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TOTAL SYNTHESIS OF NEOMYCIN B*†

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ABSTRACT

Total synthesis of neomycin B, a pseudo-tetrasaccharide aminoglycoside antibiotic, has been achieved through two key glycosylation reactions. Coupling of 3-*O*-acetyl-2,6-diazido-4-*O*-benzyl-2,6-dideoxy-L-idopyranosyl chloride with 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose under modified Koenigs–Knorr conditions gave 70% of the desired β-L disaccharide (**3**) corresponding to neobiosamine in structure. After deisopropylidenation of **3** and acetylation, 1,2-di-*O*-acetyl-3-*O*-(3-*O*-acetyl-2,6-diazido-4-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-5-*O*-benzoyl-D-ribofuranose was coupled to HO-5 of 3,2′,6′-tri-*N*-(benzyloxycarbonyl)-1-*N*:6-*O*-carbonyl-3′,4′-di-*O*-(*o*-methoxybenzoyl)neamine, using trimethylsilyl trifluoromethanesulfonate, to give 60% of the pseudo-tetrasaccharide **19** possessing the framework and masked functionality corresponding to neomycin B. Deblocking and reduction of the azido groups then gave neomycin B.

INTRODUCTION

Neomycin B is a main component of the neomycin complex which was independently discovered by H. Umezawa *et al.*[3] and Waksman *et al.*[4]. The neomycin group is a representative of pseudo-tetrasaccharide antibiotics, which provide some fascinating problems of synthesis[5,6]. The complete structures and absolute stereochemistry of the neomycins have been elucidated[7] and a total synthesis of neomycin C has been achieved[8]. Neamine (neomycin A), the pseudo-disaccharide portion of neomycin B and C, was prepared[9] by 6′-amination of paromamine and later[10] by a coupling reaction. However, the total synthesis of neomycin B has been hindered[11] by the difficulty associated with glycosylation with 2,6-diamino-2,6-dideoxy-L-idose (neosamine B), which distinguishes neomycin B from C. We now report the first total synthesis of neomycin B.

The key glycosyl chloride, 3-*O*-acetyl-2,6-diazido-4-*O*-benzyl-2,6-dideoxy-L-idopyranosyl chloride[12] (**1**) was already known and the following reactions were involved: (*1*) coupling of **1** to HO-3 of a protected ribose[13] (**2**) to give a masked

*Dedicated to Professor Hans Paulsen.
†For preliminary reports of part of this work, see refs. 1 and 2.

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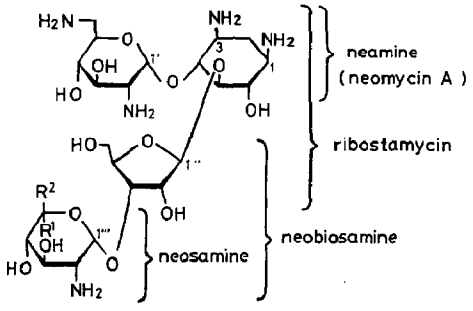
disaccharide (**3**) corresponding to neobiosamine B; and (*2*) coupling of **5**, derived from **3**, to HO-5 of a selectively protected neamine (**18**) to give a masked pseudo-tetrasaccharide (**19**), which was then converted into neomycin B.



	R^1	R^2
neomycin B :	CH_2NH_2	H
neomycin C :	H	CH_2NH_2

RESULTS AND DISCUSSION

Glycosylation of HO-3 of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose[13] (**2**) with 3-*O*-acetyl-2,6-diazido-4-*O*-benzyl-2,6-dideoxy-L-idopyranosyl chloride[12] (**1**) in the presence of mercuric cyanide, mercuric bromide, and molecular sieves (4 Å) gave 70% of the desired 1,2-*cis*(β-L) disaccharide derivative **3** together with 7% of the α-L anomer **4** after column chromatography. The β-L and α-L configurations of **3** and **4** assigned on the basis of the $[\alpha]_D$ values (+139° and +28°, respectively) were confirmed by the small $J_{2',3'}/J_{4',5'}$ values of **3** (Table I), and the long-range coupling ($J_{2',4'} \geq 1$ Hz) indicates the $^1C_4$(L) conformation almost exclusively[14,15]. The $J_{2',3'}$ and $J_{3',4'}$ values of the α-L isomer **4** are larger than those of **3**, which shows that **4** departs from the pure $^1C_4$ conformer and some contribution of the $^4C_1$ conformer is suggested[12,14–17]. However, anomeric configurations of **3** and **4** were not assigned unequivocally because of the small difference of the $J_{1',2'}$ values[18]. Therefore, the ^{1}H-n.m.r. spectra of **3** and **4** were compared with those of other idoside derivatives. The $J_{1,2}/J_{4,5}$ values for methyl 2,6-diamino-2,6-dideoxy-β-L-idopyranoside[12] (**8**) were close to those of **3**, suggesting the β configuration of **3**. On the other hand, the $J_{1',2'}$ value of **4** was essentially identical with that of the corresponding α-glycosyl acetate **7**, but different from that of the α-glycoside **9**. The *J* values of other related compounds (**10–13**) prepared in our laboratory[16] are shown in Table I for comparison.

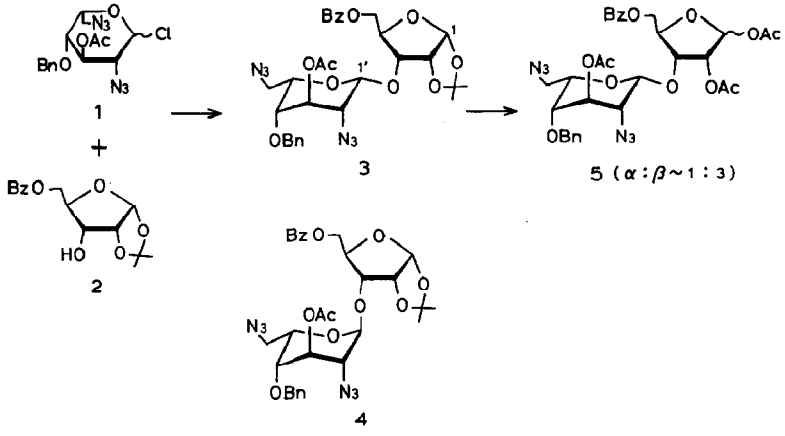
Since the ^{1}H-n.m.r. data gave no clear-cut conclusion for the anomeric configurations of **3** and **4**, the ^{13}C-n.m.r. spectra were measured (Table II). The magnitude and the sign of the chemical shift difference of C-1/C-6 of the idoside

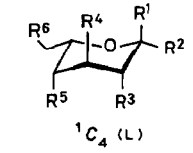
TABLE I

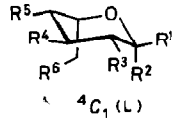
^{1}H-N.M.R. DATA ($CDCl_3$, 250 MHz) FOR THE IDOSIDE PORTION OF **3** AND **4**, AND FOR OTHER RELATED COMPOUNDS (**6–13**)

Compound	*Chemical shifts*[a]							*First-order coupling constants*							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{2,4}$	$J_{1,3}$
3(β)	5.11	3.59	5.32	3.24	3.91	2.95	3.73	2.0	3.2	3.2	2.2	3.8	8.5	$\geqslant$1	0
4(α)	4.87	3.60	5.12	3.52	4.41	3.27	3.59	3.4	~5.5	~4.5	3	5.4	8.5	0	0
6[b](β)								2.5	4.5	4.5	3	5	8	$\leqslant$1[f]	
7[b](α)								3.5	4.5	4.5	3	5	8	$\leqslant$1[f]	$\leqslant$1[f]
8[b,d](β)								2	3.5	3.5	2	4.5	8.5	~1	
9[b,d](α)								4.5	6.5	6	3.5	4.5	9		
10[c,e](β)								1	4	4	2.5	4	7.5		
11[c,e](α)								3.5	5	4	2.5	4	9		
12[c,d](β)								1.5	4	4	2			1	
13[c,d](α)								6	8.5	7.5	4.5				

[a]Confirmed by the ^{1}H–^{1}H shift-correlated 2D spectrum (for **4**), and selective decoupling (for **3**). [b]Taken from ref. 12. [c]Taken from ref. 16. [d]Measured in 20% ND_3 in D_2O. [e]Measured in pyridine-d_5–D_2O, 10:1. [f]Not reported in ref. 12.







	R^1	R^2	R^3	R^4	R^5	R^6
6	H	OAc	N_3	OAc	OBn	N_3
7	OAc	H	N_3	OAc	OBn	N_3
8	H	OMe	NH_2	OH	OH	NH_2
9	OMe	H	NH_2	OH	OH	NH_2
10	H	OMe	OH	OH	OH	N_3
11	OMe	H	OH	OH	OH	N_3
12	H	O^iPr	OH	OH	OH	NH_2
13	O^iPr	H	OH	OH	OH	NH_2

portion between **3**(β) and **4**(α) showed a similar pattern with that between **8**(β) and **9**(α). The significant up-field shift (6.6 p.p.m. between **3** and **4**, 4.3 p.p.m. between **8** and **9**) of the signals for C-5 of the α anomers compared to those of the β anomers reflect the γ-gauche effect[19] caused by H-5 and the axially oriented aglycon in the α anomer, which adopts the $^1C_4$(L) conformation. The consistent shielding of C-5 of α-aldohexopyranose derivatives compared to respective β anomers has been documented[20]. These results clearly indicate the anomeric configurations of **3** and **4** are as described.

Treatment of **3** with a mixture of acetic acid and 2M hydrochloric acid followed by conventional acetylation gave an $\alpha\beta$-mixture of glycosyl acetates (**5**, 92%, $\alpha\beta$-ratio ~1:3 as estimated from the ^{1}H-n.m.r. spectrum) which were indistinguishable on chromatography. When deisopropylidenation was carried out with HCl or H_2SO_4 in aqueous alcohol or 1,4-dioxane, the yields were markedly lower.

A suitably protected neamine derivative with HO-5 free was next sought. When tetra-*N*-(benzyloxycarbonyl)-5,6-*O*-cyclohexylideneneamine[21,22] (**15**) was treated with benzyl bromide and base, a mixture of products resulted. Benzylation under acid catalysis[23] was also unsuccessful. Acetyl and benzoyl protection was in-

TABLE II

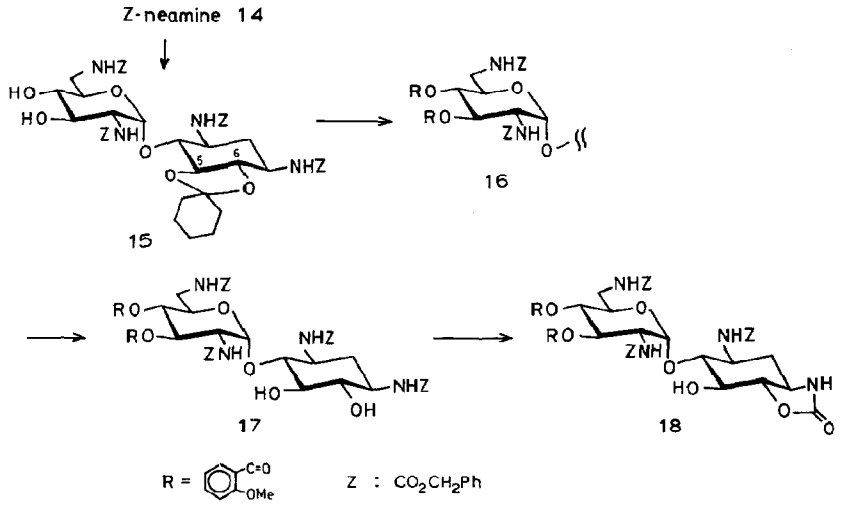
^{13}C-N.M.R. CHEMICAL SHIFT DATA ($CDCl_3$, 62.9 MHz) FOR **3** AND **4** COMPARED WITH THOSE FOR **2**, **8**, AND **9**

Carbon	**3**[a]	**4**[a]	**8**[b]	**9**[b]	$\Delta\delta$(3 − 4)	$\Delta\delta$(8 − 9)	**2**
C-1	104.7	104.2					104.1
C-2	76.7	78.8					78.5[c]
C-3	77.0	79.5					72.2
C-4	75.2	75.9					78.4[c]
C-5	63.2	62.7					63.4
C-1′	97.5	100.1	101.6	103.1	−2.6	−2.5	
C-2′	57.5	58.6	53.7	54.6	−1.1	−0.9	
C-3′	67.6	67.9	71.4	72.7	−0.3	−1.3	
C-4′	70.9	72.8	69.7	71.4	−1.9	−1.7	
C-5′	74.8	68.2	76.9	72.6	+6.6	+4.3	
C-6′	51.2	50.5	42.1	40.4	+0.7	+1.7	
$C(CH_3)_2$	113.4	113.0					112.8
$C(CH_3)_2$	26.8	26.4					26.5[d]
	26.9	26.7					
C=O	166.2	166.2					166.5
	169.0	169.6					

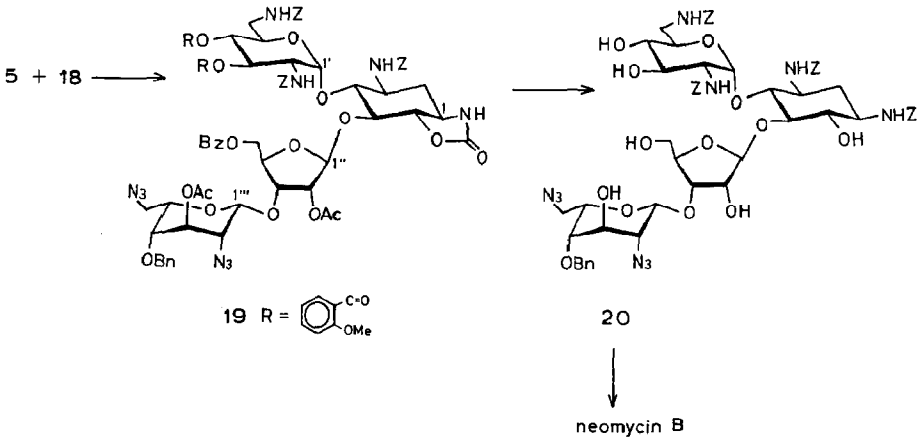
[a]Determined by the ^{13}C–^{1}H shift-correlated 2D spectrum. [b]Taken from ref. 12, measured in 20% ND_3 in D_2O. [c]Assignments may be interchanged. [d]Duplicated.

appropriate because of the use of basic conditions in the subsequent formation of the cyclic carbamate. α-Naphthoyl protection was found to improve the situation, but was still unsatisfactory. Protection by the *o*-methoxybenzoyl group proved to be suitable. Thus, **15** was acylated with *o*-methoxybenzoyl chloride in pyridine to give the 3′,4′-di-*O*-acyl derivative (**16**) in good yield. After decyclohexylidenation (→**17**), HO-6 was protected by cyclic carbamate formation with NH_2-1 (→**18**), using sodium hydride in *N*,*N*-dimethylformamide. The yield of the carbamate was ~60% when the reaction was conducted in the usual manner, but it was raised to ~80% by the addition of excess benzyltriethylammonium chloride. Without the quaternary ammonium salt, polar and faster-moving by-products appeared (t.l.c.). The ^{1}H-n.m.r. spectrum of the higher-mobility substance showed it to be benzyl *o*-methoxybenzoate.

Coupling of **5** and **18** in benzene in the presence of trimethylsilyl trifluoromethanesulfonate[24] gave 60% of the desired pseudo-tetrasaccharide derivative **19**, having the β-D configuration, as the only isolable product; formation of the α anomer was not detected. Although the resonances of H-1″ and H-1‴ of **19** were overlapped with other signals, the mechanism of the glycoside formation under Lewis-acid catalysis involving neighbouring-group participation[25] strongly suggested the formation of a β-D(1,2-*trans*) ribosyl bond, which was eventually established by the small (2.3 Hz) $J_{1'',2''}$ value of fully deblocked neomycin B. The addition of molecular sieves as reported[24] did not improve the yield of **19**. BF_3-etherate and $SnCl_4$ did not promote the glycosylation, but trityl perchlorate[26] showed a result almost identical with that obtained with trimethylsilyl trifluoromethanesulfonate.



Treatment of **19** with sodium benzyloxide in benzyl alcohol gave the 1,3,2′,6′-tetra-*N*-(benzyloxycarbonyl) derivative **20**. The *o*-methoxybenzoyl group in **19** was resistant to saponification with aqueous alkali. Catalytic hydrogenolysis of **20** over Pd/C removed both the *N*-benzyloxycarbonyl and *O*-benzyl groups, and the azido groups were converted into amino groups to give 65% of neomycin B. The ^{1}H-[27] and ^{13}C-n.m.r.[28] spectra of the free base and the specific rotation[29] of the hydrochloride accorded with the data reported for natural neomycin B. The synthetic and natural specimens showed the same activity[2] against Gram-positive and -negative bacteria.



EXPERIMENTAL

General methods. — Melting points were determined with a Kofler block and are uncorrected. Optical rotations were measured at 20–22° with a Perkin–Elmer 241 polarimeter for solutions in chloroform, unless otherwise stated. T.l.c. was performed on Kieselgel 60 F_{254} (Merck) with detection by u.v. light (254 nm), iodine vapor, and charring with sulfuric acid. Flash column chromatography was performed on Kieselgel 60 (40–63 μm) or Wakogel C-300. I.r. spectra were recorded for KBr discs with a JASCO A-202 grating spectrophotometer. ^{1}H-N.m.r. (250 MHz) and ^{13}C-n.m.r. (62.9 MHz) spectra were recorded with a Bruker WM-250 spectrometer for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated.

3-O-*(3-O-Acetyl-2,6-diazido-4-O-benzyl-2,6-dideoxy-β-* (**3**) *and -α-*L*-idopyranosyl)-5-O-benzoyl-1,2-O-isopropylidene-β-*D*-ribofuranose* (**4**). — A mixture of **1** (1.00 g, 2.62 mmol), **2** (398 mg, 1.35 mmol), $Hg(CN)_2$ (380 mg, 1.50 mmol), $HgBr_2$ (100 mg, 0.28 mmol), and powdered molecular sieves (4 Å, 2.5 g) in dry CH_2Cl_2 was stirred for 28 h at room temperature under a gentle stream of N_2. T.l.c. (toluene–ethyl acetate, 4:1) then revealed no **2** (R_F 0.22), and the presence of **3** (0.31), **4** (0.58), and some minor by-products. The mixture was filtered with $CHCl_3$ through a pad of Celite, washed successively with saturated aqueous $NaHCO_3$, H_2O, aqueous 10% KI, H_2O, and aqueous 10% NaCl, dried (Na_2SO_4), and concentrated. Column chromatography [silica gel (100 mL); toluene–ethyl acetate, 4:1] of the residue (~1.2 g) gave **3** as a glassy solid (605 mg, 70%), $[\alpha]_D$ +139° (*c* 0.8); ν_{max} 2110 (N_3), 1740 and 1720 (C=O), 1270, 1220, 1040 cm^{-1}. ^{1}H-N.m.r. (see Table I also): δ 5.87 (d, $J_{1,2}$ 3.7 Hz, H-1), ~4.75 (H-5b), ~4.73 (H-2), ~4.4 (3 H, H-3,4,5a), 1.40 and 1.60 (2 s, each 3 H, CMe_2). See Table II for ^{13}C-n.m.r. data.

Faster moving fractions containing **4** were re-chromatographed using preparative h.p.l.c. (Senshu Pak SSC-Silica-842, 5 μm, 30 × 250 mm; toluene–ethyl acetate, 9:1, 13 mL/min) to give **4** (61 mg, 7%), $[\alpha]_D$ +28° (*c* 1.1). ^{1}H-N.m.r. (see Table I also): δ 5.79 (d, $J_{1,2}$ 3.6 Hz, H-1), 4.73 (dd, $J_{2,3}$ 4.4 Hz, H-2), 4.64 (dd, $J_{4,5b}$ 3 Hz, H-5b), 4.50 (dd, J_{5gem} 12.3, $J_{4,5a}$ 5 Hz, H-5a), 4.29 (ddd, $J_{3,4}$ 9.2 Hz, H-4), 3.84 (dd, H-3), 1.36 and 1.57 (2 s, each 3 H, CMe_2). See Table II for ^{13}C-n.m.r. data.

Anal. Calc. for $C_{30}H_{34}N_6O_{10}$ (638.6): C, 56.42; H, 5.37; N, 13.16. Found: **3**, C, 56.79; H, 5.28; N, 13.12; **4**, C, 56.61; H, 5.49; N, 13.40.

*1,2-Di-*O*-acetyl-3-O-(3-O-acetyl-2,6-diazido-4-O-benzyl-2,6-dideoxy-β-*L*-idopyranosyl)-5-O-benzoyl-*D*-ribofuranose* (**5**). — To a solution of **3** in acetic acid (15 mL) was added 2M hydrochloric acid (7 mL), and the solution was left for 2.5 h at room temperature. After concentration to ~5 mL, $CHCl_3$ (50 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ and aqueous 10% NaCl, dried (Na_2SO_4), and concentrated to dryness. The residue (810 mg) was acetylated with acetic anhydride (0.8 mL) in pyridine (4 mL) in the usual manner, to give **5** as a glassy solid (910 mg, 92%), $[\alpha]_D$ +82° (*c* 0.9); ν_{max} 2110, 1740, 1270, 1220 cm^{-1}. ^{1}H-N.m.r.: δ 6.48 (d, $J_{2,3}$ 4.5 Hz, H-1α), 6.20 (s, H-1β), 5.32 (d, $J_{2,3}$ 4.5 Hz, H-2β),

5.27 (t, $J_{2',3'} = J_{3',4'} = 2.7$ Hz, H-3'β, maybe overlapped with H-3'α), 5.04 (dd, $J_{2,3}$ 7 Hz, H-2α), 5.00 (d, $J_{1',2'}$ 2 Hz, H-1'β), 4.4–4.9 (m, 6 H, CH_2Ph and H-3,4,5,5), 3.91 (ddd, $J_{4',5'}$ 2, $J_{5',6'a}$ 4, $J_{5',6'b}$ 8.2 Hz, H-5'β), 3.80 (ddd, H-5'α), 3.69 (dd, $J_{6'gem}$ 12.6 Hz, H-6'bα), 3.66 (dd, $J_{6'gem}$ 13 Hz, H-6'bβ), 3.48 (apparent t, $J_{2',4'} \geq 1$ Hz, H-2'α), 3.40 (apparent t, $J_{2',4'} \geq 1$ Hz, H-2'β), 3.20–3.22 (H-4'α,β), 3.12 (dd, H-6'aα), 3.02 (dd, H-6'aβ), 2.16–1.86 (5 s, 9 H, 3 Ac); the $\alpha\beta$-ratio varied depending on the signals integrated and was in the range 1:2.7 based on H-6'a to 1:3.7 based on H-1.

Anal. Calc. for $C_{31}H_{34}N_6O_{12}$ (682.6): C, 54.54; H, 5.02; N, 12.31. Found: C, 54.24; H, 5.11; N, 12.46.

1,3-Di-O-acetyl-2,6-diazido-4-O-benzyl-2,6-dideoxy-α-L-idopyranose (**7**). — The crystalline $\alpha\beta$-mixture[12] was repeatedly recrystallised from ether–pentane to give **7** with a constant m.p. 70–71°, $[\alpha]_D$ −8.6° (*c* 0.6). The ^{1}H-n.m.r. data agreed with those[12] of the major anomer (see Table I).

Anal. Calc. for $C_{17}H_{20}N_6O_6$ (404.4): C, 50.49; H, 4.99; N, 20.78. Found: C, 50.80; H, 4.93; N, 20.69.

1,3,2',6'-Tetra-N-(benzyloxycarbonyl)-5,6-O-cyclohexylidene-3',4'-di-O-(o-methoxybenzoyl)neamine (**16**). — The described[21] preparation of **15** was improved. To a solution of tetra-*N*-(benzyloxycarbonyl)neamine[22] (**14**; 5.20 g, 6.05 mmol) in dry *N,N*-dimethylformamide (40 mL) containing 1,1-dimethoxycyclohexane (8 mL) and anhydrous toluene-*p*-sulfonic acid (200 mg) was added dry CH_2Cl_2 (200 mL), and the cloudy solution was boiled under reflux, using a Soxhlet-type apparatus filled with molecular sieves (5 Å) to trap the methanol liberated. After 6 h, the solution became clear, and it was then washed with saturated aqueous $NaHCO_3$ and aqueous 10% NaCl, dried (Na_2SO_4), and concentrated to dryness. A solution of the residue (6.18 g, almost pure dicyclohexylidene derivative) in ethanol (65 mL) containing pyridinium toluene-*p*-sulfonate (300 mg) was kept for 16 h at 25°. Ether (~200 mL) was added to complete the precipitation and the bulk of mother liquor was removed by centrifugation. The solid was collected, washed with H_2O, and dried to give crude **15** (4.88 g, 86%; contaminated with ≤10% of **14**), which could be used for the next acylation. A portion (430 mg) of this preparation was purified by acetylation (pyridine and acetic anhydride), chromatography of the resulting diacetate (389 mg, 83%; δ_H 1.82 and 1.90 for 2 Ac), and deacetylation (28% NH_4OH–methanol, 1:9) to give **15** as needles (69% overall from **14**). Recrystallisation from methanol–H_2O (~6:1) gave material with m.p. 181–182°, $[\alpha]_D$ +6.7° (*c* 0.8), +29° (*c* 0.8, 1,4-dioxane); lit.[21] (for amorphous solid) m.p. 185.5–187°, $[\alpha]_D$ +5.3° (*c* 0.9); lit.[30] +26.67° (1,4-dioxane).

Anal. Calc. for $C_{50}H_{58}N_4O_{14}$ (939.0): C, 63.95; H, 6.23; N, 5.97. Found: C, 63.87; H, 6.21; N, 6.06.

To an ice-cold solution of crude **15** (1.95 g) in dry pyridine (10 mL) was added a solution of *o*-methoxybenzoyl chloride (1.45 g) in dry CH_2Cl_2 (20 mL) during ~5 min. The mixture was left overnight at room temperature, aqueous 50% pyridine (0.4 mL) was added and, after 0.5 h, the bulk of the solvent was

evaporated. A solution of the residue in $CHCl_3$ (40 mL) was washed successively with H_2O, saturated aqueous $NaHCO_3$, aqueous 10% $KHSO_4$, and H_2O, dried (Na_2SO_4), and concentrated to dryness. The residue (2.77 g) was chromatographed on silica gel (160 mL) with $CHCl_3$–ethyl acetate (3:1) to give **16** as foamy solid (2.11 g, 84%; 72% from **14**), $[\alpha]_D$ +22.5° (*c* 0.8); ν_{max} 1720 (broad), 1520 (Amide II), 1300, 1250, 1020 cm^{-1}. ^{1}H-N.m.r.: δ 3.72 and 3.70 (2 s, each 3 H, 2 OMe), 2.43 (H-2*e*), 1.2–1.6 (11 H, cyclohexylidene and H-2*a*).

Anal. Calc. for $C_{66}H_{70}N_4O_{18}$ (1207.3): C, 65.66; H, 5.84; N, 4.64. Found: C, 65.95; H, 5.80; N, 4.61.

1,3,2',6'-Tetra-N-*(benzyloxycarbonyl)-3',4'-di*-O-*(o-methoxybenzoyl)neamine* (**17**). — A solution of **16** (2.05 g, 1.70 mmol) in acetic acid–oxolane–H_2O (3:1:1, 35 mL) was kept for 3 h at 60° and then concentrated to dryness with several additions of toluene to give amorphous **17** (1.88 g, 98%), $[\alpha]_D$ +59° (*c* 0.8); ν_{max} 1720 (broad), 1520, 1250 cm^{-1}. ^{1}H-N.m.r.: δ 3.68 and 3.62 (2 s, each 3 H, 2 OMe), 2.22 (m, H-2*e*), 1.43 (m, H-2*a*).

Anal. Calc. for $C_{60}H_{62}N_4O_{18}$ (1127.2): C, 63.93; H, 5.54; N, 4.97. Found: C, 63.75; H, 5.57; N, 4.74.

3,2',6'-Tri-N-*(benzyloxycarbonyl)-1*-N:*6*-O-*carbonyl-3',4'-di*-O-*(o-methoxybenzoyl)neamine* (**18**). — To an ice-cold solution of **17** (330 mg, 0.29 mmol) in dry *N,N*-dimethylformamide (5 mL) was added NaH (26 mg, 60% suspension in oil, net ~0.65 mmol), and the mixture was stirred under N_2. After 20 min, a solution (10 mL) of benzyltriethylammonium chloride (550 mg, 2.42 mmol) in dry *N,N*-dimethylformamide was added at 0°, and the mixture was stirred for 2 h, then neutralised with acetic acid, and concentrated. A solution of the residue in $CHCl_3$ (20 mL) was washed successively with H_2O, saturated aqueous $NaHCO_3$, aqueous 10% $KHSO_4$, and H_2O, dried (Na_2SO_4), and concentrated to dryness. The residue (350 mg) was chromatographed on silica gel (30 mL) with $CHCl_3$–ethanol (25:1) to give amorphous **18** (232 mg, 78%), $[\alpha]_D$ +80° (*c* 1.1); ν_{max} 1770 (sh, cyclic carbamate), 1720, 1520, 1300, 1250 cm^{-1}. ^{1}H-N.m.r.: δ 3.64 and 3.68 (2 s, each 3 H, 2 OMe), 2.15 (m, H-2*e*), 1.55 (q, *J* ~12 Hz, H-2*a*).

Anal. Calc. for $C_{53}H_{54}N_4O_{17}$ (1019.0): C, 62.47; H, 5.34; N, 5.50. Found: C, 62.38; H, 5.27; N, 5.48.

5-O-*[2*-O-*Acetyl-3*-O-*(3*-O-*acetyl-2,6-diazido-4*-O-*benzyl-2,6-dideoxy-β*-L-*idopyranosyl)-5*-O-*benzoyl-β*-D-*ribofuranosyl]-3,2',6'-tri*-N-*(benzyloxycarbonyl)-1*-N:*6*-O-*carbonyl-3',4'-di*-O-*(o-methoxybenzoyl)neamine* (**19**). — To a solution of **18** (320 mg, 0.31 mmol) and **5** (325 mg, 0.48 mmol) in dry benzene (16 mL) was added trimethylsilyl trifluoromethanesulphonate (100 μL, ~0.52 mmol) under N_2, and the solution was left for 4 h at room temperature. T.l.c. ($CHCl_3$–acetone, 2:1) then showed spots of **19** (R_F 0.43), **18** (0.25, slight), and **5** (0.92). The mixture was washed with saturated aqueous $NaHCO_3$ and aqueous 10% NaCl, dried (Na_2SO_4), and concentrated to dryness. The residue (640 mg) was chromatographed on silica gel (100 mL) with $CHCl_3$–acetone (5:2) to give **19** (310 mg, 60%). An analytical sample, prepared by re-precipitation from 1,4-dioxane–H_2O, had $[\alpha]_D$ +55° (*c* 0.6);

ν_{max} 2110, 1770 (sh), 1720, 1240 cm^{-1}. ^{1}H-N.m.r.: δ 3.68 (s, 6 H, 2 OMe), ~2.2 (overlapped with Ac, H-2*e*), 2.13 and 2.11 (2 s, 6 H, 2 Ac), 1.40 (unresolved q, H-2*a*).

Anal. Calc. for $C_{82}H_{84}N_{10}O_{27}$ (1641.6): C, 59.99; H, 5.16; N, 8.53. Found: C, 59.98; H, 5.01; N, 8.33.

Elution with $CHCl_3$–acetone (1:1) gave **18** (60 mg, 19%).

5-O-[3-O-(2,6-Diazido-4-O-benzyl-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-1,3,2′,6′-tetra-N-(benzyloxycarbonyl)neamine (**20**). — To a solution of **19** (150 mg, 91 μmol) in dry *N,N*-dimethylformamide (0.25 mL) and dry benzyl alcohol (1.9 mL) was added NaH (11 mg, 60% suspension in oil) under N_2, and the solution was left for 4 h at room temperature. After neutralisation with solid CO_2, the solution was diluted with $CHCl_3$ (15 mL), washed with aqueous 10% NaCl, dried (Na_2SO_4), and concentrated to dryness. The residue (140 mg) was chromatographed on silica gel (15 mL). Elution of the faster moving by-products with 20:1 $CHCl_3$–ethanol was followed by elution with 10:1 $CHCl_3$–ethanol to give amorphous **20** (100 mg, 85%), $[\alpha]_D$ +40° (*c* 1); ν_{max} 3400 (broad), 2100, 1720 (sh), 1700 (broad), 1260, 1040 cm^{-1}. ^{1}H-N.m.r. ($CDCl_3$ + D_2O): δ 7.25 and 7.28 (2 s, 25 H, 5 Ph), 1.95 (H-2*e*), 1.25 (H-2*a*).

Anal. Calc. for $C_{62}H_{72}N_{10}O_{21}$ (1293.3): C, 57.58; H, 5.61; N, 10.83. Found: C, 57.23; H, 5.61; N, 10.62.

Neomycin B. — A solution of **20** (80 mg, 62 μmol) in 1,4-dioxane–H_2O–acetic acid (2:2:1, 7.5 mL) was hydrogenated over 10% Pd/C (150 mg) under 4 $kg.cm^{-1}$ H_2. After 26 h, t.l.c. of the mixture showed a ninhydrin-positive major spot having the same R_F (0.18; $CHCl_3$–methanol–17% NH_4OH, 1:4:3) as natural neomycin B. The catalyst was removed (Celite), and the filtrate was concentrated to dryness with several additions of toluene. A solution (~5 mL) of the residue in 5mM NH_4OH was applied to a column (15 mL) of CM-Sephadex C-25 (NH_4^+ form, equilibrated with 5mM NH_4OH) and, after washing with 5mM NH_4OH (~30 mL), elution was continued with a gradient of NH_4OH (5mM to 0.4M) to give neomycin B (25 mg, 65%). N.m.r. spectra were measured on the sample after passing through a short bed of Dowex 1-X2 (HO^-) resin with decarbonated water and lyophilising the eluate. ^{1}H-N.m.r. (D_2O, internal Me_4Si) and ^{13}C-n.m.r. (D_2O, corrected to Me_4Si by the signal of 1,4-dioxane at 67.4 p.p.m.) spectra were essentially identical with those reported[27,28]. Typical values are given below, with the respective reported values[27,28] in brackets: ^{1}H, δ 5.45 (d, *J* 3.5 Hz, H-1′) [5.45, *J* 3.9 Hz], 5.38 (d, *J* 2.3 Hz, H-1″) [5.37, 2.7 Hz], 4.97 (slightly broadened s, H-1‴) [4.96, *J* 1.8 Hz], 1.94 (dt, *J* ~4, ~4, and ≥12 Hz, H-2*e*) [1.95, *J* 4.1, 4.1, and 12.4 Hz], 1.20 (q, *J* ≥12 Hz, H-2*a*) [1.20, *J* 12.5 Hz]; ^{13}C, δ 109.1 (C-1″) [109.2], 100.3 (C-1′) [100.3], 99.8 (C-1‴) [99.8], 51.1 (duplicated, C-1 and C-3) [51.2], 36.4 (C-2) [36.5].

The hydrochloride was prepared by dissolving the free base (30 mg) in a minimum amount of H_2O, acidifying to pH ~2 with M hydrochloric acid, and trituration with ethanol (~1 mL) and acetone (~5 mL). The resulting solid was centrifuged and washed with acetone, and the solvent was evaporated. An aqueous

solution was lyophilised to give the amorphous hydrochloride (32 mg), $[\alpha]_D$ +54° (*c* 1, water); lit.[29] +54°.

Anal. Calc. for $C_{23}H_{46}N_6O_{13} \cdot 6\,HCl \cdot H_2O$ (851.4): C, 32.44; H, 6.39; N, 9.87; Cl, 24.98. Found: C, 32.48; H, 6.44; N, 9.56; Cl, 24.41.

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