112 mg

(29%) of **11**, mp 165—196 °C (dec); $[\alpha]_D^{20}$ +126° (*c* 1.0, water). ^{1}H NMR (D_2O at pD 1): δ 5.90 (d, 1, J=3.5 Hz, H-1′).

b) Compound **9** (930 mg) was heated with sodium azide (319 mg) and ammonium chloride (270 mg) in 2-methoxyethanol (7 ml) for 20 h under reflux. The mixture was evaporated and the residue was acetylated. The product was dissolved in chloroform and the solution was passed through a short column of activated alumina. The effluent was evaporated to give 923 mg of a syrup. The syrup was dissolved in 0.5M ethanolic sodium ethoxide (10 ml). After overnight, the solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin and evaporated. The residue was hydrogenated in the presence of Raney nickel in an atmosphere of hydrogen (3.4 kg/cm^2) for 3 h to give 513 mg of a product. The product was dissolved in 1: 1 (v/v) aqueous methanol containing sodium carbonate (1.2 g), and ethyl chloroformate (1 ml) was added to the solution under ice cooling. After 48 h, the solution was neutralized with 6M hydrochloric acid and evaporated. The residue was acetylated and subsequently purified by a column chromatography using 30: 1 (v/v) chloroform–ethanol as eluant. Fractions showing a single spot at R_f 0.17 on TLC in 20: 1 (v/v) chloroform–ethanol were combined and evaporated to give 187 mg of 6,3′,4′-tri-*O*-acetyl-5-amino-5-deoxy-1,3,5,2′,6′-penta-*N*-(ethoxycarbonyl)-5,6-diepineamine (**12**), mp 158—160 °C (dec); $[\alpha]_D^{19}$ +72.5° (*c* 1.0, chloroform). ^{1}H NMR: δ 1.1—1.4 (m, 15, 5×CO_2-$CH_2C\underline{H}_3$), 1.99 (s, 3, OAc), 2.02 (s, 3, OAc), 2.14 (s, 3,OAc).

Found: C, 48.30; H, 6.43; N, 8.29%. Calcd for $C_{33}H_{53}N_5O_9$: C, 48.11; H, 6.48; N, 8.50%.

Compound **12** (179 mg) was hydrolyzed in aqueous barium hydroxide as described in the preparation of **3** to give 27 mg (39%) of **11**.

1,3,5,2′,6′-Penta-N-*acetyl-5-amino-5-deoxy-5,6-diepineamine(**13**)*. Compound **11** (36 mg) was acetylated with acetic anhydride (0.1 ml) in methanol (1 ml) to give 52 mg (83%) of **13**, mp 216 °C (dec); $[\alpha]_D^{16}$ +104.9° (*c* 1.5, water). ^{1}H NMR (D_2O): δ 1.97 (s, 3, NAc), 1.98 (s, 3, NAc), 2.00 (s, 3, NAc), 2.02 (s, 6, 2×NAc), 4.85 (d, 1, J=3.3 Hz, H-1′).

Found: C, 47.50; H, 7.46; N, 12.50%. Calcd for $C_{22}H_{37}N_5O_{10}\cdot 3/2H_2O$: C, 47.30; H, 7.22; N, 12.5%.

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