TOTAL SYNTHESIS OF NEOMYCIN C

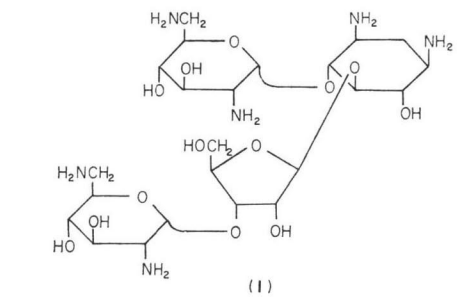
Sir:

Neomycin[1], a useful antibiotic, was independently discovered by H. UMEZAWA *et al.*[2] in 1948 and S. A. WAKSMAN *et al.*[1] in 1949. The neomycin complex consists of neomycins A, B, and C and neomycin C was isolated by DUTCHER *et al.*[3] Elucidation of the complete structures of neomycins was achieved by RINEHART and his associates[4,5] in 1963. We wish here to report the total synthesis of neomycin C (**1**). This represents the first synthesis of an antibiotic of pseudo-tetrasaccharides.

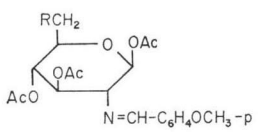
Neomycin C (**1**) is composed of two molecules of neosamine C (2,6-diamino-2,6-dideoxy-D-glucopyranose)[6], 2-deoxystreptamine[7], and D-ribose. Neamine (neomycin A), which is a pseudodisaccharide component of neomycins B and C, was synthesized by S. UMEZAWA *et al.*[8] and ribosylation of neamine was subsequently reported by ITO *et al.*[9] to afford ribostamycin which corresponds to a pseudo-trisaccharide portion common to both neomycins B and C. Hence, our approach to the synthesis of neomycin C involved pertinent protection of neosamine C and ribostamycin and regioselective α-glycosidation between them.

The α-glycosidation reaction used in the present study is based on our prior findings[10,11] that the modified KOENIGS-KNORR condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-α-D (or L)-glucopyranosyl bromide with suitably substituted sugars gave high yields ($\sim$85%) of α-glycosides. Our initial attempt to brominate 1,3,4-tri-O-acetyl-6-azido-2,6-dideoxy-2-(*p*-methoxybenzylideneamino)-β-D-glucopyranose (**2**, mp 115$\sim$116°C, $[\alpha]_D^{20}+117.6°$ (*c* 1.43, chloroform)) with hydrogen bromide in methylene chloride (2 minutes at -20°C) was not successful and resulted in the formation of 1,3,4-tri-O-acetyl-6-bromo-2,6-dideoxy-2-(*p*-methoxybenzylideneamino)-β-D-glucopyranose (**3**, mp151$\sim$152°C, $[\alpha]_D^{20}+96.8°$ (*c* 0.67, chloroform)), the azido group at C-6 being replaced by bromine.[12] In a second approach to prepare the glycosyl halide, 1,3,4-tri-O-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-O-tosyl-β-D-glucopyranose[13] was successfully converted, in 85% yield, by reaction of hydrogen bromide in methylene chloride (3 hours at 0°C), to syrupy 3,4-di-O-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-6-O-tosyl-α-D-glucopyranosyl bromide (**4**). Its structure was confirmed by ^{1}H-NMR data (in $CDCl_3$): δ 1.83, 1.95, and 2.43 (each 3H, s, OAc), 3.43 (1H, q, $J_{1,2}=4.5$ Hz and $J_{2,3}=9$ Hz, C_2-H), 3.85 (3H, s, OCH_3), 6.15 (1H, d, $J_{1,2}=4.5$ Hz, C_1-H), 6.90 and 7.68 (4H, AA′BB′, $J_o=9$ Hz, $C_6\underline{H}_4OCH_3$), 7.35 and 7.82 (4H, AA′BB′, $J_o=9$ Hz, $SO_2C_6\underline{H}_4CH_3$).

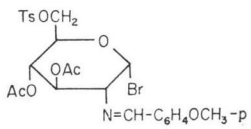
On the other hand, ribostamycin was protected in the following manner. 1,3,2′,6′-Tetra-N-benzyloxycarbonylribostamycin[14] was transformed to the 2″,3″-O-cyclohexylidene ketal (**5**), mp 119$\sim$120°C, $[\alpha]_D^{20}+6°$ (*c* 1.0, chloroform), in 80% yield, by selective cyclohexylidenation



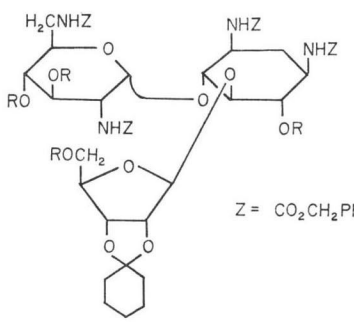
(1)



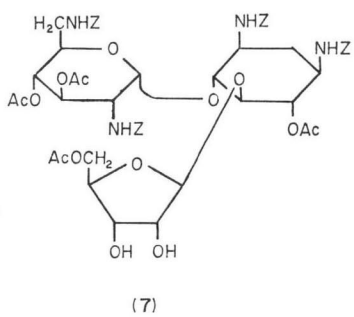
(2) R = N_3
(3) R = Br



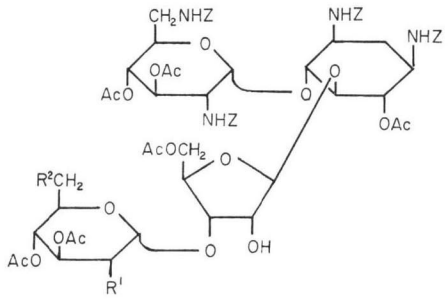
(4)



(5) R = H
(6) R = Ac



(7)



(8) R^1 = N=CH-$C_6H_4OCH_3$-p, R^2 = OTs
(9) R^1 = $NHCO_2CH_2Ph$, R^2 = OTs
(10) R^1 = $NHCO_2CH_2Ph$, R^2 = N_3

with an excess of 1,1-dimethoxycyclohexane (~2 mol) in N,N-dimethylformamide (20 hours at 70°C) in the presence of *p*-toluenesulfonic acid. The ketal was treated with acetic anhydride and pyridine (overnight at room temperature) to give the tetraacetyl derivative (**6**), mp 101~102°C, $[\alpha]_D^{21}$ +16.2° (*c* 1.54, chloroform), in 98% yield. Partial hydrolysis of **6** with 50% acetic acid (12 hours at 80°C) provided, after column chromatography on silica gel, 6,3′,4′,5″-tetra-O-acetyl-1,3,2′,6′-tetra-N-benzyloxycarbonylribostamycin (**7**), mp 102~103°C, $[\alpha]_D^{21}$ +18° (*c* 1.39, chloroform), in 35% yield.

Reaction of the protected glycosyl halide (**4**) with **7** in anhydrous chloroform in the presence of silver carbonate, silver perchlorate and Drierite (20 hours at room temperature) followed by column chromatography on silica gel, afforded a mixture (a syrup) of the condensation products containing **8** as the main product. Hydrolysis of the SCHIFF base moiety of the condensation products at pH 2 in acetone-hydrochloric acid (several minutes at room temperature) gave a syrup. The free amino group at C-2‴ was benzyloxycarbonylated by treatment with benzyloxycarbonyl chloride in aqueous acetone in the presence of sodium carbonate (20 hours at room temperature). Silica gel chromatography of the reaction mixture afforded 6,3′,4′,5″-tetra-O-acetyl-1,3,2′,6′-tetra-N-benzyloxycarbonyl-3″-O-(3,4-di-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-6-O-tosyl-α-D-glucopyranosyl)ribostamycin (**9**), a colorless solid, $[\alpha]_D^{20}$ +42.2° (*c* 0.97, chloroform), in 34% overall yield from **7**.

Treatment of **9** with sodium azide in N,N-dimethylformamide (15 minutes at 130°C) gave the 6‴-azide (**10**) as a solid, $[\alpha]_D^{20}$ +64.4° (*c* 0.71, acetone), in 85% yield. De-benzyloxycarbonylation of **10** by catalytic hydrogenolysis with palladium black and hydrogen (4 kg/cm², 14 hours at room temperature) in aqueous dioxane in the presence of acetic acid, followed by hydrolysis with 1 N barium hydroxide (5 hours at 95°C) and purification by use of Sephadex C-25 (NH_4^+) completed the synthesis, giving neomycin C (**1**), [hydrochloride: $[\alpha]_D^{20}$ +78° (*c* 0.86, water)][15], identical with that obtained from natural source as judged by the IR spectrum of the hydrochloride (KBr), ^{1}H-NMR (D_2O) (anomeric protons: δ5.52 (1H, d, $J_{1''',2'''}$ = 3.5 Hz, C_1'''-H), 5.88 (1H, d, $J_{1'',2''}$ = 1 Hz, C_1''-H), and 5.94 (1H, d, $J_{1',2'}$ = 3 Hz, C_1'-H), and ^{13}C-NMR spectra. The tentative assignment of the ^{13}C-NMR spectra (25.16 MHz, D_2O, δ = internal dioxane difference (67.4 ppm)): 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'''); 109.45 (C_1''). Identity with the natural product was further indicated by the antibacterial spectra shown in Table 1.

Table 1. Antibacterial spectra of synthetic and natural neomycin C

Test organisms*	MIC (mcg/ml)	
	Synthetic	Natural
Staphylococcus aureus FDA 209P	3.12	3.12
Bacillus subtilis NRRL B. 558	0.2	0.39
Escherichia coli K-12	6.25	6.25
Escherichia coli K-12 ML 1629	> 100	> 100
Escherichia coli K-12 LA 290 R 55	> 100	> 100
Escherichia coli K-12 LA 290 R 56	3.12	6.25
Escherichia coli K-12 W 677	1.56	1.56
Escherichia coli K-12 JR 66/W 677	> 100	> 100
Klebsiella pneumoniae PCI 602	3.12	3.12
Klebsiella pneumoniae type 22 #3038	> 100	> 100
Mycobacterium smegmatis ATCC 607**	1.56	1.56

* Agar dilution streak method (nutrient agar, 37°C, 17 hours).
** 48 hours.

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