The Total Synthesis of Neomycin C[1]

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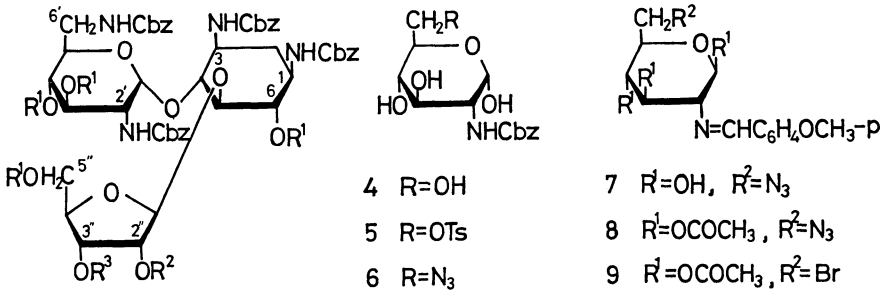
The total synthesis of the pseudo-tetrasaccharides antibiotic neomycin C is described, involving condensation of 6,3′,4′,5″-tetra-*O*-acetyl-1,3,2′,6′-tetra-*N*-(benzyloxycarbonyl)ribostamycin with 3,4-di-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-*O*-tosyl-α-D-glucopyranosyl bromide by a modified Koenigs-Knorr reaction.

Neomycin,[2] a useful antibiotic, was independently discovered by H. Umezawa *et al.*[3] in 1948 and S. A. Waksman *et al.*[2] in 1949. The neomycin complex consists of neomycin A, B, and C, and neomycin C was isolated by Dutcher *et al.*[4] Neomycins are pseudo-tetrasaccharides and show broad-spectrum antibacterial activity. Elucidation of the complete structures of neomycins are achieved by Rinehart *et al.*[5,6] in 1963. We wish now describe in full the total synthesis of neomycin C. This represents the first synthesis of an antibiotic of pseudo-tetrasaccharides.

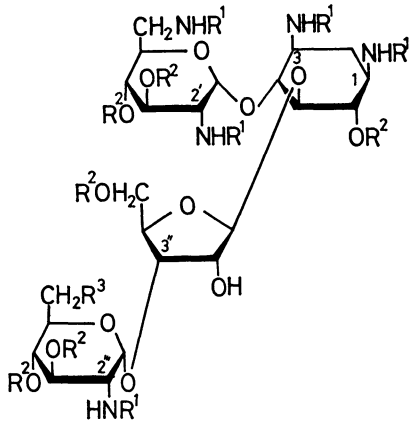
Neomycin C is composed of neosamine C (2,6-diamino-2,6-dideoxy-D-glucopyranose) and ribostamycin which is a pseudo-trisaccharide composed of D-ribose and neamine, a pseudo-disaccharide composed of neosamine C and 2-deoxystreptamine.[7] Neamine was synthesized by S. Umezawa *et al.*,[8] and ribostamycin was subsequently synthesized by Ito *et al.*[9] by ribosylation of neamine. In the present paper, we describe the synthesis of neomycin C by regio- and stereo-selective glycosylation of a pertinently protected ribostamycin (**3**) with a glycosyl halide (**11**) of glucosamine.

Ribostamycin was protected in the following manner. The selective cyclohexylidenation of 1,3,2′,6′-tetra-*N*-(benzyloxycarbonyl)ribostamycin[10] was achieved by treatment with 1,1-dimethoxycyclohexane (2 mol) in *N*,*N*-dimethylformamide (DMF) (20 h at 70 °C) in the presence of *p*-toluenesulfonic acid to give the 2″,3″-*O*-cyclohexylidene derivative (**1**) in 80% yield. Treatment of the acetal with acetic anhydride and pyridine gave the tetraacetyl derivative (**2**) in 98% yield. Decyclohexylidenation of **2** with 50% aqueous acetic acid followed by column chromatography on silica gel provided 6,3′,4′,5″-tetra-*O*-acetyl-1,3,2′,6′-tetra-*N*-(benzyloxycarbonyl)ribostamycin (**3**).

On the other hand, the synthesis of neosamine portion was carried out in the following manner. Our initial approach to the neosamine portion involved treatment of 2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranose[11] (**4**) with tosyl chloride in pyridine to give 2-(benzyloxycarbonylamino)-2-deoxy-6-*O*-tosyl-α-D-glucopyranose (**5**) in 51% yield. Displacement of the tosyl group of **5** with sodium azide in DMF gave 6-azido-2-(benzyloxycarbonylamino)-2,6-dideoxy-α-D-glucopyranose (**6**). Hydrolysis of **6** with 3 M (1 M= 1 mol dm^{-3}) aqueous hydrochloric acid followed by treatment with *p*-anisaldehyde in a mixture of 1 M aqueous sodium hydroxide solution and ethanol afforded 6-azido-2,6-dideoxy-2-(*p*-methoxybenzylideneamino)-β-D-glucopyranose (**7**). Acetylation with acetic anhydride in pyridine then gave 1,3,4-tri-*O*-acetyl-6-azido-2,6-dideoxy-2-(*p*-methoxybenzylideneamino)-β-D-glucopyranose (**8**). However, replacement of the anomeric acetoxyl group of **8** with bromine by treatment with hydrogen bromide in dichloromethane was unsuccessful and resulted in the formation of 1,3,4-tri-*O*-acetyl-6-bromo-2,6-dideoxy-2-(*p*-methoxybenzylideneamino)-β-D-glucopyranose (**9**), the azido group at C-6 being replaced by bromide.[12] A desired glycosyl halide, 3,4-di-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-*O*-tosyl-α-D-glucopyranosyl bromide (**11**), was successfully prepared by conversion of 1,3,4-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-*O*-tosyl-β-D-glucopyranose[13] (**10**) by reaction with hydrogen bromide in dichloromethane (3 h at 0 °C) in 85% yield.



Condensation of the protected glycosyl halide (**11**) with **3** in anhydrous chloroform in the presence of silver carbonate, perchlorate, and Drierite afforded a mixture of condensation products containing the desired pseudo-tetrasaccharide as the main product. Hydrolysis of the Schiff base moiety of the mixture of condensation products at pH 2 in acetone–hydrochloric acid followed by treatment with benzyloxycarbonyl chloride in aqueous acetone in the presence of sodium carbonate and silica-gel chromatography afforde d6,3′,4′,5″,3‴,4‴-hexa-*O*-acetyl-1,3,2′,6′,2‴-penta-*N*-(benzyloxycarbonyl)-6‴-deamino-6‴-tosyl oxyneomycin C (**12**), a colorless solid, in 34% overall yield from **3**. Treatment of **12** with sodium azide in DMF gave the 6‴-azido derivative (**13**) in 85% yield. Finally, de-benzyloxycarbonylation of **13** by catalytic hydrogenolysis with palladium black followed by hydrolysis with 0.5 M barium hydroxide and purification by use of Sephadex C-25 (NH_4^+) completed the synthesis, giving neomycin C (**14**), identical with that obtained from natural source as judged by optical rotations, IR spectra, and ^{1}H-NMR and ^{13}C-NMR spectra. The antibiotic spectra and minimal inhibitory concentrations[1] of the synthetic **14** against test organisms were in agreement with those of natural neomycin C.



12 R^1=Cbz, R^2=Ac, R^3=OTs
13 R^1=Cbz, R^2=Ac, R^3=N_3
14 R^1=R^2=H, R^3=NH_2 (Neomycin C)

Experimental

Thin layer (TLC) and column chromatography were carried out on silica gel of Wakogel B-5 and Wakogel C-200 (Wako Pure Chemicals Co., Ltd., Osaka), and the spots on TLC were visualized with sulfuric acid. ^{1}H-NMR (PMR) spectra were recorded at 60, 90, and 100 MHz with Hitachi R-24, Varian EM-390, and Varian XL-100 spectrometers, respectively.

1,3,2′,6′-Tetra-N-*(benzyloxycarbonyl)-2″,3″*-O-*cyclohexylideneribostamycin (**1**).* To a solution of 1,3,2′,6′-tetra-*N*-(benzyloxycarbonyl)ribostamycin[10] (52 g) in dry DMF (520 ml) were added *p*-toluenesulfonic acid (0.9 g) and 1,1-dimethoxycyclohexane (15.1 g), and the mixture was stirred at 70 °C for 20 h. On TLC of silica gel with benzene–ethyl acetate (1:6), the starting material (R_f 0.01) almost disappeared, and the products of R_f 0.51 (major), R_f 0.89 (minor), and R_f 0.72 (minor) appeared. The solvent was evaporated to half volume, and then the solution was poured into a large amount of water. The resulting precipitate (56 g) was chromatographed on a column of silica gel (2 kg, 76×1040 mm) with benzene–ethyl acetate (1:6) to give a foamy solid, 45.1 g (80%), mp 119—120 °C, $[\alpha]_D^{20}$ +6° (*c* 1.0, chloroform); IR (KBr): 3400, 1700 (broad), and 1520 (broad) cm^{-1}.

Found: C, 61.95; H, 6.13; N, 4.98%. Calcd for $C_{55}H_{66}N_4O_{18}$: C, 61.67; H, 6.21; N, 5.23%.

NMR (in $CDCl_3$): δ 1.0—1.9 (10H m, cyclohexylidene), 5.10 (8H s, 4×COOC$\underline{H}_2C_6H_5$), 7.30 and 7.35 (each s, 20H in all, 4×COOC$H_2C_6\underline{H}_5$).

6,3′,4′,5″-Tetra-O-*acetyl-1,3,2′,6′-tetra*-N-*(benzyloxycarbonyl)-2″,3″*-O-*cyclohexylideneribostamycin (**2**).* To a solution of compound **1** (5 g) in dry pyridine (75 ml) was added acetic anhydride (20 ml), and the solution was allowed to stand at room temperature overnight. On TLC of silica gel with benzene–acetone (7:1), the starting material of R_f 0.01 disappeared, and the product of R_f 0.32 appeared. The mixture was poured into a large amount of water, and the resulting precipitate was filtered and dried to give a colorless solid, 5.7 g (98%), mp 101—102 °C, $[\alpha]_D^{21}$ +16.2° (*c* 1.54, chloroform); IR (KBr): 1740, 1520 cm^{-1}.

Found: C, 60.82; H, 5.92; N, 4.51%. Calcd for $C_{63}H_{74}N_4O_{22}$: C, 61.06; H, 6.02; N, 4.52%.

NMR (in $CDCl_3$): δ 1.0—2.5 (12H m, H_{ax}-2 and H_{eq}-2 and cyclohexylidene), 1.85 and 2.02 (each 6H s, 2×COCH_3) and 7.28 (20H s, 4×C$H_2C_6\underline{H}_5$).

6,3′,4′,5″-Tetra-O-*acetyl-1,3,2′,6′-tetra*-N-*(benzyloxycarbonyl)-ribostamycin (**3**).* Compound **2** (4 g) was dissolved in a hot 50% aqueous acetic acid (320 ml), and the solution was stirred at 80 °C for 12 h. On TLC of silica gel with benzene–acetone (2:1), the mixture showed five spots of R_f 0.82 (starting material, minor), 0.65 (minor), 0.47 (major), 0.32 (minor), and 0.23 (minor). The mixture was poured into a large amount of water, and the resulting precipitate was filtered. The solid was chromatographed on a column of silica gel (160 g, 28×500 mm) with benzene–acetone (2:1) to give a colorless solid, 1.38 g (35%), mp 102—103 °C, $[\alpha]_D^{20}$ +18° (*c* 1.39, chloroform); IR (KBr): 1720, 1620 cm^{-1}.

Found: C, 58.82; H, 5.69; N, 4.84%. Calcd for $C_{57}H_{66}O_{22}N_4$: C, 59.06; H, 5.74; N, 4.83%.

NMR (in $CDCl_3$): δ 1.87, 1.90, 1.94, and 2.00 (each 3H s, COC$\underline{H}_3$) and 7.28 (20H s, $CH_2C_6\underline{H}_5$).

*2-(Benzyloxycarbonylamino)-2-deoxy-6-*O-*tosyl-α-*D-*glucopyranose (**5**).* To a solution of 2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranose[11] (**4**) (4 g) in dry pyridine (80 ml) was added dropwise a solution of *p*-toluenesulfonyl chloride (2.8 g) in dry pyridine (80 ml) at −30 °C for 1.5 h. The solution was stirred at −20 °C for 18 h. On TLC of silica gel with benzene–acetone (2:1), the starting material (R_f 0.03) almost disappeared, and the product (R_f 0.4) appeared. After adding water (20 ml), evaporation of the solvent gave a light brown solid. The solid was chromatographed on a column of silica gel (250 g, 38×450 mm) with benzene–acetone (2:1) to give a colorless solid (3.8 g). Recrystallization from a mixture of benzene–acetone (2:1) afforded colorless crystals, 3.2 g (51%), mp 100—101 °C, $[\alpha]_D^{20}$ +47.6° (*c* 0.36, methanol); IR (KBr): 3400, 1680, 1520, 1170 cm^{-1}.

Found: C, 53.71; H, 5.29; N, 2.90; S, 6.99%. Calcd for $C_{21}H_{25}NO_9S$: C, 53.95; H, 5.39; N, 3.00; S, 6.86%.

NMR (in Py-d_5): δ 2.17 (3H s, $SO_2C_6H_4C\underline{H}_3$), 3.20 (2H s, C$\underline{H}_2C_6H_5$), 5.18 (1H d, *J* 3.5 Hz, H-1), 7.10 and 7.88 (4H ABq, *J* 9 Hz, $SO_2C_6\underline{H}_4CH_3$) and 7.21 (5H s, $CH_2C_6\underline{H}_5$).

*6-Azido-2-(benzyloxycarbonylamino)-2,6-dideoxy-α-D-glucopyranose (**6**).* Sodium azide (7.2 g) was added to a solution of **5** (14.4 g) in DMF (144 ml), and the solution was stirred at 135 °C for 20 min. On TLC of silica gel with benzene–acetone (2:1) the starting material (R_f 0.38) disappeared, and the product of R_f 0.34 (major) and 0.02 (trace) appeared. Evaporation of the solvent gave a solid which was separated into soluble and insoluble parts with dioxane. The soluble part was chromatographed on a column of silica gel (700 g, 80×200 mm) with benzene–acetone (1:1) to give a colorless solid (9.2 g). Recrystallization from a mixture of benzene–acetone (2:1) gave colorless crystals, 8.1 g (78%), mp 175—176 °C, $[\alpha]_D^{20}$ +60.9° (*c* 1.0, water–methanol (1:1)); IR (KBr): 3350 (sho), 2100, 1680, and 1545 cm^{-1}.

Found: C, 50.00; H, 5.41; N, 16.34%. Calcd for $C_{14}H_{18}N_4O_6$: C, 49.70; H, 5.36; N, 16.56%.

NMR (in Py-d_5 containing a small amount of D_2O): δ 5.15 (2H s, $C\underline{H}_2C_6H_5$), 5.7 (1H d, *J* 2 Hz, H-1), and 7.24 (5H s, $CH_2C_6\underline{H}_5$).

*6-Azido-2,6-dideoxy-2-(p-methoxybenzylideneamino)-β-D-glucopyranose (**7**).* Compound **6** (620 mg) was dissolved in 3 M hydrochloric acid (62 ml), and the solution was stirred at 90 °C for 3 h. Evaporation of the solvent and coevaporation with toluene gave a solid (458 mg). The solid was dissolved in 0.5 M aqueous sodium hydroxide (2.3 ml) in ethanol (0.45 ml), and *p*-anisaldehyde (0.3 ml) was added to the solution. The mixture was allowed to stand at room temperature for 30 min and then at 0 °C to give crystalline **7**, 260 mg (44%). Recrystallization from a mixture of acetone–methanol (1:2) gave colorless crystals, 190 mg (32%), mp 168—169 °C, $[\alpha]_D^{20}$ +32.6° (*c* 0.95, methanol); IR (KBr): 2100, 1640, 1610, and 1515 cm^{-1}.

Found: C, 52.54; H, 5.31; N, 17.05%. Calcd for $C_{14}H_{18}N_4O_5$: C, 52.17; H, 5.63; N, 17.38%.

NMR (in Py-d_5 containing a small amount of D_2O): δ 3.77 (3H s, $CHC_6H_4OC\underline{H}_3$), 5.67 (1H d, *J* 7.5 Hz, H-1), 6.95 and 7.95 (4H ABq, *J* 9 Hz, $CHC_6\underline{H}_4OCH_3$), and 8.75 (1H s, $N{=}C\underline{H}C_6H_4OCH_3$).

*1,3,4-Tri-O-acetyl-6-azido-2,6-dideoxy-2-(p-methoxybenzylideneamino)-β-D-glucopyranose (**8**).* To a solution of **7** (430 mg) in dry pyridine (5 ml) was added acetic anhydride (1.5 ml), and the solution was allowed to stand at room temperature overnight. The mixture was poured into 2% aqueous sodium hydrogencarbonate solution (70 ml), and the resulting precipitate was washed with water. Recrystallization from a mixture of ethanol–water (3:1) gave colorless crystals, 400 mg (67%), mp 115—116 °C, $[\alpha]_D^{20}$ +117.6° (*c* 1.43, chloroform); IR (KBr): 2100 and 1755 cm^{-1}.

NMR (in $CDCl_3$): δ 1.88, 2.03, and 2.05 (each 3H s, $COC\underline{H}_3$), 3.43 (1H t, *J* 9 Hz, H-2), 3.0—3.8 (2H m overlapped with OCH_3, $C\underline{H}_2N_3$), 3.86 (3H s, $-OCH_3$), 3.8—4.1 (1H m, H-5), 5.10 (1H t., *J* 9 Hz, H-4), 5.43 (1H t, *J* 9 Hz, H-3), 5.95 (1H d, *J* 9 Hz, H-1), 6.93 and 7.70 (4H ABq, $-N{=}CHC_6\underline{H}_4OCH_3$), and 8.20 (1H s, $-N{=}C\underline{H}C_6H_4OCH_3$).

*1,3,4-Tri-O-acetyl-6-bromo-2,6-dideoxy-2-(p-methoxybenzylideneamino)-β-D-glucopyranose (**9**).* A solution of **8** (100 mg) in dry dichloromethane (5 ml) was saturated with dry hydrogen bromide at −20 °C (about 2 min). Evaporation of the solvent gave a syrup, which was solidified with hexane and washed three times with a mixture of dry ether and dry hexane (4:1). The solid was dissolved in chloroform (20 ml), and the solution was washed several times with phosphate buffer solution (KH_2PO_4–Na_2HPO_4, pH 6.8) and water, and dried (Na_2SO_4). Evaporation of the solvent gave a colorless syrup which was crystallized from a mixture of chloroform, ether and hexane (1:5:20) to give colorless crystals, 50 mg (46%), mp 151—152 °C, $[\alpha]_D^{20}$ +96.8° (*c* 0.67, chloroform); IR (KBr): 1760 cm^{-1}.

Found: C, 49.22; H, 5.07; N, 2.91; Br, 16.45%. Calcd for $C_{20}H_{24}NO_8Br$: C, 49.37; H, 4.98; N, 2.88; Br, 16.44%.

NMR (in $CDCl_3$): δ 1.90, 2.03, and 2.08 (each 3H s, $COC\underline{H}_3$), 3.87 (3H s, $OC\underline{H}_3$), 5.14 (1H t, *J* 9 Hz, H-4), 5.43 (3H t, *J* 9 Hz, H-3), 5.98 (1H d, *J* 9 Hz, H-1), 6.93 and 7.70 (4H ABq, *J* 9 Hz, $N{=}CHC_6\underline{H}_4OCH_3$), and 8.20 (1H s, $N{=}C\underline{H}C_6H_4OCH_3$).

*3,4-Di-O-acetyl-2-deoxy-2-(p-methoxybenzylideneamino)-6-O-tosyl-α-D-glucopyranosyl Bromide (**11**).* A solution of **10** (5 g) in dichloromethane was saturated with anhydrous hydrogen bromide at 0 °C and allowed to stand at 0 °C for 3 h. Evaporation of the solvent gave a syrup, which was solidified with hexane and washed several times with hexane. A chloroform solution of the solid was washed with 1% aqueous sodium hydrogencarbonate solution (pH 7) and several times with phosphate buffer solution (KH_2PO_4–Na_2HPO_4, pH 6.8), dried (Na_2SO_4) and filtered. Evaporation of the filtrate gave a colorless syrup, 4.5 g (85%).

NMR (in $CDCl_3$): δ 1.83, 1.95, and 2.43 (each 3H s, $COC\underline{H}_3$), 3.43 (1H q, *J* 4.5 and 9 Hz, H-2), 3.85 (3H s, OCH_3), 4.17 (2H d, *J* 3.9 Hz, H-6), 5.13 (1H t, *J* 9 Hz, H-4), 5.57 (1H t, *J* 9 Hz, H-3), 6.15 (1H d, *J* 4.5 Hz, H-1), 6.90 and 7.68 (4H ABq, *J* 9 Hz, $N{=}CHC_6\underline{H}_4OCH_3$), 7.35 and 7.82 (4H ABq, *J* 9 Hz, $SO_2C_6\underline{H}_4CH_3$) and 8.27 (1H s, $N{=}C\underline{H}C_6H_4OCH_3$).

*6,3′,4′,5″,3‴,4‴-Hexa-O-acetyl-1,3,2′,6′,2‴-penta-N-(benzyloxycarbonyl)-6‴-deamino-6‴-tosyloxyneomycin C (**12**).* A mixture of **3** (1.1 g), newly prepared Ag_2CO_3 (4 g) and Drierite (10 g) in dry chloroform was stirred at room temperature for 20 h in the dark. To the mixture was added a solution of **11** (1.8 g) in dry chloroform (20 ml) and then $AgClO_4$ under vigorous stirring. The mixture was stirred at room temperature for 20 h. On TLC of silica gel with benzene–acetone (3:1), the mixture showed four products of R_f 0.45, 0.40, 0.37, and 0.32 and some by-products. The insoluble matter was filtered, and the filtrate was thoroughly washed with chloroform. The filtrate and washings were combined, washed with three portions of 2% aqueous sodium hydrogencarbonate (20 ml), then dried (Na_2SO_4). The solution was evaporated to a syrup (2.8 g). The starting materials and by-products were removed by a short column of silica gel (120 g, 34×250 mm) with benzene–acetone (3:1). The crude product (1.7 g) thus obtained was dissolved in acetone (5 ml), and a small amount of 1 M hydrochloric acid was added to the mixture (pH 2). To the mixture was added a large amount of ether, and the resulting oily precipitate was triturated with five portions of ether (10 ml). The oil (1.5 g) was suspended in a mixture of acetone (20 ml) and water (15 ml). After adjustment of pH of the mixture with Na_2CO_3 to pH 7, Na_2CO_3 (150 mg) was added to the mixture, and then benzyloxycarbonyl chloride (0.25 ml) was added at room temperature under vigorous stirring and stirred at room temperature for 20 h. On TLC of silica gel with benzene–acetone (4:1), the mixture showed four products of R_f 0.23 (major), 0.26 (minor), 0.34 (minor), and 0.36 (minor). The insoluble matter was filtered, and the residue was washed with chloroform. The filtrate and washings were combined, and the solvent was evaporated to syrup, which was dissolved in chloroform (300 ml). The chloroform solution was washed with three portions of water (20 ml), dried (Na_2SO_4) and evaporated. The syrup (1.4 g) was chromatographed on a column of silica gel (120 g, 27×450 mm) with benzene–acetone (5:1) to give **12**, a colorless foamy solid, 555 mg (34%), mp 104—105 °C, $[\alpha]_D^{20}$ +42.2° (*c* 0.97, chloroform); IR (KBr): 3400, 1740, 1530, and 1180

cm^{-1}.

Found: C, 57.94; H, 5.56; N, 3.89; S, 2.03%. Calcd for $C_{82}H_{93}N_5O_{32}S$: C, 58.17; H, 5.54; N, 4.14; S, 1.89%.

NMR (in $CDCl_3$): δ 1.7—2.2 (18H m, $6\times COOCH_3$), 2.47 (3H s, $SO_2C_6H_4C\underline{H}_3$), 7.40 and 7.43 (10H and 15H s, respectively, $5\times CH_2C_6\underline{H}_5$), 7.77 and 7.87 (2H q, J 2 and 8 Hz, two protons of $SO_2C_6\underline{H}_4CH_3$(?)).

*6,3',4',5",3"',4"'-Hexa-O-acetyl-6"'-azido-1,3,2',6',2"'-penta-N-(benzyloxycarbonyl)-6"'-deamino-neomycin C (**13**).* To a solution of **12** (440 mg) in dry DMF (7 ml) was added sodium azido (NaN_3, 85 mg), and the mixture was stirred at 130 °C for 15 min. On TLC of silica gel with benzene–acetone (2:1), the mixture showed two products of R_f 0.59 (major) and 0.56 (trace). Evaporation of the solvent gave a light brown solid, which was dissolved in chloroform (50 ml). The chloroform solution was washed with two portions of saturated aqueous sodium chloride (10 ml) and two portions of water (10 ml), and dried. The solvent was evaporated to give a syrup, which was chromatographed on a column of silica gel (18 g, 10×540 mm) with benzene–acetone (4:1) to give a colorless foamy solid, 350 mg (85%), $[\alpha]_D^{20}$ +64.4° (c 0.71, acetone); IR (KBr): 3400, 2100, 1740, and 1520 cm^{-1}.

Found: C, 57.46; H, 5.50; N, 7.06%. Calcd for $C_{75}H_{86}N_8O_{29}$: C, 57.61; H, 5.54; N, 7.17%.

NMR (in $CDCl_3$): δ 1.7—2.1 (18H m, $6\times COOCH_3$), 7.40 and 7.42 (15H and 10H s, $5\times COOCH_2C_6\underline{H}_5$).

*Neomycin C (**14**).* The compound **13** was hydrogenated over palladium black in a mixture of dioxane (12 ml), water (4 ml), and acetic acid (0.4 ml) at room temperature under 4 kg/cm^2 pressure of hydrogen for 14 h. After removal of the catalyst, the solution was evaporated to dryness *in vacuo*. The solid was dissolved in a hot 0.5 M aqueous barium hydroxide ($Ba(OH)_2$) (6 ml), and the mixture was stirred at 95 °C for 5 h. To the solution was introduced carbon dioxide, and the resulting precipitates were removed by centrifugation. The supernatant was evaporated to give a colorless solid (150 mg), which was chromatographed on a column of CM Sephadex C-25 (NH_4^+) (30 ml, 10×400 mm). After washing with water (40 ml), the column was eluted with an aqueous ammonia by the linear gradient from 0.0 to 0.3 M to give a colorless solid of neomycin C, 75 mg (61%), hydrochloride: $[\alpha]_D^{20}$ +78° (c 0.86, water); IR (KBr): 3400 (broad), 2950 (broad), 1980 (broad), 1600, 1500, 1030 (sh) cm^{-1}.

NMR (in D_2O): δ 5.52 (1H d, J 3.5 Hz, H-1"'), 5.88 (1H d, J 1 Hz, H-1"), and 5.94 (1H d, J 3 Hz, H-1').

^{13}C NMR (in D_2O): δ 36.4, 42.4, 42.5, 51.1 (2 carbons), 55.9, 56.3, 62.4, 72.2 (2 carbons), 73.98 (4 carbons), 74.5, 75.5, 78.4, 81.7, 82.8, 85.2, 99.7 ($C_{1'}$ and $C_{1'''}$), and 109.45 ($C_{1''}$).

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