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3′ AND 4′-AXIAL AND EQUATORIAL AMINO AND HYDROXY DERIVATIVES OF NEAMINE*

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ABSTRACT

All eight of the possible amino and hydroxyl derivatives at the 3′ and 4′ positions of neamine were prepared. The compounds are either 3′-deoxy or 4′-deoxy analogs having axial or equatorial amino or hydroxyl groups at the other position. The position and configuration of the hydroxyl groups from the reduction of the two 3′, 4′-epoxides, **11** and **12**, were established. The 3′-keto and 4′-keto derivatives (**26** and **23**) and also the corresponding oximino acetates, **33b** and **35b**, were reduced and the structures of the products determined. Hydrolytic cleavage of the exo-methylene epoxides at the 3′ and 4′ positions gave the diols, **47** and **45**. N.m.r. data for the 3′ and 4′ acetates and acetamides are presented.

INTRODUCTION

During our studies directed toward the synthesis of aminoglycoside antibiotics related to kanamycin, tobramycin, and gentamicin[2], it seemed prudent to attempt to prepare portions of the molecule, namely, neamine (**1**) derivatives, which could provide meaningful data useful for the elaboration of the complete pseudotrisaccharide molecules. Other studies have been reported on transformation of pseudotrisaccharide aminoglycoside antibiotics in an attempt to modify antibacterial action[2,3]. Although neamine, nebramine, and gentamine have essentially identical biological activities *in vitro*[4], our studies were aimed at finding biological differences (such as, activity against resistant strains or improved bioactivity) in neamine derivatives by altering the hydroxyl and amino groups on the pyranose portion. Aminoglycosides related to the clinically useful compounds would then be synthesized from the precursor neamine derivatives.

This report will show the results of our studies on synthesis of 3′ and 4′-hydroxy and amino derivatives of neamine (**1**), and on the application of n.m.r.

*Aminoglycosides, Part II. For Part I, see ref. 1.

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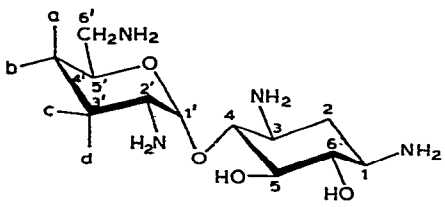


1 b = c = OH , a = d = H (neamine)
2 a = d = OH , b = c = H
3 a = OH , b = c = d = H
4 b = OH , a = c = d = H
5 c = OH , a = b = d = H
6 d = OH , a = b = c = H

spectroscopy for the assignments of configurations at the 3′ and 4′-positions of the products obtained from reduction of 3′(4′)-keto, oximino, and epoxide derivatives of **1**.

The pyranose (or primed) ring of the disaccharide **1** contains all groups equatorial. Initially, we wished to prepare "unnatural" axial hydroxyl derivatives[5] at the 3′ and 4′ positions of **1** and compare their biological activities with analogous natural products. The neamine derivatives **2–6** were synthesized for comparison of their structure–activity relationships.

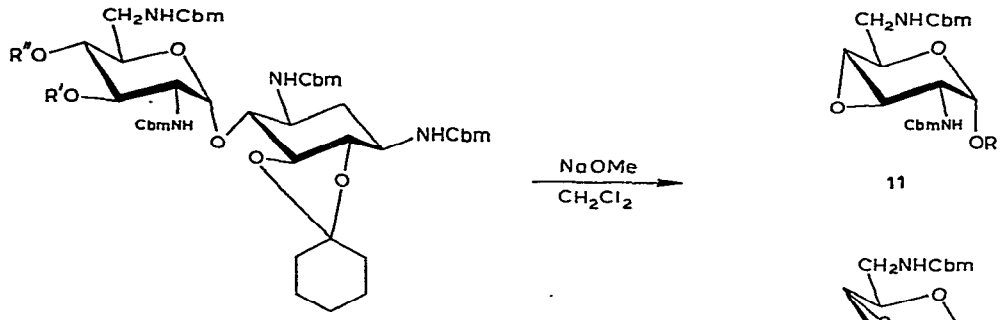
The amino groups at the 2′ and 6′ positions of certain hexoses of aminoglycoside antibiotics have been shown to exert a profound effect on antibacterial activity[4]. As no systemic study of 3′ and 4′-epimeric amino derivatives of **1** has been reported, we decided to prepare aminodeoxy derivatives from certain common precursors. In this work, all four of the monoamino derivatives of **1**, compounds **37–40**, were prepared. As examples having tertiary hydroxyl groups at the 3′ and 4′-positions of **1**, compounds **44**, **45**, and **47** were synthesized.

RESULTS AND DISCUSSION

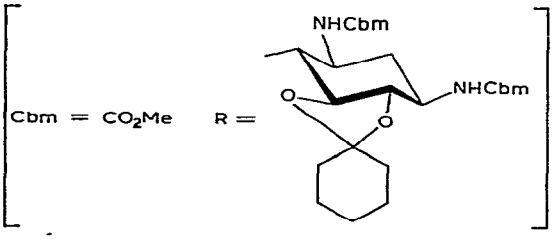
The starting material for all of the transformations was 4-*O*-[2,6-bis(methoxycarbonyl)amino-2,6-dideoxy-α-D-glucopyranosyl]-*N*,*N*′-bis(methoxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine[2a,6] (**7**).

Compound **7** was converted into the *p*-toluenesulfonate **8** according to the procedure of Umezawa *et al.*[7,8]; the isomeric sulfonate **9** was also isolated from the mixture as a minor component. Compound **8** was converted into the allo epoxide **11** with sodium methoxide and compound **9** provided the galacto epoxide **12**. Both epoxides **11** and **12** were readily separated by h.p.l.c. and t.l.c., and were shown to be homogeneous. Hydrolytic cleavage of **11** to the diol **13** was accomplished in 1,2-dimethoxyethane containing potassium carbonate in 90% yield (see Scheme I).

Acetylation of the crude product **13** provided the 3′,4′-diacetate **14**, which displayed chemical shifts for CH_3CO of δ 2.08 and 2.13 ($CDCl_3$), which are completely compatible with axial acetate resonances for pyranoid derivatives. The reported[5,9–12] chemical shifts in $CDCl_3$ for equatorial acetates range from δ 1.93 to 2.09, and for



7 $R' = R'' = H$
8 $R' =$ Ts, $R'' = H$
9 $R' = H$, $R'' =$ Ts
10 $R' = R'' =$ Ac



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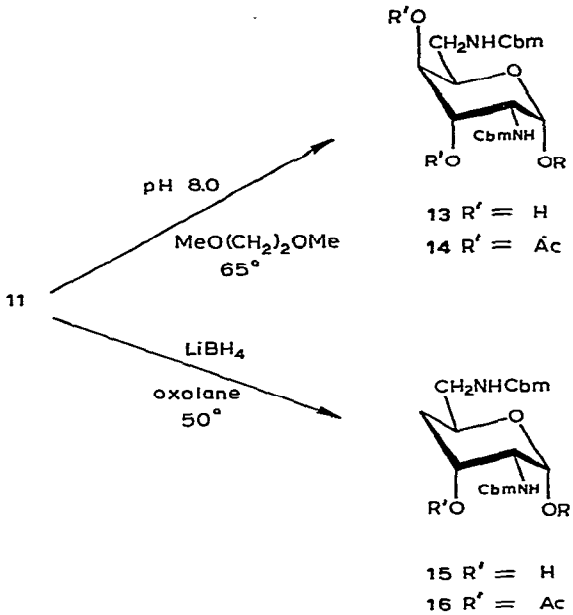
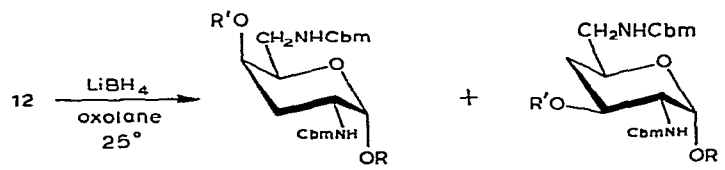
axial acetates from 2.09 to 2.22. The acetyl resonances of the diequatorial derivative **10** are δ 2.00 and 2.03.

Treatment of the epoxide **11** with lithium borohydride at 50° gave the alcohol **15** in high yield. When the reduction was attempted at room temperature, minimal reaction was observed. The derived acetate **16** showed acetyl resonances at δ 2.06 ($CDCl_3$) and 1.98 [$(CD_3)_2SO$], which correspond to an axial disposition. Final proof for the 3′-position of the hydroxyl group of **15** will be shown in the discussion later. The galacto epoxide **12** was reduced with lithium borohydride at 25° to give the two alcohols **17** and **18** in a ratio of about 4 to 1. After acetylation of the crude mixture, the major product displayed a chemical shift for CH_3CO of δ 2.15 ($CDCl_3$) and is assigned the 4′-axial acetate **19**; the minor product having δ 2.03 is the 3′-equatorial acetate **20**.

An alternative approach to either 3′ or 4′-hydroxy derivatives via reduction of the 3′- and 4′-ketone derivatives was initiated, as efficient routes to compounds **18** and **24** were needed. Displacement reactions of alcohols **15** and **17** comprise another route to the corresponding epimeric alcohols, but as it turned out, this approach was not necessary. The syntheses of the 4′-ketone **23** and the 3′-ketone **26** are shown in Scheme 2. Oxidation of the sulfonate **8** with acetic anhydride in dimethyl sulfoxide[13] gave the ketone **21**. The iodo derivative **22** was prepared from **21**, and





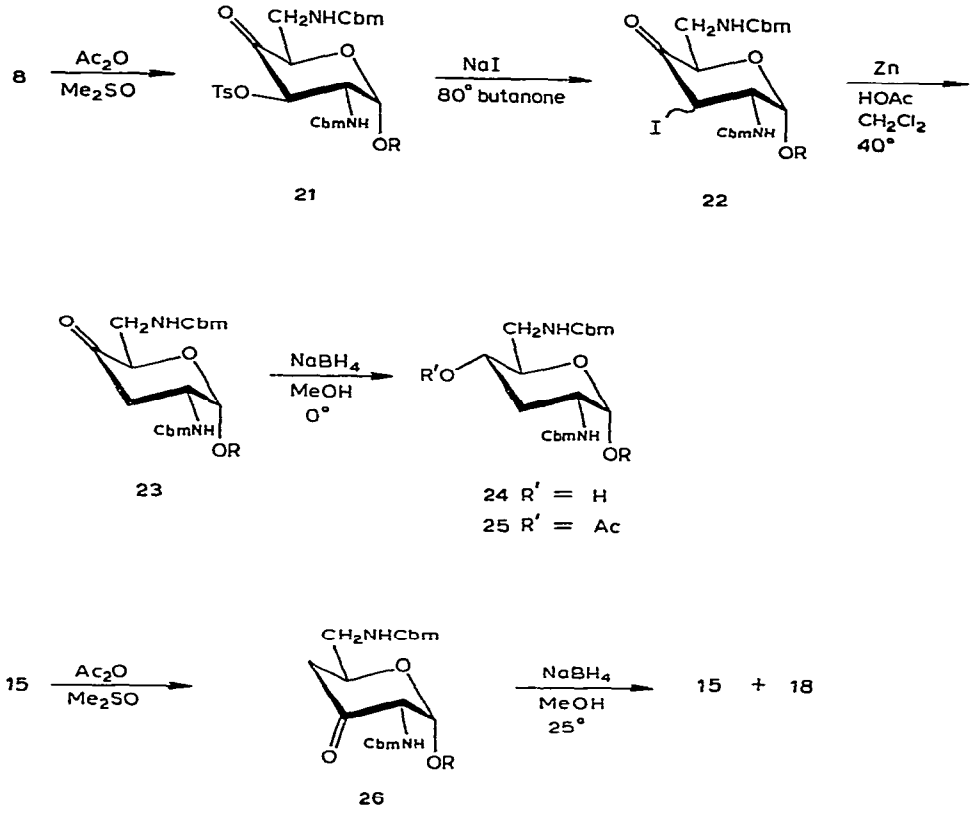
17 R' = H 18 R' = H
19 R' = Ac 20 R' = Ac

Scheme 1

reduced *in situ* to the 4′-ketone **23** with zinc–acetic acid. Reduction of **23** with sodium borohydride afforded the alcohol **24** (ref. 2a), which is a derivative of 3-deoxyneamine[14], in at least 95% configurational purity, as determined by the n.m.r. spectrum of the derived acetate **25**. The acetyl resonance at δ 2.04 ($CDCl_3$) is in complete accord with the equatorial orientation and has essentially the same chemical shift as one of the acetyl groups of the diequatorial diacetate **10**. Based on these observations, the two acetyl groups of **10** may be assigned as δ 2.00 for the 3′-acetate and 2.03 for the 4′-acetate.

The 3′-ketone **26** was prepared in 80% yield by oxidation of the 3′-axial alcohol **15** with acetic anhydride–dimethyl sulfoxide. Compound **26** was reduced with sodium borohydride at 25° to afford a 6.5 : 1 mixture of **15** and **18**. Compound **18**, a derivative of 4-deoxyneamine[15] **18**, was also the minor product from reduction of the epoxide **12** with lithium borohydride.

Further structural proof of the hydroxyl derivatives derived in Schemes 1 and 2 was shown by oxidation of the alcohols to the corresponding ketones, as t.l.c. readily differentiated ketones **23** and **26**. Compound **15**, the alcohol derived from the epoxide



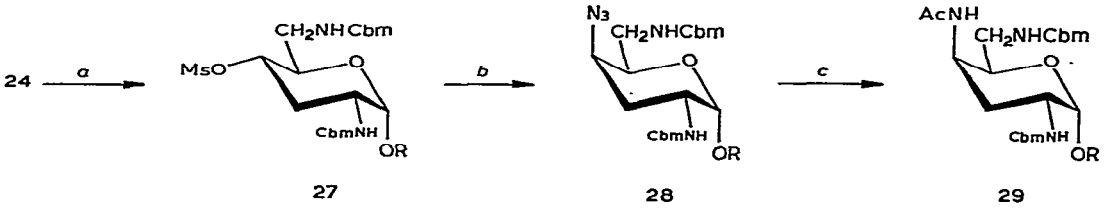
Scheme 2

11, was oxidized with acetic anhydride–dimethyl sulfoxide to **26** to confirm the position of the hydroxyl group. The crude mixture from the reduction of epoxide **12**, containing the alcohols **17** and **18** in a 4:1 ratio, gave on oxidation the ketones **23** and **26** in the same ratio.

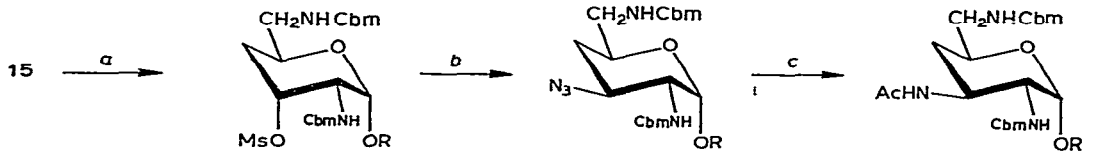
The 4′-equatorial alcohol **24** was converted into the 4′-axial acetamide **29** as shown in Scheme 3. The chemical shift of the axial *N*-acetyl methyl group of **29** was δ 2.05 ($CDCl_3$), which is in agreement with reported values for hexoses[10,11,16,17] and cyclitols[18]. In $CDCl_3$, equatorial *N*-acetyl methyl groups resonate in the range of δ 1.73–2.00 and axial ones at 2.04–2.08; in Me_2SO-d_6 the range is 1.72–1.80 for equatorial *N*-acetyl methyl groups and 1.94–2.00 for axial ones.

The conversion of the 3′-axial alcohol **15** to the 3′-equatorial acetamide **32** was entirely analogous to the preparation of **29**, and **32** displayed a resonance for an equatorial *N*-acetyl methyl group of δ 1.92 ($CDCl_3$). Displacement of the axial methanesulfonate of **30** to give the equatorial azide **31** was more facile than the displacement of **27** to give **28**, presumably because the strain of the 1,3-diaxial interaction of the sulfonate group with the anomeric substituent is relieved.

As the reduction of a 3′-oxime or oxime acetate might provide a convenient route to the axial acetamide **34**, compound **26** was converted in good yield into the



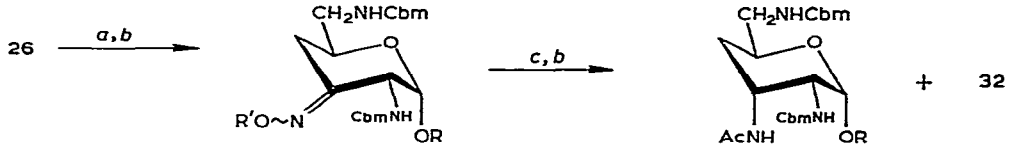
27 28 29



30 31 32

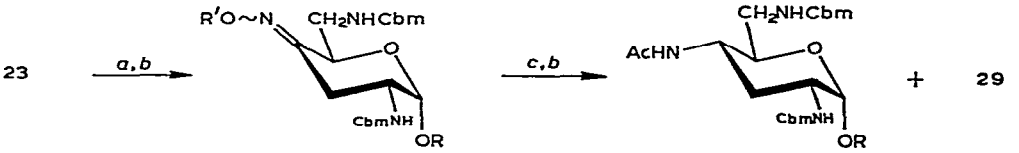
a, $MsCl—C_5H_5N$; *b*, $NaN_3—(Me_2N)_3PO$, 110° ; *c*, Raney Ni—Ac_2O, H_2.

Scheme 3



33a R′ = H
33b R′ = Ac

34



35a R′ = H
35b R′ = Ac

36

a, $NH_2OH \cdot HCl—C_5H_5N$, 70° ; *b*, $Ac_2O—C_5H_5N$; *c*, $BH_3—THF$, 25°

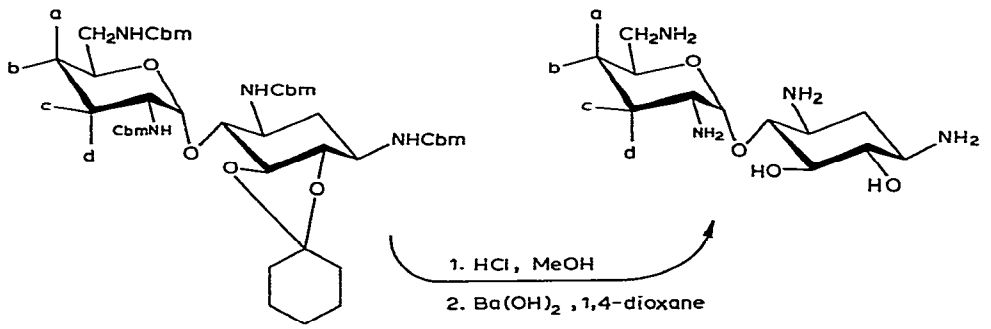
Scheme 4

TABLE I

DERIVATIVES OF NEAMINE

Cpd.	*M.p., °C*[a]	*Yield*[b]	$[\alpha]_D^{25}$, *deg*[c]	*Formula*	*Analysis*							
					Calculated				*Found*			
					C	*H*	*N*	*S*	*C*	*H*	*N*	*S*
2	250–255	90	+66.9	$C_{12}H_{26}N_4O_6 \cdot 2H_2SO_4$[d]	26.41	6.09	10.27	11.74	26.61	6.40	10.04	11.77
3	235–245	36	+62.2	$C_{12}H_{26}N_4O_5 \cdot 2H_2SO_4$[e]	26.32	6.62	10.23	11.71	26.27	6.49	10.05	11.35
4[f]	248–250	27	+63.2	$C_{12}H_{26}N_4O_5 \cdot 2H_2SO_4$[g]	25.08	6.67	9.75	11.16	24.93	6.55	9.12	11.10
5[h]	240–245	35	+81	$C_{12}H_{26}N_4O_5 \cdot 2H_2SO_4$[g]	25.08	6.67	9.75	11.16	25.28	6.11	9.63	11.35
6	220–225	33	+60.6	$C_{12}H_{26}N_4O_5 \cdot 2H_2SO_4$[i]	27.30[j]	6.11	10.61	12.14	27.10	6.41	10.03	11.80
37	260–265	41	+66.2	$C_{12}H_{27}N_5O_4 \cdot 2.5H_2SO_4$[k]	23.81[l]	5.99	11.57	13.24	23.87	5.99	11.52	13.56
38	260–264	62	+48.2	$C_{12}H_{27}N_5O_4 \cdot 2.5H_2SO_4$[m]	22.46	6.58	10.91	12.34	22.29	5.97	10.97	11.88
39	247–248	27	+62.4	$C_{12}H_{27}N_5O_4 \cdot 2.5H_2SO_4$[n]	25.23[o]	5.81	12.25	14.02	25.29	6.03	12.27	14.16
40	240–246	24	+66.2	$C_{12}H_{27}N_5O_4 \cdot 2.5H_2SO_4$[p]	23.21[q]	6.17	11.27	12.91	23.06	5.56	10.89	12.98
44	220–225	43	+64.9	$C_{13}H_{28}N_4O_5 \cdot 2H_2SO_4$[r]	26.28[s]	6.30	8.75		26.67	6.56	8.70	
45	235–238	39	+65.8	$C_{13}H_{28}N_4O_6 \cdot 2H_2SO_4$[p]	26.61	6.52	9.55	10.93	26.34	6.09	9.36	11.03
47	255–260	24	+23.5	$C_{13}H_{28}N_4O_6 \cdot 2H_2SO_4$[n]	28.78	6.13	10.32	11.71	28.69	6.26	10.58	12.32

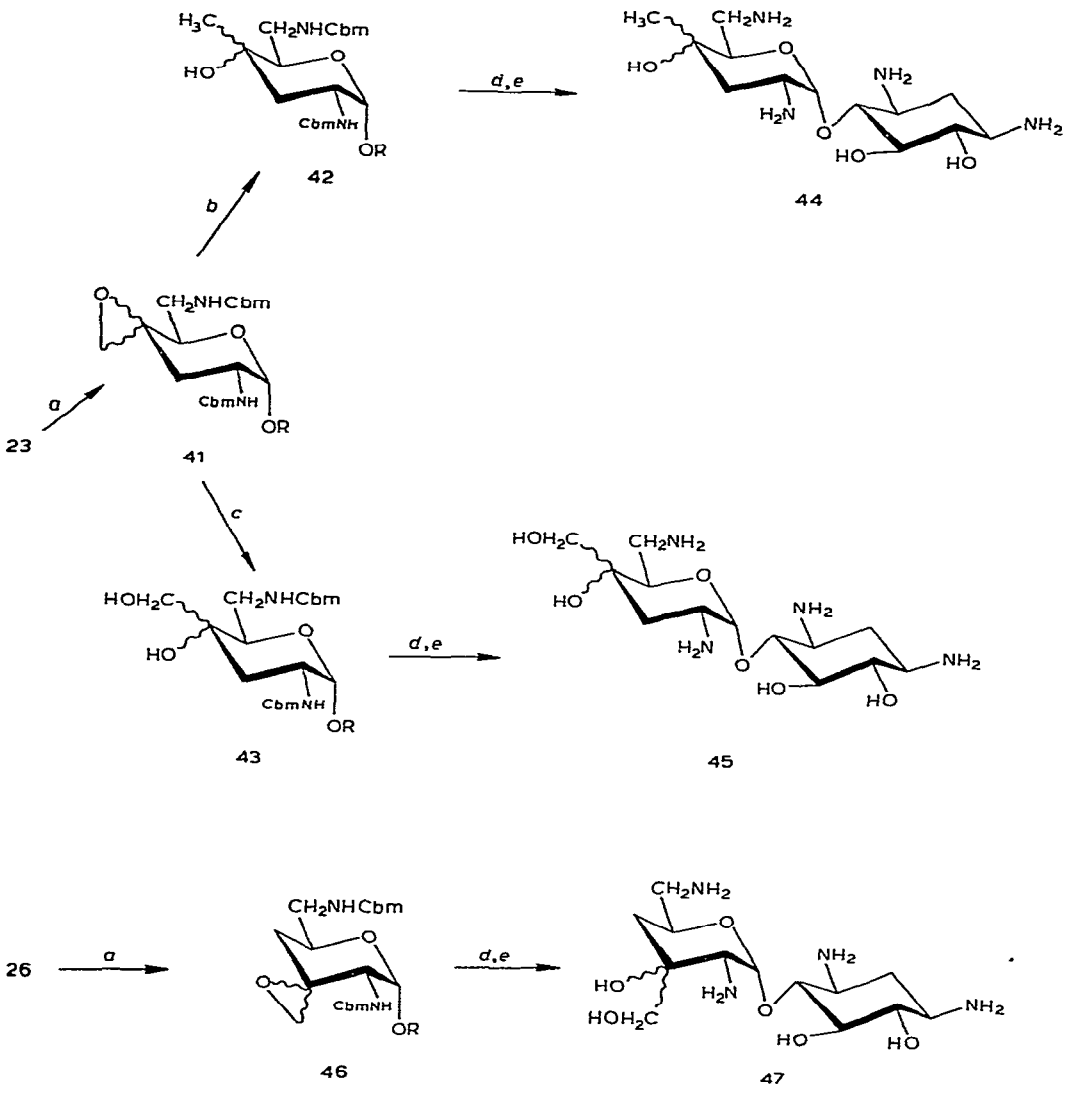
[a]All compounds were precipitated from water-methanol. [b]Yields are calculated from the completely blocked precursor and after chromatography. [c]Optical rotations determined in water at *c* 1. [d]Sesquihydrate. [e]Calc. for 2.5 mol of water of hydration. [f]Compound is nebramine, see refs. 2a, 14. [g]Tetrahydrate. [h]Compound is 4-deoxyneamine, see ref. 15. [i]Hydrate. [j]Compound contained 1.41% ash on combustion. [k]Dihydrate. [l]Compound contained 3.0% ash on combustion. [m]Pentahydrate. [n]Hemihydrate. [o]Compound contained 2.14% ash on combustion. [p]Trihydrate. [q]Compound contained 2.04% ash on combustion. [r]Dihydrate-monomethanolate. [s]Compound contained 8.61% ash on combustion, ash shown to be sodium sulfate.



29 a = NHAc , b = c = d = H
36 b = NHAc , a = c = d = H
32 c = NHAc , a = b = d = H
34 d = NHAc , a = b = c = H

37 a = NH_2 , b = c = d = H
38 b = NH_2 , a = c = d = H
39 c = NH_2 , a = b = d = H
40 d = NH_2 , a = b = c = H

oximes **33a** and **33b** (see Scheme 4). 2-Oximino derivatives of hexopyranoses have been efficiently reduced to amines with diborane[19]. The oxime acetate **33b**, on treatment with diborane followed by acetylation, gave a mixture of two isomeric acetamides in 5:1 ratio. The major product was the 3′-axial acetamide **34** having an *N*-acetyl resonance of δ 2.06 ($CDCl_3$) and the minor product was **32**. About the same axial–equatorial mixture of products was obtained by borohydride reduction of the ketone **26** and by diborane reduction of the oxime acetate **33b**. The 4′-oxime acetate **35b**, on diborane reduction, also gave a mixture of amines, and the derived acetamides **36** and **29**, obtained in 4:1 ratio, displayed resonances for the *N*-acetyl methyl group of δ 1.97 and 2.06, respectively. From the reduction of the two oxime acetates **33b** and **35b**, the major products, **34** and **36**, were the two amino epimers required to complete the preparation of all four of the epimeric 3′- and 4′-monodeoxy acetamido derivatives of **1**. Therefore, convenient syntheses of all eight of the epimeric hydroxyl and amino derivatives of **1** at the 3′- and 4′-positions are available. All of these compounds were deblocked to their parent aminoglycosides. The cyclohexylidene groups were removed with dilute methanolic hydrogenchloride and the *N*-methoxycarbonyl groups were hydrolyzed with hot barium hydroxide in aqueous 1,4-dioxane. The aminoglycosides, **2–6** and **37–40**, were purified by chromatography on a weakly acidic ion-exchange resin in the ammonium form with a gradient of dilute ammonium hydroxide. The compounds were conveniently isolated as sulfate salts (see Table I). Compounds **23** and **26** were converted into epoxides **41** and **46** by treatment with dimethyloxosulfonium methylide[20], but the stereochemistry of the epoxides was not established[21] (see Scheme 5). Compound **41** was reduced with lithium borohydride to afford the alcohol **42** as a mixture of a major product and a small-to-minor component showing chemical shifts for the angular methyl groups of δ 1.17 with a shoulder at 1.23. The configurations of these methyl groups were not established[22]. The mixture **42** was deblocked to give **44**, which displayed the angular methyl resonance as a singlet at δ 1.39 (D_2O) with a slight shoulder. Hydrolytic ring-opening of the epoxide **41** provided the diol **43**, which was deblocked to **45**, which has a tertiary and a primary



a, $Me_3\overset{+}{S}OI^-$, NaH, Me_2SO ; *b*, $LiBH_4$, oxolane, 25° ; *c*, $MeO(CH_2)_2OMe$, 65°, pH 8.0 ; *d*, HCl, MeOH, 25° ; *e*, $Ba(OH)_2$, 1,4-dioxane, 110°

Scheme 5

hydroxyl group at the 4′-position. When the 3′-exo epoxide **46** was treated with lithium borohydride at 25°, only starting material was recovered. This is in contrast to the facile opening of the 4′-exo epoxide **41**. Perhaps the incipient 1,3-diaxial interactions already mentioned have an influence. Raising the temperature of the reduction mixture did not provide the 3′-hydroxy-3′-methyl isomer of **42**, but initiated loss of the protecting groups. Similarly, the hydrolytic conditions useful for the

opening of **41** to give **43** were not efficient for opening the epoxide **46**. Only strenuous basic hydrolysis (barium hydroxide at 105°) was successful in cleaving **46** to **47**.

None of the compounds showed antibacterial activity *in vitro* that was sufficiently superior to **1** to justify synthesis of pseudotrisaccharide derivatives. Complete biological results will be published elsewhere.

EXPERIMENTAL

General methods. — The compounds were routinely checked by i.r. spectroscopy; melting points were determined in a Thomas–Hoover melting-point apparatus and are uncorrected; optical rotations were measured on a Perkin–Elmer 141 polarimeter, and n.m.r. spectra were taken on a Varian T-60 spectrometer and Me_4Si or DSS were used as the internal standards. T.l.c. was carried out on 0.25-mm Analtech silica gel GF plates (Analtech, Inc., Wilmington, Delaware). Baker silica gel (60–200 mesh) was used for column chromatography. H.p.l.c. of **11** and **12** was performed on a Waters ALC 202 instrument with a R401 differential refractometer detector and a column (250 × 32 mm) of Lichrosorb 10 micro silica gel with 19:1 of chloroform–methanol. Reaction solvents were dried over molecular sieves and organic extracts of products were dried over magnesium sulfate.

4-O-[2,6-Bis(methoxycarbonyl)amino-2,6-dideoxy-3-O-p-tolylsulfonyloxy-α-D-glucopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine (**8**). — Compound[6] **7** (50 g, 785 mmol) was azeotroped with dry pyridine and then dissolved in pyridine (150 mL) and chloroform (100 mL) (distilled from phosphorus pentaoxide). The solution was stirred at room temperature as a solution of 25.6 g (135 mmol) of freshly recrystallized *p*-toluenesulfonyl chloride in chloroform (100 mL) was added dropwise over several h. After being stirred overnight at room temperature, the mixture was diluted with iced brine, extracted well with ethyl acetate, and the combined extracts were washed with ice-cold 10% acetic acid, water, 5% sodium hydrogencarbonate solution, and brine. The dried solution was concentrated to an amorphous solid (about 65 g) and chromatographed on 2 kg of silica gel, eluting first with chloroform then with a gradient of 1–5% of methanol in chloroform. With a 2–2.5% proportion, the 4′-sulfonate **9** was obtained (10.4 g, 17%). The structure of this minor product was shown by conversion with sodium methoxide into the 3′,4′-anhydro galactoside **12**. Later fractions provided the 3′-sulfonate **8** (32.6 g, 53%). The column was monitored by t.l.c. with 1:19 methanol–chloroform. The analytical sample was crystallized from ethyl acetate–petroleum ether to provide white, crystalline **8**, m.p. 142–145°, $[\alpha]_D^{25}$ +28.6° (*c* 1, chloroform).

Anal. Calc. for $C_{33}H_{48}N_4O_{16}S$: C, 50.25; H, 6.13; N, 7.10; S, 4.06. Found: C, 50.34; H, 6.32; N, 6.88; S, 3.99.

4-O-[3,4-Anhydro-2,6-bis(methoxycarbonyl)amino-2,6-dideoxy-α-D-allopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine (**11**). — A suspension of **8** (4.3 g) in 60 mL of dry chloroform was cooled to 0° and a solution of sodium (0.5 g) in anhydrous methanol (15 mL) was added all at once. The resulting

solution was stirred for 30 h at 0 ±5°, diluted with iced brine, partitioned with ethyl acetate, and the organic extracts were washed with brine, dried, and evaporated to a white solid. Crystallization from acetone–ether gave the epoxide **11** (2.35 g, 70%), m.p. 147–149° (softening at 125°), $[\alpha]_D^{25}$ −8.8° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{40}N_4O_{13} \cdot 0.5\ H_2O$: C, 49.92; H, 6.61; N, 8.96; Found: C, 49.84; H, 6.81; N, 8.60.

4-O-[3,4-Anhydro-2,6-bis(methoxycarbonyl)amino-2,6-dideoxy-α-D-galactopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine (**12**). — The procedure to prepare **12** from **9** was exactly as described for the conversion of **8** into **11**. From 16.4 g (21 mmol) of **9** in 130 mL of dry chloroform and 0.91 g (39 mmol) of sodium in methanol (25 mL) was obtained 8.0 g (63%) of **12**, m.p. 139–140° (from ethyl acetate–ether). The separation of compounds **11** and **12** was performed by t.l.c. on silica gel G with acetonitrile–ether. The epoxide **12** was synthesized by an independent and more-efficient route and will be reported in another paper from our laboratories.

4-O-[2,6-Bis(methoxycarbonyl)amino-2,6-dideoxy-α-D-gulopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine (**13**). — The epoxide **11** (2.0 g) was dissolved in 1,2-dimethoxyethane (280 mL) and added to 280 mL of a 4% solution of potassium carbonate which was adjusted to pH 8.0 with dilute hydrochloric acid. The solution was stirred for 72 h at 65°, the dimethoxyethane evaporated off, and the aqueous residue was extracted well with ethyl acetate. The combined organic extracts were washed with brine, dried, and evaporated to a white solid that was dissolved in chloroform and added to hexane to give white, amorphous **13** (1.85 g, 90%). T.l.c. with 1:19 methanol–chloroform showed one-spot material with a trace of starting **11**. For analysis, a sample of **13** was chromatographed on silica gel with a 1–3% gradient of methanol in chloroform to give pure **13**; m.p. 165–170° (from acetone–hexane); $[\alpha]_D^{25}$ +33.9° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{42}N_4O_{14}$: C, 49.21; H, 6.67; N, 8.83. Found: C, 48.91; H, 6.94; N, 8.58.

The 3′,4′-diacetate (**14**) of **13** was prepared by stirring a solution of 1.0 g of **13** in acetic anhydride (10 mL) and pyridine (20 mL) for 18 h at 50°. The cooled mixture was stirred for 1 h with iced sodium hydrogencarbonate solution and then extracted with ethyl acetate and washed with cold, 10% acetic acid solution, water, 5% sodium hydrogencarbonate solution, and brine. The dried, concentrated solution (1.3 g) was chromatographed on silica gel, first with chloroform and then with 1% methanol in chloroform. The analytical sample was crystallized from chloroform–hexane to afford white, powdery **14**, m.p. 164–167°, $[\alpha]_D^{25}$ +18.8° (*c* 0.5, chloroform); n.m.r. ($CDCl_3$): δ 2.08 (s, 3, $OCOCH_3$) and 2.13 (s, 3, $OCOCH_3$).

Anal. Calc. for $C_{30}H_{46}N_4O_{16}$: C, 50.13; H, 6.45; N, 7.79. Found: C, 50.55; H, 6.70; N, 7.61.

4-O-[2,6-Bis(methoxycarbonyl)amino-2,4,6-trideoxy-α-D-ribo-hexopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine (**15**). — A solution of 20.0 g (32.5 mmol) of epoxide **11** in oxolane (800 mL) was treated portion-

wise with 6.2 g (287 mmol) of lithium borohydride at 25°. The mixture was stirred for 18 h at 25°, the solvent was evaporated at room temperature, and the residue partitioned between water and ethyl acetate. The extract was washed with brine, dried, and evaporated to a white solid (20 g, 100%). Crystallization from chloroform–ether gave white **15**, m.p. 135–140°, $[\alpha]_D^{25}$ +36° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{42}N_4O_{13} \cdot 0.5\ H_2O$: C, 49.76; H, 6.91; N, 8.96. Found: C, 49.70; H, 6.76; N, 8.78.

The acetate (**16**) of **15** was prepared. A solution of **15** (300 mg) in acetic anhydride (4 mL) and pyridine (8 mL) was stirred for 18 h at 40° and processed as for **14**. The crude solid was crystallized from acetone–ether to afford white, powdery **16**, m.p. 118–120°, $[\alpha]_D^{25}$ +14.2° (*c* 1, 1:1 chloroform–methanol); n.m.r. ($CDCl_3$) δ 2.06 (s, 3, $OCOCH_3$); (Me_2SO-d_6) δ 1.98 (s, 3; $OCOCH_3$).

Anal. Calc. for $C_{28}H_{44}N_4O_{14}$: C, 50.90; H, 6.71; N, 8.48. Found: C, 50.70; H, 6.66, N, 8.31.

*4-O-[2,6-Bis(methoxycarbonyl)amino-2,4,6-trideoxy-α-*D*-erythro-hexopyranosyl-3-ulose]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**26**). — The alcohol **15** (1.6 g) was dissolved in dry dimethyl sulfoxide (15 mL) and acetic anhydride (10 mL) and the solution was stirred overnight at room temperature. Iced brine was added, the aqueous layer was extracted with ethyl acetate, and the organic layer was washed repeatedly with brine, dried, and concentrated to an oil. This was dissolved in a little ether and diluted with petroleum ether to give a white solid (1.2 g, 80%). An analytical sample was prepared from acetone as the white, crystalline ketone **26**; m.p. 142–145° (shrinking at 125°), $[\alpha]_D^{25}$ +27° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{40}N_4O_{13}$: C, 50.64; H, 6.54; N, 9.09; Found: C, 50.35; H, 6.68; N, 8.79.

Reduction of **26** *to give 4-O-[2,6-bis(methoxycarbonyl)amino-2,4,6-trideoxy-α-*D*-xylo-hexopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**18**) and **15**. — The 3′-ketone **26** (100 mg) was dissolved in methanol (10 mL), the solution was cooled to 10°, and sodium borohydride (100 mg) was added in portions. The mixture was stirred for 30 min at 25°, the methanol was evaporated, the product extracted into ethyl acetate, and the extracts were washed with brine. The crude mixture of 3′-axial alcohol **15** and 3′-equatorial alcohol **18** was acetylated by using pyridine (3 mL) and acetic anhydride (1.5 mL) for 18 h at 50°. The acetates were isolated as described for **14**. N.m.r. spectroscopy showed two acetyl peaks in a ratio of 6.5:1; the major product, showing δ 2.06 (chloroform-*d*) and 1.97 (dimethyl sulfoxide-d_6) is the axial isomer **16** and the minor product, δ 2.02 ($CDCl_3$), is the equatorial isomer **20**.

Reduction of the epoxide **12** *to give 4-O-[2,6-bis(methoxycarbonyl)amino-2,3,6-trideoxy-α-*D*-xylo-hexopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**17**) *and* **18**. — A mixture of epoxide **12** (5.0 g) lithium borohydride (1.6 g) and dry oxolane (300 mL) was stirred for 18 h at 50°. The oxolane was evaporated, and the crude product extracted into ethyl acetate and washed with water. The crude solid was a mixture of **17** and **18**, as oxidation of an aliquot with

acetic anhydride–dimethyl sulfoxide gave **23** and **26**. The separation of ketones **23** and **26** was achieved on plates of silica gel G with ethyl acetate. Products were detected by careful charring after spraying with dilute sulfuric acid; ketone **23** had R_F 0.4 and burned brown-black, whereas ketone **26** had R_F 0.35 and afforded a reddish spot. Acetylation of the mixture with acetic anhydride in pyridine followed by the isolation described for **14** gave a product having two acetyl resonances in a ratio of ~4:1. The major product (δ 2.15 in $CDCl_3$) was the 4′-axial acetate **19** and the minor component (δ 2.03 in $CDCl_3$) was the 3′-equatorial acetate **20**. The mixture was chromatographed on 350 g of silica gel with a gradient of methanol in chloroform. Careful elution with 0.25, 0.5, 0.75, 1.0, and 2.0% of methanol separated the two acetates. Compound **19** was eluted first and was crystallized from ether–hexane to give 1.6 g (32%) of white crystals; m.p. 150–155°, $[\alpha]_D^{25}$ +23° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 2.15 (s, 3, $OCOCH_3$).

Anal. Calc. for $C_{28}H_{44}N_4O_{14}$: C, 50.90; H, 6.71; N, 8.48. Found: C, 51.01; H, 6.68; N, 7.93.

The minor isomer **20** was precipitated from ether–hexane to give 380 mg (7.5%) of white, amorphous solid; m.p. 145–155°, $[\alpha]_D^{25}$ +35.4° (*c* 1, chloroform); n.m.r. ($CDCl_3$) δ 2.03 (s, 3, $OCOCH_3$).

Anal. Calc. for $C_{28}H_{44}N_4O_{14}$: C, 50.90; H, 6.71; N, 8.48. Found: C, 51.28; H, 7.06; N, 7.94.

*4-O-[2,6-Bis(methoxycarbonyl)amino-2,3,6-trideoxy-α-*D*-erythro-hexopyranosyl-4-ulose]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**23**). — A solution of **8** (10.0 g) in acetic anhydride (60 mL) and dimethyl sulfoxide (90 mL) was stirred for 18 h at room temperature, poured into water (1.5 L) and extracted with ethyl acetate. The extracts were washed with water, dried, and evaporated, first at water-aspirator pressure and then at high vacuum at 25°. The residual syrup was triturated with ether (100 mL) and petroleum ether (400 mL) to afford a yellow oil. The decanted, oily **21** was stirred with dry butanone (200 mL) and dry sodium iodide (10.0 g) while heating in an oil bath kept for 2 h at 80° (or until the disappearance of the u.v.-positive starting material on t.l.c.). The mixture was cooled slightly (40–45°) and dry dichloromethane (150 mL), acetic acid (50 mL), and zinc dust (50 g) were added. After 1.5 h at 40–45°, an additional 5 g of zinc and 10 mL of acetic acid were added. After another 30 min of heating and stirring, the cooled mixture was diluted with ethyl acetate, and filtered. The filter cake was washed thoroughly with ethyl acetate and the combined filtrates were washed first with water (4 times), and then with 5% sodium hydrogencarbonate solution, and finally with brine. The dried, crude ketone **23** (5.8 g) was chromatographed on 300 g of silica gel with 1–2% methanol in chloroform to give 4.1 g (57%) of **23**. Crystallization from acetone–ether–hexane gave the white solid **23**; m.p. 140–143°, $[\alpha]_D^{25}$ +10.7° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{40}N_4O_{13}$: C, 50.64; H, 6.54; N, 9.09. Found: C, 50.42; H, 6.48; N, 8.78.

On a larger scale with the same reaction conditions, but using a slightly different

isolation procedure, the ketone **23** was prepared from 60.0 g of **8**, 100 mL of acetic anhydride and 200 mL of molecular sieve-dried dimethyl sulfoxide to give **21**. This compound, with sodium iodide (60 g), butanone (500 mL), zinc dust (300 g), acetic acid (150 mL), and 300 mL of molecular sieve-dried dichloromethane gave the crude **23**. The product was dissolved in chloroform (100 mL) and added dropwise to a stirred solution of hexane (600 mL) and ether (200 mL) to give 34.6 g (78%) of white **23**, which was sufficiently pure for further transformations.

*4-O-[2,6-Bis(methoxycarbonyl)amino-2,3,6-trideoxy-α-*D*-ribo-hexopyranosyl]-N,N'-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**24**). — The ketone **23** (1.3 g) was dissolved in methanol (50 mL), cooled to 0°, and sodium borohydride (1.3 g) was added portionwise during 20 min. The mixture was stirred for an additional 20 min at 0–10°, diluted with iced brine, extracted with ethyl acetate, and the extracts were washed with brine. An aliquot of the crude reduction mixture was acetylated with acetic anhydride in pyridine as already described, to give **25**. The n.m.r. spectrum showed only one acetyl resonance, at δ 2.04 ($CDCl_3$). The bulk of the crude reduction product, which contained the alcohol **24** in at least 95% configurational purity, was chromatographed on 100 g of silica gel with 1–2% methanol in chloroform to provide 800 mg (62%) of **24**, m.p. 150–153° (from acetone–ether), $[\alpha]_D^{25}$ +29° (*c* 1, 1:1 methanol–chloroform).

Anal. Calc. for $C_{26}H_{42}N_4O_{13}$: C, 50.48; H, 6.84; N, 9.06. Found: C, 50.15; H, 6.71; N, 8.89.

*4-O-[4-Azido-2,6-bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-α-*D*-xylo-hexopyranosyl]-N,N'-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**28**). — A solution of 4.0 g (6.5 mmol) of **24** in 60 ml of dry pyridine was cooled to 0° and 1.86 g (16 mmol) of methanesulfonyl chloride (distilled from phosphorus pentaoxide) was added dropwise. The mixture was then stirred overnight at 25°, poured into iced sodium hydrogencarbonate solution, and extracted with ethyl acetate. The extracts were washed with water, cold 10% acetic acid, water, cold sodium hydrogencarbonate solution, and brine. The dried, crude sulfonate **27** was dissolved in hexamethylphosphoric triamide (HMPA) (145 mL) and sodium azide (3.7 g) was added. The mixture was heated for 4.5 h at 105–110°, poured into 3 L of ice–water, extracted with ethyl acetate, and washed with water to give, after drying, the crude 4'-axial azide **28**, which contained some HMPA. This was applied to a column of silica gel (180 g) and the product was eluted with 1–1.5% methanol in chloroform to provide 3.2 g (77%) of azide **28**. Crystallization from chloroform–hexane afforded the white, powdery **28**, m.p. 124–132°, $[\alpha]_D^{25}$ −15.1° (*c* 1, chloroform); λ_{max}^{Nujol} 4.75 μm (N_3).

Anal. Calc. for $C_{26}H_{41}N_7O_{12}$: C, 48.52; H, 6.42; N, 15.23. Found: C, 48.23; H, 6.54; N, 14.94.

*4-O-[4-Acetamido-2,6-bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-α-*D*-xylo-hexopyranosyl]-N,N'-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**29**). — A mixture of **28** (2.3 g) acetic anhydride (60 mL) and 2 teaspoons of Raney nickel was shaken in a Parr apparatus for 18 h under 60 lb.in.$^{-2}$ of hydrogen.

The filtered mixture was evaporated under high vacuum at 40° and then cyclohexane was evaporated from the residue. The residue was dissolved in ethyl acetate and water, the layers were separated, and the organic extract was washed with brine, dried, and evaporated. The product was chromatographed on 160 g of silica gel with a gradient of 1–2% methanol in chloroform to give 1.4 g (60%) of homogeneous **29**. Crystallization from chloroform–ether afforded the white acetamide **29**, m.p. 160–166°, $[\alpha]_D^{25}$ +13.7° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 2.05 (s, 3, $NHCOCH_3$).

Anal. Calc. for $C_{28}H_{45}N_5O_{13}$: C, 50.97; H, 6.87; N, 10.61. Found: C, 50.93; H, 6.75; N, 10.83.

*4-O-[3-Azido-2,6-bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-α-*D*-xylo-hexopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**31**). — The procedure was similar to the preparation of **28**. From 8.0 g (13 mmol) of **15**, pyridine (200 mL), and methanesulfonyl chloride (15 g) was obtained 8.5 g of the crude sulfonate **30**. This was heated for 1 h at 105° with sodium azide (8.0 g) and HMPA (300 mL), and the crude product chromatographed on 750 g of silica gel. Homogeneous azide **31** was eluted with 1% methanol in chloroform to give 5.5 g (66%). Crystallization from chloroform–ether provided white, crystalline **31**, m.p. 128–130°, $[\alpha]_D^{25}$ +35.3° (*c* 1, chloroform); λ_{max}^{Nujol} 4.76 μm (N_3).

Anal. Calc. for $C_{26}H_{41}N_7O_{12}$: C, 48.52; H, 6.42; N, 15.23. Found: C, 48.45; H, 6.31; N, 15.25.

*4-O-[3-Acetamido-2,6-bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-α-*D*-xylo-hexopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**32**). — The procedure was similar to the preparation of **29**. From 4.0 g of **31** and proportional amounts of reagents, there was obtained (after chromatography on silica gel) 1.8 g (44%) of the amide **32**. Crystallization from chloroform gave white **32**, m.p. 255–258°, $[\alpha]_D^{25}$ +34° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 1.92 (s, 3, $NHCOCH_3$).

Anal. Calc. for $C_{28}H_{45}N_5O_{13}$: C, 50.97; H, 6.87; N, 10.61. Found: C, 50.63; H, 6.78; N, 10.97.

*4-O-[2,6-Bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-3-oximino-α-*D*-erythro-hexopyranosyl-3-ulose]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**33a**). — A solution of the ketone **26** (7.0 g), hydroxylamine hydrochloride (7.0 g), and pyridine (450 mL) was heated for 4 h at 70° (oil-bath temperature), and the pyridine was evaporated at vacuum-pump pressure. The residue was extracted with ethyl acetate and washed with cold 10% acetic acid solution, water, 5% sodium hydrogencarbonate solution, and water. The dried, concentrated product (7.0 g, 98%) was crystallized from chloroform–ether to give white, crystalline oxime **33a**, m.p. 158–162°, $[\alpha]_D^{25}$ +18° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{42}N_5O_{13} \cdot H_2O$: C, 48.06; H, 6.67; N, 10.78. Found: C, 48.12; H, 6.42; N, 10.29.

Reduction of oxime acetate **33b** *to give 4-O-[3-acetamido-2,6-bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-α-*D*-ribo-hexopyranosyl]-N,N′-bis(methoxycarbonyl-5,6-O-cyclohexylidene-2-deoxystreptamine* (**34**) and **32**. — The oxime **33a** (7.0 g)

was azeotropically dried several times with pyridine, dissolved in pyridine (30 ml) and acetic anhydride (15 ml), and stirred for 4 h at 25°. Conventional acid–base processing as described for **14** gave about 7 g of crude oxime acetate **33b**. This was dissolved in dry oxolane (400 mL) and the solution was cooled to 0° as excess diborane in oxolane was added dropwise under nitrogen. The solution was stirred for an additional 3 h at 25°, recooled, and an excess of methanol was cautiously added. The solvents were evaporated off and the product was azeotropically dried with additional methanol. The crude mixture of 3′-amines was acetylated as before to give the two 3′-acetamides. N.m.r. spectroscopy showed the major product to be the 3′-axial acetamide **34** (δ 2.06 in $CDCl_3$) and the minor product was the 3′-equatorial acetamide **32** (δ 1.92) in the ratio of ~5:1. Chromatography on 300 g of silica gel with 1.5–2.5% of methanol in chloroform afforded 1.5 g (21%) of homogeneous **34**, m.p. 175–178° (from chloroform–ether), $[\alpha]_D^{25}$ +25.1° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 2.06 (*s*, 3, $NHCOCH_3$).

Anal. Calc. for $C_{28}H_{45}N_5O_{13}$: C, 50.97; H, 6.87; N, 10.61. Found: C, 50.54; H, 7.02; N, 10.06.

The minor product (350 mg, 4.8%) was isolated from the column with 3% methanol in chloroform and was found identical in all respects to **32** obtained by catalytic reduction of the azide **31**.

*4-O-[2,6-Bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-4-oximino-α-*D*-erythro-hexopyranosyl-4-ulose]-N,N′-bis(methoxycarbonyl)-5,6-O-cyclohexylidene-2-deoxystreptamine* (**35a**). — The procedure was similar to the preparation of **33a**. From 3.6 g of ketone **23**, 3.6 g of hydroxylamine hydrochloride, and pyridine (200 mL), and after column chromatography on 200 g of silica gel, there was obtained 1.9 g (52%) of homogeneous oxime **35a**, m.p. 140–145° (from chloroform–ether), $[\alpha]_D^{25}$ +5.7° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{42}N_5O_{13} \cdot 0.5\ H_2O$: C, 48.67; H, 6.75; N, 10.91. Found: C, 48.45; H, 6.66; N, 10.66.

Reduction of the oxime acetate **35b** *to give 4-O-[4-acetamido-2,6-bis(methoxycarbonyl)amino-2,3,4,6-tetradeoxy-α-*D*-ribo-hexopyranosyl]-N,N′-bis(methoxycarbonyl)-5,6,-O-cyclohexylidene-2-deoxystreptamine* (**36**) and **29**. — The oxime acetate **35b** was prepared from the oxime **35a** (1.14 g) as described for **33b**, to give 1.2 g of **35b**. The conditions and proportions of reagents used to prepare **34** were followed exactly, to give a mixture of two 4′-acetamides having chemical shifts ($CDCl_3$) of δ 1.97 and 2.06 in a ratio of ~3.5:1. Column chromatography on 100 g of silica gel was accomplished with a gradient of 1–2% of methanol in chloroform. The first compound eluted from the column (184 mg, 15.7%) was identical in all respects to the 4′-axial acetamide **29**. The major product (536 mg, 46%) was eluted with 2% of methanol in chloroform and was crystallized from chloroform–ether to give the white, 4′-equatorial acetamide **36**; m.p. 158–162°, $[\alpha]_D^{25}$ +53.6° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 1.97 (s, 3, $NHCOCH_3$).

Anal. Calc. for $C_{28}H_{45}N_5O_{13} \cdot H_2O$: C, 49.55; H, 7.12; N, 10.31. Found: C, 49.57; H, 6.87; N, 10.03.

4-O-[*4,4*^{1}-*Anhydro-2,3,6-trideoxy-2,6-bis(methoxycarbonyl)amino-4*-C-*methylene-α*-D-ribo(xylo)-*hexopyranosyl*]-N,N′-*bis(methoxycarbonyl)-5,6*-O-*cyclohexylidene-2-deoxystreptamine* (**41**). — In a dry flask under nitrogen were placed 3.4 g (15.4 mmol) of trimethyloxosulfonium iodide[20] and 0.75 g (15.4 mmol) of 50% sodium hydride in mineral oil. Dry dimethyl sulfoxide (75 mL) was added and the mixture was stirred for about 20 min at 20–25° to produce a milky suspension. A solution of 7.5 g (12.1 mmol) of the 4′-ketone **23** in dimethyl sulfoxide (75 mL) was added rapidly at 25°. The mixture was stirred for 20 min at 25° and 1.5 h at 55°, poured into 2 L of ice–water and extracted well with ethyl acetate. The extracts were washed thoroughly with brine, dried, and evaporated to give 6.2 g of crude product, which was chromatographed on 650 g of silica gel with a 1–3% gradient of methanol in chloroform. The homogeneous product (2.65 g, 35%) was crystallized from acetone to give white, crystalline epoxide **41**, m.p. 142–144°, $[\alpha]_D^{25}$ +46.3° (*c* 1, chloroform).

Anal. Calc. for $C_{27}H_{42}N_4O_{13}$: C, 51.42; H, 6.71; N, 8.88. Found: C, 51.12; H, 6.90; N, 8.78.

4-O-[*3,3*^{1}-*Anhydro-2,6-bis(methoxycarbonyl)amino-3*-C-*methylene-2,4,6-trideoxy-α*-D-ribo(xylo)-*hexopyranosyl*]-N,N′-*bis(methoxycarbonyl)-5,6*-O-*cyclohexylidene-2-deoxystreptamine* (**46**). — The epoxide **46** was prepared by the procedure described for the epoxide **41**, and with use of 7.8 g of trimethyloxosulfonium iodide, 1.7 g of 50% sodium hydride in mineral oil, dimethyl sulfoxide (200 mL) and 17.5 g of the 3′-ketone **26**. The crude product (12.5 g) was dissolved in a small amount of chloroform and cooled to give 5.0 g (36%) of white, crystalline epoxide **46**, m.p. 184–185°, $[\alpha]_D^{25}$ −9.6° (*c* 1, chloroform).

Anal. Calc. for $C_{27}H_{42}N_4O_{13}$: C, 51.42; H, 6.71; N, 8.88. Found: C, 51.13; H, 6.86; N, 8.69.

4-O-[*2,6-Bis(methoxycarbonyl)amino-2,3,6-trideoxy-4*-C-*methyl-α*-D-ribo(xylo)-*hexopyranosyl*]-N,N′-*bis(methoxycarbonyl)-5,6*-O-*cyclohexylidene-2-deoxystreptamine* (**42**). — In dry oxolane (75 mL) was dissolved 1.4 g (2.24 mmol) of **41**, and 0.5 g (23 mmol) of lithium borohydride was added portionwise at 25°. The milky mixture was stirred for 3 h at 25°, evaporated to dryness, and the residue partitioned between water–ethyl acetate. The dried organic extract (1.4 g) was crystallized from chloroform–ether–hexane to give white, powdery **42**, m.p. 160–164° (sintered at 145–150°), $[\alpha]_D^{25}$ +47.1° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 1.17 (s, 3, C-CH_3) and 1.23 (shoulder).

Anal. Calc. for $C_{27}H_{44}N_4O_{13} \cdot 0.5\ H_2O$: C, 50.44; H, 7.07; N, 8.73. Found: C, 50.25; H, 6.97; N, 8.45.

4-O-[*2,6-Bis(methoxycarbonyl)amino-2,3,6-trideoxy-4*-C-(*hydroxymethyl*)-*α*-D-ribo(xylo)-*hexopyranosyl*]-N,N′-*bis(methoxycarbonyl)-5,6*-O-*cyclohexylidene-2-deoxystreptamine* (**43**). — Potassium carbonate solution (3%, 4.0 mL) was adjusted to pH 8.0 with 3M hydrochloric acid and diluted with a solution of 1.15 g of **41** in 1,2-dimethoxyethane (40 mL). The mixture was stirred and heated for 30 h at 65°, evaporated to remove the dimethoxyethane, and diluted with brine and ethyl acetate. The organic extracts were washed with water, dried, and evaporated to give 1.05 g of

crude product. This was chromatographed on 70 g of silica gel. Homogeneous fractions (760 mg, 63%) were obtained by elution with 2.0–2.5% methanol in chloroform. Crystallization from chloroform–hexane gave the white diol **43**, m.p. 150–154° (sintered at 125°), $[\alpha]_D^{25}$ +15.4° (*c* 1, chloroform); *m/e* 648 (M^+).

Anal. Calc. for $C_{27}H_{44}N_4O_{14}$: C, 49.99; H, 6.84; N, 8.64. Found: C, 49.71; H, 6.60; N, 8.36.

4-O-[2,6-Diamino-2,4,6-trideoxy-3-C-(hydroxymethyl)-α-D-ribo(xylo)-hexopyranosyl]-2-deoxystreptamine (**47**). — A solution of **46** (1.5 g), 3M hydrochloric acid (1.5 mL) and methanol (30 mL) was stirred for 75 min at 25°, the solvents were evaporated, and the residue was azeotropically dried by several evaporations of methanol. The residue was dissolved by simultaneously adding warm 1,4-dioxane (50 mL), and 50 mL of freshly filtered (under nitrogen), warm 0.5M barium hydroxide. The mixture was heated under reflux under nitrogen at 110–115° (oil-bath temperature) for 18 h. After cooling, carbon dioxide was bubbled in to precipitate barium carbonate, and the mixture was filtered with the aid of additional water. The filtrate was concentrated to low volume and applied to a column (2.25 cm, inside diameter) filled with 150 mL of Amberlite CG-50 (100–200 mesh, NH_4^+ form) ion-exchange resin. Gradient elution with 0.1–0.5M ammonium hydroxide gave homogeneous **47** (t.l.c. on silica gel with 3:1:1:1 methanol–chloroform–ammonium hydroxide–water). After evaporation of the eluates, the product was dissolved in a small amount of water, treated with charcoal, filtered, and acidified to pH 3.5 (pH meter) with dilute sulfuric acid. Methanol was added to turbidity and the mixture was chilled to give the sulfate salt of **47** (300 mg, 24%) as a white, amorphous powder. For analysis, the salt was dissolved in water and lyophilized; m.p. 255–260°, $[\alpha]_D^{25}$ +23.5° (*c* 1, water).

Anal. Calc. for $C_{13}H_{28}N_4O_6 \cdot 2H_2SO_4 \cdot 0.5\ H_2O$: C, 28.78; H, 6.13; N, 10.32; S, 11.71. Found: C, 28.69; H, 6.26; N, 10.58; S, 12.32.

The compounds listed in Table I were prepared essentially as described for **47**, and purified by chromatography on CG-50 (NH_4^+) resin by elution with 0.1–1.0M NH_4OH. Lyophilization of the sulfate salts gave more-consistent analytical data.

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