Carbohydrate Research, 52 (1976) 187–196

CHEMICAL MODIFICATION OF AMINOCYCLITOL ANTIBIOTICS

TETSUO SUAMI*, SHIGERU NISHIYAMA, YASUHIDE ISHIKAWA, AND SHINICHI KATSURA
Department of Applied Chemistry, Faculty of Engineering,
Keio University, Hiyoshi, Yokohama 223 (Japan)
(Received April 26th, 1976; accepted for publication with revisions, June 20th, 1976)

ABSTRACT

The aminocyclitol antibiotic neamine has been chemically modified at the hydroxyl group on C-6 of the 2-deoxystreptamine moiety. The partially acetylated neamine derivatives, 6,3′,4′-tri-*O*-acetyl- (**3**) and 5,3′,4′-tri-*O*-acetyl-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**4**), were prepared by random hydrolysis of the 5,6-*O*-ethoxyethylidene derivative (**2**), followed by chromatographic purification. Condensation of **4** and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride led to 6-*O*-(β-D-ribofuranosyl)neamine (**7**). Analogous condensation of **4** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide or 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide afforded the corresponding 6-*O*-(D-hexopyranosyl)neamines.

INTRODUCTION

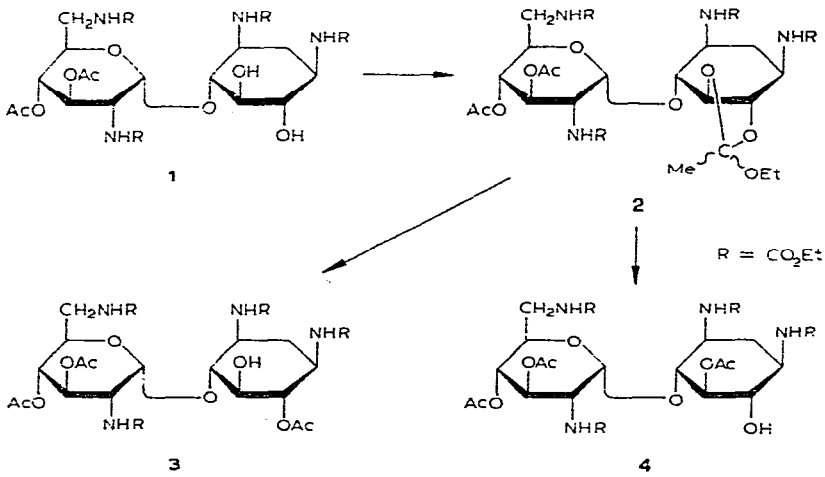
Recently, Hanessian and his coworkers[1] described a synthesis of 6-*O*-(β-D-ribofuranosyl)paromamine, which showed much lower antibacterial activity than did its positional isomer, 5-*O*-(β-D-ribofuranosyl)paromamine[2]. This might also be true in the case of neamine derivatives, and to confirm this point, 6-*O*-(β-D-ribofuranosyl)-neamine (**7**) has been synthesized by an unambiguous route. As the positional isomer of **7** [ribostamycin[3], 5-*O*-(β-D-ribofuranosyl)neamine], is sufficiently active against bacteria for clinical use, it was of interest to determine the activity of **7**. Also, to establish the effect on the antimicrobial activity of a second carbohydrate component attached to the neamine, the 6-*O*-(D-hexopyranosyl)neamines (**11**, **12**, **17**, and **18**) have been prepared.

At the outset, 3′,4′-di-*O*-acetyl-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**1**) was prepared by acetylation and subsequent hydrolysis of 5,6-*O*-cyclohexylidene-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine, a compound obtained by reactions analogous to those employed in the preparation of 5,6-*O*-cyclohexylidene-1,3,2′,6′-tetra-*N*-(methoxycarbonyl)neamine[4].

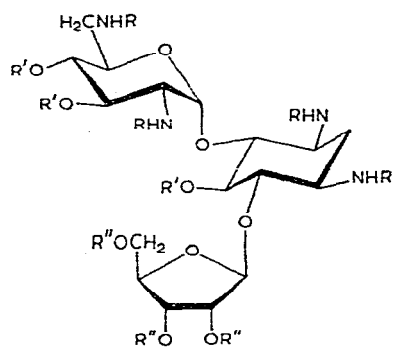
When **1** was treated with triethyl orthoacetate in the presence of *p*-toluenesulfonic acid, the 5,6-*O*-(ethoxyethylidene)derivative (**2**) was obtained as a syrup. Treatment of **2** with Amberlite IR-120 (H^+) resin in aqueous acetone afforded a

*To whom correspondence should be addressed.

mixture of three products, which were separated on a silica gel column giving 6,3′,4′-tri-*O*-acetyl- (**3**) and 5,3′,4′-tri-*O*-acetyl-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**4**), plus compound **1** in 22, 25, and 21% yields, respectively.



Compound **4** was condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[5] in benzene in the presence of mercuric cyanide and "Drierite". The condensation product was purified by column chromatography on silica gel to give 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**5**) in 59% yield. O-Deacylation of **5** gave 1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-6-*O*-(β-D-ribofuranosyl)neamine (**6**) in 91% yield. Compound **6**



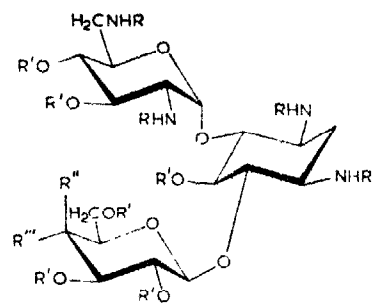
5: R = CO_2Et, R′ = Ac, R″ = Bz
6: R = CO_2Et, R′ = R″ = H
7: R = R′ = R″ = H
8: R = Ac, R′ = R″ = H

was heated in aqueous barium hydroxide and the hydrolyzate was purified on a column of Amberlite CG-50 (NH_4^+) resin to give chromatographically pure 6-*O*-(β-D-ribofuranosyl)neamine (**7**) in 54% yield. Compound **7** showed much less activity against bacteria, than had previously been expected, as compared with neamine.

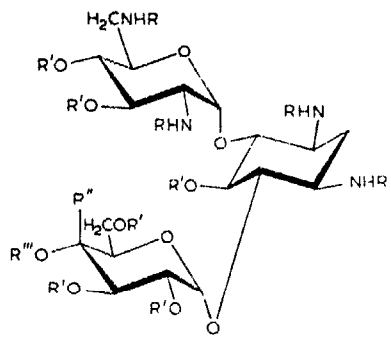
In contrast, when compound **4** was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-

glucopyranosyl bromide[6], chromatographic fractionation of the product yielded two compounds, 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- (**9**) and -6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**10**) in 64 and 13% yields, respectively. Compound **9** was hydrolyzed in aqueous barium hydroxide and the hydrolyzate was purified on a column of Amberlite CG-50 (NH_4^+) resin to give 6-*O*-(β-D-glucopyranosyl)neamine (**11**). 6-*O*-α-D-Glucopyranosyl)neamine (**12**) was obtained from **10** by an analogous procedure.

Compound **12** has already been isolated from a culture broth of *Streptomyces kanamyceticus*[7], that exhibited considerable activity against microorganisms. Synthetic **12** showed the same activity, whereas **11** showed less antibacterial activity than neamine.



9 R = CO_2Et, R′ = Ac, R″ = H, R‴ = OAc
11 R = R′ = R″ = H, R‴ = OH
13 R = Ac, R′ = R″ = H, R‴ = OH
15 R = CO_2Et, R′ = Ac, R″ = OAc, R‴ = H
17 R = R′ = R‴ = H, R″ = OH
19 R = Ac, R′ = R‴ = H, R″ = OH



10 R = CO_2Et, R′ = Ac, R″ = H, R‴ = OAc
12 R = R′ = R″ = H, R‴ = OH
14 R = Ac, R′ = R″ = H, R‴ = OH
16 R = CO_2Et, R′ = Ac, R″ = OAc, R‴ = H
18 R = R′ = R‴ = H, R″ = OH
20 R = Ac, R′ = R‴ = H, R″ = OH

6-*O*-(β-D-Galactopyranosyl)neamine (**17**) and 6-*O*-(γ-D-galactopyranosyl)-neamine (**18**) have been prepared by an analogous reaction between **4** and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[8]. Compound **18** showed activity a comparable to that of **12**, but **17** was found to be less active than neamine.

RESULTS AND DISCUSSION

Prior to the condensation, each one of the two vicinal, *trans*-hydroxyl groups in the aminocyclitol moiety of neamine must be protected by an appropriate group. This has been achieved by applying recent methods developed by three research groups[9–11]. Thus, compound **1** was treated with triethyl orthoacetate to give **2**. As the ethoxyethylidene group is attached to a *trans*-glycol in compound **2**, it was very sensitive to conventional acid hydrolysis and, in formic acid, only **1** was recovered. When **2** was hydrolyzed under mild conditions, with Amberlite IR-120 (H^+) resin, a mixture of **3** and **4** was obtained.

The structures of **3** and **4** were deduced from their n.m.r. spectra by deuterioacetylation techniques[12,13]. Compound **3** displayed three sharp signals in its n.m.r. spectrum, at δ 1.99, 2.02, and 2.10, attributable to the three acetoxyl groups. When **3** was deuterioacetylated, only one singlet (δ 2.10) was shifted (to δ 2.03) and other two signals were not changed, indicating that the signal at δ 2.03 was attributable to an acetoxyl group in the aminocyclitol moiety.

Likewise, compound **4** revealed three singlets, at δ 1.99, 2.02, and 2.05, and after deuterioacetylation, only the signal at δ 2.05 was shifted (to δ 1.99).

To differentiate between these two signals (δ 1.99 and 2.03) for the acetoxyl groups at C-5 and C-6 of the 2-deoxystreptamine moiety, n.m.r. spectra of 4,6-di-*O*-acetyl-2,5-dideoxy-1,3-di-*N*-(ethoxycarbonyl)streptamine (**21**) and DL-5,6-di-*O*-acetyl-2,4-dideoxy-1,3-di-*N*-(ethoxycarbonyl)streptamine (**22**) were determined. Compound **21** displayed a singlet at δ 2.04 (6 H) and **22** showed two singlets, at δ 2.00 (3 H) and 2.04 (3 H), for the two acetoxyl groups, respectively. Now it is obvious that the signal at δ 1.99 is caused by the acetoxyl group at C-5 and the signal at δ 2.03 is due to the acetoxyl group at C-6 of the neamine derivatives. Therefore, the proposed structures **3** and **4** may be reasonably assigned.

The configurations of the anomeric proton of the newly introduced carbohydrate in **7**, **11**, **12**, **17**, and **18** were established by n.m.r. spectra of the corresponding tetra-*N*-acetyl derivatives, **8**, **13**, **14**, **19**, and **20**. Compound **8** showed the signal of the anomeric proton of the D-ribofuranosyl group at δ 5.23 ($J \approx 1$ Hz), indicating that it was the β-anomer, as tetra-*N*-acetylribostamycin showed the corresponding signal at δ 5.20 ($J < 1$ Hz)[14]. The anomeric proton of the D-hexopyranosyl group in **13** and **19** resonated at δ 4.62 ($J = 8$ Hz) and 4.48 ($J = 8$ Hz) respectively, indicating the presence of a β-D-linkage between the hexopyranose and neamine. The anomeric proton in **14** and **20** resonated at δ 5.17 ($J = 3$ Hz) and 5.24 ($J = 3.5$ Hz), respectively establishing that it was that of the α-anomer in each instance.

Biological activities were determined by a paper-disk method and are listed in Table I.

TABLE I

ANTIMICROBIAL ACTIVITY

Compound (1,000 μg/ml)	*Diameter of inhibition zone (in mm) by the paper-disk method*			
	Staphylococcus aureus 6538 P	*Bacillus subtilis ATCC 6633*	*Escherichia coli K-12*	*Mycobacterium smegmatis ATCC 607*
7	0	—	20.2	0
11	0	12.0	16.4	0
12	18.5	30.4	31.3	18.9
17	10.4	21.6	19.1	13.1
18	20.7	29.9	32.7	22.8
Neamine	19.5	31.5	28.9	26.5

Introduction of β-D-ribose or a β-D-hexose to neamine at C-6 decreases the antimicrobial activity. In contrast, introduction of α-D-hexose at the same position did not decrease the activity, as compared with that of neamine.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes and are uncorrected. Solutions were evaporated under diminished pressure below 40°. Optical rotations were measured on a Japan Spectroscopic DIP-SL polarimeter. N.m.r. spectra were recorded at 60 MHz with a Varian A-60D spectrometer in chloroform-*d* or deuterium oxide with tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfonate as the internal standard. Peak positions are given as δ-values. Acetylation was carried out in the conventional manner with acetic anhydride in pyridine, and *O*-deacetylation was performed in methanolic ammonia or 0.1M ethanolic sodium ethoxide at room temperature. T.l.c. was performed on Wakogel B-10 (Wako Pure Chemical Co. Ltd.) plates. Silica gel (Wakogel C-300) was employed for column chromatography.

*3',4'-Di-*O*-acetyl-1,3,2',6'-tetra-*N*-(ethoxycarbonyl)neamine* (**1**). — 5,6-*O*-Cyclohexylidene-1,3,2',6'-tetra-*N*-(ethoxycarbonyl)neamine[4] (1.50 g) was acetylated to give 1.50 g (93%) of the acetylation product as amorphous powder; m.p. 209–212°, $[\alpha]_D^{21}$ +44° (*c* 1.0, methanol).

Anal. Calc. for $C_{34}H_{54}N_4O_{16}$: C, 52.70; H, 7.02; N, 7.23. Found: C, 52.51; H, 7.00; N, 6.98.

The product (0.58 g) was heated for 1.5 h at 80° in 70% aqueous acetic acid (20 ml), and the solution was evaporated. Ether was added to the residue to give 0.50 g of an amorphous solid. Recrystallization from aqueous methanol afforded **1** as crystals (0.44 g, 85%); m.p. 132–135°, $[\alpha]_D^{21}$ +52° (*c* 1.0, methanol); n.m.r. δ 1.24 (t, 12 H, *J* 7.0 Hz, $4CO_2CH_2CH_3$), 1.99 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 4.08 (q, 8 H, *J* 7.0 Hz, $4CO_2CH_2CH_3$).

Anal. Calc. for $C_{28}H_{46}N_4O_{16}$: C, 48.41; H, 6.68; N, 8.07. Found: C, 48.40; H, 6.65; N, 7.75.

*3',4'-Di-*O*-acetyl-1,3,2',6'-tetra-*N*-(ethoxycarbonyl)-5,6-*O*-(ethoxyethylidene)-neamine* (**2**). — Compound **1** (2.49 g) and triethyl orthoacetate (17.3 ml) was heated for 1 h at 70° in *N*,*N*-dimethylformamide (23 ml) in the presence of *p*-toluenesulfonic acid (78 mg). After treating with Amberlite IRA-400 (OH^-), the solution was evaporated to give 2.64 g (99%) of **2** as a crude syrup; n.m.r. δ 1.1–1.5 (m, 18 H, $C\text{-}CH_3$, OCH_2CH_3, $4CO_2CH_2CH_3$), 1.96 (s, 3 H, OAc), 1.99 (s, 3 H, OAc).

*6,3',4'-Tri-*O*-acetyl-* (**3**) *and 5,3',4'-tri-*O*-acetyl-1,3,2',6'-tetra-*N*-(ethoxycarbonyl)neamine* (**4**). — Compound **2** (2.60 g) was dissolved in 85% aqueous acetone (35 ml) and the solution was agitated in the presence of Amberlite IR-120 (H^+, 1.5 g) resin for 24 h. The mixture was filtered and the filtrate was evaporated. The residue was fractionated on a silica gel column by elution with 16:1 (v/v) chloroform–ethanol. Fractions showing a single spot at R_F 0.41 on t.l.c. in the same solvent system were

combined and evaporated. The residue was washed with cyclohexane to give **3** as an amorphous solid (0.58 g, 22%) that melted at 99–106° without showing a sharp melting point; $[\alpha]_D^{23}$ +64.1° (*c* 1.03, chloroform); n.m.r. δ 1.1–1.4 (m, 12 H, $4CO_2CH_2CH_3$), 1.99 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.10 (s, 3 H, OAc).

Anal. Calc. for $C_{30}H_{48}N_4O_{17}$: C, 48.91; H, 6.57; N, 7.61. Found: C, 48.70; H, 6.46; N, 7.23.

Fractions showing a single spot at R_F 0.33 on t.l.c. in the same solvent were combined and evaporated to give a crystalline residue. Recrystallization from 2:1 (v/v) hexane–methanol afforded 0.67 g (25%) of **4**: m.p. 206–207°, $[\alpha]_D^{21}$ +33.7° (*c* 0.83, chloroform); n.m.r. δ 1.25 (t, 12 H, *J* 7.0 Hz, $4CO_2CH_2CH_3$), 1.99 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.05 (s, 3 H, OAc).

Anal. Calc. for $C_{30}H_{48}N_4O_{17}$: C, 48.91; H, 6.57; N, 7.61. Found: C, 48.83; H, 6.43; N, 7.41.

From the fractions that showed a spot at R_F 0.21 on t.l.c. in the same solvent 0.54 g (20%) of the starting material **1** was recovered.

Condensation of **4** *with 2,3,5-tri-*O*-benzoyl-*D*-ribofuranosyl chloride.* — A mixture of **4** (0.92 g) and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[5] (10.5 g) in dry benzene (50 ml) was heated for 143 h under reflux in the presence of mercuric cyanide (2.5 g) and "Drierite" (3.0 g) with mechanical agitation. The mixture was filtered and the filtrate was evaporated. The residue was acetylated and a chloroform solution of the product was washed successively with water, sodium hydrogensulfate solution, sodium hydrogencarbonate solution, and water. The solution was dried over anhydrous sodium sulfate and evaporated. The residue was fractionated on a column of silica gel (320 g, 47 × 354 mm) with 30:1 (v/v) chloroform–ethanol as eluant. The fractions showing a single spot at R_F 0.38 on t.l.c. in 20:1 (v/v) chloroform–ethanol were conbined and evaporated to give 0.86 g (59%) of 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**5**) as an amorphous solid; m.p. 105–116°, $[\alpha]_D^{19}$ +43.9° (*c* 1.05, chloroform); n.m.r. δ 1.24 (m, 12 H, $4CO_2CH_2CH_3$), 1.98 (s, 6 H, 2 OAc), 2.00 (s, 3 H, OAc).

Anal. Calc. for $C_{56}H_{68}N_4O_{24}$: C, 56.94; H, 5.80; N, 4.74. Found: C, 56.68; H, 5.79; N, 4.58.

*1,3,2′,6′-Tetra-*N*-(ethoxycarbonyl)-6-O-(β-*D*-ribofuranosyl)neamine* (**6**). — Compound **5** (0.80 g) was *O*-deacylated in ethanolic sodium ethoxide to give 0.45 g (91%) of **6** as a powder; m.p. 242–245°, $[\alpha]_D^{20}$ +41.0° (*c* 0.8, water).

Anal. Calc. for $C_{29}H_{50}N_4O_8$: C, 46.90; H, 6.79; N, 7.54. Found: C, 46.52; H, 6.60; N, 7.19.

*6-O-(β-*D*-Ribofuranosyl)neamine* (**7**). — Compound **6** (0.43 g) was heated in a mixture of barium hydroxide (2.6 g) in water (4 ml) for 7 h at 90–94°. Carbon dioxide was bubbled into the mixture and the precipitate was filtered off. The filtrate was evaporated, and the residue was adsorbed on a column (7 × 200 mm) of Amberlite CG-50 (NH_4^+) resin. After washing with water and 0.05M aqueous ammonia, the column was eluted with 0.3M aqueous ammonia to give 0.14 g (54%) of **7**: m.p. 140°

(dec.), $[\alpha]_D^{21}$ +33.0° (*c* 1.0, water). The product showed a single spot at R_F 0.34 on t.l.c. in a 28% ammonia–butanol–ethanol–water (5:8:10:7, v/v) solvent system.

1,3,2′,6′-Tetra-N-*acetyl-6*-O-(*β*-D-*ribofuranosyl)neamine* (**8**). — Compound **7** (36 mg) was acetylated with acetic anhydride (0.8 ml) in methanol (2 ml) to give 46 mg (93%) of **8** as an amorphous powder; m.p. 251–252° (dec.), $[\alpha]_D^{20}$ +52.7° (*c* 1.16, water); n.m.r. (D_2O): δ 2.00 (s, 3 H, NAc), 2.02 (s, 3 H, NAc), 2.06 (s, 6 H, 2 NAc), 5.23 (d, 1 H, *J* 1 Hz, H-1″), 5.43 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{25}H_{42}N_4O_{14}$: C, 48.23; H, 6.80; N, 9.00. Found: C, 48.11; H, 6.60; N, 8.86.

Condensation of **4** *with 2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl bromide.* — A mixture of **4** (1.57 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[6] (9.93 g) in dry benzene was boiled for 74 h under reflux in the presence of mercuric cyanide (4.10 g) and "Drierite" (4.90 g). The mixture was processed as described in the preparation of **5**. The crude product was fractionated on a column (50 × 360 mm) of silica gel (250 g) with 1:3 (v/v) butanone–toluene as an eluant to give 3.37 g of a syrup. The syrup was again fractionated on a column (30 × 380 mm) of silica gel (100 g) with 40:1 (v/v) chloroform–ethanol as an eluant. Fractions showing a single spot at R_F 0.61 on t.l.c. in 16:1 (v/v) chloroform–ethanol were combined and evaporated to give 1.46 g (64%) of 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**9**) as an amorphous solid; m.p. 129–135°, $[\alpha]_D^{20}$ +26.5° (*c* 1.6, chloroform); n.m.r. δ 1.24 (t, 12 H, *J* 7 Hz, $4CO_2CH_2CH_3$), 1.97 (s, 6 H, 2 OAc), 2.01 (s, 6 H, 2 OAc), 2.06 (s, 6 H, 2 OAc), 2.09 (s, 3 H, OAc).

Anal. Calc. for $C_{44}H_{66}N_4O_{26}$: C, 49.53; H, 6.24; N, 5.25. Found: C, 49.55; H, 6.16; N, 4.99.

Fractions showing a spot at R_F 0.52 on t.l.c. in the same solvent were combined and evaporated to give 297 mg (13%) of 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**10**); m.p. 111–120°, $[\alpha]_D^{22}$ +84.8° (*c* 1.1, chloroform); n.m.r. δ 1.25 (t, 12 H, *J* 7 Hz, $4CO_2CH_2CH_3$), 1.97 (s, 6 H, 2 OAc), 2.01 (s, 9 H, 3 OAc), 2.09 (s, 3 H, OAc), 2.14 (s, 3 H, OAc).

Anal. Calc. for $C_{44}H_{66}N_4O_{26}$: C, 49.53; H, 6.24; N, 5.25. Found: C, 49.32; H, 6.16; N, 4.98.

6-O-(β-D-Glucopyranosyl)neamine (**11**). — Compound **9** (801 mg) was hydrolyzed in aqueous barium hydroxide and the hydrolyzate was purified on a column of Amberlite CG-50 (NH_4^+) resin as described in the preparation of **7** to give 192 mg (53%) of **11**; m.p. 200–230° (dec.), $[\alpha]_D^{21}$ +42.8° (*c* 1.04, water). The product showed a single spot at R_F 0.32 on t.l.c. in the same solvent system as described for **7**.

1,3,2′,6′-Tetra-N-*acetyl-6*-O-(*β*-D-*glucopyranosyl)neamine* (**13**). — Compound **11** (94 mg) was acetylated as described for **8** to give 111 mg (85%) of **13** as an amorphous powder; m.p. above 250°, $[\alpha]_D^{19}$ +69.8° (*c* 1.0, water); n.m.r. (D_2O): δ 1.97 (s, 6 H, 2 NAc), 2.01 (s, 3 H, NAc), 2.03 (s, 3 H, NAc), 4.62 (d, 1 H, *J* 8 Hz, H-1″), 5.37 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}\cdot H_2O$: C, 46.56; H, 6.91; N, 8.35. Found: C, 46.85; H, 6.60; N, 8.32.

6-O-(α-D-Glucopyranosyl)neamine (**12**). — Compound **10** (270 mg) was hydrolyzed by aqueous barium hydroxide as described for the preparation of **7**, to give 61 mg (50%) of **12**; m.p. 217–227° (dec.); $[\alpha]_D^{22}$ +129° (*c* 1.1, water). The product showed a single spot at R_F 0.38 on t.l.c. in the solvent system used for **7** (lit.[7] m.p. 209–216°, $[\alpha]_D^{23}$ +128° in water).

1,3,2′,6′-Tetra-N-acetyl-6-O-(α-D-glucopyranosyl)neamine (**14**). — Compound **12** (48 mg) was acetylated with acetic anhydride in methanol to give 56 mg (84%) of **14** as an amorphous powder; m.p. above 250°, $[\alpha]_D^{20}$ +118° (*c* 0.6, water); n.m.r. (D_2O): δ 1.98 (s, 3 H, NAc), 2.00 (s, 3 H, NAc), 2.03 (s, 3 H, NAc), 2.06 (s, 3 H, NAc), 5.17 (d, 1 H, *J* 3 Hz, H-1″), 5.43 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}\cdot H_2O$: C, 46.56; H, 6.91; N, 8.35. Found: C, 46.82; H, 6.62; N, 8.30.

Condensation of **4** *with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide.* — A mixture of **4** (1.71 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[8] (18.7 g) in dry benzene (80 ml) was heated for 91 h at 65° in the presence of mercuric cyanide (7.7 g) and "Drierite" (4.4 g) with mechanical agitation. The mixture was processed as described for preparation of **5**. The product was purified two times on a column of silica gel. Fractions showing a single spot at R_F 0.4 on t.l.c. developed twice in 20:1 (v/v) chloroform–ethanol were combined and evaporated to give 0.38 g (15%) of 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine (**15**); m.p. 130–140° (dec.), $[\alpha]_D^{21}$ +38.5° (*c* 0.77, chloroform); n.m.r. δ 1.25 (t, 12 H, *J* 7 Hz, $4CO_2CH_2CH_3$), 1.99 (s, 6 H, 2 OAc), 2.04 (s, 3 H, OAc), 2.08 (s, 9 H, 3 OAc), 2.16 (s, 3 H, OAc).

Anal. Calc. for $C_{44}H_{66}N_4O_{26}$: C, 49.53; H, 6.24; N, 5.25. Found: C, 49.29; H, 6.06; N, 5.16.

Fractions showing a single spot at R_F 0.3 on t.l.c. (developed twice in the same solvent) were combined and evaporated to give 126 mg (5%) of 5,3′,4′-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)-neamine (**16**); m.p. 119–123°, $[\alpha]_D^{20}$ +80.9° (*c* 0.77, chloroform); n.m.r. δ 1.27 (m, 12 H, $4CO_2CH_2CH_3$), 2.01 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.07 (s, 6 H, 2 OAc), 2.10 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 2.20 (s, 3 H, OAc).

Anal. Calc. for $C_{44}H_{66}N_4O_{26}$: C, 49.53; H, 6.24; N, 5.25. Found: C, 49.51; H, 6.16; N, 5.11.

6-O-(β-D-Galactopyranosyl)neamine (**17**). — Compound **15** (350 mg) was hydrolyzed in aqueous barium hydroxide as described for the preparation of **7**, to give 104 mg (65%) of **17**; m.p. 195–205° (dec.), $[\alpha]_D^{21}$ +63.4° (*c* 1.0, water). The product showed a single spot having R_F 0.18 on t.l.c. in the solvent system used for **7**.

1,3,2′,6′-Tetra-N-acetyl-6-O-(β-D-galactopyranosyl)neamine (**19**). — Compound **17** (54 mg) was acetylated with acetic anhydride in methanol to give 68 mg (94%) of **19**; m.p. 273° (dec.), $[\alpha]_D^{22}$ +90.6° (*c* 1.1, water); n.m.r. (D_2O): δ 1.91 (s, 6 H, 2 NAc),

1.97 (s, 3 H, NAc), 1.98 (s, 3 H, NAc), 4.48 (d, 1 H, *J* 8 Hz, H-1″), 5.30 (d, 1 H, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}$: C, 47.85; H, 6.80; N, 8.58. Found: C, 47.50; H, 6.63; N, 8.36.

6-O-(α-D-Galactopyranosyl)neamine (**18**). — Compound **16** (120 mg) was hydrolyzed in aqueous barium hydroxide as described in the preparation of **7**, to give 22 mg (40%) of **18**; m.p. 120–140° (dec.), $[\alpha]_D^{19}$ +115.2° (*c* 1.08, water). The product showed a single spot having R_F 0.18 on t.l.c. in the same solvent system described for 7.

1,3,2′,6′-Tetra-N-acetyl-6-O-(α-D-galactopyranosyl)neamine (**20**). — Compound **18** (10 mg) was acetylated with acetic anhydride in methanol to give 12 mg (87%) of **20**; m.p. 294° (dec.), $[\alpha]_D^{20}$ +126° (*c* 0.37, water); n.m.r. (D_2O): δ 2.08 (s, 6 H, 2 NAc), 2.13 (s, 6 H, 2 NAc), 5.24 (d, 1 H, *J* 3.5 Hz, H-1″), 5.49 (d, 1 H, *J* 4 Hz, H-1′).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}\cdot H_2O$: C, 46.56; H, 6.91; N, 8.35. Found: C, 46.40; H, 6.67; N, 8.22.

6,3′,4′-Tri-O-acetyl-1,3,2′,6′-tetra-N-(ethoxycarbonyl)-5-O-trideuterioacetylneamine. — Compound **3** (158 mg) was acylated with acetic anhydride-d_6 in pyridine to give 151 mg (90%) of crystalline product; m.p. 134–136°; n.m.r. δ 1.99 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 2.02 (s, 3 H, OAc).

5,3′,4′-Tri-O-acetyl-1,3,2′,6′-tetra-N-(ethoxycarbonyl)-6-O-trideuterioacetylneamine. — Compound **4** (162 mg) was acylated with acetic anhydride-d_6 in pyridine to give 162 mg (94%) of crystals; m.p. 135–136°; n.m.r. δ 1.99 (s, 6 H, 2 OAc), 2.03 (s, 3 H, OAc).

5,6,3′,4′-Tetra-O-acetyl-1,3,2′,6′-tetra-N-(ethoxycarbonyl)neamine. — Compound **1** (191 mg) was acetylated to give 129 mg (62%) of crystals; m.p. 135–138°; n.m.r. δ 1.99 (s, 6 H, 2 OAc), 2.02 (s, 3 H, OAc), 2.03 (s, 3 H, OAc).

4,6-Di-O-acetyl-2,5-dideoxy-1,3-di-N-(ethoxycarbonyl)streptamine (**21**). — A 0.84 g portion of 2,5-dideoxystreptamine dihydrochloride[15] was added to ethyl chloroformate (1.4 ml) in M sodium hydroxide solution, and the mixture was kept alkaline by adding sodium hydroxide solution during the reaction period. After being kept overnight, the solution was neutralized with M hydrochloric acid and evaporated. The residue was extracted with boiling 1,4-dioxane and the extract was evaporated. The residue was recrystallized from ethanol to give 0.82 g (73%) of 2,5-dideoxy-1,3-di-*N*-ethoxycarbonylstreptamine; m.p. 205–207°.

Anal. Calc. for $C_{12}H_{22}N_2O_6$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.75; H, 7.54; N, 9.40.

The product (47 mg) was acetylated to give 40 mg (66%) of **21**; m.p. 180–181°; n.m.r. δ 1.23 (t, 6 H, *J* 7 Hz, $2CO_2CH_2CH_3$), 2.04 (s, 6 H, 2 OAc), 4.11 (q, 4 H, *J* 7 Hz, $2CO_2CH_2CH_3$).

Anal. Calc. for $C_{16}H_{26}N_2O_8$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.47; H, 6.83; N, 7.47.

DL-*5,6-Di-O-acetyl-2,4-dideoxy-1,3-di-N-(ethoxycarbonyl)streptamine* (**22**). — DL-Tetra-*N*,*O*-acetyl-2,4-dideoxystreptamine[15] (0.46 g) was hydrolyzed in boiling

6M hydrochloric acid for 3 h under reflux, and the product was recrystallized from 3:1 (v/v) ethanol–methanol to give 0.24 g (75%) of DL-2,4-dideoxystreptamine dihydrochloride; m.p. 247–249°.

Anal. Calc. for $C_6H_{16}N_2O_2Cl_2$: C, 32.89; H, 7.36; N, 12.78; Cl, 32.36. Found: C, 32.69; H, 7.18; N, 12.72; Cl, 32.20.

The dihydrochloride (0.44 g) was treated with ethyl chloroformate (0.76 ml) in 50% aqueous acetone (24 ml) and with sodium carbonate (2.2 g). The product was recrystallized from ethanol to give 0.45 g (77%) of DL-2,4-dideoxy-1,3-di-*N*-(ethoxycarbonyl)streptamine; m.p. 180–183°.

Anal. Calc. for $C_{12}H_{22}N_2O_6$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.26; H, 7.39; N, 9.42.

The product (99 mg) was acetylated to give 70 mg (55%) of **22**; m.p. 146–148°; n.m.r. δ 1.23 (t, 6 H, J 7 Hz, $2CO_2CH_2CH_3$), 2.00 (s, 3 H, OAc), 2.03 (s, 3 H, OAc).

Anal. Calc. for $C_{16}H_{26}N_2O_8$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.35; H, 6.88; N, 7.37.

ACKNOWLEDGMENTS

This work has been supported financially by a grant of the Asahi Glass Foundation for Contribution to Industrial Technology. The authors express their appreciation to Professor Sumio Umezawa for his helpful advice and Mr. Saburo Nakada for elemental analyses.

REFERENCES

1 T. OGAWA, T. TAKAMOTO, AND S. HANESSIAN, *Tetrahedron Lett.*, 46 (1974) 4013–4016.
2 T. TAKAMOTO AND S. HANESSIAN, *Tetrahedron Lett.*, 46 (1974) 4009–4012.
3 T. ITO, E. AKITA, T. TSURUOKA, AND T. NIIDA, *Agr. Biol. Chem.*, 34 (1970) 980–981; *idem*, *Antimicrobial Agents Chemother.*, (1970) 33–37.
4 T. JIKIHARA, T. TSUCHIYA, S. UMEZAWA, AND H. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 46 (1973) 3507–3510; S. UMEZAWA, Y. OKAZAKI, AND T. TSUCHIYA, *ibid.*, 45 (1972) 3619–3624.
5 N. YUNG AND J. J. FOX, *Methods Carbohydr. Chem.*, 2 (1963) 108–112.
6 R. U. LEMIEUX, *Methods Carbohydr. Chem.*, 2 (1963) 221–222.
7 M. MURASE, T. ITO, S. FUKATSU, AND H. UMEZAWA, *Progress in Antimicrobial and Anticancer Chemotherapy*, University of Tokyo Press, Vol. 2, 1970, pp. 1098–1110.
8 J. CONCHIE AND G. A. LEVVY, *Methods Carbohydr. Chem.*, 2 (1963) 335–337.
9 J. ŽEMLIČKA, *Chem. Ind. (London)*, (1964) 581.
10 C. B. REESE AND J. E. SULSTON, *Proc. Chem. Soc.*, (1964) 214–215.
11 R. U. LEMIEUX AND H. DRIGUEZ, *J. Am. Chem. Soc.*, 97 (1975) 4069–4075.
12 D. HORTON, J. B. HUGHES, J. S. JEWELL, K. D. PHILIPS, AND W. N. TURNER, *J. Org. Chem.*, 32 (1967) 1073–1080.
13 T. SUAMI, S. OGAWA, Y. NAITO, AND H. SANO, *J. Org. Chem.*, 33 (1968) 2831–2834.
14 E. AKITA, T. TSURUOKA, N. EZAKI, AND T. NIIDA, *J. Antibiot.*, 23 (1970) 173–183.
15 T. SUAMI, S. OGAWA, H. UCHINO, AND Y. FUNAKI, *J. Org. Chem.*, 40 (1975) 456–461.