THE AMINOGLYCOSIDE ANTIBIOTICS. IV SYNTHESIS OF AMINODEOXY ANALOGS OF NEAMINE

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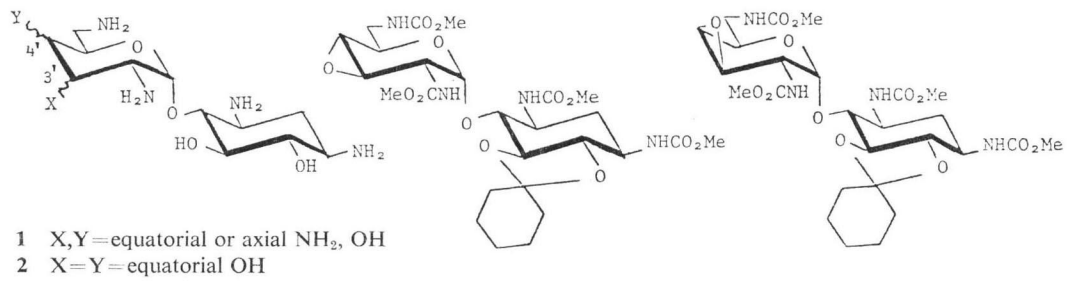
An efficient synthesis of the key 3′,4′-*galacto* epoxide intermediate **4** obtained from the known 5,6-O,O′-cyclohexylidene-N,N′-bis-(methoxycarbonyl)-4-O-[2,6-dideoxy-2,6-bis(methoxycarbonylamino)-α-D-glucopyranosyl]-2-deoxystreptamine (**5**) is described. Treatment of this epoxide with sodium azide, followed by reduction and acetylation, yielded the protected 4′-amino-4′-deoxyneamine **18** (3′,4′-diequatorial), whereas treatment with ammonia followed by acetylation yielded the protected 3′-amino-3′-deoxyneamine analog **19** with a diaxial configuration of its 3′ and 4′ positions. Reaction of the previously described protected 3′,4′-*allo* epoxide **3** with sodium azide yielded separable mixtures of the protected 3′-amino-3′-deoxyneamine **14** and the protected diaxial 4′-amino-4′-deoxyneamine isomer **13**, the ratios of products depending on the solvent and reaction temperature. Structural assignments for **13**, **14**, **18** and **19** were based on their PMR spectra. An additional 4′-amino-4′-deoxyneamine analog (**24**) with an axial configuration at its 4′ position was also prepared by azide displacement of an appropriately protected 4′-methanesulfonyl neamine intermediate **10**. The five protected isomers were deblocked to yield a series of aminodeoxyneamine analogs (**15**, **16**, **20**, **21** and **25**), all of which were less active *in vitro* than neamine against a group of Gram-positive and Gram-negative bacteria.

As part of a program designed to study the effects of structural modifications on the antibacterial activity of aminoglycosides,[1~4] we synthesized a series of analogs (**1**) of the pseudodisaccharide neamine (**2**) modified at its 3′ and 4′ positions. Other workers have shown that the removal of oxygen from these positions produces antibiotics with enhanced activity against resistant organisms containing



1 X,Y=equatorial or axial NH_2, OH
2 X=Y=equatorial OH

3 **4**

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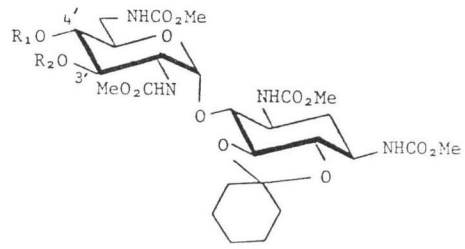
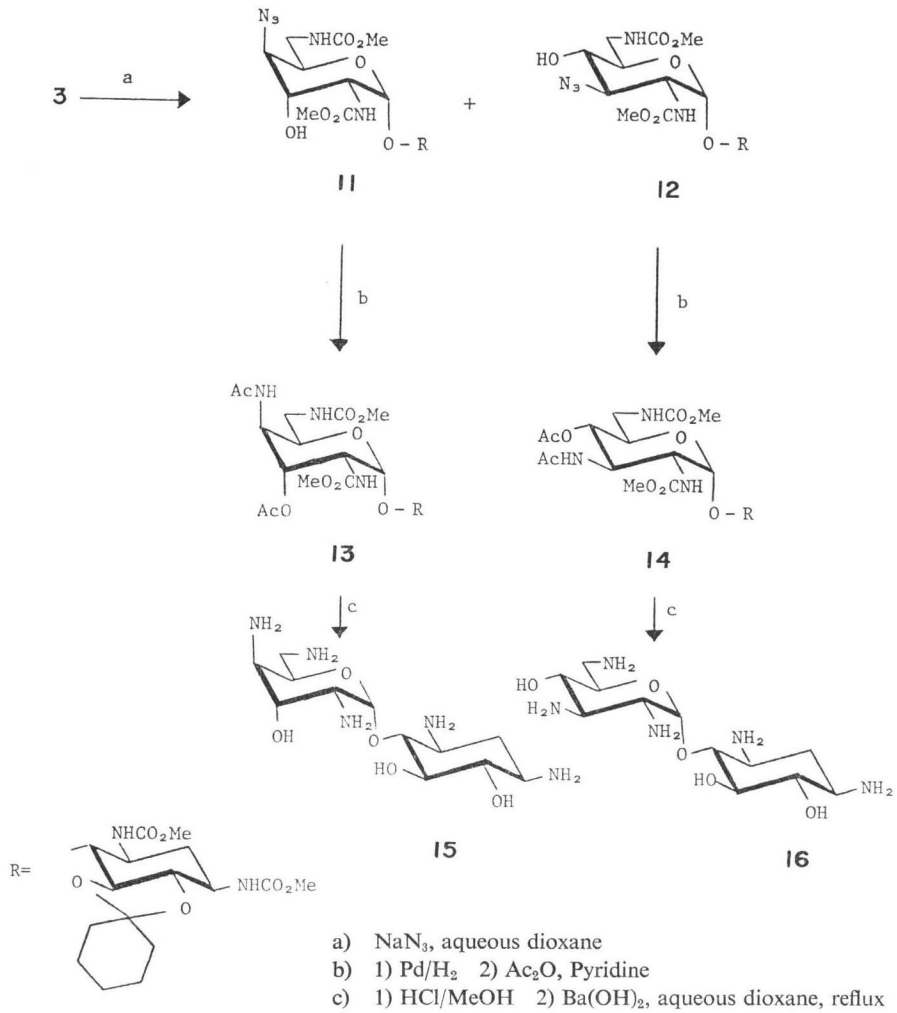
R-factor mediated phosphorylating enzymes.[5~8] Although **2** itself exhibits only weak antibacterial activity, it also displays lower toxicity than typical pseudotrisaccharides such as kanamycin or gentamicin.[8] Since the specific activity of many aminoglycosides parallels the number of amino groups, especially at the 2′ and 6′ positions,[6~9] substitution of an amino for a hydroxyl group at other positions might serve to inhibit enzymatic inactivation or improve specific activity. On this basis, a group of 3′- or 4′-amino-3′- or 4′-deoxyneamine analogs was synthesized



5 $R_1=R_2=H$
6 $R_1=H$, $R_2=Tos$
7 $R_1=Tos$, $R_2=H$
8 $R_1=H$, $R_2=C_6H_5CO$
9 $R_1=R_2=C_6H_5CO$
10 $R_1=Mes$, $R_2=C_6H_5CO$

Scheme 1.



a) NaN_3, aqueous dioxane
b) 1) Pd/H_2 2) Ac_2O, Pyridine
c) 1) HCl/MeOH 2) $Ba(OH)_2$, aqueous dioxane, reflux

both for biological evaluation and for use as intermediates for pseudotrisaccharide preparation.

Access to four of these neamine analogs was provided through two key intermediate epoxides, **3** and **4**, prepared from the known protected tetracarbomethoxyneamine **5**[5]. As described in a previous paper,[2] *allo* epoxide **3** was prepared by methoxide catalyzed elimination of the 3′-tosylate group of **6**, which was obtained by selective tosylation of diol **5**. Although *galacto* epoxide **4** could be obtained by methoxide treatment of the isomeric tosylate **7** (isolated as a minor product in the tosylation of **5**), a more efficient route was needed for large scale preparation of **4**. Consequently, **5** was reacted with an excess of benzoyl chloride in pyridine at low temperature to give mono-benzoate **8**, in good yield, along with some di-benzoate **9**. Amorphous **8** was readily separated from the more soluble **9**

Scheme 2.

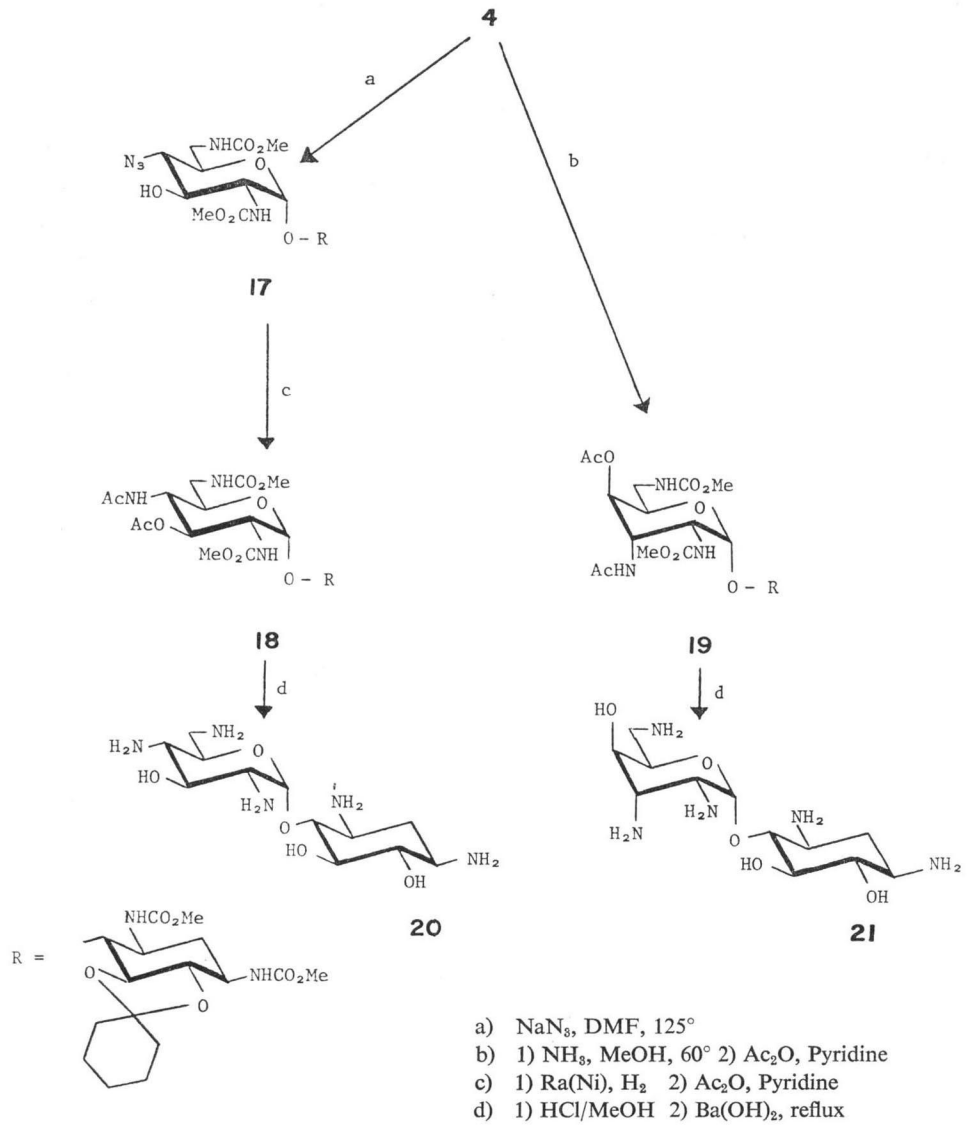
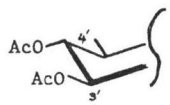
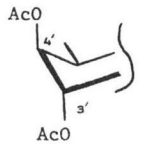
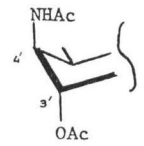
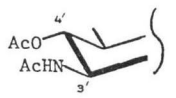
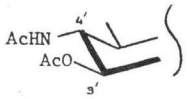
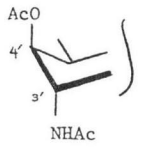
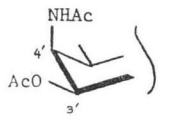


Table 1. Chemical shift assignments of acetoxy and acetamido methyl groups of 3′ and 4′ positions of tetracarbomethoxy cyclohexylidene neamines. ($CDCl_3$ relative to TMS).

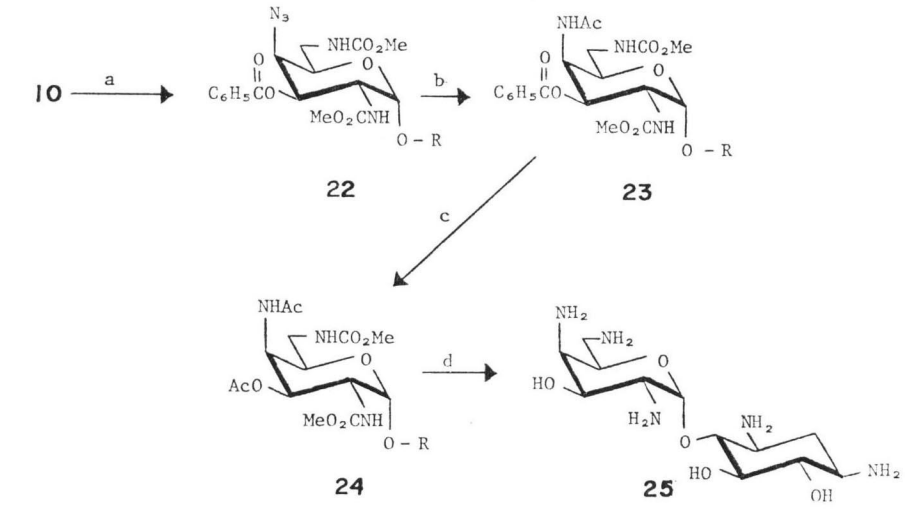
		Chemical shift 3′	4′
		2.00[2]	2.03[2]
		2.08[2]	2.14[2]
	(13)	2.10	2.06
	(14)	1.90	2.06
	(18)	2.03	1.94
	(19)	2.16	2.13
	(24)	2.01	2.03

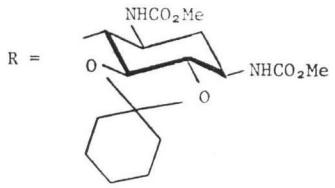
by precipitation from ether-petroleum ether. Since only one mono-ester could be detected in the product, any 4′-mono-benzoate formed in the reaction must have undergone further benzoylation to **9**. Mesylation of **8**, followed by methoxide treatment of the product (**10**), yielded the desired *galacto* epoxide **4**, readily distinguishable from the *allo* isomer (**3**) by TLC (silica, acetonitrile - ether, 1: 1) and HPLC (Microporasil, $CHCl_3$ - MeOH, 95: 5). Although none of the intermediates were crystalline, the amorphous epoxide **4**, which was obtained by precipitation, was analytically and chromatographically pure and its preparation amenable to large scale work.

Treatment of **3** with sodium azide (Scheme 1) in 80% dioxane-water at 88°C provided azido alcohols **11** and **12** in a 4: 1 ratio which were reduced and acetylated to give **13** and **14** respectively. The structures of **13** and **14** were assigned with the aid of proton magnetic resonance (PMR) (Table 1) by comparing their spectra with those of reference compounds and literature data as described in an earlier paper.[2] The diaxial structure was assigned to the isomer (**13**) with the lower field chemical shifts for its N- and O-acetyl methyl resonances. Thus, at 88°C, ring opening proceeded in the expected "normal" diaxial manner as reported by other workers for 3,4-*allo* epoxides.[10~12] However, treatment of **3** with sodium azide in 60% dioxane-water at 115°C resulted in a reversal of the product ratio to 1: 2 and allowed enhanced production of **12** with the desired diequatorial configuration. Sequential acid and base hydrolysis of **13** and **14**, followed by chromatography on CG-50 (NH_4^+) resin, yielded the aminodeoxyneamine analogs **15** and **16** which were isolated as their respective sulfates.

Treatment of *galacto* epoxide **4** (Scheme 2) with sodium azide at 125°C in DMF yielded **17** which was characterized by conversion to crystalline **18**. In contrast, the isomeric diacetyl derivative **19** was obtained when the epoxide group of **4** was opened with ammonia at 60°C and the product acetylated. Again, the diequatorial structure was assigned to isomer **18** because it exhibited higher field chemical shifts (PMR) for its acetyl methyl protons than isomer **19** (see Table 1). Examination of the mother liquors from the respective crystallizations of **18** and **19** respectively indicated that the azide opening at high temperature proceeded with high selectivity (95%) whereas the opening with ammonia at low temperature favored the diaxial "normal" product in only a 2: 1 ratio. Deblocking of **18** and **19**

Scheme 3.





a) NaN_3, DMF, reflux
b) 1) Pd/H_2 2) Ac_2O, Pyridine
c) 1) K_2CO_3/MeOH 2) Ac_2O, Pyridine
d) 1) HCl/MeOH 2) $Ba(OH)_2$, reflux

provided the aminodeoxyneamine analogs **20** and **21**, respectively, isolated as their amorphous sulfate salts.

An additional 4′-amino-4′-deoxyneamine isomer (**25**, 4′-axial, Scheme 2) was obtained *via* azide displacement of the 4′-methanesulfonyl group of the protected neamine intermediate **10** followed by reduction and acetylation to give **23**. Again, confirmation of the structure of the displacement product was based on the PMR chemical shifts (Table 1) of its corresponding diacetyl derivative **24**. Deblocking of **24** yielded **25** which was isolated as its sulfate salt.

All five analogs exhibited only modest *in vitro* activities when tested against four strains of Gram-positive (*Staphylococcus aureus*, *Streptococcus faecalis*) and eleven strains of Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Shigella paradysenteriae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Proteus morganii*, *Enterobacter aerogenes*, *Enterobacter cloacae*). The 4′-amino-4′-deoxy analog **20** was the most active. Its spectrum of activity was similar to that of neamine (**2**), but its specific activities [minimum inhibitory concentrations (MIC's) 25～200 μg/ml] were one-half to one-quarter those of neamine (MIC's 6.3～50 μg/ml). The 3′-amino-3′-deoxy analog **16**, as well as the diaxial analogs **15** and **21** and the axial-equatorial analog **25**, were significantly less active. None of them inhibited the bacterial strains tested at concentrations below 200 μg/ml.

Experimental Section

Column chromatography was carried out on J. T. Baker silica gel (60～200 mesh). Proton magnetic spectra (PMR) were run on a Varian T-60 instrument using TMS as standard. Mass spectra were

obtained from a Perkin-Elmer RMU-6 or a Varian CH5 instrument. Infrared spectra were run on a Perkin-Elmer Infracord Model 137. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was run on Uniplate precoated silica gel plates, 250 micron (Analtech, Inc. Newark, Delaware). Spots were detected by charring with H_2SO_4 or by ninhydrin where applicable. Antibacterial activities were obtained on sulfate salts by an agar dilution method in Penassay seed medium at pH 8.0.

4-O-[3-O-Benzoyl-2,6-dideoxy-2,6-bis[(methoxycarbonyl) amino]-α-D-glucopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**8**)

Benzoyl chloride (53.7 g, 0.38 mol) was added dropwise with stirring to a solution of 4-O-(2,6-dideoxy-2, 6-bis[(methoxycarbonyl)amino]-α-D-glucopyranosyl]-5, 6-O, O′-cyclohexylidene-N, N′-bis (methoxycarbonyl)-2-deoxystreptamine[5], **5** (106.5 g, 0.17 mol), in dry pyridine (600 ml) while maintaining the temperature at −10°C with external cooling. When the TLC of an aliquot ($CHCl_3$ - MeOH, 15: 1) indicated complete consumption of **5**, water (100 ml) was slowly added to the milky suspension followed by saturated sodium bicarbonate solution (1 liter). The solution was extracted twice with ethyl acetate (2 liters) and the combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was triturated with ether (500 ml) and the crude solid was dissolved in ethyl acetate (200 ml) and precipitated from ether-petroleum ether (400 ml, 1: 1) to give amorphous **8** (84.1 g, 68%); $[\alpha]_D^{25}$ +67.4° (*c* 1, MeOH); IR (mull) 1709, 714 cm^{-1}; NMR (DMSO-d_6) δ 7.5~8.0 (5H, m, aromatic).

Anal: Calcd. $C_{33}H_{46}N_4O_{15}$: C, 53.65; H, 6.28; N, 7.58%
Found: C, 53.37; H, 6.44; N, 7.43%

4-O-[3-O-Benzoyl-2, 6-dideoxy-4-O-methanesulfonyl-2, 6-bis[(methoxycarbonyl) amino]-α-D-glucopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**10**)

Methanesulfonyl chloride (11 g, 0.095 mol) was added dropwise with stirring to a cooled (−10°C) solution of **8** (27.5 g, 0.037 mol) and triethylamine (15.1 g, 0.149 mol) in methylene chloride (350 ml) and tetrahydrofuran (120 ml). The mixture was stirred for 1 hour at room temperature, quenched with saturated sodium bicarbonate solution (400 ml) and extracted twice with methylene chloride (400 ml). The organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in methylene chloride (100 ml) and precipitated from petroleum ether (500 ml) to give **10** as an amorphous solid (26 g, 86%), $[\alpha]_D^{25}$ +32.5° (*c* 1, $CHCl_3$); IR (mull): 1709, 1176, 709 cm^{-1}; NMR ($CDCl_3$) δ 7.2~8.2 (5H, m, aromatic), 2.9 (3H, s, mesylate).

Anal: Calcd. $C_{34}H_{48}N_4O_{17}S$: C, 49.91; H, 5.92; N, 6.86; S, 3.93%
Found: C, 50.23; H, 5.90; N, 6.83; S, 4.02%

4-O-[3, 4-Anhydro-2, 6-dideoxy-2, 6-bis[(methoxycarbonyl)amino]-α-D-galactopyranosyl]-5, 6-O, O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**4**)

A solution of sodium (16.1 g, 0.7 g-atom) in dry methanol (500 ml) was added at room temperature to a solution of **10** (80 g, 0.098 mol) in chloroform (700 ml). After 2 hours the reaction was quenched with solid sodium bicarbonate (50 g) and then buffered with glacial acetic acid (30 ml). The mixture was concentrated to dryness *in vacuo*, the residue was partitioned between chloroform (400 ml) and water (400 ml), and the aqueous layer further extracted with chloroform (400 ml). The combined chloroform extracts were dried (Na_2SO_4) and concentrated to dryness *in vacuo*. The residue was dissolved in methylene chloride (400 ml) and precipitated from petroleum ether (4 liters) to yield **4** (49 g, 81%) as a hygroscopic amorphous solid, $[\alpha]_D^{25}$ +2.5° (*c* 1, $CHCl_3$).

Anal: Calcd. $C_{26}H_{40}N_4O_{13}$: C, 50.64; H, 6.54; N, 9.10%
Found: C, 50.50; H, 6.39; N, 8.98%

4-O-[4-Azido-2, 4, 6-trideoxy-2, 6-bis[(methoxycarbonyl)amino]-α-D-gulopyranosyl]-5, 6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**11**) and 4-O-[3-azido-2,3,6-trideoxy-2,6-bis[(methoxycarbonyl)amino]-α-D-glucopyranosyl]-5, 6-O, O′-cyclohexylidene-N, N′-bis(methoxycarbonyl)-2-deoxystreptamine (**12**)

Method A A solution of **3** (6.2 g, 0.01 mol), sodium azide (5.2 g, 0.08 mol) in H_2O (20 ml) and

dioxane (80 ml) was heated at 88°C for 24 hours. The solvents were evaporated and the residue partitioned between brine and EtOAc. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated to an oily solid (7.1 g). TLC ($CHCl_3$ - MeOH, 95: 5) indicated a mixture of a major (**11**, Rf 0.55) and one minor product (**12**, Rf 0.45). The mixture was chromatographed on Florisil (220 g) with a $CHCl_3$ - MeOH gradient (0 to 2%). The major isomer, **11**, was precipitated from acetone-petroleum ether to give an amorphous solid (2.9 g, 44%); $[\alpha]_D^{25}-2.3°$ (*c* 1, $CHCl_3$); IR (Nujol) 2114 cm^{-1} (N_3).

Anal: Calcd. $C_{26}H_{41}N_7O_{13}$: C, 47.34; H, 6.26; N, 14.86%
Found: C, 47.16; H, 6.45; N, 14.43%

The minor isomer, **12**, was also obtained after precipitation as an amorphous solid (0.7 g, 11%); $[\alpha]_D^{25}+4.1°$ (*c* 1, $CHCl_3$); IR (Nujol) 2114 cm^{-1}.

Anal. Found: C, 47.47; H, 6.46; N, 14.61%

Method B A solution of **3** (21.5 g, 0.035 mol) in dioxane (320 ml) and water (100 ml) was heated in an oil bath at 115°C and sodium azide (36 g, 0.55 mol) dissolved in a minimum amount of water was added in one portion. The oil bath was held at 115~118 °C for 18 hours, and the reaction worked up as described in Method A, to yield **11** (4.4 g, 19%) and **12** (7.9 g, 34%).

4-O-[4-Acetamido-3-O-acetyl-2, 4, 6-trideoxy-2,6-bis[(methoxycarbonyl)amino]-α-D-gulopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**13**)

A mixture of **11** (0.5 g, 0.75 mmol), 10% palladium on charcoal (0.5 g), EtOAc (20 ml) and dioxane (20 ml) was shaken on a Parr apparatus (60 psi) for 22 hours. The filtered solution was concentrated to a semisolid which was dissolved in pyridine (5 ml) and acetic anhydride (2.5 ml) and left overnight at room temperature. The mixture was diluted with iced brine and EtOAc, and the organic extract was washed sequentially with 10% HOAc, H_2O, 5% $NaHCO_3$ solution and brine. The oily residue was chromatographed on Florisil (35 g) with a petroleum ether-EtOAc gradient to yield crystalline **13** (350 mg, 65%); m.p. 162~165°C ($CHCl_3$ - benzene - ether); $[\alpha]_D^{25}+7.5°$ (*c* 0.7, $CHCl_3$); NMR ($CDCl_3$) δ 2.06 (3H, s), 2.10 (3H, s).

Anal: Calcd. $C_{30}H_{47}N_5O_{15}$: C, 50.20; H, 6.60; N, 9.76%
Found: C, 49.80; H, 6.59; N, 9.52%

4-O-[3-Acetamido-4-O-acetyl-2, 3, 6-trideoxy-2, 6-bis[(methoxycarbonyl) amino]-α-D-glucopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**14**)

Azide **12** (0.5 g, 0.75 mmol) was reduced and acetylated as described for **11**, to give amorphous **14** (150 mg, 28%), precipitated from $CHCl_3$-ether-hexane; $[\alpha]_D^{25}+47.5°$ (*c* 1, $CHCl_3$); NMR ($CDCl_3$) δ 1.90 (3H, s), 2.06 (3H, s).

Anal: Calcd. $C_{30}H_{47}N_5O_{15}$: C, 50.20; H, 6.60; N, 9.76%
Found: C, 49.89; H, 6.68; N, 9.78%

4-O-(2,4,6-Triamino-2,4,6-trideoxy-α-D-gulopyranosyl)-2-deoxystreptamine (**15**)

A solution of **13** (710 mg, 1 mmol) and 3 N HCl (15 drops) in MeOH (15 ml) was stirred at room temperature for 3 hours and concentrated to dryness. The residue was dissolved in dioxane (30 ml) and freshly filtered 1 N barium hydroxide (30 ml). The solution was refluxed under nitrogen for 18 hours and then diluted with H_2O (50 ml). Carbon dioxide gas was introduced to precipitate barium carbonate. The resulting suspension was filtered with the aid of additional H_2O and the filtrate and washings concentrated to dryness. The residue was chromatographed on Amberlite CG-50 (NH_4^+) resin with an NH_4OH gradient (0 to 0.3 N) to yield **15** as its free base (185 mg, 57%). The sulfate salt was prepared by dissolving the product in a minimum volume of 50% aqueous MeOH, treating with charcoal, and adjusting to pH 3.5 (pH meter) with 0.5 N H_2SO_4. The white solid that resulted on cooling was collected to give **15** (145 mg), m.p. 240~250°C d; $[\alpha]_D^{25}+69.3°$ (*c* 0.8, H_2O).

Anal: Calcd. $C_{12}H_{27}N_5O_5 \cdot 2.5H_2SO_4$: C, 25.44; H, 5.69; N, 12.36%
Found: C, 25.17; H, 6.09; N, 11.54%

4-O-(2,3,6-Triamino-2,3,6-trideoxy-α-D-glucopyranosyl)-2-deoxystreptamine (**16**)

Compound **14** (900 mg, 1.25 mmol) was deblocked in the same manner as described for **13**, to yield **16** as its free base (255 mg, 63%) which was converted to its sulfate salt; $[\alpha]_D^{25}+61.5°$ (*c* 0.5, H_2O).

Anal: Calcd. $C_{12}H_{27}N_5O_5 \cdot 2.5\ H_2SO_4$: C, 25.44; H, 5.69; N, 12.36%

Found: C, 24.70; H, 6.40; N, 11.71%

4-O-[4-Azido-2,4,6-trideoxy-2,6-bis[(methoxycarbonyl)amino]-α-D-glucopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**17**)

A mixture of **4** (20 g, 0.032 mol) and sodium azide (20 g, 0.31 mol) in DMF-H_2O (9: 1, v/v, 200 ml) was stirred at 125°C (oil bath) overnight and then allowed to cool to room temperature and poured into ice-water (2 liters). The product was extracted into ethyl acetate (2×1 liter). The combined organic extracts were washed with water (200 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on silica with chloroform - methanol to give amorphous **17** (7.1 g, 33%); $[\alpha]_D^{25}$ +31.2° (*c* 1, $CHCl_3$); IR (CH_2Cl_2) 2110 cm^{-1}.

Anal: Calcd. $C_{26}H_{41}N_7O_{13}$: C, 47.34; H, 6.26; N, 14.86%
Found: C, 47.46; H, 6.41; N, 14.62%

4-O-[4-Acetamido-3-O-acetyl-2,4,6-trideoxy-2,6-bis[(methoxycarbonyl)amino]-α-D-glucopyranosyl] 5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**18**)

A solution of **17** (6.6 g, 0.01 mol) in ethanol (250 ml) was hydrogenated (50 psi) overnight at room temperature with RANEY nickel (2 g), filtered and concentrated *in vacuo*. The residue was dissolved in pyridine (35 ml) containing acetic anhydride (5 ml) and allowed to stand overnight at room temperature. The reaction mixture was concentrated to dryness *in vacuo*, the residue evaporated with toluene, and the product crystallized from ethanol to give **18** (4.0 g, 56%); m.p. 248~250°C; $[\alpha]_D^{25}$ +75.0° (*c* 1, MeOH); NMR ($CDCl_3$+DMSO-d_6) 1.95 (3H, s) and 1.83 (3H, s); MS *m/e* 717 $(M)^+$.

Anal: Calcd. $C_{30}H_{47}N_5O_{15}$: C, 50.20; H, 6.60; N, 9.76%
Found: C, 49.93; H, 6.51; N, 9.89%

4-O-[3-Acetamido-4-O-acetyl-2, 3, 6-trideoxy-2, 6-bis[(methoxycarbonyl) amino]-α-D-gulopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (**19**)

A solution of **4** (20 g, 32.4 mmol) in methanol (2 liters) and concentrated NH_4OH (600 ml) was heated at 60°C in a sealed bomb overnight and then concentrated to dryness *in vacuo*. The residue was acetylated using pyridine (130 ml) and acetic anhydride (38 ml) as described for **18** and the residue on evaporation was crystallized from ethyl acetate and then from ethanol to yield **19** (4.6 g, 26%); m.p. 191~193°C; $[\alpha]_D^{25}$ +17.9° (*c* 1, $CHCl_3$); MS *m/e* 717 $(M)^+$; NMR ($CDCl_3$) δ 2.16 (3H, s) and 2.13 (3H, s).

Anal: Calcd. $C_{30}H_{47}N_5O_{15}\cdot\frac{1}{2}H_2O$: C, 49.58; H, 6.65; N, 9.64%
Found: C, 49.43; H, 6.62; N, 9.62%

4-O-(2,4,6-Triamino-2,4,6-trideoxy-α-D-glucopyranosyl)-2-deoxystreptamine (**20**)

A suspension of **18** (2.5 g, 3.4 mmol) in methanol (100 ml) containing 3 N HCl (3 ml) was stirred at room temperature for 1 hour, and the clear solution was neutralized with saturated aqueous barium hydroxide and evaporated to dryness. The residue was dissolved in water (125 ml) and extracted twice with ether. After adding barium hydroxide octahydrate (14.3 g), the aqueous solution was refluxed overnight, neutralized with CO_2 at 100°C and filtered while hot. The precipitate was washed with hot water and the combined filtrates were concentrated *in vacuo* to 75 ml. The concentrate was neutralized to pH 6 with dilute sulfuric acid, filtered, and passed through a column of IRC-50 resin (70 ml) in the ammonium cycle. The column was eluted with an NH_4OH gradient (0~0.5 M) and fractions homogeneous on TLC (CH_3CN - H_2O - HOAc 50: 50: 2) were collected and concentrated *in vacuo*. The residue was dissolved in water (50 ml), neutralized to pH 6 with dilute sulfuric acid and lyophilized to yield (**20**) as its sulfate salt (1.0 g, 50%), $[\alpha]_D^{25}$ +79.2° (*c* 1, H_2O).

Anal: Calcd. $C_{12}H_{27}N_5O_5\cdot 1.4\ H_2SO_4\cdot 0.5\ H_2O$: C, 30.81; H, 6.63; N, 14.97%
Found: C, 31.24; H, 6.68; N, 14.96%

4-O-(2,3,6-Triamino-2,3,6-trideoxy-α-D-gulopyranosyl)-2-deoxystreptamine (**21**)

The procedure used to prepare **20** was used to deblock **19** (2.2 g, 3.1 mmol) giving **21** as its sulfate salt (1.1 g, 68%); $[\alpha]_D^{25}$ +57.0° (*c* 1, H_2O).

Anal: Calcd. $C_{12}H_{27}N_5O_5\cdot 2H_2SO_4\cdot H_2O$: C, 26.91; H, 6.21; N, 13.07%
Found: C, 26.81; H, 5.75; N, 12.90%

4-O-[4-Azido-3-O-benzoyl-2,4,6-trideoxy-2,6-bis[(methoxycarbonyl)amino]-α-D-galactopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (22)

A mixture of **10** (10.0 g, 12.2 mmol) and sodium azide (4 g, 61 mmol) was refluxed in DMF (240 ml) for 2 hours. The cooled reaction mixture was poured into water (700 ml), and the resulting precipitate was collected and dissolved in methylene chloride (200 ml). This solution was dried (Na_2SO_4), concentrated to 100 ml *in vacuo* and added dropwise to a stirred mixture of ether - petroleum ether (1: 1, 1 liter). The crude product (6.2 g) was chromatographed on silica using ethyl acetate - petroleum ether (1: 1) to give **22** as an amorphous solid after precipitation as described above (3.7 g, 40%); $[\alpha]_D^{25}$ −40.7° (*c* 1, $CHCl_3$); IR (CH_2Cl_2) 2105 cm^{-1}.

Anal: Calcd. $C_{33}H_{45}N_9O_{14}\cdot H_2O$: C, 51.29; H, 6.00; N, 12.68%
Found: C, 51.60; H, 5.97; N, 12.35%

4-O-[4-Acetamido-3-O-benzoyl-2, 4, 6-trideoxy-2, 6-bis[(methoxycarbonyl) amino]-α-D-galactopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (23)

Azide **22** (3 g, 3.9 mmol) was hydrogenated and acetylated by the procedure used in the preparation of **18**. Chromatography of the crude product on silica (chloroform - isopropanol, 98: 2) yielded amorphous **23** (2.0 g, 66%); $[\alpha]_D^{25}$ +9.9° (*c* 2, $CHCl_3$); MS *m/e* 779 $(M)^+$.

Anal: Calcd. $C_{35}H_{49}N_5O_{15}\cdot H_2O$: C, 52.69; H, 6.44; N, 8.77%
Found: C, 52.25; H, 6.65; N, 8.69%

4-O-[4-Acetamido-3-O-acetyl-2,4, 6-trideoxy-2, 6-bis[(methoxycarbonyl)amino]-α-D-galactopyranosyl]-5,6-O,O′-cyclohexylidene-N,N′-bis(methoxycarbonyl)-2-deoxystreptamine (24)

A mixture of **23** (1.6 g, 2.1 mmol) and potassium carbonate (0.5 g) in methanol (80 ml) was stirred at room temperature overnight, filtered and concentrated *in vacuo*. The residue was acetylated in pyridine (70 ml) and acetic anhydride (2 ml) at room temperature overnight. After evaporation to dryness *in vacuo*, the residue was dissolved in $CHCl_3$ and precipitated with hexane to yield **24** as an amorphous solid; $[\alpha]_D^{25}$ +58.4° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) δ 2.05 (3H, s), 2.00 (3H, s).

Anal: Calcd. $C_{30}H_{47}N_5O_{15}\cdot H_2O$: C, 48.91; H, 6.70; N, 9.50%
Found: C, 49.13; H, 6.47; N, 9.14%

4-O-(2,4,6-Triamino-2,4,6-trideoxy-α-D-galactopyranosyl)-2-deoxystreptamine (25)

The *galacto* analog **24** (0.8 g, 1.1 mmol) was deblocked by the procedure used for **18** to yield **25** (156 mg, 25%) as its sulfate salt; $[\alpha]_D^{25}$ +80.1° (*c* 1.0, H_2O).

Anal: Calcd. $C_{12}H_{27}N_5O_5\cdot 2.5H_2SO_4\cdot H_2O$: C, 24.66; H, 5.86; N, 11.98%
Found: C, 23.21; H, 5.91; N, 11.29; Ash, 5.66%
(Corrected for ash, C, 24.60; H, 6.26; N, 11.96%)

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